A MAGYAR BIOFIZIKAI TÁRSASÁG

XXIX. KONGRESSZUSA

2023. augusztus 28–31., Budapest

Program és absztrakt könyv

A Kongresszus honlapja: mbft.hu/kongresszus2023

Tartalomjegyzék

A MAGYAR BIOFIZIKAI TÁRSASÁG XXIX. KONGRESSZUSA

Budapest - 2023. augusztus 28–31.

TUDOMÁNYOS BIZOTTSÁG

KELLERMAYER MIKLÓS, ELNÖK (SE)

AZ MBFT SZEKCIÓK ELNÖKEI

CSIGE ISTVÁN (MTA ATOMKI)
HARMAT GYÖRGY
NAGY PÉTER (DE)
PANYI GYÖRGY (DE)
SÁFRÁNY GÉZA (OSSKI)
SMELLER LÁSZLÓ (SE)
SOLYMOSI KATALIN (ELTE)
VARGA ZOLTÁN (TTK)
VOZÁRY ESZTER (SZIE)

TOVÁBBI FELKÉRT SZEKCIÓSZERVEZŐK

BÓTA ATTILA (TTK)
DERÉNYI IMRE (ELTE)
GALAJDA PÉTER (SZBK)
HAJDU PÉTER (DE)
HORVÁTH RÓBERT (EK)
KELEMEN LÓRÁND (SZBK)
KRIZBAI ISTVÁN (SZBK)
LUKÁCS ANDRÁS (PTE)
SZÖLLŐSI GERGELY JÁNOS (ELTE)
VEREB GYÖRGY (DE)
VÁMOSI GYÖRGY (DE)
ZIMÁNYI LÁSZLÓ (SZBK)

SZERVEZŐBIZOTTSÁG

DERÉNYI IMRE, ELNÖK (ELTE)
BÓTA ATTILA (TTK)
GRAMA LÁSZLÓ (WEB, PTE)
HORVÁTH RÓBERT (EK)
PUSZTAINÉ HOLCZER MAGDOLNA (MBFT)
SOLYMOSI KATALIN (ELTE)

Köszöntő

Kedves Kollégák, kedves Barátaink!

Köszöntjük a Magyar Biofizikai Társaság XXIX. kongresszusának résztvevőit! E kétévente más-más hazai helyszínen megrendezett kongresszusnak idén a Természettudományi Kutatóközpont ad helyet Budapest Lágymányos nevű városrészén.

Az idei kongresszus, a hagyományokhoz híven ismét „megmozgatta” a magyar biofizikus és a biofizika után érdeklődő tudományos közösséget. A regisztrált résztvevők száma meghaladja a 150-et. Nyolc szekcióban 44 előadás hangzik el a nagyelőadóban, és 59 poszter tölti meg az aulát, ahol szép számmal vonulnak fel kiállító támogatóink is. A szekciók előadóit, közöttük számos fiatal biofizikust, a benyújtott összefoglalók alapján a szekciók elnökei választották ki.

A kongresszus meghívott plenáris előadói Vicsek Tamás és Garab Győző, akik mindketten kiemelkedő alakjai a magyar biológiai fizikai és biofizikai kutatásoknak. Vicsek Tamás az élőlények kollektív mozgásának leírásában, Garab Győző pedig a fotoszintézis folyamtának feltárásában ért el kimagasló eredményeket.

A nagyon színvonalasnak ígérkező tudományos program mellett igyekszünk vendégeink budapesti tartózkodását még kellemesebbé tenni Beri Áron gitárművész rövid koncertjével, a Zwack Múzeum meglátogatásával és a Trófea Grill Étteremben megtartandó gálavacsorával.

Szeretettel várjuk a biofizika és határterületei kutatóit, művelőit és a biofizika tudományával ismerkedő hallgatókat 2023. augusztus 28-31. között Budapesten!

Kellermayer Miklós
az MBFT elnöke

Derényi Imre
a szervezőbizottság elnöke

Támogatók

Magyar Biofizikai Társaság

Természettudományi Kutatóközpont

Eötvös Loránd Tudományegyetem

Cytosurge

Joó Katalin Kereskedelmi és Szolgáltató Kft.

Nanosurf AG

Image Science Kft.

Kvalitex Kft.

Unicam Magyarország Kft.

Zeiss Magyarország

abberior INSTRUMENTS

Superresolution STED
microscopy from its inventors

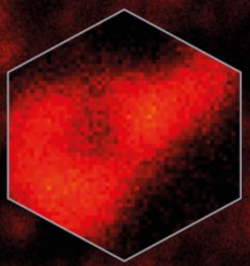
Facility Line

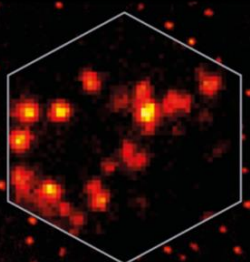
fastest time to result

Compact Line STEDYCON

expands any microscope to confocal & STED

„There are many reasons why your standard fluorescence microscope ...





... should be a STED“.

Stefan W. Hell, Nobel Laureate in Chemistry 2014

1 µm

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Információk

HELYSZÍN

A konferencia helyszíne a Természettudományi Kutatóközpont (TTK) Aulája és nagy előadóterme (Magyar Tudósok körútja 2. Budapest, 1117). A 2023. augusztus 28-i fogadás helyszíne a TTK Aulája. A Zwack Múzeum irányított vezetéssel 2023. augusztus 29-én 18:00-19:30 között látogatható a lenti részletek alapján (Cím: Dandár utca 1. Budapest, 1095). A gálavacsora 2023. augusztus 30-án 18:00-22:00 óra között kerül megrendezésre, helyszíne az Újbudai Trófea Grill Étterem (Hauszmann Alajos utca 6b. Budapest, 1117). A konferencia ebédek helyszíne az Ericcson étterme (Magyar Tudósok körútja 11, Budapest, 1117). Térképek a programfüzet utolsó oldalán találhatók.

KÖZLEKEDÉS

A TTK a belvárosból a 4-es vagy 6-os villamossal érhető el, a Petőfi híd Budai hídfő nevű megállónál kell leszállni, majd déli irányban az ELTE TTK és a TTK felé gyalogolni (300 m, kb. 4 perc), és a Magyar Tudósok körútjához érve jobbra kell fordulni, és az első piros, tégla és üveg épületig kell menni, mely az ELTE TTK mellett található.

ELŐADÁSOK

A programban jelzett időtartamok a vitát is tartalmazzák. Az előadóteremben kivetítő, illetve egy PC (Powerpoint programmal) áll az előadók rendelkezésére, melyre az előadások USB pendrive-ról átmásolhatók, legkésőbb a szekció előtti szünetben. Előzetes egyeztetés alapján saját laptopról is lehet vetíteni (HDMI és VGA csatlakozón keresztül).

POSZTERSZEKCIÓK

A posztertáblák teljes mérete 100x150 cm (álló formátumban). Az 1. poszterszekció (aug. 29. 15:30-17:00) poszterei a szekciót megelőzően bármikor kifüggeszthetők, viszont a szekció végén mindet le kell szedni.
A 2. poszterszekció (aug. 30. 15:30-17:00) poszterei pedig az 1. poszterszekció végétől (aug. 29. 17:00-től) kezdődően függeszthetők ki, és a kongresszus végéig fennmaradhatnak. Kérjük, hogy a poszterszekciók ideje alatt a szerző tartózkodjon a posztere mellett.

ÉTKEZÉS

A regisztráció három ebédet tartalmaz a 2023. augusztus 29-31. közötti időtartamra (kedd-csütörtök között). Az ebéd helyszíne Ericsson étterme (Magyar Tudósok körútja 11. Budapest, 1117), ahol menüválasztásos étkezésre van lehetőség. A menüválasztás magába foglal egy adag levest és egy adag főételt. Savanyúságot vagy desszertet az ebédjegy nem tartalmaz, ezeket igény esetén külön kell fizetni a kasszánál. Ebédelni a regisztrációkor kapott ebédjegyek leadásával lehetséges. 2023. augusztus 28-án este a nyitó fogadás a TTK Aulájában, míg a 2023. augusztus 30-i gálavacsora az Újbudai Trófea Grill Étteremben kerül megrendezésre. A részvételhez a névtáblákat a helyszínen fel kell mutatni. 2023. augusztus 29-én a vacsora egyénileg történik. A kávészünetekben cukrászati sós és édes aprósütemények, kávé, tea, szénsavas és szénsavmentes üdítő, ásványvíz fogyasztható.

TÁRSASÁGI PROGRAMOK ÉS HELYSZÍNEIK

2023. augusztus 28-án Beri Áron gitárművész ad rövid koncertet a TTK Előadótermében. 2023. augusztus 29-én a Zwack Múzeumot (Dandár utca 1. Budapest, 1095) látogatjuk meg. A Zwack Múzeum megközelíthető a mellékelt térkép szerint gyalogosan (1,7 km, kb. 25 perces séta), illetve a 4-es vagy 6-os villamossal vagy a 212-es busszal a Petőfi híd Budai hídfő megállóhelytől a Boráros tér megállóhelyig (1. megálló). Innen gyalogosan kb. 7 perc séta (600 m), vagy pedig a 2-es, 2B vagy 23-as villamossal

egy megálló a Haller utca/Soroksári út megállóig, ahonnan kb. 1 perc séta visszafelé a Boráros tér irányába. A Múzeumot egy rövid filmvetítést követően csoportokra osztva fogjuk megtekinteni. Az egyik csoport idegenvezetése angol nyelven lesz.
A gálavacsorát az Újbudai Trófea Grill Étteremben tartjuk 18-22 óra között. Az éttermet (Hauszmann Alajos utca 6b. Budapest, 1117) a mellékelt térkép szerint egyénileg kell megközelíteni. Gyalogosan 1.4 km (kb. 18 perc), de az 1-es villamossal az InfoPark megállótól 2 megálló (a Hauszmann Alajos utca/Szerémi úti megállóig).

Program

Áttekintő program

2023. AUGUSZTUS 28., HÉTFŐ

14:00 – 17:00	Regisztráció
17:00 – 19:00	Megnyitó, plenáris ülés
19:00 – 21:00	Nyitófogadás

2023. AUGUSZTUS 29., KEDD

08:30 – 10:30	1. szekció: **Molekuláris biofizika**
10:30 – 11:00	Kávészünet
11:00 – 12:30	2. szekció: **Modern biofizikai módszerek**
12:30 – 12:50	Támogatói előadás (Nanotemper)
12:30 – 14:00	Ebéd
13:20 – 14:00	Az MTA Biofizikai Bizottságának ülése
14:00 – 15:30	3. szekció: **Orvosi biofizika és sugárbiológia**
15:30 – 16:00	Kávészünet
15:30 – 17:00	**1. poszterszekció**
18:00 – 19:30	Zwack Múzeum látogatás

2023. AUGUSZTUS 30., SZERDA

08:30 – 10:30	4. szekció: **Membránok, membránfehérjék biofizikája**
10:30 – 11:00	Kávészünet
11:00 – 12:30	5. szekció: **Bioszenzorika és bio-nanotechnológia**
12:30 – 14:00	Ebéd
13:20 – 14:00	MBFT elnökségi ülés
14:00 – 15:30	6. szekció: **Sejtanalitika biofizikai megközelítéssel**
15:30 – 16:00	Kávészünet
15:30 – 17:00	**2. poszterszekció**
18:00 – 22:00	Gálavacsora (Trófea étterem)

2023. AUGUSZTUS 31., CSÜTÖRTÖK

09:30 – 11:00	7. szekció: **Bioenergetika és fotobiofizika**
11:00 – 11:30	Kávészünet
11:30 – 12:30	8. szekció: **Elméleti biofizika**
12:30 – 13:00	Záróünnepség
13:00 – 14:00	Ebéd
14:00 – 15:30	Szatellit szekció: Az ELTE Biológia Fizika Tsz. 25 éves jubileumi ülése

Részletes program

2023. AUGUSZTUS 28., HÉTFŐ

14:00 – 17:00	Regisztráció	
17:00 – 17:20	**Ünnepélyes megnyitó** Elnök: Derényi Imre Müller Viktor (ELTE TTK), Závodszky Péter (ELKH TTK), Kellermayer Miklós (MBFT)	
17:20 – 18:40	**Plenáris ülés** Elnök: Derényi Imre	
17:20 – 18:00	Vicsek Tamás *Swarms, flocks and crowds*	PE01
18:00 – 18:40	Garab Győző *The fascinating world of photosynthesis – best playground for biophysicists*	PE02
18:40 – 19:00	Művészeti program, Beri Áron gitárművész koncertje	
19:00 – 21:00	Nyitófogadás	

2023. AUGUSZTUS 29., KEDD

08:30 – 10:30	1. szekció **Molekuláris biofizika** Elnök: Smeller László, Vámosi György	
08:30 – 08:50	Kellermayer Miklós *Truncated titin is integrated into the human dilated cardiomyopathic sarcomere*	E01
08:50 – 09:10	Kele Péter *Bioorthogonally activated photoresponsive systems*	E02
09:10 – 09:30	Lukács András *Conformational flexibility in a photoactivated adenylate cyclase studied by fluorescence spectroscopy and solution X-ray scattering*	E03
09:30 – 09:45	Hajdú István *Allosteric mechanism in both the activity and regulation of Rho kinase 2*	E04
09:45 – 10:00	Balog-Molnár Andrea *Phosporylation alters the structure of surface bound titin molecules*	E05
10:00 – 10:15	Hoffka Gyula *Multiscale computational study on the binding of nirmatrelvir to SARS-CoV-2 main protease: possible pathways to resistance*	E06

10:15 – 10:30	Rehó Bálint *Investigation of the interactions of nuclear receptors by modern fluorescence microscopic methods*	E07
10:30 – 11:00	Kávészünet	
11:00 – 12:30	2. szekció **Modern biofizikai módszerek** Elnök: Lukács András, Vereb György	
11:00 – 11:15	Kelemen Lóránd *Single cell optical manipulation with deformable microtools*	E08
11:15 – 11:30	Nagy Krisztina *Emergence of bacterial phage resistance in complex stress landscapes*	E09
11:30 – 11:45	Nagy Máté *High-throughput collective animal behavior studies and their connections to artificial systems*	E10
11:45 – 12:00	Patkó Dániel *Understanding the root-microbe interactions*	E11
12:00 – 12:15	Fehér Bence *Modelling of small-angle scattering data on the absolute scale: investigation of model system NaPSS/DTAB and potentials for structural biophysics applications*	E12
12:15 – 12:30	Kovács Dóra Kinga *Nanoinjection of fluorescent nanoparticles to single live cells by robotic fluidic force microscopy*	E13
12:30 – 12:50	**Támogatói előadás**	
12:30 – 12:50	Pawel Kania (Nanotemper) *Characterize your most challenging interactions with two independent technologies. The New Monolith X.*	T01
12:30 – 14:00	Ebéd	
13:20 – 14:00	Az MTA Biofizikai Bizottságának ülése	
14:00 – 15:30	3. szekció **Orvosi biofizika és sugárbiológia** elnök: Csige István, Krizbai István	
14:00 – 14:20	Varga Zoltán *In vivo Biodistribution of Extracellular Vesicles: Developing Efficient Radiolabeling Techniques*	E14
14:20 – 14:40	Madas Balázs *Metastatic potential of HeLa-cells does not increase directly after radiation exposure*	E15

14:40 – 15:00	Lumniczky Katalin *Bone marrow-derived extracellular vesicles influence radiation-induced leukemogenesis*	E16
15:00 – 15:15	Dudás Tamás *Capillary pericytes regulate vascular tone and local blood flow in inflammation*	E17
15:15 – 15:30	Csige István *Radiation exposure of the skin from radon and its decay products*	E18
15:30 – 16:00	Kávészünet	
15:30 – 17:00	**1. poszterszekció**	
18:00 – 19:30	Zwack Múzeum látogatás	

2023. AUGUSZTUS 30., SZERDA

08:30 – 10:30	4. szekció **Membránok, membránfehérjék biofizikája** elnök: Bóta Attila, Hajdu Péter	
08:30 – 08:45	Böde Kinga *Lipid polymorphism of Photosystem II membranes – evidence of the role of isotropic lipid phase in membrane fusions*	E19
08:45 – 09:00	Gaál Szabolcs *Characterization of a novel mutation in Brugada Syndrome*	E20
09:00 – 09:20	Goda Katalin *Crosstalk between nucleotide and substrate binding in ABCG2*	E21
09:20 – 09:40	Bartók Ádám *Molecular strategies for heat detection by TRPM2 channels*	E22
09:40 – 09:55	Budavári Bálint *Development of liposomal corticosteroids*	E23
09:55 – 10:10	Tasvilla Sonallya *Systematic investigation and classification of host defence and cell penetrating peptides based on their affinity for interaction with extracellular vesicles*	E24
10:10 – 10:30	Pavela Olivér *The membrane binding mechanism and oligomerization of the antifungal protein NFAP2 with molecular dynamics simulations*	E25
10:30 – 11:00	Kávészünet	

11:00 – 12:30	5. szekció **Bioszenzorika és bio-nanotechnológia** elnök: Horváth Róbert, Kelemen Lóránd	
11:00 – 11:15	Bató Lilia *An obstacle-free microfluidic system for monitoring protein diffusion*	E26
11:15 – 11:30	Kovács Boglárka *Flagellin: a convenient protein in biosensorics*	E27
11:30 – 11:45	Kincses András *Lab-on-a-chip device for the monitoring of surface charge properties of confluent cell monolayers*	E28
11:45 – 12:00	Madarász Miklós *Transparent neural interfaces for simultaneous Ca2+ imaging and cortical electrophysiology in vivo*	E29
12:00 – 12:15	Szittner Zoltán *Label-free single-cell compatible biophysical methods in immune cell activation*	E30
12:15 – 12:30	Valkai Sándor *Could the SARS-CoV-2 S1 subunit cross the blood-brain barrier? – a lab-on-a-chip model study*	E31
12:30 – 14:00	Ebéd	
13:20 – 14:00	MBFT elnökségi ülés	
14:00 – 15:30	6. szekció **Sejtanalitika biofizikai megközelítéssel** elnök: Galajda Péter, Nagy Péter	
14:00 – 14:18	Végh Attila Gergely *The force awakens: mechanical interaction of metastatic tumor cells with the neurovascular unit*	E32
14:18 – 14:36	Nagy Péter *The effect of fluorescence labeling on the function and dynamical properties of antibodies*	E33
14:36 – 14:54	Varga Zoltán *Hollow Organosilica Beads: A Novel Reference Material for the Flow Cytometry Analysis of Extracellular Vesicles*	E34
14:54 – 15:12	Deli Mária *Modulation of brain endothelial surface charge changes the transfer of charged molecules and targeted nanoparticles*	E35
15:12 – 15:30	Czirók András *Transport kinetics of a small apolar drug candidate passing through a transwell barrier model – mathematical analysis and automated sampling*	E36

15:30 – 16:00	Kávészünet	
15:30 – 17:00	**2. poszterszekció**	
18:00 – 22:00	Gálavacsora (Trófea étterem)	

2023. AUGUSZTUS 31., CSÜTÖRTÖK

09:30 – 11:00	7. szekció **Bioenergetika és fotobiofizika** elnök: Solymosi Katalin, Zimányi László	
09:30 – 09:50	Horváth Gábor *Experimental study of the functions of zebra stripes: A new thermophysiological explanation*	E37
09:50 – 10:10	Zimányi László *Spectral and redox properties of a mouse cytochrome b561 protein suggest transmembrane electron transfer function*	E38
10:10 – 10:30	Bódis Emőke *Accelerated electron transfer and increased enzymatic activity in genetically modified photoactivable adenylate cyclase OaPAC*	E39
10:30 – 10:45	Sipos Áron *Machine learning analysis of ultrafast fluorescence kinetics of NADH conformations in solutions*	E40
10:45 – 11:00	Magyar Melinda *Rate-limiting steps in the dark-to-light transition of photosystem II: Dependence on the temperature and the lipidic environment of the reaction center*	E41
11:00 – 11:30	Kávészünet	
11:30 – 12:30	8. szekció **Elméleti biofizika** elnök: Szöllősi Gergely János	
11:30 – 11:50	Oz Kilim *Regularizing the combinatorial fitness landscape*	E42
11:50 – 12:10	Zafeiris Anna *Studying epistatic interactions in protein evolution with measurements and machine learning techniques*	E43
12:10 – 12:30	Szánthó Lénárd *Compositionally Constrained Sites Drive Long-Branch Attraction Artefact in Deep Phylogenomic Inferences*	E44

12:30 – 13:00	**Záróünnepség, poszterdíjak átadása**
13:00 – 14:00	Ebéd
14:00 – 15:30	Szatellit szekció **Az ELTE Biológia Fizika Tanszék 25 éves jubileumi ülése**

Poszterek listája

1. poszterszekció
2023. augusztus 29., kedd
15:30 – 17:00

Balázs Katalin *Acute and long-lasting immunological changes in prostate cancer patients treated with three different radiotherapy protocols*	P01
Balogh Anna *The FINmaj mutation results in hypophosporilation of the sarcomeric titin molecules*	P02
Barkó Szilvia *Direct binding of fluorescent vancomycin to MreB*	P03
Berekméri Evelin *Investigation of a red-footed falcon hub in Angola with deep learning*	P04
Bihariné Batta Ágnes *Improved estimation of the ratio of detection efficiencies of excited acceptors and donors for FRET measurements*	P05
Bőcskei-Antal Barnabás *Super-resolution investigation of liposomal nanosystems*	P06
Csányi Mária Csilla *Nanosurgical manipulation of extended von Willebrand factor multimers*	P07
Gráczer Éva Laura *Regulation of actin cytoskeleton dynamics through adhesion-molecule-mediated mechanotransduction*	P08
Győryné Galgand Kinga *Effect of ionizing radiation on the behaviour of miRNA binding proteins*	P09
Halmóczki Sarolta *A comprehensive review of various metal-containing nanoparticles in terms of their antibacterial effect and cytotoxic properties*	P10
Hársfalvi Jolán *Biophysical Characterization of Clot Retraction in Platelet Rich Plasma of Patients with Primary Anti-phospholipid Syndrome*	P11
Hoffka Gyula *Crystallographic and molecular dynamics simulations shed light on the self-inactivated conformation of the Venezuelan equine encephalitis virus (VEEV) protease*	P12
Homolya Szabolcs *Preliminary Experiments for the Rheological Study of Oleogels*	P13
Huber Tamás *Comparative Analyses of the Gelsolin Homology Domains of Gelsolin and Flightless-I*	P14

2. poszterszekció
2023. augusztus 30., szerda
15:30 – 17:00

Takács-Kollár Veronika *Investigation of actin polymerization: methodology and case study*	P31
Török György *Adaptive changes in the a-band region of the giant protein titin in diseased human cardiac sarcomere*	P32
Ujfalusi Zoltán *The effects of contrast agents on renal cell lines and on the actin cytoskeleton*	P33
Vozáry Eszter *Effect of Weight Loss on the Electrical Impedance Parameters of Lettuce and Iceberg Lettuce Stored at Room Temperature*	P34
Balogh Anna *Label-free tracking of cell adhesion kinetics as a function of various parameters*	P35
Farkasné Bebesi Tímea *Spectroscopic study of extracellular vesicles using plasmonic gold nanoparticles*	P36
Biró-Gyuris Katinka *Role of the hHv1 proton channel in vascular smooth muscle cells*	P37
Bóta Attila *Nanoerythrosome-based promising drug delivery systems*	P38
Czigléczki Janka Zsófia *Functionally important C-terminus of small GTPase Ran: exploring its nucleotide-specific conformational surface*	P39
Domingos Geraldo Jorge *Identification of inhibitors of the human hv1 proton channel*	P40
Farkas Enikő *Controlling Live Cell Adhesion through Characterization of Biofunctionalized Surfaces using Label-Free Biosensors*	P41
Ghofrane Medyouni *Role of ion channels in CAR T-cell*	P42
Jusztus Vivien *Ion channel expression of CD8+ T cells in ovarian cancer*	P43
Kashmala Shakeel *Isolation of novel peptide toxins from the venom of the scorpion Centruroides bonito which block Kv1.2 ion channel with picomolar affinity*	P44
Lambrev Petar *Light energy harvesting by photosystem I in cyanobacterial cells*	P45

Absztraktok

Előadás absztraktok

PEO1

Swarms, flocks and crowds

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Over the past 25 years collective motion has attracted increasing interest in the physics and biology communities. Motivations include the associated novel type of the “collision rule” (for physicists) as well as major technological advances regarding resources such as computational power or the collection of data. Observations of bacteria as they – thousands of them – exhibit rich group-motion patterns under the microscope in a Petri dish inspired an interpretation in terms of statistical physics.
It turned out that a simple rule could be used to interpret the behaviour and that variants of this rule could be introduced to describe group motion in a surprisingly wide variety of comoving units, from cells through animals to people, and very recently to swarms of aerial robots. The related results have been obtained by computer simulations, developing theoretical techniques and, naturally, carrying out experiments.
After an introduction to the basic examples and aspects of flocking, I shall present two recent case studies related to the i) role of random environment and ii) the dynamics of pattern formation during the segregation of two kinds of tissue cells.

PEO2

The fascinating world of photosynthesis – best playground for biophysicists

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In the 1970s scientists might have thought, paraphrasing De Gaulle, that 'biology is too serious a matter to be left to biologists'. Probably this view justified my employment in 1971, a physicist with fresh diplom, in the Laboratory of Photosynthesis of the BRC. Certainly, photosynthesis research demands teams with multidisciplinary approach and offers important mind-boggling questions. With the treasured support from my students and colleagues I had a few 'pet' topics of this kind; here, I'll dwell on two of them.

Photosystem II (PSII) uses light energy to oxidize water, providing us with an oxygenic atmosphere; by this means, it is the ultimate source of virtually all reducing equivalents in the Biosphere. Its activity is routinely monitored by recording the variable chlorophyll-a fluorescence (ChlF). The 'mainstream' interpretation of ChlF, which has never been free of controversies, is based on the 1963 two-state model ($PSII_O$, open and $PSII_C$ closed). We revealed the existence of $PSII_L$, the light-adapted closed state, with stabilized charges compared to $PSII_C$; and showed that the physical mechanism of ChlF must be laid on new grounds, in which intense steady-state and transient local electric fields and dielectric relaxation processes and protein memory effects play key roles.

The Fluid-Mosaic Model (FMM) of biological membranes – with the bulk lipid molecules organized into bilayer – provides a framework for the photosynthetic generation of proton motive force and its chemiosmotic utilization for ATP synthesis. However, FMM does not take into account that the major lipid species of thylakoid membranes (TMs) is non-bilayer lipid, and allows no room for the thoroughly documented non-bilayer lipid phases in TMs. DEM, the Dynamic Exchange Model, an extension of FMM, explains the presently available data on the basic features of the highly organized, intervowen vesicular TM system and its plasticity; DEM appears to apply also on the inner mitochondrial membranes.

E01

Truncated titin is integrated into the human dilated cardiomyopathic sarcomere

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Heteroyzgous truncating mutations in the TTN gene (TTNtv) encoding the giant protein titin are the most common genetic cause of dilated cardiomyopathy (DCM). However, the molecular mechanisms by which TTNtv mutations induce DCM are controversial. Here we investigated 118 clinically identified DCM human cardiac samples with next-generation sequencing, high-resolution gel electrophoresis, Western blot analysis and super-resolution microscopy in order to dissect the structural and functional consequences of TTNtv mutations. The occurrence of TTNtv was found to be 15% in the DCM cohort. Truncated titin proteins matching, by molecular weight, the gene-sequence predictions were detected in the majority of the TTNtv samples. The total amount of expressed titin, which includes the truncated fragments, was comparable in the TTNtv+ and TTNtv- samples, indicating that titin haploinsufficiency may not be the leading cause of the molecular pathogenesis. Proteomic analysis of washed cardiac myofibrils and STED super-resolution microscopy of myocardial sarcomeres labeled with sequence-specific anti-titin antibodies revealed that truncated titin is structurally integrated in the sarcomere. Sarcomere length-dependent anti-titin epitope position, shape and intensity analysis pointed at structural disarrangements in the I/A junction and the M-band of TTNtv+ sarcomeres, which may play a role, via faulty mechanosensor function, in the development of manifest DCM.

Acknowledgments

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EO2

Bioorthogonally activated photoresponsive systems

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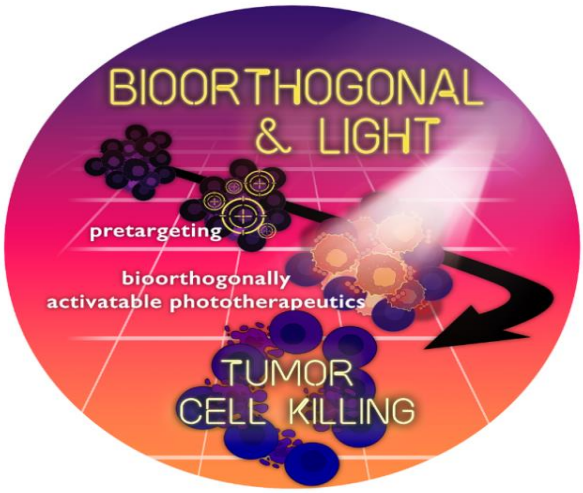
Photoresponsive materials offer excellent spatiotemporal control over biological processes and the emerging phototherapeutic methods are expected to have significant effects on targeted cancer therapies. Recent examples show that combination of photoactivatable approaches with bioorthogonal chemistry enhances the precision of targeted phototherapies and profound implications are anticipated particularly in the treatment of non-operable tumors. The extra level of on-target selectivity and improved spatial/temporal control considerably intensified related bioorthogonally assisted phototherapy research [1].

The Chemical Biology Research Group made several efforts toward the more efficient control of photoresponsivity using bioorthogonal reactions as triggers. The knowledge we gained upon the development of simple probes [2] through more complex red-emitting fluorogenic scaffolds [3] was applied to gain control of the photoresponsivity of photolabile protecting groups (photocages) that paves the way for the highly precise activation of drugs in response to a specific ligation reaction and light [4]. The concept of conditional photoactivation can be extended to more complex systems in the future. The presentation gives a brief overview of the milestones of this track.



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EO3

Conformational flexibility in a photoactivated adenylate cyclase studied by fluorescence spectroscopy and solution X-ray scattering

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The photoactivated adenylate cyclase from the photosynthetic cyanobacterium *Oscillatoria acuminata* OaPAC is a homodimeric enzyme comprising of a N-terminal domain that senses blue light using flavin (BLUF) [1] and a C-terminal class III adenylate cyclase (AC) domain that catalyses the formation of cAMP from ATP (adenosine triphosphate) [2,3]. cAMP is a universal regulator of metabolism and gene expression in all life forms [4]. Modulating the cellular concentration of cAMP has emerged in the focus of modern optogenetic applications and therapeutic approaches. Recent crystallographic studies have indicated that the activation mechanism of OaPAC involves only small movements. In this study [5], we apply small-angle X-ray scattering (SAXS) [6] and time-resolved solution X-ray scattering [7] in combination with other biophysical techniques to investigate the substrate induced-conformational changes of OaPAC in solution. The implications of our work to the function of the enzyme are discussed.

Acknowledgments

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EO4

Allosteric mechanism in both the activity and regulation of Rho kinase 2

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Rho-associated protein kinase 2 (ROCK2) is a membrane-anchored, long, flexible, multidomain, multifunctional protein. Our solution small-angle X-ray scattering (SAXS) study revealed that ROCK2 population is a dynamic mixture of folded and partially extended conformers. Binding of RhoA to the coiled-coil domain shifts the equilibrium towards the partially extended state. Enzyme activity measurements suggest that binding of natural protein substrates to the kinase domain breaks up the interaction between the N-terminal kinase and C-terminal regulatory domains. We observed, that besides RhoA, binding of bioactive lipids to the C-terminal lipid binding domains also play an important part in the activation of the enzyme.

ROCK2 phosphorylates both Amyloid-beta Precursor Protein (at Thr654) and the APP-cleaving enzyme BACE1 (at Ser498) regulating amyloidogenic processing. Using direct physical methods in combination with *in silico* approach, we found that BACE1 binds to ROCK2 with a low affinity (K_d=18 µM), while no binding of APP to ROCK2 alone could be detected. Strong association (K_d =3.5 nM) of APP to the weak ROCK2-BACE1 complex was observed, although no stable ternary complex was detected, i.e. BACE1 was displaced by APP. We constructed a sequential functional model: (1) BACE1 weakly binds to ROCK2 and induces an allosteric conformational change in ROCK2; (2) APP strongly binds to the ROCK2/BACE1 complex, and BACE1 is released (3) ROCK2 phosphorylates APP at Thr654 (leading to a longer stay in the early endosome during APP processing). Using mutational and fragmenting approach, we concluded that two binding sites are involved in the ROCK2/APP interaction: (1) The substrate binding groove, where the $APP_{646-664}$ sequence containing Thr654 sits (2) and the allosteric binding site, where the $APP_{665-695}$ sequence binds. These results open the way to attack the allosteric site to prevent APP phosphorylation without inhibiting the activity of ROCK2 towards its other substrates.

E05

Phosporylation alters the structure of surface bound titin molecules

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During muscle stretch, elastic or "passive" force develops which is mainly determined by the giant protein titin that forms the third filament system of muscle sarcomeres. The magnitude of this force is mainly dependent on the elasticity of the titin filaments, which is primarily determined by the structure of the polypeptide chain. However, it is suggested that post-translational modifications of titin, such as phosphokinase activity, regulate the sarcomeric passive force development. Mechanical studies on single myofibrils revealed that different protein kinases alter the passive tension of muscle, surprisingly, in opposite ways. This suggests that phosphorylation of sarcomeric proteins by various kinases is an essential regulatory mechanism of passive force. However, the extrapolation of these findings to titin's phosphorylation has not been studied so far at the single- molecule level. To reveal titin's structural alterations due to phosphorylation, single-molecule experiments must be carried out on individual titin molecules, where the effect of phosphorylation can be tested individually. In our work, we isolated individual titin molecules from rabbit and mouse skeletal muscle. The isolated native titin molecules showed high levels of phosphorylation, when stained with phosphoprotein gel stain. The isolated molecules were treated with lambda protein phosphatase to decrease the *in situ* phosphorylation level of the polymer. To investigate the effect of the treatment on titin's structure, we visualized surface-bound titin molecules by atomic force microscope and found that the terminal regions of dephosphorylated titins collapse into a compact, coiled structure. Our findings suggest that the structure of both I-band and terminal A-band region of titin is highly sensitive for phospohrylation which might play a role in controlling protein-titin interactions.

E06

Multiscale computational study on the binding of nirmatrelvir to SARS-CoV-2 main protease: possible pathways to resistance

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The pandemic caused by SARS-CoV-2 has resulted in millions of infections worldwide. Multiple vaccination strategies are available, but the number of the antivirals which are specific for SARS-CoV-2 is still limited. The Paxlovid is an antiviral combination that has been developed by Pfizer to treat non-hospitalized COVID-19 patients. It contains nirmatrelvir, a covalent inhibitor of the SARS-CoV-2 main protease (Mpro). Similar to the SARS-CoV-2 spike protein, the Mpro is also prone to mutations, which mutations may potentially interfere with binding of ligands, including nirmatrelvir, leading to the emergence of resistance.
In order to study the structural background of enzyme-inhibitor interactions, we examined the non-covalent interactions, as well as the reaction mechanism of SARS-CoV-2 Mpro with a peptide substrate and nirmatrelvir inhibitor. The interactions between the enzyme and the peptide substrate or nirmatrelvir were compared based on multiple crystal structures [1]. We mapped the crucial non-covalent interactions by applying molecular dynamics simulations for structural analyses. Mechanism of substrate cleavage and nirmatrelvir binding at atomic level were investigated by applying hybrid QM/MM ONIOM method. Previously described nirmatrelvir-resistant SARS-CoV-2 Mpro variants were also investigated to study the mechanism of resistance.
Our results contribute to a better understanding of the molecular mechanism underlying the substrate cleavage and nirmatrelvir binding. Based on a comprehensive analysis of our computational simulations, we propose how individual mutations of the active site may contribute to resistance development.

Acknowledgments

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EO7

Investigation the interactions of nuclear receptors by modern fluorescence microscopic methods

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Nuclear receptors are transcription factors regulating gene expression in a ligand dependent manner. They play a central role in cell differentiation, growth, and death. We investigated interactions and dynamics of retinoic acid receptor (RAR), Vitamin D receptor (VDR) and retinoid X receptor (RXR) in live cells. Their operation is described by the relatively static nuclear receptor molecular switch model [1]. Nowadays, this model is being changed for more dynamic ones (for example the “Hit and run” model [2]) due to intense investigations in the field.
We found previously that nuclear receptors compete for heterodimerization with their common partner, RXR, in a ligand-dependent manner [3]. To investigate potential competition in their DNA binding, we monitored the mobility of RAR and VDR in live cells by fluorescence correlation spectroscopy (FCS) [4][5].
Our FCS studies showed that there are two distinct populations of nuclear receptors present in the nucleus: in the fast population they are bound to the chromatin with shorter, whereas in the slow population with much longer residence times. In the case of RAR, agonist treatment or RXR co-transfection increased the fraction of the slow population, which is due to an increased stability of chromatin-binding or increased residence time. In contrast to RAR, the slow population of the VDR only increased in the presence of both agonist and RXR, so the chromatin binding of the VDR is stable only in a liganded, RXR-bound form. By the triple co-transfection of RAR, VDR and RXR, we showed that the competition between RAR and VDR for the binding of RXR appeared on the level of chromatin-binding, which may at least be partly responsible for the side effects of nuclear receptor targeted therapies.
Our results can help in the designing and optimizing nuclear receptor-based therapies and raise attention to the importance of competition between nuclear receptors.

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E08

Single cell optical manipulation with deformable microtools

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Task-specific microstructures can efficiently and versatilely support single cell research helping their actuation, deformation or even culturing. The ability to move and deform these microstructures provides an even broader spectrum of interaction with the cells. On the other hand, optical tweezers have already proven to be successful to operate such microtools with precise spatial and temporal control [1]. Here, we introduce a family of deformable microtools made by laser microfabrication and demonstrate their applicability in the manipulation of non-adherent mammalian cells. The tools are operated with optical tweezers via specific elastic elements incorporated to the otherwise rigid structure. The deformability enables them to take hold, carry and rotate the cells with the added benefit of being able to release them after their task is fulfilled. The achievable cell manipulation schemes are demonstrated with three types of structures. The first one selectively collects the cells in a suspension and transports them with minimal mechanical influence to a pre-defined location. The second one enables the precise microscopic observation of free-floating cells from any direction by holding them more firmly thus minimizing their fluctuation. The third system is shown to initiate spatio-temporally controlled cell-cell interaction applying a pair of microtools: one mounts the cell firmly to the substrate while the other carries a second cell and makes a contact between them. The elasticity of the presented microtools makes them applicable in a broad range of tasks in a microfluidic environment beyond single cell manipulation.

Acknowledgments

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E09

Emergence of bacterial phage resistance in complex stress landscapes

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Bacteriophages are the most abundant organisms on Earth in terms of particle numbers. As viruses, they coexist with microbes, playing a fundamental role in microbial diversity, population dynamics, and evolution. Understanding the interaction between phages and bacteria gives us essential information on ecological and evolutionary processes.
Here we used a microfabricated environment to reveal the importance of spatial structure and bacterial clustering in the evolution of resistance in *E. coli* against bacteriophage T4r. We created a stress landscape where phage titers are distributed across an array of localized metapopulations. Motile bacteria were able to move around and explore the precisely controlled landscape. The growth and distribution of a population were monitored by fluorescence time-lapse microscopy.
In this structured environment, resistant subpopulations emerged and spread from biofilm-like pockets within 1-2 days. After the experiments resistant bacteria were collected and selected by the replica plating method. Whole genome sequencing of insensitive bacterial clones was performed, and characteristic mutations were identified. Changes in the bacteriophage receptor ompC and several biofilm-related genes were found.

E10

High-throughput collective animal behavior studies and their connections to artificial systems

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Spectacular aerial displays of birds, the mesmerizing swirling of giant schools of fish or the rumbling gallop of hundreds of horses are fascinating examples of group behavior occurring in nature. During the presentation, we examine how these interesting phenomena are created through the interactions of individuals with each other and their environment, and how we can understand them with the help of physics and emerging technologies. What ways can we measure and quantify behavior using high-throughput methods and deep learning. Virtual reality for animals provides a tool to study decision making, and to test hypotheses through animals interacting with computational models. Can robots using artificial intelligence achieve similar (or even better) performance as animals? Let's see what we can learn from the biological insights and how we can use them to design improved artificial systems.

E11

Understanding the root-microbe interactions

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Modern agriculture is enabled by the heavy usage of irrigation, herbicides, pesticides and fertilisers. Such practises are not sustainable due to their ecological impact, and it is believed that engineering microbial interactions in soil could reduce the need for synthetic agrochemicals while preserving soil quality. Candidate microorganisms are known, but the challenge is to maintain lasting root colonisation. Hence, it is essential to gain a fundamental understanding on how root-microbe interactions evolve during the development of the root system.
Here we present various approaches for the screening of plant roots and rhizosphere interactions. We combine polymer matrix based artificial soil system, environmental control, a live quantitative imaging system and paper based microfluidic system for chemical sensing. The soil is designed to match the refractive index of its liquid solution to create an optically clear environment for imaging under realistic conditions [1,2]. A plastic waste based sensing soil was also developed to investigate pH changes in the vicinity of the root [3]. Patterned paper microfluidic system provides a simple, cost effective, easy to use way to investigate root exudates spatially and temporally. Using our system, we reveal the fascinating complexity of bacterial mobility in soil and reveal how chemical heterogeneity [3,4,5] created by the exudation of the root might explain these complex migrations. The combined application of optical and microfluidic technologies to crops is recent. Results in this study show there is great potential for such technologies to assist with the transition towards sustainable agriculture.

Acknowledgments

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E12

Modelling of small-angle scattering data on the absolute scale: investigation of model system NaPSS / DTAB and potentials for structural biophysics applications

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Polyelectrolyte / surfactant (P/S) complexes are important model systems of biologically relevant complexes such as DNA, RNA, protein / surfactant complexes. Due to the structural complexity of biologically relevant polyelectrolytes they are often modelled by synthetic polyelectrolytes in order to investigate the fundamental thermodynamics of the system. There are several studies aiming at describing the structural and phase properties of P/S systems. In the phase-separation region, examples of hexagonal, lamellar, and cubic structure have been identified. At the same time, there is little information about the structure of the complexes in the one-phase region and only a few studies were published dealing with the effect of surfactant concentration on the morphology of P/S aggregates.

In our study we prepared PSS/DTAB samples in the equilibrium and phase separation range. Since in the recent decades it has been clearly shown that the high charge density P/S mixtures are prone for the formation of kinetically arrested non-equilibrium aggregates, we used a novel sample preparation method to facilitate the formation of the equilibrium non-aggregated P/S complexes in the entire investigated surfactant concentration range. We measured the binding isotherm and simultaneously we performed small-angle X-ray scattering measurements and performed data modelling with least-square fitting method on absolute scale. We showed that by increasing the binding ratio the formed complexes exhibit an elongated to spherical transition which ends with well-defined 3 dimensional hexagonal precipitate with spherical particles in the crystal points. We also showed that the addition of indifferent electrolyte (NaCl) changes the structural behavior of the system and yields amorphous structure. Summarizing, first in the literature we connected the binding isotherm to the structural features of complexes in the equilibrium and phase separation regime.

Acknowledgments

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E13

Nanoinjection of fluorescent nanoparticles to single live cells by robotic fluidic force microscopy

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Direct injection of fluorescent nanoparticles into the cytoplasm of living cells can provide new insights into the intracellular fate of various different fluorescently labelled biologically active particles. Here we used fluorescent nanoparticles to prove the feasibility of nanoinjection into single live HeLa cells by using robotic fluidic force microscopy (FluidFM). This injection platform offers the advantage of high cell selectivity and efficiency. We confirmed the successful injection of both GFP encoding plasmids and GFP tagged fluorescent nanoparticles to the cells by confocal microscopy. We were able to track the nanoparticles in the living cells for 20 hours. The injected nanoparticles were initially localized in concentrated spot-like regions within the cytoplasm. Later, they were transported towards the periphery of the cells. Based on our proof-of-principle data, the FluidFM platform is suitable for targeting single living cells by fluorescently labelled biologically active particles and may lead to information about the intracellular cargo delivery at a single-cell level.

E14

In vivo Biodistribution of Extracellular Vesicles: Developing Efficient Radiolabeling Techniques

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The understanding of extracellular vesicle (EV) biodistribution plays an important role in advancing circulating biomarker research. Nuclear imaging techniques like single-photon emission computed tomography (SPECT) hold potential, but the literature on radioisotope labeling of EVs for in vivo studies remains scarce. This presentation explores the evolution of novel radiolabeling methods, focusing on the development and comparative evaluation of various Tc99m radiolabeling strategies.
Our initial method involved the radioisotope labeling of erythrocyte-derived EVs using the Tc99m-tricarbonyl complex [1]. In vivo SPECT/CT biodistribution studies in mice showed that intravenously administered Tc99m-labeled EVs primarily accumulated in the liver and spleen. Our observations suggested good in vivo stability, with a minor fraction of the radioactive label detaching from the EVs. Next, we explored an alternative approach using Tc99m-HYNIC-Duramycin to label cell-derived EVs [2]. Duramycin, a membrane-active peptide, specifically labels EVs, resulting in higher labeling efficiency. Following previous observations, significant uptake of EVs in the liver and the spleen was observed.
The latest experimental focus is on the use of recombinant proteins with His-tag in conjunction with the Tc99m-tricarbonyl complex to label EVs. Preliminary investigations indicate promising superior performance with this method compared to the previous techniques.
This presentation will provide an in-depth comparison of these methods, emphasizing their development process and potential implications for advancing in vivo EV imaging studies. Through a critical evaluation of their advantages and potential limitations, we aim to foster a greater understanding of efficient tracking of EV biodistribution for future research.

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E15

Metastatic potential of HeLa-cells does not increase directly after radiation exposure

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While radiation therapy increases local tumor control, it remains controversial whether ionizing radiation increases the metastatic potential of cancer cells. One of the potential mechanisms of radiation-induced metastasis is the direct release of tumor cells into the circulation requiring the detachment of the cells. The objective of the present study was to directly measure how ionizing radiation affects the kinetics of cellular adhesion, especially its initial stage after cell attachment on a biomimetic surface.
For this purpose, an automatic irradiation facility with gamma-radiation from Cs-137 has been developed providing parallel irradiation opportunity of 96 wells of a biosensor microplate with different doses. The employed optical biosensor records the wavelength shift of reflected light from a nanostructured waveguide, being proportional to the cell adhesion strength. Absorbed doses were measured by thermoluminescent dosimeters (TLDs) in each well. As a model system, a cervical cancer cell line (HeLa) was studied.
Three different experimental setups have been used distinguished by the sequence of irradiation and cellular attachment to the surface. The wavelength shift as the function of time was measured for different absorbed doses. The maximum wavelength shift as the function of dose was also analyzed.
The results show that adhesion of HeLa cells is not affected by ionizing radiation in the first hours after irradiation. The result is independent of whether cells are exposed during the adhesion process, in suspension, or attached. This suggests that radiation therapy does not directly increase the metastatic potential of cancer cells by decreasing their adhesion.
The experimental setup can be used to quantify the effects of ionizing radiation on cell adhesion as the function of time at different absorbed doses. It has been shown that ionizing radiation does not affect the adhesion of HeLa cells in the first hours after exposure, while experiments with longer follow-up are required to see whether adhesion changes at later time points.

E16

Bone marrow-derived extracellular vesicles influence radiation-induced leukemogenesis

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Haematological malignancies are considered the main long-term consequences of bone marrow (BM) irradiation. Ionizing radiation (IR) damages the stem and progenitor cells and alters signalling between the stem cell compartment and the BM stroma. The main objective of our work was to investigate extracellular vesicles (EVs)-mediated IR effects on leukaemogenesis after irradiation and to study possible underlying mechanisms using an *in vivo* murine model. Leukaemia incidence was followed in the CBA mouse model either irradiated or treated with EVs isolated from the BM supernatant of irradiated mice or subjected to both irradiation and EV treatment. Compared to spontaneous acute myeloid leukaemia (AML) incidence (below 1%), high dose (3 Gy) irradiation increased the incidence to 19%. EV treatment resulted in 4.5-6% leukaemia incidence with no significant difference between mice treated with EVs isolated from irradiated or non-irradiated animals. The combination of irradiation and EV treatment had an additive effect. Myeloid leukemias had two distinct phenotypes: a classical myeloblastic phenotype with a quick deterioration of the health status of the mice and increased bone marrow infiltration with myeloid blasts and a myelomonocytic phenotype with a slow clinical progression. EV treatment had a significant impact on leukaemia phenotype significantly increasing the frequency of the aggressive type myeloblastic leukemia. Lymphoid malignancies were also noted after irradiation and their incidence increased with the dose, though treatment of mice with EVs did not increase the incidence of this disease. In conclusion we showed that EVs influenced both the incidence and the phenotype of radiation-induced myeloid leukaemias but not of lymphoid ones. Our results also highlight the role of intercellular signalling mechanisms in radiation-induced leukemogenesis.

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E17

Capillary pericytes regulate vascular tone and local blood flow in inflammation

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Pericytes are the only contractile cells in cerebral capillaries. However, their role in the regulation of capillary diameter, microvascular tone and local cerebral blood flow is far from being completely understood. Furthermore, a large number of CNS disorders is accompanied by inflammatory processes. Therefore, in our present study, we investigated the role of pericytes in the maintenance of capillary tone and how inflammatory mediators could regulate pericyte contractility.

Using primary human pericytes in an in vitro collagen contraction assay, we could demonstrate that TNF-alpha, IL-6 and CCL2 induce a significant pericyte contraction. In order to prove that inflammatory mediators have similar effects in vivo, we used two photon microscopy in mice with labelled pericytes. Inflammatory mediators were administered in the vicinity of identified pericytes using microinjection techniques under continuous monitoring. TNF-alpha induced a slow but significant reduction in the capillary diameter. In addition, using line scan technology, we could show a decrease in red blood cell velocity and a reduction in the number of red blood cells passing the capillary segment in the neighbourhood of the injection. Furthermore, careful ablation of pericytes using the two-photon laser led to a late onset (after 24 hours) dilation of the capillary segment belonging to the ablated pericyte.

Our results indicate that pericytes may have an important role in the maintenance of the capillary tone, and may regulate capillary flow under inflammatory conditions.

E18

Radiation exposure of the skin from radon and its decay products

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It is generally accepted that the majority of radon-related radiation exposure, around 98%, comes from the inhalation of short-lived decay products of radon, and that it affects the respiratory system, primarily the bronchi. All other organs and tissues are affected by orders of magnitude smaller doses, and their effects are negligible compared to the increased risk of lung cancer. This is also why it caused a stir that, during the statistical analysis of the Swiss cancer registry and the results of the radon surveys in apartments there, the researchers came to the conclusion that a very strong correlation can be observed between the radon content of the apartments and the increase in the frequency of skin cancer (malignant melanoma) mortality. If it is proven that this relationship is causal, the increase in the relative risk of skin cancer due to radon is similar to that observed in the case of lung cancer. In this work, we performed measurements and model calculations for the transport of radon through the skin, as well as the deposition of short-lived decay products of radon on the surface of the skin, and we also estimated the related doses. We have found that our results do not support the findings of the Swiss epidemiological study, instead we point out the role of other confounding factor.

E19

Lipid polymorphism of photosystem II membranes – evidence of the role of isotropic lipid phase in membrane fusions

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Plant thylakoid membranes (TMs), in addition to the bilayer (or lamellar, L) phase, contain at least two isotropic (I) lipid phases and an inverted hexagonal (H_{II}) phase. The non-bilayer propensity of bulk TM lipids has been proposed to safe-guard the lipid homeostasis of TMs; further, an I phase has been shown to arise from VDE:lipid assemblies (VDE is a luminal photoprotective enzyme) [1]. Effects of proteases and lipases on the lipid polymorphism of TMs have revealed that the H_{II} phase originates from lipids encapsulating stroma-side proteins and that the non-bilayer phases are to be found in domains outside the protein-rich regions of TM vesicles; an I phase is proposed to be involved in the fusion of membranes and thus in the self-assembly of the TM network [2].
The aim of the present study was to test the hypothesis on the role of I phase in the membrane fusion.
We capitalize on the fact that wheat-germ lipase (WGL) selectively eliminates the ^{31}P-NMR-spectroscopy detectable I phases while it exerts no effect on the L and H_{II} phases and does not perturb the structure and function of the photosynthetic machinery [2].
Our data show that (i) Photosystem II (BBY) subchloroplast particles, compared to intact TMs, display weaker L and I phases and no H_{II}phase – in accordance with the diminished lipid content of these particles and the absence of stroma TM; (ii) similar to intact TMs, WGL has no effect on the molecular organization and functional activity of BBY particles but (iii) eliminates their I phase; and (iv) parallel with the diminishment of the I phase, it disintegrates the large (>10 μm diameter) sheets of the BBY membranes, which are composed of stacked membrane pairs of granum thylakoids of ~500 nm diameter. These data provide evidence on the involvement of I phase in the lateral fusion of stacked Photosystem II membranes.

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E20

Characterization of a novel mutation in Brugada Syndrome

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Voltage-gated sodium channels (Na_V) play a key role in the initiation and propagation of cardiac action potential essential for the rhythmic beating of the heart. Therefore, alterations of the sodium current (I_{Na}) in cardiomyocytes can lead to diseases responsible for cardiac arrhythmias, such as Brugada Syndrome (BrS) that has an increased risk for sudden cardiac death due to ventricular fibrillation. The major disease gene for BrS is SCN5A encoding the primary alpha-subunit of the cardiac $Na_V1.5$ channel. Exploring SCN5A mutations in patients with inherited arrhythmogenic syndromes is critical for the deeper understanding the pathogenesis of BrS.

Accordingly, we aimed at fully characterizing the biophysical properties of $Na_V1.5$ containing a novel heterozygous mutation of R893C localized in the P-loop of domain II identified in a patient with BrS. We subsequently compared the main gating parameters of R893C channels to wild-type $Na_V1.5$ channels (WT). The mutation was introduced by site-directed mutagenesis. The channels were transiently expressed in CHO cells and I_{Na} was measured using the standard whole cell patch-clamp technique.

We found that the peak current density is substantially reduced by the R893C mutation compared to WT channels. We also observed slower activation kinetics of I_{Na} current in R893C channels, although the mutation had no significant effect on the steady-state activation. All observations confirmed the loss-of-function of R893C channels. Pharmacological studies revealed that the reducing agent Dithiothreitol might restore the normal function of $Na_V1.5$ containing R893C by reducing the cysteine bridges that may be responsible for the loss of conduction.

Our conclusion is that the analysed mutation in the SCN5A gene result in biophysical changes in the $Na_V1.5$ current and thus, may be responsible for BrS. Understanding the structure-function relationship of $Na_V1.5$ will shed new light on exploiting new therapeutic drugs for SCN5A channelopathies.

E21

Crosstalk between nucleotide and substrate binding in ABCG2

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ABCG2 is an exporter type ABC protein that can expel numerous chemically unrelated xeno- and endobiotics from cells. When expressed in tumor cells and tumor stem cells, it may cause multidrug resistance contributing to the failure of chemotherapy. A better understanding of the molecular mechanism of ABCG2 may provide new therapeutic targets to improve the treatment of drug resistant tumors. In the present work, we designed fluorescence-based assays to investigate the affinity of ABCG2 to transported drugs and nucleotides in live or semi-permeabilized cells. Using the conformation-sensitive antibody 5D3, we show that the switch from the 5D3-reactive inward-facing (IF) to a 5D3-dim outward-facing (OF) conformation is induced by nucleotide binding, and this conformational transition is accelerated by substrates and hindered by the known inhibitor Ko143. The drop of 5D3 binding occurred simultaneously with the decrease of substrate binding, suggesting that the high-to-low switch in drug binding affinity might coincide with the transition from the IF to the OF conformation. Low substrate binding persists in the vanadate-trapped post-hydrolysis state, indicating that dissociation of the ATP hydrolysis products is required to reset the high substrate affinity IF conformation of ABCG2.

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E22

Molecular strategies for heat detection by TRPM2 channels

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TRPM2, a Ca^{2+} permeable non-selective cation channel co-activated by cytosolic Ca^{2+} and ADP ribose (ADPR), plays key role in the central regulation of body temperature. The central thermostat must contain a temperature sensor, which is able to differentiate temperature fluctuations as small as ±1°C around 37°C. To address whether heat-activation of TRPM2 gating in intact cells is an intrinsic property of the TRPM2 protein, temperature dependence of TRPM2 currents was studied in inside-out patches between 15°C and 40°C, across broad ranges of concentrations of both agonists. For fully liganded TRPM2 pore opening is intrinsically endothermic, the enthalpy of opening is ~180 kJ/mol. However, the TRPM2 temperature threshold is too high (>40°C) for unliganded, but too low (<15°C) for fully liganded channels. Calculations based on a mechanistic gating model indicate that TRPM2 warmth sensitivity around 37°C is restricted to narrow ranges of agonist concentrations. For ADPR that range (submicromolar-to-micromolar) matches, but for Ca^{2+} (>1 µM) it exceeds bulk cytosolic values, suggesting that a Ca^{2+}nanodomain drives TRPM2 activation in vivo. TRPM2 is Ca^{2+}permeable, and the binding sites for activating Ca^{2+} are near the cytosolic pore entrance. We therefore investigated how the presence of a physiological extracellular [Ca^{2+}] affects temperature dependence of TRPM2 gating between 37°C and 40°C, while bulk cytosolic [Ca^{2+}] was buffered to 100 nM and [ADPR] was set to 2 µM. Under such quasi-physiological conditions Po was ~0.04 and ~0.34, respectively, at 37°C and 40°C. These findings demonstrate and quantitate the positive feedback provided by Ca^{2+} influx. The larger Po at 40°C elevates local [Ca^{2+}] around the activating sites, which in turn further enhances Po. That positive feedback provides strong amplification to the TRPM2 temperature response (Q10~1000), enabling the TRPM2 protein to autonomously respond to tiny temperature fluctuations around 37°C. [1].

Acknowledgments

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E23

Development of liposomal corticosteroids

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Introduction Liposomes are nanoscale drug delivery systems that offer many pharmacokinetic advantages. Corticosteroids as lipophilic active agents integrate into the lipid bilayer. This novel approach can improve the efficacy of several anti-inflammatory therapies.
Aims Our goals were to create long-term stable liposomes, which can incorporate and release corticosteroids at inflamed body temperature. Considering the possible future application in asthma therapy, we also aimed at reaching high entrapment efficacy (EE%) and slight drug leakage of the vesicles.
Methods Two kinds of liposomes were prepared from 3 different phospholipids by thin layer hydration method and subsequent extrusion to get small unilamellar vesicles (SUVs). Prednisolone (Pred) and budesonide (Bud) were used as active agents. Stability tests were executed by dynamic light scattering while EE% was determined by size-exclusion gel chromatography. The effect of liposomal drugs on cell viability was measured on EBC-1 human lung carcinoma cells. The internalization of the liposomes was studied by flow cytometry (FC) and confocal microscopy (CM).
Results SUVs with 100 nm in diameter were successfully prepared. Their hydrodynamic diameter has remained in the desired range through 6 months in case of the vast majority of the samples. It turned out that the presence of corticosteroid has a bigger impact on stability, than its type. The EE% was exceptionally high in both cases (above 90%) and the drug leakage was 35-40% for Pred and 6-8% for Bud in the first 30 min. Neither the free corticosteroids nor their liposomal form affected significantly the cell viability. CM images and FC results proved equally the internalization of the vesicles by the cells.
Conclusion We have successfully created corticosteroid-loaded liposomes with long-term stability and excellent EE% due to the lipophilic character of the applied drugs. Furthermore, they are not toxic and can be internalized by the investigated pulmonary cells.

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E24

Systematic investigation and classification of host defence and cell penetrating peptides based on their affinity for interaction with extracellular vesicles

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Host defence peptides (HDP) are promising biomaterials with antimicrobial and anticancer applications. By disturbing or lysing the cell membrane, they carry out their biological role. These peptides show numerous types of membrane interaction mechanisms i.e., carpet, toroidal pore, and barrel stave. Cell penetrating peptide find application in cargo loading and uptake of small molecules and nanoparticles. The interactive mechanism of these peptides has been studied widely with model membranes however our knowledge with extracellular vesicles (EV) is scarce. There are several aspects where EV – HDP interactions could be relevant, ranging from cooperative presence on infection sites functions to EV cargo loading. Hence, based on their in-depth investigation using biophysical techniques, the binding affinity with extracellular vesicles was studied and categorised as low binding affinity, medium binding affinity and high affinity. This initial categorisation gives further insight into its specific interactive mechanism.

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E25

The membrane binding mechanism and oligomerization of the antifungal protein NFAP2 with molecular dynamics simulations

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Neosartorya fischeri antifungal protein 2 (NFAP2) is a cysteine-rich, cationic protein with potent anti-*Candida* activity, which was recently identified [1]. Its antifungal activity includes pore formation in the fungal cell membrane. Here we followed up the previous experimental results with Molecular Dynamics (MD) simulations in order to find out more about the antifungal effect and action mechanism of NFAP2. We used simple lipid bilayer composition models as well as more complex membrane models. An important complex membrane model we built aims to mimick fungal cell membranes. We studied the binding affinity of NFAP2 towards 7 membrane models. We determined which amino acids play crucial role in the binding to the membrane. In addition, we have also investigated the dimerization of NFAP2 with MD simulations. Results indicate that there is a crucial loop region which is expected to play a central role in the initial attachment of NFAP2 to the lipid membranes of the target organism.

Acknowledgments

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E26

An obstacle-free microfluidic system for monitoring protein diffusion

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Determining characteristic chemical and physical properties of proteins are essential for predicting their behaviour and interactions in Lab-on-Chip and Organ-on-Chip systems. Diffusion coefficients are widely used to estimate molecule size, hydration state or aggregation [1, 2]. Constructed diffusion is crucial to develop adequate molecular concentration distribution in these applications. Our goal is to develop a free-diffusion based obstacle-free microfluidic device to achieve fast diffusion coefficient measurements.

A specific microfluidic system was created – inspired by Taylor et al. [3] – containing two main channels connected by multiple perpendicular capillaries to ensure stable and stationary molecular concentration at the edge of the monitoring channel. The device was fabricated by soft lithography in polydimethylsiloxane and was bonded to a glass slide. The main channels were filled with test puffer and adequate fluorescent protein solutions, respectively. The symmetry of the layout minimizes the pressure differences between the channels.

Diffusion coefficient of various, fluorescently labelled proteins (bovine serum albumin, immunoglobulin G, rhodamine B) were determined using time dependent fluorescent microscopy. To acquire the evolving intensity profiles along the channels 30 s time-lapse images were taken. Python evaluation code was developed to fit model complementary error functions to the experimental profiles and calculate the diffusion coefficients. The defined diffusion coefficients for the certain proteins were in accordance with the values found in literature and estimated by the Stokes-Einstein equation.

Easy-to-use free-diffusion based microfluidic system were developed and proved to be applicable for fast determination the diffusion coefficients of characteristic proteins, using time-lapse fluorescent imaging and efficient evaluation algorithm.

Acknowledgments

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E27

Flagellin: a convenient protein in biosensorics

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Flagellin is the main building block of bacterial flagellar filaments. Since the filaments are located outside of the cells, cell lysis is not required to purify flagellin. Flagellin consists of 4 domains: D0, D1, D2, and D3. The D0 domain contains amphipathic helical regions with hydrophobic amino acids on one side of the helix. This part of flagellin is disordered in solution, but can be used to anchor the protein on hydrophobic surfaces with the D3 domain pointing towards the solution [1]. The hypervariable D3 domain situated on the filament surface is a largely independent part of the flagellin that can be removed or replaced without disturbing filament formation.
During our work we in-depth characterized the coatings created from flagellin, and influenced the adsorption of the protein with Hofmeister salts [1]. We applied genetically modified high affinity Ni-binding variant as receptor, and demonstrated the unique sensitivity of grating-coupled interferometry [2].
The monolayer of wild-type flagellin mimics the surface of the bacterial flagellar filament, and we hypothesized that oriented flagellin layers have bacteria-repellent properties. To prove this, we studied the adhesion of bacterial *E. coli* and human cancer cells on oriented wild-type flagellin layers [3,4].
Through genetic modification, specific oligopeptide segments can be also inserted into the D3 domain of flagellin, which can induce cell adhesion through integrin receptors. We studied cancer cell adhesion on the genetically engineered protein layers with label-free optical biosensors [4]. Mammalian cells can recognize flagellin in solution through Toll-like receptors, and the protein can cause innate immune system response. We are studying the above biological mechanisms and its consequences in the adhesion of the flagelljn exposed cells. Our results prove, that flagellin can be used in many ways in creating capture layers in biosensors.

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E28

Lab-on-a-chip device for the monitoring of surface charge properties of confluent cell monolayers

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Lab-on-a-chip devices emerged to play pivotal role in the in vitro modelling of biological barriers. The microfluidic channels combined with integrated electrodes provide controlled environment for the tightly interconnecting cell monolayers of intestinal, pulmonary and vascular models. The convenient and fast measurement of the trans-endothelial/epithelial resistance (TEER) and passive permeability provide important information about the integrity of the cell monolayer under healthy and pathological conditions. We developed a versatile lab-on-a-chip device that can measure the TEER and the passive permeability and also enables the visual monitoring of the cell monolayer via phase contrast microscopy and immunohistochemistry [1]. The device was standardized under static and dynamic condition (without and with fluid flow, respectively) using epithelial and endothelial barrier models. We studied how the shear stress effects the blood-brain barrier properties and the glycocalyx [2]. The latter is especially important, since it contributes to the high negative surface charge of the luminal surface of the barrier forming cells. The negative surface charge plays crucial role in transport processes, infections and other pathologies, so it is very important to investigate the relationship between the surface charge and the overall barrier function. There are very few studies focusing on the surface charge and all of these measures the zeta potential of cell-suspensions. We upgraded our lab-on-a-chip device with a pair of Ag/AgCl electrodes to monitor the surface charge of confluent monolayers via the measurement of transient streaming potential signals [3].

Acknowledgments

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E29

Transparent neural interfaces for simultaneous Ca2+ imaging and cortical electrophysiology in vivo

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Multimodal electrophysiological and neuroimaging approaches hold great potential for revealing the anatomical and functional connectivity of neuronal ensembles in the intact brain. Creating devices that provide high-resolution, artifact free neural recordings while facilitating the interrogation or stimulation of underlying anatomical features is currently one of the greatest challenges in the field of neuroengineering. Optically transparent micro – electrocorticography devices enable the simultaneous recording of brain activity with electrocorticography and fluorescent Ca2+ signals with two-photon imaging. There are numerous trade-offs in the design and development of transparent neural interfaces, in the electrical, optical, and mechanical properties, the stability and longevity of the integrated features and biocompatibility in vivo. We present transparent devices designed for chronic, multimodal interrogation of brain circuits and demonstrate in vivo viability through long term implantation [1-3]. We characterise the electrochemical and mechanical properties, photoartefacts and photodegradation of the materials and show that single neurons remained active and distinctive even 22 weeks after implantation. Simultaneous examination of neural networks through transparent ECoG devices provides an opportunity to better understand the physiological and pathological states of experimental animals and guide the application of these devices toward future human disease diagnostics and medication.

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E30

Label-free single-cell compatible biophysical methods in immune cell activation

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Recent advances in biophysical methods provide a novel approach to characterize immune cell activation. Here, we present an experimental platform for studying adhesion kinetics, adhesion force, and cell morphology at a single-cell level. These techniques enable the precise characterization of complex cellular mixtures and the testing of the effects of various compounds on immune cell activation(1). The adhesion force, measured by computer-controlled micropipette and fluidic force microscopy, serves as a proxy for cellular activation induced by various compounds(2). The resonant wavelength grating technique exploits the sensitivity of the surface-bound optical evanescent field to changes in the local refractive index, enabling the study of cell adhesion kinetics in single cells at subminute time resolutions(3). Moreover, digital holographic microscopy records the morphology of single cells during their activation and extracts multiple features, such as cell area, optical thickness, and motility, to characterize their activation state(4). Importantly, these biophysical methods enable the characterization of cellular processes in a label-free manner, reducing the complexity and material demand of each measurement and enabling the investigation of single cells in their native state. Comparing and evaluating these techniques carefully can enhance our understanding of immune cell activation and lead to the development of diagnostic approaches and novel therapies for immune system-related questions.

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E31

Could the SARS-CoV-2 S1 subunit cross the blood-brain barrier? – a lab-on-a-chip model study

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The outbreak of the global pandemic caused by severe acute respiratory coronavirus 2 (SARS-CoV-2) has pulled several clinical aspects of the disease into attention. Besides its primary route of infection through the respiratory system, SARS-CoV-2 is known to have neuroinvasive capacity, causing multiple neurological symptoms with increased neuroinflammation and blood–brain barrier (BBB) damage. The viral spike protein disseminates via circulation during infection, and when reaching the brain could possibly cross the BBB, which was demonstrated in mice. Therefore, its medical relevance is of high importance. The aim of our study was to evaluate the barrier penetration of the S1 subunit of spike protein in model systems of human organs highly exposed to the infection. For this purpose, *in vitro* human BBB and intestinal barrier cell-culture model systems were applied, in combination with an optical biosensing method.

We found that spike protein crossed the human brain endothelial cell barrier effectively. Additionally, spike protein passage was found in a lower amount through the intestinal barrier cell layer too. These observations were corroborated with parallel specific ELISA tests.

The findings on the BBB model could provide a further basis for studies focusing on the mechanism and consequences of spike protein penetration across the BBB to the brain. [1]

Keywords

biosensor; coronavirus spike-protein permeability; tissue barriers; human brain endothelial cells; Caco-2 cells; integrated optics; Mach–Zehnder interferometer

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E32

The force awakens: mechanical interaction of metastatic tumor cells with the neurovascular unit

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The central nervous system has prominent defense lines, however, most of the malignancies detected within the brain parenchyma are of metastatic origin. As the brain lacks classical lymphatic circulation, the primordial way for metastasis relies on hematogenous routes. The first and probably the most crucial step for invading tumor cells relies on their interaction with the neurovascular unit. The neurovascular unit plays crucial role in the maintenance of the proper homeostasis of the central nervous system [1]. Endothelial cells and pericytes are the most exposed to mechanical stresses their mechanobiology is of primordial importance [2]. Furthermore, tumor cell derived extracellular vesicles might play key role in pre-metastatic niche formation and might be involved in metastatic organotropism [3].
Single cell-force spectroscopy was applied to investigate the adhesive properties of living breast adenocarcinoma cells to confluent layers of brain endothelial cells and pericytes. Cell type dependent adhesion characteristics were found as well as the existence of metastatic potential related nanomechanical differences between the studied tumor cells, relying partly on membrane tether dynamics. Apparent mechanical properties such as elasticity, maximal adhesion force, number, size and distance of individual rupture events have been found cell type dependent, correlating with their metastatic abilities. Additionally, tumor cell derived extracellular vesicles alter the adhesive properties of tumor cells to brain endothelial layer. Exploring the mechanobiology of constituent of the neurovascular unit could not only lead to a better understanding of their function but could also help to identify novel targets for the improvement of its barrier function.

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E33

The effect of fluorescence labeling on the function and dynamical properties of antibodies

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Fluorescent antibodies have been the cornerstone of cell biological investigations in the last couple of decades due to their relatively straightforward application. While fluorescence labeling has been shown to deteriorate the affinity of antibodies and the fluorescence properties of the dyes, an effect that may significantly affect the reliability of quantitative biophysical measurements [1], the background and further implications of these findings have not been explored. Here we show that fluorescence labeling of antibodies not only deteriorates their epitope binding capability, but functions linked to other IgG domains, and the extent of these effects reveals remarkably similar dependence on the degree of labeling. The melting temperature of the Fab and Fc domains of unlabeled and fluorescently-tagged antibodies were identical according to differential scanning fluorometry implying that the overall stability of antibody domains is not affected by fluorescence labeling. According to time-dependent measurements, the decay rate of fluorescence anisotropy increased by the degree of labeling suggesting that the wagging motion of antibody domains in accelerated by the presence of the fluorophores. This conclusion is corroborated by FRET measurements between the Fc domain and the IgG-bound epitope in which the steady-state energy transfer efficiency was higher in antibodies with a high degree of labeling implying that the Fc and epitope-binding domains approach each other more closely in highly labeled antibodies on average over time. The investigations suggest that the effect of fluorescence labeling on all antibody functions may be due to altered antibody dynamics.

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E34

Hollow organosilica beads: A novel reference material for the flow cytometry analysis of extracellular vesicles

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Extracellular vesicles (EVs) are increasingly recognized as key biomarkers with significant potential in diagnostic and therapeutic applications. Their precise concentration measurement in body fluids is crucial, for which flow cytometry is currently the clinically most applicable method. This technique often uses solid polystyrene reference beads for calibration, but the disparity in refractive indices between these beads and EVs can distort accurate size determination. In response to this challenge, this study aims to prepare, characterize, and test hollow organosilica beads (HOBs) with different diameters as reference beads to set EV size gates in flow cytometry investigations.

HOBs were produced through the hard template sol-gel method, followed by a thorough analysis of their morphology, size, and colloidal stability. Their suitability as reference particles was then examined using flow cytometry. Findings indicated that HOBs exhibit a uniform size distribution and shell thickness. Further, two-angle light scattering measurements showed that HOBs scatter far less light than comparable solid silica beads due to differences in refractive indices. The scattering intensity of HOBs aligns with that expected from EVs of similar sizes.

Moreover, the study demonstrated that HOBs can be used to standardize EV concentration measurements, independent of the light scattering collection angles of the flow cytometer. Concentration measurements of platelet-derived EVs, conducted using size gates established by HOBs, showed the smallest percentage difference relative to the mean concentration, pointing to HOBs' superiority over solid beads.

In conclusion, HOBs, due to their similar structure and light scattering properties to EVs, can set size gates in nanometers, regardless of the flow cytometer's optical configuration.

E35

Modulation of brain endothelial surface charge changes the transfer of charged molecules and targeted nanoparticles

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The highly negative surface charge of brain endothelial cells is part of the blood-brain barrier (BBB)defense systems. It is derived from charged membrane lipids and the endothelial surface glycocalyx. We showed that physiological factors inducing BBB properties (co-culture, fluid flow, targeting signaling pathways) increase the surface glycocalyx thickness and makes the zeta potential of the cells more negative measured by laser-Doppler velocimetry (LDv).
Zeta potential by LDv can only be measured on cells in suspension, so we designed and fabricated a novel lab-on-a-chip (LOC) device to monitor streaming potential parallel to the surface of confluent cell layers. Streaming potential measured on brain endothelial cell monolayers in the LOC device were recorded and verified by comparing to zeta potential results measured by LDv and model simulations. Changes in the negative surface charge of the BBB model by neuraminidase (cleaving negatively charged sialic acid residues from the glycocalyx) or lidocaine (interacts with lipid membranes) could be measured by both the LOC device and LDv.
Lidocaine, a cationic and lipophilic anesthetic and antiarrhythmic drug turned more positive the negative zeta potential of brain endothelial cells. It also decreased the flux of a cationic lipophilic molecule (rhodamine 123) across the BBB model without changing the penetration of hydrophilic neutral or negatively charged markers. Neuraminidase and the cationic lipid TMA-DPH, which elevated the surface charge, increased the uptake of vesicular nanoparticles targeted by alanine and glutathione in brain endothelial cells.
In conclusion, the negative surface charge of brain endothelial cells is a fundamental BBB property. It is important in the transfer of charged molecules and the uptake mechanism of charged nanoparticles and can be modulated by modification of plasma membrane lipid composition or the glycocalyx.

E36

Transport kinetics of a small apolar drug candidate passing through a transwell barrier model -- mathematical analysis and automated sampling

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In the preclinical phase of drug development, it is necessary to determine how the pharmacological compound can pass through the biological barriers surrounding the target tissue. In vitro barrier models provide a reliable, low-cost, high-throughput solution for screening substances at an early stage of the drug development process, thus reducing more complex and costly animal studies.
The transport properties of an *in vitro* 3D barrier model were determined using a drug candidate. The drug was delivered into the apical chamber of the transwell device, and the concentration of the drug passing through the barrier layer was determined by automated liquid sampling and subsequent spectroscopic analysis. The measurement system replaces the media in the basolateral compartment every 30 minutes for 6 hours and stores the collected samples for further analysis. During the experiment more than half of the compound loaded into the apical compartment passes through the barrier into the basolateral compartment, binds to the filter membrane, accumulates in the cells, or gets metabolized.
Comparison of the time-dependent concentration profiles obtained from both the cellular barrier and membranes saturated with serum proteins reveals the extent the cell layer functions as a diffusion barrier to the compound. Due to the large number of collected samples a detailed mathematical model of the diffusive currents can be fitted to the measured concentration profiles. Based on the fitted parameters, one can determine the diffusivity of the drug in the cell layer, the affinity of the drug binding to the cell membrane as well as the rate by which the cells metabolize the compound. This novel sampling and quantitative analysis approach goes beyond the standard permeability coefficient obtained from transwell inserts and thus offers more detailed pharmacokinetic characterization of the transwell barrier model.

E37

Experimental study of the functions of zebra stripes: A new thermophysiological explanation

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With a thermodynamic field experiment we refuted the wide-spread hypothesis of the cooling effect of zebra stripes. Using Schlieren optics in laboratory, we showed that convective air eddies do not form above sunlit zebra stripes that could cool the zebra's body. In field experiments we demonstrated that female horseflies prefer to suck blood on sunlit dark (warm) and shiny (strongly polarizing) host animals. In field experiments we showed that the escape success of horseflies decreases with decreasing target temperature, that is escape success is driven by temperature. This explains the behaviour of biting horseflies that they prefer warmer hosts against colder ones. Our results also explain why horseflies prefer sunlit dark hosts against bright ones, and why these parasites attack their hosts usually in sunshine, rather than under shaded conditions. In field experiments we corroborated our new hypothesis explaining why biting horseflies avoid host animals with striped pelages: Since the temperature gradients at the borderlines of sunlit white and black stripes can hamper the thermal vessel detection by blood-seeking female horseflies, striped host animals are unattractive to these parasites which prefer hosts with homogeneous coat, on which the temperature gradients above blood vessels can be detected more easily. This 5-year research project was supported by the grant NKFIH K-123930 received from the National Research, Development and Innovation Office.

E38

Spectral and redox properties of a mouse cytochrome *b*561 protein suggest transmembrane electron transfer function

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Cytochrome *b*561 proteins (CYB561s) are integral membrane proteins with 6 trans membrane domains, two heme *b* redox centers, one on each side of the host membrane. The major characteristics of these proteins are their ascorbate reducibility and transmembrane electron transferring capability. More than one CYB561 can be found in a wide range of animal and plant phyla and they are localized in membranes different from the membranes participating in bioenergization. Two homologous proteins, both in humans and rodents, are thought to participate via yet unidentified way in cancer pathology. The recombinant forms of the human tumor suppressor 101F6 protein (Hs_CYB561D2 and its mouse ortholog (Mm_CYB561D2 have already been studied in some details. However, nothing has yet been published about the physical- chemical properties of their homologues (Hs_CYB561D1 in humans and Mm_CYB561D1 in mice). The tissue specificity, location and function of this protein is unknown. Here we present optical, redox and structural properties of the recombinant Mm_CYB561D1 obtained based on various spectroscopic methods and homology modeling. In a new model calculation we show that traditional evaluation of the redox titration of these proteins cannot unequivocally provide the midpoint redox potentials of the individual heme centers without further assumptions. The interaction with ascorbate, the redox properties, the (limited) sequence homology and the modelled 3D structure all substantiate the function of Mm_CYB561D1 as a transmembrane electron transporter with ascorbate as the primary electron donor [1].

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E39

Accelerated electron transfer and increased enzymatic activity in genetically modified photoactivable adenylate cyclase OaPAC

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Photoactivable adenylate cyclases (PAC) are light activated enzymes that combine blue light sensing capacity with the ability to convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and pyrophosphate (PPi) in a light-dependent manner. In most of the known PACs blue light regulation is provided by a BLUF domain which undergoes a structural reorganization after blue-light absorption. This minor structural change then is translated towards the C-terminal of the protein, inducing a larger conformational change that results in the ATP conversion to cAMP. As cAMP is a key second messenger in numerous signal transduction pathways regulating various cellular functions, photoactivable adenylate cyclases are of great interest in optogenetic studies. The optimal optogenetic device must be "silent" in the dark and highly responsive upon light illumination. OaPAC is a very good candidate as its basal activity is very small in the dark and the conversion rates increase 20-fold upon light illumination. In this paper, we studied the effect of replacing D67 to N, in the BLUF domain. This mutation was found to accelerate the primary electron transfer process in the photosensing domain of the protein, as has been predicted. Furthermore, it resulted in a longer lived signalling state, which was formed with a lower quantum yield. We hypothesized that the more effective electron transfer correlates with a more efficient cAMP production. Our studies show that D67N OaPAC mutant has a slightly higher conversion of ATP to cAMP compared to the wild-type OaPAC which points in the direction that by fine tuning the electron transfer process more responsive PACs and optogenetic devices can be generated.

Acknowledgments

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E40

Machine learning analysis of ultrafast fluorescence kinetics of NADH conformations in solutions

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The structure of many different enzyme-bound forms of the essential coenzyme nicotinamide adenine dinucleotide is well characterized by X-ray diffraction data. Due to the limitations of this technique in solution, the unbound forms of the molecule need to be characterized by alternative methods, such as time-resolved fluorescence spectroscopy. In NADH the relative position of the nicotinamide and adenine groups has primary impact on the fluorescence kinetics of the excited nicotinamide group. In aqueous solution the molecule exists in an equilibrium of closed and open conformations, while the presence of methanol favors the latter.
The fluorescence kinetics of NADH were measured in water and methanol environments using fluorescence upconversion and time-correlated single photon counting in a large, 50 fs – 10 ns time window at different wavelengths for both environments.
To avoid the uncertainties of exponential fitting, the experimental data were fitted by a quasi-continuous set of time constants, applying regularization terms for favoring sparse solutions, i.e., a minimum number of nonzero amplitudes. For fine tuning the level of sparsity we developed a machine-learning method based on cross-validation and Bayesian optimization. This approach was found to be a powerful method for fluorescence kinetics analysis, avoiding any arbitrary or random parameters.
According to the above analysis the fast (<100 ps) part of the kinetics can be characterized by an unusually complex, three-step vibrational relaxation process. The slow part is well modelled either by the conventional distinct exponential terms or by distributed kinetics, corresponding to an equilibrium of a very high number of conformational states [1], as shown by the improved version of the analysis method.

Acknowledgments

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E41

Rate-limiting steps in the dark-to-light transition of photosystem II: Dependence on the temperature and the lipidic environment of the reaction center

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Photosystem II (PSII) is the redox-active pigment–protein complex embedded in the thylakoid membrane (TM) that catalyzes the oxidation of water and the reduction of plastoquinone. We performed single-turnover saturating flash-induced (STSF) variable chlorophyll-*a* fluorescence transient measurements on PSII, and we have identified rate-limiting steps in the dark-to-light transition of PSII [1]. It was demonstrated in diuron-treated samples that the first STSF – generating the closed state ($PSII_C$) – induces only an F_1($<F_m$) fluorescence level, and additional excitations with sufficiently long $\Delta\tau$ waiting times between them are required to reach the maximum (F_m) level. We also revealed that the F_1-to-F_m transition is linked to the gradual formation of the light-adapted charge-separated state, $PSII_L$, which possesses an increased stabilization of charges [2]. Recently, we studied the effects of different physicochemical environments of PSII on the half-rise time ($\Delta\tau_{1/2}$) and probed its presence during later steps (F_2, F_3 *etc.*) [3, 4]. In particular, we investigated the influence of the lipidic environment [3] and the temperature dependence of $\Delta\tau_{1/2}$ of PSII core complexes (CC) of *Thermosynechococcus (T.) vulcanus* and pea TMs [4]. We showed that (i) while non-native lipids has no effect, TM lipids shorten the $\Delta\tau_{1/2}$of PSII CCs of *T. vulcanus* to that of TMs – uncovering the role of lipid matrix in the rate limiting steps; (ii) PSII CCs of *T. vulcanus* and spinach TMs exhibit very similar temperature dependences, with enhanced values at low temperatures; and (iii) the $\Delta\tau_{1/2}$ values in PSII CC are essentially independent at all temperatures on the number of the STSF-induced increments. These data suggest that the same physical mechanism is involved during the $PSII_C$-$PSII_L$transition.

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E42

Regularizing the combinatorial fitness landscape

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High throughput sequencing and large scale biophysical measurements such as deep mutational scanning [1] coupled with the ability to learn high dimensional complex functions with neural networks opens an exciting window of opportunity for sequence to function mapping. However, due to the sparsity of real world measurements possible from the full combinatorial sequence space we must develop some heuristics for regularizing such deep networks to avoid overfitting or “shrinking” over the subspaces of measurements. Such regularization would allow for model generalization and the ability to make predictions of the function of unseen sequences tractable. We explore two potential avenues to accomplish such regularization to enable distant exploration of the fitness landscape In-silico.
We expire the heuristic of “factorization of the combinatorial space” with a geometric proof. We use this concept on NK model [2] generated data for the saturation of the combinatorial space of sequences with letter length 5. We present the theoretical bounds for fitness landscape reconstruction based on the theory of compressed sensing and pose open questions about DMS library design for optimal generalizability. We explore the Walsh Hadamard transform for regularizing the fitness landscape and present the idea that a key concept for progression in the topic would be to define optimal strategies for collecting data on ensembles of genotypes that is sufficient for discovering the biologically relevant epistatic structure of systems [3].
We take a real world manifestation of this problem: SARS-CoV-2 variant-of-concern prediction. Such models would allow for development of vaccines ahead of time and drastically reduce severity of epidemics. We explore how “early” receptor binding domain RBD sequence’s deep mutational scanning measurements [4] can act as a training set for models to make phenotypic predictions about Omicron variants such as antibody escape and ACE2 binding.

Acknowledgments

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E43

Studying epistatic interactions in protein evolution with measurements and machine learning techniques

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Epistasis is the phenomenon that a mutation in a gene or amino acid sequence modifies the effect of another mutation in the same sequence. Since this phenomenon limits the viable evolutionary paths between sequences, it can serve as an important tool in studying evolution and reconstructing evolutionary trajectories. However, due to its combinatorial complexity – originating from the vast amount of possible mutation orders – full studies on its structure and extension is scarce. In the present talk I shall overview some recent advances on protein language model based machine learning techniques along with experimental results.

E44

Compositionally constrained sites drive long-branch attraction artefact in deep phylogenomic inferences

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Accurate phylogenies are fundamental to our understanding of the pattern and process of evolution. Yet, phylogenies at deep evolutionary timescales, with correspondingly long branches, have been fraught with controversy resulting from conflicting estimates from models with varying complexity and goodness of fit. Analyses of historical as well as current empirical datasets, such as alignments including Microsporidia, Nematoda, or Platyhelminthes, have demonstrated that inadequate modeling of across-site compositional heterogeneity, which is the result of biochemical constraints that lead to varying patterns of accepted amino acids along sequences, can lead to erroneous topologies that are strongly supported. Unfortunately, models that adequately account for across-site compositional heterogeneity remain computationally challenging for an increasing fraction of datasets. Here we introduce "compositional constraint analysis", a method to investigate the effect of site-specific constraints on amino acid composition on phylogenetic inference. We show that more constrained sites with lower diversity and less constrained sites with higher diversity exhibit ostensibly conflicting signals under models ignoring across-site compositional heterogeneity that lead to long-branch attraction artifacts and demonstrate that more complex models accounting for across-site compositional heterogeneity can ameliorate this bias. We present CAT-posterior mean site frequencies (PMSF), a pipeline for diagnosing and resolving phylogenetic bias resulting from inadequate modeling of across-site compositional heterogeneity based on the CAT model. CAT-PMSF is robust against long-branch attraction in all alignments we have examined. We suggest using CAT-PMSF when convergence of the CAT model cannot be assured. We find evidence that compositionally constrained sites are driving long-branch attraction in two metazoan datasets and recover evidence for Porifera as the sister group to all other animals.

T01

Characterize your most challenging interactions with two independent technologies. The New Monolith X.

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Knowing the strength of the interactions between key players is crucial to get the insights you need to understand the details behind how a given biological event occurs.
The new Monolith is the latest solution we provide to our customers who wish to quantify their biomolecular interactions of any kind in any experimental conditions. Monolith utilizes two proprietary technologies- MST as well as our newest addition to the portfolio- isothermal spectral shift.
MST technology allows for quantification of molecular interactions between a target and ligand by detecting changes in fluorescence intensity while a temperature gradient is applied over time. The fluorescent signal comes from the target that is either fluorescently labeled or has intrinsic fluorescence and becomes an extremely sensitive reporter for the interaction.
Isothermal spectral shift in order to quantify a molecular interactions utilizes an experimental procedure during which a fluorescently labelled target generates a particular emission spectrum, and if a ligand binds to this target, the fluorophore's local chemical environment is changed, causing a shift in its fluorescence spectrum. This Monolith detector exploits this phenomenon by performing ratiometric measurements at two emission wavelengths of a labelled target in the presence of various concentrations of a ligand. [1]
In both of the detectors types which can be combined in a Monolith instrument- the binding affinity is automatically determined at the end of each run without additional and lengthy data analysis. (figure 1.)

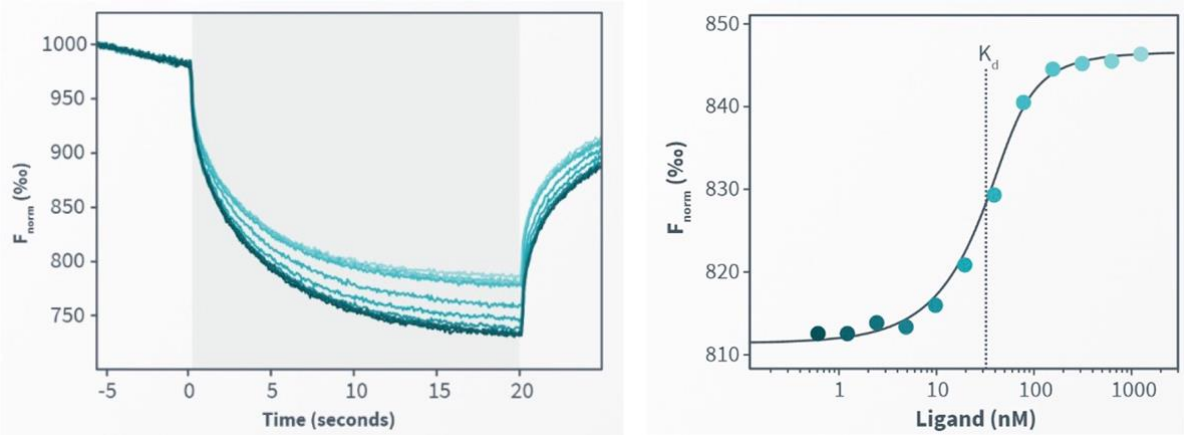

Figure 1. The affinity constant (Kd) is calculated from a fitted curve that plots normalized fluorescence against concentration of ligand.

Monolith enables characterization of in solution interactions for a wide range of biomolecules, even for challenging samples such as membrane proteins, intrinsically disordered proteins, small molecules and cell lysates. Since the binding partners are in solution, there is no lost activity due to immobilization, and evaluation is size independent. Measurements can be performed in any buffer, including detergents, using low sample volumes and concentrations. The collected data analysis also facilitates the evaluation of competition assays and ternary binding events.
Monolith provides a valuable orthogonal method to validate your results from other biophysical methods and to characterize your most challenging interactions.

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Poszter absztraktok

P01

Acute and long-lasting immunological changes in prostate cancer patients treated with three different radiotherapy protocols

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Radiotherapy can modify systemic immune responses of cancer patients. However, little is known about how long these alterations persist in patients successfully cured of cancer. We investigated how prostate cancer and radiotherapy impact the innate and adaptive immune system of cancer patients treated with various radiotherapy protocols in order to mark potential immune-related biomarkers for patient follow-up.
Blood samples were collected from 63 patients treated with three different type of radiotherapy protocols (LDR brachytherapy: n=21; HDR brachytherapy: n=22; LINAC-based teletherapy: n=20 patients) before and at 7 time points after the therapy up to 36 months. Phenotypical changes in peripheral blood mononuclear cells were analysed by multicolor flow cytometry.
Natural killer (NK) cells were among the most strongly altered in all prostate cancer patients. LDR therapy induced very moderate changes within the NK subpopulation, however teletherapy caused a strong and persistent shift toward immature NK cells. The proportion of non-senescent and senescent CD4+ and CD8+ T cells changed very similarly in HDR and teletherapy groups; the balance shifted strongly to the senescent populations during the follow-up. We further investigated the amount of PSA-containing macrophages which level increased several folds in cancer patients before therapy compared to control and found positive correlation between the plasma PSA level and the level of circulating PSA+ macrophages before therapy.
Our studies demonstrate that prostate cancer patients show long-lasting immunological changes. Furthermore different radiotherapy protocols lead to different long-term immune alterations highlighting the importance of deposition kinetics of ionizing radiation energy in modulating systemic immune responses.

Acknowledgments

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P02

The FINmaj mutation results in hypophosphorilation of the sarcomeric titin molecules

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The giant sarcomeric protein titin has central mechanistic and developmental roles in striated muscle. Mutations in the TTN gene cause a wide spectrum of skeletal and cardiac myopathies. The dominant tibial muscular dystrophy (TMD) and recessive limb griddle muscular dystrophy (LGMD2J) in Finnish patients are caused by the FINmaj mutation, changing four amino acids (EVTW > VKEK) in the most C-terminal Ig-domain M10 of titin, in the sarcomeric M-line, which domain harbors binding sites for obscurin/obscurin-like 1 and myospryn. Overall, the FINmaj induces a pathological cleavage and degradation of a 70–80 kDa portion from the titin C-terminus, soon after its synthesis.
To investigate the effect of the mutation on the sarcomeric structure of titin we isolated titin molecules from FINmaj murine skeletal muscle samples and from skeletal muscle biopsies of TMD/LGMD2J patients homozygous for the FINmaj mutation. The isolated titin molecules were visualized using atomic force microscopy. We found that the isolated titin molecules maintained their oligomeric structures, in which the overlapping C-termini of titins from neighboring sarcomeres remain bound. The oligomers showed a collapsed structure with an M-band complex that both increased in area and topographical height. Overall, the appearance of the FINmaj oligomers resembled the structure of hypophosphorylated titin molecules.
Our findings suggest that the FINmaj mutation does not destruct the M-band complex in titin oligomers but decreases the level of phosphorylation in the A-band segment of titin.

Acknowledgments

We thank for the FINmaj samples for prof. Udd Bjarne at University of Helsinki

P03

Direct binding of fluorescent vancomycin to MreB

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The discovery of antibiotics is one of the greatest discoveries in human history. It is also known that bacteria have developed a serious arsenal of weapons to resist antibiotics. As a result, despite the availability of many antibiotics with very different mechanisms of action, the number of antibiotic-resistant bacterial species is increasing. The result is a worldwide crisis which mankind currently seems powerless to tackle.

In our study we describe a novel potential target in bacteria, which is essential for bacterial survival. MreB, which has major role in organising cell wall synthesis and is found in every bacterium, can be inactivated with a MreB-specific drug A22. This target, although it affects a component of the cell wall, is fundamentally different from the antibiotics used so far.

In our studies, we confirmed the binding of Bodipy-vancomycin to the MreB protein by steady-state anisotropy measurements, which showed an affinity on the order of micromolar. The anisotropy further increased upon exposure to native vancomycin, confirming the previous observation that vancomycin molecules can form supercomplexes with each other and with target proteins. Our light-scattering measurements suggest that vancomycin, like other proteins, can induce aggregation of MreB, but this effect is not observed for ATP-bound protein. This supports our previous observations that nucleotide binding plays a crucial role in MreB stabilisation. Our microbiological and fluorescence microscopy results show that A22 promotes the uptake of vancomycin into cells, which may explain why a synergistic effect between A22 and vancomycin in inhibiting *E. coli* cell division is observed.

All these observations suggest that antimicrobials based on the mechanism of action of A22 could play a key role in the future in the fight against resistant bacterial strains.

P04

Investigation of a red-footed falcon hub in Angola with deep learning

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Automating the perception of our environment with object detection has an increasing number of applications, not only in everyday life, such as for self-driving cars or in security, but also in scientific research. Object detection is a deep learning-based computer vision technique that involves recognising predefined classes of objects in visual recordings and locating them within the frame of the recording.
In this study, we focus on a recently discovered, yet unpublished migratory bird hub in Angola, where red-footed falcons gather from different points of the world. These hubs play a crucial role in connecting remote locations worldwide, potentially assisting in disease transmission, and acting as indicators of global environmental changes due to the birds' sensitivity to environmental variations**.**
Our aim is to identify and track birds on video recordings from the hub providing insight into their population size and preparing further collective behaviour studies such as identifying when they are making use of thermals to gain altitude during their flights. We utilise state-of-the-art object detection frameworks and utilize the latest developments in deep learning and computer vision, taking into consideration that we also aim to deploy our method for real-time, on-board detection on drones.
Currently our understanding of the population size of this species, based on traditional counting methods, has multiple orders of magnitude uncertainty. Our approach offers numerous advantages over traditional procedures, being not only faster and more cost-efficient but also can be more accurate and it also offers real-time monitoring. Such approaches have been used to study the population of other species, such as bat colonies.

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P05

Improved estimation of the ratio of detection efficiencies of excited acceptors and donors for FRET measurements

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Förster resonance energy transfer (FRET) is a radiationless interaction between a donor and an acceptor whose distance dependence makes it a sensitive tool for studying the oligomerization and the structure of proteins. When FRET is determined by measuring the sensitized emission of the acceptor, a parameter characterizing the ratio of detection efficiencies of an excited acceptor versus an excited donor is invariably involved in the formalism. For FRET measurements involving fluorescent antibodies or other external labels, this parameter, designated by α, is usually determined by comparing the intensity of a known number of donors and acceptors in two independent samples leading to a large statistical variability if the sample size is small. Here, we present a method that improves precision by applying microbeads with a calibrated number of antibody binding sites and a donor-acceptor mixture in which donors and acceptors are present in a certain, experimentally determined ratio. A formalism is developed for determining α and the superior reproducibility of the proposed method compared to the conventional approach is demonstrated. Since the novel methodology does not require sophisticated calibration samples or special instrumentation, it can be widely applied for the quantification of FRET experiments in biological research. [1]

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P06

Super-resolution investigation of liposomal nanosystems

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Dynamic light scattering is a method used for a long time in the study of nano-sized lipid vesicles (~100 nm in diameter). Up until now, no imaging methodology has been used that directly reveals the inner structure of vesicles in this size range. However, the use of a method that makes the structure of nanovesicles visible under physiological conditions would have significant advantages in the planning and development of therapeutic use. Several research teams have already imaged liposomes using confocal microscopy, but this microscopic method does not have a sufficiently high resolution to enable a clear separation of the membrane and the internal fluid space. Our aim was to develop a methodology that the structure of liposomal systems can be visualized in a high-resolution, robust and reproducible manner.

For our work, small unilamellar vesicles produced from DPPC molecules were labeled with fluorescent (Alexa Fluor 594) lipopolysaccharide molecules, and this system was examined by dynamic light scattering and STED microscopy.

The diameter of the liposomes extruded through the 100 nm pore opening was found to be almost the same both during the light scattering measurement and in the confocal reconstruction images (~100-110 nm). After finding the right DPPC/LPS ratio and finding the optimal STED settings, we managed to record images with a good signal-to-noise ratio. Through the deconvolution processing of the STED images, the internal aqueous phase of the liposomes can also be successfully explored.

Our research group previously dealt with photoreactive damage of the membranes. To test our methodology, we also followed this process using our developed STED approach.

Abbreviations

DPPC – Dipalmitoylphosphatidylcholine
LPS – Lipopolysaccharide
STED – Stimulated Emission Depletion

P07

Nanosurgical manipulation of extended von Willebrand factor multimers

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The von Willebrand factor (VWF) is a varying-length multimeric chain of glycoprotein dimers/protomers with distinct domain structures. Conformational response of domains upon exposure to shear- and elongational forces ensures that VWF is able to mediate platelet adhesion to and aggregation on an injured vessel wall where it is explored or immobilized from the circulation. Human plasma-derived VWF multimers stretched by molecular combing extend through a specific hierarchy of structural intermediates of protomers as we have recently shown by using atomic force microscopy (AFM) [1]. However, the full scope of local domain stabilities and extensibilities remained hidden.
To uncover these cryptic structural details, in the present work, we used an *in situ* nanosurgical approach to probe whether targeted domains of VWF could be further extended and unfolded. To achieve this, surface-stabilized and pre-extended VWF multimers were manipulated at distinct spatial locations with the tip of the AFM cantilever. By moving the AFM tip in a direction perpendicular to the longitudinal axis of the VWF multimer, protein loops could be pulled out of the chain, the local extension of which was assessed following the acquisition of a subsequent topographic AFM image.
The extension resulted in ruptured and non-ruptured protein loops, with and without the appearance of hairpin-like thin sections and nodules, while the adjacent domains were non-displaced. Ruptures occurred in 27% of the VWF. Extension, which is the ratio of the length of the section following and prior to manipulation was 2.7 vs. 1.6, p= 0.0001 in non-ruptured and ruptured multimers. Nanomanipulation of protomers in which all the 5 nodules are separated, resulted in a mean final length of 345±69 nm, compared to their pre-manipulation length of 168±54 nm. None of the above results correlated with the protomer's structural hierarchy showing the main role of the adhesion of certain domains to the mica surface. The nanosurgical manipulation used here demonstrates the VWF mechanical properties at the single domain level via extending further the C1-C6 and A1-A2-A3 domains or eventually rupturing them. The observed conformational changes indicate that VWF may have a large conformational force response potential in order to fine-tune the opening up of hidden epitopes for the different functions of the VWF.

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P08

Regulation of actin cytoskeleton dynamics through adhesion-molecule-mediated mechanotransduction

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Endothelial cells are continuously exposed to external forces. In addition, during inflammation or metastasis a leukocyte or a cancer cell can exert tension on the endothelial monolayer. Change in tension is sensed by cell adhesion molecules, such as ICAM-1 or PECAM-1, and is translated to specific cellular responses, such as actin reorganization, in a process called mechanotransduction. To model leukocyte or cancer cell mediated mechanotransduction, we used magnetic beads coated with an antibody against a specific adhesion molecule. After adhesion of the beads, we applied a continuous force (about 40 pN) with a permanent magnet on the endothelial monolayer and followed reorganization of the actin cytoskeleton by confocal microscopy. We have found that during PECAM-1-mediated mechanotransduction adhesion increased peripheral actin ring formation, which was followed by stress fiber formation upon application of force. VE-cadherin junctions were subsequently reorganized, weakening cell-cell junctions. Thus, the force applied by a cancer cell might help its transmigration by weakening endothelial cell-cell junctions. At the same time, the strength of cell-extracellular matrix (ECM) adhesion was increased, which might play a role in the regulation of cell-cell adhesion. During inflammation on the apical side of endothelial cells filopodia-like protrusions are formed, which help not only in the adhesion of immune cells, but also guide them where to pass the endothelial monolayer. We have found that adhesion through ICAM-1 increases the size of these filopodia, and their size is decreased upon force application. We could also visualize filopodia formation during the transmigration step of A375 melanoma cells. The molecular details of the regulation of filopodia size are not known and we propose that the activity of the Yes-associated protein, YAP might play a role. Our integrative biomechanical and cell signaling approach might reveal new targets to inhibit metastasis.

Acknowledgments

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P09

Effect of ionizing radiation on the behaviour of miRNA binding proteins

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RNA-binding proteins play an important role in the regulation of miRNAs and affect a number of intracellular processes (e.g. DNA repair). miRNA binding proteins may also affect the regulation of intercellular communication by shaping the content of EVs, which are key players in the transmission of information between cells and are also responsible for mediating the ionizing radiation-induced bystander effect [1-4].
We investigated the effects of ionizing radiation on miRNA-binding proteins. Our aims are to analize the behaviour of proteins that influence the composition of the miRNA content of EVs after exposure and to explore their role in mediating the bystander effect.
We studied the behaviour of miRNA binding proteins in mouse bone marrow cells, which are highly sensitive to ionizing radiation. The experiments were performed in a control (0 Gy) and two X-ray irradiated groups (0.1 Gy and 3 Gy) 24 hours after exposure. Changes in the expression level of miRNA binding proteins were examined by Real-Time PCR, and intracellular protein levels by Western blot. we compared the results to the post-irradiation data of miRNAs detected by them and also present in EVs.
The results show significant differences in the expression levels of 4 out of 17 proteins tested after 3 Gy irradiation. Based on a combined interpretation of the results and the miRNA measurement data, it can be concluded that the abundance of certain proteins and the EV abundance of their bound miRNAs show a similar trend upon exposure to radiation. According to this information, it can be concluded that certain miRNA binding proteins are able to modulate the miRNA composition of extracellular vesicles in response to radiation, and thus are likely to play an important role in the bystander effect.

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P10

A comprehensive review of various metal-containing nanoparticles in terms of their antibacterial effect and cytotoxic properties

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One of the main reasons, why the healing of chronic wounds is highly challenging is the emergence of multidrug-resistant bacteria. These wounds usually cannot be treated effectively with conventional antibiotics, therefore, alternative methods and agents are investigated to replace them. [1] [2] In this regard, metal nanoparticles (MeNPs) could be promising substances, because they possess significant antimicrobial activity, with the advantage that bacteria are unlikely to develop resistance against them. [3] On the other hand, application of MeNPs may be challenging, since their toxic effect against microorganisms is not selective, and they additionally carry cytotoxic effect. [2] Numerous research articles have been published, which examine the potential of using different MeNPs for wound healing applications. However, it would be invaluable to have a review article that compares the antimicrobial and cytotoxic properties of the most promising MeNPs.

More than 50 scientific articles have been studied, regarding the wound healing potential of silver, gold, copper-, iron- and zinc containing nanoparticles. In the comparison, the main focus was on the antimicrobial activity and cytotoxic effect. Numerous types of Gram-positive and Gram-negative bacteria was treated with metal nanoparticles. In most of the cases, MeNPs were not on their own, rather in a hydrogel, or along with other agents, such as traditional antibiotics. Cytotoxicity was tested on various malignant and normal cell lines. Several cytotoxicity-reducing methods were exhibited, but in general, the effect of the MeNPs is dose-, shape- and size-dependent, but exposition time and the exact cell line used are also not negligible factors.

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P11

Biophysical characterization of clot retraction in platelet rich plasma of patients with primary anti-phospholipid syndrome

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Anti-phospholipid syndrome (APS) is an autoimmune process leading to thrombotic disorders [1], but with poorly understood mechanisms.
By using atomic force microscopy (AFM)-based nano-thrombelastography (nTEG) [2], we investigated the biophysical characteristics of the fibrin network during clot formation and degradation in platelet-rich plasma (PRP) of 38 APS patients compared with 18 controls. Patients with APS and venous thromboembolism (VTE) were selected as case and control, respectively.
Citrated blood was centrifuged at room temperature (150 g, 10 min) to obtain PRP, so that platelet count was set to 50G/L. An AFM cantilever was submerged in a 0.3-mL sample and cyclically moved up and down with an amplitude of 1 μm and a speed of 1 μm/s. In addition to PRP the sample was completed with 10mM Ca^{2+}, and clotting was initiated with thrombin at a final activity of 1 IU/ml. As the sample clotted, the cantilever deflected progressively during its vertical travel, reflecting the onset and increase in the elastic and viscous properties of the clot. The onset of clot formation was determined by measuring the time delay until the first non-zero force signal appeared. Clot contractility was assessed by measuring the rate of force increase. Finally, the viscoelastic response of the clot was obtained by measuring the force hysteresis area and the peak force difference in the datasets collected in the opposite cantilever directions (up versus down). The median parameter values of the APS and control samples were compared.
We found that in the APS group, the delay until the first force signal increased 2-3-fold [sec]; the slope of the force generation decreased to about 1/2 [nN/sec]; and the maximal force difference in the mechanical cycles decreased to about 1/3 [nN]. These results compare well with recent observations in which macroscale methods were used [3].
In sum, we were able to characterize quantitatively the nanoscale changes in the viscoelastic properties during platelet contractility and fibrin network formation in human PRP in a distinct pathology. We expect that the rich dataset provided by the AFM-based measurement employed here will provide insights into the molecular mechanisms associated with the pathology of APS.

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P12

Crystallographic and molecular dynamics simulations shed light on the self-inactivated conformation of the Venezuelan equine encephalitis virus (VEEV) protease

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The application of computational simulations in molecular biology allows us to examine a wide range of properties of enzymes, which would otherwise be challenging using experimental methods. Molecular dynamics are exceptionally useful to study conformational landscapes, substrate binding patterns, as well as interaction networks.
The Venezuelan equine encephalitis virus (VEEV) is responsible for causing mild to severe disease in both humans and livestock. The non-structural protein 2 protease (nsP2pro) of VEEV is considered as a drug target due to its crucial role in the viral life-cycle. Therefore, investigation of this viral protein may provide valuable information for structure-based drug design.
We aimed to study the structure of VEEV nsP2pro using both *in vitro* and *in silico* approaches. The structure, containing the wild-type N475 N-terminal residue at the active site, was determined experimentally at high resolution (1.46 Å), using X-ray crystallography [1]. The protein exhibited an unexpected conformation wherein the N-terminus mimics substrate binding. This self-inactivated conformation was previously observed only for a N475A mutant enzyme.
To compare the active and inactive conformers, we have used the Amber16 software for molecular dynamics simulations. We investigated the dynamic hydrogen bond networks at the active site, and compared the differences between the dynamic properties of the active and self-inactivated conformers as well as between the N475 and A475 containing variants. The comparison sheds light on the interactions that are crucial to the stabilization of the conformers.

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P13

Preliminary experiments for the rheological study of oleogels

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Due to deforestation to expand oil palm plantations and the many negative health effects of palm oil, the food industry needs other alternative sources of fats to replace palm oil. In our research, we investigated blends of fully hydrogenated rapeseed oil and non-hydrogenated high oleic sunflower oil, as well as beeswax and high oleic sunflower oil as possible oleogel alternatives. Oleogels are gelled forms of vegetable oils. Gelling agents can be waxes or proteins. The rheological properties of these oleogels were compared with the rheological properties of palm oil and a commercially available confectionery fat.
The rheological measurements were performed on the following materials: Unfractionated palm oil, Chocofill confectionery fat, Blends of fully hydrogenated rapeseed oil and non-hydrogenated high oleic sunflower oil: 25 m/m% rapeseed oil + 75 m/m% sunflower oil; 30 m/m% rapeseed oil + 70 m/m% sunflower oil; 35 m/m% rapeseed oil + 65 m/m% sunflower oil and Beeswax and high oleic sunflower oil mixture: 85 m/m% sunflower oil + 15 m/m% beeswax.
The flow curve in the shear rate range 0,1 to 100 1/s, as well storage (G') and loss (G") modules of oleogels, palm oil and confectionery fat were determined with an Anton-Paar MCR302 oscillatory rheometer. The flow curves were approximated with the Hershel-Bulkley and Casson model using the Excel Solver program. The linear viscoelastic limit was determined from the curves obtained by amplitude sweeping.
The confectionary fat showed rheological similarity with palm oil, however, this type of sample was the furthest from the rapeseed-sunflower and beeswax-sunflower blends in rheological properties. Based on the data analyses and the characteristics of the substitute blends, the sample containing 35% fully hydrogenated rapeseed oil and beeswax oleogel might be suitable as palm oil substitutes, but further studies are needed to determine this statement.

P14

Comparative analyses of the gelsolin homology domains of gelsolin and flightless-I

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Flightless-I is a unique member of the gelsolin (GSN) superfamily alloying six gelsolin homology (GH) domains and leucine-rich repeats. Flightless-I is an established regulator of the actin cytoskeleton. However, its biochemical activities in actin dynamics regulation are still largely elusive. To better understand its biological functioning, we performed a comparative analysis of GSN and Flightless-I by in vitro fluorescence spectroscopy and single filament TIRF microscopy approaches. We found that Flightless-I can interact with actin and affect actin dynamics in a calcium-independent fashion. Notably, our functional analyses indicate that GSN and Flightless-I respond to calcium differently implying different conformational characteristics of the GH domains in the two proteins. Bioinformatics analyses predict that the sequence elements responsible for calcium activation of GSN are not conserved in the GH domains of Flightless-I. Consistently, the use of intrinsic and extrinsic fluorescent probes revealed that unlike that of GSN the conformational behavior of the GH domains Flightless-I was not significantly affected by calcium-binding. Altogether, our work reveals different calcium-response and predicts distinct modes of activation of GSN and Flightless-I.

Acknowledgments

Activities of Flightless I revealed by acto-myosin based in vitro motility techniques, 2021-4.1.2-NEMZ_KI-2022-00025 (TH); New National Excellence Program of the Ministry for Innovation and Technology ÚNKP-21-3-II-PTE-997 (PG). We thank József Mihály (Institute of Genetics, Biological Research Centre) for the *Drosophila* Flightless-I plasmids, Tomohito Higashi (Fukushima Medical University) for the *human* Flightless-I plasmids and Robert C. Robinson (Okoyama University) for the *human* GSN plasmid.

P15

Co-assemblies of cationic antimicrobial peptides with anionic small molecules: unique thermophoretic behaviour

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Peptidic supramolecules formed via self- and co-assembly show emerging applications from materials chemistry to biomedicine. Recently we have demonstrated for a couple of cationic, amphiphilic antimicrobial peptides (AMPs) the ability to form co-assemblies with various anionic, aromatic small molecule (SM) binding agents. Their interaction results in modulating their activity in vitro. Herein we addressed interaction systems of four selected AMPs (CM15, Dhvar4, LL-37, and FK-16, coupled to carboxyfluorescein) with four selected SMs (suramin, tartrazine, biliverdin, and bilirubin ditaurate) exploiting the sensitivity of microscale thermophoresis (MST). MST is a solution phase method utilizing the phenomenon of induced thermophoresis where movement of the molecules is monitored via the fluorescence of the target partner. Besides substantial similarities, various scenarios of thermophoretic responses were revealed. Interestingly, negative thermophoresis was frequently observed, and maximal MST responses were typically found not at the highest SM concentrations applied. Findings suggest substantial contributions of charge and hydration effects. Variations in the thermophoretic behaviour could also be attributed to the oligomeric state of the individual components, and the dynamic nature of the association process. Results demonstrate that the MST method is an excellent additional technique for identifying and studying peptidic assemblies.

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P16

Conformational Dynamics of Human Calmodulin at Low Ca^{2+} Saturations

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Calmodulin is an extremely conservative key protein of intracellular signal transduction. It can bind four Ca^{2+}-ions by two EF-hand motifs in its N- and C-terminal domains. During Ca^{2+}-binding hidden hydrophobic surfaces become available, which take part in recognition of a broad range of target proteins. These observations led to the idea of calmodulin being a molecular switch, activating its partners when Ca^{2+}-saturated at elevated intracellular calcium levels. It is known that calmodulin can adopt numerous conformational states, however, the change of conformers along Ca^{2+}-binding is not known. Importantly, considering the intracellular calmodulin and Ca^{2+}-levels, and the Ca^{2+}-affinities of calmodulin EF-hands, it is unlikely that calmodulin gets Ca^{2+}-saturated in physiologic signaling conditions. What is then the mechanism of target protein selection? The Ca^{2+}-binding of calmodulin displays cooperativity which can be described by a Perutz binding model to explain Ca^{2+}-saturation experiments *in vitro*. The function/conformation of calmodulin at low Ca^{2+}-loads has not been explored yet in details, whereas all physiologic Ca^{2+}-signaling happens at low Ca^{2+}/calmodulin concentration ratio. Compartmentalization of target proteins and canonic binding modes of Ca^{2+}-saturated calmodulin cannot account for why the spatiotemporal information content of intracellular Ca^{2+}-signaling is not lost during calmodulin signal transduction.
Our experiments uncovered an increased conformational diversity at low Ca^{2+}/calmodulin concentration ratio, and that the binding of model peptide melittin to calmodulin happened much before the Ca^{2+}-saturation of the protein. We carried out molecular dynamics simulations of calmodulin with adding two Ca^{2+}-ions to the C-terminal EF-hands of apocalmodulin and also by removing two Ca^{2+}-ions from the N-terminal EF-hands of holocalmodulin. We observed striking differences in the structural dynamics of the N-terminal apo–C-terminal holo calmodulin, depending on the initial state from which we have reached this state. Based on our experiments and molecular dynamics calculations we hypothesize that the spatiotemporal changes in intracellular Ca^{2+}-concentrations can be coded into the structural/conformational dynamics of calmodulin.

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P17

Single amino acid mutation decouples photochemistry of the BLUF domain from the enzymatic function of OaPAC and drives the enzyme to a switched-on state

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Photoactivated adenylate cyclases (PACs) are light-activated enzymes that combine a BLUF (blue-light using flavin) domain and an adenylate cyclase domain that are able to increase the levels of the important second messenger cAMP (cyclic adenosine monophosphate) upon blue-light excitation [1-3]. The light-induced changes in the BLUF domain are transduced via a mechanism that has not been established yet to the adenylate cyclase domain. One critical residue in the vicinity of the flavin in BLUF proteins is the glutamine amino acid close to the N5 of the flavin[4-6]. The role of this residue has been investigated extensively both experimentally and theoretically. However, its role in the activity of the photoactivated adenylate cyclase, OaPAC has never been addressed. In this work, we have applied ultrafast transient visible and infrared spectroscopies to study the photochemistry of the Q48E OaPAC mutant. This mutation decelerated the primary electron transfer process but switched the enzyme in to a permanent 'on' state, able to increase the cAMP levels under dark conditions compared to the wild-type OaPAC. Differential scanning calorimetry measurements pointed out to a less compact structure for the mutant. These findings provide insight into the important elements in PACs and how their fine tuning may result to the design of optogenetic devices.

Acknowledgments

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P18

How nanoparticles affect aggregation and cytotoxicity of the amyloid-beta peptide?

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Using nanoparticles is becoming common in medicine and biotechnology. Additionally, nanoparticles are also present in our environment as nano-pollution which might enter living organisms. Proteins which get into contact with nanoparticles, might undergo structural changes. Aggregation and amyloid formation of proteins and peptides can also be affected by nanoparticles. Our group is interested in investigating the aggregation and amyloid formation of the amyloid-beta (Aβ) peptide which is known for creating amyloid fibrils in the brain in Alzheimer's disease. We used in-house expressed recombinant Aβ(1-42) to study the structure, aggregation kinetics, and cytotoxicity of the peptide. We characterized nanoparticles of different materials (CaF_2, silica, polystyrene) and studied their effects on Aβ. Based on thioflavin T fluorescence, we found that nanoparticles typically reduce lag time of the aggregation. Polystyrene nanoparticles bind peptide monomers up to a saturation point and let only excess peptides to form fibrils. CaF_2 and silica do not show an effect similar to this, but they influence the secondary structure of the fibrils. We used circular dichroism (CD) spectroscopy and the BeStSel method to determine secondary structure composition. Aβ fibrils formed alone have a high content of parallel β-sheets. Nanoparticles, depending on their type, affect the secondary structure composition of Aβ aggregates altering the fractions of the different type of β-sheet structures. Toxicity measurements on mouse hippocampal cells suggest that Aβ is highly cytotoxic, but nanoparticles might reduce cytotoxicity of Aβ if applied in appropriate concentrations.

Acknowledgments

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P19

Investigation of antibody-mediated adhesion force of individual immune cells using computer-controlled micropipette and fluidic force microscopy

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Cell adhesion is a fundamental process that plays a critical role in various biological phenomena, such as the immune response. To understand the mechanisms of cell adhesion and develop new therapies, it is necessary to measure the forces involved.Computer-controlled micropipettes (CCMP) and Fluidic Force Microscopy (FluidFM) techniques have become effective instruments for the accurate determination of cell adhesion in recent years.

A CCMP is a tool that can be used to probe single cell interactions with specific macromolecules and surfaces. The micropipette is mounted onto a micromanipulator on a normal inverted microscope and is controlled by a computer. The adhesion force of surface-attached cells can be accurately probed by repeating a pick-up process on the examined cells while increasing a vacuum applied through a pump system in the pipette positioned above the cell. Using this methodology, high number of cells adhered to specific macromolecules, treated surfaces can be measured one by one in a short period of time. Additionally, the probed single cells can be easily picked up and separated for further examinations by other techniques. This is a definite advantage of the CCPM.[1]

FluidFM, on the other hand, uses a hollow cantilever with a small opening at the tip that can be filled with liquid using a fluid reservoir that is attached to a pressure control system, allowing for precise fluid dispensing and manipulation at the nanoscale. This technology enables the precise measurement of adhesion forces between cells and their targets and makes it possible to record the entire cell detachment process quickly and accurately. [2]

The combination of these two technologies provides high accuracy and resolution in the measurement of cell adhesion forces and in addition to measuring adhesion forces. We used these techniques to study the adhesion strength of immune cells on different surfaces.

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P20

Salt loaded fibrous meshes from polysuccinimide for medical applications

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Silver is increasingly being pushed out of medicine, and other ions with antibacterial effect are coming to the forefront. This is because since silver ions have huge environmental burden.
The aim of our research is to create a bicomponent polymer network by electrospinning, which contains zinc and strontium salts in addition to the polymer, which according to the literature, have antibacterial properties. If such a new type of biocompatible wound dressing could be created, not only would be a mechanical barrier to infection but also have antibacterial activity against microorganisms.
The first step was to synthesize the polymer, then mix it with the selected inorganic salts in dimethylformamide, and then optimize the electrostatic fiber formation parameters. The inorganic salts used in the experiments were $Zn(O_2CCH_3)_2$ and $Sr(NO_3)_2$. Next, the chemical and mechanical properties of the complete polymer networks were investigated by FTIR spectroscopy, SEM images, and their mechanical behavior through specific load capacity. In the next step, I investigated the antibacterial effect of polymer networks containing inorganic salts on four different bacterial species.
Based on FTIR spectroscopy, the salts formed a physical bond with polysuccinimide, but there was no chemical bonding between them. SEM of a polymer mesh containing inorganic salts showed a big difference between the fiber diameters in the presence of the salts. However, no difference was found in their specific load capacity. In antibacterial experiments, a more significant clearance zone appeared for the polymer containing $Zn(O_2CCH_3)_2$, and a lesser inhibition zone for those containing $Sr(NO_3)_2$ salts. The next step was to find out if the polymer meshes with the salts have cytotoxic activity against human tumor and fibroblast cells, because these fibrous structures are going to use as wound dressing, so they must interact with the human cells.

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P21

Nanoparticle uptake of living cells with digested glycocalyx

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In biomedical imaging and targeted drug delivery, functionalized nanoparticles are widely used due to their penetration into living cells. The glycocalyx is a surface sugar layer of the cells, which presumably plays an essential role in any uptake process. However, its exact function in nanoparticle uptake is still uncovered. We *in situ* monitored the penetration of positively charged gold nanoparticles into adhered cancer cells with or without preliminary glycocalyx digestion. During the experiments, the components of glycocalyx of HeLa cells were digested by chondroitinase ABC enzyme. The measurements were performed by applying a high-throughput label-free resonant waveguide grating biosensor. The positively charged gold nanoparticles were used with different sizes (S, M, L). Negatively charged citrate-capped tannic acid nanoparticles, and other types of glycocalyx digesting enzymes were also applied in control experiments. The biosensor data confirmed the cellular uptake of the functionalized nanoparticles with an active process, which was verified by transmission electron microscopy [1,2]. Based on the findings we conclude that the components of gylcocalyx control the uptake process in size- and charge-dependent manner, and the possible roles of various glycocalyx components were highlighted.

Acknowledgments

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P22

Mathematical modelling of low dose hyper-radiosensitivity and induced radioresistance

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The surviving fraction of cells decreases exponentially with the increase of the absorbed dose at high doses. At low doses however, experiments show that in many different cell lines the surviving fraction differs from this, due to the effects of hyper-radiosensitivity and induced radioresistance [1]. The result is a function that starts steeper and after a local minimum starts to increase to a local maximum as the dose increases before following the exponential decrease at higher doses.
The aim of this study was to test if the principle of minimum mutation load [see e.g., 2] can describe both hyper-radiosensitivity and induced radioresistance at low doses. In this case the principle means that the most damaged cells in a vicinity use apoptosis to reduce the mutation rate in the tissue.
To test this hypothesis a mathematical model was developed and an experimental database constructed from published articles for validation.
For the model validation a database was used, consisting of various experimental data featuring low dose hyper-radiosensitivity [3]. The model has been developed in Python. A total of 600 cells are placed randomly in a circle with a given radius. The cells are able to communicate their DNA damage by a signal, its concentration following normal distribution, centered on the cell. The DNA damage of the cells follow Poisson distribution for any given dose.
The fit parameters for the model were acquired by fitting with the Nelder-Mead method to the experimental data in each case. The parameter calculation consisted first a preliminary calculation (using the starting slope and the local minimum of each dataset) to acquire the initial parameters, then a two dimension fit with the Nelder-Mead method was calculated to reach the best values for them. The results then were evaluated and compared to the induced repair model where possible (where there was a fit in the original article).

Acknowledgments

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P23

Single-cell adhesion measurements using fluidic force microscopy

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Nowadays single-cell techniques are becoming valuable tools in the field of biology and biophysics. By investigating at a cellular level, we can better understand the cellular heterogeneity and the possible subpopulations in a tissue. This knowledge can bring novel applications and solutions to health and medicine. Fluidic Force Microscopy (FluidFM) is similar to atomic force microscopy (AFM), but uses a hollow, microfabricated cantilevers connected to a liquid reservoir and pressure controller system [1]. Through its precise force control, the cantilever is ideal to approach individual cells gently and reproducibly. Depending on the applications, the end of the cantilever can be different. For cell adhesion measurements 2-8 µm circular openings are ideal [2], while for live cell sampling and injections [3][4], so-called nanosyringes are used. These cantilevers have pyramidal tips with a 600 nm opening at their side. The cantilevers used for cell adhesion measurements can have different spring constants, which also determine some applications of the device. The short lecture will introduce a robotic version of the technique by presenting experimental results on the adhesive and mechanical properties of both cancerous and healthy cell types.

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P24

G-quadruplexes: Thermodynamic characterization, identifying the physicochemical parameters influencing the stability

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Besides of the well-known double-helical structures nucleic acids can form non-canonical motifs as well. The most important one is the G-Quadruplex (GQ), which is formed by guanine-rich sequences of the genome. Potentially GQ-forming sequences were found in crucial loci of the human genome, where formation of GQs can take part in the regulation of important processes like cell proliferation and cell death. Their appearance in the oncogene promoter regions made GQs an attractive target of the cancer research.
Here we report the results we obtained on two oligos, both DNA and RNA ones [1-3]. They were chosen from the genome of the hepatitis B and SARS-CoV-2 viruses. We selected a short oligo that was suggested by computer analysis of the genome to form GQ with high probability.
Infrared and fluorescence spectroscopic methods have been used to determine the conformation of the GQs. Förster resonance energy transfer allowed following the unfolding of the oligos.
Since GQs were considered as targets of the cancer therapy, several ligands were developed for stabilization of the human GQs. We investigated three of these ligands, whether they can stabilize the viral GQs as well.
As conclusion, we have proven that both HepB (DNA) and C19/1 (RNA) viral oligos form G-quadruplex structure however with different temperature stability. The unfolding mid-points were 53 and 37 °C respectively.
All the ligands we investigated (TMPyP4, BRACO19, PhenDC3) were proven to bind and stabilize these oligos (except BRACO19 which did not bind C19/1).
These results might have important impact in the fighting against viruses, since stabilization of the GQs will influence the proliferation of the virus.

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P25

Variation of electrical impedance parameters of dry crops during soaking

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When processing nutritionally important dry pulses such as beans, soaking is one of the most important preparatory steps. As this process is relatively time-consuming. However, this long soaking time can be reduced by ultrasound.
The experiments were performed on red kidney beans (Phaseolus vulgaris cv. Rampart). Prior to the actual sample preparation, beans were manually sorted into four groups according to their size (S - small ≤ 13 mm; M - medium = 13 - 15 mm; L – large = 15 - 17 mm; X - extra-large ≥ 17 mm).
For the ultrasound treatments we used an ultrasonic bath (HBM Machines, The Netherlands). The treatments applied were performed at 40 kHz 300 W at 20 °C.
The magnitude and phase angle of the impedance of the beans were measured in the frequency range of 30 Hz - 30 MHz with an HP4284A and HP4285A precision LCR meters at a measuring voltage of 1 V in an HP16451 B test fixture. To achieve a good electrical contact, an ECG electrically conductive gel was placed between the bean shell and the electrode.
As the soaking time increased, the magnitude of the impedance decreased, which may indicate that the increased water content reduced the viscosity and thus increased the mobility of the charges, resulting in a decrease in resistance and impedance.
This reduction is also clearly visible in the ultrasonic treatments compared to the control samples.
The phase angle spectrum shifted towards lower frequencies, which in turn may be a result of the destructive effect of ultrasound. The effect of the treatments shifts the minimum point around 31 kHz towards lower frequencies. The longer the soaking time, the lower the impedance value and the more the ultrasound-induced structure damage was well detected. These suggest that it may be possible to qualify the structural condition of the soaked dry structures.

P26

Anisotropy imaging using RCM

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Both the differential polarization attachments for LSMs and Re-scan Confocal Microscopy (RCM) facilitate revealing structures at the molecular level [1]. For subcellular structures, increasing the available resolution can be fundamental. The re-scan confocal microscopy (RCM) provides 4 times better signal to noise ratio (compared to conventional PMTs) using an sCMOS camera, moreover, due to the second scanner, the image resolution increases by the factor of 1.4, while the axial resolution is identical with the LSMs [2].

With additional polarization elements, the RCM enables the 2D and 3D microscopic mapping of the anisotropy of samples via measuring fluorescence-detected linear dichroism (FDLD). The usage of the liquid crystal modulator for the RCM and the high frequency modulation (photoelastic modulator – PEM) are synchronised with the imaging. The DP-LSMs and the pRCM (RCM equipped with polarization attachment) are suitable for obtaining unique structural information on the anisotropic molecular organization of biological samples and intelligent materials [3, 4] in 2D and 3D.

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P27

Fibrillin-1 microfibrils in Marfan syndrome: nanoscale structural characterization using atomic force microscopy

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Fibrillin-1 microfibrils are essential elements of the extracellular matrix serving as a scaffold for the deposition of elastin and endowing connective tissues with tensile strength and elasticity. Mutations in the fibrillin-1 gene (FBN1) are linked to Marfan syndrome (MFS), a systemic connective tissue disorder that usually manifests in life-threatening aortic complications. The aortic involvement may be explained by a dysregulation in microfibrillar function and, conceivably, alterations in the microfibrils' supramolecular structure.
The aim of the study was to perform a nanoscale structural characterization of fibrillin-1 microfibrils isolated from human aortic samples with different FBN1 gene mutations and to compare them with microfibrillar assemblies purified from non-MFS human aortic tissue.
Aortic wall samples were obtained from patients undergoing specific cardiovascular surgical interventions. Fibrillin-rich microfibrils were extracted by bacterial collagenase digestion and purified by size-exclusion chromatography. Atomic force microscopy was employed to visualize and study the microfibrillar assemblies.
Fibrillin-1 microfibrils displayed a characteristic "beads-on-a-string" appearance. The microfibrillar assemblies were investigated for bead geometry (height, length, and width), interbead region height, and periodicity. MFS fibrillin-1 microfibrils had a slightly higher mean bead height, but the bead length and width, as well as the interbead height, were significantly smaller in the MFS group. The mean periodicity varied around 50–52 nm among samples.
In conclusion, the data suggest an overall thinner and presumably more frail structure for the MFS fibrillin-1 microfibrils, which may play a role in the development of MFS-specific aortic symptomatology.

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P28

Automated single cell isolation on a microscope

Bálint Szabó

CellSorter Kft.

Current robots for single cell manipulations can only handle surface attached cells limiting the field of applications. CellSorter developed a computer vision-based robot to automatically recognize and gently isolate intact individual cells (for subsequent analysis, e.g., DNA/RNA sequencing) from suspension without immobilizing cells on the surface of the Petri dish. We used a motorized microscope and robotic micropipette controlled by the computer vision of cells. Combination of 1 µm positioning precision, adaptive cell targeting and < 1 nl liquid handling precision resulted in an unprecedented accuracy in robotic cell isolation. Single cells could be picked up in a volume of 1.4 ±0.6 nl without removing neighboring cells. Sorting process did not affect the viability of cells. We compared the efficiency of our method to that of single cell entrapment in microwells and subsequent sorting with the automated micropipette: the recovery rate of single cells was greatly improved. Our straightforward technique needs minimal sample preparation and can be applied for virtually any tissue cell type. We expect new applications in circulating tumor cell (CTC) detection and isolation for molecular characterization and personalized treatment.

P29

Finite element modelling and analysis of fluid dynamic phenomena in two-phase droplet based microfluidic systems

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Recently the development and application of Lab-on-a-Chip and microfluidic devices have been spreading, enabling not only the extension of biological sample preparation and sensing solutions, but the comprehensive analysis and understanding of microscale fluid dynamic phenomena. The high performance finite element modelling (FEM) environments combined with experimental methods are enable solid description of microfluidic processes and reliable prediction the behaviours of novel architectures. However, the modelling of multiple-phase flows in microscale geometries is still a challenge.
Finite element Modelling code – COMSOL Multiphysics – was applied to analyse the process of droplet formation in 2D and 3D two-phase models to achieve more accurate comprehension of the behaviour of microfluidic systems depending on geometries and fluid parameters [1]. The analysis is based on the numerical solution of the governing Navier-Stokes and continuity equations. The fluidic environment and boundary conditions in these specific capillary systems can be characterised by the Capillary-number, which describes the relationship between the surface tension and the viscous forces. In this work the influence of channel geometries, the volume flow ratio at the inlets, the viscosity of the fluids, and the interface tension were studied in successive parametric sweep simulations. For handling multiphase fluids in 2D and 3D models a robust Level Set calculation method was used [2].
The droplet generation phenomena was studied by experimental methods, to verify FEM results, proving that the droplet formation processes as well as droplet sizes and generation frequencies are significantly affected accordingly can be effectively controlled by the geometric and flow parameters. The microfluidic systems were manufactured by soft lithography techniques in Polydimethylsiloxane (PDMS) polymer and tested using water / oil two phase fluid system.

Acknowledgments

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P30

Structural features of a disordered protein motif

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SALS (sarcomere length short) is a Drosophila-specific sarcomeric protein that regulates sarcomere length and organization. Lack of SALS is lethal in embryonic age, possibly due to the shortening of sarcomeric actin filament length or the disruption of their order.
Our bioinformatics analysis suggests that SALS is an intrinsically disordered protein (IDP). IDPs are biologically active proteins, however, do not have a well-defined three-dimensional structure. They possess specific physicochemical properties different from those characteristics for ordered proteins (e.g., amino acid composition, thermal stability, electrophoretic mobility). There is growing attention to studying IDPs for their key roles in diseases or cellular processes. SALS contains two intrinsically disordered protein regions (IDRs), the Wiscott-Aldrich syndrome homology 2 (WH2) domains, composed of a few tens of amino acids. Proteins containing WH2 domains possess actin-binding properties and can exhibit multifunctional character depending on the number and sequence of WH2 domains.
Based on our functional analysis of the SALS WH2 domains (SALS-WH2), both WH2 domains interact with actin and influence actin homeostasis by shifting the monomer:filament ratio towards monomeric actin. The structural properties and conformational dynamics of SALS-WH2 have not yet been described. Therefore, we further aim to characterise these features using in silico and experimental approaches. Our prediction-based results were experimentally verified by fluorescence spectroscopy and thermal analysis.

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P31

Investigation of actin polymerization: methodology and case study

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Actin is one of the most important components of the cytoskeleton that plays a crucial role in many cellular processes. The organization of the actin cytoskeleton is under the control of actin binding proteins (ABPs). Total internal reflection microscopy (TIRFM) is a proper technique for the observation and characterization of actin dynamics from single molecules to a complex system in vitro, it works as a bridge between in vitro and in vivo actin polymerization assays. Furthermore, it is suitable to investigate the role of ABPs in modifying the structural and dynamic properties of actin. Flightless-I (Fli-I) is a member of gelsolin superfamily. It has a unique structure including gelsolin-like domains (GH) and leucine-rich repeats (LRR). Fli-I can interact with actin, however, its biochemical activities in actin dynamics regulation are largely elusive.
The aim of our study is to provide an overview of actin polymerization experiments by fluorescence spectroscopy and TIRFM from basic principles to data/image analysis. We test the applicability of different fluorophores and specify the advantages and limitations of each approach. As a case study, we investigate the effect of wild-type Fli-I and its disease-causing mutations and truncations on actin dynamics. Pyrene-actin-based fluorescence spectroscopy reveals a biphasic effect of Fli-I on actin assembly kinetics, complementary TIRFM assays enlightens the molecular mechanism underlying the activities of Fli-I and its disease-related variants at the single filament level.

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P32

Adaptive changes in the A-band region of the giant protein titin in diseased human cardiac sarcomere

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Titin, the largest protein known, spans the half-sarcomere, the contractile unit of skeletal and cardiac muscle through its Z-disk to M-line and interacts with thin and thick filaments in the I- and A-band of the muscle sarcomere, respectively. Titin's A-band segment is not well understood but is shown to be orders of magnitude less extensible than the I-band region of the molecule. Heterozygous truncating mutations (TTNtv) affecting A-band titin are often associated with dilated cardiomyopathy (DCM). Marfan syndrome (MFS), a connective tissue disorder caused by mutations of the matrix glycoprotein fibrillin is also associated with impaired cardiac contractility but its exact pathomechanism is largely unknown.
Here, we performed STED super-resolution microscopy on sections of stretched and fixed demembranated human cardiac myofibrils carrying heterozygous TTNtv mutations or on samples originated from MFS patients. Sequence-specific anti-titin antibodies included the 1) MIR, which labels titin at the ends of the thick filaments, 2) A170, labeling titin close to the M-line (this epitope is missing in TTNtv+). Sarcomere length-dependent anti-titin epitope position, shape and intensity analysis pointed at structural defects in the I/A junction and the M-band of TTNtv+ sarcomeres. Our experiments indicate that truncated titin is able to integrate into the cardiac sarcomere. We propose that the truncated titin cannot precisely register the ends of the thick filaments, and this can ultimately lead to the manifestation of DCM by disrupting the overlapping of thin and thick filaments. In MFS sarcomeres a pronounced, ~30 nm shift away from the M-line was found in the case of the A170 titin epitope suggesting that alterations in the M-band ultrastructure might be important contributors of the impaired cardiac contractility of MFS patients.

P33

The effects of contrast agents on renal cell lines and on the actin cytoskeleton

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Medical images may show some degree of contrast loss in most imaging techniques. In such cases contrast materials are the best tools to enhance the density and intensity of the given area. Nowadays, radiologists can choose from a wide range of contrast agents. The active material of these contrast media penetrates in cells and because of their limited ability of depletion these molecules can accumulate in cells of different tissues. In many cases, contrast agents are used in relatively high volumes, which places a heavy burden on kidney function. A few years ago, contrast agent treatment was identified as the third leading cause of hospital-acquired acute kidney injury (after surgery and hypotension), accounting for 12% of all cases. Today, ~5% of hospitalized patients who develop acute renal failure have normal renal function before contrast administration. We believe that contrast agents exert a significant proportion of their cell-damaging effects by affecting the actin cytoskeleton. The overview of the corresponding literature clarifies that the effects of the clinically applied contrast materials expressed directly on the actin cytoskeleton and its detailed molecular mechanisms are unknown. Our aim is to investigate all possible effects contrast materials can express on human renal cells and the actin protein inside, especially the dynamic organization/rearrangement of the actin network.
Our results clearly show that the applied contrast materials greatly affect the polymerization properties of actin. The examined contrast compounds changed other parameters of actin too and caused dramatic changes on the examined cell lines as well. The DSC results show significantly decreased thermal stability for the treated actin filaments.

P34

Effect of weight loss on the electrical impedance parameters of lettuce and iceberg lettuce stored at room temperature

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We bought the lettuce and iceberg lettuce at the local market. The leaves were stored at room temperature. The weight loss of the leaves was measured with a scale. The electrical impedance spectra were determined with HP4284A and 4285A precision LCR meters in the frequency range of 30 Hz to 30 MHz. At a measuring voltage of 1 V, the magnitude and phase angle of the electrical impedance were measured. The measured spectra were corrected with the stray inductance and capacitance values. A homemade needle electrode and ECG electrodes were used for the measurement. The initial moisture content of the leaves was determined from samples dried to constant weight in an oven at 110°C. Both the impedance measurement and the weight measurement were performed at room temperature for each leaf for 10 minutes over 4-5 hours until the leaf completely withered. The measured impedance spectra were approximated with three distributed elements connected in series. We determined the resistance and capacity of the elements of the model circuit, as well as the relaxation time. The change in the obtained parameters followed well the weight loss, i.e. the change in the water content of the leaf.

P35

Label-free tracking of cell adhesion kinetics as a function of various parameters

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Most tissue cells cannot survive for more than a few hours without adherence, if we were able to prevent the adhesion of malignant cells, metastasis could be prevented [1] [2]. The data used for analysis was recorded by a surface sensitive, label-free, resonant waveguide grating based optical biosensor. Due to the force calibration of the optical signal provided by the sensor for individual cells, this technique is suitable for determining the force curves of a large number of cells [3]. To analyse the adhesion signals at the molecular level, we applied and developed different kinetic models. These models take into account relevant molecular parameters, such as dissociation and association rates, the two dimensional kinetic dissociation constant which describes the integrin-ligand binding strength [4].
We investigated the extent to which the adhesion kinetics of cells are affected by the surface density of the cells, the density of echistatin, and the effect of gold nanoparticles on cell adhesion. Echistatin is a potent inhibitor of αIIβ3, αvβ3 and α5β1 receptors [5]. Functionalized nanoparticles can penetrate into living cells, Peter et. al. demonstrated from the recorded kinetic adhesion data that the uptake of the functionalized nanoparticles is an active process [6]. Peter et. al. also investigated the role of glycocalyx components in the cellular uptake of nanoparticles [7]. Subjecting these results to further analysis, we developed procedures and codes that enable the study of molecular parameters influencing adhesion in living cells, in their real environment, without isolating the relevant molecules. Our results potentially open the way for further analysis of the kinetic data obtained from the adhering cells.

Acknowledgments

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P36

Spectroscopic study of extracellular vesicles using plasmonic gold nanoparticles

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Extracellular vesicles (EVs), spontaneously released by cells, play an important role in intercellular communication. Due to their special size and composition (lipid bilayer-bounded nanosystems, usually smaller than 200 nm, containing both proteins and RNA), they play diagnostic, prognostic and therapeutic roles, for example, they can be "new generation" biomarkers of various diseases.
IR spectroscopy, especially attenuated total reflection (ATR), is rapidly emerging as a label-free promising tool for molecular profiling of EVs. However, the relative low number of extracellular vesicles (~10^{10} particle/mL) and possible impurities (protein aggregates, lipoproteins, buffer molecules, etc.) present in EV samples might result in poor signal-to-noise (S/N) ratio. The plasmonic properties of gold nanoparticles (AuNPs) are used in many characterization techniques, inclusive characterization and testing of EVs. Surface-enhanced infrared spectroscopy (SEIRA – Surface-enhanced IR absorption) using plasmonic nanoparticle, however, is still an unexploited method.
Nanosized gold nanoparticles and tailored nanostructures with confined electromagnetic near-fields were prepared, characterized and tested with model-EVs (EV-like liposomes) and red blood cell derived EVs. A concentration dependent interaction was established between the citrate-stabilized gold nanoparticles and the lipid bilayers, which strongly affected both the plasmonic behaviour of AuNPs and the bilayers lipid organization. At appropriate extracellular vesicle – gold nanoparticle ratio a 6-fold maximum enhancement was obtained in the lipid spectral signatures. Exploiting the fine details of EV – gold nanoparticles interaction, further surface modifications of gold nanoobjects are planned, enhancing the sensitivity and specificity of EV detection enabling a strong platform for IR spectroscopic investigations of EVs.

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P37

Role of the hHv1 proton channel in vascular smooth muscle cells

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The hHv1 voltage-dependent proton channel is a passive transporter that selectively transfers protons across the membrane, and thus plays an important role in pH regulation of many cell types. Vascular smooth muscle cells (VSMCs) found in the arterial wall have a resting or contractile phenotype under physiological conditions. When the vessel wall is damaged, the cells switch to a synthetic, migratory and proliferative phenotype, which allows tissue regeneration. Failure of migrating/proliferating cells to switch back to a contractile phenotype induces pathogenic vascular remodelling leading to atherosclerosis.
In our work, we aim to demonstrate the presence of hHv1 on vascular smooth muscle cells and to elucidate the role of the channel in normal and pathological cellular activities through the regulation of intracellular pH. We investigate the role of hHv1 in VSMC survival, differentiation and matrix production.
If hHv1 plays a key role in the pathological activity of VSMCs during atherosclerosis but not in normal function, the channel may become an important pharmacological target to inhibit the pathological activity of VSMCs in atherosclerosis.
To confirm this hypothesis, we performed viability assays using MTT assay, motility experiments using scratch assay method and impedance measurement, PCR and Western blot experiments to detect hHv1, alizarin red staining to visualize the effect on calcified extracellular matrix production, and microscopic studies.
Our results support the hypothesis that hHv1 is expressed in VSMCs and plays a role in their ability to migrate and influences their viability. This conclusion may provide a basis for further experiments to quantify the difference in hHv1 expression between contractile and differentiated proliferative cells and to identify related functional differences.

P38

Nanoerythrosome-based promising drug delivery systems

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Nanoerythrosomes are artificial vesicle-like objects formed from erythrocyte-membranes, named ghosts, by physical processes, such as extrusion or sonication. Phosphatidylcholines (PCs) and sphingomyelins (SMs) are outer membrane constituents, while phosphatidylserines (PSs) and phosphatidylethanolamine (PEs) generally take place on the inner side of the membrane-bilayer. By addition of different artificial lipids, very different size-ranges of nanoerythrosomes can be achieved, therefore proper reference materials and drug delivery systems with adequate surface chemical behaviour can be prepared [1]. The presence of dipalmitoyl-phosphatidylethanolamine (DPPE) results in the formation of larger nanoerythrosomes, while the addition of dipalmitoylphosphatidylcholine (DPPC) induces the formation in a middle-range (140 -160 nm). The presence of the mixture of DPPC - LPC (lysophosphatidylcholine) causes bicelle – micelle type nanoparticles [2]. Here we show that in the complex physic-chemical study, among the different experimental methods (transmission electron-microscopy combined with freeze-fracture (FF-TEM), Microfluidic Resistive Pulse Sensing (MRPS), dynamic light scattering (DLS), the small-angle X-ray scattering (SAXS) turned out to be a powerful tool in the complex physic-chemical study of this drug delivery system.

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P39

Functionally important C-terminus of small GTPase Ran: exploring its nucleotide-specific conformational surface

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As a member of the Ras superfamily of small GTPases, Ran (Ras-related Nuclear protein) is the main regulator of the nucleo-cytoplasmic transport through the nuclear core complex. It functions as a molecular switch cycling between the GDP-bound inactive or “off” and GTP-bound active or “on” state. Since deregulation of Ran is linked to numerous cancers from the stage of cancer initiation to metastasis, understanding the complexity of its interaction, especially the regulatory mechanism, is critical for drug discovery.

Ran consists of a globular (G) domain and a C-terminal region, which is bound to the G-domain in the inactive, GDP-bound states. The crystal structures of the GTP-bound active form complexed with Ran binding proteins (RanBP) show that the C-terminus undergoes a large conformational change, embracing Ran binding domains (RanBD), whereas in the crystal structures of macromolecular complexes not containing RanBDs the structure of the C-terminal segment remains unresolved, indicating its large conformational flexibility. This movement could not have been followed either by experimental or simulation methods. Here, by using molecular dynamics (MD) and MDeNM (Molecular Dynamics with excited Normal Modes) simulation methods, we present how rigid the C-terminal region is in the inactive RanGDP form and for the first time in the literature, we were able to follow its conformational flexibility in the GTP-bound form. This conformational mapping allows us to envisage how the C-terminus can embrace RanBDs during the function of Ran.

The simulations were carried out by JC, and were analyzed and interpreted by JC, PR, BD, HJ, RN, DP, and EB.

P40

Identification of inhibitors of the human h_V1 proton channel

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The human voltage-gated proton channel (hH_V1) plays an important role in immune system and cancer cells being involved in functions such as proliferation, migration, and oxidative burst. H_V1 does not have a conventional ion-conducting pore, the conduction occurs through the voltage-sensing domain. This difference may be the reason for the lack of selective hH_V1 inhibitors. Currently, 5-chloro-2-guanidinobenzimidazole (ClGBI) is the most widely used inhibitor of H_V1 but it has low selectivity for the channel. This could lead to misinterpretation of functional assays addressing the role of H_V1 with the use of ClGBI. Thus, our aim was to find potent and more selective inhibitors for hH_V1, which could be useful research tools and serve as lead molecules for the development of drug molecules targeting H_V1.
We used patch-clamp to test the affinity and selectivity of potential inhibitors of H_V1 on CHO and HEK cells expressing hH_V1 and other channels. Seven “hit” molecules were identified among the NZ family of compounds of which NZ-13 has the best selectivity profile.
The widely-used HV1 inhibitor ClGBI blocks various ion channels and therefore is not a selective HV1 blocker. This must be considered in functional tests investigating the role of HV1 in healthy and pathological conditions.
We have identified a new family of hHV1 inhibitors, which have comparable affinities for the channel to ClGBI.
Most NZ molecules have low selectivity for hHV1, but NZ-13, the one with the highest selectivity, may be better suited for functional tests than ClGBI as it inhibits T cell proliferation less.

P41

Controlling live cell adhesion through characterization of biofunctionalized surfaces using label-free biosensors

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Biomaterial coatings that possess cell-repellent or cell-adhesive properties have a significant interest in medical and biotechnological applications [1-4]. However, conventional approaches lack in-depth analysis and quantitative comparison of these coatings for regulating adhesion, particularly for bacterial cell adhesion. Label-free Optical Waveguide Lightmode Spectroscopy (OWLS) can offer a solution for the detailed analysis of biomaterial coatings. OWLS biosensors detect the optical properties of the adhesive surface using evanescent waves with a penetration depth of 100-150 nm [5-7]. This surface-sensitive technique enables a thorough evaluation of biomaterial coatings for regulating adhesion. Uniquely, OWLS enables the in situ measurement of both the coating process and subsequent cell adhesion.
The present study utilizes the OWLS method for in-depth characterization of biomaterial surfaces with regard to bacterial adhesion. Initially, adhesion blocking biomaterials, namely bovine serum albumin, I-block, PAcrAM-g-(PMOXA, NH2, Si), (PAcrAM-P), and PLL-g-PEG, with varying coating temperatures, were screened. PAcrAM-P exhibited the best blocking capability against bacterial concentrations up to 10^7 cells/mL. Subsequently, different immobilization methods, such as Mix&Go (AnteoBind) films, protein A, avidin-biotin based surface chemistries, and simple physisorption, were employed to capture*Escherichia coli* specific antibodies. Bacterial cell adhesion was then tested on immobilized antibodies with various blocking agents. The OWLS analysis allowed for the determination of the parameters of the applied agents by considering the kinetic data of adhesion, the surface mass density, and the protein orientation. Based on the experimental results, surfaces were created and tested for controlling both bacterial and mammalian cell adhesion. [8]

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P42

Role of ion channels in CAR T-cell

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Cancer immunotherapy partly relies on the reprogramming of host immune cells to eliminate cancer cells. Genetic modification of T cells to express chimeric antigen receptors (CARs) is utilized in the treatment of hematological malignancies. Despite its success, many challenges remain to improve the efficacy and safety of this therapy. Ion channels in T-cells participate in the regulation of Ca^{2+}-dependent activation pathway and play a role in various effector functions inevitable for target cell abolition. Hence, modification of ion channels' function can contribute to successful immune therapy. However, no study has been reported about functional role of CAR T-cell ion channels yet.
We established a 3rd-generation CAR expressing cell line (CD19-CAR cells) from Jurkat cells. We used the whole-cell patch-clamp technique and FURA-2-based Ca^{2+}-imaging to determine the biophysical properties of Kv1.3 and Ca^{2+}-response of CD19-CAR cells, respectively. We adapted a Calcein Red based killing assay to test CD19-CAR cells' target cell killing capacity. We assessed the localization of Kv1.3 in standalone and in the CAR-synapse engaged CD19-CAR cells.
We showed that Kv1.3 activation and inactivation kinetics are the same in non-transfected and CD19-CAR cells, while voltage-dependence of activation was different. Thapsigargin-induced Ca^{2+}-response of CD19-CAR cells was lower as compared to the control. We showed that Kv1.3 channels are co-localized with CARs in standalone CD19-CAR cells, and they redistribute to the contact region between a CD19-CAR cell and a target cell (Raji B cell). Upon Vm24 addition (specific Kv1.3 inhibitor, 1 nM) the target cell ability of CD19-CAR cells was impaired. Based on these results, we suppose that ion channels can affect the outcome of immunotherapy, and further experiments are needed to clarify their functional role.

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P43

Ion channel expression of $CD8^+$ T cells in ovarian cancer

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The ion channels of T lymphocytes have an important role in effector functions such as activation, cytokine production and tumor cell elimination. T cells recognise and kill cancer cells during continuous monitoring. Although tumor infiltrating lymphocytes (TILs) are able to penetrate the tumor, they cannot fulfil their effector function due to the suppressive nature of the tumour microenvironment. K^+channels, such as Kv1.3 and KCa3.1, stabilize the negative membrane potential of T cells to control Ca^{2+}-influx through CRAC channels and Ca^{2+}-dependent signaling. In the present study, we determined the expression of T cell ion channels from peripheral blood of untreated ovarian cancer patients and healthy donors.

PBMCs were isolated from blood of ovarian patients and healthy donors using Ficoll-Paque density gradient method. Cells were activated with CD3/CD28 antibodies. Whole-cell current was measured in activated $CD8^+$ T cells using patch-clamp technique. Ca^{2+} response of $CD8^+$ cells was evaluated with FURA-2 Ca^{2+}-imaging method.

KCa3.1 expression level in blood $CD8^+$ cells from malignant tumor patients were lower than in healthy and benign tumor groups. Contrary, the Kv1.3 conductance of $CD8^+$ T cells were significantly higher in malignant tumor patients as compared to other two groups. To asses Ca^{2+} response of cells, we determined the quotient of FURA-2 ratios measured in 2 mM Ca^{2+} and 0 mM Ca^{2+} after thapsigargin addition: there was no differences between the groups.

In summary, we suppose that down-regulation of KCa3.1 expression and Kv1.3 level upregulation in blood $CD8^+$ cells could be a reporter on the presence of malignancy, as we reported before for head and neck cancer patients [1].

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P44

Isolation of novel peptide toxins from the venom of the scorpion *Centruroides bonito* which blocks Kv1.2 ion channel with picomolar affinity

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Seven new peptide toxins named as CboK1 to CboK7 were isolated from the venom of the Mexican scorpion *Centruroides bonito* by liquid chromatography. The primary structure of these peptides were determined by Edman degradation. Mass spectrometry analysis was used to determine the molecular weights which range between 3760.4 Da to 4357.9 Da, comprising 32 to 39 amino acid residues cross-linked with three tightly folded disulfide-bridges. The amino acid sequence alignment with known potassium scorpion toxins (KTx) and phylogenetic tree analysis unveiled that CboK1 (α-KTx 10.5) and CboK2 (α-KTx 10.6) belong to α-KTx 10 subfamily, whereas CboK3 (α-KTx 2.22), CboK4 (α-KTx 2.23), CboK6 (α-KTx 2.21), CboK7 (α-KTx 2.24) bears more than 95% amino acid similarity with the members of α-KTx 2 subfamily, and CboK5 is 100% identical with previously described Ce3 toxin (α-KTx 2.10). The electrophysiological assays (whole-cell patch clamp) revealed that except CboK1, all other six peptide toxins blocked the voltage-gated potassium channel Kv1.2 with high affinity, having Kd values in the picomolar range (24-763 pM) and inhibited the Kv1.3 ion channel with comparatively less potency (Kd values between 20-171 nM). Moreover, CboK2 and CboK3 inhibited ~10% and CboK7 inhibited ~50% of Kv1.1 currents at 100 nM concentration. Among all CboK7 (α-KTx 2.24) has the highest affinity for Kv1.2 ion channel with Kd value of 24 pM, and reasonable selectivity over Kv1.3 (~1000-fold) and Kv1.1 (~6000-fold) ion channels. These distinguishable characteristics of the CboK7 toxin may provide a framework for developing tools to treat Kv1.2-related gain of function channelopathies.

P45

Light energy harvesting by photosystem I in cyanobacterial cells

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Photosystem I (PSI) is a crucial component of the light-dependent reactions of oxygenic photosynthesis occurring in cyanobacteria, algae and plants. It is a multi-subunit pigment-protein complex binding more than a hundred pigment – chlorophylls (Chls) and carotenoids, as well as cofactors carrying out photoinduced electron transport with a quantum yield of near unity. Whereas PSI in plants and eukaryotic algae is attached to peripheral membrane-intrinsic light-harvesting complexes, cyanobacteria utilize the membrane extrinsic phycobilisomes (PBS) as the main light-harvesting antenna complex[1]. While the structural and energetic interaction between the PBS and photosystem II (PSII) is well established, less is known about the connectivity of PBS and PSI and the ability of the PBS to transfer energy directly to PSI is debated. We investigated the pathways and dynamics of energy transfer from PBS to the photosystems in *Synechocystis sp.* PCC 6803. The excitation kinetics of PBS and PSI were followed by picosecond time-resolved fluorescence spectroscopy in the wild-type strain and in a mutant devoid of PSII[2]. We found that PBS are capable of directly transferring energy to PSI in the PSII-deficient mutant, in a time scale of about 20 ps at room temperature. Based on an earlier model of energy transfer in *Synechocystis sp.* PCC 6803[3] and simultaneous fitting to the measured data of isolated complexes and intact cells, a detailed model of energy transfer between different PBS, PSI and PSII chromophore groups was obtained.

Many cyanobacterial species, when exposed to iron limitation conditions, produce a specialized pigment-protein complex, IsiA, that is known to associate into rings around PSI[4]. The physiological function of IsiA is not fully understood. In isolated PSI-IsiA complexes IsiA efficiently transfers absorbed photon energy to the PSI core[5], which can extend the absorption cross-section of the photosystem and help reduce the number of iron-rich PSI core complexes in the cells. However, IsiA has also been proposed to have a photoprotective, energy-dissipating role or to serve as a Chl depot[6]. To find more about the light-harvesting role of IsiA in vivo, we followed the cellular content of IsiA in cells of *Synechocystis sp.* PCC 6803 under iron limitation and investigated the energy transfer from IsiA to PSI by time-resolved spectroscopy. IsiA formed PSI-IsiA supercomplexes in vivo having similar energy transfer characteristics as isolated supercomplexes – confirming the primary role of IsiA as an accessory light-harvesting antenna to PSI. However, a significant fraction (40%) remained unconnected to PSI, supporting the notion of a dual functional role of IsiA. Moreover, we found that *Synechocystis* mutants containing only monomeric PSI contained far fewer IsiA units per PSI compared to the wild-type strain. We conclude that the trimeric organization of PSI in wild-type *Synechocystis*has role both in the accumulation and the energy transfer capabilities of IsiA under iron stress.

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P46

Role of glycocalyx in cancer cell adhesion: kinetics of interactions from label-free optical biosensor measurements

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The glycocalyx is a sugar rich layer covering the surface of the cells [1]. It is composed of glycoproteins and proteoglycans. The cellular glycocalyx plays an important, but not yet understood, role in cellular signaling and metabolism, its disorders generate pathological process [2]. Interestingly, the thickness of the glycocalyx layer of cancer cells is significantly larger compared to that of healthy cells. This fact further highlights the importance of glycocalyx in tumor progression and treatment. In an earlier work, a regulatory mechanism of cellular glycocalyx in cancer adhesion was revealed using label-free optical biosensor, fluorescent microscopy, and cell surface charge measurements [3].
The primary goal of our work is to study the role of glycocalyx components in cellular adhesion by employing various types of digesting methods. In these initial measurements we use a label-free, high-throughput, resonant waveguide grating-based optical biosensor. The instrument is well suited for monitoring of cellular adhesion kinetics in real-time, even at the single-cell level [4].

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P47

The effect of light and age of the leaves on plastid differentiation and essential oil composition of spearmint (*Mentha spicata* L.)

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Spearmint (*Mentha spicata* L.) is a widely used spice, aromatic and medicinal plant with a characteristic fragrance. Its valuable active substances are monoterpene and sesquiterpene essential oil components produced by exogenous secretory structures, i.e. the various glandular hairs located on the shoot, especially on the leaves. The biosynthesis of the terpenoid essential oils requires isoprenoid biosynthesis of peculiar plastids present in the glandular hairs.
In this work, we have investigated 1) whether the prolamellar body-like membrane structures observed in the neck cells of peltate glandular hairs of spearmint can be considered as homologues with the prolamellar bodies present in etioplasts of dark-grown plants, 2) whether the presence and the activity (essential oil production) of the glandular hairs depends on the developmental stage of the leaves, 3) whether light conditions (e.g. light or dark development) affect plastid differentiation and the essential oil production of the leaves.
We have described in detail the various plastids and the organization of their inner membranes in the different cells of the exogenous secretory structures of spearmint, i.e. in capitate and peltate glandular hairs. We found clear structural differences between the prolamellar bodies of spearmint etioplasts of dark-grown leaves and of the similar structures found in secretory cells, which rather ressembled regularly arranged clusters of plastoglobuli, and not tubuloreticular membranes. Dark-growth did not influence much the structure of the plastids in the secretory cells, and our data obtained with solid-phase microextraction followed by gas chromatography and mass spectrometry (GC/MS) confirmed that not the illumination conditions but rather the developmental stage of the leaves influences the essential oil composition of spearmint.

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P48

Cm39 (α-KTx 4.8): A novel scorpion toxin that inhibits voltage-gated K^+ channel Kv1.2 and small- and intermediate-conductance Ca^{2+}-activated K^+ channels KCa2.2 and KCa3.1

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A novel peptide toxin, Cm39, was identified in the venom of the Colombian scorpion *Centruroides margaritatus*. It is composed of 37 amino acid residues with a MW of 3980.2 Da and folded by three disulfide bonds. The Cm39 sequence also contains the Lys-Tyr (KY) functional dyad required to block voltage-gated K^+ (Kv) channel. Amino acid sequence comparison with previously known K^+ channel inhibitor scorpion toxins (KTx) and phylogenetic analysis revealed that Cm39 is a new member of α-KTx 4 family and registered with systematic number of α-KTx4.8. The full chemical synthesis and proper folding of Cm39 was obtained. The pharmacological properties of the synthetic peptide were determined using patch-clamp electrophysiology. Cm39 inhibits the voltage-gated K^+ channel hKv1.2 with high affinity (K_d = 65 nM). The conductance-voltage relationship of Kv1.2 was not altered in the presence of Cm39, the analysis of the toxin binding kinetics was consistent with a bimolecular interaction between the peptide and the channel, and therefore the pore blocking mechanism is proposed for the toxin-channel interaction. Cm39 also inhibits the Ca^{2+}-activated KCa2.2 and KCa3.1 channels, with K_d = 575 nM, and K_d = 59 nM, respectively, however, the peptide does not inhibit hKv1.1, hKv1.3, hKv1.4, hKv1.5, hKv1.6, hKv11.1, mKCa1.1 potassium channels or the hNav1.5 and hNav1.4 sodium channels at 1 μM concentration. Understanding the unusual selectivity profile of Cm39 motivates further experiments to reveal novel interactions with the vestibule of toxin-sensitive channels [1].

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P49

A synthetic flavonoid derivate modulates the fluorescent signal of voltage-gated proton channels

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The voltage-sensing domain (VSD) of voltage-gated proton channel (Hv1) serves as a pore for protons as well as a voltage sensor, which makes this channel unique among voltage-gated channels. Natural flavonoids, which are widely distributed and act as chemical messengers and physiological regulators in plants, modulate the function of some voltage-gated ion channels (EAG1, HCN2. etc.) in animal cells. We have designed synthetic flavonoid derivatives to inhibit the current of Hv1. We produced and tested tens of flavonoid derivatives on Ciona intestinalis Hv1 using the voltage-clamp fluorometry (VCF). The most potent compound, molecule #109, changed the originally negative VCF signal to positive and altered the biphasic VCF signal shape to monophasic. Also, this molecule caused a rightward shift in the conductance-voltage relationship in a concentration dependent manner. This flavonoid derivative quenched the TAMRA-MTS fluorescence in cuvette and on frog oocytes decreasing the baseline fluorescence, independently the oocyte expressed Hv1 or not. Furthermore, #109 could stain the membrane of HEK cells. These results indicate that #109 binds not directly to CiHv1 but to the cell membrane and in this way, it indirectly modifies the gating of Hv1 and the VCF signal, as a strong quenching molecule in close vicinity of the fluorophore. The latter was confirmed by our model calculations, in which we assumed that molecule #109, as a strong quencher, is embedded in the cell membrane close to TAMRA and during the conformational change due to depolarization, TAMRA is continuously moving away from this strong quencher molecule. Therefore, the VCF signal becomes a continuously increasing fluorescence change from the originally complex shape, which was overall a decreasing fluorescence change.

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P50

Remote detection of life through full-Stokes spectropolarimetry

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Homochirality is a generic and unique property of all biochemical life as we know it. There is a growing concensus that homochirality is a universal prerequisite of life and therefore as a biosignature free from the assumptions made by using terrestrial life as a benchmark [1]. Spectropolarimetry and, in particular, the detection of non-zero signatures in circular polarization is an indicator of the homochiral nature of molecular and supramolecular organic matter and is thus a direct and intuitive proxy for the remote detection of life using unpolarized incident light such as from the Sun [2]. We will describe the ongoing effort to characterize and quantify the nature of these chiroptical signals resulting from living organisms. While various studies have been performed to this end on eukaryotic photosynthetic organisms, in both the laboratory and in the field, including aerial observations (see e.g. [3][4]), relatively little systematic observations have been made of prokaryotic life. The results gathered so far on microbial mats and pure cultures show a remarkable variety in terms of both polarimetric spectral shape and magnitude [5]. Within the framework of the SenseLife project, we aim to further characterize and quantify the nature of these signals including the polarimetric signal response to external factors and physiology. We will further present novel results demonstrating the potential of characterization aerobiology using spectropolarimetry in the visible. Within this context we will describe the performance of FlyPol [2], a fast and sensitive spectropolarimetric instrument dedicated to the remote detection of linear and circular polarizance. In addition, we will present the results of ongoing aerial and field measurement campaigns probing the polarizance resulting from natural habitats, providing an outlook on the endeavor of measuring especially circular polarizance from space using Earth as a benchmark.

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P51

Assessing the effect of photobleaching on morphometric parameters of different cell types in the central nervous system

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The sensitivity of immunofluorescence to the illumination time and intensity is well known; however, its effects on morphological parameters are less considered. Morphometric evaluation of different cell types could indicate the condition of the region of interest; for example, thickness and number of branches in microglia can imply their activation state.
Fluorescent detection using immunohistochemistry (IHC) targeting microglia/macrophages (fine structures) and neurons (bulk structures) was performed on neural tissues using different types of fluorophores while IHC with photostable diamonibenzidine was served as standard. Time series (0.5, 1, 2, 5, 10 and 15 minutes of illumination after taking the first image) were acquired from each spinal cord sections with the initial microscope settings. After standard mean intensity measurements, morphological parameters were extracted. Dynamic and relative total area microglial density were measured with a macro developed in our lab used routinely for quantifying glial activation. Fractal geometrical parameters were measured with the help of the Fiji plugin FracLac, and changes were examined between the initial image and the images containing faded structures.
Standard mean intensity measurements show different fading properties of different fluorophores. Dynamic and relative total area density comparisons show that in some cases all structures can fade to the point of becoming non-detectable after 2 minutes of illumination, while in some fractal geometrical parameters, more than 35 % differences can be observed.
Our results suggest that while fluorescent detection using IHC is an excellent method for localization and co-localization, for proper fine structure morphological measurements a photostable staining method is essential.

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P52

Label-free immune cell analysis using optical biosensor

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Understanding of activation processes at the single-cell level in response to different stimuli is essential for the diagnosis of certain diseases.
External stimuli induced differences are reflected in the dynamic changes of cell biophysical parameters, such as cell motility, shape, spreading and adhesion properties [1]. Novel highly sensitive optical biosensors allow the monitoring of changes in these parameters in a label-free manner. The advantage of label-free detection is that cells can be examined in an intact/organism-specific manner without modification.
The above parameters can be studied using microplate-based, high-throughput systems [2]. Surface functionalisation provide the opportunity to create an organism-specific environment [3]. In classical measurements, such as microscopy, dye-conjugated antibody labelling is essential and can be combined with label-free data [4].
Our aim is to interpret the activation mechanisms of various cell types and their function triggered by different stimuli and compare the results with conventional testing methods.

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P53

Effect of salt stress on etioplast and chloroplast membranes of thylakoid transporter mutants of *Arabidopsis thaliana*

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Soil salinity is an increasing problem for agriculture worldwide. Salinity has a complex effect on plants and influences the structure of plastids in different ways. Most often the effect of salt stress is studied in leaf chloroplasts, and in several cases swelling of the intrathylakoidal space of chloroplast inner membranes is reported under such conditions. However, it is yet unclear what causes the swelling of these membranes, and whether it has any relation to ion transport processes across these membranes. In this work, plastid ultrastructure was compared in the cotyledons and leaves of *Arabidopsis thaliana* plants of different developmental stages and grown under different light regimes under control conditions as well as under salt stress (30 min treatment with 200 or 300 mM NaCl or 600 mM NaCl:KCl, 1:1). In addition to the wild-type (WT) plants, we also analysed the thylakoid membrane structure and photosynthetic activity in single, double and triple mutants of the thylakoid-located voltage-gated chloride ion channel VCCN1, chloride ion channel CLCe and potassium proton exchanger KEA3. The above salt treatment did not affect the structure of the photosynthetic apparatus of mature chloroplasts in old leaves, however, it influenced the structure of chloroplasts in cotyledons in various ways, indicating the sensitivity of the young seedlings to the stress, and the potential presence of protective mechanisms that stabilize chloroplast structure at later developmental stages even under the above stress conditions. Salt stress also had an effect on the etioplasts of both WT and some mutant plants.

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P54

Effect of rational modification of disordered domains of the epidermal growth factor receptor on its biophysical characteristics

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The epidermal growth factor receptor (EGFR) belongs to the ErbB receptor tyrosine kinase family. The extracellular domain of EGFR consists of four subdomains (I-IV). Upon ligand binding, the extracellular (EC) domain of the receptor is rearranged, resulting in exposition of the dimerization arm of subunit II providing an opportunity to form homodimers or heterodimers. The 3D structure determined by the amino acid sequence affects protein function. Proteins in which the formation of an ordered structure is not complete are called intrinsically disordered proteins. Recently, an algorithm (FuzPred) was developed that predicts the tendency of proteins to form disordered regions and their structural changes upon interacting with other proteins. The amino acid sequence of the EGFR was analyzed by FuzPred, which determines the tendency of distinct sequence regions to constitute globular (assuming ordered conformations), disordered (intrinsically disordered adopting ordered conformation upon binding), or fuzzy regions (remaining disordered even in their bound state). The following mutations were designed based on the software prediction that alter the fuzziness and the molecular interactions of EGFR domain II. 1. Mutation T274G is predicted to result in increased dynamics and destabilization of the dimerization site. It still forms a dimer, but the interaction is weak. 2. Mutation Q276F is expected to decrease dynamics of the dimerization arm and create a rigid binding site. As a result, it is unable to form a dimer due to lack of flexibility. 3. Mutation K284Q is expected to decrease dynamics while strengthening the binding site. This mutation promotes dimerization. We successfully generated all three mutants (dark and EGFP tagged on the C terminal of the proteins) by site-directed mutagenesis and stably transfected CHO cells expressing the EGFP-tagged mutant EGFRs. We investigated the ligand binding and the cooperativity of the wild-type EGFR and its mutant variants. CHO cells transfected with the wild-type or the mutant EGFRs were labeled with a concentration series of fluorescently labeled EGF (TAMRA-EGF). Our results suggest that those mutants that were predicted to be less prone to dimerization bind EGF less cooperatively and with a slightly lower affinity. In our further experiment we would like to examine the dimerization process (FRET, N&B) itself and consequent transmembrane signaling.

P55

Analysis of unilateral Walker A and A-loop mutants indicate that a single active catalytic site is sufficient to promote transport in ABCB1

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The human ABCB1 is a full transporter with two nucleotide binding domains (NBDs) and two pseudo-symmetric transmembrane domains (TMDs). The two NBDs form two symmetrically arranged composite nucleotide binding sites (NBSs). Each NBS is formed by the A-loop, H-loop, Walker A, Walker B and Q-loop of one NBD, and the X-loop and signature sequence of the other NBD. The conserved tyrosine of the A-loop aligns the adenine ring of the bound ATP, contributing to nucleotide binding affinity through stacking interactions. The Walker A lysine interacts with the α and β phosphate of ATP. The two nucleotide binding domains were shown to be functionally equivalent, and the integrity of both catalytic centers is generally believed to be needed for transport. Consistently with the widely accepted models predicting that the two NBDs hydrolyze ATP in a strictly alternating order, unilateral mutation of these residues have been described to disrupt ATP hydrolysis and even affect ATP binding.
Here we demonstrate that while ABCB1 variants carrying bilateral A-loop or Walker A mutations are completely inactive, the unilateral exchange of the A-loop tyrosine to alanine or the unilateral mutation of the Walker A lysine to methionine is compatible with both ATP hydrolytic activity and transport function. Characterization of the single mutants revealed the significant (about 10-fold) reduction of the apparent ATP binding affinity compared to wild-type ABCB1. Stabilization of the post-hydrolytic complex by phosphate mimicking anions, such as vanadate or BeFx also occurred at higher ATP concentrations compared to wild-type, supporting that the mutated site probably has an effect on the overall conformation of the NBD dimer. Although the basal catalytic activity was strongly reduced in accordance with the decreased ATP binding affinity of the single mutants, the degree of ATPase stimulation by verapamil was almost identical to that of the wild-type, showing that drug-stimulation of the ATPase activity is preserved in the single mutants. Location of the mutation in the N or C terminal NBD did not affect the extent of ATPase stimulation by verapamil. Taken together, our data indicate that, in contrast to prevailing views, single-site NBD mutant ABCB1

P56

Evaluation of peptide carrier candidates using tissue barrier models

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Targeting peptides represent a promising approach to improve uptake and efficiency of pharmacological compounds. In vitro barrier models are valuable screening tools to evaluate peptide transport, uptake and toxicity. Here we characterize a number of readily available lung and kidney epithelial cell lines in a transwell barrier model. After forming a monolayer and a subsequent maturation phase of cell-type specific duration, the epithelial cells develop tight junctions on the surface of polycarbonate inserts as evidenced by beta-catenin and ZO1 immunolabeling as well as delayed transport of targeting peptides. Daily monitoring of transepithelial electrical resistance (TEER) values reveal a cell line specific, characteristic time course. Thus, the TEER method with this calibration data offers a non-destructive and agent-free procedure to time pharmacological transport measurements, and to evaluate cytotoxicity of the transported agents. To characterize peptide targeting efficiency, a detector cell layer was cultured in the basolateral compartment of the barrier tissue model. Uptake of fluorescein-labelled peptides was evaluated by flow cytometry. We demonstrate that a derivative of the well-known peptide penetratin that also contains the neuropilin-binding sequence from tuftsin, a naturally occurring tetrapeptide produced by enzymatic cleavage from immunoglobulin G, is better able to pass through barrier layers that expresses its receptor, NRP-1.

P57

Molecular characterization of pathological and tissue-specific TRPM2 cation channel variants

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TRPM2 is a temperature-sensitive, Ca2+-permeable, non-selective cation channel, showing high level of expression in cells of the central nervous system, bone marrow, granulocytes and pancreatic β-cells. Activation of the channel requires the simultaneous intracellular presence of adenosine diphosphate ribose (ADPR), Ca2+ ions and phosphatidylinositol 4,5-bisphosphate (PIP2). In these cells, TRPM2 contributes to Ca2+ influx resulting in important physiological and pathological functions, such as body temperature regulation, cytokine production, oxidative stress response, inflammation or controlled cell death. Moreover, certain TRPM2 point mutations show close genetic connection with bipolar disorder (D543E, R755C) [1] or amyotrophic lateral sclerosis and Parkinson's dementia (P1018L) [2]. Interestingly, alternative splice products were isolated from healthy neutrophil granulocytes (ΔC-TRPM2) [3] and from striatum (SSF-TRPM2) [4], which presumably modify the ligand specificity and function of the channel in a cell-specific manner.

Until now, the mentioned variants have only been investigated using fluorescent imaging techniques or whole-cell electrophysiological methods providing limited opportunities to study the ligands acting intracellularly. Our aim is to examine the listed ion channel variants in molecular details. To this end, expression vector encoding TRPM2 variants have been produced and expressed transiently in HEK cells. Functional measurements are performed by inside-out patch clamp configuration enabling reliable recordings of micro- and macroscopic currents and fast exchange of intracellular ligands. With our method, it is possible to map crucial biological and biophysical parameters of the channel variants: ADPR and Ca2+ sensitivity, gating parameters, inactivation kinetics, temperature dependence. This detailed knowledge is essential for a comprehensive understanding of the role of these mutants in pathomechanisms and tissue-specific variant functions.

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P58

Substrate selectivity of sulfotransferase isoenzymes, results based on molecular dynamics and ensemble docking

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Sulfotransferase enzymes (SULTs) are a family of cytosolic globular proteins in the chain of metabolism. By catalysing a sulfate transfer from their co-factor, 3′-Phosphoadenosine 5′-Phosphosulfate (PAPS), they eliminate a large variety of small molecules like drugs, hormones and neurotransmitters. Even though the tertiary structure across the family is very similar, their substrates vary considerably in size and complexity. The aim of our project is to better understand the reasons of selectivity between the different SULT isoenzymes, by comparing the broad targeting hepatic detoxifier SULT1A1, and the ileum located, dopamine selective SULT1A3.
Based on our previous results and Molecular Dynamics (MD) and Molecular Dynamics with excited Normal Modes (MDeNM), an extended conformational space of the PAPS-bound SULT1A1 was explored. Further developments of our method utilising ensemble docking with categorised ligands, a method known as Virtual Screening was achieved. Moreover, we have broadened our scope to use the same approach for the SULT1A3.
Based on our new results, we identified the key differences, that are responsible for changing the protein dynamics and binding mechanisms, by opening the binding pocket to an unfavourable conformation for the most common ligands of 1A1, thus acting as efficient selectors. These results can be helpful in the future to develop an algorithm for machine learning, that could differentiate and even predict new substrates of the different isoforms, thus helping in the development of ADME-Tox profiling of novel drug candidates and xenobiotics.

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P59

Examinations of cellular uptake of cell penetrating peptides *in vitro* and *in vivo*

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Cell-penetrating peptides (CPPs) are peptides that enter cells by endocytosis and/or directly through the cell membrane. CPPs in general have been considered potential carriers of molecules that have difficulties entering cells. This is the feature that we would like to exploit and thereby establishing the opportunity for CPPs to have therapeutic applications in the long term. Our previously published results have shown that we can increase the cellular uptake and endosomal release of CPPs with statins. Our goal was to modify them and test if it is possible to make them enter the cells more efficiently. We also aimed to test the biodistribution of CPPs in mice after intravenous administration. We examined the cellular uptake and endosomal release by flow cytometry and confocal microscopy in SKBR-3 and MDA-MB-231 cell lines, while for the *in vivo* experiments a mouse model was applied. Fluorescently-labeled CPPs were used both in the in vivo and in vitro experiments. We compared the differences in the biophysical properties of the original and the modified CPPs, and we found that the cellular uptake of the modified version is more effective. There is a difference between the enhancement in the uptake of CPPs labeled by the pH-sensitive naphthofluorescein or Alexa Fluor 532. In the case of *in vivo* experiments, we found that peptides enter the mouse organs, including the liver, for which we have shown that CPPs is present in the intracellular space of hepatocytes. CPPs hold promise for increasing the efficiency and specificity of drug delivery to cells.

Művészeti program

Beri Áron gitárművész koncertje

Beri Áron egész fiatal korában, nagyjából 7-8 évesen kezdte a gitározást. Több gitárversenyen vett részt és ért el eredményeket, számos koncertet adott Magyarországon és néhányszor külföldön is. Jelenleg a Miskolci Egyetem hallgatója klasszikus gitár tanár szakon. Zenéjét próbálja egyedivé tenni, legfőbb ismertetői a klasszikus, a jazz, némi népzenei vonal összeszövése saját érzéseivel és improvizációival.

MŰSOR

Beri Áron:
Victoria
Felhők

Vincente Amigo:
Roma

Harold Arlen:
Over the rainbow

Szerzők névsora

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G

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N

O

P

Q

R

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T

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V

W

Z

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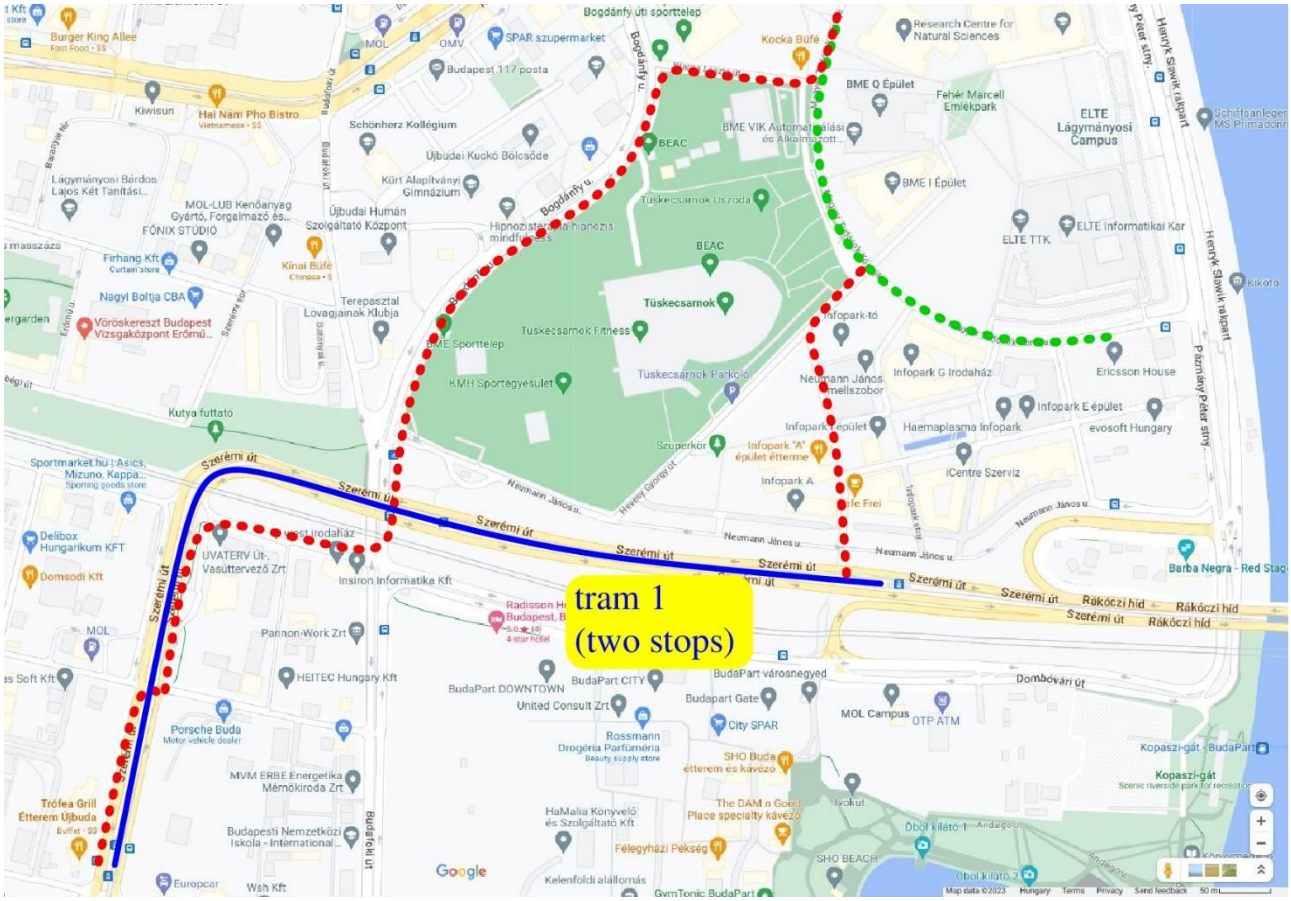

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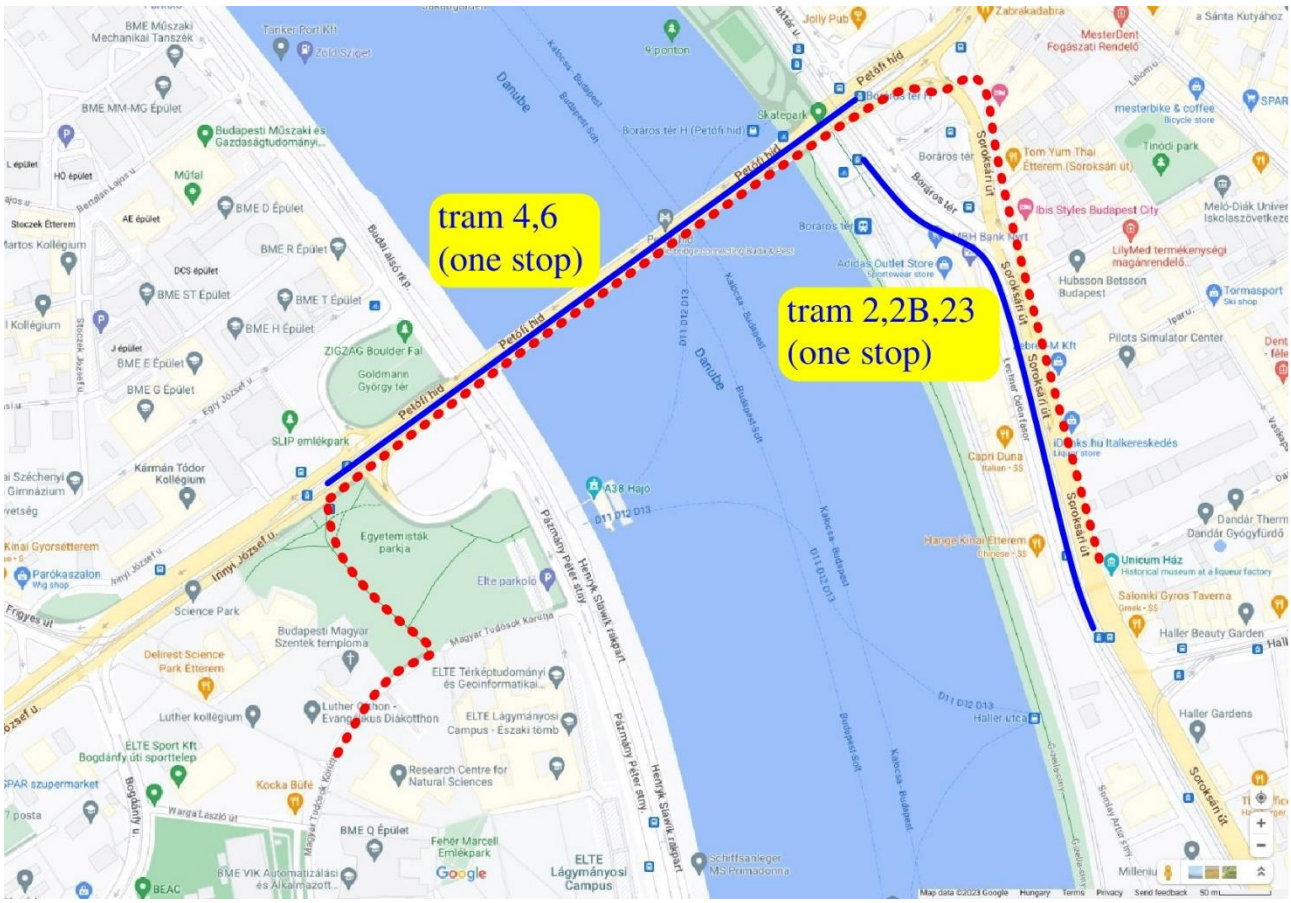

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