Biophysical chemistry

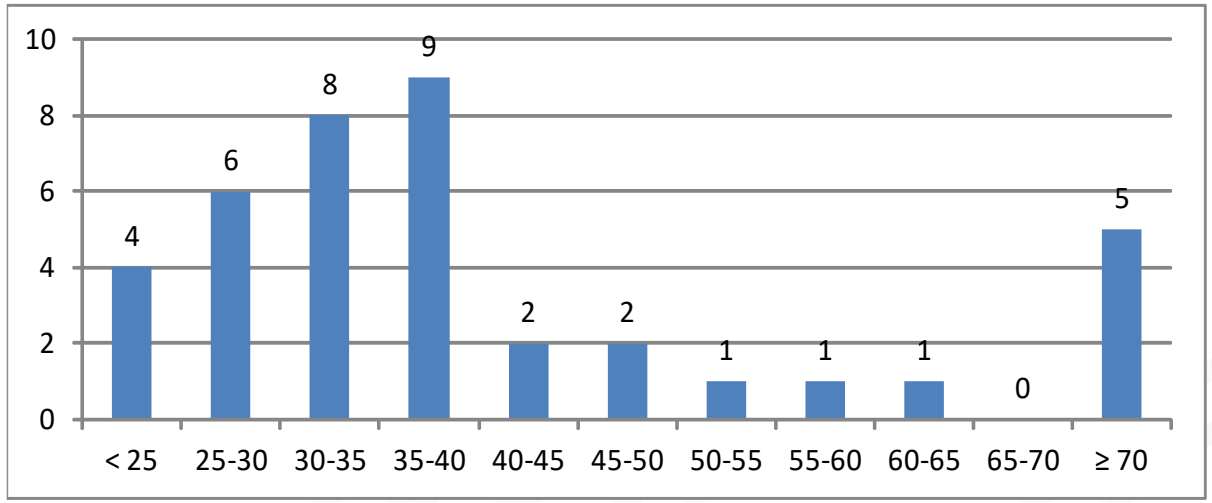
Presentation by the Head of Department (team leader)
RNDr. Radek Šachl, PhD.

"Retrospective (2015-2019) and Perspective (2019-…) "

Team Members

Age structure of the team

Age category	< 25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	≥ 70
Number of members	4	6	8	9	2	2	1	1	1	0	5



Team members (02/2021)

- Head, deputy head and the institute Director (founder of the team) with 0.7 FTE out of 2.5 FTE covered by grants

Dr. Šachl (CZ), Dr. Amaro (PT) and Prof. Hof (D)

- 7 key Scientists (6.5 FTE) with 59 % of FTE covered by grants

Prof. Samec (CZ), Prof. Vlček (CZ), Dr. Cebecauer (SK), Dr. Jurkiewicz (PL), Dr. Petráková (CZ), Dr. Sýkora (CZ), Dr. Záliš (CZ)

- 12 Scientists and Post-docs (8.8 FTE) with 56 % of FTE covered by grants

- 10 PhD students and 2 research assistants (6.8 FTE) with 72 % of FTE covered by grants

Focus of the team: **mission** and **areas of interest**

Mission: To understand biologically relevant processes at the molecular level with primary focus on the processes taking place on cellular membranes and their synthetic models.

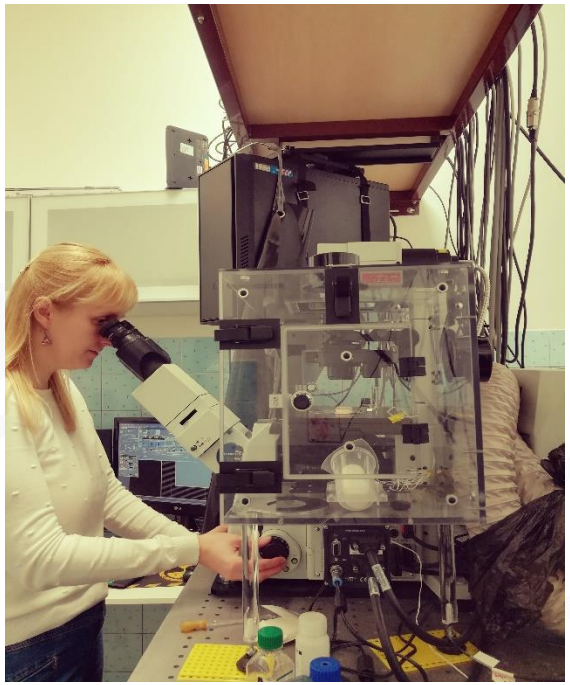
The main areas of scientific interest during the period 2015-2019

1. Understanding **membrane biophysics at an atomistic level by fluorescence spectroscopy and microscopy.**
2. Elucidating relationships between **dynamics - hydration and function** of proteins
3. **Relation between structure, membrane nanoscale organisation and protein function** in cells and synthetic models of a cell
4. **Ultrafast photophysics and photoinduced electron transfer** in complex systems
5. **Biomimetic electrochemistry** at the polarized liquid/liquid interfaces

Focus of the team: **key methods and instruments**

The key experimental methods and instruments used by the team are (most of the instruments are **home-assembled**):

- Time-resolved fluorescence spectroscopy and Fluorescence Lifetime Imaging (FLIM)
- Fluorescence Correlation and Cross-Correlation Spectroscopy (**FCS and FCCS**)
- **3D nanoscope** with photoactivated localization microscopy (PALM)
- Basic molecular biology approaches
- Fast transient electrochemical techniques for the polarization of liquid/liquid interfaces
- Dynamic interfacial tension measurements using video-image pendant-drop method



Science at the department

1. **Understanding membrane biophysics at an atomistic level by fluorescence spectroscopy and microscopy**
2. **Elucidating relationships between dynamics - hydration and function of proteins**
3. **Relation between structure, membrane nanoscale organisation and protein function in cells and synthetic models of a cell**
4. **Ultrafast photophysics and photoinduced electron transfer in complex systems**
5. **Biomimetic electrochemistry at the polarized liquid/liquid interfaces**

1. Understanding Membrane Biophysics at an Atomistic Level

Demonstrative example: Phospholipid mediated oligomerization of β-amyloid (Aβ) monomers studied by (z-scan/cross correlation FCS + MC-FRET + MD simulations).

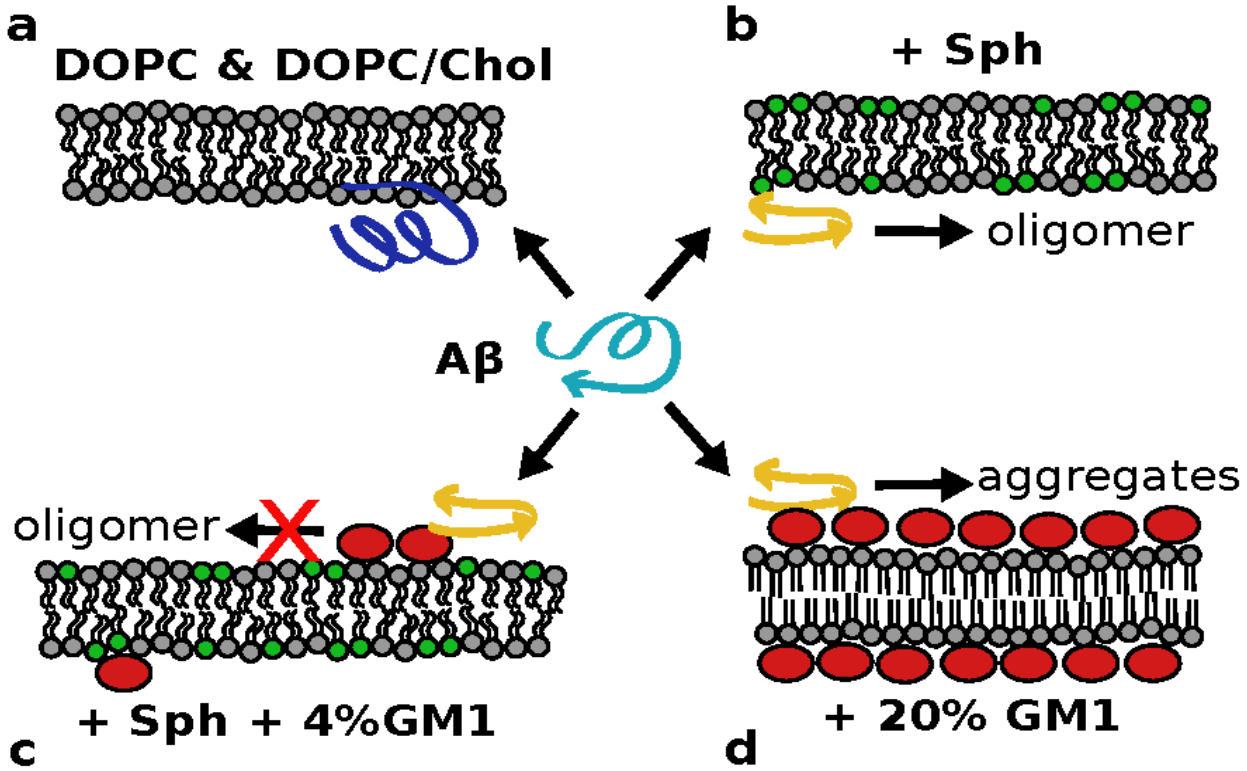
Aβ amyloids play a significant role in the onset of Alzheimer disease in the brain.

Sphingomyelin specific triggering of in-membrane oligomerization of β-amyloid.

Strong inhibitory effect of ganglioside GM1.

Future aims: The role of other gangliosides in Aβ oligomerisation?

Amaro et al. Angew. Chem 2016



1. Understanding Membrane Biophysics at an Atomistic Level

Membrane lipid nanodomains

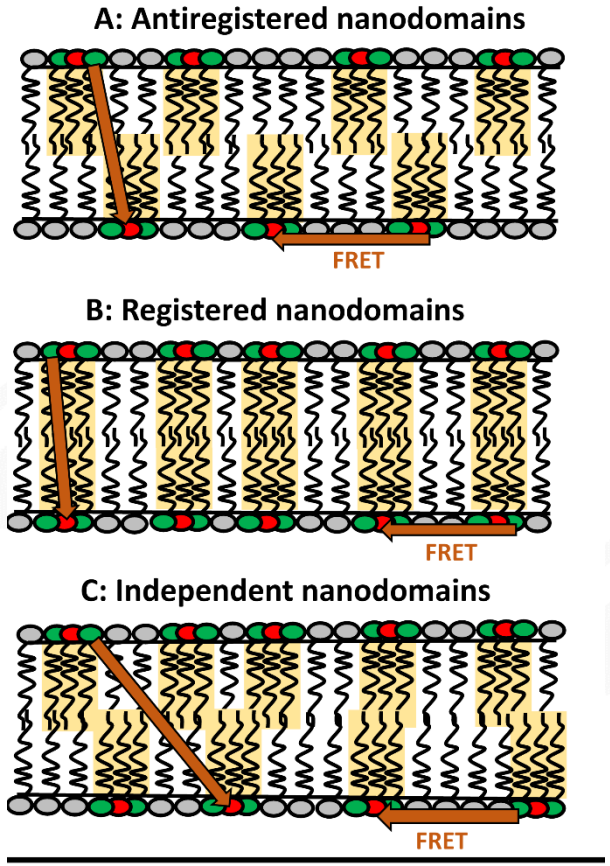
Lipid membranes can spontaneously organize their components into domains of different sizes and properties, forming a plattform for various cell functions.

Development of new approaches enabling detection and characterization of lipid nanodomains:

- MC-FRET – determination of nanodomain sizes concentration and inter-leaflet organisation of the domains (**Vinklárek et al. J. Phys. Chem. Lett. 2019**)
- The analysis of nanoscale lipid diffusion in the presence of lipid nanodomains.

Future aims:

- The role of inter-leaflet coupling in signal transduction?
- Modulation of the strength of inter-leaflet coupling?
- The role of membrane asymmetry?



Cebecauer et al. Chem. Rev. 2018

2. Elucidating relationships between dynamics/hydration/function in proteins

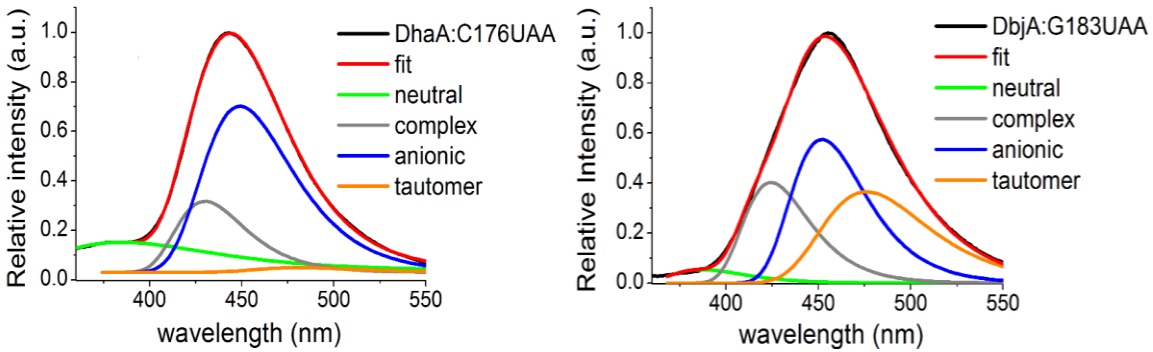
Protein hydration is important in enzymatic catalysis because of its influence on enzymes' kinetics and enantioselectivity, protein folding and even ligand-binding.

Amaro et al., JACS 2015

Development of a simple and general steady-state fluorescence spectroscopy method for site-specific analysis of protein hydration based on the in vivo incorporation of fluorescent unnatural amino acids.

DhaA
narrow tunnel mouth

DbjA
wide tunnel mouth

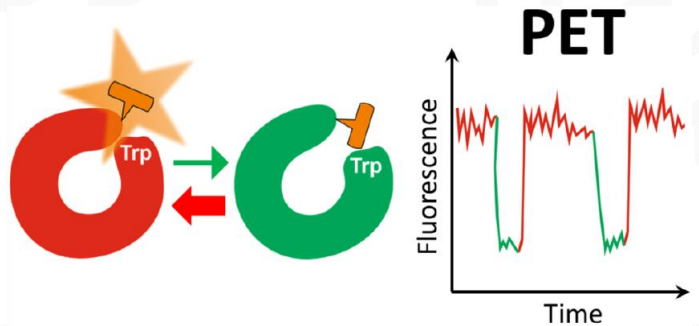


Kokkonen et al., JACS 2018

Application of PET-FCS to study conformational dynamics of a molecular gate and identification of dynamical reasons for the superior activity of the most efficient (engineered) haloalkane dehalogenase.

Perspective

Combination of transient kinetics, molecular modelling, organic synthesis and single molecule spectroscopy for **rational design of enzymes with improved catalytic properties.**



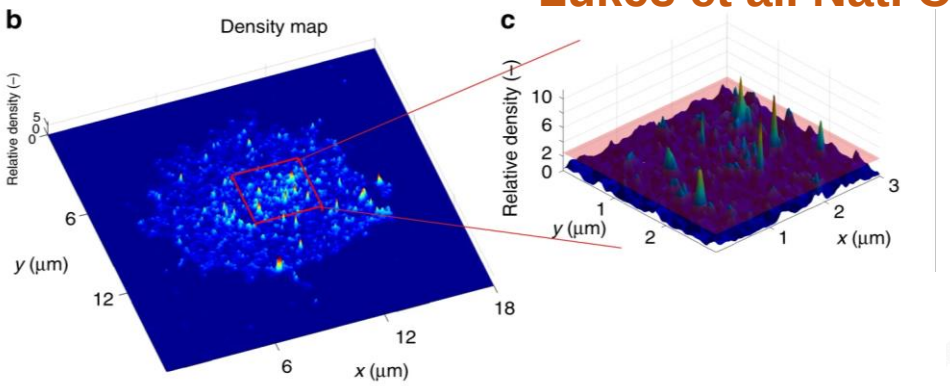
3. Relation between structure, membrane nanoscale organisation and protein function in cells and synthetic models of a cell

Example from cellular plasma membranes:

Development of a robust, model-free, quantitative clustering analysis (**SOFI**) to determine the distribution of membrane molecules on cellular plasma membranes.

Investigation of nanoscale distribution of CD4 glycoprotein in the plasma membrane of T cells.

Lukeš et al. Nat. Comm. 2017

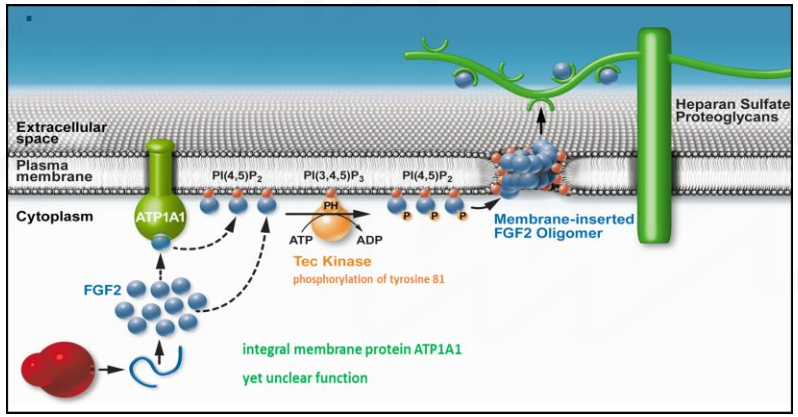


Future: Map and classify receptor distributions under normal conditions and compare those to the distributions undergoing pathological transformation.

Example from model membranes:

Exploring the structure function relationships of membrane-pore-forming FGF2 oligomers **GAČR-DFG (2014-2016 and 2020-2022).**

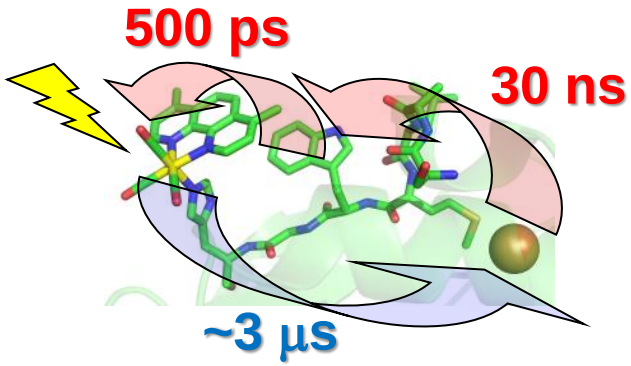
In-membrane oligomerisation of FGF2 and opening of membrane pores occurs through functional hepta- and octamers of FGF2 (**Steringer et al, elife 2017**)



- Lifetime of FGF2 pores?
- The role of heparan sulfates and ATP1A1?

Future: the correlation of membrane pore formation with protein oligomerization in one experiment.

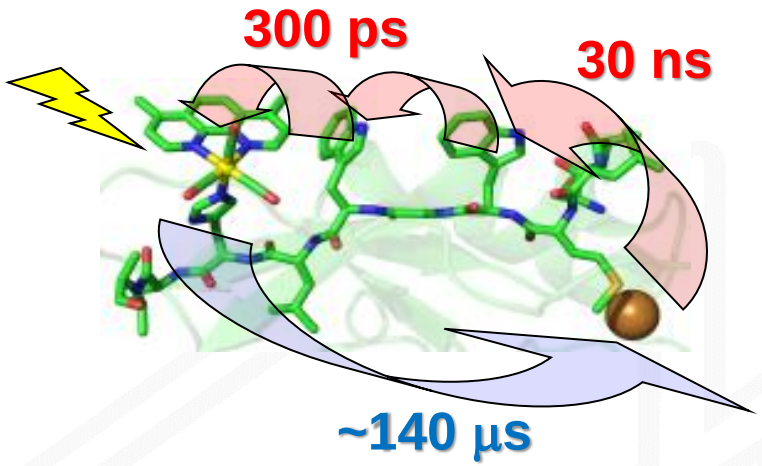
4. Ultrafast photophysics and photoinduced electron transfer in complex systems



300-fold acceleration over 19 Å

We aim to understand kinetics and mechanisms of photoinduced electron transfer in proteins with covalently appended organometallic photosensitizers. **Accomplished (Takematsu et al. ACS Centr. Sci. 2019):**

- Electron transfer over 19 and 23 Å is accelerated to nanoseconds by inserting one or two tryptophans into the redox pathway, thereby changing the mechanism from tunneling to incoherent hopping
- Operates intramolecularly as well as across protein interfaces



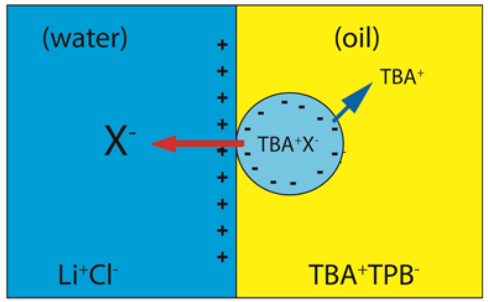
10,000-fold acceleration over 23 Å !!

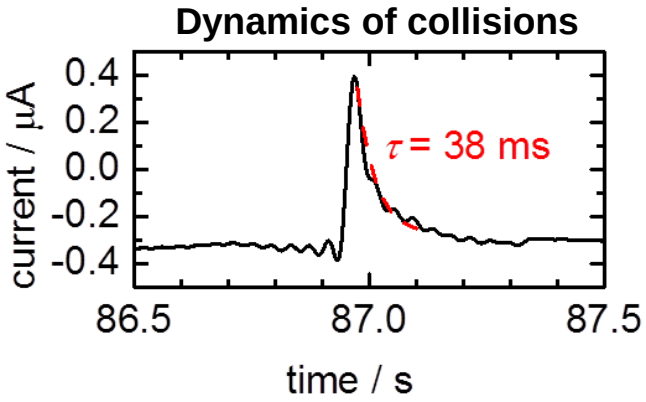
Perspective:

- **Develop a comprehensive theoretical model of electron-hopping** processes
- Characterize intermediates and explore different hopping pathways
- Search for coupling between electron- and structural dynamics
- **Unravel design principles** for constructing (bio)molecular systems capable of directional, long-lived photoinduced charge separation

5. Biomimetic electrochemistry at the polarized liquid/liquid interfaces

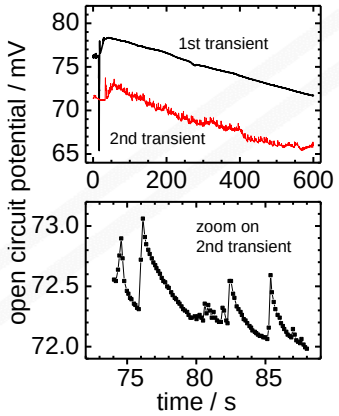
Emulsion droplet collision and fusion within a liquid/liquid interface: Electrochim. Acta, 299 (2019) 875

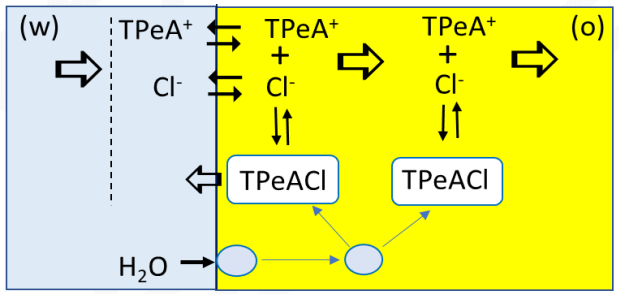




Anomalous salt extraction driven by hydrated salt cluster formation in the organic phase (o)

Electrochim. Acta, 361 (2020) 137059





TPeACl = tetrapentylammonium chloride

These studies improve understanding of spontaneous emulsification encountered in numerous practical applications in the fields of foods, cosmetics, medicine, paints, hydraulic fluids etc.

Retrospective

- Current or open circuit potential (OCP) transient measurements were used to detect collisions of single emulsion droplets at a liquid/liquid interface.
- Analysis of transients provided information about the droplet size distribution, and the collision mechanism and dynamics.

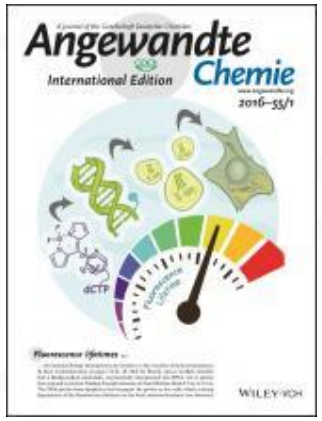
Perspective

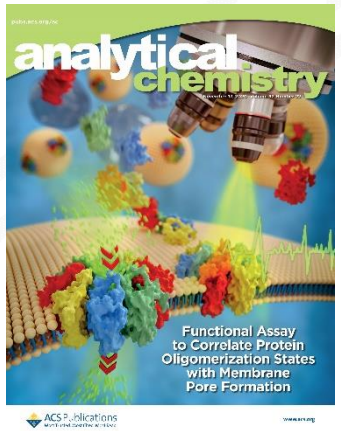
- Effects of interfacial instabilities on the ion transport in two-phase liquid systems
- Catalysis in collision electrochemistry at a polarized liquid-liquid interface

Publications: 118 in impacted journals (2015-2019)

Journal Classification	Number of publications
D1	22
Q1	35
Q2	38

Note: Publications of the former Dep. of Biomimetic Electrochemistry are not included in this statistics





Selected publications:

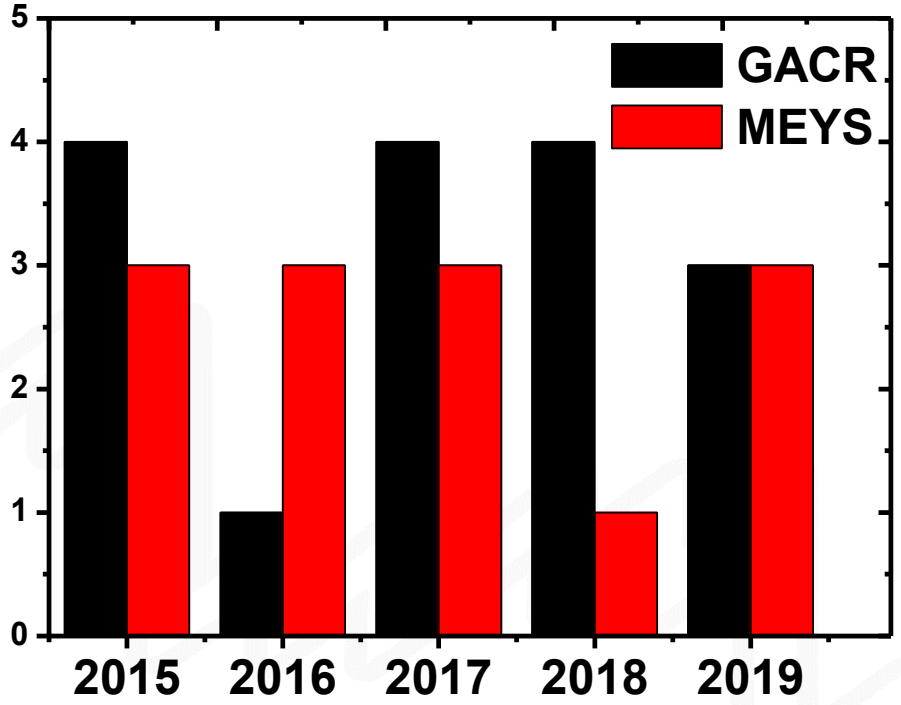
Chemical Reviews, Coord. Chem. Rev	2
Nature Comm, Methods, Protocols	4
ACS Nano, Central Science, JACS, PNAS	8
Angewandte, Chemical Science	4
J.Phys.Chem.Lett.	3
eLife, Frontiers in cell and dev. Biology/Immunology, Free Rad.Bio. Med., J.Cell Sci	6
Electrochemistry Comm, Electrochem Acta	5
Nanoscale	1
BBA, Biophysical J.	12
Langmuir, PCCP, Scientific. Rep, Soft Matter	11
Inorganic Chem.	1
Journal of Phys.Chem.	4

Note: Publications of the former Dep. of Biomimetic Electrochemistry are not included in this statistics

Grants (active 2015-2019)

25 grants were running at the department (most of them funded by GAČR and MEYS) during 2015-2019

- **Praemium Academiae** of the Czech Academy of Sciences (**end 2017**, PI M. Hof)
- **EXPRO GAČR:** Concert of lipids, ions, and proteins in cell membrane dynamics and function (**2019 – 2023**, PI M. Hof).
- **European Commission**: Solar Energy for a Circular Economy (**2019**). PI A.Vlček



- **1 advanced** and **1 starting ERC** grant and **5 ITN** proposals were submitted. **1 ITN** received funding.

Societal relevance

All research topics of the team are motivated by clear needs of our society:

- Understanding function of proteins in cells and the biology of disease.
- The needs in protein engineering. The findings can serve as a basis for further development of enzymes that will find application in biomedicine, biotechnology and pharmaceutical industries.
- A need for information about nanoscale organization of plasma membranes playing a role in immune cell communication or misfolding in neurodegenerative diseases.
- Development photoenzymes that would perform energy-saving transformations upon solar irradiation (water splitting to hydrogen or reducing N_2 to NH_3.
- Understanding electro-separation processes taking pace at electrochemically polarizable liquid/liquid interfaces

Collaborations

Selected international collaborations (backed up by a grant):

- **GACR-DFG** project ”Exploring the structure function relationship of membrane-pore-forming FGF2 oligomers - a single molecule approach” **2020-2022**, PI: R. Šachl and W.Nickel)
- **Marie Skłodowska-Curie ITN**: Proton transport and proton-coupled transport. **2019-2023,** PI P. Pohl, Co-PI M. Hof)
- **Horizon 2020 (SUNRISE)** Coordination and Support Action for the Future Emerging Technologies **2017-2019**, PI: A. Vlček)
- **COST Action** CM1405 "Molecules in Motion", 2015-2018, PI: A. Vlček
- **COST Action CM1202** "Supramolecular photocatalytic water splitting", **2013-2016**.
- **FET proactive (ONEM):** Optical near field electron microscopy, **2021-2024**, co-PI: M.Amaro, PI: T. Juffmann, Wien)

Collaborations between departments

- **Department of Computational Chemistry**: several publications with Prof. Cwiklik
- **Department of Low-dimensional Systems:** Kovaricek et al ACS Nano 2018, or in the framework of FET proactive - ONEM)
- **Department of Chemistry and Ions in Gaseous Phase:** Shestivska et al, Rapid Com Mass Spect 2018

Invited Lectures

Members of the department have been invited to give **26 invited lectures at international conferences** and **21 research seminars at universities**:

Examples:

Amaro: Biophysics Joint Meeting 2017 - Hünfeld (Germany)

Cebecauer: Winter Seminar in Biophysical Chemistry 2017, Klosters (Switzerland)

Hof: Joint 12th EBSA, 10th ICBP-IUPAP Biophysics Congress, Madrid (Spain) or 14th MAF Conference 2015, Würzburg (Germany);

Šachl: Alzheimer disease Consortium meeting 2019

Vlček: Invited seminars at Univ. of North Carolina, (USA) or Univ. of California San Diego (USA)

Activity Plans for 2020-2024:

Research Strategy of the department:

Emphasize the physiological and medical relevance of the biomembrane studies carried out at the department.

Planned departmental activities

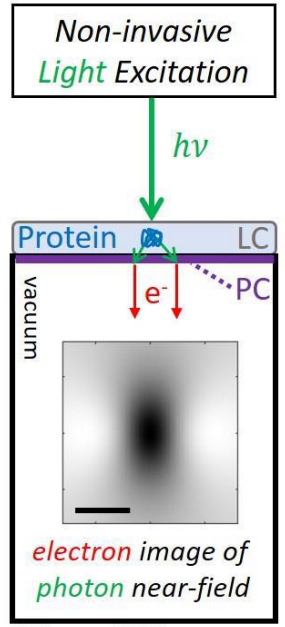
Thematic goals set for the previous evaluation period will be continued. The activities are currently supported by several **GAČR/MEYS** grants, larger **EXPRO GAČR (2019-2023) and EC grants: EU-FET proactive (2021-2024), Horizon 2020 (2019-2020) and ITN (2019-2023)**.

Research topics:

- Concert of lipids, ions and proteins in cell membrane dynamics and function.
- Nanoscale organisation and protein function in cells
- Photoinduced charge separation in complex systems.
- Biomimetic electrochemistry at liquid-liquid interfaces.
- Development of new membrane supports for electron and optical microscopy - ONEM and GMIET (GACR).
- Elucidating Dynamics-Function Relationships in Proteins

Activity Plans for 2020-2024:

One example for all: Optical near-field electron microscopy – ONEM (FET Proactive Horizon 2020)

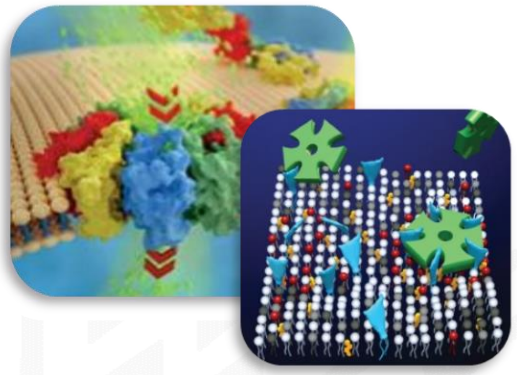


Develop and establish a new hybrid imaging technique that combines the best of two worlds:

- **non-invasiveness** of probing a sample with light
- **high spatial and temporal resolution** offered by electron optical read-out

Label- and damage-free imaging at 3 nm resolution, high frame rates (up to kHz), over extended periods

Applications: Investigation of electrochemical phenomena, plasmonic properties of nanostructured materials in liquid environment (biosensors), and **studies of membrane biology (pore formation, oligomerization, protein diffusion,...)**



Our role:

- Development of technology for creation of special sample chambers based on graphene and liquid cells.
- Development of technology for tethering lipid membranes on the interface which will allow to study membrane biology processes (oligomerisation of proteins) at high resolution and frame rates.

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 101017902

Awards (2015-2019)

- **M. Amaro: 2017:** Otto Wichterle Award
- **Z. Samec: 2019:** Memorial medal of Faculty of Sciences Charles university in Prague; **2018:** The Jaroslav Heyrovský Honorary Medal for Merit in the Chemical Sciences; **2016:** Prize METROHM CR
- **R. Šachl: 2017:** Otto Wichterle Award
- **J. Heyda: 2016**: Danubius Young Scientist Award

Teaching and supervision (2015-2019)

14 different courses with focus on light spectroscopy and microscopy, physical chemistry and electrochemistry are thought at **Charles University, UCT Prague, Palacký University in Olomouc and University of South Bohemia** by the members of the department.

Type of study	No. of supervisors	No. of consultants	Theses defended 2015-2019
Bachelor	2	1	7
Master	2	2	4
Doctoral	8	11	6

Outreach

The members of the team have positive attitude towards presentation of the most interesting results to general public.

National media

- Czech Television, 2016: GM_1 ganglioside inhibits β-amyloid oligomerization induced by sphingomyelin (Angew. Chemie. **2016**, 55)
- Czech Television, 2017: Quantifying protein densities on cell membranes using super-resolution optical fluctuation imaging. (Nat. Comm. **2017**, 8, 1)
- Czech Television, 2018: Molecular Gating of an Engineered Enzyme Captured in Real Time *(J. Am. Chem. Soc.* **2018** 140,17999-18008)

Educational activities

- Participation of the department in the program Open Science focused on training high school students in our laboratories.
- Contributions (oral presentations on the basics of fluorescence) to annual Summer school NANO (1 week) for high school students.

SWOT analysis

Strengths:
- Friendly working environment
- Good international contacts
- Good international standing
- Focus on original ideas
- Modern instrumentation
- Age structure of the team
- Strong core team of scientists acting as PI
- Multidisciplinary team members

Weaknesses:
- Lack of laboratory and office space.
- Difficulties in recruiting highly qualified people
- high bureaucratic burden

Opportunities:
- Recruiting motivated people
- Growth in carrier supported at the department
- Collaborations within the department and the institute
- Obtaining ERC or similar funding
- Obtaining funding for a new instrument from CAS

Threats:
- Insufficient funding
- Irreparable failure of the instruments
- Difficult access to students at universities
- Departure of talented people abroad

Thank you for your attention