WP1.7. Targeting interactions of membrane receptors with other proteins and peptidomimetics (Lazar)

Research aims

- To expand the capabilities of two-photon polarization microscopy (2PPM).
- To apply 2PPM to the identification and development of drugs targeting interactions of membrane G-protein coupled receptors and insulin/IGF-1 receptors with other proteins and peptidomimetics.

Research plan and methodology

(i) Expanding the capabilities of two-photon polarization microscopy.

While 2PPM has proven to be a valuable tool of research into molecular mechanisms of cell signalling events[1,2], 2PPM should also be valuable for pharmaceutical drug discovery and drug development. In order for 2PPM to become a tool of drug discovery, it needs to be developed into a high-throughput (HT) technique, and better 2PPM probes need to be developed. The main obstacle to implementing 2PPM as a HT technique is that useful information is present only in the parts of 2PPM images containing cell outlines. Although it should be possible to develop computer algorithms that automatically recognize cell outlines in 2PPM images, we envision an easier, more robust and reliable approach, relying on cells cultured on micropatterned support[3]. As our preliminary data show (Fig. 2), microstructures can create arrays of highly regular cell membrane outlines. The outlines allow observations of fluorophore orientation by 2PPM. The regularity of the shape and distribution of the microstructure-guided outlines should allow facile development of computerized algorithms that will recognize these outlines in 2PPM images and automatically process the information the outlines contain. Within the scope of the project, we will optimize the properties (shape, size, density) of the microstructures used for cell growth and imaging, and develop algorithms allowing fully automated processing of the corresponding 2PPM images. The work will be an extension of an existing collaboration with J. Jacak and B. Plochberger at J. Kepler University in Linz, experts in microstructure preparation and applications[4,5].

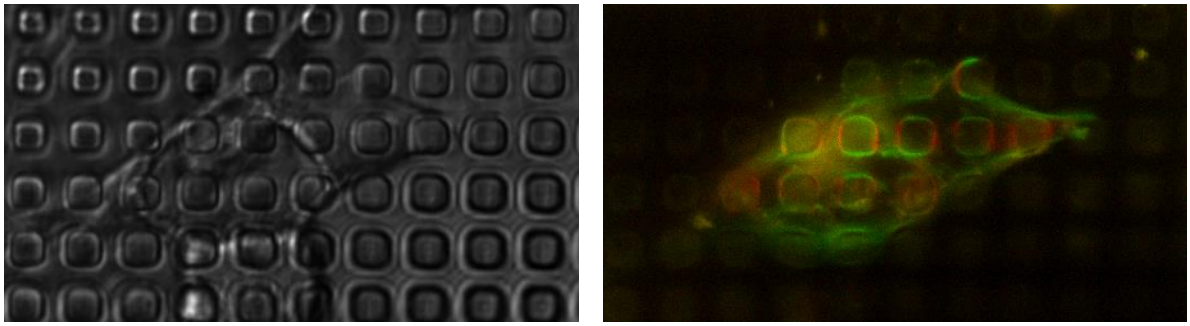

Fig. 2: An HEK293 cell, grown on micropatterned support, expressing a cell membrane-anchored eGFP construct. Left: a transmission microscopy image, showing the regularly shaped microstructures. Right: a 2PPM image, showing the cell membrane outlines induced by the presence of the microstructured support. Fluorescence elicited by excitation light polarized horizontally and vertically has been colored red and green, respectively. The microstructure-induced outlines show a red/green pattern indicative of a well-defined orientation of the present fluorophores with respect to the cell membrane.

In order to apply 2PPM to the development of drugs targeting interactions of membrane receptors with other proteins, we will apply existing 2PPM probes of GPCR:G-protein interactions (developed by us) and non-2PPM probes of GPCR:arrestin interactions. However, we will also improve the existing probes and develop new genetically encoded 2PPM probes of activation of insulin- and IGF-1 receptors, applicable to development of non-peptide insulin mimetics (in collaboration with J. Jiráček and U. Jahn, WP2.6.).

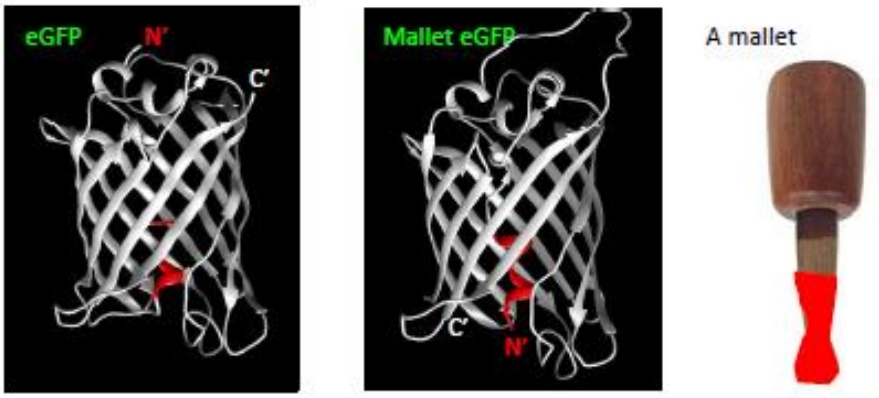

Fig 3. Design and function of the mallet eGFP. Left: known structure of eGFP, with the N' and C' termini shown and an internal a-helical domain marked in red. Middle: proposed structure of mallet eGFP, created by circular permutation and engineering of eGFP. New N' and C' termini shown, and an N'- terminal α-helical 'handle' marked in red. Right: a carpenter's mallet. A rigid handle marked in red.

An important part of our effort will involve creating a novel general approach towards development of 2PPM probes, relying on modified fluorescent proteins ('mallet FPs', Fig. 3). New N' and C'-termini will be introduced in the mallet FPs so that an existing α-helix can be extended to the outside of the fluorescent protein and used as a 'handle' for a rigid attachment to a protein of interest. Rigid attachment should allow efficient transduction of conformational changes in the protein of interest to changes in orientation of the FP moiety, observable by 2PPM. Therefore, mallet FPs will allow creation of highly sensitive 2PPM probes. Extending the α-helix will allow predictable changes in FP orientation, a property favourable for development of both 2PPM and FRET probes. Thus, mallet FPs will allow development of a wide range of genetically encoded fluorescent probes usable in both fundamental biological research and in pharmaceutical drug development. Successful development of mallet FPs will require collaboration with experts in protein engineering (J. Vondrášek, IOCB) and protein structure (P. Řezáčová, IOCB; A. Royant, ESRF Grenoble). We will use the resulting mallet FPs for the identification and development of drugs targeting interactions of membrane receptors with other proteins.

(ii) Applying 2PPM to identification and development of drugs targeting interactions of membrane receptors with other proteins and non-peptide mimetics.

The molecular probes developed in Aim (i), allowing sensitive observations of interactions between GPCRs and other proteins, will be used for the observations and investigations of such interactions. What will be of particular interest will be interactions between adrenergic, glutamatergic, and cannabinoid receptors and the corresponding G-proteins (Gαi1, Gαi2, Gαi3, Gαs, Gαo, Gαq) and arrestin-2 and -3, in response to existing, commercially available pharmaceutical drugs. Investigations of the molecular basis of particular GPCR-effector interactions will yield information useful in targeted development of novel pharmaceutical drugs. Apart from GPCRs, will also develop and apply 2PPM probes of activation/inhibition of the insulin and IGF-1 receptor pathways with non-peptide insulin mimetics, investigated and being developed by the research groups of J. Jiracek and U. Jahn.

The project will be realized in a close collaboration with P. Řezáčová (protein structure analysis), J. Jiráček (insulin/IGF-1 receptors), J. Vondrášek (protein design).

[1] Lazar, J., Bondar, A., Timr, S., Firestein, S. J., *Nature Methods* **2011**, *8*(8), 684-690.
[2] Bondar, A., Lazar, J., *J. Biol. Chem.* **2007**, *289*(3), 1271-1281.
[3] Sevcsik, E., Brameshuber, M., Fölser, M., Weghuber, J., Honigmann, A., & Schütz, G. J. *Nature Commum.*, **2015**, *6*.
[4] Buchegger, B., Kreutzer, J., Plochberger, B., Wollhofen, R., Sivun, D., Jacak, J., & Klar, T. A., *ACS nano* **2016**, *10*(2), 1954-1959.
[5] Plochberger, B., Wollhofen, R., Jacak, J., Axmann, M., Motsch, V., Schütz, G. J., & Klar, T. *Biophys. J.* **2016**, *3*(110), 40a.

Research schedule

2017-2019

- Development of 2PPM into HT technique (microstructure optimization, software development).
- Development of mallet FPs.

2017-2022

- Development of 2PPM probes of interactions of GPCRs with other proteins, interactions of insulin and IGF-1 receptors with insulin mimetics
- Observations of receptor-other protein/peptide mimetics interactions.
  Testing of series of compounds for their interactions with receptors.

Publications and patents

Publications (Jimp)

We plan to publish our results in several peer-reviewed scientific journals with a high impact factor during the course of the grant project and we expect to publish about 5 papers per 5-year period of the project and about 5 papers during the following 5-year period.

	Jimp
2017	0
2018	1
2019	1
2020	1
2021	1
2022	1
Total	5

Journal of Biological Chemistry
Journal of Medicinal Chemistry
PNAS U.S.A.

Cooperation with foreign institutions

- Dr. S. Firestein, Columbia University, New York,
- Dr. A. Royant, European Synchrotron Radiation Facility, Grenoble,
- Drs. B. Plochberger and J. Jacak (J. Kepler University, Linz, Austria.