CSC-RUB PhD Project Proposal

Title: Development of 2nd generation optically-controllable glutamate receptors for *in vivo* applications

Sector of research: Neuroscience, Optogenetics, Chemical Biology

Degree awarded: PhD in Neuroscience

Keywords:

synaptic signaling; optogenetic methods; ionotropic glutamate receptors; protein design; optopharmacology; photoswitchable tethered ligands; electrophysiology;

Supervisor of PhD project:

Prof. Andreas Reiner, Cellular Neurobiology, Department of Biology and Biotechnology

Research focus of supervisor:

My research focuses on molecular and cellular aspects of signaling at glutamatergic synapses, which play a key role for central nervous system function and disease. To dissect glutamate receptor signaling and the functional diversity of this receptor family, my lab combines state-of-the-art chemical, biochemical, electrophysiological and imaging approaches. A key part of our research is the development and application of novel chemo-optogenetic approaches, as these tools allow us to control and probe neuronal communication and the contribution of specific receptor complexes with light and high precision. Our findings have important mechanistic implications for receptor gating, pharmacological manipulation, and synaptic function/dysfunction, i.e. during ischemic conditions.

Publications:

1. Subunit-selective iGluR antagonists can potentiate heteromeric receptor responses by blocking desensitization. Pollok S. & Reiner A. # (2020). *Proc. Natl. Acad. Sci. USA* 117: 25851-25858.

2. Glutamatergic signaling in the central nervous system: Ionotropic and metabotropic receptors in concert. Reiner A. # & Levitz J. # (2018). *Neuron* 98: 1080-1098.

3. A family of photoswitchable NMDA receptors. Berlin S.*/Szobota S.*, **Reiner A.**, Carroll E.C., Kienzler M.A., Guyon A., Xiao T., Trauner D. & Isacoff, E.Y. (2016). *eLife* 5: e12040.

4. Tethered ligands reveal glutamate receptor desensitization depends on subunit occupancy. Reiner A. & Isacoff E.Y. (2014). *Nat. Chem. Biol.* 10: 273-280

5. A red-shifted, fast-relaxing azobenzene photoswitch for visible light control of an ionotropic glutamate receptor. Kienzler M.A.* / Reiner A.*, Trautman E., Yoo S., Trauner D. & Isacoff E.Y. (2013). *J. Am. Chem. Soc.* 135: 17683-17686.

For a full publication list see PubMed:
https://www.ncbi.nlm.nih.gov/pubmed/?term=andreas+reiner+not+winker

Summary of research plan

Background: Optogenetic approaches have become important tools to interrogate neuronal function in a cell type-specific manner. A particular powerful approach is the use of tethered photoswitchable ligands, which can be used to control ionotropic glutamate receptors (iGluRs) and other neuro-transmitter-gated receptors with ultimate specificity and high spatio-temporal resolution. The 1st generation of these photoswitchable receptors has relied on a cysteine-based labelling approach, but *in vivo* applications would benefit from alternative labelling schemes for photoswitch attachment that can provide higher efficacy, specificity, as well as compatibility with CRISPR/Cas-based editing.

Study objective: Aim of this project is to design and develop new labelling schemes to equip different iGluRs with functional, photoswitchable ligands. The project will focus on placing a portable tagging domain (SNAP, CLIP, Halo etc.) at suitable positions remote from the binding pocket (PORTL approach). Specifically synthesized photoswitches will be used to achieve efficient photoagonism or antagonism.

Expected Results: Based on preliminary work and work on related receptor families, we expect that PORTLs will provide an efficient way for iGluR labelling and photoswitching in tissues and *in vivo*. Screening of different receptor/attachment/photoswitch combinations will provide specific photo-agonists and antagonists – e.g. to study the role of distinct NMDA receptors and temporal activation patterns for inducing long-term changes in synaptic plasticity. Most importantly, N-terminally placed tagging domains would allow for CRISPR/Cas-based editing in postmitotic cells and thereby circumvent problems due to overexpression of light-switchable iGluRs.

Methods: Protein design and molecular biology techniques will be used for engineering of new receptor constructs and chemical synthesis to obtain the respective azobenzene-based photoswitches. Screening and characterization will be performed using patch-clamp electrophysiology in HEK cells and organotypic slice cultures, supported by calcium imaging. Expression of genetically-modified iGluRs is performed with AAVs and will be extended to CRISPR/Cas-mediated manipulations. The project will be supported by collaborations with organic chemists and neurophysiologists.

Candidate Requirements: Required is an excellent university degree in Biology, Chemical Biology, Biochemistry, Molecular Medicine, or a related discipline. A very high motivation to engage in independent experimental research, the ability to work in an interdisciplinary team, and very good English skills are mandatory. For this project we specifically seek candidates with experience in the area of neurobiology, as well as electrophysiology or chemical biology/synthesis.

Motivation for CSC application: We offer excellent training and research possibilities in a collaborative and interdisciplinary research environment. The candidate will perform hands-on, cutting-edge research in the field of chemo-optogenetics, receive training in patch-clamp electrophysiology and state-of-the-art optogenetic experimentation, and will have opportunity for collaboration and publication on an international level. Participation in international meetings is strongly encouraged. Further training will be provided through integration into the *International School of Neuroscience (IGSN)* and the *Rub Research School* (part of the *Research Academy Ruhr*), which provides guidance and supports interdisciplinary skill development.