

Technology Offer

Innovative chemical inducers of dimerization (CID) for studying protein interactions in cells

Ref.-No.: 0803-5308-MSG

An innovative chemical inducer of dimerization for studying protein interactions in cells, that overcomes challenges of available dimerizers.

Background

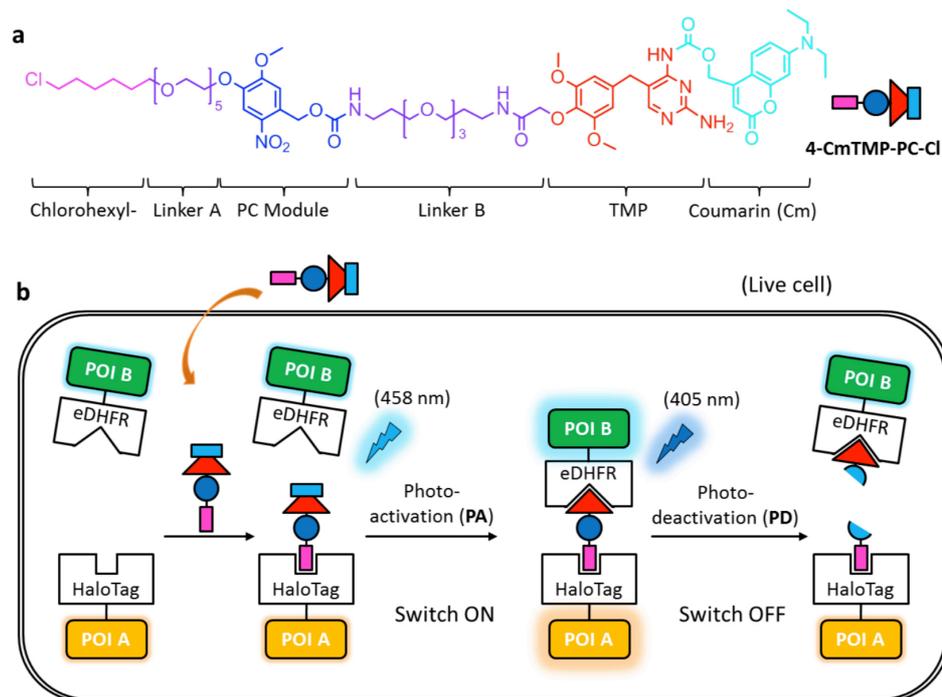
Chemical and optogenetic tools to modulate protein activity in cells are a robust approach to elucidate the dynamics of biological systems and to dissect the complexity of regulatory networks. All known chemical inducers of dimerization require a very long time, up to several minutes, to cross the plasma membrane and distribute to the entire cell, before action. They therefore lack spatial and temporal resolution, making it difficult to study acute and local cellular processes without affecting other regions in the cell. Optogenetic approaches using photosensitive proteins, although powerful, suffer from several inherent problems:

- 1) Optogenetic systems cannot readily be tuned by light intensity, as photosensitive proteins exist in equilibrium between active and inactive conformations
- 2) Broad absorption spectra of photosensitive proteins (e.g. LOV: 400-500 nm, Cry2: 390-500nm, PhyB: 550-800nm) overlap with commonly used fluorescent proteins (FPs); and as a result, the number of FPs that can be used in optogenetic experiments is greatly limited
- 3) Photosensitive proteins require continuous illumination, which is experimentally challenging and may cause photo-toxicity to cells
- 4) They suffer from non-zero affinity in the dark state, leading to pre-activation of the protein of interest (POI).

Technology

The photoswitchable chemically induced dimerization (psCID) approach developed by our scientists overcomes several key limitations of canonical optogenetic systems.

- 1) The degree of dimerization and dedimerization is fine-tuned by the dose of illumination. Therefore, the number of molecules in the on/off state can be quantitatively controlled. This is useful to emulate cellular states that are controlled by the concentrations of the active protein
- 2) This system can be operated by a single pulse of laser light of distinct wavelengths (405 nm and 458 nm) or laser light of a single wavelength (405 nm) with different intensities, unlike the PhyB-PIF system
- 3) Optical perturbation at 405nm using the psCID system can be combined with multi-color imaging, including mTurquoise2, mCitrine, mCherry and Atto740 dye
- 4) The signal-to-noise ratio in the chemo-optogenetic perturbation is significantly improved.



a Chemical structure of the psCID, 4-CmTMP-PC-Cl, featuring a chlorohexyl- ligand for covalent binding with HaloTag, a linker containing a photocleavable (PC) 2-nitrobenzyl module, and a diethylaminocoumarinyl-caged TMP ligand.

b Schematic view of 4-CmTMP-PC-Cl for reversible control of protein dimerization in a live cell using light. 4-CmTMP-PC-Cl is readily cell permeable and first pre-localizes to the protein of interest (POI) A fused with HaloTag. Afterwards, a first light illumination decages the diethylaminocoumarinyl group and exposes TMP ligand to binding with eDHFR-fused POI B. This step is called photoactivation (PA). Upon a second light illumination (e.g. using a different wavelength of light), the photocleavable linker is cleaved, leading to the dissociation of the dimerization process. This step is called photodeactivation (PD).

In summary, the psCID system is reversible, tunable, acute with high spatial resolution at the micrometer scale. Both PA and PD using this approach are very rapid. The fast on/off speed makes it highly valuable for studying fast biological processes. We are now looking for a licensing partner for this exciting technology.

Publication

<https://doi.org/10.1002/anie.201800140>

Patent Information

EP Prio 17180401.6; filed on July 17, 2017

Contact

Dr. Matthias Stein-Gerlach

Senior Patent- & License Manager
Biologist

Phone: +49 (0)89 / 29 09 19 - 18
eMail: stein-gerlach@max-planck-innovation.de