Life and Science imaging

In-situ Visualization of Uniform Rectangular Platelet Micelles using Structured Illumination Microscopy

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The realization of complex self-assembled structures in solution requires access to more advanced analytical techniques that permit the study of systems in their native environment. Current methods for micelle characterization either provide ensemble measurements (e.g. static light scattering or bulk fluorescence), possess limited resolution (e.g. laser scanning confocal microscopy, LSCM), or require the invasive removal of solvent prior to imaging (e.g. electron microscopy). Super-resolution fluorescence microscopy techniques offer an attractive solution to these problems.

We have successfully employed Structured Illumination Microscopy (SIM) to image nanostructures prepared from the living Crystallisation Driven Self-Assembly of fluorescent block copolymers. This technique enabled the structures to be studied in their native environment at higher resolution than afforded by LSCM.

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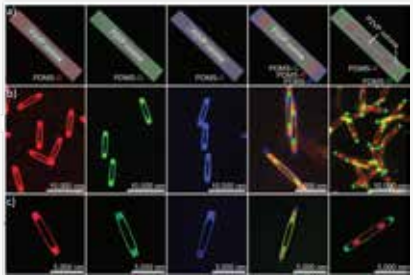

Uniform multiblock rectangular platelets selectively functionalized using fluorescent poly(ferrocenyldimethylsilane) BCPs. a) Schematic representations b) LSCM and c) SIM images of typical rectangular platelet block comicelles, with segregated regions composed of nonfluorescent poly(2-vinylpyridine) coronas and multiple dye-functionalized fluorescent poly(dimethylsiloxane) (PDMS) coronas. The PDMS coronas with red, green, and blue fluorescence are denoted as PDMS-R, PDMS-G, and PDMS-B, respectively. Figure reproduced with permissions from Science, 2016, 352, 697.

Structured illumination microscopy (SIM) as an approach to functionally dissect periodic membrane structures in neuronal axons

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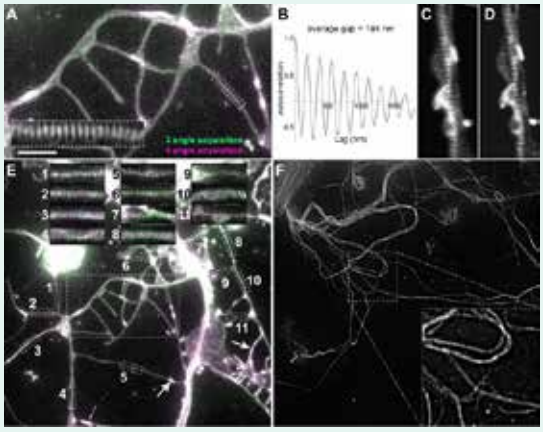
Axons contain parallel bundles of microtubules (MTs) that form their structural backbones and serve as life-sustaining transport routes. MT bundles are surrounded by regularly spaced actin rings termed periodic membrane skeleton (PMS) which were initially described in mammals. Their function was not known. We functionally dissected the structure and role of PMS by combining versatile Drosophila genetics with super-resolution microscopy.

Our combined approach using efficient super-resolution SIM imaging with versatile Drosophila genetics enabled us to functionally dissect PMS in axons. The advantage of SIM imaging is that it is fast and reliable and therefore ideally suited for quantitative analysis with very high sample numbers. This enabled us to apply a wide range of genetic and pharmacological manipulations and classify their affects using PMS abundance as readout. We could then apply our knowledge by showing a first functional relevance of PMS for MT bundles, MT dynamics, and even axon maintenance.



Superresolution images of Drosophila axons display PMS. (A) SIM image of SiR-actin–stained Drosophila primary neurons at 10 DIV; (B) Autocorrelation analysis showing the regular periodicity of the actin staining with a lag of 184 nm. (C, D) Axon visualized via STED shown as raw (C) and deconvolved (D) images. (E) Full SIM image of SiR-actin–stained neurons at 10 DIV

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Determining the geometry of EGFR oligomers via fluorophore localization imaging with photobleaching

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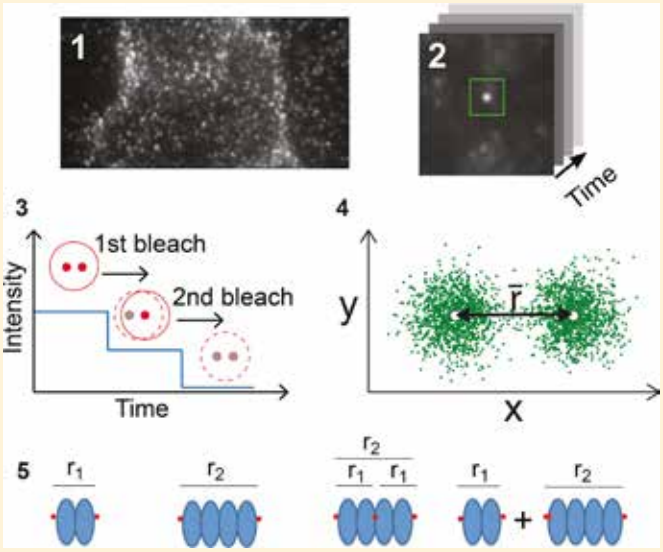
Dimerisation, oligomerisation and clustering of cell surface receptors are recognized as important in the control of signalling processes. The epidermal growth factor receptor (EGFR, or HER1/ErbB1) is a cell surface receptor involved in many signalling pathways and which if dysregulated can lead to cancer. Using Fluorophore Localisation Imaging with Photobleaching (FLImP), a method developed by members of the OCTOPUS team, we probe the structure of inactive and ligand-induced EGFR oligomers measuring discrete pairwise separations between fluorophore conjugated HER1 Affibody or EGF.

Using FLImP we find that at physiological EGF concentrations, EGFR assembles into oligomers, as indicated by pairwise distances of receptor-bound fluorophore-conjugated EGF ligands. The pairwise ligand distances correspond well with predictions from structural models of EGFR dimers, tetramers, hexamers, octamers and decamers.



How to obtain separations with the FLImP method. (1) TIRF images are collected of fluorophore tagged ligand bound receptors and (2) spots of individual complexes are tracked to give intensity time courses. (3) Representation of a spot containing two fluorophore-conjugated ligands (red dots) where the intensity vs time trace shows two intensity levels which decays to zero in two bleaching steps. When one fluorophore bleaches (grey spot) the spots centroid position shifts. (4) A global least-squares seven-parameter-fit is used to identify the best intensity, x-y positions and the full-width at half-maximum of the point spread function for each fluorophore, from which their separation is calculated with a precision determined by the localization error; (5) Examples of the separations collected from a two-ligand dimer and tetramer, a three-ligand tetramer, and a mixture of a dimer and a tetramer.

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