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motion of the probe thus the target molecules of interest. Herein, we present an integrated imaging platform based on the combination of birefringent gold nanorod probes and differential interference contrast (DIC) microscopy that can achieve single particle orientation and rotation tracking (SPORT). This new technique allows us to resolve translational and rotational motions of gold nanorod probes at each stage throughout the clathrin-mediated endocytosis process, leading to the disclosure of their binding status on the cell membrane and characteristic rotational motions generated by protein modules.

2196-Pos Board B182
Particle Tracking of Membrane VAMP2 within Secretory Cells in the Nanometer Regime
Daniel Boening, Martin Wiemhoefer, Julia Trahe, Cora Sandra Thiel, Jurgen Klingauf.
In the past we suggested that a pre-sorted and pre-assembled pool of SV proteins on the presynaptic membrane might support a first wave of clathrin-mediated endocytosis. Recent evidence using IsoSTED nanoscopy of surface vesicular proteins indicates, that such pre-assembled patches exist at the periactive zone in hippocampal boutons.
Here we analyzed the diffusion kinetics of the surface-stranded vesicular protein Vamp2 coupled to the photoactivatable proteins Dendra and Eos by 3D particle tracking in combination with photo activation localization microscopy (PALM). The z-position information of the fluorophores was estimated by measuring the widths of the elliptical PSF caused by a cylindrical lens in the detection pathway. The elliptical PSF is translated into axial positions by a least squares fit of the ellipticity. The axial position estimation can then be enhanced by Gaussian image filtering with a resolution enhancement of at least 30%.
With 3D particle tracking in living secretory cells like PC12 or hippocampal boutons nanostructures of faster and slower diffusion or transport of a VAMP2-EOS complex were identified. From 3D particle tracking maps we can show highly heterogeneous diffusion behaviour of Vamp2 within PC12 cells and synapses. This enables us to resolve and characterize areas of endocytosis within living cells.

2197-Pos Board B183
Superresolution Analysis of syb2 Distribution in Membranes of Live and Fixed Cells
Martin Wiemhoefer, Julia Trahe, Cora Thiel, Jürgen Klingauf.
Imaging of continous biological structures is diffraction limited in far field microscopy due to the overlap of the point spread functions (PSFs) of the single fluorescent emitters. Superresolution beyond the diffraction limit by photoactivation localization microscopy (PALM) has therefore become indispensible for studying biological processes.
By combining PALM with Total Internal Reflection Microscopy (TIRF) we were able to localize single molecules involved in secretion in fixed and live neurosecretory cells. The fusion of secretory vesicles with the membrane is mediated by the assembly of low-energy complexes formed by the coil-coiling of three members of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein family: SNAP-25, syntaxin, and synaptobrevin 2 (syb2).
Now a dimerization of the syb2 via transmembrane domains (TMDs) in intact PC12 cells has been reported [1]. However it is not clear if such interactions have an effect on the kinetic properties of fast vesicle fusion. To this end we expressed syb2 fused to Dendra2 in HeLa and PC12 cells and mapped the protein distribution in membranes of live and fixed cells. We found evidence for a dimerization of syb2/Dendra2 by comparison with cotransfection of untagged syb2 in HeLa cells and quantified the protein distribution in the cellular membranes.
TMD mutants display slight inhibitory and enhancing effects on membrane fusion as described in the literature [1]. To further characterize the TMD-mediated interactions between syb2 molecules in intact cells we studied the distribution of TMD mutants in live and fixed cells and quantified density and distance to study the role for the syb2 TMD in exocytosis.
[1] Fdez et al, 2010.

2198-Pos Board B184
Contributions of CryoeM to Visualize Membrane-Curvature Generation - Mechanisms and Implications
Vinzenz M. Unger, Carsten Mim.
Protein mediated recognition, maintenance and modulation of membrane curvature plays critical roles in many cellular processes. Based on a growing number of crystal structures from the BAR-superfamily of proteins, MD simulations and spectroscopic work, it now is widely accepted that scaffolding and/or insertion of amphiphatic wedges play important roles in the curvature related processes. What had been missing from the picture was a direct visualization of BAR-domain proteins in their membrane bound states. Addressing this need through use of electron cryomicroscopy, our previous work on F-BAR domains confirmed that these modules induce curvature by scaffolding, but also showed that these domains can engage flat bilayers through an alternate binding interface that is physiologically relevant. Extending our work to the N-BAR family, it now becomes apparent that these modules function very differently from their F-BAR cousins, and that the specific design principles of N-BAR lattices have direct implications for the interaction of these scaffolding components with downstream interaction partners.

2199-Pos Board B185
Membrane Deformation Caused by Clathrin and Associated Adaptor Proteins In Vitro
Mohammed Saleem, François-Damien Delapierre, Laurent Malaquin, Aurelien Roux.
Clathrin-dependent endocytosis is a common mode of cellular trafficking. The formation of clathrin-coated vesicles is preceded by deformation of membrane due to the interacting coat proteins comprising of clathrin and various adaptors. However, the precise mechanism behind the deformation of membrane and existence of diverse shapes, sizes and symmetry remains unknown. We intend to develop a bio-mimetic system to allow the study of the precise biochemical and physical parameters that control the rate of clathrin polymerization and to determine the membrane deforming forces. Clathrin binding to giant unilamellar vesicles was followed using fluorescent clathrin and the coat polymerization was confirmed by fluorescent recovery after photobleaching (FRAP). Interestingly, clathrin binding and polymerization on membrane takes place only when AP180 is present in the injected mixture of proteins. We also observed significant differences in the membrane deformation caused by various adaptor proteins (AP180 and AP 1, 2) and clathrin by differential interference contrast fast video microscopy (DICM), on a micron scale. Additionally, interesting insights on the topology of the membrane deformations caused by various adaptor/clathrin mixtures on membrane sheet assemblies were obtained by means of atomic force microscopy (AFM). Our findings suggest that AP180/clathrin lead to the generation of extensive bud like structures that are ~100nm in diameter and ~20nm in height. Further, high resolution imaging will be used to differentiate various polygonal transformations in clathrin lattices under varying conditions of adaptor/clathrin, which may define various shapes and sizes of buds. In addition, precise forces and actors generating the forces behind membrane deformation and the role of pre-induced membrane curvature in the clathrin polymerization and varying lattice structure will be investigated.

2200-Pos Board B186
Quantitative Analysis of Membrane Deformation and Fission Induced by Dynamin GTPase Activity
Sandrine Morlot, Martin Lenz, Jacques Prost, Jean-François Joanny, Aurélien Roux.
Dynamin is widely used by cells to sever lipid bilayers. During this process a short helical dynamin polymer (1 to 3 helical turns) assembles around a membrane tubule and reduces its radius and pitch upon GTP hydrolysis. This deformation is thought to be crucial for dynamin's severing action and results in an observable twisting of the helix [1]. Here we quantitatively study the factors determining the dynamics of this deformation by studying long dynamin. We perform in vitro experiments where we attach small beads to the dynamin helix and track their rotation in real time, thus collecting information about the space and time dependence of the deformation. Longer helices deform more slowly as predicted by a generalized hydrodynamics theoretical model [2]. Further agreement between experiments and theory indicates that the concerted deformation dynamics is dominated by the draining of the membrane out of the helix, allowing us to quantitatively characterize helix-membrane interactions [3]. We also study the dynamics of tube fission induced by dynamin GTPase activity. Membrane nanotubes are pulled from Giant Unilamellar Vesicles (GUV) using optical tweezers and membrane tension is set by aspirating the GUVs within a micropipette. Dynamin and GTP are injected near the tube. Tubes always break few seconds after dynamin starts polymerizing around the tube. We show that probability of fission depends on GTP concentration, no global depolymerization occurs during GTP hydrolysis and membrane geometry affects fission.