

reconstruction methods (Zhang and Hinshaw, 2001, Chen et al., 2004). Crystal structures of the GTPase and PH domains from various species have been fitted to the structure of the  $\Delta$ PRD dynamin phospholipid tube in its constricted and non constricted states (Mears et al., 2007). The PRD interacts with the SH3 domains of several proteins involved in signaling pathways. We are using cryo-EM and a single particle approach to solve the structures of the full length protein-lipid tubes in the constricted and non-constricted states. In both states, we have observed a decrease in the number of subunits per turn compared to previous structures of  $\Delta$ PRD dynamin tubes. This suggests that the presence of the PRD changes the arrangement of the dynamin domains around the phospholipid tube. Further evidence of a direct interaction between the GTPase domain and the PRD is provided by simultaneous immunogold labeling of the two terminal domains.

## References:

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**393-Pos Board B272****Structural Basis For HIV-1 DNA Integration in the Human Genome**

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Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein that requires the lens epithelium-derived growth factor (LEDGF), a cellular transcriptional coactivator. In the presence of LEDGF, integrase forms a stable complex *in vitro* and importantly becomes soluble by contrast with integrase alone which aggregates and precipitates. Using cryo-electron microscopy (EM) and single-particle reconstruction, we obtained three-dimensional structures of the wild type full length integrase-LEDGF complex with and without DNA. The stoichiometry of the complex was found to be (integrase)<sub>4</sub>-(LEDGF)<sub>2</sub> and existing atomic structures were unambiguously positioned in the EM map. *In vitro* functional assays reveal that LEDGF increases integrase activity likely in maintaining a stable and functional integrase structure. Upon DNA binding, IN undergoes large conformational changes. Cryo-EM structure underlines the path of viral and target DNA and a model for DNA integration in human DNA is proposed.

**394-Pos Board B273****Structural Studies of a Phycobilisome**

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Phycobilisomes are protein complexes present in cyanobacteria and red algae; they are involved in light harvesting and conduction of light and the aim of this study has been to understand the high efficiency observed in these processes. The structure of the phycobilisome from an eukaryotic algae *Gracilaria chilensis* was studied by biochemical methods in order to obtain intact phycobilisomes and to obtain their phycobiliprotein components, phycoerythrin, phycocyanin and allophycocyanin. The structure of phycobilisomes has been studied by electron microscopy and electrophoresis and by theoretical methods; the structure of phycobiliproteins has been studied by protein crystallography and because they are chromophorylated, their properties also were studied by absorption and emission spectroscopy. We have also built a theoretical docking model for an antenna formed by two units of phycoerythrin and two units of phycocyanin. This model was used to obtain the  $k_T$  for the transfer in resonance of the light; the pathway of the light was calculated through the antenna. An evaluation of the model was performed by comparison with the electron microscopy images. The effect the protein environment on the spectroscopic properties of the chromophoric groups was also considered and analysed. FONDECYT 108.0267.

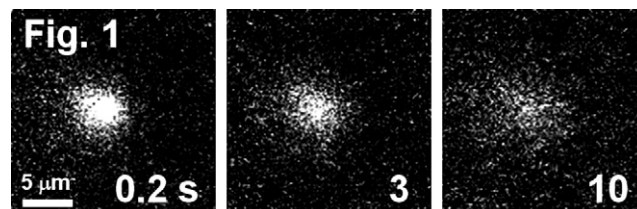
**395-Pos Board B274****Quantification of the Exchange of Subunits from Membrane Protein Complexes Using Foerster Transfer Recovery (FTR)**

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To quantify the exchange of subunits of membrane protein complexes cells expressing CFP/YFP-tagged phospholamban (PLB) were observed by total internal reflection fluorescence (TIRF) microscopy. We performed YFP-selective photobleaching of spots, lines, or larger regions of interest on the basal surface

of the cells. This resulted in enhanced CFP fluorescence, indicating CFP-YFP fluorescence resonance energy transfer (FRET). Subsequent spatial broadening of this "pseudo-photoactivated" CFP fluorescence was analyzed as a measure of the lateral diffusion of PLB complexes away from the target region. In addition, exchange of bleached and unbleached YFP-PLB from complexes restored FRET over time. This process of Foerster transfer recovery (FTR) was taken to indicate the rate of exchange of fluorescently-labeled subunits of the membrane protein complex. Diffusion and exchange processes were quantified by image analysis using a custom MatLab application for 2-dimensional Gaussian fitting. In addition to its application to FTR, this approach may be useful for cytoplasmic proteins as a way of quantifying dynamic membrane recruitment and lateral diffusion on the plane of the bilayer. Fig. 1 shows the diffusion of acceptor-photobleached CFP/YFP-PLB complexes from a target region, followed by subunit exchange.

**396-Pos Board B275****Ligand Binding and Sick Cell Hemoglobin Polymerization Kinetics: Implications for Therapies**

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Sickle Cell Disease results from a point mutation on the beta subgroups of hemoglobin. When hemoglobin releases its four ligands it changes from a relaxed (R) structure to a tense (T) structure and the mutation causes polymer chains to grow. Typical *in vitro* experiments measure this through complete photolysis of a COHbS sample with a laser and then quantify the scattered light from growing polymers. However, *in vivo*, many molecules are partially liganded due to the incomplete transfer of oxygen from red blood cells to the surrounding tissue. Liganded T state molecules could contribute to polymer growth, although until now the effect on the kinetics of fractional saturation was unknown. We examined the effects of introducing NO into COHbS samples. The strong binding of NO to HbS keeps its ligand distribution unchanged during the COHbS experiment. We found that the NOHbS caused the polymerization rate to decrease by 50% due to tertiary inhibition of the partially bound T state hemoglobin. We ruled out the possible effects of non-polymerizing R state NO Hb through a flash photolysis experiment, where photolysis curves were analyzed for an initial fast recombination of CO to R state Hb. Only an insignificant possible amount of R state was found (<3%), and could not account for the effects recorded. The effect of partial ligation on polymerization is important in analyzing possible therapies for sickle cell disease. One possible therapy would be to alter the oxygen affinity of Hb, thereby decreasing the number of fractional intermediates and decreasing the number of T state HbS overall.

**397-Pos Board B276****Fiber Depolymerization: Fracture, Fragments, Vanishing Times and Stochastics in Sick Cell Hemoglobin**

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Polymerization of sickle cell deoxyhemoglobin (HbS) into stiff fibers lies at the root pathology in sickle cell disease. It induces red cell rigidification, cell membrane damage with myriad pathophysiological consequences, and hemolysis and anemia. The well characterized polymerization kinetics bear intimate relation to pathogenesis, but the role of the less well characterized fiber depolymerization remains to be defined. Its rates may be important in at least 3 ways: i) they govern whether residual polymers fail to dissolve in the lungs and pass into the systemic circulation, facilitating repolymerization; ii) they may affect resolution of vaso-occlusion in sickle cell crises; iii) delayed dissolution might exacerbate cellular damage. Here we observe depolymerization experimentally and develop a theoretical model that encompasses fiber fracture, fragment formation, stochastics and the probabilistic distribution of fiber vanishing times. We use Monte Carlo simulations to show when dissolution is rapid and when slow. Experimentally, we demonstrate fracture in real time and show that dissolution of a fiber does not proceed uniformly in time and space and thus is stochastic. We derive an analytic equation for the distribution of