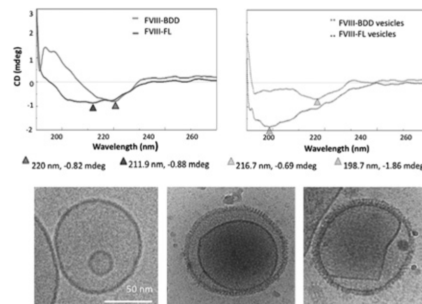
modeling algorithms for the analysis of multi-scale data sets. Such an integrated package has various advantages - the user-interface not only eases the typically steep learning curve of pure algorithmic techniques, but it also permits an instantaneous analysis and post-processing of the results. In addition, we show that such a system is also highly beneficial in the case of low-resolution data sets, which are often difficult to interpret by fully-automatic docking tools. Therefore, we propose a peak-selection that enables the user to explore the score landscape of a black-box docking algorithm. This semi-interactive technique is accompanied in Sculptor by a novel simultaneous, multi-body refinement procedure. An integrated modeling approach also demands high-quality, special purpose visualization techniques, that are able to represent complex molecular assemblies in real-time, using modern GPU programming techniques. The proposed software system is freely available on our website, http://sculptor.biomachina.org.

1755-Pos Board B665
Visualizing Ribosome Biogenesis: Parallel Assembly Pathways for 30S Subunit
Anke M. Mulder.
Ribosomes are self-assembling macromolecular machines that translate DNA into proteins, and an understanding of ribosome biogenesis is central to cellular physiology. Previous studies on the E. coli 30S subunit suggest that ribosome assembly occurs via multiple, parallel pathways rather than through a single rate-limiting step, but little mechanistic information is known about this process. Discovery Single-particle Profiling (DSP), an application of time resolved electron microscopy, was used to obtain over 1 million snapshots of assembling 30S subunits, identify and visualize the structures of 14 assembly intermediates, and monitor the population flux of these intermediates over time. DSP results were integrated with mass spectrometry data to construct the first ribosome assembly mechanism that incorporates binding dependencies, rate constants, and structural characterization of populated intermediates.

1756-Pos Board B666
Structural and Functional Role of INI1, a Cellular Cofactor of HIV-1 integrase in the Early Step of HIV Infection
Benoit Maillot, Corinne Crucifix, Sylvia Eiler, Karine Pradeau, Nicolas Levy, Yves Mely, Dino Moras, Patrick Schultz, **Marc Ruff**.
Integration of the human immunodeficiency virus type 1 (HIV-1) cDNA into the human genome is catalyzed by the viral integrase protein (IN) that requires cellular cofactors for viral infectivity. IN catalyzes a two step reaction, first the 3' processing removes two nucleotides on the 3' end of the viral DNA and second the strand transfer reaction performs the integration. A cryo-EM structure at 14 Å resolution of the HIV-1 integrase in complex with the lens epithelium-derived growth factor (LEDGF) has been solved in presence and absence of DNA (1). Recently we solve a cryo-EM structure of the ternary complex of HIV-1 Integrase, LEDGF and the integrase binding domain of the integrase interactor 1 protein (INI1) in absence (16 Å) and presence (18 Å) of DNA. *In vitro* functional and binding assays using fluorescence anisotropy have been performed showing the effect of INI1 on DNA binding and on the first catalytic activity of integrase, the 3'processing. We show that INI1 decreases the affinity of viral DNA for the IN/LEDGF complex by a factor of 2 and inhibits completely the 3'processing reaction. The structure shows that INI1 prevent the viral DNA to access the catalytic site of integrase. The functional role of INI1 could be to prevent the auto integration of the viral DNA. Theses structures could led the basis for the design of 3'processing inhibitors.
(1) Michel, F., Crucifix, C., Granger, F., Eiler, S., Mouscadet, J.F., Korolev, S., Agapkina, J., Ziganshin, R., Gottikh , M., Nazabal, A., Emiliani, S., Benarous, R., Moras, D., Schultz, P. and Ruff, M. (2009). Structural basis for HIV-1 DNA integration in the human genome, role of the LEDGF/P75 cofactor. EMBO J., 28, 980-991.

1757-Pos Board B667
An Improved Model for Dynamin Assembly Revealed by Cryo-EM
Joshua S. Chappie, Jason A. Mears, Shunming Fang, Marilyn Leonard, Sandra L. Schmid, Ronald A. Milligan, Jenny E. Hinshaw, Fred Dyda.
Dynamin is a multidomain GTPase that assembles into collar-like structures at the necks of deeply invaginated coated pits during the final stages of clathrin-mediated endocytosis (CME) and catalyzes membrane scission. Assembly of purified dynamin tetramers in vitro yields helical structures comparable to those observed in vivo. The formation of these oligomers stimulates dynamin's basal GTP hydrolysis >100-fold. Mutational analysis indicates that dynamin's stimulated GTP hydrolysis is required for CME; however, mounting evidence suggests that this activity causes disassembly of the dynamin collar rather than direct membrane severing. Despite recent structural studies showing that stimulated hydrolysis arises from the transition-dependent dimerization of dynamin's catalytic G domains, little is known about the conformational changes that precede and/or result from this interaction in the context of the polymer. Specifically, it is unclear how the G domains are properly oriented, which subunits associate, and how catalysis triggers dissociation of the pleckstrin homology (PH) domain at the membrane surface. Much of this ambiguity can be attributed to the low resolution (>20Å) of previous dynamin polymer models and the absence of a complete dynamin tetramer crystal structure. To clarify these issues, we have used cryo-EM and iterative helical real space refinement to generate an 11Å reconstruction of a truncated form of dynamin (ΔPRD) in the assembled, GMPPCP-bound state. This map reveals new structural characteristics including a twisted, interlacing interaction that stabilizes the middle/GED stalk and a previously uncharacterized density feature adjacent to the exterior GTPase head. Computational docking of crystallized dynamin fragments reveals the location and connectivity of different domains within the assembled polymer. Chemical crosslinking experiments also provide new insights into the architecture and organization of dynamin tetramer. These data have important implications regarding the conformational changes associated with dynamin catalyzed GTP hydrolysis and membrane fission.

1758-Pos Board B668
Membrane-Bound Organization of Coagulation Factor VIII
Svetla Stoilova-McPhie.
The focus of this study is to follow the structural changes of two human recombinant Factor VIII (FVIII) forms: full length (FVIII-FL) and B domain deleted (FVIII-BDD) upon binding to phosphatidylserine (PS) containing phospholipid (PL) vesicles and nanotubes (LNT) by combining Cryo-electron microscopy (Cryo-EM) and circular dichroism (CD) studies. FVIII or antihemophilic factor is a multidomain plasma glycoprotein of 280 kDa, essential for blood coagulation. Activated FVIII (FVIIIa) is a cofactor to the serine protease Factor IXa in the Tenase complex assembled onto the negatively charged platelet surface. Cryo-EM is unique in its ability to visualize the structure of macromolecules within an ensemble at subnanometer resolution and closest to physiological conditions. The Cryo-EM data show strong protein-membrane and protein-protein interactions. The CD data show a shift in the CD spectra towards the shorter wavelengths upon binding of the FVIII to the PL vesicles, more pronounced for the FVIII-FL form. The above data confirm our hypothesis that the FVIII conformation in solution is significantly altered upon attaching to the platelet membrane and assembly of the Tenase complex. This work is supported by a National Scientist Development Grant from the American Heart Association: 10SDG3500034 to SSM.



Biophysics Education

1759-Pos Board B669
Netflux: Biological Network Modeling for Biologists and Students
Stephen T. Dang, **Jeffrey J. Saucerman**.
Cell signaling and gene regulatory networks are remarkably complex, hindering intuitive understanding of how these networks regulate cell function. This presents a challenge both for biological researchers and students learning about these networks for the first time. Here, we introduce an open-source software package, Netflux, which allows the user to simulate a biological network using only the pathway structure (e.g. from a review article). The mathematical framework uses logic-based differential equations, but these are hidden behind a user-friendly graphical interface. We tested this software with 2 groups of high school students, finding that they are able to quickly build and simulate complex signaling networks. The models create a platform for inquiry-based learning, where students can design and perform virtual experiments to test their understanding. For example, high school students were challenged to identify an optimal combination of