representative for the class. The traditional cross correlation method searches a large space of rotations and translations since information about the alignment is not known a priori. Furthermore, that method requires an initial reference image to be provided by the user. In contrast, in our new version of the cross correlation method, the size of the search space is reduced by preprocessing class images as described below, and this peprocessing step circumvents the need for a reference image.
During preprocessing, the centers of mass and the principal axes of images within a class are aligned, resulting in a blurred version of the underlying image. This blurry image is then used in place of an initial reference image. Even though the initial alignment is coarse, the statistics of the resulting misalignment can be estimated well based on the ergodic properties of the additive background noise. Using the statistical properties of the misalignments, a targeted search within the set of all translations and rotations of images is performed, resulting in reduced computational time and increased alignment accuracy. Using synthetic data, we compare the new method to both the classical cross correlation approach and the maximum likelihood method, and demonstrate the improvement in performance that results when using our method.
This work was supported by NIH Grant R01GM075310.

**1750-Pos Board B660**
**Novel Methods for Rapid Comparison and Multimeric Protein Complex Fitting for Low-Resolution Electron Microscopy Data**
Lee Sael, Juan Esquivel-Rodriguez, **Daisuke Kihara**.
Recent advancements of experimental techniques for determining protein tertiary structures raise significant challenges for protein bioinformatics. Previously, we have introduced a method for protein surface shape representation using the 3D Zernike descriptors (3DZDs). The 3DZD enables fast structure database searches, taking advantage of its rotation invariance and compact representation. The 3DZD has been successfully applied for global protein surface shape comparison, local pocket shape comparison, protein docking prediction, and rapid small ligand molecule search. Here, we apply the 3DZD for comparing low-resolution structure data from the electron microscopy (EM). Two applications are presented. First, we use the 3DZD for rapid comparison for an EM density map of a protein structure to a database of EM data. We examined EM maps of varying resolutions and found that the method has good performance in identifying the structures of the same fold even for EM maps at a resolution of 15 Angstroms (Sael, Kihara , BMC Bioinformatics, in press). Next, we applied the 3DZD for fitting multiple component proteins into an EM map. The method integrates a multiple protein docking procedure and the 3DZD, which compares surface shape of docking conformation to the EM map. The multiple docking is performed by the Multi-LZerD algorithm, which starts by computing pairwise docking prediction of component chains by using the LZerD docking program, which our group have developed recently (Venkatraman et al., BMC Bioinformatics, 2010). Multi-LZerD combines the pairwise docking results generating a couple of hundreds solutions. Then the fitness of the multiple docking decoys and the EM map is quantified by using the 3DZD. Overall, we show that the 3DZD is powerful in comparing low-resolution structure data for comparison and multiple-docking guided by the low-resolution data.

**1751-Pos Board B661**
**Helex: an Evolutionary Tabu Search Strategy for the Identification of Helical Regions in cryo-Electron Microscopy Reconstructions**
**Mirabela Rusu**, Stefan Birmanns.
Cryo-electron microscopy (cryo-EM) enables the imaging of macromolecular complexes in near-native environments at resolutions that in many instances approach atomic level of detail. Already covering 9.8% of the EBI database for cryo-EM reconstructions, the maps at intermediate- to high-resolution (better than 8A) potentially allow the identification of secondary structure elements. Especially, alpha helices which frequently show consistent patterns in volumetric maps, may be annotated using pattern matching methods. Previously introduced approaches predict secondary structure elements in cryo-EM datasets by applying multi-step heuristics to characterize the geometric features of the volumetric data. Here, we introduce helex (Helix Extractor) - a novel technique for the identification of helical regions in cryo-EM data sets. Helex is a hybrid optimization technique that combines a genetic algorithm, a tabu-search strategy, and a one-dimensional iterative optimization to locate and characterize helical regions. Our method takes advantage of the stochastic nature of genetic algorithms to identify optimal placements for a template helix. These placements are then used to characterize the length of the helical region, using an adaptive one-dimensional search that allows suboptimal steps during the optimization. Moreover, the tabu-search strategy prevents further exploration of already characterized helical regions. The method was extensively evaluated using maps at various resolutions, sampling rates and system complexity. Helex reliably identified medium to large helices (> 6 amino acids) in both synthetic and experimental test cases, placing them frequently with root mean square deviations of 1A or less from the correct helical axes. Helex is distributed in our molecular modeling software Sculptor, freely available at http://sculptor.biomachina.org.

**1752-Pos Board B662**
**Scanning Transmission Electron Microscopy of Eukaryotic Cells in Liquid**
**Niels de Jonge**, Madeline J. Dukes, Elizabeth A. Ring, Diana B. Peckys.
We have recently introduced liquid scanning transmission electron microscopy (STEM) [1-3], a novel electron microscopy technique for the imaging of whole cells in liquid. Eukaryotic cells in liquid were placed in a microfluidic chamber with a thickness of ~5 μm enclosed between two ultra-thin electron-transparent windows. Images were obtained by scanning a focused electron beam over the sample and detecting the elastically scattered electrons with an annular dark field detector. On account of the atomic number (Z) contrast of the STEM, nanoparticles of a high-Z material, e.g. gold, can be detected within the background signal produced by a micrometers-thick layer of a low-Z liquid, e.g. water, or cellular material. Nanoparticles specifically attached to proteins can be used to study protein distributions in whole cells in liquid, similar as proteins tagged with fluorescent labels can be used to study cellular function with fluorescence microscopy. COS7 fibroblast cells were labeled with gold nanoparticles conjugated with epidermal growth factor (EGF). Intact fixed cells in liquid were imaged with STEM with a spatial resolution of 4 nm and a pixel dwell time of 20 μs [1]. In addition, proteins were labeled with quantum dots (QDs), fluorescent nanoparticles visible both with light- and with electron microscopy. STEM images showed individual QDs, and their locations were correlated with the cellular regions, as imaged with fluorescence microscopy [4]. Liquid STEM also obtains contrast on the native structure of live cells. Wild type S. pombe cells and several mutants were imaged with a spatial resolution of 30 nm.
[1] N. de Jonge et al., Proc. Natl. Acad. Sci., 106, 2159 (2009).
[2] D.B. Peckys et al., PLoS One, 4, e8214 (2009).
[3] E.A. Ring & N. de Jonge, Microsc. Microanal. 16, 622 (2010).
[4] Dukes et al., ACS Nano 4, 4110 (2010).

**1753-Pos Board B663**
**Orbweaver: A New Graphical User Interface for Particle Selection in Single-Particle Reconstruction with Cryo-Em**
**Robert Ezra Langlois**, Joachim Frank.
Computer algorithms play a key role in nearly every step of single-particle reconstruction of data collected by cryo-electron microscopy (cryo-EM). A three-dimensional (3D) reconstruction of a macromolecular complex requires tens of thousands of 2D experimental projections to be handpicked from hundreds of film micrographs (or thousands of CCD micrographs). To handle this deluge of data, semi-automated particle-picking algorithms have been developed to assist in locating particles on a micrograph, but only in a few easy cases do these algorithms perform a significant portion of the work. In most cases, particle picking comprises most of a researcher's time in building a 3D reconstruction, often taking weeks to months.
Two current trends in cryo-EM substantially increase the load on particle picking. One is the need to improve the resolution of the density maps, to aid in the interpretation in terms of atomic structures. The other is the need to characterize heterogeneous samples by an entire array of reconstructions, one for every class encountered.
We are working on optimizing every step of the particle-picking process, developing new machine-learning algorithms and designing a better graphical user interface. To this end, we have recently developed an unsupervised learning algorithm to extract potential particles from the micrograph, in combination with an active learning algorithm aimed to drastically reduce the number of manually selected particles required to build an accurate supervised classifier. Since active learning requires a new type of manual particle picking interface, we now present a new graphical user interface to improve both the speed and accuracy in manual selection through active learning.

**1754-Pos Board B664**
**Using Sculptor for the Simultaneous Assembly of Atomic Components into Volumetric Maps**
**Stefan Birmanns**.
An important challenge, especially from a computational standpoint, is the integration of data from different biophysical sources, by providing efficient algorithms and user-friendly software tools. We propose a new, integrated software system that combines extensive visualization capabilities with molecular

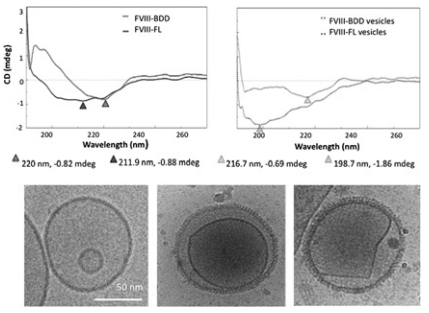
modeling algorithms for the analysis of multi-scale data sets. Such an integrated package has various advantages - the user-interface not only eases the typically steep learning curve of pure algorithmic techniques, but it also permits an instantaneous analysis and post-processing of the results. In 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