International Symposium on Cryo-EM 3D Image Analysis

March 12 – 15, 2014
Granlibakken Conference Center & Lodge
Lake Tahoe, CA USA

Program Schedule
Abstracts
List of Participants

International Symposium on Cryo-3D Image Analysis

March 12-15, 2014

The Symposium focuses on cutting edge techniques for 3D image analysis in Cryo-EM. Novel algorithm and methodological developments are required for obtaining a comprehensive and quantitative molecular understanding of nano-machines, complexes, and organelles within the cell. The meeting includes a mix of established investigators, postdocs and students to actively discuss existing and new algorithms and to discover what improvements will be required to achieve the desired biological results. Posters will be presented throughout the symposium and several abstracts have been selected from among the posters for short presentations.

Dorit Hanein, Chair
Sanford-Burnham Medical Research Institute

Steven J. Ludtke, Co-Chair
Baylor College of Medicine

Organizing Committee

Wah Chiu
Baylor College of Medicine

Edward H. Egelman
University of Virginia Medical School

Ben Hankamer
University of Queensland

Masahide Kikkawa
University of Tokyo

David N. Mastronarde
University of Colorado

Pawel A. Penczek
University of Texas-Houston Medical School

Henning Stahlberg
University of Basel

Fei Sun
Chinese Academy of Sciences

Niels Volkmann
Sanford-Burnham Medical Research Institute

Meeting Coordinator

Laura Nelson
Sanford-Burnham Medical Research Institute

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Program

WEDNESDAY, MARCH 12

4:00-6:00 Meeting Registration / Check-in

5:00-6:00 Reception (Cedar House)

6:00-7:30 Dinner (Granhall)

7:30-7:40 Welcome and Introduction, **Dorit Hanein**, Program Chair
Professor of Bioinformatics and Systems Biology, Sanford-Burnham Medical Research Institute, La Jolla, USA

7:40-8:40 **David DeRosier, PLENARY TALK**
Professor Emeritus of Biology, Brandeis University, Waltham, USA
If you don't know where you're going, you might not get there

8:40-11:00 Cash bar (Cedar House)

THURSDAY, MARCH 13

7:00-8:00 Breakfast (Granhall)

Session I (Morning), Map Validation

8:00-8:05 Introduction, **Catherine Lawson**, Session Chair
Associate Research Professor, Rutgers University, New Brunswick, USA

8:05-8:30 **Wah Chiu**, Platform Speaker
Professor of Biochemistry & Molecular Biology, Molecular Virology & Microbiology, Molecular Physiology & Biophysics, and Molecular & Cell Biology, Baylor College of Medicine, Houston, USA
Validating map and model at near atomic resolution

8:30-8:55 **Michael Radermacher**, Platform Speaker
Professor of Molecular Physiology and Biophysics, University of Vermont, Burlington, USA
Structures, ambiguities and methods

8:55-9:20 **Richard Henderson***, Platform Speaker
Programme Leader, MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, UNITED KINGDOM
Using tilt pairs and high resolution noise substitution for map validation

9:20-9:45 **Michael G. Rossmann**, Platform Speaker
Hanley Professor of Biological Sciences, Purdue University, West Lafayette, USA
Limiting the structural parameters to a reasonable number in the interpretation of a cryoEM map

9:45-10:00 **Javier Vargas**, Short Talk
Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN
Efficient initial volume determination using RANSAC algorithm

10:00-10:30 Coffee/Tea Break and **Group Photo**

Session II, Single Particle Approaches to Helical Symmetry and 2D Crystals

10:30-10:35 Introduction, **Masahide Kikkawa***, Session Chair
Professor of Cell Biology & Anatomy, Graduate School of Medicine, The University of Tokyo, JAPAN

10:35-11:00 **Edward H. Egelman**, Platform Speaker
Professor of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, USA
Cryo-EM of helical polymers at near-atomic resolution

11:00-11:25 **Akihiro Narita***, Platform Speaker
Associate Professor of The Structural Biology Research Center and Division of Biological Science, Graduate School of Sciences, Nagoya University, JAPAN
Structural analysis for the actin filament in vitro and in vivo

11:25-11:50 **Henning Stahlberg***, Platform Speaker
Professor of C-CINA, Biozentrum, University of Basel, SWITZERLAND
Image processing for 2D crystals

*Speakers from outside the U.S.

11:50-12:05 **Alexis Rohou**, Short Talk
HHMI Janelia Farm, Virginia, USA
Frealix: model-based refinement of helical filament structures from electron micrographs

12:15-1:15 Lunch (Granhall)

Poster Session I (Afternoon) – Mountain Lake Room

3:50-4:30 Vendor Roundtable

4:30-6:00 Poster Session I

6:00-7:30 Dinner (Granhall)

Session III (Evening), DDD Movie Processing – Alignment, dose weighting…

7:30-7:35 Introduction, **Fei Sun***, Session Chair
Professor of Structural Biology and Leader & Chief Scientist of Center for Biological Imaging, Institute of Biophysics, Chinese Academy of Sciences, CHINA

7:35-8:00 **Benjamin Bammes**, Platform Speaker
Molecular & Cell Biology, Baylor College of Medicine, Houston, USA
Methods to account for dynamic specimen processes in TEM using a Direct Detection Device

8:00-8:25 **Yifan Cheng**, Platform Speaker
Associate Professor of Biochemistry and Biophysics, University of California, San Francisco, USA
Motion correction with the K2 Summit camera

8:25-8:50 **Fred J. Sigworth**, Platform Speaker
Professor of Cellular and Molecular Physiology, Yale University, New Haven, USA
High-precision alignment in DDD movies

*Speakers from outside the U.S.

8:50-9:05 **Jose-Maria Carazo**, Short Talk
Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN
Alignment of Direct Detector Device micrographs using a local least-squares approach

9:05-11:00 Cash bar (Cedar House)

FRIDAY, MARCH 14

7:00-8:00 Breakfast (Granhall)

Session IV (Morning), Tomogram Segmentation / Annotation and Filtration

8:00-8:05 Introduction, **David N. Mastronarde**, Session Chair
Professor and Co-director of Boulder Laboratory for 3D EM of Cells, Department of MCD Biology, University of Colorado, Boulder, USA

8:05-8:30 **Niels Volkmann**, Platform Speaker
Associate Professor of Bioinformatics and Systems Biology, Sanford-Burnham Medical Research Institute, La Jolla, USA
Automatic feature detection in electron cryo-tomograms

8:30-8:55 **Ben Hankamer***, Platform Speaker
Professor, Institute of Molecular Bioscience
The University of Queensland, AUSTRALIA
Development of processes for edge detection and tomogram segmentation

8:55-9:20 **Achilleas Frangakis***, Platform Speaker
Prof. Dr. of Cluster of Excellence Macromolecular Complexes, Institute of Biophysics, Goethe University, Frankfurt, GERMANY
Quality assessment of electron tomograms

9:20-9:45 **Friedrich Förster***, Platform Speaker
Group Leader, Department of Structural Biology, Max-Planck Institute for Biochemistry, Martinsried, GERMANY
Structural analysis of macromolecular complexes by cryo-electron tomography

*Speakers from outside the U.S.

9:45-10:00 **Jing He**, Short Talk
Computer Science, Old Dominion University, Virginia, USA
Detection of Beta-strands from the cryo-EM density maps at medium resolutions

10:00-10:30 Coffee/Tea Break

Session V , Single Particle – Resolution Barriers (ewald sphere, beam tilt, oversampling, tomography averaging, etc.)

10:30-10:35 Introduction, **Steven Ludtke**, Session Chair
Professor, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA

10:35-11:00 **Wen Jiang** Platform Speaker
Associate Professor of Markey Center for Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, USA
High resolution refinement: beyond 5 parameters

11:00-11:25 **Z. Hong Zhou**, Platform Speaker
Professor of Microbiology, Immunology & Molecular Genetics, Director of Electron Imaging Center for Nanomachines , University of California, Los Angeles, USA
"Super resolution" cryoEM reconstructions, ions and intermediate states

11:25-11:50 **Sriram Subramaniam**, Platform Speaker
Senior Investigator Head of Biophysics Section in the Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH Bethesda, USA
Application of sub-volume averaging and related methods in cryo-electron tomography

11:50-12:15 **Marin van Heel***, Platform Speaker
Professor of Cryo-EM Data Analysis at the Faculty of Science, Centre for Electron Nanoscopy (NeCEN), Leiden University of the Netherlands , Leiden Netherlands
The highs and lows of atomic-resolution cryogenic Electron Microscopy

*Speakers from outside the U.S.

12:15-12:40 **Kenneth A. Taylor** , Platform Speaker
Donald L.D. Caspar Professor of Biological Sciences, Institute of Molecular Biophysics, Florida State University, Tallahassee, USA
Discovering unexpected structures in different contexts using multivariate data

12:40-12:55 **Alberto Bartesaghi**, Short Talk
Laboratory of Cell Biology, Center for Cancer Research, NCI/NIH, Maryland, USA
Use of direct electron detectors to achieve near-atomic resolution of small (<500 kDa) complex by cryo-EM

12:55-1:55 Lunch (Granhall)

Poster Session II (Afternoon) – Mountain Lake Room

4:30-6:00 Poster Session II

6:00-7:30 Dinner (Granhall)

Session VI (Evening), Heterogeneous Data (could apply to both 2-D and 3-D data)

7:30-7:35 Introduction, **Jose-Maria Carazo***, Session Chair
Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

7:35-8:00 **Steven Ludtke**, Platform Speaker
Professor, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, USA
Classification accuracy for individual particles from a heterogenous population

8:00-8:25 **Holger Stark***, Platform Speaker
Professor, Max-Planck Institute for Biophysical Chemistry, GERMANY
Camera versus Biochemistry

*Speakers from outside the U.S.

8:25-8:50 **Dmitry Lyumkis**, Platform Speaker
National Resource for Automated Molecular Microscopy and Department of Cell Biology, The Scripps Research Institute, La Jolla, USA
Multi-step 3D classification facilitates identification and characterization of cofactors involved in translation quality control

8:50-9:15 **Pawel Penczek**, Platform Speaker
Professor of Biochemistry & Molecular Biology and Director of Structural Biology Imaging Center, The University of Texas-Houston Medical School, Houston, USA
Ab initio EM map validation: convergence and parameter consistency

9:15-9:30 **Slavica Jonić**, Short Talk
IMPMC, Sorbonne University, French National Centre for Scientific Research – CNRS, Paris, FRANCE
HEMNMA: Hybrid Electron Microscopy Normal Mode Analysis to fully explore macromolecular dynamics

9:30-9:35 Closing Remarks, **Steve Ludtke**, Program Co-Chair

9:35-12:00 Cash Bar/Social Time (Cedar House)

SATURDAY, MARCH 15

7:00-9:00 Breakfast (Granhall)

Departures

*Speakers from outside the U.S.

Short Talk Abstracts

Efficient initial volume determination using RANSAC algorithm
Javier Vargas, Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

Frealix: model-based refinement of helical filament structures from electron micrographs
Alexis Rohou, HHMI Janelia Farm, Virginia, USA

Alignment of Direct Detector Device micrographs using a local least-squares approach
Jose-Maria Carazo, Instruct Image Processing Center, Spanish National Center for Biotechnology CNB-CSIC, Madrid, SPAIN

Detection of Beta-strands from the cryo-EM density maps at medium resolutions
Jing He, Old Dominion University, Virginia, USA

Use of direct electron detectors to achieve near-atomic resolution of small (<500 kDa) complex by cryo-EM
Alberto Bartesaghi, Center for Cancer Research, NCI/NIH, Maryland, USA

HEMNMA: Hybrid Electron Microscopy Normal Mode Analysis to fully explore macromolecular dynamics
Slavica Jonić, Sobornne University,French National Centre for Scientific Research – CNRS, Paris, FRANCE

Efficient initial volume determination using RANSAC algorithm

J. Vargas*[1], R. Marabini[2], J. M. Carazo[1], C. O. S. Sorzano[1]

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[2] *Escuela Politécnica Superior, Universidad Autónoma de Madrid,* C/ Francisco Tomás y Valiente, 28049 Cantoblanco (Madrid), Spain

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In this work, we present a fast and efficient approach to obtain a reliable low resolution initial volume of a macromolecule. The proposed method is based on an initial non-lineal dimensionality reduction approach, which permits to automatically select representative very small sets of class average images that capture most of the structural information of the particle under study. These reduced sets are then used to generate multiple volumes from random orientation assignments. The best volume is determined from these guesses using a random sample consensus (RANSAC) approach. We have tested our proposed algorithm with simulated and experimental data obtaining very satisfactory results under the low signal to noise conditions typical of cryo-electron microscopy. The algorithm is freely available as part of the Xmipp 3.1 package [http://xmipp.cnb.csic.es].

Keywords: Initial Volume; Single Particle Analysis; Electron Microscopy

Frealix: model-based refinement of helical filament structures from electron micrographs

Alexis Rohou [a,b], Nikolaus Grigorieff [a,b]

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[b] Janelia Farm Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA

The structures of helical protein filaments can be derived from cryo-EM images by treating short segments of filaments as independent “single particles”. This approach can yield near-atomic resolutions for rigid and well-ordered filaments and sub-nanometer resolutions for more flexible filaments. However, in the case of thin and flexible filaments, such as some amyloid-β (Aβ) fibrils, it can fail because helical segments may be curved or otherwise distorted and their alignment inaccurate due to low contrast in the micrographs. We developed new software called Frealix that allows us to use arbitrarily short filament segments during alignment to approximate even high curvatures. All segments in a filament are aligned simultaneously with constraints that ensure that they connect to each other in space to form a continuous helical structure. We have benchmarked the algorithm against datasets of Aβ(1-40) fibrils and tobacco mosaic virus (TMV), and match results obtained in earlier work. However, in the case of Aβ(1-40) fibrils, we are also able to obtain reliable alignments and ~8-Å reconstructions from curved filaments. Our algorithm also offers a detailed characterization of filament deformations in three dimensions and enables a critical evaluation of the worm-like chain model for biological filaments.

Alignment of Direct Detector Device micrographs using a local least-squares approach

V. Abrishami*[1], J. Vargas*[1], R. Marabini[2], J. M. Carazo[1], C. O. S. Sorzano[1]

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Abstract:

Direct Detector Devices (DDDs) represent a big step forward for Three-dimensional electron microscopy (3DEM). As expected, the quality of these DDD images/frames is much better than the one obtained from CCD's, but, additionally, their fast image/frame acquisition rate allows the study of how frozen hydrated specimens behave as a function of electron dose/rate. Indeed, biological specimens in a solid matrix of amorphous ice do move as they are being imaged, resulting in Beam Induced Movement (BIM). It turns out that BIM is a very serious experimental "resolution barrier" in cryo-electron microscopy. However, the alignment of the different micrographs/frames is not an easy task, and several methods have been proposed. In this work, we present an alignment method to combine DDD frames based on a local least squares approach that can further extend the resolution of the combined image, besides providing a valuable tool for a better characterization of BIM. The algorithm is freely available as part of the Xmipp package [http://xmipp.cnb.csic.es].

Keywords: Direct Detector Devices; Single Particle Analysis; Electron Microscopy

Detection of Beta-strands from the Cryo-EM Density Maps at Medium Resolutions

Dong Si & **Jing He**
Department of Computer Science, Old Dominion University, Norfolk, VA

Although more and more near atomic structures have been derived from high-resolution density maps that are obtained from the electron cryo-microscopy (cryo-EM), it is still challenging to derive the atomic structures from the density data at the medium resolutions such as 5-10Å. The major secondary structure features such as α-helices and β-sheets can be computationally detected from such cryo-EM density maps. However, a critical piece of information for modeling the atomic structures is missing, since there are no tools to detect β-strands from the cryo-EM maps at the medium resolutions. β-strands are visible only when the resolution is better than 4.7Å, the approximate spacing of β-strands. We propose a new method, *StrandTwister*, to detect the β-strands from cryo-EM density maps at the medium resolutions. It does not require the visualization of β-strands and therefore can be used for density maps at lower resolutions. *StrandTwister* has been tested using 100 sheets simulated to 10Å resolutions and 40 sheets computationally detected from the cryo-EM density maps at 4.2-7.4Å resolutions. Although the experimentally-derived cryo-EM maps contain errors, *StrandTwister* was able to detect 82.4% of the β-strands with an overall 1.65Å 2-way distance between the detected and the observed β-traces, if the best of the top ten detections is considered. *StrandTwister* detected the four strands of a β-sheet of gp10 protein from the bacteriophage epsilon15 cryo-EM density map at 7.3Å resolution. Our results suggest that it is possible to detect β-strands from the cryo-EM density data at medium resolutions when the separation of β-strands is not visible.

Use of direct electron detectors to achieve near-atomic resolution of small (<500 kDa) complex by cryo-EM

Alberto Bartesaghi[1], Doreen Matthies[1], Soojay Banerjee[1], Alan Merk[1] and Sriram Subramaniam[1]

[1]Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

The adoption of direct electron detectors in cryo-EM has unlocked the prospects of achieving near-atomic resolution of complexes in a size range that was out of reach as recent as a year ago. In addition to the improved DQE characteristic of these detectors, the ability to acquire dose fractionated series of exposures provides many opportunities to improve image quality and accuracy of image processing routines in single-particle cryo-EM. While the most obvious benefit is the ability to correct for beam induced motion during the course of an exposure, intermediate frames may also be used to improve accuracy of CTF estimation and minimize radiation damage effects while still providing enough low resolution contrast to accurately align the images. Taking advantage of these features, we solved the structure of Escherichia coli β-galactosidase (~ 465 kDa) at ~ 3.2 Å resolution by single particle cryo-electron microscopy. The majority of the side- chains, the N-termini, and the geometry of the active sites, including a catalytic Mg2+ ion, can be clearly discerned in the density map.

HEMNMA: Hybrid Electron Microscopy Normal Mode Analysis to fully explore macromolecular dynamics

Qiyu Jin[1], Carlos Oscar S. Sorzano[2], Jose Miguel de la Rosa-Trevín[2], José Román Bilbao-Castro[3], Rafael Núñez-Ramírez[4], Oscar Llorca[4], Florence Tama[5], and **Slavica Jonić**[1,*]

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Present address: Qiyu Jin, Institute of Image Processing and Pattern Recognition, Shanghai Jiao Tong University, 200240 Shanghai, China; Rafael Núñez-Ramírez, Instituto de Estructura de la Materia, IEM-CSIC, 28006 Madrid, Spain.

Abstract: Structural changes are critical for biological functions of proteins and describing conformational changes in large macromolecular complexes is a major challenge. To fully allow studying gradual conformational changes, we have recently developed Hybrid Electron Microscopy Normal Mode Analysis (HEMNMA) [1]. This method combines transmission electron microscopy (EM), normal mode analysis (NMA), and image analysis [1]. Normal modes are used within HEMNMA to elastically align EM images with a reference structure, in order to determine the conformations present in images and evaluate their pertinence. HEMNMA allows analyzing gradual changes more extensively than other EM methods, as HEMNMA gives full dynamics while other EM methods give only a few conformations. The computed conformational distribution allows modeling of transition pathways. This poster shows examples of HEMNMA applications and our current work on building a user-friendly graphical interface to the method within Xmipp 3.1.

[1] Qiyu Jin, Carlos Oscar S. Sorzano, José Miguel de la Rosa-Trevín, José Román Bilbao-Castro, Rafael Núñez-Ramírez, Oscar Llorca, Florence Tama, Slavica Jonić, “Iterative Elastic 3D-to-2D Alignment Method Using Normal Modes for Studying Structural Dynamics of Large Macromolecular Complexes,” *Structure*, Available online 6 February 2014, ISSN 0969-2126, http://dx.doi.org/10.1016/j.str.2014.01.004.

Poster Abstracts

Posters will be displayed throughout the conference at all times.

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5. Cardone, Giovanni	25. Llaguno, Marc C.
6. Chang, Yi-Wei	26. Louder, Robert
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18. Kaushal, Prem	38. Zhou, Hong
19. Rohou, Alexis*	49. Bartesaghi, Alberto*
20. Vargas, Javier*	40. Carazo, Jose-Maria*
	41. Jonić, Slavica*
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*see Short Talk Abstracts

A structural perspective of EspB, substrate of ESX-1 secretion in Mycobacteria

Pavel Afanasyev[1,2], Musa Sani[1], Florence Pojer[3], Nicole van der Wel[1], Stewart Cole[3], Marin van Heel[2] and Peter J. Peters[1]

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A novel secretion system designated as type VII secretion (T7S) system was recently identified in mycobacteria. Once internalized by macrophages, this secretion system is indispensable to Mycobacterium tuberculosis, which uses it to secrete substrates that aid its escape from phagosomes to the cytosol leading to host cell death. Strains that lack the locus encoding this secretion system are not able to escape from the phagosome and are subsequently non-pathogenic. In an effort to unravel the functioning of the T7S system we performed single particle cryo-EM analysis on purified EspB, a substrate of the secretion machinery. EspB is itself secreted and acts as a chaperone making it a critical protein for the secretion machinery. The EspB oligomer was found to have a 7-fold symmetry organisation (“C7”). With this relatively low symmetry the EspB oligomer manages to form a cage-like quasi-spherical structure. We are currently obtaining higher resolution information in order to gain more mechanistic insight.

Optimizing TEM image quality using a Direct Detection Device

Benjamin Bammes

Multiple factors reduce the resolution and signal-to-noise ratio (SNR) of TEM images, including the microscope instrumentation, dynamic specimen processes (e.g., drift, beam-induced motion, charging, radiation damage, etc.), and inefficient electron detectors. With the goal of overcoming many of these obstacles, Direct Electron (San Diego, CA, USA) introduced the first large-format Direct Detection Device (DDD®) in 2008 (called the “DE-12”), followed by its newest 20-megapixel “DE-20” camera system in 2012. Performance evaluations of the DE-12 and DE-20 cameras show significantly improved performance compared to traditional electron detectors such as film or CCD cameras. In addition to improved detection efficiency and resolution, the architecture of DDD cameras allows for continuous streaming of unbinned full-frame images at >30 frames per second, with no dead time between consecutive frames. This “movie” acquisition provides a large field-of-view and improved resolution for visualizing dynamic specimens in experiments where this of interest, such as in Situ TEM. However, many TEM methods require a static specimen image, such as low-dose electron cryo-microscopy of biological specimens. In these methods, dynamic specimen processes are detrimental, causing either non-isotropic resolution loss (i.e., specimen drift) or overall degradation of the SNR in each image (e.g., beam-induced motion, charging, radiation damage, etc.). We have developed methods and algorithms including “motion correction” and “damage compensation” for exploiting the “movie mode” output from DDD cameras to correct for these dynamic processes and maximize the isotropic resolution and SNR of each image. We have demonstrated the benefits of these methods using several biological specimens.

Conformational Shift of a Major Poliovirus Antigen Confirmed by Immuno-Cryogenic Electron Microscopy

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‡ Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115 USA
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Small, interfacial conformational changes occur in some antigen-antibody interactions. Using cryogenic electron microscopy (cryo-EM), we have demonstrated such changes in a major antigenic site of a poliovirus capsid protein. During cell entry, native human poliovirus (160S particle) converts to a cell-entry intermediate (135S particle) and later to an RNA-released (80S) particle. By mixing particles with Fabs of the neutralizing C3 monoclonal antibody, we labeled the external BC loop of the capsid protein VP1 (residues 95–105) in the 160S and 135S states. We then determined three-dimensional structures by cryo-EM and enhanced their interpretability by fitting high-resolution coordinates of C3 Fab and the capsid proteins into the density maps. Binding of C3 to either 160S or 135S particles caused residues of the BC loop, located on the tip of a prominent peak known as the "mesa", to move by an estimated 5 Å. C3 antibodies are neutralizing and can bind bivalently. The orientation of the bound Fabs in our reconstructions suggests that C3 neutralizes poliovirus by binding two adjacent BC loops on the same mesa and inhibiting conformational changes in the viral capsid.

Published in *J. Immunol.* **191**, 884-891 (2013)

Architecture of the Large Subunit of the Mammalian Mitochondrial Ribosome

Daniel Boehringer[1], Basil J. Greber[1], Alexander Leitner[2], Philipp Bieri[1], Felix Voigts-Hoffmann[1], Jan P. Erzberger[1], Marc Leibundgut[1], Ruedi Aebersold[2,3], and Nenad Ban[1]

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Keywords: mammalian mitochondrial ribosome, 39S large ribosomal subunit, translation, ribosomal proteins, rRNA, polypeptide exit site, membrane association

Mitochondrial ribosomes synthesize a number of highly hydrophobic proteins encoded on the genome of mitochondria that are responsible for energy conversion by oxidative phosphorylation. The ribosomes in mammalian mitochondria have undergone massive structural changes throughout their evolution, including rRNA shortening and acquisition of mitochondrial-specific ribosomal proteins. Here, we present the three-dimensional structure of the 39S large subunit of the porcine mitochondrial ribosome determined by cryo-electron microscopy at 4.9 Å resolution [1]. We combined data from chemical crosslinking and mass spectrometry experiments to assign and build the structure of mitochondrial ribosomal proteins. The reduction in rRNA content is architecturally compensated for by mitochondrial ribosome-specific proteins, some of which bear structural and functional homology to soluble and membrane-associated proteins or enzymes. The structure provides detailed insight into the architecture of the polypeptide exit site which provides a specialized platform for the synthesis and membrane insertion of the highly hydrophobic protein components of the respiratory chain.

[1] Greber BJ *et al*. Nature. **505** (2014) p. 515

Approaching a 3D microscope: High-throughput single particle analysis oficosahedral viruses at the microscope

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Advancements in cryo-electron microscopy instrumentation and automated acquisition techniques have made it possible to record in a few days enough high quality images to potentially provide very detailed 3D structures, including ones that approach atomic resolution. Currently these images are analyzed and processed only after all microscopy is completed, by means of multi-step, semi-automatic procedures. This delay in the determination of the final structure represents a bottleneck not only for highthroughput analysis, but also for validating the experiments themselves. This is particularly true when imaging samples that, more typically than not, are reticent to high-resolution studies. To overcome this limitation, we are currently developing approaches to integrate single particle imaging with microscopy, intially focusing on icosahedral viruses, with the goal of determining a 3D reconstruction *while* the sample is in the microscope. We have identified two specific, but separate situations, where real-time, automatic processing could offer significant advantages over current practices: 1) while performing preliminary analysis of a sample at the microscope, it would help to glean as much structural information as possible, even at low resolution, from the few micrographs acquired; 2) after sample quality is assessed and deemed suitable for acquiring a large data set, a 3D reconstruction of the sample provided during acquisition, and continuously updated as more data are recorded, would help to drive the acquisition and to monitor the quality of the data in terms of their ability to generate a high resolution structure.

To address the challenges in the first scenario, we have developed AutoRTM, a program that, by integrating and automating all the processing, is able to compute a low-resolution 3D reconstruction from particle images in a single micrograph, typically in one minute or less. The advantage of this approach was demonstrated on two different samples, baboon reovirus and adeno-associated virus, where reconstructions obtained from just one micrograph of these samples were able to answer questions regarding the effect of preparation conditions on gross morphology and binding properties of antibodies, respectively.

For the second scenario, we have designed and implemented a software prototype, SPRINT, which can generate a high-resolution reconstruction by processing micrographs *while* they are acquired at the microscope. The system handles autonomously all the processing steps, from defocus estimation to particle picking, *ab-initio* modeling, and orientation determination. As a proof of concept we simulated the acquisition process for three available data sets of known good quality: aquareovirus GCRV and bacteriophages P22 and CUS-3. Results show that the processing of single micrographs can keep pace with an acquisition rate of about two images per minute. By the time the acquisition of all the micrographs was completed, the reconstructions had achieved resolutions of 4.4 (GCRV), 6.1 (P22), and 7.5 (CUS-3) Å, which could be further improved after a few additional iterations of orientation refinement. The quality of the final maps is very close to that obtained by expert users who processed semi-automatically all the micrographs, after the acquisition was completed and in a time frame of weeks.

The results presented will highlight some remaining challenges that must be overcome to achieve realtime imaging under more general conditions, but also will illustrate the advantages of coupling automated 3D image analysis with automated cryoEM data acquisition.

Correlated cryogenic photoactivated localization microscopy and electron cryotomography for *in situ* identification of molecular structures

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ABSTRACT

Electron cryotomography (ECT) produces three-dimensional images of intact cells in a near-native, "frozen-hydrated" state and is therefore a powerful technique for revealing cellular structures *in vivo*. Identifying objects within the complex environment of the cell can be challenging, however. One solution is to correlate cryotomograms with light micrographs in which cellular objects of interest are localized with fluorescent tags. Unfortunately, the significantly lower resolution of light microscopy in cryogenic conditions precludes precise localization of targets. Here we describe a super-resolution microscopy method, "cryo-PALM," to image samples with increased precision at 80K. We use correlated cryo-PALM and ECT to identify multiple and new conformations of the highly dynamic type VI secretion system sheath in the crowded cellular interior of *Myxococcus xanthus*.

Autofocussed 3D Classification of Cryo-electron Subtomograms

Yuxiang Chen

Subtomogram averaging in cryo-electron tomography (CET) is a powerful approach to resolve the structures of macromolecular complexes in situ on the nanometer scale. An important step in subtomogram averaging is classification of the subtomograms into homogeneous subsets, which allows assessing the conformational ensemble of the macromolecule under scrutiny and improving the resolution of subtomogram averages. Major challenges of subtomogram classification are the low signal-to-noise ratio of CET, the incomplete sampling of the tomogram and the unknown number of classes and quantities. Here we propose a clustering algorithm that bases on a similarity measure, which automatically focuses on the areas of major structural discrepancy between respective subtomogram averages. Furthermore, we incorporate a spherical harmonics based fast subtomogram alignment algorithm, which provides a significant speedup for the clustering. The evaluations on the simulated and experimental datasets indicate substantially increased classification accuracy of the presented method compared to state-of-the-art approaches.

Structural analysis of a helical plant virus

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Barley stripe Mosaic virus (BSMV) is a helical rod shaped plant virus of the Hordeivirus genus which is distantly related to the well studied Tobamovirus, Tobacco mosaic virus (TMV). BSMV virons are made up of both RNA and a 22.5kDa capsid protein. A recent structural study combining X-ray fibre diffraction and cryo-em has suggested that there are 23.2 capsid proteins per 25.6Å turn of the 224Å diameter virion with the asymmetric unit consisting of 116 subunits in 5 turns (Kendall and co-authors, 2013).

We have used cryo-em and single particle analysis to determine the structure of the BSMV virion. Helical parameters of BSMV were initially assessed using negatively stained virions: 22.2 subunits per 26.2Å turn with the asymmetric unit consisting of 111 subunits in 5 turns. However during data processing it became apparent that the structure, using these helical parameters, could not be refined. Using multivariate statistical analysis (MSA) it became clear that the data set contained two types of virions. After separation using MSA and competitive alignment it was possible to obtain 2 reconstructions of the BSMV viron at 4.1Å resolution. The wide BSMV virion (224Å diameter) did contain 22.2 subunits per 26.2Å turn but the narrow BSMV virion (216Å diameter) contained one subunit less per turn. In combination with homology modelling, model building and EM refinement we revealed the first near atomic resolution structure of the BSMV capsid protein. From this we can see that the BSMV capsid protein is very similar to that of TMV but contains a long insertion loop protruding from its hydrophobic core and has a much longer inner loop which is disordered in the cryo-em density.

Denoising Cryo EM Images by Representation Theoretic Decomposition

James Delfeld, University of Texas, Austin

We present a method of denoising Cryo-EM images without class averages. Collections of images are viewed as a section of a fiber bundle over the set of orientations, SO(3).

Representation theoretic considerations find a low dimensional subspace containing the signal of the section, the images are denoised by projecting onto this subspace. Under assumptions the method scales the snr linearly with the number of images and recovers true representations of the images.

Automated High-Resolution Ab Initio Single-Particle Cryo EM 3D Reconstruction Using SIMPLE

Hans Elmlund

We introduce a probabilistic ab initio 3D reconstruction algorithm that in a single step, without any user intervention or input other than the noisy individual particle images and the microscope- dependent parameters associated with them, can generate a high-resolution (< 10 A) map. The novelty of our approach lies in the stochastic optimization strategy applied to determine a sparse continuous distribution of weights that relates the continuous distribution of orientation and CTF parameters to the 3D reconstruction.

Towards Automated Single Particle Reconstruction for EPU

Erik Franken, FEI Company

We are aiming at easy to use processing workflows that automate the process of single particle reconstruction as much as possible, and that allow to already start processing micrographs already during the automatic acquisition with EPU (FEI's single particle acquisition software). To study the details of single particle reconstruction (SPR) techniques, we made a software prototype.

Using a number of predefined workflows that can be fully customized, we can generate *initial reconstructions* directly from data acquired by EPU. New micrographs from a running EPU session are processed on-the-fly, leading to improving results over time. This helps to increase experiment throughput by assessing the quality of the ongoing EPU run. The software has a graphical user interface to provide manual input or corrections, set parameters, and inspect intermediate and final results.

Algorithms 'under the hood' are mainly developed in-house. The CTF estimation is based on fitting of elliptical Thon rings using the Radon transform and CTF model fitting [Vulovic2013]. The particle picking is done by maximum local variance or template matching followed by a clustering step on calculated particle image features, to remove false positives. Classification is achieved MSA by steerable PCA or normal PCA [Ponce2011], followed by K-means or hierarchical classification. The final steps to generate the initial model reconstruction are under development. In the meantime, we experiment by wrapping third-party implementations (EMAN2 e2initialmodel).

The software is developed in python and C++, and is platform independent (Windows or Linux). It uses the EMX (Electron Microscopy Exchange) standard that is under development. First experiments have shown successful initial reconstructions on various particles such as Worm Hemoglobin and GroEL.

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Imaging of Isolated Disk Architecture from Rod Outer Segments of the Retina by Cryo-Electron Tomography

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Rod cells of the retina are critically involved in dim-light vision. These cells are composed of an inner segment, which contains organelles and the nucleus, and an outer segment, primarily responsible for phototransduction. Rod outer segments are made up of thousands of membrane disks filled with rhodopsin, the key protein involved in rod phototransduction. Understanding the architecture of these light-sensitive disks and the outer segment of rod cells is important for understanding the mechanisms involved in vision as well as problems of vision, including retinitis pigmentosa, a group of hereditary retinal degenerative diseases primarily affecting photoreceptor cells. While much is known on the general organization of these components, they have been difficult visualize in order to obtain higher-resolution details. In particular, details of the disk architecture and composition within the rod outer segment remain a significant challenge. To visualize these key cellular components and their organization, our lab utilizes cryo-electron tomography, a structural technique that involves taking several two-dimensional images at different angles of a sample in non-crystalline ice in order to get a three-dimensional tomogram reconstruction. Subtomogram averaging, a process of averaging data between and within tomograms, can enhance detailed structural information of the disks alone. This will give us a starting point to compare to structural changes of isolated disks in disease-state rod outer segments.

ATOM: A GPU Powered Package for Electron Tomography Reconstruction

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Electron tomography (ET) has been developed rapidly and applied widely in recent years, as it provides the prospects of reconstructing non-uniform cells or macromolecules in nano scale. We have developed a Graphic Processor Unit (GPU) powered software package, ATOM.

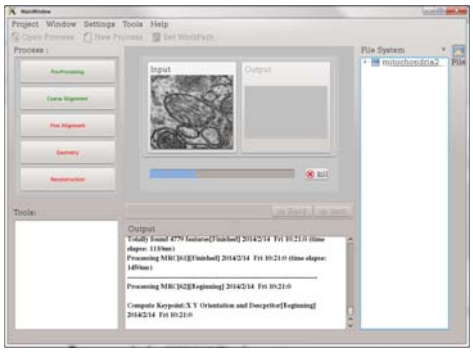

Fig1: GUI of ATOM. The procedure of reconstruction is managed as a project, to provide a user friendly interaction.

The main functions include: tilt series alignment, reconstruction parameters estimation, 3D reconstruction, and 2D image visualization etc.

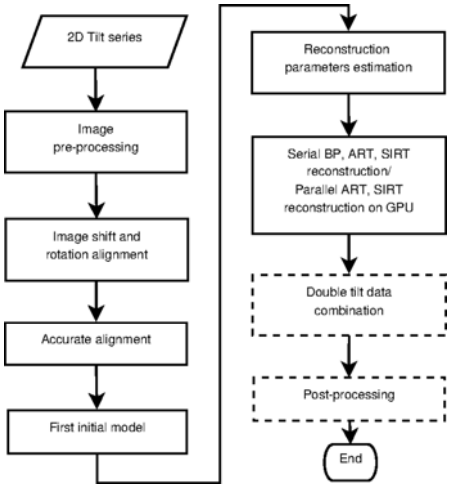

Fig2: The flowchart of ATOM; Procedures in solid frames have been implemented in ATOM.

Our package features in the following three aspects: First, in addition to cross-correlation alignment method, we provide a feature-based marker free alignment method [1], whose achieved accuracy is comparable with fiducial marker alignment and could finish a typical alignment (2048×2048) in 30 minutes. Second, we provide an adaptive simultaneous algebraic reconstruction technique (ASART) in which a modified multilevel access scheme and an adaptive relaxation parameter adjustment method are developed to improve the quality of the reconstructed 3D structure [2]. Third, our package support Back Projection, several iterative reconstruction methods, and their parallel versions on GPU platform. Furthermore, we propose a multilevel parallel strategy combined with an asynchronous communication scheme and a blob-ELLR data structure to efficiently perform blob-based iterative reconstructions on multi-GPUs [3].

Fig3 illustrates a pair of central x-y section of reconstruction of a mitochondria specimen, with the similar depth. We should notice that there are no fiducial markers here. The left is reconstructed by WBP with procedure of IMOD and the right is reconstructed by ASART with procedure of ATOM. We could find that in our result, the sharpness is similar in both reconstructions, while the reconstruction based on our method shows clearer details of membranes.

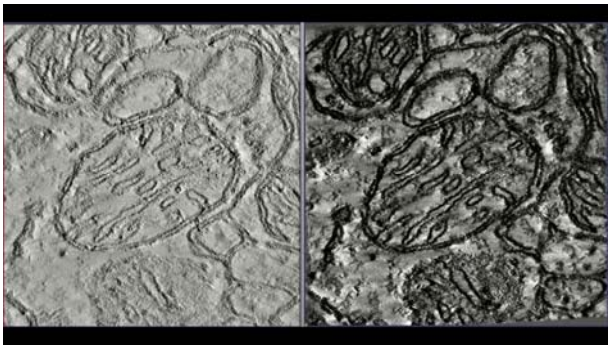

Fig3: A comparison of reconstruction. Left: output of IMOD (reconstructed by WBP); Right: output of ATOM (reconstructed by ASART)

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Edge detection and segmentation of macromolecules and organelles

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The intricate photosynthetic machinery of plants is designed to tap the huge solar energy resource of the sun (>1300 ZJ yr^{-1} photosynthetic reactive radiation) which is 2600x greater than our total global energy demand (the 0.5 ZJ yr^{-1}). Consequently single celled green algae (microalgae) which can be produced on non-arable land using saline or waste water sources are being developed as advanced solar fuel systems that do not compete with food production. The importance of such systems is that they can theoretically reduce CO_2 emissions, increase energy security and provide a secure basis for economic development.

The first step of all biofuel production is light capture and it's optimisation is therefore essential for the development of high-efficiency solar fuel production processes. Microalgae have approximately 20 light harvesting proteins associated with photosystems I and II which undergo complex and dynamic rearrangements in the thylakoid membranes in 4D (3D space and time). To fully understand this process requires tomographic cellular reconstructions at different time points and the ability to model macromolecular assemblies within them a ‘pseudo-atomic’ resolution.

We have been working on methods to integrate x-ray crystallographic data into single particle reconstructions and to dock these into the contours of macromolecular assemblies within cellular tomograms. This requires accurate 3-D edge detection of macromolecules and organelles.

All the images suffer from some degree of noise contamination. Algorithms capable of high quality edge detection are therefore required and these must be able to accurately distinguish between signal and noise, and be fast as well as robust in their ability to detect objects of differing contrast, shape and size. Here a range of algorithms developed to detect the contours of single particle macromolecules as well as macromolecules and organelles within tomograms will be presented. The performance, strengths and weaknesses of these algorithms will be summarised to illustrate the potential and limitation of using them for high throughput segmentation with the ultimate aim of generating a ‘pseudo-atomic’ resolution 3-D reconstruction of the photosynthetic machinery by docking x-ray crystallographic, single particle and tomographic data.

Whole Cell Cryo-Electron Tomography Reveals Mitochondria Divide by Budding

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Eukaryotes rely on mitochondrial division so that new generation of cells can acquire adequate number of mitochondria to maintain their physiological functions. Mitochondrial division has long been thought to occur by binary fission which has lately been considered to be mediated by Drp1[1]and ER[2]. However, the ultimate verification of the process of mitochondrial division has so far depended heavily on the visualization by fluorescent microscopy or conventional electron microscopy (EM). The resolution limit of Fluorescent microscopy (~200 nm) essentially prevents observers from seeing sufficient details as mitochondrial division involves very small distance (low to 0) between two separating mitochondrial bodies. Specimen in conventional EM usually goes through extensive chemical and mechanical treatments (such as fixation, dehydration, staining and sectioning) which could readily disrupt critical structure features that may define mitochondrial division. Meanwhile, in conventional electron microscopy, overlapping of three-dimensional (3D) structural information in two-dimensional (2D) projection images always causes blurring, embedding even loss of contrast of fine features. Moreover, knockout Drp1 cells still showed mitochondrial replication which suggests mitochondrial replication be charged by a process different from mitochondrial fission. In addition, there were contradicting results for Drp1 role in mitochondrial dynamics[3]. On the other hand, it has been gradually recognized that the process of mitochondrial fission does not create new mitochondrial mass thus jeopardizing the hypothesis itself. In order to solve the enigmas in mitochondrial division, I utilized Cryo-Electron Tomography to visualize mitochondrial division in frozen hydrated intact cells which were grown on EM grids. A large amount of three-dimensional reconstructed maps of cellular regions which presented mitochondria were obtained. Small mitochondria (less than 200 nm) were clearly observed protruding from large ones. Relatively larger mitochondrial buds of various sizes from ~200 nm to several hundred nanometers were also captured connected by long tethers or stalk-like structures to large mitochondria, which resembles the reproductive budding process of alpha-peoteobacteria[4]. As suggested by the genomics, mitochondria are believed to be evolved from alpha-peoteobacteria[5]. Therefore, the resemblance indicates the inheritance. High contrast densities observed inside each of the small mitochondrial buds are believed to be mitochondrial DNA. Taken together, the results have revealed mitochondria divide by budding. This work challenges prevailing opinion and textbook. It suggests the molecular mechanism regulating the mitochondrial reproduction is to be uncovered.

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Time-resolved cryo-EM study of ribosome subunit association by fast mixing-spraying

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The emergence of a high-throughput data processing pipeline and efficient classification algorithms has revived the idea of time-resolved cryogenic electron microscopy (cryo-EM), i.e., capturing time-dependent structures in a biological specimen using cryo-EM. The blotting method now routinely used to prepare cryo-EM specimen is sufficient only to study reactions that are longer than a second. Capturing faster reactions, in the sub-second range, has been a practical challenge, due to the requirement for depositing the specimen on the grid without blotting. To address this challenge, Lu et al. (2009) have developed a novel method to prepare time-resolved cryo-EM specimens, by using a nano-fabricated mixing-spraying chip. This chip allows a two-component reaction to proceed inside the chip for tens to hundreds of milliseconds (ms), which is then stopped by fast freezing. Here we improved the mixing-spraying method, and used it for studying ribosome subunit association, with the idea of capturing possible intermediates in this reaction. Ribosome subunit association is a pivotal step in translation initiation. Previous ensemble kinetic studies suggested that ribosome subunit association is a multi-step process in tens of millisecond time range, with the ribosomal inter-subunit bridges being formed successively. Using time-resolved cryo-EM, we were able to capture the association reaction in a pre-equilibrium state. By mixing the two subunits and reacting for 140 ms, we observed 45% of formed ribosome (in the total ribosomal large subunit-containing particles), compared with 89% of formed ribosome in a long-incubation control experiment. However, the proportion of the ribosomes in non-rotated (NR), and rotated (RT) conformations appeared stable from 140 ms to 75 min, suggesting that the associated ribosome may form in the non-rotated conformation first upon subunit association, then undergo conformational changes faster than the 140 ms time frame. Further we discuss the preliminary results from 60 ms reaction time for subunit association which will enhance our understanding on the conformational dynamics of this process. Thus, we have demonstrated that the mixing-spraying method of time-resolved cryo-EM is able to visualize the states of macromolecules in a reaction within a sub-second time frame. Many other biological processes, such as translation initiation, decoding, and ribosome recycling, can also be studied using time-resolved cryo-EM method.

Cryo-EM structure of the small subunit of the mammalian mitochondrial ribosome

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*equal contributions

Mitochondrial ribosome (mitoribosome) is a macromolecular machine that plays a central role in mitochondrial protein synthesis. Defects in mitochondrial translation are known to cause numerous human diseases. The mammalian mitoribosome contains significantly smaller sized ribosomal RNAs (rRNAs), but a large mass of mitochondrial ribosomal proteins (MRPs), including mito-specific amino acid extensions and insertions in MRPs that are homologous to bacterial RPs, and an additional 35 mito-specific MRPs. In this work, we present the cryo-EM structure of the small subunit of the mammalian mitoribosome (mito-SSU) at 7 Å resolution, as determined using 0.5 cutoff of Fourier Shell Correlation. The mito-SSU contains a 12S rRNA molecule and 31 MRPs. Structural features in the map allowed us to model the complete 12S rRNA, all of the 15 homologous MRPs of the mito-SSU and their mito-specific extensions. For the localization of 16 mito-specific MRPs, we have used a comprehensive approach including, immune-EM, multiple segmentation using Segger, by establishing a correlation between the size (voxel volume) of each segmented density and the molecular mass of the mito-specific MRPs, as well as by matching the structural features of each segmented density with the *ab initio* model of the MRP. This approach allowed us to position 12 of the 16 mito-specific MRPs within the mito-SSU map. We find that the mito-specific extensions/insertions in the homologous MRPs are involved in inter-MRP contacts as well as contacts with mito-specific MRPs, suggesting a stepwise evolution of the current mitoribosome structure. While most of the mito-specific MRPs and extensions of homologous MRPs are situated on the peripheral regions, they also contribute significantly to the formation of linings of the mRNA and tRNA paths, suggesting a tailor-made structural organization of the mito-SSU for the recruitment of mito-specific mRNAs most of which do not possess a 5’ leader sequence.

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Cutting edge: Collaboration gets the most out of software

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The SBGrid Consortium is a computing collaboration of over 240 structural biology labs at more than 75 academic research institutions and 3 companies in 17 countries. Consortium members benefit from automatic dissemination of a comprehensive collection of over 270 scientific software applications used in structural biology research, including more than 25 titles in the area of electron microscopy. By sharing the costs of research computing support among its members, the SBGrid Consortium expands access, facilitates communication, reduces costs and lowers barriers to biomedical computational research. In addition, SBGrid facilitates access to national cyber-infrastructure for large-scale computing via the XSEDE program. We offer tools for scientific software development and spearhead outreach, education, policy and advocacy efforts on behalf of the research computing community. SBGrid operates as a member supported, NIH-compliant non-profit service center based at Harvard Medical School.

Near-atomic resolution cryo-EM structure of Dengue serotype 4 virus

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Dengue virus (DENV), a mosquito-borne virus, is responsible for millions cases of infections worldwide. There are four DENV serotypes (DENV1 - 4). After a primary DENV infection, the antibodies elicited confer lifetime protection against that DENV serotype. However, in a secondary infection with another serotype, the pre-existing antibodies may cause antibody-dependent enhancement (ADE) of infection of macrophage cells leading to the development of the more severe form of disease, dengue hemorrhagic fever. Thus a safe vaccine should stimulate protection against all dengue serotypes simultaneously. To facilitate the development of a vaccine, good knowledge of different DENV serotype structures is crucial. Structures of DENV1 and DENV2 had been solved previously. Here we present a near-atomic resolution cryo-electron microscopy (cryo-EM) structure of mature DENV4. Comparison of the DENV4 structure with similar resolution cryo-EM structures of DENV1 and DENV2 showed differences in surface charge distribution, which may explain their differences in binding to cellular receptors such as heparin. Also, observed variations in amino acid residues involved in interactions between envelope and membrane proteins on the virus surface correlate with their ability to undergo structural changes at higher temperatures.

A Replisome Structure: Multiple Interactions Coordinate DNA Synthesis

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A model of the replication fork proposed by Bruce Alberts thirty years ago provides an elegant solution for coupling of leading- and lagging-strand synthesis. Here we present the first structure of a megadalton-sized functional replisome of bacteriophage T7 assembled on DNA resembling a replication fork. Structure of the complex consisting of DNA helicase, RNA primase, and two DNA polymerase molecules was determined by single-particle cryo-electron microscopy. The two molecules of DNA polymerase adopt different spatial arrangement at the replication fork reflecting their roles in leading- and lagging-strand synthesis. The structure reveals molecular mechanisms for coordination of leading- and lagging-strand synthesis, recycling of DNA polymerases, formation and release of the lagging-strand replication loop, and stabilization of the RNA primer on the lagging-strand for subsequent extension to an Okazaki fragment. Since mechanisms of DNA replication are highly conserved, the observations are relevant to other replication systems.

Finding the 3D variance-covariance density map in Cryo-EM

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[3]Howard Hughes Medical Institute

We discuss a way to estimate the 3D covariance matrix from Cryo-EM images. A recent approach [1] first estimates the Fourier transform of the covariance matrix and then solves its inverse Fourier transform. We instead attempt to do all the computations in the image domain, using a series expansion method with constraints. Our approach solves iteratively a system of equations and imposes constraints during the iterations. We discuss some of the advantages and limitations of this approach and show the results on a small example.

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Chemically Functionalized Carbon Films for Single Molecule Imaging

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We describe a new method to produce chemically functionalized carbon films ("ChemiC" films) for cryoEM imaging of biological complexes. This new method has four immediate advantages. First, the new method is modular and allows covalent attachment of different bioactive ligands to the surface, including Ni-NTA, Protein G and oligonucleotides. The introduced biomass is very minimal, almost negligible in many cases. The second is that for complexes smaller than one MDa, it allows ice thickness to be decreased to 20-40 nm without losing the capability of retaining enough macromolecular complexes for imaging, which reduces background noise from ice scattering significantly and allows us to visualize 200-400 kDa complexes easily. The third is that with high affinity ligands it enables the selective enrichment of biological complexes at (sub)-nanomolar concentrations, which are two to three orders of magnitude lower than that required for the conventional method. It thus allows the cryoEM imaging of those complexes that are difficult to produce in large quantities. Fourth, it enables the selection of biological complexes based on their specific functions, for example certain binding states or specific conformations, and may help decrease intrinsic heterogeneity. As a practical application, we used ssRNA-ChemiC grids to assemble a small biological complex (~240 kDa) for cryoEM imaging and calculated its 3D structure. Our results revealed a new mechanism of ssRNA-guided assembly of the active enzyme and an unconventional way for ssRNA to access the active site of the enzyme. We therefore believe that the ChemiC films will be useful for the cryoEM imaging of various biological samples.

Structural visualization of promoter recognition by human TFIID

Robert K. Louder[1], Michael A. Cianfrocco[1], Jie Fang[2], and Eva Nogales[2,3,4]
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The general transcription factor TFIID is the core promoter recognition factor and is responsible for transmitting gene regulatory cues from regulatory cofactors to the general transcription machinery during initiation of transcription by RNA polymerase II in eukaryotes. TFIID is a ~1.2 MDa complex comprised of the TATA-binding protein (TBP) and 13-14 TBP-associated factors (TAFs) capable of making sequence-specific interactions with promoter DNA. Here, single particle electron microscopy was used to examine the role of structural plasticity and modularity in the ability of TFIID to interact with diverse promoter architectures and regulatory cofactors. Three-dimensional maximum-likelihood classification and sorting of single-particle images of negatively-stained purified human TFIID complexes reveals a dynamic flexibility in TFIID's lobe A (comprising ~⅓ of the total mass of the complex) with respect to a conformationally rigid core comprised of lobes B and C. In TFIID's canonical state, lobe A is stably-anchored to lobe C. A large conformational rearrangement involving a >100 A translocation of lobe A allows the formation of a new interaction between lobes A and B on the opposite end of the BC-core. Similar analysis of purified TFIID-promoter complexes revealed a shift in conformational preference of TFIID towards the rearranged state in the presence of DNA. Addition of TFIIA to the TFIID-promoter complex resulted in further stabilization of the rearranged state and the appearance of additional density attached to TFIID's lobe B, agreeing with TFIIA's proposed role in stabilizing the DNA-bound conformation of TBP. The existence of various structurally and functionally distinct states of TFIID may play a role in the ability of TFIID to both recognize a range of promoter architectures and be targeted by an array of regulatory transcriptional cofactors.

EMAN 2.1
Faster, Simpler and with Validation

S. Murray, J. Flanagan, J. Montoya and **S. Ludtke**

We are pleased to announce EMAN2.1, the first major update to EMAN2. This version focuseson validation, automatic refinement with gold standard resolutions and dramatically simplifies data storage. Due to the large number of changes, the testing period of this release has been extended. Alpha releases have been available for the last 8 months and the first beta release will be out in the spring. The current versions are now quite stable and functional, and offer significant improvements over EMAN2.0. New features include:

- For EMAN2.0 users, the BDB data storage system is no longer used. Now uses (chimera compatible) HDF files and human readable text files for metadata.
- e2refine_easy - evaluates your data, autoselects most parameters, and provides a written analysis of your data and the refinement. “Gold standard” resolution automatically determined and maps automatically filtered.
- Tilt validation - Including GUI tilt-picker!
- Single Particle Tomography - Dramatically expanded and improved tools.
- Relion, Frealign - Use EMAN2 refinement to automatically seed refinements in other software for comparison.
- Speed - Typical refinement speed ~2-10x faster than EMAN2.0x due to parameter optimizations and algorithm efficiency.
- Direct-detector movie-mode support!
- New file formats - DM4, FEI Falcon, other improvements.
- 3-D Viewer - Rewritten with muli-model support, slices, annotations and much more. Animate sequences of isosurfaces.

Structural analysis of a helical plant virus

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Barley stripe Mosaic virus (BSMV) is a helical rod shaped plant virus of the Hordeivirus genus which is distantly related to the well studied Tobamovirus, Tobacco mosaic virus (TMV). BSMV virons are made up of both RNA and a 22.5kDa capsid protein. A recent structural study combining X-ray fibre diffraction and cryo-em has suggested that there are 23.2 capsid proteins per 25.6Å turn of the 224Å diameter virion with the asymmetric unit consisting of 116 subunits in 5 turns (Kendall and co-authors, 2013).

We have used cryo-em and single particle analysis to determine the structure of the BSMV virion. Helical parameters of BSMV were initially assessed using negatively stained virions: 22.2 subunits per 26.2Å turn with the asymmetric unit consisting of 111 subunits in 5 turns. However during data processing it became apparent that the structure, using these helical parameters, could not be refined. Using multivariate statistical analysis (MSA) it became clear that the data set contained two types of virions. After separation using MSA and competitive alignment it was possible to obtain 2 reconstructions of the BSMV viron at 4.1Å resolution. The wide BSMV virion (224Å diameter) did contain 22.2 subunits per 26.2Å turn but the narrow BSMV virion (216Å diameter) contained one subunit less per turn. In combination with homology modelling, model building and EM refinement we revealed the first near atomic resolution structure of the BSMV capsid protein. From this we can see that the BSMV capsid protein is very similar to that of TMV but contains a long insertion loop protruding from its hydrophobic core and has a much longer inner loop which is disordered in the cryo-em density.

Molecular mechanism of ESCRT-III filament formation

Marissa G. Saunders, John McCullough, Leremy A. Colf, Wesley I. Sundquist* and Adam Frost*

Dept. of Biochemistry, University of Utah

The Endosomal Sorting Complexes Required for Transport (ESCRT) machinery is a highly conserved, modular set of protein assemblies that can facilitate membrane deformation and fission toward the cytoplasm. ESCRT proteins function in a diverse set of pathways including the final separation of daughter cells during cytokinesis, the biogenesis of intralumenal endosomal vesicles in the multi-vesicular body pathway, and the budding of many enveloped viruses, including HIV. ESCRT-III subunits, which constitute the functional core of the ESCRT machinery, assemble into membrane-bound filaments that then constrict and sever membranes. An important step towards understanding how this occurs is to understand the molecular structure of the filaments. Many ESCRT-III proteins can form helical filaments in vitro, and low resolution electron cryomicroscopic (cryoEM) reconstructions of ESCRT-III assemblies have been published previously, but the resolution has not been sufficient to reveal the orientation of individual subunits, the interactions between subunits, or the mechanism of subunit activation. Here, we present a sub-4 Å cryoEM reconstruction of a heterocopolymer formed by two different ESCRT-III subunits, CHMP1B and the N-terminal domain of IST1 ($IST1_{NTD}$). This filament forms a one-start helix comprising distinct inner and outer strands of subunits. The CHMP1B subunits that form the inner strand adopt highly extended, “open” conformations whereas the $IST1_{NTD}$ subunits that form the outer strand adopt closed conformations that resemble the crystal structure of the IST_{NTD} monomer. Intersubunit interactions in the inner CHMP1B layer are more extensive than those in the outer $IST1_{NTD}$ layer, suggesting that filament constriction requires variations in contacts between the open, inner-layer subunits. By changing assembly conditions, we have also seen that the ESCRT filament can spontaneously spiral into cones, an observation with implications for the mechanism of membrane remodeling. We are currently developing models for filament constriction, and testing the consequences of interface mutations for $IST1_{NTD}$–CHMP1B tube assembly in vitro and IST1 functions in cytokinesis.

The Structure of the TLR4/MD2, Revealed by Cryo-electron Microscopy

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Toll-like receptor TLR4 is the cell-surface receptor primarily responsible for initiating the innate immune response to lipopolysaccharide (LPS), a major component of the bacterial cell envelope. Myeloid differentiation factor 2 (MD-2) associates with the extracellular domain of TLR4 and binds LPS. It was shown that LPS has strong specificity for TLR4/MD2 and causes dimerization of TLR4/MD2. Stability of the TLR4/MD2 hetero-tetramer is influenced by constraints inherent in the membrane-associated state. We studied the structure of heterodimers of membrane-bound TLR4/MD2 with LPS by cryo-EM. His-tagged ectodomain TLR4/MD2 was bound to liposomes containing metal-chelated lipids followed by plunge-freezing in liquid ethane. The flash frozen samples were imaged in a JEOL 2200FS electron microscope at 80,000x nominal magnification using a 4k x 4k CCD camera The particles have a mushroom appearance with the stalk attached to the membrane and mushroom cup being distant from the lipid bilayer. From ~12,000 images we have obtained 1500 classes, from which the best ~4000 particles images corresponding to side views were selected. This set was subjected to statistical analysis; 478 best classes were used for the final 3D reconstruction. The effective resolution of the map was 18 Å (0.5 FSC criterion).

We observed formation of unusual dimers of TLR4/MD2 that were different from crystal structures. These changes in formation of dimers were probably caused by presence of lipid membrane. The atomic model of TLR4-MD2 heterodimer (PDB 2Z64) was fitted into the EM map as rigid body. The overall organization of dimers (back-to-back) is similar to one of the schemes suggested in the originally but not observed so far experimentally. LPS was not visible in our reconstruction because of lower resolution. It is unclear if LPS was present in dimers since its binding site as determined by X-rays would be outside of the (TLR4/MD2) - (TLR4/MD2) interface.

Near-atomic resolution cryo-EM structure of a parasite ribosome -- the *Plasmodiumfalciparum* ribosome

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Plasmodium falciparum is a species of the Apicomplexan parasites and responsible for the most severe form of human malaria disease [1]. However, our knowledge of the parasite protein translation system, which is an absolute prerequisite for the design of highly specific and effective drugs targeting this essential process, is incomplete. The translation process is performed by ribosomes, complex macromolecules composed of RNAs and proteins. To date, few eukaryotic ribosome structures are available, however, and the structure of the ribosome in *Plasmodia* is still unknown.
The ribosomal RNA (rRNA) of *P. falciparum* is unique in several aspects. Secondary structure predictions of the 18S (small subunit) rRNA point to the unusual large size of several rRNA expansion segments. In addition, two types of *P. falciparum* rRNA genes are expressed specifically during different life stages, and such stage-specific expression patterns are not found in most eukaryotes [1].
Here, we present the near-atomic cryo-EM structure of the *P. falciparum* asexual-stage ribosomes at about 4.3Å resolution (gold-standard; FSC = 0.143). The reconstruction reveals the *P. falciparum*-specific rRNA expansion segments and the dynamic properties of the ribosome. We are currently in the process of building a comprehensive atomic model of the *P. falciparum* ribosome. The rich structural information revealed in the density map will fill a gap in current parasite studies and expand our understanding of the evolution of eukaryotic translation system. Additionally, the methodology used in this project will advance the limit of cryo-EM toward the atomic level of resolution and expand the application of structural biology to a broad range of specimens.

1. K. E. Jackson et al., Protein translation in Plasmodium parasites. Trends in parasitology 27, 467 (Oct, 2011

The Structure of the Actin-Aldolase Interaction Determined by Electron Tomography of 2-D Paracrystals

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Apicomplexan parasites such as plasmodium (malaria parasite) lack locomotive organelles and employ a unique form of locomotion called gliding motility to promote their migration across biological barriers and to power host-cell invasion and egress. In these organisms, aldolase is used to bind both F-actin and the cytoplasmic tails of adhesins such as TRAP (TRAPt) and this may facilitate bridging of the adhesins to the cytoskeleton during gliding motility.

We have used electron tomography and lipid monolayer technology to study the F-actin-aldolase complex. The actin rafts are unipolar, which is consistent with the *in vivo* situation. 3D volume alignment and classification reveals that the neighboring F-actins are cross-linked either by single aldolase tetramer or by a pair of tetramers within every 37 nm cross over distance. The aldolase-actin cross-links usually involve 3 instead of the possible 2 or 4 binding sites, which means that not all interactions are quasiequivalent. The distribution of aldolase crosslinks is demonstrated using “mapback” technology in which global averages of actin and aldolase or density maps generated from their atomic structures are individually placed into a model tomogram where the raw subtomograms were positioned. No obvious pattern in either single or paired crosslinks was observed.

The class averages of aldolase and actin were aligned to a reference generated from the corresponding atomic structures and the alignment parameters applied to the raw motifs in each class. In this way, each raw motif of aldolase is aligned to the reference generated from the pdb file. Then the reverse alignment parameters for each aldolase motif and actin subunit were applied to the corresponding atomic structure and thus an atomic model for each individual aldolase-actin contact could be generated. Combination of the actin and aldolase model represents the raw motifs with two actin filaments cross-linked by one or a pair of aldolase tetramers. The contact regions between aldolase and actin were then analyzed by a residue-to-residue distance calculation using VMD. The two contact regions on actin consists of residues 5-10, 20-30, 90-105 and residues 350-365. Aldolase residues 240-260, 200-210 constitute one contact region and residues 310-330, 45-60 constitute the other.

The aldolase-actin contacts are highly heterogeneous, but show some preferences. Some aldolase tetramers contact only a single actin filament at either one or two sites, an observation that can be used to determine the most likely contacts and their orientation. For example, for a single actin contact, there is a 5:2 preference for contact region one over a combination of regions one and two. The polarity of the interaction is distinct. On the contrary, there is only a 3:2 polarity preference in the aldolase contact site when crosslinking two neighboring actin filaments via a single contact on each actin subunit. Although the crosslinking appears chaotic, there is a pattern to the contacts that suggests that aldolase may be capable of performing its crosslinking role by rolling between contact sites as actin filaments slide in a dynamic system. This research supported by the NIH.

Camera Characteristics Determined A Posteriori from Large Image Data Sets

Marin van Heel

Cryo-EM is becoming the method of choice for elucidating the 3D structures of large macromolecular complexes. The NeCEN facility at Leiden University houses two of the worlds most advanced ‘KRIOS’ electron microscopes with the associated equipment and expertise. Our in-house research is aimed at obtaining sets of atomic-resolution 3D reconstructions (‘4D’ structures) from very large, noisy EM datasets. In this context, we found the standard a priori flat-field correction schemes to be insufficient for basic statistical reasons and we designed a straightforward a posteriori correction scheme that fully resolves the problem.

Toward High-accuracy *de Novo* Protein Structure Determination from Near-atomic-resolution Cryo-EM Maps

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De novo protein structure determination from near atomic-resolution (3.3-5 Å) electron density data is a challenging but important problem. At these resolutions, sidechain density is at best ambiguous, and at worst indiscernible; correctly assigning sequence into these maps is difficult. We present an automated *de novo* model building approach that uses representative local backbone conformations derived from local sequence, in order to simultaneously trace the backbone and assign sequence into near-atomic-resolution density maps. Monte Carlo sampling is then applied to identify a set of backbone segments consistent with each other and with maximum agreement to the data. High-confidence partial models are then produced, covering 30 to 80 percent of the full-length protein. The procedure described above may be run iteratively to improve coverage. On 8 experimental cryo-EM maps, varying in resolution from 3.3-4.8 Å, containing proteins of size 148-386 residues, our method yields partial models which, on average, have 60% coverage, with 1.0-2.5 Cα RMSd to the experimental structures in all cases. In many cases, these partial models may be further rebuilt and refined to completion using density-guided comparative modeling in Rosetta. In one such case – a 4.8 Å cryo-EM map of the 221-residue 20S proteasome α-subunit – we obtain a model with Cα RMSd of 1.09 Å and all-atom RMSd of 1.79 Å compared to the crystal structure. These results suggest it is possible to directly build atomic models from near-atomic resolution density maps. By using backbone conformations predicted from local sequence, we may accurately identify sidechains even at resolutions where sidechain density is ambiguous.

An atomic model of BMV using direct electron detection and real-space optimization

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Recent advances in cryo-EM have enabled structure determination of macromolecules at near-atomic resolution. However, *de novo* structure determination remains susceptible to model bias and overfitting, potentially yielding inaccurate structures even when resolution appears high. Here, we describe a complete workflow for data acquisition, image processing, all-atom modeling, and validation of a RNA virus, brome mosaic virus (BMV). Data was collected using “movies” with a direct electron detector in integrating mode with a cumulative exposure beyond the traditional radiation damage limit. Reconstruction based on randomly generated initial models yielded density maps at 3.3 and 3.8 Å resolution, with and without subunit averaging within an asymmetric unit, respectively. We used these maps to compute a *de novo* Cα backbone model, which was converted to an all-atom model and optimized with a newly implemented real-space refinement protocol. The validity of the final model was verified by its match with the density map and a previously published model from X-ray crystallography, as well as the internal consistency of models from independent maps. Furthermore, the final *de novo* atomic model ranks in the 99th percentile when compared to structures at an equivalent resolution using statistical scores routinely used in X-ray crystallography.

Tools for structure determination of small macromolecular complexes by cryo-electron microscopy

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The combination of hardware advances in transmission electron microscopes together with automated data collection and streamlined image processing routines have transformed the pace at which samples can be converted into 3D structures by single-particle cryo-EM. In this work, we present a number of tools to further systematize the process of data production both for the purpose of structure determination and validation. Automated tracking of instrument usage provides a rational basis for managing resources allowing continuous adaptation of microscope time allocation according to specific project needs. Automated data collection of large single-particle datasets using intermediate frames to take advantage of new direct electron detectors and tilt-pair data collection, enables effective operation in high-throughput production mode. Using these tools together with image processing routines to correct for beam induced motion and improve the quality of cryo-EM images, we determine and validate the structure of the 464-KDa β-galactosidase enzyme at ~6Å resolution.

ATOM: A GPU Powered Package for Electron Tomography Reconstruction

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Electron tomography (ET) has been developed rapidly and applied widely in recent years, as it provides the prospects of reconstructing non-uniform cells or macromolecules in nano scale. We have developed a Graphic Processor Unit (GPU) powered software package, ATOM.

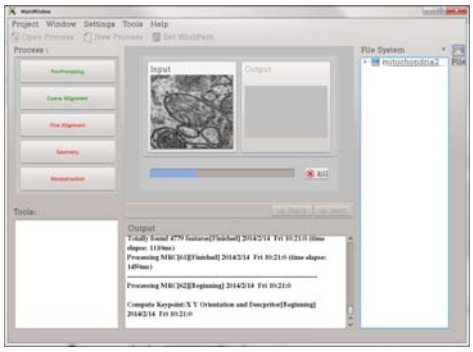

Fig1: GUI of ATOM. The procedure of reconstruction is managed as a project, to provide a user friendly interaction.

The main functions include: tilt series alignment, reconstruction parameters estimation, 3D reconstruction, and 2D image visualization etc.

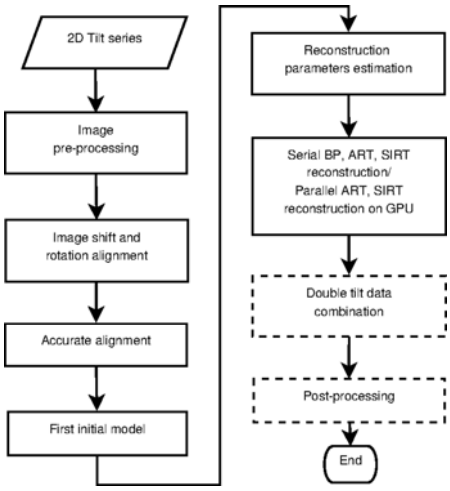

Fig2: The flowchart of ATOM; Procedures in solid frames have been implemented in ATOM.

Our package features in the following three aspects: First, in addition to cross-correlation alignment method, we provide a feature-based marker free alignment method [1], whose achieved accuracy is comparable with fiducial marker alignment and could finish a typical alignment (2048×2048) in 30 minutes. Second, we provide an adaptive simultaneous algebraic reconstruction technique (ASART) in which a

modified multilevel access scheme and an adaptive relaxation parameter adjustment method are developed to improve the quality of the reconstructed 3D structure [2]. Third, our package support Back Projection, several iterative reconstruction methods, and their parallel versions on GPU platform. Furthermore, we propose a multilevel parallel strategy combined with an asynchronous communication scheme and a blob-ELLR data structure to efficiently perform blob-based iterative reconstructions on multi-GPUs [3].

Fig3 illustrates a pair of central x-y section of reconstruction of a mitochondria specimen, with the similar depth. We should notice that there are no fiducial markers here. The left is reconstructed by WBP with procedure of IMOD and the right is reconstructed by ASART with procedure of ATOM. We could find that in our result, the sharpness is similar in both reconstructions, while the reconstruction based on our method shows clearer details of membranes.

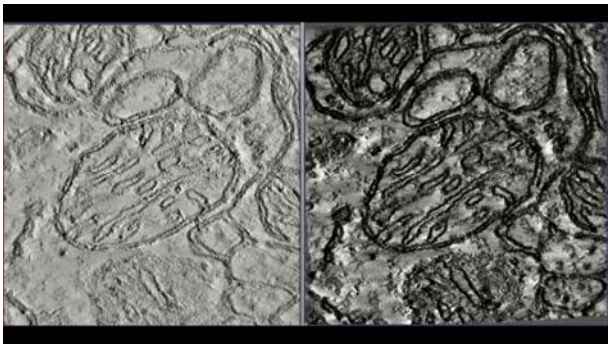

Fig3: A comparison of reconstruction. Left: output of IMOD (reconstructed by WBP); Right: output of ATOM (reconstructed by ASART)

[1] Han, R., Zhang, F., Wan, X., Fernandez, J-J., Sun, F., Liu, Z., (in publishing). A Marker-free Automatic Alignment Method Based on Scale-Invariant Features.

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[3] Wan, X., Zhang, F., Chu, Q., Liu, Z., 2012, High-performance blob-based iterative three dimensional reconstruction in electron tomography using multi-GPUs. BMC Bioinformatics, 13(Suppl 10):S4.

3.8 Angstrom resolution structure of microtubule by cryo-EM

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Microtubules (MTs) are hollow tubes assembled from α/ß tubulin heterodimers, which align in a polar head-to-tail manner. In living cells, the “plus-end” of MTs are highly dynamic and undergo rapid episodic switches between growth and shrinkage.

The intrinsic dynamic property of MTs is carefully regulated by a large family of Microtubule Associated Proteins (MAPs). Of particular importance is a set of MAPs called plus-end tracking proteins (+TIPs), which selectively localize to the MT growing ends and mediate interactions with various cellular structures such as kinetochores and cell cortex.

End-binding proteins (EBs) are the central hub of the +TIPs interaction networks. Previous studies (Surrey et al., 2011) showed that EBs and their yeast homologue Mal3 can recognize MTs at different nucleotide states, with high affinity for GTPγS MTs, which is believed to be a good mimic of the GTP-cap of MT.

With the start-of-the-art technology of K2 direct detector (Gatan Inc) and improved helical-reconstruction programs, we determined the cryo-EM structure of EB-decorated GTPγS microtubule to 3.8 Angstrom resolution, which allowed us to de novo build a complete atomic model of microtubule at high level of accuracy. This model is a big step towards our detailed understanding of the two key questions in microtubule biology, i.e. the molecular mechanism of MT dynamic instability and MT +TIPs’ end tracking behavior.

Biochemical and structural characterization of an archaeal flagellum

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The Archaea produce a variety of cell motility and adhesion surface apparatus, such as pili, hami, cannulae, and flagella which are genetically and biochemically unique to this domain of life. To explore the identity and properties of flagella from the model methanogenic archaean *Methanospirillum hungatei* strain JF1, we isolated the polar flagellar filaments by cell shearing and differential centrifugation. Gently curved, ~11 nm in diameter and up to 10 μm in length, this archaeal flagellum differs significantly from the larger diameter bacterial sinusoidal filaments. We identify the major flagellin proteins by SDS PAGE and mass spectrometry analyses. Unlike the flagella of other described archaeal species, *M. hungatei* contains only one major flagellin protein, Mhun_3140, one of the three FlaB paralogs present in the genome. On SDS gel, this protein exhibited a molecular weight significantly higher than that predicted from its amino acid sequence, suggesting post-translational modifications. A glycostain and subsequent glycan analysis confirmed the presence of a glycan modification. We have determined a three-dimensional reconstruction of the archaeal flagellar filament at 7.5Å resolution by cryo electron microscopy. The structure reveals a core α helix in each flagellin subunit that contributes to the formation of the filament that is similar to that observed the bacterial type IV pili. Despite this similarity, the archaeal flagella structure exhibits two additional domains that bear little resemblance to the bacterial type IV pili.

Vendor Roundtable

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GATAN, Inc.

"Cryo-EM Imaging with the K2 Direct Detection Camera"

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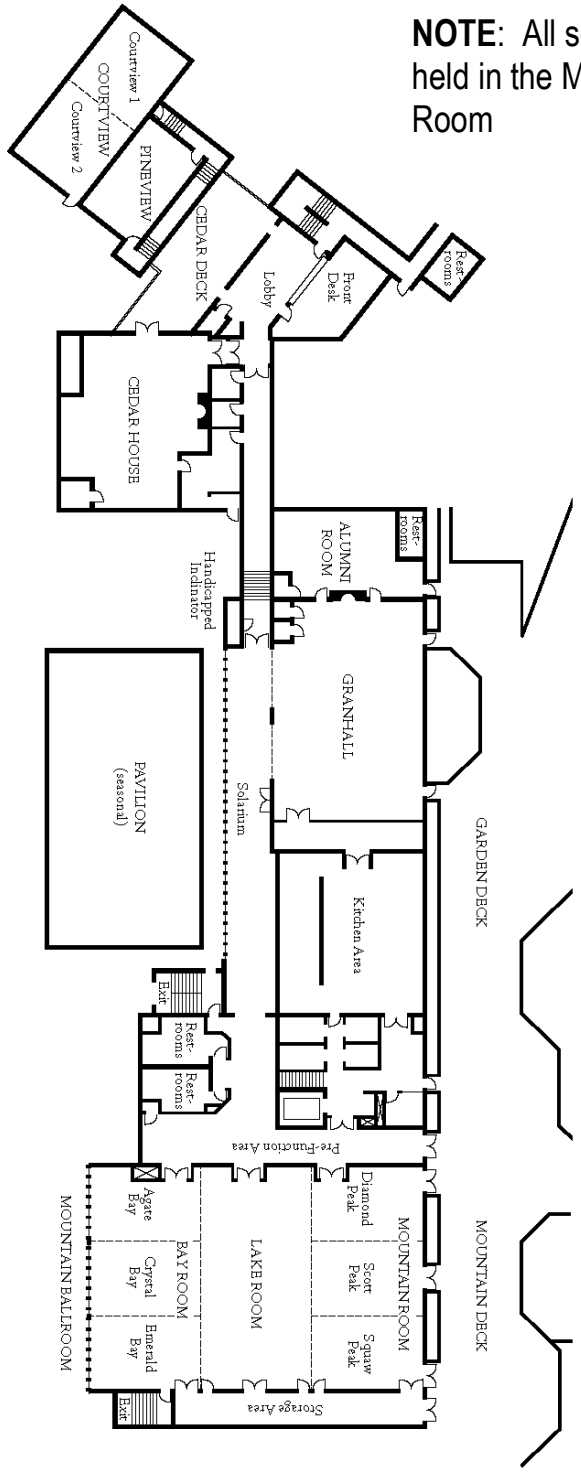
Meeting Room Diagram

NOTE: All sessions will be held in the Mountain Lake Room



MEETING ROOM DIAGRAM

Meeting Room:	Size	Room Dimensions			Seating Capacity			
		Sq. Footage	Ceiling Height	Theatre	Classroom	Banquet	Reception
Granhall	57 x 40 ft.	2,280	9-14 ft.	200	150	250	250
Solarium	54 x 13 ft.	702	sloping	-	-	45	70
Alumni Room	20 x 30 ft.	600	9-14 ft.	50	32	40	-
Cedar House	45 x 25 ft.	1,125	A-frame	100	75	100	100
Courtview (opt'ns)	48 x 22 ft.	1,056	16-17 ft.	80	60	-	-
Courtview1	24 x 22 ft.	528	16-17 ft.	40	32	-	-
Courtview2	24 x 22 ft.	528	16-17 ft.	40	32	-	-
Pineview	18 x 30 ft.	540	16-17 ft.	40	32	-	-
Decks:							
Garden & Mtn Decks	freeform	7,000	-	-	-	-	-
Cedar Deck	30 x 60 ft.	1,800	-	-	-	450	700
						100	150

Meeting Room:	Size	Room Dimensions			Seating Capacity			
		Sq. Footage	Ceiling Height	Theatre	Classroom	Banquet	Reception
Mountain Ballroom (opn)	60 x 80 ft.	9,600	12-14 ft.	900	540	900	900
Mountain Room	60 x 30 ft.	1,800	12-14 ft.	180	120	120	180
Diamond Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Scott Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Squaw Peak	20 x 30 ft.	600	14 ft.	50	32	32	50
Lake Room	60 x 30 ft.	1,800	12-14 ft.	180	120	120	180
Bay Room	60 x 30 ft.	1,800	12-14 ft.	180	120	120	180
Agate Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Crystal Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Emerald Bay	20 x 30 ft.	600	14 ft.	50	32	32	50
Pavilion (seasonal)	40 x 80 ft.	3,200	16-20 ft.	180	120	120	180

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