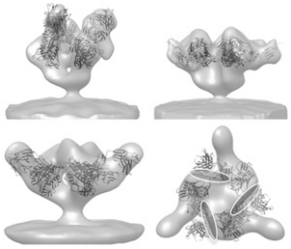
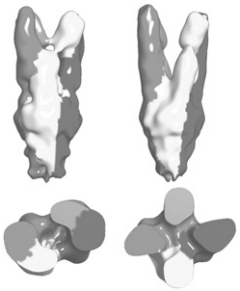
1803-Pos Board B695
Binding of Neutralizing Antibodies Results in Distinct Quaternary Conformations of Trimeric HIV-1 Envelope Glycoproteins
Erin E.H. Tran, Oleg Kuybeda, Alberto Bartesaghi, Jacqueline L.S. Milne, Sriram Subramaniam.
National Institutes of Health, Bethesda, MD, USA.
The HIV surface glycoprotein, Env, initiates HIV infection by binding to cell-surface CD4 and a co-receptor molecule on T cells. Understanding the interactions of these molecules with native, trimeric Env is crucial for effective vaccine design. using cryo-electron tomography of intact HIV, we show that binding to either the co-receptor mimic 17b or soluble CD4 is sufficient for formation of an open quaternary conformation of Env. In contrast, binding of the broadly neutralizing, CD4-binding-site antibody, VRC01, locks Env in the native, closed conformation, preventing 17b binding and formation of the open Env conformation. The CD4-binding-site antibody, b12, requires a partial opening in the quaternary structure of Env and cannot bind in the conformation that binds to the VRC antibodies due to steric clashes. Our results show that, despite general similarities in regions of the HIV-1 gp120 polypeptide that contact CD4, VRC01 and b12, important differences exist in the quaternary structures of these complexes. These findings shed new mechanistic insight into the HIV entry process and potentially explain differences in the neutralizing breadth and potency of antibodies with similar specificities.

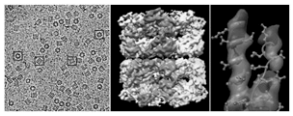


1804-Pos Board B696
Glutamate Receptor Desensitization Mediated by Changes in Quaternary Structure of the Ligand Binding Domain
David M. Schauder[1], Oleg Kuybeda[2], Jinjin Zhang[3], Katherine Klymko[1], Alberto Bartesaghi[1], Mario J. Borgnia[1], Mark L. Mayer[3], **Sriram Subramaniam**[1].
[1]National Cancer Institute, NIH, Bethesda, MD, USA, [2]National Library of Medicine, NIH, Bethesda, MD, USA, [3]NICHD, NIH, Bethesda, MD, USA.
Glutamate receptor ion channels are membrane proteins that mediate excitatory synaptic transmission in the central nervous system of vertebrates. Insight into the molecular mechanisms underlying their gating is limited by lack of structural information for receptors trapped in different conformational states. Here, we report the use of single particle cryo-electron tomography to determine the structures, at ~ 25 Å resolution, of full-length GluK2 kainate receptors trapped in resting and desensitized states. The resting state structure, stabilized by the competitive antagonist LY466195, closely resembles the crystal structure of the AMPA receptor GluA2, with well-resolved proximal and distal subunits exhibiting cross over between the amino terminal (ATD) and a 2-fold symmetric ligand binding domain (LBD) dimer of dimers assembly. In the desensitized state, the LBD regions undergo major rearrangements resulting in a remarkable separation of the four subunits by ~ 30 Å. From fits of the ATD and LBD domains into the density map of the desensitized state, we have derived a structural model for the differences in quaternary conformation between resting and desensitized states of glutamate receptor ion channels.

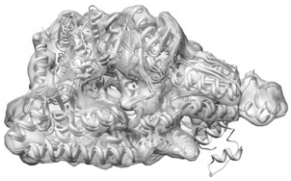


1805-Pos Board B697
Multiple Subunit Fitting into a Low-Resolution Density Map of a Macromolecular Complex using Gaussian Mixture Model
Takeshi Kawabata.
Osaka University, Osaka, Japan.
Recently,electron microscopy measurement of single-particles has enabled us toreconstruct a low-resolution 3D density map of large bio-molecular complexes. If structures of the complex subunits can besolved by X-ray crystallography at atomic resolution, fitting these models intothe 3D density map can generate an atomic resolution model of the entire largecomplex. The fitting of multiple subunits,however, generally requires large computational costs. We developed a new fast fitting program, "*gmfit*", which employs a Gaussian mixture model (GMM) to represent approximated shapes of the 3D density map and the atomic models. A GMM is a distribution function composed by the sum of several 3D Gaussian density functions (GDFs). Because our model analytically provides an integral of a product of two distribution functions, it enables us to quickly calculate the fitness of the density map and the atomic models. using the integral, two types of potential energy functions are introduced: the attraction potential energy between a 3D density map and each subunit, and the repulsion potential energy between subunits. The restraint energy for symmetry is also employed to build symmetrical origomeric complexes. To find the optimal configuration of subunits, we randomly generated initial configurations of atomic models, and performed a steepest descent method using forces and torques of the three potential energies. We then performed test fitting calculations for simulated low-resolution density maps of atomic models of homo dimer, trimer and hexamer, using different search parameters. The results indicated that our method was able to rebuild atomic models of complex even for 30 Å resolution maps, if sufficient numbers of GDFs were employed for each subunit, and the symmetric restraints were assigned for complexes with more than three subunits. (*Biophys.J.*95,4643-4658 (2008))

1806-Pos Board B698
Automated Workflows for Structure Determination by Single-Particle Cryo-EM at Sub-Nanometer Resolutions
Alberto Bartesaghi[1], Jason Pierson[2], David Schauder[1], Mario Borgnia[1], Sriram Subramaniam[1].
[1]National Institutes of Health, Bethesda, MD, USA, [2]FEI, Eindhoven, Netherlands.
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 automated workflows for data collection and processing of large single-particle datasets. Efficient micrograph screening routines together with automatic particle picking strategies enable processing of entire datasets in high-throughput mode in a routine manner. We use this platform as a framework to perform comparative studies of the effects of different types of detector, acceleration voltages and different dose fractionation schemes on the resolution of reconstructions. We applied these workflows to multiple datasets of GroEL imaged under a variety of conditions showing that reconstructions at better than 7A resolution can be consistently achieved using this framework.



1807-Pos Board B699
Macromolecular Structure Modeling and Electron Microscopy Fitting using 3D Zernike Descriptors
Daisuke Kihara, Juan Esquivel-Rodriguez.
Purdue University, West Lafayette, IN, USA.
A novel computational method for fitting high-resolution structures of multiple proteins into a cryoelectron microscopy (EM) map is presented. The method named EMLZerD generates a pool of candidate complex conformations of component proteins using a novel multiple protein docking method, Multi-LZerD, which are later compared with a provided EM map to select the ones that fit well into the EM map (Esquivel-Rodriguez & Kihara, J. Phys Chem B, 2012). Multi-LZerD (Esquivel-Rodriguez & Kihara, Proteins, 2012) builds models of multimeric protein complexes by effectively reusing pairwise docking predictions of component proteins. The comparison of docking conformations and the EM map is performed using the 3D Zernike descriptor (3DZD), a mathematical series expansion of three-dimensional functions. The 3DZD provides a unified representation of the surface shape of multimeric protein complex models and EM maps, which allows a convenient, fast quantitative comparison of the three-dimensional structural data. Out of 19 multimeric complexes tested, near native complex structures with a RMSD of less than 2.5 Å were obtained for 14 cases while medium range resolution structures with correct topology were computed for the additional 5 cases.



1808-Pos Board B700
Photorhabdus Luminescens Toxins use a Novel Syringe-Like Injection Mechanism for Cell Entry
Christos Gatsogiannis[1], Alexander E. Lang[2], Dominic Meusch[1], Oliver Hofnagel[1], Vanda Pfaumann[2], Roland Benz[3], Klaus Aktories[2], **Stefan Raunser**[1].