Axel Brunger

Professor of Molecular and Cellular Physiology, of Neurology and Neurological Sciences, of Photon Science and, by courtesy, of Structural Biology

Molecular & Cellular Physiology

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Bio

Axel Brunger received his Physics Diploma at the University of Hamburg in 1980, and his Ph.D. degree from the Technical University of Munich in 1982 working with Klaus Schulten. He held a NATO postdoctoral fellowship and subsequently became a research associate with Martin Karplus at the Department of Chemistry, Harvard University. In 1987 he joined the faculty in the Department of Molecular Biophysics and Biochemistry at Yale University. In 2000, he moved to Stanford University where he is Professor and Chair of the Department of Molecular and Cellular Physiology. He also holds an appointment as Investigator in the Howard Hughes Medical Institute. In 1995 he was awarded the Röntgen Prize for Biosciences from the University of Würzburg. In 2003, he received the Gregori Aminoff Award of the Royal Swedish Academy. In 2005 he was elected member of the National Academy of Sciences. In 2011 he received the DeLano Award of the American Society for Biochemistry and Molecular Biology, and in 2014 he received the Bernard Katz Award of the Biophysical Society and the Carl Hermann Medal of the German Crystallographic Society.

Academic Appointments

- Professor, Molecular & Cellular Physiology
- Professor, Stanford Synchrotron Radiation Lightsource
- Professor, Neurology & Neurological Sciences
- Professor (By courtesy), Structural Biology
- Member, Bio-X
- Member, Stanford Neurosciences Institute

Administrative Appointments

- Chair, Department of Molecular and Cellular Physioogy (2013 - Present)

Honors & Awards

- Carl Hermann Medal, German Crystallographic Society (DGK) (2014)
- Katz Award, Exocytosis & Endocytosis Group, Biophysical Society (2014)
- DeLano Award, American Society for Biochemistry and Molecular Biology (2011)
- Elected Member, National Academy of Sciences (2005)
- Gregori Aminoff Prize, The Royal Swedish Academy of Sciences (2003)
- Röntgen Prize in Biosciences, University of Würzburg, Germany (1995)

Boards, Advisory Committees, Professional

Organizations

- Investigator, Howard Hughes Medical Institute (1987 - Present)

Professional Education

- Diplom, University of Hamburg, Physics (1980)
- Ph.D., Technical Univ. of Munich, Biophysics (1982)

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Links

My Lab Site

Research & Scholarship

Current Research and Scholarly Interests

Neurotransmitter Release
Neuronal communication is made possible by the release of neurotransmitters, which in turn depends on the fusion of neurotransmitter-laden synaptic vesicles at the ends of nerve cells. Synaptic vesicle fusion is triggered by an influx of Ca2+ ions into the neuron upon depolarization of the neuron, a process that initiates neurotransmission. Neurotransmitter release is quantized; that is, at most one synaptic vesicle fuses in the active zone upon an action potential. This process is controlled by several proteins, including SNAREs (soluble NSF [N-ethylmaleimide-sensitive factor] attachment protein receptors), the Ca2+sensor synaptotagmin-1, Munc18, Munc13, complexin, and the ATPase NSF, among others. Thus, neurotransmitter release is a biological phenomenon controlled by

complex interactions between individual molecules. An understanding of the underlying molecular mechanisms requires methods that are capable of observing single vesicles and molecules.

Ideally, observations of single vesicles and molecules would be performed in live neurons. Although such studies have been under way (in our laboratory and others), they currently provide limited information, largely because the genetic manipulations and labeling techniques used do not provide the spatial and time resolution required for studying the molecular mechanism of neurotransmitter release. Thus, reconstituted (in vitro) systems are needed that mimic the neurotransmitter release characteristics observed in neurons and that allow manipulations and observations not possible in vivo. Such in vitro systems will set the stage for deciphering of the effect of other factors on the process. They could also become screening tools for the development of therapeutic leads to modulate neurotransmitter release and combat neurological disorders.

Our approach to understanding the molecular basis for neurotransmitter release consists of a combination of structural and biophysical studies of the synaptic vesicle fusion machinery. Structural information about complexes between the individual molecular components is primarily obtained by x-ray crystallography and electron cryo-microscopy. This hybrid approach provides the framework for investigations targeted at the functional and dynamic aspects of the system, using single-molecule and single-particle fluorescence microscopy techniques.

Teaching

2014-15 Courses

- Independent Studies (10)
  - Directed Reading in Biophysics
    BIOPHYS 399 (Aut, Win, Spr, Sum)
  - Directed Reading in Molecular and Cellular Physiology
    MCP 299 (Aut, Win, Spr, Sum)
  - Directed Reading in Neurosciences
    NEPR 299 (Aut, Win, Spr, Sum)
  - Graduate Research
    BIOPHYS 300 (Aut, Win, Spr, Sum)
  - Graduate Research
    MCP 399 (Aut, Win, Spr, Sum)
  - Graduate Research
    NEPR 399 (Aut, Win, Spr, Sum)
  - Medical Scholars Research
    MCP 370 (Aut, Win, Spr, Sum)
  - Out-of-Department Advanced Research Laboratory in Experimental Biology
    BIO 199X (Aut, Win, Spr, Sum)

- Out-of-Department Graduate Research
  BIO 300X (Aut, Win, Spr, Sum)
- Undergraduate Research
  MCP 199 (Aut, Win, Spr, Sum)

Graduate and Fellowship Programs

- Biophysics (Phd Program)
- Molecular and Cellular Physiology (Phd Program)
- Neurosciences (Phd Program)
- Structural Biology (Phd Program)

Publications

All Publications

- ATG14 promotes membrane tethering and fusion of autophagosomes to endolysosomes *NATURE* Diao, J., Liu, R., Rong, Y., Zhao, M., Zhang, J., Lai, Y., Zhou, Q., Wilz, L. M., Li, J., Vivona, S., Pfuetzner, R. A., Brunger, A. T., Zhong, Q. 2015; 520 (7548): 563-?

  ### Abstract

  Autophagy, an important catabolic pathway implicated in a broad spectrum of human diseases, begins by forming double membrane autophagosomes that engulf cytosolic cargo and ends by fusing autophagosomes with lysosomes for degradation. Membrane fusion activity is required for early biogenesis of autophagosomes and late degradation in lysosomes. However, the key regulatory mechanisms of autophagic membrane tethering and fusion remain largely unknown. Here we report that ATG14 (also known as beclin-1-associated autophagy-related key regulator (Barkor) or ATG14L), an essential autophagy-specific regulator of the class III phosphatidylinositol 3-kinase complex, promotes membrane tethering of protein-free liposomes, and enhances hemifusion and full fusion of proteoliposomes reconstituted with the target (t)-SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) syntaxin 17 (STX17) and SNAP29, and the vesicle (v)-SNARE VAMP8 (vesicle-associated membrane protein 8). ATG14 binds to the SNARE core domain of STX17 through its coiled-coil domain, and stabilizes the STX17-SNAP29 binary t-SNARE complex on autophagosomes. The STX17 binding, membrane tethering and fusion-enhancing activities of ATG14 require its homo-oligomerization by cysteine repeats. In ATG14 homo-oligomerization-defective cells, autophagosomes still efficiently form but their fusion with endolysosomes is blocked. Recombinant ATG14 homo-oligomerization

mutants also completely lose their ability to promote membrane tethering and to enhance SNARE-mediated fusion in vitro. Taken together, our data suggest an autophagy-specific membrane fusion mechanism in which oligomeric ATG14 directly binds to STX17-SNAP29 binary t-SNARE complex on autophagosomes and primes it for VAMP8 interaction to promote autophagosome-endolysosome fusion.

View details for DOI 10.1038/nature14147

View details for Web of Science ID 000353334500048

View details for PubMedID 25686604

- Munc18a Does Not Alter Fusion Rates Mediated by Neuronal SNAREs, Synaptotagmin, and Complexin. *journal of biological chemistry* Zhang, Y., Diao, J., Colbert, K. N., Lai, Y., Pfuetzner, R. A., Padolina, M. S., Vivona, S., Ressl, S., Cipriano, D. J., Choi, U. B., Shah, N., Weis, W. I., Brunger, A. T. 2015; 290 (16): 10518-10534

Abstract

Sec1/Munc18 (SM) proteins are essential for membrane trafficking, but their molecular mechanism remains unclear. Using a single vesicle-vesicle content-mixing assay with reconstituted neuronal SNAREs, synaptotagmin-1, and complexin-1, we show that the neuronal SM protein Munc18a/nSec1 has no effect on the intrinsic kinetics of both spontaneous fusion and Ca(2+)-triggered fusion between vesicles that mimic synaptic vesicles and the plasma membrane. However, wild type Munc18a reduced vesicle association ∼50% when the vesicles bearing the t-SNAREs syntaxin-1A and SNAP-25 were preincubated with Munc18 for 30 min. Single molecule experiments with labeled SNAP-25 indicate that the reduction of vesicle association is a consequence of sequestration of syntaxin-1A by Munc18a and subsequent release of SNAP-25 (i.e. Munc18a captures syntaxin-1A via its high affinity interaction). Moreover, a phosphorylation mimic mutant of Munc18a with reduced affinity to syntaxin-1A results in less reduction of vesicle association. In summary, Munc18a does not directly affect fusion, although it has an effect on the t-SNARE complex, depending on the presence of other factors and experimental conditions. Our results suggest that Munc18a primarily acts at the prefusion stage.

View details for DOI 10.1074/jbc.M114.630772

View details for PubMedID 25716318

- Structures of C1q-like Proteins Reveal Unique Features among the C1q/TNF Superfamily *STRUCTURE* Ressl, S., Vu, B. K., Vivona, S., Martinelli, D. C., Suedhof, T. C., Brunger, A. T. 2015; 23 (4): 688-699

Abstract

C1q-like (C1QL) -1, -2, and -3 proteins are encoded by homologous genes that are highly expressed in brain. C1QLs bind to brain-specific angiogenesis inhibitor 3 (BAI3), an adhesion-type G-protein coupled receptor that may regulate dendritic morphology by organizing actin filaments. To begin to understand the function of C1QLs, we determined high-resolution crystal structures of the globular C1q-domains of C1QL1,

C1QL2, and C1QL3. Each structure is a trimer, with each protomer forming a jelly-roll fold consisting of 10 β strands. Moreover, C1QL trimers may assemble into higher-order oligomers similar to adiponectin and contain four Ca(2+)-binding sites along the trimeric symmetry axis, as well as additional surface Ca(2+)-binding sites. Mutation of Ca(2+)-coordinating residues along the trimeric symmetry axis lowered the Ca(2+)-binding affinity and protein stability. Our results reveal unique structural features of C1QLs among C1q/TNF superfamily proteins that may be associated with their specific brain functions.

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View details for Web of Science ID 000352507400012

View details for PubMedID 25752542

- Capture and X-ray diffraction studies of protein microcrystals in a microfluidic trap array *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Lyubimov, A. Y., Murray, T. D., Koehl, A., Araci, I. E., Uervirojnangkoorn, M., Zeldin, O. B., Cohen, A. E., Soltis, S. M., Baxter, E. L., Brewster, A. S., Sauter, N. K., Brunger, A. T., Berger, J. M. 2015; 71: 928-940

Abstract

X-ray free-electron lasers (XFELs) promise to enable the collection of interpretable diffraction data from samples that are refractory to data collection at synchrotron sources. At present, however, more efficient sample-delivery methods that minimize the consumption of microcrystalline material are needed to allow the application of XFEL sources to a wide range of challenging structural targets of biological importance. Here, a microfluidic chip is presented in which microcrystals can be captured at fixed, addressable points in a trap array from a small volume (<10 µl) of a pre-existing slurry grown off-chip. The device can be mounted on a standard goniostat for conducting diffraction experiments at room temperature without the need for flash-cooling. Proof-of-principle tests with a model system (hen egg-white lysozyme) demonstrated the high efficiency of the microfluidic approach for crystal harvesting, permitting the collection of sufficient data from only 265 single-crystal still images to permit determination and refinement of the structure of the protein. This work shows that microfluidic capture devices can be readily used to facilitate data collection from protein microcrystals grown in traditional laboratory formats, enabling analysis when cryopreservation is problematic or when only small numbers of crystals are available. Such microfluidic capture devices may also be useful for data collection at synchrotron sources.

View details for DOI 10.1107/S1399004715002308

View details for Web of Science ID 000352507200019

View details for PubMedID 25849403

- Enabling X-ray Free Electron Laser Crystallography for Challenging Biological Systems from a Limited Number of Crystals *ELIFE* Uervirojnangkoorn, M., Zeldin, O. B., Lyubimov, A. Y., Hattne, J., Brewster, A. S., Sauter, N. K., Brunger, A. T., Weis, W. I. 2015; 4

Abstract

There is considerable potential for X-ray free electron lasers (XFELs) to enable determination of macromolecular crystal structures that are difficult to solve using current synchrotron sources. Prior XFEL studies often involved the collection of thousands to millions of diffraction images, in part due to limitations of data processing methods. We implemented a data processing system based on classical post-refinement techniques, adapted to specific properties of XFEL diffraction data. When applied to XFEL data from three different proteins collected using various sample delivery systems and XFEL beam parameters, our method improved the quality of the diffraction data as well as the resulting refined atomic models and electron density maps. Moreover, the number of observations for a reflection necessary to assemble an accurate data set could be reduced to a few observations. These developments will help expand the applicability of XFEL crystallography to challenging biological systems, including cases where sample is limited.

View details for Web of Science ID 000351865600006

View details for PubMedID 25781634

- Mechanistic insights into the recycling machine of the SNARE complex *NATURE* Zhao, M., Wu, S., Zhou, Q., Vivona, S., Cipriano, D. J., Cheng, Y., Brunger, A. T. 2015; 518 (7537): 61-?

Abstract

Evolutionarily conserved SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptors) proteins form a complex that drives membrane fusion in eukaryotes. The ATPase NSF (N-ethylmaleimide sensitive factor), together with SNAPs (soluble NSF attachment protein), disassembles the SNARE complex into its protein components, making individual SNAREs available for subsequent rounds of fusion. Here we report structures of ATP- and ADP-bound NSF, and the NSF/SNAP/SNARE (20S) supercomplex determined by single-particle electron cryomicroscopy at near-atomic to sub-nanometre resolution without imposing symmetry. Large, potentially force-generating, conformational differences exist between ATP- and ADP-bound NSF. The 20S supercomplex exhibits broken symmetry, transitioning from six-fold symmetry of the NSF ATPase domains to pseudo four-fold symmetry of the SNARE complex. SNAPs interact with the SNARE complex with an opposite structural twist, suggesting an unwinding mechanism. The interfaces between NSF, SNAPs, and SNAREs exhibit characteristic electrostatic patterns, suggesting how one NSF/SNAP species can act on many different SNARE complexes.

View details for DOI 10.1038/nature14148

View details for Web of Science ID 000349098000031

View details for PubMedID 25581794

- Data Exploration Toolkit for serial diffraction experiments *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Zeldin, O. B., Brewster, A. S., Hattne, J., Uervirojnangkoorn, M., Lyubimov,

A. Y., Zhou, Q., Zhao, M., Weis, W. I., Sauter, N. K., Brunger, A. T. 2015; 71: 352-356

Abstract

Ultrafast diffraction at X-ray free-electron lasers (XFELs) has the potential to yield new insights into important biological systems that produce radiation-sensitive crystals. An unavoidable feature of the `diffraction before destruction' nature of these experiments is that images are obtained from many distinct crystals and/or different regions of the same crystal. Combined with other sources of XFEL shot-to-shot variation, this introduces significant heterogeneity into the diffraction data, complicating processing and interpretation. To enable researchers to get the most from their collected data, a toolkit is presented that provides insights into the quality of, and the variation present in, serial crystallography data sets. These tools operate on the unmerged, partial intensity integration results from many individual crystals, and can be used on two levels: firstly to guide the experimental strategy during data collection, and secondly to help users make informed choices during data processing.

View details for DOI 10.1107/S1399004714025875

View details for Web of Science ID 000349439900018

View details for PubMedID 25664746

- Goniometer-based femtosecond crystallography with X-ray free electron lasers *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Cohen, A. E., Soltis, S. M., Gonzalez, A., Aguila, L., Alonso-Mori, R., Barnes, C. O., Baxter, E. L., Brehmer, W., Brewster, A. S., Brunger, A. T., Calero, G., Chang, J. F., Chollet, M., Ehrensberger, P., Eriksson, T. L., Feng, Y., Hattne, J., Hedman, B., Hollenbeck, M., Holton, J. M., Keable, S., Kobilka, B. K., Kovaleva, E. G., Kruse, A. C., Lemke, H. T., Lin, G., Lyubimov, A. Y., Manglik, A., Mathews, I. I., McPhillips, S. E., Nelson, S., Peters, J. W., Sauter, N. K., Smith, C. A., Song, J., Stevenson, H. P., Tsai, Y., Uervirojnangkoorn, M., Vinetsky, V., Wakatsuki, S., Weis, W. I., Zadvornyy, O. A., Zeldin, O. B., Zhu, D., Hodgson, K. O. 2014; 111 (48): 17122-17127

Abstract

The emerging method of femtosecond crystallography (FX) may extend the diffraction resolution accessible from small radiation-sensitive crystals and provides a means to determine catalytically accurate structures of acutely radiation-sensitive metalloenzymes. Automated goniometer-based instrumentation developed for use at the Linac Coherent Light Source enabled efficient and flexible FX experiments to be performed on a variety of sample types. In the case of rod-shaped CpI hydrogenase crystals, only five crystals and about 30 min of beam time were used to obtain the 125 still diffraction patterns used to produce a 1.6-Å resolution electron density map. For smaller crystals, high-density grids were used to increase sample throughput; 930 myoglobin crystals mounted at random orientation inside 32 grids were exposed, demonstrating the utility of this approach. Screening results from cryocooled crystals of β2-adrenoreceptor and an RNA polymerase II complex indicate the potential to extend the diffraction

resolution obtainable from very radiation-sensitive samples beyond that possible with undulator-based synchrotron sources.

View details for DOI 10.1073/pnas.1418733111

View details for Web of Science ID 000345920800042

View details for PubMedID 25362050

- Direct visualization of trans-synaptic neurexin-neuroligin interactions during synapse formation. *journal of neuroscience* Tsetsenis, T., Boucard, A. A., Araç, D., Brunger, A. T., Südhof, T. C. 2014; 34 (45): 15083-15096

Abstract

Neurexins and neuroligins are synaptic cell-adhesion molecules that are essential for normal synapse specification and function and are thought to bind to each other trans-synaptically, but such interactions have not been demonstrated directly. Here, we generated neurexin-1β and neuroligin-1 and neuroligin-2 fusion proteins containing complementary "split" GFP fragments positioned such that binding of neurexin-1β to neuroligin-1 or neuroligin-2 allowed GFP reconstitution without dramatically changing their binding affinities. GFP fluorescence was only reconstituted from split-GFP-modified neurexin-1β and neuroligin-1 if and after neurexin-1β bound to its neuroligin partner; reassociation of the split-GFP components with each other did not mediate binding. Using trans-cellular reconstitution of GFP fluorescence from split-GFP-modified neurexin-1β and neuroligins as an assay, we demonstrate that trans-synaptic neurexin/neuroligin binding indeed occurred when mouse hippocampal neurons formed synapses onto non-neuronal COS-7 cells expressing neuroligins or when mouse hippocampal neurons formed synapses with each other. This visualization of synapses by neurexin/neuroligin binding prompted us to refer to this approach as "SynView." Our data demonstrate that neurexin-1β forms a trans-synaptic complex with neuroligin-1 and neuroligin-2 and that this interaction can be used to label synapses in a specific fashion in vivo.

View details for DOI 10.1523/JNEUROSCI.0348-14.2014

View details for PubMedID 25378172

- Deformable elastic network refinement for low-resolution macromolecular crystallography *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Schroeder, G. F., Levitt, M., Brunger, A. T. 2014; 70: 2241-2255
View details for DOI 10.1107/S1399004714016496

View details for Web of Science ID 000341819500001

- Complexin inhibits spontaneous release and synchronizes Ca2+-triggered synaptic vesicle fusion by distinct mechanisms. *eLife* Lai, Y., Diao, J., Cipriano, D. J., Zhang, Y., Pfuetzner, R. A., Padolina, M. S., Brunger, A. T. 2014; 3

Abstract

Previously we showed that fast Ca(2+)-triggered vesicle fusion with reconstituted neuronal SNAREs and synaptotagmin-1 begins from an initial

hemifusion-free membrane point contact, rather than a hemifusion diaphragm, using a single vesicle-vesicle lipid/content mixing assay (Diao et al., 2012). When complexin-1 was included, a more pronounced Ca(2+)-triggered fusion burst was observed, effectively synchronizing the process. Here we show that complexin-1 also reduces spontaneous fusion in the same assay. Moreover, distinct effects of several complexin-1 truncation mutants on spontaneous and Ca(2+)-triggered fusion closely mimic those observed in neuronal cultures. The very N-terminal domain is essential for synchronization of Ca(2+)-triggered fusion, but not for suppression of spontaneous fusion, whereas the opposite is true for the C-terminal domain. By systematically varying the complexin-1 concentration, we observed differences in titration behavior for spontaneous and Ca(2+)-triggered fusion. Taken together, complexin-1 utilizes distinct mechanisms for synchronization of Ca(2+)-triggered fusion and inhibition of spontaneous fusion.DOI: http://dx.doi.org/10.7554/eLife.03756.001.

View details for DOI 10.7554/eLife.03756

View details for PubMedID 25122624

- Model morphing and sequence assignment after molecular replacement *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Terwilliger, T. C., Read, R. J., Adams, P. D., Brunger, A. T., Afonine, P. V., Hung, L. 2013; 69: 2244-2250

Abstract

A procedure termed `morphing' for improving a model after it has been placed in the crystallographic cell by molecular replacement has recently been developed. Morphing consists of applying a smooth deformation to a model to make it match an electron-density map more closely. Morphing does not change the identities of the residues in the chain, only their coordinates. Consequently, if the true structure differs from the working model by containing different residues, these differences cannot be corrected by morphing. Here, a procedure that helps to address this limitation is described. The goal of the procedure is to obtain a relatively complete model that has accurate main-chain atomic positions and residues that are correctly assigned to the sequence. Residues in a morphed model that do not match the electron-density map are removed. Each segment of the resulting trimmed morphed model is then assigned to the sequence of the molecule using information about the connectivity of the chains from the working model and from connections that can be identified from the electron-density map. The procedure was tested by application to a recently determined structure at a resolution of 3.2 Å and was found to increase the number of correctly identified residues in this structure from the 88 obtained using phenix.resolve sequence assignment alone (Terwilliger, 2003) to 247 of a possible 359. Additionally, the procedure was tested by application to a series of templates with sequence identities to a target structure ranging between 7 and 36%. The mean fraction of correctly identified residues in these cases was increased from 33% using phenix.resolve sequence assignment to 47% using the current procedure. The procedure is simple to apply and is available in the Phenix software package.

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View details for Web of Science ID 000326648900011

View details for PubMedID 24189236

- Complexin-1 Enhances the On-Rate of Vesicle Docking via Simultaneous SNARE and Membrane Interactions *JOURNAL OF THE AMERICAN CHEMICAL SOCIETY* Diao, J., Cipriano, D. J., Zhao, M., Zhang, Y., Shah, S., Padolina, M. S., Pfuetzner, R. A., Brunger, A. T. 2013; 135 (41): 15274-15277

Abstract

In synaptic terminals, complexin is thought to have inhibitory and activating roles for spontaneous "mini" release and evoked synchronized neurotransmitter release, respectively. We used single vesicle-vesicle microscopy imaging to study the effect of complexin-1 on the on-rate of docking between vesicles that mimic synaptic vesicles and the plasma membrane. We found that complexin-1 enhances the on-rate of docking of synaptic vesicle mimics containing full-length synaptobrevin-2 and full-length synaptotagmin-1 to plasma membrane-mimicking vesicles containing full-length syntaxin-1A and SNAP-25A. This effect requires the C-terminal domain of complexin-1, which binds to the membrane, the presence of PS in the membrane, and the core region of complexin-1, which binds to the SNARE complex.

View details for DOI 10.1021/ja407392n

View details for Web of Science ID 000326125200002

- Disassembly of All SNARE Complexes by N-Ethylmaleimide-sensitive Factor (NSF) Is Initiated by a Conserved 1:1 Interaction between alpha-Soluble NSF Attachment Protein (SNAP) and SNARE Complex *JOURNAL OF BIOLOGICAL CHEMISTRY* Vivona, S., Cipriano, D. J., O'Leary, S., Li, Y. H., Fenn, T. D., Brunger, A. T. 2013; 288 (34): 24984-24991

Abstract

Vesicle trafficking in eukaryotic cells is facilitated by SNARE-mediated membrane fusion. The ATPase NSF and the adapter protein α-SNAP disassemble all SNARE complexes formed throughout different pathways, but the effect of SNARE sequence and domain variation on the poorly understood disassembly mechanism is unknown. By measuring SNARE-stimulated ATP hydrolysis rates, Michaelis-Menten constants for disassembly, and SNAP-SNARE binding constants for four different ternary SNARE complex and one binary complex we found a conserved mechanism, not influenced by N-terminal SNARE domains. α-SNAP and ternary SNARE complex form a 1:1 complex as revealed by multi-angle light scattering. We propose a model of NSF-mediated disassembly, where the reaction is initiated by a 1:1 interaction between α-SNAP and the ternary SNARE complex, followed by NSF binding. Subsequent additional α-SNAP binding events may occur as part of a processive disassembly mechanism.

View details for DOI 10.1074/jbc.M113.489807

View details for Web of Science ID 000330612300061

View details for PubMedID 23836889

- Processive ATP-driven Substrate Disassembly by the N-Ethylmaleimide-sensitive Factor (NSF) Molecular Machine *JOURNAL OF BIOLOGICAL CHEMISTRY* Cipriano, D. J., Jung, J., Vivona, S., Fenn, T. D., Brunger, A. T., Bryant, Z. 2013; 288 (32): 23436-23445

Abstract

SNARE proteins promote membrane fusion by forming a 4-stranded parallel helical bundle that brings the membranes into close proximity. Post fusion, the complex is disassembled by an AAA+ ATPase called N-ethylmaleimide sensitive factor (NSF). We present evidence that NSF uses a processive unwinding mechanism to disassemble SNARE proteins. Using a real-time disassembly assay based on fluorescence dequenching, we correlate NSF-driven disassembly rates with the SNARE-activated ATPase activity of NSF. Neuronal SNAREs activate the ATPase rate of NSF by ~26-fold. One SNARE complex takes an average of ~5 seconds to disassemble in a process that consumes ~50 ATP. Investigations of substrate requirements show that NSF is capable of disassembling a truncated SNARE substrate consisting of only the core SNARE domain, but not an unrelated four-stranded coiled coil. NSF can also disassemble an engineered double-length SNARE complex, suggesting a processive unwinding mechanism. We further investigated processivity using single turnover experiments, which show that SNAREs can be unwound in a single encounter with NSF. We propose a processive helicase-like mechanism for NSF in which ~1 residue is unwound for every hydrolyzed ATP molecule.

View details for DOI 10.1074/jbc.M113.476705

View details for Web of Science ID 000330598200050

View details for PubMedID 23775070

- Ultrahigh-resolution imaging reveals formation of neuronal SNARE/Munc18 complexes in situ. *Proceedings of the National Academy of Sciences of the United States of America* Pertsinidis, A., Mukherjee, K., Sharma, M., Pang, Z. P., Park, S. R., Zhang, Y., Brunger, A. T., Südhof, T. C., Chu, S. 2013; 110 (30): E2812-20

Abstract

Membrane fusion is mediated by complexes formed by SNAP-receptor (SNARE) and Secretory 1 (Sec1)/mammalian uncoordinated-18 (Munc18)-like (SM) proteins, but it is unclear when and how these complexes assemble. Here we describe an improved two-color fluorescence nanoscopy technique that can achieve effective resolutions of up to 7.5-nm full width at half maximum (3.2-nm localization precision), limited only by stochastic photon emission from single molecules. We use this technique to dissect the spatial relationships between the neuronal SM protein Munc18-1 and SNARE proteins syntaxin-1 and SNAP-25 (25 kDa synaptosome-associated protein). Strikingly, we observed nanoscale clusters consisting of syntaxin-1 and SNAP-25 that contained associated Munc18-1. Rescue experiments with syntaxin-1 mutants revealed that Munc18-1 recruitment to the plasma membrane depends on the Munc18-1 binding to the N-terminal peptide of

syntaxin-1. Our results suggest that in a primary neuron, SNARE/SM protein complexes containing syntaxin-1, SNAP-25, and Munc18-1 are preassembled in microdomains on the presynaptic plasma membrane. Our superresolution imaging method provides a framework for investigating interactions between the synaptic vesicle fusion machinery and other subcellular systems in situ.

View details for DOI 10.1073/pnas.1310654110

View details for PubMedID 23821748

- Ultrahigh-resolution imaging reveals formation of neuronal SNARE/Munc18 complexes in situ *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Pertsinidis, A., Mukherjee, K., Sharma, M., Pang, Z. P., Park, S. R., Zhang, Y., Brunger, A. T., Suedhof, T. C., Chu, S. 2013; 110 (30): E2812-E2820
  View details for DOI 10.1073/pnas.1310654110

  View details for Web of Science ID 000322112300011

- Studying protein-reconstituted proteoliposome fusion with content indicators in vitro *BIOESSAYS* Diao, J., Zhao, M., Zhang, Y., Kyoung, M., Brunger, A. T. 2013; 35 (7): 658-665

Abstract

In vitro reconstitution assays are commonly used to study biological membrane fusion. However, to date, most ensemble and single-vesicle experiments involving SNARE proteins have been performed only with lipid-mixing, but not content-mixing indicators. Through simultaneous detection of lipid and small content-mixing indicators, we found that lipid mixing often occurs seconds prior to content mixing, or without any content mixing at all, during a 50-seconds observation period, for Ca(2+) -triggered fusion with SNAREs, full-length synaptotagmin-1, and complexin. Our results illustrate the caveats of commonly used bulk lipid-mixing fusion experiments. We recommend that proteoliposome fusion experiments should always employ content-mixing indicators in addition to, or in place of, lipid-mixing indicators.

View details for DOI 10.1002/bies.201300010

View details for Web of Science ID 000320394000012

- Properties of native brain a-synuclein. *Nature* Burré, J., Vivona, S., Diao, J., Sharma, M., Brunger, A. T., Südhof, T. C. 2013; 498 (7453): E4-6
  View details for DOI 10.1038/nature12125

  View details for PubMedID 23765500

- Properties of native brain alpha-synuclein *NATURE* Burre, J., Vivona, S., Diao, J., Sharma, M., Brunger, A. T., Suedhof, T. C. 2013; 498 (7453): E4-E6
  View details for DOI 10.1038/nature12125

  View details for Web of Science ID 000320283400001

- Native alpha-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2 *ELIFE* Diao, J., Burre, J., Vivona, S., Cipriano, D. J., Sharma, M., Kyoung, M., Suedhof, T. C.,

Brunger, A. T. 2013; 2
View details for DOI 10.7554/eLife.00592

View details for Web of Science ID 000328614700004

- Studying calcium-triggered vesicle fusion in a single vesicle-vesicle content and lipid-mixing system. *Nature protocols* Kyoung, M., Zhang, Y., Diao, J., Chu, S., Brunger, A. T. 2013; 8 (1): 1-16

Abstract

This protocol describes a single vesicle-vesicle microscopy system to study Ca(2+)-triggered vesicle fusion. Donor vesicles contain reconstituted synaptobrevin and synaptotagmin-1. Acceptor vesicles contain reconstituted syntaxin and synaptosomal-associated protein 25 (SNAP-25), and they are tethered to a PEG-coated glass surface. Donor vesicles are mixed with the tethered acceptor vesicles and incubated for several minutes at a zero-Ca (2+) concentration, resulting in a collection of single interacting vesicle pairs. The donor vesicles also contain two spectrally distinct fluorophores that allow simultaneous monitoring of temporal changes of the content and membrane. Upon Ca(2+) injection into the sample chamber, our system therefore differentiates between hemifusion and complete fusion of interacting vesicle pairs and determines the temporal sequence of these events on a sub-100-millisecond time scale. Other factors such as complexin can be easily added. Our system is unique in that it monitors both content and lipid mixing and starts from a metastable state of interacting vesicle pairs before Ca(2+) injection.

View details for DOI 10.1038/nprot.2012.134

View details for PubMedID 23222454

- Advances, Interactions, and Future Developments in the CNS, Phenix, and Rosetta Structural Biology Software Systems *ANNUAL REVIEW OF BIOPHYSICS, VOL 42* Adams, P. D., Baker, D., Brunger, A. T., Das, R., DiMaio, F., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C. 2013; 42: 265-287

Abstract

Advances in our understanding of macromolecular structure come from experimental methods, such as X-ray crystallography, and also computational analysis of the growing number of atomic models obtained from such experiments. The later analyses have made it possible to develop powerful tools for structure prediction and optimization in the absence of experimental data. In recent years, a synergy between these computational methods for crystallographic structure determination and structure prediction and optimization has begun to be exploited. We review some of the advances in the algorithms used for crystallographic structure determination in the Phenix and Crystallography & NMR System software packages and describe how methods from ab initio structure prediction and refinement in Rosetta have been applied to challenging crystallographic problems. The prospects for future improvement of these methods are discussed.

View details for DOI 10.1146/annurev-biophys-083012-130253

View details for Web of Science ID 000321695700013

View details for PubMedID 23451892

- Studying calcium-triggered vesicle fusion in a single vesicle-vesicle content and lipid-mixing system *NATURE PROTOCOLS* Kyoung, M., Zhang, Y., Diao, J., Chu, S., Brunger, A. T. 2013; 8 (1): 1-16
  View details for DOI 10.1038/nprot.2012.134

  View details for Web of Science ID 000313051300001

- Native a-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. *eLife* Diao, J., Burré, J., Vivona, S., Cipriano, D. J., Sharma, M., Kyoung, M., Südhof, T. C., Brunger, A. T. 2013; 2

Abstract

?-Synuclein is a presynaptic protein that is implicated in Parkinson's and other neurodegenerative diseases. Physiologically, native ?-synuclein promotes presynaptic SNARE-complex assembly, but its molecular mechanism of action remains unknown. Here, we found that native ?-synuclein promotes clustering of synaptic-vesicle mimics, using a single-vesicle optical microscopy system. This vesicle-clustering activity was observed for both recombinant and native ?-synuclein purified from mouse brain. Clustering was dependent on specific interactions of native ?-synuclein with both synaptobrevin-2/VAMP2 and anionic lipids. Out of the three familial Parkinson's disease-related point mutants of ?-synuclein, only the lipid-binding deficient mutation A30P disrupted clustering, hinting at a possible loss of function phenotype for this mutant. ?-Synuclein had little effect on Ca(2+)-triggered fusion in our reconstituted single-vesicle system, consistent with in vivo data. ?-Synuclein may therefore lead to accumulation of synaptic vesicles at the active zone, providing a 'buffer' of synaptic vesicles, without affecting neurotransmitter release itself. DOI:http://dx.doi.org/10.7554/eLife.00592.001.

View details for DOI 10.7554/eLife.00592

View details for PubMedID 23638301

- Synaptic proteins promote calcium-triggered fast transition from point contact to full fusion *ELIFE* Diao, J., Grob, P., Cipriano, D. J., Kyoung, M., Zhang, Y., Shah, S., Amie Nguyen, A., Padolina, M., Srivastava, A., Vrljic, M., Shah, A., Nogales, E., Chu, S., Brunger, A. T. 2012; 1
  View details for DOI 10.7554/eLife.00109

  View details for Web of Science ID 000328584600005

- Improved crystallographic models through iterated local density-guided model deformation and reciprocal-space refinement *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Terwilliger, T. C., Read, R. J., Adams, P. D., Brunger, A. T., Afonine, P. V., Grosse-Kunstleve, R. W., Hung, L. 2012; 68: 861-870

Abstract

An approach is presented for addressing the challenge of model rebuilding after molecular replacement in cases where the placed template is very different from the structure to be determined. The approach takes advantage of the observation that a template and target structure may have local structures that can be superimposed much more closely than can their complete structures. A density-guided procedure for deformation of a properly placed template is introduced. A shift in the coordinates of each residue in the structure is calculated based on optimizing the match of model density within a 6?Å radius of the center of that residue with a prime-and-switch electron-density map. The shifts are smoothed and applied to the atoms in each residue, leading to local deformation of the template that improves the match of map and model. The model is then refined to improve the geometry and the fit of model to the structure-factor data. A new map is then calculated and the process is repeated until convergence. The procedure can extend the routine applicability of automated molecular replacement, model building and refinement to search models with over 2?Å r.m.s.d. representing 65-100% of the structure.

View details for DOI 10.1107/S0907444912015636

View details for Web of Science ID 000305968400015

View details for PubMedID 22751672

- Improving the accuracy of macromolecular structure refinement at 7 Å resolution. *Structure* Brunger, A. T., Adams, P. D., Fromme, P., Fromme, R., Levitt, M., Schröder, G. F. 2012; 20 (6): 957-966

Abstract

In X-ray crystallography, molecular replacement and subsequent refinement is challenging at low resolution. We compared refinement methods using synchrotron diffraction data of photosystem I at 7.4 Å resolution, starting from different initial models with increasing deviations from the known high-resolution structure. Standard refinement spoiled the initial models, moving them further away from the true structure and leading to high R(free)-values. In contrast, DEN refinement improved even the most distant starting model as judged by R(free), atomic root-mean-square differences to the true structure, significance of features not included in the initial model, and connectivity of electron density. The best protocol was DEN refinement with initial segmented rigid-body refinement. For the most distant initial model, the fraction of atoms within 2 Å of the true structure improved from 24% to 60%. We also found a significant correlation between R(free) values and the accuracy of the model, suggesting that R(free) is useful even at low resolution.

View details for DOI 10.1016/j.str.2012.04.020

View details for PubMedID 22681901

- Application of DEN refinement and automated model building to a difficult case of molecular-replacement phasing: the structure of a putative succinyl-diaminopimelate desuccinylase from Corynebacterium glutamicum *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Brunger, A. T., Das, D., Deacon, A. M., Grant, J., Terwilliger, T. C., Read, R.

J., Adams, P. D., Levitt, M., Schroeder, G. F. 2012; 68: 391-403

Abstract

Phasing by molecular replacement remains difficult for targets that are far from the search model or in situations where the crystal diffracts only weakly or to low resolution. Here, the process of determining and refining the structure of Cgl1109, a putative succinyl-diaminopimelate desuccinylase from Corynebacterium glutamicum, at ?3?Å resolution is described using a combination of homology modeling with MODELLER, molecular-replacement phasing with Phaser, deformable elastic network (DEN) refinement and automated model building using AutoBuild in a semi-automated fashion, followed by final refinement cycles with phenix.refine and Coot. This difficult molecular-replacement case illustrates the power of including DEN restraints derived from a starting model to guide the movements of the model during refinement. The resulting improved model phases provide better starting points for automated model building and produce more significant difference peaks in anomalous difference Fourier maps to locate anomalous scatterers than does standard refinement. This example also illustrates a current limitation of automated procedures that require manual adjustment of local sequence misalignments between the homology model and the target sequence.

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View details for Web of Science ID 000302138400008

View details for PubMedID 22505259

- A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis *EMBO JOURNAL* Arac, D., Boucard, A. A., Bolliger, M. F., Nguyen, J., Soltis, S. M., Suedhof, T. C., Brunger, A. T. 2012; 31 (6): 1364-1378

Abstract

The G protein-coupled receptor (GPCR) Proteolysis Site (GPS) of cell-adhesion GPCRs and polycystic kidney disease (PKD) proteins constitutes a highly conserved autoproteolysis sequence, but its catalytic mechanism remains unknown. Here, we show that unexpectedly the ?40-residue GPS motif represents an integral part of a much larger ?320-residue domain that we termed GPCR-Autoproteolysis INducing (GAIN) domain. Crystal structures of GAIN domains from two distantly related cell-adhesion GPCRs revealed a conserved novel fold in which the GPS motif forms five ?-strands that are tightly integrated into the overall GAIN domain. The GAIN domain is evolutionarily conserved from tetrahymena to mammals, is the only extracellular domain shared by all human cell-adhesion GPCRs and PKD proteins, and is the locus of multiple human disease mutations. Functionally, the GAIN domain is both necessary and sufficient for autoproteolysis, suggesting an autoproteolytic mechanism whereby the overall GAIN domain fine-tunes the chemical environment in the GPS to catalyse peptide bond hydrolysis. Thus, the GAIN domain embodies a unique, evolutionarily ancient and widespread autoproteolytic fold whose function is likely relevant for GPCR signalling and for multiple human diseases.

View details for DOI 10.1038/emboj.2012.26

View details for Web of Science ID 000302131600005

View details for PubMedID 22333914

- A grid-enabled web service for low-resolution crystal structure refinement *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* O'Donovan, D. J., Stokes-Rees, I., Nam, Y., Blacklow, S. C., Schroeder, G. F., Brunger, A. T., Sliz, P. 2012; 68: 261-267

Abstract

Deformable elastic network (DEN) restraints have proved to be a powerful tool for refining structures from low-resolution X-ray crystallographic data sets. Unfortunately, optimal refinement using DEN restraints requires extensive calculations and is often hindered by a lack of access to sufficient computational resources. The DEN web service presented here intends to provide structural biologists with access to resources for running computationally intensive DEN refinements in parallel on the Open Science Grid, the US cyberinfrastructure. Access to the grid is provided through a simple and intuitive web interface integrated into the SBGrid Science Portal. Using this portal, refinements combined with full parameter optimization that would take many thousands of hours on standard computational resources can now be completed in several hours. An example of the successful application of DEN restraints to the human Notch1 transcriptional complex using the grid resource, and summaries of all submitted refinements, are presented as justification.

View details for DOI 10.1107/S0907444912001163

View details for Web of Science ID 000300444300008

View details for PubMedID 22349228

- Beltless Translocation Domain of Botulinum Neurotoxin A Embodies a Minimum Ion-conductive Channel *JOURNAL OF BIOLOGICAL CHEMISTRY* Fischer, A., Sambashivan, S., Brunger, A. T., Montal, M. 2012; 287 (3): 1657-1661

Abstract

Botulinum neurotoxin, the causative agent of the paralytic disease botulism, is an endopeptidase composed of a catalytic domain (or light chain (LC)) and a heavy chain (HC) encompassing the translocation domain (TD) and receptor-binding domain. Upon receptor-mediated endocytosis, the LC and TD are proposed to undergo conformational changes in the acidic endocytic environment resulting in the formation of an LC protein-conducting TD channel. The mechanism of channel formation and the conformational changes in the toxin upon acidification are important but less well understood aspects of botulinum neurotoxin intoxication. Here, we have identified a minimum channel-forming truncation of the TD, the "beltless" TD, that forms transmembrane channels with ion conduction properties similar to those of the full-length TD. At variance with the holotoxin and the HC, channel formation for both the TD and the beltless TD occurs independent of a transmembrane pH gradient. Furthermore, acidification in solution

induces moderate secondary structure changes. The subtle nature of the conformational changes evoked by acidification on the TD suggests that, in the context of the holotoxin, larger structural rearrangements and LC unfolding occur preceding or concurrent to channel formation. This notion is consistent with the hypothesis that although each domain of the holotoxin functions individually, each domain serves as a chaperone for the others.

View details for DOI 10.1074/jbc.C111.319400

View details for Web of Science ID 000299321000008

View details for PubMedID 22158863

- Synaptic proteins promote calcium-triggered fast transition from point contact to full fusion. *eLife* Diao, J., Grob, P., Cipriano, D. J., Kyoung, M., Zhang, Y., Shah, S., Nguyen, A., Padolina, M., Srivastava, A., Vrljic, M., Shah, A., Nogales, E., Chu, S., Brunger, A. T. 2012; 1

Abstract

The molecular underpinnings of synaptic vesicle fusion for fast neurotransmitter release are still unclear. Here, we used a single vesicle-vesicle system with reconstituted SNARE and synaptotagmin-1 proteoliposomes to decipher the temporal sequence of membrane states upon Ca(2+)-injection at 250-500 ?M on a 100-ms timescale. Furthermore, detailed membrane morphologies were imaged with cryo-electron microscopy before and after Ca(2+)-injection. We discovered a heterogeneous network of immediate and delayed fusion pathways. Remarkably, all instances of Ca(2+)-triggered immediate fusion started from a membrane-membrane point-contact and proceeded to complete fusion without discernible hemifusion intermediates. In contrast, pathways that involved a stable hemifusion diaphragm only resulted in fusion after many seconds, if at all. When complexin was included, the Ca(2+)-triggered fusion network shifted towards the immediate pathway, effectively synchronizing fusion, especially at lower Ca(2+)-concentration. Synaptic proteins may have evolved to select this immediate pathway out of a heterogeneous network of possible membrane fusion pathways.DOI:http://dx.doi.org/10.7554/eLife.00109.001.

View details for DOI 10.7554/eLife.00109

View details for PubMedID 23240085

- Post-Translational Modifications and Lipid Binding Profile of Insect Cell-Expressed Full-Length Mammalian Synaptotagmin 1 *BIOCHEMISTRY* Vrljic, M., Strop, P., Hill, R. C., Hansen, K. C., Chu, S., Brunger, A. T. 2011; 50 (46): 9998-10012

Abstract

Synaptotagmin 1 (Syt1) is a Ca(2+) sensor for SNARE-mediated, Ca(2+)-triggered synaptic vesicle fusion in neurons. It is composed of luminal, transmembrane, linker, and two Ca(2+)-binding (C2) domains. Here we describe expression and purification of full-length mammalian Syt1 in insect cells along with an extensive biochemical characterization of the purified protein. The expressed and purified protein is properly folded and has

increased ?-helical content compared to the C2AB fragment alone. Post-translational modifications of Syt1 were analyzed by mass spectrometry, revealing the same modifications of Syt1 that were previously described for Syt1 purified from brain extract or mammalian cell lines, along with a novel modification of Syt1, tyrosine nitration. A lipid binding screen with both full-length Syt1 and the C2AB fragments of Syt1 and Syt3 isoforms revealed new Syt1-lipid interactions. These results suggest a conserved lipid binding mechanism in which Ca(2+)-independent interactions are mediated via a lysine rich region of the C2B domain while Ca(2+)-dependent interactions are mediated via the Ca(2+)-binding loops.

View details for DOI 10.1021/bi200998y

View details for Web of Science ID 000296893700007

View details for PubMedID 21928778

- A New Generation of Crystallographic Validation Tools for the Protein Data Bank *STRUCTURE* Read, R. J., Adams, P. D., Arendall, W. B., Brunger, A. T., Emsley, P., Joosten, R. P., Kleywegt, G. J., Krissinel, E. B., Luetteke, T., Otwinowski, Z., Perrakis, A., Richardson, J. S., Sheffler, W. H., Smith, J. L., Tickle, I. J., Vriend, G., Zwart, P. H. 2011; 19 (10): 1395-1412

Abstract

This report presents the conclusions of the X-ray Validation Task Force of the worldwide Protein Data Bank (PDB). The PDB has expanded massively since current criteria for validation of deposited structures were adopted, allowing a much more sophisticated understanding of all the components of macromolecular crystals. The size of the PDB creates new opportunities to validate structures by comparison with the existing database, and the now-mandatory deposition of structure factors creates new opportunities to validate the underlying diffraction data. These developments highlighted the need for a new assessment of validation criteria. The Task Force recommends that a small set of validation data be presented in an easily understood format, relative to both the full PDB and the applicable resolution class, with greater detail available to interested users. Most importantly, we recommend that referees and editors judging the quality of structural experiments have access to a concise summary of well-established quality indicators.

View details for DOI 10.1016/j.str.2011.08.006

View details for Web of Science ID 000296125100009

View details for PubMedID 22000512

- In vitro system capable of differentiating fast Ca2+-triggered content mixing from lipid exchange for mechanistic studies of neurotransmitter release *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Kyoung, M., Srivastava, A., Zhang, Y., Diao, J., Vrljic, M., Grob, P., Nogales, E., Chu, S., Brunger, A. T. 2011; 108 (29): E304-E313

Abstract

Understanding the molecular principles of synaptic vesicle fusion is a long-

sought goal. It requires the development of a synthetic system that allows manipulations and observations not possible in vivo. Here, we report an in vitro system with reconstituted synaptic proteins that meets the long-sought goal to produce fast content release in the millisecond time regime upon Ca (2+) triggering. Our system simultaneously monitors both content and lipid exchange, and it starts from stable interacting pairs of donor and acceptor vesicles, mimicking the readily releasable pool of synaptic vesicles prior to an action potential. It differentiates between single-vesicle interaction, hemifusion, and complete fusion, the latter mimicking quantized neurotransmitter release upon exocytosis of synaptic vesicles. Prior to Ca (2+) injection, the system is in a state in which spontaneous fusion events between donor and acceptor vesicles are rare. Upon Ca(2+) injection, a rapid burst of complete fusion events emerges, followed by a biphasic decay. The present study focuses on neuronal SNAREs, the Ca(2+) sensor synaptotagmin 1, and the modulator complexin. However, other synaptic proteins could be added and their function examined. Ca(2+) triggering is cooperative, requiring the presence of synaptotagmin, whereas SNAREs alone do not produce a fast fusion burst. Manipulations of the system mimic effects observed in vivo. These results also show that neuronal SNAREs alone do not efficiently produce complete fusion, that the combination of SNAREs with synaptotagmin lowers the activation barriers to full fusion, and that complexin enhances this kinetic control.

View details for DOI 10.1073/pnas.1107900108

View details for Web of Science ID 000292876900007

View details for PubMedID 21705659

- Reintroducing Electrostatics into Macromolecular Crystallographic Refinement: Application to Neutron Crystallography and DNA Hydration *STRUCTURE* Fenn, T. D., Schnieders, M. J., Mustyakimov, M., Wu, C., Langan, P., Pande, V. S., Brunger, A. T. 2011; 19 (4): 523-533

Abstract

Most current crystallographic structure refinements augment the diffraction data with a priori information consisting of bond, angle, dihedral, planarity restraints, and atomic repulsion based on the Pauli exclusion principle. Yet, electrostatics and van der Waals attraction are physical forces that provide additional a priori information. Here, we assess the inclusion of electrostatics for the force field used for all-atom (including hydrogen) joint neutron/X-ray refinement. Two DNA and a protein crystal structure were refined against joint neutron/X-ray diffraction data sets using force fields without electrostatics or with electrostatics. Hydrogen-bond orientation/geometry favors the inclusion of electrostatics. Refinement of Z-DNA with electrostatics leads to a hypothesis for the entropic stabilization of Z-DNA that may partly explain the thermodynamics of converting the B form of DNA to its Z form. Thus, inclusion of electrostatics assists joint neutron/X-ray refinements, especially for placing and orienting hydrogen atoms.

View details for DOI 10.1016/j.str.2011.01.015

View details for Web of Science ID 000289592600011

View details for PubMedID 21481775

- Towards Structural Biology with Single Molecules Brunger, A., Strop, P., Vrljic, M., Chu, S., Weninger, K. FEDERATION AMER SOC EXP BIOL. 2011
  View details for Web of Science ID 000310708402359

- Three-dimensional molecular modeling with single molecule FRET *JOURNAL OF STRUCTURAL BIOLOGY* Brunger, A. T., Strop, P., Vrljic, M., Chu, S., Weninger, K. R. 2011; 173 (3): 497-505

  ## Abstract

  Single molecule fluorescence energy transfer experiments enable investigations of macromolecular conformation and folding by the introduction of fluorescent dyes at specific sites in the macromolecule. Multiple such experiments can be performed with different labeling site combinations in order to map complex conformational changes or interactions between multiple molecules. Distances that are derived from such experiments can be used for determination of the fluorophore positions by triangulation. When combined with a known structure of the macromolecule(s) to which the fluorophores are attached, a three-dimensional model of the system can be determined. However, care has to be taken to properly derive distance from fluorescence energy transfer efficiency and to recognize the systematic or random errors for this relationship. Here we review the experimental and computational methods used for three-dimensional modeling based on single molecule fluorescence resonance transfer, and describe recent progress in pushing the limits of this approach to macromolecular complexes.

  View details for DOI 10.1016/j.jsb.2010.09.004

  View details for Web of Science ID 000287681200010

  View details for PubMedID 20837146

- A smooth and differentiable bulk-solvent model for macromolecular diffraction *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Fenn, T. D., Schnieders, M. J., Brunger, A. T. 2010; 66: 1024-1031

  ## Abstract

  Inclusion of low-resolution data in macromolecular crystallography requires a model for the bulk solvent. Previous methods have used a binary mask to accomplish this, which has proven to be very effective, but the mask is discontinuous at the solute-solvent boundary (i.e. the mask value jumps from zero to one) and is not differentiable with respect to atomic parameters. Here, two algorithms are introduced for computing bulk-solvent models using either a polynomial switch or a smoothly thresholded product of Gaussians, and both models are shown to be efficient and differentiable with respect to atomic coordinates. These alternative bulk-solvent models offer algorithmic improvements, while showing similar agreement of the model with the observed amplitudes relative to the binary model as monitored using R, R (free) and differences between experimental and model phases. As with the standard solvent models, the alternative models improve the agreement primarily with lower resolution (>6 A) data versus no bulk solvent. The

models are easily implemented into crystallographic software packages and can be used as a general method for bulk-solvent correction in macromolecular crystallography.

View details for DOI 10.1107/S0907444910031045

View details for Web of Science ID 000281635500008

View details for PubMedID 20823553

- Iterative Structure-Based Peptide-Like Inhibitor Design against the Botulinum Neurotoxin Serotype A *PLOS ONE* Zuniga, J. E., Hammill, J. T., Drory, O., Nuss, J. E., Burnett, J. C., Gussio, R., Wipf, P., Bavari, S., Brunger, A. T. 2010; 5 (6)

Abstract

The botulinum neurotoxin serotype A light chain (BoNT/A LC) protease is the catalytic component responsible for the neuroparalysis that is characteristic of the disease state botulism. Three related peptide-like molecules (PLMs) were designed using previous information from co-crystal structures, synthesized, and assayed for in vitro inhibition against BoNT/A LC. Our results indicate these PLMS are competitive inhibitors of the BoNT/A LC protease and their K(i) values are in the nM-range. A co-crystal structure for one of these inhibitors was determined and reveals that the PLM, in accord with the goals of our design strategy, simultaneously involves both ionic interactions via its P1 residue and hydrophobic contacts by means of an aromatic group in the P2' position. The PLM adopts a helical conformation similar to previously determined co-crystal structures of PLMs, although there are also major differences to these other structures such as contacts with specific BoNT/A LC residues. Our structure further demonstrates the remarkable plasticity of the substrate binding cleft of the BoNT/A LC protease and provides a paradigm for iterative structure-based design and development of BoNT/A LC inhibitors.

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View details for Web of Science ID 000279370000009

View details for PubMedID 20614028

- Polarizable Atomic Multipole X-Ray Refinement: Hydration Geometry and Application to Macromolecules *BIOPHYSICAL JOURNAL* Fenn, T. D., Schnieders, M. J., Brunger, A. T., Pande, V. S. 2010; 98 (12): 2984-2992

Abstract

We recently developed a polarizable atomic multipole refinement method assisted by the AMOEBA force field for macromolecular crystallography. Compared to standard refinement procedures, the method uses a more rigorous treatment of x-ray scattering and electrostatics that can significantly improve the resultant information contained in an atomic model. We applied this method to high-resolution lysozyme and trypsin data sets, and validated its utility for precisely describing biomolecular electron density, as indicated by a 0.4-0.6% decrease in the R- and R(free)-values, and a corresponding decrease in the relative energy of 0.4-0.8 Kcal/mol/residue. The re-

refinements illustrate the ability of force-field electrostatics to orient water networks and catalytically relevant hydrogens, which can be used to make predictions regarding active site function, activity, and protein-ligand interaction energies. Re-refinement of a DNA crystal structure generates the zigzag spine pattern of hydrogen bonding in the minor groove without manual intervention. The polarizable atomic multipole electrostatics model implemented in the AMOEBA force field is applicable and informative for crystal structures solved at any resolution.

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View details for Web of Science ID 000278913500027

View details for PubMedID 20550911

- The Longin SNARE VAMP7/TI-VAMP Adopts a Closed Conformation
JOURNAL OF BIOLOGICAL CHEMISTRY Vivona, S., Liu, C. W., Strop, P., Rossi, V., Filippini, F., Brunger, A. T. 2010; 285 (23): 17965-17973

Abstract

SNARE protein complexes are key mediators of exocytosis by juxtaposing opposing membranes, leading to membrane fusion. SNAREs generally consist of one or two core domains that can form a four-helix bundle with other SNARE core domains. Some SNAREs, such as syntaxin target-SNAREs and longin vesicular-SNAREs, have independent, folded N-terminal domains that can interact with their respective SNARE core domains and thereby affect the kinetics of SNARE complex formation. This autoinhibition mechanism is believed to regulate the role of the longin VAMP7/TI-VAMP in neuronal morphogenesis. Here we use nuclear magnetic resonance spectroscopy to study the longin-SNARE core domain interaction for VAMP7. Using complete backbone resonance assignments, chemical shift perturbations analysis, and hydrogen/deuterium exchange experiments, we conclusively show that VAMP7 adopts a preferentially closed conformation in solution. Taken together, the closed conformation of longins is conserved, in contrast to the syntaxin family of SNAREs for which mixtures of open and closed states have been observed. This may indicate different regulatory mechanisms for SNARE complexes containing syntaxins and longins, respectively.

View details for DOI 10.1074/jbc.M110.120972

View details for Web of Science ID 000278133400072

View details for PubMedID 20378544

- Neurexins Physically and Functionally Interact with GABA(A) Receptors
NEURON Zhang, C., Atasoy, D., Arac, D., Yang, X., Fucillo, M. V., Robison, A. J., Ko, J., Brunger, A. T., Sudhof, T. C. 2010; 66 (3): 403-416

Abstract

Neurexins are presynaptic cell-adhesion molecules that form trans-synaptic complexes with postsynaptic neuroligins. When overexpressed in nonneuronal cells, neurexins induce formation of postsynaptic specializations in cocultured neurons, suggesting that neurexins are synaptogenic. However, we find that when overexpressed in neurons,

neurexins do not increase synapse density, but instead selectively suppressed GABAergic synaptic transmission without decreasing GABAergic synapse numbers. This suppression was mediated by all subtypes of neurexins tested, in a cell-autonomous and neuroligin-independent manner. Strikingly, addition of recombinant neurexin to cultured neurons at submicromolar concentrations induced the same suppression of GABAergic synaptic transmission as neurexin overexpression. Moreover, experiments with native brain proteins and purified recombinant proteins revealed that neurexins directly and stoichiometrically bind to GABA(A) receptors, suggesting that they decrease GABAergic synaptic responses by interacting with GABA(A) receptors. Our findings suggest that besides their other well-documented interactions, presynaptic neurexins directly act on postsynaptic GABA(A) receptors, which may contribute to regulate the excitatory/inhibitory balance in brain.

View details for DOI 10.1016/j.neuron.2010.04.008

View details for Web of Science ID 000277825200009

View details for PubMedID 20471353

- Super-resolution biomolecular crystallography with low-resolution data
  NATURE Schroeder, G. F., Levitt, M., Brunger, A. T. 2010; 464 (7292): 1218-U146

Abstract

X-ray diffraction plays a pivotal role in the understanding of biological systems by revealing atomic structures of proteins, nucleic acids and their complexes, with much recent interest in very large assemblies like the ribosome. As crystals of such large assemblies often diffract weakly (resolution worse than 4 A), we need methods that work at such low resolution. In macromolecular assemblies, some of the components may be known at high resolution, whereas others are unknown: current refinement methods fail as they require a high-resolution starting structure for the entire complex. Determining the structure of such complexes, which are often of key biological importance, should be possible in principle as the number of independent diffraction intensities at a resolution better than 5 A generally exceeds the number of degrees of freedom. Here we introduce a method that adds specific information from known homologous structures but allows global and local deformations of these homology models. Our approach uses the observation that local protein structure tends to be conserved as sequence and function evolve. Cross-validation with R(free) (the free R-factor) determines the optimum deformation and influence of the homology model. For test cases at 3.5-5 A resolution with known structures at high resolution, our method gives significant improvements over conventional refinement in the model as monitored by coordinate accuracy, the definition of secondary structure and the quality of electron density maps. For re-refinements of a representative set of 19 low-resolution crystal structures from the Protein Data Bank, we find similar improvements. Thus, a structure derived from low-resolution diffraction data can have quality similar to a high-resolution structure. Our method is applicable to the study of weakly diffracting crystals using X-ray micro-diffraction as well as data from new X-

ray light sources. Use of homology information is not restricted to X-ray crystallography and cryo-electron microscopy: as optical imaging advances to subnanometre resolution, it can use similar tools.

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View details for Web of Science ID 000276891100043

View details for PubMedID 20376006

- Molecular mechanism of the synaptotagmin-SNARE interaction in Ca2+-triggered vesicle fusion *NATURE STRUCTURAL & MOLECULAR BIOLOGY* Vrljic, M., Strop, P., Ernst, J. A., Sutton, R. B., Chu, S., Brunger, A. T. 2010; 17 (3): 325-U92

Abstract

In neurons, SNAREs, synaptotagmin and other factors catalyze Ca(2+)-triggered fusion of vesicles with the plasma membrane. The molecular mechanism of this process, especially the interaction between synaptotagmin and SNAREs, remains an enigma. Here we characterized this interaction by single-molecule fluorescence microscopy and crystallography. The two rigid Ca(2+)-binding domains of synaptotagmin 3 (Syt3) undergo large relative motions in solution. Interaction with SNARE complex amplifies a particular state of the two domains that is further enhanced by Ca(2+). This state is represented by the first SNARE-induced Ca(2+)-bound crystal structure of a synaptotagmin fragment containing both domains. The arrangement of the Ca(2+)-binding loops of this structure of Syt3 matches that of SNARE-bound Syt1, suggesting a conserved feature of synaptotagmins. The loops resemble the membrane-interacting loops of certain viral fusion proteins in the postfusion state, suggesting unexpected similarities between both fusion systems.

View details for DOI 10.1038/nsmb.1764

View details for Web of Science ID 000275182700013

View details for PubMedID 20173762

- Single-molecule FRET-derived model of the synaptotagmin 1-SNARE fusion complex *NATURE STRUCTURAL & MOLECULAR BIOLOGY* Choi, U. B., Strop, P., Vrljic, M., Chu, S., Brunger, A. T., Weninger, K. R. 2010; 17 (3): 318-U84

Abstract

Synchronous neurotransmission is triggered when Ca(2+) binds to synaptotagmin 1 (Syt1), a synaptic-vesicle protein that interacts with SNAREs and membranes. We used single-molecule fluorescence resonance energy transfer (FRET) between synaptotagmin's two C2 domains to determine that their conformation consists of multiple states with occasional transitions, consistent with domains in random relative motion. SNARE binding results in narrower intrasynaptotagmin FRET distributions and less frequent transitions between states. We obtained an experimentally determined model of the elusive Syt1-SNARE complex using a multibody docking approach with 34 FRET-derived distances as restraints. The Ca (2+)-binding loops point away from the SNARE complex, so they may

interact with the same membrane. The loop arrangement is similar to that of the crystal structure of SNARE-induced Ca(2+)-bound Syt3, suggesting a common mechanism by which the interaction between synaptotagmins and SNAREs aids in Ca(2+)-triggered fusion.

View details for DOI 10.1038/nsmb.1763

View details for Web of Science ID 000275182700012

View details for PubMedID 20173763

- Warren L. DeLano 21 June 1972-3 November 2009 OBITUARY *NATURE STRUCTURAL & MOLECULAR BIOLOGY* Brunger, A. T., Wells, J. A. 2009; 16 (12): 1202-1203
  View details for Web of Science ID 000272609200002

  View details for PubMedID 19956203

- Neuroligin-1 performs neurexin-dependent and neurexin-independent functions in synapse validation *EMBO JOURNAL* Ko, J., Zhang, C., Arac, D., Boucard, A. A., Brunger, A. T., Suedhof, T. C. 2009; 28 (20): 3244-3255

Abstract

Postsynaptic neuroligins are thought to perform essential functions in synapse validation and synaptic transmission by binding to, and dimerizing, presynaptic alpha- and beta-neurexins. To test this hypothesis, we examined the functional effects of neuroligin-1 mutations that impair only alpha-neurexin binding, block both alpha- and beta-neurexin binding, or abolish neuroligin-1 dimerization. Abolishing alpha-neurexin binding abrogated neuroligin-induced generation of neuronal synapses onto transfected non-neuronal cells in the so-called artificial synapse-formation assay, even though beta-neurexin binding was retained. Thus, in this assay, neuroligin-1 induces apparent synapse formation by binding to presynaptic alpha-neurexins. In transfected neurons, however, neither alpha- nor beta-neurexin binding was essential for the ability of postsynaptic neuroligin-1 to dramatically increase synapse density, suggesting a neurexin-independent mechanism of synapse formation. Moreover, neuroligin-1 dimerization was not required for either the non-neuronal or the neuronal synapse-formation assay. Nevertheless, both alpha-neurexin binding and neuroligin-1 dimerization were essential for the increase in apparent synapse size that is induced by neuroligin-1 in transfected neurons. Thus, neuroligin-1 performs diverse synaptic functions by mechanisms that include as essential components of alpha-neurexin binding and neuroligin dimerization, but extend beyond these activities.

View details for DOI 10.1038/emboj.2009.249

View details for Web of Science ID 000271008200016

View details for PubMedID 19730411

- Receptor and substrate interactions of clostridial neurotoxins *TOXICON* Brunger, A. T., Rummel, A. 2009; 54 (5): 550-560

Abstract

The high potency of clostridial neurotoxins relies predominantly on their neurospecific binding and specific hydrolysis of SNARE proteins. Their multi-step mode of mechanism can be ascribed to their multi-domain three-dimensional structure. The C-terminal H(CC)-domain interacts subsequently with complex polysialo-gangliosides such as GT1b and a synaptic vesicle protein receptor via two neighbouring binding sites, resulting in highly specific uptake of the neurotoxins at synapses of cholinergic motoneurons. After its translocation the enzymatically active light chain specifically hydrolyses specific SNARE proteins, preventing SNARE complex assembly and thereby blocking exocytosis of neurotransmitter.

View details for DOI 10.1016/j.toxicon.2008.12.027

View details for Web of Science ID 000269965400002

View details for PubMedID 19268493

- Polarizable atomic multipole X-ray refinement: application to peptide crystals *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Schnieders, M. J., Fenn, T. D., Pande, V. S., Brunger, A. T. 2009; 65: 952-965

Abstract

Recent advances in computational chemistry have produced force fields based on a polarizable atomic multipole description of biomolecular electrostatics. In this work, the Atomic Multipole Optimized Energetics for Biomolecular Applications (AMOEBA) force field is applied to restrained refinement of molecular models against X-ray diffraction data from peptide crystals. A new formalism is also developed to compute anisotropic and aspherical structure factors using fast Fourier transformation (FFT) of Cartesian Gaussian multipoles. Relative to direct summation, the FFT approach can give a speedup of more than an order of magnitude for aspherical refinement of ultrahigh-resolution data sets. Use of a sublattice formalism makes the method highly parallelizable. Application of the Cartesian Gaussian multipole scattering model to a series of four peptide crystals using multipole coefficients from the AMOEBA force field demonstrates that AMOEBA systematically underestimates electron density at bond centers. For the trigonal and tetrahedral bonding geometries common in organic chemistry, an atomic multipole expansion through hexadecapole order is required to explain bond electron density. Alternatively, the addition of interatomic scattering (IAS) sites to the AMOEBA-based density captured bonding effects with fewer parameters. For a series of four peptide crystals, the AMOEBA-IAS model lowered R (free) by 20-40% relative to the original spherically symmetric scattering model.

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View details for Web of Science ID 000269350000009

View details for PubMedID 19690373

- Mechanistic insights into active site-associated polyubiquitination by the ubiquitin-conjugating enzyme Ube2g2 *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Li, W.,

Tu, D., Li, L., Wollert, T., Ghirlando, R., Brunger, A. T., Ye, Y. 2009; 106 (10): 3722-3727

Abstract

Lys-48-linked polyubiquitination regulates a variety of cellular processes by targeting ubiquitinated proteins to the proteasome for degradation. Although polyubiquitination had been presumed to occur by transferring ubiquitin molecules, one at a time, from an E2 active site to a substrate, we recently showed that the endoplasmic reticulum-associated RING finger ubiquitin ligase gp78 can mediate the preassembly of Lys-48-linked polyubiquitin chains on the catalytic cysteine of its cognate E2 Ube2g2 and subsequent transfer to a substrate. Active site-linked polyubiquitin chains are detected in cells on Ube2g2 and its yeast homolog Ubc7p, but how these chains are assembled is unclear. Here, we show that gp78 forms an oligomer via 2 oligomerization sites, one of which is a hydrophobic segment located in the gp78 cytosolic domain. We further demonstrate that a gp78 oligomer can simultaneously associate with multiple Ube2g2 molecules. This interaction is mediated by a novel Ube2g2 surface distinct from the predicted RING binding site. Our data suggest that a large gp78-Ube2g2 heterooligomer brings multiple Ube2g2 molecules into close proximity, allowing ubiquitin moieties to be transferred between neighboring Ube2g2s to form active site-linked polyubiquitin chains.

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View details for Web of Science ID 000264036900017

View details for PubMedID 19223579

- SINGLE MOLECULE STUDIES OF THE SYNAPTIC VESICLE FUSION MACHINERY Brunger, A. T., Weninger, K., Vrljic, M., Choi, U. B., Bowen, M. A., Chu, S. WILEY-BLACKWELL. 2009: 55-55
  View details for Web of Science ID 000263336800116

- X-ray structure determination at low resolution *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Brunger, A. T., DeLaBarre, B., Davies, J. M., Weis, W. I. 2009; 65: 128-133

Abstract

As an example of structure determination in the 3.5-4.5 A resolution range, crystal structures of the ATPase p97/VCP, consisting of an N-terminal domain followed by a tandem pair of ATPase domains (D1 and D2), are discussed. The structures were originally solved by molecular replacement with the high-resolution structure of the N-D1 fragment of p97/VCP, whereas the D2 domain was manually built using its homology to the D1 domain as a guide. The structure of the D2 domain alone was subsequently solved at 3 A resolution. The refined model of D2 and the high-resolution structure of the N-D1 fragment were then used as starting models for re-refinement against the low-resolution diffraction data for full-length p97. The re-refined full-length models showed significant improvement in both secondary structure and R values. The free R values dropped by as much as 5% compared with the original structure refinements, indicating that refinement is meaningful at low resolution and that there is information in the diffraction data even at

approximately 4 A resolution that objectively assesses the quality of the model. It is concluded that de novo model building is problematic at low resolution and refinement should start from high-resolution crystal structures whenever possible.

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View details for Web of Science ID 000263557900004

View details for PubMedID 19171967

- Single-Molecule Studies of the Neuronal SNARE Fusion Machinery *ANNUAL REVIEW OF BIOCHEMISTRY* Brunger, A. T., Weninger, K., Bowen, M., Chu, S. 2009; 78: 903-928

Abstract

SNAREs are essential components of the machinery for Ca(2+)-triggered fusion of synaptic vesicles with the plasma membrane, resulting in neurotransmitter release into the synaptic cleft. Although much is known about their biophysical and structural properties and their interactions with accessory proteins such as the Ca(2+) sensor synaptotagmin, their precise role in membrane fusion remains an enigma. Ensemble studies of liposomes with reconstituted SNAREs have demonstrated that SNAREs and accessory proteins can trigger lipid mixing/fusion, but the inability to study individual fusion events has precluded molecular insights into the fusion process. Thus, this field is ripe for studies with single-molecule methodology. In this review, we discuss applications of single-molecule approaches to observe reconstituted SNAREs, their complexes, associated proteins, and their effect on biological membranes. Some of the findings are provocative, such as the possibility of parallel and antiparallel SNARE complexes or of vesicle docking with only syntaxin and synaptobrevin, but have been confirmed by other experiments.

View details for DOI 10.1146/annurev.biochem.77.070306.103621

View details for Web of Science ID 000268069200032

View details for PubMedID 19489736

- A Potent Peptidomimetic Inhibitor of Botulinum Neurotoxin Serotype A Has a Very Different Conformation than SNAP-25 Substrate *STRUCTURE* Zuniga, J. E., Schmidt, J. J., Fenn, T., Burnett, J. C., Arac, D., Gussio, R., Stafford, R. G., Badie, S. S., Bavari, S., Brunger, A. T. 2008; 16 (10): 1588-1597

Abstract

Botulinum neurotoxin serotype A is the most lethal of all known toxins. Here, we report the crystal structure, along with SAR data, of the zinc metalloprotease domain of BoNT/A bound to a potent peptidomimetic inhibitor (K(i)=41 nM) that resembles the local sequence of the SNAP-25 substrate. Surprisingly, the inhibitor adopts a helical conformation around the cleavage site, in contrast to the extended conformation of the native substrate. The backbone of the inhibitor's P1 residue displaces the putative catalytic water molecule and concomitantly interacts with the "proton shuttle" E224. This mechanism of inhibition is aided by residue contacts in the conserved S1' pocket of the substrate binding cleft and by the induction of

new hydrophobic pockets, which are not present in the apo form, especially for the P2' residue of the inhibitor. Our inhibitor is specific for BoNT/A as it does not inhibit other BoNT serotypes or thermolysin.

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View details for Web of Science ID 000259930800017

View details for PubMedID 18940613

- Highly specific interactions between botulinum neurotoxins and synaptic vesicle proteins *CELLULAR AND MOLECULAR LIFE SCIENCES* Brunger, A. T., Jin, R., Breidenbach, M. A. 2008; 65 (15): 2296-2306

Abstract

Despite its extreme toxicity, botulinum neurotoxin is widely utilized in low doses as a treatment for several neurological disorders; higher doses cause the neuroparalytic syndrome botulism. The toxin blocks neurotransmitter release by preferentially attaching to pre-synaptic membrane receptors at neuromuscular junctions and subsequently delivering a Zn2+-dependent protease component to presynaptic neuronal cytosol. These highly specialized enzymes exclusively hydrolyze peptide bonds within SNARE (soluble N-ethylmaleiamide sensitive factor attachment protein receptor) proteins. In this review we discuss the structural basis for botulinum toxin's exquisite specificity for its neuronal cell-surface receptors and intracellular SNARE targets.

View details for DOI 10.1007/s00018-008-8088-0

View details for Web of Science ID 000258275900005

View details for PubMedID 18425411

- Botulinum neurotoxin interactions with substrate Brunger, A. PERGAMON-ELSEVIER SCIENCE LTD. 2008: 2-2
  View details for Web of Science ID 000257192200004

- Improved structures of full-length p97, an AAA ATPase: Implications for mechanisms of nucleotide-dependent conformational change *STRUCTURE* Davies, J. M., Brunger, A. T., Weis, W. I. 2008; 16 (5): 715-726

Abstract

The ATPases associated with various cellular activities (AAA) protein p97 has been implicated in a variety of cellular processes, including endoplasmic reticulum-associated degradation and homotypic membrane fusion. p97 belongs to a subgroup of AAA proteins that contains two nucleotide binding domains, D1 and D2. We determined the crystal structure of D2 at 3.0 A resolution. This model enabled rerefinement of full-length p97 in different nucleotide states against previously reported low-resolution diffraction data to significantly improved R values and Ramachandran statistics. Although the overall fold remained similar, there are significant improvements, especially around the D2 nucleotide binding site. The rerefinement illustrates the importance of knowledge of high-resolution structures of fragments covering most of the whole molecule. The structures suggest that nucleotide hydrolysis is transformed into larger conformational changes by

pushing of one D2 domain by its neighbor in the hexamer, and transmission of nucleotide-state information through the D1-D2 linker to displace the N-terminal, effector binding domain.

View details for DOI 10.1016/j.str.2008.02.010

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View details for PubMedID 18462676

- Accessory proteins stabilize the acceptor complex for synaptobrevin, the 1 : 1 syntaxin/SNAP-25 complex *STRUCTURE* Weninger, K., Bowen, M. E., Choi, U. B., Chu, S., Brunger, A. T. 2008; 16 (2): 308-320

Abstract

Syntaxin/SNAP-25 interactions precede assembly of the ternary SNARE complex that is essential for neurotransmitter release. This binary complex has been difficult to characterize by bulk methods because of the prevalence of a 2:1 dead-end species. Here, using single-molecule fluorescence, we find the structure of the 1:1 syntaxin/SNAP-25 binary complex is variable, with states changing on the second timescale. One state corresponds to a parallel three-helix bundle, whereas other states show one of the SNAP-25 SNARE domains dissociated. Adding synaptobrevin suppresses the dissociated helix states. Remarkably, upon addition of complexin, Munc13, Munc18, or synaptotagmin, a similar effect is observed. Thus, the 1:1 binary complex is a dynamic acceptor for synaptobrevin binding, and accessory proteins stabilize this acceptor. In the cellular environment the binary complex is actively maintained in a configuration where it can rapidly interact with synaptobrevin, so formation is not likely a limiting step for neurotransmitter release.

View details for DOI 10.1016/j.str.2007.12.010

View details for Web of Science ID 000253219400015

View details for PubMedID 18275821

- Rab and arl GTPase family members cooperate in the localization of the golgin GCC185 *CELL* Burguete, A. S., Fenn, T. D., Brunger, A. T., Pfeffer, S. R. 2008; 132 (2): 286-298

Abstract

GCC185 is a large coiled-coil protein at the trans Golgi network that is required for receipt of transport vesicles inbound from late endosomes and for anchoring noncentrosomal microtubules that emanate from the Golgi. Here, we demonstrate that recruitment of GCC185 to the Golgi is mediated by two Golgi-localized small GTPases of the Rab and Arl families. GCC185 binds Rab6, and mutation of residues needed for Rab binding abolishes Golgi localization. The crystal structure of Rab6 bound to the GCC185 Rab-binding domain reveals that Rab6 recognizes a two-fold symmetric surface on a coiled coil immediately adjacent to a C-terminal GRIP domain. Unexpectedly, Rab6 binding promotes association of Arl1 with the GRIP domain. We present a structure-derived model for dual GTPase membrane attachment that highlights the potential ability of Rab GTPases to reach binding partners at a significant distance from the membrane via their

unstructured and membrane-anchored, unstructured and membrane-anchored, hypervariable domains.

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View details for Web of Science ID 000253427700014

View details for PubMedID 18243103

- The structure of the yeast plasma membrane SNARE complex reveals destabilizing water-filled cavities *JOURNAL OF BIOLOGICAL CHEMISTRY* Strop, P., Kaiser, S. E., Vrljic, M., Brunger, A. T. 2008; 283 (2): 1113-1119

Abstract

SNARE proteins form a complex that leads to membrane fusion between vesicles, organelles, and plasma membrane in all eukaryotic cells. We report the 1.7A resolution structure of the SNARE complex that mediates exocytosis at the plasma membrane in the yeast Saccharomyces cerevisiae. Similar to its neuronal and endosomal homologues, the S. cerevisiae SNARE complex forms a parallel four-helix bundle in the center of which is an ionic layer. The S. cerevisiae SNARE complex exhibits increased helix bending near the ionic layer, contains water-filled cavities in the complex core, and exhibits reduced thermal stability relative to mammalian SNARE complexes. Mutagenesis experiments suggest that the water-filled cavities contribute to the lower stability of the S. cerevisiae complex.

View details for DOI 10.1074/jbc.M707912200

View details for Web of Science ID 000252128100051

View details for PubMedID 17956869

- Structures of neuroligin-1 and the Neuroligin-l/Neurexin-1 beta complex reveal specificprotein-protein and protein-Ca2+ interactions *NEURON* Arac, D., Boucard, A. A., Ozkan, E., Strop, P., Newell, E., Sudhof, T. C., Brunger, A. T. 2007; 56 (6): 992-1003

Abstract

Neurexins and neuroligins provide trans-synaptic connectivity by the Ca2+-dependent interaction of their alternatively spliced extracellular domains. Neuroligins specify synapses in an activity-dependent manner, presumably by binding to neurexins. Here, we present the crystal structures of neuroligin-1 in isolation and in complex with neurexin-1 beta. Neuroligin-1 forms a constitutive dimer, and two neurexin-1 beta monomers bind to two identical surfaces on the opposite faces of the neuroligin-1 dimer to form a heterotetramer. The neuroligin-1/neurexin-1 beta complex exhibits a nanomolar affinity and includes a large binding interface that contains bound Ca2+. Alternatively spliced sites in neurexin-1 beta and in neuroligin-1 are positioned nearby the binding interface, explaining how they regulate the interaction. Structure-based mutations of neuroligin-1 at the interface disrupt binding to neurexin-1 beta, but not the folding of neuroligin-1 and confirm the validity of the binding interface of the neuroligin-1/neurexin-1 beta complex. Our results provide molecular insights for understanding the role of cell-adhesion proteins in synapse function.

View details for DOI 10.1016/j.neuron.2007.12.002

View details for Web of Science ID 000252138000009

View details for PubMedID 18093522

- Transglutaminase 2 undergoes a large conformational change upon activation *PLOS BIOLOGY* Pinkas, D. M., Strop, P., Brunger, A. T., Khosla, C. 2007; 5 (12): 2788-2796

Abstract

Human transglutaminase 2 (TG2), a member of a large family of enzymes that catalyze protein crosslinking, plays an important role in the extracellular matrix biology of many tissues and is implicated in the gluten-induced pathogenesis of celiac sprue. Although vertebrate transglutaminases have been studied extensively, thus far all structurally characterized members of this family have been crystallized in conformations with inaccessible active sites. We have trapped human TG2 in complex with an inhibitor that mimics inflammatory gluten peptide substrates and have solved, at 2-A resolution, its x-ray crystal structure. The inhibitor stabilizes TG2 in an extended conformation that is dramatically different from earlier transglutaminase structures. The active site is exposed, revealing that catalysis takes place in a tunnel, bridged by two tryptophan residues that separate acyl-donor from acyl-acceptor and stabilize the tetrahedral reaction intermediates. Site-directed mutagenesis was used to investigate the acyl-acceptor side of the tunnel, yielding mutants with a marked increase in preference for hydrolysis over transamidation. By providing the ability to visualize this activated conformer, our results create a foundation for understanding the catalytic as well as the non-catalytic roles of TG2 in biology, and for dissecting the process by which the autoantibody response to TG2 is induced in celiac sprue patients.

View details for DOI 10.1371/journal.pbio.0050327

View details for Web of Science ID 000251874900010

View details for PubMedID 18092889

- Combining efficient conformational sampling with a deformable elastic network model facilitates structure refinement at low resolution *STRUCTURE* Schroeder, G. F., Brunger, A. T., Levitt, M. 2007; 15 (12): 1630-1641

Abstract

Structural studies of large proteins and protein assemblies are a difficult and pressing challenge in molecular biology. Experiments often yield only low-resolution or sparse data that are not sufficient to fully determine atomistic structures. We have developed a general geometry-based algorithm that efficiently samples conformational space under constraints imposed by low-resolution density maps obtained from electron microscopy or X-ray crystallography experiments. A deformable elastic network (DEN) is used to restrain the sampling to prior knowledge of an approximate structure. The DEN restraints dramatically reduce over-fitting, especially at low resolution. Cross-validation is used to optimally weight the structural information and experimental data. Our algorithm is robust even for noise-added density maps and has a large radius of convergence for our test case. The DEN

restraints can also be used to enhance reciprocal space simulated annealing refinement.

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View details for PubMedID 18073112

- Structure and function of the yeast U-box-containing ubiquitin ligase Ufd2p *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Tu, D., Li, W., Ye, Y., Brunger, A. T. 2007; 104 (40): 15599-15606

Abstract

Proteins conjugated by Lys-48-linked polyubiquitin chains are preferred substrates of the eukaryotic proteasome. Polyubiquitination requires an activating enzyme (E1), a conjugating enzyme (E2), and a ligase (E3). Occasionally, these enzymes only assemble short ubiquitin oligomers, and their extension to full length involves a ubiquitin elongating factor termed E4. Ufd2p, as the first E4 identified to date, is involved in the degradation of misfolded proteins of the endoplasmic reticulum and of a ubiquitin-beta-GAL fusion substrate in Saccharomyces cerevisiae. The mechanism of action of Ufd2p is unknown. Here we describe the crystal structure of the full-length yeast Ufd2p protein. Ufd2p has an elongated shape consisting of several irregular Armadillo-like repeats with two helical hairpins protruding from it and a U-box domain flexibly attached to its C terminus. The U-box of Ufd2p has a fold similar to that of the RING (Really Interesting New Gene) domain that is present in certain ubiquitin ligases. Accordingly, Ufd2p has all of the hallmarks of a RING finger-containing ubiquitin ligase: it associates with its cognate E2 Ubc4p via its U-box domain and catalyzes the transfer of ubiquitin from the E2 active site to Ufd2p itself or to an acceptor ubiquitin molecule to form unanchored diubiquitin oligomers. Thus, Ufd2p can function as a bona fide E3 ubiquitin ligase to promote ubiquitin chain elongation on a substrate.

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View details for Web of Science ID 000249942700004

View details for PubMedID 17890322

- Structural and biochemical studies of botulinum neurotoxin serotype C1 light chain protease: Implications for dual substrate specificity *BIOCHEMISTRY* Jin, R., Sikorra, S., Stegmann, C. M., Pich, A., Binz, T., Brunger, A. T. 2007; 46 (37): 10685-10693

Abstract

Clostridial neurotoxins are the causative agents of the neuroparalytic disease botulism and tetanus. They block neurotransmitter release through specific proteolysis of one of the three soluble N-ethylmaleimide-sensitive-factor attachment protein receptors (SNAREs) SNAP-25, syntaxin, and synaptobrevin, which constitute part of the synaptic vesicle fusion machinery. The catalytic component of the clostridial neurotoxins is their light chain (LC), a Zn^{2+} endopeptidase. There are seven structurally and

functionally related botulinum neurotoxins (BoNTs), termed serotype A to G, and tetanus neurotoxin (TeNT). Each of them exhibits unique specificity for their target SNAREs and peptide bond(s) they cleave. The mechanisms of action for substrate recognition and target cleavage are largely unknown. Here, we report structural and biochemical studies of BoNT/C1-LC, which is unique among BoNTs in that it exhibits dual specificity toward both syntaxin and SNAP-25. A distinct pocket (S1') near the active site likely achieves the correct register for the cleavage site by only allowing Ala as the P1' residue for both SNAP-25 and syntaxin. Mutations of this SNAP-25 residue dramatically reduce enzymatic activity. The remote alpha-exosite that was previously identified in the complex of BoNT/A-LC and SNAP-25 is structurally conserved in BoNT/C1. However, mutagenesis experiments show that the alpha-exosite of BoNT/C1 plays a less stringent role in substrate discrimination in comparison to that of BoNT/A, which could account for its dual substrate specificity.

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View details for Web of Science ID 000249433100028

View details for PubMedID 17718519

- Botulinum neurotoxin heavy chain belt as an intramolecular chaperone for the light chain *PLOS PATHOGENS* Brunger, A. T., Breidenbach, M. A., Jin, R., Fischer, A., Santos, J. S., Montal, M. 2007; 3 (9): 1191-1194
  View details for DOI 10.1371/journal.ppat.0020113.g001

  View details for Web of Science ID 000249768300002

  View details for PubMedID 17907800

- Crystal structure of a hyperactive Escherichia coli glycerol kinase mutant Gly230 -> Asp obtained using microfluidic crystallization devices *BIOCHEMISTRY* Anderson, M. J., DeLaBarre, B., Raghunathan, A., Palsson, B. O., Brunger, A. T., Quake, S. R. 2007; 46 (19): 5722-5731

Abstract

The crystal structure of an Escherichia coli glycerol kinase mutant Gly230 --> Asp (GKG230D) was determined to 2.0 A resolution using a microfluidics based crystallization platform. The crystallization strategy involved a suite of microfluidic devices that characterized the solubility trends of GKG230D, performed nanoliter volume free interface diffusion crystallization experiments, and produced diffraction-quality crystals for in situ data collection. GKG230D displays increased enzymatic activity and decreased allosteric regulation by the glycolytic pathway intermediate fructose 1,6-bisphosphate (FBP) compared to wild-type GK (GKWT). Structural analysis revealed that the decreased allosteric regulation is a result of the altered FBP binding loop conformations in GKG230D that interfere with the wild-type FBP binding site. The altered FBP binding loop conformations in GKG230D are supported through a series of intramolecular loop interactions. The appearance of Asp230 in the FBP binding loops also repositions the wild-type FBP binding residues away from the FBP binding site. Light scattering analysis confirmed GKG230D is a dimer and is resistant to tetramer formation in the presence of FBP, whereas GKWT dimers are converted into

putatively inactive tetramers in the presence of FBP. GKG230D also provides the first structural evidence for multiple GK monomer conformations in the presence of glycerol and in the absence of a nucleotide substrate and verifies that glycerol binding is not responsible for locking GK into the closed conformation necessary for GK activity.

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View details for Web of Science ID 000246283600010

View details for PubMedID 17441732

- A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate *NATURE* Li, W., Tu, D., Brunger, A. T., Ye, Y. 2007; 446 (7133): 333-337

Abstract

In eukaryotic cells, many short-lived proteins are conjugated with Lys 48-linked ubiquitin chains and degraded by the proteasome. Ubiquitination requires an activating enzyme (E1), a conjugating enzyme (E2) and a ligase (E3). Most ubiquitin ligases use either a HECT (homologous to E6-associated protein C terminus) or a RING (really interesting new gene) domain to catalyse polyubiquitination, but the mechanism of E3 catalysis is poorly defined. Here we dissect this process using mouse Ube2g2 (E2; identical at the amino acid level to human Ube2g2) and human gp78 (E3), an endoplasmic reticulum (ER)-associated conjugating system essential for the degradation of misfolded ER proteins. We demonstrate by expressing recombinant proteins in Escherichia coli that Ube2g2/gp78-mediated polyubiquitination involves preassembly of Lys 48-linked ubiquitin chains at the catalytic cysteine of Ube2g2. The growth of Ube2g2-anchored ubiquitin chains seems to be mediated by an aminolysis-based transfer reaction between two Ube2g2 molecules that each carries a ubiquitin moiety in its active site. Intriguingly, polyubiquitination of a substrate can be achieved by transferring preassembled ubiquitin chains from Ube2g2 to a lysine residue in a substrate.

View details for DOI 10.1038/nature05542

View details for Web of Science ID 000244892900049

View details for PubMedID 17310145

- Inhibition of metalloprotease botulinum serotype A from a pseudo-peptide binding mode to a small molecule that is active in primary neurons *JOURNAL OF BIOLOGICAL CHEMISTRY* Burnett, J. C., Ruthel, G., Stegmann, C. M., Panchal, R. G., Nguyen, T. L., Hermone, A. R., Stafford, R. G., Lane, D. J., Kenny, T. A., McGrath, C. F., Wipf, P., Stahl, A. M., Schmidt, J. J., Gussio, R., Brunger, A. T., Bavari, S. 2007; 282 (7): 5004-5014

Abstract

An efficient research strategy integrating empirically guided, structure-based modeling and chemoinformatics was used to discover potent small molecule inhibitors of the botulinum neurotoxin serotype A light chain. First, a modeled binding mode for inhibitor 2-mercapto-3-phenylpropionyl-RATKML

(K(i) = 330 nM) was generated, and required the use of a molecular dynamic conformer of the enzyme displaying the reorientation of surface loops bordering the substrate binding cleft. These flexible loops are conformationally variable in x-ray crystal structures, and the model predicted that they were pivotal for providing complementary binding surfaces and solvent shielding for the pseudo-peptide. The docked conformation of 2-mercapto-3-phenylpropionyl-RATKML was then used to refine our pharmacophore for botulinum serotype A light chain inhibition. Data base search queries derived from the pharmacophore were employed to mine small molecule (non-peptidic) inhibitors from the National Cancer Institute's Open Repository. Four of the inhibitors possess K(i) values ranging from 3.0 to 10.0 microM. Of these, NSC 240898 is a promising lead for therapeutic development, as it readily enters neurons, exhibits no neuronal toxicity, and elicits dose-dependent protection of synaptosomal-associated protein (of 25 kDa) in a primary culture of embryonic chicken neurons. Isothermal titration calorimetry showed that the interaction between NSC 240898 and the botulinum A light chain is largely entropy-driven, and occurs with a 1:1 stoichiometry and a dissociation constant of 4.6 microM.

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View details for Web of Science ID 000244482000083

View details for PubMedID 17092934

- Ab initio molecular-replacement phasing for symmetric helical membrane proteins *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Strop, P., Brzustowicz, M. R., Brunger, A. T. 2007; 63: 188-196

Abstract

Obtaining phases for X-ray diffraction data can be a rate-limiting step in structure determination. Taking advantage of constraints specific to membrane proteins, an ab initio molecular-replacement method has been developed for phasing X-ray diffraction data for symmetric helical membrane proteins without prior knowledge of their structure or heavy-atom derivatives. The described method is based on generating all possible orientations of idealized transmembrane helices and using each model in a molecular-replacement search. The number of models is significantly reduced by taking advantage of geometrical and structural restraints specific to membrane proteins. The top molecular-replacement results are evaluated based on noncrystallographic symmetry (NCS) map correlation, OMIT map correlation and R(free) value after refinement of a polyalanine model. The feasibility of this approach is illustrated by phasing the mechanosensitive channel of large conductance (MscL) with only 4 A diffraction data. No prior structural knowledge was used other than the number of transmembrane helices. The search produced the correct spatial organization and the position in the asymmetric unit of all transmembrane helices of MscL. The resulting electron-density maps were of sufficient quality to automatically build all helical segments of MscL including the cytoplasmic domain. The method does not require high-resolution diffraction data and can be used to obtain phases for symmetrical helical membrane

proteins with one or two helices per monomer.

View details for DOI 10.1107/S0907444906045793

View details for Web of Science ID 000243495700008

View details for PubMedID 17242512

- Version 1.2 of the Crystallography and NMR system *NATURE PROTOCOLS* Brunger, A. T. 2007; 2 (11): 2728-2733

Abstract

Version 1.2 of the software system, termed Crystallography and NMR system (CNS), for crystallographic and NMR structure determination has been released. Since its first release, the goals of CNS have been (i) to create a flexible computational framework for exploration of new approaches to structure determination, (ii) to provide tools for structure solution of difficult or large structures, (iii) to develop models for analyzing structural and dynamical properties of macromolecules and (iv) to integrate all sources of information into all stages of the structure determination process. Version 1.2 includes an improved model for the treatment of disordered solvent for crystallographic refinement that employs a combined grid search and least-squares optimization of the bulk solvent model parameters. The method is more robust than previous implementations, especially at lower resolution, generally resulting in lower R values. Other advances include the ability to apply thermal factor sharpening to electron density maps. Consistent with the modular design of CNS, these additions and changes were implemented in the high-level computing language of CNS.

View details for DOI 10.1038/nprot.2007.406

View details for Web of Science ID 000253140000009

View details for PubMedID 18007608

- Single molecule studies of SNARE-dependent fusion Brunger, A., Chu, S., Bowen, M., Weninger, K., Vrljic, M. CELL PRESS. 2007: 375A-375A
View details for Web of Science ID 000243972402305

- Botulinum neurotoxin B recognizes its protein receptor with high affinity and specificity *NATURE* Jin, R., Rummel, A., Binz, T., Brunger, A. T. 2006; 444 (7122): 1092-1095

Abstract

Botulinum neurotoxins (BoNTs) are produced by Clostridium botulinum and cause the neuroparalytic syndrome of botulism. With a lethal dose of 1 ng kg(-1), they pose a biological hazard to humans and a serious potential bioweapon threat. BoNTs bind with high specificity at neuromuscular junctions and they impair exocytosis of synaptic vesicles containing acetylcholine through specific proteolysis of SNAREs (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors), which constitute part of the synaptic vesicle fusion machinery. The molecular details of the toxin-cell recognition have been elusive. Here we report the structure of a BoNT in complex with its protein receptor: the receptor-binding domain of botulinum neurotoxin serotype B (BoNT/B) bound to the luminal

domain of synaptotagmin II, determined at 2.15 A resolution. On binding, a helix is induced in the luminal domain which binds to a saddle-shaped crevice on a distal tip of BoNT/B. This crevice is adjacent to the non-overlapping ganglioside-binding site of BoNT/B. Synaptotagmin II interacts with BoNT/B with nanomolar affinity, at both neutral and acidic endosomal pH. Biochemical and neuronal ex vivo studies of structure-based mutations indicate high specificity and affinity of the interaction, and high selectivity of BoNT/B among synaptotagmin I and II isoforms. Synergistic binding of both synaptotagmin and ganglioside imposes geometric restrictions on the initiation of BoNT/B translocation after endocytosis. Our results provide the basis for the rational development of preventive vaccines or inhibitors against these neurotoxins.

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View details for Web of Science ID 000242971100061

View details for PubMedID 17167421

- Structural and functional comparisons of nucleotide pyrophosphatase/phosphodiesterase and alkaline phosphatase: Implications for mechanism and evolution *BIOCHEMISTRY* Zalatan, J. G., Fenn, T. D., Brunger, A. T., Herschlag, D. 2006; 45 (32): 9788-9803

Abstract

The rapid expansion of the amount of genomic and structural data has provided many examples of enzymes with evolutionarily related active sites that catalyze different reactions. Functional comparisons of these active sites can provide insight into the origins of the enormous catalytic proficiency of enzymes and the evolutionary changes that can lead to different enzyme activities. The alkaline phosphatase (AP) superfamily is an ideal system to use in making such comparisons given the extensive data available on both nonenzymatic and enzymatic phosphoryl transfer reactions. Some superfamily members, such as AP itself, preferentially hydrolyze phosphate monoesters, whereas others, such as nucleotide pyrophosphatase/phosphodiesterase (NPP), preferentially hydrolyze phosphate diesters. We have measured rate constants for NPP-catalyzed hydrolysis of phosphate diesters and monoesters. NPP preferentially catalyzes diester hydrolysis by factors of 10(2)-10(6), depending on the identity of the diester substrate. To identify features of the NPP active site that could lead to preferential phosphate diester hydrolysis, we have determined the structure of NPP in the absence of ligands and in complexes with vanadate and AMP. Comparisons to existing structures of AP reveal bimetallo cores that are structurally indistinguishable, but there are several distinct structural features outside of the conserved bimetallo site. The structural and functional data together suggest that some of these distinct functional groups provide specific substrate binding interactions, whereas others tune the properties of the bimetallo active site itself to discriminate between phosphate diester and monoester substrates.

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View details for Web of Science ID 000239596600016

View details for PubMedID 16893180

- Ensemble molecular dynamics yields submillisecond kinetics and intermediates of membrane fusion *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Kasson, P. M., Kelley, N. W., Singhal, N., Vrljic, M., Brunger, A. T., Pande, V. S. 2006; 103 (32): 11916-11921

Abstract

Lipid membrane fusion is critical to cellular transport and signaling processes such as constitutive secretion, neurotransmitter release, and infection by enveloped viruses. Here, we introduce a powerful computational methodology for simulating membrane fusion from a starting configuration designed to approximate activated prefusion assemblies from neuronal and viral fusion, producing results on a time scale and degree of mechanistic detail not previously possible to our knowledge. We use an approach to the long time scale simulation of fusion by constructing a Markovian state model with large-scale distributed computing, yielding an understanding of fusion mechanisms on time scales previously impossible to simulate to our knowledge. Our simulation data suggest a branched pathway for fusion, in which a common stalk-like intermediate can either rapidly form a fusion pore or remain in a metastable hemifused state that slowly forms fully fused vesicles. This branched reaction pathway provides a mechanistic explanation both for the biphasic fusion kinetics and the stable hemifused intermediates previously observed experimentally. Our distributed computing and Markovian state model approaches provide sufficient sampling to detect rare transitions, a systematic process for analyzing reaction pathways, and the ability to develop quantitative approximations of reaction kinetics for fusion.

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View details for Web of Science ID 000239701900019

View details for PubMedID 16880392

- Considerations for the refinement of low-resolution crystal structures *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* DeLaBarre, B., Brunger, A. T. 2006; 62: 923-932

Abstract

It is often assumed that crystal structures have to be obtained at sufficiently high resolution in order to perform macromolecular refinement. In several recent structures, the threshold of what is considered ;acceptable' has been pushed to lower diffraction resolutions. Here, considerations and modifications to standard refinement protocols are described that were used to solve and refine a particular set of low-resolution structures for the ATPase p97/VCP. It was found that reasonable R(free) values and good geometry can be achieved upon refinement that includes experimental phase information along with judicious use of restraints at diffraction limits as low as 4.7 A. At this resolution, the topology and the backbone-chain trace are mostly defined, some side-chain positions can be unambiguously assigned and ligands within known binding sites can be identified.

Furthermore, large conformational changes can be discerned when structures in different states are available, information that is not easily obtainable by other means.

View details for DOI 10.1107/S0907444906012650

View details for Web of Science ID 000239119800012

View details for PubMedID 16855310

- Conformation of the synaptobrevin transmembrane domain *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* Bowen, M., Brunger, A. T. 2006; 103 (22): 8378-8383

Abstract

The synaptic vesicle protein synaptobrevin (also called VAMP, vesicle-associated membrane protein) forms part of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex, which is essential for vesicle fusion. Additionally, the synaptobrevin transmembrane domain can promote lipid mixing independently of complex formation. Here, the conformation of the transmembrane domain was studied by using circular dichroism and attenuated total reflection Fourier-transform infrared spectroscopy. The synaptobrevin transmembrane domain has an alpha-helical structure that breaks in the juxtamembrane region, leaving the cytoplasmic domain unstructured. In phospholipid bilayers, infrared dichroism data indicate that the transmembrane domain adopts a 36 degrees angle with respect to the membrane normal, similar to that reported for viral fusion peptides. A conserved aromatic/basic motif in the juxtamembrane region may be causing this relatively high insertion angle.

View details for DOI 10.1073/pnas.0602644103

View details for Web of Science ID 000238206800016

View details for PubMedID 16709671

- Central pore residues mediate the p97/VCP activity required for ERAD *MOLECULAR CELL* DeLaBarre, B., Christianson, J. C., Kopito, R. R., Brunger, A. T. 2006; 22 (4): 451-462

Abstract

The AAA-ATPase p97/VCP facilitates protein dislocation during endoplasmic reticulum-associated degradation (ERAD). To understand how p97/VCP accomplishes dislocation, a series of point mutants was made to disrupt distinguishing structural features of its central pore. Mutants were evaluated in vitro for ATPase activity in the presence and absence of synaptotagmin I (SytI) and in vivo for ability to process the ERAD substrate TCRalpha. Synaptotagmin induces a 4-fold increase in the ATPase activity of wild-type p97/VCP (p97/VCP(wt)), but not in mutants that showed an ERAD impairment. Mass spectrometry of crosslinked synaptotagmin . p97/VCP revealed interactions near Trp551 and Phe552. Additionally, His317, Arg586, and Arg599 were found to be essential for substrate interaction and ERAD. Except His317, which serves as an interaction nexus, these residues all lie on prominent loops within the D2 pore. These data support a model of substrate dislocation facilitated by interactions with p97/VCP's D2 pore.

View details for DOI 10.1016/j.molcel.2006.03.036

View details for Web of Science ID 000237813300007

View details for PubMedID 16713576

- Towards an understanding of the molecular mechanism of neurotransmitter release Brunger, A. T. FEDERATION AMER SOC EXP BIOL. 2006: A1309-A1309
  View details for Web of Science ID 000236326204248

- The structure of nucleotide pyrophosphatase/phosphodiesterase and implications for enzyme evolution in the alkaline phosphatase superfamily Zalatan, J., Fenn, T., Brunger, A., Herschlag, D. FEDERATION AMER SOC EXP BIOL. 2006: A477-A477
  View details for Web of Science ID 000236206504055

- Neuronal SNAREs do not trigger fusion between synthetic membranes but do promote PEG-mediated membrane fusion *BIOPHYSICAL JOURNAL* Dennison, S. M., Bowen, M. E., Brunger, A. T., Lentz, B. R. 2006; 90 (5): 1661-1675

Abstract

At low surface concentrations that permit formation of impermeable membranes, neuronal soluble N-ethyl maleimide sensitive factor attachment protein receptor (SNARE) proteins form a stable, parallel, trans complex when vesicles are brought into contact by a low concentration of poly (ethylene glycol) (PEG). Surprisingly, formation of a stable SNARE complex does not trigger fusion under these conditions. However, neuronal SNAREs do promote fusion at low protein/lipid ratios when triggered by higher concentrations of PEG. Promotion of PEG-triggered fusion required phosphatidylserine and depended only on the surface concentration of SNAREs and not on the formation of a trans SNARE complex. These results were obtained at protein surface concentrations reported for synaptobrevin in synaptic vesicles and with an optimally fusogenic lipid composition. At a much higher protein/lipid ratio, vesicles joined by SNARE complex slowly mixed lipids at 37 degrees C in the absence of PEG, in agreement with earlier reports. However, vesicles containing syntaxin at a high protein/lipid ratio (>or=1:250) lost membrane integrity. We conclude that the neuronal SNARE complex promotes fusion by joining membranes and that the individual proteins syntaxin and synaptobrevin disrupt membranes so as to favor formation of a stalk complex and to promote conversion of the stalk to a fusion pore. These effects are similar to the effects of viral fusion peptides and transmembrane domains, but they are not sufficient by themselves to produce fusion in our in vitro system at surface concentrations documented to occur in synaptic vesicles. Thus, it is likely that proteins or factors other than the SNARE complex must trigger fusion in vivo.

View details for DOI 10.1529/biophysj.105.069617

View details for Web of Science ID 000235235600020

View details for PubMedID 16339880

- Automated crystallographic ligand building using the medial axis transform of an electron-density isosurface *ACTA CRYSTALLOGRAPHICA SECTION D-BIOLOGICAL CRYSTALLOGRAPHY* Aishima, J., Russel, D. S., Guibas, L. J., Adams, P. D., Brunger, A. T. 2005; 61: 1354-1363

Abstract

Automatic fitting methods that build molecules into electron-density maps usually fail below 3.5 A resolution. As a first step towards addressing this problem, an algorithm has been developed using an approximation of the medial axis to simplify an electron-density isosurface. This approximation captures the central axis of the isosurface with a graph which is then matched against a graph of the molecular model. One of the first applications of the medial axis to X-ray crystallography is presented here. When applied to ligand fitting, the method performs at least as well as methods based on selecting peaks in electron-density maps. Generalization of the method to recognition of common features across multiple contour levels could lead to powerful automatic fitting methods that perform well even at low resolution.

View details for DOI 10.1107/S0907444905023152

View details for Web of Science ID 000232353500006

View details for PubMedID 16204887

- A hybrid machine-learning approach for segmentation of protein localization data *BIOINFORMATICS* Kasson, P. M., Huppa, J. B., DAVIS, M. M., Brunger, A. T. 2005; 21 (19): 3778-3786

Abstract

Subcellular protein localization data are critical to the quantitative understanding of cellular function and regulation. Such data are acquired via observation and quantitative analysis of fluorescently labeled proteins in living cells. Differentiation of labeled protein from cellular artifacts remains an obstacle to accurate quantification. We have developed a novel hybrid machine-learning-based method to differentiate signal from artifact in membrane protein localization data by deriving positional information via surface fitting and combining this with fluorescence-intensity-based data to generate input for a support vector machine.We have employed this classifier to analyze signaling protein localization in T-cell activation. Our classifier displayed increased performance over previously available techniques, exhibiting both flexibility and adaptability: training on heterogeneous data yielded a general classifier with good overall performance; training on more specific data yielded an extremely high-performance specific classifier. We also demonstrate accurate automated learning utilizing additional experimental data.

View details for DOI 10.1093/bioinformatics/bti615

View details for Web of Science ID 000232596100012

View details for PubMedID 16091410

- SNARE complex between reconstituted vesicles does not trigger but does promote PEG-triggered fusion Lentz, B., Dennison, M., Bowen, M. E.,

Brunger, A. T. AMER CHEMICAL SOC. 2005: U1075-U1075
View details for Web of Science ID 000236797302158

- Single-molecule studies of synaptotagmin and complexin binding to the SNARE complex *BIOPHYSICAL JOURNAL* Bowen, M. E., Weninger, K., Ernst, J., Chu, S., Brunger, A. T. 2005; 89 (1): 690-702

Abstract

The assembly of multiprotein complexes at the membrane interface governs many signaling processes in cells. However, very few methods exist for obtaining biophysical information about protein complex formation at the membrane. We used single molecule fluorescence resonance energy transfer to study complexin and synaptotagmin interactions with the SNARE complex in deposited lipid bilayers. Using total internal reflectance microscopy, individual binding events at the membrane could be resolved despite an excess of unbound protein in solution. Fluorescence resonance energy transfer (FRET)-efficiency derived distances for the complexin-SNARE interaction were consistent with the crystal structure of the complexin-SNARE complex. The unstructured N-terminal region of complexin showed broad distributions of FRET efficiencies to the SNARE complex, suggesting that information on conformational variability can be obtained from FRET efficiency distributions. The low-affinity interaction of synaptotagmin with the SNARE complex changed dramatically upon addition of Ca2+ with high FRET efficiency interactions appearing between the C2B domain and linker domains of synaptotagmin and the membrane proximal portion of the SNARE complex. These results demonstrate that single molecule FRET can be used as a "spectroscopic ruler" to simultaneously gain structural and kinetic information about transient multiprotein complexes at the membrane interface.

View details for DOI 10.1529/biophysj.104.054064

View details for Web of Science ID 000230114500067

View details for PubMedID 15821166

- X-ray scattering from unilamellar lipid vesicles *JOURNAL OF APPLIED CRYSTALLOGRAPHY* Brzustowicz, M. R., Brunger, A. T. 2005; 38: 126-131
View details for DOI 10.1107/S0021889804029206

View details for Web of Science ID 000226414500014

- Structure and function of SNARE and SNARE-interacting proteins *QUARTERLY REVIEWS OF BIOPHYSICS* Brunger, A. T. 2005; 38 (1): 1-47

Abstract

This review focuses on the so-called SNARE (soluble N-ethyl maleimide sensitive factor attachment protein receptor) proteins that are involved in exocytosis at the pre-synpatic plasma membrane. SNAREs play a role in docking and fusion of synaptic vesicles to the active zone, as well as in the Ca2+-triggering step itself, most likely in combination with the Ca2+ sensor synaptotagmin. Different SNARE domains are involved in different processes, such as regulation, docking, and fusion. SNAREs exhibit multiple configurational, conformational, and oliogomeric states. These different

states allow SNAREs to interact with their matching SNARE partners, auxiliary proteins, or with other SNARE domains, often in a mutually exclusive fashion. SNARE core domains undergo progressive disorder to order transitions upon interactions with other proteins, culminating with the fully folded post-fusion (cis) SNARE complex. Physiological concentrations of neuronal SNAREs can juxtapose membranes, and promote fusion in vitro under certain conditions. However, significantly more work will be required to reconstitute an in vitro system that faithfully mimics the Ca2+-triggered fusion of a synaptic vesicle at the active zone.

View details for DOI 10.1017/S0033583505004051

View details for Web of Science ID 000236307800001

View details for PubMedID 16336742

- Structure of a human A-type potassium channel accelerating factor DPPX, a member of the dipeptidyl aminopeptidase family Strop, P., Bankovich, A. J., Hansen, K. C., Garcia, K. C., Brunger, A. T. CELL PRESS. 2005: 456A-456A
View details for Web of Science ID 000226378502221

- X-ray scattering from unilamellar lipid vesicles Brzustowicz, M. R., Brunger, A. T. CELL PRESS. 2005: 17A-17A
View details for Web of Science ID 000226378500074

- Deformable modeling for improved calculation of molecular velocities from single-particle tracking *2005 IEEE COMPUTATIONAL SYSTEMS BIOINFORMATICS CONFERENCE, PROCEEDINGS* Kasson, P., Davis, M. M., Brunger, A. T. 2005: 208-211

Abstract

Single-particle tracking provides a powerful technique for measuring dynamic cellular processes on the level of individual molecules. Much recent work has been devoted to using single particle tracking to measure long-range movement of particles on the cell surface, including methods for automated localization and tracking of particles [1-3]. However, most particle tracking studies to date ignore cell surface curvature and dynamic cellular deformation, factors frequently present in physiologically relevant situations. In this report, we perform quantitative evaluation of single-particle tracking on curved and deforming cell surfaces. We also introduce a new hybrid method that uses non-rigid cellular modeling for improved computation of single-particle tracking trajectories on the surfaces of cells undergoing deformation. This method combines single-molecule and bulk fluorescence measurements in an automated manner to enable more accurate and robust characterization of dynamic cell physiology and regulation.

View details for Web of Science ID 000231800100025

View details for PubMedID 16447978

- Observation of single liposome-bilayer fusion induced by SNARE proteins Weninger, K. R., Bowen, M. E., Brunger, A. T., Chu, S. AMER CHEMICAL SOC. 2004: U207-U207
View details for Web of Science ID 000223713801003

- Single molecule studies of SNARE effector binding Bowen, M. E., Chu, S.,

Weninger, K., Brunger, A. T. CELL PRESS. 2004: 237A-238A
View details for Web of Science ID 000187971201222

- Quantitative analysis of membrane protein localization and signalling Kasson, P. M., Huppa, J. B., DAVIS, M. M., Brunger, A. T. IEEE COMPUTER SOC. 2004: 540-541
View details for Web of Science ID 000224127800077

- Effect of SNARE complex on PEG-mediated membrane fusion Dennison, S. M., Bowen, M., Brunger, A., Lentz, B. R. CELL PRESS. 2004: 518A-518A
View details for Web of Science ID 000187971202673

- Structure of N-ethyl maleimide sensitive factor by electron cryo-microscopy at 11 angstrom resolution Furst, J., Sutton, R. B., Chen, J., Brunger, A. T., Grigorieff, N. CELL PRESS. 2004: 78A-78A
View details for Web of Science ID 000187971200405

- Quantitative analysis of lymphocyte membrane protein redistribution from fluorescence microscopy Kasson, P. M., Huppa, J. B., DAVIS, M. M., Brunger, A. T. IEEE. 2004: 2933-2936
View details for Web of Science ID 000228043504023

- A structural analysis of full length VCP, a AAA protein. DeLaBarre, B., Rouiller, I., May, A. P., Weis, W. I., Wilson-Kubalek, E. M., Brunger, A. T. CELL PRESS. 2003: 356A-356A
View details for Web of Science ID 000183123801742

- SNARE proteins alter poly(ethylene glycol) (PEG)-mediated fusion of model membranes Dennison, S. M., Bowen, M., Brunger, A., Lentz, B. CELL PRESS. 2003: 195A-195A
View details for Web of Science ID 000183123800956

- Conformational changes of the multifunction VCP/p97 AAA ATPase during the ATPase cycle Rouiller, I., DeLaBarre, B., May, A. P., Weiss, W. I., Brunger, A. T., Milligan, R. A., Wilson-Kubalek, E. M. AMER SOC CELL BIOLOGY. 2002: 263A-263A
View details for Web of Science ID 000179569101479

- Macromolecular assemblages machines and networks - Editorial overview *CURRENT OPINION IN STRUCTURAL BIOLOGY* Saibil, H. R., Brunger, A. T. 2002; 12 (2): 215-216
View details for Web of Science ID 000175043000011

- Mutational analysis of synaptobrevin transmembrane domain oligomerization Bowen, M. E., Engelman, D. M., Brunger, A. T. AMER SOC CELL BIOLOGY. 2001: 473A-473A
View details for Web of Science ID 000172372502601

- The 1.0 angstrom crystal structure of Ca+2-bound calmodulin: An analysis of disorder and implications for functionally relevant plasticity Wilson, M. A., Brunger, A. T. CELL PRESS. 2001: 398A-398A
View details for Web of Science ID 000166692201813

- Biophysical methods - New approaches to study macromolecular structure and function - Editorial overview *CURRENT OPINION IN STRUCTURAL BIOLOGY* Brunger, A. T., Laue, E. D. 2000; 10 (5): 557-557

View details for Web of Science ID 000089983100008

- Algorithmic challenges in computational molecular biophysics *JOURNAL OF COMPUTATIONAL PHYSICS* Schlick, T., Skeel, R. D., Brunger, A. T., Kale, L. V., Board, J. A., Hermans, J., Schulten, K. 1999; 151 (1): 9-48
  View details for Web of Science ID 000080181500002
- Phase improvement by multi-start simulated annealing refinement and structure-factor averaging *JOURNAL OF APPLIED CRYSTALLOGRAPHY* Rice, L. M., Shamoo, Y., Brunger, A. T. 1998; 31: 798-805
  View details for Web of Science ID 000077183100019
- New applications of simulated annealing in crystallographic refinement *DIRECT METHODS FOR SOLVING MACROMOLECULAR STRUCTURES* Brunger, A. T., Adams, P. D., Rice, L. M. 1998; 507: 143-157
  View details for Web of Science ID 000074740800014
- Site-directed dichroism as a method for obtaining rotational and orientational constraints for oriented polymers *JOURNAL OF THE AMERICAN CHEMICAL SOCIETY* Arkin, I. T., MacKenzie, K. R., Brunger, A. T. 1997; 119 (38): 8973-8980
  View details for Web of Science ID A1997XX82000025
- Crystallographic refinement by simulated annealing: Methods and applications *MACROMOLECULAR CRYSTALLOGRAPHY, PT B* Brunger, A. T., Rice, L. M. 1997; 277: 243-269
  View details for Web of Science ID A1997BJ57S00013

  View details for PubMedID 18488313
- STRUCTURAL MODEL OF THE PHOSPHOLAMBAN ION-CHANNEL IN MEMBRANES *PROTEIN ENGINEERING* Arkin, I. T., Adams, P. D., LUDLAM, C. F., Aimoto, S., Rothschild, K. J., Brunger, A. T., Engelman, D. M., Smith, S. O. 1995; 8: 44-44
  View details for Web of Science ID A1995RL74200068

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