Fall Workshop
for
BioXFEL Students and Postdocs
2015

How do you make a movie of a single particle jiggling and wiggling with atomic resolution and ultra-fast temporal resolution? How do you calculate the energy landscape or the kinetics/dynamics of a sequence of conformational changes? What do you do with millions and millions of snapshots, each for a random orientational and/or conformational state, noisy and with artifacts? How can I use symmetry to my advantage? We will answer all these questions and more.

Tuesday, September 29, 2015

8:00a – 8:30a	Registration/ Breakfast/ Coffee	KEN 2/F Foyer
8:30a – 8:45a	Introduction	KEN 2175

Morning Session

Manifold Embedding – From Single-Particle Snapshots to Movies of Molecular Machines and Energy Landscapes

8:45a – 9:25a	Manifold Embedding Part 1 Peter Schwander	KEN 2175
9:25a – 9:55a	Q&A/ Coffee	
9:55a – 10:25a	Manifold Embedding Part 2 Ali Dashti	KEN 2175
10:25a – 10:55a	Q&A/ Coffee	
10:55a – 11:25a	Manifold Embedding Part 3 Ahmad Hosseinizadeh	KEN 2175
11:25a – 11:55a	Q&A	
11:55a – 12:55p	Lunch	???

Afternoon Session

12:55p – 1:35p	Protein Dynamics George Phillips Jr	KEN 2175
1:35p – 2:05p	Q&A/ Coffee	
2:05p – 2:45p	Structure based protein dynamics Marius Schmidt	KEN 2175
2:45p – 3:15p	Q&A/ Coffee	
3:15p – 3:55p	Finding the structures of randomly oriented particles: method of angular correlations Dilano Saldin	KEN 2175
3:55p – 4:25p	Q&A/ Coffee	
4:25p – 6:00p	Practical	???
6:30p	Dinner	???

Morning Session: Manifold Embedding – From Single-Particle Snapshots to Movies of Molecular Machines and Energy Landscapes

Presenters: Peter Schwander, Ali Dashti, Ahmad Hosseinizadeh

Biological molecular machines cycle through many conformational states, and each state is only slightly different structurally from the previous one. Single-particle methods are ideally suited for studying conformations as they take individual snapshots of molecules, thus avoid averaging over different states. When successfully applied, single-particle approaches have the capability to compile 3D movies of molecular machines at work.

A novel analytic approach, based on manifold embedding, allows model-free, quantitative analysis of the degrees of freedom and the energy landscape underlying continuous conformational changes. This approach has been successfully applied to cryoEM snapshots. More recently, XFEL has been used to take snapshots from single molecules before they are destroyed by the intense beam. XFEL thus provides a new source of single-particle snapshots for studying conformations and will complement the more established cryoEM in the near future.

This session is divided into three lectures:

A) Overview of Single-Particle Methods, Manifold Embedding and Conformations (Peter Schwander).
B) Application to cryoEM data of ribosome, including practical demos to retrieve molecular movies and energy landscapes (Ali Dashti).
C) Application to XFEL data of viruses. Incorporation of symmetry and elimination of experimental artifacts of XFEL (Ahmad Hosseinizadeh).

Afternoon Session B: Structure based protein dynamics

Presenter: Marius Schmidt

Ideally, it should be possible to make a movie of a single particle jiggling and wiggling with atomic resolution and ultra-fast temporal resolution. However, no method to date, even the X-ray FEL, is able to do this. A movie must be made by patching together tiny contributions from many molecules. Consequently the movie consists of the dynamical behavior of an ensemble of molecules. Although each and every molecule does what it wants in a more or less random fashion, one can still expect to extract some non-random information from the movie. In crystallography, the ensemble is formed by the roughly 10^{13} molecules inside the crystal. Experiments are of the pump-probe type where an ultrafast laser flash initiates a reaction and an equally ultrafast X-ray pulse probes the electron density distribution a delay Δt after the laser pulse. The experimental results are diffraction patterns with a time-stamp, the delay, attached.

In a crystallographic experiment the integrated intensities of the Bragg reflections are determined in more or less complicated ways depending on how the data are collected. The result of a pump-probe experiment is a time-dependent data set consisting of indices h k l, integrated intensities and some errors attached to them. Since photolysis rates are usually small on the order of 10%, difference electron density maps need to be employed. These maps show the differences between the time-dependent electron density and a reference electron density, which is typically the one found in crystals kept in the dark. Difference maps are calculated directly from the time-dependent data set of intensities and the intensities measured from the reference. Errors in the intensities may have specific effects on the shape of the difference maps. This will be briefly discussed, and ways to reduce these effects are presented.

A movie consists of a series of difference maps aligned in consecutive temporal order. It can be produced by molecular viewers such as ‘Chimera’ in standard movie formats such as ‘avi’, ‘mp4’ or ‘mov’, and played back with ‘QuickTime’ or ‘RealPlayer’ or embedded in ‘PowerPoint’. However, the information provided by the movie cannot be assessed by simply viewing it. In analogy to conventional electron density maps, which need to be interpreted with atomic models to understand their meaning, a movie needs a kinetic interpretation to extract the dynamics of the crystalline ensemble. It will be shown how such a kinetic interpretation can be performed. The initial steps of this interpretation are based on a component analysis to extract the main constituents of the movie. Kinetic modeling is usually necessary to assemble the difference electron densities of the reaction intermediates from the main components. Note the difference electron

densities of the reaction intermediates are time-independent in contrast to the time-dependent difference electron densities of the movie. Finally, the molecular structures of the intermediates are determined from their respective time-independent difference electron densities. This process is greatly facilitated by using extrapolated, conventional maps, which are calculated with difference amplitudes and structure factors of the reference state.

The kinetic interpretation needs software. However, no standard software is available. It must be self-written in a chosen programming language. The 'Fortran' programs 'SVD4TX' and 'GetMech' are introduced here that support a component analysis of difference maps and the subsequent kinetic modeling, respectively. As a result the structures of the reaction intermediates and the chemical kinetic mechanism is extracted. From the mechanism, time-dependent concentrations of all intermediates called the concentration profile can be calculated. By using both the concentration profile and the molecular structures, time-dependent conventional electron density maps are calculated for each intermediate and assembled as a movie. In this movie the electron density of each particular intermediate appears (gets stronger) and disappears (fades away) in favor of the electron density of another intermediate. Rather than showing the trajectory of the electronic ensemble along the reaction coordinate, the movie shows a sequence of time-independent states that are populated by a varying (time-dependent) fraction of molecules of the crystalline ensemble.

The conceptual differences between single molecule dynamics and the dynamics of the ensemble, which is called the kinetics, become clear now. There is a chance, though, that the molecular dynamics and the ensemble dynamics can be unified. This happens on ultrafast time-scales when the concept of chemical kinetics breaks down. Here the crystalline ensemble has no time to de-phase. All molecules march essentially synchronously down a common energy surface. The entire crystal is one large reacting molecule. Of course, only very fast processes play a role, but these are the elemental processes of chemistry such as bond breaking and isomerization. The ultra-fast pulses of the X-ray FEL will be of extreme value to access and assess these time-scales.

Afternoon Session C: Finding the structures of randomly oriented particles: method of angular correlations

Presenter: Dilano Saldin

Particles are incident into an XFEL in random unknown orientations. One approach to structure determination in this case is to evaluate from each diffraction pattern a quantity that depends on the structure but not on its orientation, namely the angular correlation. Because each individual measured diffraction pattern is usually very weak, it is sensible to take the average of these orientationally independent quantities. For ease of understanding, we will initially demonstrate the method at a lower dimension than in the usual XFEL application, which may allow structure determination of membrane proteins where their orientations are random about a single axis normal to the membrane. This allows their structure determination *in situ* in an experiment with a *black lipid* membrane. Due to its statistically robust properties, the angular correlations converge to a sensible limit, and it can be shown it is often possible to recover the 3D structure of the particle from these average correlations. How this structure determination can be done is still the subject of research. Viruses are amongst the first biological structures experimentally studied at x-ray free electron lasers (XFELs). We have already demonstrated solutions in cases of common virus symmetries, namely those of icosahedral and helical cases. Recently a more general solution has been proposed, which exploits the fact that the angular correlation function, measurable by experiment, may be written as a sum over the squared moduli of the spherical harmonic expansion coefficients of the diffraction volume. This allows both their magnitudes and phases to be found by an iterative phasing algorithm. Finally, a solution is also demonstrated for when a closely related structure is known, as in the difference Fourier method of time-resolved crystallography, except that it is actually applicable to determining fast structural changes for molecules in an ensemble of randomly oriented particles.

Reading list to prepare for the workshop:

Morning Session: Manifold Embedding – From Single-Particle Snapshots to Movies of Molecular Machines and Energy Landscapes

"Conformations of Macromolecules and their Complexes from Heterogeneous Datasets", P. Schwander, R. Fung, A. Ourmazd, Philosophical Transactions B, p. 20130567-20130574 (2014).
doi: 10.1098/rstb.2013.0567

"Trajectories of a Brownian Machine: The Ribosome", A. Dashti, P. Schwander, R. Langlois, R. Fung, W. Li, A. Hosseinizadeh, H. Liao, J. Pallesen, G. Sharma, V.A. Stupina, A. E. Simon, J. Dinman, J. Frank, A. Ourmazd, PNAS,PNAS, A 111, 17492-17497 (2014)).
doi: 10.1073/pnas.1419276111

"High-resolution structure of viruses from random diffraction snapshots", A. Hosseinizadeh, P. Schwander, A. Dashti, R. Fung, R.M. D'Souza,and A. Ourmazd, Philosophical Transactions B, p. 20130326-20130331 (2014).
doi: 10.1098/rstb.2013.0326

"Single-particle structure determination by X-ray free-electron lasers: Possibilities and challenges"
A. Hosseinizadeh, A. Dashti, P. Schwander, R. Fung, and A. Ourmazd
Struct. Dyn. 2, 041601 (2015);
doi:10.1063/1.4919740

Afternoon Session B: Structure based protein dynamics

M. Schmidt, S. Rajagopal., Z. Ren., K. Moffat (2003) Application of Singular Value Decomposition to the Analysis of Time-Resolved Macromolecular X-ray Data. Biophys. J. 84, 2112-2129 (ground breaking paper, concepts and software used in a multiple of following projects by numerous research groups).

M. Schmidt (2008) Structure Based Enzyme Kinetics by Time-Resolved X-ray crystallography, in: Ultrashort Laser Pulses in Medicine and Biology (W. Zinth, M. Braun, P. Gilch eds.), Springer-Verlag (nice review).

J. Tenboer, S. Basu, N. Zatsepin, K. Pande, D. Milathianaki, M. Frank, M. Hunter, S. Boutet, G. Williams, J. E. Koglin, D. Oberthuer, M. Heymann, C. Kupitz, C. Conrad, J. Coe, S. Roy-Chowdhury, U. Weierstall, D. James, D. Wang, T. Grant, A. Barty, O. Yefanov, J. Scales, C. Gati, C. Seuring, V. Srajer, R. Henning, P. Schwander, R. Fromme, A. Ourmazd, K. Moffat, J. Van Thor, J. H. C. Spence, P. Fromme, H. Chapman, M. Schmidt (2014) Time Resolved Serial Femtosecond Crystallography Captures High Resolution Intermediates of Photoactive Yellow Protein, Science 346 (6214): 1242-1246 (recent breakthrough at LCLS)

Afternoon Session C: Finding the structures of randomly oriented particles: method of angular correlations

D. K. Saldin. V. L.Shneerson, R. Fung, and A. Ourmazd, J. Phys.: Condens. Matter 29, 234024 (2009).

D. K. Saldin, H.-C. Poon, P, Schwander, M. Uddin, and M. Schmidt, Optics Express 19, 17318 (2011).

H.-C. Poon and D. K. Saldin, Struct. Dyn. 2, 041716 (2015).