# “Making the Molecular Movie”: Quest for the Structure-Function Correlation of Biology

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## I. Introduction

### “Molecular Movies”: First Frames

One of the great scientific challenges of our times is to directly observe atoms during the primary processes governing physical phenomena. The concept of the atomic structure of matter has been established well over a century ago and is now part of our collective consciousness. With the development of field ion microscopes, electron microscopes, and scanning tunneling microscopes we can even directly observe atoms in real space. However, static pictures of matter don’t tell us how things happen. The world is dynamic. It would be a very boring place if there were no anharmonic interactions (coupled to a continuum) to give rise to dynamics and breathe life into otherwise inanimate matter. The world as we know it is an open system in which the system and all its constituent components evolve in time. It is not a system rigorously defined by an orthogonal basis set of eigenfunctions. We use approximate basis sets of eigenfunctions with anharmonic interactions or couplings to try to understand processes. In this exercise, there is a natural tendency to try to understand phenomena at higher and higher levels of refinement, with an atomic level understanding being the ultimate limit (weak force limit).

Let me give you some concrete examples. Biologists routinely discuss how ligands bind at active sites, how DNA unwinds, or how protein regulation occurs. These concepts typically involve picturing how atoms move relative to one another in the process. Similarly chemists love to talk about atomic motions during chemical processes. Imagine being able to directly witness two atoms moving along a dissociative coordinate involved in bond breaking. If you could do this, you would directly witness the “death” of a molecule and the “rebirth” as two new molecules, a kind of molecular reincarnation. Physicists likewise discuss phenomena such as phonons, phase transitions *etc.* in terms of collective coordinates involving atomic descriptions. Wouldn’t it be great if we could directly observe atoms move during a phase transition, measure the absolute atomic motions to directly determine so called electron-phonon couplings and associated lattice dynamics? If at any point, you found yourself picturing atoms move relative to one another during the above descriptions, you just performed a classic thought experiment. You made a “Molecular Movie” albeit in your mind’s eye.

For the most part, the concept of “Making a Molecular Movie” has been thought to be one of the purest forms of a gedanken experiment, outside the realm of direct observation in the lab frame. Irrespective, the above thought processes have been used to formulate one of the central tenets of chemistry and biology — the concept of a system evolving through the so-called transition state region (vide infra). This single class of thought experiments has given our current map of the world at the atomic level of detail. In scientific exploration, one does not have to directly observe a particular phenomenon to rationalize it. There is an over whelming body of observations that have lead to our present picture and logical deductions follow. However, it is also well appreciated that within this map metaphor, the higher the resolution, the better, and the more complete the map will be. For this reason, there is an inherent desire to directly observe atoms move during dynamical processes.

Why do I say that "Molecular Movies" were thought to be the exclusive domain of thought experiments? Consider, as my research group did many years ago, the challenge to make a "Molecular Movie Camera". What is the shutter speed one would need to capture atomic motions in real time? Take for example the event of bond breaking. What is the fastest time scale involved in breaking chemical bonds? First, we have do define when a chemical bond is broken. Take your favourite interatomic potential as a starting point. Interestingly enough, the specific functional form of most interatomic/molecular potentials are very similar. If two masses are separated by twice the equilibrium bond length, the potential is no longer attractive within the thermal energy, kT, of the constituent atoms. At this point, the bond can be said to be broken. Within thermally sampled coordinates, the fastest two atoms could move along a bond breaking coordinate would be at the speed of sound. To keep things simple, assume an equilibrium bond length of 1 Å and a speed of sound typical for organic materials, of approximately $10^5$ cm/sec. Then the time it would take the bond to break is the time it takes the two masses to move 1 Å along the bond dissociation coordinate at the speed of sound ($t_{\text{bond break}} = 1$ Å x $10^{-8}$ cm/Å / $10^5$ cm/sec = $10^{-13}$ seconds) or 100 femtoseconds (1 femtosecond (fs) = $10^{-15}$ second). More rigorous analysis can be made using transition state theory to discuss the displacements of the most anharmonic modes that contribute most to the reaction energetics. These modes are always typically in the 10 – 100 $\text{cm}^{-1}$ range, again giving approximate timescales of 100 fs. Also, typical prefactors in the Arrhenius expression for rate constants (unimolecular processes) are typically $10^{13}$ $\text{sec}^{-1}$, once again 100 fs sampling frequencies of the reaction coordinate. To be sure, there are faster atomic motions. For example, the OH stretch vibration of water molecules has a period of 10 fs. However, the rms motion is on the order of .05 Å not the 1 Å scale needed for irreversible structural changes. There are faster and slower time scales for different reactions but this 100 fs window serves as the canonical shutter speed required for making so called "Molecular Movies". The relevant timescale has been known since Arrhenius' time. This timescale of one ten millionth of a millionth of a second, would have seemed unapproachable even up to 25 years ago. However, with modern laser technology, we now push the boundaries of monitoring molecular dynamics down to the attosecond ($10^{-18}$ sec) time scale. Femtosecond laser systems are commercially available with time resolution superior to the aforementioned required effective shutter speed of 100 fs. It is not the time resolution that remains illusive. As all directors know in order to make a great movie, one must catch the actors with the correct lighting. In our movie, the "actors" are the atoms. In order to spatially resolve the atomic positions requires the use of a source of lighting with wavelengths on the atomic length scale, i.e. one must use either hard x-rays in the 10 KeV range or alternatively electrons with de Broglie wavelengths of 1 Å or less. The real challenge from an experimental point of view is develop a source with enough x-ray photons or electrons to light up the atomic motions. This statement is made as the process of directly observing atomic motions requires a perturbation or excitation step that synchronizes the event of interest. Generally speaking, the experiments require excitation levels sufficient to excite 1-10% of the atoms/molecules involved in order to clearly resolve the dynamics above background. The excitation process, not the monitoring process, makes the process irreversible, i.e., the system in general does not return exactly back to the starting conditions. Thus, one must come up with a lighting source that is sufficiently bright to obtain full structures at atomic resolution in a single shot or time frame for the movie. If not, the amount of sample required (for irreversible processes) becomes intractable. This statement is made as a generalization of sample conditions. There are a precious few samples that can be excited repeatedly, as will be discussed, and then low brightness sources can be used.

So the real challenge, I put forward to my group many years ago was to make a molecular movie camera with enough flux to essentially capture structures at the atomic level in a single shot — using electrons. For economical reasons, we needed to work with table top systems and the prospect of a table-top x-ray source with sufficient brightness is many decades in the future yet. Now you have to consider the fundamental problem of using electrons. We needed to have enough electrons in a pulse of subpicoseond duration and cross sectional area to approach single shot structure determination without using up huge amounts of material for the "molecular movie film". Here you have to realize that the sample thickness has to be on the order of 10-100 nm thick, depending on the electron energy, so that the electron diffraction process to monitor atomic motions occurs in the single electron scatter limit for simple inversion of the diffraction pattern. It is extremely difficult to make samples this thin and certainly not with the required thickness and flatness and sufficient surface area. It is extremely important to be as economical as possible in the number of laser shots per time point taken to capture a molecular movie. The number of electrons has to be sufficient to get as close to single shot structure determinations as possible. Here it is important to recall that electrons are charged particles that undergo electron-electron repulsion and will be hard to coerce into a very short pulse. The natural tendency is for electron-electron repulsion, or space-charge effects, to lead to pulse broadening. It was thought at the time we embarked on this quest that it would be impossible to have sufficient electron densities to achieve this "high water mark", as I will explain below. Suffice it to say, we did figure this out and were able to achieve the first femtosecond time domain picture of structural changes with atomic resolution.

In Figure 1, I show you a picture of our "3rd Generation Electron Gun" in the parlance of the field (left panel) that enabled the first femtosecond time resolution to structural dynamics. The size of the "camera" is approximately that of a soccer/foot ball. To the right is a diffraction pattern of polycrystalline Al captured using femtosecond electron pulses. In my opinion, electron diffraction is one of the most beautiful manifestations of quantum mechanics there is. The fact that you see a diffraction pattern demonstrates that matter has wave properties. It appears as if you threw a rock into water to observe the ripples or wave properties. The difference is that the phase of the electron wavefunction as it scatters off the coulomb potential (charge density) of the atoms leads to interference effects. It is this effect that unambiguously illustrates the wave properties of matter. It is akin to watching waves scatter off support pylons of ocean piers. You can use the interfere effect of the water waves to calculate the spacing of supporting pylons by this interference pattern. Likewise, one can invert electron diffraction to the positions of atoms within a lattice unit cell.

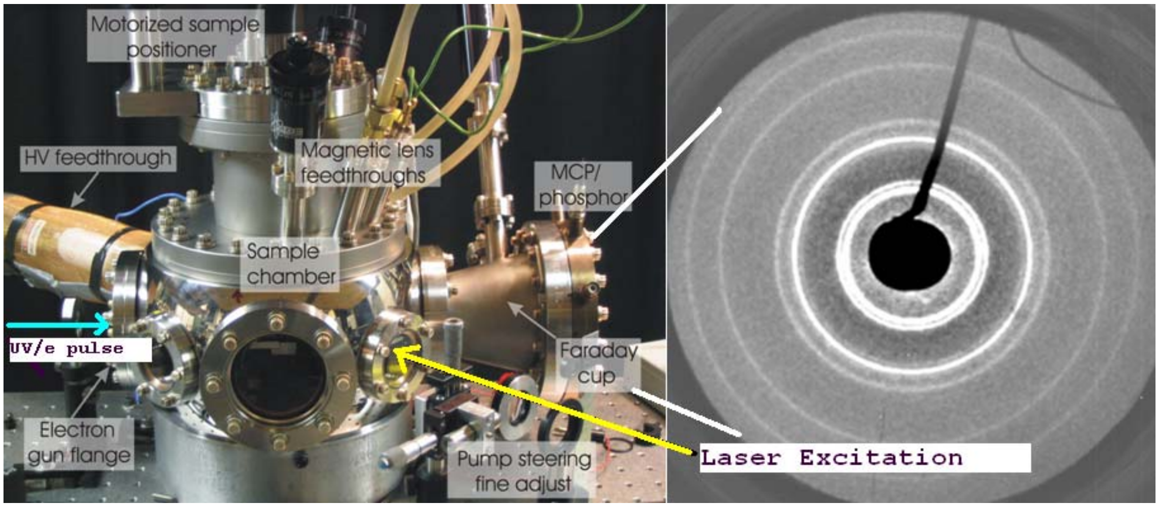


**Figure 1**. Left panel shows the experimental setup. The laser excitation used to trigger the structure changes goes through an optical port to the sample as shown. A perfectly synchronized fs UV pulse is used to generate a replica electron pulse through the photoelectric effect. The electrons are accelerated up to 30 KeV and spatially overlap with the laser excited volume of the sample. The ensuing diffraction pattern is visualized using a microchannel plate (MCP) intensifier/phosphor. The diffracted electron image for polycrystalline Al is shown on the right panel. The atomic motions are imaged by changing the time delay between the excitation pulse prodding the atoms into action and the electron viewing pulse in stroboscopic fashion. Between laser shots the sample is moved to provide a fresh sample for a new time point in the movie being constructed by storing diffraction images as a function of the electron probe delay.

I will give a simple explanation of the experimental method from which you can understand the basics of the experimental concept. It is possible to observe the diffraction pattern effective by eye at the position of the phosphor detector. The femtosecond laser pulse used to induce the structure changes comes into a port intersecting the sample that sits in the middle of the chamber (yellow arrow in Figure 1). This laser pulse is used to effectively poke the “actors” (atoms) into motions as part of the movie you wish to record. The lighting for observing the atoms is obtained by using a UV pulse, or 2-photon excitation process, of a photocathode to generate a replica femtosecond electron pulse through the well known photoelectric effect. The resulting electron pulse is directed to the sample to light up the atomic motions. Now please picture the following events. My students, Brad Siwick, Jason Dwyer, Robert Jordan and myself, looking at this pattern to the right, in the wee hours of the morning as usually occurs in these moments, and observing the fringes to the diffraction pattern change. We knew the time step and immediately knew we were observing atomic motions faster than diffusive motions and collisions could blur the details. Each laser shot destroyed the sample so we quickly ran out of film. However, we were able to record enough frames to stitch together a movie of how a material melts at the atomic level. We could literally watch a lattice, under strongly driven conditions, shake itself apart at the atomic level of inspection. This was the first direct observation of a structural transition with enough orders of diffraction to invert the data back to the atomic motions. This was a very exciting moment for me personally and for my group.

I should add, as a way to provide some commentary on the sociology of science, that the idea to build up such a Molecular Movie Camera came to me back in 1989. I was in a sleep deprived state, due to parental duties involving my youngest daughter who was up all night. In this susceptible state of mind, I convinced myself that we could build up an electron source capable of single shot structures based on some simple approximations for the space charge effects. It took 13 years to realize this objective and 14 years till our first publication. I started this research while I was at the University of Rochester. Many of the students who started this topic with me had to go onto to other topics. I relocated my group to the University of Toronto for the expressed purpose to focus on this problem (and use the excellent, cost free, shops to build up the instrument). There were still many failures that lay ahead as I will explain below. However, the final realization of this "dream experiment" was quite gratifying to say the least. (One of the hardest questions I get when I give this talk is how did I keep my research funding? This research program was a truly "skunk-works" project. We always did this work on the side.)

## Operational Definition of "Molecular Movies"

The term "molecular movie" has been used in a number of different contexts. Here I argue for a specific definition that I hope will be adopted by the field. Movies allow motions to be recorded so that the causal events of interest can be fully observed. In a number of applications, the term "molecular movie" has been used to describe the determination of transient structures on the 1 ns to even second time scales. These structures are valuable for determining intermediates and reducing the number of *possible pathways* connecting structures but they do not enable an unambiguous determination of the actual mechanism or specific pathway. I would liken structural information collected in this way to time lapse photography. The details connecting changes are missing. As a crude analogy, you can take photos of someone undergoing weight loss but not determine how the change occurred. *To qualify as a true "molecular movie", the time and space resolution must be sufficient to follow the atomic motions faster than collisions lead to diffusive motions that wash out or blur the force correlations between atoms that lead to the change in structure.* If not, one has simply a before laser excitation and after laser excitation structure with no clear pathway connecting the two structures. The information on transient intermediates is important in its own right but does not provide sufficient mechanistic information. One is left to models to fill in the details. To follow the key motions leading from one structure to another requires nominally subpicosecond time resolution to atomic motions as discussed above. In the case of isolated gas phase molecules (no collisions), the time resolution must be as high as the half period motions of the reactive modes.

## The Big (Motion) Picture — Direct Observation of Transition States

As stated above, one of the most important concepts in chemistry is the notion of a transition state. This concept describes the intersection between a reactant and product surface along a reaction coordinate. The idea that there is a singularity in this surface at which point small fluctuations take the system from nuclear configurations defining the reactant to those of the product state goes back to the days of Eyering. There are fairly detailed microscopic models of such processes in which specific structures are proposed for motion along the reaction coordinate. There is a strong desire to connect reaction pathways to structural changes as this gives some insight into how to control barrier height and thereby chemical reactions.

The transition state is structurally depicted as the halfway point along the displacement of some principle reaction mode. For example, in the case of trans to cis isomerization, the reaction mode is the torsion or rotational motion around a double bond. The transition state is defined to be at the $90^0$ point in the rotation about this bond. Even in the simplest case, there is also a coupling mode such as the stretching or softening mode of the double bond in the above example. Reaction coordinates need to be depicted by multidimensional surfaces. In principle, all the atomic motions of the reactants are coupled and affect the reaction forces leading to product formation. For small molecular systems, say 10 atoms or less, there are generally well defined reaction modes so the dimensionality of the problem, as in the case of isomerization, can be reduced to a manageable level. For such systems, we have very good descriptions of transition states and pictures of nuclear passage through the transition state region. However, the complexity of the problem quickly scales with the number of atoms involved and the dimensionality does not lend itself well to even highly simplified potential energy surfaces. Reactions occurring in solution phase or within proteins are just a few examples where simple pictures need to be challenged. There are not unique pathways connecting the reactant surface to the product surface. There are numerous, even innumerable pathways, through the transition state region. In this case, the reaction coordinate is better represented as a free energy surface for which the different barrier crossing configurations are lumped into the entropic terms of the free energy surface. Even with this representation, there is still a strong motivation to connect the free energy surface to a narrow distribution of structures.

Effectively, for all but the simplest chemical reaction, we have only a rudimentary understanding of transition state processes. We know that the reactions are governed by the barriers separating reactant and product surfaces and that different barrier heights determine which reaction pathway dominates over competing pathways. All possible combinations of putting the atoms together are possible; yet only one or a few reaction products are observed. This is due to the exponential dependence of reaction rates on barrier heights. One reaction pathway wins out dynamically over the others. To control the barrier crossing processes, it is essential to know the structures so that chemical means of controlling charge distribution (and thereby barrier height) at that point can be made to give the desired reaction in preference over other possible side reactions. In other words, we need a structural basis for transition state dynamics to advance the field of chemistry. In this regard, the ability to directly observe atomic motions during a chemical event will have enormous impact on the field of chemistry.

Here, we can further reflect on the importance on determining the potential energy surface for a chemical reaction. Enormous efforts have been expended in the chemical physics community using molecular beam methods in combination with spectroscopy and theory to derive such surfaces. For small molecular systems, where this is possible, there is an enormous amount of information in such diagrams. It is possible for any given position in nuclear configuration to visualize the potential energy gradients or forces on the atoms, pushing them along the reaction coordinate. We can get a nearly visceral feel for the reaction and how it occurs. However, as discussed, this depiction is not possible for molecules beyond a few atoms. In contrast, if you could directly visualize atomic motions during a chemical reaction, you would have the same fundamental information of interest as embedded in the most rigorous potential energy surfaces for small molecule reactions. You would have the mass and time dependent velocities (forces) for even the most complex molecular systems. Most important the entire multidimensional reaction coordinate is reduced to the principle modes involved in directing the process and thereby define the barrier region.

The recent development of ultrabright electron, and soon hard x-ray sources, to directly observe atomic motions on the femtosecond timescale is opening up the exploration of reaction dynamics of complex molecules and providing the same level of rigor previously only obtainable for small molecule systems using a multitude of different experimental and theoretical methods. This information content is obtained in a single measurement. This experimental methodology will revolutionize chemistry.

## Mother Nature and the Molecular Big Bang: The Structure-Function Correlation Problem in Biology

One of the central tenets of biology is that biomolecules have optimally evolved to direct chemistry by control over barriers or the transition state. Mother Nature is the grand master of controlling chemical processes to do work on the surroundings as part of a biological function.

In figure 2, I am showing you my favourite part of the Universe. It represents a special moment in time and space that happens in each and every one of us. I have zoomed in on the structure of hemoglobin by a factor of $10^7$ in which the star like objects represent heavy atoms in the active site of hemoglobin. The giant red star depicts the iron binding site within the porphyrin ring. The supernovae event is to convey the rarified moment in time; the very moment the Fe-$O_2$ bond breaks, i.e. the momentary crossing of the transition state region. This figure is given to capture your imagination in terms of thinking about the length scales of the forces involved in transducing chemical energy such as a bond breaking event into a biological function. The initial event of bond breaking clearly occurs over atomic length scales and is quantum in nature. However, this relatively minute amount of energy must couple to some thousands of other

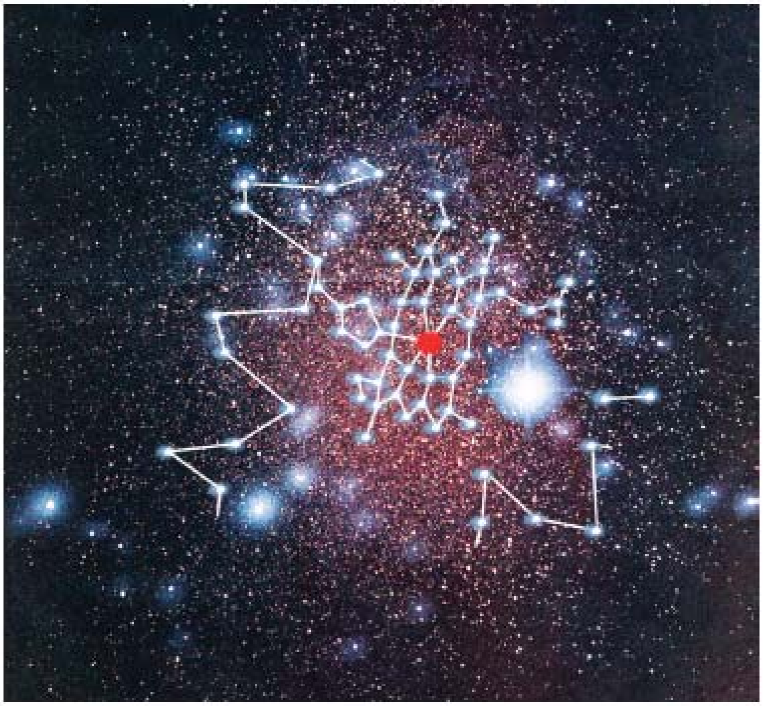


**Figure 2**. Artistic view of the transition state for the Fe-$O_2$ bond breaking event involved in oxygen transport mediated by hemoglobin. The carbon and nitrogen heavy atoms are shown as star like objects and the Fe binding site for oxygen depicted as a giant red star. The bond breaking energy is shown as a supernova event at the molecular level and the diatomic oxygen is just to the right to convey coupling of bond breaking to translational motion. This view of the transition state is meant to illustrate the challenge in understanding how localized bond breaking events at the atomic level couple to the mesoscale length scale of biological functions. The specific structure of the surrounding protein directs these motions. The connection between protein structure and functional response represents the most fundamental understanding of biological processes. The structure-function correlation will be directly observed using femtosecond structural probes (from Miller, Can. J. Chem. 2002).

atomic degrees of freedom to execute a biological function over mesoscale dimensions. Herein lays the wonder of biological processes. The bond breaking event is clearly quantum mechanical in nature and involves fluctuations on the Å length scale. The quantum singularity of bond break must propagate out to the nm or mesoscale and couple to thousands of atoms to perform a biological function, i.e. do work on the surroundings. The number of degrees of freedom coupled to the single act of a bond breaking process or other chemical reaction is enormous. The amount of energy distributed over these degrees of freedom is marginally larger than the background thermal energy yet these processes work nearly unerringly. Mother Nature clearly understands quantum mechanics and the Correspondence Principle in optimally channeling chemical energy into biological functions — coupling quantum systems to the classical mechanics continuum limit.

How do we understand this energy transduction process? Figure 3 shows the protein structures for hemoglobin and myoglobin with and without ligand binding to the Fe active site.

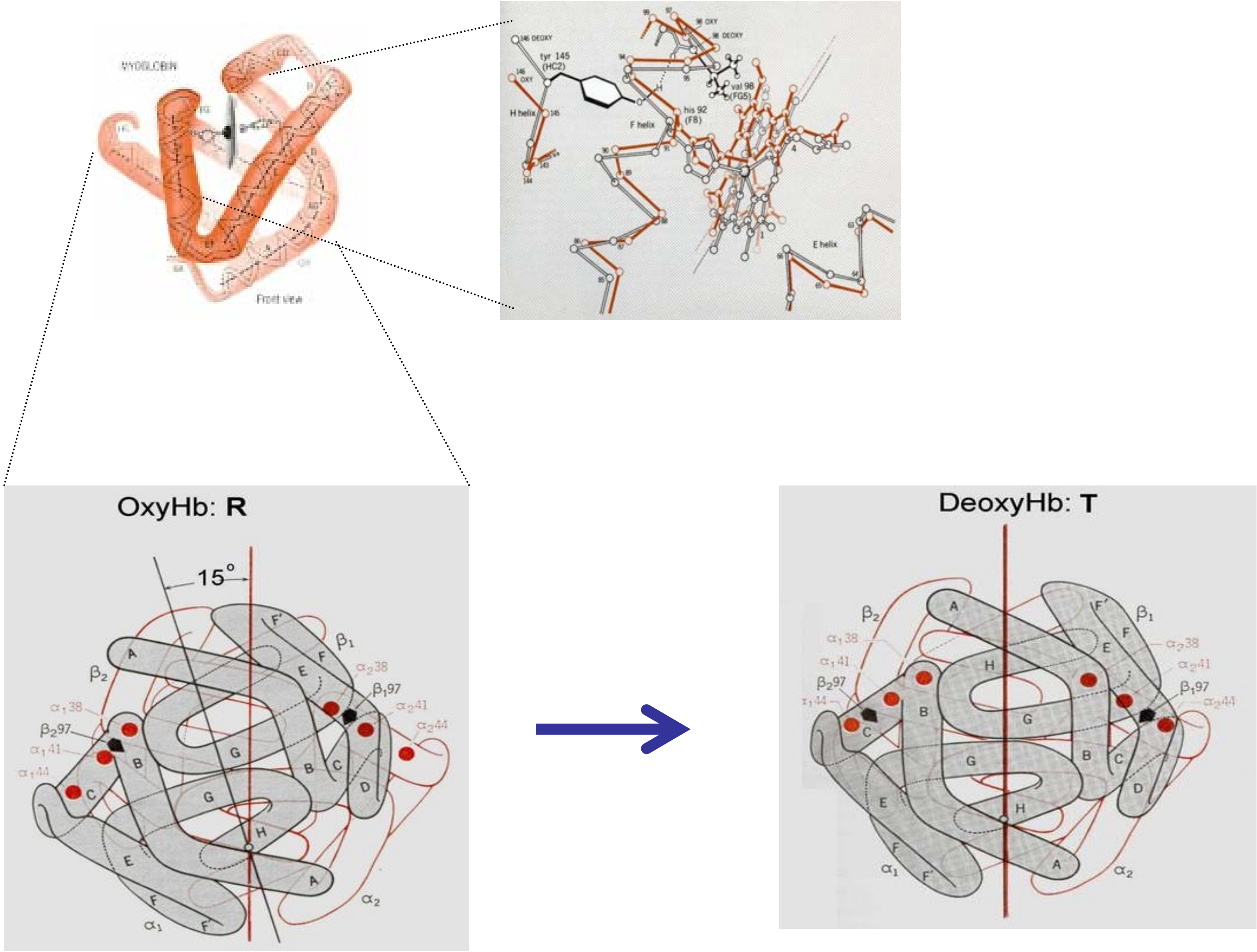


**Figure 3**. Schematic represention of the structural changes in heme proteins following ligand dissociation based on static difference structures determined from x-ray crystallography. The lower panels show the quaternary structure changes of hemoglobin following ligand dissocation involving a ball and socket type motion of the 4 heme proteins. At the individual protein level, the tertiary structural changes are depicted by myoglobin (top panel) with an expanded view of the iron binding site. This section of the protein structure should look familiar to that of figure 2. The structural changes localized at the Fe or heme binding site are shown in the top right panel. Upon ligand dissociation at the Fe, the iron moves out of plane in what is referred to as doming. This motion leads to the motion of the proximal histadine and helical motions. It is the helical motions that change the force balance at the quaternary interface in hemoglobin and modifies the activation barrier for ligand binding. This is the "communication" coordinate between heme proteins and serves as one of our best model systems for understanding molecular cooperativity in biological systems. (Figure is a modified version of original artworkt in R.E. Dickerson and I. Geis. Hemoglobin: Structure, function, evolution, and pathology. The Benjamin/Cummings Publishing Company, Inc., Menlo Park, California. 1983.).

In the process of oxygen transport in the body, somewhere between the dissociation of 1 to 2 oxygen molecules, this molecule "decides everyone out of the pool". Hemoglobin then undergoes a remarkable 15 degree rotation between the α and β protein subunits and a 6 Å translation in at type of ball and socket motion about the quaternary contacts, involving some 10,000 degrees of freedom. This change in quaternary structure causes a change in binding efficacy of nearly a factor of 100 for diatomic oxygen through structural modification of the barriers to ligand binding. With these static structures, obtained with and without a ligand bound to the iron, we have developed a fairly detailed picture of the sequence of events leading to molecular cooperativity in the binding of oxygen. In this context, hemoglobin serves as our cornerstone in the understanding of molecular cooperativity in general. But is the picture correct?

The top panel in figure 3 shows the structural changes of myoglobin as a model system for what transpires at the tertiary level. The top right panel shows a close up of the motions local to the Fe binding site (inspiration for figure 2). Basically the breaking of the bond leads to homolytic cleavage with an electron becoming localized on the d-orbital of the iron. The iron effectively becomes bigger, experiences repulsive forces that force it out of plane (doming) and pushes against the proximal histidine. A series of cascaded events localized at the proximal histidine are invisaged, akin to a series of falling dominoes, the size of single amino acids, that lead to the overall motion of the EF helix. It is this motion of the helical sections that ultimately lead to changes in the contact forces of hemoglobin and drive the quaternary structure changes. Effectively, it is the coupling of the heme doming to the helical motions that affects the barrier height for ligand binding. Changes in structure change the force needed for motion along the doming coordinate and thereby give a through space coupling of reaction coordinates to affect molecular cooperativity, the simplest form of molecular feedback and signaling.

If one examines this model for the motions based on static x-ray structures, it would appear that the key motions are all along the normal to the heme ring. If so, it is a simple matter to provide a structural basis for the transition state and think about means to control the barrier height (or how Nature does it). The transition state would simply be the iron motion out of plane to a position half way between the fully domed configuration and all motions would be along the heme normal. If you thought this, you would be dead wrong (dead in the sense the protein wouldn't work this way). By photodissociating the Fe-CO bond of carboxymyglobin with few cycle, 6 fs, pulses, we were able to determine that the bond dissociation occurs within 26 fs along a repulsive surface. This motion is comparable to a half cycle of the Fe-CO stretch coordinate and is fast enough to impulsively excite all the reaction modes coupled to ligand dissociation in this far from equilibrium, optically accessed, configuration (see Armstrong et al, PNAS 2003). It turns out that in order for the bond to break and heme to dome, the in-plane motions of the heme are also involved. The heavy atoms in the ring have to move to accommodate the Fe doming motion. These porphyrin ring modes are in turn coupled to the low frequency helical motions through a series of cascaded motions over different length scales that are subharmonics of one another. The problem with the simple picture of the transition state can be readily exposed by applying a uniaxial stress to a finite elastic object. Try squeezing a ball, along one axis, and you will see the orthogonal direction dilate. This is Poisson's ratio for the off diagonal elasticity tensor elements of finite mechanical systems. This simple demonstration illustrates the problem with inferring structural dynamics from static pictures, especially transiton state structures where a singularity must be considered within a free energy landscape not cartesian space coordinates. This problem of "guesstimating" structures from static pictures is well known. Based on before and after pictures of someone losing weight, you would not hazard a guess at what dietary regime was taken or intermediate steps to the final fit figure. This point is made to drive home the importance of real time structural determinations. We need this information if we are to really refine our understanding of transition state processes for problems of interest in chemistry and biology. We need structural information on barrier crossing to optimally design control strategies.

The problem is further complicated by the enormous number of degrees of freedom in typical biological problems. If the protein were to function through a statistical sampling of all possible degrees of freedom, it would take longer than the age of the Universe for it to function. This statement is similar to arguments presented for the so-called Levinthal's paradox in protein folding (see K.A. Dill. Curr. Opin. Struct. Biol. **3**, 99 (1993)). If a protein were to sample all possible configurations at the fastest possible sampling frequency, it would take eons for a protein to converge to its active structure. Clearly proteins do not sample all possible nuclear configuration phase space. There are potential gradients due to interactions with its surroundings (water, other proteins, chaperons) that guide the search. Same for proteins executing their functions. The problem is how do we connect the active structure of a protein to its function. If I give you a particular protein structure; can you tell me how the protein works? This is the fundmantal connection we need in order to understand how protein structures have evolved to optimally control chemical processes to perform their functions. There is a kind of periodic table of biology in which specific structural features lead to a particular property with respect to directing chemical energy. The problem must be reduced in complexity. We know from straight forward kinetic arguments that proteins coarse grain sample their potential eneryg surfaces so there must be topologically distinct elements to this sampling process that reduce the complexity of the problem. To date, we have approximately 20,000 protein structures determined. Of these, one can argue that there are on the order of 100 to 1000 topologically distinct structures. However, a rudimentary examination of these structures suggests the number of topological units can be further reduced to include helices, beta sheets, loops, and interconnection of topological units through tertiary contacts. How can we connect these topological units to visualize how the protein undergoes thermal fluctations and samples its built-in reaction coordinate/response to environmental changes? This connection will give us the most fundamental level understanding possible for biology.

To further get across the marvel of biochemical processes, consider the following analogy. I invite you to attend a symphony. This is a magnificent symphony for which some 10,000 different musicians have been assembled and who are all warming up their instruments as you enter a massive concert theatre. The sounds you would hear would be quite chaotic (although I much enjoy the warm hum up of the orchestra pit). Then the mistro steps forward, taps his baton, and this marvellous music, the music of life (to over extend the metaphor), bursts forward. What just happened? The different degrees of freedom, the muscicians, all agreed to be correlated through space by visual inspection of the conductor's baton. The scaling of this analogy is nearly correct. The motion and energy expended by the conductor is similar to that of the other musicians yet he/she is able to correlate some 10,000 degrees of freedom. At the molecular level, the energy released with bond breaking is a small fraction of the total thermal energy of the protein and the root mean square (rms) motions are not similar for all degrees of freedom. In order for the relatively small amount of energy to orchestrate biological functions, there must be correlations imposed by the 3D structure of the protein. In other words to understand this problem, we are looking for the conductor's baton nestled within the various topological features of the protein's structure. In effect, biological molecules are examples of strongly correlated atoms. There is some incredible physics occuring that leads to such strong correlations and enormous speed up in reaction rates as observed in Nature. We are effectively looking for the director within the various structural elements.

The pursuit of the structure-function correlation in biology has been the driving force for me my entire research career. In my opinion, my group has solved how protein's coarse grain sample their potential energy surfaces. We proposed the Collective Mode Coupling Model some years ago based on direct observations of global protein motions (see Genberg et al. Science 1991, Miller, Accounts 1994, and Miller Can J. Chem 2002). This idea was also put forward by Seno and Go (J. Mol. Biol. **216**, 95 (1990); ibid p. 111). about the same time based MD calculations and modal analysis of the displacements. After many years of work, we showed that the global motions of heme proteins following ligand dissociation occurred on the same timescale as motions local to the bond dissociation coordinate. The different length scales of motion were 1:1 correlated. The correlation in motion over different length scales is the definition of a collective response. Furthermore, we developed new methods for measuring reaction energetics that showed this collective or inertial relaxation phase was the dominant component to the structural relaxation, i.e. the deterministic process (see review of Miller, Can. J. Chem. 2002). The work by Armstrong et al (PNAS 2003) mentioned above was able to observe more specifically the cascaded length scales of motion. In terms of the bond breaking event of heme proteins, as depicted in figures 2 and 3, the doming of the heme is coupled to in-plane motions and further changes in contact points within the heme pocket that creates a force displacing the helices surrounding the heme. It is a collective coordinate rather than a local coordinate as previously envisaged.

If one thinks of this mechanically, proteins are effectively hybrid states of matter, with solid-like and fluid-like compositional elements. The helical sections are rigid structural elements relative to the large rms motions of the loop regions. The motion of these sections leads to the atom-atom correlations. In this respect, the loops are probably the most important topological elements. The rms motions of some loops can be larger than liquids. These topological units are the "grease" that enables functionally relevant motions. In the heme problem, the reaction forces from the bond breaking event couple to the helical motions that move in relation to the loops like a forearm moving relative to an elbow joint. It is the combination of structural fluctuations of differentially rigid sections to soft sections within the nanoscale elasticity tensor of proteins that leads to coarse grain sampling. Again, specifically for the myglobin problem, one can map to greater than 60% accuracy the deoxy tertiary structure to that of the oxy tertiary structure through the displacment of just a few collective modes (Seno and Go). These modes are superpositions of displacements of the helical sections. These modes have frequencies in the 10 $cm^{-1}$ to 100 $cm^{-1}$ range and are strongly damped (also shown experimentally through studies of vibrational energy relaxation – see Miller, Ann. Rev. Phys. Chem. 1991) and very efficiently propagate the system from reactant to product surfaces along a reaction coordinate. Another way to say this is that all reaction coordinates are coupled to all possible degrees of freedom in the molecule-bath (surroundings) reference frame. The coupling coefficients are larger for some types of thermally sampled degrees of freedom than others. For solution phase chemistry, there appears to be no evidence for strong propensities; reactions seem to be dictated by density of states and damping terms. However, one would expect mode selective coupling in biological systems. The structures of biological moleculese surrounding reaction site are highly anisotropic in contrast to the homogeneous, isotropic nature of solvation shells surrouding reaction partners in solution. We have indeed found this to be the case. The largest coupling coefficients, accounting for up to 80% of the structural relaxation/reorganization energetics along a reaction coordinate are associated with a discrete number of collective modes (Miller, Can. J. Chem. 2002 and Walther et al. JPC 2005). In my opinion, we have demonstrated the Collective Mode Coupling Model. There has also been a number of theoretical studies (e.g. John Straub et al) and experiments that show the involvement of collective modes (recent work by P. Anfinrud et al., Mitzutani et al, P. Champion et al.) However, we can't tell you from the present experiments which motions are involved. We have to resort to MD based methods and various approximations to cast out the key modes coupled to the reaction coordinate. Furthermore, most biologists will resist this picture unless we can show that the atomic motions are indeed correlated and do in fact map onto easily defined topological features.

Here is the crux of the Structure-Function Correlation problem in Biology. The Collective Mode Coupling mechanism provides a nice framework to visualize strongly correlated motions. However, what motions are involved? As discussed in the introduction, the system is so complex that we can not inferr this information from any form of spectrosopy. The density of states and dimensionality of the problem is too enormous for such renderings. However, if we could directly observe atomic motions during the barrier crossing event along a reaction coordinate, we would in a single measurement be able to cast out the principle motions, i.e. we would have a direct observation of the Structrure-Function Correlation. To my mind, this is the single most important problem to be undertaken by these next generation ultrabright electron and x-ray sources capable of directly viewing atomic motions. The key statement here again is that the time and space resolution must be sufficient to be able to resolve the relative atomic motions faster than collisions and diffusive sampling washes out the inherent correlations imposed by the protein structure re: impulsively excited reaction coordinations.

There are only a few biological systems that can be optically excited and projected onto barrierless surfaces from which to observe the structure-function correlation. The photodissociation of heme proteins represents one class of such proteins. There are other systems that serve as photosensors such as photoactive yellow proteins, rhodopsins, and bacterial analogues. However, there are precious few that lend themselves to the required phototriggering step needed to synchronize the atomic motions and enable making so called molecular movies. These systems will have to serve as a our basis for understanding thermally sampled barrier crossing events in general. Again, recall that one of the central tenets of biology is that the biomolecular structures have experienced strong evolutionary pressures to optimize their structures to control barrriers. We will soon have our first glimpses in how Nature, the ultimate master of chemistry, has found solutions to controling atomic motions from the atomic to the mesoscale to perform functions. This information will transform both chemistry and biology.

The key to this information is to directly observe structural changes with atomic resolution on the timescale of the strongly damped collective modes. The relevant time resolution required is nominally subpicosecond for 10 -100 $cm^{-1}$ modes as inferred from vibrational energy relaxation and collisional transfer to the water layer. This spatial-temporal time resolution will enable a direct determination of the atom-atom correlation functions to cast out the key modes coupled to the reaction coordinate. The discussion that follows is related to the development of the necessary technology to pursue this scientific objective.

## II. Technology Developments

### X-Rays vs. Electrons

The emphasis of this lecture is on the development of high number density ("brightness") electron sources for real time structural studies. There is a parallel effort on-going in the development of x-ray sources.

By far the largest investment is being made in the development of so called 4th Generation Light Sources based on X-ray Free Electron Lasers (X-FELs). Figure 4 shows an aerial view of a few of the slated X-FELs being built around the world. These are huge facilities requiring linear accelerators up to 3 kms long. A short, high bunch charge, electron pulse is generated with a laser pulse. This electron pulse is then accelerated up to 9 GeV and run through an undulator to generate coherent x-rays. The machine physics and engineering involved is remarkable. The X-FEL pulses can be sychronized to the laser used to photoinitiate structural changes with timing jitter on the order of 1 ps. The timing jitter can be reduced by measuring optically the arrival of the relativistic electron pulse near the experimental end station using the electrooptic effect. This measurement is conducted with the same laser used to excite the samples of interst. The effective timing jitter can be reduced to approximately 100 fs in this way. This represents the time resolution for the X-FELS and is sufficient as outlined above for making molecular movies. The fact that the time resolution can be made as high as this after a 3 kilometers of travel is really an engineering feat. The overall pathlength variations over 3 km must be kept to 300 microns so the timing jitter is not greater than the time window of interest.

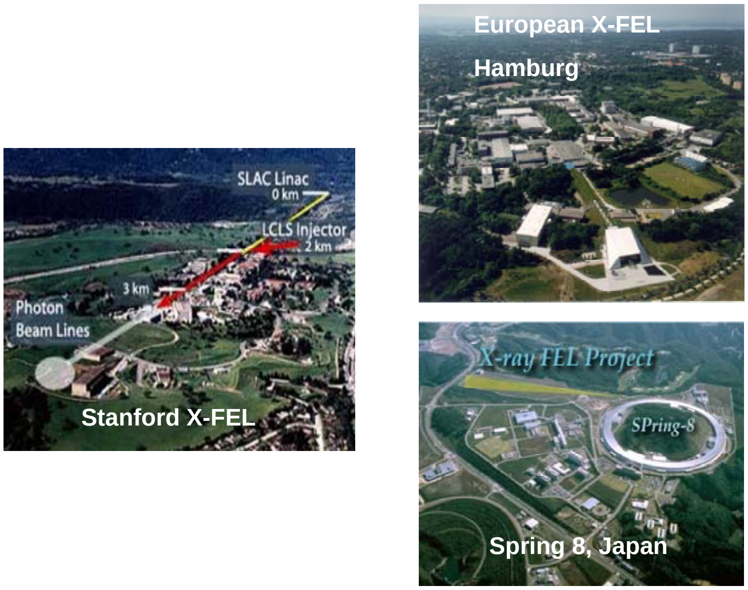


**Figure 4.** The various 4th Generation Light Sources being constructed aroud the world. Left: LCLS at Stanford, USA. Upper right: European X-FEL at DESY Hamburg. Lower Right: Japan's Spring 8 Facility. Images taken from: http://www-ssrl.slac.stanford.edu/lcls/downloads/lcls_brochure_screen.pdf, http://www.xfel.eu/XFELpresse/en/hintergrund/flash/index.html, and http://www-xfel.spring8.or.jp/, respectively.

The Linac Coherent Light Source (LCLS) announced in April 2009 that it produced "first laser light" — the first coherent x-ray laser. The current configuration produces soft x-rays. In order to resolve atomic positions requires wavelengths in the 0.1 nm range. The LCLS will be online with hard x-rays for this purpose by September 2010. The European X-FEL being built in Hamburg will be online with hard x-rays by 2014.

From figure 4, it can be appreciated that the 4th Generation Light Sources are clearly not table top systems. These machines carry $1B price tags. There is no doubt that these new light sources will have a major impact in Science. Is there another way?

The other way is to use electrons. It is relatively simple to accelerate electrons up to the prerequisite energy to have short enough de Broglie wavelengths to resolve atomic structures. Table 1 gives a comparison of electron and x-rays in this application.

**Table 1. Electrons vs. X-rays**

| Electrons | X-rays |
|---|---|
| Strongly scattered | Weakly scattered |
| Table top experiment | Syncrotron/X-FELs/large frame laser |
| Number density dependent pulse parameters | Number density independent pulses |
| Excellent match between excited and electron probed volume | Poor match between excited and x-ray probed volume/large background |
| Multidiffraction orders facilitate structure determinations | Potential for Laue Diffraction (not for narrow band X-FELs) |
| Energy deposited in sample per scattering event 400-1000 times less (1.5 A X-rays) | x-ray induced damage limits crystal lifetime/diffract and destroy |
| *Incoherent (for > 1 micron beam diameters) | Coherent (X-FELs only) |

The above table reflects that electrons interact much more strongly with matter than x-ray photons. For the same energy, electrons scatter $10^6$ more strongly than x-rays. This difference means a factor of $10^6$ less electrons are needed to give the same resolving power for the same energy electrons and x-rays. The challenge as stated above is how to develop high bunch number electron pulses without having coulomb repulsion or space charge effects ruin the spatio-temporal resolution. My group has developed some relatively very "bright" sources for this application. For calibration, the number of x-ray photons/pulse from a 3rd Generation Light Source Sycrotron, such as the ESRF in Grenoble, is $10^9$ in a nominally subnanecond pulse. We have been able to achieve $10^4$ electron per pulse in a 200 fs pulse. Taking into account the much higher diffraction efficiency with electrons, the diffracted particle flux per sample pathlenth (experimental figure of merit) is approximately 1000 to 10,000 times higher than a 3rd Generation Light Source. Only the 4th Generation X-FELs will be brighter when they come on line with hard x-rays (about 100x). As will be discussed below, this difference in experimental brightness, re: diffracted particle flux, will soon be reduced with next generation electron guns.

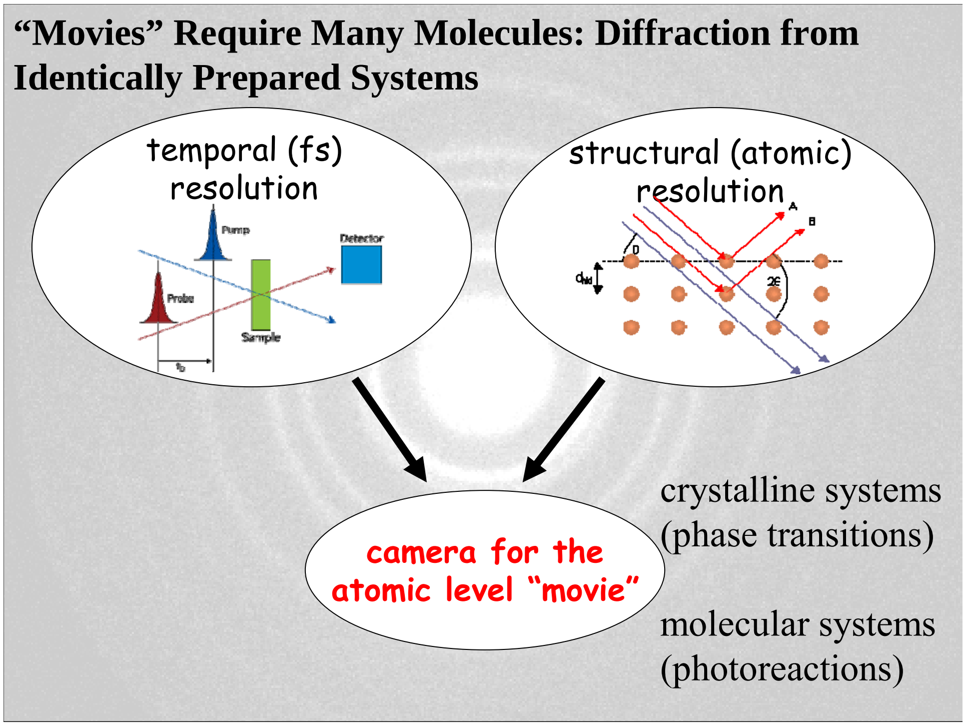
The main message of the above table is that anything that one can do with x-rays, one can do with electrons as well. There are trade offs depending on the application in which one source may be better than the other in extracting structural dynamics. The one property of the new x-ray sources that would seem to be insurpassable is the coherence of the 4th Generation Light Sources. These are truly spatially coherent laser sources. In the electron case, the electron pulse is derived from photoemission from a photocathode. Each electron in the bunch has a random phase and are sources more closely related to light from a tungsten light bulb. However, the asterix (*) label by incoherent in this table is intended to draw attention to the fact that it is possible to make fully coherent electron sources analagous to x-rays. Standard field emission transmisssion electron microscopes have effectively fully coherent electron beams. With field emission tips, the electrons are emitted from a small enough source that the transverse coherence is akin to that of an electron "laser". One of the most important scientific objectives of 4th Generation Light Sources is to be able to exploit the high coherence of the x-ray source to do single molecule imaging. Most protein drug targets are membrane bound proteins that may never be crystallized. The prospect of being able to solve protein structures of membrane bound proteins to guide drug design made a very compelling case for X-FELs. The problem is that in order to ensure enough coherently scattered x-ray photons, the x-ray beam must be focused down to provide $10^6$ x-ray photons/$Å^2$ to give one coherent scatttering event per atom. At these focusing conditions, the electric field of the laser radiation well exceeds the Coulombic attractive field of the atom. The molecules will be ionized down to nearly bare nucleii. The x-ray pulse must be made short enough to provide a coherent scattering pattern prior to the molecule blowing up. This effect has been referred to as "diffract and destroy". Even in the event of 10 fs or even shorter x-ray pulses, it is the electron distribution that scatters the x-ray photons. The diffraction process is occuring in the strong perturbation limit and will need major technological advances in both theory and experiment to succeed.

Here is one application where the much lower induced damage and higher scattering cross section may give electrons a distinct advantage over x-rays. New electron sources specifically designed for this application will open up the study of both biological and nanoscale materials. However, it is not possible to obtain short pulses of electrons for such an application as electron-electron repulsion will limit electron pulses to microsecond durations or longer. This application is primarily for static structure determinations. (In order to have femtosecond time resolution with such a source, there would have to be only a single electron emitted per excitation of the emitter. The sample must be photoinitiated at the required excitation to see structural changes. This requires finding a sample that is fully reversible over $10^7$ -$10^9$ photon cycles. Such systems have not been found to date. This approach would not lend itself to a general technique. This point will be discussed below.)

At this point, it is important to emphasize that structural dynamics at atomic resolution will require the use of crystals. In principle, if single molecule imaging can be achieved, one could excite single molecules with a laser and follow the changes. However, even in this limit, there is not enough information from a single projection to reconstruct the structure. The use of the term "single molecule structures" is misleading as each coherently sampled molecule only reveals a cross sectional view of the atomic positions. It takes nearly 1 million randomly sampled angular orientations to reconstruct a molecule in this manner with atomic resolution. Rather than try to repeat the experiment 1 million time for a single time point, it is much simpler to have 1 million copies of the molecules arranged in a regular manner, i.e. in a crystal. The basic concept for making Molecular Movies is shown in Figure 5. This figure shows the case for solid state samples. Liquids and gas phase samples can also be used in which case there is no periodic order to enhance the structural refinement.

## Ultrabright Electron Source Development

As can be inferred from Figure 5,  a significant fraction of the atoms or molecules will need to be excited to get the changes in diffraction above the background contributions of the unexcited parts of the sample.  The very act of exciting a crystal normally leads to strain and cracking of crystals due to the very induced structural changes.  Even without this consideration, the process of laser excitation generally causes changes in the sample through other possible side reactions and nonradiative relaxation mechanisms for energy disposal of the absorbed photon energy into the lattice. It can be readily appreciated that one may quickly run out of sample or film to be able to resolve the motions of interest. The major technological advance that led to the first studies of atomic motions in real time (aka molecular movie) was based on a new concept for creating high density electron bunches.



**“Movies” Require Many Molecules: Diffraction from Identically Prepared Systems**

crystalline systems (phase transitions)

molecular systems (photoreactions)

**Figure 5.  Basic experimental layout for electrons or x-rays**.  In the top left, the pulse sequence is shown.  A femtosecond laser pulse is needed to induce a structural change.  This pulse synchronizes the motions.  The subsequent atomic motions in response to the photostimulus is followed stroboscopically using an equally short pulse of either electrons or x-rays to diffract off the lattice of atoms/molecules.  The time delay between the laser excitation and structural probe pulse can be controlled with $10^{-15}$ second precision using variably pathlengths using motorized translation states. By using multiple sequences of excitation and probe delay times, a Molecular Movie is made of the ensuing structural dynamics.

The major technological challenges to making Molecular Movies with electron sources are the following:

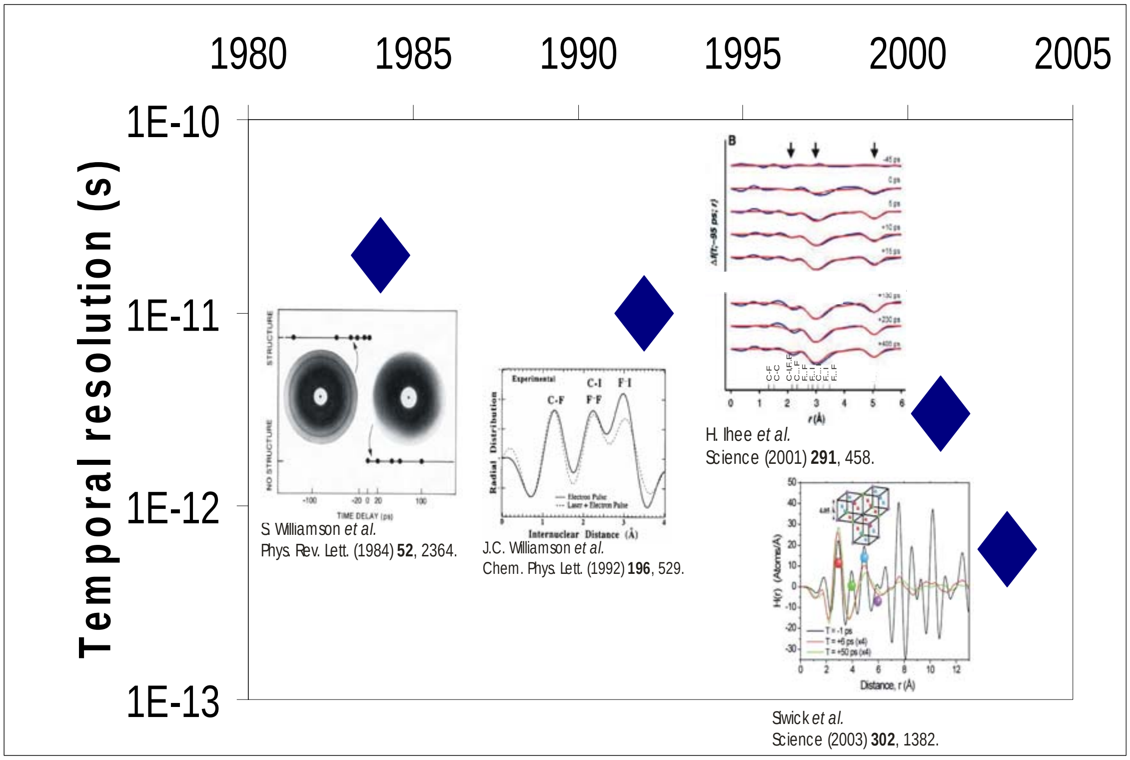
- recognition that most photoinduced structural changes are irreversible and require sample exchange and constraints on the total possible laser shots ⇒ general solution requires near single shot structure determination with atomic resolution.
- One must generate more than $10^{12}$ electrons $sec^{-1}$ $cm^{-2}$ to enable single shot structure determination in the presence of electron-electron repulsion. How can we generate such high number density pulses without incurring significant space charge broadening of the electron pulse?
- If we can solve the above, how can we characterize 100 femtosecond electron pulses? Strong space charge effects/electron-electron repulsion lead to rapidly changing electron pulse durations with propagation. Characterization requires 100 femtosecond time resolution with a spatial resolution along the beam path of 1 mm or less.
- How to characterize t = 0 timing between electron and light pulses?

The above challenges have been presented at numerous workshops and conferences as serious if not insurmountable barriers to the use of electron sources for structural dynamics. Prior to our work, there was no means to generate pulses with sufficient electron density for single shot structures. Similarly there was no detector fast enough to measure the electron pulses. In order to have any chance of reducing space charge effects, the electron beam size must be on the order of 100 microns and the beam rapidly changes during propagation. Streak cameras only afford 1-2 picosecond instrument response times for such beam parameters and need 4+ cm deflection plates, well short of the requirements. Similarly, there was no means to determine the relative time of arrival of the electron pulse and laser excitation pulse as needed to sychronize the filming of atomic motions. The effective shutter speed or time resolution of the Molecular Movie camera is determined by both the pulse duration of the electrons (convolved to the laser excitation pulse) and the accuracy in t = 0 position. It is also necessary to take into account the difference in velocity of electrons and laser light in the sample region as this also affects the instrument response time. If we are to reach the canonical 100 fs time resolution required for making Molecular Movies as discussed in the introduction, it is essential that we be able to fully characterize the pulses with 10 fs -100 fs time resolution and mm spatial resolution along the beam propagation.

We were at the same point in development of ultrafast electron pulsed sources as we were with femtosecond laser systems in the 1980's. There was no way to characterize such short laser pulses. No detector was fast enough and new ways of using nonlinear correlations between laser pulses needed to be developed in order to achieve the time resolution promised by such short laser pulses. In the last 3 years, all of the above technical hurdles have been solved by my group as I will detail below.

## Historical Progress in Ultrafast Electron Diffraction for Structural Dynamics

There are a number of key developments that led to the present technology. This historical progression is shown in Figure 6 as a means to properly cite previous work and show the rapid progress in the last few years. The first use of electrons for time resolved structural studies of short lived gas phase intermediates was the work of Ischenko et al (Appl. Phys. B 32, 161–163) using electric field deflection plates of an electron beam. This streaking of the diffracted beams gave microsecond time resolution.



S Williamson *et al.*
Phys. Rev. Lett. (1984) **52**, 2364.

J.C. Williamson *et al.*
Chem. Phys. Lett. (1992) **196**, 529.

H. Ihee *et al.*
Science (2001) **291**, 458.

Siwick *et al.*
Science (2003) **302**, 1382.

**Figure 6. Progress of Ultrafast Diffraction for Structural Dynamics**. The above time line shows the major milestones with respect to following changes in structure. The first use of time resolve diffraction was by Ischenko *et al* that used streak plates to obtain microsecond time resolution and is not shown here. Most electron diffraction experiments use the modified streak camera technology introduced by Gerard Mourou's group (1984 time point). There is an enormous body of work by Ahmed Zewail's group that can't be adequately depicted here. The philosophy in their work to date is to use small numbers of electrons to single electrons per pulse to avoid space charge broadening. Such low electron numbers require fully reversible samples, gas phase (1992) and reflected electrons from surfaces (2001). The first study capable of subpicosecond time resolution to solve a structural dynamics problem was from the R. J. Dwayne Miller group (2003 time point). Not shown is recent studies that have captured electronically accelerated atomic motions of Bi and phase transition within 200 fs (Sciaini et al, Nature 2009).

The seminal work in terms of technology development was the work from Gerard Mourou's group. All present designs of electron guns are based on this work with the exception of next generation guns to be described below and relativistic electron sources (see J. Hastings 2006). My own group was inspired by the work of Mourou et al. The first experiments of this kind basically took a streak camera apart and put a sample before the streak plates. They were the first to use a pump pulse and electron probe pulse delay for high time resolution. It is now clear that Williamson et al (1984) were able to illustrate lattice heating but not true structural changes (and possibly should not be listed in this figure). The diffraction dynamics were thought to be do to melting but it is now known from the work of Siwick et al (Science 2003) that the excitation was too low to see melting. Nevertheless this work achieved nominally 20 picosecond time resolution and set the stage for the work to follow. The next major milestones were accomplished by the Zewail group. They adopted the use of single electrons or low electrons per pulse to study structural dynamics. There is a large body of work from this group that I can not do justice to in this brief lecture. This work has been extensivley reviewed (see Zewail, Ann. Rev. Phys. Chem. 2006). Suffice it to say that their philosophy in solving space charge broadening of the time resolution limited them to the study of fully reversible systems. In order to obtain a good diffraction pattern, with multiple time points or movie frames, requires approximately $10^6$ electrons minimum. To be in the single electron/pulse limit actually requires that each laser pulse impinging on the photocathode only generate 1 electron every 100 excitation events to statistically avoid multiple electron emission and associated space charge events. The sample must be excited every laser shot irrespective of whether an electron is photoemitted. The problem from a time resolution point of view is that it is then necessary to find a sample that is capable of undergoing $10^6$ to $10^8$ photon cycles without changing in the excitation process. Nearly all molecular systems have quantum yields for nonradiative relaxation and side reactions that well exceed the limit ($<10^{-6}$) to avoid build up of other contaminating structures from complicating the diffraction analysis. It is not impossible in principle to find fully reversible systems. There are a few candidates but certainly the subset of potential systems do not lend themselves to a general approach. By going to gas phase and using molecular beams solves this aspect of the problem as it permits rapid sample exchange between laser shots and use of high repetition lasers to achieve reasonable data collection times (see the work of Peter Weber et al). However, the molecular beam dimensions are on the order of several 100 microns to millimeters. Typically 10 KeV electrons are used in these studies to take advantage of the higher scattering cross section at lower energies. The velocity of the electrons at this energy is approximately 1/5th the speed of light. This difference in velocity between nonrelativistic electrons and light is referred to as the velocity mismatch problem. Over the typical molecular beam dimensions, it takes the electrons 10 picoseconds to traverse the beam in the same time the light takes only 2 picoseconds. Here, there is a common misconception that femtosecond time resolution has been obtained as the electron pulses in such low numbers should have femtosecond durations. The electron and laser pulses can both be of femtosecond duration. However, the effective time resolution or shutter speed is on the order of 10 picoseconds (re: Williamson 1992) due to the velocity mismatch between the excitation and probe pulses as they propagate through the sample. This confusion in time resolution arises from the typical practice of reporting pulse durations rather than instrument response functions. For those in the field, the effective time resolution is understood but it is worth pointing out this important distinction in the present context. It is also possible to study surface dynamics in reflection (with higher electron numbers/pulse) where the underlying lattice helps the system relax back to the equilibrium structure. Here the projection of the electron beam at the required glancing angles again means an effective sample pathlength on the 100 micron scale and velocity mismatch limited time resolution to several picoseconds. Recently, Baum et al (Science 2007) have reported the use of tilted excitation pulses to better match the velocities and have shown nominally subpicosecond time resolution (see footnote 40 for a qualification on the time resolution). This work has surface charging effects that need to be sorted out in order to determine if the changes observed are due to structural changes or fields (see below in summary section).

The first work to break the picosecond barrier and enable direct observation of atomic motions faster than diffusive motions wash out details was the work of Siwick et al (Science 2003). This was accomplished by going to studies of solid state samples using electron transmission where the samples must be between 20 nm to 100 nm thin. The ultrathin sample pathlength ensures that velocity mismatch between the electron and laser pulses is negligible. However, for such thin samples, the laser excitation needed to excite a significant fraction of unit cells to induce structural changes, as opposed to simple lattice heating, will lead to irreversible changes in the sample. The work of Siwick et al used a novel compact electron gun design, to be discussed below, that provided 600 fs electron pulses with enough electrons to obtain atomically resolved structures with as few as 10 averaged shots (100 shots used for best compromise in surface area and signal to noise). This was a major advance in electron source "brightness" for structural studies. Since these studies, the basic compact gun design has been advanced to provide pulses as short as 200 fs with enough electrons to do truly single shot structures (Sciaini et al. Nature 2009).

With the latter time point, it is clear that the camera for Making Molecular Movies is now in hand. One can argue about the "molecular" nature of such observations for solid state systems. Here it needs to be born in mind that crystals can be thought of as giant molecules. The atoms are all bonded and bonds have to be broken to undergo the structural changes. There are even large biomolecules with as many atoms as the nanocrystal grains in such films. However the real distinction between say a solid state and a molecular descriptions is when one talks about discrete atomic positions rather than collective coordinates of a periodic lattice. The observed dynamics involve nucleation sites that are truly nm in size and befit a description of the relative motions of atoms. It is in this sense the term making molecular movies is used. It is important to emphasize that this accomplishment was achieved by going to high brightness or more accurately high electron number density pulses to achieve single shot structure capabilities. Although it is possibe to find fully reversible systems and use single electron pulses to avoid space charge effects, subpicosecond time resolution to structural dynamics (as opposed to simple lattice heating) has not been achieved to date with this approach. In all cases, high electron number pulses will increase the signal to noise over low number electron pulses. The benefits are similar to trying to collect spectra detecting a single photon per pulse *vs* the entire spectra in a single shot. The improvements in signal to noise are improved by orders of magnitude and can make the difference in resolving spectra. Similar issues pertain to fully resolving structural dynamics. It is essential to have enough light or rather electrons in this case to light up the relative atomic motions.

## Development of High "Brightness" Electron Sources for Structural Dynamics

In the less formal nature of this lecture, I think it is useful to relay some of the trials and tribulations that my group experienced before solving this problem. It helps depict the execution of science in its unpolished form. It is my hope that this account may provide some resolve to those reading these lecture notes see a problem to the end. This statement is made with the assumption that the problem is worth doing. Deciding what problem is worth this kind of effort is half the battle.

I can vividly recall the day that Brad Siwick came to my office to discuss his latest results on electron pulse characterization. After a 2 year effort building up the experimental setup with Jason Dwyer, and another year building a zero jitter streak camera (nontrivial in its own right), Brad informed me that all his measurements indicated that it would not be possible to get subpicosecond electron pulses with sufficient electron numbers per pulse to do the experiments we planned. Further he showed me "in a back of the envelope" calculation that the 1D liquid theory for electron propagation in the literature, that we used to design the electron gun, could not get the proper asymptotic limit to the pulse duration. He had no confidence in the theory we used to design the gun. I am glossing over some of the discussion. At one point, he exclaimed that "This $#@! experiment was never going to &%# work!" . This was a very dark day for me in my scientific career as I had invested all my research funds given to me by the University of Toronto as part of my start up package there to build this machine. Here was one of my top students telling me it was not going to ever work (at least if we continued on the same path). Can you imagine. All your efforts over many years and all the funding seemingly misdirected. A daunting thought to be sure. Then Brad asked me the one question I think all frustrated experimentalists ask themselves. Could he do theory? (This is one of my favourite stories.). What could I do? I of course conceded that we neeed to look into the electron propagation problem more carefully.

Brad Siwick was very enterprising. He took it upon himself to master the Barnes-Hut Code used by astronomers to study early galaxy formation. It is a tree algorithm that enables the optimal allocation of computation time to solve the equations of motion for many body systems. The computation time scales as NlogN rather than $N^2$ for solving the equations of motion for N interacting bodies, a great saving in computation time as the system increases in size. Brad's first group meeting, armed with this new approach, showed the formation of a pinwheel galaxy involving some 10,000 planetary masses moving with weak gravitatation forces (with the simulation clock advancing at $10^{+15}$ sec steps). This was a simple test of the code. Four months later he gave another group meeting in which he changed the particle masses to electron masses and interaction to repulsive Coulombic forces. This was yet another dark day in my scientific career as the true nature of the challenged was laid bare. Figure 7 shows the basic configuration for these tree code calculations and nicely illustrates the main features of the technology.

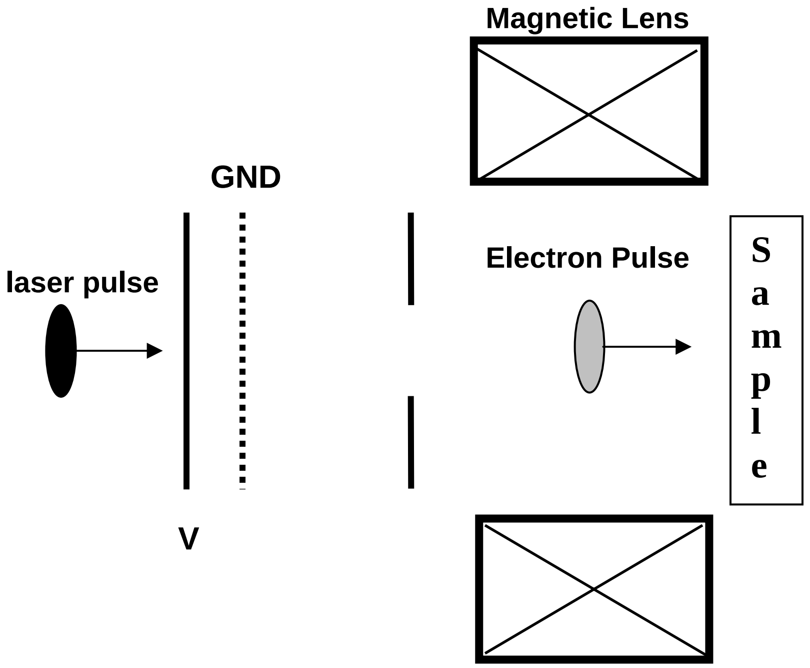


**Figure 7. Modeling of Electron Propagation Dynamics**. The basic layout of an electron gun for diffraction studies is shown. A fs laser pulse (from left) excites a photocathode that leads to photoemission of a replica fs electron pulse through the Einstein photoelectric effect. The photocathode is held at -30 KV in this calculation. The extraction grid is held at ground. The electrons are accelerated up to 30 KeV energy, isolated with an iris then pass a magnetic lens for focusing the electron beam onto the detector plane (not shown). The de Broglie wavelength of the electrons at 30 KeV is .06 Å and the average separation is 10 microns for the densities modeled. There is virtually no wavefunction overlap and the free propagation of the electrons from the photocathode to the sample can be treated classically. The quantum aspects of the problem show up when the electrons impinge upon the sample at which point the electron de Broglie wavelength is comparable to the interatomic spacings and gives rise to diffraction (a pure wave phenomenon).

The equations of motion for 10,000 electrons interacting through repulsive Coulombic forces could be solved numerically effectively exactly in the classical limit. The extraction field has to be as high as possible to minimize energy spread effects on pulse broadening. It is important to get the electrons up to the final energy as quickly as possible. We modeled our experimental parameters, using 100 KV/cm extraction for 150 fs electron pulses created in an approximately 100 micron beam size with an initial energy spread of .6 eV at birth (Siwick et al. JAP 2002). By modeling the electron bunch as a thin 2D disc (initial dimensions at birth are 10 microns by 100 microns), it was possible to derive a mean field approximation to the electron propagation dynamics, i.e.,

$$\boxed{\frac{d^2 l}{dt^2} = \frac{Ne^2}{m\varepsilon_o \pi r^2}\left[1 - \frac{l}{\sqrt{l^2 + 4r^2}}\right]} \qquad (1)$$

where m is the mass of the electron, l (t) is the propagation distance (time) from the photocathode, r is the electron beam radius, and N is the number of electrons.

It turns out we had badly underestimated the space charge effects in the design of our, at that time, 2nd generation electron gun. Figure 8 shows why this was such a stark realization.

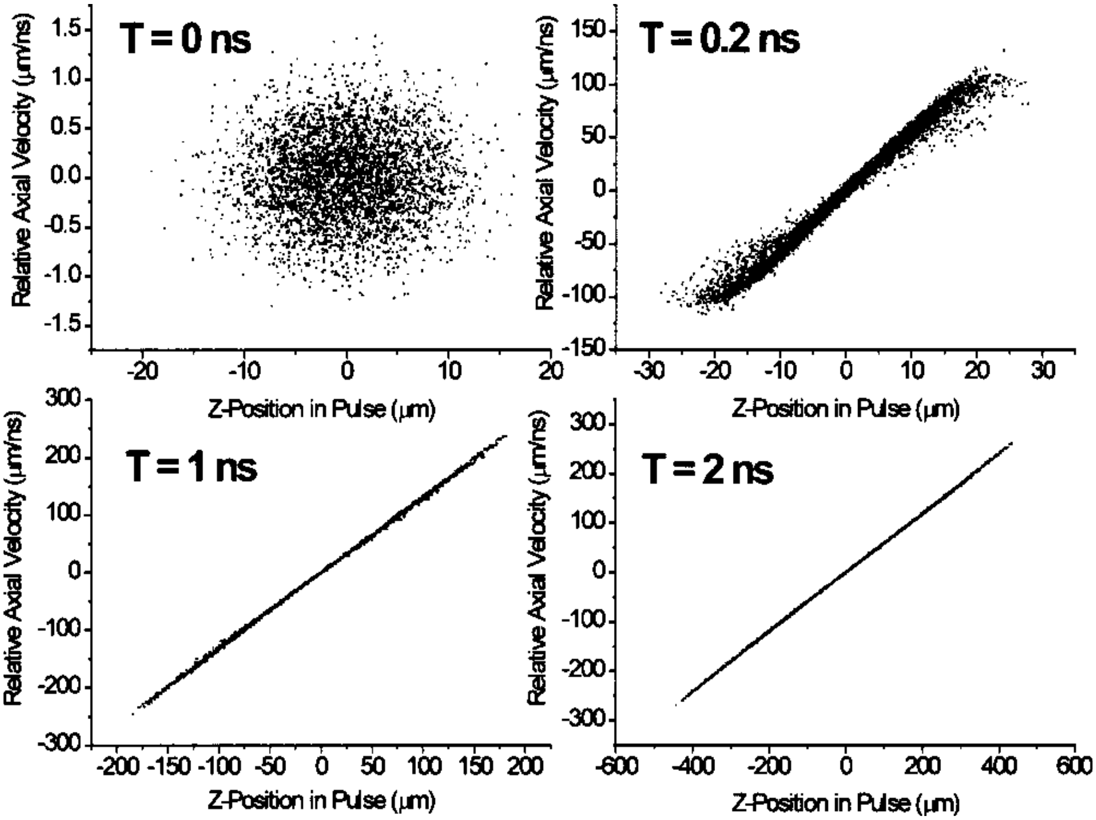


**Figure 8. Electron Pulse Propagation Dynamics**. The panels show a dot density plot of the electron pulse as it propagates for times T (note .1 ns = 1 cm propagation distance for 30 KeV electrons). The initial pulse of 150 fs, 10,000 electron, r =75 microns undergoes rapid changes in duration as a function of propagation time. The effects of space charge broadening are clearly observable from the increase in the kinetic energy distribution as seen in axial velocity (y axis). The energy spread increases by a factor of 100 and the pulse increases by a factor of approximately 2 in just 2 cm propagation distance. The initial distribution of electrons at the front edge of the pulse experiences a large negative charge density accelerating the front edge; while the back edge experiences a large negative charge density in front of it that decelerates the back edge. The electron pulse is born at high potential energy that is transduced to translation motion through Coulomb repulsion (from Siwick et al, JAP 2002).

It turns out that the electron pulse would increase its pulse duration by an order of magnitude within just a few 10 centimeters of propagation. This feature was also faithfully reproduced by our analytical solution (Eq. 1). Our electron gun at the time was the standard 20 cm length. No wonder, it was not providing bright enough pulses for Molecular Movies.

I have to say that my jaw dropped when I saw these plots. In order to achieve the canonical 100 fs time window needed for Molecular Movies, we would need to build an electron gun of just a few centimeters. The thought of designing such a compact gun holding off 30 KV without a lightening storm (arcing in the chambre) seemed to be insurmountable problem. However, the group was invigorated as we finally knew exactly what we needed to do to make a Molecular Movie Camera.

The first Molecular Movie Camera was based on the compact gun design shown in figure 9. This gun design was a significant departure from the low electron number density guns of Mourou et al. The challenge was to keep all the surfaces, not just the photocathode region, free of sharp edges and points of high voltage breakdown. There were also significant challenges with respect to preventing ions produced during laser excitation of samples from backtracking into the photocathode region. By using small extraction pinholes and photocathode exchange it was possible to minimize this problem to an acceptable level.

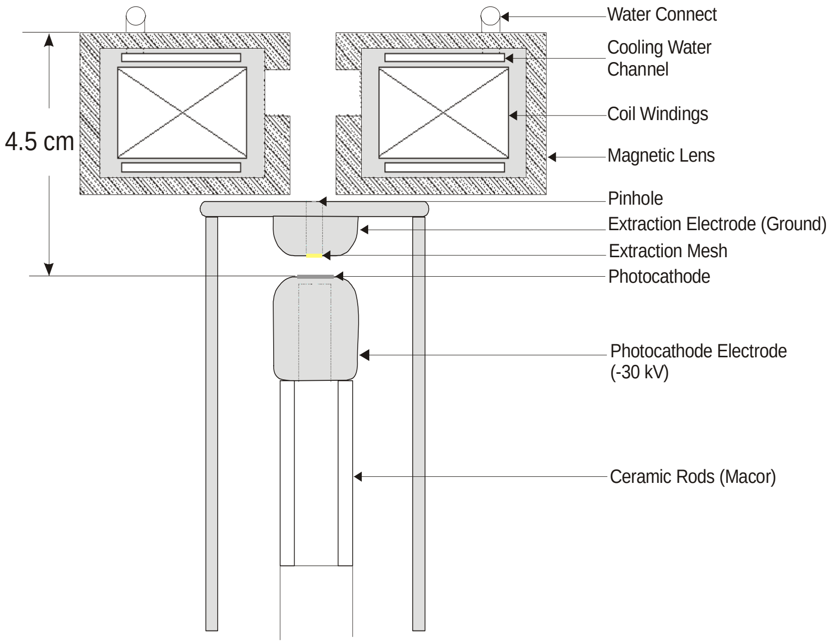
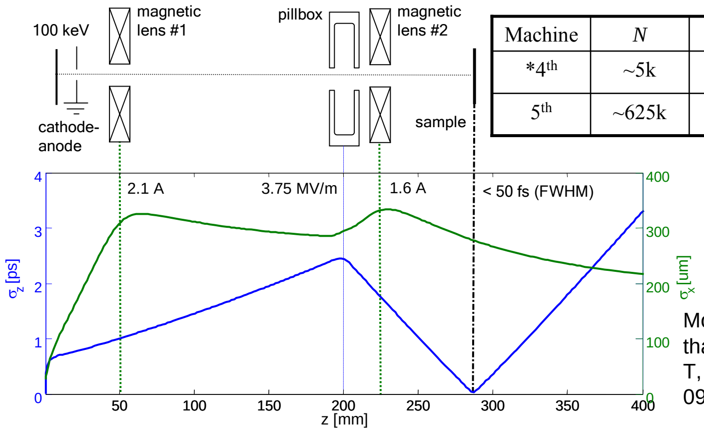


**Figure 9. The 3rd Generation Electron Gun**. By putting a water cooled magnetic lens directly after the extraction pinhole it was possible to reduce the propagation distance to the sample to approximately 4 cm. With 6000 electrons, this gun provided approximatetly 600 fs pulses in a small enough area to atomically resolve structural dynamcs near the single shot limit. Subsequent 4th Generation versions have been able to further reduce the compact design concept to approximately 1-2 cm by use of wrap around magnetic lens designs and pulse shortening down to less than 200 fs.

The other very significant feature to come out of this study that was not anticipated prior to the simulations is that nonrelativistic electron bunches very rapidly develop almost perfectly linear chirps (see lower panels in Figure 8). The space charge induced increase in the kinetic energy spread is more than a factor of 100 more than the initial 0.6 eV energy spread. This means that the energy distribution is determined by the spatial position of the electron in the bunch. The initial energy spread is negligible. In this regard, vacuum is “dispersive” for nonrelativistic electrons; higher energy electrons travel faster than lower energy electrons. As the electrons then propagate, the high energy electrons separate from the lower energy electrons to eventually form a linear spatial chirp. This feature to nonrelativistic electron bunches is being specifically exploited now for 5th Generation Electron Guns (Figure 10) that will be capable of 30 fs electron pulse durations and factors of 100 more electron per bunch. All the results I will present will be improved by a factor of $10^3$ with these new gun designs, that will compete head to head with 4th Generation Light Sources. In this regard, there will be some problems better suited to study with electrons and some with x-rays.

## Electron Pulse Compression



| Machine | $N$ | $t_p$ (fs) | $L_c$ (nm) |
|---|---|---|---|
| *4th | ~5k | 100 | 3 |
| 5th | ~625k | <50 | 7 |

Modified design from that of van Oudheusden T, et al. JAP 102 (9) 093501 , 2007

**⇒Expect another factor of 1000 increased “brightness” re: re-bunching**

**⇒ less than 50 femtosecond time resolution**

**Figure 10. 5th Generation Electron Guns**. The basic concept involves the use of a second cavity to invert the velocity chirp acquired during space charge broadening during pulse propagation. The rf field in the box marked pill lens is such that the front edge of the pulse sees a smaller electric field than the back edge as the rf field of approximately 3GHz frequency changes enough during the pulse propagation through the lens to invert the so called phase space (momentum:position) of the electron pulse. The electron is effectively time focused to a minimum pulse duration (285 mm) where the sample needs to be located.

## Pulse Characterization

As can be readily appreciated from even a cursory inspection of Figures 8 and 10, high number density electron pulses rapidly change with propagation distance. Until recently it was not possible to characterize electron pulses on the 10 fs time scale now approachable with 5th Generation Electron Guns nor could one make such measurements with the required millimeter spatial accuracy. My group was able to solve this problem using a bit of well known physics. Through the laser pondermotive effect it is possible to spatially deflect electrons. The Lorenz force provides the displacive force; effectively electrons move in response to the magnetic and electric field gradients of an intense laser pulse. This interaction would give us the ability to time stamp the electron bunch by a known laser pulse, and thus determine t = 0 and also determine the electron pulse duration through a cross correlation in time of the unknown electron pulse profile with a known reference laser pulse. The pondermotive force is well known but it was not known what field intensities would be needed for the laser pulse to give an observable effect. Armed with the ability to accurately calculate the electron propagation dynamics, we could now include a laser field to the propagation problem in order to estimate the required laser intensity. The basic measurement concept is shown in Figure 11.

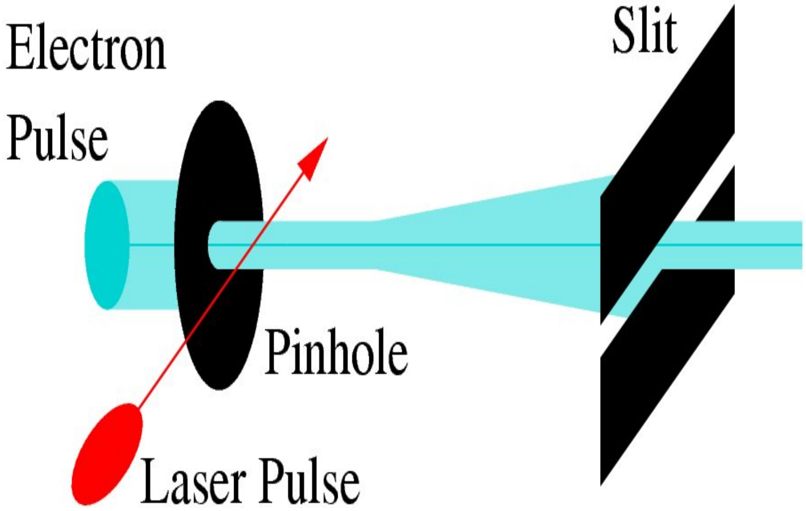


**Figure 11. Pondermotive Laser Scattering for Full Electron Pulse Characterization with Micron Spatial Resolution.** An intense laser pulse intercepts the electron pulse at right angles creating a transverse force to deflect electrons. The hole drilled in the electron beam can be directly visualized in an expanded beam or by using an aperature to prevent secondary electron scattering of the deflected electron, the deflected electron themselves can be imaged. The laser beam can be focused down to less than 10 microns. The intrinsic time resolution is given by the transit time of the electrons across the focused laser beam. For 100 KeV range electrons, this time resolution is on the order of 10 fs for both t=0 position and pulse characterization, well good enough to characterize electron pulses capable of following the fastest nuclear motions. (The slit shown is typically replaced with a phosphor to directly image the deflected electrons.)

Specifically, the gradient in the laser pondermotive potential ($U_{pond}$) gives rise to a pondermotive force ($F_{pond}$),

$$F_{pond} = -\nabla U_{pond};\ U_{pond} = (e^2\lambda^2 I)/(2\pi m c^3) \qquad (2)$$

where $\lambda$ is the laser wavelength and I is the laser intensity. Including this term in the electron propagation, we calculated that we would need approximately 10-15 mj, 100 fs, laser pulses to obtain an observable deflection at the detector plane (microchannel plate amplifier/phosphor and CCD camera combination).

These calculations were born out. The agreement both in terms of the measured electron pulse duration at a particular position and magnitude of the laser interaction were exactly that calculated (within error bars).

Figure 12 shows a dramatic capture of the electron pulse in free flight to illustrate how well this method works.

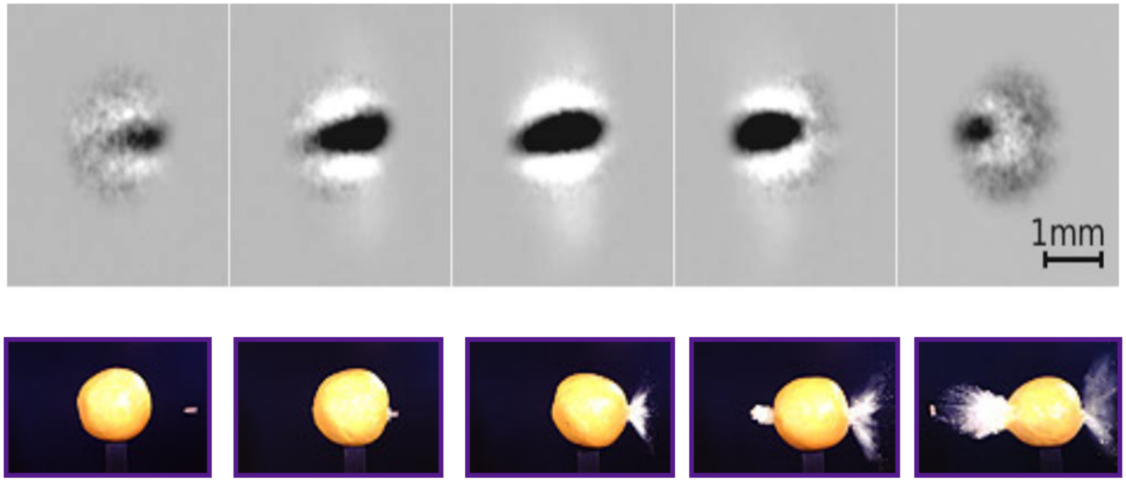


**Figure 12: Capturing an electron bullet in free flight**. The electron beam impinges on an MPC/phosphor combination for imaging. The top panel shows the passage of a 15 mj laser pulse of 90 fs duration through an electron pulse at time steps of approximately 100 fs (Hebeisen et al Optics Letter 2006). The observations show that the electron pulse is comparable in duration to the 90 fs laser pulse. For effect, this rendering is compared to time sequence of phographs of a bullet passing through a lemon (courtesy Andrew Davidhazy RIT)

This measurement is nontrivial and requires a major laser facility to deliver 10 – 100 fs laser pulses in the 10-100 mj range. We used this method to calibrate our camera and demonstrate how accurate our numerical simulations of the electron propagation were. This study illustrated we now had an excellent handle on how the electrons propagate and thereby a means to advance electron source technology. However, any changes to our e gun would require sending the camera to a major laser installation to be recalibrated. The bigger problem is the spatial sensitivity of the electron pulse duration. It is very difficult to ensure the machine pulse parameters are constant at the sample position. We have now recently greatly improved the methodology. By going to counterpropagating laser pulses to make a standing intensity grating, we have increased the intensity gradient enormously (re: Eq. 2) and increased the number of scattering centres. Each fringe in the interference pattern represents a scattering centre. We have increased the laser pondermotive scattering efficiency by approximately a factor of 100 (see Hebeisen *et al*, Optics Express 2007). These measurements are now routine and can be conducted with microjoule pulsed systems. This reduces the laser requirements to conduct atomically resolved structural dynamics to that of what could be a hand held amplified femtosecond fibre laser system.

The above discussion documents the historical path to high brightness electron sources that are now capable of 10-100 fs time resolution, sufficient to capture the fastest atomic motions, and most important with sufficient brightness for single shot structure determinations. These advances have been discussed for nonrelativistic electrons as only these sources have demonstrated femtosecond time resolution to structrural dynamcs to date. There are equally promising developments based on relativistic electron sources but these machines have not been employed in time resolved measurements as yet (see J.

Hastings et al. 2006 and papers citing this work). All the technical hurdles to making a Molecular Movie Camera based on nonrelativistic electron diffraction have been overcome. These technical advances have served as the basis for the first atomically resolved structural dynamics that qualify as a Molecular Movie.

As a last comment for this section, the tools are now in place for generating and fully characterizing femtosecond electron pulses. Just as in femtosecond optical studies with the advent of nonlinear correlation methods, it is no longer acceptable to estimate pulse durations or time resolution. *Pulses need to be fully characterized. It is now essential to use the above described methods to properly characterize electron pulses and accurately report the instrument response function.*

## III. The Science: First Frames

### Atomically Resolved Phase Transitions

As with any new method, one starts with the simplest systems for study first. In terms of structural dynamics, one of the simplest structural changes is melting. Everyone has experienced this phenomenon first hand. This is an order to disorder phase transition. The simple question is how does something go from an ordered structure such as a Face Centred Cubic (FCC) lattice with a coordination number of 12 to the disordered shell like structrure of a liquid with an effective coordination number of 10 (in the case of aluminum)? The length scales of motion, collapse of the transverse barrier to highly anharmonic liquid motion and rearrangement to a shell like liquid structure depends on the how strongly driven the phase transition is.

To make the problem a little more graphic, we all know how ice melts. If you imagine a block of ice melting, you know it melts from the surface inwards. The melting point of the surface layer is lower than the bulk as the atoms have much smaller barriers to motions along the normal. Also the temperature gradient exists at the ice interface. So melting occurs at the interface and propagates inward in a process known as heterogeneous nucleation. Under 1 atm pressure, the inward propagating liquid melt zone is pinned at a temperature of 0 $^0$C. The other bit of information gained from everyday experiences, that we all know, is that if we were to increase the rate of heating of the ice it would melt faster. If I was to direct a blow torch towards the ice, the melt zone would propagate inward to the bulk faster. The temperature of the melt zone would still be 0 $^0$C at 1 atm. There is however clearly a relation between the rate of heating and the propagation of the melt zone that can be described mathematically. Now here is the other intriguing question. What if I had a special kind of “blow torch” with which I could heat up the ice so fast that no matter what theory you used, you would predict the ice would melt faster than the atoms could move? This question was pondered by Born, among others, back in the 1930’s with respect to the fleeting state of superheated matter. At some point, the granularity of the interatomic potential should factor into the problem. Now in a manner of speaking, I actually do have a special kind of “blow torch”. With femtosecond lasers, it is possible to deposit energy into a lattice and have it thermalized at heating rates of $10^{15}$ $^0$C/sec. We can approach this intriguing limit to how fast materials can lose structural correlations.

There is another important consideration with the application of femtosecond laser pulses with respect to exciting materials. In the last 10 years, it has been determined that there is a unique and special attribute to femtosecond laser excitation, not seen with longer pulses. If the laser pulse excites approximately 10% of the material's electrons, the material will appear to melt on time scales faster than the absorbed energy can be thermalized. This process is referred to as electronic induced melting or nonthermal melting. With longer pulses, the energy is thermalized faster than material excitation so the effect is not observed. Basically, on the femtosecond time scale the lattice is frozen. The use of femtosecond excitation creates a new charge distribution that alters the bonding, generally softening the lattice. At some point the lattice potential is no longer a bound state and the lattice appears to "melt" from various optical signatures. This phenomenon appears to be completely general. To drive home the highly unusual nature of this effect, imagine I was to shine a femtosecond laser on you and excite 10% of your electrons. You would appear to melt in under a picosecond. The visual effect would be similar to the famous Salvador Dali painting of a melting watch, e.g. work entitled Persistence of Memory, 1931. (Of course with so much absorbed energy, you would burst into a fire ball a few picoseconds later, or more acccurately experience laser ablation physics first hand, but you would appear to melt first.)

The notion of nonthermal melting had never been rigorously tested with structural probes. The observation of so called nonthermal melting was always done through changes in the frequency dependence of the material linear and nonlinear susceptibility or optical properties. Under such strong electronic excitation, these parameters are not well known. The effect of nonthermal melting could simply be disordering of the periodic electronic structure describing the valence electrons or population of uncorrelated excited states in a molecular basis that leads to the changes in optical properties. Irrespective, the use of femtosecond laser pulses to change the charge distribution within an initially frozen lattice description allows for the first time the manipulation of interatomic forces and energy landscapes of materials. It is this aspect of femtosecond laser driven phase transitions that is so intriguing. The time scale is so short that we can literally go into the material, rearrange the electron distribution, and watch how this affects the fundamental forces of bonding. To access this information, we need to directly observe the atomic motions in response to the induced change in bond strength. This problem was the first problem addressed using structurally sensitive probes or first Molecular Movie.

At the time of these experiments, my group was inspired to study the issue of nonthermal melting of Al by the work of Tony Taylor and co-workers at Los Almos. They observed what appeared to be the onset of nonthermal melting for absorbed excitation fluences corresponding to approximately 1.5 times the energy required to melt the lattice or a superheating, $\theta$, paramter of 1.5. Under these excitation conditions, the lattice optically appeared to take on the dielectric properties of liquid Al within 600 fs. This conclusion was based on observing the changes in optical reflectivity at various angles for a single colour and comparison of the derived parameters to that of liquid vs solid Al. This observation was remarkable in that Al is viewed as the classic, jellium, model of a metal. The crystal structure of Al is FCC with no directional bonding per se. The band structure can be well described by an isotropic distribution of electrons bound by the collective periodic potential of the underying positively charge nucleii. The melting of Al within 600 fs is also remarkable in that this nominally isotropic electron distribution, would have to lead to statistical variations leading to bond breaking on a time scale of just a few phonon periods or effective lattice collisions. How is this possible? The basic idea is that the optical excitation leads to an interband transition in which the band structure involves weaker bonds. At excitation levels of approximately 10% of the valence electrons, the lattice is no longer a bound state and it collapses to a disordered structure. This notion is shown schematically in Figure 13.

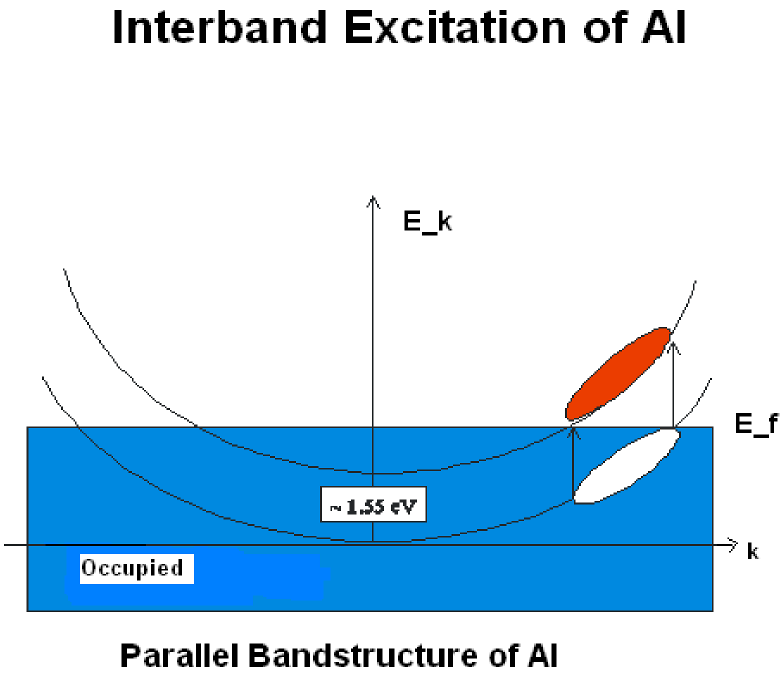


**Figure 13. Schematic of optical excitation of an interband transition in Al**. The upper lying conduction band has more antibonding character and leads to bond softening. At excitation levels exceeding 10%, the lattice was thought to no longer be a bound state.

You don't need to know anything about band structure or molecular orbitat theory to understand the basic concept portrayed here. If you consider the model quantum mechanical problem of a particle-in-a-box (electron in retangle potential), you know that as you go from a lower level to a higher level, the nodes in the electron's wavefunction increase by one. In terms of chemical bonding, the energy of the system is lowered if the electrons involved in bonding experience the increased attractive coulombic force of two nucleii relative to just one. This lower energy state that we call a bond involves a build up of electron density between the two bonded nucleii so the electrons feel a larger net $Z_{eff}$ of the two nucleii. As the number of nodes increases, there is a depletion of electron probability distribution between the nucleii and the bond becomes weaker. So it is easy to imagine at some point the increased antibonding character leads to sufficient softening of the lattice to cause melting through this purely electronic effect. However, what is unusual for Al is that there is no directional bonding. So why would the change in distribution affect bonding? The electrons are moving in a sea of positive charge. Also in considering the electron excitation process, the excited electrons would have to remain in the new electron distribution corresponding to the initially accessed electronic band for this degree of antibonding or bond softening to persist long enough to cause melting. However, the electron-electron scattering rate with the underlying cold, unexcited electrons, is exceedingly fast, faster than the reported 600 fs (see work of C. Schmuttenmaer *et al* on fs relaxation rates of electrons in metals, Phys. Rev B 1994 and subsequent papers). Finally, and this was the most interesting point to me, in order to melt within 600 fs, the lattice would have to undergo sufficient collisions to sample the disordered potential energy surface of a liquid. On the 600 fs timescale, the lattice is effectively frozen. How could the system sample sufficient phase space and exchange in positions to go to the shell like structure of liquid? There is a demo I like to do on this problem using coins positioned as a 2D cross section of an FCC lattice, confined in a box to approximate the periodic hard wall conditions of the problem. There is no attactive forces between the coins and the potential is completely flat. It is easy to see that it would take many collisions for even a nominally completely unbounded (flat) lattice potential to sufficiently sample the disordered state that is needed to undergo melting. This demonstration nicely shows that within an atomistic or localized atomic basis it is hard to reconcile the observed dynamics as a true ordered to disordered phase transition.

I hope it is apparent that this problem contains some deep fundamental issues. First, the use of femtosecond laser pulses to rearrange electron distributions within a frozen lattice allows us to explore the fundamental connection between electron distribution and bonding. In order to access this information we need to directly observe the atomic motions in response to the induced changes in the potential energy surface of the lattice to report on the changes in forces. The new ability to directly observe atomic motions on the prerequisite time scale and spatial resolution using fs electron diffraction opens up this possibility. Prior to this development, we could only measure material properties as given. Now we can manipulate electron distributions and observe the change in interatomic forces. In principle, studies of optically induced changes in the phonon dispersion could give this information but the optical phonons typically become strongly damped at such excitation conditions and the entire phonon branch would have to be determined to invert to changes in the lattice potential energy surface. This information is obtained directly from the determination of the displacement of the atoms from their equilibrium positions. Secondly, this class of experiments enables us to probe electron-electron correlation effects. We have deeply ingrained views of lattice structure in terms of an atomistic view. However, the minimum energy position giving rise to this structure is related to the minimum energy for a given electronic distribution or electronic state and this is a true many body effect. We can now view the consequences of electron-electron correlations as will be described below.

The specific experimental setup used for the studies of strongly driven phase transitions in Al is shown in Figure 14. This setup serves to point out the general features of the experiment.

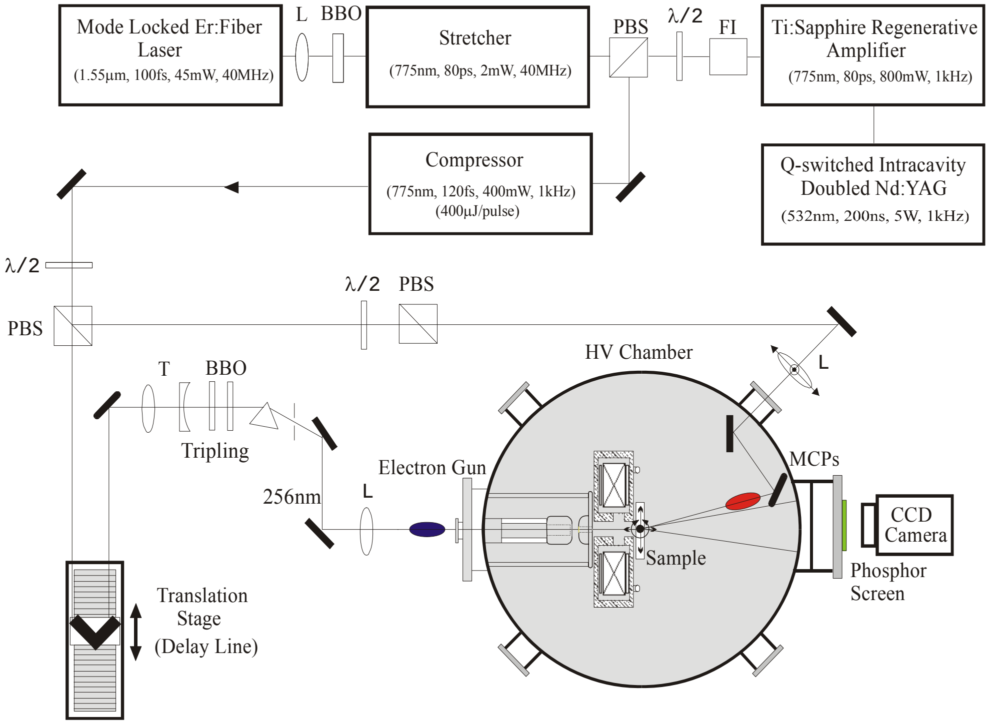


**Figure 14. General Experimental Layout for Femtosecond Electron Diffraction.** A femtosecond laser system serves to provide both the pulses to excite the sample (red) and to generate the electron probe pulse (blue). In this manner, there is no timing jitter between the laser excitation and electron probe pulse. The sample needs to be on the order of 10-100 nm thin to enable electron transmission for the highest time resolution without velocity mismatch between the light and electron pulses to broaden the time resolution. The time sequence of structural changes is stitched together to make a movie by exciting the sample and probing the changes for a given relative time delay between the excitation and electron probe pulses. The translation stage varies the pathlength of the laser pulse used to generate the electron pulse and thereby varies the time delay. With optical encoders, it is no problem to vary the time delay with subfemtosecond accuracy. Upon each laser excitation, the sample must be moved to bring a fresh spot to the excitation area as the sample has been irreversibly changed or damaged. The electron diffraction pattern is detected in the present case by the use of a microchannel plate image intensifier with a gain of $10^6$ and CCD readout.

The key experimental details are the use of a compact electron gun design to minimize space charge broadening of high number electron bunches, as discussed above, and nanofabrication of large surface area samples. Of the two, it is now apparent that the sample is the most imporant experimental consideration. The machine physics for generating sufficient short, bright, electron pulses is now in hand. Sample problems are not amenable to a systematic approach (as any crystallographer will tell you). To give you some sense of the sample challenges, the first work on Al required fabricating free standing films of 20 nm thickness with a surface area of 2-3 cm$^2$ in order to have enough "movie film" to capture a full time sequence. If this was not enough, the sample has to be mirror flat over the entire area to avoid errors in t=0 position, that would appear at timing jitter. If there is a sample ripple of say 10 microns, at one position the electron pulse and laser pulse could be overlapped in time and define t=0 but at the position displaced by 10 microns, a 30 KeV electron pulse would arrive 100 fs offset from the other position This variation in t=0 amounts to timing jitter and loss of time resolution.

This problem puts enormous constraints on the sample. The yaw and pitch of the sample position must be adjusted to be perfectly normal to the beams, or corrected on the fly, and the sample must be mirror flat over this sampled region. I have to single out Jason Dwyer for solving this problem. The fabrication of the first samples involved floating 20 nm films of Al off polished salt crystals, then catching them on large area, home made, Cu TEM grids. The tension on the different Cu wires of the grid were adjusted with a specially designed holder, under an interferometer, to make the sample mirror flat. A contact probe in the electron diffractometer was then used to align the sample's yaw and pitch. You have to visualize a 20 nm free standing, mirror flat, sample of several centimeters area to appreciate this accomplishment. This process used to take several months to qualify a sample. We have refined the procedure by using Si nanofabrication methods to make the sample support grids. The Si wafers are extremely flat. We can now prepare samples of up to 25 $cm^2$ area and larger that are mirror flat. We have also introduced the use of SiN window supports with each window looking very much like a frame in traditional 8 mm movie film. We can now simply put the sample in the chamber and be collecting data within an hour of pumping down the chamber.

The first experimental study of structural dynamics to achieve subpicosecond time resolution, as required to solve a problem, is shown in Figure 15. These studies were conducted under the exact same excitation conditions used in the optical studies that reported nonthermal melting of Al. The results are rather dramatic and don't need a high level of analysis to arrive at the most important conclusions of the work. It is immediately apparent that the crystal still shows diffraction from long range order at an electron probe delay of t = 500 fs. At t = 1.5 ps, the higher orders of diffraction indicative of the solid state order are still present but dimmer. This decrease in diffraction from the higher diffraction orders is due to a well known effect of lattice heating and decrease in coherence of the diffraction process, or Debye-Waller effect. The larger rms atomic motions decreases the correlation in atomic positions and thus leads to decreases in constructive interfernce leading to diffraction at a given order. The amplitude of the higher order diffractions fall off quadratically with scattering wavevector. This effect is used to determine the amplitude of rms atomic motions and lattice heating dynamics. It is not until t = 2.5 ps that one sees the onset of the diffraction at low scattering wavevectors indicative of liquid formation. At t = 3.5 ps, it is apparent the entire sample has been converted to the liquid state. Based on this simple pattern recognition of the general features of solid state and liquid state, one can arrive at one of the most important conclusions of this work. The solid to liquid phase transition is not nonthermal but rather can be completely understood as a thermally driven phase transition. The melting dynamcs are occuring on the picosecond timesecale and not the 100 fs timescale required for a purely electronically driven process. Second, and perhaps equally important, it is clear the nucleation process and lattice collapse occurs with a propagation time across the entire sample of approximately 2 ps. This propagation time is faster than the speed of sound (getting back to our original question). For a 20 nm thick sample, it would take at least 8 ps for the melt zone to propagate through the sample at the speed of sound if it was to occur through heterogeneous nucleation. Rather, the mechanism of melting clearly involves <u>homogeneous nucleation</u> (see papers of B. Rethfeld, Phys. Rev. B 65, 214 303 and Z. Lin, L.V. Zhigilei, PRB 73, 184113 (2006) for the theory). Rather than melting from the outside-in, as in the normal heterogeneous nucleation process, it is melting from the inside-out. The degree of superheating (θ = 1.5) is such that there is enough thermal energy to overcome barriers to forming liquid nucleation sites. The nucleation sites must be on the order of nm to not subtend the whole sample, i.e. the nucleation sites are truly molecular in scale.

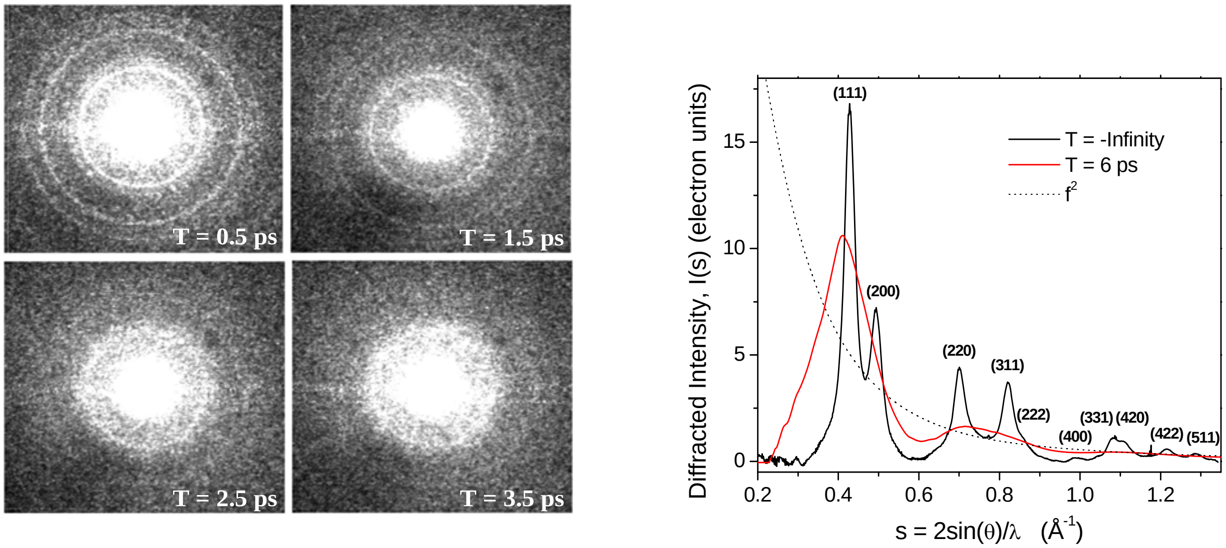


**Figure 15. First Frames**. The left panel shows the raw electron diffraction data for polycyrstalline Al under excitation conditions corresponding to a superheating of approximately 1.5. The polycrystalline sample gives the classic ring structure for diffraction from randomly oriented crystal projections. The long range solid state order is clearly visible up to 1.5 ps time delays after the excitation pulse through the higher order diffraction rings. Between 2.5 ps and 3.5 ps, the lattice is seen to collapse to the shell like diffraction pattern with intensity build up at low scattering wavevectors corresponding to the shell like structure of liquids. The right panel shows the radially averaged data and peak assignments. The data shown for 6 ps illustrates that the diffraction is from liquid Al. The signal did not signficantly change between 3.5 ps and 6 ps (Siwick et al. Science 2003).

The tranition to the liquid state can be seen more clearly by looking at the difference-diffraction data and comparing the decay in the high order diffraction intensities with expected lattice heating effects. These comparisons are shown in Figure 16. The build up of diffracted electron density at low scattering wavevector (*s*) indicative of longer range density changes in forming a liquid structure is apparent. There is also an abrupt departure from simple lattice heating as can be seen in the right hand panel of Figure 16. It is clearly a thermally driven phase transition with an abrupt collapse to the liquid state in which large amplitude thermal motions ultimately lead to bond breaking and creation of liquid state nucleation sites. Once the nucleation sites form there is very rapid collapse of the entire lattice. Here the importance of obtaining a sufficient number of diffraction orders to invert the diffraction to structure needs to be emphasized. Previous time resolved diffraction experiments were only able to resolve a single rocking curve for a particular diffraction order (e.g., see the work of von der Linde et al, Rousse et al using laser based x-ray plasma sources circa 2000). These early studies did not have the brightness sufficient for more orders. Note, if we were to just examine the (111) diffraction order we would have concluded the sample did not melt. This peak overlaps with the liquid peak and only decays to a common amplitude. In contrast if we were to monitor the highest order diffractions, (331) or (420), we might have concluded that the lattice was undergoing nonthermal melting as the decay in amplitude is subpicosecond. The decay in this order nicely demonstrates the time resolution of this experiment was in fact in the 100 fs range (600 fs instrument response) as required to resolve the issue of thermal vs. nonthermal melting. Multiple diffraction orders are needed to invert to structure.

Here it is also important to emphasize the need for structure sensitive probes such as electron or x-ray diffraction. The femtosecond optical studies indicated melting occurred on a 600 fs time scale. The problem is that the optical signature is determined by the electronic band structure changes with electron density. At room temperature, the electron mean free path is on the order of 10 nm. This mean free path defines the induced polarization volume through interactions of the electrons with the applied optical field. At very high excitation, the increased electron scattering and electron-hole scatttering processes will reduce this and more localize the electron wavefunction, similar to the effect of loss of periodicity in forming the liquid state. It is simply not possible to connect changes in optical properties, over a limited frequency spectrum, to structure. There needs to be signficant theoretically modeling of the correlation between changes in spectra and structure. This statement holds true for all frequency ranges. It is possible, however, to use a very broad spectrum to access multiple transitions to discern collapse of bandstructure and give qualitative statements about the process. The electron diffraction studies have been further confirmed by such a broadband spectral analysis that shows melting occuring on a 2 ps timescale indicative of a thermally propagated phase transition (see Kandyal *et al*. PRB 2007)

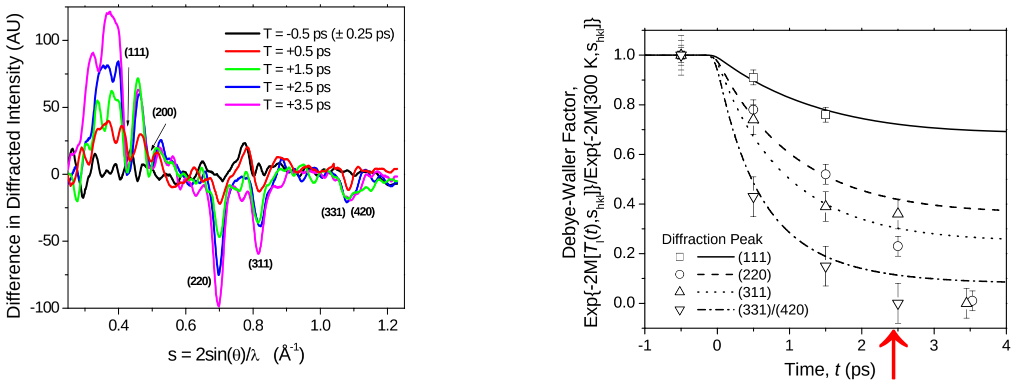


**Figure 16. Projection of the Onset of Melting**. From the difference in diffraction intensity with and without laser excitation, it is readily observed that the diffraction intensity builds up at low s, in regions below that of the (111) diffraction order of the crystalline phase, showing longer range density changes. The loss in the higher order diffraction is also apparent and due to the loss of periodic lattice structure to support higher order diffraction. The right panel shows the expected trends in the time dependent diffraction if simple lattice heating was involved. The data can clearly be explained as simple heating up to the time point marked by the red arrow. At this moment, the signal undergoes a precipitous drop from the (220), (311) and 331) diffraction orders in relation to the continuous decay expected from increased lattice heating. At this point, the average thermal energy and rms motions lead to bond breaking and large

amplitude motions involved in collapse of the FCC lattice to the liquid shell structure. (Siwick et al, Chem. Phys. 2004).

A more detailed analysis of the actual mechanics in the disordering process can be gleaned from the diffraction data. This analysis is shown in Figures 17 and 18.

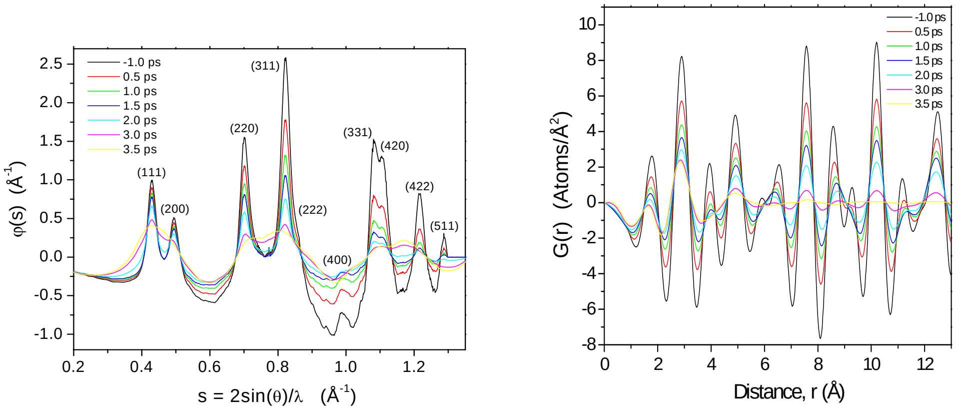


**Figure 17. Diffraction analysis and inversion from reciprocal to real space.** The data on the left is the baseline corrected, radially averaged data normalized for scattering wave vector, s, dependence at the various time delays. The normalized diffraction intensity can then be converted through a sine transform to the reduced density function (right panel). This plot was made with cyclic boundary conditions to give the impression of the time dependent changes in atomic positions through the entire crystal

The time dependent structure factors, φ(s), in the diffraction pattern are determined, after background subtraction, from the relation:

$$\varphi(s) = s\left(\frac{I(s)}{Nf^{2}} - 1\right) \tag{3}$$

where s is the scattering wave vector in reciprocal space, N is a normalization constant, I is the diffracted intensity, and f is the atomic form factor. This can be used to calculate the reduced density function, G(r) using:

$$G(r) = 4\pi r\left(\rho(r) - \rho_o\right) = 8\pi \int_0^\infty \varphi(s)\sin(2\pi sr)ds \tag{4}$$

in which ρ(r) is the position dependent change in atomic density relative to the average density $\rho_0$. This time dependent function gives directly the two time atom-atom pair correlation function. At any position r, this function gives the angularly averaged probability of an atom at this position. Since we know the relative positions of the atoms at t =0, we can track the changes in the atomic position relative to one another by examining G(r, t). The important feature of this experiment is that the time resolution was sufficient to follow the relative atomic motions on a timescale faster than atomic diffusion over a single bond length. In this case, we can literally colour the atoms and discern the origins of the atomic displacements as shown in Figure 18.

By integrating the area of the probability distribution for the different time steps we were able to discern the change in coordination from a solid state lattice to the simple shell like structure of a fluid. Inspection of the different time steps shows uniform broadening of the probability distribution at all sites. The lattice is literally shaking itself apart in this thermally propagated phase transition.

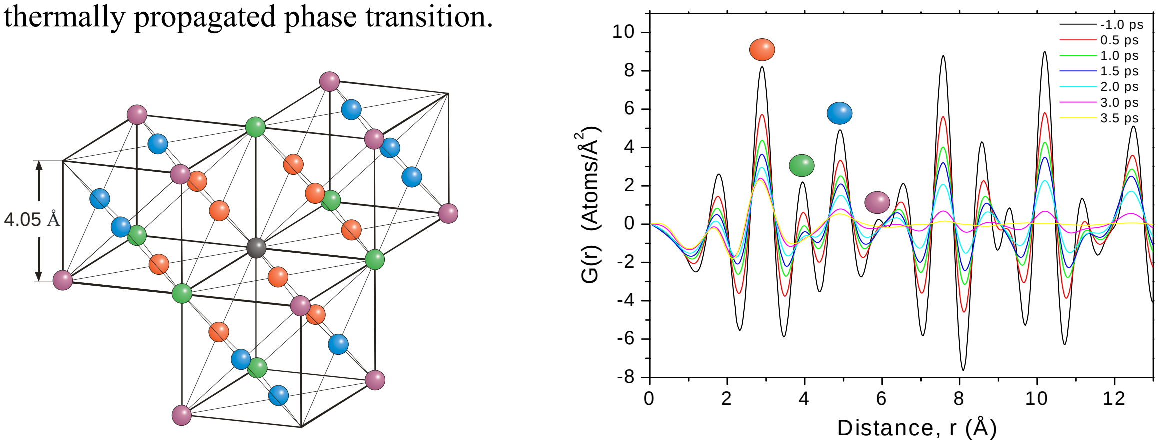
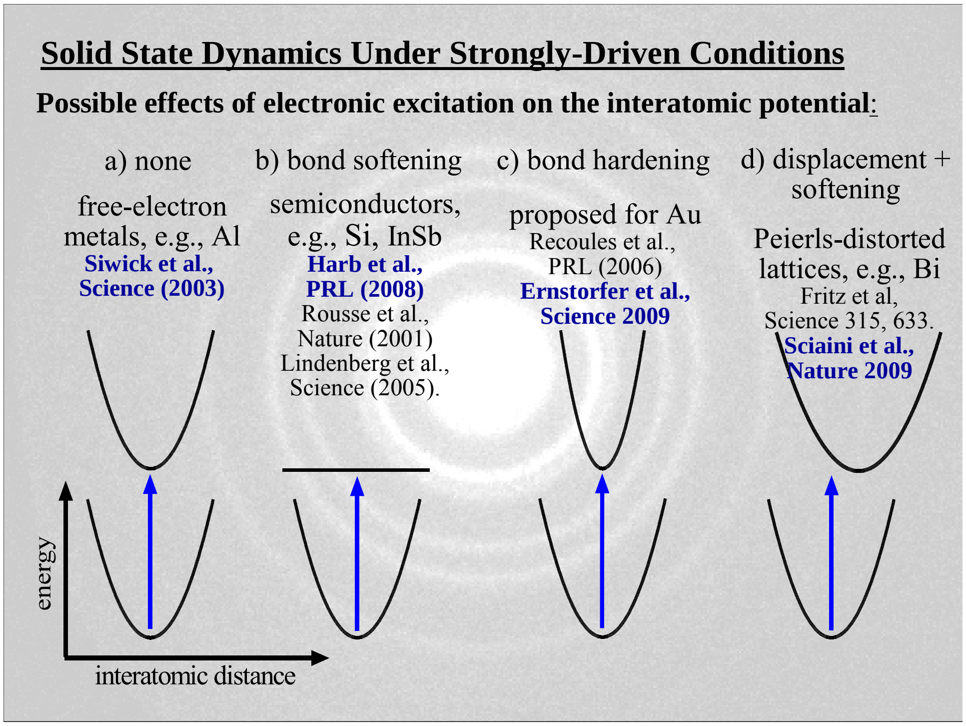


**Figure 18. Time Dependent Reduced Density Function — Atomic Pespective of Melting.** The left figure shows the FCC lattice structure of Al. The different atomic positions relative to the origin of the unit cell are coloured. In the right panel, the time dependent reduced density function is shown. It is the motion of the apex atoms that undergo the largest angular diffusion at the various time steps. These shear or transverse tmotions are central to the tranformation from a coordination of 12 for the FCC lattice to a coordination number of 10 for liquid Al, with more density build up in the interstitial regions of the initial lattice structure.

The above study represents the first to attain enough diffraction orders with sufficient time resolution to provide a real time picture of atomic motions on time scales faster than collisions and onset of diffusive motions wash out the details. It gave the first time dependent atom-atom correlation function during a structural tansition. Equally important this work involved a thermally propagated structural change along the ground electronic surface as the initially excited electrons relaxed back the ground state configuration by this time. There has been a great deal of effort expended in theoretically modeling liquids. The decay in the two time atom pair correlation function demarkates liquid behaviour. It has till now been exclusively a theoretical construct. In the above study, we know the initial relative atomic position prior to t=0 (lattice points) and can follow the statistically signficant displacements from these positions as the rms motions become large enough to project onto the liquid state. It is exactly this kind of picture, we would like to obtain for functionally relevant protein motions. In this latter case, the process would retain some overlying order. We would know the effective mass of the displacements and the time dependent velocities or forces. This information can be inverted to give the principle components to the potential energy landscape, encoded in the protein structure, that is responsible for function. The work on Al is the first experiment of its kind as a first step to this objective.

Since this initial work, a number of other systems have been explored. These are summarized along with other relevant work in Figure 19. This figure is intended to show the connection between band structure and dynamics of strongly driven phase transitions.



**Solid State Dynamics Under Strongly-Driven Conditions**

**Possible effects of electronic excitation on the interatomic potential:**

a) none
free-electron metals, e.g., Al
**Siwick et al., Science (2003)**

b) bond softening
semiconductors, e.g., Si, InSb
**Harb et al., PRL (2008)**
Rousse et al., Nature (2001)
Lindenberg et al., Science (2005).

c) bond hardening
proposed for Au
Recoules et al., PRL (2006)
**Ernstorfer et al., Science 2009**

d) displacement + softening
Peierls-distorted lattices, e.g., Bi
Fritz et al, Science 315, 633.
**Sciaini et al., Nature 2009**

**Figure 19. Schematic of the Effects of Different Crystal Symmetries and Band Structures on the Dynamics of Strongly Driven Phase Transitions.** The interatomic potential as modified by optical excitation (blue arrow) is shown to distinguish the different possible relationships. The cited literature in blue used high brightness femtosecond electron diffraction to fully resolve the problem

## Thermally Driven Phase Transitions in Free Electron Metals.

For free electron metals, there is no directional bonding. We studied the phase transition dynamics in Au, another FCC lattice, but with a factor of 10 smaller electron-phonon coupling constant, as a further check on the Al studies. It could be argued that the dynamics for melting were fast enough that we may have missed some electronic contributions to the dynamics. One normally likes an order of magnitude difference in dynamics for two potential mechanisms to clearly resolve the problem. In the Al case, there was only a factor of 3. Here the studies of Au were an important extension as the much smaller electron-phonon coupling for Au would give the factor of 10 difference in phase transition dynamics and clearly separate the timescales between any electronic effect and simple lattice heating. This study was conducted to further examine the possibility of electronic factors in free electron metals. Under the same experimental conditions as Al with respect to superheating, the phase transition was found to occur between 30-50 picoseconds (see Dwyer et al., Phil Trans 2006). There was absolutely no indication of any faster disordering dynamics indicative of electronically driven bond softening and lattice collapse at a critical density. It was possible to extend the excitation up to extremely high superheating conditions, θ > 10, all the way up to warm density matter (vide infra) with no indication of subpicosecond dynamics as signatures of electronically driven atomic motions. It is now clear that directional bonding is needed for any change in bonding to manifest itself as repulsive terms in the optically induced potential energy landscape that would lead to disordering. The system is already in a high symmetry state. In order to change coordination numbers to that of the liquid shell structure requires multiple collisions and acquisition of thermal energy to drive the sampling process.

## Electronically Driven Phase Transitions in Semiconductors

The prospect of observing electronically driven changes in bond order were first suggested by optical studies of Si by C. V. Shank and co-workers in the early 1980's. The notion that one could modify the chemical bonds in Si gained considerable attention as it opened up the possibilty for optical control of the band structure of Si for high speed applications. Also given the technological importance of Si, we have the most highly refined understanding of this crystal than any other. The initial experimental observations were backed up by theoretical predictions of lattice collapse at excitation levels corresponding to exciting 10% of the valence electrons. This theoretical prediction was consistent with the optical observations of the onset of liquid-like properties of Si. The notion that one could excite a material and cause it to undergo a structure change by manipulating the chemical bonding was intriguing. Also, the observation was that the melting of Si appeared to occur within 150 fs, effectively just one phonon period. How could the transition occur within a single collision period?

In relation to other studies, there was some very nice work by Lindenberg *et al* (Science 2006) using a proof of principle femtosecond x-ray beam line at Stanford's SPPS facility to study electronically driven atomic motions in another semiconductor, InSb. This system was used for its high atomic number and associated scattering cross section to better match the optical absorption depth to x-ray probed volume. A number of diffraction orders and rocking curves were sampled. Within the time resolution of this experiment, it appeared that the atoms had moved inertially from their initial positions at their thermal velocity at room temperature. They posited that the bonds had been effectively snipped and the atoms moved freely within an optically induced flattened potential characteristic of the liquid state. This process occurred within the 200 fs time resolution of the experiment.

Silicon is considered to be the cornerstone to the concepts of nonthermal melting and issues of the mechanism were still open to debate. With the development of our 4th generation electron gun using a wrap-around-magnetic lens design and factor of approximately 2 times shorter propagation length, we were able to reduce the pulse duration below 200 fs to provide enough time resolution to study this problem. We also developed Si nanofabrication methods to make large area samples with free standing 40 nm polycrystalline Si windows for each time frame. This development enabled us to average a large number of time steps to improve the signal to noise ratio (SNR). Also we were able to further increase the SNR by using single crystals of Si. This latter work was done in collaboration with Max Lagally and Mark Eriksons' groups at the University of Wisconsin at Madison.

We found that that Si did indeed "melt" on a subpicosecond timescale, indicative of electronically induced bond softening. This result is in stark contrast to our studies of free electron metals where there is no directional bonding. The onset of the photoinduced phase transition was not well defined but rather occurred over a range between 3-6 % valence excitation. Most important the dynamics did not show a single relaxation time, as in the reported InSb studies, but rather multiexponential relaxation dynamics with 1/e decay components as short as 300 fs for the highest excitations. The onset of liquid Si is rather obvious in the diffraction data. The observed structure factors for Si are rather unusual with a rather flat distribution in relation to other liquids. The results agree very well with other steady state diffraction of liquid Si and on this basis we consider the disordered state to be a liquid. The nonexponential relaxation dynamics in the disordering process shows that the process occurs through a diffusive sampling of the phase transition coordinate space. It is not a concerted effect as implied in the studies of InSb. This interpretation can be further substantiated from the dynamics of the different higher order diffraction peaks observed for Si. Rather than showing a quadratic dependence in the decay time with scatttering vector, as expected for Debye-Waller type effects on the diffraction process, each diffraction order showed the same decay profile. The diffraction from the higher orders thus reported on the fraction of solid state lattice remaining as a function of time.

Here we need to make an important distinction between this work and the InSb studies. A liquid does not have a flat potential energy surface as asserted in the InSb work. Rather it is a bound state of matter with small barriers separating local configurations that rapidly interconvert. The key distinguishing feature of the liquid state is its inability to support shear motions, or collapse of the transverse acoustic branch of the corresponding solid state phase. This occurs when the system has enough thermal energy to overcome the barriers within the potential energy surface to shearing motions. It was exactly this class of motions in the Al studies that leads the system from a coordination number of 12 for the FCC lattice to the liquid shell structure with an average coordination number of 10.

The interesting feature of this observation is that it is the first direct observation of electronically induced nucleation centres. There will be electronically induce lattice instabilities that lead to nucleation sites, as in the thermally sampled case of Al, but on a much faster time scale. Also, and more intriguing, one has to consider the fluctuating electron-hole carrier spatial distribution in the process. The photoexcited electrons will be rapidly exchanging spatial positions through various scattering mechanisms at high excitation. There will be regions with higher densities of photoexcited electrons and this will lead to additional lattice softening. The highly directional nature of the bonding is Si is ultimately responsible for the electronically induced bond softening, and bond breaking for rms atomic motions at the background ambient thermal energy. The increased antibonding character of the photoinduced charge distribution necessary takes the system to a softer potential. The changes in charge distribution necessarily introduce potential gradients or forces displacing the atoms from the equilibrium minima of the solid state and propagate the system on to a liquid potential energy surface (that is not flat).

These particular studies are important in that Si owes its special properties due to its highly directional bonding and associated diamond-like structure. This work showed that we could optically manipulate the lattice potential for such a well defined system. We now have the ability to manipulate electron density and directly observe the effect on chemical bonding. This class of experiments will rigorously test various approximations made in first principle calculations of Si and serve as an important benchmark for further development of time dependent methods for treating structural dynamics.

## Warm Dense Matter: Evidence for Bond Hardening

In the studies of strongly driven phase transitions, there is the obvious question. How hard can you drive the structural dynamics? It is a simple matter to just increase the excitation intensity, turn up the knob on the laser power so to speak. At the exteme end, the peak power of the laser excitation leads to dielectric breakdown and plasma formation at which point the lattice is no longer considered to be in the solid or liquid state. Intermediate to this regime, there is a state of matter that is referred to as Warm Dense Matter. It exists at extremely high temperatures and pressures. There has been a great deal of interest in this state of matter as it is relevant to the composition of planetry cores and is a precursor state to nuclear fusion.

In our aforementioned studies of Au, we found the experimentally observed dynamics for the phase transition were in reasonably good agreement with the atomistic MD calculations of Lin and Zhigilei (PRB 06). The melting dynamics appear to occur on a 30-50 ps timescale whereas the MD simulations predicted approximately 10 ps for superheating conditions of 1.6. Given the sensitivity of the lattice stability to the interatomic potential, this degree of agreement can be considered very good. The MD calculations predict a superlinear dependence on the melting dynamics, i.e. increasing the degree of superheating by 2 would increase the rate of melting by more than 2. At the opposite end of the scale, there were experimental observations of melting that appeared to show an anomolous persistent phase that existed for up to several picoseconds for Au and represents the onset of warm dense matter. The exact equation of state was unknown and there has been a great deal of discusion on this point that is outside the scope of this lecture.

Recoules et al (PRL 2006) performed high level ab initio calculations for the Au lattice as a function of electron temperature. They came to the rather suprising conclusion that the bonds of Au get stronger as the electronic temperature increases with laser excitation. The effect is a purely many body electron-electron correlation effect. As the electrons populate higher electronic states (d to f atomic orbital basis), there is an increase in the maximum in the radial probability distribution that in turn decreases electron screening of the nucleus. This prediction of bond hardening with increased excitation is completely counterintuitive. One of the definitions of metals is that they are maleable. As we all know, metals become more maleable as they are heated up. However our everday experience has not held materials at temperatures approaching 1M degrees. At these temperaratures, the electron distribution is highly perturbed and electron correlation energies could play a deciding role that we would not expect based on extrapolations from minor perturbations for comparison.

Figure 20 shows the most salient theoretical results:

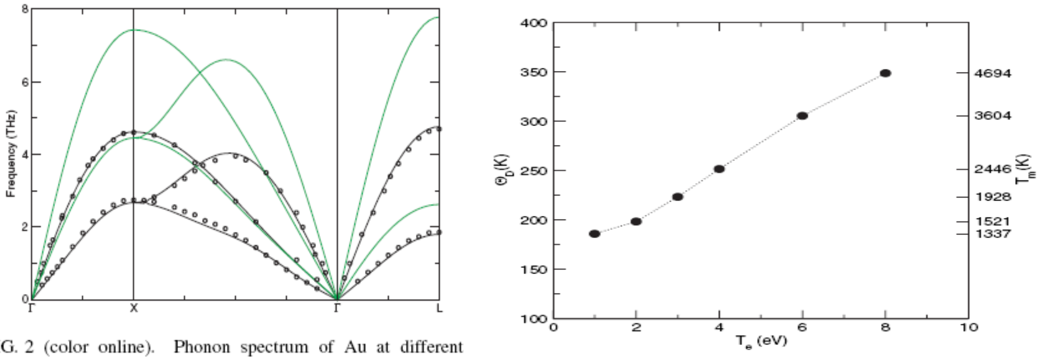


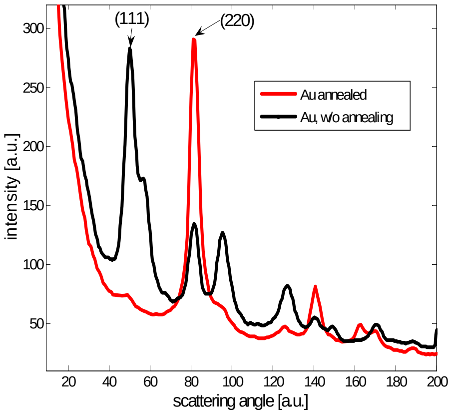
FIG. 2 (color online). Phonon spectrum of Au at different electronic temperatures. The black curves are the spectrum for room temperature. The green curves are for *Te* = 6 eV. Open circles are experimental results from [27].

FIG. 3. Debye temperature variation as a function of electronic temperature for gold. On the right-hand side is the corresponding melting temperature.

**Figure 20. Calculations from Recoules et al. PRL 2006 showing evidence of bond hardening**.

As seen in the left panel of Figure 20, the phonon dispersion curves for Au become more harmonic, or stiffer, as the elecronic temperature is increased. The black points and corresponding curves are for room temperature. The calculations recover the ambient Au lattice properties, however, there was no experimental evidence to support the accuracy of the calcuations at higher electronic temperatures. The predicted change in melting point or lattice stability is shown in the right panel. The fs electron diffraction studies were performed at electronic temperatures corresponding to 4.5 eV at the highest excitation, where the melting point is predicted to increase by a factor of approximately two.

The experiments on Au (Ernstorfer et al., Science 2009) were conducted using oriented polycrystalline films. This development in sample preparation was key to this experiment as it removed the (111) peak from overlapping with the peak in the liquid structure factor. We no longer had to convert from reciprocal space to real space to determine if the liquid state was observed. It can be readily observed directly in reciprocal (s-)space as show in Figure 21.



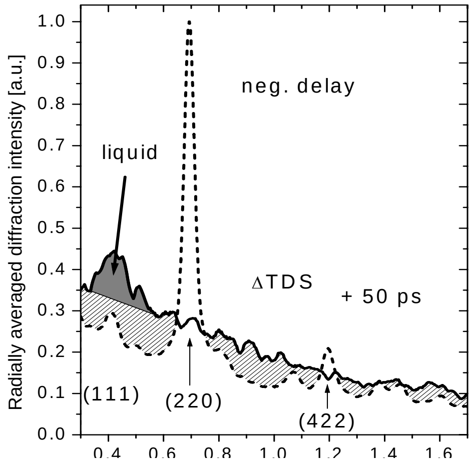


**Figure 21. Electron Diffraction Results for (111) Oriented Au**. The left panel showing removal of (111) peak as diffraction only samples the plane projections perpendicular to the surface normal. The right hand panel shows the results after laser excitation leading to melting at a delay of 50 ps where the Au is well described as a liquid. The appearance of the signature liquid peak clearly shows up at .45 Å$^{-1}$, unobstructed by the (111) diffraction order with the oriented samples. The decay in the (220) and (422), as well as increase in the thermal diffuse scatttering (TDS) can be used to follow lattice heating up to the onset of melting (Ernstorfer et al. Science 2009).

We now have a direct view of the onset of the liquid state. We were able to make a 2D diagram depicting the lattice heating through decay of the higher order diffraction peaks, Thermal Diffuse Scattering (TDS), and the rise in the liquid state. The surprising finding is, that as we increased the excitation energy, the onset of melting dramatically slowed down and not sped up as predicted by the MD calculations. This was highly unusual as numerous studies of laser induced phase transitions show the opposite, expected trend. Further as we approached the conditions for Warm Dense Matter, Au was clearly in a liquid state that took picoseconds to form. By comparing the rate of lattice heating and the decay in the higher order diffractions, it was clear that the lattice temperature had well exceeded the Au lattice temperature and lattice stability point but it retained its periodic lattice structure for superheating conditions many times the ambient lattice melting point. Based on a number of theoretical models (see papers by B. Rethfeld mentioned above), confirmed by experiment, once the lattice temperature reaches a superheating of more than 1.4 the system exceeds the thermal barrier to nucleation. Thermal fluctations should lead to rapid nucleation and lattice collapse in a matter of 1-2 picoseconds as observed for Al. This was clearly not the case as shown in Figure 22.

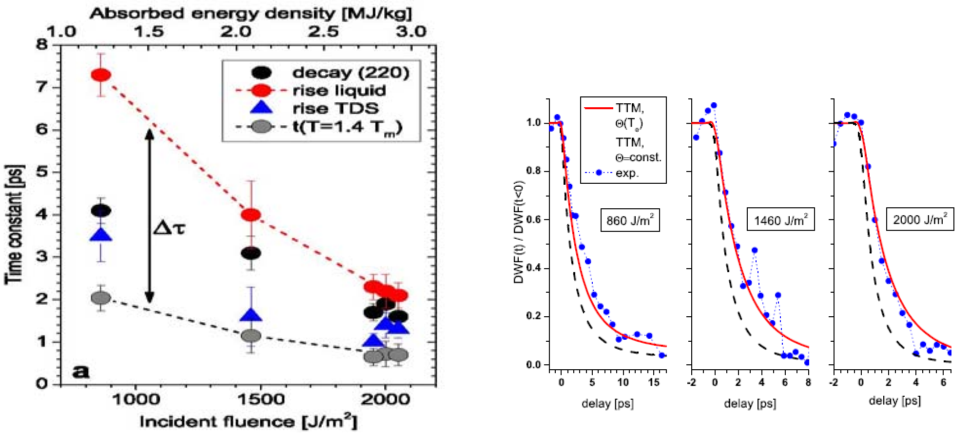


**Figure 22. Melting Dynamics in the Warm Dense Matter Regime**. The left panel depicts the rate of lattice heating through the decay in the (220) peak of Au(111) and the rise of the TDS that are reasonable well correlated. The onset of the liquid state is significantly retarded with respect to the rate of heating and time the lattice surpasses a superheating condition of 1.4 assuming the ambient Au lattice stability. Using an electronic temperature dependent Debye lattice temperature (figure 20) obtained from the ab initio calculations of Recoules et al. (right panel), excellent agreement is obtained in the decay of the Debye-Waller Factors (Ernstorfer et al. Science 2009).

The dynamics of melting is a direct measurement of lattice stability. The sub linear dependence of the melting dynamics on excitation or electronic temperature is a direct demonstration the lattice is getting stiffer or experiencing bond hardening. By simply plugging in the predicted lattice Debye temperature from the ab initio calculations into the electron-phonon coupling relation for lattice heating (courtesy Lin & Zhigilei), we get excellent agreement over a relatively broad range of excitation conditions where the lattice potential is predicted to significantly stiffened by reduced nuclear screening by the higher energy electrons whizzing about in the lattice. The Debye-Waller factors for a given lattice temperature are related to the rms motion of the atoms. This comparison illustrates the rms motion is strongly reduced at higher electronic temperatures in relation to the ambient electronic distribution. The bonds of Au have gotten harder as we more strongly excited the material.

As stated above in relation to the simple coin analogy for atomistic potentials, such an effect would not be observed. This lattice stiffening at high electronic temperatures is clearly a many body effect. The simplest example I can provide to give some insight to the operating physics is to consider the bonding in diatomic oxygen. Threshold photoionization of $O_2$ would remove an electron from the highest lying molecular orbital that is antibonding and is responsible for the bond order of $O_2$ being 2 instead of 3 as in the case of $N_2$. Excitation to a higher orbital would similarly decrease the antibonding character or screening of the $\pi$ molecular orbital and lead to bond stiffening. In the case of Au, the effect is more complicated as it involves correlated electron motions of a large number of electrons per atom centre. However, the basic physics behind the bond hardening effect are similar. The total energy of the system has increased. It is still a higher excited state of matter. It is just that the energy resides in the electron degrees of freedom at the expense of the lower lattice potential energy. In addition, this work has illustrated that is possible to use femtosecond excitation pulses to manipulate potential energy landscapes. In this case, we have shown that we can make the lattice stiffer or increased the depth of the valleys in the interatomic potential. We have likely also increased the barriers to nucleation in the process that would also have contributed to the retarded melting dynamics at high electronic temperatures.

To give a different perspective on this problem, we did not know what the state for Warm Dense Matter was prior to this work. It was not clear whether it was best to describe this as a liquid, a high density of interacting ions or pre-plasma state, or some other description of the mass-charge distribution. At the excitation levels use, the electronic temperature would correspond to lattice temperatures of over 100,000 degrees. Given the surface temperature of the sun is 5000 $^0$C, it was if we reached into the sun (if it was made of Au) and pulled out a handful of material a few kilometers from the surface, examined it, and found it to be in the liquid state. We now know what the equation of state is for Au under these rarified conditions. In our hands, these are fleeting states of matter; yet their properties are highly relevant to the extreme physics key to understanding important phenomena ranging from planetary physics to fusion processes as an energy source. Until the development of structural probes with femtosecond time resolution, it was not possible to fully characterize these states of matter. With femtosecond electron diffraction, we can now obtain an atomic perspective on such states of matter. We can also characterize the spatial distribution of charge as the system evolves into a plasma state by using electron pulses to image field lines (Hebeisen et al.

PRB 2008). Femtosecond electron diffraction seems very well suited to significantly advance our understanding of warm dense matter and extreme physics.

### Photodriven Relaxation of Peierls Distortions in Bi: Approaching the Speed Limit for Atomic Motions

Another important system we have examined with respect to the ability of femtosecond pulses to modify potential energy landscapes is the study of Bi. This system is a semi-metal with a very narrow gap in the band structure near the Fermi surface. The lattice acquires an extra degree of electronic stability by adopting a slightly distorted FCC lattice along the (111) direction. The larger wavefunction overlap between Bi pairs offsets the increased cost in lattice energy to form this distorted lattice in what is referred to as a Peierls distortion. It is this effect that is responsible for Bi's unique properties that manifest as a rather complex coexistence of metallic and covalent bond character [Madelung, Springer, 1998]. The lattice forms a kind of intermixed 1D lattice with alternating pairs of Bi atoms as shown schematically in Figure 23.

Optical excitation in this case leads to decreased stability of the Peierls' interaction and elongation of the Bi bonds along the (111) direction, denoted as motion involving the $A_{1g}$ lattice phonon mode. The electronically modified potential creates a repulsive surface that drives the atoms towards the higher symmetry FCC structure. This process could be considered an order to order phase transition, at least at low excitation. As the excitation conditions are increased past the lattice stability point, Bi is expected to undergo a melting phase transition. There have been a number of studies in which the anharmonicity of the photoinduced potential have been discussed. The discussion has pertained to the degree of anharmonicity effects on the observed phonon period (see Murray et al., PRB 2005 and Fritz et al., Science 2007) with some evidence for affects on the transverse phonon branch at relatively low excitation levels (E. Zijlstra et al., PRL 2008). However, there was no prior work either experimental or theoretical that had treated this transition and the coupled dynamics between relaxation of the Peierls Distortion and destabilization of the lattice potential towards complete disorder or melting.

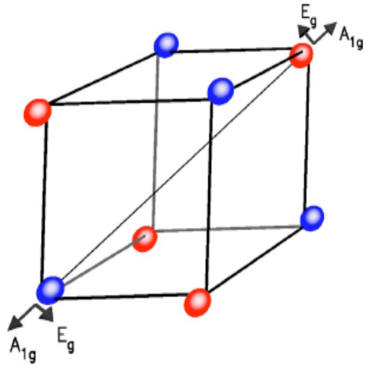


**Figure 23. Schematic of the Bi unit cell.** The lattice is a slightly skewed FCC lattice along the (111) direction that leads to the formation of slightly closer pairs of Bi atoms in a form of Peierls Distortion (from Zilstra et al., PRB 06). This distortion leads to a lattice doubling.

The importance of the development of high bunch number electron pulses is particularly highlighted in this study. Figure 24 shows a photo of the sample after making a "molecular movie". You can see the effect of the laser excitation pulses. Each hole in the sample represents a laser shot. Using a 10% quantum efficient lens-coupled-CCD detector, we needed only 10 shots per time point. Since “every electron detectors” (100% quantum efficient detection) are available with fibre coupled CCD detectors, this study illustrates we achieved single shot structure conditions with electron pulse durations of 200 fs, as characterized with the crossed beam pondermotive scattering measurement. You can see the importance of high electron number density pulses in Figure 24. If not for this development, it would simply be impractical to try to attain 20 nm free standing samples with sufficient area. We are at the practical limits now.

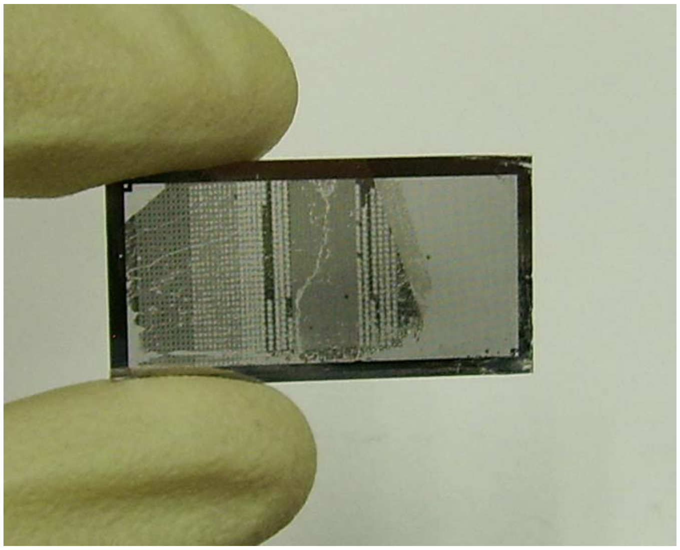


**Figure 24. Photo of Bi film after Experiment**.
The holes are due to single shot laser excitation. Sample damage is only observed after the laser absorbed pulse energy exceeds the sample melting point. No detectable damage is induced by the electrons after many hours of data collection. The peak current is high but the average current is orders of magnitude less than a conventional TEM. The film is very homogeneous. The apparent difference in thickness is coming from the different sizes among the holes depending on the laser excitation level. The only remaining undamaged area appears darker. The holes in the central part are too small to be seen with the resolution of the photo.

Figure 25 shows the dramatic changes observed for Bi on an incredibly short time scale. We observed at approximately 10% electron excitation levels that the lattice became a completely isotropic liquid in less than 1 ps. Moreover, the 1/e formation time for the liquid was found to get faster and faster with excitation, showing an approximately linear excitation dependence. Not only was the excitation causing relaxation of the Peierls’ stabilization energy but it was actually driving that atomic motions.

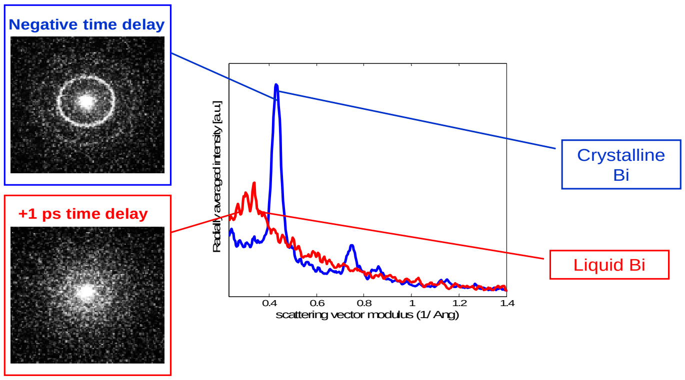


**Figure 25. Ultrafast Isotropic Displacement of Bi atoms.** This data shows the results for Bi under 11% valence electron excitation. It has clearly formed a completely isotropic liquid in less than 1 ps (Sciaini et al Nature 2009).

The specific details of the atomic displacements can be seen in Figure 26. We have also characterized the thermalization rate between the excited hot electron gas and the initially cold lattice up to equilibration temperatures closed to that of the melting point. The lattice temperature after equilibration was determined *in-situ* by implementing a parameterized Debye-Waller model (Gao and Peng, Acta Crystallogr. A 1999) and the thermalization rate was obtained from exponential fits to the increase of the inelastic scattering signal. The thermalization process was found to occur in several picoseconds (~ 3 ps). For excitation levels well above the melting point, in particular at the highest excitation (~ 15 % of excited valence electrons), the solid-to-liquid phase transition is clearly occurring on the 100 fs time scale reflecting that the process is electronically driven in nature.

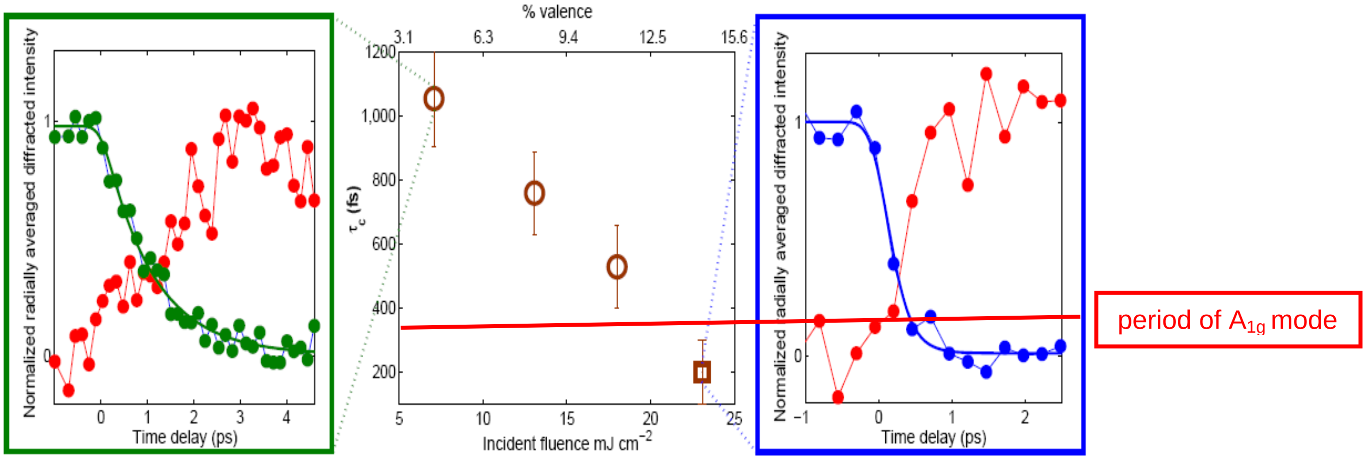


**Figure 26. Melting of Bi in less than Half the Phonon Period.** The red curves show time slices for the formation of the liquid peak that are 1:1 correlated to the decay in lattice order (Sciaini et al Nature 2009).

The amazing observation is that at the highest excitation the lattice forms a completely isotropic liquid within less than ½ phonon period of the $A_{1g}$ phonon mode of Bi that is coupled to this Peierls' relaxation coordinate. This distinction is even more dramatic when the decrease in phonon period with excitation is taken into account. It has been well documented that the phonon period becomes more anharmonic and slower with increased excitation (Fritz et al. Science 2007 and K Sokolowski-Tinten et al., Nature 2003). At excitation levels near 1.5 %, the mode is nearly a factor of two slower relative to the unperturbed $A_{1g}$ mode of Bi at ambient conditions. Here it is important to realize that in order for the lattice to form an isotropic disordered state the $A_{1g}$ mode must couple to transverse motions defined by displacements along the $E_g$ mode shown in Figure 23. This type of motion requires anharmonic mixing between the $A_{1g}$ and $E_g$ basis modes. The fact that this motion occurs within less than half a period means the motion is ballistic. There are essentially no collisions possible during the sampling process of the nuclear configuration phase space on such a time scale. The barrier to transverse displacements has completely collapsed. On this basis we can state that Bi has truly melted. Similar statements could not be made for the case for Si. A glassy description could equally hold for description of the electronically driven disordering in Si (the assignment to the liquid state was based comparison to the liquid structure factors). In the Bi case, the barrier to transverse motion has collapsed and thus defines a liquid state. Furthermore, the diffraction pattern corresponds to that of liquid Bi. There is negligible change in the signal out to 1 ns indicating that the system has completely sampled the potential energy surface of the liquid state within ½ phonon period at the highest excitation. A schematic of the optically induced changes in the potential energy landscape depicting this effect is shown in Figure 27.

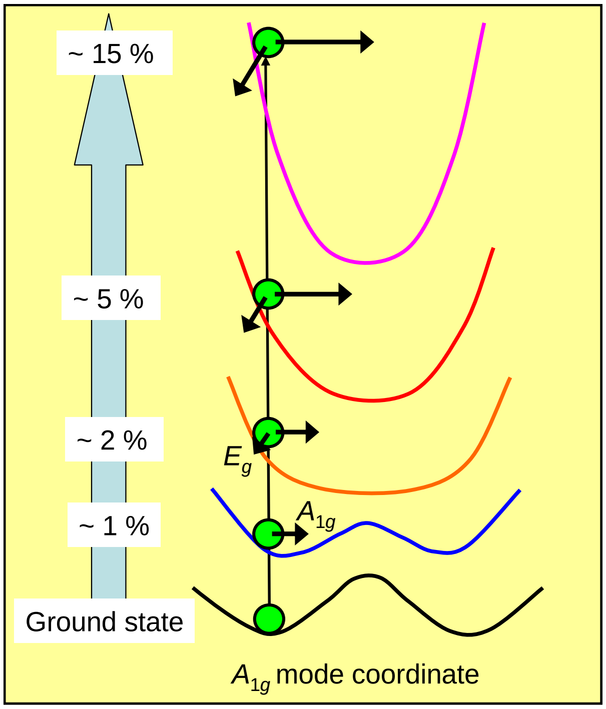


**Figure 27. Schematic representation of the optically induced changes in the potential energy landscape of Bi.** Increasing the degree of electronic excitation increases the degree of antibonding character of the Bi-Bi Peierls distortion. The motion along this coordinate becomes correspondingly more and more repulsive, driving the system to the higher symmetry structure. The potential becomes highly anharmonic (as depicted as curvatures) and strongly mixes the $A_{1g}$ motion with the transverse $E_g$ mode. At high enough excitation, the background thermal energy overcomes the modified transverse barrier and the system collapses onto the liquid state potential energy surface (from Sciaini et al., Nature 2009).

This is the first example of ballistic melting. The observed time scales being less than a collision and the linear dependence on the excitation dynamics illustrates the electronic nature of this driven process. As an analogy, these observations are akin to the accelerator peddle on a car. Increasing the laser excitation is like pressing down on the peddle making the atoms go faster and faster. The observed time scale for this full scale structural change is extraordinary. For calibration, the fastest chemical reaction known that involves nuclear motion is the cis to trans photoisomerization of the retinal chromophore in rhodopsin. This is a highly optimized reaction coordinate and corresponds to similar concepts of approaching a half period motion of the torsional motion (transverse like motion) around the double bond. The torsional mode is referred to as the reaction mode. The heavy atom motions involved in this reaction are only on the order of 0.1 Ǻ. The potential energy surface of the protein is highly optimized to direct this process. In the case of Bi, bonds must break and the motions exceed 0.1Ǻ to sample sufficient nuclear configuration space to correspond to the liquid state. These motions are occurring faster than even photoisomerization of rhodopsin. This is a very strongly driven process at the highest excitation levels and corresponds to the fastest structural transition observed to date.

The question is how fast could this process be made? At the moment the femtosecond pulse is absorbed, the atoms have initial trajectories defined by their thermal population at room temperature. These random motions map on to the photoinduced changes in the potential energy landscape of the lattice in which there are repulsive gradients along the $A_{1g}$ mode, anharmonic terms mixing the $A_{1g}$ and $E_g$ modes, and reduction in barriers to transverse motions to interstitial regions. If one considers the instantaneous velocity of the atoms moving within the initial, approximately harmonic motion of the $A_{1g}$ mode, this process could be considered to be going at approximately the mean speed of sound or mach 1. Could it be driven even faster and redefine the limits to nuclear motions involved in structural rearrangements? We were unable to characterize the motions at higher excitation to find this fundamental limit for Bi (before plasma formation) as the time resolution was insufficient. Next generation electron and x-ray sources will be able to resolve this issue.

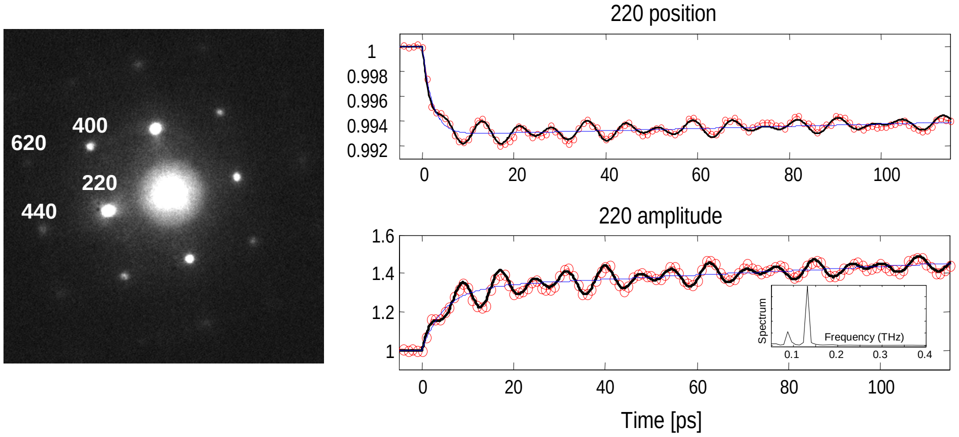
Finally in terms of picturing the overall mechanism, consider the initial electron distribution. The electron distribution follows the 1D Peierls distortion of the lattice. It is the increased orbital overlap and electron density at these alternating pairs of Bi atoms that gives rise to the Peierls' stability. At high excitations, the electronic states are more diffuse, depleting electron density between the Bi dimers, and there would be strong electron-electron correlation effects leading to a more spatially isotropic charge distribution. It is this change in charge distribution that modifies the lattice potential. The atoms are driven to the isotropic liquid structure ballistically through this many body electron-lattice coupling. It is a beautiful example of a many body effect in both the electronic and nuclear coordinates.

## Direct Observation of Collective Modes: First Step to Proteins

This section follows up on the study of Si but under low excitation conditions. Single crystals make a significant improvement in SNR over polycrystalline samples as the signal amplitude is localized in a single spot, giving amplification above diffuse background scatter. It is also possible to Fourier filter out the diffuse scatter. The challenge is obtaining high quality single crystals of the correct thickness. This work exploited the recent advances in fabricating sheets of nano-Si using sacrificial release layers in Si nanofab (see the work of M. Roberts et al. *Nature Materials* **5**, 388 (2006)). It is possible to obtain single crystals of Si that have 10-100 nm thicknesses and surface areas of 25 $cm^2$. These crystals are so thin they can be bent but are still very strong. They are now being touted as the template for high speed "plastic electronics". The experimental fs electron diffraction result for low photocarrier injection into Si(110) is shown in Figure 28.

## Direct Observation of Key Shear Modes: Low Excitation

Kinetics of the 220 Diffraction Spot



Two oscillation modes at periods of 7.6 and 11.4 ps

**Figure 28. Direct Observation of Collective Modes**. The left panel shows the observed diffraction pattern and assignment of some of the main orders. The right hand panels show the time resolved changes in the (220) diffraction order following photocarrier injection with low intensity fs 400 nm pulses. The photocarriers relax via optical phonons ultimately into acoustic phonons. This thermalized energy leads to lattice expansion along the surface normal. Since the spatially Gaussian excitation pulses are nonuniform in the plane, there is also a surface deformation. The longitudinal expansion and surface deformation couple to excite longitudinal and transverse acoustic modes of the 40 nm thick membrane that then oscillate. The Fourier transform of the oscillations is shown in the inset and corresponds exactly to the lowest frequency longitudinal and transverse modes normal to the crystal (40 nm boundary condition on the half wavelength).

The collective modes excited by uniform relaxation of the photocarriers into heat (statistical relaxation into acoustic phonons) are clearly observable by eye. The surprise here is that one sees both the longitudinal and transverse acoustic modes. It is usually not possible to excite, or detect transverse acoustic modes, by optical excitation. The planar profile of the excitation couples selective to longitudinal acoustic modes. Only in the case of an extremely tight focus, on the order of a micron, is it possible to have significant strain fields in the transverse direction to observe the acoustics with comparable amplitude to longitudinal phonons. This observation by itself is important as the full characterization of the mechanical properties of materials requires the speed of sound/elasticity measurements in both the longitudinal and transverse directions. Contact methods are normally required. The big virtue of optical excitation of sound fields is that it provides a contactless method of characterizing materials but until now could not provide information on the full elasticity tensor. With fs electron diffraction, we can now fully characterize the mechanical properties of nanoscale objects.

The new observation of transverse acoustic modes is due to the fact that electron diffraction is much more sensitive to transverse displacements than optical measurements. The diffraction process is most sensitive to motions transverse to the surface normal. There was an earlier study of acoustic phonons in Al where only the longitudinal modulation was observed (see J. Cao et al, APL 2003). This effect should only be observed with single crystals as the accumulated phase differences for displacements of the atoms in transverse directions cancels out for randomly oriented polycrystalline samples. It was difficult to understand this earlier work as one should not observe motion along the surface normal in electron diffraction. From the above work on Si, we showed that the observation of coherent acoustic phonons arises from the coupling of the longitudinal and transverse modes through the surface deformation from nonuniform excitation and the intrinsically higher sensitivity of electron diffraction to transverse atomic motions.

Apart from providing a new approach to characterizing nanoscale objects, these results illustrate the high sensitivity of electron diffraction to the displacement of collective modes. From the change in the spot positions, the rms motions of the atomic motions in phase with the acoustic mode are on the order of $10^{-3}$ Å. This amplitude is more than an order of magnitude less than the displacement of collective modes involved in protein relaxation process and functionally relevant motions. This is yet another step towards studies of the structure-function correlation of biological systems. The low frequency modes in proteins are strongly damped and it would be difficult to so clearly demonstrate the diffraction sensitivity to such displacements. These observations clearly demonstrate the high sensitivity of electron diffraction to the correlated motions of collective modes. These motions are more difficult to detect as the forces are distributed over more atoms so the net displacements with respect to relative atomic positions is much smaller than localized molecular modes. Given the large number of atoms involved in any such mode the amount of stored or released energy through the coupling to collective modes can be appreciable on the molecular scale but not lead to apparent large amplitude changes in atomic positions. The sensitivity of electron diffraction is high due the inherently very short de Broglie wavelength of electrons such that even small changes in atomic position affect the phase of the propagating electron through the lattice. Femtosecond electron diffraction has the potential to become a very powerful probe of strongly correlated motions in biological systems and other collective phenomena.

## III. Summary

### Remaining Challenges, Pitfalls, and Future

So far, the limitations of high brightness electron sources for femtosecond electron diffraction studies, in the enterprise of making molecular movies, have not been discussed. The biggest problem by far, for all electron based probes, is the samples. There has been a long history of dealing with this problem in the field of electron microscopy. The samples must be made between 10-100 nm thin to enable electron transmission. The samples generally need to be made thin enough to be in the single electron scattering event limit to avoid secondary or multiple electron scattering from distorting the diffraction process and lead to errors in structure determination in the inversion process. There are standard approaches to making samples this thin. However, the real challenge to make large area samples as required for movie mode data acquisition. Figure 24 graphically illustrates this problem. In this regard, the advent of nanotechnology and associated methods of nanofabrication will be invaluable. We have only begun to tap this methodology in sample preparation. Our work in using Si nanofabrication methods to make sample support grids with each frame representing a time point or movie frame is one example. The other approach to solve this problem is to go to higher energy electrons. We are now online with 100 KeV machines and soon 300 KeV machines. The design of the higher energy beams requires additional conditioning of the beam to ensure adequate spatial resolution. With the development of accurate codes that give effectively an exact solution to the electron propagation, this aspect of the problem is well in hand. The next generation of machines with factors of 100 more electrons, 10 time higher detection efficiency with "every electron detectors", and factor of 10 shorter pulses using rf pulse compression cavities, will give an overall gain in effective brightness of $10^4$ over the results presented above.

Every method has its pitfalls and artifacts and electron diffraction is no exception. At the high excitations required to observe nonreversible structure changes, the short excitation pulses will lead to resonant multi-photon ionization of the sample. Lattice charging from the excitation, not the electrons, will be limiting in many cases. The charging of insulating materials from the electron beam is also a standard problem and is normally solved using conductive carbon coated grids to help discharge the sample. Photoinduced charging is more difficult to control as it is an intrinsic effect. For metals, the surface charge is localized at the surface to within 1 nm and thus does not contribute significantly to the diffraction process if done in transmission. This, however, is not the case for surface sensitive probes such as ultrafast electron diffraction conducted in reflection mode. Recent studies show that this leads to artifacts and erroneous conclusions of fast structural dynamics that are actually related to the development of space charge fields with the motion of the electrons away from the surface and not lattice dynamics (See J. Zou *et al*. APL **94**, 251103(2009)). A number of studies attributing unusual effects involving correlated atomic motions normal to the surface using electron diffraction in reflection are most likely artifacts (see Zou et al for a discussion on this point). The lattice charging effects can also lead to structural changes that have nothing to do with excited lattice electrons but rather the photoemitted electrons. Charging effects generate fields along the surface normal. Changes involving motion solely along the surface normal could arise from this source and need to be checked. Similarly there are surface deformations and trivial thermal effects that must be distinguished from the photoinduced dynamics of interest. Control experiments are needed to assess both the photoinduced charging and the thermally related signals.

The above cited problems have been solved for transmission mode geometries. The cited studies have illustrated that the technology for directly observing atomic motions on the femtosecond time scale has been achieved. The camera for the molecular movie is now in hand. The first applications of this newly developed methodology have been to study the simplest order to disorder transitions albeit with femtosecond time resolution. We have been able to distinguish thermally propagated structural phase transition from electronically driven structure changes. Through these studies we have determined that the mechanisms involve nucleation sites on the nm, or molecular scale. The notion of something undergoing homogeneous nucleation, melting from the inside out, is even more intriguing when viewed at this atomic scale of inspection. In the case of electronically driven structural changes, the most important generalization is that directional bonding within the nascent material is needed in order for the optically induced changes in electron density to create gradients/forces for the displacement. In the case of Si, we have observed diffusive sampling of what can be considered electronically induced nucleation. The observations of Bi really capture the imagination. We have observed a ballistically driven phase transition that involves a many body effect to evade the requirements of multiple collisions to sufficiently sample the nuclear configuration space for liquid formation. The changes in electronic density provide the forces that map the system on to the disordered liquid potential energy landscape. This work in particular has clearly demonstrated that it is possible to optical modify the potential energy landscape of materials. As the time resolution and brightness of next generation technology improves, it will be interesting to see how far this concept can be pushed. The concept of optically modifying the fundamental forces between atoms reached the ultimate limit with the creation of Warm Dense Matter in which bond stiffening was observed for Au (before it became an unbound plasma). Higher excitation conditions and time resolution will undoubtedly lead to much needed new information for understanding the extreme physics of materials under high pressure and temperature, with laser driven nuclear fusion as one potentially important application.

With respect to optically manipulating interatomic forces, I fully expect that Coherent Control protocols will be soon be used in conjunction with structurally sensitive x-ray and electron probes. This will be an extension of the basic methodology now demonstrated with Bi. By explicitly exploiting phase in the optically prepared wavefunctions, on time scales faster than decoherence, it is possible to control chemical reactions and direct atoms along preferred paths. The current method of observing Coherent Control of matter is based on optical methods and, as discussed above, do not have sufficient information to unambiguously invert the control pulse shape to the actually induced atomic motions. With fs electron diffraction probes, it will be possible to directly observe the laser-matter interaction and induced coherent steering of matter waves. It will be very analogous to watching waves on water in which we control the wave amplitude, phase, and period.

In terms of the future, I will again emphasize the importance of sample preparation. (Did I mention samples are the key?) There are literally thousands of problems that could be solved through an atomic level inspection of the structural dynamics. Whether one uses electrons or x-rays to study the structural dynamics, it will be nontrivial to fabricate the samples. Clever new ways of exploiting nanofabrication and handling of nanoscale materials will be needed to bridge the gap from making the molecular movie film to shooting the movie. The most general solution to this problem will be to use samples that can be easily replenished between laser shots, i.e., the simplest solution is to use gas phase and liquid phase samples with flow. Due to the extremely small scattering cross section for x-rays, electrons will be the preferred probe for gas phase studies; whereas x-rays are the best probe for solution phase studies. In this respect, it is clear that the recent advances in ultrabright electron source technology and x-ray source technology will provide complementary approaches to atomically resolved structural dynamics.

## The Grand Challenge – The Structure Function Correlation of Biology

As I emphasized during the introduction, the transition state is the unifying concept connecting chemistry and biology. This grand thought experiment has now become subject to direct experimental observation. The simple example I gave with respect to the well defined static differences in structure in the binding of oxygen to hemoglobin was to illustrate that our understanding of what physically constitutes a critical point or transition state region in reaction coordinates is poorly developed for complex molecules. As with all scientific endeavors, the finer the resolution of an observation the better our understanding of the phenomenon of interest becomes. So far we have only very coarse views of transition state processes. Our vision has now improved dramatically. We now have the necessary spatio-temporal resolution to watch chemistry as it happens. My feeling is this new field of atomically resolved structural dynamics will revolutionize our understanding of chemistry. After all we are approaching the fundamental limit in time and space by which we can observe chemical processes. In so doing, we will better appreciate what forces are at play in the barrier crossing region and better design synthetic means to control barrier crossings once this connection is made.

In this pursuit, the grand master of controlling chemical processes is Mother Nature. Through evolutionary pressures, Nature has orchestrated a preponderance of structures that are hybrid states of matter between solid and liquid state. The structurally rigid components provide conserved structure and function whereas the topological loops provide the flexibility to sample the constrained phase space. The charge distribution around active sites is fully optimized to reduce barrier heights and strongly coupled to the protein fluctuations. In short Nature has optimized the dynamical variables for thermally sampling of reaction coordinates, mastered heterogeneous chemistry, and coupled the two factors through nonCondon effects to direct chemical energy into functions. It is in this respect that I believe the single most important scientific objective of this field is to determine the structure-function correlation of biological systems. By directly observing atomic motions during a biological function, we will arrive at a direct determination of the structure-function correlation. The Collective Mode Coupling Model will be uniquely tested and the key modes coupled to reaction coordinates will be revealed. It will only be possible to study a few model systems, at least initially. These systems include heme proteins, photoactive receptors and energy storage systems such as the family of rhodopsins and light harvesting systems. This relative small group will provide structural details for a certain subgroup of topological features from which it should be possible to generalize. Of course, obtaining sufficient numbers of crystals to conduct such studies is a challenge. However, the brightness of the next generation electron and x-ray sources is sufficient to bring this aspect of the problem to manageable level. The problem is important enough that the sample limitations will be solved.

In this respect, I close with a final thought. Recall the Mars landing of the Rover a few years ago during which time we were all treated to these wonderful images of the Mars landscape beamed back to Earth. As the Rover moved about and mapped out the surface, we developed a more detailed, visceral feel, for this landscape so much so that we affectionately called certain rocks “Barney”. We are now at a turning point in science where we can similarly send probes into the transition state region to beam back pictures of what the energy landscape looks like. I believe our understanding of the transition state region is going to be revolutionized by these new structurally sensitive probes. After all, we will be at the fundamental limit in resolving chemical processes. I can see a day when this information allows us to take complex multidimensional processes and convert it to a reduced reaction landscape, projecting out the key modes coupled to reaction coordinates. At this point, we will be able to rival Mother Nature in the control of chemical processes.

## Questions/Food for Thought

Here I give some of the questions I ask myself as well as questions I have fielded during question period following my lectures on this topic. I hope these help reinforce the concepts and point to new directions that this field may take.

Q: In chemical reactions, the system thermally samples a reaction surface. These experiments use optical excitation such that the system is prepared well away from the real transition state or barrier crossing region. How can we be certain that the observed structural dynamics forthcoming from such studies are relevant to chemistry on ground state surfaces?
A: It is true that optical excitation prepares the system at far from equilibrium coordinates, even far from the thermally sampled crossing points. However, there are a few systems where it is the photoexcited reaction and not the ground state reaction that is of interest (e.g. photoisomerization of rhodopsins, photosynthesis, photoreceptors). The question really being asked here is how do we connect photoinduced structural dynamics to thermally sampled reaction coordinates? This connection is essential to the argument that these measurements constitute a direct probe of the primary events of chemistry and biology. In order to do so, the system has to be large enough that the atomic displacements all occur within or near linear response (the overall response of the global coordinate can be collectively nonlinear). For such systems, the dynamics and relative motions will be the same as thermally sampled coordinates. This problem has been addressed in MD simulations of bond breaking where similar issues arise (see the work of J. Straub on the Fe-CO bond breaking dynamics of myoglobin). Biological molecules are examples of large systems where this connection won’t be a problem. Similar arguments can be made for solution phase chemistry. Small molecule gas phase dynamics most likely can only be discussed in the context of the optically accessed electronic states with little inference to ground state processes possible.

Note, one of the challenges not discussed here is that we will need new means to optically prepare systems under barrierless conditions so that the structural dynamics are not simply limited by the statistics of thermally sampling the barrier crossing region. This is key. Coherent Control protocols may play a very important role in expanding the structural dynamics that can be studied.

Q: The reaction rates of biological processes are in the microsecond or millisecond time scale. How can femtosecond structural dynamics tell me anything about the relevant biological processes?
A: First we have to be sure we are optically preparing the biological system of interest at a barrierless crossing that involves the reaction driving the biological process. This can be done for a few cases as discussed above. Here one has to realize that the microsecond and longer dynamics are solely due to the barrier height. Of course the dynamics will be slow if the barrier is high. We are interested in how the system samples and crosses the barrier. In the case of biological systems, it must coarse grain sample its dynamic potential energy landscape. We would like to know how the system constrains the search. All degrees of freedom are coupled to the reaction coordinate to a certain extent. We can assign a coupling coefficient to all possible motions. There will be specific modes or types of motions (intramolecular, intermolecular, underdamped, damped, diffusive, density fluctuations over different length scales etc) with much larger coupling coefficients than others. In the Collective Mode Coupling Model, the largest coefficients involve segmental fluctuations of helices and other relatively rigid topological features in relation to loops. It is these same motions that take the system over large barriers. The probability of thermally populating these modes is small in this case so the reaction is slow. However, the sampling process is the same (re: answer to the above question). We need to understand what modes undergo the largest displacements in sampling the barrier (what modes have the largest coupling coefficient).

Here it is important to realize that the key concept is the barrier crossing event and this process occurs on femtosecond time scales once the displacements of the key modes are sufficient to propagate the system onto the product surface. The time it takes to thermally sample nuclear configurations to get within "striking distance" of sampling the product surface is determined by the activation barrier. The barrier induces a diffusive rate limited search to thermally induced reaction processes. By optically preparing the system at barrier crossing regions, we get around this limit in our observation of the actual dynamics associated with transition state processes.

Q: In the melting studies, how do you know the system has actually melted as opposed to disordering and is better described as a glass?
A: I discuss this point a bit in the above lecture. There is the Lindeman criterion for describing a liquid as the point when the bond length is modified by 10% of the solid state lattice value. This is not really sufficient as we are studying systems under high temperature and effective pressure. The equation of state for the system is not so well known at such extreme conditions to know if this criterion still holds. The main difference between a liquid and solid is that a liquid does not support shear. The transverse acoustic phonon branch collapses. Glasses, although disordered like a liquid, still support shear acoustic modes. The best argument I can give in the case of Al, Au, and Si is that the diffraction pattern corresponds very closely to the liquid structure factors obtained under steady state conditions. In the Al case, the SNR was good enough and there were no significant problems with diffuse background, so we can determine that in real space the coordination number had changed from 12 to 10 of the liquid state.

The transverse acoustic branch must have collapsed to allow such rearrangements. The Au and Si follow from this line of reasoning. The Si case is not so well defined though as its liquid structure factors show strong correlations out to many shells. In the case of Bi, it is very clear. We see a completely isotropic shell like structure in less than ½ collision of the primary A1g mode governing the motions of the Bi atoms along the repulsive excited potential. The random thermal rms motions at the moment of the excitation are enough to completely sample an isotropic distribution. The transverse acoustic phonon branch must have collapsed to permit such excursions.

Q: What is the uncertainty principle limit to the time and space resolution to electron diffraction?
A: The initial thermal emittance defines the energy spread and transverse momentum spread. You can exploit space charge to increase $\Delta E$. With proper fields it would be possible to compress nonrelativistic electrons down to the attosecond range (with very few electrons.) re: $\Delta E \, \Delta t = h/2\pi$. However, in the process, you would sacrifice transverse coherence or spatial resolution as the effect of space charge couples both longitudinal and transverse components to electron propagation. The best is use the minimum thermal emittance possible for highest spatial resolution. With an initial .6 eV spread, it is possible to obtain pulses of less than 5 fs with sufficiently small momentum spread for .1 nm resolution and still have sufficient electron numbers for close to single shot structure determination.

Q: Isn't chemistry determined by the electrons? Wouldn't you rather watch the electrons rather than the atoms to understand chemical processes?
A: Chemistry does involve changes in electron charge distribution and I have discussed how optical excitation enables manipulation of bonding and potential energy surfaces. However, by far the largest fraction of chemistry involves thermal fluctuations along a reaction coordinate. The relative portion of energy related to the stochastic fluctuations of the valence electron distribution (electronic contribution to the heat capacity) versus that of the atomic fluctuations (nuclear heat capacity contribution) is minor. There are not large changes associate with charge fluctuations until the system samples a nuclear configuration (Franck Condon factors) allowing resonant electron transfer or energy transfer. It is the atomic fluctuations that direct traffic through a reaction coordinate. The specific details of the charge distribution in this region do determine the barrier heights and coupling coefficients to the barrier crossing.

Another way to respond to this question is that the changes in electron distribution create potential energy gradients (reaction forces) that displace the atoms. So by watching directly the atomic motions you are in fact accessing information on the charge distribution and its time evolution, with the correct reporter --- the atom.

This being said there are efforts to use attosecond spectroscopy via high harmonic generation and correlations to map electronic wavefunctions. This is not a 1 electron problem and core electron correlations are likely involved (not so different than watching atomic motions in information content). It would be very neat to see the electron dynamics in this way in any case. Alternatively one would like information on the quantum state dynamics to connect atomic motions to the electronic wavefunctions. We are setting up to do both using multidimensional coherent spectroscopies in conjunction with fs electron diffraction. The information content of correlating atomic motions to quantum state dynamics/frequency correlations provides the full picture. It is interesting times.

I have many more questions driving my research efforts and many more technical questions have been asked of me. The above give the main intellectual challenges in charting new territory.

## Suggested Readings

1. Jason R. Dwyer, Christoph T. Hebeisen, Ralph Ernstorfer, Maher Harb, Vatche B. Deyirmenjian, Robert E. Jordan and R. J. Dwayne Miller, Femtosecond Electron Diffraction: 'Making the molecular Movie', Phil. Trans. R. Soc. A (2006) **364**, 741–778. This paper gives an account of the recent advances in dynamic electron probes and includes more experimental details.

2. W. E. King WE, G. H. Campbell GH, A. Frank A, et al., Ultrafast Electron Microscopy in Materials Science, Biology and Chemistry. J. Appl. Phys. (2005) **97** (11),111101. This paper gives a very nice account of the fundamental challenges to using high number density electron pulses for studying structural dynamics and gives the fundamental limits to spatial temporal resolution in using electrons for microscopy/real space imaging.

3. A. H. Zewail, Ann. Rev. Phys. Chem. (2006) **57**, 65-103, for an alternative account of the progress in the field using single electrons/pulse or low brightness sources.

4. C.V. Schmising, M. Bargheer, M. Woerner, et al. Real-time studies of Reversible Lattice Dynamics by Femtosecond X-ray Diffraction, Zeit. Kristallographie (2008) **223** (4-5) 283-291. This paper gives an example of excitation of an intramolecular charge transfer process under reversible conditions. These studies show lattice deformation in response to the dipole field of the charge separation process as a solid state example similar to solvation dynamics. This study gives the first microscopic picture of molecular polarization. It is also an example of reversible system. The collective effective of the molecular reorientations gave an anomalously larger change in diffraction parameters. Higher excitation is needed to resolve the intramolecular structural changes.

5. G. Hummer, F. Shotte, and P. A. Anfinrud, Unveiling of Functional Protein Motions with Picosecond X-ray Crystallography and Molecular Dynamics

Simulation, PNAS (2004), 101 (43) 15330; F. Shotte et al. Picosecond Time-Resolved X-Ray Crystallography: Probing Protein Function in Real Time, J. Structural Biology (2004) **147**, 235; D. Bourgeois et al. PNAS (2006), Extended Subnanosecond Structural Dynamics of Myoglobin Revealed by Laue Crystallography. PNAS (2006) **103,** 4924. This series of papers describes seminal work on the ESRF beam line in which our first glimpses of important intermediate structural transients are revealed. It is clear from this work that the scientific objective of determining the structure-function correlation will be achieved with the higher time resolution of next generation x-ray and electron sources.

6. H. Park and J. M. Zou, Direct Measurement of Transient Electric Fields Induced by Ultrafast Pulsed Laser Irradiation of Silicon, Applied Physics Letters (2009), **94**, 251103. This paper repeats the geometry of electron diffraction experiments in reflection mode, without the beam actually touching the surface, and observes the fast transients typically seen for experiments conducted with this geometry. The dynamics are due to photoemitted electrons and not structural dynamics. This effect needs to be considered in reflection experiments and draws into question the fast dynamics observed in electron diffraction experiments conducted in reflection (see cited references therein to this regard). The field effect cancels out in transmission. This paper gives a good example of the kinds of controls needed to rule out artifacts.

## Acknowledgments

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200 fs electron pulses with single shot capabilities. He also introduced the use of counter propagating pulses to enable full electron pulse characterization with microjoule level pulses and conventional small frame laser systems. In addition, he did the work on exploiting the unique charge distribution properties of electrons to study the onset of plasma formation with 100 fs time scale resolution. This latter work was not discussed. Maher Harb's PH. D. work led to our first view of electronically driven structural changes using the classic Si system. He also worked on laser based electron acceleration (not discussed) and designed our 5th Generation Electron Gun that is just coming online now. He further advanced the nanofabrication methods that are key to our current experiments on novel systems and was responsible for interfacing all aspects of our present data collection system. Thibault Dartigalongue figured out the "every electron detector" for our current system. Ralph Ernstorfer was involved in developing the experimental methods for our first laser pondermotive measurements and most of the innovations involved in detection and elimination of background scattered ions. The latter accomplishment was essential to allowing the necessary excitation levels for the study of strongly driven structural dynamics. This work led to our studies of Warm Dense Matter of Au as well as a number of other systems. German Sciaini figured out how to mount large area Bi nanofilms by using slow evaporation methods to prevent rupture of the film. He was responsible for all the experimental protocols in the Bi studies and leads the present work. Sergei Kruglik was too briefly with us but he left his mark in building up the laser infrastructure for our 5th Generation Electron Gun. Mariko Yamaguchi methodically figured out how to fabricate 100 nm thin organic and biological crystals. Her contributions will make their impact in our next series of experiments. We also enjoyed some wonderful collaborations. In particular, the studies of single crystal Si were made possible through the always enthusiastic efforts of Weina Peng , Mark A. Eriksson, and Max G. Lagally at the University of Wisconsin at Madison. The Bi samples were provided by a very fruitful collaboration with Th. Payer, Michael Horn-von Hoegen and F.-J. Meyer zu Heringdorf at the Universität Duisburg-Essen. My group has greatly benefited from the discussions both in terms of material preparation and physics with our collaborators at both Madison and Duisberg-Essen for which we are very grateful. I should add that although I cited particular contributions, all this work has been a team effort and it is impossible for me to give full credit where credit is due. It has been a wonderful experience for me to work with such colleagues and friends.