Femtosecond Time Resolution in the Study of Protein Hydration

by

Greg Sollenberger

of

Western Illinois University

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Ohio State University

Advisor: Dongping Zhong

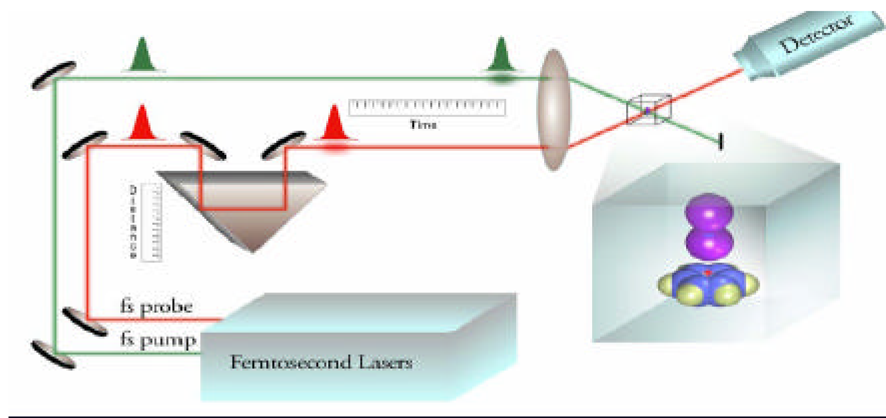
Introduction

Time resolution has increased by about twenty-two orders of magnitude since the beginning of mankind. Four thousand years ago, an accurate calendar was developed that could be used to accurately gage the length of a year. Around 1500 B.C., a sundial was constructed which could accurately scale the hours in a day. About three thousand years later, in 1300 A.D., and accurate clock was made that could resolve seconds with precision. In the twentieth century, we leaped ahead by 15 orders of magnitude. Around the turn of the century, we could resolve events with resolution in the millisecond scale with fast cameras. Fast electronics instruments could be used to resolve events faster than a millisecond. When lasers were invented, optics jumped ahead and we reached the nanosecond and picosecond regimes. Finally, in the early 1980s, the femtosecond regime was reached using ultra fast laser pulses.

As the time resolution got smaller and smaller, a clearer picture of events in chemistry and biology was formed. Professor Dongping Zhong's group studies the dynamics of protein hydration on a time scale that will help form a new model of the hydration of proteins.

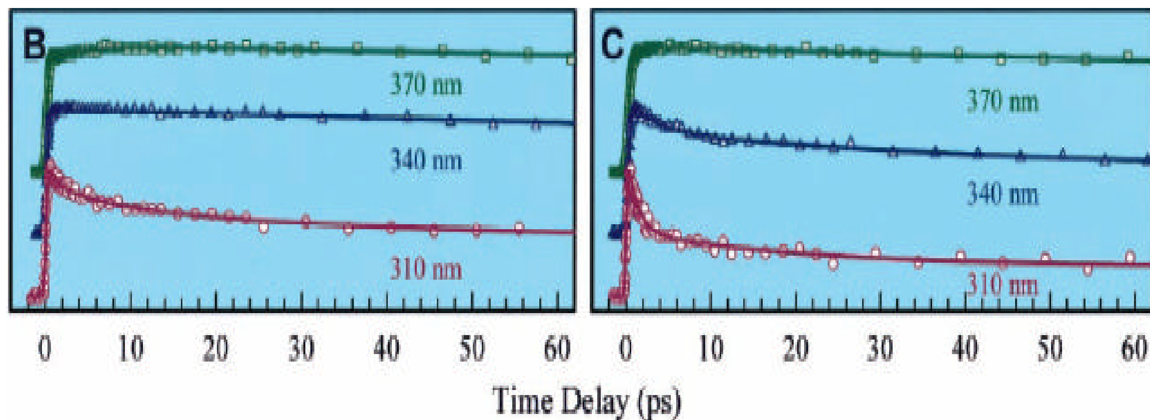
Femtosecond Studies

The pump-probe technique is an experimental method utilized by the Zhong group in its study of protein hydration. This technique uses two laser pulses from the same source. One is called the pump pulse, which excites the sample to a higher energy state.



The Pump-Probe Technique

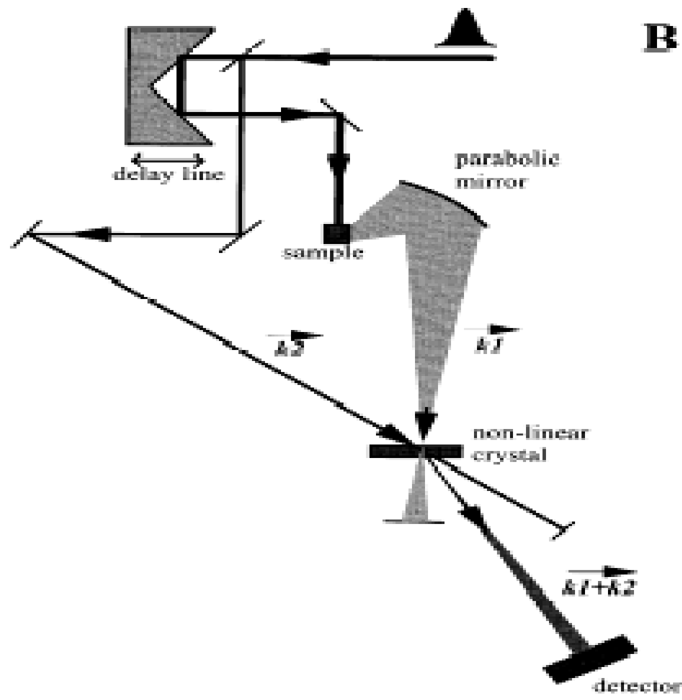
The other is called the probe pulse, and it is used as a reference beam to establish a time dependant picture. The triangle-shaped object in the picture is the delay stage. By moving the stage one micrometer, which can be easily done, the probe pulse is delayed by 3.3 fs. Each successive point on the decay curves



below is a successive movement of the translation stage. The y-axis of the graph is intensity.

The pump pulse excites the sample, and the fluorescence intensity is measured using the fluorescence up-conversion technique. In the diagram, the fluorescence is emitted by the sample as though it was a point source, and a parabolic mirror focuses the fluorescence on a non-linear crystal. The photons from the fluorescence of a certain

wavelength combine with the photons of the probe pulse, and a photomultiplier tube is set to measure the intensity of photons at the up-converted wavelength.



Fluorescence up-conversion

Using the data from the decay of the fluorescence, we can fit an exponential graph and find the decay time of the excited state we are studying.

Experimental Setup

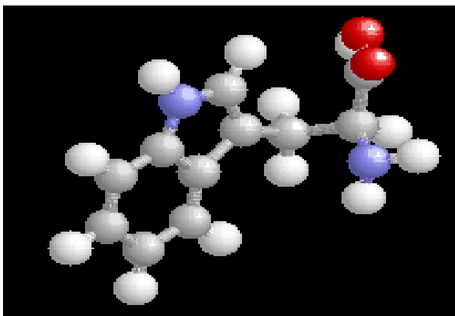
A diagram of Dr. Zhong's optics table has been attached to the end of this report. A series of lasers, amplifiers, lenses, mirrors, and other elements are aligned to deliver a 290 nm pulse to the sample. Several BBO non-linear crystals are used to up-convert photons to the desired wavelength. When two photons go through a BBO crystal, then, depending on the angle they make with the crystals optical axis, they may combine. Since energy is conserved, with input photons of frequency ?$_1$ and ?$_2$, the photons emitted

will have a frequency of $\nu_1 + \nu_2 = \nu_f$. We use BBO crystals and pulses of several different wavelengths to select the needed wavelengths to excite the sample. Filters are used to filter out the original wavelengths, since not all the incoming photons are up-converted.

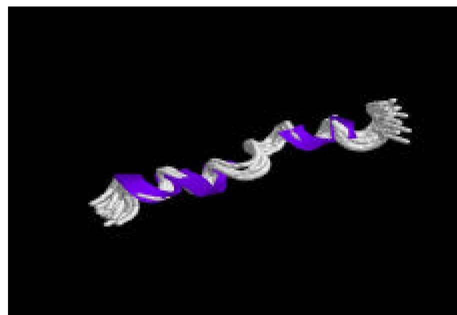
A double-prism setup is used to compress the pulse width. When the pulse leaves the laser, it has a positive group velocity dispersion (GVD), which means it is spread out. The prisms allow you to tune the GVD, which is the second derivative of the phase angle, to zero. The GVD has a positive component, which is proportional to the distance traveled through the prism, and a negative component, which is proportional to the distance between the tips of the two prisms. By adjusting these two factors, the GVD can be made zero.

Protein Dynamics

The structure and function of proteins is relatively easy to determine. What is much more difficult is discovering the dynamics by which the proteins function. Since atoms move very quickly and on such a small distance scale, picosecond to femtosecond time resolution is needed to resolve their motions. With new ultra fast techniques, such



Tryptophan



Mellitin

as the one mentioned above, we can form a coherent picture of protein dynamics.

Hydration of Proteins

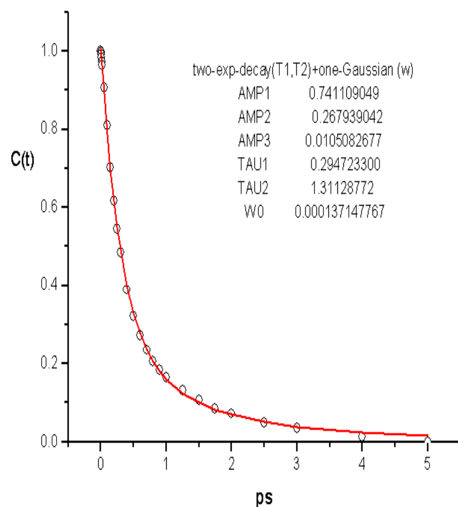
Professor Zhong's group has done work recently that should help elucidate the process of protein hydration. Using the fluorescence up-conversion technique, his group has studied the hydration of proteins using tryptophan, myoglobin, and mellitin.

A pump pulse is used to excite a residue on a protein. The residue is excited to a higher energy state, and it gives off fluorescence. The fluorescence starts out at a particular spectrum, then starts to red-shift. The red-shift is due to the excited state's loss of energy. The difference between the absorption and emission spectra is called the Stokes Shift. The fluorescence up-conversion technique gives us a graph of the intensity of the fluorescence of a given wavelength as a function of time. Using points from the fluorescence intensity graphs for several wavelengths, we can construct a spectrum. Since we have time dependent data, we can construct a spectrum for several different times. This gives us a progression of Stokes Shifts with time, or the time resolved Stokes Shifts.

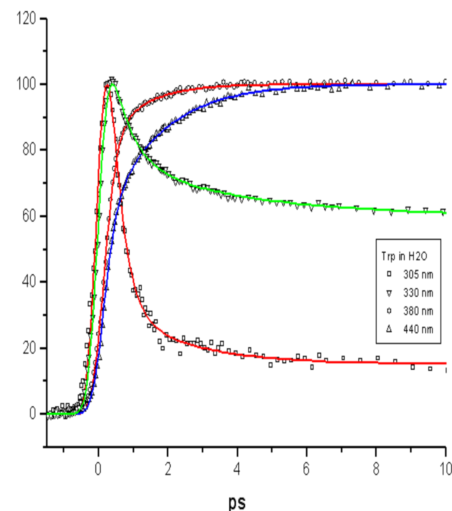
Normally, when a particle is excited, its energy state decays to the lowest vibrational state of the excited state before it emits. However, since we are dealing with such a small time scale, the vibrational energy of the protein's excited state does not have time to dissipate its energy by diffusion. On this time scale, the protein, having no other way to use the energy it's received, becomes a dipole. As the water molecules surrounding the excited protein align themselves with the dipole, the protein goes to a lower energy state. This movement toward a lower energy state causes the emission

spectrum to become red-shifted. By looking at the time-resolved Stokes Shifts, we can tell on what time scale the protein becomes hydrated. To do this, we use the hydration correlation function, which is $C(t) = \frac{\boldsymbol{n}(t) - \boldsymbol{n}(\infty)}{\boldsymbol{n}(0) - \boldsymbol{n}(\infty)}$. The variables ?(0), ?(8), and ?(t) are the peak (or average) frequencies at times t=0, t=8, and t, respectively. This usually appears as a multiple exponential decay function.

Curve Fits for Tryptophan



Hydration Correlation Function



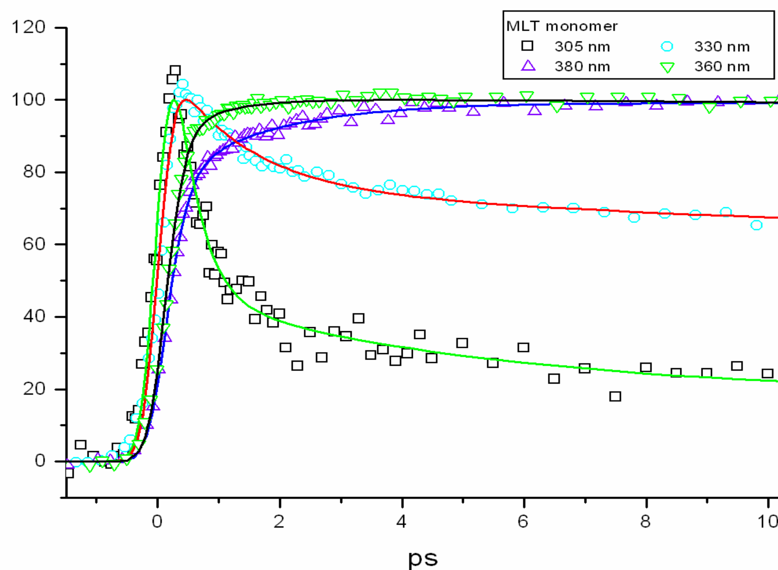
Fluorescence up-conversion

A better fit is almost always accomplished with a multiple exponential. This means there are multiple characteristic times for protein hydration. Most hydration will occur within the first characteristic time, and afterwards, hydration occurs more slowly. The graphs above are a hydration correlation function for the amino acid tryptophan in water and the fluorescence up-conversion for tryptophan at four different wavelengths. These graphs were fitted with data collected this summer.

We can study specific areas where hydration occurs, or "hydration sites", on myoglobin. Dr. Zhong's research group creates mutant structures of myoglobin with tryptophan inserted into the area of study. When we fit the data, we get a multiple exponential function that tells us the characteristic times of hydration. Since tryptophan is the only amino acid that absorbs appreciably at the excitation wavelength (290 nm), the characteristic times of hydration can be specified to the location on the protein where tryptophan was placed. Older models of protein hydration showed there to be a crystal-ice structure surrounding the molecule. With faster time resolution, this model is shown to be inaccurate.

We study mellitin, which is a residue found in bee venom. It is a monomer that can be easily turned into a tetramer structure. By studying both structures, we can see the effect of structural differences on hydration time.

As of the end of this summer, Dr. Zhong's group is still fitting data from several months' experiments.



Fluorescence Up-conversion Data for Mellitin

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