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Using Fluorescence Correlation Spectroscopy to Measure Partial Unfolding of Variants of Cytochrome c

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Abstract

The protein Cytochrome *c (Cytc)* has been known to be a regulatory on/off switch for apoptosis, or programmed cell death, when unfolded. Using Guanidine Hydrochloride (GuHCl) of different concentrations to denature 3 variants of Zinc Cyt*c* (ZnCyt*c*), the unfolding of this protein can be measured. Measuring is performed through different methods such as single- and dual-focus Fluorescence Correlation Spectroscopy (FCS) and Circular Dichroism (CD). FCS is used to measure the change in lifetime of the protein to determine if the protein has unfolded. The change in lifetime is a characterization of partially unfolded proteins. Data are recorded and analyzed through several different Matlab codes to fully understand the folding code of the protein

Introduction

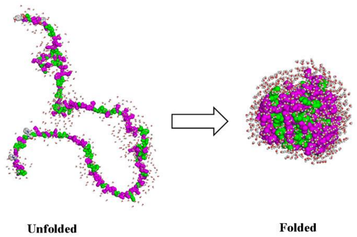

Figure 1: Protein in the unfolded state to the folded state. Image is from https://biomedical.closeupengineering.it/en/the-profax-project-and-the-protein-folding-algorithm-based-monte-carlo/8176/

- A protein is a molecule that is a polymer of amino acids that are joined together in peptide bonds.
- To assume its functional state or conformation, the protein's structure must go through a process known as protein folding (figure 1).
- Protein folding causes the long amino acid chain to curl into a globular shape (figure 1, figure 2).

- Cytochrome *c* (Cyt*c*) (figure 2) is a protein that is important in cellular respiration as well as apoptosis (cell death).
- We are able to study Cyt*c* because it a small globular protein when folded allowing us to observe it unfold through a denaturing process using different concentrations of Guanidine Hydrochloride (GuHCl).
- Cyt*c* has a heme core of iron that was replaced with a zinc core (ZnCyt*c*) in order to produce stronger emission signals.

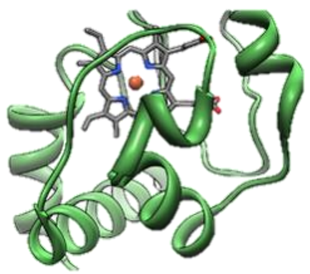

Figure 2: Image of Cytochrome *c* in its folded state, the green corresponds to the amino acid chain and the grey and red corresponds to the heme the amino acids fold around. Image taken form https://en.wikipedia.org/wiki/Cytochrome_c

Fluorescence Correlation Spectroscopy

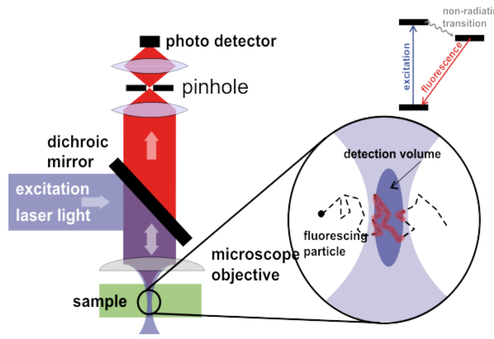

Figure 3: Experimental setup of FCS. Using a beam of horizontally polarized UV light and a beam of vertically polarized UV light to excite the protein we can determine lifetime, what folding state the protein is in and the amount of folded and unfolded proteins in a sample. Image taken from https://www.dkfz.de/Macromol/research/fcs.html

- The Translational Diffusion Coefficient is the distance it takes a fluorophore to rotate. When a fluorophore is folded, the coefficient should be smaller compared to when the fluorophore is unfolded.

- Fluorescence Correlation Spectroscopy (FCS) uses correlation analysis of time fluctuations of fluorescence intensities.
- These fluctuations help us observe the diffusion behavior and concentrations of the particles being studied.
- The use of the following two equations is need:

$$G(\tau) = G_o \frac{1}{\sqrt{(1+\frac{\tau}{\tau_D})(1+a^{-2}\frac{\tau}{\tau_D})}} + G_\infty \quad (1)$$

and

$$D = \frac{RT}{6\pi\eta R_H} \quad (2)$$

- Equation (1) is known as the auto-correlation function, where *G* is the amount of particles in the system, τ is the time, τ_D is the characteristic residence time, a is the ratio of axial to radial radii in the measurement volume and is equal to $a = \frac{\omega_z}{\omega_{xy}}$
- Equation (2) is used to find the Translational Diffusion Coefficient, where *R* is the Boltzmann's constant, *T* is the temperature in kelvin, η is the viscosity of the liquid, and R_H is the hydrodynamic radius.

Percentage of Bound vs Unbound Proteins and Lifetime of the Proteins

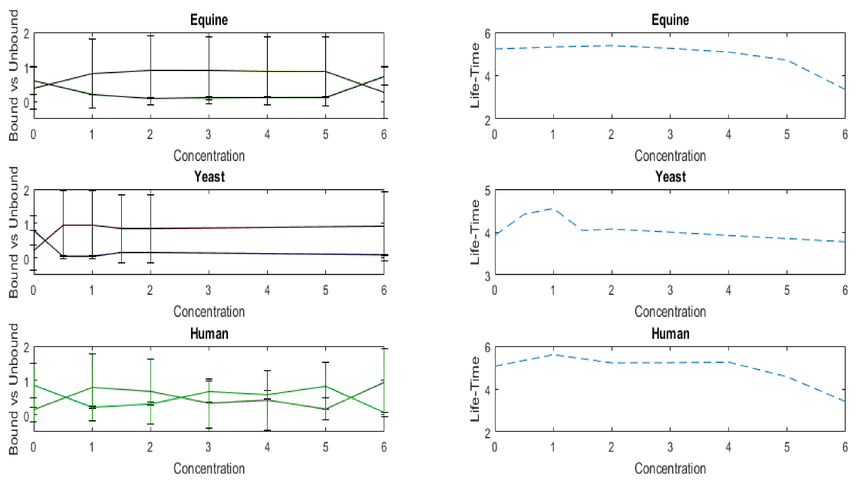

Figure 4: Plotted on the left is the percentage of bound and unbound proteins found in the solution per concentration, as be seen, as the concentration increases, the number of bound proteins decreases because the proteins are unfolding. Error bars are included to show accuracy. On the right the lifetime of the 3 protein variations can be viewed. As the concentration of GuHCl increases, the lifetime of the protein decays, showing that the proteins are in fact unfolding as a result of the changing concentrations.

Comparison of 0M and 6M Concentration on Bound vs Unbound Proteins

Variant	0M	6M
Equine	61% folded	27% unfolded
Yeast	79% folded	92% unfolded
Human	86% folded	94% unfolded

Table 1: Comparison of bound vs unbound proteins (respectfully) at 0M and 6M concentrations of GuHCl.

Table 1 shows a clear distinction between the bound and unbound proteins at specific concentrations of GuHCl. As shown in figure 3, as the concentration of GuHCl increases, the number of unbound proteins increases which was expected. When compared to the lifetime of the protein we see a drop as the concentration goes up.

Variant	0M	6M
Equine	5.2295 ns	3.353 ns
Yeast	3.9205 ns	3.771 ns
Human	5.061 ns	3.409 ns

Table 2: Comparison of the lifetime for 0M and 6M concentrations of GuHCl, as we can see as the concentration increases, the lifetime decreases.

Summary

- From the data that was recorded we are able to characterize the translational diffusion of a folded and unfolded protein.
- We can relate these both to a shift in the lifetime of the protein as seen in figure 2 and referenced in table 2.
- We these findings we can elucidate where a bound protein is folded or unfolded during an interaction.

Future Work

- While the results agree with what was expected, future work to refine the experiment in order to lower the error is planned.
- The next step in the experiment is to use the findings from the lifetime measurements In order to monitor protein unfolding during lipid membrane binding (figure 5).

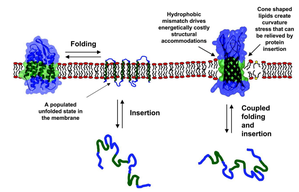

Figure 5:Example of both protein folding and unfolding on a lipid bilayer. Image taken from http://www.pnas.org/content/101/12/3995/F1.expansion.html