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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 (Cyt c) 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

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).

• 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.

Fluorescence Correlation Spectroscopy

Equation (1) is known as the auto-correlation function, where G is the amount of particles in the system, r is the time, $\tau_c$ is the characteristic residence time, a is the ratio of axial to radial radii in the measurement volume and is equal to $a = \frac{R_z}{R_n}$.

$G(r) = G_0 - \frac{1}{\tau_c} \left( 1 + \omega r \right) + a G_0 (1)$

and

$D = \frac{RT}{\omega m R_n} (2)$

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

<table>
<thead>
<tr>
<th>Variant</th>
<th>0M</th>
<th>6M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equine</td>
<td>61% folded</td>
<td>27% unfolded</td>
</tr>
<tr>
<td>Yeast</td>
<td>79% folded</td>
<td>92% unfolded</td>
</tr>
<tr>
<td>Human</td>
<td>86% folded</td>
<td>94% unfolded</td>
</tr>
</tbody>
</table>

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

We are able to characterize the transnational 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.

Summary

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).