

Paper to be discussed in Class Wed April 2, 2008

“Characterization of the active intermediate of a GroEL-GroES-mediated protein folding reaction”

by JS Weissman et al. (1996) Cell **84**:481-490.

For each figure (this semester), the following protocol of discussion should be followed:

- 1) Goal of the experiment. What are the questions that are being addressed?
- 2) Set-up of the experiment. Why were the reagents used? Describe the technique(s) employed.
- 3) Go through the actual data.
- 4) Author's conclusions.
- 5) Personal interpretation and opinions.

Figure #1.

Explain anisotropy?

What does a decrease in anisotropy indicate?

Explain the basis for the SR1 mutation?

How is the rhodanese labeled?

Figure #2.

Explain a Hummel-Dreyer experiment?

Explain gel filtration chromatography?

Why is there a decrease in [³⁵S]GroES at the GroES elution volume?

Is folded rhodanese protected from proteolysis?

Figure #3.

How is the half time of folding calculated?

What is it in A compared to B for SR1?

Why is the Wild type activity so low in B?

Figure #4.

What is GFP? Why use GFP here?

What exactly are the two different peaks designated GFP and SR1?

What are the half times for each peak in A, and how was this calculated?

Is GroEL/ES acting as a foldase here?

In C what does the WT peak at 2.5 min signify?

Figure #5.

Why does having a similar emission spectra and fluorescent lifetime indicate that the structures are similar?

Figure #6.

Why is using AMP-PNP with WT similar to using ATP with the SR1 mutant?
Why does it only reach 40% activity with AMP-PNP?

Figure #7.

How does this model exhibit cooperativity between subunits? between rings?
When is the ring space large?
What initiates the release of substrate?