

Musse et al. J. Biol. Chem. 2006, 281, 885.

**Figure 1. Table 1**

How many Trp residues are present in the protein?

Where are they located in the 3D structure?

What are they exactly measuring by determining the Trp  $\lambda_{\max}$  for the mutants?

What other non-destructive spectroscopic technique would you use to obtain information about the folding state of the mutants?

**Figure 2**

How many Cys are present in the wt protein? And in the mutants?

What information is the  $\lambda_{\max}$  of the probe providing? Red/Blue shifted; water exposed, hydrophobic environment?

Are the size of the probe and the length of the linker considered in the accessibility area calculations?

How do the residues that have a low accessibility surface area got labeled? Don't they need to be accessible to be labeled?

Any info about % of labeling? How the % of labeling of different mutants would affect the obtained data?

**Figure 3**

What different is the information provided in Fig 2 and Fig 3?

Why the  $\lambda_{\max}$  for the probes exposed to the solvent in the water-soluble monomer differ from the ones on the membrane bound protein?

Is the used fluorescent probe hydrophobic or hydrophilic?

**Figure 4**

How does anisotropy values relate to mobility of the probe?

What anisotropy value (aprox.) do you expect for a free probe in solution based on the values obtained with the labeled mutants?

What kind of interactions with the acyl chains of neighbor amino acids will decrease the mobility of the probe?

Does fig 4C provide any additional information?

**Table 2 Figure 5**

What factors affect the quantum yield of the fluorescence probe?

Are the quantum yield measurements useful for the structural analysis?

**Figure 6**

How does a charged amino acid located close to the fluorescent probe will affect the observed quenching data? Will the charges affect the 10-Doxylnonadecane quenching? Why?

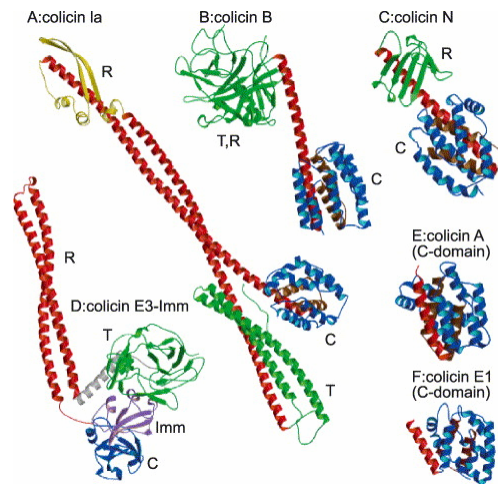
Will the addition of IK affect the ionic strength of the sample (and the structure of the protein)? How do you correct by this effect?

What fluorescence pattern do you expect for an amphipathic  $\beta$ -hairpin laying on the surface of the membrane?

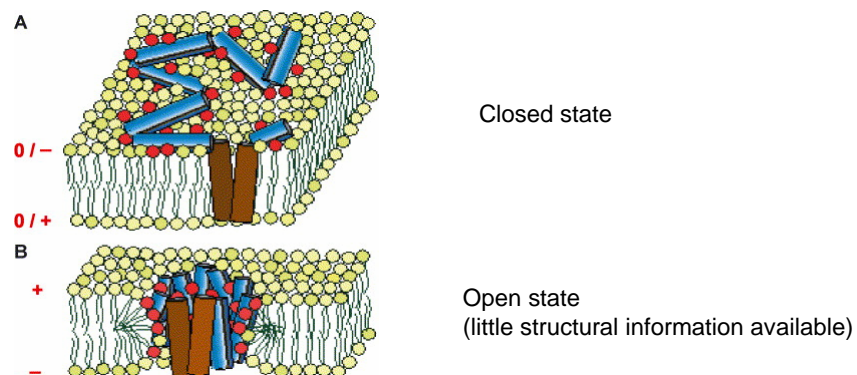
In general for the all the figures.

If a particular sample is not homogeneous (e.g., not all the protein is bound to the membrane), how the determined parameters will be affected? How would you check for homogeneity?

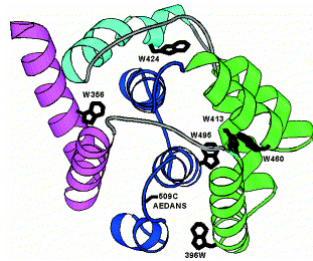
# X-ray crystal structures of Colicins



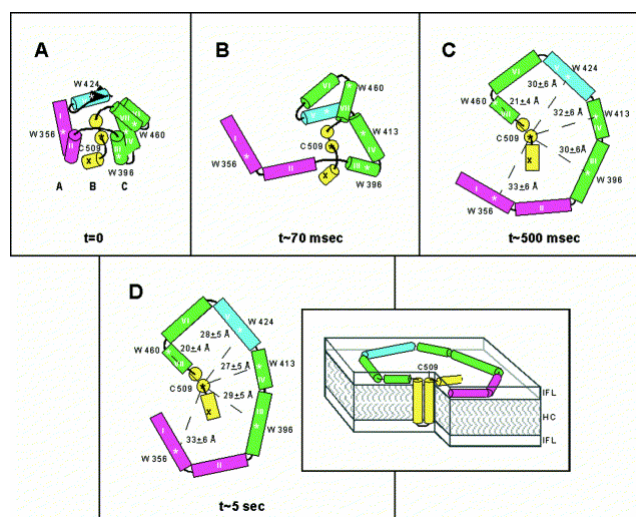
# Model of colicin membrane binding and pore formation



# FRET analysis between Trp and Cys-labeled Dansyl

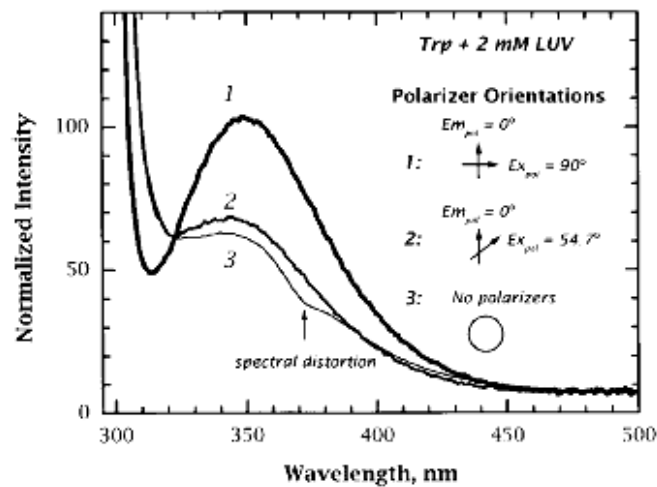


## Model from FRET data

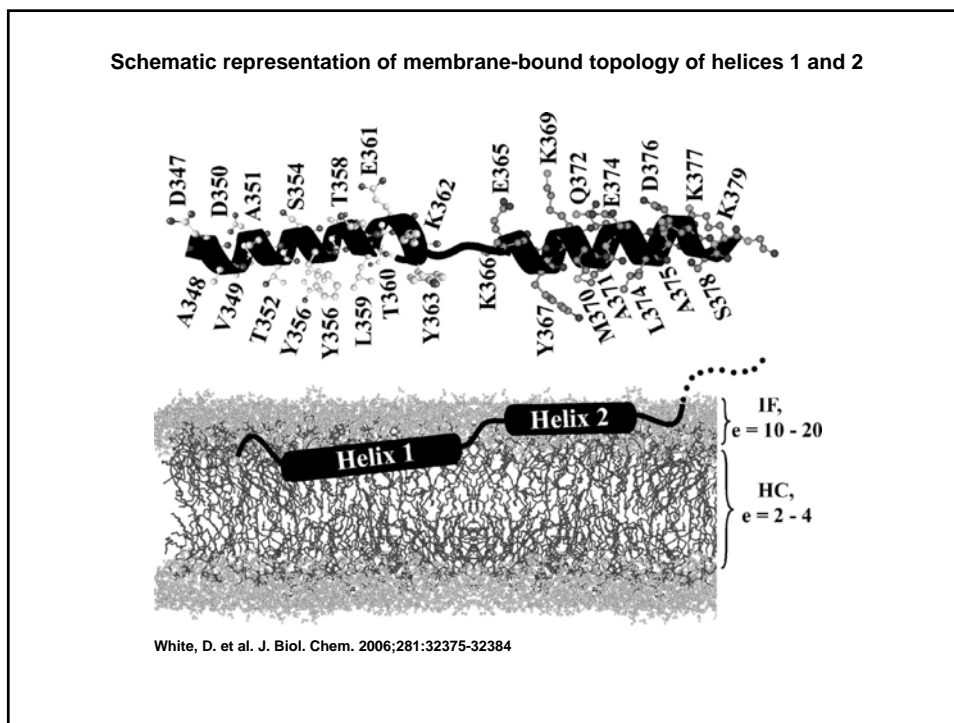
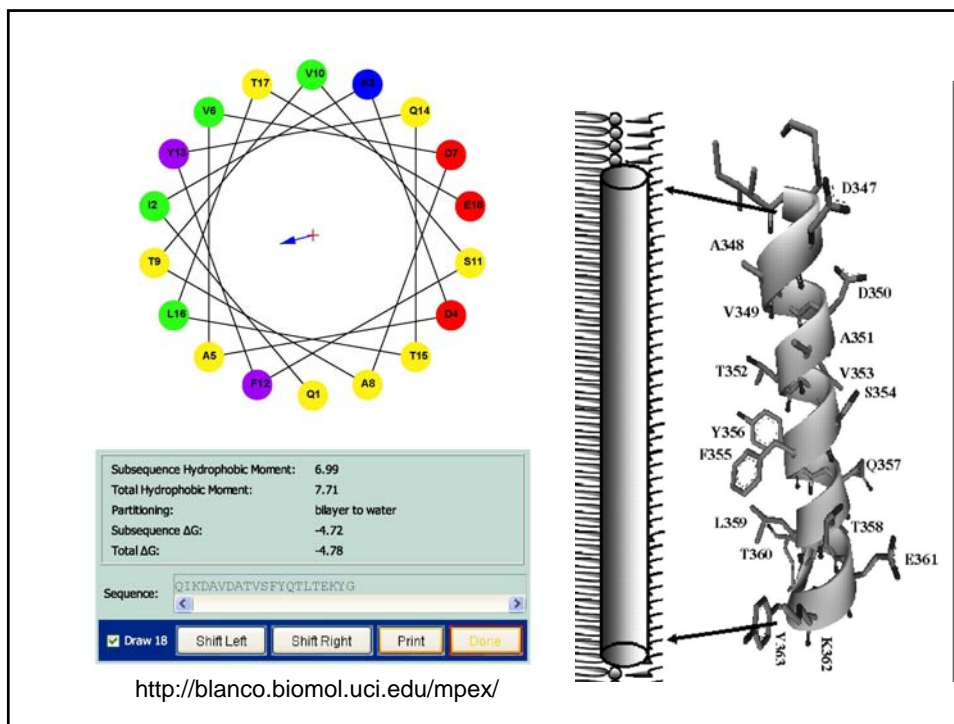


- Membrane-protein interaction triggered by acidic pH
  - Creates attractive electrostatic interactions between cationic CP(E1) and liposome membranes (30 mol%) anionic lipid
  - Facilitates the unfolding of the protein on the membrane surface

How to reduced the scatter contribution to the signal



Ladokhin et al (2000) Anal. Biochem. 285, 235-245



# Labeling reaction

