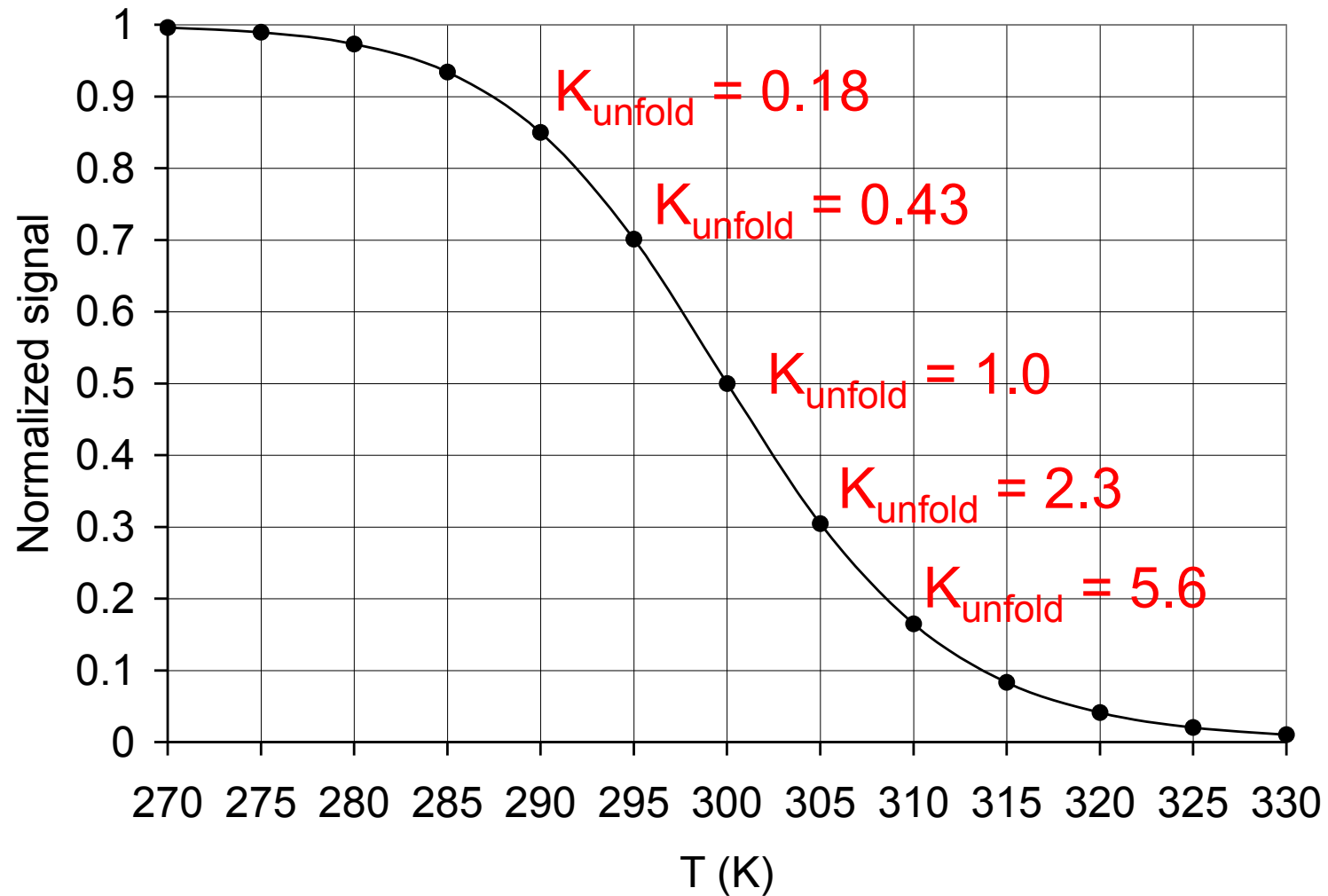


van' t Hoff Analysis

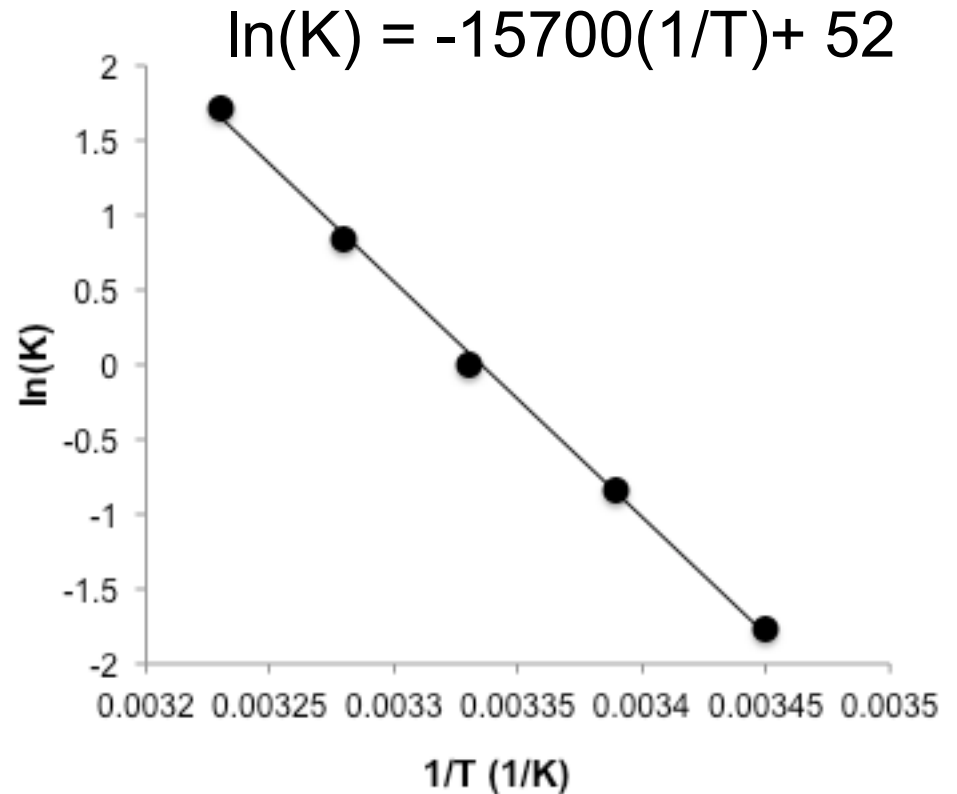


van't Hoff Plot

| T (K) | K_{unfold} | $1/T$ (K^{-1}) | $\ln(K)$ |
|-------|---------------------|---------------------------|----------|
| 290 | 0.18 | .00345 | -1.7 |
| 295 | 0.43 | .00339 | -.83 |
| 300 | 1 | .00333 | 0 |
| 305 | 2.3 | .00328 | +.84 |
| 310 | 5.6 | .00323 | +1.7 |

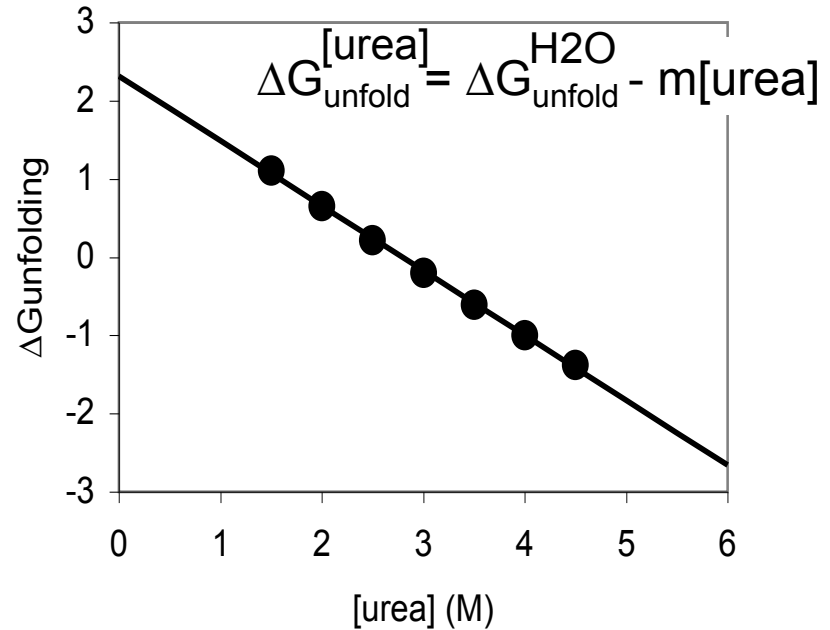
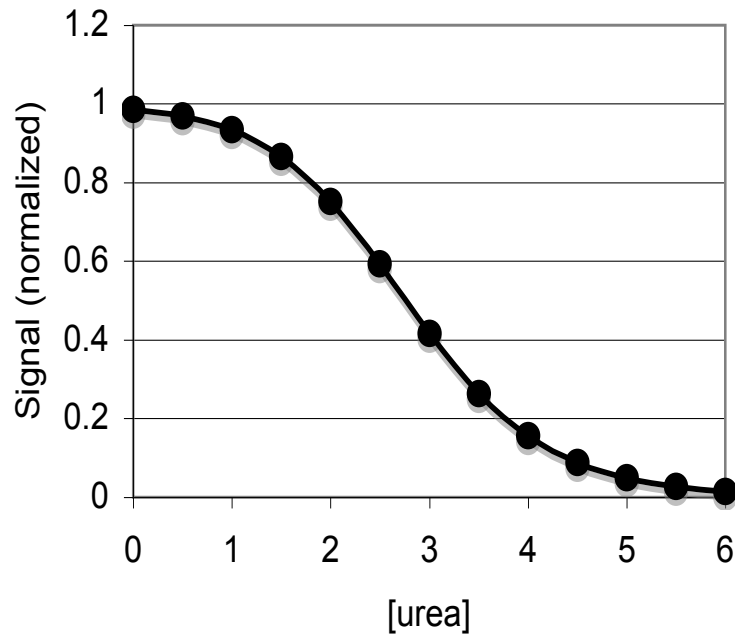
$$\Delta H^\circ = -\text{slope} \cdot R = +31 \text{ kcal/mol}$$

$$\Delta S^\circ = \text{intercept} \cdot R = +100 \text{ cal/molK}$$



Equilibrium Denaturation

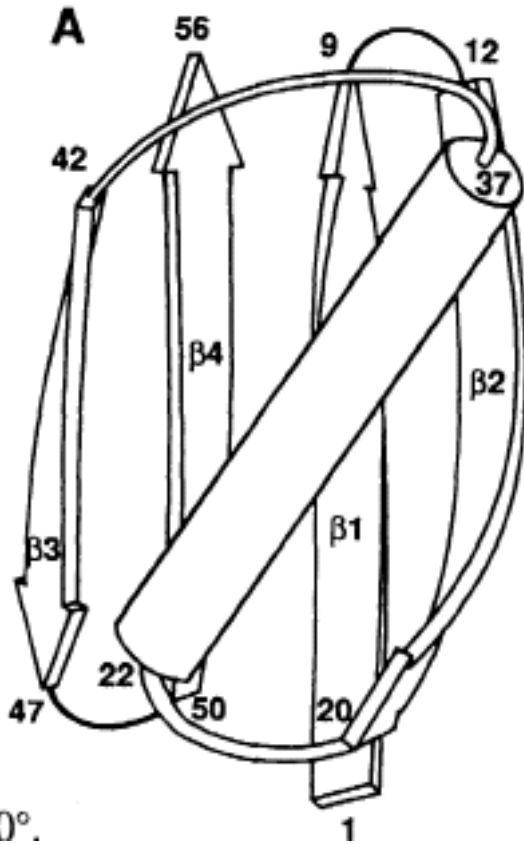
Measure equilibrium constants at each concentration of denaturant, which are used to get ΔG_{unfold} at each conc.



slope = "m" (\propto non-polar SA)

intercept = $\Delta G_{\text{unfold}}^{\text{H}_2\text{O}}$

Value of the Hydrophobic Core in Protein Stability



Protein G - B1 domain

Guessing at Enthalpy

Assume each H-bond in 2° Structure is 0.5 kcal/mol more energetic than w/ water.

50 H-bond provides $\Delta H_{\text{unfold}} = +25 \text{ kcal/mol stability}$

Guessing at Entropy

Assume gain of 9 conformations/residue in unfolding:

$$\begin{aligned} \Delta S_{\text{unfold}} &= R \ln 9^{56} \text{ res.} = 240 \text{ cal/mol}\cdot\text{K} \\ -T\Delta S_{\text{unfold}} &= -72 \text{ kcal/mol} \end{aligned}$$

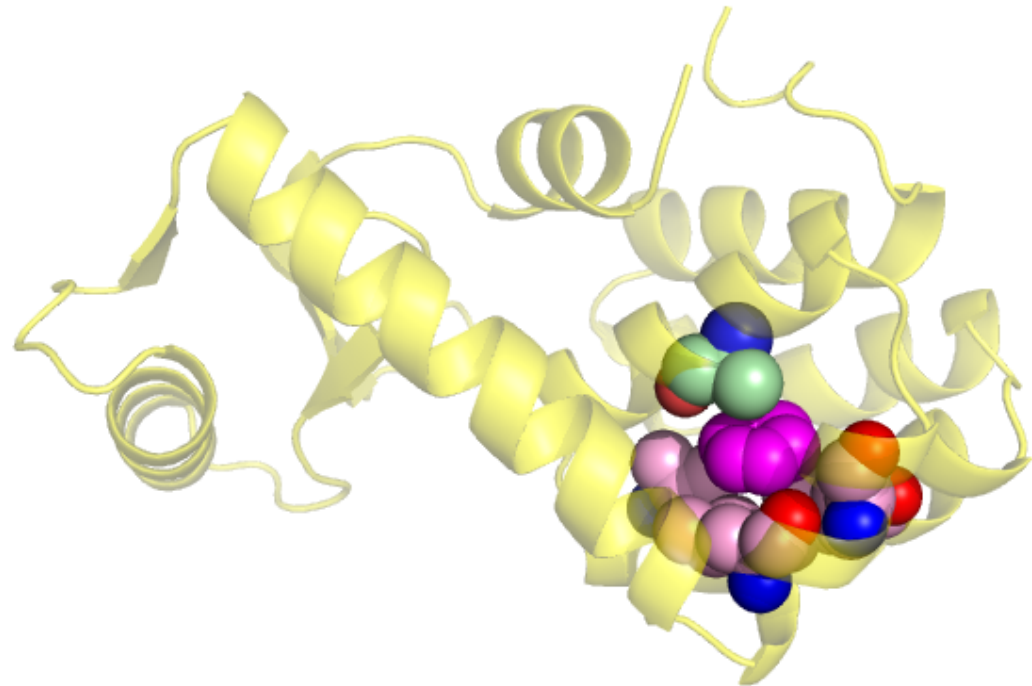
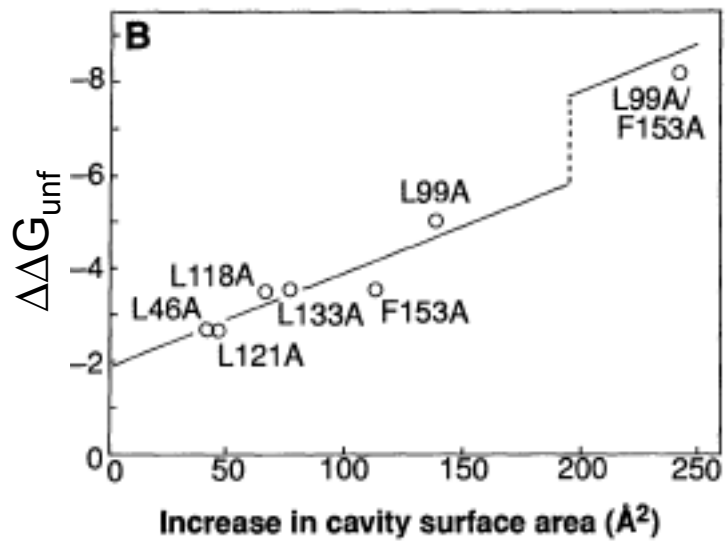
Actual

$$\Delta H_{\text{unfold}} = +64 \text{ kcal/mol}$$

$$\Delta S_{\text{unfold}} = +148 \text{ cal/mol}\cdot\text{K} \quad (-T\Delta S = -42 \text{ kcal/mol})$$

vdW contribute bulk of enthalpic stabilization
H-phobic effect counters unfavorable entropy of folded protein.

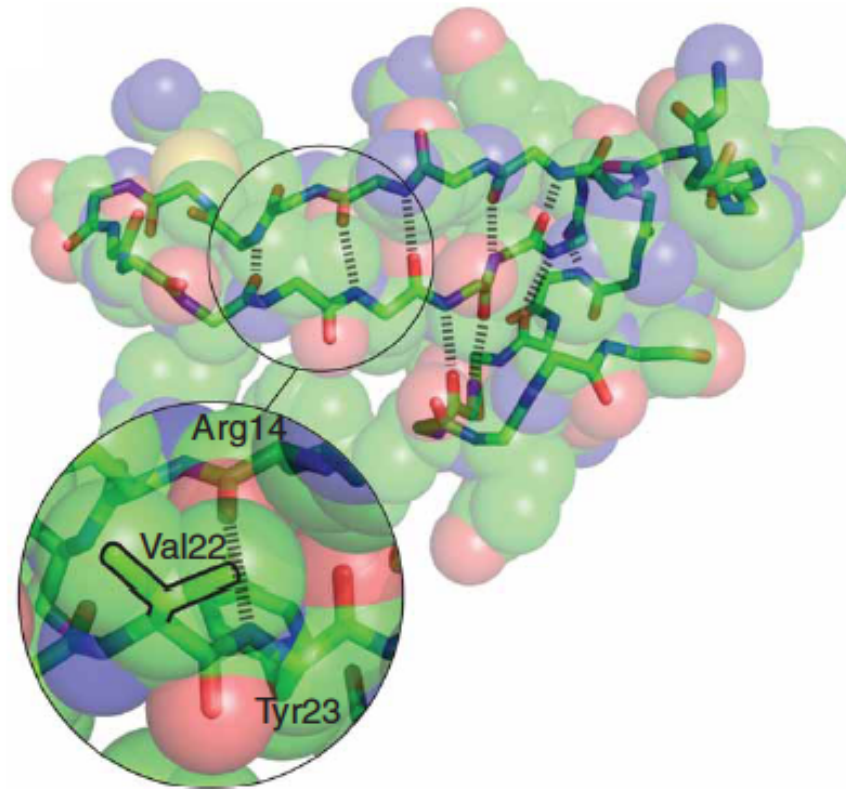
Hole in T4 Lysozyme



Creates 150\AA^3 hole

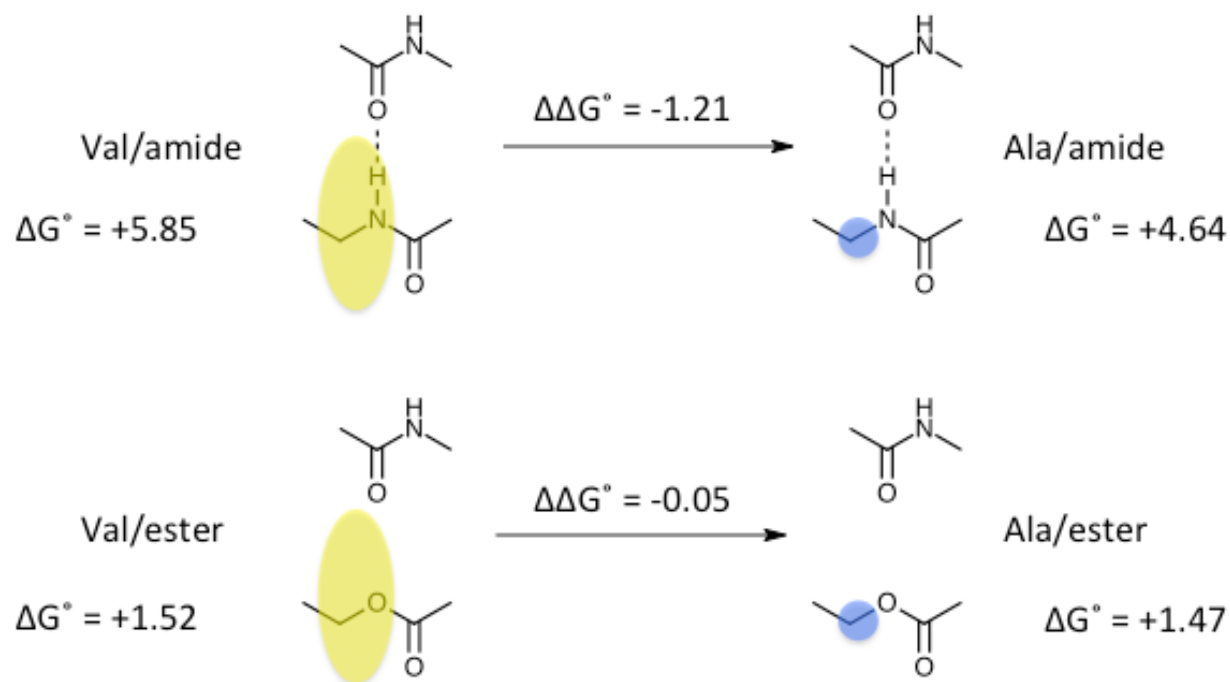
Environment Plays A Role in Energetics of H-bonding

PIN1



Nat. Mol. Struct. Biol., **16**, 684 (2009)

Environment Plays A Role in Energetics of H-bonding



Nat. Mol. Struct. Biol., **16**, 684 (2009)

Replacing a Charged Triad

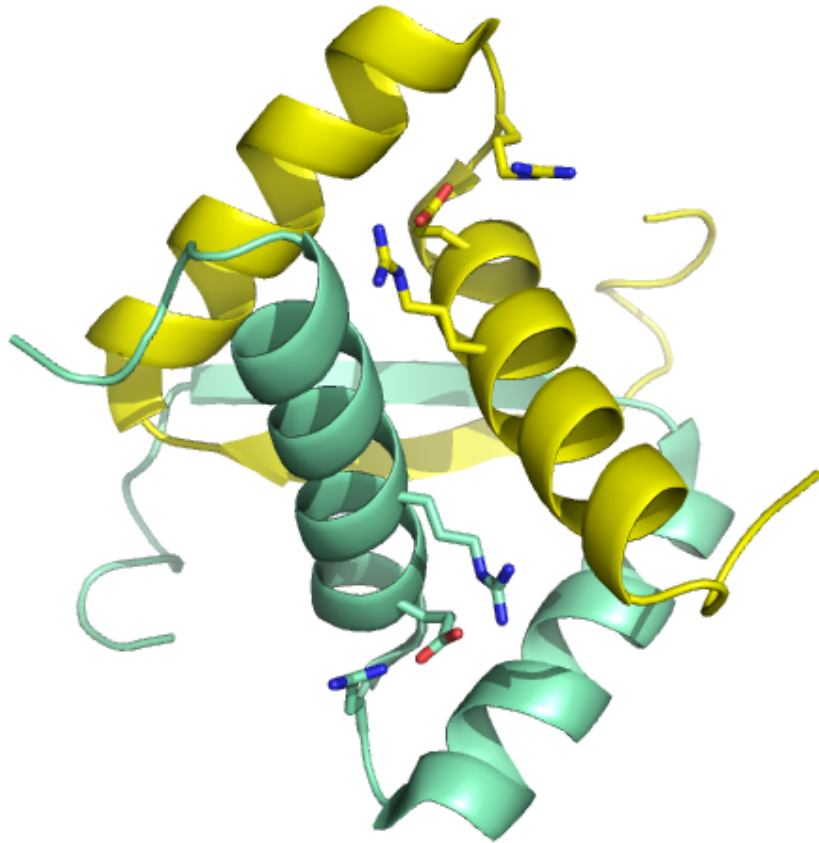
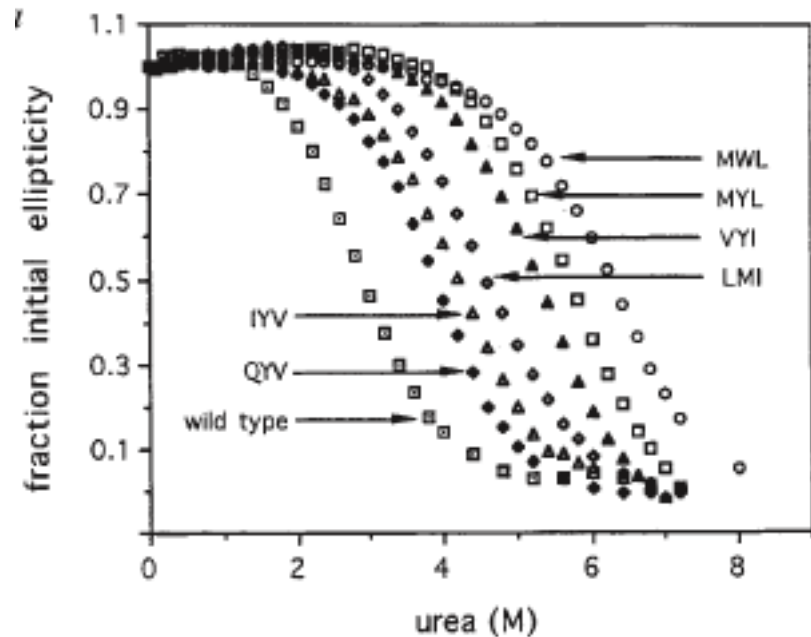
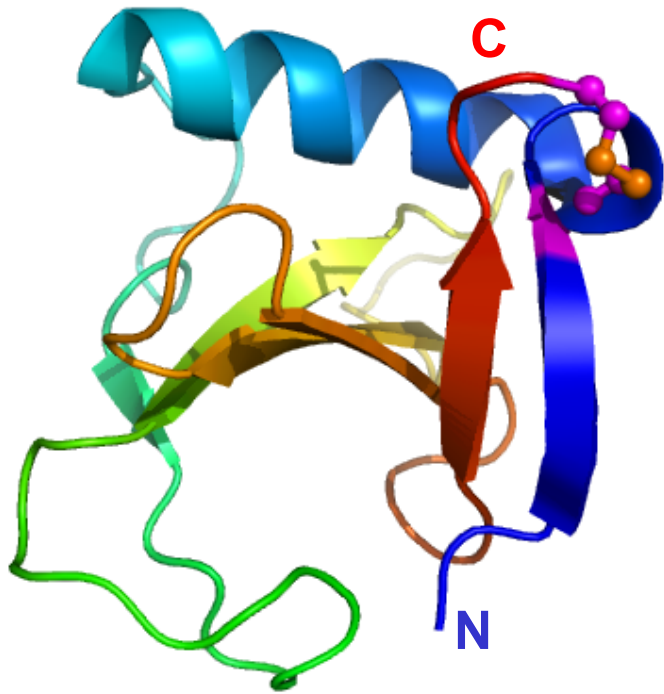


Table 1 Stabilities of wild-type Arc and active variants

| protein | ΔG_u^1 kcal per mole of dimer | $\Delta\Delta G_u^2$ kcal per mole of dimer | m kcal per mole of dimer per M |
|-----------------|---|---|--|
| 31-36-40 | | | |
| RER (wild type) | 10.3 (± 0.3) | — | 1.36 |
| MYL | 14.2 (± 0.2) | 3.9 (± 0.5) | 1.33 |
| MWL | 14.8 (± 0.2) | 4.5 (± 0.5) | 1.30 |
| VYI | 13.8 (± 0.1) | 3.5 (± 0.4) | 1.36 |
| IYV | 12.5 (± 0.4) | 2.2 (± 0.7) | 1.42 |
| QYV | 12.4 (± 0.2) | 2.1 (± 0.5) | 1.49 |
| LMI | 11.9 (± 0.2) | 1.6 (± 0.5) | 1.20 |



Disulfide in RNase Sa

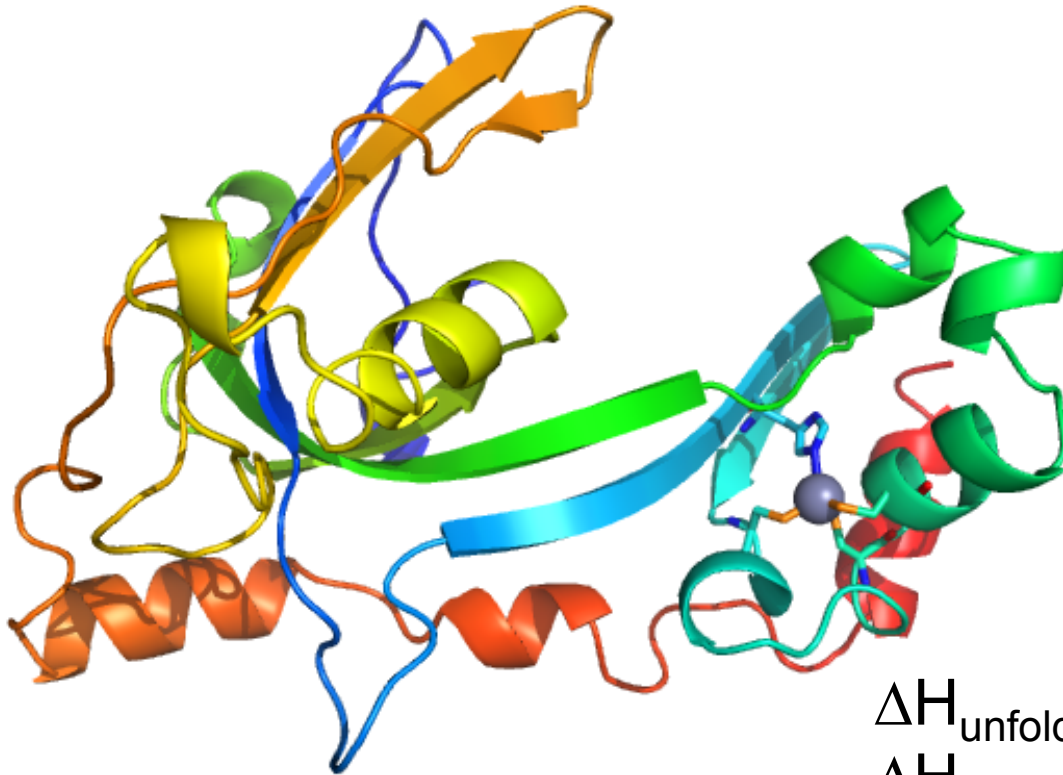


Cys7-Cy96

$\Delta G_u = 6.1 \text{ kcal/mol} + \text{disulfide}$

$\Delta G_u = 0.3 \text{ kcal/mol} - \text{disulfide}$

Gene 32 Protein: A Structural Zn^{2+}



$$\begin{aligned}\Delta H_{\text{unfold}} &= + 139 \text{ kcal/mol} \quad +Zn^{2+} \\ \Delta H_{\text{unfold}} &= + 84 \text{ kcal/mol} \quad -Zn^{2+}\end{aligned}$$

Distribution of 4° Structures

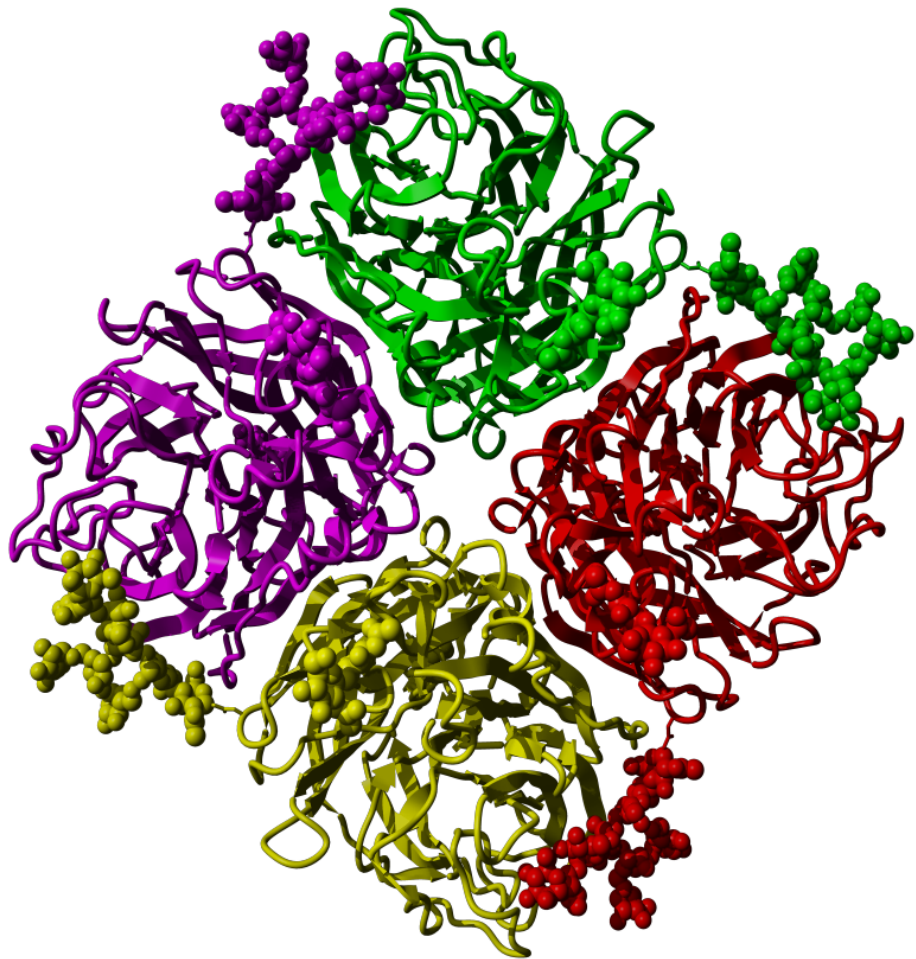
TABLE 1 Natural occurrence of oligomeric proteins in *Escherichia coli*^a

| Oligomeric state | Number of <u>homooligomers</u> | Number of heterooligomers | Percent |
|------------------|--------------------------------|---------------------------|---------|
| Monomer | 72 | | 19.4 |
| Dimer | 115 | 27 | 38.2 |
| Trimer | 15 | 5 | 5.4 |
| Tetramer | 62 | 16 | 21.0 |
| Pentamer | 1 | 1 | 0.1 |
| Hexamer | 20 | 1 | 5.6 |
| Heptamer | 1 | 1 | 0.1 |
| Octamer | 3 | 6 | 2.4 |
| Nonamer | 0 | 0 | 0.0 |
| Decamer | 1 | 0 | 0.0 |
| Undecamer | 0 | 1 | 0.0 |
| Dodecamer | 4 | 2 | 1.6 |
| Higher oligomers | 8 | | 2.2 |
| Polymers | 10 | | 2.7 |

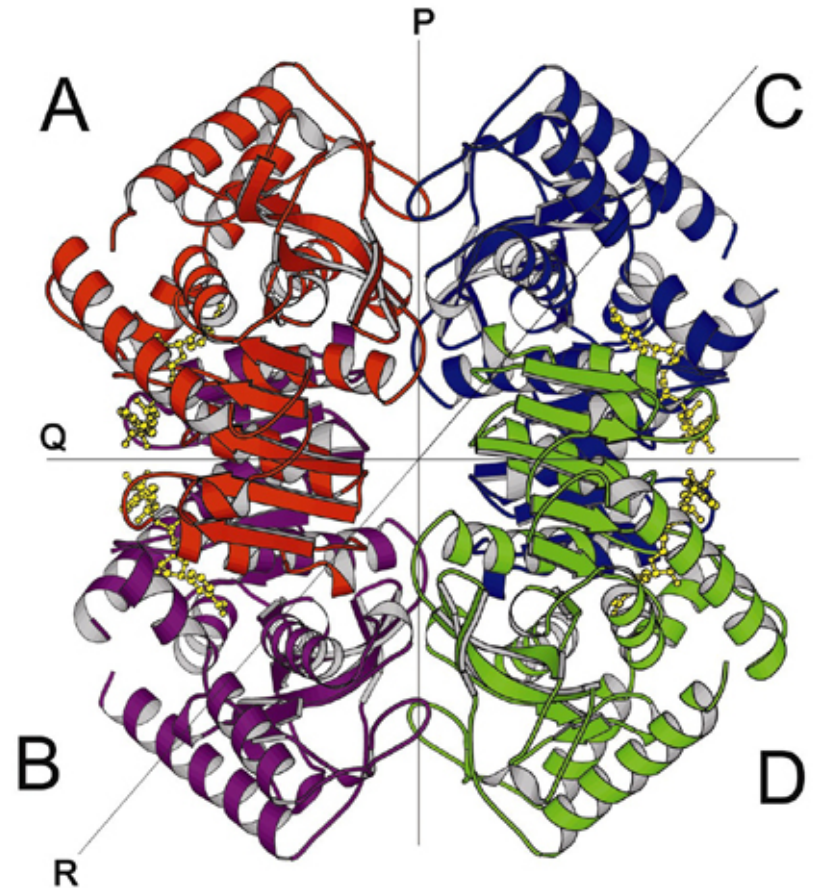
Survey of *E. coli* protein structures including soluble, membrane-bound and structural.

Goodsell & Olson, Ann. Rev. Biophys. Biomol. Struct. **26**, 105 (2000).

α_4 Tetramers

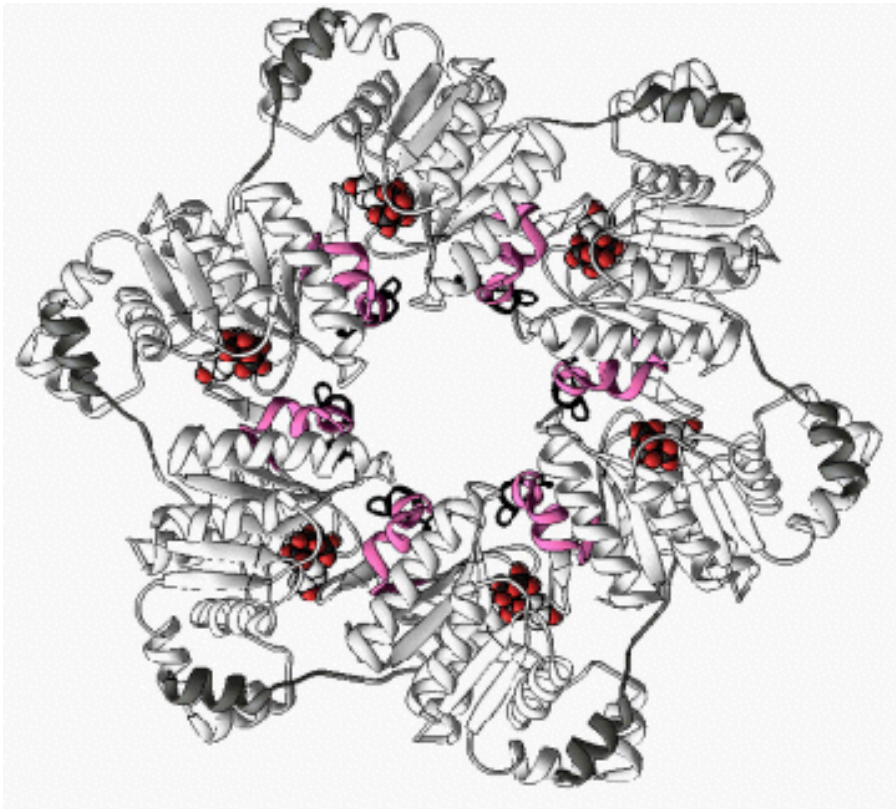


C₄ Tetramer

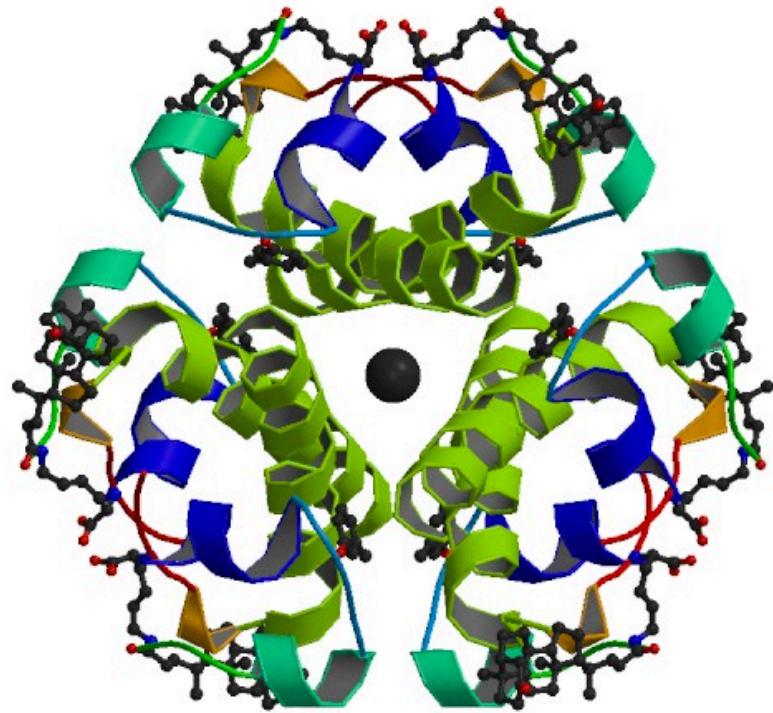


D₂ Tetramer

α_6 Hexamers

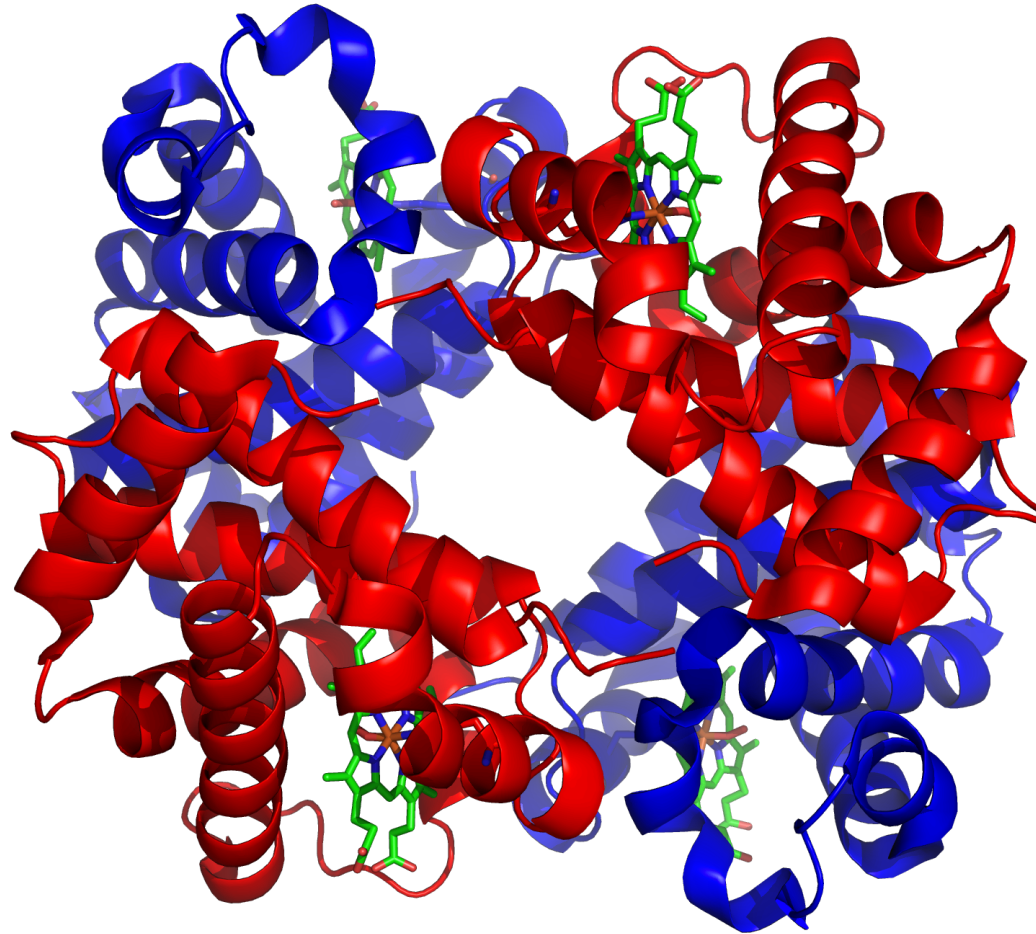


C₆ Hexamer

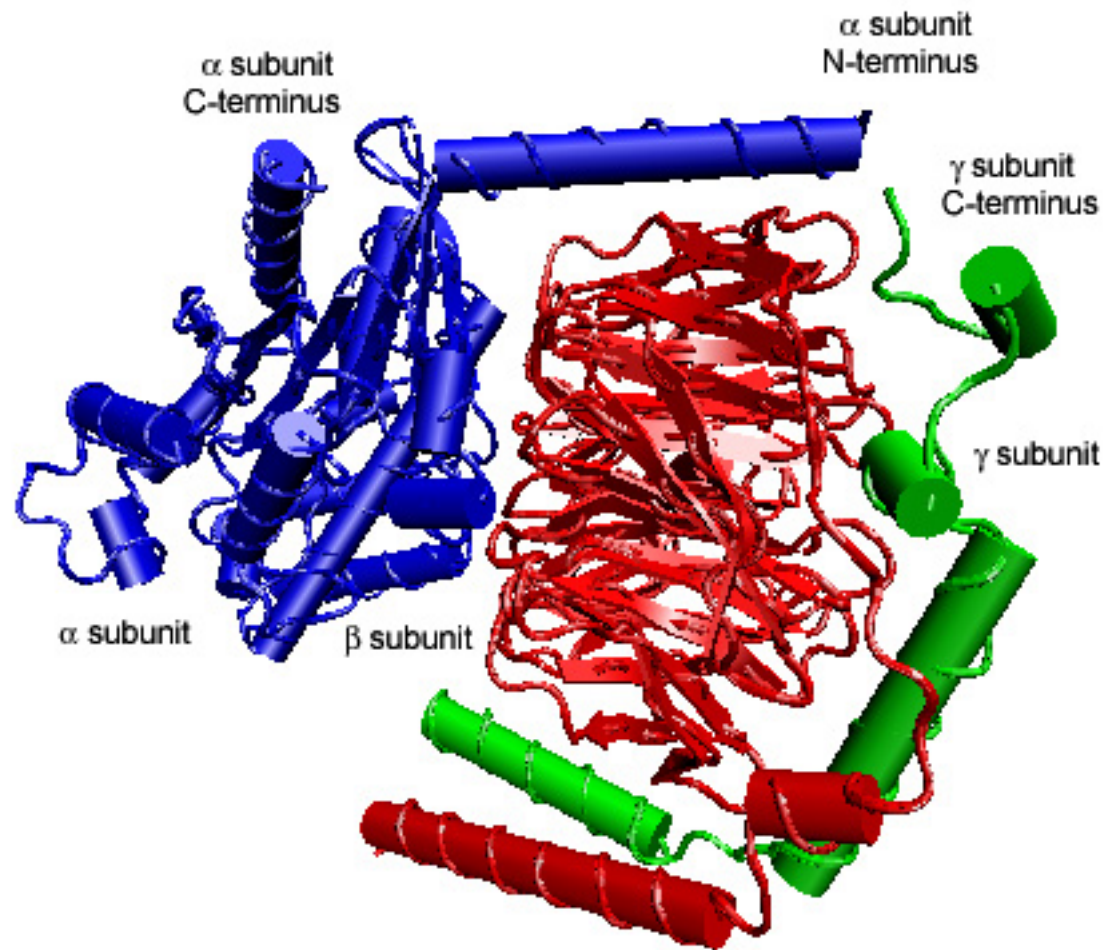


D₃ Hexamer

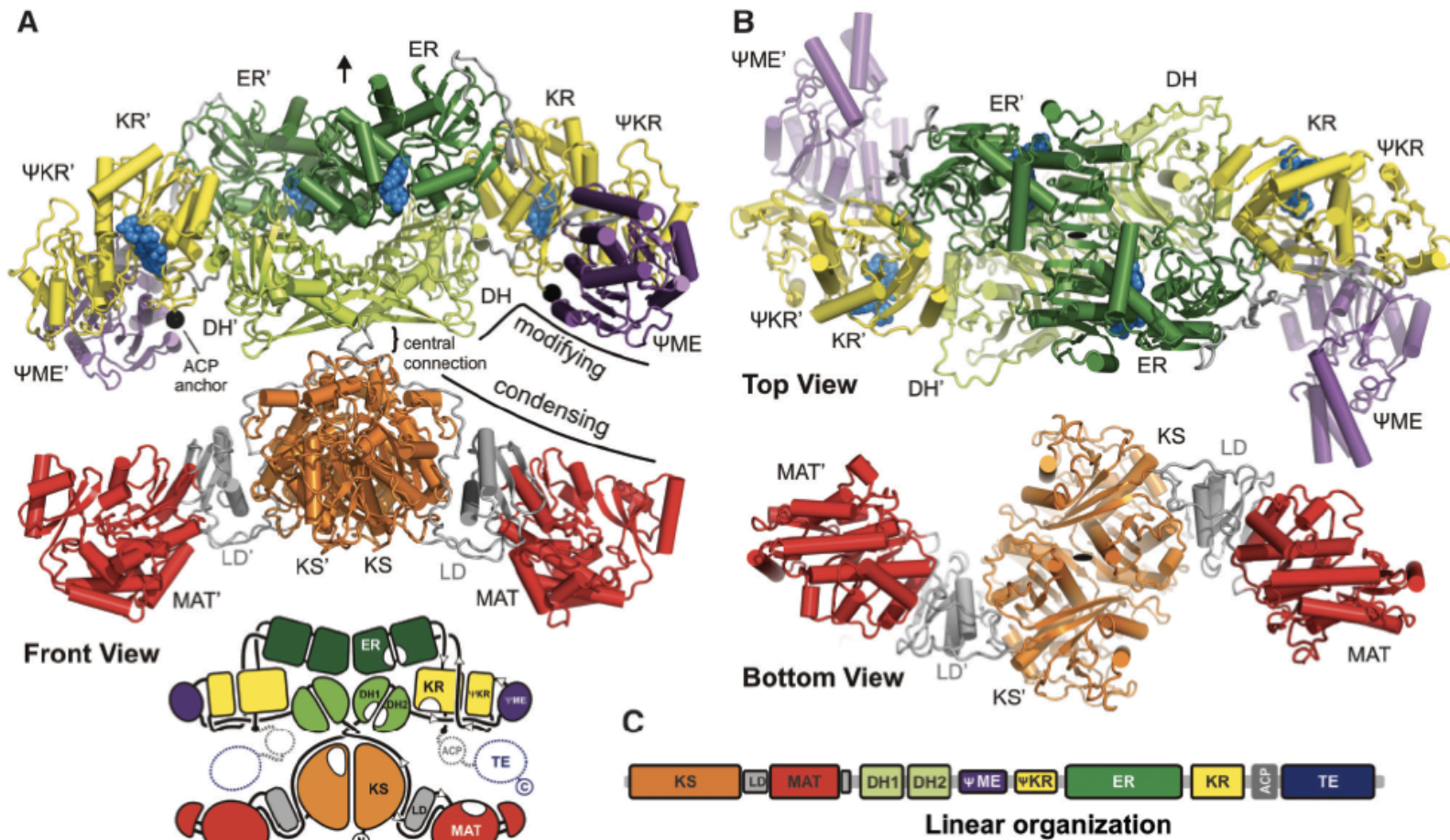
Hemoglobin - $\alpha_2\beta_2$ Tetramer



Heterotrimeric G Protein

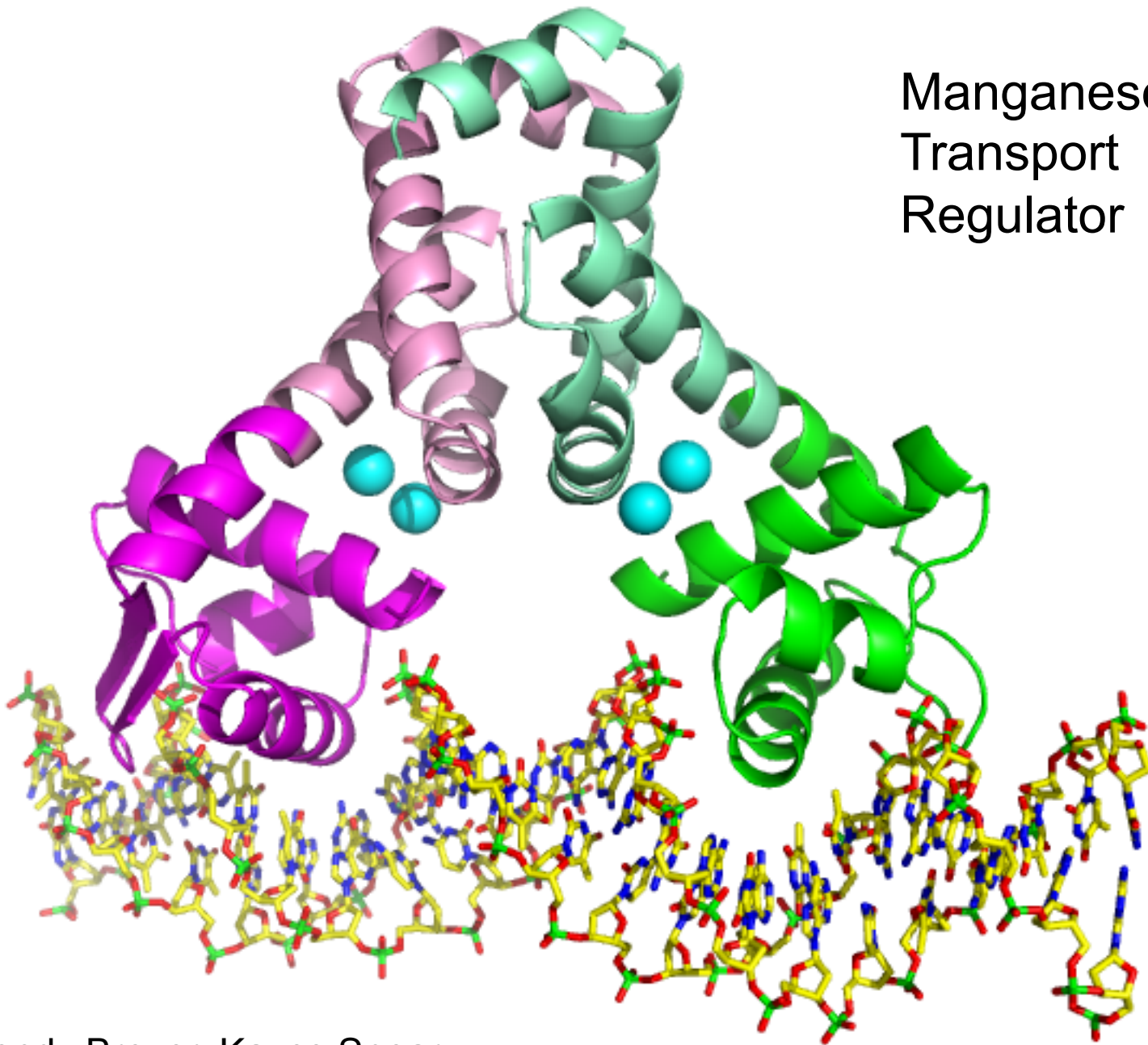


Functionally Distinct Domains



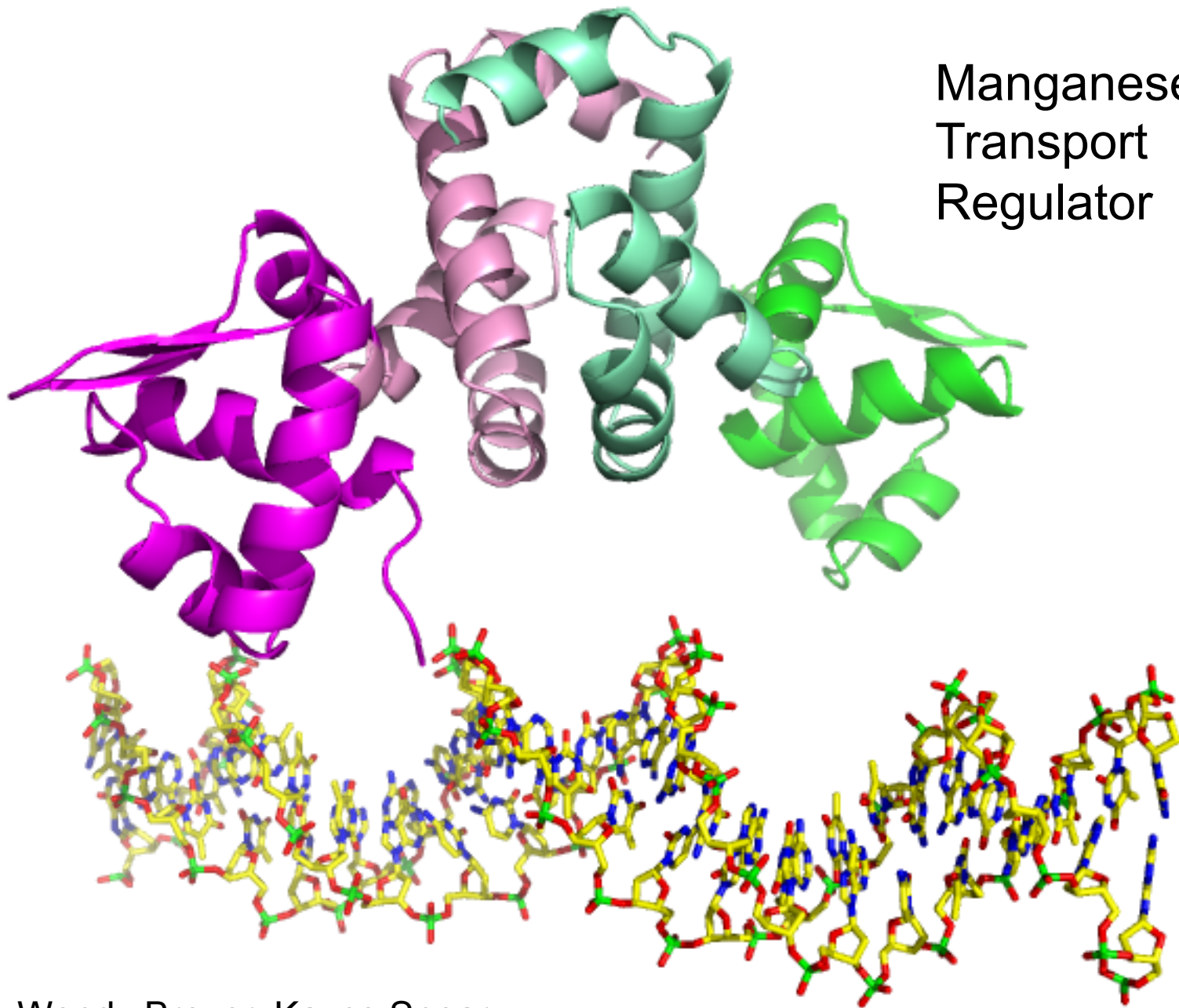
Mammalian Fatty Acid Synthase

Manganese
Transport
Regulator



Thanks Wendy Breyer, Kayce Spear

Manganese
Transport
Regulator



Thanks Wendy Breyer, Kayce Spear

Tobacco Mosaic Virus

