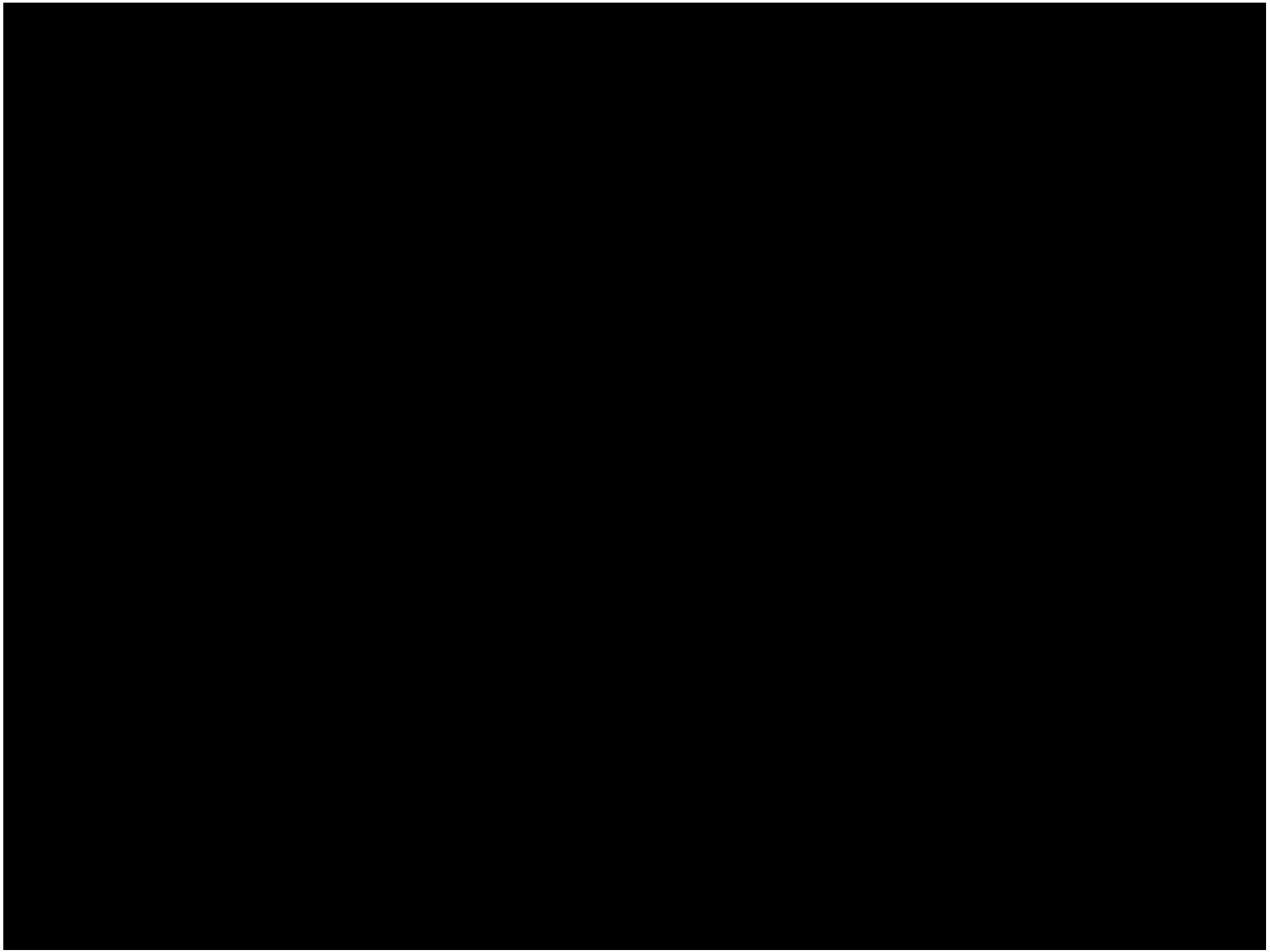


"Biological systems are necessarily metastable. They are created, modulated, and destroyed according to a temporal plan that meets the survival needs of the cell, organism, and species...Clearly, no biological system is close to true equilibrium or it would be dead...

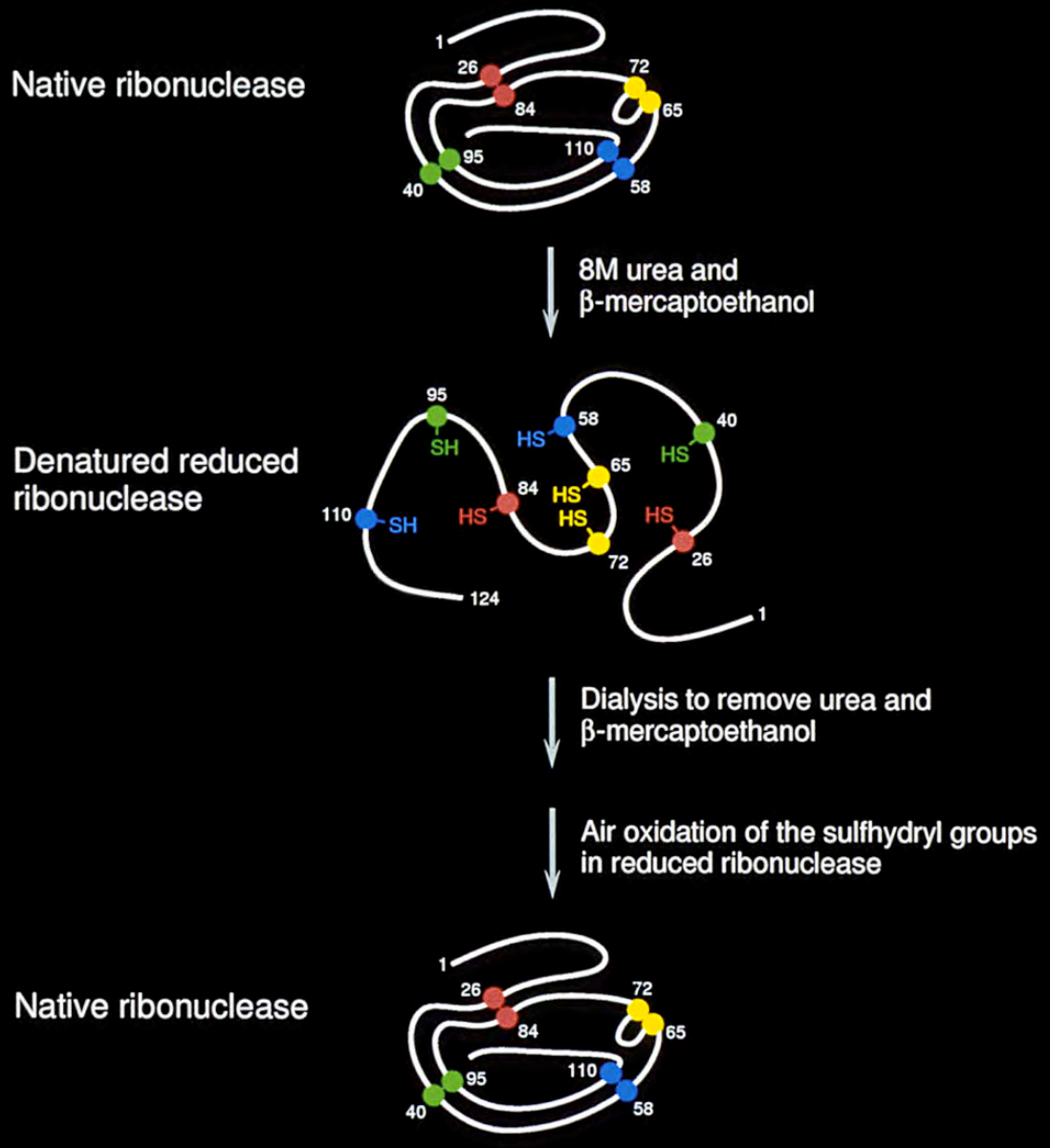
Systems survive by consuming free energy and regulating rates…a human turns over 40 kg of ATP daily, and 90% of the information in a genome encodes biological catalysts…

The more quickly something is done, the more difficult it is to do it accurately. Accuracy and specificity often are sacrificed in the name of speed…This balance is especially important in optimizing the rate of information transfer from the gene to the structures of functional nucleic acids and proteins.

The entropic activation barrier to prompt and accurate genetic expression…is overcome by the lavish use of nucleotide triphosphate to proofread and edit…"

Paul Sigler et al, 1998.

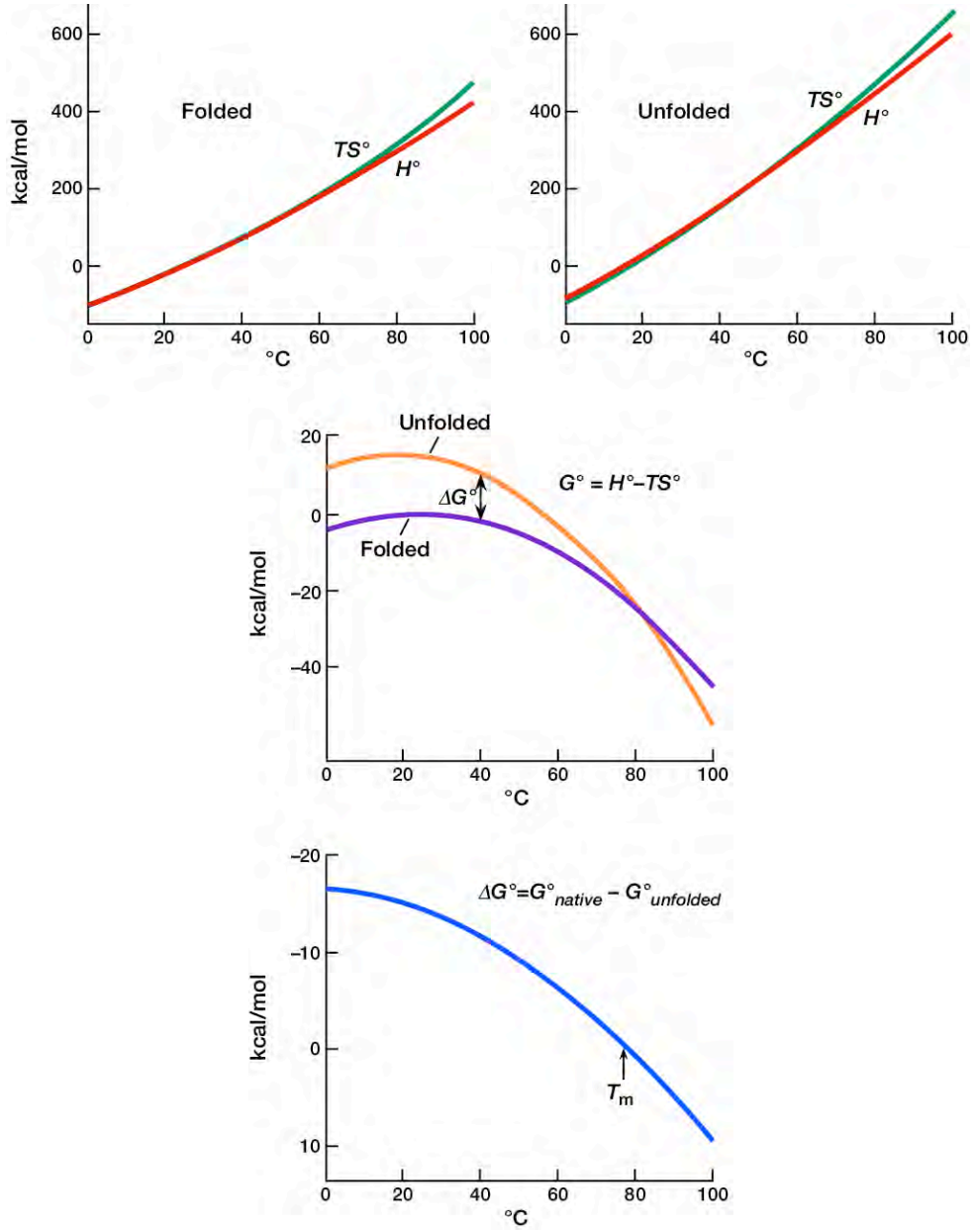
The amino acid sequence of a protein contains all of the information for folding into the unique native state, presumed to lie at the energetic minimum



Anfinsen et al, 1961

Thermodynamic Measurements

Enthalpic (H) and entropic (TS) contributions to the free energies of the folded and unfolded states of the enzyme lysozyme as a function of temperature—Large changes over T but net stability is only a few kcal/mole



Pfeil and Privalov, 1976,
adapted from Creighton text, 1997

Major Forces Contributing to the Conformational Stability of RNase T1

Stabilizing

Hydrogen bonding (104 HB)	=	166 kcal/mole
Hydrophobic groups buried (entropy of dehydration)	=	94

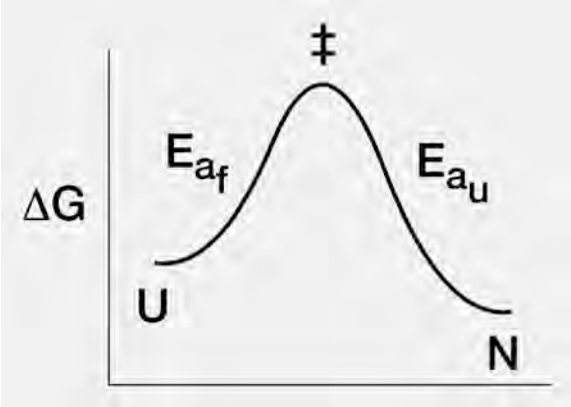
Destabilizing

Conformational entropy	=	177 kcal/mole
Peptide groups buried	=	81

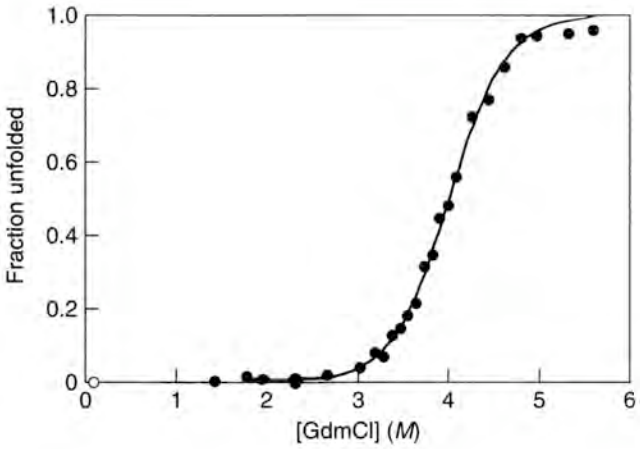
Adapted from Pace *et al.,* 1996

Behavior of Two-state Proteins, Typically Fewer Than 100 Amino Acids

$$U \underset{k_u}{\overset{k_f}{\rightleftharpoons}} N$$



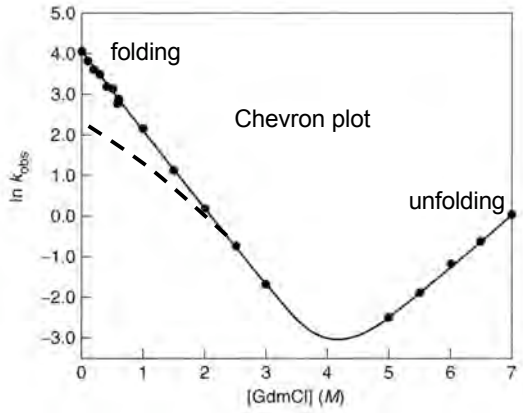
Equilibrium studies



Cooperative transition

$\Delta G_{(U \rightarrow N)} = -RT\ln K_{eq}$

Kinetic studies



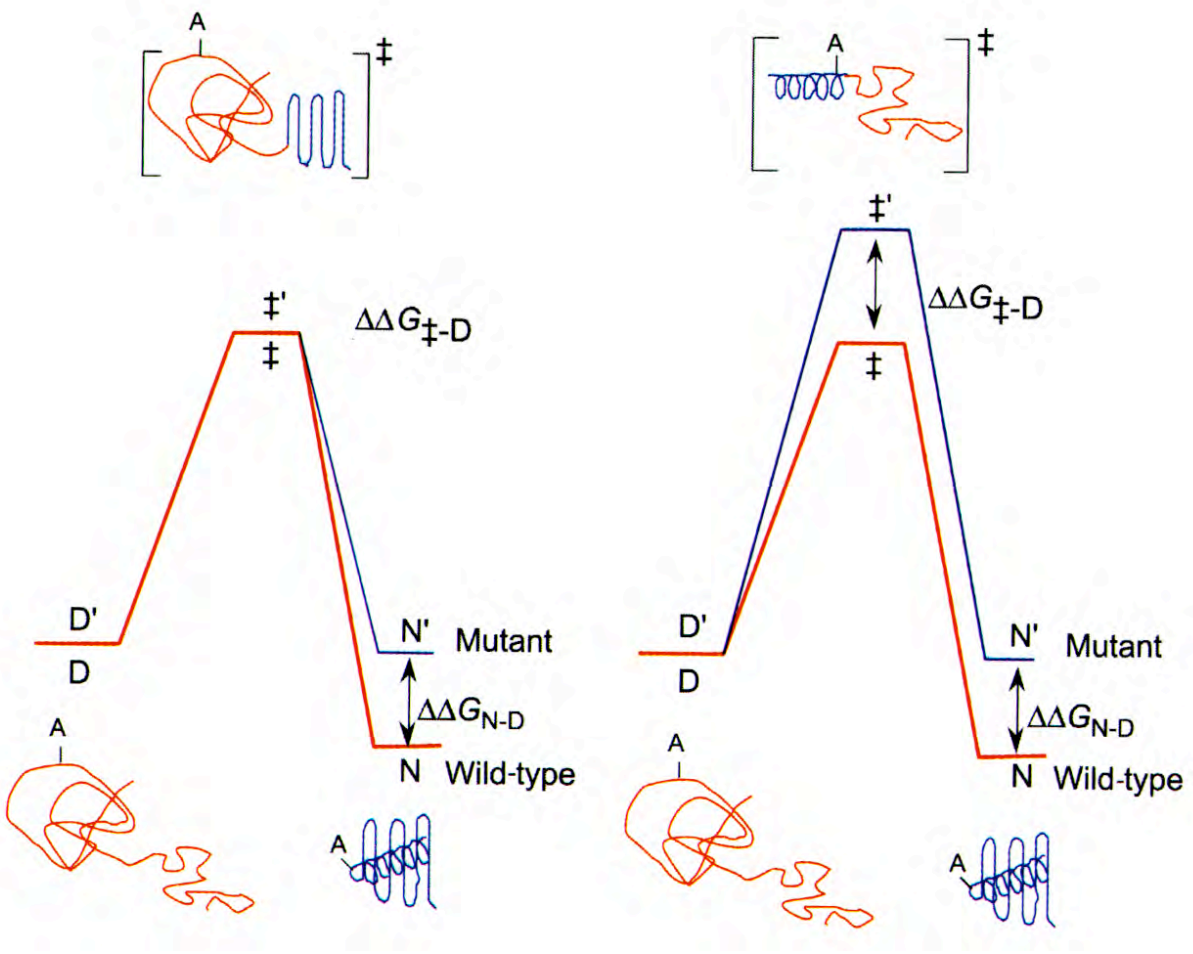
$$\ln k_{obs} = \ln((k_f^{H_2O})\exp(-m_{k_f}[\text{denaturant}]) + k_u^{H_2O}\exp(m_{k_u}[\text{denaturant}]))$$

E_a RT ln k (but non-Arrhenius behavior)

Deviation from "V" indicates formation of an intermediate or multimolecular aggregation

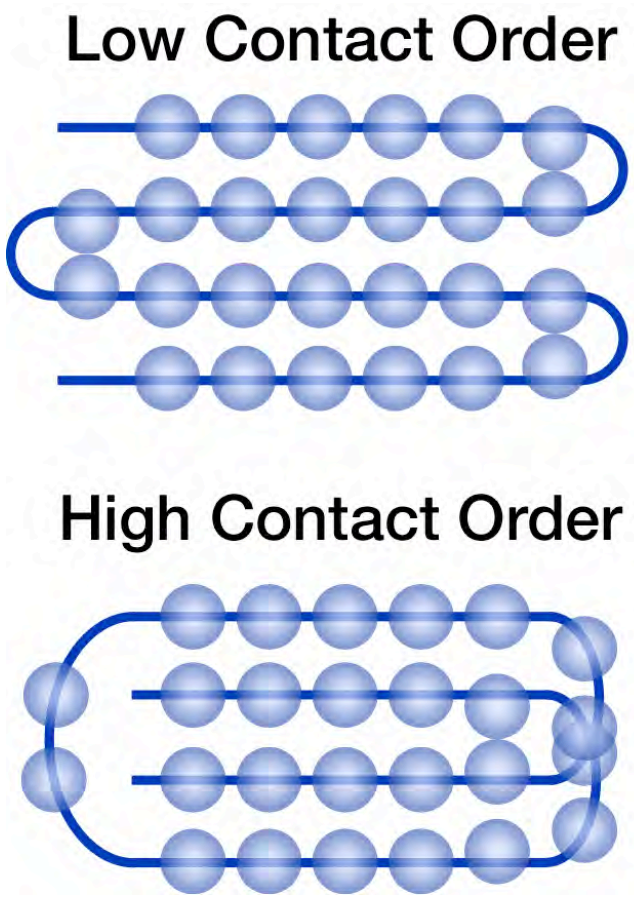
Adapted from Fersht text, 1999

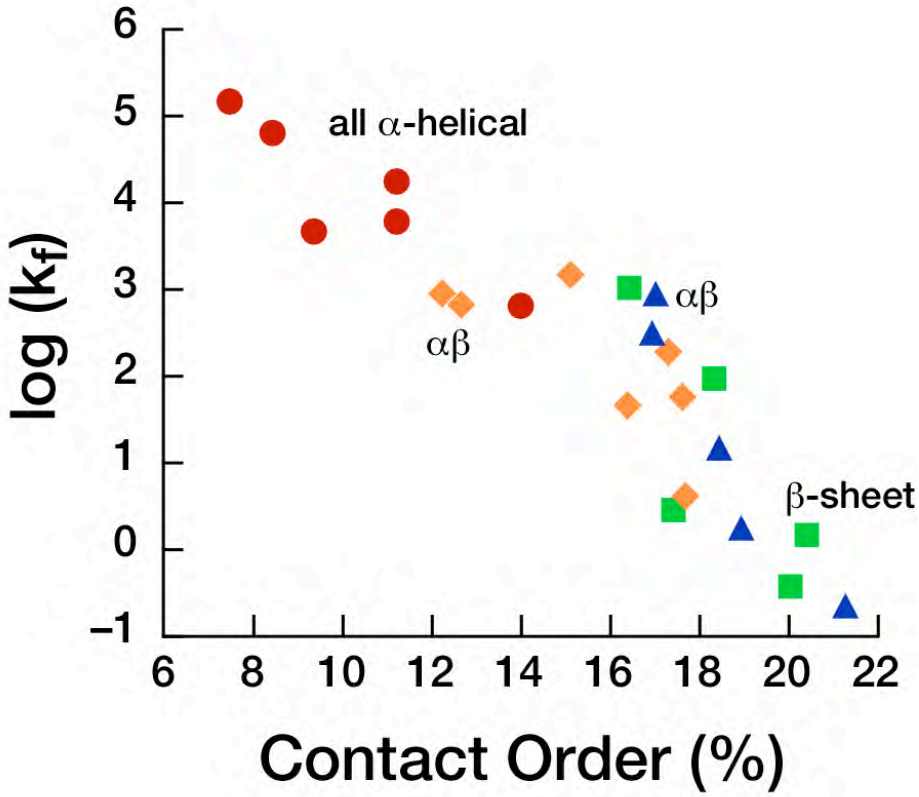
Deriving structure of transition state of folding for 2-state proteins by mutational analysis by comparing rates of folding and unfolding of wt and mutant



from Fersht and Daggett, 2002

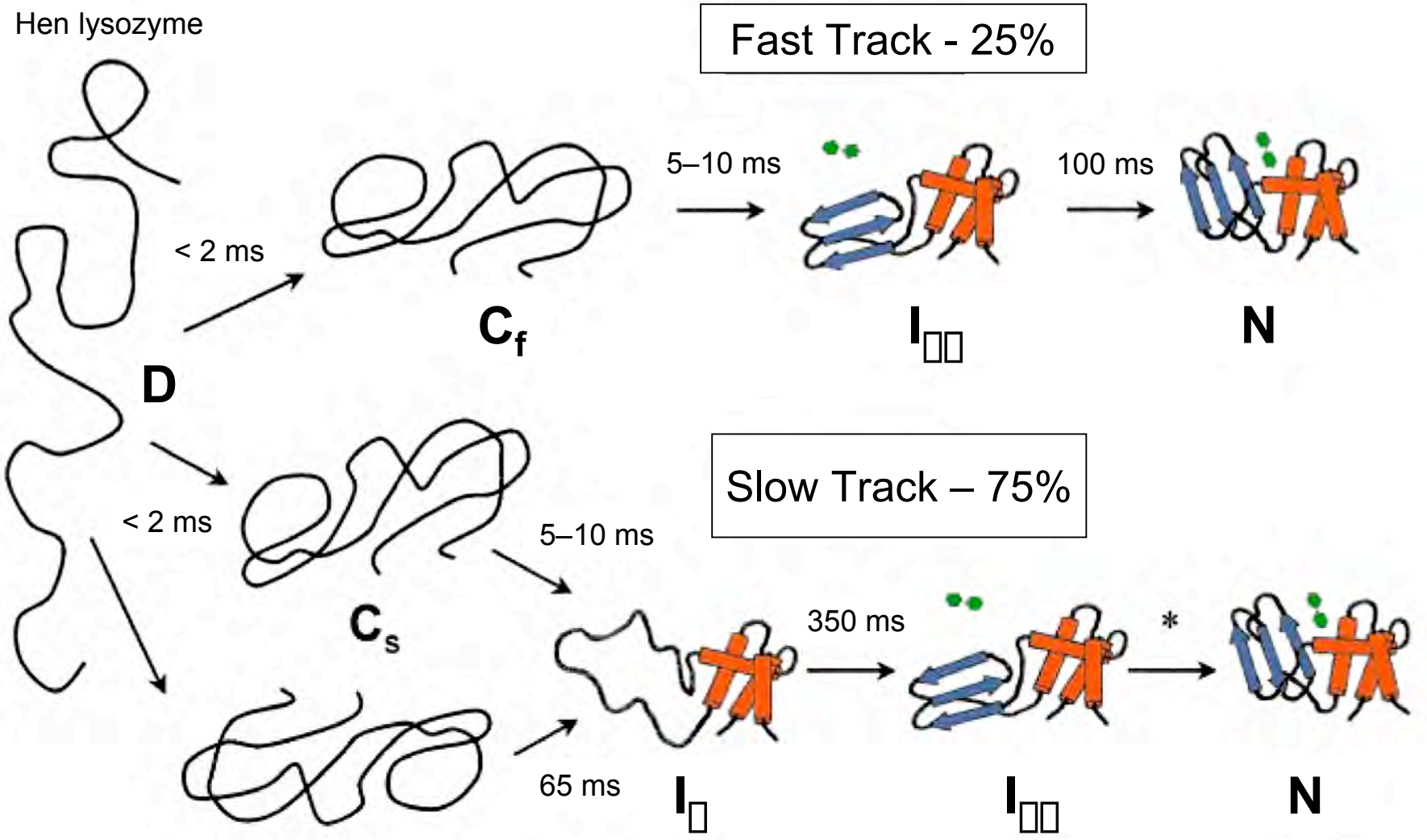
Native topology plays a key role in determining rate of folding of two-state proteins, reflecting that there is topology “searching”





From D. Baker, 2000

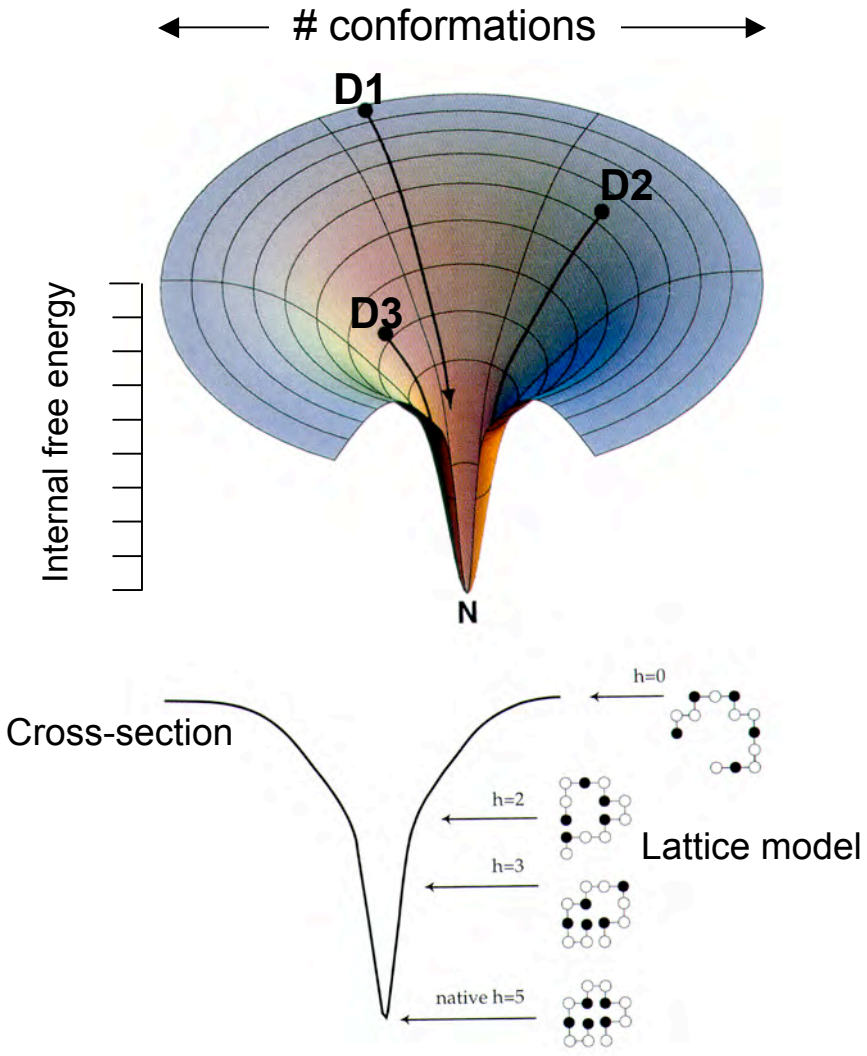
Larger proteins fold in a more complex manner, involving intermediates and multiple routes



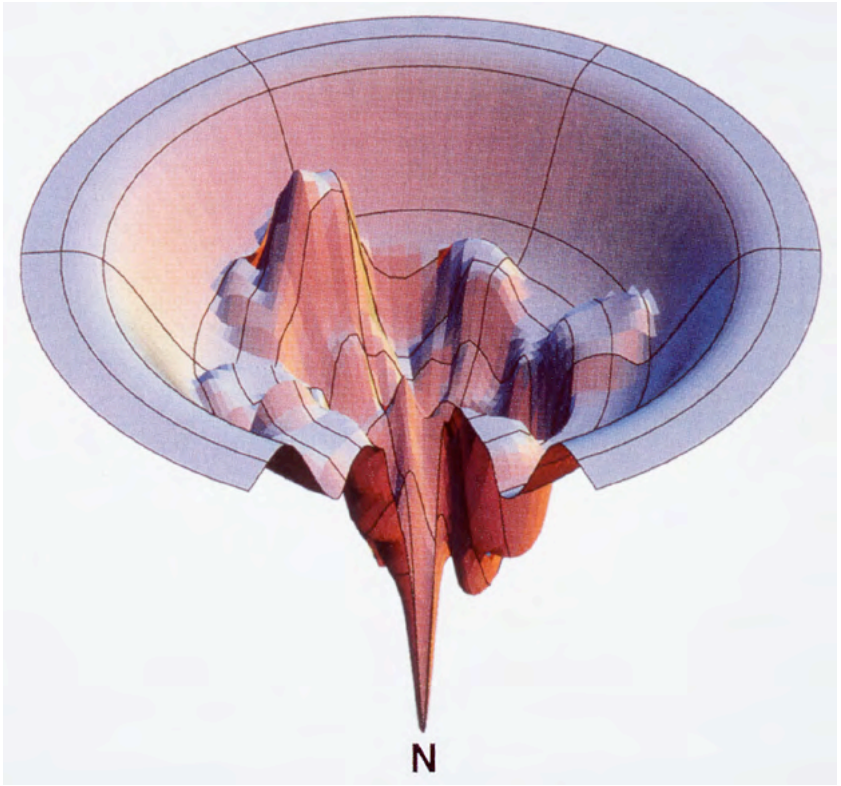
From Matagne, Dobson, & coworkers, 2000

Energy “landscapes” connect single-polypeptide microscopics with experimental macroscopics

Two-state Landscape

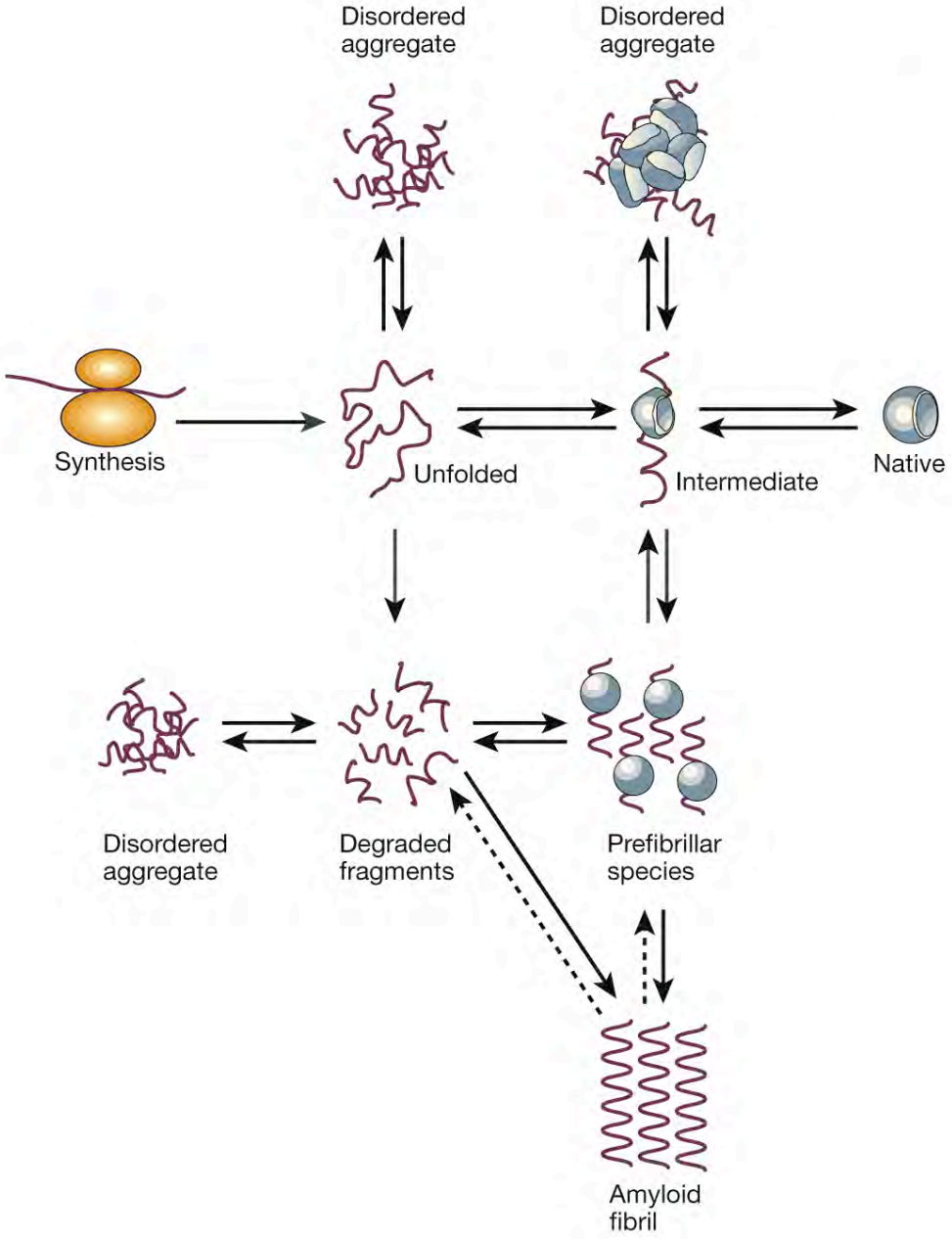


Rugged landscape replete with kinetic “traps”



Dill & Chan, 1997

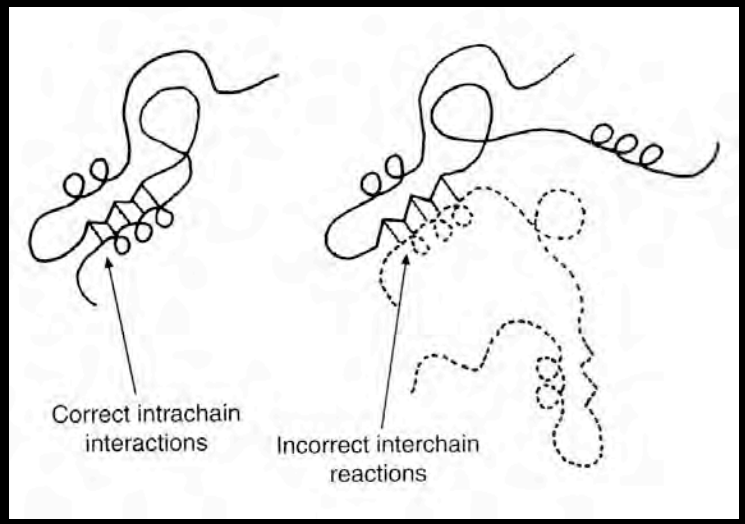
Fate of misfolded polypeptide chains in the cell – multimolecular "disordered" aggregates and, in ~20 cases, "ordered" amyloid fibrils



Adapted from Dobson, 2003

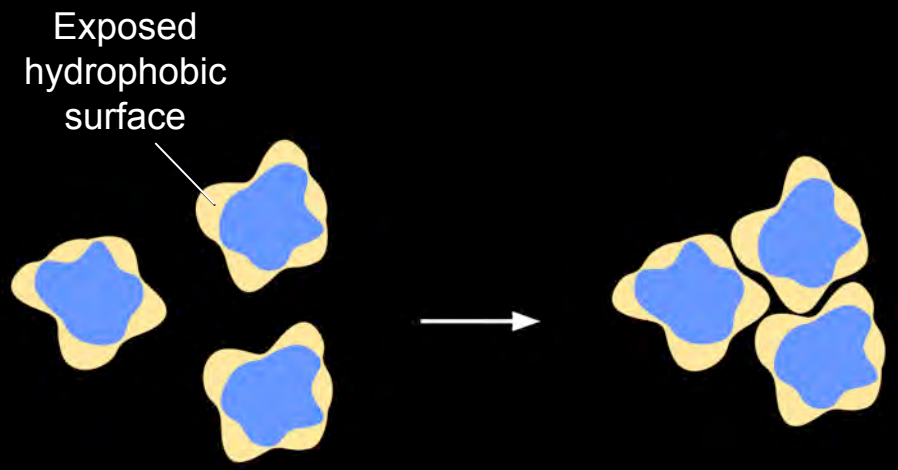
Mechanisms of Protein Aggregation

Domain Swapping



M.E.Goldberg and coworkers, 1974

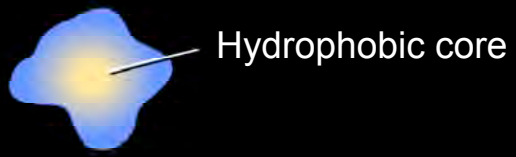
Hydrophobic Interactions



NON-NATIVE PROTEIN

AGGREGATE

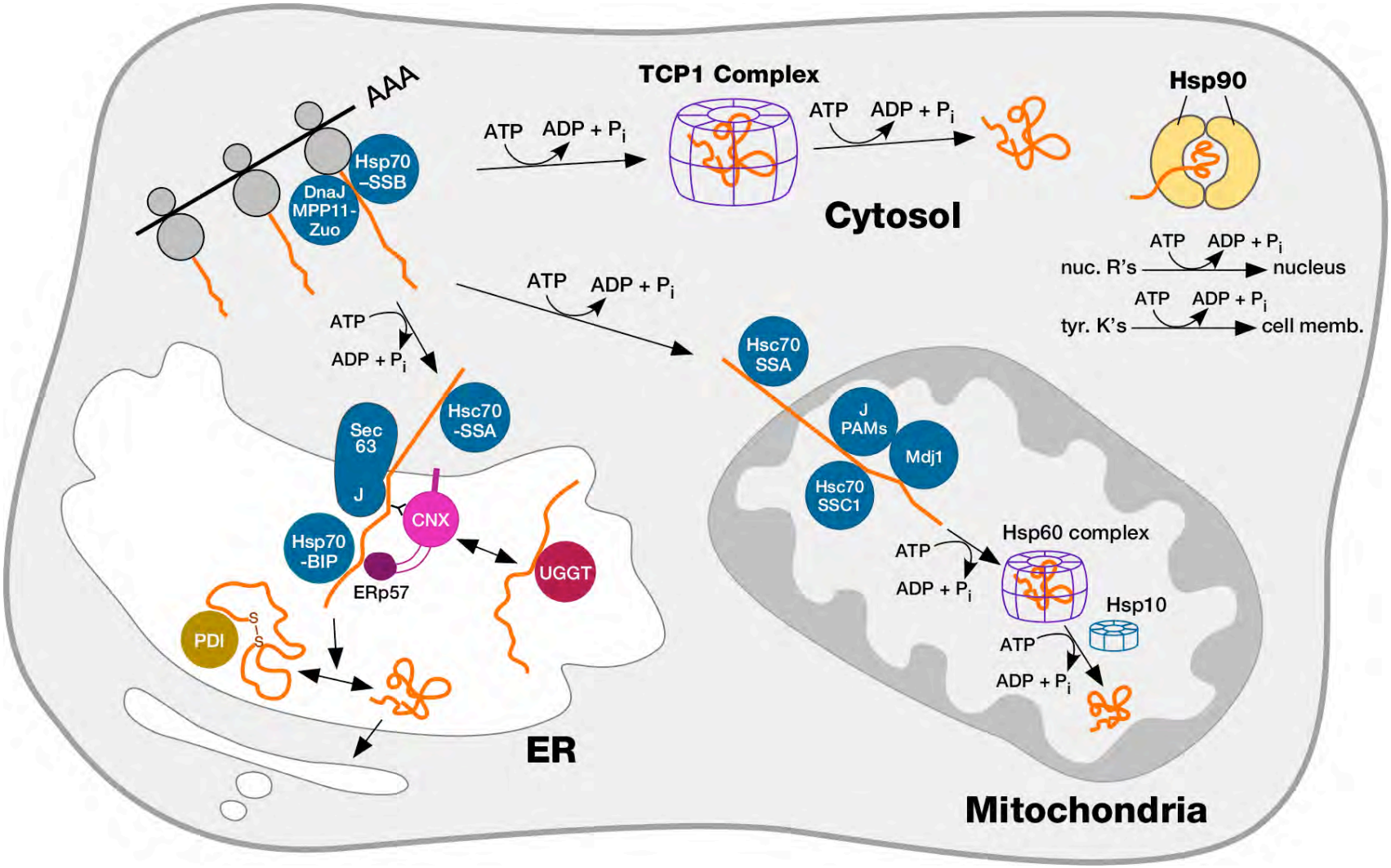
NATIVE PROTEIN

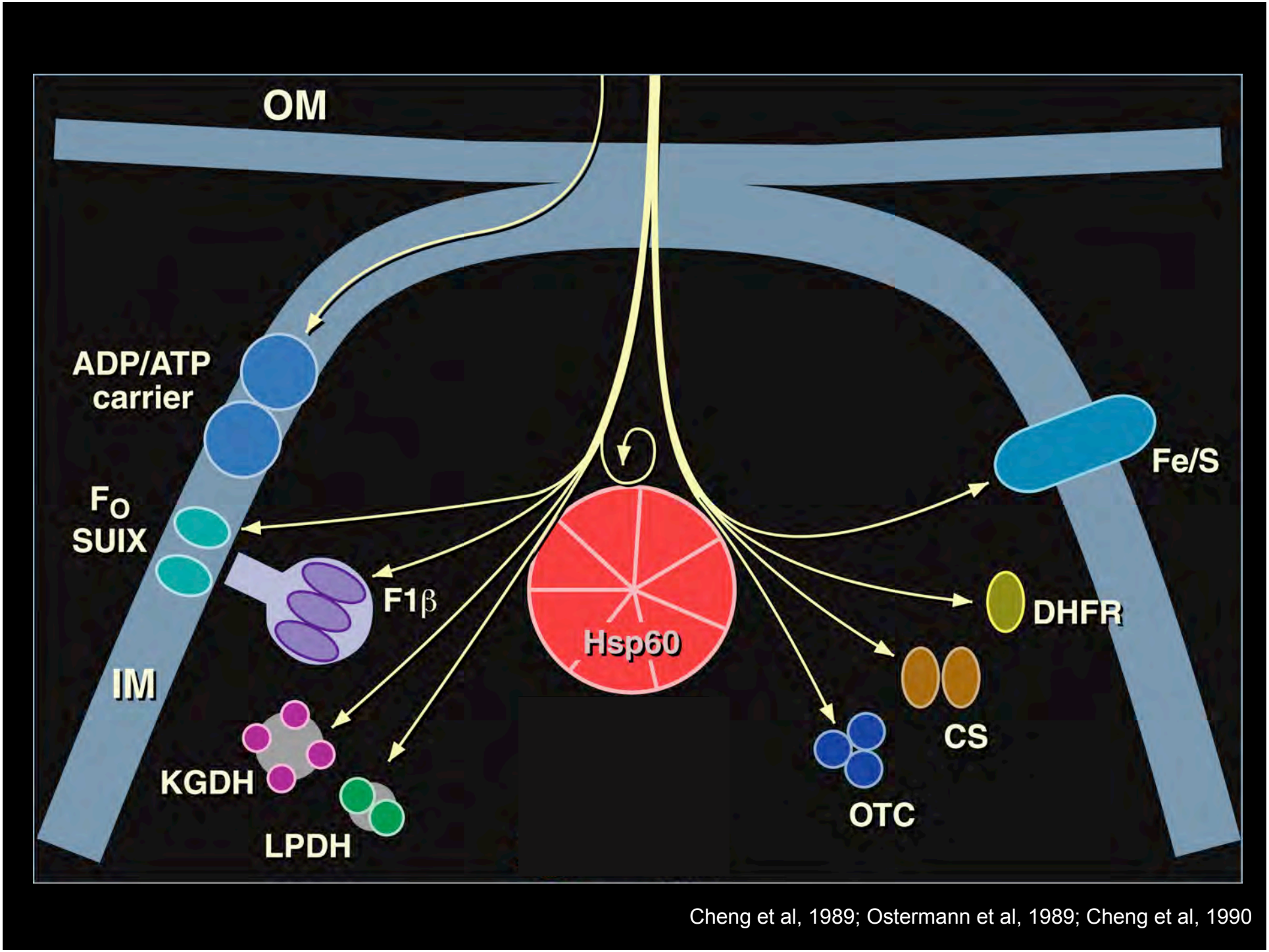


Lewis & Pelham, 1985

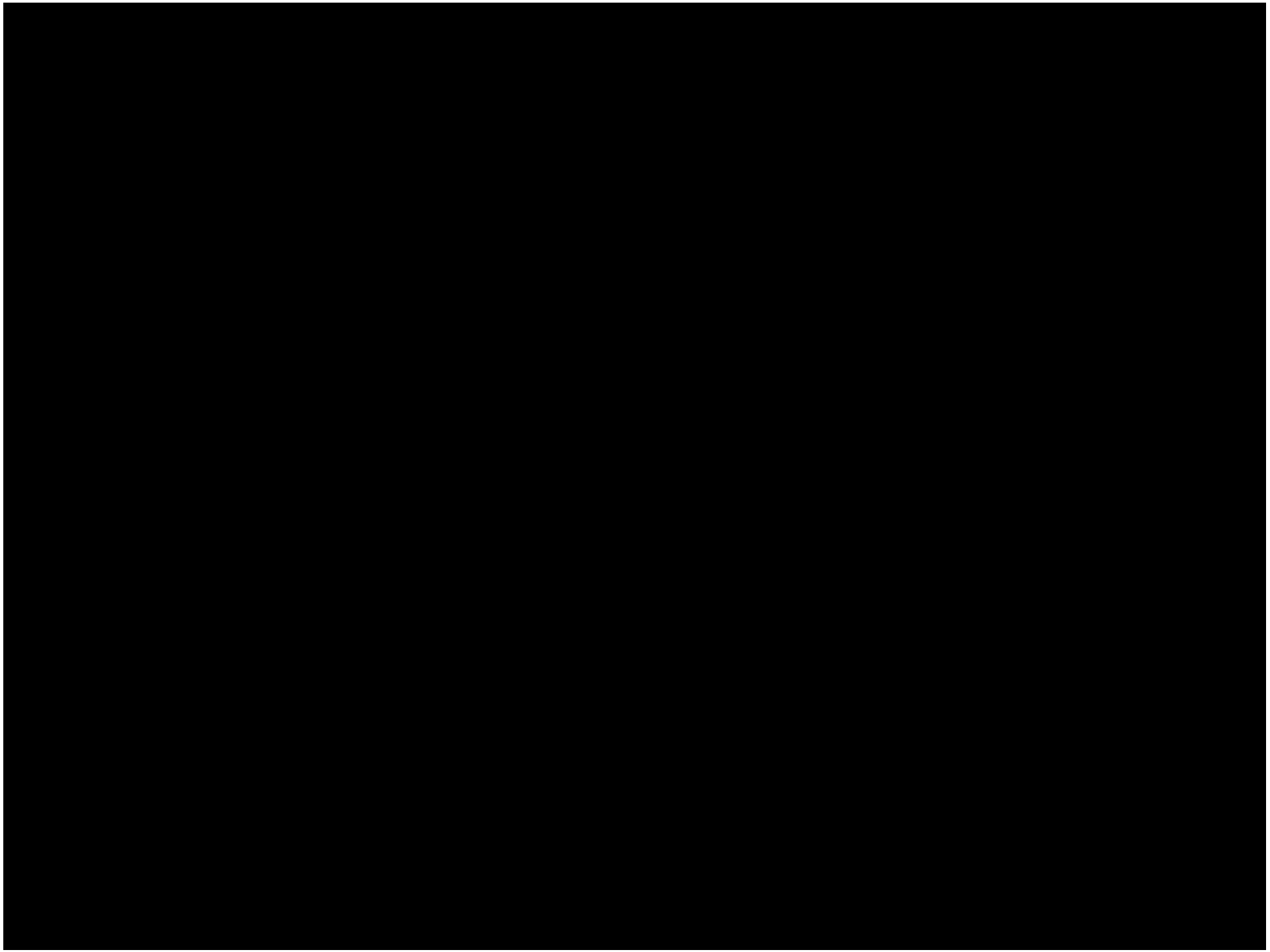
Specialized Proteins that Prevent Misfolding and Aggregation - Molecular Chaperones

Binding Exposed Hydrophobic Surfaces - Release Mediated by ATP

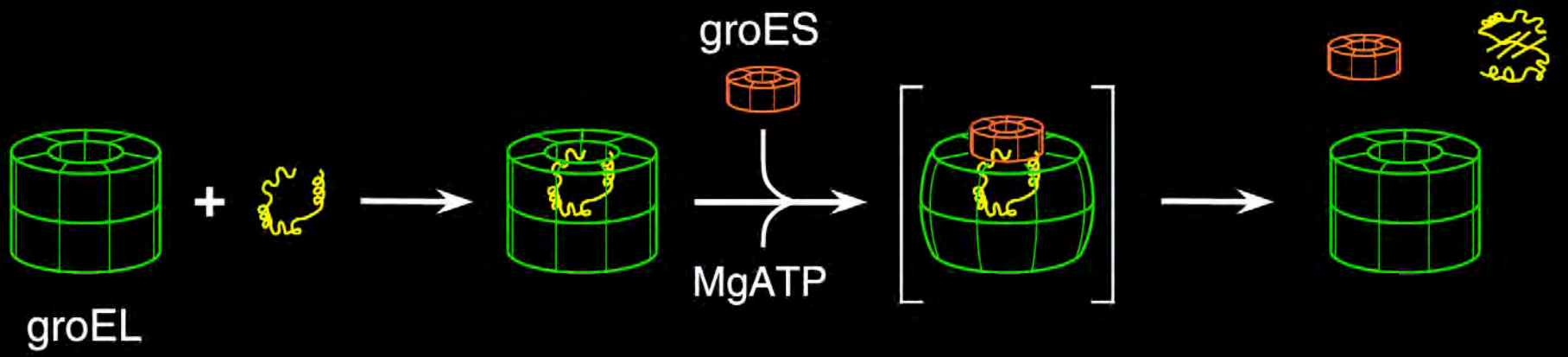




Cheng et al, 1989; Ostermann et al, 1989; Cheng et al, 1990



GroEL-GroES-mediated folding reaction

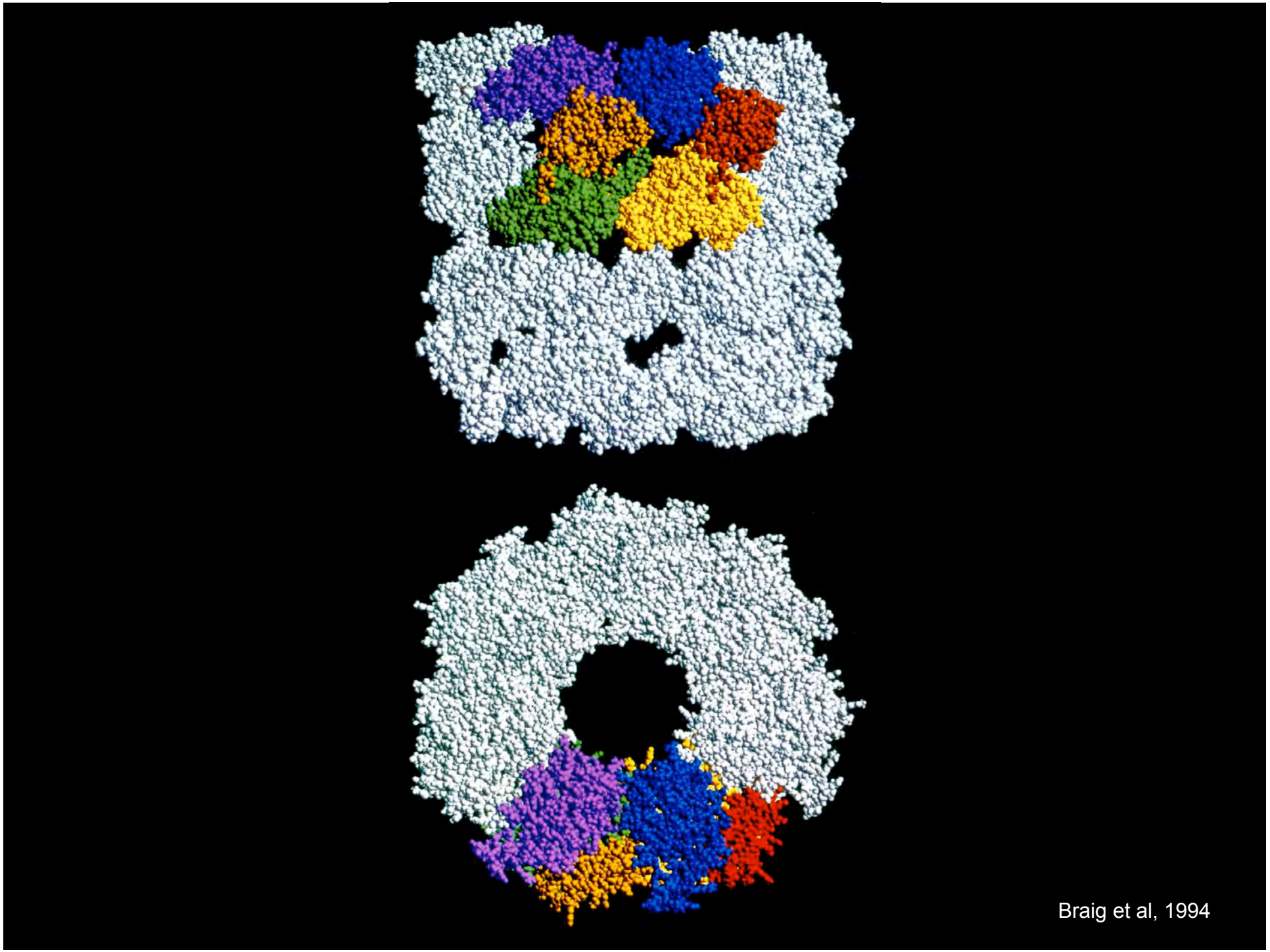


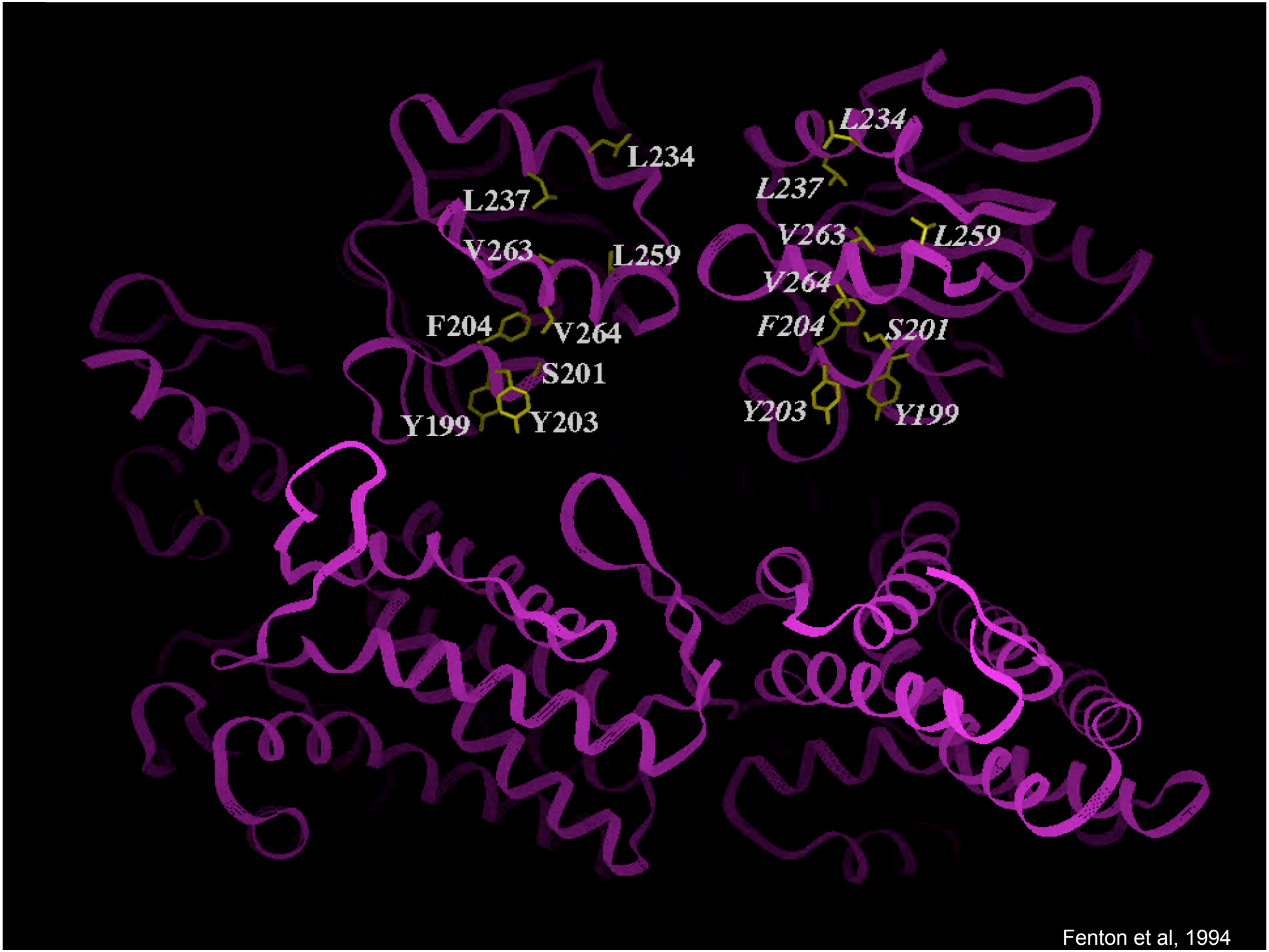
Goloubinoff et al, 1989
Martin et al, 1991

<u>NTP consumption for production of the enzyme rhodanese:</u>

Translation at the ribosome - 4 ~P per peptide bond x 297 aa = 1188 ~P consumed

GroEL/GroES-mediated folding – ~130 ATP hydrolyzed/rhodanese molecule folded =
~10% amount employed for synthesis;
implies multiple turnovers of 7-subunit rings, and amount energy consumed is ~100X $\Delta G_{U\text{-}N}$

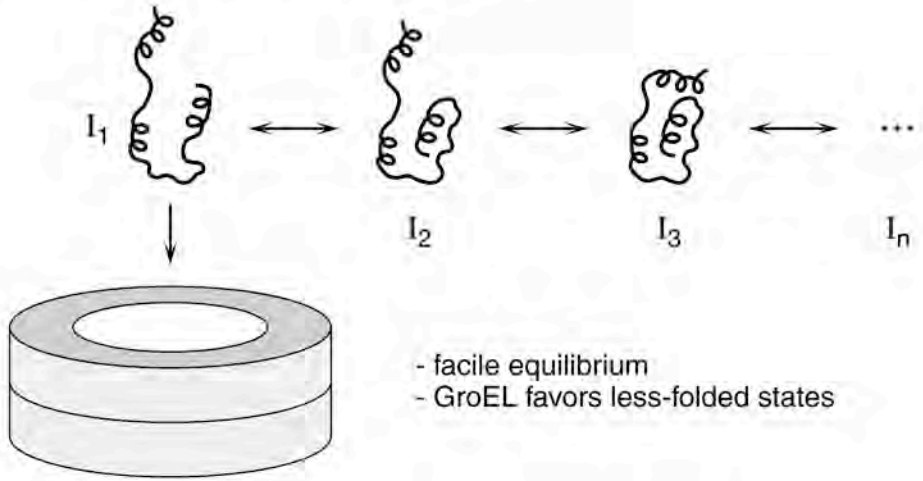

Braig et al, 1994


L234
L237
V263
L259
F204
V264
S201
Y199
Y203
L234
L237
V263
L259
V264
F204
S201
Y203
Y199
Fenton et al, 1994

POTENTIAL ACTIONS OF BINDING IN RESCUING MISFOLDED STATES

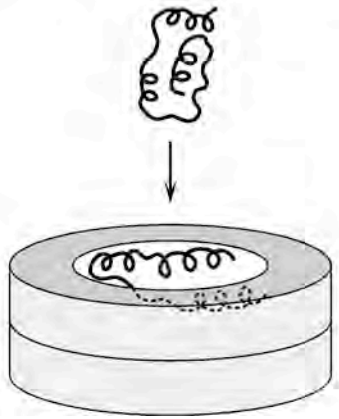
(PULLING NON-NATIVE PROTEINS UP THE ENERGY LANDSCAPE)

Thermodynamic Partitioning

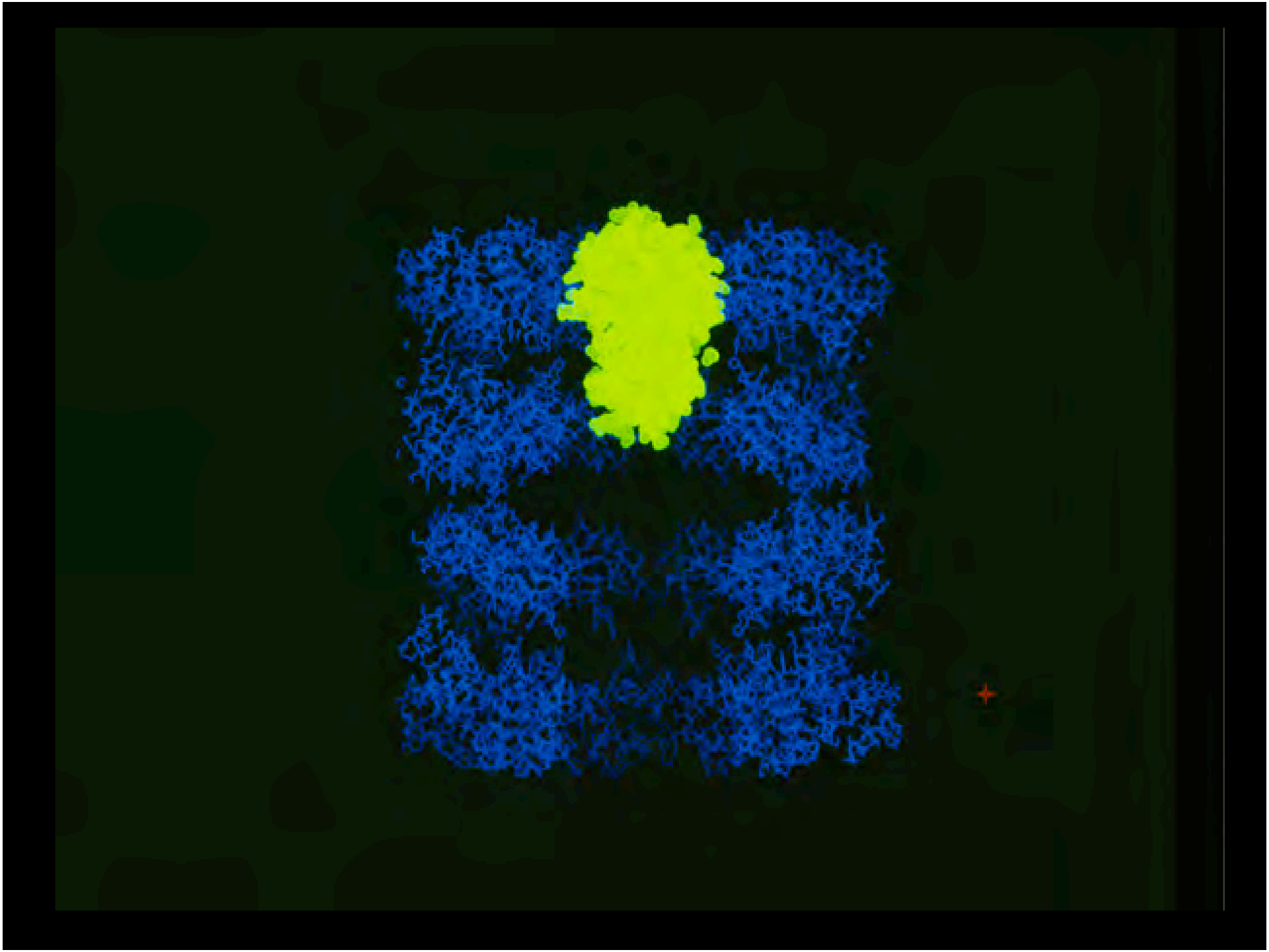


- facile equilibrium
- GroEL favors less-folded states

Catalyzed Unfolding



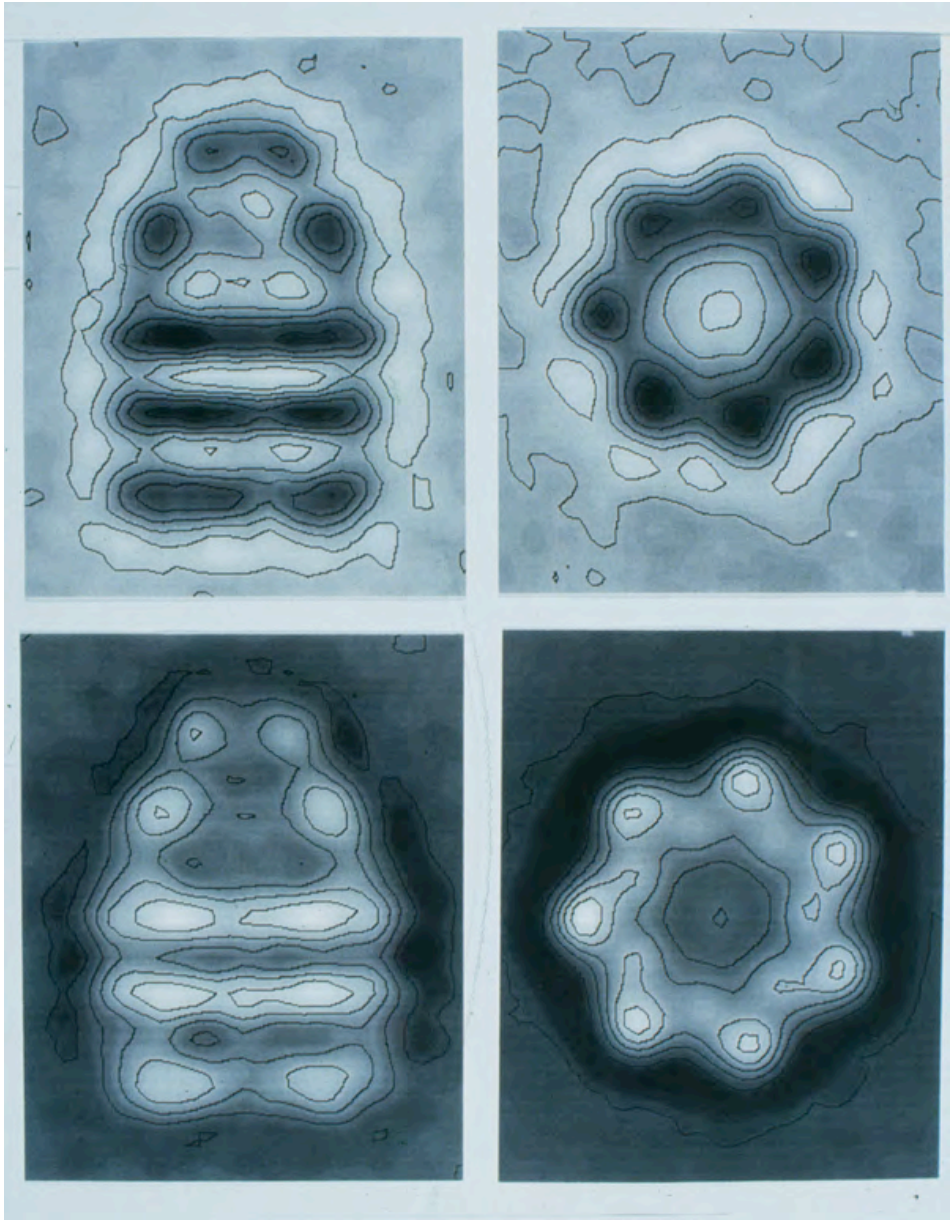
Zahn and Pluckthun, 1994; Walter et al, 1996
Zahn et al, 1996



side end

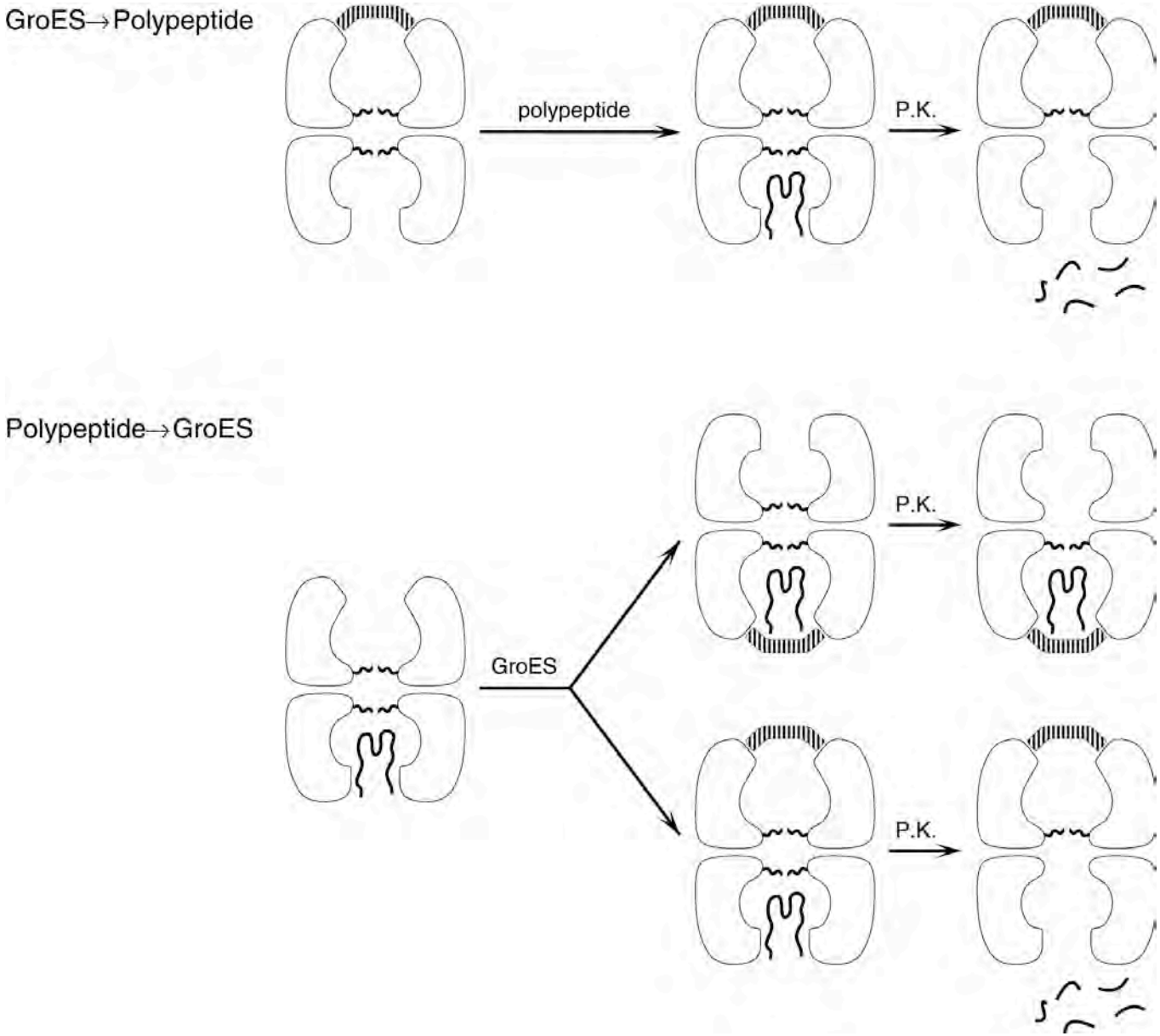
CryoEM

Negative stain

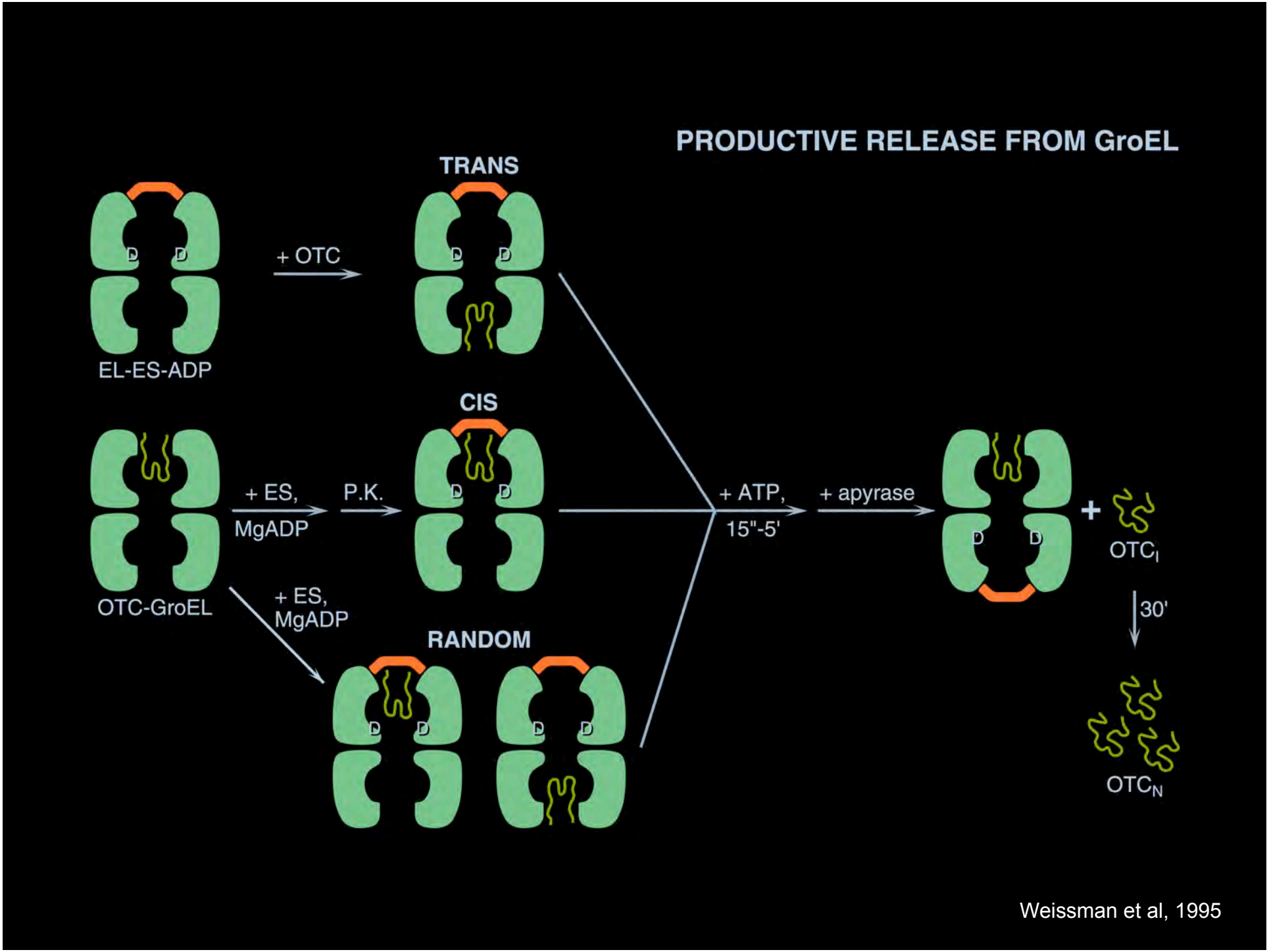


GroEL-GroES-ATP

Chen et al, 1994

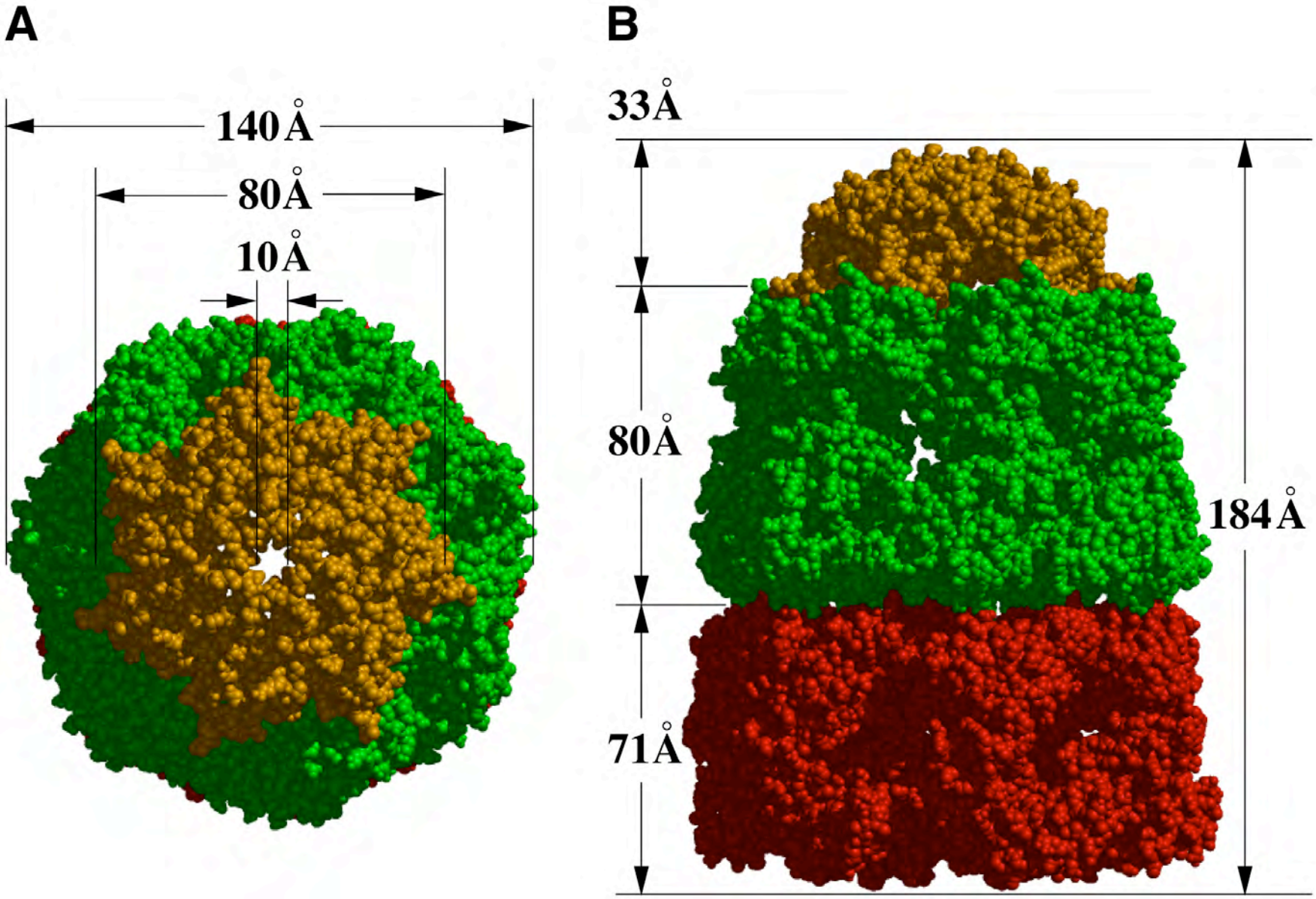


Weissman et al, 1995

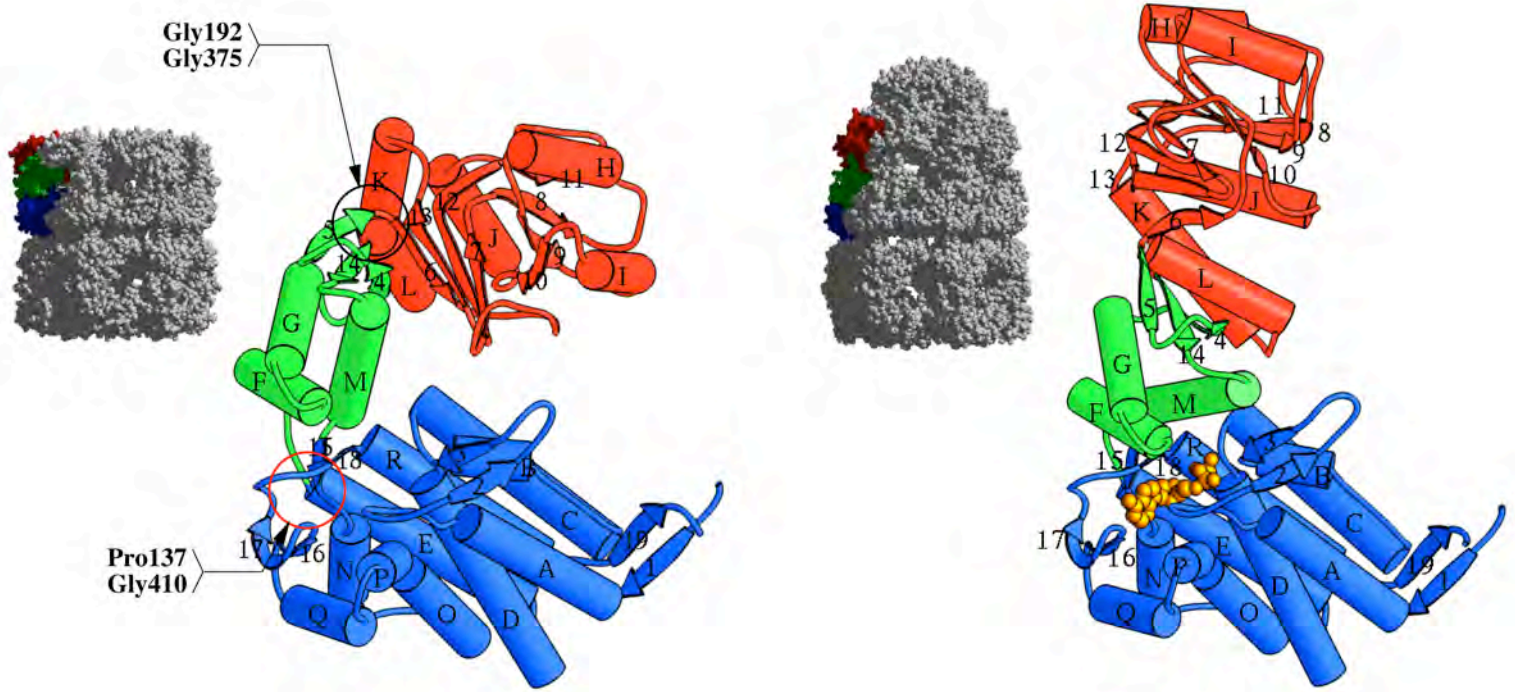


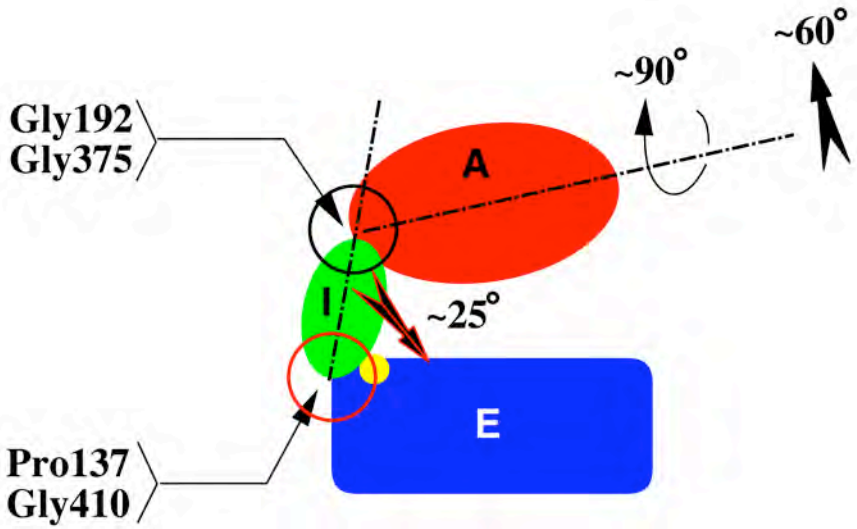
Weissman et al, 1995

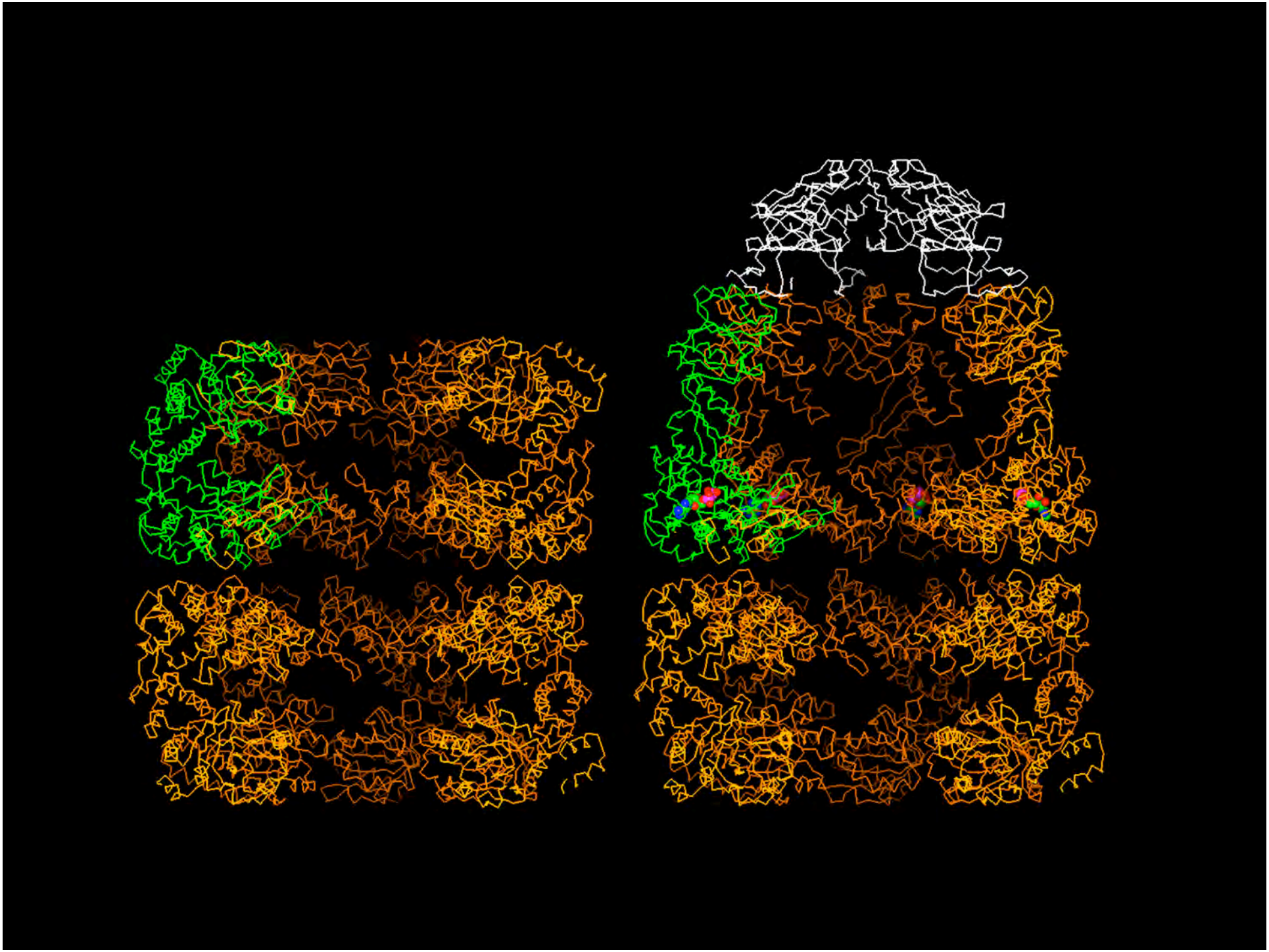
Weissman et al, 1996

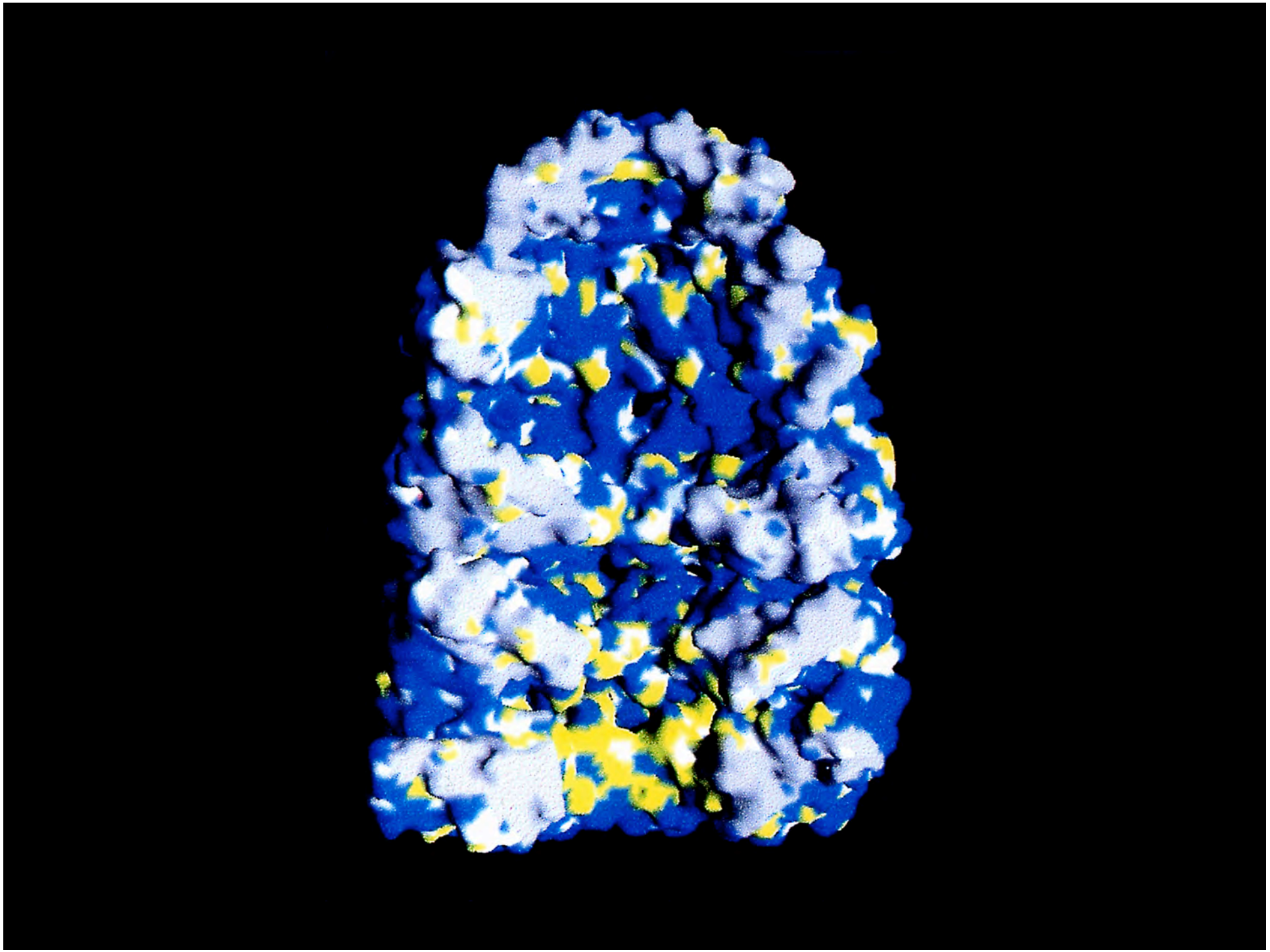


Xu et al, 1997


Gly192
Gly375
Pro137
Gly410

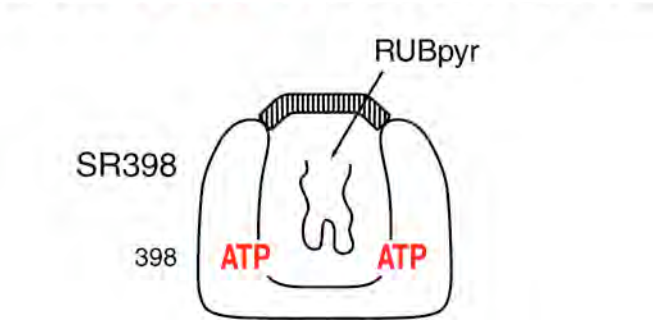

~60°
~90°
Gly192
Gly375
A
I
~25°
E
Pro137
Gly410





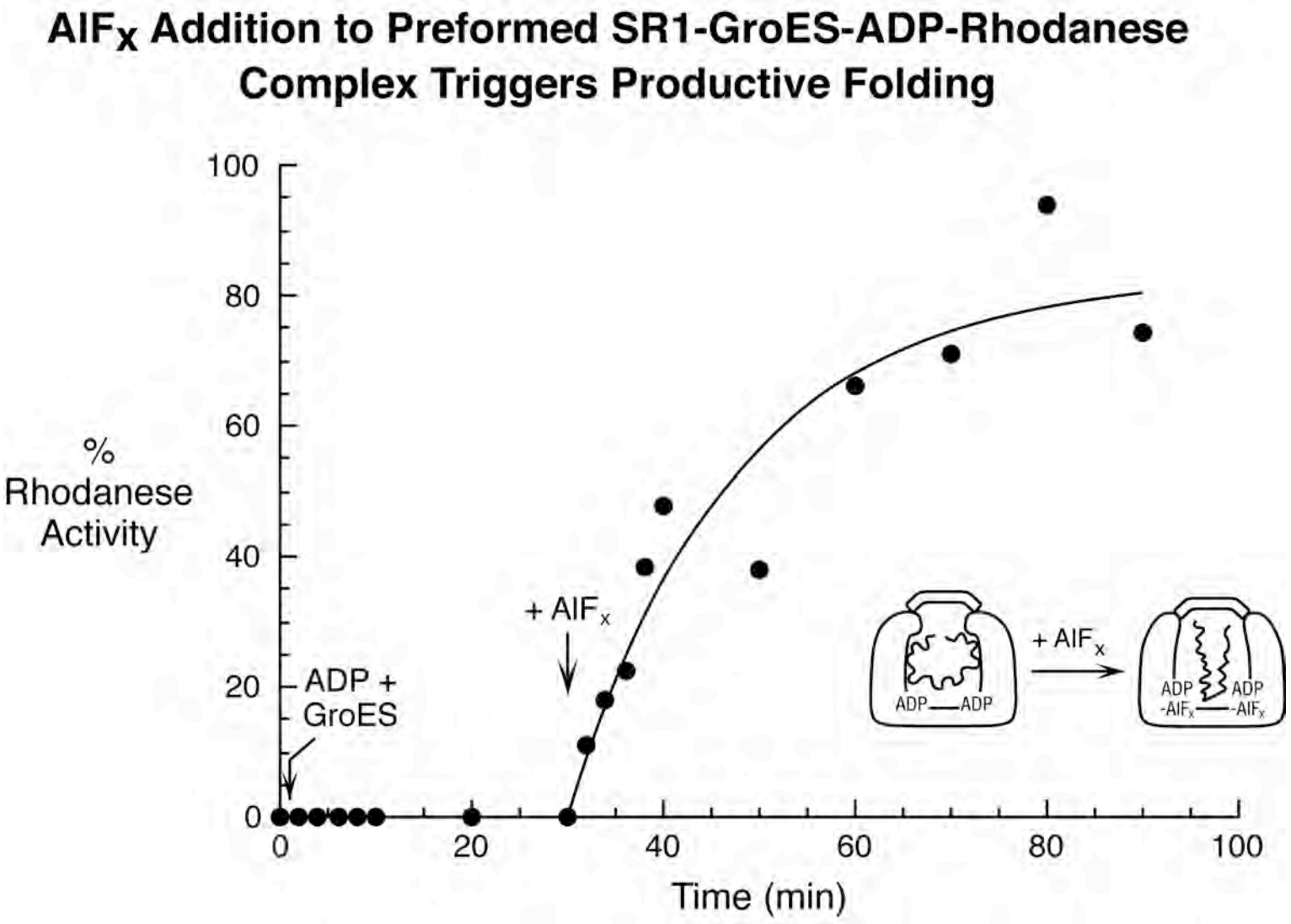
ATP binding, not hydrolysis, triggers productive folding

SR398-GroES-ATP is folding-active



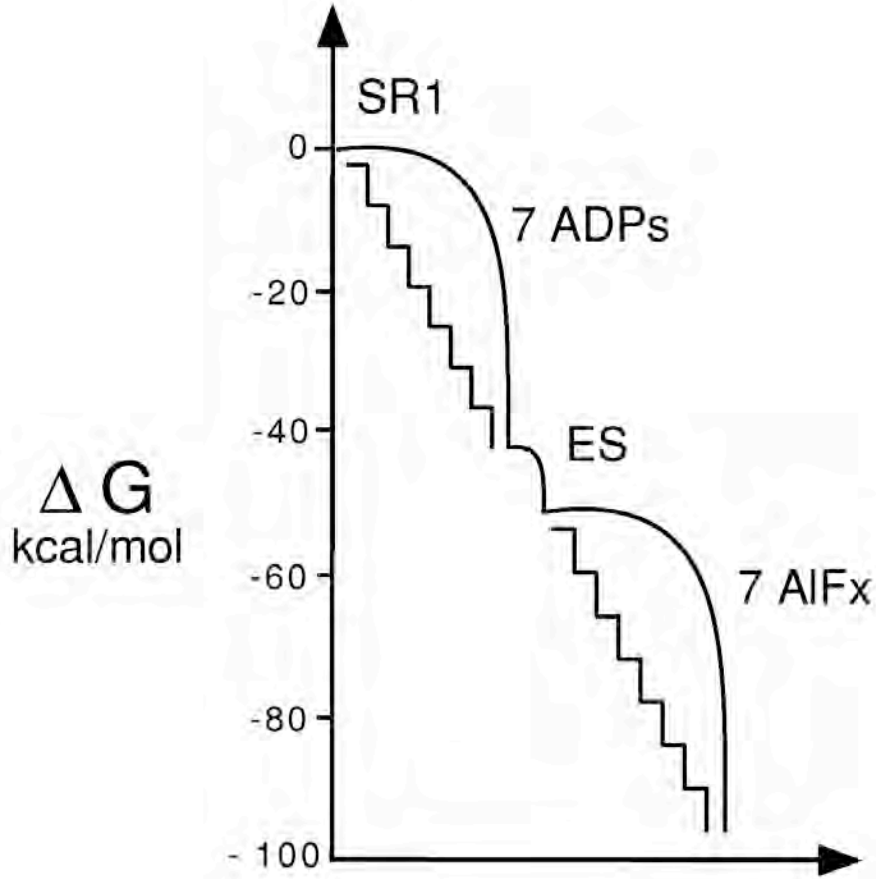
Rye et al, 1997

The γ-phosphate of ATP plays an essential role in triggering folding



Chaudhry et al, 2003

Estimated free energy transitions during activation of SR1



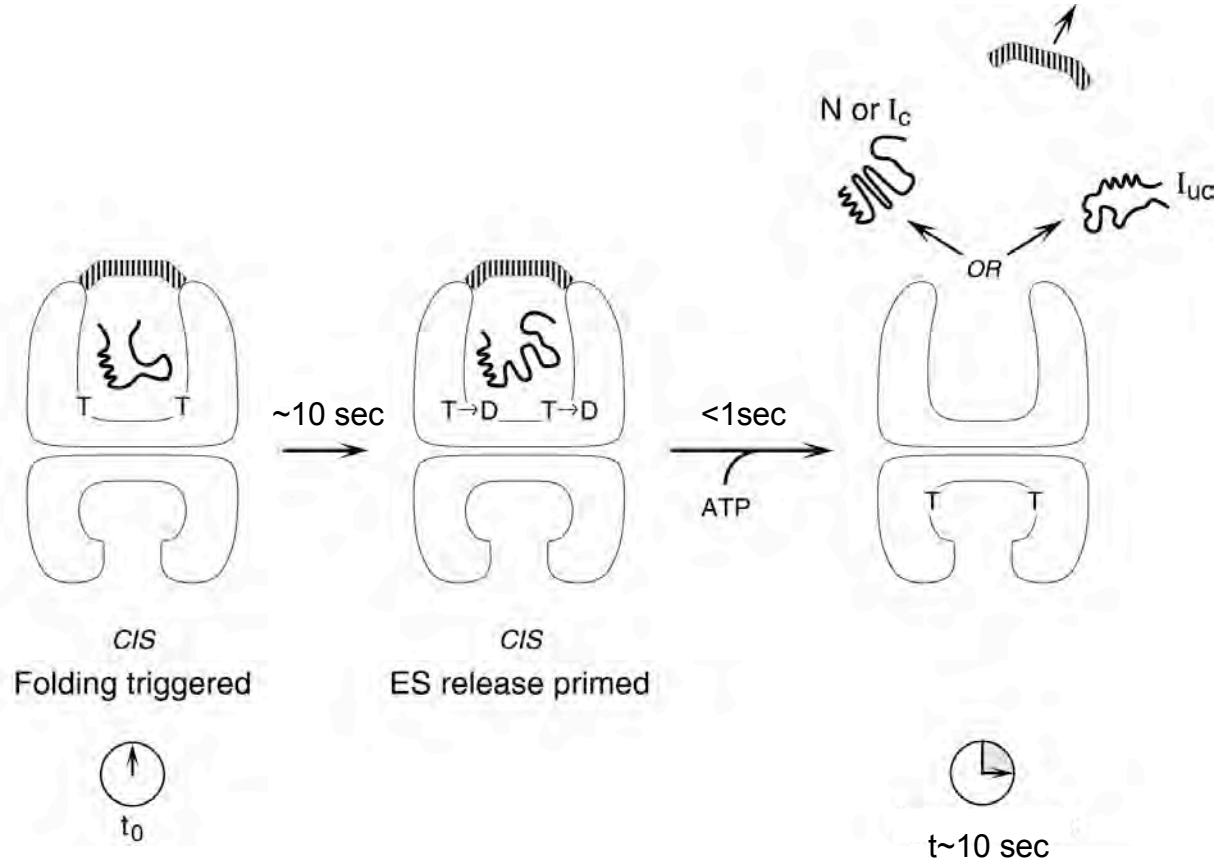
ADP binding K_D= 30 μM

GroES binding K_D= 0.45 μM

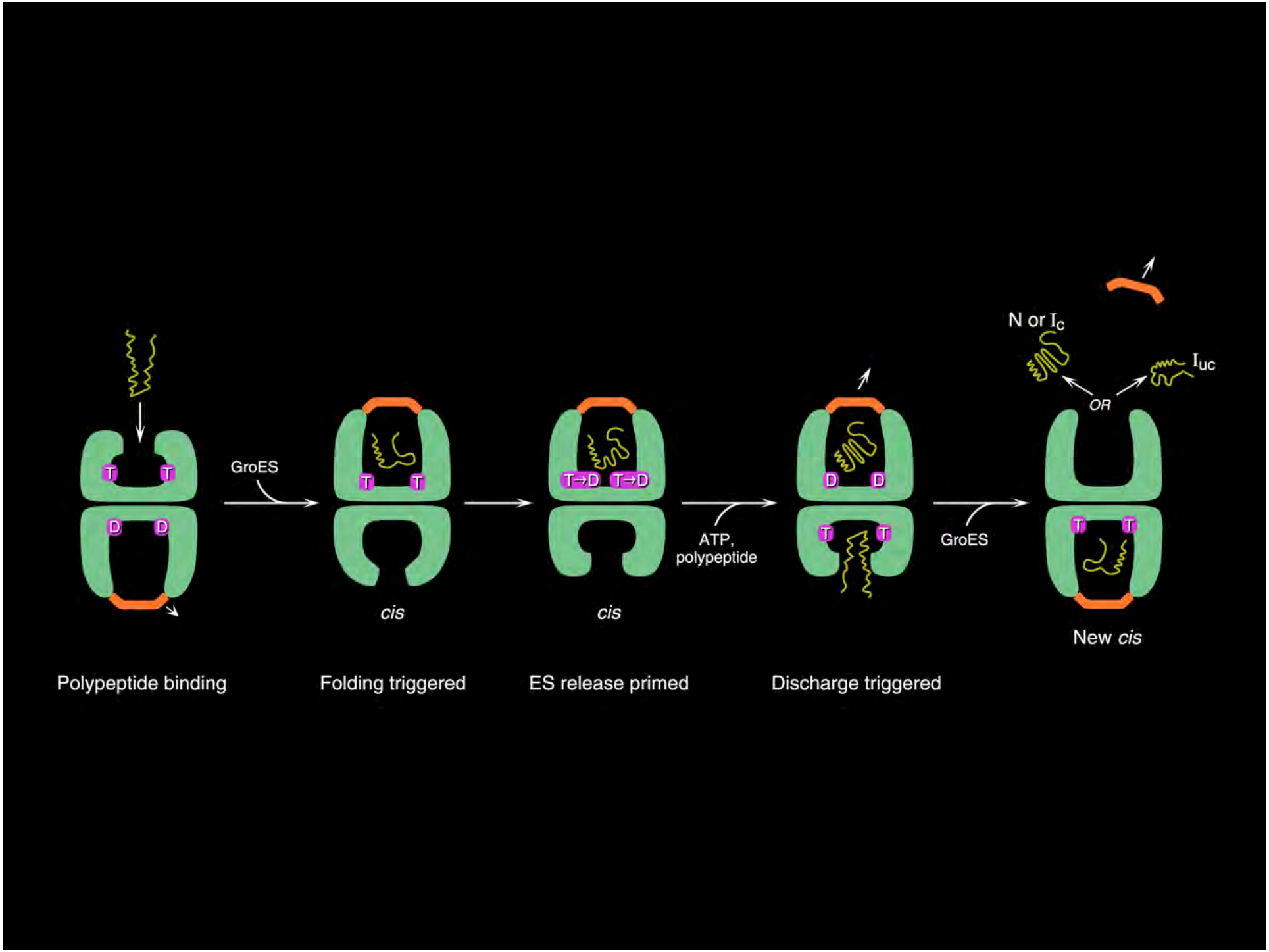
AlFx binding K_D= 16 μM

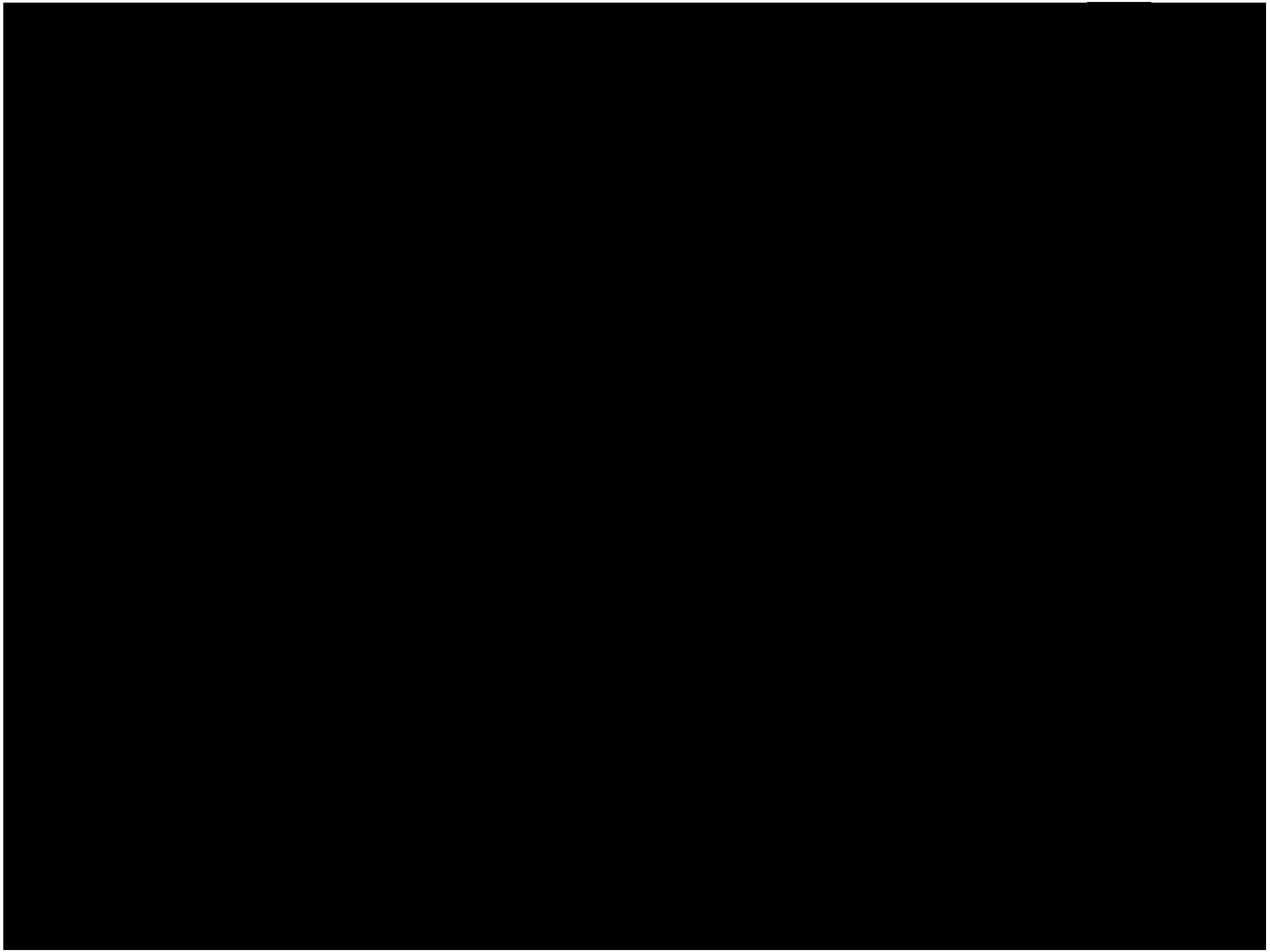
Chaudhry et al, 03

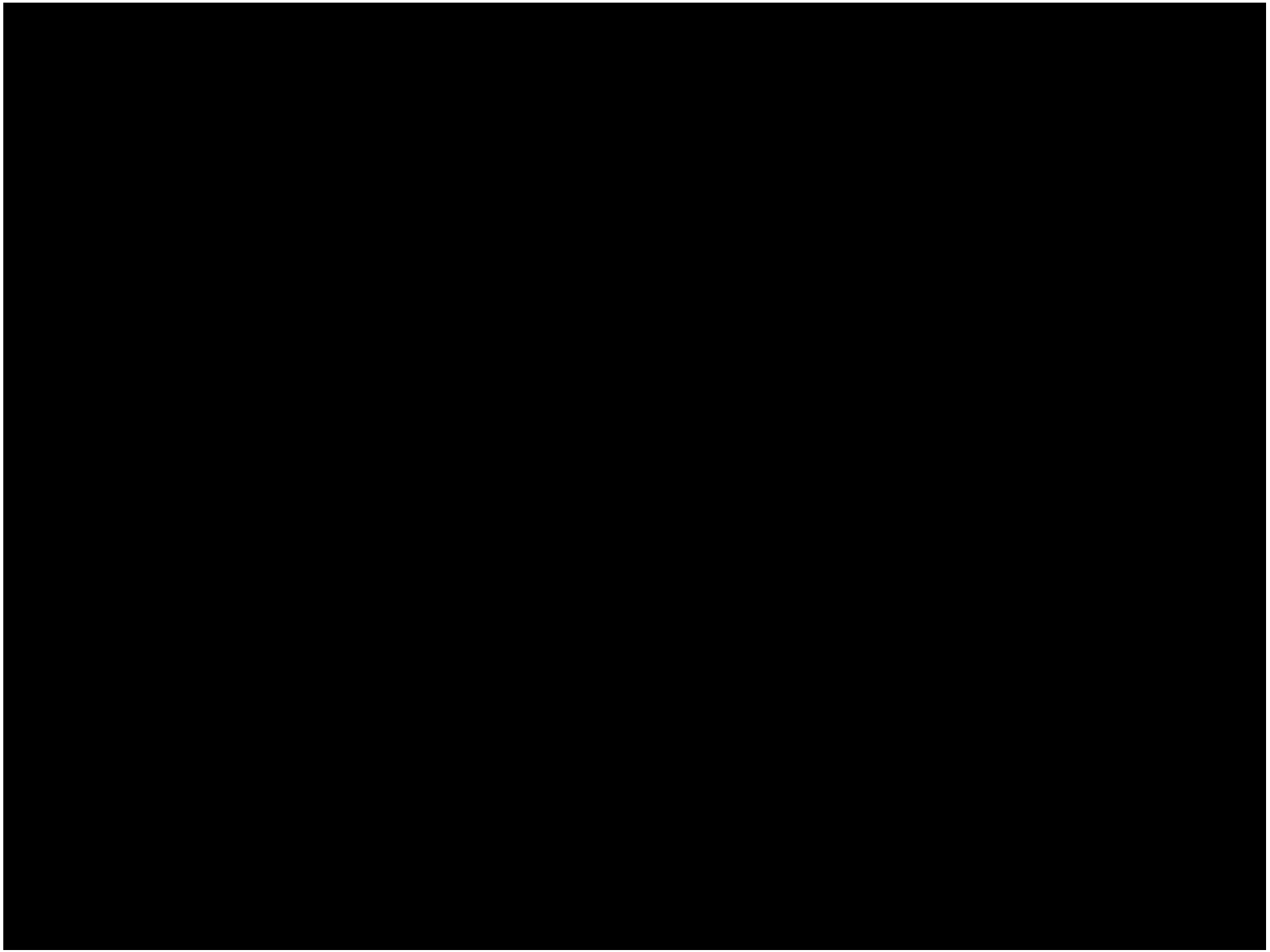
ATP hydrolysis at GroEL serves to advance the reaction forward, acting as a "timer"



Rye et al, 1997, 1999


T
T
D
D
GroES
T
T
cis
T→D
T→D
cis
ATP,
polypeptide
D
D
T
T
GroES
N or Ic
Iuc
OR
T
T
New cis
Polypeptide binding
Folding triggered
ES release primed
Discharge triggered





Hsp60/GroEL	Ming Cheng Ulrich Hartl (Munich)
X-ray and EM work	Kerstin Braig Charu Chaudhry Zbyszek Otwinowski Zhaouhui Xu Helen Saibil (Birkbeck) Paul Sigler
Topology and ATP cycle	George Farr Wayne Fenton Hays Rye Jonathan Weissman
NMR studies	Eric Bertelsen Jocelyne Fiaux Reto Horst (Scripps) Peter Wright (Scripps) Kurt Wüthrich (Scripps)