PROTEIN PHYSICS

LECTURES 17-18

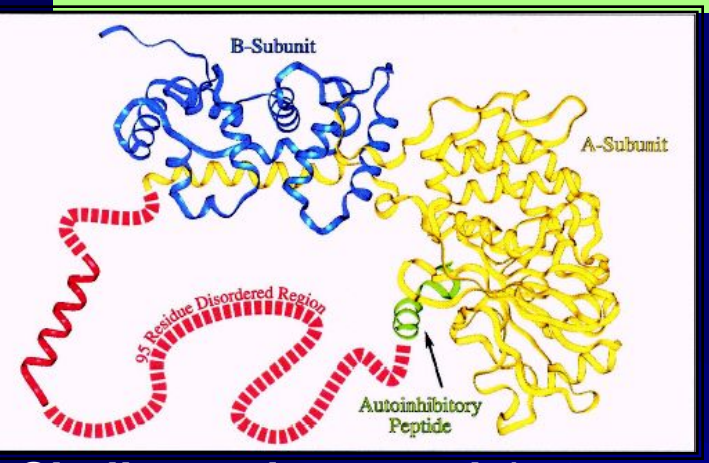
Protein Structures: Thermodynamic aspects

- Unfolded proteins *in vivo* and *in vitro*
- Cooperative transitions of protein structures
- Thermodynamic states of protein molecules
- Why protein denaturation is an “all-or-none” phase transition?
- “Energy gap” and “all-or-none” melting

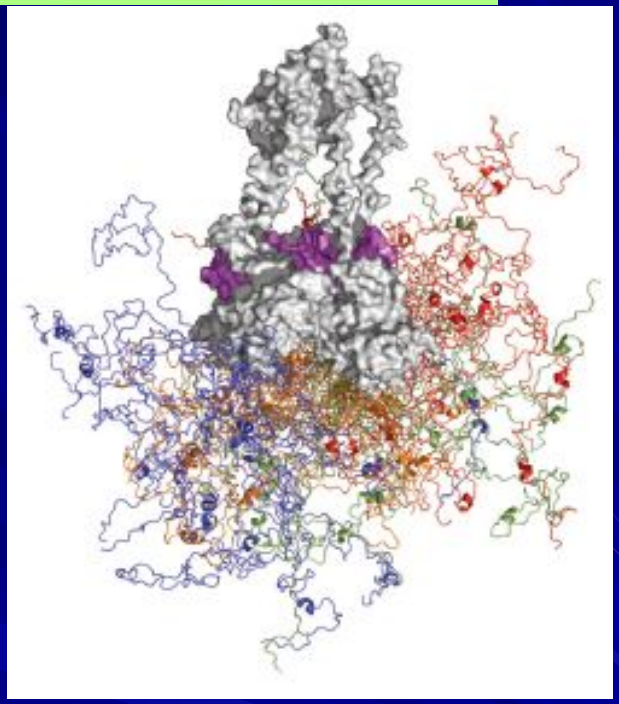
Natively disordered proteins *in vivo* - no 3D structure under physiological conditions

(Wright & Dyson, 1999; Uversky *et al.*, 2000; Dunker *et al.*, 2001; Tompa, 2002 ; Uversky, 2002--)

- Disordered states can be compact (molten globule) or extended (random coil);
- Protein can be completely disordered or contain large disordered regions



Many proteins
(>600 are now known)
display
functions *requiring*
the disordered state.



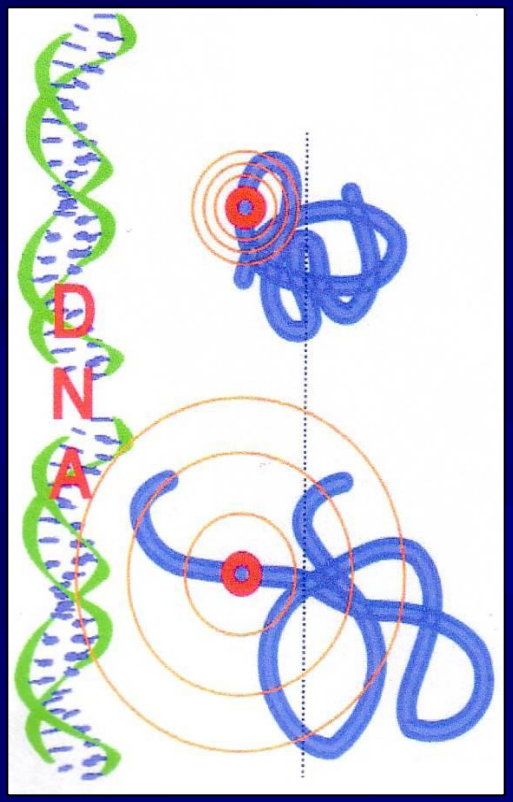
X-ray + SAXS + NMR + MD

Similar to denatured, but more extended (many PPII)
Less hydrophobic, more charges
Not enzymes, not transport proteins
Involved in recognition, signaling, regulation; in some diseases; in amyloidigenesis; in chaperone activity

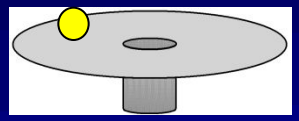
Владимир Николаевич Уверский, 1963

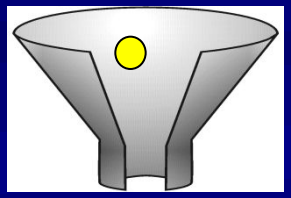
Plasticity: multi-functional
Induced folding
Rapid evolution
Post-translational modifications
Shorter half-life *in vivo*
Especially many in eukaryotes

Acceleration of molecular recognition

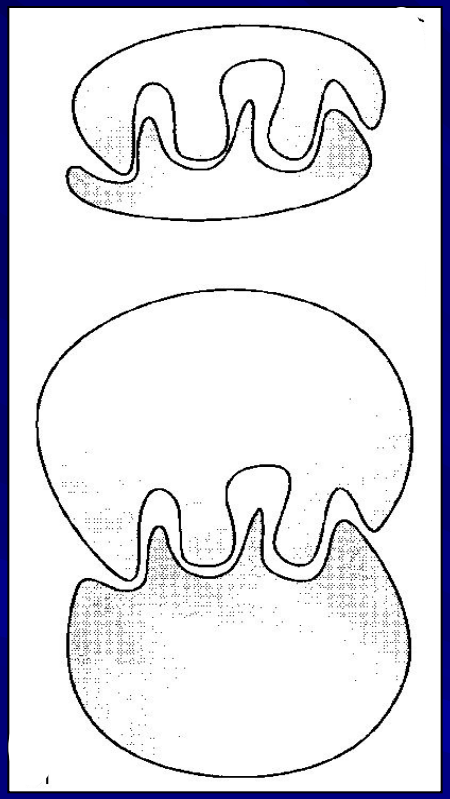


'Fly-casting mechanism'
Shoemaker *et al.*, 2000, *PNAS,* 97: 8868





Large interface at smaller size



High specificity without ultra-strong binding

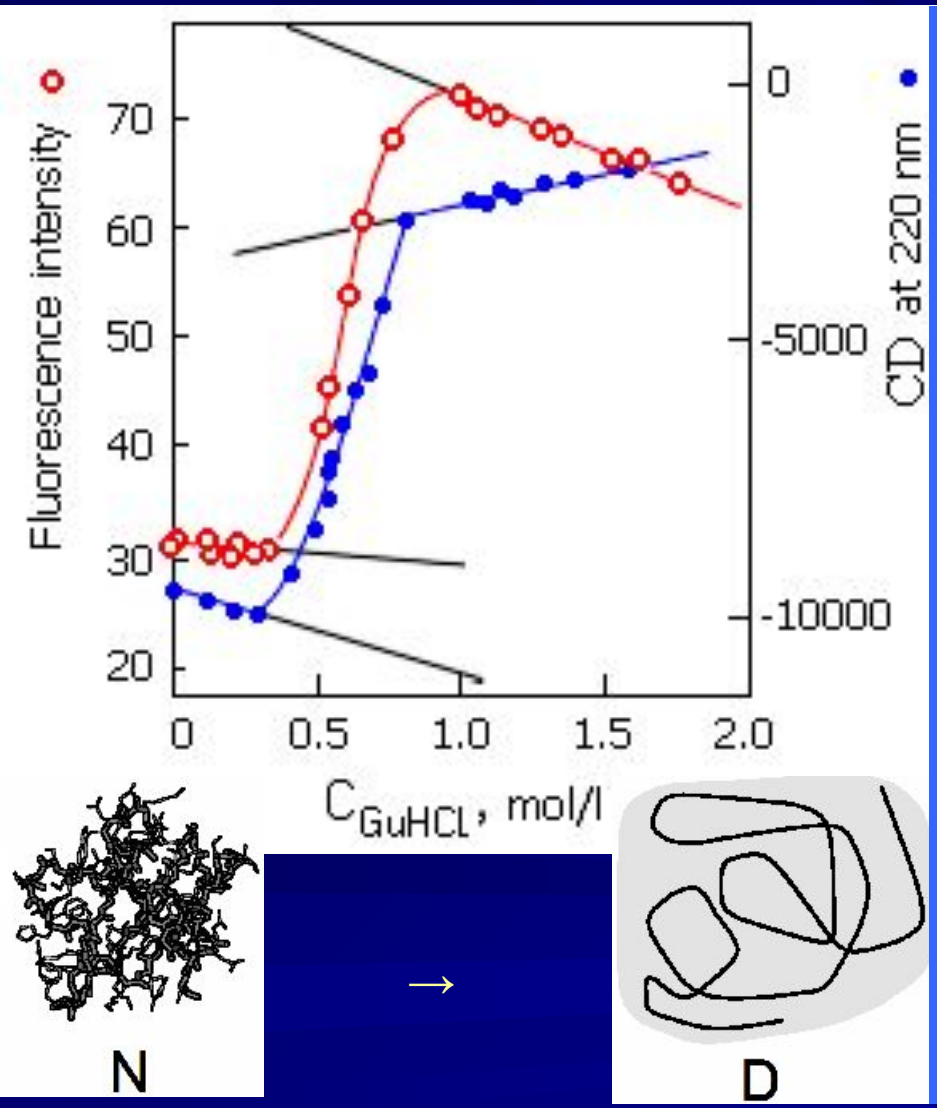
Schulz, Schirmer, 1979

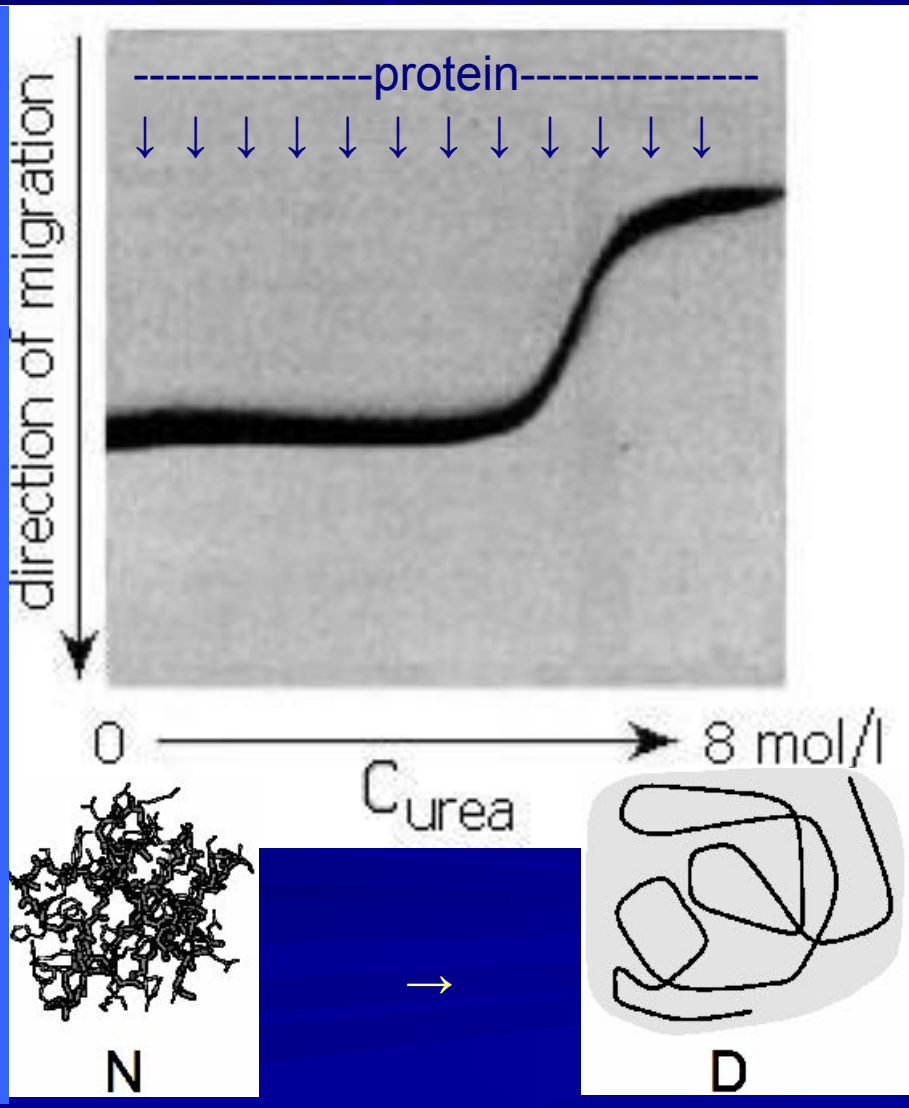
One protein – several functions

Protein's conformation is determined by the interaction partner, not only by protein's amino acid sequence itself, as it is typical for globular proteins.

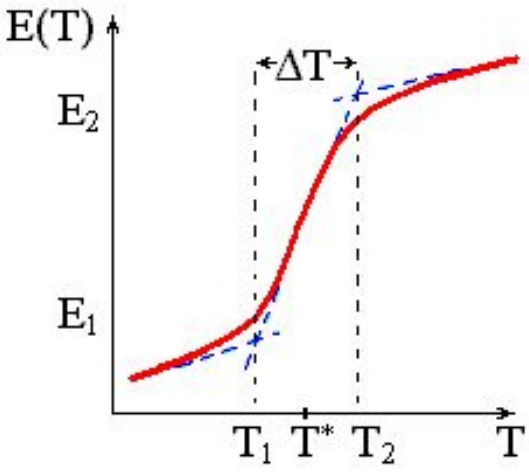
Solid protein structures can denaturate (decay), and then re-nature (fold) both *in vivo* (e.g., when protein is synthesized or transported through a membrane), and *in vitro*

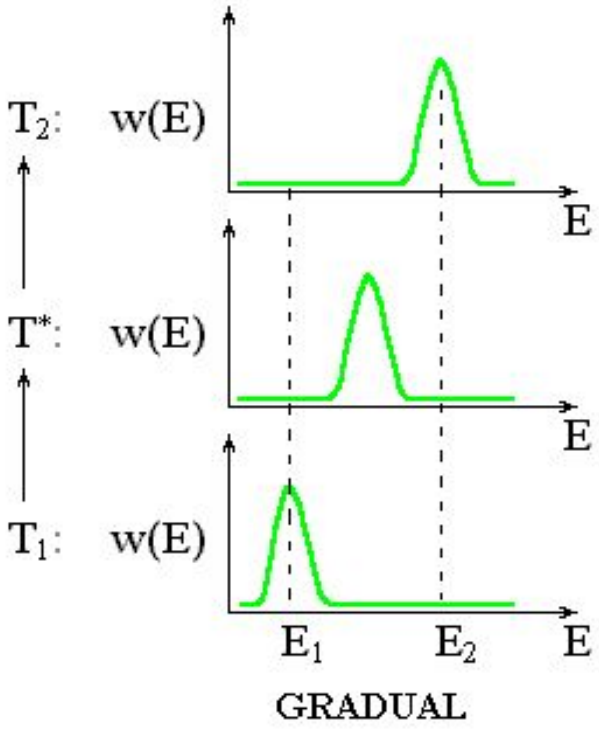
Protein denaturation ***in vitro***: cooperative transition

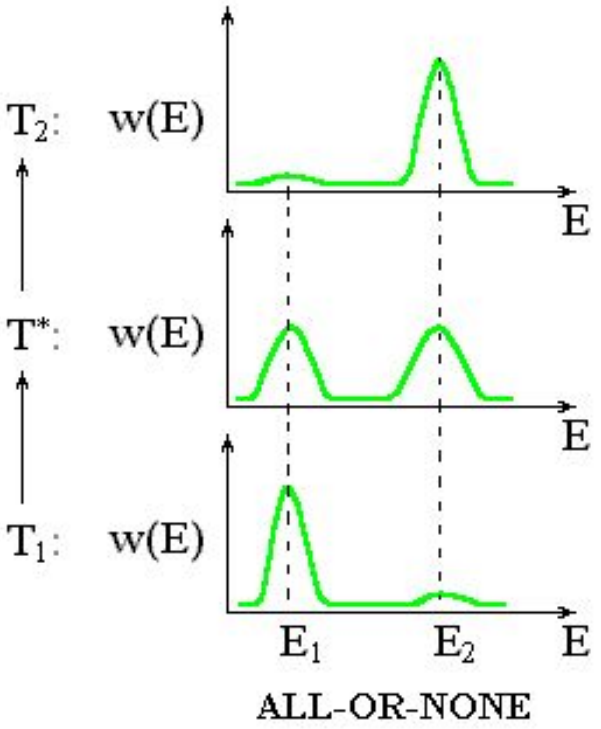


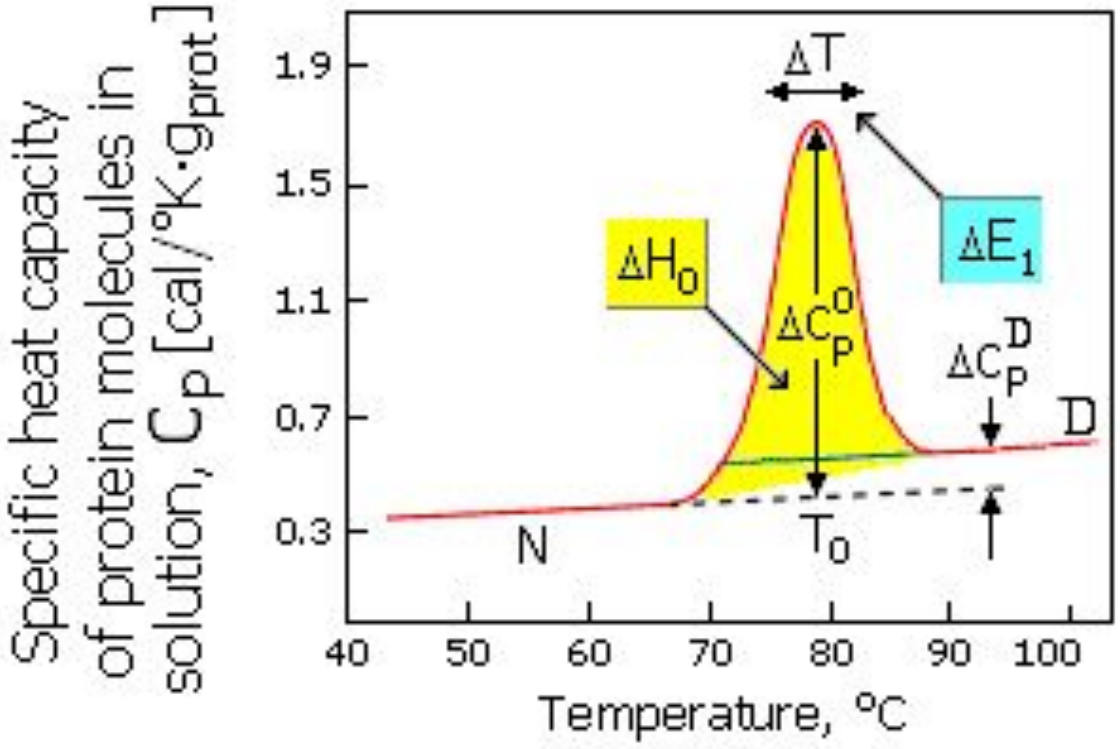


transition


E(T)
E2
E1
ΔT
T1
T*
T2
T


T2:
w(E)
E
T*:
w(E)
E
T1:
w(E)
E
E1
E2
E
GRADUAL


T2:
w(E)
E
T*:
w(E)
E
T1:
w(E)
E
E1
E2
E
ALL-OR-NONE



ΔH_0 [cal/g_{prot}] - specific heat of protein denaturation

Van't Hoff criterion for existence of the "all-or-none" (1-st order) transition:

$$\Delta E_1 \equiv 4kT_0^2 / \Delta T = \Delta H_0 / NUMB_{mol}$$

melting unit — 1 molecule

For a melting unit:

$T_0\Delta S_1 = \Delta E_1$

Transition:

$|\Delta G_1| = |-\Delta S_1 \times \Delta T| =$

$= \Delta E_1 \times |\Delta T / T_0| >> kT_0$

Denaturation: "**all-or-none**" transition in small (single-domain) proteins

(Privalov, 1969)

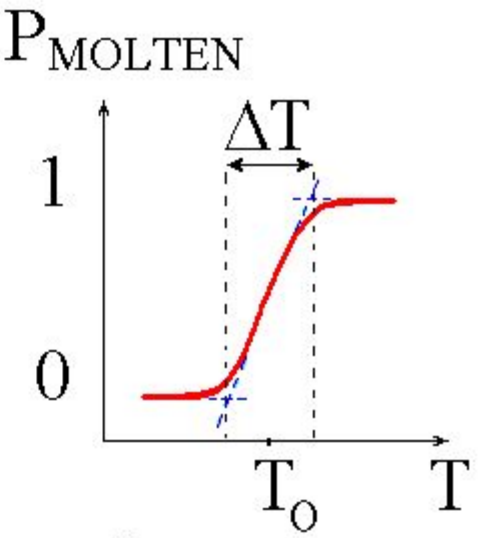
NATIVE (SOLID) ↔ DENATURED ("MOLTEN")

$E=\mathbf{0}$ $\quad E=\Delta E$

$S=\mathbf{0}$ $\quad S=\Delta S$

$\Delta S/k >> 1$

$$P_{MOLTEN} = \frac{\exp[-(\Delta E - T\Delta S)/kT]}{1+\exp[-(\Delta E - T\Delta S)/kT]}$$



$$P_{SOLID} = 1 - P_{MOLTEN}$$

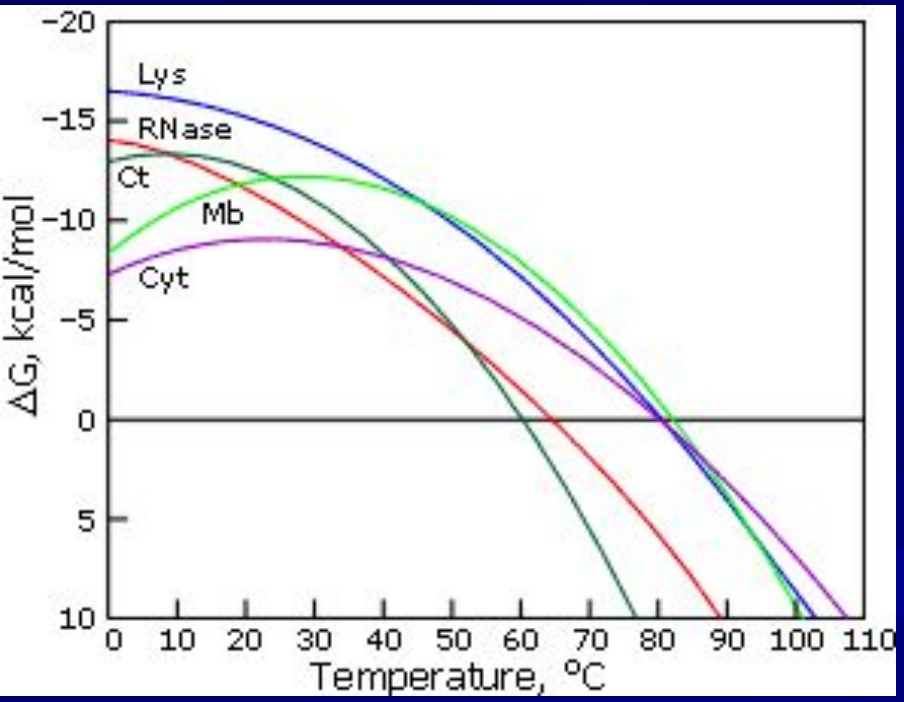
$$dP_{MOLTEN}/dT = P_{MOLTEN}(1-P_{MOLTEN})\cdot(\Delta E/kT^2)$$

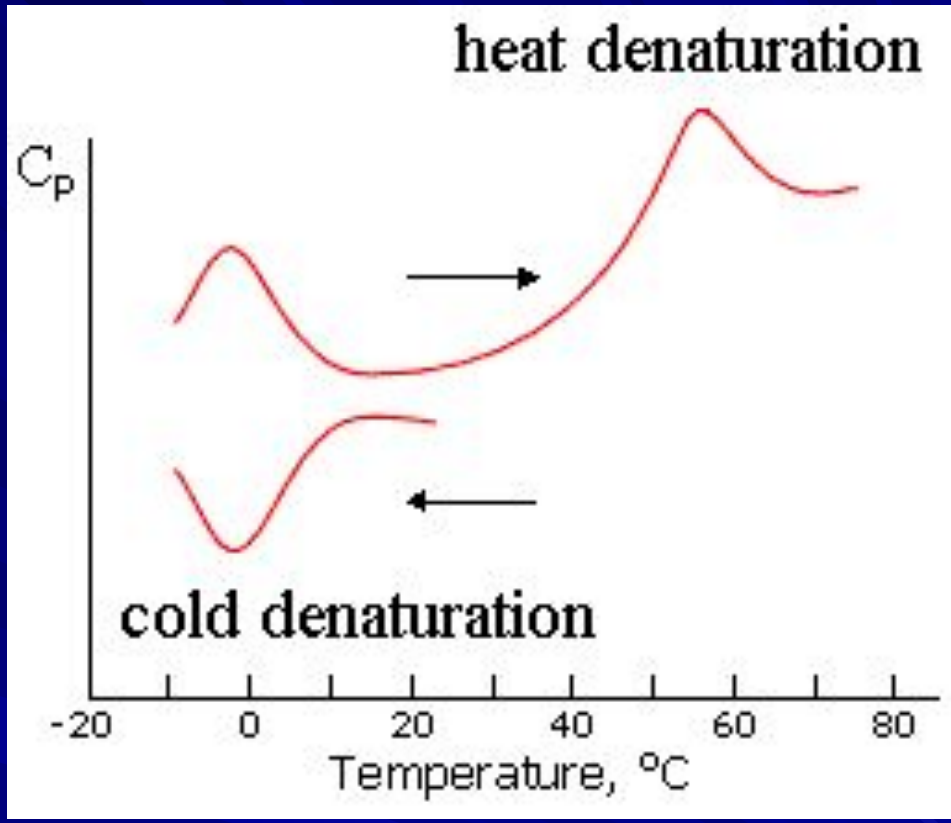
$T_0=\Delta E/\Delta S$

Mid-transition: $1/\Delta T = 0.5\cdot 0.5\cdot(\Delta E/kT_0^2)$ Van't Hoff

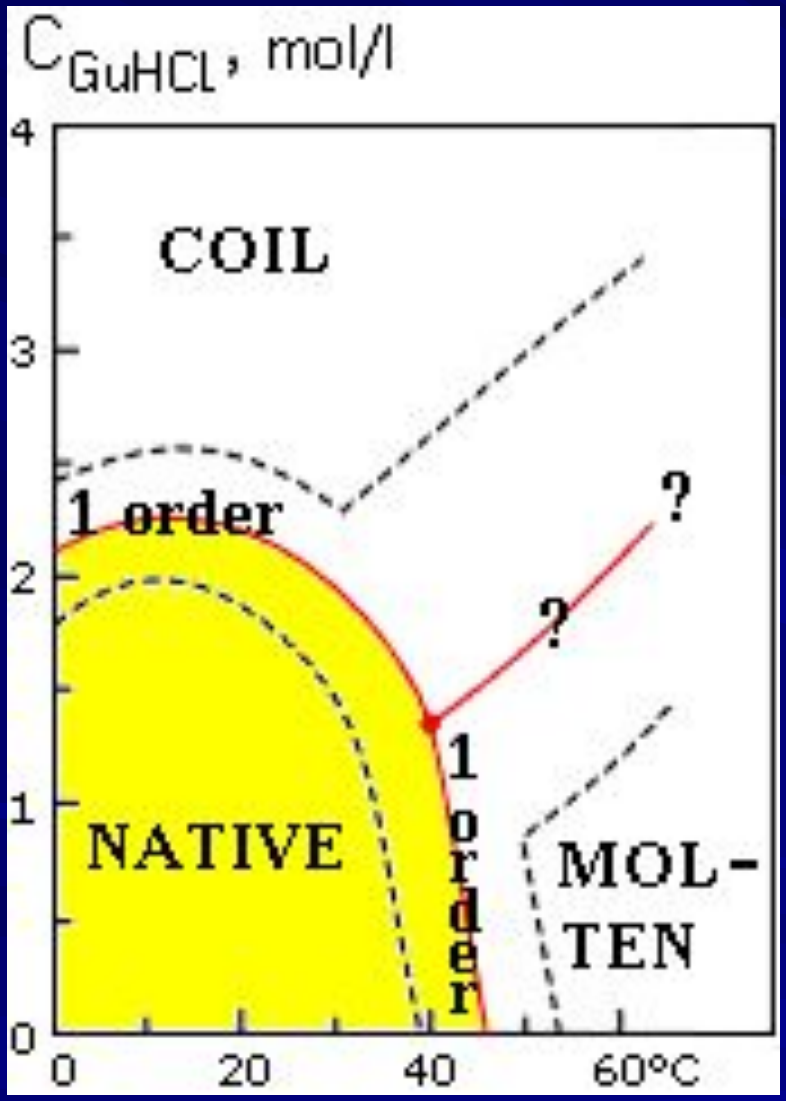
Jacobus Henricus
van 't Hoff, Jr.
(1852 –1911)
The first Nobel prize
in Chemistry, 1901

Петр Леонидович
ПРИВАЛОВ,
1932


-20
-15
-10
-5
0
5
10
ΔG, kcal/mol
Lys
RNase
Ct
Mb
Cyt
0 10 20 30 40 50 60 70 80 90 100 110
Temperature, °C


heat denaturation
C_p
cold denaturation
-20 0 20 40 60 80
Temperature, °C

Solid native state, unfolded coil, “more compact molten state” and cooperative transitions between them



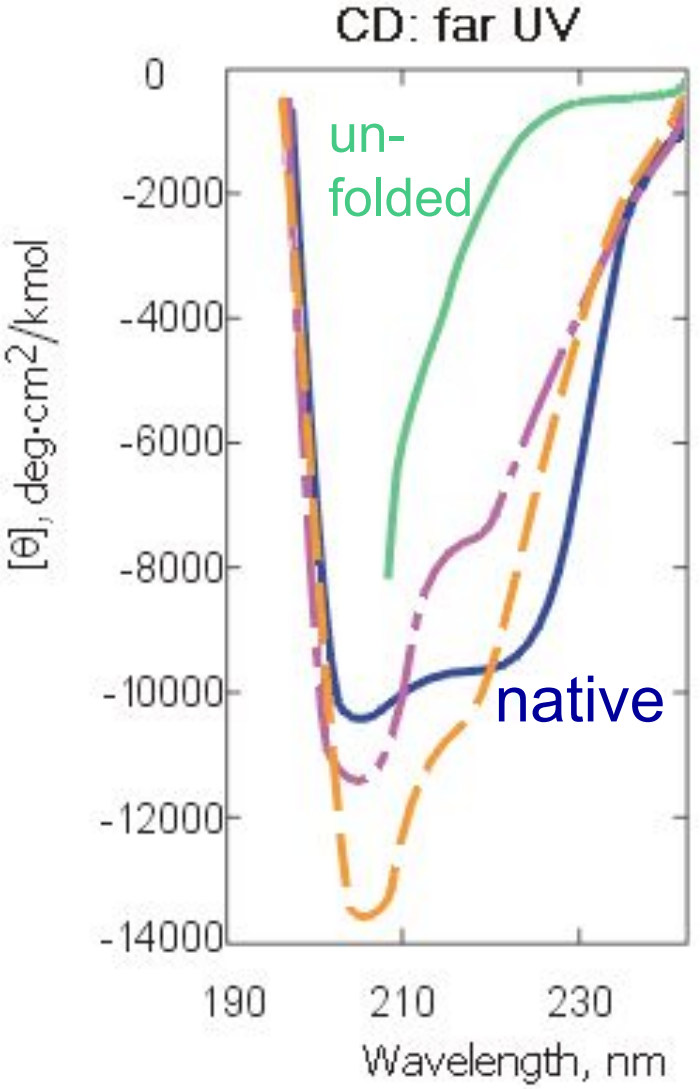
(Tanford, 1968; Ptitsyn et al., 1981)

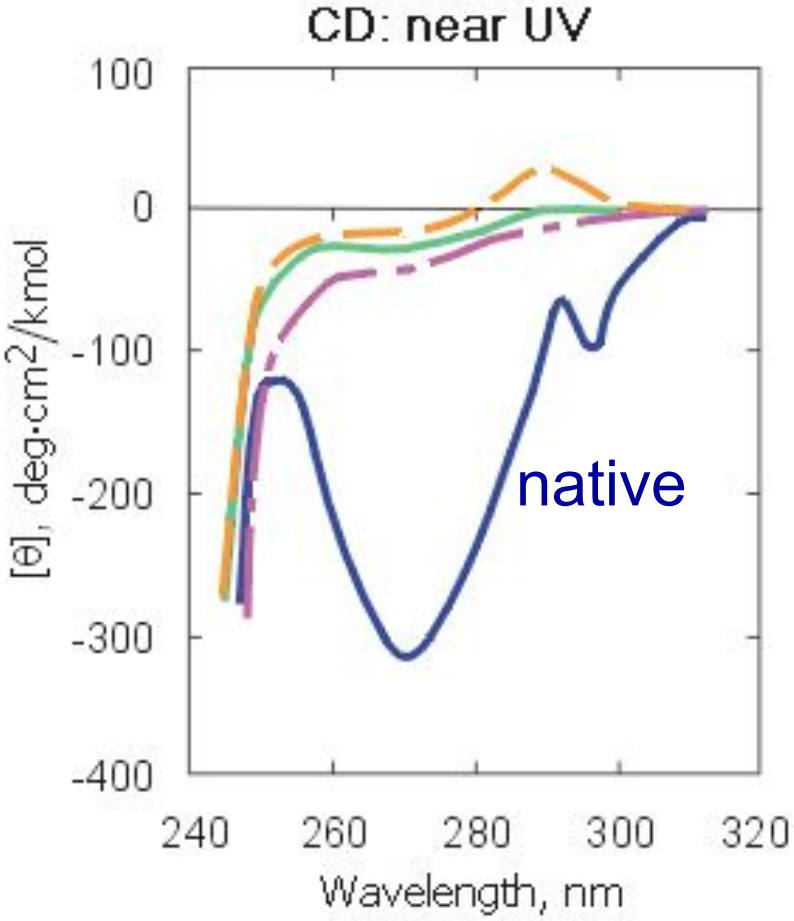
“All-or-none” decay of native protein structure:

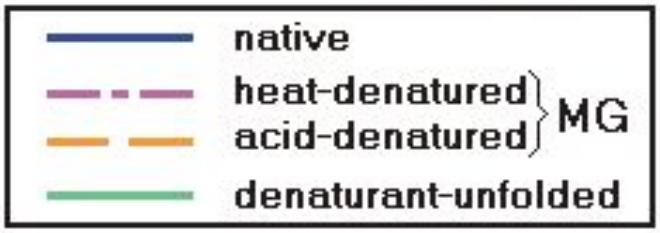
Ensures reliability and robustness of protein functioning

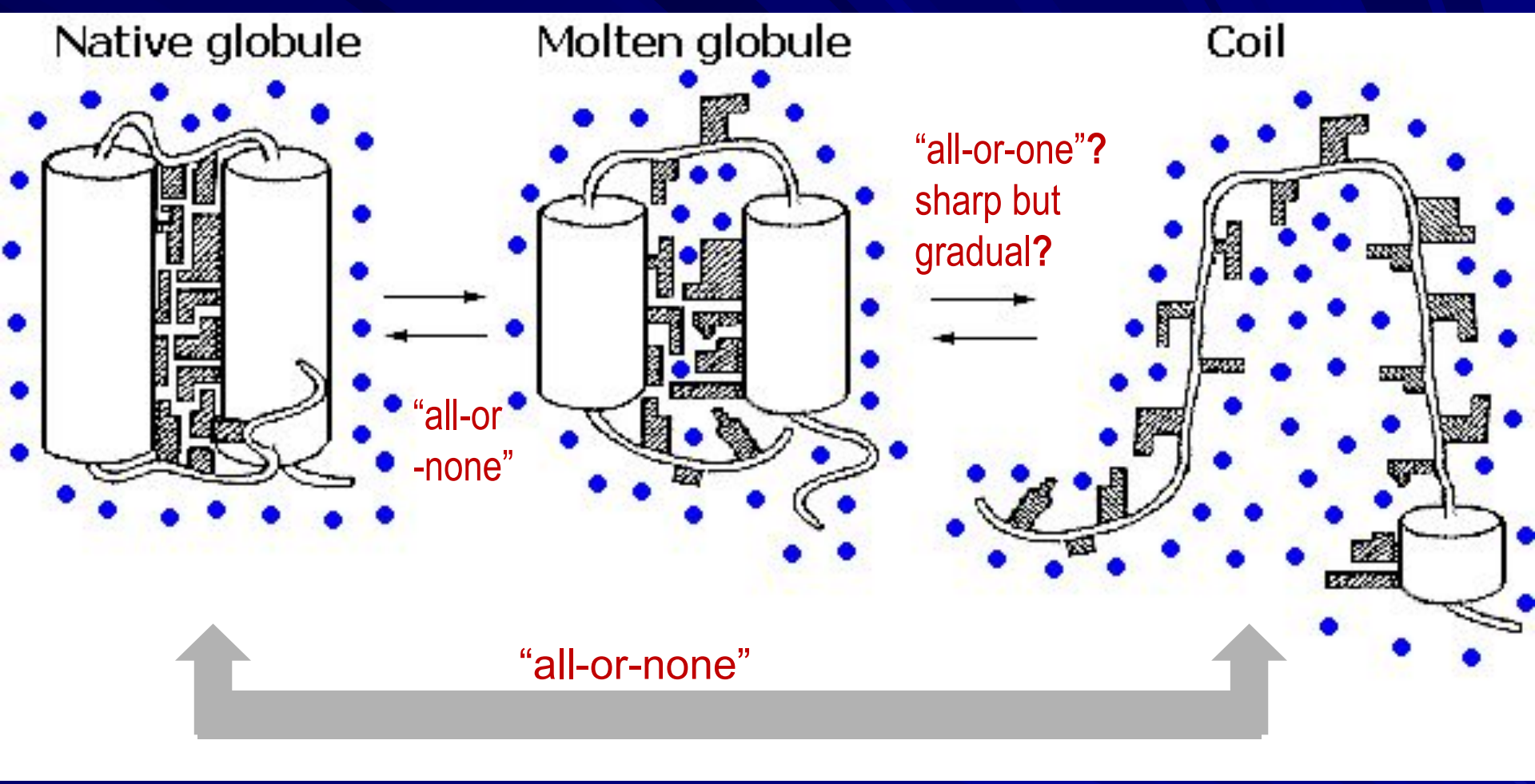
IN VARIOUS STATES:

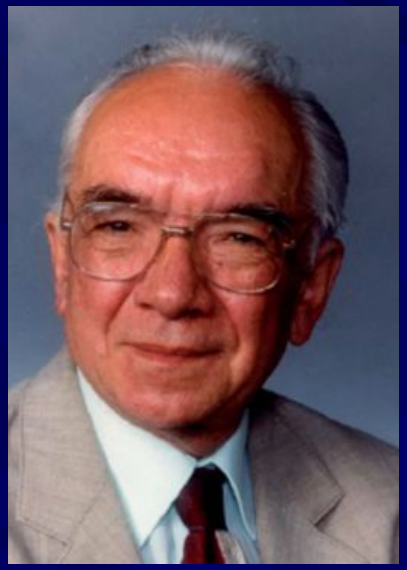
Secondary structure Side chain packing








Native globule
Molten globule
Coil
"all-or-one"?
sharp but
gradual?
"all-or
-none"
"all-or-none"



Олег Борисович **Птицын** (1929-99)

Валентина Егоровна **Бычкова**, 1934

Геннадий Васильевич **Семисотнов**, 1947

Дмитрий Александрович **Долгих**, 1954

Рудольф Ирикович **Гильманшин**, 1957

Евгений Исаакович **Шахнович**, 1957

Why protein denaturation is an “all-or-none” phase transition?

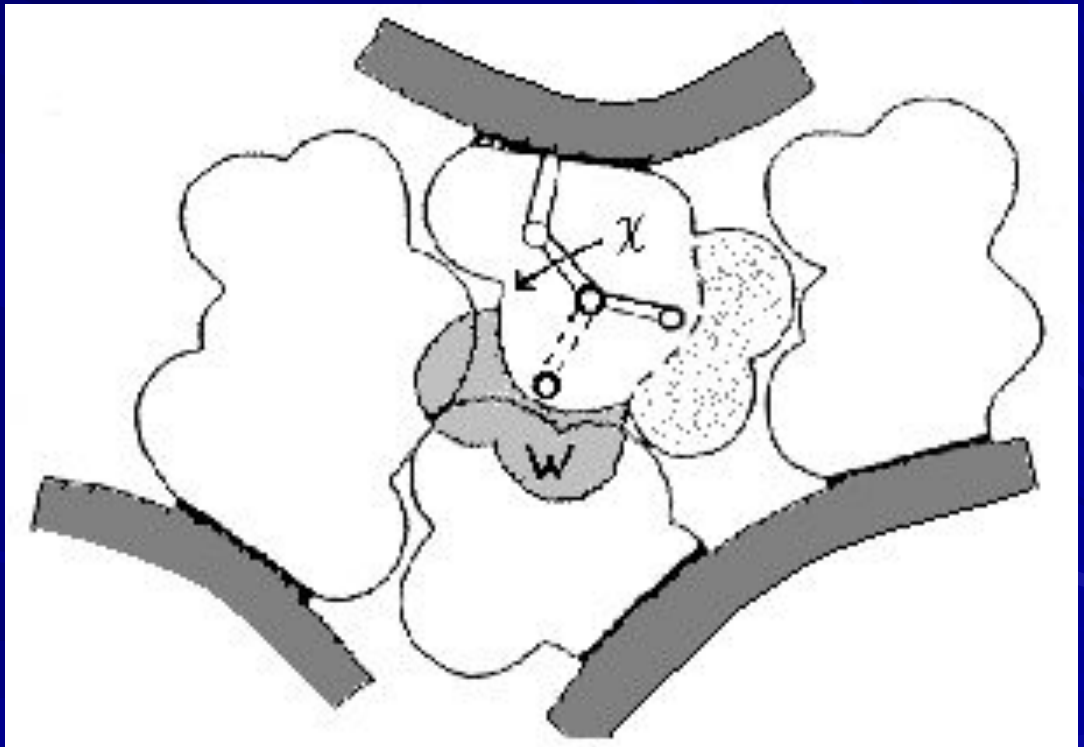
Peculiarities of protein structure:

- Unique fold;
- Close packing;
- Flexible side chains at rigid backbone
- Side chains rotamers

Impossible to create a pore to rotate only one side chain

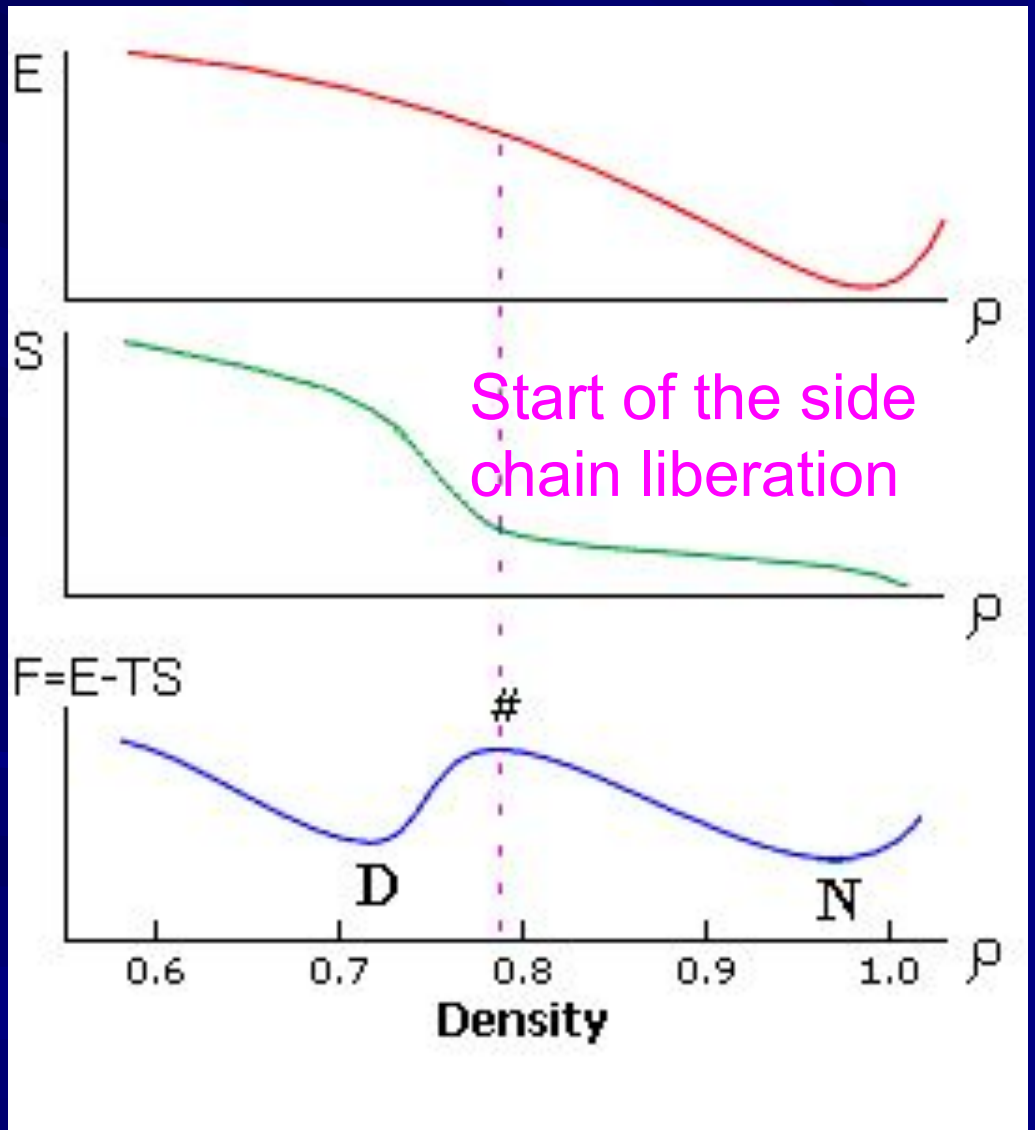
↓

energy gap

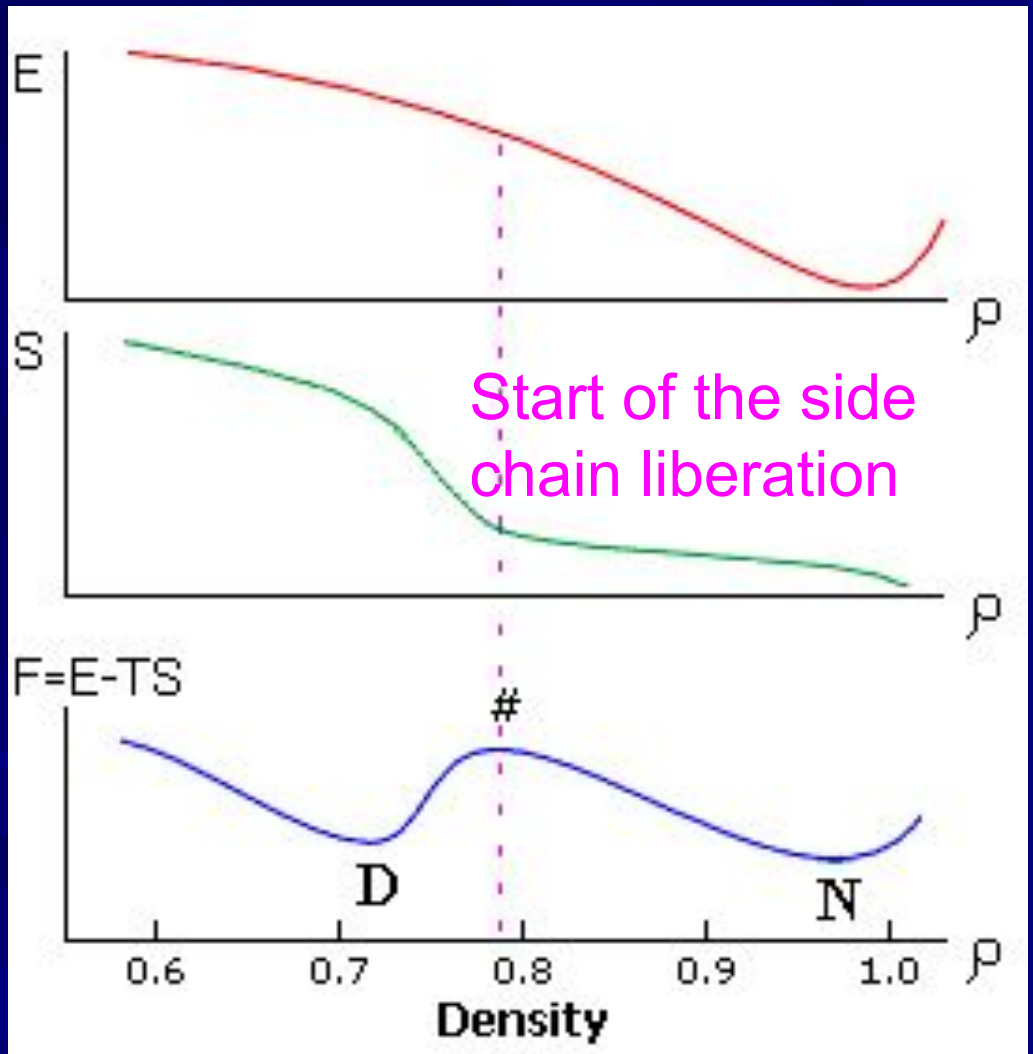


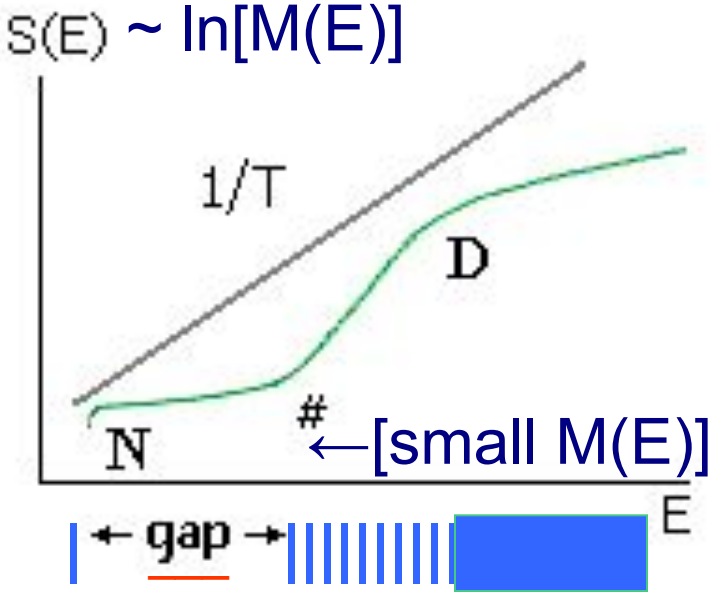
close side chain packing

“All-or-none” melting:

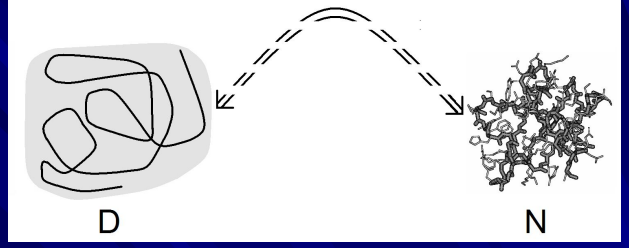


“All-or-none” melting: a result of the “ENERGY GAP”



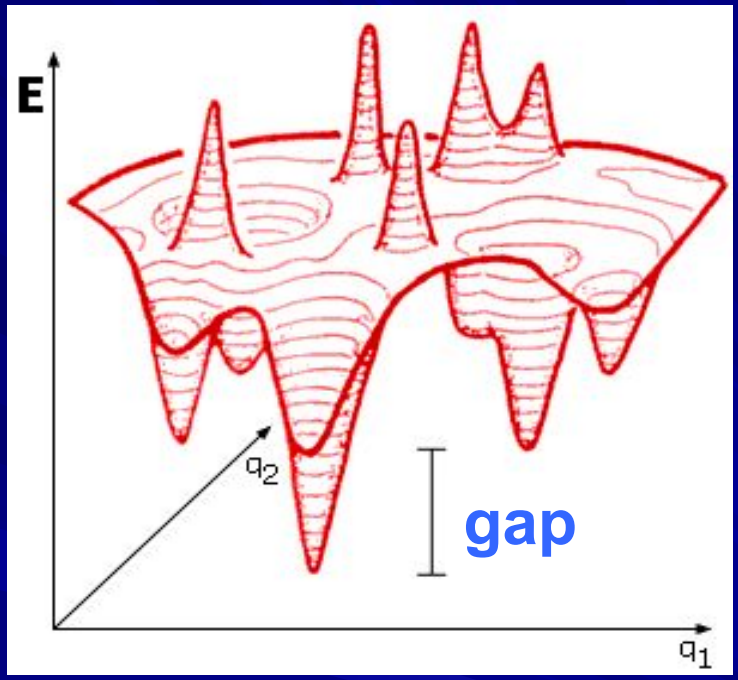


IS THE GAP “NATURAL”?



"all-or-none" transition results from the "energy gap"

Energy landscape



The "energy gap" is:
- necessary for unique protein structure
- necessary for fool-proof protein action
- necessary for fast folding
- produced by very rare sequences

GAP WIDTH: MAIN PROBLEM OF EXPERIMENTAL PROTEIN PHYSICS

PHYSICAL ESTIMATE: =???

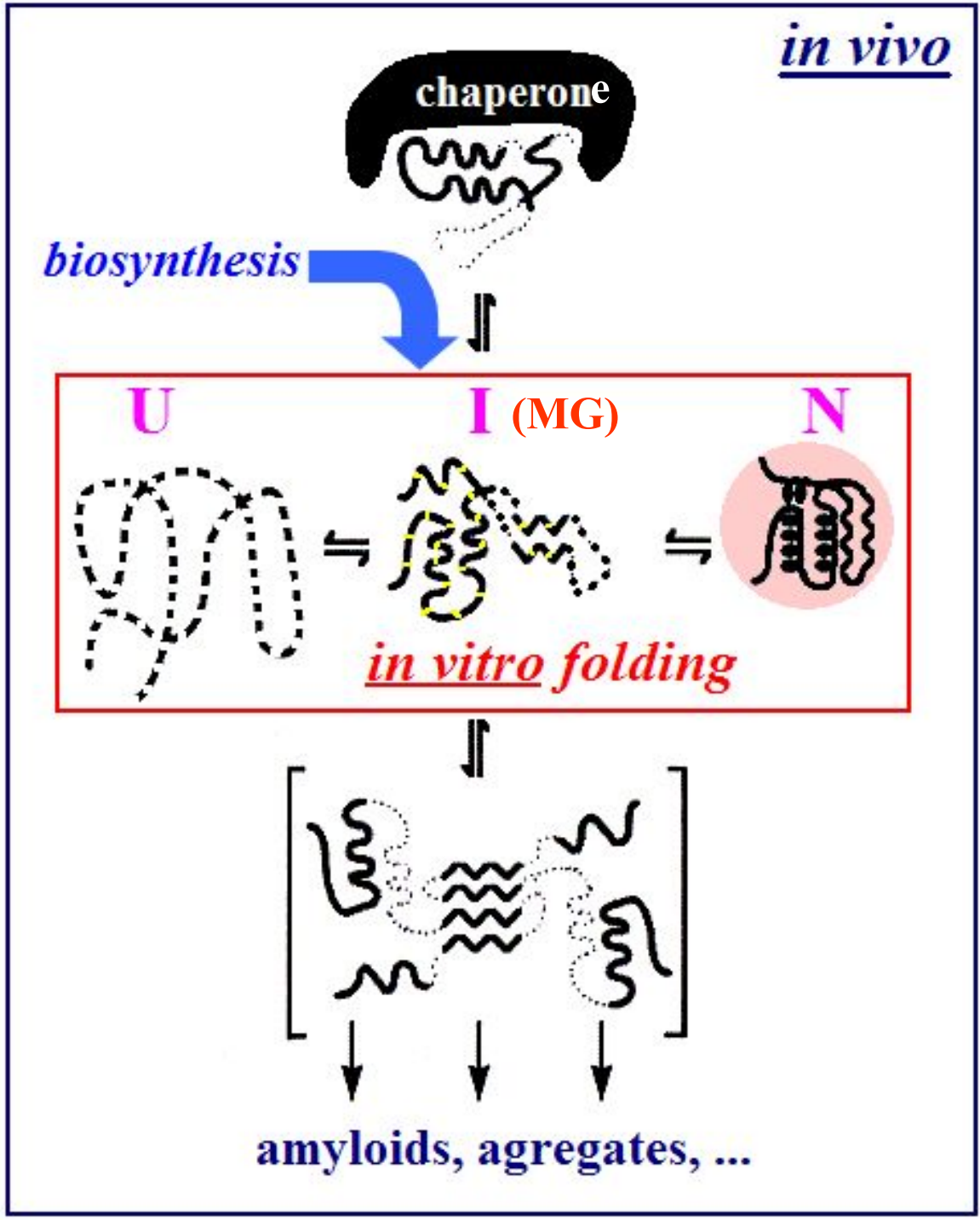
BIOLOGICAL ESTIMATE:

1 OF ~10^{10} (NOT 1 0F ~10^{100}!) RANDOM SEQUENCES MAKES A "PROTEIN-LIKE" STRUCTURE (SOLID, WITH A SPECIFIC BINDING: PHAGE DISPLAY).

THIS IMPLIES THAT $\Delta E \sim 20\ kT_0$

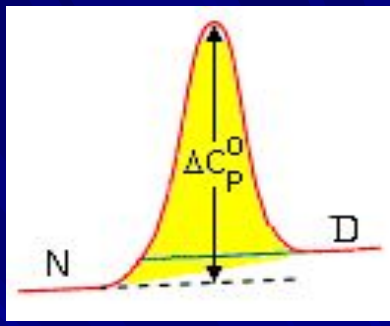
ΔE is **small** relatively to the meting energy **$\Delta H \approx 100\ kT_0$**: **narrow energy gap**

PROTEIN FOLDING:
current picture
(Dobson, 2003)

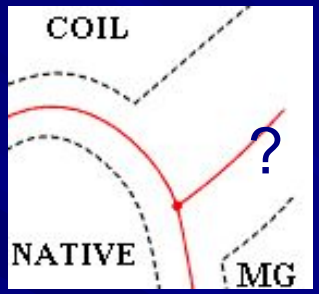


Protein Structures: Thermodynamics

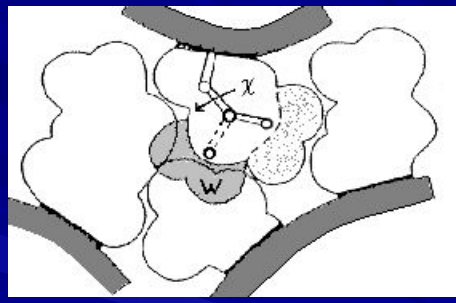
- Protein denaturation: cooperative and, moreover, an “all-or-none” transition in small proteins and separate domains.



- Solid native state, unfolded coil & “molten globule”.



- Why protein denaturation is an “all-or-none” phase transition?



- “Energy gap” and “all-or-none” melting. “Protein-like” heteropolymers.