

PROTEIN PHYSICS

LECTURES 19-21

- *In vivo* folding
- *In vitro* folding: spontaneously
- Levinthal paradox: spontaneously - how?
- Protein folding intermediates
- Two-state folding
- Transition state and protein folding nucleus
- Folding rate theory: solution of Levinthal's paradox

BASIC FACTS:

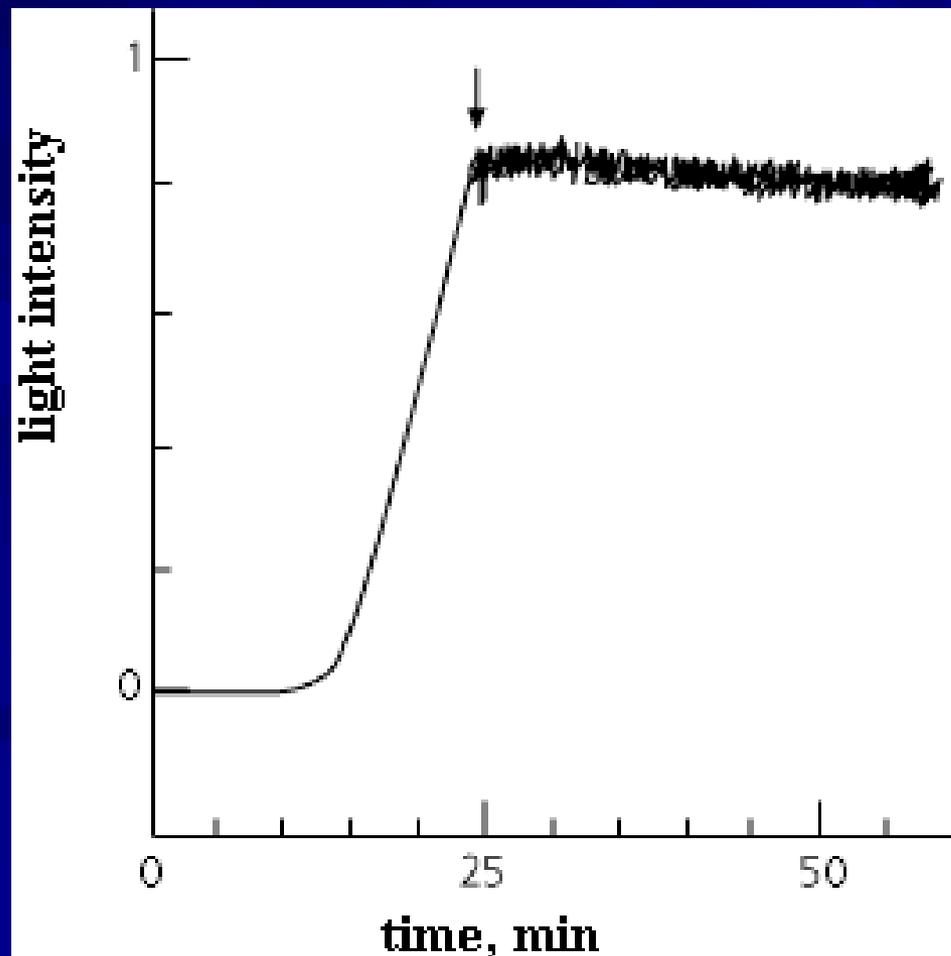
- *In vivo* (in the cell):
 - RNA-encoded protein chain is synthesized at a ribosome.
 - Biosynthesis + Folding < 10 – 20 min.
 - Folding of large (multi-domain) protein: during the biosynthesis.
 - Folding is aided by special proteins “chaperons” and enzymes like disulfide isomerase.
 - The main obstacle for *in vivo* folding experiments:
nascent protein is small, ribosome (+ ...) is large.

¹⁵N, ¹³C NMR: Eichmann et al., *PNAS* **107**, 9111 (2010):
Polypeptides remain unstructured during elongation but fold into a compact, native-like structure when the entire sequence is available.

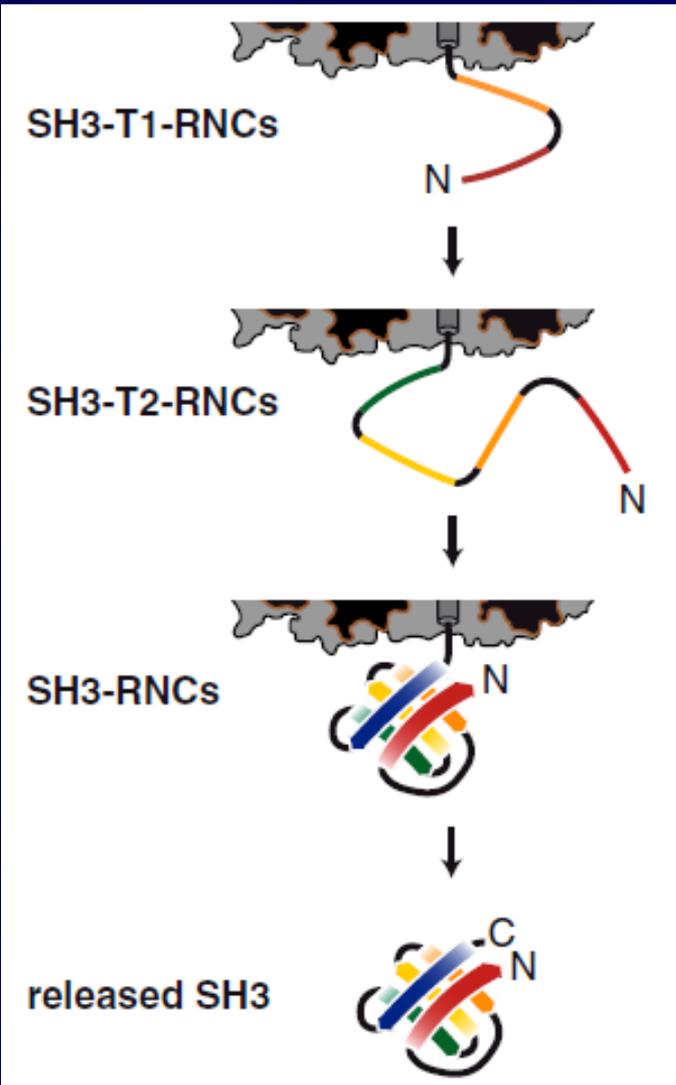
The main obstacle for *in vivo* folding experiments:
nascent protein is small, ribosome (+ ...) is large.
However, one can follow some “rare” protein activity,
and use a “minimal” cell-free system

Luciferase activity

(Kolb, Makeev,
Spirin, 1994)



Protein folding *in vivo* (at ribosome – at least for small proteins) ≈ as *in vitro*



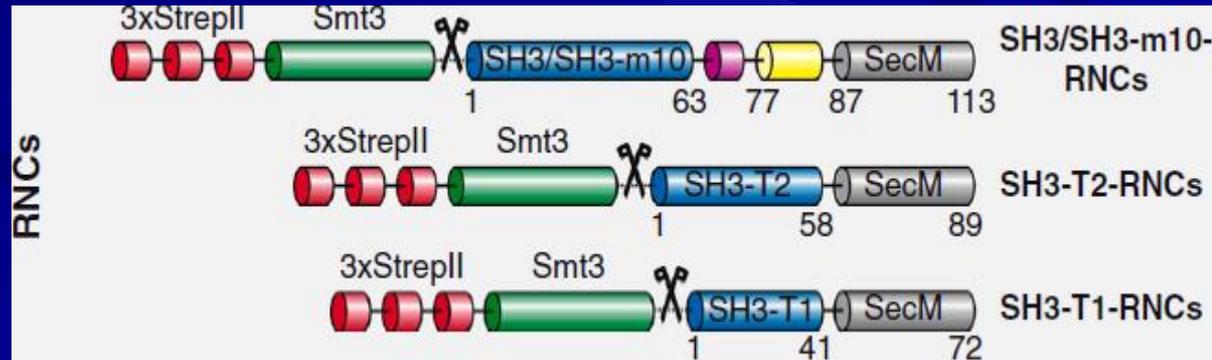
^{15}N , ^{13}C NMR:

Cotranslational structure acquisition of nascent polypeptides monitored by NMR spectroscopy.

Eichmann C, Preissler S, Riek R, Deuerling E.

PNAS 107, 9111 (2010):

«Polypeptides [at a ribosome] remain unstructured during elongation but fold into a compact, native-like structure when the entire sequence is available.»



Protein folding *in vivo* (at ribosome)

¹⁵N, ¹³C NMR:

Monitoring cotranslational protein folding in mammalian cells at codon resolution.

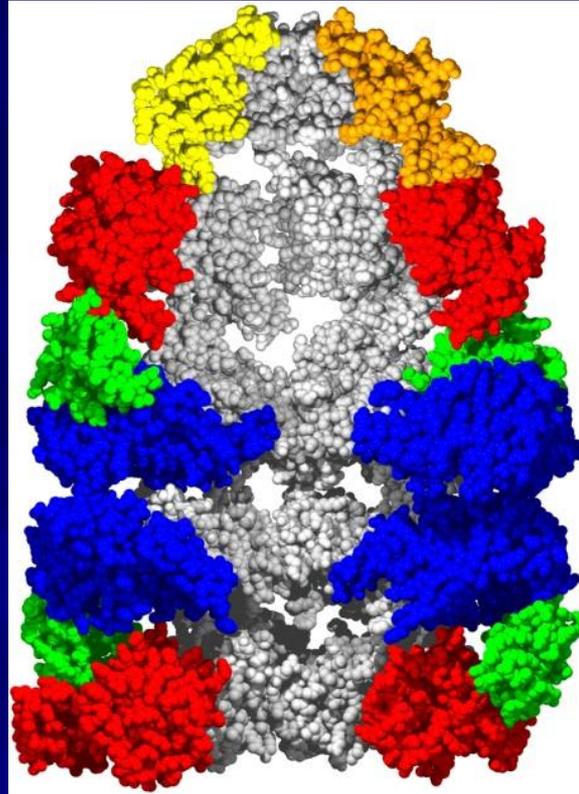
Hana Y., David A., Liu B., Magadán J.G., Bennink J.R., Yewdell J.W., Qian S.-B.

PNAS **109**, 12467 (2012):

«...folding immediately after the emergence of the full domain sequence.»

«... displaying two epitopes simultaneously when the full sequence is available.»

Chaperone



Folding:
inside or outside

GroEL/ES?

- OUTSIDE

GroEL/ES

«Active action»? -- NO

«Anfinsen cage»?

Ellis R.J. 2003
Curr. Biol. 13:R881-3

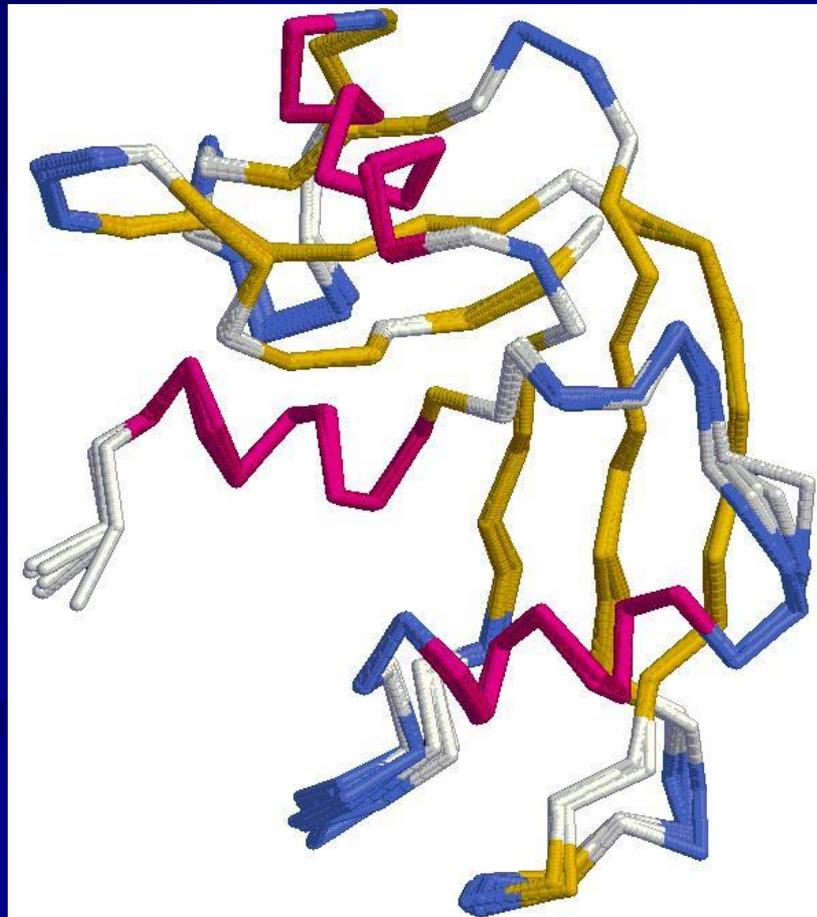
Passive and even
superpassive action –
GroEL/ES only decreases
protein concentration of
not-yet-folded protein in
solution

(Marchenkov & Semisotnov,
2009, *Int. J. Mol. Sci.*, 10: 2066-83)

“ambidextrous chaperone activity“

(Weinstock, Jacobsen, Kay, 2014,
PNAS 111(32):11679-84)

**PROTEIN CHAIN
CAN FORM ITS UNIQUE 3D STRUCTURE
SPONTANEOUSLY *IN VITRO*
(Anfinsen, 1961: Nobel Prize, 1972)**



BASIC FACTS:

- *In vitro* (in physico-chemical experiment):
 - Unfolded globular **protein is capable of renaturation** (if it is not too large and not too modified chemically after the biosynthesis), i.e., its 3D structure is capable of spontaneous folding [Anfinsen, 1961].
 - Chemically synthesized protein chain achieves its correct 3D structure [Merrifield, 1969].
 - The main obstacle for *in vitro* folding is aggregation.

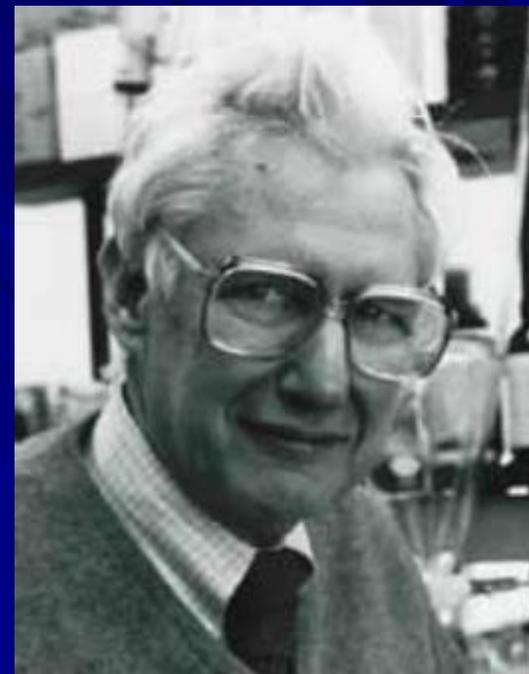
Conclusion: Protein structure is determined by its amino acid sequence;
cell machinery is not more than an “incubator” for protein folding.



Christian Boehmer
Anfinsen, Jr.
(1916 –1995)
Nobel Prize 1972



Robert Bruce
Merrifield
(1921 – 2006)
Nobel Prize 1988



Cyrus **Levinthal**
(1922 –1990)

HOW DOES PROTEIN FOLD?

and even more:

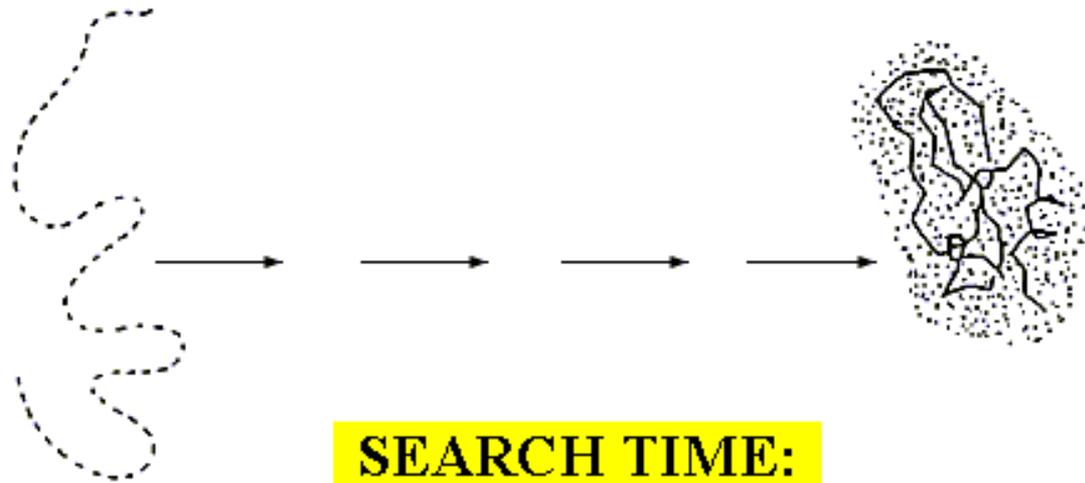
How CAN protein fold spontaneously?

Levinthal paradox (1968):

unfolded chain:
at least $\sim 2^{100}$
conformations

(for 100-residue chain)

native structure:
1 conformation



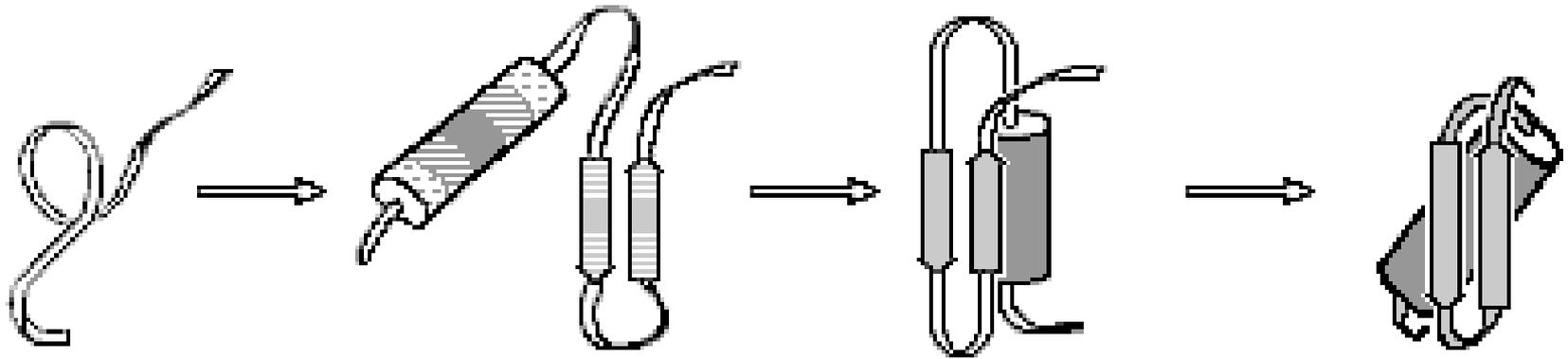
SEARCH TIME:
 $2^{100} \text{ conf.} \times 10^{-13} \text{ sec/conf.} = \sim 10^{10} \text{ years...}$

Native protein structure reversibly refolds from various starts, i.e., it is thermodynamically stable.

But how can protein chain find this unique structure - within seconds - among zillions alternatives?

SPECIAL PATHWAYS?? FOLDING INTERMEDIATES??

“Framework model” of **stepwise** folding (Ptitsyn, 1973)



**Unfolded
chain**

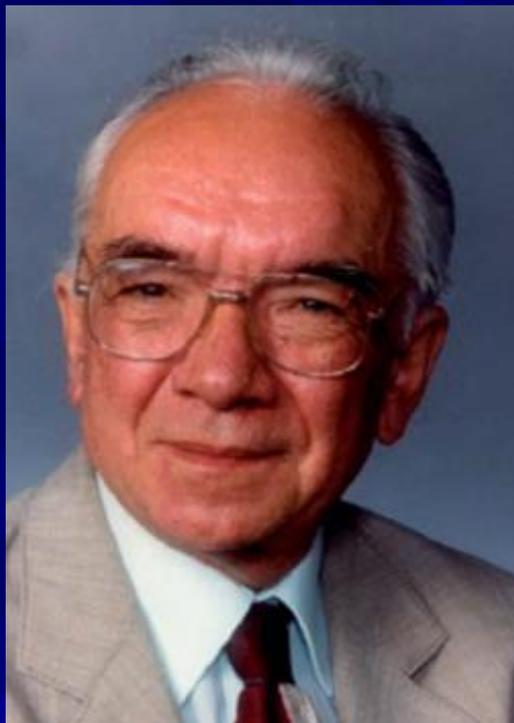
**Secondary
structure
fluctuating
around its
native position**

**Native-like
secondary
structure
and folding
pattern**

**Native
3D structure**

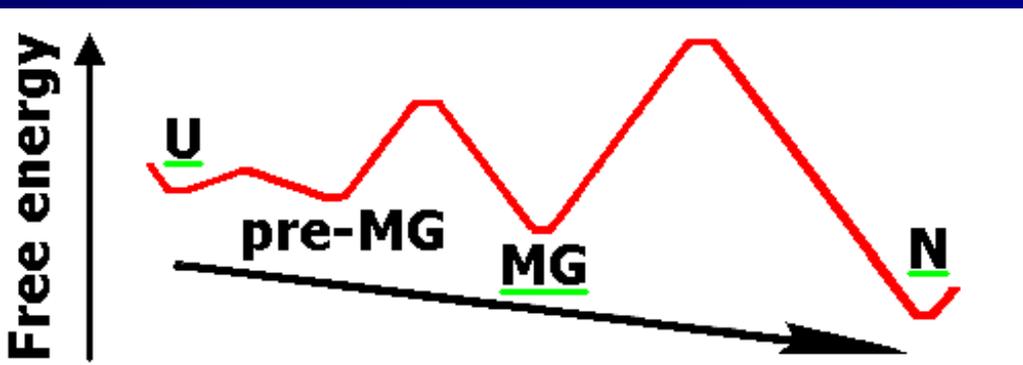
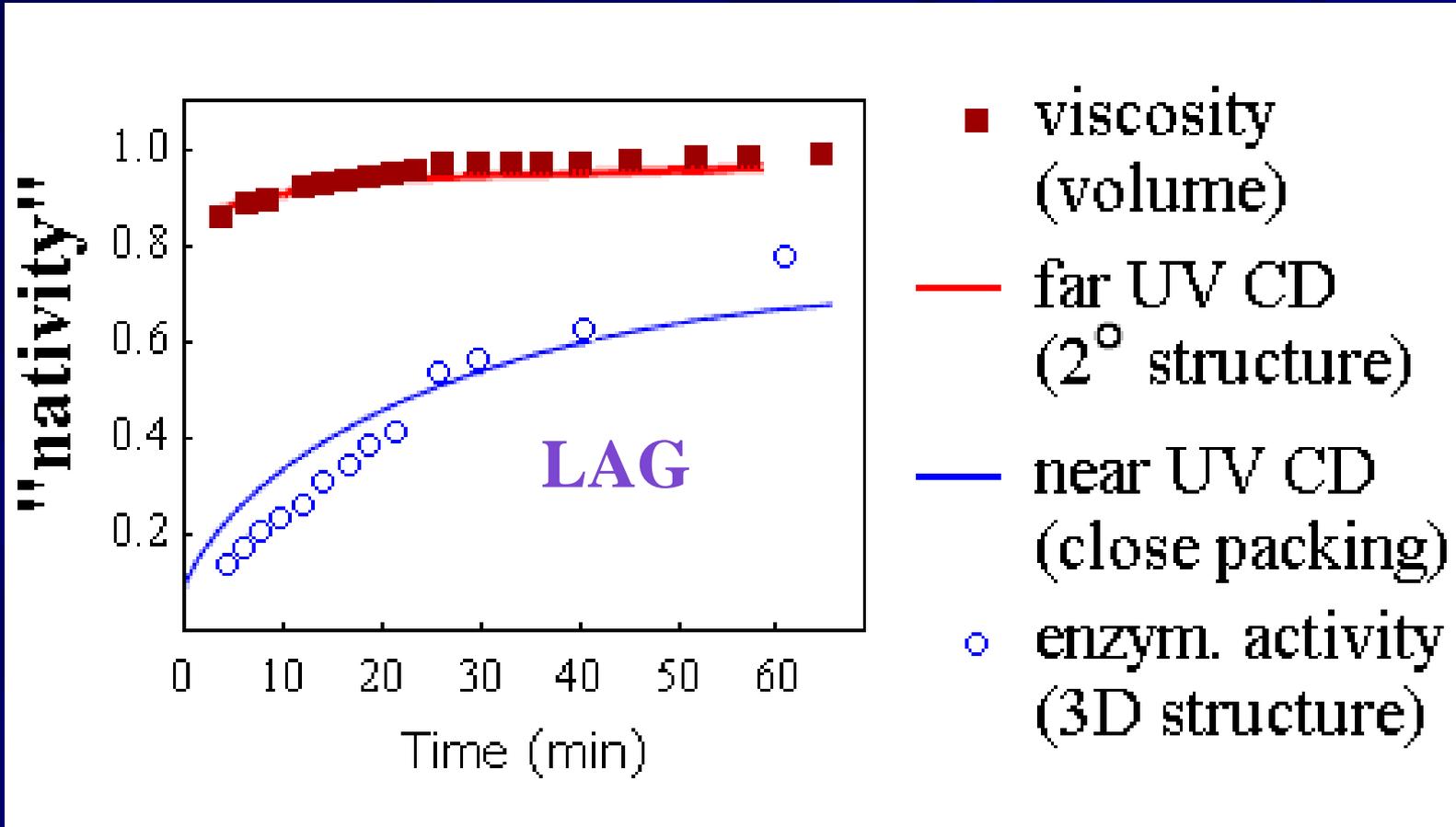
**Now:
Pre-molten
globule**

**Now:
Molten
globule**



Олег Борисович
Птицын
(1929-99)

Kinetic intermediate (molten globule) in protein folding



(Doldikh, ..., Ptitsyn, 1984)

Multi-state folding

Found: metastable (“accumulating”, “directly observable”) folding intermediates.

The idea was: intermediates will help to trace the folding pathway, - like intermediates in a biochemical reaction trace its pathway.

This was a “chemical logic”.

However, although protein folding intermediates (like MG) were found for many proteins, the main question as to how the protein chain can find its native structure among zillions of alternatives remained unanswered.

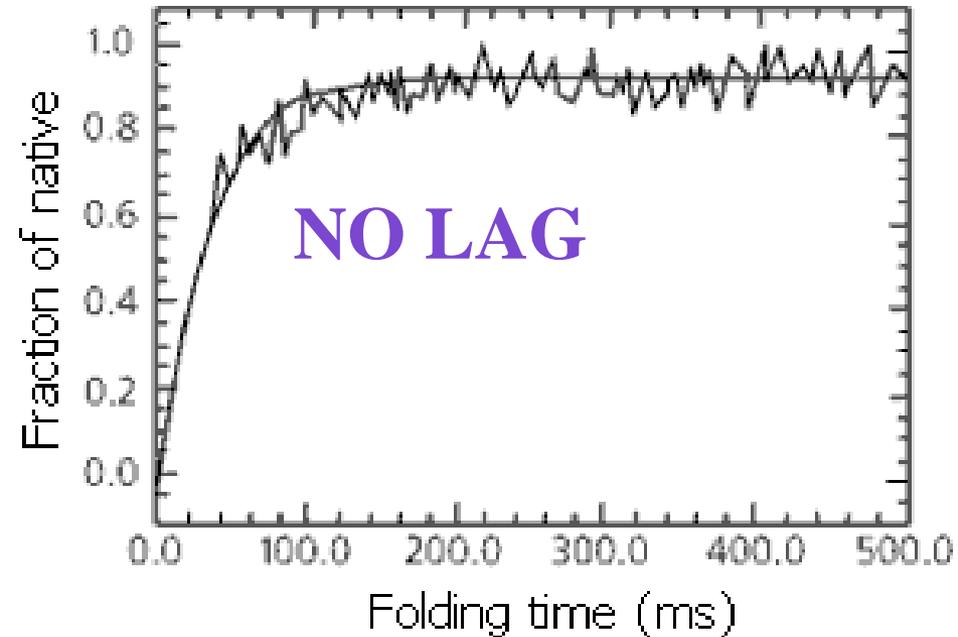
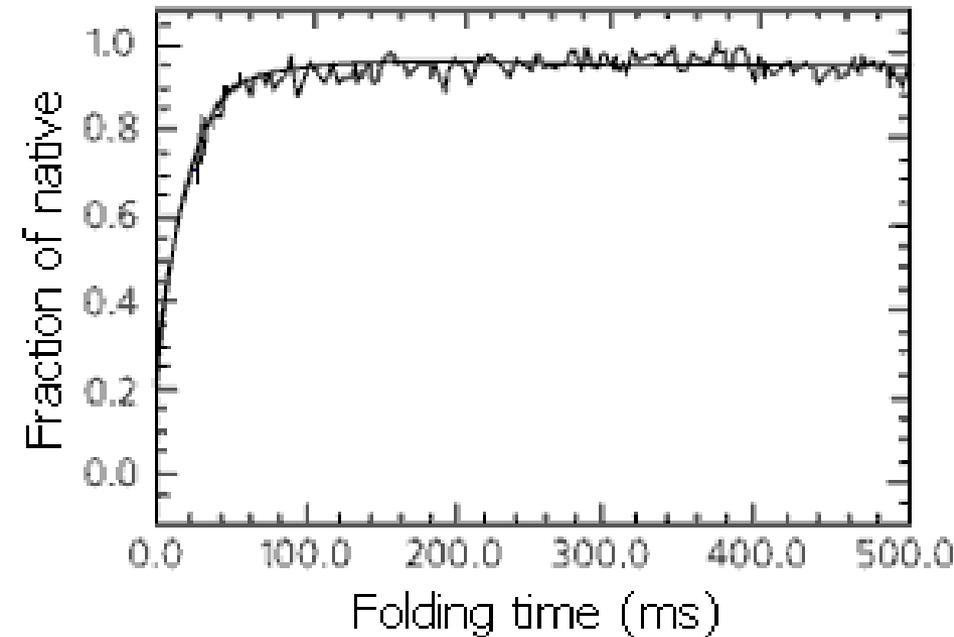
A progress in the understanding was achieved when studies involved small proteins (of 50 - 100 residues).

Many of them are “**two-state folders**”: they fold *in vitro* without any observable (accumulating) intermediates, and have only **two** observable states: the **native** fold and the **denatured** coil.

“Two-state” folding: without any observable (accumulating in experiment) intermediates

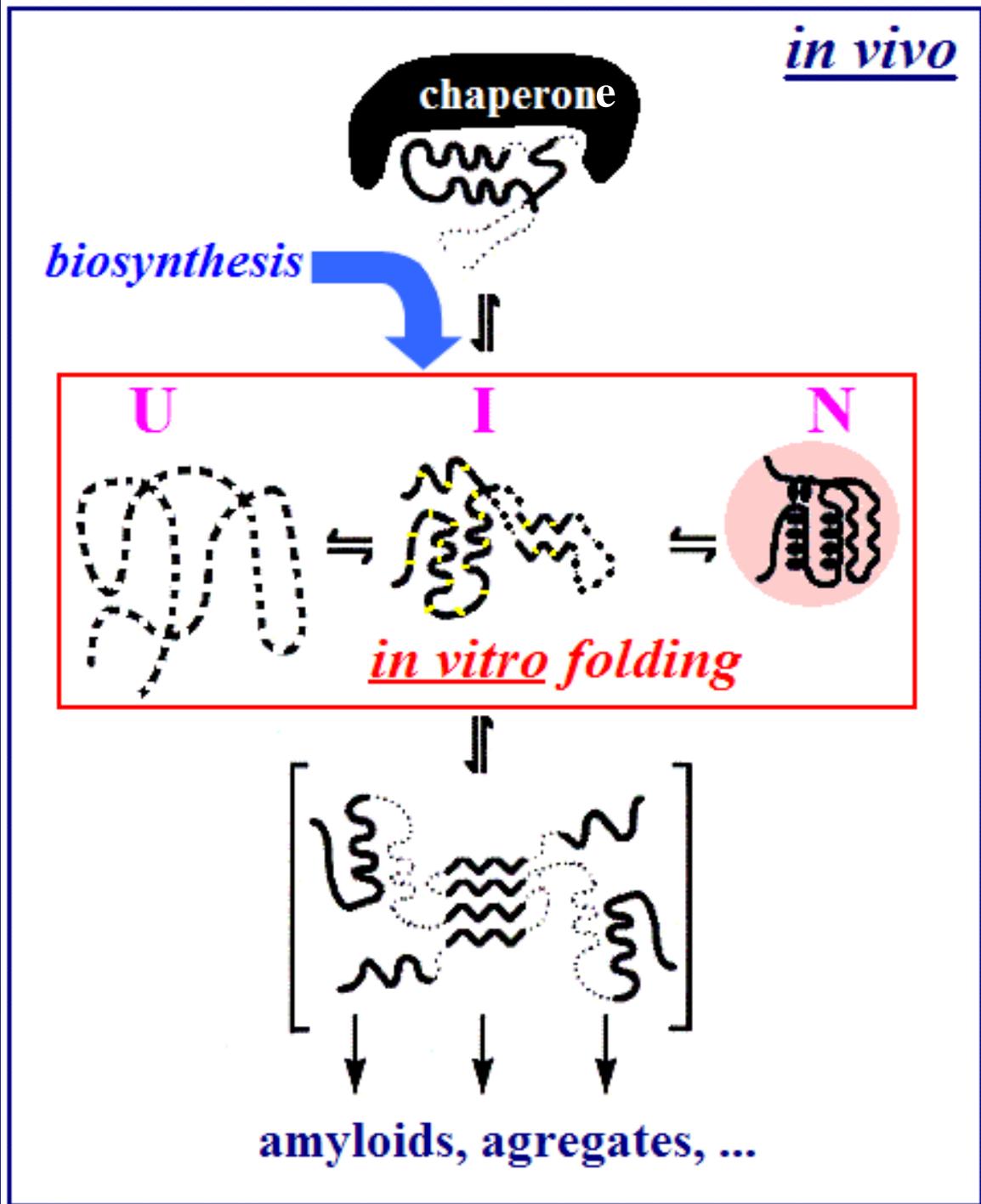
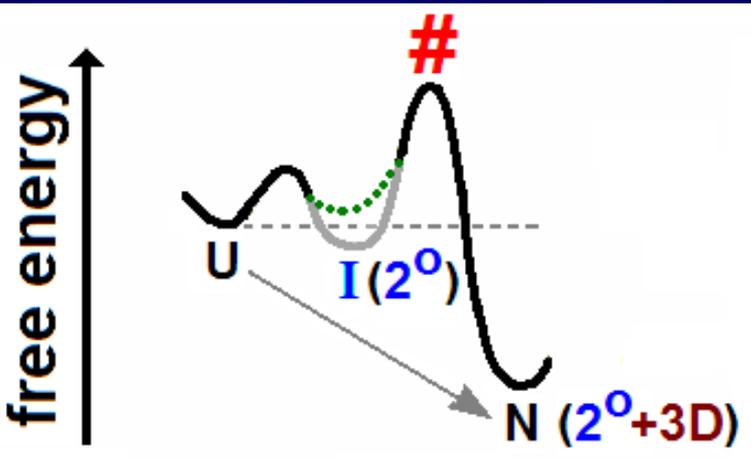
a: secondary structure

b: side chain packing



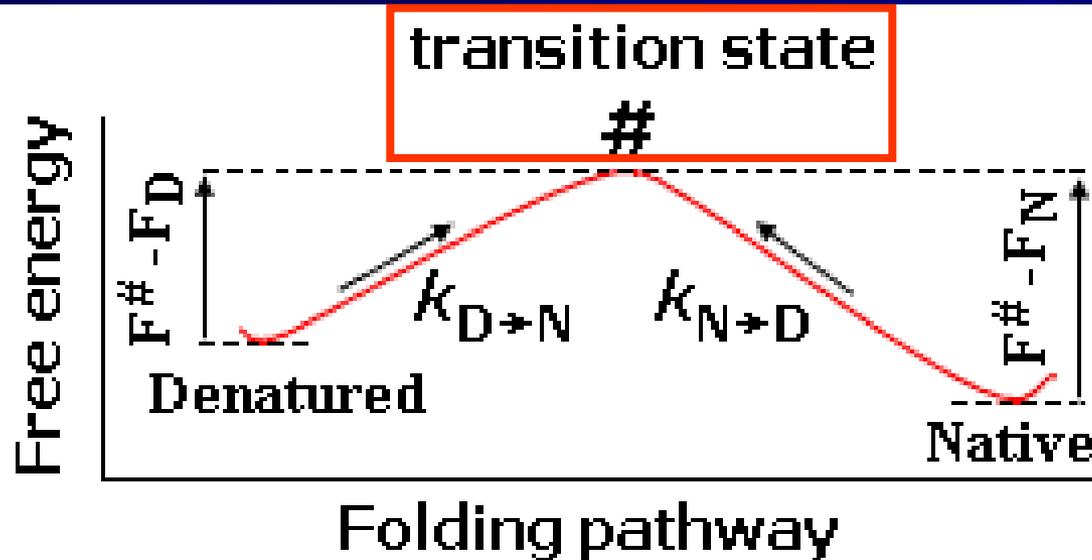
The “two-state folders” fold rapidly: not only much faster than larger proteins (not a surprise), but as fast as small proteins having folding intermediates (that were expected to accelerate folding...)

PROTEIN FOLDING: current picture



What to study in the “two-state” folding where there are no intermediates to single out and investigate?

Answer: just here one has the best opportunity to study the *transition state*, the bottleneck of folding.



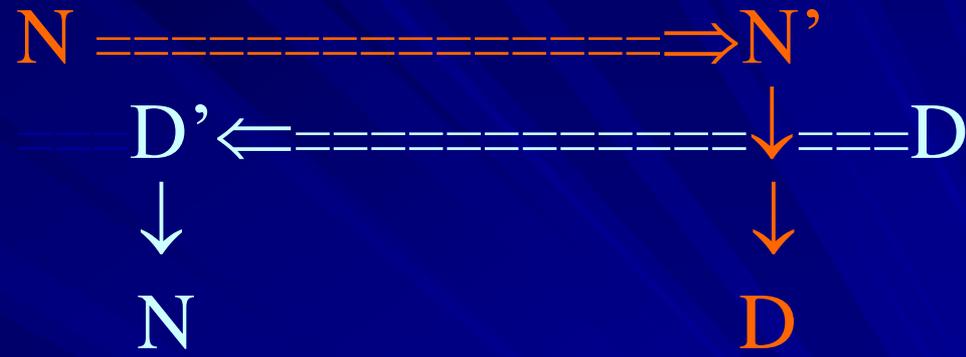
“detailed balance”:
the same pathways
for $D \rightarrow N$
and $N \rightarrow D$

$$k_{D \rightarrow N} = k_0 \exp[-(F^\# - F_D)/RT], \quad k_{N \rightarrow D} = k_0 \exp[-(F^\# - F_N)/RT]$$

EXPERIMENT GIVES: $k_{\text{apparent}(D \leftrightarrow N)} = k_{D \rightarrow N} + k_{N \rightarrow D}$
to equilibrium

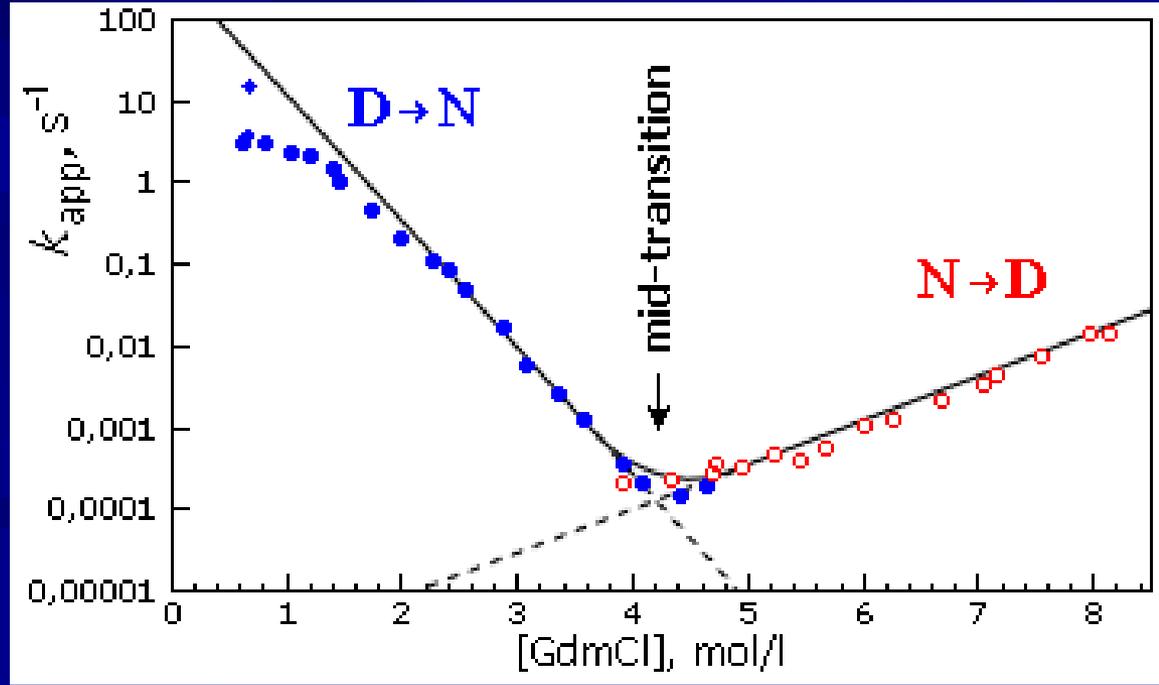
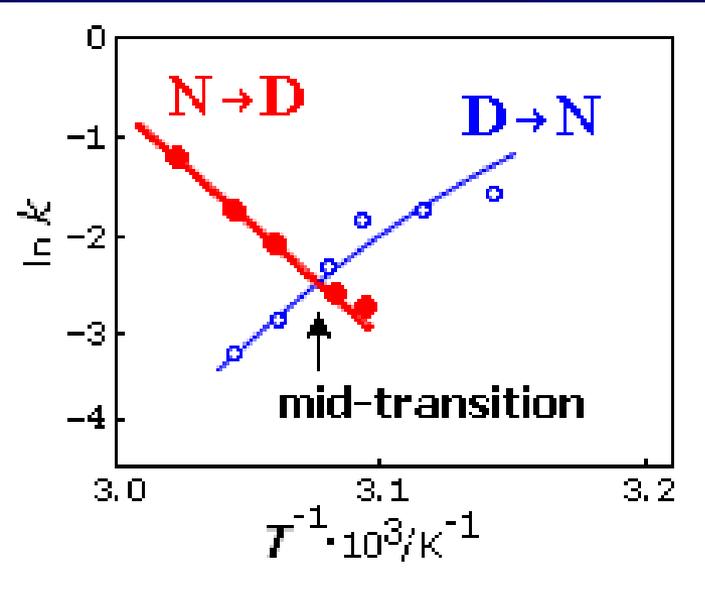
$$-\ln(k_{D \rightarrow N}/k_{N \rightarrow D}) = (F_N - F_D)/RT$$

“Chevron plots”:
*Reversible folding
 and unfolding even
 at mid-transition,*
 where $k_{D \rightarrow N} = k_{N \rightarrow D}$



(b) “Chevron plot”

(a)



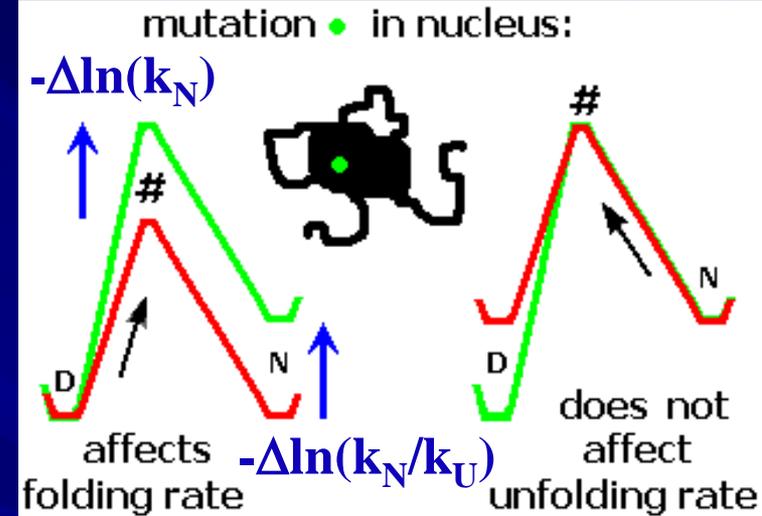
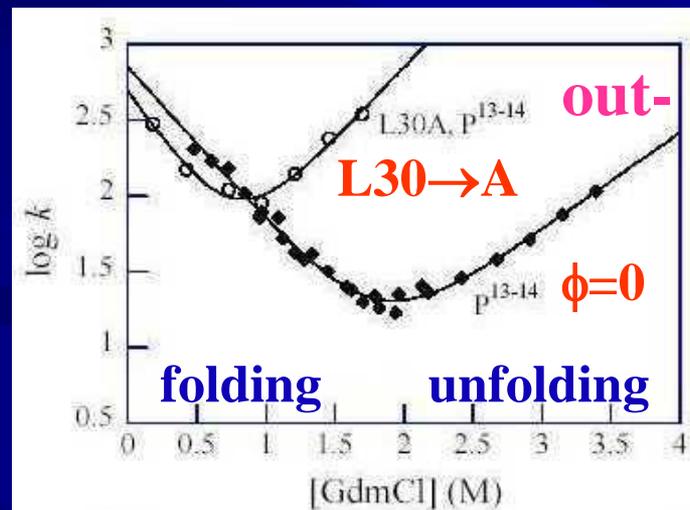
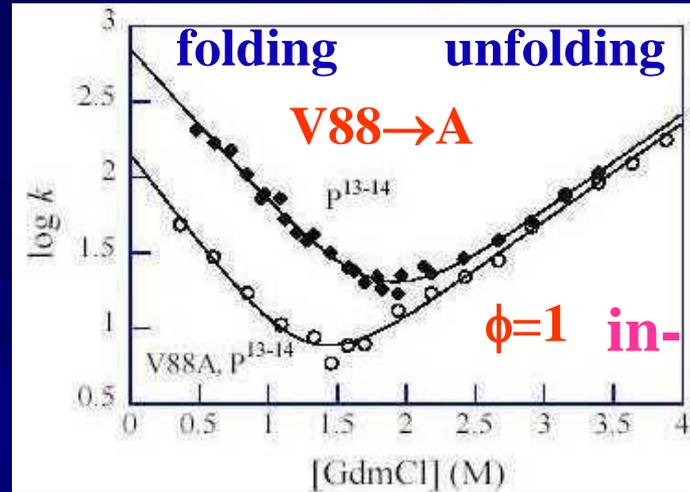
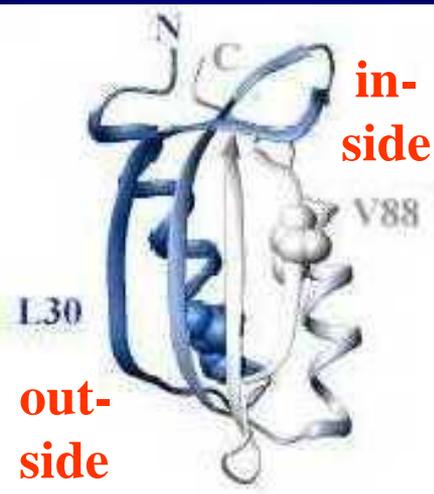


Sir Alan Roy Fersht, 1943

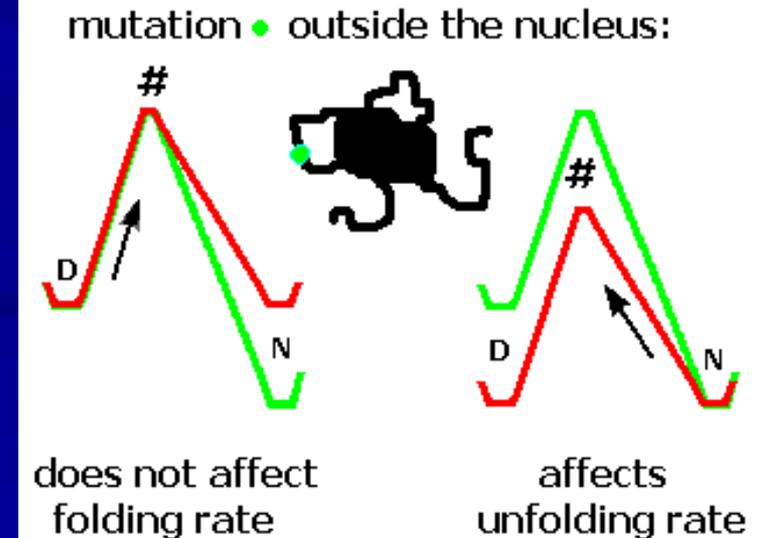
Protein engineering

Folding nucleus

Folding nucleus: Site-directed mutations show residues belonging and not-belonging to the “nucleus”, the native-like part of transition state (Fersht, 1989)



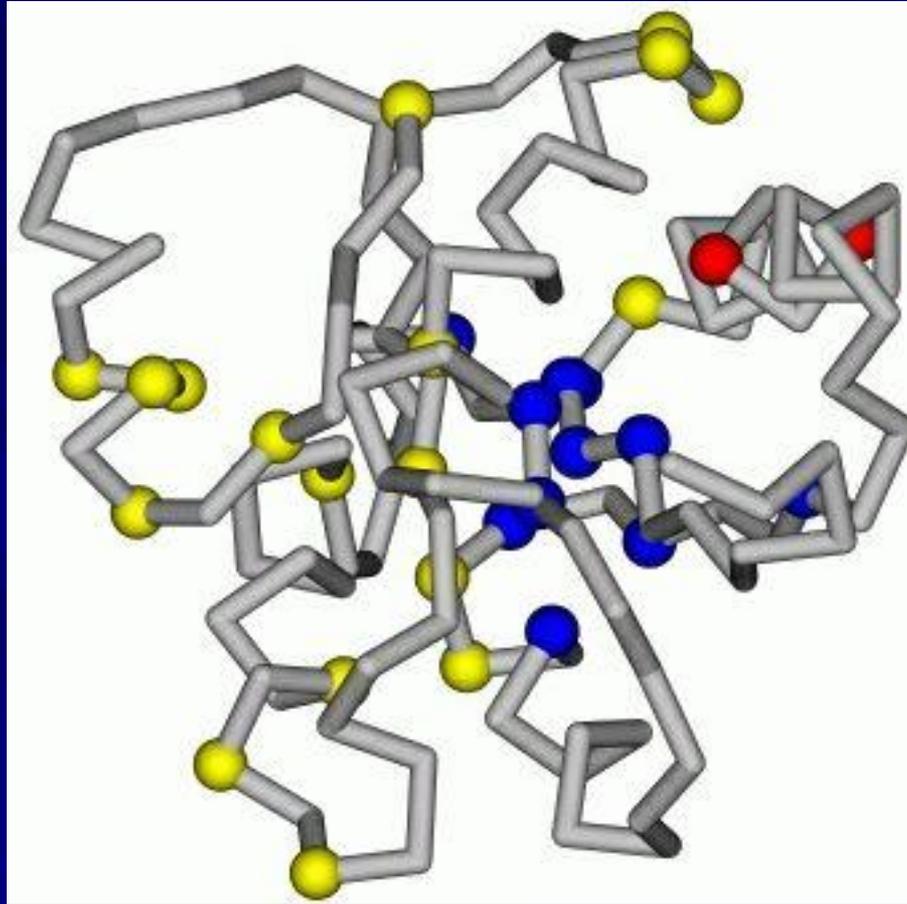
— "wild type" — mutated



$$\phi = \frac{\Delta \ln(k_N)}{\Delta \ln(k_N/k_U)}$$

Folding nucleus in CheY protein

(Lopez-Hernandes & Serrano, 1996)



● In nucleus

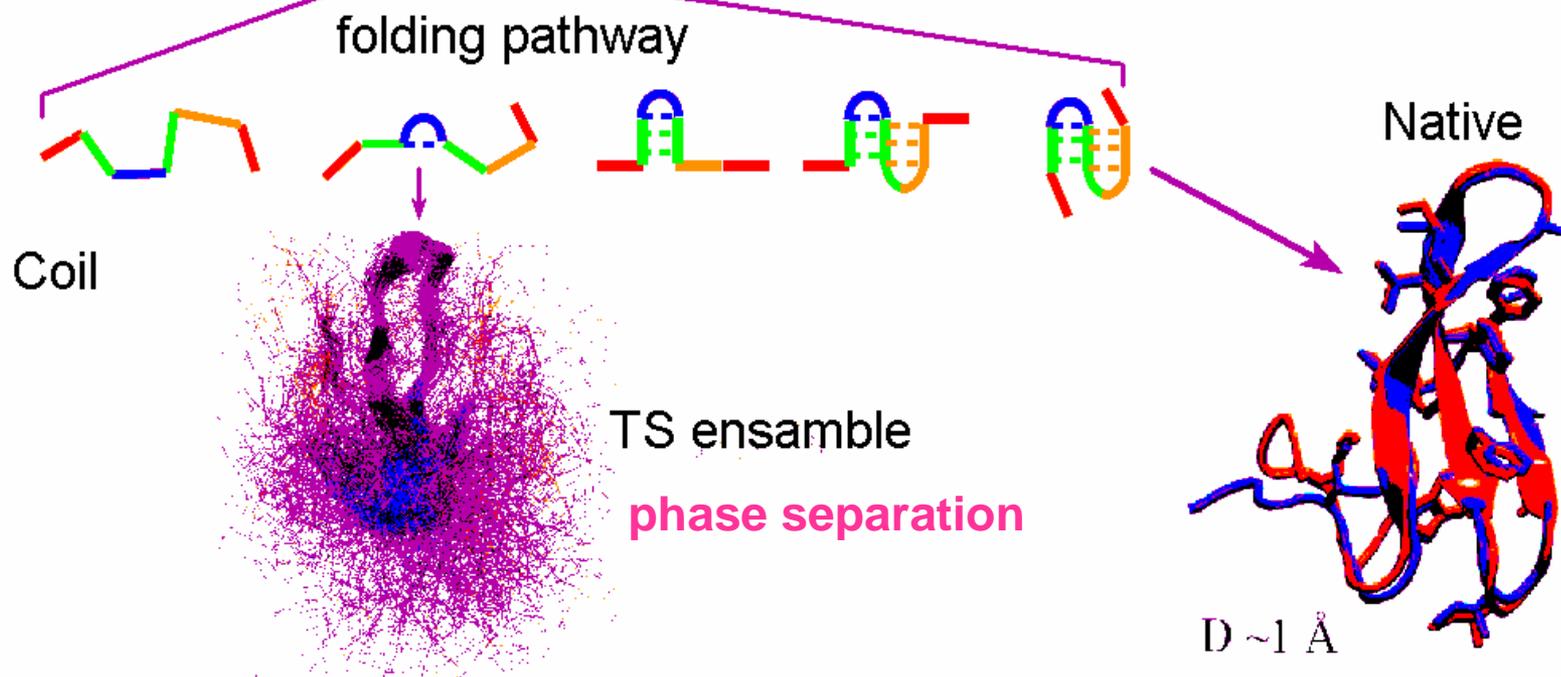
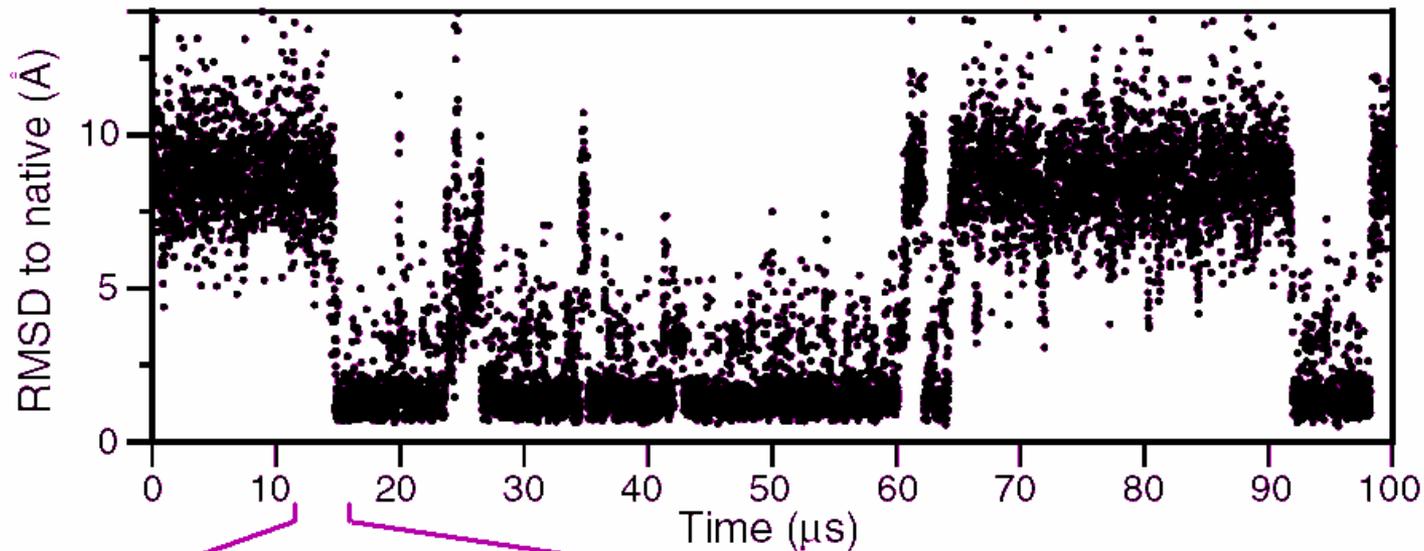
● Outside

● “difficult”

Folding nucleus is often shifted to some side of protein globule and does not coincide with its hydrophobic core; folding nucleus is **NOT** a molten globule

FIP35 protein: simulation of folding

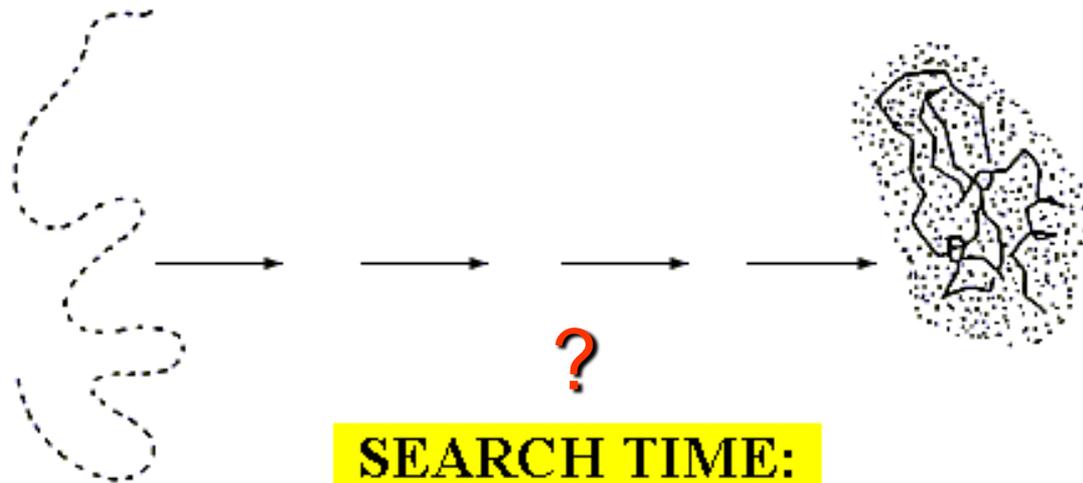
D.E. Shaw et al., Oct. 2010, *Science* **330**, 341



Back to Levinthal paradox

unfolded chain:
at least $\sim 2^{100}$
conformations
(for 100-residue chain)

native structure:
1 conformation



SEARCH TIME:

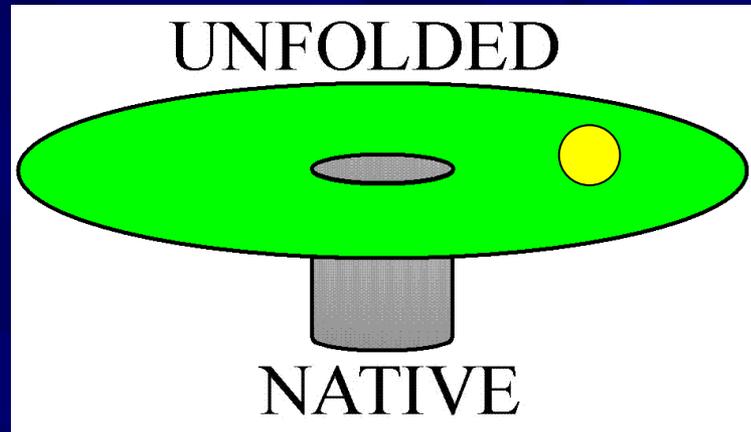
$$2^{100} \text{ conf.} \times 10^{-13} \text{ sec/conf.} = \sim 10^{10} \text{ years...}$$

Native protein structure reversibly refolds from various starts, i.e., it is thermodynamically stable.

But how can protein chain find this unique structure - within seconds - among zillions alternatives?

However, the same problem – how to find one configuration among zillions – is met by crystallization and other 1-st order phase transitions.

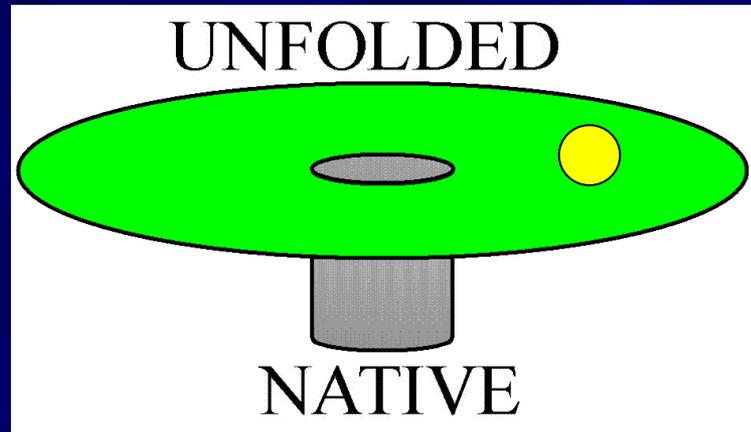
Is “Levinthal paradox” a paradox at all?



L-dimensional
“Golf course”

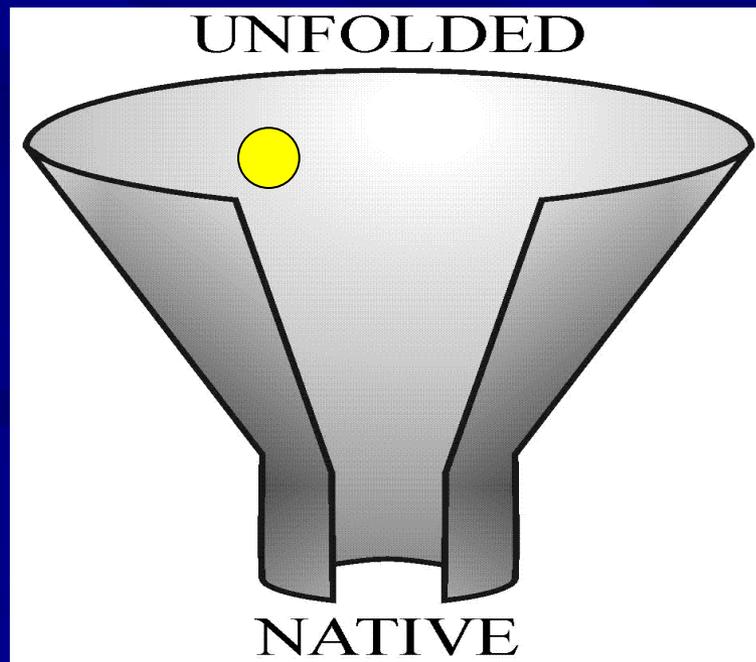


Is “Levinthal paradox” a paradox at all?



**L-dimensional
“Golf course”**

...any tilt of energy surface solves this “paradox”... (?)



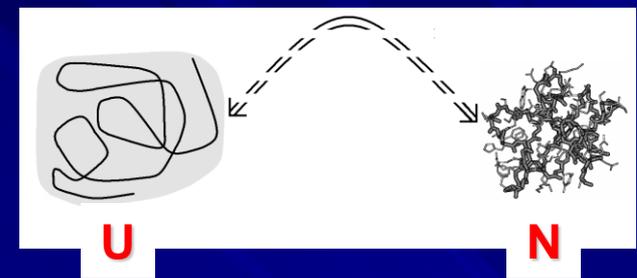
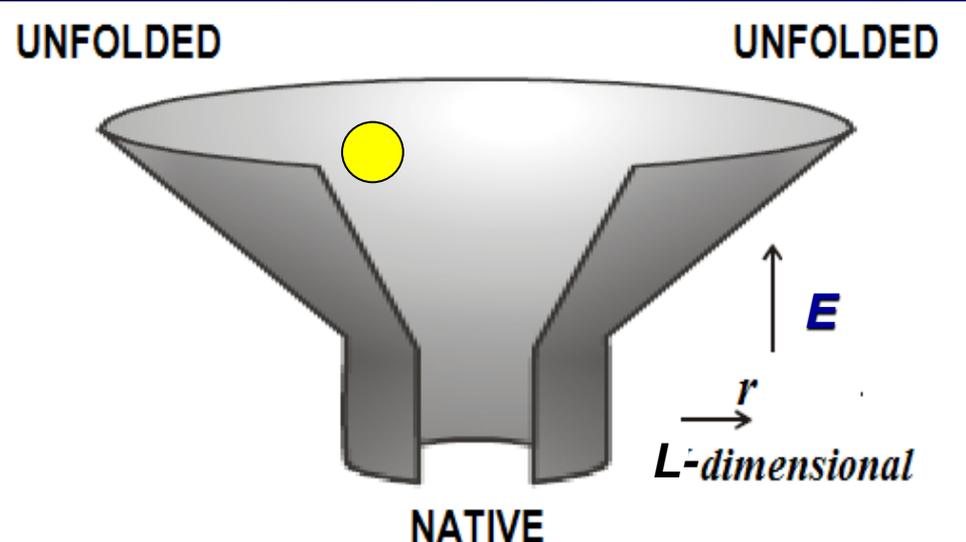
**Simple
L-dimensional
“funnel”
(without phase
separation)**

**“Funnel”:
entropy_by_energy
compensation**

Zwanzig, 1992;
Bicout & Szabo, 2000

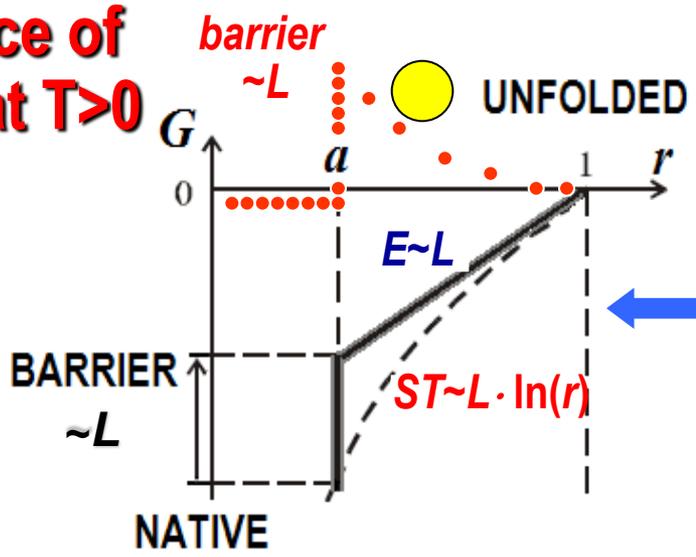
L-dimensional "folding funnel"?

Sly simplicity of a "folding funnel" (without phase separation)



All-or-none transition for 1-domain proteins
 (in thermodynamics: Privalov, 1974;
 in kinetics: Segava, Sugihara, 1984)

Resistance of entropy at $T > 0$



- NO simultaneous explanation to
 (I) "all-or-none" transition
 (II) folding within non-astron. time at mid-transition

Funnel helps, but ONLY when T is much lower than $T_{mid-tr.}$!!

A special pathway?

Phillips (1965) hypothesis:

folding nucleus is formed by the N-end of the nascent protein chain, and the remaining chain wraps around it.

for single-domain proteins: **NO:**

Goldenberg & Creighton, 1983:

circular permutants:

N-end has no special role in the *in vitro* folding.

However, for many-domain proteins:

Folding from N-end domain, \approx domain after domain

DO NOT CONFUSE N-END DRIVEN FOLDING WITHIN DOMAIN

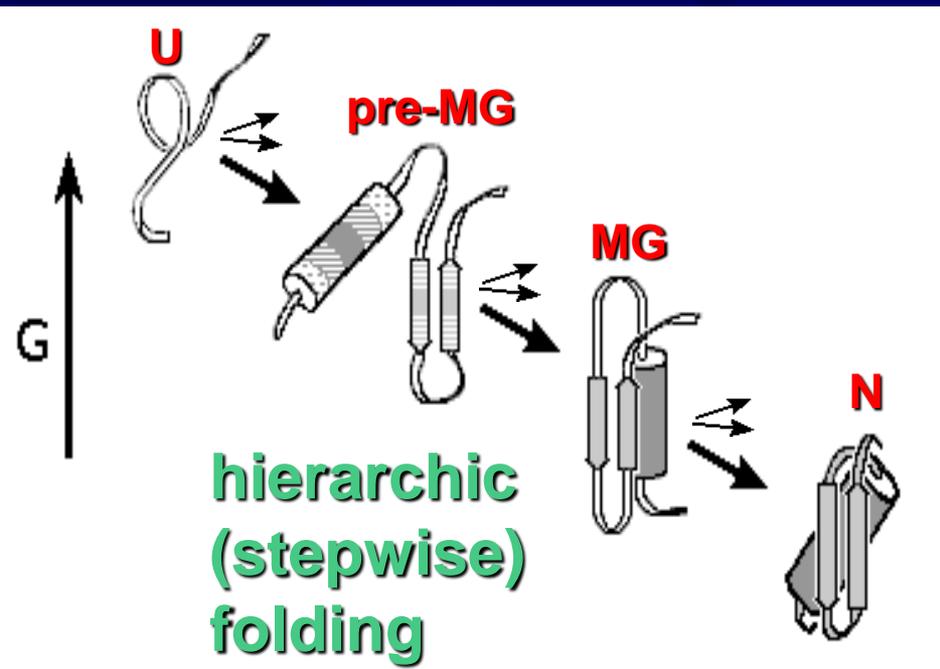
(which seems to be absent)

and

N-DOMAIN DRIVEN FOLDING IN **MANY-DOMAIN PROTEIN**

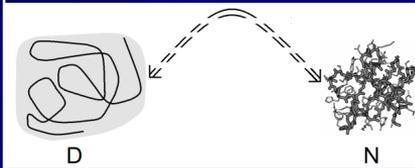
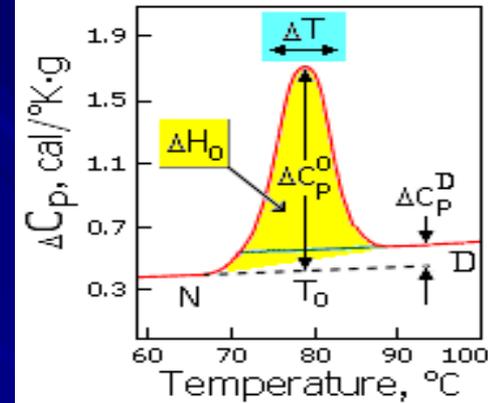
(which is observed indeed)

Sly simplicity of hierarchic folding as applied to resolve the Levinthal paradox

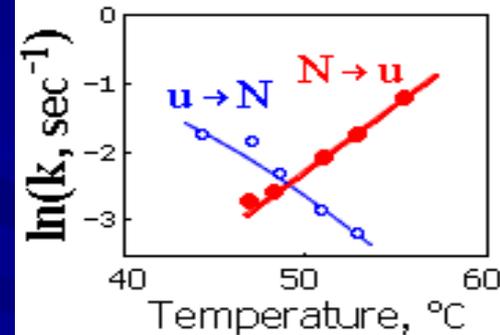


All-or-none transition:

In thermodynamics



In kinetics



Folding intermediates

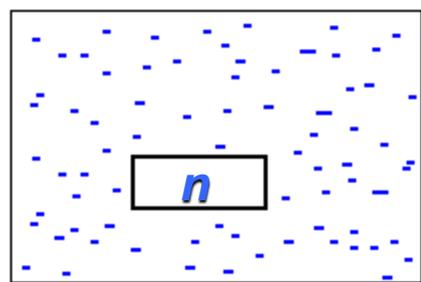
must become more and more stable for hierarchic folding.

This cannot provide a *simultaneous* explanation to

- (i) folding within non-astronomical time;
- (ii) “all-or-none” transition, i.e., co-existence of only native and denatured molecules in visible amount;
- (iii) the same 3D structure resulting from different pathways

1-st order phase transition: rate of nucleation

Crystallization, classic theory



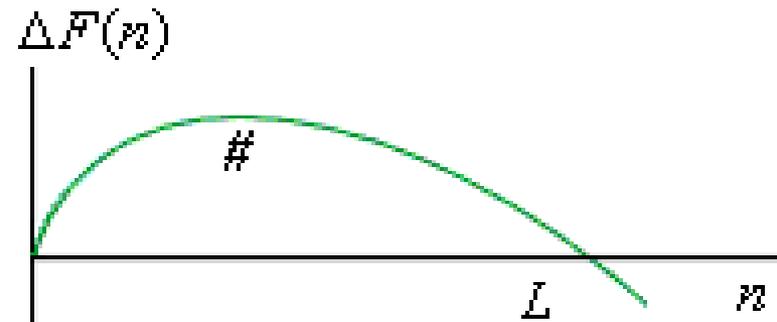
$$\text{INITIATION TIME} \sim \tau \times \exp(+\Delta F^{\#}/RT)$$

$\Delta F^{\#}$: free energy of critical nucleus

τ : time of 1 step (\sim ps \div ns)

CONSECUTIVE REACTIONS:

TRANSITION TIME \cong SUM OF TIMES \approx Max. barrier TIME



$\Delta F(n) = \Delta\mu n + Bn^{2/3}$: free energy of n -particle nucleus. New phase: $\Delta\mu < 0$.

Critical nucleus [from $d(\Delta F)/dn = 0$]: Max. ΔF : $\Delta F^{\#} = (4/27)B^3/(\Delta\mu)^2$

$$n^{\#} = (8/27)B^3/(-\Delta\mu)^3$$

Min. size L of stable new phase [from $\Delta F(L) = 0$]: $L = B^3/(-\Delta\mu)^3$

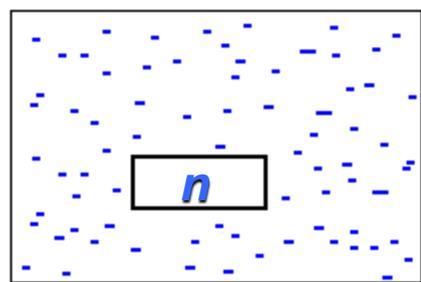
Then $\Delta F^{\#} = (4/27)BL^{2/3}$,

$$n^{\#} = (8/27)L$$

$$\text{INITIATION TIME} \sim \tau \times \exp(\text{const} \times L^{2/3})$$

1-st order phase transition: rate of nucleation

Crystallization, classic theory



INITIATION TIME $\sim \tau \times \exp(+\Delta F^\ddagger/RT)$

ΔF^\ddagger : free energy of critical nucleus

τ : time of 1 step (\sim ps \div ns)

CONSECUTIVE REACTIONS:

TRANSITION TIME \cong SUM OF TIMES \approx Max. barrier TIME

$\Delta\mu \approx -\Delta T \cdot (H_m/T_m)$ $B \sim H_m$

$\Delta F(n) = \Delta\mu n + Bn^{2/3}$: free energy of n -particle nucleus. New phase: $\Delta\mu < 0$.

Critical nucleus [from $d(\Delta F)/dn = 0$]: Max. ΔF : $\Delta F^\ddagger = (4/27)B^3/(\Delta\mu)^2$

$$n^\ddagger = (8/27)B^3/(-\Delta\mu)^3$$

Min. size L of stable new phase [from $\Delta F(L) = 0$]: $L = B^3/(-\Delta\mu)^3 \approx (T_m/\Delta T)^3$

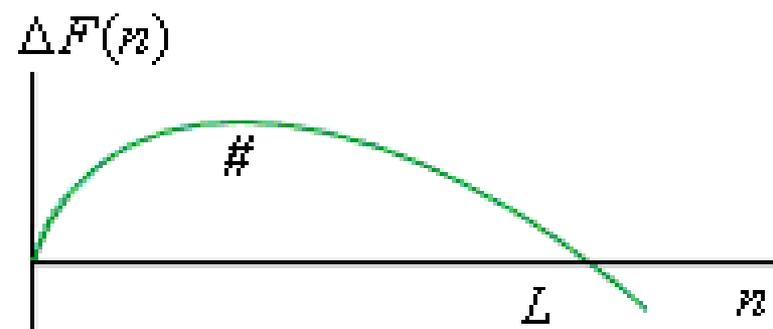
Then $\Delta F^\ddagger = (4/27)BL^{2/3}$,

$n^\ddagger = (8/27)L$ **ALL $\rightarrow \infty$ at $\Delta T \rightarrow 0$**

INITIATION TIME $\sim \tau \times \exp(const \times L^{2/3})$

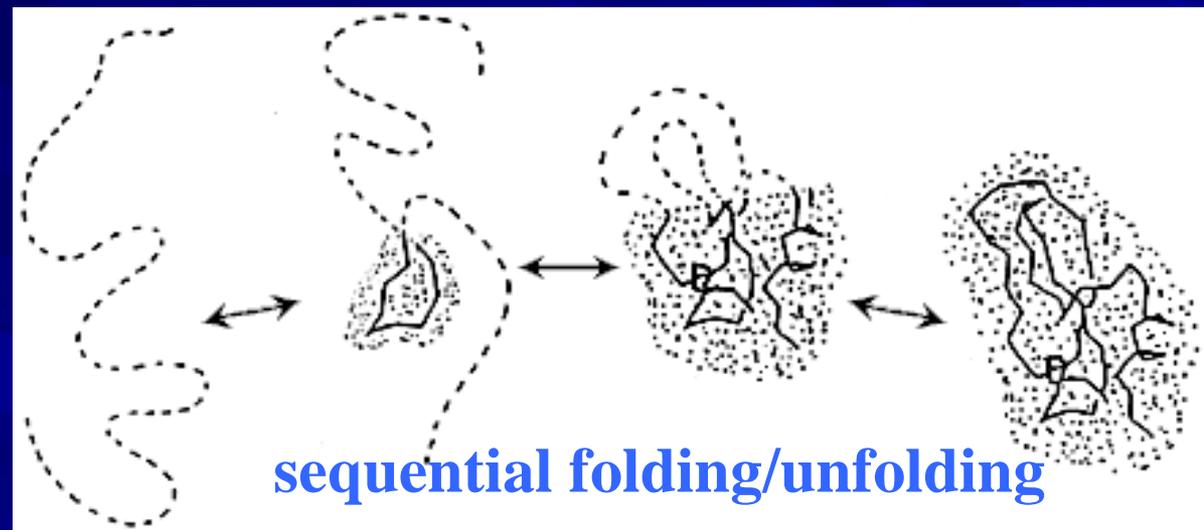
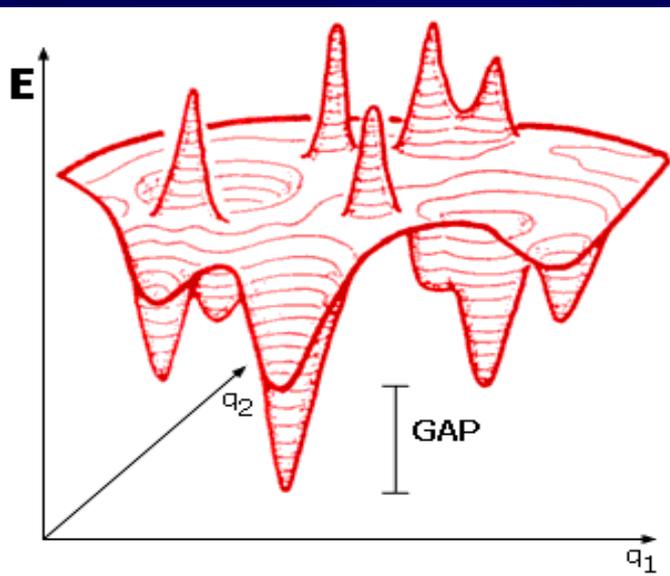
For macroscopic bodies ↓

ACTUALLY: hysteresis... INITIATION at walls, admixtures, ...



For proteins, the microscopic bodies ↓

Let us consider sequential folding (**or unfolding**) of a chain that has a large **energy gap** between the most stable fold and the bulk of the other ones; and let us consider its folding close to the thermodynamic **mid-transition**



The same pathways: “detailed balance”

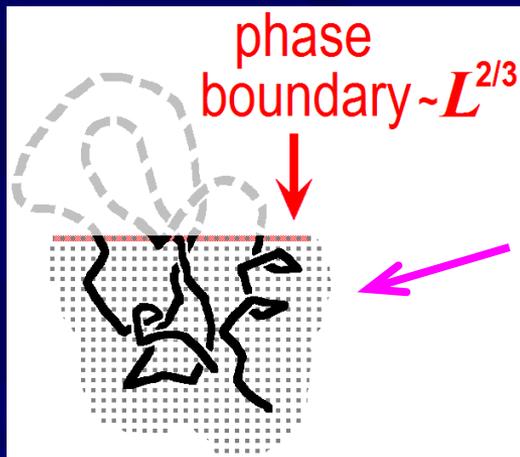
How fast the most stable fold will be achieved?

Note. Elementary rearrangement of 1 residue takes 1-10 ns. Thus, 100-residue protein would fold within μs , if there were no free energy barrier at the pathway...

HOW FAST the most stable state is achieved?

free energy barrier →

$$F(U) = F(N)$$



$$\rightarrow \Delta F^\# \sim L^{2/3} \cdot \text{surface_tension}$$

a) micro-; b) loops

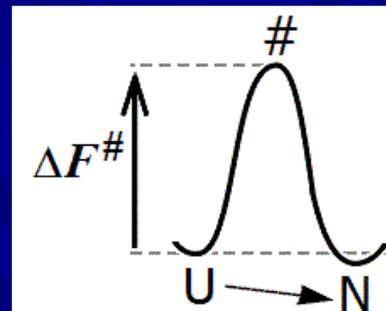
$\max\{\Delta F^\#\}$: when compact folded nucleus: $\sim 1/2$ of the chain

micro: $\Delta F^\# \approx L^{2/3} \cdot [\epsilon/4]$; $\epsilon \approx 2RT$ [experiment]

loops: $\Delta F^\# \leq L^{2/3} \cdot \frac{1}{2} [\frac{3}{2} RT \cdot \ln(L^{1/3})] + L / (\sim 100)$
 [Flory] [knots]

$$\Delta F^\# / RT \sim \left(\frac{1}{2} \div \frac{3}{2} \right) L^{2/3}$$

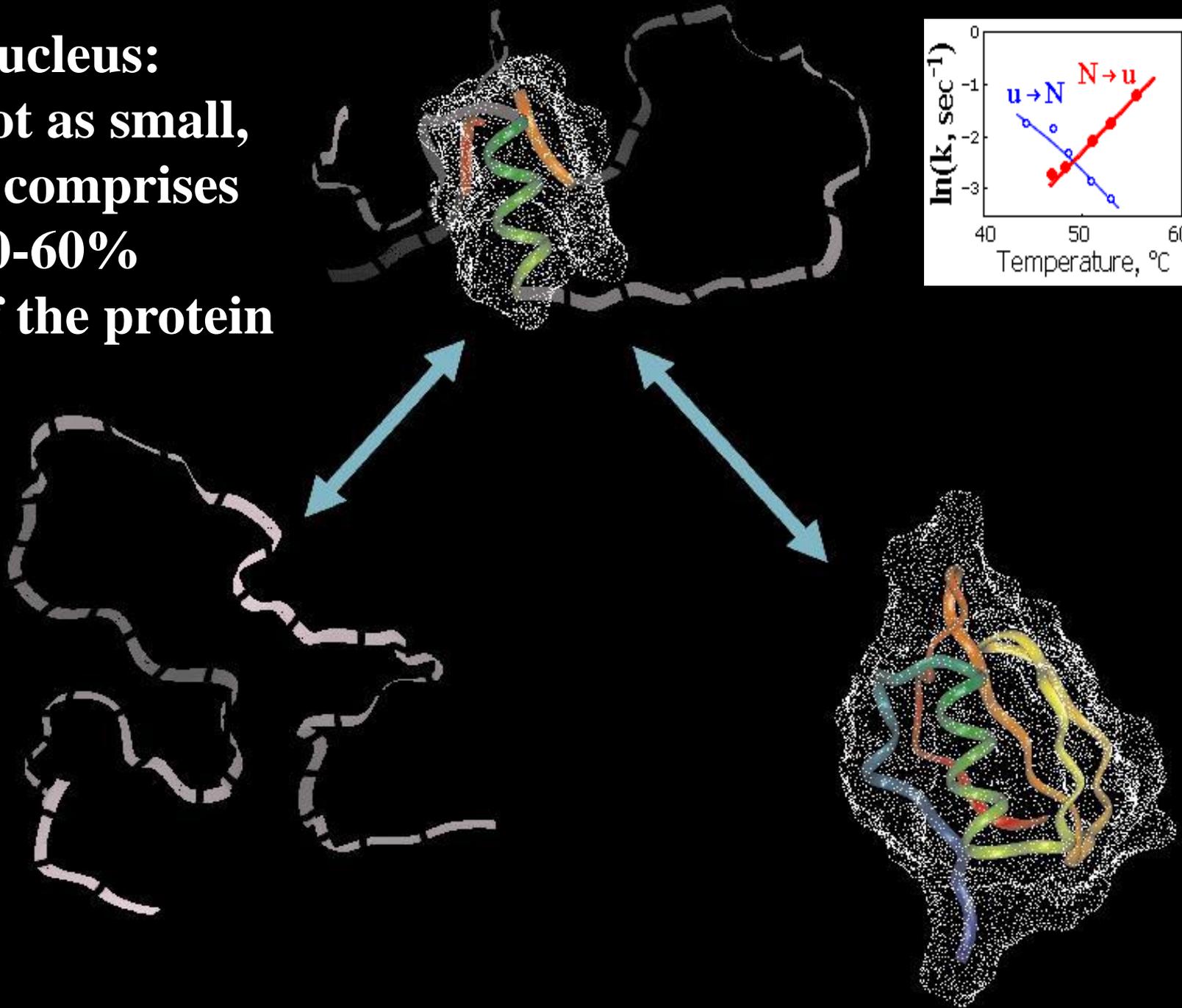
micro loops



rate $\sim \exp(-\Delta F^\# / kT)$ $\frac{1}{\text{ns}}$

Any stable fold is automatically a focus of rapid folding pathways:
“Folding funnel” with phase separation. No “special pathway” is needed.

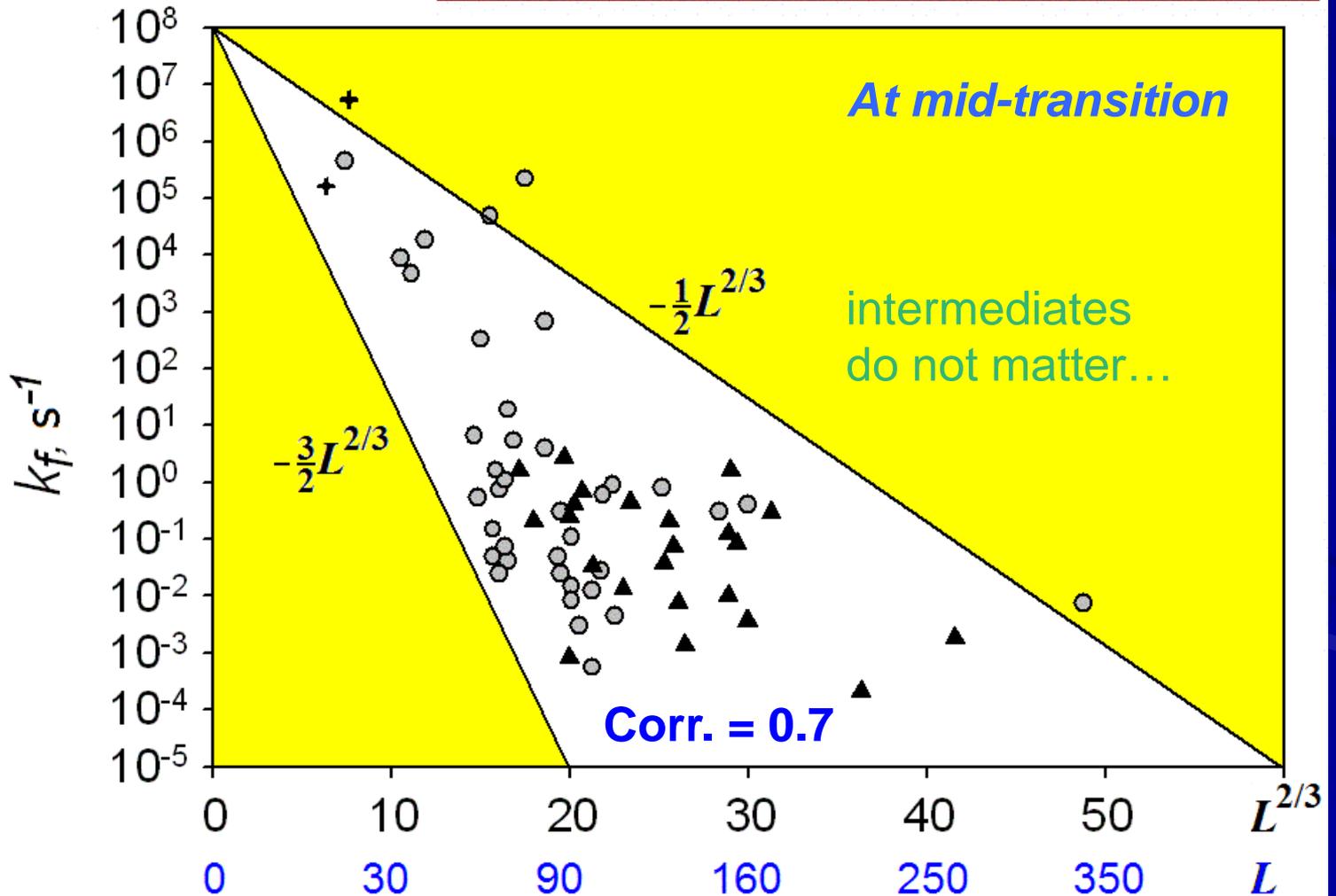
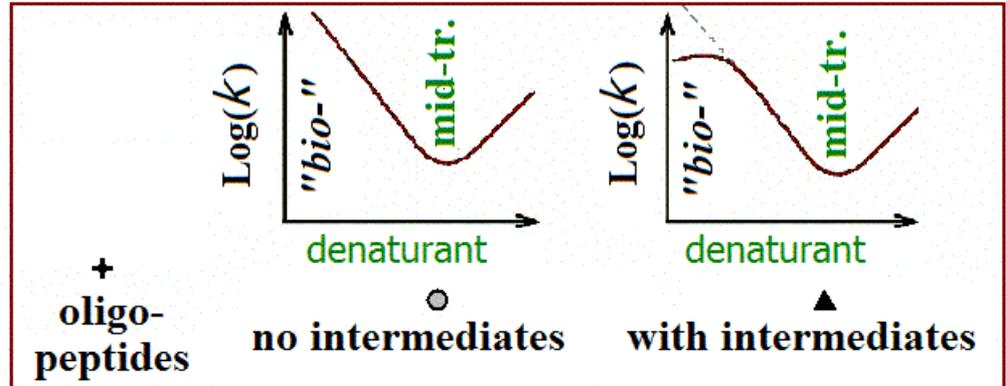
Nucleus:
not as small,
it comprises
30-60%
of the protein



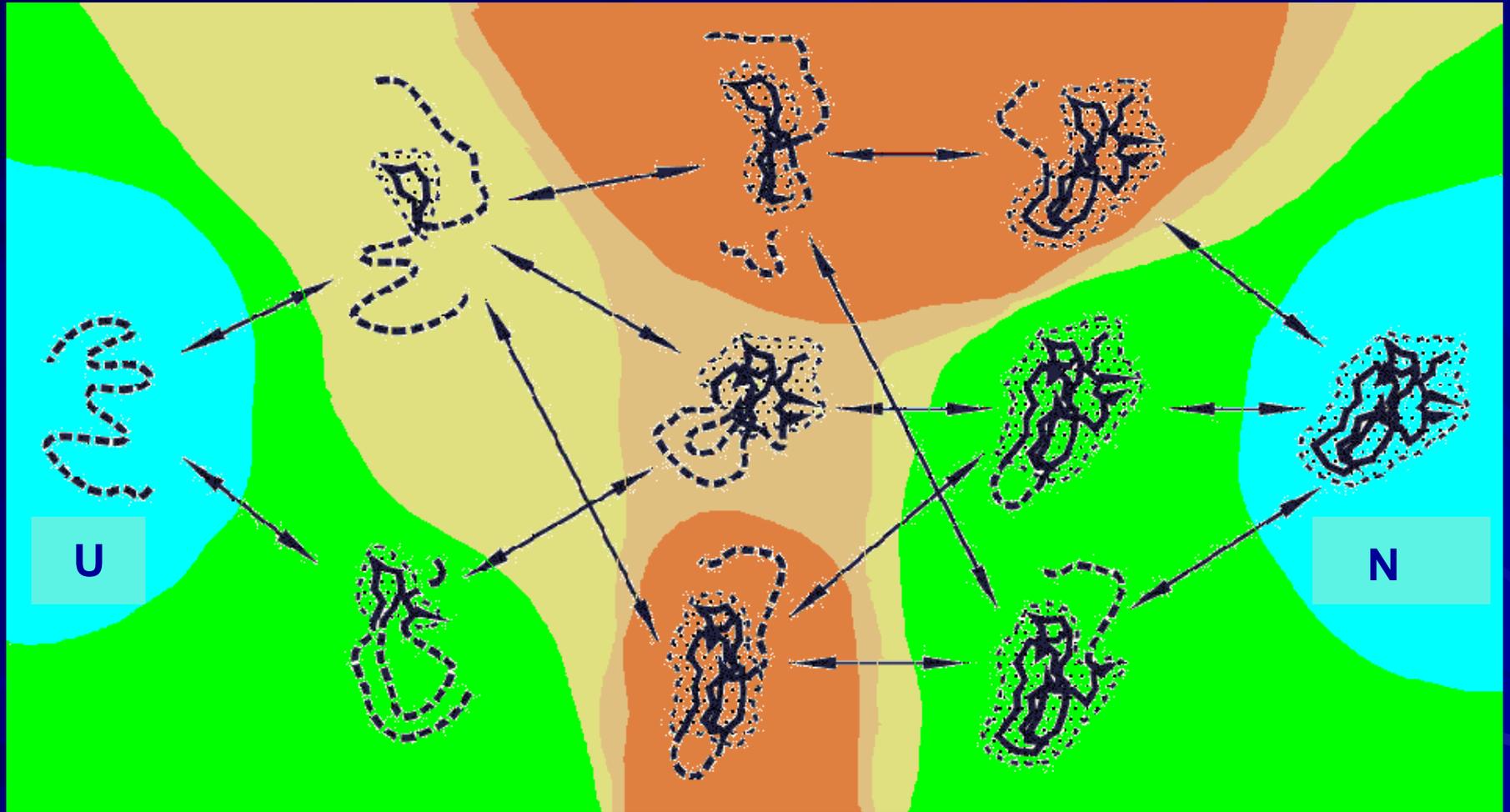
FOLDING RATE:

$$k_f \sim \exp\left[-\left(\frac{1}{2} \div \frac{3}{2}\right)L^{2/3}\right] \text{ ns}^{-1}$$

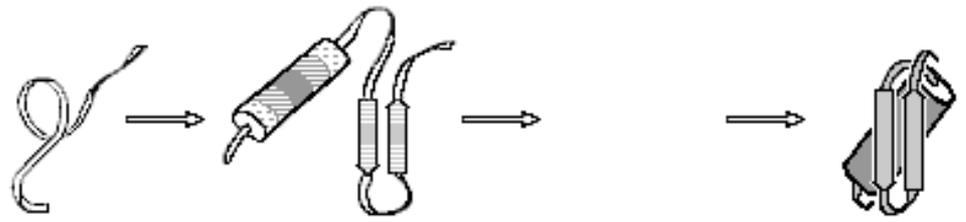
form-factor
loops



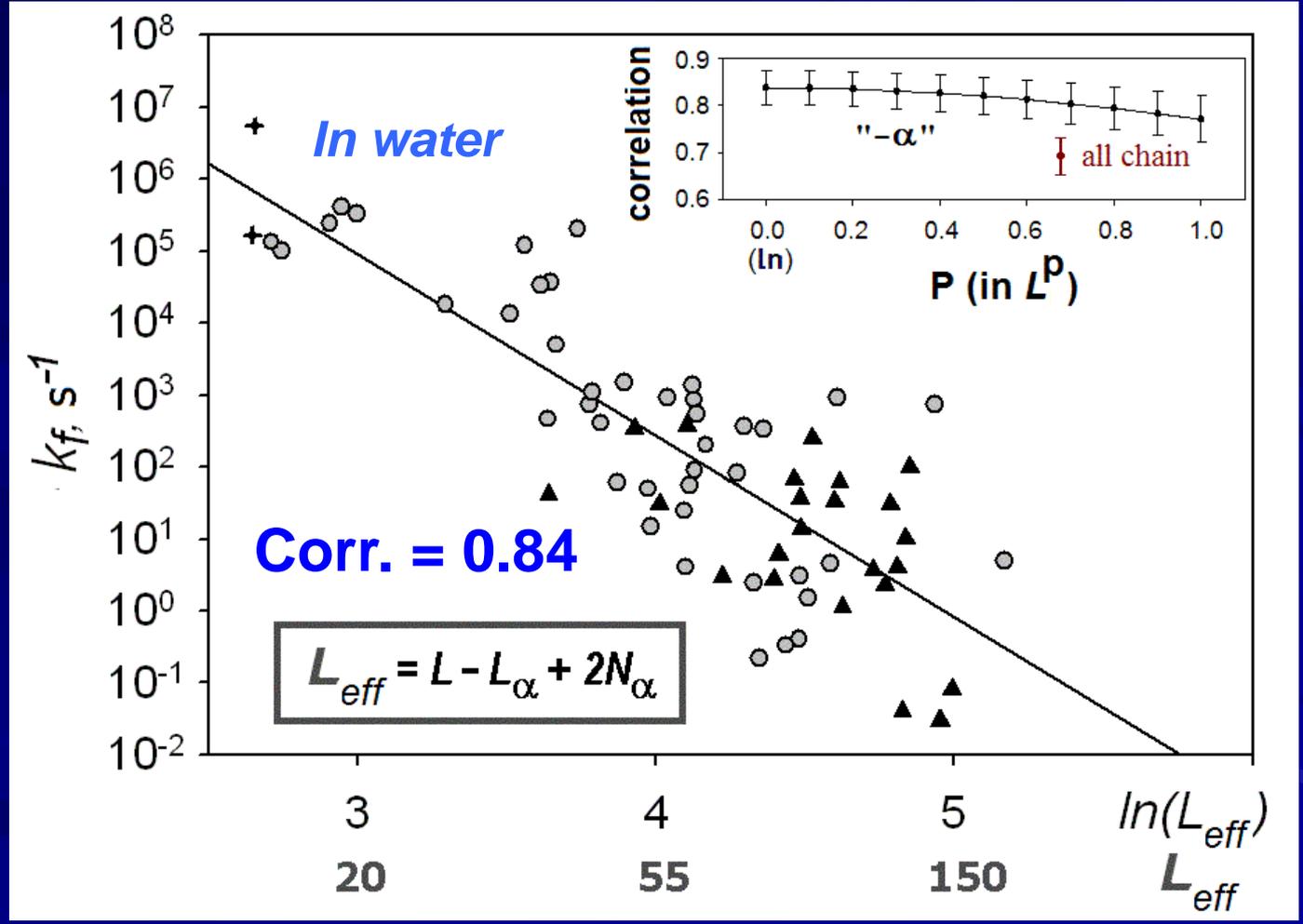
Any stable fold is automatically a focus of rapid folding pathways. No “special pathway” is needed.



α -helices decrease effective chain length. THIS HELPS TO FOLD!



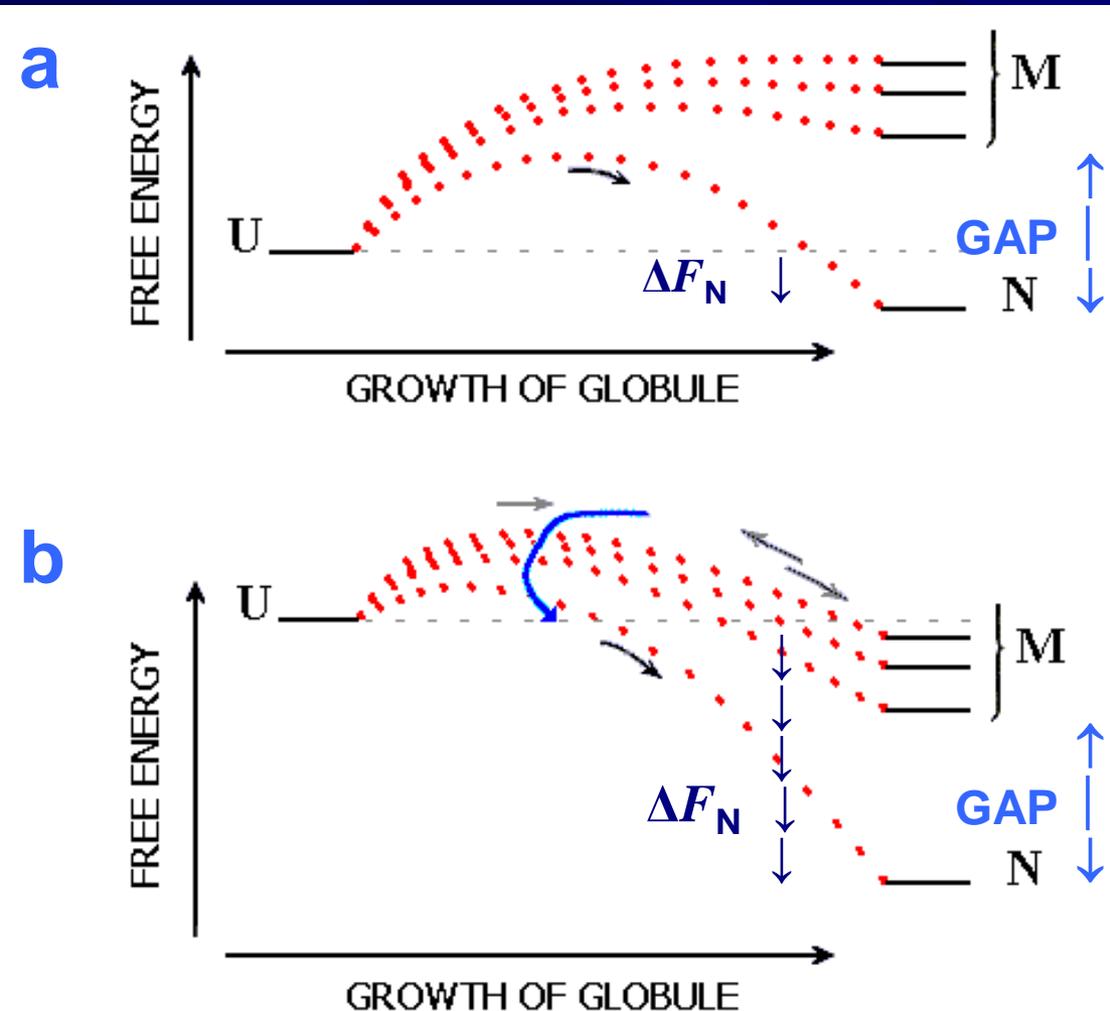
α -HELICES ARE PREDICTED FROM THE AMINO ACID SEQUENCE



Ivankov D.N., Finkelstein A.V. (2004) Prediction of protein folding rates from the amino-acid sequence-predicted secondary structure. - *Proc. Natl. Acad. Sci. USA*, 101:8942-8944.

Up to now, a vicinity of mid-transition has been considered.

When globules become more stable than U:

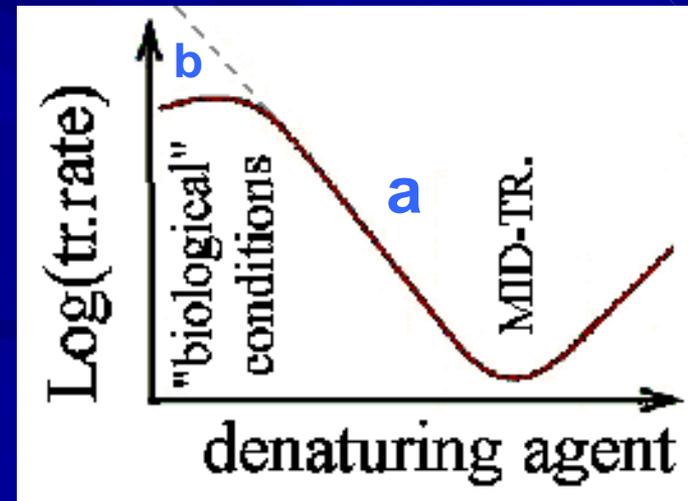


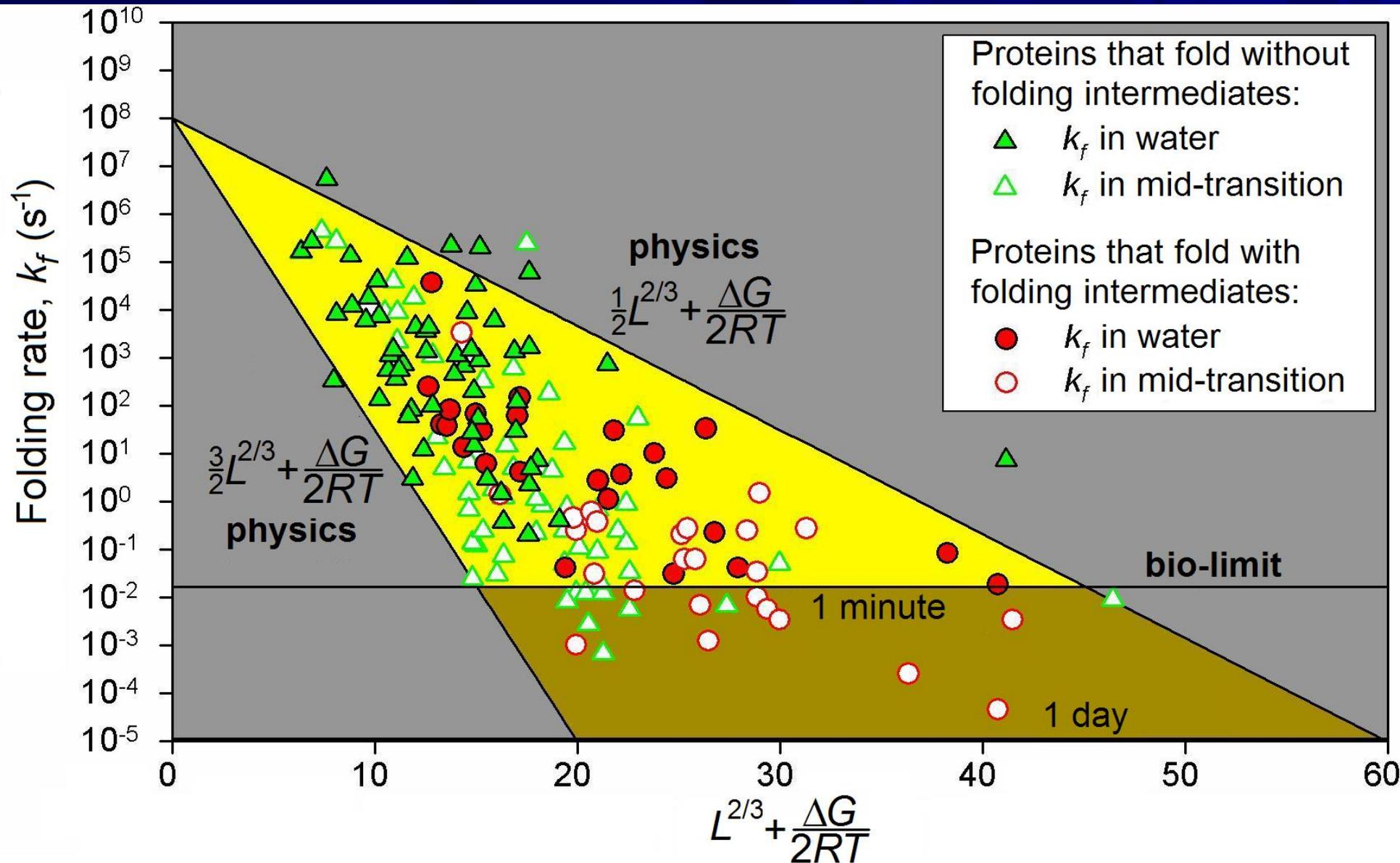
1) Acceleration:

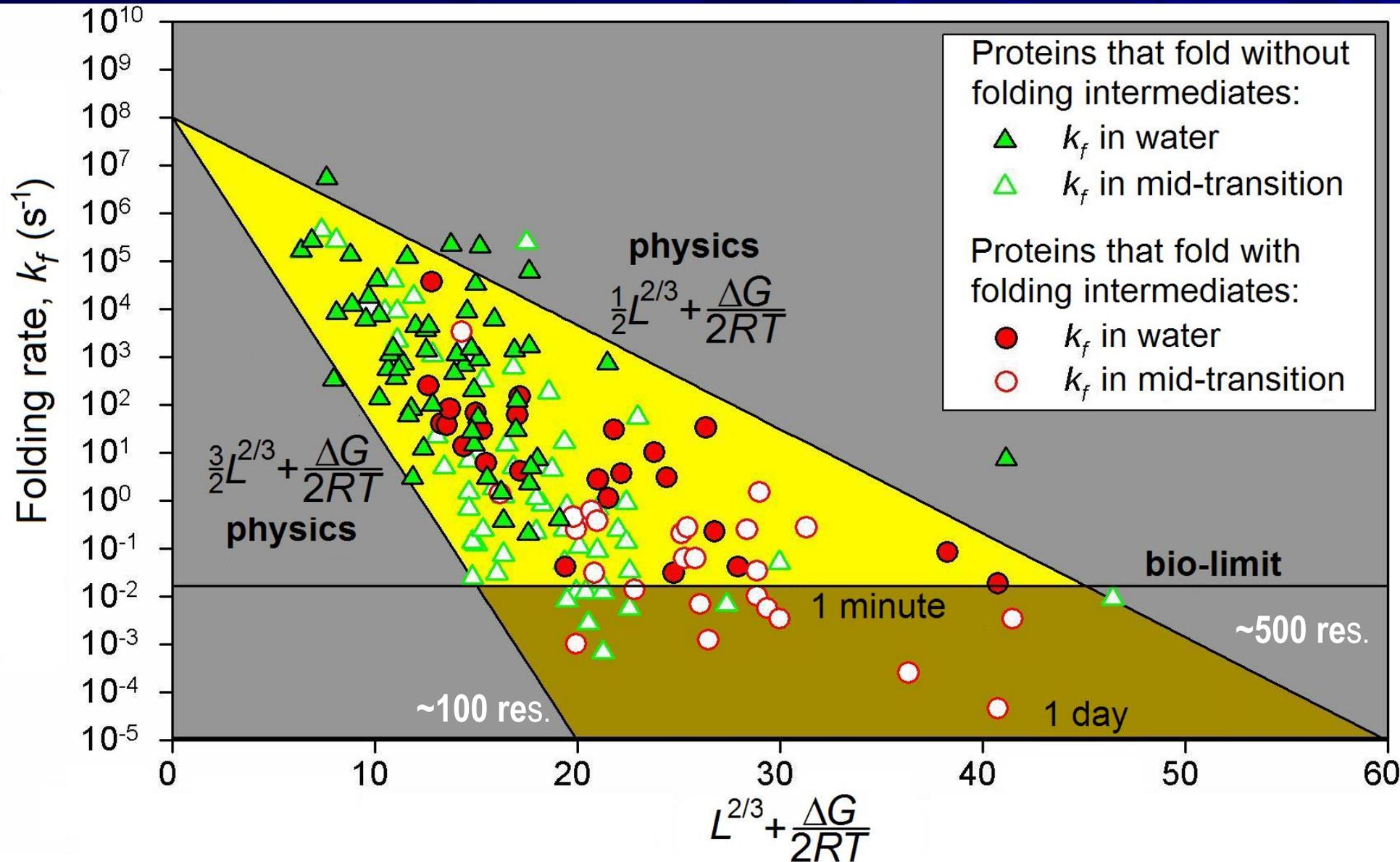
$$\Delta \ln k_f \approx -1/2 \Delta F_N / RT$$

2) Large gap \rightarrow large acceleration due to ΔF_N before

“rollover” caused by stability of intermediates M at “bio-conditions”





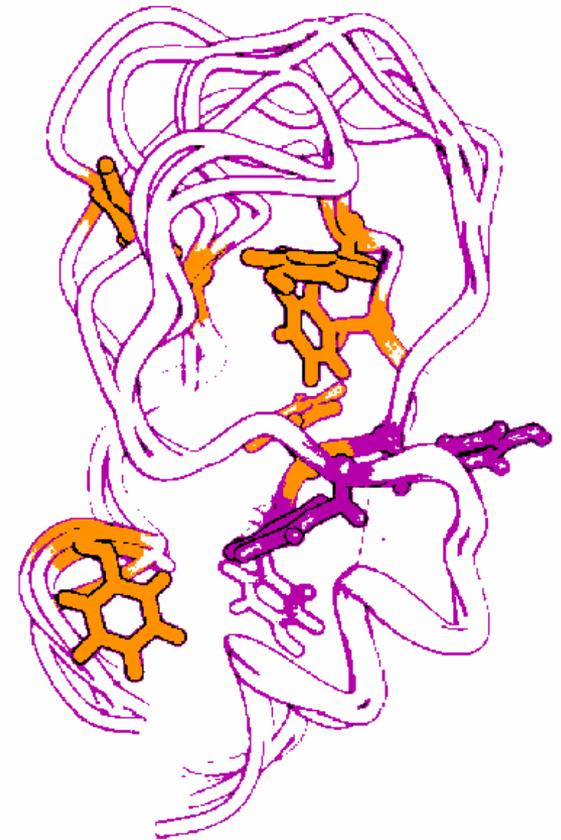
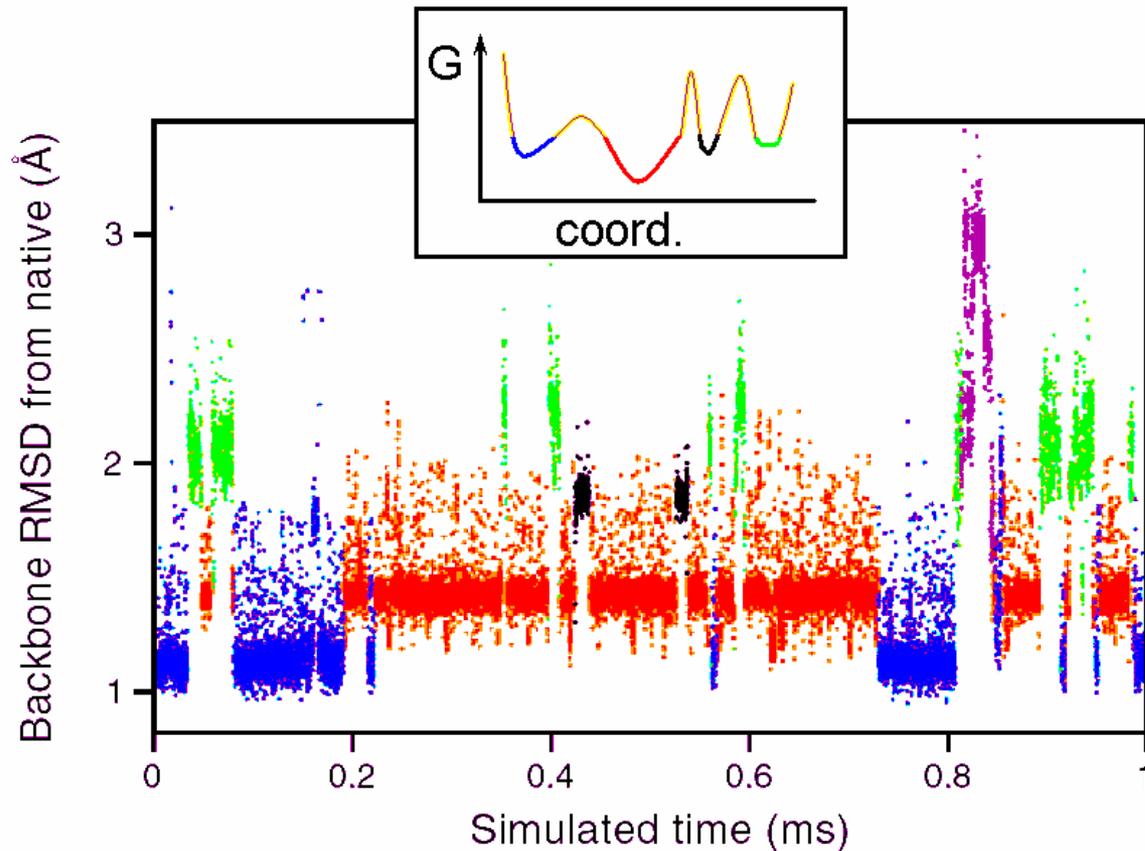


D.E. Shaw et al., Oct. 2010, *Science* **330**, 341

modified version of the Amber ff99SB force field:

K. Lindorff-Larsen *et al.*, *Proteins* **78**, 1950 (2010)

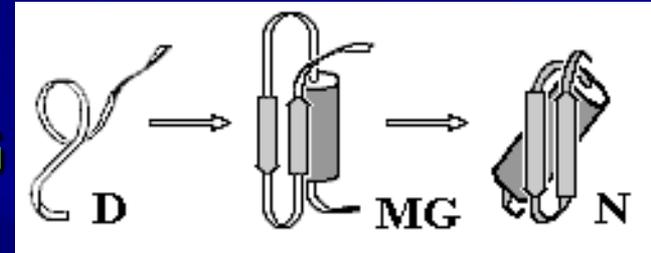
Native-state dynamics of BPTI (modeling)



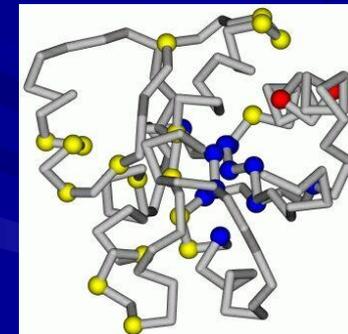
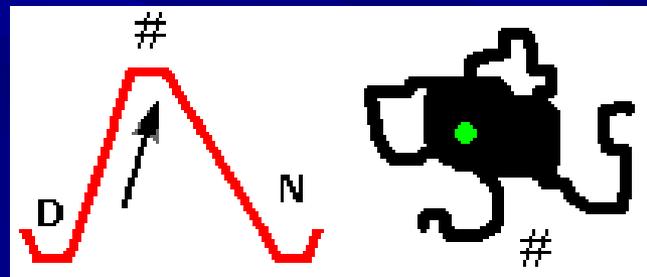
Protein Structures: Kinetic Aspects

- *In vivo* folding & *in vitro* folding
- Protein folds spontaneously: how can it?

- Protein folding intermediates; MG



- Transition state & folding nucleus



- Protein folding rate theory: solution of Levinthal's paradox

$$t \sim \exp\left[\left(\frac{1}{2} \div \frac{3}{2}\right) L^{2/3}\right] \text{ ns}$$