

PROTEIN PHYSICS

LECTURES 22-23

**PROTEIN STRUCTURE:
PREDICTION
ENGINEERING
DESIGN**

Homology

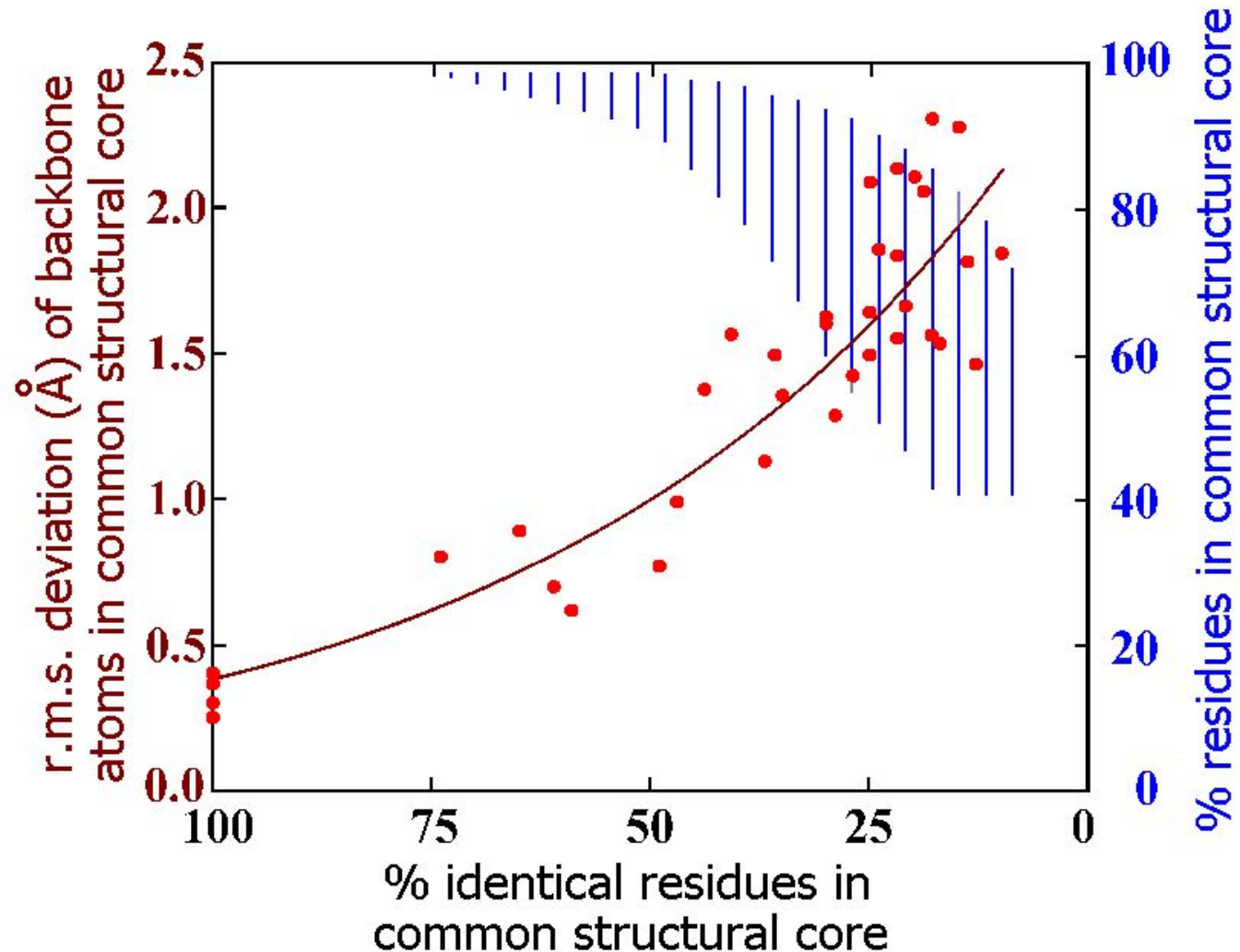
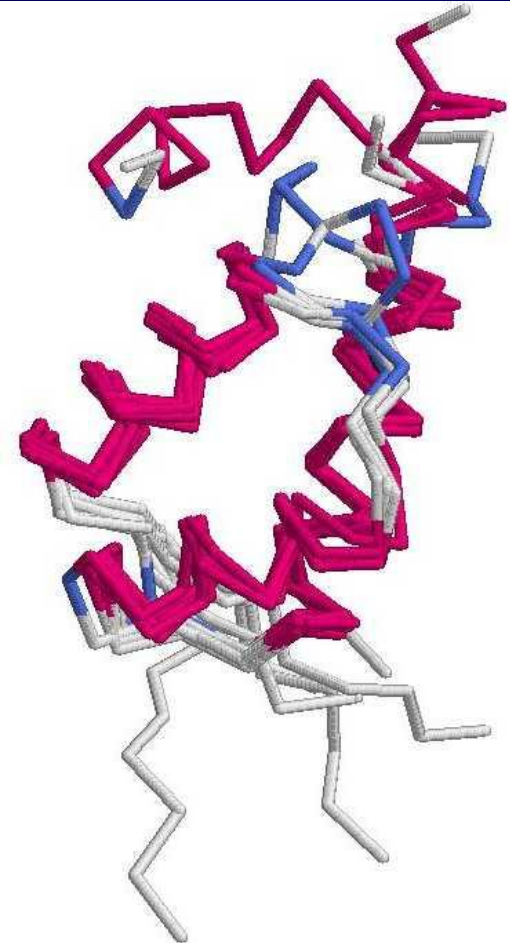
Human

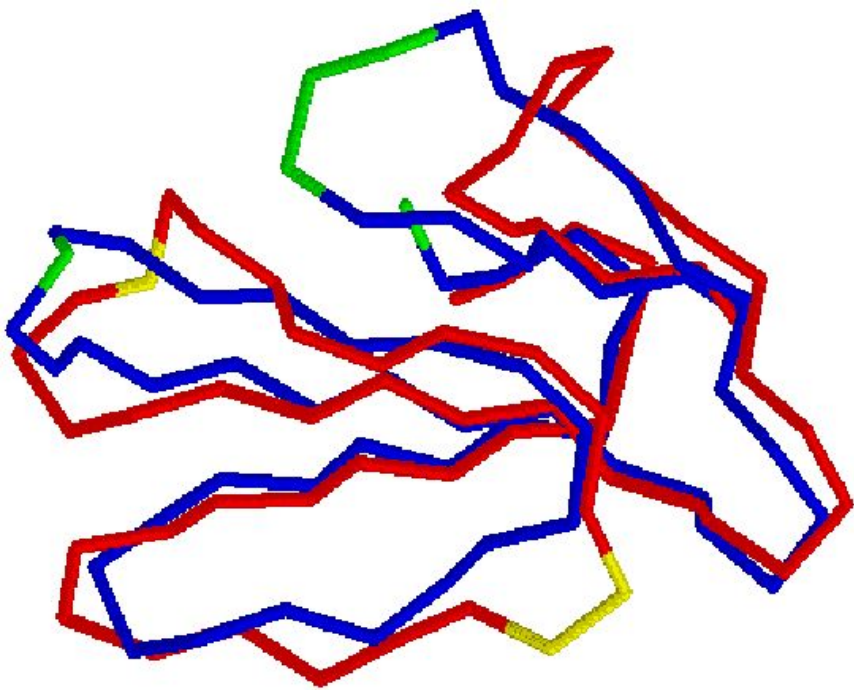
1 10
GDVEKGKKIF...

Rust fungus

GFEDGDAKKGARIF...

Sequence identity: 60%





PREDICTION FROM HOMOLOGY

SIMILAR SEQUENCES



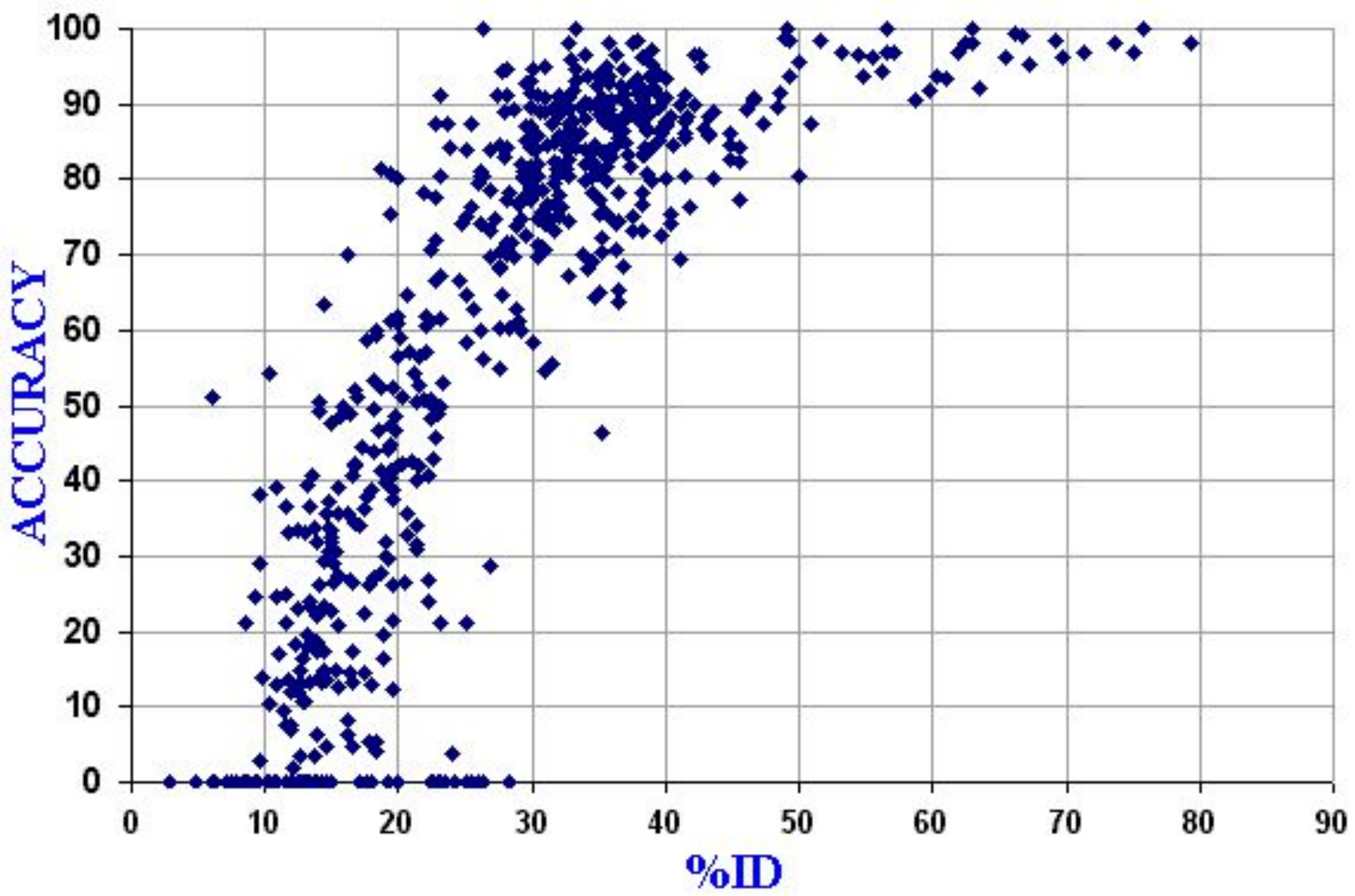
SIMILAR FOLDS

STRUCTURAL ALIGNMENT. SEQ. ID. = 32% ↓

1	lkCnqri---	PpfwkTCpkGknlCYkmtmraap	-mvpvkRGC...
2	<u>riCfnhqssq</u>	<u>PqttkTCspGessCYhkqwsdf</u>	<u>-rgtiieRGC...</u>
			////////
2	-----R	IcfnhqssqPqttKTCspGessCYhkqwsdf	rgtiieRGC...
1	lkcncqRI-----	PpfwKTCpkGknlCYkmtmraap	mvpvkRGC...

SEQUENCE ALIGNMENT ↑

SEQUENCE ALIGNMENT: BIOINFORMATICS



NO

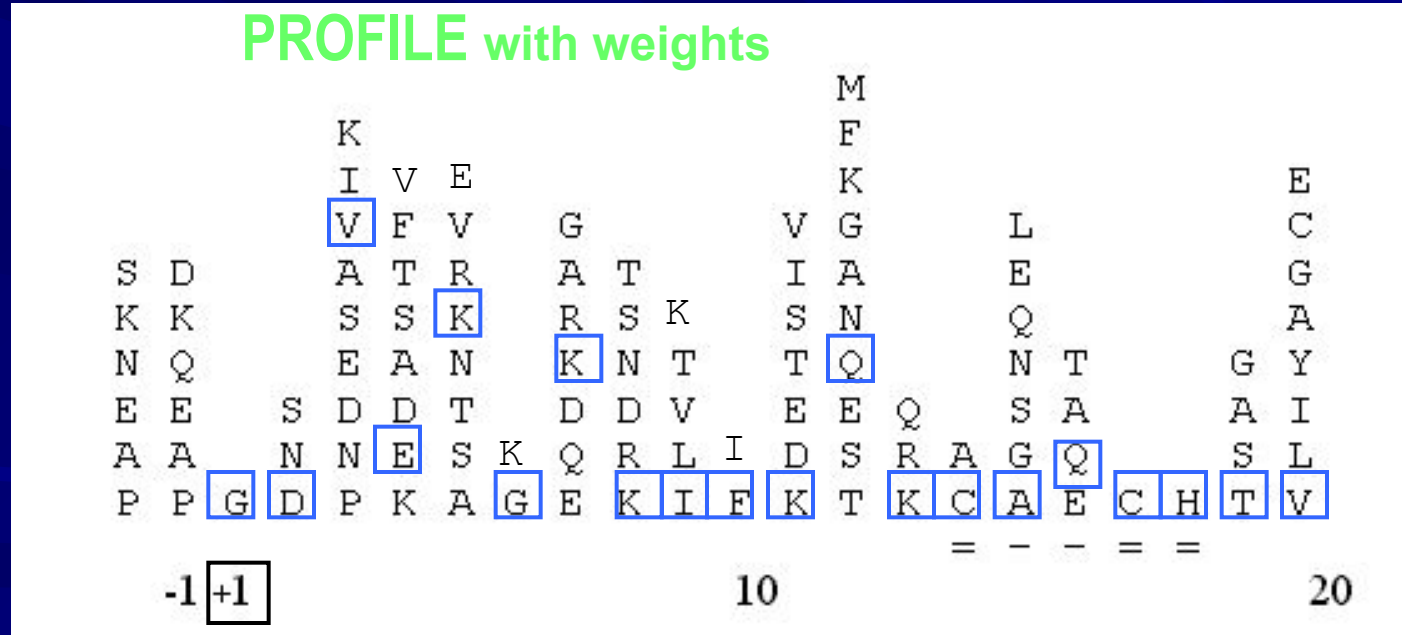
□ TWILIGHT □

===== GOOD PREDICTION =====

Multiple homology

	I	10	20
Human, chimpanzee	GDVEKGKK	IFIMKCSQ	CHTV...
Pig, bovine, sheep	GDVEKGKK	IFVQKCAQ	CHTV...
Chicken, turkey	GDIVEKGKK	IVQKCSQ	CHTV...
Puget sound dogfish	GDVEKGKK	VFVQKCAQ	CHTV...
Screw-worm fly	GVPAGDVEKGKK	IFVQRCAQ	CHTV...
Rust fungus	GFEDGDAKKGAR	IFKTRCAQ	CHTL...
Rape, cauliflower	ASFDEAPPGNSKAGEK	IFKTKCAQ	CHTV...

PROFILE with weights



TARGET
SEQUENCE

...A P G D E F G - - H I K K L M A A T C H A L...

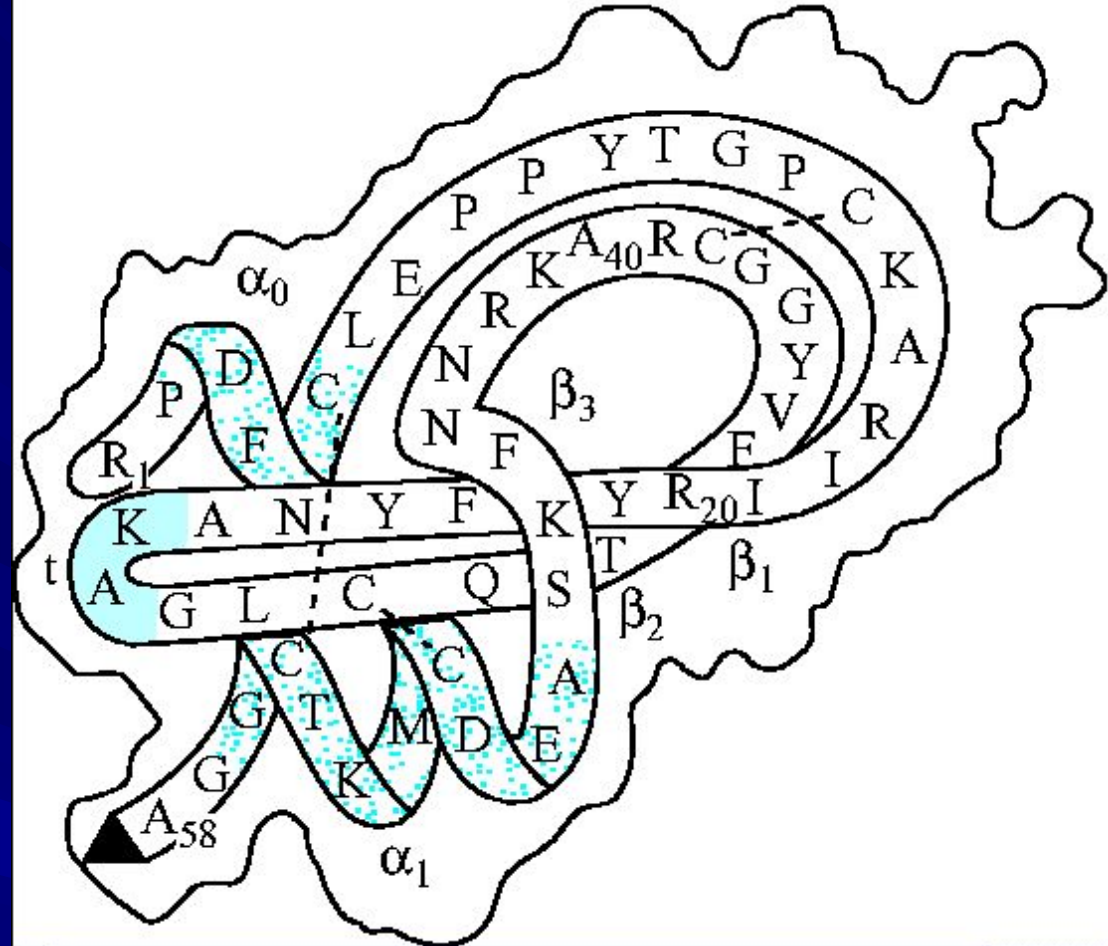
**PREDICTION
FROM
PHYSICS:**

**PROTEIN CHAIN
FOLDS**

SPONTANEOUSLY
□ **SEQUENCE HAS
ALL INFO TO**

PREDICT:

**2° STRUCTURE,
3D STRUCTURE,
SIDE CHAIN ROTAMERS,
S-S BONDS, etc.**



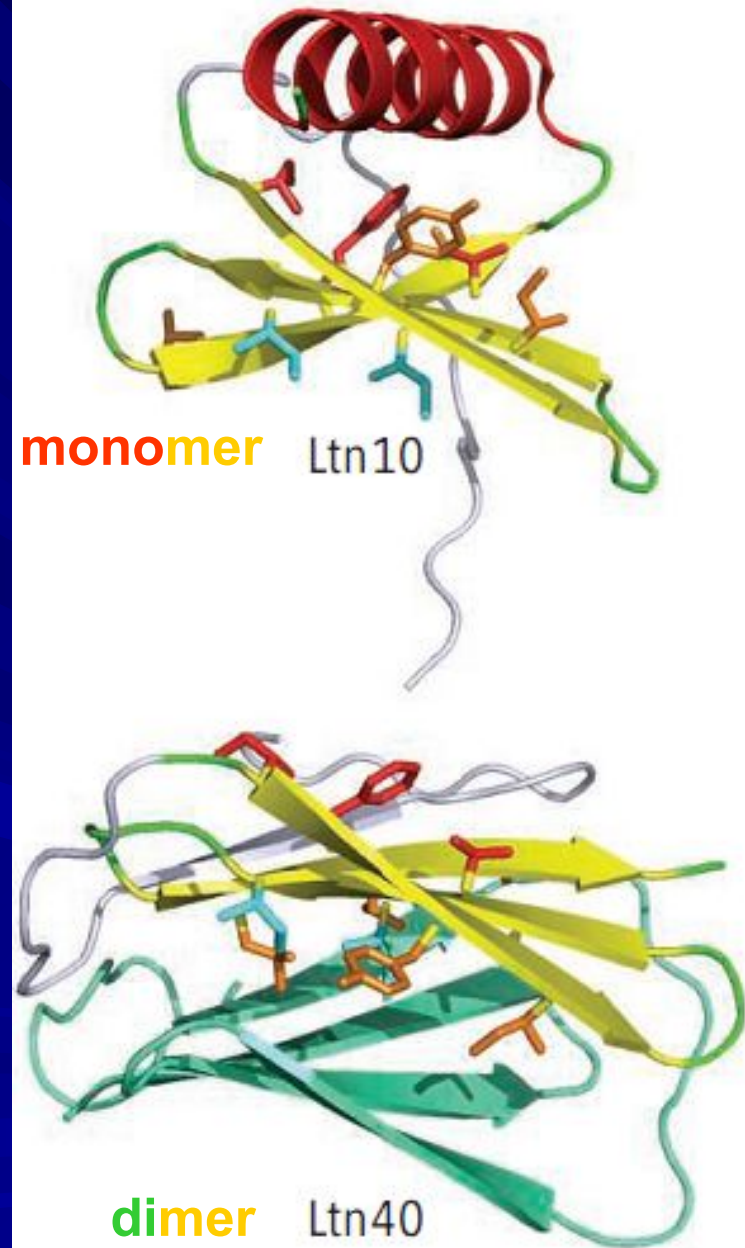
“Unique” fold?

Dimerization

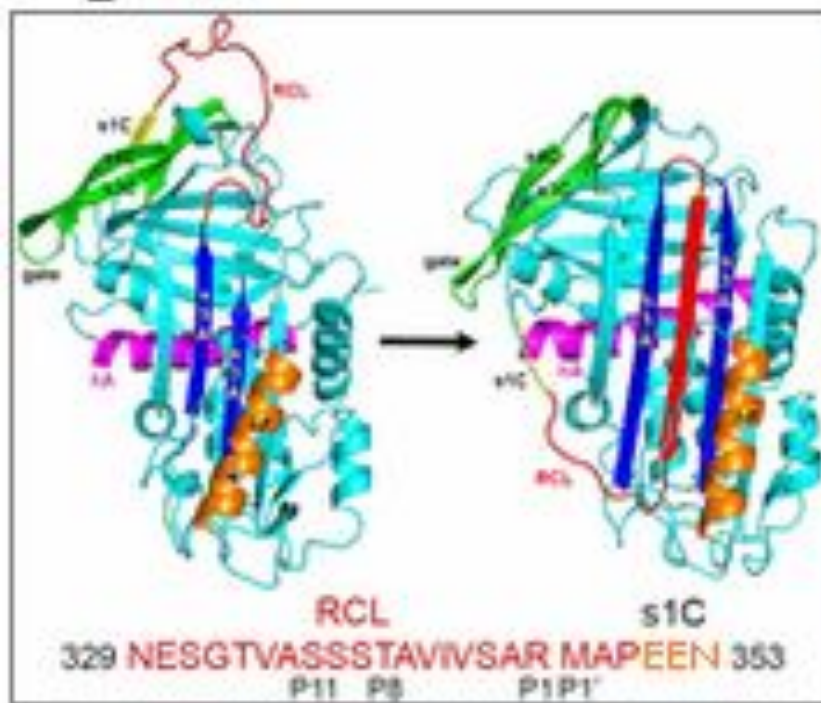
involves an isomerization of the β -sheet. Structurally equivalent residues are few and contribute either to the Ltn10 core (red) or to the dimeric interface of Ltn40 (cyan).

Other nonpolar residues (orange) change sides, such that the formation of the dimeric interface on one side of the β -sheet destroys the hydrophobic core on the other side and vice versa.

Lymphotactin



“Unique” fold?



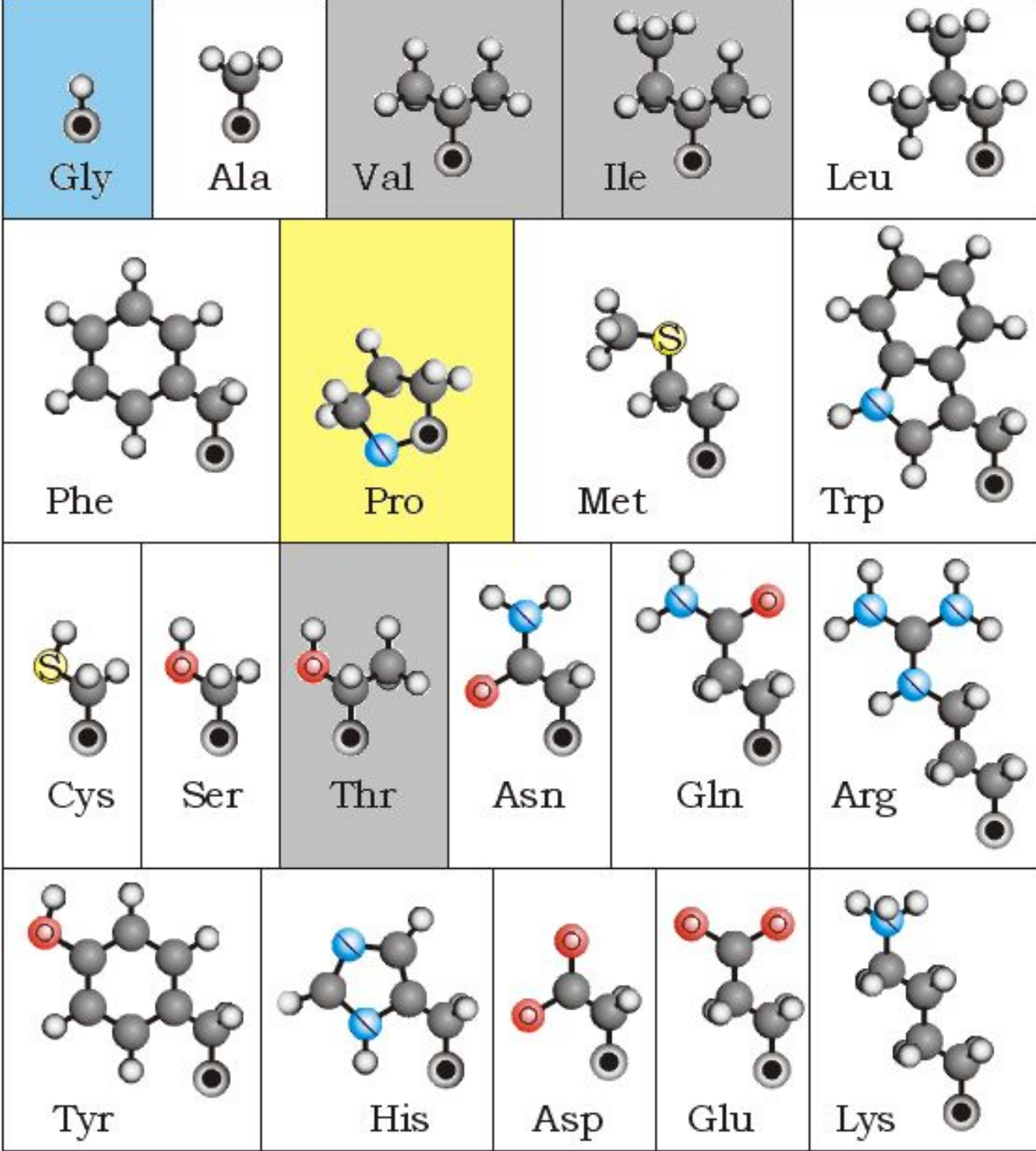
Serpin latency transition at atomic resolution

*G. Cazzoli, F. Wang,
S. a Beccara, A. Gershenson,
P. Faccioli, P. L. Wintrode*

PNAS October 28, 2014 vol. 111
no. 43 15414-15419

active
METASTABLE
form
(~ 30 min.)

INactive
STABLE
form



Side chains

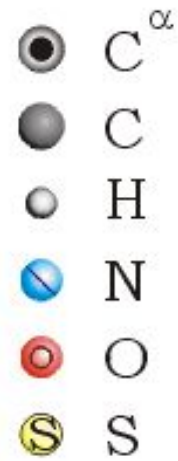
no C^β
C^β , \leq one γ
C^β , two γ
imino-acid

no C^β : coil

C^β , ≤ 1 γ : α , β , coil

C^β , 2 γ : β

imino: coil, turn



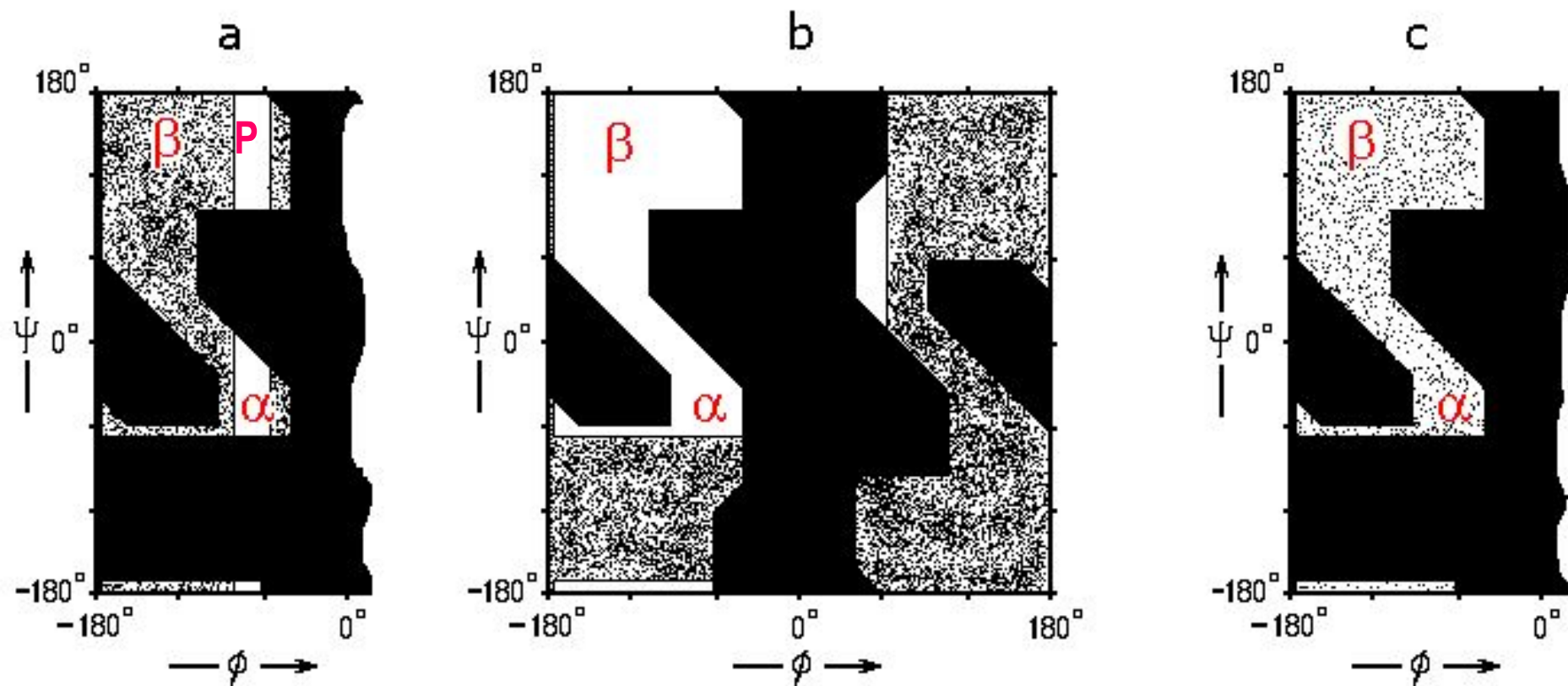
Pro

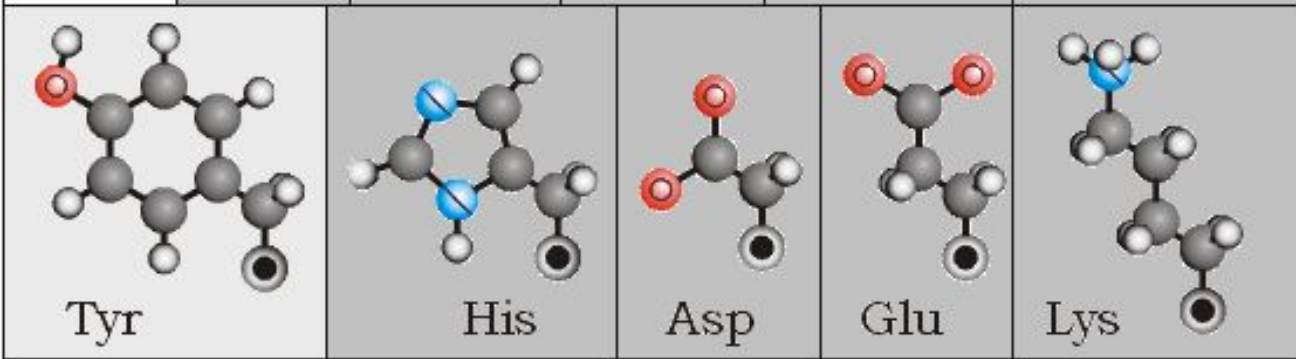
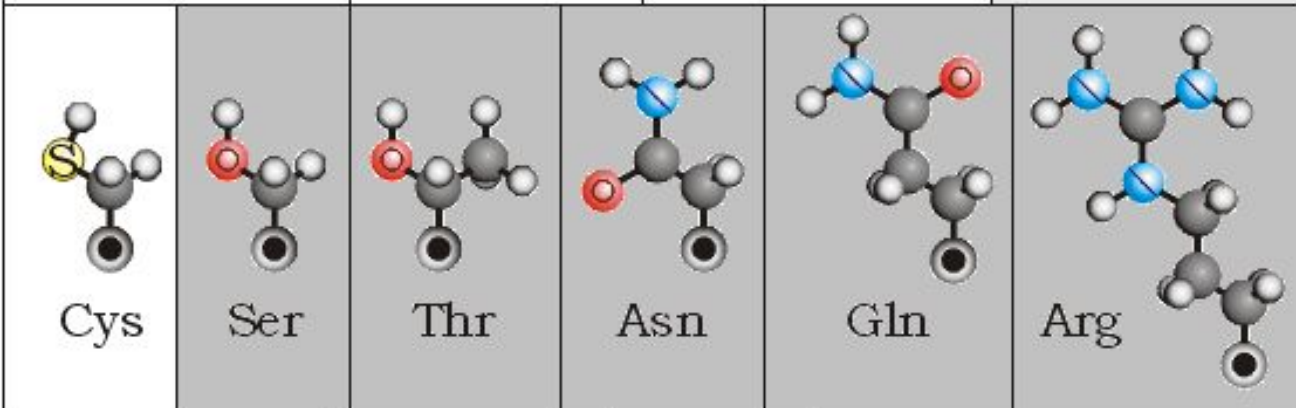
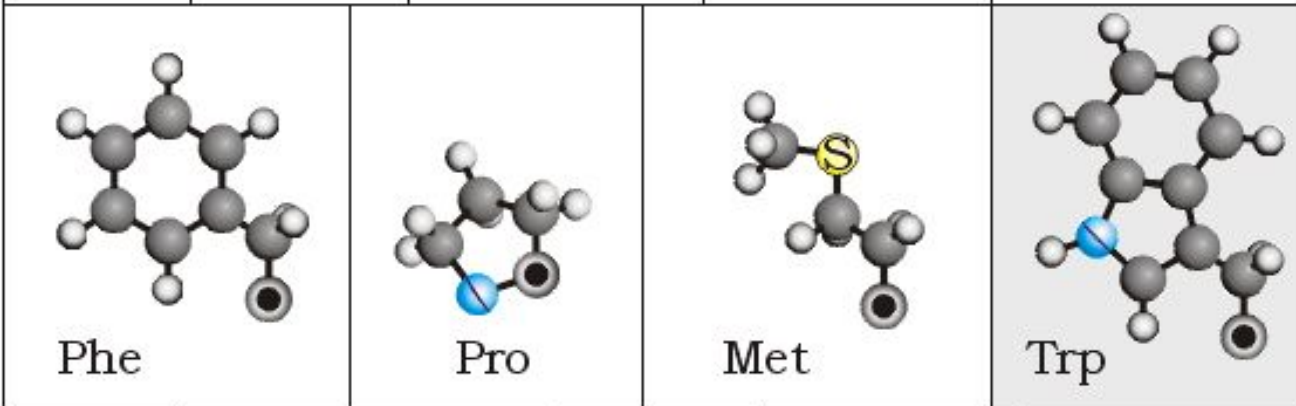
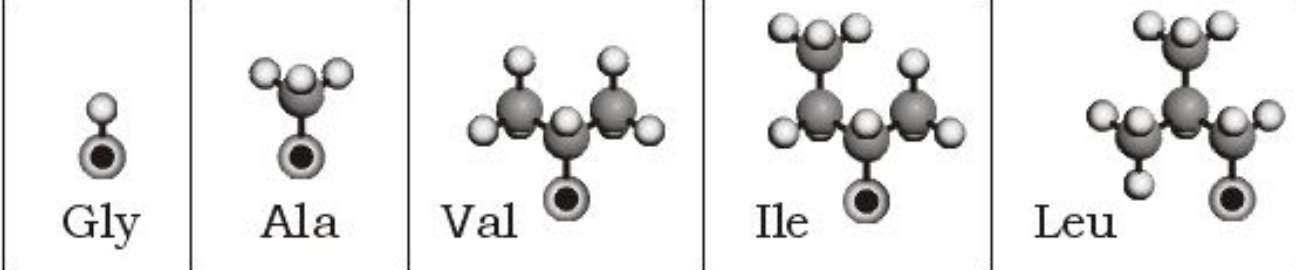
1,2,3 rot.

imino:
coil, turn, α_N

no C^β : coil
 $C^\beta, \leq 1 \gamma$: α, β , coil

$C^\beta, 2 \gamma$: β



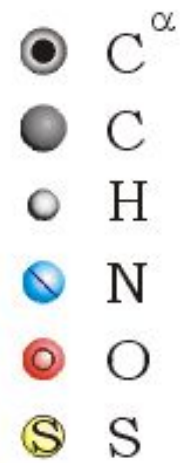


Side chains

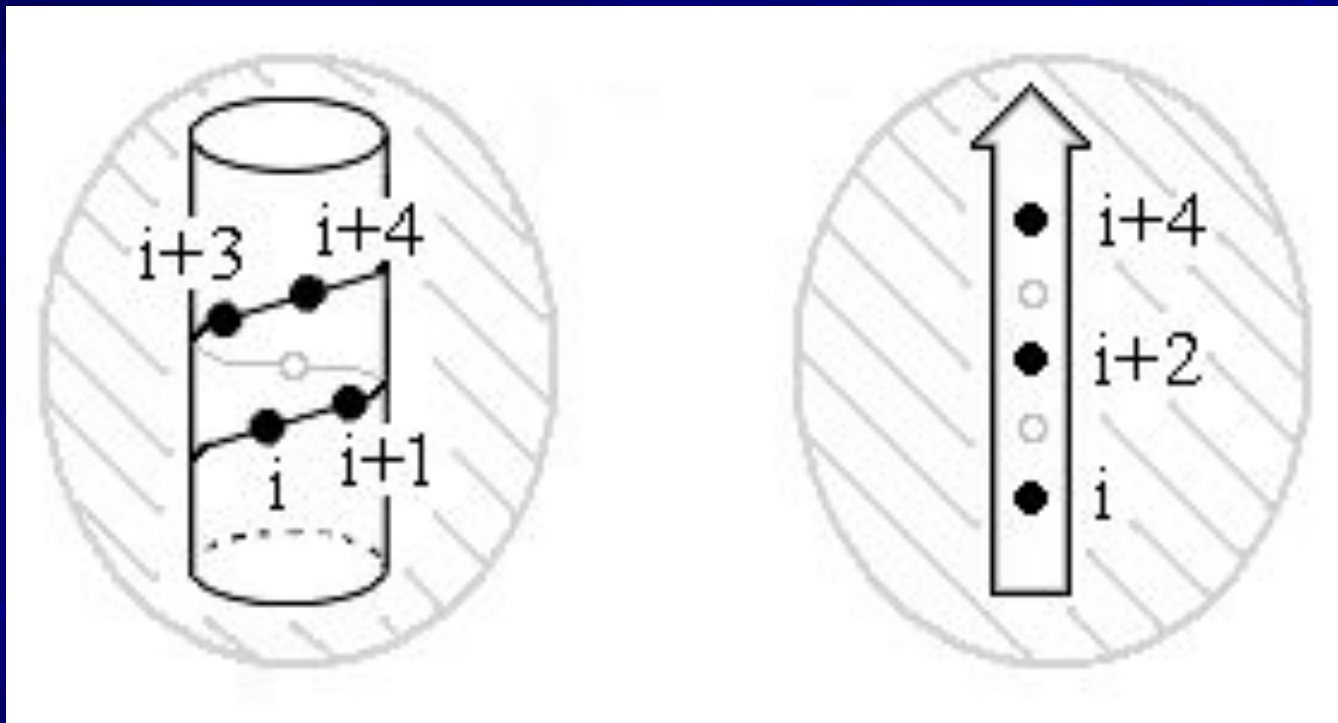
non-polar

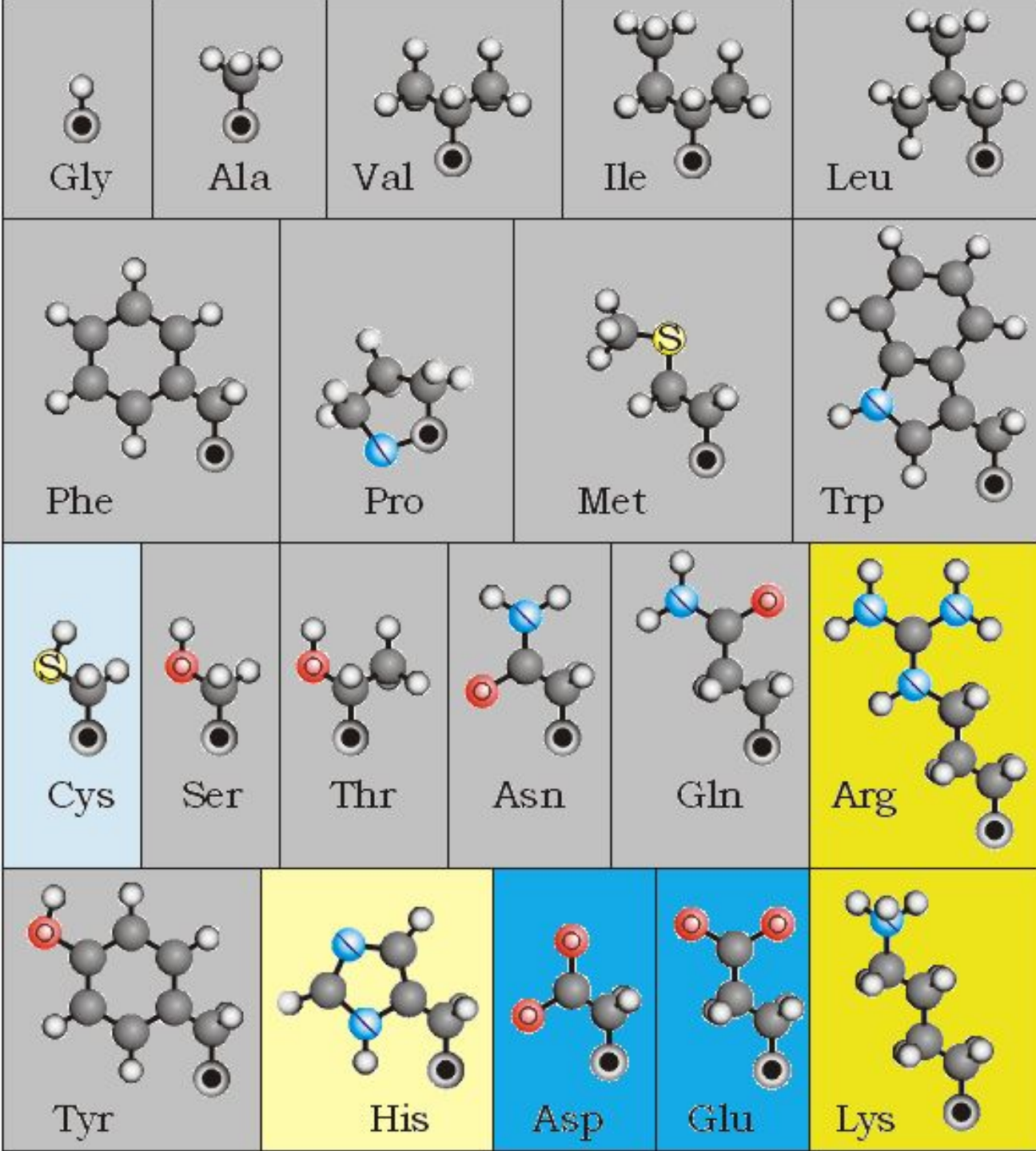
polar

non-polar: core
polar: surface



non-polar: in the core
polar: at the surface





Side chains

charged -

charged +

charged -: coil,
 α_N

charged +: coil,
 α_C

Half-charged:
active sites



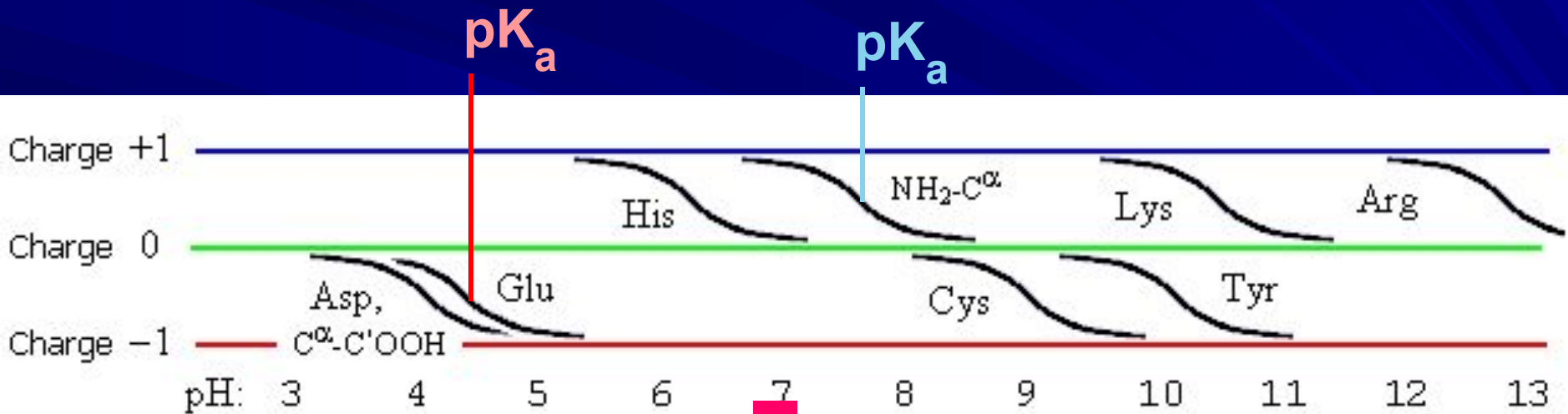
$$P_{\text{charged}} + P_{\text{uncharged}} = 1$$

$$\frac{P_{\text{charged}}}{P_{\text{uncharged}}} = 10^{-(\text{pK}_a - \text{pH})}$$

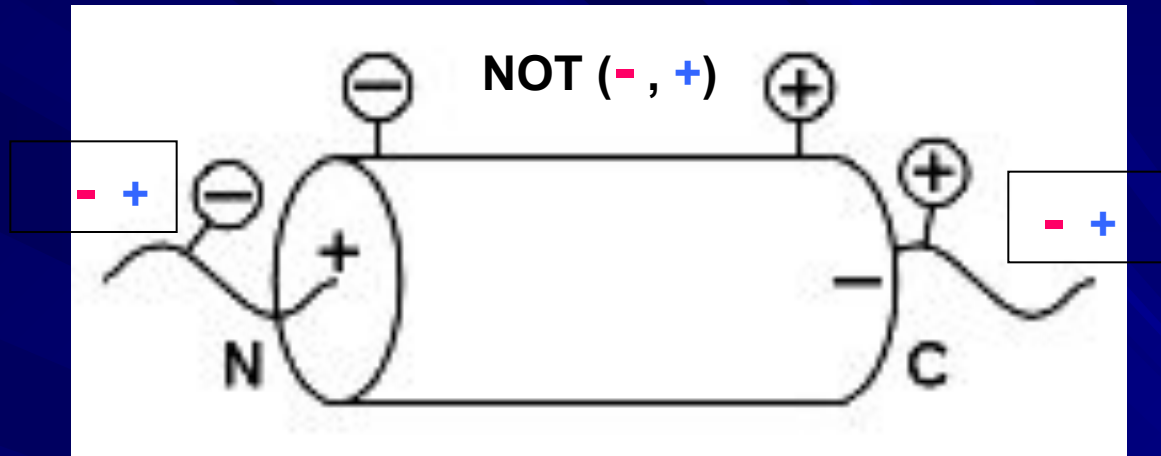
$$\frac{P_{\text{charged}}}{P_{\text{uncharged}}} = 10^{+(\text{pK}_a - \text{pH})}$$

Acids (charge -)

Bases (charge +)



Half-charged: active sites



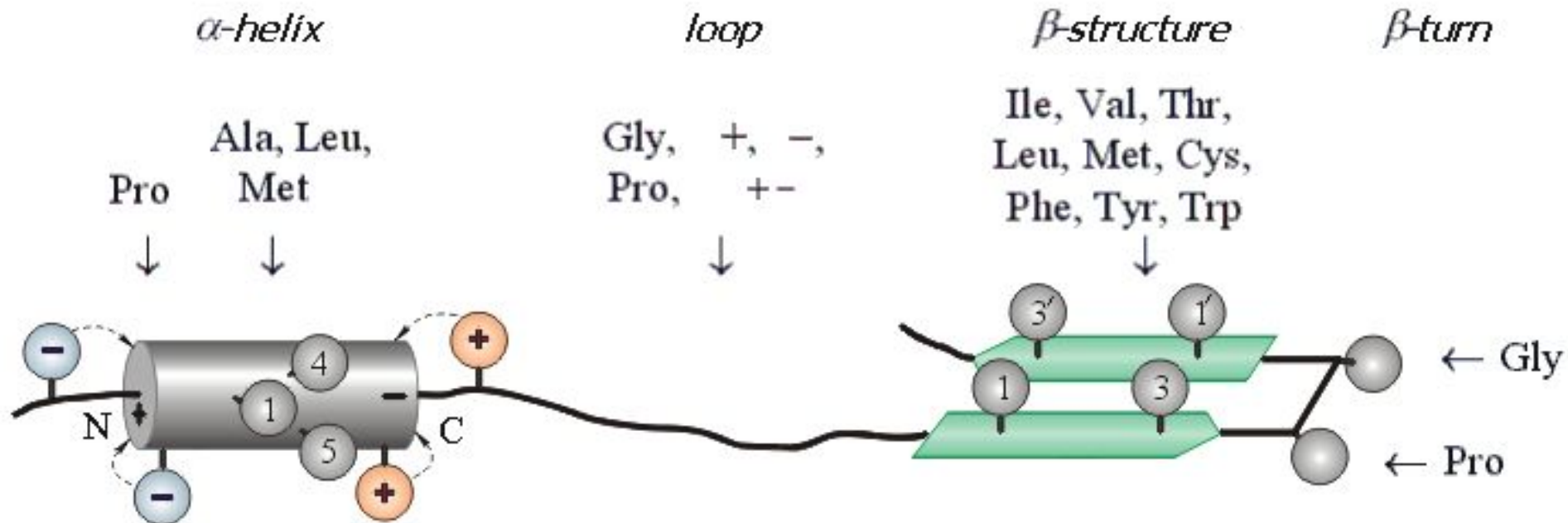
charged -: coil, α_N

====

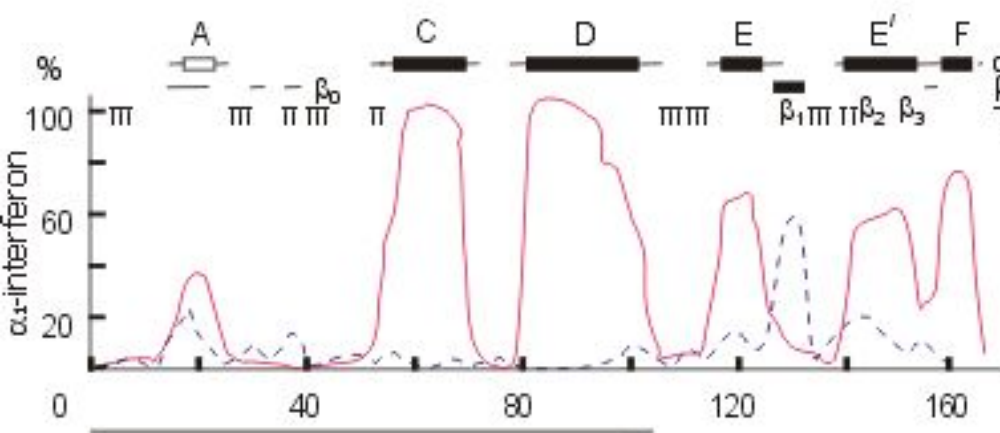
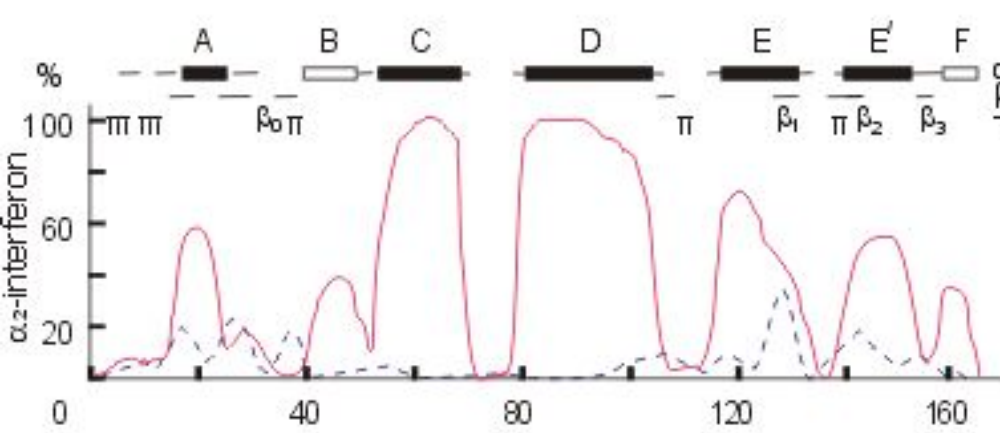
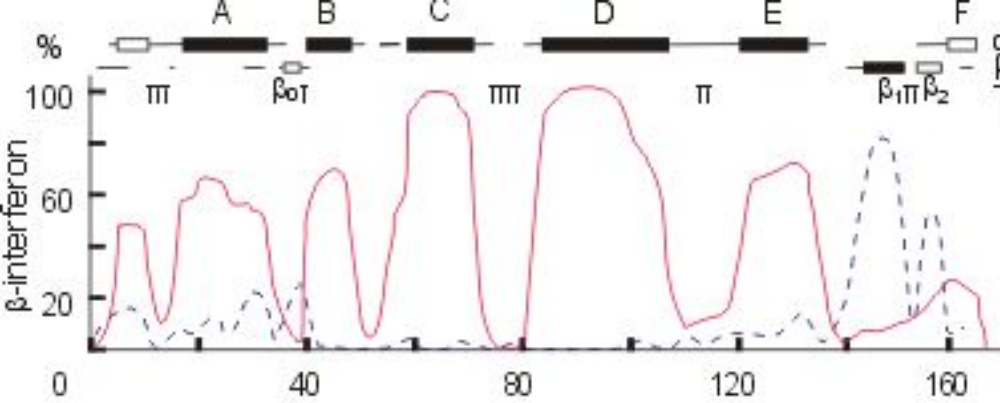
charged +: coil, α_C

====

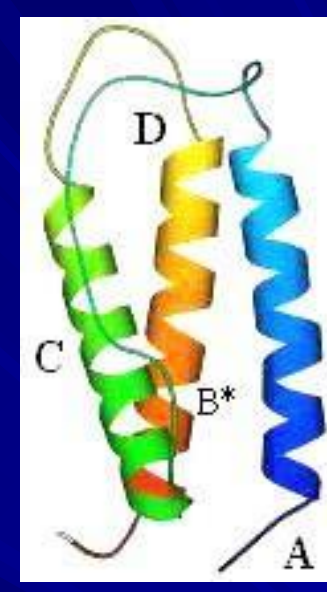
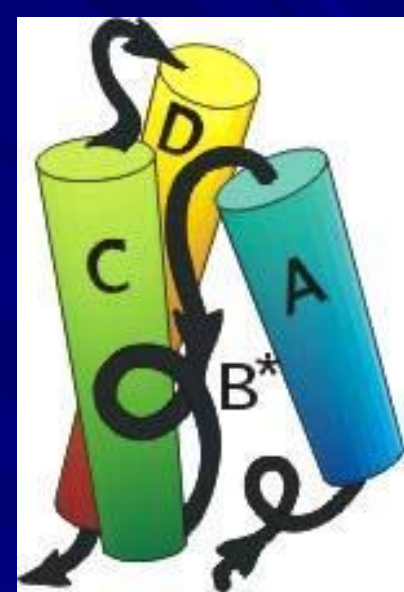
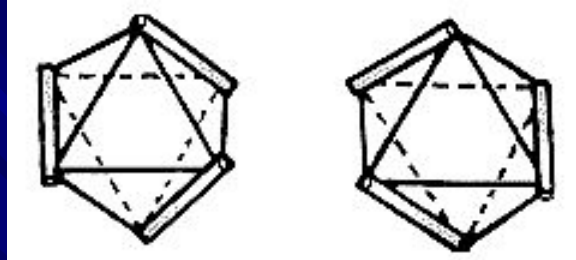
PREDICTION FROM PHYSICS (OR PROTEIN STATISTICS) 2^o STRUCTURES



USUALLY, THIS WORKS WELL, BUT...

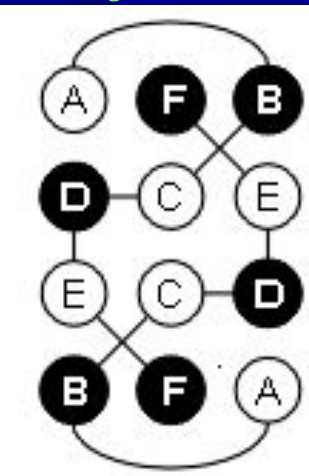
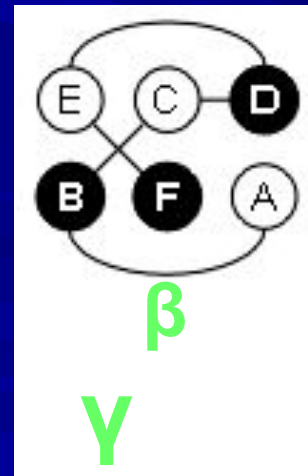


A B C D .---different---



Prediction, 1985

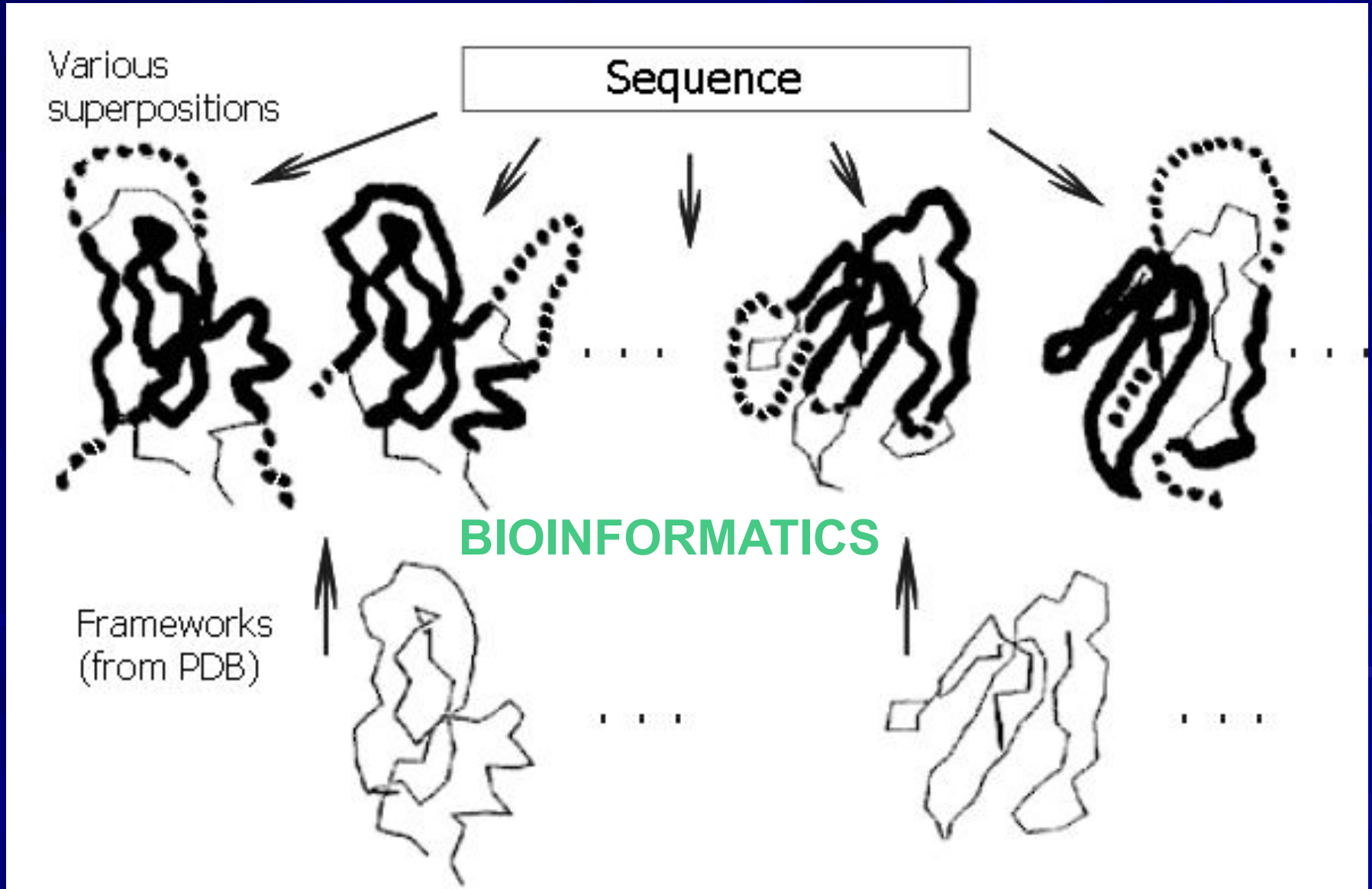
X-ray str., 1990



THREADING

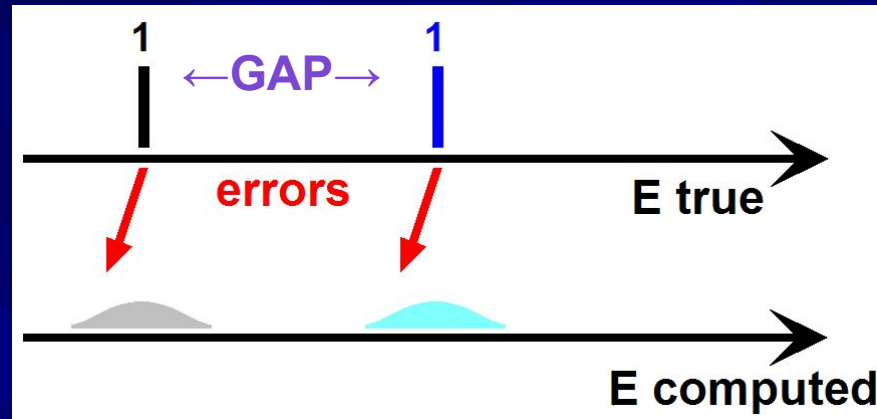
helps, when sequence identity is low (<10-20%)

Finkelstein, Reva, 1990-91 (*Nature*); Bowie, Lüthy, Eisenberg, 1991 (*Science*)

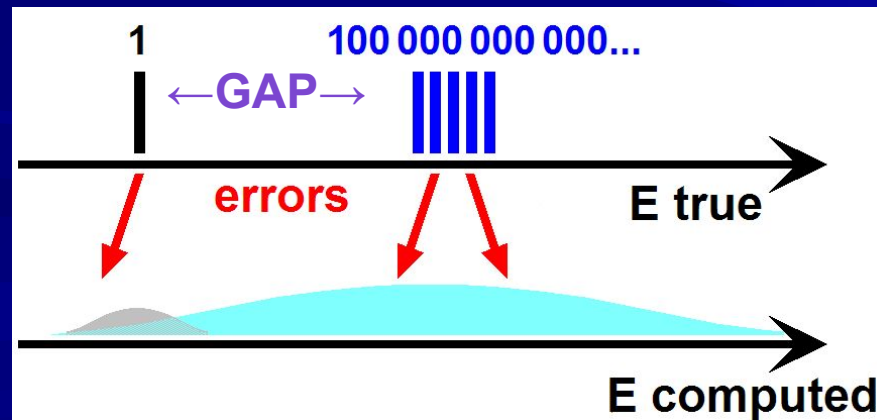


... but one still cannot reliably predict 3D protein structure from the a. a. sequence without homologues... **WHY??**

choice of one structure out of two:
DOES NOT require too precise estimate of interactions



choice of one structure out of zillions:
REQUIRES very precise estimate of interactions



HOT POINTS IN PROTEIN PHYSICS

The Nobel Prize in Chemistry 2013



Martin Karplus,
1930



Michael Levitt,
1947



Arieh Warshel,
1940

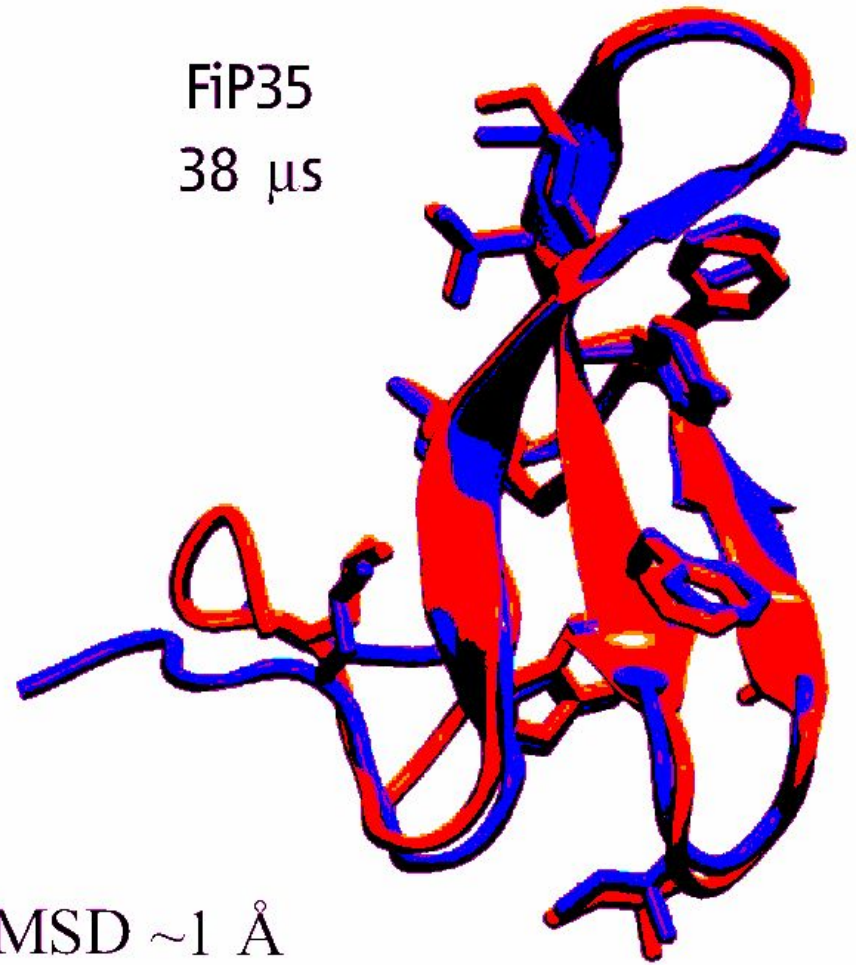
***"for the development of multiscale models
for complex chemical systems"***

Predicting 3D structures of small proteins

villin
68 μ s



FiP35
38 μ s



RMSD \sim 1 Å

modified version of the Amber ff99SB force field:
K. Lindorff-Larsen *et al.*, *Proteins* **78**, 1950 (2010)
D.E. Shaw *et al.*, Oct. 2010, *Science* **330**, 341

HOT POINTS IN PROTEIN PHYSICS



David E. Shaw, 1951

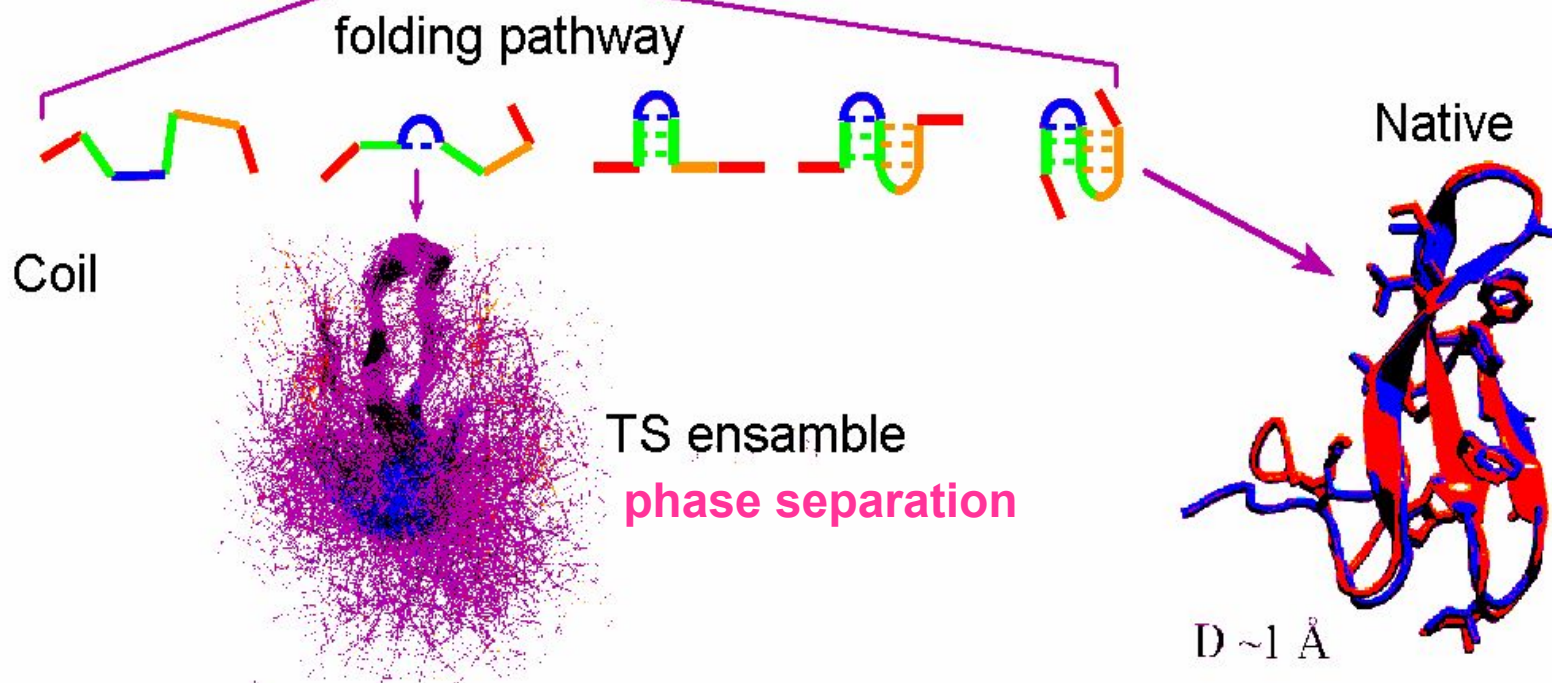
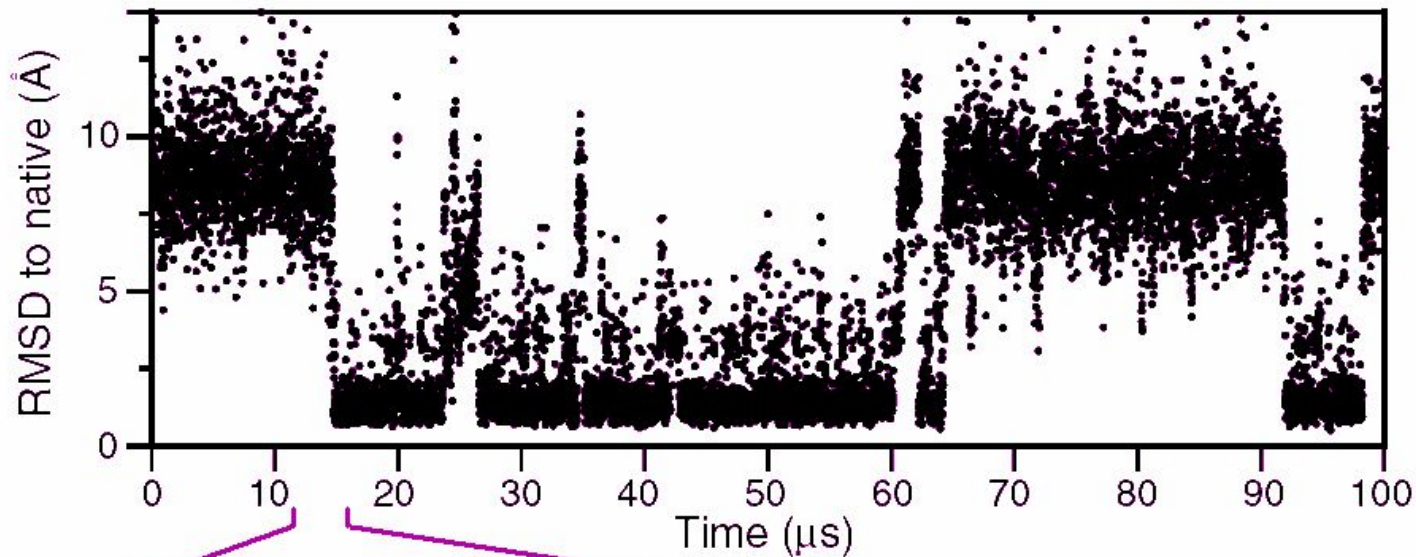
“D. E. Shaw Research”

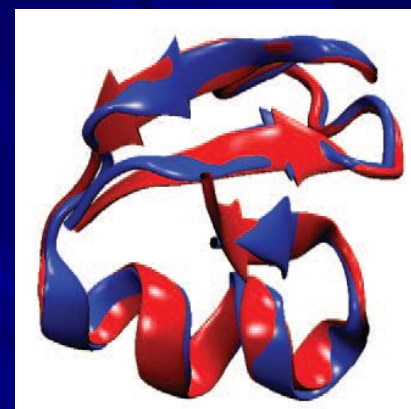
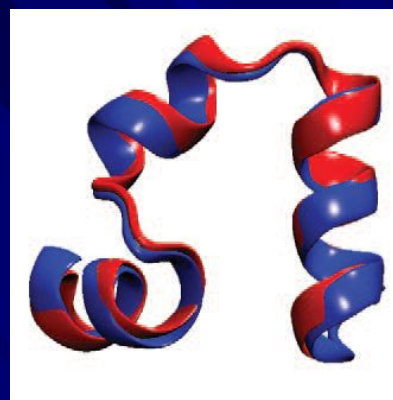
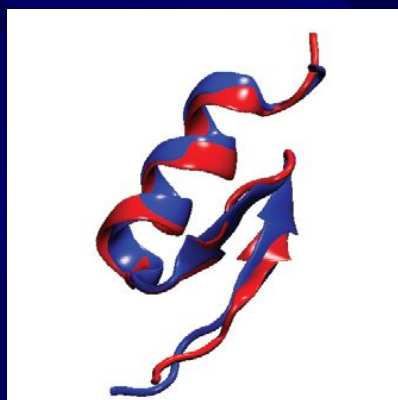
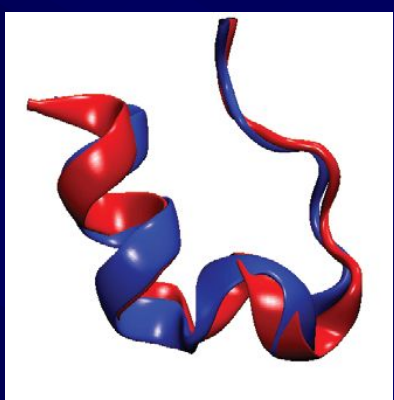
US\$ 3.5 billion

Supercomputer “Anton”

FIP35 protein: simulation of folding

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





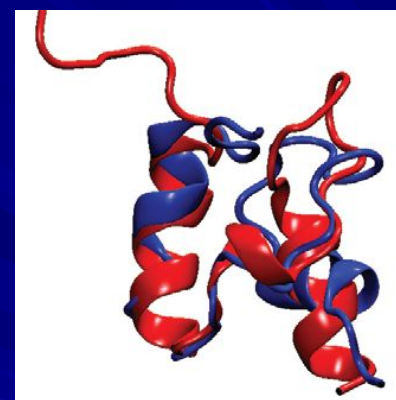
Trp-cage 208 μ s
1.4Å 14 μ s

BBA 325 μ s
1.6Å 18 μ s

Villin 125 μ s
1.3Å 2.8 μ s

NTL9 3936 μ s
0.5Å 29 μ s

In total - 12 proteins



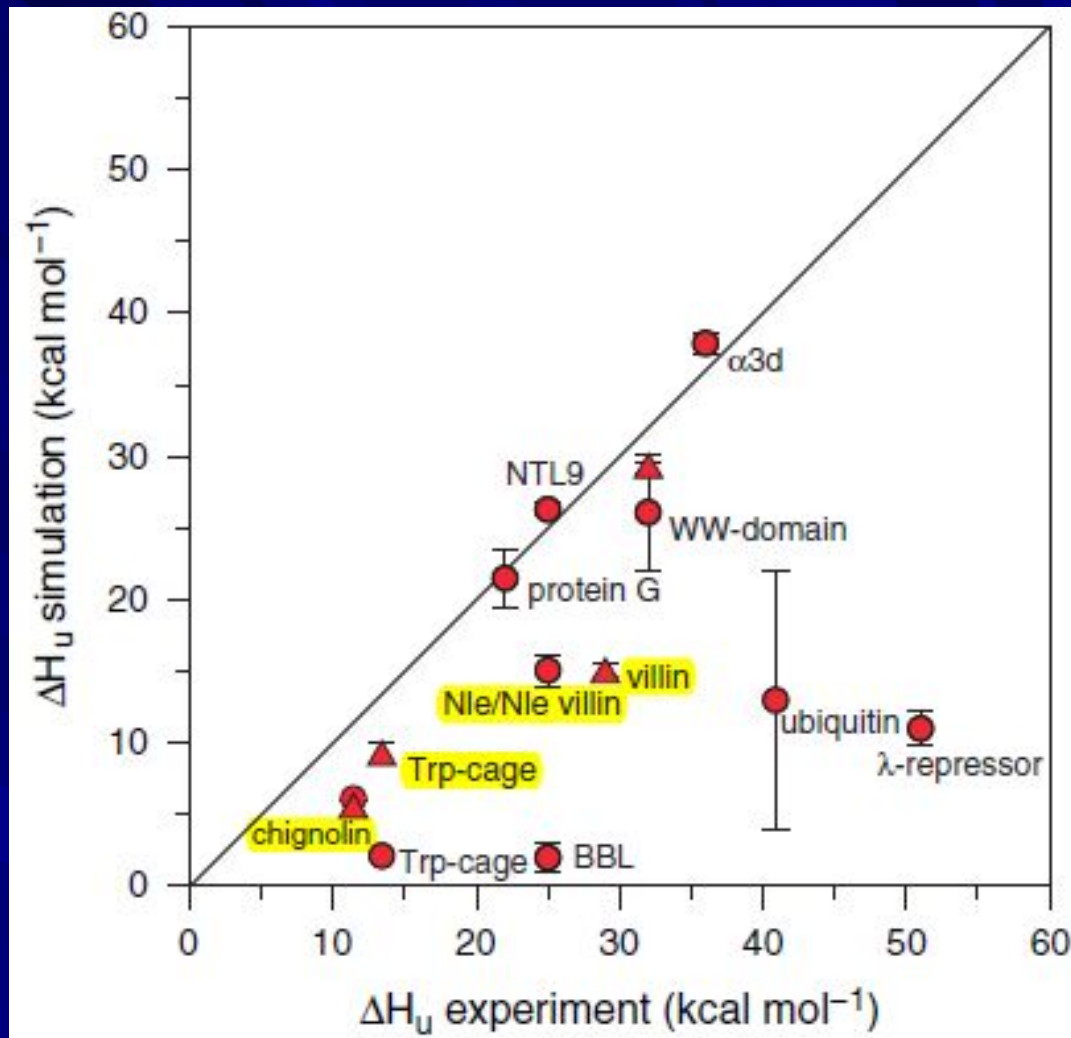
BBL 429 μ s
4.8Å 29 μ s

K. Lindorff-Larsen, S. Piana, R.O. Dror, D. E. Shaw (2011)

How Fast-Folding Proteins Fold. *Science* 334, 517

BUT:
comparison of experimental
and simulation-derived
unfolding enthalpies
shows very large differences...

**Improvement in the
potential-energy
function
is needed!**



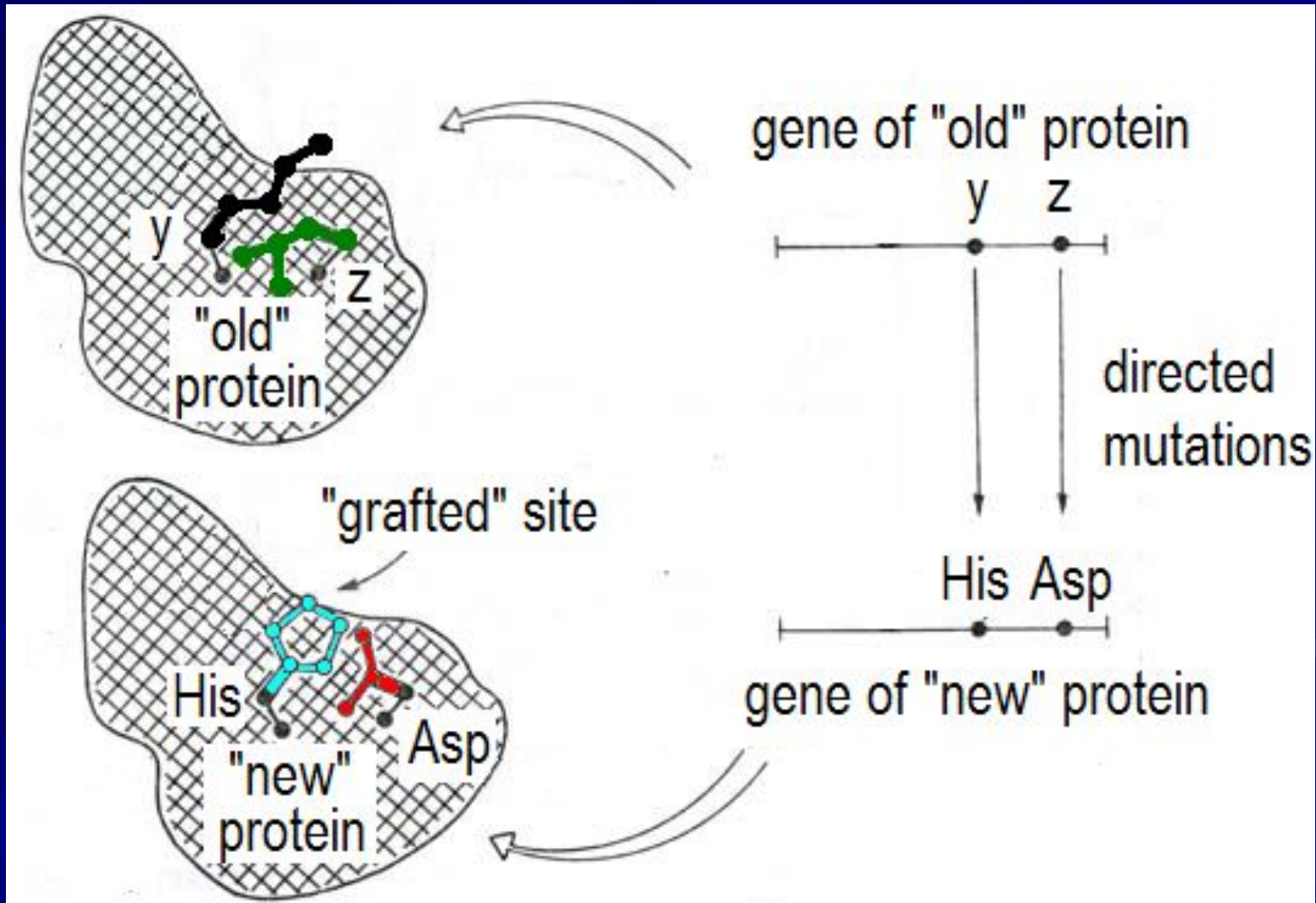
S. Piana, J.L. Klepeis, D.E Shaw

Assessing the accuracy of physical models used in protein-folding simulations: quantitative evidence from long molecular dynamics simulations

Current Opinion in Structural Biology 2014, 24:98–105

Protein engineering

Wanted: new protein with additional salt bridge
(e.g., His⁺:::Asp⁻)



Daniela Röthlisberger^{1*}, Olga Khersonsky^{4*}, Andrew M. Wollacott^{1*}, Lin Jiang^{1,2}, Jason DeChancie⁶, Jamie Betker³, Jasmine L. Gallaher³, Eric A. Althoff¹, Alexandre Zanghellini^{1,2}, Orly Dym⁵, Shira Albeck⁵, Kendall N. Houk⁶, Dan S. Tawfik⁴ & David Baker^{1,2,3}

Kemp elimination catalysts by computational enzyme design

David Baker

doi:10.1038/nature06879

2008

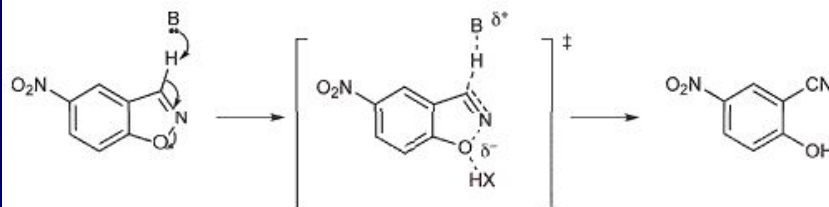
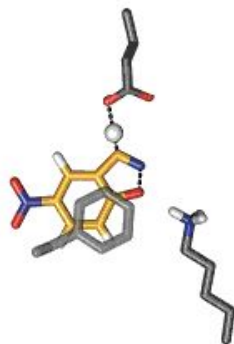
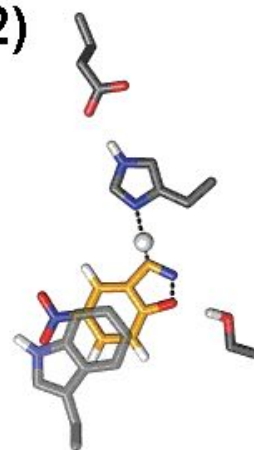


Figure 1 | Reaction scheme and catalytic motifs used in design.

1)



2)



$$k_{\text{cat}}/k_{\text{uncat}} \sim 10^6$$

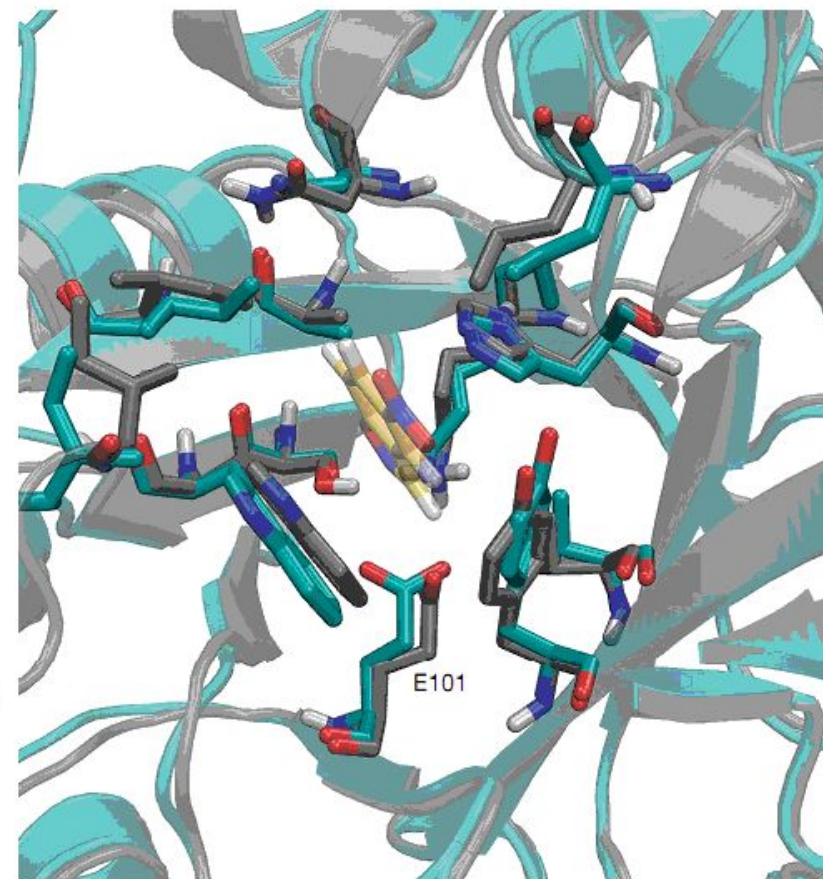


Figure 4 | Comparison of the designed model of KE07 and the crystal structure.

DESIGN

1-st step

4



Tetramer
of 4 helices

2-nd step

2



Dimer

3-rd step



Monomer

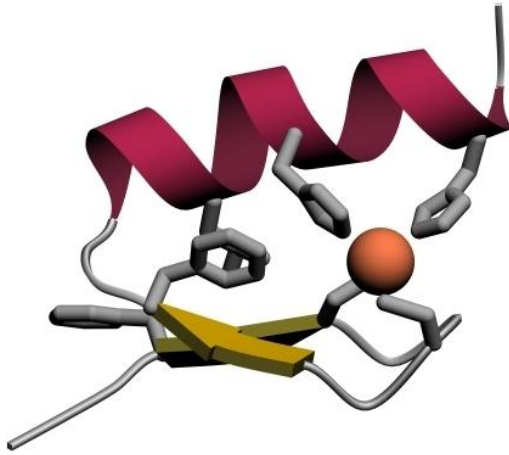
DeGrado, 1989

**DOES NOT MELT !
MOLTEN GLOBULE...**

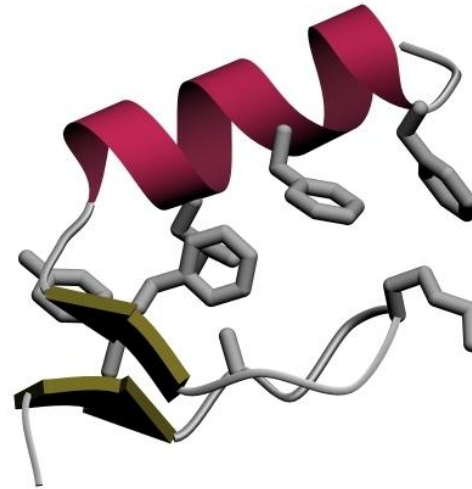
+ ION BINDING SOLID

DESIGN

a Natural protein
(with Zn ion)



b Designed without
ion: Mayo, 1997



Stephen L. Mayo



Later, in 2003,
David Baker (1962) *et al.*
designed and made a new,
„unnatural“ fold

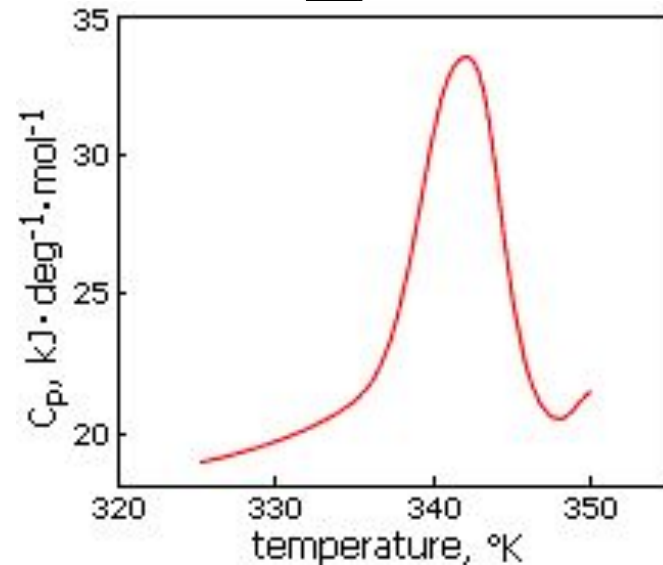
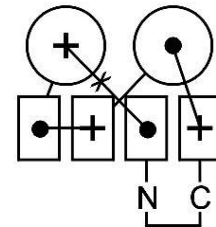
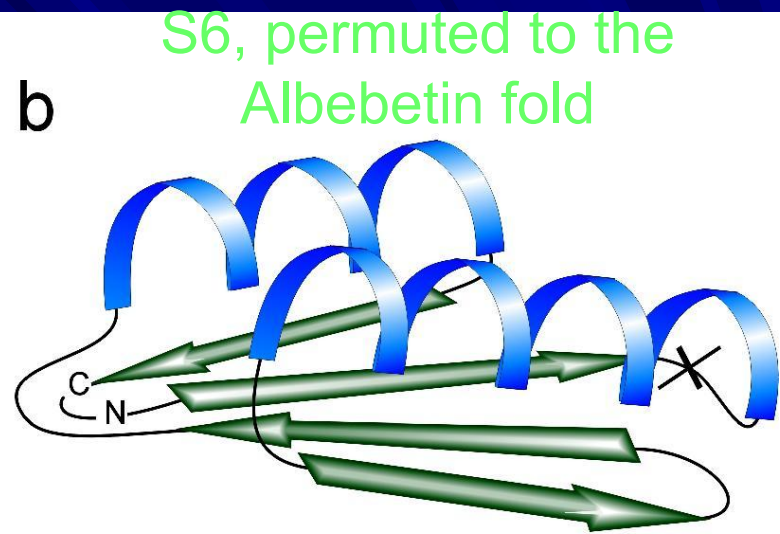
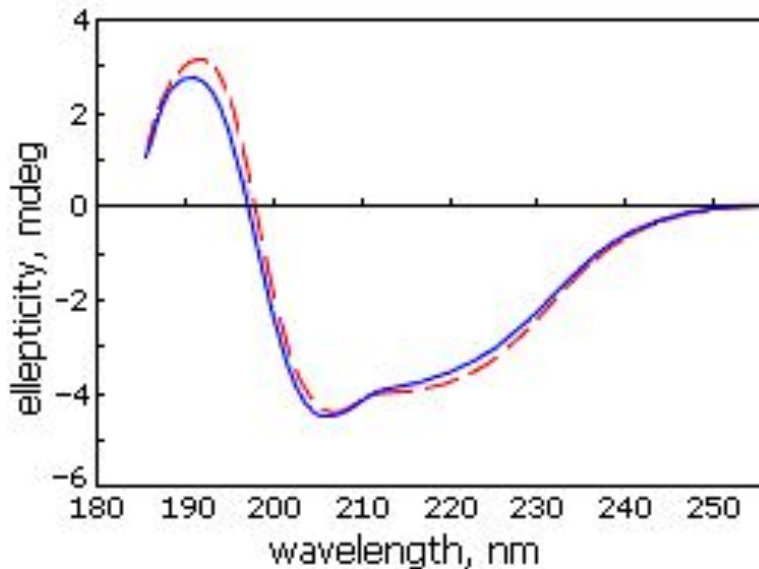
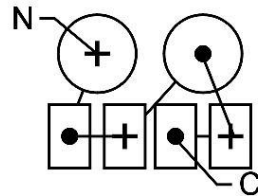
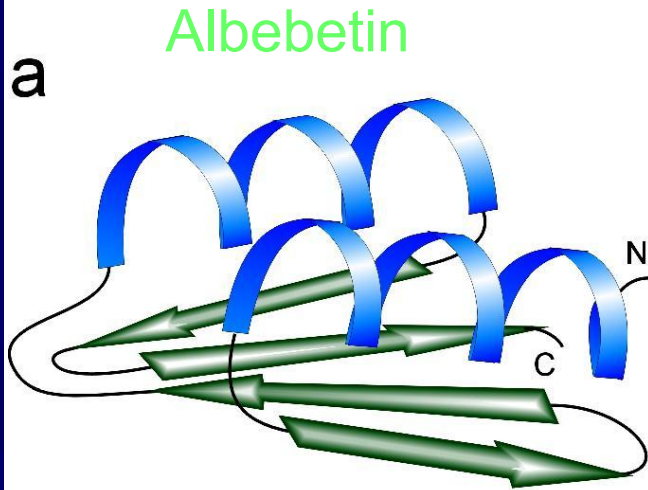
DESIGN

Ptitsyn
Dolgikh
Finkelstein
Fedorov
Kirpichnikov
1987-97

Albebetin;

↓
Albeferon,

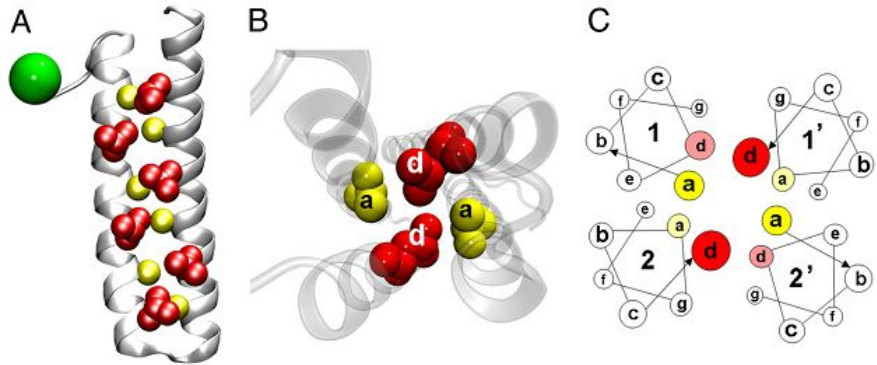
...
(grafting
functional
groups)



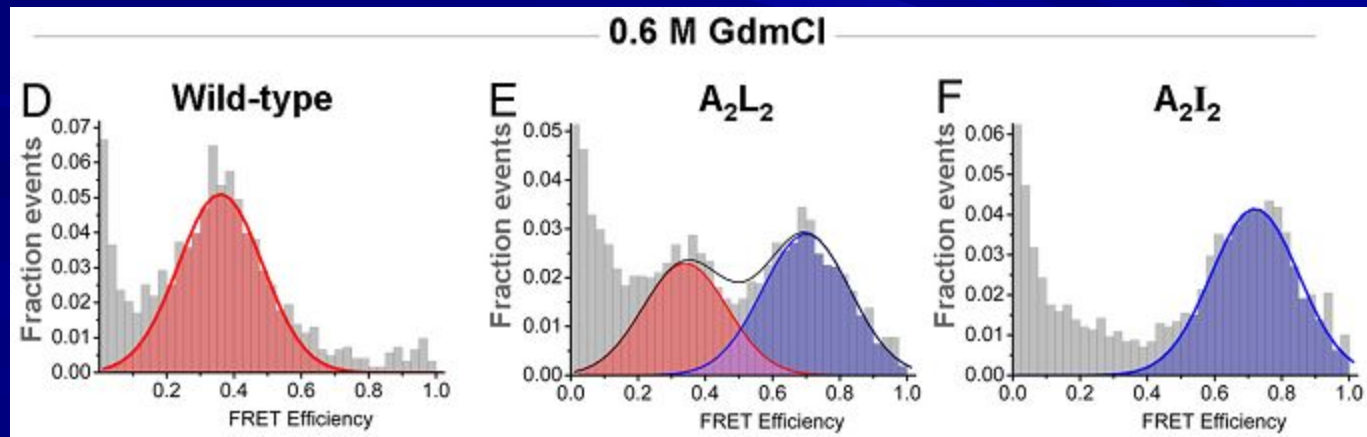
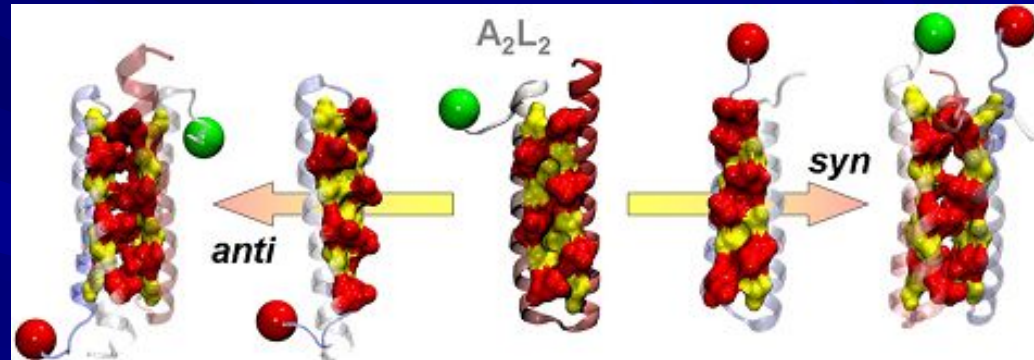
DESIGN OF A "HAMELION" PROTEIN:

Direct single-molecule observation of a protein living in two opposed native structures

Y.Gambin, A.Schug, E.A.Lemke, J.J.Lavinder, A.C.M.Ferreon, T.J.Magliery, J.N.Onuchic, A.A.Deniz
PNAS, 2009 v.106, 10153–8



ROP-wt	GTKQEKALNMFARFIRSQTLTILEKLNELDADEQADICE	SLHDHADELYRS	CLARFGDDGEN	C
A ₂ L ₂	GTKQEKTLNMFARFLRSQALTILEKANELDADELADIAE	SLHDHADELYRS	ALARFGDDGEN	C
A ₂ I ₂	GTKQEKTLNMFARFIRSQALTILEKANELDADEIADIAE	SIHDHADELYRS	ALARFGDDGEN	C



Protein design

Wanted:

new protein fold

P.A.Alexander, Y.He, Y.Chen,
J.Orban, P.N.Bryan

PNAS, 2007, **104**, 11963-8

The design and characterization
of two proteins with 88%
sequence identity but different
structure and function

Y.He, Y.Chen, P.Alexander,
P.N.Bryan, J.Orban

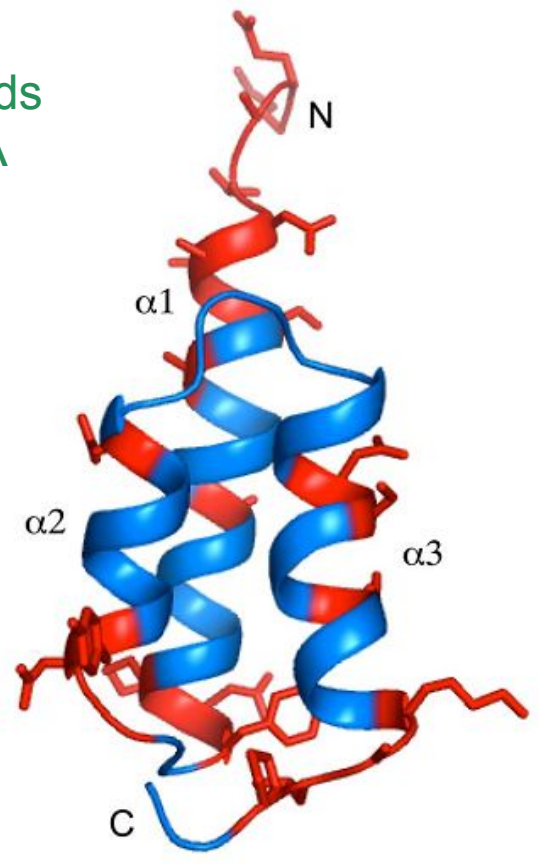
PNAS, 2008, **105**, 14412-7

NMR structures of two designed
proteins with high sequence
identity but **different
fold and function**

2012 (*Structure*, **20**, 283-91):
one-residue difference

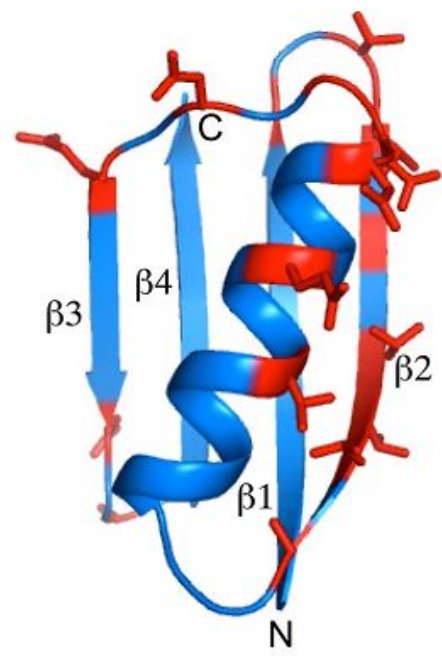
A

G_A binds
to HSA

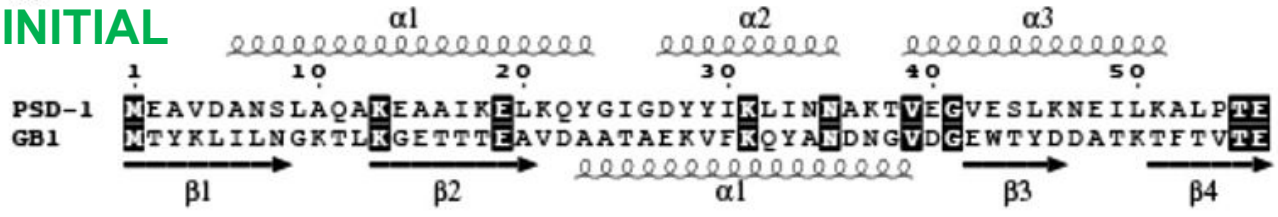


B

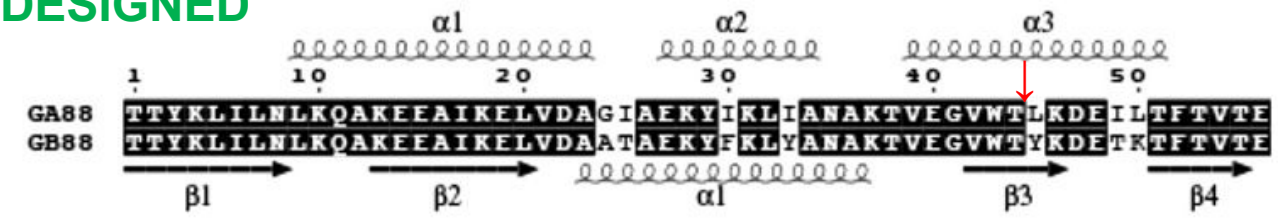
G_B binds to
IgG Fc region



C
INITIAL



DESIGNED



PROTEIN STRUCTURE:
PREDICTION
ENGINEERING
DESIGN