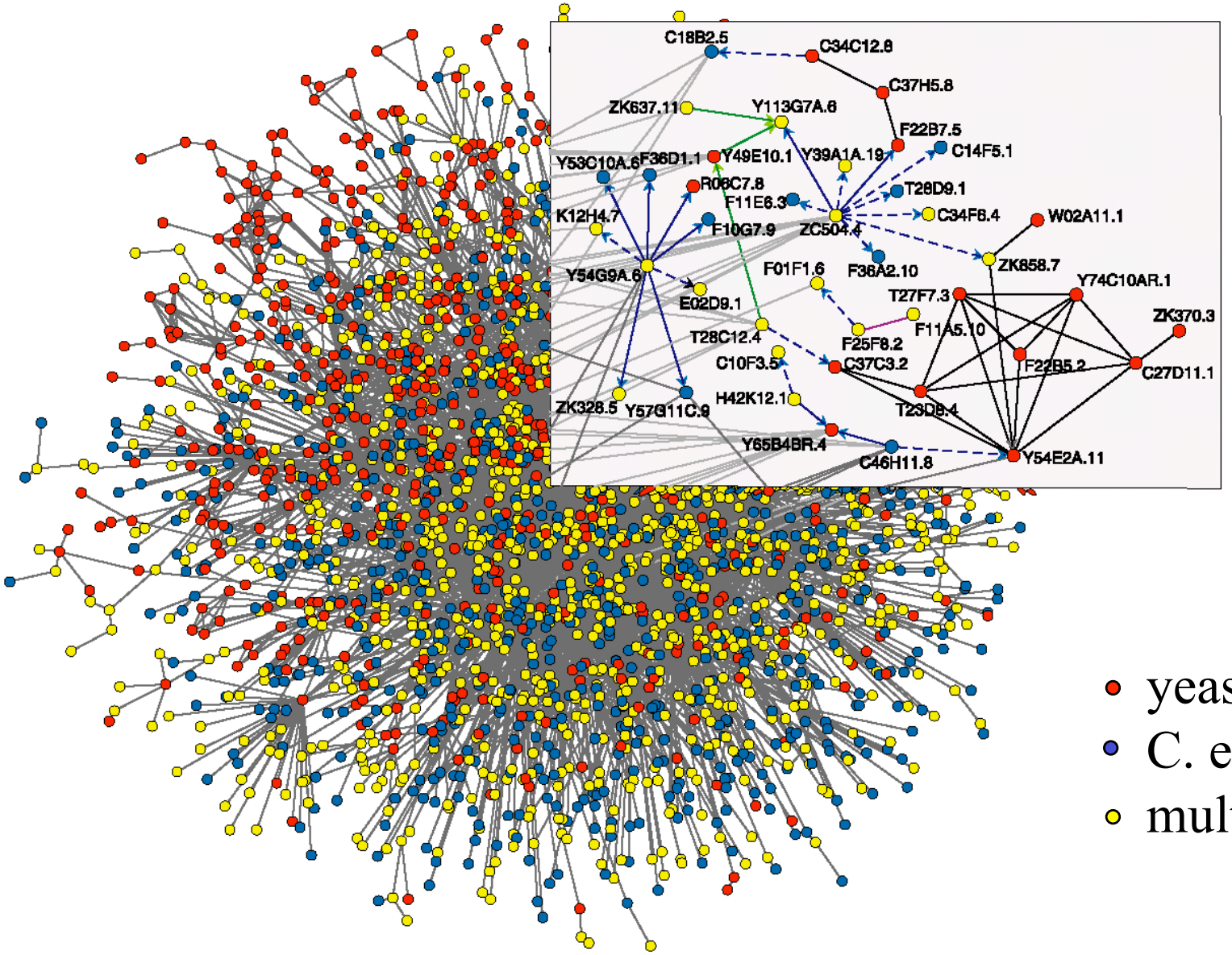
Protein Protein Interactions

- functional association (dimers, oligomers)
- amyloid formation
- aggregation

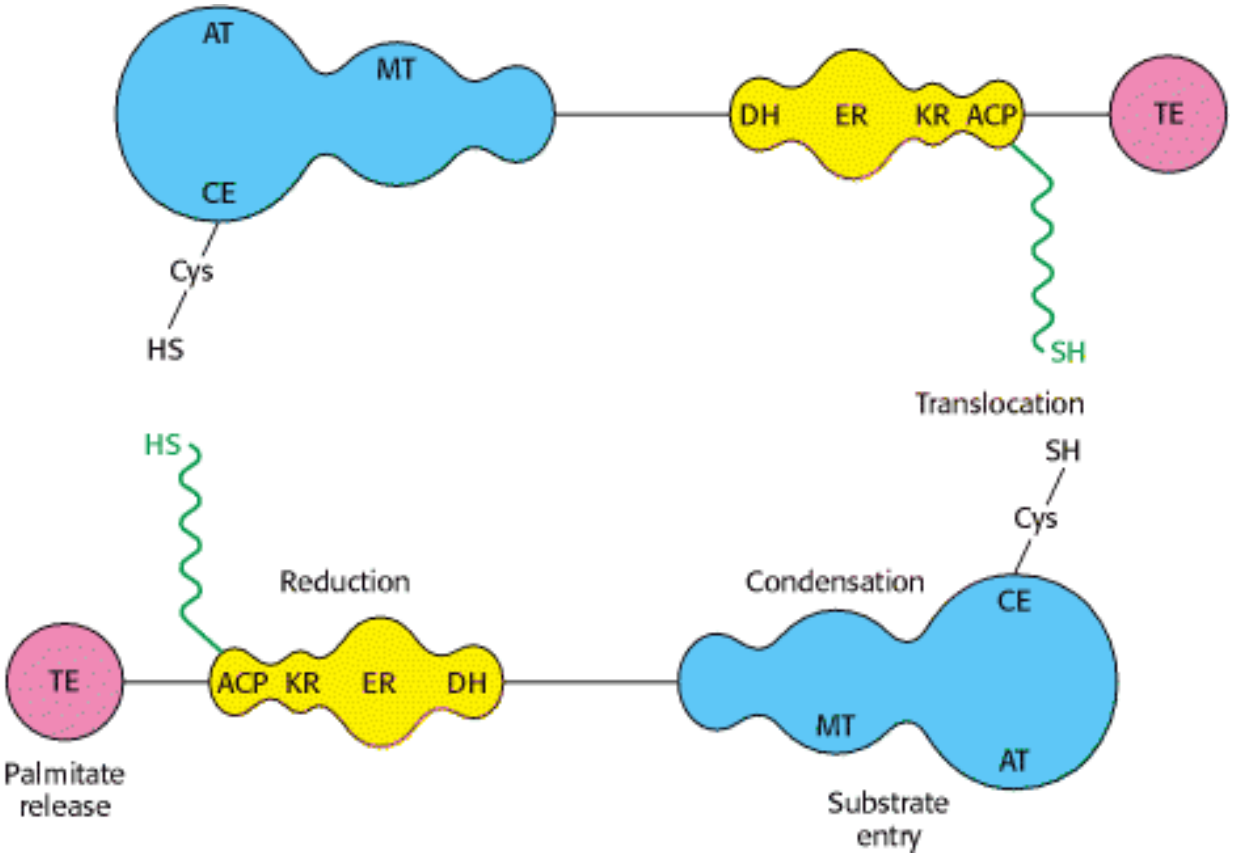
Interactome - protein interactions within a cell



- yeast
- C. elegans
- multicell.

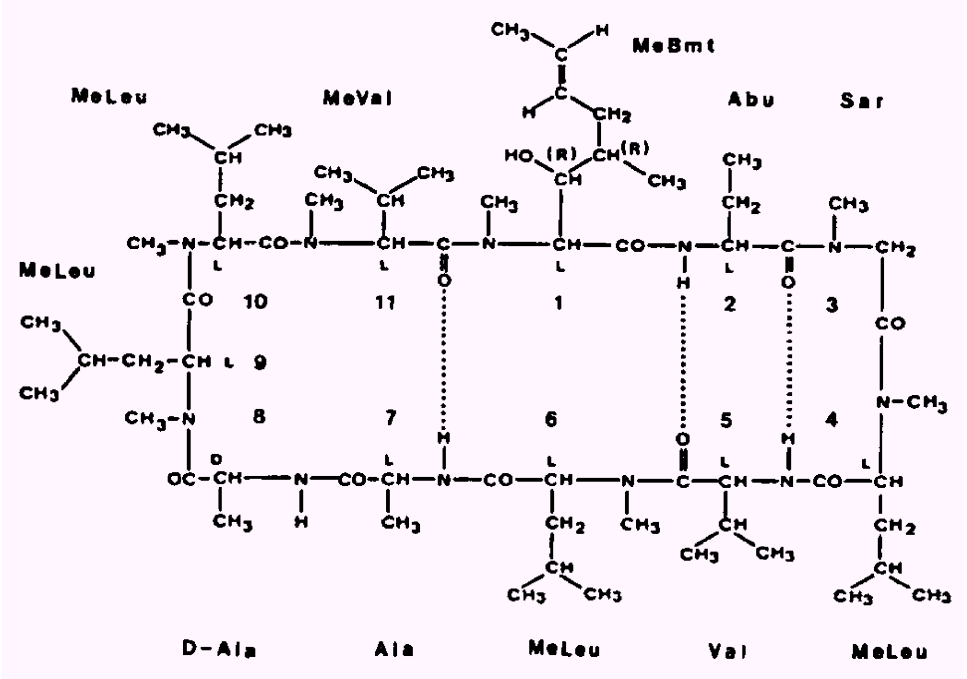
Alternative to Protein Association - Giant Proteins

Fatty acid synthase:	- E. coli	single proteins
	- yeast	$\alpha_6\beta_6$ oligomer (2 200 kDa)
	- mammals	dimer

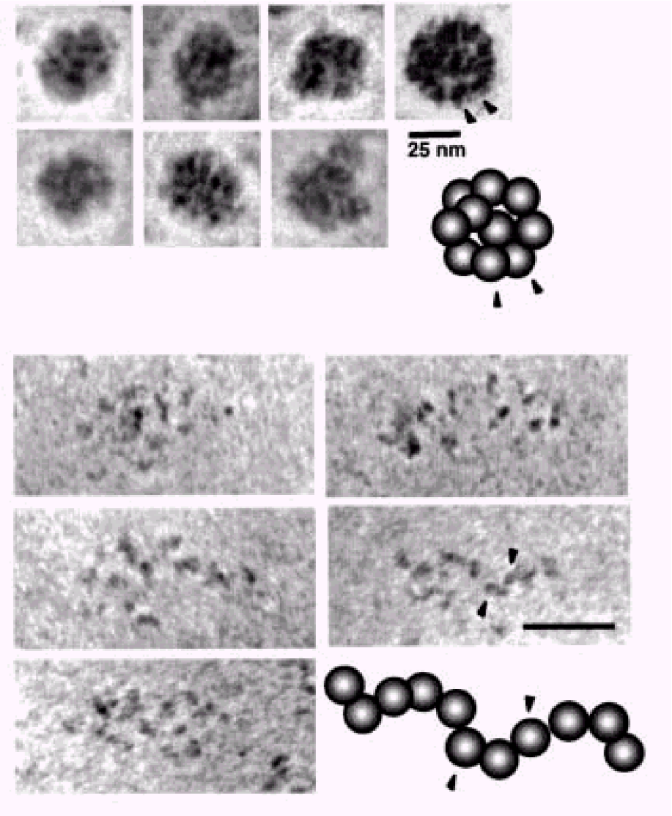


Giant Proteins - Cyclosporin synthetase

- non-ribosomal peptide synthetases often large monomeric proteins
- Cyclosporin synthetase: 1.7 MDa
  11 amino acid activating domains
  and transferases



cyclosporin

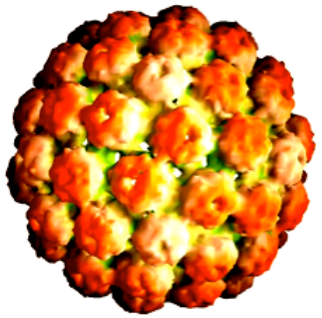


Why oligomers ?

Functional level
- allosteric regulation
- catalytic efficiency (substrate channeling)

Structural level
- errors during protein synthesis
- domain folding in large proteins slower than in isolated domains

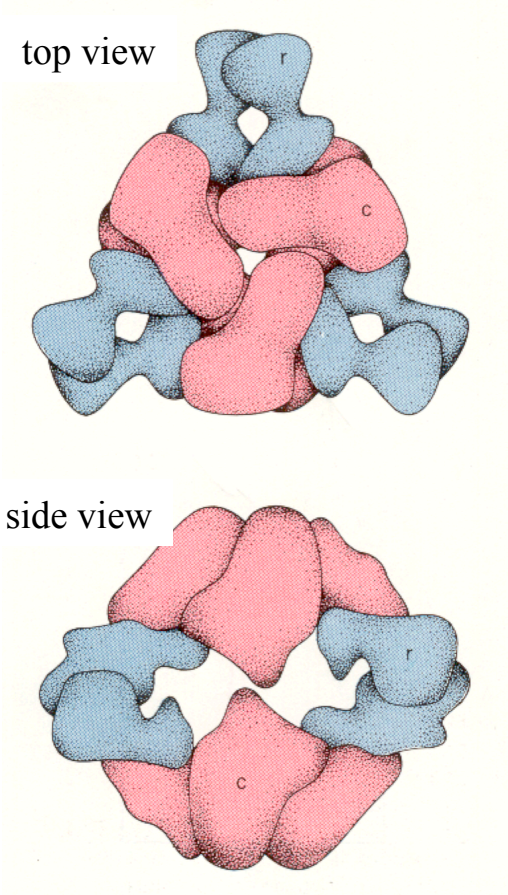
Genetic level
- oligomerization far more economical (e.g. virus capsid)



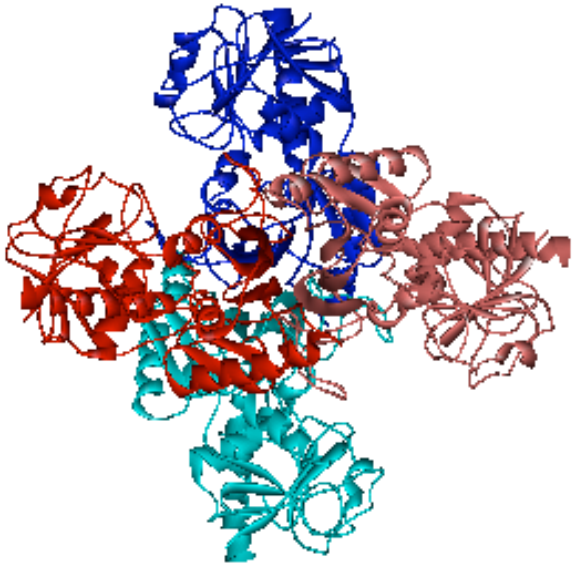
Virus shell of Polyomavirus: 72 pentamers 15 MDa

Topology of oligomers

ATCase:
- 2 catalytic trimers
- 3 regulatory dimers



GAPDH
- a tetramer as dimer of dimers



4 M ⟶ 2 D ⟶ T

alternative (unlikely):

4 M ⟶ D + 2 M ⟶ Trimer + M ⟶ T

Characteristics of inter-subunit interfaces

surfaces of inter-subunit interfaces: 1000 - 3000 Å

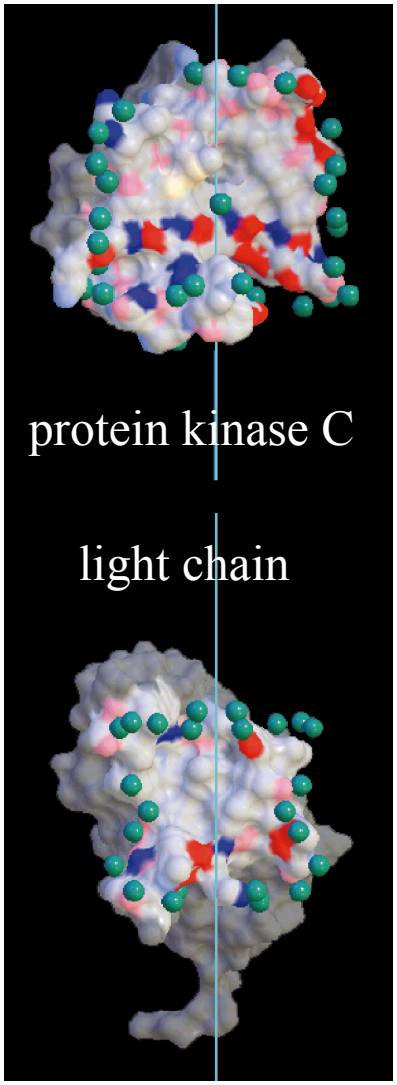
partially hydrophobic ⟶ stability

polar interactions and complementarity ⟶ specificity

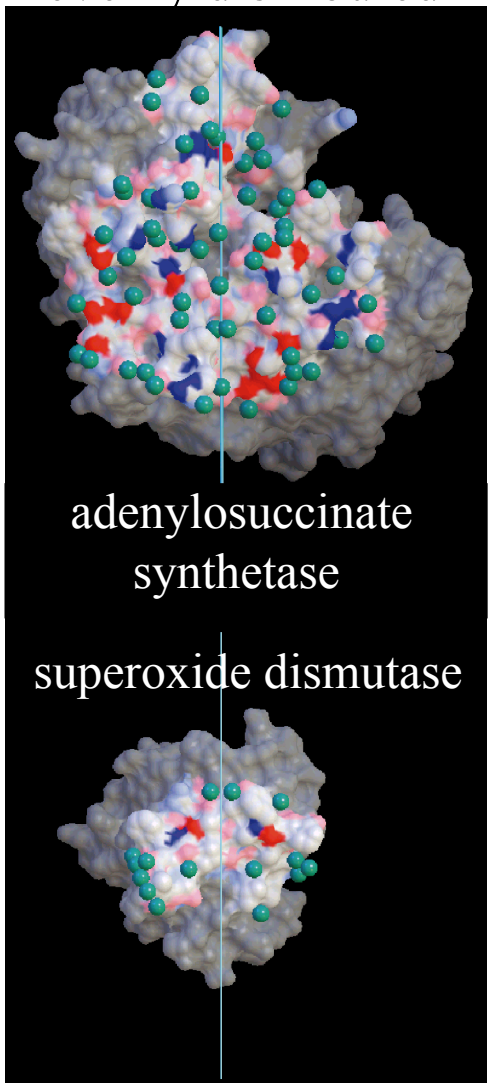
packing density of side chains within interfaces
comparable to hydrophobic core (in case of stably associated proteins)

Three classes of inter-subunit interfaces

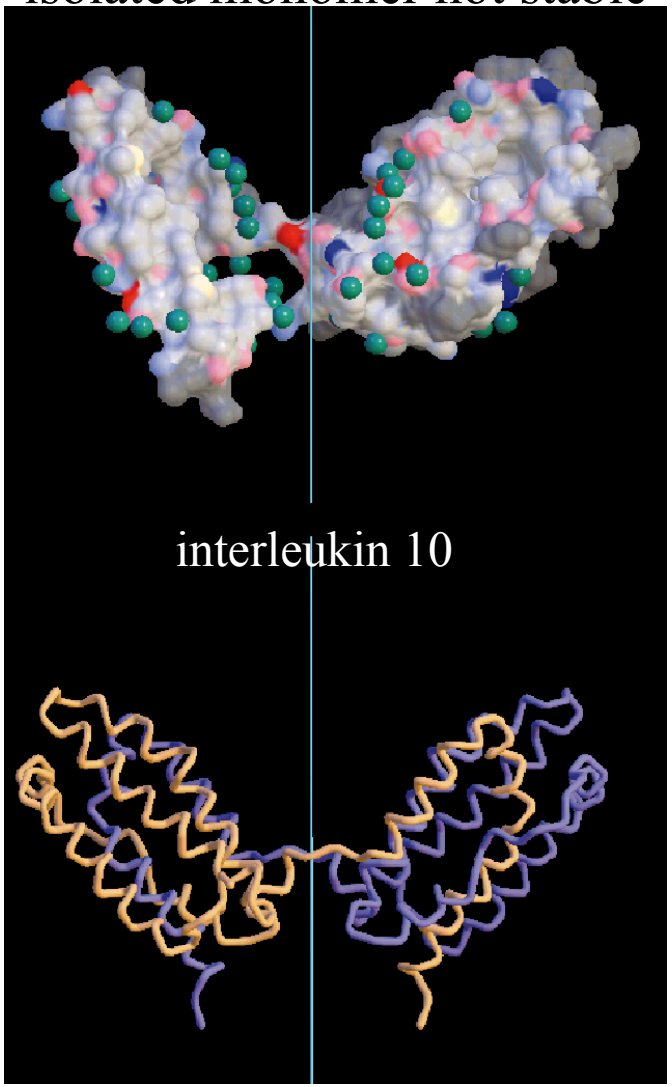
hydrophobic patch
surrounded by polar groups



small hydrophobic areas,
polar groups and water
evenly distributed



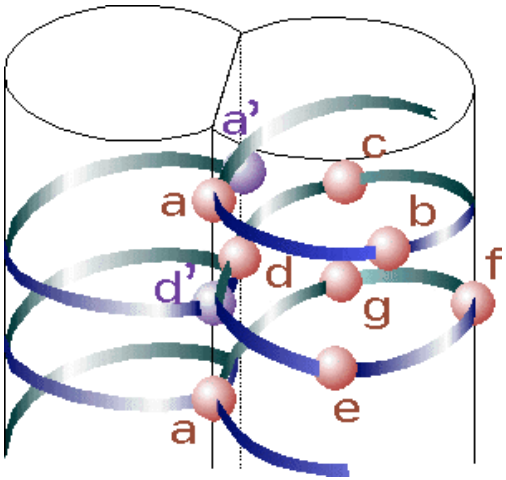
intertwined interface
resemble hydrophobic core
isolated monomer not stable

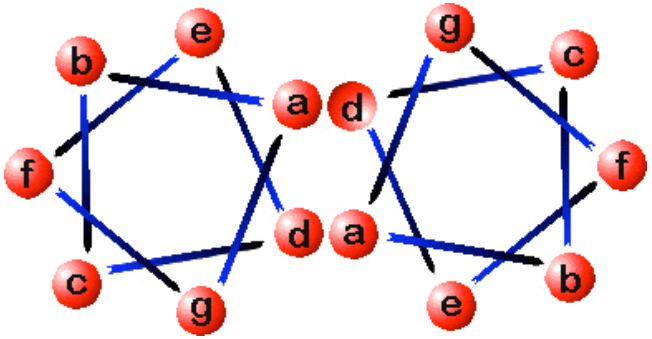


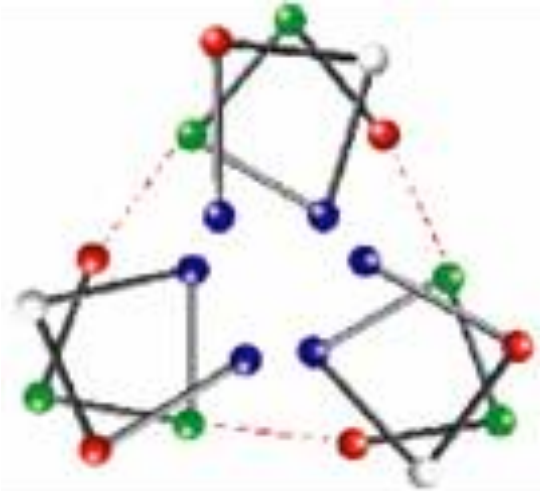
Special motifs of protein protein association

Coiled coil - Leucine zipper

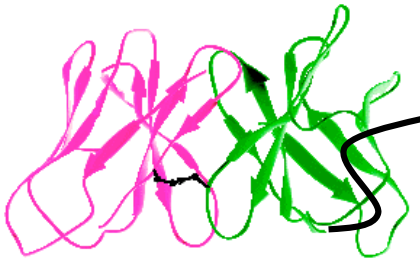
Heptad repeat: HPPHPPP coiled coils: dimers, trimers, tetramers







Artificially engineered polyionic peptides



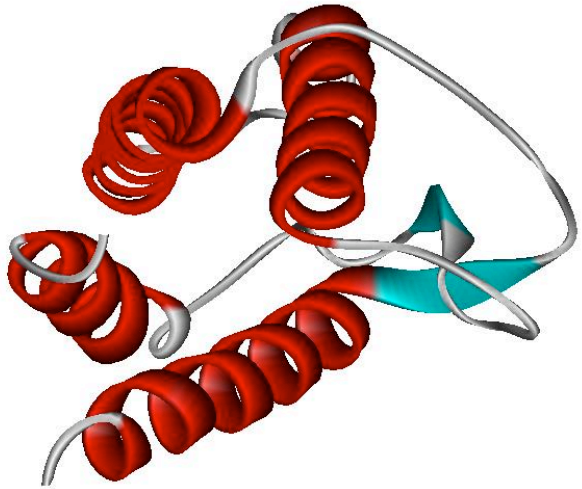
(V_H)-Val-Ser-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys-Pro

Gly-Ser-Pro-Glu-Phe-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Glu-Cys-Ala-Ser

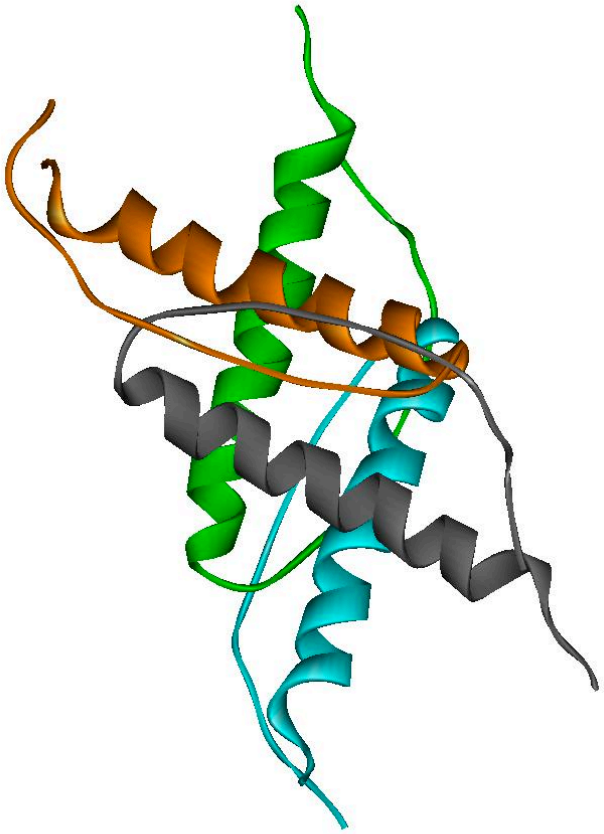
Special motifs of protein protein association

Four-helix bundle

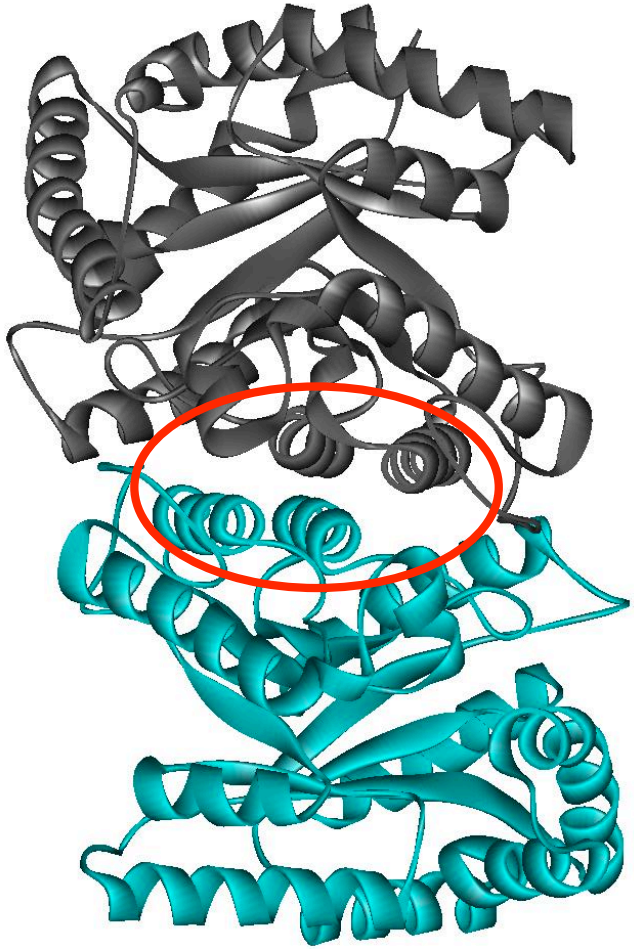
Monomeric IL4



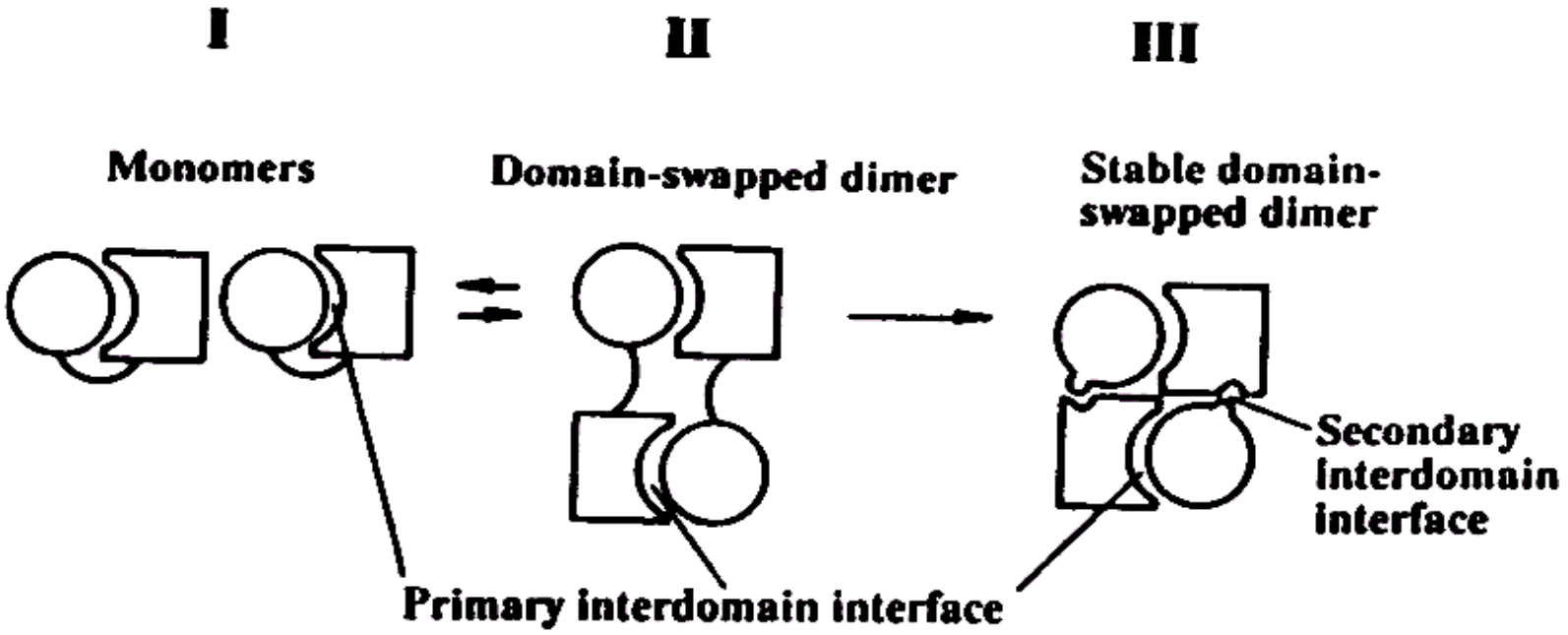
Tetramerization domain of p53



Dimeric bacterial luciferase



Domain swapping

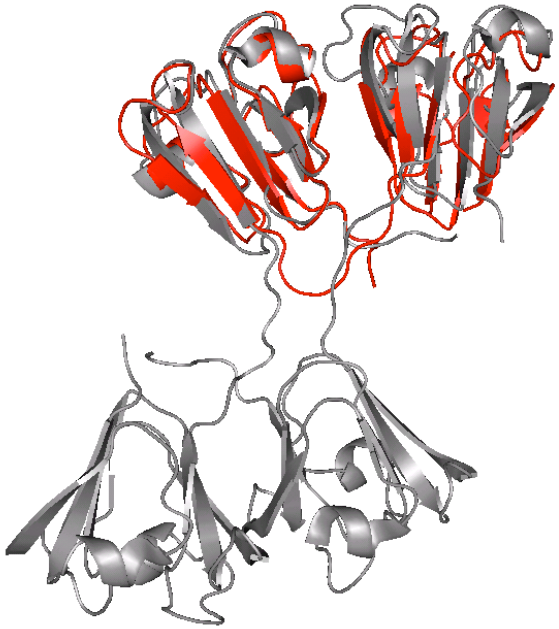


- Domain swapping ⟶ evolution from monomers to dimers
- product of domain swapping ⟶ dimers,
  but also higher oligomers ⟶ virus capsids
  ⟶ aggregation of antithrombin

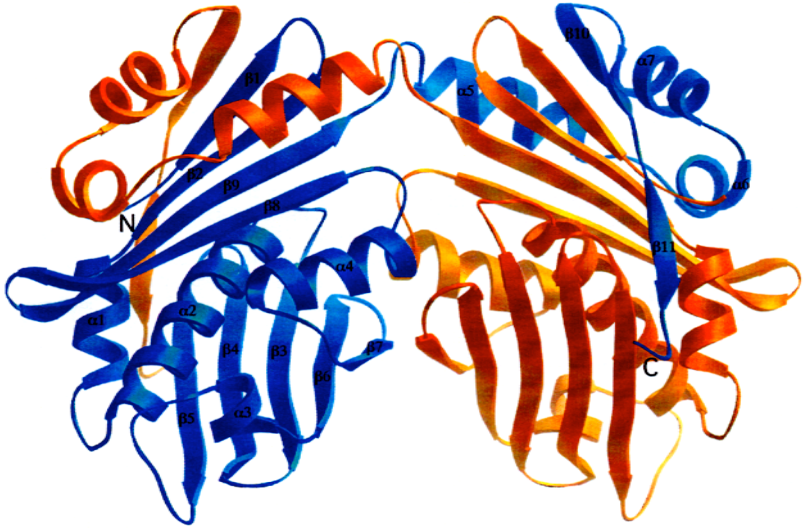
Domain swapping

swapping

of domains **of super-secondary structure** **of single strands**



Crystallins



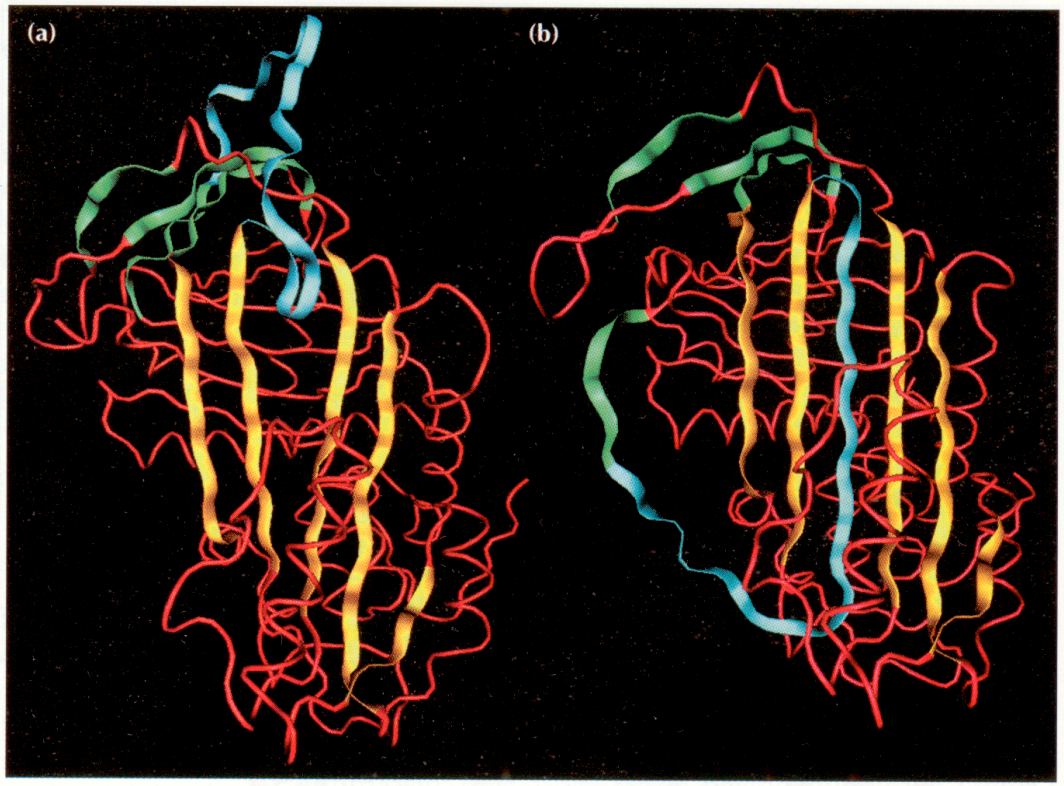
Hsp 33

RNase A

Domain swapping as cause of aggregation

Antithrombin

active inactive form



Antithrombin

- Protease inhibitor of the Serpine class

- spontaneous inactivation by inserting the active site loop as ß-strand into a pre-formed ß-sheet

- inter- instead of intra-molecular insertion

⟶ aggregation

Stability of oligomers - dissociation equilibrium

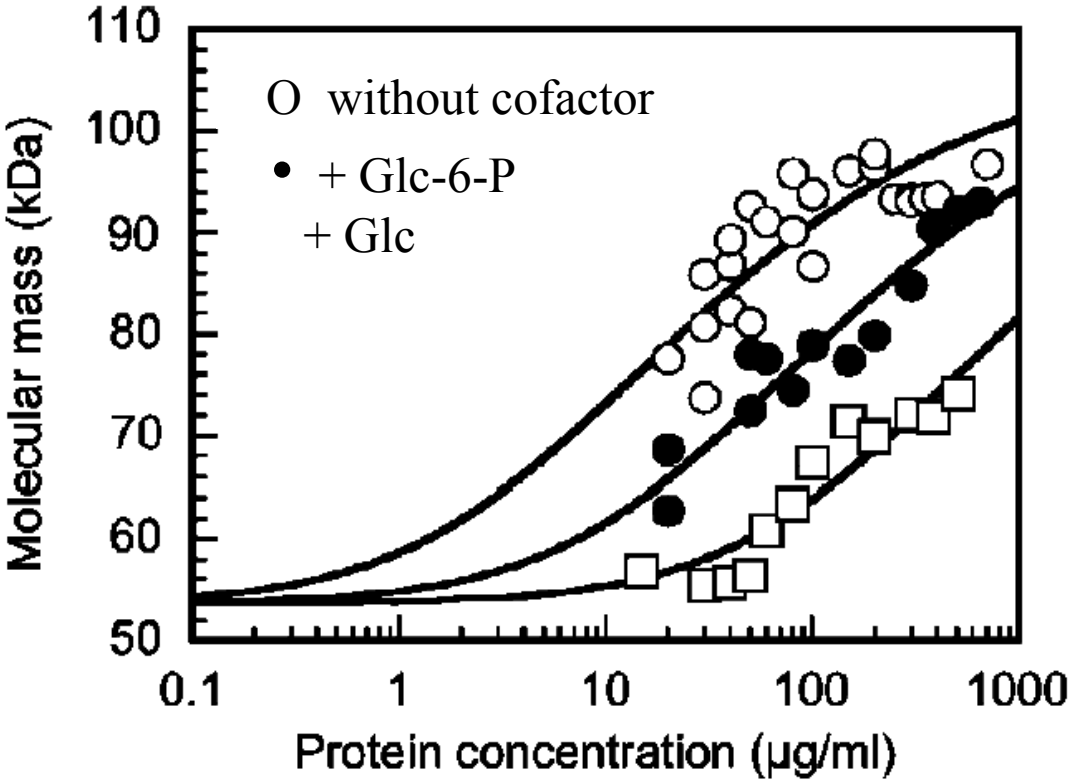
Dimerization of Hexokinase

$$2\,M \rightleftharpoons D$$

Reaction:

Glucose + ATP $\rightleftharpoons$ Glc-6-P

$K_D = 0.15 \times 10^{-6}$ M (without cofactor)



$$K_D = M^2 / D$$

$$D = M^2 / K_D$$

$$D = \frac{M_{tot} - M}{2}$$

$$M^2 + \underbrace{0.5\,K_D}_{p}\,M - \underbrace{0.5\,K_D\,M_{tot}}_{q} = 0$$

$$M = -p/2 + \sqrt{(-p/2)^2 - q}$$

$$S = S_0 + dS_{max} * 2D/M_{tot}$$

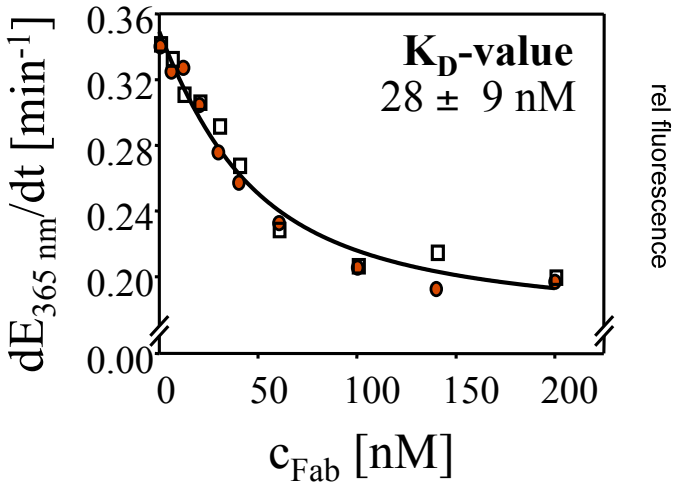
Stability of oligomers - dissociation equilibrium

Fab - antigen

antigen: creatine kinase

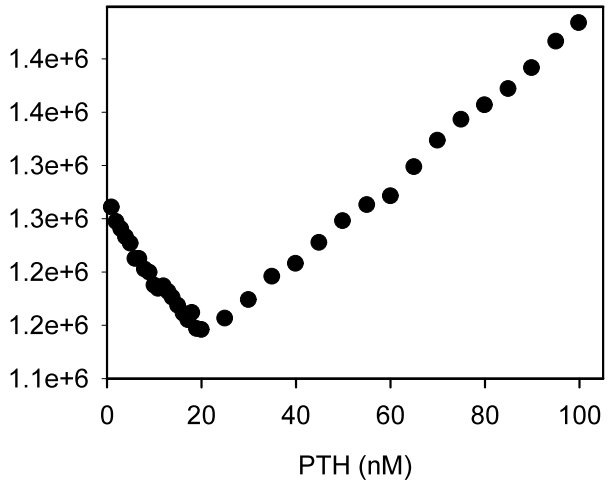
measurement:

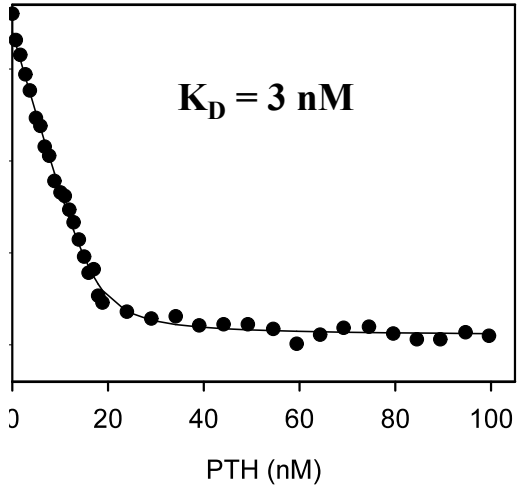
enzyme activity



PTH - PTH receptor

measurement: fluorescence titration





$$R + L \rightleftharpoons RL$$

$$RL = -p / 2 + \sqrt{(-p / 2)^2 - q}$$

$$p = (R_0 + L_0 + K_D) / 2$$

$$q = R_0 L_0$$

$$S = S_0 + dS_{max} * RL/R_0$$

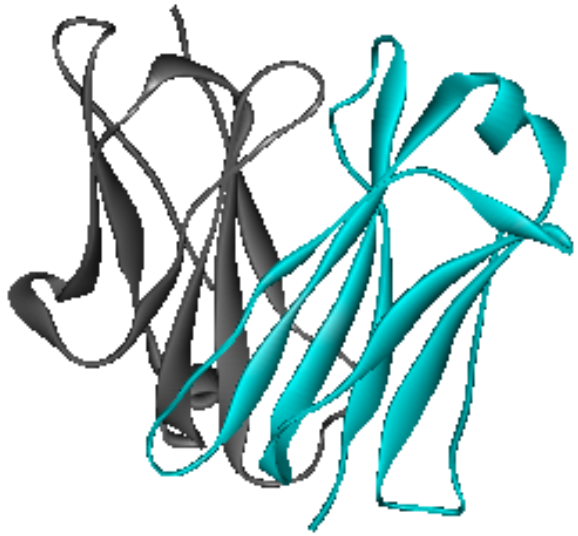
Stability of oligomers - unfolding

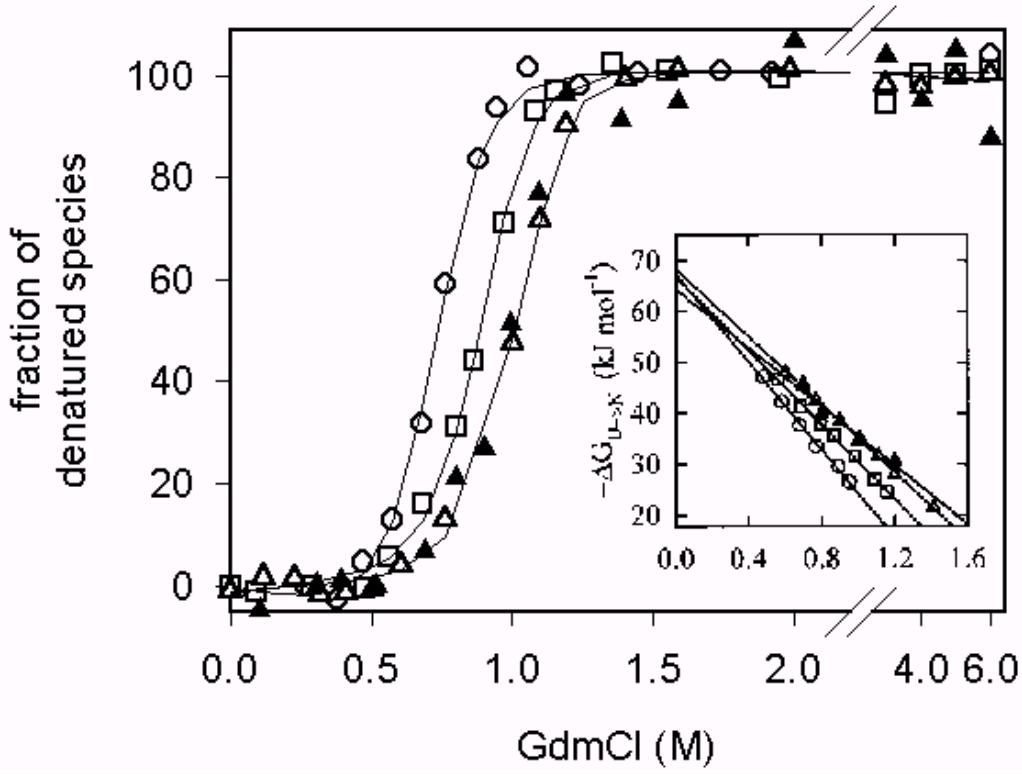
$$N_2 \rightleftharpoons 2\,U$$

$$K_U = [U]^2/[N_2] = 2P_t[f_d^2/(1-f_d)]$$

$$\Delta G^D = -RT\ \ln K_U$$

Dimeric antibody C_H3 domain





Rates of association

Diffusion limit of protein association (random diffusional collision):
10^9 $M^{-1}s^{-1}$ (chaperone-substrate interaction)

not every collision leads to complex formation (structural constraints)
⟶ maximum association rate ca. **10^6 $M^{-1}s^{-1}$**

Protein	**Subunit mass (kDa)**	**Reaction**	**k ($M^{-1}s^{-1}$)**
Triosephosphate isomerase	27	2M -> D	3×10^5
Malate dehydrogenase (mitochondrial)	33	2M -> D	3×10^4
Invertase	76	2M -> D	1×10^4
β-Galactosidase	116	2M -> D	4×10^3
Alcohol dehydrogenase (liver)	40	2M -> D	2×10^3
Phosphoglycerate mutase	27	2M -> D	6×10^3
		2D -> T	3×10^4
lactate dehydrogenase	36	2D -> T	2×10^4
Arc repressor	62	2M -> D	1×10^7
Trp aporepressor	12	2M -> D	3×10^8

Homo- versus hetero-dimerization

only relevant if the two subunits are highly homologous (gene duplication)

Bacterial Luciferase

black: ß2 homodimer
green/red: α/ß heterodimer

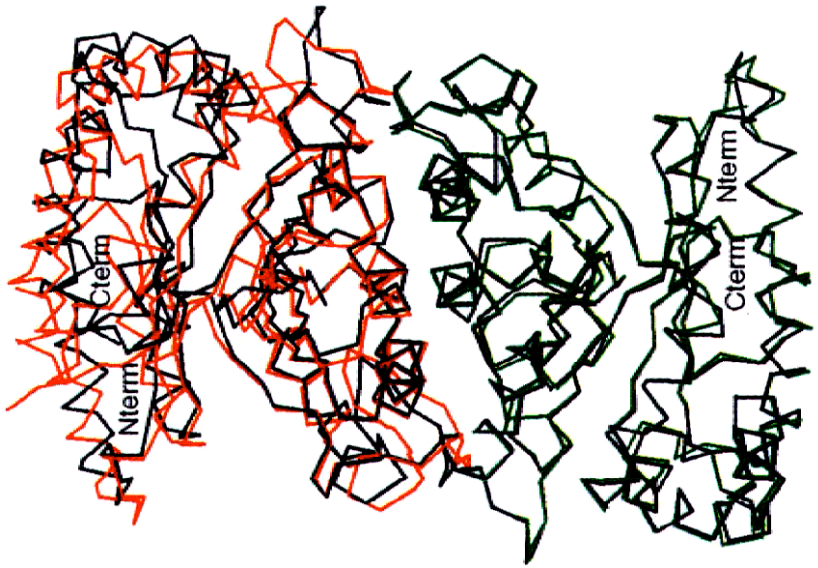


Table 2. *Intersubunit hydrogen bonds in the β_2 homodimer and the equivalent interactions in the $\alpha\beta$ heterodimer*

Subunit 1		Subunit 2		Bond Distance (Å)	α subunit	
Residue	Atom	Residue	Atom		Residue	Atom
Ser 17	O^{γ}	His 161	$N^{\delta 1}$	3.0	[b]	
Asp 18[a]	$O^{\delta 1}$	Gln 95	$N^{\epsilon 2}$	2.7	Thr 18	O^{γ}
Asp 18[a]	$O^{\delta 1}$	Gln 95	$O^{\epsilon 1}$	3.2	Thr 18	O^{γ}
His 45[a]	$N^{\delta 1}$	Glu 88	$O^{\epsilon 1}$	2.7	His 45	$N^{\delta 1}$
His 45[a]	$N^{\delta 1}$	Glu 88	$O^{\epsilon 2}$	3.3	His 45	$N^{\delta 1}$
Thr 80[a]	O	Arg 85	$N^{\eta 2}$	2.9	Thr 80	O
Thr 80[a]	O^{γ}	Arg 85	$N^{\eta 2}$	2.6	Thr 80	O^{γ}
Phe 116	O	His 82	N^{ϵ}	2.6	Val 116	O
Ser 47[a]	O	Asn 159	$N^{\delta 2}$	3.1	None	

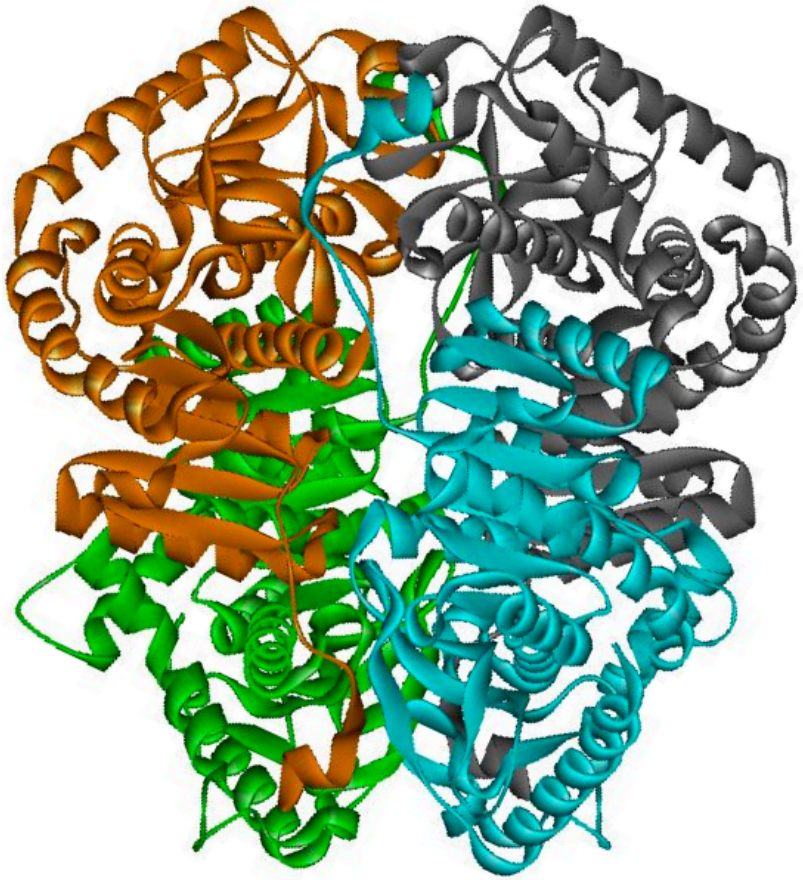
[a]The two-fold related hydrogen bonding interaction is also observed at the subunit:subunit interface for these pairs of atoms with comparable geometry and hydrogen bond distance.

[b]The equivalent side chain in the α subunit (Gln 17) forms a structurally nonequivalent hydrogen bond to the amide nitrogen of His 161.

Reason for heterodimerization:
kinetics of a/ß association
10 times faster than ß2 association

Homo- versus hetero-dimerization

only relevant if the two subunits are highly homologous (gene duplication)



Lactate dehydrogenase (LDH)

2 isoforms in sceletal muscle and heart (LDH-M and LDH-H)

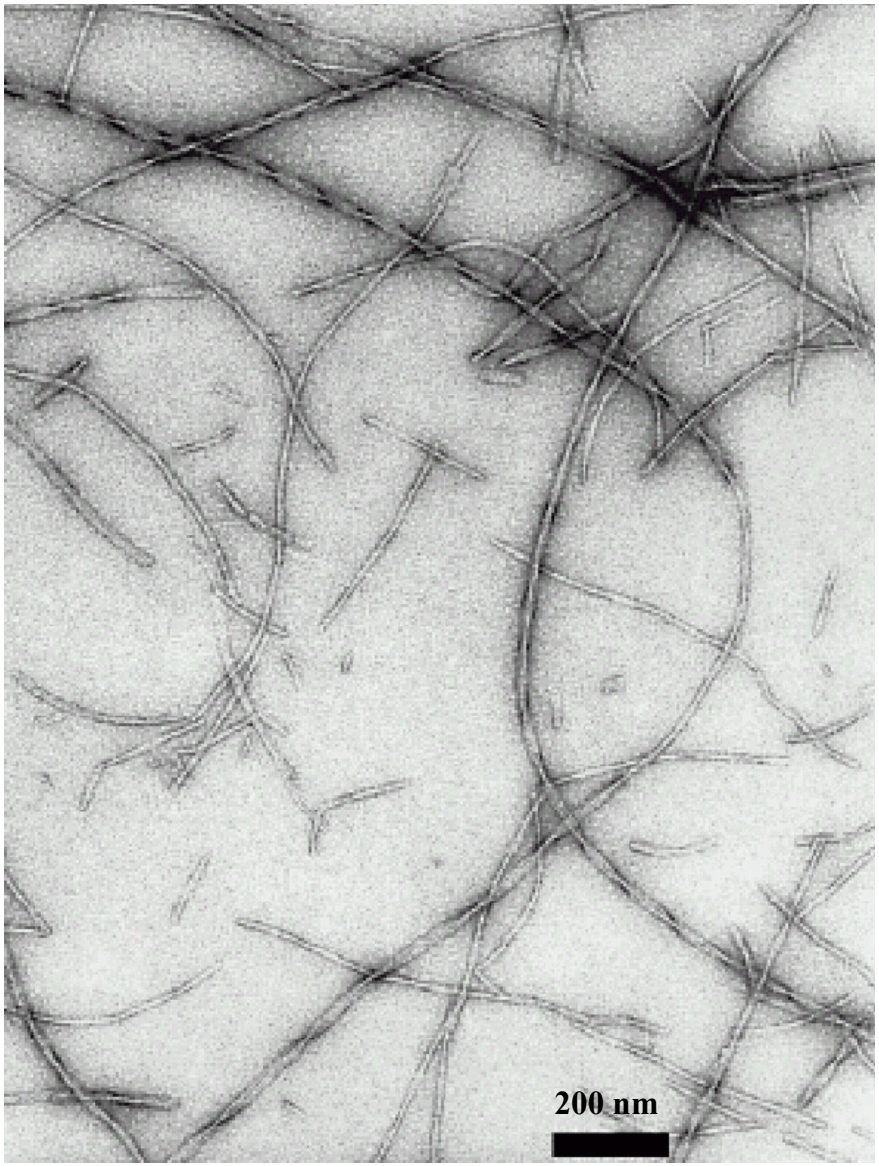
Hybrid formation of M- and H-type upon association of equimolar conc.:

M_4 M_3H_1 M_2H_2 M_1H_3 H_4

1 : 4 : 6 : 4 : 1

Amyloid structures - fibril formation

- structure of amyloids
- mechanism of fibril formation
- inhibition of fibril formation
  ⟶ therapy



Amyloid structures - diseases

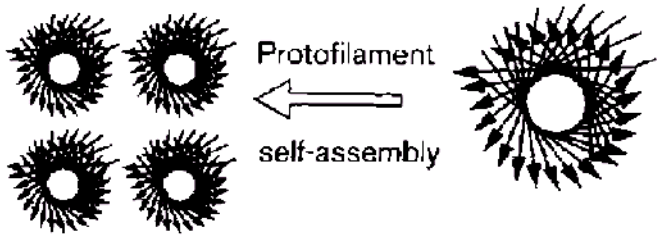
Disease	Protein
Morbus Alzheimer	APP/Alzheimer-β-Peptid (1-40, 1-42, 1-43); Tau-Protein
Transmissible Spongiforme Enzephalopathie (CJD, Kuru, BSE, Scrapie) - TSE	Prion-Protein
Chorea Huntington	Huntingtin
Morbus Parkinson	α-Synuclein

Amyloid structures - diseases

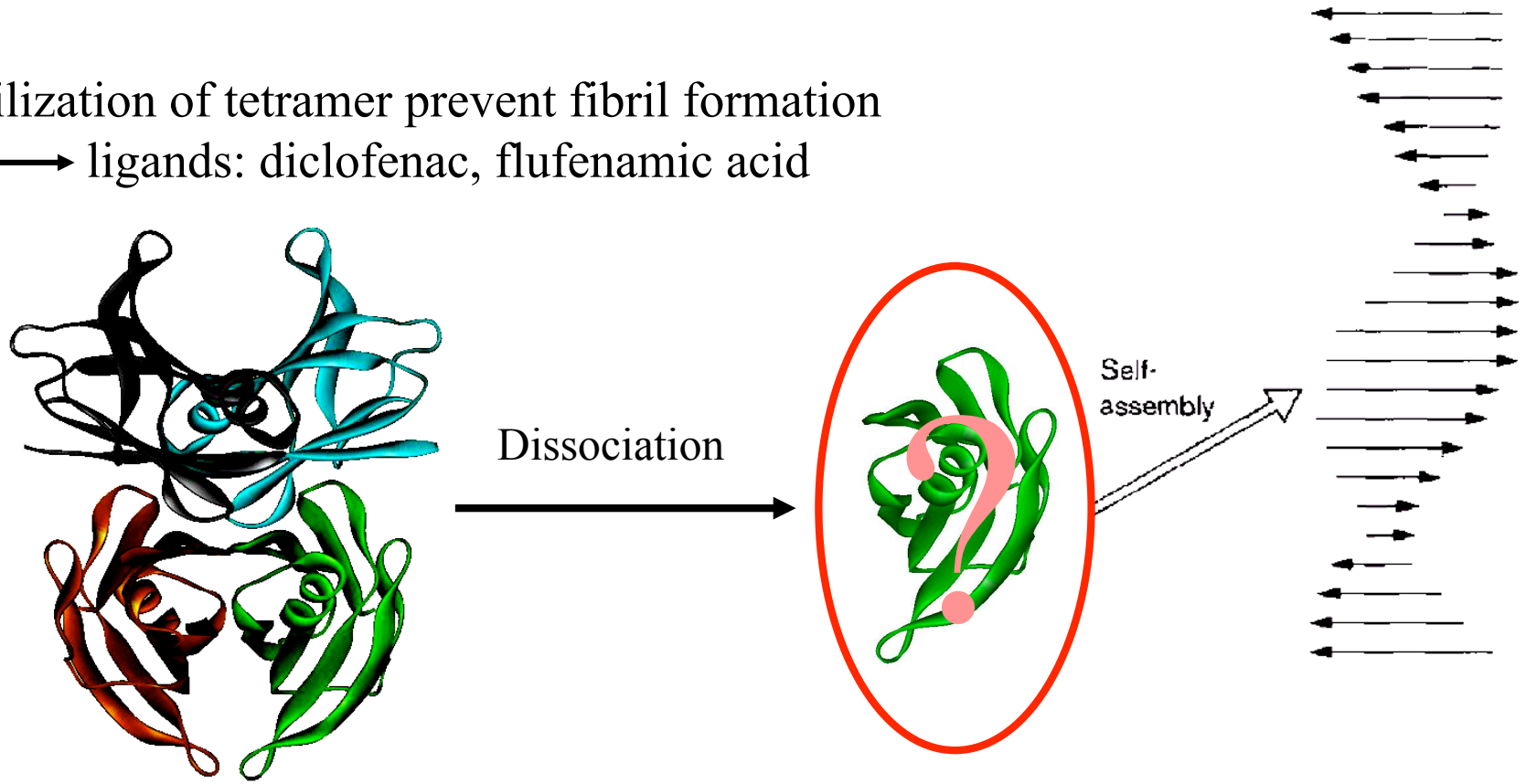
Disease	Protein
Injektionslokalisierte Amyloidose	Insulin
β-2-Mikroglobulin-Amyloidose	β-2-Mikroglobulin
Vererbbare Cerebrale Amyloide Angiopathie	Cystatin C
Primäre Asystemische Amyloidose	Immunglobulin
Finnische Vererbte Systemische Amyloidose	Gelsolin
Atriale Amyloidose	Atrial Natriuretic Factor
Familiäre Amyloide Polyneuropathie	Transthyretin
Medullaria-Carcinom der Schilddrüse	Calcitonin
Vererbbare Nichtneuropathische Amyloidose	Lysozym
Diabetes mellitus Typ II	Islet-Amyloid-Polypeptid
Reaktive Asystemische Amyloidose	Lipoproteine
Cleidocraniale Dysplasie	Transkriptionsfaktor CBFA1
Vererbte Renale Amyloidose	Fibrinogen
Okularpharyngeale Muskeldystrophie	Poly(A)-Bindungsprotein II

Fibrillation of Transthyretin

Homotetramer
transporting thyroxine
fibrillation - pH <4.5 (wt)
- pH 7 (mutants)



stabilization of tetramer prevent fibril formation
⟶ ligands: diclofenac, flufenamic acid



Fibrillation-competent state of Transthyretin

H/D exchange experiment:

completely deuterated protein

↓

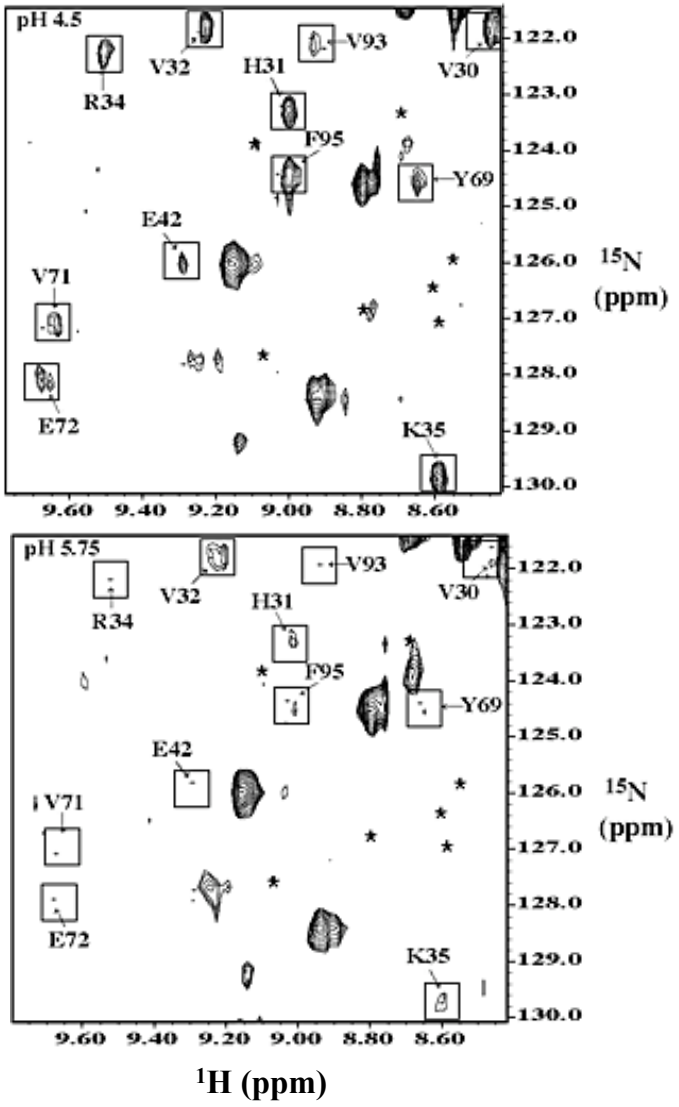
dilution into buffer at 8 µg/ml
pH either pH 4.5 or pH 5.8

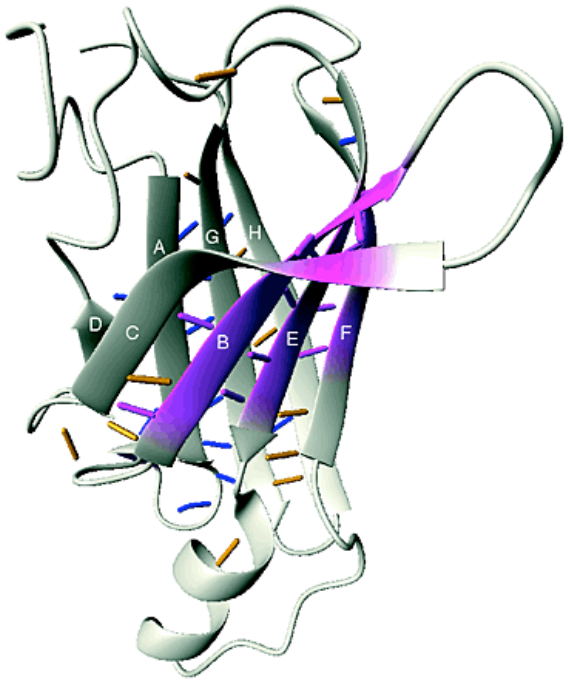
↓

shift to native conditions,
concentrating to 10 mg/ml

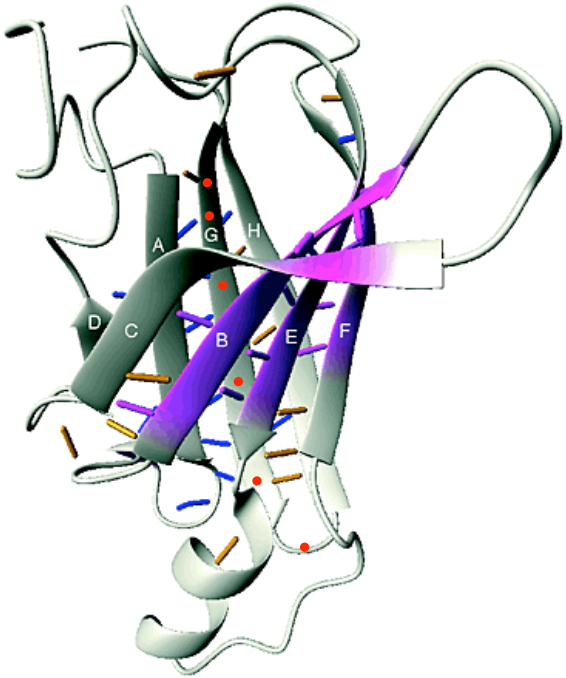
↓

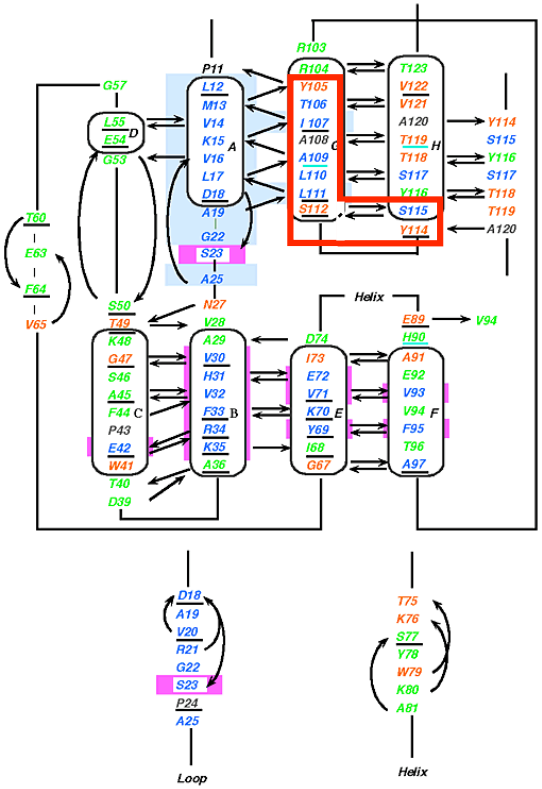
2D NMR





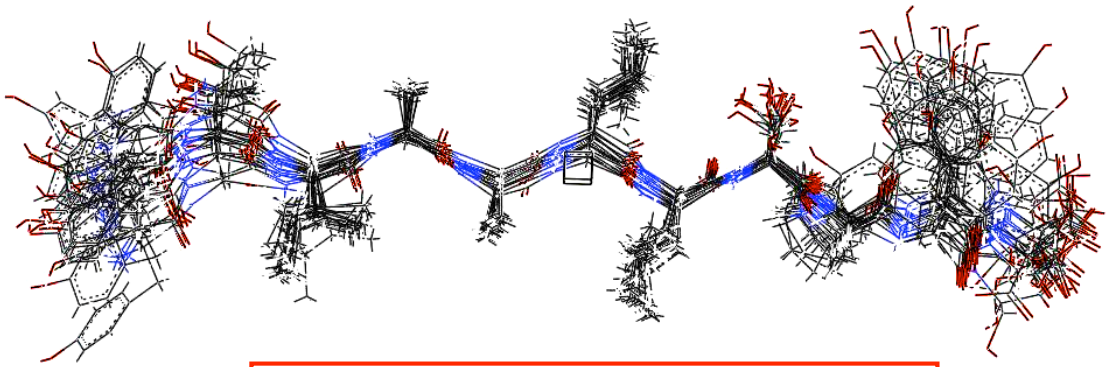
Amyloid state of a Transthyretin peptide





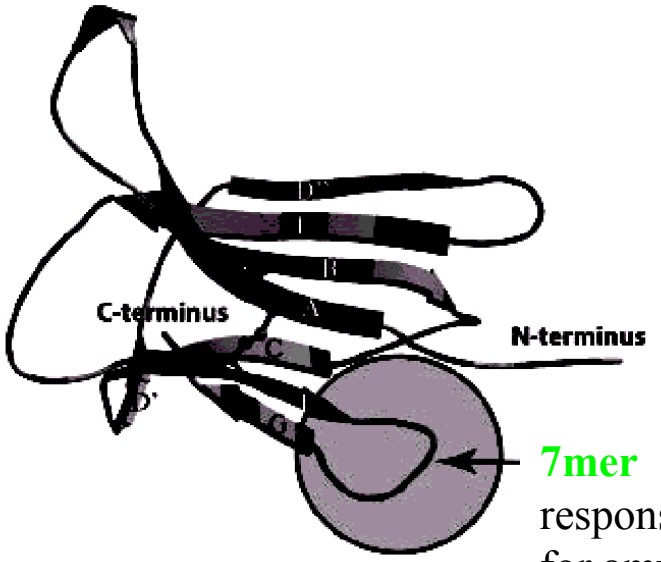
Solid state NMR (magic angle)
of a transthyretin peptide
within fibrils

⟶ elongated structure (ß-sheet)

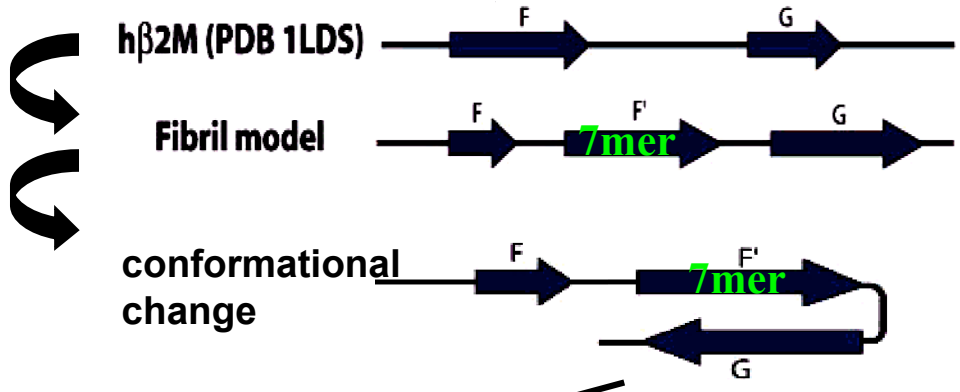


peptide 105-115 of transthyretin

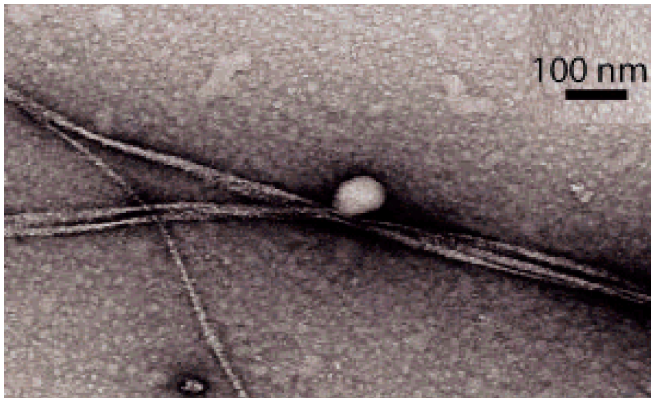
Amyloid model of ß2-microglobulin (ß2M)

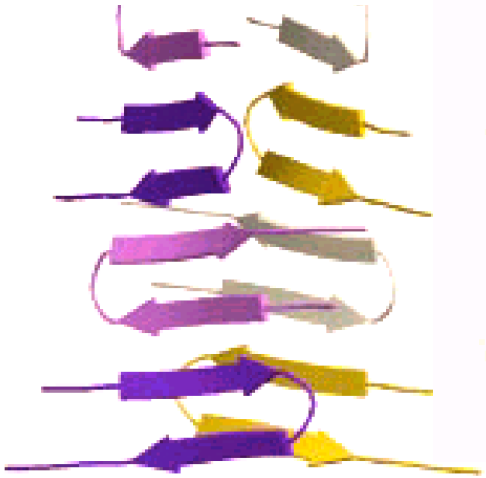


7mer responsible for amyloid formation



Fibrillation

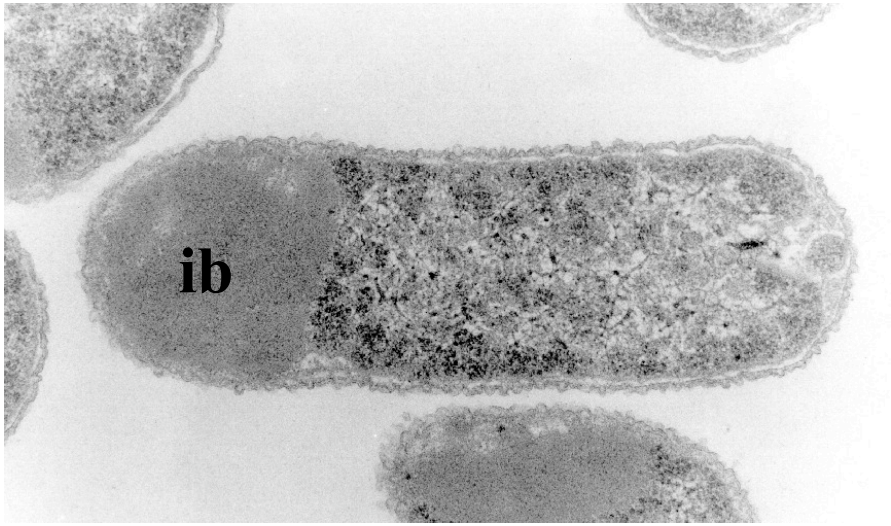




Model of complete fibril (top view)

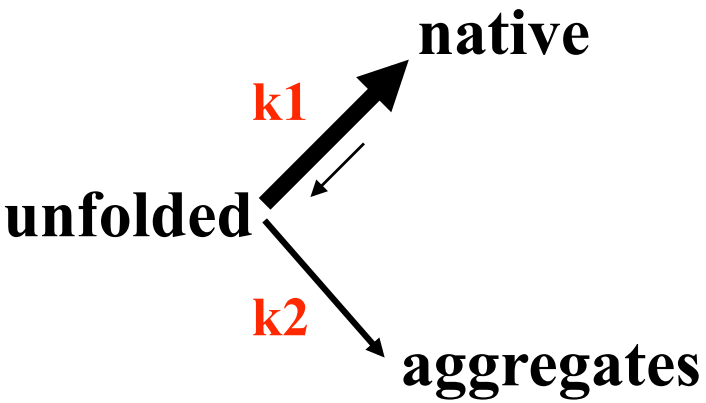
Aggregation of proteins

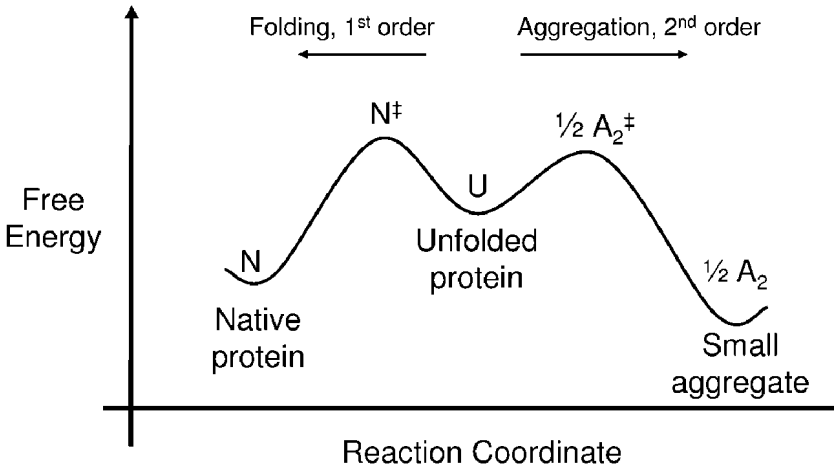
High level production of recombinant proteins in *E. coli* often leads to formation of inclusion bodies (ib‘s)



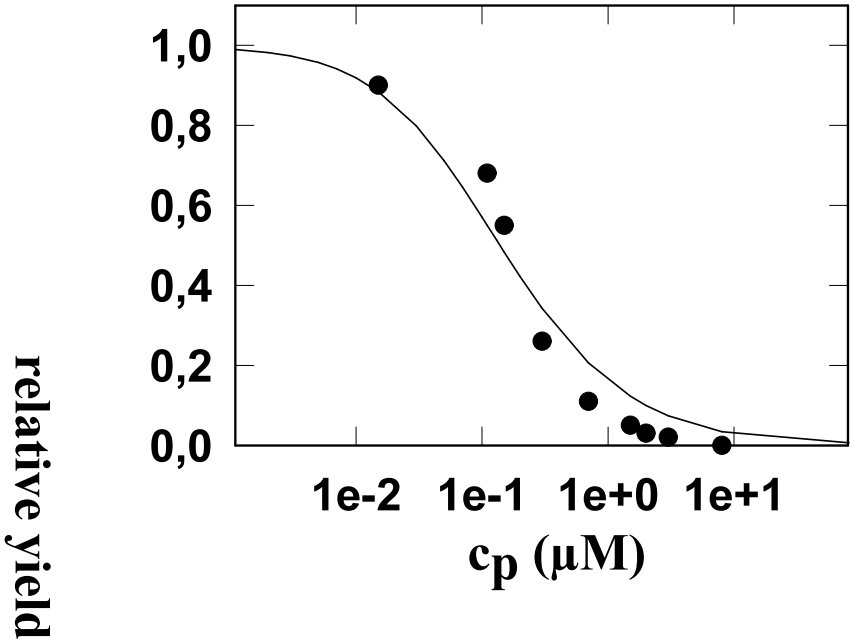
Electron micrograph of antibody producing E. coli

Kinetic competition of folding and aggregation

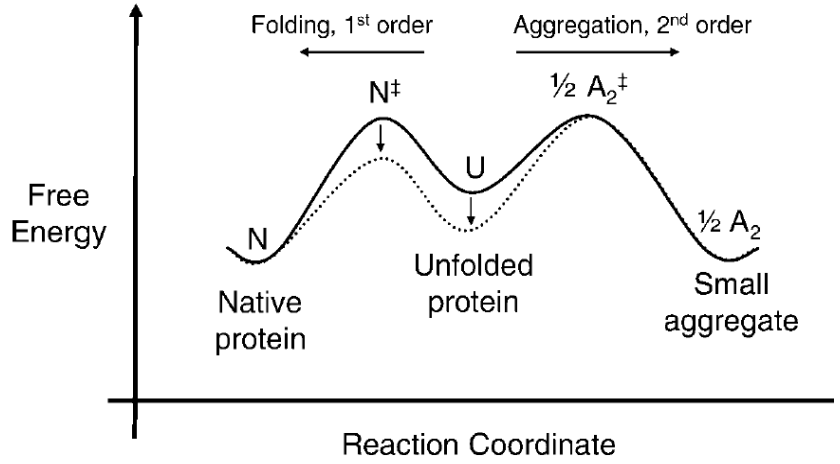




Yield = k1 / (k2 x [U]) x ln(1 + k2 x [U])

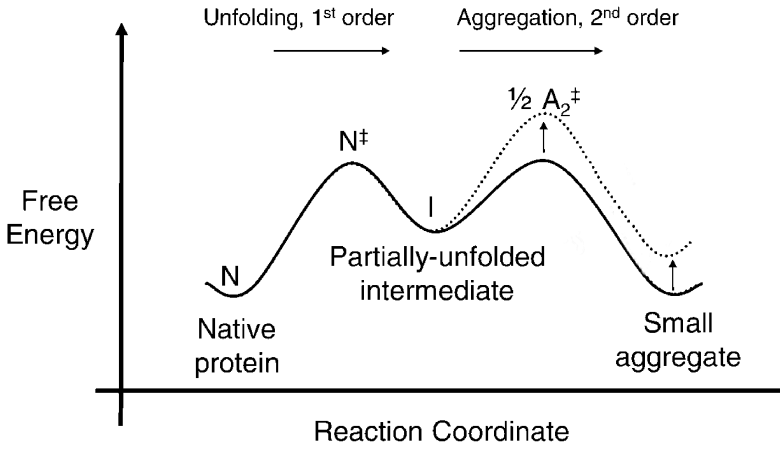


Effect of PEG on IFNγ



Kinetic competition of folding and aggregation

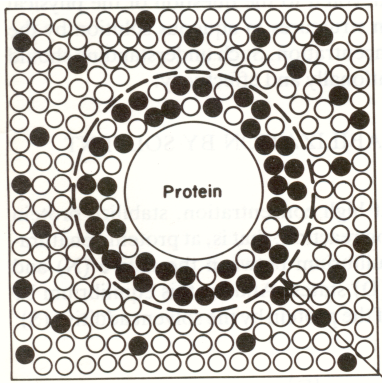
Effect of Arginine on protein association / aggregation



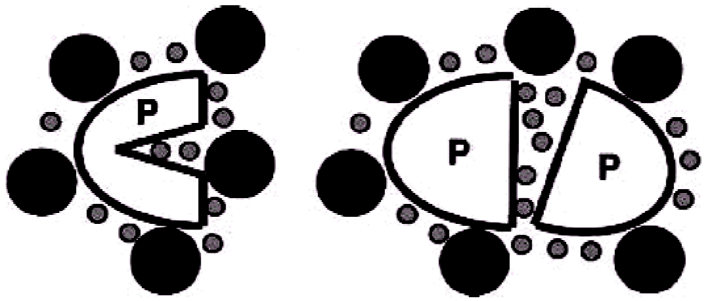
preferential binding

↓

prevent aggregation
but
destabilize structure



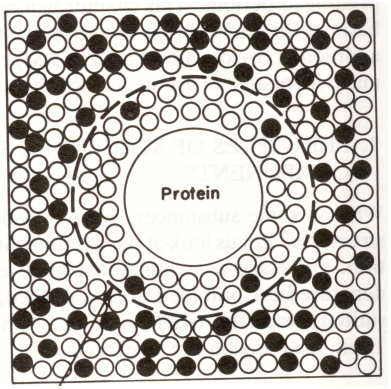
„gap effect“ slows aggregation kinetics



preferential hydration

↓

stabilize structure
but
often induce aggregation
upon renaturation



○ water ● arginine

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