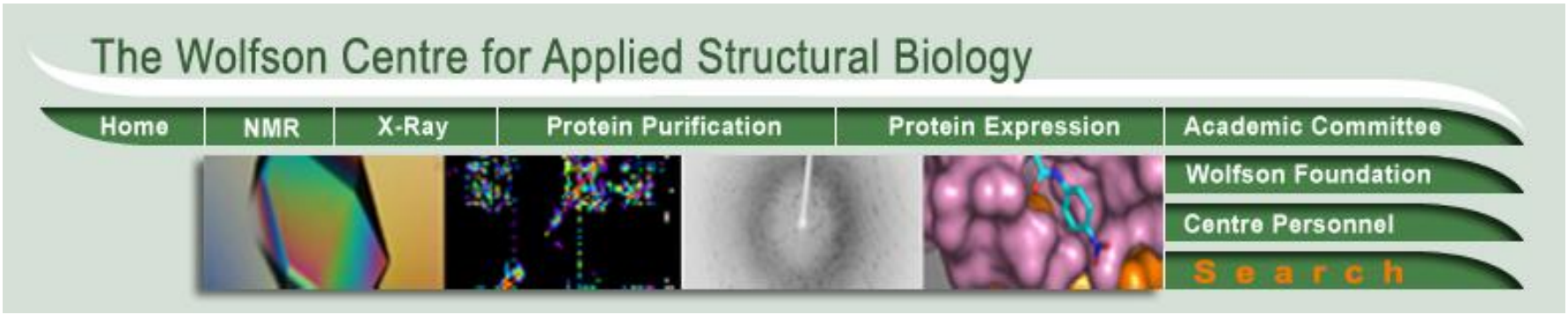


The Protein Expression and Purification Facilities

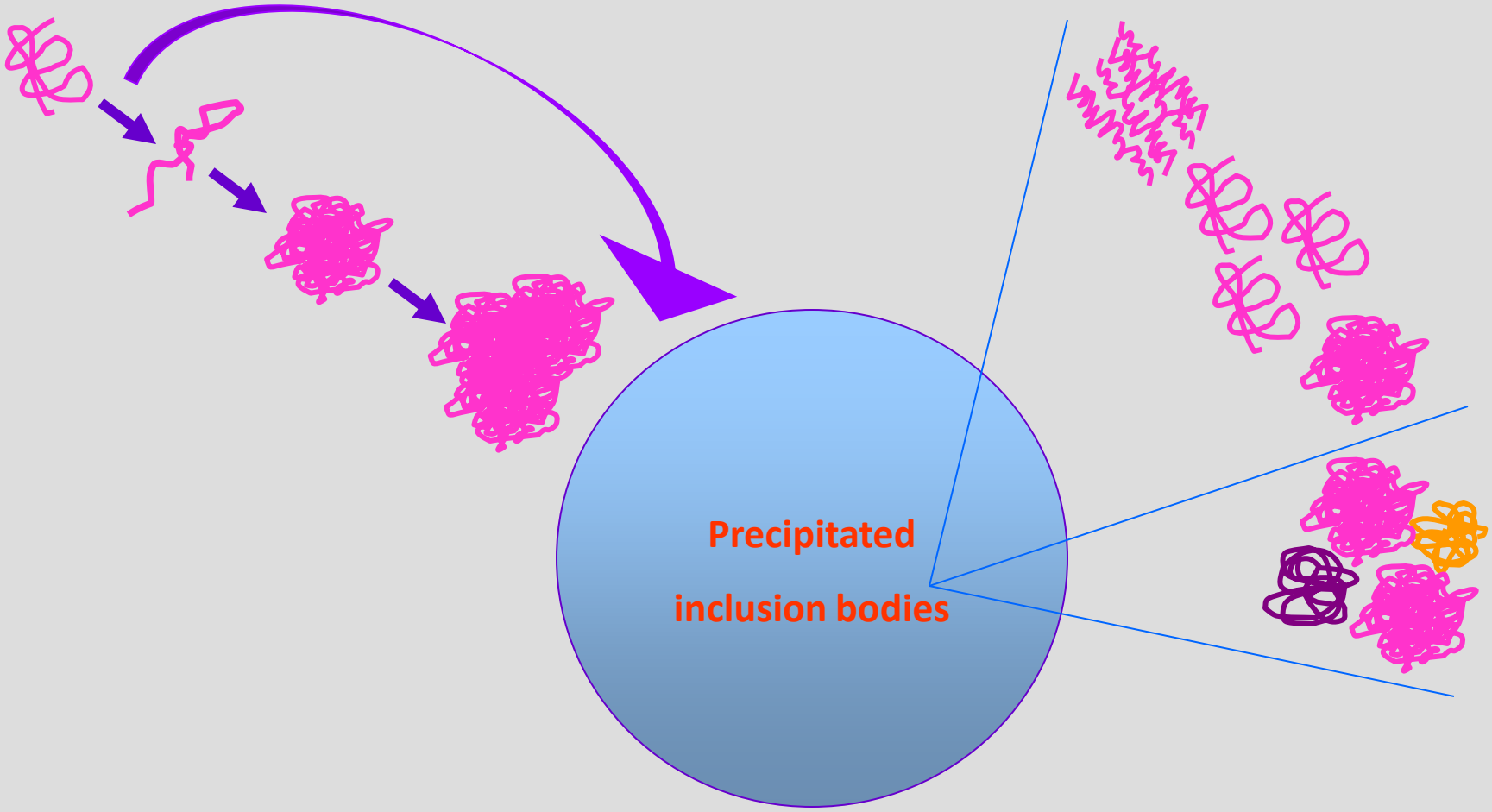
http://wolfson.huji.ac.il/expression

http://wolfson.huji.ac.il/purification

Main headache : Aggregation!!

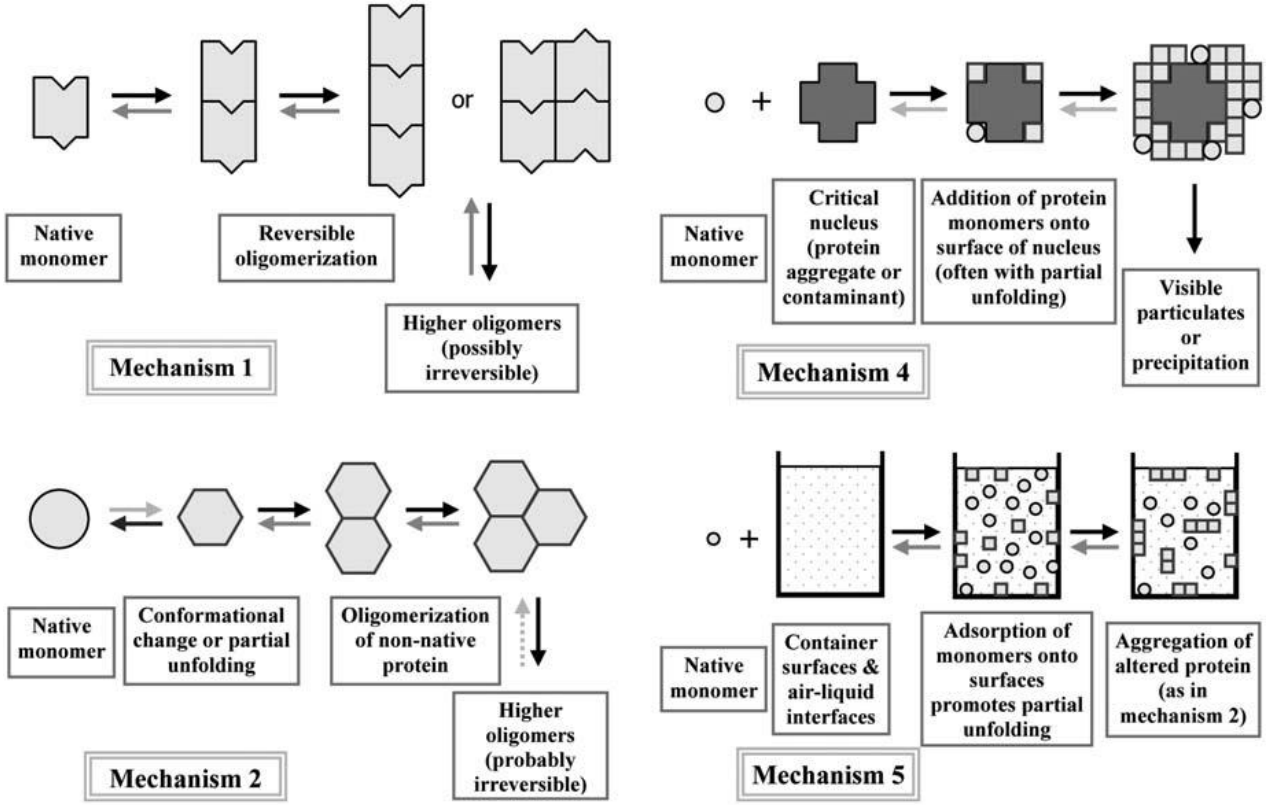
- **But where can aggregation start?**
- Expression
- Cell lysis and sample preparation
- Purification
- Storage

Protein aggregates – structure models



Mechanisms of Protein Aggregation

Philo J. *Current Pharmac. Biotech. (2009), 10, 348-351*



Nucleation!!
Continuos Progretion

How can we identify aggregates?

Insoluble aggregates

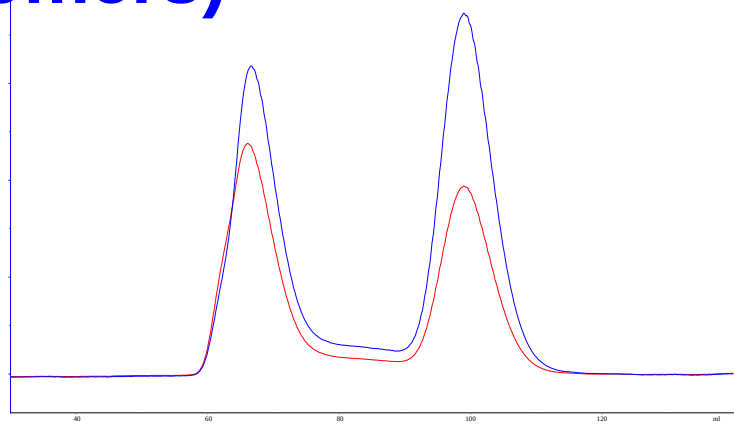
- 100µm – 1mm or more: Can be detected by eye
- 1 µm - ~100µm: Can be detected by absorbance (340, 490 or 600nm)

= Removed by filtration or centrifugation

Soluble aggregates (dimers-oligomers)

- 10nm - ~ 1 µm. Can be detected by GF, light scattering, native gels

= Removed by chromatography (mainly GF) & ultrafiltration



Aggregation = several types of interactions

- Exposure of hydrophobic surfaces

Denaturation or small perturbations

- Covalent aggregates

Incorrect intermolecular disulfide bridges, bityrosine

- Reversible protein aggregation

Weak non-covalent interactions. (Due to changes in solution conditions: protein concentration / change in pH / conductivity / polarity)

Soluble – Insoluble

Covalent – Non-covalent

Reversible – Irreversible

Folded – Unfolded

The Challenge:

Intrinsically Disordered Proteins (IDP)

Should get even MORE special treatment

Intrinsically disordered protein and unstructured domains

Also known as: Natively unfolded proteins

- Protein-protein & domain-domain interactions
- Structural flexibility that enables binding to a large number of partner ligands
- Implications for regulation, activity and function

Commonly used affinity Tags and fusion proteins

Tag	Size (aa)	Resin
His	5-15	Ni/Co
Streptag II	8	StrepTactin
Chitin-binding domain	52	Chitin
SUMO	~100	Ni/Co
6His-Thioredoxin	~115	Ni/Co
GST	~200	Glutathion
MBP	~400	Amylose
6His-NusA	~500	Ni/Co

Lipoyl domain fusion tag (HLT)

Dr.Mark Allen: MRC-CPE, Cambridge, England
mda201@mrc-lmb.cam.ac.uk

Lipoyl domain:

N-terminal part of the lipoyl domain in B. Stearothermophilus E2p

→ Extremely soluble and often prevent proteins to form IB

- Extremely resistant to proteases
- Known NMR structure
- Difficult to crystallize
- 109 amino acids
- MW: 11994.3 Da
- Theoretical pI: 4.53

- Over-expression of IDP
- NMR studies

Additives and Stabilizers

- **Reduce protein-protein interaction**
- Keep protein at lower concentration
- High salt concentration
- Protect correct disulfide bonds
- Chaotropes as Urea or aggregation suppressors as Arginine
- Protect exposed hydrophobic sites with PEG,

  citrate, and non-ionic detergents (bellow the CMC)

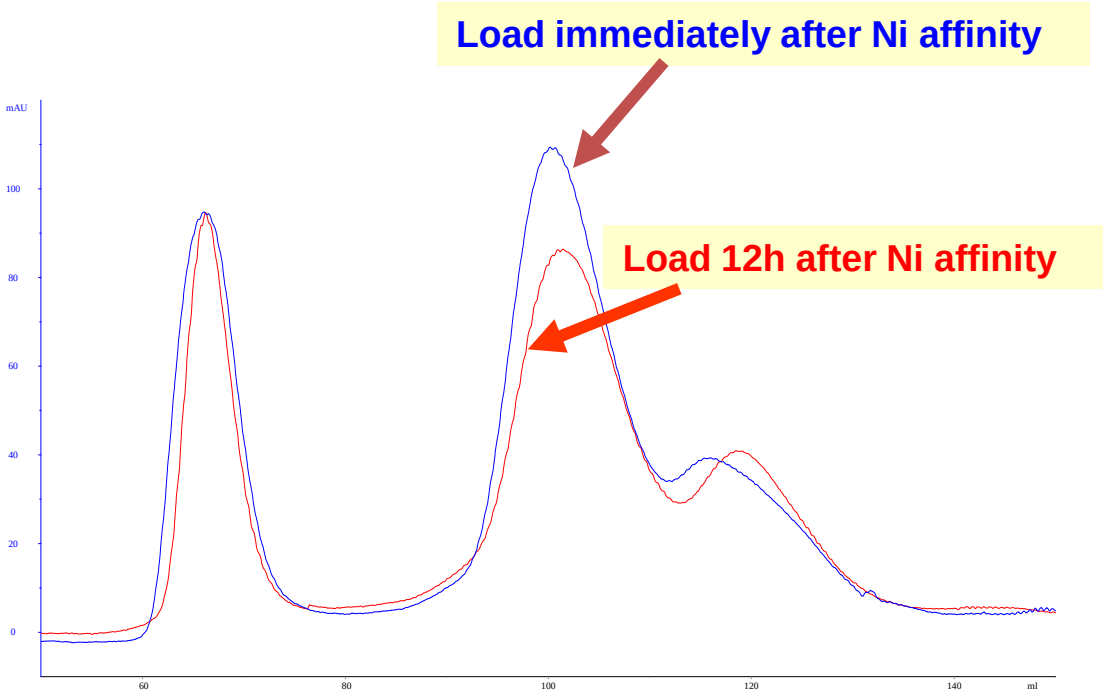
- **More Additives to stabilize the native state:**
- Glycerol, Sucrose, Sorbitol, Trehalose, Glycine, etc

Agents that may promote protein solubility

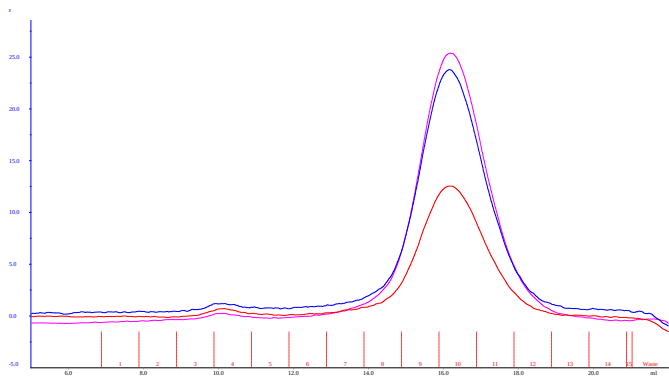
	Additive	Recommended concentration range
Kosmotropes	$MgSO_4$	0–0.4 M
	$(NH_4)_2SO_4$	0–0.3 M
	Na_2SO_4	0–0.2 M
	Cs_2SO_4	0–0.2 M
Weak kosmotropes	NaCl	0–1 M
	KCl	0–1 M
Chaotropes	$CaCl_2$	0–0.2 M
	$MgCl_2$	0–0.2 M
	LiCl	0–0.8 M
	RbCl	0–0.8 M
	NaSCN	0–0.2 M
	NaI	0–0.4 M
	$NaClO_4$	0–0.4 M
	NaBr	0–0.4 M
	Urea	0–1.5 M
Amino acids	Glycine	0.5–2%
	L-arginine	0–5 M
Sugars and polyhydric alcohols	Sucrose	0–1 M
	Glucose	0–2 M
	Lactose	0.1–0.5 M
	Ethylene glycol	0–60% v/v
	Xylitol	0–30% w/v
	Mannitol	0–15% w/v
	Inositol	0–10% w/v
	Sorbitol	0–40% w/v
	Glycerol	5–40% v/v
Detergents	Tween 80	0–0.2% w/v
	Tween 20	0–120 μM
	Nonidet P-40	0–1%

Sarah E. Bondos and Alicia Bicknell
Analytical Biochemistry (2003) 316 (2) pg 223-231

Should TEV protease be cleavage before or after GF separation of soluble aggregates?



Only monomer undergoes TEV protease cleavage (aggregates can not be cleaved)



After TEV cleavage, monomers are stable

Try to eliminate your soluble aggregates as soon as possible!!!

Possible Strategy to avoid further aggregation

Ni column

↓

TEV protease + dialysis

↓

Negative Ni column

↓

Concentration

↓

GF

Ni column

↓

GF immediately

↓

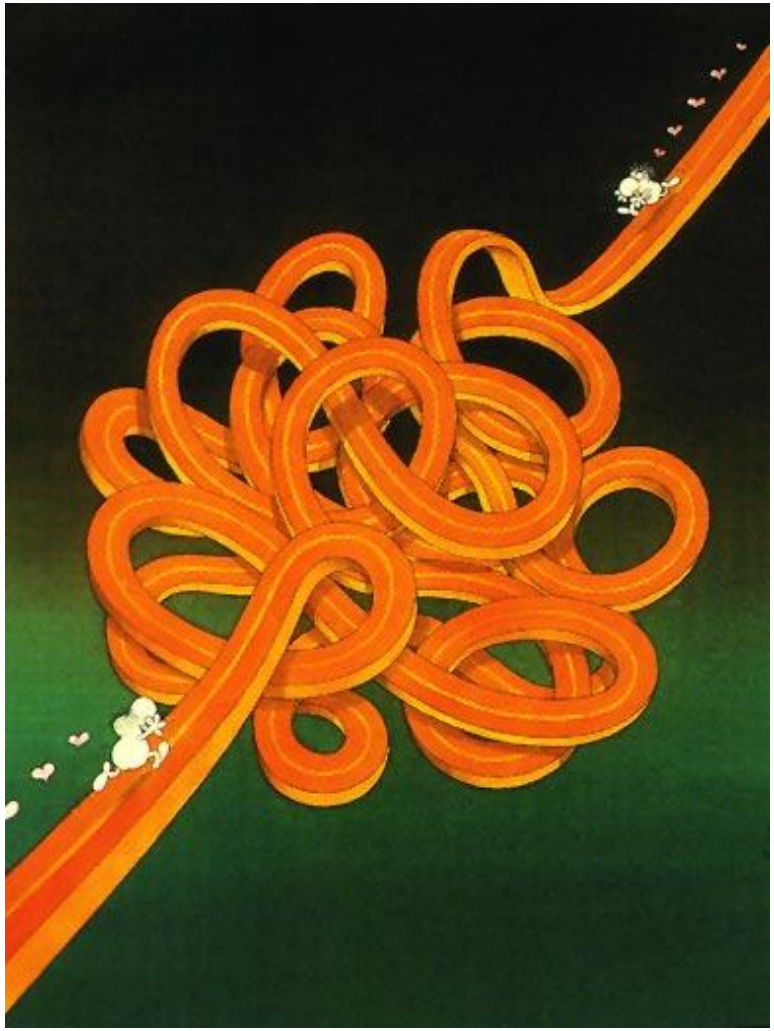
TEV protease

↓

Negative Ni column

↓

and/or Ion exchange



REVIEW *Nature Methods* - 5, 135 - 146 (2008)
Protein production and purification
Structural Genomics Consortium

Thanks!!

Dr. Tsafi Danieli
(Expression Facility
Hebrew University)

Collaborators:
Prof. Assaf Friedler
(Hebrew Univ.)
Michal Maes
Hadar Rafaely
Anat Iosub
Chen Katz
Shahar Rotem
Tzvika Hayouka
Ronen Gabizon
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2009/3/11

Gilboa