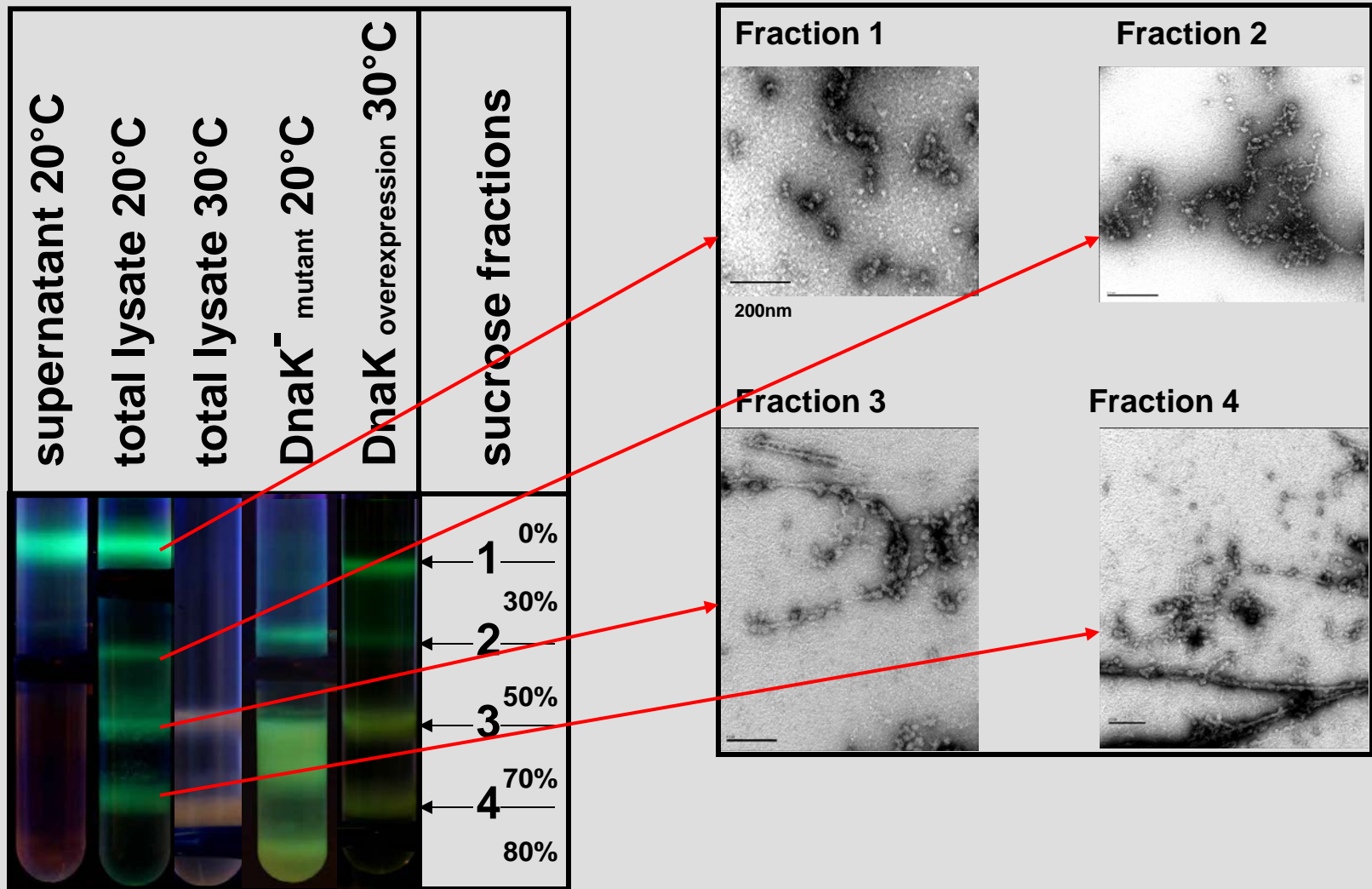


Protein Core Facilities – Network Meeting - Halle 23.02.2011

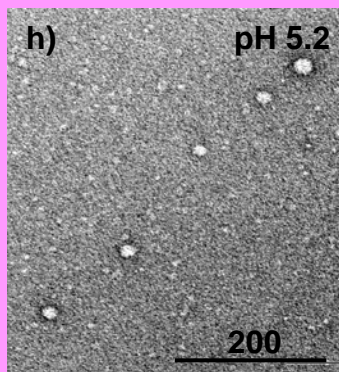
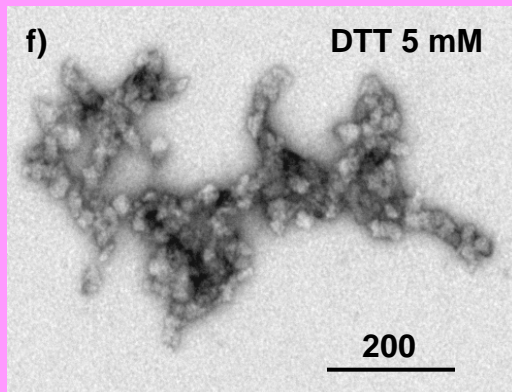
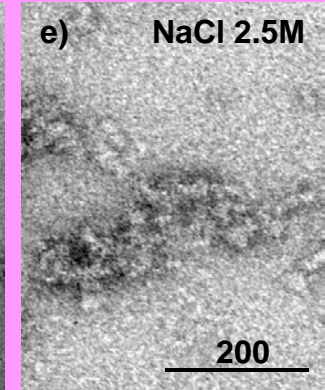
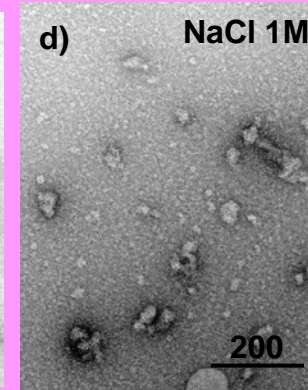
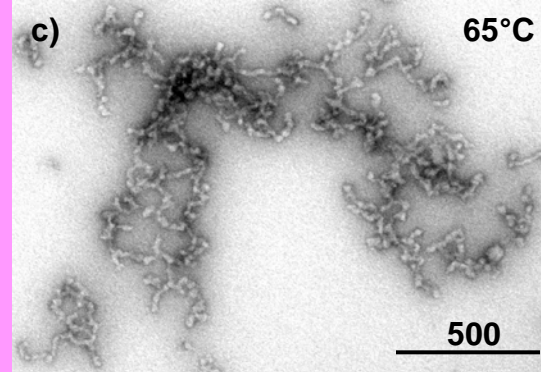
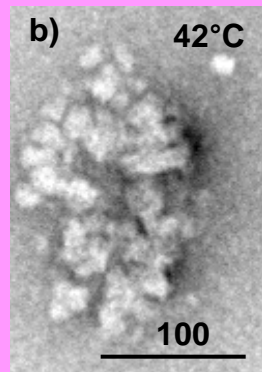
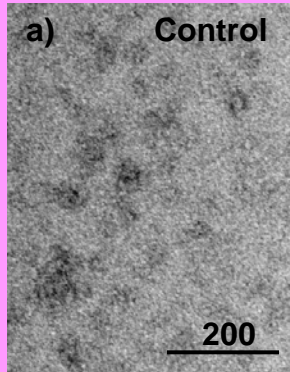
Protein aggregates, refolding, quality control

Ario de Marco – University of Nova Gorica, Slovenia

Aggregate complexity – separation in sucrose gradient and microscopic structure



Protein aggregates – shape, dimension, functionality



Treatment	Aggregate features
No treatment (Control)	Soluble, green
42°C x 20 min	Soluble, green aggregates
65°C x 20 min	Soluble, non-colored aggregates
100°C x 20 min	Insoluble, white precipitates
pH 5.2	Insoluble, white precipitates
NaCl (1 M)	Insoluble, green precipitates
NaCl (2.5 M)	Insoluble, green precipitates
DTT (5 mM)	Soluble, green aggregates

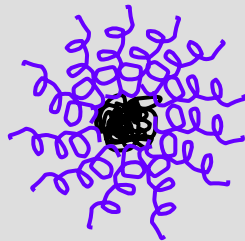
One protein (GFP-GST), many fates

Development of the soluble protein aggregates

GST - GFP

GST

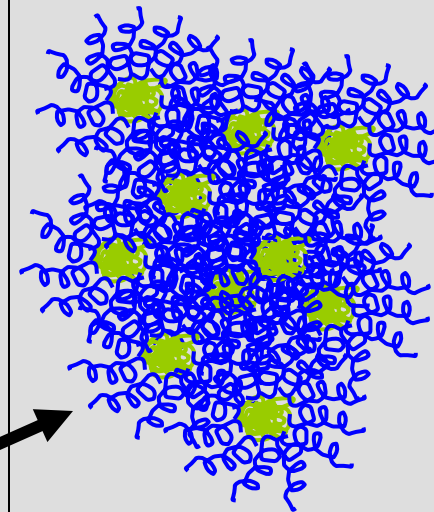
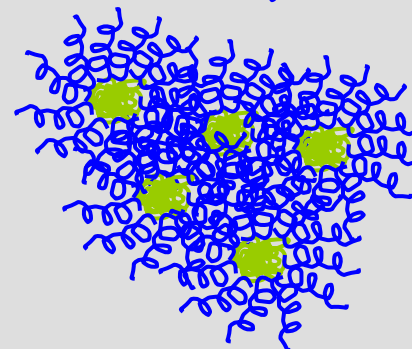
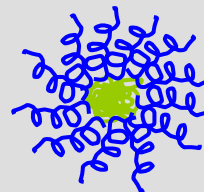
target



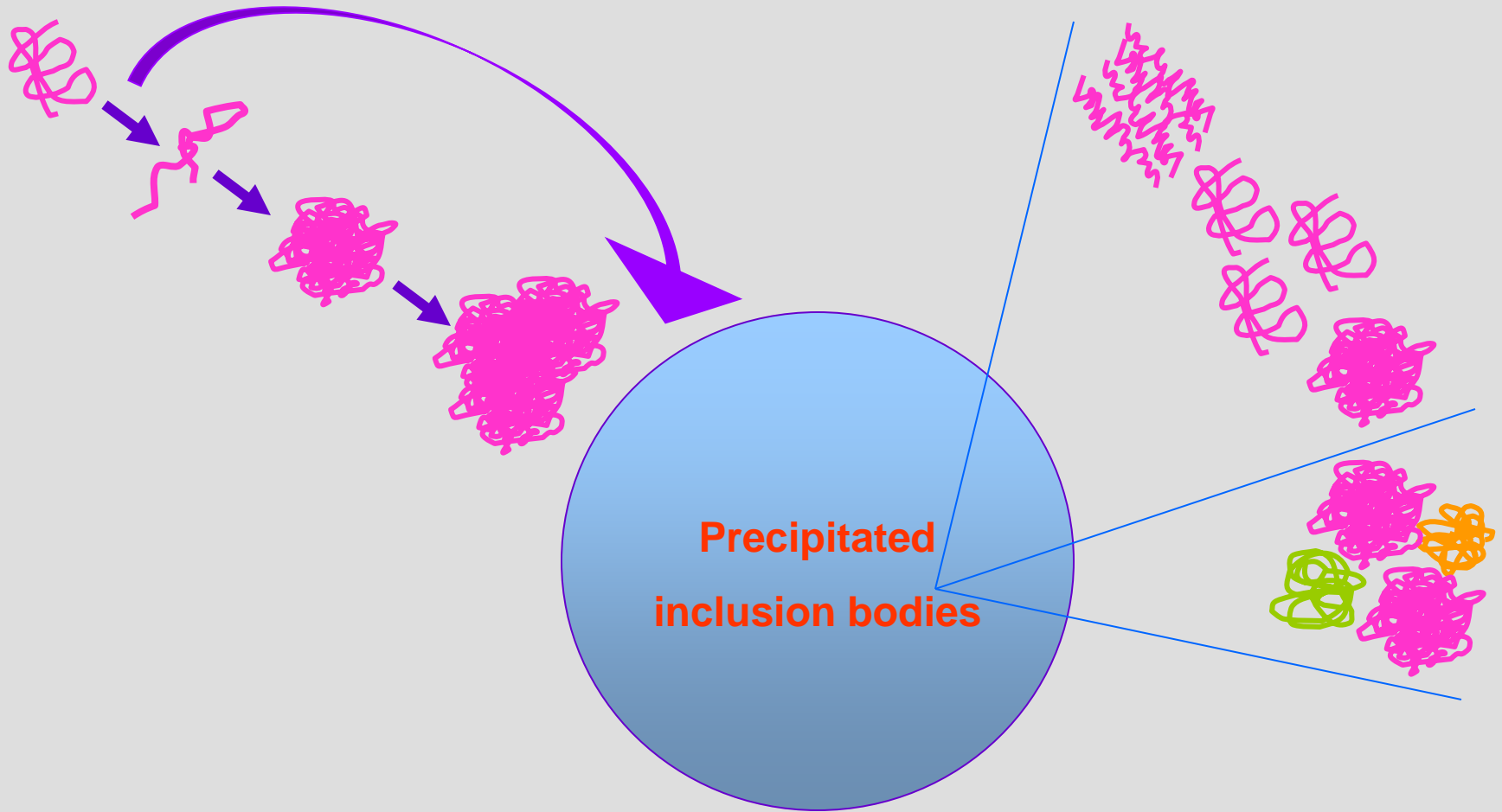
Soluble aggregates

Soluble

Insoluble

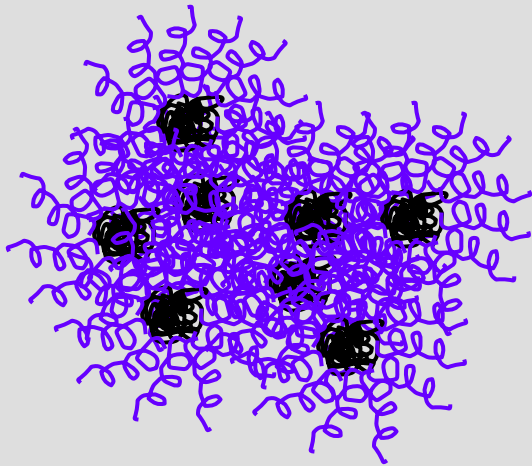


Protein aggregates – structure models

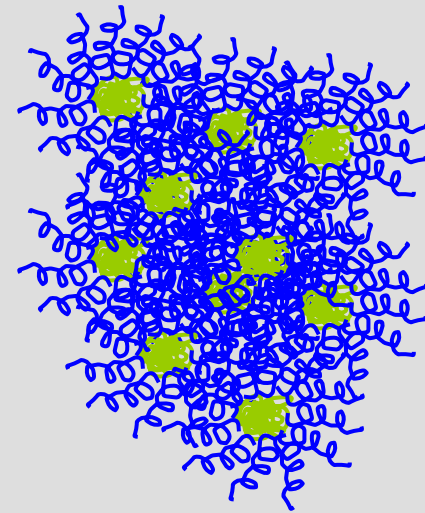


Inclusion bodies: intermediate or final products?

**Inactive protein:
to refolding trials**



**Active protein: direct
technological applications**



Inclusion bodies: solubilization or refolding?

- Technical problem: how to get the IB protein monodispersed and correctly refolded**
- Analytical problem: how to evaluate the read-out**
- Semantic problem: solubility does not correspond to native folding**

Inclusion bodies: the quality control

- Refolding evaluation: what are the available analytical tools?
- Refolding evaluation: what is the feasible analytical practice?
- Refolding evaluation: how much does it cost and need in term of time and material? (Stephan' s presentation!)

Identity and role of the Facilities

- **Relationship with the users: what they need, what we can offer. A never clear relationship**
- **For what the users pay: awareness of offered expertise and cost/benefit analysis**
- **Follow the requests or propose innovation?**

Identity and role of the Facilities

- **The role of the Facilities: technical and educational**
- **Technical role: be updated on the state-of-the-art and communicate the innovation to the users**
- **Educational: explain the GLP and the need for data correct annotation and recording**

Metadata and win-to-win situation

- **Collecting standardized data: the advantages for the user**
- **Collecting standardized data: the advantages for the facility**
- **Collecting standardized data: the advantages for the scientific community**