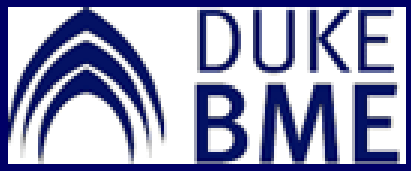
Elastin-like Polypeptides as Purification Tags: Orientation Controls Expression Levels and Activities of the Fusion Proteins

Trine Christensen, Kimberly Trabbic-Carlson, Sue Dagher, Mohammed F. Shamji, Miriam Amiram, Lori A. Setton, and **Ashutosh Chilkoti**



Elastin-Like Polypeptides (ELPs)

ELP ≡ (Val-Pro-Gly-Xaa-Gly)$_n$

- The guest residue (Xaa) in the fourth position can be any amino acid except Pro
- *n* is the number of repeats: *n* = 20 – 360

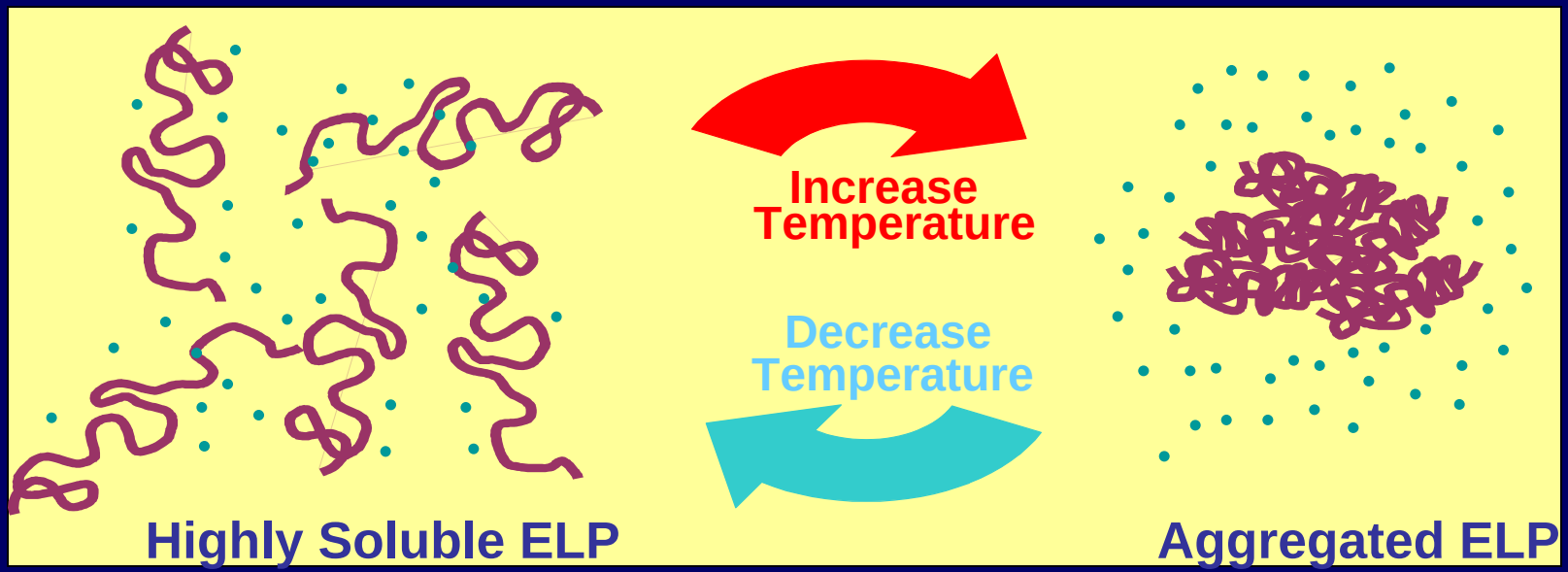
Inverse Phase Transition

Characteristic transition temperature T_t depens on:

ELP length and concentration
salt concentration
nature and stoichiometry of the guest residue

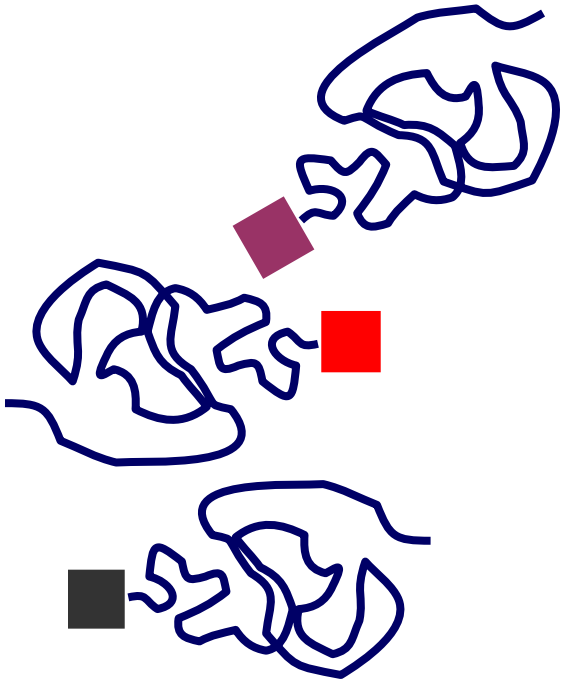
$T < T_t$

$T > T_t$



ELP Fusion Proteins

The target protein and the ELP are fused at gene level

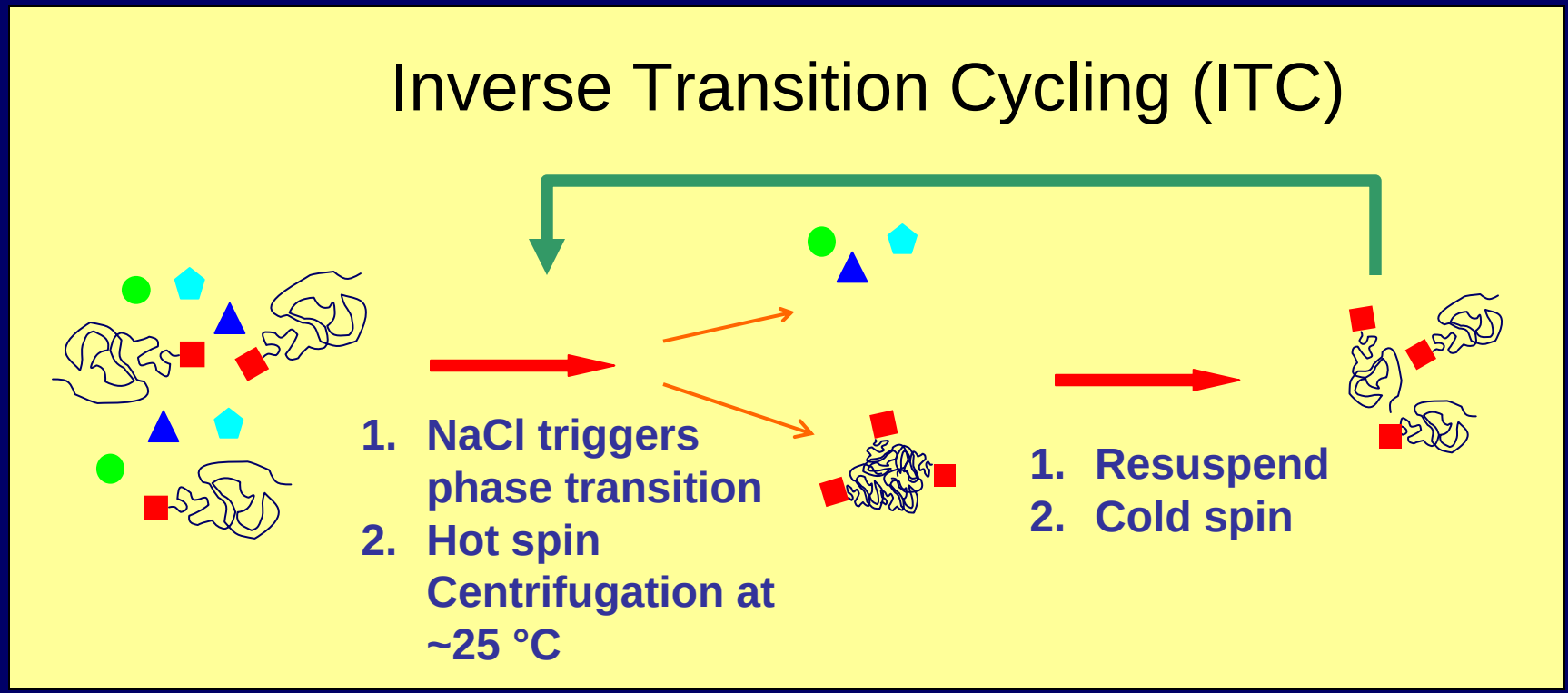


Target Proteins:

Blue/green flourescent protein
Interleukin-1 receptor antagonist
Chloramphenicol acetyltransferase
Thioredoxin
Calmodulin
Tendamistat

Purification of ELP Fusion Proteins:

The phase transition properties are retained in ELP fusion proteins



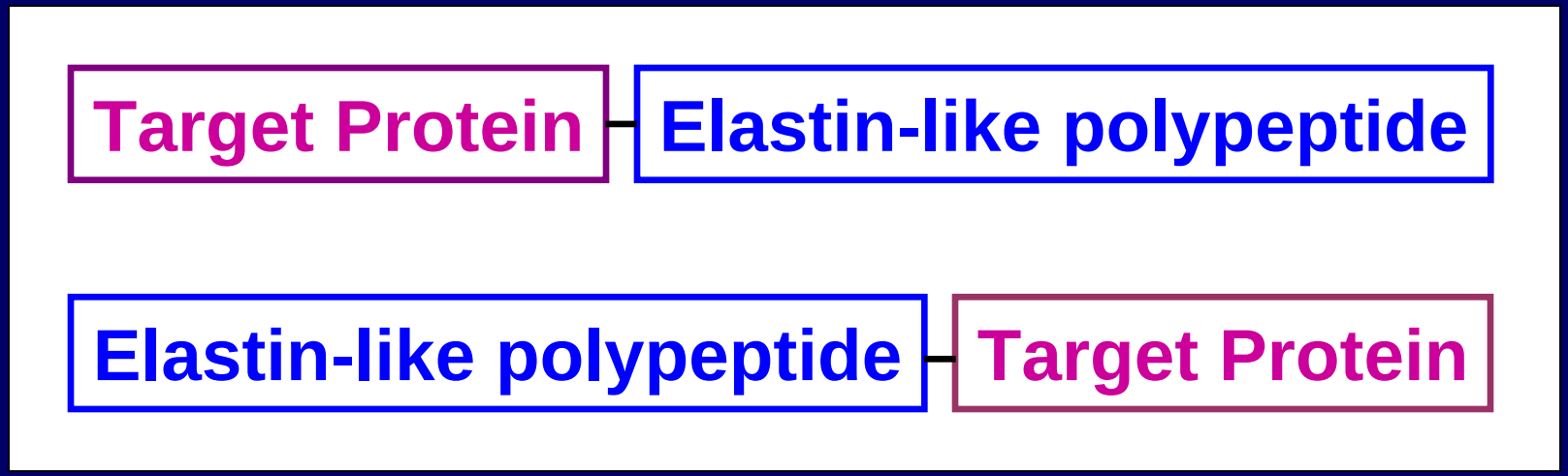
Advantages of Protein Purification by ITC:

- **TIME EFFICIENT** – it takes less than a day to purify hundreds of milligrams of fusion protein starting from lysing the cells to the final pure protein
- **INEXPENSIVE** – this purification method completely avoids column chromatography

Investigations Carried Out On Fusion Proteins

- Yields are often 50-150 mg fusion protein per liter growth
- Fusion proteins with longer ELP tags have lower yields than fusion proteins with shorter ELP tags
- Fusion proteins with longer ELP tags have lower transition temperatures

Does Fusion Order Matter?



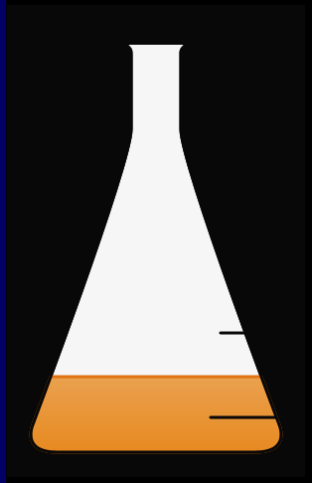
Target proteins:

1. **blue fluorescent protein (BFP)** 29.5 kDa
2. **chloramphenicol acetyltransferase (CAT)** 26.6 kDa
3. **thioredoxin (Trx)** 14 kDa
4. **interleukin-1 receptor antagonist (IL1Ra)** 18.2 kDa

ELP: $[Val_5Ala_2Gly_3]$, n = 90, 36 kDa

Strategy

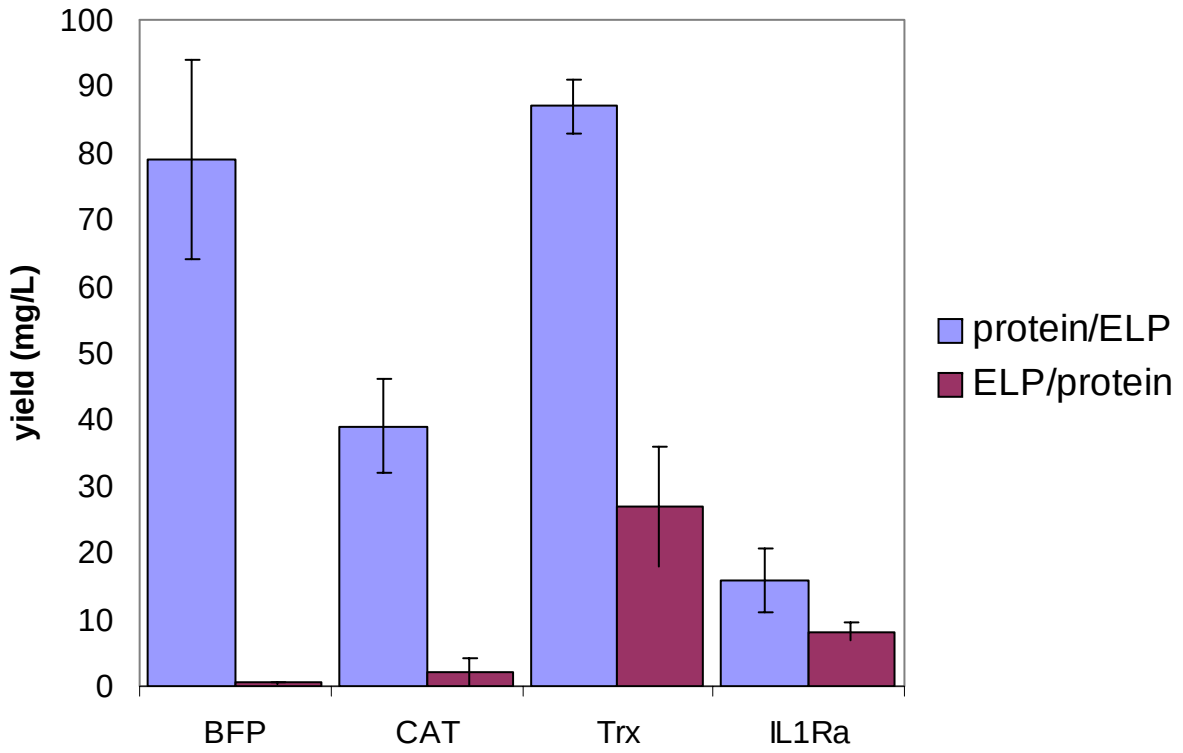
8 different fusion proteins:



purification

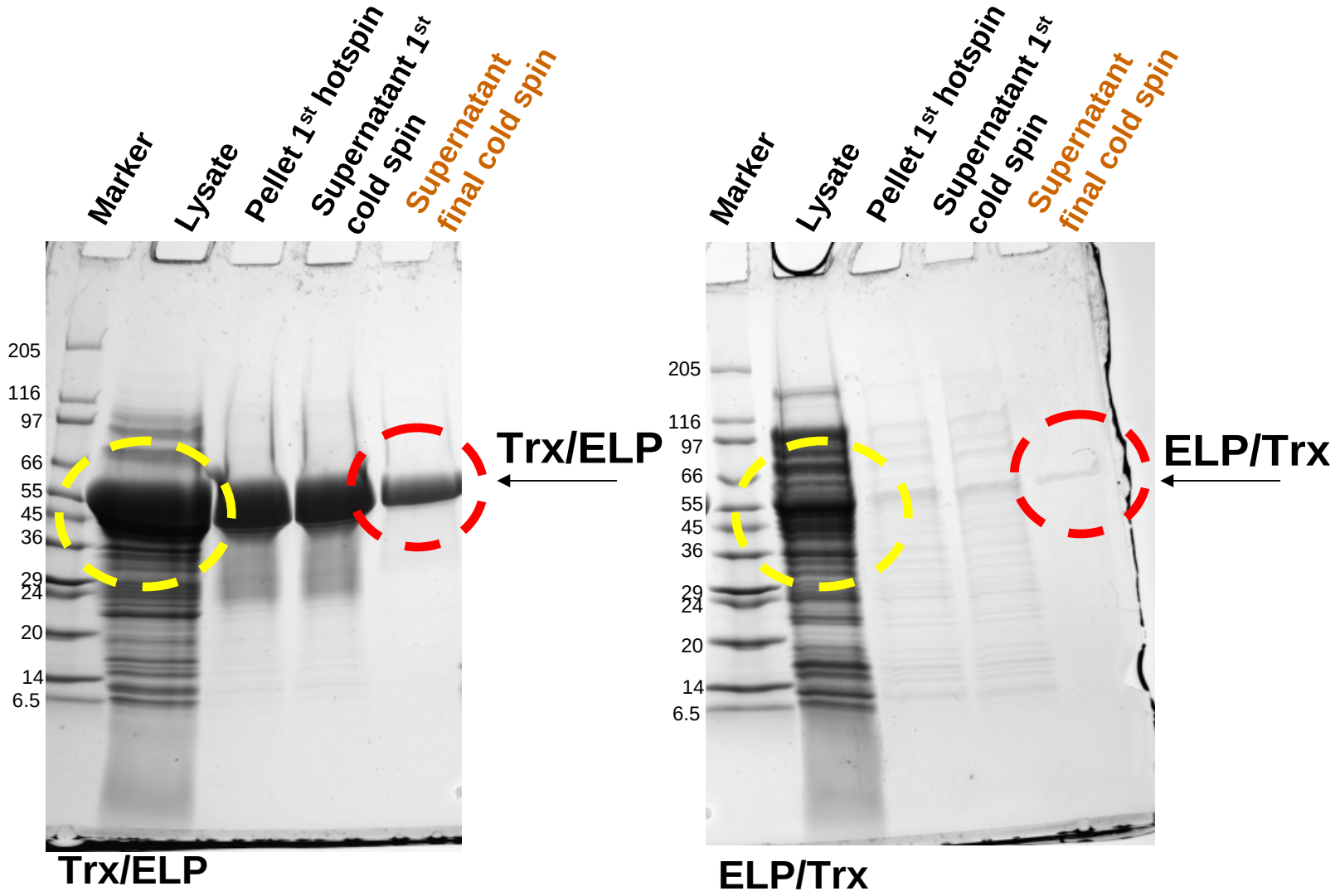
activity measurements

After Purification: Yields of Fusion Proteins



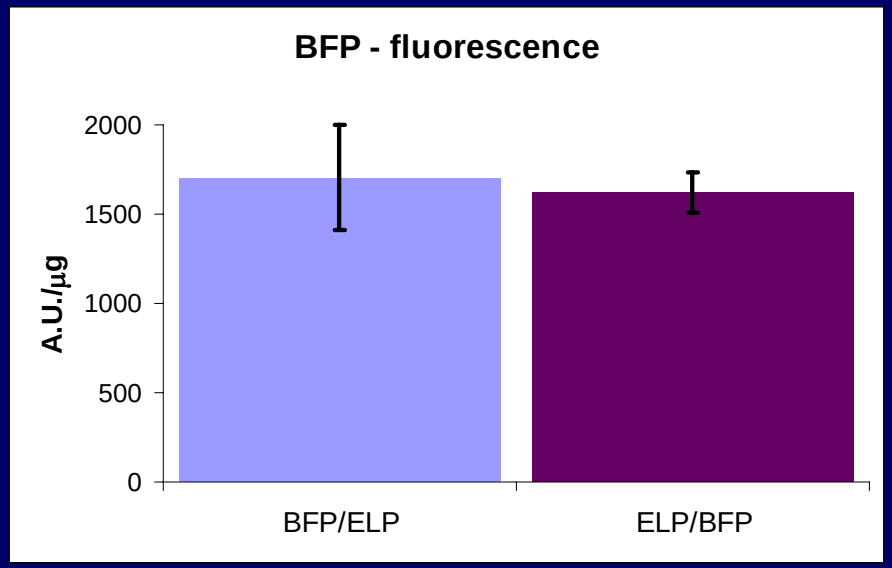
The yields of protein/ELP are always higher than the yields of ELP/protein

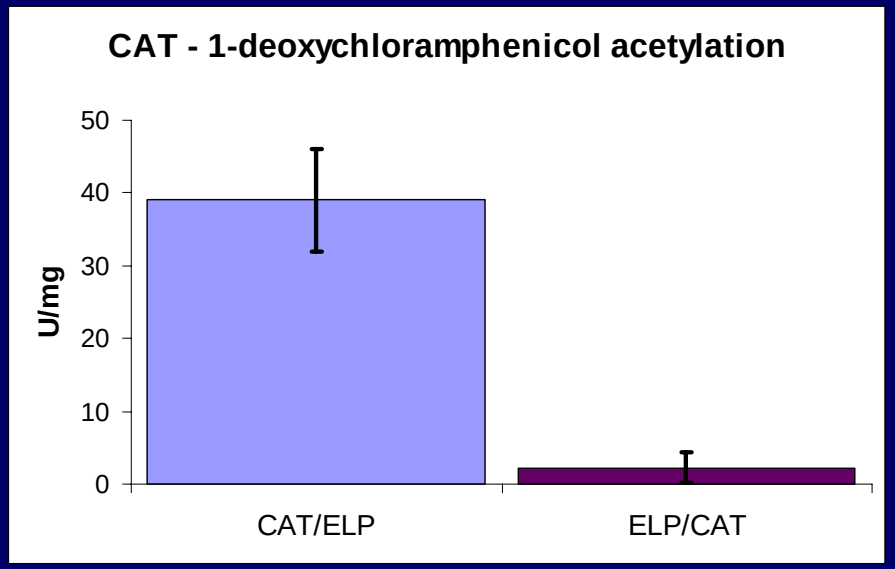
Expression Levels Are Lower for ELP/protein Fusions

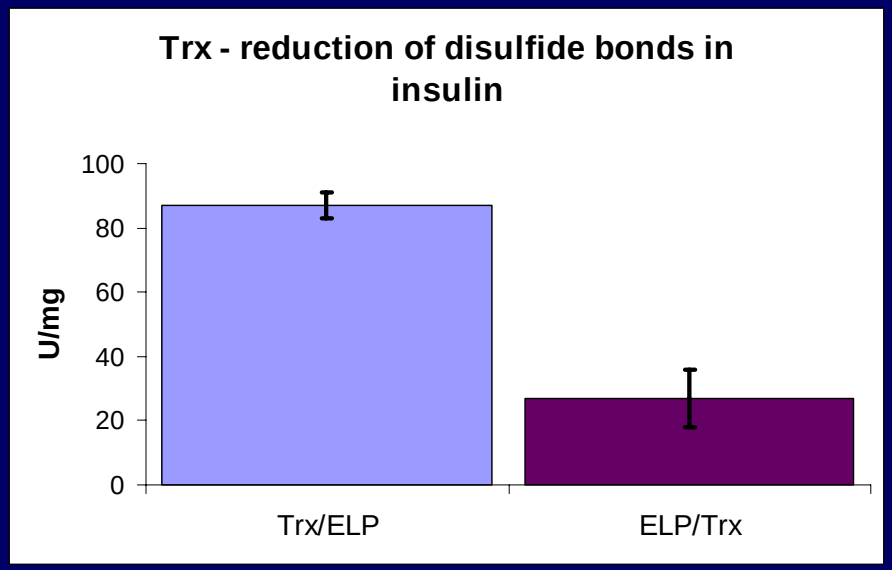


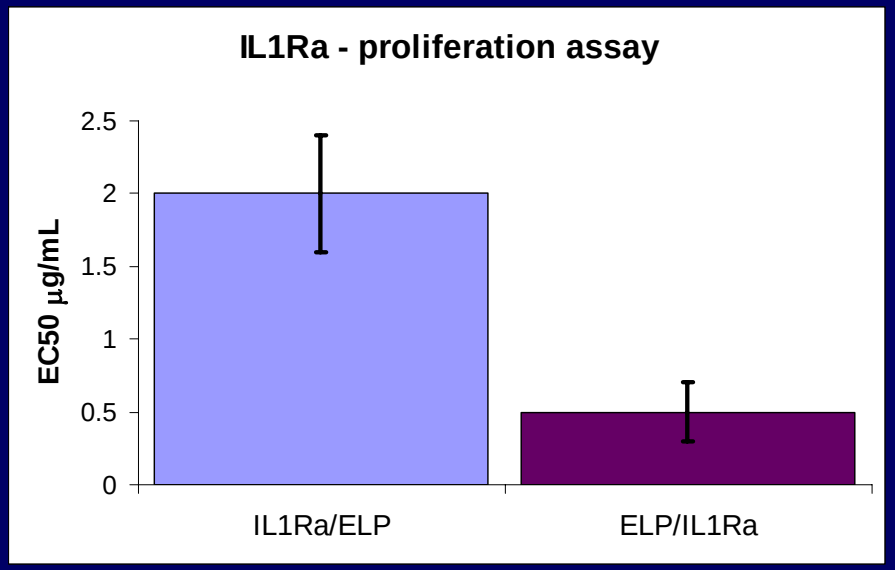
Concentration of ELP/protein lower in the soluble cell lysate

Activities of the Fusion Proteins





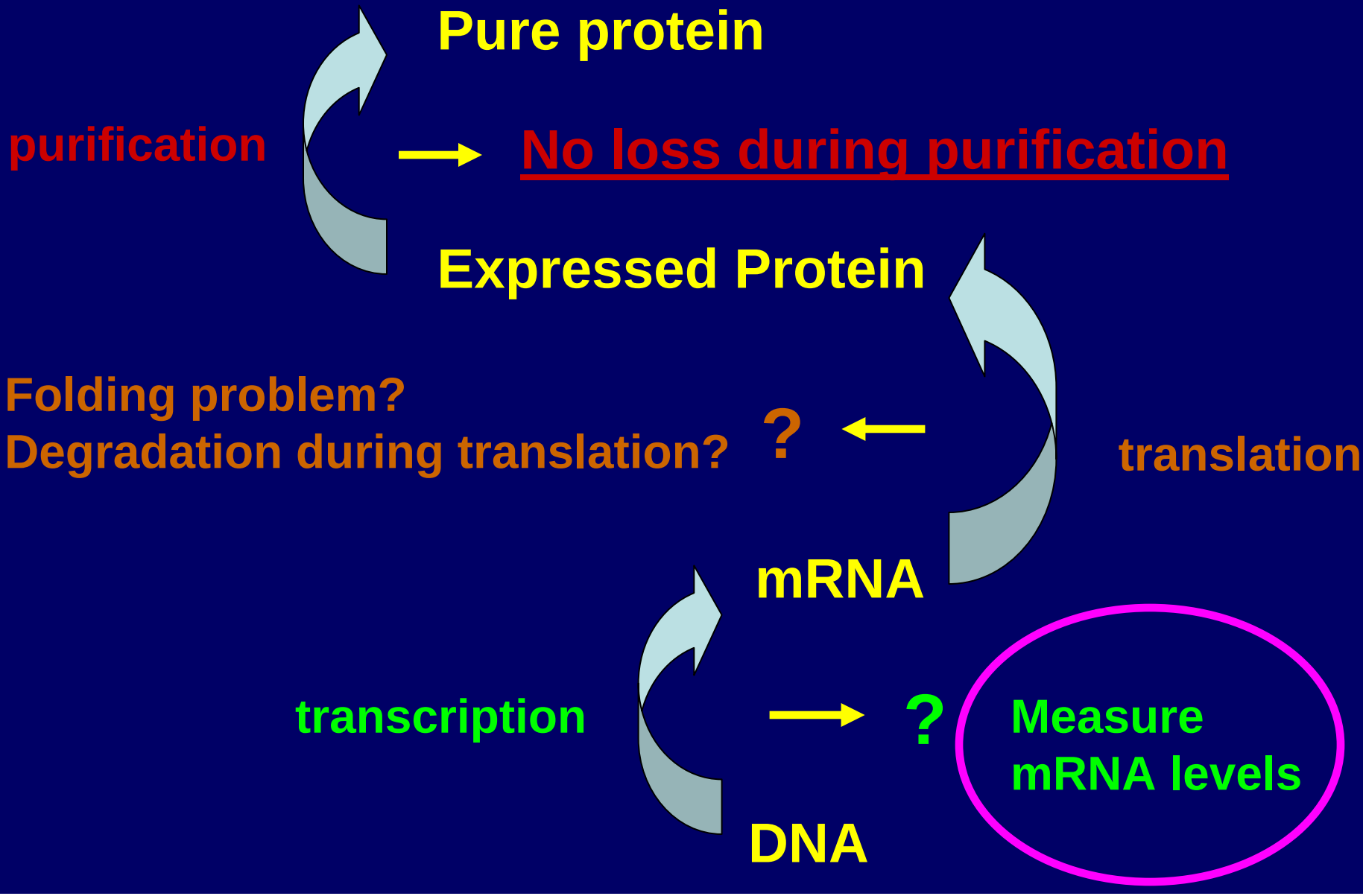




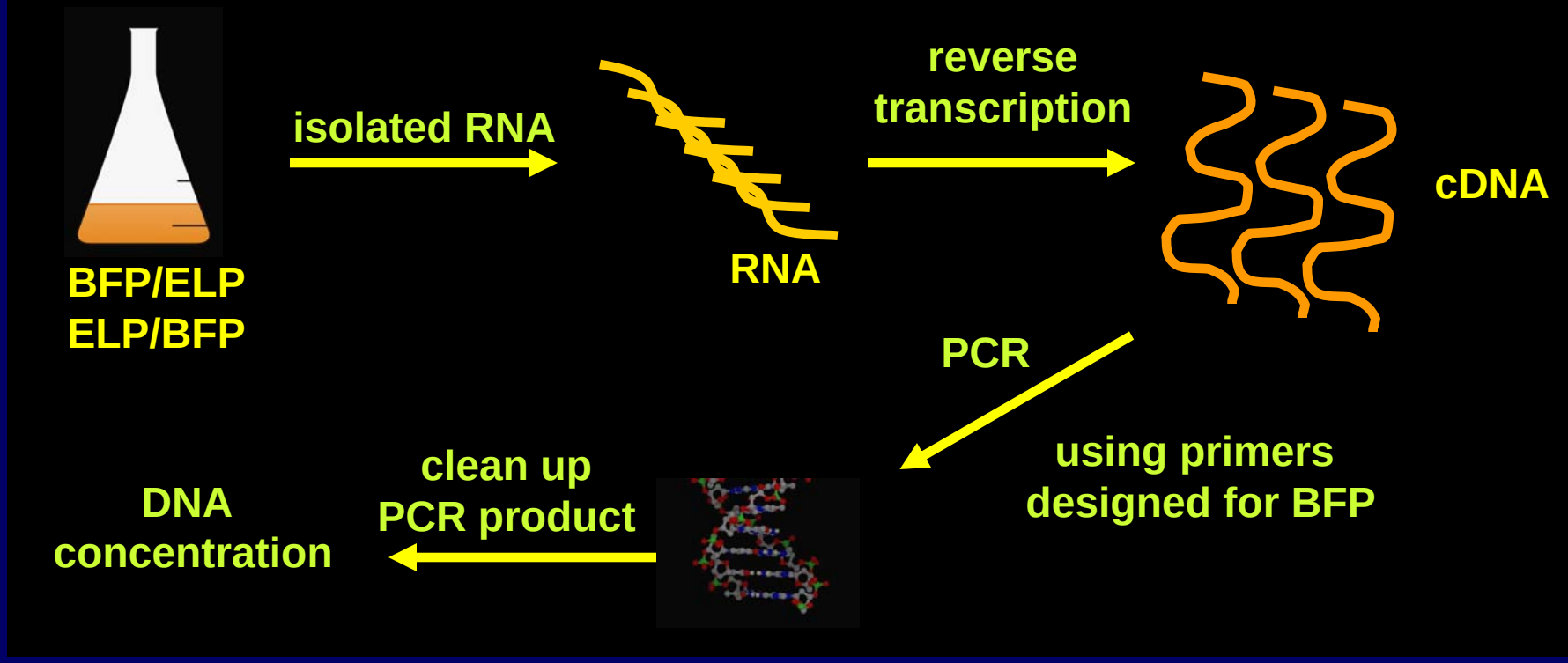
Results:

- Yields of ELP/protein fusions are significantly lower than yields of the reverse fusion order
- The lower yields are not due to loss during purification
- Activities of ELP/proteins are lower than those of protein/ELPs

Can We Explain the Lower Yields?



Preliminary Experiments: mRNA Levels Measured by Reverse Transcriptase PCR



Results From RT-PCR Experiments

- No difference in mRNA levels were observed for the two BFP constructs BFP/ELP and ELP/BFP

- The most likely explanation for the lower yields is protein degradation during translation

Can We Explain the Lower Activities of ELP/proteins?

Pure protein

- **Protein folding problem**
  - BFP – n/a
  - CAT – can be
  - Trx – can be
  - IL1Ra – can be
- **Steric hindrance due to the large ELP tag**
  - BFP – n/a
  - CAT – not likely
  - Trx – not likely
  - IL1Ra – can be

Conclusions

- Fusion order matters – target proteins should be fused to the *N*-terminus of the ELP (protein/ELP) for higher yields and specific activities
- No differences are observed on transcription level between BFP constructs
- The target proteins in the ELP/protein constructs are highly likely misfolded to a certain degree resulting in degradation during translation

Acknowledgment

Professor Ashutosh Chilkoti
Professor Lori A. Setton
Dr. Kimberly Trabbic-Carlson
Dr. Sue Dagher
Dr. Mohammed Shamji
Miriam Amiram

NIH