

# Growth and Purification of Human Growth Hormone



# Presenter Info:

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# What is Human Growth Hormone (hGH)?

Protein for pharmaceutical interest

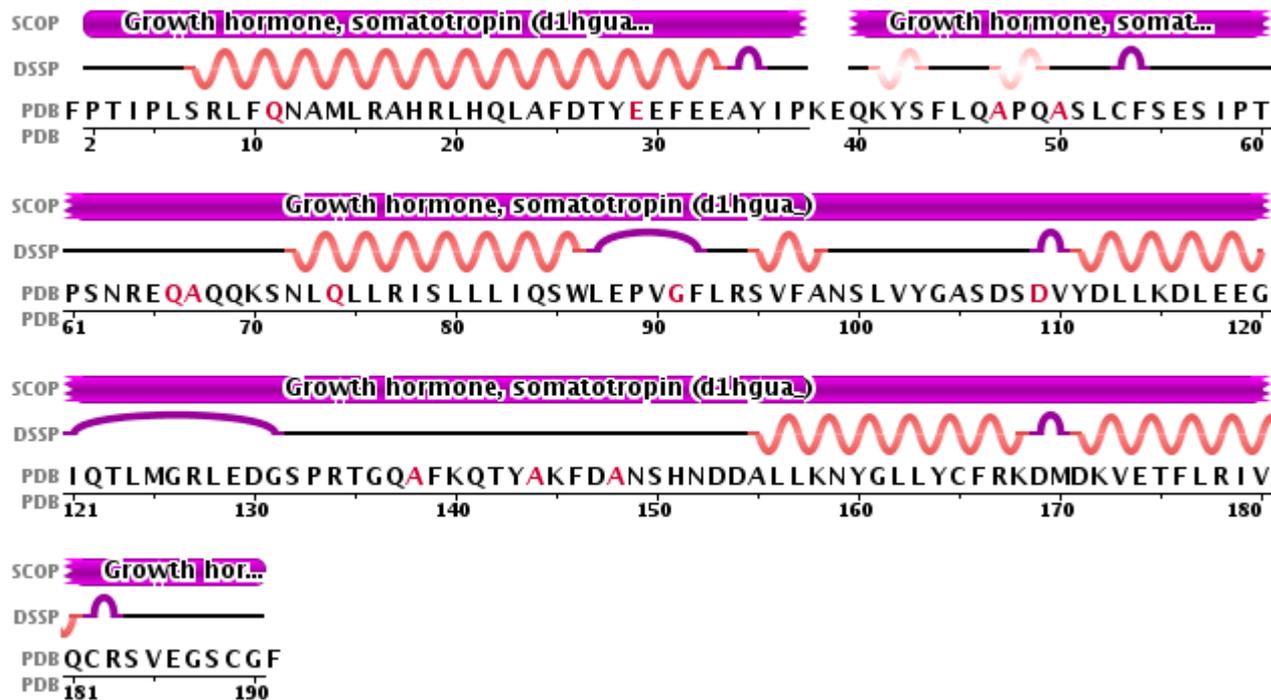
**191 Amino Acids**

**Mw 22KD**

Alpha-helices mostly

Study stability in different environments

Use neutron scattering



# Small Angle Neutron Scattering

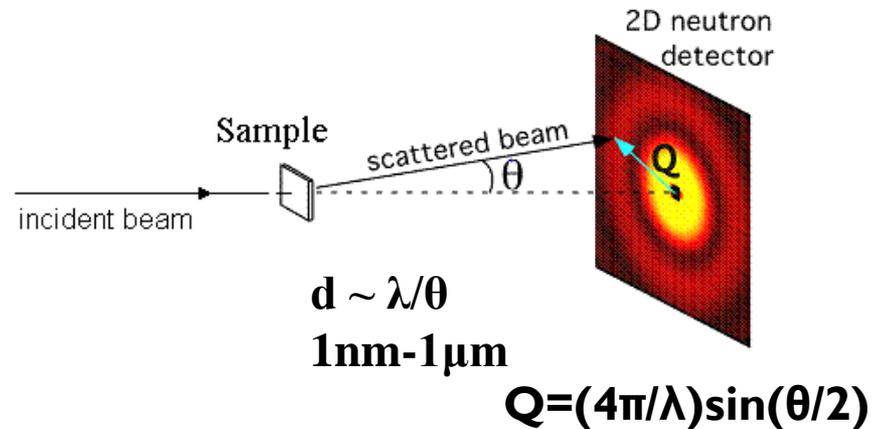
average structure factor:

$$I(q) = \frac{N}{V} (\Delta\rho)^2 V_p^2 P(q) S(\vec{q})$$

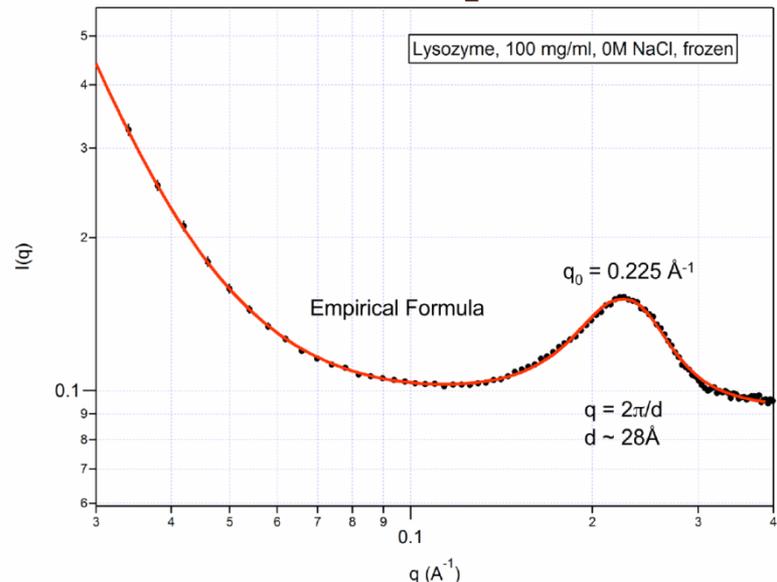
where

$$P(q) = |F(q)|^2$$

depends on particle shape

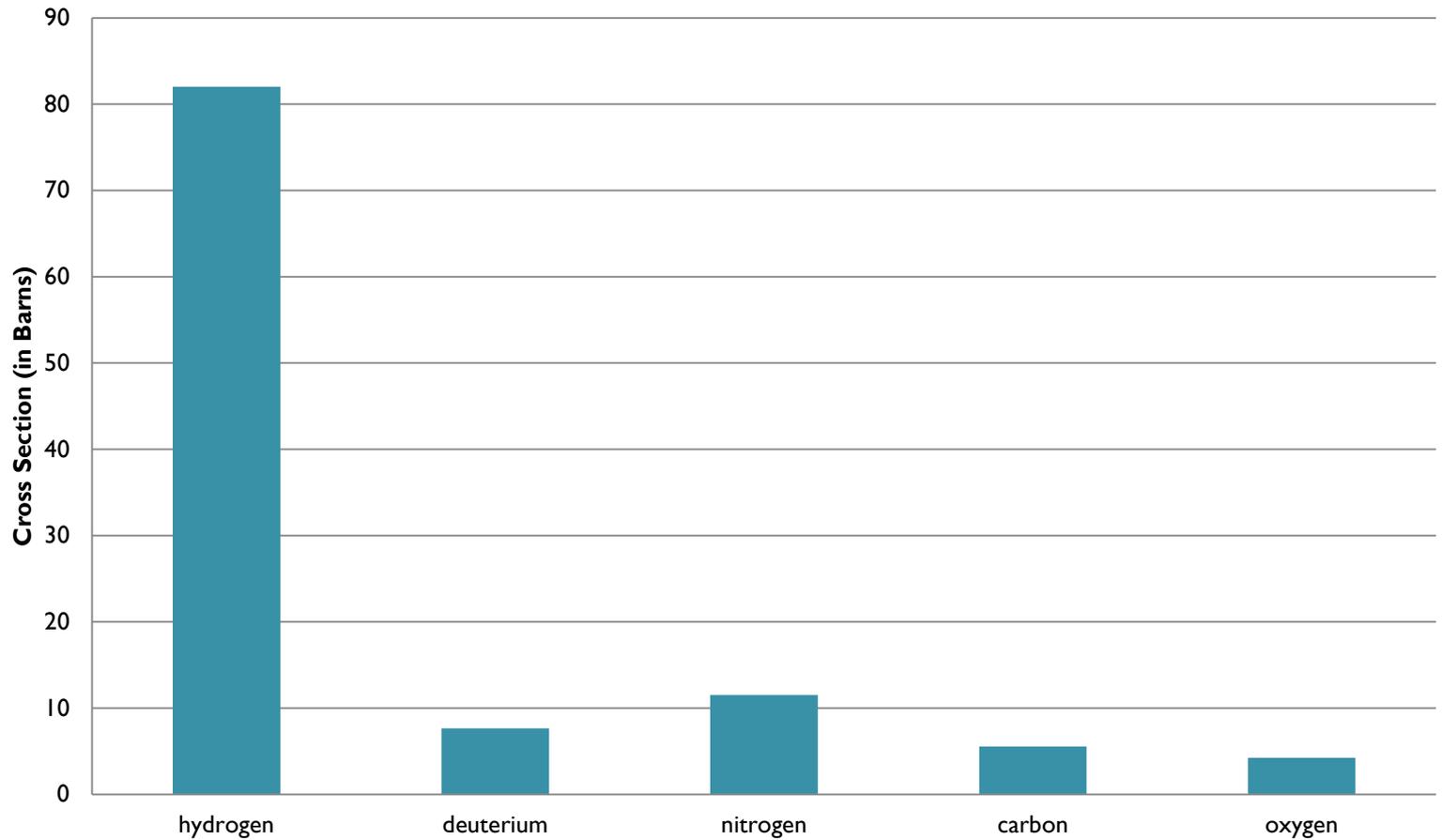


**Hard sphere fitting: estimate interaction peak**



# Neutron Scattering

## Scattering Cross Sections



# Working at IBBR and BL<sup>2</sup>

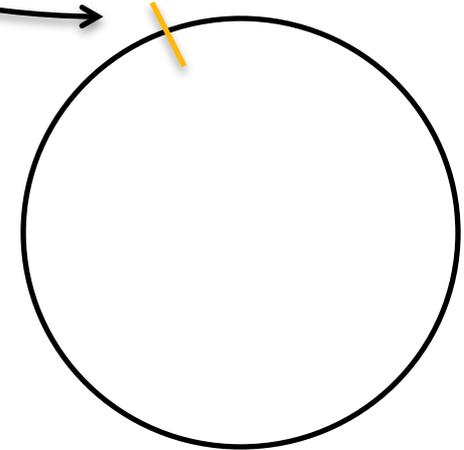
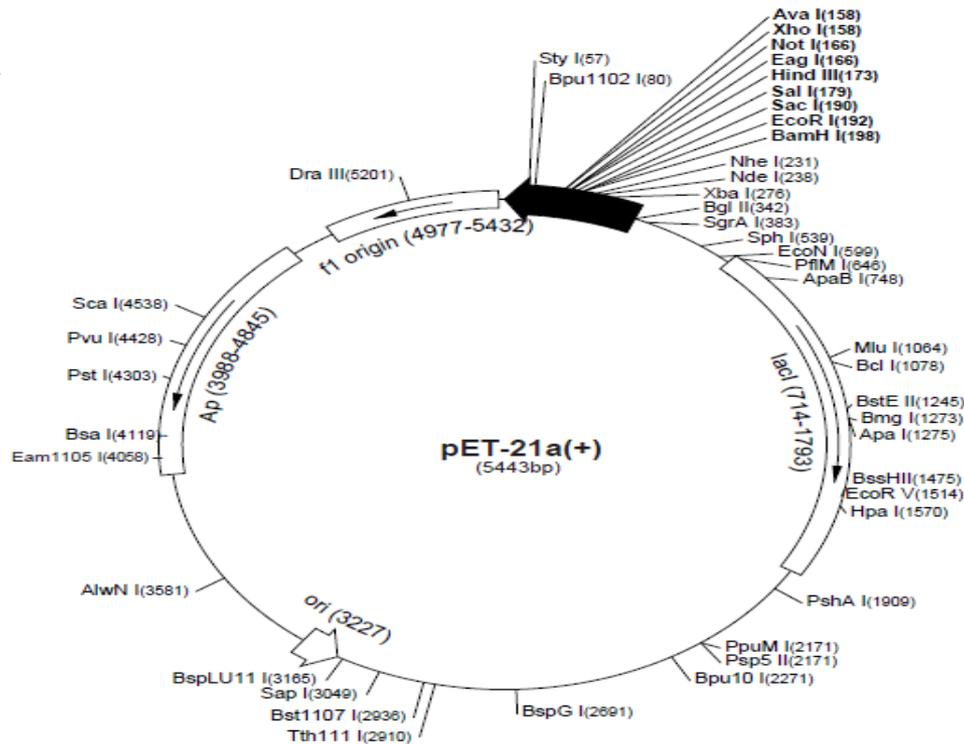


# The Process

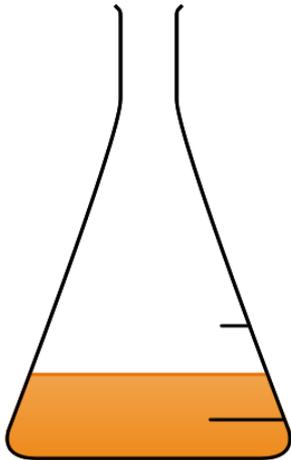
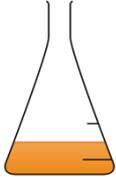
- Making a plasmid
- Grow cells
- Express the protein
- Lysing the cells
  - Unfolding and Refolding
- Purifying protein

# Making the Plasmid

- Gh2 gene
- 2.1 kb
- Replicates at single position



# Cell Growth

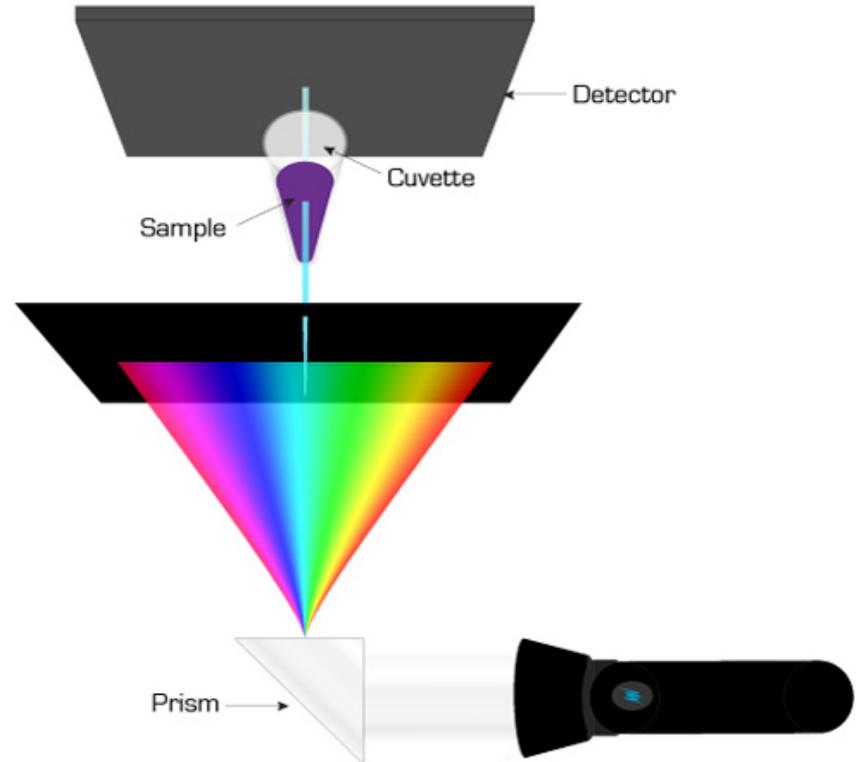


- Inoculate 50 mL LB 50  $\mu$ L Ampicillin (AMP) and 100  $\mu$ L Chloramphenicol media with 200  $\mu$ L E. Coli cells
- Let cells grow to an OD of roughly 3 and inoculate in larger 1.5 L of LB
- Why 3?

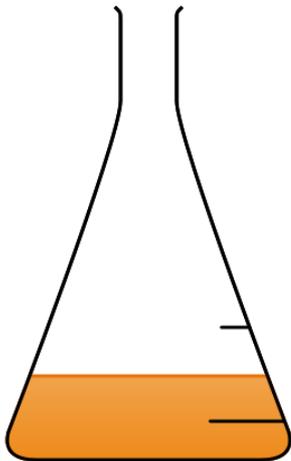
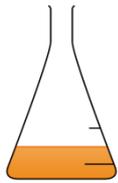
# Spectrophotometry

- Optical Density

$$OD = -\log \frac{I_0}{I}$$

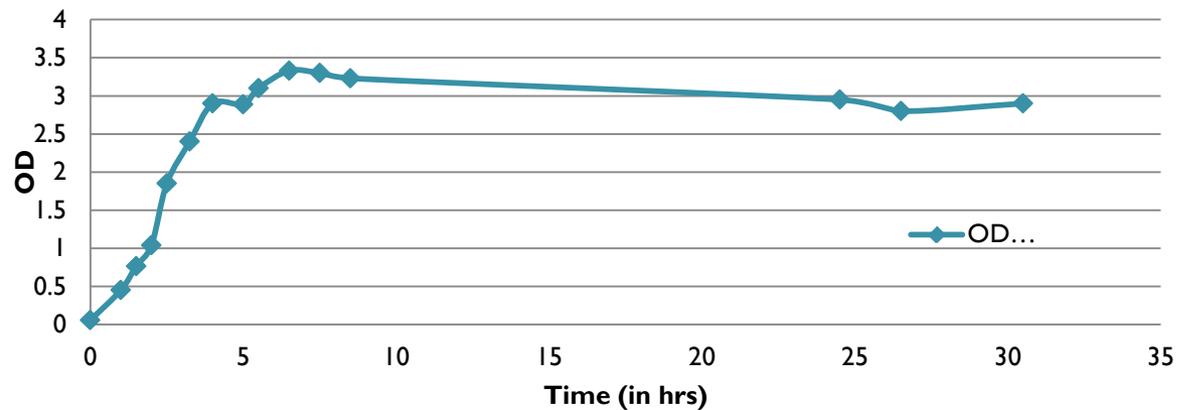


# LB growth curves



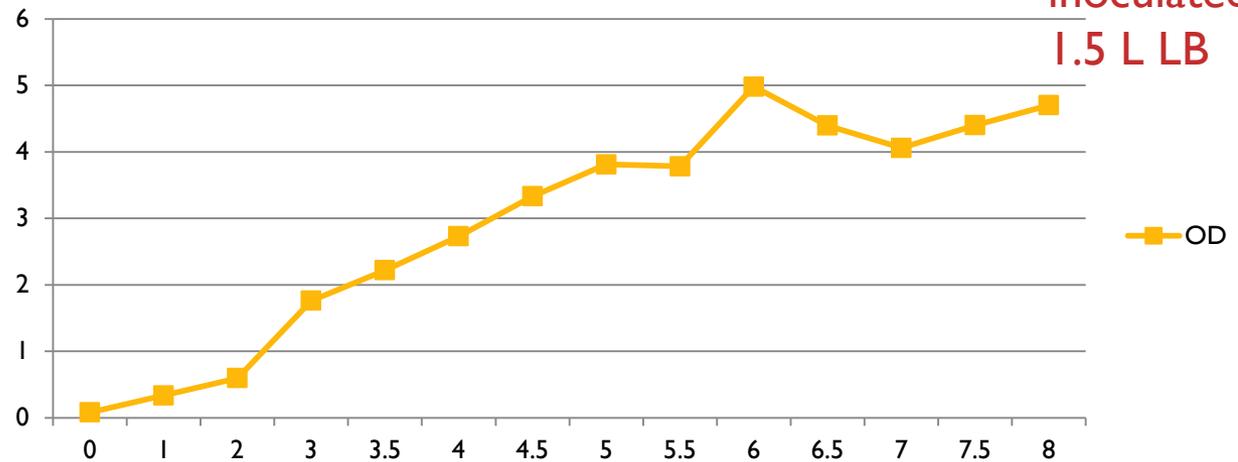
**OD growth 6/14**

100 mL LB  
inoculated



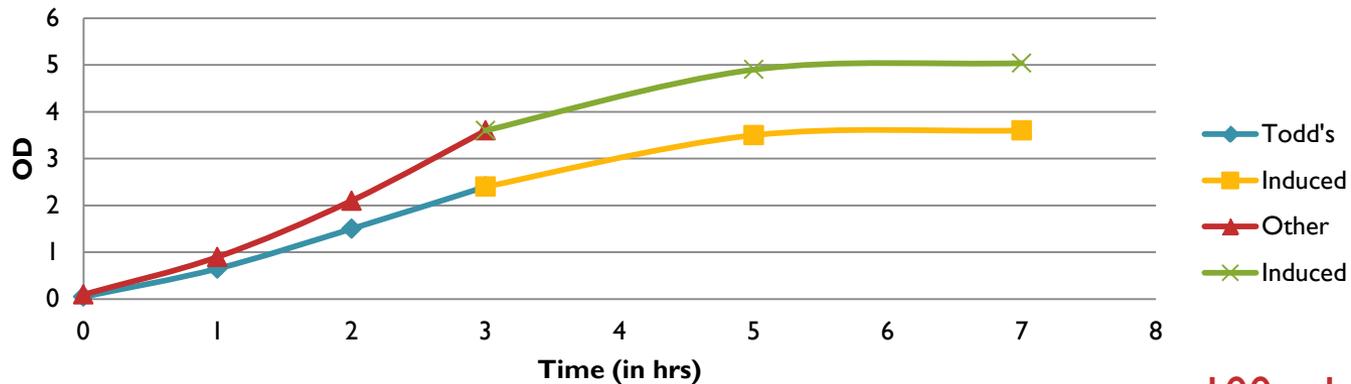
**OD**

100 mL LB  
inoculated into  
1.5 L LB



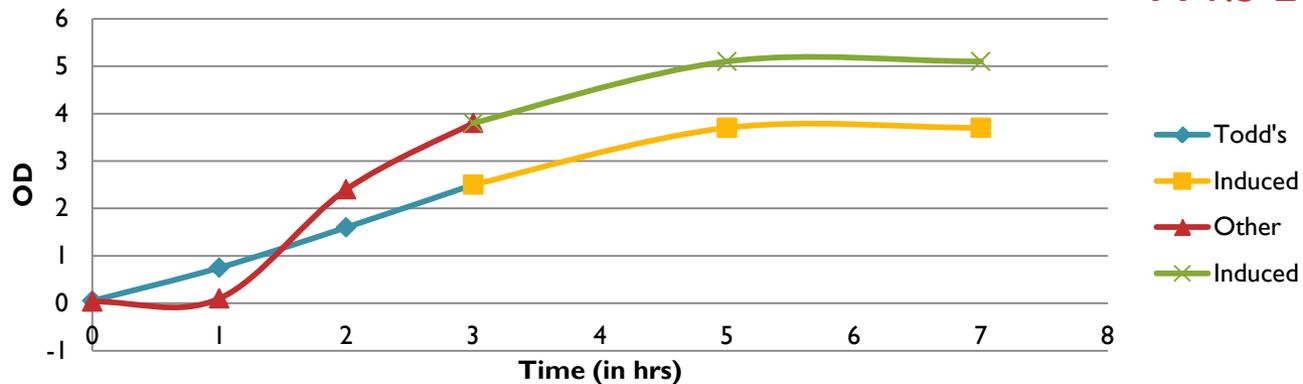
# OD Standardization

## Left Induction Curve 6-15



100 mL LB  
inoculated into 2  
X 1.5 L LB

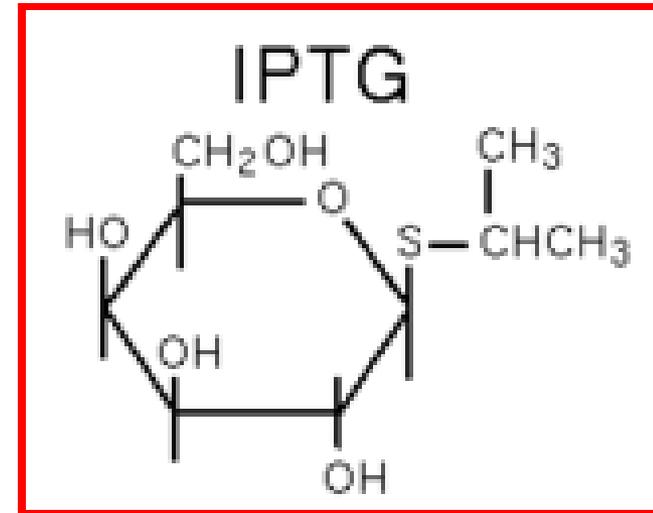
## Right Induction Curve 6-15



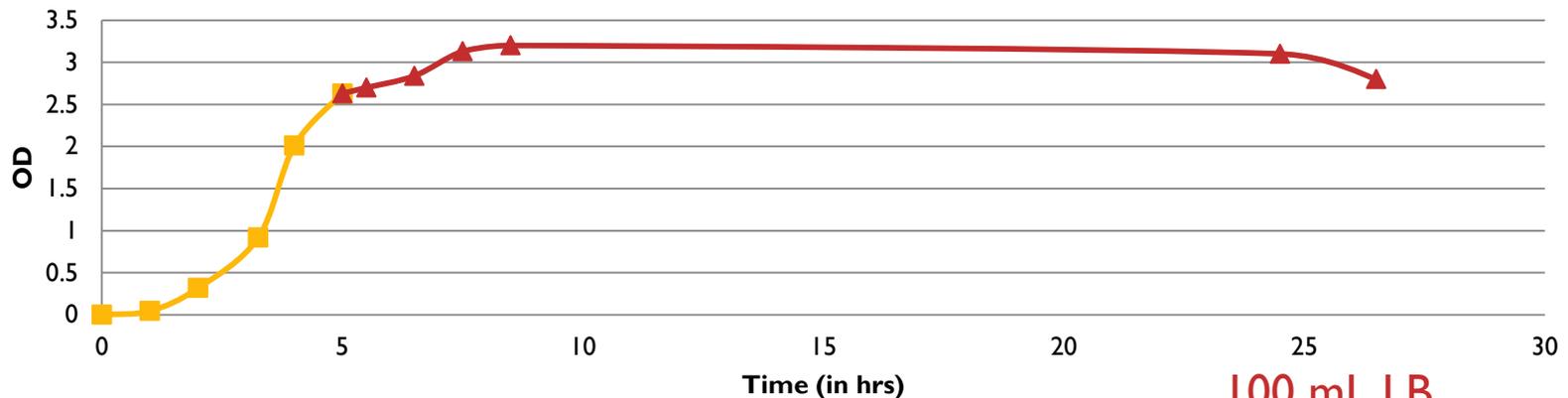
# Protein Expression

## ***IPTG***

- Isopropyl  $\beta$ -D-1-thiogalactopyranoside
- Viral promoter that allows for expression of genes
- Not metabolized by cell



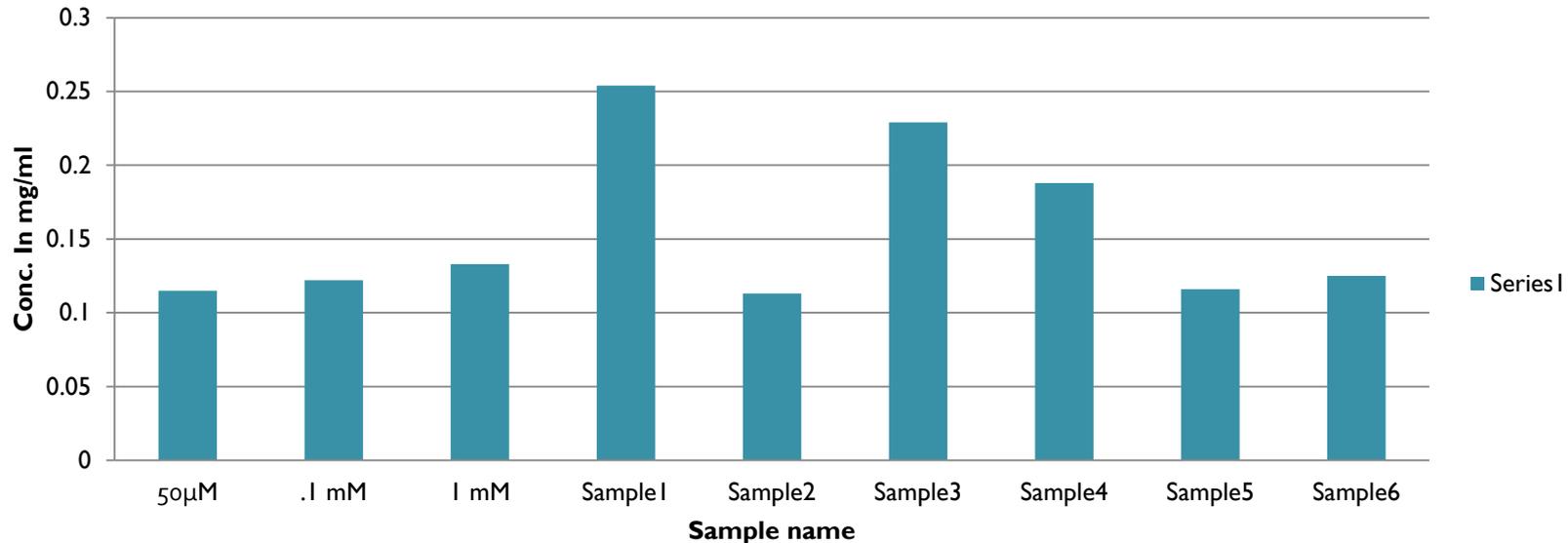
**Induction Curve 6/14**



**100 mL LB  
inoculated into  
1.5 L LB**

# IPTG Induction

## Protein Concentrations



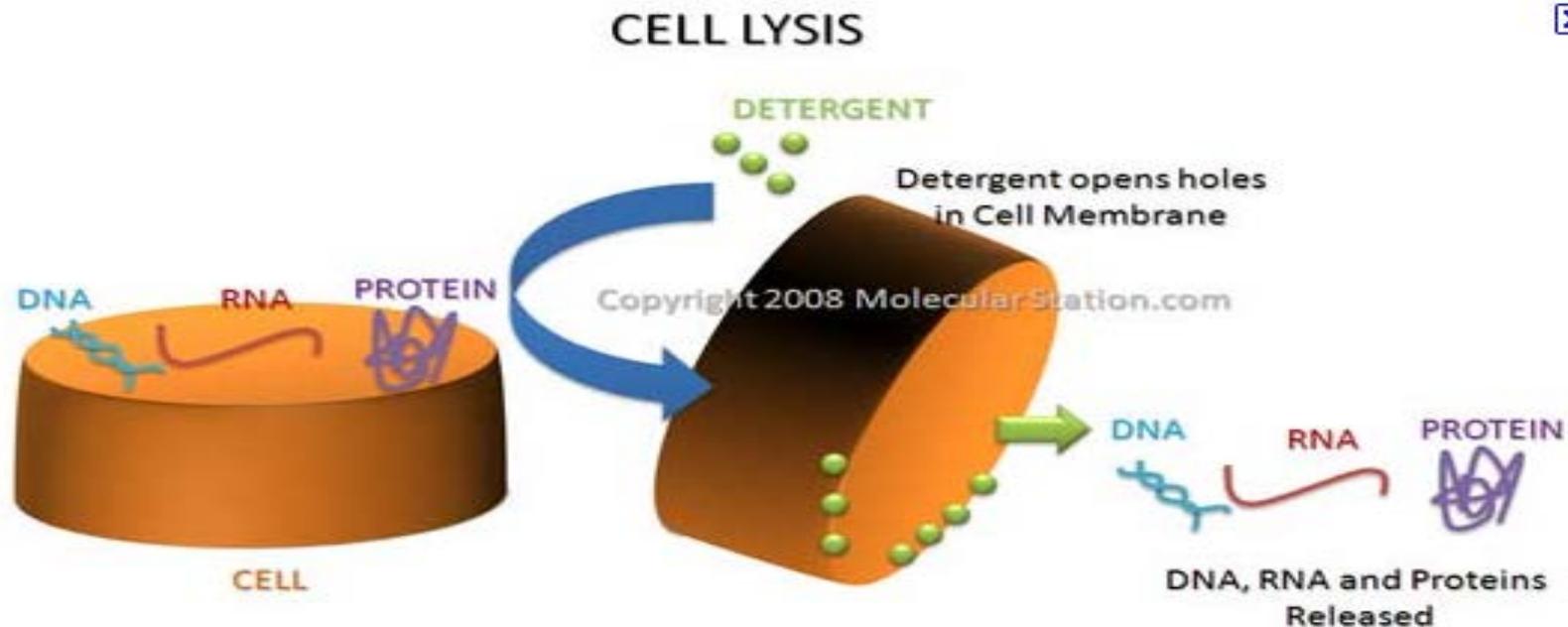
50 µM	50 µM IPTG concentration	Sample1	Normal M9 media
		Sample2	M9 media + 2X Glucose
.1 mM	.1 mM IPTG concentration	Sample3	M9 media + 2X Thiamine
		Sample4	M9 media +2X Glucose + 2X Thiamine
1 mM	1 mM IPTG concentration	Sample5	M9 media + 3X Glucose
		Sample6	M9 media + 5X Glucose

# Protein Purification

- Cell lyse
  - Sonication
  - French Press
  - **Chemical Lysing**
- Unfolding/ Refolding
  - High urea and cysteine
  - Low urea and more cysteine

# Protein Purification- I

- B-Per
  - Bacterial Protein Extraction Reagents



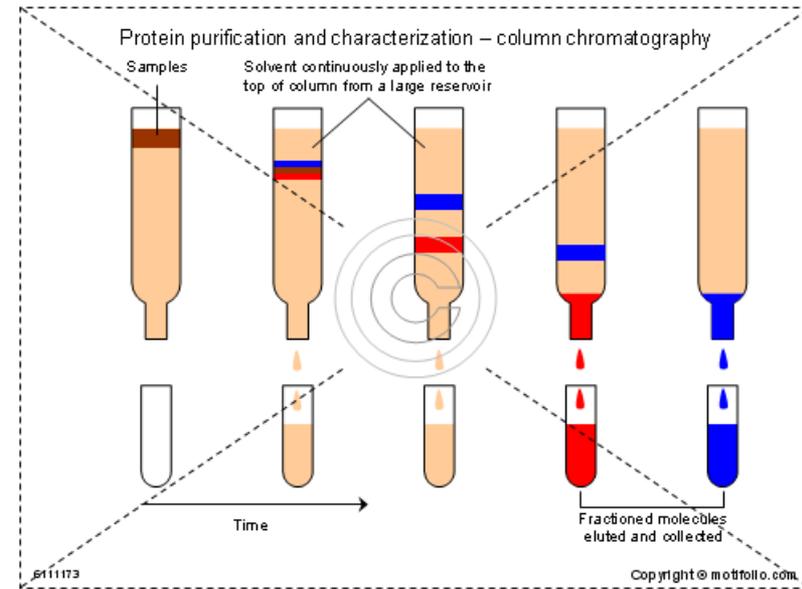
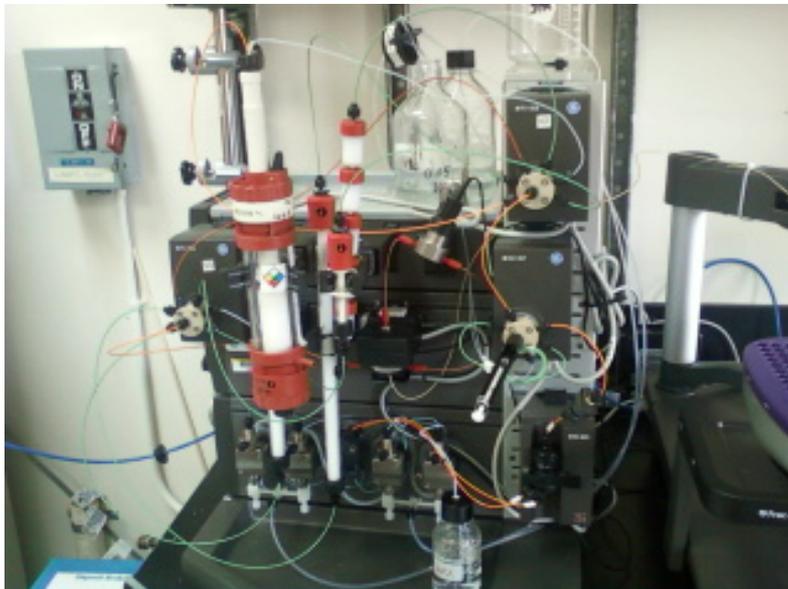
# Protein Purification-2

## ***Different chromatography columns***

Ion exchange column (IEX)

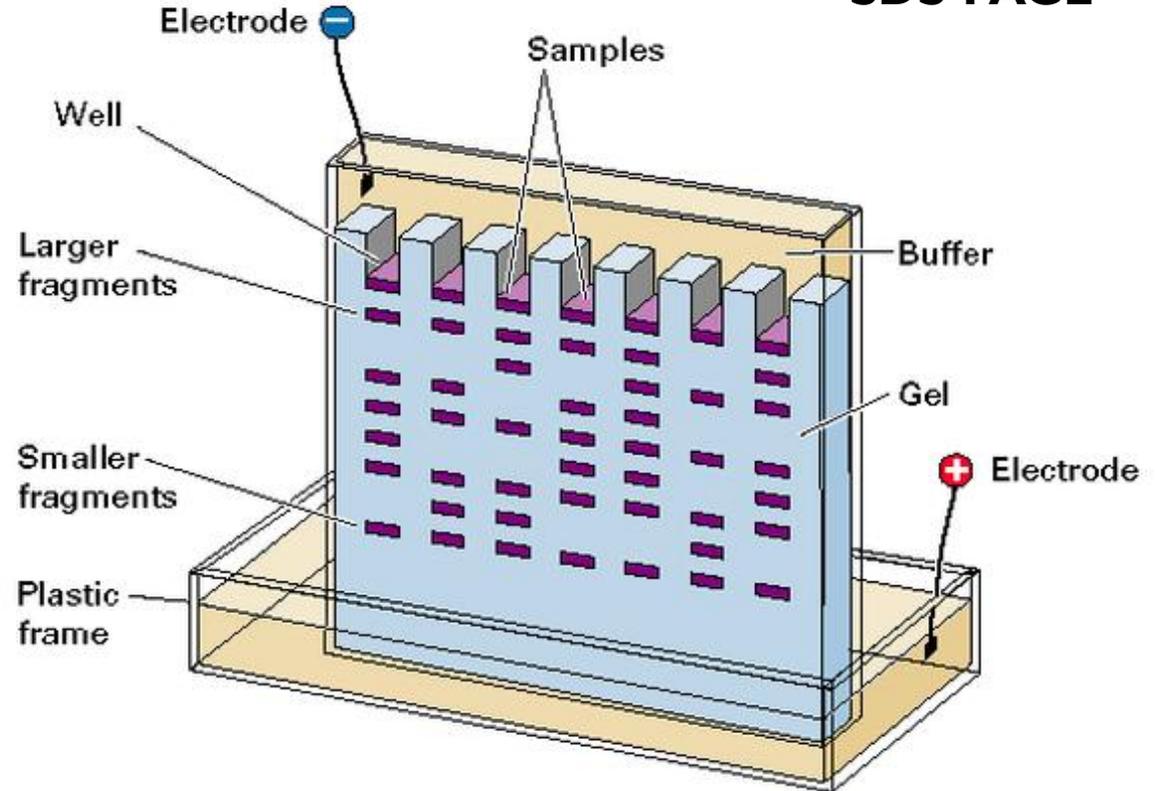
Hydrophobic column (HIC)

Size exclusion column



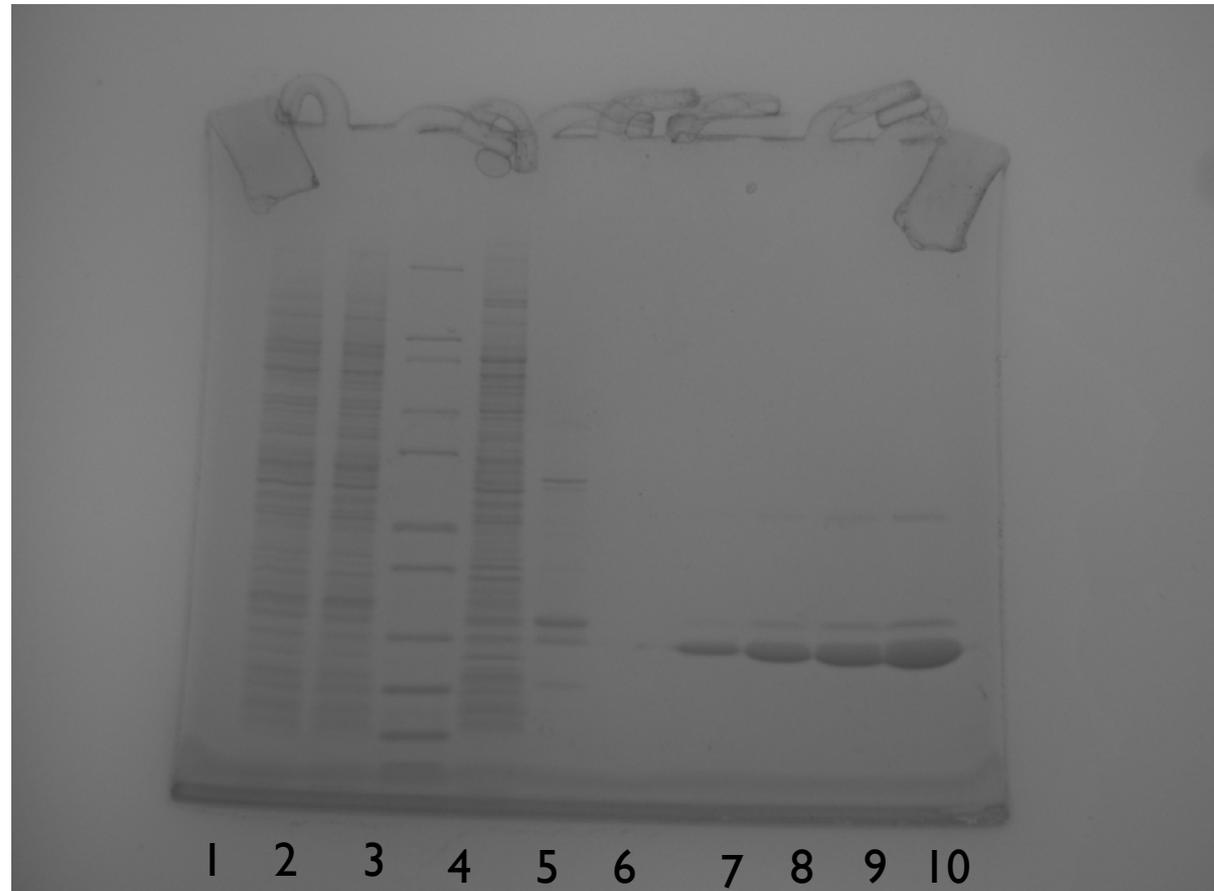
# Characterization

- Separates proteins by molecular weight
- Applies negative charge to each protein by mass.
- Tracking dye added.



# Where the protein is?

- 1: LB/ Bper/s
- 2: LB/ B-per/p
- 3: M
- 4: Enriched /B-per/s
- 5: Enriched /B-per/p
  
- 7: hGH 0.25mg/ml
- 8: hGH 0.5 mg/ml
- 9: hGH 1 mg/ml
- 10: hGH 2 mg/ml

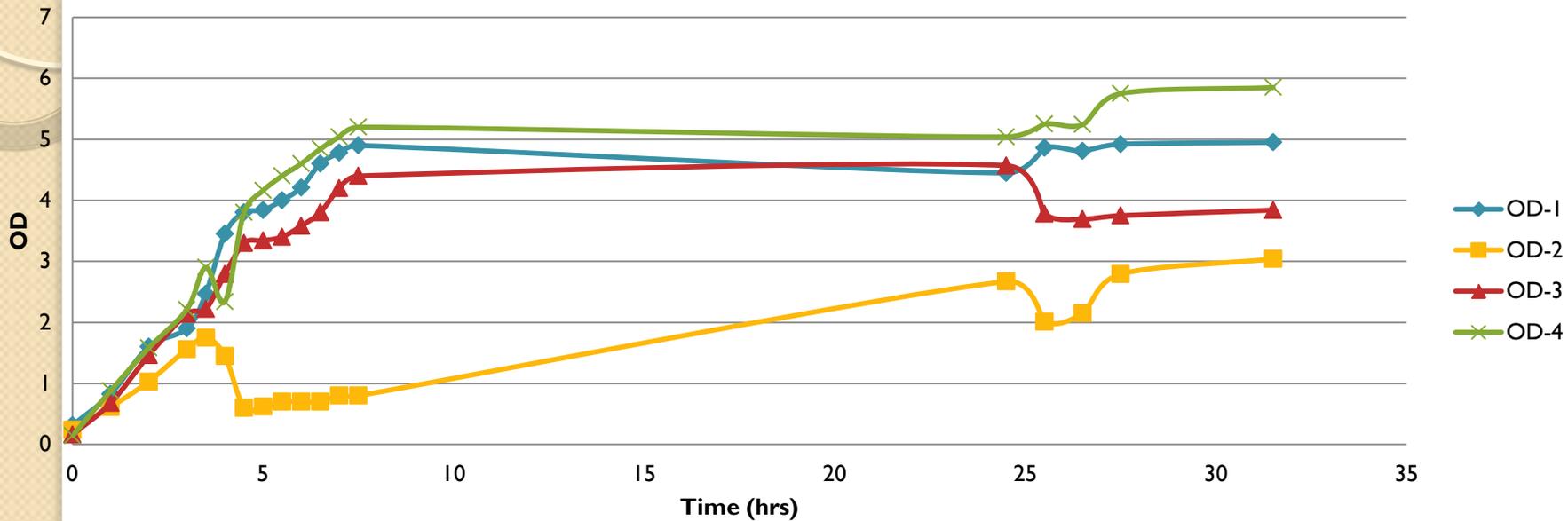


# M9 Optimization

- Be able to have labeled protein for neutron scattering
  - Slower growth rates than other media
  - Contains:
    - $\text{Na}_2\text{HPO}_4$
    - $\text{KH}_2\text{PO}_4$
    - $\text{NaCl}$
    - $\text{NH}_4\text{Cl}$
    - 1M  $\text{MgSO}_4$
    - 1M  $\text{CaCl}_2$
    - 20% Glucose
- To enrich:
- 1% Thiamine hydrochloride
  - 1.38 mg/ml  $\text{FeCl}_2$

# Growth

## M9 OD Growth

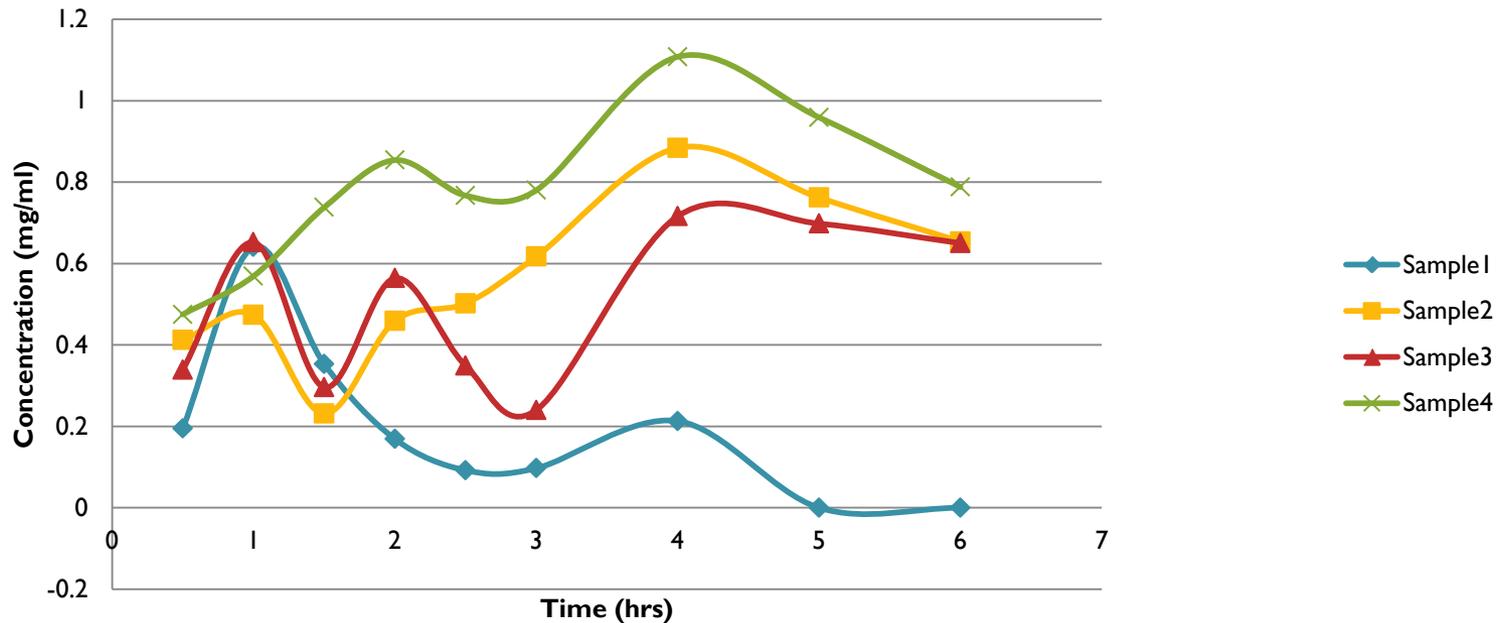


OD-1	Normal M9 media
OD-2	M9 media + 2X Glucose
OD-3	M9 media + 4X Glucose
OD-4	M9 media +2X Glucose+ 2X Vitamins

Inoculated 50 ml M9 with 10 ml LB

# Protein Expression

## Protein Concentration



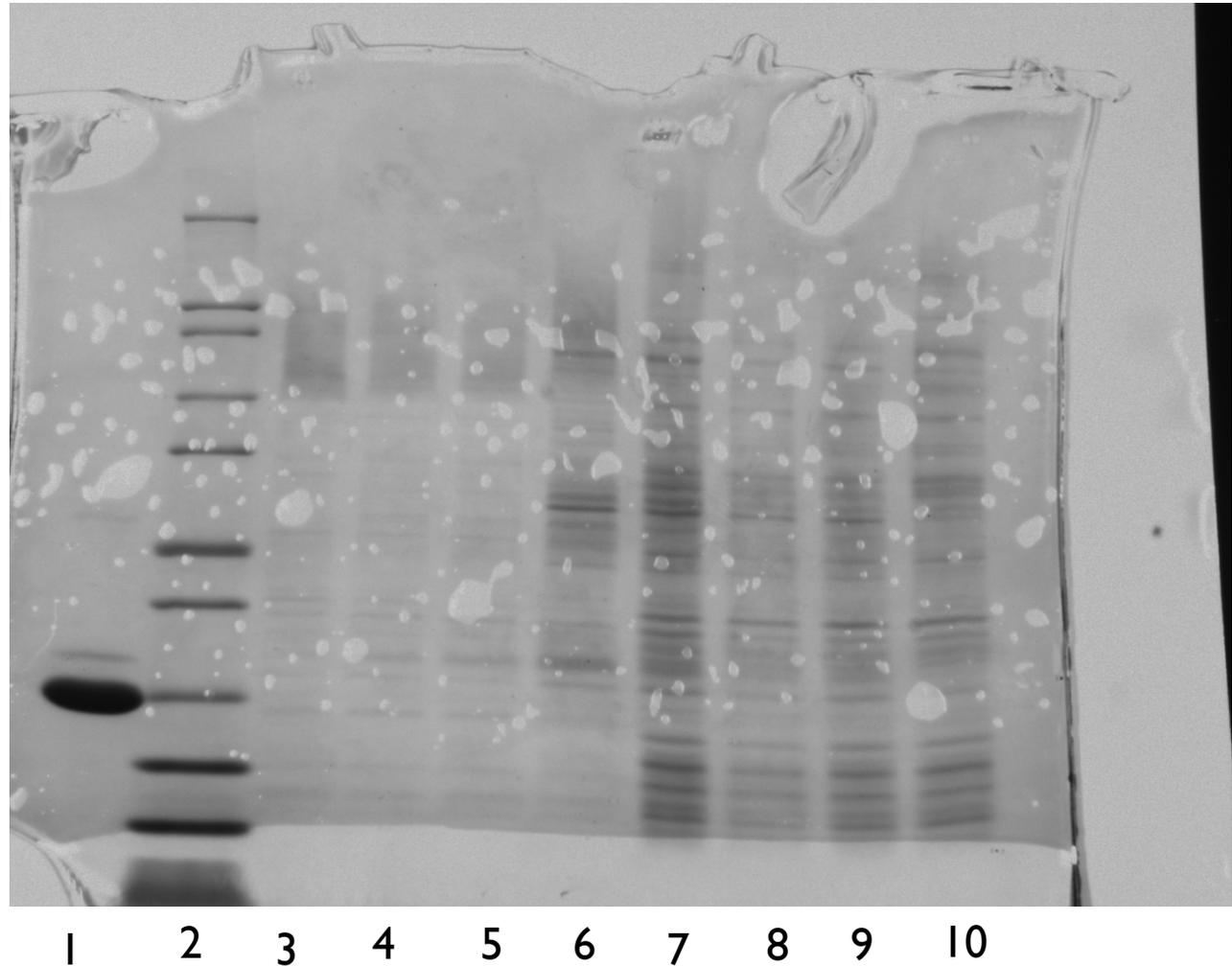
Inoculated 50 ml M9 with 10 ml LB

Sample1	Normal M9 media
Sample2	M9 media + 2X Glucose
Sample3	M9 media + 4X Glucose
Sample4	M9 media +2X Glucose + 2X Vitamins

# M9 media

Sample1	Normal M9 media
Sample2	M9 media + 2X Glucose
Sample3	M9 media + 4X Glucose
Sample4	M9 media +2X Glucose + 2X Vitamins

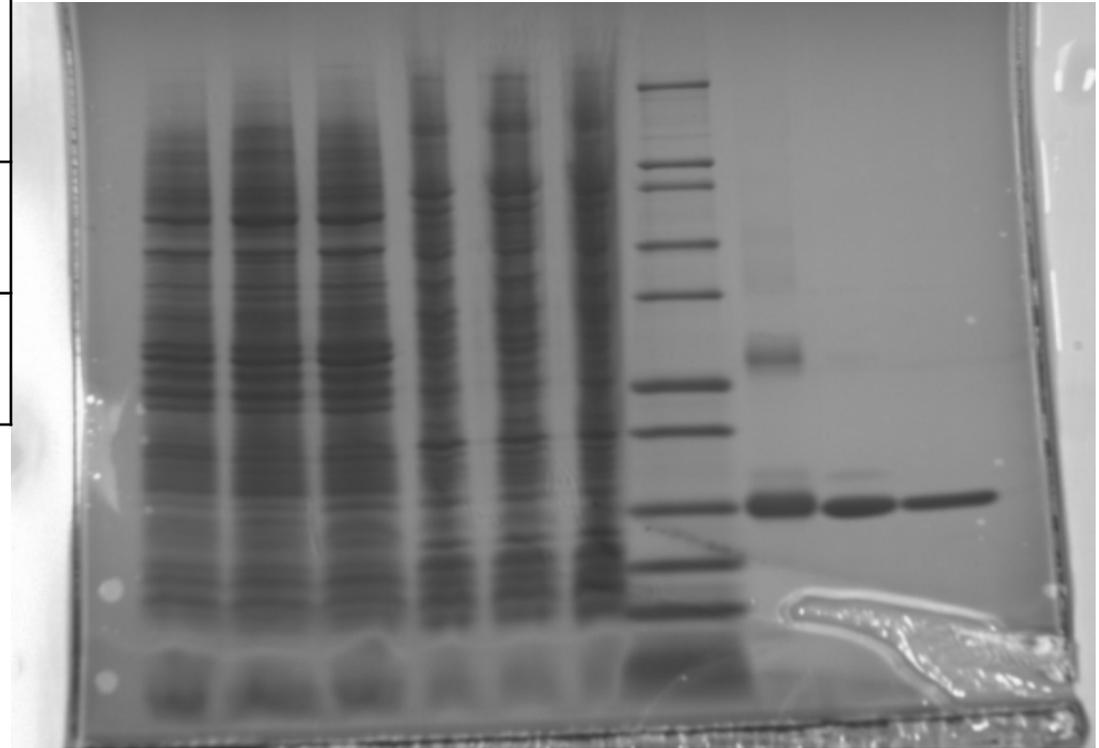
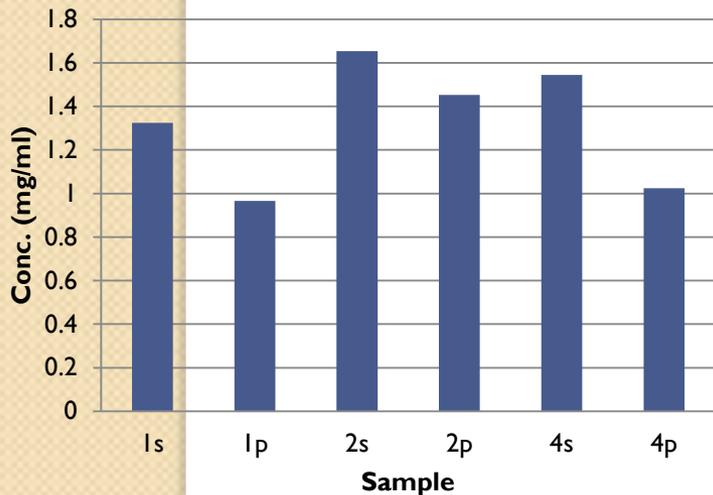
- 1: hGH 1.3 mg/ml
- 2: M
- 3: 1-Bper/P
- 4: 2-Bper/P
- 5: 3-Bper/P
- 6: 4-Bper/P
- 7: 1-Bper/s
- 8: 2-Bper/s
- 9: 3-Bper/s
- 10: 4-Bper/s



# Where the protein is And which media to choose!

1	M9 media +2X Glucose+ 2X vitamins+2.5% glycerol
2	M9 media+ different inoculants
4	M9 media+2X Glucose+ 2X vitamins

**Protein Concentration**



**1 2 4 | 1 2 4 | M | hGH**

Supernatant | pellet | Marker | purified hGH

# Further Research

- Use of D<sub>2</sub>O for growth and optimization
- Continued optimization
- Protein decay improvements
- SANS of hGH and d-hGH in different pharmaceutical relevant formulations

# Acknowledgements

- **NIST**

- Julie Borchers
- SURF Program
- Sheila Khodadadi

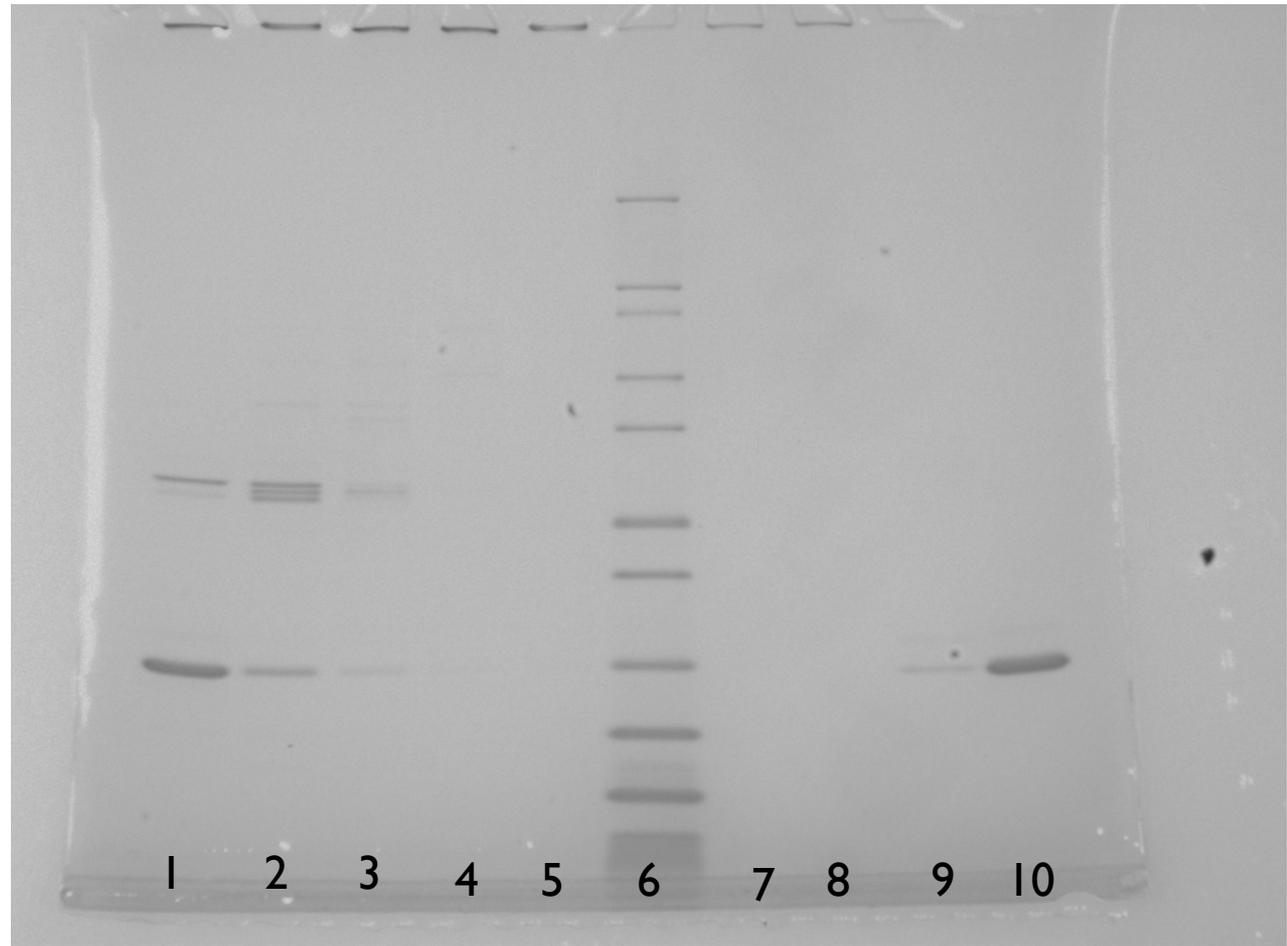
- **IBBR**

- Todd Hoopes
- John Marino



# IXC (I)

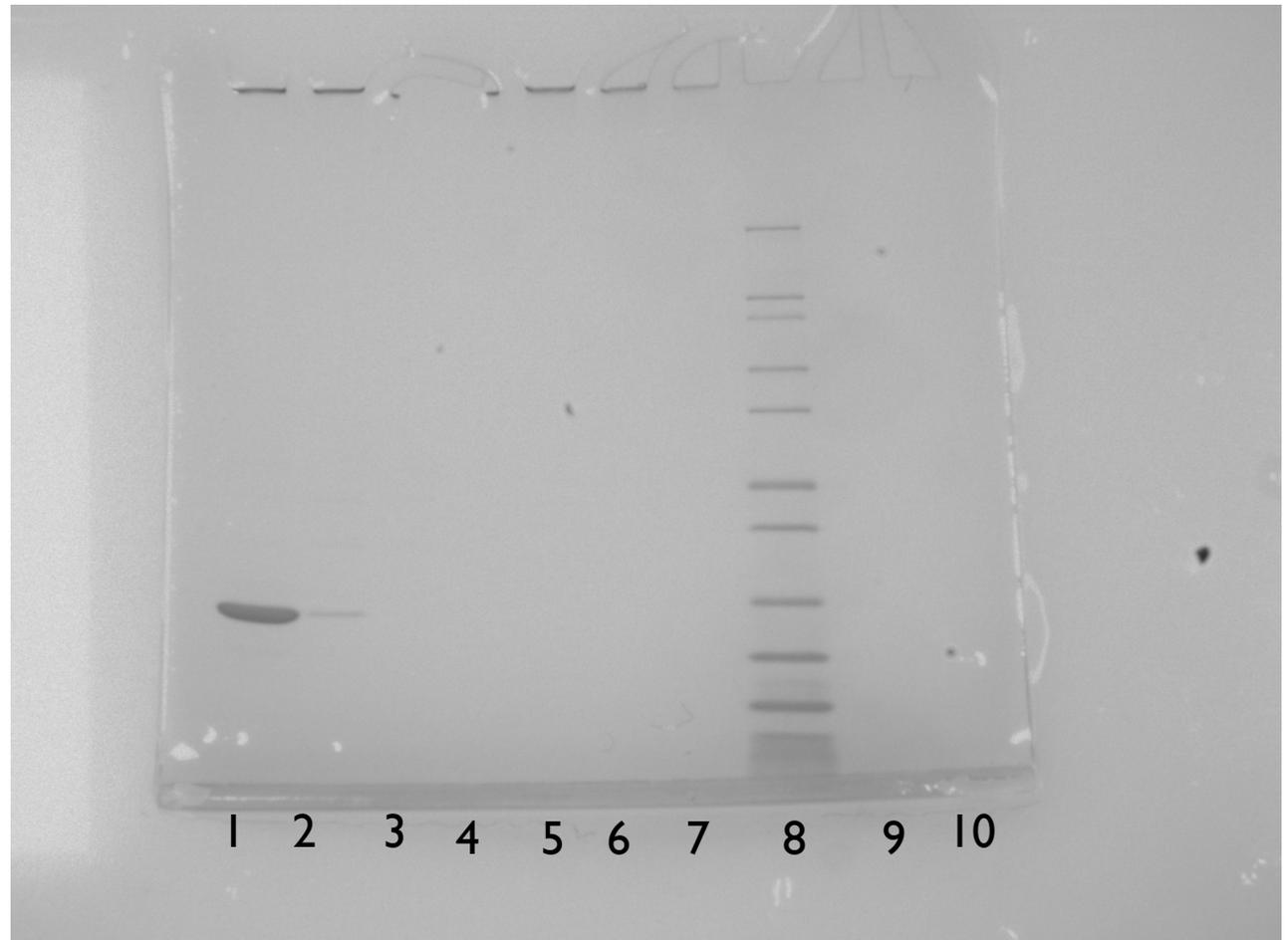
- 1: B10
- 2: B12
- 3: C2
- 4: C4
- 5: C6
- 6: M
- 7: E8
- 8: E10
- 9: Refolding (2 day old)
- 10: hGH 0.5 mg/ml



Pooled B8-B12

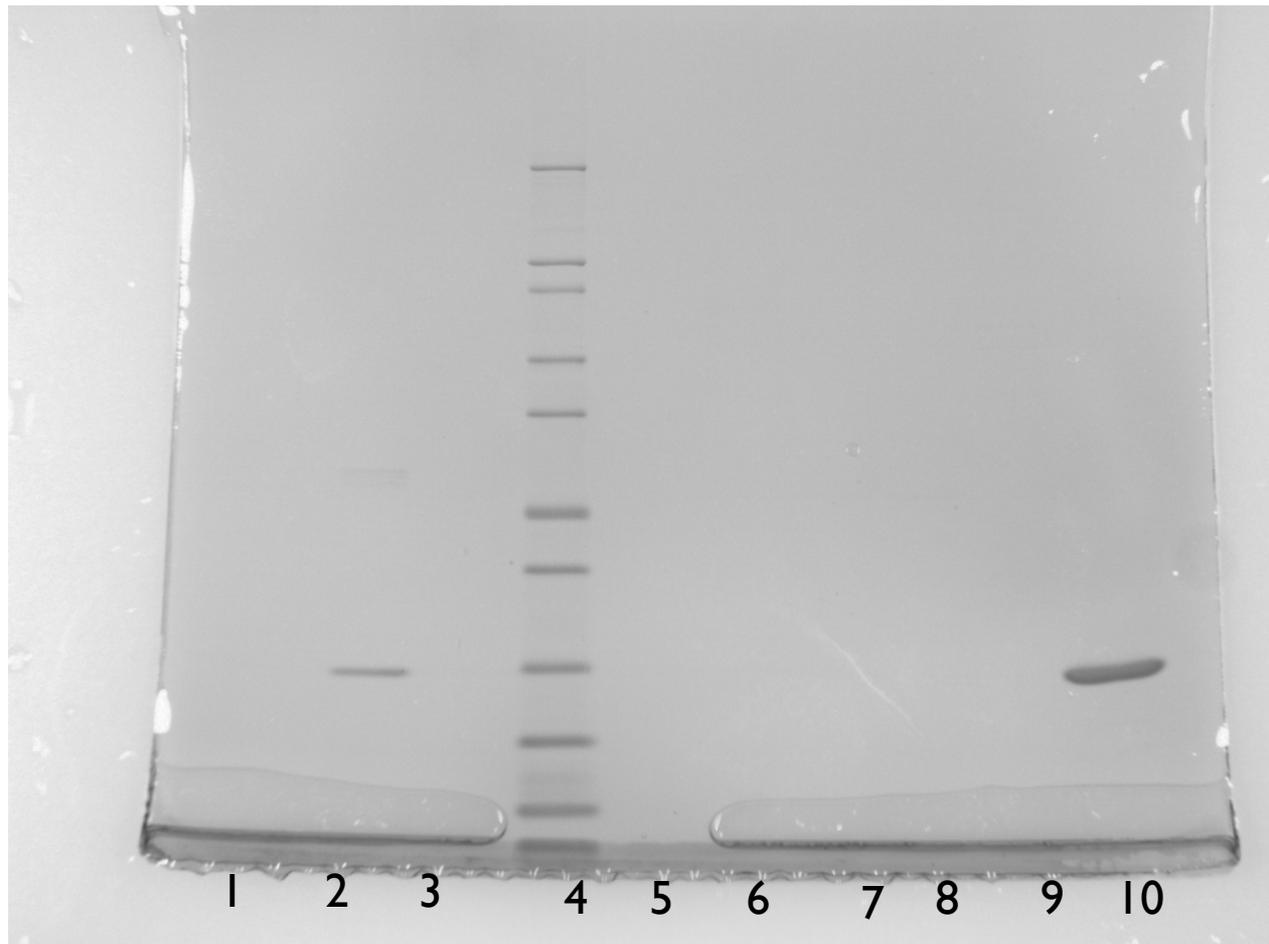
# IXC (2)

- 10: Load
- 9: Wash
- 8: M
- 7: A8
- 6: A10
- 5: A12
- 4: B2
- 3: B4
- 2: B6
- 1: B8



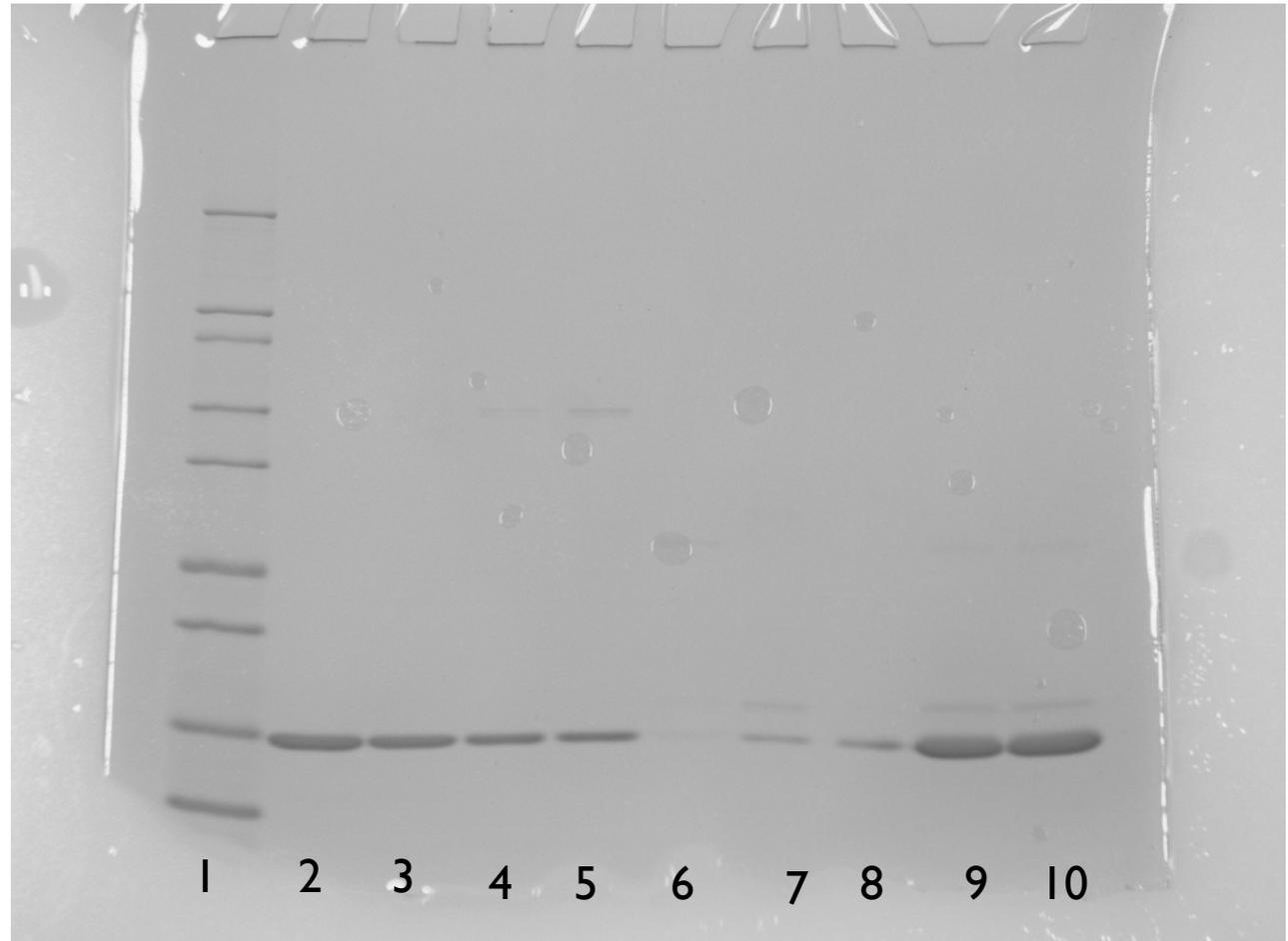
# HIC (I)

- 1: Load
- 2: P-column (after 1 day)
- 3: Wash
- 4: M
- 5: A2
- 6: A10
- 7: B1
- 8: B4
- 9: B6
- 10: B8



# HIC (2)

- 1: M
- 2: B10
- 3: B12
- 4: C1
- 5: X1
- 6: X2
- 7: Q- column (after 3 days)
- 8: spill!!
- 9: Std/ 0.3 mg/ml
- 10: Std/ 0.6 mg/ml



Pooled B8-B12