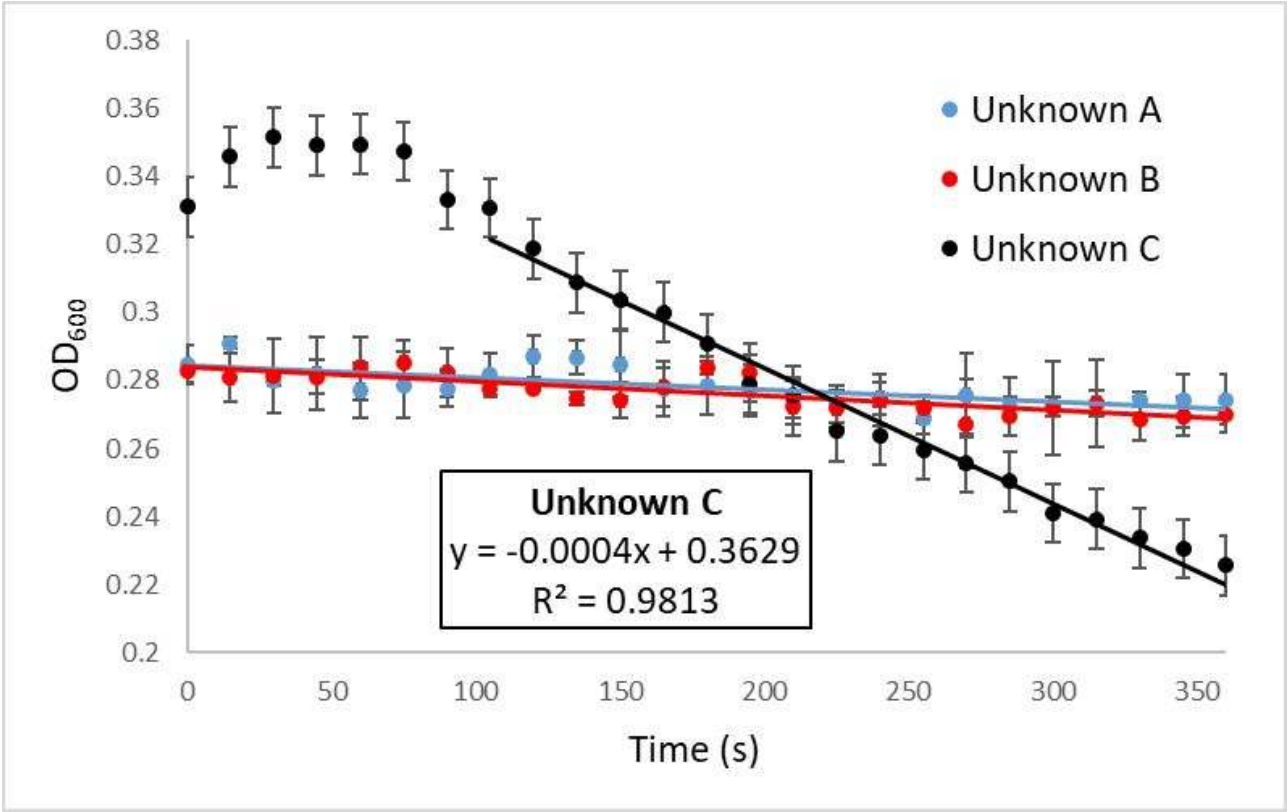
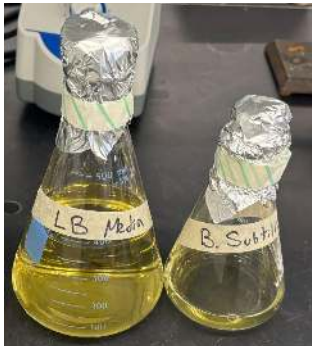
pUCK - 19
Lab Challenge

BIOC4001

Enzymatic Assay

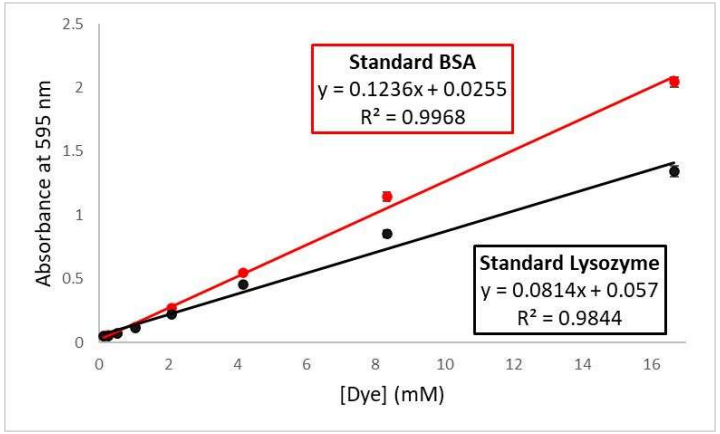


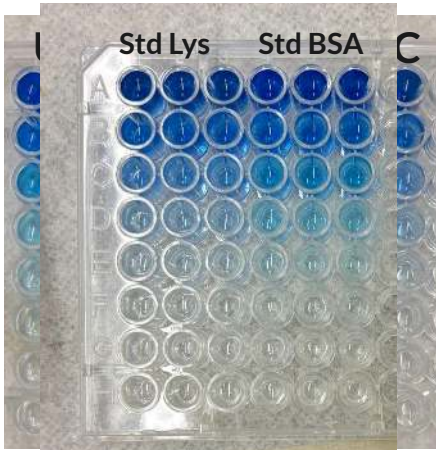
Yeast



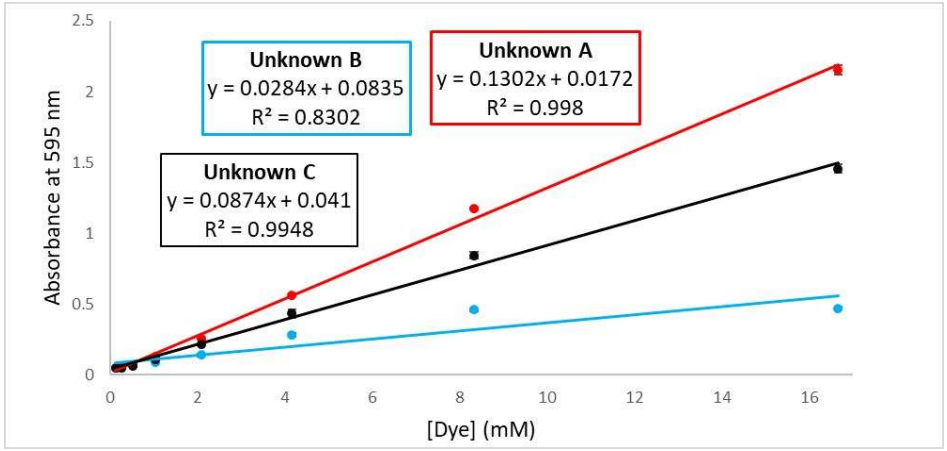
B. Subtilis

Dye Ligand Binding Assay and E1% Measurements





Fraction (0.1 mg/mL)	E1% (280 nm) $\left(\frac{L}{g \cdot cm}\right)$
Std Lys	21.0 ± 2.2
Std BSA	6.63 ± 1.00
Unk A	6.27 ± 0.25
Unk B	-1.63 ± 0.67
Unk C	21.8 ± 0.92



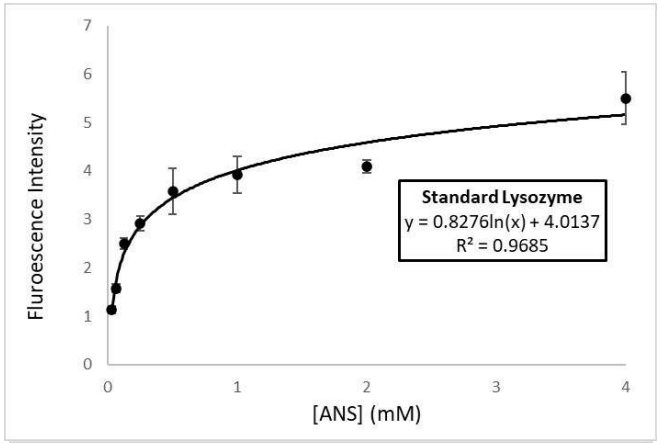
E1% is absorbance of 10 mg/mL solution

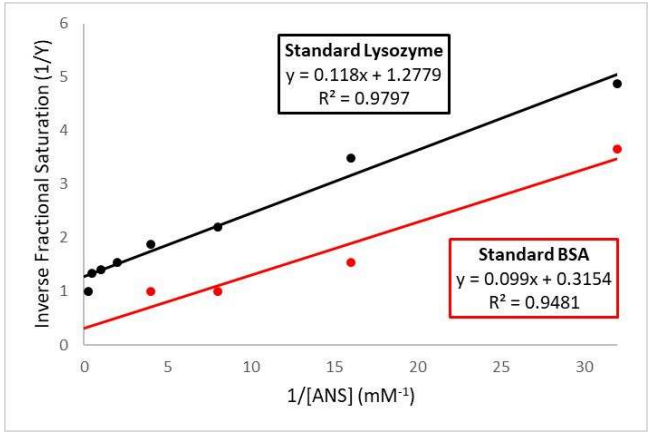
Lysozyme has a higher ratio of tyrosine and tryptophan in its structure than BSA

Unknown B had a negative absorption at 280 nm, indicating likely absence of protein, however had small absorbance at 260 nm range

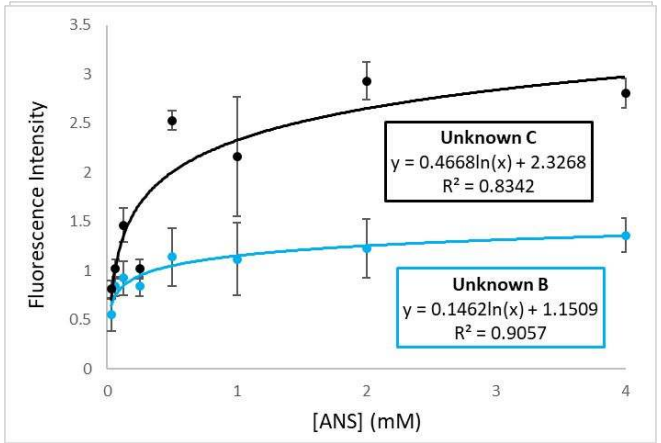
ANS Fluorescence Binding Assay

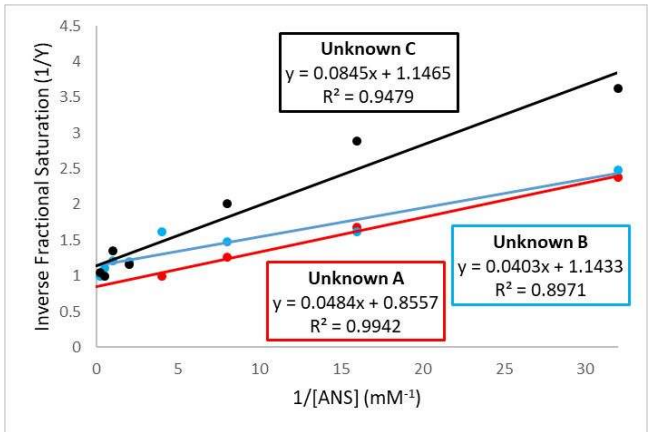
$$\frac{1}{Y} = \frac{K}{n} \cdot \frac{1}{[L]} + \frac{1}{n}$$





	Dissociation Constant, K (mol/L)	Binding Sites (n)
Std BSA	0.31383	3.171
Std Lys	0.09234	0.7826
Unk A	0.05656	1.1686
Unk B	0.03522	0.8747
Unk C	0.07372	0.8722





MW BSA = 66.4 kDa
MW Lys = 14.4 kDa

Correction = 4.61

	Corrected Dissociation Constant, K (mol/L)
Std BSA	0.31383
Std Lys	0.02002

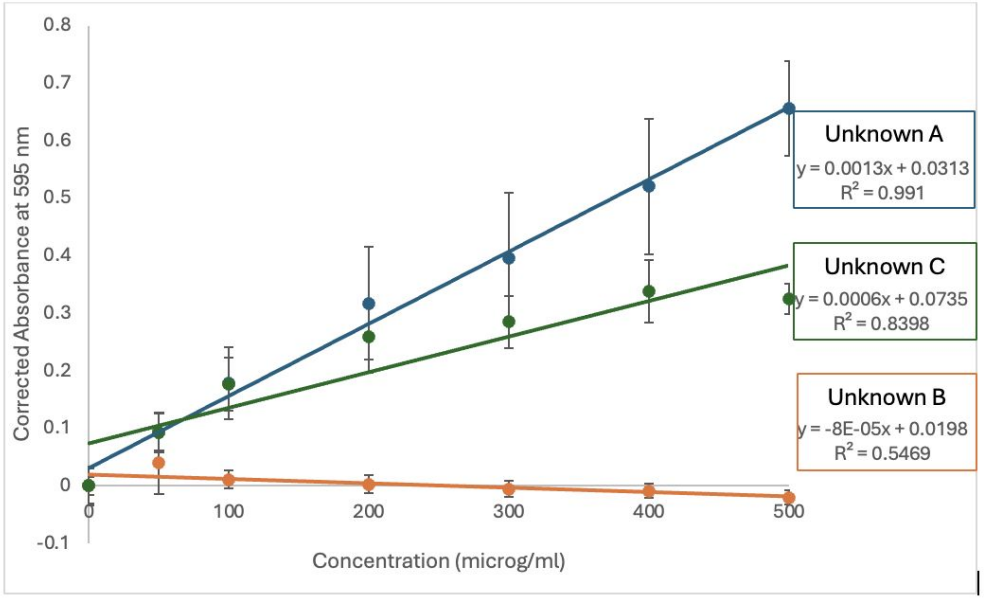
Bradford Assay

BSA would have a steeper slope and broader linear range:

-More arginine, lysine and histidine residues that strongly interact with the dye

Lysozyme may have a shorter linear range:

-high pI (11.4) makes it more prone to aggregation in the acidic conditions



Size-Exclusion Chromatography

The molecular weight cut-off (MWCO) for the spin column is 40 kDa

-BSA (MW=66 kDA) is expected to flow through the column and have a high amount of recovered protein

-Lysozyme (MW= 14.4 kDa) does not meet the cut-off

Sample	Amount of Protein (μg)
Unknown A	898.91± 599.77
Unknown B	86.739± 66.093
Unknown C	367.46± 245.43
BSA Standard	1034.5± 689.8

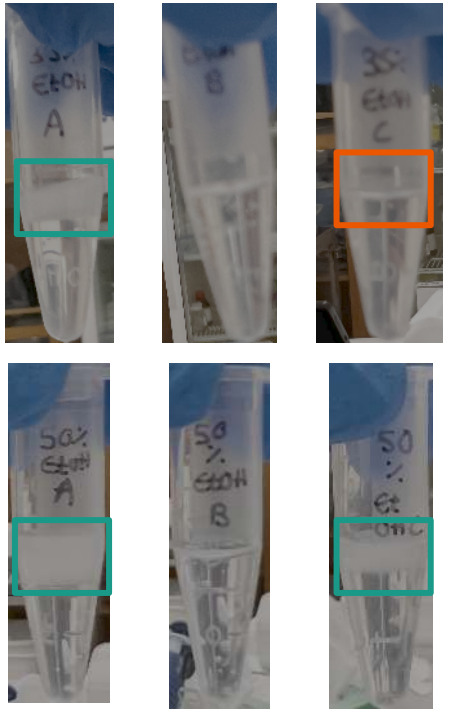
Semi-Qualitative (Organic Solvent Precipitate)

- Organic solvents exclude water from proteins and cause flocculation/aggregation.
- Lysozyme is Hydrophilic and interact more strongly with water
- BSA has hydrophobic surface and hydration shell is less stable
- BSA is expected to precipitate at lower organic solvent concentration
- pH precipitation did not work and made no use of salting out

Literature Precipitation Thresholds (%)

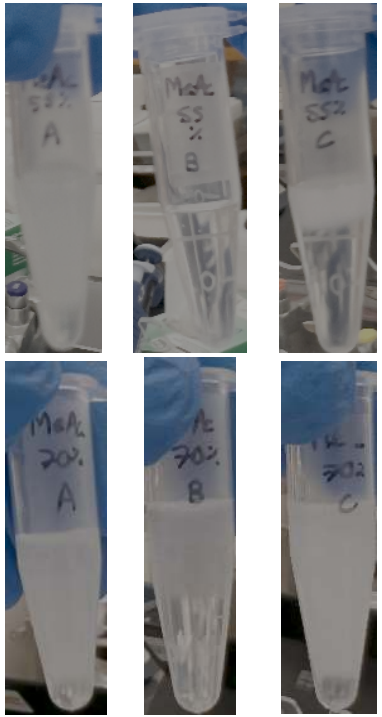
Solvent	Protein	Ppt Starts	Major	Complete
EtOH	BSA	**35**	50	60
	Lys	**50**	60	70
MeAc	BSA	45	**55**	70
	Lys	60	**70**	80

Ppt Start Test



EtOH
Top 35%
Bottom 50%
A is BSA
C is Lys

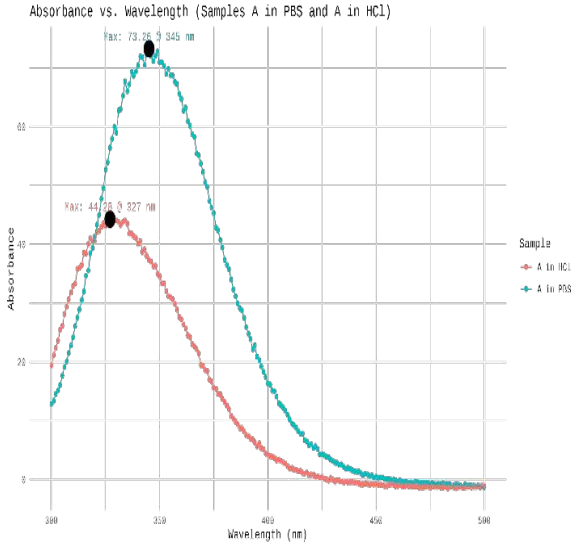
Major Ppt Test



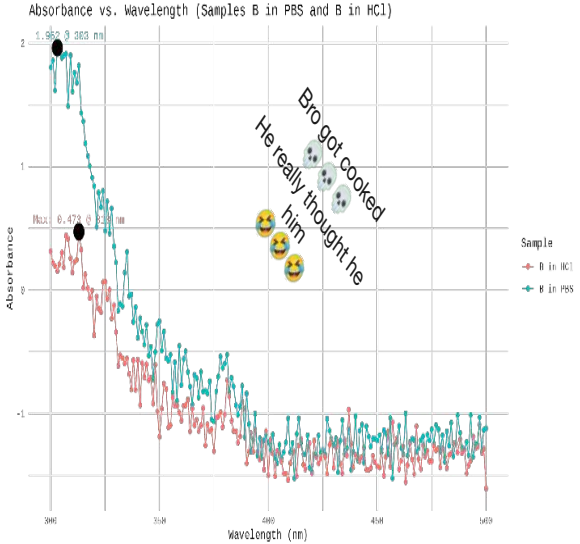
MeAc
Top 55%
Bottom 70%
A is BSA
C is Lys

Quantitative (UV-Vis)

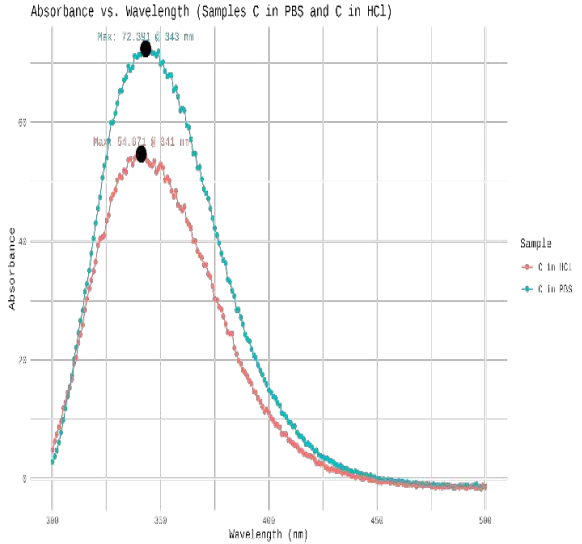
A is BSA



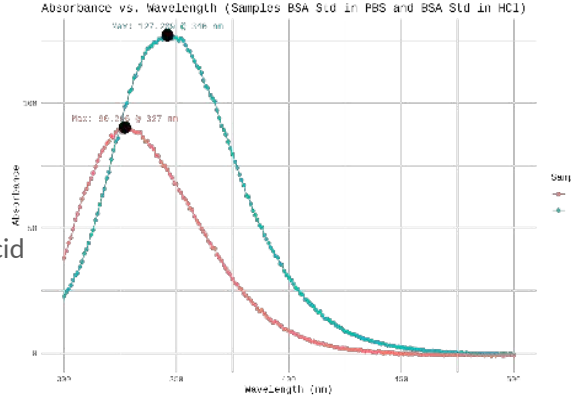
B is Unknown

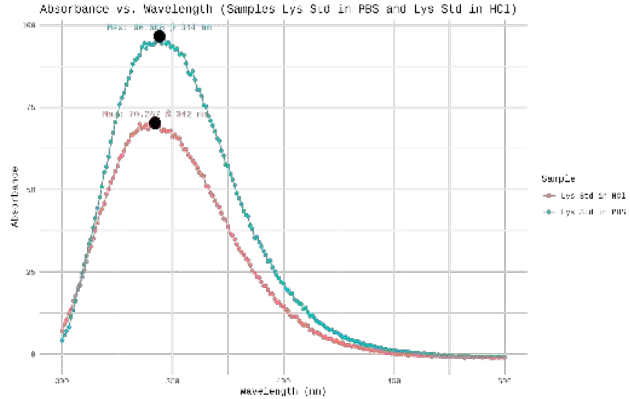


C is Lysozyme



- PBS (pH 7.4) and HCl (pH 2)
- BSA is larger and more complex, held by ionic interactions
- Lysozyme is small and compact, held by covalent interactions
- Conformational change more important in BSA in Acid
- Tryptophan Fluorescence affected by environment
- Blue-Shift as Trp residues in BSA are more shielded from solvent.





Summary of Results of Each Fraction

Fraction
Std BSA
Std Lys
Unk A
Unk B
Unk C

References

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