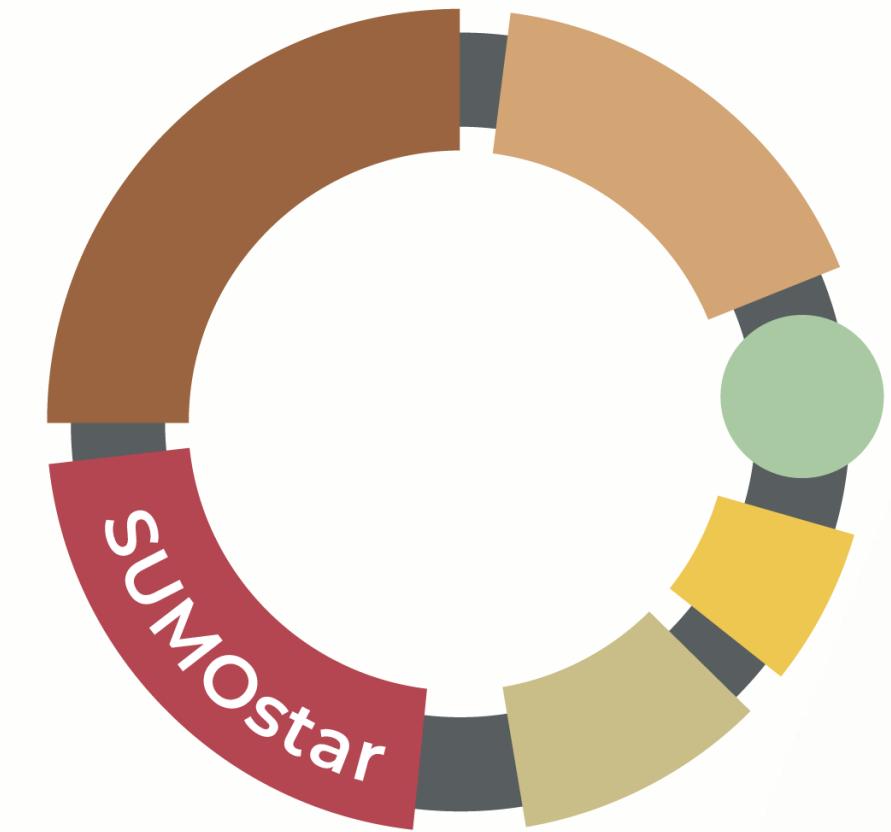


# Manufacturing Single Chain Antibodies, Nanobodies with SUMO Expression Platform



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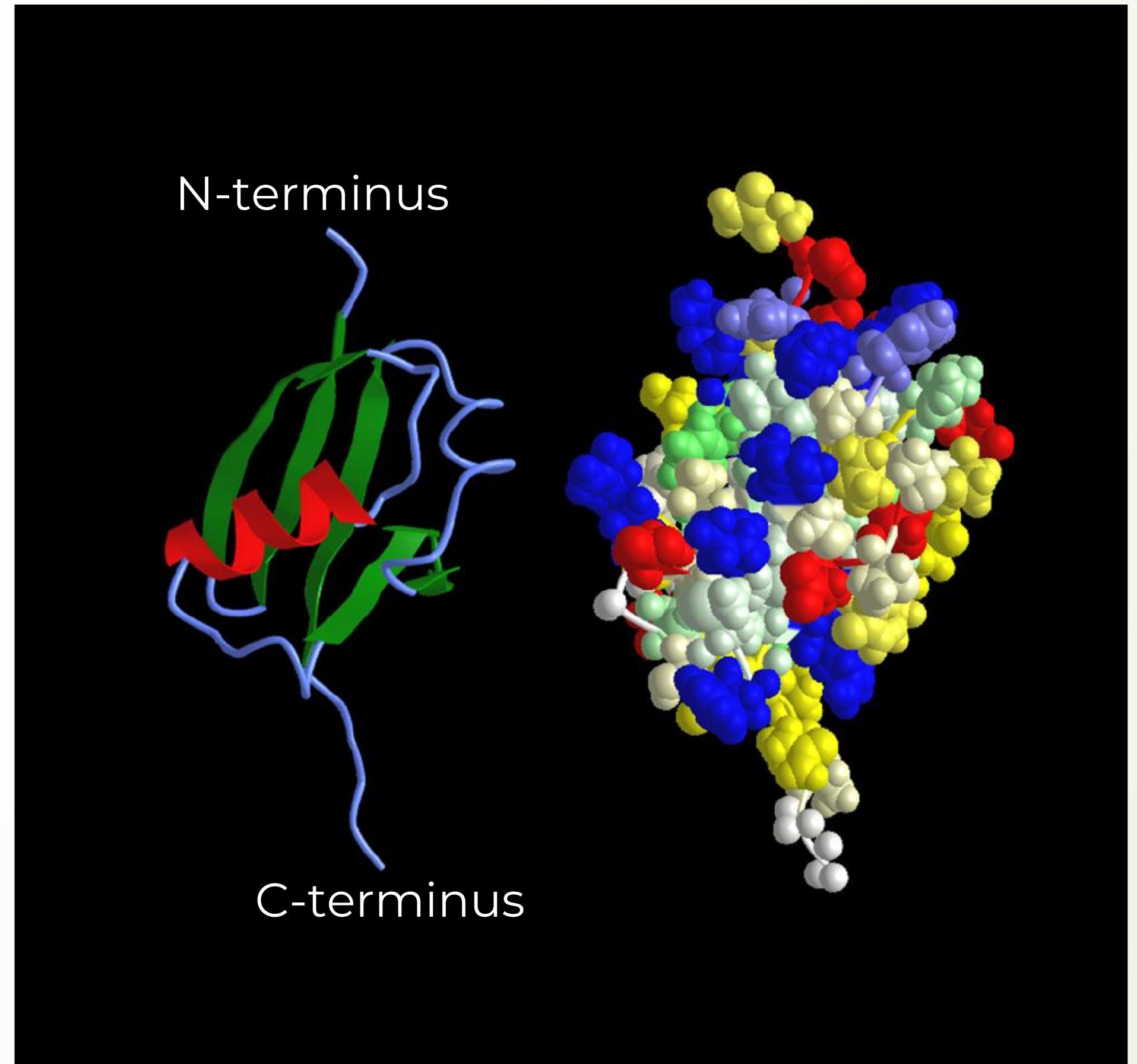
# SUMO Advantage

- Nanobodies or engineered single chain antibodies don't express well in E.coli
- Clinical programs are abandoned due to poor expression
- More than 90 proteins expressed in E.coli have been approved by FDA †
- SUMO system dramatically enhances expression of soluble nanobodies and decreases the cost of goods

† Full-length recombinant antibodies from E.coli --- clinical evaluation. MABS, 14, 1, e2111748 (20 pages)

# How Does SUMO Help Enhance Nanobody Expression?

- Nature designed SUMO to act as a chaperone for proteins
- SUMO, or Small Ubiquitin-like MOdifier, is a member of the ubiquitin family
- Flexible N-terminal region followed by a compact ball-shaped ubiquitin-like fold
- SUMO improves solubility due to hydrophilic outer surface and hydrophobic core



# SUMO Platform for Nanobodies

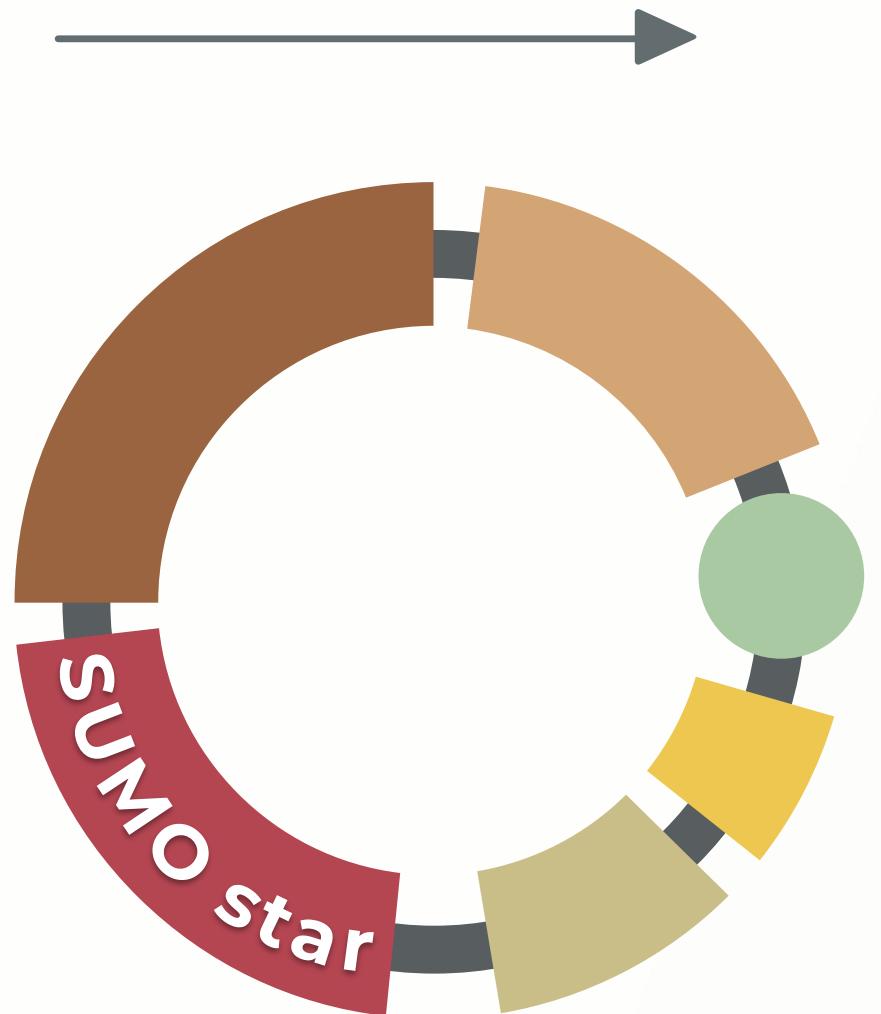
## PROBLEMS

Unstable protein,  
low expression

Insoluble Nanobodies

Miss-folded nanobodies

High cost of goods



## SOLUTIONS

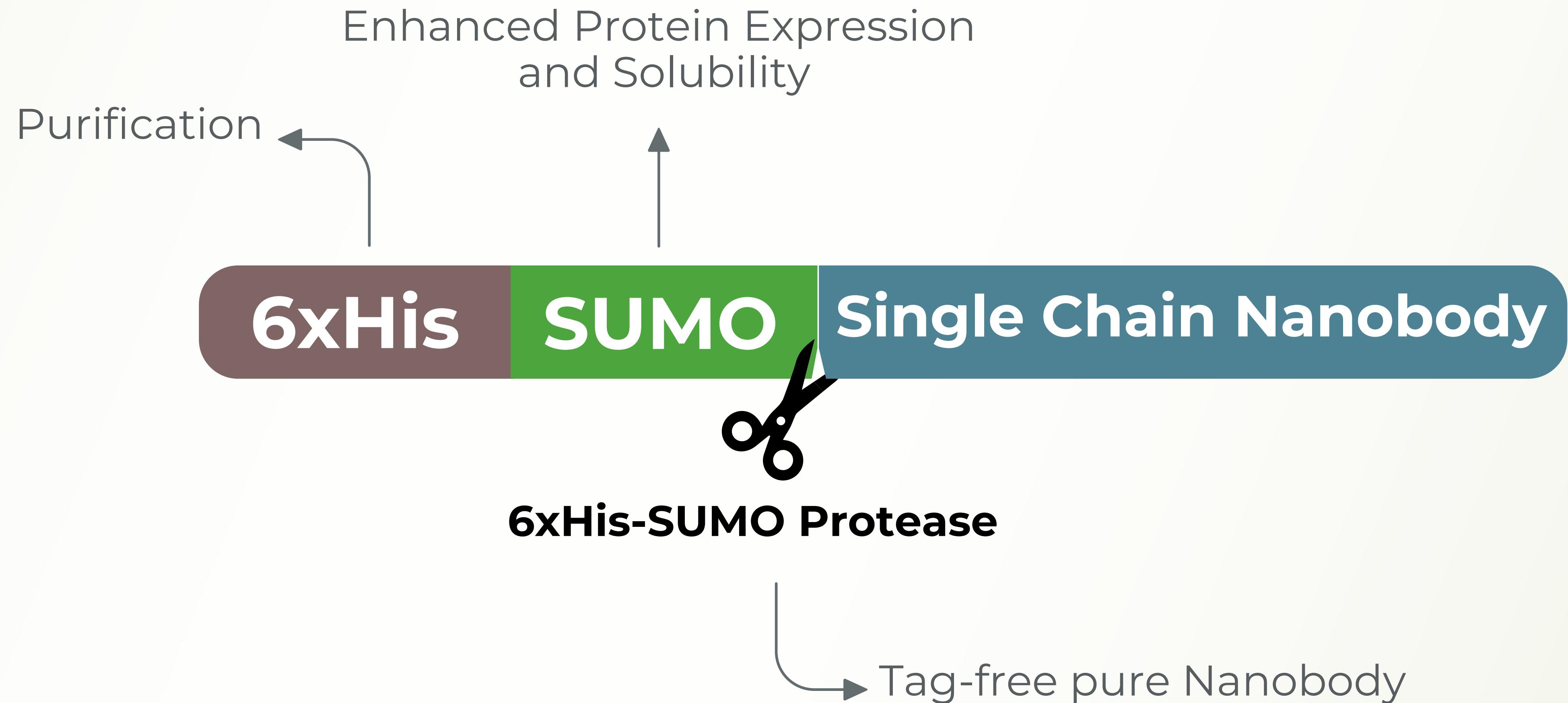
SUMO chaperoning,  
enhanced expression

SUMO derived solubility

SUMO-driven folding

High yield, saves cost

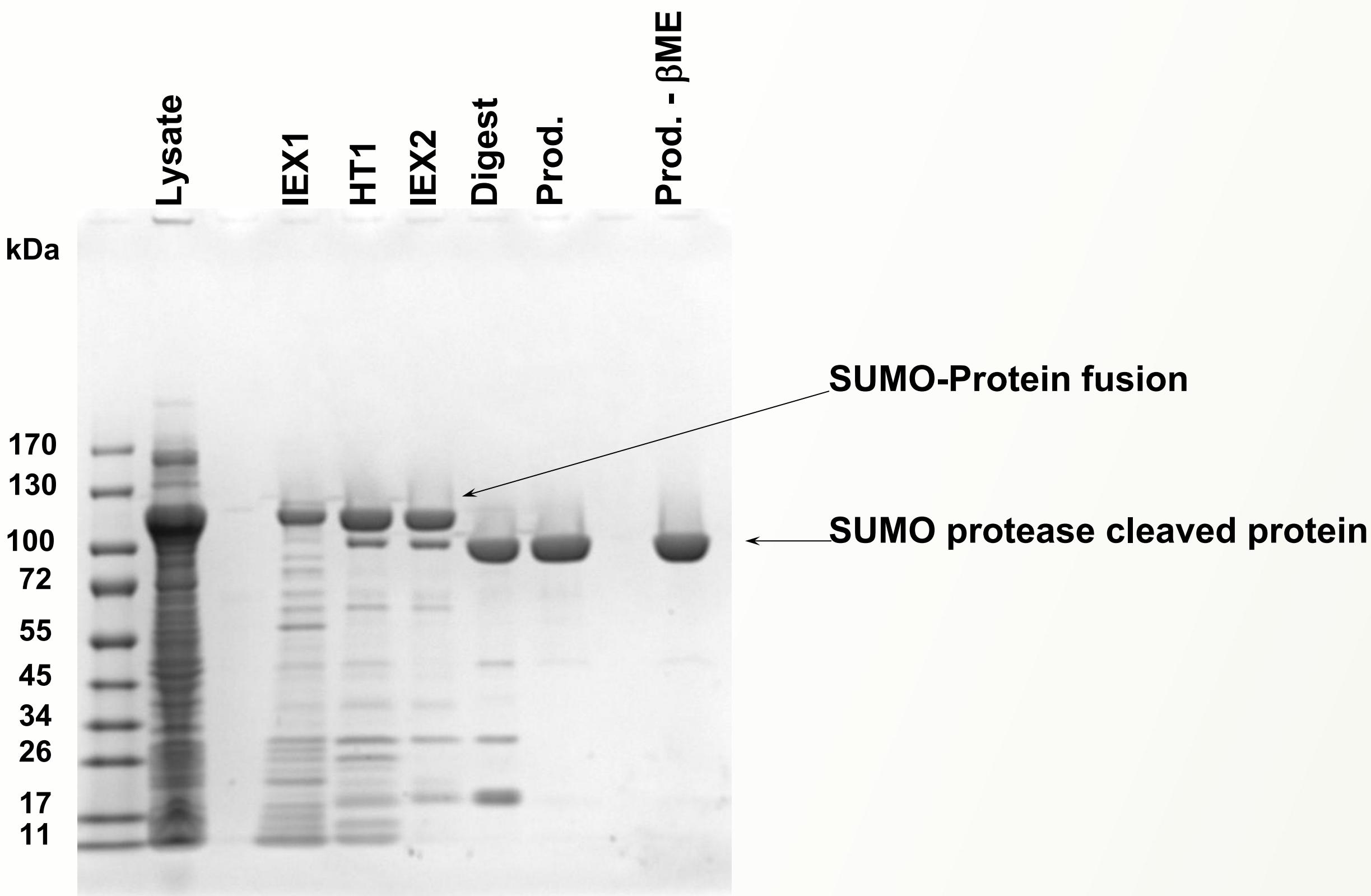
# SUMO Platform For Native Nanobodies





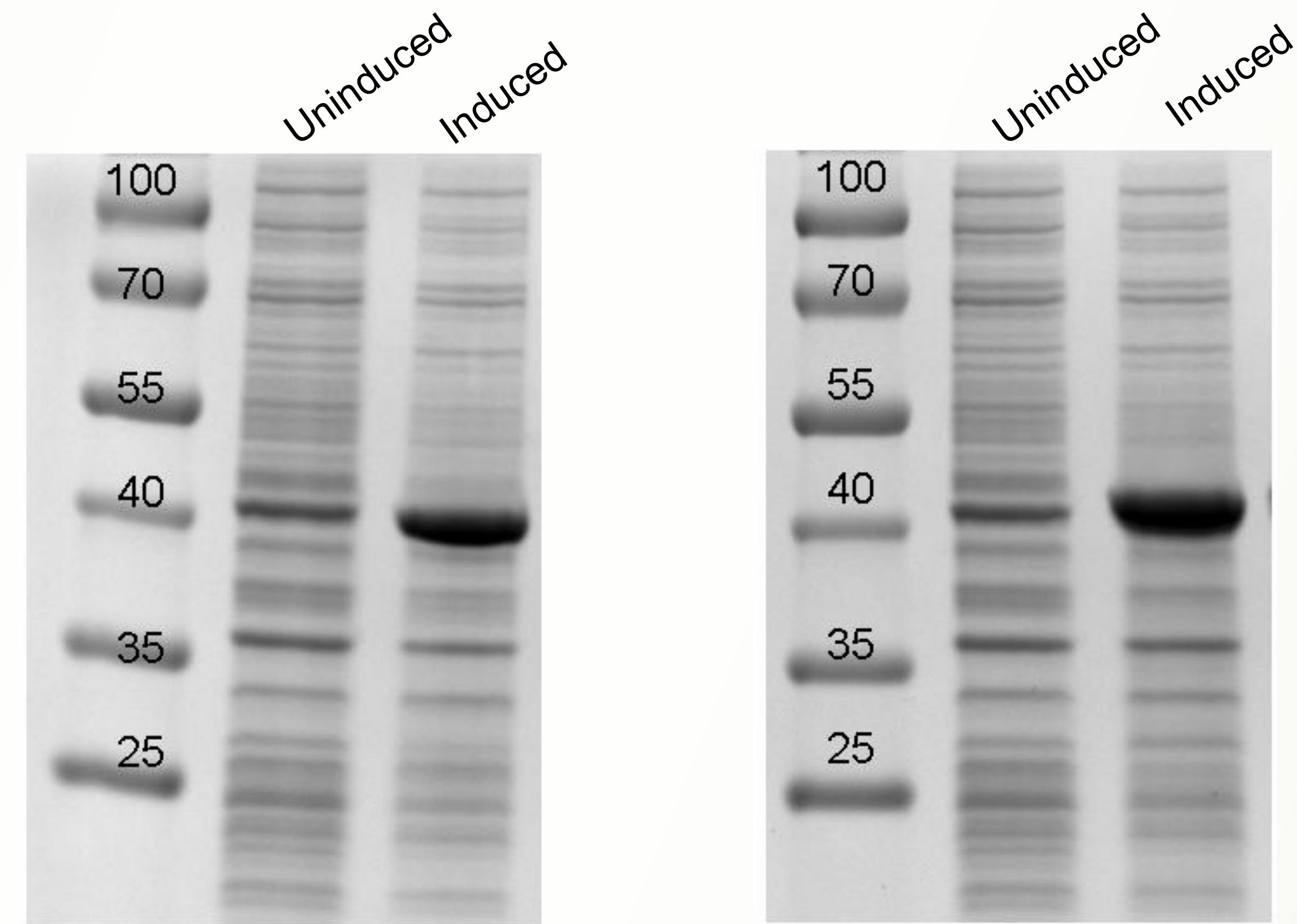
# Expression of Candidate Protein in *E. coli*

- Improved expression of Protein from 8% TCP to >20% TCP
- Purification reduced from 5 column steps to 4
- Overall yield improved from ~16% to >40%

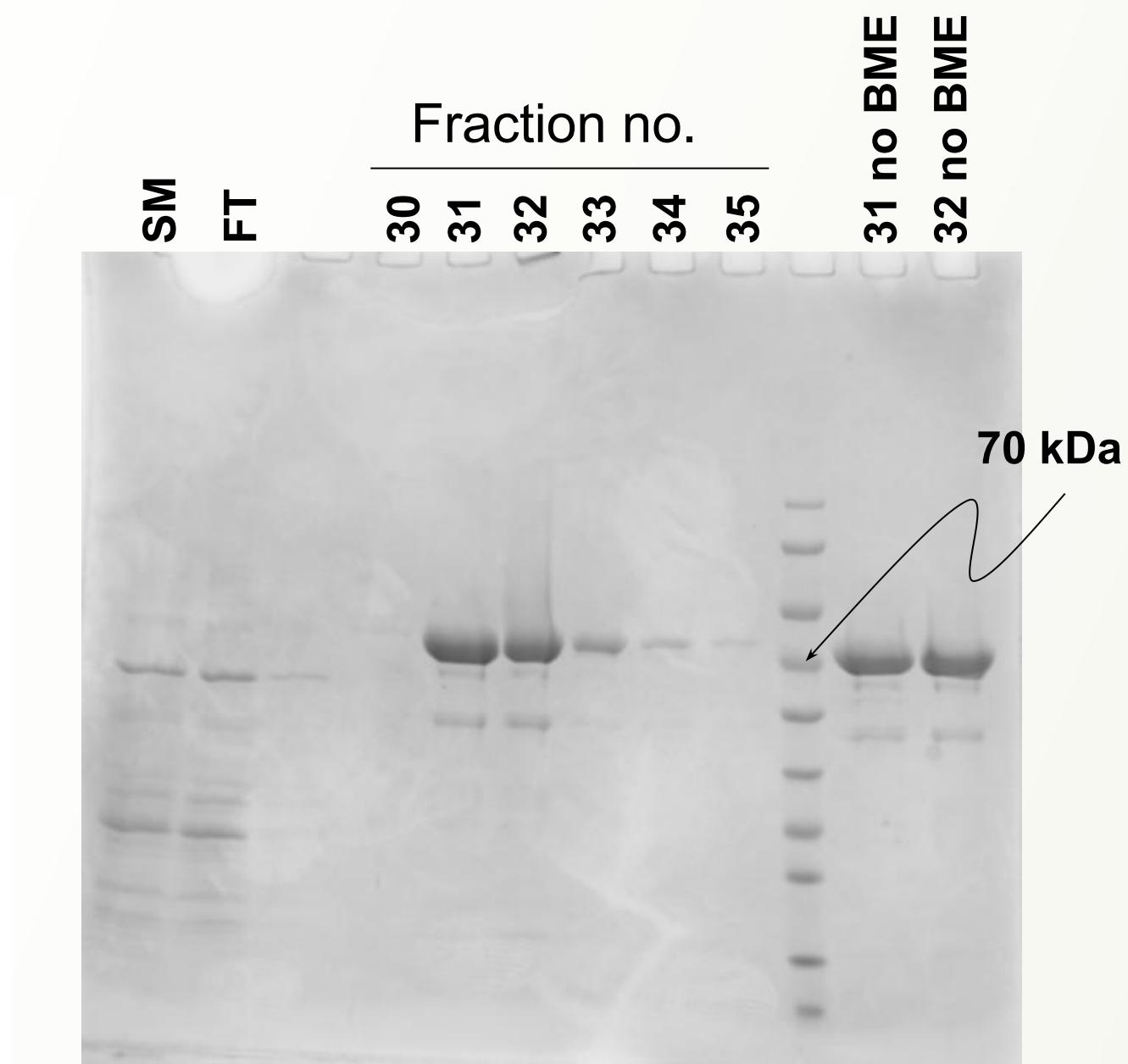
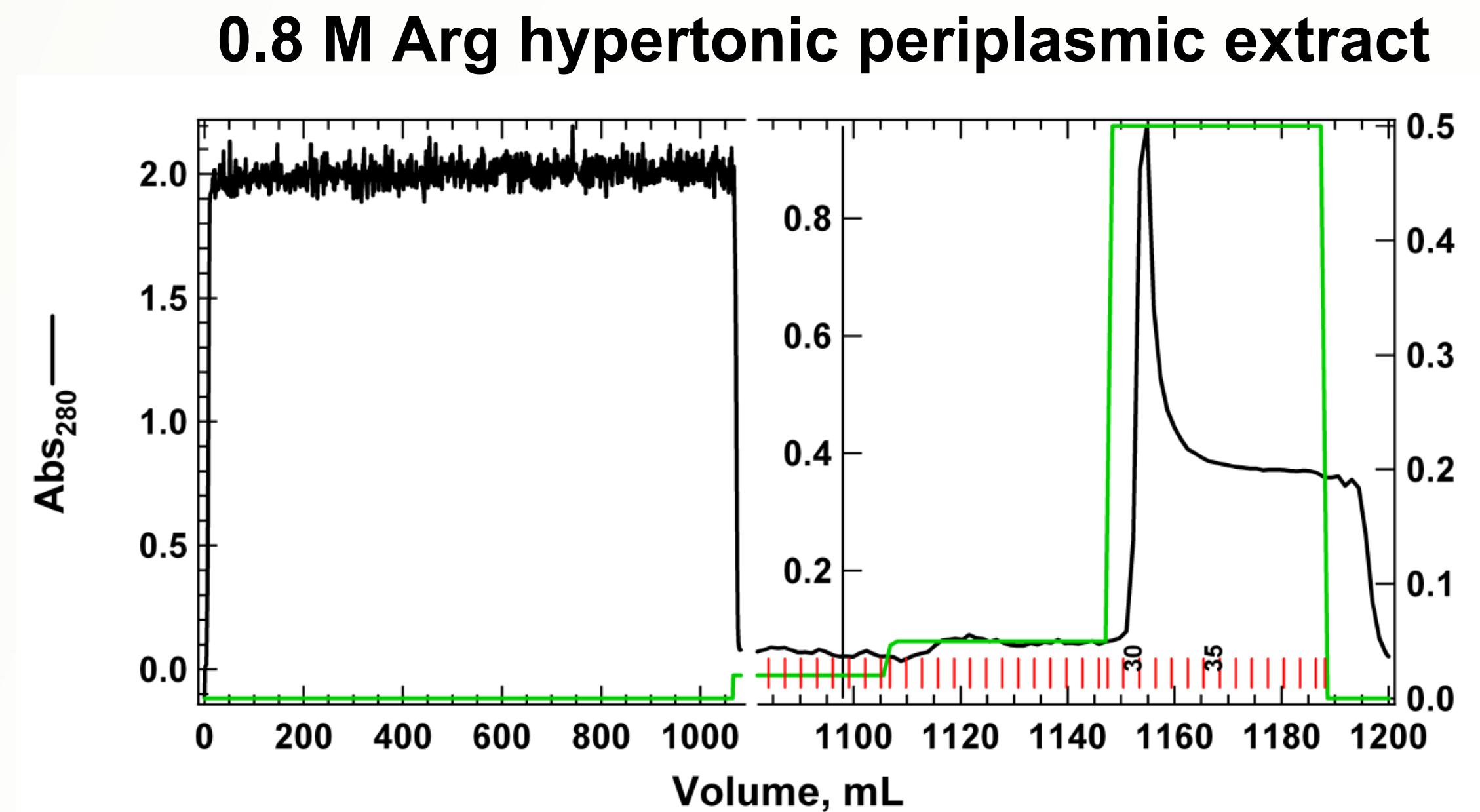




# Expression of single chain antibodies as SUMO fusions in *E. coli*



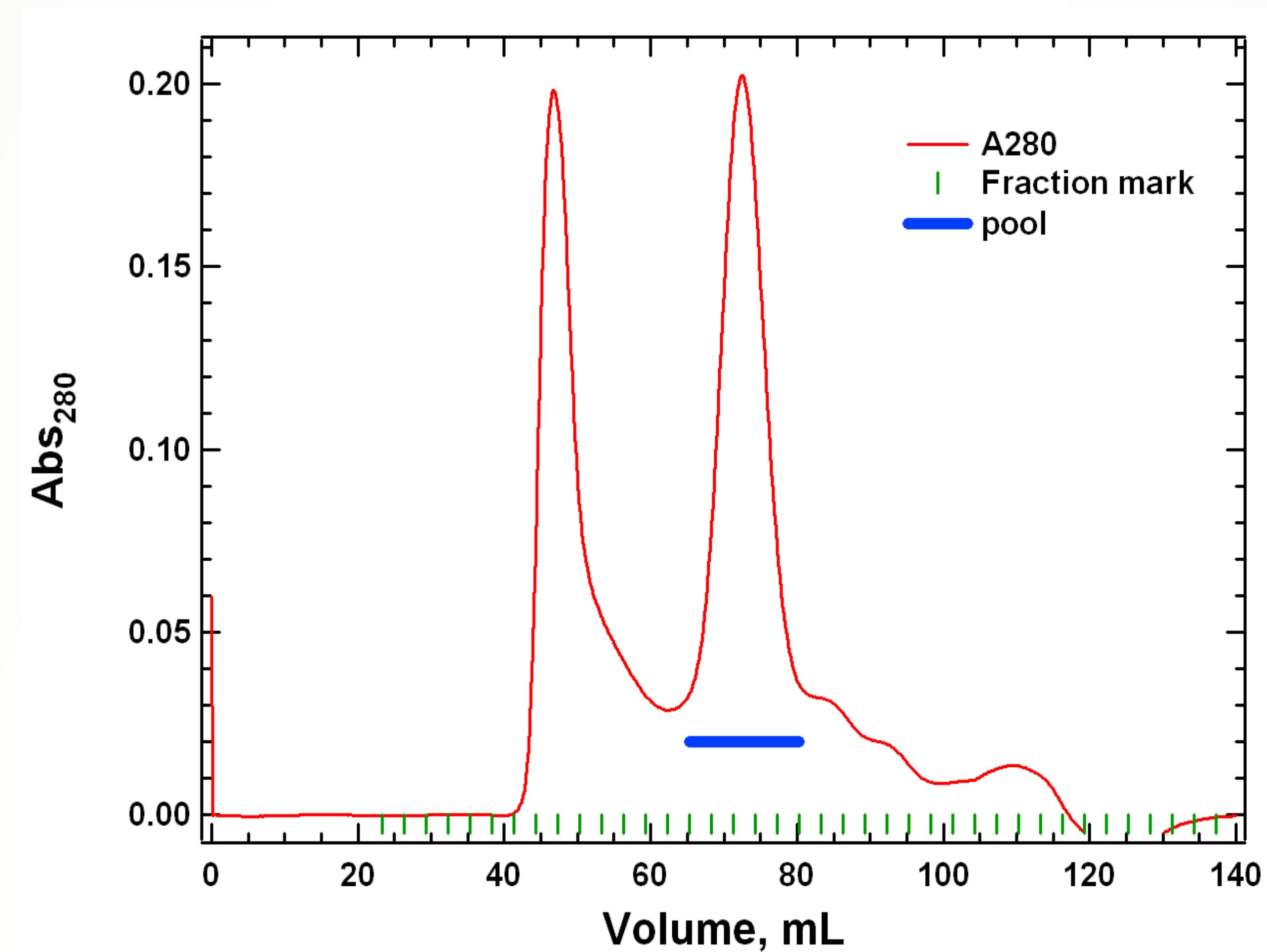
# scFv<sub>2</sub> IMAC purification



Coomassie blue stained gel.  
8  $\mu\text{L}$  of 3 mL fraction analyzed

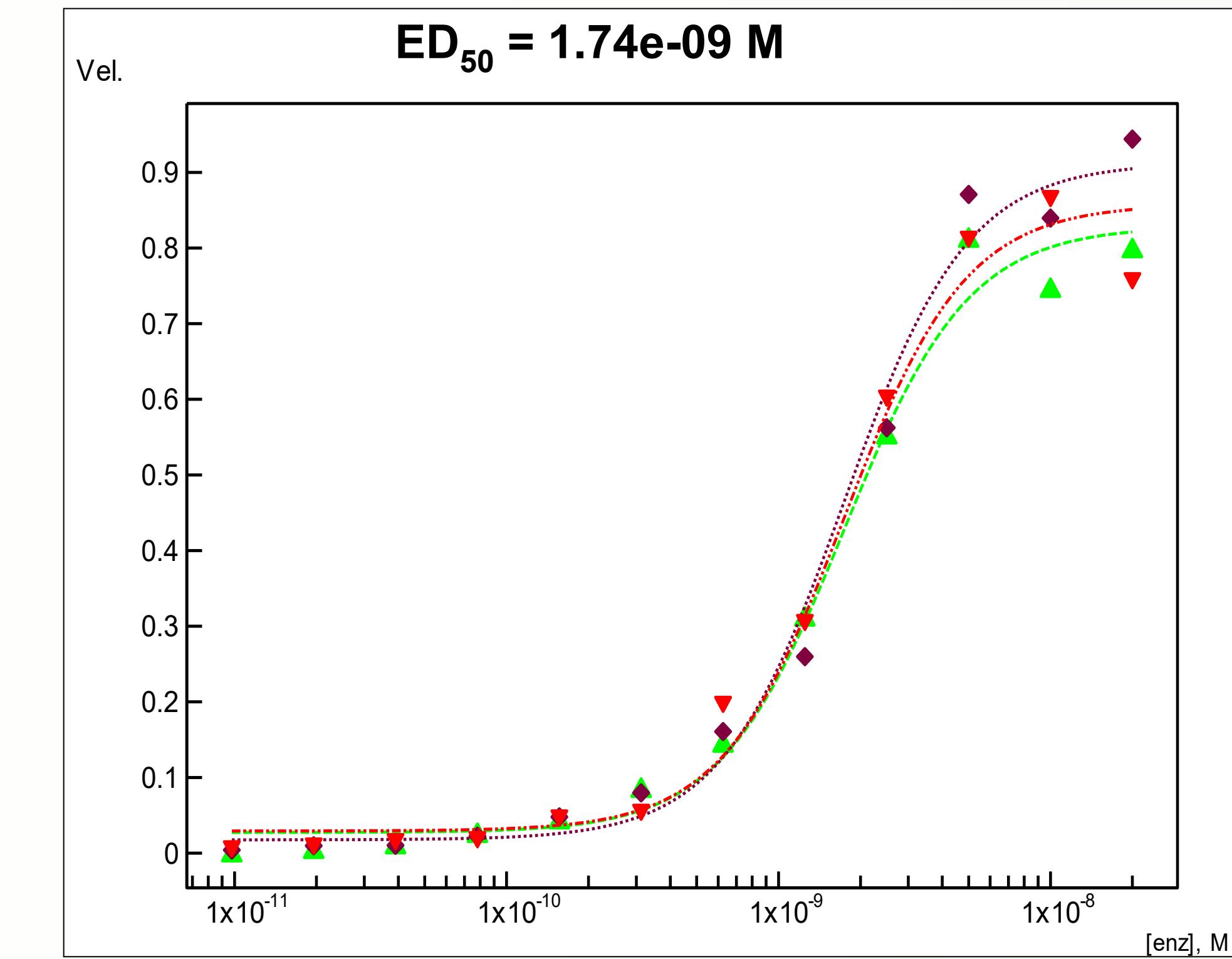
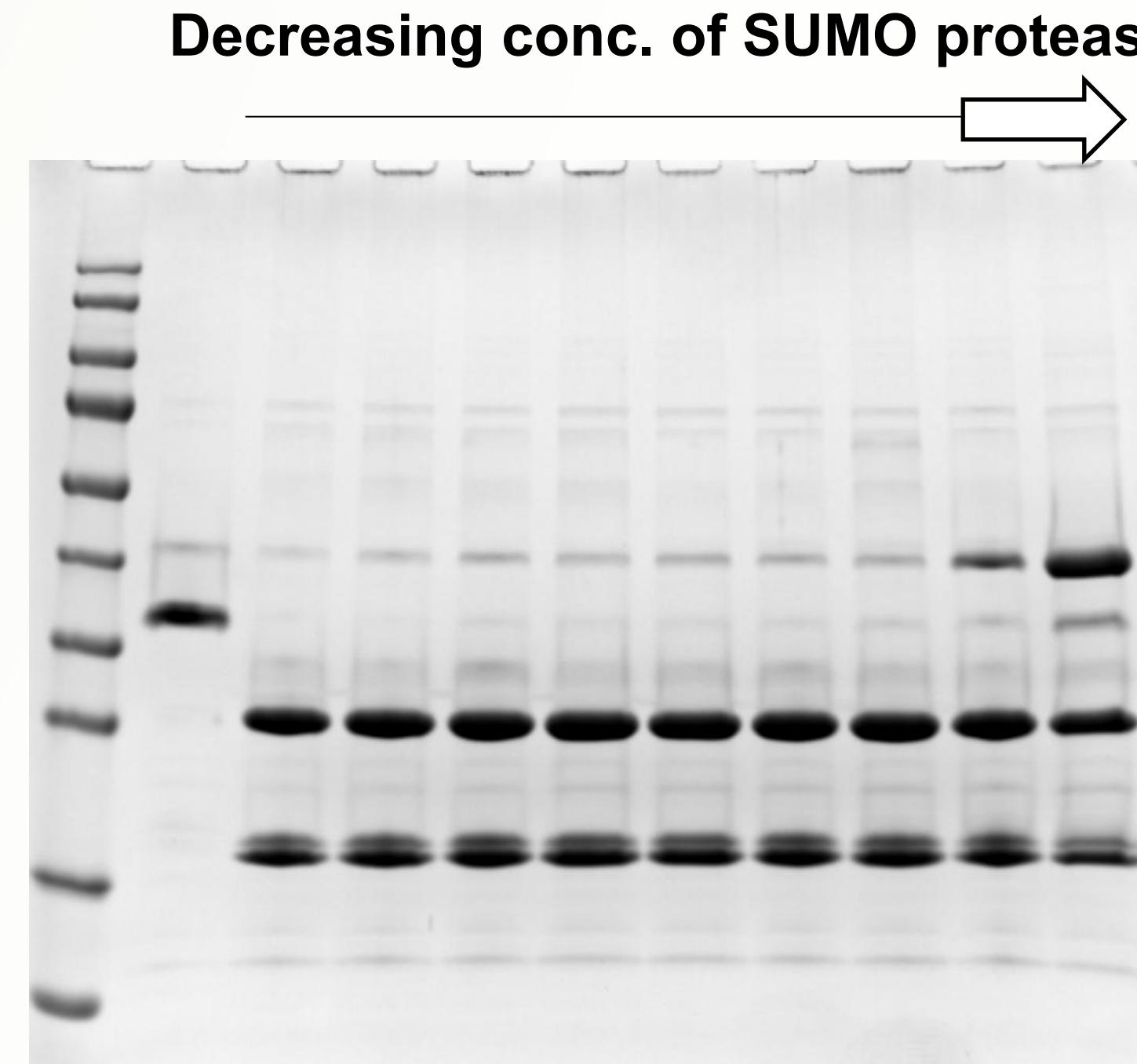


## SEC analysis of SUMO-scFv<sub>2</sub> (S200 10/30)



*SUMO-scFv<sub>2</sub> elution profile consistent with dimer formation*

# Remarkably Efficient Removal of SUMO by SUMO-protease



10 ug SUMO-GFP was incubated with 01-1.00 unit of [SUMO protease](#) for 37oC for one hour. SDS PAGE was stained with commissive blue. Data shows that LifeSensors SUMO protease is highly active. See next slide for comparison ion between different proteases

# Commonly used Tag removal enzymes, need to re-calculate the cost and compression (Shahzad in progress)



Protease	Cleavage site	Location	Residual AAs	pH range/ optimum	Chaotropic Sensitivity	Salt Sensitivity	Enz:target	\$/mg
TEV, AcTEV, ProTEV	ExxYxQ/(G/S)	N C	G/S ExxYxQ	5.5-8.5/7	2 M urea	$\leq 0.1$ M	1-3% (wt/wt)	45.00
Thrombin (flla)	LVPRG	N C	G LVPR	6-9	$\leq 0.1$ M urea	$\leq 0.15$ M	1-10% (wt/wt)	4.00
Factor Xa (fXa)	IEGR/x	N C	none IEGR	6-9	$\leq 0.1$ M urea	$\leq 0.15$ M	1-10% (wt/wt)	4.00
Enterokinase (EK)	DDDDK/x	N C	none DDDDK	7-8			0.1% (wt/wt)	45.00
<b>SUMO proteases</b>	<b>C-terminus</b>	<b>N only</b>	<b>none</b>	<b>6-9</b>	<b>0.1 M GnCl &lt; 2 M Urea</b>	<b>&lt; 0.5 M</b>	<b>0.1% (wt/wt)</b>	<b>3.15</b>
TagZyme	XaaYaaGln	N only	none	6-8	$\leq 4$ M Urea		3 enz.	46.20
Immobilized Subtilisin BPN	C-terminus of	N only	none	7.2	$\leq 4$ M Urea		1:1 (mol/mol)	26.00

# Examples of Nanobodies Expressed as SUMO

- **Fibronectin type III domain based monobodies:** Monobodies were expressed and purified using SUMO fusion system in E.coli (Gorman K et al, Curr Protoc Chem Biol. 2018; Hussain M et al, Biochemistry. 2018)
- **Fab fragments:** Cytosolic expression of functional fab fragments in Escherichia coli using a novel combination of dual SUMO expression cassette and EnBase® cultivation mode (Rezaie F et al, J Appl Microbiol. 2017)
- **Single-chain variable fragment (scFv):** High-level expression of an anti-VEGF165 scFv in E. coli achieved by SUMO fusion (Sillen M et al, Int J Mol Sci. 2020)
- **Nanobodies (Nbs):** Nbs against a GPCR, PAI and SARS-CoV-2 RBD were expressed and purified in E.coli (Koehl A et al, Nature. 2019; Sillen M et al, Int J Mol Sci. 2020; Shi Z et al, Structure. 2022; Sillen M et al, J Thromb Haemost. 2020; Sillen M et al, Int J Mol Sci. 2021)
- **VHH antibodies:** Single-Domain VHH antibodies against FMDV proteins and Chikungunya E2 protein were expressed and purified with SUMO fusion system (Li H et al, Vaccines (Basel). 2021; Li Q et al, J Nanobiotechnology. 2022; Wang D et al, BMC Vet Res. 2015)
- **Heavy-chain-only antibodies (HCabs):** HCabs against influenza HA protein were produced in E.coli fused with SUMO.

# SUMO Advantage

- **Dramatic Enhancement of Nanobodies yield as SUMO-fusion**
- **Application of the most efficient SUMO protease to generate native proteins**
- **More than 90 proteins expressed in E.coli have been approved by FDA †**
- **Application of SUMO system decreases the cost of goods**

† Full-length recombinant antibodies from E.coli --- clinical evaluation. MABS, 14, 1, e2111748 (20 pages)

# Thank You

We are your partner in Manufacturing Difficult to Express Proteins

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