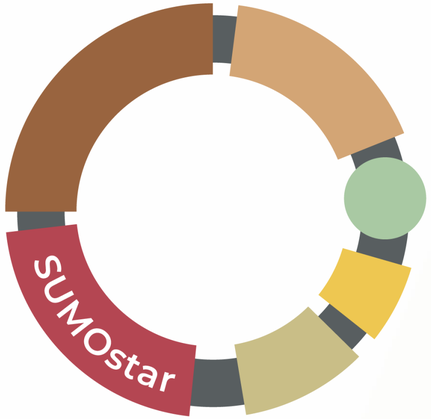
Manufacturing Single Chain Antibodies, Nanobodies with SUMO Expression Platform



LifeSensors Inc.
271 Great Valley Parkway
Malvern PA 19355

610-644-8845
bd@lifesensors.com
www.lifesensors.com

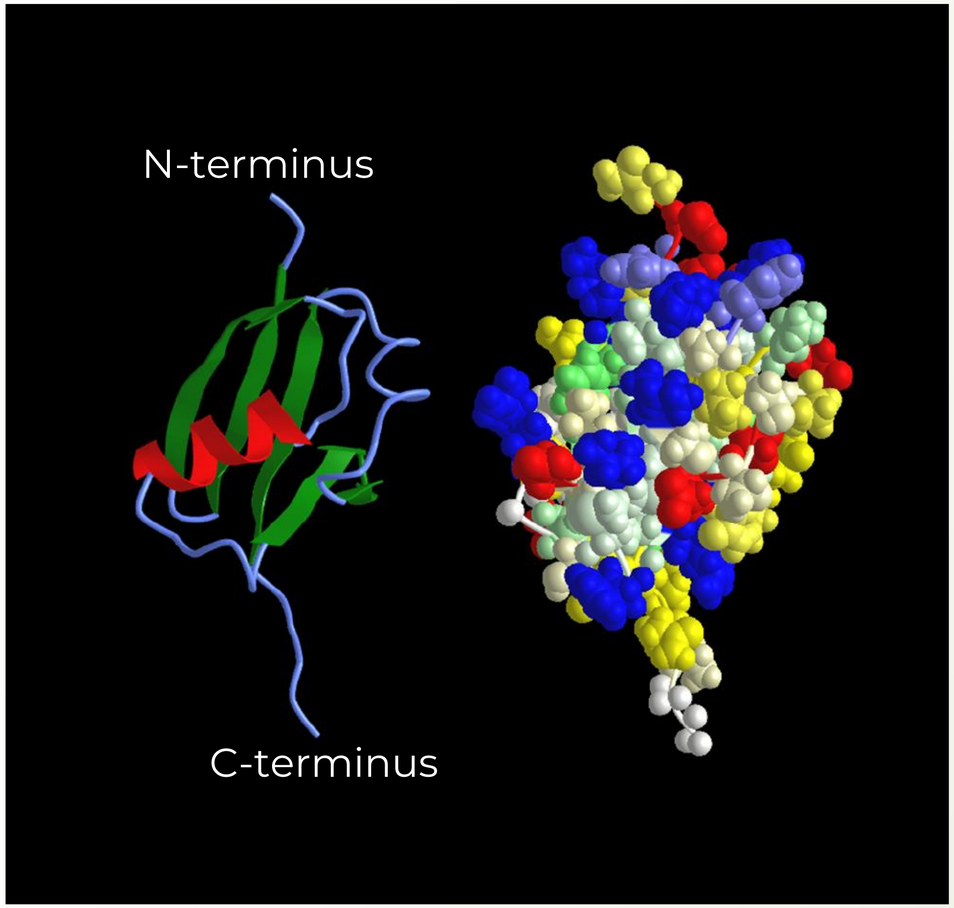
SUMO Advantage

- 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)

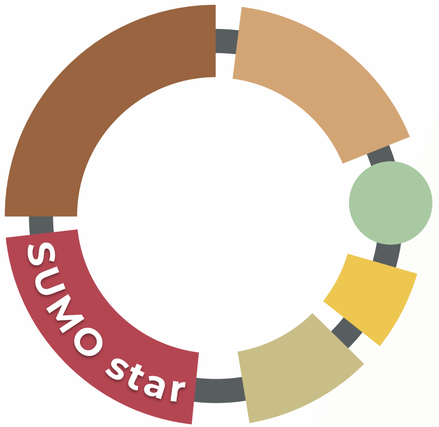
How Does SUMO Help Enhance Nanobody Expression?

- 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

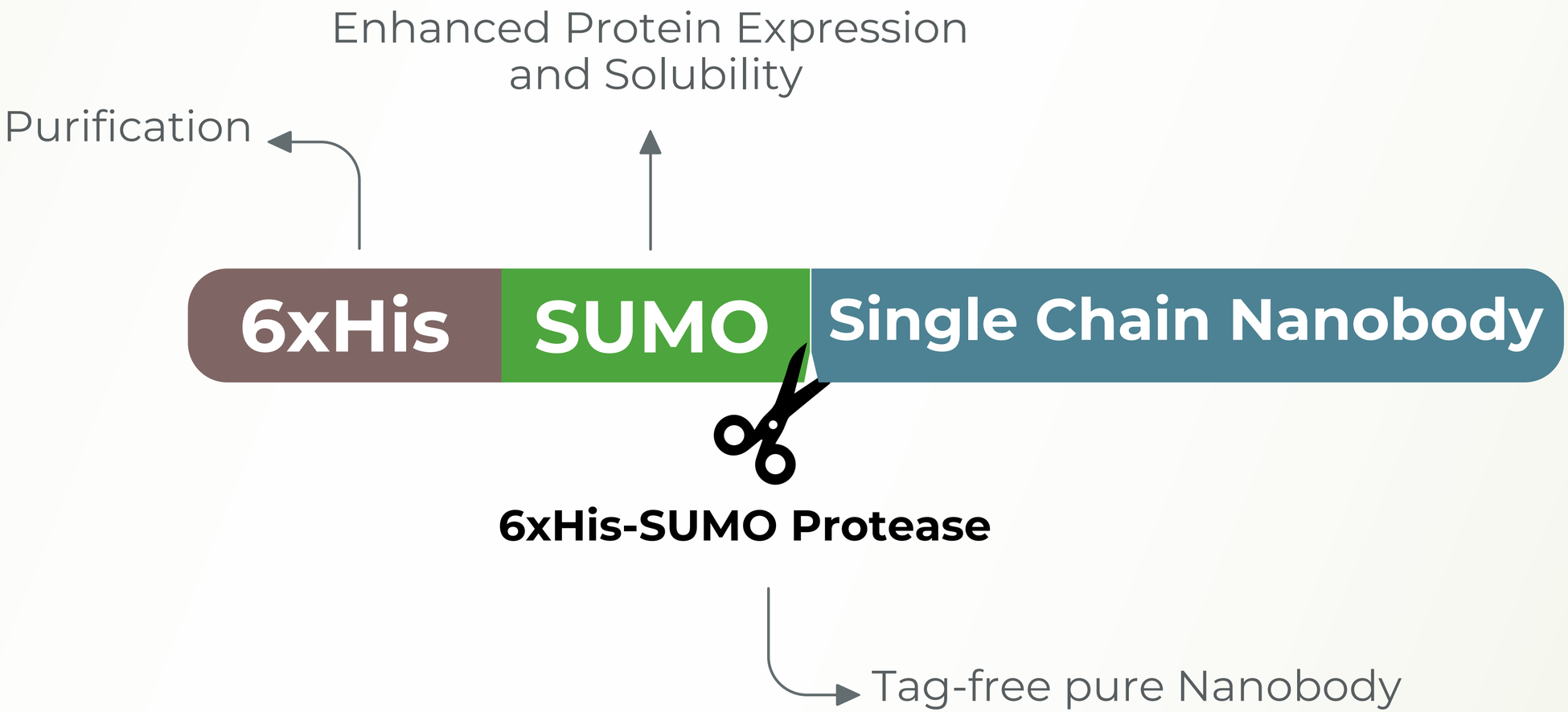


SUMO Platform for Nanobodies

PROBLEMS	SOLUTIONS
Unstable protein, low expression	SUMO chaperoning, enhanced expression
Insoluble Nanobodies	SUMO derived solubility
Miss-folded nanobodies	SUMO-driven folding
High cost of goods	High yield, saves cost

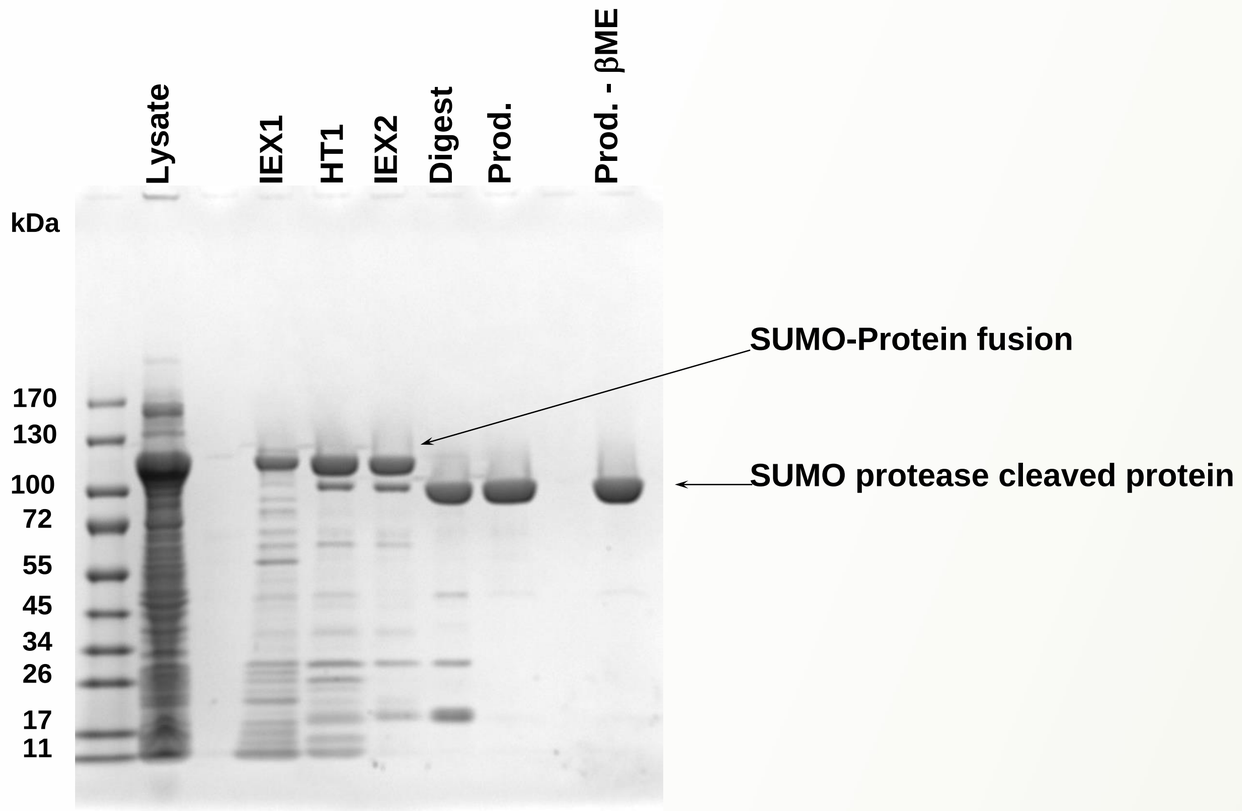


SUMO Platform For Native Nanobodies

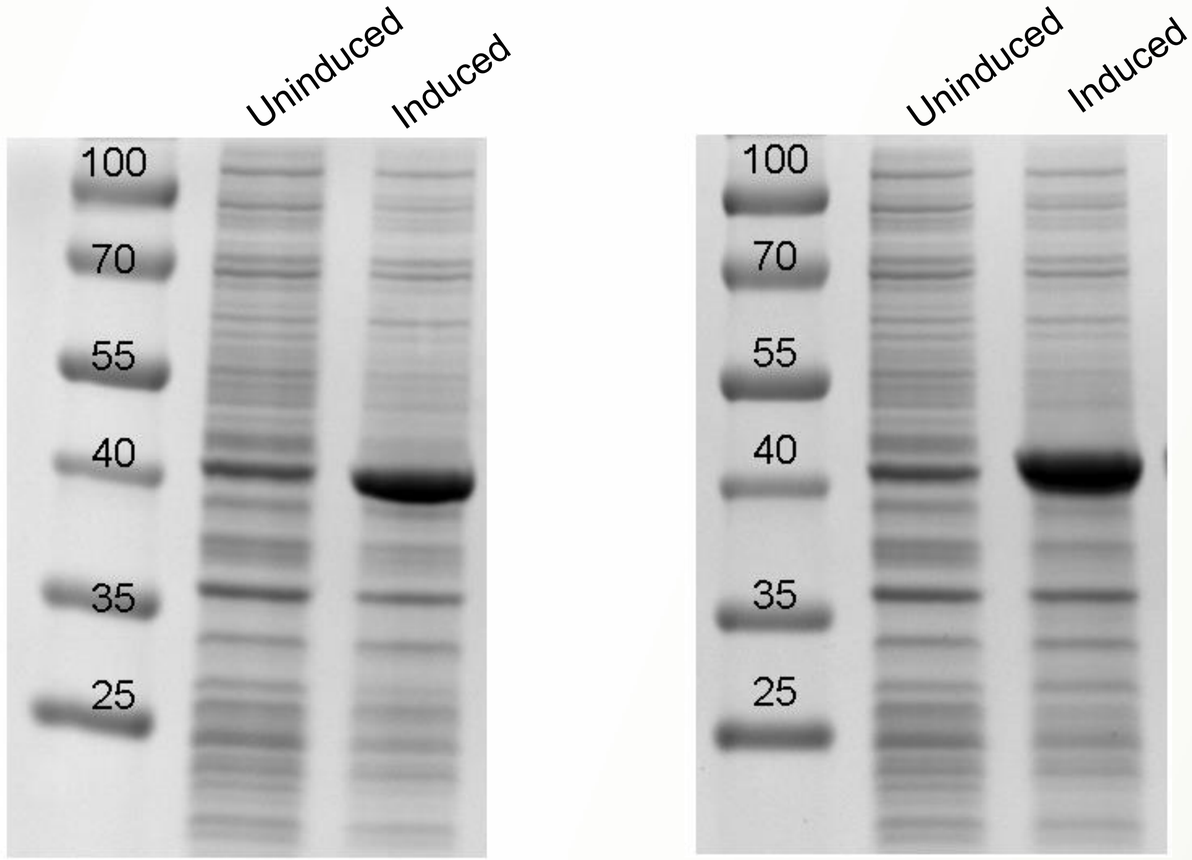


Expression of Candidate Protein in *E. coli*

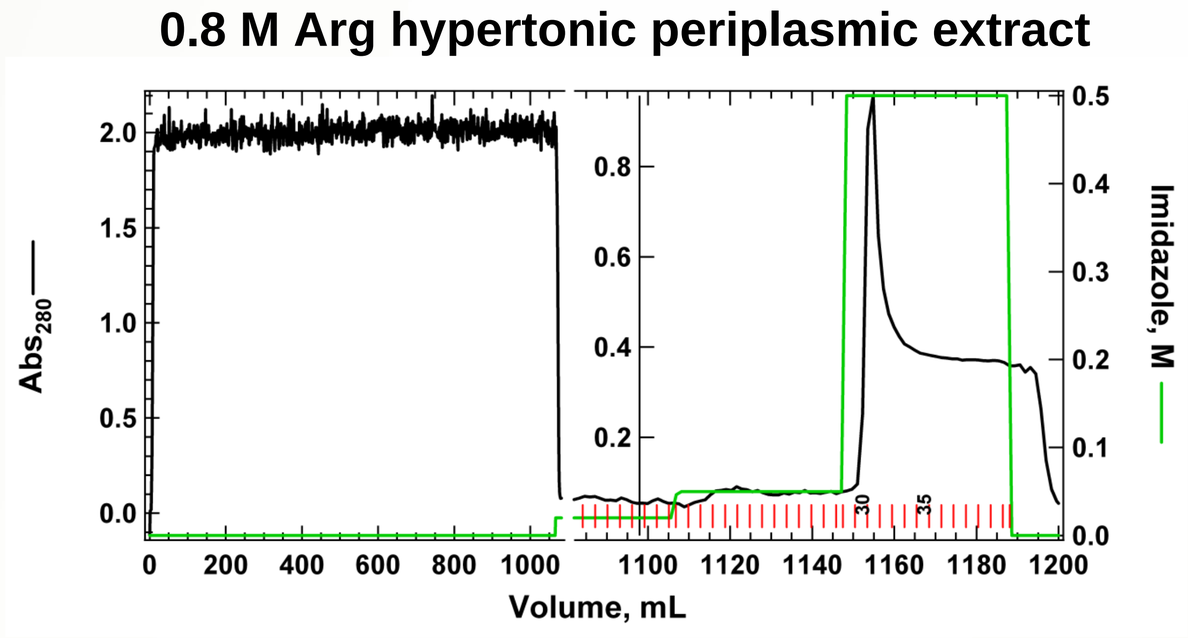
- Improved expression of Protein from 8% TCP to >20% TCP
- Purification reduced from 5 column steps to 4
- Overall yield improved from ~16% to >40%

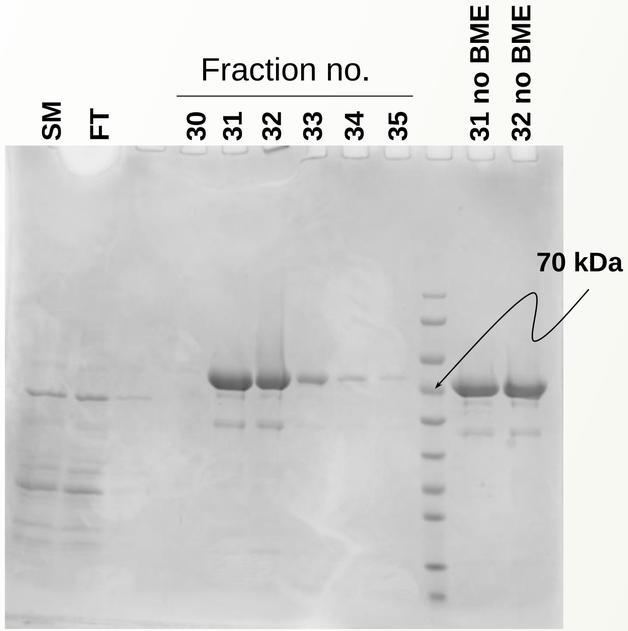


Expression of single chain antibodies as SUMO fusions in *E. coli*



$scFv_2$ IMAC purification

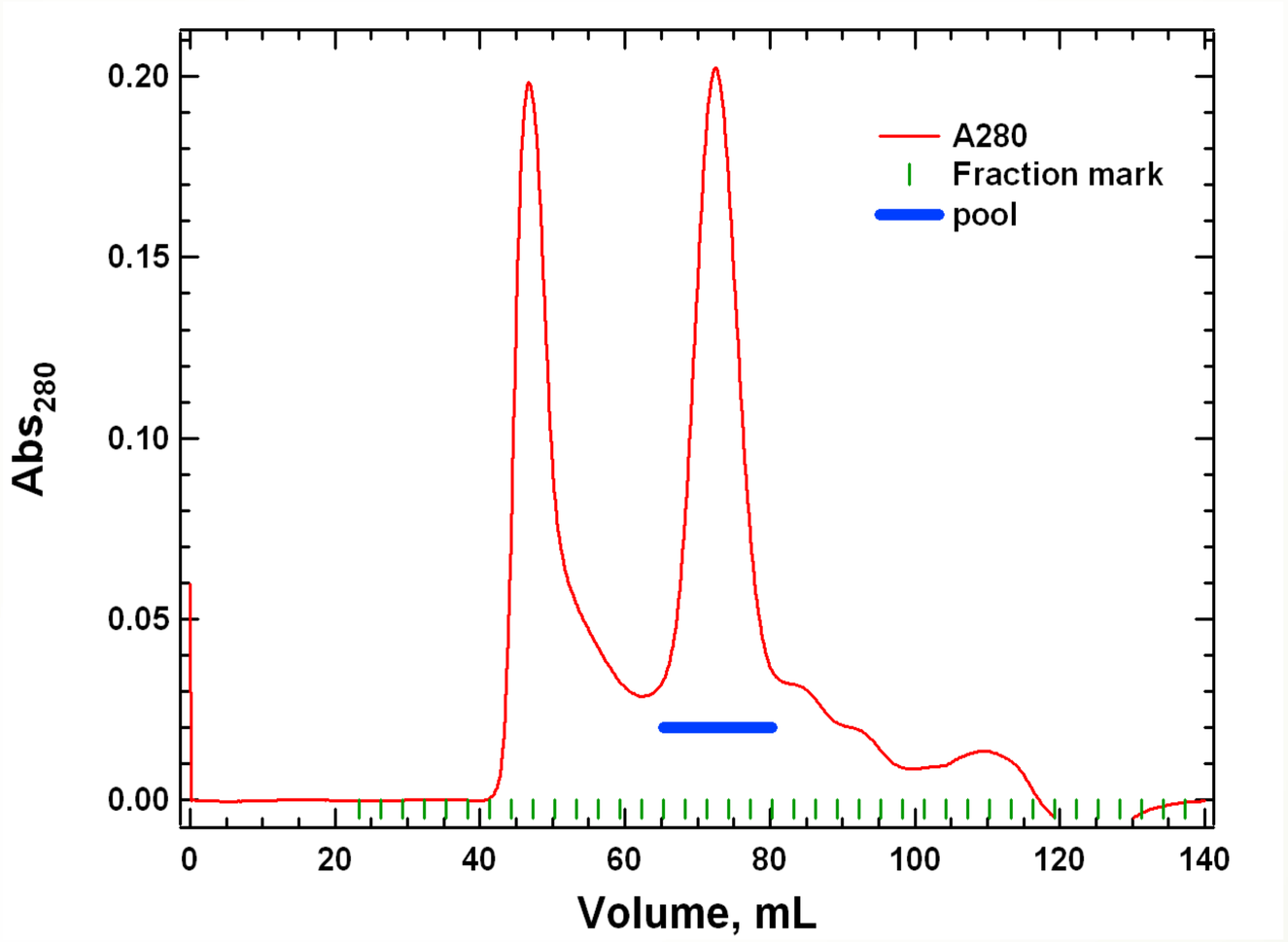




Coomassie blue stained gel.

8 μL of 3 mL fraction analyzed

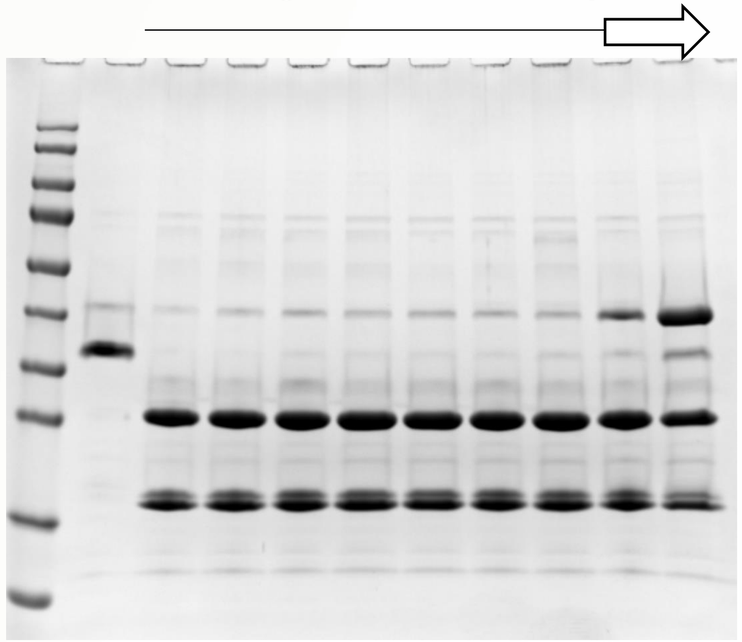
SEC analysis of SUMO-scFv$_2$ (S200 10/30)

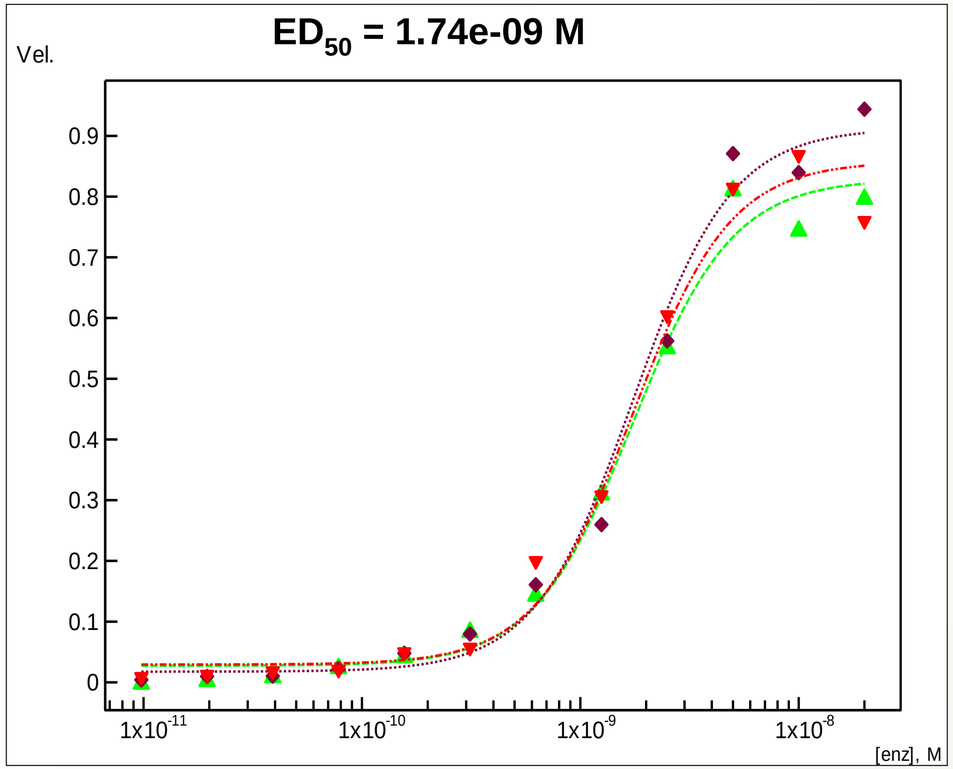


SUMO-scFv$_2$ elution profile consistent with dimer formation

Remarkably Efficient Removal of SUMO by SUMO-protease

Decreasing conc. of SUMO protease





10 ug SUMO-GFP was incubated with 01-1.00 unit of SUMO protease for 37 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

Comparison Between SUMO Protease and Commonly used Tag Removal Enzymes

Protease	Cleavage site	Location	Residual AAs	pH range/ optimum	Chaotrope Sensitivity	Salt Sensitivity	Enz: target	$/mg
TEV, AcTEV, ProTEV	ExxYxQ/(G/S)	N	G/S	5.5-8.5/7	2 M urea	≤ 0.1 M	1-3% (wt/wt)	36[1]
		C	ExxYxQ					
Thrombin (fIIa)	LVPRG	N	G	6-9	≤ 0.1 M urea	≤ 0.15 M	1-10% (wt/wt)	3.5[2**]
		C	LVPR					
Factor Xa (fXa)	IEGR/x	N	none	6-9	≤ 0.1 M urea	≤ 0.15 M	1-10% (wt/wt)	26.9[3*]
		C	IEGR					
Enterokinase (EK)	DDDDK/x	N	none	7-8	N/A	N/A	0.1% (wt/wt)	8.2[4**]
		C	DDDDK					
SUMO proteases	**No sequence preference**	**N only**	**Encode desired seq**	**6-9**	**0.1 M GnCl** **< 2 M Urea**	**< 0.5 M**	**0.1% (wt/wt)**	**10**[5]

1. https://www.fishersci.com/shop/products/promega-protev-plus-protease-2/p-4389612
2. https://www.sigmaaldrich.com/US/en/product/sigma/ge27084601
3. https://www.neb.com/products/p8010-factor-xa-protease#Product%20Information_Properties%20&%20Usage
4. https://www.neb.com/products/p8070-enterokinase-light-chain#Product%20Information
5. https://lifesensors.com/product/sp4010-sumo-protease-1/

*Assumes a **6 hour** reaction as opposed to a 1 hour reaction.

Assumes a **16 hour reaction as opposed to a 1 hour reaction

Yields are based upon shake flask growth and are unoptimized.

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

Contact Us!

Research & Product Inquiries	**R&D**	**info@lifesensors.com**	**610-644-8845 (ext 339)**
Custom Service & Assays	**BD**	**bd@lifesensors.com**	**610-644-8845 (ext 310)**