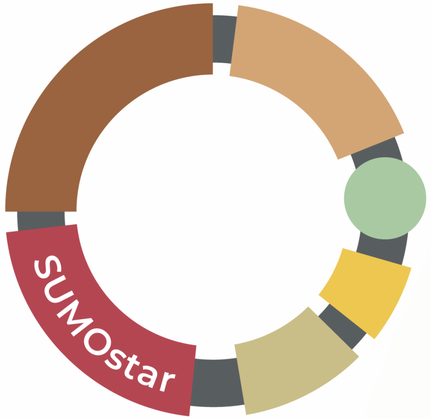
Therapeutic Proteins Manufacturing with SUMO Expression Platform



LifeSensors Inc.
271 Great Valley Parkway
Malvern PA 19355

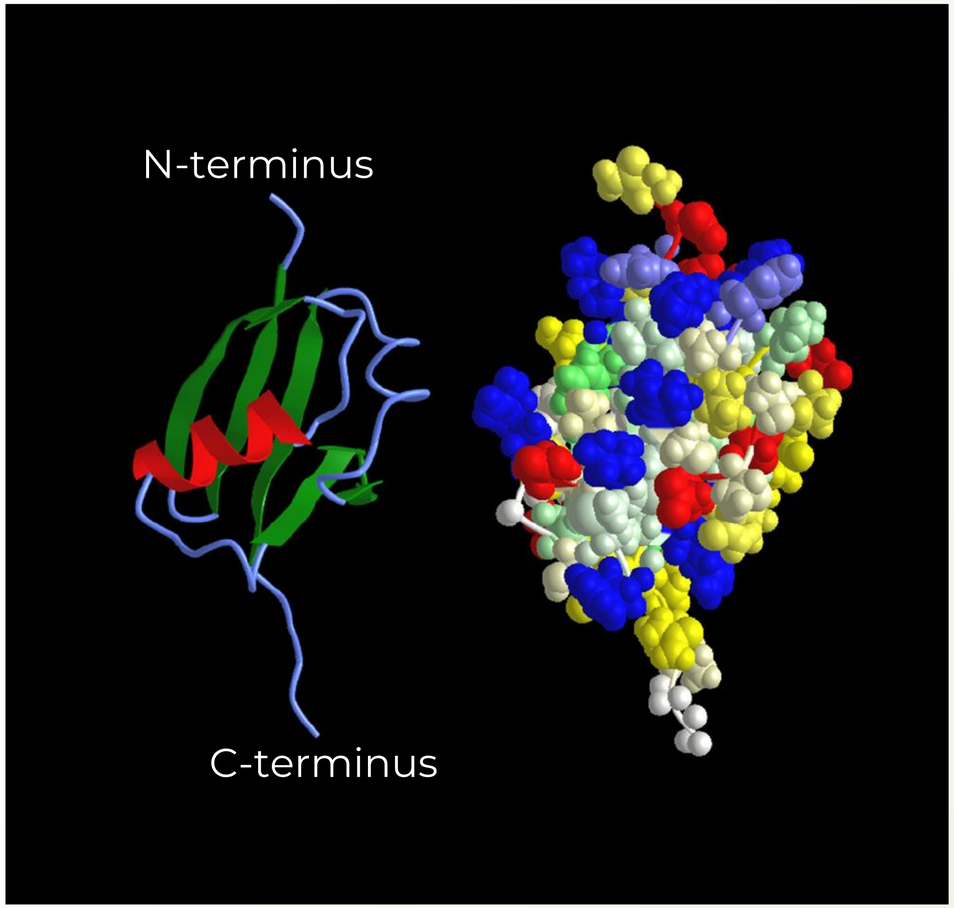
610-644-8845
bd@lifesensors.com
www.lifesensors.com

SUMO Advantage

- Efficient translation of proteins is key to the success of therapeutics, gene and cell therapy and mRNA therapy
- Poor expression of engineered genes leads to failure in the clinic
- SUMO system dramatically enhances expression, and provides unique features not possible with traditional systems
- More than 150 references of therapeutic proteins expressed as SUMO-fusion in E.coli and mammalian cells

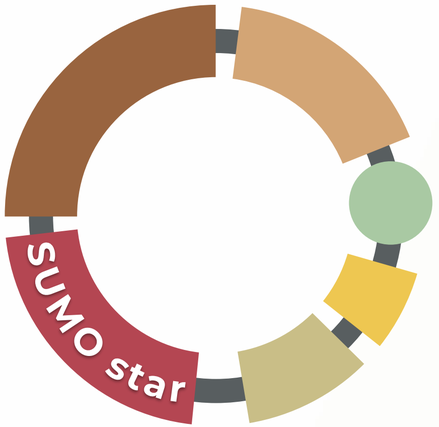
What is SUMO?

- **SUMO, or Small Ubiquitin-like modifier, is a member of the ubiquitin family**
- **SUMO is *not involved* in targeting proteins for degradation**
- **Flexible N-terminal region followed by a compact ball-shaped ubiquitin-like fold**
- **Nature designed SUMO to act as a chaperone for proteins**
- **SUMO improves solubility due to hydrophilic shell and hydrophobic core**

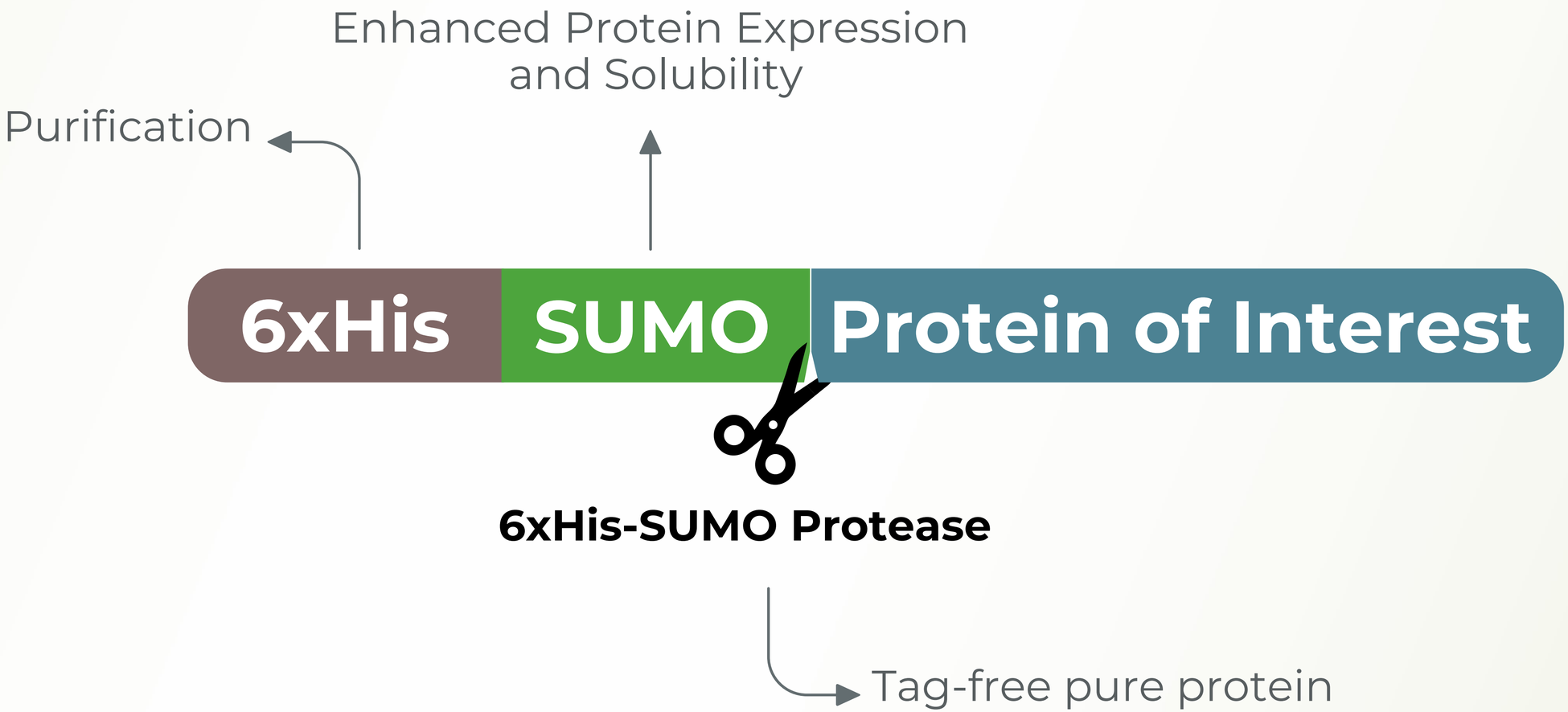


Benefits of the SUMO Platform

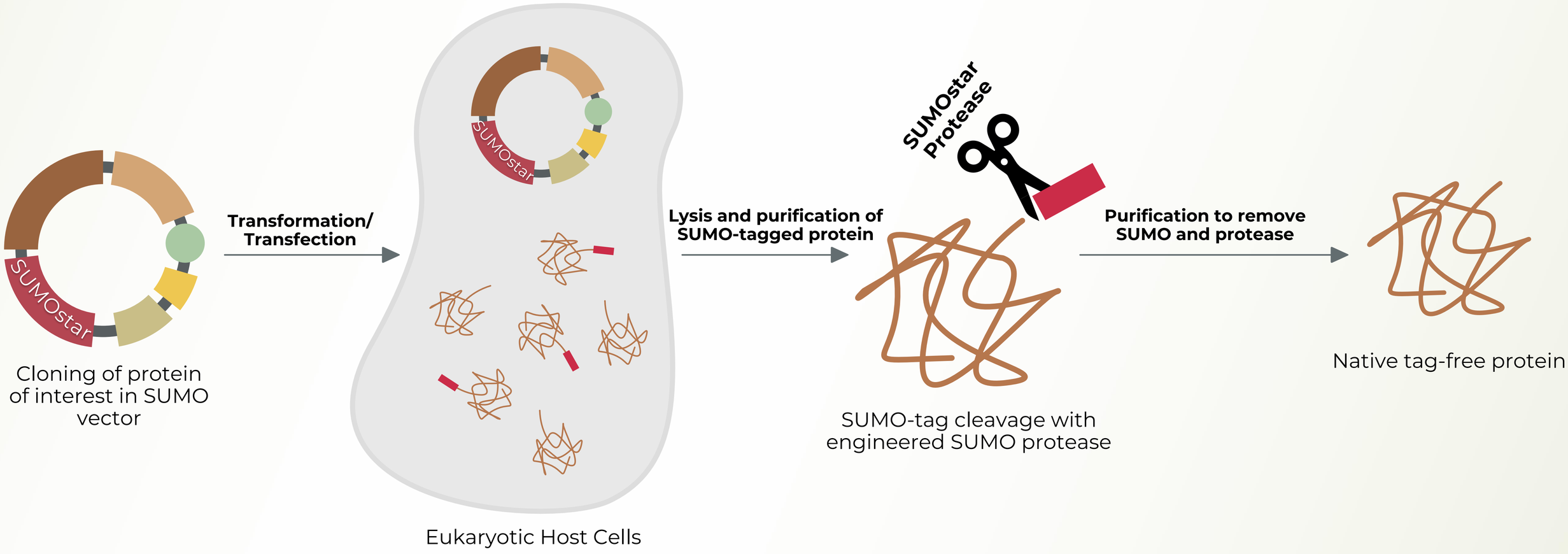
PROBLEMS	SOLUTIONS
Unstable protein, low expression	SUMO chaperoning, enhanced expression
Specific N-terminus required	SUMO-specific processing
Insoluble protein	SUMO-driven folding
High cost of goods	High yield, saves cost



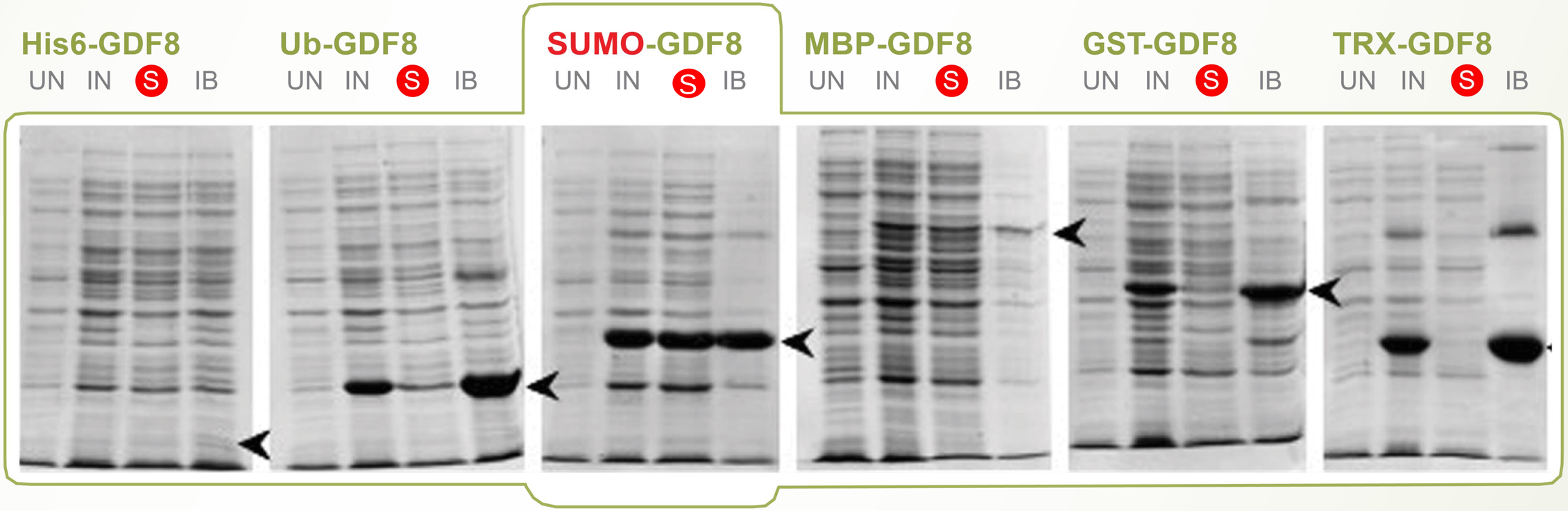
SUMO Fusion Tag Platform For E.coli



Protein Expression and Purification Process Using SUMO-tag



SUMOpro® fusion enhances expression and solubility in *E. coli*



Conditions: UN – Uninduced, IN – Induced, S – Soluble Fraction, IB – Inclusion Bodies

Marblestone et. al, Protein Sci. 2006 Jan;15(1):182-9

Therapeutic Cytokines/chemokines/GFs in the clinic

Therapeutic Protein/Polypeptide	Production Method	Company	Indication
G-CSF (Filgrastim/Neupogen, Sargramostim (Leukine)	Recombinant (E.coli, Yeast)	Amgen, Sanofi	low neutrophil count
Erythropoietin (Epoetin alfa/Epogen, Procrit)	Recombinant (E.coli, yeast)	Pfizer, Amgen	Chronic Renal Failure
Interferon alfa-2b (Intron A)	Recombinant (CHO), HEK293	Biogen (Avonex), EMD Serono/Merck (Rebif), Sigma	Multiple Sclerosis
RANTES (regulated on activation, normal T cell expressed and secreted)	Recombinant (E.coli)	StemCell Tech	**In development**
IP-10 (interferon-inducible protein 10)	Recombinant (E.coli)	Sigma	**In development** (potent inhibitor of angiogenesis and displays thymus-dependent anti-tumor effects)
SDF-1 (stromal cell-derived factor 1)	Recombinant (E.coli)	Acro Biosystems	**In development**
Eotaxin	Recombinant (HEK293)	Acro Biosystems	**In development**
MCP-1 (monocyte chemoattractant protein-1)	Recombinant (E.coli)	R&D Systems	**In development**
Platelet-derived growth factor (PDGF)	Recombinant (E.coli, yeast)	BioLegend, Regeneron (becaplermin)	diabetic neuropathic ulcers
Insulin-like growth factor 1 (IGF-1)	Recombinant (E.coli, yeast)	Ipsen Biopharmaceuticals (Mecasermin/Increlex)	growth failure and short stature in children with severe primary IGF-1 deficiency
Epidermal growth factor (EGF)	Recombinant (E.coli, HEK293)	BPS, Abcam	Skin care, In development for clinical use

Examples of SUMO-Enhanced Production of Therapeutic Proteins

	Peptide Name	Host	Yield (mg/L)
Cytokines	Human interleukin-7 (hIL-7) [1]	E.coli	135-140 mg/L
	TNF-alpha[2]	E.coli	97 mg/L
	IL-36[3]	E.coli	10 mg/L
	IL-6[4]	Nicotiana benthamiana leaves	18.5 mg/kg fresh weight
Growth Factors	Human Epidermal Growth Factor (hEGF) [5,6]	E.coli	281 mg/L
	Heparin-binding epidermal growth factor (HB-EGF) [7,8]	E.coli	104 mg/L
	FGF20[9]	E.coli	-
	FGF21[10]	E.coli	3.9 mg/L

1. Devi N et al, Appl Microbiol Biotechnol, 2016
2. Hoffman A et al, Protein Expr Purif., 2010
3. Clancy DM et al, FEBS Open Bio. 2016
4. Islam MR et al, Plant Biotechnol J. 2019
5. Su Z et al, Protein Pept Lett. 2006;
6. Ma Y et al, Appl Microbiol Biotechnol, 2016
7. Lu, W. et al, Mol Biotechnol, 2010;
8. Ferreira AS et al, Sci Rep. 2022
9. Niu J et al Drug Deliv. 2018
10. Xu P et al, PLoS One. 2016

Superior SUMO fusion enhanced the production of hIL-7 in E.coli

Compression Between. SUMO, Trx and NUS Fusion

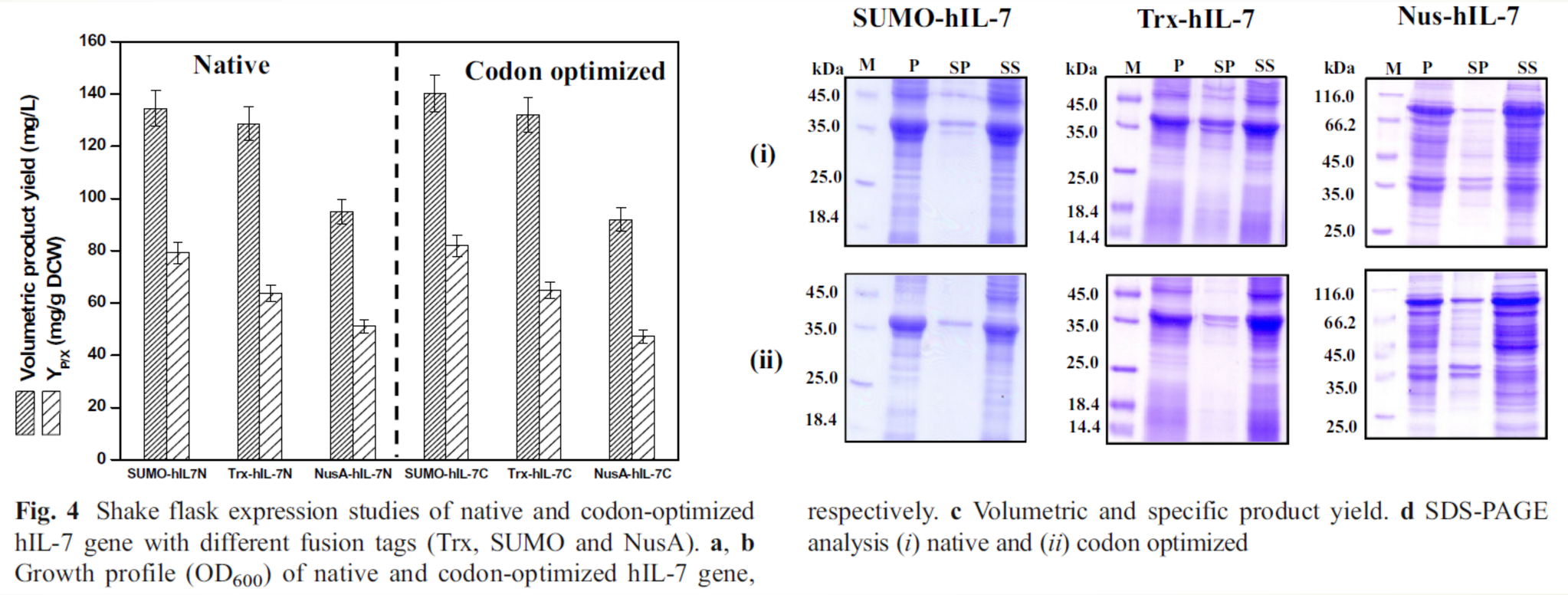
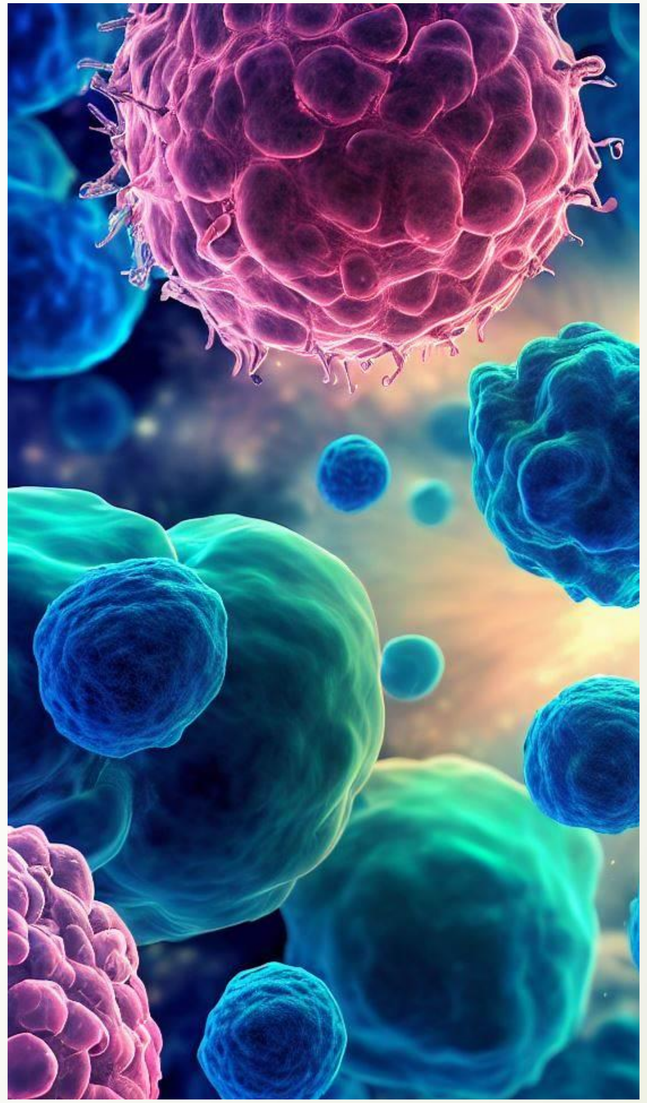


Fig. 4 Shake flask expression studies of native and codon-optimized hIL-7 gene with different fusion tags (Trx, SUMO and NusA). **a**, **b** Growth profile (OD_{600}) of native and codon-optimized hIL-7 gene, respectively. **c** Volumetric and specific product yield. **d** SDS-PAGE analysis (*i*) native and (*ii*) codon optimized

Devi, N., Adivitiya & Khasa, Y.P. *Appl Microbiol Biotechnol* **100**, 9979–9994 (2016).

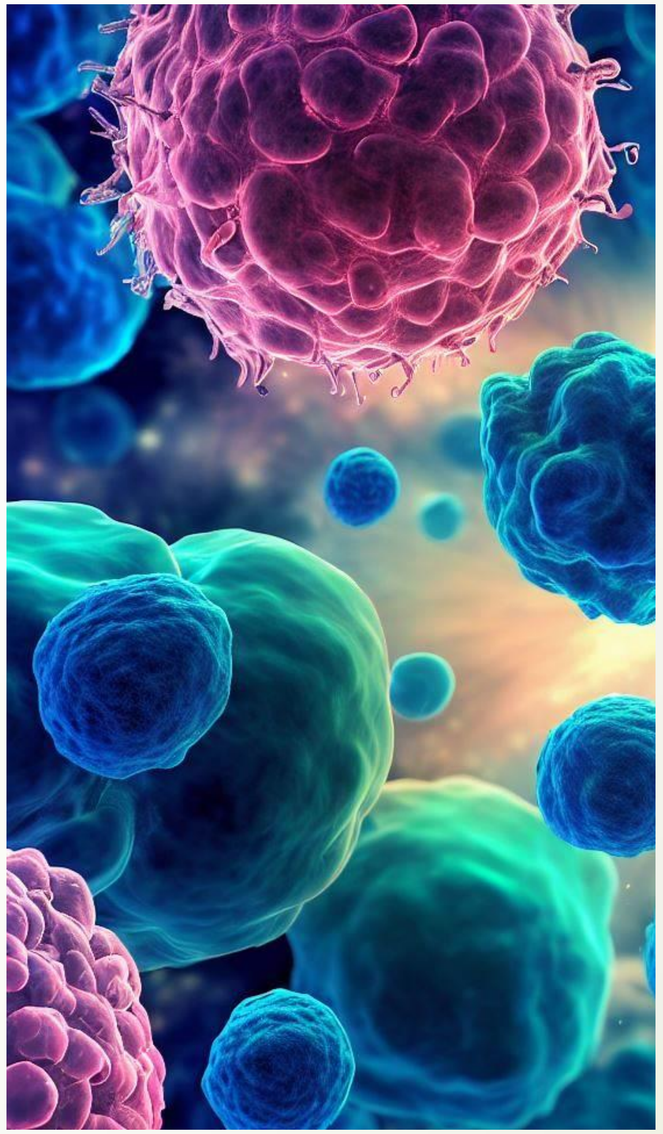
https://doi.org/10.1007/s00253-016-7683-5

Challenges in expression of Cytokines, Chemokines and Growth Factors, Solutions offered by SUMO System



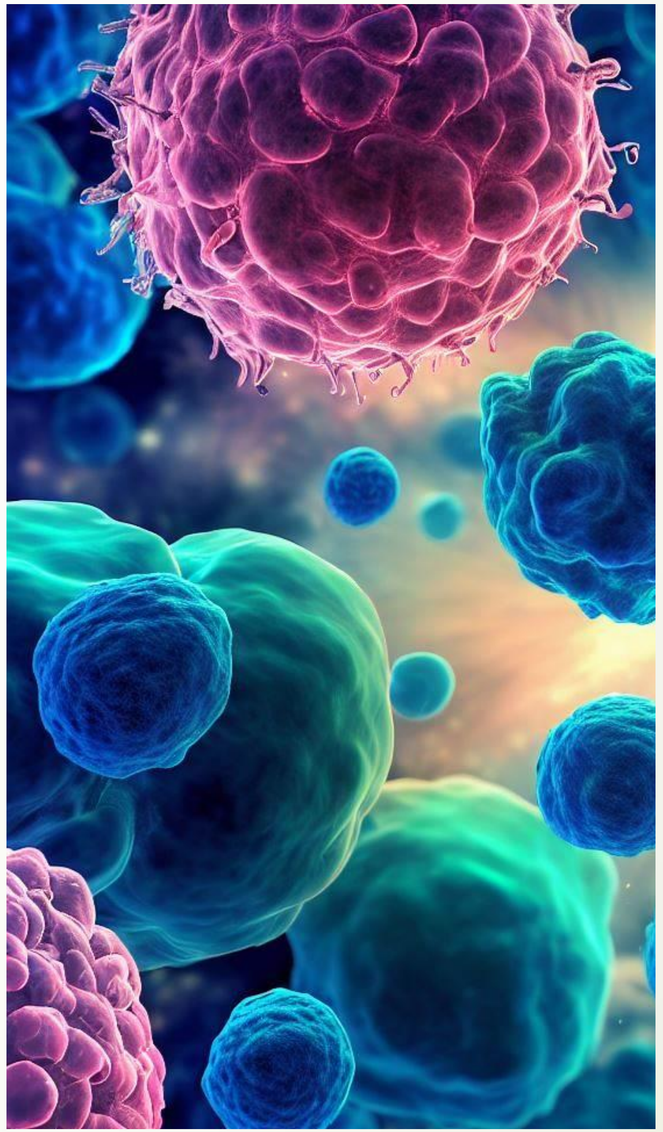
Cytokines

- Families of cytokines expressed as SUMO-fusion, just bullets, no more than five bullets per slide.
- Need 1-2 real data slides, that will show impact of SUMO, no western blots or research centric data, just stained gel or manufacturing yield per liter
- Important references, 2-3, small font, at the end of the page, such as reviews can be listed



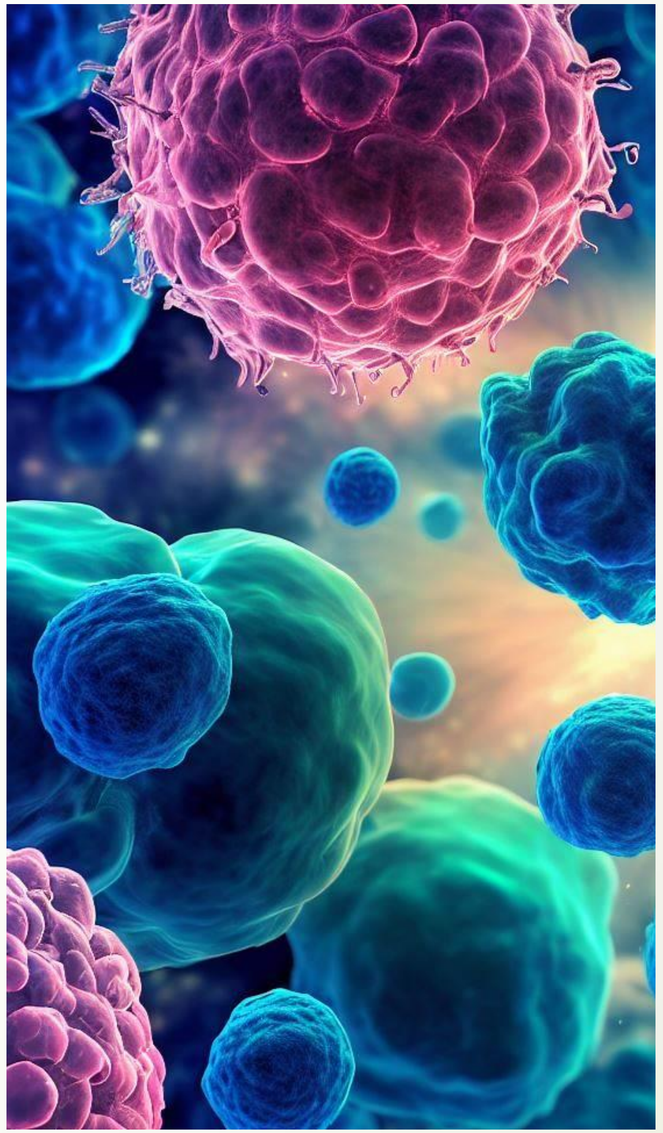
Chemokines

- One line of intro re Chemokines
- Families of Chemokines expressed as SUMO-fusion, just bullets
- Need 1-2 real data slides, that will show impact of SUMO, no western blots or research centric data, just manufacturing
- Important references, 2-3, small font, at the end of the page, such as reviews can be listed



Growth factors

- One line of intro re growth factor clinical value
- Families of growth factors expressed as SUMO-fusion, just bullets
- Need 1-2 real data slides, that will show impact of SUMO, no western blots or research centric data, just manufacturing
- Important references, 2-3, small font, at the end of the page, such as reviews can be listed



Advantages of SUMO Mediated Enhanced Expression in Mammalian Cells

- Chemokines, cytokines and growth factors and other proteins are expressed as precursor proteins.
- SUMO facilitates expression of mature protein or peptide with desired N-terminus that is extremely important for biological activity
- Manufacturing proteins with stabilizing N-termini restores biological activity and improves yield
- Post-translational modifications are preserved with enhanced yield and biological activity
- Native SUMO is cleaved in mammalian cells, LifeSensors has developed an engineered SUMO that preserves SUMO chaperoning properties to enhanced expression while keeping the SUMO tag intact
- The engineered SUMO for mammalian cell is called SUMOstar. SUMOstar fusion can only be cleaved with SUMOstar protease

SUMOstar, Preserving SUMO Enhanced Production in Eukaryotic Cells. Generating Post-translationally Modified Proteins

Proteins that require post-translational modification, need to be produced in eukaryotic cells

PROBLEM
Eukaryotic cells have endogenous SUMO proteases

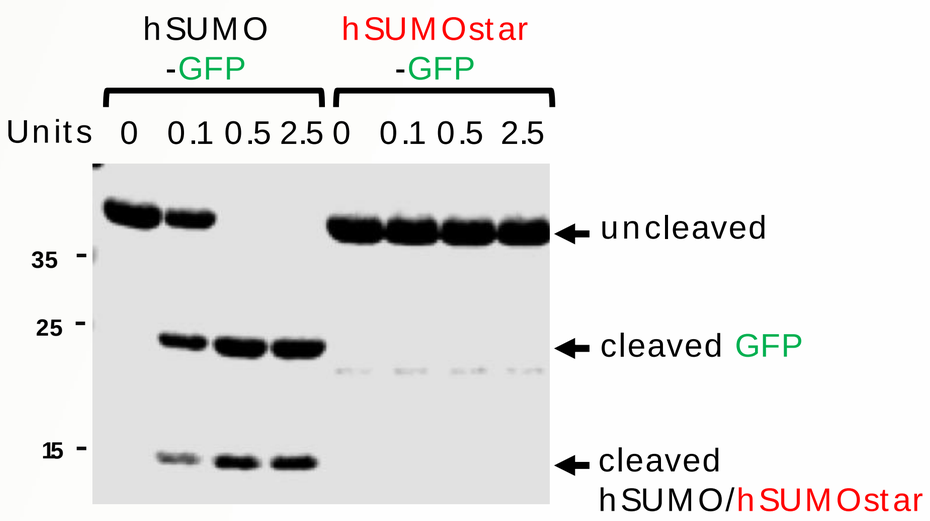
SOLUTION
Engineered SUMOstar tag & SUMOstar protease

Mammalian cells contain de-SUMOylases that cleave native SUMO-fusions

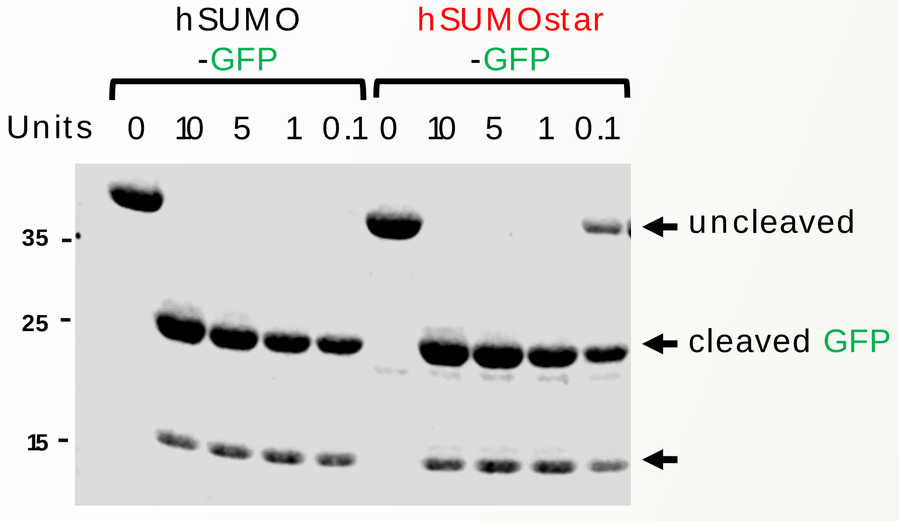
Pichia
S.cerveisea
Insect cells
Mammalian cells

hSUMOStar Protease Efficiently Cleaves the hSUMOStar Tag

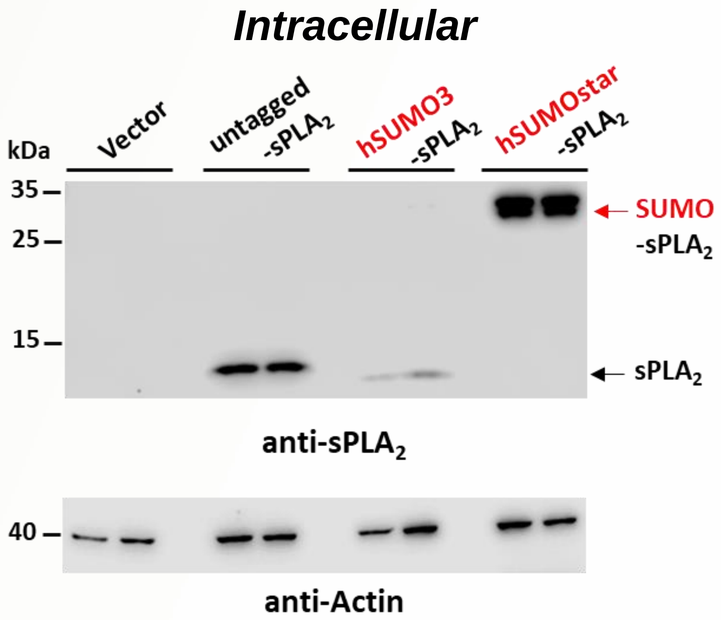
hSUMO protease

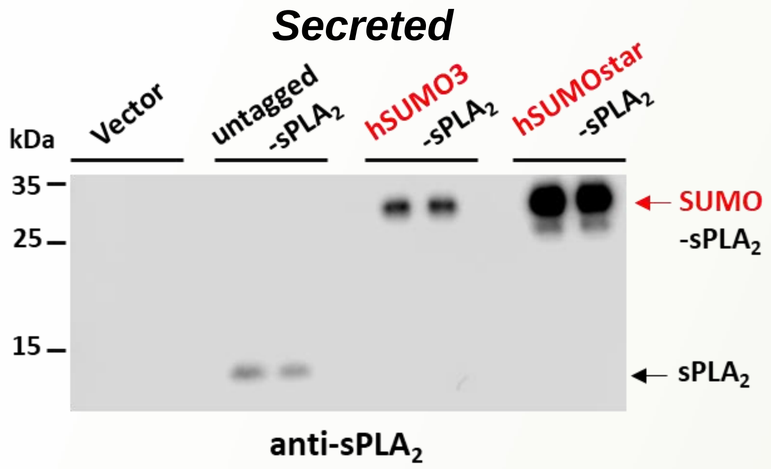


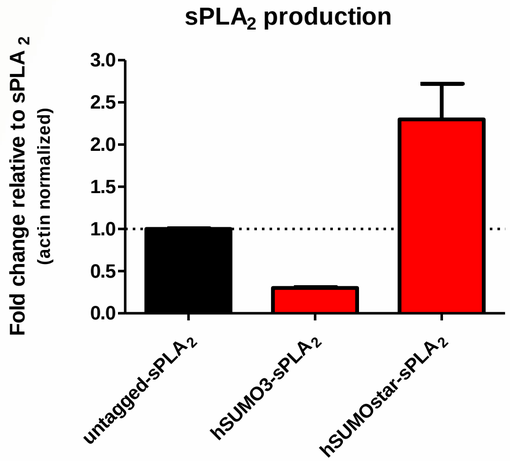
hSUMOstar protease

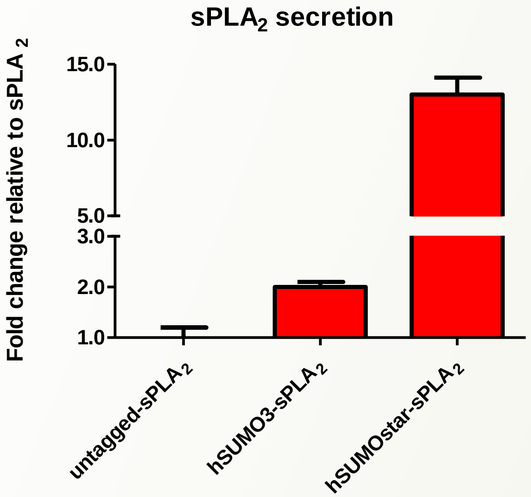


Human SUMOstar Dramatically Enhances $sPLA_2$ Production & Secretion in HEK293 cells









Advantages of SUMO Mediated Enhanced Expression in Mammalian Cells, CAR-T and Gene Therapies

- Chimeric Antigen Receptors (CAR) are engineered genes that do not express well.
- Fusion with SUMO dramatically enhances their expression level and therapeutic function
- CAR system can be incorporated with human SUMO or hSUMOstar

SUMOstar for Eukaryotic Expression

- Human SUMO and human SUMOstar dramatically enhances expression, and provides unique features not possible with traditional systems
- More than 150 publications describe therapeutic proteins expressed as SUMO or SUMOstar in E.coli and mammalian cells

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)**