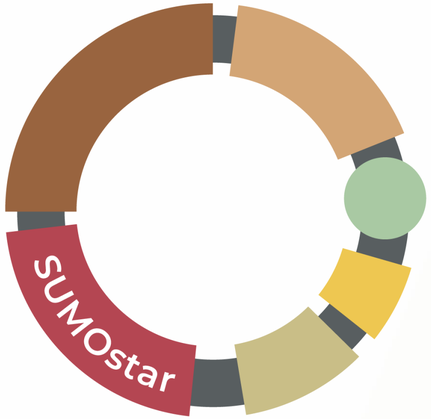
Peptide Manufacturing: Chemical Synthesis vs Recombinant Expression with SUMO-Fusions



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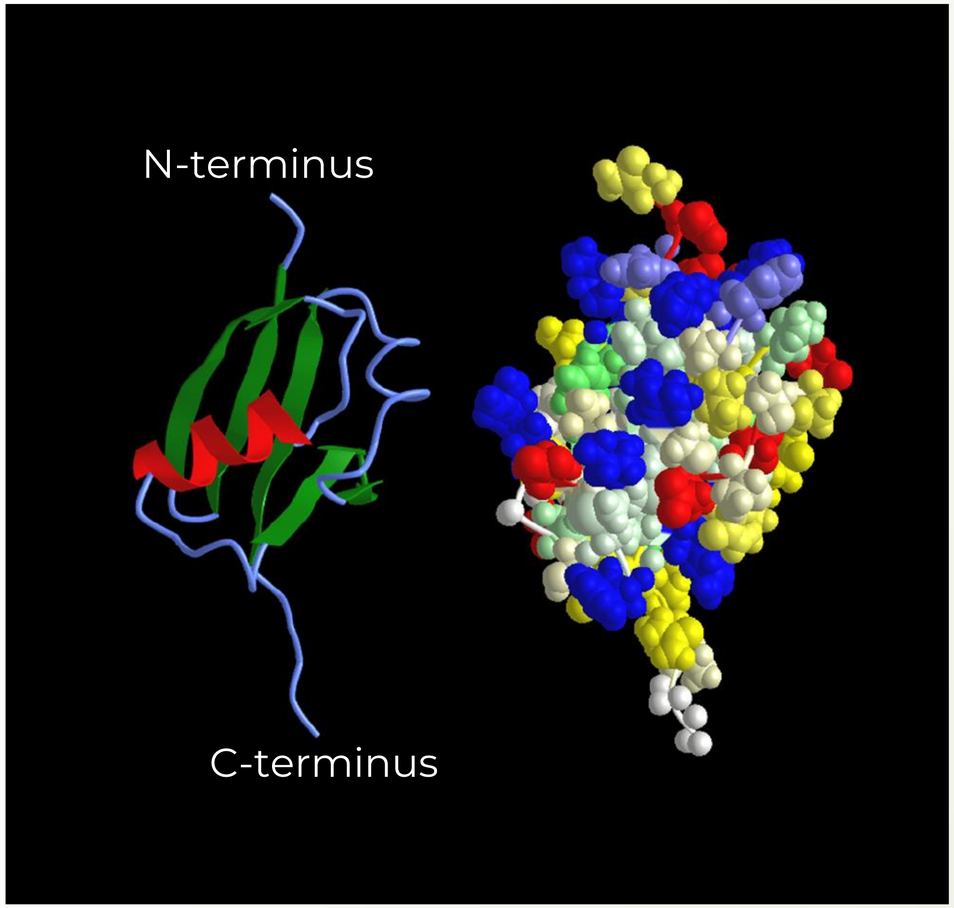
SUMO Polypeptide Factories

- LifeSensors is the developer of SUMO protein expression platform
- Chemical synthesis of poly-peptides beyond 30 amino acids is costly and suffers from poor quality, especially for therapeutic peptides
- Recombinantly expressed peptides are rapidly degraded in E.coli
- SUMO system improves quality of polypeptides, increases the yield and decreases cost

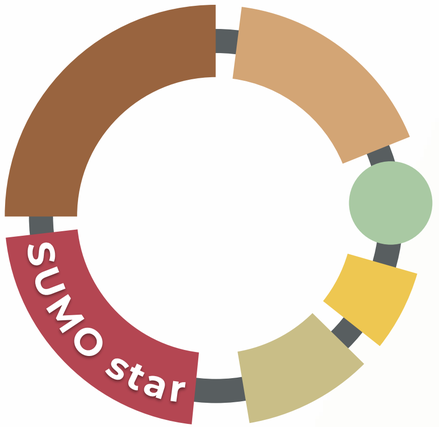
Factors that Enhance SUMO Poly-peptide Manufactuing

- 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

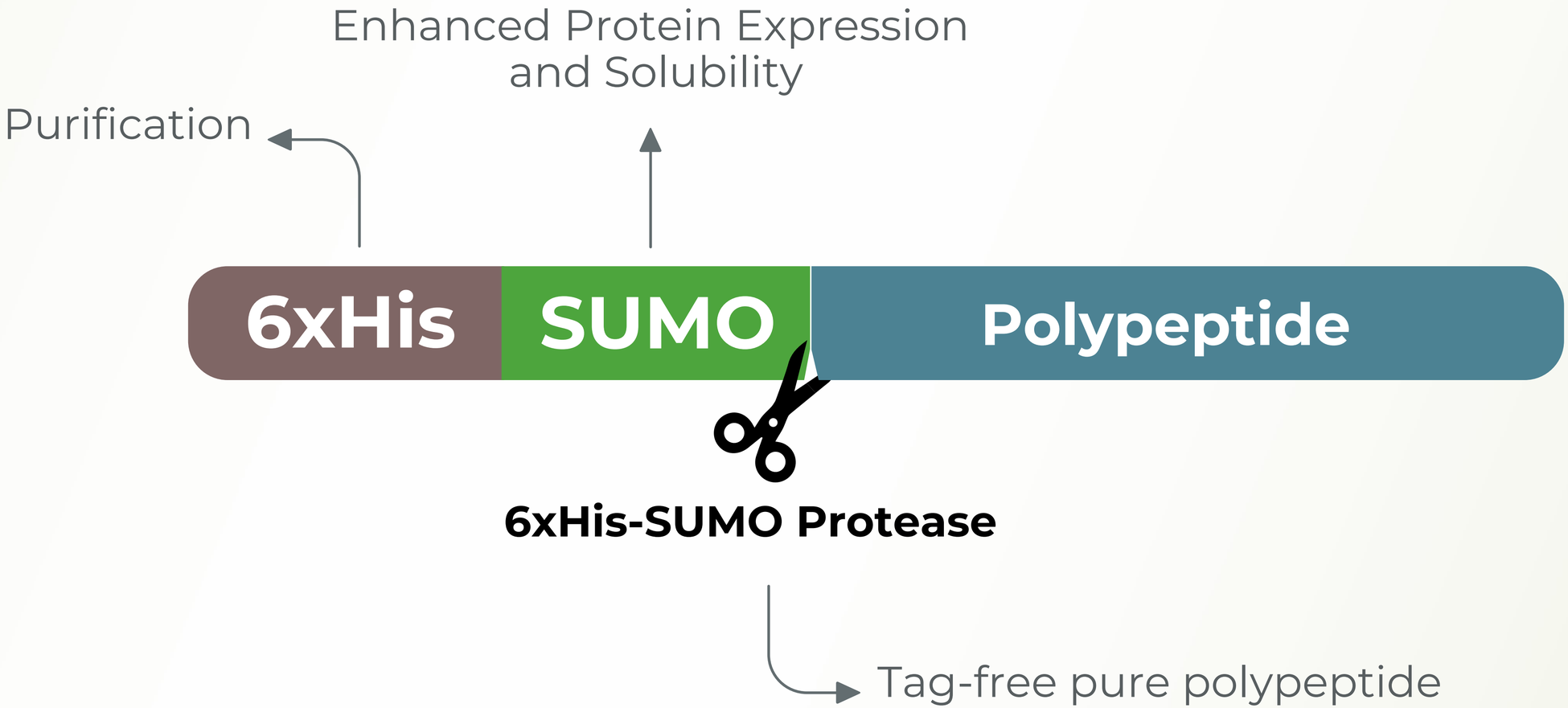


SUMO Platform for Polypeptide Manufacturing

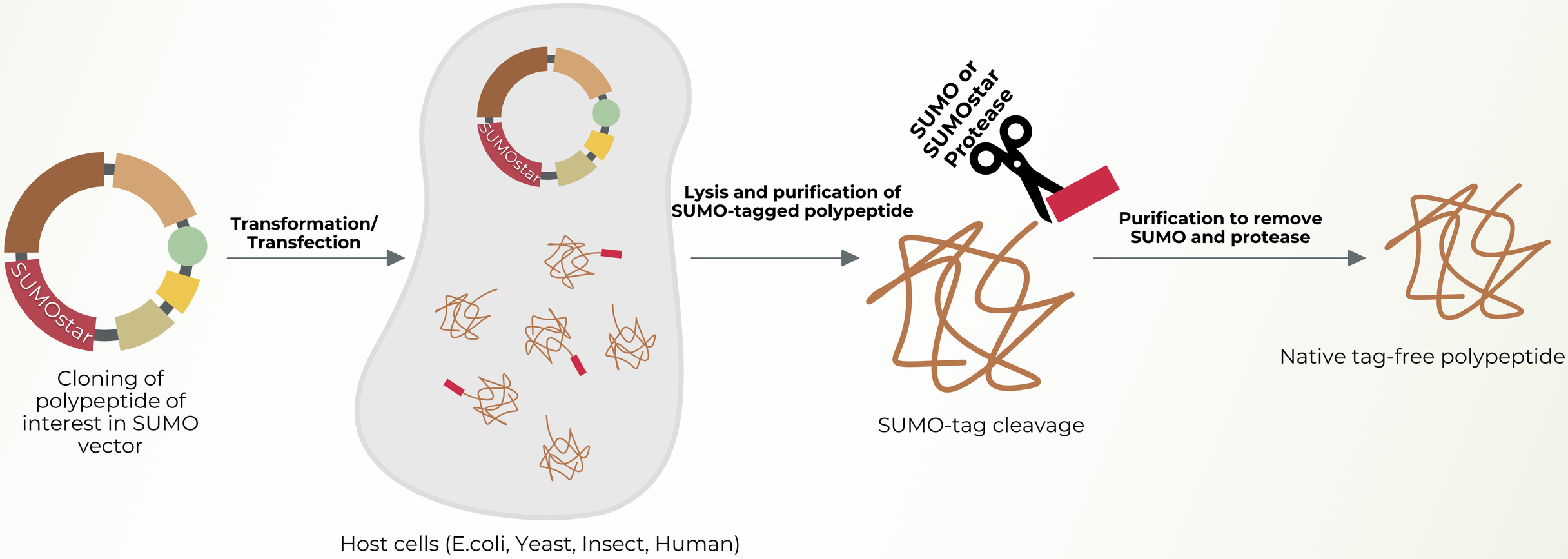
PROBLEMS	SOLUTIONS
Unstable polypeptide, low expression	SUMO chaperoning, stabilizes polypeptides
Insoluble polypeptide	SUMO derived solubility
Miss-folded polypeptide	SUMO-driven folding
High cost of goods	High yield, saves cost



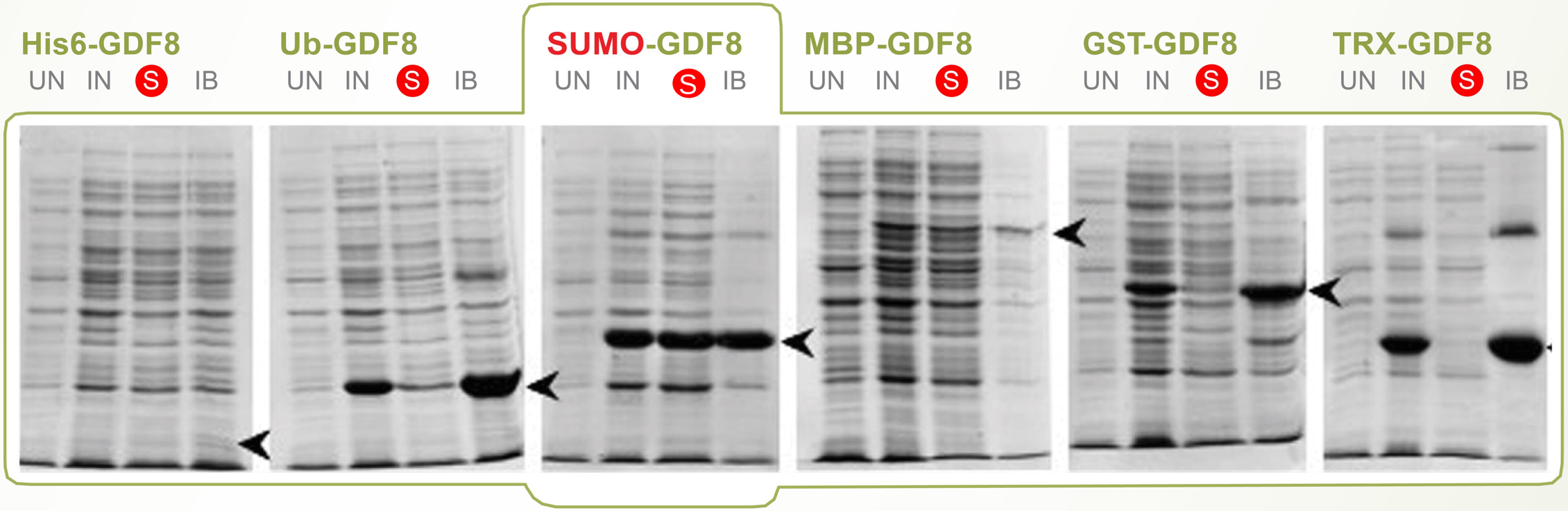
SUMO Platform For Polypeptides



SUMO Polypeptide Expression and Purification Process



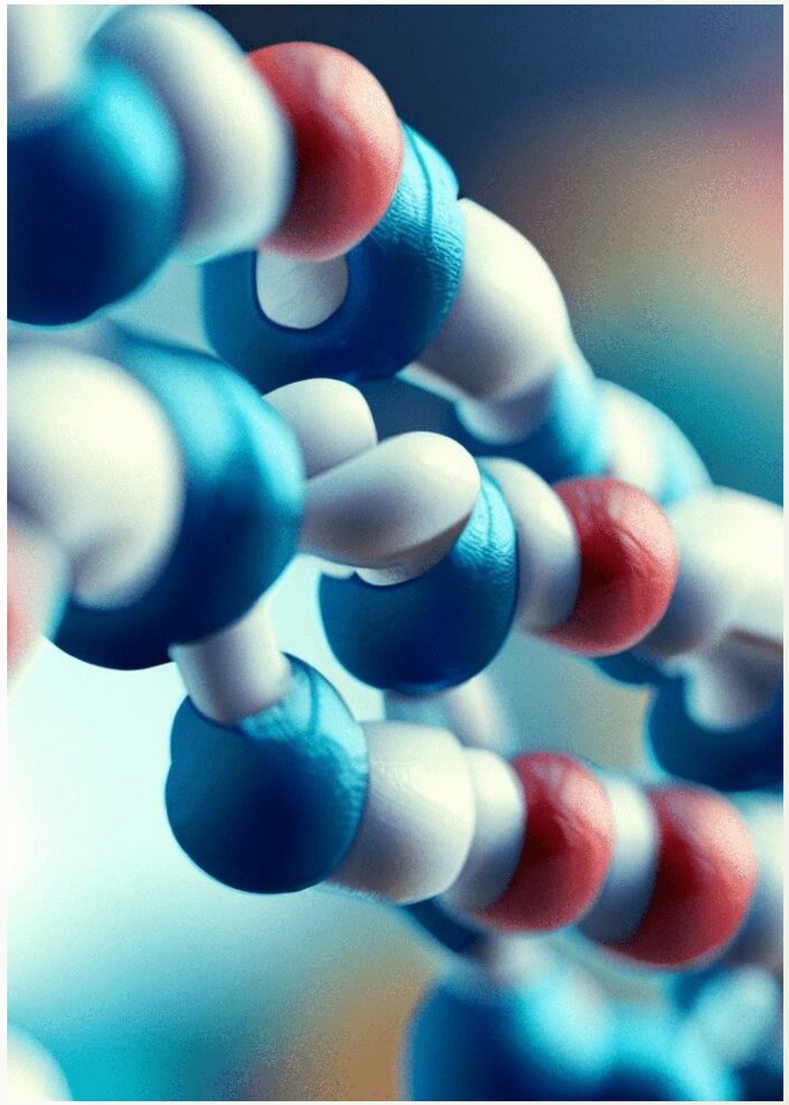
SUMOpro® fusion enhances expression and solubility in *E. coli*



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

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

Dramatic increase in yield, improved quality and decrease cost of SUMO-mediated polypeptide manufacturing



Polypeptide Manufacturing: Chemical vs Recombinant

Chemical Synthesis

- Chemical synthesis is error prone and costly beyond 30 amino acid in length
- Polypeptide yield decreases with increasing length and quality suffers
- Not all the chemically synthesized peptides fold correctly

SUMO Manufacturing

- Recombinantly expressed peptides are **rapidly degraded** in E.coli. Fusion with **SUMO provides remarkable stability** thus increasing the yield.
- Desired N-terminal of peptides are generated that is **not possible with traditional expression** systems
- Correct folding in cells provides biologically/structurally active peptides, low cost

Therapeutic peptides in the Clinic

Therapeutic peptide	Production method	Company	Indication
Insulin	Recombinant (E.coli)	Lilly, Novo Nordisk, Sanofi	Diabetes
Growth hormone	Recombinant (E.coli, yeast)	Genentech, Pfizer, Sandoz	Growth disorders
GnRH analogues	Recombinant (E.coli)	AbbVie	Prostate cancer, endometriosis, precocious puberty
Abaloparatid/Etelcalcetide (PTHrP)	Recombinant (E.coli)	Radius Health	Osteoporosis
Teriparatide (PTH)	Recombinant (E.coli)	Eli Lilly	Osteoporosis
Lixisenatide (GLP-1 analog)	Recombinant (E.coli)/Chemical synthesis	Sanofi, Amylin	Type 2 diabetes
Enfuvirtide	Chemical synthesis	Roche	HIV-1 infection
LL-37	Chemical synthesis	R&D, Sigma	**Under development**, Antimicrobial
TRAIL/Apo2L	Recombinant (E.coli)	Sigma	Cytotoxic, for use in cancer

Examples of SUMO-enhanced production of therapeutic peptides

	Peptide Name	Host	Yield (mg/L)
Antimicrobial Peptides	ABP-CM4[1]	E.coli	24 mg/L
	Bacteriophage-encoded endolysin, LysP11[2]	Nicotiana benthamiana leaves	8 mg/kg
	Abaecin[3]	E.coli	-
	Hepcidin25[5]	E.coli	3.9 mg/L
	Cationic antimicrobial host defense peptides (HDPs)[6]	E.coli in fermentation flask	16-80 mg/L
	AP-64, a human anti-microbial peptide[7]	E.coli	-
Cytotoxic Peptides	Edema Toxin from Bacillus anthracis[8]	E.coli	-
	Recombinant cytotoxin II (rCTII) from Naja naja Oxiana venom[9]	E.coli	-
	Phospholipase D from the spider Loxosceles gaucho[10]	E.coli	24 mg/L
	Scolopin 1 from centipede venom[11,12]	E.coli	16 mg/L
Anti-thrombotic Peptides	Tsetse fly salivary protein[13]	E.coli	-
	Ixodes ricinus Salivary Serpin Iripin-8[14]	E.coli	23 mg/L

1. Li, J.F. et al, Appl Microbiol Biotechnol, 2009
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7. Zhong K et al, Biomolecules. 2021
8. Yang NJ et al, Front Immunol. 2021
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10. Shimokawa-Falcão LH, Toxins (Basel). 2017
11. Chu Y et al, Toxins (Basel). 2020
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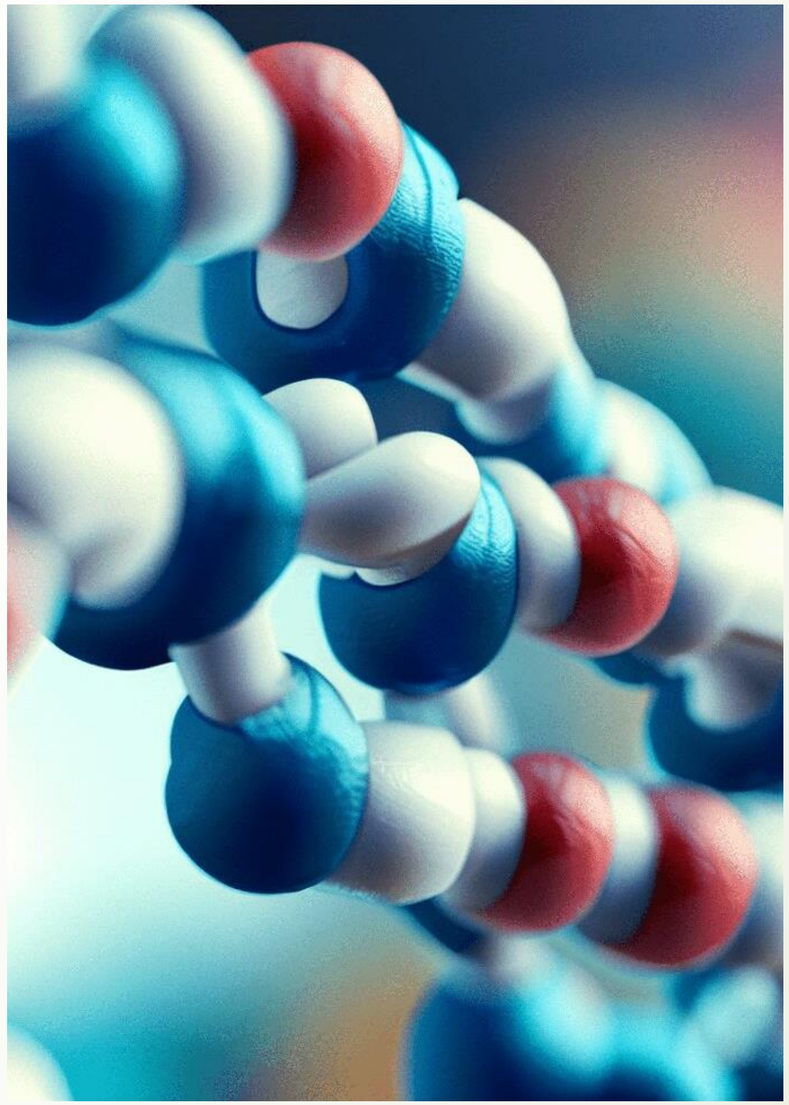
Cationic antibacterial peptide ABP-CM4

Table 2 Comparison of the SUMO fusion partner with Trx or Intein fusion partners

Fusion protein	Time cost (days)	Cleavage efficiency (%)	Yield (mg/l)
Trx–CM4	4–5	<70	1.2–12
Intein–CM4	3	80	4.2
SUMO–CM4	2	>90	24



Li, J.F. et al, Appl Microbiol Biotechnol, 2009

SUMO system is superior to chemical synthesis

- SUMO dramatically enhances the yield of polypeptides, preserving biological activity and maintaining the QC.
- SUMO generates desired N-terminus of the peptide.
- SUMO system reduces the cost of peptide synthesis.

Thank You

We are your partner in Manufacturing Needs

Contact Us!

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