SUMO Technology For Peptides

NOVEL METHODS FOR RAPID RECOMBINANT PEPTIDE EXPRESSION AND PURIFICATION

Peptides for Therapeutic, Diagnostics, Vaccines and Industrial Applications

cytokinespeptidelibrarieschemokines
growthfactorsviralpeptidesenzymes
serinekinasesexpressionpurification
cleavagesuccessSUMOpro™Technology

White Paper

October 2004

Contact Person:
John Hall
techsupport@lifesensors.com
sales@lifesensors.com
610.644.8845 x23 (phone)
610.644.8616 (fax)

LifeSensors Inc.
271 Great Valley Parkway
Malvern, PA 19355

"Nobel" Ubiquitin

In 2004, the Nobel Prize in chemistry was awarded to Drs. Aaron Ciechanover, Avram Hershko, and Irwin Rose for their contributions towards the discovery of the ubiquitin pathway, which regulates protein degradation. We would like to thank them and others, including Drs. Alex Varshavsky, Keith Wilkinson, and Arthur Haas, for their seminal work in this fascinating field. The following technology is based on their life's work.

Nobel Prize Winners in Chemistry
Aaron Ciechanover, Avram Hershko, and Irwin Rose

Introduction

Expression and purification of tractable quantities of active and properly folded protein is a major bottleneck in structural and functional genomics. Difficulties include poor protein expression as well as insoluble and/or improperly folded protein. To overcome these difficulties, scientists usually opt for gene fusion technologies. Gene fusion tags enhance expression and chaperone correctly folded protein; however, removal of these tags with high efficiency and specificity is difficult and time consuming. One solution to overcome this dilemma was the introduction of the ubiquitin (Ub) gene fusion system. In this paradigm, three steps are involved: cloning, expression, and purification. In the first step, a gene is cloned into a recombinant vector as a direct fusion with the C-terminus of the ubiquitin gene. In the next step, the ubiquitin tag generally enhances expression and chaperones correctly folded protein. In the final step, de-ubiquitinating enzymes (DUBs) remove Ub to produce native protein. However promising, the ubiquitin gene fusion system was not efficient because DUBs are neither robust nor capable of cleaving all protein fusions *in vitro*. Moreover, DUBs are unstable and difficult to produce, thus precluding their successful use in biotechnology. Since then, other gene fusion systems such as Glutathione-S-Transferase (GST), NusA, Maltose Binding Protein (MBP), and thioredoxin (Trx) have been created. To a certain extent, all of these systems enhance expression but are innately hampered by two fundamental flaws, 1) The proteases for these tags (thrombin, EK, or TEV protease) require linkers between the fusion tag and the protein of interest, leading to protein with an artificial N-terminus

LifeSensors: Company Profile

Founded in 1996, LifeSensors is a biotechnology company located 35 miles west of Philadelphia. LifeSensors technology platform is based on an important family of proteins called ubiquitin or ubiquitin-like proteins (UBL), such as SUMO (Small Ubiquitin-like MOdifer). LifeSensors has filed patents to cover the use of UBL and SUMO as gene fusions to enhance expression and facilitate rapid purification of recombinant proteins. Currently, LifeSensors is expanding its protein expression and purification capabilities to develop human receptor based protein chips for drug discovery and diagnostics.

Introduction (continued)

after cleavage; and 2) Within the linker, the recognition sequences for the proteases are small and degenerate and hence, identical amino acid sequences within the protein of interest are also cleaved.

To date, the only known solution to the aforementioned problems is the SUMO (Small Ubiquitin-like MOdifier) gene fusion system. SUMO, like Ub, dramatically enhances expression and chaperones correctly folded protein; however, unlike DUBs, SUMO proteases are extremely efficient at cleaving fusion proteins. Furthermore, unlike thrombin, EK, or TEV protease, whose recognition sequences are short and degenerate, SUMO protease recognizes the tertiary sequence of SUMO. As a result, SUMO protease never cleaves within the fused protein of interest.

LifeSensors has developed a SUMO-fusion system for peptide production to overcome the problems faced by traditional gene-fusion technologies to enhance the expression and reduce the cost of peptide production. SUMO belongs to the ubiquitin-like (UBL) family of proteins. The SUMO-fusion system dramatically enhances the expression and stability of peptides in prokaryotes. It promotes solubility and proper folding of insoluble and miss-folded peptides thus increasing the yield and reducing the cost of peptide production. For comparison of recombinant and chemical synthesis of peptide please see table 1.

SUMOpro™ Technology for Peptides

Ubiquitin-like proteins are small (~100 residues), heat-stable proteins with a highly compact globular structure. Most of them are very well expressed in E.coli and, when fused with other proteins or peptides, improve peptide production, solubility, and biological activity. The system developed by LifeSensors offers a set of E.coli vectors that allow simple unidirectional cloning of PCR-amplified genes immediately after 6xHis-SUMO. The cloning design precludes the introduction of undesirable amino-acid residues at the junction. Following initial capture, the 6xHis-SUMO portion can be cleaved with SUMO Protease.

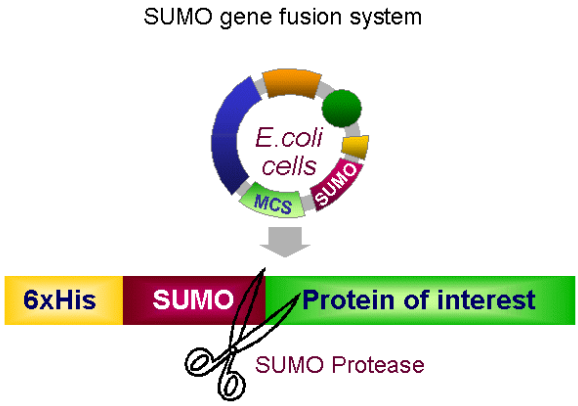

Figure 1.

Table 1. Cost Comparison
SUMO Technology vs. Chemical Synthesis

Peptide	SUMO Technology		Custom Chemical Synthesis	
	10 mg	100 mg	10 mg	100 mg
70 aa peptide	$$	$$$	$$	$$$$$
40 aa peptide	$$	$$$	$	$$$$

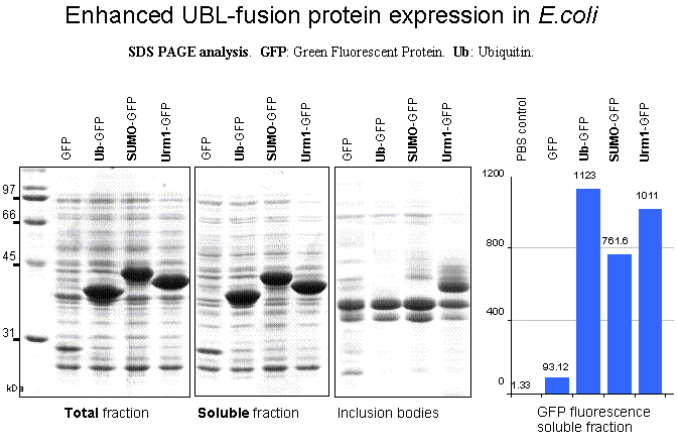

Figure 2.

SUMOpro™ for Peptides (continued)

SUMO protease is extremely durable (see Figure 3), cleaving consistently over a broad range of temperature and ionic strength. Excellent cleavage is achieved at pH values from 6 to 10.5. Moderate concentrations of chaotropic salts and non-ionic detergents can be tolerated, and the presence of up to 20% glycerol does not inhibit proteolytic activity. Generally, cleavage can be accomplished in 30-60 minutes, and cleavage failure rate is zero. Out of about 50 peptides ranging from 7 to 100 kDa tested, not one was uncleavable.

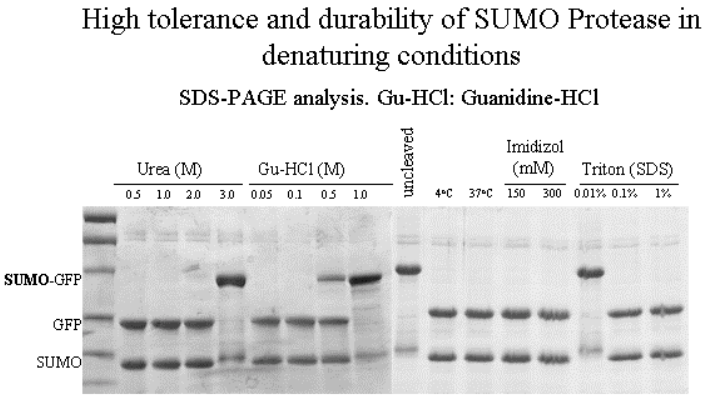

Figure 3.

An important advantage of SUMO Protease is its ability to cleave fusions at the junction, releasing peptides or proteins with any desired N-terminal residue except proline. Figure 4 demonstrates the cleavage of 20 fusion peptides, each with a different amino-acid residue following the cleavage site. It has been demonstrated that cleavage always occurs at the predicted site. This feature permits the generation of desired amino-termini for enhanced physiological activity.

Furthermore, unlike Thrombin, EK or TEV protease (whose recognition sites are short and degenerate), Ulp1 requires the entire SUMO sequence for recognition and never cleaves within the fused protein of interest. It is also worth mentioning that Ulp1 is easy to manufacture, and its cost is lower than other proteases commercially available.

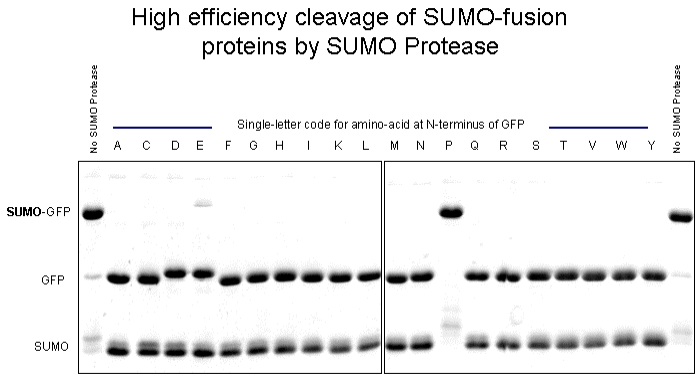

Figure 4.

A typical purification protocol involves the following five steps:

1. Homogenize cells, remove debris
2. Absorb 6xHis-SUMO-fused peptide to Ni-resin
3. Wash resin and elute 6xHis-SUMO fusion (e.g. with Imidazole)
4. Cleave fusion with SUMO Protease (6xHis-SUMO Protease) and remove imidazole (dialysis or desalting column)
5. Apply to Ni-column: subtract 6xHis-SUMO portion, 6xHis-SUMO Protease, and

SUMOpro™ for Peptides (continued)

contaminating Ni-binding peptide. Collect flow-through.

The intrinsic ability of SUMO to renature rapidly upon withdrawal of denaturing compounds allows its use in high throughput peptide purification applications such as the simultaneous isolation of peptides from multiple samples representing the clones of different peptides:

1. Lyse cells in Guanidine-HCl, remove debris
2. Absorb 6xHis-SUMO fused peptides to Ni-resin
3. Wash resin with decreasing concentrations of Guanidine-HCl and elute 6xHis-SUMO fusion with low pH buffer
4. Neutralize the eluate and cleave the fusion with SUMO Protease
5. Apply to Ni-column: subtract 6xHis-SUMO portion, 6xHis-SUMO Protease, and contaminating Ni-binding proteins. Collect flow-through.

Various classes of peptides have been expressed as SUMO-fusions and shown to have improved expression and/or solubility. These include the following:

1. Peptide libraries, ranging 37-70 AA
2. Chemokines and Cytokines (see Figure 5.)
3. Growth factors
4. Viral Peptides for antigen presentation
5. Several classes of enzymes
6. An assortment of viral proteins and
7. Serine kinases and variety of receptors

A therapeutically important cytokine (IL-X) which was not expressed using traditional methods was successfully expressed with improved quality, increased quantity and reduced overall costs of production (see Figure 5).

The use of SUMO-fusion technology in yeast and insect cells also demonstrates that fusion of SUMO enhances protein expression and solubility. Fusions produced in eukaryotes are rapidly cleaved by endogenous SUMO-

Purification & cleavage of IL-X yields ~20 mg/L
SDS PAGE analysis, IL-X.

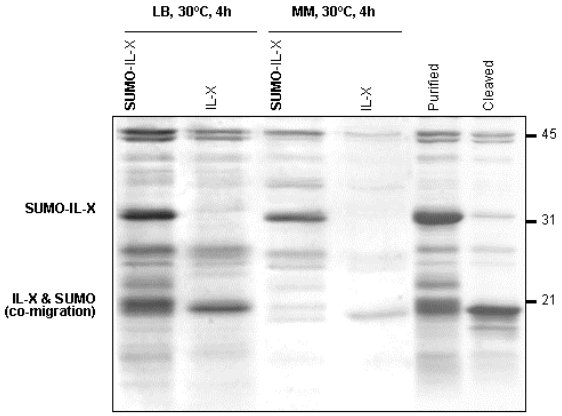

Figure 5.

SUMOpro™ for Peptides (continued)

specific proteases. For peptide expression in eukaryotic systems, we refer you to the Split SUMO White Paper.

Frequently Asked Questions

Q: Does LifeSensors have patent(s) on this technology?
A: LifeSensors owns worldwide patent applications pending approval for the use of SUMO-fusion and applications of proteases to generate novel N-termini.

Q: Is LifeSensors marketing a vector system or a specific protease?
A: LifeSensors has developed a complete solutions approach for expressing challenging proteins. Depending on the nature of the problem, LifeSensors employs the correct fusion with an appropriate host to deliver a high yield of protein. LifeSensors offers a broad range of services and support for applications of the SUMO system for a variety of purposes.

Q: How specific is the enzyme and what are the costs involved?
A: SUMO Protease 1 is a highly specific enzyme – it recognizes the entire SUMO structure and consistently cleaves at the junction to deliver a protein with designed N-terminus. A variety of protein fusions have been investigated and the enzyme never cleaves within the protein of interest. The cost of SUMO removal from a fusion is approximately ten times lower than that of traditional proteases.

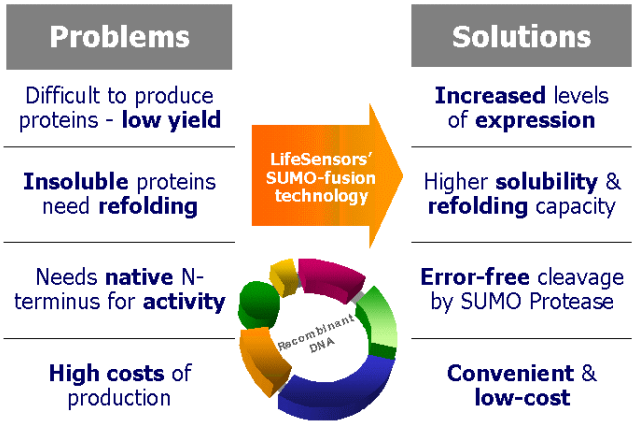

Figure 6