

SUMO Gene Fusion Technology

NEW METHODS FOR ENHANCING PROTEIN EXPRESSION
AND PURIFICATION IN PROKARYOTES

*Applications to Express and Purify Drug Targets, Therapeutics,
Vaccines and Industrial Proteins and Peptides*

White Paper

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“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.

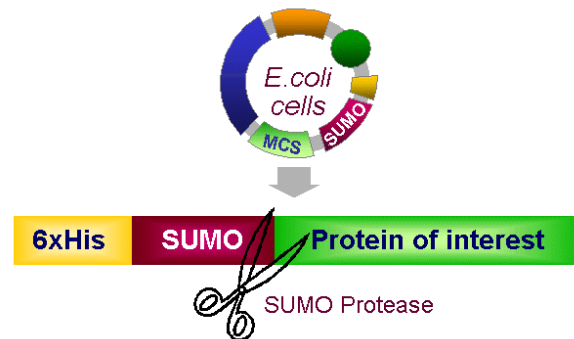
To date, the only known solution to the aforementioned problems is the SUMO gene fusion system. SUMO, like Ub, dramatically enhances expression and chaperones correctly folded protein; however, unlike DUBs, SUMO proteases are extremely efficient at cleaving fusion proteins. Furthermore, unlike thrombin, 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.

Herein, we introduce a novel protein expression and purification system, which utilizes the enhanced expression and solubility capabilities of SUMO fusions and the cleaving capabilities of SUMO Proteases. SUMO Protease 1 cleaves purified recombinant SUMO-fusion proteins with any amino acid except proline at the +1 position of the cleavage site. Therefore, the SUMO system is a powerful platform to express high quantities of soluble protein with desired amino-termini.

SUMOpro™ Gene Fusion Technology

Gene fusion technology is a powerful tool in protein expression and purification. In this methodology, a recombinant plasmid is constructed to express a fusion between the amino-terminal of a protein and the carboxy-terminal of the SUMO protein tag. The primary purpose of the protein tag is to facilitate purification but the protein tag may also enhance solubility by chaperoning proper protein folding (see Figure 2). Once expressed, protein fusions are lysed from cells and purified by adding a protease that cleaves the fusion. Ideally, the last step performed should give a high yield of properly folded and active protein. However, to date, there is no gene fusion technology that has optimised each step for all proteins in every host system.

SUMO gene fusion system



Enhanced UBL-fusion protein expression in *E. coli*

SDS PAGE analysis. GFP: Green Fluorescent Protein. Ub: Ubiquitin.

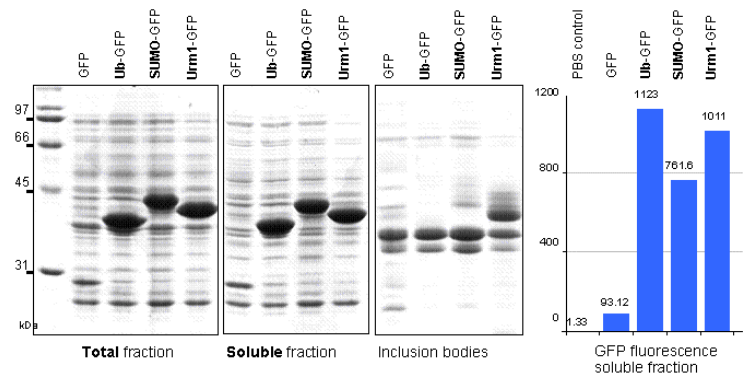


Figure 2.

SUMOpro™ Technology (continued)

Difficulties that still arise include poor protein expression, insoluble protein, improperly folded protein, and/or unselective cleavage of the fusion. Herein, we introduce the SUMO fusion technology, which utilizes the chaperoning characteristics of SUMO and the ultra selectivity of SUMO Protease 1. SUMO, a member of the Ubiquitin-like (UBL) protein family, is a small (~100 residues), heat stable protein with a highly compact globular structure. The precise chaperoning mechanism of SUMO has not been elucidated but studies have shown increased solubility and biological activity over other gene fusion technologies.

The SUMO system offers a set of E.coli, yeast, and baculovirus vectors that allow simple unidirectional cloning of PCR-amplified genes. The gene of interest is placed immediately after the SUMO gene in order to preclude the introduction of undesirable amino-acid residues at the junction. Therefore, no linker between SUMO and the protein of interest is necessary for proper cleavage. Following expression of the fusion protein, the SUMO portion can be cleaved with SUMO Protease 1.

SUMO Protease 1 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 less than 30 minutes with a cleavage failure rate of zero. Out of 12 proteins ranging from 7 to 200 kDa tested, all were cleaved.

An important advantage of SUMO Protease 1 is its ability to selectively cleave fusions, releasing peptides or proteins with any desired N-terminal residue, except proline. Figure 4 demonstrates the cleavage of 20 fusion proteins, each with a different amino-acid residue following the cleavage site. It has been demonstrated that cleavage always occurs at the predicted site. Unlike Thrombin, EK, or TEV protease (whose recognition sites are short and degenerate), SUMO

High tolerance and durability of SUMO Protease in denaturing conditions

SDS-PAGE analysis. Gu-HCl: Guanidine-HCl

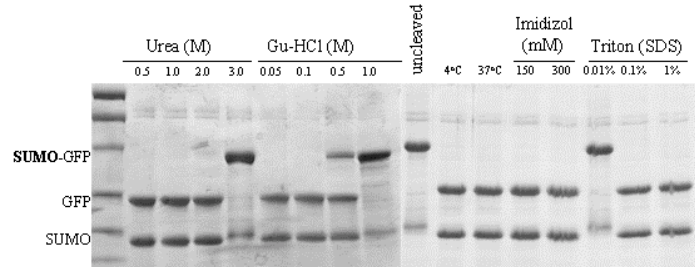


Figure 3.

High efficiency cleavage of SUMO-fusion proteins by SUMO Protease

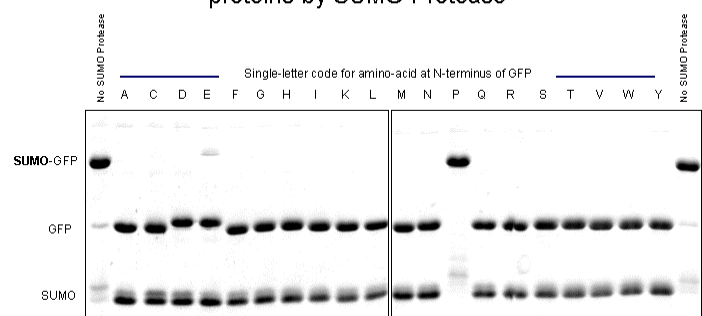


Figure 4.

SUMOpro™ Technology (continued)

Protease 1 requires the entire SUMO sequence for recognition and never cleaves within the fused protein of interest. This feature permits the generation of desired amino-termini for enhanced physiological activity. Furthermore, SUMO Protease 1 is easy to manufacture, and its cost is significantly lower than other proteases commercially available.

A typical purification protocol involves the following five steps:

1. Homogenize cells, remove debris
2. Absorb SUMO fusion to Ni-resin
3. Wash resin, elute SUMO fusion
4. Cleave fusion
5. Subtraction

Application of the system to express and purify a wide range of proteins in prokaryotes has been demonstrated. SUMO Protease 1 is a robust enzyme that rapidly cleaves fusion proteins of various structure and molecular weight. Thus, the SUMO technology lends itself to efficient and inexpensive process development and production of various classes of proteins and peptides. Various classes of proteins have been expressed as SUMO-fusions and shown to have improved expression and/or solubility. These include the following:

- Serine and Threonine kinases (see Figure 5)
- TGFβ
- Membrane proteins (see Figure 6)
- G-Protein family
- Nuclear receptors
- Cytokines and growth factors
- Several classes of enzymes
- Viral proteins and antigens
- Random peptide libraries

Expression, purification and cleavage of SUMO-kinase fusion in *E.coli*

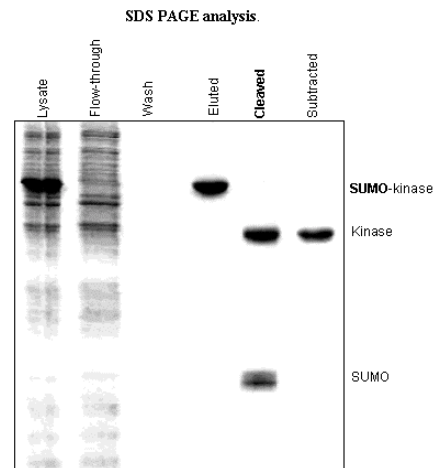


Figure 5.

SUMO-MMP13 expression and purification from *E.coli*

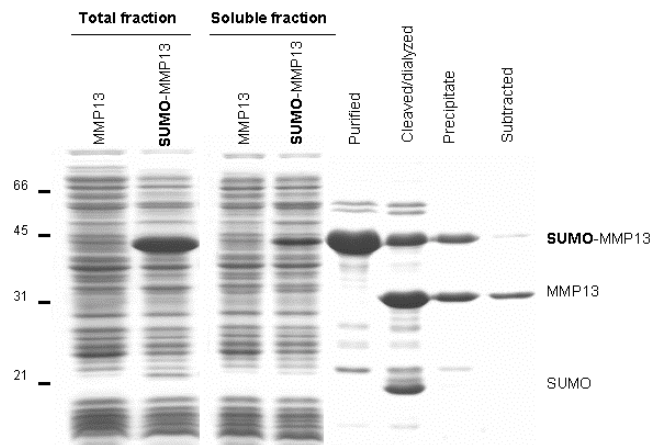


Figure 6.

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.

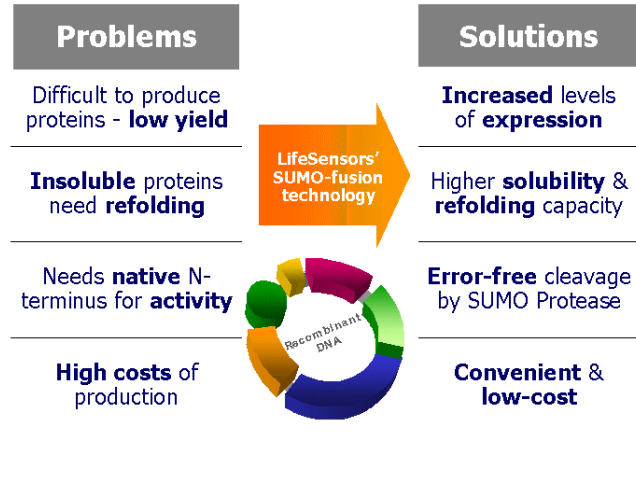


Figure 7.