Nucleics

Z-Tag

Z-Tag is a novel and versatile protein purification system developed by Nucleics P. This system has a number of superior properties that distinguish it from currently available protein purification methods. These properties include –

- *Direct one-step purification without affinity columns*
- *Ability to perform multiple purification cycles allowing extremely high levels of protein purity to be obtained*
- *Easy removal of the Z-Tag domain via a specific protease fusion*
- *High solubility of the Z-Tag fusion protein*
- *High-level of protein expression*
- *Completely scalable from the lab bench to an industrial level*

The Z-Tag principle

The Z-Tag system employs protein fusion technology to link the target protein of choice to a specific protein tag (the "Z-Tag"). The key property of the protein tag is its ability to perform a reversible self-polymerisation induced by defined and controllable physical/ chemical conditions (Fig. 1). Consequently, within the Z-Tag system the protein tag, fused to the desired protein target, can be altered between two physical states:

1. A polymerised, multimeric state of high molecular weight and size.

and

2. A soluble, depolymerised state of relatively low molecular weight and size.

These two states allow separation from each other by simple physical techniques such as centrifugation, filtration, dialysis or differential sedimentation. More importantly, the fusion protein can be purified from other protein or molecules that are either larger or smaller in weight/ size by alternating between these two states. This is best illustrated in the following example: In a crude protein solution (e.g. cell extract) polymerisation of the fusion protein is induced and the multimeric form is collected in a pellet by centrifugation. The soluble or small contaminating proteins in the supernatant are then removed by decantation and the fusion protein is depolymerised. After another centrifugation step, the fusion protein stays in solution and the insoluble proteins pelleted. This process of polymerisation and physical separation can be repeated until the desired level of protein purity is achieved.

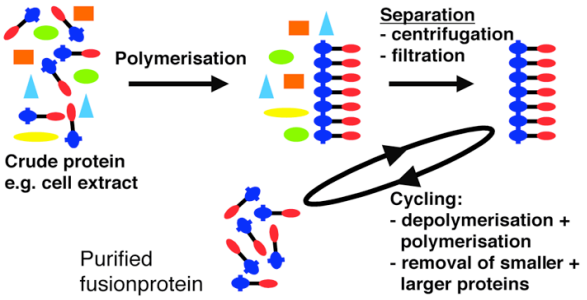

Figure 1. Z-Tag Protein Purification System

The Z-Tag system

Nucleics has developed and utilised a naturally occurring, self-polymerising protein for the Z-Tag system. This protein is the *Escherichia coli* cell-division protein, FtsZ, which polymerises reversibly, in the presence of divalent cations and guanosine triphosphate (GTP), to form high

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molecular weight filaments. Nucleics has developed variants of FtsZ with different polymerisation properties or features (e.g. independence of GTP, different temperature ranges, eukaryotic systems) to increase the flexibility of the system. Nucleics has incorporated an efficient system to release the target protein from the Z-Tag domain. Release is mediated by a specific protease (human rhinovirus protease 3C) that recognises and cleaves a unique linker-sequence located between the target and the Z-Tag. The protease itself is linked to a Z-Tag domain allowing easy removal of the protease after cleavage. Furthermore, Nucleics has generated a range of cloning vectors for easy cloning of target genes and efficient production of the fusion proteins.

The strengths of Z-Tag

High production levels & purity

For the economical production of recombinant proteins high expression levels and high solubility are desirable. FtsZ fusion proteins can be overexpressed in extremely high levels (up to 50% of the cellular proteins) and exhibit good solubility characteristics. Nucleics has overexpressed a range of FtsZ fusion proteins with exceptional yield. In addition, the ability for repetitive cycling will allow the adjustment of protein purity to the demands of the protein usage (e.g. crude preparation for some industrial purposes or highly purified for medical applications).

Scalability

Large scale protein production is developed in a stepwise fashion, from lab scale via several pilot-stages, to the final industrial scale. Each scale-up requires re-optimisation of the protein chromatographic procedures (e.g. column and absorption bed dimensions). Z-Tag is a matrix-independent purification procedure (i.e. no chromatography) and therefore is fully scalable with at most only minor adjustments.

Simplicity

Z-Tag utilises very simple, rapid steps (pipetting and centrifugation/ dialysis, etc) to purify proteins. This makes Z-Tag not only attractive for the individual protein researcher, but also amendable for automation or robotic operation. High-throughput and parallel (microtitre-plate format) Z-Tag protein purification applications should prove invaluable for large-scale screening in the pharmaceutical and biotechnology industry.

Nucleics has protected the intellectual property of Z-Tag. Nucleics is currently seeking commercial partners to further develop the Z-Tag technolgy and to establish a marketing and distribution strategy.

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