

Protein purification with stimulus responsive tags

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A technically easy and inexpensive method for recombinant protein purification is reported, which is based on imparting the protein that one wishes to purify, with stimulus responsive behavior. The recombinant protein to be purified is fused to an elastin-like polypeptide (ELP), which is able to undergo reversible inverse phase transition. A switchable protein based self-cleaving linker (intein) is placed in, between the ELP tag and the target protein. This tripartite fusion system enables rapid isolation of the target protein without the need for affinity chromatography for purification, or the need of proteases for cleavage of the target protein from the fusion. The method is based on the ability to sequentially and independently activate the ELP moiety and then the self-cleaving linker. The fusion protein can be selectively aggregated in cell lysate by the addition of NaCl, which will cause the ELP tags to selectively aggregate and it is shown that these aggregates can be recovered using microfiltration. These aggregates can be resolubilized by reversal of the phase transition using a low ionic strength buffer. Following this initial separation step, the conditions in the solution are adjusted so that the self-cleaving linker is activated resulting in the release of the target protein from the tripartite fusion. The attractive features of this self-contained purification system are its extraordinary technical simplicity and low cost: the only reagents and equipments required to purify mg levels of protein at the lab bench are NaCl and DTT, a 0.2 μ m membrane filter and a syringe.

More recent work has focused on exploiting the phase transition ability of ELP to recover proteins that are present in cells at extremely low concentrations. It is based on the use of an ELP as a co-aggregant for the capture an ELP tagged recombinant protein. This co-aggregation process results high levels of recovery (>90%) of the tagged protein, and it was found to be independent of the concentration at which the tagged protein is present, at least for concentrations as low as 10pM. The co-aggregation process is highly specific and this was demonstrated by spiking crude cell lysate with the ELP tagged recombinant protein to a final concentration of 1nM and recovering more than 80% of it to a high level of purity. This method is particularly useful for recovering poorly expressed proteins. Furthermore, the concept presented here accounts for a method that should be useful in areas other than recombinant protein purification.