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Obtaining a Pure Protein Using an ELP-Tagged TEV Protease

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Abstract

Elastin-like polypeptides (ELPs) reversibly aggregate and phase separate above and solubilize below a specific transition temperature (T_t). ELPs are composed of repeats of the structural sequence of the elastin protein, Gly-Xaa-Gly-Val-Pro $(GXGVP)_n$. Xaa is a "guest residue" in the sequence and can be any amino acid, with the exception of proline, P, in order to alter the temperature response. ELPs can then be attached to recombinant target proteins as a tag to facilitate protein purification.

We present here use of GLGVP for tagging and purifying a gadolinium binding protein domain. Leucine is used as the guest residue in order to lower the T_t below room temperature, enabling purification to take place over a more manageable temperature range. After protein purification, it is ideal to remove the ELP tag from the target protein. To accomplish this, a tobacco etch virus (TEV) protease cleavage site was inserted between the target protein and the ELP tag. The TEV protease is also tagged with an ELP so that after proteolytic cleavage the ELP tag from the target protein, a single round of thermal cycling can remove both the free ELP tag and the ELP-tagged TEV protease, leaving the purified target protein.