Removal of Thrombin recognition tags (suspension)

Aim
Following purification of protein of interest, there are cases in which a specific tag needs to be removed for downstream applications; in many cases, a tag is preceded by a Thrombin recognition site (Leu-Val-Pro-Arg-Gly-Ser)\(^1\).

This protocol details the procedure in which Thrombin, a serine protease, is added to the protein sample (<1.5ml for this protocol) and digests the linker sequence thus releasing the tag from the target protein.

Purification of protein is then conducted via suspension of protein sample with Benzamidine beads\(^2\) followed by several wash steps with sample concentrator that removes the tag peptide/protein from the sample.

Materials & Equipment
- Eppendorf tube
- 50-80µl of Benzamidine Sepharose 4 Fast Flow (GE Cat#17-5123-10)
- Bovine Thrombin (1000U/ml) (Fisher scientific, Cat#BP25432)
- Protein sample at volume<1.5ml
- 10ml of Protein’s buffer
- Sample concentrator with appropriate cutoff\(^1\)

Experiment procedure
1. Add 10µl of Thrombin for every mg of pure protein and incubate at RT for a minimum of 30’ (2hr to be on the safe side).
2. Remove 50-80µl of Benzamidine beads into eppendorf tube and washX3 with protein’s buffer (spin 1’ at 8000rpm).
3. Resuspend beads with protein sample and incubate rotating at RT for 5’.
4. Spin 1’ at 8000rpm at 4°C and keep supernatant.

\(^1\) See appendix A for detailed Thrombin recognition sites
5. Resuspend beads with 1ml of protein’s buffer and spin 1’ at 8000rpm, 4°C; keep supernatant.
6. Repeat step 4 one more time; discard of beads.

Note: In case the tag is large, it is advisable to use an additional column that binds the tag and purify the protein sample.

7. Pool supernatants from steps 4-6 and load on a sample concentrator, fill to half the concentrator volume and centrifuge at 4000rpm 4°C.
8. Repeat wash with similar volume and concentrate to volume of choice.
Appendix A - Thrombin recognition sites

Thrombin can cleave at the following wide sequence consensus:
Hydrophobic-Hydrophobic-Proline-Basic-Nonacidic-Nonacidic (cleavage between basic and nonacidic residues). For example:

- Leu-Val-Pro-Arg-Gly-Ser (used in plasmids)

Note that there are optional sequences that can be recognized by Thrombin (note the Arginine residue):

- DEEAEEERLA cutting between R and L
- FLLEHIRILK cutting between R and I
- GSIRQFAACL cutting between R and Q

It is highly recommended to check your protein’s sequence for such consensus sequences before commencing any cloning.