ELASTIN-LIKE PROTEIN-BASED ADHESIVES FOR SOFT TISSUE APPLICATIONS

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Tissue adhesives are a promising alternative method to traditional wound closure techniques, such as sutures or staples as these suffer from drawbacks, e.g. tissue tearing. The ideal adhesive should adhere under physiological conditions, allow dynamic movement and protect against microbial colonization. This project explores the use of elastin-like proteins (ELPs) as the foundational material for protein-based hydrogels. These ELPs will be combined with catechol groups, inspired by mussel foot protein-5 (Mefp-5), to introduce adhesiveness. After bacterial expression of Mefp-5-ELPs, conversion of both the lysines in the ELPs and tyrosines in the Mefp-5 will result in respectively hydrazine side groups and the desired catechol groups. The hydrazine-modified protein will be crosslinked using an aldehyde to obtain hydrazone bonds, which offer dynamic covalent behavior. Lastly, mechanical properties (shear adhesion and peel tests) and biocompatibility will be assessed to study the adhesive's performance.

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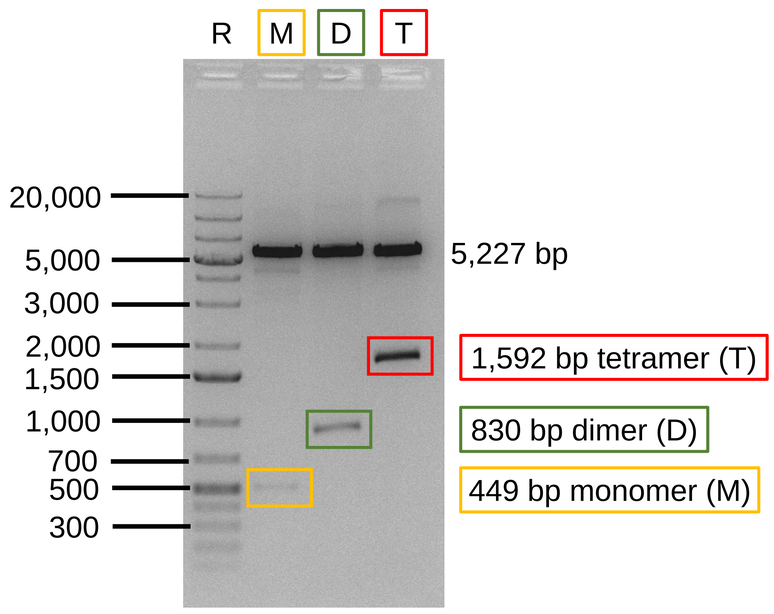
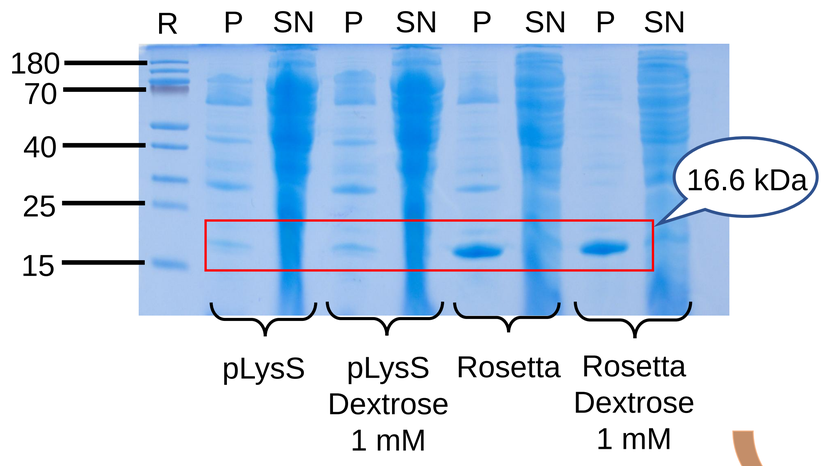
Design and expression of adhesive elastin-like proteins

Plasmid design in pET28a

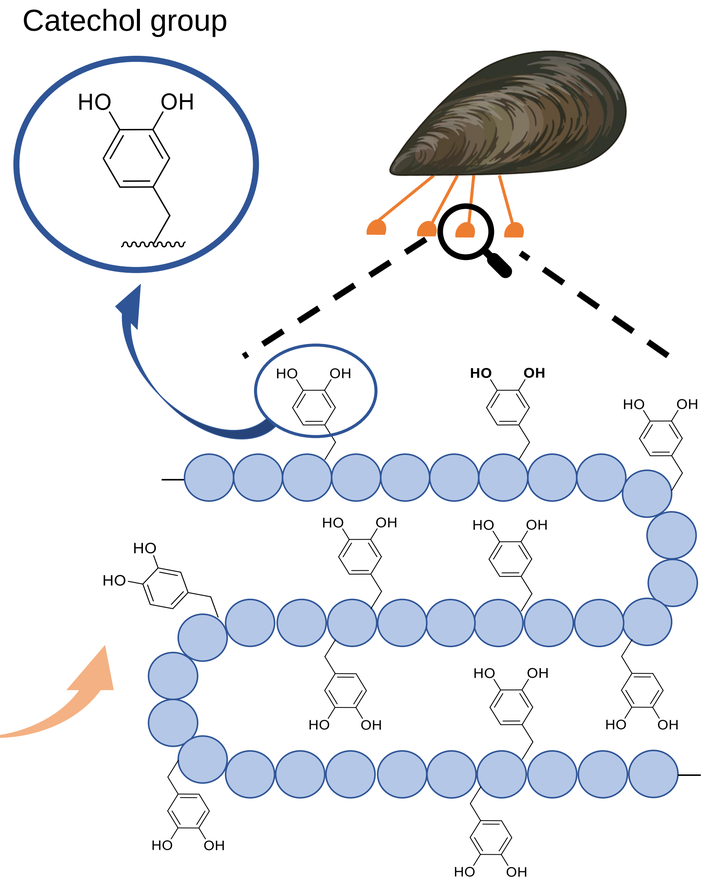
Mefp-5 — ELP_3



Protein expression of monomer

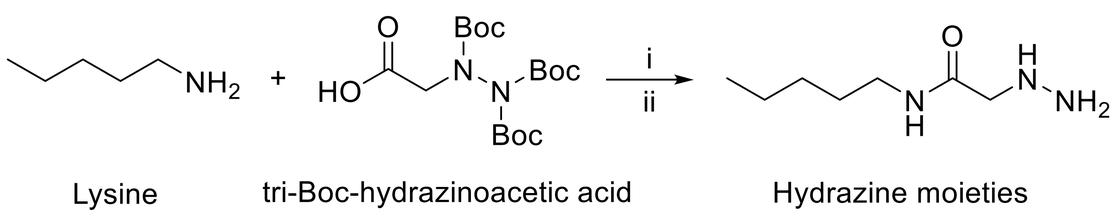


SN = supernatant after cell lysis
P = cell pellet after cell lysis
pLysS: tight control of toxic protein expression
Rosetta: better expression performance in presence of rare codons
1 mM dextrose was added to the grow medium as an additional energy source



DOPA residues in Mefp-5 are key to adhesiveness

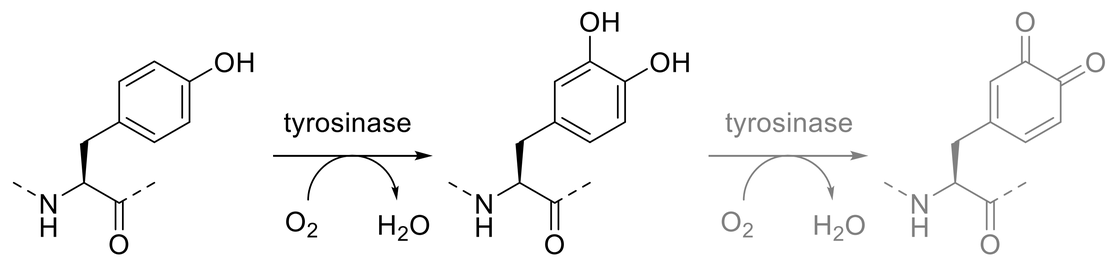
Converting lysines to hydrazines



Lysine — tri-Boc-hydrazinoacetic acid — Hydrazine moieties

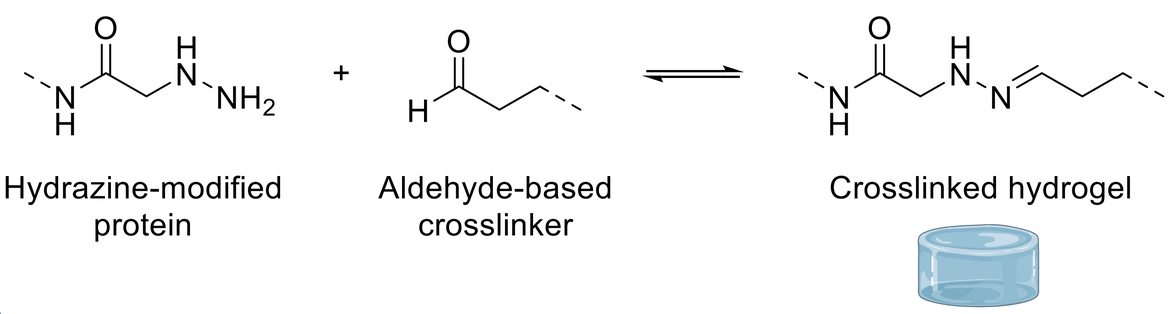
i: HATU/4-methylmorpholine in DMF; ii: tri-isopropylsilane in TFA-DCM (50/50)

Converting tyrosine to DOPA



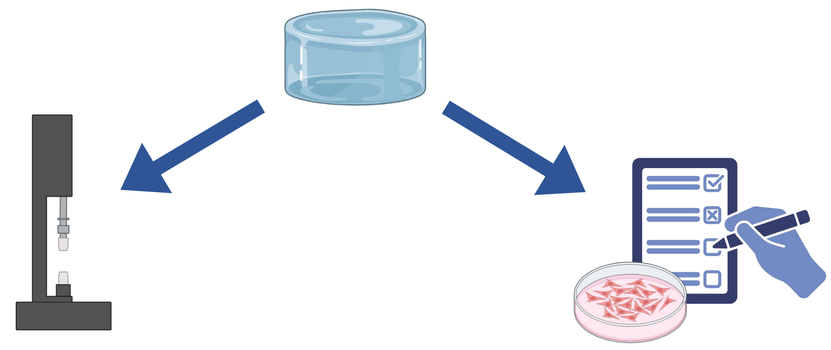
Tyrosine — DOPA — DOPA-quinone

Crosslinking ELPs using DCC



Hydrazine-modified protein + Aldehyde-based crosslinker ⇌ Crosslinked hydrogel

Assessing mechanical properties and biocompatibility



Conclusion

The plasmid design of monomeric, dimeric and tetrameric Mefp-5-ELP has been successfully performed in pET28a. According to SDS-PAGE analysis, there is an indication of bacterial expression of the monomer. This will be further assessed by Western Blot evaluation and purification using IMAC chromatography.

Acknowledgments

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