Recombinant biomaterials are they worth the trouble?

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Context

Proteins play a crucial role in the field of biomaterials due to their inherent biocompatibility, bioactivity, and ability to interact with biological systems. However, the biological origin of these materials also raises questions about the risk of disease transfer or other ethical considerations. As a result, recombinant proteins are often proposed as a workaround. However, designing and expressing recombinant proteins is not straightforward and requires a good understanding of the necessary steps to translate a gene of interest into purified proteins that can be used as biomaterials. The numerous interdependent experimental parameters make this field challenging for biomaterials scientists new to recombinant proteins. Are these materials worth the trouble?

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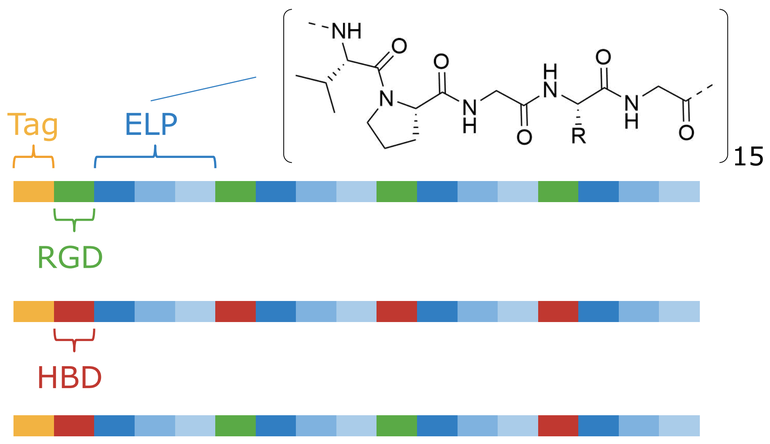
Gene construction

Heparin-binding domains (HBD) for improved tissue integration

LCST behaviour



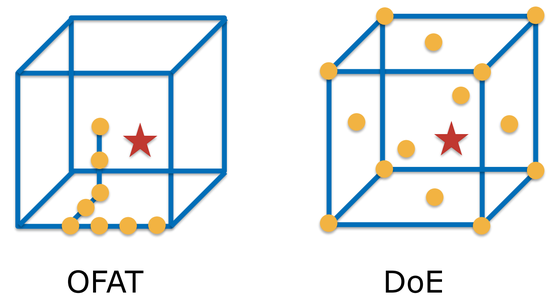
RGD: YAVTG**RGD**SPASSAA

HBD: GSSSG**WQPPRARI**

Points of attention
Repeats
Codon bias
Vector selection

Protein expression

Points of attention
Host cell selection
Culture conditions
Protein of interest

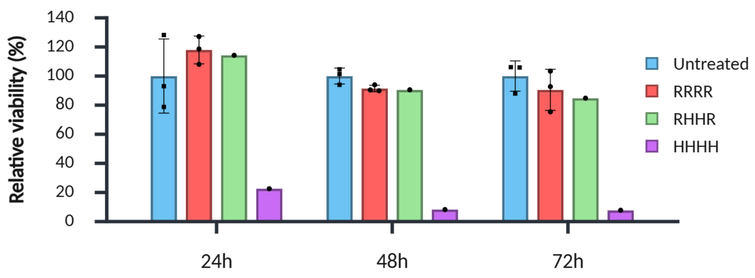


➔ Identified ideal expression conditions for each of the constructs

➔ 12 fold increase in yields

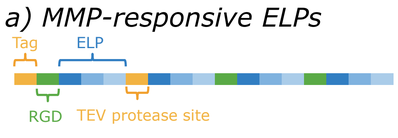
	Strain	Temp (°C)	IPTG (mM)	OD (-)	Yield (mg/l culture)
RRRR	pLysS	32	0.5	0.4	**69**
RHHR	pLysS	32	0.1	1	**72**
HHHH	BLR	37	0.4	1	**60**

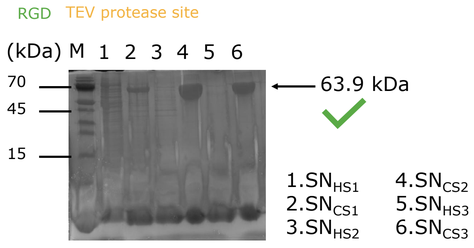
Preliminary biocompatibility



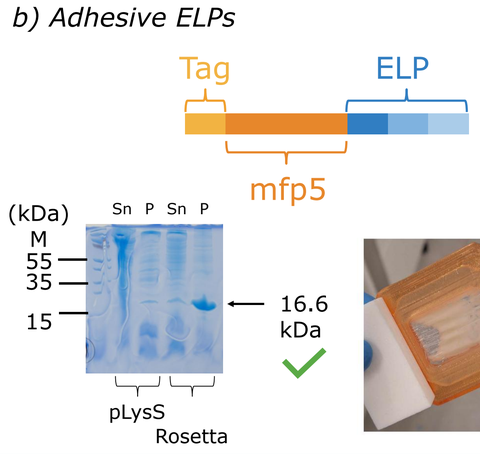
Translation of 'ideal' conditions to other ELPs

a) MMP-responsive ELPs

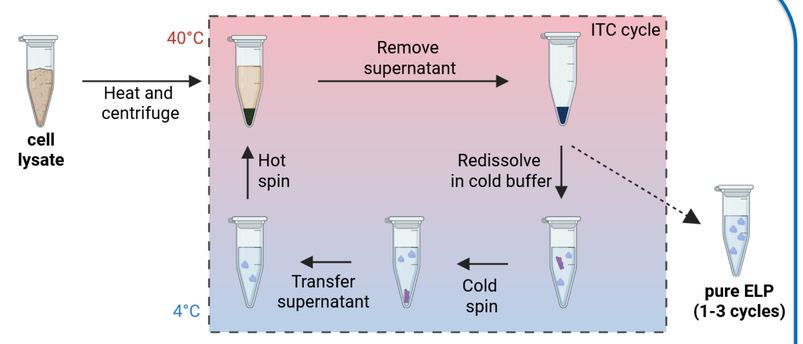




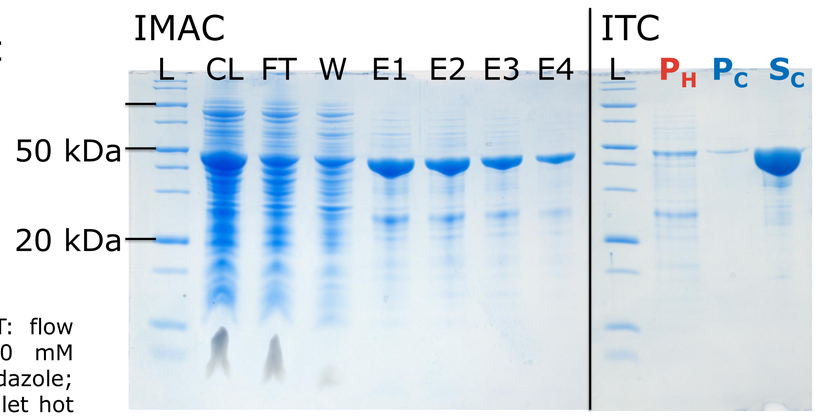
b) Adhesive ELPs



Moving forward



ITC outperforms IMAC
- More cost-effective
- Higher recovery



L: ladder; CL: cell lysate; FT: flow through; W: wash; E1: 100 mM imidazole; E2-E3: 250 mM imidazole; E4: 400 mM imidazole, P_H: pellet hot spin; P_C: pellet cold spin; S_C: supernatant cold spin;

Protein purification

Conclusions

Recombinant DNA technology allows unparalleled control over a biomaterial's structure and properties. They are definitely ***worth the effort***.

functional · modular · defined · degradable · ethical

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