Heparin-binding domains in elastin-like proteins: a way towards improved tissue integration?

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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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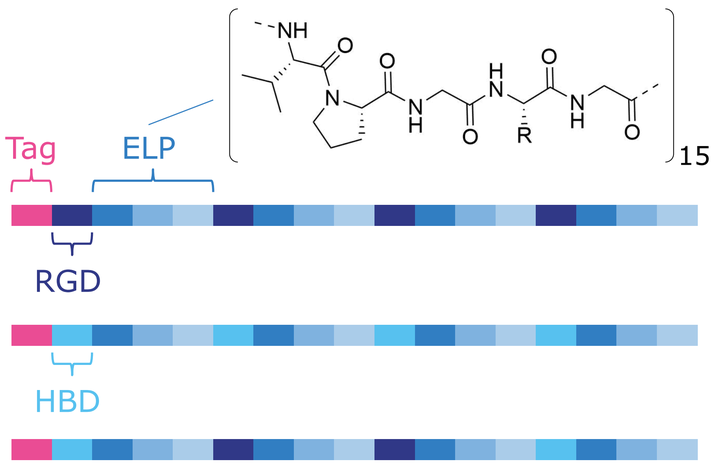
Gene construction

Heparin-binding domains (HBD) for improved tissue integration

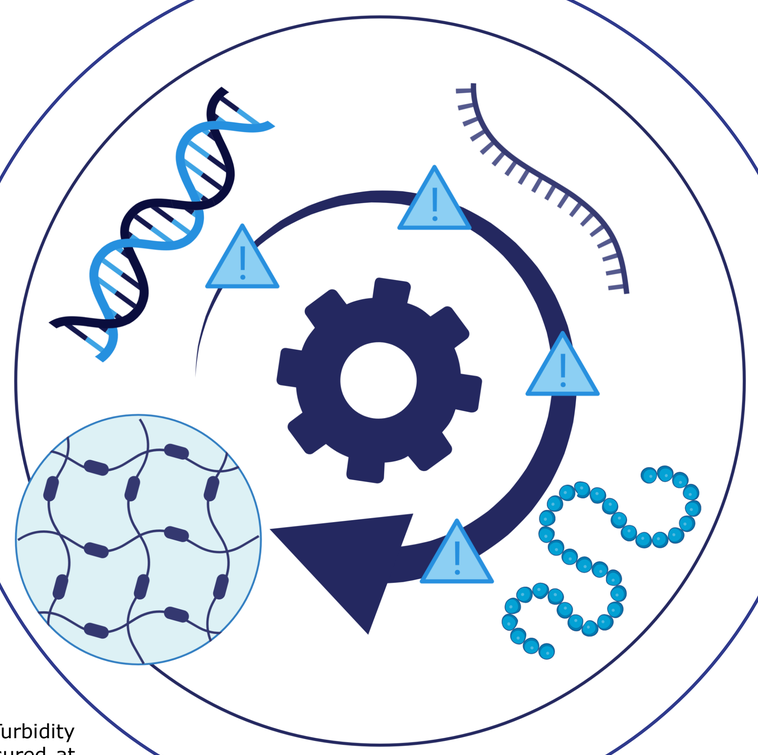
LCST behaviour



RGD: YAVTG**RGD**SPASSAA

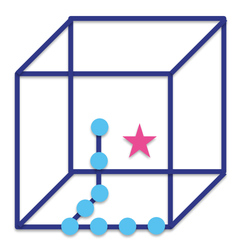
HBD: GSSSG**WQPPRARI**

Points of attention
Repeats
Codon bias
Vector selection

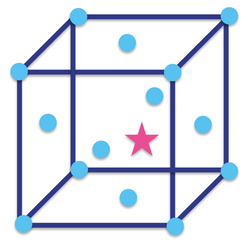


Points of attention
Host cell selection
Culture conditions
Protein of interest

Protein expression



OFAT



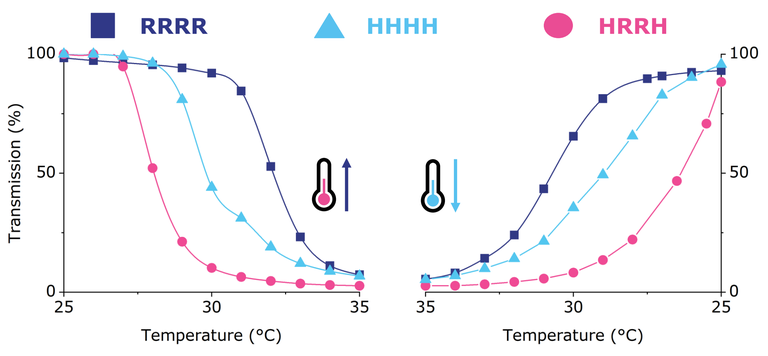
DoE

➔ Identified ideal expression conditions for each of the constructs

➔ 12 fold increase in yields

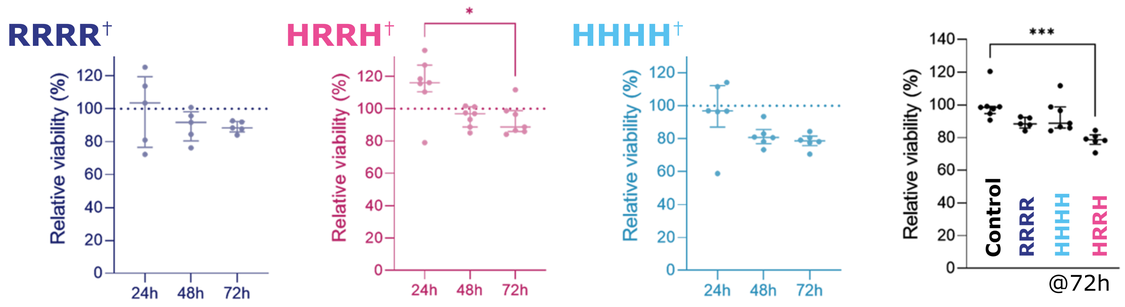
	Strain	Temp (°C)	IPTG (mM)	OD (-)	Yield (mg L^{-1})
RRRR	pLysS	32	0.5	0.4	**69**
RHHR	pLysS	32	0.1	1	**72**
HHHH	BLR	37	0.4	1	**60**

LCST behaviour

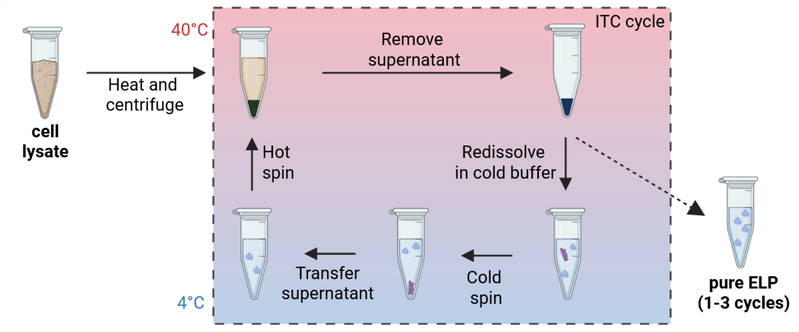


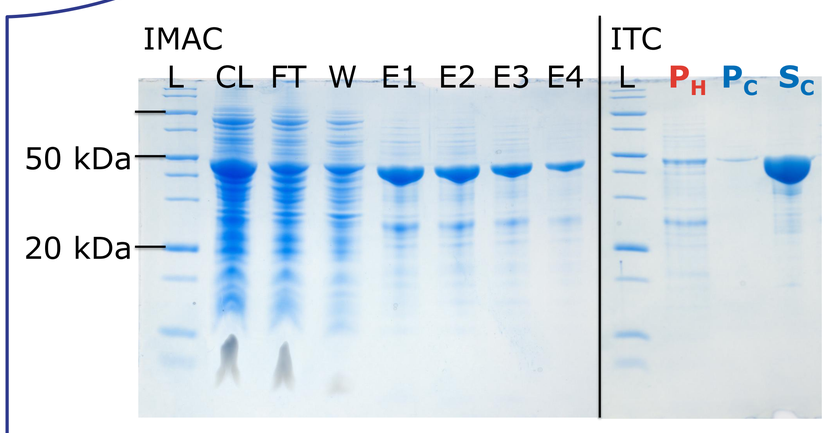
< Turbidity measured at 350 nm for ELP samples (2 mg mL^{-1} in PBS)

Cell viability (HUVECs)



^ HUVECs of passage 4 to 8 were exposed to either 10 mg/mL of ELP-RRRR (n=7), ELP-RHHR (n=7), ELP-HHHH (n=5) or to EGM-2 + 10% FCS (control) (n=7). Data are represented as median ± interquartile range (IQR). * denotes $p < 0,05$, *** denotes $p < 0,001$.





< 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;

ITC outperforms IMAC
- More cost-effective
- Higher recovery

ELP characterisation

Protein purification

Conclusions and future perspectives

Recombinant DNA technology comes with a steep learning curve, but allows unparalleled control over a biomaterial's structure and properties. A DoE approach allowed us to increase protein yield in a systematic and reproducible way. The studied constructs showed favourable LCST transition temperatures and high cell viability. Future work will look into the mechanical properties of ELP-based hydrogels and their interaction with ECM components.

functional modular ethical

Funding information

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