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Adhesion Miniproteins for Tissue Engineering – From Molten Globule to Active Metalloprotein

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Introduction

Tissue Engineering requires extracellular matrix (ECM)-like scaffold materials to support the formation of tissue from cells. Our goal is to create a biomimetic material that uses immobilized designed miniproteins mimicking the Ca^{2+}-carbohydrate-interaction of Laminin or other LG domain-containing ECM proteins and the cell-surface receptor Dystroglycan [1].

As designed peptides tend to bind their targets with a weaker affinity, we chose a tight-binding natural model – calmodulin [2] – to achieve a micromolar binding affinity similar to the LG domain-calcium interaction [3].

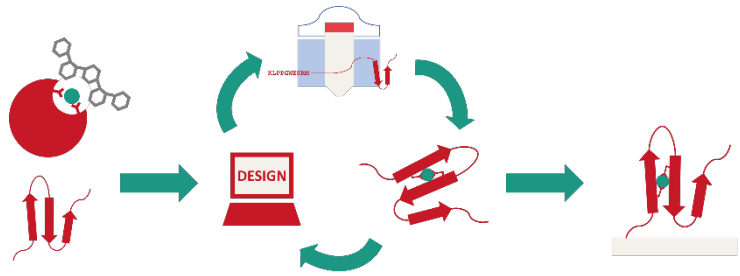


Fig. 1. Design of an ECM-mimetic material.

Design Strategy

The peptides were designed intuitively from a natural model and a small β-peptide scaffold (Figure 1). For this, the active center of the natural model protein – calmodulin – was overlaid with the crystal structure of the scaffold domain (Figure 2). Once a fitting position was found, the Scaffold was mutated to imitate the binding site of the natural model. Rosetta Relax for geometry optimization and evaluation.

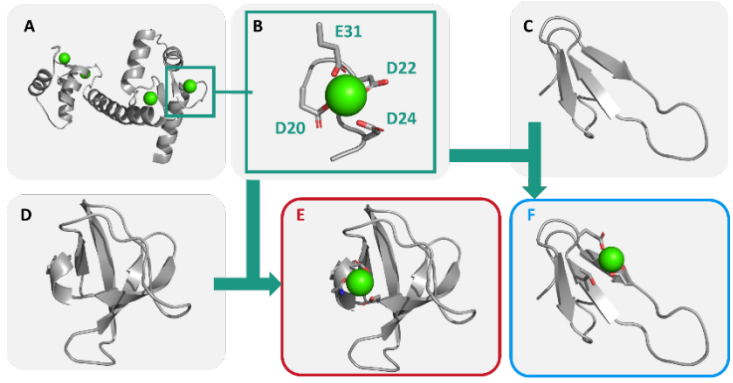


Fig. 2. Concept of the intuitive Design of adhesion miniproteins. ***(A)*** *Natural model: calmodulin,* ***(B)*** *active center,* ***(C)*** *WW domain,* ***(D)*** *SH3 domain and designed peptides* ***(E)*** *Scan 1 and* ***(F)*** *WWcalm 3.1.*

Structural Analysis

CD spectroscopy was used to determine the secondary structure of the newly designed peptides Scan and WWcalm, based on calmodulin and the SH3 and WW domains respectively (Figure 3).

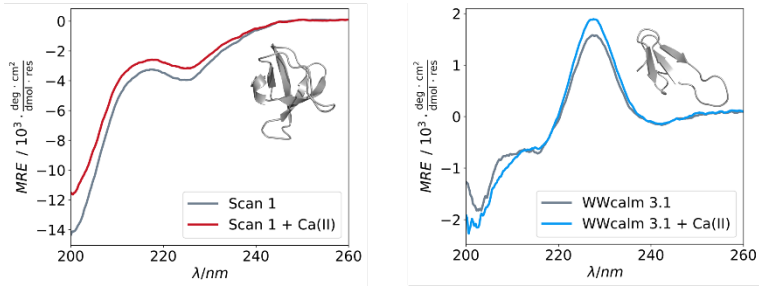
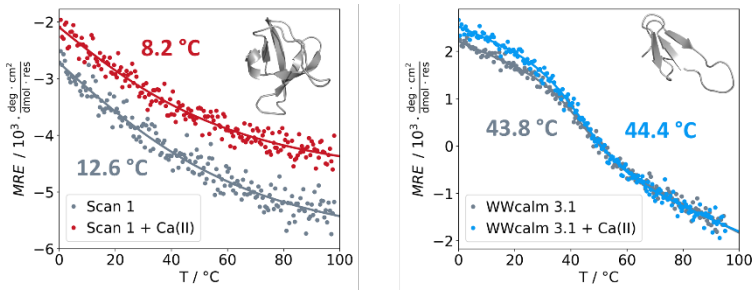


Fig. 2. CD spectra of (left) Scan 1 and (right) WWcalm 3.1 in the absence (grey) and presence of $Ca2^{+}$ (colored) in buffered solution (NEM, pH 7.2).

Fig. 3. Thermal denaturation curves of (left) Scan 1 and (right) WWcalm 3.1 in the absence (grey) and presence of Ca^{2+} (colored) in buffered solution (NEM, pH 7.2).



Stability Improvement

A more stable mutant of WWcalm 3.1 was designed using our WW domain library. The Design of a stable Scan peptide was unsuccessful (Figure 4 and 5).

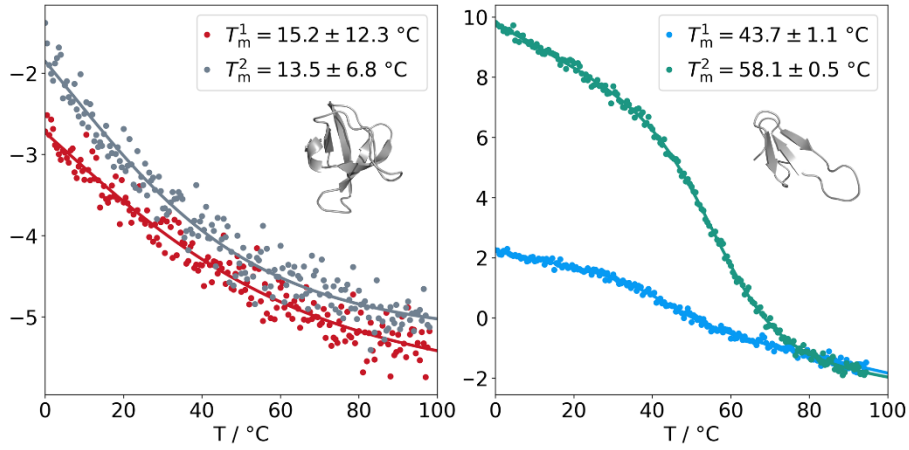


Fig. 4. Left: Thermal denaturation curves of Scan1-2. Right: Thermal denaturation curves of WWcalm 3.1-2.

Table 1. Protein domains or designed Peptides and their dissociation constants for Ca^{2+}.

	Calmodulin	*Laminin*	*Neurexin*	*Scan1*	*WWcalm3.1*	*WWcalm3.2*
K_d [μM]	0.53 - 1.18	5 – 300	400	108 ± 94	14.5 ± 6.5	80 ± 210
Ca^{2+}-binding domain	EF-hand	LG3-5	LG2	EF-hand	EF-hand	EF-hand
Reference	[2]	[3]	[5]	Design	Design	Design

Competitive Binding Assay

The UV-signal of Br_2-BAPTA at 263 nm is calcium-dependent (Figure 6). With its K_d identified, we used the dye to determine our peptides' K_d in a competitive binding experiment (Figure 7) based on a protocol by Linse et al. [4].

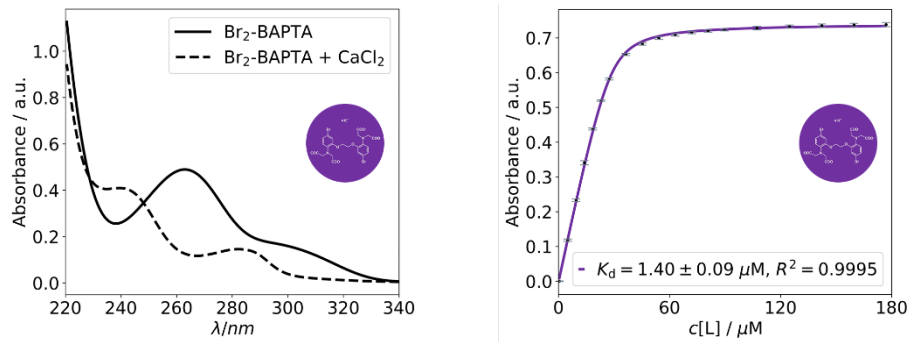


Fig. 5. Left: UV spectrum of Br2-BAPTA in the presence (dashed) and absence (straight) of Ca2+.

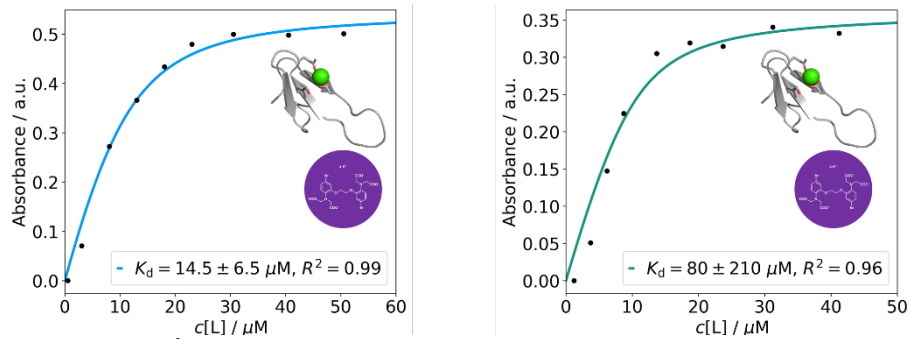


Fig. 6. Competitive Ca^{2+}-Binding assays of WWcalm3.1 (left) and WWcalm3.2 (right) versus Br_2-BAPTA.

Conclusions

We designed three Ca^{2+} -binding peptides based on the WW and SH3 domains. Structural analysis showed correct folding and structural changes upon Ca^{2+} addition. Stabilization of the metastable Scan was unsuccessful.

We determined K_d values for Scan 1 (108 μM), WWcalm 3.1 (14.5 μM) and 3.2 (80 μM) in the range of LG domains. The carbohydrate-binding will be investigated next.

Acknowledgments

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References

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