Rational Design of Fc-Binding Peptide Ligands as Next-Generation Alternatives to Protein A for Antibody Purification

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INTRODUCTION

Staphylococcal Protein A (SpA) is a 42 kDa cell wall-associated virulence factor that contributes to bacterial immune evasion by binding to human IgG1[1,2]. Owing to its high affinity and specific interaction with the Fc region of IgG, SpA is widely used as an affinity ligand for antibody purification[3]. However, its high cost and limited stability under harsh purification and regeneration conditions have created a need for alternative affinity ligands. In this study, a computational peptide engineering strategy was employed to design novel Fc-binding peptide ligands derived from the Protein A–IgG interaction interface.

MATERIAL & METHODS

Peptide Ligands Prediction

Novel peptide ligand candidates were predicted using Rosetta PeptiDerive[4] based on the Protein A–human IgG1 Fc complex (PDB ID: 4WWI), with chains A and D selected for peptide prediction. Each amino acid in the original peptide sequence was screened against all 20 possible amino acids using FoldX[5], and substitutions predicted to improve stability (ΔΔG < −0.5 kcal/mol) were selected, resulting in 15 novel peptide ligand candidates.

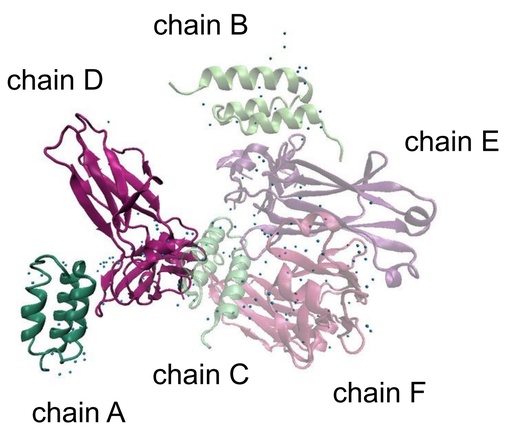


Figure 1. The crystal structure of the C domain of staphylococcal protein A in complex with the Fc fragment of human IgG (PDB ID: 4WWI).

Together with pep0, all 16 peptides were modeled using PEP-FOLD 4.0 and evaluated by molecular docking, interaction analysis, and MD simulations. Based on their similarity to the Protein A–IgG1 interaction, pep9, pep15, and pep16, along with pep0, were selected and synthesized by solid-phase peptide synthesis (SPPS).

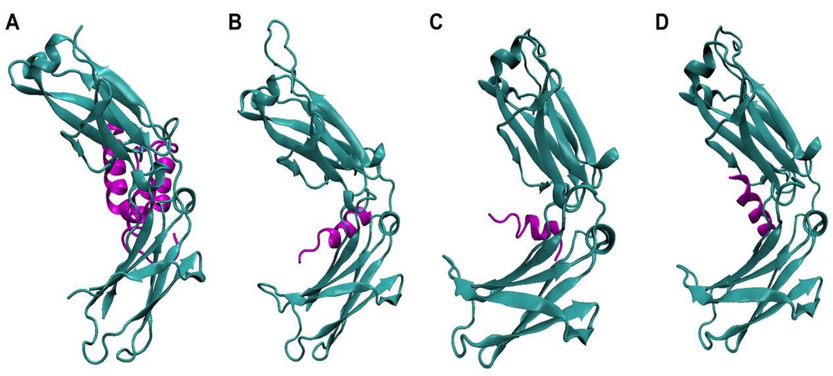


Figure 2. HADDOCK based local docking showing the interaction between the Fc fragment of human IgG1 and **A.** the C domain of protein A (positive control) **B.** pep9, **C.** pep15, and **D.** pep16.

Peptide - Human IgG Interaction

The binding affinities of the synthesized peptide candidates were experimentally evaluated by Microscale Thermophoresis (MST; NanoTemper). Humira® (adalimumab) and Herzuma® (trastuzumab), both human IgG1 monoclonal antibodies, were used as target molecules. Eculizumab, a humanized IgG2/IgG4κ hybrid monoclonal antibody, was included as a non-IgG1 control to assess potential IgG subclass-dependent binding.

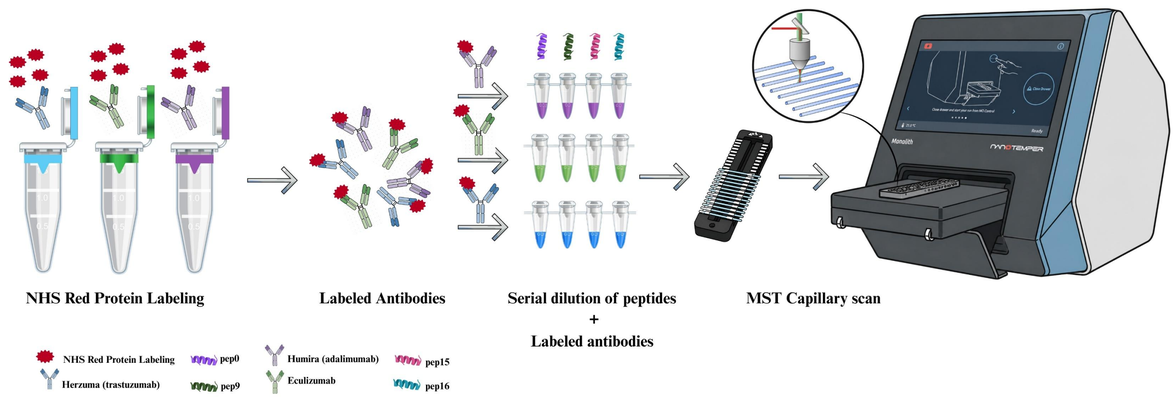


Figure 3. Schematic representation of the MST workflow.

RESULTS

The MST results demonstrated that the rationally optimized peptide candidates exhibited improved binding to human IgG1 compared with the original peptide sequence, pep0, which was directly derived from the Protein A–human IgG1 interaction interface. In particular, pep9, pep15, and pep16 showed higher binding affinities than pep0. In contrast, no detectable binding was observed between the peptide ligands and eculizumab, a humanized IgG2/IgG4κ hybrid monoclonal antibody used as a non-IgG1 control, suggesting preferential binding of the designed peptides toward IgG1.

The dose–response curves obtained from MST measurements are presented in Figure 4, and the corresponding KD values are summarized in Table 1. Overall, pep15 exhibited the lowest KD value and therefore the highest binding affinity toward human IgG1, followed by pep9 and pep16. These findings indicate that the computationally guided sequence modifications successfully enhanced the IgG1-binding properties of the parent peptide and support the effectiveness of the rational peptide optimization strategy in identifying improved Protein A-mimetic affinity ligands.

Table 1. KD values of peptide ligands for Humira and Herzuma determined by MST analysis.

Peptide	KD value (µM)	
	Humira (adalimumab)	Herzuma (trastuzumab)
Pep0	98	45.46
Pep9	0.027	1.47
Pep15	0.009	0.008
Pep16	41.72	1.16

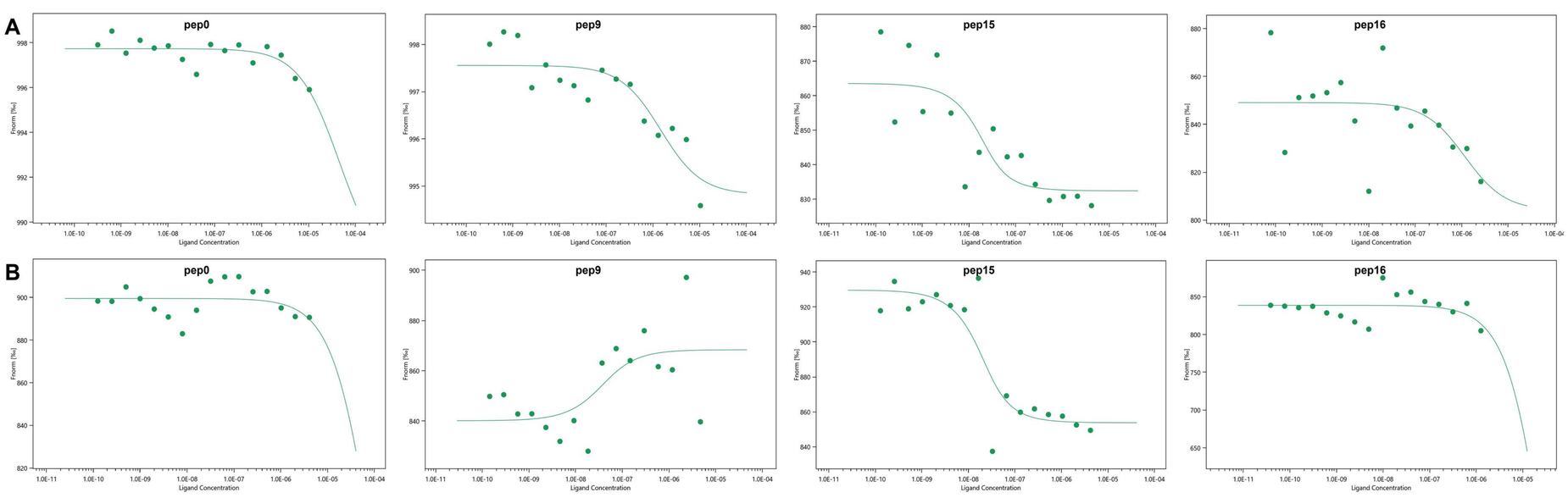


Figure 4. MST dose–response curves showing the binding interactions of the peptide ligands (pep0, pep9, pep15, and pep16) with human IgG1 monoclonal antibodies: **A.** Herzuma and **B.** Humira.

REFERENCES

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