

## Structure of the artificial protein AF.p17 developed by adaptive assembly

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### 1 Introduction

Protein engineering that exploits known functional peptides holds great promise for generating novel functional proteins. We have proposed a combinatorial approach termed adaptive assembly that provides a tailor-made protein scaffold for a given functional peptide [1]. A combinatorial library was designed to create a tailor-made scaffold which was generated from  $\beta$ -hairpins derived from a 10-residue minimal protein “chignolin” and randomized amino acid segments extended at the N- and C-terminus of a functional peptide. We applied adaptive assembly to a peptide with low-affinity for the Fc region of human immunoglobulin G, generating a 54-residue protein AFFinger p17 (AF.p17) with a 40,600-fold enhanced affinity. Here, we report on the crystal structure of AF.p17 complexed with the Fc region, and discuss the enhancement mechanism at the atomic level.

### 2 Experiment

The complex of AF.p17 and the Fc region was dialyzed against 20 mM Tris-HCl (pH 7.4) containing 10% dimethyl sulfoxide (DMSO), and then concentrated to 10 mg/ml. A crystal of the complex was obtained by using sitting drop vapor diffusion under 40% polyethylene glycol 4000, 0.1 M sodium citrate (pH 5.6), 0.2 M ammonium acetate. The diffraction data were collected under cryogenic conditions (100 K) at Photon Factory NW-12 and processed by HKL-2000. The crystal structures were determined by molecular replacement (Molrep) in the CCP4 suite by using a search model (PDB code, 1DN2), and refined by using CNS, REFMAC5 and Coot. The atomic coordinates and structural factors have been deposited in the Protein Data Bank (code 3WKN).

### 3 Results and Discussion

The crystal structure of AF.p17 complexed with the Fc region was determined at 2.9 Å resolution [1]. The asymmetric unit contained the four complexes. The overall structure of AF.p17 consisted of four  $\beta$ -strands, three  $\beta$ -hairpins, a loop-like region, and a short  $\alpha$ -helix (Figure 1). The C- and N-terminal extended segments adopted different conformations. The C-terminal segment formed a short  $\alpha$ -helix with protruding side chains that directly contacted with the binding region (Figure 1a). We term this  $\alpha$ -helix a “tail-helix-latch” that worked as a latch to support the active conformation of the binding region. Particularly, the hydrophobic side-chain of Met48 in the tail-helix-latch stabilized the conformation of the Trp30 aromatic ring. The side-chain of Trp45 contacted

with Met48, resulting in a small hydrophobic cluster of Trp30, Met43, and Trp45. Compared to the C-terminal segment, the N-terminal segment formed a loop-like structure that contacted with non-functional residues in the two  $\beta$ -hairpin corresponding to the base of the binding region (Figure 1b). This indicates that the N-terminal segment supported the overall structure of the binding region, in contrast to the C-terminal segment that directly fixed the active conformation of the binding region. To evaluate the effects of these residues on binding function, we performed alanine scanning mutagenesis. All of the alanine-substituted mutants showed decreases in affinity. In particular, Trp45Ala and Met48Ala resulted in more than 100-fold decreases in affinity, indicating that these residues critically impacted the binding function by supporting the functional segment.

In conclusion, adaptive assembly provided a functional peptide with a tailor-made protein scaffold. We propose adaptive assembly as a useful methodology for functional enhancement of a given functional peptide.

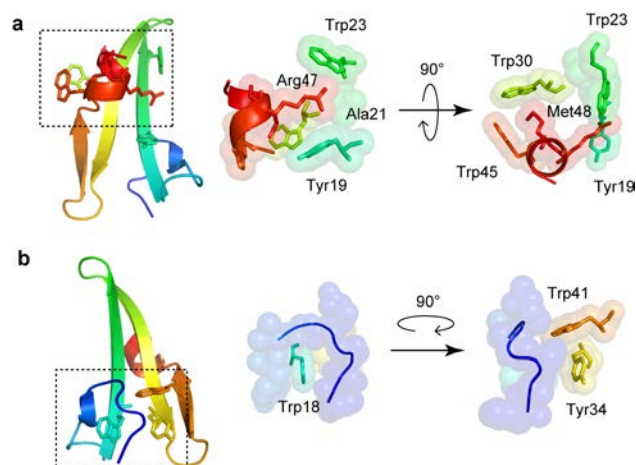


Fig. 1: The crystal structure of AF.p17. (a) The C-terminal segment formed the tail-helix-latch (red) to support the conformation of the binding region (green). (b) The N-terminal segment formed a loop-like structure (blue) to support the base of the binding region.

### References

- [1] H. Watanabe *et al.*, *Chemistry & Biology*, in press (2015).

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