Discovery of a cyclotide-based coagulation Factor XIIa inhibitor by mRNA display

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Cyclotides are plant-derived disulfide-rich cyclic peptides characterized by their head-to-tail cyclic backbone and cystine knot core, which make them attractive pharmaceutical candidates with promising enzymatic stability and cell permeability.[1] Engineering potent bioactivity against human pharmaceutical targets into cyclotides is of interest, however, has proven to be challenging due to the structural complexity.

In this work, we report the rapid discovery of potent and selective cyclotide-based coagulation factor XIIa (FXIIa) inhibitors using mRNA display. A cyclotide-based peptide library containing 12 randomized amino acids was synthesized and screened against FXIIa by mRNA display, which affords high library diversity (more than 10^{12} compounds) (Figure 1a). 5 of the most abundant selected peptides were synthesized by solid phase peptide synthesis (SPPS). Surface plasma resonance (SPR) and *in vitro* inhibitory assay revealed the most potent binder, cMCoFx1, which exhibits exceptional binding affinity (K_D = 0.9 nM) and inhibitory activity (K_i = 0.37 nM) towards FXIIa. Further inhibitory profiling with a panel of structurally and functionally related serine proteases showed high selectivity of cMCoFx1 towards FXIIa. X-ray analysis of co-crystal of cMCoFx1 and FXIIa revealed tight binding of the peptide to the enzyme in a substrate-like manner, with insertion of Arg at the peptide P1 site to the enzymatic S1 pocket (Figure 1b). These results elucidate the capability of mRNA display for identifying cyclotide-based FXIIa inhibitors, which are appealing candidates for therapeutic development.

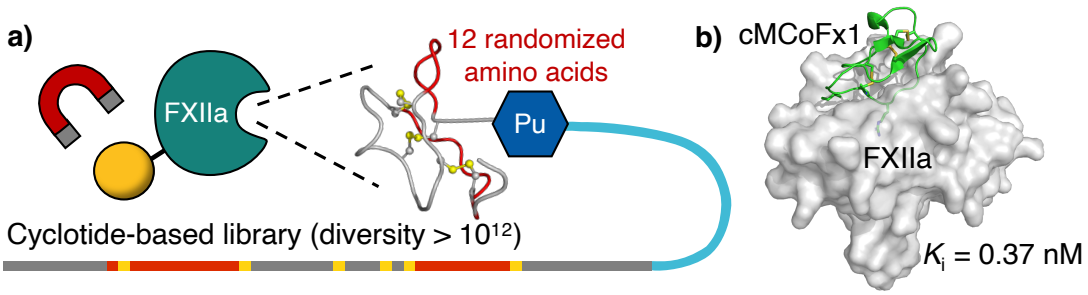

Figure 1 a) Discovery of cyclotide-based FXIIa inhibitors using mRNA display. b) Co-crystal structure of cMCoFx1 binding to FXIIa.

1) S. J. De Veer, J. Weidmann, D. J. Craik, *Acc. Chem. Res.* **2017**, *50*, 1557.