SUPPLEMENTARY MATERIAL

An engineered tryptophan zipper-type peptide as a molecular recognition scaffold

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Supplementary Methods

Library construction for FRET-based screening

The initial library of 24mers with all possible amino acid substitutions at cross-strand positions 5 and 20 was constructed by an oligonucleotide extension reaction of two oligonucleotides with 23 bases of complementary sequence (italicized) to produce a dsDNA fragment with a 5' SacI site and a 3' Kpn1 site (underlined). The two oligonucleotides were 5'-GT GGA GCT CAG AAG GCC TGG ACC NNK ACT TGG ACA T*GG AAT CCC GCT ACG GGC AAA TGG*-3' and 5'-TGC GGT ACC CTC GTT CTT TCT MNN GGT CCA TGT *CCA TTT GCC CGT AGC GGG ATT CC*-3'. To produce the dsDNA fragment, a 1:1 mixture of these oligonucleotides (1.5 μM) was subjected to 10 thermal cycles (30 s at 95 °C, 30 s at 55 °C, 45 s at 72 °C) in the presence of dNTPs (0.2 mM each), Taq polymerase (2.5 units), and the buffer provided by the manufacturer. The procedure for preparing the second library of 24mers with all possible amino acid substitutions at positions 6 and 19 was similar to that described above. The primary difference was that a mixture of 3 forward primers and 2 reverse primers was used in order to introduce the desired degeneracy. The forward primer mix included equimolar amounts of 5'-GT GGA GCT CAG AAA GCT TGG ACA TGG NNK TGG ACT TGG AAC *CCC GCC ACA GGC AAA TGG*-3', 5'-GT GGA GCT CAG AAA GCT TGG ACA SRC NNK TGG ACT TGG AAC *CCC GCC ACA GGC AAA TGG*-3', and 5'-GT GGA GCT CAG AAA GCT TGG ACA AAG NNK TGG ACT TGG AAC *CCC GCC ACA GGC AAA TGG*-3'. The reverse primer mix included equimolar amounts of 5'-TGC GGT ACC CTC GTT CTT CCT CCA MNN CCA CGT *CCA TTT GCC TGT GGC GGG*-3' and 5'-TGC GGT ACC CTC GTT CTT CCT GSK MNN CCA CGT *CCA TTT GCC TGT GGC GGG*-3'.

To prepare libraries of the 24mer peptide with random substitutions, pZC1 containing **xxtz0.5** (or **xxtz1**) was used as a template for error prone PCR with primers 5'-GGG ATC ACT ACT AGT GGA GCT CAG-3' and 5'-G CTC CTC TGC AGA GGT ACC-3'. Four reactions, each one with one dNTP at 0.025 mM and three dNTPs at 0.25 mM, were performed under otherwise standard error prone PCR conditions using Taq DNA polymerase in the presence of 0.15 mM Mn^{2+} [1].

The dsDNA resulting from either oligonucleotide extension or error prone PCR was separated on 3% agarose gel, purified by gel extraction, and digested with Sac1 and Kpn1 to produce sticky ends. The digested and purified dsDNA was ligated with the pZC1 vector that had been previously digested with the same two enzymes. The ligation mixture was used to transform *E. coli* DH10B (Invitrogen) by electroporation and transformed bacteria were plated on 10 cm Petri dishes containing LB/agar supplemented with ampicillin (0.1 mg/mL) and arabinose (0.2% w/v) at a dilution that provides several hundred colonies per dish.

Library construction for phage display

The phagemid vector used in phage display was constructed as follows. The phagemid pComb3HTT was digested with SfiI to prepare the linear vector with sticky ends [2] and subsequently ligated with a synthetic dsDNA that encoded a linker region with multiple restriction sites and had sticky ends compatible with Sfi1. The linker dsDNA was assembled by slow cooling of the mixed oligonucleotides 5'-*CG GCC GAG CTC CAT GGT ACC GGC GGT AGC TCT AGA GCG CTC GAG GGT GGC TCT GGG CCC TCT ACT AGT GGC CA*G GC -3'and 5'-*TG GCC ACT AGT AGA GGG CCC AGA GCC ACC CTC GAG CGC TCT AGA GCT ACC GCC GGT ACC ATG GAG CTC GGC CG*C CT-3'. The resulting vector, designated pComb3ZC, had a convenient set of restriction sites (SacI/KpnI/XbaI/XhoI/ApaI/SpeI) for library insertion.

To generate the molecular diversity of the **xtz1**-peptide-based '**face**' phage display library, six residues of the **xtz1**-peptide β-hairpin were randomized. The dsDNA fragment encoding the peptide library was constructed by primer extension reaction with the two partially complementary oligonucleotides 5'- GTT GAG CTC AAG GCC TGG NNK TGG NNK T*GG AAT CCC NNK NNK GGC AAA TGG* -3' and 5'- ACC CTC GAG CTC GTT CTT MNN CCA MNN *CCA TTT GCC MNN MNN GGG ATT CC* -3'.

To generate the molecular diversity of the **xxtz1/loop**-peptide-based '**loop**' phage display library, a sequence of 7 randomized residues was inserted in the turn region of the **xxtz1**-peptide. To generate the dsDNA fragment encoding the peptide library, ssDNA encoding the desired randomized residues 5'-*C TGG ACT TGG AAT CCC GGC* $(NNK)_7$ *GGT GGC AAG TGG ACA TGG C*-3' was PCR amplified with the primers 5'-GGT GAG CTC AAA GCT TGG ACC CAC GA*C TGG ACT TGG AAT CCC GGC*-3' and 5'-AAC CTC GAG TTT GTT CTT TCT CCA CA*G CCA TGT CCA CTT GCC ACC*-3'.

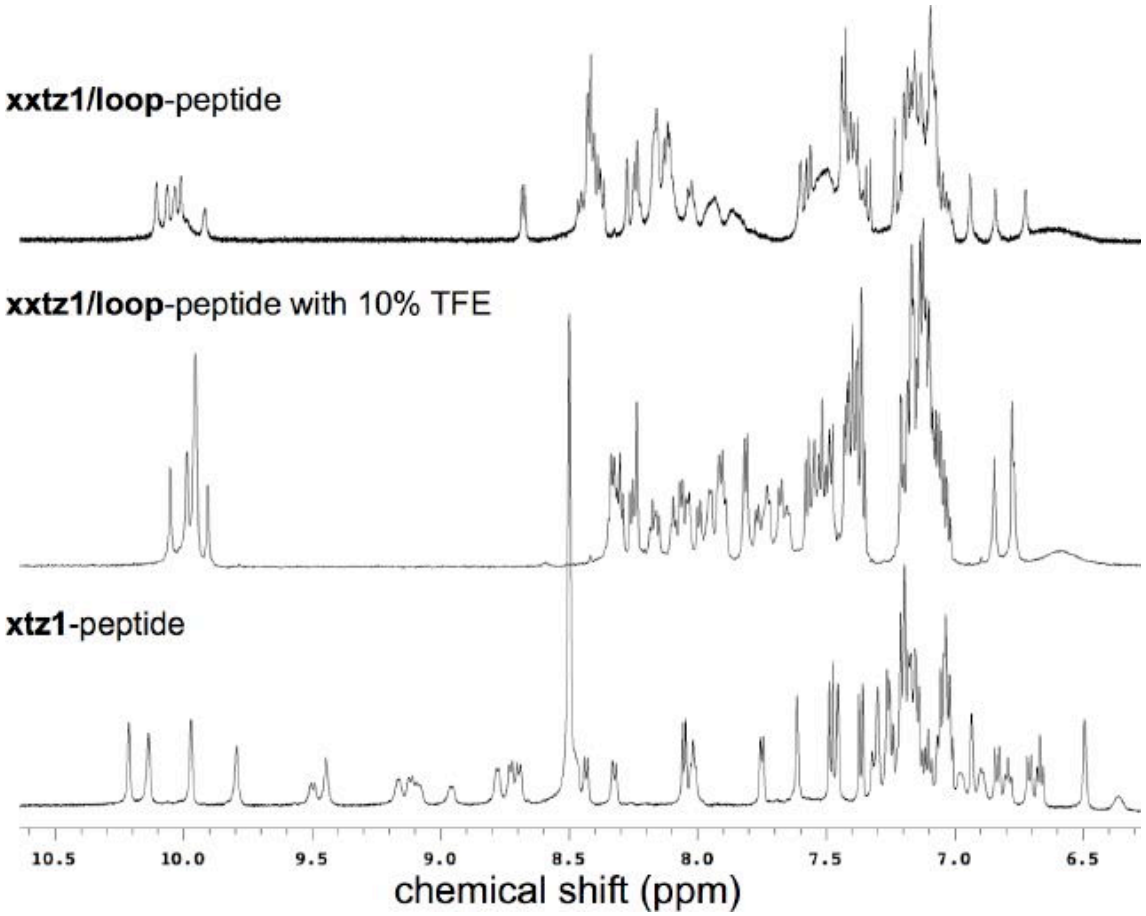
For both the **face** and **loop** libraries, the dsDNA fragment was purified by QIAEX II Gel Extraction Kit (Qiagen) and digested with SacI and XhoI. The digested product was ligated into the pComb3ZC phagemid that had been digested with the same two restriction enzymes. The ligation was performed with a molar ratio of 3:1 insert:vector for a total of 20 μg DNA in a 2 mL reaction volume. The DNA was precipitated with addition of 0.2 mL of 3 M sodium acetate (pH 5.2) and 4.4 mL of ethanol, resuspended in water, and later used for the transformation by electroporation of *E. coli* XL1-blue (Stratagene). In order to determine library size, appropriate dilutions of the transformed bacteria were plated on LB/agar plates containing carbenicillin (0.1 mg/mL) and incubated at 37 °C overnight. Based on colony counts, the **face** library contained 4×10^8 unique clones, and the **loop** library consisted of 7×10^7 unique clones. The theoretical gene diversity of these libraries is $32^6 = 1.07 \times 10^9$ and $32^7 = 34.4 \times 10^9$, respectively.

The transformed XL1-blue *E. coli* cells were cultured for two hours at 37 °C in super broth medium (SB) containing carbenicillin (50 μg/mL) and tetracycline (10 μg/mL). VCSM13 helper phage (Stratagene) was then added to the culture and, following an additional incubation period of 1.5 hours at 37 °C, kanamycin (70 μg/mL) was also added. The culture was incubated overnight at 37 °C with shaking at 300 rpm. The next day, bacteria and phage were separated by centrifugation (15 min, 8000 rpm) and the phage particles were precipitated by adding PEG-8000 (4% w/v) and NaCl (3% w/v). The phage pellet was harvested by centrifugation (30 min, 10,000 rpm), resuspended in PBS buffer, and used immediately for library panning.

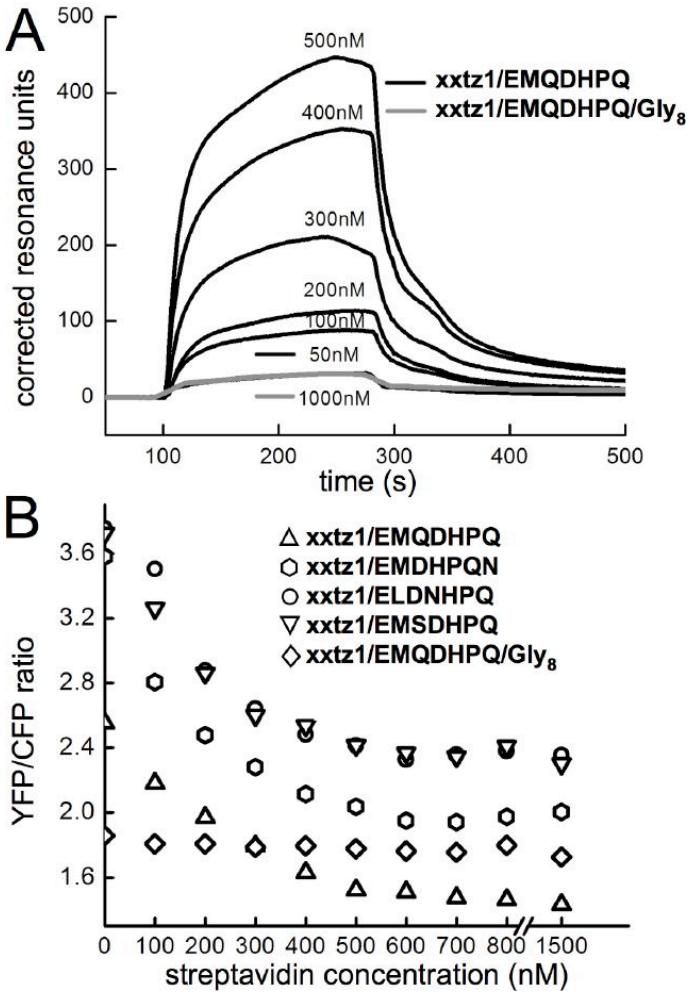
Plasmids for production of CFP-peptide-YFP proteins

Peptide sequences selected by phage display were PCR amplified from their purified phagemeids using the primers 5'-GT TGA GCT CAG AAA GCT TGG ACG CAC-3 and 5'-CC AGA GCC ACC GGT ACC TTT GTT CTT TCT CC-3'. To construct the peptide portion of the

xxtz1/EMQDHPQ/Gly$_8$ protein a primer extension reaction with the two partially complementary oligonucleotides, 5'-GT GGA GCT CAG AAG GCC TGG ACT CAC GAC TGG ACA TGG AAT CCC GGC *GGA GGT GGG GGA GGC G*-3' and 5'-TGC GGT ACC CTT GTT CTT TCT CCA CAG CCA GGT CCA TTT GCC CC*C GCC TCC CCC ACC TCC*-3', was performed as described above. The resulting dsDNA fragments were purified, digested with Kpn1 and Sac1, and ligated into the the appropriate sites of pZC1 as described above. These plasmids were used for the production of proteins of the general structure CFP-peptide-YFP which were, in turn, used for *in vitro* characterization of streptavidin binding affinity.



Supplementary Figure 1. ^{1}H NMR spectra of **xxtz1/loop**-peptide at room temperature both with and without TFE. The previously acquired ^{1}H NMR spectrum of the highly structured **xtz1**-peptide is shown for reference [3].



Supplementary Figure 2. Analysis of peptide binding to streptavidin. (A) SPR sensorgrams for the **xxtz1/EMQDHPQ** and **xxtz1/EMQDHPQ/Gly$_8$** proteins binding to a streptavidin-coated chip. (B) FRET-based analysis of proteins (50 nM) binding to streptavidin in solution.

Supplementary References

1. Cirino PC, Mayer, KM, Umeno, D. Generating mutant libraries using error-prone PCR. *Methods Mol. Biol*. 2003; **231**: 3-9.

2. Barbas III CF, Wagner, J. Synthetic Human Antibodies: Selecting and Evolving Functional Proteins. *Methods* 1995; **8**: 94-103.

3. Cheng Z, Miskolzie, M, Campbell, RE. In vivo screening identifies a highly folded beta-hairpin peptide with a structured extension. *Chembiochem* 2007; **8**: 880-883.