Supplementary Information

Towards identifying preferred interaction partners of fluorinated amino acids within the hydrophobic environment of a dimeric coiled-coil peptide

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Experimental:

Peptide synthesis, purification and characterization

Table S1. *Peptides used in this study synthesized by SPPS.*

peptide	sequence
Bio-VPK	Biotin-GSGKVSALKEKVASLKEKVSALKEEVASLEEKVSALK-OH
Bio-VPK-Asn$_{16}$	Biotin-GSGKVSALKEKVASLKEKNSALKEEVASLEEKVSALK-OH
Bio-VPK-DfeGly$_{16}$	Biotin-GSGKVSALKEKVASLKEK**DfeGly**SAEKEEVASLEEKVSALK-OH
Bio-VPK-DfpGly$_{16}$	Biotin-GSGKVSALKEKVASLKEK**DfpGly**SAEKEEVASLEEKVSALK-OH
Bio-VPK-TfeGly$_{16}$	Biotin-GSGKVSALKEKVASLKEK**TfeGly**SAEKEEVASLEEKVSALK-OH
VPK	Abz-KVSALKEKVASLKEKVSALKEEVASLEEKVSALK-OH
VPK-Asn$_{16}$	Abz-KVSALKEKVASLKEK**N**SALKEEVASLEEKVSALK-OH
VPK-DfeGly$_{16}$	Abz-KVSALKEKVASLKEK**DfeGly**SAEKEEVASLEEKVSALK-OH
VPK-DfpGly$_{16}$	Abz-KVSALKEKVASLKEK**DfpGly**SAEKEEVASLEEKVSALK-OH
VPK-TfeGly$_{16}$	Abz-KVSALKEKVASLKEK**TfeGly**SAEKEEVASLEEKVSALK-OH
VPE	Abz-EVSALEKEVASLEKEVSALEKKVASLKKEVSALE-OH
VPE-L$_{16}$	Abz-EVSALEKEVASLEKE**L**SALEKKVASLKKEVSALE-OH
VPE-L$_{15}$I$_{16}$	Abz-EVSALEKEVASLEKLISALEKKVASLKKEVSALE-OH
VPE-L$_{15}$I$_{16}$Y$_{19}$	Abz-EVSALEKEVASLEKLISAYEKKVASLKKEVSALE-OH
VPE-Y$_{15}$I$_{16}$	Abz-EVSALEKEVASLEKYISALEKKVASLKKEVSALE-OH

Abz: o-Aminobenzoic acid.

Table S2. *Identification of the synthesized peptides by ESI-TOF mass spectrometry.*

Peptide	calc.$[M+4H]^{4+}$	obs $[M+4H]^{4+}$
Bio-VPK	1024.822	1024.830
Bio-VPK-Asn$_{16}$	1028.565	1028.580
Bio-VPK-DfeGly$_{16}$	1030.313	1030.334
Bio-VPK-DfpGly$_{16}$	1033.817	1033.838
VPK	947.801	947.803
VPK-Asn$_{16}$	951.536	951.5469
VPK-DfeGly$_{16}$	953.292	953.287
VPK-DfpGly$_{16}$	956.796	956.790
VPK-TfeGly$_{16}$	957.789	957.793
VPE	948.267	948.772
VPE-L$_{16}$	951.770	951.781
VPE-L$_{15}$I$_{16}$	947.781	947.809
VPE-L$_{15}$I$_{16}$Y$_{19}$	960.276	960.306
VPE-Y$_{15}$I$_{16}$	960.276	960.307
	calc.$[M+5H]^{5+}$	obs $[M+5H]^{5+}$
Bio-VPK-TfeGly$_{16}$	828.045	828.065

Molecular Biology

Construction of the phage displayed VPE-libraries was carried out as described before.[1] The following randomized oligonucleotides were purchased from *biomers.net GmbH* (Ulm, Germany) and applied for library construction (codons are in reading frame; phosphate at the 5'-end of each oligonucleotide):

Library 1 – positions **d'$_{12}$**, **g'$_{15}$**, **a'$_{16}$** and **d'$_{19}$** randomized:

sense-strand: 5'-CG GCC GAG GTT AGC GCG CTG GAA AAG GAG GTG GCC AGT NNK GAG AAA NNK NNK AGT GCC NNK GAA AAG AAA GTA GCG AGC CTG AAA AAG GAG GTA AGT GCG TTA GAA GGC CAG GC-3'

anti-sense-strand: 5'-TG GCC TTC TAA CGC ACT TAC CTC CTT TTT CAG GCT CGC TAC TTT CTT TTC MNN GGC ACT MNN MNN TTT CTC MNN ACT GGC CAC CTC CTT TTC CAG CGC GCT AAC CTC GGC CGC CT-3'

Library 2 – positions **d'$_{12}$**, **a'$_{16}$** and **d'$_{19}$** randomized:

sense-strand: 5'-CG GCC GAG GTT AGC GCG CTG GAA AAG GAG GTG GCC AGT NNK GAG AAA GAG NNK AGT GCC NNK GAA AAG AAA GTA GCG AGC CTG AAA AAG GAG GTA AGT GCG TTA GAA GGC CAG GC-3'

anti-sense-strand: 5'-TG GCC TTC TAA CGC ACT TAC CTC CTT TTT CAG GCT CGC TAC TTT CTT TTC MNN GGC ACT MNN CTC TTT CTC MNN ACT GGC CAC CTC CTT TTC CAG CGC GCT AAC CTC GGC CGC CT-3'

N stands for A, G, C, and T
K stands for G and T
M stands for A and C

Phage Display Results

Sequencing of randomly picked clones after the last round of panning against the different VPK variants resulted in the following amino acid pattern in the randomized positions of VPE:

Table S3. *Amino acid pattern in the variable amino acid positions of the VPE peptides selected by panning using different substituted VPK variants as target.*

	frequency	**position d'$_{12}$**	**position g'$_{15}$**	**position a'$_{16}$**	**position d'$_{19}$**
VPK wild type	3×	Leu	Tyr	Ile	Leu
	1×	Leu	Tyr	Val	Leu
	1×	Leu	Leu	Ile	Tyr
VPK-Asn$_{16}$	3×	Leu	Glu	Leu	Leu
	1×	Leu	Ala	Leu	Leu
	1×	Leu	Val	Leu	Leu
VPK-DfeGly$_{16}$	5×	Leu	Leu	Ile	Tyr
	1×	Leu	Ala	Ile	Leu
	1×	Leu	Tyr	Ile	Leu
	1×	Leu	Val	Ile	Leu
	1×	Leu	Tyr	Val	Leu
VPK-DfpGly$_{16}$	4×	Leu	Leu	Ile	Tyr
	3×	Leu	Tyr	Ile	Leu
	1×	Leu	Val	Ile	Leu
	1×	Tyr	Leu	Ile	Leu
VPK-TfeGly$_{16}$	4×	Leu	Leu	Ile	Tyr
	2×	Phe	Leu	Ile	Leu
	1×	Leu	Tyr	Ile	Leu
	1×	Leu	Tyr	Val	Leu

The results which were obtained after panning using the truncated VPE library with the positions **d'$_{12}$**, **a'$_{16}$** and **d'$_{19}$** randomized are listed in table S4.

Table S4. *Amino acid pattern in the variable amino acid positions of the VPE peptides selected from the shortened VPE library.*

	frequency	**position d'$_{12}$**	**position a'$_{16}$**	**position d'$_{19}$**
VPK wild type	6×	Leu	Leu	Leu
	2×	Leu	Ile	Leu
	1×	Thr	Ile	Leu
VPK-DfeGly$_{16}$	4×	Leu	Ile	Leu
	2×	Leu	Leu	Leu
	1×	Ile	Leu	Leu
VPK-DfpGly$_{16}$	2×	Leu	Ile	Leu
	1×	Leu	Leu	Leu
	1×	Ile	Leu	Leu
VPK-TfeGly$_{16}$	8×	Leu	Ile	Leu
	2×	Leu	Leu	Leu
	1×	Trp	Ile	Leu
	1×	Leu	Arg	Leu

References

[1] T. Vagt, C. Jäckel, S. Samsonov, M.T. Pisabarro, B. Koksch, *Bioorg. Med. Chem. Lett.* 2009, **19**, 3924-3927.