Electronic Supplementary Material (ESI) for RSC Advances

SUPPORTING INFORMATION

Accommodating Fluorinated Amino Acids in Helical Peptide Environments

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Peptide synthesis:

Peptide synthesis was carried out in a 0.05 mM scale on a Syro-XP-1 peptide synthesizer (MultiSynTech GmbH, Witten, Germany) using standard Fmoc/tBu chemistry as described previously.[1] The coupling mixture contained 0.23 M $NaClO_4$ to prevent on-resin aggregation. Fluorinated amino acids as well as the first subsequent amino acid were activated by means of DIC/HOAt 1:1.The molar excess of amino acid and coupling reagents was reduced for fluorine-containing residues to 1.5-fold for the first and 0.8-fold for the second coupling. These couplings were performed manually until completion was indicated by a negative Kaiser test.[2] Prior to Fmoc deprotection of these amino acids possibly non-acylated N-termini were capped by adding a mixture of acetic anhydride and DIEA (10 % each) in DMF (3×10 min). A mixture of DBU and piperidine (2 % each) in DMF was used for Fmoc deprotection (4×5 min). N-terminal coupling of biotin (Acros) was performed as double coupling using DIC/HOBt as coupling reagents and a fourfold excess of relative to the resin loading. Non biotionylated peptides were cleaved from the resin by treatment with 4 mL TFA/TIS/H_2O (95:2.5:2.5). Biotinylated peptides were cleaved from the resin with TFA–TIS–EDT–H_2O (94:2.5 :2.5 :1) to prevent oxidation of biotin. Purification was carried out by RP-HPLC (Phenomenex®, Luna C_8,10 mm, 250 nm*21.2 mm). Purity and identity of the products was determined by analytical HPLC (Phenomenex®, Luna C_8, 5 µm, 250 nm*4.6 mm) combined with high resolution mass spectra (Agilent 6210 ESI-TOF mass spectrometer, Agilent Technologies, Santa Clara, CA, USA).

Identification of the peptides by ESI-TOF mass spectrometry[a]

Peptide	**calc. $[M+4H]^{4+}$**	**obs. $[M+4H]^{4+}$**
VPE	948.274	948.274
VPE-$L_{16}L_{23}$	955.273	955.786
Bio-VPK-Abu_{19}	1017.556	1018.315
VPK-Abu_{19}	940.793	940.792
VPK-$MfeGly_{19}$	945.292	945.346
Bio-VPK-$MfeGly_{19}$	1022.311	1022.316
VPK-$DfeGly_{19}$	949.788	949.789
Bio-VPK-$DfeGly_{19}$	1026.810	1026.830
VPK-$TfeGly_{19}$	954.286	954.285
Bio- VPK-$TfeGly_{19}$	1031.868	1031.328
VPK-$DfpGly_{19}$	956.796	956.790
Bio-VPK-$DfpGly_{19}$	1030.874	1030.3311

[a] All non-biotinylated peptides bear an N terminal Abz label for exact concentration determination.

Construction of the VPE-library

Library construction was performed by annealing two complementary VPE encoding oligonucleotides in which the library codons are randomized applying the NNK-strategy.[3, 4] 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 sense strand:

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

Library antisense strand:

5'-GC CTG GCC TTC TAA CGC ACT TAC CTC CTT TTT CAG GCT CGC MNN TTT CTT TTC MNN GGC ACT MNN CTC TTT CTC TAA ACT GGC CAC CTC CTT TTC CAG CGC GCT AAC CTC GGC CG-3'

The library DNA was cloned into the pComb3H27 phagemid vector (GenBank database accession number: AF268280, Barbas laboratory, TSRI) by SfiI sites and transformed into E.coli K12 ER2738 (New England Biolabs #E4104S) as described previously.[5] The Library size was calculated to be 1.2×10^7. Production of the phage library was carried out as previously described.[5]

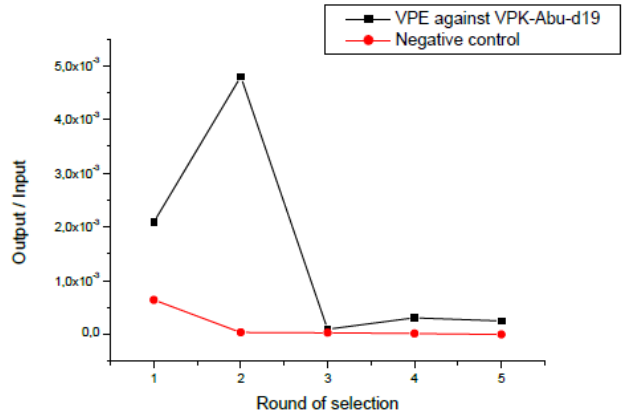
Phage selection and amplification

For phage selection, approximately 10 nmol biotinylated target peptide was immobilized on 30 µL streptavidin-coated magnetic particles (M-280, Dynal Biotech). Particles were washed twice with 500 µL 0.1% Tween20 in PBS. 500 µL 5% non-fat dried milk in PBS was added and the sample was incubated at RT for 45 min. After removing the milk–PBS suspension, 500 µL phage solution were added and phage–target binding was performed for 1.5 h at RT. Subsequently, the particles were washed four times with 500 µL Tween20 in PBS (PBS buffer contained 0.1% Tween 20 in round 1; 1% Tween 20 in rounds 2–5; in round 5 two washing steps with 1 M GndHCl in PBS were added) and once with 500 µL TBS. Bound phages were eluted from magnetic particles by adding 25 µL freshly prepared trypsin solution (10 mg/mL in TBS) and incubation at RT. After 30 min the reaction was quenched with 75 µL SB medium. For reinfection the received phage suspension (100 µL) was transferred to 5 mL E. coli culture. After 30 min at 37°C/200 rpm, 10 µL of the cell culture were removed for outputtitering and 5 mL prewarmed SB medium containing 2.5 µL carbenicillin (100 mg/mL) were added. Cells were incubated for 1 h at 37°C/200 rpm and then transferred to 90 mL prewarmed SB medium to which 46 µL carbenicillin (100 mg/mL) and 1 mL VCSM13 helper phage (Stratagene #200251) were added. After another 1.5 h at 37°C/200 rpm 140 µL kanamycin (50 mg/mL) were added and phages were produced over night. The culture was centrifuged for 30 min at 4°C and 3000 g, and phages were precipitated by the addition of 20 vol% PEG–NaCl [20% (w/v) PEG 8000, 2.5 M NaCl] to the supernatant. After incubation for 30 min on ice phages were centrifuged for 30 min at 4°C and 12 000g. Isolated phages were

suspended in PBS buffer and used in the following round of panning after sterile filtration (0.22 mm).

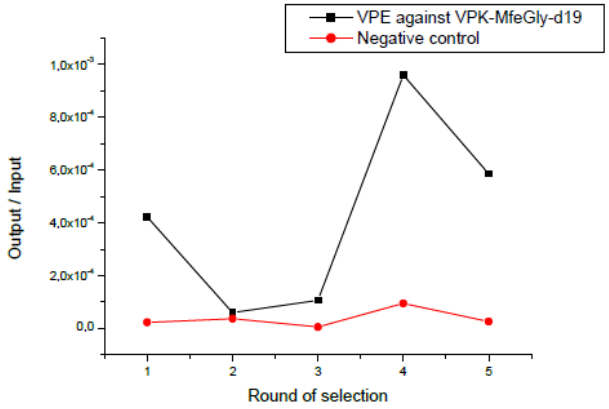
In the following, phage enrichment is depicted as the quotient of selected phages (output) and deployed phages (input) of the respective panning round. Moreover, the phenotypes and genotypes of the three randomized VPE positions (a'_{16}, d'_{19} and a'_{23}) after the 5th panning round against the different VPK-X_{19}-variants are presented.

Selection against VPK-Abu$_{19}$:



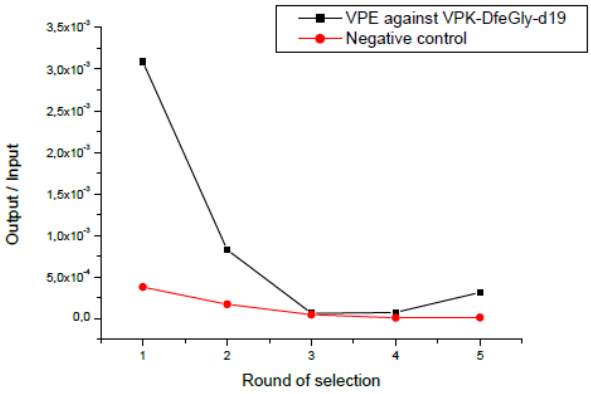
Clone	Pos. a'_{16}	Pos. d'_{19}	Pos. a'_{23}
1	Leu (TTG)	Leu (CTT)	Ile (ATT)
2	Leu (CTT)	Leu (CTG)	Leu (CTG)
3	Val (GTG)	Leu (CTG)	Leu (TTG)
4	Leu (CTT)	Leu (CTG)	Leu (CTT)
5	Leu (CTT)	Leu (CTT)	Ile (ATT)
6	Leu (CTT)	Leu (CTT)	Leu (TTG)
7	Leu (CTG)	Leu (CTG)	Leu (TTG)
8	Leu (CTG)	Leu (CTG)	Leu (TTG)
9	Leu (CTT)	Leu (CTT)	Leu (CTT)
10	Ile (ATT)	Leu (CTT)	Ile (ATT)

Selection against VPK-MfeGly$_{19}$:



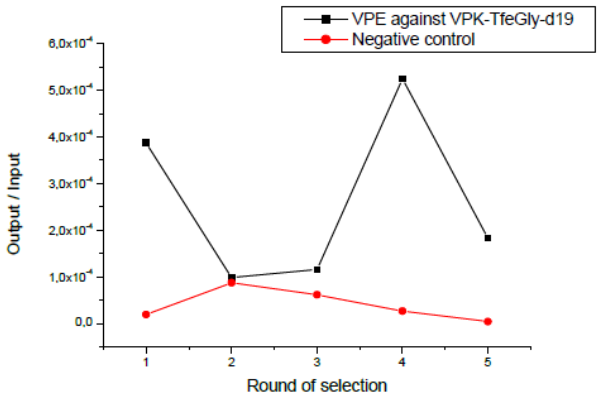
Clone	Pos. a'_{16}	Pos. d'_{19}	Pos. a'_{23}
1	Leu (CTT)	Leu (CTG)	Leu (CTG)
2	Leu (CTT)	Leu (CTG)	Leu (CTG)
3	Leu (CTG)	Leu (TTG)	Leu (CTT)
4	Leu (TTG)	Leu (CTG)	Leu (CTT)
5	Leu (CTG)	Leu (TTG)	Ile (ATT)
6	Leu (CTG)	Leu (CTG)	Leu (TTG)
7	Leu (CTG)	Leu (CTT)	Leu (CTT)
8	Leu (CTG)	Leu (CTG)	Ile (ATT)

Selection against VPK-DfeGly$_{19}$:



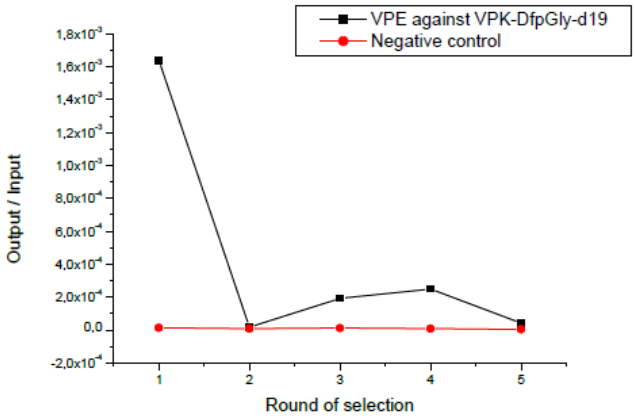
Clone	Pos. a'_{16}	Pos. d'_{19}	Pos. a'_{23}
1	Leu (TTG)	Leu (CTT)	Leu (CTG)
2	Leu (CTT)	Leu (TTG)	Leu (CTG)
3	Leu (CTG)	Leu (CTG)	Leu (TTG)
4	Leu (CTG)	Leu (CTG)	Ile (ATT)
5	Leu (CTT)	Leu (CTT)	Leu (TTG)
6	Leu (CTG)	Leu (CTT)	Ile (ATT)
7	Leu (CTG)	Leu (CTT)	Leu (TTG)
8	Leu (CTT)	Leu (CTG)	Leu (CTG)
9	Leu (TTG)	Leu (CTT)	Leu (CTT)

Selection against VPK-TfeGly$_{19}$:



Clone	Pos. a'$_{16}$	Pos. d'$_{19}$	Pos. a'$_{23}$
1	Leu (TTG)	Leu (CTG)	Leu (CTG)
2	Leu (CTT)	Leu (CTG)	Leu (CTG)
3	Leu (CTT)	Leu (TTG)	Leu (CTT)
4	Leu (CTT)	Leu (CTT)	Leu (CTG)
5	Leu (CTT)	Leu (CTT)	Leu (CTG)
6	Leu (CTT)	Leu (CTT)	Leu (CTG)
7	Leu (TTG)	Leu (TTG)	Leu (CTT)
8	Leu (CTT)	Leu (CTT)	Leu (CTT)
9	Leu (CTG)	Leu (CTT)	Leu (TTG)

Selection against VPK-DfpGly$_{19}$:



Clone	Pos. a'$_{16}$	Pos. d'$_{19}$	Pos. a'$_{23}$
1	Leu (CTG)	Leu (CTT)	Leu (CTG)
2	Leu 2(TTG)	Leu (CTG)	Leu (CTG)
3	Leu (TTG)	Leu (CTG)	Ile (ATT)
4	Leu (CTG)	Leu (CTT)	Leu (CTG)
5	Leu (TTG)	Leu (CTT)	Leu (TTG)
6	Leu (CTT)	Leu (CTG)	Leu (CTG)
7	Leu (TTG)	Leu (CTG)	Leu (TTG)
8	Leu (CTT)	Leu (CTG)	Leu (CTT)
9	Leu (TTG)	Leu (CTT)	Leu (CTT)

SE/SLS:

Static light scattering data were collected on a Dawn Heleos 8 light scattering instrument (Wyatt Technology) coupled with an analytical gel filtration (workstation: La Chrom, VWR, Hitachi, L-2130; column: WTC-015S5; 5µm, 150Å,7.8 x 300 mm, Wyatt Technology) at λ = 220 nm. All measurements were performed at room temperature with a flow rate of 0.3 ml/min. Data were analyzed using the ASTRA software version 5.3.4.20 (Wyatt Technology).

Measurements were taken at pH 7.4 from a 1:1 mixture of **VPK(X$_{19}$)/VPE$_{selected}$** and repeated two times to confirm reproducibility and give standard deviations. Peptide concentrations were determined using the absorbance of *o*-aminobenzoic acid (λ_{max}=320 nm at pH 7.4) as previously described.[1]

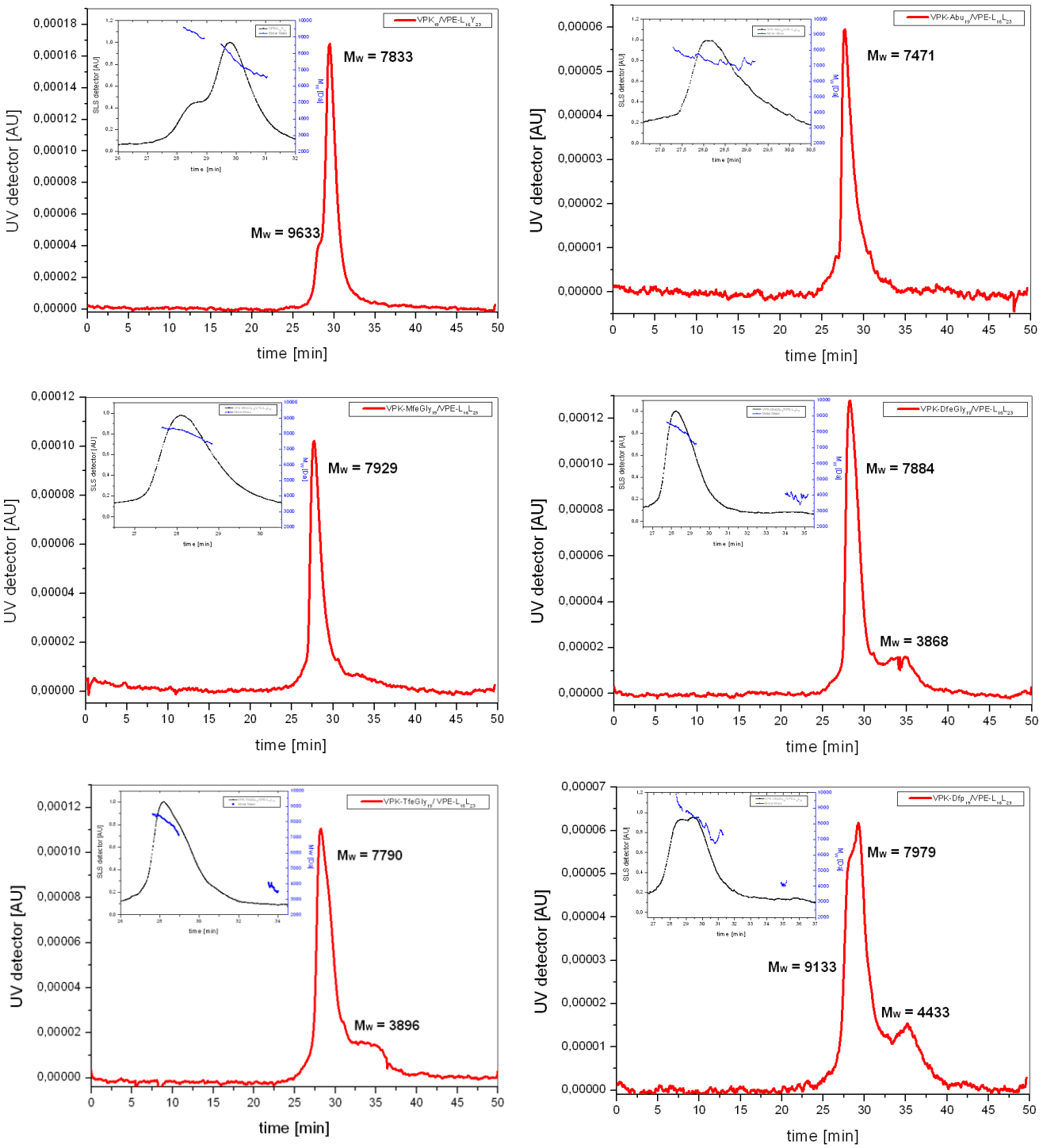


Figure 1: SEC/SLS chromatograms depicted as the mean of 3 experiments. Peptide conc.:35 µM; eluent: 10mM PBS; flow rate: 0.3 ml/min; pH 7.4. Measurements were taken from a 1:1 mixture of **VPK(X_{19})/VPE$_{selected}$** and repeated two times to confirm reproducibility and give standard deviations.

CD spectroscopy

CD spectra were recorded on a Jasco J-715 spectropolarimeter at 20°C (Jasco PTC-348 WI peltier thermostat). Peptide concentrations were determined using the absorbance of *o*-aminobenzoic acid (λ_{max}=320 nm at pH 7.4).

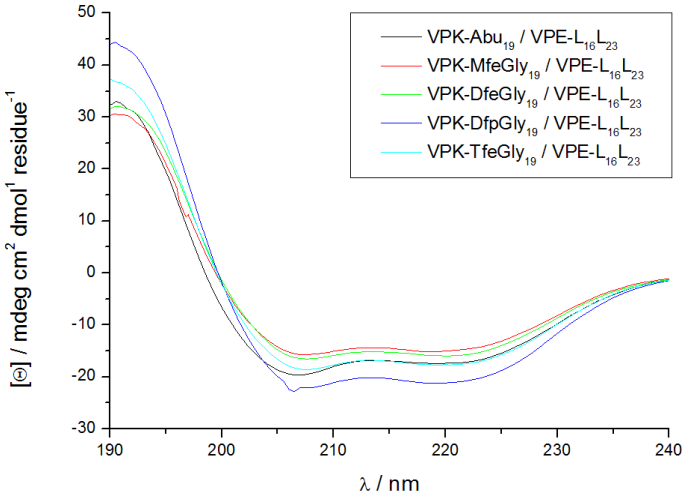


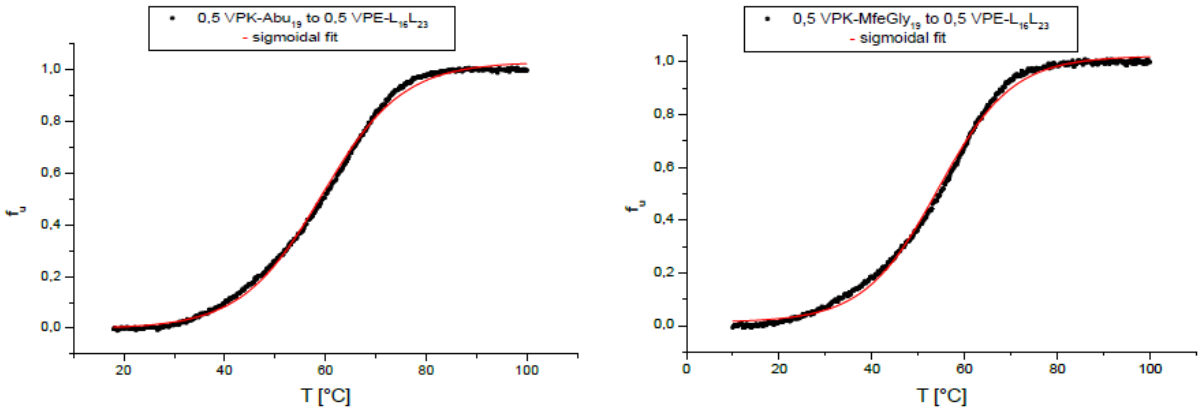
Figure 2: CD spectra of investigated **VPK(X_{19})/VPE$_{selected}$** heteromers depicted as the mean of 3 experiments. Peptide conc.:20 µM; 100mM Phosphate buffer; Measurements were taken from a 1:1 mixture of **VPK(X_{19})/VPE$_{selected}$** and repeated two times to confirm reproducibility and give standard deviations.

helical content $\int_{helix}$ [a]

	helical content [%]
VPK-Abu$_{19}$/VPE-L$_{16}$L$_{23}$	83
VPK-MfeGly$_{19}$/VPE-L$_{16}$L$_{23}$	63
VPK-DfeGly$_{19}$/VPE-L$_{16}$L$_{23}$	76
VPK-TfeGly$_{19}$/VPE-L$_{16}$L$_{23}$	62
VPK-DfpGly$_{19}$/VPE-L$_{16}$L$_{23}$	79

[a] $\int_{helix} = \Theta_{222nm}*100/[-40000*(1-(4,6/n))]$, where n=35

For melting curves, the CD signal at 222 nm was recorded applying a heating rate of 3K/min from 20 to 95°C. All spectra were baseline corrected and each sample was prepared three times. The determination of T_M was carried out as described previously.[1]



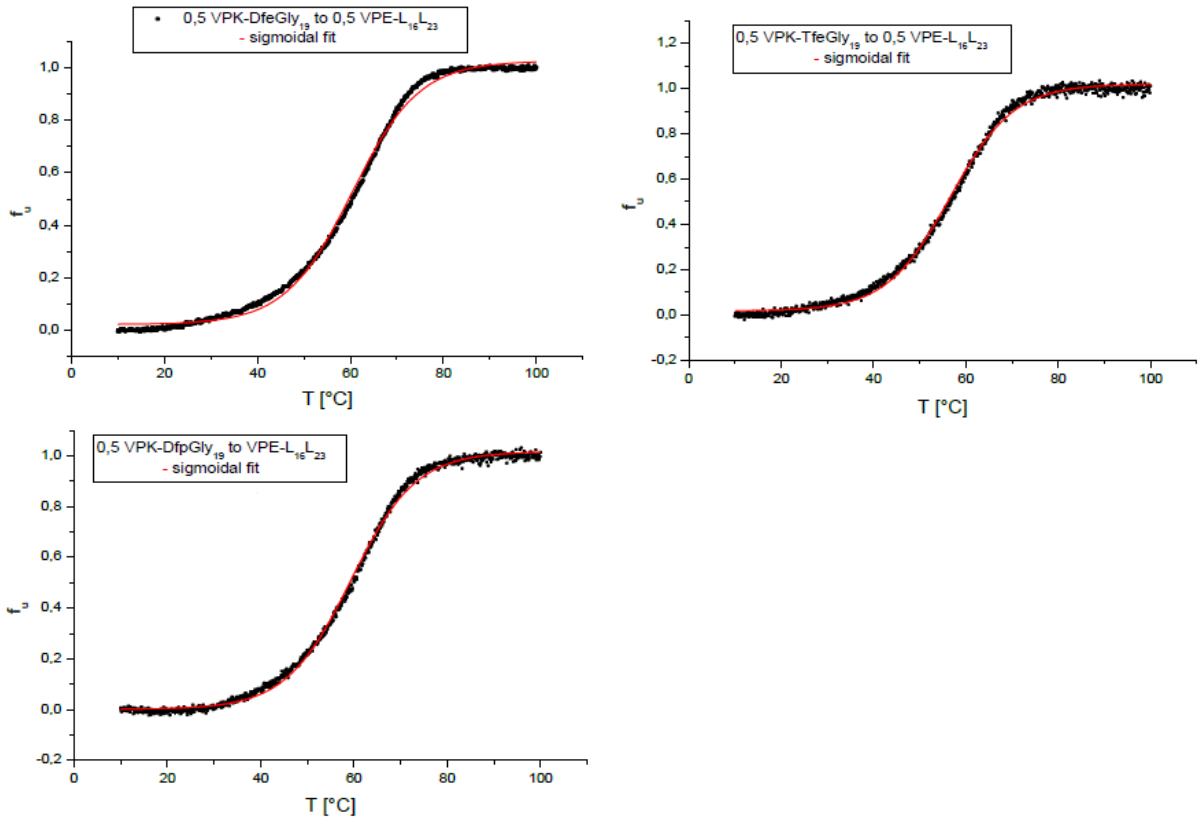


Figure 3: Denaturation curves for the investigated heteromers of **VPK(X_{19})/VPE$_{selected}$** depicted as the mean of 3 experiments. Peptide conc.:10 µM; 100mM Phosphate buffer; Measurements were taken from a 1:1 mixture of **VPK(X_{19})/VPE$_{selected}$** and repeated two times to confirm reproducibility and give standard deviations.

1. Salwiczek, M., et al., *Position-Dependent Effects of Fluorinated Amino Acids on the Hydrophobic Core Formation of a Heterodimeric Coiled Coil.* Chemistry – A European Journal, 2009. **15**(31): p. 7628-7636.
2. Kaiser, E., et al., *Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides.* Analytical Biochemistry, 1970. **34**(2): p. 595-598.
3. Reidhaar-Olson, J.F. and R.T. Sauer, *Combinatorial cassette mutagenesis as a probe of the informational content of protein sequences.* Science, 1988. **241**(4861): p. 53-57.
4. Barbas, C.F., et al., *Phage display: A Laboratory Manual*. Vol. Chapter 4. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
5. Vagt, T., et al., *Towards identifying preferred interaction partners of fluorinated amino acids within the hydrophobic environment of a dimeric coiled coil peptide.* Organic & Biomolecular Chemistry. **8**(6): p. 1382-1386.