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Supplementary information

Precision compatibilizers for composites: In-between self-aggregation, surfaces recognition and interface stabilization

Soft Matter

Authors: V. Samsoninkova, N. L. Venkatareddy, W. Wagermaier, A. Dallmann

Corresponding author: H.G. Börner*

To whom correspondence should be addressed:

Prof. Dr. Hans G. Börner

Humboldt-Universität zu Berlin

Department of Chemistry, Laboratory for Organic Synthesis of Functional Systems

Brook-Taylor-Str. 2, Room 2.115, D-12489 Berlin, Germany

Phone: +49 (0)30-2093 7348

Fax:+49 (0)30-2093-7500

E-Mail: h.boerner@hu-berlin.de

Web: http://www.boernerLab.de

Materials and Methods

Materials

L-amino acid derivatives: Fmoc-Gly-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Ala-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-His(Trt)-OH, Fmoc-Asp(*t*Bu)-OH, Fmoc-Met-OH, bromotrimthylsilane (TMSBr), *N*-methyl-2-pyrrolidone (NMP, 99.9+%, peptide synthesis grade), 2-(1H-benzotriazole-1-yl)-1,1,3,3- tetramethyluronium hexafluorophosphate (HBTU), 1-Hydroxybenzotriazole (HOBt), dichloromethane (DCM, peptide grade), used for peptide synthesis, were purchased from IRIS Biotech (Marktredwitz, Germany). NMP was filtrated before use, DCM was distilled, other chemicals were used as received. *N*,*N*-diisopropylethylamine (DIPEA, peptide grade), piperidine (peptide grade), trifluoroacetic acid (TFA, peptide grade), 2,5-dihydroxybenzoic acid (DHB), were purchased by Acros Organics (Thermo Fisher Scientific) (Waltham, MA, USA) and α-Cyano-4-hydroxycinnamic acid (αCHCA) (MALDI MS matrix) by Sigma Aldrich (St. Louis, U.S.A). Guanidinium chloride (99.5%) was obtained from Carl Roth (Karlsruhe, Germany). TentaGel® PAP resin (PEG attached peptide resin, loading: 0.24 mmol g⁻¹; Mn = 3200) was obtained from Rapp Polymere (Tübingen, Germany).

DMSO d₆ with 99,9% purity and Methyl alcohol-d₃ 99,5% purity were obtained in ampules from Deutero GmbH (Kastellen, Germany).

PEO, used as a matrix with M_n =900.000, methanol (\geq 99.6%) and magnesium turnings (99.98%) for magnesium fluoride synthesis were obtained from Sigma Aldrich (St. Louis, U.S.A). Hydrogen fluoride (>99%) was obtained from Solvay Fluor (Hannover, Germany)) and used as a methanolic solution. Methanol (LC-MS grade) was bought from Acros Organics.

Equipment

Conjugate synthesis: Solid-phase peptide synthesis (SPPS) was performed on an ABI 433A peptide synthesizer by Applied Biosystems (Darmstadt, Germany) as described previously.¹

Mass spectrometry (MS): Mass spectrometry was performed on a Bruker autoflex III smart beam with matrix-assisted laser desorption/ionization (MALDI) and time of flight detector (MALDI-ToF-MS) (Rheinstetten, Germany) and Axima Confidence (Shimadzu Biotech Kyoto, Japan). For MALDI-MS the samples were dissolved in water. Matrix and sample solutions were mixed directly on the plate in a ratio of 3:1. It was measured in linear positive mode.

Nuclear Magnetic Resonance (NMR) Spectroscopy: NMR titrations experiments were performed on a Bruker AVANCE 600 in methanol d₃. Standard ¹H and 2D as DOSY NMR spectra were measured on a Bruker AVANCE II 500. NMR samples depending on the solubility were dissolved in DMSO d₆ 99,8% or methanol d₃.

Circular dichroism (CD) spectroscopy: Circular dichroism spectra were recorded on a JASCO 700 (JASCO Germany, Gross-Umstadt, Germany) spectrometer using quartz cuvettes of 1 cm path length at 25 °C. All measurements were performed in water.

Hot pressing: Hot pressing of the composite materials was performed on the SPECAC machines from Specac Limited (Orrington, UK) at 70°C. The composites were heated without load for 3 min, and then 1 min pressed at 1 ton, followed by 2 tons pressure for 3 min. The composites are cooled down for 2 min. To prevent sticking of the composite to the pressing plates and to ensure quick release from the pressing plates, Polyethylene terephthalate (PET) foil is used. The average thickness of the composite ranges from 80-120 μ m. The thickness of the composite can be varied using different rings.

Tensile testing: For the evaluation of the tensile toughness, samples were tested on the Zwick machine with testing speed 200 mm/min. The energy to failure is calculated as the area under the stress-strain curve. The stress is calculated as the engineering stress, or maximum force divided by initial effective cross-sectional area. Eight samples were tested per concentration in each type of experiment. Samples showed very high sensitivity to the defects. T-Test (Excel) was used to evaluate results and calculate statistical significance of value differences. All the p-values below 0.05, will be considered as significantly different.

FT-IR: Attenuated total reflection Fourier-Transform-Infrared Spectroscopy (ATR-FT-IR) analysis were recorded in a vacuum on an FT-IR spectrometer Vertex 70 from Bruker Optik GmbH (Leipzig Germany) with diamond ATR and resolution of 4 cm⁻¹. Conjugates were measured as solids and composites after hot pressing.

Methods

MgF₂ synthesis: The synthesis of MgF₂ was performed under inert atmosphere (Ar) using Schlenk techniques. Mg turnings (2.43 g, 100 mmol) were dissolved in 500 mL dried methanol to yield Mg(OMe)₂ at a concentration of 0.2 M. To initiate fluorolysis a stoichiometric amount of HF dissolved in methanol was slowly added under vigorous stirring to give the desired product. Aging for 2-3 weeks resulted in optically clear sols. ¹⁹F NMR 300 Hz, locked in CDCl₃ in methanol, δ): -154 (BF₄⁻ from the reaction of HF with glass vessel), -187 (HF adsorbed), -198 ppm (MgF₂).

Solid-phase supported peptide synthesis (SPPS): Peptide-PEO conjugates were synthesized according to standard Fmoc procedures with side-chain protected amino acids (AA). ABI-Fastmoc protocols (single coupling for 1st to 10th AA, double coupling afterwards, capping; *N*-terminal acetylation followed in NMP using TentaGel® PAP resin. AA coupling was facilitated by HBTU/DIPEA. After final deprotection, the resin was washed

thoroughly with NMP and DCM. The cleavage was done with TFA/TMSBr/thioanisol = 94:1:5 (v/v/v) for 2 x 1 h. The conjugates were precipitated in cold diethyl ether and centrifuged. The dried conjugates were dissolved in water with guanidine hydrochloride (0.1% w/v). Conjugates are then dialyzed in Spectra Por cellulose ester dialysis membrane with molecular weight cut-off 500 Da against Milli-Q® water for ca. 4 days with change of solvent at least three times per day.

Selection of peptide sequences

Analysis of a portion of hydrophobic, charged and resides capable of forming extensive intermolecular hydrogen bonding was performed according to Hopp and Woods. The amino acids residues were considered as following: E,D,R,K,H – charged amino acids; S,T;E,D,K,R,H,N,Q,Y - amino acids capable of forming extensive intermolecular hydrogen bonding, V, I, L, F, M, C, A, G – hydrophobic amino acids.

Preparation of composites: The preparation procedure of the composites consisted of two steps: solution casting and hot pressing. For 15wt% MgF2 filled composites stabilized with 0.5 mol% of conjugate: 11.3 mg of the peptide-polymer conjugate was dissolved in methanol (LC-MS grade), after which 2.5 mL of MgF₂ sol was added. The mixture was stirred for a minimum of 4 hours to allow the conjugate to adhere to the inorganic surface. 3.5 mL of a 5 wt.% aqueous solution of PEO (900 kg mol⁻¹) was added and all components were mixed for another 30 min. The solution was cast into a small bowl and the material was dried under an extraction hood for 12 hours. The material that was obtained after the solution casting step was not suitable for the mechanical testing experiments, which require even and homogeneously thick composites. A hot pressing procedure was applied to prepare composite materials in order to eliminate unevenness in thickness of composites. With an average thickness of 80-120 μm, samples were cut in a bone form of 18 mm length and 6 mm width.

Analysis of conjugates

Mass spectrometry (MS)

Pep-I-PEG

Ac-GTQYYAYSTTQKS-PEG



Figure S 1. MALDI-ToF-MS of Pep-I-PEG.

MS (MALDI-ToF) m/z: [M + H]⁺ calc, 4579.06 Da; found, 4579.56 Da; calculated with 69 EO (m=44.05 Da) units.

The mass can be assigned within ± 0.5 m/z accuracy.



Figure S 2. NMR spectra of pep-I-PEG in DMSO-d6.

¹H NMR (500 MHz, DMSO-*d*₆, δ): 0.97 (3H, γ CH₃ T₂), 1.02-1.10 (6H, γ CH₃ T₉, T₁₀), 1.17 (2H, γ , δ K12), 1.28-1.37 (3H, γ , δ K12), 1.72-1.65 (2H, β H A6, δ K12), 1.94-1.72 (4H, β H Q₃, G₁₁), 1.98-2.18 (4 γ H Q₃, G₁₁), 2.56-2.98 (8H, β H + CH S₈, Y₄; Y₅, Y₇), 3.21 (2H, β H S₁₃), 3.51 (326H, CH₂ PEO), 3.59 (1H, β H S₈), 3.63-3.67 (3H, CH Y₄; Y₅, Y₇), 3.78 (1H, α H G₁), 3.97 (1H, β H T₂), 4.01-4.12 (2H, β H T₉, T₁₀), 4.37-4.15 (9H, α H T₂ Q₃ Q₁₁ A₆ T₁₀ K₁₂ S₁₃ Y₄ T₉), 4.39-4.46 (2H, α H Y₅ S₈), 4.50 (1H, α H Y₇), 5.03- 5.14 (2H, OH S₈ S₁₃) 6.58-6.66 (6H, ϵ H Y₄ Y₅ Y₇), 6.76-6.83 (2H, δ NH₂ Q₃ Q₁₁), 6.92-6.98 (2H, δ CH Y₄), 6.99-7.05 (4H, δ CH Y₅ Y₇), 7.22-7.29 (2H, δ NH₂ Q₃ Q₁₁), 8.21—8.13 (2H. NH G₁ S₈), 8.07-7.94 (5H, NH K₁₂, A6 Q₃ Q₁₁ Y₅), 7.79-7.71 (3H, NH Y₇ T₁₀ T₂), 7.94-7.81 (6H, NH S₁₃ T₉ Y₄; 1H ϵ NH³⁺ K₁₂, 2 H δ NH2 Q₃ Q₁₁)

IR spectroscopy (IR)



Figure S 3. FT-IR spectrum of pep-I-PEG.

ATR-IR: *v* = 3283 (*m*; Amid A); 2890 (*w*, C-H); 1624; 1665; 1697 (*s*, Amid I); 1518 (*m*, Amid II); 1467 (m), 1343 (m), 1280 (m). 1241 (m), 1203 (w, Amide III); 1145 (w); 1104 (s); 1062 (w); 962 (m); 842 (m); 722 (vw, Amide IV).

Pep-VI-PEG

GGLNQVLRIPSFI - PEG



Figure S 4. MALDI-ToF-MS of Pep-VI-PEG.

MS (MALDI-ToF) m/z: $[M + H]^+$ calc, 3968.57 Da; found, 3968.57 Da; calculated with 58 EO (m=44.05 Da) units.

The mass can be assigned within ± 0.0 m/z accuracy.

NMR

 $G_1G_2L_3N_4Q_5V_6L_7R_8I_9P_{10}S_{11}F_{12}I_{13}$

[ppm] Figure S 5. NMR spectrum of pep-VI-PEG in MeOD ¹H NMR (600 MHz, MeOD) δ 8.29 – 8.26 (m, 1H) NH L₃ , 8.15 – 7.96 (m, 9H) NH S₁₁ NH R₈ I₁₃ F₁₂ N₄ I₉ Q₅ V₆ L₇, 7.89 (tt, J = 62.1, 18.5 Hz, 2H) NH G₁G₂, 7.69 (s, 1H) γ NH₂ N₄, 7.56 (s, 1H) δ NH2 Q5, 7.27 – 7.16 (m, 7H) 5H Ar F12,) δNH2 Q5 γNH2 N4 7.05 (s, 1H) γNH2 N4, 6.89 (m, 1H) δ NH2 Q5, 5.41 – 5.15 (m, 1H) OH S11 4.62 (s, 1H) H α I9, 4.46 – 4.29 (m, 6H) Hα S₁₁ R₈ F₁₂ L₃ Q₅ P₁₀, 4.17 (t, *J* = 8.4 Hz, 2H) Hα I₁₃ N₄, 4.11 – 4.06 (m, 1H) Hα L₇, 3.88 – 3.75 (m, 3H) Hβ S₁₁ Hβ N₄ Hδ P₁₀,3.75 – 3.69 (m, 4H) Hβ S₁₁ Hβ N₄ , 3.62 (s, *J* = 4.5 Hz, 470H), CH₂ PEO , 3.56 - 3.47 (m, 4H) H α G₁ G₂ H δ R₈ , 3.17 (dd, J = 14.6, 4.9 Hz, 1H)

Hß F₁₂, 2.97 (m, J = 14.3, 8.7 Hz, 1H) Hß F₁₂, 2.84 – 2.66 (m, 2H) Hß P₁₀ 2.59 (s, 1H), 2.50 – 2.41 (m, 2H) H γ Q5 2.37 – 2.25 (m, 1H) Hß L₃, 2.17 – 1.75 (m, 10H) Hß I₁₃ I₉ Q5 V₆ L₇

 $H\gamma L_{3} L_{7 (2)} P_{10} H\gamma P_{10}, 1.67 - 1.41 (m, 8H) H\beta R_{8} Q_{5}, H\delta L_{3} H\gamma I_{13} I_{9}, 1.27 (s, 2H), H\gamma R_{8}, 1$

 $1.20-1.07\ (m,\,2H)\ H\gamma\ I_{13},\ I_{9}\ ,\ 0.99-0.80\ (m,\ 6H)\ H\delta\ I_{13}\ I_{9}\ (2)L_{7}\ (2)H\gamma\ V_{6}.$

IR spectroscopy (IR)



Figure S 6. FT-IR spectrum of pep-VI-PEG.

ATR-IR: *v* = 3275(*m*; Amid A); 2884 (*m*, C-H); 1627; 1663; (*s*, Amid I); 1537 (*m*, Amid II); 1464 (m), 1358 (m), 1344 (m), 1281 (m). 1241 (m), 1202 (vw, Amide III); 1095 (m); 962 (m); 863 (m)

Pep-VII-PEG

GSPKHNLDMVKMM-PEG



Figure S 7. MALDI-ToF-MS of Pep-VII-PEG

MS (MALDI-ToF) m/z: $[M + K]^+$ calc, 4565.27 Da; found, 4566.21 Da; calculated with 69 EO (m=44.05 Da) units.

The mass can be assigned within ± 0.94 m/z accuracy.

NMR (G1S2P3K4H5N6L7D8M9K11M12M13 – PEG)



¹H NMR (600 MHz, MeOD) δ 8.85 (s, 1H) H₅, 8.65 (s, 1H) NH S₂, 8.48 (s, 1H) NH N₆, 8.36 (d, J = 5.0 Hz, 1H) NH L₇, 8.26 (dd, J = 14.0, 4.9 Hz, 2H) NH K₄ D₈, 8.00 (d, J = 5.6 Hz, 1H) NH H₅, 7.92 (d, J = 5.0 Hz, 2H) NH M₉ K₁₁, 7.79 (dd, J = 11.2, 6.0 Hz, 1H) V₁₀, 7.76 – 7.70 (m, 2H) M₁₂M₁₃, 7.59 (t, J = 5.9 Hz, 1H) G₁, 7.46 (s, 1H) H₅, 7.36 – 7.28 (m, J = 11.3 Hz, 1H) Hγ N₆, 6.80 (s, 1H) γNH₂ N₆, 4.48 – 4.34 (m, 4H) Hα H₅ M₁₃ N₆ D₈, 4.33 – 4.28 (m, 1H) Hα M₁₂, 4.26 – 4.21 (m, 1H) Hα M₉, 4.20 – 4.15 (m, 1H) Hα S₂, 4.14 – 4.06 (m, 2H) Hα K₁₁ L₇, 4.06 – 3.95 (m, 2H) Hα K₄ P₃ , 3.95 – 3.89 (m, 1H) Hδ P₃ 3.80 – 3.72 (m, 2H) Hα V₁₀ Hδ S₂, 3.71 – 3.59 (m, J = 8.5 Hz, 301H) CH₂ PEO , 3.48 – 3.38 (m, 3H) Hβ H₅ Hα G₁ Hδ P₃ , 3.29 (s, 1H) Hβ H₅ , 3.05 – 2.94 (m, 5H) Hβ N₆ D₈. Hε K₄ K₁₁, 2.85 (d, J = 4.2 Hz, 1H) Hβ M₁₂ , 2.61 – 2.57 (m, J = 8.7, 4.9 Hz, 1H) Hγ M₁₃ , 2.52 (dd, J = 13.3, 6.2 Hz, 1H) Hγ M₉, 2.46 – 2.35 (m, 1H) Hβ M₉, 2.30 – 2.25 (m, 1H) Hβ V₁₀, 2.22 – 2.13 (m, 5H) Hβ P₃. Hε M₉M₁₂M₁₃ , 2.11 – 2.03 (m, 5H) Hε M₉M₁₂ M₁₃, 1.98 (dd, J = 15.5, 7.6 Hz, 2H) Hλ K₄ Hε M₉, 1.89 – 1.42 (m, 9H) Hβ K₄ K₁₁L₇ Hγ K₄ K₁₁ L₇ Hδ K₄ 1.36 – 1.27 (m, 2H) Hγ L₇ K₁₁, 1.12 (d, J = 6.9 Hz, 2H) Hγ V₁₀, Hδ L₇ 1.02 – 0.89 (m, 4H) Hγ V₁₀ Hδ L₇ Hγ K₄K₁₁



IR spectroscopy (IR)

Figure S 9. FT-IR spectrum of pep-VII-PEG.

ATR-IR: *v* = 3273(*m*; Amid A); 2884 (*m*, C-H); 1627; 1665; (*s*, Amid I); 1539 (*m*, Amid II);

1468 (m), 1415 (m), 1344 (m), 1279 (m). 1243 (m), 1202 (vw, Amide III); 1097 (m); 962 (m);

841 (m).

CD spectroscopy



Figure S 10. CD spectra of conjugates: pep-I, VI&VII-PEG measured in water. Molar elipticity is expressed in $\times 10^{5}$ (deg \times cm²/dmol). Spectra evidence random coil structures for all considered conjugates.

NMR titration experiments

Titrations experiments were realized in methanol-d₃ used in ampules of 0.75 mL in volume. At first, 5 mg of each of the studied conjugate were mixed with the methanol d₃. From the obtained samples 2D spectra (TOSCY and ROESY) were measured to be able to assign protons. The same amount of sol particle was stepwise titrated to each conjugate sample. These experiments were performed to analyze the amount of particles, which can be accommodated by the system indicating the availability of particular conjugate for an interaction. After each addition of conjugate, ¹H NMR spectra were recorded. The amount of particles which can be titrated was evaluated based on two factors: broadening of the spectra to such extent that the signals disappear and the possibility to perform shimming for the measurements. After a certain amount, the system became so inhomogeneous that shimming was impossible. It is worth to note that these experiments are not correctly designed to evaluate the residue involved in the interaction due to the dilution effect causing shifts in NMR spectra. Below there are titration series for the considered sequences.



Figure S 11. ¹H NMR spectra (NH and aromatic region) of pep-I-PEG representing titration series of MgF_2 NP in the sol form added to the conjugate in 10µl and corresponding changes in the spectra.



Figure S 12. ¹H NMR spectra (NH and aromatic region) of pep-VI-PEG representing titration series of MgF₂ NP in the sol form added to the conjugate in 10μ l and corresponding changes in the spectra.



Figure S 13. ¹H NMR spectra (NH and aromatic region) of pep-VII-PEG representing titration series of MgF_2 NP in the sol form added to the conjugate in 10µl and corresponding changes in the spectra.

All presented spectra indicate that all three considered conjugates have different behavior in relation to the particle interaction. For all conjugates, spectra broaden after particle addition evidencing the presence of interaction, while different amounts are needed to cause this broadening. For pep-I-PEG, even 5 μ L of particles cause strong broadening of the spectra, where further addition does not impact significantly spectra. The same 5 μ L does not cause extreme broadening for pep-VI-PEG pep-VII-PEG. Even though after the first titration, signals become extremely broad for pep-VI-PEG. In the case, pep-VII-PEG signals broaden with each titration consequently up to 20 μ L. The higher amount is accommodated by pep-VII-PEG – 90 μ L, followed by pep-I-PEG– 70 μ L and pep-VI-PEG – 40 μ L. The difference in the behavior of conjugate in relation to the amount of NP needed to cause broadening apart aggregation effects may be also related to the speed of peptide particle interaction.

Study of peptide interaction with NP surface by NMR

NMR experiments were performed to address the question: which amino acid residues are involved and responsible for binding of peptides to the surface of nanoparticles and potentially identify the type of interaction, which drives the binding itself. All experiments were performed in deuterated methanol d₃. Selection of this solvent was predetermined for several reasons. Original nanoparticles exist and are stable in the methanolic solution in a form of sol. This fact defined selection of the solvent media for the experiments for composites development. Having all experiments with composites performed in methanol and to study interactions existing in composites, methanol was the solvent of choice. Apart from that prior to NMR studies, we have checked the behavior of the system with solid nanoparticles. It turned out that solid particles, obtained from the original sole and dried are stable in methanolic solution and do not sediment, while sedimentation took place in water. It

was a strong argument to choose methanol. On the other side addition of particles may cause changes in pH, which can significantly impact the interaction. For that purpose, changes of pH were monitored during the addition of nanoparticles, which took place but in the moderate range: from 4.7 to 3.7. In buffered water solution, which would allow to maintaining pH, the sedimentation of NPs prohibited measurements. Analysis of pKa values for residues showed that only Aspartic acid could be influenced in this range and a shift in NH region for this residue could be additionally caused by pH changes.

TOCSY measurements were done prior and after addition of nanoparticles and superimposed view of the spectra revealed significant changes as in the NH region as in aliphatic regions. Changes in the spectra in NH region are presented in the Figure S8, while changes in the aliphatic region in the Figure S9 using the same TOCSY spectra. TOCSY spectra are an identical and separate representation of NH and aliphatic shifts are done for clarity purposes.



Figure S 14. Superimposed TOCSY spectra of conjugate pep-VII-PEG in methanol d3, before (blue) and after addition of NP (red). NH region of the spectra is presented. Dotted lines represent signals in TOCSY spectra belonging to the spin system of amino acids and arrows shows the movement and its direction for signals in the NH region.



Figure S 15. Superimposed TOCSY spectra of conjugate pep-VII-PEG in methanol d3, before (blue) and after addition of NP (red). NH region of the spectra is presented. Dotted lines represent signals in TOCSY spectra belonging to the spin system of amino acids. Black triangles represent shifts in the aliphatic region for indicated residues.



Amino Acids Figure S 16. Chemical shift perturbation in NH region for pep-VII-PEG after addition of NP showing shifts for each residue.

Most significant shifts in the NH region experience Ser and Lys4, both shift to lower ppm over 0.1 ppm. As these residues should be not affected by slight pH change and therefore these changes can be considered as a sign for the involvement of these residues into the binding event. All other residues except Gly, Pro and Val evidence small shifts 0.01-0.04 ppm and seem to provide a small contribution from their NH protons. Nearly all residues with smaller shifts shift also to lower ppm, only Met9 and Leu show very minor shift to higher ppm. NH₂ protons of Lys4 are not visible in the original TOCSY spectra but become visible after addition of nanoparticles. This fact would suggest that Lys is involved in the interaction and upon binding becomes fixated in its position which makes his NH₂ side chain protons visible due to the hindered rotation. The same phenomena was not observed for Lys11, suggesting that no significant contribution to binding. Also, close proximity to PEG 3200 could also explain this fact, that PEO hinders side chains of Lys11 to interact with the surface.



Figure S 17. Chemical shift perturbation in aliphatic region for pep-VII-PEG after addition of NP showing shifts for each residue.

All the residues apart from Gly, Pro and Val show again chemical shift perturbation in the aliphatic region upon addition of nanoparticles, which again supports the statement that many of residues in the sequences are involved in the interaction and cause the shift in TOCSY spectra. Shifts cannot be caused by small changes in pH, especially taking into account the fact that aliphatic protons are much less sensitive to pH changes. Contrary to the shift in the NH region, where Ser and Lys showed the most prominent shift, in the aliphatic region, most of the proton shifts come from Met9 followed by comparably equal shifts by Lys4, His5, Asn6, and Asp8. The chemical shift perturbation in the aliphatic region in overall smaller than observed for NH region and is in the range of 0.04-0.09 ppm. It is worth to note that the same amino acids show shifts in NH and aliphatic region, suggesting that the shifts have its origin in the interaction with the surface and are not originating from pH sensitivity.

Only two residues contribute to the interaction with their side chain protons, Asn and His. Both experienced cumulative shifts up to 0.04 ppm. The involvement of His in the interaction is very predictable, as it was already mentioned in several publications that His is one of the residues, which were mentioned quite often being involved in the binding. The sum of a shift in NH and aliphatic region significantly defines the total chemical shift perturbation.



Figure S 18. Chemical shift perturbation for side chain protons for pep-VII-PEG after addition of NP showing shifts for each residue.

References

1. D. Eckhardt, M. Groenewolt, E. Krause and H. G. Börner, *Chem. Commun.*, 2005, 2814-2816.