The Impact of Structural Variation in Simple Lanthanide Binding Peptides

Electronic Supplementary Information

Density Functional Theory (DFT) modelling with DMol ³	2
Synthesis of 1,3-Dioxo-1H-benz[d,e]isoquinoline-2(3H)-acetic acid (naph)	2
General methods for solid phase peptide synthesis	3
Naphthalimide modified peptides	4
Effect of the naphthalimide tag	5
HypSpec modelled speciation plots from luminescence titrations	7
Luminescence decay rates	8
Circular Dichroism (CD)	9
Nuclear Magnetic Resonance (NMR) Titration	12
Density Functional Theory (DFT) modelling	12
Solid State NMR	14
CHN Microanalysis	14
Zeta Potential Measurements	15
Sorption	15
References	16

Density Functional Theory (DFT) modelling with DMol³

DMol³ uses a basis set of numeric atomic functions for the atoms. For this work, the double numeric polarised (DNP) basis set with a cutoff radius of 3.5 Å was chosen and the generalised gradient approximation (GGA/PBE) functional¹ was used. Core electron potentials were represented using DFT semi-core pseudopotentials. Thermal smearing of 0.01 Hartree (Ha) was used. Atom-centred grids were used for the numerical integration with increasing numbers of grid points for the 'coarse' and 'medium' geometry optimisations, respectively. Spins were unrestricted for structures containing Eu. All self-consistent field (SCF) calculations were performed with a final convergence criterion of 2×10^{-5} Ha for the energy.

All calculations were performed using the conductor-like screening model (COSMO) with water as the solvent.² After a coarse geometry optimisation at 0 K, simulated annealing at 300 K for 0.5 ps was performed to eliminate very strained conformations.³ This was followed by coarse and medium geometry optimisation at 0 K to refine and predict the structure and 'structural energy' of each dipeptide. In this instance, structural energy is defined as the difference between the total energy of the molecule and the combined energy of the atom fragments constituting the molecule.

Simulated annealing was performed using molecular dynamics, again as part of the DMol³ program within Materials Studio. The coarse geometry optimised structures of the peptide:Eu complexes were subjected to 500 temperature cycles from 0 to 300 K using constant volume/constant temperature (NVT) conditions. At the end of each annealing cycle the structures were again energy minimised to converge below 10⁻⁴ Ha and the energy minimised structures used for further modelling. Thermal smearing of 0.01 Ha and a simple NH thermostat were also used for the molecular dynamics simulated annealing.

Attempts to correlate the NMR and DFT data by predicting NMR chemical shifts based on the modelled DFT structures using CASTEP were unsuccessful as the energy would not converge.

Synthesis of 1,3-Dioxo-1H-benz[d,e]isoquinoline-2(3H)-acetic acid (naph)



A mixture of glycine (0.28 g, 3.7 mmol) and 1,8-naphthalic anhydride (0.59 g, 3.0 mmol) in 3:1 v/v ethanol (EtOH)/water (20 mL) was heated in a pressure tube at 130 °C for 70 h. The solvent was then removed under vacuum, affording the crude product as a brown solid. The crude product was washed with EtOH and filtered. The filtrate was reduced in volume under vacuum and the resulting precipitate again filtered, washing with EtOH, to give the desired product as a light brown solid (0.63 g, 82 % yield). m.p. 272-275 °C; ¹H NMR (300 MHz, DMSO-d₆) δ (ppm): 8.53 (m, 4H), 7.91 (t, *J* = 7.8 Hz, 2H), 4.73 (s, 2H). Characterisation data was consistent with previously reported results.⁴

General methods for solid phase peptide synthesis

Solid phase peptide synthesis was performed in polypropylene syringes equipped with Teflon filters (Torviq).

Resin loading: Wang resin (0.10 g, 1.1 mmol/g, 0.11 mmol) was allowed to swell in dimethylformamide (DMF, 2 mL) for 30 min. Meanwhile, Fmoc-Glu(*t*Bu)-OH (0.42 g, 1.0 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and *N*,*N'*-diisopropylcarbodiimide (DIC, 79 µL, 0.5 mmol) added. The resulting solution was immediately placed in an ice bath and stirred at 0 °C for 20 min. The solvent was then removed under vacuum to afford the asymmetric anhydride as a white solid. This residue was redissolved in DMF (4 mL) and added to the swollen Wang resin with 4-dimethylaminopyridine (DMAP, 1.2 mg, 0.01 mmol). The resin was then shaken at room temperature for 1 h before washing with DMF (5 x 3 mL), CH₂Cl₂ (5 x 3 mL) and DMF (5 x 3 mL).

Deprotection: Fmoc deprotection was performed by washing the resin three times with 1:4 v/v piperidine/DMF (3 mL) for 5-10 min. Measurement of the uv-visible absorbance of the washings at λ = 301 nm showed that the amino acid loading onto the resin was quantitative (ϵ = 7800 for the fulvene-piperidine adduct). The resin was then again washed with DMF (5 x 3 mL), CH₂Cl₂ (5 x 3 mL) and DMF (5 x 3 mL).

Amino acid coupling: Couplings were performed by adding a solution of either L- or D- Fmoc-Glu(*t*Bu)-OH (0.25 g, 0.6 mmol), O-benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluorophosphate (HBTU, 0.21 g, 0.5 mmol) and *N*,*N*'-diisopropylethylamine (DIPEA, 200 μ L, 1.1 mmol) in DMF (3 mL) to the resin and shaking at room temperature for at least 1 h. The resin was then washed with DMF (5 x 3 mL), CH₂Cl₂ (5 x 3 mL) and DMF (5 x 3 mL). After the final coupling, the resin was washed with DMF (5 x 3 mL) and CH₂Cl₂ (5 x 3 mL) then dried under vacuum for 1 h before being sealed with parafilm and stored under refrigerated conditions.

Naphthalimide coupling: a solution of naphthalimide **7** (56 mg, 0.22 mmol), benzotriazol-1yloxytri(pyrrolidino)-phosphonium hexafluorophosphinate (PyBOP, 116 mg, 0.22 mmol) and DIPEA (76 μ L, 0.44 mmol) in DMF (6mL) was added to the peptide loaded resin (100 mg, 0.11 mmol). The resin was then shaken at room temperature for 2 h before washing with DMF (5 x 5 mL), CH₂Cl₂ (5 x 5 mL) and DMF (5 x 5 mL).

Cleavage: The naphthalimide tagged peptides were cleaved from the resin and the side chains concomitantly deprotected by adding a mixture of 90:5:5 v/v/v trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/water (2.0 mL) to the resin (0.1 g, 0.11 mmol) and shaking at room temperature for 2 h. The cleaved products were separated from the resin by filtration and combined with TFA washings (2 x 2 mL). Addition of cold diethyl ether (50 mL) afforded a white precipitate which was extracted into distilled water (1 x 50 mL, 2 x 10 mL). After washing the aqueous phase with diethyl ether (2 x 10 mL), the volume was reduced under vacuum and the remaining liquid removed by lyophilisation to afford the modified peptides.

Naphthalimide modified peptides

Naph-D-Glu(OH)-L-Glu(OH)-D-Glu(OH)-L-Glu(OH)-OH (naph-DLDL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired tetra-peptide as a white powder (88 mg, 100%); m.p. 179-182 °C; $[\alpha]_D^{20}$ +21.5° (*c* 0.1, MeOH); ¹H NMR (300 MHz, MeOD) δ (ppm): 8.56 (d, *J* = 7.2 Hz, 2H), 8.35 (d, *J* = 8.2 Hz, 2H), 7.81 (dd, *J* = 7.2, 8.2 Hz, 2H), 4.74 (s, 2H), 4.37 (m, 4H), 2.38 (m, 8H), 2.05 (m, 8H); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 174.4, 174.3, 174.3, 174.1, 173.5, 171.6, 171.5, 171.4, 163.9, 162.8, 135.1, 131.8, 131.4, 128.0, 127.8, 122.4, 52.7, 52.2, 51.5, 42.9, 31.2, 30.5, 30.3, 30.2, 28.3, 27.9, 27.9, 26.9 (1 signal obscured or overlapping); HRMS (ESI) calcd for C₃₄H₃₇N₅O₁₆ [M+Na]⁺ *m/z* 794.2134, found 794.2128.

Naph-L-Glu(OH)-L-Glu(OH)-L-Glu(OH)-L-Glu(OH)-OH (naph-LLLL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired tetra-peptide as a white powder (52 mg, 61%); m.p. 200-203 °C; $[\alpha]_D^{20}$ -43.6° (*c* 0.1, MeOH); ¹H NMR (200 MHz, DMSO-d₆) δ (ppm): 8.49 (m, 4H), 8.17 (d, *J* = 7.6 Hz, 1H, NH), 8.01 (br s, 1H, NH), 7.89 (dd, *J* = 7.6, 7.9 Hz, 2H), 4.72 (s, 2H), 4.24 (m, 4H), 2.27 (m, 8H), 1.83 (m, 8H); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 174.5, 174.4, 174.2, 173.5, 171.6, 171.3, 167.3, 163.8, 135.0, 131.8, 131.4, 128.0, 127.8, 122.4, 52.3, 51.7, 42.8, 30.6, 30.5, 28.0, 27.7, 26.6 (6 signals obscured or overlapping); HRMS (ESI) calcd for C₃₄H₃₇N₅O₁₆ [M+Na]⁺ *m/z* 794.2134, found 794.2128.

Naph-L-Glu(OH)-D-Glu(OH)-L-Glu(OH)-OH (naph-LDL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired tri-peptide as a white powder (69 mg, 100%); m.p. 185-191 °C; $[\alpha]_D^{20}$ -21.5° (*c* 0.1, MeOH); ¹H NMR (200 MHz, DMSO-d₆) δ (ppm): 8.54 (m, 4H), 8.12 (d, *J* = 8.3 Hz, 2H, NH), 7.92 (dd, *J* = 7.8, 8.3 Hz, 2H), 4.75 (s, 2H), 4.29 (m, 3H), 2.23 (m, 6H), 1.88 (m, 6H); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 174.3, 174.3, 174.1, 171.4, 171.4, 167.6, 163.8, 135.0, 131.5, 131.4, 128.0, 127.8, 122.4, 52.7, 52.2, 51.5, 42.9, 30.4, 30.3, 30.2, 28.0, 27.8 (2 signals obscured or overlapping); HRMS (ESI) calcd for C₂₉H₃₀N₄O₁₃ [M+Na]⁺ *m/z* 665.1708, found 665.1702.

Naph-L-Glu(OH)-L-Glu(OH)-L-Glu(OH)-OH (naph-LLL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired tri-peptide as a white powder (51 mg, 74%); m.p. 201-207 °C; $[\alpha]_D^{20}$ -50.9° (*c* 0.1, MeOH); ¹H NMR (300 MHz, DMSO-d₆) \bar{o} (ppm): 8.51 (m, 4H), 8.16 (d, *J* = 7.7 Hz, 1H, NH), 7.97 (d, *J* = 7.6 Hz, 1H, NH), 7.90 (dd, *J* = 7.6, 7.7 Hz, 2H), 4.73 (s, 2H), 4.27 (m, 3H), 2.29 (m, 6H), 1.85 (m, 6H); ¹³C NMR (100 MHz, DMSO-d₆) \bar{o} (ppm): 174.5, 174.4, 174.2, 173.5, 171.6, 171.3, 167.3, 163.8, 135.0, 131.8, 131.4, 128.0, 127.8, 122.4, 52.3, 51.7, 42.8, 30.6, 30.5, 28.0, 27.8, 26.7 (2 signals obscured or overlapping); HRMS (ESI) calcd for C₂₉H₃₀N₄O₁₃ [M+Na]⁺ *m/z* 665.1708, found 665.1702.

Naph-D-Glu(OH)-L-Glu(OH)-OH (naph-DL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired di-peptide as a white powder (41 mg, 73%); m.p. 145-148 °C; $[\alpha]_D^{20}$ +23.3° (*c* 0.1, MeOH); ¹H NMR (300 MHz, DMSO-d₆) δ (ppm): 8.48 (m, 4H), 8.06 (d, *J* = 8.0 Hz, 1H, NH), 7.89 (dd, *J* = 7.6, 8.0 Hz, 2H), 4.73 (s, 2H), 4.30 (m, 2H), 2.25 (m, 4H), 1.89 (m, 4H); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 174.3, 174.1, 173.5, 171.5, 167.3, 163.9, 135.1, 131.8, 131.4, 128.0, 127.8, 122.4, 52.4, 51.6, 42.9, 31.2, 30.4, 28.3, 26.9; HRMS (ESI) calcd for C₂₄H₂₃N₃O₁₀ [M+Na]⁺ *m*/z 536.1282, found 536.1276.

Naph-L-Glu(OH)-LGlu(OH)-OH (naph-LL-Glu-OH) was synthesised on Wang resin utilising the general methods for for SPPS to give the desired di-peptide as a white powder (55 mg, 97%); m.p. 188-194 °C; $[\alpha]_D^{20}$ -34.3° (*c* 0.1, MeOH); ¹H NMR (200 MHz, DMSO-d₆) δ (ppm): 8.54 (m, 4H), 8.21 (d, *J* = 7.4 Hz, 1H, NH), 7.92 (dd, *J* = 7.4, 7.9 Hz, 2H), 4.76 (s, 2H), 4.31 (m, 2H), 2.32 (m, 4H), 1.90 (m, 4H); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 174.6, 174.3, 173.7, 171.8, 167.4, 164.0, 135.2, 132.0, 131.5, 128.1, 127.9, 122.5, 52.2, 51.9, 43.0, 30.7, 30.6, 28.3, 26.7; HRMS (ESI) calcd for C₂₄H₂₃N₃O₁₀ [M+Na]⁺ *m/z* 536.1282, found 536.1276.

Effect of the naphthalimide tag

Titration of a non-buffered, 200 µM aqueous solution of LLLL-Glu-OH, without a naphthalimide tag, at pH 6 with up to 10 equiv. of 10 mM Eu nitrate was performed in phosphorescence mode from 550 to 750 nm using an excitation wavelength of 282 nm (the observed absorbance peak in the uv-visible spectrum of the peptide:Eu complex). The PMT voltage was set at 800 V, excitation slit 20 nm, emission slit 10 nm, emission filter open, excitation filter on automatic. The scan averaging time was 0.03 s and the data interval was 0.5 nm. A Savitzky-Golay smoothing factor of 9 was applied.

The characteristic narrow emission bands of Eu phosphorescence were evident during the titration of LLLL-Glu-OH, but the complexes formed were poorly luminescent and resulted in noisy data. Direct excitation of Ln is very inefficient due to the forbidden f-f transitions of Ln,⁵ hence the poor luminescence observed. Nevertheless it is clear that the luminescence increased at a decreasing rate as the Eu was added (Figure S1).



Figure S1: Luminescence titration using 200 µM LLLL-Glu-OH in aqueous solution at pH 6. The legend indicates the equiv. of Eu that have been added.

Since the luminescence titration with LLLL-Glu-OH showed only a weak phosphorescence signal, a strongly absorbing chromophore was incorporated into the peptides to allow transfer of the absorbed energy of the chromophore to the Ln excited state and hence sensitise emission.⁶ This is known as the "antenna effect". The chromophore 1,8-naphthalimide was utilised as it is an effective sensitising antenna for both Eu and Tb and has been previously shown not to affect the affinity of peptides for Ln.⁴ The synthesis of the 1,8-naphthalimide chromophore is described above.

Phosphoresence emission spectra were recorded upon addition of 0 to 10 equivalents of 3 mM Eu nitrate to a 30 μ M solution of naph-LLLL-Glu-OH in pH 6.9 10 mM HEPES buffer and the results for addition of up to 2.6 equiv. of Eu are shown in *Figure S2*. The fluorimeter settings were the same as for the titration in in *Figure S1* except an excitation wavelength of 345 nm was used as the naphthalimide antenna absorbs light at this wavelength via a n- π * transition and is able to effectively transfer this absorbed energy to populate the excited state of Eu(III) (${}^{5}D_{0}$ = 17200 cm⁻¹).⁴ Naph-LLLL-Glu-OH in pH 6.9 10 mM HEPES buffer demonstrated a luminescence signal more than 60 times stronger than that of LLLL-Glu-OH in pH 6 water, despite the peptide concentration of naph-LLLL-Glu-OH being more than six times lower. Thus, luminescence was substantially enhanced when Eu was complexed by peptides with the naph chromophore.



Figure S2: Luminescence titration of naph-LLLL-Glu-OH in pH 6.9 10mM HEPES with Eu.

HypSpec modelled speciation plots from luminescence titrations



Figure S3: Speciation diagrams from HypSpec modelling of luminescence titrations with Eu nitrate in 10 mM HEPES buffer of naph-DLDL- and naph-LDDL-Glu-OH at pH 4.1 (top) and naph-LLLL-Glu-OH at pH 4.7 (bottom).



Figure S4: Speciation diagrams from HypSpec modelling of luminescence titrations with Eu nitrate in 10 mM HEPES buffer of naph-LDL- and naph-LLL-Glu-OH at pH 4.9.



Figure S5: Speciation diagrams from HypSpec modelling of luminescence titrations with Eu nitrate in 10 mM HEPES buffer of naph-DL- and naph-LL-Glu-OH at pH 4.9.

Luminescence decay rates

Luminescence decay rates were measured on a Cary Eclipse Fluorescence Spectrometer in phosphorescence mode using 50 cycles with an excitation wavelength of 345 nm and an emission wavelength of 592 nm. The PMT voltage was 800 V, excitation slit was 20 nm, emission slit was 10 nm, delay time was 0.1 ms, flash count was 1, total decay time was 0.005 s and gate time was 0.02 s.

Luminescence decay rates of the tetra-peptides naph-LDDL- and naph-DLDL-Glu-OH with between 0.5 and 4 equivalents of Eu added were measured at pH 4 in 10 mM HEPES H_2O/D_2O solutions (Figure S8) to determine the number of coordinated water molecules. The hydration number q was calculated using equation (1) given by Parker et al., which accounts for the contribution of closely diffusing OH and NH amide oscillators.⁷

$$q = A \left(k_{H_2 O} - k_{D_2 O} \right) \tag{1}$$

In equation (1), A is a proportionality constant which has the value 1.2 ms for Eu and k is the rate constant for de-excitation, ie the luminescence decay rate. The value of Δk was corrected by a factor of -0.25 ms⁻¹ to account for the effect of outer sphere water molecules on Eu and by -0.075*4 = -0.3 ms⁻¹ to account for the effect of the four carbonyl-bound amide NH oscillators in naph-LDDL- and naph-DLDL-Glu-OH.⁷ Since the solid peptides synthesised in this work were hydrated, it was impossible to prepare a solution of the peptide:Eu complex in 100% D₂O. Instead the luminescence decay rate of the complex in 100% D₂O was extrapolated from the decay rates in solutions with increasing fractions of D₂O (Figure S8).



Figure S6. Luminescence decay rates of 1:1 peptide:Eu complexes with naph-LDDL-Glu-OH and naph-DLDL-Glu-OH in 10mM HEPES buffer pH 4.1 with increasing fraction 'x' of H₂O.

For naph-LDDL-Glu-OH in pH 4.1 10mM HEPES buffer, $\tau_{H2O} = 0.11$ ms and $\tau_{D2O} = 1.60$ ms and for naph-DLDL-Glu-OH the results were very similar, with $\tau_{H2O} = 0.11$ ms and $\tau_{D2O} = 1.54$ ms. For both tetra-peptides, these lifetimes corresponded to $q_{Eu} = 9.2$ using equation (1). Little difference was seen in the measured lifetimes upon addition of between 0.5 and 4 equivalents of Eu.

Circular Dichroism (CD)

The CD spectra of the three tetra-peptides at pH 4-4.5 in 10 mM HEPES buffer (Figure S9) were different, indicating that the differing stereochemistry of these tetra-peptides had a measurable effect on their secondary structure. In previous work, the CD spectrum of LLLL-Glu has been assigned as random coil.⁸ However, the CD spectrum of naph-LLLL-Glu-OH (Figure S9) did not correspond to this random coil structure, suggesting that the presence of the naph group altered the secondary structure. No changes in the CD spectra of the tetra-peptides were observed upon addition of Eu, most likely because the predominant peptide species was still the uncoordinated peptide due to the weak Eu binding at this acidic pH.



Figure S7: CD spectra of tetra-peptides in 10 mM HEPES buffer (naph-DLDL- and naph-LDDL-Glu-OH at pH 4, naph-LLLL-Glu-OH at pH 4.5)

The CD spectra of 60 µM tri-peptide solutions at pH 4.9 and 60 µM di-peptide solutions at pH 5.9 in 10 mM HEPES buffer upon addition of Eu are shown in Figure 5. Both tri-peptides exhibited similar shaped CD spectra in the absence of Eu, but the molar ellipticity of naph-LDL-Glu-OH was lower. The di-peptides reached equilibrium earlier in the CD titrations than the tri-peptides as only 2 equiv. Eu were added to reach equilibrium, rather than 4. This could be due to the higher pH of the titrations or the shorter length of the peptides. Unlike the tri-peptides, naph-DL- and naph-LL-Glu-OH had different shaped CD spectra before Eu addition, indicating different secondary structures. Upon addition of Eu, changes in the CD spectra of the di- and tri-peptides were observed (Figure 5), indicating a change in secondary structure upon Eu binding. The changes seen in the CD spectra during addition of Eu to the tri-peptides were similar, indicating that similar changes in their secondary structures occurred upon Eu binding. However this was not the case for the di-peptides. The final CD spectra of the tripeptides after addition of 4 equiv. Eu and naph-LL-Glu-OH after addition of 2 equiv. Eu, with a positive band at 215-220 nm, were indicative of an extended structure.⁸ Given that the 1:1 peptide:Eu complex predominated under these conditions, it was assumed that these final CD spectra were representative of this 1:1 species.



Figure S8: CD spectra in 10 mM HEPES buffer upon addition of Eu nitrate. Top left: naph-LLL-Glu-OH, pH 4.9; Top right: naph-LDL-Glu-OH, pH 4.9; Bottom left: naph-DL-Glu-OH, pH 5.9; Bottom right: naph-LL-Glu-OH, pH 5.9.

Nuclear Magnetic Resonance (NMR) Titration

NMR titrations were performed using solutions containing 300 μ M di-peptide and 10 mM HEPES buffer (pH 6) in D₂O in order to obtain a NMR signal but minimise precipitation upon Eu addition.



Figure S9: Changes in chemical shift of ¹H NMR signals from naph-DL-Glu-OH upon Eu addition.



Density Functional Theory (DFT) modelling

Figure S10: Geometry optimised structures of naph-LL-Glu-OH (left) and naph-DL-Glu-OH (right) calculated by DFT modelling. All of the carboxylic acids are deprotonated, simulating a pH value of approximately 6.



Figure S11: Optimised geometry of 1:1 naph-DL-Glu-OH:Eu complexes. Non-binding water molecules are not shown. Structural energy of E = -411.85 eV, F = -411.75 eV, G = -411.80 eV and H = -411.94 eV

Solid State NMR



Figure S12: ¹³C-¹H CP MAS NMR spectra of TiO₂-alkyl and TiO₂-peptide

CHN Microanalysis

It was assumed that for every molecule of undecene-LDDL-Glu-OH ($C_{31}H_{48}N_4O_{14}$) present there were nine molecules of 1-undecene ($C_{11}H_{22}$) also present, so that the overall molecular formula of the organic functional groups was $C_{130}H_{246}N_4O_{14}$. This molecular formula and the total measured %CHN were used to determine the calculated %CHN values, which agreed with the experimentally measured values to within 0.2 %. However, the fact that the calculated %C was higher than measured and the calculated %N was lower than measured suggests that the ratio of undecene-LDDL-Glu-OH to 1-undecene present on the surface of the titania nanoparticles may have in fact been higher than 1:10. The total organic content of TiO₂-peptide was calculated as 1.2 % based on the percentage weight of CHNO. Assuming a 1:9 undecene-LDDL-Glu-OH: 1-undecene molecular ratio on the surface of these materials, the undecene-LDDL-Glu-OH accounts for 33.6 % of the total organic mass and the 1-undecene 66.4 %. For TiO₂-peptide, this equates to 0.001 mol% undecene-LDDL-Glu-OH and 0.005 mol% 1-undecene in terms of 55 m²/g) was 1.6 nm² for TiO₂-peptide.

Measured	TiO ₂ -peptide
%C	0.7
%Н	0.2
%N	0.2
Total	1.1
Calculated	$C_{130}H_{246}N_4O_{14}$
Calculated %C	C ₁₃₀ H ₂₄₆ N ₄ O ₁₄ 0.9
Calculated %C %H	C ₁₃₀ H ₂₄₆ N ₄ O ₁₄ 0.9 0.1
Calculated %C %H %N	C ₁₃₀ H ₂₄₆ N ₄ O ₁₄ 0.9 0.1 0.0(3)

Table S1: Elemental CHN microanalysis of functionalised titania nanoparticles (weight %)

Zeta Potential Measurements



Figure S13: Measured zeta potential of TiO₂-NF and TiO₂-peptide in aqueous nitrate media

Sorption

The results of batch sorption experiments in 10 mM HEPES buffer at at pH 4.8 are shown in Figure S15. As at pH 4.2, Ca, Ni, Sr and Cs were not sorbed by any of the materials. For TiO₂-NF, Al and U were again sorbed strongly, but a small amount of Ln was also sorbed at this higher pH, presumably due to the higher negative surface charge of TiO₂-NF. TiO₂-alkyl again showed enhanced sorption of Al and Ln relative to TiO₂-NF at pH 4.8, although U was similarly sorbed by both materials at pH 4.8 because sorption by TiO₂-NF was already quantitative. The sorption behaviour of TiO₂-peptide at pH 4.8 also showed the same trends relative to TiO₂-NF and TiO₂-alkyl as were observed at pH 4.2. Namely, Ln sorption was enhanced, U sorption was decreased and Al sorption was similar to TiO₂-NF and less than TiO₂-alkyl. However, sorption of Al, Ln and U by TiO₂-peptide were all higher at pH 4.8 than at pH 4.2, as expected given the higher negative surface charge at pH 4.8 resulting from deprotonation of more of the carboxylic acid side chains of the peptide ligand.



Figure S14: Percentage extraction of 1 ppm Al, Ca, Ni, Sr, Cs, Ln and U from pH 4.8 10 mM HEPES buffer by TiO₂-NF, TiO₂-alkyl and TiO₂-peptide.

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