Ag-Nanoparticle Formation Induced by Peptides Identified Within Split-and-Mix Libraries – Generation of Ag-Nanoparticles in Different Sizes

Kirsten Belser,[a] Tünde Vig Slenters,[b] Conelious Pfumbidzai,[a] Grégory Upert,[a] Laurent Mirolo,[b] Katharina M. Fromm,*[b] Helma Wennemers*[a]

[a]Department of Chemistry, University of Basel
St. Johanns-Ring 19, CH-4056 Basel (Switzerland)
Fax: +41-61-267-0976
e-mail: Helma.Wennemers@unibas.ch

[b]Department of Chemistry, University of Fribourg
Chemin du Musée 9, CH-1700 Fribourg (Switzerland)
Fax: +41- 26-300-9738
e-mail: katharina.fromm@unifr.ch

1. General aspects and materials

Materials and reagents were of the highest commercially available grade and used without further purification. Inorganic reagents and solvents were purchased from Fluka and Acros, amino acids from Bachem, IRIS Biotech and Neomps. Tentagel (130µm) was purchased from Rapp Polymers and Rink amide resin from Novabiochem. For all the solution and the washing steps only MilliQ bidistillated water was used. Silver nitrate and ascorbic acid sodium salt solutions were prepared daily. The irradiation was carried out in plastic bottle using an ordinary 15W light-bulb (Philips, EU). ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX 500 and DPX 400 spectrometers. Chemical shifts are reported in ppm using TMS as a reference. Finnigan MAT LCQ and TSQ 700 instruments were used for electrospray ionization (ESI) mass spectrometry measurements. Analytical HPLC was performed on a Shimadzu LC10 series using a LiChrospher 100 RP-18e 5 µm (250 mm x 4 mm) column from Merck. For automated peptide synthesis, a Syro I Peptide Synthesizer (MultiSynTech GmbH, Witten, Germany) was employed. Combinatorial assays were analyzed with an Olympus SZX12 microscope. GC analyses were performed on a Hewlett Packard HP 6890 from Agilent. SEM measurements were performed using Hitachi S4800 and ESEM XL 30 FEG instruments. UV-Vis measurements were performed on a Perkin Elmer Lambda Bio UV/Vis spectrometer. Silver uptake measurements were done using a Shimadzu AA-6300 Atomic Absorption Spectrometer (AAS) instrument. X-ray powder diffraction for all samples was measured on a STOE STADI P automated diffractometer, CuK source, monochromator graphite, by using quartz sample holders; for the pure reagents 10-25 mg of substance were employed.

2. General protocols for solid phase synthesis

General procedure for peptide couplings: Fmoc-Xxx-OH (3 eq.) and HCTU (3 eq.) dissolved in DMF followed by $^{i}Pr_2NEt$ (9 eq.) were added to the amino-functionalized resin in DMF (≈100 mM concentration). The mixture was agitated for 1.5 h before washing with DMF (3x) and CH_2Cl_2 (5x). For couplings on the peptide synthesizer, 4 eq. of the reagents were used, $^{i}Pr_2NEt$ was dissolved in N-Methylpyrrolidone (3 M) and the washing steps were carried out only with DMF (5x).

General procedure for Fmoc-deprotections: A solution of piperidine:DMF (1:4) was added to the resin (preswollen in DMF) and the reaction mixture was agitated for 3 min, drained and the piperidine treatment was repeated for 10 min. Finally the resin was washed with DMF (7x) and CH_2Cl_2 (5x). On the peptide synthesizer only DMF was used for the washing.

General procedure for acetylation: NEt_3 (20 eq.) and Ac_2O (20 eq.) were added to the resin in CH_2Cl_2 (≈120 mM). The mixture was agitated for 1 h and then washed with CH_2Cl_2 (7x).

General procedure for cleavage of peptides from the solid support: The solid supported peptides were cleaved off the Rink Amide resin by stirring in a mixture of TFA:TIS:H_2O 95:2.5:2.5 for 1.5 h and a second time for 20 min. Pooling of filtrates and removal of all volatiles under reduced pressure followed by precipitation in Et_2O, filtration and drying *in vacuo* to afford the peptides as TFA salts.

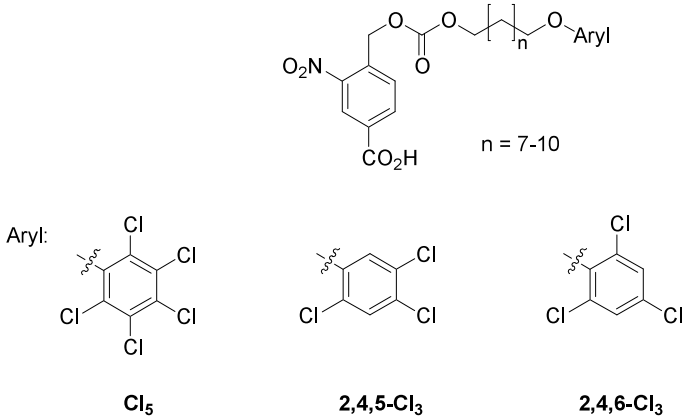
3. Library synthesis [1]

Splitting and encoding: Amino-functionalized Tentagel resin (2.1 g, 0.92 mmol, loading 0.44 $mmol.g^{-1}$) was split into seven equal portions of 300 mg (0.13 mmol) each and placed into fifteen 25 mL Merrifield shaking vessels. Each portion was then suspended in dry CH_2Cl_2 (5 mL). 2 mol% of each tag (Figure 1, see below) were dissolved in 1 mL DMF and added into the fifteen reaction vessels together with HOBt (20 mg, 0.13 mmol, 1 eq. per tag) dissolved in DMF (0.5 mL). The mixtures were shaken for at least 5 min to ensure an equal distribution of the tags. After the addition of DIC (20 µL, 0.20 mmol, 1.5 eq. per tag) to each reaction vessel the mixture was shaken immediately and the reaction allowed to proceed overnight. The fifteen portions of resin were then washed with DMF (3x) and with CH_2Cl_2 (3x).

Decoding of tags: In order to check the success of the tag coupling, three beads from each of the fifteen vessels were isolated and placed into 25 µL micropipettes. The beads were washed with DMF (2x), then DMF (2.5 µL) was added and the micropipettes were sealed. The tag alcohols were released by photolysis using a UV lamp (366 nm, 15W) for 2 h and analyzed by EC-GC. If EC-GC detection of the tags was successful, the resin was carried on to the amino acid coupling, if not, the tag coupling process was repeated.

Coupling of the Amino Acids: The amino acid couplings and the Fmoc-deprotections were performed as described in the general protocol. After drying the resin for at least 2 hours; the processes of splitting, encoding of the resin and coupling of each amino acid cycles were repeated using the protocols described above until the tripeptides were assembled (see Figure 1 and Table 1). Finally, the *N*-termini were acetylated following the general protocol for acetylation.

Tag molecules used for the synthesis:



Step 1:	$(CH_2)_{10}$-Cl_5 (646.74 g/mol, 2.64 µmol, 8.5 mg) = tag Nr. 1 (**T_1**)
	$(CH_2)_{11}$-Cl_5 (660.77 g/mol, 2.64 µmol, 8.7 mg) = tag Nr. 2 (**T_2**)
	$(CH_2)_{12}$-Cl_5 (674.80 g/mol, 2.64 µmol, 9.0 mg) = tag Nr. 3 (**T_3**)
Step 2:	$(CH_2)_{12}$-2,4,6-Cl_3 (604.90 g/mol, 2.64 µmol, 8.0 mg) = tag Nr. 1 (**T_4**)
	$(CH_2)_{12}$-2,4,5-Cl_3 (604.90 g/mol, 2.64 µmol, 8.0 mg) = tag Nr. 2 (**T_5**)
	$(CH_2)_9$-Cl_5 (632.72 g/mol, 2.64 µmol, 8.4 mg) = tag Nr. 3 (**T_6**)
Step 3:	$(CH_2)_{10}$-2,4,5-Cl_3 (576.85 g/mol, 2.64 µmol, 7.6 mg) = tag Nr. 1 (**T_7**)
	$(CH_2)_{11}$-2,4,6-Cl_3 (590.88 g/mol, 2.64 µmol, 7.8 mg) = tag Nr. 2 (**T_8**)
	$(CH_2)_{11}$-2,4,5-Cl_3 (590.88 g/mol, 2.64 µmol, 7.8 mg) = tag Nr. 3 (**T_9**)

Figure 1: Tags and amount used for each encoding step

Table 1: Scheme of encoding

Step 1	M [g mol^{-1}]	m [mg]	Tag	Code		
				T_1	T_2	T_3
Fmoc-L-Asp(O^{t}Bu)-OH	411.45	163	T_3	0	0	1
Fmoc-D-Asp(O^{t}Bu)-OH	411.45	163	T_2	0	1	0
Fmoc-L-Ser(O^{t}Bu)-OH	383.44	152	$T_2 + T_3$	0	1	1
Fmoc-D-Ser(O^{t}Bu)-OH	383.44	152	T_1	1	0	0
Fmoc-L-His(Trt)-OH	619.72	245	$T_1 + T_3$	1	0	1
Fmoc-D-His(Trt)-OH	619.72	245	$T_1 + T_2$	1	1	0
Fmoc-L-Tyr(O^{t}Bu)-OH	459.54	182	$T_1 + T_2 + T_3$	1	1	1
Step 2	**M [g mol^{-1}]**	**m [mg]**	**Tag**	**Code**		
				T_4	T_5	T_6
1) Fmoc-Aib-OH (Pro-Aib)	325.36	129	T_6	0	0	1
2) Fmoc-L-Pro-OH (Pro-Aib)	337.37	134				
Fmoc-ε-Ahx-OH	353.40	140	T_5	0	1	0
Fmoc-Cl (no motif)	258.70	102	$T_5 + T_6$	0	1	1
Fmoc-Gly-OH	297.31	118	T_4	1	0	0
1) Fmoc-Gly-OH	297.31	118	$T_4 + T_6$	1	0	1
2) Fmoc-L-Pro-OH	337.37	134				
Fmoc-ß-Alanin-OH	311.30	123	$T_4 + T_5$	1	1	0
Fmoc-rac-Achc-OH	365.00	145	$T_4 + T_5 + T_6$	1	1	1
Step 3	**M [g mol^{-1}]**	**m [mg]**	**Tag**	**Code**		
				T_7	T_8	T_9
Fmoc-L-Asp(O^{t}Bu)-OH	411.45	163	T_9	0	0	1
Fmoc-D-Asp(O^{t}Bu)-OH	411.45	163	T_8	0	1	0
Fmoc-L-Ser(O^{t}Bu)-OH	383.44	152	$T_8 + T_9$	0	1	1
Fmoc-D-Ser(O^{t}Bu)-OH	383.44	152	T_7	1	0	0
Fmoc-L-His(Trt)-OH	619.72	245	$T_7 + T_9$	1	0	1
Fmoc-D-His(Trt)-OH	619.72	245	$T_7 + T_8$	1	1	0
Fmoc-L-Tyr(O^{t}Bu)-OH	459.54	182	$T_7 + T_8 + T_9$	1	1	1

4. Combinatorial screening results

Approximately 10 mg of the library were suspended in a solution of $AgNO_3$ (0.05 M, 660 µL, ~6 equiv.), sonicated for 5 min and allowed to incubate for another 10 min. After washing 5x with deionized water (1 mL each), the beads were either irradiated with an electric lamp for 8 h (light reduction assay) or incubated with a solution of sodium ascorbate (0.05 M, 660 µL, ~6 eq.) for 5 min (chemical reduction assay) before washing with deionized water (5x). The combinatorial screening assays were evaluated using a light microscope, single beads were isolated and the peptide sequences analyzed.

Table 2: Consensus sequences of library **1** after light reduction

Color	AA2	Linker	AA1	No. of analyzed beads bearing this sequence
	D-His	Achc	L-Tyr	7
	L-His	Achc	L-Tyr	3
	D-His	Pro-Aib	L-Tyr	4
	D-His	Pro-Gly	L-Tyr	1
	L-Ser	Achc	L-Tyr	3
	D-Ser	Achc	L-Tyr	4
	L-Tyr	Achc	L-Tyr	2
	D-Ser	Pro-Aib	L-Tyr	1
	L-Ser	Pro-Aib	L-Tyr	2
	D-Ser	Pro-Gly	L-Tyr	1
	L-Tyr	Achc	D-Ser	5
	D-Ser	Pro-Aib	L-Ser	1
	L-Ser	Pro-Aib	L-Ser	1
	D-Ser	Pro-Gly	D-Ser	2
	L-Tyr	Pro-Gly	D-Ser	1
	L-His	Pro-Gly	D-Ser	1
	D-His	Achc	L-Ser	1
Red	D-His	Achc	L-Ser	1
	L-His	Gly	D-Ser	1
	D-His	Ahx	L-Ser	1
	L-His	Gly	L-Ser	1
	D-His	Pro-Aib	L-Ser	1
	D-Ser	Pro-Aib	L-His	2
	D-Ser	Pro-Gly	L-His	1
	D-Ser	Pro-Gly	D-His	1
	L-Ser	β-Ala	D-His	1
	L-Ser	Gly	L-His	1
	L-Tyr	Achc	L-His	1
	L-Tyr	Pro-Aib	D-His	2
	L-Tyr	Pro-Gly	D-His	1
	L-Tyr	Ahx	D-His	1
	L-His	Gly	L-His	1
	D-His	β-Ala	L-His	1
	L-His	Gly	D-His	1
	L-Ser	Pro-Aib	D-Asp	1

Table 3: Consensus sequences of library **1** after chemical reduction

Color	AA2	Linker	AA1	No. of analyzed beads bearing this sequence
Dark orange Red	D His	Pro-Aib	D Asp	1
	D His	Pro-Aib	L Asp	2
	L His	-	L Asp	1
	L His	Achc	D Asp	1
	D Asp	Ahx	D His	1
	D Asp	Achc	D His	1
	D Asp	-	L His	1
	D Asp	Pro-Gly	L His	1
	L Asp	Pro-Aib	L His	1
	L Asp	Achc	L His	1
	L Asp	Ahx	D His	1
	D His	-	D Ser	1
	L His	Achc	L Ser	1
	L Ser	Gly	L His	1
	L Ser	Ahx	D His	1
	L Ser	Achc	L His	1
	D Ser	Ahx	D His	1
	L His	Ahx	L Tyr	1
	L His	Achc	L Tyr	1
	L Tyr	Achc	D His	1
	L Tyr	Gly	D His	1
	L Tyr	Ahx	L His	1
	L Tyr	Achc	L Tyr	1
	D His	Pro-Gly	D His	1
	L His	-	D His	1
	D His	β-Ala	L His	1
	D His	Gly	L His	1
	D His	Pro-Aib	L His	1
	L His	Gly	D His	1
	L His	Gly	D His	1
	L His	Achc	D His	1
Light orange Yellow	L-Tyr	Achc	D-Ser	1
	L-Tyr	Ahx	D-Ser	1
	D Asp	Pro-Aib	D Asp	1
	D Asp	Pro-Gly	L Asp	1
	L Asp	β-Ala	D Asp	1
	L Asp	Pro-Gly	L Asp	1
	L Asp	β-Ala	L Asp	1
	L Asp	Gly	L Asp	1
	D Asp	Pro-Gly	L Ser	1
	L Asp	Ahx	D Ser	1
	D Ser	Pro-Aib	D Asp	1
	D Asp	Pro-Gly	D His	1
	D Asp	-	D His	1
	D His	Ahx	D Asp	1
	D Ser	-	D His	1
	D Ser	-	L His	1
	D His	-	L His	1
	L Tyr	-	D His	1

5. Synthesis and analytical data of peptides 2a-8a and 2b-8b

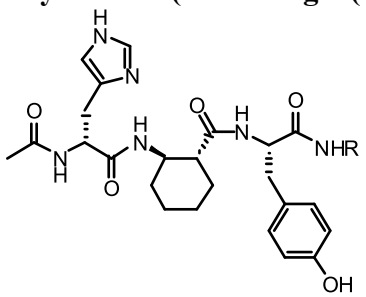
Synthesis:

All peptides were prepared in parallel on amino-functionalized TentaGel and Rink amide resin to verify that the syntheses of peptides **2a-8a** proceeded fine. The peptides were prepared on Tentagel (TG) **(2a-8a)** and Rink amide resin **(2b-8b)** in a 86 µmol and 142 µmol scale, respectively. The syntheses were accomplished following the general procedures for solid phase peptide syntheses.

Desalting of peptides

A solution of sodium hydroxide (1 M) was passed through the ion exchange resin loaded in a column. When the pH of the eluent was above 12, the resin was washed with nanopure water till eluent pH was below 9. This was followed by eluting a solution of acetic acid (1 M) to get the pH of the eluent to below 2. After washing the resin with nanopure water to pH > 6, the peptide dissolved in nanopure water was passed through the column and eluted with nanopure water. The elution process was monitored using ninhydrin test. Fractions with peptide were combined, concentrated and dried under *vacuum*.

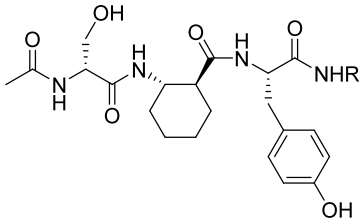
a. Ac-D-His-Achc-L-Tyr-NH-R (R=Tentagel (2a) and R=H (2b))



R = Tentagel, TG) **(2a)**
R = H **(2b)**

^{1}H-NMR (400 MHz, CD_3OD, 25 °C): δ (ppm) = 8.76 (s, 2H), 7.3 (s, 2H), 7.2 (s, 2H), 7.05 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.3 Hz, 2H), 6.68 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 8.6 Hz, 2H), 4.66 (dd, J = 8.1 Hz, 6.1Hz, 1H) 4.55 (dd, J = 8.8 Hz, 5.6 Hz, 2H), 4.49 (dd, J = 9.8 Hz, 4.8 Hz, 1H), 3.99 (dt, J = 11.2 Hz, 4.3 Hz, 1H), 3.85 (dt, J = 11.6 Hz, 4.8 Hz, 1H), 3.19 (dd, J = 15.4 Hz, 6.1 Hz, 1H), 3.06 (m, 3H), 2.96 (dd, J = 15.2 Hz, 7.8 Hz, 1H), 2.78 (m, 2H), 2.69 (dd, J = 15.1 Hz, 6.1 Hz, 1H), 2.26 (m, 2H), 1.99 (s, 3H), 1.96 (s, 3H), 1.88 (m, 3H), 1.71 (m, 5H), 1.33 (m, 8H); ^{13}C-NMR (101 MHz, CD_3OD, 25 °C): δ (ppm) = 177.1, 176.2, 173.4, 171.1, 170.9, 168.5, 165.7, 157.4, 134.8, 134.7, 131.3, 131.4, 131.2, 130.5, 129.6, 128.9, 118.7, 118.6, 116.2, 55.5, 53.7, 53.1, 51.9, 50.9, 50.5, 38.5, 38.2, 33.5, 31.3, 30.5, 28.7, 28.2, 26.0, 26.0, 25.9, 25.8, 22.7, 22.5; ESI-MS: 485.2 $[M+H]^+$ (calcd.: 485.2).

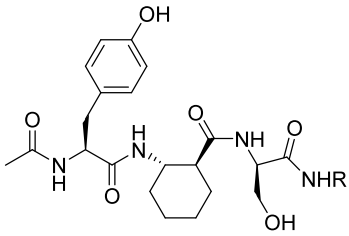
b. Ac-D-Ser-Achc-L-Tyr-NH-R (R=Tentagel (3a) and R=H (3b))



R = TG **(3a)**
R = H **(3b)**

^{1}H-NMR (400.0 MHz, d_6-DMSO, 25 °C): δ (ppm) = 7.05 (d, J = 7.1 Hz, 4H), 6.67 (dd, J = 8.6 Hz, 2.5 Hz, 4H), 4.39 (s, 2H) 4.35 (dd, J = 9.1 Hz, 5.1 Hz, 1H), 4.24 (m, 3H), 3.51 (m, 4H), 2.92 (ddd, J = 13.3 Hz, 7.1 Hz, 5.3 Hz, 2H), 2.71 (m, 2H), 2.26 (dt, J = 11.1 Hz, 3.6 Hz, 1H), 2.17 (dt, J = 12.1 Hz, 3.6 Hz, 1H), 1.97 (s, 3H), 1.89 (s, 3H), 1.80 (m, 2H), 1.61 (m, 6H), 1.20 (m, 8H); ^{13}C-NMR (100.6 MHz, d_6-DMSO, 25°C): δ (ppm) = 174.4, 173.9, 173.8, 173.7, 170.8, 170.6, 169.8, 169.5, 155.9, 130.6, 130.4, 128.3, 115.2, 62.2, 62.0, 55.7, 55.1, 54.8, 54.1, 50.1, 49.8, 49.3, 48.6, 37.0, 36.9, 32.7, 32.5, 29.3, 24.8, 24.7, 23.0, 22.8; ESI-MS: 457.2 $[M+Na]^+$ (calcd.: 457.2).

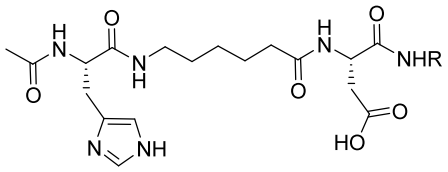
c. Ac-L-Tyr-Achc -D-Ser-NH-R (R=Tentagel (4a) and R=H (4b))



R=TG **(4a)**
R=H **(4b)**

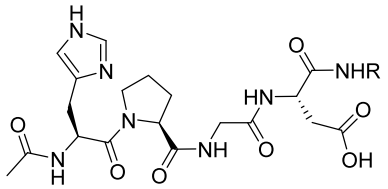
^{1}H-NMR (400.0 MHz, d_6-DMSO, 25°C): δ (ppm) = 7.04 (dd, J = 8.6 Hz, 6.3 Hz, 4H), 6.68 (dd, J = 8.6 Hz, 2.2 Hz, 4H), 4.30 (s, 3H), 4.14 (m, 1H), 3.66 (m, 2H), 3.60 (m, 4H), 2.87 (dd, J = 13.6 Hz, 5.8 Hz, 1H), 2.78 (dd, J = 14.1 Hz, 3.5 Hz 1H), 2.51 (m, 2H), 2.30 (m, 2H), 1.79 (2 s, 9H) 1.67 (m, 5H), 1.41 (m, 2H), 1.20 (m, 6H); ^{13}C-NMR (100.6 MHz, d_6-DMSO, 25°C): δ (ppm) = 174.9, 174.9, 173.8, 173.6, 171.8, 171.4, 155.9, 130.9, 128.9, 115.7, 62.2, 62.1, 55.7, 55.1, 54.8, 54.5, 50.0, 49.9, 49.9, 49.6, 37.8, 37.6, 33.0, 29.7, 27.5, 25.1, 24.9, 24.4, 24.1, 23.9, 23.1, 22.9; ESI-MS: 457.2 $[M+Na]^+$ (100), 435.7 $[M+H]^+$ (50) (cacd.: 457.2 ; 435.2).

d. Ac-L-His-Ahx-L-Asp-NH-R (R=Tentagel (5a) and R=H (5b))



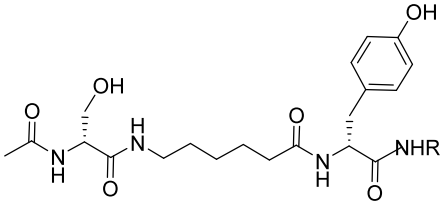
^{1}H-NMR (400 MHz, D_20, 25 °C): δ (ppm) = 8.49 (s, 1H), 7.16 (s, 1H), 4.45 (m, 2H), 3.04 (m, 4H), 2.58 (dd, J = 5.01 Hz, 15.98 Hz, 2H), 2.46 (dd, J = 8.76 Hz, 15.98 Hz, 2H), 2.15 (t, J = 7.18 Hz, 14.18 Hz, 2H), 1.86 (s, 3H), 1.43 (m, 2H) 1.29 (m, 2H), 1.07 (m, 3H); ^{13}C-NMR (100.6 MHz, D_20, 25°C): δ (ppm) = 177.3, 176.7, 174.4, 171.9, 133.9, 129.0, 117.5, 53.3, 51.6, 39.5, 39.0, 35.6, 28.2, 26.9, 25.6, 25.0, 22.0; ESI-MS: 424.81 $[M+H]^+$ (calcd.: 424.21).

e. Ac-L-His-Pro-Gly-L-Asp-NH-R (R=Tentagel (6a) and R=H (6b))



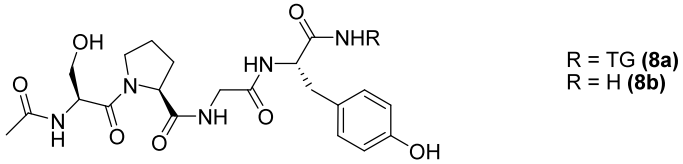
^{1}H-NMR (400.0 MHz, D_20, 25°C): δ (ppm) = 8.79 (s, 1H), 7.43 (s, 1H), 4.97 (t, J = 6.8 Hz, 1H), 4.80 (dd, J = 8.3 Hz, 5.6 Hz, 1H), 4.35 (t, J = 7.1 Hz, 1H), 4.05 (d, J = 16.6 Hz, 1H), 3.83 (m, 1H), 3.77 (d, J = 16.6 Hz, 1H), 3.50 (m, 1H), 3.20 (dd, J = 15.1 Hz, 6.8 Hz, 1H), 3.07 (dd, J = 15.1 Hz, 6.8 Hz, 1H), 2.94 (dd, J = 16.9 Hz, 5.3 Hz, 1H), 2.83 (dd, J = 16.7 Hz, 8.3 Hz, 1H), 2.25 (m, 1H), 2.08 (m, 1H), 1.97 (m, 5H); ^{13}C-NMR (100.6 MHz, CD_3OD, 25°C): δ (ppm) = 175.7, 174.1, 173.1, 171.6, 170.9, 135.2, 130.4, 119.1, 62.4, 51.7, 51.1, 43.9, 36.7, 30.7, 27.6, 26.3, 25.0, 22.3; ESI-MS: 466.2 $[M+H]^+$ (calcd.: 465.4).

f. Ac-L-Ser-Ahx-L-Tyr-NH-R (R=Tentagel (7a) and R=H (7b))



^{1}H-NMR (400.0 MHz, D_2O, 25 °C): δ (ppm) = 7.03 (d, J = 8.44 Hz, 2H), 6.71 (d, J = 8.48 Hz, 2H), 4.43 (m, 1H), 4.20 (t, J = 5.38 Hz, 10.72 Hz, 1H), 3.69 (d, J = 5.38 Hz, 2H), 3.01 (m, 4H), 2.70 (m, 1H), 2.04 (m, 2H), 1.24 (m, 4H), 0.86 (m, 2H); ^{13}C-NMR (100.6 MHz, D_20, 25°C): δ (ppm) = 177.3, 176.7, 174.8, 171.9, 154.7, 130.8, 128.9, 115.8, 61.5, 56.3, 54.9, 39.5, 36.6, 35.7, 28.3, 25.2, 22.1; ESI-MS: 445.3 $[M+Na]^+$ (calcd.: 445.2)

g. Ac-L-Ser-ProGly-L-Tyr-NH-R (R=Tentagel (8a) and R=H (8b))



^{1}H-NMR (400.0 MHz, D_2O, 25°C): δ (ppm) = 7.01 (d, J = 8.34 Hz, 2H), 6.71 (d, J = 8.36 Hz, 2H), 4.60 (m, 1H), 4.40 (dd, J = 6.22 Hz, 8.37 Hz, 1H), 4.30 (dd, J = 5.88 Hz, 8.32 Hz, 1H), 3.65 (m, 6H), 2.97(dd, J = 6.10 Hz, 14.07 Hz, 1H), 2.82 (dd, J = 8.44 Hz, 14.13 Hz, 1H), 2.13 (m, 1H), 1.88 (m, 5H), 1.74 (m, 1H); ^{13}C-NMR (100.6 MHz, D_20, 25°C): δ (ppm) = 176.1, 175.1, 174.5, 171.4, 170.8, 154.8, 130.9, 128.6, 115.8, 61.3, 60.9, 55.1, 54.1, 48.4, 42.6, 36.5, 29.6, 25.0, 21.9; ESI-MS: 486.6 $[M+Na]^+$ (calcd.: 486.2).

6. Ag-nanoparticle formation by solid supported peptides 2a-8a

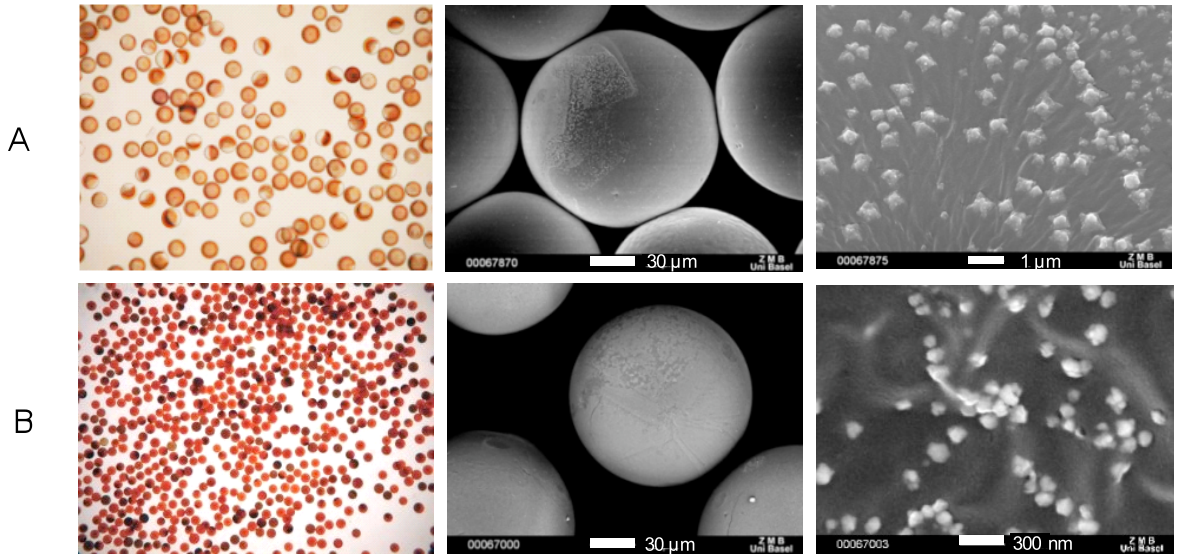
Peptides **2a-8a** bound to TentaGel resin were treated under the same conditions as the library for the light and chemical reduction processes. Images were taken using a light microscope and formation of Ag-NPs was confirmed by SEM studies (see below).

Light microscope and SEM pictures:

A- Light reduction

B- Chemical reduction

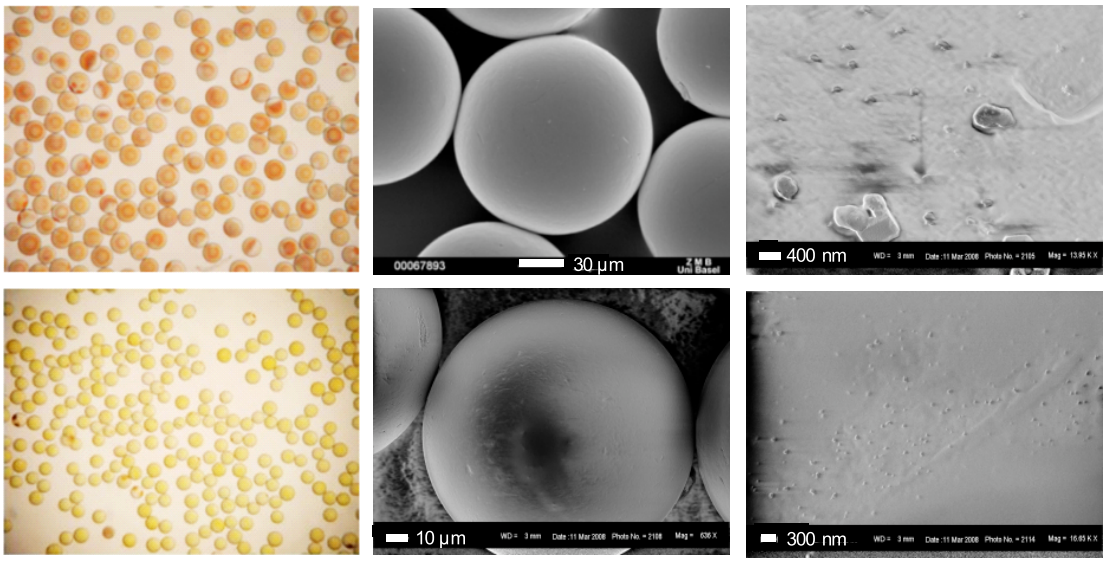
Peptide **2a**



Peptide **3a**

A

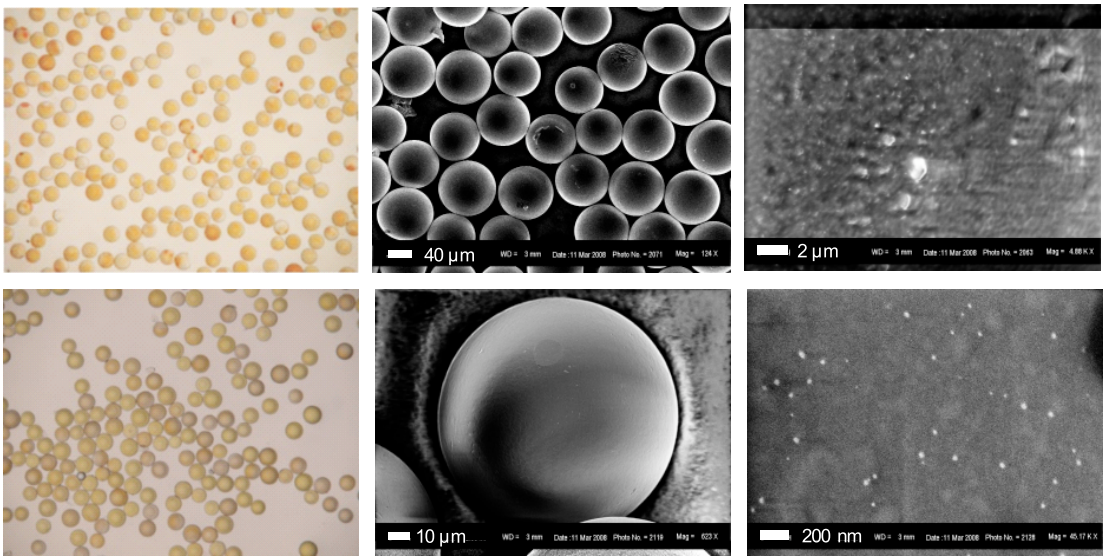
B



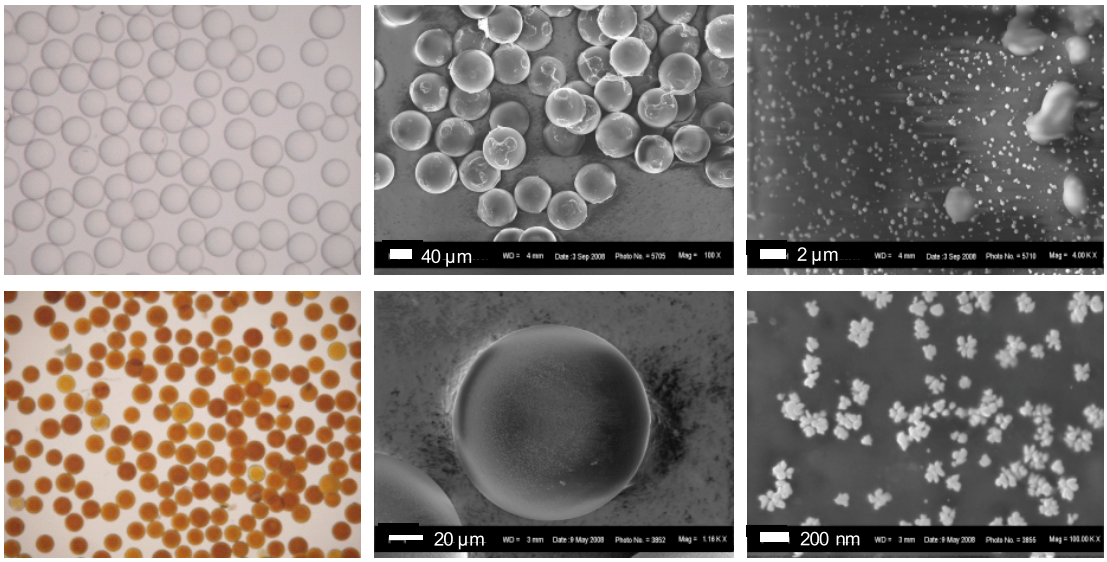
Peptide **4a**

A

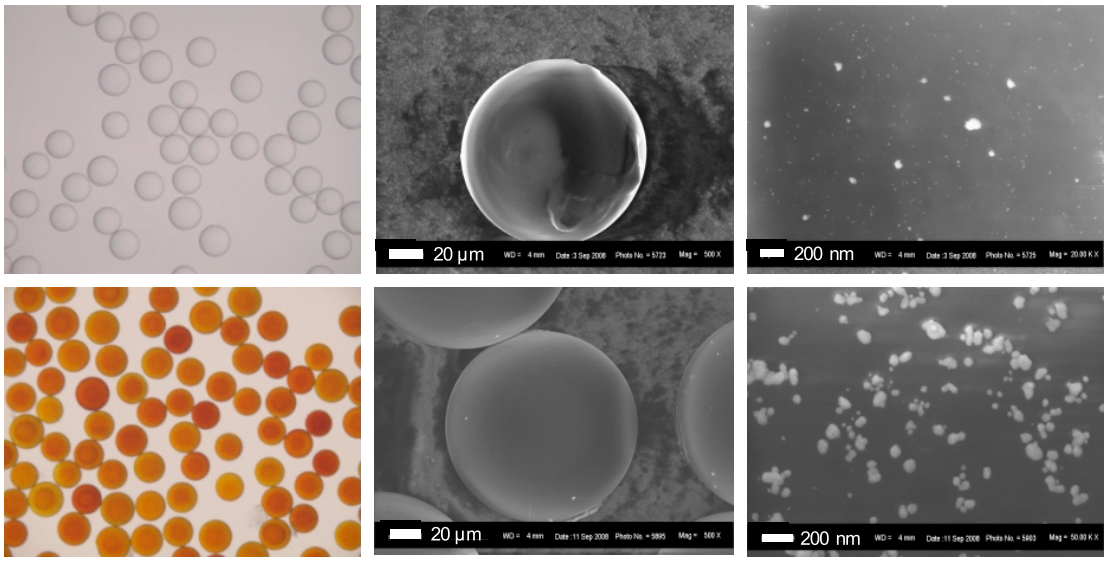
B



Peptide **5a**



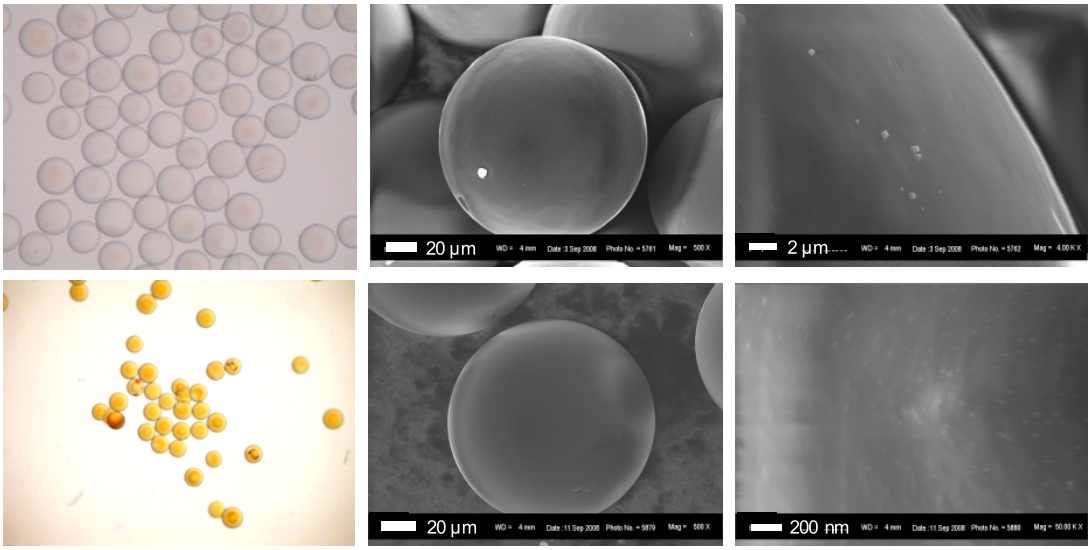
Peptide **6a**



Peptide **7a**

A

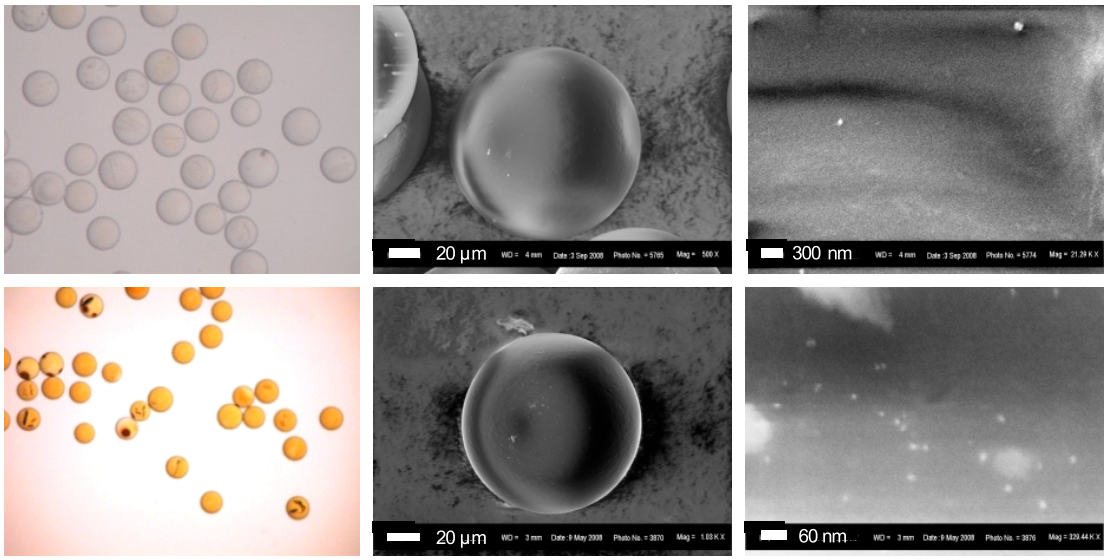
B



Peptide **8a**

A

B



7. Ag uptake studies of solid supported peptides 2a-8a

Sample preparation: 10.0 mg of dry resin (exact weight) were suspended in a solution of $AgNO_3$ (0.05 M, 660 µL) solution and sonicated for 15 min. Then, 500 µL of the solution was removed and diluted to 50 mL. 5 mL of the obtained solution was diluted again to 100 mL after addition of 2 mL of 10% HNO_3. Then the Ag concentration of this solution was measured by atom absorption spectroscopy (AAS) measurements and compared with the concentration of the reference solution prepared from the starting $AgNO_3$ solution (0.05 M) diluted in the same way.

The uptakes of the different peptides **2a-8a** are summarized in Table 2.

Table 2: Silver ions uptake for peptide bound resin **2a-8a**

Peptide	Uptake in mol(Ag)/mol(Peptide)
2a	0.58
3a	0.37
4a	0.30
5a	0.55
6a	0.47
7a	0.26
8a	0.19

8. X-Ray Powder diffraction (Figure 2)

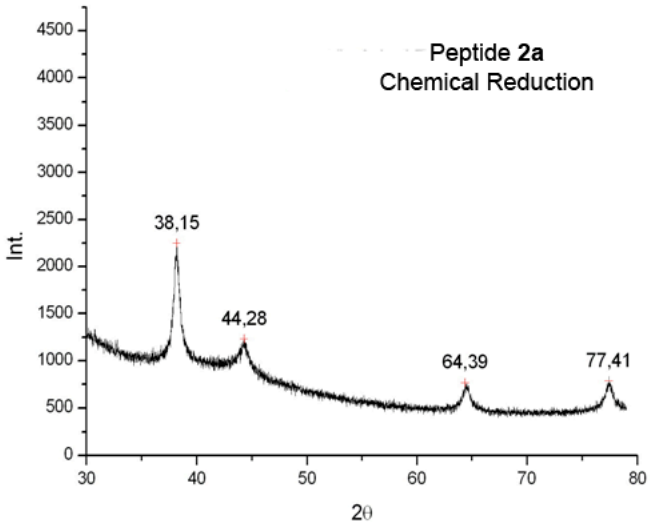


Figure 2: Example of a X-Ray powder diffraction spectrum of Ag-NPs on solid supported peptide **2a**

9. Solution phase experiments

Solutions of peptides **5b** and **6b** (5 mM) were adjusted to pH 8.5 with a solution of NaOH (2 M) in deionized water. After incubating the mixture with a solution of $AgNO_3$ (0.5 mM) in the absence of light for 15 minutes, sodium ascorbate (0.6 mM) was added. The formation of AgNPs was followed by UV-Vis spectroscopy.

10. References

[1] M. H. J. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. H. Wigler, W. C. Still, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922-10926.