Supplementary Information

Highly Efficient Cell Adhesion on Beads Functionalized with Clustered Peptide Ligands

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Materials and equipments

Protected amino acids were obtained from Activo tec (Cambrigde, UK), Bachem Biochimie SARL (Voisins-les-Bretonneux, France), France Biochem SA (Meudon, France), Merck Eurolab (Fontenay-sous-Bois, France) or Calbiochem-Novabiochem (Merck Biosciences - VWR, Limonest, France). PyBOP® was purchased from Calbiochem-Novabiochem and PyAOP® from PerSeptive Biosystems (Foster City, Canada). NovaSyn® TGR resin were obtained from Calbiochem-Novabiochem, Fmoc-Gly-SASRIN® resin from Bachem Biochimie SARL and 2-chlorotritylchloride® resins from Advanced ChemTech Europe. Other reagents were obtained from Aldrich (Saint-Quentin Fallavier, France) and Acros (Noisy-le-Grand, France).

RP-HPLC analyses were performed on Waters equipment consisting of a Waters 600 controller, a Waters 2487 Dual Absorbance Detector and a Waters In-Line Degasser. The analytical column used was the Nucleosil 120 Å 3 µm C18 particles, 30×4 mm^2 operated at 1.3 mL.min^{-1} with linear gradient programs in 15 min run time (classical program 5 to 100 % B in 15 min). UV monitoring was performed most of the time at 214 nm and 250 nm. Solvent A consisted of H_2O containing 0.1% TFA and solvent B of CH_3CN containing 9.9% H_2O and 0.1% TFA. Water was of Milli-Q quality and was obtained after filtration of distilled water through a Milli-Q® cartridge system. CH_3CN and TFA were of HPLC use quality. RP-HPLC purifications were performed on Waters equipment consisting of a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. The preparative column, Delta-Pak™ 300 Å 15 µm C18 particles, 200×25 mm^2 was operated at 22 mL.min^{-1} with linear gradient programs in 30 min run time. Solvents A and B were the same than the ones used in RP-HPLC analysis.

Electron spray ionization (ESI-MS) mass spectra were obtained on an Esquire 3000 (Bruker). NMR spectra were recorded on BRUKER Avance 300 spectrometers. Chemical shifts are expressed in ppm and calculated taking the solvent peak as an internal reference.

General procedures for peptide syntheses

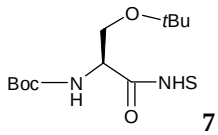
Assembly of all linear protected peptides was performed manually or automatically by solid-phase peptide synthesis (SPPS) using the standard 9-fluorenylmethoxycarbonyl/tertiobutyl (Fmoc/*t*Bu) protection strategy. In manual SPPS, device consisted in a glass reaction vessel fitted with a sintered glass frit. The latter allowed elimination of excess reagents and solvents under compressed air. Before use, the vessel was treated for 12 h (typically overnight) with $(CH_3)_2SiCl_2$ as lubricant to prevent resin beads from sticking to the glass inner wall during the synthesis. It was then carefully washed with CH_2Cl_2 until complete acid trace clearance. At the beginning of the synthesis and after each ether washing, the resin was washed and swollen twice with CH_2Cl_2 (20 mL/g resin) for 15 min and once with DMF (20 mL/g resin) for 15 min. Coupling reactions for **12** and **16** were performed using, relative to the resin loading, 1.5-2 eq. of N_α-Fmoc-protected amino acid *in situ* activated with 1.5-2 eq. PyBOP and 3-4 eq. DIPEA in DMF (10 mL/g resin) for 30 min. The resin was then washed twice with DMF (20 mL/g resin) for 1 min and twice with CH_2Cl_2 (20 mL/g resin) for 1 min. The completeness of amino acid coupling reaction was checked by two tests: Kaiser and TNBS. *N*α-Fmoc protecting groups were removed by treatment with piperidine/DMF (1:4) (10 mL/g resin) for 10 min. The process was repeated three times and the resin was further washed five times with DMF (10 mL/g resin) for 1 min. The completeness of the deprotection was checked by UV measurement (λ = 299 nm, ε = 7800 $M^{-1}.cm^{-1}$).

Automated syntheses of peptide are performed on ABI 433 A1 Peptide Synthesizer (Applied Biosystems) using standard solid-phase methods or on 348 Ω Synthesizer (Advance ChemTech) using the same conditions as manual procedure.

Cyclization reactions of peptides **12** and **16** in solution are performed using protected linear peptides. There were dissolved in DMF (0.5 mM) and the pH of the solution was adjusted to 8-9 by addition of DIPEA. PyBOP (1.1 or 1.2 eq.) was added and solution was stirred at r.t. for at least 30 min. Solvent was removed under reduced pressure and residue dissolved in the minimum of CH_2Cl_2. Ether was added to precipitate the peptide. The latter was triturated and washed three times with ether affording crude material, which was, most of the time, further used without additional purification.

Syntheses of modified amino acid for SPPS

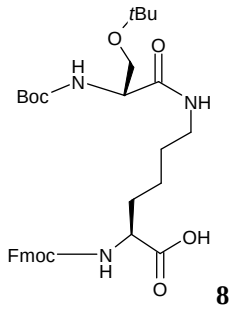
Boc-Ser(*t*Bu)-*N*-hydroxysuccinimide ester



7

To a stirred solution of Boc-Ser(*t*Bu)-OH (2.8 g, 10 mmol) and *N*-hydroxysuccinimide (1.2 g, 10.7 mmol) in EtOAc/dioxan (1:1, 100 mL) cooled on ice bath were added DCC (2.2 g, 10.7 mmol). After 5 hr stirring at r.t. the reaction mixture was filtered through a pad of Celite with EtOAc and the collected filtrate was concentrated in vacuum. The obtained oily residue was taken into 150 mL ethyl acetate and washed with 5 % aqueous $NaHCO_3$ (2 x 70 mL), water (2 x 70 mL) and brine (2 x 70 mL). The organic fraction was then dried over Na_2SO_4. Boc-Ser(*t*Bu)-*N*HS **7** was obtained as a white powder after evaporation of the solvent (3.5 g, 9.8 mmol, 98%). MS (ESI-MS, positive mode): $C_{16}H_{26}N_2O_7$: Calcd MW = 358.2 g.mol^{-1}, Found MW = 359.3 g.mol^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.41 (1H, d, J = 9.0 Hz), 4.78 (1H, d, J = 9.0 Hz), 3.92 (1H,m), 3.66 (1H, m), 2.82 (4H, s), 1.46 (9H, s), 1.20 (9H, s).

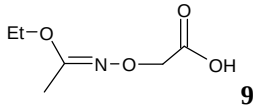
Fmoc-Lys[Boc-Ser(*t*Bu)]-OH



8

The above Boc-Ser(*t*Bu)-*N*HS **7** (8.00 g, 22.3 mmol) in CH_2Cl_2 (75 mL) was added dropwise over 30 min to a stirred suspension of finely powdered TFA-salt of Fmoc-lysine (10.90 g, 22.6 mmol) and DIPEA (15.6 mL, 90.0 mmol) in CH_2Cl_2 (130 mL). After 5 hr reaction, the solvent was evaporated off in vacuum and the remaining residue was dissolved in EtOAc (400 ml), followed by washing with 10 % aqueous citric acid solution (70 ml), water (2 x 100 ml), and finally brine (100 mL). The organic fraction was dried over Na_2SO_4 and evaporation under vacuum gave 11.85 g of a white solid. This solid was recrystallized from CH_2Cl_2 with a hexane-diethyl ether solution (95/5) giving Fmoc-Lys[Boc-Ser(*t*Bu)]-OH **8** as a pure white powder (8.94 g, 14.6 mmol, 66%). MS (ESI-MS, positive mode): $C_{33}H_{45}N_3O_8$: Calcd MW = 611.3 g.mol^{-1}, Found MW = 611.2 g.mol^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (2H, d, J = 7.5 Hz), 7.55 (2H, d, J = 7.5 Hz), 7.33-7.18 (4H, m), 6.84 (1H, broad s), 6.25 (1H, broad d), 5.62 (1H, broad s), 4.34-4.06 (5H, m), 3.63 (1H, m), 3.36 (1H, m), 3.20-3.05 (2H, m), 1.84 (1H, m), 1.65 (1H, m), 1.47-1.32 (14H, m),1.09 (9H, s).

2-(1-ethoxyethylideneaminooxy)acetic acid

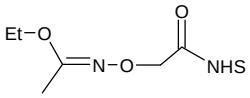


9

To a stirred solution of iodoacetic acid (9.00 g, 48.4 mmol) in water (19 mL) at 0 °C was added aqueous NaOH (3.0 ml, 40 % w/w). The resulting solution was allowed to heat to r.t., where after ethyl *N*-hydroxyacetimidate (6.0 g, 58.3 mmol) was added followed by aqueous NaOH (4.5 mL, 40 % w/w) and water (19 mL) (pH of solution >12). After 4.5 hr stirring at 80 °C and cooling to r.t., water was added (70 mL) and the aqueous mixture was washed with CH_2Cl_2 (3 x 50 mL). The water phase was brought to pH = 2-3 with a 1 M hydrochloride solution. The acidified water phase was then extracted with CH_2Cl_2 (4 x 50 mL), and the combined organic phases from this last extraction was washed with brine (50 mL), dried over Na_2SO_4 and concentrated in vacuum, providing the product **9** as a colourless oil (5.69 g, 35.3 mmol,

73%). MS (ESI-MS, positive mode): $C_6H_{11}NO_4$: Calcd MW = 161.2 g.mol^{-1}, Found MW = 161.0 g.mol^{-1}; NMR ^{1}H (300 MHz, $CDCl_3$): δ 4.48 (2H, s), 4.00 (2H, q, J = 7.2 Hz), 2.01 (3H, s), 1.27 (3H, t, J = 7.2 Hz).

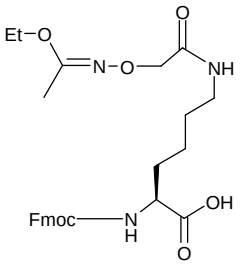
***N*-hydroxysuccinimidyl 2-(1-ethoxyethylideneaminooxy)acetate**



10

To a stirred solution of **9** (5.69 g, 35.3 mmol) and *N*-hydroxysuccinimide (4.06 g, 35.3 mmol) in ethyl acetate/dioxan (120 mL, 1/1) at 0 °C was added in one portion DCC (7.28 g, 35.3 mmol). The resulting mixture was stirred at r.t. for 5 hr The formed DCU was filtered off and the filtrate concentrated under vacuum. The obtained residue was dissolved in ethyl acetate (300 mL) and the solution was washed with 5 % aqueous $NaHCO_3$ (2 x 75 mL), water (75 mL) and brine (75 mL). The organic solution was dried over Na_2SO_4 and evaporated under vacuum to oil **10** (8.69 g, 33.7 mmol, 95%) which was used without further purification. NMR ^{1}H (300 MHz, $CDCl_3$): δ 4.78 (2H, s), 4.01 (2H, q, J = 7.2 Hz), 2.84 (4H, s), 1.98 (3H, s), 1.28 (3H, t, J = 7.2 Hz).

Fmoc-Lys[*N*-EEI-Aoa]-OH

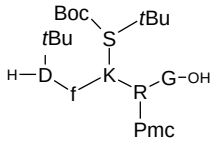


11

To a stirred mixture of Fmoc-lysine (6.22 g, 16.9 mmol) and DIPEA (3.0 ml, 17.3 mmol) in CH_2Cl_2 (100 ml) at r.t. was added dropwise over 20 min a solution of the above prepared *N*HS-ester **10** (4.35 g, 16.9 mmol) in CH_2Cl_2 (40 mL). The pH of the resulting mixture was regularly adjusted to pH 8-9 by further additions of DIPEA. After 1 hr reaction the unreacted Fmoc-lysine was filtered off and the filtrate was concentrated under vacuum providing an oily residue. After addition of CH_2Cl_2 (100 mL) the organic phase was washed with a concentrated citric acid solution. The aqueous phase was then extracted with methylene chloride (3 x 80 mL). The combined organic phases were then washed with brine (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure. To the residue were added 60 mL of acetonitrile and 60 mL of water. The product was lyophilized, thereby providing the protected amino acid **11** as a white powder (7.47 g, 14.6 mmol, 86%). MS (ESI-MS, positive mode): $C_{27}H_{33}N_3O_7$: Calcd MW = 511.2 g.mol^{-1}, Found MW = 511.3 g.mol^{-1}; NMR ^{1}H (300 MHz, $CDCl_3$): δ 7.75 (2H, d, J = 7.4 Hz), 7.60 (2H, d, J = 7.4 Hz), 7.38 (2H, t, J = 7.4 Hz), 7.29 (2H, t, J = 7.4 Hz), 6.50 (1H, t, J = 5.6 Hz), 5.70 (1H, d, J = 7.8 Hz), 4.38-4.36 (5H, m), 4.20 (1H, t, J = 6.9 Hz), 3.96 (2H, q, J = 7.1 Hz), 3.34 (2H, m), 1.96 (3H, s), 1.81 (2H, m), 1.58 (2H, m), 1.45 (2H, m), 1.24 (3H, t, J = 7.1 Hz).

Syntheses of cyclopentapeptidic ligands

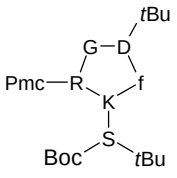
H-D(*t*Bu)-f-K(BocS(*t*Bu))-R(Pmc)-G-OH



12

The linear pentapeptide **12** was assembled on Fmoc-G-SASRIN™ resin (2.0 g, loading of 0.69 mmol/g) using the general procedure with amino acid **8**. The peptide was released from the resin using cleavage solution of TFA/CH_2Cl_2 (1/99). The free linear protected peptide **12** was obtained as a white solid powder (1.31 mg, 1.0 mmol) after precipitation, triturating and washing with diethyl ether. This crude material was used without further purification. MS (ESI-MS, positive mode): $C_{57}H_{90}N_{10}O_{15}S$: Calcd MW = 1186.6 g.mol^{-1}, Found MW = 1186.2 g.mol^{-1}.

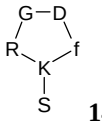
c[-R(Pmc)-G-D(*t*Bu)-f-K(BocS(*t*Bu)-]



13

The crude cyclic peptide **12** (142 mg, 0.14 mmol) was dissolved in 16 mL of DMF and the pH was adjusted to 9 with DIPEA. PyBOP (125 mg, 0.24 mmol) and Boc-Ser(*t*Bu)-OH (63 mg, 0.24 mmol) were added and the reaction mixture was stirred for 30 min at r.t. The solvent was removed under reduced pressure and the peptide was precipitated, triturated and washed with diethyl ether. Crude product **13** (163 mg, 0.14 mmol) was obtained as a white powder and further used without additional purification. MS (ESI-MS, positive mode): $C_{57}H_{88}N_{10}O_{14}S$: Calcd MW = 1168.6 g.mol^{-1}, Found MW = 1168.2 g.mol^{-1}.

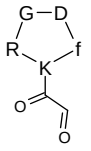
c[-R-G-D-f-K(S)-]



14

The cyclopeptide **13** (770 mg, 0.66 mmol) was treated in 66 mL of TFA/TIS/H_2O (95/2.5/2.5) solution. The reaction mixture was stirred for 1 hr at r.t. The product was concentrated under reduced pressure. Precipitation and work-up in diethyl ether afforded peptide **14** as a white solid powder (603 mg, 0.66 mmol). This crude material was used without further purification. MS (ESI-MS, positive mode): $C_{30}H_{46}N_{10}O_9$: Calcd MW = 690.3 g.mol^{-1}, Found MW = 690.1 g.mol^{-1}.

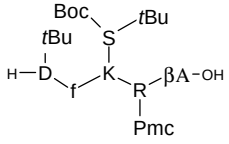
c[-R-G-D-f-K(COCHO)-]



15

Peptide **14** (120 mg, 0.131 mmol) and $NaIO_4$ (282 mg, 1.32 mmol) were dissolved in 12 mL of water. The mixture was stirred for 20 min and the product was purified by RP-HPLC affording pure aldehyde-containing cyclopeptide **15** as a white powder (85 mg, 0.110 mmol). MS (ESI-MS, positive mode): $C_{29}H_{41}N_9O_9$: Calcd MW = 659.3 g.mol^{-1}, Found MW = 659.4 g.mol^{-1}.

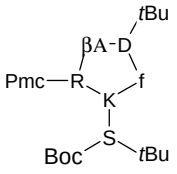
H-D(*t*Bu)-f-K(BocS(*t*Bu))-R(Pmc)-βA-OH



16

The linear decapeptide was assembled on 2-chlorotritylchloride® resin (2 g, loading of 1.4 mmol/g) using the general procedure and amino acid **8**. The anchoring of the first amino acid (Fmoc-βAla-OH) through nucleophilic substitution was performed following the procedure given by Advanced ChemTech and yielding to a convenient resin loading of 0.9 mmol/g. The peptide was released from the resin using cleavage solution of TFA/CH_2Cl_2 (1/99). The free linear protected peptide **16** was obtained as a white solid powder (1.26 g, 0.96 mmol) after precipitation, triturating and washing with diethyl ether. This crude material was used without further purification. MS (ESI-MS, positive mode): $C_{58}H_{92}N_{10}O_{15}S$: Calcd MW = 1200.6 g.mol^{-1}, Found MW = 1200.2 g.mol^{-1}.

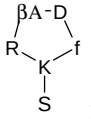
c[-R(Pmc)-βA-D(*t*Bu)-f-K(BocS(*t*Bu)-]



17

The crude cyclic peptide **16** (303 mg, 0.29 mmol) was dissolved in 30 mL of DMF and the pH was adjusted to 9 with DIPEA. PyBOP (194 mg, 0.37 mmol) and Boc-Ser(*t*Bu)-OH (93 mg, 0.35 mmol) were added and the reaction mixture was stirred for 1 hr at r.t. The solvent was removed under reduced pressure and the peptide was precipitated, triturated and washed with diethyl ether. Crude product **17** (340 mg, 0.29 mmol) was obtained as a white powder and further used without additional purification. MS (ESI-MS, positive mode): $C_{58}H_{90}N_{10}O_{14}S$: Calcd MW = 1182.6 g.mol^{-1}, Found MW = 1182.2 g.mol^{-1}.

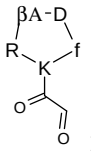
c[-R-βA-D-f-K(S)-]



18

The cyclopeptide **17** (1093 mg, 0.92 mmol) was treated in 66 mL of TFA/TIS/H_2O (95/2.5/2.5) solution. The reaction mixture was stirred for 1 hr at r.t. The product was concentrated under reduced pressure. Precipitation and work-up in diethyl ether afforded peptide **18** as a white solid powder (857 mg, 0.92 mmol). This crude material was used without further purification. MS (ESI-MS, positive mode): $C_{31}H_{48}N_{10}O_9$: Calcd MW = 704.4 g.mol^{-1}, Found MW = 704.3 g.mol^{-1}.

c[-R-βA-D-f-K(COCHO)-]



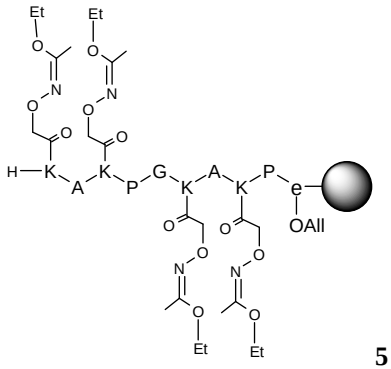
19

Peptide **18** (80 mg, 0.086 mmol) and $NaIO_4$ (184 mg, 0.86 mmol) were dissolved in 8 mL of water. The mixture was stirred for 20 min and the product was purified by RP-HPLC affording pure aldehyde-containing cyclopeptide **19** as a white powder (51 mg, 0.065 mmol). MS (ESI-MS, positive mode): $C_{30}H_{43}N_9O_9$: Calcd MW = 673.3 g.mol^{-1}, Found MW = 673.6 g.mol^{-1}.

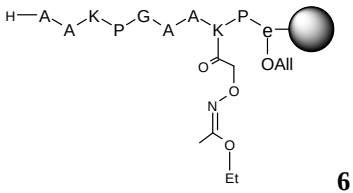
Decapeptide syntheses on resin beads

All peptides were analysed from samples of resin beads treated by a TFA/H_2O solution (9/1).

Solid phase decapeptide syntheses

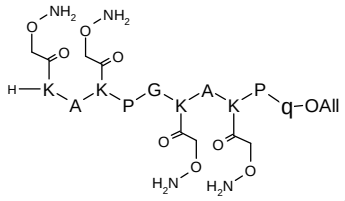


5



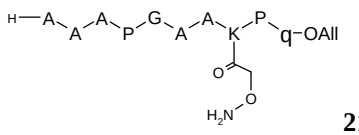
6

The assemblies of fully protected peptides **5** and **6** on resin were carried out on 100 mg NovaSyn TGR, swelled in CH_2Cl_2 (6 mL, 15 min) and DMF (6 mL, 3 min). Stirred on a flask, the first coupling reaction was performed, by using 1 eq. of Fmoc-e-Allyl amino acid (relative to the resin loading targeted between 0.2 mmol/g and 0.2 pmol/g) activated *in situ* in DMF (1.5-3 mL) for 1 hr with 0.4 mmol/g of PyBOP and DIPEA to adjust pH to 8-9. Then the resin was capped with a solution of pyridine/Ac_2O/DMF (2/1/7). Other coupling reactions were performed manually on a glass reaction vessel fitted with a sintered glass frit by using 0.4 mmol/g of classical Fmoc-AA-OH amino acids or building block **11** activated *in situ* in DMF (6 mL) for 30 min with 0.4 mmol/g PyBOP and DIPEA to adjust pH to 8-9. Fmoc protecting groups were removed by 3 treatments with a piperidine/DMF solution (1/4) for 10 min (10 mL/g resin). Between each coupling or deprotection steps, the resins were washed with DMF (6 mL x 4) and CH_2Cl_2 (6 mL).



20

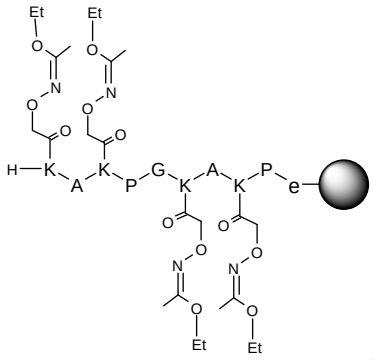
Sample of resin beads **5** was treated by TFA/H_2O (9/1) affording peptide **20**. **MS** (ESI-MS, positive mode): $C_{58}H_{100}N_{18}O_{21}$: Calcd MW = 1384.7 g.mol^{-1}, Found MW = 1383.8 g.mol^{-1}.



21

Sample of resin beads **6** was treated by TFA/H_2O (9/1) affording peptide **21**. MS (ESI-MS, positive mode): $C_{43}H_{20}N_{12}O_{15}$ **:** Calcd MW = 994.5 g.mol^{-1}, Found MW = 994.3 g.mol^{-1}.

Removal of allyl protecting group



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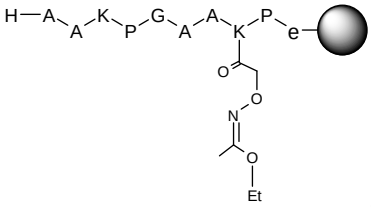
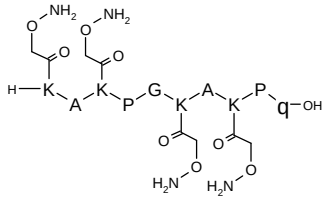


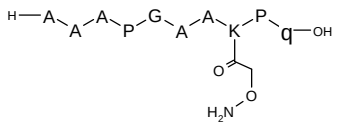
23

The linear decapeptides on resin **5** and **6** (50 mg), previously swelled in dry CH_2Cl_2 under argon (6 mL 15 min), were treated in dry CH_2Cl_2 (6 mL) under argon by adding successively phenylsilane (4.92 mL, 40 mmol) followed after 3 min by $Pd(PPh_3)_4$ (46 mg, 0.04 mmol). The reaction mixtures were stirred for 30 min at r.t. The linear peptides on resin **22** and **23** were washed in CH_2Cl_2 (6mL x 2), in dioxane/H_2O (9/1), then in DMF (6mL) and CH_2Cl_2 (6 mL).



24

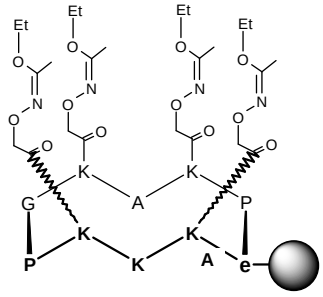
Sample of resin beads **22** was treated by TFA/H_2O (9/1) affording peptide **24**. MS (ESI-MS, positive mode): $C_{55}H_{97}N_{19}O_{20}$ **:** Calcd MW = 1343.7 g.mol^{-1}, Found MW = 1343.5 g.mol^{-1}.



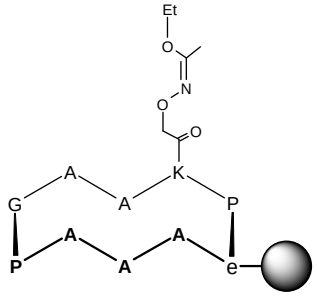
25

Sample of resin beads **23** was treated by TFA/H_2O (9/1) affording peptide **25**. MS (ESI-MS, positive mode): $C_{40}H_{67}N_{13}O_{14}$ **:** Calcd MW = 953.5 g.mol^{-1}, Found MW = 953.5 g.mol^{-1}.

Peptide cyclization on solid support

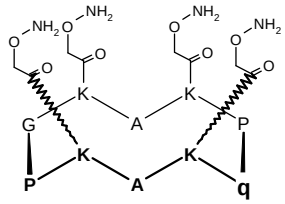


26



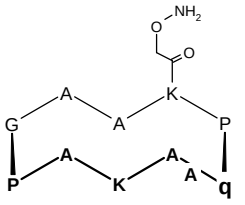
27

The linear decapeptide on resin (50 mg), previously swelled in dry CH_2Cl_2 (6 mL, 15 min), was solvated in DMF (6 mL) and the pH value was adjusted to 8-9 by addition of DIPEA. PyAOP (0.4 mmol/g) was added and the mixture was stirred at r.t. for 1 hr. The cyclodecapeptide resin was washed with DMF (6 mL x 4) and CH_2Cl_2 (6 mL).



28

Sample of resin beads **26** was treated by TFA/H_2O (9/1) affording peptide **28**. MS (ESI-MS, positive mode): $C_{55}H_{95}N_{19}O_{19}$ **:** Calcd MW = 1325.7 g.mol^{-1}, Found MW = 1325.8 g.mol^{-1}.

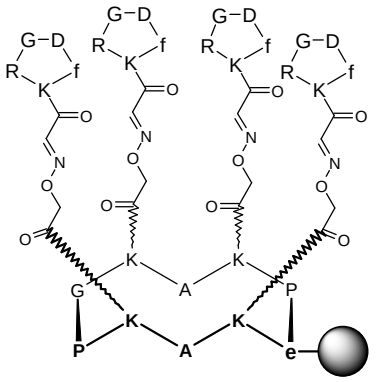


29

Sample of resin beads **27** was treated by TFA/H_2O (9/1) affording peptide **29**. MS (ESI-MS, positive mode): $C_{40}H_{65}N_{13}O_{13}$ **:** Calcd MW = 935.5 g.mol^{-1}, Found MW = 935.3 g.mol^{-1}.

Molecular assemblies of compounds 1-3

Tetravalent RGD-containing resin beads



1

The cyclodecapeptide on resin **26**, previously swelled in dry CH_2Cl_2 (6 mL, 15 min), was treated by TFA/H_2O/CH_2Cl_2 (94/3/3) solution for 30 min, under vigorous stirring and then washed with DMF. Solution of peptide **15** (1 mmol/g) in dry DMF was added to the resin. The mixture was stirred at r.t. for 2 hr. The cyclodecapeptide on resin **1** was washed with DMF (6 mL x 4), MeOH (6 mL), H_2O 0.1 % TFA (6 mL x 40) and MeOH (6 mL). Sample of resin **1** was treated by TFA/H_2O (9/1) solution affording free peptide for analysis.

Figure S1: RP-HPLC: RT = 12.0 min (C_{18}, 214 nm, 5-60% B in 30 min)

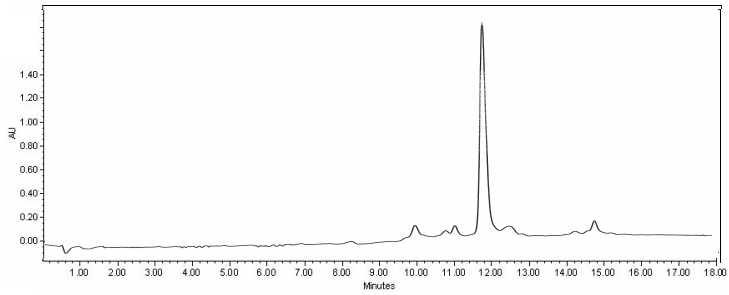
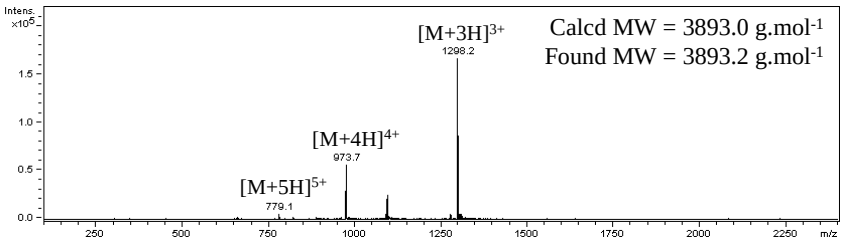
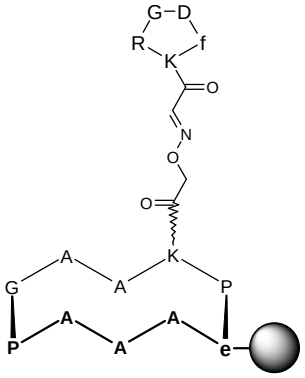


Figure S2: MS (ESI-MS, positive mode) for $C_{171}H_{251}N_{55}O_{51}$



Monovalent RGD-containing resin beads



2

The cyclodecapeptide on resin **27**, previously swelled in dry CH_2Cl_2 (6 mL, 15 min), was treated by TFA/H_2O/CH_2Cl_2 (94/3/3) solution for 30 min, under vigorous stirring and then washed with DMF. Solution of peptide **1**5 (0.4 mmol/g) in dry DMF was added to the resin. The mixture was stirred at r.t. for 2 hr. The cyclodecapeptide on resin **2** was washed with DMF (6 mL x 4), MeOH (6 mL), H_2O 0.1 % TFA (6 mL x 40) and MeOH (6 mL). Sample of resin **2** was treated by TFA/H_2O (9/1) solution affording free peptide for analysis.

Figure S3: RP-HPLC: RT = 6.4 min (C_{18}, 214 nm, 5-100% B in 30 min)

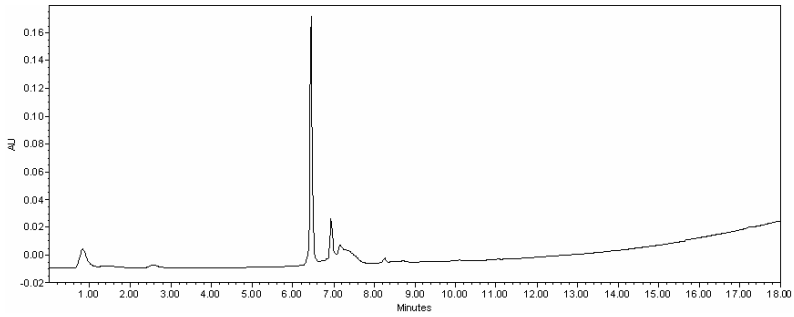
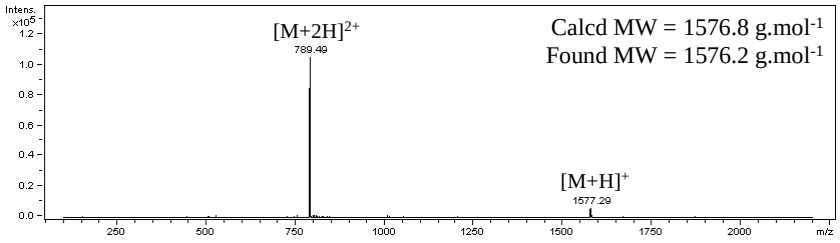
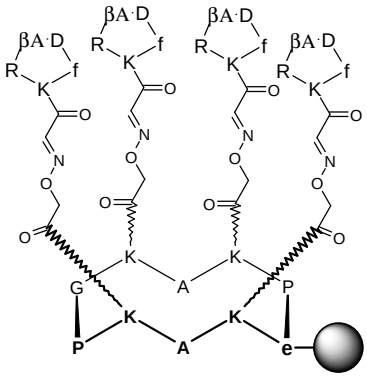


Figure S4: MS (ESI-MS, positive mode) for $C_{69}H_{104}N_{22}O_{21}$



Tetravalent RβAD-containing resin beads



3

The cyclodecapeptide on resin **26**, previously swelled in dry CH_2Cl_2 (6 mL, 15 min), was treated by TFA/H_2O/CH_2Cl_2 (94/3/3) solution for 30 min, under vigorous stirring and then washed with DMF. Solution of peptide **19** (1 mmol/g) in dry DMF was added to the resin. The mixture was stirred at r.t. for 2 hr. The cyclodecapeptide on resin **3** was washed with DMF (6 mL x 4), MeOH (6 mL), H_2O 0.1 % TFA (6 mL x 40) and MeOH (6 mL). Sample of resin **3** was treated by TFA/H_2O (9/1) solution affording free peptide for analysis.

Figure S5: RP-HPLC: RT = 7.2 min (C_{18}, 214 nm, 5-100% B in 30 min)

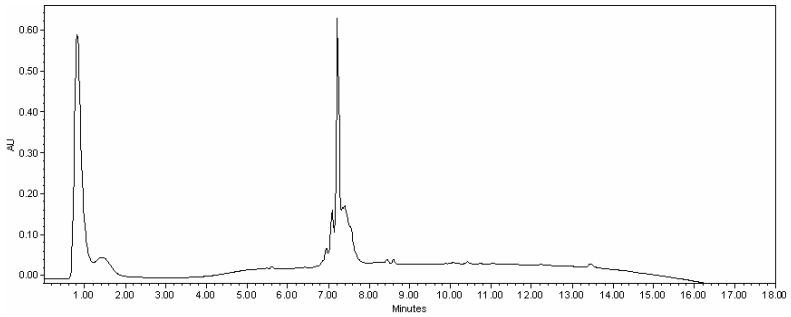
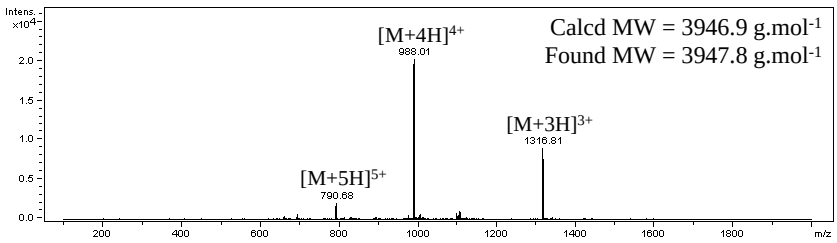


Figure S6: MS (ESI-MS, positive mode) for $C_{175}H_{259}N_{55}O_{51}$



Cell adhesion procedure

HEK293(β_3) and HEK293(β_1) cells are subclones of the human embryonic kidney HEK293 cell line, stably transfected by a plasmid encoding the human β_3 and β_1 subunits respectively. They were cultured in DMEM enriched with 4.5 $g.L^{-1}$ glucose and supplemented with 1 % glutamine, 10 % FBS, penicillin (50 $U.mL^{-1}$), streptomycin (50 $\mu g.mL^{-1}$) and G418 (700 $\mu g.mL^{-1}$). 3-LL cells are a subclone of murine Lewis lung carcinoma, not expressing $\alpha_V\beta_3$ integrin. They were cultured in DMEM enriched with 4.5 $g.L^{-1}$ glucose and supplemented with 1% glutamine, 10 % FBS, penicillin (50 $U.mL^{-1}$), streptomycin (50 $\mu g.mL^{-1}$) and G418 (700 $\mu g.mL^{-1}$). All cells were maintained at 37°C under an atmosphere of 5 % CO_2.

An amount of 2-20 mg of beads was washed 5 min with water and PBS before equilibration in PBS (15 min) and finally suspension in DMEM w/o FBS. Beads were incubated with media for 30 min at room temperature. HEK293(β3), HEK293(β1) and 3-LL cells were removed from culture flasks using trypsin treatment, washed and re-suspended in DMEM w/o FBS at a final density of 1 million cells/mL. 1 mL of cell suspension was mixed with the beads and incubated at 37 °C with gentle shaking for 30 min. Beads were gently washed 3 times with DMEM w/o FBS and cells were fixed with ethanol. After 20 min of fixation, beads were dried (2 hr at 37°C), then stained with methylene blue 1 % in borate buffer (30 min). The dye was eliminated and the beads were washed 3 times with PBS before microscopy observation in moviol. Optical microscopy was performed with an Olympus BX41 laboratory microscope.

Optical microscopy images of cell adhesion using 0.2 mmol/g-beads

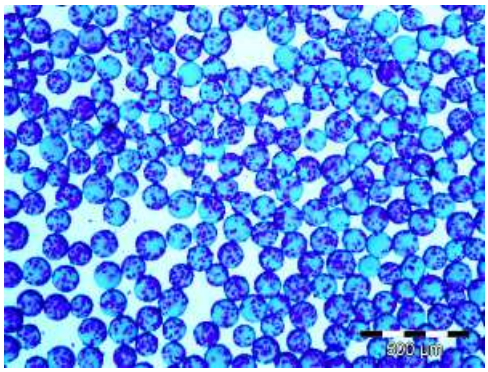


Figure S7: HEK 293 (β3) incubated with **1**

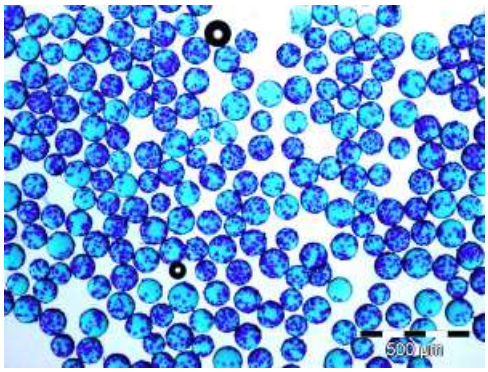


Figure S8: HEK 293 (β3) incubated with **2**

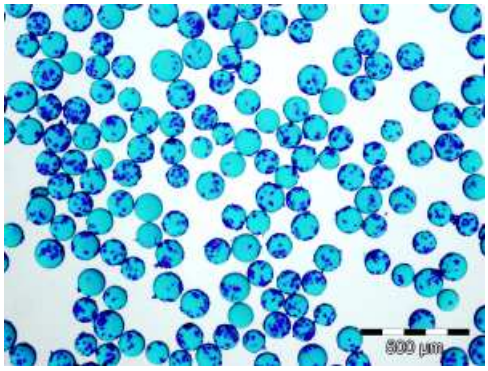


Figure S9: HEK 293 (β1) incubated with **1**

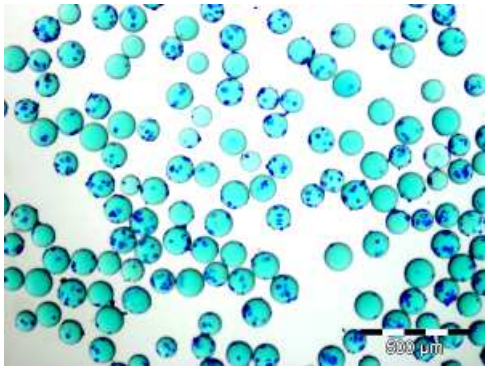


Figure S10: HEK 293 (β1) incubated with **2**

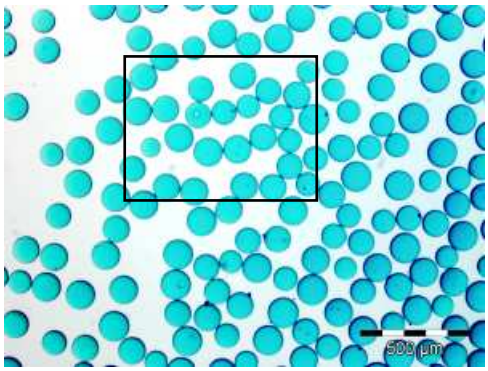


Figure S11: 3LL incubated with **1**

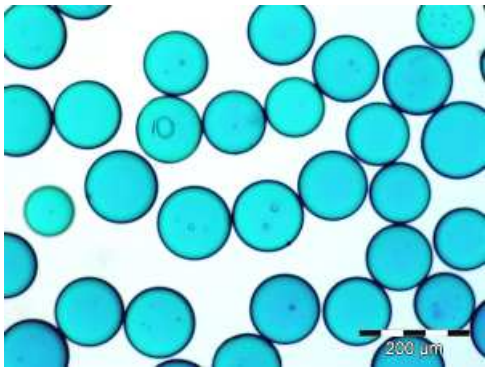


Figure S12: enlargement of Figure S11

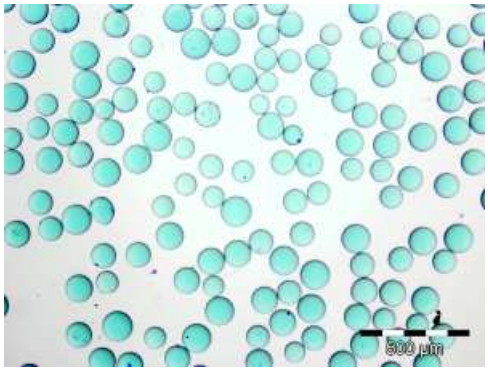


Figure S13: 3LL incubated with **2**

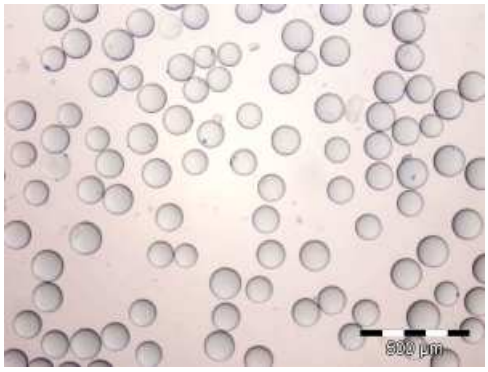


Figure S14: HEK 293 (β3) incubated with **3**

Optical microscopy images of cell adhesion using 20 μmol/g-beads

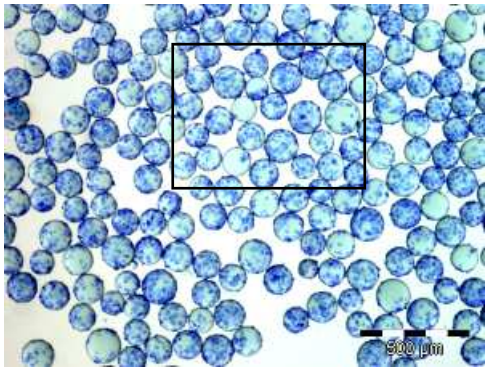


Figure S15: HEK 293 (β3) incubated with **1**

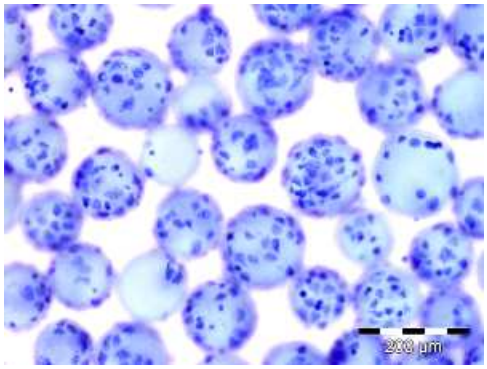


Figure S16: enlargement of Figure S15

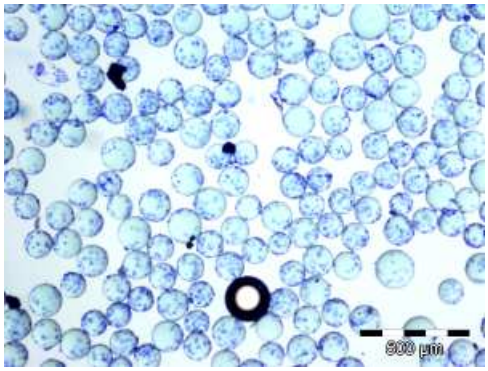


Figure S17: HEK 293 (β3) incubated with **2**

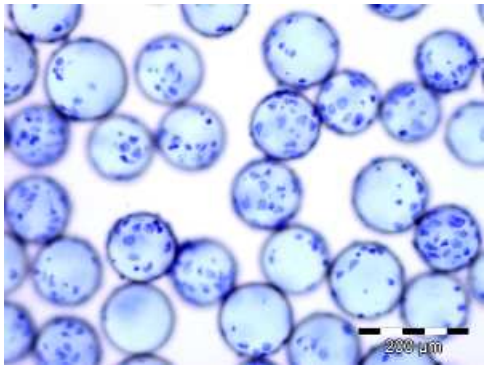


Figure S18: HEK 293 (β3) incubated with **2**

Figure S19: 3LL incubated with **1**

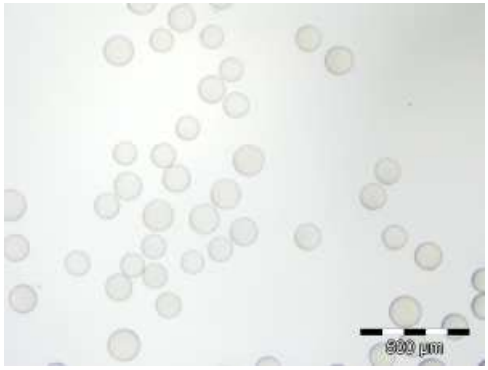


Figure S20: HEK 293 (β3) incubated with **3**

Optical microscopy images of cell adhesion using 2 μmol/g-beads

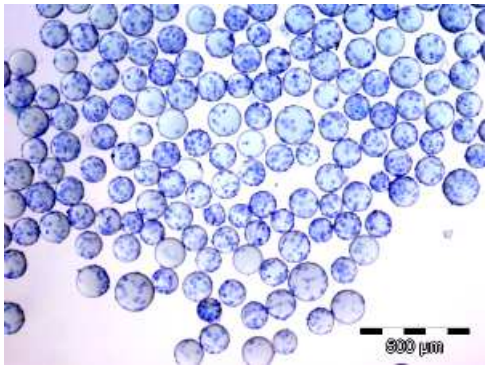


Figure S21: HEK 293 (β3) incubated with **1**

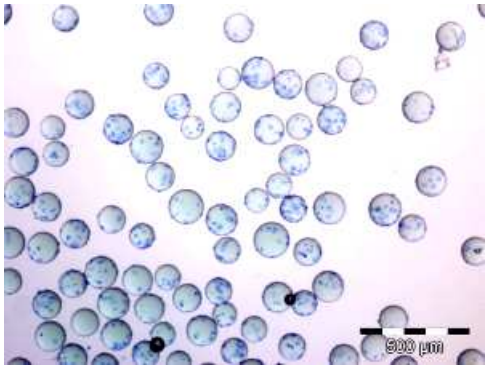


Figure S22: HEK 293 (β3) incubated with **2**

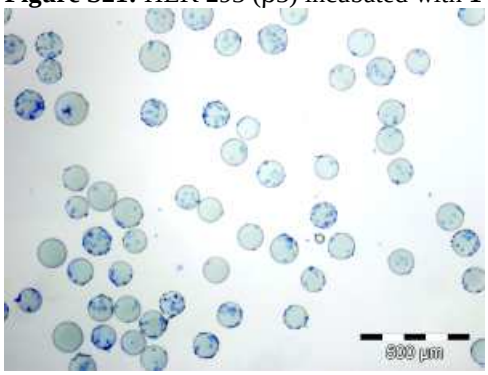


Figure S23: HEK 293 (β1) incubated with **1**

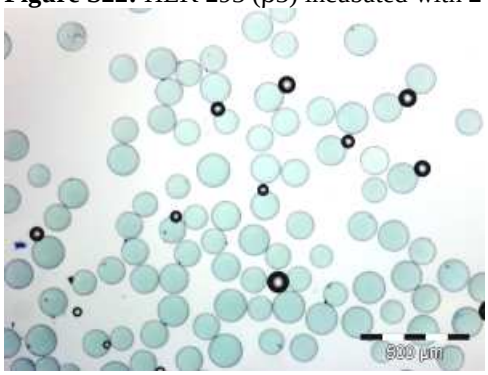


Figure S24: HEK 293 (β1) incubated with **2**

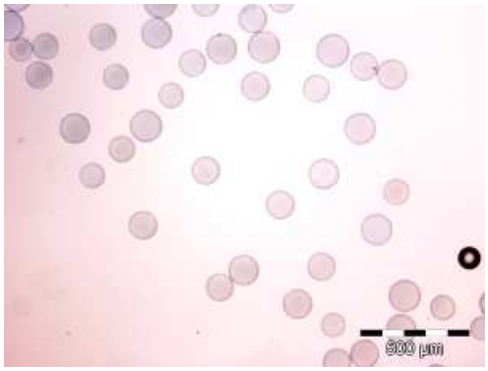


Figure S25: HEK 293 (β3) incubated with **3**

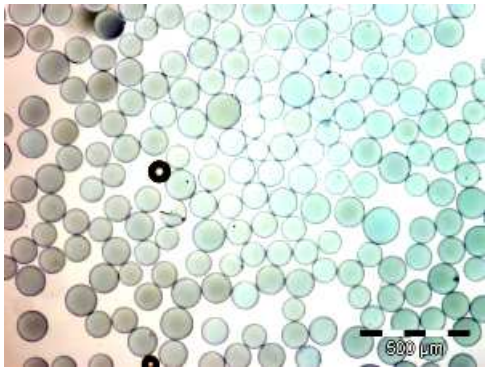


Figure S26: HEK 293 (β1) incubated with **3**

Optical microscopy images of cell adhesion using 2 nmol/g-beads

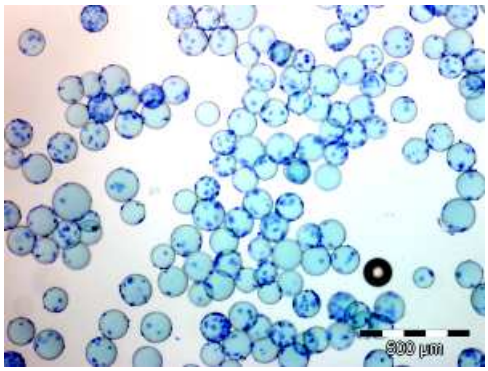


Figure S27: HEK 293 (β3) incubated with **1**

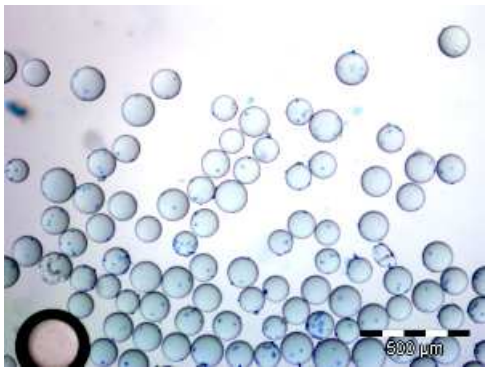


Figure S28: HEK 293 (β3) incubated with **2**

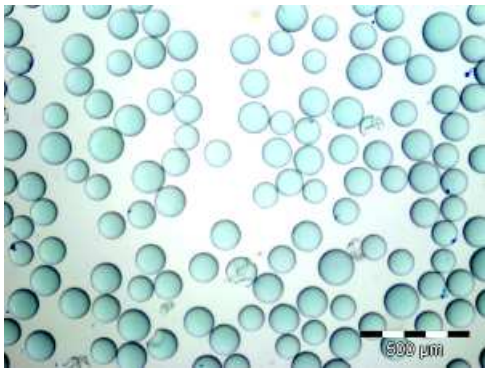


Figure S29: HEK 293 (β1) incubated with **1**

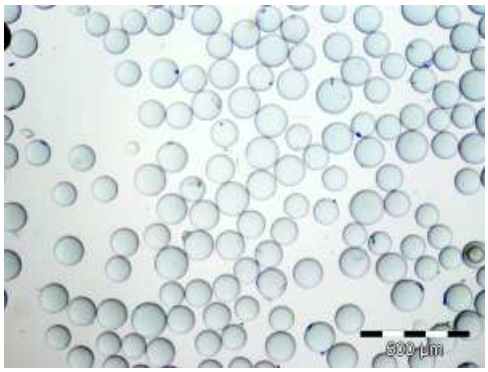


Figure S30: HEK 293 (β1) incubated with **2**

Optical microscopy images of cell adhesion using 2 pmol/g-beads

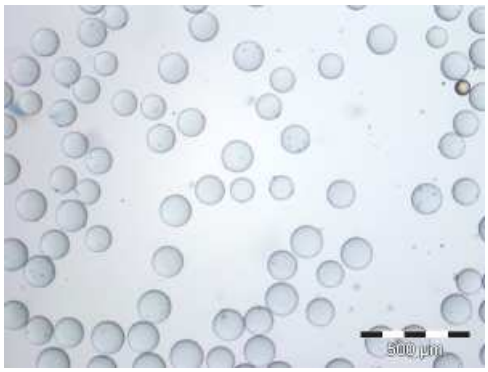


Figure S31: HEK 293 (β3) incubated with **1**

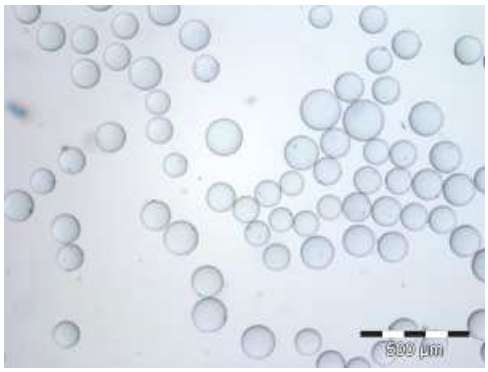


Figure S32: HEK 293 (β3) incubated with **2**

Optical microscopy images of live and fixed cell adhesion using cell mixture

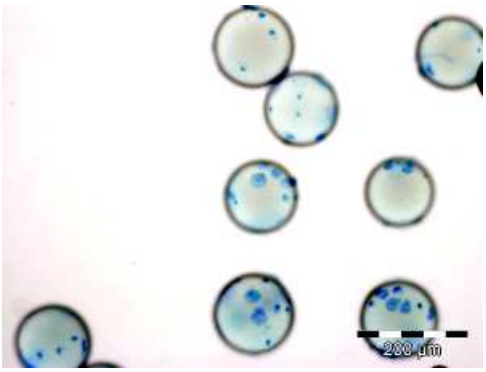


Figure S33: HEK 293 (β3)/3LL (1:9) cell mixture was treated with **1** (20 µmol/g_{resin}) for 30 min at 37°C, then fixed and stained with methylene blue.

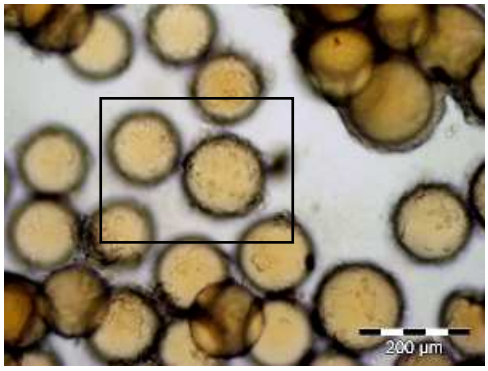


Figure S34: HEK 293 (β3)/3LL (1:9) cell mixture was treated with **1** (20 µmol/g_{resin}) for 30 min at 37°C. Beads were washed with PBS and incubated in cell culture medium for 96 hours at 37°C in a humidified 5% CO_2 atmosphere.

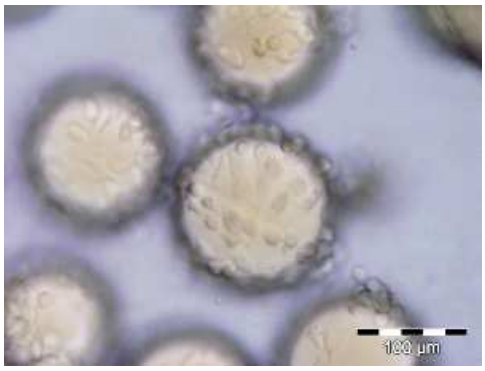


Figure S35: enlargement of Figure S34.

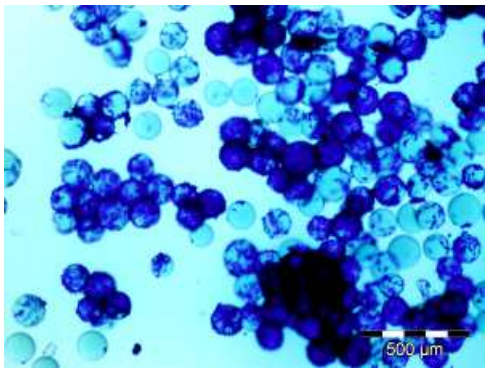


Figure S36: HEK 293 (β3)/3LL (1:9) cell mixture was treated with **1** (20 µmol/g_{resin}) for 30 min at 37°C. Beads were washed with PBS and incubated in cell culture medium for 96 hours at 37°C in a humidified 5% CO_2 atmosphere, then fixed and stained with methylene blue.

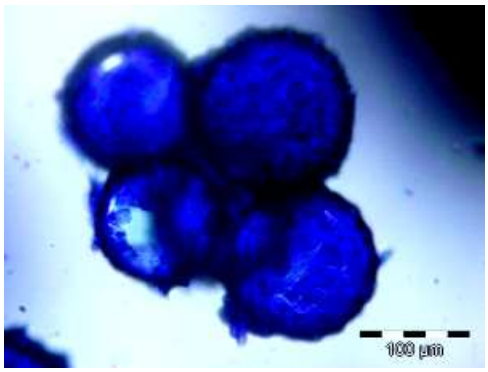


Figure S37: details of cell coating.

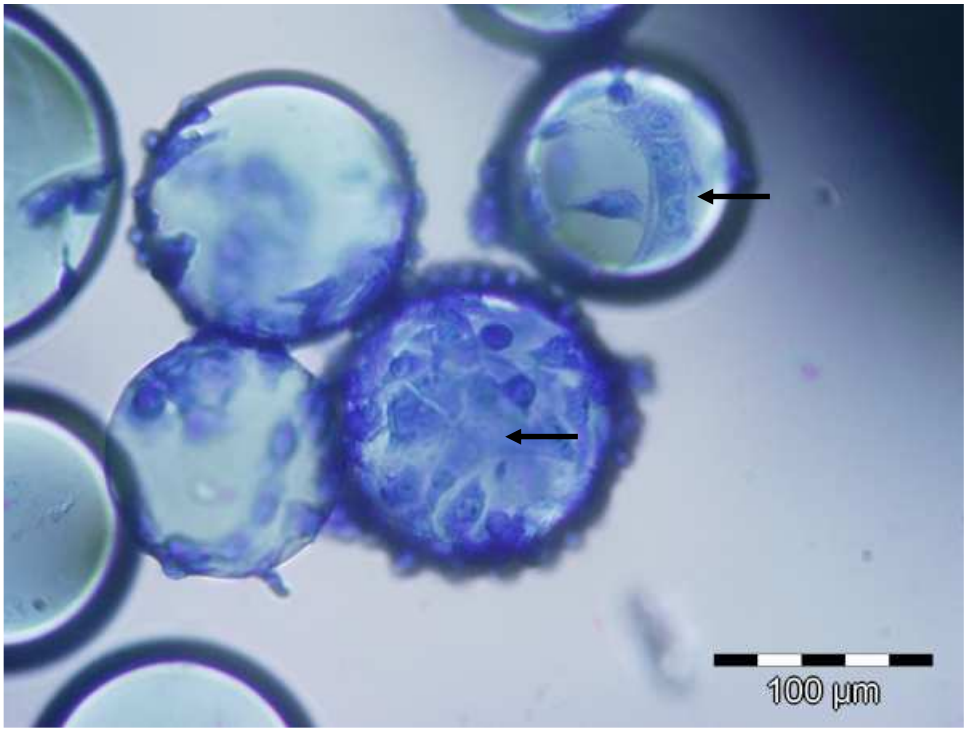


Figure S38: details of extended and flattened cells.

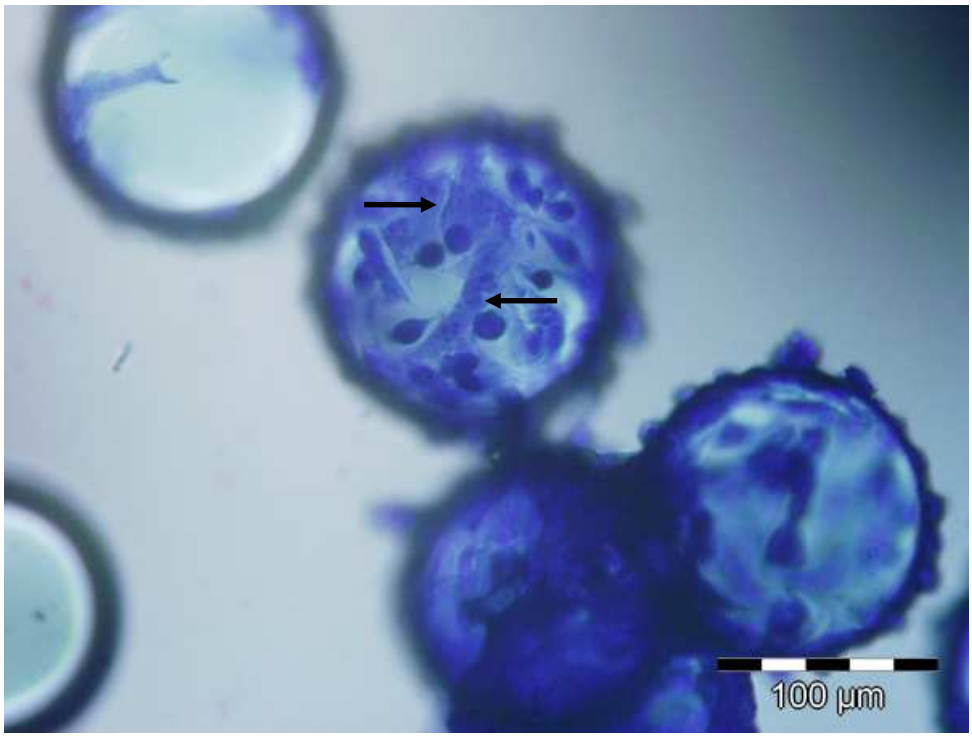


Figure S39: details of extended and flattened cells.

Vinculin staining of flattened cells.

Cells attached to the resin beads **1** (20 μmol/g_{resin}) were fixed in a 4% solution of formaldehyde/PBS. After fixation the cells were rinsed twice with PBS. Cells were then treated for 3 min with 0.2% Triton X-100/ 4% formaldehyde/PBS, rinsed with PBS and then treated with a blocking solution of 3% bovine serum albumin. After 15 min the resin beads were washed with PBS. A mouse monoclonal anti-vinculin antibody was diluted with 1% BSA/PBS (1/50°). Beads were incubated in a humidity chamber at 37°C for 1 h. with the mAb solution. Once this incubation was complete the beads were washed in three 3-min changes of 1% BSA/PBS. These resin beads were then incubated for 30 min at room temperature with secondary antibody conjugated to Alexa488 (1/500° in 1% BSA/PBS). The stained cells on resin were rinsed in three 5-min changes of PBS and then stained with Hoechst 33342. The stained cells on resin were again rinsed with PBS and mounted with mowiol onto glass slides.

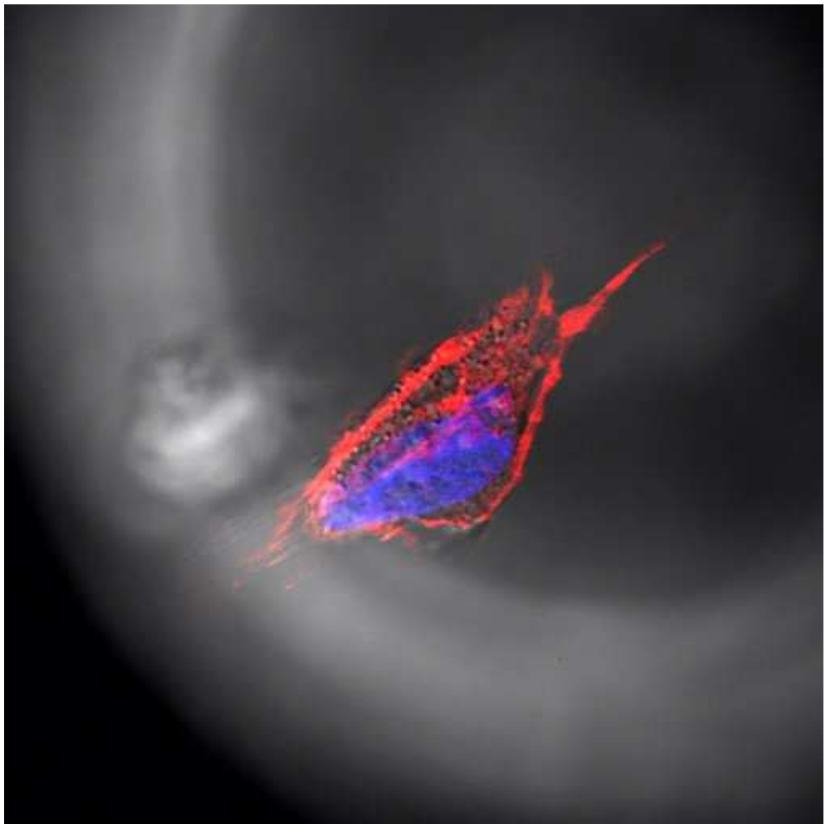


Figure S40: details of extended and flattened cell. Vinculin proteins were stained by Alexa488 (red color). Nucleus was stained by Hoechst dye (blue color).