Supporting Information

Peptide-Polymer Bioconjugates via Atom Transfer Radical Polymerization and Their Solution Aggregation into Hybrid Micro/Nanospheres for Dye Uptake

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Synthesis of Boc-Tyr(1)-Val(2)-OH

Synthesis of Boc-Tyr(1)-Val(2)-OH involves following steps:

(i) Synthesis of Boc-Tyr(1)-OH: The synthesis of this compound was carried out according to our previously reported method.[1]

(ii) Synthesis of $HCl.H_2N$-Val(2)-OMe: For the synthesis of methyl ester of valine, 40 ml dry methanol was taken in a dried R.B capped with $CaCl_2$ guard tube and kept it in a ice bath for 15 minute. After that, 4 ml $SOCl_2$ was added drop wise to it and then valine (4.68g, 40 mmol) was added to it. The reaction was continued for 12 hour. Solvent was removed by rotary evaporator and washed two times with diethyl ether to remove excess $SOCl_2$ and finally dried in vacuum.

(iii) Synthesis of Boc-Tyr(1)-Val(2)-OMe: Boc-Tyr(1)-OH (3.64 g, 15 mmol) were taken in an ice-water bath. The methyl ester of valine (H_2N-Val-OMe) (2.62 g, 20 mmol) isolated from the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate was then added to this solution. Finally, DCC (2.06 g, 10 mmol) and HOBt (1.35 g, 10 mmol) were added to the above reaction mixture. The reaction mixture was stirred for three days in a magnetic stirrer. The resultant product was taken in ethyl acetate (30 mL) and DCU was filtered off. The organic layer was washed with HCl (2N, 3× 20 mL), brine (1×20 mL), sodium

carbonate (1M, 3× 20 mL), and brine (2×20 mL). The organic layer was then dried over anhydrous sodium sulfate and evaporated under vacuum. Purification was carried out by silica-gel column chromatography (100–200 mesh) by using ethyl acetate/toluene as the eluent.

(iv) Synthesis of Boc-Tyr(1)-Val(2)-OH: Boc-Tyr(1)-Val(2)-OMe was dissolved in minimum volume of MeOH. Then 15 mL of 1M NaOH was added dropwise and the progress of the reaction was monitored by TLC. The reaction mixture was stirred. After completion of the reaction, methanol was removed under vacuum and the residue was taken in 50 mL of water and washed with ethyl acetate (2 × 50 mL). The pH of the aqueous layer was then adjusted to 2-3 using 1(N) HCl, the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The extract was then dried over anhydrous sodium sulfate and evaporated under vacuum.

Synthesis of H_2N-Tyr-OMe

For the synthesis of methyl ester of tyrosine, 40 ml dry methanol was taken in a dried R.B capped with $CaCl_2$ guard tube and kept it in an ice bath for 15 minute. After that, 4 ml $SOCl_2$ was added drop wise to it and then tyrosine (7.2g, 40 mmol) was added to it. The reaction was continued for 12 hour. Solvent was removed by rotovap and washed two times by diethyl ether to remove excess $SOCl_2$ and finally dried in vacuum. The methyl ester of tyrosine (H_2N-Tyr-OMe) was isolated from the corresponding methyl ester hydrochloride by neutralization and subsequent extraction with ethyl acetate. The extract was then dried over anhydrous sodium sulfate and evaporated under vacuum before reaction.

N-Protected Peptide-1 [Boc-Tyr(1)-Val(2)-Tyr(3)-OMe]

Yield = 4.57 g (8.2 mmol, 82%). ^{1}H NMR (300 MHz, d_6-DMSO, TMS): δ = 9.19 & 9.16 (s, 2H, tyrosinate proton), 8.39-8.37 (d, 1H, *J* = 6.79 Hz, Tyr(3) NH), 7.60-7.57 (d, 1H, *J* = 8.95 Hz,

Tyr(1) NH), 7.02-6.99 (d, 2H, *J* = 8.4 Hz, Tyr(1)/Tyr(3) ring hydrogen), 6.98-6.95 (d, 2H, *J* = 8.4 Hz, Tyr(1)/Tyr(3) ring hydrogen), 4.38-4.31 (m, 1H, Tyr(3) C^{α}-H) , 4.26-4.21 (m, 1H, Tyr C^{α}-H), 4.09-4.04 (m,1H, Val(2) C^{α}-H), 3.58 (s, 3H, -OCH_3), 2.91-2.77 (m, 4H, Tyr(1)/Tyr(3) C^{β}-H), 1.95-1.88 (m, 1H, Val(2) C^{β}-H), 1.28 (s, 9H, Boc-CH_3), 0.83-0.81 ppm (d, 6H, *J* = 7.02, Val(2) C^{γ}-H).

MS (ESI) (35 eV): m/z (%): 580 (100) [M+Na^+].

N-Protected Peptide-II [Boc-Leu(1)-Aib(2)-Phe(3)-OMe]

Yield = 3.72 g (7.8 mmol, 78%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): 7.35-7.33 (d, 1H, *J* = 7.02 Hz, -NH), 7.29-7.13 (m, 5H, aromatic ring protons), 5.79 (b, 1H, -NH), 5.23 (b, 1H, -NH), 4.74-4.68 (m, 1H, *J* = 6.84, C^{α}-H of Leu(1)), 4.13-4.04 (m, 1H, C^{α}-H of Phe(3)), 3.65 (s, 3H, -OCH_3), 3.14-3.0 (m, 2H, C^{β}-H of Phe(3)), 1.65-1.54 (m, 2H, C^{β}-H of Leu(1)), 1.46 (s, 6H, C^{β}-H of Aib(2)), 1.43 (s, 9H, Boc-CH_3) 1.22 (m, 1H, C^{γ}-H of Leu(1)), 0.93-0.91 ppm (m, 6H, C^{δ}-H of Leu(1)).

MS (ESI) (35 eV): m/z (%): 500 (100) [M+Na^+].

Peptide-I [NH_2-Tyr(1)-Val(2)-Tyr(3)-OMe]

Yield = 1.6 g (3.5 mmol, 70%). ^{1}H NMR (300 MHz, d_6-DMSO, TMS): δ = 9.20 & 9.15 (s, 2H, tyrosinate proton), 8.42-8.40 (d, 1H, *J* = 7.07 Hz, Tyr(3) NH), 7.92-7.89 (d, 1H, *J* = 9.46 Hz, Tyr(1) NH), 7.01-6.98 (d, 2H, *J* = 8.08 Hz, Tyr(1)/Tyr(3) ring hydrogen), 6.66-6.64 (d, 2H, *J* = 8.25 Hz, Tyr(1)/Tyr(3) ring hydrogen), 4.37-4.32 (m, 1H, Tyr(3) C^{α}-H), 4.25-4.20 (m, 1H, Tyr C^{α}-H), 4.06-3.99 (m, 1H, Val(2) C^{α}-H), 3.55 (s, 3H, -OCH_3), 2.93-2.73 (m, 4H, Tyr(1)/Tyr(3) C^{β}-H), 1.99-1.90 (m, 1H, Val(2) C^{β}-H), 0.81-0.74 ppm (dd, 6H, J = 6.65,val(2) C^{γ}-H).

MS (ESI) (35 eV): m/z (%): 480 (100) [M+Na^+].

Peptide-II [NH_2-Leu(1)-Aib(2)-Phe(3)-OMe]

Yield = 1.32 g (3.5 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 7.64 (s, 2H, -NH_2), 7.29-7.27 (d, 1H, *J* = 7.02 Hz, -NH), 7.22-7.12 (m, 5H, aromatic ring proton), 7.08-7.06 (d, 1H, *J* = 6.27 Hz, -NH), 4.74-4.7 (m, 1H, *J* = 6.03, C^{α}-H of Leu(1)), 3.64 (s, 3H, -OCH_3), 3.23-3.19 (m, 1H, C^{α}-H of Phe(3)), 3.14-2.96 (m, 2H, C^{β}-H of Phe(3)), 1.65-1.54 (m, 2H, C^{β}-H of Leu(1)), 1.43 (s, 6H, C^{β}-H of Aib(2)), 1.22 (m, 1H, C^{γ}-H of Leu(1)), 0.90-0.84 ppm (m, 6H, C^{δ}-H of Leu(1)).

MS (ESI) (35 eV): m/z (%): 378 (100) [$M+H^+$].

Synthesis of Model Tyr-initiator ($Me_2C(Br)$-CO-NH-Tyr-OMe)

The model initiator i.e. tyrosine based amino acid initiator was synthesized from NH_2-Tyr-OMe with 2-bromoisobutyric acid (BIBA) via a standard coupling procedure using DCC/HOBT as the coupling agent. Briefly, a sample of BIBA (0.334g, 2mmol) in DMF (10 mL) was cooled in an ice-water bath. NH_2-Tyr-OMe (0.780 g, 4 mmol) was then added to this solution. Next, DCC (0.412 g, 2 mmol) and HOBt (0.540 g, 4 mmol) were simultaneously added to the above reaction mixture. The reaction mixture was stirred for three days with a magnetic stirrer. After completion of the reaction, ethyl acetate (30 mL) was added to the final mixture and the precipitated DCU was filtered off. The organic layer was washed with HCl (2N, 3× 20 mL), brine (1×20 mL), sodium carbonate (1M, 3× 20 mL), and brine (2×20 mL). The organic layer was then dried over anhydrous sodium sulfate and evaporated under vacuum. Further purification of tyrosine based amino acid initiator was done via silica-gel column chromatography (100–200 mesh) using a mixture of ethyl acetate/toluene as the eluent.

Yield = 0.55 g (1.6 mmol, 80%). ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 7.11 & 7.09 (d, 1H, *J* = 7.4, -NH), 7.0-6.97 & 6.75-6.72 (d, 4H, *J* = 8.34, tyrosine ring protons), 4.80-4.73 (m, 1H, C^{α}-H

of Tyr), 3.75 (s, 3H, -OCH_3), 3.15-2.99 (m, 2H, C^{β}-H of Tyr), 1.91 & 1.87 ppm (s, 6H, C^{β}-H of isobutyric acid)

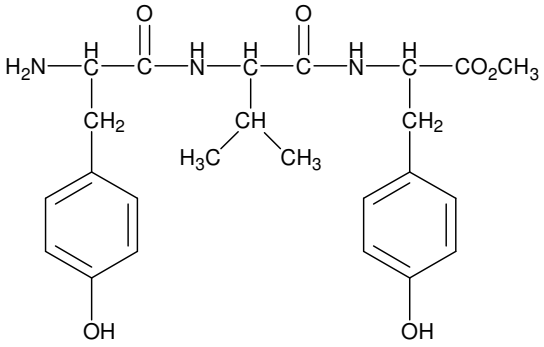
MS (ESI) (35 eV): m/z (%): 367(100) [$M+Na^+$]

Synthesis of Phe-initiator ($Me_2C(Br)$-CO-NH-Phe-OMe)

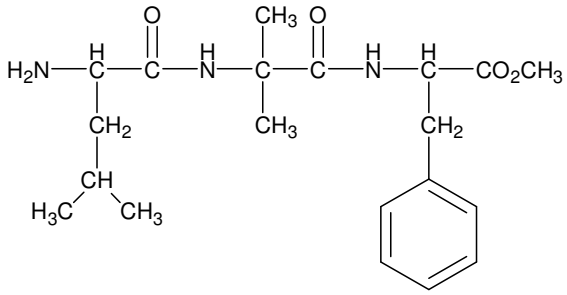
Phenylalanine based model initiator was synthesized from NH_2-Phe-OMe and 2-bromoisobutyric acid following the above-mentioned procedure that used for the synthesis of model Tyr-initiator. Purification of the synthesized phenylalanine based initiator (Phe-initiator) was done via silica-gel column chromatography (100–200 mesh) using a mixture of ethyl acetate/toluene as the eluent.

Yield = 0.52 g (1.6 mmol, 80%). ^{1}H NMR (500 MHz, $CDCl_3$, TMS): δ = 7.31-7.23 & 7.14-7.12 (m, 5H, phenylalanine ring protons), 7.05-7.04 (d, 1H, *J* = 6.5 Hz, -NH), 4.82-4.78 (m, 1H, C^{α}-H of Phe), 3.75 (s, 3H, -OCH_3), 3.21-3.08 (m, 2H, C^{β}-H of Phe), 1.91 & 1.86 ppm (s, 6H, C^{β}-H of isobutyric acid)

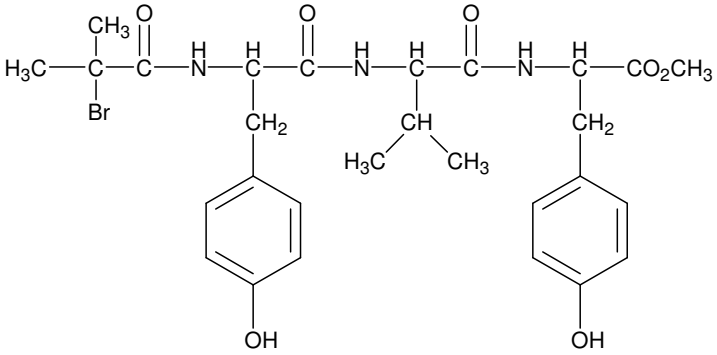
MS (ESI) (35 eV): m/z (%): 350(100) [$M+Na^+$]



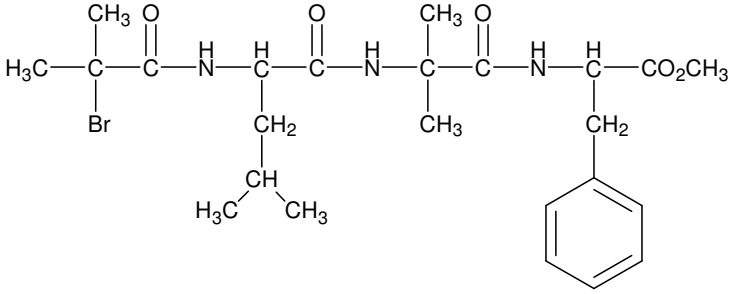
Peptide-I



Peptide-II



Peptide-Initiator-I



Peptide-Initiator-II

Figure S1. Chemical structures of peptide-I, peptide-II, peptide-initiator-I and peptide-initiator-II.

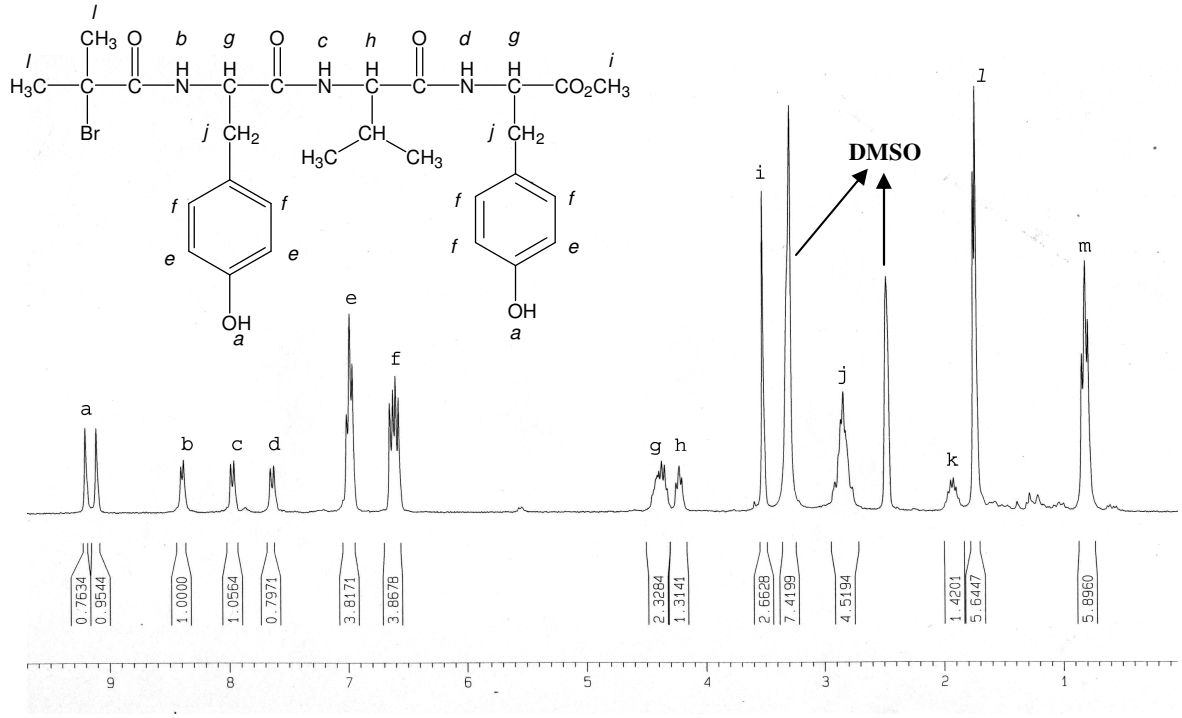


Figure S2. ^{1}H NMR spectrum of the peptide-initiator-I in d_6-DMSO.

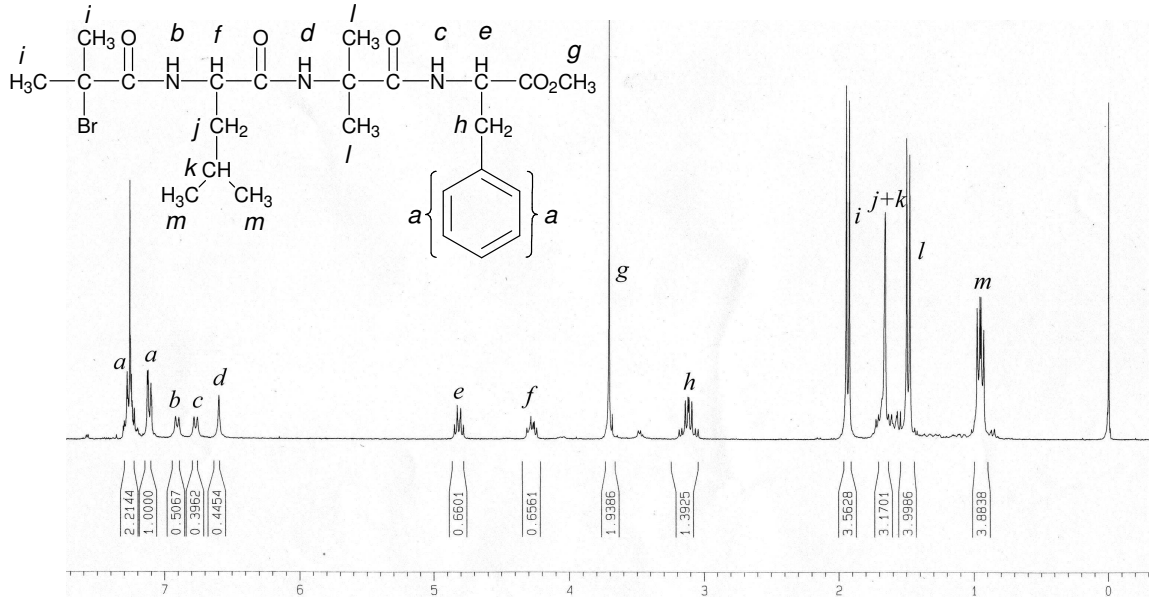


Figure S3. ^{1}H NMR spectrum of the peptide-initiator-II in $CDCl_3$.

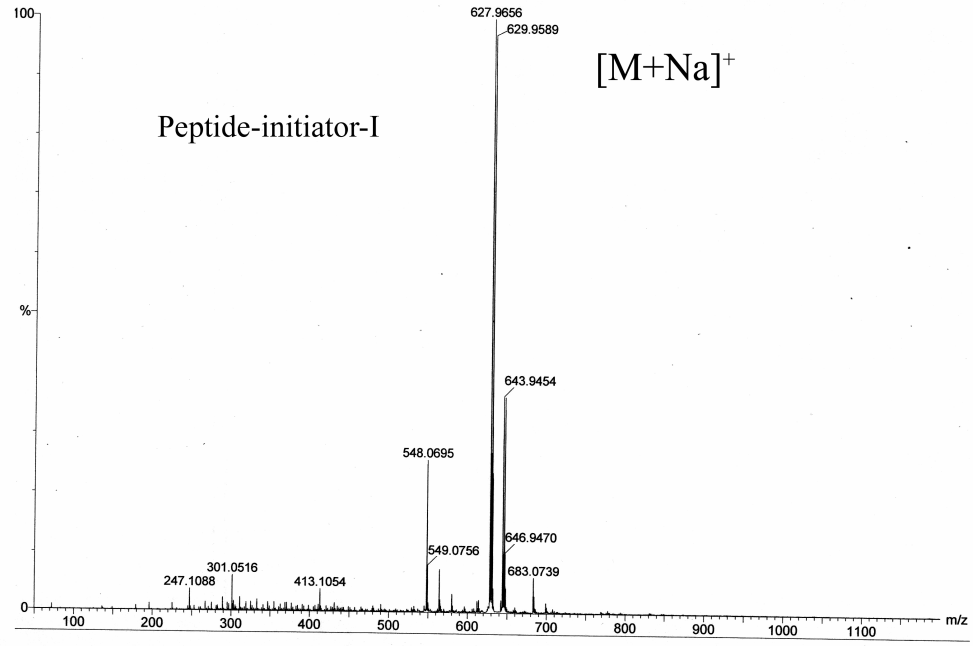


Figure S4. ESI-MASS spectrum of the peptide-initiator-I.

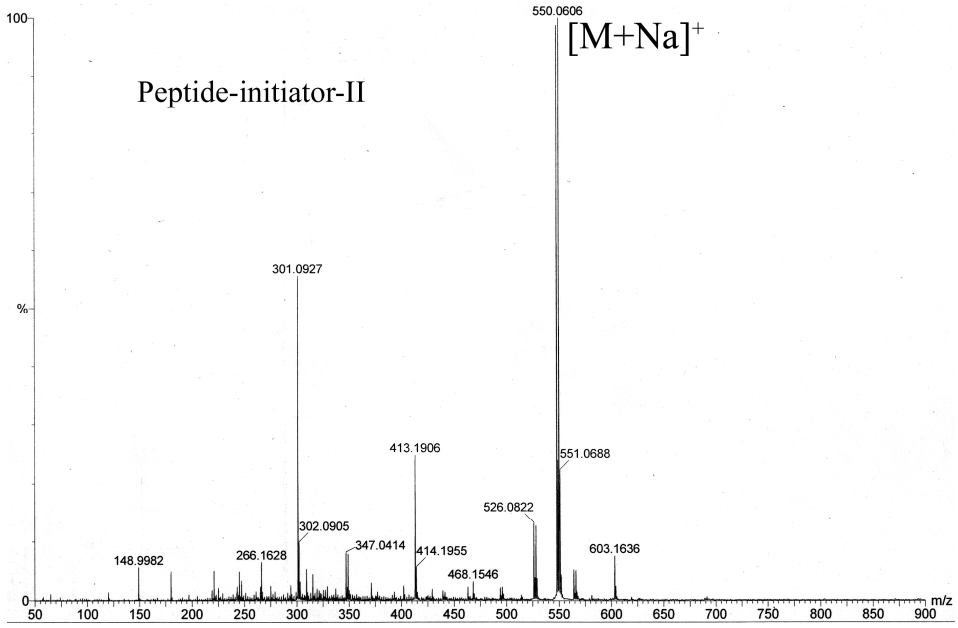


Figure S5. ESI-MASS spectrum of the peptide-initiator-II.

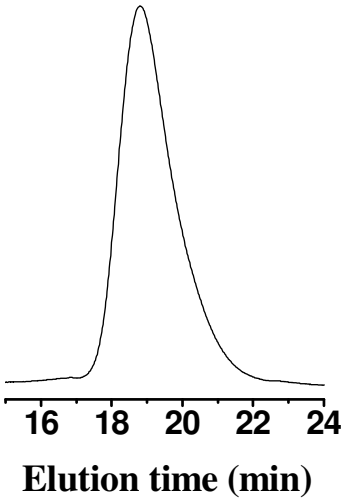


Figure S6. GPC trace of the PMMA synthesized using CuCl/Boc-Tyr-Val-Tyr-OMe as catalyst system and ethyl-2-bromoisobutyrate as the initiator in DMSO at 90ºC.

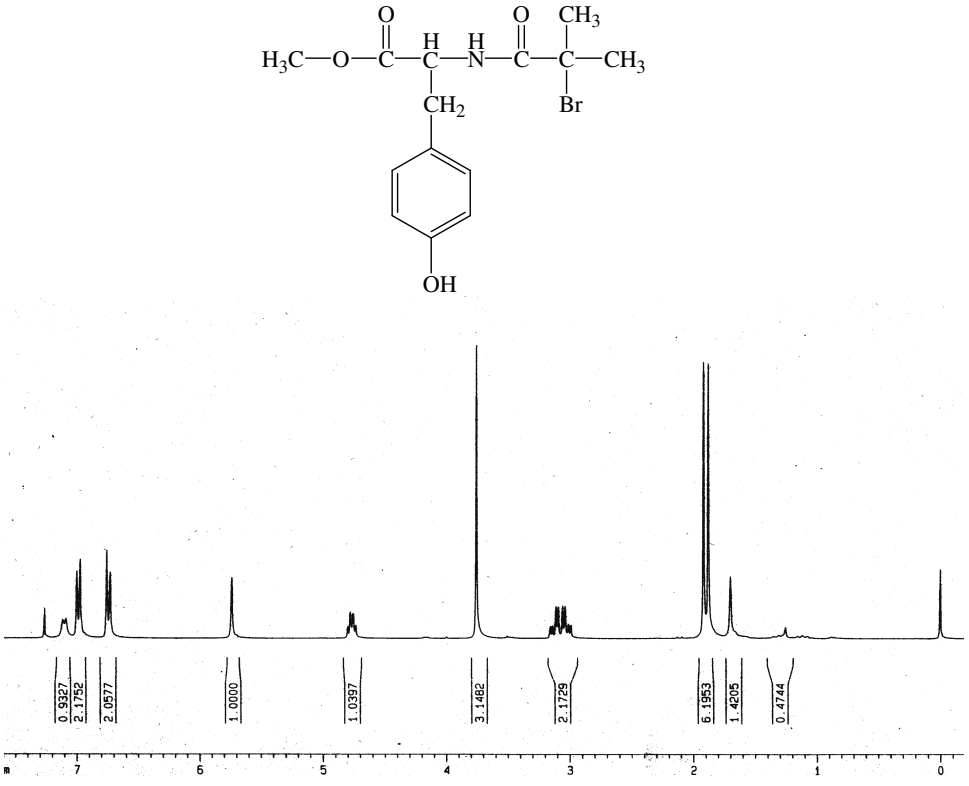


Figure S7. ^{1}H NMR spectrum of the Tyr-initiator [$Me_2C(Br)$-CO-NH-Tyr-OMe] in $CDCl_3$.

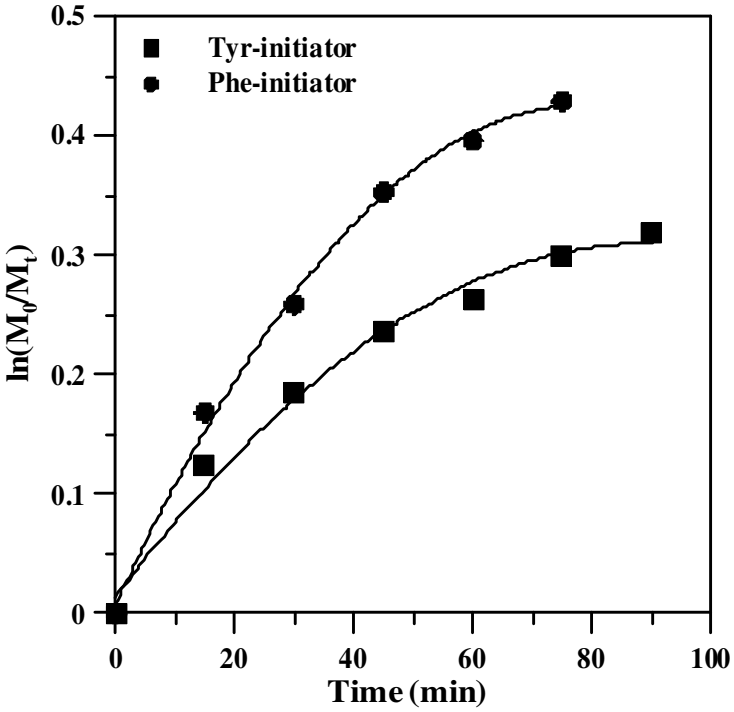


Figure S8. Semi-logarithm kinetics plot for the polymerization of MMA in DMSO at 90ºC initiated by the model Tyr-initiator (▪) and Phe-initiator (•) initiators in presence of the catalyst CuCl/PMDETA. Conditions: $[MMA]_0/[Tyr\text{-}initiator/Phe\text{-}initiator]_0/[CuCl]_0/[PMDETA]_0$ = 421:1:1:1.

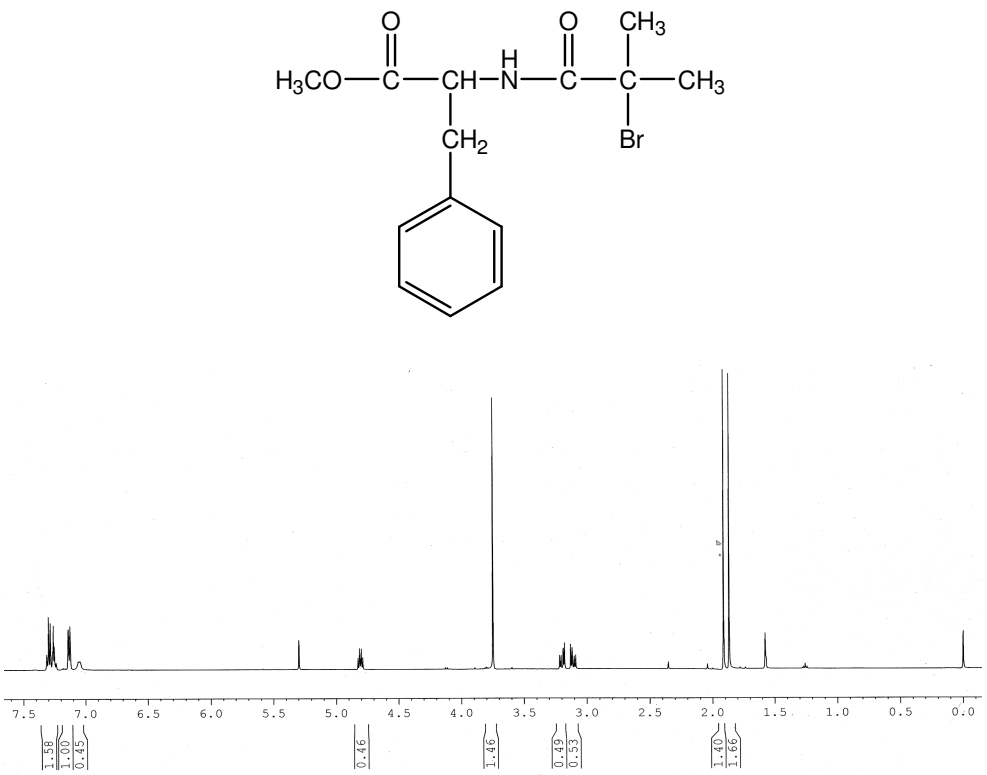


Figure S9. ^{1}H NMR spectrum of the Phe-initiator [$Me_2C(Br)$-CO-NH-Phe-OMe] in $CDCl_3$.

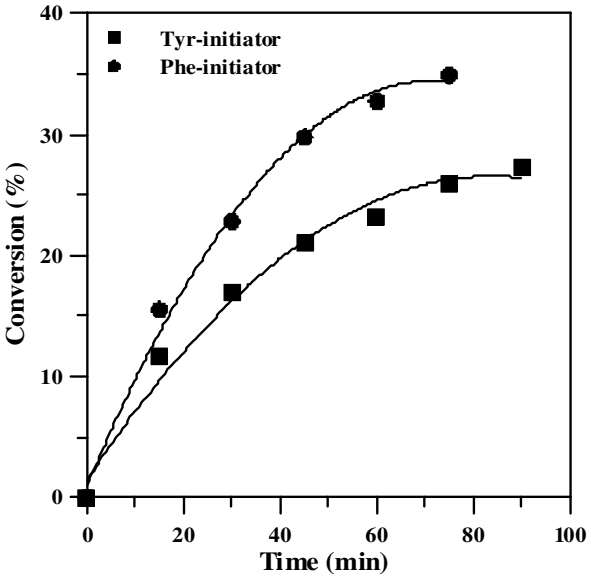


Figure S10. Plot of monomer-to-polymer conversion versus time in the polymerization of MMA using model Tyr-initiator (▪) and Phe-initiator (●) initiators in presence of the catalyst CuCl/PMDETA in DMSO at (90ºC). Conditions: $[MMA]_0/[Phe\text{-}initiator]_0/[CuCl]_0/[PMDETA]_0$ = 421:1:1:1.

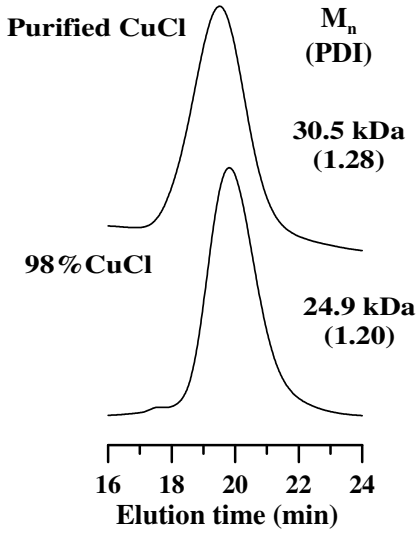


Figure S11. GPC traces of the PMMA synthesized using purified CuCl (purified)/PMDETA and CuCl (98%)/PMDETA catalyst systems and peptide-initiator-I as the initiator in DMSO at 90ºC.

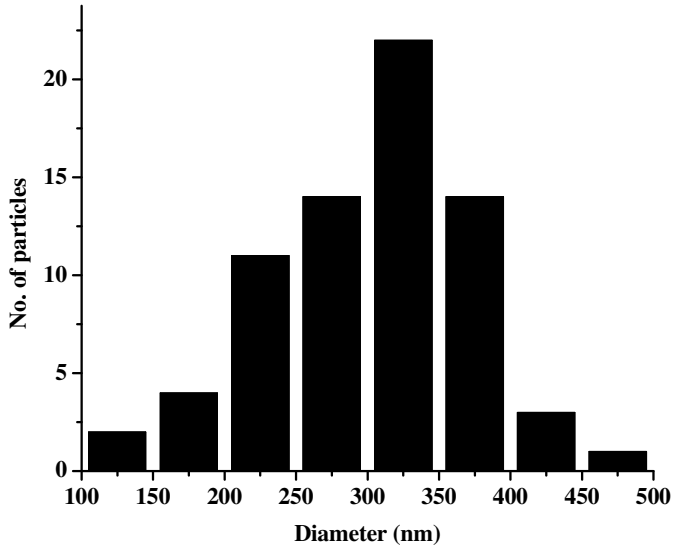


Figure S12. Histogram of particle size distribution of the aggregated micro/nanospheres obtained from peptide-PMMA-Ia in DMF. Particle sizes were determined from FESEM image.

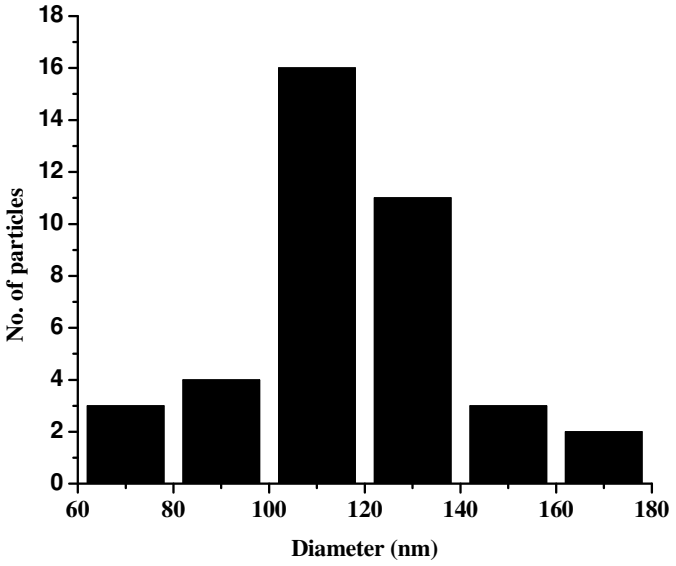


Figure S13. Histogram of particle size distribution of the aggregated micro/nanospheres obtained from peptide-PMMA-Ia in DMSO. Particle sizes are determined from FESEM image.

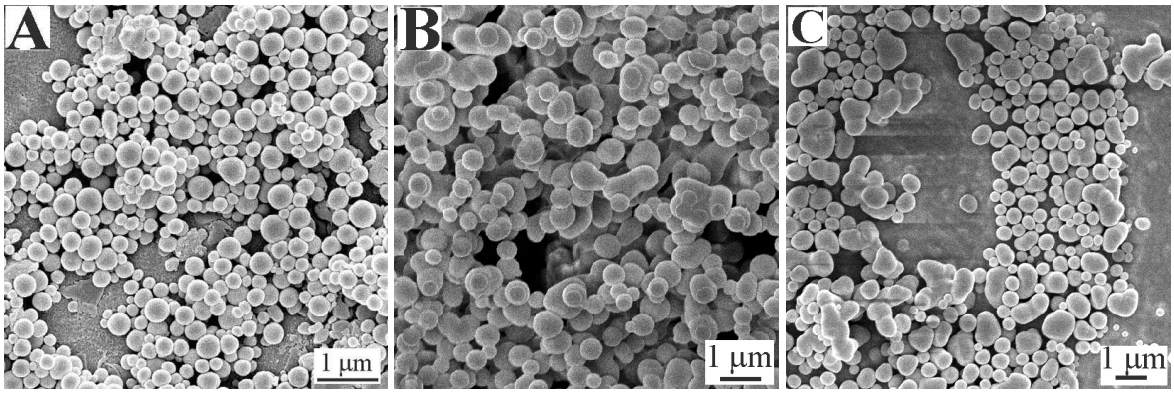


Figure S14. FESEM images of aggregated hybrid nanospheres obtained by aggregation of different hybrid conjugates in DMF; A) peptide-PMMA-Ia (M_n = 24.9 kDa); B) peptide-PMMA-Ib (M_n = 33.0 kDa); C) peptide-PMMA-If (M_n = 75.4 kDa).

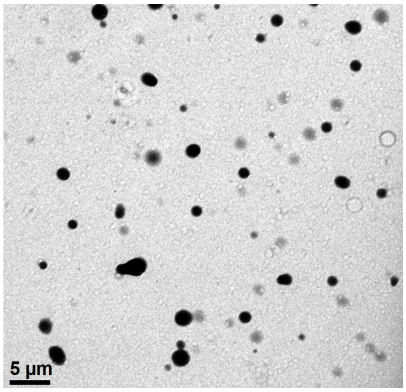
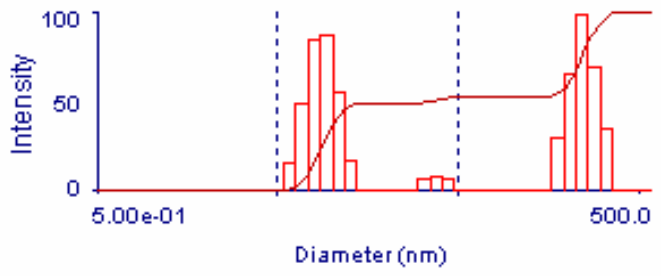
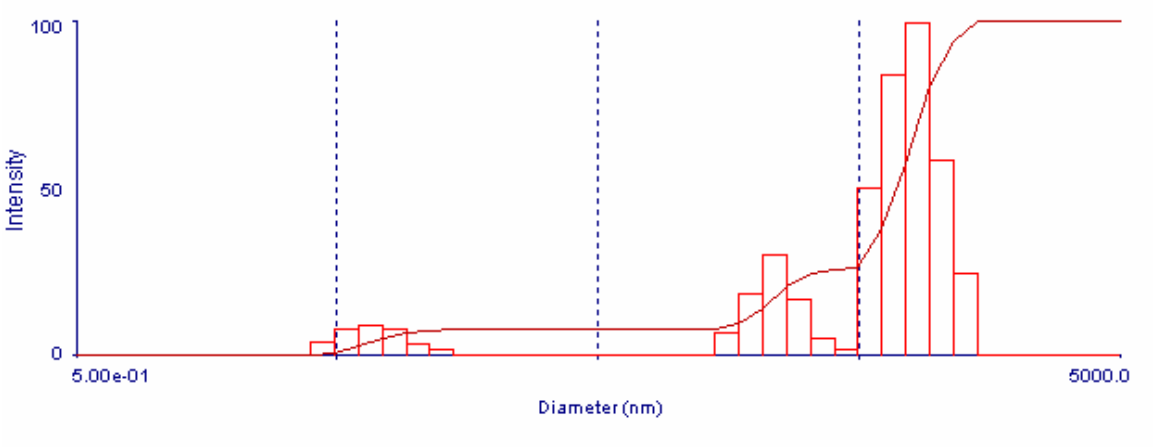


Figure S15. TEM image of aggregated hybrid micro/nanospheres obtained by the aggregation of peptide-PMMA-Ia in CH_3CN.



d	G(d)	C(d)	d	G(d)	C(d)	d	G(d)	C(d)
4.25	0	0	23.32	0	49	127.83	0	53
4.96	0	0	27.22	0	49	149.22	0	53
5.80	16	2	31.77	7	50	174.18	31	57
6.76	50	10	37.09	9	52	203.32	67	68
7.90	87	24	43.29	7	53	237.33	100	83
9.22	89	38	50.53	0	53	277.03	71	94
10.76	57	46	58.99	0	53	323.38	36	100
12.56	17	49	68.85	0	53	377.47	0	100
14.66	0	49	80.37	0	53	440.62	0	100
17.11	0	49	93.82	0	53	514.33	0	100
19.98	0	49	109.51	0	53	600.38	0	100

Figure S16. NNLS distribution of peptide-PMMA-Ia hybrid nanospheres in CH_3CN after 30 minutes as obtained from DLS measurement (Figure 5).



d	G(d)	C(d)	d	G(d)	C(d)	d	G(d)	C(d)
2.87	0	0	28.83	0	8	289.40	17	25
3.54	0	0	35.56	0	8	356.90	5	26
4.37	4	1	43.85	0	8	440.16	2	27
5.39	8	3	54.08	0	8	542.84	50	38
6.64	9	5	66.69	0	8	669.46	85	58
8.19	8	7	82.25	0	8	825.63	100	81
10.11	4	8	101.44	0	8	1018.23	59	94
12.46	2	8	125.10	0	8	1255.75	25	100
15.37	0	8	154.28	7	10	1548.68	0	100
18.96	0	8	190.27	19	14	1909.94	0	100
23.38	0	8	234.66	30	21	2355.48	0	100

Figure S17. NNLS distribution of peptide-PMMA-Ia hybrid micro/nanospheres in CH_3CN after 7 days as obtained from DLS measurement (Figure 5).

Reference

(1) Si, S.; Bhattacharjee, R. R.; Banerjee, A.; Mandal, T. K. *Chem. Eur. J.* **2006**, *12*, 1256.