Supplementary material

Synthesis and Characterization of Poly(RGD) Proteinoid Polymers and NIR fluorescent Nanoparticles of Optimal D,L-Configuration for Drug Delivery Applications – In Vitro Study

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Supporting information including:

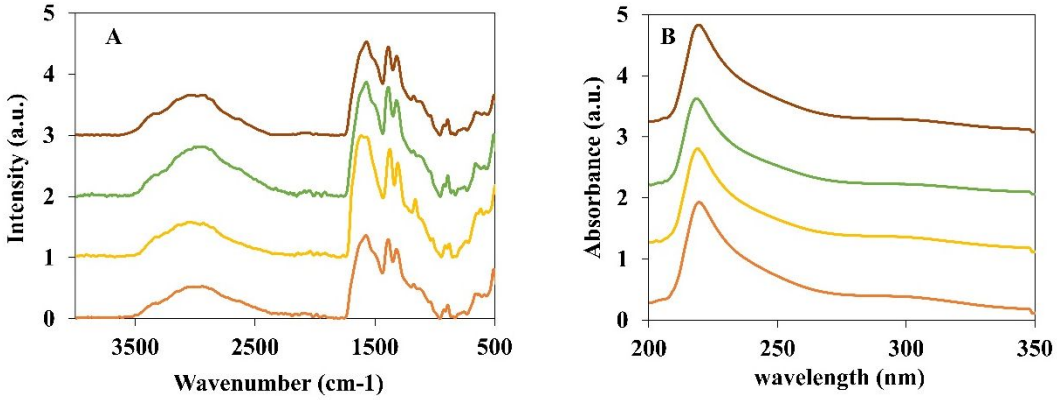

Figure S1. FTIR spectrum (A) and UV-Vis absorption spectra (B) of, P($R^{D}GD$ $R^{D}GD^{D}$, RGD, RGD^{D}) proteinoids (orange, yellow, green, brown) respectively.

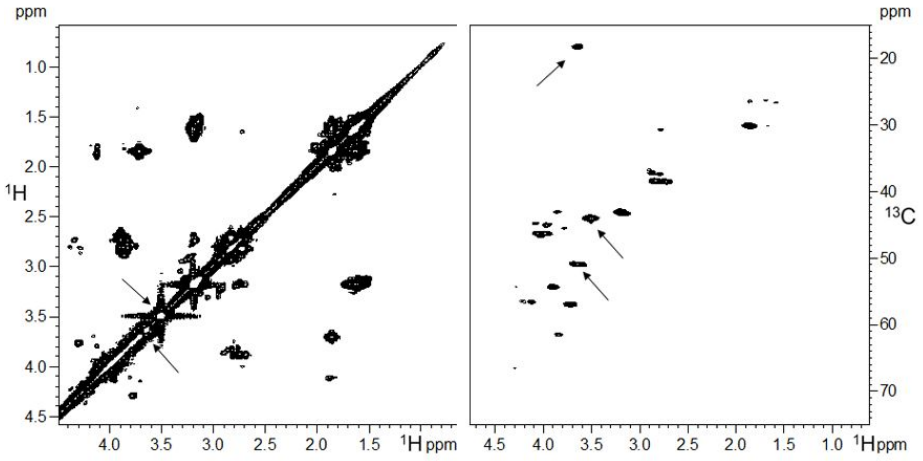

Figure S2. 2D-NMR analysis of P($R^{D}GD$) proteinoids. Left, 2D homonuclear COSY spectrum of P($R^{D}GD$), and **right**, 2D ^{1}H-^{13}C-HMQC spectrum of P($R^{D}GD$), both acquired for a 10 mg/ml sample in $^{2}H_2O$ at 300 K and 16.4 T. Analysis of these spectra and comparison to expected correlation cross-peaks of the three amino acids allowed specific peaks (marked with arrows) to be identified as non-proteinoid signals and to be excluded from the integration analysis.

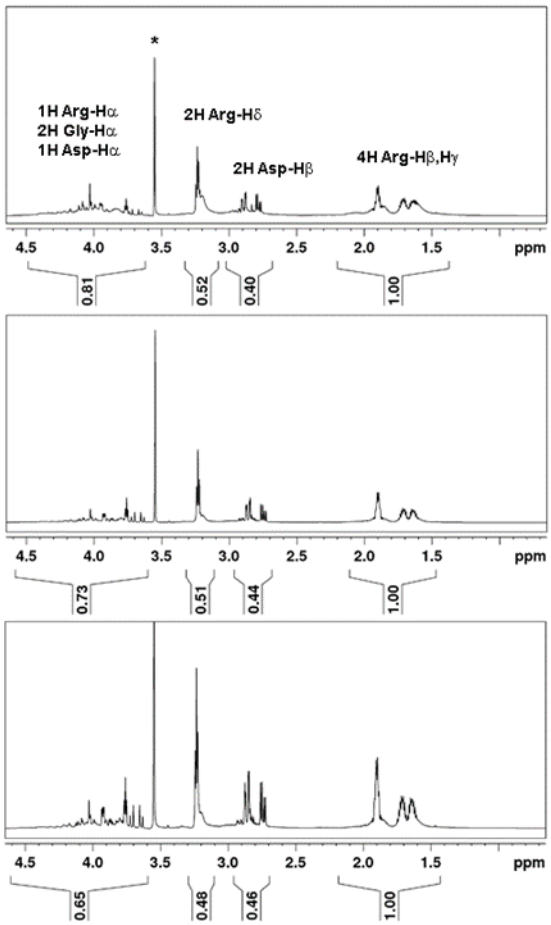

Figure S3. NMR analysis of P(R^{D}GD) proteinoid amino acid composition. Similarly to Figure 1, ^{1}D ^{1}H-NMR spectrum of 10 mg/ml proteniod in $^{2}H_2O$ were acquired at 300 K and 16.4 T. Signals emanating from arginine, glycine and aspartate protons as well as integrated signal areas are shown. Asterisks denote non-proteinoid peaks excluded from the integration analysis. **Top**, P(R^{D}GD^{D}), **Middle**, P(RGD^{D}), **Bottom**, P(RGD).

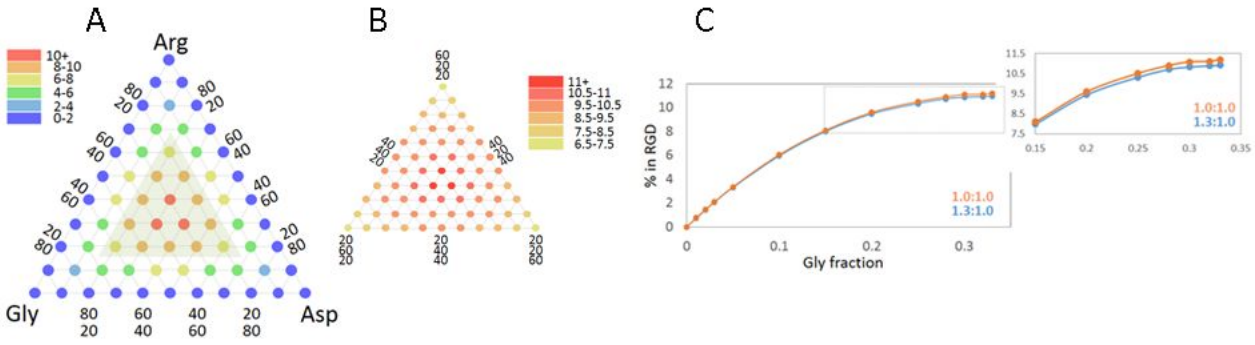

Figure S4. Simulations for prediction of RGD content using amino acid composition. Based on the NMR-derived amino acid composition, random polypeptides were simulated and RGD occurrences counted. These are presented in a ternary component graph. **(A)** Percentage of residues involved in RGD triads as a function of the amino acid composition. **(B)** Detailed presentation of central region (shaded grey triangle) of the graph in **(A)**. The color scale shows the %RGD for each point in the ternary graph. **(C)** Expected levels of residue involvement in RGD triads for conditions prevalent in this study as a function of the Gly mol:mol fraction when Arg:Asp is 1:1 (blue) and when Arg:Asp is 1.3:1 (orange), representing the range of ratios seen in our P(RGD)s.

Table S1a: NMR analysis of amino-acid composition in P(R^DGD^D)

ppm-1	ppm-2	^{1}H nuclei	Relative intensity	Normalized intensity[a]
1.25	2.21	4H $Arg^D(H^\beta,H^\gamma)$	1.00	0.25
2.60	3.02	2H $Asp^D(H^\beta)$	0.40	0.20
3.04	3.32	2H $Arg^D(H^\delta)$	0.52	0.26
3.56	4.41	1H $Arg^D(H^\alpha)$, 2H Gly(H^α), 1H $Asp^D(H^\alpha)$	0.81[b]	Arg^D – 0.25[c] Gly – 0.18 Asp^D – 0.20[c]

Table S1b: NMR analysis of amino-acid composition in P(RGD^D)

PPM-1	PPM-2	^{1}H nuclei	Relative intensity	Normalized intensity[a]
1.25	2.21	4H Arg(H^β,H^γ)	1.00	0.25
2.60	3.02	2H $Asp^D(H^\beta)$	0.44	0.22
3.04	3.32	2H Arg(H^δ)	0.51	0.255
3.56	4.41	1H Arg (H^α), 2H Gly(H^α), 1H $Asp^D(H^\alpha)$	0.73[b]	Arg – 0.25[c] Gly – 0.13 Asp^D – 0.22[c]

Table S1c: NMR analysis of amino-acid composition in P(RGD)

PPM-1	PPM-2	^{1}H nuclei	Relative intensity	Normalized intensity[a]
1.25	2.21	4H Arg(H^β,H^γ)	1.00	0.25
2.60	3.02	2H Asp(H^β)	0.46	0.23
3.04	3.32	2H Arg(H^δ)	0.48	0.24
3.56	4.41	1H Arg(H^α), 2H Gly(H^α), 1H Asp(H^α)	0.65[b]	Arg – 0.25[c] Gly – 0.085 Asp – 0.23[c]

[a] Signal intensity per proton nucleus.

[b] Excluding the signals from solvent impurities at 3.57/3.65 ppm.

[c] Relative contributions of Arg/Gly/Asp were based on the appropriate stoichiometric ratios.

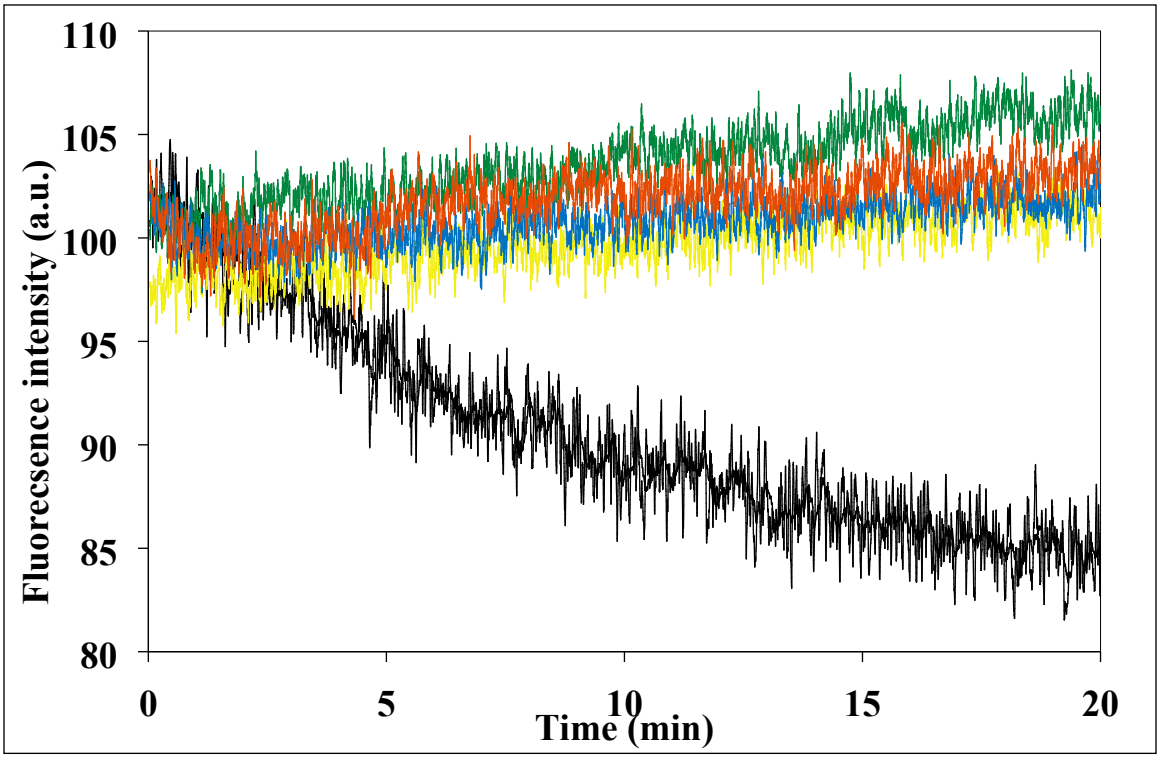

Figure S5. Photostability of the encapsulated ICG P(R^{D}GD R^{D}GD^{D}, RGD, RGD^{D}) NPs and Free ICG. Illumination was performed continuously at 780 nm for a period of 30 minutes, for all the four different configurations of the ICG-encapsulated P(R^{D}GD, R^{D}GD^{D}**,** RGD**,** RGD^{D}) NPs and were compared to the photostaibility of the free ICG. (**Red)** ICG-encapsulated P(R^{D}G^{D}D) NPs, (**blue)** ICG-encapsulated P(RGD) NPs, (**green)** ICG-encapsulated P(R^{D}GD) NPs, (**yellow)** ICG-encapsulated P(RG^{D}D) NPs, (**black**) free ICG.