Supporting Information: Monitoring aggregation of a pH-responsive polymer via proton exchange

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I. EXPERIMENTAL

Material synthesis

Boc-L-isoleucine (Boc-L-Ile-OH, 99%) and trifluoro acetic acid (TFA, 99.5%) were purchased from Sisco Research Laboratories Pvt. Ltd., India, and The 2, 2'-azobisisobutyronitrile used as received. (AIBN, Sigma, 98%) initiator was purified by recrystallization from methanol, anhydrous N. N'dimethylformamide (DMF, 99.9%, Sigma) were purchased and used without further purification. 4-Cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (CDP)[2] and Boc-protected amino acid based monomer Boc-L-isoleucine acryloyloxyethyl ester (Boc-L-IIe-HEMA) were synthesized according to the earlier reported procedure.[1]

Instrumentation

Molecular weights and molecular weight distributions (dispersity (D)) of polymers were determined by Waters ACQUITY Advanced Polymer Chromatography (APC). The instrument contains a 1500 series HPLC pump, an ACQUITYTM refractive index (RI) detector, one ACQUITY APCTM XT 2002.5 μ m (4.6 × 7.5 mm) column in DMF at 45^oC at 0.25 mL/min flow rate. Poly(methyl methacrylate) (PMMA) standards were used to calibrate the instrument.

RAFT polymerization of Boc-L-Ile-HEA.

A typical polymerization procedure is described as follows: Boc-Ile-HEA (0.5 g, 1.5 mmol), CDP (12.2 mg, 30.3 μ mol), AIBN (1.0 mg, 6.07 μ mol; 0.5 g stock solution of 4.0 mg AIBN in 2.0 g DMF) and DMF (2.0 g) were sealed in a 20 mL vial equipped with a magnetic stir bar. The vial was purged with dry N₂ for 20 min and was placed in a preheated reaction block at 70^oC. After 12 h, the polymerization reaction was quenched by cooling the vial in an ice-water bath and exposing the solution to air. The solution was diluted with acetone and precipitated into cold hexanes. The polymer, P(Boc-Ile-HEA), was reprecipitated four times from acetone/hexanes and dried under vacuum at 40^oC for 6 h. The purified polymer was isolated as yellowish white powder.



FIG. S1. ¹H NMR spectrum of P(Boc-Ile-HEA)₄₇ in CDCl₃



FIG. S2. ¹H NMR spectra of (A) P(Boc-Ile-HEA)₄₇-*b*-PMMA₂₅ in CDCl₃ and (B) P(⁺H₃N-Ile-HEA)₄₇-*b*-PMMA₂₅ in D₂O(B).

Synthesis of Block Copolymer

A typical block copolymerization procedure is described as follows: MMA (40 mg, 0.4 mmol), P(Boc-Ile-HEA)macroCTA ($M_{n,NMR} = 15800$ g/mol, 200 mg, 12.6 μ mol), AIBN (0.41 mg, 2.5 μ mol; 0.21 g stock solution of 4.0 mg AIBN in 2.0 g DMF), and DMF (1.0 g) were

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added in a 20 mL polymerization vial equipped with a magnetic bar and purged with dry N_2 gas for 15 min. The reaction vial was put in a preheated reaction block at 70^oC for 6 h. The resulting block copolymer, P(Boc-Ile-HEA)-b-PMMA, was purified as mentioned above for the homopolymer.

Preparation of polymers with free $-NH_2$ pendants through successive Boc group expulsion via TFA treatment

Typically, 2.0 mL TFA was added to the solution containing 0.2 g of polymer in 1.0 mL DCM in a 20 mL glass vial. The solution was stirred for 2 h at room temperature, precipitated four times in hexanes from acetone solutions, and finally dried under vacuum at 40° C for 8 h. [1]

Dynamic light scattering (DLS) measurements

Intensity-size distributions of the sample solutions at six different pH were determined using a dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instrument Ltd., UK) instrument equipped with a He-Ne laser beam at 658 nm. All Samples were sonicated during 3-5 minutes before the mesurement. The results are shown in FIG. S3.



FIG. S3. Plots of intensity of scattered light versus average diameter (nm) of the particles in aqueous media at six different pH. It shows that with the increase of pH, mean value of the size of the particle increases.

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The samples for FE-SEM analysis were prepared as follows: an aliquot of sample solution was drop casted on a cover slip, vacuum dried, coated with gold:palladium (20:80) alloy and then images of samples, preapared at four different pH were captured using a Carl Zeiss-Sigma instrument. The recorded set of images clearly indiacates how the aggregate size increases as pH incearses. The subfigure (a) shows small (possibly single) micelles, whereas, subfigures (b-d) show the increasing size of aggregates. Subfigure (b) shows the longer rod like stucture of the aggregates, and (c) shows the result of compactificaton and lastly (d) shows the very large size of the aggregates. So, all the images are in qualitative agreement with the main results, outlined in the manuscript.

Scanning Electron Microscope (SEM) studies



FIG. S4. SEM images of aggregates of polymer prepared at four different pH

II. REFERENCES

- [1] K. Bauri, S. Ghosh Roy, S. Pant and P. De, *Langmuir* 2013, **29**, 2764-2774.
- [2] U. Haldar, M. Nandi, B. Ruidas and P. De, *Eur. Polym. J.* 2015, 67, 274-283.