Supplementary Material (ESI) for Polymer Chemistry

Covalently Incorporated Protein-Nanogels using AGET ATRP in Inverse Miniemulsion

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Experimental

Materials. Oligo(ethylene oxide) monomethyl ether methacrylate (average molecular weight ~300 g/mol, $OEO_{300}MA$), tris(2-pyridylmethyl)amine (TPMA), Span-80, ascorbic acid, and $Cu(II)Br_2$ (98%, Acros) were purchased from Aldrich. Inhibitor was removed from $OEO_{300}MA$ by passing through a basic alumina column. poly(ethylene glycol)isobutyryl bromide ($PEG_{2000}MI$, M_n = 2000) and poly(ethylene oxide) dimethacrylate ($PEG_{400}DM$, M_n=4000) were prepared as previously described.[1] Green fluorescent proteins were prepared as previously described.[2] All other reagents and solvents were purchased from Aldrich and used as received.

Characterization. Particle size measurements were conducted using a Zetasizer Nano from Malvern Instruments. Confocal microscopy was carried out using a Carl Zeiss LSM 510 Meta NLO Confocor 3 Inverted Spectral Confocal Microscope using an excitation of 488 nm. UV-vis spectroscopy was conducted on a Cary 5000 spectrophotometer and fluorescence spectra were collected on a Cary Eclipse fluorescence spectrophotometer.

Nanogel Synthesis: GFP-NGs were prepared through a water-in-oil inverse miniemulsion utilizing AGET ATRP. The inverse miniemulsion was composed of a water phase consisting of $Cu(II)Br_2$ (2.79 mg, 0.013 mmol) / tris(2-pyridylmethyl)amine (TPMA) (3.63 mg, 0.013 mmol), GFP1 (52.5 mg, 0.002 mmol), $PEO_{2000}iBBr$ (50 mg, 0.025 mmol, M_n=2000), oligo(ethylene oxide)methacrylate

($OEO_{300}MA$, 900 mg, 3 mmol), PEO_{4000}-dimethacrylate (400 mg, 0.1 mmol) were dissolved in 1.46 ml 0.1M phosphate buffered saline (PBS) (pH 7.4) and emulsified with a 0.05% (w/w) of Span-80 in cyclohexane using ultrasonication to form stable droplets**.** After degassing, ascorbic acid 200μL (27.5 mg/ml degassed) was injected to initiate AGET ATRP which was stopped after 15 hours at 30°C. The NG were purified by precipitation into THF followed by dialysis (50000 MWCO membrane) into water to remove all unreacted reagents

Reference

1. J.K. Oh *et al Biomacromolecules* **2009,** 10, 2300–2309
2. J.C. Peeler *et al Journal of the American Chemical Society* **2010** 132, 13575-1357