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Light-mediated intracellular polymerization

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Light Mediated Intracellular Polymerization

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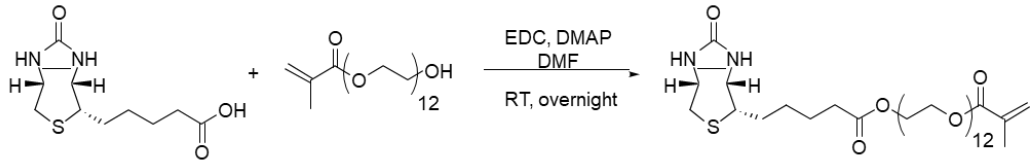
Synthesis of biotinylated methacrylate poly(ethylene glycol)[1]



A mixture of biotin (1.0 g, 4.1 mmol), poly(ethylene glycol) methacrylate (1.5 g, 4.1 mmol, average moleculear weight = 500), EDC (0.78 g, 4.1 mmol), and 4-DMAP (0.05 g, 0.22 mmol) in DMF (50 mL) was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude product was re-dissolved in DCM and washed with 5% $NaHCO_3$ (aq), 1% HCl, and brine (three times each). The solvent was removed *in vacuo* and the crude product was purified by column chromatography eluting with DCM/MeOH (from100:2 to 100:10), which gave the product as a viscous oil in 30% yield (0.75 g)

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.15 (s, 1H), 5.60 (s, 1H), 5.00 (s, 1H), 4.72 (s, 1H), 4.53 (m, 1H), 4.35 (m, 1H), 4.31 – 4.26 (m, 5H), 3.80 – 3.63 (m, 48H), 2.39 (t, *J* = 5.1 Hz, 2H), 1.98 (s, 3H), 1.49 – 1.71 (m, 6H); 13C NMR (500 MHz, $CDCl_3$): δ = 173.5, 167.4, 162.7, 136.2, 125.7, 70.6, 69.1, 64.1, 63.9, 63.5, 61.8, 60.0, 55.2, 50.9, 40.5, 33.7, 28.2, 24.7, 18.3; IR λ_{max} (cm^{-1}) 3350, 2868, 1703, 1456, 1298, 1254, 1101, 952; ESI-MS ($C_{40}H_{72}N_2O_{17}S$): $[M+H]^+$: 885.16, found: 885.20.

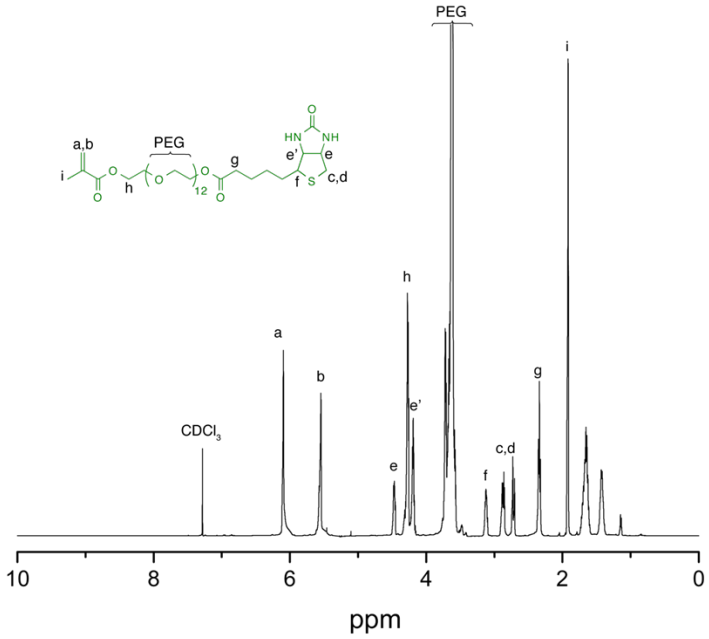
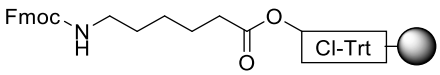


Fig. S1. ^{1}H NMR spectrum of biotinylated methacrylate poly(ethelene glycol) in CDCl3.

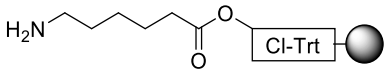
Synthesis of His-CTA[2]
(Fmoc-amino) hexanoic acid linker bound resin S3



In an SPE filter cartridge (12 mL, fitted with a polystyrene resin functionalised with a 2-chlorotritylchloride linker (Cl-Trt) with 20 µm pores, Sigma-Aldrich), thionyl chloride (40 µL, 0.55 mmol) was added to preswollen (in anhydrous DCM) 2-chlorotrityl chloride resin (500 mg, reported loading 0.95 mmol/g) in anhydrous DCM under a N_2 atm, and the reaction mixture was stirred for 1 h. The solvent was drained, and the resin was washed with anhydrous DCM (3 × 5 mL) and anhydrous DMF (3 × 5 mL). The re-activated resin was swollen in anhydrous DCM for 10 min, followed by the addition of Fmoc-Ahx-OH (237 mg, 1.8 mmol) and DIPEA (275 µL, 1.7 mmol) in anhydrous DMF (5 mL), and was shaken for 1 h. The resin was washed with anhydrous DCM (3 × 5 mL) and anhydrous DMF (3 × 5 mL), and then treated twice with DCM/MeOH/DIPEA (80:15:5, 5 mL) and washed with DCM (3 × 5 mL) and DMF (3

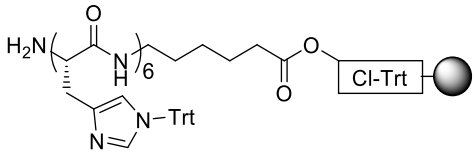
× 5 mL).

6-Aminohexanoic acid linker bound resin S4



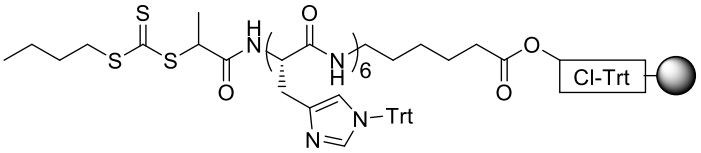
To the resin **S3** (500 mg, pre-swollen in DCM), piperidine (5 mL, 20% *v*/*v* in DMF) was added and the resin was shaken for 2 × 10 min. The solvent was drained, and the resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), MeOH (3 × 5 mL) and diethyl ether (3 × 5 mL).

Hexahistidine tag resin S5



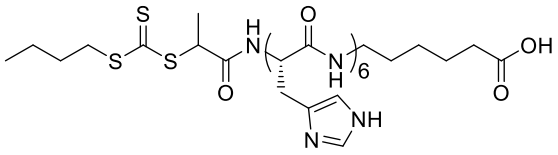
Fmoc-His(Trt)-OH (930 mg, 1.5 mmol) and ethyl cyano(hydroxyimino) acetate (Oxyma) (213 mg, 1.5 mmol) were dissolved in DMF (5 mL) and stirred for 10 min. *N*,*N*′-Diisopropylcarbodiimide (232 µL, 1.5 mmol) was added and stirred for further 2 min. The mixture was added to resin **S4** (500 mg, pre-swollen in DCM) and stirred for 3 h. The solution was drained, and the resin was washed with DCM (3 × 5 mL) and DMF (3 × 5 mL). The resulting resin was swollen in DCM, drained, and piperidine (5 mL, 20% *v*/*v* in DMF) was added and shaken for 2 × 10 min before the solvent was drained and the resin was washed with DCM (3 × 5 mL), DMF (3 × 5 mL), MeOH (3 × 5 mL) and Et_2O (3 × 5 mL). This procedure was repeated six times to generate the hexahistidine tag moiety. The coupling reactions were monitored by a ninhydrin test.[3]

Hexahistidine tagged RAFT agent bound resin S6



CA-CTA (358 mg, 1.5 mmol), and ethyl cyano(hydroxyimino) acetate (213 mg, 1.5 mmol) were dissolved in DMF (5 mL) and stirred for 10 min. *N*,*N*′-diisopropylcarbodiimide (232 µL, 1.5 mmol) was added and stirred for further 2 min. The mixture was added to resin **S5** (500 mg, pre-swollen in DCM) and reaction mixture was shaken for 3 h. The solution was drained, and the resin was washed with DCM (3 × 5 mL) and DMF (3 × 5 mL).

Hexahistidine tagged His-CTA



2

The resin **S6** (500 mg, pre-swollen in DCM) was shaken in TFA/water (95:5, *v*/*v*, 5 mL) for 2 h. The filtrate was collected and the resin was washed with TFA/water (3 × 5 mL). The solutions were combined and evaporated *in vacuo*. The crude product was purified by reverse phase column chromatography using a gradient of acetonitrile (5% to 95%) and water as the eluent (170 mg, 63%).

^{1}H NMR(400 MHz, DMSO-d_6) δ = 8.97–8.73 (m, 6H), 8.68 (m, 1H), 8.59–8.38 (m, 3H), 8.30 (m, 1H), 8.13 (m, 1H), 7.42–7.08 (m, 6H), 4.81–4.41 (m, 7H), 3.34 (t, J = 7.2 Hz, 2H), 3.16–2.81 (m, 14H), 2.18 (t, J = 7.4, 2H), 1.61 (m, 2H), 1.54–1.28 (m, 8H), 1.28–1.13 (m, 3H), 0.88 (t, J = 7.4 Hz, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ = 223.1, 174.9, 170.6, 170.5, 170.4, 170.4, 170.2, 170.1, 170.0, 159.6, 159.3, 159.1, 158.9, 134.4, 134.3, 130.3, 130.2, 130.0, 129.9, 120.5, 118.5, 117.3, 117.2, 117.1, 116.5, 114.6, 52.6, 52.5, 52.5, 52.3, 50.1, 49.8, 39.0, 38.7, 36.6, 35.8, 34.1, 30.0, 29.0, 27.6, 26.4, 26.3, 25.5, 24.7, 24.6, 21.8, 18.5, 18.0, 13.9.

HRMS (ESI) for $C_{50}H_{67}N_{19}O_9S_3$ $[M+H]^+$: *calcd.*: 1174.4604; *found*: 1174.4599.

HPLC (ELSD) t_R = 1.49 min (purity > 98%).

Data in agreement with the literature.[2]

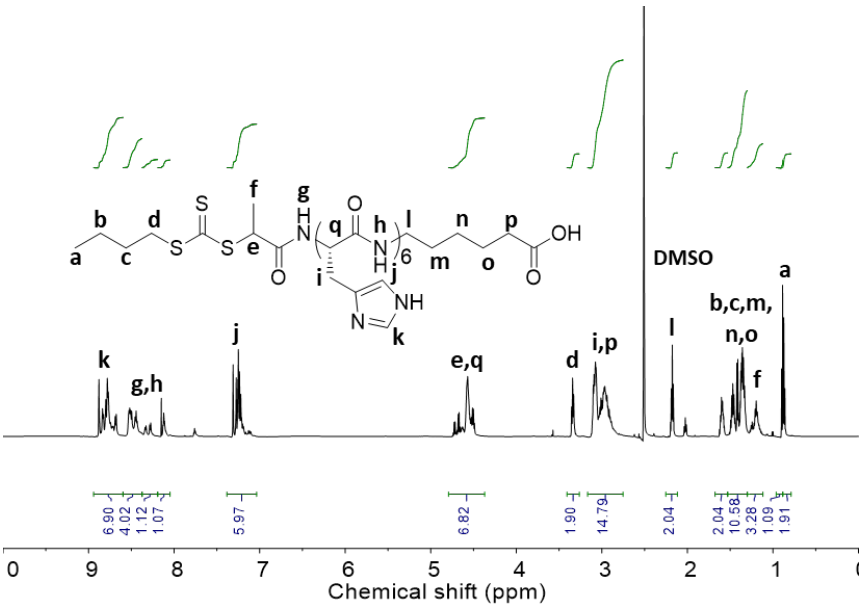


Fig. S2. ^{1}H spectrum of His-CTA recorded in DMSO-d_6.

Table S1. Summary of intracellular polymerization strategies.

Polymerization method	Stimuli	Location	Applications	Conditions	Structure	M_n (kDa)	*Đ*	Ref
UV-Mediated Free Radical Polymerization	UV light (365 nm, 5 mW cm^{-2})	Cytoplasm, nucleus, lysosomes	Modulate cellular function, enhance actine polymerization, bioimaging	FMMA (10 mM) and initiator (1 mM)/ 5 min UV illumination	Nanoparticles (50–70 nm)	6.2	1.52	3
	UV light (365 nm, 5 mW cm^{-2})	Inner cell membrane interface (Cytoplasm)	Support the cell membrane interface against rupture and disintegration and preserving its fluid function	(PEG-DA-Mn = 700 Da (4 to 40 wt%), Irgacure D-2959 (1 wt%), and 0.05 wt% of fluorescein O,O'-diacrylate	hydrogel	–	–	4
Visible-Mediated PET-RAFT Polymerization	Visible light (470 nm, 100 mW/cm^{-2})	Cytoplasm	Cancer treatment	(DMA) (5.0 mM) or (HPMA) (50.0 mM), (His-CTA) (1000 μM), (eosin Y) (100 μM)	Linear-polymer	His-PDMA-1 (13.2 kDa) and His-PHPMA-1 (20.9 kDa)	His-PDMA-1 (1.07) and His-PHPMA-1 (1.11)	5
Redox reaction-Mediated Free Radical Polymerization	GSH-reduction of Cu(II)−histidine to Cu(I) which activate alkyl bromide to	Cytoplasm	Formation of Intracellular polymer bearing paclitaxel for cancer treatment	FMMA (1 mM), TEG-Br (10 μM), Cu-His (400 μM), NaAsc (800 μM) and intracellular GSH	Nanoparticles of poly-FMMA (20−40 nm)	N/A	N/A	6

	initiate the polymerization reaction							
Addition Polymerization	spontaneous amino-yne click polymerization of primary amine and terminal diyne activated by adjacent carbonyl group	Cytoplasm	Cancer cell death and imaging	diamine monomer 1 (10 μM), diyne monomer 2 (10 μM), and DMEM (10% FBS) incubated with HeLa cells for 160 min	Aggregates of poly(β-aminoacrylate)	7.3	N/A	7
Condensation Polymerization	Glutathione induces cyanobenzothiazole (CBT)-cysteine condensation	Cytoplasm	Bioimaging	Fluorescent Probes 1 (1 nmol in 30 μL of PBS buffer) and probe 2 (1 nmol in 30 μL of PBS buffer) were then injected in mice	Linear-polymer	N/A	N/A	8
	pH change, Furin activity, and Glutathione effect induces cyanobenzothiazole (CBT)-cysteine condensation	At trans side of the Golgi apparatus	Bioimaging the proteolytic activity of furin and a trans-Golgi protease	MDA-MB-468 cell incubated with monomer 8 (200 μM) for 8 hrs and stained with streptavidin-gold nanoparticles (15 nm)	Aggregates	N/A	N/A	9

Oxidative Polymerization (ROS-Mediated Polymerization)	ROS induces the polymerization of organo-tellurides ((HO−EG_4−C6)$_2$−Te)) to produce Te−O polymers	Not clear	The interaction with selenoproteins disrupts the in cellulo antioxidant system, increases the oxidative stress, and leads to selective apoptosis of cancer cell	Te nanoreservoirs of 10 and 20 μg mL^{-1} composed of (HO−EG4−C6)$_2$−Te loaded on Au by coordination bond (Te/Au= 6:1)	Linear-polymer	N/A	N/A	10
	ROS induced disulfide polymerization of a dithiol-bearing molecule by the oxidation reaction	Mitochondria	Dysfunction of the mitochondria leads to cell necroptosis	Mito-1-NBD bearing dithiole groups (30 μM) incubated with HeLa cell	[a]Nano aggregates	N/A	N/A	11
Enzyme-Mediated Polymerization	Transglutaminase enzyme (TGase) forms the isopeptide bond between glutamine and lysine side chain.	Cytoplasm	In cellulo elastin-like polypeptides induces intracellular protease degradation and cell death	FTIC labeled peptide 4 (600 μM) Containing peptide sequence QRLGVGFPK incubated with HeLa cells	Nanoparticles by decreasing the temperature from 37 °C to 4 °C	28	1.25	12
	Transglutaminase enzyme (TGase)	Cytoplasm	Bioimaging of hypoxic neuroblastoma	FITC-Pep 9 (600 mM) with peptide sequence	[b]Nanostructure at 4 °C	N/A	N/A	13

	polymerizes the peptide monomer with active sites on the N- and C-terminals and elastin-based repeat units (Xaa-Gly-Val-Gly-Pro: XGVGP or Gly-Val-Gly-Xaa-Pro: GVGXP)		cells	QGVGFPK incubated with HeLa cells to produce FITC-$7P_F$ assemblies				
	Overexpressed tyrosinase induces oxidative polymerization	Cytoplasm	Melanoma therapy by capture the immunogenic functional proteins released from ribosome, nucleus, and mitochondria. Introducing tumor antigenic properties	FITC labelled tripeptide Asp-Phe-Tyr (DFY) (30 μM) was incubated woth B16 cells	Nanofibers (300−600 nm)	N/A	N/A	14
	Cathepsin protease B catalyzes CBT-cysteine condensation	Lysosome	Bioimaging and cancer therapy	AIEgen-peptide conjugate (D2P1) (20 μM) and cyanobenzothiazole-cysteine (3CBT)	[c]Nanoaggregates	N/A	N/A	15

	polymerization in reducing environment containing glutathione (GSH)			(20 μM) incubated with MDA-MB-231 and HT-29				

[a]The size of nanoaggregates were recorded as 786 nm in PBS (not in cells) by using Mito-1 (10 mM). [b]Decreasing the temperature to 4 °C lead to a collapse in the polypeptide chains to form nanoparticles with a size distribution around at 872 ± 190 nm (*in vitro*). [c]The size of nanoaggregates wasn't recorded in/out the cell.

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