Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information to:

"Polymer Design and Component Selection Contribute to Uptake, Distribution & Trafficking Behaviours in Live Breast Cancer Cells"

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Small Molecule Characterisation

*2-(((phenylethylthio)carbonothioyl)thio)isobutyronitrile (**1**)*

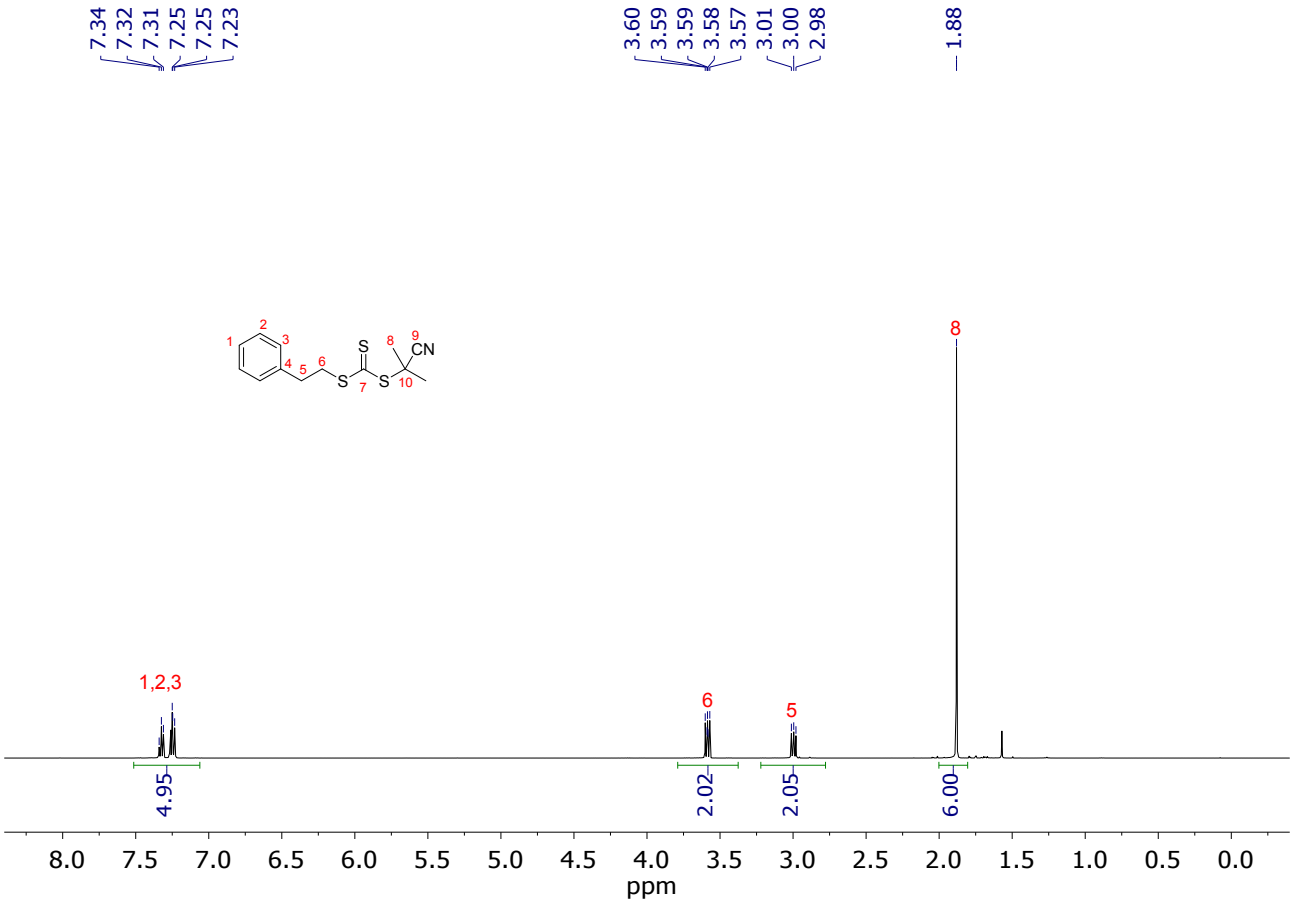

Figure S1. ^{1}H NMR spectrum of **1** (500 MHz, $CDCl_3$).

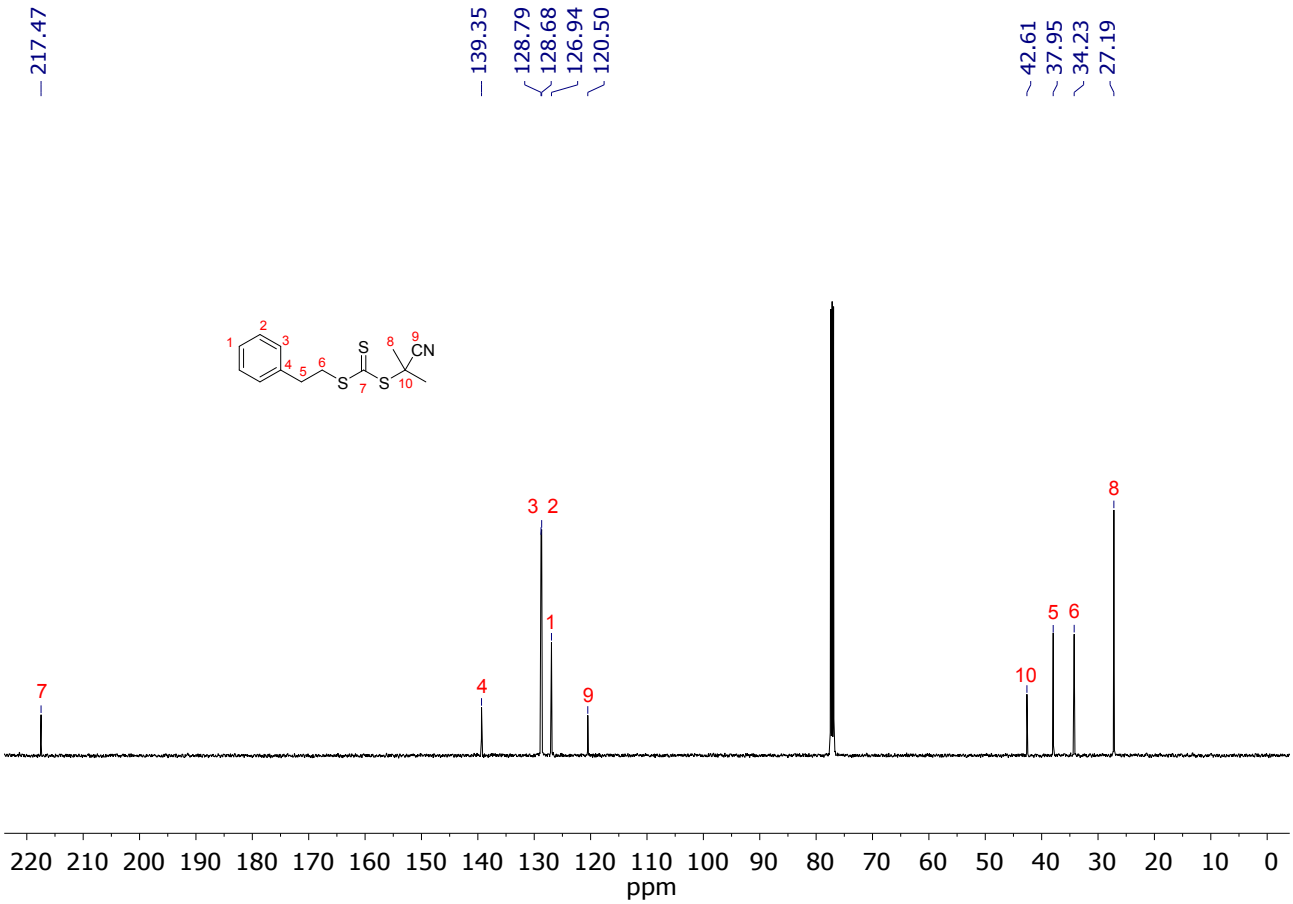

Figure S2. ^{13}C NMR spectrum of **1** (125 MHz, $CDCl_3$).

2-((tert-butoxycarbonyl)amino)ethyl 4-cyano-4-(((phenylethylthio)carbonothioyl)thio) pentanoate

(2)

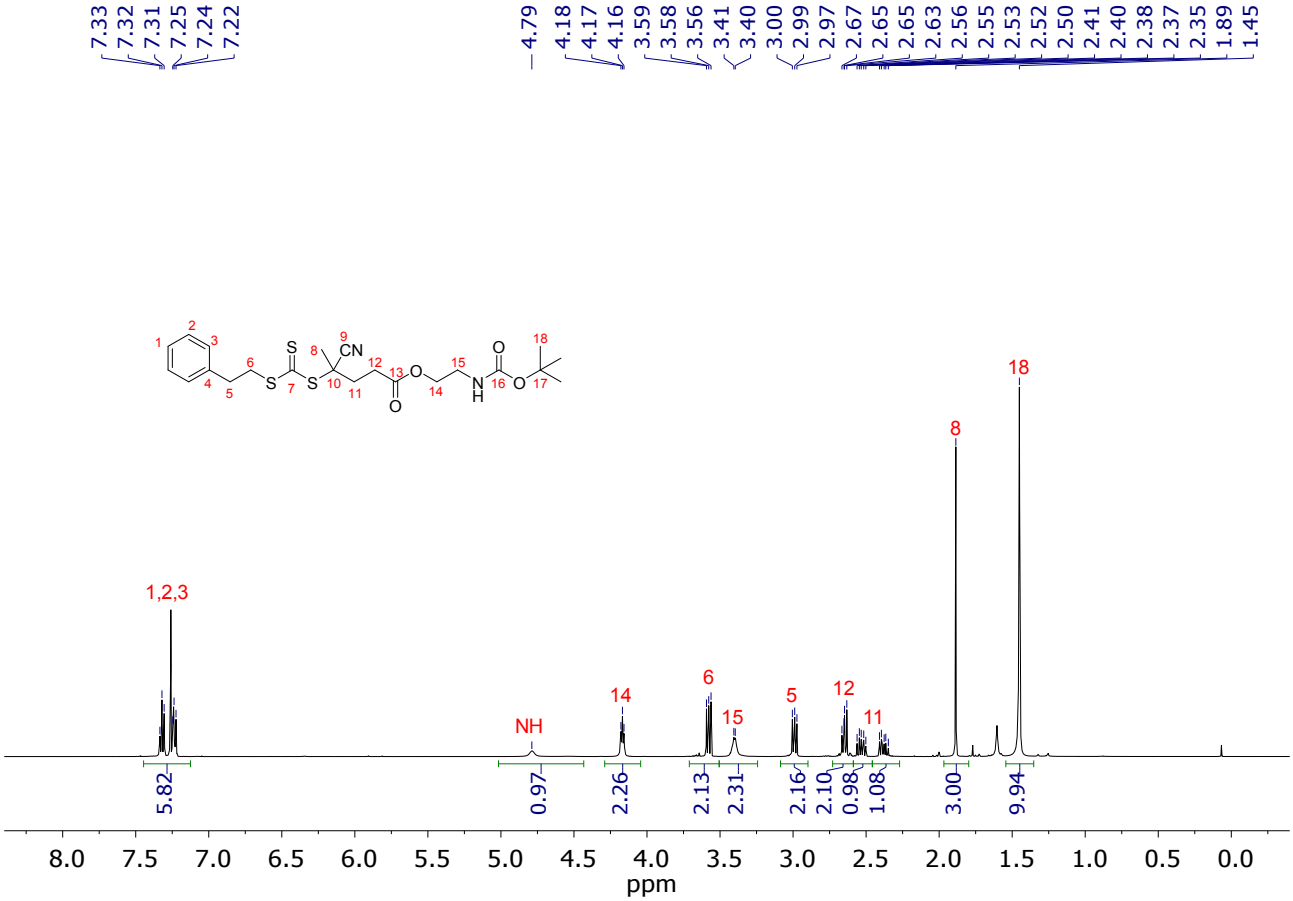

Figure S3. ^{1}H NMR spectrum of **2** (500 MHz, $CDCl_3$).

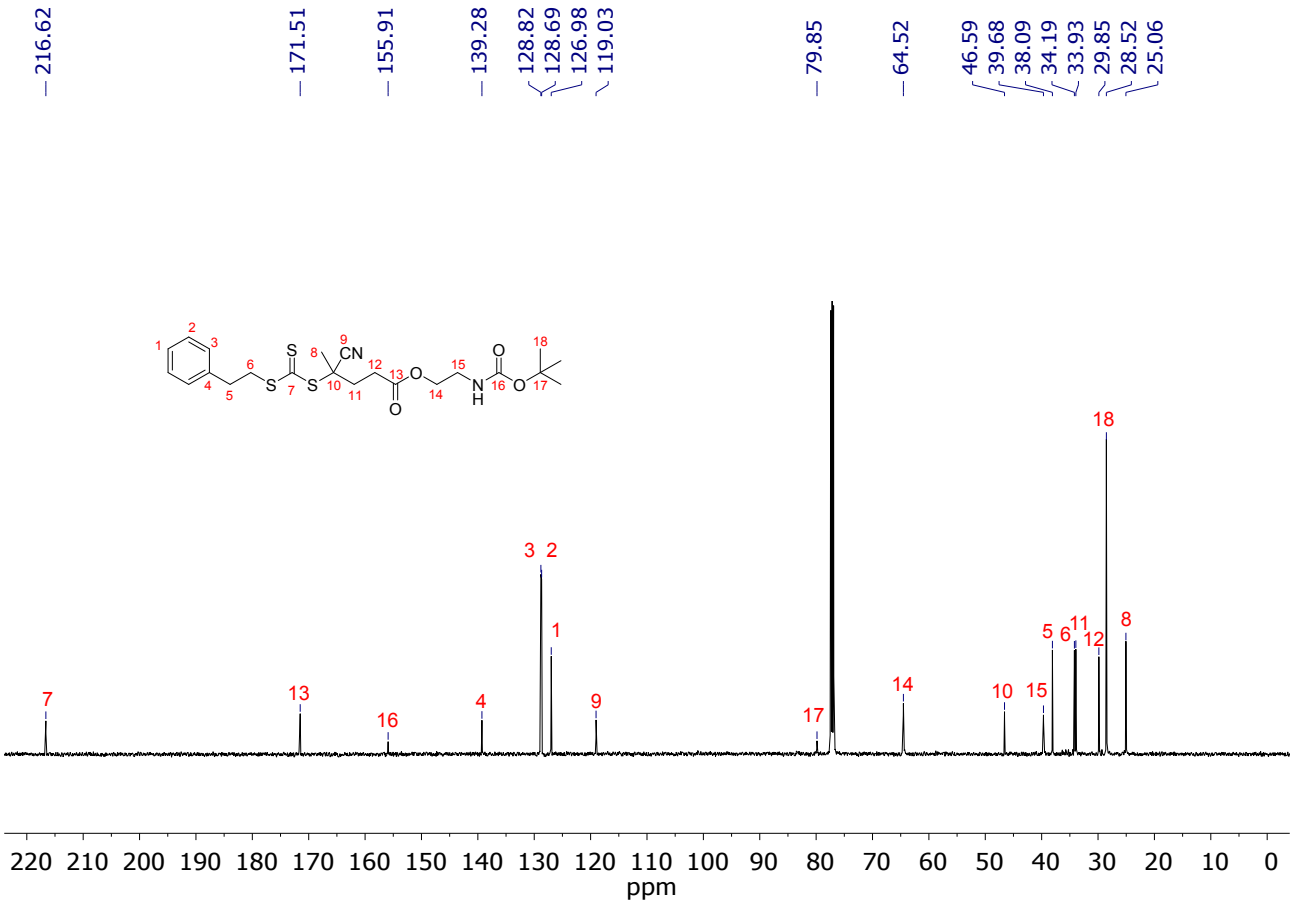

Figure S4. ^{13}C NMR spectrum of **2** (125 MHz, $CDCl_3$).

*FAM methacrylate (**3**)*

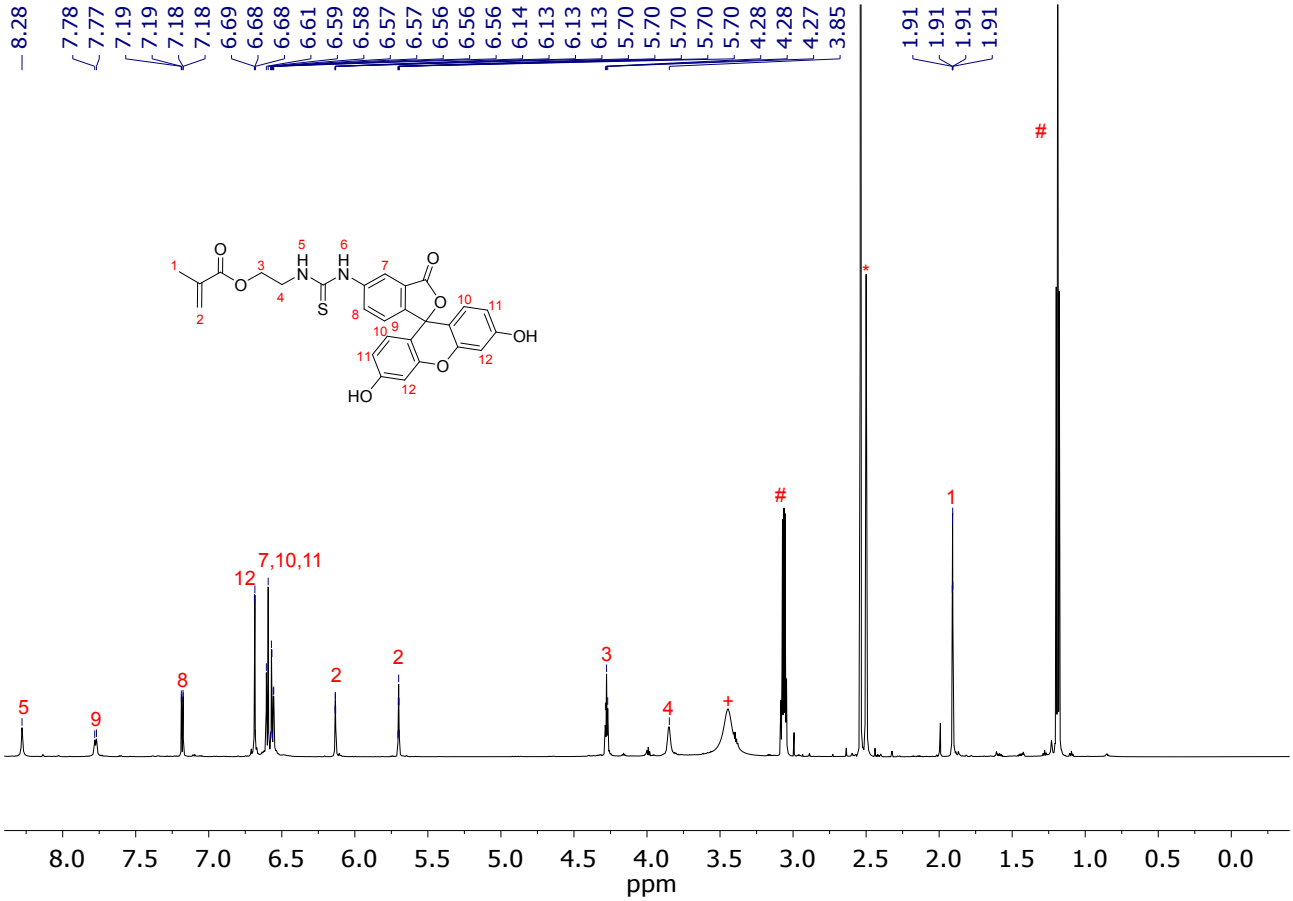

Figure S5. ^{1}H NMR spectrum of FAM methacrylate (**3**) (700 MHz, DMSO-d_6). * - DMSO; + - H_2O; # - Et_3NHCl.

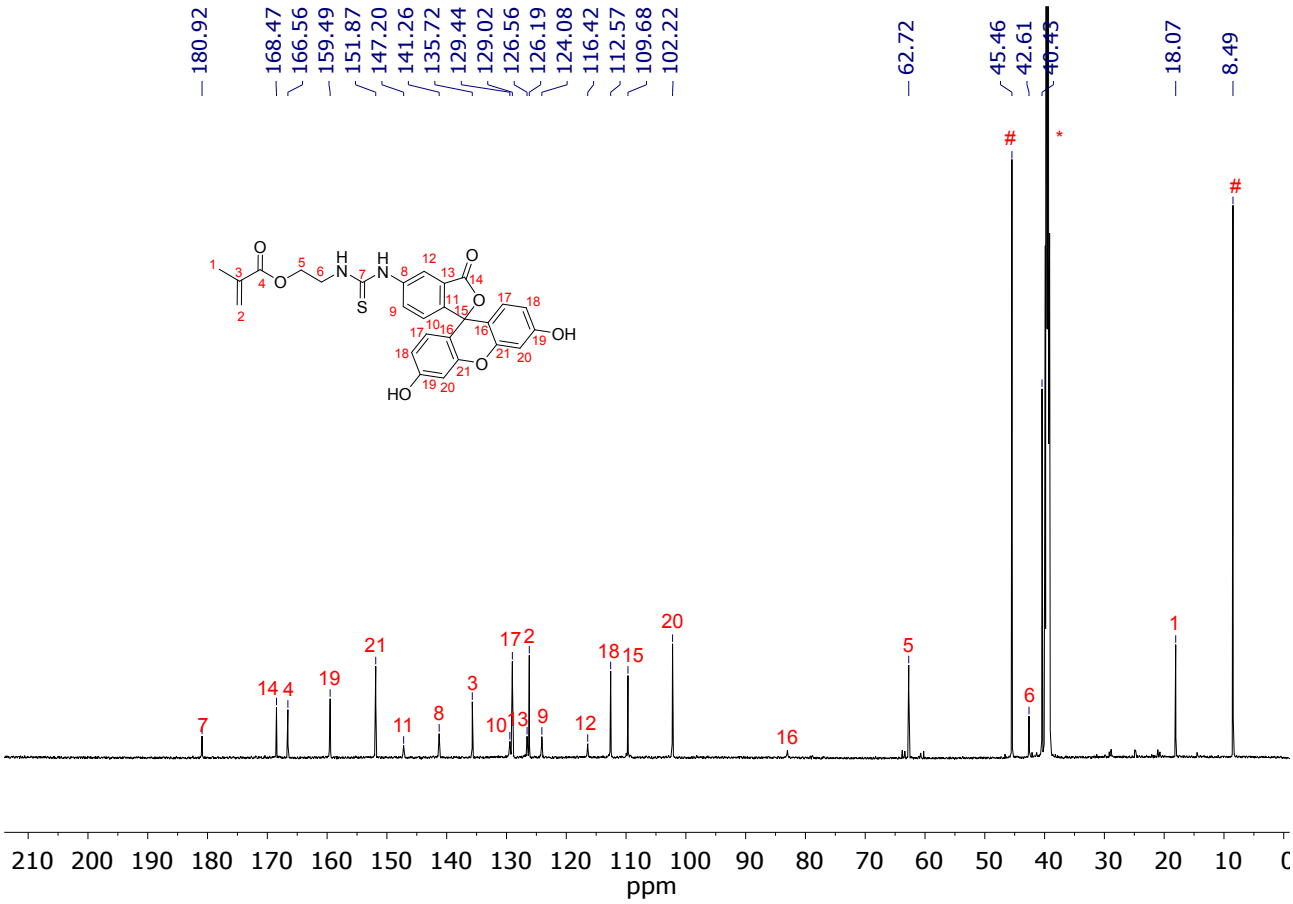

Figure S6. ^{13}C NMR spectrum of FAM methacrylate (**3**) (175 MHz, DMSO-d_6). * - DMSO; # - Et_3NHCl.

*BODIPY FL methacrylate (**4**)*

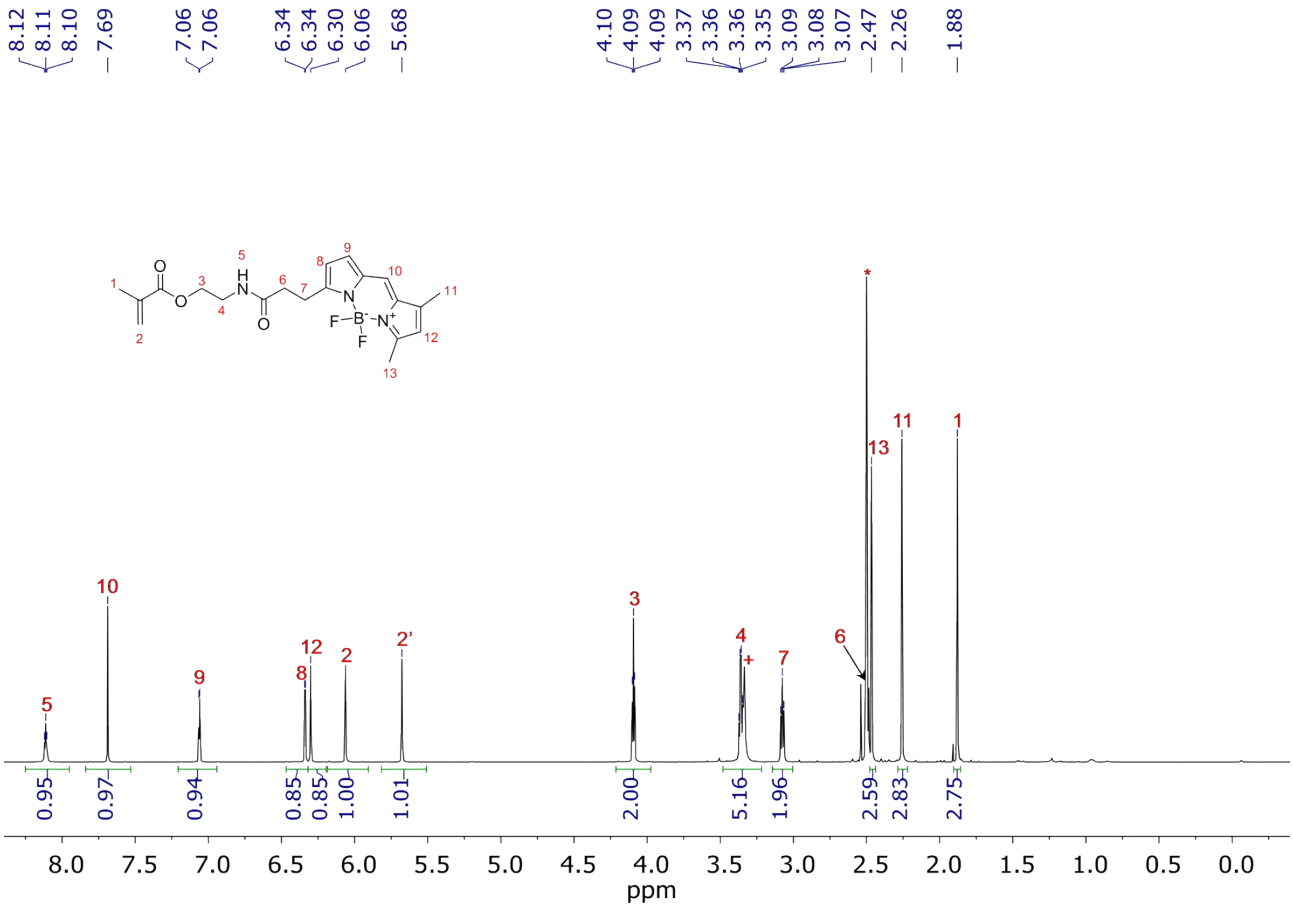

Figure S7. ^{1}H NMR spectrum of BODIPY FL methacrylate (**4**) (700 MHz, DMSO-d_6). * - DMSO; + - H_2O.

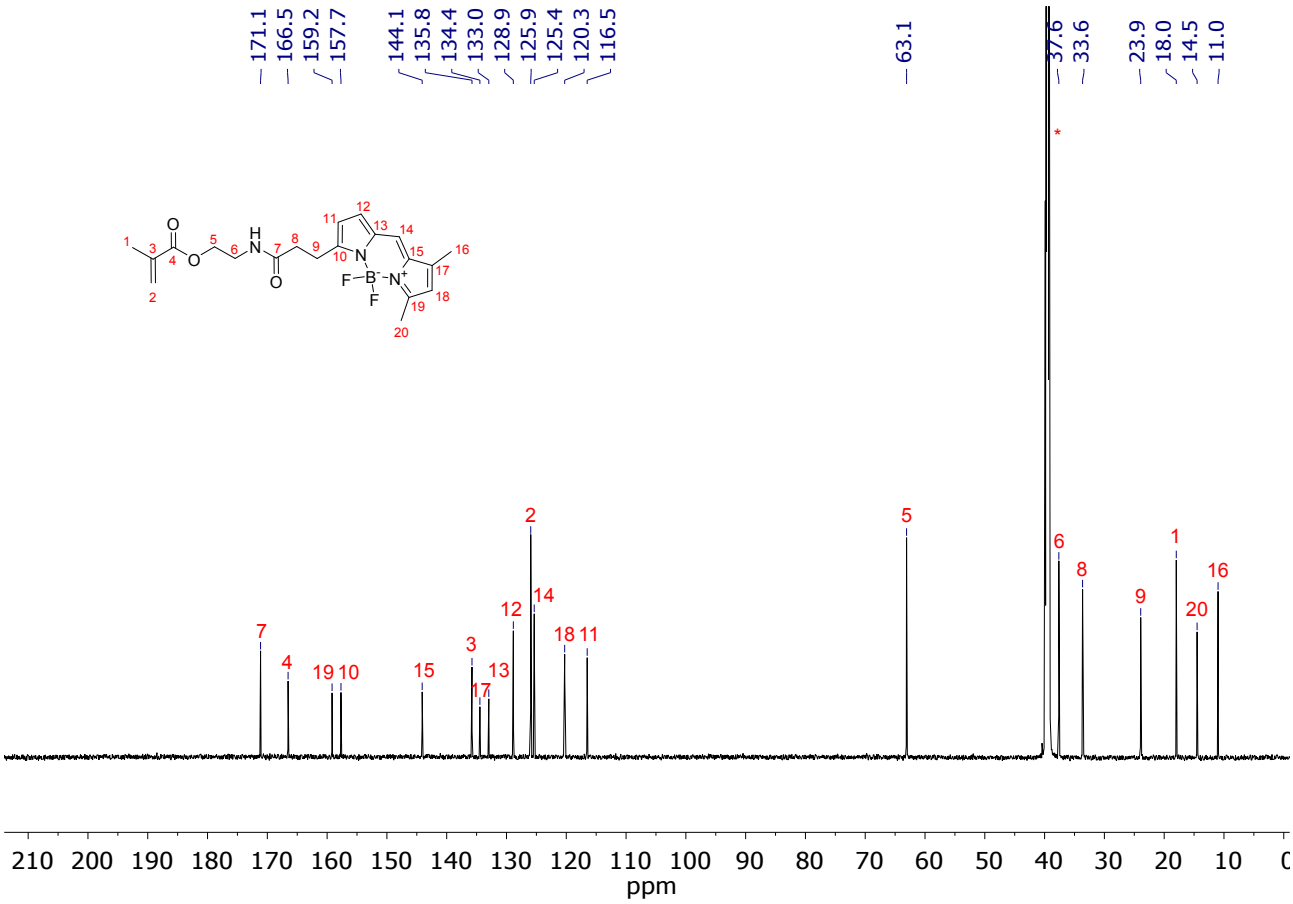

Figure S8. ^{13}C NMR spectrum of BODIPY FL methacrylate (**3**) (175 MHz, DMSO-d_6). * - DMSO.

Polymer Characterisation

*Characterisation of PEGMA HBPs for the external attachment of fluorophores (**5-6**)*

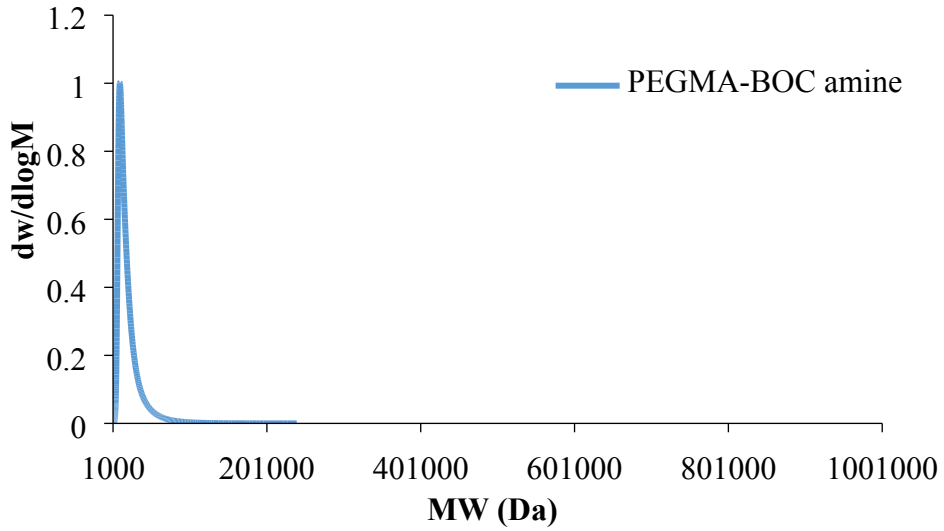

Figure S9. SEC chromatogram of BOC-amine functional PEGMA HBP (**5**).

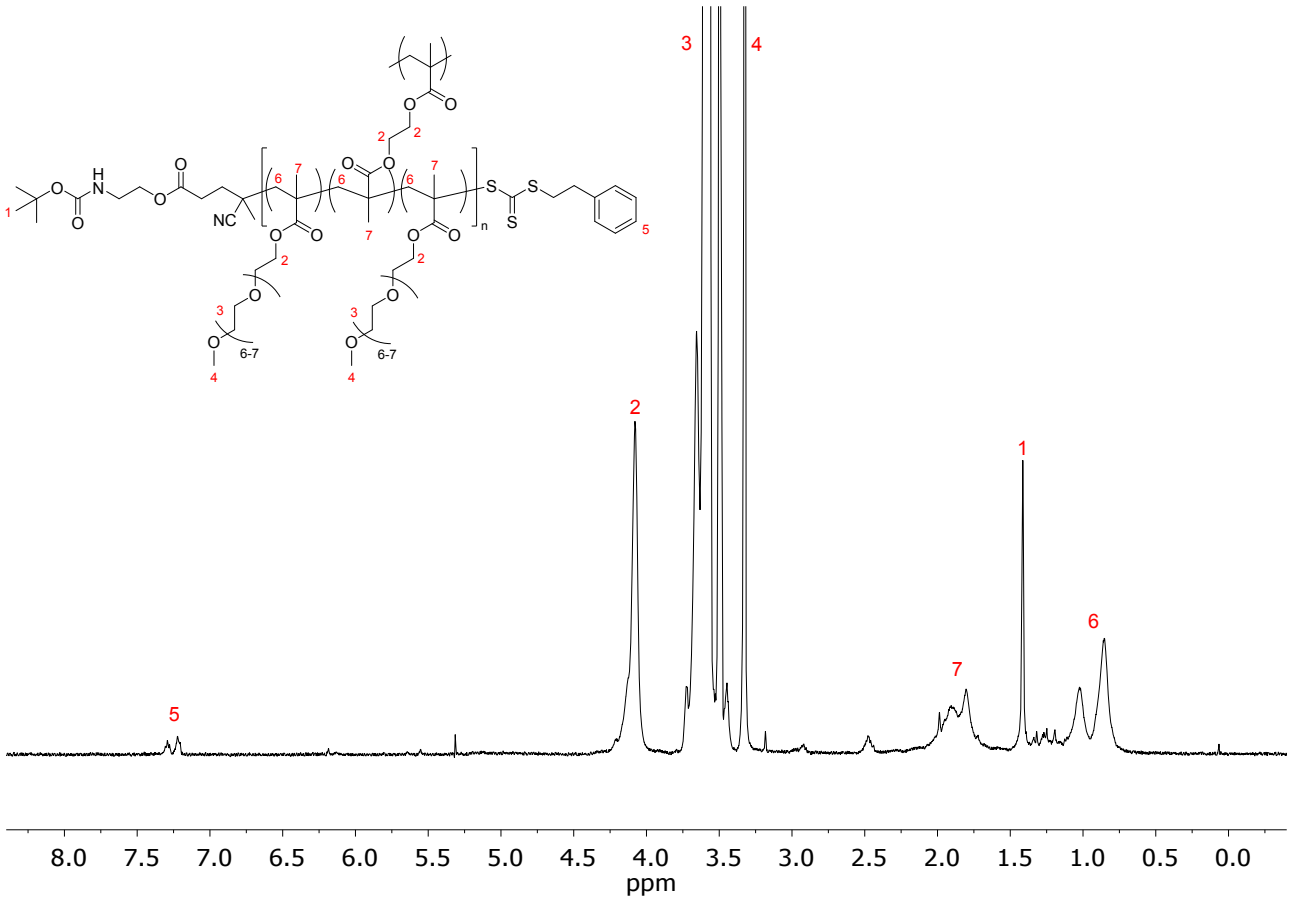

Figure S10. ^{1}H NMR spectrum of BOC-amine functional PEGMA HBP (**5**) (500 MHz, CD_2Cl_2).

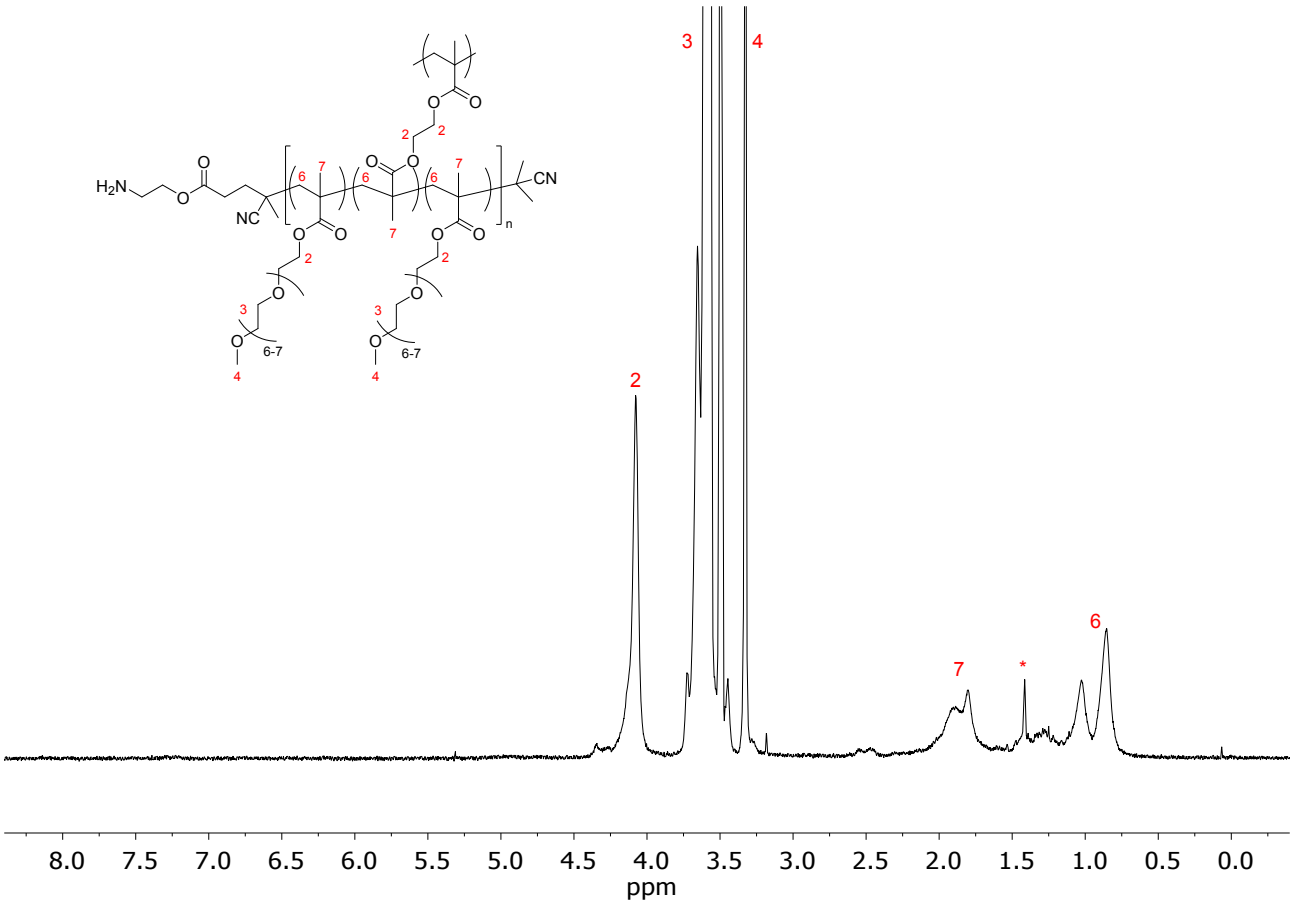

Figure S11. ^{1}H NMR spectrum of amine functional PEGMA HBP (**6**) (500 MHz, CD_2Cl_2). * - residual amount of BOC protection (16% by integration).

*UV-Vis spectroscopy of HBPs with externally attached fluorophores (7**A-D**)*

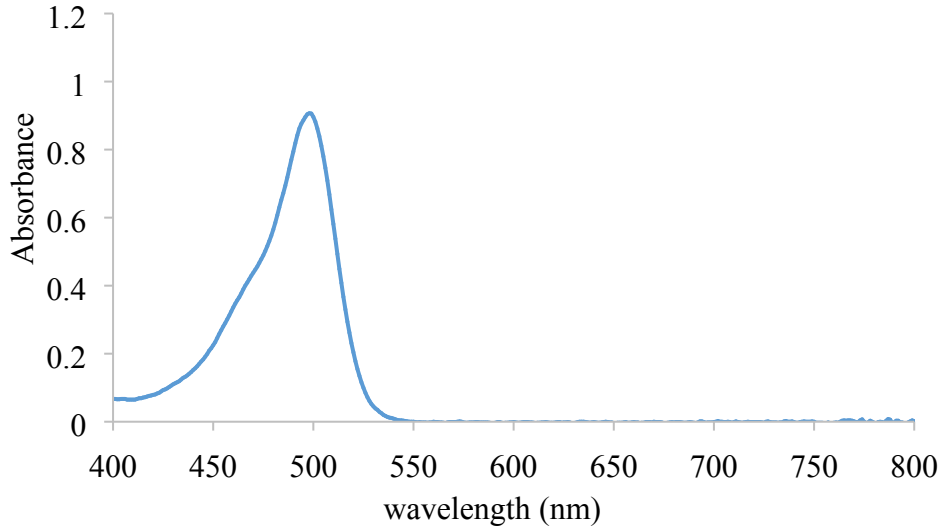

Figure S12. UV-Vis chromatogram of PEGMA-FAM (**7A**) in PBS at 3.0 mg/mL.

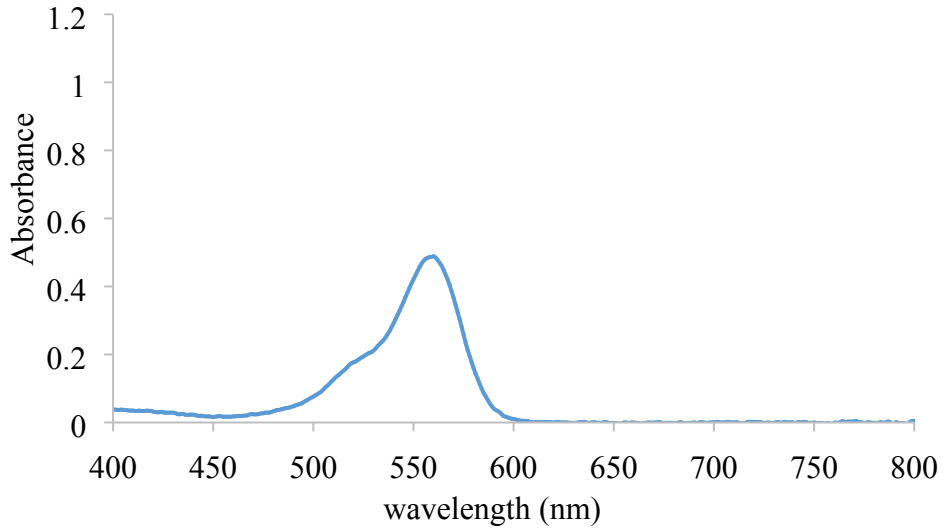

Figure S13. UV-Vis chromatogram of PEGMA-RhB (**7B**) in PBS at 5.0 mg/mL.

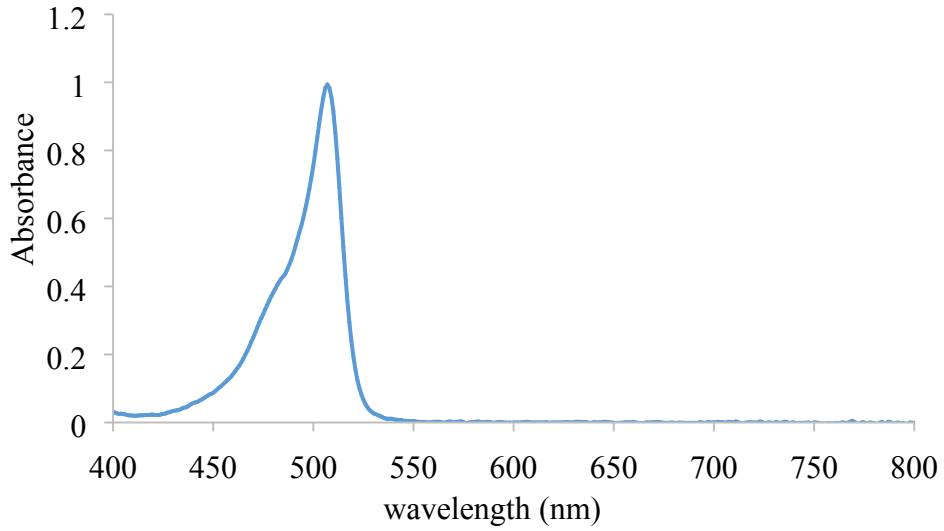

Figure S14. UV-Vis chromatogram of PEGMA-BDP-FL (**7C**) in PBS at 1.5 mg/mL.

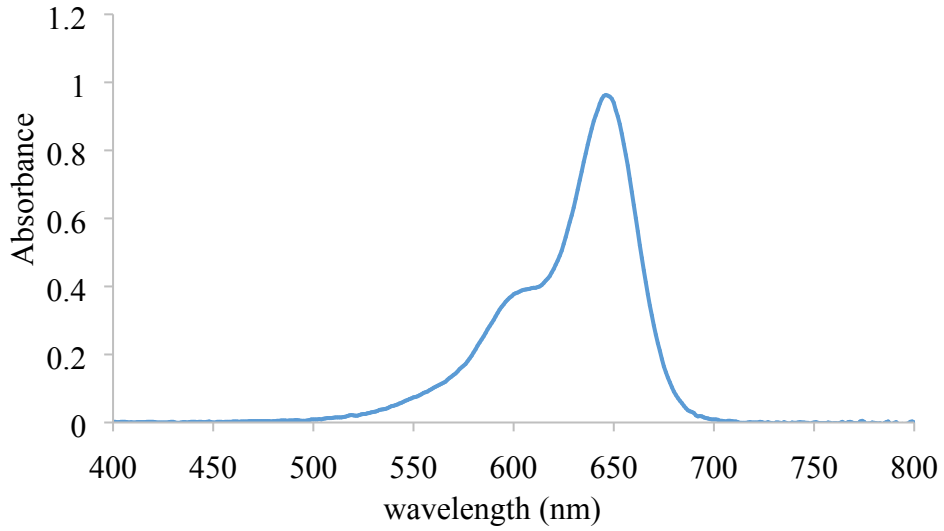

Figure S15. UV-Vis chromatogram of PEGMA-Cy5 (**7D**) in PBS at 0.4 mg/mL.

*PEGMA-co- FAM MA HBP (**8A**)*

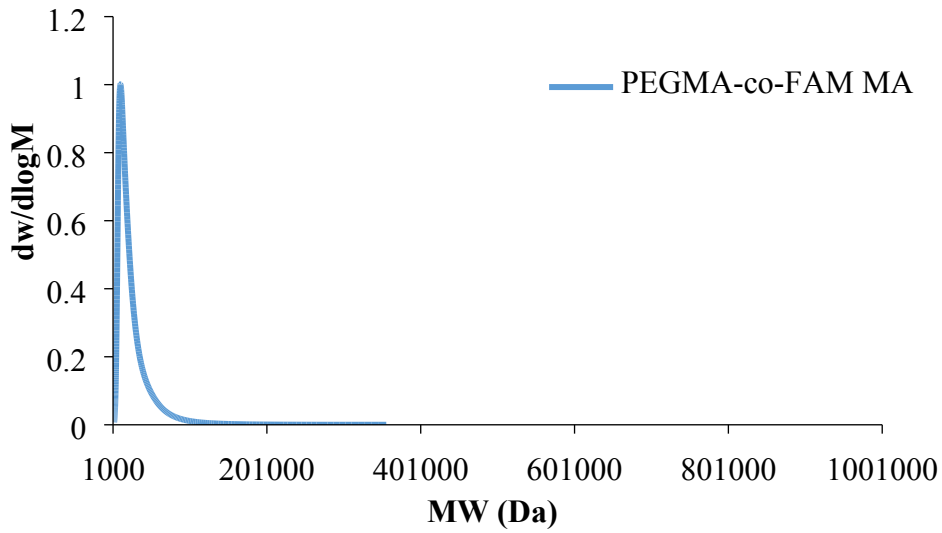

Figure S16. SEC chromatogram of PEGMA-*co*-FAM MA HBP (**8A**).

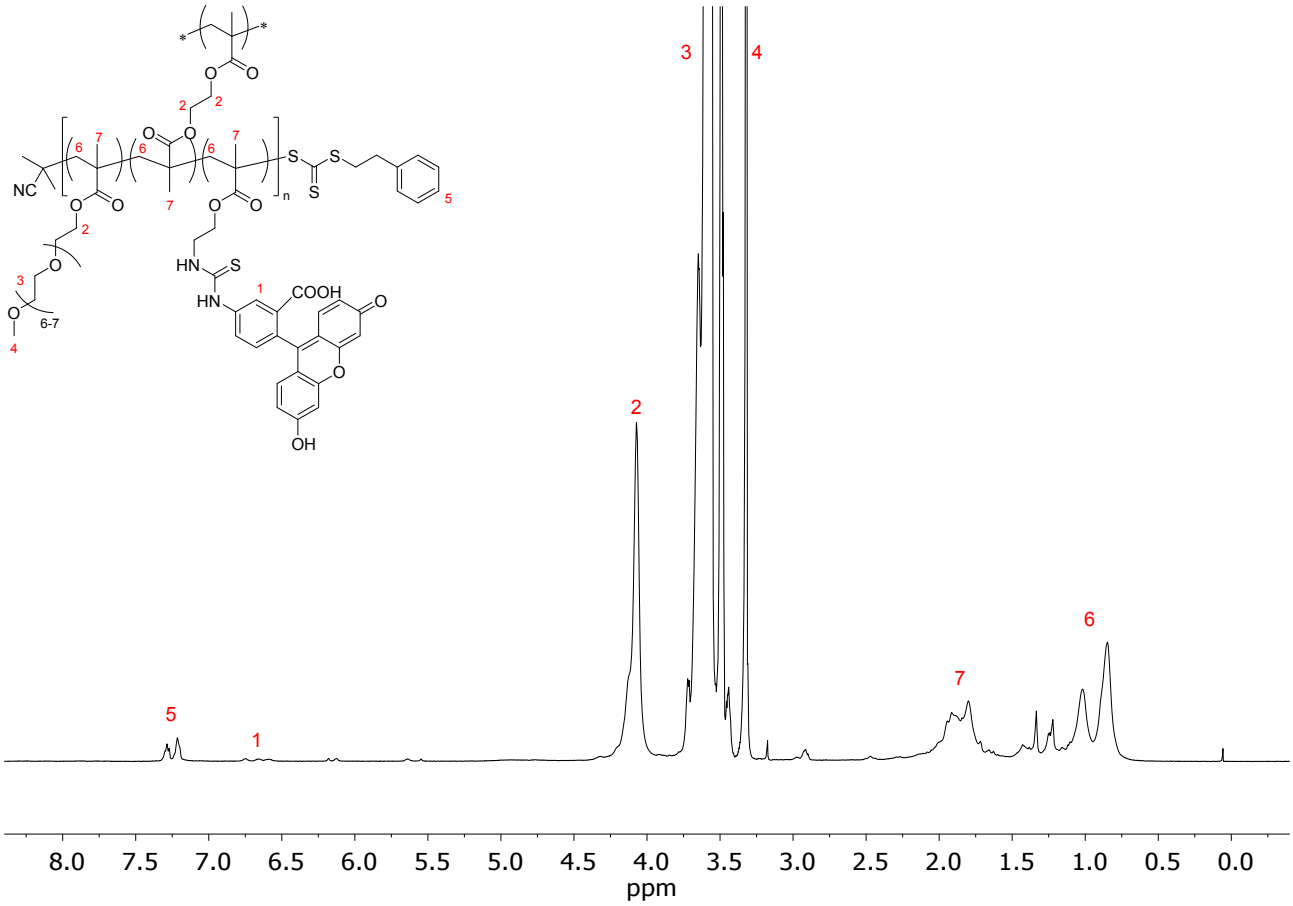

Figure S17. ^{1}H NMR spectrum of PEGMA-*co*-FAM MA HBP (**8A**) (500 MHz, CD_2Cl_2).

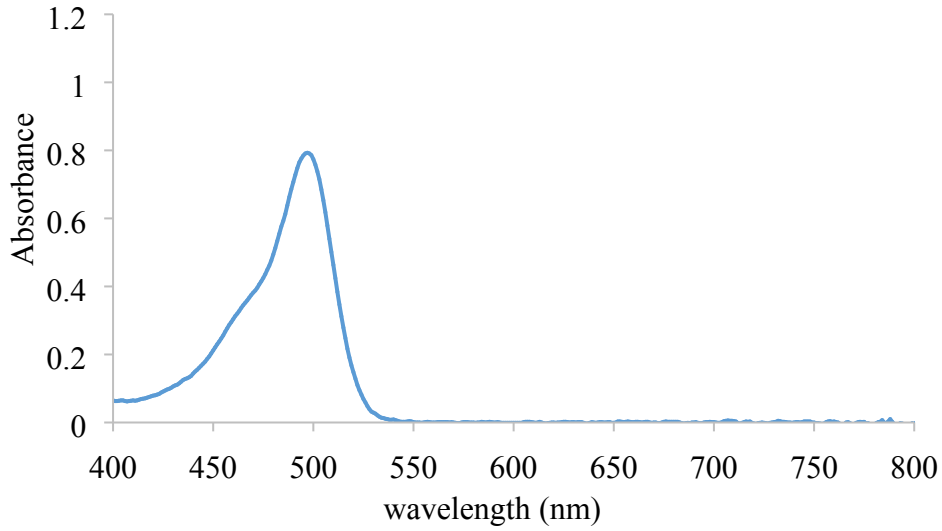

Figure S18. UV-Vis chromatogram of PEGMA-*co*-FAM MA (**8A**) in PBS at 2.2 mg/mL.

*PEGMA-co-RhB MA HBP (**8B**)*

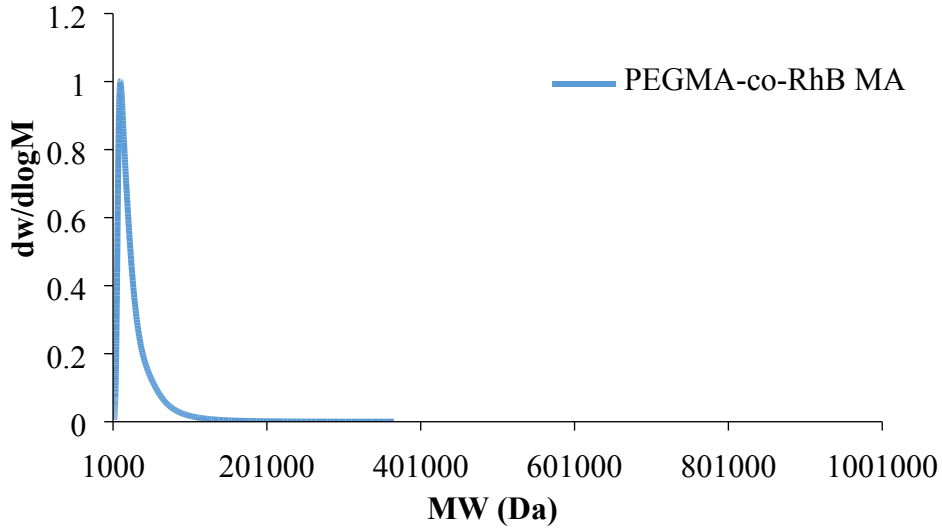

Figure S19. SEC chromatogram of PEGMA-*co*-RhB MA HBP (**8B**).

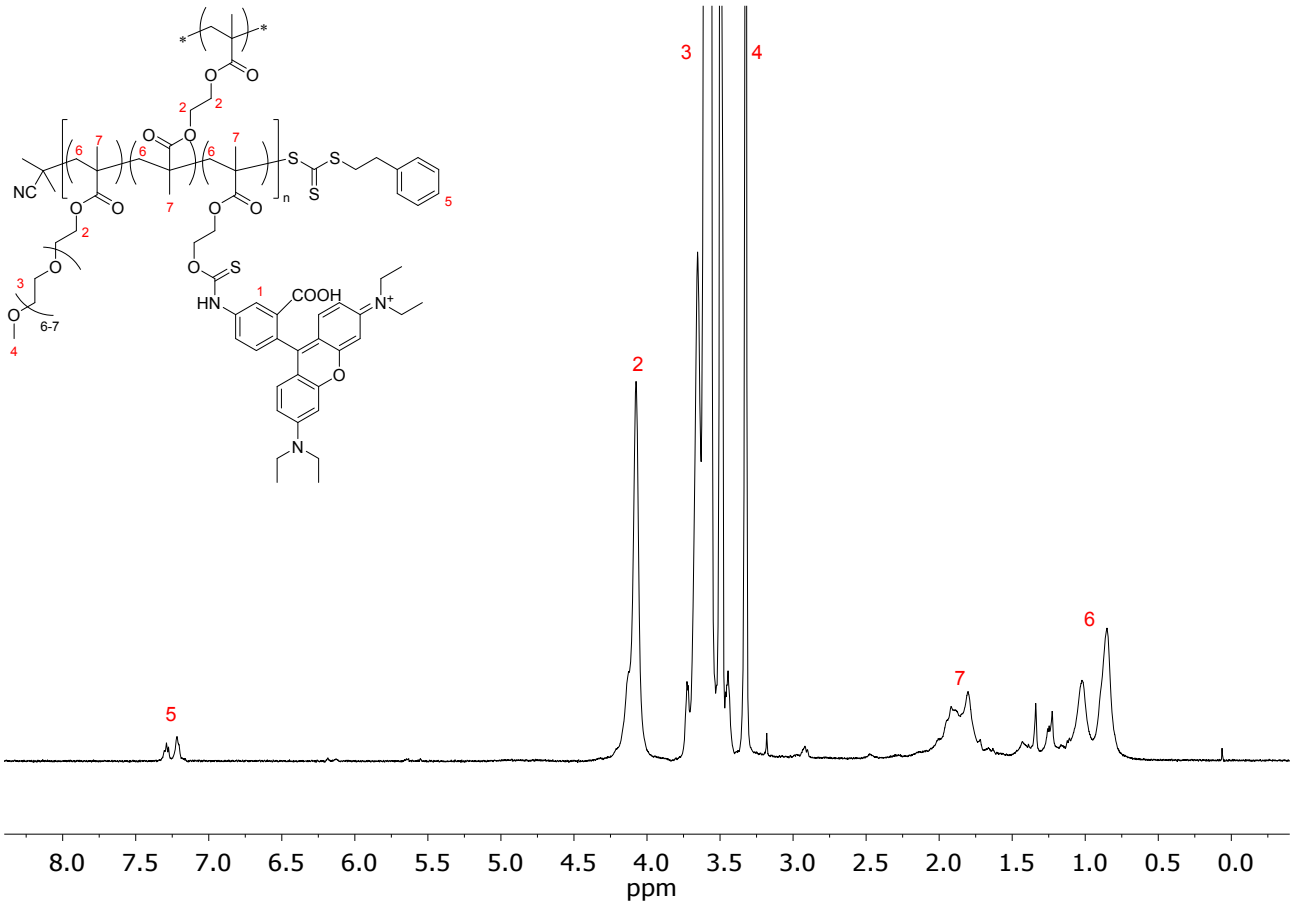

Figure S20. ^{1}H NMR spectrum of PEGMA-*co*-RhB MA HBP (**8B**) (500 MHz, CD_2Cl_2).

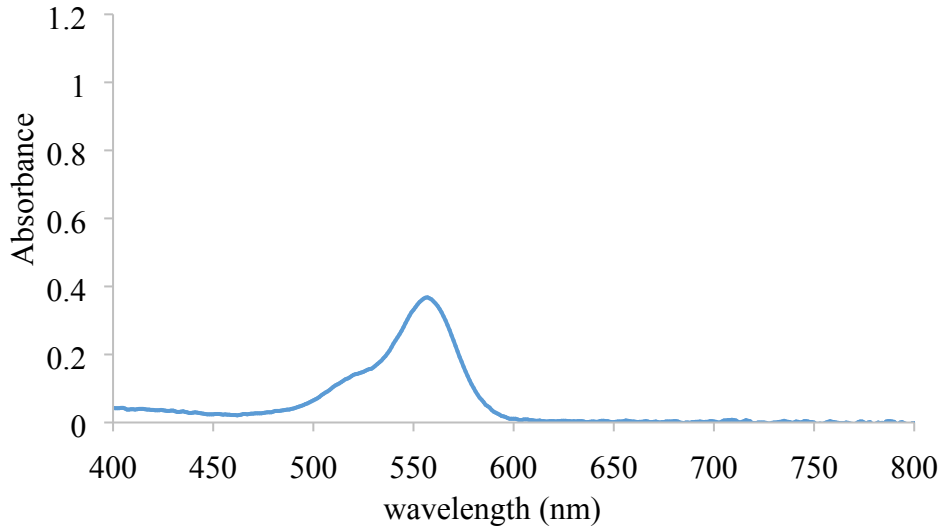

Figure S21. UV-Vis chromatogram of PEGMA-*co*-RhB MA (**8B**) in PBS at 5.3 mg/mL.

*PEGMA-co-BDP-FL MA HBP (**8C**)*

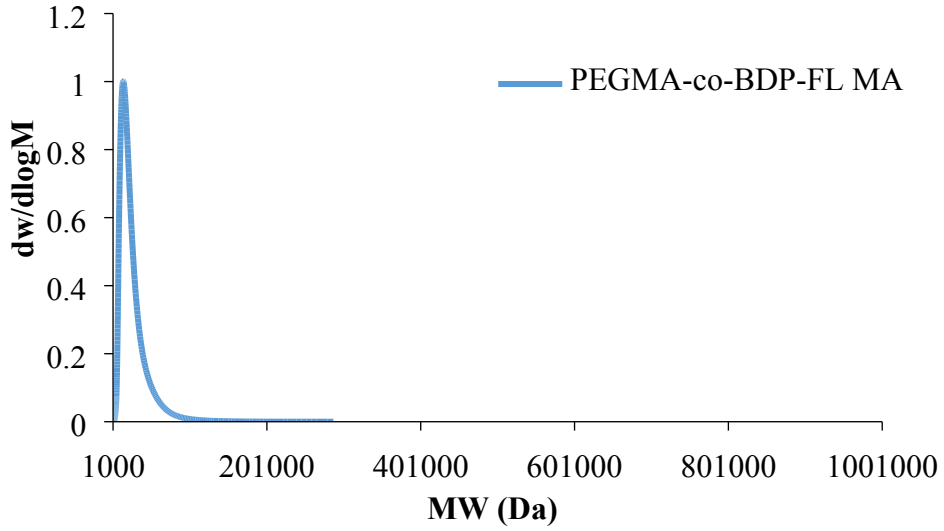

Figure S22. SEC chromatogram of PEGMA-*co*-BDP-FL MA HBP (**8C**).

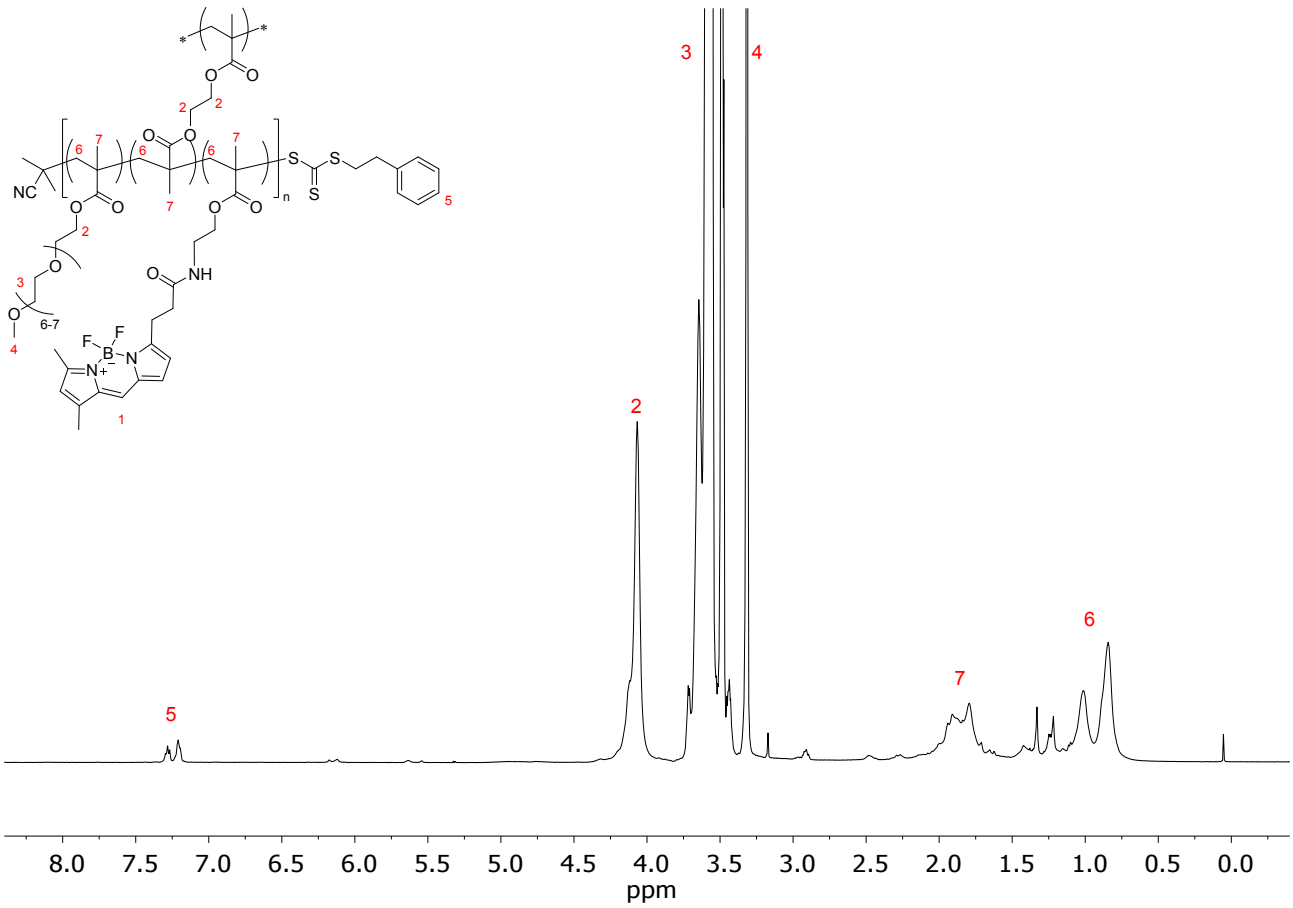

Figure S23. ^{1}H NMR spectrum of PEGMA-*co*-BDP-FL MA HBP (**8C**) (500 MHz, CD_2Cl_2).

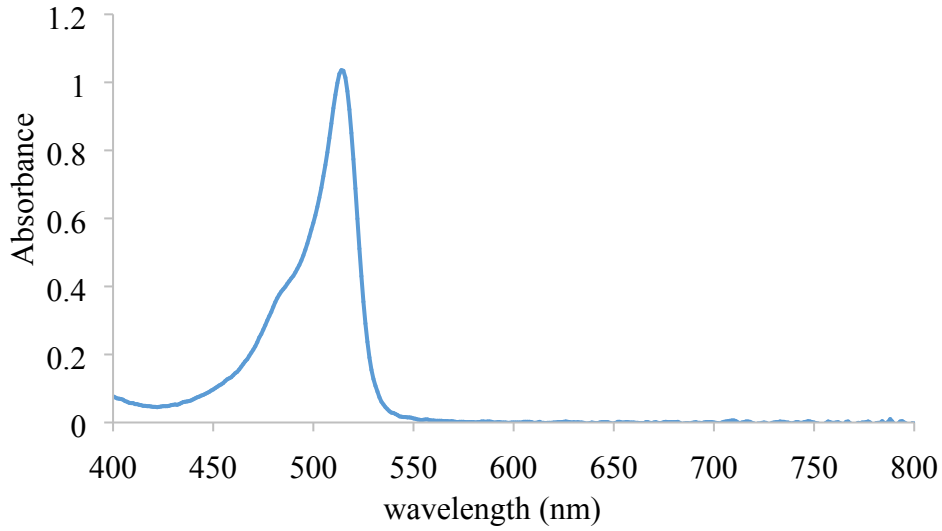

Figure S24. UV-Vis chromatogram of PEGMA-*co*-BDP-FL MA (**8C**) in PBS at 2.1 mg/mL.

*PEGMA-co-Cy5 MA HBP (**8D**)*

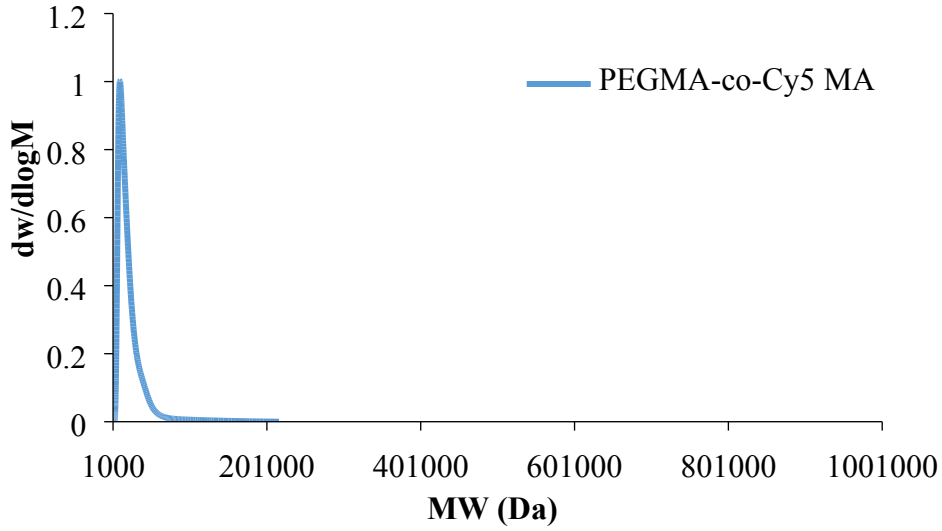

Figure S25. SEC chromatogram of PEGMA-*co*-Cy5 MA HBP (**8D**).

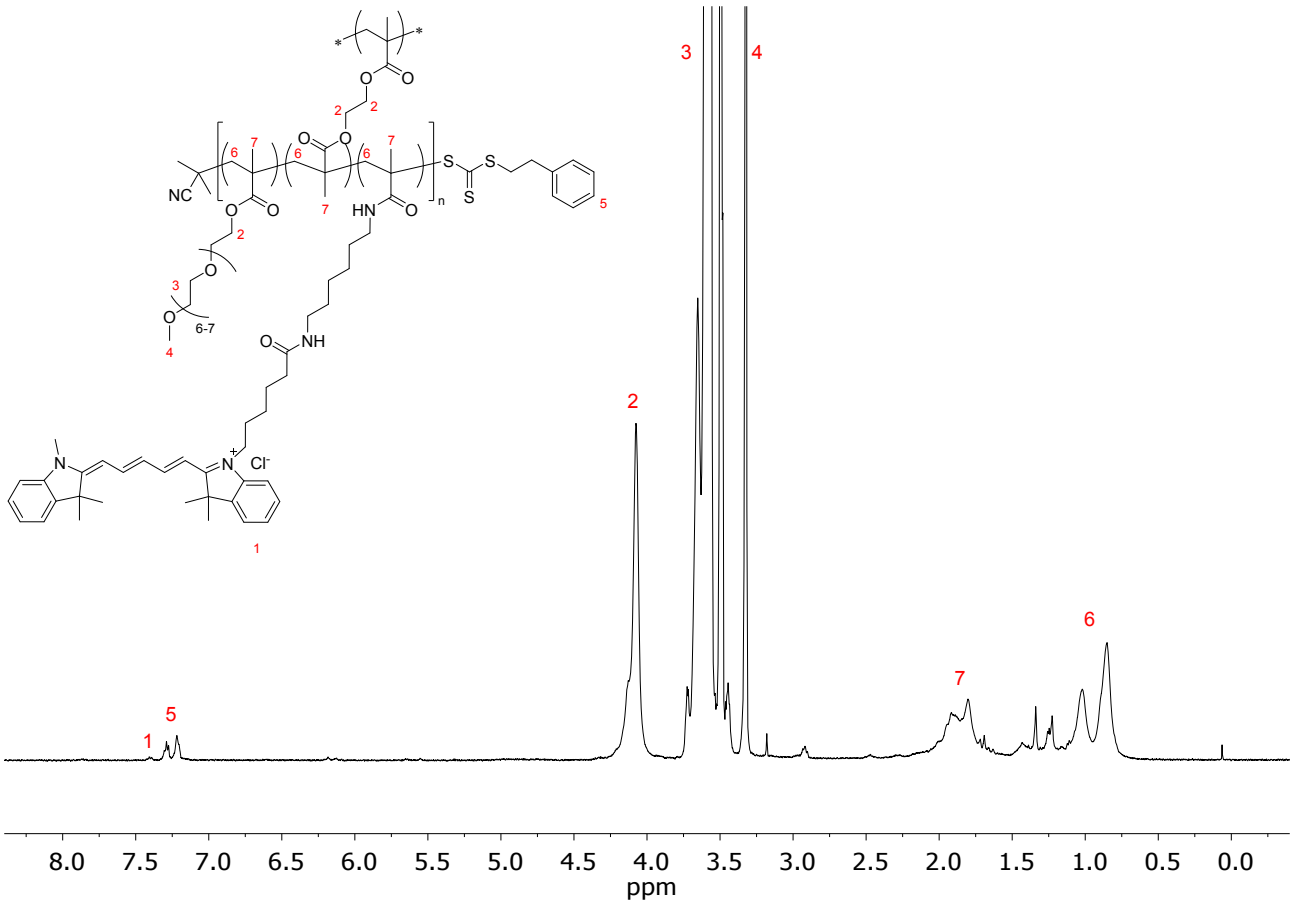

Figure S26. ^{1}H NMR spectrum of PEGMA-*co*-Cy5 MA HBP (**8D**) (500 MHz, CD_2Cl_2).

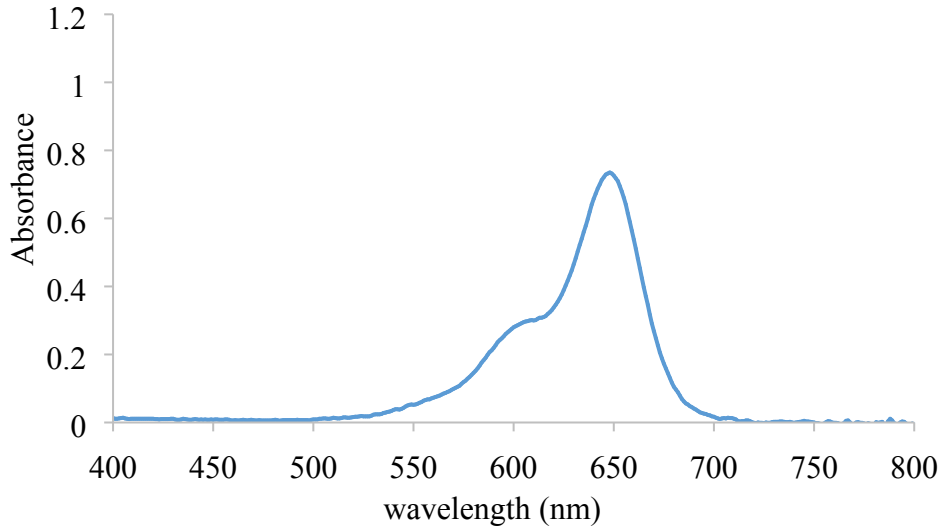

Figure S27. UV-Vis chromatogram of PEGMA-*co*-Cy5 MA (**8D**) in PBS at 1.0 mg/mL.

Table S1. Calculations to determine the labelling of fluorophore per particle.

	MW	conc (mg/mL)	conc (M)	A	e	conc (fluoro)	ratio (fluoro/polymer)
FL	30300	3	9.90E-05	0.907	73000	1.24E-05	0.13
Rh	30300	5	1.65E-04	0.489	106000	4.61E-06	0.03
BDP	30300	1.5	4.95E-05	0.994	80000	1.24E-05	0.25
Cy5	30300	0.4	1.32E-05	0.963	250000	3.85E-06	0.29
FITC int	26700	2.2	8.24E-05	0.793	73000	1.09E-05	0.13
RITC int	51300	5.3	1.03E-04	0.368	106000	3.47E-06	0.03
BDP int	58900	2.1	3.57E-05	1.036	80000	1.30E-05	0.36
Cy5 int	58700	1	1.70E-05	0.735	250000	2.94E-06	0.17

Additional Fluorophore Internalization Comparison

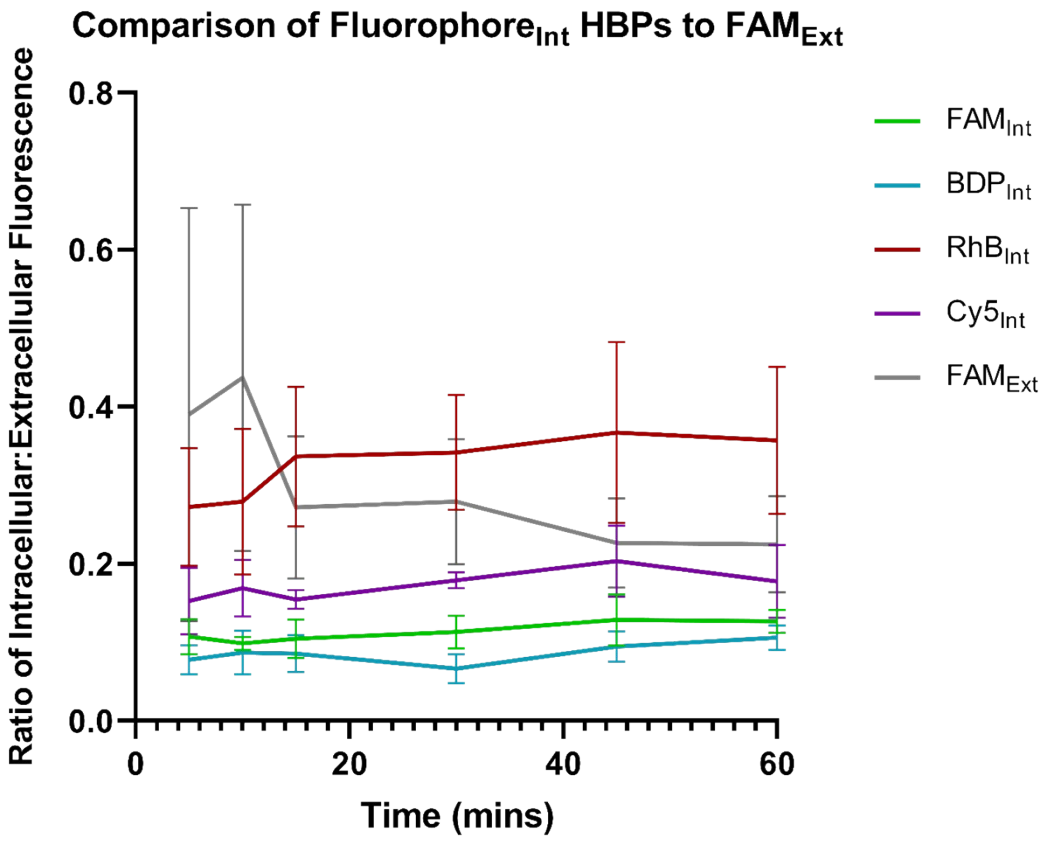

Figure S28. Internalization rates of co-polymerized fluorophore HBPs to chain-end modified reacted fluorescein derivative.

Flow cytometry of PEGMA HBPs that differ by fluorophore and labelling strategy

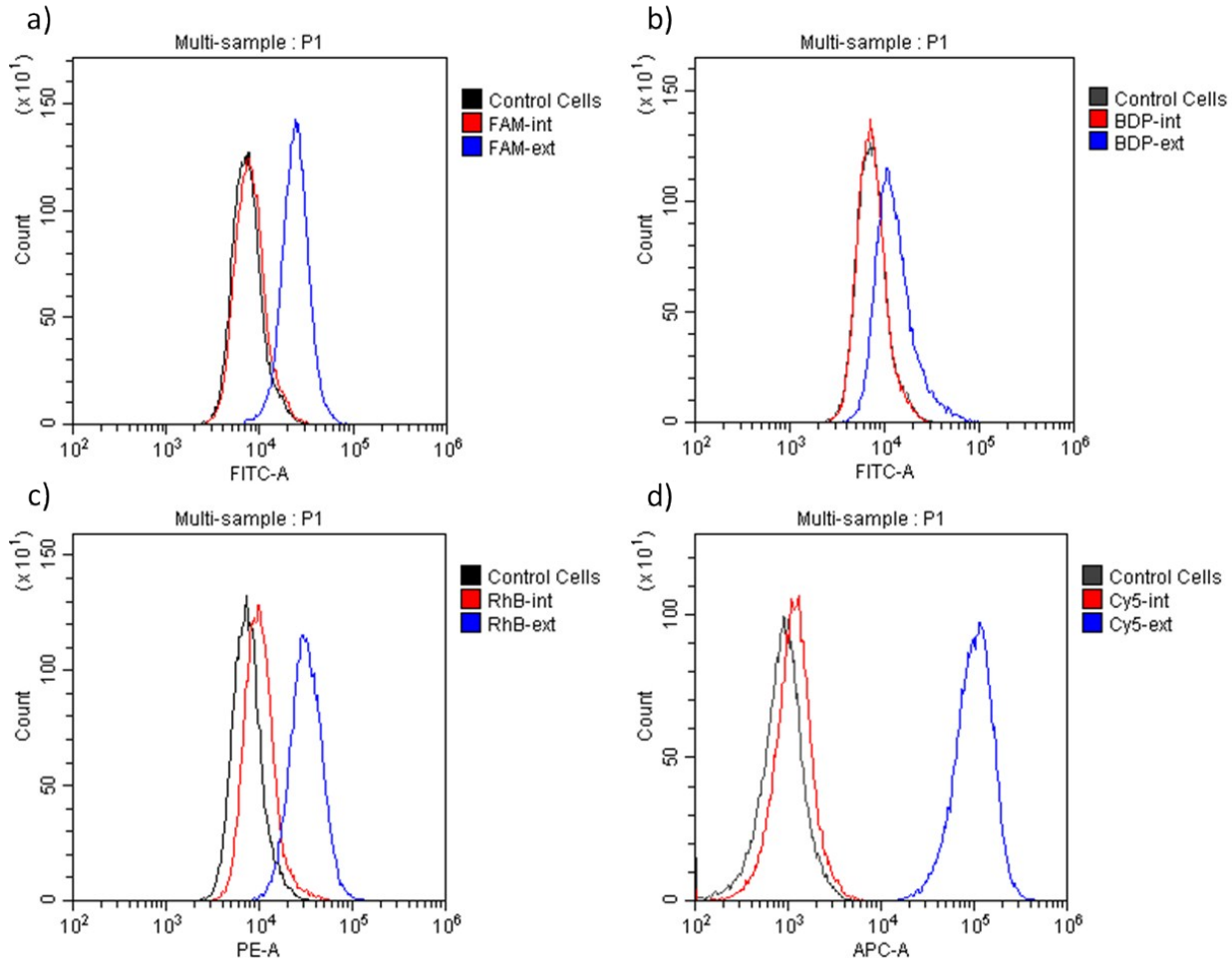

Figure S29. Cellular association of fluorophore HBPs. (a) fluorescein derivatives (b) BODIPY derivatives (c) Rhodamine B derivatives and (d) Cyanine-5 derivatives. Black lines represent unstained control cells, red shows the distribution of internal fluorescent monomer derivatives and the materials labelled to produce external fluorophore HBPs by post-synthesis modification are shown in blue.

Additional Confocal Data

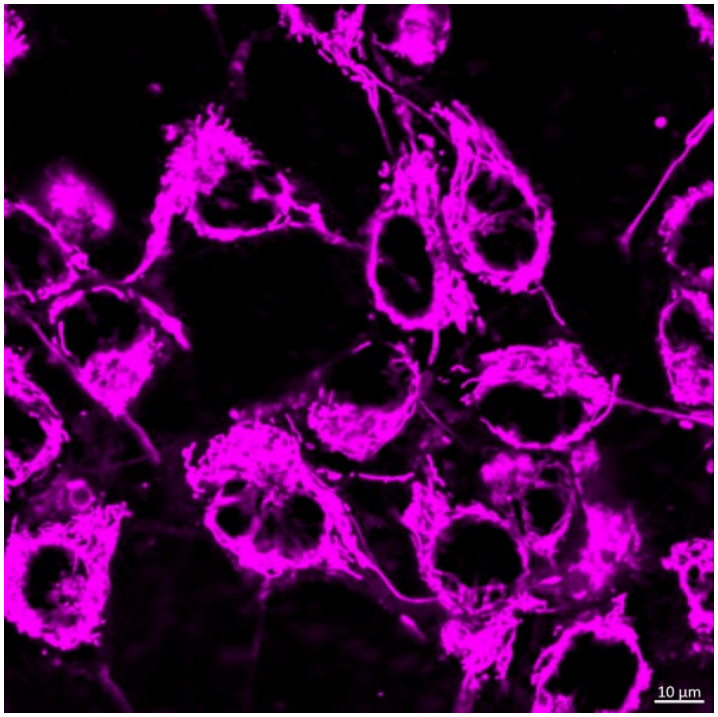

Figure S30. Confocal micrograph of live MDA-MB-468 cells exposed to free Cyanine-5 dye, note the mitochondrial staining.

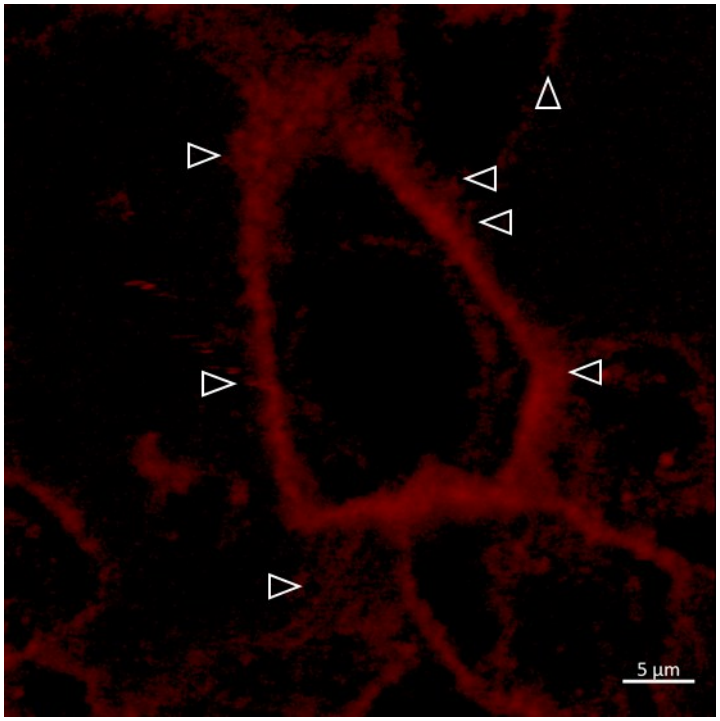

Figure S31. Confocal micrograph of live MDA-MB-468 cells exposed to EGFR targeted-RhB_{Ext} adjacent the coverslip at 15 min, showing filopodial staining (marked with arrowheads). Rhodamine fluorescence (Red), scale bar as labelled.

Minimum Information Reporting in Bio–Nano Experimental Literature

Checklist

Minimum Information Reporting in Bio–Nano Experimental Literature

The MIRIBEL guidelines were introduced here: https://doi.org/10.1038/s41565-018-0246-4

The development of these guidelines was led by the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology: https://www.cbns.org.au/. Any updates or revisions to this document will be made available here: http://doi.org/10.17605/OSF.IO/SMVTF.

The MIRIBEL guidelines were developed to facilitate reporting and dissemination of research in bio–nano science. Their development was inspired by various similar efforts:

- MIAME (microarray experiments): *Nat. Genet.* **29** (2001), 365; http://doi.org/10.1038/ng1201-365
- MIRIAM (biochemical models): *Nat. Biotechnol.* **23** (2005) 1509; http://doi.org/10.1038/nbt1156
- MIBBI (biology/biomedicine): *Nat. Biotechnol.* **26** (2008) 889; http://doi.org/10.1038/nbt.1411
- MIGS (genome sequencing): *Nat. Biotechnol.* **26** (2008) 541; http://doi.org/10.1038/nbt1360
- MIQE (quantitative PCR): *Clin. Chem.* **55** (2009) 611; http://doi.org/10.1373/clinchem.2008.112797
- ARRIVE (animal research): *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412
- *Nature*'s reporting standards:
    - Life science: https://www.nature.com/authors/policies/reporting.pdf; e.g., *Nat. Nanotechnol.* **9** (2014) 949; http://doi.org/10.1038/nnano.2014.287
    - Solar cells: https://www.nature.com/authors/policies/solarchecklist.pdf; e.g., *Nat. Photonics* **9** (2015) 703; http://doi.org/10.1038/nphoton.2015.233
    - Lasers: https://www.nature.com/authors/policies/laserchecklist.pdf; e.g., *Nat. Photonics* **11** (2017) 139; http://doi.org/10.1038/nphoton.2017.28
- The "TOP guidelines": e.g., *Science* **352** (2016) 1147; http://doi.org/10.1126/science.aag2359

Similar to many of the efforts listed above, the parameters included in this checklist are not intended to be definitive requirements; instead they are intended as 'points to be considered', with authors themselves deciding which parameters are—and which are not—appropriate for their specific study.

This document is intended to be a living document, which we propose is revisited and amended annually by interested members of the community, who are encouraged to contact the authors of this document. Parts of this document were developed at the annual International Nanomedicine Conference in Sydney, Australia: http://www.oznanomed.org/, which will continue to act as a venue for their review and development, and interested members of the community are encouraged to attend.

After filling out the following pages, this checklist document can be attached as a "Supporting Information" document during submission of a manuscript to inform Editors and Reviewers (and eventually readers) that all points of MIRIBEL have been considered.

Table S2. Material characterization*

Question	Yes	No
1.1 Are "**best reporting practices**" **available** for the nanomaterial used? For examples, see *Chem. Mater.* **28** (2016) 3535; http://doi.org/10.1021/acs.chemmater.6b01854 and *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235	Y	
1.2 If they are available, **are they used**? If not available, ignore this question and proceed to the next one.		
1.3 Are extensive and clear instructions reported detailing all steps of **synthesis** and the resulting **composition** of the nanomaterial? For examples, see *Chem. Mater.* **26** (2014) 1765; http://doi.org/10.1021/cm500632c, and *Chem. Mater.* **26** (2014) 2211; http://doi.org/10.1021/cm5010449. Extensive use of photos, images, and videos are strongly encouraged. For example, see *Chem. Mater.* **28** (2016) 8441; http://doi.org/10.1021/acs.chemmater.6b04639	Y	
1.4 Is the **size** (or **dimensions**, if non-spherical) and **shape of** the nanomaterial reported?	Y	
1.5 Is the **size dispersity** or **aggregation** of the nanomaterial reported?	Y	
1.6 Is the **zeta potential** of the nanomaterial reported?	Y	
1.7 Is the **density (mass/volume)** of the nanomaterial reported?		N/A
1.8 Is the amount of any **drug loaded** reported? 'Drug' here broadly refers to functional cargos (e.g., proteins, small molecules, nucleic acids).	Y	
1.9 Is the **targeting performance** of the nanomaterial reported, including **amount** of ligand bound to the nanomaterial if the material has been functionalised through addition of targeting ligands?		N/A
1.10 Is the **label signal** per nanomaterial/particle reported? For example, fluorescence signal per particle for fluorescently labelled nanomaterials.		N/A
1.11 If a material property not listed here is varied, has it been **quantified**?	Y	
1.12 Were characterizations performed in a **fluid mimicking biological conditions**?		N/A
1.13 Are details of how these parameters were **measured/estimated** provided?	Y	
Explanation for **No** (if needed): Many of the components of the checklist are not relevant to this study. 1.7 Not applicable to PEG-based HBPs. 1.10 Labelling efficiency is described. 1.12 Materials were characterised in the solvents indicated as biological conditions would interfere with measurements.		

Table S3. Biological characterization*

Question	Yes	No
2.1 Are **cell seeding details**, including **number of cells plated, confluency at start of experiment,** and **time between seeding and experiment** reported?	Y	
2.2 If a standardised cell line is used, are the **designation and source** provided?	Y	
2.3 Is the **passage number** (total number of times a cell culture has been subcultured) known and reported?		N
2.4 Is the last instance of **verification of cell line** reported? If no verification has been performed, is the time passed and passage number since acquisition from trusted source (e.g., ATCC or ECACC) reported? For information, see *Science* **347** (2015) 938; http://doi.org/10.1126/science.347.6225.938		N
2.5 Are the results from **mycoplasma testing** of cell cultures reported?		N
2.6 Is the **background signal of cells**/**tissue** reported? (E.g., the fluorescence signal of cells without particles in the case of a flow cytometry experiment.)		N/A
2.7 Are **toxicity studies** provided to demonstrate that the material has the expected toxicity, and that the experimental protocol followed does not?		N/A
2.8 Are details of media preparation (**type of media, serum,** any **added antibiotics**) provided?	Y	
2.9 Is a **justification of the biological model** used provided? For examples for cancer models, see *Cancer Res.* **75** (2015) 4016; http://doi.org/10.1158/0008-5472.CAN-15-1558, and *Mol. Ther.* **20** (2012) 882; http://doi.org/10.1038/mt.2012.73, and *ACS Nano* **11** (2017) 9594; http://doi.org/10.1021/acsnano.7b04855		N
2.10 Is characterization of the **biological fluid** (*ex vivo*/*in vitro*) reported? For example, when investigating protein adsorption onto nanoparticles dispersed in blood serum, pertinent aspects of the blood serum should be characterised (e.g., protein concentrations and differences between donors used in study).		N/A
2.11 For **animal experiments**, are the ARRIVE guidelines followed? For details, see *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412		N/A
Explanation for **No** (if needed): 2.3 & 2.4 The cells were between passages 20-25 since receipt from ATCC. 2.5 Cells were mycoplasma tested regularly and were last reported as negative as of 12/04/2019; these experiments predate that time. 2.6 Cell autofluorescence was not pertinent, being accounted for during experimental set-up. 2.7 Toxicity studies were not relevant to this study. 2.9 The cell line (MDA-MB-468) was selected due to being a common EGFR+ breast cancer model.		

*For *in vitro* experiments (e.g., cell culture), *ex vivo* experiments (e.g., in blood samples), and *in vivo* experiments (e.g., animal models). The questions above that are appropriate depend on the type of experiment conducted.

Table S4. Experimental details*

Question	Yes	No
3.1 For cell culture experiments: are **cell culture dimensions** including **type of well**, **volume of added media**, reported? Are cell types (i.e.; adherent vs suspension) and **orientation** (if non-standard) reported?		N
3.2 Is the **dose of material administered** reported? This is typically provided in nanomaterial mass, volume, number, or surface area added. Is sufficient information reported so that regardless of which one is provided, the other dosage metrics can be calculated (i.e. using the dimensions and density of the nanomaterial)?	Y	
3.3 For each type of imaging performed, are details of how **imaging** was performed provided, including details of **shielding, non-uniform image processing**, and any **contrast agents** added?		N/A
3.4 Are details of how the dose was administered provided, including **method of administration, injection location, rate of administration,** and details of **multiple injections**?		N/A
3.5 Is the methodology used to **equalise dosage** provided?		N/A
3.6 Is the **delivered dose** to tissues and/or organs (in vivo) reported, as % injected dose per gram of tissue (%ID g^{-1})?		N/A
3.7 Is **mass of each organ/tissue measured** and **mass of material** reported?		N/A
3.8 Are the **signals of cells/tissues with nanomaterials** reported? For instance, for fluorescently labelled nanoparticles, the total number of particles per cell or the fluorescence intensity of particles + cells, at each assessed timepoint.	Y	
3.9 Are **data analysis details**, including **code used** for analysis provided?	Y	
3.10 Is the **raw data** or **distribution of values** underlying the reported results provided? For examples, see *R. Soc. Open Sci.* **3** (2016) 150547; http://doi.org/10.1098/rsos.150547, https://opennessinitiative.org/making-your-data-public/, http://journals.plos.org/plosone/s/data-availability, and https://www.nature.com/sdata/policies/repositories		N/A
Explanation for **No** (if needed): 3.1 The μ-dishes reported were 35 mm width and the final volume for experimental procedures was 1 mL. MDA-MB-468 cells are an adherent cell line. 3.3-3.7 No animal imaging performed.		

* The use of protocol repositories (e.g., *Protocol Exchange* http://www.nature.com/protocolexchange/) and published standard methods and protocols (e.g., *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235, and *Chem. Mater.* **29** (2017) 475; http://doi.org/10.1021/acs.chemmater.6b05481) are encouraged.