

Grafting of Cyclodextrin to Theranostic Nanoparticles Improve Blood-Brain Barrier Model Crossing

Antonino Puglisi ^{1,*}, Noemi Bognanni ^{1,2}, Graziella Vecchio ², Ece Bayir ³, Peter van Oostrum ¹, Dawn Shepherd ⁴, Frances Platt ⁴ and Erik Reimhult ¹

¹ Department of Bionanosciences, Institute of Biologically Inspired Materials, University of Natural Resources and Life Sciences (BOKU), 1190 Vienna, Austria

² Dipartimento di Scienze Chimiche, Università degli Studi di Catania, 95125 Catania, Italy

³ Central Research Testing and Analysis Laboratory Research and Application Center, Ege University Bornova, Izmir 35100, Turkey

⁴ Department of Pharmacology, University of Oxford, Oxford OX1 3QT, UK

* Correspondence: antonino.puglisi@boku.ac.at

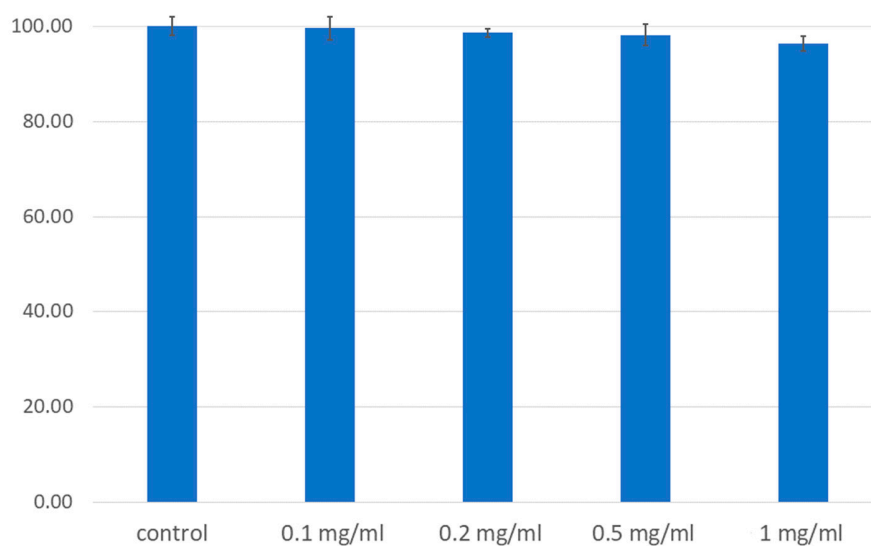


Figure S1-Resazurin viability assay on *Npc1*-deficient CHO cells after 24 h incubation with different high concentrations of CySPION and 48 h regeneration compared to a control, showing no significant cytotoxicity.

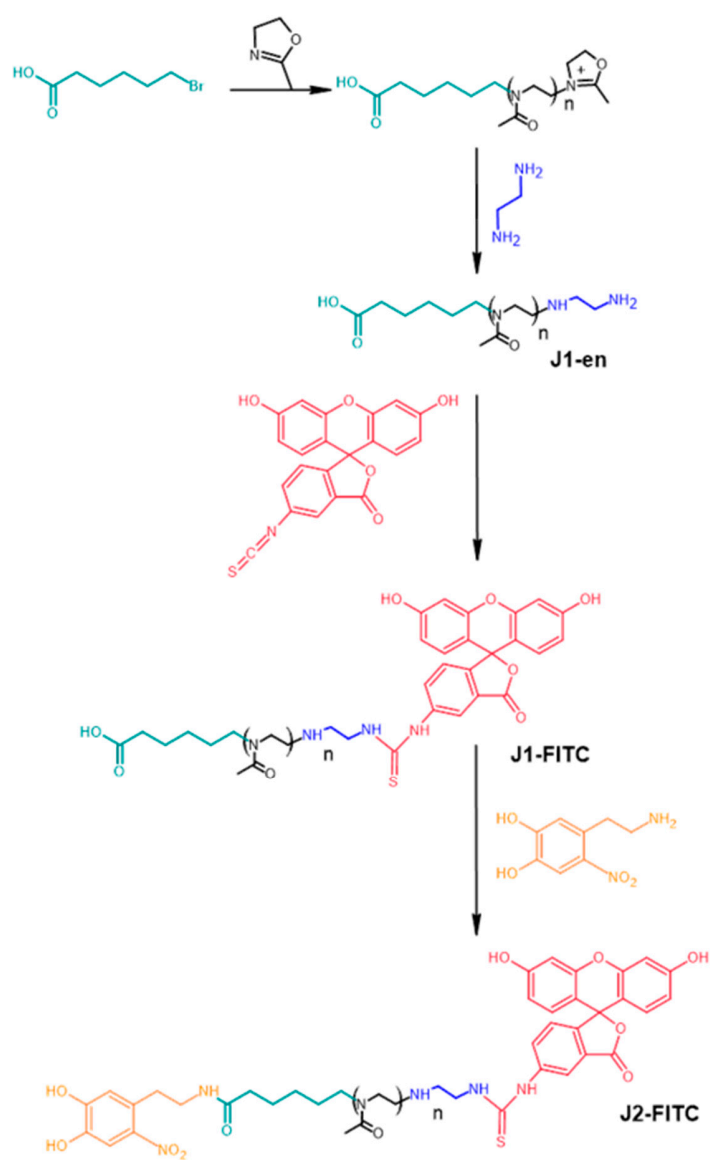


Figure S2-General scheme of synthesis of the fluorescein-labelled polymer

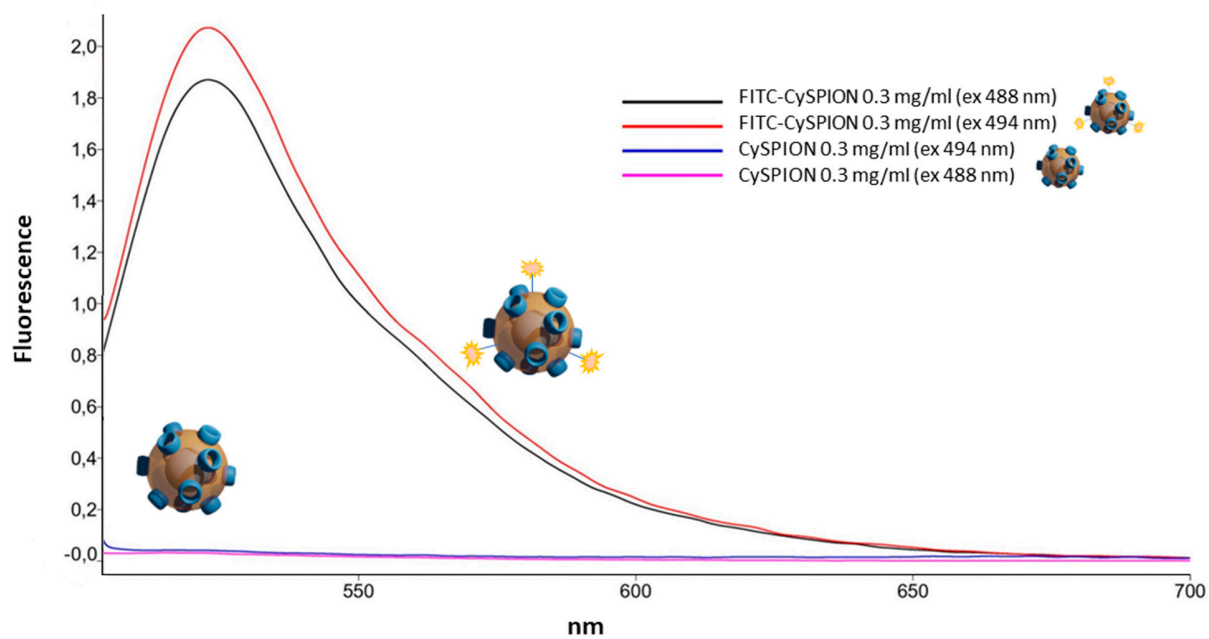


Figure S3 Fluorescence of CySPION and FITC- CySPION at 488 nm and 494 nm

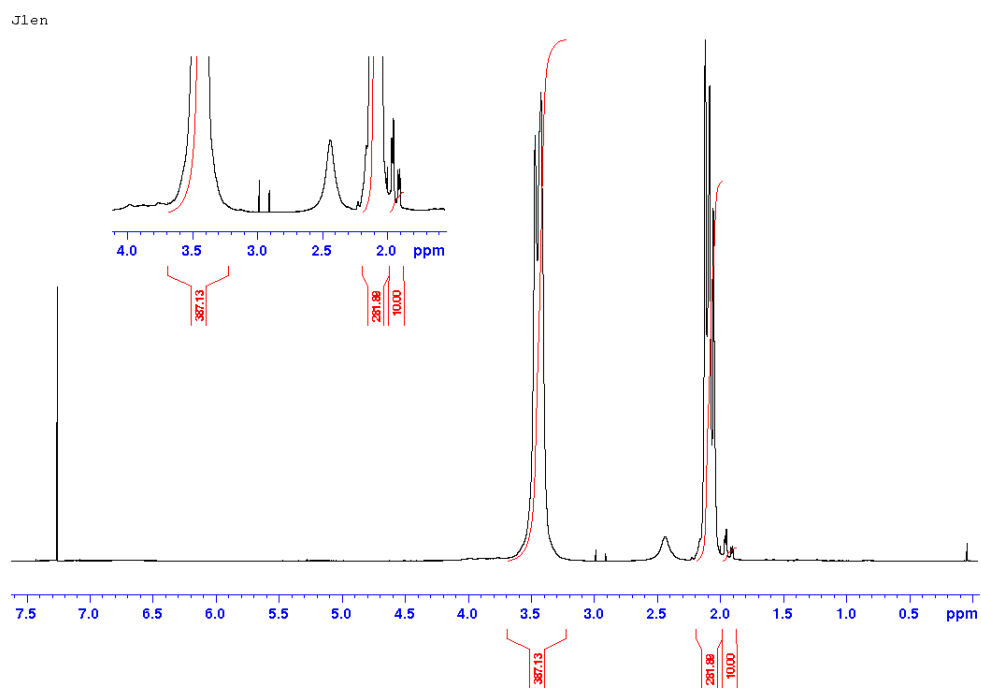


Figure S4 - ^1H NMR (300 MHz) of J1-en in CDCl_3

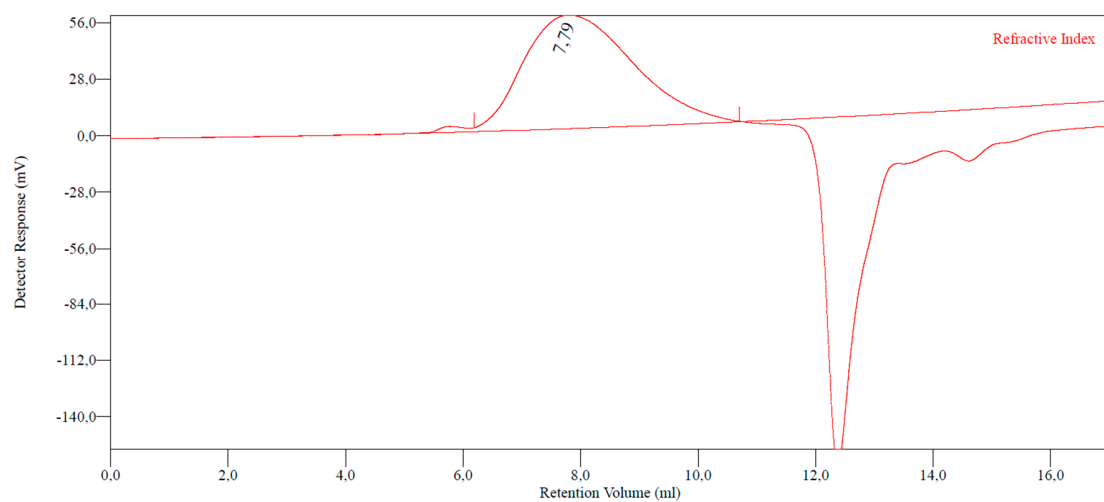


Figure S5 - GPC of J1-en

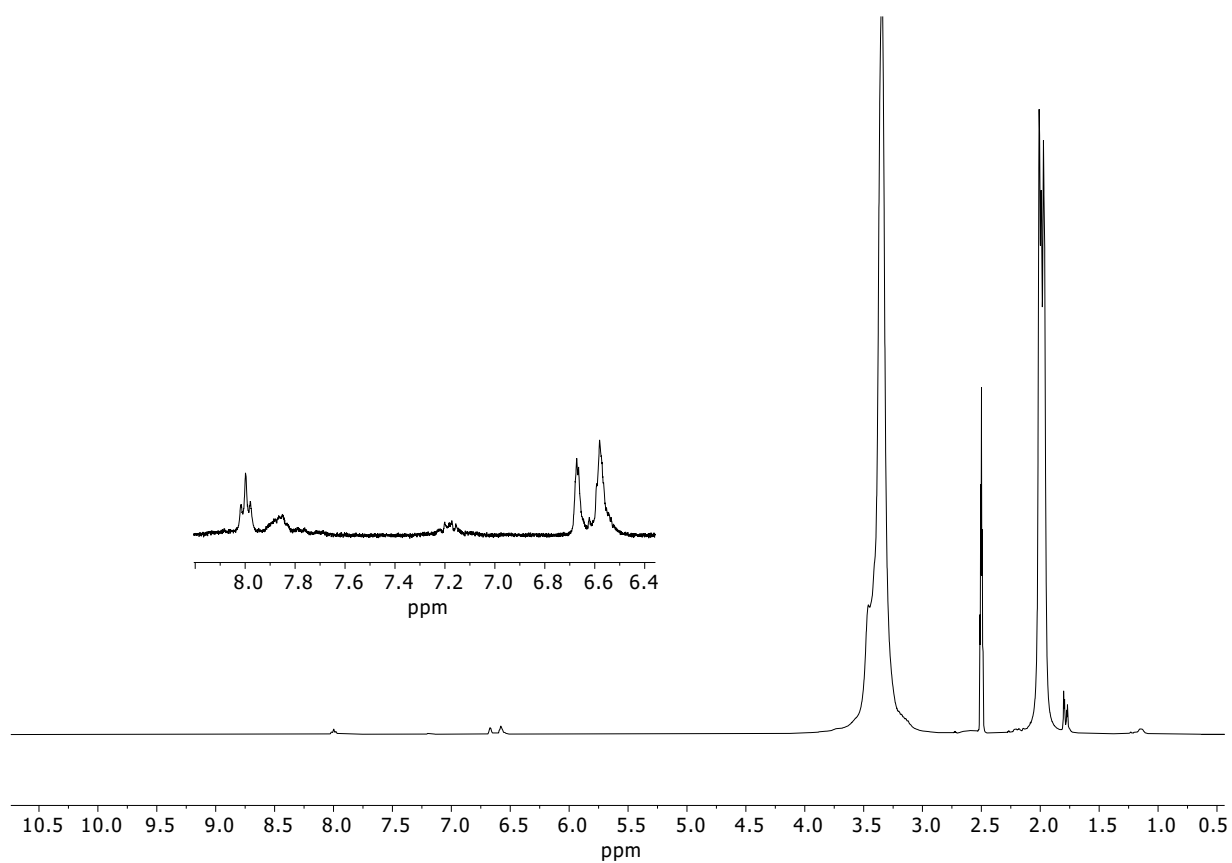


Figure S6 - ^1H NMR (300 MHz) of J1-FITC in DMSO

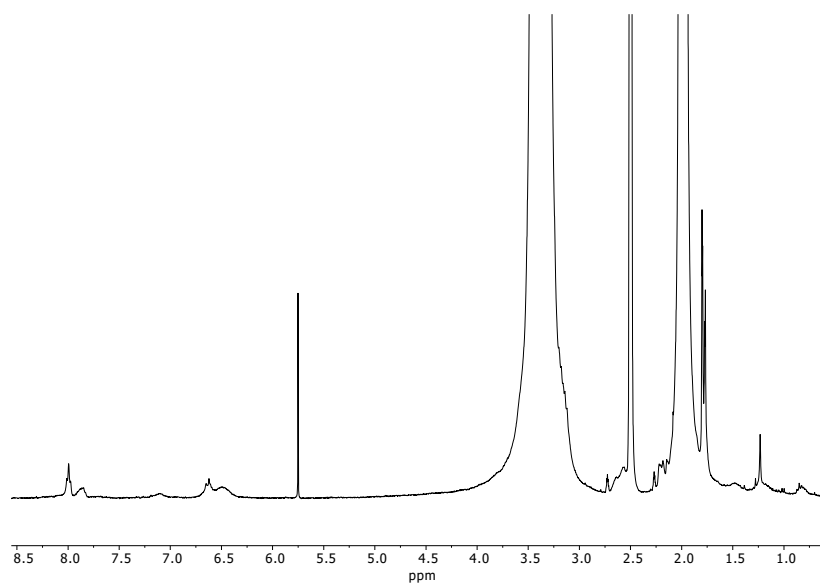


Figure S7 - ^1H NMR (300 MHz) of FITC-PMOXA in DMSO

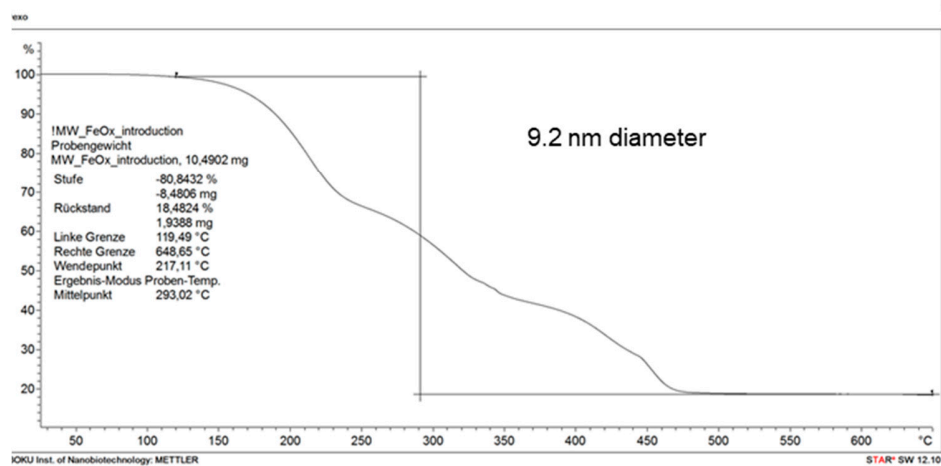
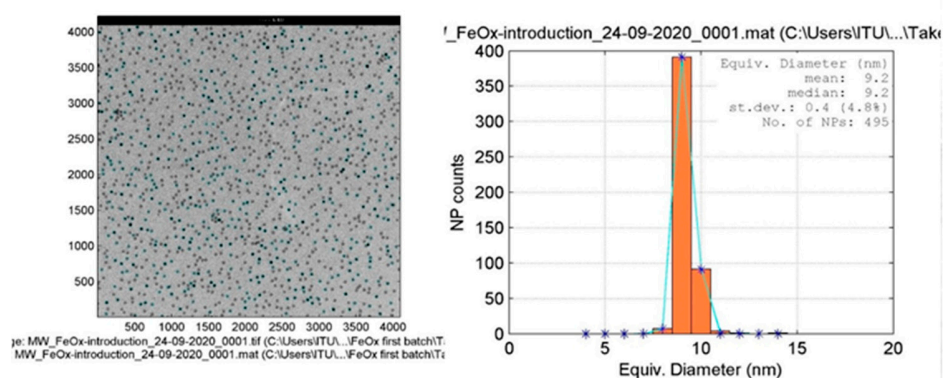


Figure S8 - TEM and TGA of oleic acid-coated SPIONs obtained via heat-up method

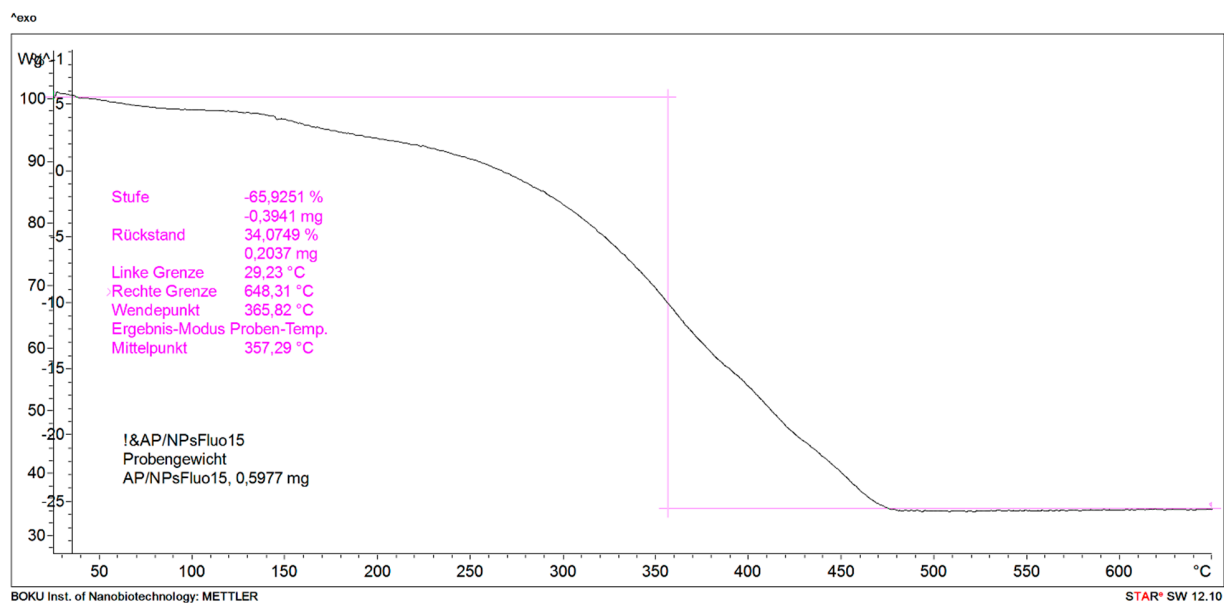


Figure S9 - TGA CySPIONs doped with 15% fluorescein-labelled polymer

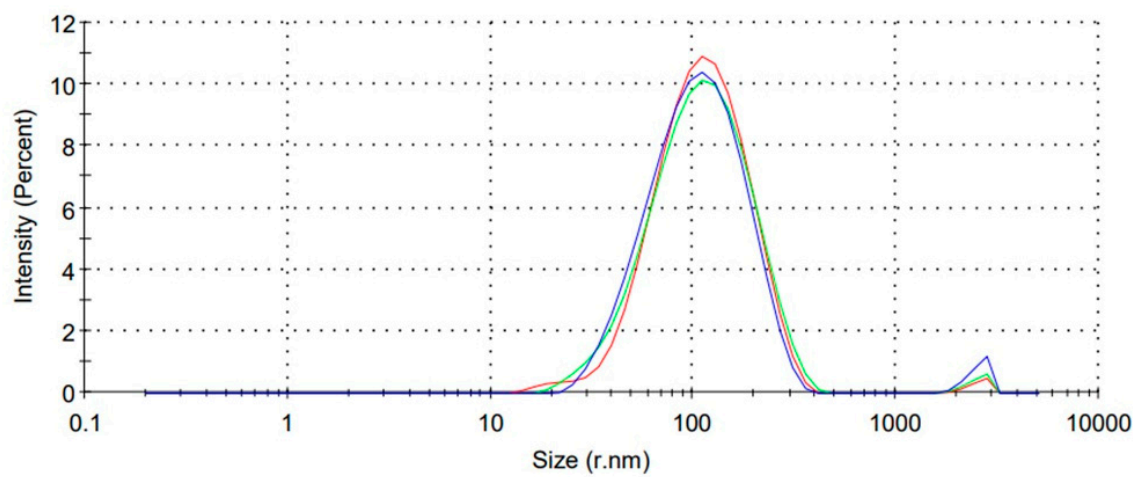


Figure S10 - Size distribution by intensity for FITC-CySPION. Radius is 120 nm

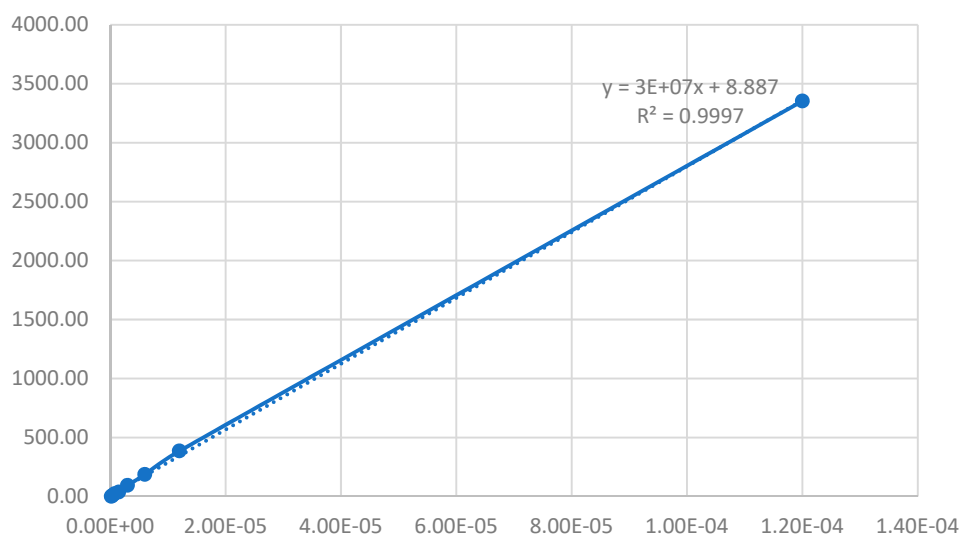


Figure S11 - Calibration curve of LY

Table S1 - Calibration curve of LY at 530nm

	Concentration (M)	Fluorescence (530nm)
1	1.20E-04	3353.10
2	1.20E-05	385.40
3	6.00E-06	186.40
4	3.00E-06	93.00
5	1.50E-06	37.10
6	7.50E-07	22.20
7	3.75E-07	7.00
8	1.88E-07	0.00

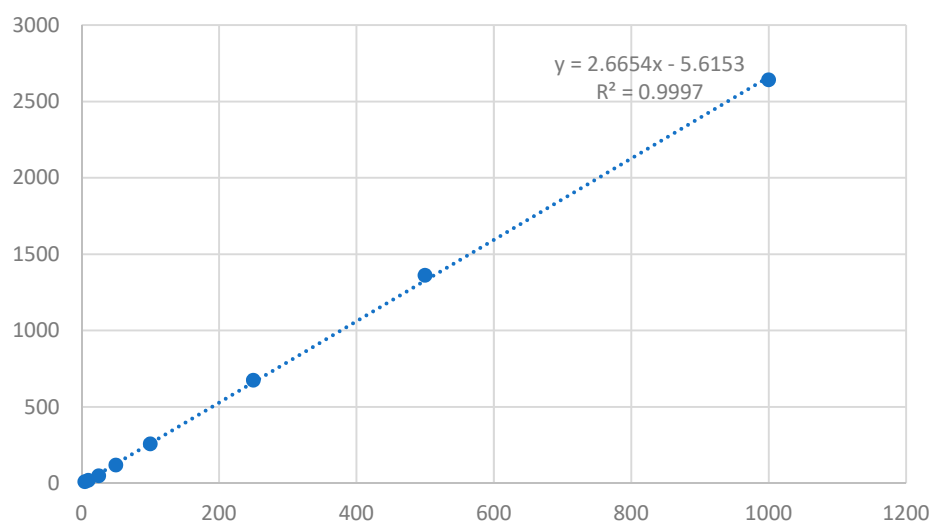


Figure S12 - Calibration curve of FITC-PMOXA

Table S2 - Calibration curve of FITC-PMOXA at 520nm

	Concentration FITC-PMOXA (µg/mL)	RFU (520 nm)
1	1000	2640.9
2	500	1361
3	250	673.6
4	100	256.4
5	50	118.3
6	25	48.3
7	10	18.4
8	5	9.1

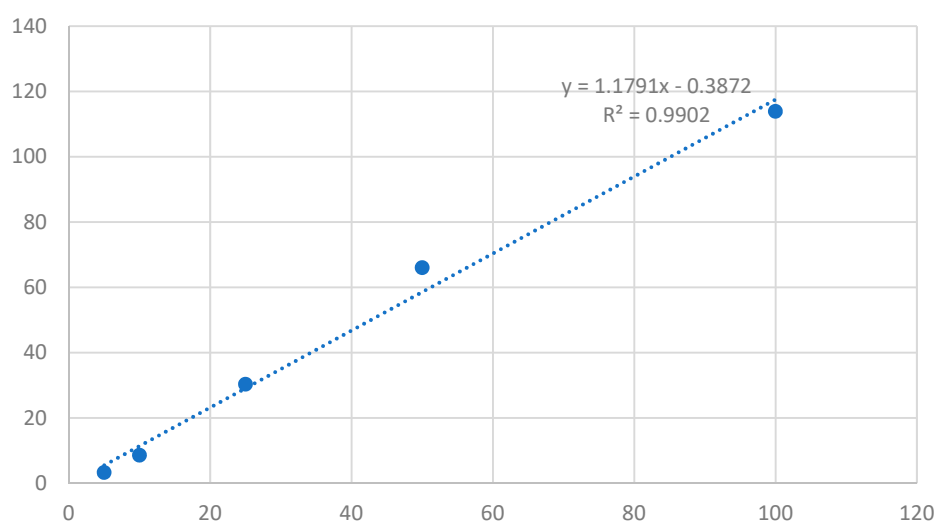


Figure S13 - Calibration curve of FITC-CySPION

Table S3 - FITC-CySPION at 520nm

	Concentration FITC-CySPION (µg/mL)	RFU (520 nm)
1	100	113.9
2	50	66
3	25	30.3
4	10	8.6
5	5	3.3

Table S4 - Permeability values for LY, FITC-PMOXA and FITC-CySPION

Lucifer Yellow

Time (min)	Permeability (cm/s)
30	1.38536E-06
60	1.65806E-06
90	2.048E-06

FITC-PMOXA

Time (min)	Permeability (cm/s)	Standard deviation
30	1.25396E-06	6.88833E-07
60	2.4988E-06	4.56429E-07
90	1.66836E-06	3.23723E-07

FITC-CySPION

Time (min)	Permeability (cm/s)	Standard deviation
30	3.77893E-06	8.27035E-07
60	3.06986E-06	7.23057E-07
90	2.27464E-06	2.62875E-07