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Potential applications and design of protein delivery systems

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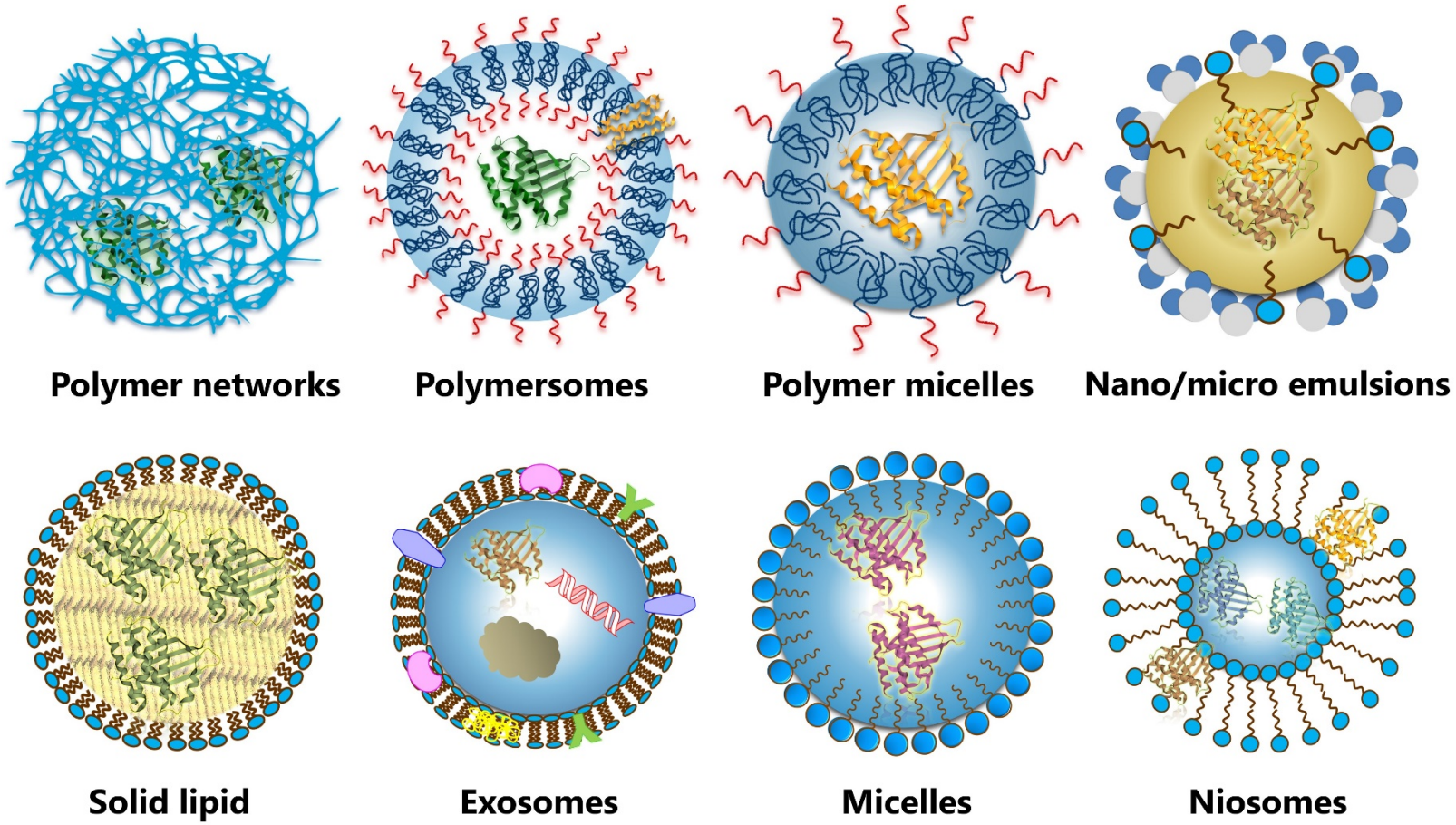
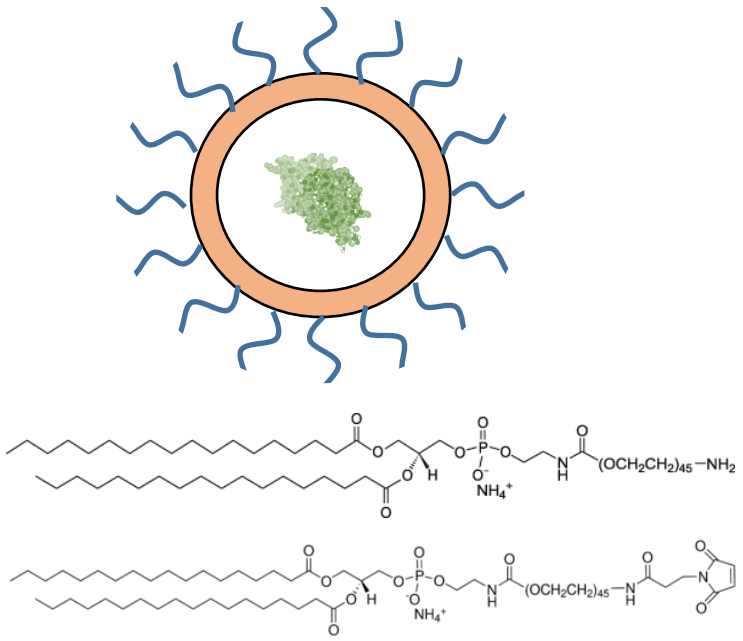
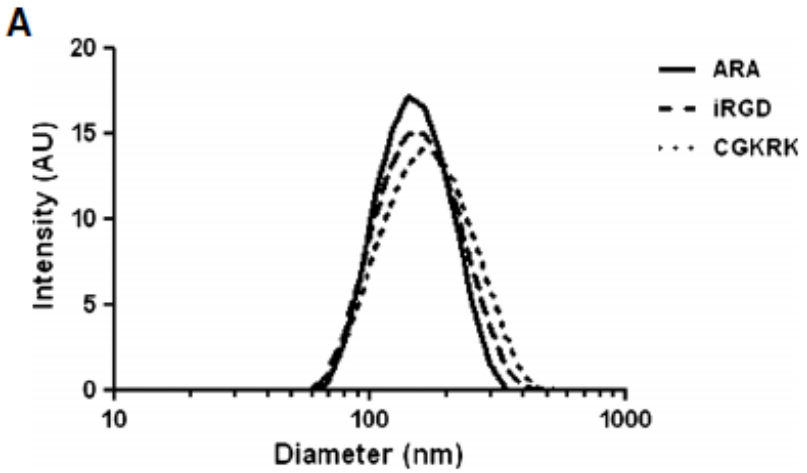
Nanosized systems for protein delivery

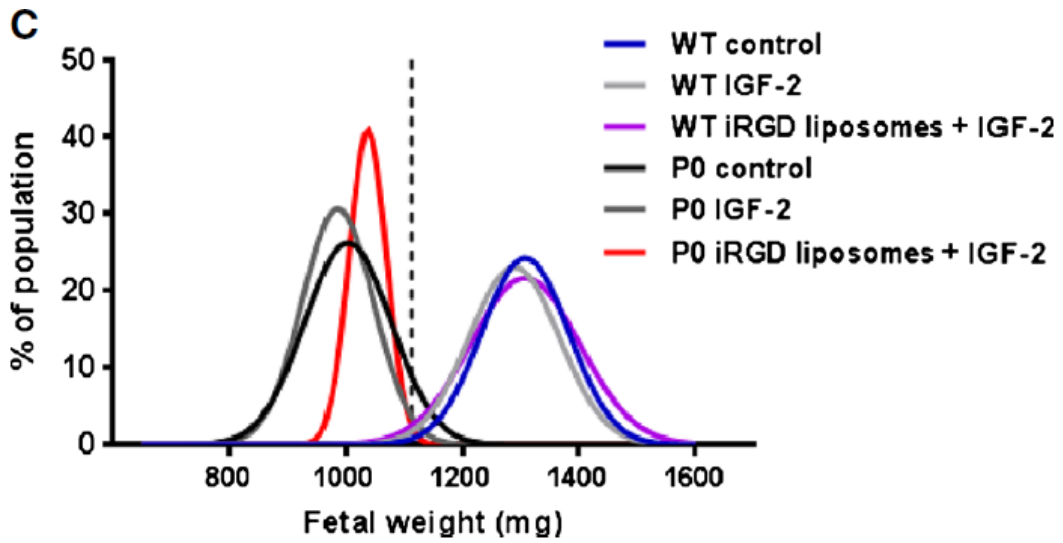


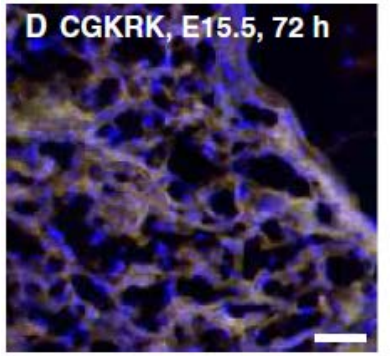
Moncalvo F. et Al., Frontiers in Bioengineering and Biotechnology 2020

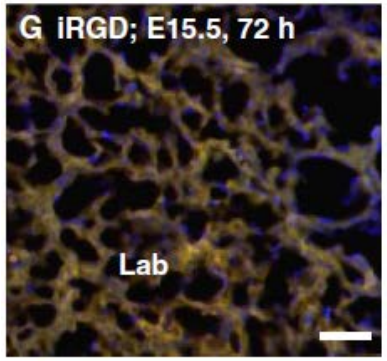
Liposomal nanocarriers

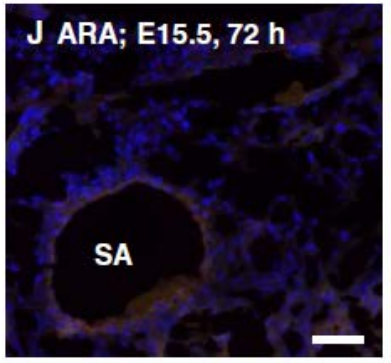










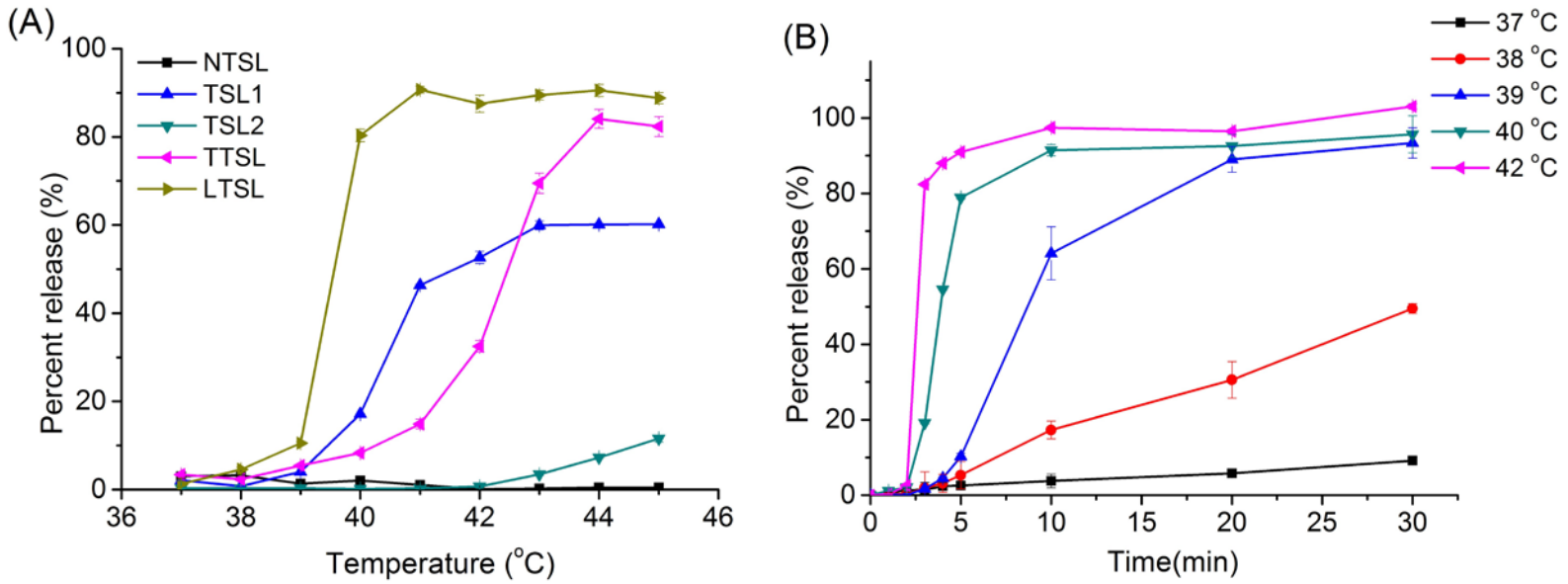


King et al. Sci. Adv. 2016; 2 : e1600349

Dr. Lynda K Harris, Pharmacy, Uni Manchester (UK)

Thermosensitive liposomes

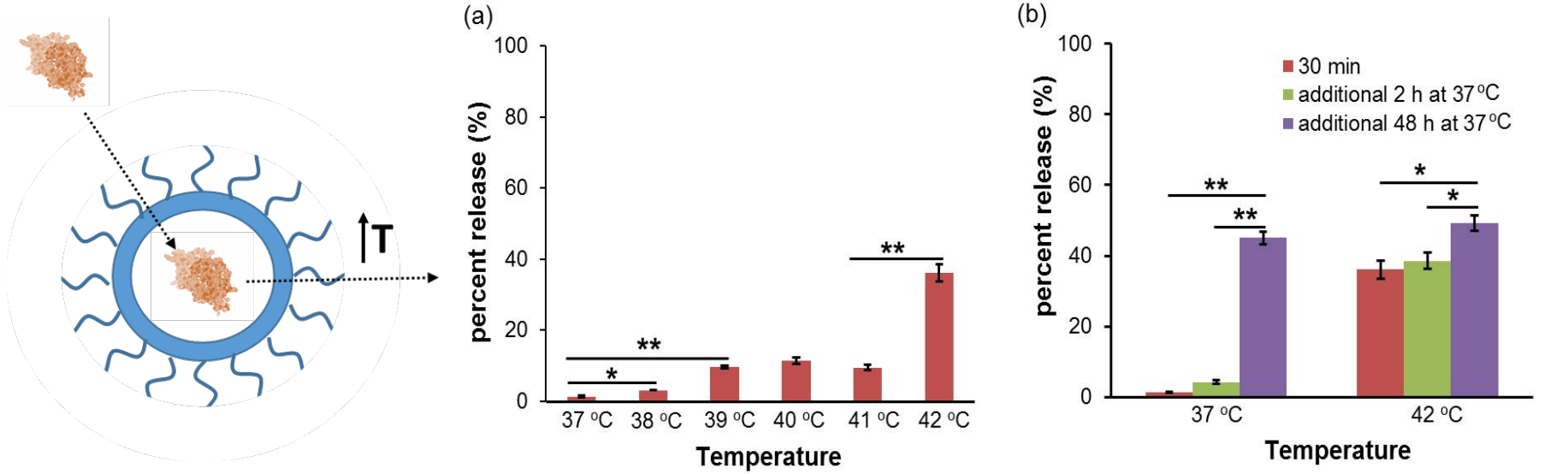
Liposome	Lipid composition (mol/mol)	Thermo-sensitivity	T_m (°C)	d_h (nm)[a]	PDI[a]	ζ-potential (mV)[a]
NTSL	DSPC/Chol/DSPE-PEG(2000) = 65/30/5	No	n/a*	155 ± 2	0.05 ± 0.02	−2.1 ± 0.4
TSL1	DPPC/DSPE-PEG(2000) = 95/5	YES	42.5	152 ± 1	0.08 ± 0.01	−2.2 ± 0.6
TSL2	DPPC/DSPC/DSPE-PEG2000 = 80/15/5	YES	43.0	134 ± 1	0.05 ± 0.02	−5.4 ± 0.2
TTSL	DPPC/DSPC/Chol/DSPE-PEG(2000) = 50/25/15/3	YES	40.9	147 ± 2	0.03 ± 0.01	−1.8 ± 0.2
LTSL	DPPC/Lyso-PC/DSPE-PEG2000 = 90/10/4	YES	40.9	148 ± 1	0.08 ± 0.02	−2.0 ± 0.2



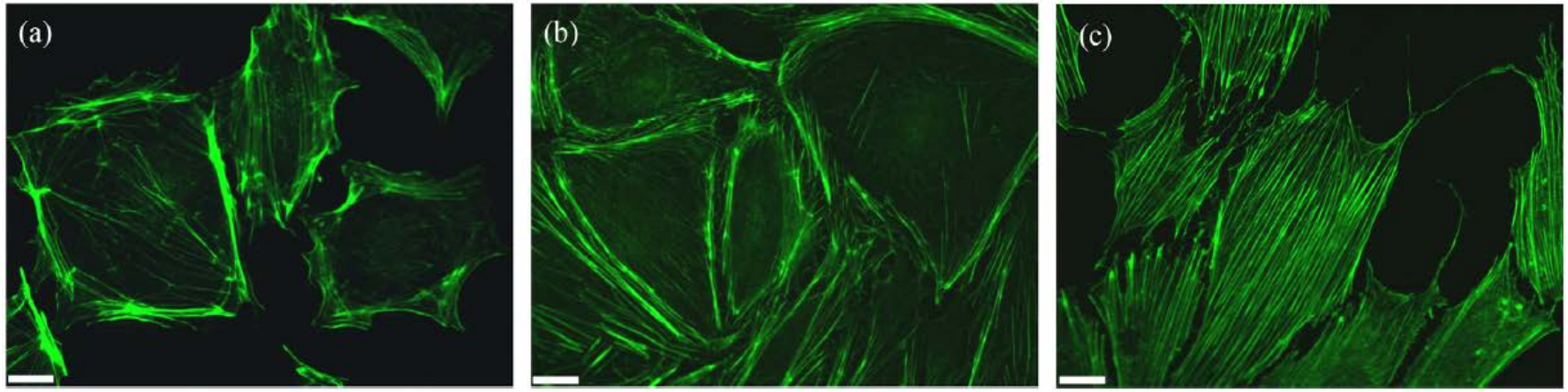
A) Temperature dependent release of CF from liposomes (lipid concentration 5 mM) in PBS (10 mM, pH= 7.4). The samples were incubated at the desired temperature (37-45 ° C) for 5 min. The CF release was measured for each formulation. B) Time dependent CF release from LTSL at different temperature (37-42° C). Results are given as mean of three measurements, ±SD.

X. Huang et Al., Int J Pharm. 2017

BDNF - Thermosensitive liposomes



(a) Temperature-dependent percent release of BDNF from LTSL in 30 min (n = 3, *p < 0.05 and **p < 0.01). (b) Time-dependent percent release of BDNF in 30 min at 37 C and 42 C. After 30 min incubation at set temperature, further incubation at 37 C for additional 2 h and 48 h was carried out.



Morphology of podocytes stained by green phalloidin (scale bar 20 mm); (a) cells treated with ADR for 24 h; (b) cells damaged by ADR and incubated for 48 h with blank LTSL (c) cells damaged by ADR and incubated for 48 h with BDNF-loaded LTSL (BDNF concentration 200 ng/mL).

X. Huang et Al., Int J Pharm. 2017

Macromolecules loading in liposomes

Table 3
Characterization of FITC-albumin encapsulated liposomes (0.5 mM lipid suspension in PBS 10 mM, pH = 7.4, 25 °C).[a] Mean ± SEM, N = 3.

Liposome	Z-average size (nm)[a]	PDI[a]	ζ-potential (mV)[a]	LE (%)[a]	EE (%)[a]
TSL1	153 ± 1	0.08 ± 0.01	−2.1 ± 0.4	0.023 ± 0.004	15.0 ± 2.7
TSL2	154 ± 3	0.13 ± 0.04	−6.5 ± 0.1	0.038 ± 0.008	25.0 ± 5.3
TTSL	150 ± 1	0.14 ± 0.03	−0.6 ± 0.1	0.013 ± 0.001	9.0 ± 0.7
LTSL	145 ± 1	0.12 ± 0.03	−6.8 ± 0.4	0.062 ± 0.007	41.3 ± 4.7

Table 4
Characterization of TRITC-dextran and Rho-A4PEG5 encapsulated liposomes (0.5 mM lipid suspension in PBS buffer 10 mM, pH = 7.4, 25 °C).[a] Mean ± SEM, N = 3.

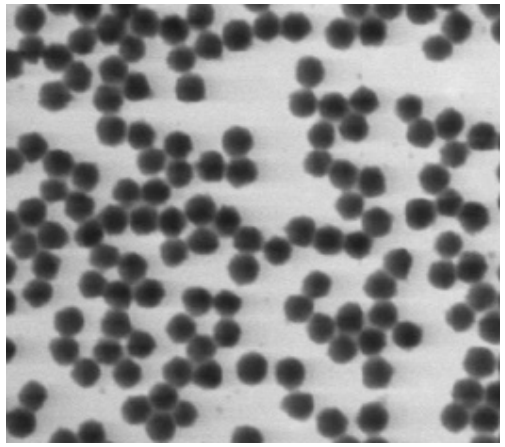
Liposome	Payload	Z-average (nm)[a]	PDI[a]	ζ-potential (mV)[a]	LE (mol%)[a]	EE (%)[a]
TSL2	TRITC-dextran	128 ± 6	0.09 ± 0.02	−1.3 ± 0.3	0.012 ± 0.001	5.1 ± 0.6
	Rho-A4PEG5	132 ± 2	0.08 ± 0.01	−1.5 ± 0.2	0.052 ± 0.004	5.6 ± 0.4
TTSL	TRITC-dextran	156 ± 3	0.03 ± 0.01	−1.0 ± 0.3	0.009 ± 0.001	3.7 ± 0.7
	Rho-A4PEG5	150 ± 2	0.06 ± 0.01	−0.9 ± 0.3	0.049 ± 0.005	5.3 ± 0.5
LTSL	TRITC-dextran	134 ± 2	0.08 ± 0.01	−1.0 ± 0.1	0.015 ± 0.001	6.0 ± 0.7
	Rho-A4PEG5	133 ± 3	0.09 ± 0.01	−1.5 ± 0.2	0.063 ± 0.005	6.8 ± 0.5

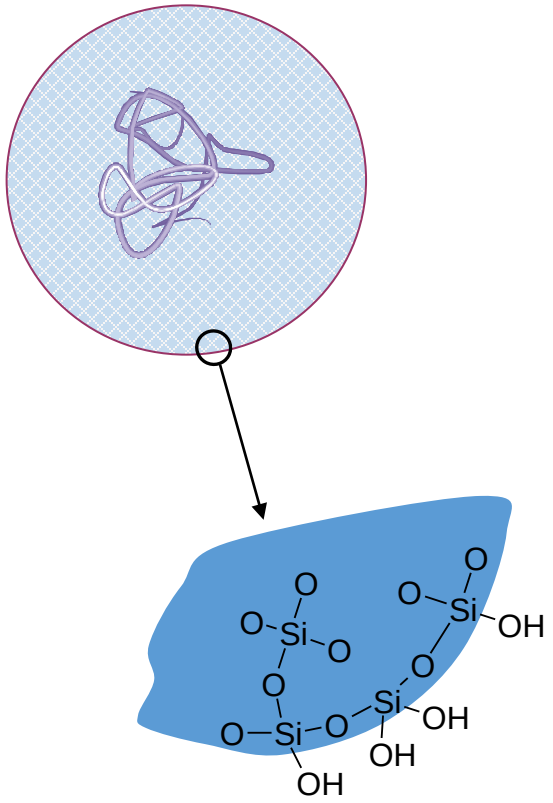
Table 5
Characteristics of FITC-lysozyme (LZ) and BDNF loaded liposomes with different formulations. [a] Mean ± SEM, N = 3. Liposomal vesicles were diluted to 0.5 mM with PBS buffer (10 mM, pH = 7.4) and the particle size, size distribution and ζ-potential were measured by DLS techniques at 25 °C.

Protein	Lipid formulation	Initial protein/lipids ratio (mol/mol)	Average size (nm)[a]	PDI[a]	ζ-potential (mV)[a]	LE (mol%)[a]	EE (%)[a]
LZ	TSL1	7.1×10^{-4}	134 ± 1	0.10 ± 0.02	−2.02 ± 0.20	0.011± 0.006	15.4 ± 0.8
LZ	TSL2	7.1×10^{-4}	136 ± 3	0.09 ± 0.03	−2.25 ± 0.29	0.011± 0.003	15.1 ± 0.4
LZ	LTSL	7.1×10^{-4}	133 ± 1	0.09 ± 0.01	−1.96 ± 0.35	0.010 ± 0.001	14.4 ± 0.2
LZ	LTSL	1.4×10^{-3}	138 ± 2	0.06 ± 0.02	−1.44 ± 0.19	0.030 ± 0.001	21.2 ± 0.6
LZ	LTSL	7.1×10^{-3}	134 ± 2	0.10 ± 0.01	−1.40 ± 0.30	0.069 ± 0.005	9.6 ± 0.7
BDNF	LTSL	3.7×10^{-4}	142 ± 5	0.10 ± 0.03	−1.78 ± 0.01	0.004 ± 0.001	11.9 ± 0.1

X. Huang et Al., Int J Pharm. 2017

SiO_2 gel nanoparticles

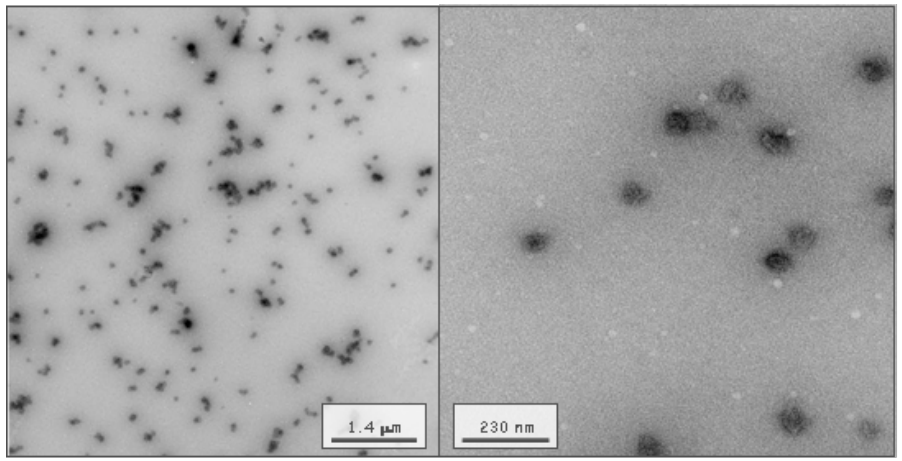




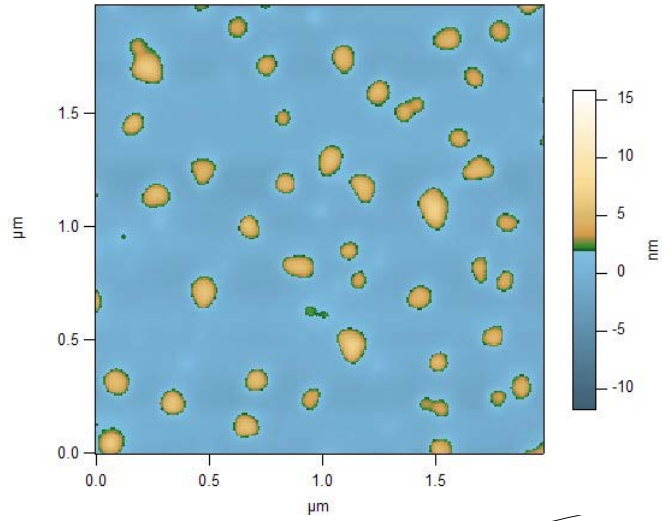
- **Non toxic** with acceptable in vivo biocompatibility.
- **No swelling and no change in porosity** in a physiologically acceptable range of temperature and pH.
- Preparation conditions based on **mild sol-gel synthesis** that can retain the activity of entrapped enzymes

Enzyme encapsulation in SiO_2 NPs

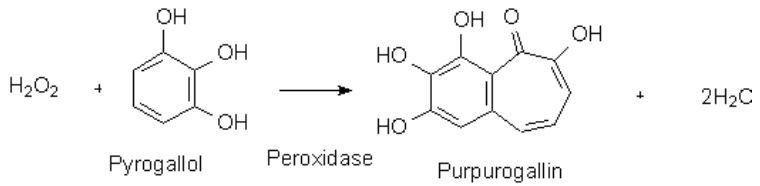
Synthesis performed in W/O microemulsion



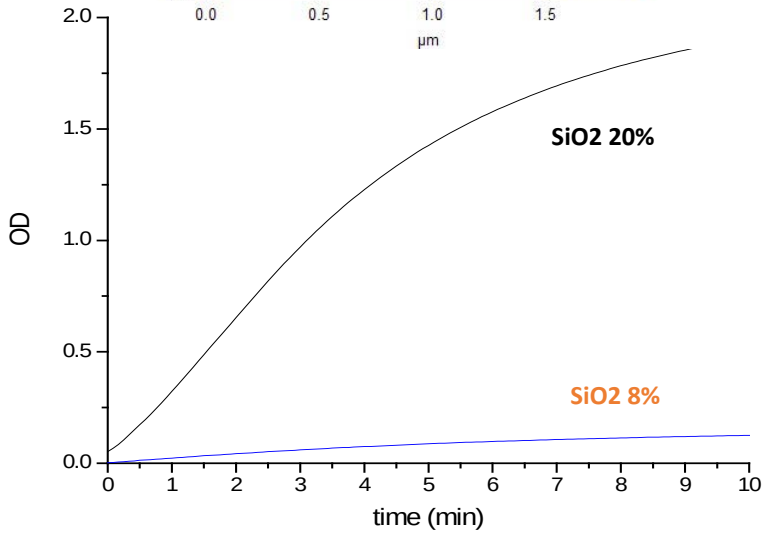
Synthesis performed in buffer (SiO_2 nucleation in presence of a positively charged enzyme)



Horseradish Peroxidase (HRP) encapsulation



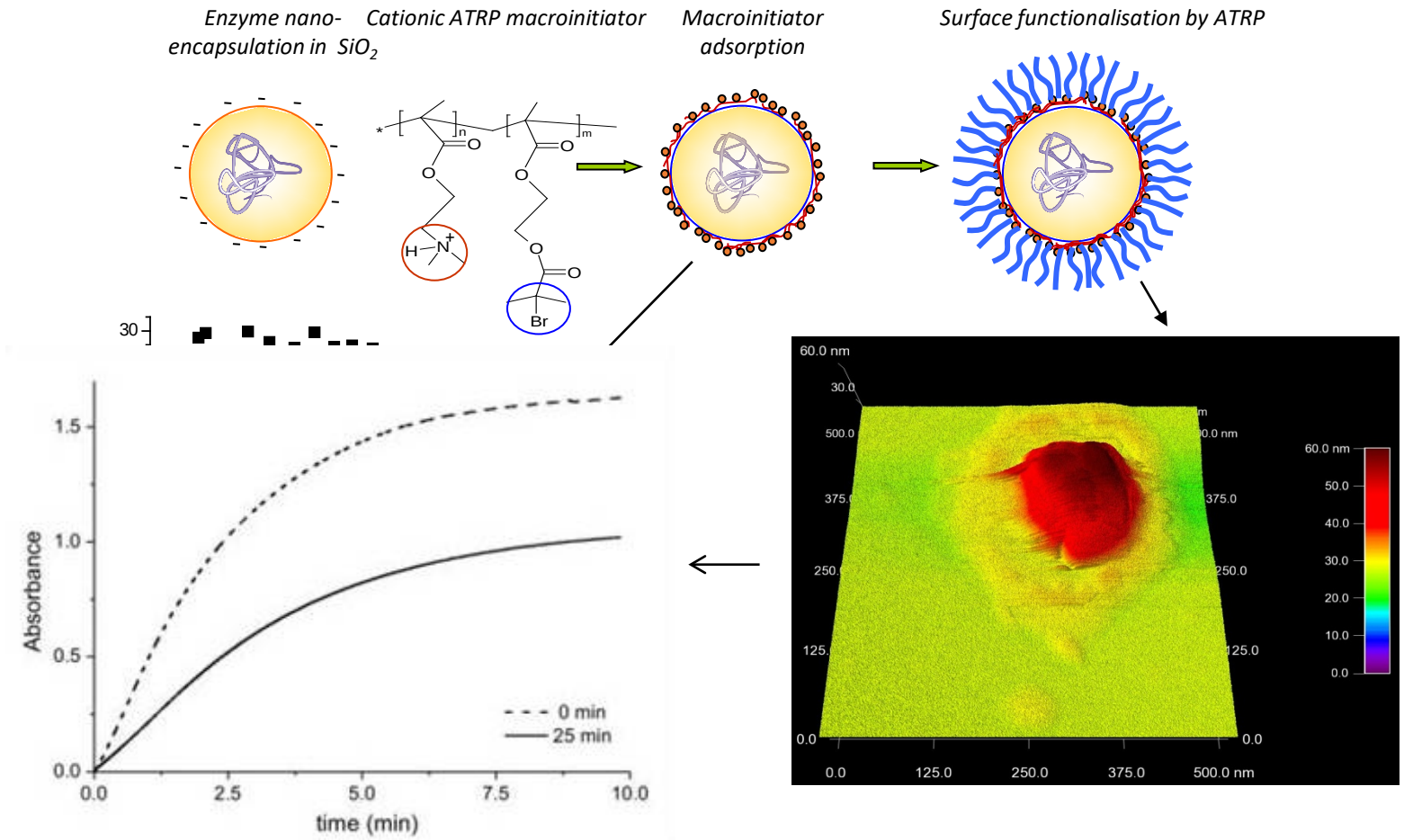
	U/mL
Free HRP (0.03 mg/mL)	4.20
20% SiO2 NPs	0.320
8% SiO2 NPs	0.023



F.Cellesi, N.Tirelli, *Colloids and Surfaces A* (288), 2006, 52-61

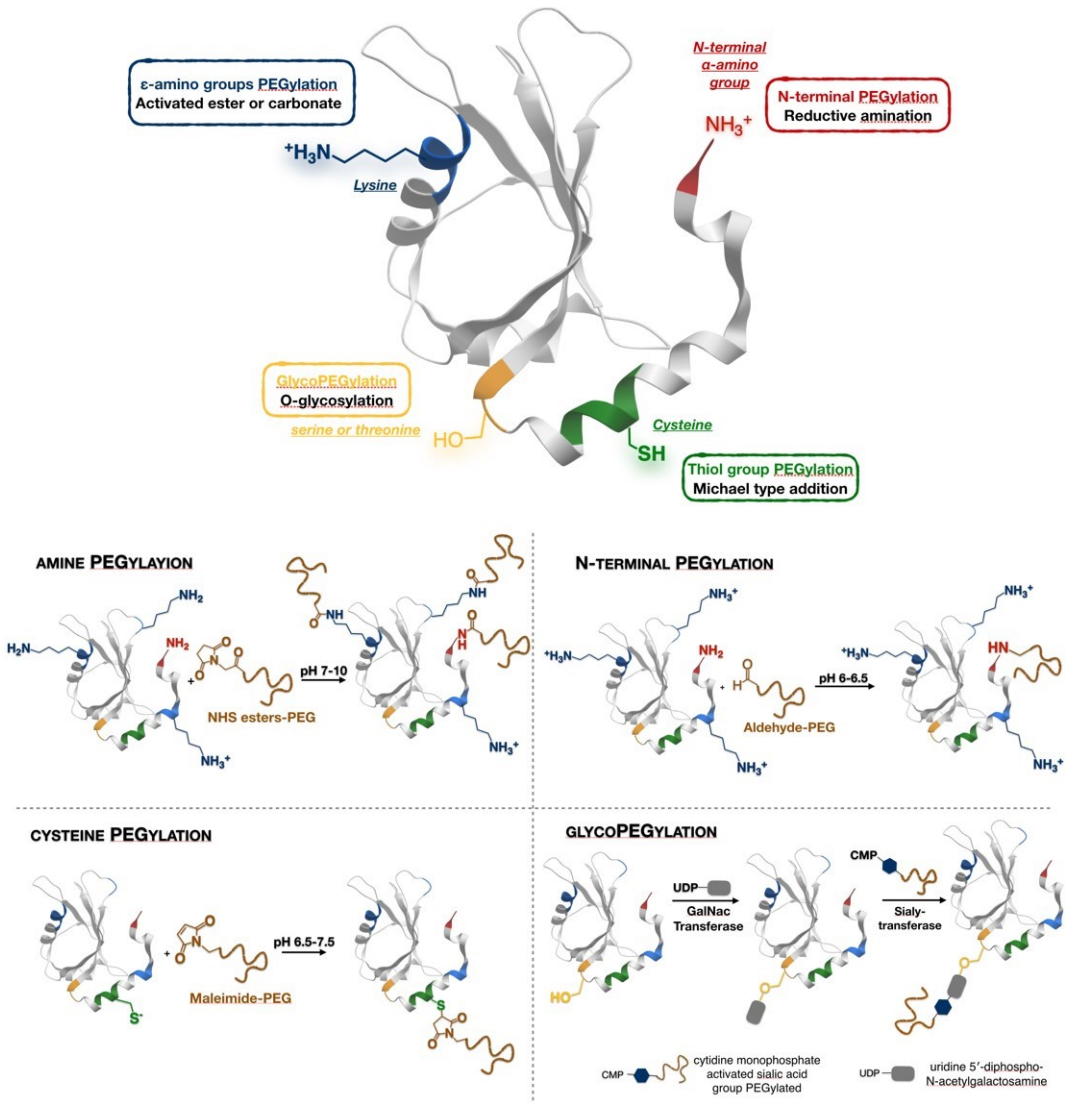
P. De leonardis et Al., Colloids and Surfaces A (580) 2019, 123734

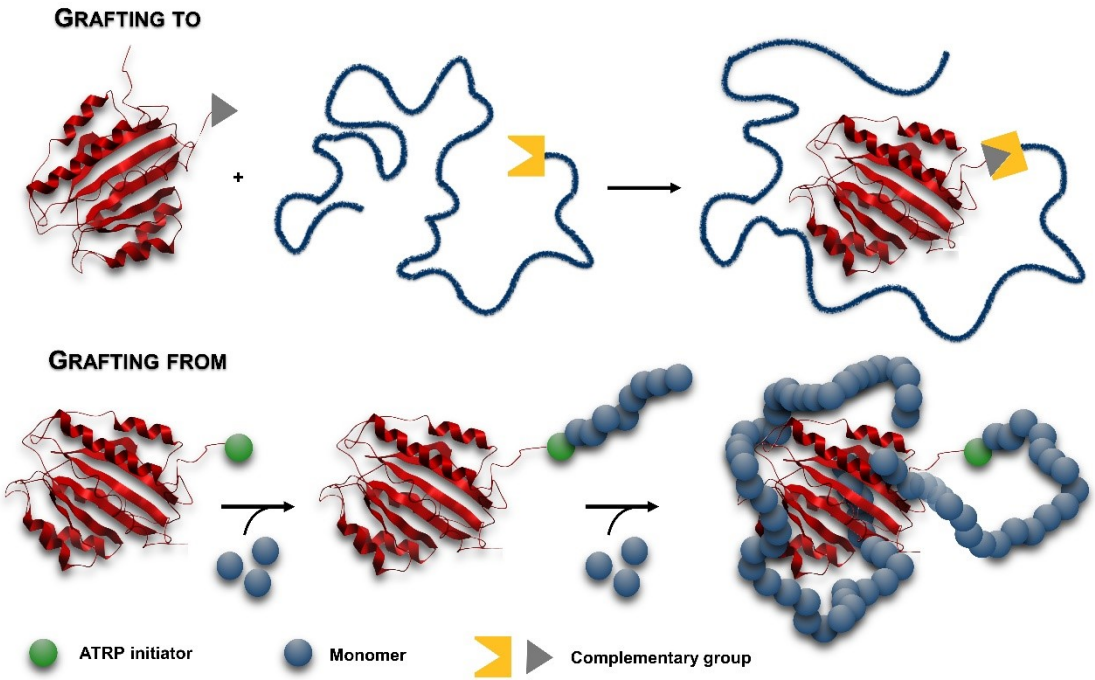
Surface polymerisation



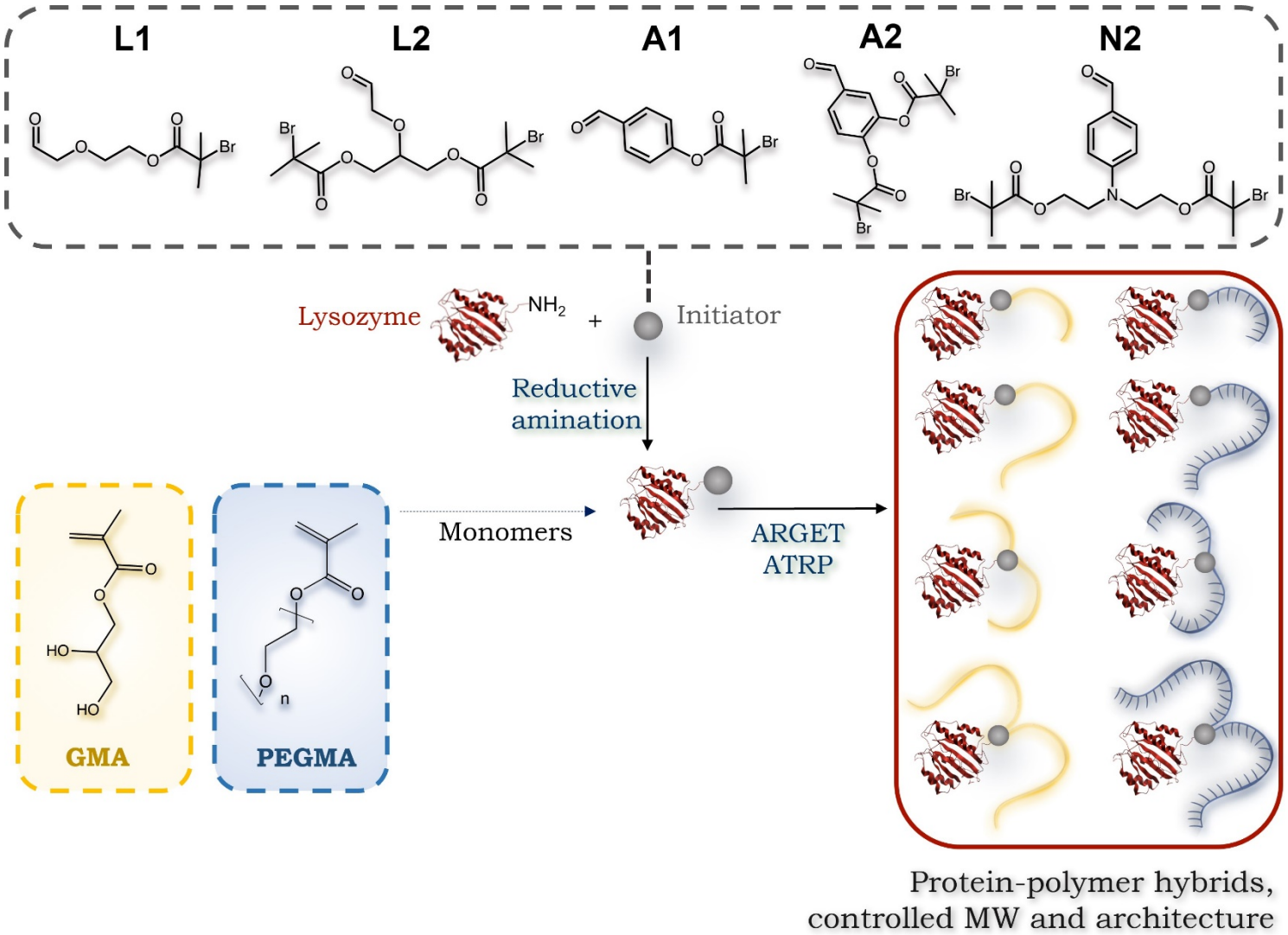
P. De leonardis et Al., Colloids and Surfaces A (580) 2019, 123734

Protein - polymer conjugates



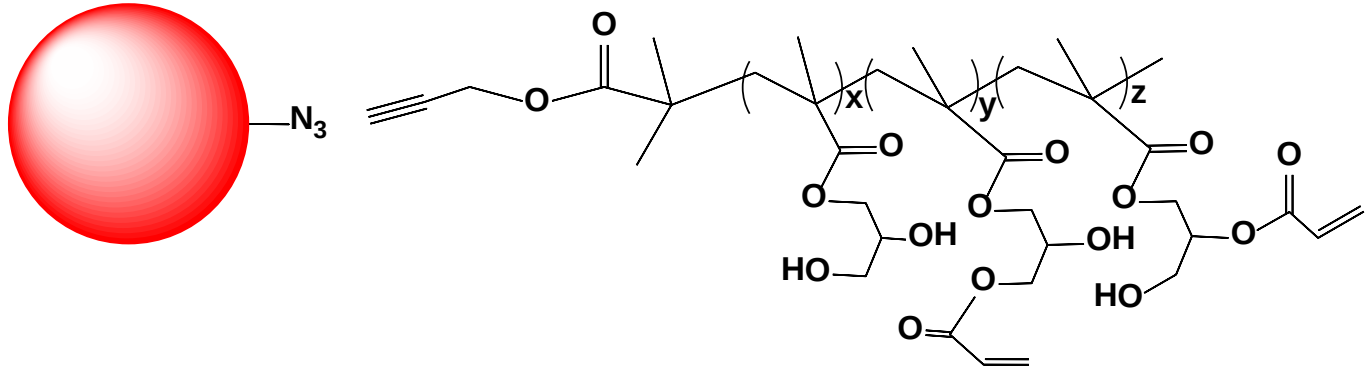


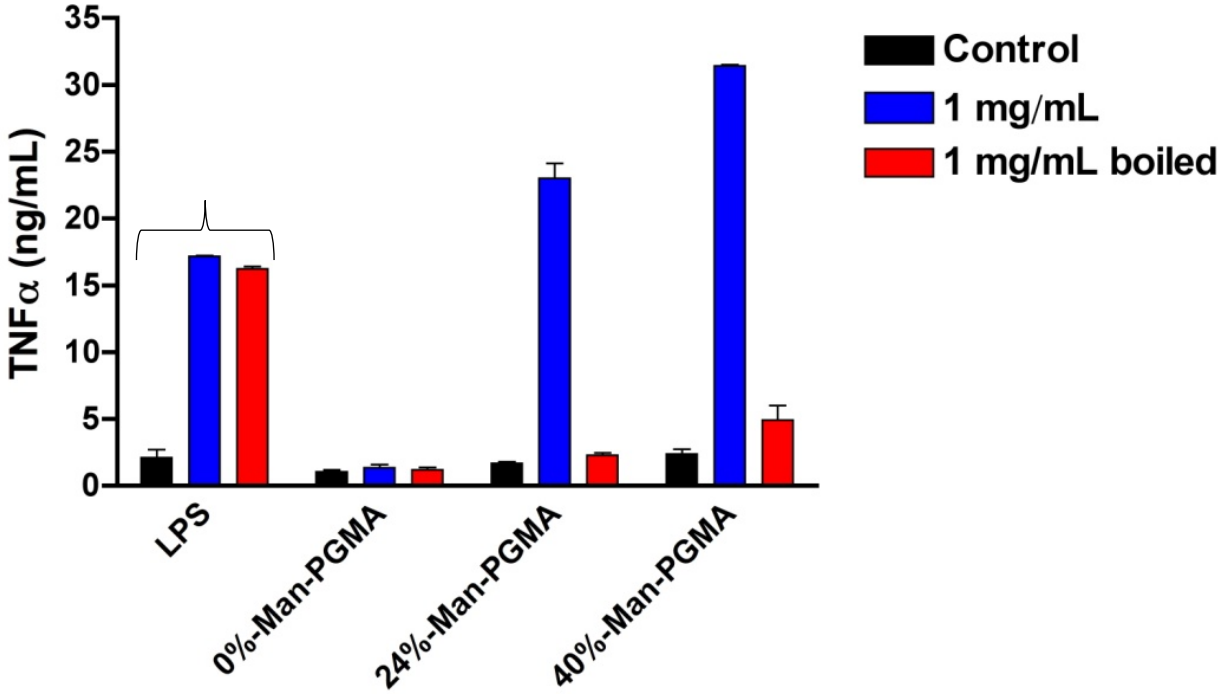
Grafting-from approach via ARGET ATRP

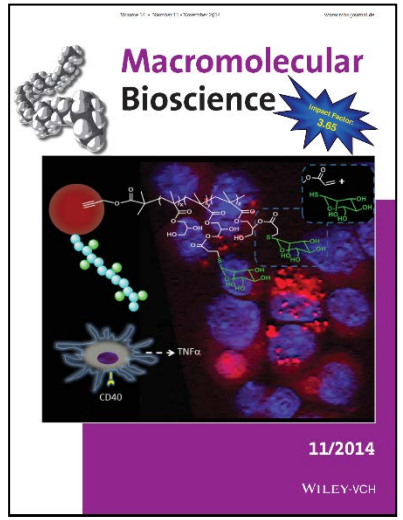


Moncalvo F. et A., Biomacromolecules 2022, submitted

Immunoactive PGMA

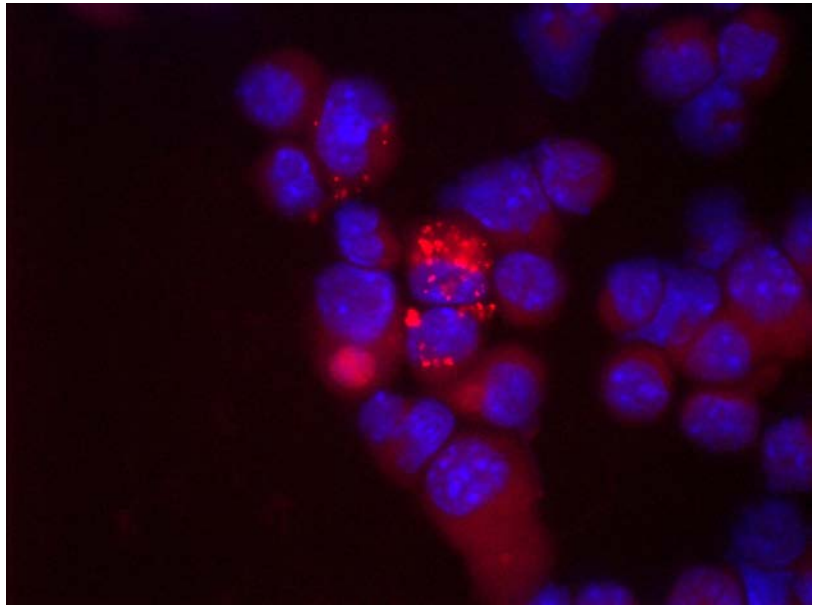


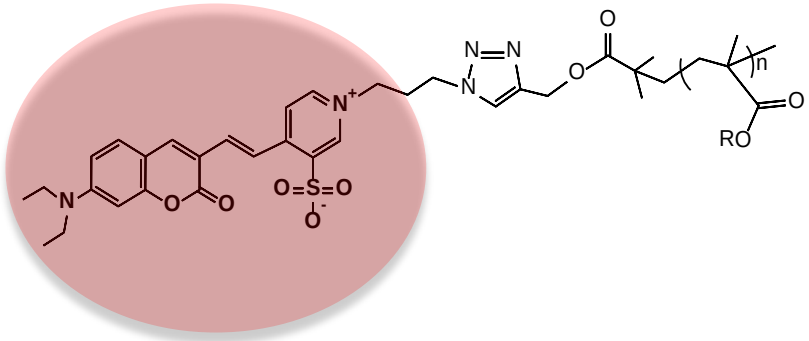


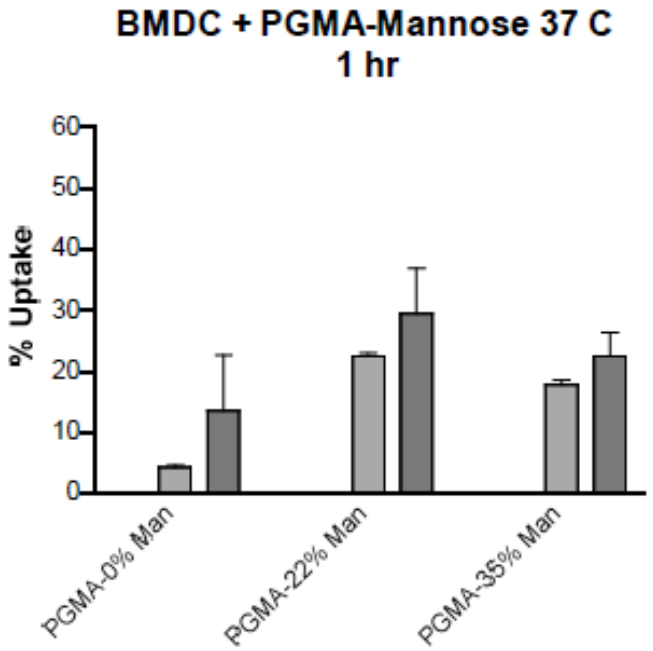


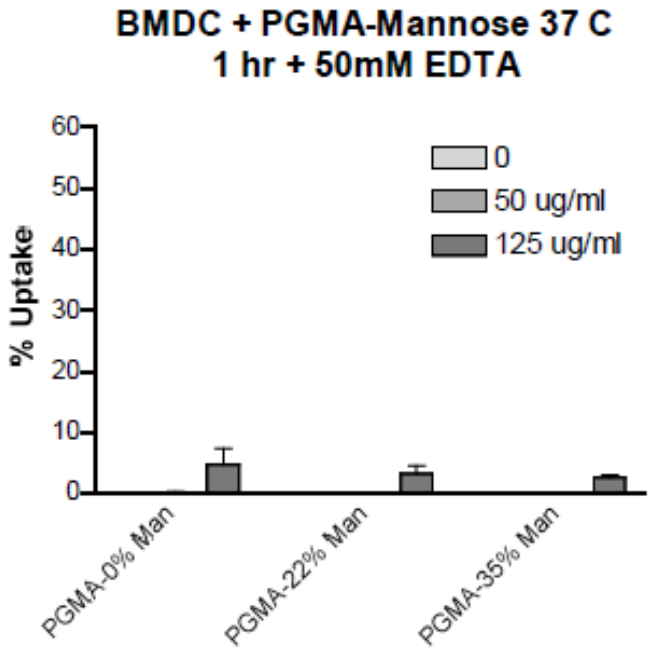
Macromol. Biosci., 2014

Mannose-PGMA Uptake



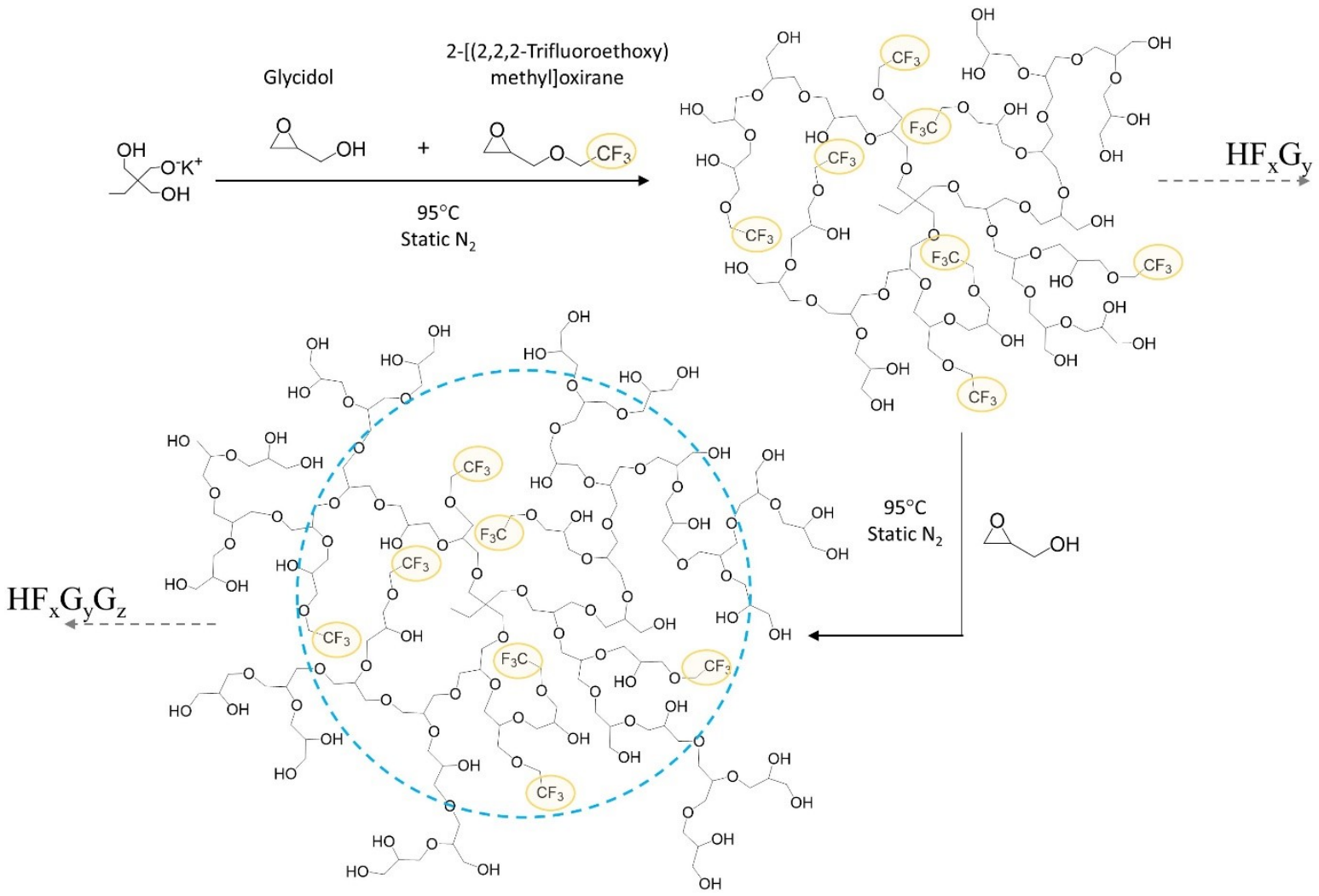






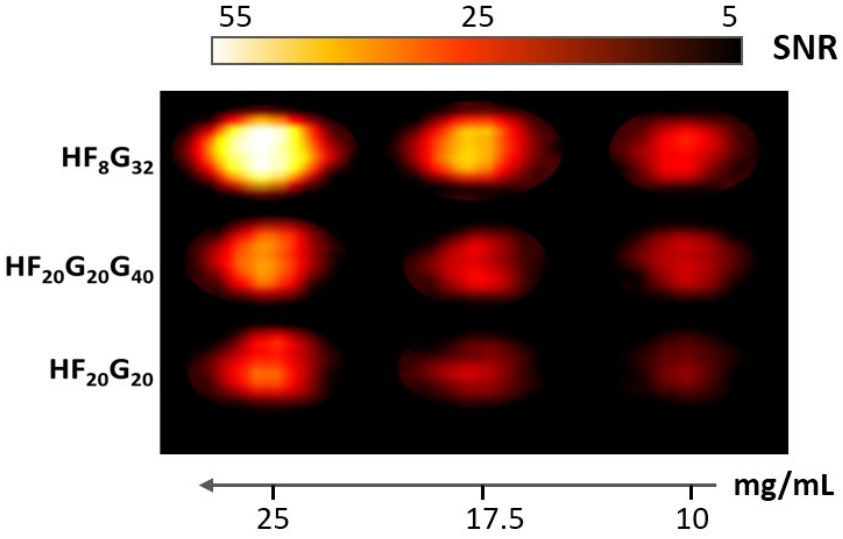
L. Ragupathy at Al, *Macromol. Biosci., 2014*

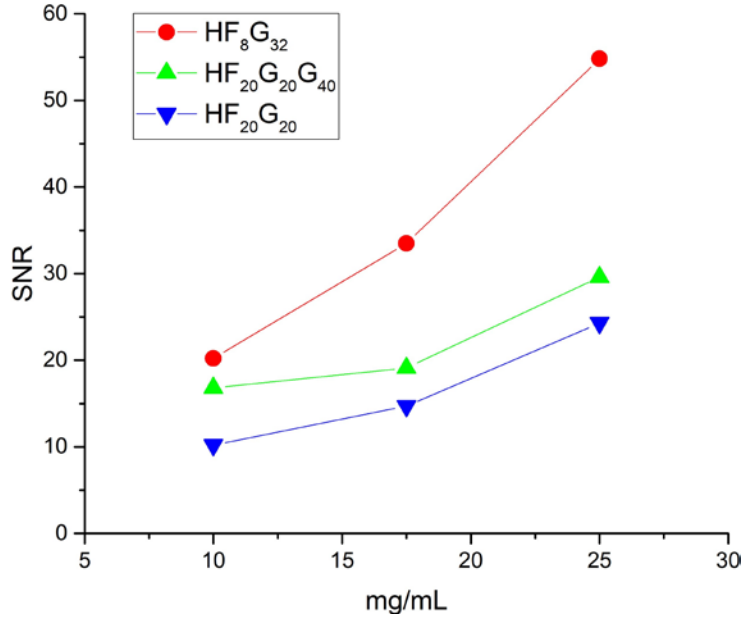
Fluorinated hyperbranched polyglycerols

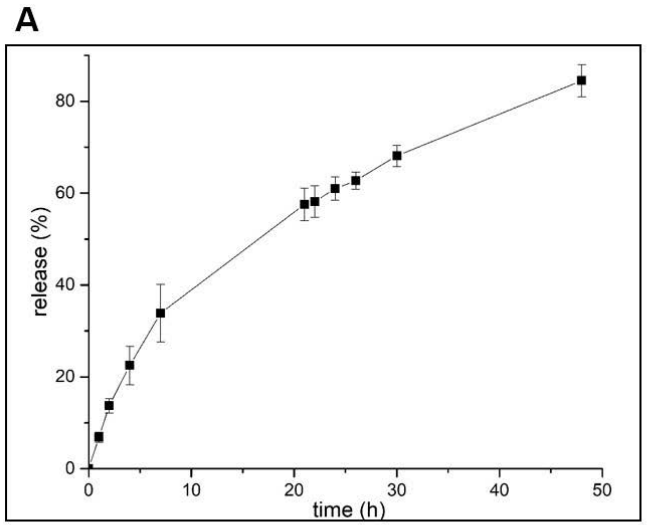


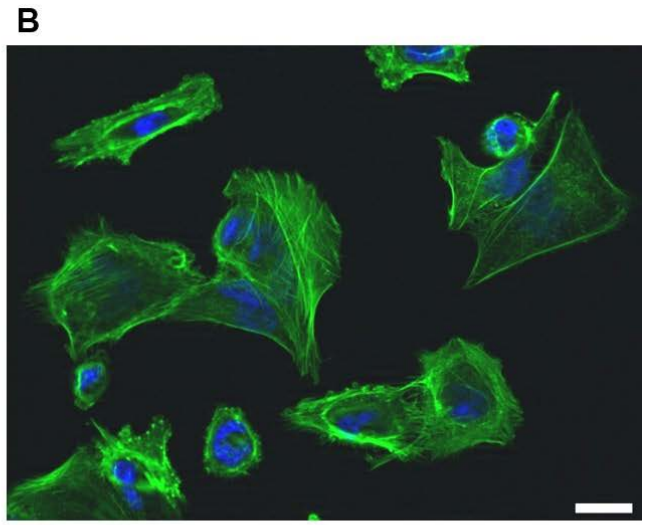
W. Celentano et Al., Polymer Chemistry 2020

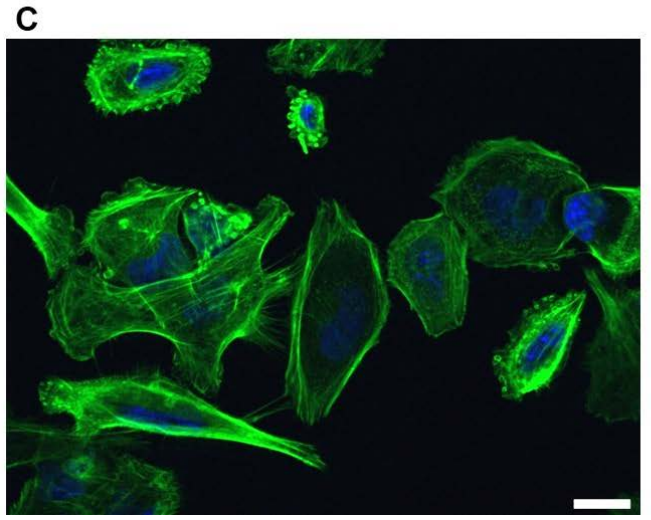
^{19}F-HPG for MRI and DEX delivery

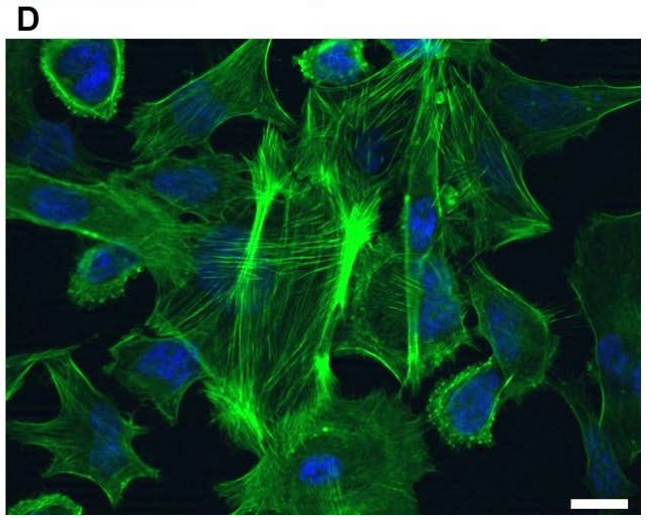






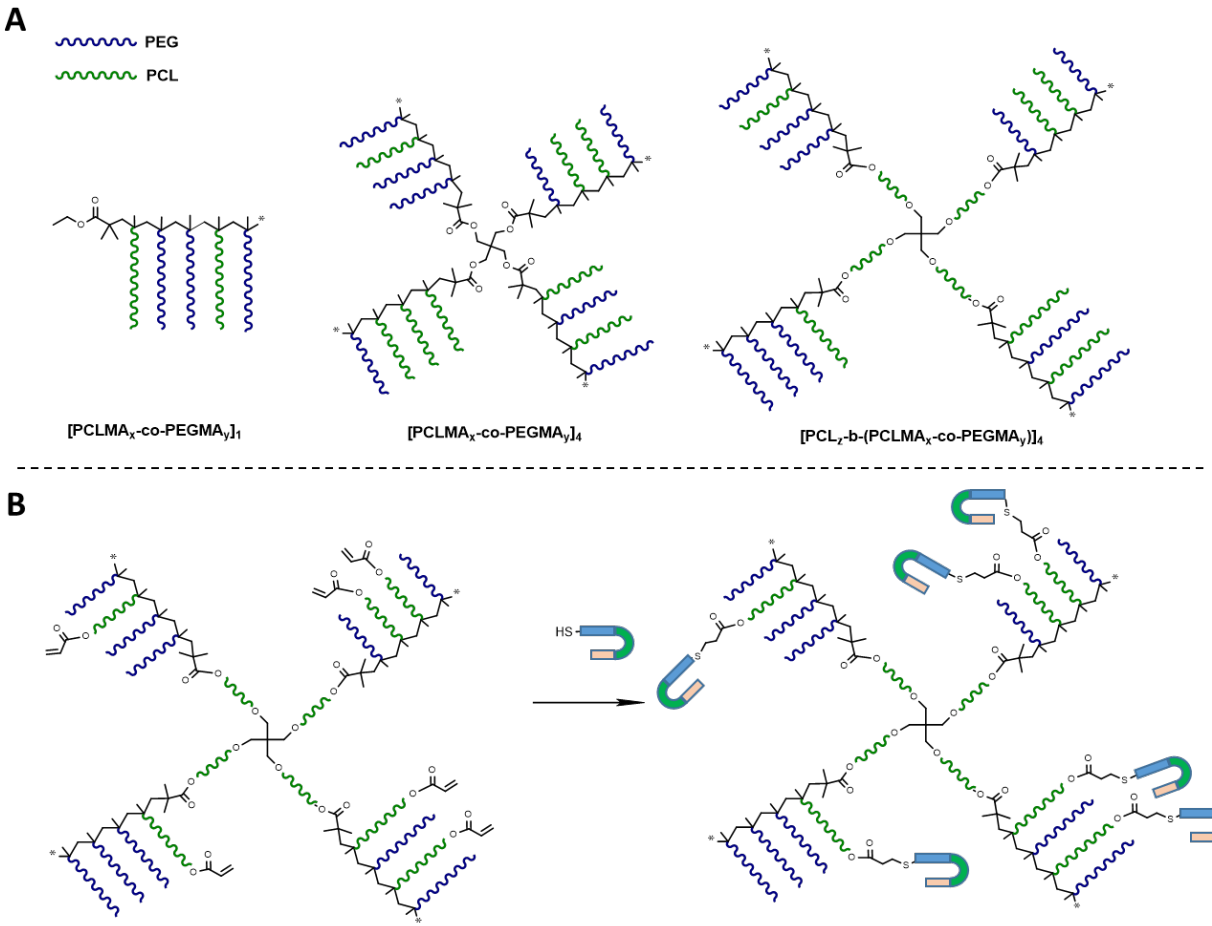


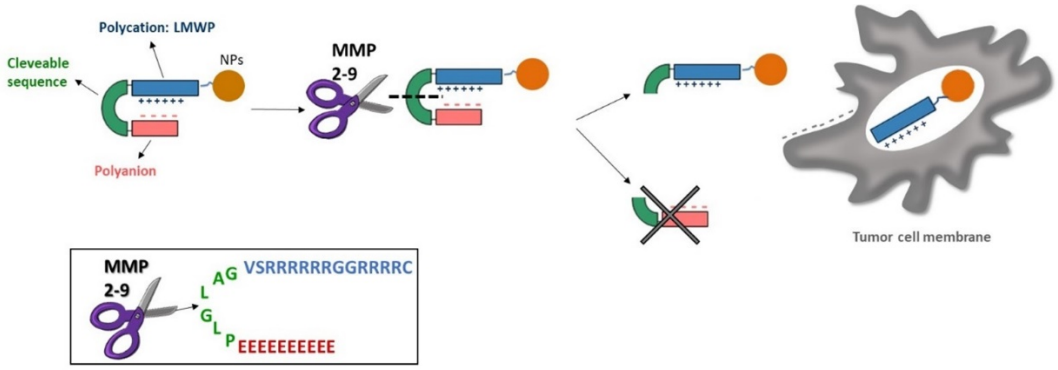




A) DEX release from HF8G32 polymeric nanoparticles in water at 37 °C (error bars indicate± SD from experiments run in triplicate). HCiPodo cells (stained by green phalloidin and DAPI, scale bar 20 um) damaged by ADR (B) and incubated for 24h (C) and 48h (D) with DEX-loaded HF8G32 (DEX concentration 10 uM).

Complex copolymers for proteinase rich tumors





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W. Celentano, M Pizzocri et Al., in preparation

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SOLVAY

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