Supporting Information for:

Multisensitive drug-loaded polyurethane/polyurea nanocapsules with pH-synchronized shell cationization and redox-triggered release

Cristina Cuscó,*[1,2] Jordi Garcia,[2] Ernesto Nicolás,[2] Pau Rocas[1] and Josep Rocas*[1]

[1] Nanobiotechnological Polymers Division, Ecopol Tech, S.L., El Foix Business Park, Indústria 7, 43720 L'Arboç del Penedès, Tarragona, Spain

[2] Organic Chemistry Section, Inorganic and Organic Chemistry Department, Faculty of Chemistry, CIBERobn and IBUB, University of Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain

1. Acronyms

IPDI: isophorone diisocyanate. It is an organic aliphatic diisocyanate. Interestingly, aliphatic isocyanates are less pulmonary sensitizing and not carcinogenic compared to the aromatic ones. It is particularly noteworthy that fully reacted isocyanate polymers to form their respective urethane or urea bonds do not contain toxicity referred to the isocyanate group.[1] In addition, degradation products of IPDI, such as isophorone diamine are rapidly eliminated via urinary excretion.[2]

DEDS: 2-hydroxyethyl disulfide.

YMER N-120: polymeric non-ionic hydrophilic building block containing two primary hydroxyl groups and a long capped ethoxylated side chain. From a synthetic point of view, YMER N-120 was selected because of its low melting point and viscosity.

Genamin TAP 100D: *N*-tallow-1,3-propylenediamine. It is a hydrophobic building block containing C18 fatty chain and two reactive amines for polymerization.

Jeffcat DPA: *N*-(3-dimethylaminopropyl)-*N,N*-diisopropanolamine. It contains two cationizable tertiary amines and two alcohols for polymerization.

THF: tetrahydrofurane.

DETA: diethylenetriamine. It is a water-soluble molecule used as a crosslinker and it contains two primary amines and one secondary amine.

HCl: hydrochloric acid.

DiO: 3,3’-dioctadecyloxacarbocyanine perchlorate (lipophilic tracer).

DiI: 1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate (lipophilic tracer).

MWCO: molecular weight cut off.

FT-IR (ATR): Fourier transform infrared spectroscopy (attenuated total reflectance).

DLS: dynamic light scattering.

TEM: transmission electron microscopy.

AFM: atomic force microscopy.

UV-Vis: ultraviolet-visible spectroscopy.

DL: drug loading.

EE: encapsulation efficiency.

FRET: Förster resonance energy transfer.

PBS: phosphate buffered saline.

HSA: human serum albumin.

BSA: bovine serum albumin.

L-GSH: reduced L-glutathione.

HLB: hydrophilic lipophilic balance.

EPR: enhanced permeability and retention.

2. Figures

Composition (% by weight) of each polymer

Synthesis of the amphiphilic cationic prepolymer (P1)

Monomer	% by weight
DEDS	3.524
Ymer N-120	40.820
Jeffcat DPA	3.432
IPDI	29.322
TAP 100 D	22.903

Synthesis of the amphiphilic prepolymer (P2)

Monomer	% by weight
DEDS	4.098
Ymer N-120	47.467
Jeffcat DPA	0
IPDI	27.149
TAP 100 D	21.286

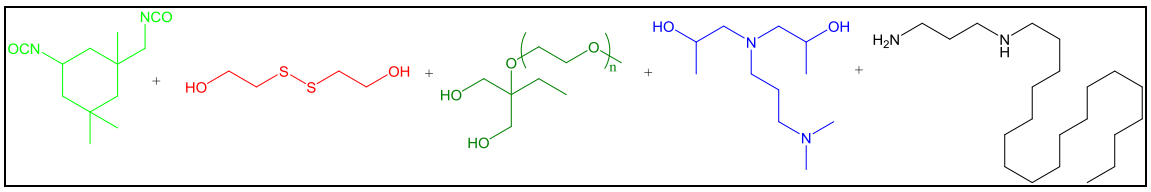
Synthesis of non-labile amphiphilic and amphoteric prepolymer (P3)

Monomer	% by weight
1,6-hexanediol	2.705
Ymer N-120	41.166
Jeffcat DPA	3.461
IPDI	29.571
TAP 100 D	23.097

Figure S1. Composition of each polymer in % by weight.

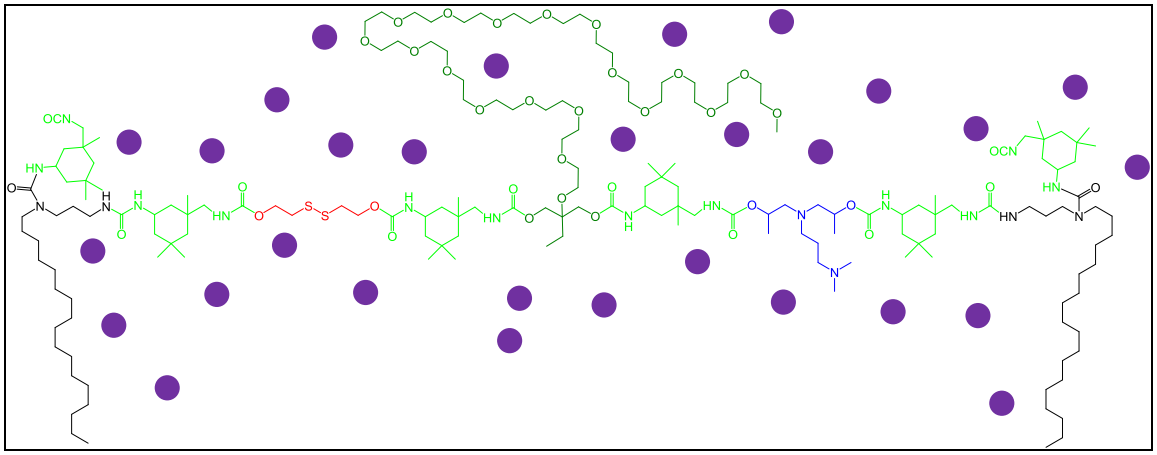
Nanoencapsulation process

(A)

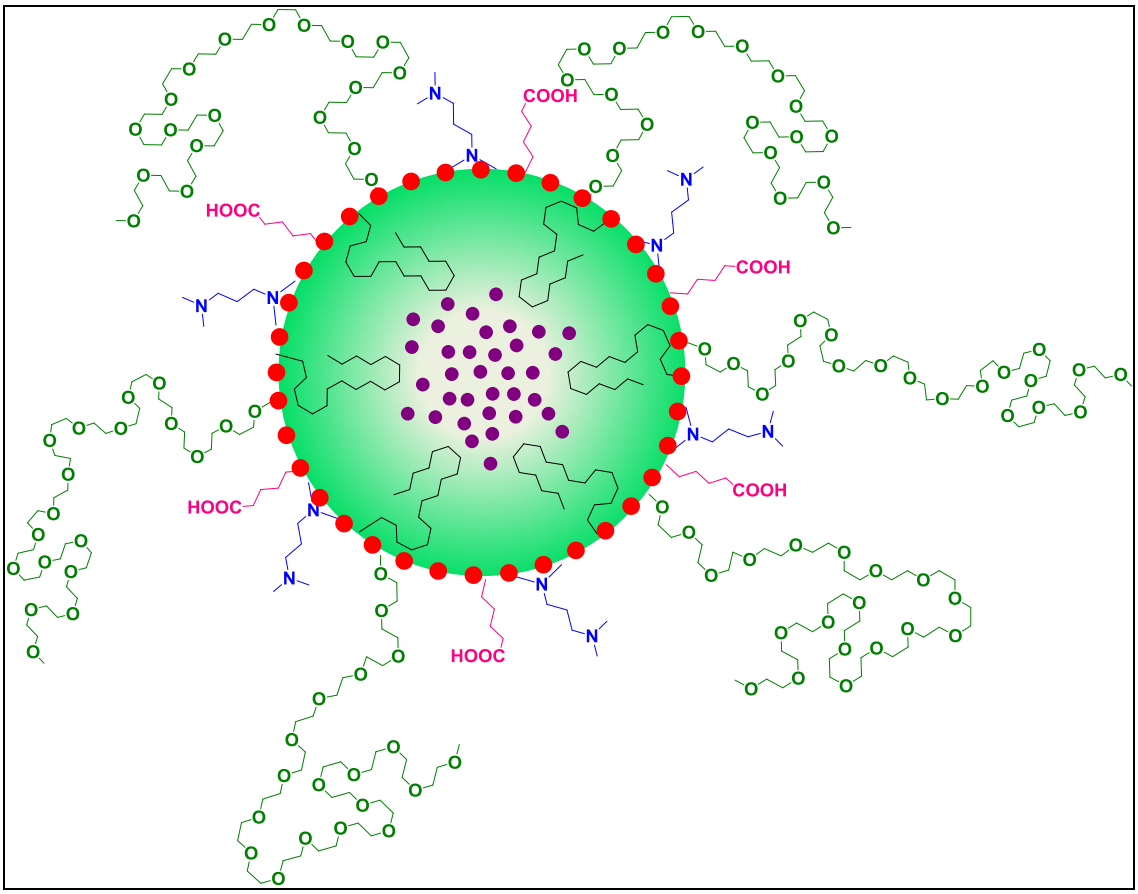


(B)

(C)



(D)



(E)

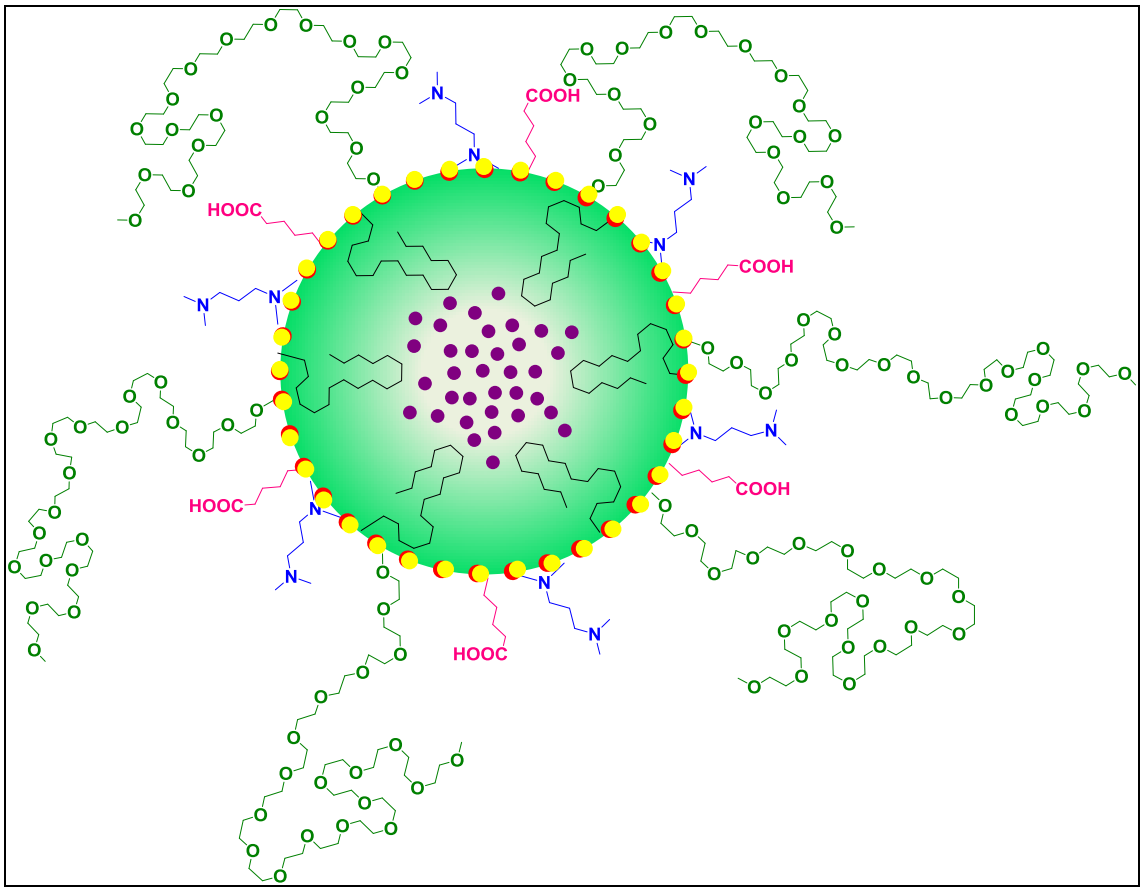


Figure S2. Main species present in each step. (A) The monomers are loaded; (B) the amphiphilic prepolymer is formed; (C) the desired hydrophobic drug (purple dots) is added into the polymeric mixture; (D) an aminic ionomer is introduced and cold water is added and the nanoemulsion is formed; (E) a polyamine (yellow dots) is added to crosslink the nanocapsules.

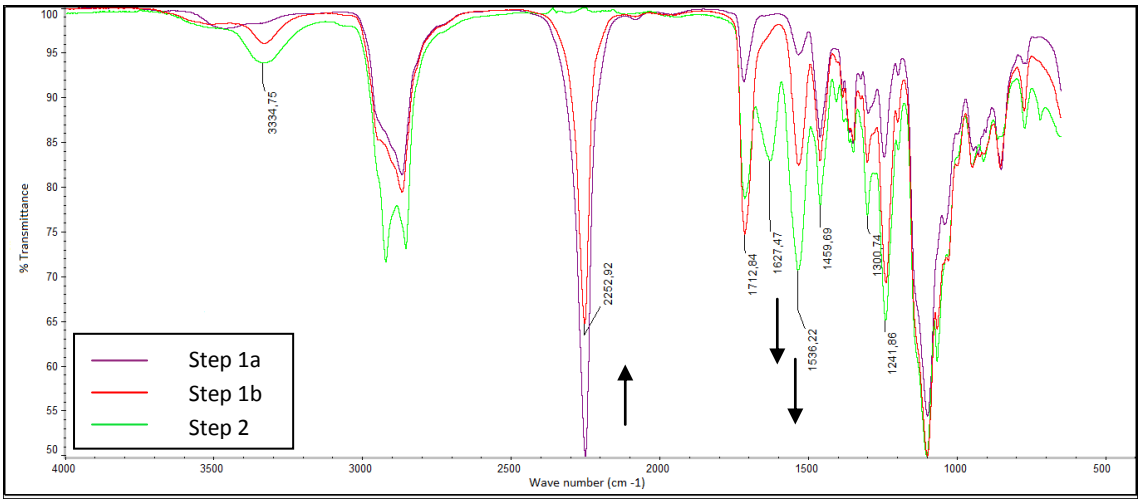


Figure S3. FT-IR spectrum corresponding to steps 1a, 1b and 2 of the synthetic process (amphiphilic prepolymer P1 preparation).

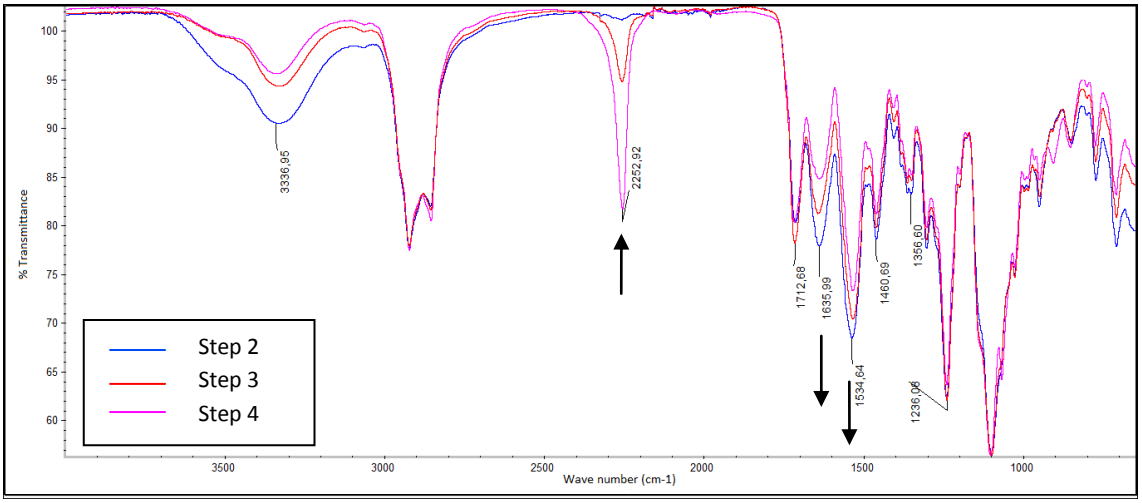


Figure S4. FT-IR spectrum corresponding to steps 2, 3 and 4 of the nanoencapsulation process (amphiphilic prepolymer P1 preparation).

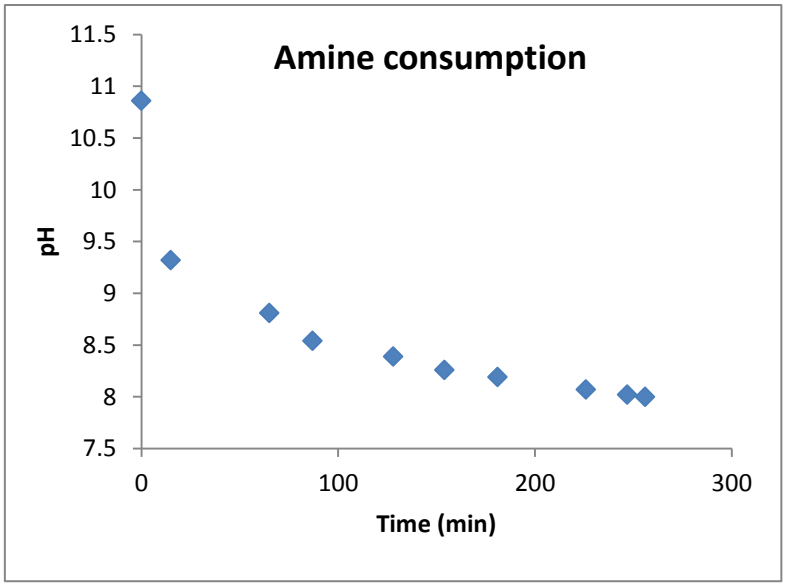


Figure S5. Measurement of pH over time after crosslinker addition.

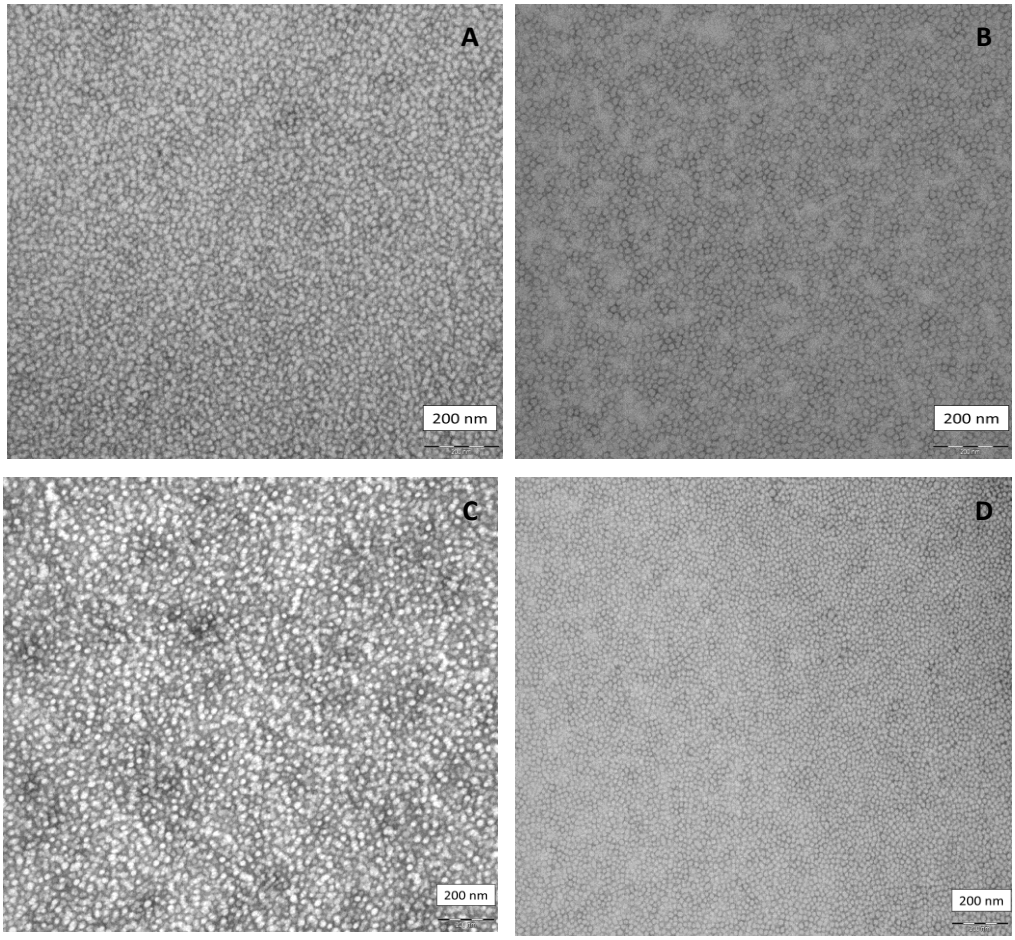


Figure S6. TEM micrographs of nanocapsules loaded with: (A) paclitaxel, (B) curcumin, (C) HL3 and (D) HL4.

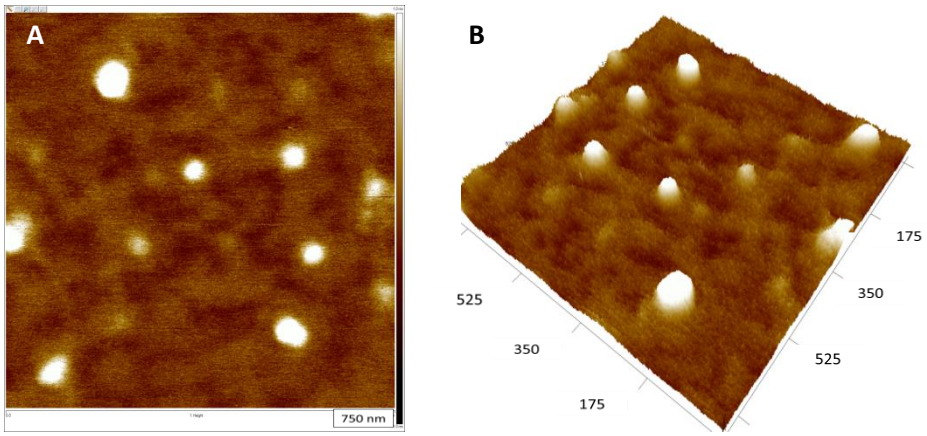


Figure S7. AFM micrographs of nanocapsules loaded with paclitaxel in: (A) 2D and (B) 3D; scale bar in nanometres.

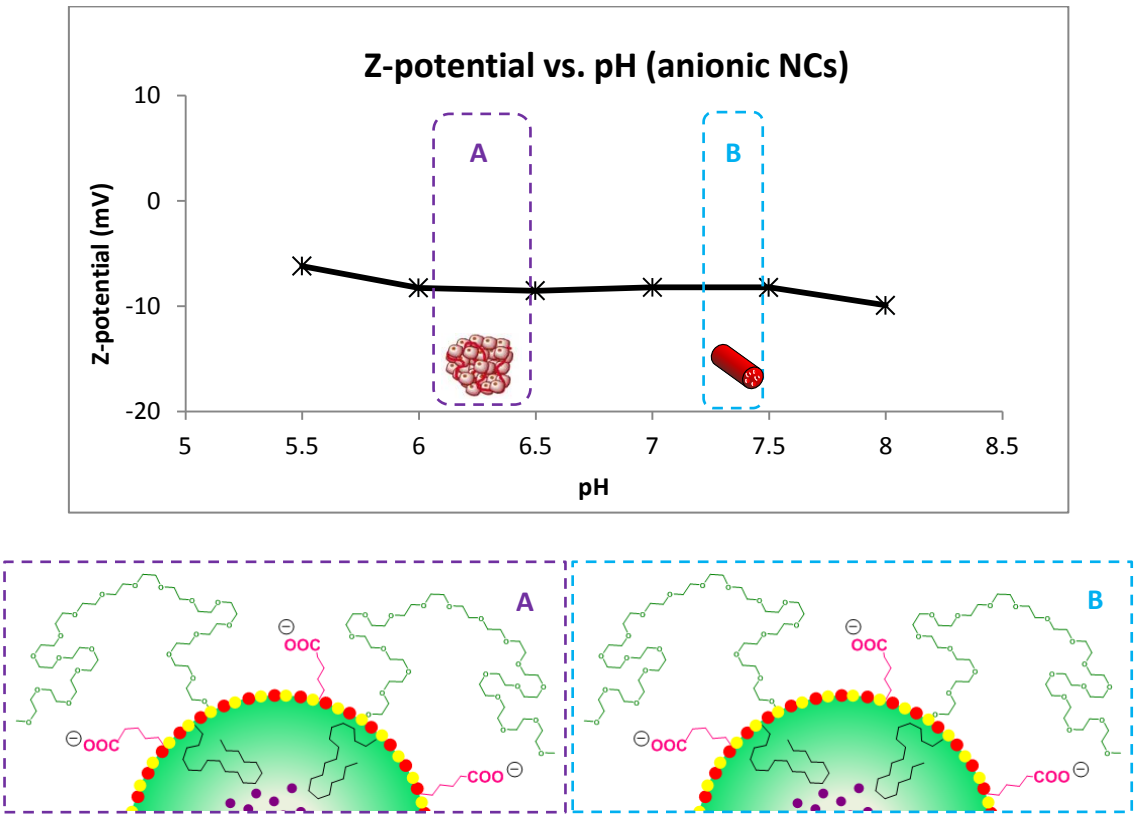


Figure S8. Z-potential measurements at different pH conditions of amphiphilic anionic nanocapsules (P2-drug loaded anionic NCs) and surface charge at: (A) extracellular microenvironmental pH and (B) blood pH.

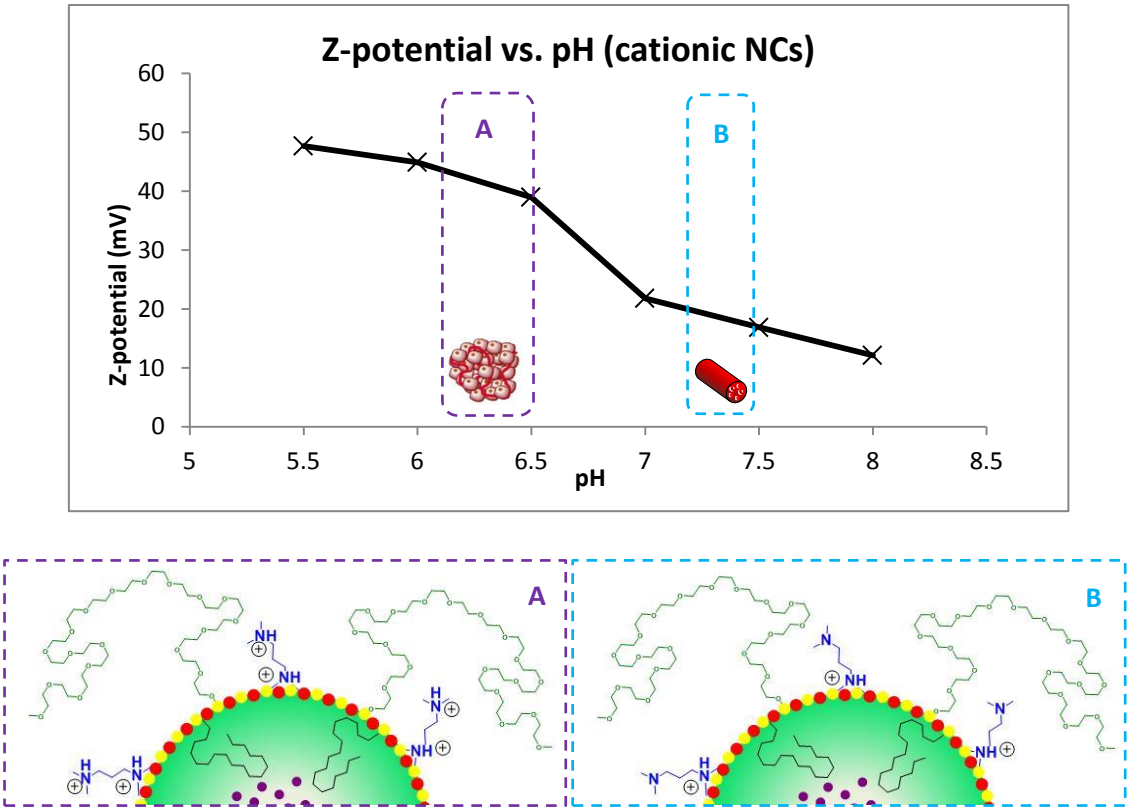


Figure S9. Z-potential measurements at different pH conditions of amphiphilic cationic nanocapsules (P1-drug-loaded cationic NCs) and surface charge at: (A) microenvironmental extracellular pH and (B) blood pH.

$$\% EE = \frac{amount\ of\ drug\ incorporated\ in\ the\ nanocapsules}{total\ amount\ of\ drug\ added\ in\ the\ aqueous\ dispersion} \cdot 100$$

$$\% DL = \frac{amount\ of\ drug\ incorporated\ in\ the\ nanocapsules}{total\ amount\ of\ freeze-dried\ nanocapsules} \cdot 100$$

Figure S10. Formulae to calculate %EE and %DL of the nanocapsules.

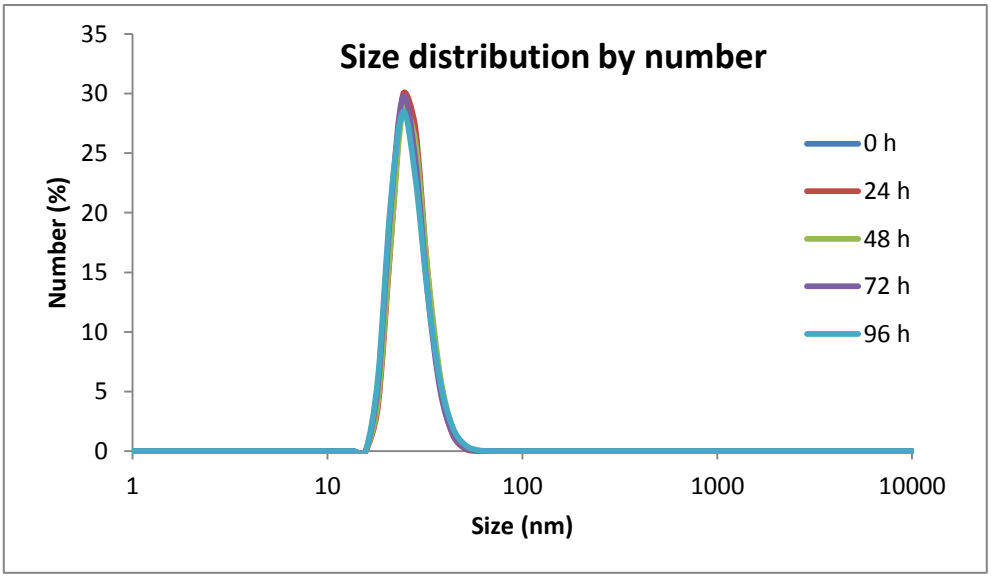


Figure S11. Size distribution by number of nanocapsules under control conditions (PBS), measured at different periods of time.

Fluorescence studies

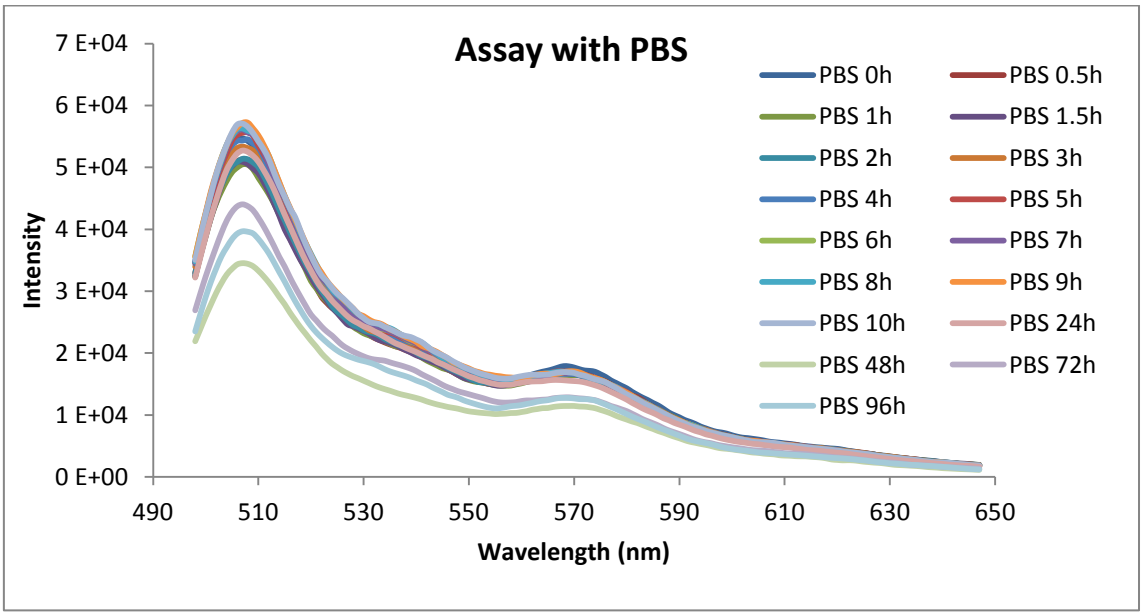


Figure S12. Fluorescence studies with DiO- and DiI-loaded NCs in control conditions (PBS).

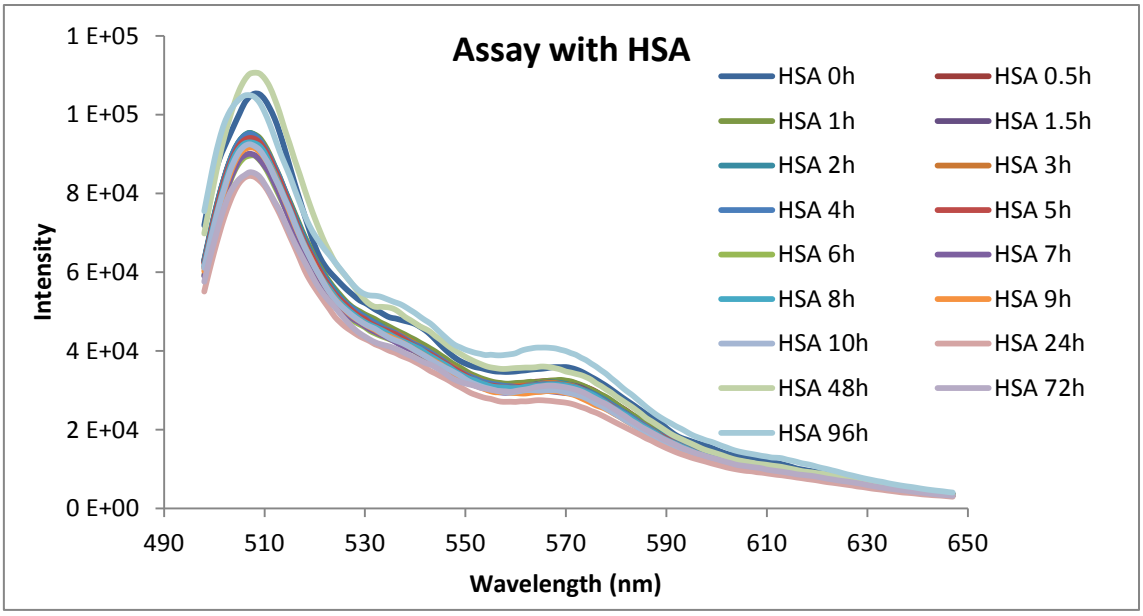


Figure S13. Fluorescence studies with DiO- and DiI-loaded NCs in HSA.

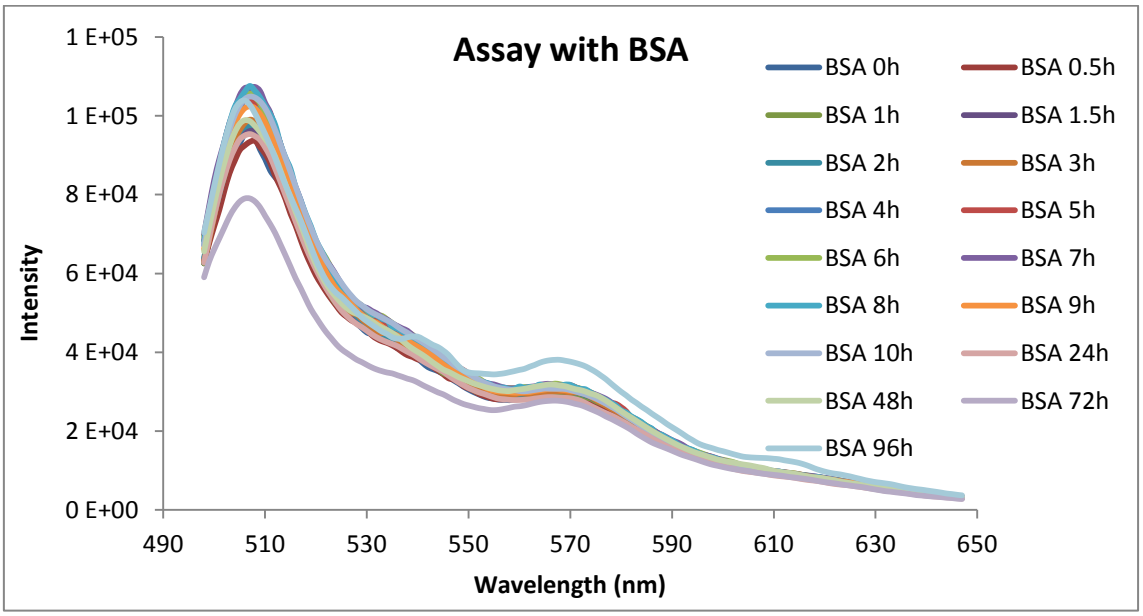


Figure S14. Fluorescence studies with DiO- and DiI-loaded NCs in BSA.

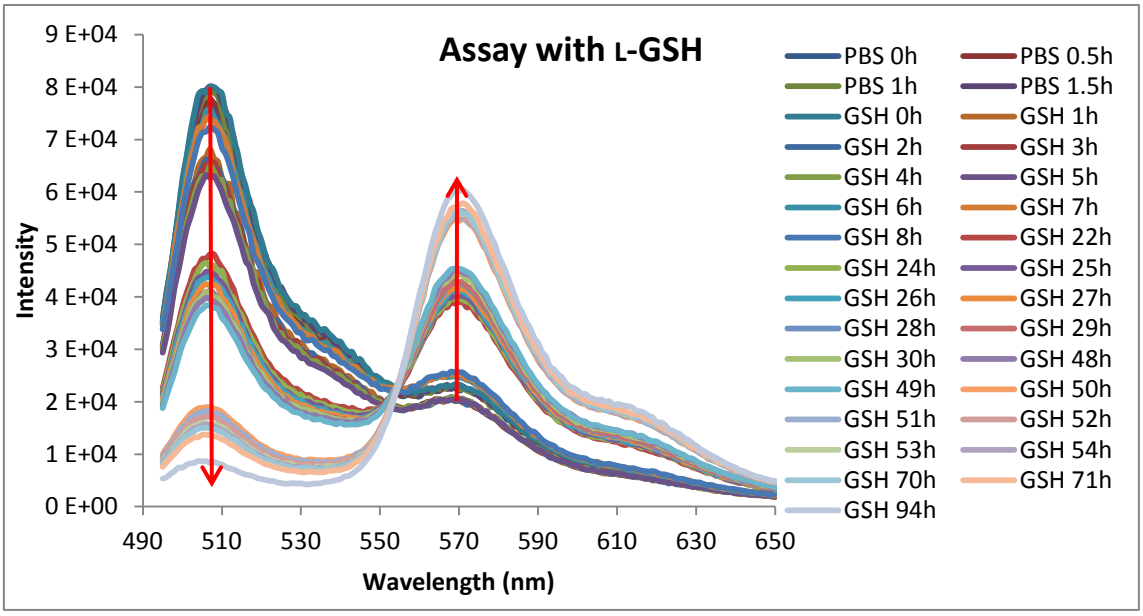


Figure S15. Fluorescence studies with DiO- and DiI-loaded NCs in reductive conditions (L-GSH 10 mM).

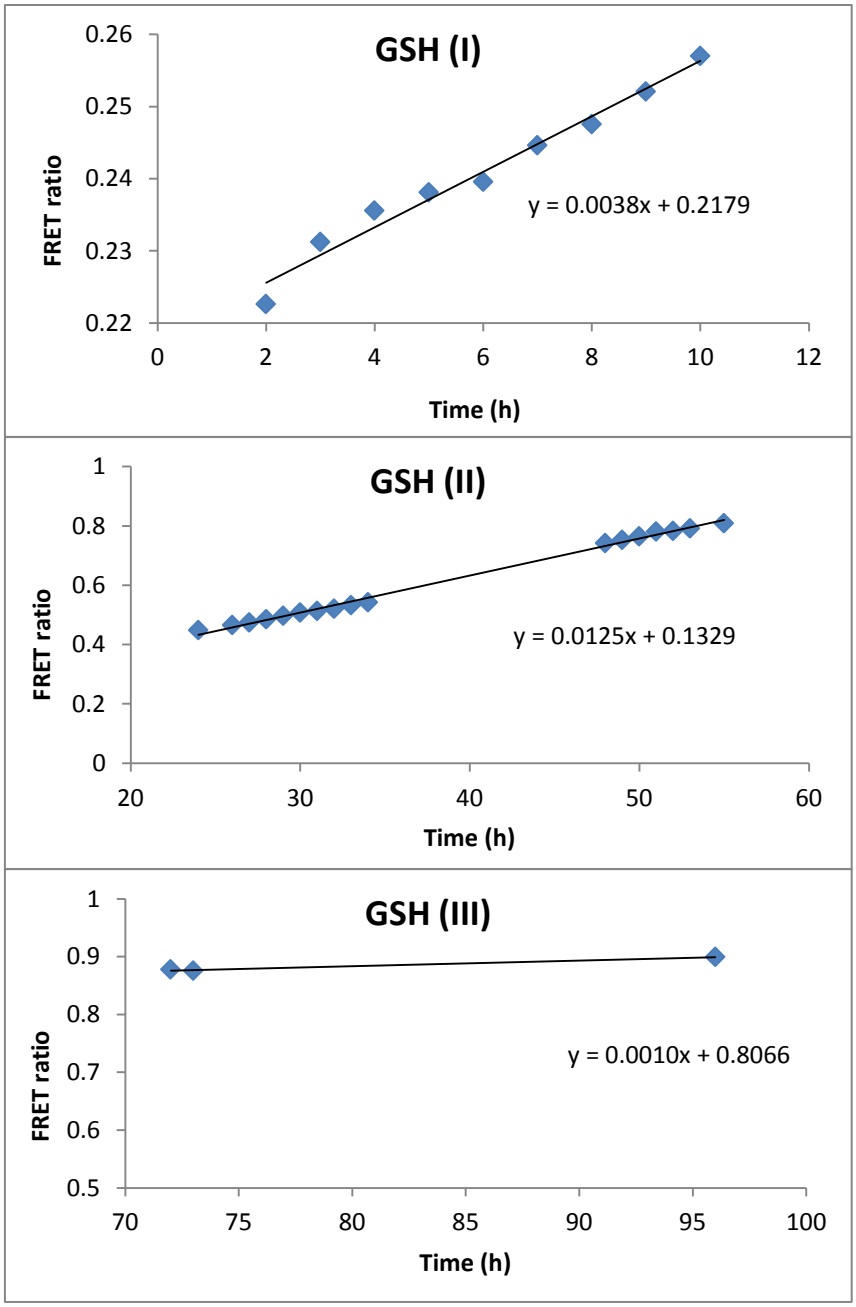


Figure S16. Slope calculated from FRET ratio over time with DiO- and DiI-loaded NCs in reductive conditions (L-GSH 10 mM)

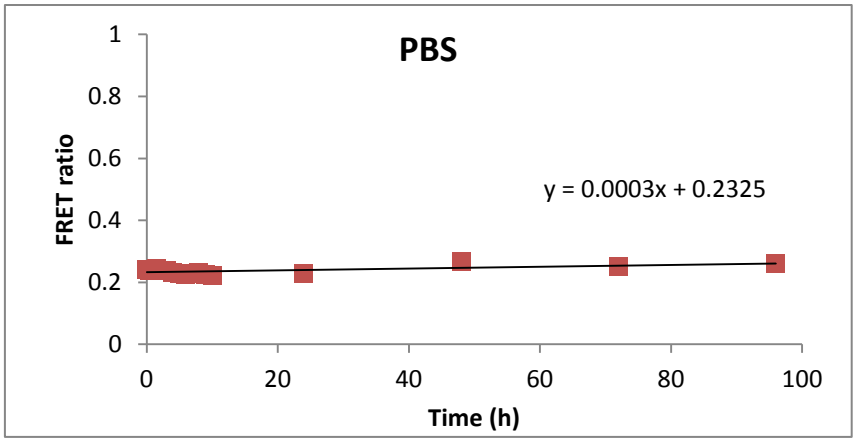


Figure S17. Slope calculated from FRET ratio over time with DiO- and DiI-loaded NCs under control conditions (PBS).

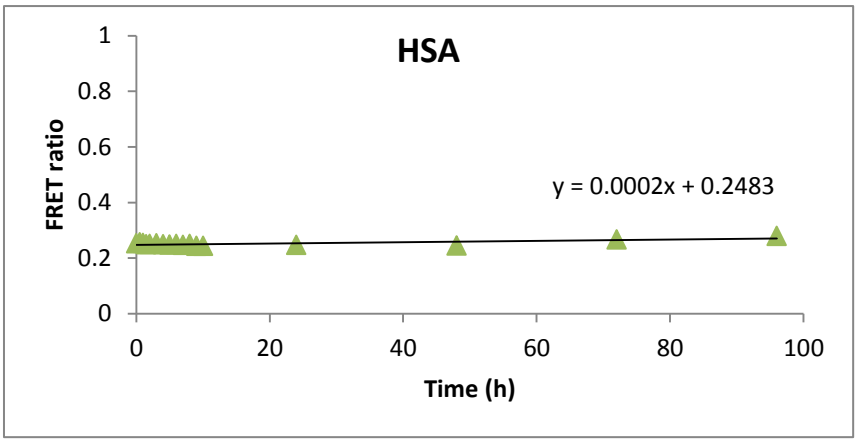


Figure S18. Slope calculated from FRET ratio over time with DiO- and DiI-loaded NCs in HSA.

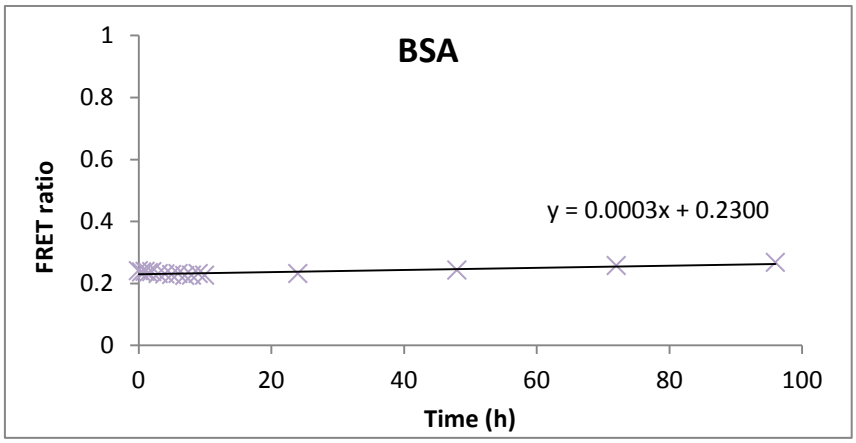


Figure S19. Slope calculated from FRET ratio over time with DiO- and DiI-loaded NCs in BSA.

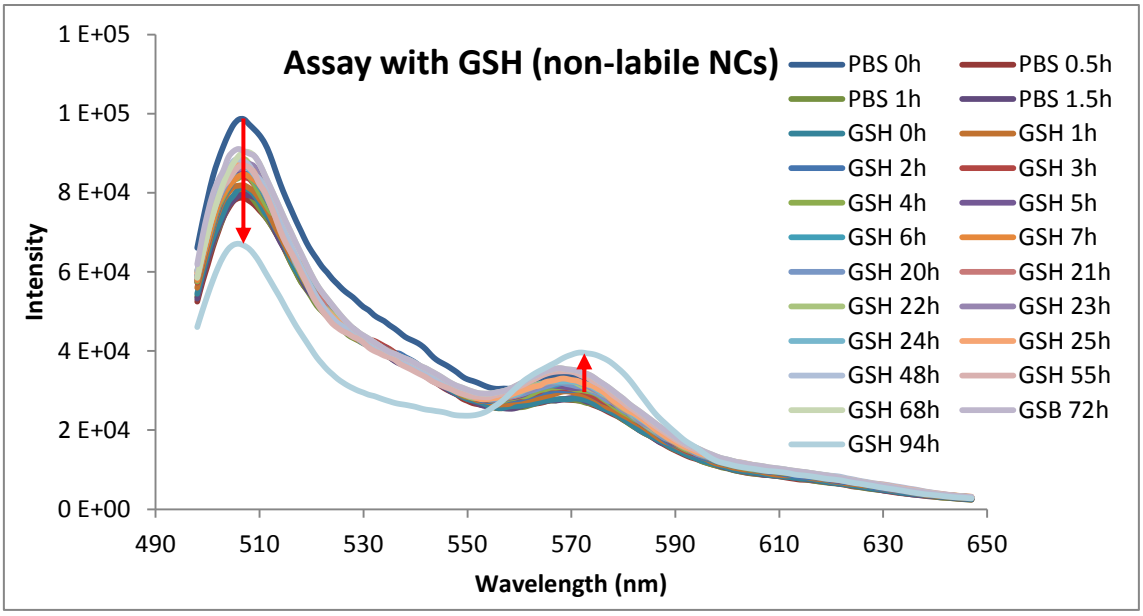


Figure S20. Fluorescence studies with non-labile DiO- and DiI-loaded NCs under reductive conditions (L-GSH 10 mM).

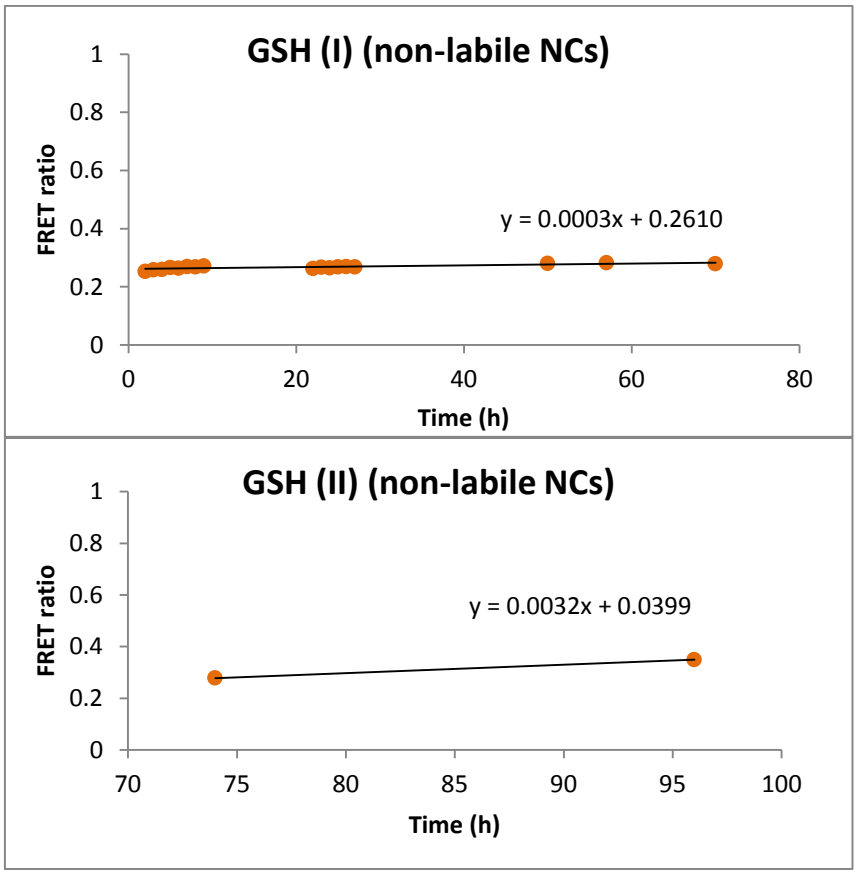


Figure S21. Slope calculated from FRET ratio over time with non-labile DiO- and DiI-loaded NCs under reductive conditions (L-GSH 10 mM).

3. References

[1] US Environmental Protection Agency_Diisocyanates Toxicology.

http://www.epa.gov/oppt/auto/profile/toxicology1a.pdf (accessed May 20, 2016).

[2] L.T. Budnik, D. Nowak, R. Merget, C. Lemiere and X. Baur, *J. Occup. Med. Toxicol.,* **2011**, *6*, 9.