Supporting Information

Environment-responsive aza-BODIPY dyes quenching in water as potential probes to visualize the i*n vivo* fate of lipid-based nanocarriers

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Methods

*Materials*

Precirol ATO 5 was kindly provided by Gattefosse´ Co. (Saint Priest, Cedex, France). Lecithin (Lipoid E100, containing 100% of egg phosphatidylcholine) was purchased from Lipoid GmbH Company (Ludwigshafen, Germany). Sodium taurocholate was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Lipase from porcine pancreas and Tris-maleate were purchased from Sigma-Aldrich Co. (St. Louis, USA). SIF powder was purchased from Biorelevant.com (Croydon, United Kingdom). Tween 80 and other inorganic salts were supplied by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). DiR iodide was purchased from Fanbo biochemicals Co. (Beijing, China). Deionized water was prepared by a Milli-Q purification system (Millipore, USA). All solvents used for UV and fluorescence measurements were of either spectroscopic grade or HPLC grade and purchased from Acros, Aldrich or Fluka.

*ACQ effects in water*

For preparation of the test solutions, 0.5 mM P1 and 1 mM P2 / P3 / P4 solution were first prepared by dissolving in acetonitrile. A series of test solvents were prepared by adding different volumetric percentage of water (1%, 10%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, and 99%) to the acetonitrile solution. Then, the test solutions were prepared by diluting 50 μL 0.5 mM P2 or 1 mM P2 / P3 / P4 solution to 5 mL using series of test solvents.

*ACQ effects in various aqueous environments*

Stock solution of the dyes was prepared by dissolving each of the dyes in acetonitrile to a concentration of 20 μg/mL. Then 20 μL of the stock solution was diluted by 1 mL of the following media: ultrapure water, pH 1.2 hydrochloric acid solution (HCI), pH 4.5 acetate buffer solution (ABS), pH 6.8 and pH 7.4 phosphate buffer solutions (PBS), 1% poloxamer 188 solution, 1% Tween 80 solution, 15 mM sodium taurocholate (STC) solution, 3.75 mM lecithin, fasted-state simulated gastric fluid (FaSSGF), simulated gastric fluid (SGF), simulated intestinal fluid (SIF), fasted-state simulated intestinal fluid (FaSSIF), fed-state simulated intestinal fluid (FeSSIF), respectively. After vortex mixing for 10 s，all the dispersed dye solution were kept in a water bath at 37±0.5°C, and the fluorescence intensity was measured at 0, 4, 8, 12 and 24 h by Cary Eclipse fluorospectrophotometer (Agilent, USA). All the buffers, SGF and SIF were prepared according to USP35/NF30, the bio-relevant media FaSSGF, FaSSIF and FeSSIF were made from a patented SIF Powder (biorelevant.com) with vender proposal. Fed-state simulated gastric fluid (FeSSGF) was not included in our investigation because its formulation was comprised of considerable amount of milk which could disturb fluorescence measurement.

*Preparation and characterization of SLNs*

P2-SLNs were prepared by a modified hot high-pressure homogenization method using Precirol ATO 5 (mainly glycerol distearates) as the lipid and Tween 80 as the surfactant.ATO 5 (2.5 g ) was heated to melt at 70°C, then 0.1 mM dichloromethane (DCM) solution of P2 (1.5 mL) was added into the melt lipid and mixed homogeneously. After the evaporation of DCM, the melted mixture was dispersed into 50 mL water (70°C, containing 1 g Tween-80) under high shear at 5000 r/min for 1 min by a XHF-D high-speed inner cutting homogenizer (SCIENTZ biotechnology co., LTD, Zhejiang, China). Then, the obtained primary emulsion was homogenized using a micro-jet homogenizer (Nano DeBEE, USA) under 20,000 psi for ﬁve cycles. The resulting hot O/W nanoemulsions were cooled down to room temperature by a water bath, then the lipids was solidiﬁed and P2-SLNs was formed. DiR-SLNs were prepared and characterized using the same methods, with P2 replaced by equimolar DiR, a commercial NIR dye.

The particle size was measured by Zetasizer Nano® (Malvern Instruments, Malvem, UK) equipped with a 4-mW He-Ne laser (633 nm) at 25°C.The SLN suspension was diluted by 5-folds with ultrapure water before measurement. The entrapment efficiency ( EE% ) was measured using ultrafiltration cells (Amicon®Ultra-0.5, USA). Briefly, 0.4 mL of the SLN suspension was added into the ultrafiltration cell. After centrifuging at 12000 g for 5 min, 0.2 mL of outer aqueous phase was taken out and processed with a conventional liquid-liquid extraction method, using coumarin 6 as internal standard and diethyl ether as extract solvent. Finally, the re-dissolved acetonitrile solution of P2 was determined by Cary Eclipse fluorospectrophotometer, and the amount of free P2 (*W*f) was calculated. The total amount of P2 in the formulation was represented with *W*t, Then EE% could be calculated as EE (%) = (*W*t - *W*f)/*W*t × 100%. The morphology of the NLCs was observed using a transmission electron microscope (TEM) (JEM-1230 Electron microscope, JEOL, Japan). The samples were negatively stained with 1% (w/v) uranyl acetate and placed on copper grids for TEM observation. DiR-SLNs were prepared and characterized using the same methods, with P2 replaced by equimolar DiR.

*In vitro lipolysis of SLNs*

*In vitro* lipolysis study of SLNs was performed according to a reported method with modifications in three digestive media of different bile salts and phospholipid content. The media were composed of pH 7.5 buffer (50 mM Tris-maleate, 150 mM NaCl, 5 mM CaCl2) and STC/lecithin in the ratios of 6.25 mM / 1.563, 25 mM / 6.25 mM and 0 mM / 0 mM for medium A, B and C, respectively. Briefly, add 10 mL P2-SLNs or DiR-SLNs suspension into 30 mL medium, adjust pH to 7.50±0.05 and preheat to 37±0.5°C. Then 10 mL porcine pancreatic lipase solution (1000 tributyrin units/mL) was added to initiate digestion. The reaction process was continuously monitored using a pH-stat titrator (TitraLab® 854, Radiometer Analytical) under stirring at 37±0.5°C, maintaining the pH at 7.5 by automatic compensation of 0.2 M NaOH. At definite time intervals, 1mL reaction sample was withdrawn, and the lipase activity was immediately terminated by addition of 10 μL 0.5 M 4-bromophenylboronic acid methanol solution. Then the samples were transferred to 96-well plates with a pipette, 200 μL per cell, three wells per sample. Immediately, the fluorescence of the samples were measured by the IVIS spectrum live imaging system (Caliper LifeSciences) with excitation/emission wavelength set at 710/760 nm for P2-SLNs and 745/800 nm for DiR-SLNs, respectively. Photos were captured under automatic exposure mode, regions of interest (ROI) were drawn over the fluorescent signals in photos, and average radiant efficiency (ARE) in the unit of (p/sec/cm2/sr)/(μW/cm2) within the ROIs were measured by vendor software for subsequent quantitative analysis. Each experiment was repeated three times and data were presented as mean ± SD.

**Table S1** Fluorescence intensity at different time after dispersing P1, P2, P3 or P4 acetonitrile solution into different media (Mean±SD, *n*=3)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Media | Dye | Time (h) | | | | |
| 0 | 4 | 8 | 12 | 24 |
| Acetonitrile | P1 | 230.7±2.6 | 228.8±1.9 | 224.9±3.1 | 229.5±2.5 | 226.4±2.9 |
|  | P2 | 275.0±3.2 | 272.2±2.6 | 269.7±3.8 | 273.5±3.6 | 272.0±2.5 |
|  | P3 | 280.4±2.4 | 283.7±2.5 | 288.4±2.9 | 285.1±2.0 | 285.8±1.7 |
|  | P4 | 775.3±9.4 | 777.1±10.3 | 772.6±8.7 | 779.5±7.9 | 774.8±7.3 |
| Water | P1 | 0.435±0.025 | 0.521±0.031 | 0.509±0.025 | 0.514±0.018 | 0.418±0.028 |
|  | P2 | 0.528±0.042 | 0.404±0.021 | 0.462±0.031 | 0.211±0.096 | 0.313±0.057 |
|  | P3 | 0.787±0.037 | 0.625±0.035 | 0.945±0.084 | 0.897±0.064 | 1.023±0.097 |
|  | P4 | 2.174±0.187 | 3.192±0.201 | 2.644±0.208 | 2.801±0.145 | 2.783±0.215 |
| 1%Tween80 | P1 | 56.3±7.7 | 52.8±8.2 | 53.9±6.9 | 51.9±7.6 | 57.4±8.1 |
|  | P2 | 88.1±15.4 | 88.5±11.2 | 89.5±10.8 | 83.6±8.9 | 87.1±8.2 |
|  | P3 | 99.4±10.6 | 103.5±9.7 | 105.3±8.8 | 104.7±7.2 | 103.4±6.6 |
|  | P4 | 457.8±18.7 | 462.4±23.1 | 459.2±21.9 | 468.2±28.3 | 465.8±22.8 |
| FaSSIF | P1 | 16.2±5.4 | 21.4±3.7 | 22.8±2.8 | 20.8±2.1 | 21.7±2.6 |
|  | P2 | 24.7±5.8 | 26.0±5.1 | 27.2±5.4 | 28.4±4.5 | 29.2±4.0 |
|  | P3 | 30.8±4.8 | 32.7±5.4 | 29.9±4.2 | 33.3±3.9 | 30.7±2.8 |
|  | P4 | 136.5±12.6 | 133.9±10.8 | 138.4±9.7 | 140.2±8.8 | 139.4±6.9 |
| FeSSIF | P1 | 60.2±5.3 | 61.5±3.8 | 59.9±4.4 | 60.8±4.6 | 62.3±3.4 |
|  | P2 | 95.2±9.3 | 97.7±10.2 | 100.2±8.8 | 102.2±7.4 | 102.9±6.5 |
|  | P3 | 114.5±7.9 | 116.8±7.9 | 119.4±6.2 | 116.8±7.7 | 118.6±6.3 |
|  | P4 | 480.2±16.4 | 476.8±17.8 | 482.3±20.4 | 481.4±22.3 | 483.1±19.8 |



**Figure S1** Fluorescence emission (a) and visible absorption (b) spectra of P1 in acetonitrile-water binary solution at different ratios (5×10-6 M at 25°C upon illumination at 690 nm). The inset shows the peak value of fluorescence intensity changes upon dilution by water.

**Figure S2** Fluorescence emission (a) and visible absorption (b) spectra of P3 in acetonitrile-water binary solution at different ratios (1×10-5 M at 25°C upon illumination at 630 nm). The inset shows the peak value of fluorescence intensity changes upon dilution by water.

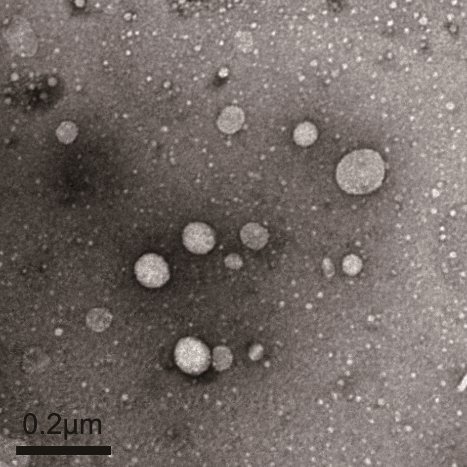
**Figure S3** Fluorescence emission (a) and visible absorption (b) spectra of P4 in acetonitrile-water binary solution at different ratios (1×10-5 M at 25°C upon illumination at 600 nm). The inset shows the peak value of fluorescence intensity changes upon dilution by water.



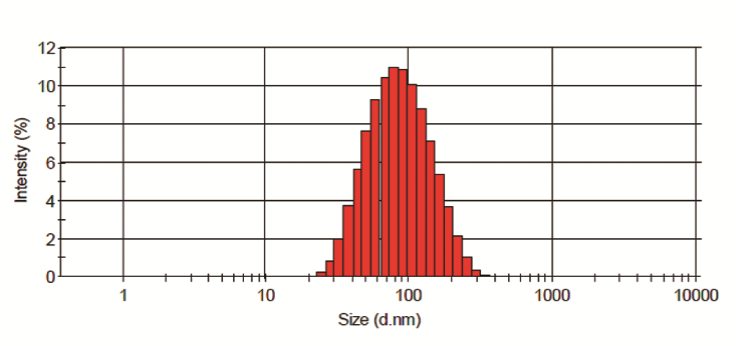
**Figure S4** Fluorescence quenching after dispersing P1,P2,P3 or P4 acetonitrile solution into different aqueous media.



**Figure S5** Fluorescence rekindling after dispersing quenched (a) P1 (b) P3 (c) P4 solution into different media (final concentrations of P1, P3, P4 are all 6.85×10-7 M at 25°C).



**Figure S6** TEM picture of P2-SLNs.



**Figure S7** Particle size distribution of P2-SLNs by intensity.



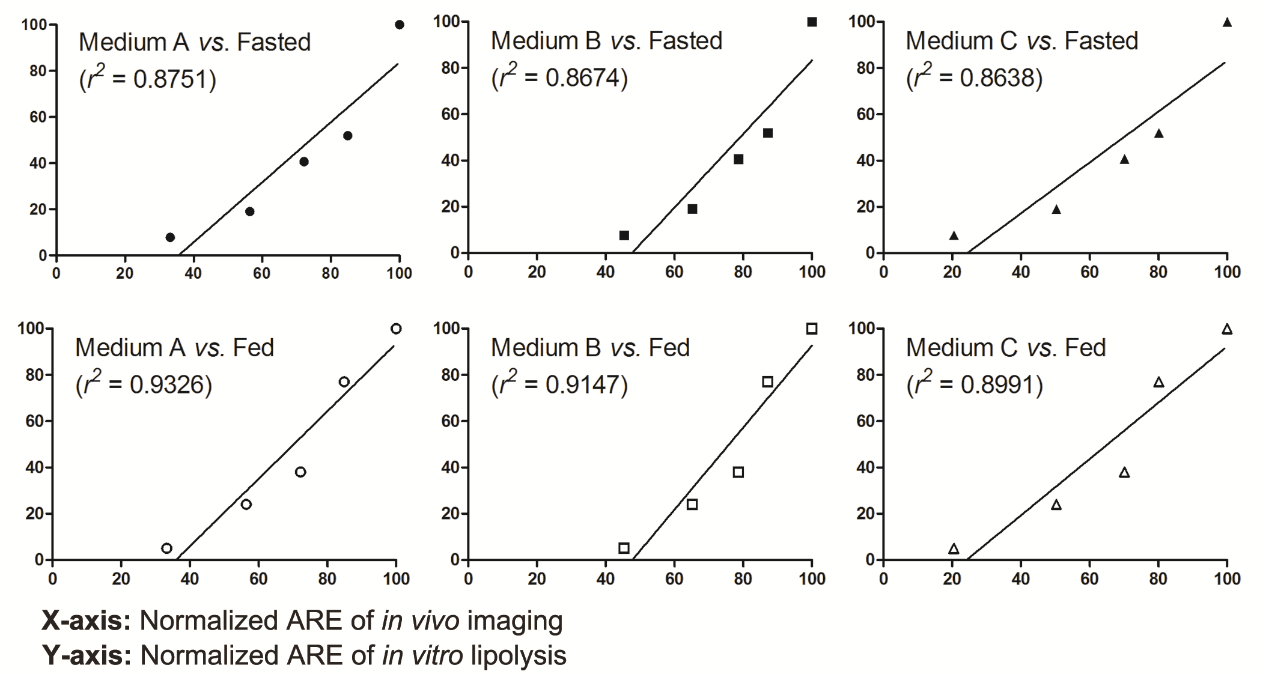
**Figure S8** PL intensity changes of P2-SLNs incubated in (a) buffer solutions of different pH and (b) different surfactant solutions.



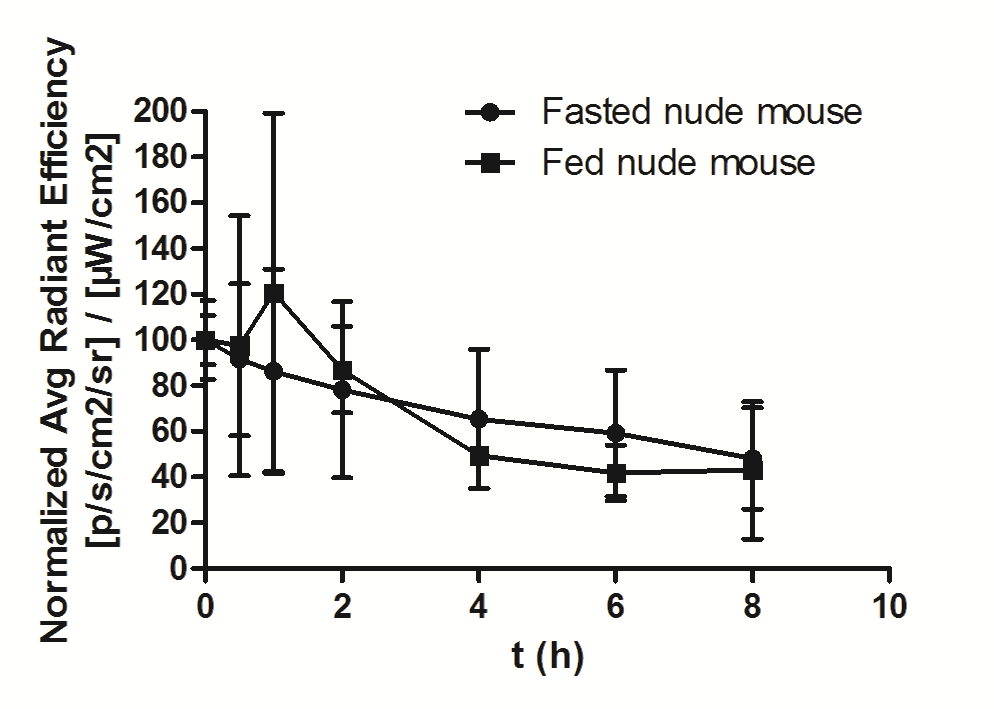
**Figure S9** The real time consumption NaOH during in vitro lipolysis of P2-SLNs.



**Figure S10** Chemical structure of DiR iodide.



**Figure S11.** The point-to-point linear correlation between ARE of *in vitro* lipolysis in different media and that of *in vivo* imaging.



**Figure S12.** The ARE of nude mice gavaged with DiR-SLNs *versus* time.