Electronic Supplementary Information

Selective Recognition of Isozymes by Polymerized Liposomes Incorporating Lanthanide Ions

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Synthesis of polymerizable inhibitor lipid 3:

4-Carboxy benzenesulfonamide (605 mg, 3 mmol) was dissolved in CHCl₃/DMF (30/20 mL). BOP reagent (1.329 g, 3 mmol) was added to it followed by triethylamine (1.5 mL, 10.7 mmol). The reaction mixture was stirred at room temperature for 10 min and the mono-BOC protected amine¹ (1.056 g, 3.3 mmol) was added. The reaction mixture was stirred overnight. The reaction was quenched with brine and the solvent was evaporated under reduced pressure. More water (75 mL) was added and aqueous phase extracted with ethyl acetate. The organic layer was washed with 4% citric acid, 4% aqueous sodium bicarbonate and finally with water. Evaporation of the solvent and purification through silica gel column chromatography (10% MeOH in CHCl₃, Rₚ = 0.3) afforded the pure compound (1.46 g, 97%) as a colourless syrup.

¹H NMR (CDCl₃/CD₃OD, 500 MHz): 7.90-7.96 (m, 4H), 3.63-3.66 (m, 6H), 3.58-3.60 (m, 2H), 3.54-3.57 (m, 2H), 3.50-3.51 (m, 2H), 3.45-3.48 (t, 2H, J = 12 Hz), 3.12-3.15 (m, 2H), 1.89-1.93 (m, 2H), 1.68-1.72 (m, 2H), 1.41 (s, 9H).

The BOC-protected amine sulfonamide (400 mg, 0.79 mmol) was taken in 4 N HCl (10 mL) in dioxane and stirred for 2 hours at room temperature. The solvent was evaporated under reduced pressure and the resultant waxy solid was dried under vacuum. This compound was found to be sufficiently pure (Yield: 347mg, 99%) and was taken to the next step directly.

¹H NMR (D₂O, 500 MHz): 7.87 (d, 2H, J = 8.5 Hz), 7.77 (d, 2H, J = 8.5 Hz), 3.45-3.52 (m, 12H), 3.34-3.36 (m, 2H), 2.93-2.96 (m, 2H), 1.76-1.79 (m, 4H).

The compound 6 (300 mg, 0.68 mmol) was reacted with the diamide of 2,3-diaminopropanoic acid ⁷ (557 mg, 0.68 mmol) using BOP as the coupling reagent. The reaction and the workup procedures were the same as described for 5. The crude product was purified by silica gel column chromatography with 10% MeOH in CH₂Cl₂ as the eluant (Rₚ = 0.4) to afford the pure lipid 3 (465 mg, 57%) as a white solid, m.p. 94-97 °C. The solid was stored under nitrogen at - 20 °C, protected from light. ¹H NMR (CDCl₃, 400 MHz) δ: 7.92-7.98 (m, 4H), 7.74-7.75 (m, 1H), 7.47 (d, 1H, J = 6.4 Hz), 7.10-7.12 (m, 1H), 6.81-6.83 (m, 1H), 6.17(s, 2H), 4.37-4.39 (m, 1H), 3.46-3.69 (m, 16H), 3.23-3.26 (m, 2H), 2.18-2.25 (m, 12H).
1.90-1.92 (m, 2H), 1.65-1.68 (m, 2H), 1.57-1.60 (m, 2H), 1.46-1.53 (m, 8H), 1.20-1.39 (m, 54H), 0.88 (t, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 175.98, 174.89, 170.45, 165.97, 145.08, 138.57, 128.11, 126.56, 71.10, 70.44, 70.33, 70.09, 69.16, 65.52, 65.44, 55.23, 42.16, 39.56, 37.4, 36.70, 36.60, 32.11, 29.84, 29.80, 29.68, 29.53, 29.41, 29.37, 29.29, 29.14, 29.08, 29.01, 28.66, 28.56, 28.54, 25.85, 25.69, 22.89, 19.41, 14.31. Anal. Calcd. for C$_{70}$H$_{115}$N$_5$O$_9$S$_2$O: C, 68.87; H, 9.66; N, 5.74. Found: C, 69.03; H, 9.52; N, 5.61.

**Preparation and polymerization of the liposomes:**

Appropriate volumes of chloroform solution containing 4.75 mg of 1-palmitoyl-2-(10,12-tricosadiynoyl)$\text{-sn}$-glycero-3-phosphocholine and 0.25 mg each of the BS lipid (lipid 3), IDA-Cu$^{2+}$ lipid (lipid 4) and lipid 2.Eu$^{3+}$ were transferred to a round bottom flask. A 9:1 mixture of spectroscopic grade chloroform:methanol (total volume = 20 mL) was added to achieve complete dissolution of the lipids. The organic solvents were removed under vacuum in a rotary evaporator (Yamato® RE 450) at 42°C with rotation of the flask at 220 rpm. After the formation of the thin film, it was kept under vacuum for 24 hr to remove the residual organic solvent.

Buffer (20 mM HEPES; pH = 7.0) was added to the flask to yield total lipid concentration of 0.5 mg/mL. The thin lipid film was detached by continuous rotation at 220 rpm for 1 hr in a water bath at 70°C. The resulting hydrated lipid suspension was sonicated for 1 hr in a heating block at 70°C using a probe sonicator (Branson® 150 continuous pulse level #3). A clear and uniform dispersion of the lipid vesicles was obtained. This solution was filtered through a 220 µm filter to remove any metal particles introduced during probe sonication.

After one hour at room temperature, the suspension of lipid vesicles became hazy, indicating their aggregation into larger vesicles. At this stage, they were extruded through a 100 nm polycarbonate filter (Avanti® Polar Lipids, AL) to obtain large unilamellar vesicles (LUVs). These liposomes were incubated at 85°C for one hour to achieve mixing of the lipid components and then cooled in an ice bath. Polymerization was achieved by UV irradiation at 254 nm for 30 minutes using a 400 W lamp resulting in a color change from clear colorless to pink-violet color. The average size of the polymerized liposomes was determined by transmission electron microscopy to be 50 nm.
**Estimation of Eu^{3+}** concentration on the outside surface of the liposomes:

The concentration of Eu^{3+} (lipid 2. Eu^{3+}) in the final liposome preparation was determined by calculating the molar ratio of specific lipid as follows:

$$0.5 \times \frac{\text{[weight of 2.Eu}^{3+}\text{ added (g)] \times 1000}}{\text{Molar mass of 2.Eu}^{3+}(g) \times \text{volume of liposome solution (mL)}} \quad (1)$$

Where 0.5 reflects the fraction of the total Eu^{3+}-lipid in the outer leaflet of the liposomes. This is the amount of Eu^{3+}-lipid available for binding to the enzyme (based on the assumption that our liposomes have transversely symmetrical bilayers). For a unilamellar liposome of 120 nm diameter, considering the bilayer thickness typically being ~ 5 nm, the ratio of inner surface area ($4\pi R^2$) to the outer surface area ($4\pi r^2$) is estimated to be $> 0.9$. Hence, the fraction of the lipid molecules on the outer leaflet for 120 nm diameter liposomes was taken as being equal to 52% of the total lipid. Given that the weight of Eu^{3+}-lipid added was 0.25 mg and the molar mass of this lipid is 1581 g/mol, the concentration in the final liposome preparation was determined to be 7.9 μM. For the spectroscopic experiments, this was diluted in buffer to a concentration of 131 nM.

**Cloning, expression and purification of carbonic anhydrase isozymes:**

Human Carbonic anhydrase isozymes I, II and XII were purified as previously described.\(^2\)\(^3\) The cloning of human Carbonic anhydrase VII was done in our laboratory and will be detailed in an upcoming publication. It was subsequently purified by serial dialysis as described for CA I and II.

**Luminescence titration and determination of excited state lifetime for Eu^{3+}**:

The steady state luminescence experiments were performed using a Photon Technologies International (PTI) Easy Life® instrument with double excitation and emission slits, a 75 W Xenon lamp as excitation source and a gated photo multiplier tube (PMT) detector with wavelength range from 150 to 650 nm.

The interaction of the liposomes with carbonic anhydrase isozymes was monitored by following the increase in luminescence intensity due to the $^5D_0$ to $^7F_2$ transition of the liposomal Eu^{3+} at 615 nm upon sensitization by the ene-yne moiety in the polymerized lipid backbone (excited at 320 nm).

In a typical experiment, liposome solutions containing 8 µg/mL total lipid concentration (260 nM of EDTA-Eu^{3+} lipid) was taken in a 4 mL cuvette and titrated with increasing concentrations of carbonic anhydrase isozymes. The emission spectrum was collected with an integration time of 1 ms after a 150 µs delay to eliminate background organic fluorescence (Figure S1). Protein concentration in all steady state luminescence experiments was kept below 7 µM to avoid inner filter effect.
**Luminescence decay measurements:**

The same instrumental set-up was used for the lanthanide luminescence decay measurements. Luminescence decay of the liposome incorporated Eu³⁺ was measured with liposomes only in standard buffer (25 mM HEPES, pH 7.0). Liposome concentration was maintained at 8.0 µg/mL total lipid. The decays were then measured in the presence of different isozymes of CA.

In these experiments, saturating concentrations of the respective CA isozymes were used, corresponding to 10 times the observed $K_d$ (see Table 1). The excitation wavelength was set at 320 nm while the emission signal at 615 nm was collected in 20 µs increments for 800 µs after an initial delay of 170 µs. The average of one hundred decay curves was taken. The decay curves were fit using the inbuilt exponential decay equations in the PTI analysis software. Single exponential decay equation (equation 1) was attempted for each decay curve, and where the resulting residuals were high, the bi-exponential decay equation (equation 3) was used.

$$y = y_o + A e^{-x/t} \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots
Figure S 1. Time-resolved emission spectra of Eu$^{3+}$-incorporated polymerized liposomes ($\lambda_{ex} = 320$ nm) as a function of increasing concentrations of CA I, CA II, CA XII and bovine erythrocyte CA (0 – 10 μM) are shown.

References: