Photo-induced molecular-recognition-mediated adhesion of giant vesicles†

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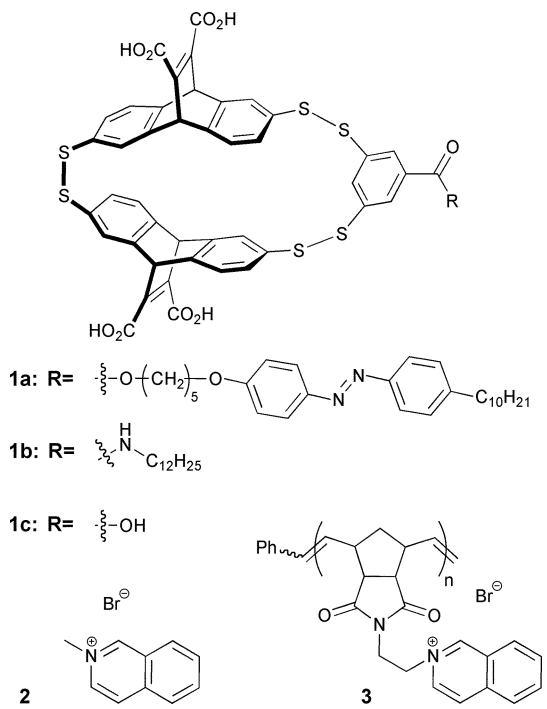
Few methods currently exist for controlling vesicle–vesicle adhesion. We now report a new system, based upon a multivalent guest and an amphiphilic receptor with a photo-isomerisable anchor that can be incorporated into lipid vesicles of different sizes. Large unilamellar vesicles containing our receptor were found to aggregate upon addition of the multivalent guest, independently of photoswitching between the two conformations of the anchor. However, for giant vesicles immobilised on a platinum wire, guest-mediated adhesion only occurred upon photo-isomerisation of the anchor. This behaviour was attributed to the dynamics introduced into the system through the conformational changes caused by irradiation.

Introduction

Tissue organisation and growth depends on well-defined adhesion and aggregation between cells. As cells are vastly complex entities, probing the basics of cellular interactions remains challenging. Large unilamellar vesicles (LUVs; 100–200 nm diameter) provide, in a first approximation, adequate models for biological membranes. The principles behind cellular aggregation have been studied in liposomes using two different strategies. The first employs recognition between vesicles bearing complementary moieties at their surfaces[1] while the other utilises the interaction of vesicle-embedded receptors with molecules in solution.[2] The latter approach is also useful for studying multivalent interactions[3] which occur frequently in biological systems as they allow for tighter binding and more diverse mechanisms.[4]

Although insights have been gained using LUVs, such systems have limitations: only bulk properties are easily studied and direct observation of single events is not straightforward. Giant unilamellar vesicles (GUVs; 5–200 μm diameter) overcome this difficulty as they can be observed directly under a light microscope. They also provide a more realistic model as their size and membrane curvature resemble those of living cells.[5] They have been used successfully for investigating a wide range of events, among them adhesion and fusion.[6] A previous study including both LUVs and giant vesicles found a discrepancy in behaviour between the two systems, that was attributed to the difference in membrane curvature.[1b]

We now report a study of the molecular recognition between a photoswitchable synthetic receptor **1a** incorporated in LUVs and GUVs and monovalent (**2**) and multivalent (**3**) guests. Receptor **1a** is equipped with an azobenzene moiety which rapidly undergoes a conformational change on excitation with long wavelength ultraviolet light and can relax thermally or upon irradiation with visible light. While our system was designed with the aim of achieving photo-induced vesicle fusion, we have thus far not observed any evidence of fusion (*vide infra*). However, we unexpectedly found that the multivalent guest was able to mediate the adhesion between receptor-containing GUVs, but only upon photo-irradiation. In contrast, adhesion between LUVs did not require photo-irradiation.



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Results and discussion

Design of receptor 1a

The design for receptor **1a** is based on disulfide macrocycle **1c** identified using dynamic combinatorial chemistry that was found to bind ammonium ions with micromolar affinity.[7] In order to enable incorporation of this macrocycle into a lipid bilayer it was equipped with a hydrophobic tail containing a photo-isomerisable azobenzene moiety. Although in many cases it has been found to be advantageous to separate the receptor unit from the anchor by a poly(ethylene glycol) chain to facilitate access of the guest, it was unnecessary here, probably due to the high polarity and bulkiness of the receptor.

The tail of **1a** will align well with the lipid alkyl chains in the membrane if the azobenzene moiety resides in the thermodynamically more stable *trans*-conformation. Photo-isomerisation to the *cis*-form, however, will introduce a kink into the hydrophobic anchor of **1a**, likely to disturb the packing of the lipid bilayer.§ Azobenzene-containing amphiphiles have found use in controlling the permeability of membranes[8] and inducing morphological changes in both synthetic[9] and lipid[10] membranes. For example, Hamada *et al.* showed that, under certain circumstances, photo-isomerisation of an azobenzene-containing amphiphile was able to cause the reversible formation of buds in giant vesicles.[10b]

We reasoned that the change in anchor shape upon photo-irradiation in combination with receptor clustering might cause a macroscopic effect that can be observed directly in giant vesicles. Previously reported **1b**[11] was used as a control compound lacking the azobenzene unit.

Aggregation of 1a-containing LUVs

As the binding pocket of receptor **1a** is known to bind isoquinolinium ions like **2**, a polymeric version **3** of this guest was synthesised. The average number of binding units per polymer chain was 81 as determined by gel permeation chromatography, exhibiting a narrow polydispersity (1.05).

LUVs (200 nm diameter) were prepared in phosphate buffer at pH 8 by mixing egg phosphatidylcholine (egg PC) with 5 mol% of amphiphilic receptor **1a**, followed by extrusion through a polycarbonate membrane. The yellow colour of the turbid solution was a first indication of the successful incorporation of water-insoluble **1a** into the lipid bilayer. Subsequent gel filtration showed elution profiles of egg PC (monitored by UV/Vis spectroscopy at 700 nm) and **1a** (monitored by HPLC) that overlay well (see Fig. S1, ESI†), verifying the incorporation of **1a** into the lipid bilayer.

Dynamic light scattering (DLS) showed virtually no difference in size distribution of extruded egg PC liposomes with and without receptor **1a** in either the *trans*- or the *cis*-conformation (see Table 1). Repeated isomerisation cycles could be performed reliably and did not compromise liposome stability (Fig. 1).

Addition of monovalent guest **2** did not induce any change in vesicle size as observed by DLS. However, addition of multivalent **3** resulted in a significant increase in aggregate size only in egg PC liposomes containing **1a**; solutions of egg PC-only liposomes remained unchanged. The contribution of electrostatics to the aggregation process is negligible, as egg PC vesicles containing 5 mol% of the negatively charged palmitoyl-oleoyl-phosphatidyl glycerol (POPG) did not aggregate (Table 1). We therefore conclude that the specific interaction of **3** with membrane-bound *trans*-**1a** is strong enough to overcome repulsion between liposomes and produce larger aggregates.

The binding affinity can be assessed by titrating liposome-embedded **1a** into solutions of monomeric guest **2** or polymeric guest **3**, exploiting the quenching of isoquinolinium fluorescence upon its binding in the cavity of **1a** (Fig. 2). The previously reported binding constant of **1c** and **2** (2.5×10^5 M^{-1}),[7] although measured using different conditions, is similar to the affinity of **2** to **1a** (7.6×10^4 M^{-1}; Table 1).

To analyse the data for the titration of **3** consisting of, on average, 81 isoquinolinium units per molecule we assumed a 1:1 binding model, with 81 copies of **1a** on the lipid bilayer surface forming a multivalent binding unit that interacts with **3** (Fig. 2b).

As anticipated, the apparent binding constant of **3** with a multivalent binding unit of *trans*-**1a** is higher than that for monovalent **2** (Table 1¶).[12] However, the difference is not as

Table 1 Hydrodynamic diameter and apparent binding constants obtained by DLS and fluorescence titration, respectively, using 5 mM egg PC LUVs and, where applicable, 5 mol% **1a** or POPG

	Hydrodynamic diameter [nm]	Apparent binding constant, K_{app} [M^{-1}]
Egg PC	150	n/a
Egg PC + **3**	157	n/a
Egg PC·*trans*-**1a**	142	n/a
Egg PC·*cis*-**1a**	140	n/a
Egg PC·*trans*-**1a** + **2**	145	$(7.6 \pm 0.4) \times 10^4$
Egg PC·*trans*-**1a** + **3**	734	$(10.6 \pm 0.5) \times 10^6$¶
Egg PC·*cis*-**1a** + **3**	803	$(6.9 \pm 0.5) \times 10^6$¶
Egg PC·POPG + **3**	167	n/a

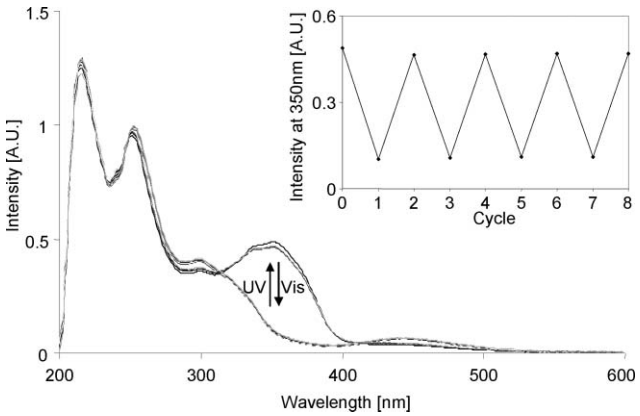

Fig. 1 Isomerisation between *cis*- and *trans*-azobenzene. UV spectra recorded after irradiation of egg PC vesicles containing 5 mol% **1a** with UV or visible light. The inset shows the absorbance change at 350 nm after each irradiation step.

§ As all experiments were performed at room temperature the lipid bilayer will be in the liquid crystalline phase [main phase transition temperature of palmitoyl-oleoyl-phosphatidylcholine (the main constituent of egg PC) is −3 °C]. However, the *cis*–*trans* isomerisation of azobenzene should still disrupt lipid packing.

¶ The values for **3** binding to lipid bilayer-embedded **1a** are given on a 'per ligand' basis. The valence-corrected values are 1.22 M^{-1} and 1.21 M^{-1} for *trans*- and *cis*-**1a**, respectively.

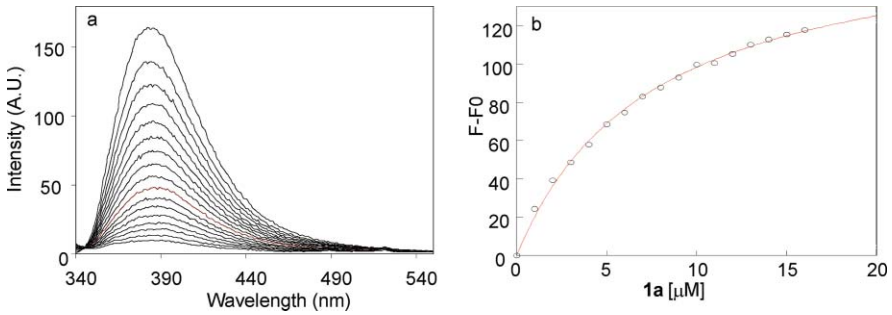

Fig. 2 Fluorescence titration for determining apparent binding constants. (a) Quenching of isoquinolinium fluorescence upon addition of egg PC vesicles containing 5 mol% *trans*-**1a** to a solution of **3**. (b) Fit of the data using a naive 1:1 binding model.

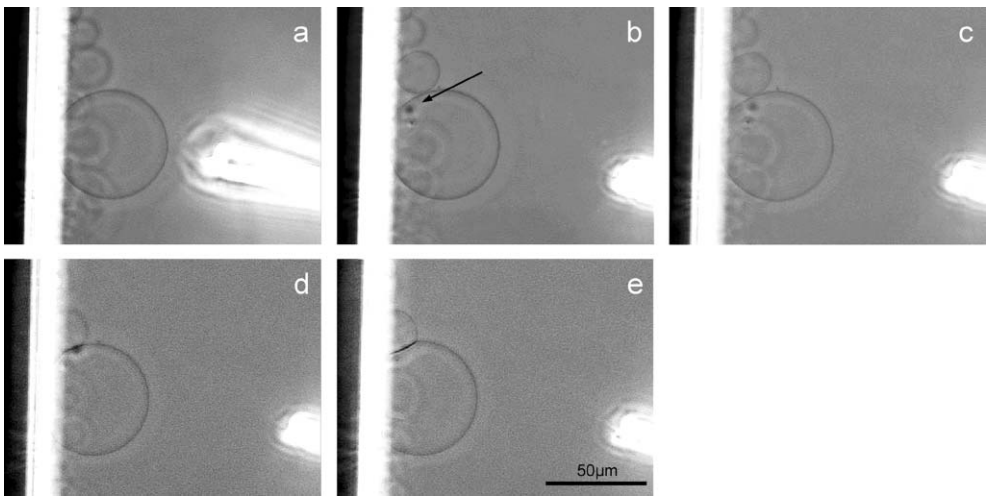

Fig. 3 Light-triggered adhesion of giant vesicles. (a) Vesicles containing 5 mol% **1a** grown on a platinum wire seen as a black shadow with white reflection on the left-hand side of the pictures. The white reflection visible in the right-hand side of the pictures is the micropipette. After injection of a solution of **3** a mobile dark spot can be seen on the vesicle surface (b; $t = 60$ s after injection of **3**, c; $t = 110$ s). Irradiation with UV light (from $t = 119$–125 s after injection of **3**, and again $t = 132$–135s) leads to adhesion of giant vesicles (d; $t = 127$ s, e; $t = 145$ s).

large as may be expected on the basis of strict additivity, partly because some of the binding energy is used to drive vesicle aggregation.[13] Moreover, binding of **3** to the vesicle surface is accompanied by significant restrictions of the polymer conformation further reducing binding affinity. Finally, it has been noted that binding at vesicle interfaces is often inherently weaker than in solution.[3d]

The binding of **3** to **1a** in the *cis*-rich photostationary state was only modestly weaker than for the *trans*-receptor (Table 1); thus the shape of the membrane anchor has little effect on multivalent recognition at the surface of the LUVs.

There are no indications that the aggregation of the vesicles containing **1a** mediated by multivalent guest **3** is accompanied by vesicle fusion. Fusion assays following the protocol established by Struck *et al.*[14] were performed on vesicles containing **1a**, but failed to provide any evidence of fusion, either before or after photo-irradiation (see ESI†).

Photo-triggered adhesion of GUVs containing 1a

Next we prepared giant vesicles consisting of egg PC and 5 mol% **1a** using the electroformation protocol adapted by Okumura *et al.*,[15] resulting in vesicles that were predominantly adhered to the platinum wire of the electroformation setup. Vesicle formation was completed after about two hours and the AC field was switched off to avoid interference of field effects with subsequent experiments.

The appearance of vesicles containing embedded **1a** did not differ from that of pure egg PC vesicles (Fig. 3a). However, irradiation with UV light induced vertical migration of vesicles, moving them out of the focus of the microscope within seconds. This movement was reversed, albeit on a much slower time-scale (within minutes) when the UV irradiation was switched off. During this process the adhesion of the giant vesicles to the platinum wire was not severed and no lateral movement along the wire was observed.

Movement of giant vesicles without exertion of mechanical force has been reported by Solon *et al.*;[16] however, this was under quite different circumstances involving negatively charged vesicles interacting with a positively charged supported lipid bilayer.

Motility was exclusive to vesicles containing **1a**; it was not observed with egg PC-only vesicles or vesicles incorporating control compound **1b**, indicating that the photoswitching of the azobenzene moiety is probably inducing vesicle motility. How exactly the motion on the molecular scale gives rise to motion of an entire GUV is as yet unclear and is the subject of further investigation.

We then proceeded to study the ability of the multivalent guest to mediate the adhesion of the GUVs. Using a micropipette, a 2 mg/mL solution of multivalent guest **3** was added near the surface of the vesicles, visible as a small dark spot by optical microscopy (see arrow in Fig. 3b). This spot was mobile but failed to induce any adhesion between vesicles. However, when the sample was irradiated with UV light (350 nm for 6 s and then a further 3 s) vesicle adhesion was observed (Fig. 3d and e). As adhesion was never observed without prior UV irradiation, we believe that this behaviour is associated with the photoswitchable azobenzene moiety. Note that it is not likely that this behaviour is caused by differences in affinity of **3** for *cis*-**1a** as compared to *trans*-**1a**; our experiments on LUVs (Table 1) indicate that *cis*-**1a** exhibits only slightly weaker binding than *trans*-**1a**.

Given the observation that irradiation with UV light induces giant vesicle motility, we speculate that the orientation between two neighbouring giant vesicles needed for their adhesion requires the extra mobility associated with *cis–trans* isomerisation of the azobenzene anchor of **1a**.

Conclusion

We have observed a large difference in **3**-mediated adhesion between LUVs in solution and the corresponding effect on already adjacent giant vesicles. In solution LUVs aggregate readily upon addition of **3**. This is not the case for the giant vesicles, despite the fact that they are already held in close proximity by the platinum wire; a situation not dissimilar to cells that form part of tissue. It appears that in order for the multivalent guest to mediate the adherence of GUVs an element of dynamics needs to be introduced into the system, provided by the *cis–trans* isomerisation of the membrane anchor. As far as we are aware these results are the first example of molecular-recognition-mediated adhesion of GUVs that can be triggered photochemically.

Experimental

Synthesis

Control compound **1b**[11] and monovalent guest **2**[17] were synthesised according to published procedures.

4-((4-Decylphenyl)diazenyl)phenol. 4-Decylaniline (2.33 g, 10 mmol) was dissolved in a 1:1 mixture of water and acetone (26 mL), and concentrated HCl (2.5 mL) was added. The mixture was cooled in an ice-bath, and a solution of sodium nitrite (0.76 g, 11 mmol) in 13 mL of cold water was added. This solution was allowed to stand for 15 min in an ice-bath and was then added slowly to a cold aqueous (26 mL) solution of phenol (1.03 g, 11 mmol), sodium hydroxide (0.44 g, 11 mmol) and sodium carbonate (1.77 g). After 30 min of stirring, the brown-yellow precipitate was filtered and recrystallised from hexane to yield 2.8 g (83%) of pure product.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84 (d, 2H, J = 8.8 Hz), 7.78 (d, 2H, J = 8.3), 7.29 (d, 2H, J = 8.2), 6.92 (d, 2H, J = 8.8), 5.32 (s, 1H), 2.66 (t, 2H), 1.64 (m, 4H), 1.31–1.25 (m, 12H), 0.87 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 159.3, 152.1, 148.4, 147.2, 130.3, 126.0, 123.7, 117.0, 37.1, 33.1, 32.5, 30.8, 30.7, 30.5, 30.5, 23.9, 15.3. Exact mass calculated: 339.2436, found: 339.2458 [M + H$^+$].

6-(4-((4-Decylphenyl)diazenyl)phenoxy)pentan-1-ol. Under a nitrogen atmosphere, a mixture of 4-((4-decylphenyl)diazenyl)-phenol (507 mg, 1.5 mmol), 5-chloro-1-pentanol (210 mg, 1.7 mmol) and K_2CO_3 (210 mg, 1.5 mmol) were refluxed in 8 mL DMF for 3 h. The mixture was allowed to cool down to room temperature and cold water (50 mL) was added. This solution was extracted with dichloromethane (3 × 50 mL) and the combined organic phases were washed with brine (3 × 20 mL), dried over Na_2SO_4, and filtered. The solvent was removed and the crude product was purified by column chromatography (silica, hexane–ethyl acetate gradient of 4:1 to 1:1) to give 598 mg (94%) of a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (d, 2H, J = 8.9 Hz), 7.79 (d, 2H, J = 8.2), 7.28 (d, 2H, J = 8.2), 6.98 (d, 2H, J = 8.9), 4.05 (t, 2H, J = 6.4), 3.69 (t, 2H, J = 6.4), 2.66 (t, 2H), 1.89–1.82 (m, 2H), 1.69–1.55 (m, 6H), 1.46 (m, 2H), 1.31–1.25 (m, 12H), 0.87 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.7, 152.2, 148.2, 147.2, 130.3, 125.9, 123.8, 116.0, 69.4, 64.1, 37.2, 33.7, 33.2, 32.6, 30.9, 30.8, 30.6, 30.6, 30.29, 24.0, 23.7, 15.4. Exact mass calculated: 425.3168, found: 425.3171 [M + H$^+$].

3,5-Bis(tritylthio)benzoic acid. 3,5-Dimercaptobenzoic acid[3] (1.0 g, 5.37 mmol) and triphenylmethyl chloride (4.5 g, 16.2 mmol) were dissolved in 15 mL dry DMF. The solution was stirred for 40 h at room temperature under a nitrogen atmosphere. Water (40 mL) was added and the mixture was extracted with chloroform (5 × 30 mL). The combined organic phases were washed with brine (30 mL) and water (20 mL), dried over Na_2SO_4 and filtered. The solvent was removed and the crude product was purified by column chromatography (silica, hexane–ethyl acetate gradient from 10:1 to 1:1) to give 3.30 g (92%) of a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31–7.29 (m, 12H), 7.26 (d, 2H, J = 1.7 Hz), 7.21–7.14 (m, 18H), 7.01 (t, 2H, J = 1.7). Exact mass calculated: 693.1892, found: 693.1926 [M + H$^+$].

(4-((4-Decylphenyl)diazenyl)phenoxy)pentyl-3,5-bis(tritylthio)-benzoate. A solution of 3,5-bis(tritylthio)benzoic acid (335 mg, 0.5 mmol) and 6-(4-((4-decylphenyl)diazenyl)phenoxy)pentan-1-ol (212 mg, 0.5 mmol) in 20 mL dichloromethane was cooled in an ice-bath. DMAP (61 mg, 0.5 mmol) was added, followed by DCC (113 mg, 0.5 mmol) and the mixture was stirred for 30 min. Then the ice-bath was removed and after stirring for 55 h at room temperature, the mixture was filtered. The filtrate was concentrated and purified by column chromatography (silica, hexane–ethyl acetate 4:1) to yield 390 mg (75%) of a yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.88 (d, 2H), 7.79 (d, 2H), 7.31–7.26 (m, 16H), 7.20–7.15 (m, 18H), 7.00 (t, 1H), 6.98 (t, 1H), 6.95 (t, 1H), 4.12 (t, 2H, J = 6.4 Hz), 4.03 (t, 2H, J = 6.3), 2.67 (t, 2H), 1.87–1.79 (m, 2H), 1.70–1.61 (m, 4H), 1.54–1.48 (m, 2H), 1.32–1.26 (m, 14H), 0.88 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.6, 164.7, 151.5, 149.6, 147.5, 146.4, 144.5, 144.09, 135.3, 135.0, 130.3, 130.2, 129.5, 128.2, 127.3, 125.1, 123.0, 115.2, 71.8, 68.4, 65.1, 38.9, 36.4, 32.4, 31.8, 30.1, 30.0, 29.8, 29.8, 29.3, 28.8, 23.2, 23.1, 14.6. Exact mass calculated: 1077.5057, found: 1077.5052 [M + H$^+$].

5-(4-((4-Decylphenyl)diazenyl)phenoxy)pentyl-3,5-dimercapto-benzoate. 5-(4-((4-Decylphenyl)diazenyl)phenoxy)pentyl-3,5-bis(tritylthio)benzoate (45 mg, 0.042 mmol) was dissolved in 5 mL dichloromethane and trifluoroacetic acid (0.2 mL) was added.

The mixture was stirred for 30 min at room temperature, then triethylsilane (15 drops) was added drop wise. After stirring for another 15 min, cold water (10 mL) was added and the reaction mixture was extracted with dichloromethane (3 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and the solvent was removed. The crude product was purified by column chromatography (silica, hexane–ethyl acetate gradient of pure hexane to 2:1) under nitrogen flow to yield 21 mg (85%) of the yellow product.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.94 (dt, 2H, J = 9.0 Hz), 7.83 (dt, 2H, J = 8.4), 7.69 (d, 2H, J = 1.7), 7.33 (t, 1H, J = 1.7), 7.29 (dt, 2H, J = 8.3), 6.99 (dt, 2H, J = 9.0), 4.34 (t, 2H), 4.07 (t, 2H), 3.52 (s, 2H), 2.66 (t, 2H), 1.93–1.82 (m, 6H), 1.68–1.60 (m, 4H), 1.31–1.25 (m, 12H), 0.87 (t, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 123.3, 152.1, 148.3, 147.8, 134.6, 14.5, 133.54, 130.7, 128.9, 126.6, 124.2, 116.4, 69.6, 66.9, 37.5, 33.6, 33.0, 31.3, 31.2, 31.1, 31.0, 30.9, 30.5, 30.1, 24.33, 24.3, 15.8. Exact mass calculated: 593.2866, found: 593.2851 [M + H$^+$].

Amphiphilic receptor (1a). A mixture of 5-(4-((4-decylphenyl)-diazenyl)phenoxy)pentyl-3,5-dimercaptobenzoate (220 mg, 0.37 mmol) and 2,6-dimercapto-9,10-dihydro-9,10-etheno-anthracene-11,12-dicarboxylic acid[7] (265 mg, 0.74 mmol) was stirred at room temperature in a mixture of DMSO (45 mL) and THF (75 mL) for 45 h. THF was removed under reduced pressure and water (200 mL) was added, followed by 3 N HCl to acidify the solution which was then extracted with dichloromethane (4 × 60 mL). The combined organic phases were washed with dilute HCl (0.01 N), dried over Na_2SO_4 and filtered. The solvent was removed and the crude product was purified by column chromatography (silica, dichloromethane–methanol (both with 0.05% formic acid) gradient from 10:1 to 1:1) which yielded 179 mg (37%) of the product as a mixture of isomers.

Characterisation of the major isomer: ^{1}H NMR (CD_3OD–$CDCl_3$ ≈ 1:5 v/v, 400 MHz): δ 8.06 (d, 1H, J = 1.6 Hz), 7.97 (d, 1H, J = 1.6), 7.85 (d, 2H, J = 8.8), 7.75 (d, 1H), 7.59 (br, 1H), 7.48–7.42 (m, 4H), 7.27 (d, 2H), 7.20–7.04 (m, 8H), 6.98 (d, 2H, J = 8.8), 5.77 (s, 1H), 5.75 (br s, 3H), 4.35 (m, 2H), 4.08 (m, 2H), 2.64 (t, 2H), 1.93–1.80 (m, 4H), 1.60–1.57 (m, 6H), 1.29–1.22 (m, 12H), 0.84 (t, 3H). ^{13}C NMR (CD_3OD–$CDCl_3$ ≈ 1:5 v/v, 125 MHz): δ 166.6, 166.5, 150.7, 149.3, 149.2, 145.8, 145.3, 141.7, 138.4, 133.3, 128.9, 124.6, 124.4, 124.3, 122.3, 122.2, 114.5, 67.8, 65.2, 52.6, 52.4, 35.6, 31.7, 31.1, 29.4, 29.4, 29.3, 29.2, 29.2, 29.1, 29.0, 28.6, 28.3, 22.4, 13.8. Exact mass calculated: 1299.2751, found: 1299.2795 [M + H$^+$].

***cis*-5-Norbornene-2,3-dicarboxylic imide.** A mixture of *cis*-5-norbornene-*endo*-2,3-dicarboxylic anhydride (8.2 g, 50 mmol) and urea (6 g, 100 mmol) was heated at 145 °C for 4 h. Water (50 mL) was added and the solution was heated until it was homogenous. A white-grey solid precipitated upon cooling. It was filtered, washed with cold water and dried to yield 7.2 g (91%) of imide.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (br, 1H), 6.18 (dd, 2H), 3.36 (m, 2H), 3.29 (m, 2H), 1.72 (d, 1H, J = 8.8 Hz), 1.50 (d, J = 8.8, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 180.5, 137.1, 54.7, 49.8, 47.4. Exact mass calculated: 164.0706, found: 164.0712 [M + H$^+$].

N-(2-Bromoethyl)-*cis*-5-norbornene-2,3-dicarboxylic imide. *cis*-5-Norbornene-2,3-dicarboxylic imide (3.26 g, 20 mmol), 1,2-dibromoethane (10 mL, 116 mmol) and K_2CO_3 (1.51 g, 11 mmol) in 20 mL acetone were refluxed for 45 h. The solvent was removed and after addition of water (40 mL) the mixture was extracted with dichloromethane (4 × 30 mL). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed to yield an off-white solid. Recrystallisation from a mixture of chloroform and hexane gave 5.0 g (93%) of pure product.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.11 (dd, 2H), 3.74 (t, 2H, J = 6.8 Hz), 3.39 (t, 2H, J = 6.8), 3.37 (m, 2H), 3.26 (m, 2H), 1.73 (m, 1H), 1.53 (d, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 179.6, 137.0, 54.6, 48.2, 47.4, 42.1, 29.3.

Poly-*N*-(2-bromoethyl)-4-vinyltetrahydrocyclopenta[*c*]pyrrole-1,3(2*H*,3a*H*)-dione. Under a nitrogen atmosphere, *N*-2-bromoethyl-*cis*-5-norbornene-2,3-dicarboxylic imide (110 mg, 0.4 mmol) was dissolved in 10 mL freshly distilled and degassed THF. A solution of 2nd generation Grubbs catalyst (6.95 mg, 0.008 mmol) in THF (2 mL) was added *via* a syringe. The resulting purple solution was refluxed for 2.5 h. During this time the solution turned cloudy yellow. Ethyl vinyl ether (1 mL) was added and the mixture was refluxed for another 15 min. The solvent was removed and chloroform was added to dissolve the residue, followed by methanol to precipitate the product which was filtered, washed with methanol and dried to yield 111 mg (100%) of product.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33–7.30 (m, 5H), 5.66–5.60 (m, br, ~164H), 3.82 (m, br, ~162H), 3.54 (m, br, ~162H), 3.17–2.88 (m, br, ~324H), 1.90–1.80 (m, br, ~81H), 1.57–1.40 (m, br, ~81H). GPC (THF, flow rate 1 mL/min): M_n = 21 985, M_w = 23 050, PDI = 1.05.

Polymeric guest (3). Under a nitrogen atmosphere, the bromide polymer (100 mg) and isoquinoline (0.5 mL) were refluxed in 3 mL chloroform for 4 days. Then diethyl ether (20 mL) was added and the solution was sonicated for a few minutes. The resulting solid was filtered and dried to yield 135 mg of a brown-yellow product.

^{1}H NMR (D_2O, 400 MHz): δ 9.78 (s, br), 8.64–7.77 (m, br), 4.97 (s, br), 4.21 (s, br), 3.23 (s, br), 2.44 (s, br), 1.47 (s, br), 0.75 (s, br). Elemental analysis calculated: C 59.56%, H 5.75%, N 6.95%, Br 19.81%, found: C 57.88%, H 4.75%, N 6.13%, Br 18.35%.

Preparation of LUVs

A solution of 25 mg of egg PC in 1 mL chloroform (purchased from Avanti Polar Lipids, Inc.) was placed in a test tube. If appropriate, **1a** (2.1 mg, 5 mol%) was added followed by 0.2 mL methanol to aid dissolution. The solvent was evaporated under a stream of nitrogen to leave a lipid film that was dried under vacuum over night.

Then 10 mM phosphate buffer pH 8 (3.29 mL) was added and the solution was vortexed for 30 s, incubated for 5 min and vortexed again for 30 s. After incubating for 20 min, 5 freeze–thaw cycles were performed, followed by extrusion, first 10 times through a polycarbonate membrane with a 400 nm pore size and finally 10 times through one with a 200 nm pore size.

Gel filtration

A pre-packed Sephadex G-25 M column (Amersham Biosciences) was saturated with lipids, then freshly prepared 5 mM egg PC vesicles containing 5 mol% **1a** (2.5 mL) were added to the column,

Table 2 HPLC elution gradient

Time (min)	THF (0.1% FA)	Water (0.1% FA)
0	40	60
20	100	0

eluted with 3.5 mL buffer and 7 fractions collected. These were analysed in a Cary 400 UV/Vis spectrometer at 700 nm to determine the amount of egg PC and by HPLC to determine the amount of **1a**.

HPLC analysis was performed on an Agilent 1050 system coupled to a UV detector by injecting 50 µL of each fraction onto a Zorbax Eclipse XBD-C8 column (150 × 4.6 mm) at room temperature. Both solvents contained formic acid (FA). The elution gradient in Table 2 was used. Data were analysed using ChemStation software.

Dynamic light scattering

Freshly prepared vesicle samples were diluted 20-fold and dynamic light scattering measurements were performed on a Malvern Zetasizer Nano ZS with a He-Ne laser operated at a wavelength of 633 nm. Each intensity graph was obtained by taking the average of fourteen measurements.

Isomerisation cycles between *trans*- and *cis*-azobenzene

A freshly prepared sample of egg PC vesicles containing 5 mol% **1a** was diluted 200-fold and the UV spectrum of this solution was recorded. This sample was irradiated alternately with UV light (365 nm wavelength) and visible light, where after each irradiation step the UV spectrum of the solution was recorded.

Fluorescence titration

A freshly prepared solution of egg PC LUVs containing 5 mol% receptor **1a** was titrated into a cuvette containing 3 mL of a solution of monovalent guest **2** or polyvalent guest **3** in 10 mM phosphate buffer pH 7. When measuring the apparent binding constant for *cis*-**1a**, the vesicle solution was irradiated with UV light of 365 nm wavelength prior to adding it to the guest solution. The background was determined by titrating egg PC-only vesicles into the guest solutions.

An excitation wavelength of 330 nm was used. The change in fluorescence intensity at 382 nm was plotted *versus* the concentration of **1a** titrated into the guest solution and this was fitted to the equation below using Kaleidagraph software,

$$\Delta F = F_0 - F = \frac{\Delta F_{max} \times [\mathbf{1a}] \times K_{app}}{1 + [\mathbf{1a}] \times K_{app}}$$

where F_0 is the fluorescence intensity of guest solution, F the fluorescence intensity after titration of a certain amount of **1a**-containing vesicles into the guest solution, ΔF_{max} the maximum change in fluorescence intensity, and K_{app} the apparent binding constant.

Fusion assay

LUVs containing 5 mol% **1a** and 1 mol% each of NBD-PE and Rh-PE were prepared using the same procedure as described above. These were mixed 1:1 with vesicles containing only 5 mol% **1a**. Polyvalent guest **3** was added and the fluorescence emission intensities of NBD (at 530 nm) and Rh (at 590 nm) were followed upon excitation at 470 nm. To test for fusion of vesicles containing *cis*-**1a**, the solution was irradiated with UV light prior to addition of **3**.

Preparation of giant vesicles

A solution containing 0.3 mg/mL egg PC and 5 mol% **1a** in a mixture of chloroform and methanol (9:1 v/v) was prepared. Under a stream of nitrogen, a 1 µL drop was carefully deposited on each of the platinum wires of the investigation chamber that was built according to the description given by Bucher *et al*.[18] The lipid film was dried under vacuum overnight.

The investigation chamber was put on the stage of an inverted microscope (Olympus IX71) equipped with a camera and micromanipulation equipment (Scientifica). The two platinum wires were connected to a frequency generator with an AC field (2 V, 10 Hz). About 1 mL of water was added to the chamber and giant vesicles could normally be observed after 2 h.

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