be attained economically (cheap template) with the aid of an additive under mild conditions (low temperature).

The solid-state ^{29}Si magic-angle spinning (MAS) NMR spectrum (see Supporting Information) of as-made mesoporous FDU-5 prepared by using MPTS as an additive under acidic conditions at room temperature exhibits four signals corresponding to T_2 ($\delta = -53$), T_3 ($\delta = -66$), Q_3 ($\delta = -101$), and Q_4 ($\delta = -111$ ppm), where T_2 and T_3 represent the species $RSi(OSi)_2OH$ and $RSi(OSi)_3$, and Q_3 and Q_4 correspond to $Si(OSi)_3OH$ and $Si(OSi)_4$ moieties. The results suggest that large-pore 3D bicontinuous mesoporous material with functional organic groups can be formed in one step under acidic conditions at room temperature. The $(T_3 + Q_4)/(T_2 + Q_3)$ ratio of 3.77 suggests a high degree of framework cross-linking.[13] The amount (4.6 %) of organic moieties incorporated in the mesostructure, calculated by the ratio $T/(T + Q)$ ($T = T_2 + T_3$, $Q = Q_3 + Q_4$), is in accordance with that calculated from the initial composition (5.1 %).

In summary, 3D bicontinuous cubic *Ia3d*-type mesoporous silica has been synthesized for the first time in acidic medium at room temperature by using a nonionic triblock copolymer as template. The resulting FDU-5 materials have extra-large pores (4.5–9.5 nm) with narrow size distributions. Such materials could have promising applications for the sorption, transport, and separation of large molecules.

Experimental Section

Typical synthesis of FDU-5: P123 (1 g, $M_w = 5800$, Aldrich) was dispersed with HCl solution (0.1 g, 2 M) and H_2O (0.9 g) in EtOH (10 g). After stirring vigorously at room temperature for 2 h, MPTS (0.106 g, 5.4 mmol) was added. After a further 2 h of stirring, TEOS (2.08 g, 10 mmol) was added. Then the mixture was stirred for 1 h. The resulting solution was dried in air for 2 d, and a solid white product was obtained. The surfactant P123 was removed from the as-synthesized material by washing with ethanol under reflux for 40 h or by calcining the product at 823 K for 6 h. The molar composition of the reaction mixture TEOS/P123/HCl/H_2O/EtOH/additive was 1/0.015–0.020/0.018–0.096/2.44–6.97/11–52/x, where x varied in the ranges of 0.046–0.058, 0.048–0.66, 0.042–0.60, 0.035–0.54, and 0.030–0.47 for MPTS, benzene, methylbenzene, 1,4-dimethylbenzene, and 1,3,5-trimethylbenzene as additive, respectively.

Characterization: TEM photographs were obtained on a JEOL 2010 microscope operated at 200 kV. XRD patterns were obtained on a Bruker D4 X-Ray diffractometer with $Cu_{K\alpha}$ radiation. Nitrogen adsorption–desorption isotherms were acquired on a Micromeritics Tristar 3000 system at 77 K. ^{29}Si NMR experiments were performed on a Bruker DSX300 spectrometer with a frequency of 59.63 MHz, a recycling delay of 600 s, and a radiation frequency intensity of 62.5 KHz.

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Transmembrane Signalling**

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Cells are separated from their surroundings by a lipid bilayer membrane, yet their survival depends on their ability to sense and respond to changes in the environment such as the levels of extracellular hormones, growth factors, cytokines, nutrients, and pathogens. One way in which these molecular signals can change the intracellular chemistry is by being transported into the cell through active transport or pores in the membrane. To date, synthetic approaches to communication across lipid membranes have relied on mimicking this direct physical transport of the molecular signal across the

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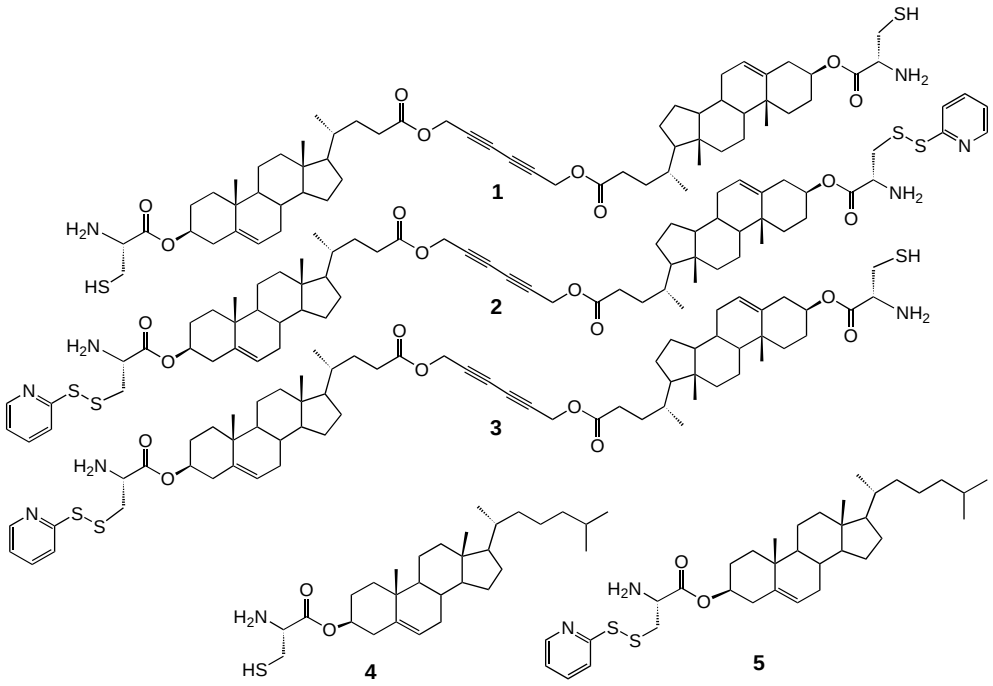

Scheme 1. Membrane-spanning molecules **1**, **2**, and **3**, with the control compounds **4** and **5**.

membrane. However, this approach means that the external and internal chemistry are not separated and that the message and response have to be intimately linked.[1–3] An alternative biological mechanism is to transduce the signal with proteins that span the cellular membrane, as exemplified by G-protein-coupled receptors and tyrosine kinase receptors; for example, an external ligand can make tyrosine kinase receptors dimerize; the dimer then autophosphorylates its intracellular domain, which initiates a cascade of events inside the cell.[4] This cascade provides a mechanism for coupling but separating the chemistry on the inside and outside of the cell—in contrast to transport mechanisms, the message and signal can be chemically unrelated and potentially harmful messenger molecules are excluded. Here we describe a synthetic system capable of molecular signal transduction without physical transport of the messenger across the membrane.

Our approach is based on the mode of action of the tyrosine kinase receptors described above and uses synthetic compounds to send and transform a signal across a unilamellar vesicle membrane. The signal-transducing molecule must span the lipid-bilayer membrane with sensor units directed towards the outside of the vesicle and signalling units on the inside. Compounds **1**, **2**, and **3** were designed for the assembly of such a system (Scheme 1). To construct the transmembrane section, we used cholenic acid, a naturally occurring bifunctional steroid from which tail-to-tail cholesterol dimers can be synthesized. Cholesterol inserts well into membranes, and the straight, relatively rigid dimer structure should favor a transmembrane conformation, leading to strong coupling between the locations of the external and internal head groups. The sensor and signalling units both exploit thiol–disulfide chemistry, allowing construction of an unsymmetrical signal transduction system from relatively simple symmetrical compounds.[5–7] The amino acid cysteine provides an ideal charged headgroup that has a thiol functional group and can be readily attached to the cholesterol dimer (Scheme 1). The sensing event is the oxidation of the thiol group to a disulfide group on the outside of the membrane, which induces dimerization of the signal-transducing molecules (Figure 1). The signalling event is the reaction of a thiol

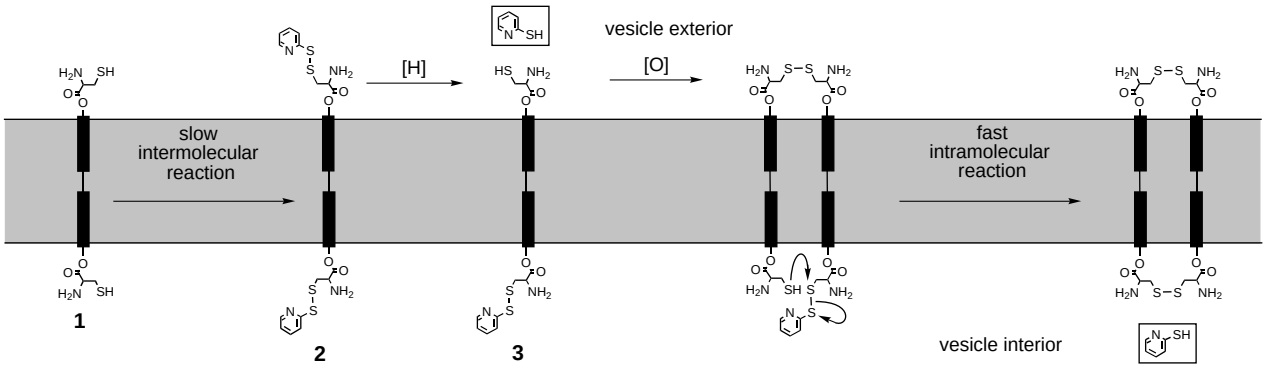

Figure 1. Chemical scheme showing the sensing and signalling reactions.

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moiety on the inside of the membrane with an activated disulfide group, which releases a colored species, pyridine-2-thiol, detectable by UV/Vis absorption spectroscopy. The idea is that the intermolecular reaction should be slow, but following dimerization of the external sensing groups, the internal signalling groups are brought into close proximity, which gives rise to fast intramolecular reaction (Figure 1).

The first requirement is clearly the identification of a thiol/disulfide pair that is unreactive under dilute conditions but can be forced to react at higher concentrations in the lipid membrane. A series of model compounds were prepared from cholesterol and cysteine, and **4** and **5** exhibited the desired behavior. Unilamellar 200 nm egg yolk phosphatidylcholine vesicles containing **4** and **5** were prepared by extrusion of a mixture of the lipid and the cholesterol derivatives through 200 nm polycarbonate membranes.[8,9] At pH 5.5 and a membrane loading of 2.5 mol%, no release of pyridine-2-thiol was observed by UV/Vis absorption spectroscopy over a period of hours. The integrity of the functional groups was confirmed by adding external reagents: addition of 2,2-dipyridyl disulfide led to an increase in the UV/Vis absorption at 341 nm, caused by the release of pyridine-2-thiol, which confirmed the presence of reactive thiol groups in the (**4** + **5**) vesicles;[10] addition of dithiothreitol also led to the release of pyridine-2-thiol, which confirmed the presence of reactive disulfide groups in the (**4** + **5**) vesicles.[11] In both cases, the magnitude of the response indicated that little reaction had taken place between **4** and **5** in the course of vesicle preparation. Thus **4** and **5** can be incorporated intact into bilayer membranes and, once inserted, they do not react under dilute conditions.

Compound **1** was synthesized from cholenic acid in four steps. The key step was formation of the propargyl ester of cholenic acid to give an analogue of cholesterol, from which tail-to-tail dimers could be prepared using Glaser–Hay coupling.[12] The dialkyne linker provides the rigidity required for close coupling of the locations of the internal and external cholesterol units. Cysteine units were attached to both ends of the dimer to give **1**. Derivatization with 2,2′-dipyridyl disulfide converted **1** into **2** (Scheme 1). Unilamellar 200 nm egg yolk phosphatidylcholine vesicles containing **1** and **2** were prepared by extrusion of a mixture of the lipid and the cholesterol derivatives. This procedure favors a membrane-spanning conformation over insertion of compounds from solution after vesicle preparation.[13] The integrity of the functional groups was again checked by adding 2,2′-dipyridyl disulfide to test for thiol groups, and dithiothreitol to test for disulfide groups, as before, and showed that both **1** and **2** were incorporated into the vesicles intact. As with the model compounds, no release of pyridine-2-thiol was observed over a period of hours, which indicates that the assembly is stable, and that no reaction takes place between **1** and **2** in the membrane.

The active signal transduction system was then revealed by deprotection of the external disulfide units using tris(3-sulfonatophenyl)phosphane, a charged reducing agent which does not cross the lipid membrane.[14] This phosphane selectively reduces the external disulfide groups, converting **2** into **3** so that the unsymmetrical product is oriented across the membrane with all of the remaining disulfide units located on the inside of the vesicle (Figure 1). Exposure of these (**1** + **3**) vesicles to potassium ferricyanide, an external oxidant that is incapable of crossing the vesicle membrane, gave an increase in the UV/Vis absorption at 341 nm, which corresponds to the release of pyridine-2-thiol on the inside of the vesicle.[15] The change in absorption is not accompanied by an increase in turbidity, which would originate from vesicle aggregation.

Thus the system appears to function in the desired manner: the inside of the vesicle responds to the presence of an external messenger molecule, ferricyanide, with the release of a second messenger, pyridine-2-thiol. The complete experiment is illustrated in Figure 2. The (**1** + **2**) vesicles can be prepared and stored with no reaction (resting, Figure 2c). These vesicles are equipped with a safety catch, the external disulfide, that renders them insensitive to the messenger molecule. Thus, addition of ferricyanide to (**1** + **2**) vesicles causes no change in the UV/Vis absorption spectrum (safety catch on, [O], Figure 2a,c). Release of the safety catch by external reduction (addition of the phosphane) causes a rapid increase in the absorbance at 341 nm over a period of about 10 min, caused by the release of pyridine-2-thiol on the outside of the vesicles ([H], Figure 2a,c). This reduction generates the active (**1** + **3**) vesicles, and when these are exposed to the messenger molecule (ferricyanide), a further jump in the absorption at 341 nm is observed, caused by the production of a second messenger molecule ("internal" pyridine-2-thiol) inside the vesicles (safety catch off, [O], Figure 2).

As a control, the (**4** + **5**) vesicles were subjected to the same conditions. The only difference between the (**1** + **2**) and (**4** + **5**) vesicles is that the connection between the inside and outside steroid units has been severed. As with the "safety catch on" (**1** + **2**) vesicles, the (**4** + **5**) vesicles are stable (resting, Figure 2c) and insensitive to oxidation by the messenger ferricyanide (control 1, [O], Figure 2b). External reduction generates pyridine-2-thiol over a period of 10 min as before ([H], Figure 2), but now addition of external oxidant causes no further change in the UV/Vis absorption spectrum (control 2, [O], Figure 2c). The failure of the messenger to trigger a response in this system is caused by the absence of a covalent connection across the membrane, so that the inside of the vesicles is isolated from the outside, and no signal transduction is possible.

In conclusion, a simple synthetic system has been constructed which successfully mimics biological signal transduction. The system consists of two membrane-spanning molecules embedded in egg yolk phosphatidylcholine vesicles. A safety catch renders the system unresponsive until it is released by addition of an external reducing agent. The vesicles then respond to the presence of an external oxidant by releasing a colored second messenger molecule on the inside of the vesicle. Thus, molecular communication is possible across the membrane without direct contact between the species involved. The flexibility inherent in our synthetic methodology will allow us to adapt both the sensor and signalling modules, retaining the same molecular framework for signal transduction, and this opens the way for the

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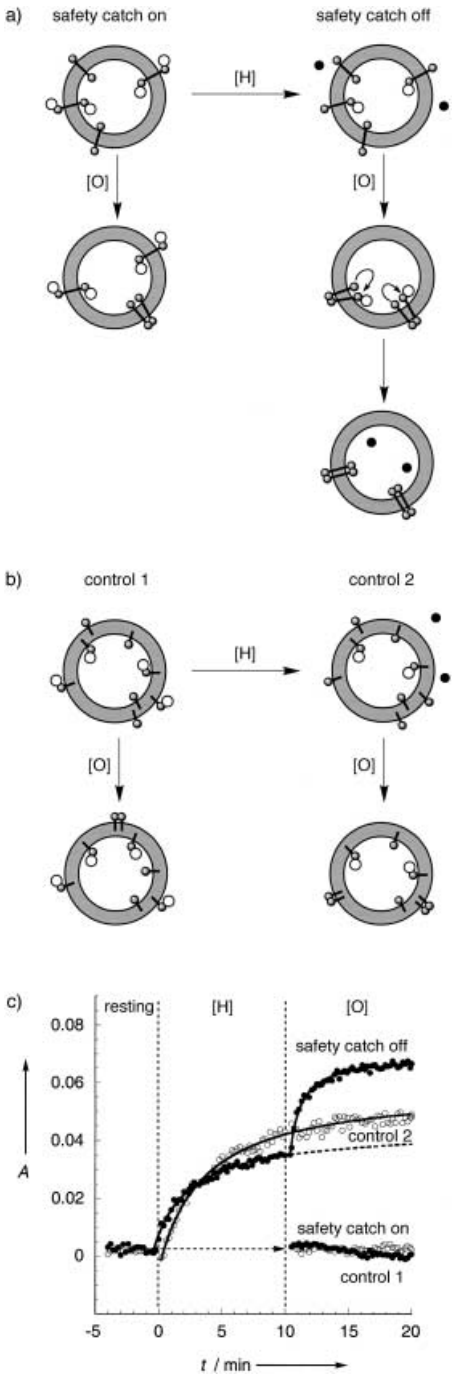

Figure 2. Mechanism of action and optical signals observed for compounds **1**–**5** embedded in vesicle membranes, for details see text. a) **1** and **2** embedded in the membrane. b) The control experiment using **4** and **5**. c) Change in absorbance at 341 nm, which indicates the release of pyridine-2-thiol (membrane-spanning molecules ●, control compounds ○). The resting period before reagents are added shows that no reaction takes place between the membrane embedded thiols and disulfides. At $t = 0$, tris(3-sulfonatophenyl)phosphane [H] was added. For both the control compounds and the membrane-spanning molecules, an increase is observed caused by the release of extravesicular pyridine-2-thiol. The kinetics of release could be fit using the same second-order rate constant $k = 100\,\text{M}^{-1}\text{s}^{-1}$ for both systems (theoretical lines shown). After 10 minutes, potassium ferricyanide [O] was added. The membrane-spanning molecules (safety catch off) generated a new signal, but no further change was observed for the control compounds. If oxidant were added without releasing the safety catch, no signal was observed for the membrane-spanning molecules (safety catch on) or the control compounds. Instantaneous jumps in absorbance caused by addition of reagents are not shown.

development of novel sensing and controlled-release technology for a wide range of messenger molecules.

Experimental Section

All compounds were made using standard synthetic procedures and gave satisfactory spectroscopic data (see Supporting Information).

Vesicles were prepared to give a final lipid concentration of 2 mM in all cases. Egg yolk L-α-phosphatidyl choline was dissolved in HPLC grade chloroform. A chloroform solution of the dopant compound was added to the lipid solution so that the concentration in the final vesicular solution was 50 μM. The lipid was deposited as a thin film by removal of the solvent under reduced pressure, followed by freeze-drying. Buffer (50 mM morpholine ethanesulfonate (MES), pH 5.5, 1 mL) was added to the thin film and the mixture stirred with a vortex mixer. The resulting suspension was passed through a 200 nm polycarbonate filter in an Avestin Lipofast extrusion apparatus 19 times, to give 200 nm diameter unilamellar vesicles.

This solution was added to a cuvette, and left to equilibrate for 10 min at 25 °C before any external reagents were added.

Changes in absorbance were monitored as a function of time at 341 nm (the absorbance wavelength of the pyridine-2-thiol leaving group) using a Cary3 UV spectrophotometer. Multiple wavelength scans were taken before and after each experiment to ensure that the changes taking place were caused by the product chromophore, and not by changes in the turbidity of the solution.

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