flexibility it allows in optimizing bilayer composition and aggregate size, at each step of the self-assembly process. □

Methods

Interior vesicle aggregates. Unilamellar vesicles were prepared by mixing dilauroylphosphatidylcholine (DLPC; Avanti Polar Lipids, Alabaster, Alabama) and dipalmitoylphosphatidylethanolamine-conjugated biotin (DPPE-biotin; Molecular Probes, Eugene, Oregon) at 0.16 mol% total lipid in chloroform, evaporating the solvent, then hydrating the lipid film with aqueous buffer (100 mM NaCl, 50 mM TES, and 0.02 wt% NaN_3 balanced to pH 7.2) for 48 h at 37 °C to form a 30 mg ml^{-1} dispersion of multilamellar vesicles. The solution was put through five freeze–thaw (liquid nitrogen–50 °C water bath) cycles followed by ten high-pressure extrusions through two stacked 100-nm-pore polycarbonate Nuclepore filters (Corning Costar, Cambridge, Massachusetts) to produce a relatively monodisperse dispersion of 100-nm unilamellar vesicles (Fig. 1a). A 100-nm vesicle of this composition contains ~80 DPPE-biotins protruding from the monolayer.

To aggregate the vesicles, sufficient (0.63 mg ml^{-1}) streptavidin (Molecular Probes, Eugene, Oregon) in the same buffer was added to produce an overall biotin–streptavidin ratio of 15:1; however, the ratio of biotin on the outside of the vesicle available for binding to streptavidin was 8:1. As streptavidin has four distinct binding sites for biotin, the ratio of exposed biotins to binding sites was 2:1, meaning there are excess surface ligands (Fig. 1). The addition of streptavidin solution diluted the dispersion of unilamellar vesicles to 20 mg per ml total lipid. Within an hour, the 20 mg per ml ULV/streptavidin suspension changed from clear and bluish to opaque and cloudy-white, indicating that vesicle aggregates were forming[9]. The aggregates were filtered under pressure through 1.0-μm. Nucleopore filters to produce the sized aggregates shown in Fig. 2.

Cochleate cylinders. Cochleate cylinders were prepared by first making a dispersion of 100-nm unilamellar vesicles containing 10 mg ml^{-1} of 1,2-dioleolyphosphatidylserine (DOPS; Avanti Polar Lipids, Alabaster, Alabama) with 0.16 mol% DPPE-biotin as previously described[9]. Equal (1 ml) volumes of the DOPS/DPPE-biotin vesicle solution (10 mg ml^{-1}) and a 6 mM $CaCl_2$ (Sigma, St Louis, Missouri) buffer solution were mixed. Immediately after mixing, the solution turbidity increased, indicating that cochleate cylinders had formed. Freeze-fracture transmission electron microscopy (not shown) confirmed that cochleate cylinders had formed, indicating the added DPPE-biotin did not alter the cochleate structure. 35 μl of 0.63 mg ml^{-1} streptavidin solution was injected into 1 ml of the cochleate cylinder solution and allowed to incubate for one day to fully saturate the biotin-lipids at the cylinder surface.

Vesosome assembly. The sized vesicle aggregates and the cylinders were mixed at a 1:1 DLPC:DOPS mole ratio: 1.0 ml of the 5 mg ml^{-1} DOPS cylinders (6.2 μmol of DOPS) was added to 0.19 ml of the 20 mg ml^{-1} DLPC vesicles (6.2 μmol of DLPC). To remove calcium ions, 0.44 ml of 5 mM EDTA was added to 0.5 ml of the mixture, resulting in a solution containing 4.2 mg ml^{-1} DOPS lipid and 3.2 mg ml^{-1} DLPC lipid. Freeze-fracture TEM samples were prepared by standard techniques[22] after one day of incubation before adding EDTA (Fig. 3a), and 5 h of incubation after adding EDTA (Fig. 3b).

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Temperature effects on the acidity of remote alpine lakes

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Climate variations and changes in sulphur and nitrogen deposition from the atmosphere influence the acid–base balance of sensitive lakes in a complex and site-specific way[1–3]. For example, although lakes in several regions have shown a decline in sulphate concentration following reductions in atmospheric sulphate deposition[4–6], the expected recovery of pH and alkalinity has not always taken place, implicating an additional response to changes in the local climate. Here we report a study of 57 remote alpine lakes which shows that, between 1985 and 1995, lake pH and the concentration of sulphate, base cations and silica have increased, whereas inorganic nitrogen concentrations have decreased. This contrasts with atmospheric input trends, which have led to a decrease in sulphate and a slight increase in nitrogen deposition over the same period[7,8]. We propose that the changes in lake chemistry are therefore likely to be caused by enhanced weathering and increased biological activity resulting from an increase in air temperature of about 1 °C since 1985. Our analysis of an alpine lake core covering a 200-year period provides further evidence for a strong positive correlation between pH and mean air temperatures, and thus for the high sensitivity of lakes at high altitudes and high latitudes to climate warming. In these remote locations, temperature effects, rather than acid deposition, appear to dominate changes in lake acidity.

We studied 57 low-alkalinity high-mountain lakes in glaciated and non-glaciated catchments, situated between 2,000 and 2,900 m above sea level (m.a.s.l.) on the northern (North Tyrol) and southern slope (East Tyrol, Carinthia) of the eastern Alps. The area is characterized by granites and gneisses of high sensitivity to acid deposition[9]. Soils are poorly developed with sparse vegetation, especially at very high altitudes where large portions (70–90%) of the catchments consist of bare rock. Samples were collected during the autumn overturn in 1985 and 1995 and analysed for pH,

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