The percent accessibility of the titratable lipid, NTA-DOGS, was determined by isothermal titration calorimetry,1 in vesicles prepared as SUVs (A) and LUVs (B). SUVs and LUVs were made with binary mixtures of DOPC and NTA-DOGS (Avanti Polar Lipids, Alabaster, AL) and were titrated with Ni²⁺. The interaction of Ni²⁺ with the nitrilotriacetic acid (NTA) moiety of NTA-DOGS is known to reach saturation at a 1:1 molar ratio. Titrations were carried under conditions where the vesicles were intact (○), and where all the NTA-DOGS was accessible to added Ni²⁺ (●), which was achieved by including the nonionic detergent octyl-β-glucopyranoside in the buffer (1% w/v). Arrows indicate titration endpoints (EP). The ratio of the endpoints in the absence and presence of detergent EP(minus detergent)/EP(plus detergent) was used to determine the fraction of NTA-DOGS accessible to Ni²⁺ in the intact vesicles, which was taken to be equivalent to the fraction of the lipid in the outermost leaflets of vesicle membranes. According to this method, the percent accessibilities of SUVs and LUVs were determined to be 51 and 45. Analogous experiments, conducted with multilamellar vesicles, produced percent accessibilities that were significantly lower (<10%, data not shown).

Titrations were carried out at 25 °C in a Microcal MCS-ITC (Microcal LLC, Northampton, MA). The calorimeter reaction cell (1.343 mL) was loaded with vesicle solutions in 20 mM HEPES, 100 mM NaCl, pH 7.6 that, as noted, could also contain 1% octyl-β-glucopyranoside. SUVs were present in the reaction cell at a total lipid concentration of 7.5 mM and contained one mole percent NTA-DOGS. LUVs were prepared by extrusion through filters with 1 µm diameter pores, were present at a total lipid concentration of 1.2 mM and contained sixty mole percent NTA-DOGS. NiSO₄ solutions were prepared in matching buffers and were present in the syringe at concentrations of 1.0 and 10.3 mM for titrations involving SUVs and LUVs, respectively.
Figure S2. Microscope images of vesicles (A), and vesicles with proteins (B & C), observed with phase contrast optics at 63X magnification. Samples of vesicles and CF (B) displayed significant aggregation, vesicles alone (A) and vesicles with CF, CheW and CheA (C) displayed no observable aggregation. The scale bar is 5 µm. Vesicles were composed of a 1:1 Ni²⁺-NTA-DOGS:DOPC mixture and prepared by extrusion through filters with 50 nm diameters pores. Sample compositions: A, LUVs present at a 580 µM total lipid concentration; B, LUVs (as in A) plus 30 µM CFEEEE; C, LUVs (as in A) plus 30 µM CFEEEE, 15 µM CheW and 1.2 µM CheA.
Figure S3. A negative-stain transmission electron microscope image of LUVs, without template-assembled signaling proteins present. The LUVs (1:1 DOPC:Ni²⁺-NTA-DOGS) were prepared by extrusion through a filter with 1000 nm diameter pores. The scale bar is 200 nm.

Figure S4. Electron micrograph of LUVs, which were prepared by extrusion through a 50 nm pore diameter filter, and were incubated with CFQQQQ, CheW and CheA for four hours under assembly conditions (580 µM lipid 1:1 DOPC:Ni²⁺-NTA-DOGS, 30 µM CF; 5 µM CheW, 1.2 µM CheA). The scale bar is 200 nm.
Figure S5. Electron micrograph of an LUV, from a preparation made by extrusion through a filter with 1000 nm diameter pores, which was incubated with CFEEEE, CheW and CheA for four hours under assembly conditions (580 μM lipid 1:1 DOPC:Ni^{2+}-NTA-DOGS, 30 μM CF; 5 μM CheW, 1.2 μM CheA). The scale bar is 500 nm.

References