Supporting Information

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SI Results

Recovery of GalP Protein Fluorescence and 100% Activity upon Refolding. See Figs. S1 and S2.

GalP Stability and Refolding in Guanidine Hydrochloride (GuHCl). See Figs. S3 and S4.

Refolding GalP into Lipids. See Fig. S5.

Thermal Denaturation of GalP. See Fig. S6.

SI Materials and Methods.

Circular Dichroism. CD spectra were collected at station 12.1 at the Synchrotron Radiation Source and station UV1 at the Institute for Storage Ring Facilities, as well as with an Aviv Model 410 CD spectrometer. The collector angle is optimized in all three of these setups to collect light scattered by lipid samples, thus giving data that are free from light scattering artefacts. The high intensity of the synchrotron sources also increases the spectral range attainable further into the UV than conventional CD spectrometers. Spectra were recorded at 20 °C in 0.01–0.05-cm pathlength cells between 260 and 180 nm with a data pitch of 0.5 nm and a dwell time of 1 s. The buffer-only spectrum was subtracted and the data analyzed using CDSSTR (1, 2) at the DICHROWEB Web site (3, 4).

Kinetics. Folding and unfolding kinetics were determined from changes in protein fluorescence over time using either a stopped flow spectrometer (Applied Photophysics) or a steady state fluorimeter (Fluoromax). GalP was mixed 1:5 with 10 M urea buffer to give a final concentration of 0.5 μM protein, and excited at 280 nm (1-nm bandwidth). The total fluorescence emission was collected using a >305-nm filter, which cuts off light below 305 nm. Slower refolding kinetics were recorded on the Fluoromax by diluting unfolded protein into buffer as above and monitoring the intrinsic fluorescence signal at 332 nm over time.

Reconstitution. Detergent saturation was performed by adding octyl glucoside to liposomes to a final concentration of 1% (wt/vol) and mixed for 10 min. GalP was added at a 40:1 ratio by weight and incubated for 15 min. Excess detergent was removed by a 100-fold dilution in 50 mM sodium phosphate (pH 8), 1 mM β-mercaptoethanol and liposomes recovered by centrifugation at 250,000 × *g* for 1 h and resuspended in buffer to 10 mg/mL.

1. Manavalan P, Johnson WC, Jr. (1987) Variable selection method improves the prediction of protein secondary structure from circular dichroism spectra. *Anal Biochem* 167:76–85.
2. Compton LA, Johnson WC, Jr. (1986) Analysis of protein circular dichroism spectra for secondary structure using a simple matrix multiplication. *Anal Biochem* 155:155–167.
3. Whitmore L, Wallace BA (2008) Protein secondary structure analyses from circular dichroism spectroscopy: Methods and reference databases. *Biopolymers* 89:392–400.
4. Whitmore L, Wallace BA (2004) DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res* 32 (Web Server issue):W668–673.

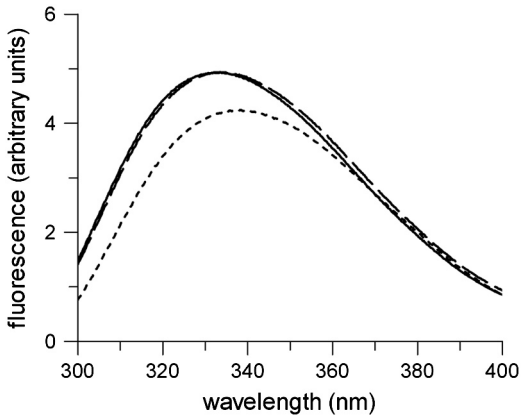

Fig. S1. Changes in GalP intrinsic fluorescence during unfolding and refolding from urea. Intrinsic fluorescence changes for unfolding and refolding GalP in urea; 0.5 μM GalP in 1 mM DDM (solid lines), unfolded in 8 M urea (short dashes), and refolded from urea into 1 mM DDM (long dashes).

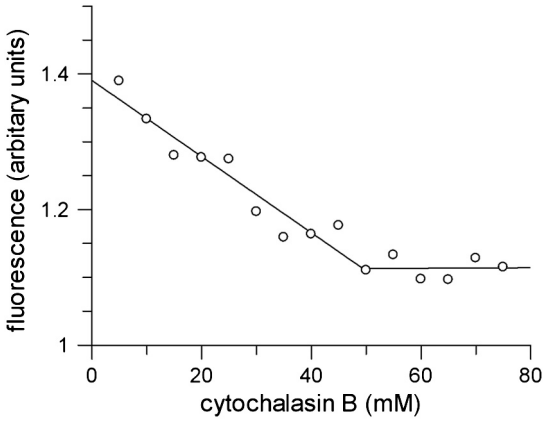

Fig. S2. Cytochalasin B ligand titration curve for refolded GalP in DDM. Quenching of intrinsic protein fluorescence upon binding of cytochalasin B to refolded GalP. GalP was refolded from 8 M urea into DDM. The GalP concentration was 50 μM, above the K_d of 24 μM, such that every cytochalasin B molecule added to GalP binds until all sites are saturated. GalP has a single binding site and 1 cytochalsin B molecule binds per GalP (1). The resulting titration curve fits to two straight lines, with the increasing linear region corresponding to GalP binding prior to saturation. The intersection of the two lines shows that 50 μM GalP binds 49 μM cytochalasin B at saturation; thus 98% of the refolded GalP is active.

1. Patching SG, Henderson PJ, Herbert RB, Middleton DA (2008) Solid-state NMR spectroscopy detects interactions between tryptophan residues of the E. coli sugar transporter GalP and the alpha-anomer of the D-glucose substrate. *J Am Chem Soc* 130:1236–1244.

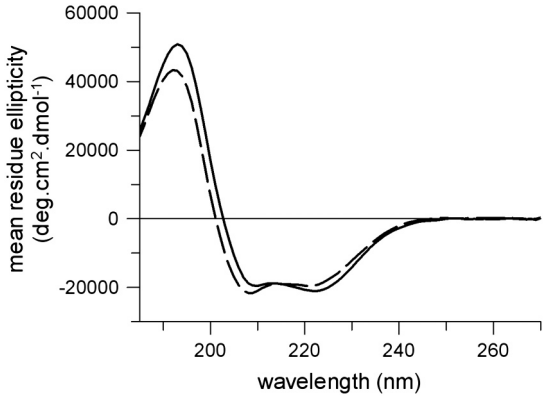

Fig. S3. Effect of SDS on GalP secondary structure. Unlike most helical membrane proteins examined to date, GalP was not significantly unfolded by sodium dodecylsulfate (SDS). Far UV circular dichroism spectra of 15 μM GalP in 1 mM DDM (solid line) and unfolded into 40 mM SDS/1 mM DDM (dashed line). There is little change in the spectra indicating a very small, or no, reduction in helical content.

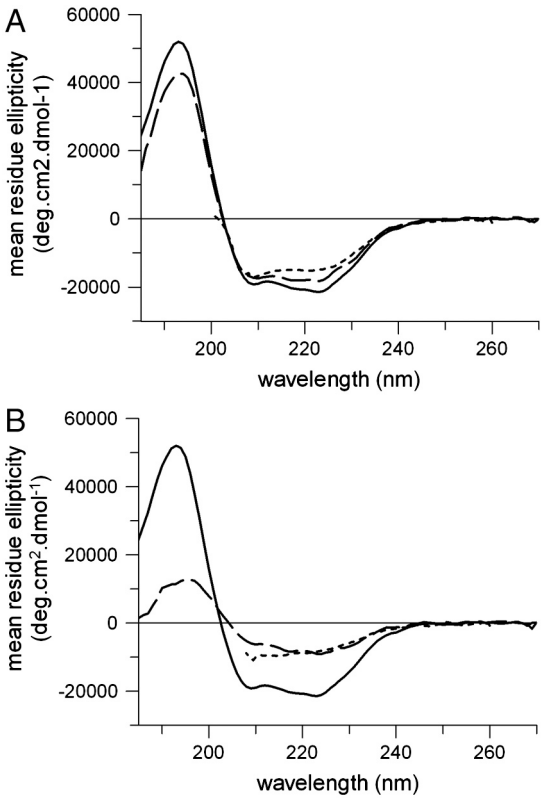

Fig. S4. GalP unfolded and refolded from GuHCl. Far UV circular dichroism spectra of GalP unfolded in (*A*) 3.5 M GuHCl or (*B*) 7 M GuHCl, then refolded from GuHCl into 1 mM DDM. Folded 15 μM GalP in 1 mM DDM (solid line), unfolded in (*A*) 3.5 M GuHCl or (*B*) 7 M GuHCl (short dashes) and refolded from GuHCl into 1 mM DDM (long dashes). Some recovery of secondary structure occurs during refolding from 3.5 M GuHCl (*A*), but no recovery is observed from 7 M GuHCl. Attempts at refolding from GuHCl concentrations above 3.5 M were unsuccessful.

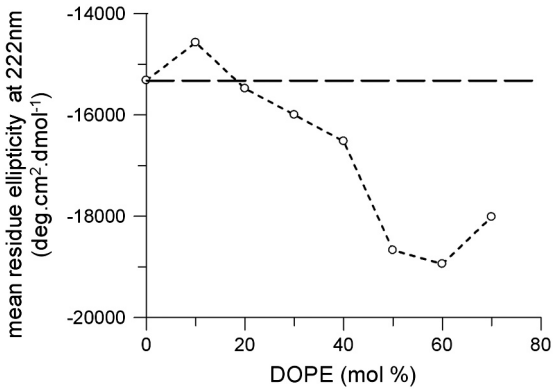

Fig. S5. GalP refolded directly from urea into DOPC/DOPE lipids. Dependence of 222-nm signal intensity of GalP refolded in DOPC/DOPE as a function of DOPE. Lipid composition had no effect on the amount of protein in the vesicle; an equal amount of protein was found to be associated with the vesicles in each lipid sample, by quantification of the protein associated with lipid vesicles following centrifugation.

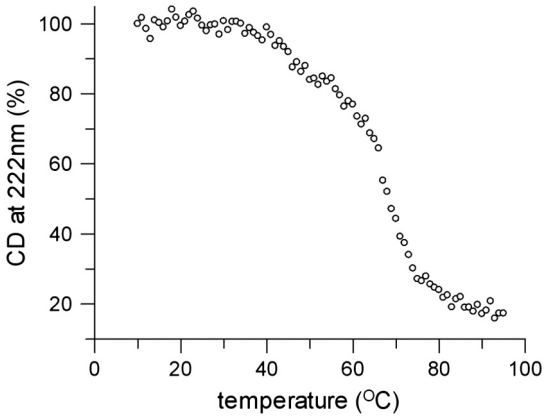

Fig. S6. Changes in GalP secondary structure upon heating. Change in the 222-nm CD intensity of 5 μM GalP in 1 mM DDM with increasing temperature, which is indicative of reduction of secondary helical structure with increasing temperature. A broad transition is observed, with structure loss commencing at about 45 °C, and a significant further reduction occurring above 60 °C.