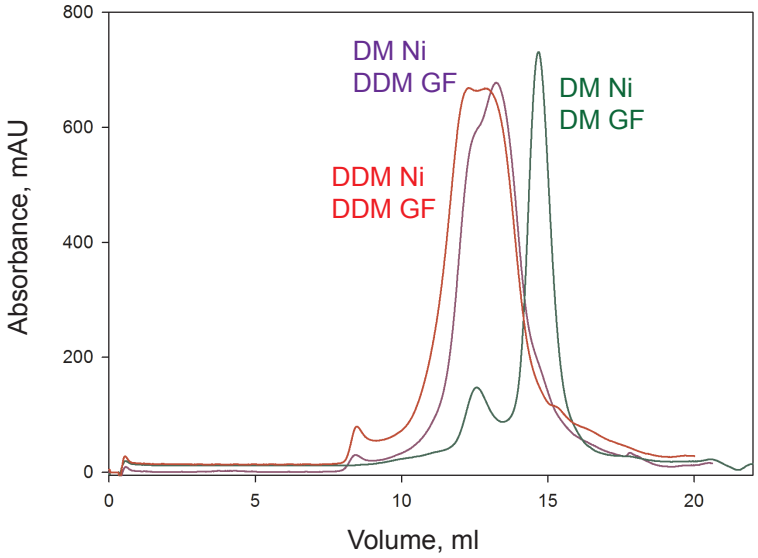
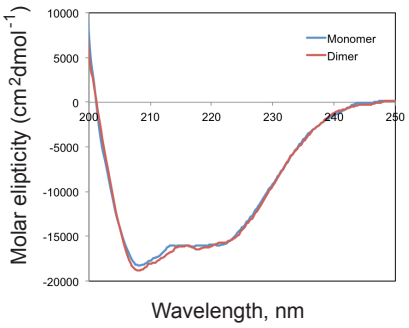
a



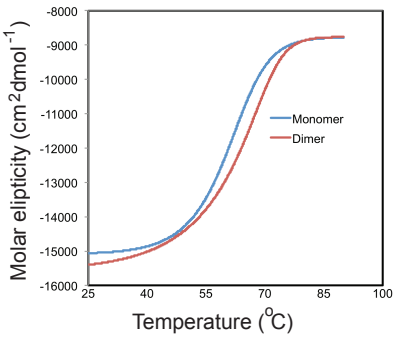
b

Protein Sample and/or buffer	Hydrodynamic Radius (nm)	MW (kDa)
hiGlpG in TBS, 0.1% DDM	5.7	197.9
TBS, 0.1% DDM	3.8	78.5
hiGlpG in TBS, 0.2% DM	3.7	75.4
TBS, 0.2% DM	3.16	49.9

c



d



Supplemental Figure S5. (a) SEC of hiGlpG rhomboid performed on Superdex 200 (10/30). hiGlpG was purified by Ni-NTA chromatography (Ni) either using DDM or DM detergents. The gel-filtration (GF) was then conducted using the buffer containing DDM (0.1%) or DM (0.2%). **(b)** hiGlpG molecular weight determination by dynamic light scattering (DLS) in various conditions. hiGlpG was purified by Ni-NTA chromatography, using either DDM and DM detergent and assessed by DLS. The molecular weight (MW) of detergent micelles was estimated using the buffer alone. The MW of hiGlpG in DDM corresponds to 2 protein molecules with detergent bound, whereas in DM buffer the MW corresponds to a single protein molecule and bound detergent. Note in this experiment the protein was not subjected to a second column, which in some scenarios, can also influence oligomeric state **(c)** Far-UV CD spectrum of hiGlpG in DDM (dimer) and DM (monomer) containing buffer. (**d**) Melting curves of hiGlpG samples in DDM and DM buffer.