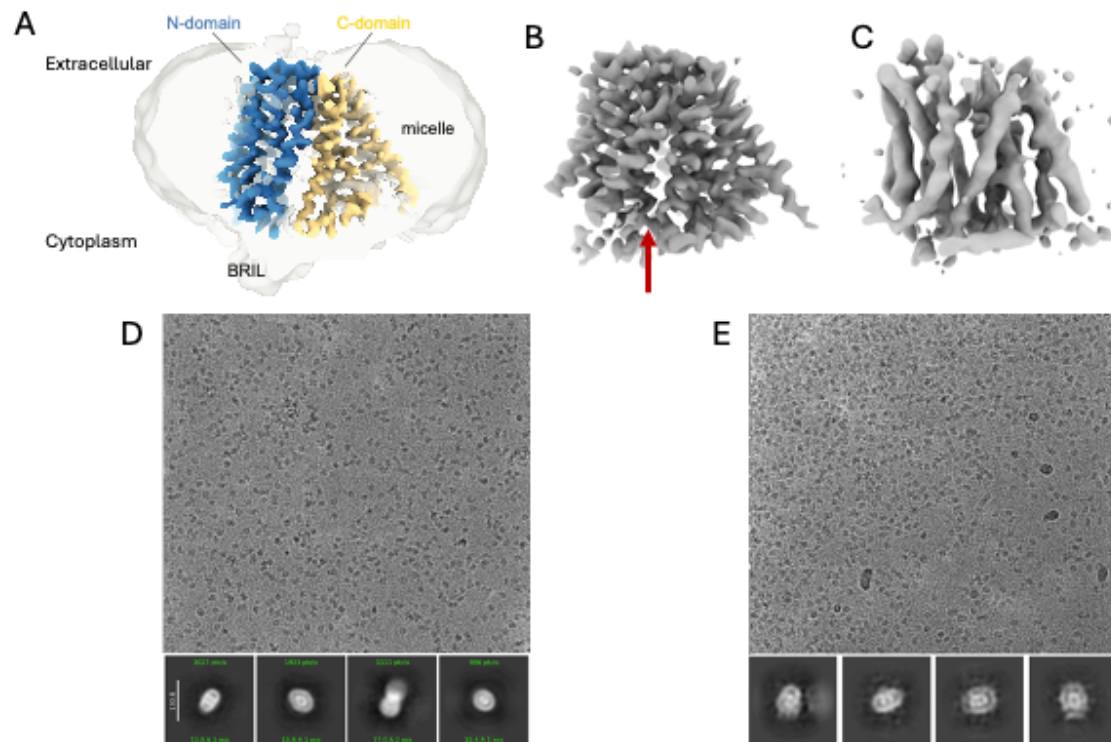


Supplemental Figure



Supplemental Figure 1: RFC structural progress

A) Structure of WT RFC with methotrexate bound (side-view) in the inward-open conformation (Wright & Fedor, *et al.*, 2022), depicting the two domains that change conformation to provide alternating access to either side of the membrane.

B) CryoEM map of RFC single mutant in the inward-occluded conformation. The loop closing cytoplasmic access is indicated by the red arrow. Pixel size 1.08Å at ~3.2Å resolution.

C) CryoEM map of the RFC triple mutant in the outward-open conformation. Pixel size 1.08Å at ~5.3Å resolution.

D-E) Representative micrographs and 2D images from grid screening of RFC triple mutant with inhibitor (**D**) and anti-folate drug methotrexate (**E**) bound.