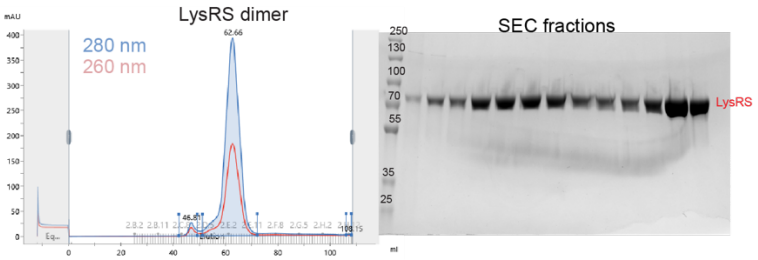

Figure 1: Purification of human LysRS. A) Size exclusion chromatography using a HiLoad superdex 200 16/600 increase in 25 mM Tris pH 7.5, 20 mM KCl, 10 mM MgCl2, 1 mM DTT showing a single species with an elution volume in agreement with a dimer. B) SDS-PAGE showing compositional homogeneity.

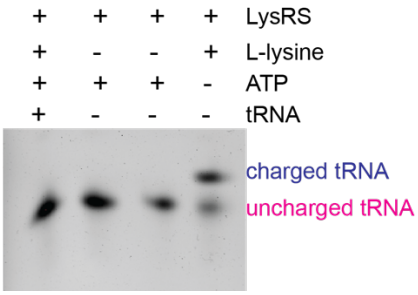

Figure 2: Purified LysRS is active. Acidic denaturing PAGE showing the aminoacylation activity of LysRS on human $tRNA^{Lys}$. The charged tRNA migrates slower and is observed only in the presence of LysRS with the additional substrates, ATP and L-lysine.

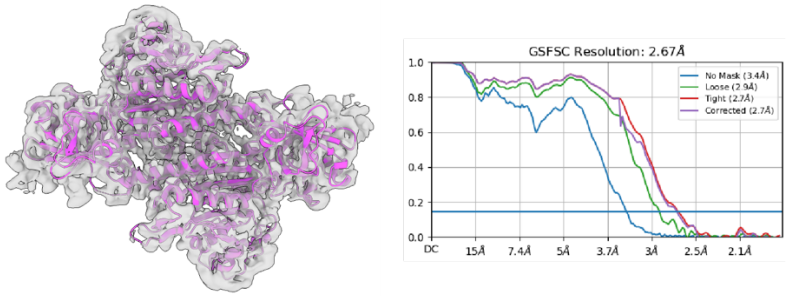

Figure 3: Preliminary cryoEM analysis leads to a 2.7 Angs cryoEM map of free LysRS. Ab initio map computed in CryoSPARC from ~2,500 movies collected on a Glacios with a direct electron detector showing a global resolution of 2.7 Angs using the gold-standard FSC of 0.143. The dimeric structure of LysRS fits well in the density.

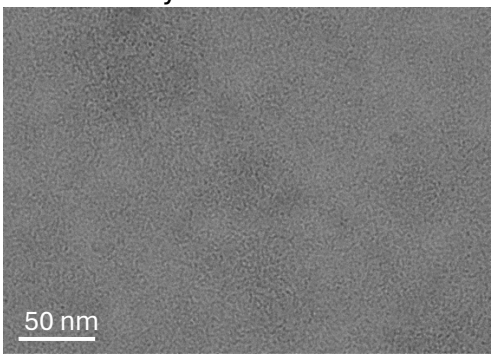

Figure 4: Preliminary cryoEM micrograph of LysRS bound to tRNA. Particles are clearly visible but ice thickness and particle distribution need further optimization.