Preliminary results and figures (1 pdf page) - High-throughput CryoEM methods, as exploited here, are ideal as it is possible to achieve sample preparation, data collection, and 3D reconstruction within one week. With sufficient microscope access, iterative rounds of development would provide high resolution structures of these evolved ribosomes.

The structural information obtained will be used to design mutants which will allow us to decipher the importance of specific residues or codons in translational kinetics.

We obtained 2D classes from negative staining and the orthogonal bisections of 50S subunit of strain S4.4 (*V.cholerae*) using Glacios. As seen in the figures below, some of the low resolution secondary structural features can be clearly observed which forms the foundation of our future experiments on Krios.

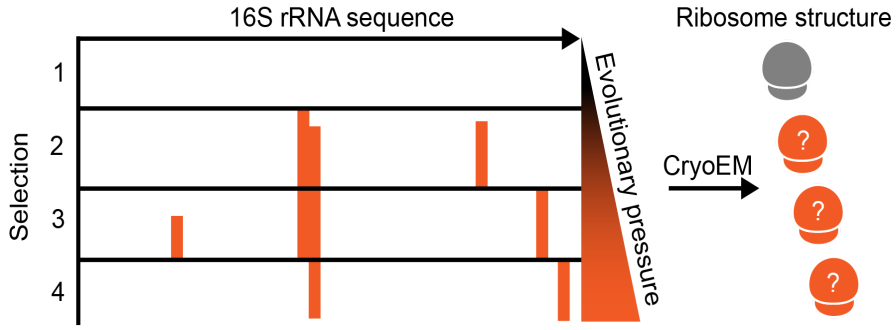

Figure 1. Schematic explaining the approach in this proposal. The ultimate aim is to structurally map the effect of evolutionary pressure on translation kinetics in the three bacterial species (*E.coli, V.cholerae, P.aeruginosa*).

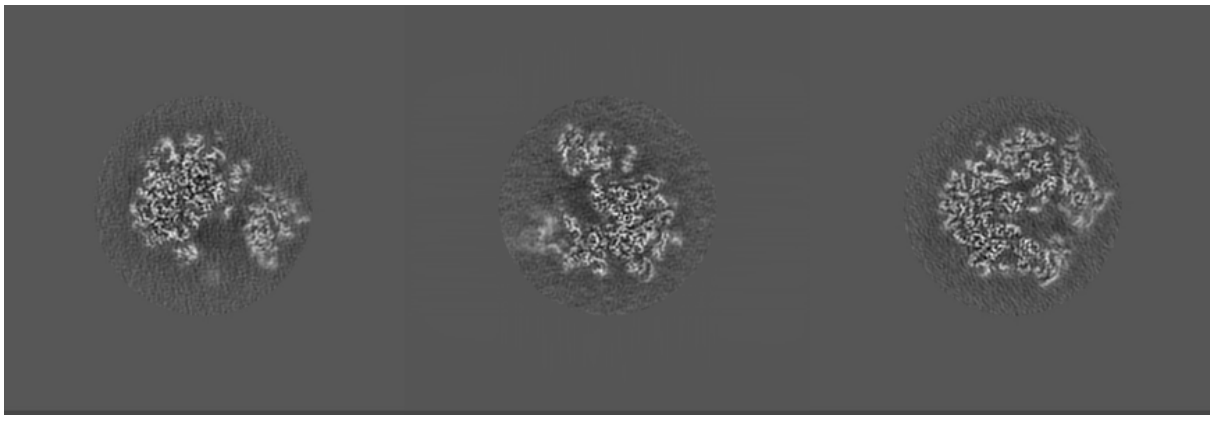

Figure 2: Example 2D classes of ribosomes from the strains S4.4 (*V. cholerae*) (A) and S3.3 (*P. aeruginosa*) (B) from negative staining and orthogonal bisections of 50S ribosomal subunit from the strain S4.4 (*V.cholerae*) (C) from half a day of data collection on Glacios equipped with a Gatan K3 camera.