SUPPLEMENTARY INFORMATION

We previously submitted a proposal in October 2019 where we proposed three separate projects. We were encouraged to submit a proposal for each project which we have now done for the January 5th, 2020 deadline. For this new application (i.e. not submitted in Oct 2019), we include more information on how samples were prepared for EM analysis and the the data collection parameters. We thank the reviewers for their suggestions.

Structural basis of hyper-accurate PheRS in response to oxidative stress.

Background. *Salmonella enterica* serovar Typhimurium is a bacterial species that is able to survive oxidative stress (reactive oxygen species) in the host. Notably, there is an increase in *ortho*-, *meta*- and *para*-tyrosine isomers due to oxidation of the phenylalanine aromatic ring[1]. Recently, our collaborators in the Ibba lab, have shown that PheRS becomes oxidized in response to stress revealing it is beneficial to reduce translation fidelity under specific stresses for survival[2]. Further, biochemical analysis reveal that PheRS undergoes a structurally rearrangement in response to a change in its oxidation state and PheRS oxidized residues were identified by mass spectrometry[2]. Our goal in this project is to determine the high-resolution structure of oxidized PheRS to determine how this modification causes PheRS to be hyper-accurate.

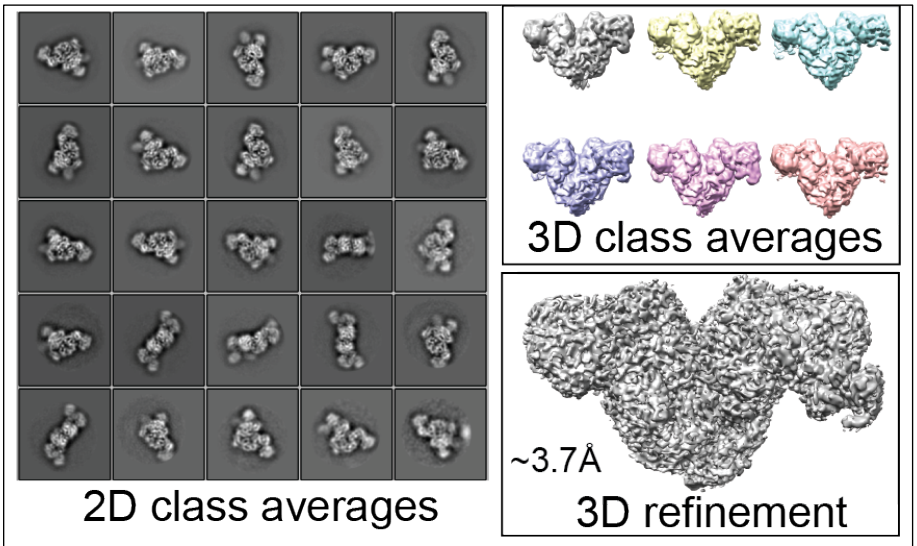

Figure 1. Preliminary cryo-EM analysis of PheRS. 2D class averages and 3D class averages of PheRS are shown. 3D refinement of the dataset to ~3.7Å.

Preliminary data. To define how the oxidative state of PheRS changes its secondary structural features to become hyper-accurate, we performed EM studies of this complex (Figure 1). First, we purified PheRS as previously published, oxidized PheRS by incubation with 20 mM H_2O_2, and excess H_2O_2 was removed by dialysis as previously described[2]. PheRS was placed on glow-discharged grids (Quantifoil 1.2/1.3 300 mesh Cu) and blotted using a FEI Vitrobot. Grids were screened using a JEOL JEM-1400 120 kV and a 1330 micrograph dataset was collected on an FEI Talos Arctica transmission electron microscope operating at 200 keV with BioQuantum/Gatan K2 direct electron detector. Micrographs were collected using a defocus range of -0.5 to -3.5 µm and a dose of 64.7 e^- per pixel and were acquired as 48-frame movies with 12s exposure time. Motion correction and dose weighting was performed with MotionCorr2 and contrast transfer function parameters determined with Gctf. 2D and 3D classifications were conducted in Relion-3.0. Semi-autonomous particle picking was used to select protein and incorrectly selected particles discarded after reference-free two-dimensional classification. Three-dimensional refinement and classification without alignment was conducted with a 45-Å low-pass filtered cryo-EM *P. aeruginosa* PheRS reference map (PDB: 4p71). Final particles were polished, CTF refined and subjected to 3D refinement to yield a reconstruction at ~3.7Å.

Proposed studies and expected results. Our goal in this project is to identify structural changes of PheRS, as revealed by our collaborators[2], undergoes in response to oxidative stress. We have a 3.7-Å dataset but we require higher resolution to confidently identify both the molecular details of these changes and amino acid modifications. **We request a half day for one dataset.**

References

1 Bullwinkle, T. J. *et al.* Oxidation of cellular amino acid pools leads to cytotoxic mistranslation of the genetic code. *eLife* **3**, doi:10.7554/eLife.02501 (2014).

2 Steiner, R. E., Kyle, A. M. & Ibba, M. Oxidation of phenylalanyl-tRNA synthetase positively regulates translational quality control. *Proc Natl Acad Sci U S A* **116**, 10058-10063, doi:10.1073/pnas.1901634116 (2019).