Supporting material for GUP1 proposal entitled: 'Characterizing the translating and hibernating ribosomes of the Lyme disease pathogen *Borrelia burgdorferi*'

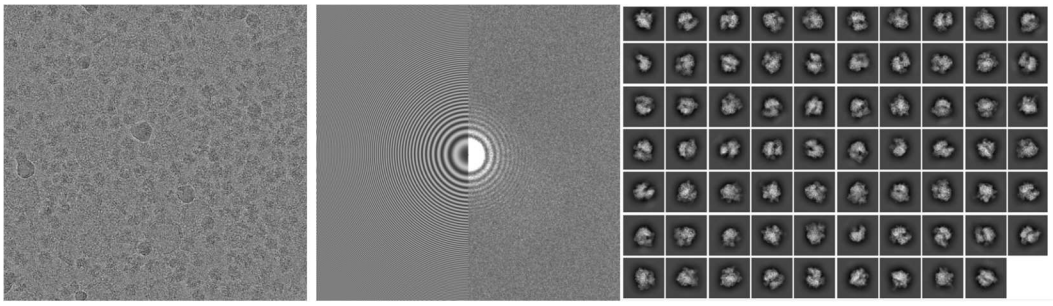

Fig. 1. Cryo-EM image processing for the *Bb* 70s hibernating ribosome. A representative micrograph (left panel), its contrast transfer function (CTF) fit (middle panel), and 2D class averages for the selected 280,018 particles from 2,993 selected micrographs collected on our in-house 300 KV JEOL 3200 FSC microscope, using a Gatan K2 direct electron detector (DED) camera (right panel).

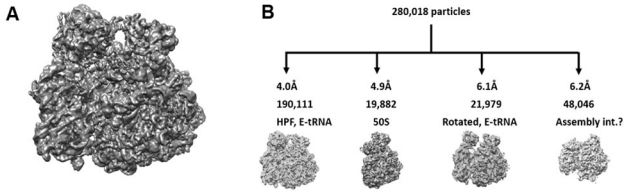

Fig. 2. Cryo-EM structures of the *Bb* 70s hibernating ribosome. (A) Cryo-EM map obtained from 280,018 particles from 2,993 selected micrographs. **(B)** 3D classification indicates the presence of at least three additional distinct sub-populations: the isolated large 50S subunit, the rotated ribosome with E-site tRNA, and a possible ribosome assembly intermediate that requires higher resolution characterization to be identified unambiguously(unpublished).

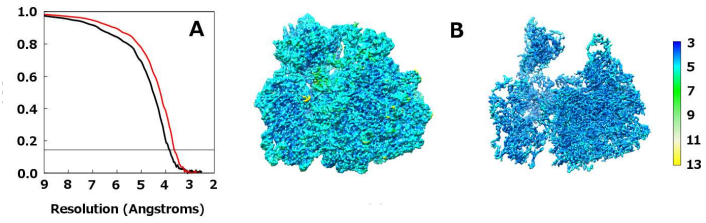

Fig. 3. Local resolution of the *Bb* 70s hibernating ribosome cryo-EM map. (**A**) FSC curve showing overall resolution of 70S ribosome (4.0 Å, black) and the 50S LSU (red, 3.7 Å). **(B)** Local resolution at low (left) and high (right) density threshold values show that only core regions approach near-atomic resolution(unpublished).

Key Personnel. Nilesh Banavali (PI) and Manjuli Sharma (primary user).

Materials and Methods. 70S ribosomes were isolated from *Borrelia burgdorferi* B31-A3 (*Bb*) [1] and grids were prepared using a Vitrobot [2]. Data was obtained on a 300 kV JEOL3200 FSC with a Gatan K2 DED camera with a defocus range of −1.0 to −3.5 µm at ×25,000 magnification with pixel size 1.25 Å, and a total dose of 10-20 e/Å2. The 40-frame movies were processed using CryoSPARC [3]. A curated selection of 2,993 movies and a 2D classification-based selection of 280,018 extracted particles (Fig. 1) resulted in a 3.9 Å resolution cryo-EM map (Fig. 2). 3D classification showed 4 distinct classes with lower individual resolutions (Fig. 2) and local resolution estimation showed only core regions with resolutions approaching 3 Å (Fig. 3).

References.

1. Li, Y., et al., Zinc depletion induces ribosome hibernation in mycobacteria. Proc Natl Acad Sci U S A, 2018. 115(32): p. 8191-8196.
2. Koripella, R.K., et al., Structures of the human mitochondrial ribosome bound to EF-G1 reveal distinct features of mitochondrial translation elongation. Nat Commun, 2020. 11(1): p. 3830.
3. Punjani, A., et al., cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. Nat Methods, 2017. 14(3): p. 290-296.