

Supplementary material:

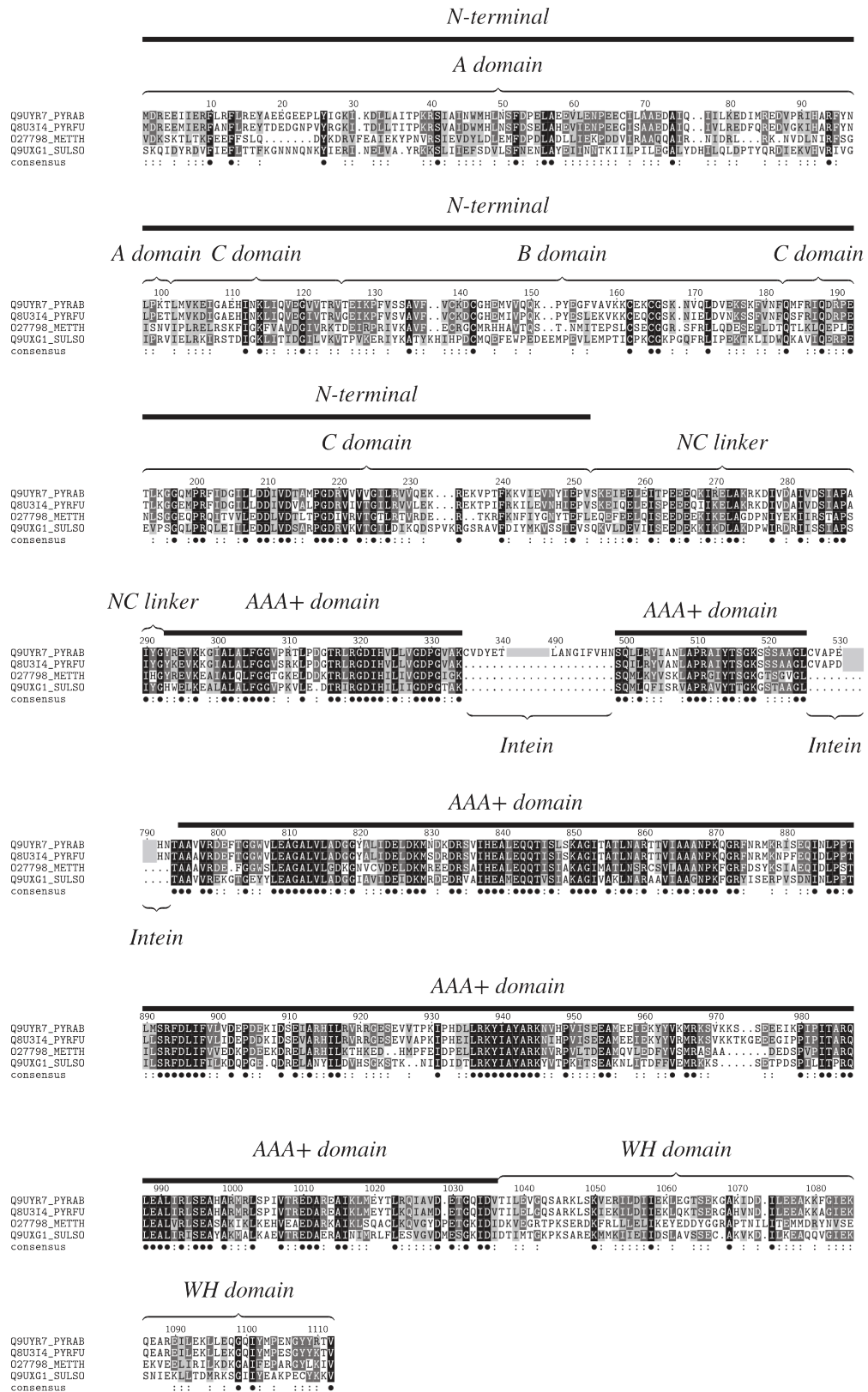
Structure of an octameric form of the minichromosome maintenance protein from the archaeon *Pyrococcus abyssi*

Giuseppe Cannone^{1,2,3} Silvia Visentin^{1,2,4}, Adeline Palud^{5,6,7}, Ghislaine Henneke^{5,6,7} and Laura Spagnolo^{1*}

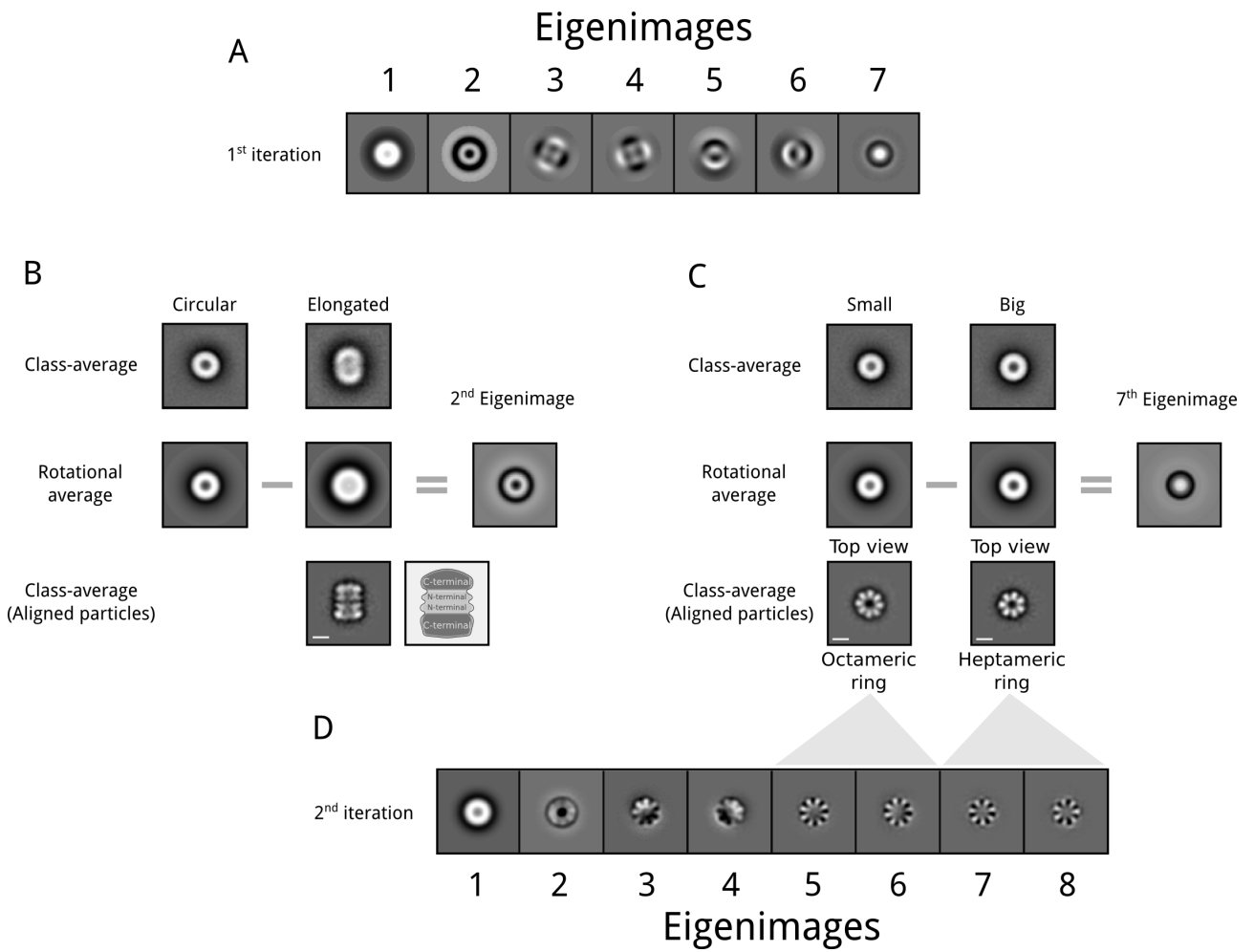
¹Institute of Molecular, Cell and Systems Biology, University of Glasgow, University Avenue, Glasgow G12 8QQ; ²School of Biological Sciences and ³Centre for Science at extreme conditions, University of Edinburgh, Max Born Crescent, Edinburgh EH9 3JR, UK; ⁴ISIS neutron source, Science and Technologies Research Council, Rutherford Appleton Laboratories, Harwell, OX11 0QX United Kingdom ⁵IFREMER, Laboratoire de Microbiologie des Environnements Extrêmes, UMR 6197, ZI de la pointe du diable CS 10070 29280 Plouzané, France; ⁶Université de Bretagne Occidentale, Laboratoire de Microbiologie des Environnements Extrêmes, UMR6197, rue Dumont d'Urville 29280 Plouzané, France ; ⁷CNRS, Laboratoire de Microbiologie des Environnements Extrêmes, UMR6197, rue Dumont d'Urville 29280 Plouzané, France

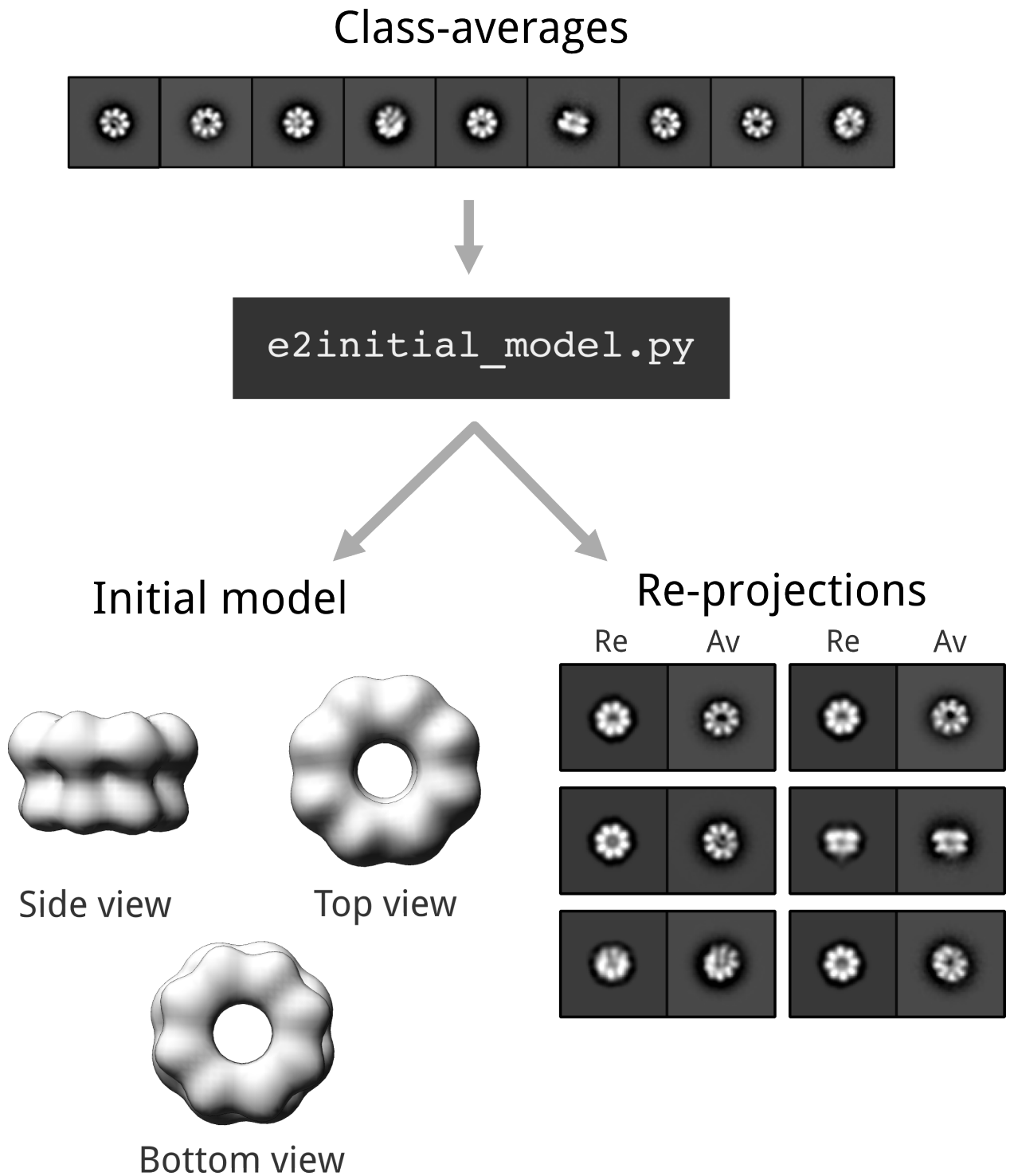
*Corresponding author: laura.spagnolo@glasgow.ac.uk

Cannone et al., Figure S1



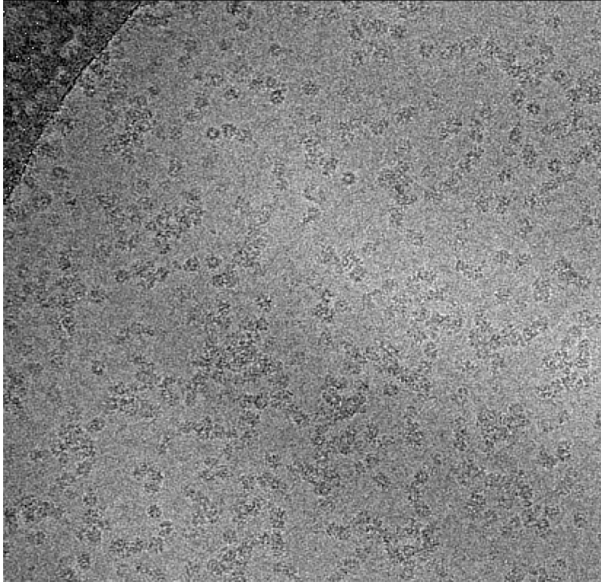
Cannone *et al.*, Figure S2



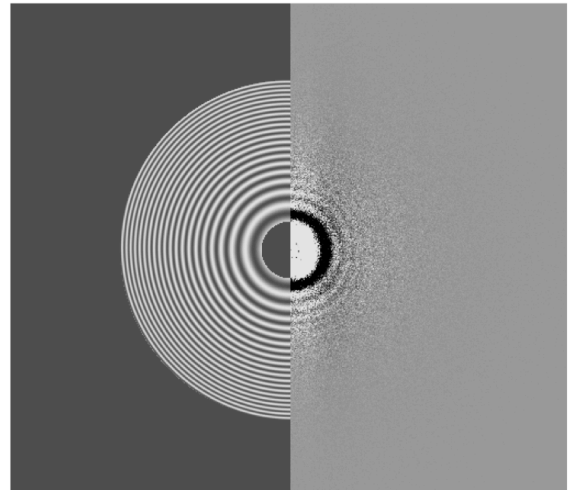


Cannone *et al.*, Figure S4

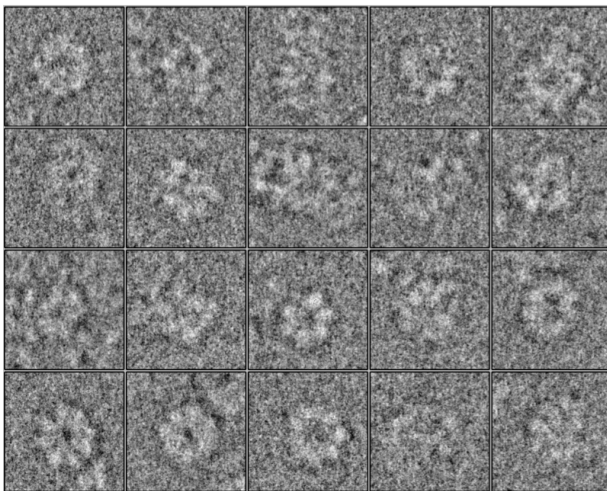
A. Micrograph



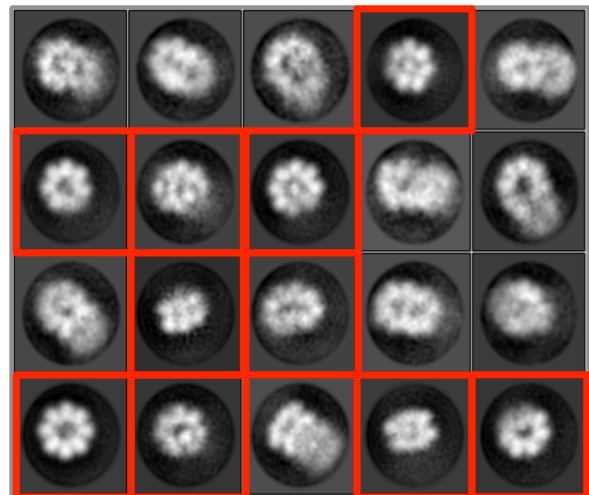
B. CTF



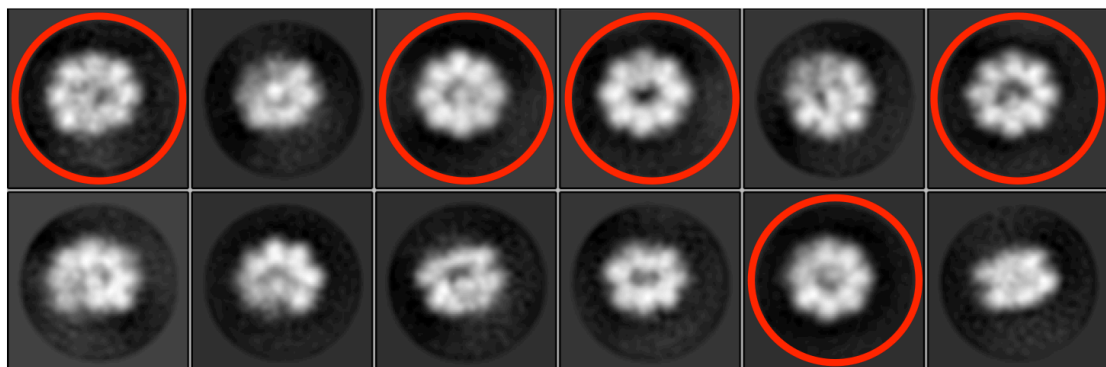
D. Individual particles



E. Classes calculated from the whole dataset



F. Classes calculated from the subset indicated in E



Supplementary Figures Legends

Figure S1: Sequence alignment of *S. solfataricus*(Q9UXG1), *M. Thermoautrophicus* (O27798), *P. furiosus*(Q8U3I4) and *P. abyssi* MCMs. Highly conserved residues (identity 100%) are shaded in black while black dots are shown in the consensus. Residues with identity >75% are shaded in light grey.

Figure S2: MSA classification of *Pab*MCM molecular images. (A) First eigen images used for the classification of the molecular images. (B) Two characteristics class-average, which show size variation. Class averages were compared by subtraction (Circular minus elongated) to verify that the eigenimage 2 was representative of the difference in size between circular and elongated particles. Further MSA classification followed by multi-reference alignment (MRA) of the molecular images belonging to the elongated class-average revealed a characteristic two-fold symmetry and four tiers structure. These features are similar to those ones previously observed for *Mth*MCM. (C) Two characteristics class-average, which show size variation. Class-averages were compared by subtraction (Small minus big) to verify that the eigenimage 7 was representative of the difference in size between small and big particles. Further MSA classification followed by multi-reference alignment (MRA) of the molecular images belonging to the both class-averages revealed a two ring-shaped class-averaged with a characteristic 8-fold and 7-fold symmetry for *Mth*MCM. Scale bar 100 Å.

Figure S3: 3D reconstruction strategy for the initial model of the full-length *Pab*MCM. From the top, 9 class averages were selected for the 3D reconstruction of an initial model of the full-length *Pab*MCM. The initial model was calculated by using the `e2initial_model.py` software from the EMAN2 image processing suite²¹. The best initial model, showed in figure, was chosen based by evaluating the correspondence between class averages (*Av*) and the re-projections (*Re*). The initial 3D model of the full-length *Pab*MCM single octamer was rendered in Chimera³⁸.

Figure S4: 2D image processing of cryo-EM images. A. Typical raw micrograph. B. Typical CTF image. D. individual particles. E. 2D classes from the full dataset. Individual images belonging to the classed in the red squares were selected and processed as a subdataset of single ring images. F. Classes calculated from the selected subset. Classes in red circles are consistent with those in Figure 2 and Figure S3. Boxsize=290 Å.

