

SUPPLEMENTARY ONLINE DATA

Archaeal flagellar ATPase motor shows ATP-dependent hexameric assembly and activity stimulation by specific lipid binding

Abhrajyoti GHOSH*, Sophia HARTUNG†‡, Chris van der DOES§, John A. TAINER†‡ and Sonja-Verena ALBERS*¹

*Molecular Biology of Archaea, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany, †Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, U.S.A., ‡Lawrence Berkeley National Laboratory, Berkeley, CA 94720, U.S.A., and §Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse 10, 35043 Marburg, Germany

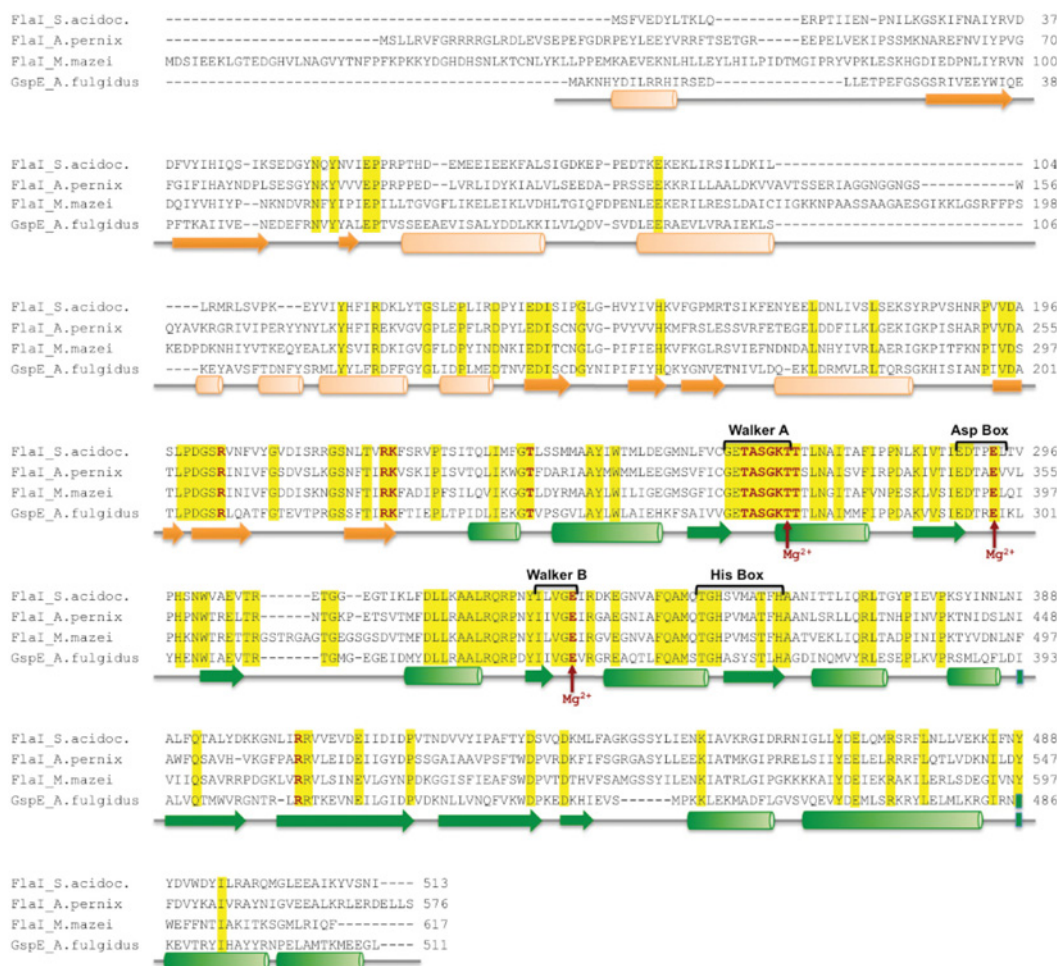


Figure S1 Multiple alignments of FlaI homologues [FlaI_ *S. acidocaldarius* (GenBank® accession number YP_255813); FlaI_ *Aeropyrum premix* (GenBank® accession number NP_148247); FlaI_ *Methanosarcina mazei* (GenBank® accession number NP_632341); and GspE_ *A. fulgidus* (GenBank® accession number NP_069493)] in different archaea

Conserved motifs involved in ATP binding and hydrolysis are assigned from the known motifs in GspE_ *A. fulgidus*, the T2S system ATPase, whose crystal structure has been solved (PDB code 2OAP). In brown and bold are residues that bind p(NH)ppA in the GspE crystal structure. Magnesium co-ordinating residues are marked in the alignment. The N-terminal domain and the C-terminal ATPase domain are shown in orange and green respectively. Conserved residues are highlighted.

¹ To whom correspondence should be addressed (email albers@mpi-marburg.mpg.de).

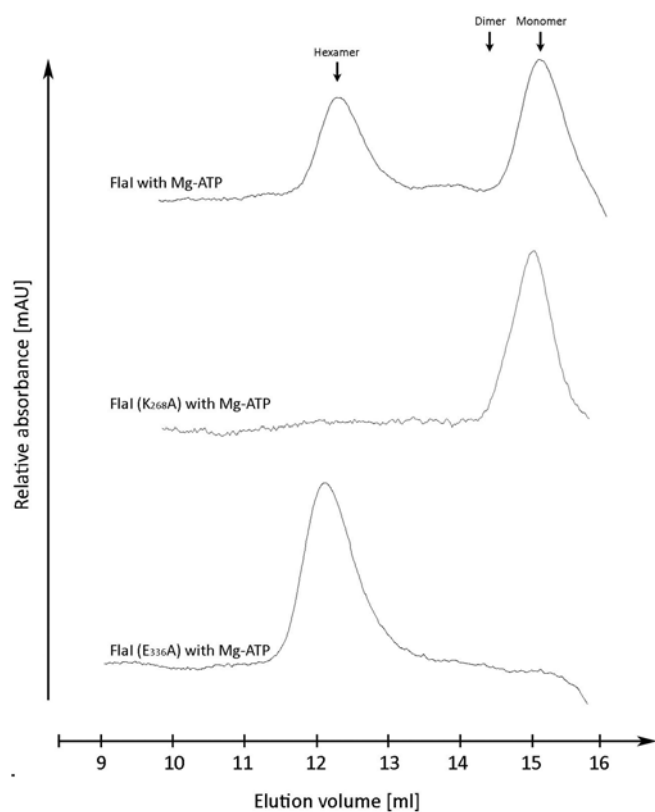


Figure S2 Size-exclusion chromatographic profiles

Comparative size-exclusion chromatographic profiles of Flal, Flal^{K268A} and Flal^{E336A} pre-incubated with ATP and Mg²⁺, on a Superdex 200 (10/300) gel-filtration column equilibrated with 50 mM Hepes (pH 7.5) containing 150 mM NaCl and 0.5 mM ATP.

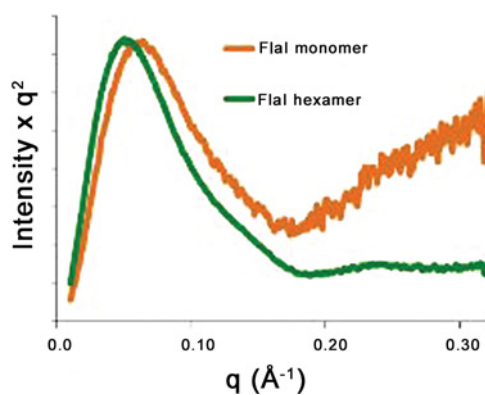


Figure S3 Comparative Kratky plot from SAXS data for Flal monomer and hexamer showing higher flexibility of the monomeric Flal

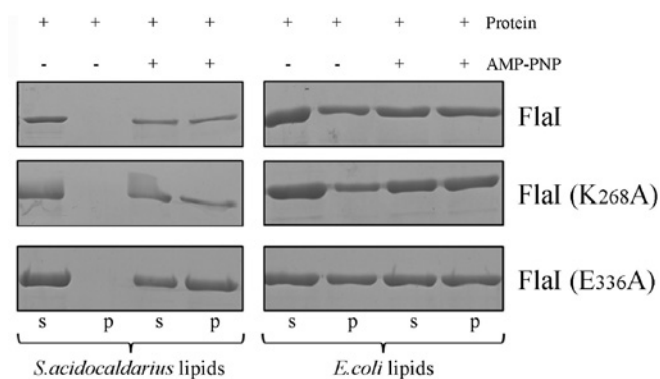


Figure S4 Liposome pull-down assay of Flal in the presence of *S. acidocaldarius* tetraether lipids and *E. coli* lipid extract

The interaction of either Flal, Flal^{K268A} or Flal^{E336A} protein was tested either with *S. acidocaldarius* tetraether lipids or lipids from *E. coli* in the presence or absence of p[NH]ppA.