Supplementary Data Figure Captions

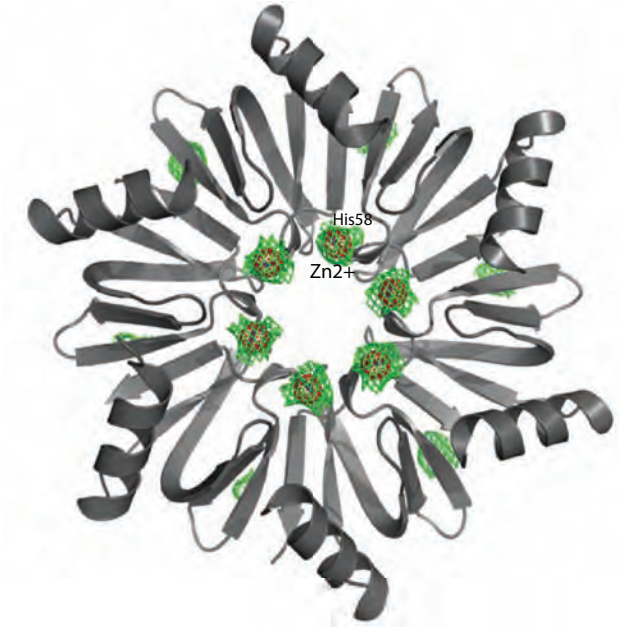
Supplementary Figure S1. *Proximal face binding by zinc*
(**Left**) Zinc binding to residue His58 of Sa Hfq. A view into the proximal face of Sa Hfq in which the hexameric protein is shown as a grey ribbon, zinc ions as red spheres and the composite omit electron density (contoured at 1 σ) as green mesh. Residue His58 and one Zn^{2+} are labelled. (**Right**) Same view as in the left figure but for the sake of clarity without the inclusion of the composite omit electron density.

Supplemental Figure S2. *The R-site can accommodate a guanosine*
Models of guanosine binding to the R-sites of the (A) Sa Hfq, (B) Bs Hfq, and (C) Ec Hfq. Each structure was generated by simple substitution of the adenine base observed in each complex with a guanine. No additional rotation was necessary to fit the guanine and as a result the protein-RNA interaction distances are the same as in Figure 3. The atom colouring is the same as used in Figure 3.

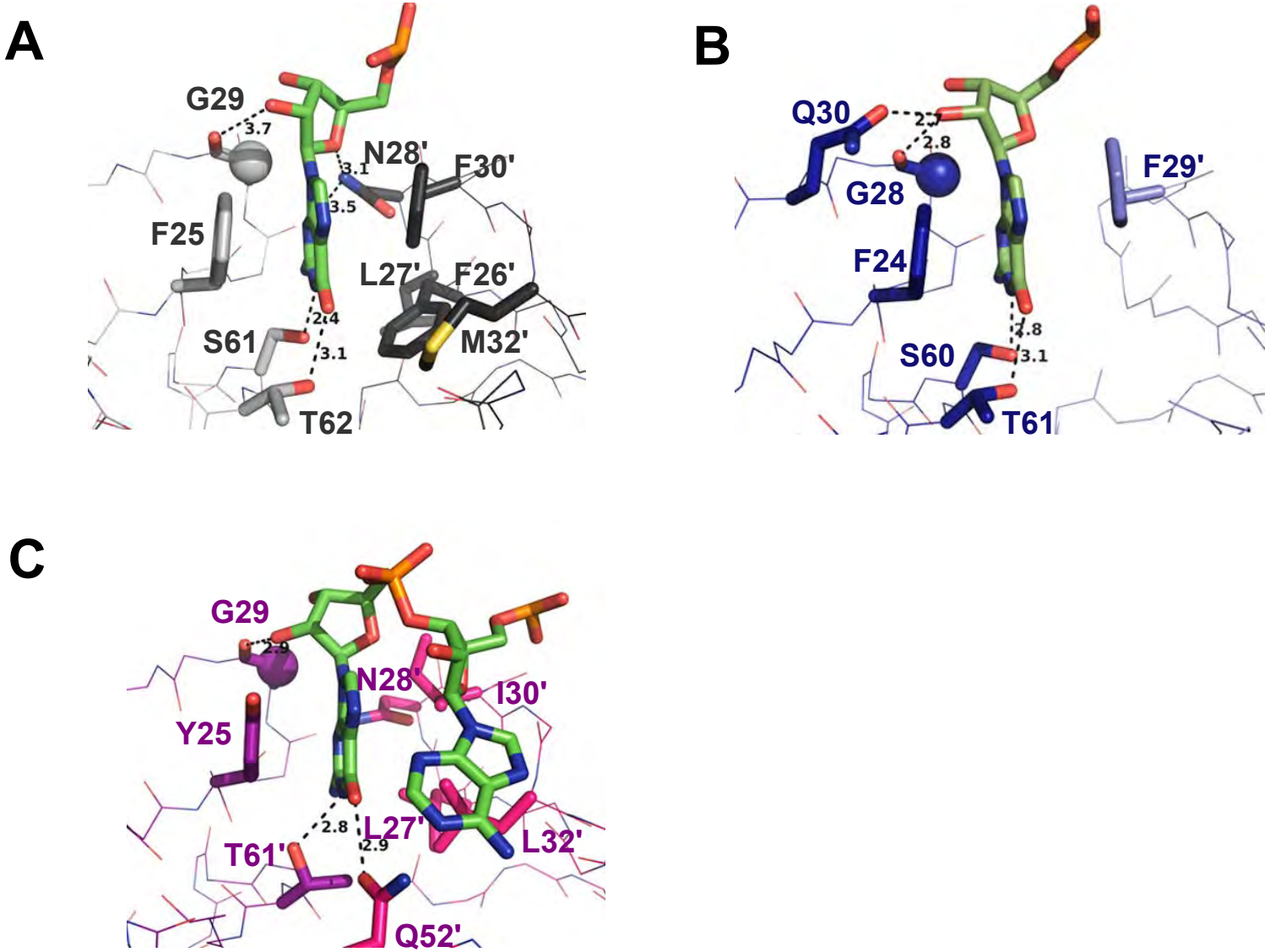
Supplemental Figure S3. *Sequence alignment of Hfq proteins from selected bacteria indicating a conserved, but different, RNA binding mechanisms for Gram-positive and Gram-negative bacteria*
Decisive factors for the R-L binding mechanism are amino acid residue 30 and the β3-loop-β4 region. Each are conserved amongst Gram-positive bacteria but differ from the respective amino acid residues in Gram-negative bacteria. Residues 30 and 50 of Sa Hfq are marked with arrows. β strands 3 and 4 are labelled below the sequence and residues constituting these β strands as well as residue 30 are highlighted in bold letters (grey for Gram-positive and magenta for Gram-negative bacteria, respectively). Sequences for which an Hfq-RNA crystal structure exists are accentuated by bold letters.

Supplemental Figure S4. *Potential Sa Hfq residue Lys33-L-site base interactions*
Modelled interaction between Sa Hfq residue Lys33 and appropriate hydrogen bond acceptors of all four RNA bases at the L-site. The coordinates of the Bs Hfq-$(AG)_3A$ complex were used (34B, PDB 3HSB) and residue Arg32 was substituted by a lysine. Each base was examined at the L-site (A) adenine (green), (B) cytosine (yellow), (C) guanine (cyan) and (D) uracil (magenta). Note that unlike the purine bases and uracil, the cytosine base must take the *anti* glycosidic conformation to hydrogen bond with Lys33. The length of the modelled hydrogen bonds, in Ångstrom, is shown in each panel. No attempts were made to optimize the interactions but care was used to avoid any bad contacts.

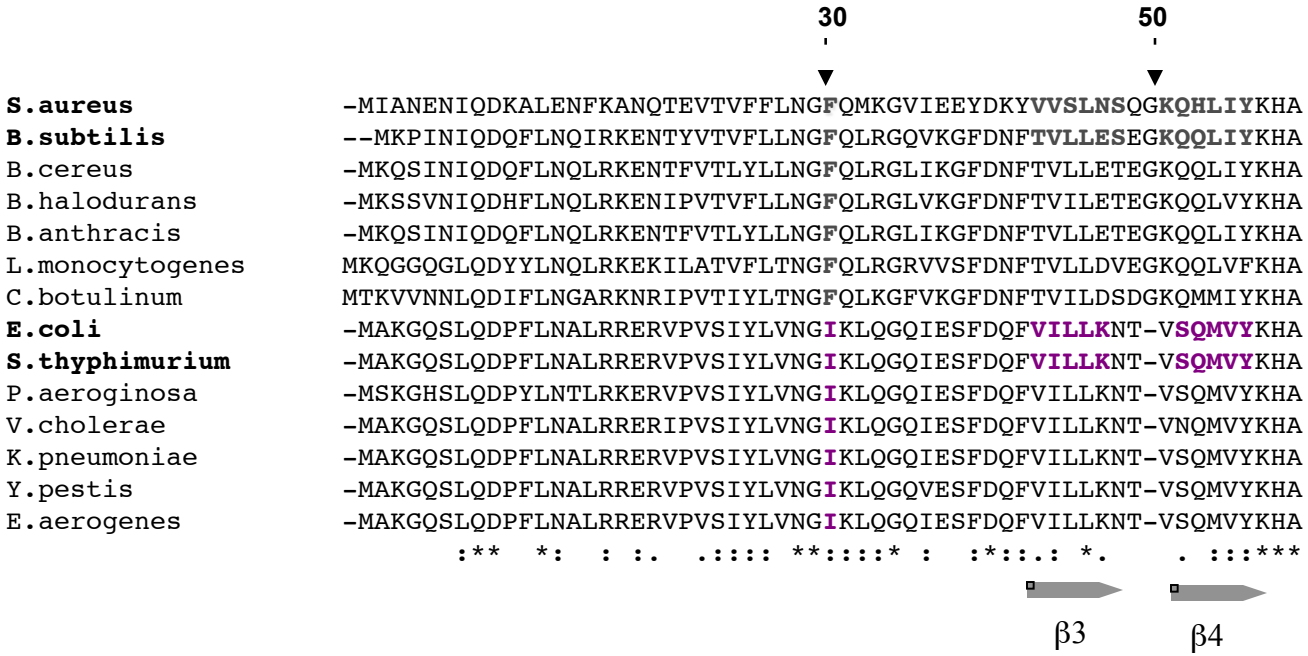
Supplementary Figure S5. *Sa Hfq binds longer RNA A-tracts more weakly than Ec Hfq*
(A) Ec Hfq–RNA A_{16} binding isotherm. (B) Ec Hfq–RNA A_{27} binding isotherm. (C) Sa Hfq–RNA A_{16} binding isotherm. (D) Sa Hfq–RNA A_{27} binding isotherm.



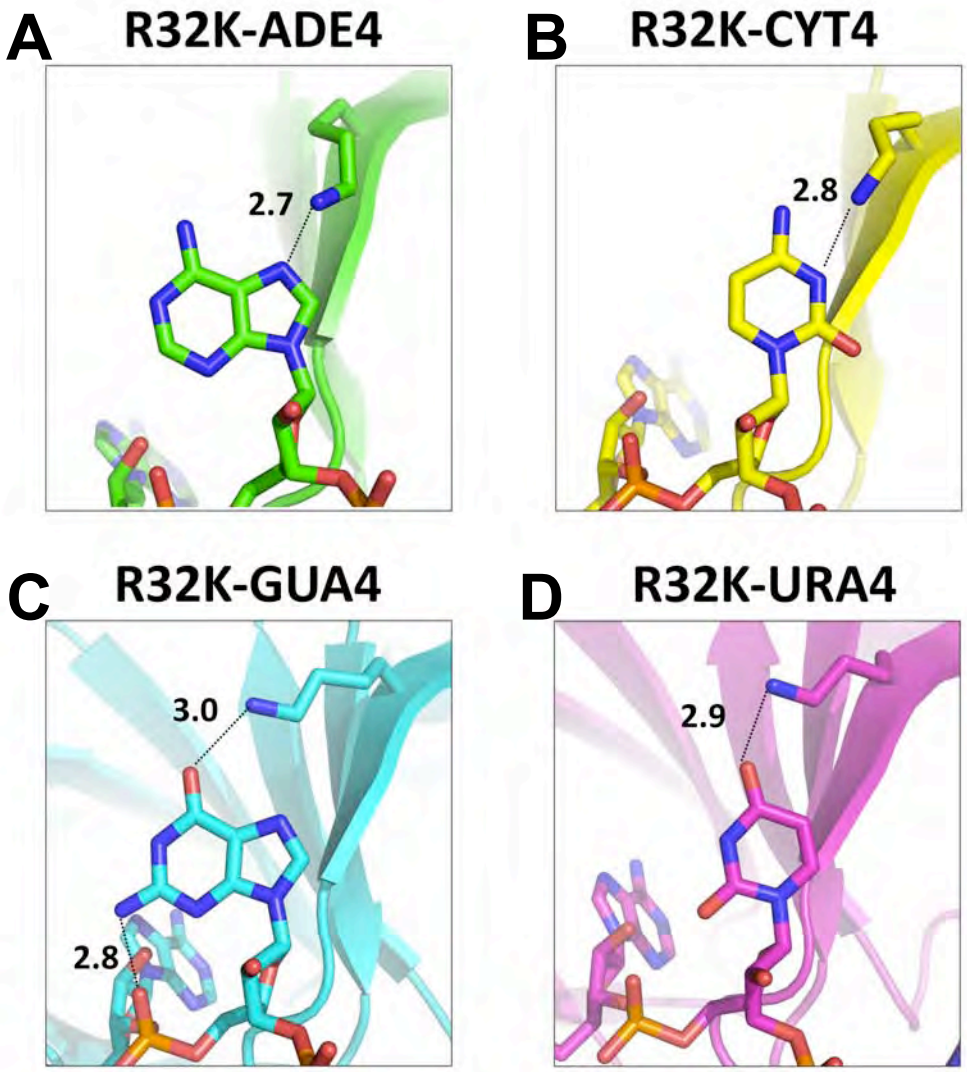
Supplemental Figure 1



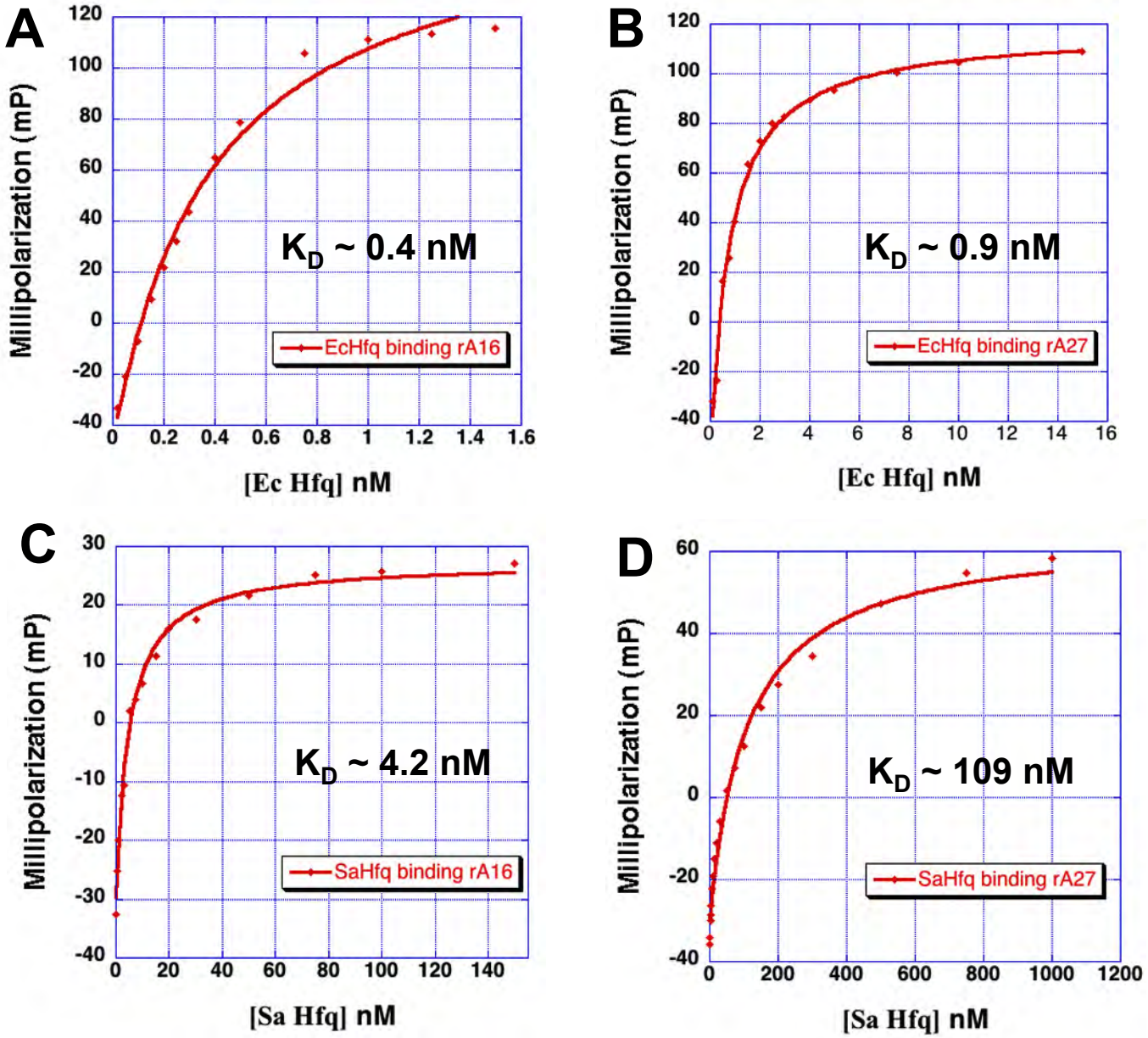
Supplemental Figure 2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5