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**The sodium-dependent D-glucose transport protein
of *Helicobacter pylori***

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Supplementary information

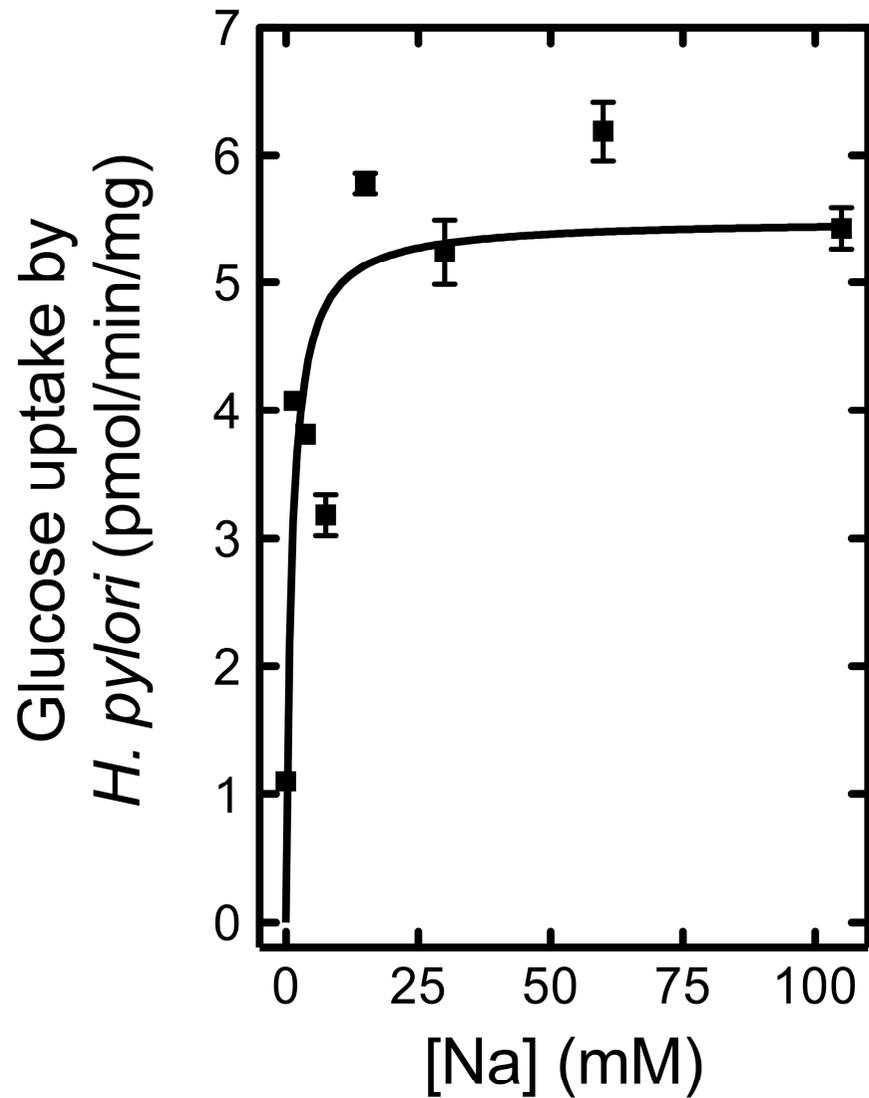


Fig. S1. Kinetics of glucose transport into *H. pylori* in the presence of varying concentrations of sodium. The initial rates of transport of radioisotope-labelled glucose into wild-type *Helicobacter pylori* glucose were measured by sedimentation in triplicate as described in Methods. The concentration of glucose was maintained at 0.88 mM, while the concentration of NaCl was varied as shown maintaining a total osmolarity equivalent to 150 mM salt by making up with appropriate complementary concentrations of KCl. The apparent K_m for sodium = 1.060 ± 0.412 mM and V_{max} 5.496 ± 0.297 nmol.mg⁻¹.min⁻¹.

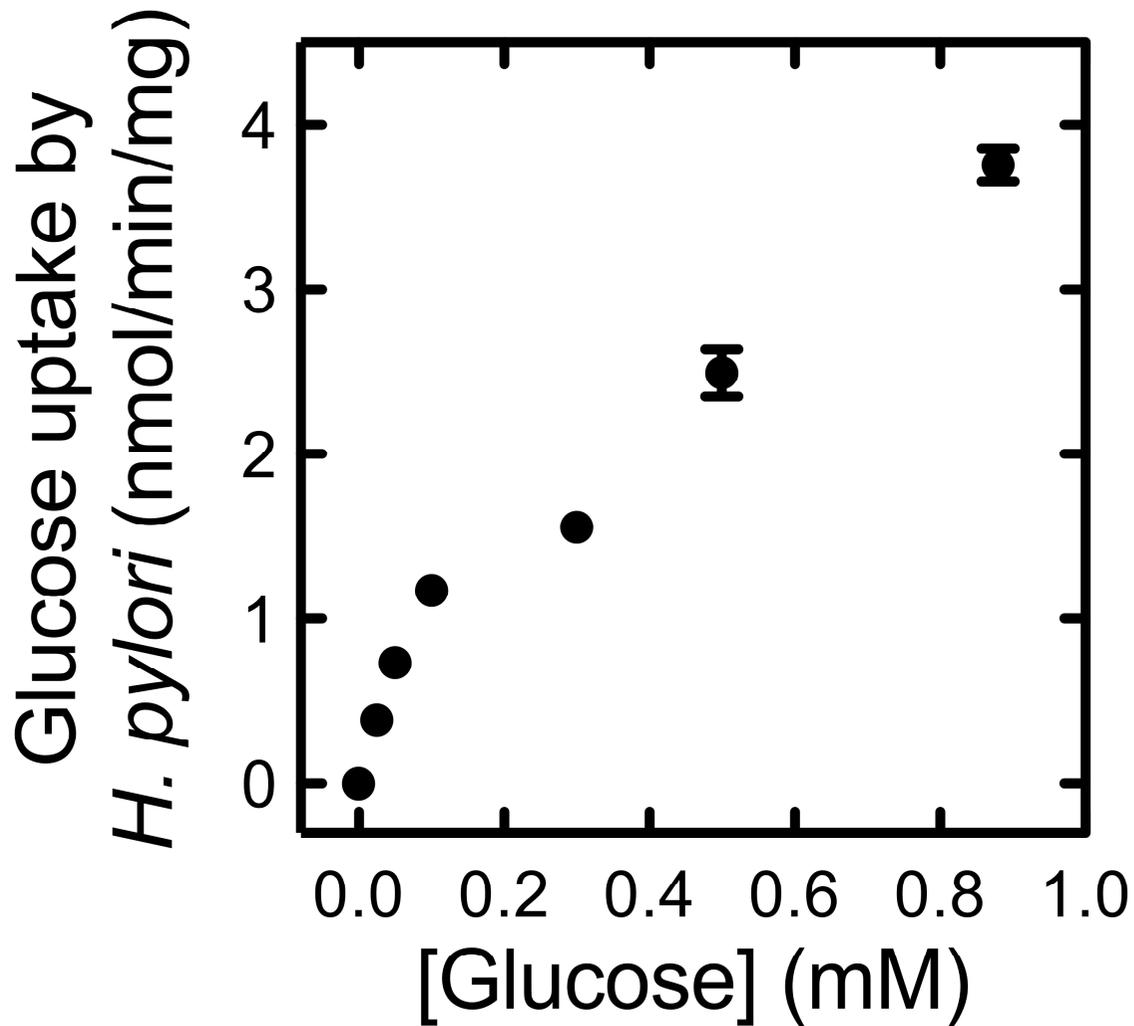


Fig. S2. Kinetics of glucose transport into *H. pylori* in the presence of excess sodium. The initial rates of transport of radioisotope-labelled glucose into wild-type *Helicobacter pylori* glucose was measured by sedimentation in triplicate as described in Methods. The supporting medium contained 150 mM NaCl, 5mM MES, pH 6.6, and the final concentration of glucose was varied as shown. The first four points fit to a single hyperbola with an apparent K_m of $0.162 \text{ mM} \pm 0.028$ and V_{max} of $3.66 \text{ nmol.mg}^{-1}.\text{min}^{-1}$. All the points can be fitted to one hyperbola with parameters apparent $K_m = 0.800 \pm 0.228 \text{ mM}$ and $V_{max} 6.886 \pm 1.135 \text{ nmol.mg}^{-1}.\text{min}^{-1}$. The points might also be fitted to the sum of two hyperbolas, but the limited specific activity of the radioisotope-labelled glucose limits the possibility of obtaining reasonable estimates of the parameters.

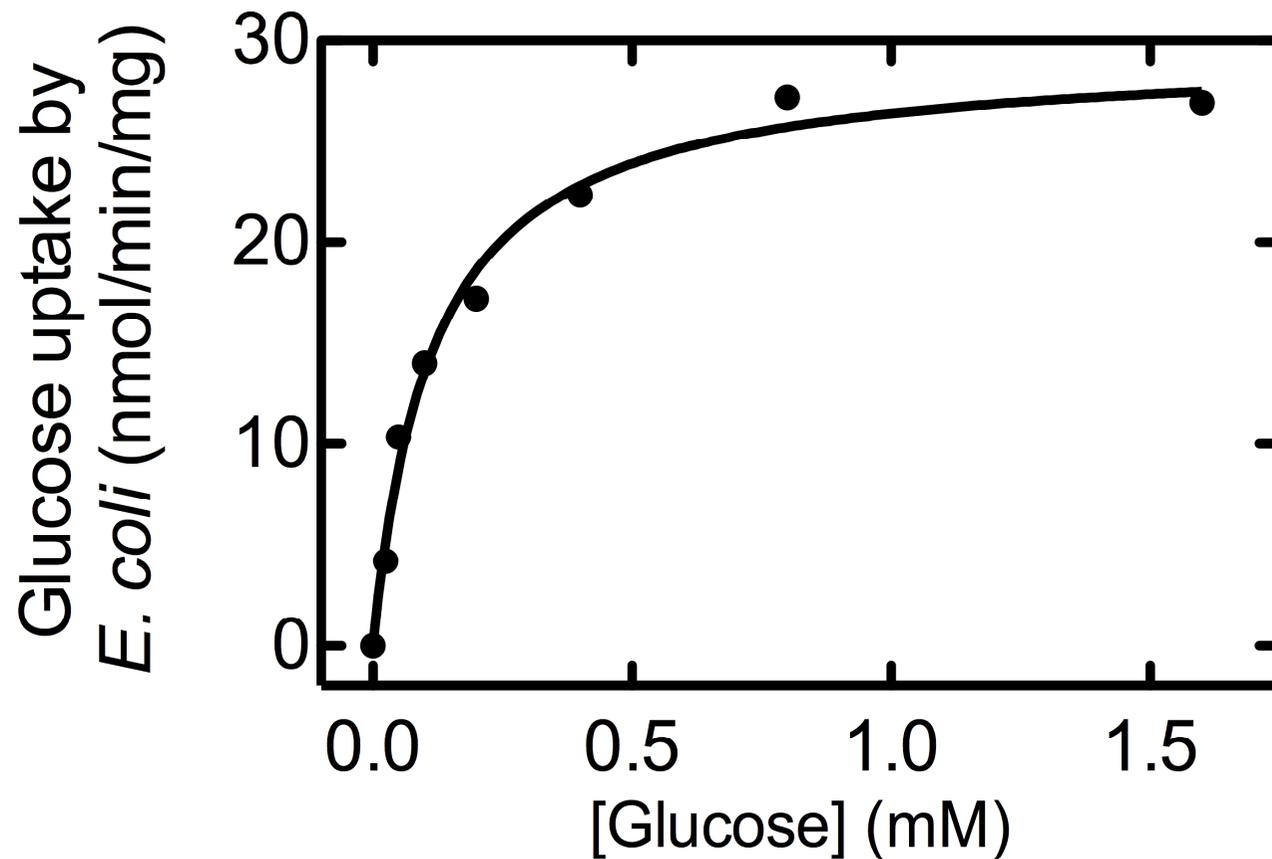


Fig. S3. Kinetics of glucose transport by GluP into *E. coli* in the presence of excess sodium. The initial rates of transport of radioisotope-labelled glucose into IPTG-induced *E. coli* cells hosting the pTTQ18hp1174H₆ plasmid was measured at 25°C by filtration in duplicate as described in Methods. The supporting medium contained 60 mM NaCl, 90 mM KCl, 0 mM glycerol, 5 mM MES, pH 6.6, and the final concentration of glucose was varied as shown. The points fit to a single hyperbola with an apparent K_m of 0.116 ± 0.015 mM and V_{max} of 29.55 ± 1.10 nmol.mg⁻¹.min⁻¹.

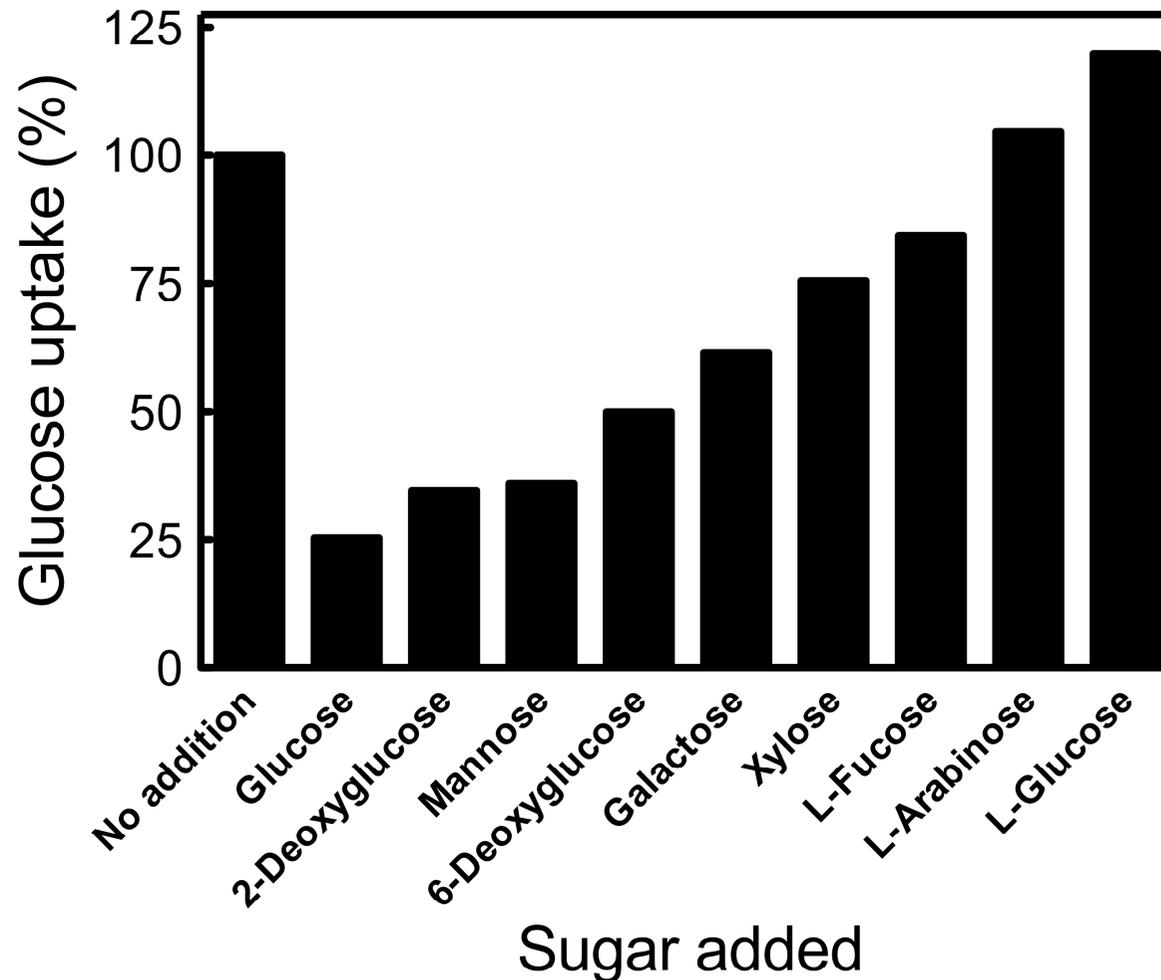


Fig. S4. Inhibition of glucose transport by GluP in the presence of sugars. The transport of radioisotope-labelled 0.05 mM glucose into IPTG-induced *E. coli* cells hosting the pTTQ18 $hp1174H_6$ plasmid was measured at 25°C by filtration in duplicate for 2 minutes as described in Methods. The supporting medium contained 60 mM NaCl, 90 mM KCl, 10 mM glycerol, 5mM MES, pH 6.6, and a final concentration of 40 mM of each sugar as indicated.

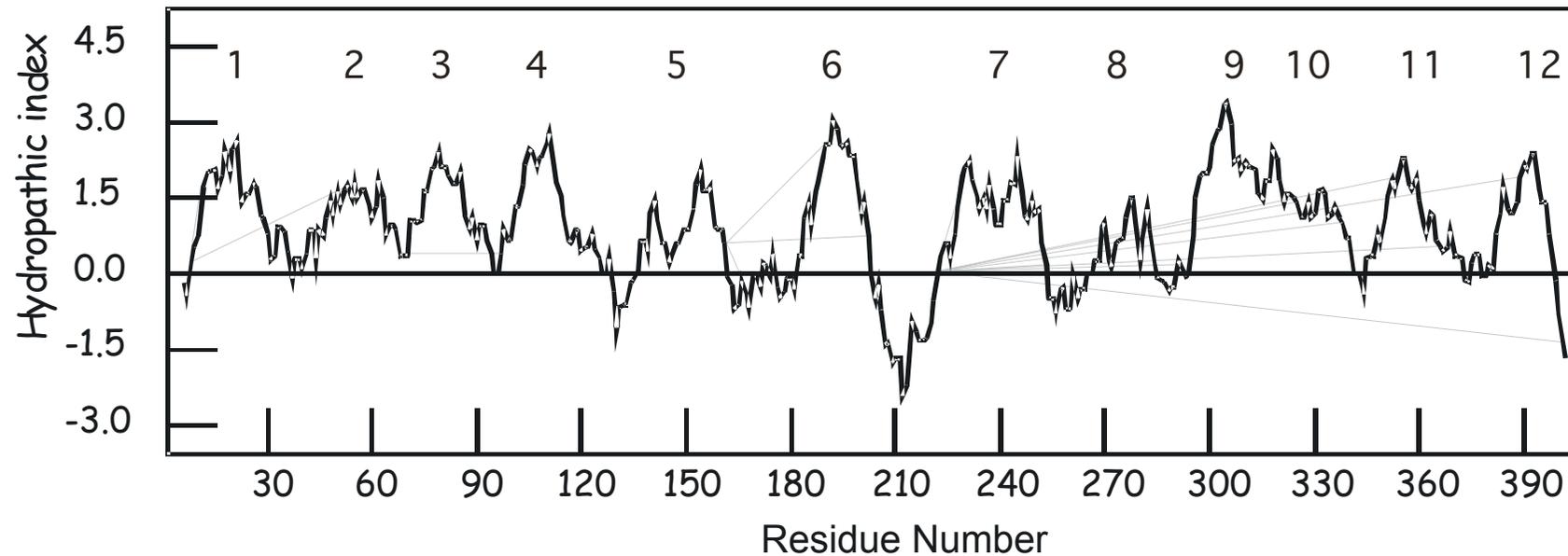


Fig. S5. Hydropathy plot of the GluP protein of *H. pylori* and model of the topology of the HP1174 protein. The algorithm of Kyte and Doolittle (1982) was used with a window size of 11 residues; the putative positions of 12 helices are indicated. **The model is compatible with von Heijne's positive-inside rule** (1992). The generation of hydrophobic profiles was carried out by the WinPep package (Hennig, 1999) (<http://www.biologie.uni-freiburg.de/data/schaefer/lhennig/winpep.html>).

Creation of a homology-based structural model of GluP from H. pylori

A homology model of GluP was made using the crystal structure of *Escherichia coli* GltP [PDB accession 1PW4; (Huang *et al.*, 2003)] as a template. Extensive comparisons indicated that GltP is a more likely paradigm than the structure of the Na⁺-galactose transport protein from *Vibrio parahaemolyticus* (Faham *et al.*, 2008) since its protein fold is very different from GltP. Because of the evolutionary distance between GluP and the GltP structural template, a combination of techniques was used to optimise their alignment. An initial alignment was created using the profile-to-profile based Multiple Mapping Method (Rai, B.K. & Fiser, A. (2006) *Proteins* **63**, 644-66). This preliminary alignment was next adjusted in the light of analysis of the aligned sequences of 91 GluP family members and, separately, of 100 GltP homologues for patterns of residue conservation (using the ConSeq method; Berezin, C., Glaser, F., Rosenberg, J., Paz, I., Pupko, T., Fariselli, P., Casadio, R. and Ben Tal, N. (2004) *Bioinformatics* **20**, 1322-1324), and of hydrophobicity. The resultant alignment is shown in Figure S5. Modeller version 8.2 (Fiser and Sali (2003) *Methods Enzymol.* **374**, 461-491) was then used to create 100 models based on this alignment, and the five of lowest energy were further analysed using MolProbity (Lovell *et al.* (2003) *Proteins: Structure, Function, and Genetics* **50**, 437-450). That selected for subsequent investigation had only 6 residues in the disallowed region of the Ramachandran plot.

Analysis of the model

In addition to validation of the structure using MolProbity, the surface of the chosen model was examined for the presence of hydrophilic side chains on the putative lipid-facing surfaces. These are shown in solid molecular representation, with the truly polar residues shown in colour (red = acidic; blue = basic; green = amide). Overall, the model is reasonably distributed in this respect - there is a band of non-polar residues girdling the protein in the likely vicinity of the bilayer, with few polar residues to be found here.

Faham, S., Watanabe, A., Besserer, G.M., Cascio, D., Specht, A., Hirayama, B.A., Wright, E.M., Abramson, J., (2008) *Science* 321:810-814.

Huang, Y., Lemieux, M.J., Song, J., Auer, M. and Wang, D-N (2003). Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. *Science* 301: 616-620.

