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Supplementary Materials for

Structural basis of Lewis^b antigen binding by the *Helicobacter pylori* adhesin BabA

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This PDF file includes:

Supplementary Materials and Methods

Secondary structure analysis

Circular dichroism spectra of BabA and BabA variants (containing N206A and D233A/S244A substitutions) was measured using a J-810 Spectropolarimeter (Jasco). Protein concentration was 1 μM in a buffer containing 20 mM tris-Cl (pH 7.4) and 3 mM NaCl. Measurements were made at 25^{o}C in a quartz cell with a 0.1 cm pathlength at a data pitch of 0.5 nm and scanning speed of 100 nm/min. Reported spectra are baseline-corrected for buffer alone and averaged from three independent scans.

Thermal stability analysis

For differential scanning fluorimetry experiments, SYPRO Orange dye (20× final concentration) was added to 10 μM BabA and BabA variants (containing N206A and D233A/S244A substitutions) in a buffer containing 20 mM Tris-Cl (pH 7.4) and 300 mM NaCl. Changes in fluorescence were measured across an increasing temperature gradient from 25^{o}C to 60^{o}C using a LightCycler 480 II (Roche) at a ramp rate of 0.01^{o}C/s. Primary data points from three independent experiments were fitted to a 6-parameter unfolding equation *(46)* using the Prism analysis package (GraphPad Software).

Supplementary Figures and Tables

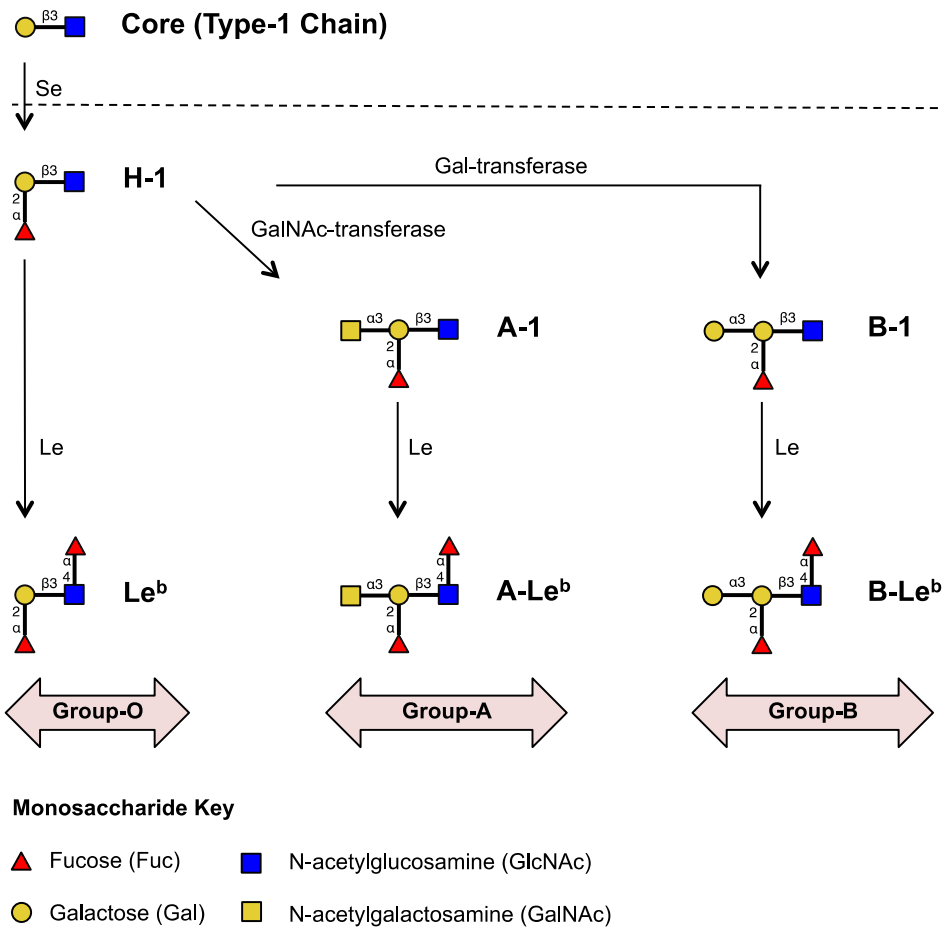


Fig. S1. Glycan symbolic representations of the fucosylated histo-blood group antigens that act as BabA receptors. The terminal glycan epitopes of the secreted ABH/Lewis antigens that act as receptors for BabA in the gastric mucosa are shown. Histo-blood group antigens are produced after the fucosylation of a type 1 lacto series core chain by an α1,2-fucosyltransferase (Se), which generates the H-1 antigen. This oligosaccharide is modified by a GalNAc-transferase in blood group A individuals and Gal-transferase in blood group B individuals, thereby producing the A-1 and B-1 antigens, respectively. All ABH antigens are subjected to further fucosylation by an α1,3/4-fucosyltransferase (Le), which produces Le^b, A-Le^b and B-Le^b in blood group O, A and B individuals, respectively *(6)*.

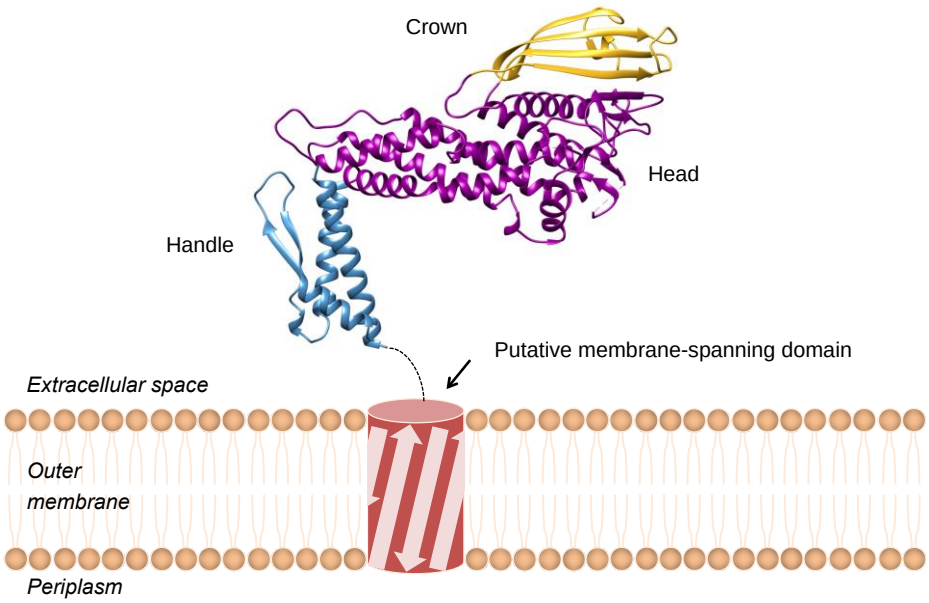
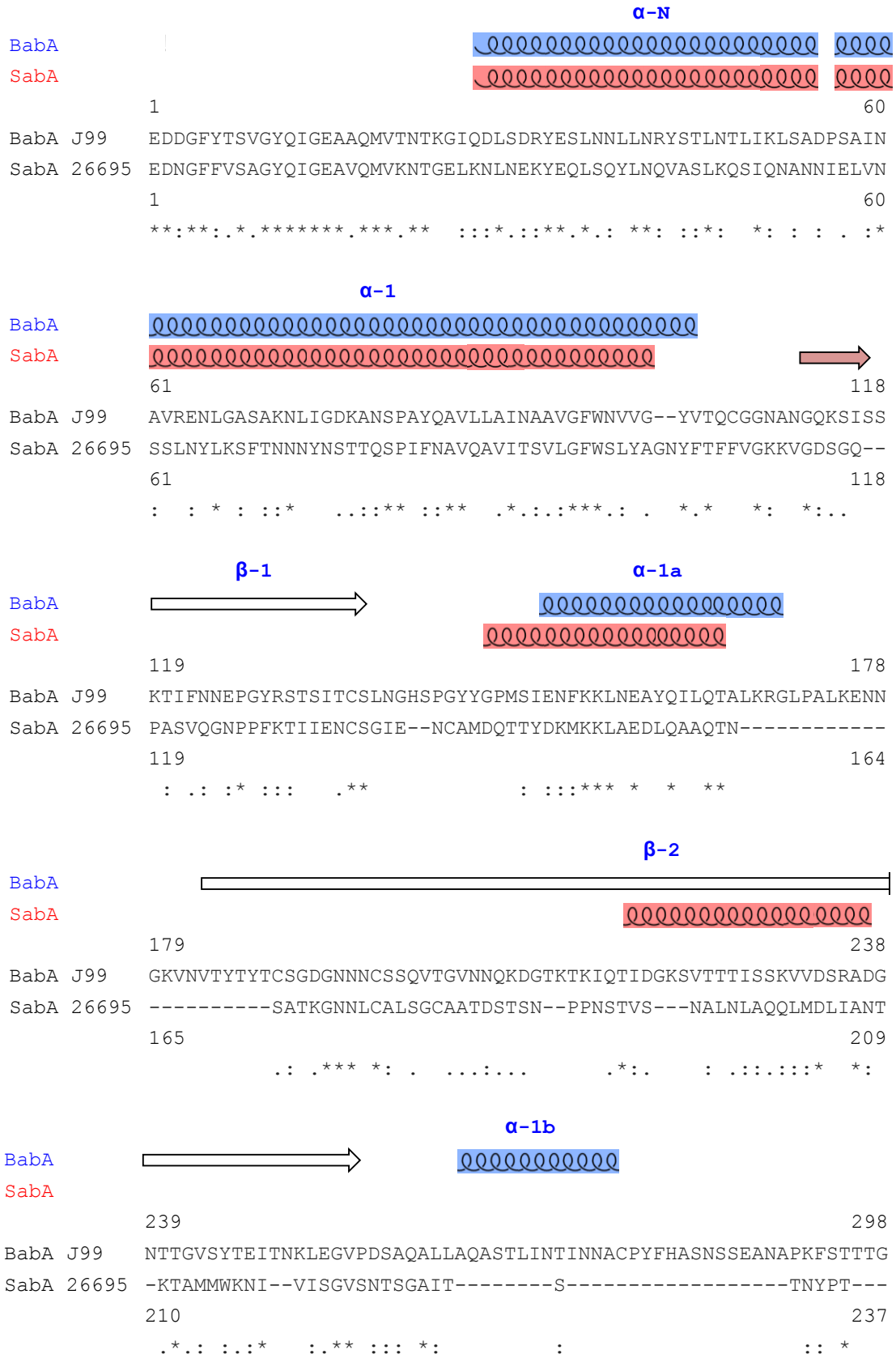
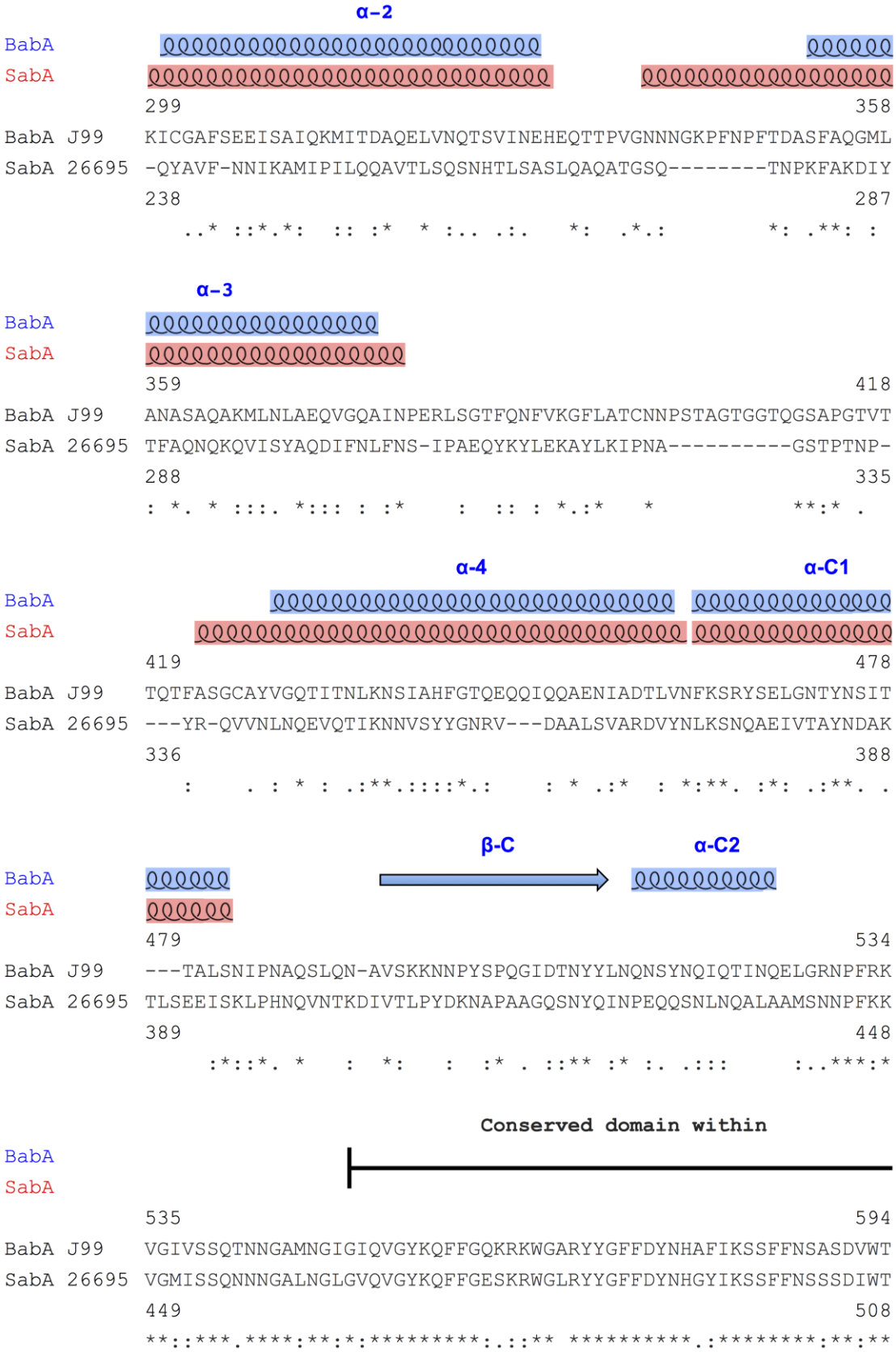


Fig. S2. **Schematic illustration of the predicted domain structure of BabA.** Indicated are the handle (blue) and head regions (dark magenta), and the crown β-strand unit (gold) of the extracellular domain. BabA is anchored to the *H. pylori* outer membrane through a putative C-terminal transmembrane β-barrel domain.


α-N
BabA
SabA
1 60
BabA J99 EDDGFYTSVGYQIGEAAQMVTNTKGIQDLSDRYESLNNLLNRYSTLNTLIKLSADPSAIN
SabA 26695 EDNGFFVSAGYQIGEAVQMVKNTGELKNLNEKYEQLSQYLNQVASLKQSIQNANNIELVN
1 60
α-1
BabA
SabA
61 118
BabA J99 AVRENLGASAKNLIGDKANSPAYQAVLLAINAAVGFWNVVG--YVTQCGGNANGQKSISS
SabA 26695 SSLNYLKSFTNNNYNSTTQSPIFNAVQAVITSVLGFWSLYAGNYFTFFVGKKVGDSGQ--
61 118
β-1
α-1a
BabA
SabA
119 178
BabA J99 KTIFNNEPGYRSTSITCSLNGHSPGYYGPMSIENFKKLNEAYQILQTALKRGLPALKENN
SabA 26695 PASVQGNPPFKTIIENCSGIE--NCAMDQTTYDKMKKLAEDLQAAQTN----------
119 164
β-2
BabA
SabA
179 238
BabA J99 GKVNVTYTYTCSGDGNNNCSSQVTGVNNQKDGTKTKIQTIDGKSVTTTISSKVVDSRADG
SabA 26695 ----------SATKGNNLCALSGCAATDSTSN--PPNSTVS---NALNLAQQLMDLIANT
165 209
α-1b
BabA
SabA
239 298
BabA J99 NTTGVSYTEITNKLEGVPDSAQALLAQASTLINTINNACPYFHASNSSEANAPKFSTTTG
SabA 26695 -KTAMMWKNI--VISGVSNTSGAIT--------S----------TNYPT---
210 237


α-2
BabA
SabA
299
358
BabA J99 KICGAFSEEISAIQKMITDAQELVNQTSVINEHEQTTPVGNNNGKPFNPFTDASFAQGML
SabA 26695 -QYAVF-NNIKAMIPILQQAVTLSQSNHTLSASLQAQATGSQ--------TNPKFAKDIY
238
287
α-3
BabA
SabA
359
418
BabA J99 ANASAQAKMLNLAEQVGQAINPERLSGTFQNFVKGFLATCNNPSTAGTGGTQGSAPGTVT
SabA 26695 TFAQNQKQVISYAQDIFNLFNS-IPAEQYKYLEKAYLKIPNA----------GSTPTNP-
288
335
α-4
α-C1
BabA
SabA
419
478
BabA J99 TQTFASGCAYVGQTITNLKNSIAHFGTQEQQIQQAENIADTLVNFKSRYSELGNTYNSIT
SabA 26695 ---YR-QVVNLNQEVQTIKNNVSYYGNRV---DAALSVARDVYNLKSNQAEIVTAYNDAK
336
388
β-C
α-C2
BabA
SabA
479
534
BabA J99 ---TALSNIPNAQSLQN-AVSKKNNPYSPQGIDTNYYLNQNSYNQIQTINQELGRNPFRK
SabA 26695 TLSEEISKLPHNQVNTKDIVTLPYDKNAPAAGQSNYQINPEQQSNLNQALAAMSNNPFKK
389
448
Conserved domain within
BabA
SabA
535
594
BabA J99 VGIVSSQTNNGAMNGIGIQVGYKQFFGQKRKWGARYYGFFDYNHAFIKSSFFNSASDVWT
SabA 26695 VGMISSQNNNGALNGLGVQVGYKQFFGESKRWGLRYYGFFDYNHGYIKSSFFNSSSDIWT
449
508

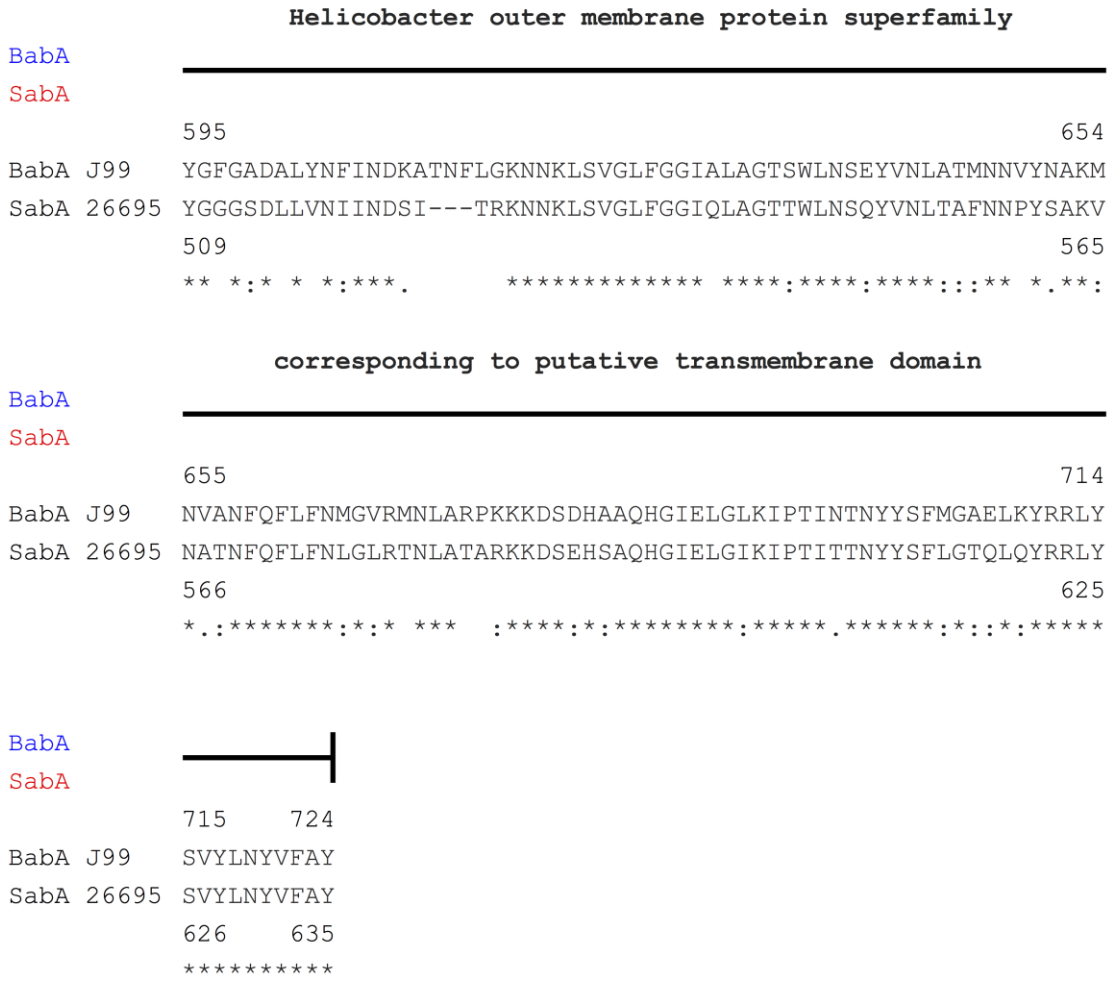


Fig. S3. Alignment of BabA J99 and SabA 26695 protein sequences annotated with secondary structure elements. Full-length BabA and SabA share 40% sequence identity and 24% sequence similarity. The extracellular domains of BabA and SabA share 26% sequence identity and 28% sequence similarity. The transmembrane domains of BabA and SabA share 73% sequence identity and 15% sequence similarity. Indicated are positions that have a fully conserved residue (*****); conservation between groups with strongly similar properties (> 0.5 in the Gonnet PAM 250 matrix) (**:**); conservation between groups with weakly similar properties (< 0.5 in the Gonnet PAM 250 matrix) (**.**). Alignment was performed with Clustal Omega.

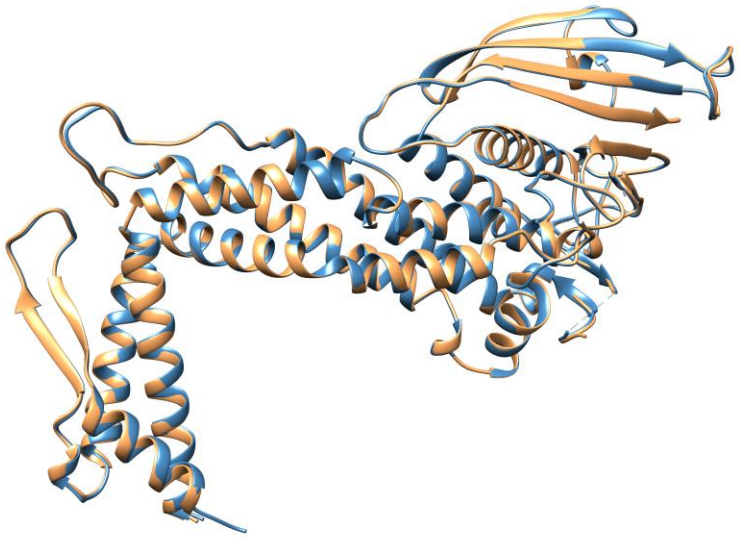


Fig. S4. Superimposition of BabA from apo and cocrystal structures. No global conformational change occurs in BabA (sandy brown) after Le^b complex formation (steel blue) - RMSD = 0.25Å for all Cα atoms *(22)*.

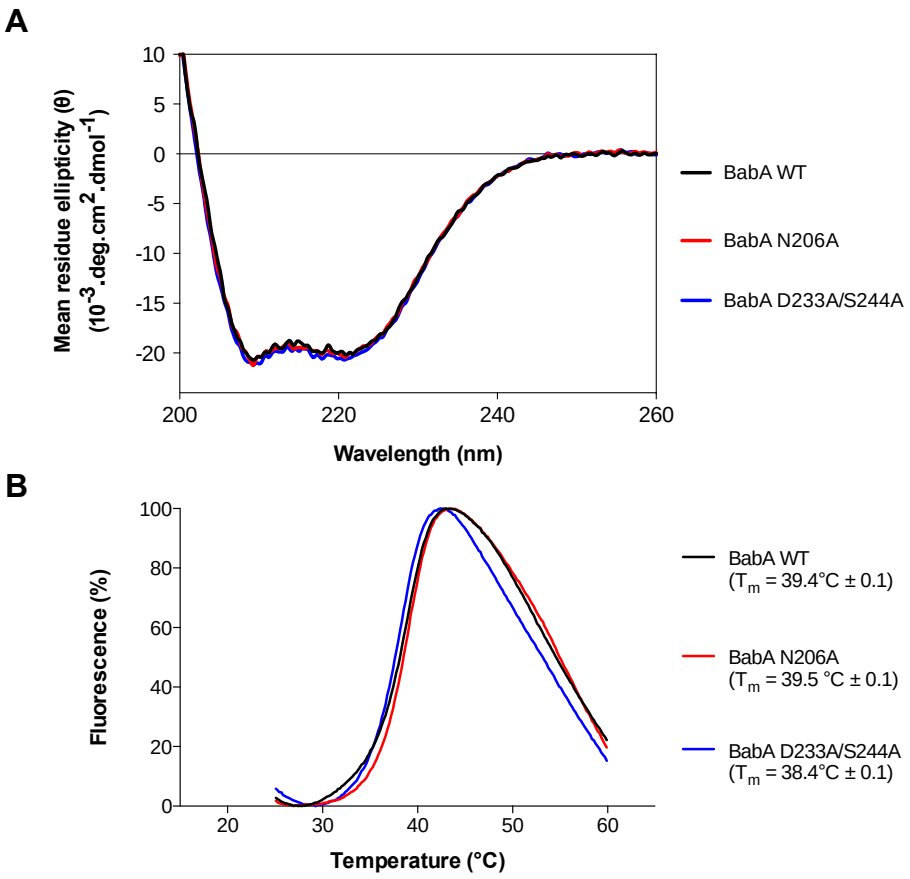


Fig. S5. Secondary structure and thermal stability of BabA and BabA variants. (**A**) Overlay of far-UV circular dichroism spectra averaged from three independent experiments. (**B**) Temperature-induced unfolding transition determined using differential scanning fluorimetry. The reported midpoint temperature of each protein unfolding transition (T_m) is the average (± SEM) from three independent experiments.

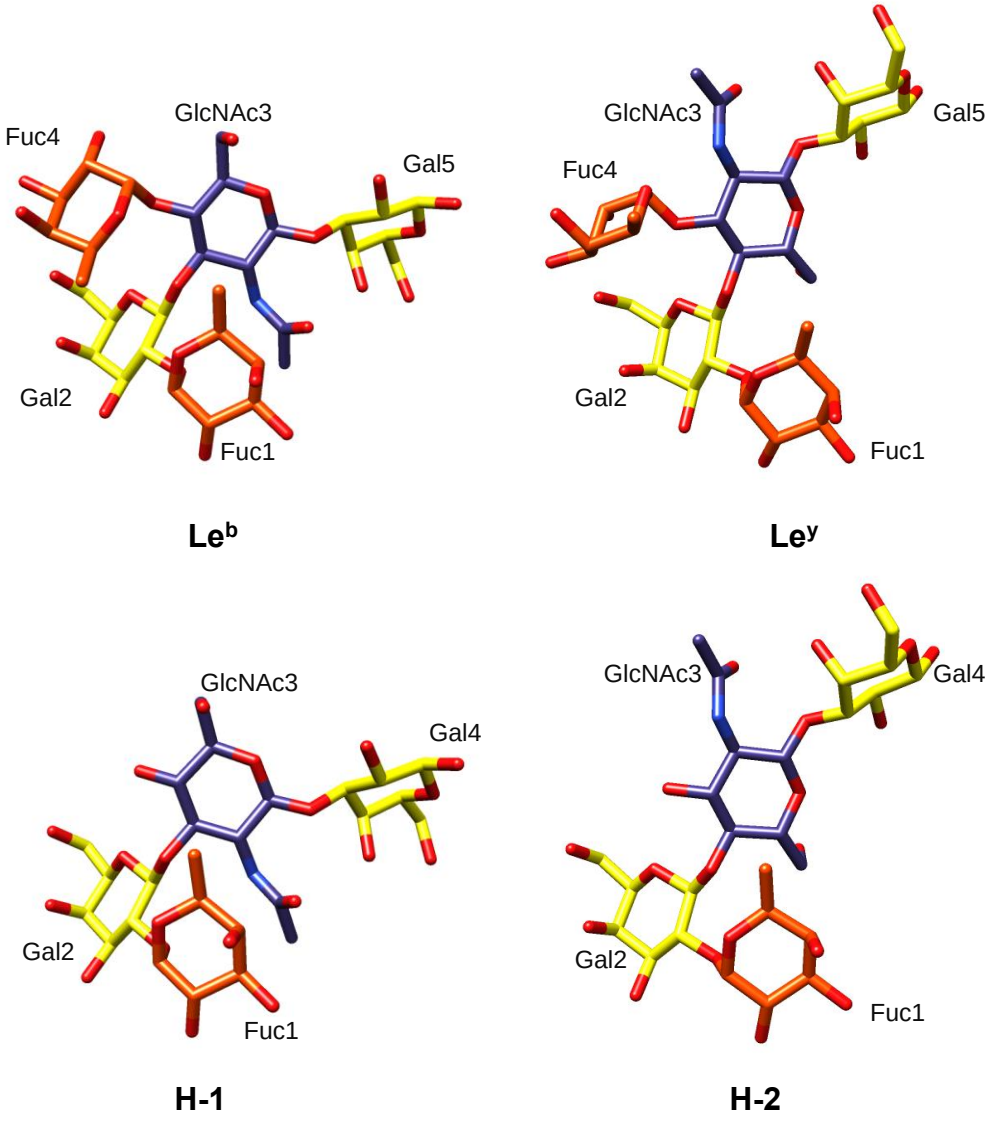


Fig. S6. Type 1 and type 2 fucosylated histo-blood group antigen molecular models. Stick models of Le^b and H-1 (type 1) antigens show a distinctly different three-dimensional orientation to Le^y and H-2 (type 2) antigens due to Galβ1-3GlcNAc and Galβ1-4GlcNAc linkages, respectively. Models were calculated in minimum energy conformations with the SWEET-II system *(47)*. Fucose, galactose and *N*-acetylglucosamine residues are colored orange, yellow and blue, respectively.

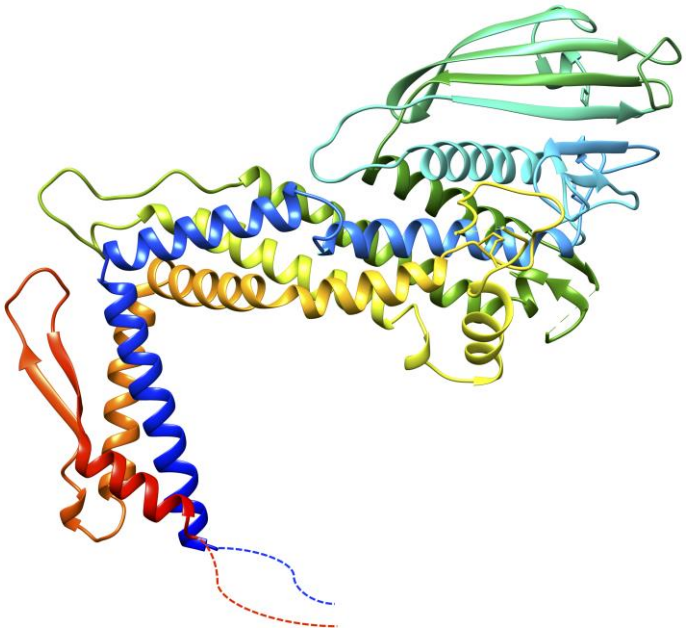


Fig. S7. Rainbow representation of apo-BabA. Recombinant BabA used in this study contains amino acids 10 to 527 of mature BabA followed by three C-terminal polypeptide tags (6xLys-c-Myc-6xHis). The residues visible in the apo-BabA electron density map run from 27-527. BabA amino acids 10-26 (blue dotted line) and the C-terminal polypeptide tags corresponding to amino acids 528-552 (red dotted line) were not modeled.

Table S1. X-ray diffraction data collection and refinement statistics.

Components	SeMet BabA (SAD data collection)	BabA:Le[b]
Data collection		
Space group	P 21 21 21	P 21 21 21
Cell dimensions		
a, *b*, *c* (Å)	60.83 93.04 96.92	60.59 91.77 96.42
α, β, γ (°)	90 90 90	90 90 90
Wavelength (Å)	0.97925 (PEAK)	0.920
Resolution (Å)	48.46-1.91 (1.98-1.91)	44.78-2.12 (2.18-2.12)
$R_{merge\ (all\ I+\ and\ I-)}$	0.236 (3.427)	0.141 (0.643)
$I/\sigma I$	13.2 (0.9)	11.8 (3.0)
Completeness (%)	98.7 (89.2)	99.1 (99.4)
Multiplicity[1]	23.1 (13.8)	6.6 (6.5)
Refinement		
Resolution (Å)	48.46-1.91 (1.98-1.91)	44.78-2.12 (2.18-2.12)
No. reflections	39877 (96.7%)	29319 (98.9%)
R_{work}/ R_{free}	0.189/0.231 (0.378/0.331)	0.171/0.223 (0.224/0.271)
No. atoms		
Protein	3662	3654
Ligand/ion	-	57
Water	132	156
B-factors		
Protein	25.327	20.97
Ligand/ion		35.413 (31.9)[§]
Water	34.124	30.034
R.m.s deviations		
Bond lengths (Å)	0.0184	0.0165
Bond angles (°)	1.7756	1.726

[§]Excluding the partially visible galactose (Gal5) moiety

Table S2. Thermodynamic parameters of BabA:Le^b interaction at pH 4.5 and 7.4. The upper panels in each ITC trace show a representative calorimetric response obtained by titrating BabA with Le^b. The lower panels depict the binding isotherm obtained where the continuous line represents the least-squares fit of the data to a single-site binding model. The reported thermodynamic parameters are the average (± SEM) of three independent experiments. There are no significant differences between the thermodynamic parameters and dissociation constants of BabA:Le^b binding at pH 4.5 and 7.4 (unpaired two-tailed Welch's t-test, $P > 0.05$).

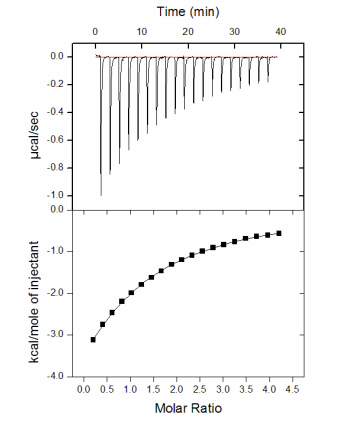
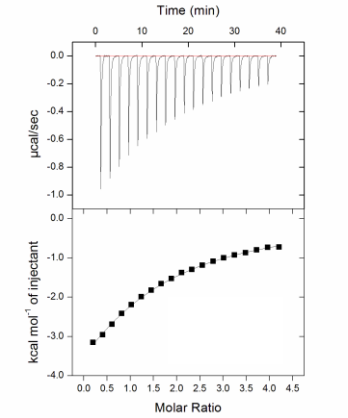
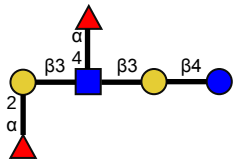
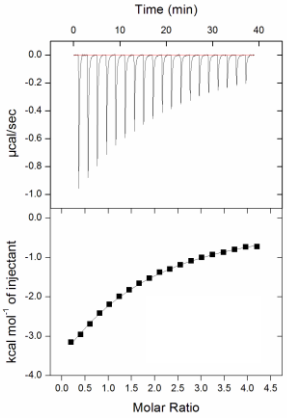
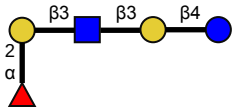
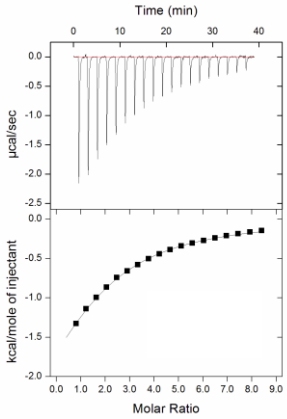
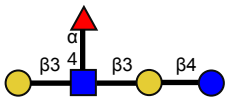
Thermodynamic parameters	ITC trace
pH = 4.5 K_D = 227 ± 22 μM N = 0.91 ± 0.15 ΔH = -12.2 ± 1.8 kcal/mol $-T\Delta S$ = 7.2 ± 1.8 kcal/mol	
pH = 7.4 K_D = 252 ± 15 μM N = 1.07 ± 0.03 ΔH = -10.9 ± 0.5 kcal/mol $-T\Delta S$ = 6.0 ± 0.5 kcal/mol	

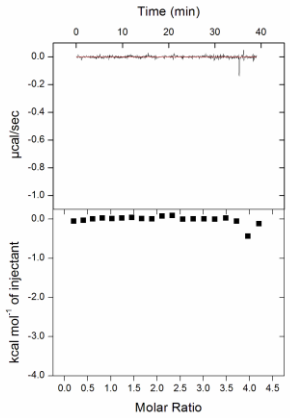
Table S3. Binding affinity of BabA to various histo-blood group antigens. Glycan structural representations can be interpreted with the following key: fucose (Fuc, ▲), galactose (Gal, ●), *N*-acetylglucosamine (GlcNAc, ■), glucose (Glc, ●), *N*-acetylneuraminic acid (Neu5Ac, ◆). The upper panels in each ITC trace show a representative calorimetric response obtained by titrating BabA with the respective histo-blood group antigens. The lower panels depict the binding isotherm obtained where the continuous line represents the least-squares fit of the data to a single-site binding model (where applicable). Calorimetric titrations were performed at pH 7.4.

Histo-blood group antigen and binding affinity	ITC trace
Le^b antigen (hexasaccharide form) Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc  K_D = 252 μM[a]	
H-1 antigen (pentasaccharide form) Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc  K_D = 617 μM[b]	

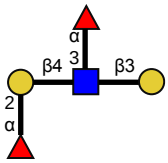
Le^{a} antigen (pentasaccharide form)
Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc



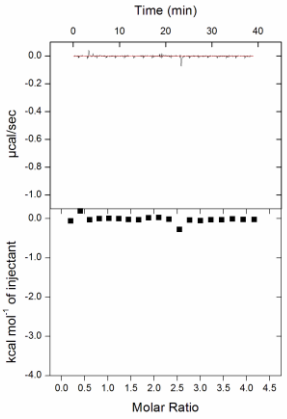
No binding detected



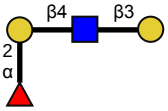
Le^{y} antigen (pentasaccharide form)
Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal



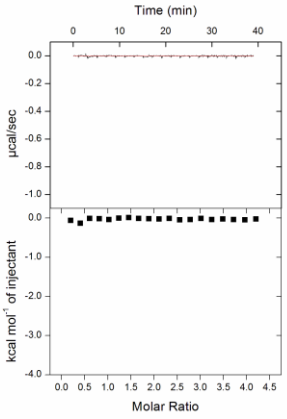
No binding detected



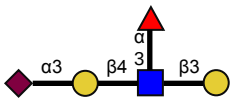
H-2 antigen (tetrasaccharide form)
Fucα1-2Galβ1-4GlcNAcβ1-3Gal



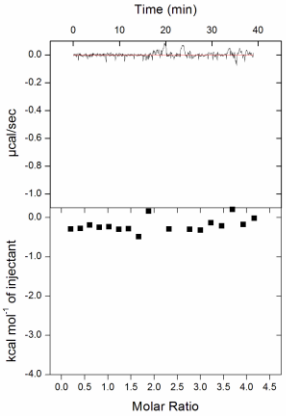
No binding detected



SLe^x antigen (pentasaccharide form)
Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal



No binding detected



[a] Average of three independent experiments (SEM = ± 15µM).
[b] Average of two independent experiments (Range = ± 45µM).

Table S4. Oligonucleotides used in BabA cloning and site-directed mutagenesis. Where applicable, the recognition sites for the stated restriction enzymes are underlined. [FOR] and [REV] denote sense and antisense primers, respectively. [Phos] denotes a 5' phosphorylation.

Designation	**Sequence (5' – 3')**
BabA [FOR] 1- NcoI	TCGGATCCATGGAAGACGACGGCTTTTAC
BabA [REV] -527 BamHI	TCTGCTGGATCCCTTCTTCTTCTTCTTCTTGAGTTCTTG GTTGATGG
BabA D233A [FOR]	[Phos]GTTCAAAAGTGGTTGCTAGTCGTGCAGATG
BabA D233A [REV]	[Phos]TGATCGTGGTGGTTACGCTTTTGCCGTCTATG
BabA D233A/S244A [FOR]	[Phos]GTAATACAACAGGGGTGGCCTACACCGAAATCA C
BabA D233A/S244A [REV]	[Phos]CATCTGCACGACTAGCAACCACTTTTGAAC
BabA N206A [FOR]	[Phos]GAACCAAGACTAAAATCCAAACCATAGAC
BabA N206A [REV]	[Phos]CGTCTTTTTGAGCATTTACACCTGTGAC