Supporting Information

Vik et al. 10.1073/pnas.0809504106

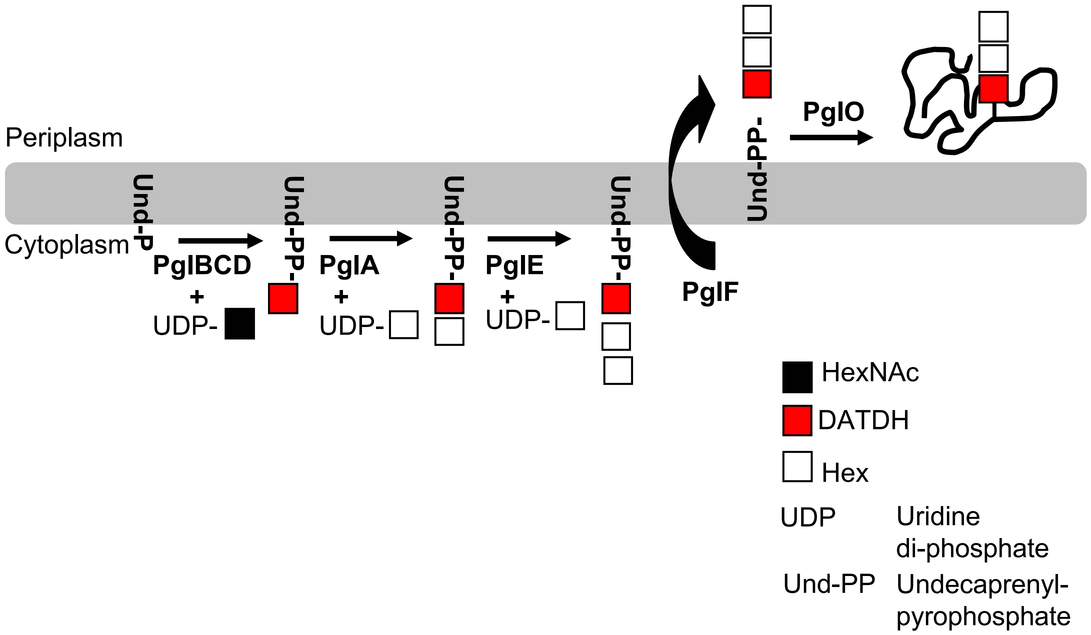

Fig. S1. Current model of the O-linked protein glycosylation pathway in *N. gonorrhoeae*. In the wild-type background used in this work, the *pglE* allele is in an off (out-of-frame) configuration [Aas FE, Vik A, Vedde J, Koomey M, Egge-Jacobsen W (2007) *Neisseria gonorrhoeae* O-linked pilin glycosylation: Functional analyses define both the biosynthetic pathway and glycan structure. *Mol Microbiol* 65:607–624]. Note that O-acetylation of the oligosaccharide mediated by PglI is not depicted here.

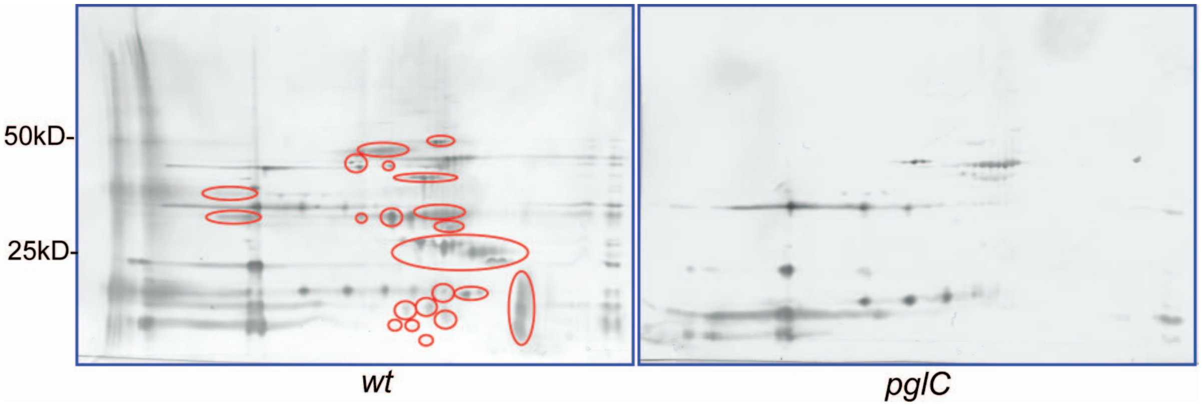

Fig. S2. Identification of potential glycoproteins in detergent extracts from wild-type and *pglC* backgrounds using 2D gel electrophoresis and immunoblotting. Signals unique to the wild-type background are circled in red and were used as a template to guide excision of equivalent protein spots from 2D gels run in parallel.

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NGO1717        MKSRHLALALGVAALFALAACDSKVQTSVPADSAPAASAAAAPAGLVEGQNYTVLANPIP
NGO1548        MKFKHLLPLLLSAVLS-------------------------AQAYALTEGEDYLVLDKPIP
               ** :**   *  *.*                         * . .*.**::* ** :***

NGO1717        QQQAGKVEVLEFFGYFCPHCARLEPVLSKHAKSFKDDMYLRTEHVVWQKEMLPLARLAAA
NGO1548        QEQPGKIEVLEFFGYFCVHCHHFDPLLLKLGKALPSDTYLRTEHVVWRPEMLGLARMAAA
               *:*.**:********** ** :::*:* * .*:: .* *********: *** ***:***

NGO1717        VDMAAAESKDVANSHIFDAMVNQKIKLQEPEVLKKWLGEQTAFDGKKVLAAYESPESQAR
NGO1548        VKLSGLKYQ--ANSAVFKAVYEQKIRLENRAVAGKWALSQKGFDGKKLMRAYDSPEAAAV
               *.::. : :  *** :*.*: :***:*::  *  **  .*..*****:: **:***: *

NGO1717        AGKMQELTETFQIDGTPTVIVGGKYKVEFAD-WESGMNTIDLLADKVREEQKAAQ
NGO1548        ALKMQKLTEQYGIDSTPTVIVGGKYRVIFNNGFDGGVHTIKELVAKVREERKRQTPAVQK
               * ***:*** : **.**********:* * : ::.*::**. *. *****:*

CLUSTAL W (1.83) multiple sequence alignment
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Fig. S3. Sequence alignment of Ng1717 and Ng1548 DsbA paralogues. The Ng1717 ASP-rich LCR and associated glycopeptide 27-TSVPADSAPAASAA-40 (green) have no counterparts in Ng1548. The mature N-terminal Cys residue of Ng1717 is shown in red.

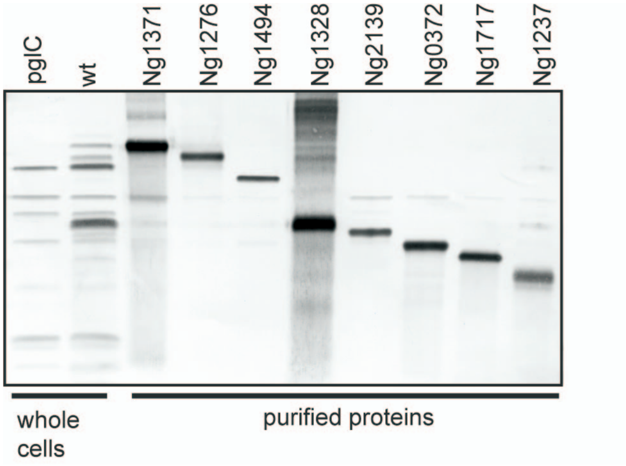

Fig. S4. Identification of glycoproteins detected in immunoblots of whole-cell lysates (in a *pilE* background) by comparison with the migration of purified glycoproteins. Ng0994, Ng1043, and Ng1225 were not included here.

Table S1. LCRs found in glycoproteins for which glycopeptides have yet to be identified.

Protein	LCR sequence
Ng0372	19-CGGSEGGSGASSAPAQSAISGS-40
Ng0994	18-CSQEPAAPAAEATPAGEAPASEAPAAEAAPADAAEAPAAGNCAA-61
Ng1494	29-AGSAPAANAEAAA-41
Ng1225	25-EAAPASASEPAAASAA-40

For Ng 0372 and Ng0994, LCR sequences are shown starting at the lipoprotein cleavage site Cys only.

Table S2. Strains

Strain name	Parental strain	Relevant genotype	Reference
N400	VD300	*recA6**	(1)
KS104/GGC	N400	*pglC::kan*	(2)
N400 pilE S63A	N400	*pilE(S63A)*	(2)
4/3/1	VD300	$pilE_{ind}$	(3)
KS105	4/3/1	*pglC::kan*	This study
KS127	4/3/1	$pglE_{on}$	This study
KS123	4/3/1	*pglO::kan*	This study
KS122	4/3/1	*pglA::kan*	This study
KS153	4/3/1	*pglF::kan*	This study
KS154	4/3/1	*pglD::kan*	This study
KS102	4/3/1	*NG1371–6xHis*	This study
KS103	4/3/1	*pglC::kan NG1371–6xHis*	This study
KS109	4/3/1	*NG0372–6xHis*	This study
KS108	4/3/1	*pglC::kanNG0372–6xHis*	This study
KS110	4/3/1	*NG1237–6xHis*	This study
KS111	4/3/1	*pglC::kan NG1237–6xHis*	This study
KS112	4/3/1	*NG1328–6xHis*	This study
KS113	4/3/1	*pglC::kan NG1328–6xHis*	This study
KS120	4/3/1	*pglA::kan NG1328–6xHis*	This study
KS121	4/3/1	*pglO::kan NG1328–6xHis*	This study
KS128	4/3/1	*pglF::kan NG1328–6xHis*	This study
KS138	4/3/1	*NG1276–6xHis*	This study
KS139	4/3/1	*pglC::kan NG1276–6xHis*	This study
KS152	4/3/1	*NG2139–6xHis*	This study
KS132	4/3/1	*pglC::kan NG2139–6xHis*	This study
KS133	4/3/1	*NG1548–6xHis*	This study
KS134	4/3/1	*NG1494–6xHis*	This study
KS135	4/3/1	*pglC::kan NG1494–6xHis*	This study
KS136	4/3/1	*NG1717–6xHis*	This study
KS137	4/3/1	*pglC::kan NG1717–6xHis*	This study
KS155	4/3/1	*NG1043–6xHis*	This study
KS156	4/3/1	*pglC::kan NG1043–6xHis*	This study
KS157	4/3/1	*NG0994–6xHis*	This study
KS158	4/3/1	*pglC::kan NG0994–6xHis*	This study
KS159	4/3/1	*NG1225–6xHis*	This study
KS160	4/3/1	*pglC::kan NG1225–6xHis*	This study

*recA6 is an IPTG-inducible allele of recA.

1. Tonjum T, Freitag NE, Namork E, Koomey M (1995) Identification and characterization of *pilG*, a highly conserved pilus-assembly gene in pathogenic Neisseria. *Mol Microbiol* 16:451–464.

2. Hegge FT, et al. (2004) Unique modifications with phosphocholine and phosphoethanolamine define alternate antigenic forms of *Neisseria gonorrhoeae* type IV pili. *Proc Natl Acad Sci USA* 101:10798–10803.

3. Wolfgang M, van Putten JP, Hayes SF, Dorward D, Koomey M (2000) Components and dynamics of fiber formation define a ubiquitous biogenesis pathway for bacterial pili. *EMBO J* 19:6408–6418.

4. Aas FE, Vik A, Vedde J, Koomey M, Egge-Jacobsen W (2007) *Neisseria gonorrhoeae* O-linked pilin glycosylation: Functional analyses define both the biosynthetic pathway and glycan structure. *Mol Microbiol* 65:607–624.