

Structure-based design of chimeric antigens for multivalent protein vaccines

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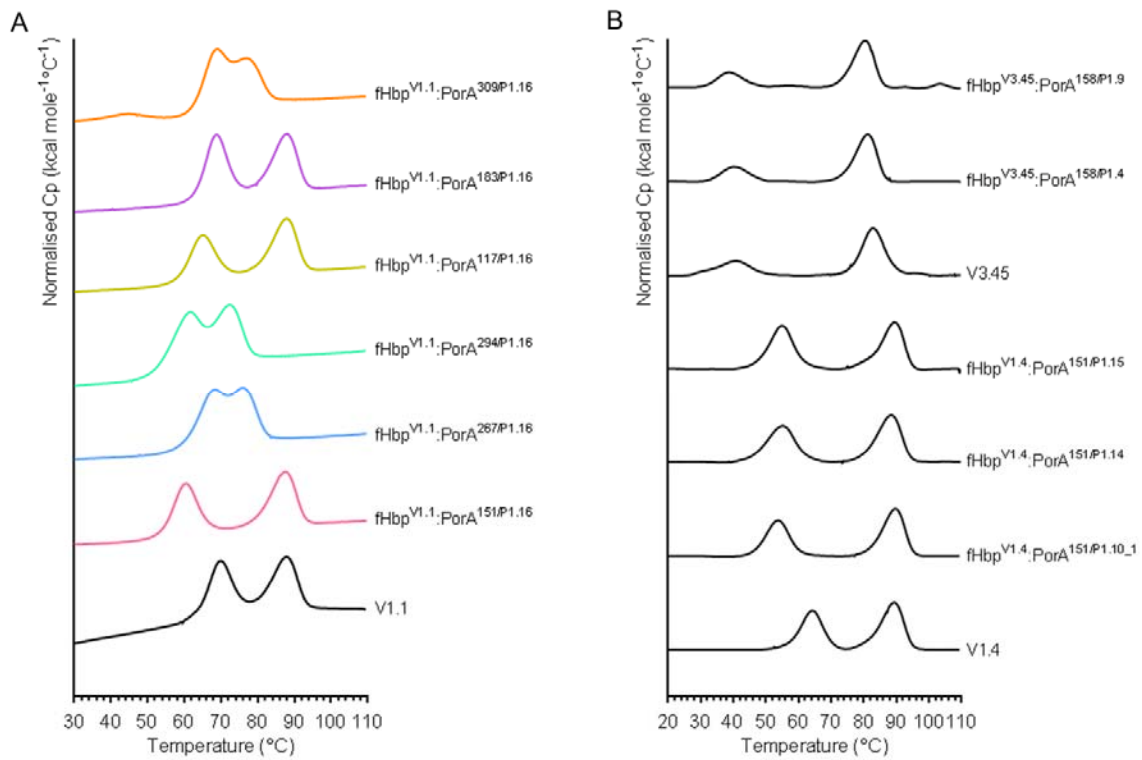
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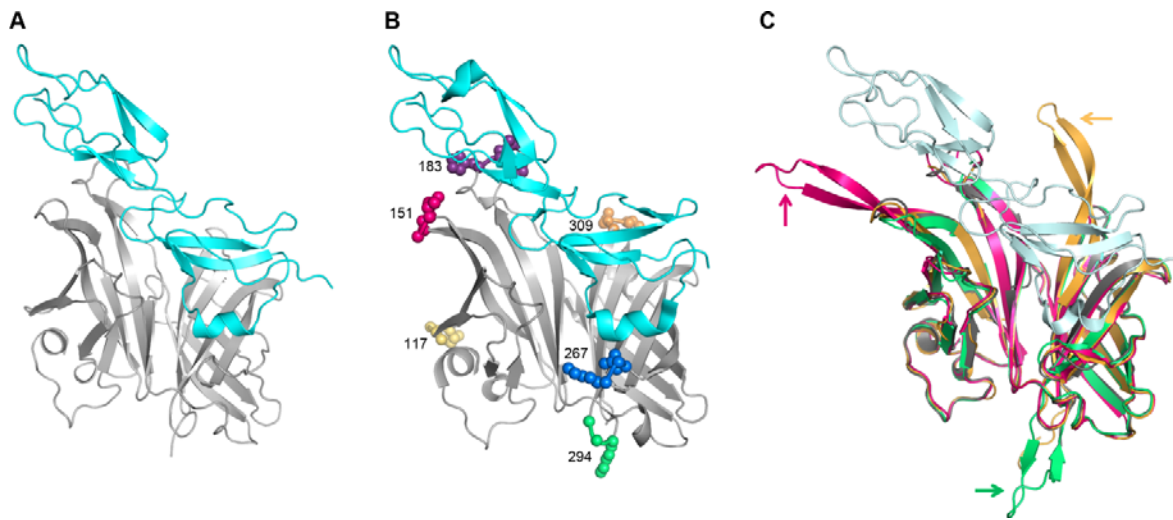
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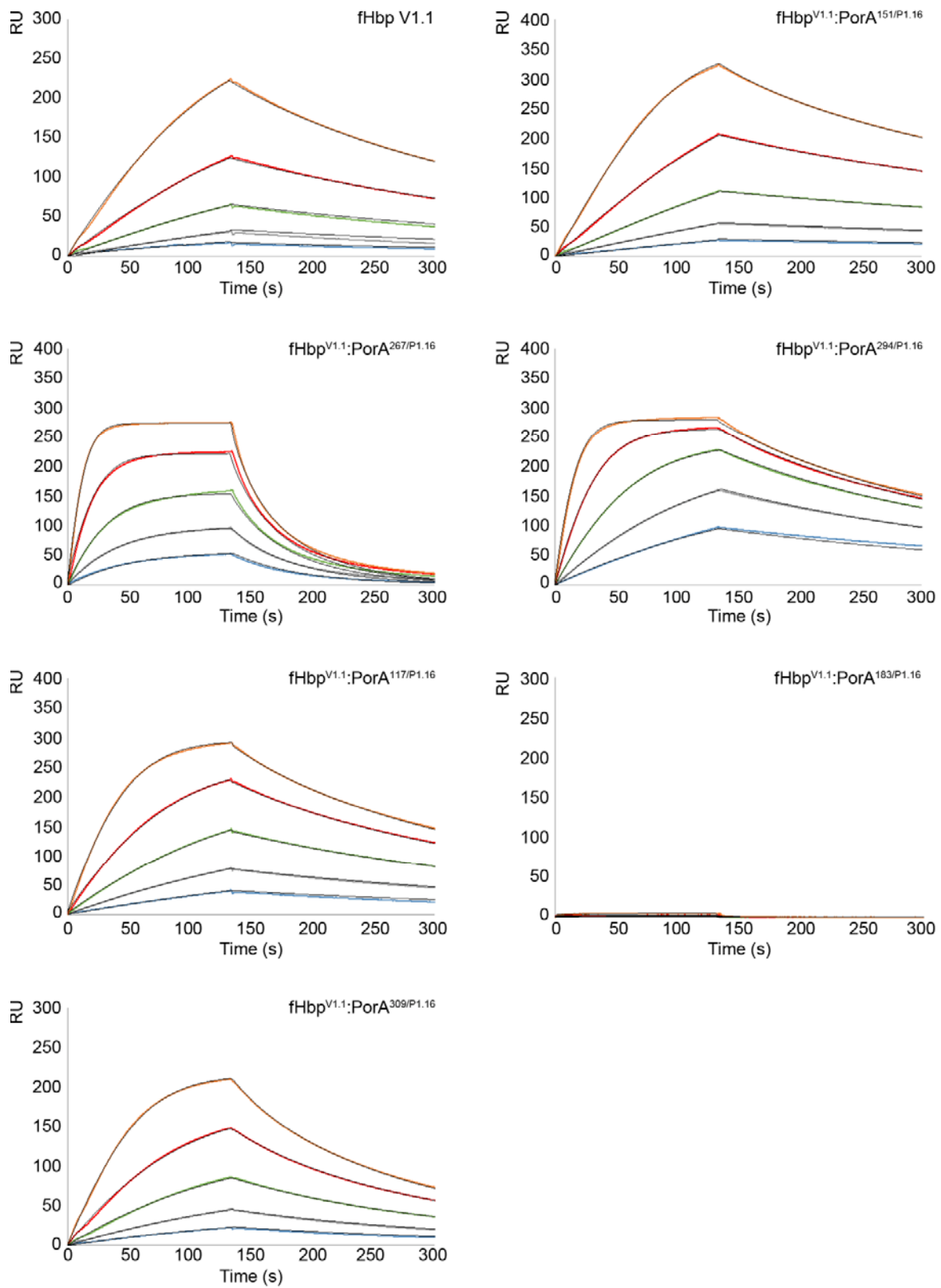
Supplementary Figure 1. Differential scanning calorimetry.

Normalised thermal melt traces of ChAs composed of **(A)** fHbp V1.1 and PorA VR2 P1.16, or **(B)** fHbp V1.4 and VR2 peptides P1.10_1, P1.14 and P1.15 inserted at position 151, or fHbp V3.45 and VR2 peptides P1.4 or P1.9 inserted at position 158.



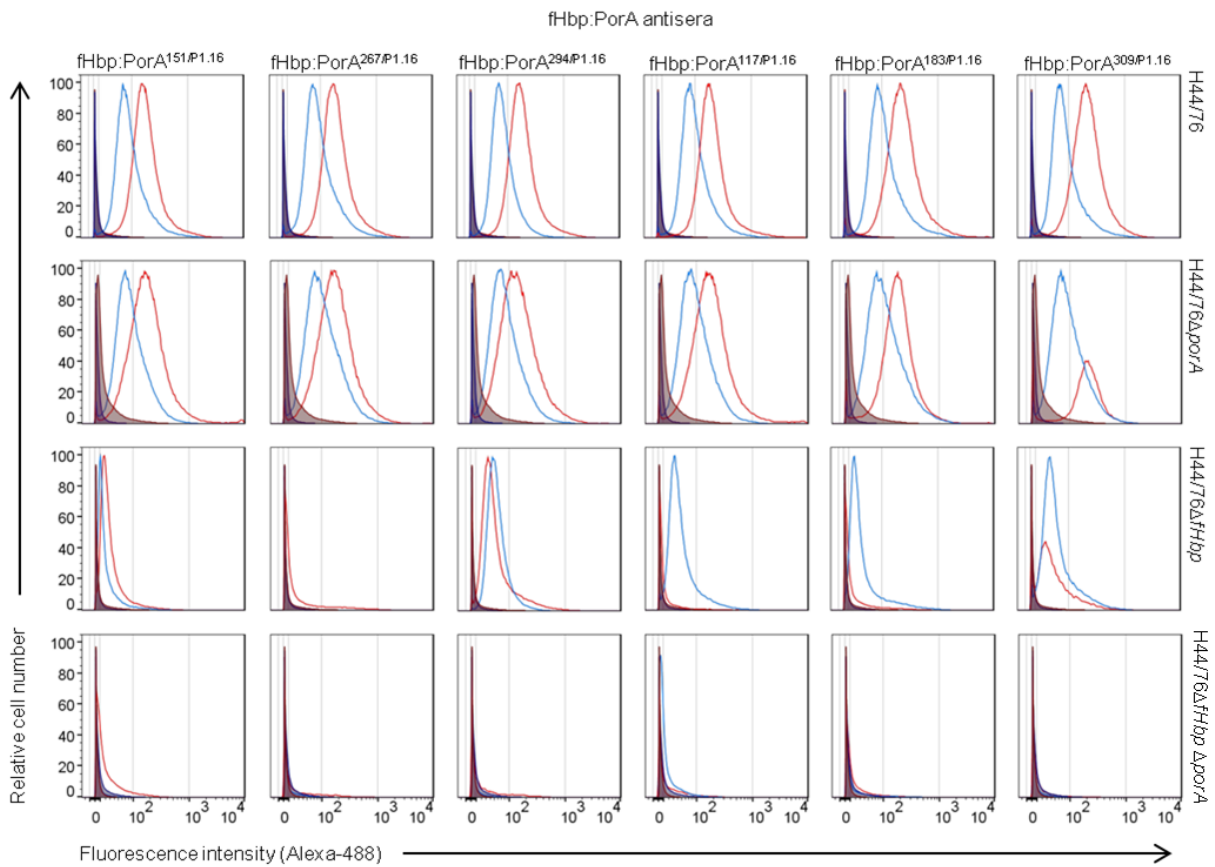
Supplementary Figure 2. Structural alignments of ChAs with wild type fHbp.

(A) Complex formed by V1.1 fHbp (grey) and CFH_{6/7} (cyan, PDB ID 2W80). (B) Locations of the six fHbp residues replaced with VR2 P1.16 in relation to the CFH_{6/7}. (C) Secondary structure alignment of ChAs with fHbp V1.1 (grey) in a complex with CFH_{6/7} (light blue); fHbp^{V1.4}:PorA^{151/P1.16} (pink), fHbp^{V1.1}:PorA^{294/P1.16} (green), fHbp^{V1.4}:PorA^{309/P1.16} (orange), VR2 P1.16 is indicated by the correspondingly coloured arrows.

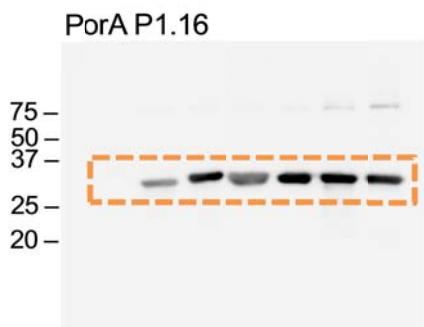
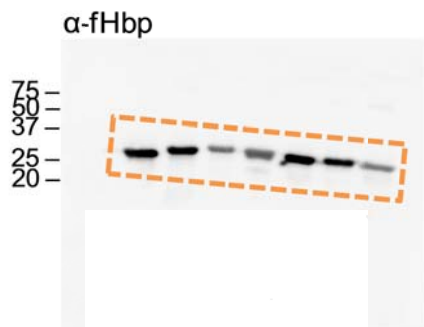
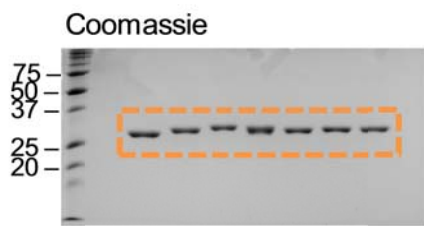


Supplementary Figure 3. Surface plasmon resonance of CFH₆₇ interacting with ChAs.

The ChA was immobilized on the chip surface and increasing concentrations of CFH⁶⁷ flowed over the surface: 1nM (blue), 2nM (grey), 4nM (green), 8nM (red), 16nM (orange). Binding was analyzed using the BiaEvaluation software and the fits to the curves are shown as black lines. Kd values are reported in **Table 1**.



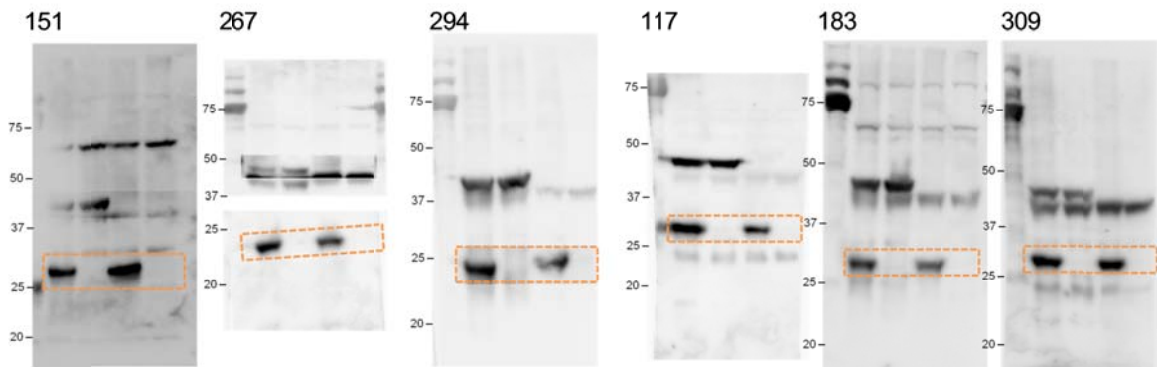
Supplementary Figure 4. Flow cytometry histograms showing detection of fHbp and PorA by ChA antisera. Histograms of flow cytometry experiments conducted with ChA/alum (blue) and ChA/MPLA (red) antisera binding to fixed *N. meningitidis* H44/76 strains: wild type, $\Delta fHbp$, $\Delta porA$ and $\Delta fHbp\Delta porA$. fHbp and PorA antibodies bound to *N. meningitidis* were detected using Alexa-488 conjugated IgA/G/M secondary antibody. Alum PBS and MPLA PBS antisera are shown in dark blue and dark red shaded profiles, and secondary IgAGM control is shown in black. Comparisons of the geometric means from these histograms, and the histograms from two other independent repeats, are shown in **Figures 2C** and **2D**.



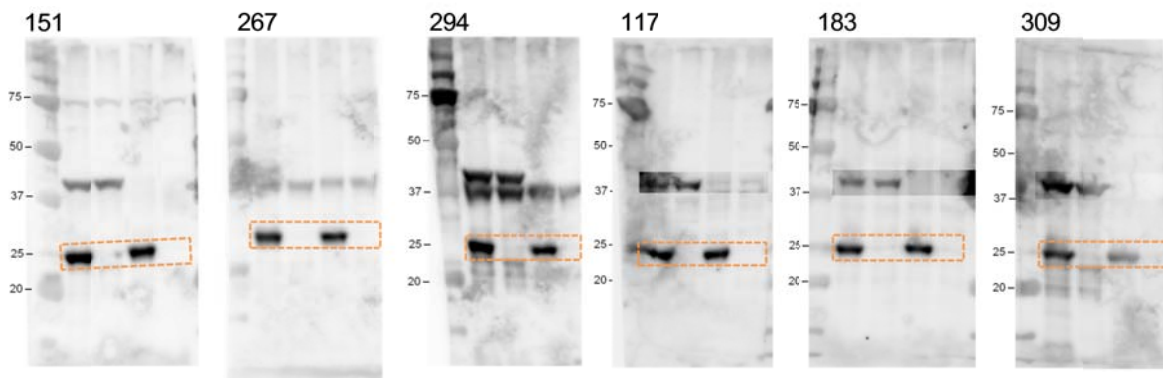
Supplementary Figure 5. Complete gel and Western blots from Figure 1C.

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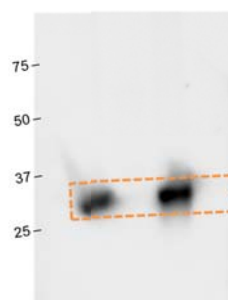
Alum + fHbp:PorA^{P1.16}



MPLA + fHbp:PorA^{P1.16}

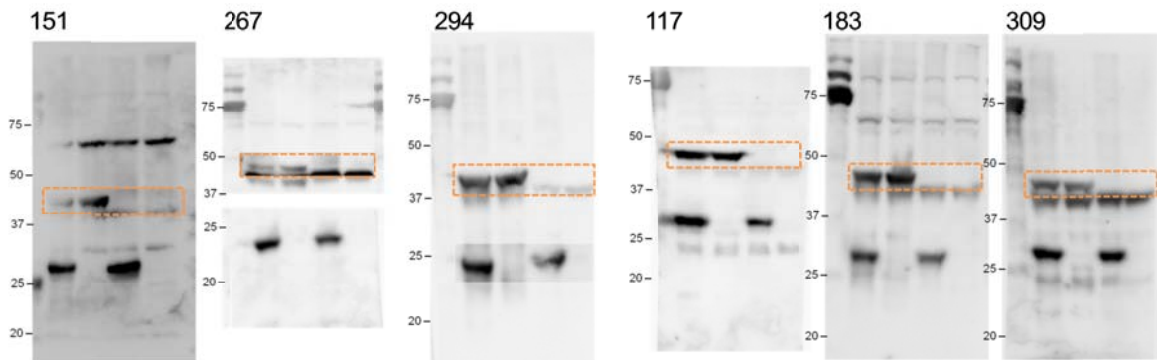


α -V1.1 fHbp

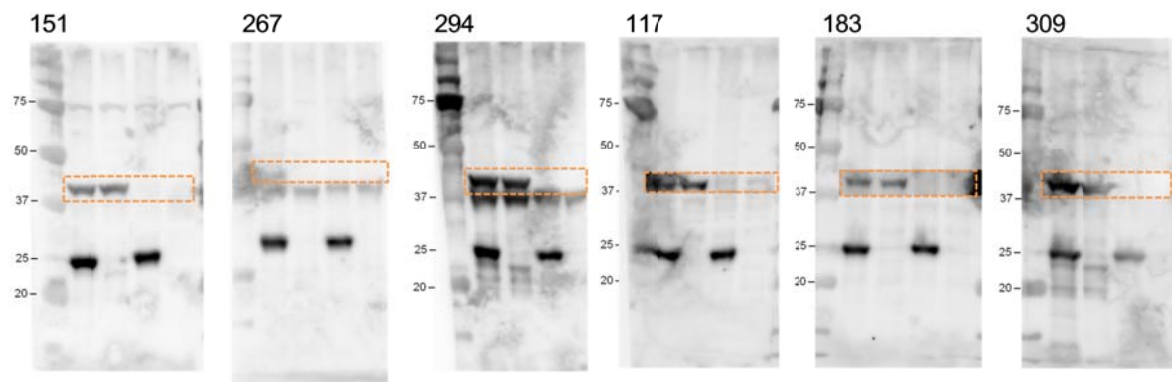


Supplementary Figure 6. Complete Western blots from Figure 2B.

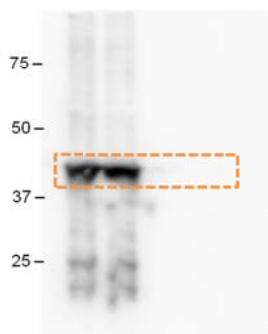
Alum + fHbp:PorA^{P1.16}



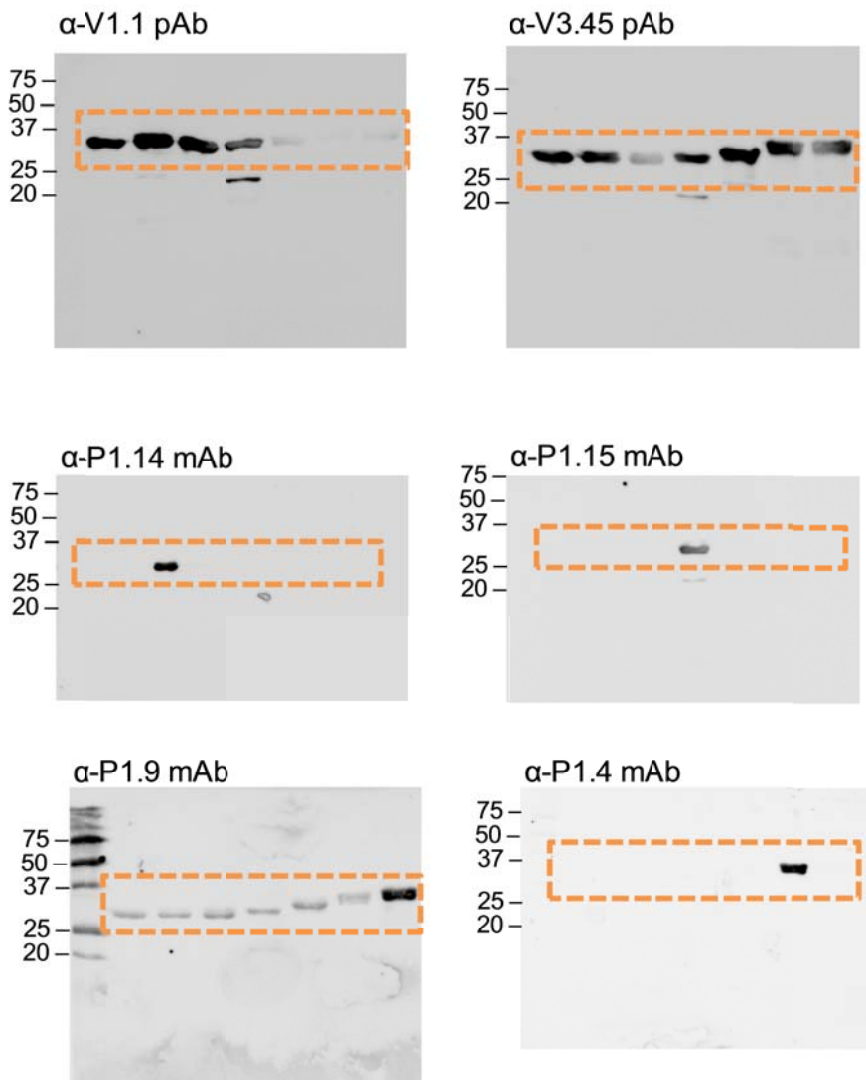
MPLA + fHbp:PorA^{P1.16}



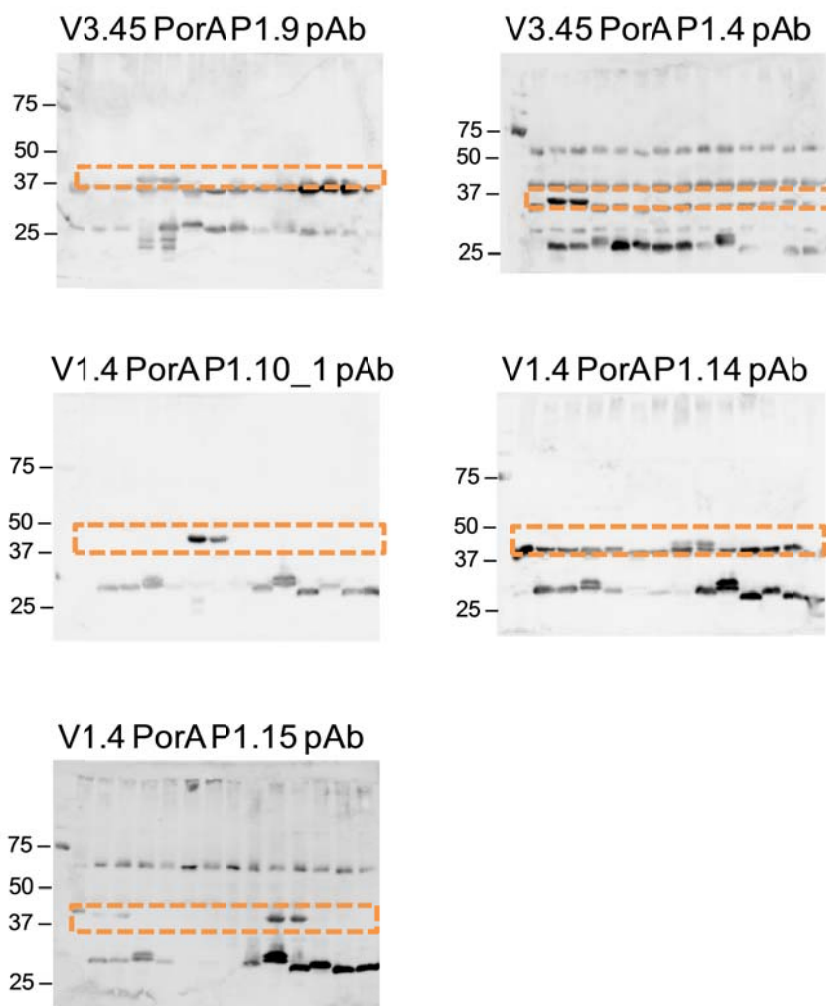
α -PorA P1.16



Supplementary Figure 7. Complete Western blots from Figure 2D.



Supplementary Figure 8. Complete Western blots from Figure 4E.



Supplementary Figure 9. Complete Western blots from Figure 4F

Supplementary Table 1: ChA nomenclature

fHbp scaffold	PorA VR2 loop inserted	Residue position of PorA VR2 loop insertion	ChA
V1.1	P1.16	151	fHbp ^{V1.1} :PorA ^{151/P1.16}
V1.1	P1.16	267	fHbp ^{V1.1} :PorA ^{267/P1.16}
V1.1	P1.16	294	fHbp ^{V1.1} :PorA ^{294/P1.16}
V1.1	P1.16	117	fHbp ^{V1.1} :PorA ^{117/P1.16}
V1.1	P1.16	183	fHbp ^{V1.1} :PorA ^{183/P1.16}
V1.1	P1.16	309	fHbp ^{V1.1} :PorA ^{309/P1.16}
V1.4	P1.16	151	fHbp ^{V1.4} :PorA ^{151/P1.16}
V1.4	P1.16	309	fHbp ^{V1.4} :PorA ^{309/P1.16}
V1.4	P1.10_1	151	fHbp ^{V1.4} :PorA ^{151/P1.1.10_1}
V1.4	P1.14	151	fHbp ^{V1.4} :PorA ^{151/P1.14}
V1.4	P1.15	151	fHbp ^{V1.4} :PorA ^{151/P1.15}
V3.45	P1.4	158	fHbp ^{V3.45} :PorA ^{158/P1.4}
V3.45	P1.9	158	fHbp ^{V3.45} :PorA ^{158/P1.9}

Supplementary Table 2: *Neisseria meningitidis* strains

Strain	fHbp	PorA VR1	PorA VR2	Year	Serogroup	Source
H44/76	V1.1	P1.7	P1.16	1976	B	80
H44/76ΔfHbp	deleted	P1.7	P1.16	1976	B	
H44/76ΔPorA	V1.1	P1.7	deleted	1976	B	
H44/76ΔfHbp ΔPorA	deleted	P1.7	deleted	1976	B	This study
M10.0240129	V1.92*	P1.7_2	P1.4	2010	B	
M10.0240123	V1.92*	P1.7_2	P1.4	2010	B	
M11 240428	V1.13	P1.22	P1.9	2011	B	
M11 240431	V2.19	P1.22	P1.9	2011	B	
M11 240189	V3.84	P1.5_1	P1.10_1	2011	B	Meningococcal Reference Unit.
M11 240287	V2.584	P1.5_1	P1.10_1	2011	B	Public Health England
M15 240917	V3.45	P1.22	P1.14	2015	B	
M15 240923	V1.4	P1.18_1	P1.14	2015	B	
M07 240902	V1.220	P1.19	P1.15	2007	B	
M08 240404	V1.1	P1.19	P1.15	2008	B	
M11 240382	V1.4	P1.12_1	P1.16	2011	B	
M11 240406	V1.1	P1.7	P1.16	2011	B	

*fHbp truncated at residue 242

Supplementary Table 3: *Escherichia coli* strains

Strain	Plasmid	Protein expressed
B834	pET21b-fHbp ^{V1.1}	V1.1 fHbp
B834	pET21b-fHbp ^{V1.1} :PorA ^{151/P1.16}	fHbp ^{V1.1} :PorA ^{151/P1.16}
B834	pET21b-fHbp ^{V1.1} :PorA ^{267/P1.16}	fHbp ^{V1.1} :PorA ^{267/P1.16}
B834	pET21b-fHbp ^{V1.1} :PorA ^{294/P1.16}	fHbp ^{V1.1} :PorA ^{294/P1.16}
B834	pET21b-fHbp ^{V1.1} :PorA ^{117/P1.16}	fHbp ^{V1.1} :PorA ^{117/P1.16}
B834	pET21b-fHbp ^{V1.1} :PorA ^{183/P1.16}	fHbp ^{V1.1} :PorA ^{183/P1.16}
B834	pET21b-fHbp ^{V1.1} :PorA ^{309/P1.16}	fHbp ^{V1.1} :PorA ^{309/P1.16}
B834	pET21b-fHbp ^{V1.4} :PorA ^{151/P1.16}	fHbp ^{V1.4} :PorA ^{151/P1.16}
B834	pET21b-fHbp ^{V1.4} :PorA ^{309/P1.16}	fHbp ^{V1.4} :PorA ^{309/P1.16}
B834	pET21b-fHbp ^{V1.4} :PorA ^{151/P1.1.10_1}	fHbp ^{V1.4} :PorA ^{151/P1.1.10_1}
B834	pET21b-fHbp ^{V1.4} :PorA ^{151/P1.14}	fHbp ^{V1.4} :PorA ^{151/P1.14}
B834	pET21b-fHbp ^{V1.4} :PorA ^{151/P1.15}	fHbp ^{V1.4} :PorA ^{151/P1.15}
B834	pET21b-fHbp ^{V3.45} :PorA ^{158/P1.4}	fHbp ^{V3.45} :PorA ^{158/P1.4}
B834	pET21b-fHbp ^{V3.45} :PorA ^{158/P1.9}	fHbp ^{V3.45} :PorA ^{158/P1.9}

Supplementary Table 4: Primers

Primer Name	Sequence
fHbp F1	GCGCATATGGTCGCCGCCGACATCG
fHbp R4	GCGCTCGAGTTGCTTGGCAGCAAGACCGATATGG
fHbp ^{V1.1} :PorA ^{151/P1.16} R2	CGCCAAATCGAAGTGGACTACTACACCAAAGACACCAACAACAACCT GACCCTGGTCCAGCTCATTACCTTGGAG
fHbp ^{V1.1} :PorA ^{151/P1.16} F3	CTCCAAGGTAATGAGCTGGACCAGGGTCAGGTTGTTGTTGGTGTCTT TGGTGTAGTAGTCCACTTCCGATTTGGCG
fHbp ^{V1.1} :PorA ^{267/P1.16} R2	AAGCCGATTACTACACCAAAGACACCAACAACAACCTGACCCTGGT CAAACGCCAT
fHbp ^{V1.1} :PorA ^{267/P1.16} F3	ATGGCGTTTGACCAGGGTCAGGTTGTTGTTGGTGTCTTTGGTGTAGT AATCCGGCTT
fHbp ^{V1.1} :PorA ^{294/P1.16} R2	TACTACACCAAAGACACCAACAACAACCTGACCCTGGTCAAAGCCCA GGAA
fHbp ^{V1.1} :PorA ^{294/P1.16} F3	GACCAGGGTCAGGTTGTTGTTGGTGTCTTTGGTGTAGTAGCCAAAGA TACC
fHbp ^{V1.1} :PorA ^{117/P1.16} R2	GACCAGGGTCAGGTTGTTGTTGGTGTCTTTGGTGTAGTATTGTGCCG CCAGCTTCAGCTTT
fHbp ^{V1.1} :PorA ^{117/P1.16} F3	TACTACACCAAAGACACCAACAACAACCTGACCCTGGTCGCGGAAAA AACTTATGGAAACGGTGAC
fHbp ^{V1.1} :PorA ^{183/P1.16} R2	GACCAGGGTCAGGTTGTTGTTGGTGTCTTTGGTGTAGTACGAATCTT GTATTTGCTCGGTCTGAAAGG
fHbp ^{V1.1} :PorA ^{183/P1.16} F3	TACTACACCAAAGACACCAACAACAACCTGACCCTGGTCCATTCCGG GAAGATGGTTGCG
fHbp ^{V1.1} :PorA ^{309/P1.16} R2	GACCAGGGTCAGGTTGTTGTTGGTGTCTTTGGTGTAGTATACGGTTT TCACTTCCGCGCTGC
fHbp ^{V1.1} :PorA ^{309/P1.16} R4	GTTCTCGAGTTGCTTGGCAGCAAGACCGATATGGCGTATGCCGACC AGGGTCAGGTTGTTGTTGG
fHbp ^{V1.4} :PorA ^{151/P1.16} R1	CGGGACCAGCGTCAGGTTGTTATTTGTATCCTTGGTATAGTAGTCCA CTTCGATTTGACGGATAAAGTCG
fHbp ^{V1.4} :PorA ^{151/P1.16} F2	TACTATACCAAGGATACAAATAACAACCTGACGCTGGTCCCGCAGCT CATTACCTTGGAGAGCGGAG
fHbp ^{V1.4} :PorA ^{309/P1.16} R1	CGGGACCAGCGTCAGGTTGTTATTTGTATCCTTGGTATAGTATGCCG TTTCCACTTCTGCGCTGC
fHbp ^{V1.4} :PorA ^{309/P1.16} R2	ATACTCGAGTTGCTTTGCAGCAAGACCAATATGTCGTATTCCCGGGA CCAGCGTCAGG
fHbp ^{V1.4} :PorA ^{151/P1.1.10_1} R1	CGGCACCAGGGTTGGCGGTTGGTTCTGTTTATTCTGGTCCACTTCGA TTTGACGGATAAAGTCG
fHbp ^{V1.4} :PorA ^{151/P1.1.10_1} F2	CAGAATAAACAGAACCAACCGCCAACCCTGGTGCCGCAGCTCATTAC CTTGGAGAGCGGAG
fHbp ^{V1.4} :PorA ^{151/P1.14} R1	AGCGTGAACCATTTTCTTTTCATCCACATAGTCCACTTCGATTTGACG GATAAAGTCG
fHbp ^{V1.4} :PorA ^{151/P1.14} F2	TATGTGGATGAAAAGAAAATGGTTCACGCTCAGCTCATTACCTTGA GAGCGGAG
fHbp ^{V1.4} :PorA ^{151/P1.15} R1	CGGTACAAAAACATCTGCGTTATTTTGGACGGGTATAATGGTCCACTTC GATTTGACGGATAAAGTCG
fHbp ^{V1.4} :PorA ^{151/P1.15} F2	CATTATACCCGTCAAATAACGCAGATGTTTTTGTACCGCAGCTCATT ACCTTGGAGAGCGGAG
fHbp ^{V3.45} :PorA ^{158/P1.4} R1	CGGTACATGGGTTGCCACTTTATTATTACAACAACATGGTCCACTTC GATTTTTTGCACAAAGTCG
fHbp ^{V3.45} :PorA ^{158/P1.4} F2	CATGTTGTTGTGAATAATAAAGTGGCAACCCATGTACCGCAAACCAT CACGCTGGCAAGC
fHbp ^{V3.45} :PorA ^{158/P1.9} R1	TGCGTGGTATTTAGACTGTTCTGTCTACGTAGTCCACTTCGATTTTTT CACAAAGTCG
fHbp ^{V3.45} :PorA ^{158/P1.9} F2	TACGTAGACGAACAGTCTAAATACCACGCACAAACCATCACGCTGGC AAGC

PorA KO F1	GCTTCAGCACCTTAGAGAATCG
PorA KO R1	CGCTATCAGGATCCAATCACTAGTGCTGGTAGTTCCTGCCTTCC
PorA KO F2	GGAGATAGTTATTATAAACTACAAGTGGATATTGTGGGACGACCGAA
PorA KO R2	ATTGCCGCCAC
PorA(tet) KO F1	CGTGGGAATGACAATTCGAGACCT
PorA(tet) KO R1	CCAGGACATATGGATTTCTTAGCAGAAGTA
PorA(tet) KO R1	GGCTCTTTTAGTTCTATCTCCACATGATACT

Supplementary Table 5: Data collection and refinement statistics.

ChA	fHbp ^{V1.4} PorA ^{151/P1.16}	fHbp ^{V1.1} PorA ^{294/P1.16}	fHbp ^{V1.4} PorA ^{309/P1.16}	fHbp ^{V1.1} PorA ^{309/P1.16}
PDB Code	5nqp	5nqx	5nqy	5nqz
Data collection statistics				
Beamline	Diamond I02	Diamond I02	ESRF ID29	Diamond I04-1
Wavelength (Å)	0.97949	0.97858	0.97623	0.92818
Space group	C222 ₁	P22 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	C2
Resolution limits (Å) ^a	40.74-2.86 (2.93-2.86)	81.65-3.66 (3.76-3.66)	51.17-2.60 (2.67-2.60)	58.55-1.63 (1.67-1.63)
Unit cell dimensions (Å, °)	100.7,171.4,131.5 90,90,90	94.6,114.9,160.3 90,90,90	56.9,62.6,88.7 90,90,90	91.4,76.3,83.9 90,91.3,90
Unique reflections	26403 (1923)	19820 (1431)	10208 (743)	70772 (4967)
Multiplicity ^a	4.5 (4.4)	4.4 (4.6)	6.4 (6.6)	3.0 (2.2)
Completeness (%) ^a	99.1 (99.4)	99.3 (99.9)	99.6 (99.4)	98.6 (93.9)
$\ \langle I \rangle \ $ ^a	17.1 (2.0)	7.6 (1.9)	16.7 (2.3)	12.7 (1.5)
R_{merge} (%) ^{a,b}	0.074 (0.645)	0.187 (0.833)	0.073 (0.845)	0.062 (0.615)
R_{pim} (%)	0.038 (0.340)	0.101 (0.439)	0.032 (0.356)	0.041 (0.534)
Refinement statistics				
Resolution limits (Å)	40.74-2.86 (2.97-2.86)	81.65-3.66 (3.85-3.66)	51.17-2.60 (2.86-2.60)	58.55-1.63 (1.67-1.63)
Number of reflections in working set	25137 (2746)	18807 (2650)	9665 (2369)	63880 (4735)

Number of reflections in test set	1238 (134)	975 (121)	498 (120)	3445 (225)
<i>R</i> factor of working set ^{a,c}	0.198 (0.303)	0.232 (0.321)	0.226 (0.351)	0.162 (0.289)
<i>R</i> _{free} ^{a,d}	0.239 (0.351)	0.282 (0.368)	0.271 (0.420)	0.194 (0.297)
Number of atoms (protein/water/other)	5895/28/84	9572/0/0	1994/4/4	4046/540/45
Residues in Ramachandran favoured region (%)	95.2	97.8	95.3	97.0
Ramachandran outliers (%)	0.1	0.1	0.0	0.2
r.m.s.d. bond lengths (Å)	0.002	0.002	0.002	0.020
r.m.s.d. bond angles (°)	0.58	0.42	0.45	1.98

^aNumbers in parentheses refer to the appropriate outer shell.

^b $R_{\text{merge}} = 100 \times (\sum_{hkl} \sum_i |I(hkl;i) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I(hkl;i))$, where $I(hkl;i)$ is the intensity of an individual measurement of a reflection and $\langle I(hkl) \rangle$ is the average intensity of that reflection.

^c $R_{\text{factor}} = (\sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|)$, where $|F_{\text{obs}}|$ and $|F_{\text{calc}}|$ are the observed and calculated structure factor amplitudes.

^d R_{free} equals the *R*-factor of test set (5% of the data removed prior to refinement).

r.m.s.d.: root mean square deviation from ideal geometry.