Supporting information for article:

Crystal structures of human Fabs targeting the Bexsero™ meningococcal vaccine antigen NHBA

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Table S1  Apo-Fab 10C3 structures solved in this work

Column I: starting material used for the crystallization experiment; Column II: identification number of the dataset; Column III: crystallization conditions; Column IV: resolution of each dataset; Column V and VI: cell dimensions and space group (SG); column VII: rmsd values obtained by SSM of each dataset superposed by SSM onto the reference structure from dataset #15.

| starting crystallization material | # dataset | Mother liquor composition | Resolution | cell dimension (a b c) | SG | SSM r.m.s.d. (Å) |
|----------------------------------|-----------|---------------------------|------------|------------------------|----|-----------------|
| (1) Crystals of apo-Fab 10C3     | 1         | 0.2 M LiSO₄, 0.1 M BIS-TRIS 5.5 pH, 25 %w/v PEG 3350 | 1.9 Å      | 69.30 76.58 82.14      | P 2₁ 2₁ 2₁ | 0.33            |
|                                  | 2         | 0.1 M KSCN, 30 %w/v PEG MME 2000 | 1.8 Å      | 69.67 76.78 82.29      | P 2₁ 2₁ 2₁ | 1.01            |
|                                  | 3         | 0.1 M KSCN, 30 %w/v PEG MME 2000 | 1.9 Å      | 69.72 77.69 82.49      | P 2₁ 2₁ 2₁ | 0.76            |
|                                  | 4         | 0.2 M NH₄ Acet, 0.1 M BIS-TRIS 5.5 pH, 25 %w/v PEG 3350 | 1.9 Å      | 69.65 77.91 82.35      | P 2₁ 2₁ 2₁ | 0.67            |
|                                  | 5         | 0.2 M (NH₄)₂H Cit, 20 %w/v PEG 3350 | 1.8 Å      | 69.65 77.31 82.05      | P 2₁ 2₁ 2₁ | 0.88            |
|                                  | 6         | 37.5 %w/v M1K3350, 0.1 M MB1 6.5 pH, 10 % MAA | 2.2 Å      | 69.61 74.24 82.41      | P 2₁ 2₁ 2₁ | 0.97            |
|                                  | 7         | 0.2 M NH₄ Acet, 0.1 M Na₃ Cit 5.6 pH, 30 %w/v PEG 4000 | 1.7 Å      | 69.74 78.69 82.89      | P 2₁ 2₁ 2₁ | 0.23            |
| (2) Crystals of complexed Fab 10C3 | 8         | 0.2 M Ammonium sulfate 0.1 M Sodium acetate pH 4.6, 25 % w/v PEG 4000 | 1.89 Å     | 69.17 76.80 82.53      | P 2₁ 2₁ 2₁ | 0.56            |
|                                  | 9         | 0.2 M Ammonium sulfate 30 % w/v PEG 4000 | 1.53 Å     | 67.93 75.54 81.87      | P 2₁ 2₁ 2₁ | 0.44            |
|                                  | 10        | 0.2 M Magnesium chloride hexahydrate 0.1 M MES pH6.0 20 % w/v PEG 6000 | 1.69 Å     | 69.33 76.81 82.28      | P 2₁ 2₁ 2₁ | 0.76            |
|                                  | 11        | 0.2 M Magnesium chloride hexahydrate 0.1 M MES pH6.0 20 % w/v PEG 6000 | 1.28 Å     | 69.49 78.11 82.86      | P 2₁ 2₁ 2₁ | 0.36            |
|                                  | 12        | 0.2 M Calcium chloride dihydrate 0.1 M MES pH6.0 20 % w/v PEG 6000 | 1.44 Å     | 76.77 82.04 68.80      | P 2₁ 2₁ 2₁ | 0.58            |
| (3) Crystals of soaked Fab 10C3  | 13        | 0.2 M NaCl, 0.1 M Na Phos Cit 4.2 pH, 20 %w/v PEG 8000 | 2.2 Å      | 70.19 78.15 83.86      | P 2₁ 2₁ 2₁ | 0.43            |
|                                  | 14        | 0.2 M NaCl, 0.1 M Na Phos Cit 4.2 pH, 20 %w/v PEG 8000 | 1.5 Å      | 69.51 78.52 82.73      | P 2₁ 2₁ 2₁ | 0.24            |
|                                  | 15        | 0.17 M (NH₄)₂SO₄, 15 %v/v Glycerol, 25.5 %w/v PEG 4000 | 1.5 Å      | 69.91 79.83 82.58      | P 2₁ 2₁ 2₁ | reference       |
Table S2  Binding affinities of Fabs 12E1 and 10C3 towards NHBA variants

Kinetic parameters of Fabs 12E1 and 10C3 towards NHBA peptides 2, 3 and 20 calculated by SPR experiments are shown. SPR data were analysed using the Biacore T200 Evaluation software (GE Healthcare) and the U-values for all the measurements were below the value of 15, therefore indicating uniqueness in the $K_D$ determination.

| Fab  | Protein  | Kon 1/M*s | Koff 1/s | U-value | $K_D$ (M)   |
|------|----------|-----------|----------|----------|-------------|
| 12E1 | NHBA p2  | 6.1 x 10^{-4} | 2.0 x 10^{-5} | 15        | 3.3 ± 0.8 x 10^{-10} |
|      | NHBA p3  | 8.4 x 10^{-4} | 3.1 x 10^{-4} | 5         | 5.1 ± 0.17 x 10^{-10} |
|      | NHBA p20 | 4.9 x 10^{-4} | 3.0 x 10^{-4} | 1         | 6.2 ± 1.5 x 10^{-9}  |
| 10C3 | NHBA p2  | 2.37 x 10^{-5} | 9.2 x 10^{-4} | 2         | 5.5 ± 0.5 x 10^{-9}  |
|      | NHBA p3  | 4.34 x 10^{-5} | 5.41 x 10^{-4} | 1         | 1.2 ± 0.16 x 10^{-10} |
|      | NHBA p20 | No binding       |            |           |             |
Table S3  CDRs loop conformation of Fabs 12E1 and 10C3

The CDRs of Fab 12E1 (a) and Fab 10C3 (b) are shown as identified and classified according to the cluster definition introduced by (North et al., 2011). Loop conformation is assigned on the basis of the region of the Ramachandran plot for every CDR residue. B stands for β-sheet region, P for polyproline II, A for α-helix, D for δ region (near α-helix but at more negative values of ϕ), L for left-handed helix, and G for γ region (ϕ>0° excluding the L and B regions). Cis conformation is indicated by lower case letters.

(a) Fab 12E1

| CDR loop | Residues     | Cluster | Loop Conformation      |
|----------|--------------|---------|------------------------|
| CDR-H1   | Lys23–His35  | H1-13-1 | PPBLBPAAABPBB          |
| CDR-H2   | Trp50–Lys59  | H2-10-1 | BBPAADLPBB             |
| CDR-H3   | Ile97-Pro104 | Not assigned | BPAGLLBp          |
| CDR-L1   | Arg24–Asn39  | L1-16-1 | BPBLPBPAPLLPPBBBPB     |
| CDR-L2   | Tyr54–Ser61  | L2-8-1  | BLLDPPPP               |
| CDR-L3   | Met94–Thr102 | L3-9-cis7-1 | BBDABPpPB          |

(b) Fab 10C3

| CDR loop | Residues     | Cluster | Loop Conformation      |
|----------|--------------|---------|------------------------|
| CDR-H1   | Lys23-His55  | H1-13-1 | PPBLPAABBPBB          |
| CDR-H2   | Trp50-Asn59  | H2-10-1 | BBPDDDPBB              |
| CDR-H3   | Ala96-Tyr103 | H3-7-1  | BPGADLAB               |
| CDR-L1   | Thr23-Ser36  | L1-14-2 | BBBAADDABDPBB          |
| CDR-L2   | Tyr51-Ser58  | L2-8-1  | BLLDPPPP               |
| CDR-L3   | Ser91-Val100 | L3-10-1 | BBPDGLLPPB             |
Table S4  Amino-acidic composition of the CDRs of Fabs 12E1 and 10C3

The contribution of single amino acids or groups of amino acids to the total solvent accessible area (ASA) as calculated by PISA (Krissinel & Henrick, 2007) are expressed both in percentage and Å², and are clustered on the basis of their biochemical properties. The total ASA is calculated as the sum of the ASA of each residues belonging to the CDRs.

(a)

**Fab 12E1**

| Type            | % on ASA | Amino acid | ASA (Å²) | % on ASA |
|-----------------|----------|------------|----------|----------|
| Aromatic        | 18.93    | Tyr        | 373.35   | 9.70     |
|                 |          | Trp        | 240.42   | 6.25     |
|                 |          | Phe        | 114.77   | 2.98     |
| Positive        | 28.84    | Lys        | 513.73   | 13.35    |
|                 |          | His        | 144.41   | 3.75     |
|                 |          | Arg        | 451.67   | 11.74    |
| Negative        | 7.72     | Asp        | 239.15   | 6.21     |
|                 |          | Glu        | 57.85    | 1.50     |
| Polar uncharged | 28.97    | Ser        | 378.39   | 9.83     |
|                 |          | Asn        | 290.85   | 7.56     |
|                 |          | Gln        | 103.75   | 2.70     |
|                 |          | Thr        | 225.57   | 5.86     |
|                 |          | Pro        | 116.60   | 3.03     |
|                 |          | Cys        | 0.00     | 0.00     |
| Non polar       | 15.54    | Ile        | 262.38   | 6.82     |
|                 |          | Gly        | 176.57   | 4.59     |
|                 |          | Leu        | 88.66    | 2.30     |
|                 |          | Val        | 36.67    | 0.95     |
|                 |          | Ala        | 8.99     | 0.23     |
|                 |          | Met        | 24.99    | 0.65     |
| Total           | 100.00   |            | 3848.77  | 100.00   |
(b) Fab 10C3

| Type            | % on ASA | Amino acid | ASA (Å²) | % on ASA |
|-----------------|----------|------------|----------|----------|
| Aromatic        | 18.16    | Tyr        | 422.48   | 11.71    |
|                 |          | Trp        | 218.67   | 6.06     |
|                 |          | Phe        | 14.05    | 0.39     |
| Positive        | 13.47    | Lys        | 101.79   | 2.82     |
|                 |          | His        | 134.04   | 3.72     |
|                 |          | Arg        | 249.96   | 6.93     |
| Negative        | 8.14     | Asp        | 227.42   | 6.30     |
|                 |          | Glu        | 66.07    | 1.83     |
| Polar uncharged | 46.53    | Ser        | 718.75   | 19.92    |
|                 |          | Asn        | 547.36   | 15.17    |
|                 |          | Gln        | 0.00     | 0.00     |
|                 |          | Thr        | 381.09   | 10.56    |
|                 |          | Pro        | 31.46    | 0.87     |
|                 |          | Cys        | 0.00     | 0.00     |
| Non polar       | 13.71    | Ile        | 51.88    | 1.44     |
|                 |          | Gly        | 202.27   | 5.61     |
|                 |          | Leu        | 51       | 1.41     |
|                 |          | Val        | 185.44   | 5.14     |
|                 |          | Ala        | 3.86     | 0.11     |
|                 |          | Met        | 0.00     | 0.00     |
| Total           | 100.00   |            | 3607.59  | 100.00   |
Figure S1  NHBAp2 and secondary structures predictions. (a) The NHBAp2 domain organization and structural coverage are depicted with a colored bar. Blue and yellow are for the N- and C-terminal domains, respectively, while orange and grey boxes show the location of the IB-insertion and the Arg-rich motif. (b) Secondary structure predictions on NHBAp2 sequence were performed using: Phyre2 (Kelley et al., 2015), SABLE (Adamczak et al., 2005), PSIPRED (Buchan et al., 2013) and JPred (Drozdetskiy et al., 2015). Putative epitopes of 12E1 and 10C3 mapped by Giuliani et al. (Giuliani et al., in preparation) are boxed with a dashed line and a dotted line respectively. Cys18 (underlined) is
the NHBA lipidation site and residues preceding such Cys are cleaved in the mature surface-exposed form of the protein.

**Figure S2** SPR sensorgrams of Fab10C3 and Fab12E1 and NHBA peptides 2, 3, and 20. Surface plasmon resonance (SPR) was used to determine the dissociation constants ($K_D$), using the single cycle kinetic (SCK) approach, for the NHBA variants p2, p3 and p20. The titrations included NHBA concentrations from 6.25 to 100 nM. Sensograms referring to Fab12E1 and 10C3 are plotted in the first (a) and the second (b) column respectively. Colored curves represent the experimental data, black lines represent the fitted curves. Note that the $K_D$ of Fab10C3 towards NHBAp20 could not be measured due to lack of recognition of this variant.