Structural Basis for Iron Piracy by Pathogenic *Neisseria*

Of the 11 species of *Neisseria* bacteria that colonize humans, 9 of them coexist peacefully with us. However, two can cause serious diseases: *N. gonorrhoeae*, responsible for the sexually transmitted disease gonorrhea, and *N. meningitidis*, which causes septicemia and meningitis. While both pathogenic species can lead to significant long-term consequences, normally only *N. meningitidis* causes significant fatalities. Onset of infection is quick, resulting in about a 15% mortality rate and leaving 20% of those who survive with lifelong complications. Antibiotics are prescribed against these pathogens post-infection, but increasing resistance has spurred an immediate push for vaccine development (1).

Commercially available vaccines exist that work against four of the five disease-causing serogroups of *N. meningitidis* that have been isolated (A, B, C, Y, W135) but there is no vaccine against serogroup B (menB), nor is there a vaccine available against *N. gonorrhoeae*. One approach for vaccine development against menB and *N. gonorrhoeae* is to target the iron transporters found on the surface of the pathogens*. Without iron, these pathogens cannot survive; therefore the transporters are usually well-conserved across Neisserial strains and generally do not undergo significant genetic variation, making them ideal targets for vaccine development.

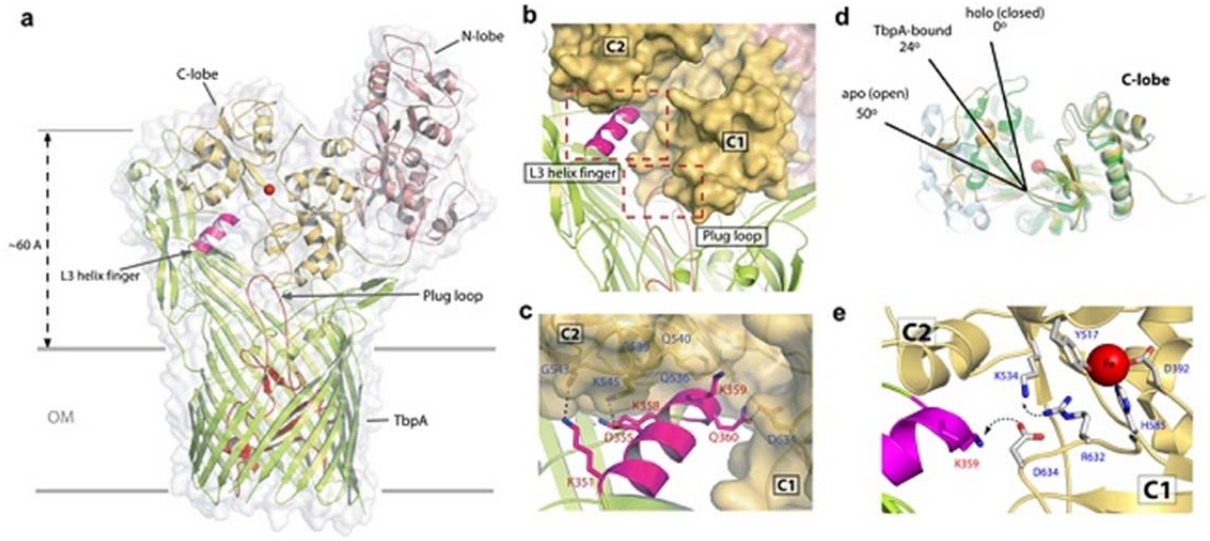

Figure 1. Interactions between Neisserial TbpA and human transferrin. **a**. The crystal structure of Neisserial TbpA (β-barrel domain in green, plug domain in red) in complex with human transferrin (N-lobe shown in pink and C-lobe in gold). Only the C-lobe interacts with TbpA. **b**. Zoomed view of the interactions between the TbpA helix finger and plug loop with transferrin. **c**. Molecular interactions between the TbpA helix finger with transferrin. **d**. Alignment of conformations of the C-lobe of transferrin comparing apo, holo, and TbpA-bound states. TbpA locks the C-lobe of transferrin in a conformation roughly halfway between that observed for apo and holo. **e**. Proposed mechanism for how TbpA catalyzes the release of iron from transferrin. TbpA residue K359 is believed to hijack the pH sensing triad mechanism of transferrin which leads to repulsion between transferrin residues K534 and R632 and a conformational change that releases iron.

In this study, the crystal structures of two of the surface receptors of menB, TbpA and TbpB, were determined. These receptors are used specifically by *Neisseria* to pirate iron from the abundant human iron binding protein, transferrin, during pathogenesis. TbpA is an essential TonB-dependent transporter (22-stranded β-barrel membrane protein) that is responsible for transporting iron across the outer membrane. Remarkably, this structure was crystallized in complex with human transferrin, which allowed a precise description of the interactions between the Neisserial TbpA and the human transferrin protein (Fig. 1a), enabling the identification of a helix finger and plug loop that are crucial for function (Fig. 1b,c). Neisserial TbpA was seen to lock transferrin in a slightly open conformation, sufficient to allow iron release from the cleft (Fig. 1d). These results led to a plausible mechanism for how Neisserial TbpA can catalyze the release of iron from transferrin at neutral pH for internalization (Fig. 1e). Further, antibodies based on this structure were developed against Neisserial TbpA that could effectively block transferrin binding using *in vitro* assays. MD simulations designed to mimic interactions with the Ton system also revealed a mechanism for how the iron, once extracted, is transported across the outer membrane through the β-barrel domain of TbpA.

Also reported in this study was the structure of Neisserial TbpB (Fig. 2a), a lipoprotein co-receptor that significantly increases the efficiency of iron uptake by specifically binding only iron-containing transferrin and concentrating it on the Neisserial surface. TbpB then shuttles the iron-loaded transferrin to TbpA which subsequently extracts and imports the iron across the Neisserial outer membrane. While attempts to crystallize the TbpB-transferrin complex were unsuccessful, SAXS analysis based on data collected at the BL4-2 beam line at SSRL was instrumental in constructing a model for how the Neisserial co-receptor was able to interact with human transferrin at the cell surface (Fig. 2b), revealing that TbpA and TbpB could simultaneously bind transferrin at distinct sites (Fig. 2c). The SAXS model was further verified when Calmettes, *et al.* later reported the structure of the TbpB-transferrin complex using X-ray crystallography.

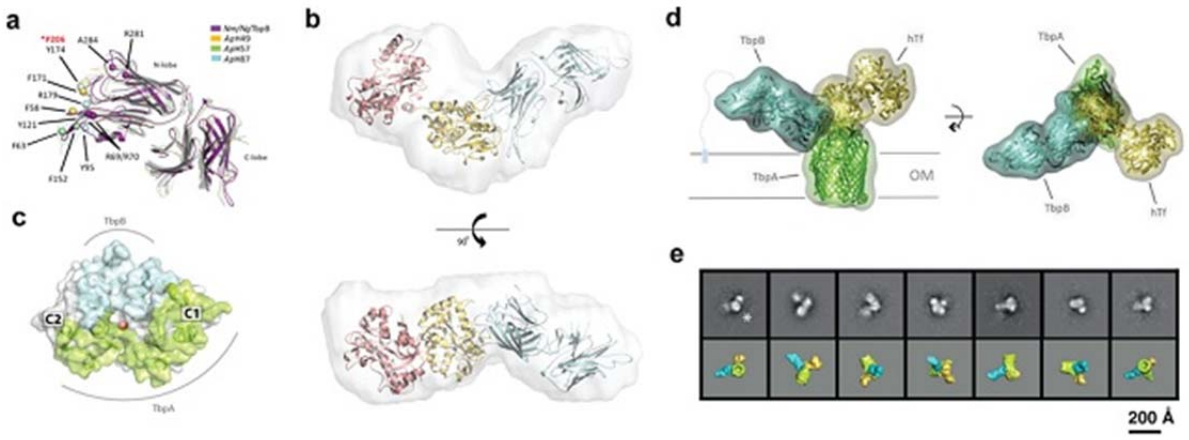

Figure 2. Models for the Neisserial TbpB and human transferrin complex and for the fully assembled iron import complex from *Neisseria*. **a**. The crystal structure of Neisserial TbpB (purple) aligned to known TbpB structures from porcine pathogens. Residues shown to affect transferrin binding are indicated and were used when modeling the complex with transferrin. **b**. SAXS analysis was used to model the TbpA-transferrin complex. Shown here is the model fit into the SAXS envelope. **c**. TbpA (green) and TbpB (cyan) were seen to have distinct non-overlapping binding sites on transferrin. **d**. Based on the X-ray crystallography and SAXS model, a model for the fully assembled iron import complex was formed (TbpA in green, TbpB in cyan, and transferrin in gold). **e**. EM analysis of the fully assembled iron import complex was shown to be consistent with our model (panel d).

Based on the X-ray crystallography and SAXS results, the fully assembled Neisserial transferrin-iron import complex (TbpA-TbpB-transferrin) was modeled (Fig. 2d) and EM studies performed to look at it experimentally. Here, purified complex was used for negative-stain EM analysis to produce class averages of the complex (Fig. 2e). These class averages were found to be consistent with the model for the fully assembled Neisserial transferrin-iron import complex, allowing the first look at what the transferrin-iron import complex looks like at the Neisserial surface during its pathogenesis.

References

1. C. Calmettes, J. Alcantara, R. H. Yu, A. B. Schryvers and T. F. Moraes, "The structural basis of transferrin sequestration by transferrin-binding protein B", *Nat. Struct. Mol. Biol*. **19**, 358 (2012)

Primary Citation

N. Noinaj, N. C. Easley, M. Oke, N. Mizuno, J. Gumbart, E. Boura, A. N. Steere, O. Zak, P. Aisen, E. Tajkhorshid, R. W. Evans, A. R. Gorringe, A. B. Mason, A. C. Steven, S. K. Buchanan, "Structural Basis for Iron Piracy by Pathogenic *Neisseria". Nature* **483,** 53 (2012) [DOI: 10.1038/nature10823]

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SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences and the National Center for Research Resources.