

The application of Structural Vaccinology in the development of a meningococcal antigen inducing broad protective immunity

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Background: prevention of meningitis

- *Neisseria meningitidis* is a major cause of bacterial septicemia and meningitis, diseases that can kill children and young adults in hours.
- Five *N. meningitidis* serogroups (A,B,C,W-135 & Y) cause most of the disease burden.
- Preventative vaccines using a carrier protein conjugated to capsular polysaccharides from serogroups A,C,W-135 & Y are now available.
- A vaccine against serogroup B has been more difficult to develop, partly due to the low immunogenicity, and potential human cross-reactivity, of the B capsular polysaccharide.

Introduction: protein-based vaccines

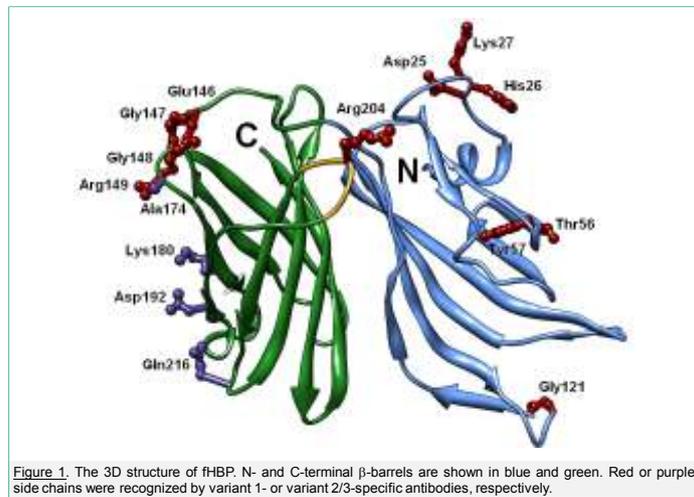
- In vaccine design, an alternative to using capsular polysaccharide conjugates is to use surface-exposed proteins (antigens) of the pathogen.
- Vaccine design is often hampered by sequence variability in such antigens; *i.e.* an antigen is protective against its own pathogenic strain but not against other strains.
- *N. meningitidis* serogroup B is an extreme case: circa 300 sequence variants of the antigen fHBP (factor H binding protein) have been identified.
- Serogroup B fHBPs can be grouped in 3 variant classes, that are not cross-protective.

Key question:

- Is it possible to design a single antigenic fHBP molecule conferring protection against all possible MenB strains from the 3 different classes?

Experimental Methods

- We analyzed the fHBP structure [1], which exhibits two β -barrels & a short linker (Fig. 1).
- Epitope-mapping analyses revealed that residues contributing to the immunogenicity of variant 1 or variants 2 and 3 were located in non-overlapping areas (Fig. 1).



- We tested whether residues from fHBP variants 2 and 3 could be substituted onto the scaffold of fHBP variant 1, in order to engineer a fully cross-protective antigen.
- Single, double or triple mutants were not cross-protective.
- 54 different chimeric variant 1 proteins were designed, each engineered to display a portion of a variant 2 and/or 3 protein on its surface.
- Each protein was purified and tested for ability to induce protective immunity in mice.
- The structural integrity of each engineered protein was verified by biophysical methods.
- The structure of the most efficacious chimera was determined by X-ray crystallography.

References

- [1] F. Cantini et al., *J Biol Chem*, 2009; A. Mascioni et al., *J Biol Chem*, 2009; and M. Schneider et al., *Nature*, 2009.
 [2] M. Scarselli et al., *Sci Trans Med*, 2011.

Results

- The fHBP C-terminal β -barrel domain surface was divided computationally into 11 partially overlapping areas ("patches") of 900-2000Å² (Fig. 2A).
- 54 different mutant 'patch' proteins were produced and purified. Size-exclusion chromatography, CD and NMR spectroscopy (Fig. 2B) were used to verify foldedness.

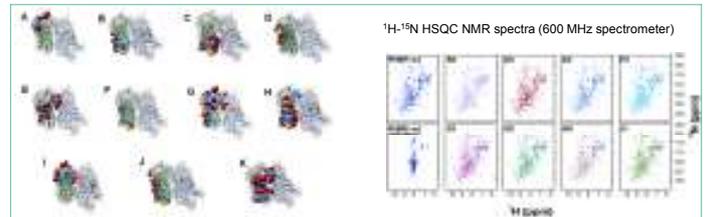


Figure 2A. In each of the 11 patches, several point mutations from variants 2 or 3 were introduced, making a total of 54 different engineered proteins. All 'patch' proteins were expressed in *E. coli*.

Figure 2B. NMR spectroscopy was used to confirm the structural integrity of each mutant. All HSQC spectra (except for the 'unfolded' control) have well-dispersed peaks, indicating a folded sample.

- The 'patch' mutations did not disrupt protein architecture, indicating that the designed conformational epitopes were successfully grafted onto the molecular scaffold of fHBP.
- After immunization of mice with each of the 54 mutants, one patch protein ("G1"), that induced sera bactericidal against all MenB strains tested, was identified.
- We crystallized the fHBP G1 patch protein and a single strongly-diffracting crystal yielded readily interpretable electron-density maps (Fig. 3).

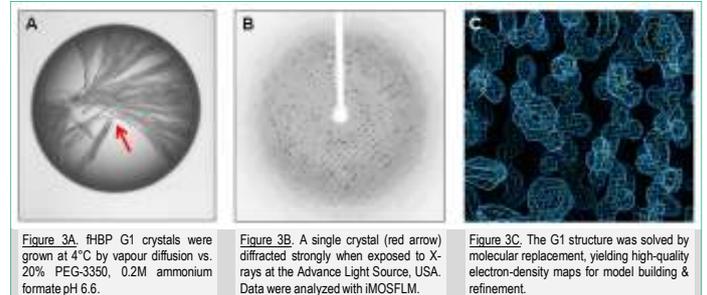


Figure 3A. fHBP G1 crystals were grown at 4°C by vapour diffusion vs. 20% PEG-3350, 0.2M ammonium formate pH 6.6.

Figure 3B. A single crystal (red arrow) diffracted strongly when exposed to X-rays at the Advance Light Source, USA. Data were analyzed with IMOSFLM.

Figure 3C. The G1 structure was solved by molecular replacement, yielding high-quality electron-density maps for model building & refinement.

- We determined the X-ray crystallographic structure of the G1 fHBP mutant [2] (Fig. 4).
- The immunogenicity of G1 has been further optimized by additional formulation strategies, see poster V57 (M. Pallao et al.)

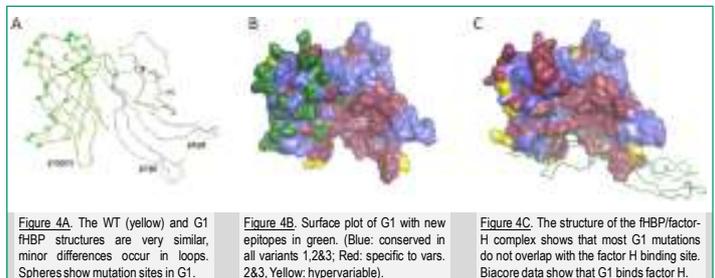


Figure 4A. The WT (yellow) and G1 fHBP structures are very similar, minor differences occur in loops. Spheres show mutation sites in G1.

Figure 4B. Surface plot of G1 with new epitopes in green. (Blue: conserved in all variants 1,2&3; Red: specific to vars. 2&3; Yellow: hypervariable).

Figure 4C. The structure of the fHBP/factor-H complex shows that most G1 mutations do not overlap with the factor H binding site. Biacore data show that G1 binds factor H.

Conclusion and Outlook

- An fHBP antigen was engineered to display epitopes from all three MenB variants.
- Structural Vaccinology, as the rational design of multiple immunodominant surfaces on a single protein scaffold, is an effective way to create broadly protective vaccines.