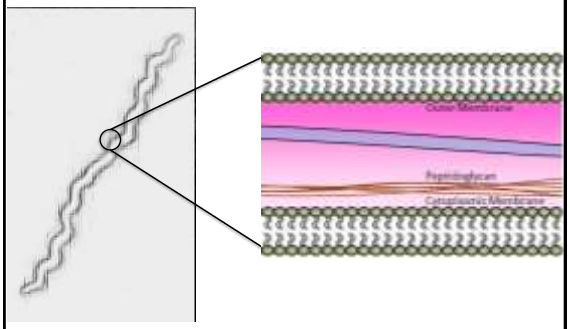
The Discovery of a family of transmembrane proteins with Factor-H binding activity in *Borrelia*

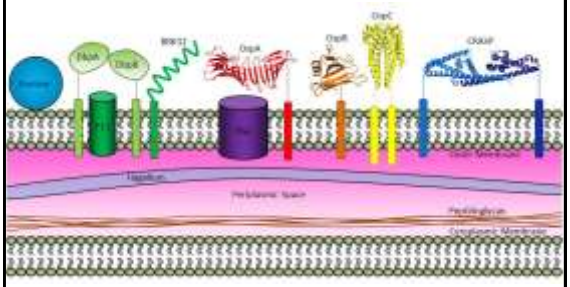
Richard Bingham
Adam Dyer
The University of Huddersfield

Talk Outline

- Background
  - The Borrelial outer membrane
  - An overview of selected known surface proteins
- Results
  - Discovery of Borrelia OmpA/W like proteins
  - Recombinant protein expression/purification
  - Structural analysis (CD, X-ray, SAXS)
- Conclusions

The outer membrane of *Borrelia*





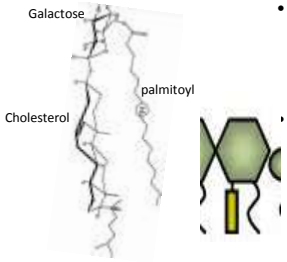
Borrelia outer membrane morphology

- The basic structure consists of a cytoplasmic membrane surrounded by peptidoglycan and a loosely associated outer membrane (OM).

  Unique characteristics:
  - Abundance of lipoproteins
  - Glycolipids replace lipopolysaccharide
  - The presence of cholesterol

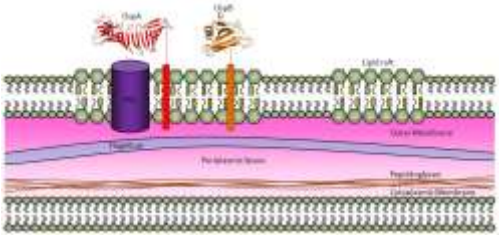
(Ben-Menachem et al., 2003; Schroder et al., 2003; Stubs et al., 2009)

Glycolipids and cholesterol in the membrane

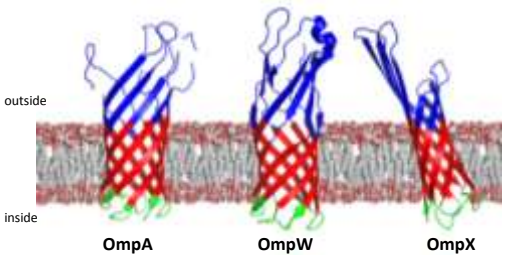


- Glycolipids and cholesterol make up a significant proportion of the Borrelia membrane.
- Cholesterol rich domains form lipid rafts

- OspA, OspB and P66 associate together in cholesterol rich lipid rafts.
- There is evidence that OspA and OspB shield underlying antigens such as p66 (LaRocca et al 2010)



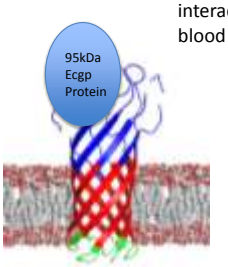
An introduction to Beta-barrels



These proteins are virulence factors in many other bacteria

- *E. coli* OmpX
  - promotes bacterial adhesion to and entry into mammalian cells, resistance to complement
- *N. meningitidis* OpcA
  - Role in adherence to and invasion of human epithelial and endothelial cells
  - binds vitronectin, which in turn binds integrins (Virji, 1993).
- *E. coli* OmpA - neonatal meningitis
  - Role in penetration of the blood brain barrier (Huang, 1995, Selvaraj, 2007)

E coli OmpA makes a specific interaction with a protein on the human blood brain barrier



Prasadarao,N.V. (2002) Identification of Escherichia coli outer membrane protein A receptor on human brain microvascular endothelial cells *Infect. Immun.* **70**(8):4556–4563.

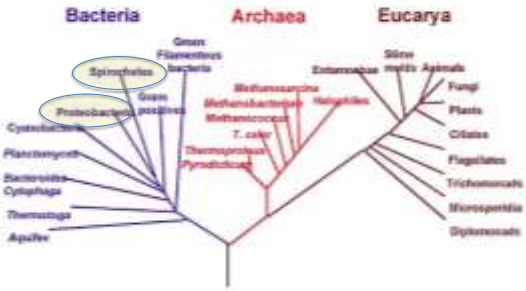
Research at Huddersfield

- Very few beta-barrels are known in Borrelia
  - P66, BamA (BB0795), P13?
- Our aim is to identify and characterise novel membrane spanning beta-barrel proteins in Borrelia
- The major target is the highly conserved **OmpA-type membrane-spanning domain**

Why look for OmpA

- The Enterobacteriaceae are a large family of Gram-negative bacteria forming part of the phylum of proteobacteria.
- OmpA is highly conserved among the *Enterobacteriaceae* family of Gram-negative bacteria but unknown in spirochetes
- OmpA has numerous roles
  - Membrane channel
  - Maintenance of cell shape/membrane integrity
  - Roles in virulence

Phylogenetic Tree of Life



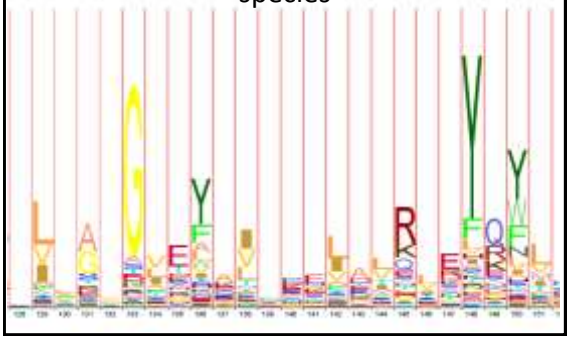
OmpA as a vaccine target

- Exposed outer loops
- High sequence conservation between different Borrelial strains
- OmpA from several other bacterial species have been shown to induce specific humoral and cytotoxic responses in the absence of adjuvant.
-
- OmpA has been proposed in the design of vaccines for numerous other bacteria
  - *Klebsiella pneumoniae, Chlamydia, Neisseria gonorrhoeae, Salmonella*
- Further reading: Jeannin *et al*, 2002

Searching for the Beta Barrel Proteins in *Borrelia*

- Lots of lipoproteins, no known membrane spanning beta barrel proteins resembling OmpA
- We searched the available genome sequences using two strategies
  - BLAST searches
  - Hidden Markov models (HMM)
- To begin we need a HMM PROFILE, this is based on known Omps from other bacteria

HMM Profile Profile generated from ~900 sequences from 451 bacterial species



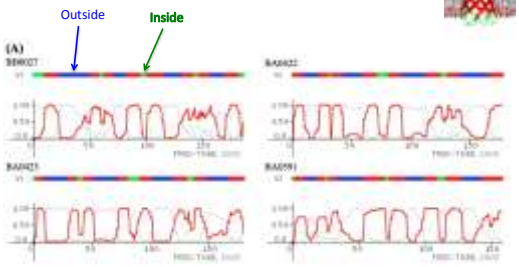
Results of HMM searches

- The results were cross checked with Fold & Function Assignment Server (FFAS)
  - http://ffas.ljcrf.edu/
- This identified the following proteins as potential OmpA-like membrane spanning domains
  - BB0027, BG0027, BA0026
  - BB0405, BG0407*, BA0422
  - BB0406, BG0408, BA0423
  - BB0562, BG0572, BA0591

At least one of these protein binds human Factor H

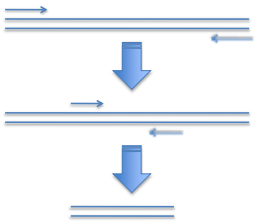
- BG0407 is a protein that was identified as binding to human factor H (Bhide *et al*, 2009)
- Factor H is a regulator of the complement immune response
- It is highly likely that the other members of this protein family also bind to human factor H or FHL protein based on their similar amino acid sequence.

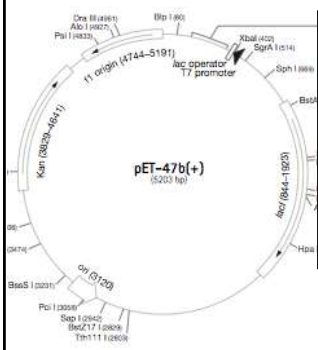
Topology Prediction



Cloning

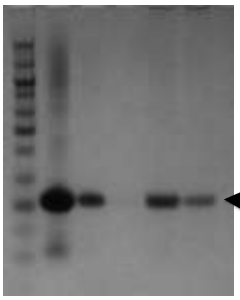
- DNA provided by Dr Gabi Margos (University of Bath)
- We used nested-PCR to clone the Borrelia target sequences.





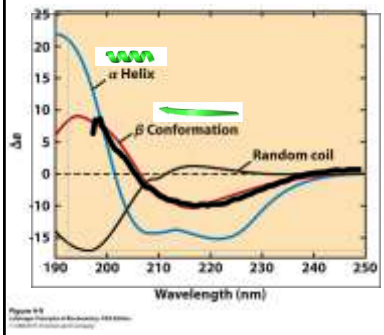
- Borrelia DNA was inserted into a circular plasmid.
- This was introduced into *E. coli* for protein expression
- Purified by affinity chromatography and size exclusion

SDS-PAGE



- Nice single band
- No contamination

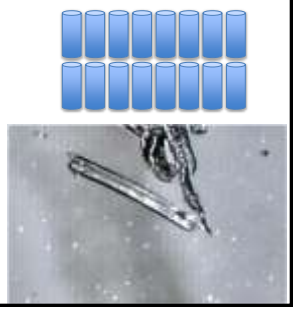
Circular Dichroism Spectroscopy



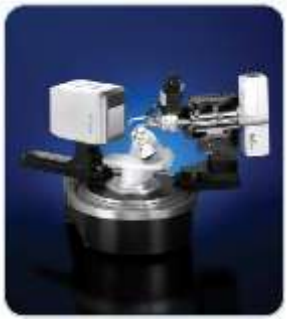
Small Angle X-ray Scattering

Curious Results

- Protein molecules appear to associate together to form larger complexes
- Disc-like structure only 2 molecules thick
- Also some small crystals



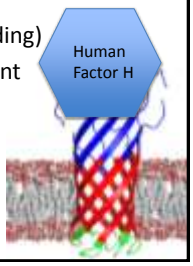
Protein X-ray Diffraction



- The University of Huddersfield has recently purchased a Bruker diffractometer.
- This will allow high resolution structure determination

Conclusion

- We have identified a family of novel outer membrane proteins in Borrelia
- Potential vaccine target
- Potential role in virulence (FH binding)
- Cloned, produced pure recombinant protein
- Basic structural characterisation
  - Circular dichroism
  - SAXS



Current Objectives

- X-ray crystal structure- high resolution model
- Experimental evidence of Factor H binding
- Functional characterisation

Acknowledgments

All the work…
- Adam Dyer MSc (University of Huddersfield)

Borrelia DNA
- Dr Gabriele Margos (University of Bath)

SAXS
- Dr Peter Laity (University of Huddersfield)

Circular Dichroism
- Dr Andrew Leech (University of York)

References

- Bhide et al, (2009) Complement factor H binding by different Lyme disease and relapsing fever *Borrelia* in animals and human BMC Research Notes, **2**:134
- LaRocca (2010) Cholesterol lipids of *Borrelia burgdorferi* form lipid rafts and are required for the bactericidal mechanism of a complement- independent antibody. Cell Host Microbe. 8(4): 331–342.
- Jeannin et al (2002) Outer membrane protein A (OmpA): a new pathogen-associated molecular pattern that interacts with antigen presenting cells—impact on vaccine strategies Vaccine 20: A23–A27

Composition of the Borrelia outer membrane

- Phospholipids
  - Phosphatidylcholine
  - Phosphatidylglycerol
- Cholesterol
- Glycolipids
  - cholesteryl 6-O-acyl-β-D-galactopyranoside(ACGal)
  - cholesteryl-β-D-galacto-pyranoside (CGal)
  - mono-α-galactosyl-diacylglycerol (MGalD) (Ben-Menachem et al., 2003; Schroder et al., 2003; Stubs et al., 2009)
-