Bacterial Surface Receptors and Transporters – Methods for studying their interactions
Structure and Function of Protein and Ion Translocation Systems:

• Iron acquisition
  – Bacterial Transferrin Receptor

• Surface Lipoprotein (SLP) Translocation
  – SLPs including: TbpB, bacterial transferrin receptor
  – FHbp, Factor H binding Protein

• Phosphate Transport System
  – OprP, PstS in Pseudomonas aeruginosa

• Sulfate/Bicarbonate transporters
  – SLC26 SulP transporters, Mammalian -> Archaea
• Structure and Function of Protein and Ion Translocation Systems:
  – Gram negative bacterial pathogens
    • *Pseudomonas*
    • *Salmonella*
    • *EPEC, EHEC*

    • *Neisseria meningitidis*
    • *Neisseria gonnorhea*
    • *Actinobacillus pleuropneumoniae*
Neisseria Meningitidis

*Neisseria meningitidis*:
- Obligate Human restricted pathogen (Gram negative)
- Asymptomatic nasopharyngeal carriage (10-30%)
- Major cause of infant meningococcal disease and sepsis
- Meningococcal disease
  - 2,600 cases of bacterial meningitis (US)/yr
  - Fatality rate ranges between 10-20%
Host-Restricted Gram negative Bacterial Pathogens

Eg: Neisseria meningitidis

- Obligate commensal bacteria
  - Asymptomatic in 10% of the population
  - Sporadically cross the epithelial barrier

- Meningococcal disease
  - 2,600 cases of bacterial meningitis (US)/yr
  - Fatality rate ranges between 10-20%

CDC – March 2004
Gram-negative Outer membrane transport systems
Iron is Essential for Cell Viability

### Biological Roles for Iron
- Major redox mediator
  - Iron-sulfur clusters
  - Heme
  - Catalytic center for enzymes and redox reactions
- Central to cellular processes
  - Electron transport, Amino acid and nucleoside synthesis...

### Iron
- Iron (Fe) can adopt two positively charge ionic forms
- Fe II (Ferrous)
  \[
  \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^-
  \]
  - Generation of radicals
- Fe III (Ferric)
  - Predominant form in aerobic environment
  - Insoluble, aggregates as oxy-hydroxide polymers
Mammalian Iron Sequestration

Intracellular Fe is sequestered by Ferritin and subsequently by other proteins, enzymes or cofactors...

Extracellular Fe is sequestered by Transferrin and Lactoferrin.
Transferrin: Mammalian Iron Retrieval System

Extracellular Space (pH 7.4)

Fe$_2$-Tf

Apo-Tf

TfR

Clathrin-coated Vesicle

Clathrin-coated Pit

Endosome

Cytoplasm

Acidified Endosome (pH 5.5)

Released Fe

DMT1

H$^+$

Proton Pump

T.Waltz Lab
Bacterial Transferrin Receptor, TbpA/B

Bacterial transferrin Receptor

Bipartite:
TbpA  - TonB dependent outer membrane protein
TbpB  - Surface lipoprotein
Bacterial Transferrin Receptor, TbpA/B

Objective:
Understand how TbpB and TbpA bind and acquire iron from transferrin.

Long term Research Goal:
Provide insight into the design and implementation of a vaccine or other form of therapeutic.
Structure of TbpB
Structure of TbpB

Moraes et al. Mol Cell 2009
How does TbpB bind Transferrin?

[Diagram of protein structure and transferrin binding]
Variable Regions on the N-lobe surface of TbpBs
TbpB N-lobes Vary in Structure

A  Cap area  

B  Cap area  

C  Cap area  

ApH49 TbpB  

ApH87 TbpB  

AsH57 TbpB  

Calmettes et al. JBC 2011
Pull-down and ITC illustrates TbpB N-lobe binds transferrin

Protein Input

Affinity Capture

Binding Assay

TbpB + Tf

N-lobe + Tf

Kd = 44nM

Kd = 35nM
What Common Features Reside in the Variable TbpB N-lobes?

Common binding elements:
(i) Positively charged surface
(ii) Solvent exposed hydrophobic residues

Calmettes et al. JBC 2011
Targeted Mutagenesis and Binding Assays provide a more Accurate Model of TbpB-Transferrin

1. Made mutants of surface residues within N-lobe Cap region - measured affinities by SPR

Calmettes et al. JBC 2011
TbpB-Transferrin Model

Model Generation
1. Defined binding determinants by side-directed mutagenesis and SPR.

2. Rosetta docked models using residues as constraints.

3. Validated the model with mutagenesis on pTf and by Mass Spectrometry

Silva et al. JBC 2011  Calmettes et al. JBC 2011
TbpB-Transferrin Model

Model Generation
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Silva et al. JBC 2011  Calmettes et al. JBC 2011
Despite sequence and structural variation, TbpBs bind transferrin through a conserved mechanism.
Confirmation of Model via the Co-crystal Structure of TbpB bound to Transferrin
Analyzing TbpB Point Mutants by Biolayer Interferometry

Calmettes et al. Submitted Infection & immunity
## Comparison of ITC, SPR, and BLI data

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mutation</th>
<th>Loop</th>
<th>Kd</th>
<th>Method</th>
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<td>ITC</td>
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<td>NDB</td>
<td>BLI</td>
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</table>

TBD – To Be Determined  
NDB – No Detectable Binding  
ITC – isothermal titration calorimetry  
SPR – surface plasmon resonances  
BLI – biolayer interferometry
BLI for Ongoing Analysis of TbpB-Transferrin interactions

• Point mutations and biomolecular interaction data provide us information for docking /interaction surfaces

• NEEDED COST EFFECTIVE METHOD TO EVALUATE BINDING-BLI
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