# Design of an electrochemical biosensor for detecting P.69 pertactin associated with *B. pertussis*

Marissa Allen and Andrew J. Bonham, Department of Chemistry and Biochemistry

Metropolitan State University of Denver

Metropolitan State University of Denver
Department of Chemistry and Biochemistry

## Introduction

Whopping cough caused by *Bordetella pertussis* can cause serious and prolonged health affects in people of all ages but is especially deadly in infants. We are developing an aptamer-based electrochemical biosensor for the detection of P.69 pertactin, a well-known adhesion factor present on the outside of *B. pertussis.* A biosensor that detects this protein can therefore be used to diagnose *B. pertussis* infections more rapidly and accurately than current testing standards.

## P.69 Pertactin

Figure 1. A 2d rendering of P.69 pertactin courtesy of Protein Data Bank. Molecular weight of P.69 pertactin: 69,000 g/mol. Length: 910 aa.

## Methods

Thus far we have obtained purified P.69 pertactin and biotinylated the protein: incorporating a covalently attached biotin molecule through reaction with primary amino groups present on P.69 pertactin. Biotinylation results were confirmed via HABA Assay. Currently we are continuing our development of an electrochemical biosensor through SELEX, the systematic evolution of ligands by exponential enrichment. SELEX is a process by which unique DNA sequences called aptamers are introduced to target molecules and screened for selective binding to the target, in our case P.69 pertactin.

## Results

### Biotinylation and HABA ASSAY

Figure 2. Chemical reaction diagram of protein biotinylation courtesy of Thermo Scientific. In this case the Sulfo-NHS is the leaving group and then the target protein attaches via a primary amine making a covalently bonded biotinylated molecule with a stable amide.

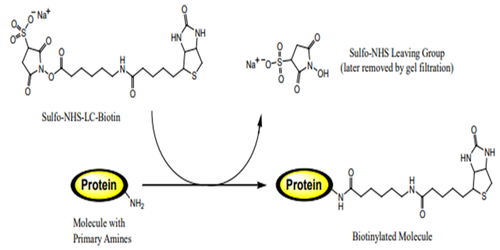
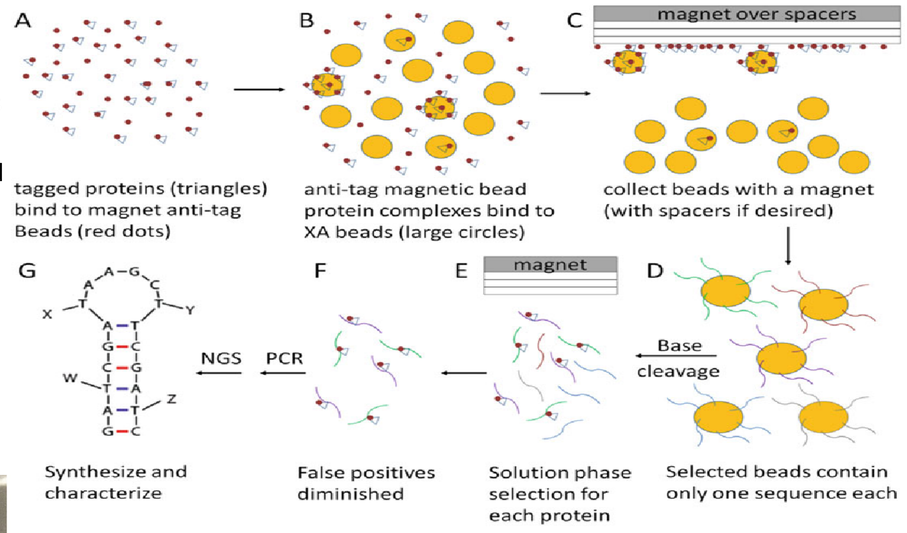



Figure 3. Formula used to calculate the average of 42 biotin molecules attached to each P.69 pertactin protein.

$$\frac{\left(\frac{mmol\ biotin}{mL\ reaction\ mixture}\right)(10)(dilution\ factor)}{mmol\ protein\ per\ mL} = mmol\ biotin/mmol\ protein$$

### Selex and PCR

Figure 4. Diagram of the SELEX process showing biotinylated molecules binding to magnetic beads, aptamers are then introduced and selectively bind to the protein. PCR is performed to amplify aptamer sequences that are complimentary to the target protein and then aptamers are sent for next-generation sequencing.



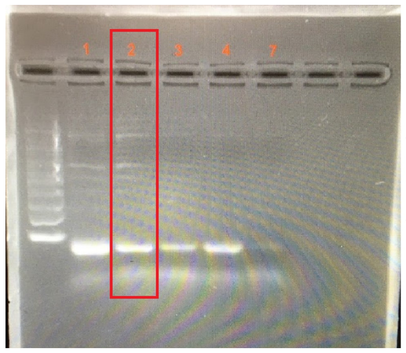
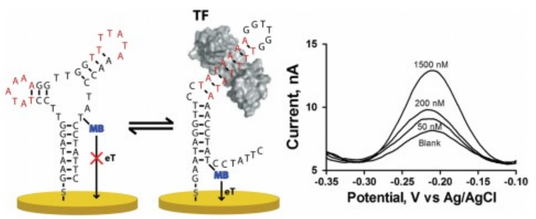




Figure 4. PCR results.

The leftmost column shows the DNA ladder used as a reference to measure the weight/length of DNA molecules. Column 1: positive control, Column 2: P.69 pertactin aptamer, Column 3 & 4: samples for other experiments in the Bonham lab, Column 7: negative control.

## E-DNA Biosensor Example

Figure 5. E-DNA biosensors use small sequences of DNA that can adopt 2 different folding conformations to detect the presence of a target molecule. The target molecule, P.69 pertactin, will only bind to one folder DNA conformation of the aptamer, first identified via SELEX and next-generation sequencing. Our lab will modify the DNA sequence to also fold into a non-binding sequence. The modified DNA sequence is then attached to a gold electrode system, and binding of the target cause it to adopt the binding conformation. A potentiostat is used to detect changes in electrical current caused by this conformational change when there is binding to the aptamer by the target protein, allowing sensitive detection of target. Image is strictly an example and not an illustration of the actual DNA sequences for P.69 pertactin.




## Conclusion

After identifying and obtaining purified P.69 pertactin , we were able to biotinylate the protein and confirmed success of that procedure via HABA assay. This process laid the ground work for the next process SELEX. In the Selex process the biotinylated protein has aptamers introduced and a specific aptamer binds to the protein.

We were able identify successful binding of an aptamer to P.69 pertactin, which will be characterized by next-generation sequencing. We will then utilize that sequence to construct an electrochemical, DNA aptamer-based biosensor specific to P.69 pertactin.

## Literature Cited


- Emsley, P., Charles, I. G., Fairweather, N. F., & Isaacs, N. W. (1996). Structure of bordetella pertussis virulence factor P.69 pertactin. *Nature, 381*(6577), 90-2. doi:http://dx.doi.org.aurarialibrary.idm.oclc.org/10.1038/381090a0
- Melvin JA, Scheller EV, Miller JF, Cotter PA. Bordetella pertussis pathogenesis: current and future challenges. Nature reviews. Microbiology. 2014 Apr;12(4):274-288. DOI: 10.1038/nrmicro3235.
- Leininger, E., et al. "Pertactin, an Arg-Gly-Asp-Containing Bordetella Pertussis Surface Protein That Promotes Adherence of Mammalian Cells." *Proceedings of the National Academy of Sciences*, vol. 88, no. 2, 1991, pp. 345–349., doi:10.1073/pnas.88.2.345.
- Bonham, Andrew J., et al. "Quantification of Transcription Factor Binding in Cell Extracts Using an Electrochemical, Structure-Switching Biosensor." *Journal of the American Chemical Society*, vol. 134, no. 7, 2012, pp. 3346–3348., doi:10.1021/ja2115663.
- Lokesh GL, Wang H, Lam CH, Thiviyanathan V, Ward N, Gorenstein DG, Volk DE. X-Aptamer Selection and Validation. Methods Mol Biol. 2017;1632:151-174. doi: 10.1007/978-1-4939-7138-1_10. PMID: 28730438.
- Bank, RCSB Protein Data. "1DAB: The Structure of Bordetella Pertussis Virulence Factor P.69 Pertactin." *RCSB PDB*, www.rcsb.org/structure/1dab.
- Thermo Scientific, EZ-Link Sulfo-NHS –LC Biotinylation Kit, https://assets.fishersci.com/TFS-Assets/LSG/manuals/MAN0011567_EZ_Micro_Sulfo_NHS_Biotinylation_UG.pdf
- LI, J., et al. "Cloning, Nucleotide Sequence and Heterologous Expression of the Protective Outer-Membrane Protein P.68 Pertactin from Bordetella Bronchiseptica." *Journal of General Microbiology*, vol. 138, no. 8, 1992, pp. 1697–1705., doi:10.1099/00221287-138-8-1697.


## Acknowledgements

This research would not be possible with out support from the MSU Denver Department of Chemistry and Biochemistry, and grant support from the MSU Denver Undergraduate Research Program. A special thank you to Dylan Poch and Andrew Smith for their help and support during this process.

## Contact

Dr. Andrew J. Bonham
Metropolitan State University of Denver

Email: abonham@msudenver.edu
Website: http://bonhamlab.com
Phone: 303615-0278