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The Role of Glycosylation in the Bactericidal Pore Forming Protein Perforin-2

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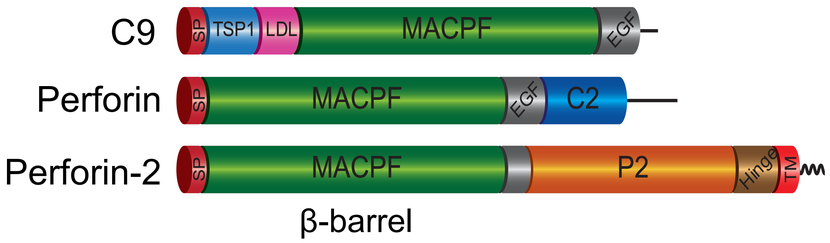
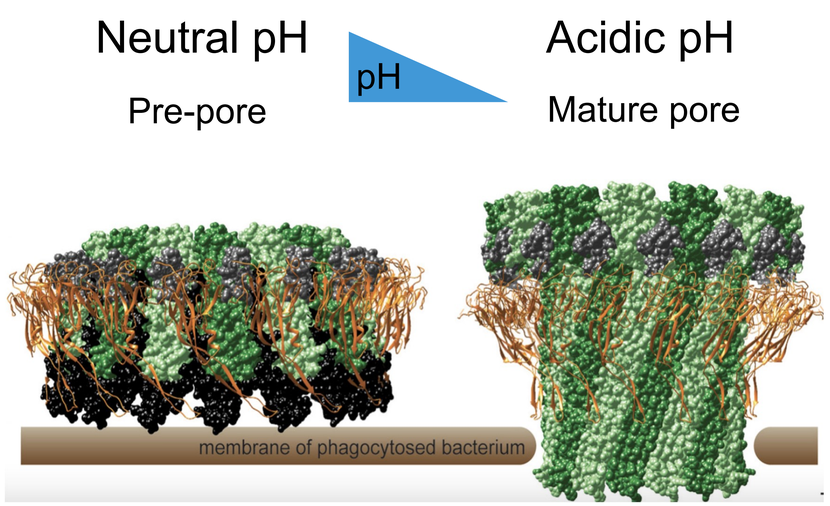
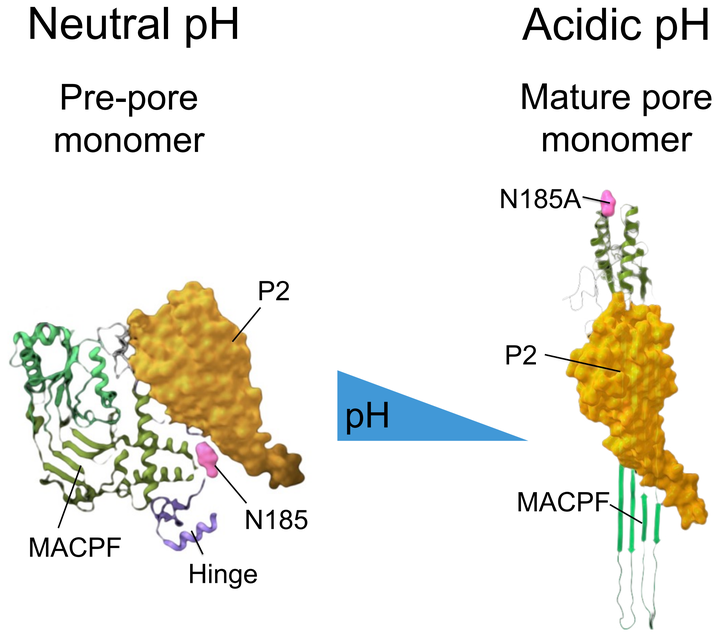
Introduction



- Perforin-2 (P2) is a member of a family of immune proteins that employs it MACPF domain to form a β-barrel pore in bacterial membranes
- The P2 gene, *Mpeg1*, is evolutionarily ancient and found in early metazoan phyla such as Porifera and Cnidaria[1]
- P2 is constitutively expressed in innate immune cells such as macrophages where it co-localizes in the phagosome with phagocytized bacteria



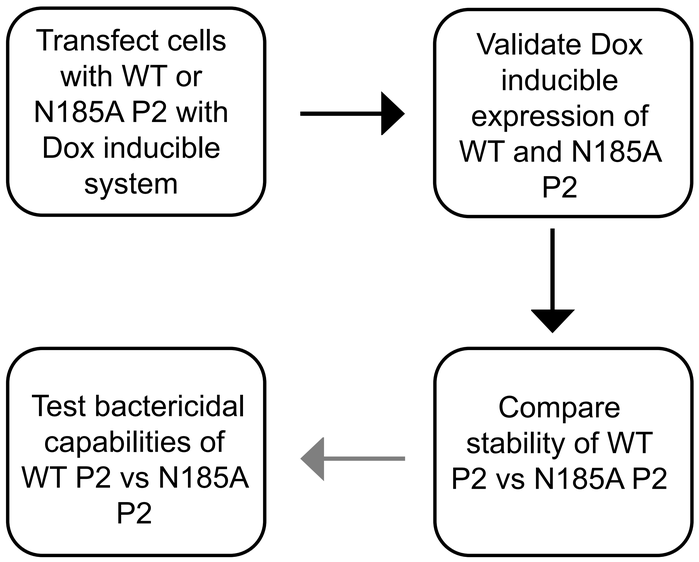
- Cyro-EM analysis has revealed P2 exists in two conformations, a pre-pore and a mature pore
- The transition from pre-pore to pore results from a rotational reorganization of the MACPF that increases the length of the pore and can cross the bacterial membrane[1]
- This transition is triggered by acidification - a hallmark of phagosomes- and provides a channel for osmotic imbalance and/or chemical destruction of the target



- The transition of the MACPF domain occurs around a rotational axis known as the hinge[1]
- The tip of the MACPF domain is glycosylated at residue N185 and is close proximity to the hinge

Hypothesis: The N185 glycan prevents rotation of the MACPF domain at neutral pH

Methods



Results

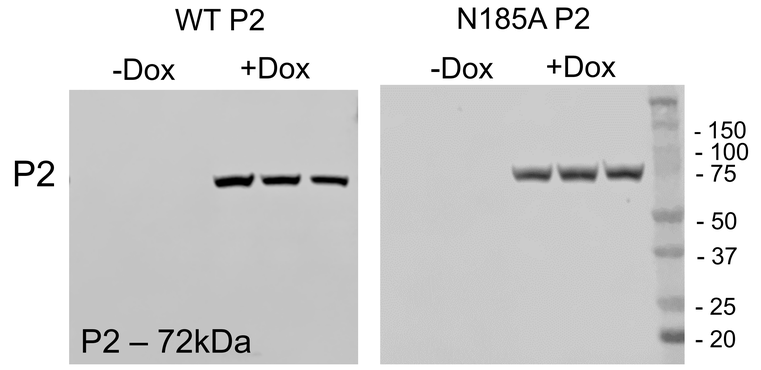


Figure 1: Murine embryonic fibroblasts transfected with either WT P2 or P2 with an N185A mutation are both doxycycline (Dox) inducible and stably expressed

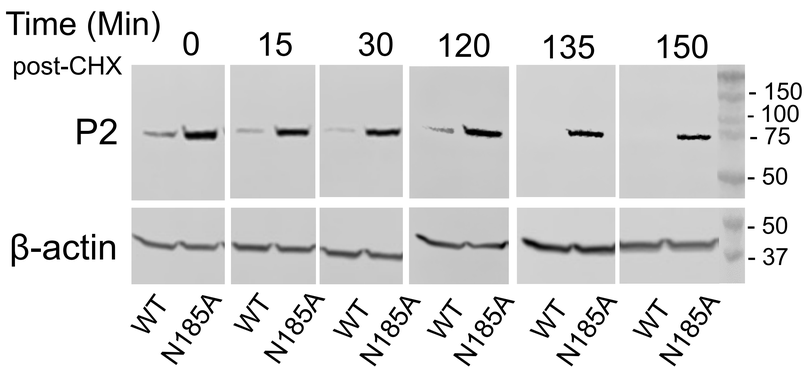


Figure 2: De novo translation of WT and N185A P2 was inhibited with cycloheximide (CHX) addition. WT P2 is not detected after 120 minutes. In contrast P2 with the N185A mutation is detected throughout the experiment.

Discussion

- Acidification of the phagosome may disrupt electrostatic interactions between the N185 glycan and the hinge allowing rotation to occur
- Does P2 with the N185A mutation have the same functional capabilities as WT P2?

Acknowledgements: Thank you to Dr George P Munson for supporting and oversight of this research. Thank you to PhD candidates Zachary P Rivas and Kacey M. Talbot for their guidance and support in and outside of the laboratory.
Funding: NIH/NIAID R01A1110810, R21AI128164, R21AI156567, R21AI159794

References: [1]Merselis LC, Rivas ZP and Munson GP (2021) Breaching the Bacterial Envelope: The Pivotal Role of Perforin-2 (MPEG1) Within Phagocytes. *Front. Immunol.* 12:597951. doi: 10.3389/fimmu.2021.597951