Determining the structure and catalytic mechanism of the membrane protein PagP using novel isotope labeling strategies

One limitation of solution NMR is that rapid signal decay worsens with larger systems. This can be partially circumvented through selective deuteration as well as with transverse relaxation-optimized (TROSY) spectroscopy. We are developing cost-effective methods to selectively deuterate protein amino acids by exploiting the biosynthetic pathways of E. coli.

The labeling methods we develop will be used to produce a high-resolution NMR structure of the membrane protein, PagP. A high-resolution structure is needed to determine the catalytic mechanism of PagP. PagP catalyzes the palmitoylation of lipopolysaccharide (LPS), the major lipid component of the Gram-negative bacterial outer membrane. The modification alters the immunogenicity of LPS, a major culprit molecule in precipitating septic shock. Moreover, the palmitoylation of LPS also confers resistance to antimicrobial peptides.

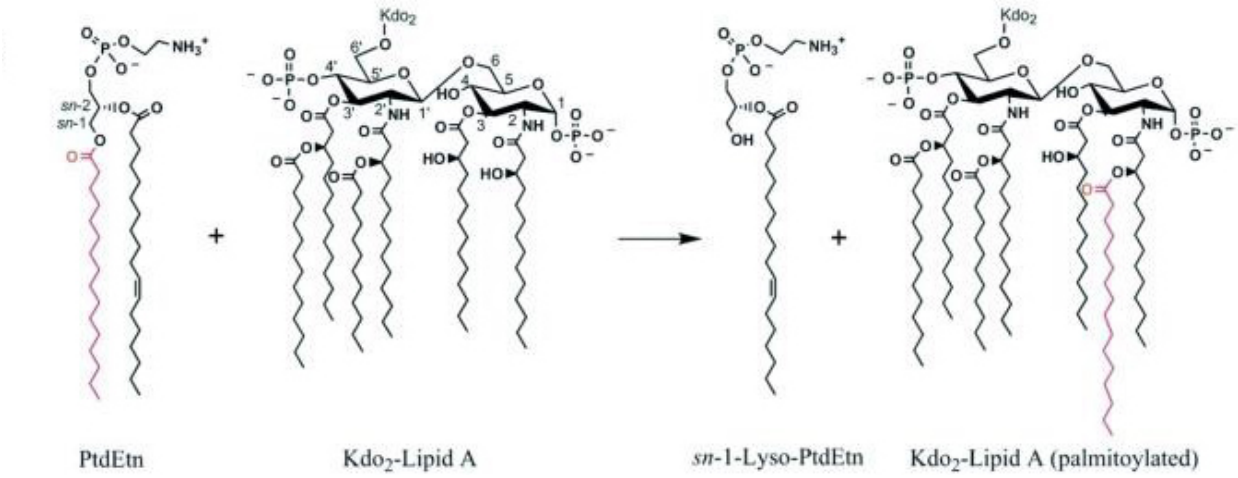

Figure 1. PagP catalyzes the palmitoylation of lipid A or LPS.

Figure reproduced from Hwang PM, Choy WY, Lo EI, Chen L, Forman-Kay JD, Raetz CR, Prive GG, Bishop RE, and Kay LE. 2002. Solution structure and dynamics of the outer membrane enzyme PagP by NMR. *Proc. Natl. Acad. Sci. USA* **99(21)**, 13560-13565

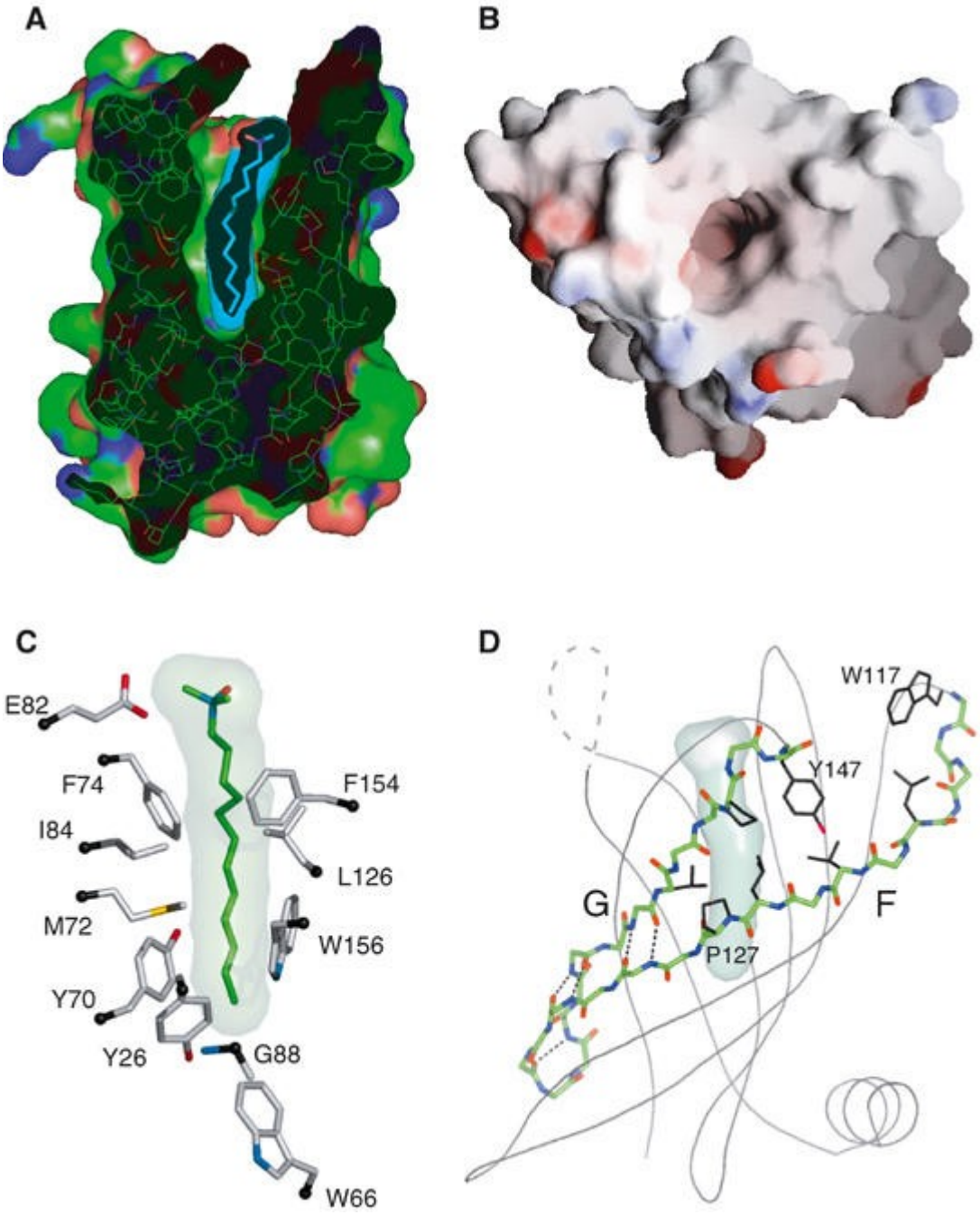

Figure 2. The X-ray crystal structure of PagP in LDAO detergent micelles shows an LDAO molecule occupying the substrate phospholipid binding site.

Figure reproduced from Ahn VE, Lo EI, Engel CK, Chen L, Hwang PM, Kay LE, Bishop RE, and Privé GG. 2004. A hydrocarbon ruler measures palmitate in the enzymatic acylation of endotoxin. *EMBO J.* **23(15)**, 2931-2941

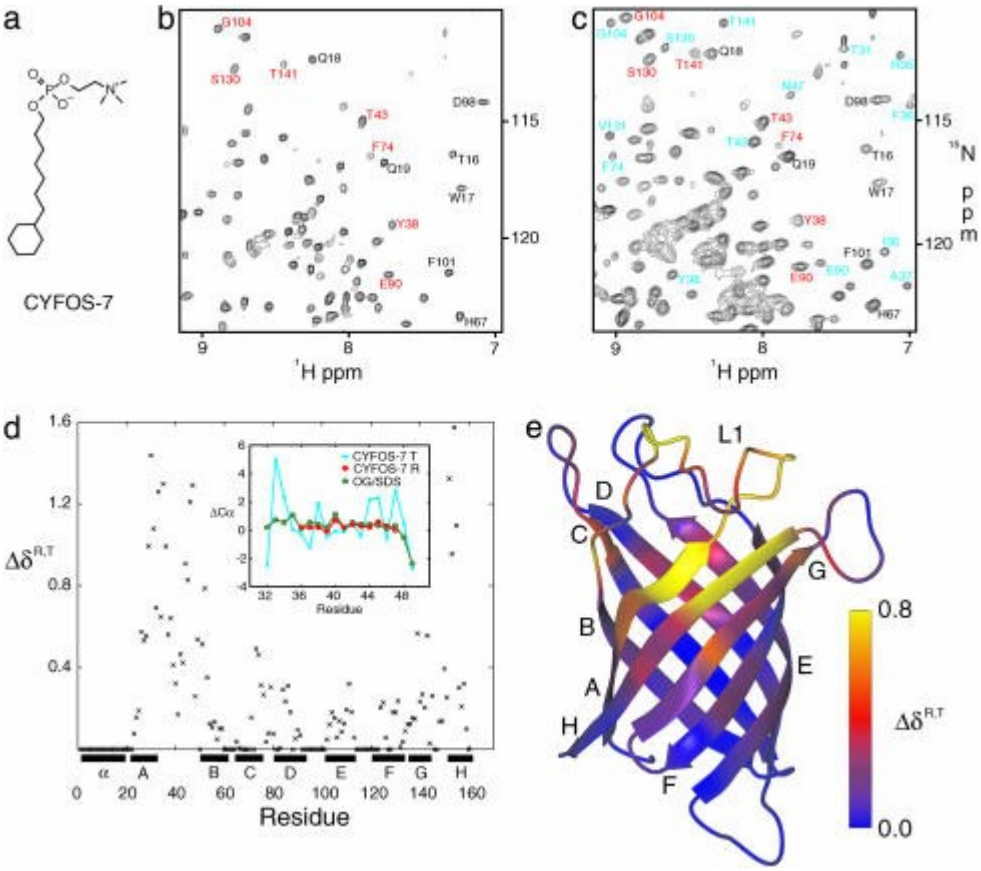

Figure 3. NMR studies of enzymatically active PagP in CYFOS-7 detergent reveals that PagP alternates between two conformations. The relaxed "R" state is more mobile and facilitates substrate entry to the barrel interior. The tense "T" state is highly structured and is believed to form the catalytic active site.

Figure reproduced from Hwang PM, Bishop RE, and Kay LE. 2004. The integral membrane enzyme PagP alternates between two dynamically distinct states. *Proc. Natl. Acad. Sci. USA.* **101(26)**, 9618-9623.