Site-Directed Spin Labeling Studies of Protein–Membrane Interactions: Activation of the Bacterial Phospholipase, ExoU

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Site-directed spin labeling (SDSL) in conjunction with electron paramagnetic resonance (EPR) spectroscopy is a powerful approach for structural analysis of proteins under physiological conditions. SDSL is applicable to proteins that are too large for analysis by nuclear magnetic resonance or that fail to crystallize, and to proteins incorporated into large macromolecular complexes, embedded in a lipid bilayer, or associated with the membrane surface. We are currently using SDSL to investigate the membrane association and protein-protein interactions of ExoU, a secreted phospholipase that plays a key role in infections by the opportunistic pathogen, *Pseudomonas aeruginosa* [1-3]. ExoU is activated by noncovalent interaction with the small eukaryotic accessory protein, ubiquitin [2]. Ubiquitin binding facilitates ExoU association with the membrane, leading to phospholipid hydrolysis, disruption of the lipid bilayer, and ultimately cell death. Using SDSL, we have uncovered one of the mechanisms used by ExoU to bind to the surface of the target membrane [3] and identified the primary ubiquitin binding site. These studies may lead to the development of peptides or small molecules that block the activation of ExoU as a novel approach to preventing tissue damage and sepsis arising from infection by *P. aeruginosa*.

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