

A STUDY OF SECA, THE MOTOR OF THE BACTERIAL SECRETION SYSTEM,
BY SITE-DIRECTED SPIN LABELING AND EPR

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ABSTRACT

For more than 3 decades, the biochemical details of the Sec system of protein export have been teased out of *Escherichia coli*. Through these efforts we know that SecA, the ATPase of the Sec system, interacts with several entities: unfolded precursor polypeptides, the molecular chaperone SecB, membrane phospholipids, and the membrane-embedded translocase SecYEG. Functional studies implied that some of these interactions occurred simultaneously. However, little is known about the details of these binding sites, and it was not clear to what extent SecA interacted with these diverse ligands at distinct, adjacent or overlapping surfaces along its extended structure.

We investigated these issues using electron paramagnetic resonance (EPR) spectroscopy and site-directed spin labels at a multitude of sites on the surface of the 102 kDa SecA protein. EPR spectroscopy is sensitive to changes in the local environment of the spin label, and thus our survey of the SecA surface provided a map of interaction sites with its partners in the Sec pathway of protein export. Strikingly, we found that SecA utilizes a single interactive surface to bind its multiple partners during protein export.

Knowing the locations and relationships of these binding sites on the surface of SecA represents significant progress in revealing the mechanistic steps of passing an unfolded polypeptide chain from SecB to SecA and ultimately driving it through the secretion pore.