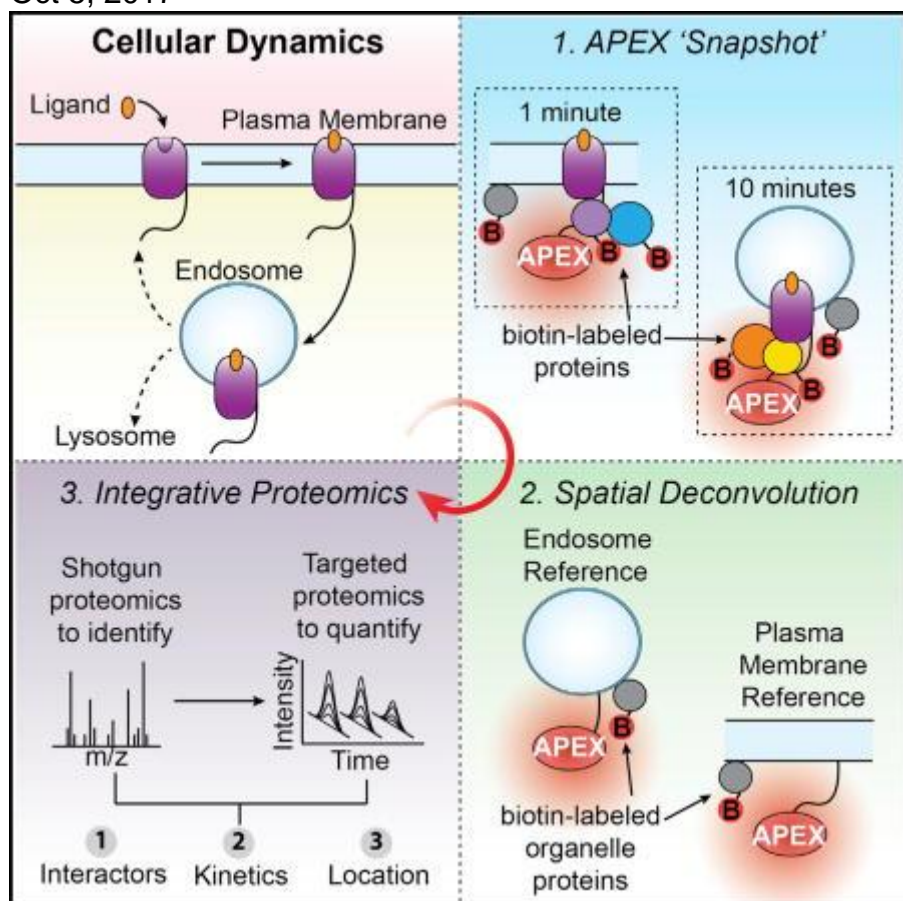


An Approach to Spatiotemporally Resolve Protein Interaction Networks in Living Cells

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Cells operate through protein interaction networks organized in space and time. Here, we describe an approach to resolve both dimensions simultaneously by using proximity labeling mediated by engineered ascorbic acid peroxidase (APEX). APEX has been used to capture entire organelle proteomes with high temporal resolution, but its breadth of labeling is generally thought to preclude the higher spatial resolution necessary to interrogate specific protein networks. We provide a solution to this problem by combining quantitative proteomics with a system of spatial references. As proof of principle, we apply this approach to interrogate proteins engaged by G-protein-coupled receptors as they dynamically signal and traffic in response to ligand-induced activation. The method resolves known binding partners, as well as previously unidentified network components. Validating its utility as a discovery pipeline, we establish that two of these proteins promote ubiquitin-linked receptor downregulation after prolonged activation.

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[1] <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5616215/>