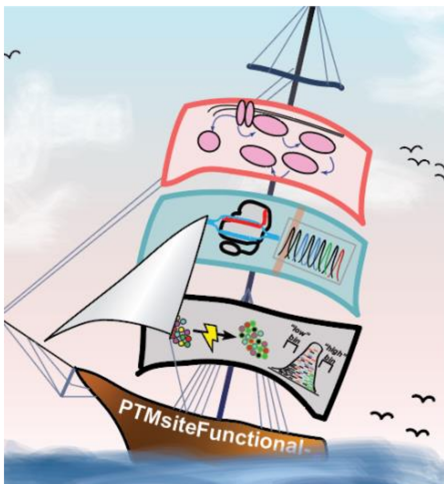
Dredging function from the depths of the phosphoproteome.

Fastening together post-translational modification site-centric base editing with phenotypic screens raises the anchor that has moored the functional assessment of phosphorylation sites with contemporary throughput, enabling the study of expansive signaling networks at a speed abreast with functional genomics.



The problem

Post-translational modifications (PTMs), particularly protein phosphorylation, are at the helm of nearly every process in eukaryotic cells. Modern mass spectrometry has revealed that signalling pathways form vast and intricate biochemical circuits, comprised of tens of thousands of dynamically regulated phosphorylation sites (phosphosites). Unfortunately, our functional understanding of the overwhelming majority of these sites has remained elusive[1]. This is largely due to the lack of technologies to causally link phosphorylation events to their downstream functions. Thus, we often describe incredibly complex signalling systems through a handful of flagship biochemical events. Understanding individual phosphosite function within the phosphoproteome, with a resolution commensurate to its complexity, is pivotal to elucidate the precise molecular mechanisms underlying essential cellular functions in health and disease.

The solution

We present an experimental and computational framework for investigating the functional atlas of PTM sites in high-throughput. We combined quantitative phosphoproteomics, "proteome-wide" base editing of individual phosphosites, and phenotypic screens, to systematically assess a vast number of previously unexplored phosphorylation events with respect to their distinct influences on cell survival or proliferations, and transcriptional responses (**Figure 1**). Analogous to CRISPR-Cas knock out screens, individual cells receive single and specific mutations, amongst a sea of engineered mutations, and are enriched or depleted within specific phenotypes of interest. Here, instead of knocking out an entire gene, CRISPR-mediated base editors mutate codons of endogenous genes to amino acids that cannot be phosphorylated[2].

By coupling PTM-centric base editor screens to transcriptional reporters, we uncovered new kinase activities and linked hundreds of unstudied phosphorylation events to regulation of NFAT transcriptional responses downstream of T cell activation. Because the results from PTM-centric base editors screens are often immediately interpretable, our approach allowed us to define a distinct phosphosite on the Ser/Thr phosphatase PHLPP1 as a novel regulator of NFAT and NFκB activities during T cell activation. Transcriptomic analysis of various phosphosite mutant T cells demonstrated subtle but significant changes in the expression of genes in the T cell transcriptional response, charting a possible course for the eventual mapping of individual phosphorylation events and their contribution to the expression of specific genes. PTM-centric base editor screens offer a framework for functionally exploring the intricate network of biochemical signalling events and systematically deciphering their functions.

Future directions

With the increasing sensitivity and robustness of mass spectrometry-based approaches to study post-translational modifications proteome-wide, the approaches presented in this study will empower the research community. These tools offer the capability to establish the *functional significance* of novel signalling events, reducing the risk of being marooned with a costly site-specific antibody or mouse model that proves to be irrelevant. As the cell signalling field grapples more and more with the system's complexity, our methodology will provide a valuable framework for comprehensive exploration. This will allow researchers to delve into previously uncharted territories and gain a deeper understanding of intricate signalling networks in a variety of biological contexts or phenotypes of interest.

Our PTM-centric base editor screen workflow was designed to be widely applicable and easily adopted by other crews, using infrastructure available at most research institutions. Other PTMs such as acetylation/ubiquitination (Lys), O-GlcNAcylation (Ser/Thr), or site-specific proteolysis (e.g. caspases) should all be amenable to this approach. As should other model systems such as post-mitotic and primary cells.

In our laboratory, we see this as the final rigging to launch the “Measure-Model-Predict-*and now*-Perturb” voyages that propel science forward, now amenable for PTMs. Traditional approaches have shown us the importance and expansiveness of PTMs, but technological limitations have left us with an incomplete survey of signalling pathways. Just as Jacques Cousteau said, "The ocean, once it casts its spell, holds one in its net of wonder forever," so too does the phosphoproteome. With PTM site-centric base editing coupled to phenotypic screens, we can navigate the functional landscape of PTM sites with the power comparable to that of GPS rather than a compass.

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