cells as well as the barcoding substrate (for example, beads or hydrogels). Bues et al. now design, develop and implement a sophisticated droplet-microfluidics system that enables a user to determine when and which cells should be co-encapsulated and subsequently processed, either manually or using quantitative parameters. The technique, dubbed DisCo (deterministic, mRNA-capture bead and cell co-encapsulation), includes an imaging module such that the co-flow of a cell and barcoding bead into the same droplet can be actively observed and assessed, stimulating a decisive action to include the droplet into or exclude it from downstream pipelines. In this method, two particles are stopped at the encapsulation site; the two particles can be ejected into one droplet, and the droplet can be selectively extracted into a sample channel. The power of this approach is that most of the cells within a single small specimen could, in principle, be captured, selected and analyzed.

The authors applied this methodological innovation to explore cell heterogeneity of individual mouse intestinal organoids (Fig. 1). These intestinal organoids are derived from adult stem cells that reside within the crypt of the small intestine. Once isolated, each stem cell can give rise to a complex epithelial structure composed of diverse cell types, including nutrient-absorptive enterocytes, hormone-secreting enteroendocrine cells and mucous-secreting goblet cells[6]. Previous work showed that the composition of the cell types within individual organoids can vary, and this variability can be extracted through image-based methods and used to resolve quantitative phenotypic landscapes from hundreds of thousands of mouse intestinal organoids[7]. Image-based phenotypic screening allows sampling a large number of objects at high spatial resolution; it is, however, currently limited in the number of features that can be extracted from the same specimen. In a study by Lukonin et al.[7], phenotypic classification was based on the measurement of molecular features, including markers of absorptive (enterocyte) and secretory (Paneth cell) lineages as well as their spatial arrangements. Researchers observed a palette of phenotypes, including organoids called 'enterocysts' consisting exclusively of cells of absorptive lineage. Bues et al., using single-organoid sequencing, were able to observe the phenotypes seen in image-based screening, but also to detect the presence of organoids composed entirely of mucus-producing goblet cells ('gobloids'), marked by expression of mucin. This gene was not among the readouts of the phenotypic screen, and hence the phenotype could not be captured, highlighting the importance of the depth of profiling enabled by sequencing.

The study by Bues et al. paves a way for addressing many questions in organoid biology; however, there is still a tradeoff between depth and throughput, sampling and determinism, in the measurement of phenotypic landscapes. Questions remain around how easily the current DisCo technology can be distributed and become adopted by other laboratories. Also, cell loss during the generation of a single-cell suspension and during transfer and capture within the device remains a concern when working with small samples and aiming to sequence all cells in a population. DisCo in combination with imaging would, however, help bridge the gap by giving the ability to deterministically profile organoids selected by imaging a larger pool, thus dissecting individual phenotypes and avoiding readout bias.

We envision a future where methods for deterministic single-cell sequencing, together with single-cell-resolved and multimodal imaging approaches, will be available to researchers so they may understand the diverse and dynamic phenotypic landscapes prevalent in each and every biological nook, no matter how small it is. ❐

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References
1. Hellman, G. *Philos. Sci.* **58**, 132 (1991).
2. Bues, J. et al. *Nat. Methods* https://doi.org/10.1038/s41592-021-01391-1 (2022).
3. Klein, A. M. et al. *Cell* **161**, 1187–1201 (2015).
4. Macosko, E. Z. et al. *Cell* **161**, 1202–1214 (2015).
5. Bioč anin, M., Bues, J., Dainese, R., Amstad, E. & Deplancke, B. *Lab Chip* **19**, 1610–1620 (2019).
6. Sato, T. et al. *Nature* **459**, 262–265 (2009).
7. Lukonin, I. et al. *Nature* **586**, 275–280 (2020).

Competing interests
J.G.C. and I.L. are employed by F. Hoffmann La Roche.

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PROTEOMICS

Uncovering an overlooked consequence of phosphorylation: change in cysteine reactivity

Global profiling of changes in the reactivity of cysteine residues in response to phosphorylation during mitosis identifies cysteine residues as potential regulatory and drug binding sites on proteins.

Markus Lakemeyer and Matthew Bogyo

Protein phosphorylation, in which a phosphate group is added to a specific amino acid residue on a protein, is arguably the most extensively studied form of post-translational modification. It results in a dramatic change in the physiochemical properties of the modified amino acid, which often leads to a change in its affinity for specific domains found on other proteins or within the phosphorylated protein itself. A large percentage of known signaling events involve transmission of a signal through phosphorylation-induced changes in protein structure or interactions with other proteins

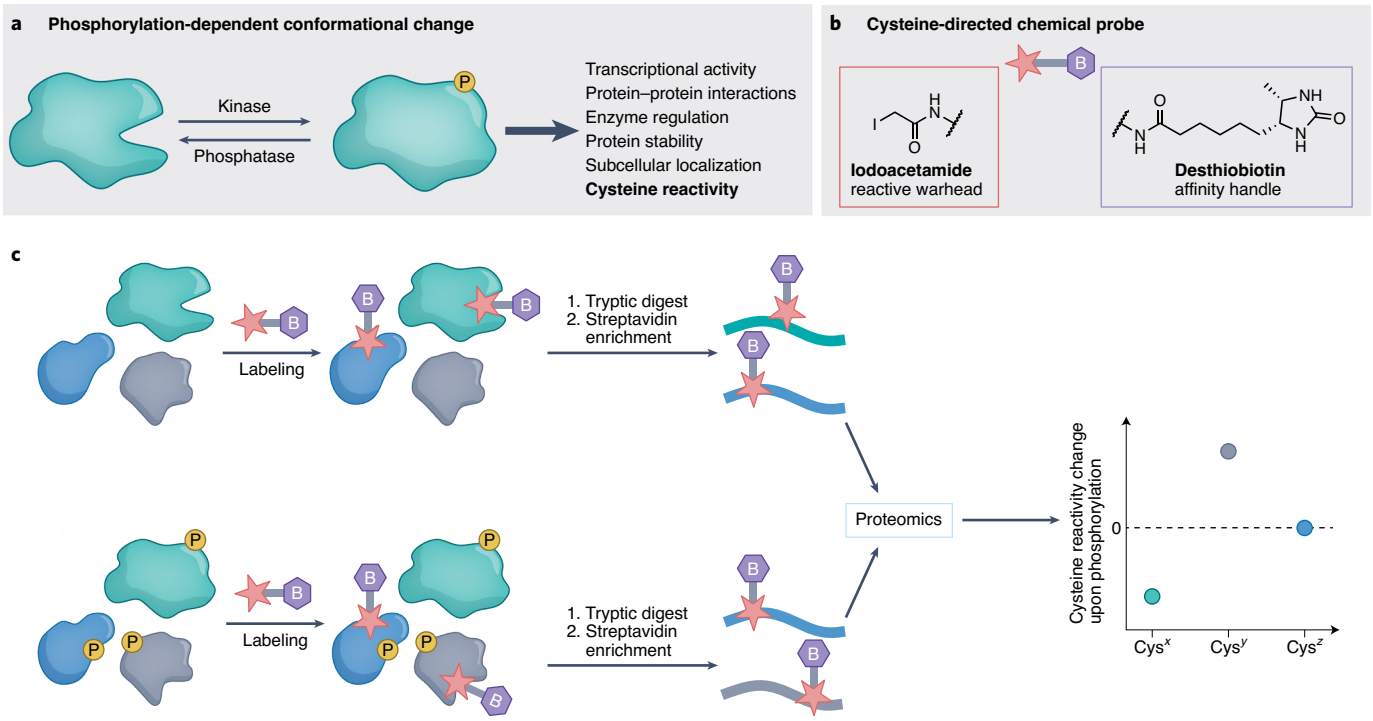

Fig. 1 | Chemical profiling of changes in cysteine reactivity upon protein phosphorylation. a, Overview of phosphorylation-mediated protein functions. **b**, The cysteine-reactive probe used and chemical structures of the reactive warhead and affinity handle. **c**, The chemoproteomic workflow applied by Kemper et al. to identify cysteine residues with altered reactivity upon protein phosphorylation by comparing cell lysates of asynchronous (top) versus mitotic (bottom) cells, or mitotic cell lysate with (top) versus without (bottom) global dephosphorylation by lambda phosphatase.

to propagate a signaling cascade (Fig. 1a). But what if phosphorylation has other, less well-understood impacts on protein structure and function? For example, how does the addition of a highly negatively charged phosphate group on a protein affect not just the shape and fold of the protein but the chemical environment of other amino acids in the vicinity of this site?

One amino acid residue in particular, cysteine, has garnered attention owing to its generally high level of chemical reactivity and its potential to be a key regulator of protein structure and function. Recent work using cysteine-reactive chemical labels has enabled global studies to quantify reactivity of all cysteine residues within complex proteomic samples[1–3]. This reactivity profiling method has highlighted the importance of cysteine residues for protein function, as well as helped to correlate the overall chemical reactivity of a given cysteine residue with its likelihood of having regulatory functions in that protein. Any type of protein modification that modulates the chemical or structural microenvironment surrounding a cysteine residue has the potential to alter the function of that protein. Given that phosphorylation is a common and dynamic protein modification that results in a substantial change of charge state and electronegativity of a defined amino acid residue, it may influence the chemical properties of cysteine residues nearby.

In this issue of *Nature Methods*, Kemper et al[4]. perform global proteomic profiling studies that catalog the impact of serine/threonine phosphorylation on cysteine reactivity. The work builds on a foundation of studies using probes that contain a cysteine-specific reactive electrophile (for example, iodoacetamide) and an affinity handle (for example, biotin or desthiobiotin) for enrichment of covalently labeled cysteine residues (Fig. 1b). The individual reactive sites can be resolved in proteomic workflows, often using isotopic labeling strategies that allow sample multiplexing and relative quantification[1,2,5]. By labeling cysteines in a proteomic sample at low and high concentrations of the probe, it is possible to get a relative measure of the reactivity of each cysteine. In previous applications, this method has been used to identify potential functional hotspots on proteins without prior knowledge of their functions, as well as to identify specific ligandable sites on proteins. It also allows global assessment of changes in reactivity upon induction of a specific stimulus or during a defined biological process. Because the method enriches and identifies specific cysteine residues within a known protein sequence, it is ideally suited to measure changes in a cysteine residue's reactivity in response to specific phosphorylation events and then mapping its position relative to the phosphorylation site(s). Kemper et al. focused on changes in cysteine reactivity that occur during cell division. This is a logical starting place for the application of the technology as there are over 30,000 phosphosites that have been identified during mitosis, with many being nearly quantitatively converted to the phosphorylated state[6].

To accomplish the analysis of mitosis-specific changes in cysteine reactivity, the authors compared highly synchronous cells in mitosis to asynchronous cells. They first performed labeling with the cysteine reactivity probe to produce an initial list of cysteines whose activity was increased or decreased during initiation of the cell cycle (Fig. 1c). One of the keys to the study is the use of the general dephosphorylating enzyme lambda phosphatase to globally remove phosphates from proteins while maintaining their folded states. This allowed filtering for cysteine reactivity changes that were the direct result of serine/threonine phosphorylation

events and not due to, for example, changes in protein abundance. However, for this approach to provide relevant information, it must take into consideration many possible artifacts, including changes in the recovery and ionization of tryptic peptides that contain a cysteine and a phosphorylation site on the same peptide. To control for these issues, the authors developed a protocol in which samples were treated with lambda phosphatase both before and after labeling with the probe for cysteine reactivity. This allowed them to exclude artifacts and identify cysteines whose altered reactivity is a direct result of phosphorylation.

The Kemper study provides both a useful new method and a valuable dataset for further analysis. The results strongly support the conclusion that cysteine reactivity is in fact altered in many proteins when they become phosphorylated. This change can result from direct changes to the chemical microenvironment near the cysteine or could result from structural rearrangements induced by allosteric phosphorylation events that are far from the cysteine in question. The changes can also result in increased or decreased reactivity, and these differences seem to depend on the location of the cysteine residue in either folded or disordered sites. Furthermore, because the impact on cysteine reactivity seems to be specific to certain residues and not others, even within the same protein, the results suggests that the changes may have implications in our understanding of how phosphorylation regulates protein function.

Perhaps the bigger question is: what is the consequence of phosphorylation-induced changes in cysteine reactivity? Are these changes important for protein function or are they simply a downstream consequence of the phosphorylation event? These questions can only be answered by careful experimental studies of specific cysteines for which phosphorylation impacts their reactivity, something that is now possible thanks to the Kemper et al. dataset. In addition, it will be interesting to determine whether other types of post-translational modifications such as acylation, methylation or glycosylation can similarly affect cysteine (or other amino acid) reactivity to regulate protein structure and function. Regardless of whether the changes in cysteine reactivity have defined biological consequences, it is clear that identification of distinct proteoforms defined by their reactivity state of specific cysteine residues has the potential to open up a new direction in covalent drug targeting by cysteine-reactive ligands. It is not difficult to envision the value of a therapeutic agent that only targets proteins during the process of cell division. The results reported by Kemper et al. give us the first inkling that this type of proteoform-specific targeting may be possible. ❐

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References

1. Vinogradova, E. V. et al. *Cell* **182**, 1009–1026.e29 (2020).
2. Backus, K. M. et al. *Nature* **534**, 570–574 (2016).
3. Maurais, A. J. & Weerapana, E. *Curr. Opin. Chem. Biol.* **50**, 29–36 (2019).
4. Kemper, E. K. et al. *Nat. Methods* https://doi.org/10.1038/s41592-022-01398-2 (2022).
5. Zanon, P. R. A., Lewald, L. & Hacker, S. M. *Angew. Chem. Int. Edn Engl.* **59**, 2829–2836 (2020).
6. Sharma, K. et al. *Cell Rep.* **8**, 1583–1594 (2014).

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Competing interests

The authors declare no competing interests.