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A Tale of Two Tyrosines

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Protein phosphorylation is a ubiquitous posttranslational modification used for regulating cellular processes, including DNA replication and repair, cell division, metabolism and cell signaling, and thus also a frequent drug target (1). It occurs when serine, threonine, or tyrosine residues in substrate proteins are phosphorylated by one of over 500 protein kinase enzymes with different substrate specificity (2). Because phosphorylation requires the residues in question to be accessible to the enzyme, it makes sense that kinase recognition motifs are frequently located within solvent-exposed, intrinsically disordered or loop regions (3) where they can be easily added or removed in response to kinase or phosphatase activity. More counterintuitive are the many phosphorylation sites that have been predicted by large-scale bioinformatics analysis to be either partially or completely buried within folded domains (4), many of which have been verified to be functionally significant. This observation leads one to ask first how kinases could access such occluded sites and second, whether their burial may have functional advantages.

An exciting new study in the current issue of *Biophysical Journal* by Henriques and Lindorff-Larsen (5) addresses aspects of both of these questions, as well as raising new ones. The authors use all-atom molecular simulations with enhanced sampling methods to investigate the phosphorylation of the protein p27 bound to the complex between cyclin-dependent kinase 2 (CDK2) and cyclin A. Although p27 in isolation is intrinsically disordered, apon-binding CDK2/cyclin A, it wraps around the complex in a specific, folded structure. The activity of CDK2/cyclin A (a kinase itself) is inhibited by p27 binding, but phosphorylation of p27 at Y88 and Y74 (by BCR-ABL and Src kinases, respectively) relieves the inhibition, leading to cell cycle progression. It is these two tyrosine residues that are buried when p27 is folded and bound. This study showed that unbiased simulations of the unphosphorylated protein remained stable in the native state on a microsecond timescale. However, metadynamics simulations (6), with a parallel bias on several collective variables, revealed a rare "open" state in which Y88 was released from its binding pocket. This finding qualitatively matches with the lowly populated open state inferred from nuclear magnetic resonance and single-molecule Förster resonance energy transfer experiments (7). Transient fluctuations to this open state could allow access to a kinase, providing a plausible answer to how phosphorylation can occur in this case. A similar mechanism has also been suggested for other buried phosphorylation sites (8). Such a transient exposure mechanism also provides one reason why the target tyrosine residues may be buried—it helps to slow the rate of phosphorylation such that CDK2/cyclin A is not too easily activated.

A second, intriguing, consequence of the tyrosines being buried was revealed by running simulations with the phosphorylated Y88 (pY88). As expected, phosphorylation caused pY88 to be expelled from its binding pocket and populate an open state similar to that seen in the metadynamics simulations of the unphosphorylated p27. In addition, however, this open state was then shown to be strongly coupled to the opening of the second residue to be phosphorylated, Y74, facilitating a phenomenon known as "hierarchal phosphorylation" (9). Even though the open state of Y74 was found to be even more unfavorable than that of Y88 in the absence of Y88 phosphorylation, once Y88 was phosphorylated, the open state of Y74 became more populated than the buried state. The simulations also provided a structural mechanism for this cooperativity: the conformational change brought about by phosphorylation of Y88 created strain in an intermolecular β-sheet linking p27 to the CDK2/cyclin A complex, which lowered the free energy for opening of Y74. This positive cooperativity between the two phosphorylation sites

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would be expected to create a sharper switch in the rate of p27 inactivation as a function of BCR-ABL/Src kinase activity, potentially providing an additional functional role for the burial of this pair of tyrosine residues.

Not considered in the discussion so far is any explicit consideration of the kinase itself. The substrate tyrosines need to be accessible, but how much accessibility is sufficient for the substrate sequence to bind to the relevant kinase? Studies of recognition of substrates by kinases have historically focused on peptides binding to the active site cleft, but it is increasingly understood that the context of the cognate sequence also matters (2), in this case that the location of the substrate in an folded structure. Explicitly considering how the substrate dynamics needed to expose the active site is coupled to recognition by kinases would be an interesting avenue for future exploration in this and similar examples. In a trivial "conformational selection" scenario, the kinase recognizes the substrate only when fully exposed, a reasonable mechanism if the population of the substrate in the accessible state is large enough. Alternatively, the kinase may be able to bind weakly to closed or partially open states, facilitating the opening itself. This mechanism could potentially also be used to improve the selectivity of phosphorylation, provided that noncognate kinases did not also facilitate opening. In addition, kinases such as Src and ABL have specific substrate recognition domains (SH3 and SH2) that may bind either the substrate itself or other proteins in complex with the substrate. The availability of more accurate force fields (10) and enhanced sampling methods (6) is making such problems increasingly accessible to all-atom simulations, as the study by Henriques and Lindorff-Larsen nicely demonstrates (5).

REFERENCES

1. Albanese, S. K., D. L. Parton, …, J. D. Chodera. 2018. An open library of human kinase domain constructs for automated bacterial expression. *Biochemistry.* 57:4675–4689.
2. Miller, C. J., and B. E. Turk. 2018. Homing in: mechanisms of substrate targeting by protein kinases. *Trends Biochem. Sci.* 43:380–394.
3. Iakoucheva, L. M., P. Radivojac, …, A. K. Dunker. 2004. The importance of intrinsic disorder for protein phosphorylation. *Nucleic Acids Res.* 32:1037–1049.
4. Mok, J., P. M. Kim, …, B. E. Turk. 2010. Deciphering protein kinase specificity through large-scale analysis of yeast phosphorylation site motifs. *Sci. Signal.* 3:ra12.
5. Henriques, J., and K. Lindorff-Larsen. 2020. Protein dynamics enables phosphorylation of buried residues in Cdk2/Cyclin A-bound p27. *Biophys. J.* 119:2010–2018.
6. Valsson, O., P. Tiwary, and M. Parrinello. 2016. Enhancing important fluctuations: rare events and metadynamics from a conceptual viewpoint. *Annu. Rev. Phys. Chem.* 67:159–184.
7. Tsytlonok, M., H. Sanabria, …, R. Kriwacki. 2019. Dynamic anticipation by Cdk2/Cyclin A-bound p27 mediates signal integration in cell cycle regulation. *Nat. Commun.* 10:1676.
8. Li, P., I. R. S. Martins, …, M. K. Rosen. 2008. Internal dynamics control activation and activity of the autoinhibited Vav DH domain. *Nat. Struct. Mol. Biol.* 15:613–618.
9. Roach, P. J. 1991. Multisite and hierarchal protein phosphorylation. *J. Biol. Chem.* 266:14139–14142.
10. Zerze, G. H., W. Zheng, …, J. Mittal. 2019. Evolution of all-atom protein force fields to improve local and global properties. *J. Phys. Chem. Lett.* 10:2227–2234.