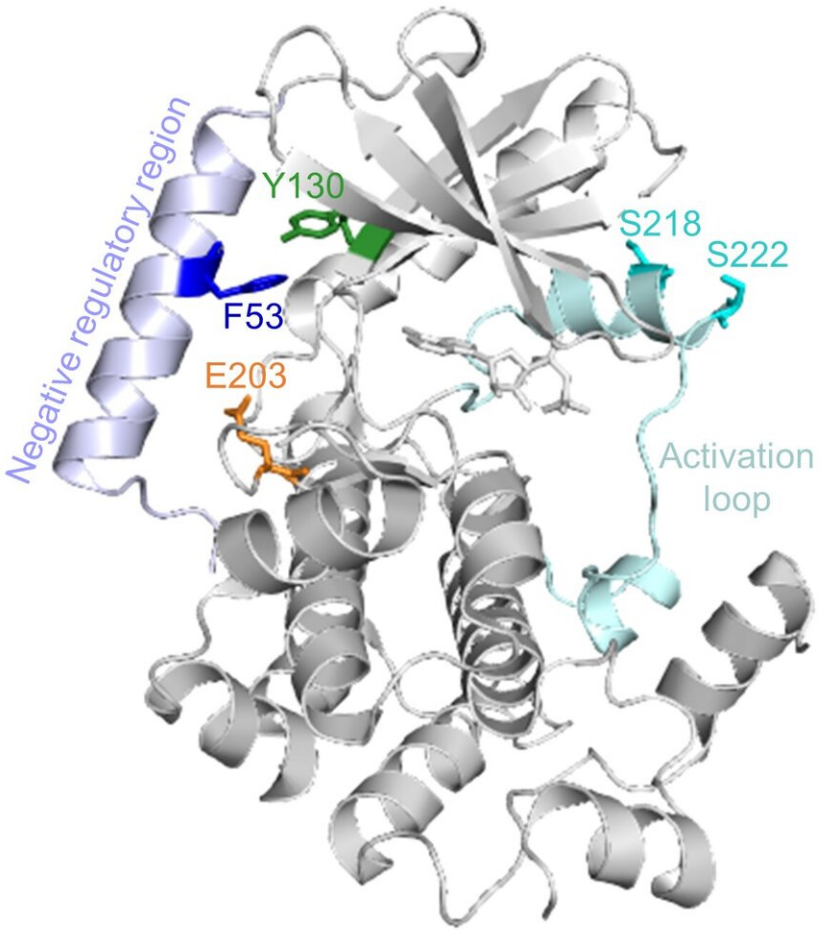
Mathematical model reveals behavior of cellular enzymes

February 13 2020



A ribbon diagram shows the structure of the enzyme MEK and the position of three mutations (called F53, Y130, and E203) that cause disease. Credit: Eyan Yeung and Martin Wühr, Princeton University

Everything a cell does, from dividing in two to migrating to a different part of the body, is controlled by enzymes that chemically modify other proteins in the cell. Researchers at Princeton University have devised a new mathematical technique to describe the behavior of many cellular enzymes. The approach, which will be published February 13 in the journal *Current Biology*, will help researchers determine how genetic mutations change the behavior of these enzymes to cause a range of human diseases, including cancer.

Enzymes called kinases can add phosphate molecules to multiple sites on other proteins (including other kinases), altering their activity within the cell. Studying these "multisite phosphorylation reactions" is complicated because the phosphate groups can be added rapidly and in different orders, which may affect how the modified proteins behave within the cell. This makes it difficult to understand exactly what goes wrong when a kinase is mutated.

A team of Princeton researchers led by Martin Wühr, an assistant professor of molecular biology, and Stanislav Shvartsman, a professor of chemical and biological engineering at Princeton and an Investigator at the Flatiron Institute, developed a mathematical model of how a kinase called MEK adds two phosphate molecules to a kinase called ERK. This double phosphorylation activates ERK so that it can drive numerous cellular processes, including cell growth and division. Mutations in MEK and ERK can cause several diseases, including cancer.

"There are many mutations in MEK that affect the overall levels of dually phosphorylated ERK," Wühr said. "But the effects of these mutations on the mechanism of ERK activation remain unknown."

The researchers' model revealed how fast each phosphate group is added

and how often both phosphates are added by the same enzyme. Most of the time, a single MEK enzyme binds to ERK and adds one phosphate molecule before it detaches and allows a second MEK enzyme to bind and add the second phosphate.

The researchers then used their model to analyze a mutant version of MEK that is found in human cancers. This mutant MEK was twice as fast at adding the first phosphate to ERK, and was much more likely to remain attached and add the second phosphate group itself. Together, this enhances ERK activation and accelerates cancer cell growth.

The researchers then analyzed two other MEK mutations that cause a variety of developmental abnormalities, including congenital heart defects and stunted growth. These mutations did not affect MEK's ability to add phosphate molecules to ERK. Instead, they enhance the activation of MEK by another kinase, called Raf, which adds two phosphate molecules onto MEK.

"Our analysis therefore reveals which of the multiple steps in this cascade of multisite phosphorylation are affected by each mutation," Shvartsman said. "We expect that our mathematical models will allow a deeper, more quantitative understanding of cell regulation systems, including their responses to mutations of constituent proteins."

Uncovering exactly how mutations alter enzyme function can help researchers develop new therapeutic strategies that restore their function back to normal.

"Our approach is not limited to kinases and is applicable to a broad class of biochemical mechanisms where one enzyme modifies multiple sites on its substrate," Wühr said.

More information: Eyan Yeung et al, Inference of Multisite

Phosphorylation Rate Constants and Their Modulation by Pathogenic Mutations, *Current Biology* (2020). DOI: 10.1016/j.cub.2019.12.052