Speeding 'fingertip' discovery -- 20 years of protein info in 1 place

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Researchers at Johns Hopkins took advantage of a new technique that reads the makeup of proteins to identify nearly all chemical changes nature makes by adding phosphate to proteins manufactured in human cells.

The Hopkins team then added its list of these so-called phosphorylation events to lists compiled by others and created a publicly available database on the Web - PhosphoMotif Finder [http://www.hprd.org/PhosphoMotif_finder] - to help speed the work of researchers around the world.

“Finding so many at one time is a huge advance,” says Akhilesh Pandey, M.D., Ph.D., an associate professor at the McKusick-Nathans Institute of Genetic Medicine at Hopkins. “Phosphorylation is essential for controlling chemical reactions in our cells’ protein factories, and phosphorylation gone awry has been implicated in several diseases. The ability to study more than one phosphorlyation at a time will help us understand some of these diseases - including cancers - sooner.

“What we have here is about 20 years’ worth of lots of work in one searchable list,” says Pandey. A report on all of the newly identified protein alterations is published in the Feb. 13 issue of the *Proceedings of the National Academy of Sciences*, while a report on the database appears in the March issue of *Nature Biotechnology*.

Pandey’s team used electron transfer dissociation (ETD) tandem mass

spectrometry, a technology that breaks apart proteins into small fragments, separates them by size and identifies the fragments based on their mass - their size and weight. The process improves on previous techniques by breaking up proteins more gently and keeping chemical modifications like phosphorylation intact. Previous spectrometry methods were “just too rough” on the delicate protein alterations and sheared them right off, he says. “We had to guess where they might be and nobody wants to chase false leads based on wrong guesses.”

Pandey says the original goal of the research was to identify accurately as many protein changes as possible using the new technology. “But to see how well we measured up, we had to compare our findings to what already was published, and there was just no clean, easy way of doing that because there were reports all over the place.

“That’s when we decided to go through and consolidate just about everything on phosphorylation that was out there.”

Working with human kidney cells, the researchers fished out the thousands of different proteins and analyzed them by ETD, resulting in a net total of 1,435 phosphorylations. Comparing these 1,435 to the 20 years’ of published data, they discovered that about 80 percent of what they found never had been reported.

The team then constructed an online search tool, PhosphoMotif Finder, which was incorporated into their previously established Human Protein Reference Database. Human Protein Reference Database now contains about 16,000 phosphorylation sites described in the literature and the PhosphoMotif Finder tool allows any researcher to find potential phosphorylation sites in any protein of interest.

“The power of this technique is not just in the numbers,” says Pandey. “Rather, we’ve found what you might call new information about old

proteins, and we hope the new data will help researchers study their favorite proteins in greater depth. After all, there's no sense in reinventing the wheel."

Pandey and his team now are curious about other chemical modifications of proteins, which are the "business-end" products of our genes. "There is evidence of other, more fragile modifications that until now no one has been able to get a handle on because they're way too hard to work with. Now we have the tools to probe further," he says.

Source: Johns Hopkins Medical Institutions