PROTEOMICS

An affinity for motifs

Antibodies targeting short sequence motifs found in multiple proteins can be used in a discovery array–based platform.

Antibody reagents are one of the most important tools in a biologist's toolbox. They are used for detecting proteins in western blots, for immunoprecipitating target proteins from a lysate and for tracking cellular proteins with fluorescence, just to name a few applications. They are even now being used in proteomics studies.

In all of these applications, the potent selectivity of antibodies for single-protein targets is key for the success of the experiment. But this unique selectivity limits the use of antibodies in proteomics to targeted platforms: researchers can follow only those proteins to which they have antibodies.

Carl Borrebaeck, of Lund University in Sweden, and his colleagues now report a new concept with the power to transform the antibody microarray into a discovery platform. Contrary to the 'one antigen, one antibody' paradigm, they generate renewable human recombinant single-chain variable fragment (scFv) antibodies selective for short sequence motifs of four to six amino acids. These motifs can be found in 50–100 proteins in a proteome. After the proteome sample is digested into peptides, the motif-specific antibodies will pull down all peptides containing these motifs. The immuno-captured peptides, and by extension the proteins from which they came, can then be identified using standard mass spectrometry techniques. Borrebaeck calls this method "global proteome survey."

With motif-specific antibodies, not only are discovery-based applications possible, but the need for highly purified antigens is circumvented. "The limitation with conventional approaches," Borrebaeck says, "is you need antibodies, and to make the antibodies you need antigens, and there's a limit on how much purified antigen is available."

In their recent proof-of-principle report, the researchers chose 27 peptide motifs consisting of four to six residues. They selected scFv antibodies from a phage-display library, generating 91 nonredundant scFvs. They chose 14 antibodies directed to 8 motifs to characterize. This limited set of reagents bound to 113 unique proteins on a human protein microarray. The 14 antibodies also enriched peptides, via microcolumns, from human liver, mouse liver and yeast, revealing the species-independence of the method. The microcolumn setup could also be multiplexed to capture more than one set of peptide targets at the same time.

The researchers detected yeast proteins with concentrations varying over four orders of magnitude, demonstrating that the method enriches very low abundance proteins, an ongoing challenge in the proteomics field. Antibodies targeting short peptide motifs containing post-translational modifications could also be theoretically generated.

Other antibody enrichment approaches, such as the 'stable-isotope standards and capture by anti-peptide antibodies' (known as SISCAPA) method developed by Leigh Anderson (of the Plasma Proteome Institute in Washington, DC), also use peptide-specific antibodies for enrichment and mass spectrometry for detection. But SISCAPA antibodies target longer peptide sequences unique to only a single antigen, so although the approach is highly sensitive, it does not allow for discovery-based applications.

The global proteome survey approach may also have advantages over the use of extensive sample fractionation and high-resolution mass spectrometry, which, though a discovery-based platform, is not that sensitive and requires a heroic experimental effort. In addition to the higher sensitivity of this approach, once motif-specific antibodies are selected, "this will be a very quick assay," notes Borrebaeck. "It's not very expensive to do, and the throughput is going to be high, though that remains to be seen."

With about 100 motif-specific antibodies, Borrebaeck estimates, about 50% of the human proteome could be captured, at least in theory. His group has the capacity to produce hundreds of antibodies in just a month or two, so he anticipates that they could select antibodies to cover the whole proteome in a relatively short time period.

Borrebaeck is most interested in applying the method for clinical applications. "The challenge now is to work with more complex proteomes, such as the serum proteome in humans, and do clinically relevant studies," he says.

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RESEARCH PAPERS

Olsson, N. *et al.* Proteomic analysis and discovery using affinity proteomics and mass spectrometry. *Mol. Cell. Proteomics* advance online publication (14 June 2011).

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