Research into synthetic antibodies offers hope for new diagnostics

January 24 2011, by Richard Harth

Antibodies are watchdogs of human health, continuously prowling the body and registering minute changes associated with infection or disease with astonishing acuity. They also serve as biochemical memory banks, faithfully recording information about pathogens they encounter and efficiently storing this data for later use.

Stephen Albert Johnston, Neal Woodbury and their colleagues at the Biodesign Institute at Arizona State University have been exploring mechanisms of antibody activity, particularly the ability of these sentries to bind -- with high affinity and specificity -- to their protein targets. A more thorough understanding of the antibody universe may lead to a new generation of rapid, low-cost diagnostic tools and speed the delivery of new vaccines and therapeutics.

Borrowing a script from nature, the group has been working to construct synthetic antibodies or synbodies, through a simple method developed in Johnston's Center for Innovations in Medicine. They have also examined the broad portrait of antibody activity revealed in a sample of blood, harnessing this information for the presymptomatic diagnosis of disease. These immunosignatures, as Johnston has named them, provide a dynamic report card on human health.

In a pair of new papers, the group demonstrated a simple means of improving the binding affinity of synbodies, which are composed of 20 unit chains of amino acids, strung together in random order. They also used random peptide sequences spotted onto glass microarray slides to

mine information concerning the active regions or epitopes of naturally occurring antibodies. These two projects recently appeared in the journals *PloS ONE* and Molecular and Cellular Proteomics, respectively.

While antibodies have been in use for biomedical research for a long time, conventional techniques for producing them have been time consuming and expensive. Normally, antibodies used for research are produced in animals, which respond to a given injected protein by producing a protein-specific antibody, which may then be extracted.

In earlier work, Johnston's group showed that high-affinity antibody mimics can be produced synthetically by simple means. Their technique turns the traditional production approach on its head. Rather than beginning with a given protein and trying to generate a corresponding antibody, the new method involves building a synthetic antibody first, later determining the protein it effectively binds with, by screening it against a library of potential protein mates.

The first step in this process is to generate random strings of 20 amino acids. Roughly 10,000 such random peptides are then spotted onto a glass microarray slide. The protein one is seeking an antibody to is screened against this random sequence array and peptides with high binding affinity are identified. Two such peptides can be linked together to form a synbody, whose binding affinity is the product of each separate peptide. In this way, two weakly binding peptides join forces to form a high affinity unit, useful for investigations into the proteome, the vast domain of proteins essential to virtually all biological processes.

In the *PloS ONE* study, lead author Matthew Greving and his collegues describe a strategy for further refinement of binding affinity in random sequence peptides. "The problem," Johnston explains, is that the microarray contains about 10,000 peptides, but that is less than a quadrillionth of the possible peptides by sequence. So we're sampling a

very small part of the sequence space. " A consequence of this is that the probability of generating a 20 amino acid sequence, that binds with optimal affinity, is pretty low.

To improve sequence affinity, a lead sequence is first selected. In the study, one such sequence was the 17 amino acid peptide TNF-1, a key regulator of immune cells. The lead sequence is then used as a template from which to generate additional peptide sequences in which a single amino acid at each subsequent position along the peptide chain is replaced with a different amino acid.

Using this method, 96 variant peptides are constructed on a microarray plate. These enhanced variants are screened against a desired protein for binding affinity and a map is produced displaying this affinity from low to high. The most successful variants can then be assembled into a new high affinity peptide, whose binding strength is the sum of the components.

This simple, algorithmic process can rapidly optimize random sequence peptides, improving their binding affinity by 100 to 1000 times. The method can also be used to improve the specificity of peptides, enabling the construction of binding agents able to attach to a given protein while excluding unwanted binding targets.

The MCP study asked whether a similar random peptide microarray could assist in the process of epitope mapping, in which the active binding regions of antibodies are identified. Epitope mapping is one method for determining if a given antibody is suitable for a particular application, and a faster, more cost-effective method would be of significant biomedical value.

For these experiments, antibodies of known epitope were screened against random sequence peptides on a microarray. High affinity

peptides were identified and bioinformatics techniques were used to see if the random peptides could help identify the antibody epitopes.

Two techniques were applied; one in which high affinity random sequence peptides were compared side by side with the antibody epitopes they bound with and similarities statistically analyzed. The other method searched the peptides for signature "motifs"—consisting of at least 7 amino acids (or two shorter motifs in combination). Lead author Rebecca Halperin and colleagues were able to show that statistically useful information on epitopes could indeed be gleaned from such bioinformatic probing, bringing the prospect of high throughput, inexpensive exploration of natural antibodies a step closer.

Johnston stresses the importance of this research. "The paper asks if there are mechanisms to transfer from random sequence space to real sequence space based on antibody binding. No one has explored this as deeply as Rebecca has." Further refinement should allow diagnosis of the precise protein sequence causing a given illness, based purely on analysis of immune response.

Provided by Arizona State University