form (free molecular pathogenic prion protein). They found that the non-membrane-associated molecular spike partitioned differently from the three membrane-bound spikes. Removal is now being evaluated with two spiking agents – one membrane-bound and the other unbound, so that results can be expressed as a log range between the 'best case' and 'worst case' scenario for prion reduction [6]. Other companies, such as Bayer and ZLB (Bern, Switzerland), are beginning to evaluate purified forms of prion innocula as spiking agents.

Combining both methods

Although different companies have used different methods for prion detection, this might not represent a disadvantage. 'When you correlate Bayer's and Baxter's work on [the] western blot together with Aventis' work with CDI, this produced independent verification that was enormously reassuring to the FDA,' says Steve Petteway, Director of Pathogen Safety and Research at Bayer.

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Novel templates for rapid protein separation

Sharon Dorrell, freelance writer

Scientists are under pressure to provide innovative drug treatments fast. A new molecular imprinting technique developed at Aspira Biosystems (San Francisco, CA, USA) could speed up protein separation and, therefore, target molecule characterization. The technique, which is called ProteinPrint™, enables the generation of cavities complementary to a portion of a protein of interest. The cavities can be synthesized on tiny beads approximately 10 μm in diameter or on microarrays. The cavities selectively bind to protein that fits the mould, similar to the way in which antibodies bind antigen, but with the ability to distinguish even a single amino acid mismatch. Each bead can bind an estimated one million protein molecules, although the company is working on increasing the capacity of the beads.

Molecular imprinting

ProteinPrint™ is based on a process known as molecular imprinting, in which synthetic monomers are allowed to self-assemble around a template peptide to create a synthetic mould of the peptide. A signature sequence of amino acids acts as the template molecule. 'The signature sequence of a protein could be any portion of the protein that is unique to that protein,' explains Casey Lynch, President of Aspira Biosystems. The signature sequence might be derived experimentally or drawn from a database of predicted sequences. 'In our case, we usually use the C-terminal 7–9 amino acids, which provides a unique signature for 80–90% of proteins in the proteome and is easier to predict for novel proteins because of stop codons,' says Lynch.

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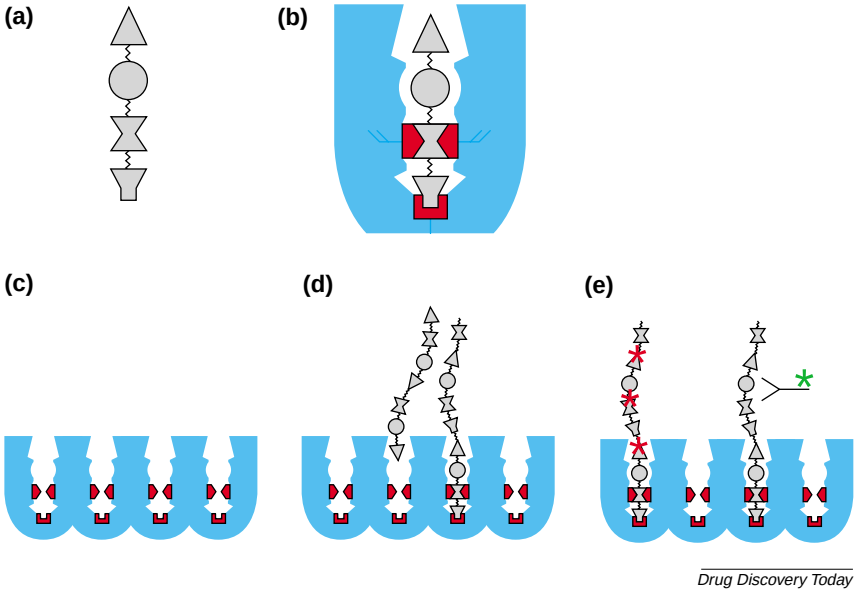

Figure 1. The five stages involved in the ProteinPrint™ process. **(a)** Phase 1: peptide synthesis. **(b)** Phase 2: self assembly. **(c)** Phase 3: extraction. **(d)** Phase 4: capture. **(e)** Phase 5: detection (the red crosses and green asterix represent staining and tagging techniques, respectively. Figure kindly provided by Aspira Biosystems (San Francisco, CA, USA). (see http://www.aspirabio.com/2.3_protein_print.html).

The process

The first stage of the process is to synthesize a template peptide that corresponds to a portion of the target protein (Fig. 1a). The next step is to assemble the molecular mould around the template. Polymerizable synthetic polar monomers are allowed to self assemble in the prescence of the template peptide and then cross-linked into place (Fig. 1b). The template peptide is then washed away (Fig. 1c). 'The result is a synthetic material with cavities that are complementary to the template peptide,' explains Lynch.

The penultimate step is to expose the beads to a mixture of denatured proteins to enable them to selectively bind target proteins with the same signature sequence as the template (Fig. 1d). Finally, the unwanted proteins can be washed away leaving the beads bound to the target protein (Fig. 1e). Staining and tagging techniques are used to assay protein expression levels and post-translational modifications.

An alternative approach

Lynch expects the ProteinPrint™ technique to be an alternative to both antibody arrays and two-dimensional (2D) gel electrophoresis protein separation techniques. She adds that it has advantages over 2D gels in that the ProteinPrint™ technique provides more rapid results. Moreover, Lynch says, 'Direct comparison to monoclonal antibodies shows ProteinPrint™ is cleaner, more specific and easier to use.'

Lynch believes that the new technique could have a wide variety of applications, including the analysis of protein expression, protein purification, analysis of post-translational modifications, domain mapping of protein–protein interactions, and small-molecule screening. Currently, the company is planning to use the ProteinPrint™ technique to identify, select, and validate new drug targets for development either in-house or by other pharmaceutical companies.