A look back: adventures in the matrix

Decades before they would be lionized by the rise of glycobiology and demonized by the Atkins diet, carbohydrates were quietly doing wonders for biological research. In 1955, Oliver Smithies published a groundbreaking *Biochemical Journal* article, introducing the world to gel electrophoresis, using potato starch–based slabs[1]—a considerable step forward from the filter paper–based approaches that had prevailed previously. It was also around this time that starch helped launch another important nascent separation technology, size exclusion chromatography.

The idea of using starch as a chromatographic substrate was hardly a new one. In several articles from the 1940s, Richard Synge, a pioneer in partition chromatography, William Stein and Stanford Moore—all future Nobel laureates for their protein research—demonstrated the effectiveness of starch columns for the analysis of amino acids and protein fragments[2,3]. But the 1956 work of Grant Lathe and Colin Ruthven brought the technology an important step forward, demonstrating that by applying solutions of mixed proteins to such columns, one could separate them by size. The authors theorized that this happens because of the varying extent to which differently sized proteins can penetrate the porous, hydrated starch grains; because bigger (and therefore heavier) proteins will travel around, rather than through, pores in the starch matrix, they will therefore exit the column earlier than smaller proteins[4].

This was a promising start, but starch was a less than ideal material to work with for several reasons, including instability and particle heterogeneity. Fortunately, this technique soon benefited from a happy accident in the laboratory of Arne Tiselius, one of the forefathers (with mentor Theodor Svedberg) of protein electrophoresis. While at the University of Uppsala, Tiselius was approached by the Swedish Sugar Manufacturers' Association to resolve a sticky situation: dealing with a mysterious slime that was accumulating during the processing of sugar beets. His investigation revealed that the substance contained the polysaccharide dextran, produced by bacteria in the beet preparations[5]. Jerker Porath and Per Flodin, colleagues of Tiselius, saw potential in this gooey compound for filtration protocols, and developed a method for purifying and cross-linking dextran to generate stable matrices for chromatography, in which porosity could be controlled to alter the range of molecular weights that can be sorted[6]. They subsequently demonstrated dextran's effectiveness for the size separation of proteins and small organic molecules, and showed that one could fairly reliably predict the size of a molecule by identifying the fraction in which it elutes from the column[7].

It wasn't long before other alternatives appeared, and another alumnus of the Tiselius lab, Stellan Hjertén, soon developed an alternative filtration approach using the synthetic polymer acrylamide. Hjertén poured acrylamide gels, freeze-dried them and ground them through a mesh, generating granular particles of a size determined by that of the mesh openings. These particles also proved effective for column-based protein separation, and like Porath and Flodin, Hjertén and coauthor Rolf Mosbach found that the rate of migration through their columns could be correlated quite accurately with the size of the migrating particle[8]. Another useful alternative arose from the work of Alfred Polson[9], who also generated particles for filtration by mashing gels through sieves, but used agarose instead of acrylamide. By itself, the agarose suffers from stability issues, but the addition of certain cross-linking agents dramatically enhances the durability of the gel matrix, and cross-linked agarose has also come to be used widely in many applications. All of these gel materials—dextran, acrylamide and cross-linked agarose—have since become commercially available as beads, optimized for experimental consistency. Gel-filtration chromatography has also benefited from the work of scientists like Patrick Andrews, who put great effort into analyzing how different proteins behave during gel filtration and into identifying factors that can muddle the accuracy of size determination[10].

Today, column-based separation generally has gone automatic, incorporated into more sophisticated systems such as high-performance liquid chromatography (HPLC), which use size-exclusion—as well as more precise column-based strategies—for analysis of protein mixtures. Nevertheless, traditional gel filtration remains popular for several applications—including the widely used method for the purification of radiolabeled probes. Gel beads also got a new lease on life with the introduction of techniques that allow them to be functionalized for the attachment of chemical groups, making them a suitable scaffolding for far more specialized purification procedures, like immunoprecipitation and other protein-protein interaction–based schemes.

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