Breakthrough technology quickly separates large proteins and viruses from their surroundings

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Researchers looking to isolate individual proteins from complex environments usually turn to chromatography, a technique where mobile solutions of biomolecules flow through columns packed with solid, porous particles. Separation occurs when attractive chemical forces

cause the molecules to adsorb onto the solid while contaminants pass through. Despite major progress, however, chromatographic purification of viruses and other large biomolecules remains challenging: their spatial heft makes it hard for them to diffuse through columns in a reasonable amount of time.

Pete Gagnon and co-workers at the A*STAR Bioprocessing Technology Institute in Singapore have discovered a new chromatography approach that can boost the capacity and resolution of large-scale biological purifications. Instead of relying on chemical attraction, the team's 'steric exclusion chromatography' (SXC) technique exploits the physical distribution of biomolecules and a dissolved polymer to drive adsorption at a chromatography surface—a strategy that generates extremely fast binding kinetics and virus purification efficiencies thousands of times greater than current techniques.

No two compounds dissolved in a solution can occupy the same space. In addition, random movements and collisions create narrow zones adjacent to surfaces where smaller dissolved molecules are statistically absent. As these zones create excess free energy, materials in the solution spontaneously rearrange themselves to reduce the excess.

Gagnon and his team exploited this effect by dissolving proteins into polyethylene glycol (PEG), creating PEG-free zones around the biomolecules and an inert chromatography surface. When the biomolecules randomly encounter the surface, their PEG-deficient zones fuse together to reduce the system's free energy and they become stabilized on the solid support. Because larger biological species are more affected by this phenomenon, they tend to associate with the chromatography surface, whereas smaller compounds are swept through the column and eliminated.

By performing the separation in special monolithic columns that

transport dissolved materials through convection, not diffusion, the researchers were able to purify viruses with unprecedented efficiency. They achieved binding capacities of 10 trillion virus particles per milliliter of monolith, despite the passage time through the column being only six seconds. Some 99.8% of E. coli proteins and 93% of DNA contaminants were removed. Virus recovery was 90%, and critically, the viruses retained full biological activity.

Gagnon notes that this unexpected discovery drastically improves upon the sluggishness and low efficiency problems currently associated with size-based chromatography methods. "Steric exclusion chromatography provides process developers with a rapid, high-precision tool needed to support effective and economical industrial purification."

More information: Lee, J., Gan, H. T., Latiff, S. M. A., Chuah, C., Lee, W. Y. et al. Principles and applications of steric exclusion chromatography. *Journal of Chromatography A* 1270, 162–170 (2012). dx.doi.org/10.1016/j.chroma.2012.10.062

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