All in the mix

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A team of Canadian, Japanese and German chemists has successfully demonstrated how a newly developed capillary electrophoresis method can be used to screen potential enzyme inhibitors. Termed transverse diffusion of laminar flow profiles (TDLFP), it represents the first effective method for mixing together different reactants within a capillary tube.

Capillary tubes are in many ways ideal places to conduct chemical and biological reactions. For a start, as capillaries are fairly narrow, only small amounts of the reactants (potentially just nanolitres) would be needed. Also handy is the fact that any products of the reaction could be separated from the reactants by simply conducting electrophoresis. While identifying the products would simply require hooking the capillary up to some form of detector, like a mass spectrometer.

But the big problem with conducting reactions within a narrow capillary has always been getting the various reactants to mix together. The only realistic way is often simply to wait for the different reactants to mix by diffusion. But this can take hours if the reactants are inserted as individual, sausage-shaped plugs. This is because the plugs can only diffuse together at the point where they meet, which is usually the width of the capillary (around 50µm).

Then, in 2005, **Sergey Krylov** and his colleagues at York University, Ontario, came up with TDLFP, which involves injecting reactant plugs one after another at high velocities. 'Due to the laminar nature of flow inside the capillary, at a relatively high injection velocity the injected plugs do not have time to diffuse considerably and the non-diffused plugs have parabolic profiles with predominantly longitudinal interfaces between the plugs,' explains Krylov.

In other words, the high velocity injection causes the plugs to form long points that pierce the plugs in front, causing them to look like a stack of wizards' hats. This shape means that the plugs are in contact with one another along the whole length of their points, giving them a much larger area over which they can diffuse together. As a result, the various reactants are able to mix together in a much shorter period of time, often only a few minutes.

More recently, Krylov and his colleagues have started to

explore some of the practical applications of TDLFP, starting with its most promising application: screening potential enzyme inhibitors. As enzyme inhibitors make very good drug candidates.

As a first test, they used TDLFP to screen six different compounds for their ability to inhibit a key regulatory enzyme found in the protozoa *Entamoeba histolytica*. This unicellular parasite is the second leading cause of death from parasitic disease in humans, able to cause dysentery, colitis and liver abscess.

They chose to focus on an enzyme known as farnesyltransferase (FT), which is involved in cell reproduction. Specifically, it catalyses the transfer of a lipid compound known as a farnesyl group from farnesyl pyrophosphate to a protein known as Ras.

So Krylov and his colleagues designed a TDLFP procedure that involved sequentially injecting five plugs into a capillary tube. These plugs comprised farnesyl phosphate followed by a fluorescently-labelled peptide-mimic of the Ras protein followed by FT followed by one of six potential inhibitors followed by farnesyl phosphate again.

The idea was that the five plugs would quickly mix together within the capillary tube, allowing FT to work its magic unless it was prevented by one of the potential inhibitors. The reactants and any products would then be separated by electrophoresis, with the fluorescently-labelled peptide, either with or without an attached farnesyl group, identified by laser-induced fluorescence detection. The modified and unmodified peptides would then show up as two separate peaks in the subsequent electropherogram.

And this is exactly what happened. Krylov and his team found that the plugs mixed together in around a minute and that the respective sizes of the two peptide peaks revealed whether the potential inhibitor worked or not. In this way, the chemists were able show that four known inhibitors of mammalian FT also inhibit FT in *E. histolytica*.

Krylov and his team are now using TDFLP to screen short DNA strands known as DNA aptamers for their binding affinity and have also recently developed a mathematical model of the TDFLP process.

Related links:

- ***Journal of the American Chemical Society*, 2008, 130, 11862 - 11863**: "'Inject-mix-react-separate-and-quantitate' (IMReSQ) method for screening enzyme inhibitors"
- ***Analytical Chemistry*, 2005, 77, 5925 - 5929**: "Transverse diffusion of laminar flow profiles to produce capillary nanoreactors"

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