PROTEIN BIOCHEMISTRY

Protein production: no cells required

DNA hydrogels improve the efficiency of cell-free protein production.

Escherichia coli, the recombinant protein production workhorse, is not always the best creature for specialized jobs. Cell-free protein synthesis systems, consisting of cell extracts containing all of the necessary protein production machinery, are particularly useful for making proteins that are challenging to produce in cells, such as toxic proteins or those that undergo rapid proteolysis. Dan Luo and his group at Cornell University recently reported a method that makes cell-free protein production even more efficient.

Luo's group has been in the business of using DNA as both a "genetic and a generic material," as Luo describes it. They previously developed a method to make hydrogels entirely from branched DNA molecules. Now they have put the hydrogel to practical use for cell-free protein synthesis. They form a hydrogel by ligating branched DNA and linear plasmids, using a flat 1-millimeter square mold to make 20-micrometer-thick gel pads. They then drop the gene-containing gel pads into a test tube containing a commercial cell-free system and wait for the magic to happen.

Luo's group synthesized 16 different proteins using the hydrogel approach, which was up to 300 times more efficient than solution-based cell-free synthesis. They believe that the increased efficiency of transcription is a result of three possible mechanisms. First, the gel matrix caps the free ends of the gene and protects it from nucleases. Second, the local concentration of the gene in the gel can be greatly increased without resulting in DNA precipitation, unlike in solution. Third, enzyme turnover is likely much more efficient because the enzyme does not need to diffuse very far to find its next substrate.

"High throughput is definitely an advantage of this technology," says Luo. "We can synthesize proteins on a very large scale because we don't need to culture cells." The hydrogel system should also be useful for making membrane proteins because solubilizing detergent can be added directly to the expression system. "It is very difficult to do that to a live cell because detergent would disrupt the cell membrane," explains Luo. Notably, the researchers also synthesized a 'glue' protein from mussels, which would typically form inclusion bodies in *E. coli* or even kill the cell.

Though making the gel pads requires a clean room and a specialized micromold, Luo aspires to translate the method into a commercial product. For the time being, he is happy to help others make DNA hydrogels for protein production and other applications.

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RESEARCH PAPERS

Park, N. *et al.* A cell-free protein-producing gel. *Nat. Mater.* advance online publication (29 March 2009).