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New technology for protein microarrays

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Abstract

Nanodroplets of active, solvated protein can be printed onto treated glass slides for protein microarray experiments.

Significance and context

Modern biochemistry often involves a search through a whole cell's worth of proteins for a new function. To find new binding partners for a known test molecule, biochemists usually opt for time-tested *in vivo* experiments (namely the yeast two-hybrid screen). MacBeath and Schreiber have now developed a new *in vitro* protein screen. They deposit tiny droplets of protein solution onto glass slides, then screen the slides for binding or enzymatic activity. This is the first step toward the ultimate goal: screening a slide full of pure concentrated samples, one of each protein in the genome. Such a 'protein microarray' may have significant advantages over *in vivo* experiments: it does not require good protein expression or strong binding in a living host, and it can probe enzymatic function.

Key results

MacBeath and Schreiber carried out three types of controls on their microarray slides. In one, they detected the binding of three protein pairs, each previously known to interact. In this experiment they deposited a 100 µg/ml solution of one member of the pair onto a glass slide, then incubated it with 0.1-0.5 µg/ml of the other protein in the pair. The latter was fluorescently labeled, so the slide was passed under a fluorimeter to detect binding. Specific binding was observed, even in a single droplet of one target protein on a slide containing 10,799 droplets of another protein. In the second control, the authors detected phosphorylation activity in three protein pairs, each known to be a kinase and complementary substrate. Kinase solutions and labeled ATP were incubated with glass slides dotted with substrate droplets (concentrations are not given in the article). Labeled phosphate was observed in the droplets, and phosphorylation was specific. In the third control, binding of three pairs of receptors and their small-molecule ligands was detected. This experiment probed droplets of receptor proteins with fluorescently labeled small molecules at 10 µg/ml. The authors found specific binding in the slide format, in complexes with affinities as low as 2.6 µM.

Methodological innovations

MacBeath and Schreiber first covered glass slides with an aldehyde-containing silane reagent. The aldehyde can link with lysines and amino termini on the surfaces of proteins. A robot covered these treated slides with droplets of protein solution, each 150-200 μm in diameter; the solutions contained glycerol to prevent evaporation of water. Proteins in the droplets then bound to the surface of the slides, but were also available for physiologically relevant binding or function in an aqueous environment.

Conclusions

The authors propose that the glass slide format may be a viable alternative to *in vivo* protein screens.

Reporter's comments

Some remarkable nanotechnology is described here, which may be useful in making protein microarrays. But the paper does not report results from droplets of many different proteins in parallel. That is the Holy Grail, and it seems that neither MacBeath and Schreiber, nor any other group, has quite achieved it yet.

Table of links

Science

References

1. MacBeath G, Schreiber SL: Printing proteins as microarrays for high-throughput function determination. Science. 2000, 289: 1760-1763. 0036-8075