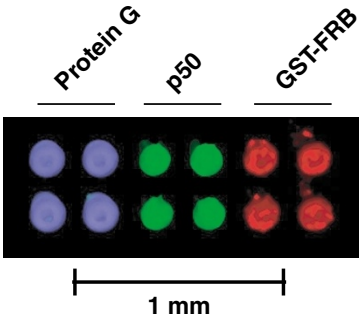
A protein microarray

The sequencing phase of the human genome project is now near completion, but then what? An even harder challenge remains — to identify the structures and functions of all proteins encoded in the genome. One approach to answer this challenge is to identify functions based on structures; on page 903 of this issue of *Nature Structural Biology*, Edwards, Arrowsmith and coworkers present progress on a large scale structural genomics project. Although structures can help to identify biochemical functions, they are not as useful in defining biological functions — that is, the interaction partners of a protein and the process in which it is involved. New methods for determining functions or functional interactions on a large scale are required.

Microarrays have been developed for large scale genome-wide applications. By arranging small amounts (a few nanoliters) of thousands of different molecules in an array on a glass support (at a density of ~1,000 spots per square centimeter), microarray technology allows many different experiments to be performed in parallel. For example, a microarray carrying immobilized DNA fragments that are complementary to the protein coding regions (cDNAs) can be used to detect and quantify the entire content of cellular messenger RNAs; therefore, it is possible to monitor the gene expression pattern in a cell.

Microarrays are attractive high throughput devices because they require very small sample volumes (typically a couple of microliters). Some of these attributes have now been adapted in a protein microarray, constructed by Gavin MacBeath and Stuart Schreiber (*Science*, **289**, 1760–1763), which demonstrates potential for high throughput determination of protein functions.



The microarray is constructed by covalent attachment of proteins onto a chemically derivatized microscope glass slide. This format is compatible with instrumentation, such as commercially available imaging scanners, for detection and for robotic printing of the array. One example application — the screening of protein–protein interactions — is demonstrated by the simultaneous detection of three sets of proteins that are known to interact: protein G and immunoglobulin G (IgG); p50 of the nuclear NF-κB complex and the NF-κB inhibitor IκBα; and the human immunophilin FK506 binding protein (FKBP12) and a FKBP12–rapamycin binding domain (FRB).

In the example shown, protein G (left four spots), p50 (middle four spots) and FRB (right four spots) were immobilized, and the microarray was probed with a mixture of fluorescently labeled IgG (labeled with BODIPY-FL; pseudocolor blue), IκBα (labeled with Cy3; pseudocolor green), FKBP12 (labeled with Cy5; pseudocolor red) and rapamycin. The formation of protein complexes resulted in the localization of the fluorophores at the specific sites of the immobilized proteins.

In addition to screening for protein–protein interactions, the microarray method can also be used to identify substrates for enzymes such as protein kinases, or to screen small molecule–protein interactions, and MacBeath and Schreiber provided a proof-of-principle example for each of these applications. However, the potential of the device has yet to be realized in experiments to identify previously unknown interactions. Moreover, the design of the current experiments requires that purified proteins be used in constructing the array, which may be difficult to obtain in some cases and thus may restrict the systems that can be studied using this method. Nonetheless, the protein microarray is a significant step toward systematic, genome-wide biochemical characterization of protein functions.

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