SPECTROSCOPY

A need for speed

A new target-immobilized NMR technique for screening binding interactions greatly reduces the required target quantity and experimental time.

Fast, reliable and sensitive screens of large libraries of compounds for target binding are crucial in drug discovery. Nuclear magnetic resonance (NMR) spectroscopy is gaining importance in finding drug leads because it is more sensitive to weak binding interactions than traditional high-throughput screening methods. The screening method favored by the pharmaceutical industry, structure-activity relationships by NMR (SAR by NMR), screens for binding of small-molecule ligands to a protein by observing changes in the NMR spectrum of the isotopically labeled protein. This method, however, is limited to use with small proteins (less than 40 kDa) and is often time-consuming. Reverse methodologies, based on observing changes in the spectra of the ligands rather than the protein, are gaining popularity. But these methods have a limited range of targets and usually require large quantities of protein.

In a recent study, Gregg Siegal and colleagues of the Leiden Institute of Chemistry at Leiden University introduced an NMR method of screening compound libraries for binding to an immobilized target. Target-immobilized NMR screening (TINS) allows a diverse array of targets to be screened; the target does not need to be soluble or even be a protein. The method also greatly reduces the quantity of target required, as a single sample of the target is sufficient for a flow-through screen. Most notably for drug discovery, TINS takes up substantially less experimental time. "Other NMR methods can take 1 to 3 months to screen a 10,000-compound library. Using TINS it can be done in less than a week," says Siegal.

Siegal and colleagues immobilize targets on a new gel-based solid support. By using the gel supports, researchers avoid the problems associated with conventional glass supports, which result in poorly defined spectra of dissolved compounds owing to the large difference in magnetic susceptibility of solids versus liquids. By effectively subtracting the spectrum of potential ligands in the presence of the target from the control spectrum of the ligand compounds in the absence of the target, ligands that bind to the target can be identified.

Is TINS the next SAR by NMR? "SAR by NMR has changed the view of the pharmaceutical industry away from a narrow focus of high-throughput screening and therefore has had tremendous impact," says Siegal. "However, many pharmaceutical companies are now using methods that focus on the NMR spectrum of the compounds to be tested. Therefore, ultimately there may be more users of the TINS methodology than the original SAR by NMR method."

Allison Doerr

RESEARCH PAPERS

Vanwetswinkel, S. *et al.* TINS, target immobilized NMR screening: an efficient and sensitive method for ligand discovery. *Chem. Biol.* **12**, 207–216 (2005).

MICROARRAYS

Getting quantity and quality

A new strategy for the analysis of single nucleotide polymorphisms (SNPs) permits investigators to conduct accurate, multiplexed genotyping of large, customized variant panels.

SNP genotyping is both big science and big business; as such, it's no surprise that substantial progress happens where the two intersect. In this vein, collaboration between researchers at ParAllele Bioscience, Inc. and the Baylor College of Medicine, both participants in the ongoing International HapMap Project, has produced a new system for the large-scale, multiplexed analysis of hand-picked SNP arrays (Hardenbol *et al.*, 2005).

"With our technology... you can choose 10,000 of the most relevant SNPs for your particular study," explains ParAllele researcher Tom Willis, "and make a multiplexed assay that would allow you to score those particular SNPs." The centerpiece of this technology is the molecular inversion probe (MIP), a reagent previously developed by members of this group (Hardenbol *et al.*, 2003). A single MIP is designed for each SNP; after hybridization, separate enzymatic reactions are carried out to identify any possible nucleotide variant. In the subsequent microarray analysis, SNPs are identified using a MIP-specific, thermodynamically optimized bar-code sequence: for each of the four reactions, bar codes are PCR-amplified in the presence of a different fluorophore, and by analyzing the array data, one can assess the ratio of nucleotide variants at each site.

This system was used to genotype sets of 6,000 and 12,000 SNPs against genomic samples from the HapMap collection; these specimens were collected from families, allowing the accuracy of the process to be confirmed by checking the mendelian inheritance of each SNP. The MIP strategy proved suitable for recognizing more than 85% of the SNPs the researchers selected, and the data consistently achieved levels of accuracy that surpassed 99.6% and were highly repeatable. With less stringent standards, higher SNP recognition was possible, although accuracy was compromised slightly as a result.

Willis indicates that this system is easily scalable and suggests that chips capable of analyzing 100,000 SNPs should be possible. "There are no large obstacles today, other than just building the resource of MIPs and optimizing the label to get sufficient signal off of the arrays." ParAllele is continuing to develop this technology and participate in the HapMap Consortium, but other applications are also being considered for this system, and Willis sees great potential in the development of chips specific for polymorphisms that affect protein sequence or alter gene expression. "We've developed one panel... that's got 10,000 amino acid–changing SNPs in one assay," he says. "[And] one obvious way to expand that is to go from just amino acid–changing SNPs to other categories of functional SNPs, like splice-site SNPs and promoter SNPs... We think this represents an important, complementary genetic strategy to HapMap-type approaches."

Michael Eisenstein

RESEARCH PAPERS

Hardenbol, P. *et al.* Highly multiplexed molecular inversion probe genotyping: over 10,000 targeted SNPs genotyped in a single tube assay. *Genome Res.* **15**, 269–275 (2005).

Hardenbol, P. *et al.* Multiplexed genotyping with sequence-tagged molecular inversion probes. *Nat. Biotechnol.* **21**, 673–678 (2003).