thermal hypersensitivity produced by cutting the L5 and L6 spinal nerves in rats. In addition, these effects were ablated by an antagonist selective for CB_2 receptors, but not by a CB_1-receptor-specific antagonist. Furthermore, AM1241 reversed allodynia in mice lacking CB_1 receptors, establishing that CB_2 is the crucial receptor.

Some of the current treatment options for neuropathic pain have unwanted side effects on the central nervous system (CNS), and so drugs directed against targets not found in the CNS would make attractive agents. CB_2 receptors, which are not expressed in the brain or CNS, fit the bill.

Daniel Jones

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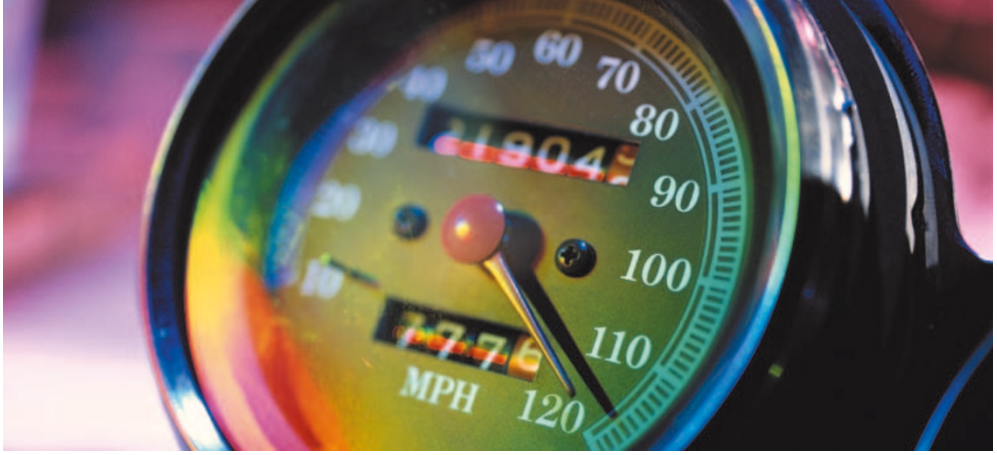

LEAD DISCOVERY

RAMPing up NMR screening

Nuclear magnetic resonance (NMR)-based screening approaches that directly detect the binding of compounds to a target are becoming increasingly popular in lead discovery, as they have several valuable advantages over established high-throughput functional assays. First, they can be used for highly novel targets for which functional assays are not readily available; second, they can detect weak-binding 'fragments' that might be more easily developed into potent lead compounds; and last, they can yield detailed structural data that indicate where on the protein the compound is binding, thereby aiding compound optimization. However, the NMR spectra resulting from such screens are often complex and hard to analyse, leading to relatively low throughput. As described in the *Journal of the American Chemical Society*, Zartler *et al.* have now developed a new NMR-based screening approach that yields greatly simplified spectra that not only allow increased throughput owing to easier spectral analysis, but also allow — for the first time in any format — multiple proteins to be screened simultaneously in one tube.

Like several other NMR-based screening methods, such as 'SAR by NMR', the authors approach — dubbed 'RAMPED-UP NMR' — is based on labelling the protein target with ^{15}N, an NMR-active nitrogen isotope (^{14}N, which has a natural abundance of 99.6%, is not NMR-active, whereas the most common isotope of hydrogen, ^{1}H, is NMR-active). Ligand binding is then assessed by observing the movements induced in the chemical shifts of ^{1}H–^{15}N resonances in the protein NMR spectra; resonances that shift will usually originate from residues in or close to the binding site.

However, typically only a relatively small percentage of the ^{1}H–^{15}N resonances in a uniformly ^{15}N-labelled protein will be significantly affected by ligand binding; the many resonances that do not shift merely add complexity to the spectra and provide no useful information. Zartler *et al.* decided to address this issue by only labelling specific amino acids with ^{15}N, which can be achieved by expressing the protein in bacteria grown in media containing the chosen labelled amino acid, or by using *in vitro* expression systems and supplying the chosen labelled amino acid.

The authors selected three unrelated targets to illustrate their methodology: protein tyrosine phosphatase 1B (PTP1B), kRAS and green fluorescent protein (GFP). A key issue with selective labelling is which amino acids should be labelled so as to maximize the likelihood of detecting a binding event. In the case of PTP1B, the location of the binding site is known, and it was decided to label Trp residues with ^{15}N, as a Trp residue is close to this site. For the other two targets — as would probably be done for targets for which the position of the binding site is uncertain — the authors chose to label Ile and Ala, respectively, as these provide a good sampling of the primary protein sequence, meaning that there is a good probability that a labelled residue is close to any binding sites.

The authors then showed that the NMR spectra of a solution containing these three targets could be easily used to quickly and cleanly discriminate the binding of known weak ligands for PTP1B and kRAS, highlighting the power of the approach. In this case, the targets are not related, but the strategy would clearly also be valuable when aiming to discover ligands that are selective for particular members of a family with highly homologous active sites — for example, kinases — a scenario in which the ability to analyse potential binding events for multiple family members simultaneously in the same tube would be especially desirable.

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