

Multiplexed Assays: The Way Forward



Dr David Grainger Chief Scientific Officer Pronostics Ltd

For more than a decade now, the potential impact of multiplexing on early stage drug discovery and compound screening in particular, has been obvious. Screening compound libraries against many related targets simultaneously offers the prospect of selecting hits on the basis of particular patterns of binding to multiple targets, rather than trying to control such selectivity during subsequent hit-to-lead medicinal chemistry programmes.

Until very recently, however, multiplexing technology has been unable to deliver this vision in practice. Dye-encoded beads, for example, are incompatible with most reporter systems used in high throughput screening, and the bead substrate itself insufficiently robust for many assays. Similarly, micro arrays are useful for analysing a few samples in many assays, but screening generally requires analysis of very many samples.

However, UltraPlex™, from Pronostics, is able to meet these challenges and deliver the promise of straightforward, robust multiplexed screening. The UltraPlex™ platform is based around aluminium microparticles, smaller than the breadth of a human hair, which each carry a unique barcode (Figure 1). Each barcoded microparticle, or UltraCode, carries a different assay.

The real advantage of UltraPlex™ comes from the robust nature of the UltraCode particles. Made of metal, they can be subjected to a wide range of assay conditions from live cell conditions to organic solvents. And because the multiplex coding is a physical barcode (and not dependent on fluorescent dyes), UltraPlex is also compatible with well-established reporters such as scintillation proximity and fluorescence polarisation.

With a wide range of attachment surfaces available, the UltraCode particles can be used as the physical substrate for almost any kind of assay. Live cells can be grown on the particles, allowing gene expression reporters to be assayed; active enzymes can be bound to the surface for rapid screening of inhibitors against multiple enzymes in a family all in the same tube; recombinant receptors or antibodies can be attached for binding assays; DNA can even be hybridized to probes attached to the particles – the possibilities are almost limitless.

It is even possible to deliver true three-dimensional multiplexing in a high-throughput screening setting. Consider taking 20 different recombinant cell lines, each expressing a different G-protein coupled receptor, and growing each line on a different UltraCode. Now take 20 different ligands for the

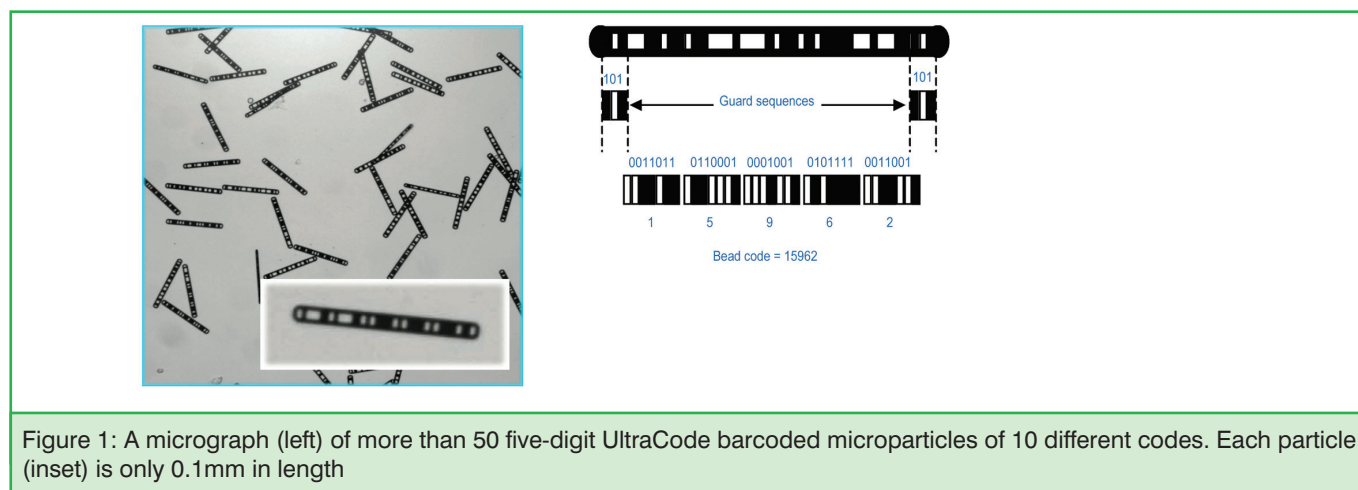
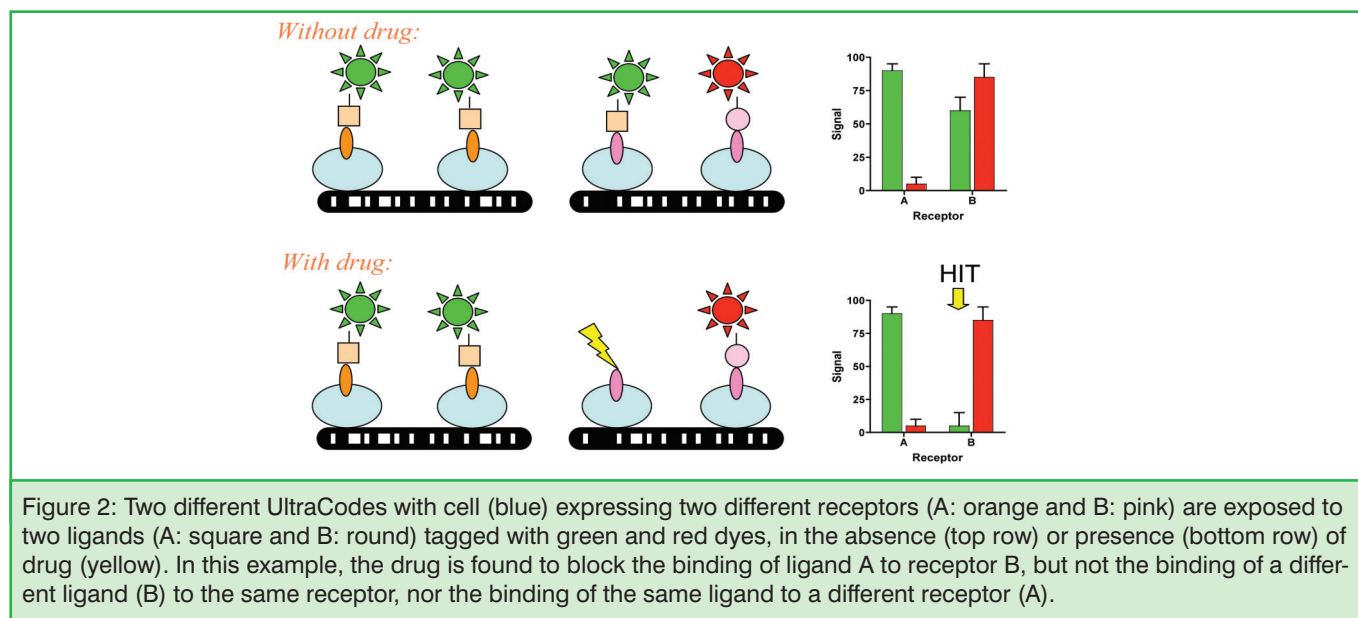


Figure 1: A micrograph (left) of more than 50 five-digit UltraCode barcoded microparticles of 10 different codes. Each particle (inset) is only 0.1mm in length



receptors, each labelled with a spectrally-unique reporter dye. Expose the cells to the ligands in the presence of each compound in the library, and the result is a matrix of the effects of each compound on up to 400 ligand:receptor interactions in a single tube (Figure 2 illustrates this approach using just two receptors and ligands).

Using UltraPlex™, an experiment of this kind can be set up today, with straightforward transfer of existing assay protocols onto the coded particle substrate. In most instances, no special optimisation is required, and the assay reproducibility is as good as using conventional one-analyte-at-a-time technologies. The resulting output can be captured using the medium-throughput SmartReader™ from Pronostics (designed for clinical laboratory applications) or with a number of third-party systems running our dedicated software. The fastest systems can deliver 100,000 data points an hour compatible with the highest throughput screening environments.

The other unique advantage of UltraPlex™ is its scalability. Our 5-digit UltraCode Secure particle design can multiplex 100,000 assays into a single tube (if you have that many well validated assays ready to port across). The only hurdle to screening for binding to every protein in the human proteome in a single tube is the availability of the purified proteins. With UltraPlex™, for the first time it's not multiplexing technology which is limiting access to ultra-high dimensional screening.

UltraPlex™ is already powering an expanding range of multiplexed clinical tests for the hospital laboratory (see www.ultraplex.com for the range of products available today). Some of these products port existing clinical tests and allow them to be performed in parallel in a single tube, saving the healthcare provider time and money. Future tests, such as Pronostics' flagship profiling diagnostic tests, are designed to allow the diagnosis of diseases (such as heart disease) which cannot currently be diagnosed from a simple blood sample alone. In these tests, thousands of analytes could be

measured simultaneously to create an immunological fingerprint of the subject, which could then be compared to libraries of profiles from healthy and diseased individuals – an approach only made possible by the scalability of UltraPlex™ and its robust, low cost microparticles.

Other multiplexing platforms have promised much, but they have been slow to deliver. The principles are accessible and straightforward, but making your assays work in practice has always been considerably more challenging. With UltraPlex™ it's a different experience, and even technically demanding assays, such as those behind most high throughput screens, can be ported across quickly and easily. Even live cell reporter assays are within reach. Equally importantly, the underlying platform technology is as robust as the particles themselves: decoding errors are essentially absent, the signal-to-noise ratio excellent and the sensitivity of most assays meets or exceeds expectations.

Multiplexing is ready to provide a much needed shot in the arm for conventional small molecule screening paradigms. With the ability to select hits with a particular specificity profile against many targets, it should be possible to identify agents with exciting and novel pharmacologies which today depend often on serendipity to find them. After all, learning more about a compound family at the very earliest stages of the discovery process is one of the key drivers of successful innovation. ■

CONTACT DETAILS

Pronostics Ltd
 Babraham Hall, Babraham, Cambridge, CB22 3AT
 United Kingdom
 Tel: +44 1223 496730
 Fax: +44 1223 496731
 E-mail: info@pro-nostics.com
 Websites: www.pro-nostics.com and www.ultraplex.com