Target engagement: Shining a light

The ability to measure binding of a compound to its intended target in live cells or tissue is a critical parameter for drug discovery. A new method using polarised light microscopy adds to the current toolbox by enabling monitoring of target engagement *in vitro* and *in vivo* at single cell resolution.

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Retrospective analyses on positive outcomes in drug development programmes have identified a number of parameters which appear key to enable the successful transition from identification of a target to clinically efficacious agents and ultimately drug approval. These parameters, sometimes also referred to as "the four pillars"[1] or "the five R's"[2], include the ability to demonstrate that a given compound engages, that is binds to the target of interest in cells or tissue. A majority of the currently available methods provide an average view of target engagement across a cell population or tissue sample and have limited applicability to *in vivo* settings. In this issue, Dubach *et al.*[3] present an approach based on fluorescence polarisation microscopy which measures alterations in anisotropy due to the displacement of "companion imaging probes" (CIPs) from protein targets by test compounds (**Fig. 1**). Thanks to the spatial resolution of microscopy the CIP strategy allows for monitoring of drug target binding in cells and animal models in single cells.

Cellular target engagement assays aim to provide evidence that a compound binds to its designated target in live cells. Some approaches determine direct physical binding (e.g. chemical proteomics) whereas others such as fluorescence- or bioluminescence resonance energy transfer (FRET/BRET)-based assays use spatial proximity as a surrogate (for a recent review of general target engagement approaches see ref[4]). All these strategies have individual benefits as well as limitations such as they either require chemical modification of the compound of interest, tagging of proteins, or both. Furthermore, transferring these assays into tissue or even animal models represents a substantial challenge. The cellular thermal shift assay (CETSA)[5] provides a label-free method that addresses some of these potential issues. The power and potential scope of this approach has recently been demonstrated by the successful combination of CETSA and quantitative protein mass spectrometry[6-8]. However, more data is needed to better estimate the CETSA-compatible fraction of the proteome. Considering the heterogeneity and context-dependence of cellular responses to various stimuli[9] an ideal method would also enable the measurement of drug target engagement on the single cell level.

Building on previous efforts to monitor drug target engagement by utilizing fluorescence polarisation microscopy in combination with drug derivatives conjugated to fluorophores[10], Dubach *et al.*[3] hypothesised that the approach could be extended to unlabelled compounds if these compete with target-specific companion imaging probes (CIPs) for binding to the respective target (**Fig. 1b,c**). Detection is enabled by changes in anisotropy when the CIP is bound to the target (i.e. free movement is restrained) versus the CIP being displaced from the protein due to competitive binding of the compound under investigation. In the latter case the fluorescence properties of the CIP become more isotropic due to free rotation, thus enabling the differentiation of unbound and bound states.

The authors demonstrate the feasibility of the approach by investigating two covalent BTK kinase inhibitors as well as two reversible PARP inhibitors (see example Fig 1a) with a single CIP for each respective target highlighting the potential for broadening of the method towards new target classes provided a compatible CIP can be identified. Dubach *et al.*[3] were also able to derive apparent intracellular K_d values for the unmodified compounds and, notably, measure target engagement of a reversible (PARP) inhibitor in live xenografts. Taking advantage of the single cell resolution provided by the microscopy approach, the results also confirmed expectations that target occupancy can vary significantly between subpopulations of cells. This observation supports the notion that methods based on bulk measurements of cells and tissue are likely to provide a blurred view of compound action.

The method developed by Dubach and colleagues nicely complements the existing suite of target engagement assays suitable for *in vivo* applications. Whilst covalent inhibitors can be studied in resected tumours after dosing of animals, reversible inhibitors can be evaluated via intravital microscopy which - albeit currently being limited to the use of window chambers – represents a significant advantage over previous approaches. Although this is a label-free approach in theory, the method still relies on the need to use optimised CIPs that require validation of the potency and specificity. Particularly the latter is important since on- versus off-target engagement cannot be distinguished if the CIP and the test compound have common off-targets. Furthermore, other aspects such as the nature of the fluorophore which may affect CIP uptake, distribution and/or intracellular localization need to be considered in the design of CIP probes. In general, chemistry as well as overall cellular abundance of the respective targets of interest could be limiting and provide challenges in some cases. Nevertheless, future efforts providing CIPs for other target classes such as the vinblastine probe used by Dubach *et al.*[3] will further expand the scope of the technology. In combination with other single cell approaches such as single cell RNA sequencing and mass cytometry, this method may provide scientists with an improved, more physiologically relevant view of compound action in living systems.

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Competing financial interests

The author declares no competing financial interests.

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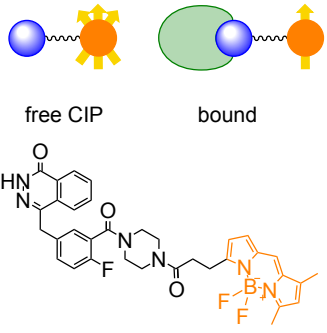
Figure legends

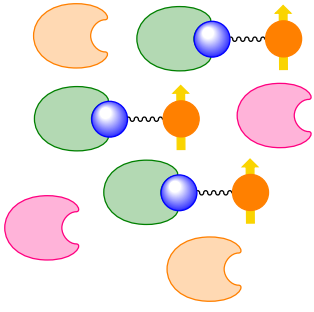
Figure 1. Measuring cellular target engagement of unlabelled drugs by using fluorescence polarisation microscopy in combination companion imaging probes (CIPs). (**a**) *Top:* Free rotation of the unbound CIP confers low anisotropy upon excitation whereas restrained movement increases anisotropy. *Bottom:* Example for a PARP CIP with the BODIPY fluorophore highlighted in orange. (**b**) In absence of competitor/target engagement by the compound of interest the CIP is fully bound to the target leading to high anisotropy. (**c**) Displacement of the CIP from the target by binding of unlabelled compound decreases anisotropy.

Figure 1

a



b



c