Harnessing the power of Acoustic Mist Ionisation MS to identify redox cycling compounds in HTS outputs.

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High-throughput screening (HTS) outputs routinely contain false positive hits caused by samples acting via undesirable mechanisms of action. Pursuit of these 'hits' can result in wasted time and resource, making their early identification highly desirable. Redox cycling compounds (RCCs) produce reactive oxygen species, such as H_2O_2, which can damage protein function and falsely appear as hits. The high throughput assays available to identify RCCs in are indirect, have limited sensitivity or are prone to flagging false positives. The direct measurement of tris(2-carboxyethyl)phosphine (TCEP) oxidation was demonstrated to be a sensitive and accurate measure of redox cycling. However, the current NMR based detection method is not compatible with the magnitude required for triage of an HTS campaign (Tarnowski et al. 2018). The development of Acoustic Mist Ionisation MS (AMI-MS) has made it possible to rapidly measure oxidation of TCEP at large scale. AMI-MS uses acoustic energy to fire nanolitre volumes of ionised sample directly from microtitre plates into the mass spectrometer, allowing for multiple ion species to be measured rapidly and simultaneously. Utilising this technique we are now able to accurately flag RCCs that catalyse the oxidation of TCEP, in a high throughput manner (2 samples per second). Applying this assay to HTS outputs revealed that 80% of hits from an Open Innovation project and 18% of hits from a dehyrdogenase target were capable of redox cycling. Incorporating this triaging assay into our standard HTS screening cascade has facilitated the delivery of high-quality data that facilitates faster prioritisation of robust lead series.

Tarnowski, M., A. Barozet, C. Johansson, P. O. Eriksson, O. Engkvist, J. Walsh, and J. W. M. Nissink. 2018. 'Utility of Resazurin, Horseradish Peroxidase, and NMR Assays to Identify Redox-Related False-Positive Behavior in High-Throughput Screens', *Assay Drug Dev Technol*, 16: 171-91.