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**Title**: Activity based profiling for drug discovery.

**Abstract**: Activity based protein profiling (ABPP) is emerging as a game-changing tool for drug discovery, target validation and basic biology. In this issue Chang et al. (2011) report the ABPP-facilitated discovery of JW480, a highly selective, potent and orally bioavailable inhibitor of monoalkylglycerol ether hydrolase KIAA1363 that dramatically impairs *in vivo* growth of human prostate cancer cell lines. Venturing beyond the limits of conventional approaches, competitive and quantitative ABPP permitted direct quantification of JW480 on- and off-targets *in vitro* and *in vivo*. This groundbreaking study emphasizes the growing role of ABPP as a key tool in modern drug discovery.

Identification and characterization of the functionally diverse enzyme complexes that coordinate and control all processes in the cell is among the most important challenges of the post-genomic era. Quantitative understanding of dynamic enzyme activity, integrated from cell up to whole organism, is an essential step towards a unified model of life, and a powerful tool in the increasingly complex search for viable drug targets across all diseases. To understand enzyme function we must decipher the emergent chemistry of proteins, and the application of chemical technologies to this challenge has proven particularly powerful, giving rise to the vibrant multidiscipline of *chemical proteomics*. An ultimate objective of this emerging field is to profile all types of enzymatic activity in whole organisms, a process commonly termed Activity-Based Protein (or Proteome) Profiling (ABPP), presenting some fascinating challenges in chemical biology (Heal et al., 2011). ABPP has origins in work from the 1980s labeling the active site of proteases, but it is only recently that it has matured into a versatile and powerful platform technology. The Cravatt laboratory is a recognized ABPP pioneer, having demonstrated profiling across a remarkably broad range of enzyme classes. In a notable recent study a search for selective inhibitors of members of the serine hydrolase (SH) superfamily (Bachovchin et al., 2010) was implemented using competitive ABPP, in which synthetic molecules compete with the probe for binding to the target enzyme. In this case, a SH-directed fluorophosphonate-rhodamine (FP-Rh) probe was used to profile over 70 hydrolases against more than 150 carbamate inhibitors, ultimately resulting in compounds selective towards single or small groups of SHs. Competitive ABPP is a perfect fit to the aspirations of modern drug discovery, allowing fine tuning of inhibitor selectivity and potency against numerous enzymes in parallel, directly in the native complexity of the proteome. Furthermore, even inhibitors that are selective for/against uncharacterized enzymes for which substrates have not yet been reported can be developed utilizing this methodology. The implications for understanding inhibitor on- and off-targets *in vivo* during drug development are evident, and ABPP holds great promise for avoiding drug attrition due to toxicity or efficacy failures in late-stage clinical trials.

In the current issue Ben Cravatt and co-workers report the development of JW480, a potent and selective carbamate-based inhibitor of KIAA1363 (aka AADACL1), which is a membrane-bound 2-acetyl monoalkylglycerol ether hydrolase. KIAA1363 is a member of the aforementioned abundant and diverse SH superfamily, which includes esterases, thioesterases, lipases, amidases, and proteases. Several SHs are implicated in the development and progression of tumors (Nomura at al., Nat Rev Cancer 2010), but unfortunately the biological and physiological functions for many of these potential pharmacological targets remain poorly understood (Simon and Cravatt, 2010). Increased activity of KIAA1363 results in overproduction of monoalkylglycerol ethers (MAGEs), that in turn are converted into lysophospholipids that stimulate survival, mobility, and aggressiveness of cancer cells (Chiang et al., 2006). KIAA1363 is the second SH enzyme reported recently by the same group to lead to overproduction of pro-tumourigenic lipids. In the former study a combination of ABPP, proteomic, and lipidomic analyses revealed a key role of monoacylglycerol lipase (MAGL) in levelling these fats (Nomura et al., Cell 2010).

The current study evolves from this work, focusing on the KIAA1363-MAGE pathway in prostate cancer cells with recently discovered lead compounds (Bachovchin et al., 2010) providing a starting point for rational design of more potent and selective analogues. In initial experiments increased activity of KIAA1363, measured with the FP-Rh probe and 1D PAGE-fluorescence readout (Figure 1) was shown to correlate with elevated levels of MAGE in aggressive cancer cell lines. Next a sizable library of carbamates was designed and tested, ultimately resulting in JW480, a highly selective and potent (IC50 = 6 nM) mechanism-based inhibitor of KIAA1363 in living aggressive prostate cancer cells. The authors leveraged competitive and quantitative variants of ABPP technology to demonstrate that the inhibitory action of JW480 (1M) is maintained in these cells for at least 48 hours with no signs of off-target reactivity, with the exception of carboxylesterase ES1, a cross-reactivity target common for carbamates. Furthermore, highly selective inhibition of mouse brain KIAA1363 by JW480 can be achieved *in vivo*, which has striking potential implications in the context of brain tumor progression (Albert and Anderson, 1977) and lipid signaling in the CNS. Finally, in an aggressive prostate cancer model (Figure 1) severe reduction, but not complete blockage, of tumor growth was observed in mice to which JW480 was administered orally.

The data presented make a strong case for JW480 as a near-ideal tool for further studies on the cellular biochemistry of hydrolase KIAA1363, and the lipid signaling network within which it is embedded. The authors have delivered a useful and practical compound that is readily synthesized, orally bioavailable and CNS penetrant, with important potential applications extending beyond cancer to CNS and metabolic disorders (Homan et al., 2011). Most importantly to scientists trying to understand lipid signaling, by exploiting the power of ABPP the authors have gone much further than traditional biochemical approaches permit to validate the on-target effect of their compounds, and eliminate potential off-target effects. In future investigations it will be interesting to see whether bio-orthogonally tagged or fluorescent analogues of JW480 can be used to cross-confirm selectivity in a manner not biased towards the SH superfamily, and for in vivo imaging studies of KIAA1363 activity. The other standout feature of this work is the elegant combination of activity-based proteomics with targeted lipidomics, resulting in new information about the role of the target enzyme in the network as a whole. Taking this a step further, potential feedback of aberrant lipid metabolism into changes in post-translational lipidation of proteins is an intriguing prospect for future exploration, since aberrant protein lipidation is also implicated in the progression of cancer (Heal and Tate, 2010). As ABPP becomes integrated with other high-throughput post-genomic and post-proteomic tools, a multi-dimensional analytical platform is now on the horizon that has the potential to revolutionize both modern drug discovery and our fundamental understanding of living systems.

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**Figure 1**. ABPP assisted discovery of selective inhibitor JW480 against serine protease KIAA1364, the activity of which is up-regulated in aggressive cancer cells. Orally administered JW480 also significantly impaired the growth of tumor in a mouse xenograph model.