Bioluminescent Kinase Profiling Systems For Characterizing Small Molecule Kinase Inhibitors

Hicham Zegzouti, Jacquelyn Hennek, Tracy Worzella, Monse Contreras, Cristopher Cowan and Said Goueli

Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

1. Introduction

In order to profile compounds against a broad panel of kinases, in-house profiling requires rigorous kinase assay development. Most importantly, it requires an optimization for each kinase in the panel, which can be costly and time consuming. On the other hand, outsourcing kinase profiling is fraught with obstacles such as requirements of agreements, long timelines and lack of control over the whole process. Thus, a profiling system with simple and rapid in-house implementation would obviate such logistical inconveniences and concerns.

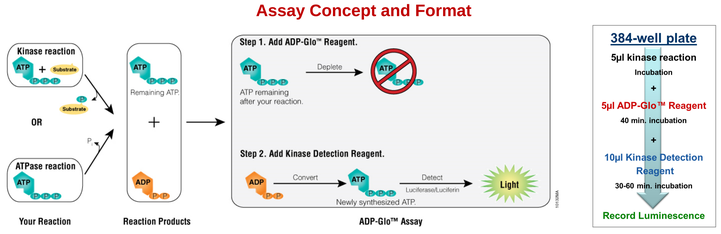
We created new kinase profiling systems based on the luminescent ADP-Glo™ kinase assay platform. The kinase profiling systems have the following features and advantages:

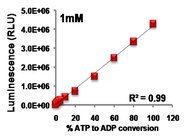
- Set of kinases organized by kinase families, presented in easy to use multi-well strips, and standardized for optimal kinase activity.
- The strip system provides flexible kinase inhibitor profiling, as each strip can be used to profile compounds at a single dose or used for a dose response against 8 kinases at once.
- Easily automated with fast and simple reaction assembly.

The data generated with this novel set-up are concordant with published inhibitor potency profiles produced by radioactivity assays. Using this technology we created profiles for 16 small molecule compounds that are approved for different cancers and inflammatory diseases. Medicinal chemists and chemical biologists can easily adopt this novel approach for regular in-house kinase inhibitor profiling and gain more control over the data for fast progression into developing lead compounds.

2. Positive Detection Assay for Product Formation

Assay Concept and Format

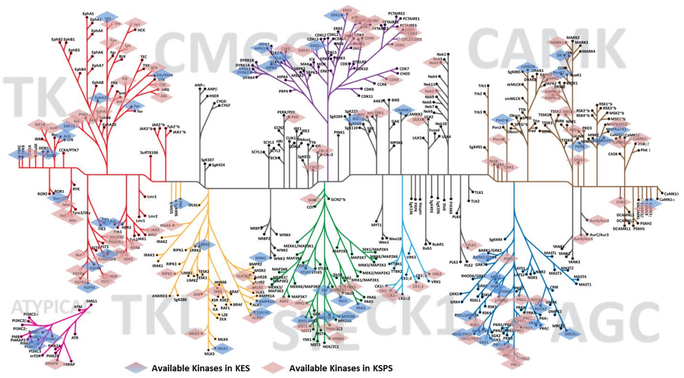




- Universal: Any kinase-substrate combination.
- Wide dynamic range: High sensitivity at low % ATP to ADP conversion allows use of lower amount of enzyme.
- Broad range of [ATP]: (µM to mM) allows distinction between ATP competitive and non competitive inhibitors.

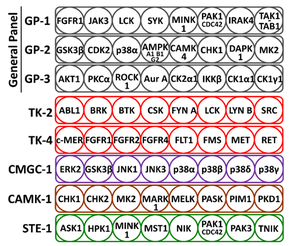
3. Promega Validated Kinase Panel Covers the Human Kinome

Broad Human Kinome Coverage with 174 Protein Kinase Enzyme Systems (KES), Lipid Kinases and 112 Kinases for profiling

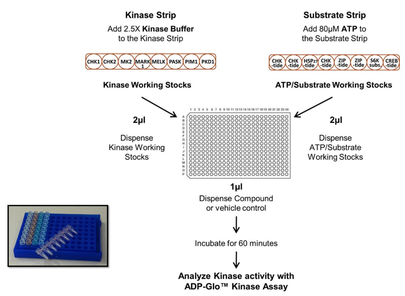


4. Kinase Strips Make Profiling Simple

Kinase Selectivity Profiling Systems Family-Based Strips or General Panel



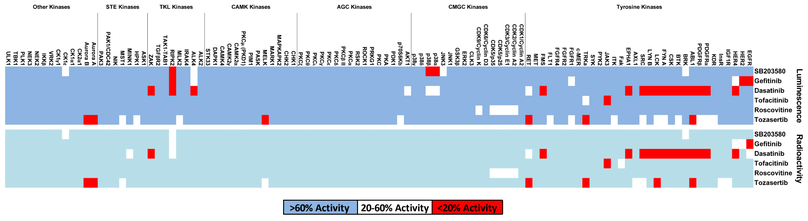
Streamlined Profiling Protocol



- Important kinase targets organized in multi-well strip panels (112 kinases)
- Simple protocol for flexible and targeted inhibitor profiling

5. Enabling Small or Large Selectivity Profiles In-House

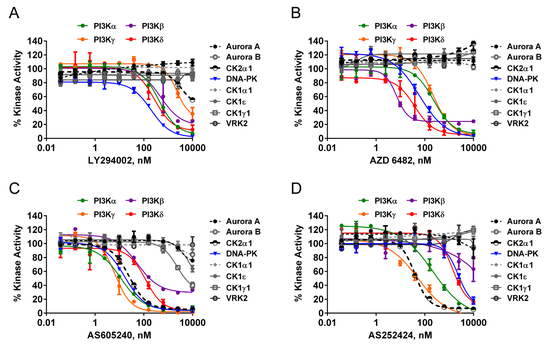
Large Single Dose Profiling (106 kinases)



- Dose response or single dose profiling against 8 or more kinases at once
- Data generated with ADP-Glo™ platform consistent with published potencies of radioactivity-based kinome profiling[1]

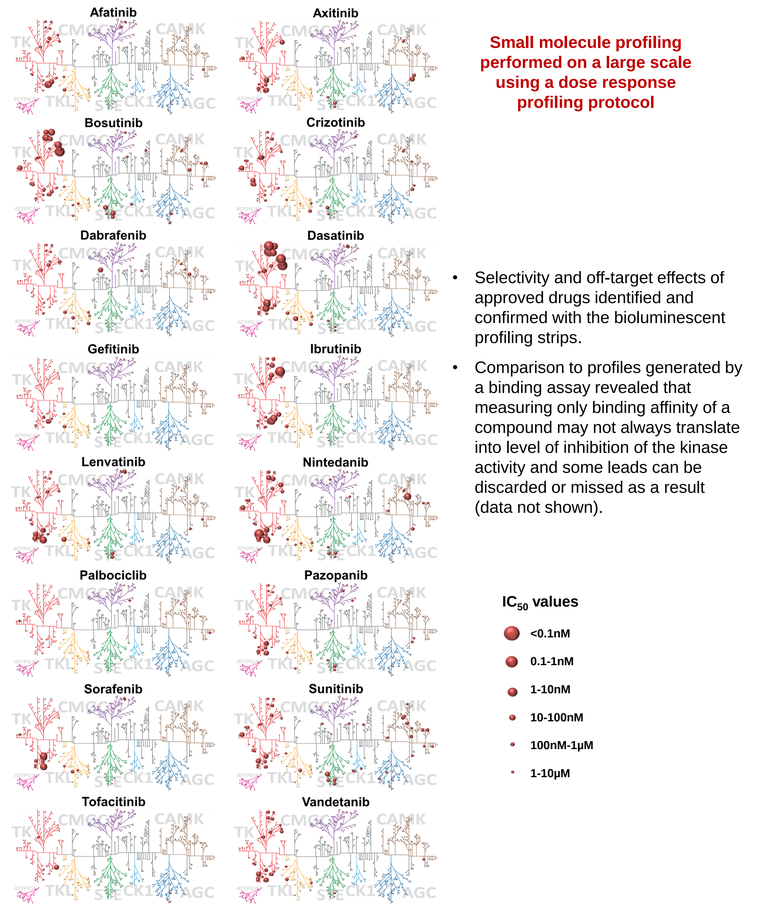
6. Simultaneous Compound Profiling Against Protein and Lipid Kinases

Assembling Lipid Kinases in Strips and Profiling with Protein Kinases



- Profiling kinases in strips to identify compounds' selectivity towards members of PI3 Kinase family.
- Two specific PI3K inhibitors have off-target effects on CK2 that weren't reported before and would have been missed if compounds were not profiled against lipid and protein kinases simultaneously[2].

7. Creating Selectivity Profiles for Approved Drugs Using Kinase Strip-Tubes



Small molecule profiling performed on a large scale using a dose response profiling protocol

- Selectivity and off-target effects of approved drugs identified and confirmed with the bioluminescent profiling strips.
- Comparison to profiles generated by a binding assay revealed that measuring only binding affinity of a compound may not always translate into level of inhibition of the kinase activity and some leads can be discarded or missed as a result (data not shown).

8. Conclusions

ADP-Glo™ Kinase Profiling Systems have the following advantages:

- Fast and simple in house profiling: Two quick dilutions provide working stocks of kinase and substrate/co-factor solutions sufficient for 25 kinase reactions.
- Formatted strips provide access to eight kinases at a time: Kinases from singular kinase families are grouped together for more relevant selectivity profiles.
- One-time use design: Eliminating multiple freeze/thaw cycles ensures optimal kinase activity for each experiment.
- Optimized kinase activity for inhibitor profiling: All kinases have been optimized to provide optimal ADP production with >10-fold S/B.

1. Anastassiadis, T. et al; *Nat. Biotechnol.* 29 (2011), 1039-1045.
2. Hennek, J. et al; *Analytical Biochemistry* 495 (2016), 9-20.

Corresponding author: hicham.zegzouti@promega.com