A simple, direct, and automatable assay for kinase inhibitor binding affinities using fluorescent probe compounds

Sonya M. Hanson,[1] Lucelenie Rodriguez,[1] Mehtap Isik,[1] Jan-Hendrik Prinz,[1] Nick Levinson,[2] and John D. Chodera[1,*]

[1]*Computational Biology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY 10065, United States*[†]

[2]*Department of Pharmacology, University of Minnesota, Minneapolis, MN, 55455, United States*[‡]

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Understanding the specificity of kinase inhibitors has tremendous therapeutic significance. To predict inhibitor selectivity computationally, an iterative approach incorporating experimental measurements is ideal. This work describes the development of a high-throughput label-free fluorescent ligand-binding assay to measure inhibitor affinities. Taking advantage of the intrinsic fluorescence increase of a group of FDA-approved kinase inhibitors upon binding kinases, we are able to measure inhibitor binding affinity with small amounts of protein and without any fluorescent labels. This facilitates rapid characterization of a wide range of kinase inhibitors and kinase resistance mutants, within a system that can be reproduced identically in molecular dynamics simulations.

Keywords: kinase inhibitors, Src kinase, Abl kinase, imatinib, bosutinib, fluorescence

INTRODUCTION

Wolf chartreuse beard [1], paleo bushwick locavore tumblr selvage health goth narwhal post-ironic meggings cronut DIY etsy. Tote bag viral craft beer migas, brooklyn keffiyeh shabby chic wayfarers godard scenester affogato pabst. Humblebrag chartreuse schlitz, post-ironic wolf ethical narwhal salvia everyday carry gastropub venmo kale chips. You probably haven't heard of them cornhole tilde readymade mixtape irony. Sriracha occupy yuccie, green juice roof party fap tumblr hammock mumblecore ramps pabst. Artisan listicle truffaut kogi, shabby chic kombucha distillery etsy cronut +1 pabst mustache VHS vinyl green juice. Before they sold out brooklyn yuccie, gluten-free sriracha lumbersexual four loko kombucha semiotics letterpress biodiesel kale chips art party normcore slow-carb.

I. METHODS

These are the Methods.

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II. RESULTS

These are the Results.

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[1] N. M. Levinson and S. G. Boxer, PLoS ONE **7**, e29828 (2012).

[†] sonya.hanson@choderalab.org
[‡] nml@umn.edu

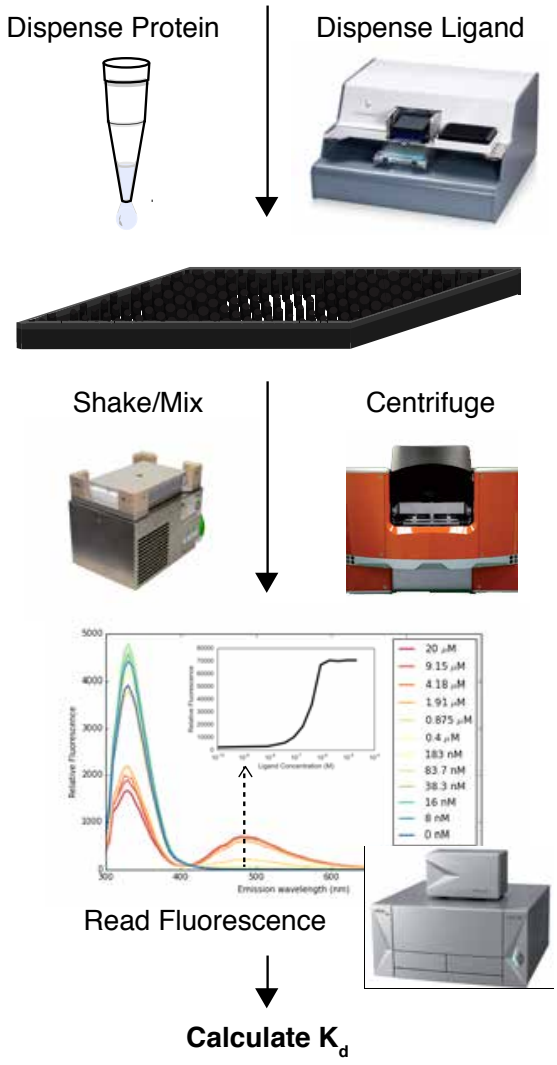

FIG. 1. Overview of the assay protocol. This figure illustrates the assay protocol of this simple, direct, and automatable assay for kinase inhibitor binding affinities using fluorescent probe compounds.

* Corresponding author; john.chodera@choderalab.org

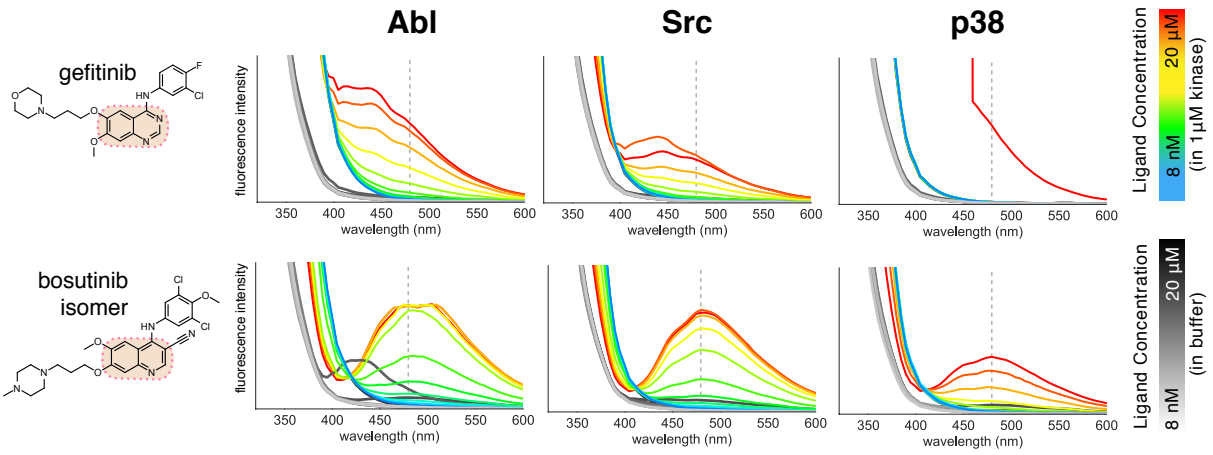

FIG. 2. Example spectra of complex fluorescence when varying ligand concentration. Here it is clear how saturation binding curves from which ligand binding affinities can be calculated can be measured using the fluorescence of the complex of kinases with fluorescent ligands like bosutinib.

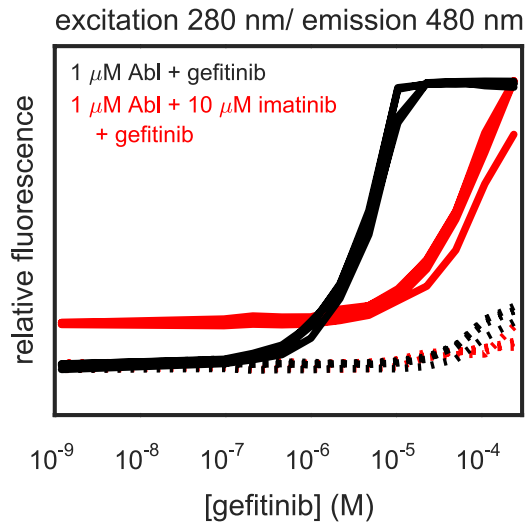

FIG. 3. Competition assays expand the relevance of this assay to innumerable other kinase inhibitors. Here imatinib is used to compete off the fluorescent ligand gefitinib. Thus binding affinities can be measured for even non-fluorescent ligands, expanding the applicability of this simple, direct, cheap, and automatable assay to innumerable other kinase inhibitors.