Functional Interrogation of the Kinome Using Nucleotide Acyl Phosphates

Matthew P. Patricelli,‡ A. Katrin Szardenings,‡ Marek Liyanage, Tyzoon K. Nomanbhoy, Min Wu, Helge Weissig, Arwin Aban, Doris Chun, Stephen Tanner, and John W. Kozarich*  
ActivX Biosciences, 11025 North Torrey Pines Road, La Jolla, California 92037  
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ABSTRACT: The central role of protein kinases in signal transduction pathways has generated intense interest in targeting these enzymes for a wide range of therapeutic indications. Here we report a method for identifying and quantifying protein kinases in any biological sample or tissue from any species. The procedure relies on acyl phosphate-containing nucleotides, prepared from a biotin derivative and ATP or ADP. The acyl phosphate probes react selectively and covalently at the ATP binding sites of at least 75% of the known human protein kinases. Biotinylated peptide fragments from labeled proteomes are captured and then sequenced and identified using a mass spectrometry-based analysis platform to determine the kinases present and their relative levels. Further, direct competition between the probes and inhibitors can be assessed to determine inhibitor potency and selectivity against native protein kinases, as well as hundreds of other ATPases. The ability to broadly profile kinase activities in native proteomes offers an exciting prospect for both target discovery and inhibitor selectivity profiling.

Protein kinases represent the single largest mammalian enzyme family with more than 500 members in the human proteome. These enzymes have been implicated in a wide array of complex cellular functions and pathways, ranging from metabolic regulation to tumorigenesis. Assessing kinase function in vivo is complicated by a high degree of post-translational regulation, generally low expression levels, and overlapping substrate selectivity. Thus, despite intense efforts, the physiological function of the majority of protein kinases remains unknown.

Considerable effort in the pharmaceutical industry is currently directed at the generation of novel protein kinase inhibitors (1). Several kinase inhibitors have been approved for clinical indications, including the anticancer drugs Gleevec (2) and Iressa (3). These drugs, as well as the majority of other kinase inhibitors in development, are designed to bind to the kinase ATP-binding site, thereby preventing substrate phosphorylation. Since the ATP-binding sites of protein kinases are highly conserved, the identification of inhibitors that are both potent and selective is paramount. At present, even the most comprehensive kinase selectivity screens cover less than 30% of the predicted kinome, leaving the majority of kinases unexplored. Recent reports have demonstrated that unexpected activities of protein kinase inhibitors can be found in kinase families structurally remote from the primary target (4, 5), highlighting the need for comprehensive screening. Additionally, due to the high degree of post-translational regulation of kinase activity, it is not clear whether assays of recombinant enzymes address the physiologically relevant state of each kinase. These observations, and the fact that many other families of ATP binding proteins exist with potential affinity for protein kinase inhibitors, highlight the need for improved methods for the comprehensive screening of protein kinases and other ATP binding proteins.

Here we describe a probe-based technology (6, 7) that is uniquely capable of profiling the selectivity of kinase inhibitors against a broad range of protein kinases and other nucleotide binding proteins directly in native proteomes. This method also enables researchers to identify protein kinases and other ATPases with altered expression or activity in human disease or cell models. The method is based on novel biotinylated acyl phosphates of ATP or ADP that irreversibly react with protein kinases on conserved lysine residues in the ATP binding pocket. To date, more than 400 different protein kinases (>80% of the predicted kinome) have been identified and, in most cases, functionally assayed in various mammalian tissues and cell lines using this method.

MATERIALS AND METHODS

Synthesis of (+)-Biotin-Hex-Acyl-ATP (BHAcATP).† To a stirred suspension of N- (+)-biotinyl-6-aminohexanoic acid (30 mg, 0.085 mmol) in 3 mL of a dioxane/DMF/DMSO mixture (1:1:1) were added triethylamine (47 µL, 0.34 mmol) and isobutyl chloroformate (33 µL, 0.255 mmol) at 0 °C. The cloudy mixture was kept at that temperature for 20 min, allowed to warm up to room temperature, and then stirred for an additional 1.5 h. A solution of ATP triethylammonium salt (69 mg, 0.085 mmol) in anhydrous DMSO (1 mL) was added to the mixture described above to give a clear solution. The reaction was monitored by 31P NMR and MALDI. After 18 h, the reaction was quenched with water (4 mL) and the solution quickly extracted with ethyl acetate (3 x 4 mL).

† Abbreviations: BHAcATP, (+)-biotin-Hex-acyl-ATP; BHAcADP, (+)-biotin-Hex-acyl-ADP; Xsite, ActivX Biosciences Inc. kinase profiling platform; MALDI, matrix-assisted laser desorption ionization mass spectrometry.
The aqueous layer was immediately frozen and lyophilized. The resulting solid was suspended in water (1 mL), transferred to a pre-equilibrated C18 column (14 mm x 45 mm), and eluted with water. Fractions containing product were immediately frozen and lyophilized to yield 14 mg (15% yield) of a white powder. The product, which was obtained as a triethylammonium salt (2.5 equiv), was characterized by MALDI (C_{26}H_{41}N_{8}O_{16}P_{3}S, calcd 846.16, seen 845, 846, 847) and NMR (see the Supporting Information): 1H NMR (400 MHz, D_{2}O) δ 8.57 (s, 1H, purine 8-H), 8.28 (s, 1H, purine 2-H), 6.12 (d, J = 6.0 Hz, 1H, ribose 1-H), 4.56 (m, 2H), 4.36 (m, 2H), 4.22 (m, 2H), 3.22 (m, 2H), 3.21 (q, J = 7.2 Hz, 15H, triethylamine-CH_{3}), 3.19 (m, 2H), 3.09 (m, 2H), 2.95 (m, 1H), 2.74 (m, 1H), 2.37 (m, 2H), 2.20 (m, 2H), 1.50 (m, 6H), 1.38 (m, 4H), 1.26 (m, J = 7.2, 22.5 Hz, triethylamine-CH_{2}^{-}); 31P NMR (162 MHz, D_{2}O) δ -10.44 (d, J = 19.8 Hz, 1P), -18.71 (d, J = 19.6 Hz, 1P), -22.66 (dd, J = 19.6, 19.8 Hz, 1P).

Synthesis of (+)-Biotin-Hex-Acyl-ADP (BHAcADP). To a stirred suspension of N-(+)-biotinyl-6-aminohexanoic acid (40 mg, 0.111 mmol) in 3 mL of a dioxane/DMF/DMSO mixture (1:1:1) were added triethylamine (47 μL, 0.34 mmol) and isobutyl chloroformate (31 μL, 0.222 mmol) at 0 °C. The cloudy mixture was kept at that temperature for 20 min, allowed to warm up to room temperature, and then stirred for an additional 1.5 h. A solution of ADP triethylammonium salt (70 mg, 0.111 mmol) in anhydrous DMSO (1 mL) was added to the mixture described above to give a clear solution. The reaction was monitored by 31P NMR and MALDI. After 18 h, the reaction was quenched with water (4 mL) and the solution quickly extracted with ethyl acetate (3 x 4 mL). The aqueous layer was immediately frozen and lyophilized. The resulting solid was suspended in water (1 mL), transferred to a pre-equilibrated C18 column (14 mm x 45 mm), and eluted with water. Fractions containing product were immediately frozen and lyophilized to yield 14 mg (13% yield) of a white powder. The product, which was obtained as a triethylammonium salt (1.5 equiv), was characterized by MALDI (C_{26}H_{40}N_{8}O_{13}P_{2}S, calcd 766.19, seen 765, 766, 767) and NMR (see the Supporting Information): 1H NMR (400 MHz, D_{2}O) δ 8.54 (s, 1H, purine 8-H), 8.28 (s, 1H, purine 2-H), 6.14 (d, J = 6.0 Hz, 1H, ribose 1-H), 4.75 (m, 2H), 4.52 (m, 2H), 4.37 (m, 2H), 4.22 (m, 2H), 3.22 (m, 2H), 3.17 (q, J = 7.2 Hz, 9H, triethylamine-CH_{3}), 3.05 (m, 2H), 2.95 (m, 1H), 2.74 (m, 1H), 2.37 (m, 2H), 2.20 (m, 2H), 1.50 (m, 6H), 1.45 (m, 2H), 1.32 (m, 3H), 1.26 (m, J = 7.2, 13.5 Hz, triethylamine-CH_{2}^{-}), 1.17 (m, 2H); 31P NMR (162 MHz, D_{2}O) δ -10.73 (d, J = 22.0 Hz, 1P), -18.73 (d, J = 22.0 Hz, 1P).

Cell Lysate Preparation. Cell pellets were resuspended in 4 volumes of lysis buffer (20 mM Hepes, 150 mM NaCl, 0.1% Triton X-100, and 20 mM MnCl_{2}) and sonicated using a tip sonicator. Lysates were cleared by centrifugation at 10000g for 60 min. The cleared lysates were filtered through a 0.22 μm filter and gel filtered into fresh lysis buffer using Bio-Rad 10DG columns. Gel filtration was performed to remove endogenous nucleotides, particularly ATP which inhibits probe reaction with kinases.

General Labeling and Sample Preparation. Gel-filtered cell lysate at a total protein concentration of 5 mg/mL (0.5
mL) was labeled with either BHAcATP or BHAcADP at 20 μM, for 5 min. For drug selectivity analysis, the inhibitors were added to the proteomes to the desired concentration and preincubated for 5 min prior to probe addition. The remaining sample preparation steps were essentially as previously described (8). Briefly, samples were reduced with DTT, and cysteines were blocked with iodoacetamide and gel filtered to remove excess reagents and exchange the buffer. The gel-filtered sample was digested with trypsin, and then probe-labeled peptides were isolated with streptavidin-agarose beads (Pierce, catalog no. 20349). Following washing, the labeled peptides were eluted at 65 °C with a 50% CH3CN/water mixture with 0.1% TFA.

**Mass Spectrometry Analysis.** Samples were analyzed by LC–MS/MS on Finnigan LCQ Deca XP ion trap mass spectrometers essentially as described previously (8). Data were searched using the Sequest algorithm with searching and scoring modifications described previously (8, 9). A modification mass of 339 Da was used as a variable modification on lysine for search purposes.

**Sequence Alignments and Generation of Sequence Logos.** Protein kinase sequences were obtained from http://www.kinase.com/kinome (10, 11) and aligned using ClustalX (12). Sequence logos shown in Figure 1 were generated with the Delila suite of programs (13).

**RESULTS**

**Probe Design and Synthesis.** Irreversible, active site-directed probes for various enzyme families have been developed by us (14) and others (15). These probes typically consist of a recognition element or scaffold that provides specificity for the enzyme class and a reactive group that forms a covalent bond in the active site of the target. For detection and quantification of the labeled proteins, a biotin or fluorophore tag must also be attached through a linker at a position that does not interfere with binding. The development of a protein kinase probe faced several challenges. First, kinases do not have an activated catalytic nucleophile in their ATP binding site that can be specifically targeted by an electrophile and used to discriminate against other enzymes, as is the case with serine (16) or cysteine hydrolases (17). Selectivity of the probe, instead, must be imparted by a structural scaffold that binds to the kinase active site and properly positions a reactive group that generates an irreversible bond. Second, the scaffold must be broadly specific to capture the majority of the >500 members of the kinase family in human proteomes. Finally, the biotin or fluorophore tag must be attached to the probe in a way that would not interfere with binding.

The obvious choice of a scaffold for a kinase-directed probe is ATP. First, all kinases bind to ATP and/or ADP with reasonable affinity and in a predictable orientation. Sequence comparisons have shown that virtually all protein kinases have at least one conserved lysine residue within their active sites (Figure 1). One lysine is found in the ATP binding loop region of the kinase primary sequence in all "typical" protein kinases with few exceptions (10, 11). A second lysine is found two residues to the C-terminus of the catalytic aspartic acid in the majority of serine/threonine kinases. Cocrystal structures of protein kinase catalytic
domains bound to ATP reveal that these lysine residues are positioned in the proximity of the $\beta$- and $\gamma$-phosphates of bound ATP (Figure 1) (18). Consequently, by using ATP or ADP as a scaffold, the probe should bind to all protein kinases in an orientation that would place a portion of the probe in the proximity of an $\epsilon$-lysine amine. To convert ATP and/or ADP into a covalent probe, a reactive group had to be positioned near the location of the ATP $\beta$- or $\gamma$-phosphate group without significant disruption of the key binding elements of the nucleotide. In light of the binding specificity for the adenosine moiety, we chose the relatively unexplored direct attachments of an electrophilic acyl group directly to the terminal phosphate of the nucleotide. Among the number of chemically reactive phosphates, acyl phosphate has the most appropriate reactivity with amines and at the same time sufficient stability in an aqueous environment. Acyl phosphates have been known for more than 50 years; the parent congener acetyl phosphate was extensively studied by Jencks (19, 20), and acyl phosphates of amino acids and AMP are well-studied intermediates in the aminoaacylation of tRNAs (21). Most notably, acetyl phosphate is particularly reactive toward primary amines via addition of the amine nitrogen to the carbonyl group and facile elimination of the phosphate by C=O bond cleavage, resulting in the formation of the corresponding stable acetamide (19, 20). Methyl acetyl phosphate is known to inactivate several enzymes by acylation (22). The electrophilic properties of acyl phosphates have also been used in a number of applications such as cross-linking reagents for hemoglobins (23) and the affinity modification of DNA-recoginizing proteins (24). Little research has been reported for acyl phosphates of di- and triphosphates. Kluger and Huang prepared acyl pyrophosphates and demonstrated their ability to acylate pyrophosphate binding enzymes, such as farnesyl synthetase (25). The acyl phosphates of ADP and ATP containing a fatty acid side chain have been synthesized as potential lipophilic nucleotide analogues by Kreimeyer et al. (26). In our case, their reaction conditions needed only minor modifications for the introduction of the more polar biotin group.

To minimize interference of the biotin tag with the binding of the probe for targeting proteins, a C-6 linker was inserted between the acyl phosphate and the biotin to distance the biotin from the nucleotide. Probes based on ADP (BHAcADP) and ATP (BHAcATP) were synthesized and tested to determine the optimal position for the acyl group to react with one or both of the lysines in the ATP binding site (Figure 2A). On the basis of crystal structures of ATP analogues in kinases, we predicted that the acyl phosphate reactive group on these probes would be positioned near one of the two conserved lysine amines in the kinase active site. The elevated effective concentration of the active site lysine would accelerate reaction with the activated acyl phosphate to form a stable amide bond with the biotin tag, releasing ATP or ADP (Figure 2B).

**Proteome Coverage.** The ability of BHAcATP to profile the adenosine nucleotide-interacting proteome was initially assessed by labeling MDA-MB-435 breast cancer cell lysates and analyzing the samples using our Xsite platform (8, 9). Briefly, proteins were labeled with BHAcATP, followed by proteolytic digestion with trypsin and purification of the probe-labeled peptides with streptavidin—agarose beads. The labeled peptides were subsequently analyzed by LC—MS/MS to determine the identity of the labeled protein as well as the precise site of labeling. Results from a typical experiment are summarized in Figure 3A. In this single experiment, 136 distinct proteins were identified with >95% confidence. Of these proteins, two-thirds (90 proteins) were ATP phosphohydrolases, including 41 protein kinases, metabolic kinases, and other ATPases, such as heat shock proteins. Many of the remaining targets have nucleotide binding functions, including NADH utilization, GTP binding, or DNA/RNA binding. (B) Sequences of the peptides identified for the protein kinases. An asterisk denotes the lysine acylated by the probes. As expected, most protein kinases were labeled on one of two lysines conserved in protein kinases.
function and 15 proteins that are well-characterized but have no known nucleotide recognition ability. Many members of the latter group are abundant proteins and were possibly labeled by the probe in a nonselective manner involving surface lysines. Similar results were observed with BHAcADP, consistent with the dual recognition of ATP and ADP by most of these proteins.

A deeper analysis of the results obtained in the experiment described above yielded even stronger evidence that the probes are acting by the anticipated mechanism of labeling (Figure 3B). Analysis of the labeling sites of BHAcATP on the 41 distinct protein kinases identified in the above experiment revealed that 33 of these kinases were labeled on one of the two highly conserved lysine residues known to make contact with the $\gamma$-phosphate of the substrate ATP. Five of the remaining eight kinases were labeled in regions that are predicted to be in the ATP binding domain on the basis of primary structure analysis. Only three kinases were labeled on sites that could not be confirmed to be directly related to the probe binding in the ATP binding pocket.

To evaluate the overall coverage of the acyl phosphates, an analysis of approximately 4000 MS runs in more than 100 human, mouse, rat, and dog proteomes was conducted with the acyl phosphate probes. Using a probability cutoff of 75% (8, 9), a total of 394 protein kinases were found in this data set (Figure 4). Approximately 73% (288 kinases) of these were labeled at one of the two conserved active site lysine residues, with the remaining 27% labeled on nonconserved lysine residues predicted to reside in or near the ATP binding pocket. Using a more stringent probability cutoff of 95%, 322 different protein kinases were found with 77% (247 kinases) being labeled on one of the two conserved active site lysines. Interestingly, the probes were able to detect 31 of the 39 “atypical” protein kinases, which lack a conserved kinase domain (11). These kinases were labeled on nonconserved lysine residues within the catalytic domain. Thus, consistent with the designed properties, these acyl phosphates appear to be capable of reacting with the majority of protein kinases.

**Kinase Profiles of Cell Lines.** To identify a concise set of proteomes covering a large number of kinases, a set of 10 human cancer cell lines was analyzed using both acyl phosphate probes. An automated algorithm (8, 9) was used to determine the MS signal intensity (signal to noise) for each identified kinase peptide. From this analysis, a total of 110 unique human protein kinases were identified, 88 of which exhibited a robust signal that would allow a quantitative analysis (signal-to-noise ratio of both replicates of >2).
From the 20 conditions that were analyzed (10 cell lines × 2 probes), a set of four was selected that provided an efficient screen: MDA-MB-435 and SKMEL5 cells with BHAcADP and Molt 4 and K562 cells with BHAcATP (Figure 5A). For several kinases, the relative expression levels between the proteomes as indicated by the probe were confirmed by Western blotting (Figure 5B). Thus, the probes can be used to inventory the identities and levels of protein kinases across tissues and/or cells.

Inhibitor Profiling with Staurosporine. To observe changes in kinase activities due to the presence of an inhibitor or changes in biological function, a method for rapidly and accurately quantifying the mass spectrometric signals was required. Historically, quantification based on the MS signal, particularly for complex protein/peptide mixtures, has been difficult due to the inherent instability of signals from electrospray ionization MS data. Methods for dynamic normalization of data from run to run are required to eliminate drifts in signal due to spray inconsistency and detector instability. To this end, the broad coverage of the acyl phosphate probes proved to be very useful for accurate quantification. In a typical LC−MS/MS analysis, 150−300 individual peptides are observed with BHAcADP and BHAcATP. Thus, at any given point in an analysis run, there are invariably multiple peptides either coeluting or eluting very near the peptide of interest. If one assumes that addition of an inhibitor will affect only a small fraction of the peaks in a given run (<20%), the signals of peaks in the proximity of a peak of interest can be compared between runs (i.e., control vs inhibited runs) to generate precise normalization factors. An automated program was developed that performed quantification on the basis of this general idea. More information about the method used for this program will be published elsewhere.

To test the ability of the Xsite platform and MS-based quantification program to profile kinase inhibitors, the broad-spectrum kinase inhibitor staurosporine was added to the four chosen cell lysates and analyzed for inhibition of protein kinases as well as all other targets of the probes. In this experiment, 74 protein kinases were identified, of which 70 were observed with sufficient signal to permit quantification (Figure 6). At 10 μM, 39 targets of staurosporine were identified (Figure 6, red shaded boxes). One cell line, K562, was chosen for the determination of the IC50 of staurosporine against all the identified targets (Figure 7). In the majority of cases, clean dose responses were observed and IC50 values could be determined. In some cases, the signal from the kinase was too weak to observe a smooth dose response.
The results of the MS-based Xsite analysis of the BHAcATP- and BHAcADP-labeled proteomic samples strongly support the hypothesis that these probes interact broadly and specifically with the protein kinase enzyme superfamilies. As predicted by the probe structural features, the majority of the protein kinase labeling events were observed on one of the two conserved lysine residues that are located in the proximity of the γ-phosphate of ATP. Thus, the probes function as intended. Further, on the basis of the structural features of the probes in relation to conserved structural elements of protein kinases, it can be assumed that these probes could potentially label all protein kinases since all protein kinases bind ATP and have at least one lysine residue positioned in the vicinity of the acyl phosphate moiety of the bound probes. The cumulative results of thousands of experiments strongly support this hypothesis with current coverage exceeding 70% of the known kinome. Because some kinases have very restricted expression patterns and/or very low expression levels, it may be difficult to detect these in natural proteomes.

Considering the difficulty in developing a comprehensive kinase profiling platform, the probes described here are remarkably simple. One advantage of the design is that a number of different tags and linkers can be attached without affecting the critical binding of adenosine nucleotide to the enzyme. Further, the relatively large binding group (ATP/ADP) is released in the reaction, leaving the uncharged biotin tag on the protein, facilitating MS analysis. Similarly, adenosine can be replaced by other nucleosides or recognition elements, and the same tag and/or linker would be transferred to the protein. This potentially provides a powerful tool for investigating the binding differences of adenosine and guanosine phosphates, for example, and could further increase the coverage of the nucleotide binding proteome.

Recent work with GTP/GDP probe analogues supports this, revealing significant labeling of GTP-dependent proteins not observed with the ATP/ADP probes. These studies will be reported elsewhere.

Perhaps the only disadvantage with the use of ATP-based probes is the presence of numerous, highly abundant ATP binding proteins such as actin and heat shock proteins, which could interfere with kinase detection. However, as shown here, the use of the Xsite profiling method has circumvented this issue. Critical advantages of this method are the ultrahigh resolution and sensitivity, combined with precise and accurate identification of the labeling site on all target proteins. Thus, the significance of each labeling event with respect to the target protein function can be assessed in most cases. As a case in point, even in an extremely large data set (>4000 MS analyses, >1000000 MS/MS spectra searched) and with a rather modest confidence filter cutoff, approximately 73% of the protein kinases were identified with labeling on one of the two conserved lysine residues. It is important to note, however, that these probes label a large number of enzymes in proteomic samples (>200 in many cases), and only methods with extremely high resolution are suitable for analysis. Gel-based methods (16, 27), for example, are not useful for examining kinases with these probes.

Inhibitors of protein kinases can be screened for selectivity in cell or tissue lysates, as long as the inhibitors are ATP competitive. The strength of this platform for protein kinase inhibitor profiling was demonstrated by the analysis of staurosporine, a nonselective kinase inhibitor, in a variety of cell lysates. A comparison of the IC50 values obtained using our method to a recent literature report using recombinant enzymes expressed as T7 bacteriophage fusions and an inhibitor affinity column for kinase screening (5) is presented in Figure 7 (last column in the table). Our conditions were optimized for both probe concentration and labeling time to ensure that the kinase labeling was in an initial rate phase with subsaturating probe binding. Thus, the IC50 values that are obtained should closely reflect the binding constants of the inhibitor that is analyzed. Most of the IC50 values that are obtained are similar (within approximately 10-fold) to those determined by the T7 fusion method (5), such as the IC50 values of AurA, CAMK2g, CDK2, CDK5, Fer, JNK1, Mark3, and Lyn, while some are substantially different, such as those of RSK2 and SYK. The differences in IC50 values may reflect the normal cellular milieu present in our method, and missing in other methods, that may affect the properties of the kinase and/or the inhibitor. One interesting observation is that, for kinases that contain more than one competent active site, such as the RSK group of kinases (RSK1–RSK3), the two kinase active sites...
can be independently analyzed. This is due to the fact that the peptide sequences surrounding the conserved lysines are distinguishable between the two active sites. In the case of RSK1, it can be seen that staurosporine interacts with the N-terminal kinase domain (IC50 = 0.3 μM) but not with the C-terminal kinase domain. In recombinant enzyme assays, RSK kinases are typically expressed as single-domain kinases; thus, this information cannot be obtained in a single assay and from a single protein.

When considering the results from both inhibitor selectivity analysis and general kinase profiling using these probes, it is important to consider the potential differences between the properties of native and recombinant kinases. Using the method presented here, native protein kinases are analyzed directly in complex protein mixtures, where important post-translational modifications and protein-protein interactions will be preserved. Because of the highly variable nature of kinase activity regulation, it is not possible to accurately predict the extent to which these probes will reflect physiological changes in kinase activation; however, it is expected that many types of regulation will be detectable. Because the probes require a functional ATP binding site, any regulation of kinase activity that prevents or changes ATP binding affinity would be detectable as a decrease or change in the level of probe labeling. Further, because the site of probe labeling is identified for all targets, structural changes in the active site may be observable by a change in labeling site. Additional work involving complementary kinase analysis methods is ongoing to assess the impact of post-translational regulation mechanisms on probe labeling and will be reported.

Overall, these results demonstrate that the probes and analysis methods described here provide an unprecedented ability to broadly profile protein kinase activities in cell lysates for both overall activity and/or expression level and sensitivity to candidate inhibitors without the need for protein expression or substrate assay development. In addition, the acyl phosphate probes provide the framework for a species-independent kinase profiling platform that will enable studies in the comparative biochemistry of kinases that cannot be achieved by recombinant approaches.

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SUPPORTING INFORMATION AVAILABLE

1H NMR and 31P NMR spectra for BHAcATP (and 31P NMR spectra for ATP) and BHAcADP (and 31P NMR spectra for ADP). This material is available free of charge via the Internet at http://pubs.acs.org.
REFERENCES


