DOI: 10.1002/cbic.200800212

An Improved Mechanism-Based Cross-Linker for Multiplexed Kinase Detection and Inhibition in a Complex Proteome

Kai Liu,[a] Karunakaran A. Kalesh,[b] Li Bing Ong,[b] and Shao Q. Yao*[a, b, c]

Protein phosphorylation is the most prevalent event in cell signaling.[1] Despite decades of intensive research, there exist several major challenges in the field of phosphoproteomics: 1) how does one identify new kinase–substrate pairs in the ever-expanding phosphosignaling cascades, 2) how can one detect multiple kinases in their native environment, and subsequently 3) look for potent and selective small-molecule inhibitors?[2] Existing biological and chemical methods have offered invaluable tools for the identification of phosphorylated proteins, as well as sites of phosphorylation.[3] Shokat et al. recently developed a mechanism-based cross-linker, OPA-AD (**1**, Figure 1), that potentially allows researchers to use known phosphoproteins/phosphopeptides to identify their upstream kinases.[4] To detect kinases in their native environments, protein-based biosensors have routinely been used but offer limited success.[5] Imperiali et al. recently developed a homogeneous fluorescence-based assay that enables multiplexed kinase detection in cell lysates.[6] Activity-based probes (ABPs), based on either reversible small-molecule kinase inhibitors or irreversible ATP analogues, have also demonstrated good utilities in large-scale kinase detection and identification.[7] None of these kinase-detecting methods, however, has thus far been expanded to the screening and identification of inhibitors against specific kinase–substrate pairs in their native states. Herein, we report an improved mechanism-based cross-linker, NDA-AD (**2**, Figure 1), based on the originally reported **1**, for multiplexed detection and inhibition of kinase–substrate pairs in a complex proteome.

When **1** was initially used in a complex proteome, we unexpectedly discovered that it produces a large number of nonspecific cross-linking bands (Figure 1C and in the Supporting Information) in addition to the one corresponding to the desired kinase–pseudosubstrate pair (i.e., a kinase peptide substrate in which the S/T/Y phosphorylation site was replaced by cysteine). This severely limits its potential applications.[8] Thus we aimed to modify the highly reactive *o*-phthaldialdehyde (OPA) moiety in **1** and make it compatible with proteomic experiments. Consequently, **2** was designed to covalently trap the transient kinase–substrate–ATP ternary complex formed during the phosphorylation.[4] With an adenosine moiety guiding **2** to the ATP-binding pocket of a kinase, the naphthalene-2,3-dicarboxaldehyde (NDA) group serves as a bifunctional chemical that cross-links the proximal catalytic lysine residue (from the kinase) and the cysteine residues (from the pseudosubstrate); this generates a stable isoindole linkage between the kinase–substrate pair (Figure 1B).[9] We reasoned that NDA would cross-link kinase–substrate pairs more specifically than OPA, because of its more desirable chemical properties and better structural fit in the kinase active site. In the current study with **2** we demonstrate, for the first time, that 1) the cross-linking strategy is compatible with both tyrosine and serine/threonine kinases, 2) it can be used to cross-link the desired kinase–substrate pairs in a crude proteome with high specificity and sensitivity, 3) multiplexed detection of kinases in their native environment is possible, and 4) screening of potent and selective inhibitors of a given kinase–substrate pair can be done in a complex proteome.

We first assessed whether **2** could serve as a general mechanism-based cross-linker for both tyrosine and serine/threonine kinases.[8] The cross-linking reactions were tested with a set of six purified kinases, of which three are Tyr kinases (Csk, Src, and Abl) and the other three Ser/Thr kinases (Erk1, Erk2, and Pka). All kinases were recombinantly expressed and tested to ensure their purity as well as enzymatic activities. Fluorescein-labeled, cysteine-containing kinase pseudosubstrates were chemically synthesized based on their known peptide substrate sequences (Table 1).[10] As shown in Figure 2A, incubation of each of the six kinases, regardless of whether they are Tyr or Ser/Thr kinases, with their cognate pseudosubstrates in the presence of **2** led to the successful cross-linking of the kinase–substrate complex, as indicated by a fluorescent band on the SDS-PAGE. All three components (i.e., kinase, pseudosubstrate, and **2**) were necessary as labeling was not observed in the absence of any of them. No cross-linking was seen with heat-denatured kinases; this indicates that cross-linking was dependent on the active conformation of kinases. To test the tolerance of the cross-linking towards exogenous thiols or amines, the Pka–PKAtide pair was incubated with **2** with increasing concentrations of β-mercaptoethanol (BME) or lysine. Similar to previous reports with OPA-AD, NDA-AD guided cross-linking reactions were not affected by 200-fold excess of exogenous thiols or 1000-fold excess of exogenous amines (Supporting Information). Competition experiments were performed with ATP and LRRASLG-OH (a Pka peptide substrate); a 1000-fold excess of either ATP or LRRASLG-OH was necessary to completely block the cross-linking. To assess whether the degree of cross-linking depends proportionally on the availa-

[a] *K. Liu, Prof. Dr. S. Q. Yao*
Deptartment of Biological Sciences, National University of Singapore
14 Science Drive 4, Singapore 117557 (Singapore)
Fax: (+65) 67791691
E-mail: chmyaosq@nus.edu.sg

[b] *K. A. Kalesh, L. Bing Ong, Prof. Dr. S. Q. Yao*
Department of Chemistry, National University of Singapore
3 Science Drive 3, Singapore 117543 (Singapore)

[c] *Prof. Dr. S. Q. Yao*
NUS MedChem Program of the Office of Life Sciences
National University of Singapore
3 Science Drive 3, Singapore 17543 (Singapore)

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

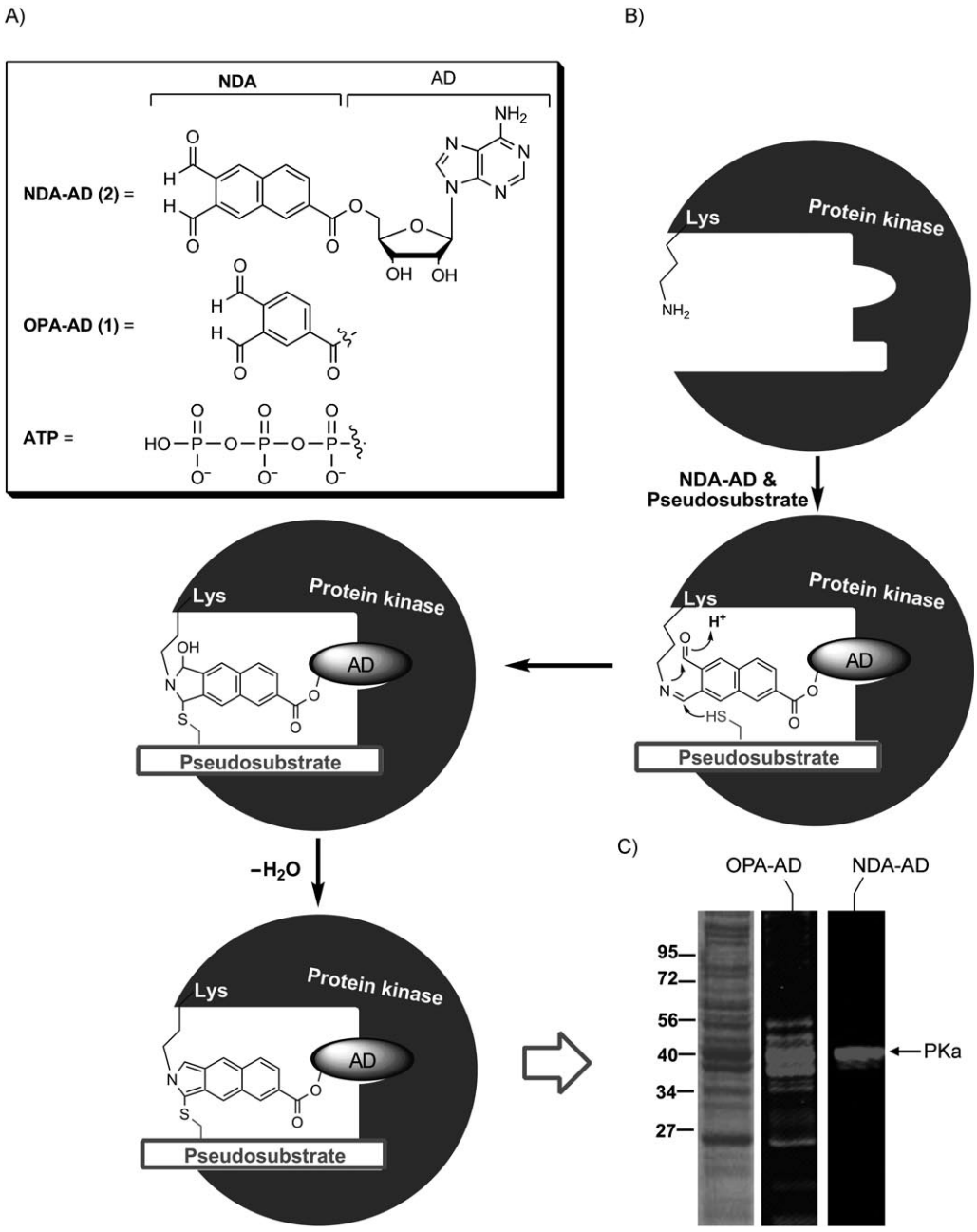

Figure 1. A) Structures of ATP, OPA-AD, and NDA-AD. B) Scheme showing the cross-linking reaction of the kinase–pseudosubstrate–**2** ternary complex. C) Improved cross-linking specificity of a kinase–substrate pair in a crude proteome by **2** versus **1**. Reactions were performed with Pka–PKAtide under identical conditions (Supporting Information); left: Coomassie stained gel.

Table 1. Sequences of kinase pseudosubstrates used in our studies.

Name	Sequence
CSKtide	fluorescein–GG-KKKKEEI**C**FFF
SRCtide	fluorescein–GG-KVEKIGEG**TC**GVVYK
ABLtide	fluorescein–GG-EAI**C**AAPFAKKK
PKAtide	fluorescein–GG-LRRA**C**LG
ERKtide	fluorescein–GG-ELVEPL**C**PSGEAPNQ

[a] The fluorescein-labeled, cysteine-containing pseudosubstrate for each kinase was denoted "XXXtide" after the name of each kinase. [b] The sequences of the pseudosubstrates were based on reported optimal peptide substrates for the kinases, with a cysteine mutation in the phosphorylation site (bold).

bility of the kinase active site, different amounts of the kinase were cross-linked. In addition, the same amount of a kinase was cross-linked in the presence of different amounts of staurosporine (ST)—a general kinase inhibitor.[11] Both results confirmed the dose-dependent nature of the cross-linking against the active kinase. Also shown in Figure 2B, the gel-based inhibition results could be conveniently plotted to generate the corresponding IC_{50} curves so as to obtain quantitative data of the tested kinases against ST; results gave an IC_{50} value of 1016 and 19.9 nM for Csk and Pka, respectively, which is in close agreement with previous literature values.[11] All these

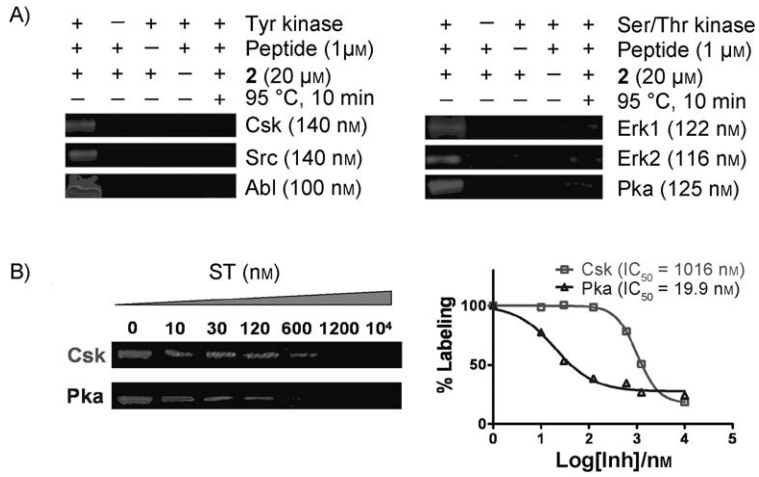

Figure 2. Cross-linking of **2** with purified kinases. A) Fluorescence-scanned gels showing cross-linking profiles of **2** against three different Tyr kinases (left) and three different Ser/Thr kinases (right). B) Cross-linking profiles of Csk and Pka with their pseudosubstrates in the presence of various amounts of staurosporine and the corresponding IC_{50} curves (right). ST: staurosporine; % labeling: the relative fluorescence of cross-linked kinase in the presence of inhibitor (100%: no inhibitor). All experiments were performed in duplicate.

lines of evidence indicate the robustness of the cross-linking reaction by **2** and its potential applications to study kinase–substrate interactions and inhibition in a complex proteome.

To determine the specificity of the cross-linking, Csk and Pka were taken as model kinases and tested against the full set of five fluorescein-labeled pseudosubstrates. As shown in Figure 3, the strongest labeling was observed for a kinase with its cognate pseudosubstrate. Some noticeable "cross-talk", however, was observed between the kinase and other pseudosubstrates. For example, as much as 30–40% cross-linking was observed between Csk and SRCtide (assume 100% cross-linking for Csk/CSKtide). Similarly, up to 20% cross-linking was observed between Pka and SRCtide (assume 100% cross-linking for Pka/PKAtide). We wondered if this was due to the well-documented promiscuous nature of the kinases in their substrate recognition.[2] We therefore performed an in situ phosphorylation assay using the commercially available Kinase-Glo™ Plus kit.[12] Both results from the cross-linking experiments and the phosphorylation assay were compared (Figure 3, graphs). In general, the substrate preferences of the two kinases showed good consistency across the set of substrates (or pseudosubstrates) from the two independent kinase-screening platforms. The high fidelity of **2**-guided cross-linking of specific kinase–substrate pairs again indicates the potential of its application in real proteomic experiments.

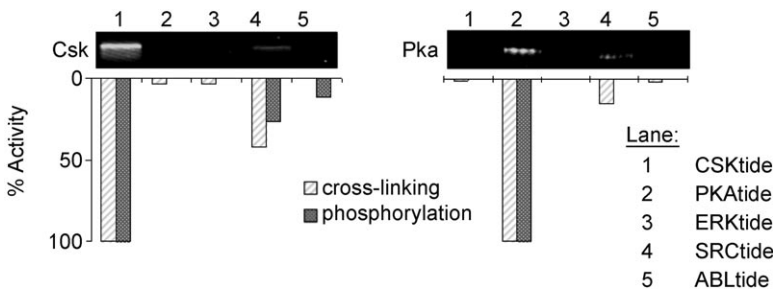

Figure 3. Specificity of **2**-guided cross-linking. Top panels: fluorescence gel images of Csk (left) and Pka (right) cross-linked with five pseudosubstrates. Lower panels: comparison of kinase substrate preferences as determined by cross-linking and phosphorylation assays. % Activity: the relative extent of cross-linking or phosphorylation of a kinase versus different substrates (100%: kinase with its cognate substrate).

We next assessed the **2**-guided cross-linking of specific kinase–substrate pairs in a crude proteome. Lysates from the *E. coli* DE3 strain were used as the crude proteome and spiked with the target kinase (i.e., Csk or Pka). Subsequently, the corresponding fluorescein-labeled pseudosubstrate together with **2** was added. For comparison, identical experiments were performed with **1**, OPA-AD. As shown in Figure 4A, highly specific cross-linking between Csk–CSKtide and Pka–PKAtide pairs was observed with **2**, but not with **1** (Supporting Information) in the presence of the bacterial proteome. It should be noted that no known kinases are present in the *E coli* DE3 proteome. The detection limit of the cross-linking in the crude proteome was further determined by incubating **2**, the pseudosubstrate, and serial dilutions of the kinase; as little as 20 ng (0.3% of total proteome) for Pka and 200 ng (3% of total proteome) for Csk were detectable. The tenfold higher detection limit of Csk might be due to its intrinsically lower enzymatic activity.[13] Our results thus provide the first documented example of cross-linking kinase–substrate pairs in a proteomic experiment.

Many kinases are proven therapeutic targets. Yet highly potent and specific inhibitors against select kinases are relatively scarce because of their propensity to inhibit multiple kinases.[14] Therefore, much effort in kinase research has been spent on developing strategies capable of rapidly screening potential kinase inhibitors that address issues related to efficacy, selectivity, and safety.[15] Most kinase inhibitors developed thus far have been identified from in situ assays that involve the use of recombinant enzymes. As a result, off-targets of the inhibitors often escape unnoticed. Recent advances in activity-based protein profiling (ABPP) have shown that with suitably designed activity-based probes that target specific enzymes, one can screen inhibitors against multiple enzymes in their native environment; this ensures that both the potency and selectivity of these inhibitors are concurrently evaluated.[16] In our study, the highly efficient and specific cross-linking ability of **2** has presented a good opportunity for the strategy to be used for inhibition studies of kinases in the crude proteome. To confirm this, we performed dose-dependent inhibition of Csk–CSKtide and Pka–PKAtide pairs in the bacterial proteome. As shown in Figure 4B, a concomitant decrease in the fluorescent band was observed with increasing amounts of staurophorine. The cross-linking results were further quantified and plotted to generate the

corresponding inhibition curves. The obtained IC_{50} values (6.49 and 40.24 nM for Csk and Pka, respectively) were only 2–5-times higher than that obtained with pure enzymes (e.g., Figure 2B); again this indicates the feasibility and reliability of our strategy for inhibitor screening of kinases in crude lysates.

We next assessed whether the cross-linking strategy could be used for multiplexed detection of kinase activities, as well as their inhibition, in their native environment. Specifically, we asked whether this approach can selectively identify the activity of one kinase in the presence of another, and whether the quantification of the activity of this kinase can be affected by another more abundant kinase. As shown in Figure 5A, by spiking increasing amounts of Pka (0 to 200 ng) and a fixed amount of Csk (400 ng) into the same bacterial proteome, followed by cross-linking with **2** and either PKAtide or CSKtide individually, or together, we were able to detect highly specific cross-linking of Pka–PKAtide and Csk–CSKtide pairs separately and/or together. Furthermore, the presence of the more abundant Csk (400 ng in this case) did not appear to have any noticeable effect on the detection of the less abundant Pka (40 ng in lanes 2, or 10% of Csk). This is a crucial feature of multiplexed experiments in a crude proteome in which multiple endogenous kinases are inevitably present at different expression levels.[6] Next, to assess how the multiplexed kinase detection strategy could be extended for inhibitor discovery, the above experiments were repeated with increasing amounts of staurosporine (Figure 5B). Between 10 to 500 nM of ST was sufficient to completely abolish the Pka–PKAtide cross-linking; this is consistent with its earlier determined IC_{50} value of ~40 nM against Pka. Partial cross-linking of Csk–CSKtide pair was still observed even with 25 μM of ST, which further confirms the poor potency of ST against Csk. Taken together, results herein indicate multiplexed kinase detection and inhibition in a complex proteome is possible with **2** and our strategy described herein.

Finally, we tested whether **2**-guided cross-linking experiments could be extended to the detection of kinases expressed endogenously in cells. Pka was again used as our model kinase as it is ubiquitously expressed in most mammalian cells. To do this, CHO-K1 lysate was treated with **2** and PKAtide, followed by SDS-PAGE analysis, fluorescence scanning, and Western blotting with an anti-Pka antibody. As shown in Figure 6 (left), a number of cross-linked bands were detected, one of which was attributed to the endogenous Pka and was unambiguously confirmed by both Western blot (right) and Pka-spiked experiments. Other fluorescent bands were likely cross-linked products between other endogenous kinases and PKAtide. This is possible because PKAtide might be the substrate of multiple kinases in addition to Pka.[10] Work is underway to further characterize the identity of these unknown bands and results will be reported in due course.

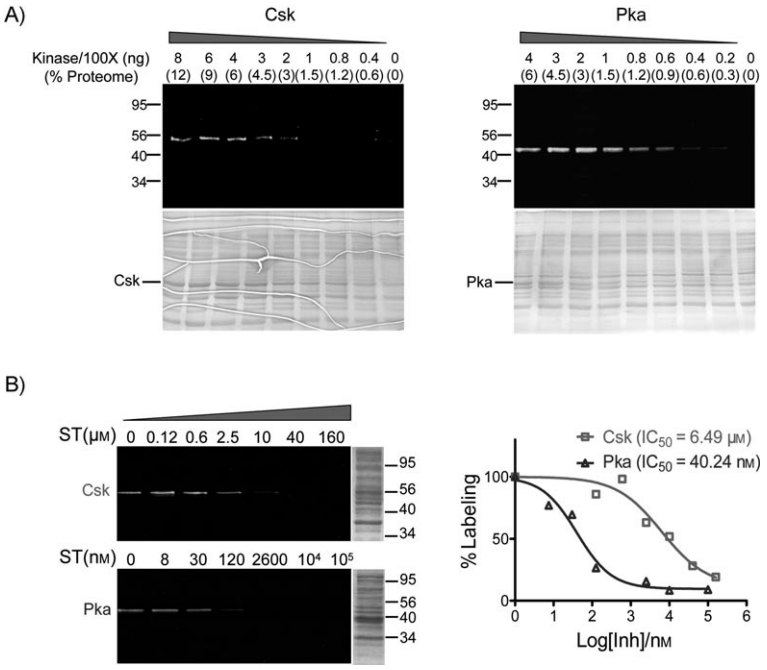

Figure 4. The **2**-guided cross-linking in crude lysates. A) Fluorescent gels showing cross-linking in a bacterial proteome spiked with different amounts of a kinase. B) Kinase inhibition by ST in a complex proteome and the corresponding IC_{50} curves (right). % Labeling: the relative fluorescence of cross-linked kinase in the presence of the inhibitor (100%: no inhibitor).

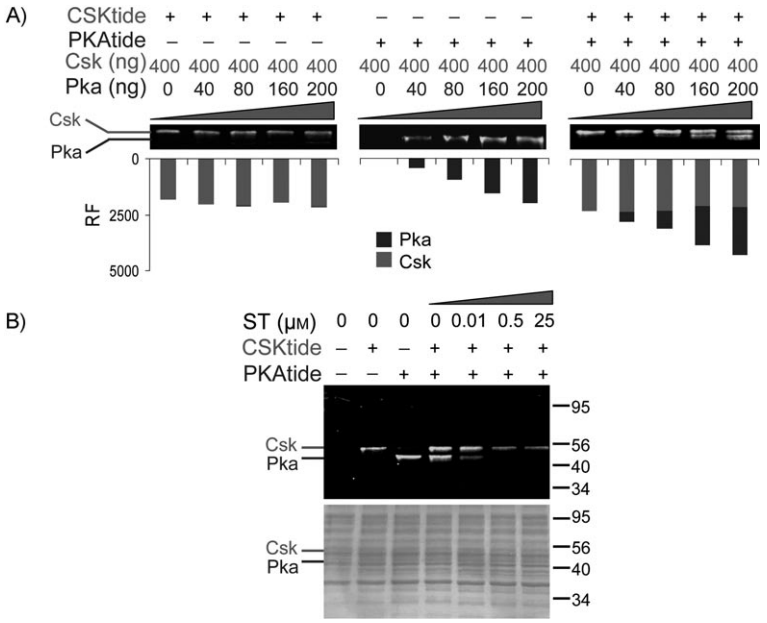

Figure 5. A) Multiplexed kinase detection in a complex proteome. An increasing amount of Pka (0 to 200 ng) and a fixed amount of Csk (400 ng) were used. Gels (left to right) represent cross-linking with CSKtide, PKAtide, or a CSKtide/PKAtide mixture, respectively. Graphs: quantification of fluorescent bands from the corresponding lanes (above). B) Multiplexed kinase inhibition in a complex proteome with increasing amounts of staurosporine (ST); see the Supporting Information for details.

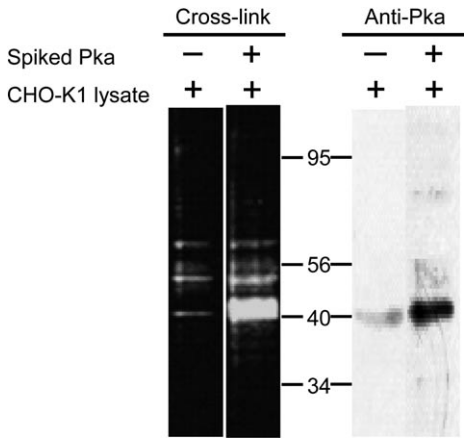

Figure 6. Detection of endogenous Pka expression in CHO-K1 cell lysate by **2**-guided cross-linking. CHO-K1 cell lysates (6 μg), with or without spiked Pka (400 ng), pseudosubstrate (2 μM), NDA-adenosine (20 μM) in the reaction buffer were incubated for 40 min at room temperature before SDS-PAGE analysis. After fluorescence scanning, proteins were transferred to PVDF membrane and probed with anti-Pka antibody.

To conclude, using an improved mechanism-based cross-linker, **2**, we demonstrated for the first time a general chemical approach for the identification of multiple kinase activities directly from the whole proteome. With both purified enzymes as well as kinases present in a crude lysate, we showed unequivocally that the strategy is compatible with not only serine/threonine kinases, but also tyrosine kinases. Preliminary results indicated that the method is robust enough to cross-link endogenous kinases in mammalian cells.[17] Our results also indicated that the approach was useful for multiplexed detection and kinase activities present in a proteome, and is amenable for potential screenings of potent and selective inhibitors of kinases in their native environments. The establishment of the highly specific and sensitive NDA-adenosine guided cross-linking reactions with desired kinase–substrate pairs in their native states represents a step forward towards the creation of novel chemical tools in cell signaling and drug discovery. Studies are in progress to extend the use of this method for the cross-linking of other kinases and their protein substrates.

Experimental Section

Chemical synthesis of NDA-adenosine and general peptide synthesis procedures are reported in the Supporting Information. OPA-adenosine, **1**, was synthesized as previously reported.[4]

Unless otherwise indicated, standard cross-linking reactions were performed as follows: fluorescein-labeled, cysteine-containing, peptide pseudosubstrate (1.0 μM), NDA-adenosine **2** (20 μM), and a desired amount of the kinase (100 to 140 nM) were incubated in 20 μL of the reaction buffer (25 mM HEPES at pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$) for 20 min at room temperature before SDS-PAGE analysis and fluorescence scanning. For inhibition experiments, cross-linking experiments were carried out with various amounts of the general kinase inhibitor, staurosporine.

In situ kinase assay was carried out by using a Kinase-Glo™ Plus Kit (Promega). Briefly, the kinase (12.5 nM for Pka and 14 nM for Csk), an original peptide substrate (100 μM), and ATP (100 μM) in the kinase reaction buffer (20 μL; 25 mM HEPES at pH 7.5, 10 mM $MgCl_2$, 0.1% β-mercaptoethanol, 100 μM Na_3VO_4) were incubated for 20 min at room temperature. Subsequently, Kinase-Glo™ Plus reagent (20 μL) was added. The resulting luminescence was detected by using a Tecan microplate reader, and the amount of phosphorylation was calculated for each kinase–substrate pair by following the manufacturer's instructions.

For the cross-linking of kinase–pseudosubstrate pairs in crude lysates, the bacterial proteome was prepared as described in the Supporting Information and spiked with the desired amount of kinase, with or without staurosporine. Subsequently, pseudosubstrate (1 μM) and **2** (20 μM) were added and the reaction (20 μL; in 25 mM HEPES at pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$) was incubated for 20 min at room temperature before SDS-PAGE analysis and fluorescence scanning.

For the mammalian proteome, CHO-K1 cells (American Type Culture Collection) were grown in DMEM (90%), fetal bovine serum (10%), glutamine (2 mM), and penicillin and streptomycin (100 mg mL^{-1} each) to confluence. Cells were trypsinized and centrifuged. Cell pellets were resuspended in homogenization buffer (10 mM HEPES at pH 7.4, 1 mM EDTA, 150 mM NaCl, 10% glycerol, and complete protease inhibitor mixture). The cells were sonicated (complete lysis: 10 rounds of 1 s on, and 4 s off, at 25% amplitude). The protein concentration of whole lysates were quantified by using the Bradford assay (Biorad) and adjusted to 10.0 mg mL^{-1}. Samples were divided into aliquots, stored at −80 °C, and used for subsequent cross-linking experiments. For the detection of endogenous Pka expressed in CHO-K1 cells, cell lysates (6 μg) with or without spiked Pka (400 ng), PKAtide (2 μM), and **2** (20 μM) in reaction buffer (25 mM HEPES at pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$) were incubated for 40 min at room temperature before SDS-PAGE analysis. After fluorescence scanning, proteins in SDS-PAGE gels were then transferred to a PVDF membrane and subsequently blocked with BSA (5%) in PBST. Membranes were incubated, overnight, at 4 °C with anti-Pka antibody (1:500, Santa Cruz Sc-28892) in 5% (*w/v*) BSA/PBST, together with the SuperSignal West Pico kit (Pierce).

Acknowledgements

We thank Dr. John Kuriyan (UC, Berkeley) for providing the expression construct for recombinant Src and Abl, Dr. Melanie H. Cobb (UTSW) for providing constructs needed for Erk1 and Erk2 expression, Dr. Garnesh ANAND (NUS, DBS) for providing the expression construct for Pka. Funding was provided by National University of Singapore and Agency for Science, Technology, and Research (A STAR), Singapore.*

Keywords: active site · affinity labeling · phosphorylation · protein kinase · proteomics

[1] G. Manning, R. M. D. Whyte, T. Hunter, S. Sudarsanam, *Science* **2002**, *298*, 1912–1934.
[2] J. A. Ubersax, J. E. Ferrell Jr. , *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 530–541.
[3] a) D. T. McLachlin, B. T. Chait, *Curr. Opin. Chem. Biol.* **2001**, *5*, 591–602; b) Z. A. Knight, B. Schilling, R. H. Row, D. M. Kenski, B. W. Gibson, K. M. Shokat, *Nat. Biotechnol.* **2003**, *21*, 1047–1054.
[4] D. J. Maly, J. A. Allen, K. M. Shokat, *J. Am. Chem. Soc.* **2004**, *126*, 9160–9161.

[5] J. Zhang, R. E. Campbell, A. Y. Ting, R. Y. Tsien, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 906–918.
[6] M. D. Shults, K. A. Janes, D. A. Lauffenburger, B. Imperiali, *Nat. Methods* **2005**, *2*, 277–283.
[7] a) M. J. Evans, B. F. Cravatt, *Chem. Rev.* **2006**, *106*, 3279–3301; b) M. C. Hagenstein, J. H. Mussgnug, K. Lotte, R. Plessow, A. Brockhinke, O. Kruse, N. Sewald, *Angew. Chem.* **2003**, *115*, 5793–5796; *Angew. Chem. Int. Ed.* **2003**, *42*, 5635–5638; c) M. P. Patricelli, A. K. Szardenings, M. Liyanage, T. K. Nomanbhoy, M. Wu, H. Weissig, A. Aban, D. Chun, S. Tanner, J. W. Kozarich, *Biochemistry* **2007**, *46*, 350–358; d) J. A. Blair, D. Rauh, C. Kung, C. H. Yun, Q. W. Fan, H. Rode, C. Zhang, M. J. Eck, W. A. Weiss, K. M. Shokat, *Nat. Chem. Biol.* **2007**, *3*, 229–238.
[8] In their original work, that is, in ref. [4], Shokat et al. reported the labeling of **1** with exclusively purified Ser/Thr kinases and peptide pseudosubstrates. No results were disclosed either with kinases spiked in a crude proteome, or with the labeling of Tyr kinases.
[9] S. P. Haris, Y. Zhang, B. Le Bourdonnec, C. R. McCurdy, P. S. Portoghese, *J Med. Chem.* **2007**, *50*, 3392–3396.
[10] a) J. E. Casnellie, *Methods Enzymol.* **1991**, *200*, 115–120; b) H. C. Cheng, H. Nishio, O. Hatase, S. Ralph, J. H. Wang, *J. Biol. Chem.* **1992**, *267*, 9248–9256; c) F. A. Gonzalez, D. L. Raden, R. J. Davis, *J. Biol. Chem.* **1991**, *266*, 22159–22163; d) B. E. Kemp, D. J. Graves, E. Benjamini, E. G. Krebs, *J. Biol. Chem.* **1977**, *252*, 4888–4894; e) H. Sekimoto, C. M. Boney, *Endocrinology* **2003**, *144*, 2546–2552.
[11] F. Meggio, A. Donella Deana, M. Ruzzene, A. M. Brunati, L. Cesaro, B. Guerra, T. Meyer, H. Mett, D. Fabbro, P. Furet, *Eur. J. Biochem.* **1995**, *234*, 317–322.
[12] http://www.promega.com.
[13] D. Sondhi, W. Xu, Z. Songyang, M. J. Eck, P. A. Cole, *Biochemistry* **1998**, *37*, 165–172.
[14] a) J. Baselga, *Science* **2006**, *312*, 1175–1178; b) Z. A. Knight, K. M. Shokat, *Chem. Biol.* **2005**, *12*, 621–637.
[15] M. W. Karaman, S. Herrgard, D. K. Treiber, P. Gallant, C. E. Atteridge, B. T. Campbell, K. W. Chan, P. Ciceri, M. I. Davis, P. T. Edeen, R. Faraoni, M. Floyd, J. P. Hunt, D. J. Lockhart, Z. V. Milanov, M. J. Morrison, G. Pallares, H. K. Patel, S. Pritchard, L. M. Wodicka, P. P. Zarrinkar, *Nat. Biotechnol.* **2008**, *26*, 127–132.
[16] D. Leung, C. Hardouin, D. L. Boger, B. F. Cravatt, *Nat. Biotechnol.* **2003**, *21*, 687–691.
[17] Our system was able to detect Pka with much higher sensitivity than Csk. Although we were able to successfully detect Pka in a crude proteome, it is quite likely that, unless further optimizations are carried out, we might not be able to detect other endogenously expressed kinases, such as Csk, for example, in the crude proteome.

Received: April 2, 2008
Published online on July 15, 2008

