

## Profiling of The Nucleotide Cofactor-Binding Properties Affinities of the Cellular Kinome by Chemical Proteomics

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### OVERVIEW OF SUPPORTING INFORMATION

#### SUPPLEMENTARY FIGURES S1-S4

#### SUPPLEMENTARY TABLES S1-S5

##### Suppl. Table 1: Overview

Overview of profiling experiments including experimental variables

##### Suppl. Table 2a: Kinase abundance

MS1 data from Jurkat and SK-MEL-28 extracts

##### Suppl. Table 2b: RNA vs. protein

Comparison of mRNA and MS1 data for kinases with >10-fold difference in MS1 abundance

##### Suppl. Table 3: Affinities

Average  $IC_{50}$ ,  $pIC_{50}$ , and  $K_d^{app}$  values for MgATP, MnATP, MgADP, MgGTP in Jurkat and SK-MEL-28 cell extracts

##### Suppl. Table 4: Correction factors

Correction factors for calculation of apparent dissociation constants from  $IC_{50}$ s obtained in profiling experiments using CZK2 (ePK-directed) and CZK73 (aPK-directed) beads and Jurkat cell extracts

##### Suppl. Table 5: dasatinib

TP effect on dasatinib  $IC_{50}$  values for kinases captured using CZK2 (ePK-directed) beads

#### SUPPLEMENTARY DATASETS S1-S3

##### Suppl. Dataset 1:

MS1 data from Jurkat and SK-MEL-28 cells

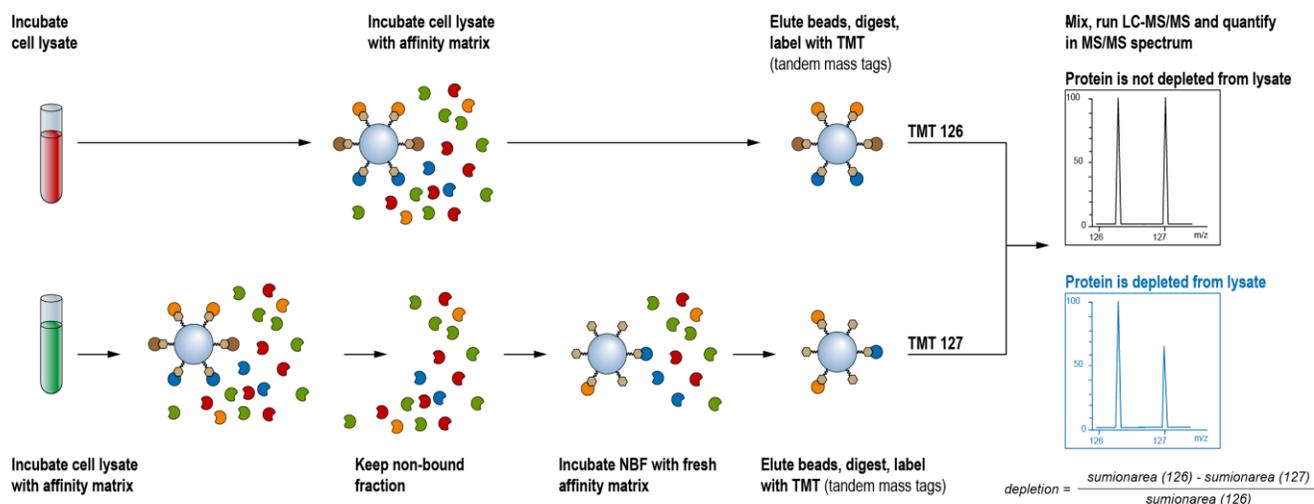
##### Suppl. Dataset 2:

$IC_{50}$ ,  $pIC_{50}$ , and  $K_d^{app}$  values for MgATP, MnATP, MgADP, MgGTP in Jurkat and SK-MEL-28 cells

##### Suppl. Dataset 3:

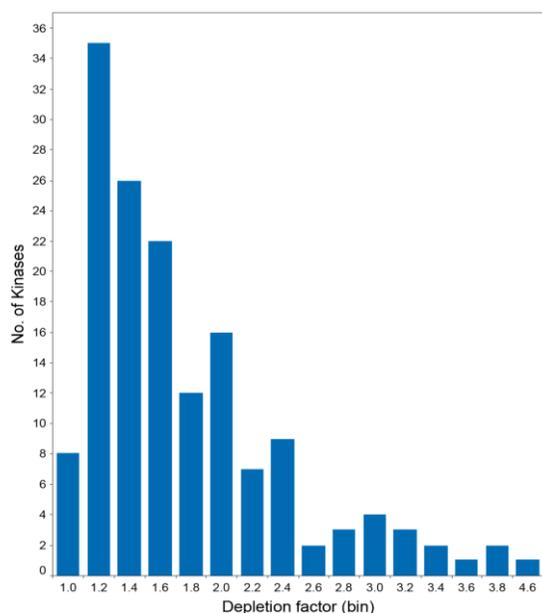
ATP effect on dasatinib  $IC_{50}$  values for kinases captured using ePK/directed beads

## Suppl. Figure S1 (A)

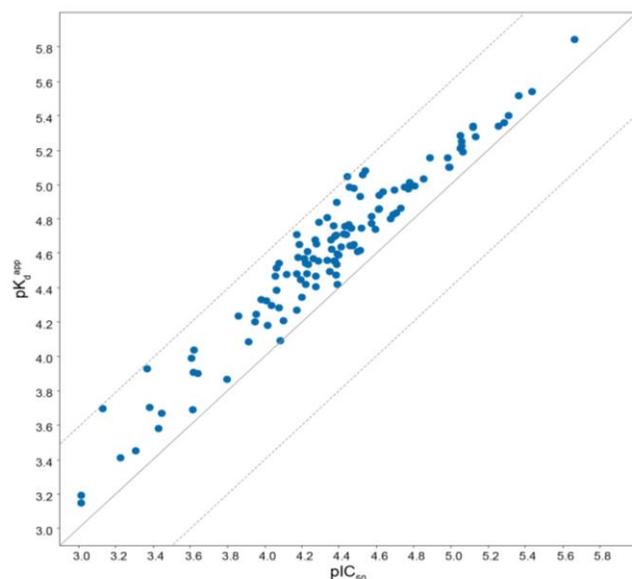


**(A) Determination of protein “depletion factors” required for the determination of  $K_d^{\text{app}}$  from  $IC_{50}$  values**

In order to avoid a substantial deviation of  $IC_{50}$  values from true affinities, it is advantageous to use sufficient amounts of cell extract such that depletion of proteins from the extract is limited (see Bantscheff *et al.*, *Nat. Biotechnol.* 25, 2007, 1035-44, and Supplementary Information therein). This can be ensured by the experimental determination of protein depletion. Cell extract is incubated with the affinity matrix (kinobeads), and captured proteins are eluted, digested and labeled with TMT6plex. At the same time, a second aliquot of cell extract is incubated with the beads, and the non-bound fraction is subjected to a second incubation with fresh affinity matrix. From this second incubation the bound proteins are eluted, digested and labeled with TMT6plex. The relative abundance of retained proteins is determined by the ratios of reporter ion intensities. A protein that is not depleted will have same signals in both samples. The depletion of a protein from the extract by the beads is indicated by a lower reporter ion signal.

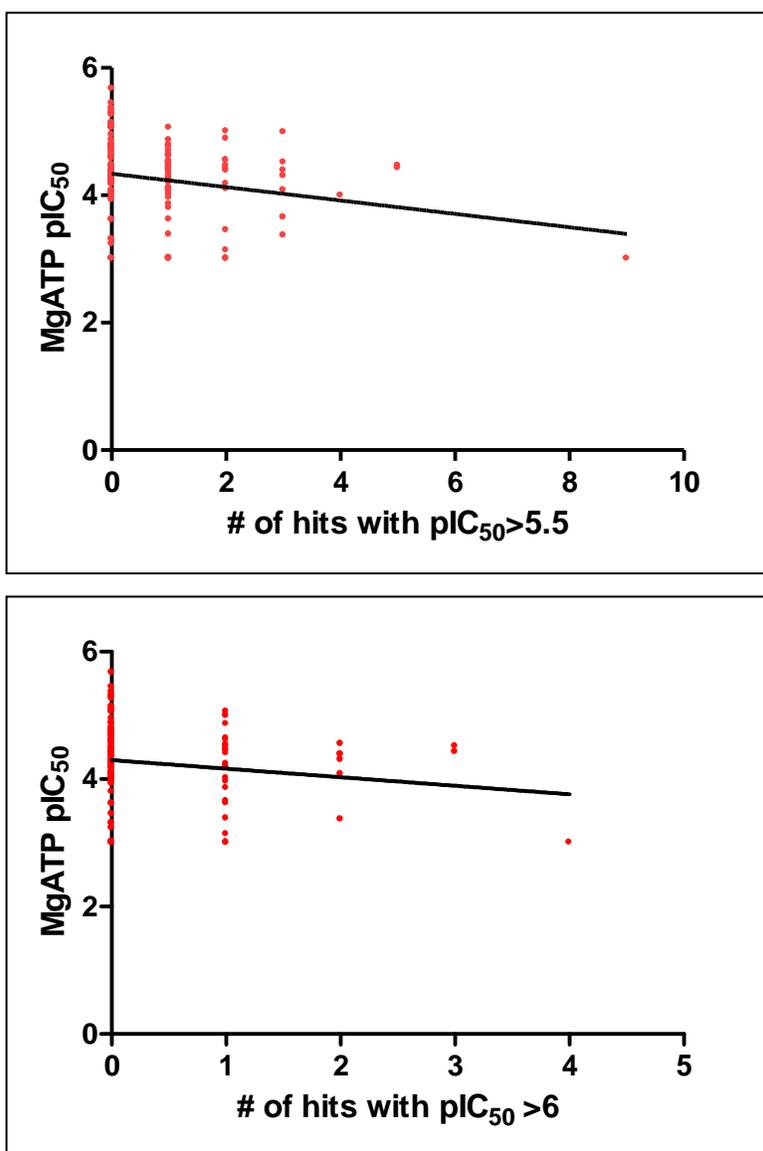
**Suppl. Figure S1 (B,C)**

**(B) Depletion factors for captured kinases are distributed over a narrow range.** Each bar represents the number of kinases with a depletion factor (indicating fold depletion) in the binned range (bins of 0.2). Depletion factors were determined for more than 150 kinases, of which 80% exhibited lower than 2.2-fold depletion.



**(C) Deviation between  $K_d^{app}$  and  $IC_{50}$  values was less than threefold for all kinases;** comparison of  $pIC_{50}$  and  $pK_d$  determined for Mg-ATP,  $K_d^{app}$  values are calculated by dividing  $IC_{50}$  by the depletion factor. Each circle represents a kinase, where on the y-axis the  $pK_d^{app}$  and on the x-axis the  $pIC_{50}$  is displayed. The dashed lines show the 3-fold region.

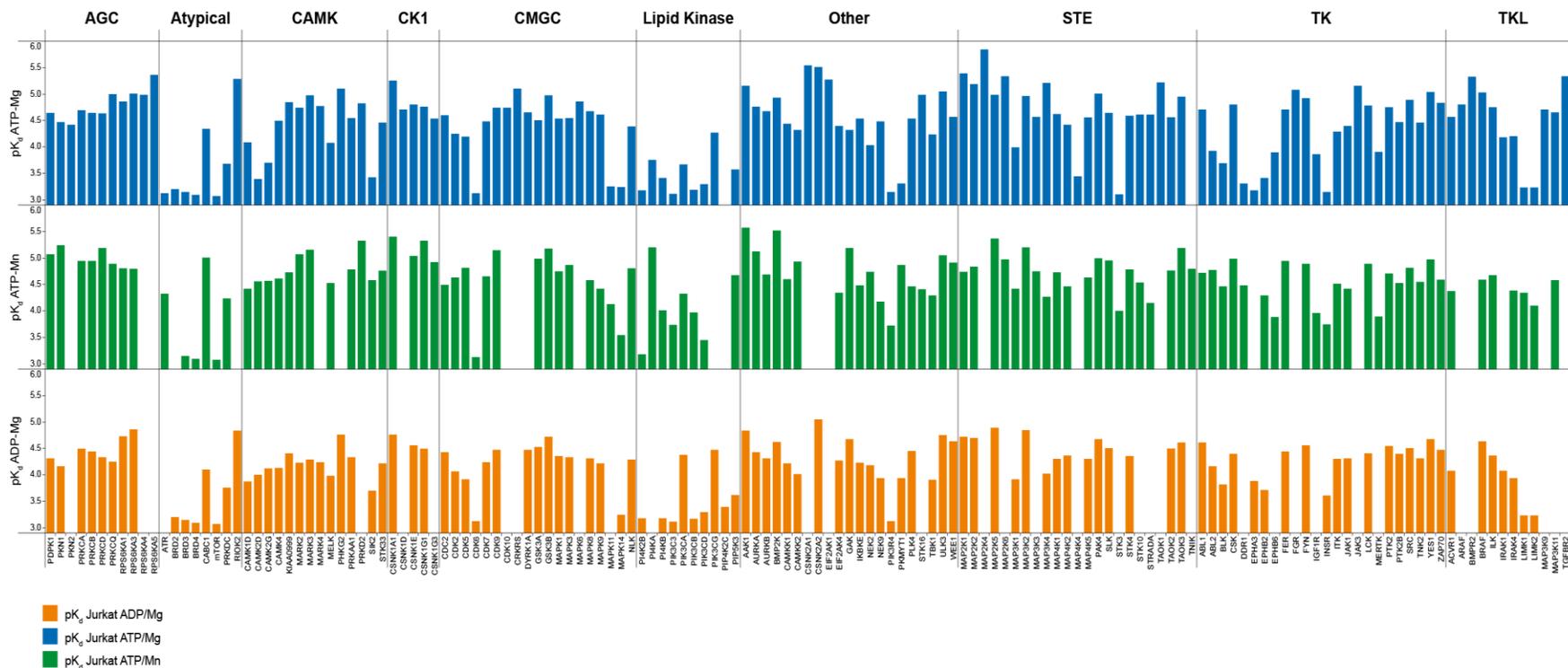
Suppl. Fig. S2



**No correlation was observed between ATP affinity and inhibitor “promiscuity”.**

Kinase inhibitor selectivity data were taken from the most extensive public dataset (Davis *et al.*, *Nat. Biotechnol.* 29, 2011, 1046-51.). pIC<sub>50</sub> values for MgATP were compared to the number of hits with pIC<sub>50</sub>>5.5 or pIC<sub>50</sub>>6 as reported by Davis *et al.* for a given kinase using a total of 72 inhibitors. The correlation values are below 0.05 in both cases.

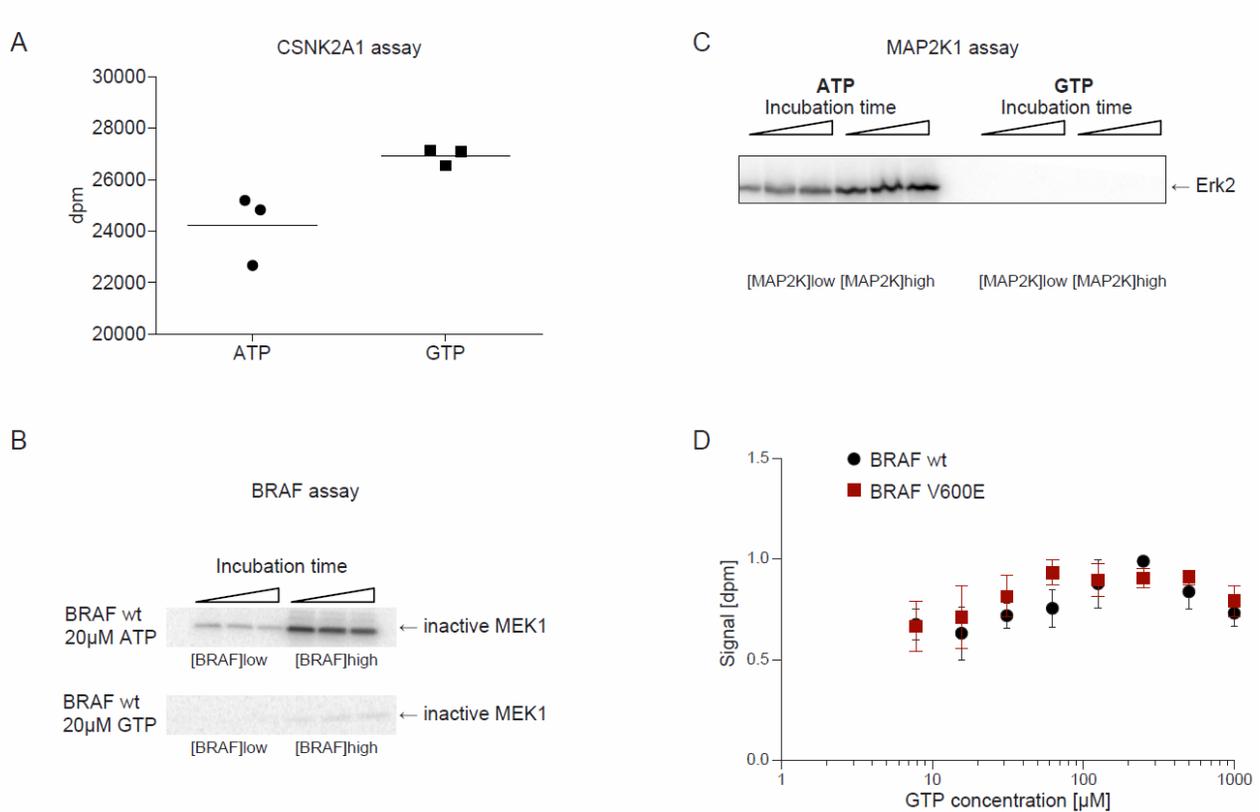
Suppl. Fig. S3



**MnADP binds to most kinases with lower affinity than MgATP. Replacement of  $Mg^{2+}$  with  $Mn^{2+}$  leads has a comparably small effect on most affinity values.**

Comparison of  $pK_d^{app}$  values for the kinase cosubstrates MgATP (upper panel, blue), MnATP (mid panel, green), and Mg-ADP (lower panel, orange). Each bar represents a  $pK_d^{app}$  value. Kinases are grouped in subclasses. The affinity profile for ADP (orange) shows generally a good agreement with the ATP profile (blue). c

## Suppl. Fig. 4



**CK2 efficiently utilized GTP as phosphate donor, whereas BRAF showed low activity with GTP, and MAP2K1 was inactive.** We tested to what extent GTP can be utilized as phosphate donor using recombinant kinases in a kinase enzyme assay with either peptides or inactive kinase as substrate.

(A) Incorporation of labeled phosphate in CK2-tide (a CK2 substrate peptide) was determined after a 10 min incubation at 25 °C by scintillation counting. The signals detected are comparable for ATP and GTP.

For BRAF (B) and MAP2K1 (C) purified inactive kinase protein was used as substrate at two concentrations (indicated as low and high) and either [ $\gamma^{32}$ P]ATP or [ $\gamma^{32}$ P]GTP for 5, 10 or 15 min. (D) Activation of BRAF and BRAF mutant with GTP; substrate MAP2K1 (inactive form), 200μM [ $\gamma^{32}$ P]ATP, over a range of GTP concentrations (1:2 dilutions).