

SUPPLEMENTARY DATA

Fig. S1. Purification of recombinant A-CAT constructs. The Coomassie blue-stained SDS gels show wild-type (WT) and mutant constructs of A-CAT purified from *E. coli*. Constructs were purified as described in Materials and Methods. The $\Delta 831$, $\Delta 823$ and $\Delta 809$ constructs are truncated at the indicated C-terminal residue, 5xA has Ser553, Thr612, Thr613, Thr614 and Thr634 converted to alanine, RRGT and RRGS have Gln822 and Gln823 mutated to arginine and RRGS has Thr825 mutated to serine, $\Delta 3$ has residues 812-814 deleted, $\Delta 6$ has residues 810-815 deleted and BT is the A-CAT-MHCK B chimera. None of the truncations or mutations significantly altered the expression of A-CAT or its ability to be purified.

Fig. S2. Detailed view of the Pi binding site and its connection to the active-site loop. The $2F_o - F_c$ electron density map, contoured at 2σ level (gray mesh), of Pi, Lys684, Arg734 and Asp762 in the Pi-pocket of A-CAT- $\Delta 809$. Thr736, Asp756 and Asp766 are shown as sticks. Arg734 bridges the Pi molecule to Asp762 to anchor the active site loop containing the invariant Asp756 and Asp766 catalytic residues.

Fig. S3. Top-scoring FloppyTail and FlexPepDock models of the C-tail at the Pi-pocket. (A) The two top-scoring models of the C-tail from the Floppy-Tail protocol, out of 5500 that were generated, are shown. The pThr825 residue was constrained to occupy the Pi-pocket. The two models were selected since they had nearly identical Rosetta energy, and moreover, were nearly identical structurally. This is remarkable considering that the only bias on the sampling was over the position of pThr285. (B) Residues 823-830 for the two top-scoring FloppyTail protocol models are shown in transparent magenta and red sticks, respectively. The two top-scoring models following refinement using the FlexPepDock protocol are shown in blue and cyan sticks. Polar bonds are highlighted in green. The two models converged indicating a strong signal for this binding mode.

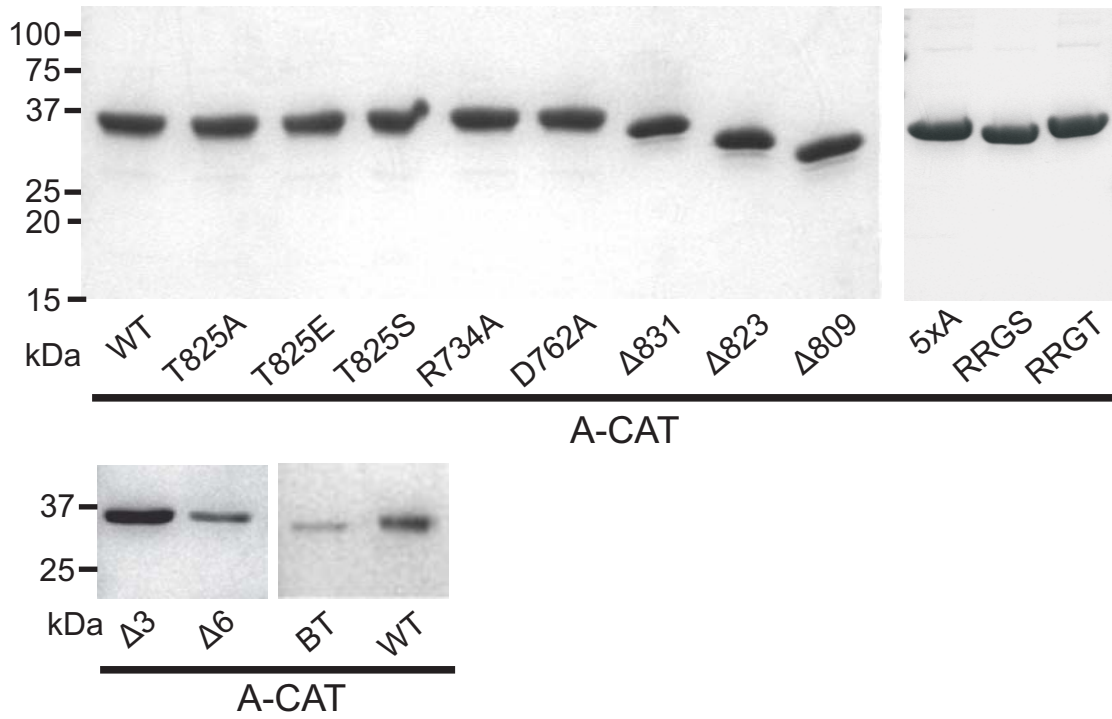
Fig. S4. The top 10 scoring models of the C-tail at the active site. An ensemble of the ten top-scoring FloppyTail and FlexPepDock models of the C-tail at the active site is shown. The Thr825 residue was loosely constrained to occupy a position close to Asp756, which is predicted to be the catalytic base. ATP is shown as sticks in the active site. The models show that the C-tail can adopt a number of different conformations that allows Thr825 to access the active site.

Fig. S5. A multiple sequence alignment of 20 MHCK/eEF2K family members. The top panel shows that the residues that comprise the Pi-pocket (Lys684, Arg734 and Thr736 in MHCK A) and the catalytic loop (Asp756, Asp762 and Asp766 in MHCK A) are invariant in the MHCK/eEF2K branch of the α -kinases. The bottom panel shows that a threonine-hydrophobic sequence corresponding to Thr825 in MHCK A is present in the C-tail regions of all MHCK/eEF2K family members. The full names of the organisms and the NCBI protein accession numbers are: *Caenorhabditis elegans* eEF2K (NP_001022572); chicken eEF2K (XP_414920); *Ciona intestinalis* eEF2K (XP_002124480); *Danio rerio* eEF2K (NP_001002740); *Dictyostelium discoideum* AK1 (XP_629868), MHCK A (XP_635119), MHCK B (XP_636368), MHCK C (XP_635600), and MHCK D (XP_640080); *Dictyostelium purpureum* AK1 (DPU0059919), MHCK A (DPU0073191) and MHCK D (PU0070677); *Hydra magnipapillata* eEF2K (XP_002162308), mouse eEF2K (NP_031934); *Phytophthora ramorum* α -kinase1 (sequence ID 72635; genome.jgi-psf.org/Phyra1_1); *Polysphondylium pallidum* AK1 (EFA84766), MHCK A (EFA85481) and MHCK D (EFA85552); *Trichoplax adhaerens* eEF2K (XP_002118108); and *Xenopus tropicalis* eEF2K (NP_001120146).

Table S1. Data collection and refinement statistics.

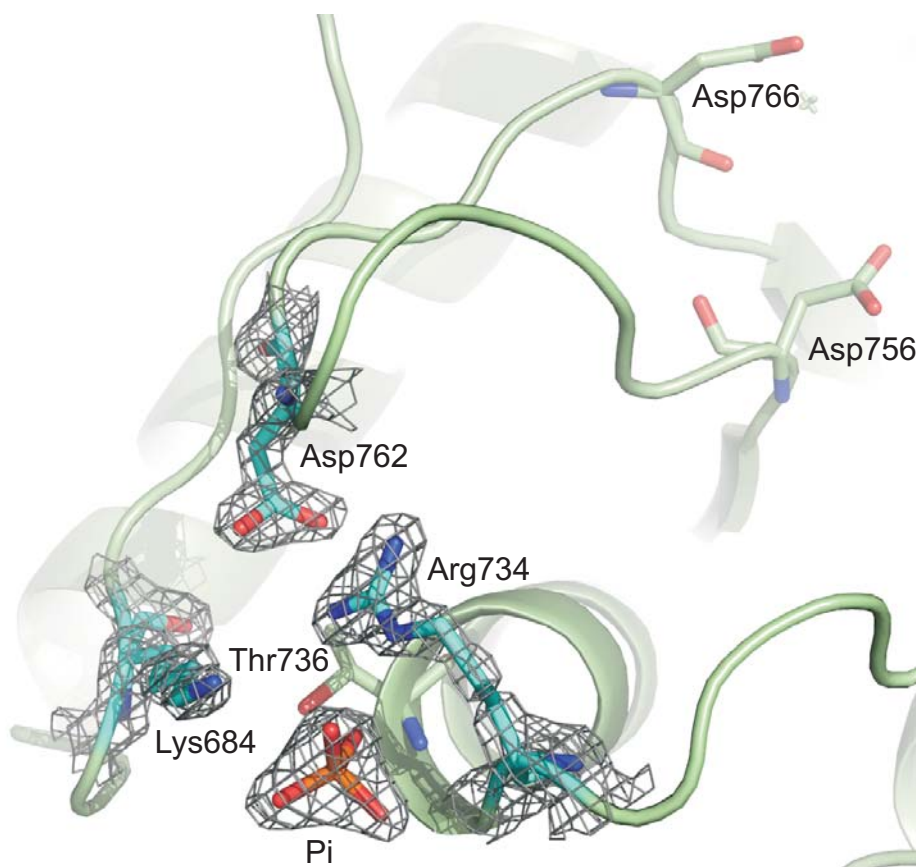
	ACAT- Δ 809 ADP
Data collection	
Space group	<i>P2₁2₁2</i>
Cell dimensions	
<i>a, b, c</i> (Å)	77.1, 83.7, 44.7
α, β, γ (°)	90, 90, 90
Resolution (Å)	30-1.8
<i>R</i> _{merge}	0.11 (0.45)
<i>I</i> / σ <i>I</i>	21.8 (4.4)
Completeness (%)	99.2(99.9)
Redundancy	5.6 (5.5)
Refinement	
Resolution (Å)	30-1.8
No. reflections	43603
<i>R</i> _{work} / <i>R</i> _{free}	0.2/0.23
No. atoms	
Protein	1988
Nucleotide/Mg ²⁺ /	26/2/
Zn ²⁺ / H ₂ PO ₄ or	1/10
SO ₄ ²⁺	
Water	299
<i>B</i> -factors	
Protein	19.8
Nucleotide/Mg ²⁺ /	24.2 /29.3 /16.3 /
Zn ²⁺ / H ₂ PO ₄	18.4
R.m.s. deviations	
Bond lengths	1.39
(Å)	
Bond angles (°)	0.007

Supplemental Data, Crawley *et al.*
Figure S1



Supplemental Data, Crawley *et al.*

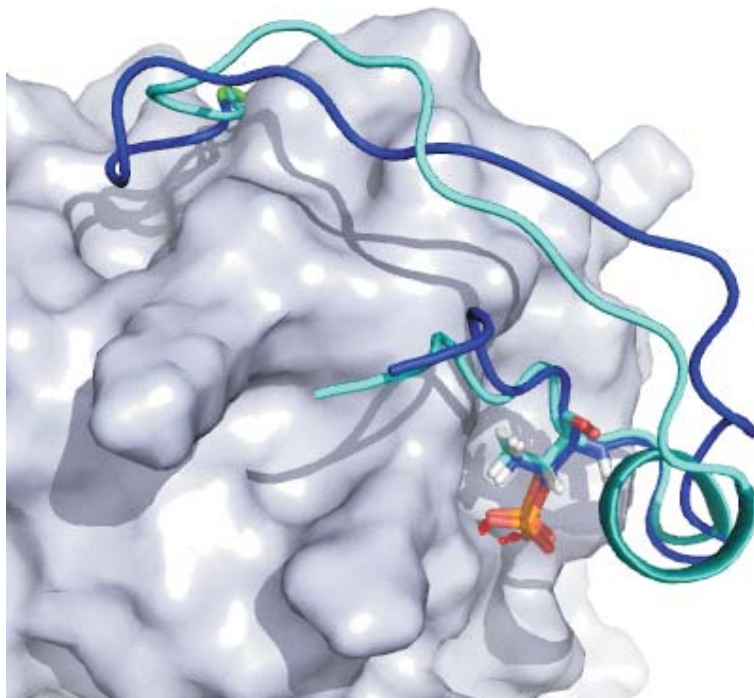
Figure S2



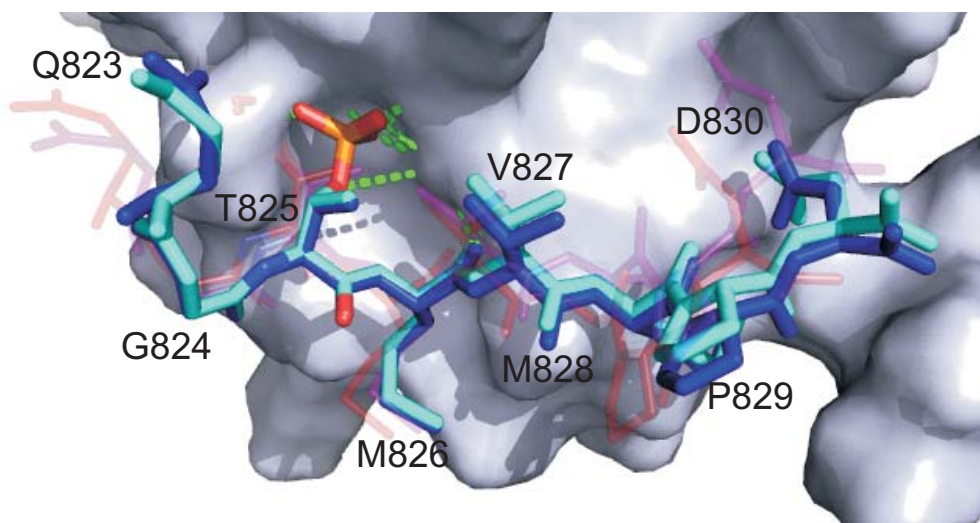
Supplemental Data, Crawley *et al.*

Figure S3

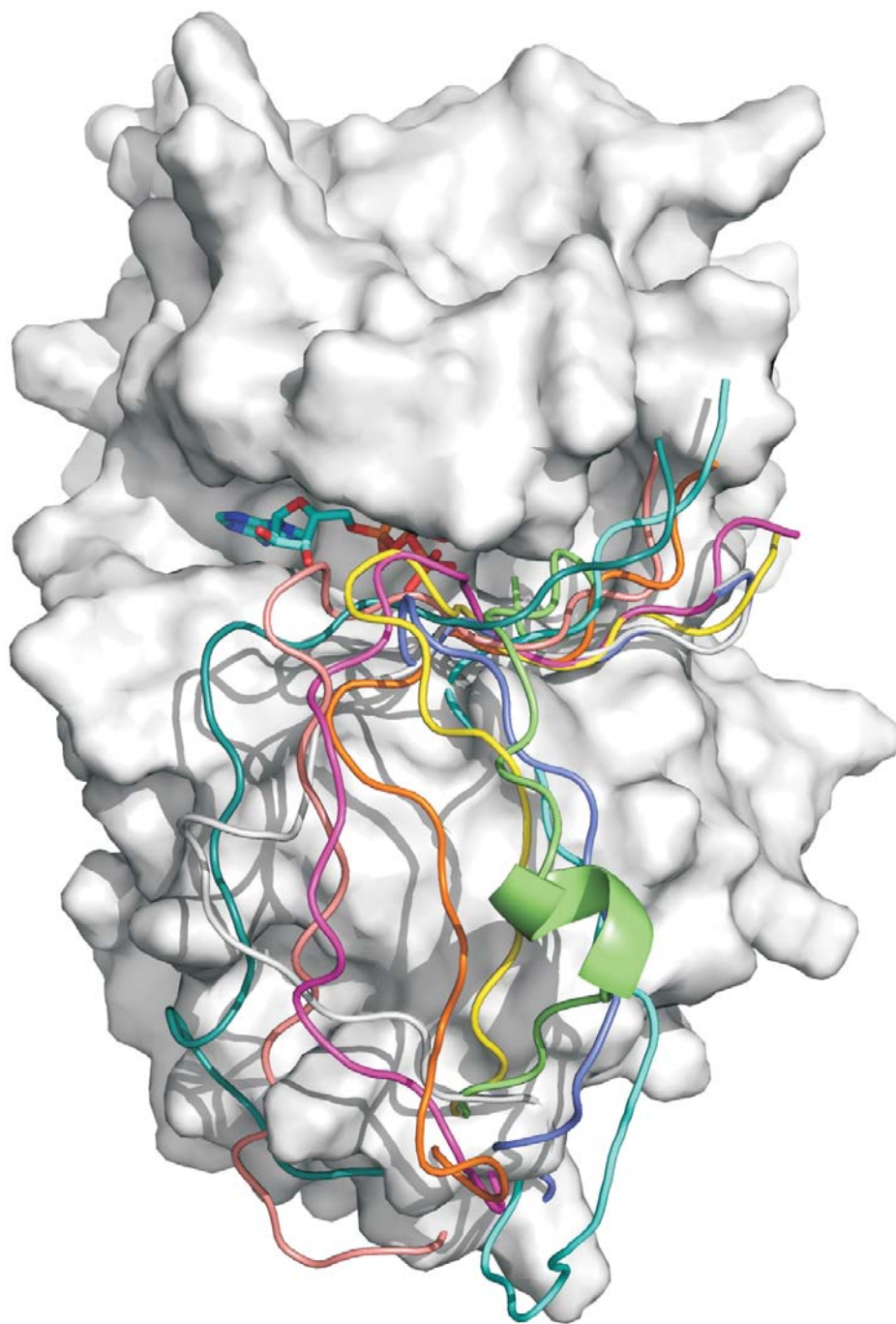
A



B



Supplemental Data, Crawley *et al.*
Figure S4



Supplemental Data, Crawley *et al.*

Figure S5

	Pi-Pocket			Catalytic Loop		
	684	734	736	756	762	766
Caee_EF2K	PPKKI	---	ARLTPQ	---	DIQGVGD	LDLYTD
Chick_EF2K	PPKQV	---	IRLTPQ	---	DIQGVGD	LDLYTD
Cioi_EF2K	PPKQV	---	ARTTPQ	---	DIQGVGD	LWTD
Danr_EF2K	PPKQV	---	IRLTPQ	---	DIQGVGD	LDLYTD
Dicd_AK1	PPKKI	---	DRNTPQ	---	DIQGVGD	VDLYTD
Dicd_MHCKA	PPKKI	---	NRSTPQ	---	DIQGVGD	LDLYTD
Dicd_MHCKB	PPKKI	---	ERNTPQ	---	DIQGVGD	DHYTD
Dicd_MHCKC	PPKPI	---	ARNTPQ	---	DIQGVND	DFYTD
Dicd_MHCKD	PPKKI	---	ERNTPQ	---	DIQGVDD	IYTD
Dicp_AK1	PPKKI	---	DRNTPQ	---	DIQGVGD	VDLYTD
Dicp_MHCKA	PPKKI	---	NRSTPQ	---	DIQGVDD	LDLYTD
Dicp_MHCKC	PPKPI	---	ARNTPQ	---	DIQGVND	DFYTD
Hydm_EF2K	PPKKI	---	VRATPQ	---	DIQGVGD	LDLYTD
Mouse_eEF2K	PPKQV	---	IRLTPQ	---	DIQGVGD	LDLYTD
Phyr_1	PPKRV	---	ERNTPQ	---	DIQGVGD	LDLYTD
Polp_AK1	PPKKI	---	DRNTPH	---	DIQGVAD	VDLYTD
Polp_MHCKA	PPKKI	---	NRSTPQ	---	DIQGVDD	LDLYTD
Polp_MHCKD	PPKNI	---	DRNTPQ	---	DIQGVDD	DHYTD
Tria_EF2K	PPKQV	---	LRATPQ	---	DVQGVGD	LDLYTD
Xent_EF2K	PPKQV	---	IRLTPQ	---	DVQGVGD	LDLYTD

	C-Tail	
	805	825
Caee_EF2K	LSNFELSPPEIEATEVAMEVA	AKQKKSCIVPPTVFEAR
Chick_EF2K	LAPFDLSSKEKDALDHSNKL	-----LESAQTI LRGT
Cioi_EF2K	LSPFDLTFHEKSNCRRASV	-----FARRGTLPTGY
Danr_EF2K	LTRFDLSPAELAQLDCTNKL	-----LQSAKTVLRGF
Dicd_AK1	LSSVNPKPA	-----NDESGTMPRPP
Dicd_MHCKA	LDVKLGGVLSGNNKK	-----QLQQGTMVMMPD
Dicd_MHCKB	LQSINPKSE	-----KSDCGTVPRPD
Dicd_MHCKC	LKPINQSKK	-----ALLRGTLPVVQ
Dicd_MHCKD	LPPIGLETGRNAH	-----RVIRGTMLLPD
Dicp_AK1	LSAVNPKPA	-----NDESGTMPKPP
Dicp_MHCKA	LDVKLGGVLSGNNKK	-----QLQQGTMVMMPD
Dicp_MHCKC	LKPINQSKK	-----ALLRGTLPVVQ
Hydm_EF2K	LKKFDLSEEEKRIDNAGSKSSS	ST--ELKSDTFSGSS
Mouse_eEF2K	LTPFDLSPREQDAVNQSTRL	-----LQSAQTI LRGT
Phyr_1	LPAVNPKSR	-----HADSGTLPVQE
Polp_AK1	LNSVNPKPL	-----TDDSGTVPKPPQ
Polp_MHCKA	LDVKLGGSSLSLTK	-----GQLKGTVMVMMPD
Polp_MHCKD	LPPIGVDLTDNRNIT	-----RVIRGTMALPD
Tria_EF2K	LGTFDLSSNGKQRLLSQFS	-----NQSSSTLSIQD
Xent_EF2K	LTPFDLSAKEKAVLDNCSKM	-----LQTAQTVVVRGI