

Drug Target Identification in Tropical Pathogens

Protein Kinase A in *Trypanosoma*

Gizem Karabıyık

ARTICLE

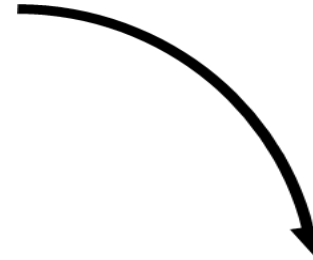
<https://doi.org/10.1038/s41467-019-09338-z>

OPEN

Nucleoside analogue activators of cyclic AMP-independent protein kinase A of *Trypanosoma*

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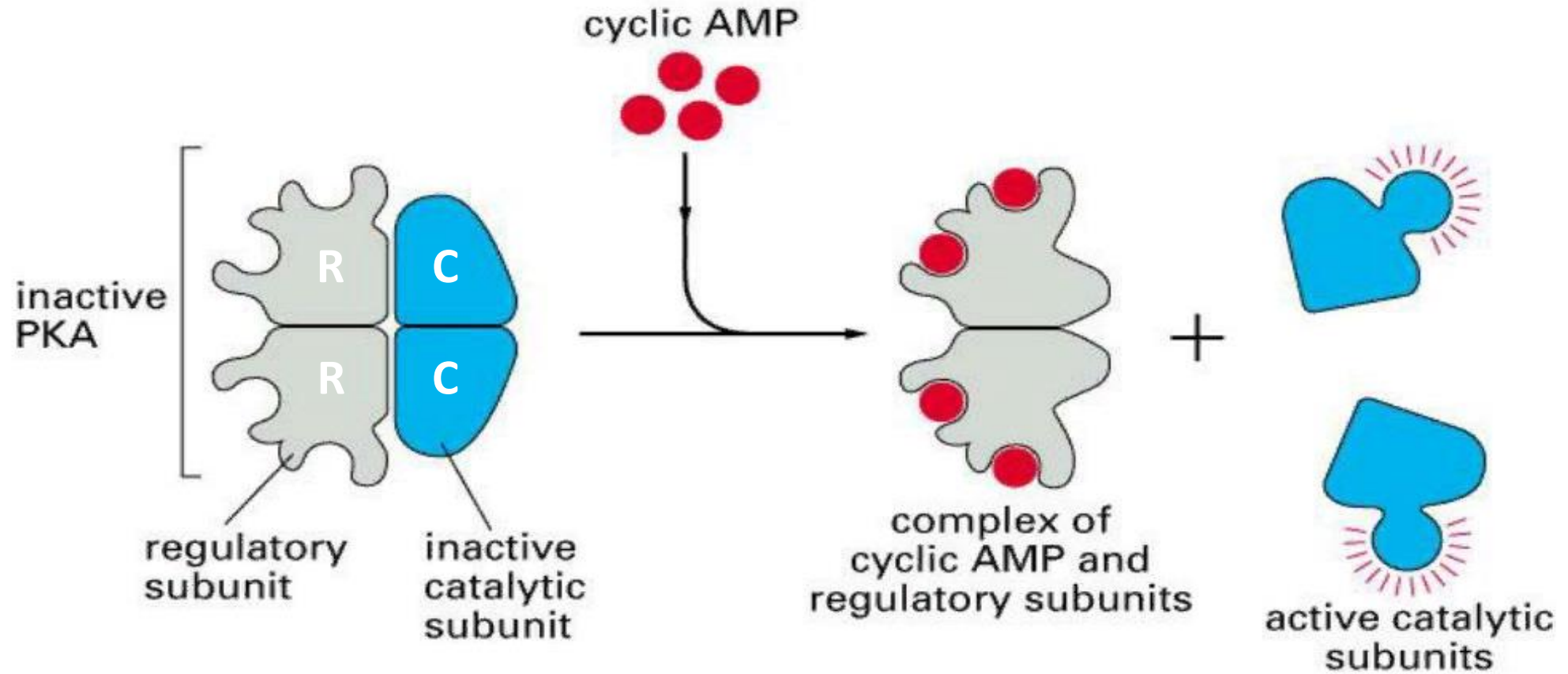
PKA (Protein Kinase A)



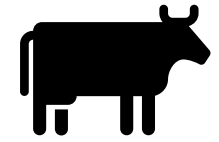
cAMP-dependent protein kinase

- in most eukaryotes
- highly conserved structure
- phosphorylates directly or indirectly proteins
- main effector of cAMP (cyclic adenosine monophosphate)
- the best studied protein kinase, numerous mutants and crystal structures
- as a model for allosteric mechanisms of protein kinases

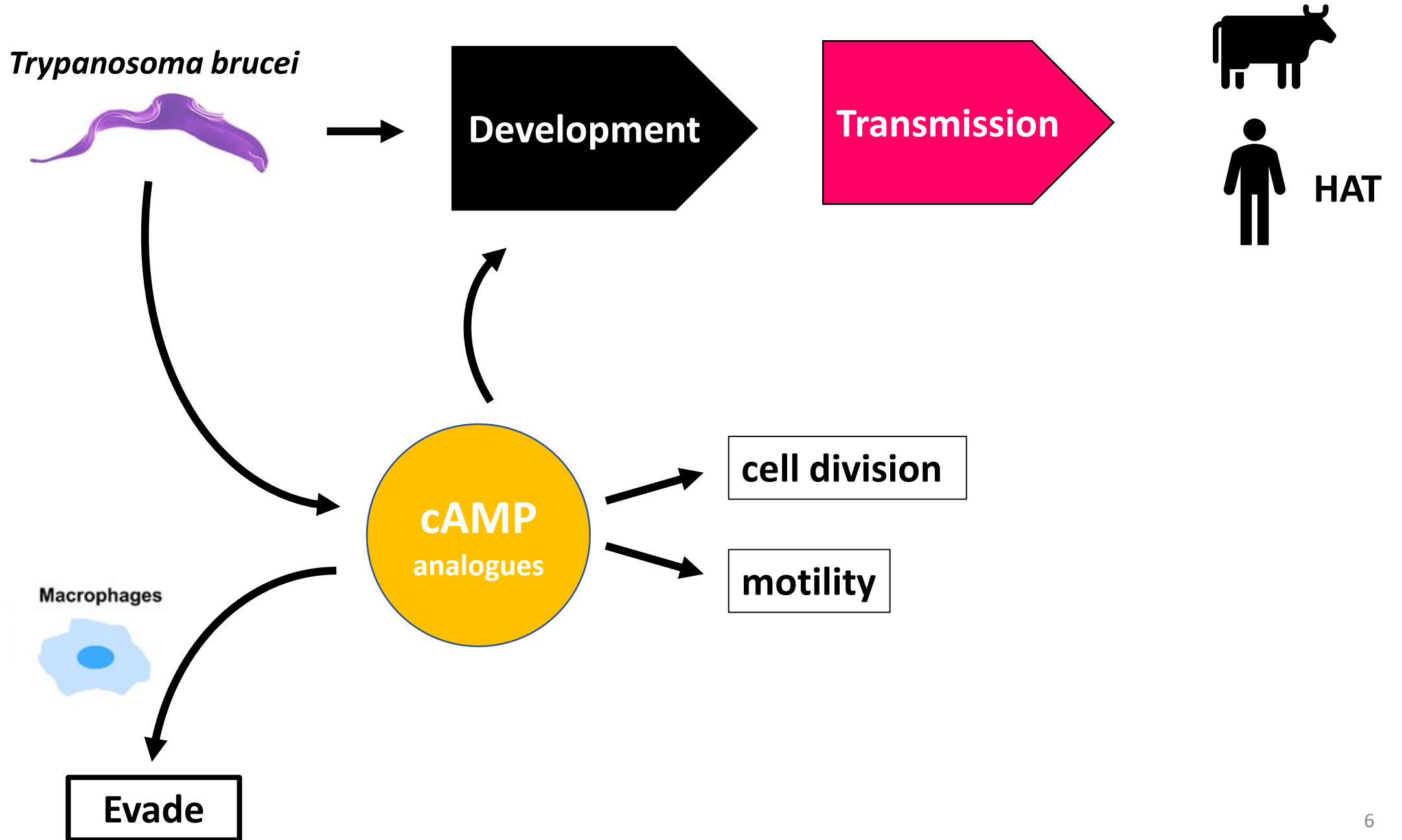
Allosteric Regulation of PKA



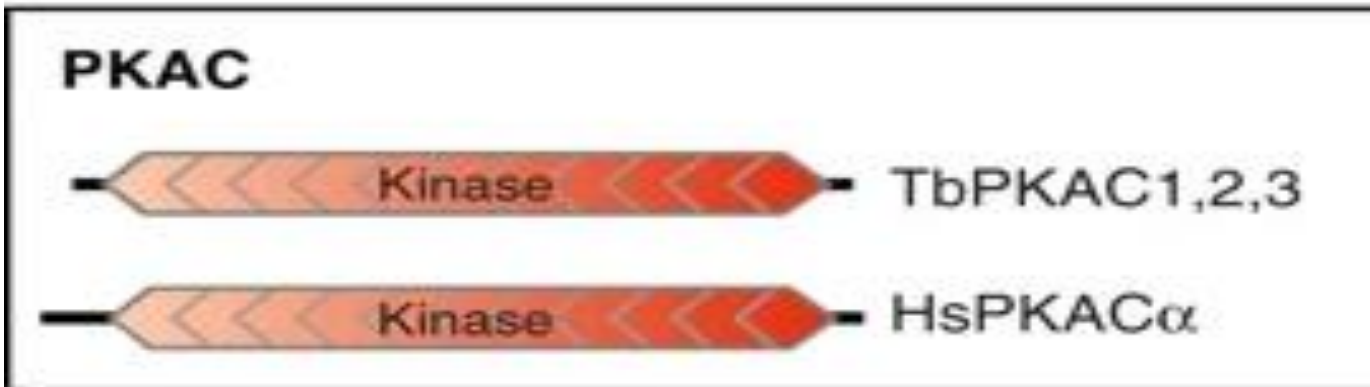
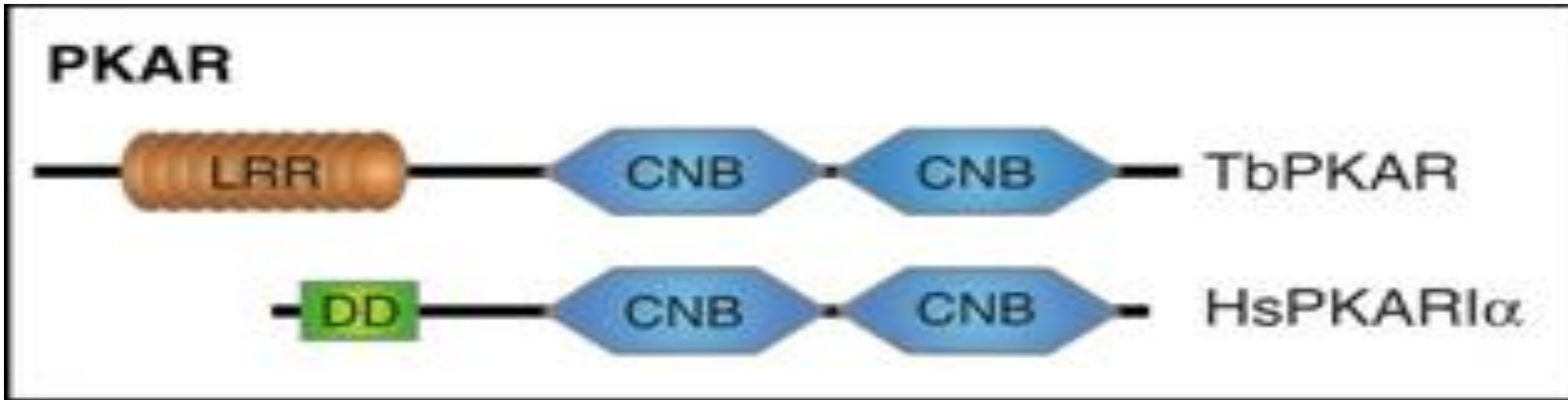
Trypanosoma brucei



HAT



Domain architecture of PKAR (top) and PKAC (bottom) orthologues



R: Regulatory subunit

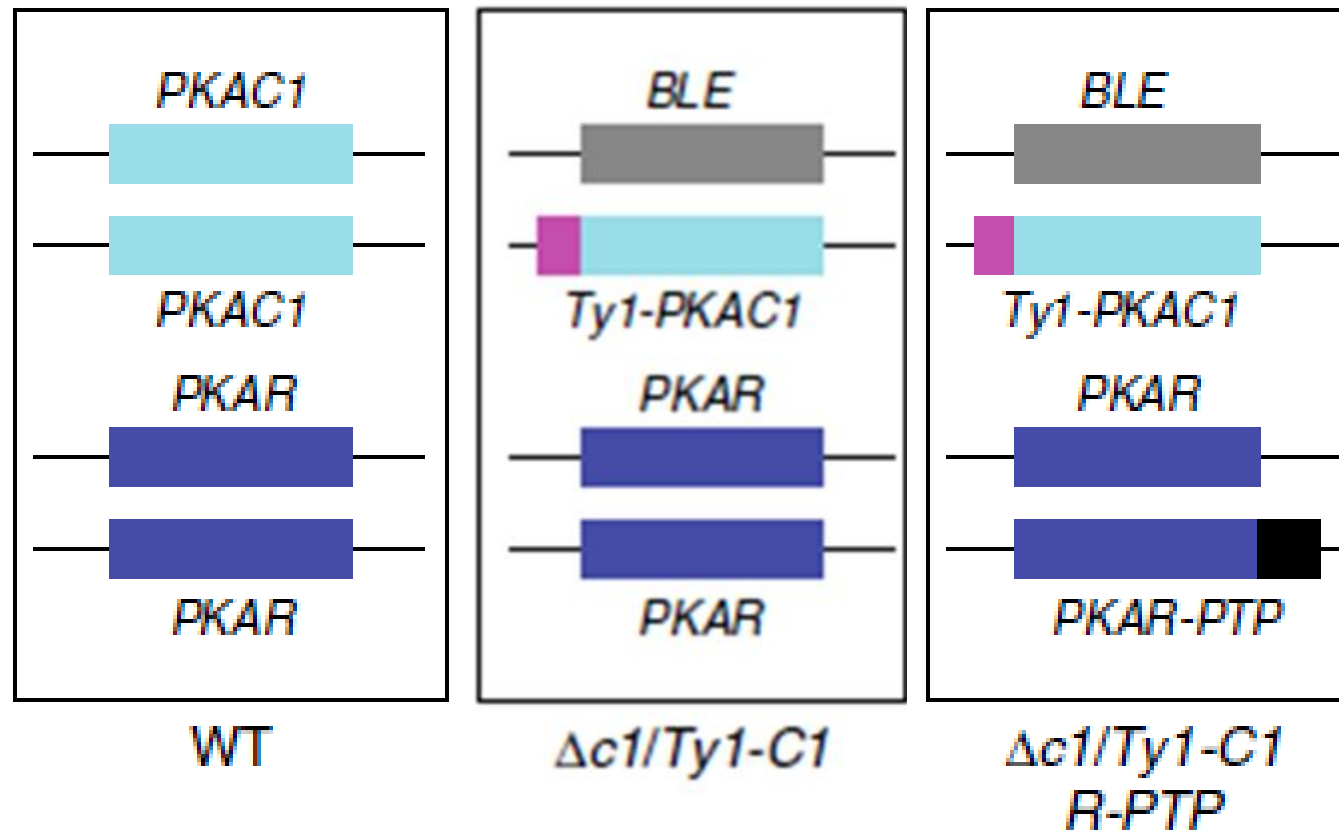
C: Catalytic subunit

CNB: Cyclic nucleotide binding

DD: dimerization/docking domain



Orthologous PKA subunit genes in *T. brucei* encode expected PKA holoenzyme complexes



BLE gene: Phleomycin resistance

PKA holoenzyme complexes of mutant cell lines were pulled down via PTP tag.

Pull-down Assay -> protein-protein interaction

1



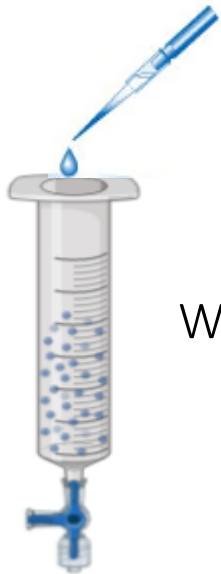
Mutant parasites lysed by sonication.

2



Supernatant is incubated with beads that are specific to PTP tag.

3



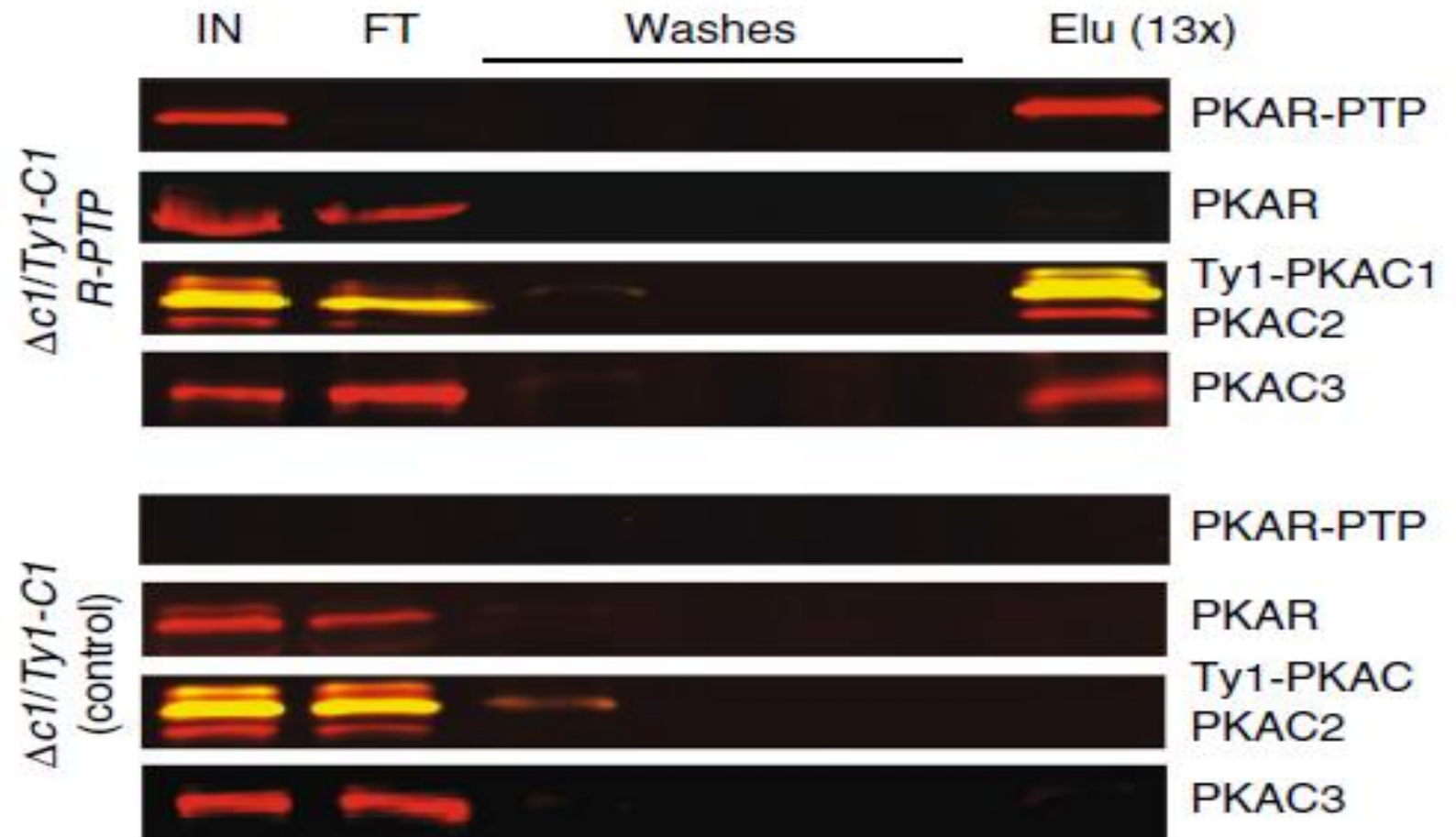
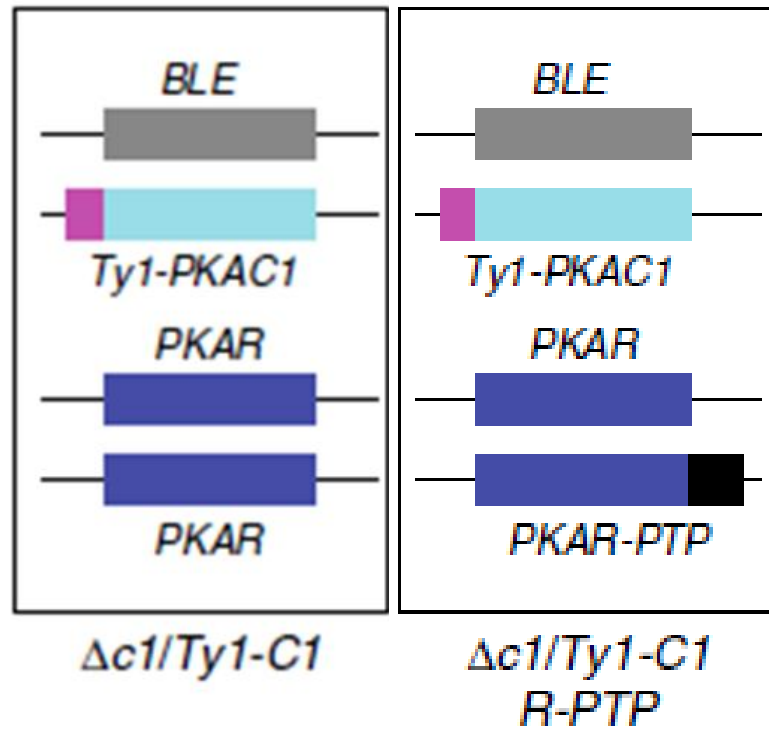
Washing steps

4



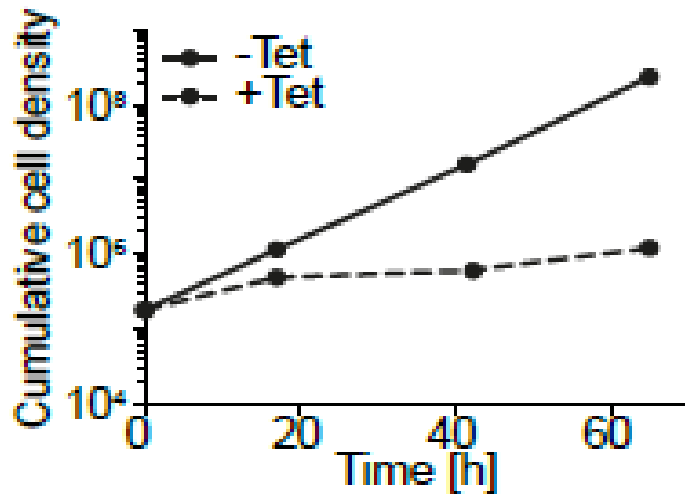
Proteins bound to the beads are eluted.

PTP affinity purification

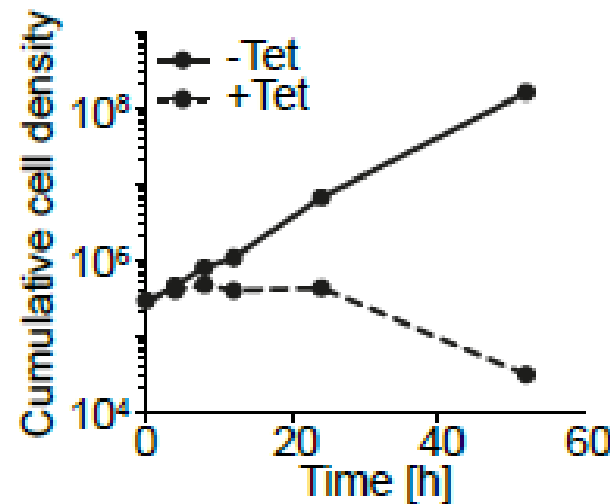


The catalytic function of the PKAR and PKAC isoforms upon inducible RNAi in *T. brucei*

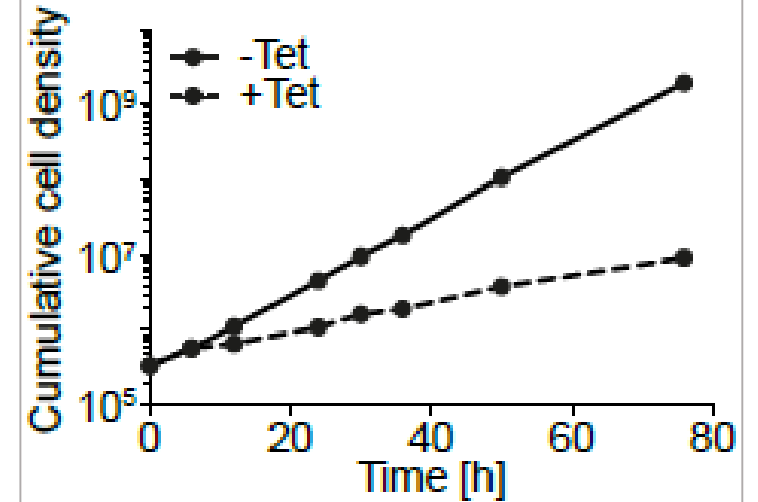
PKAR RNAi



PKAC1/2 RNAi



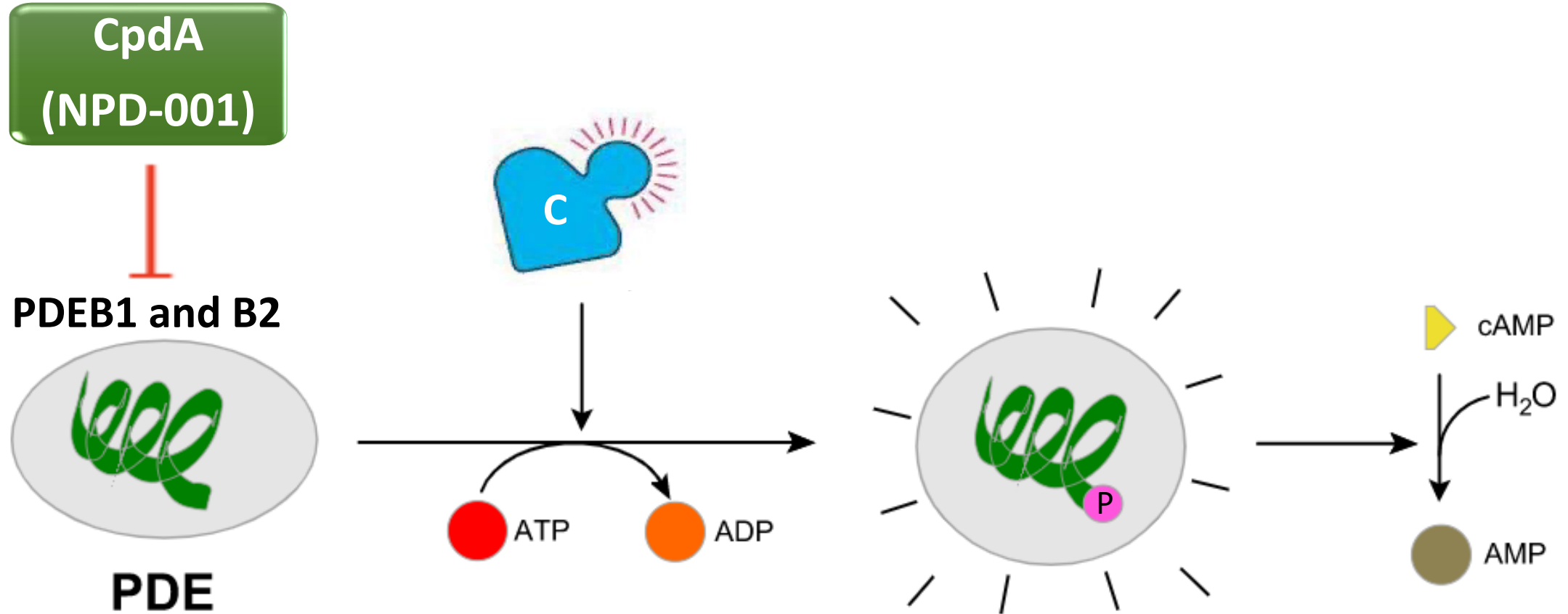
PKAC3 RNAi



PKAC1/2 -> lethal PKACR and 3 -> growth inhibitory

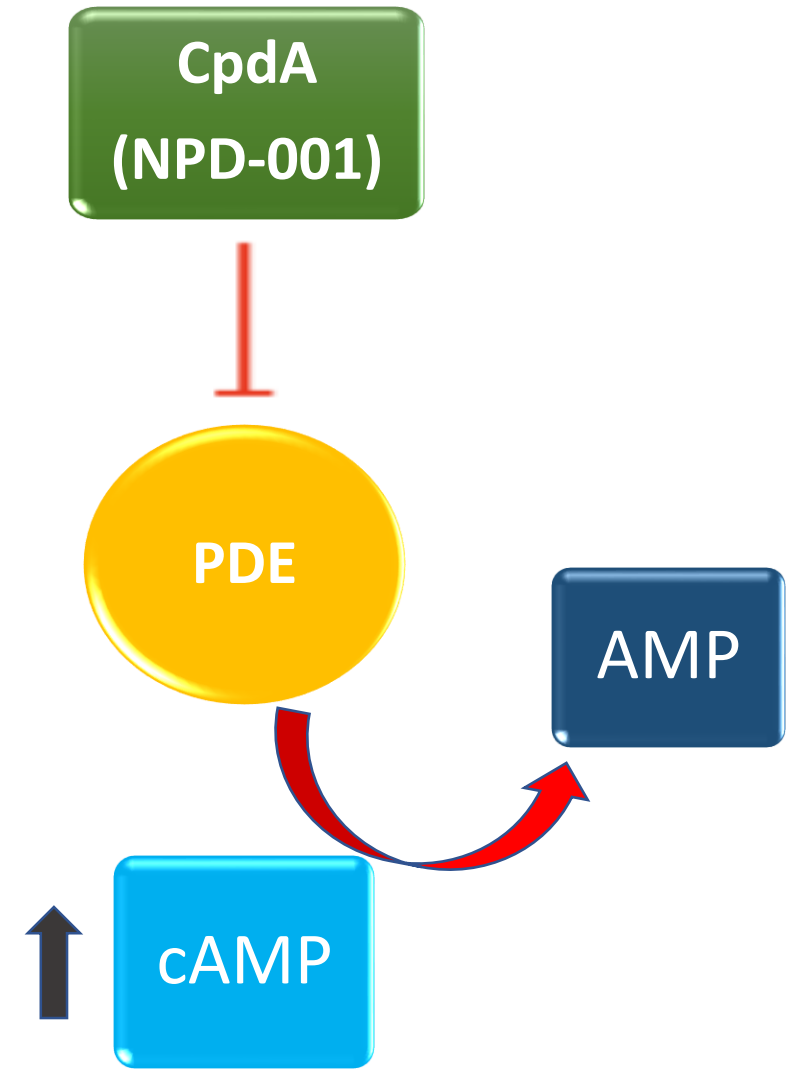
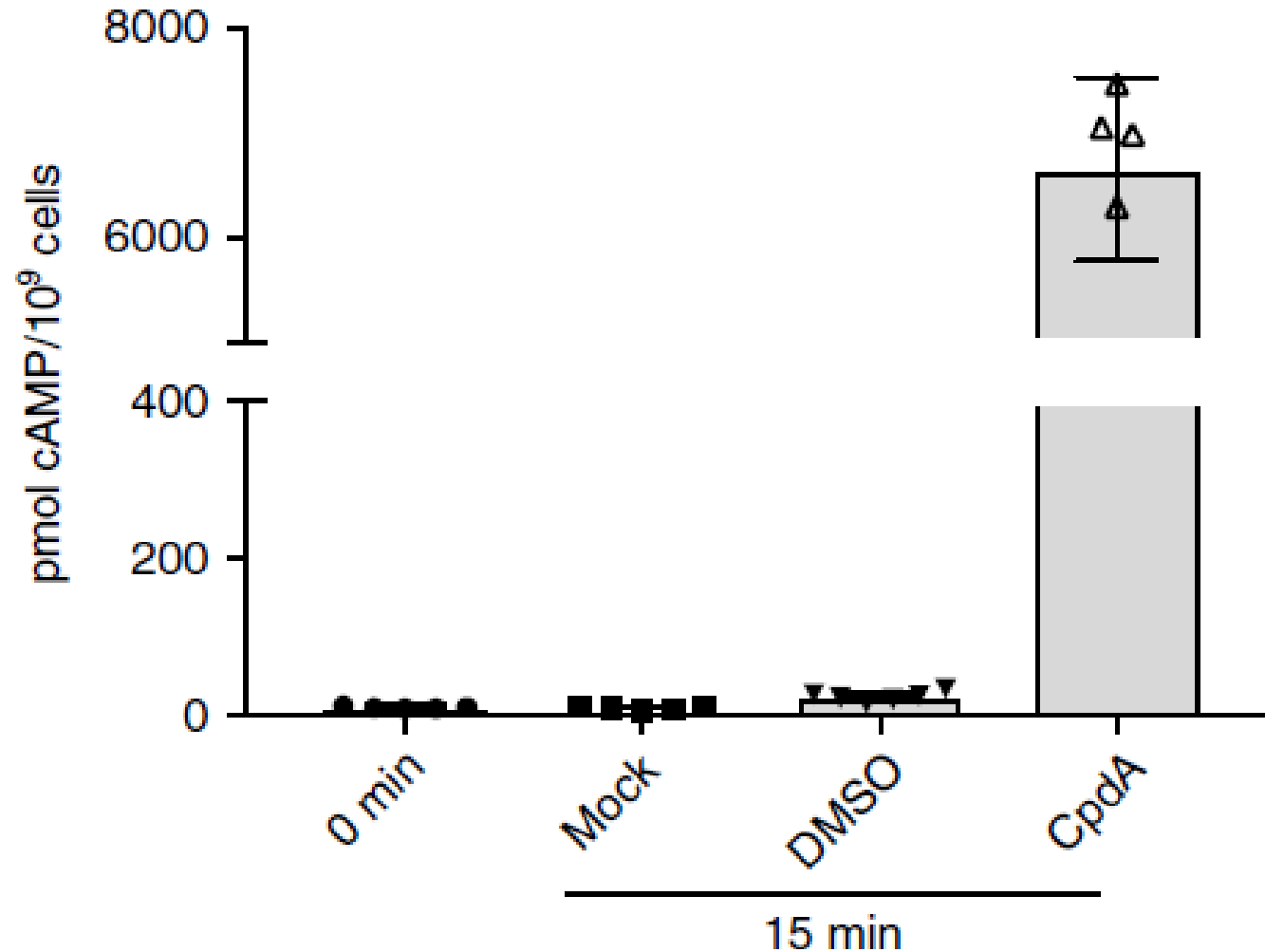
cAMP hydrolyzing PDE (Phosphodiesterase)

in *T. Brucei*.

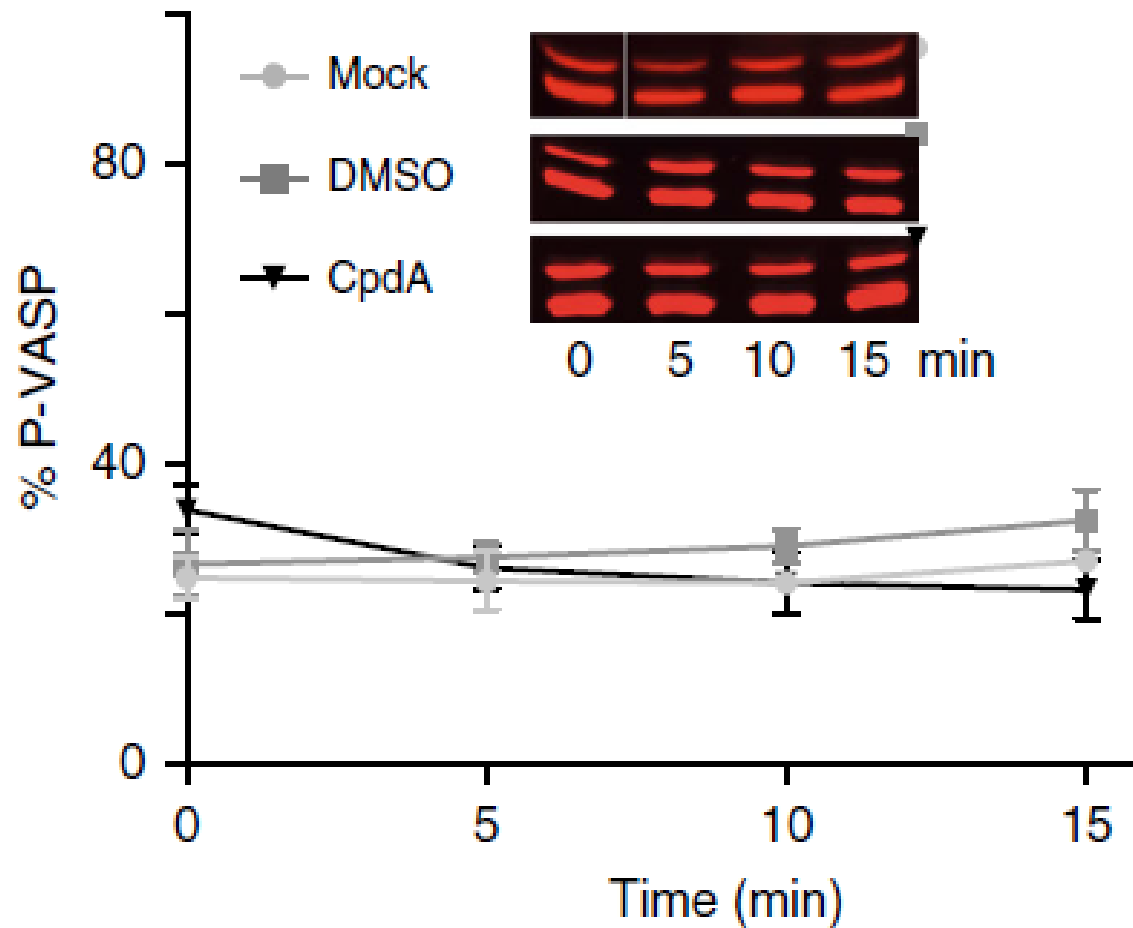


To see the effects of cAMP on PKA activity

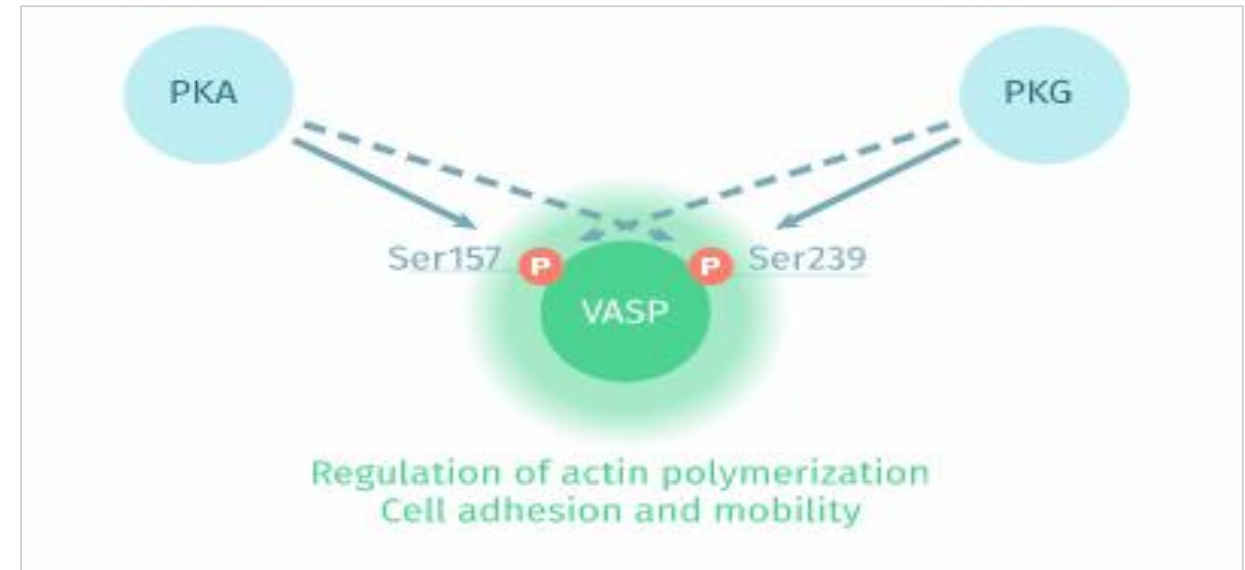
- Three independent approaches



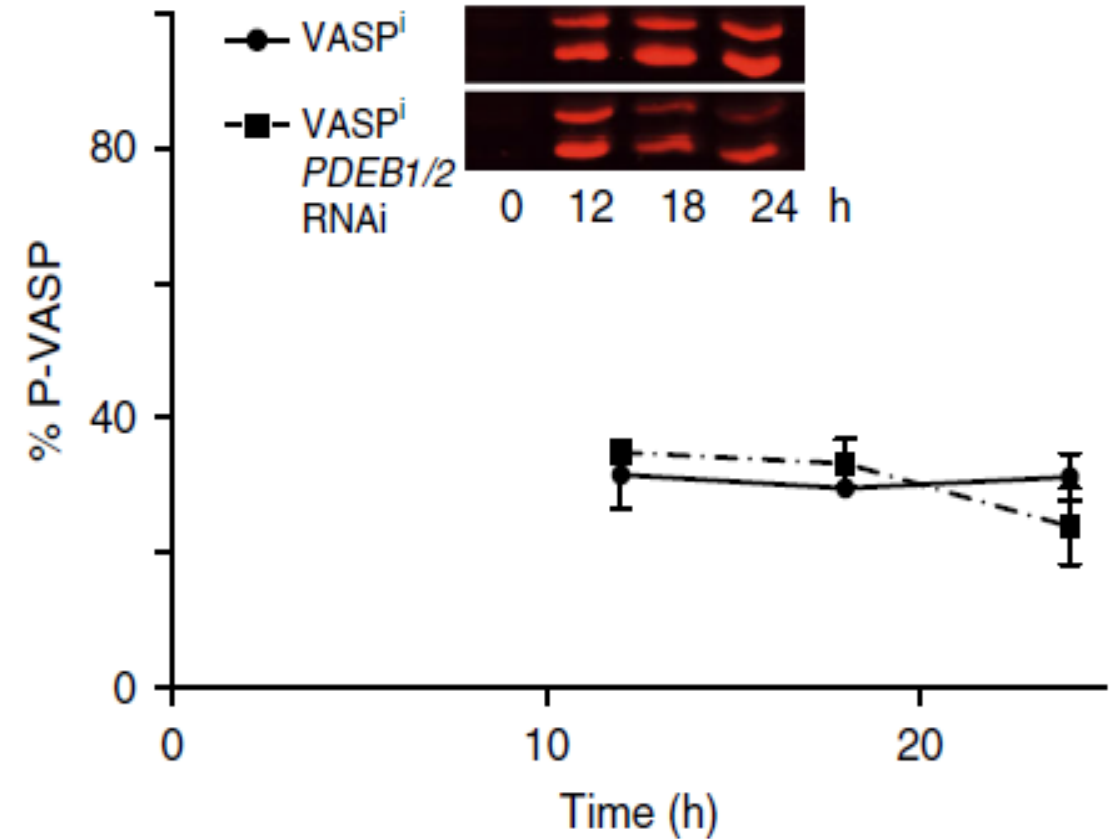
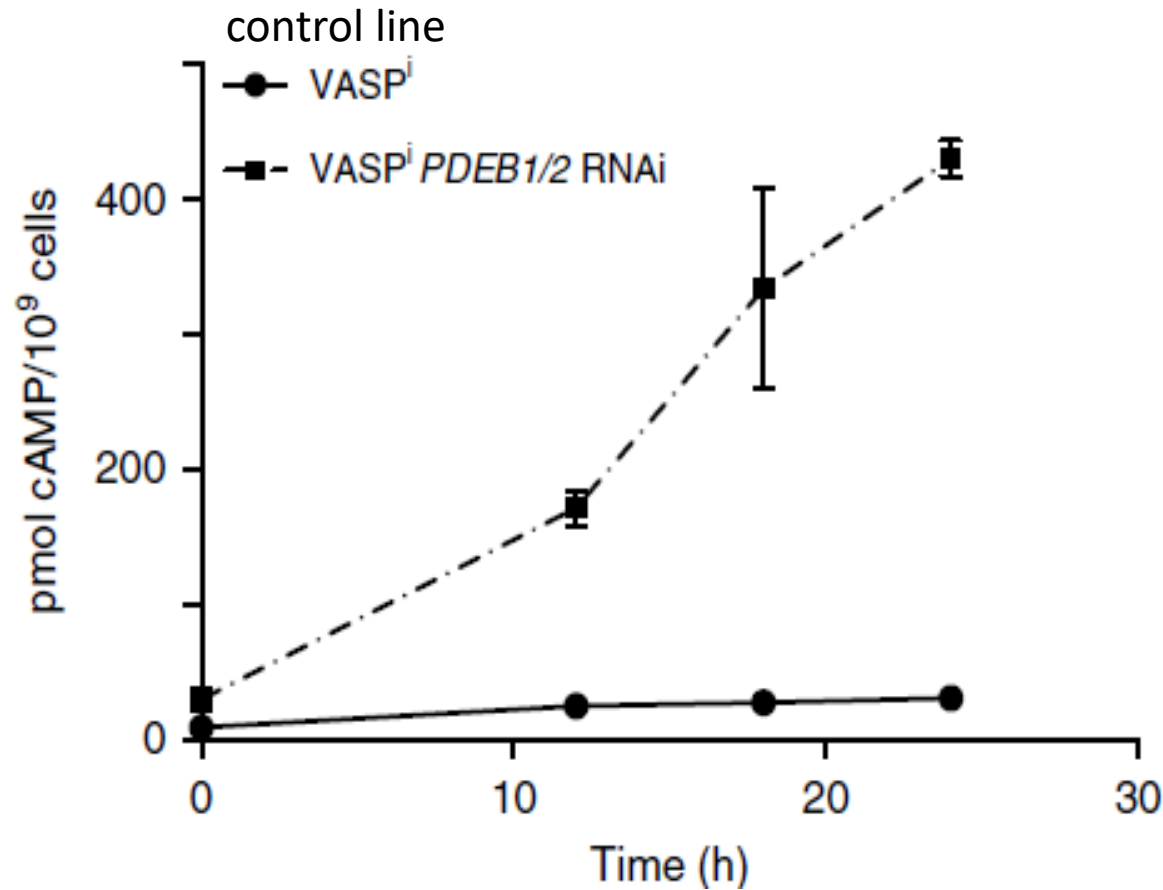
Monitoring of PKA Activation



VASP: human platelet vasodilator stimulated phosphoprotein

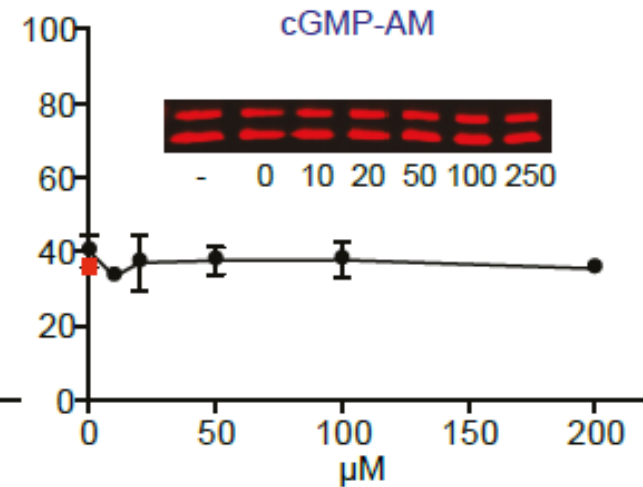
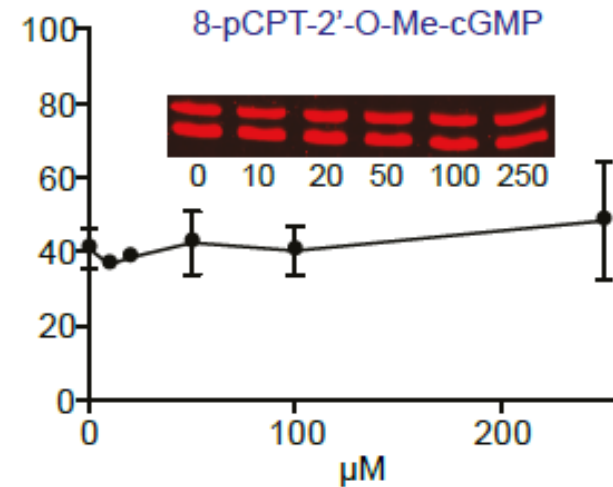
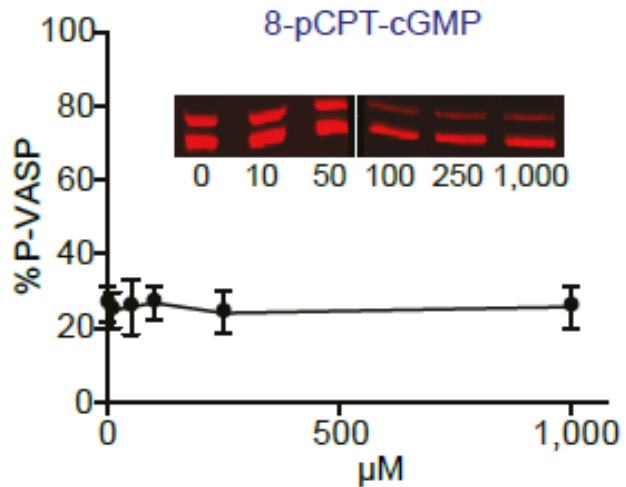
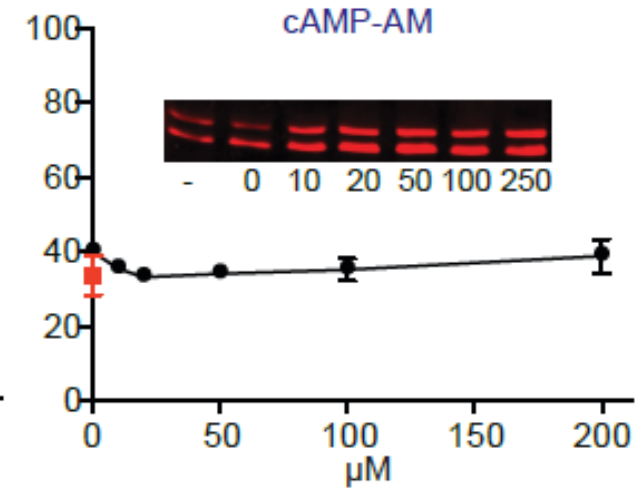
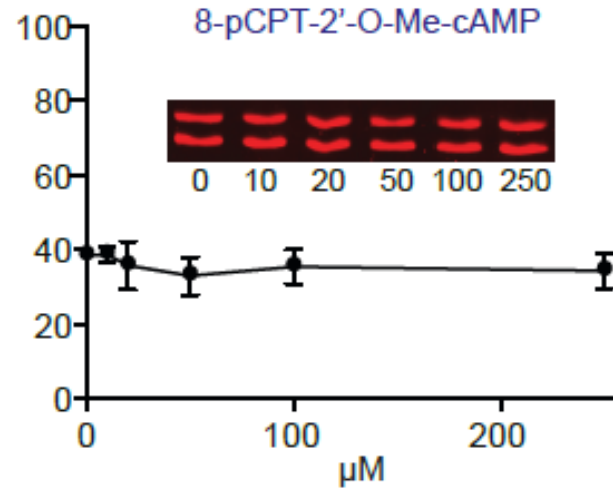
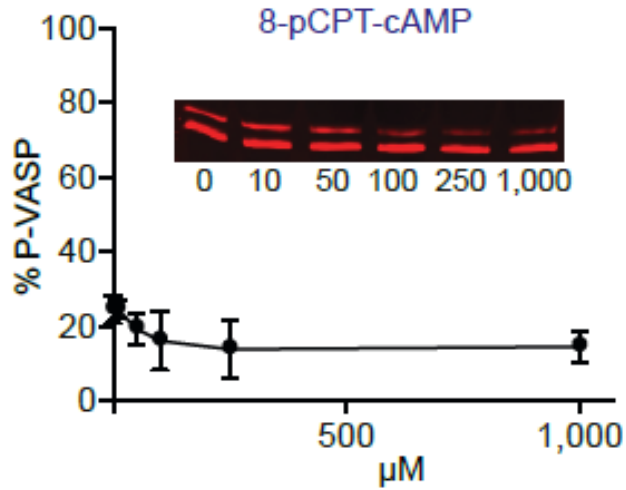


Intracellular cAMP level and PKA activity upon inducible RNAi repression of PDEB1 and PDEB2



45-fold increase of cAMP content but did not change PKA activity.

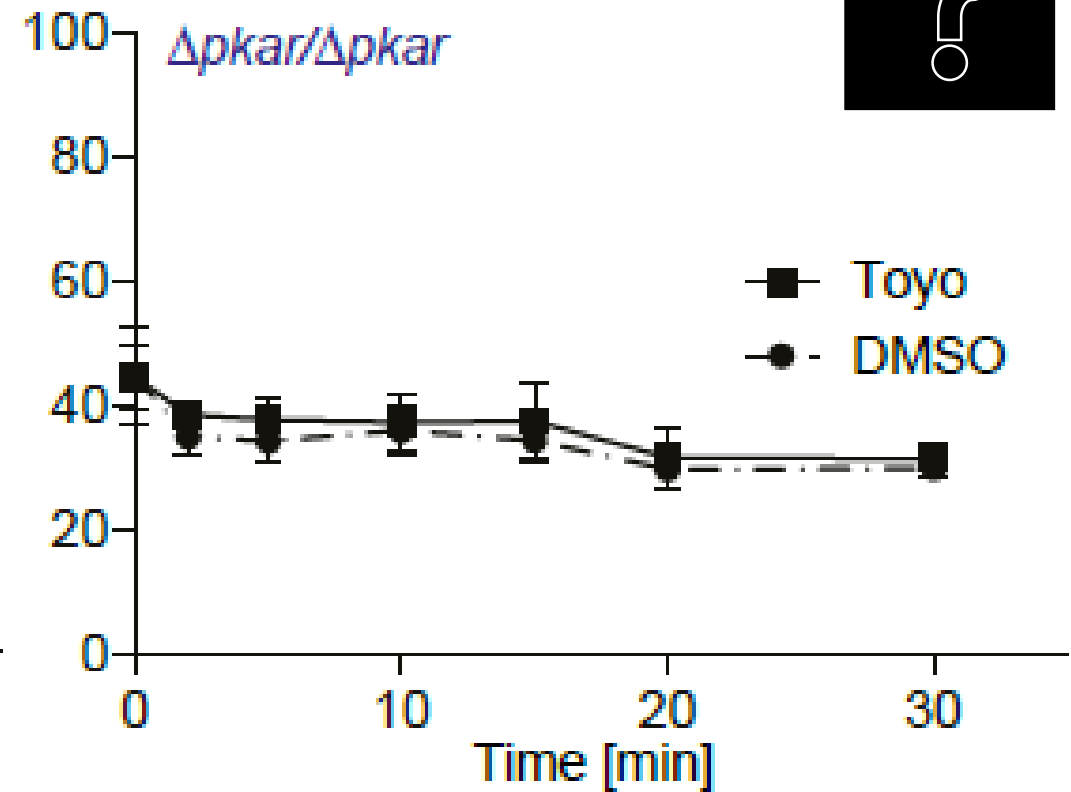
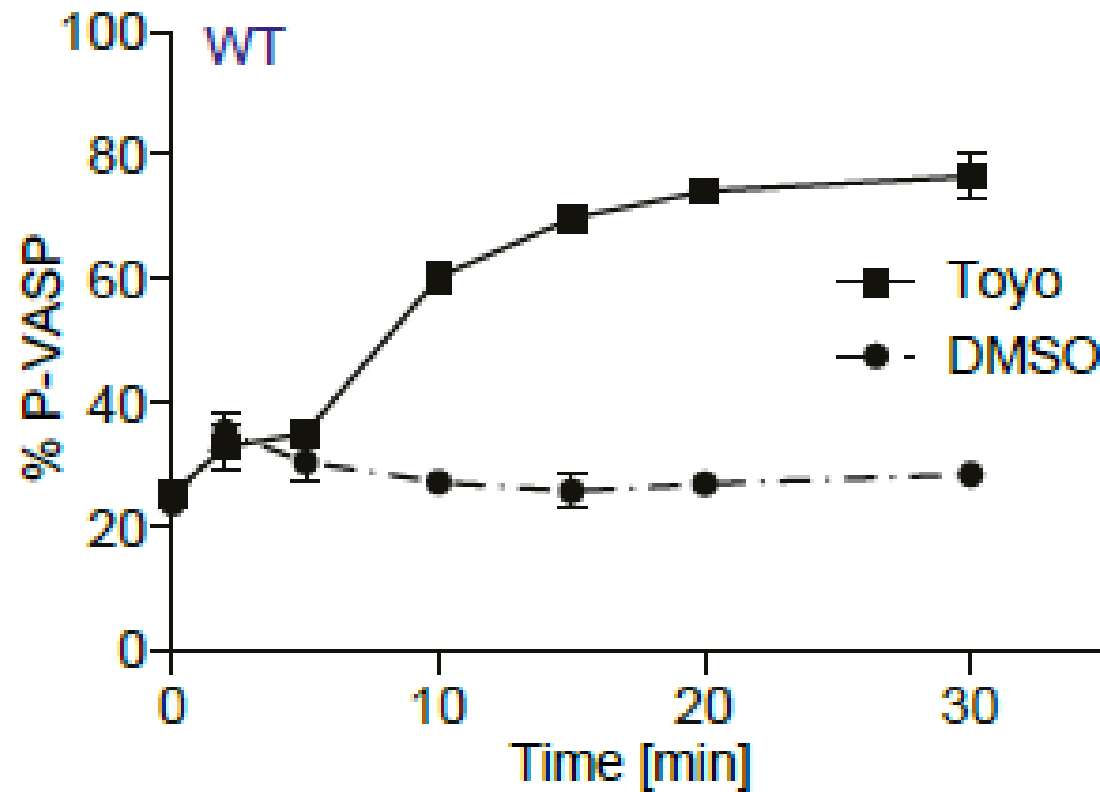
cAMP and cGMP analogues



<u>Compound</u>	<u>Abbreviation</u>
Tubercidin, 7-Deazaadenosine	Tu
5-Bromotubercidin, 7-Bromo-7-deazaadenosine	5-Br-Tu
5-Iodotubercidin, 7-Iodo-7-deazaadenosine	5-I-Tu
Toyocamycin, 7-Cyano-7-deazaadenosine	Toyo
Sangivamycin, 7-Carboxamido-7-deazaadenosine	
6-Bromotubercidin, 8-Bromo-7-deazaadenosine	6-Br-Tu
8-(4-Chlorophenylthio)adenosine	8-pCPT-Ado
8-(4-Chlorophenylthio)-2'-O-methyladenosine	8-pCPT-2'-O-Me-Ado
8-(4-Chlorophenylthio)adenine	8-pCPT-Ade
8-(4-Chlorophenylthio)guanosine	8-pCPT-Guo
8-Bromoadenosine	8-Br-Ado
2-Chloroadenosine	2-Cl-Ado
6-Chloropurine riboside	6-Cl-PuR

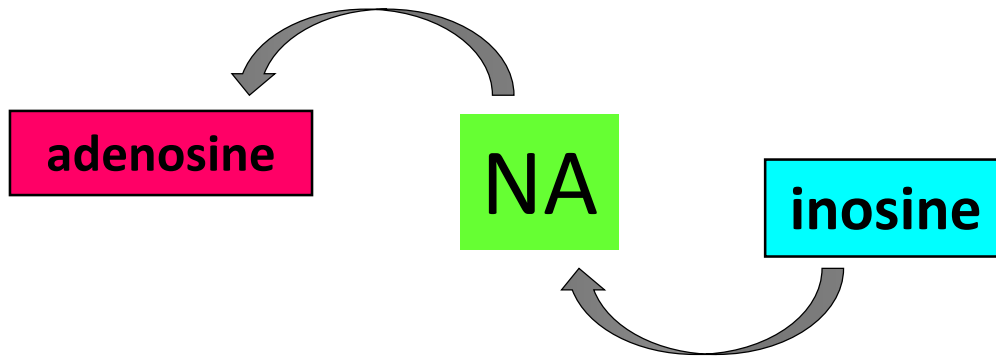
Four 7-deazaadenosine
(tubercidin, Tu)
analogues induced PKA
activity.

The most potent activator: Toyocamycin



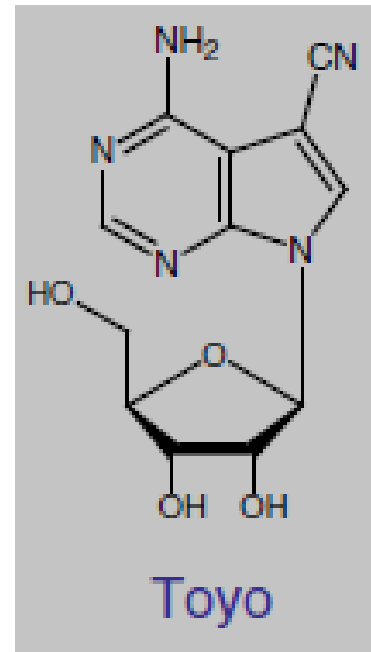
Development of a trypanosome-specific PKA activator

Less Toxic

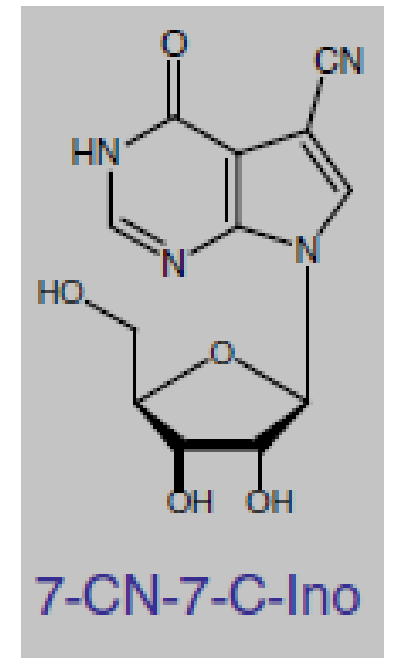


In Mammals

Toyocamycin
(7-cyano-7-deazaadenosine)



7-cyano-7-deazainosine
(7-CN-7-C-Ino, Jaspamycin)

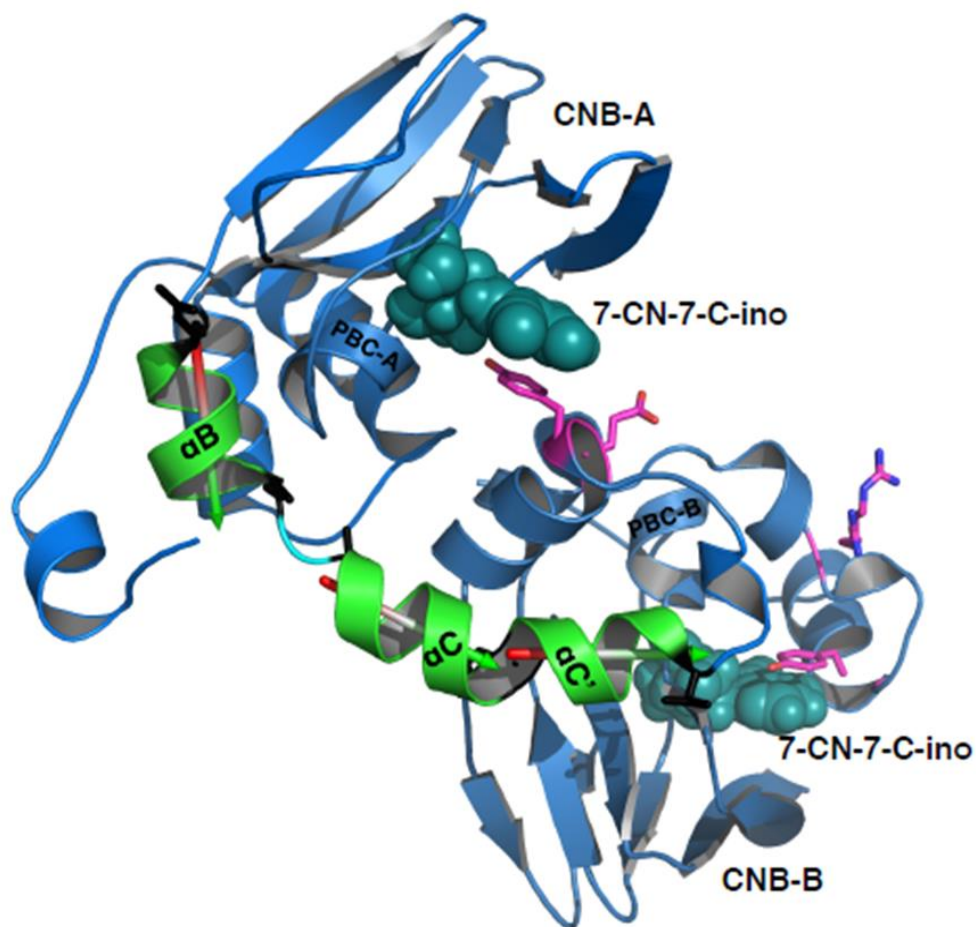


- ✓ Nontoxic
- ✓ Potent
- ✓ Specific

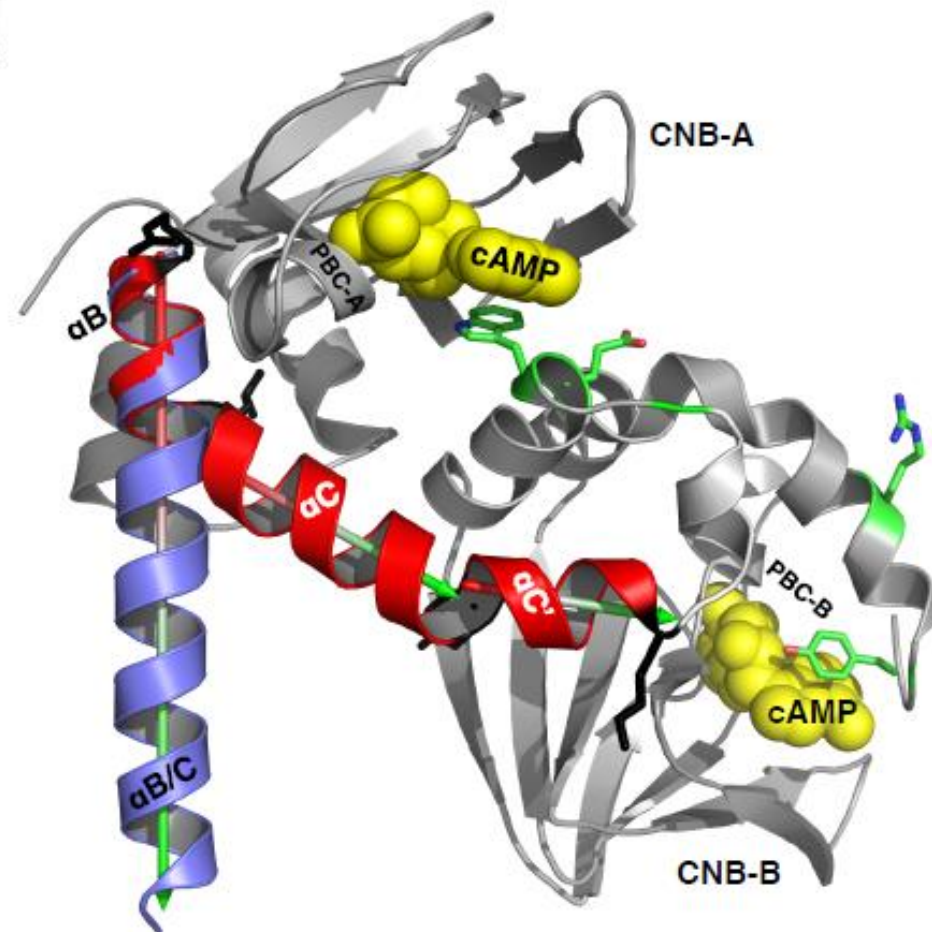
Structure of parasite PKAR with Jaspamycin (7-cyano-7-deazainosine)

Binding
Selectivity

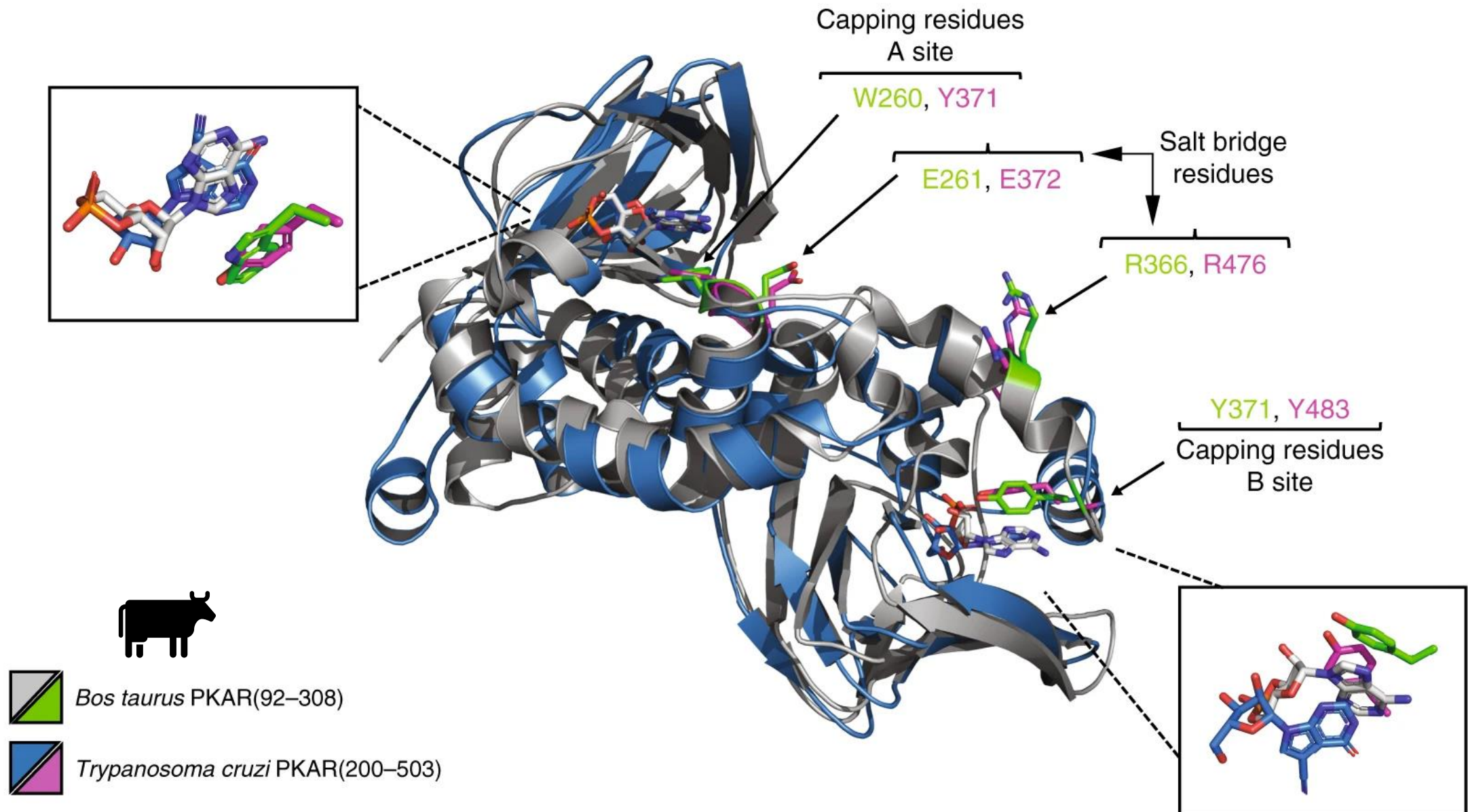
T. cruzi
(79% identity)



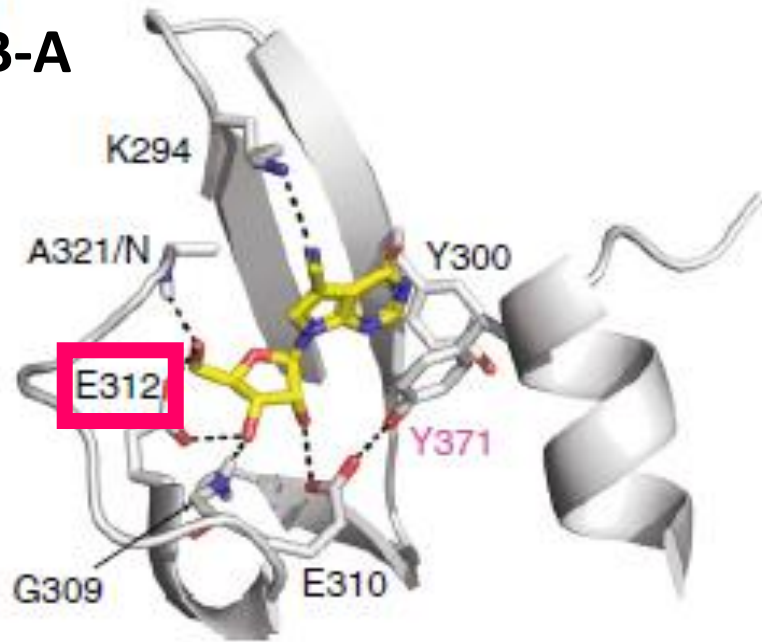
Structure of mammalian PKAR bound to cAMP (yellow)



Overlay of *T. cruzi* PKAR (in blue) and *Bos taurus* PKAR (in grey)



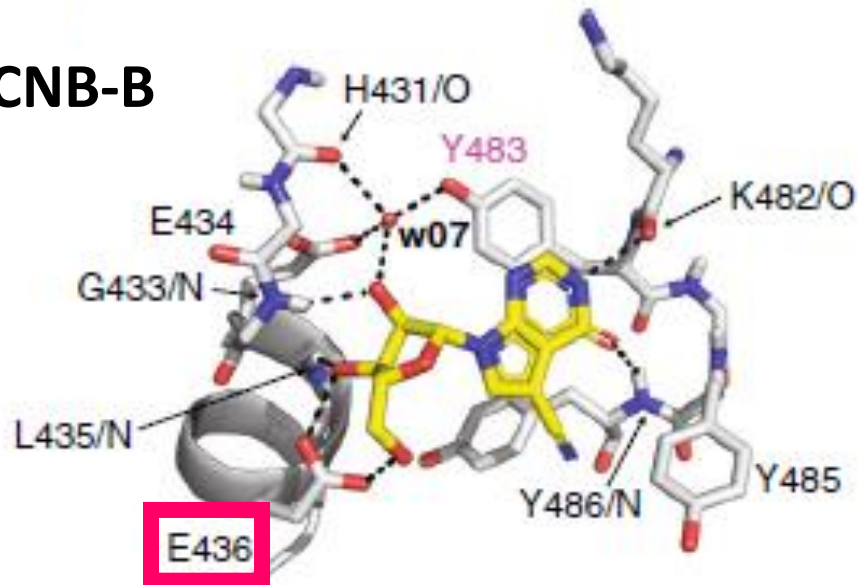
CNB-A



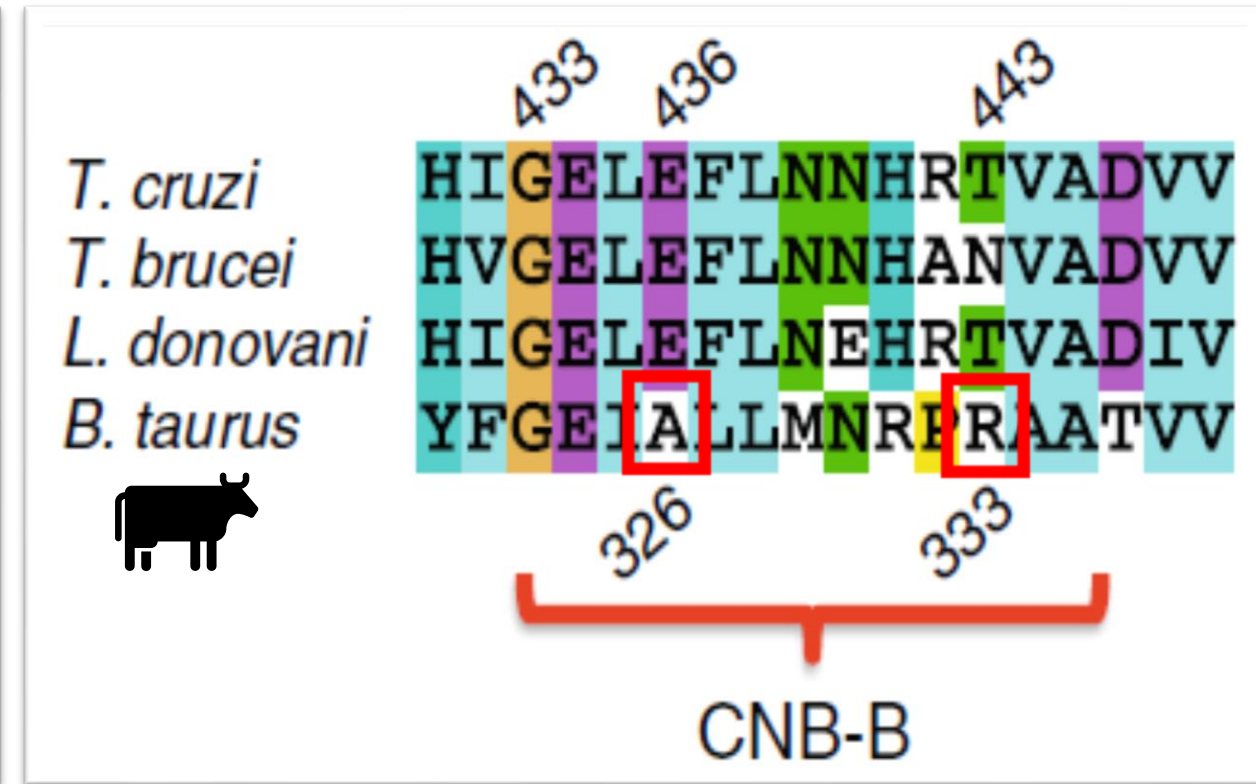
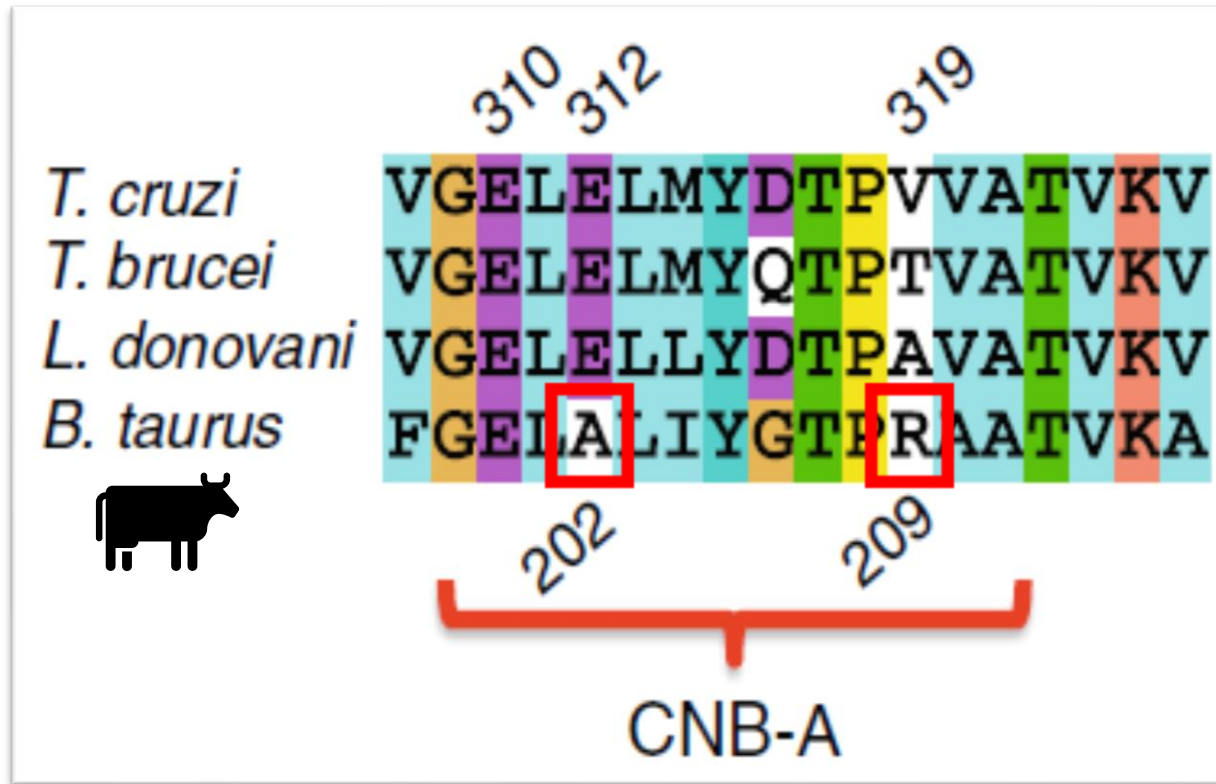
H-bonding network of 7-CN-7-C-Ino bound to CNB-A and CNB-B of TcPKAR

The cocrystal structure shows that glutamate residues (E312/E436) present in both CNB pockets of TcPKAR may clash with the negatively charged phosphate of cAMP but strengthen the interaction with 7-CN-7-C-Ino.

CNB-B

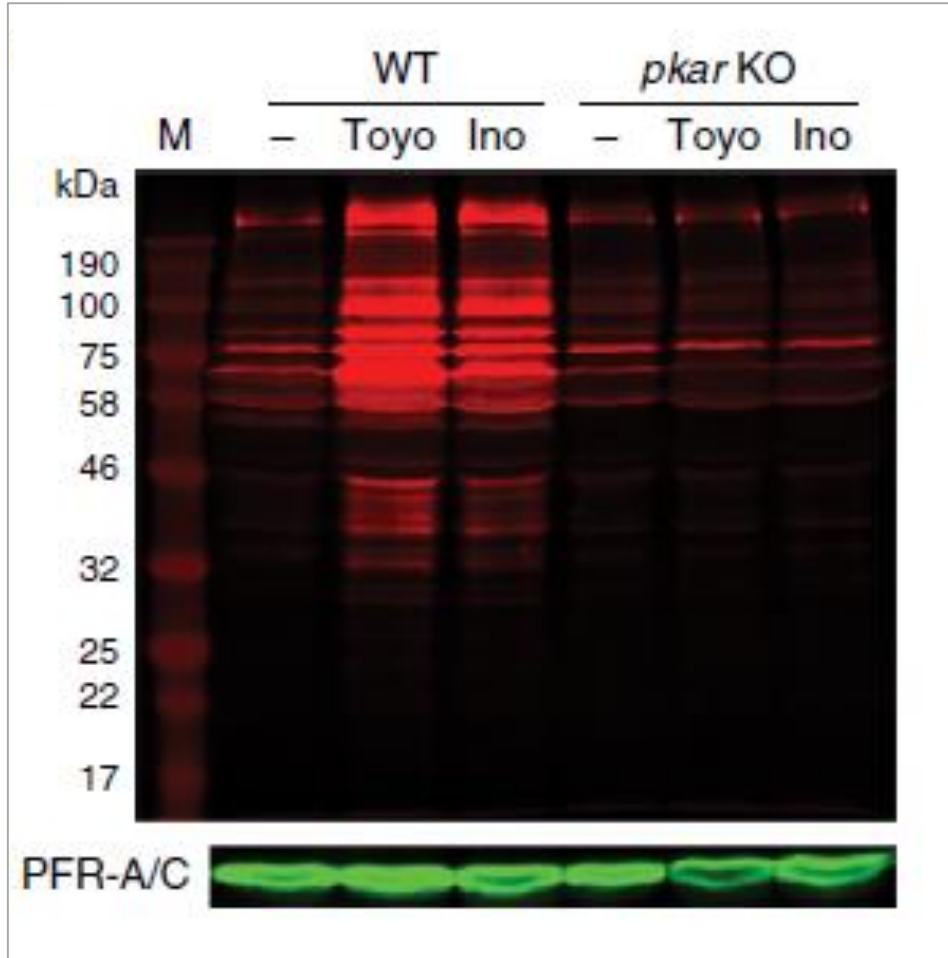


Sequence alignment of PKAR fragment



A: Alanine, E: Glutamate, R: Arginine (+), V: Valine, T: Threonine, N: Asparagine

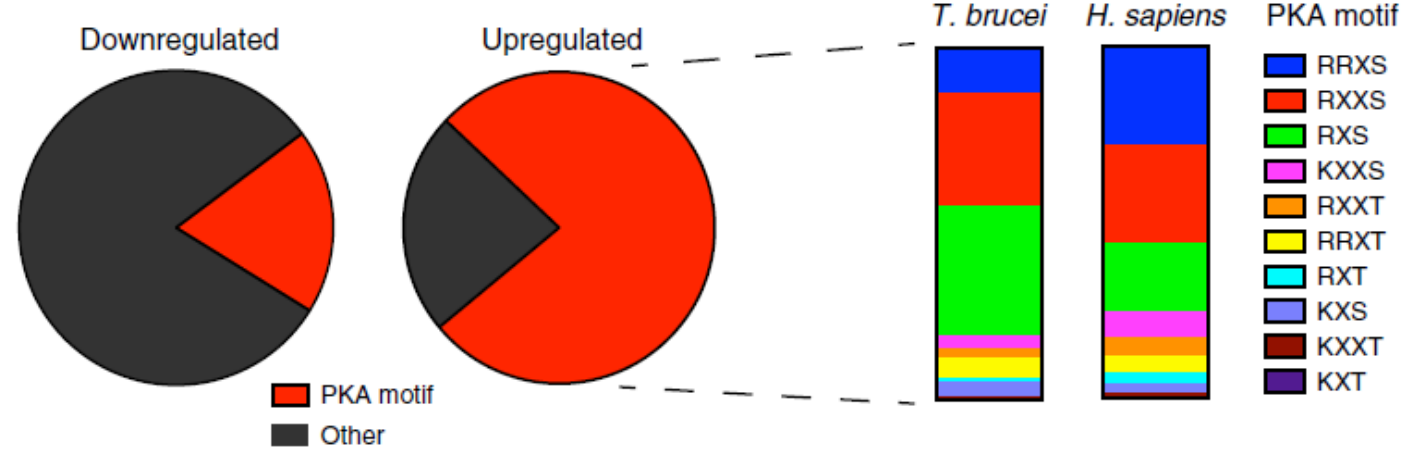
Phosphoproteomic Analysis- PKA signalling and targets in trypanosomes



M: protein molecular weight marker.

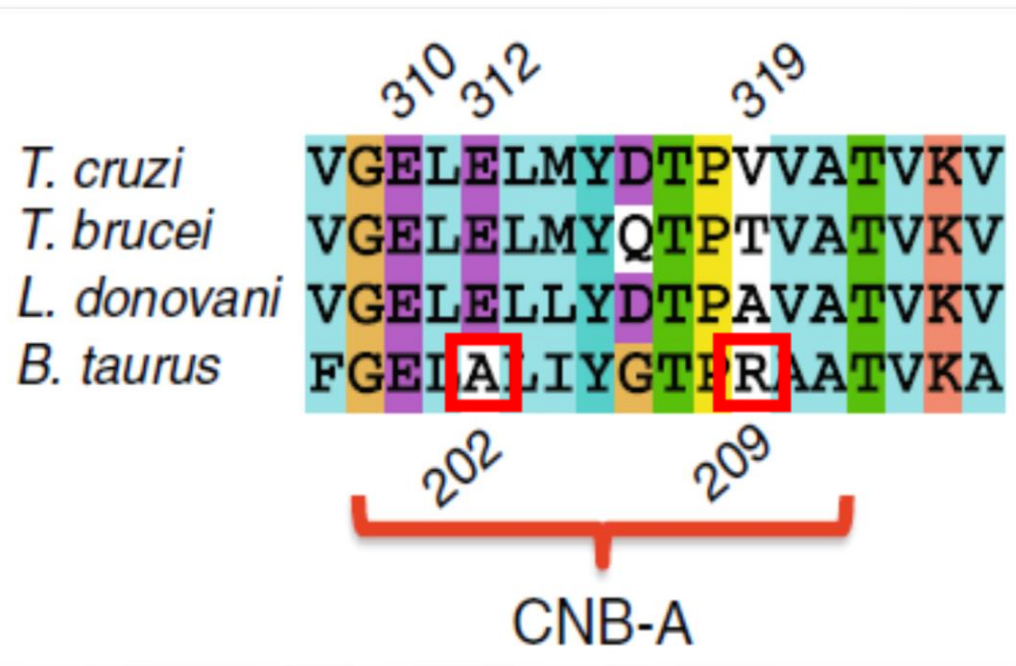


> phospho-specific PKA substrate antibody

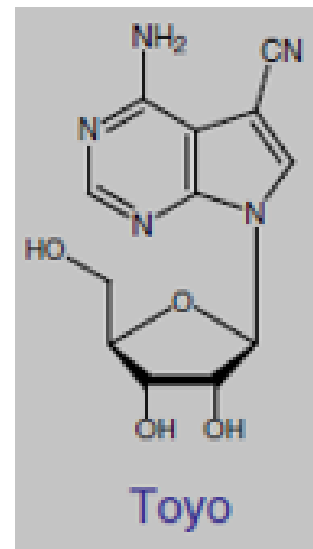


642 significantly upregulated phosphosites, mostly containing PKA motifs (77%)
84 downregulated phosphosites

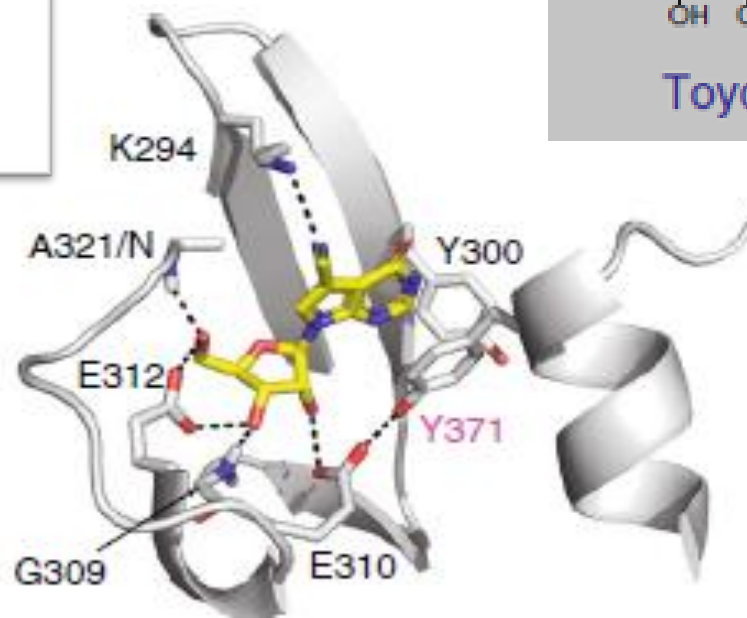
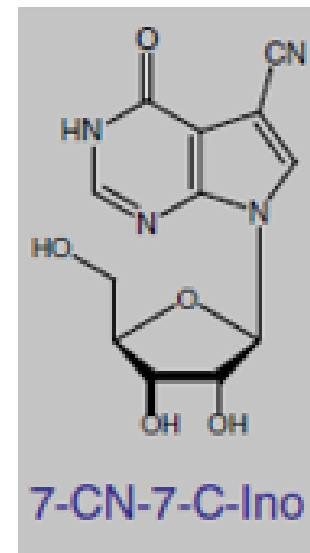
Summary



Toyocamycin
(7-cyano-7-deaza**adenosine**)



7-cyano-7-deaza**inosine**
(7-CN-7-C-Ino, Jaspamycin)

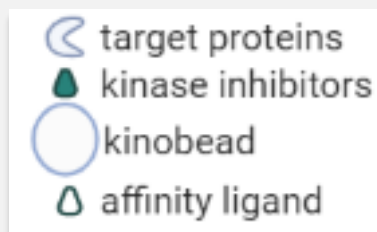
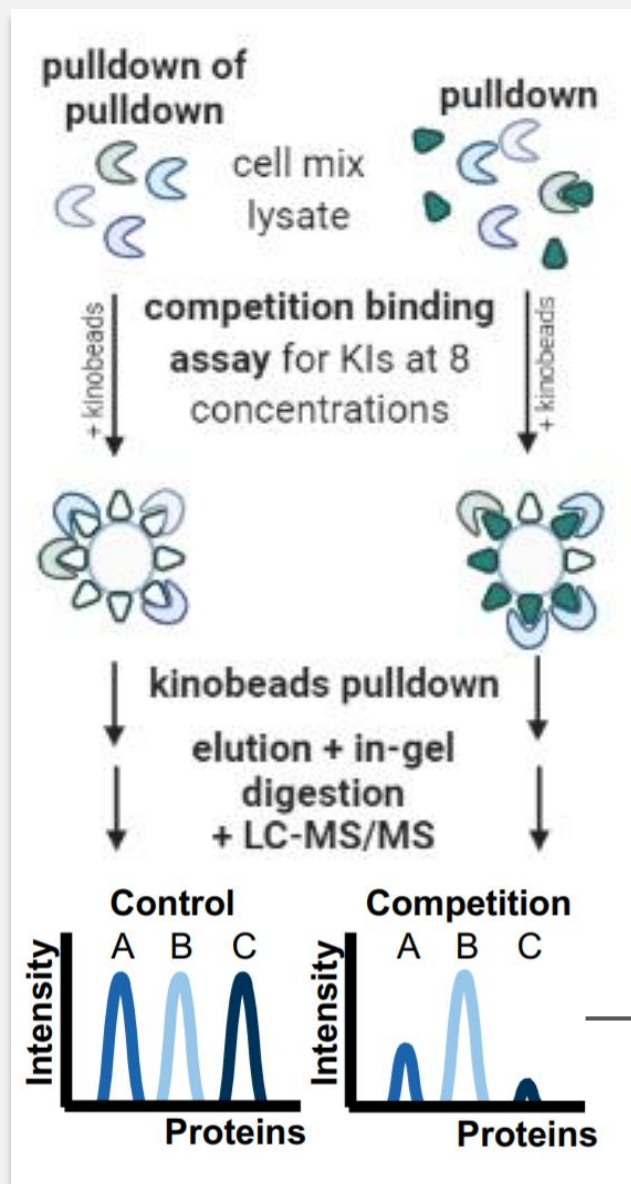


THANK YOU



Proteomic Workflow – Pulldown

Kinases as Drug Targets
by Iris Lade



Experimental background:

- K-562, COLO 205 & MV-4-11 in RPMI 1640 medium
- SK-N-BE(2) cultured in DMEM/HAM's F-12 medium

