Investigation of the metal ion dependence of Phosphodiesterases 5 & 6

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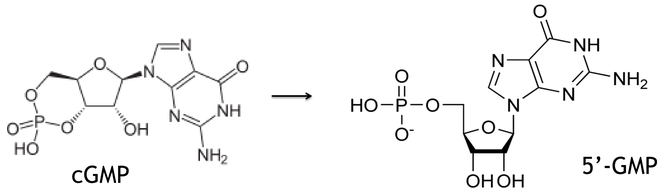
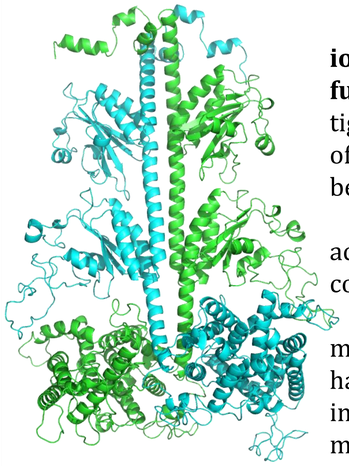
Phosphodiesterase 6 (PDE6)

Phosphodiesterases make up a superfamily of enzymes that degrade cyclic nucleotides in cells and are important in many cellular signaling mechanisms in the body. Phosphodiesterase enzyme 6 (PDE6) is involved in visual signal transduction, or turning light energy into electrical signals to be sent to the brain and processed. PDE6 has divalent cations involved in its catalytic processes.[1,2]





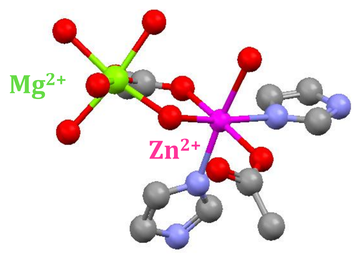
The type and concentration of metal ion can impact the enzyme's catalytic function. PDE6αβ binds two Zn(II) ions tightly per catalytic subunit, though activity of the enzyme requires additional metals to be present.[2]

Picomolar Pb(II) concentration inhibits activity in the presence of millimolar concentrations of Mg(II).[3]

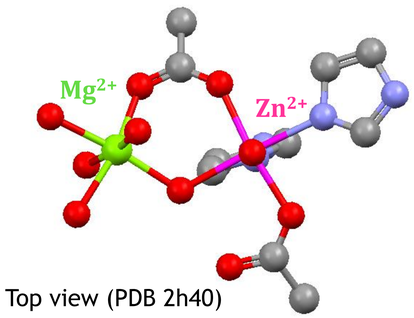
Cd(II) is not only the most similar metal on the periodic table to zinc, but it has documented toxicity, and therefore investigation of its effect on enzyme activity may be medically relevant. [4]

Phosphodiesterase 5 (PDE5)

PDE5 is the most similar family member to PDE6. Not only are the metal-binding residues of the enzymes identical, but both selectively hydrolyze cGMP over cAMP.[5]



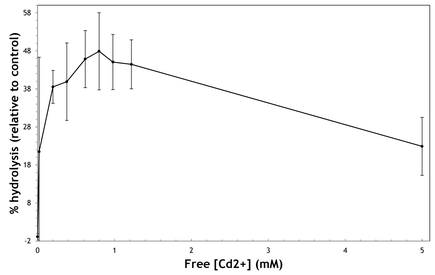
Side view (PDB 2h40)



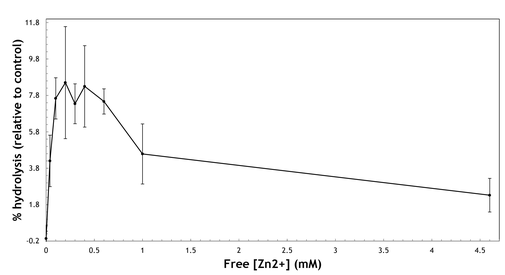
Top view (PDB 2h40)

Results

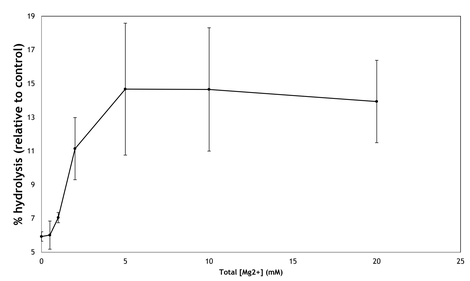
Cadmium



Zinc

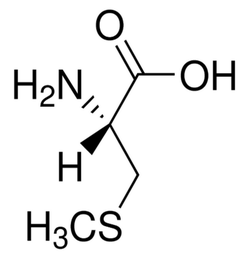


Magnesium



PDE5 and PDE6 respond very differently to metal ion concentrations despite their similar catalytic sites.

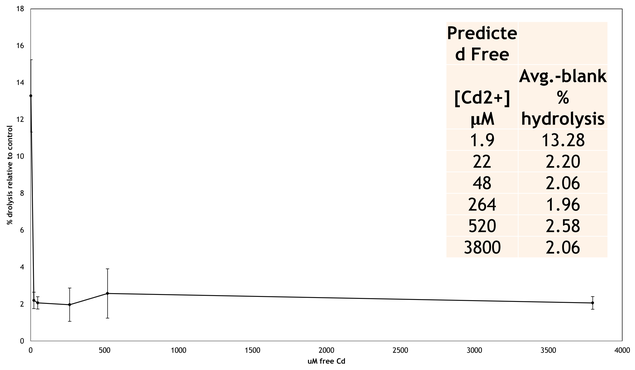
Buffering



s-methylcysteine

Metal ion buffering was employed to control free cadmium ion concentration. S-methylcysteine was used as a buffering agent due to its range of stability constants for cadmium, biological nature, and appropriate buffering region.

PDE6 activity in **Cd-s-methylcysteine** buffer at pH 7.4 using MOPS.



Predicted Free [Cd2+] µM	Avg.-blank % hydrolysis
1.9	13.28
22	2.20
48	2.06
264	1.96
520	2.58
3800	2.06

Acknowledgements & References

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(1) Francis SH, Colbran JL, McAllister-Lucas LM, Corbin JD. Zinc interactions and conserved motifs of the cGMP-binding cGMP-specific phosphodiesterase suggest that it is a zinc hydrolase. *J Biol Chem* **1994**; *269*: 22477–22480.
(2) He F, Seryshev AB, Cowan CW, Wensel TG. Multiple zinc binding sites in retinal rod cGMP phosphodiesterase, PDE6ab. *J Biol Chem*. **2000**; *275*: 20572–20577.
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(5) Cote, RH. Characteristics of Photoreceptor PDE (PDE6): similarities and differences to PDE5. *International Journal of Impotence Research*. **2004**. 16, S28-S33.