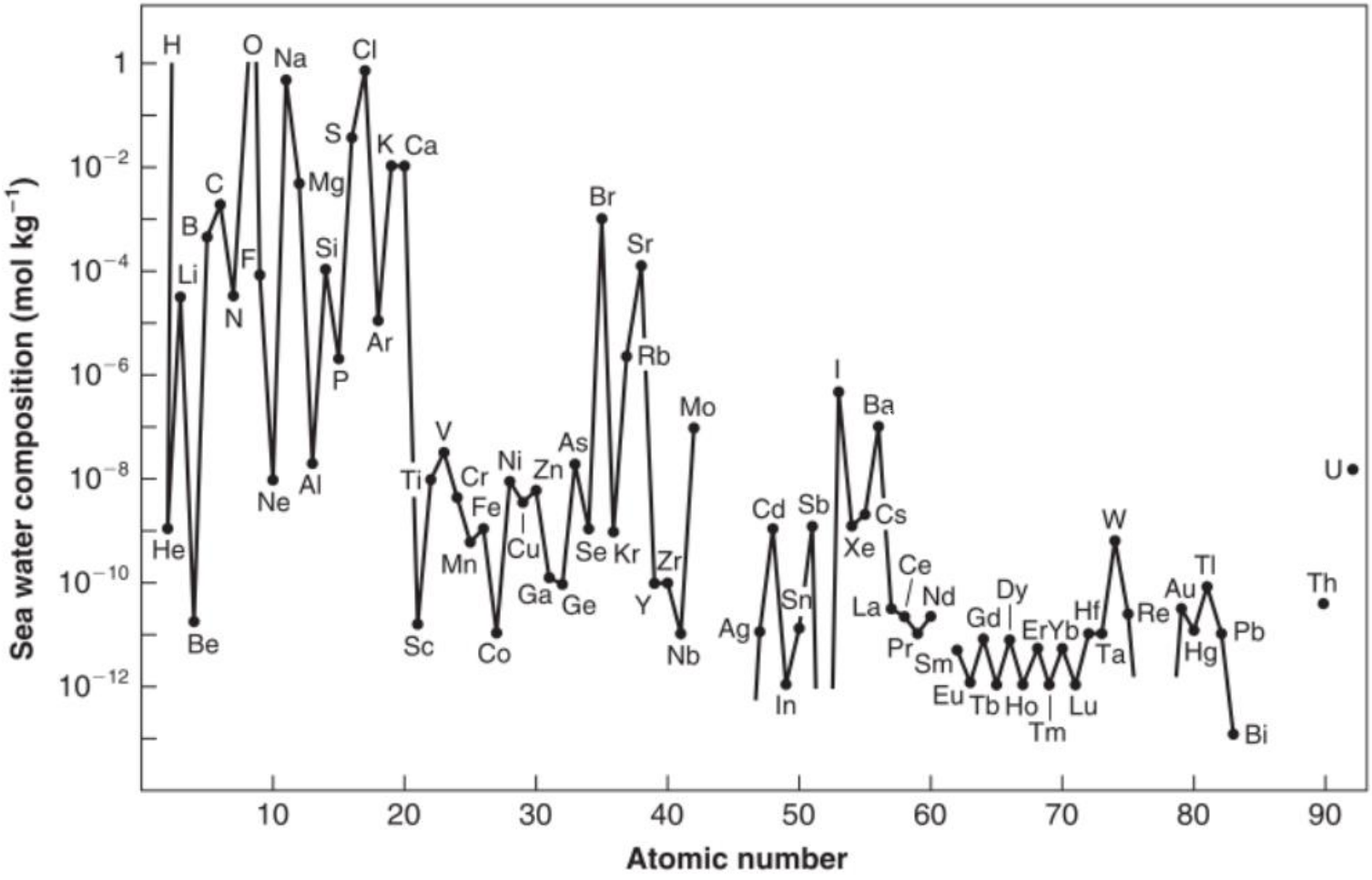
Metals in Biology and Medicine

Supplementary Reading

Prof. Arthur D. Tinoco
University of Puerto Rico, Rio Piedras Campus

1. Element Abundance in the Ocean



- The measure of element abundance in the ocean is a good indicator of bioavailability due to it being also a measure of solubility

2. Vertical Profiles of Elements in the Ocean are more telling of their Potential Biological Use

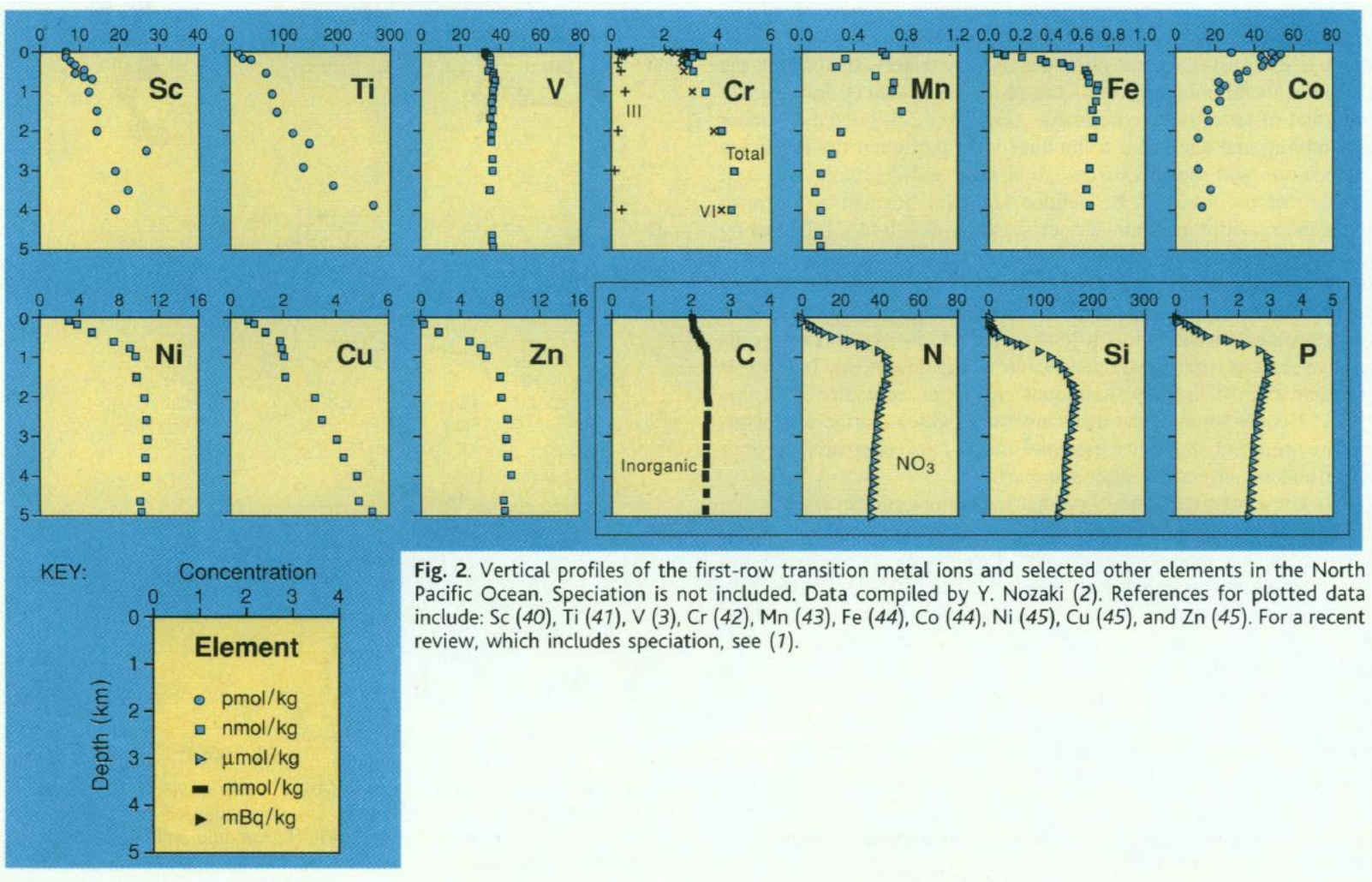
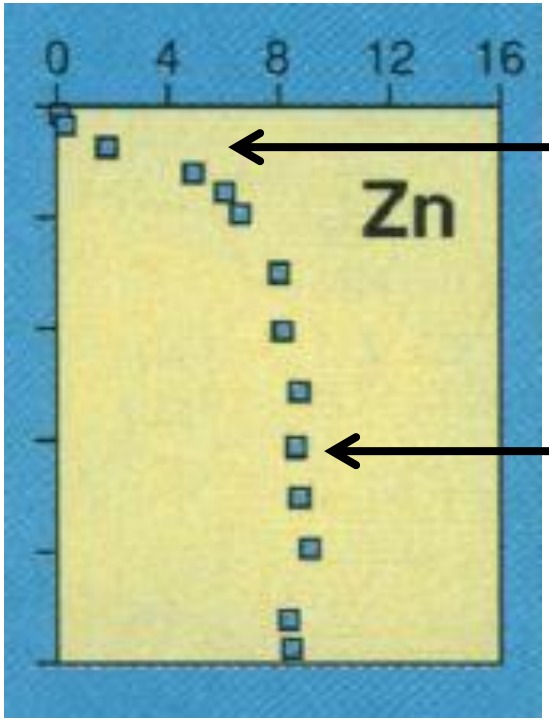


Fig. 2. Vertical profiles of the first-row transition metal ions and selected other elements in the North Pacific Ocean. Speciation is not included. Data compiled by Y. Nozaki (*2*). References for plotted data include: Sc (*40*), Ti (*41*), V (*3*), Cr (*42*), Mn (*43*), Fe (*44*), Co (*44*), Ni (*45*), Cu (*45*), and Zn (*45*). For a recent review, which includes speciation, see (*1*).

Butler, A. *Science*, **1998**, *281*, 207-210.

2. Vertical Profiles of Elements in the Ocean are more telling of their Potential Biological Use

Several transition metals show a nutrient-like distribution profile:



Depletion in surface waters possibly due to consumption by organisms

Significant increase in element concentration in greater depths of the ocean where life is more scarce

3. Zn^{2+} is commonly employed in proteins in different roles

I. Catalytic
   - Cofactor

II. Structural
   - Required for the stability of a protein structure
   - Arrangement of an active site (i.e. CuZn superoxide dismutase (CuZnSOD))

III. Regulatory
   - Zinc finger family of proteins are known to enable DNA-protein interactions

   Cys_2His_2 Zn^{2+} fingers are found in 2% of all human genes

Jamieson, A.C. *Nat. Rev. Drug. Discov.*, **2003**, *2*, 361-368.

A. Zn^{2+} properties important for the type of catalytic roles that it plays

- Zn^{2+} is the only accessible oxidation state (d^{10})
  - An important property for non-redox processes
  - Zn^{2+} is referred to as a spectroscopically silent ion and can be substituted by Cu^{2+} or Co^{2+} to probe a binding site within a protein
- Is an intermediate metal and thus binds hard and soft ligands
- Has a preferred tetrahedral geometry but is capable of expanding its coordination shell to C.N. = 5 or 6

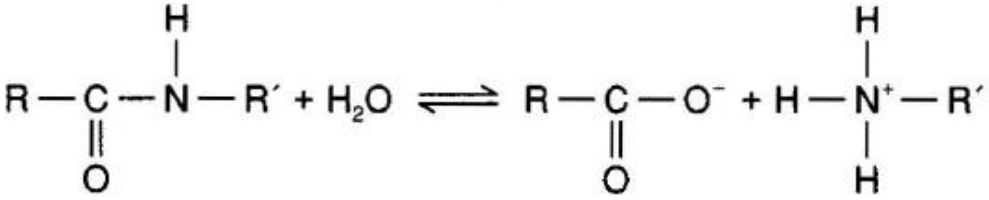
B. Zn^{2+} is commonly involved in hydrolytic and related reactions

Table 2.1
Representative metalloenzymes catalyzing hydrolytic and related reactions.

Enzyme	Metal(s)	Function
Carboxypeptidase	Zn^{2+}	Hydrolysis of C-terminal peptide residues
Leucine aminopeptidases	Zn^{2+}	Hydrolysis of leucine N-terminal peptide residues
Dipeptidase	Zn^{2+}	Hydrolysis of dipeptides
Neutral protease	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Collagenase	Zn^{2+}	Hydrolysis of collagen
Phospholipase C	Zn^{2+}	Hydrolysis of phospholipids
β-Lactamase II	Zn^{2+}	Hydrolysis of β-lactam ring
Thermolysin	Zn^{2+}, Ca^{2+}	Hydrolysis of peptides
Alkaline phosphatase	Zn^{2+}, Mg^{2+}	Hydrolysis of phosphate esters
Carbonic anhydrase	Zn^{2+}	Hydration of CO_2

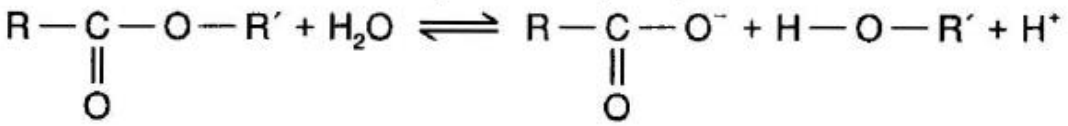
B. Zn^{2+} is commonly involved in hydrolytic and related reactions

peptide hydrolysis



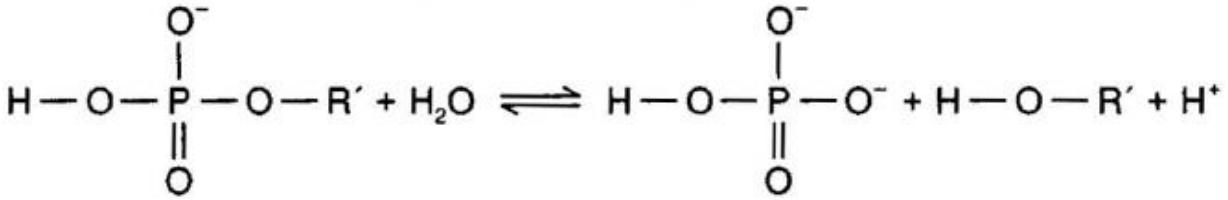
(2.1)

carboxylic ester hydrolysis



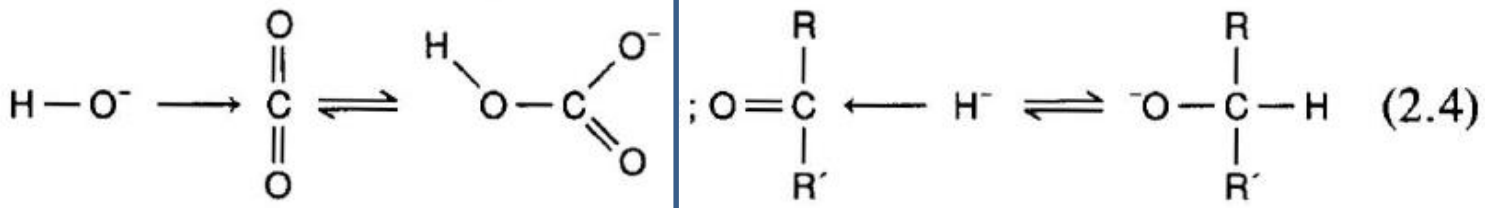
(2.2)

phosphoric ester hydrolysis



(2.3)

nucleophilic addition of OH^- and H^-



(2.4)

C. Carbonic Anhydrase

I. An enzyme that is located in red blood cells in mammals and that helps to buffer the blood by catalyzing the reaction:

$$H_2O + CO_2 \rightleftarrows HCO_3^- + H^+$$

$$k_{uncat} = 10^{-11}\ s^{-1}$$
$$k_{cat} = 10^{4}\ s^{-1}$$

Carbonic acid then buffers pH via the following equilibrium:

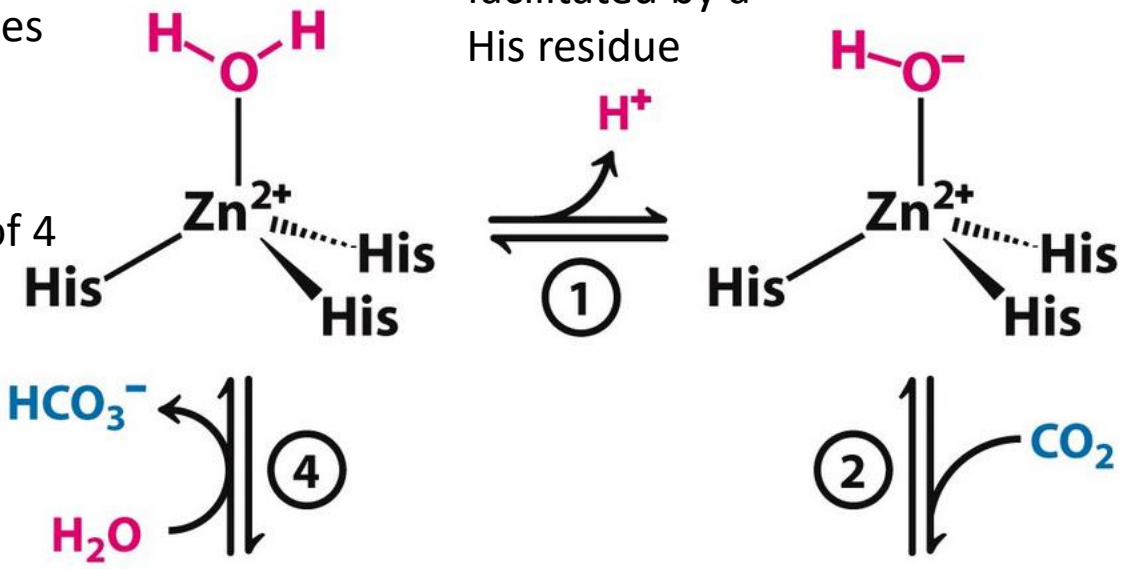
$$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

C. Carbonic Anhydrase

Deprotonation facilitated by a His residue

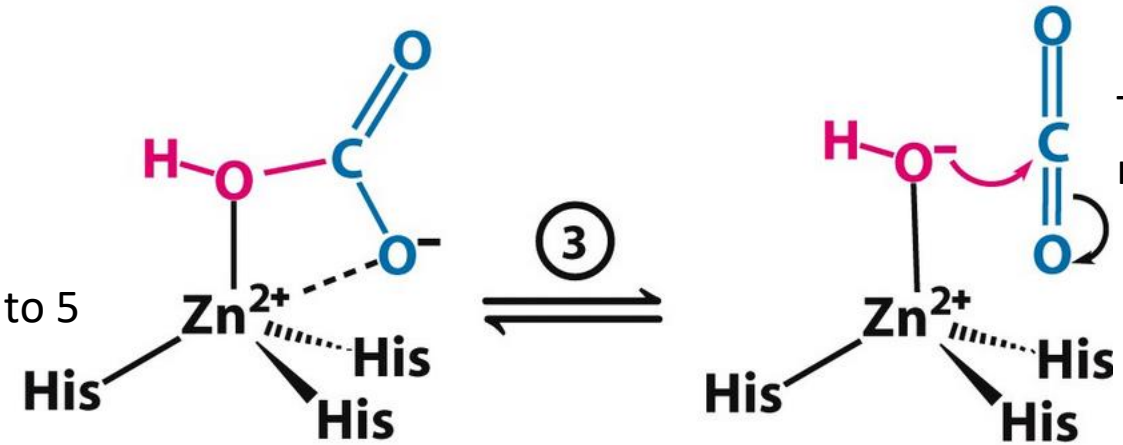
Water then displaces the bicarbonate

- A return to the preferred C.N. of 4



The bicarbonate binds to Zn^{2+} as a bidentate ligand

- C.N. increases to 5



The OH^- serves as a nucleophile

Figure 9.25
***Biochemistry*, Seventh Edition**

C. Carbonic Anhydrase

II. CA is categorized into three main classes:

α, β, and γ which share low sequence and structural homology

α and γ-CA coordinate Zn^{2+} via three histidines

β coordinates Zn^{2+} via two cysteines and one histidine

δ-CA is less commonly known but has an active site similar to α-CA

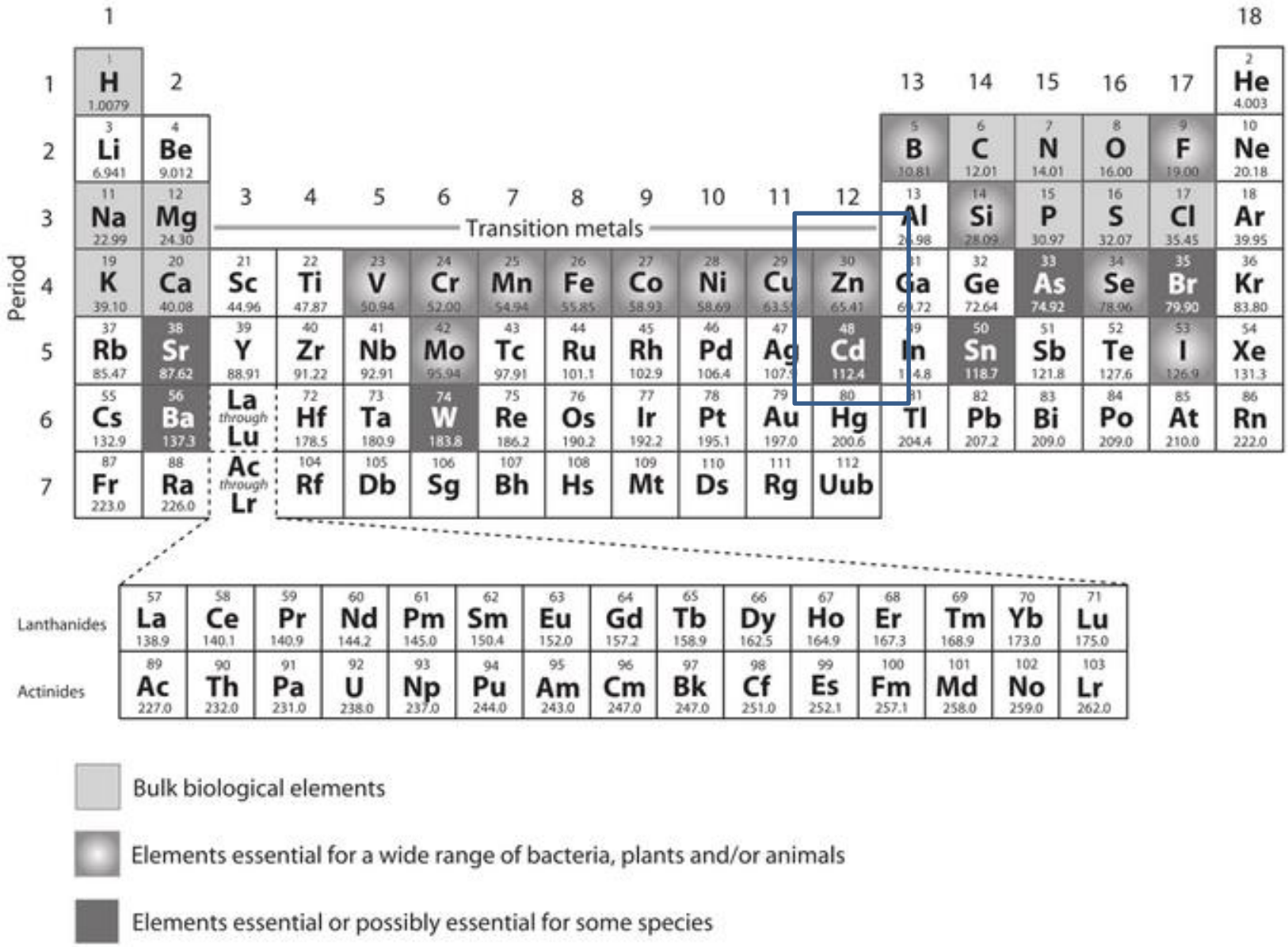
D. Not all carbonic anhydrases are Zn^{2+}-based

Several marine diatoms (unicellular algae) use Cd^{2+} as a catalytic metal ion in CA. These organisms

- Are responsible for nearly 40% of net marine primary production (the synthesis of organic compounds from a carbon dioxide source)
- Have adapted to life in an environment where the concentrations of essential metals steadily decreases

  Cd-CA is thought to be part of this adaptation

- Use CA to acquire inorganic carbon (CO_2) for photosynthesis


Period
Transition metals
Lanthanides
Actinides
Bulk biological elements
Elements essential for a wide range of bacteria, plants and/or animals
Elements essential or possibly essential for some species

I. Cadmium

- Once thought to be not only unimportant to biology but also very TOXIC.
- Nutrient-like vertical profile in oceans suggests bioactivity

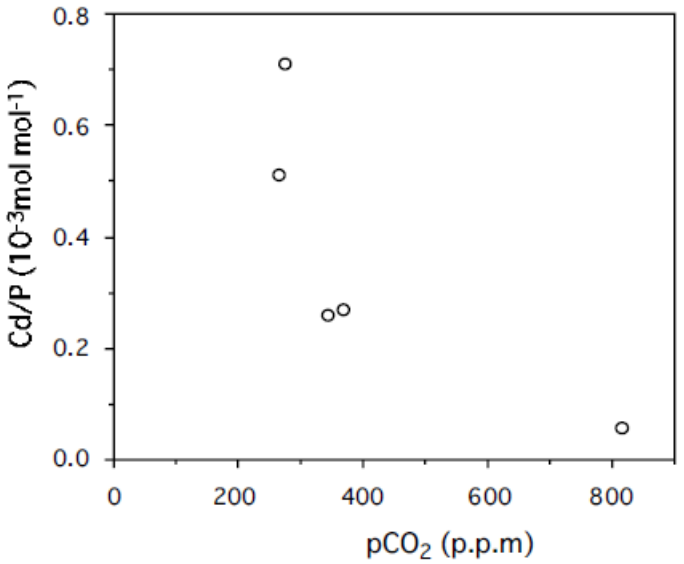


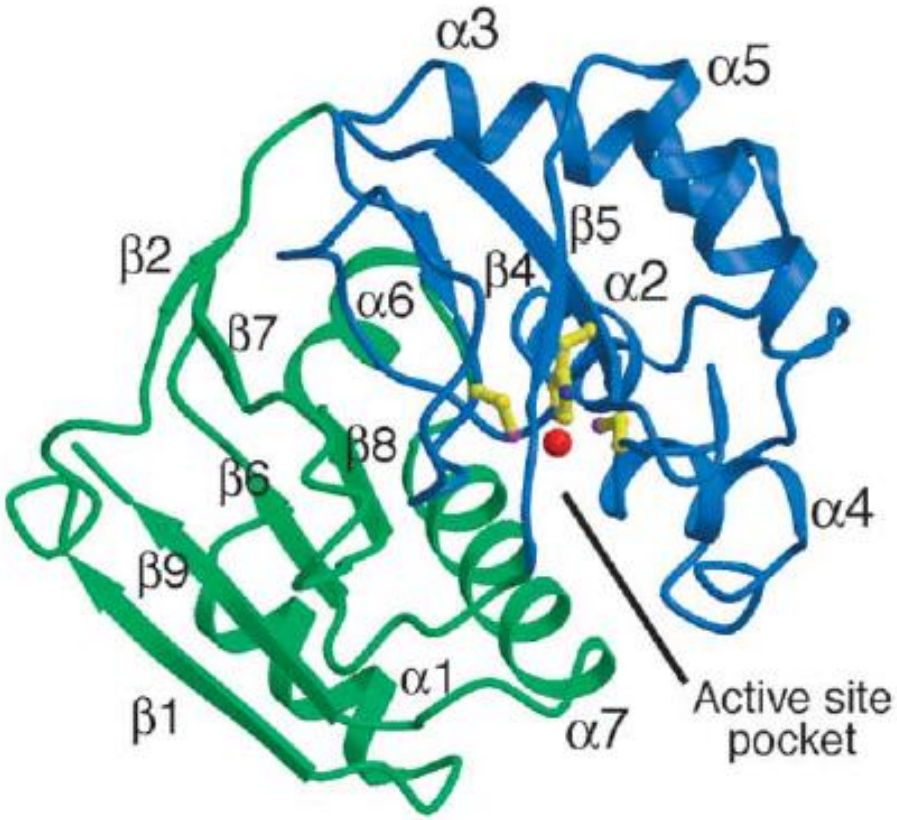
Fig. 5. Phytoplankton Cd/P ratio versus pCO_2 in field samples. Samples were collected at locations within 50 km off central California in April 1997 (Cullen *et al.*, 1999).

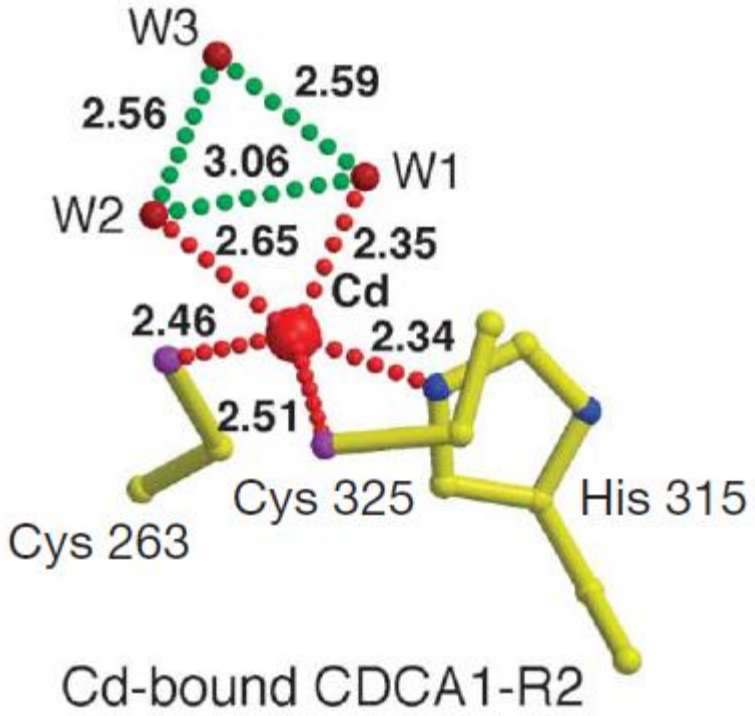
Abe, K. *Global environmental change in the ocean and on land,* **2004**, 189-203.

II. CDCA1

- ζ-CA: Has active site conformation similar to β-CA
- Three highly homologous CA repeats (R1-R3)
- Although Cd^{2+} is naturally bound to the enzyme, the enzyme is cambialistic
  - Can use Zn^{2+} or Cd^{2+} for catalysis
- Likely uses a similar catalytic mechanism for both metals

II. CDCA1





Xu, Y. *Nature*. 2008, *456*, 56-62.

III. But why Cd^{2+}?

- Cd^{2+} is more scarce than Zn^{2+}
  - But can keep Zn-limited laboratory cultures alive
  - Increase CA activity
  - Improve growth rate
- CDCA1 has lower catalytic efficiency (k_{cat}/K_M) when Cd^{2+} bound. Nonetheless,
  - It was determined that 0.6 µmol enzyme per mol C is needed to effectively acquire and transform CO_2
  - This corresponds to a cellular Cd/C ratio of 2 µmol Cd^{2+}/mol C, which is the amount that is found in the phytoplankton biomass.

Xu, Y. *Nature*. 2008, *456*, 56-62.

4. The serendipitous discovery of Vanadium Bioactivity

There was a major interest in marine seaweeds because they produce high quantities of diverse halogenated (Cl, Br, I-containing) natural products as metabolites.

- These metabolites are of pharmacological interest due to several exhibiting antifungal, antiviral, and anti-inflammatory activities

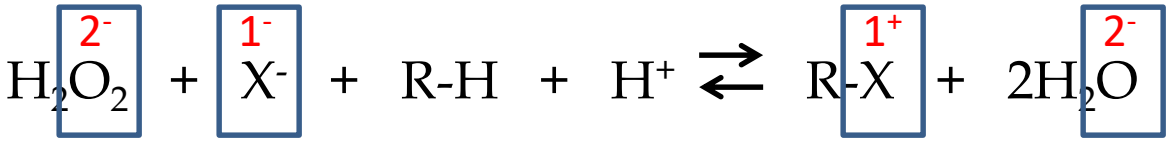
- It was believed that a haloperoxidase enzyme was responsible for the biogenesis of several of these metabolites

  -The active site of these enzymes typically contain an Fe-Heme center

  -Isolation of proteins showing haloperoxidase activity proved surprising when no Fe-Heme was present but rather stoichiometric amounts of vanadium

4A. Vanadium Haloperoxidases

These enzymes catalyze the oxidation of halides (iodide, bromide, chloride) by hydrogen peroxide and subsequent halogenation of organic substrates via a vanadate cofactor.



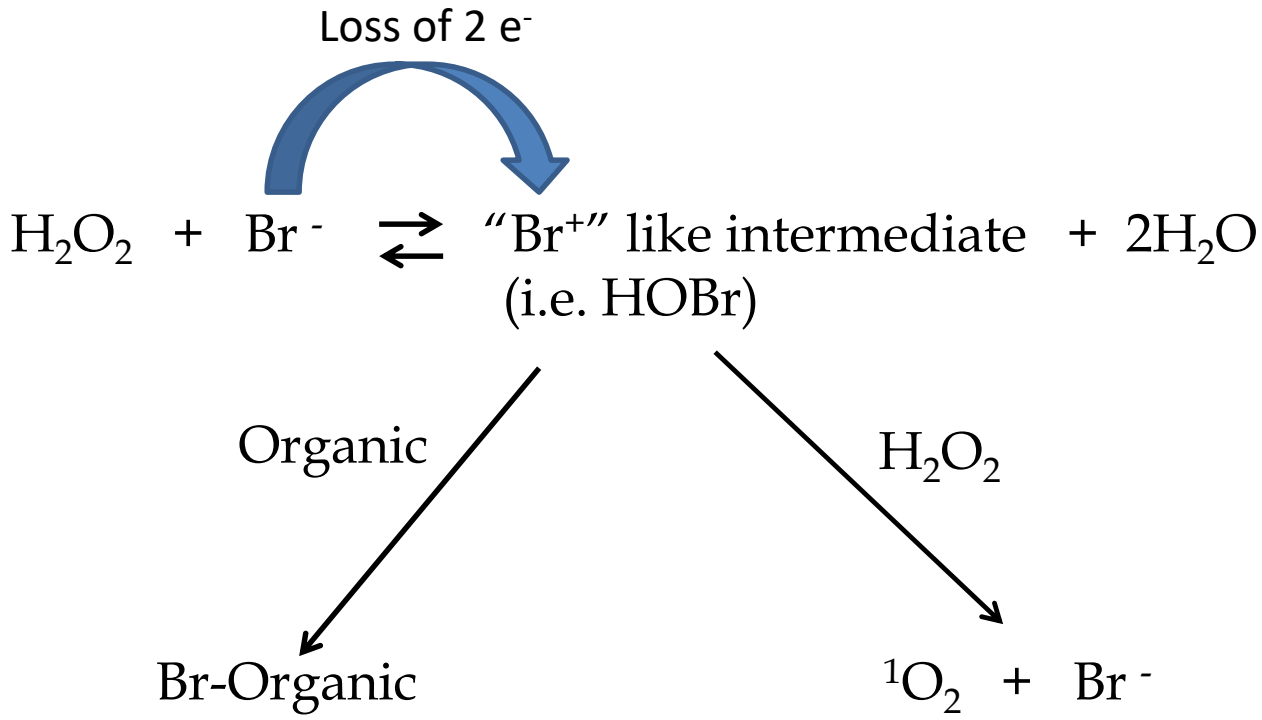
X^-: Halides
R-H: Organic Substrates

Classified according to the most electronegative halogen oxidized:

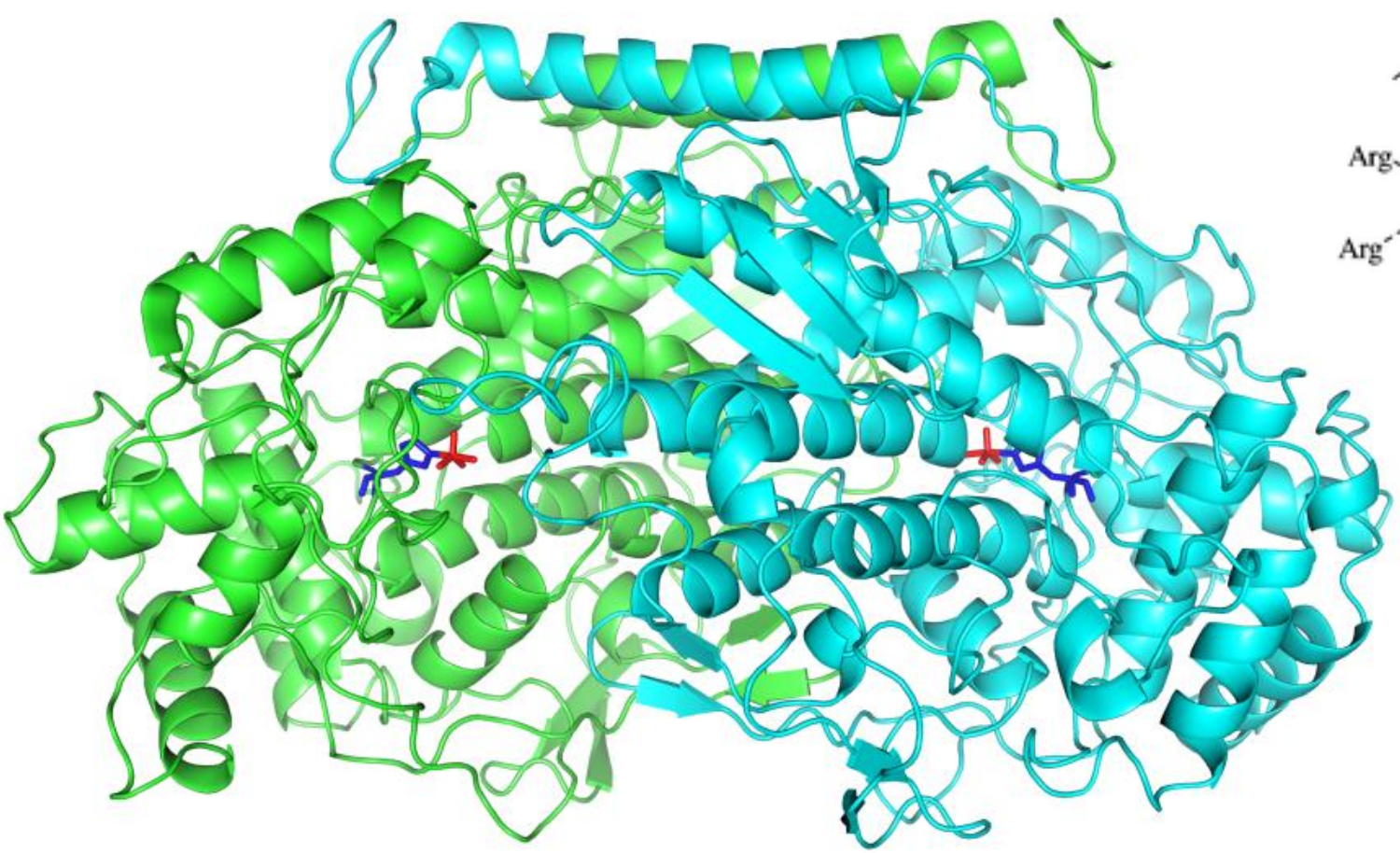
- Vanadium chloroperoxidases (V-ClPOs) can oxidize chloride, bromide, and iodide (isolated from fungi)
- Vanadium bromoperoxidases (V-BrPOs) can oxidize bromide and iodide (isolated from green, brown, red algae)
- Vanadium iodoperoxidases (V-IPOs) can oxidize iodide (isolated from brown algae)

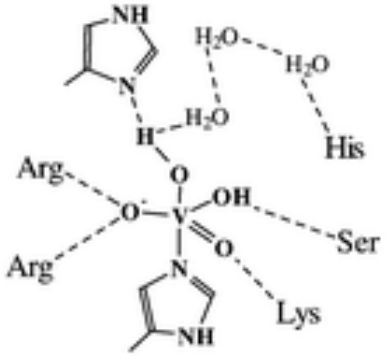
4A. Vanadium Haloperoxidases

A closer look at the catalyzed reaction:



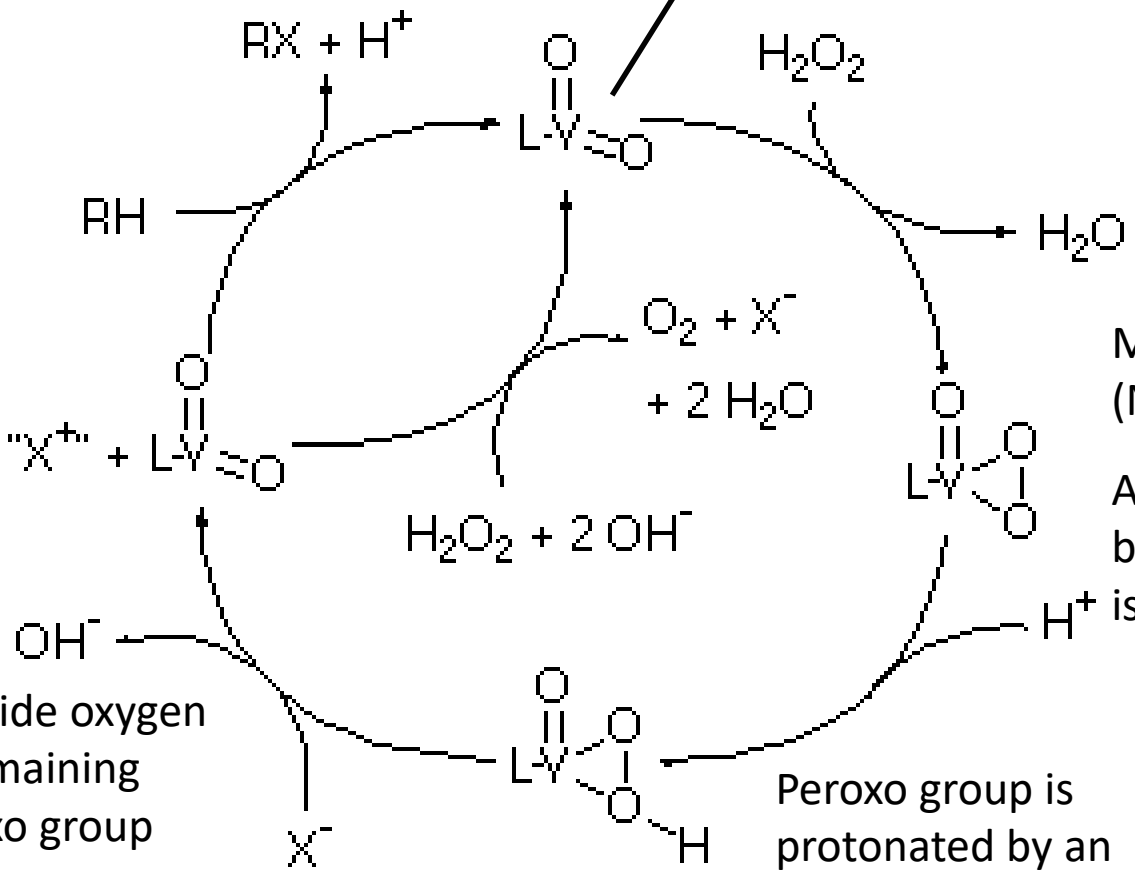
4B. Crystal Structure of Vanadium Bromoperoxidase





This is a dimeric structure of the enzyme showing two metal binding sites, in which vanadate is coordinated to one His. PDB: 1QI9

4C. The mechanism of action of Vanadium Bromoperoxidase



Metal Oxidation State: 5+
Inactive form

Metal Oxidation State: 5+
(No change)

Active form with bidentate peroxo; each O is O.S.: -1

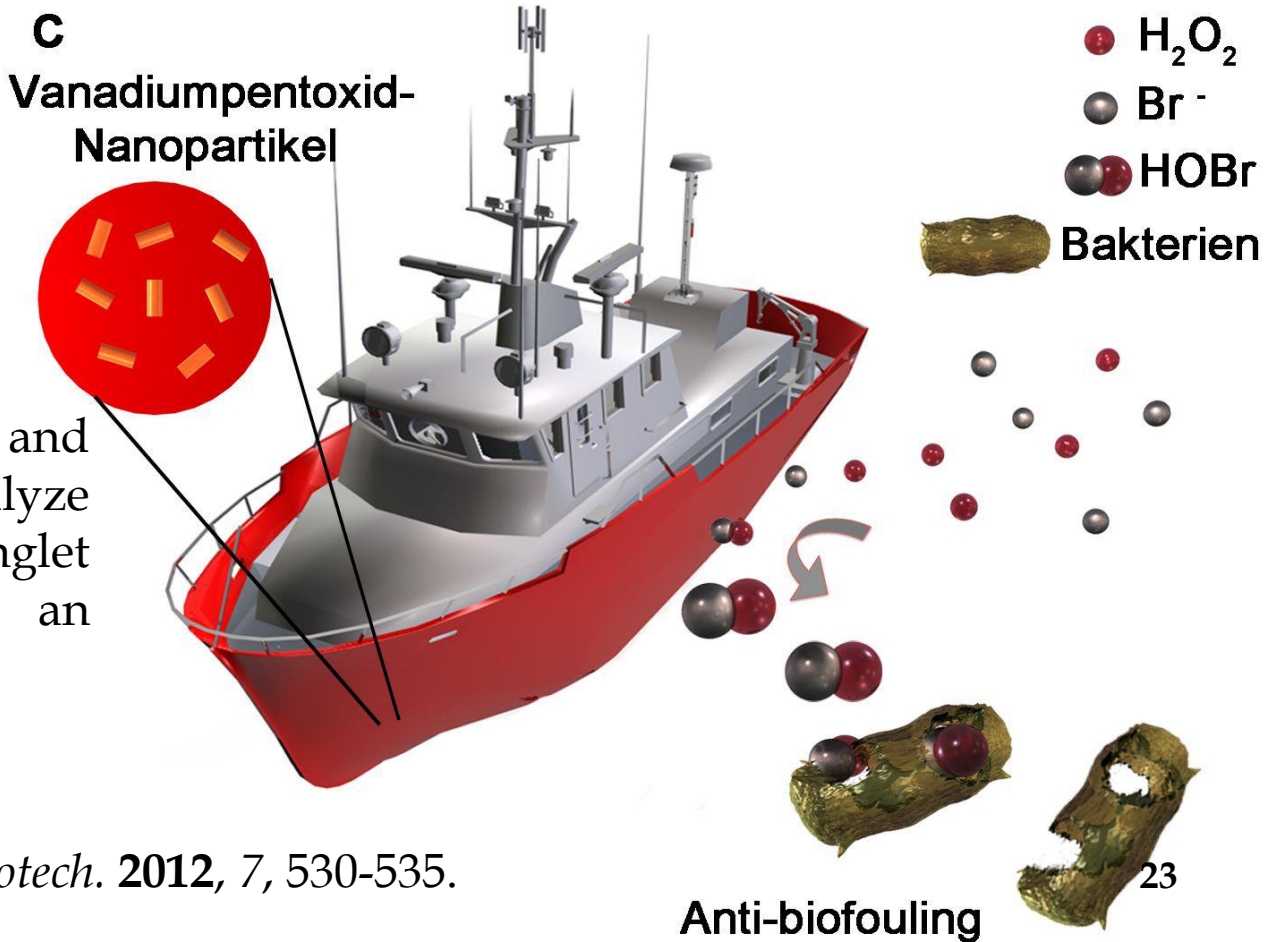
The hydroxide oxygen and the remaining terminal oxo group both gain one e^-

Peroxo group is protonated by an amino acid

4D. Why do algae employ this enzyme?

The enzyme may serve the function of a defensive mechanism. It is speculated that the production of halogenated organic molecules makes the algae "taste" bad and it wards off predators.

Bio-inspired materials design



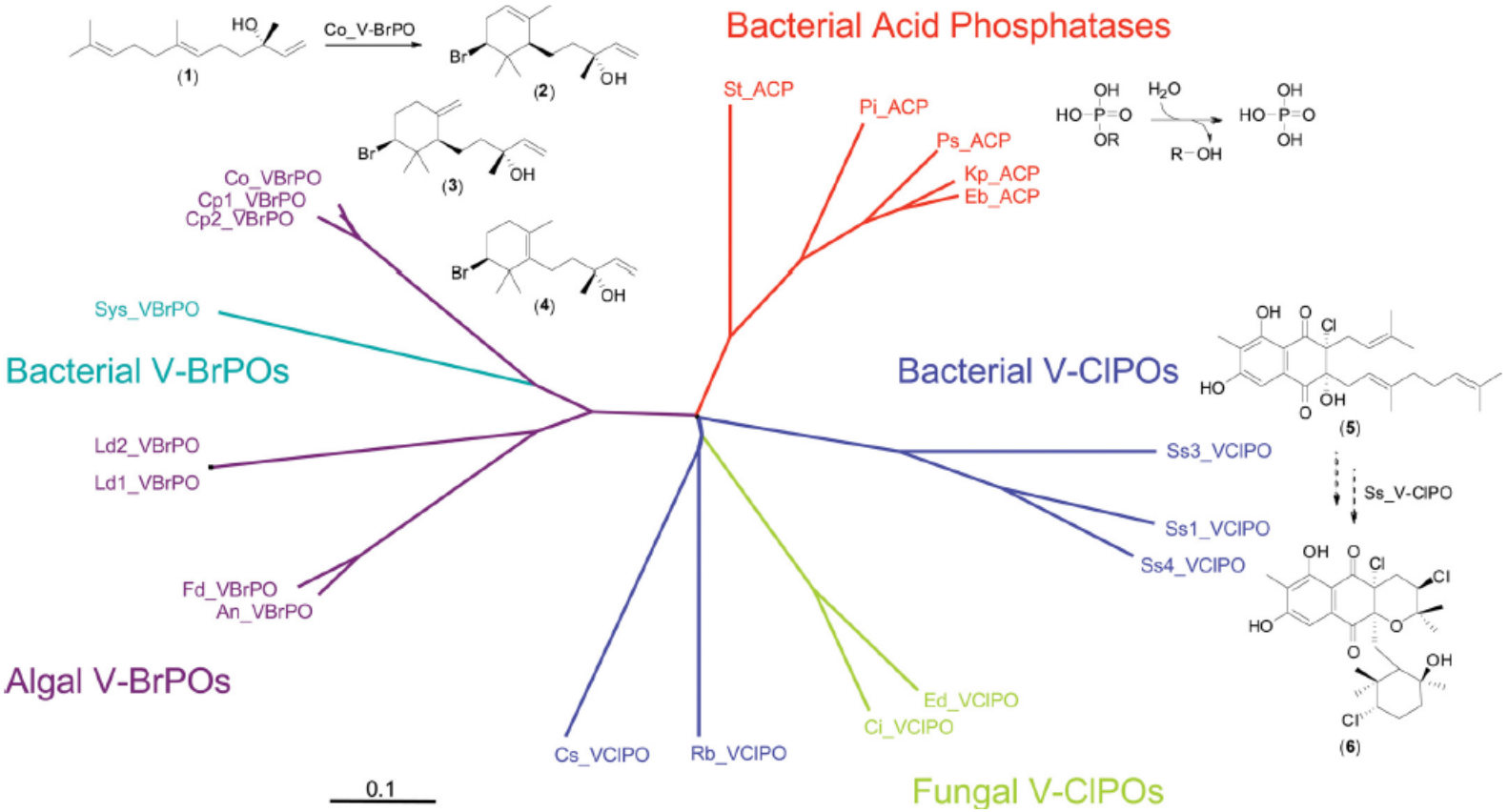
- In the presence of Br^- and H_2O_2, the nanowires catalyze the formation of singlet oxygen, which exerts an antibacterial effect.

Natalio, F. *et al. Nat. Nanotech.* **2012**, *7*, 530-535.

4E. The Evolution of the Vanadium Haloperoxidases

The vanadium haloperoxidases have not extended beyond primitive organisms and appear to be related to phosphatases

- Vanadate is an analogue of phosphate



Winter, J.M. and Moore, B.S. *J. Biol. Chem.* **2009**, *284*, 18577-18581.

4F. Vanadium's place in biology

I. Vanadium has an extensive oxygen chemistry and can exist as an oxyanion [VO_4^{3-}, V^{5+}] and as an oxycation [VO^{2+}, V^{4+}] [VO_2^{+}, V^{5+}]
   - Coordination complexes with vanadium typically have at least one oxo group bound to the metal center

II. The oxovanadium species serve as one-electron oxidizing agents
   - Vanadate (VO_4^{3-}): $E^{o'}$ = +0.5V

III. Vanadium behaves well in sulfur-rich reducing conditions forming
   - Sulfur-containing anionic species [VS_4^{3-}, V^{5+}]
   - Sulfur-containing cationic species [VS^{2+}, V^{4+}]
   - Iron-sulfur clusters [Fe, V, S cluster in a lesser studied nitrogenase]

4F. Vanadium's place in biology

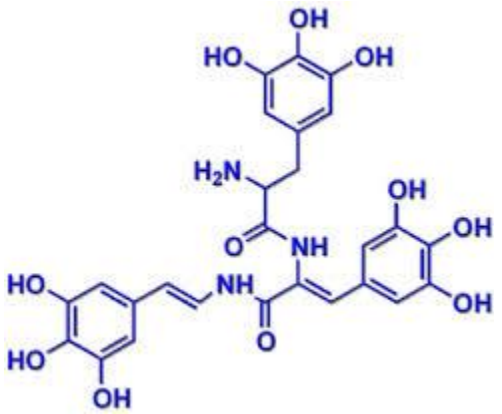
Vanadium may have been one of the most primitive metals used in biology and possibly existed as solid V_3S_2 and VS_4 and soluble sulfur complexes as VS^{2+} and $VSSH^{+}$ or mixed metal sulfur clusters but became more oxidized as the atmosphere became oxidizing

- In early times, vanadium may have been a source of reducing equivalents to capture light energy for photosynthesis
- This would account for the high vanadium concentration in marine photosynthetic species
- There are several examples of vanadium use/storage in primitive organisms

4G. Vanadium in Organisms

I. Ascidians- Invertebrate marine organisms known for their tough outer "tunic"

- Use **tunichromes** (similar to siderophores) to sequester V possibly for protection and regrowth of the tunic
- Use vanabins, vanadium-binding metalloproteins, to concentrate V(III/IV) in the blood cells (pH ~ 1.9) to more than 100 times higher than the surrounding water.
  - Their function is not known but maybe for a defense mechanism

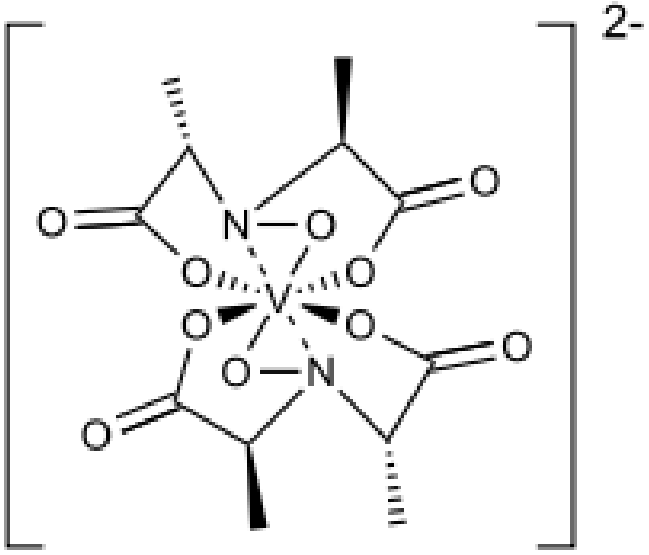


Example of a tunichrome

4G. Vanadium in Organisms

II. *Amanita* mushrooms

- Contain amavadin, a V(IV)-containing anionic species
- C.N. = 8
- No oxo ligand



- The function of this complex is unknown but it may play a peroxidase or catalase role in a defense mechanism

4H. Vanadium in medicine

- Vanadium(IV) and vanadium(V) salts and compounds demonstrate an important role in antidiabetic treatments.
  - The mechanism of action of these metals appears to be insulin enhancement by way of phosphate mimicking.

OUTLINE

1. **Introduction: Diabetes Mellitus**
   - *General Complications*
   - *Global Statistics*
2. **Insulin signaling pathway**
   - *Insulin: general facts and DMT1*
   - *Insulin signaling and Insulin resistance: DMT2*
3. **Vanadium antidiabetic properties**
   - *Vanadium inorganic salts*
   - *Vanadium chelates*
   - *Biodistribution*
4. **VO(IV)-flavonoid complexes**
   - *Flavonoid properties*
   - *In vitro and in vivo studies*

Diabetes Mellitus

- DM is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.

- DM is characterized by **persistent elevation in fasting and postprandial blood glucose levels** (*Hyperglycemia**, ≥7.0 mmol/L***). Uncontrolled hyperglycemia leads to serious multisystemic damage, especially the nerves and blood vessels,

DM type 1. Deficient insulin production and requires daily administration of insulin. Unknown cause. Not preventable.

Symptoms. Excessive excretion of urine, thirst, constant hunger, weight loss, vision changes and fatigue. These may occur suddenly.

DM type 2. Body's ineffective use of insulin. Is largely the result of excess body weight and physical inactivity

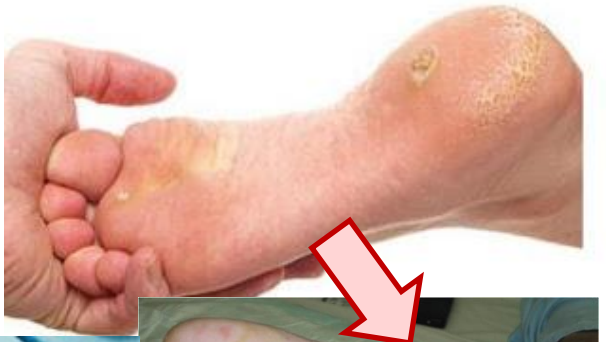
Symptoms. Similar but less marked to those of type 1. May be diagnosed several years after onset, once complications have already arisen.

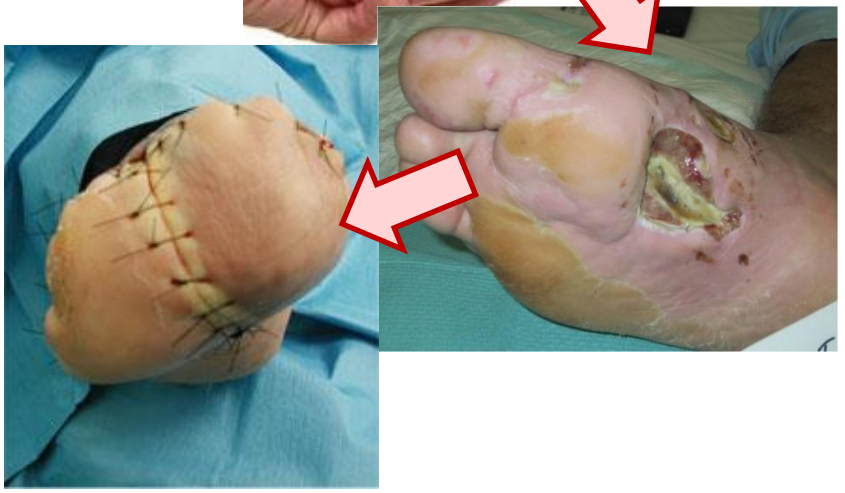
World Health Organization, Geneva, **1999**. Report Number WHO/NCD/NCS/9932.

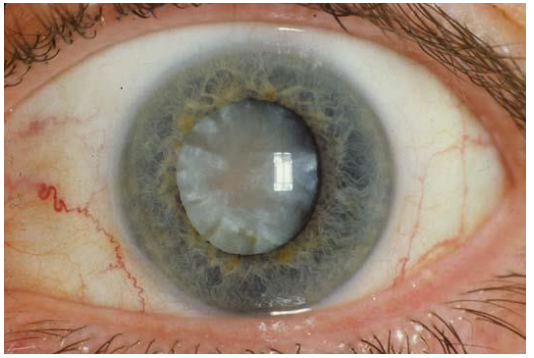
Common consequences of DM

Over time, DM can damage the heart, blood vessels, eyes, kidneys and nerves:

- Adults with DM have a 2-3-fold increased risk of **heart attacks and strokes**.
- Combined with reduced blood flow, **neuropathy** (nerve damage) in the feet increases the chance of **foot ulcers**, infection and eventual need for limb amputation.
- **Diabetic retinopathy** is an important cause of **blindness**, and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. *2.6% of global blindness can be attributed to diabetes.*
- DM is among the leading causes of **kidney failure**.

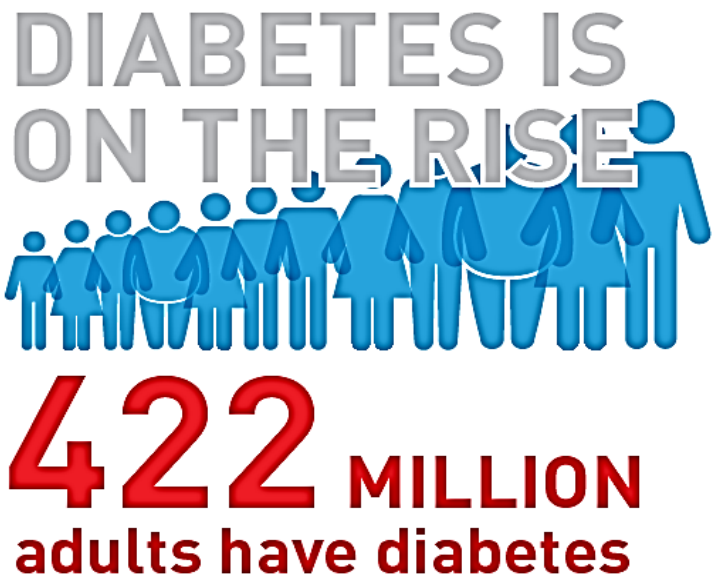






Sarwar, N.; Gao, P.; Seshasai, SR.; Gobin, R.; Kaptoge, S.; Di Angelantonio et al. *Lancet*. **2010**, 26;375, 2215-2222.
Bourne, RR.; Stevens, GA.; White, RA.; Smith, JL.; Flaxman, SR.; Price, H et al. *Lancet Global Health* **2013**, 1, 339-349
United States Renal Data System. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. **2014**, 188–210.

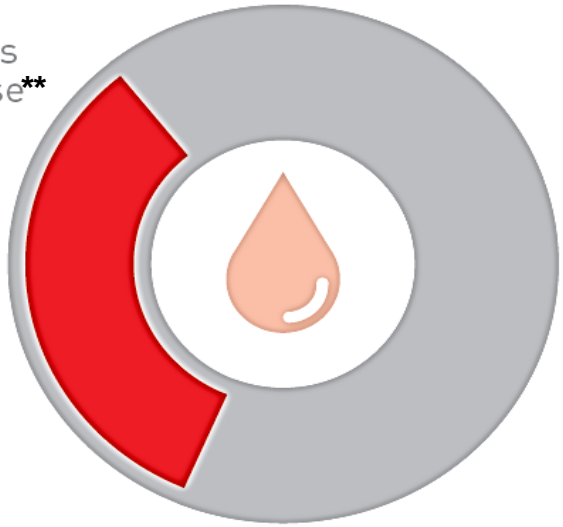
WHO: Global report on diabetes, 2016



3.7 MILLION
deaths due to diabetes
and high blood glucose**

1.5 MILLION
deaths caused
by diabetes

World Health Organization



THAT'S 1 PERSON IN 11

- The number of people with DM has risen from 108 million(1980) to 422 million (2014).
- The global prevalence of DM (population percentage) among adults over 18 years of age has risen from 4.7% (1980) to 8.5% (2014).
- WHO projects that diabetes will be the 7th leading cause of death in 2030.

***High blood glucose**: distribution of fasting plasma glucose in a population that is higher tan 4.9-5.3 mmol/L, SD 0.4-0.6. *Statistical concept, not clinical or diagnostic category*.

World Health Organization. *Global report on diabetes*. Geneva, **2016**.
Mathers, CD.; Loncar, D. *PLoS Med*. **2006**, *3*(11), 442.

WHO: Global report on diabetes, 2016

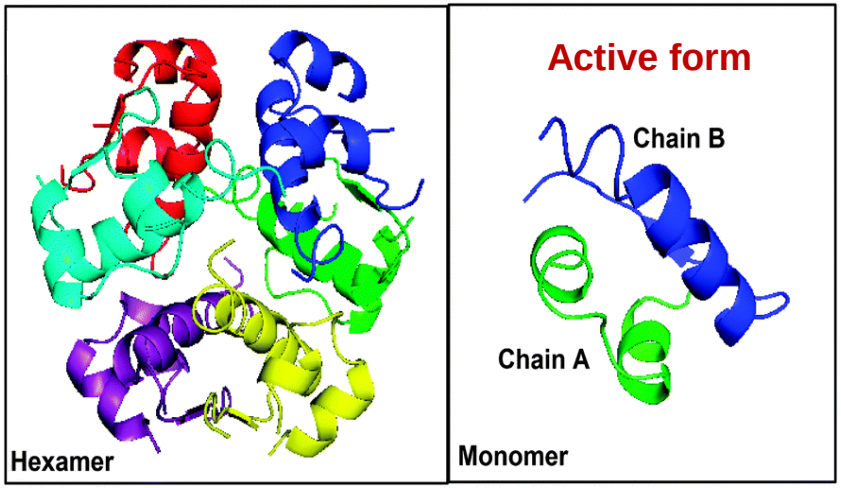
Prevalence of diabetes and related risk factors (%).

Country	Diabetes	Overweight	Obesity	Physical Inactivity
China	**9.4**	35.4	7.3	23.8
Dominic Rep.	**9.3**	54.8	23.0	35.0
USA	**9.1**	**69.6**	**35.0**	35.0
Venezuela	**8.8**	61.0	24.0	---
Costa Rica	**8.5**	59.9	24.0	---
Colombia	**8.0**	55.8	20.7	**63.5**
India	**7.8**	21.4	4.7	12.1
Haiti	**6.9**	35.5	10.7	---

Body mass index (BMI): *person's weight (kg) divided by the square of his/her height (m).*
Overweight: *BMI ≥ 25 kg/m^2*
Obesity: *BMI ≥ 30 kg/m^2*

World Health Organization. *Diabetes country profiles*. Geneva, **2016**.
American Diabetes Association. *Diabetes Care*. **2013**, 36(4), 1033-1046.

Insulin: peptide hormone

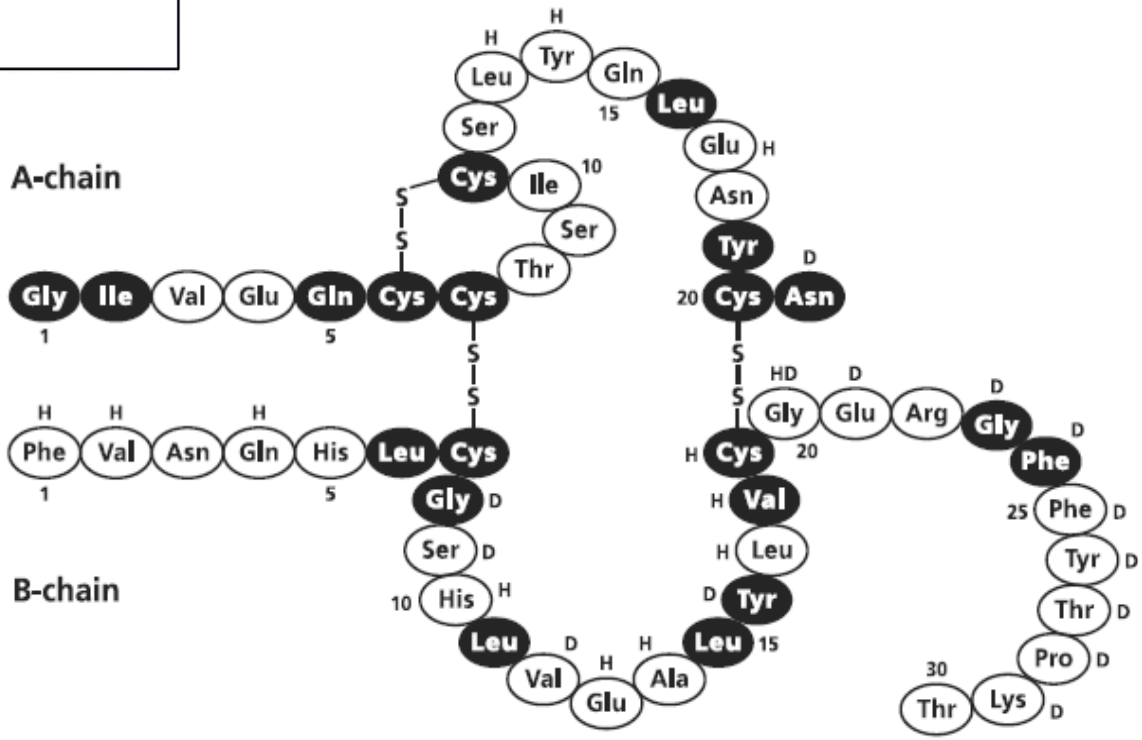


Human insulin.

- 51 amino acids
- 5808 Da.
- Dimer: A-chain and B chain linked by disulfide bonds.
- Produced and stored in the body as a hexamer stabilized by two Zn^{2+}: **high long-term stability**.

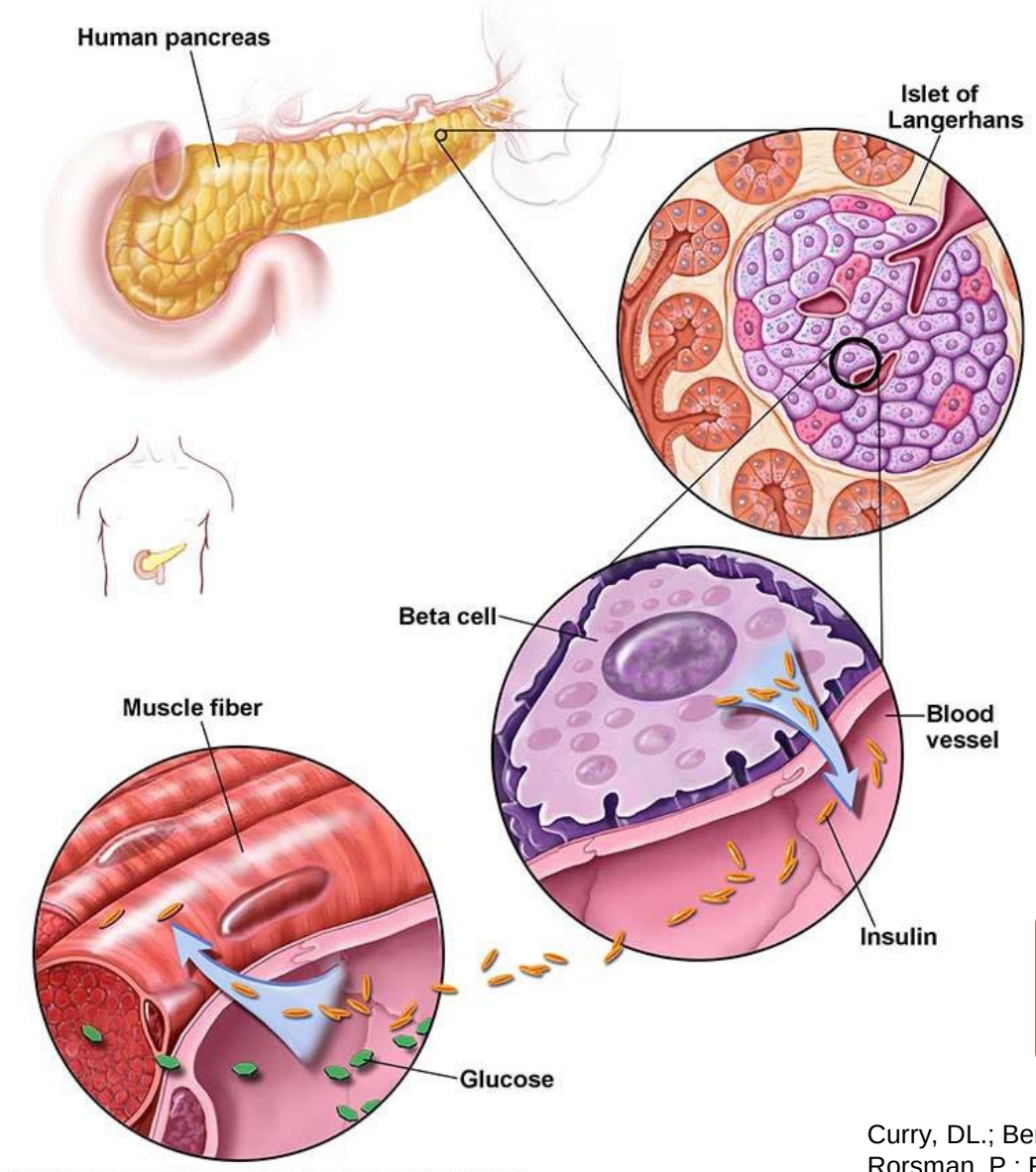
Main functions.

- Control of cellular uptake of glucose in muscle (energy) and adipose tissue (triglyceride synthesis).
- Increase of DNA replication and protein synthesis via control of amino acid uptake.
- Stimulates glycogen formation in liver.



Brange, J.; Langkjœr, L., Insulin Structure and Stability. In *Stability and Characterization of Protein and Peptide Drugs: Case Histories*;Wang, Y. J.; Pearlman, R., Eds. Springer US: Boston, MA, 1993; pp 315.

Insulin secretion in response to glucose uptake



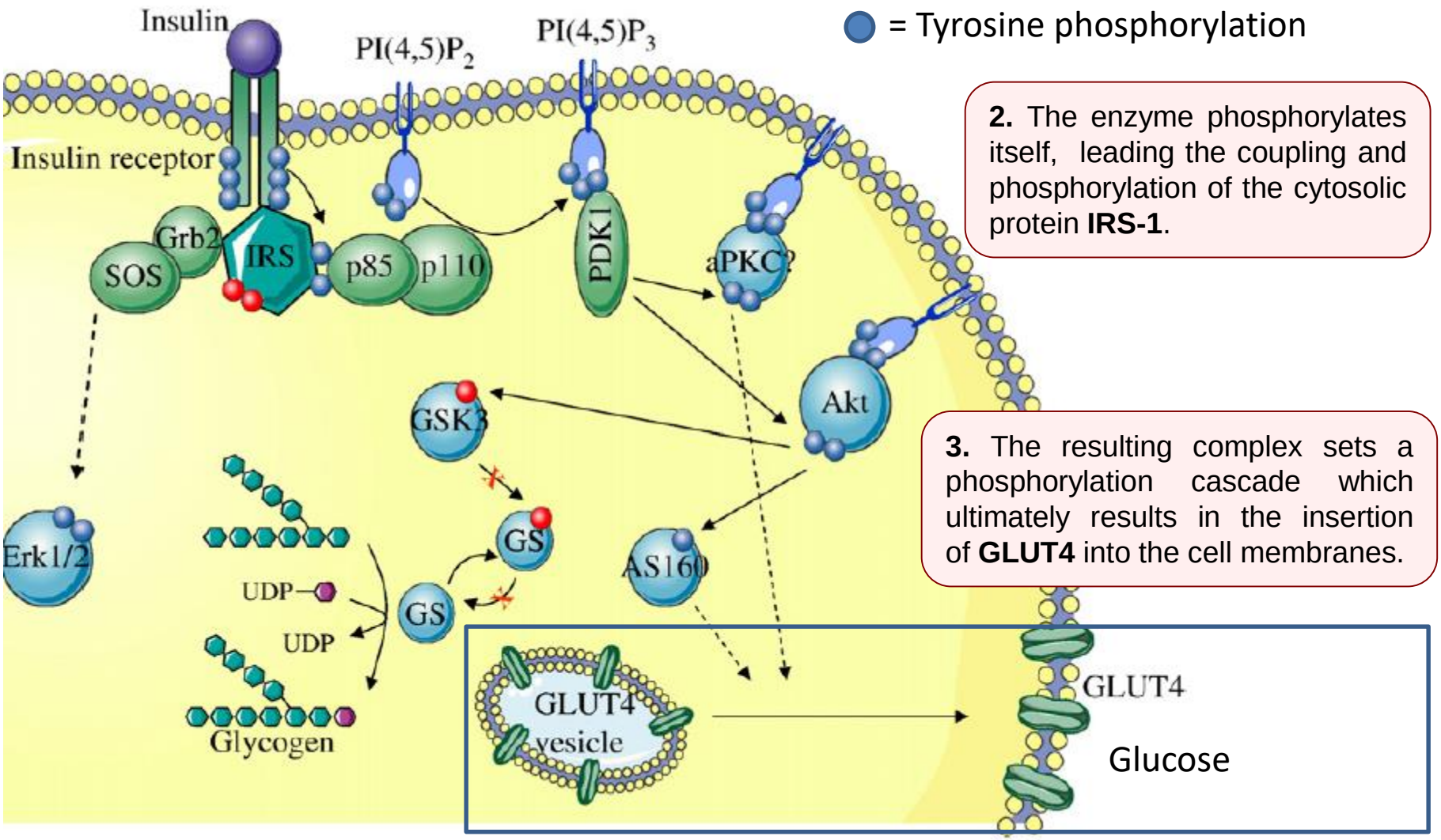
- Pancreas has hundreds of thousands of cell clusters called ***islets of Langerhans*** where ***β-cells*** live.
- β-cells release insulin in response to increased blood glucose levels in aprox. 10 minutes.
- Any alteration in β-cells functioning has a profound impact on glucose homeostasis:
  - Excessive secretion: hypoglycemia
  - Insufficient secretion: Diabetes (*type 1*).

Only treatment for type 1 DM: *insulin injections* ***for life*.**

Curry, DL.; Bennett, LL; Grodsky, GM. *Endocrinology* **1968**, *83(3)*, 572-584.
Rorsman, P.; Renstrom, E. *Diabetologia* **2003**, *46(8)*, 1029-1045.
Yoon, JW.; Jun, HS. *Am J Ther* **2005**, 12(6), 580-591.

Insulin signaling: glucose uptake

1. Insulin binding: **IR** is converted into an active enzyme, *tyrosine kinase*.

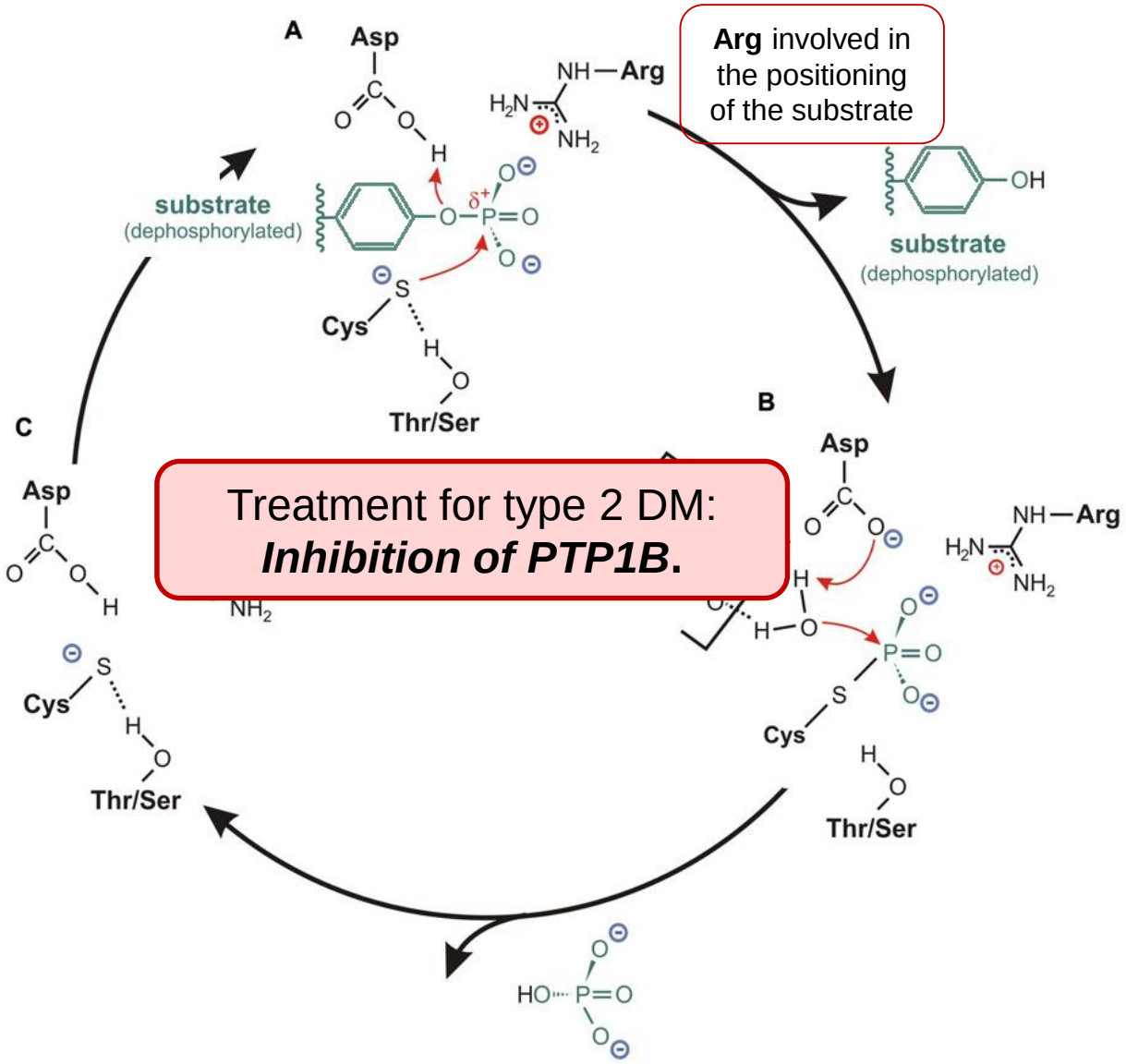


2. The enzyme phosphorylates itself, leading the coupling and phosphorylation of the cytosolic protein **IRS-1**.

3. The resulting complex sets a phosphorylation cascade which ultimately results in the insertion of **GLUT4** into the cell membranes.

Frojdo, S.; Vidal, H.; Pirola, L. *Biochimica et Biophysica Acta* **2009**, *1792*, 83-92.

Insulin Resistance: diabetes type 2



Arg involved in the positioning of the substrate

Treatment for type 2 DM: ***Inhibition of PTP1B*.**

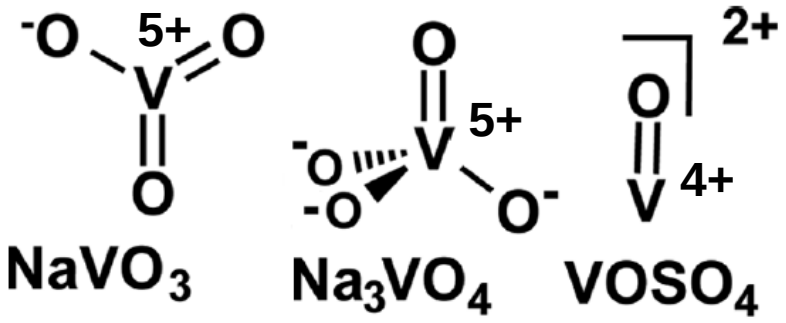
Protein tyrosine phosphatase 1B (PTP1B) shuts down insulin signaling by acting at the insulin receptor and downstream signaling components, such as **IRS1**, leading uncoupling from IR.[15] ***Expression and activity is elevated during type 2 DM***.[16]

- Nucleophilic attack at phosphorous center by a reduced **Cys** residue, followed by subsequent protonation of the tyrosine by **Asp**.
- Regeneration of active enzyme: hydrolysis of the thiophosphate intermediate, is mediated by an activated water molecule through H-bonds with **Glu**.

Goldstein, BJ.; Bittner-Kowalczyk, A.; White, MF.; Harbeck, M. *J. Biol. Chem.* **2000**, *275*, 4283–4289.
Wu, X. et al. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5973–5980.

Antidiabetic property of V(IV): background

Antidiabetic effect of vanadium salts on cells and diabetic animals: **1980s.**



Vanadium salts in drinking water resulted in normoglycemia in diabetic rats.

Mimic most of physiological effects of insulin:

- Stimulation of glucose uptake in fat cells.
- Enhancement of glycogenesis in muscles and liver.
- Stimulation of fatty acids formation in fat cells.

Shechter, Y.; Karlish, S. J. D. *Nature* **1980**, *284*, 556.
Meyerovitch, J.; Farfel, Z.; Sack, J.; Shechter, Y. *J. Biol. Chem.***1987**, *262*, 6658.
Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**,*227*, 1474.

Insulin mimetic or Insulin-enhancing?

Insulin mimetic: vanadium was believed to replace absent insulin in diabetic rats.

Biobreeding Diabetes Prone rats (BBDP rat). Vanadium was not enough to keep rats alive in total absence of insulin.

Biobreeding Diabetes Prone rats (BBDP rat).

(X)(Insulin) = Normoglycemia

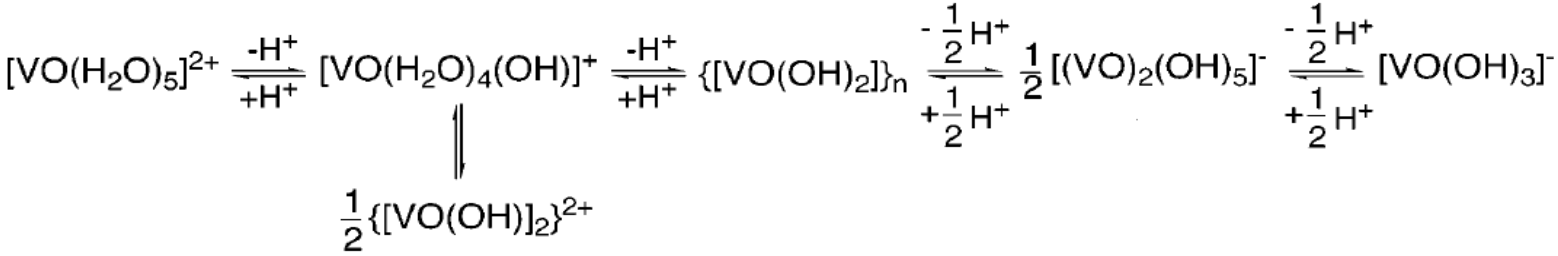
Biobreeding Diabetes Prone rats (BBDP rat).

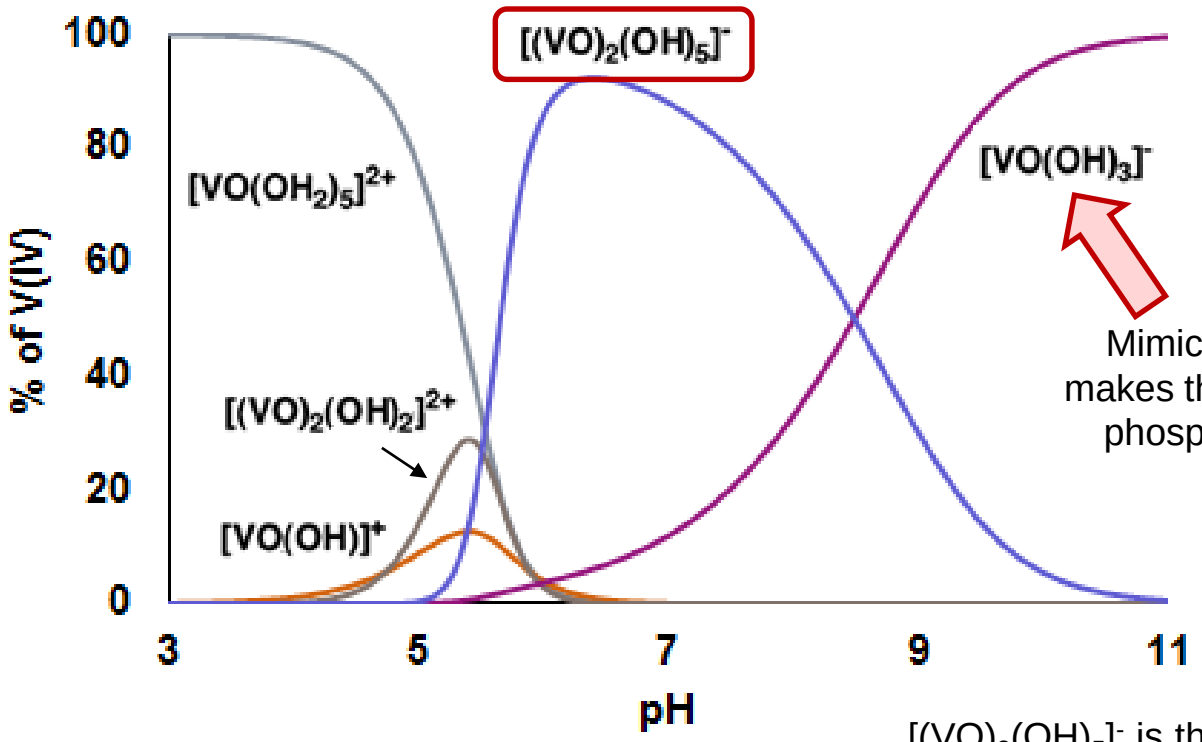
Vanadium + (X-1)(Insulin) = Normoglycemia

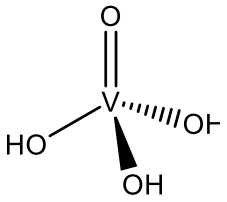
Vanadium defined as **Insulin-enhancer**

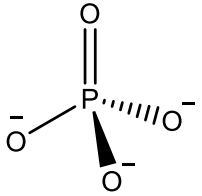
Halberstam, M.; Cohen, N.; Shlimovich, P.; Rossetti, L.; Shamoon, H. *Diabetes* **1996,** *45*, 659.

V(IV) also inhibits phosphatases







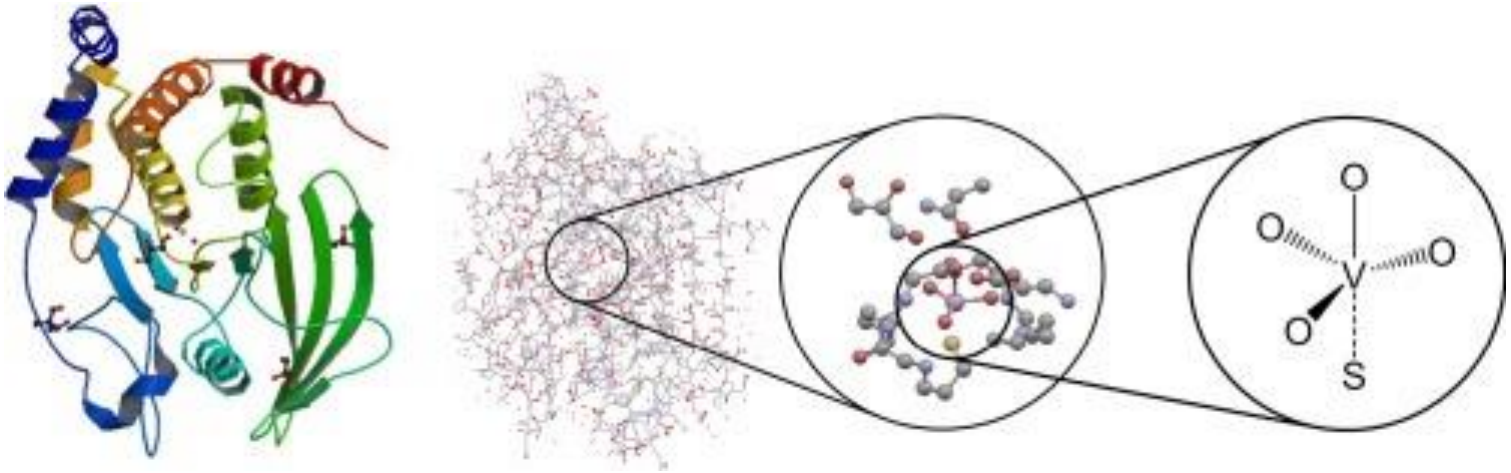


Mimicking phosphate makes the species a good phosphatase inhibitor

$[(VO)_2(OH)_5]^-$ is the predominant species at pH 7-7.5 when total V(IV) concentration of all species is in the range 1-10 µM.

Chasteen, N. D. *Struct. Bond* **1983**, *53*, 105.
Francavilla, J.; Chasteen, N. D. *Inorg. Chem.* **1975**, *14*, 2860.
Komura, A.; Hayashi, M.; Imanaga, H. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2927.

V(IV) also inhibits phosphatases



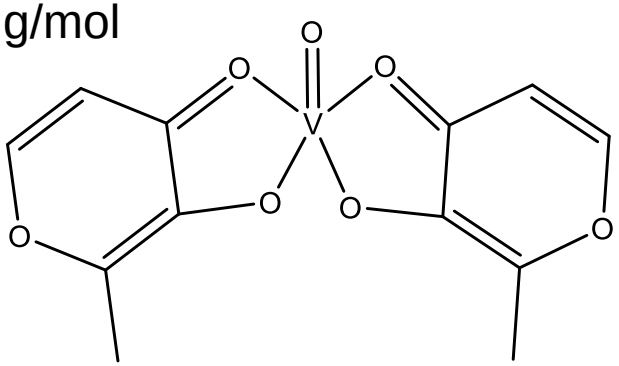
Inhibition constant. (inverse relation to *binding affinity*)
V(IV)→ K_i = *0.4 - 5 μM*
Phosphate → K_i = *10 - 30 mM*

Vanadium can form 5- to 6-coordinated highly stable complex with the phosphatase active site at the cysteine residue, resulting in the inhibition of the enzyme.

Brandao, T.: Hengge, A.: Johnson, S. *J. Biol. Chem* **2010**, *285(21)*, 15874-1583

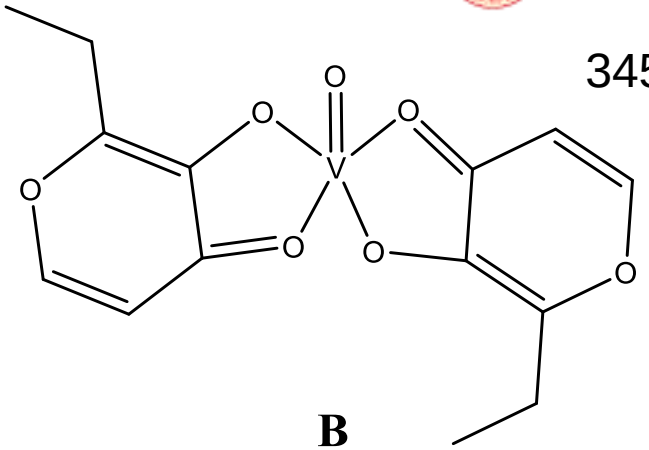
Referring V(IV) compounds : BMOV and BEOV

317.15 g/mol



A

345.2 g/mol



B

(A) bis(maltolato)oxovanadium(IV) (BMOV)

(B) bis(ethylmaltolato)oxovanadium(IV) (BEOV)

- **CHO cells:** Inhibition of overexpressed PTP1B.

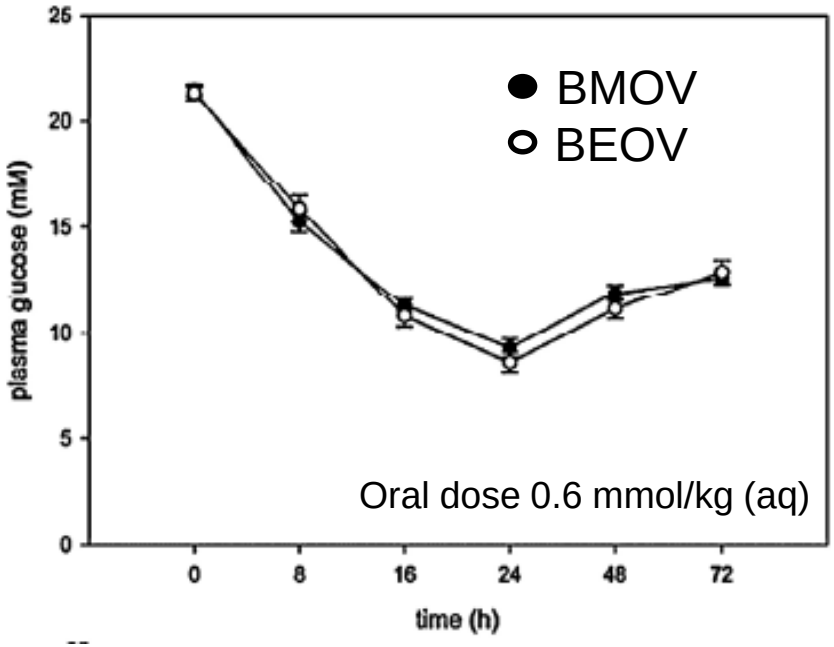
.

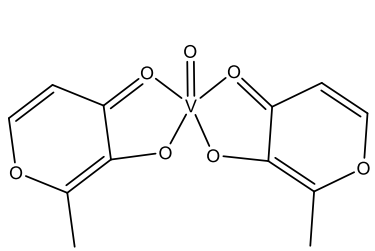
- **Male STZ Wistar rats:** oral 0.6 mmol/kg or injected 0.1 mmol/kg doses result in normoglycemia for 2 days.

- **Male STZ ddY mice**: injected 0.1 mmol/kg dose resulted in normoglycemia within 18h.

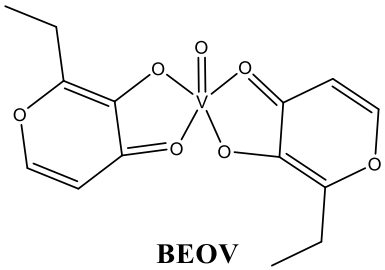
K.H. Thompson, B.D. Liboiron, Y.S.K.D.D. Bellman, I.A. Setyawati, B.O. Patrick, V. Karunaratne, G. Rawji, J. Wheeler, K. Sutton, S. Bhanot, C. Cassidy, J.H. McNeill, V.G. Yuen, C. Orvig, *J. Biol. Inorg. Chem*. **2003**, *8*, 66–74.
M.Z. Mehdi, A.K. Srivastava, *Arch. Biochem. Biophys*. **2005**, *440*, 158–164.

Referring V(IV) compounds : BMOV and BEOV

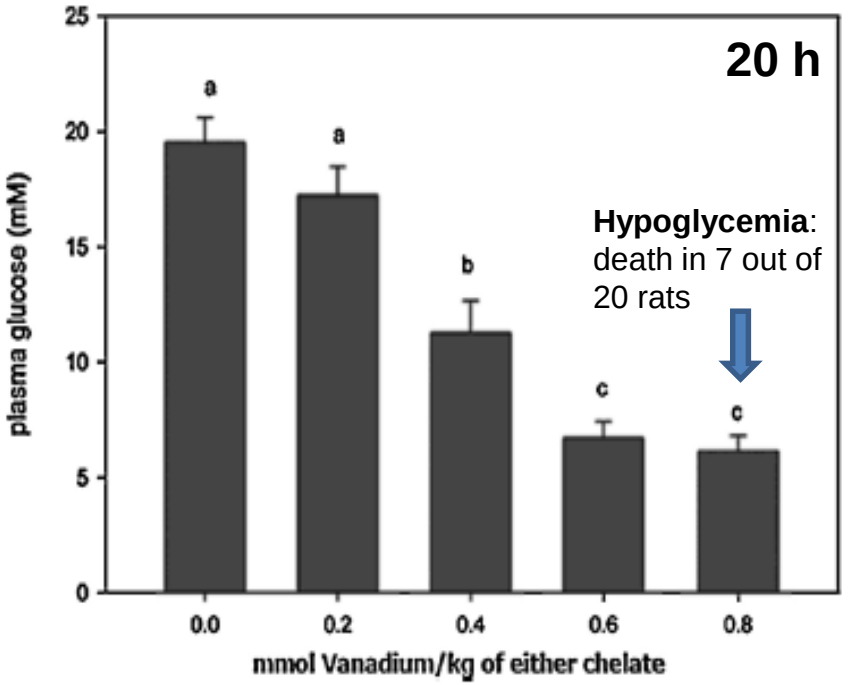




BMOV



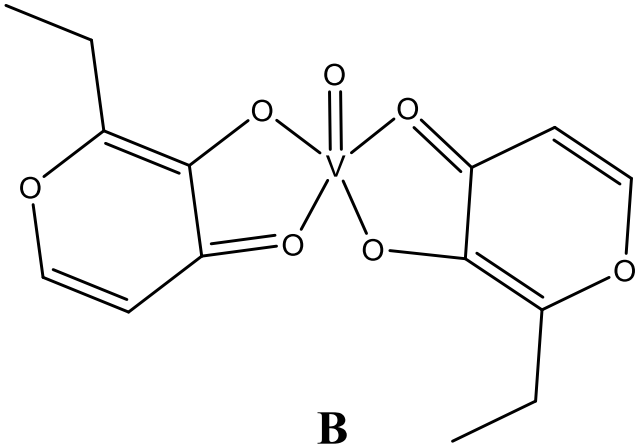
BEOV



K.H. Thompson, B.D. Liboiron, Y.S.K.D.D. Bellman, I.A. Setyawati, B.O. Patrick, V. Karunaratne, G. Rawji, J. Wheeler, K. Sutton, S. Bhanot, C. Cassidy, J.H. McNeill, V.G. Yuen, C. Orvig, *J. Biol. Inorg. Chem*. **2003**, *8*, 66–74.

M.Z. Mehdi, A.K. Srivastava, *Arch. Biochem. Biophys*. **2005**, *440*, 158–164.

BEOV in clinical trials



B

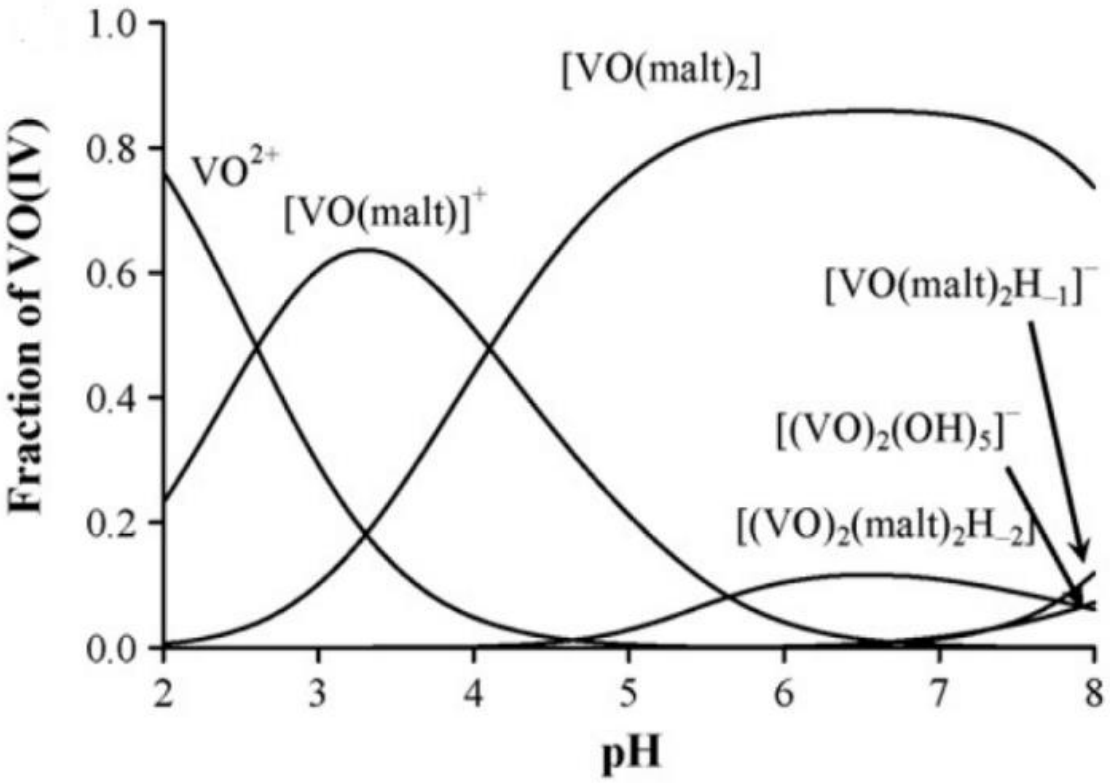
BEOV → **Phase II clinical trials**

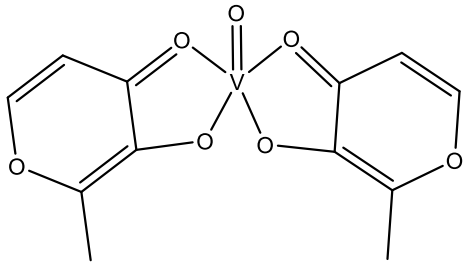
0.03 mmol/kg – 0.26 mmol/kg → No adverse effects in humans.

- **The aqueous speciation of this compound may help to explain why its metabolism in humans result in low efficacy.**

K.H. Thompson, B.D. Liboiron, Y.S.K.D.D. Bellman, I.A. Setyawati, B.O. Patrick, V. Karunaratne, G. Rawji, J. Wheeler, K. Sutton, S. Bhanot, C. Cassidy, J.H. McNeill, V.G. Yuen, C. Orvig, *J. Biol. Inorg. Chem*. **2003**, *8*, 66–74.
M.Z. Mehdi, A.K. Srivastava, *Arch. Biochem. Biophys*. **2005**, *440*, 158–164.

Biotransformation. Step 1 – gastric tract





[VO(malt)$_2$]

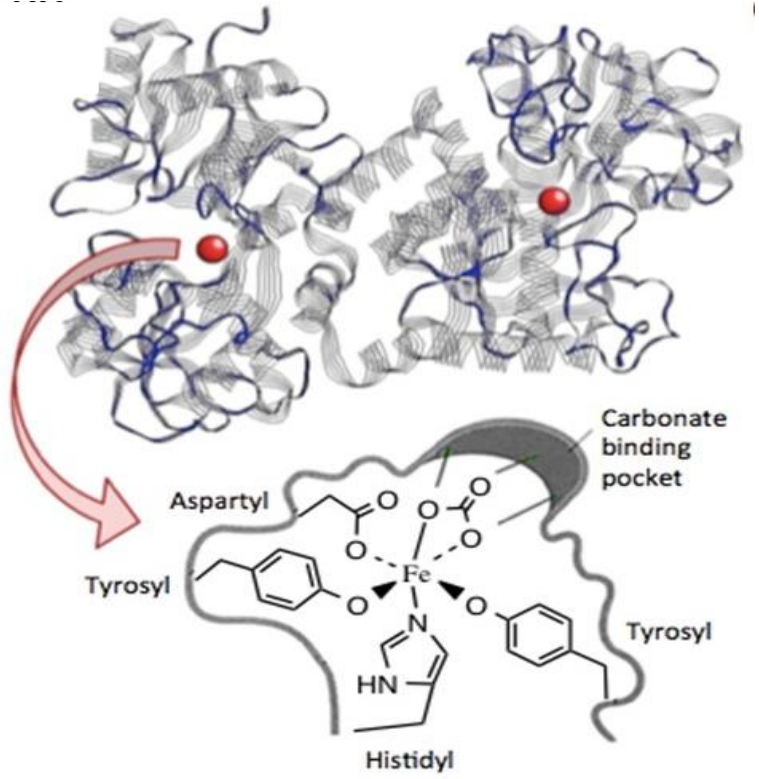
- **pH 2**: BMOV will dissociate into free VO^{2+} ions, affecting the absorption of the compound.

Encapsulation effectively increase their absorption after oral administration.

- 0.6 mmol/kg oral dose of BMOV: two to three times greater absorbed amount than the same dose of $VOSO_4$ (lower than 10%).
- BMOV and BEOV achieve the same therapeutic insulin-enhancing effect at significantly lower doses that the simple salts do

Thompson, K. H.; Lichter, J.; LeBel, C.; Scaife, M. C.; McNeill, J. H.; Orvig, C. *J. Inorg. Biochem.* **2009,** *103*, 554-558.

Biotransformation. Step 2 – Serum

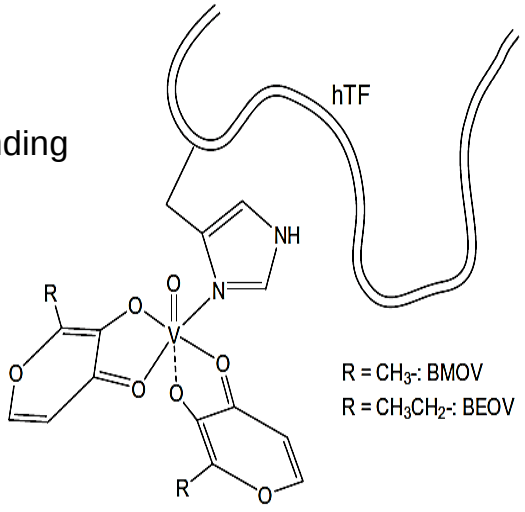


The human serum transferrin (hTf) metal binding site.

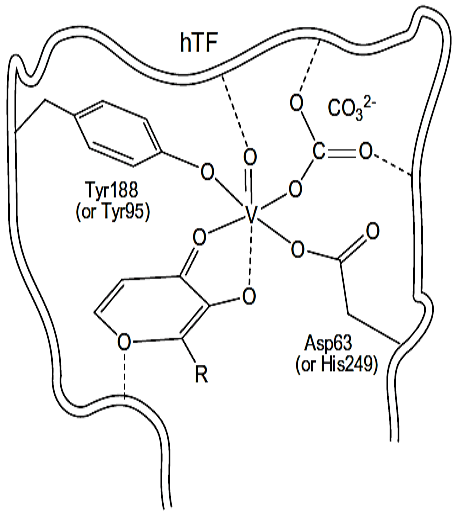
- In serum, vanadium is almost entirely bound to hTf.
- apo-hTf binds two equivalents of VO^{2+}. The presence of carbonate synergistic anion increases binding affinity.
- holo-Tf strong interactions with BMOV and BEOV at surface of the protein, via coordination of His-N, Asp-COO^- and Glu-COO^- donors.

***Type 1* formulation** (VO)(hTf)(L) when the carrier is a synergistic ligand and *cis*-VOL_2(hTf).

***Type 2* formulation** of ternary complexes (VO)(hTf)(L) and $(VO)_2$(hTf)$(L)_2$ independently on the features of the carrier and geometry assumed by the VO_2 complex in aqueous solution



Type 1



Type 2

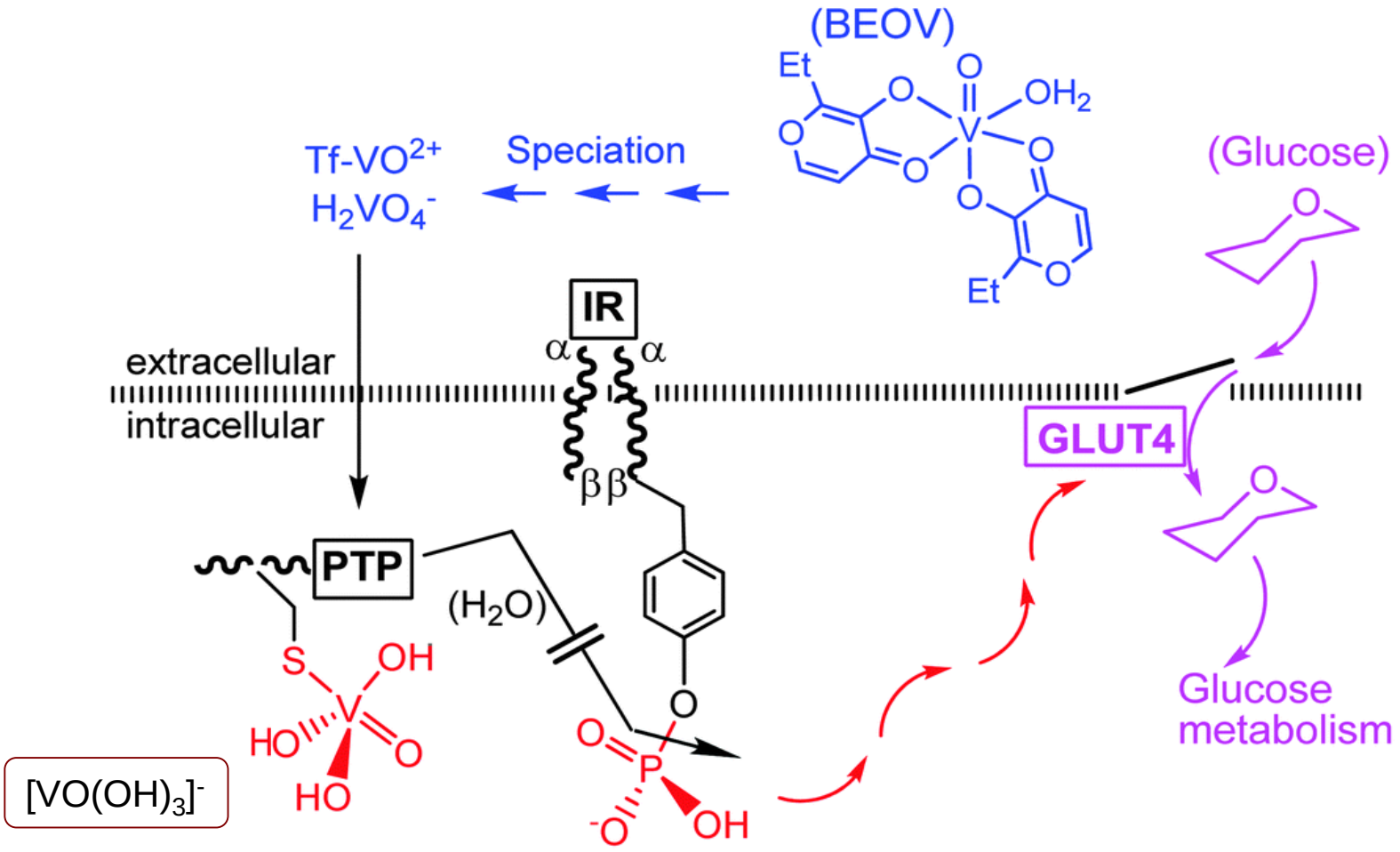
Justino, G. C.; Garribba, E.; Pessoa, J. C. *J Biol Inorg Chem* **2013,** *18*, 803-813.

Sanna, D.; Micera, G.; Garribba, E. *Inorg. Chem.* **2010,** *49*, 174-187.

Sanna, D.; Micera, G.; Garribba, E. *Inorg. Chem.* **2013,** *52*, 11975-11985.

Goncalves, G.; Tomaz, I.; Correia, I.; Veiros, L. F.; Castro, M. M. C. A.; Avecilla, F.; Palacio, L.; Maestro, M.; Kiss, T.; Jakusch, T.; Garcia, M. H. V.; Pessoa, J. C. *Dalton Trans.* **2013,** *42*, 11841-11861.

Inhibition of PTPases



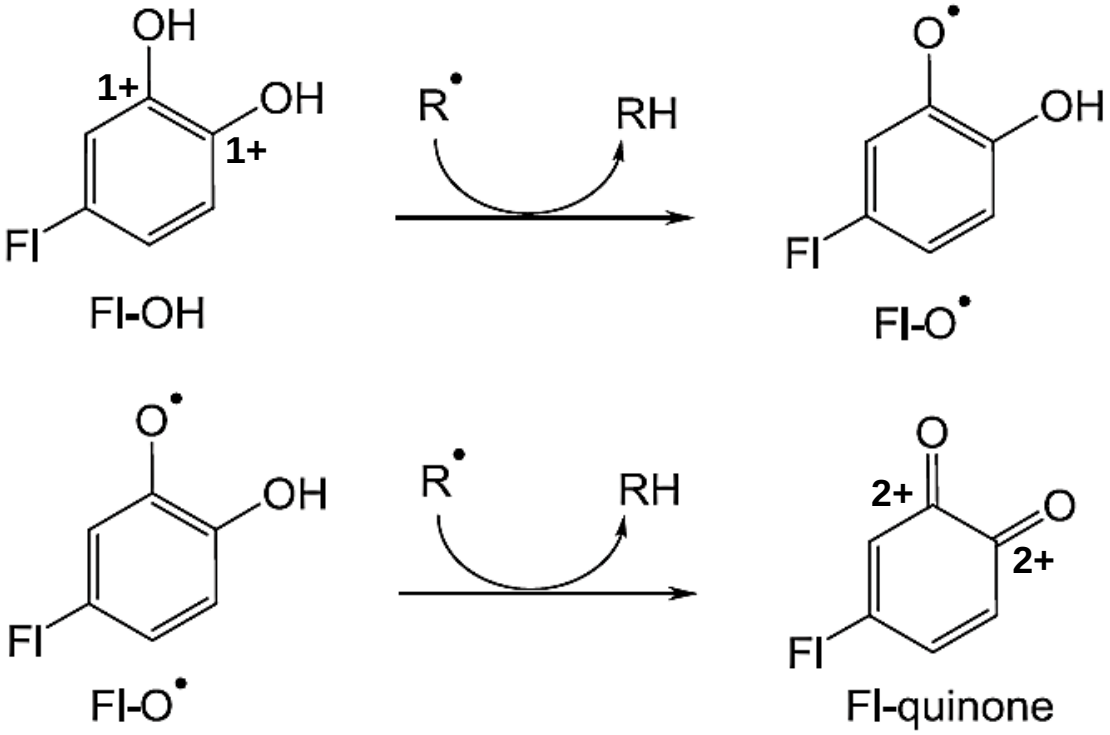
Vanadium can form 5- to 6-coordinated highly stable complex with the phosphatase active site at the cysteine residue, resulting in the inhibition of the enzyme.

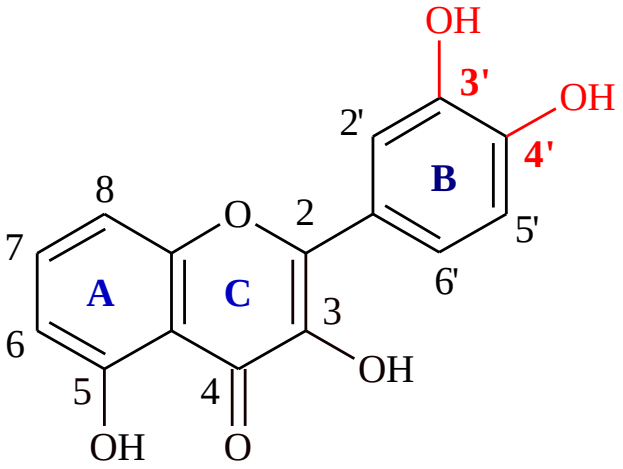
Rehder, D. *Future Med. Chem.* **2012,** *4*, 1823-1837.

Oxovanadium(IV)-flavonoid complexes: new contenders in the insulin-enhancing drug design

Flavonoids: antioxidant activity

Flavonoids can prevent injury caused by free radicals mainly by: **direct scavenging of reactive oxygen species (ROS)**





Flavonoids scavenges free radicals directly by hydrogen donation.

Low redox potentials (0.23 < E° < 0.75**):**
Flavonoids thermodynamically able to reduce free radicals with redox potentials in the range 2.13-1.0 V.

$HO^{\bullet}, H^{+}/HO$	2.310
$RO^{\bullet}, H^{+}/ROH$ (alkoxyl)	1.600
$ROO^{\bullet}, H^{+}/ROOH$ (peroxyl)	1.000

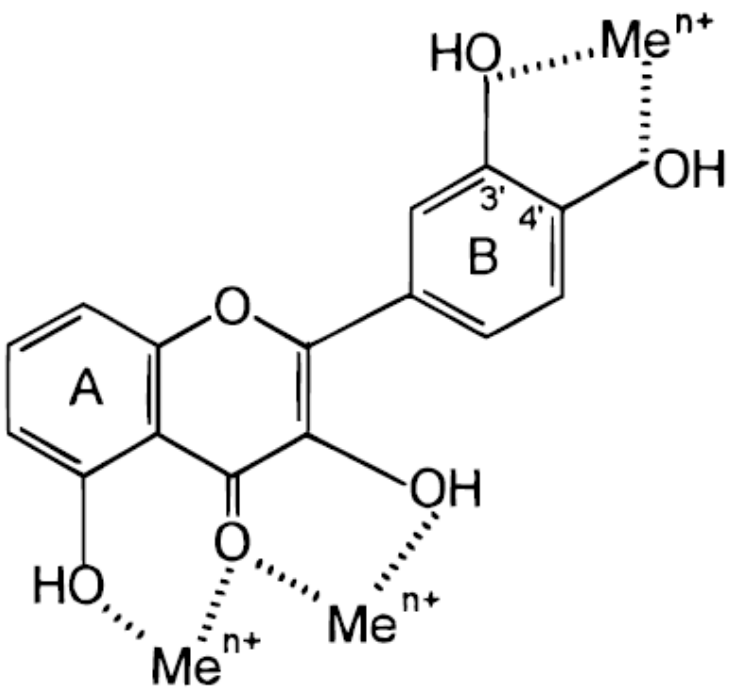
Pietta, PG. *J, Nat. Prod* **2000**, *63*, 1035-1042..

Flavonoids: antioxidant activity

Flavonoids effeciently **chelate trace metals**, which play important roles in oxygen metabolism: Free iron and copper.

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}$$

$$H_2O_2 + Cu^{+} \rightarrow {}^{\bullet}OH + OH^- + Cu^{2+}$$



Binding sites for trace metals to flavonoids:

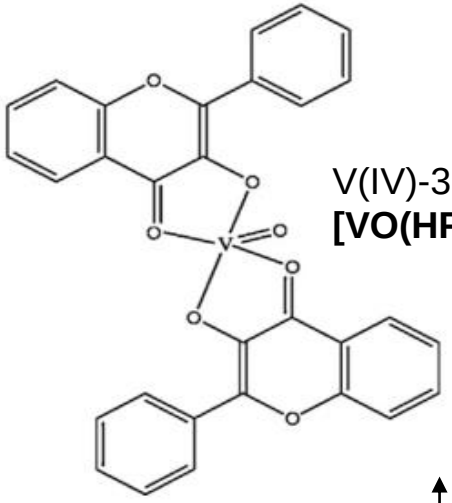
- The catechol moiety in B ring*: **the major contribution.***
- 3-hydroxyl and 4-oxo in C ring.
- 4-oxo and 5-hydroxyl in A ring.

Mitochondria can generate ROS induced by VO^{2+}.

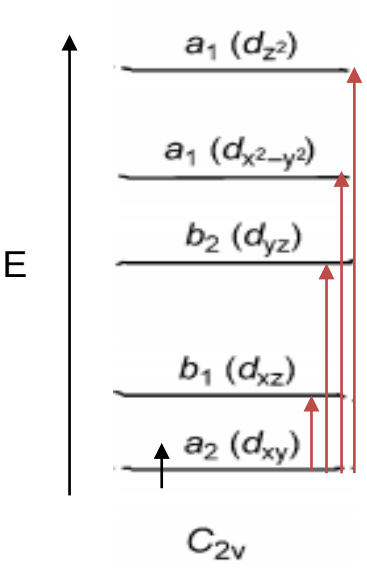
Pietta, PG. *J, Nat. Prod* **2000**, *63*, 1035-1042.
Congcong H,Gang W,Xiaogai Y. *J Chinese Phar Sc*. **2013**, *22(1)*, 77-80.

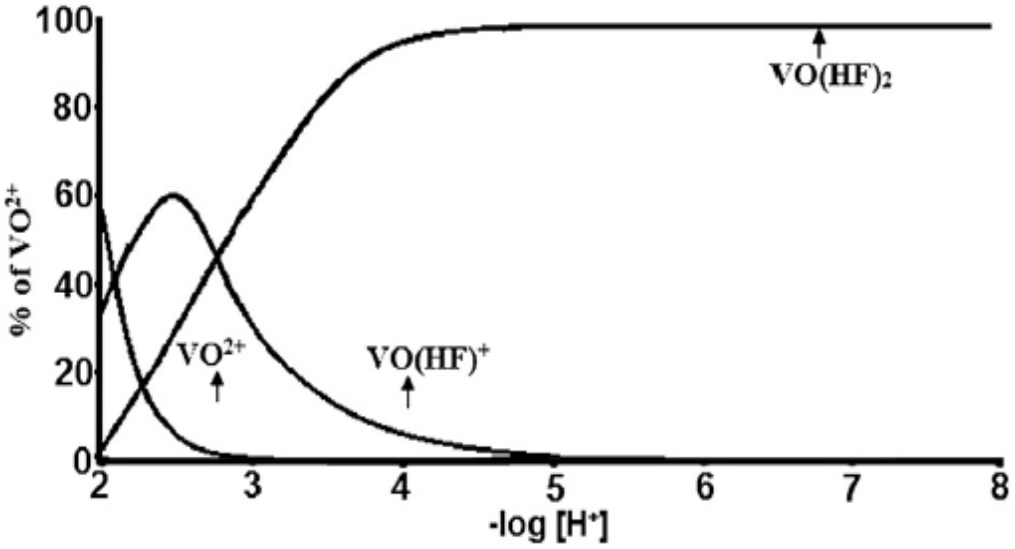
V(IV)-flavonoid complexes

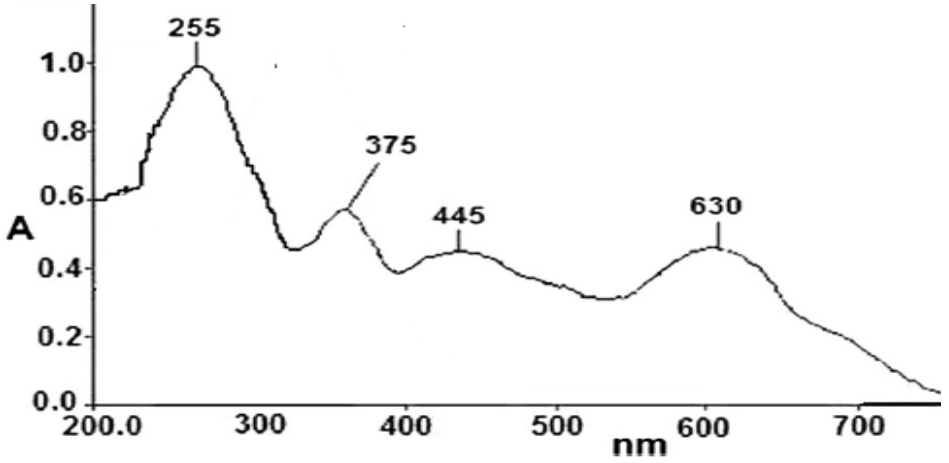
These properties are increased upon complexation with oxovanadium(IV) ion and as a result oxovanadium(IV) complexes of **rutin**, **morin**, **quercetin** and **kaempferol glycosides** have been investigated for their anti-diabetic property.



V(IV)-3-hydroxyflavone
[VO(HF)$_2$]





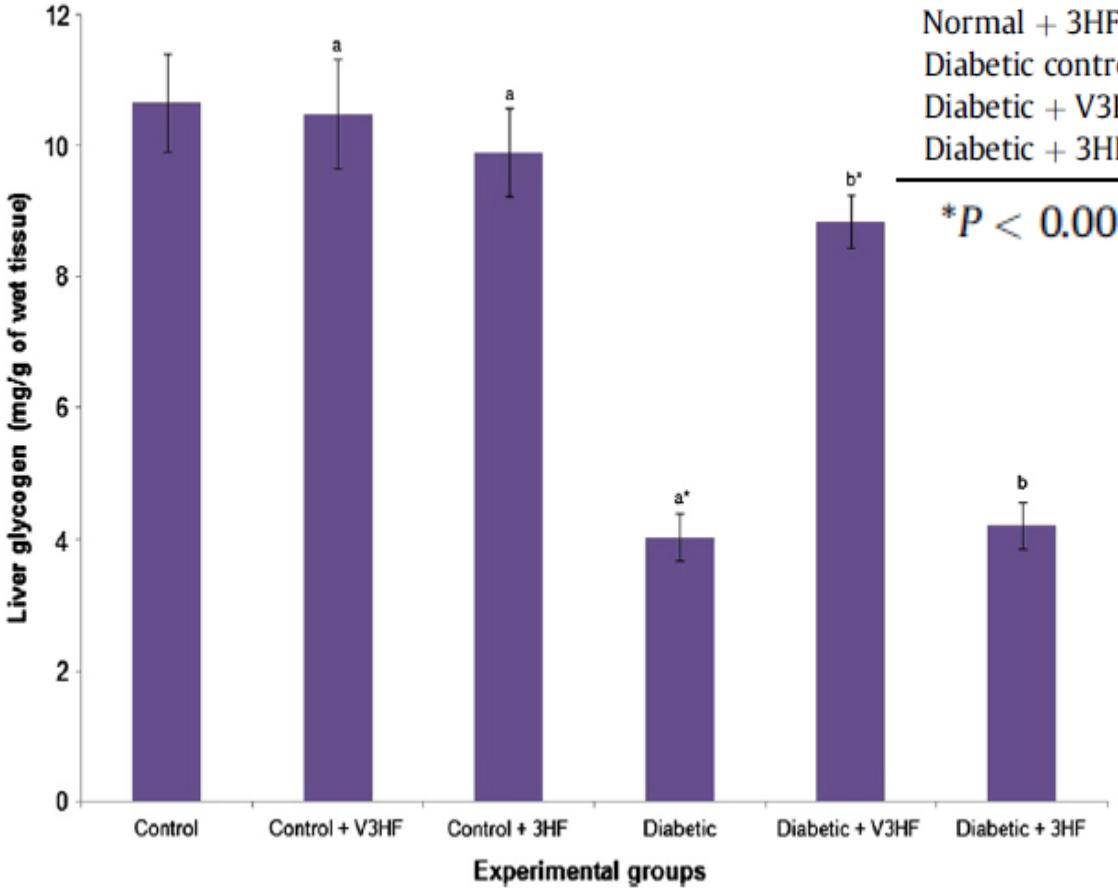


Pillai, S. I.; Subramanian, S. P.; Kandaswamy, M. *Eur. J. Med. Chem.* **2013,** *63*, 109-117.

V(IV)-flavonoid complexes

Oral dose 0.01 mmol/kg

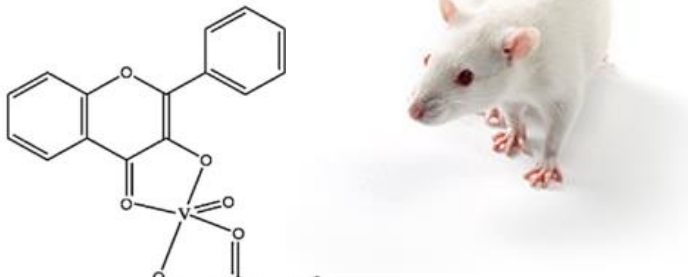
V3HF→ ***57.7%*** Blood glucose reduction
3HF → ***10.84%*** Blood glucose reduction



Levels of fating blood glucose (FBG) in control and experimental groups of rats. 30 days experiment period.

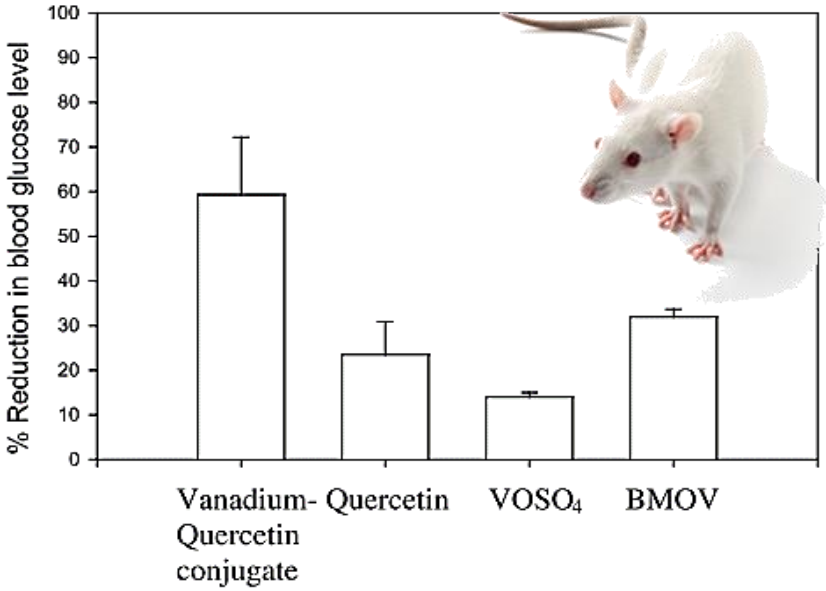
Groups	FBG (mg/dl)	Urine sugar
Normal control	83.42 ± 5.47	Nil
Normal + V3HF (5 mg/kg)	79.92 ± 6.21 a	Nil
Normal + 3HF (50 mg/kg)	88.87 ± 3.86 a	Nil
Diabetic control	322.78 ± 14.95 a*	+++
Diabetic + V3HF (5 mg/kg)	136.45 ± 5.14 b*	Nil
Diabetic + 3HF (50 mg/kg)	287.78 ± 12.78 b@	++

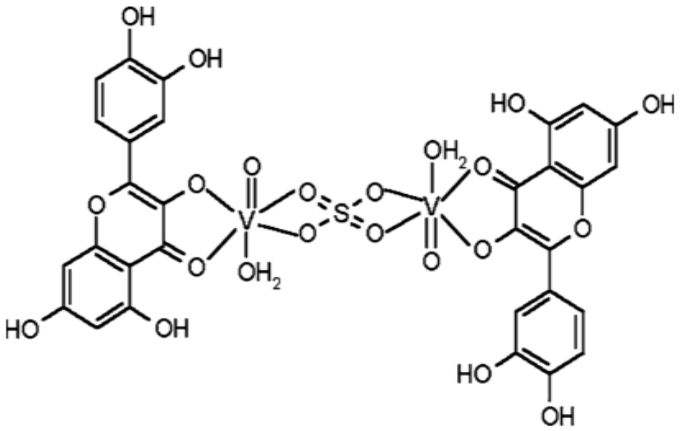
$^{*}P < 0.001$; $^{@}P < 0.05$.



Pillai, S. I.; Subramanian, S. P.; Kandaswamy, M. *Eur. J. Med. Chem.* **2013,** *63*, 109-117.

V(IV)-flavonoid complexes: relevant results



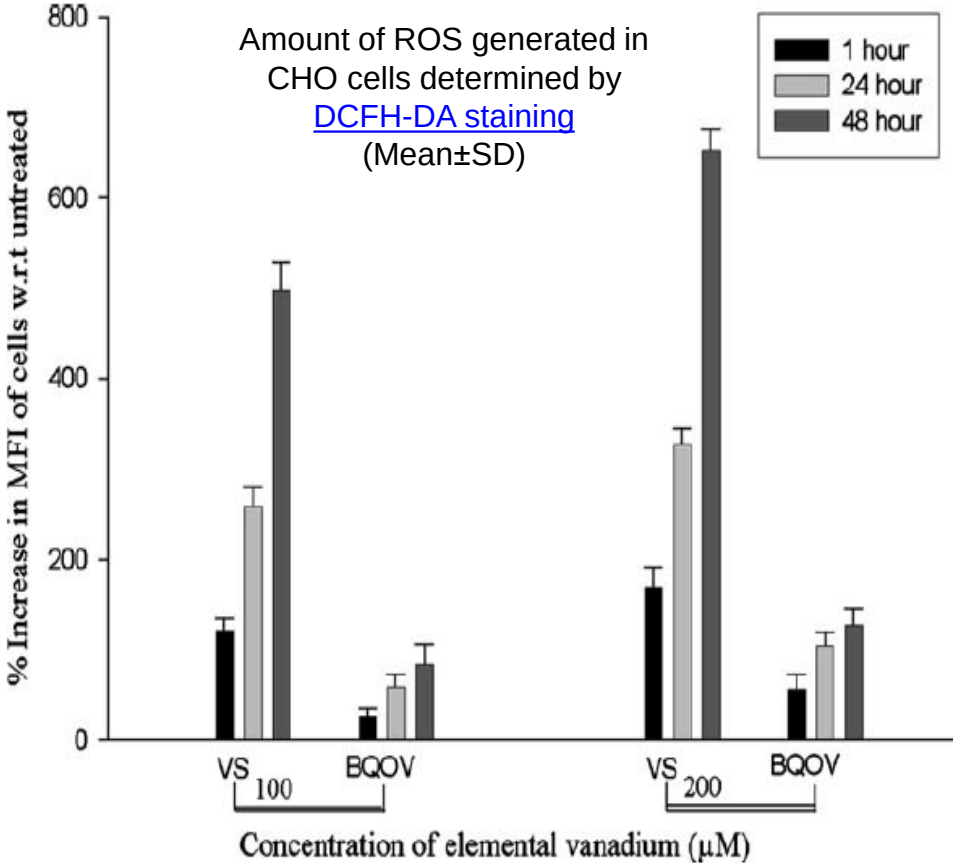


bis(quercetinato)oxovanadium(IV) **(BQOV)**

Compounds were **orally** administrated at one-time dose of **0.4 mmol/kg** to diabetic mice. Blood glucose was measured at **21 h** after dosing.

%Reduction of blood glucose = [(initial - final)/initial]*100.

Amount of ROS generated in CHO cells determined by DCFH-DA staining (Mean±SD)



Shukla, R.; Barve, V.; Padhye, S.; Bhonde, R. *Biometals* **2006,** *19*, 685-693.

Requirements for V(IV) compounds

1. Compound must enhance bioavailability of VO(IV).

2. Ligand must impart sufficient stability to prevent hydrolytic degradation prior to absorption (oral administration).

3. Since the ligand and VO(IV) must dissociate for the compound to be pharmaceutically active, the use of NON-TOXIC ligands becomes a priority.

SUMMARY

1. When compared with BMOV or $VOSO_4$, VO(IV)-flavonoids can achieve comparable antidiabetic activity at similar doses.

2. Antidiabetic activity of free flavonoids are exceeded by its VO(IV) complexes.

3. Superior insulin-enhancing effects upon intraperitoneal injection administration, suggests higher bioavailability compared with oral administration: VO(IV)-flavonoid complexes may have also high affinity to hTf.

4. Further research regarding the transport, delivery and intracellular target of VO(IV)-flavonoid complexes will render insight to the active species and developing of even effective compounds.