July 14, 2021 IRIS Public Science Meeting

Vanadium and Compounds (Inhalation Exposure) IRIS Assessment Plan (IAP)

Comments on Issue #4 Cancer mode of action (MOA) for alveolar/bronchiolar neoplasms.

By Debbie C. Crans; Colorado State University

Problem to be Assessed

The following key science issues were identified on the basis of the preliminary literature survey results (see Section 2.3.1) and review of past assessments on inhalation exposure to vanadium and compounds (see Section 2.1).

Issue #1 relates to issues surrounding chemical speciation of vanadium,

Issues #2 and #3 pertain to consideration in interpreting nonneoplastic lesions in the upper and lower respiratory tract and alveolar/bronchiolar neoplasms in rodents,

Issue #4 pertains to evaluating the MOA information relevant to potential carcinogenicity.

Issues identified in U.S. EPA. ORD Staff Handbook for Developing IRIS Assessments (Public Comment Draft, Nov 2020). U.S. EPA Office of Research and Development, Washington, DC, EPA/600/R-20/137, 2020

Issue #4 under investigation

Science Issue #4. Cancer mode of action (MOA) for alveolar/bronchiolar neoplasms.

As summarized in Section 2.1, there is some support for both a mutagenic MOA and an MOA dependent on **cellular cytotoxicity and reparative regeneration** (and potentially other undetermined mechanisms) as suggested in the EPA PPRTV assessment (U.S. EPA, 2008).

A similar lack of a clearly delineated **MOA for alveolar/bronchiolar lung tumors** with vanadium pentoxide exposure was proposed in the unfinalized draft IRIS Assessment of Vanadium Pentoxide (U.S. EPA, 2011).

As reported in these reviews, **mutagenicity data for vanadium pentoxide appears generally negative**, and some data support **a mechanism involving DNA damage and cell proliferation**.

Given the potential uncertainties in the available MOA information and the potential impact of this information on assessment conclusions, a focused evaluation of the available evidence regarding cancer MOA(s) for alveolar/bronchiolar neoplasms, including judgments regarding human relevance expected to be a key component of the vanadium (inhalation) IRIS assessment.

Key points from Issue #1, 2 and 3 relevant for #4

Science Issue from #1, 2 and 3 and literature precedence:

Specific to V_2O_5

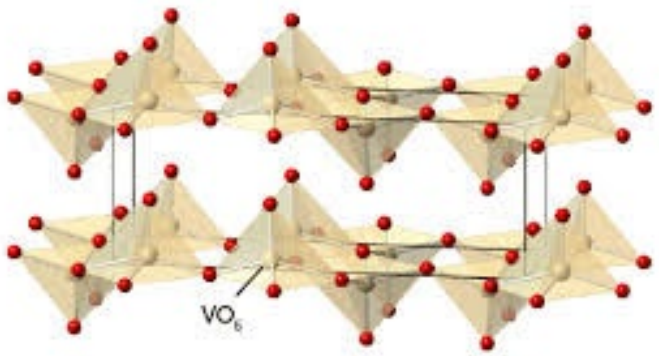
- **What form** of V_2O_5 (or V_4O_{10}) is used for treatment – **from solid or solution**?
- Include **mode of action of $V_{10}O_{28}^{6-}$ and simple salts** (vanadate and vanadyl cation)
- Methods used for **inhalation studies, aerosolizing vanadium pentoxide** (or other vanadium compound) from solution, **rather than exposure to vanadium as a dust.**

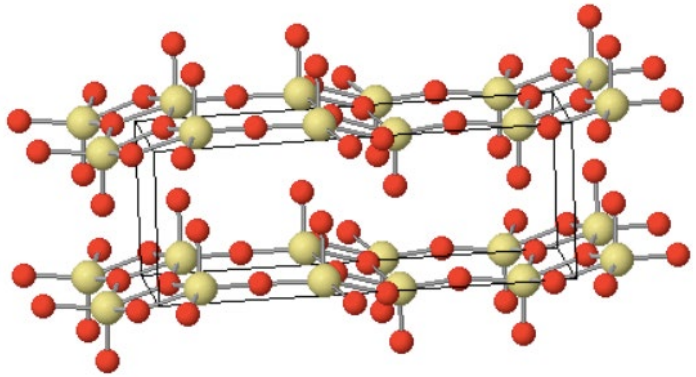
General mode of action of vanadium salts and V-compounds

- **Vanadium speciation under physiological conditions, pH, concentration, redox potential**
- **Model study of $V_{10}O_{28}^{6-}$ as V_2O_5 analog**
- **Different biological activities of vanadium compounds and general modes of action (MOA)**

Structure of V_2O_5 and $V_{10}O_{28}^{6-}$ (1c)

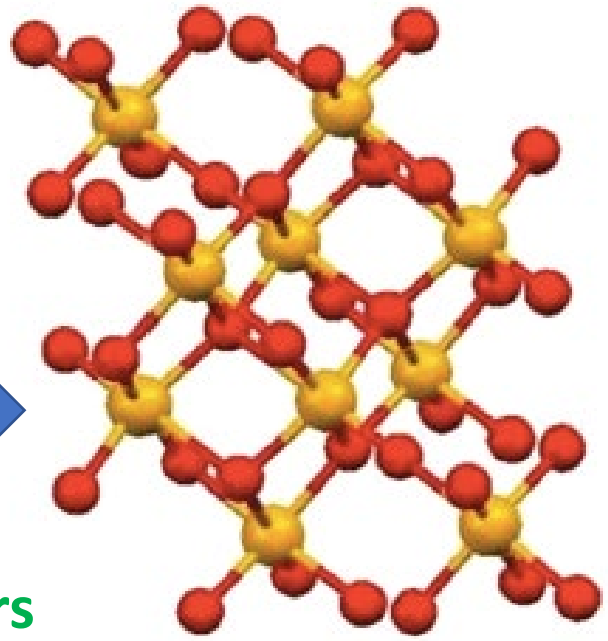
V_2O_5 have been modeled by $V_{10}O_{28}^{6-}$

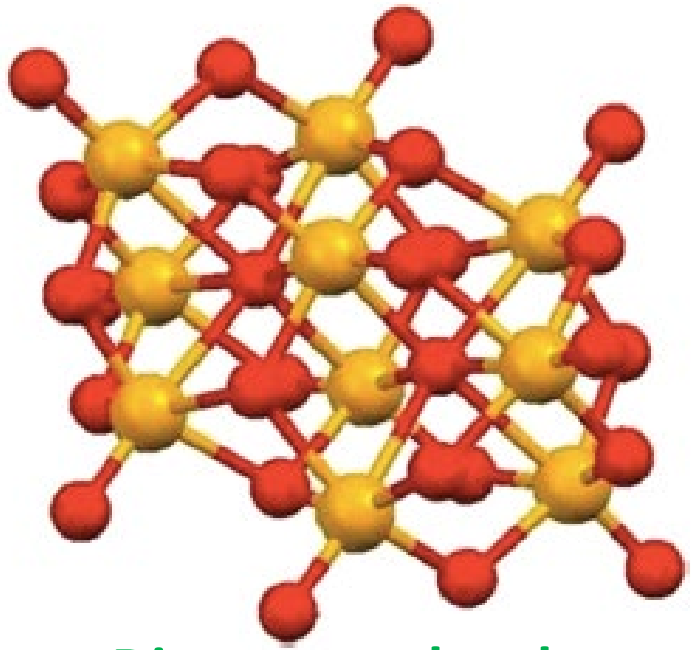




V_2O_5

Sheets - polymers





Discrete molecule

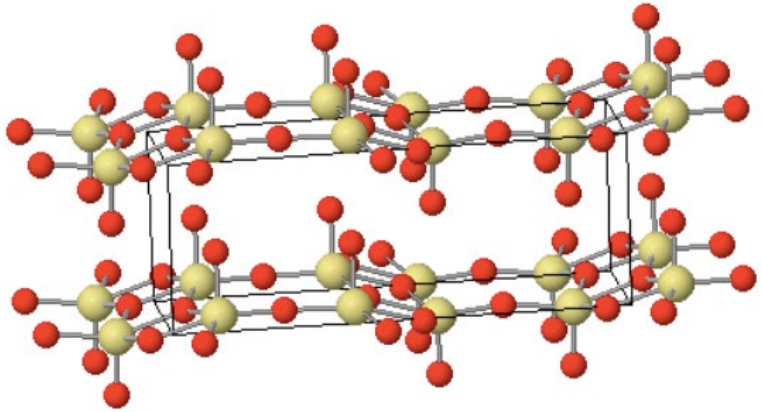
V_2O_5 - Solid state
Nick Greeves, Creative Comments Lincence

The partial structure for **V_2O_5 sheet** is shown (left). The structure for the **discrete anion (V_{10})** is shown (right). While V_2O_5 falls apart in solution, the discrete V_{10} anion retains its structure upon dissolution.

V_{10} used a s model for V_2O_5 Al-Qatati et al. *Dalton Trans.*, 2013, 42, 11912–11920

How does this translate to the biological experiments?(1c)

- Airborne V_2O_5 **are delivered in aerosols**
- What is speciation in aerosols?
- How are aerosol prepared? From solid or from solution?



V_2O_5 - Solid state
Nick Greeves, Creative Comments Lincence

Fragmenting sheets → What are the speciation in these aerosols?

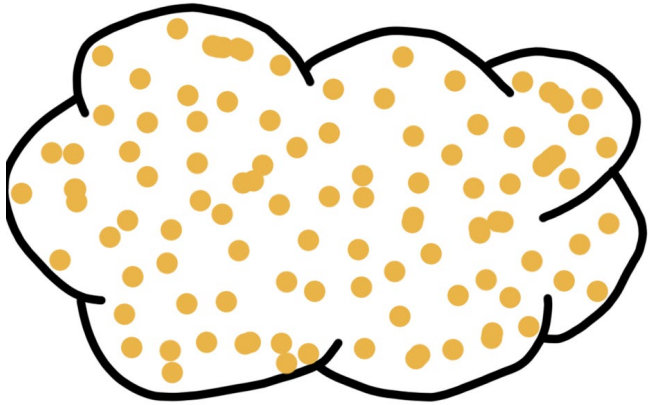
Dissolution → Various protonation states of V_{10}
So what information is available to do accessment?
Still imortant: pH, concentration, and redox potential

Speciation Studies in Confined Spaces

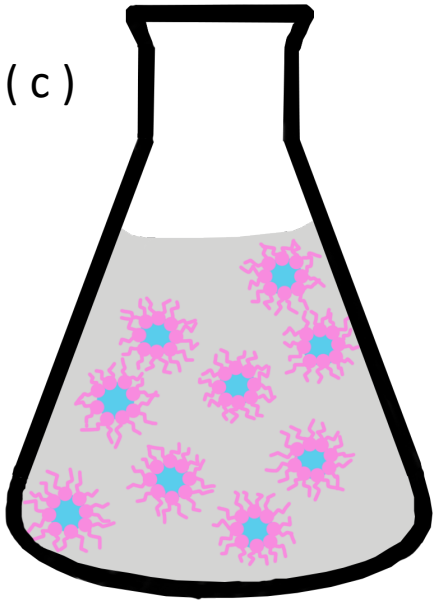
Aerosol definition: A colloidal suspension of particles dispersed in air or gas.

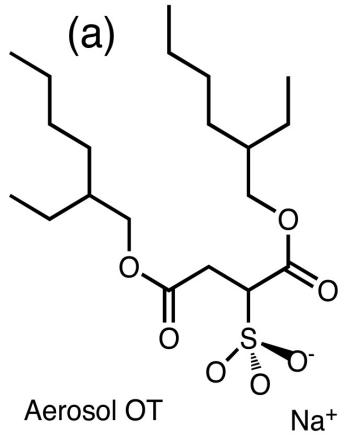
Related System: A colloidal suspension of particles dispersed in solution

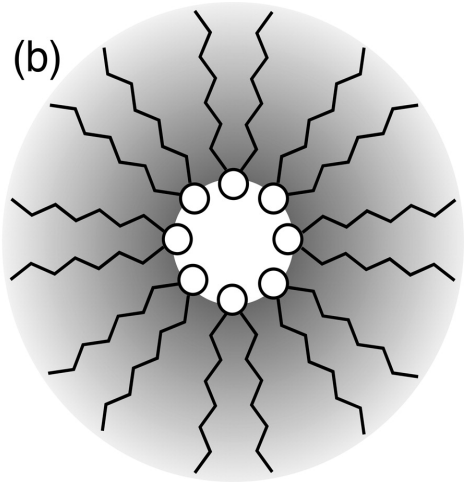
Ternary system consisting of aqueous water pool, Aerosol-OT, organic solvent



Aerosols



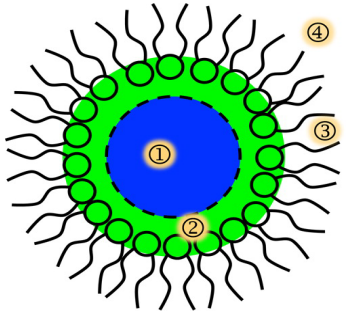




(a) Surfactant aerosol OT (AOT)
(b) Reverse micelle (RM), water pool, surrounded by AOT molecules (gray), organic solvent
(c) Solution of RMs

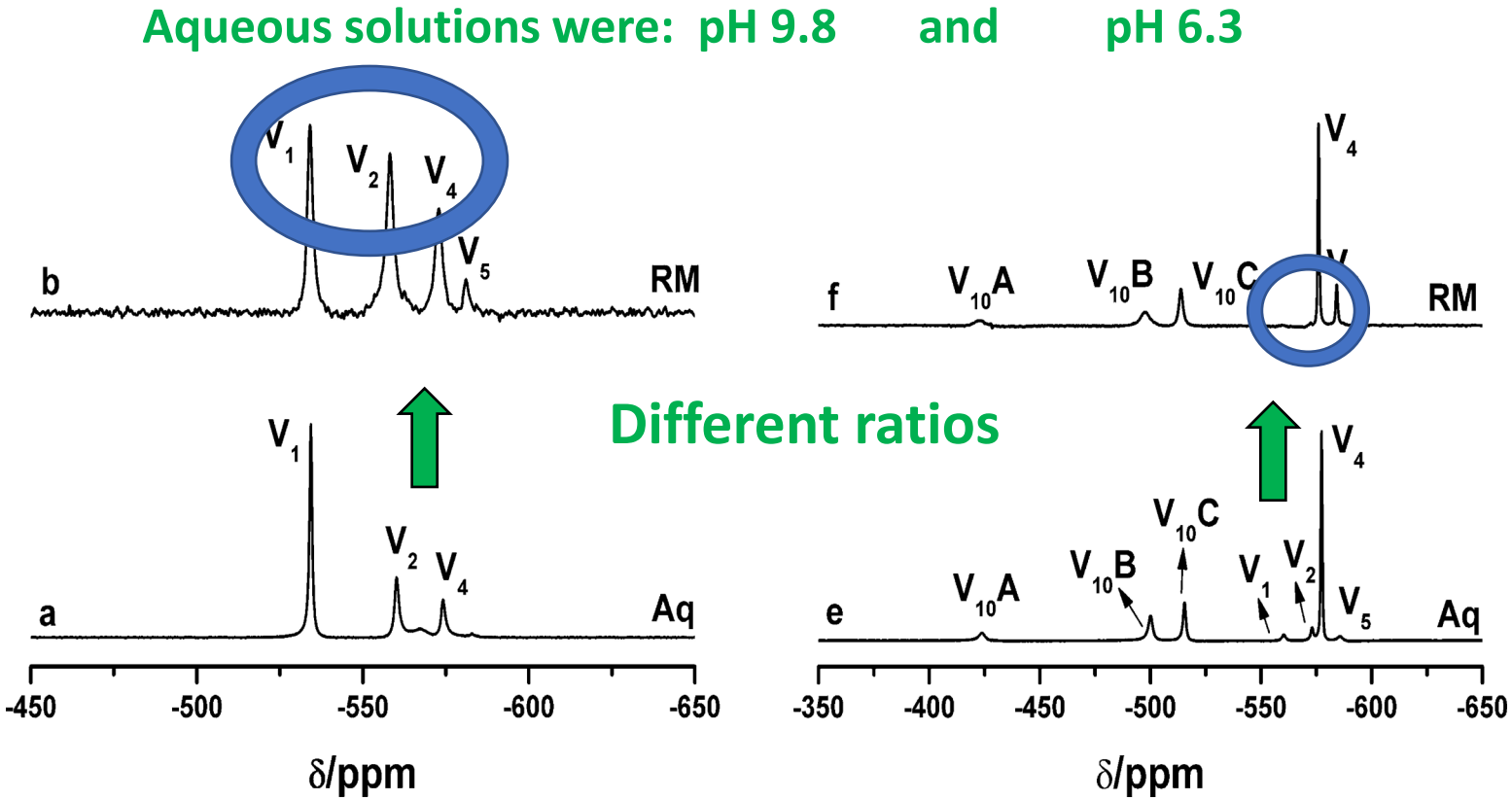
What is known about speciation in confined spaces? (1c)

Aerosol OT
Reverse micelle
RM



- Aerosols prepared from Aerosol OT
- Speciation exists in confined space
- **Speciation changed from H_2O to confined space**

Data suggest speciation will change also in aerosols containing dissolved V_2O_5



^{51}V NMR spectra of vanadate in aqueous and reverse micelle samples collected at 78.9 MHz of aqueous vanadate solution (50 mM) or in 50 mM vanadate in w_0 = 12 AOT/isooctane RM suspension.

Levinger et al. JACS, 2011, 133, 7205-7213

Different Vanadium Species have variable Biological Effects

Molecular interactions between vanadium and proteins

- **Simple forms of vanadium species are potent inhibitors for phosphatases**
- **Some forms of vanadium species facilitate signal transduction**
- Some oxovanadates (oxidovanadates) are inhibitors of enzyme activities

Effects on cells, rodents and humans

- Some forms of vanadium species are inhibiting cellular growth
- Some forms of vanadium are toxic
- Some forms of vanadium are alleviating cancer
- Some forms of vanadium are alleviating high blood glucose levels in diabetes

Crans, *J. Org. Chem.* **2015**, 80 (24), 11899-11915;. McLauchlan, et al., *Coord. Chem. Rev.*, **2015**, 301-302, 163-199; Crans, et al. *Met. Ions Life Sci,* **2018,** *18*, 251-279; Crans, et al., *Met. Ions Life Sci,* **2019**, *19*, 203-230; Samart et al. *Coord. Chem. Rev.* **2020**, *416*, 213-286; Crans, et al. Chapter 6 in Metal Toxicology Handbook, Taylor & Francis Group, 2021; Lima, et al., *Inorganics* **2021**, 9, 42.

Major modes of action of V-compounds

Phosphate analog
- Transition state analog – all phosphorylases
- Phosphatases – general, specific

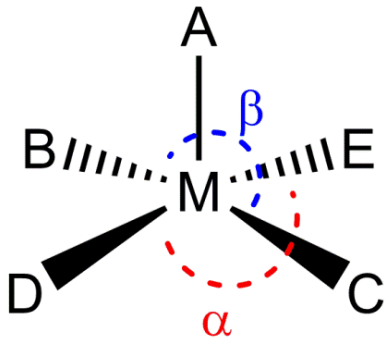
Transport proteins
- Transferrin
- Serum Albumin

Redox state
- Redox metabolites Gluthathione and Ascorbate
- Reactive Oxygen Species (ROS)

Membrane Interactions – signal transduction

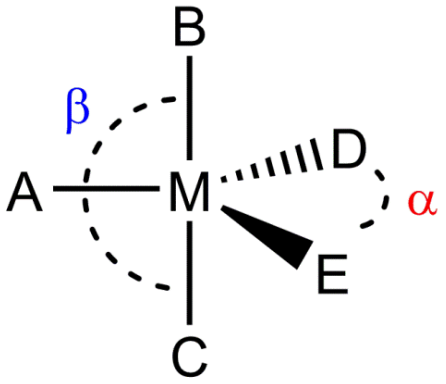
V-Protein X-Ray structures to characterize the inhibitory geometry

Five-coordinate transition state geome



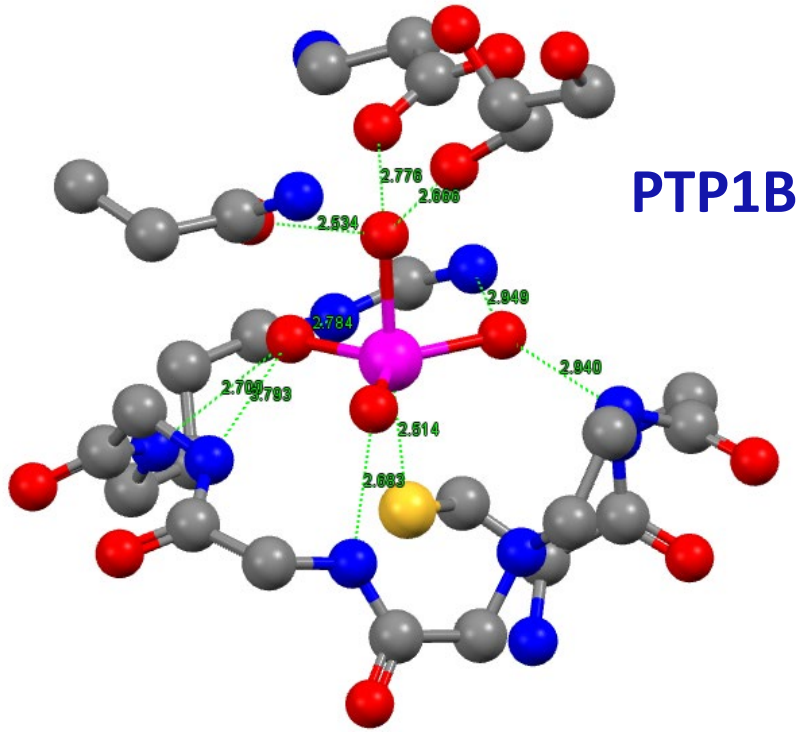
square pyramidal

$\alpha = \beta = 180°$



trigonal bipyramidal

$\alpha = 120°; \beta = 180°$



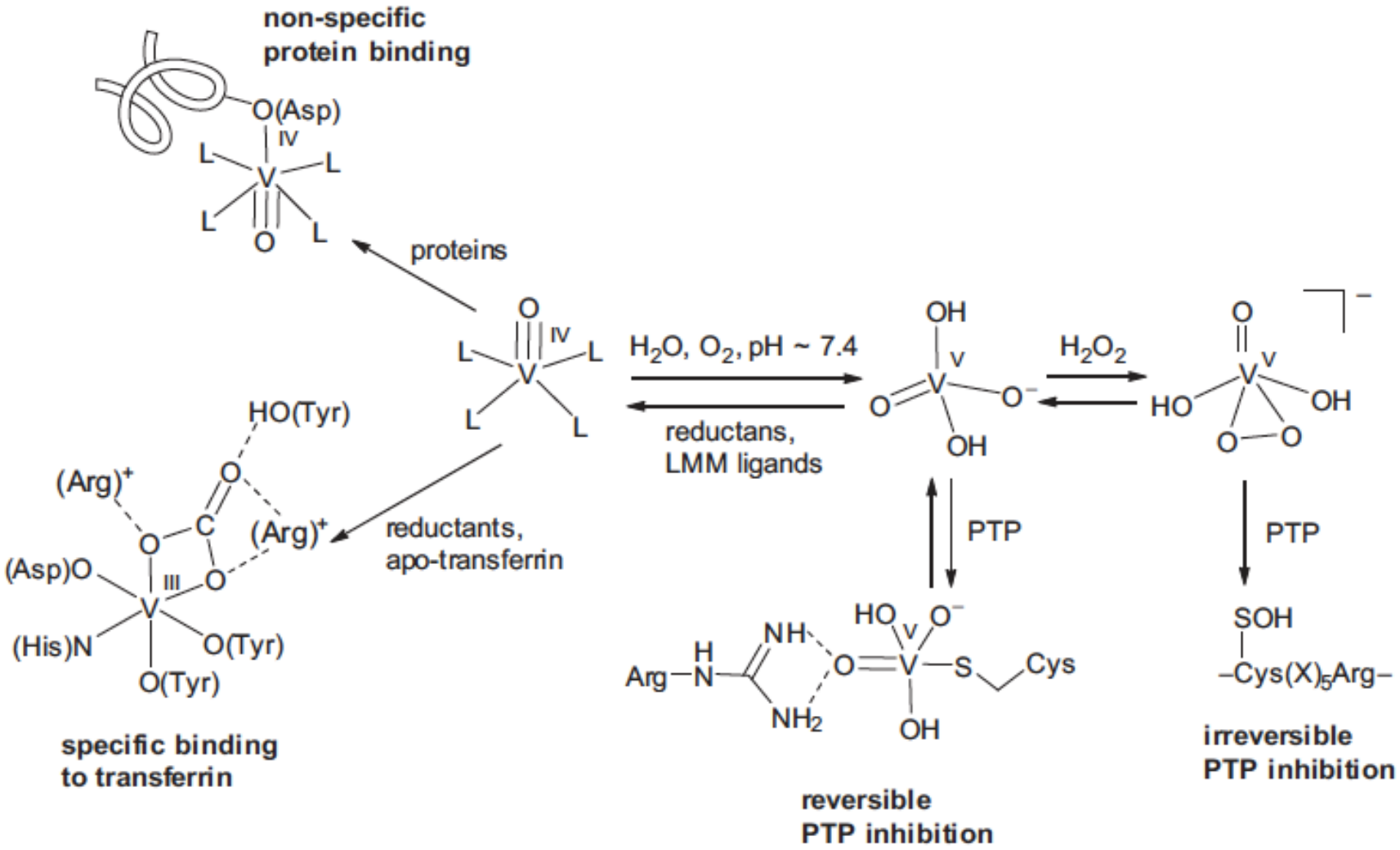
PTP1B

Data mining of phosphatase-inhibitor complexes could characterize the ideal inhibitors

Crans *et al. Eur J. Inorg. Chem.* **2014,** *27,* 4450-4468; McLauchlan et al. *Coord. Chem. Rev.* **2015**, 301-302, 163-199; Sànchez-Lombardo et al. *J. Inorg. Biochem.* **2015** 147, 153-164; Crans *J. Org. Chem.* **2015**, 80 (24), 11899-11915

PTP1B is considered an enzyme that goes by an exploded mechanism; it is the enzyme associated with the antidiabetic action. ***Close-up of details from crystal structures along the reaction pathway catalyzed by PTP1B.*** **Second transition state.**

Transport proteins



Transferrin
Serum albumin
Immunoglobins

Many groups have studies this

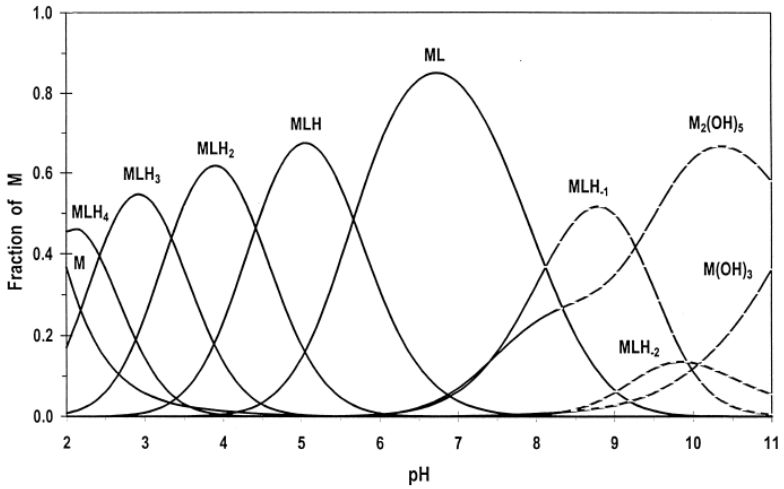
New methods are available to identify protein-V-complexes

See for example
Ugone et al. Inorg. Chem. 2020, 59, 9739−9755

Levina et al. *Coord. Chem. Rev.*, 2017, 5, 1491–1502

Redox State – Reducing Metabolites - ROS

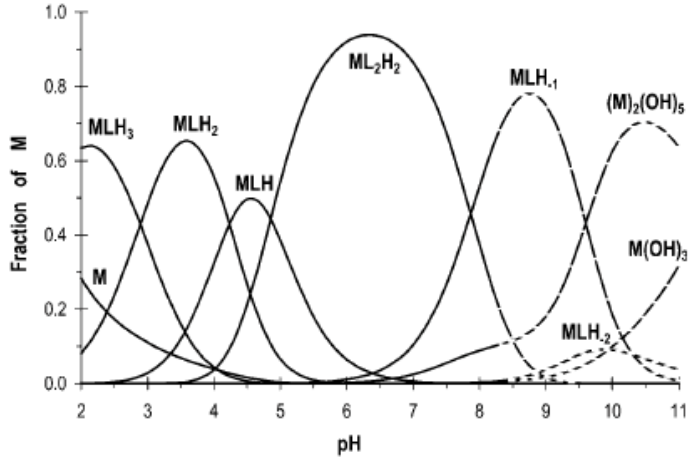
- Vanadium(V) and vanadium(IV) form both complexes with gluthathione (GSH)
- Vanadium form complexes with ascorbic acid and is reduced
- Vanadium(V) can be reduced by GSH
- Vanadium recycle in biological conditions
- Metabolites undergo redox and in the process generate ROS



The $V(IV)^{2+}$-GSSG system V(IV) 7 mM and 70 mM GSSG

Pessoa et al. *J. Inor. Biochem.* 2001, 84, 259-270

- **Relevance:** Vanadium in blood or plasma form ROS and also V-GSH complexes



The $V(IV)O^{2+}$-GSH system with V(IV) 10 mM and 250 mM GSH

Pessoa et al. *J. Biol. Chem.* 2002, 7, 225-240

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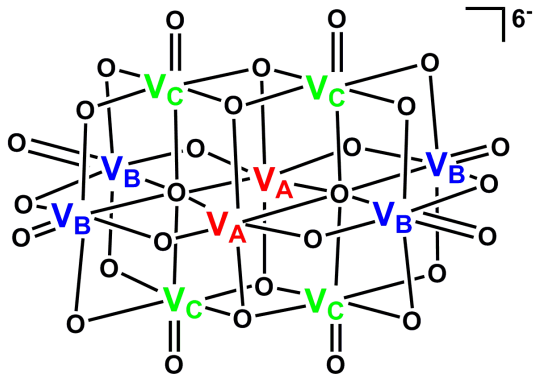
Signal transduction – interactions at the membrane

Signaling molecules on the membrane cytoplasmic face form in response to **receptor aggregation**

Samart, et al. *Coord. Chem. Rev.* **2020**, *416*, 213-286
Althumairy et al. *Metallomics,* **2020,** *12,* 1044-1061
And references there in

- Studies with membrane models
- Studies with IR, FcεRI and LHR
- Key for inhalation studies FcεRI

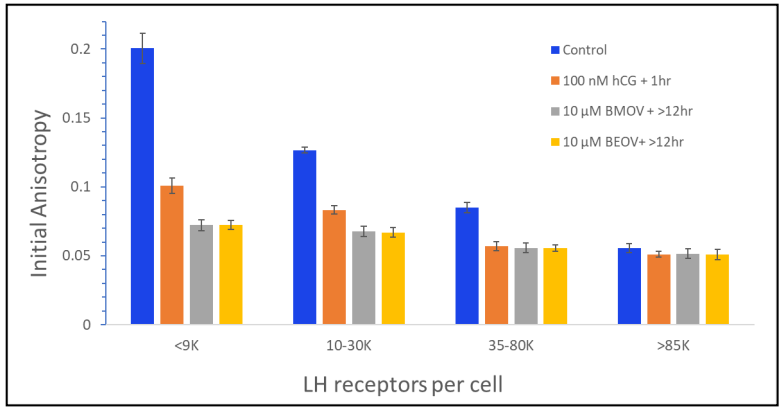


Deb Roess

Deborah Roess & George Barisas

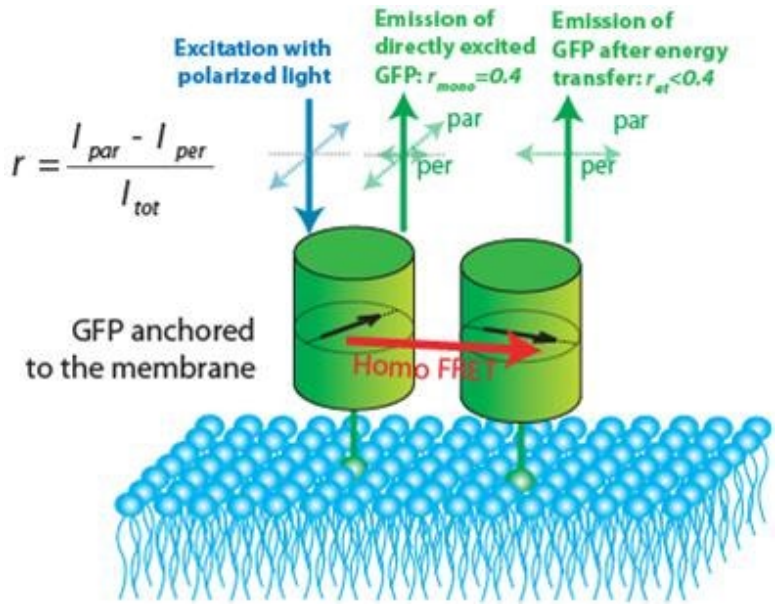
Measuring initial anisotropies indicative of receptor clustering
METHODS: Homo-transfer fluorescence resonance energy transfer

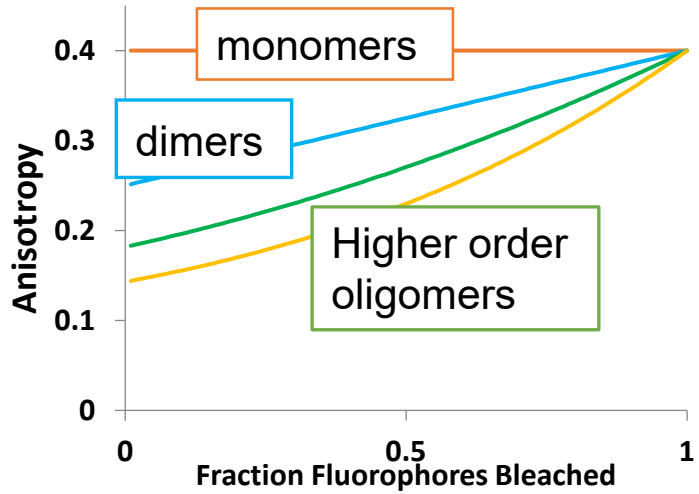
Results:



Smaller values for "Initial Anisotropy" are indicative of receptor clustering.
Increased receptor clustering when

1. higher numbers of LHR/cell.
2. hCG, BMOV and BEOV (or V_{10})





CHO cells stably express LHR-YFP

a. 9k LHR/cell
b. 10k-30k LHR/cell
c. 35k-80k LHR/cell
d. >85k LHR/cell

Homo-transfer FRET between YFPs is measured using intensity values for fluorescence emission parallel and perpendicular to the polarized exciting light.

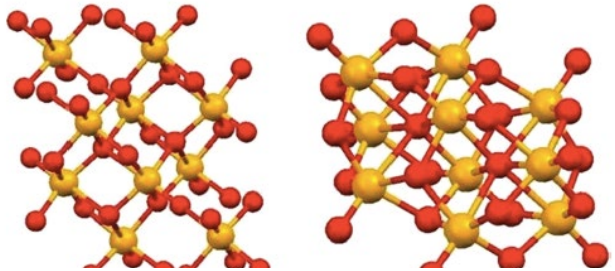
If receptors are monomers, the initial anisotropy at t=0 is expected to be 0.4.

Dimers, trimers or higher oligomers will have lower initial anisotropy values.

Althumairy et al *J. Inorg. Biochem.* 2019 submitted

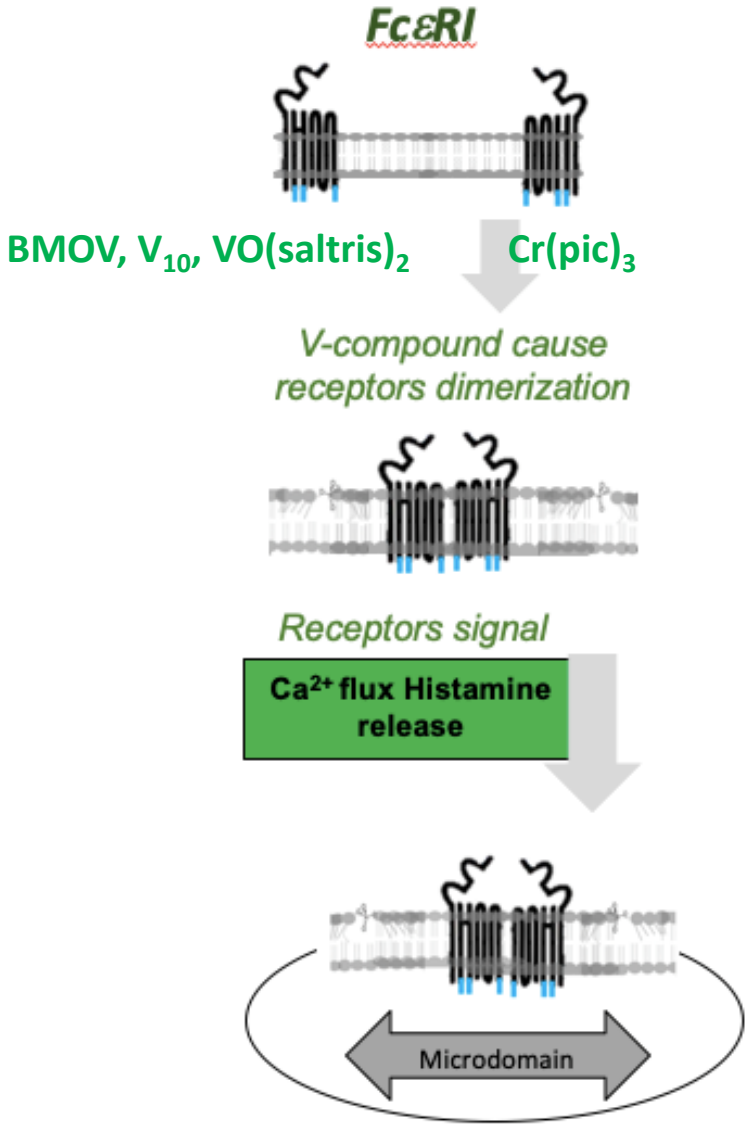
Reported results with the FcεRI membrane

V_2O_5



$V_{10}O_{28}^{6-}$

Compound	RBL-2H3 Cells			
	Type 1 Fce Receptor (tyrosine kinase receptor)			
	Lipid packing	Receptor Aggregation	Raft Translocation	Signal Transduction
$VO_2(dipic)^-$				
BMOV	decreased		Yes	Ca^{2+} flux
$VO_2(malto)_2^-$				
V_{10}		Yes	Yes	histamine release
$VO(saltris)_2$		Yes		histamine release
$Cr(pic)_3$			Yes	



Al-Qatati et al. *Dalton Trans*., 2013, 42, 11912–11920; Samart, et al. *Coord. Chem. Rev.* **2020**, *416*, 213-286 and references therein

Althumairy et al *J. Inorg. Biochem.* 2019 submitted

These modes of actions and others exist through biology and are likely to be important and should be considered when accessing toxicity

Phosphate analog

- Transition state analog – all phosphorylases
- Phosphatases – general, specific

Transport proteins

- Transferrin
- Serum Albumin

Redox state

- Redox metabolites Gluthathione and Ascorbate
- Reactive Oxygen Species (ROS)

Membrane Interactions – signal transduction