Aconitase

Chemistry 419

University of Washington

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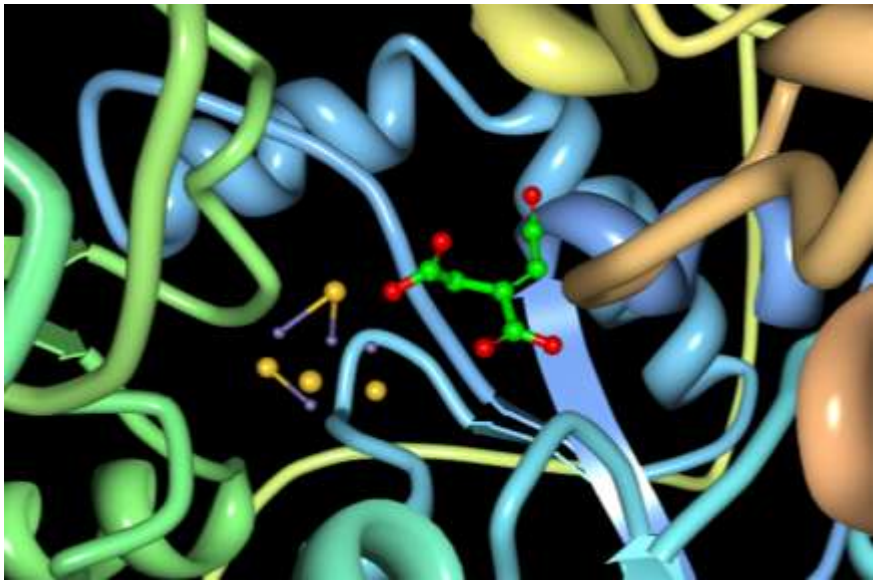
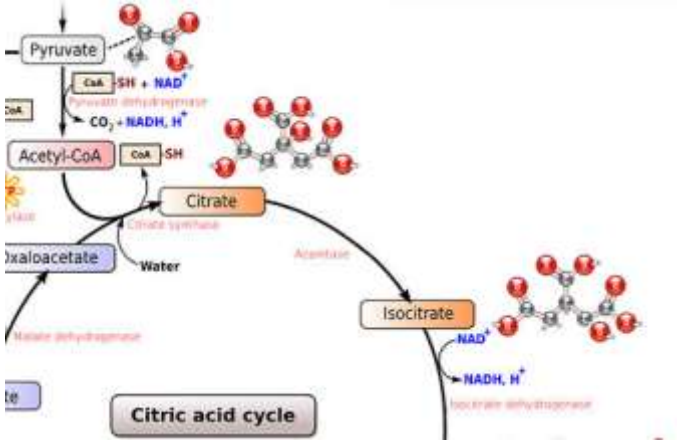
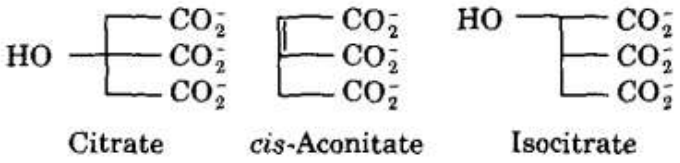


Hello Aconitase! (citrate[isocitrate] hydrolyase)

- Vital enzyme involved in the Krebs Cycle – converts citrate to isocitrate
- One of the initial proteins studied that had a $[4Fe\text{-}4S]^{2+}$ cluster
- Early studies into inactivation and studies by mutagenesis to allow crystallization to learn more about iron cluster

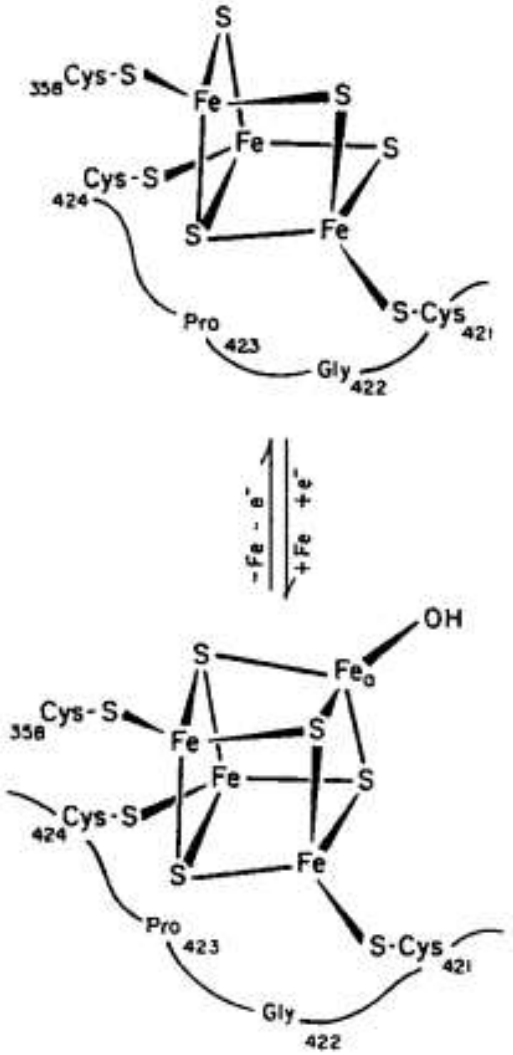




Top: Wiki: User: Narayanese
Above: Beinert, H.; Kennedy, M. C. *Eur. J. Biochem.* **1989**, *186*, 5-15

States of Aconitase

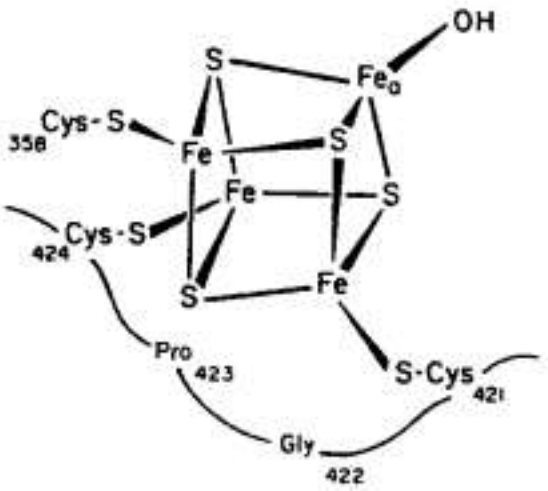
- Inactive: $[3Fe\text{-}4S]^{+}$
  - Obtained via
    - Aerobic purification
    - Treatment with H_2O_2
- Active: $[4Fe\text{-}4S]^{2+}$
  - Obtained via
    - High levels of Fe *in vivo*
    - Reduction (adding e^-)



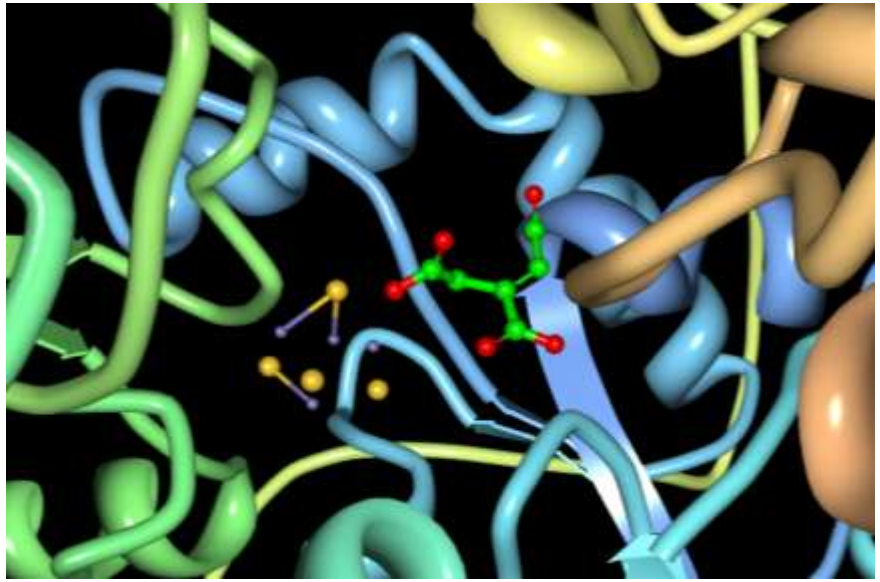
Beinert, H.; Kennedy, M. C. *Eur. J. Biochem.* **1989**, *186*, 5-15
Villafranca, J. J.; Mildvan, A. S. *J. Biol. Chem.* **1972**, *247*, 3454-3463

Aconitase - Protein

- The protein is designed to stabilize the [4Fe-4S] cluster with cystine residues and accomidate citrate



- Quick demo



PDB: 1aco, 1ami, 1amj

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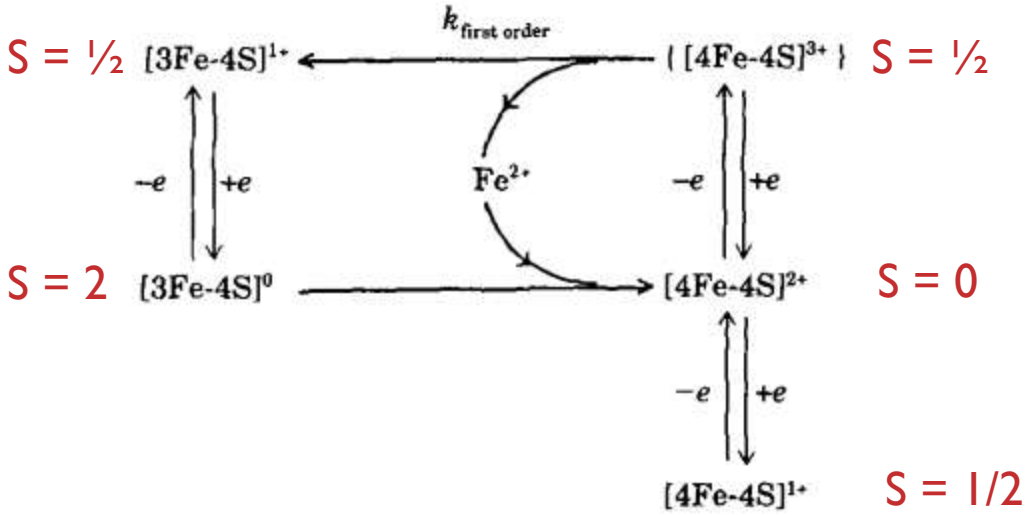
Oxidation States



- Note that the Fe_a atom leaves and rejoins.

Tong, J.; Feinberg, B.A. *J. Biol. Chem.* **1994**, *269*, 24920-24927

Spectroscopy - EPR

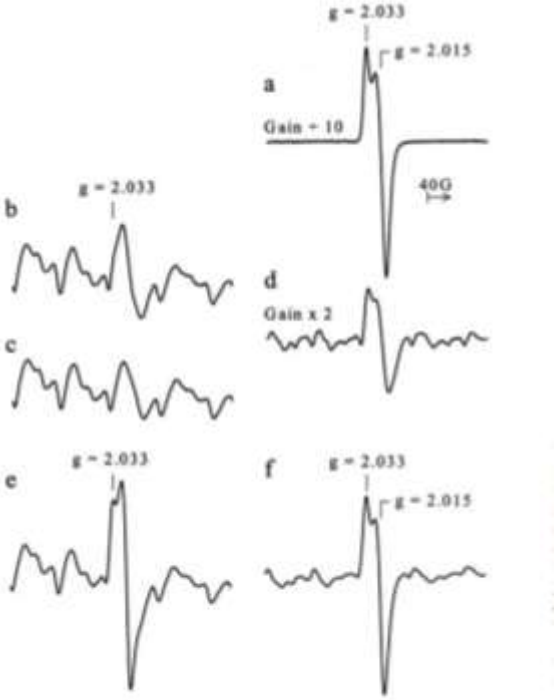


FIG. 1. **EPR spectra of yeast expressing wild-type IRP1.** Yeast cells were grown in SD-ura-his medium without or with H_2O_2 and prepared for EPR analysis as described under "Experimental Procedures." *Scans b, c,* and *e* are the spectra that resulted from EPR with intact yeast cells. Each spectrum represents the average of four scans.

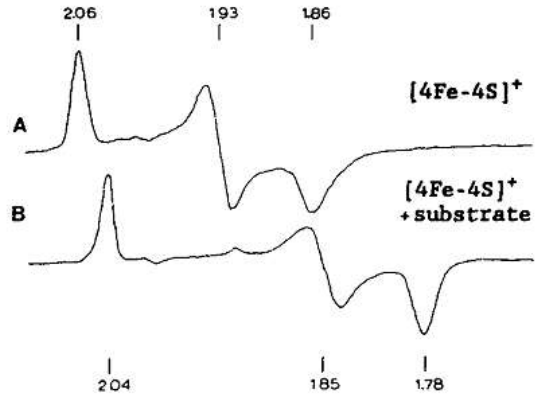


Fig. 11. *EPR spectra of the [4Fe-4S]+ cluster of aconitase in the absence (A) and the presence (B) of substrate.* Prominent features of the spectra are marked of a *g*-value scale. Prior to freezing, the samples were photoreduced anaerobically in the presence of deazaflavin and oxalate. The spectra were recorded at a frequency of 9.24 GHz, 1 mW power and 13 K

a = purified aconitase [3Fe-4S]
b = transformed *in vivo* yeast aconitase, small [3Fe-4S]
c = non-transformed *in vivo* – [4Fe-4S]
d = 1b – 1c, signal of oxidized aconitase
e = 1b plus additional 6mM H_2O_2 a
f = 1e – 1c, signal of oxidized aconitase

Brown, N. M., et al. *J. Biol. Chem.* **2002**, *277*, 7246-7254
Beinert, H.; Kennedy, M. C. *Eur. J. Biochem.* **1989**, *186*, 5-15

^1H / ^2H ENDOR

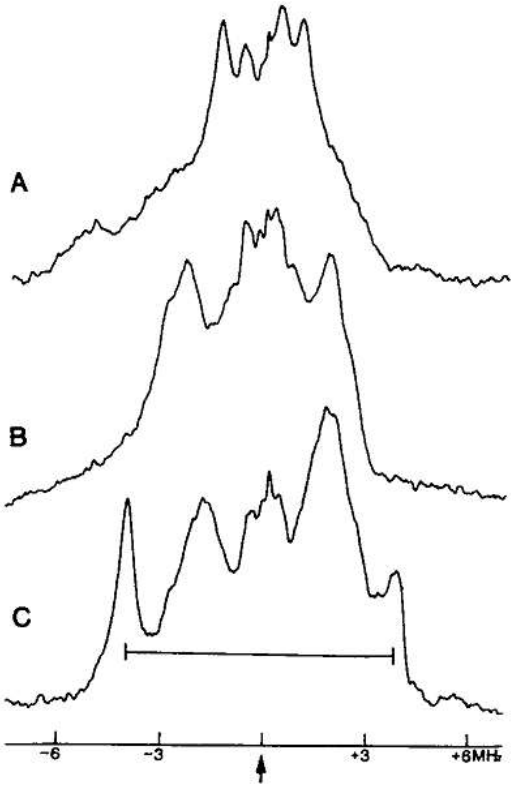


Fig. 12. ^{1}*H-ENDOR spectra of the [4Fe-4S]$^+$ cluster of aconitase at* g_{min} *without substrate (A), with perdeuterated* cis*-aconitate (B) and with ordinary citrate (C).* For (A) and (B) the solvent was ^2H_2O; for (C) it was ^1H_2O. Aconitase was photoreduced as for Fig. 11. The

- No real change from A to B..
- Huge change between B and C – only difference is solvent: ^2H_2O vs ^1H_2O, respectively
  - hyperfine coupling is large => cluster bound H_xO (x = 1, 2) to Fe_a

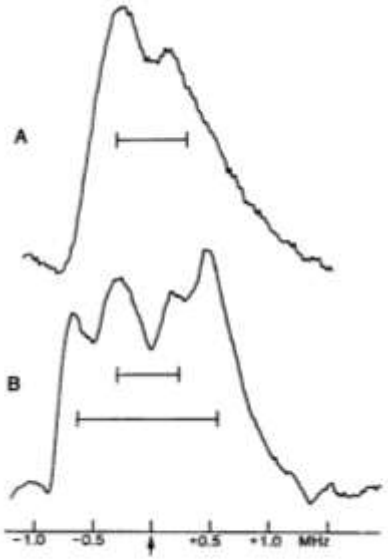


Fig. 13. ^{2}*H-ENDOR spectra of the [4Fe-4S]$^+$ cluster of aconitase in the absence (A) and presence (B) of substrate.* The other conditions

Since solvent species in left ^2H ENDOR exhibits two distinct protons, the additional ligand is H_2O

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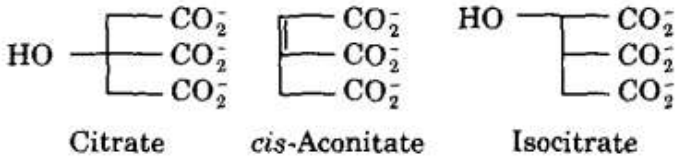
Mechanism



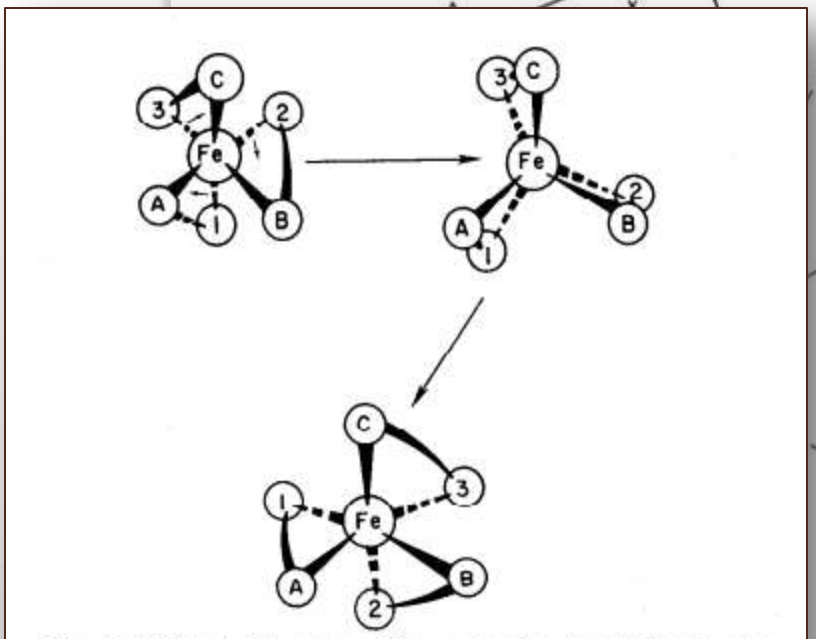
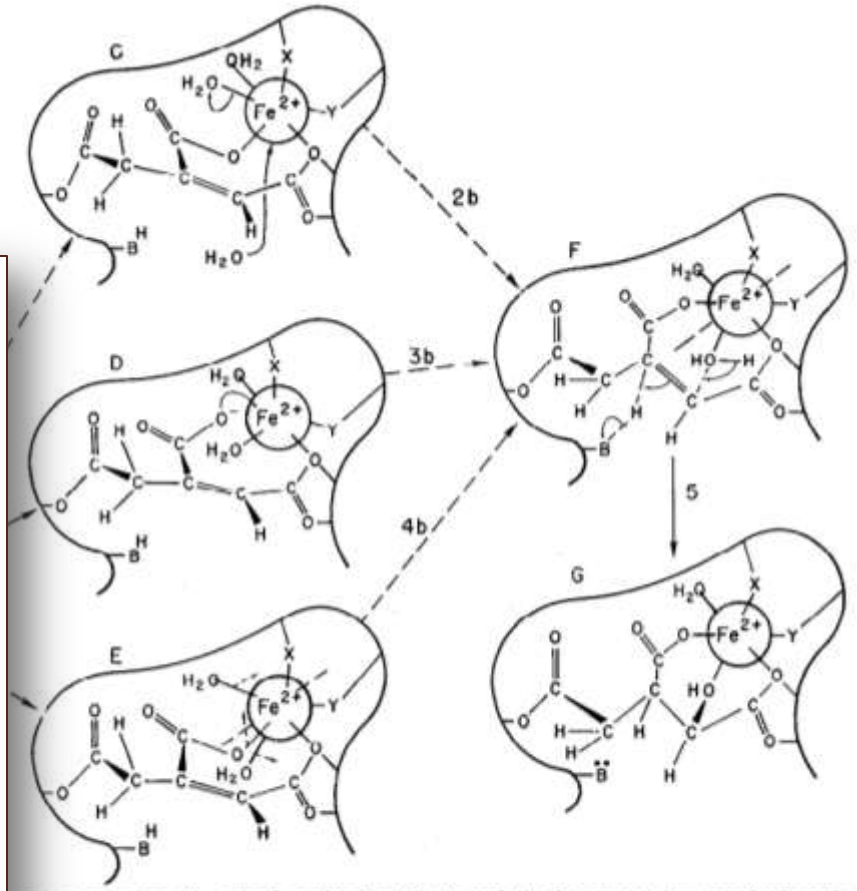


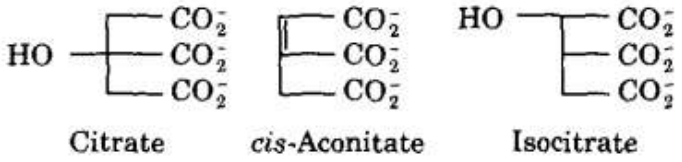
FIG. 7. Bailar twist or turnstile mechanism found for an octahedral Fe(II) complex (39). The Ligands *A*, *B*, and *C*, which point toward the reader, are fixed. The Ligands *1*, *2*, and *3*, which point away from the reader, are mobile. In the turnstile reaction the bonds to Ligands *1*, *2*, and *3* rotate 120° about the 3-fold axis, resulting in a 90° shift in the positions of Ligands *1*, *2*, and *3*.



the conversion of
hich are consistent
on of water (Step *1*)
e-like conformation
e conformation (*F*)
and *2b*, the ferrous
wheel mechanism (7); *3a* and *3b*, the reverse ferrous wheel mechanism; *4a* and *4b*, the Bailar twist mechanism. The *broken line* through the Fe^{2+} is the 3-fold axis about which the Bailar twist occurs. Ligands X, Y, and the terminal carboxyl groups of the substrate are fixed. Water then adds (Step *5*) to form isocitrate.

Villafranca, J. J.; Mildvan, A. S. *J. Biol. Chem.* **1972**, *247*, 3454-3463

Mechanism



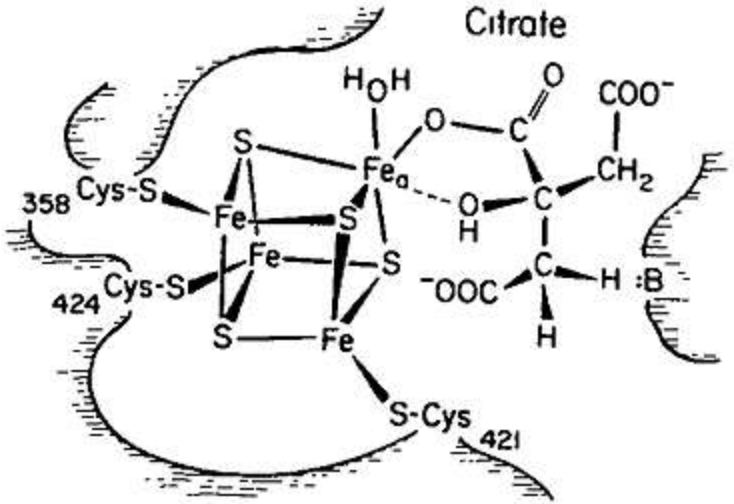


Fig. 15. *Scheme summarizing information obtained from the work described or mentioned above (cf. particularly Gahan et al. [59]) on the active site of aconitase and the binding of substrate to the Fe-S cluster.* The figure shows citrate bound. When isocitrate is bound, the molecule is flipped by 180° so that the $-CH_2-COO^-$ group points downward and the carboxyl at the bottom in the figure now becomes bound to Fe_a

- Most definitely stereospecific!

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Model Compounds

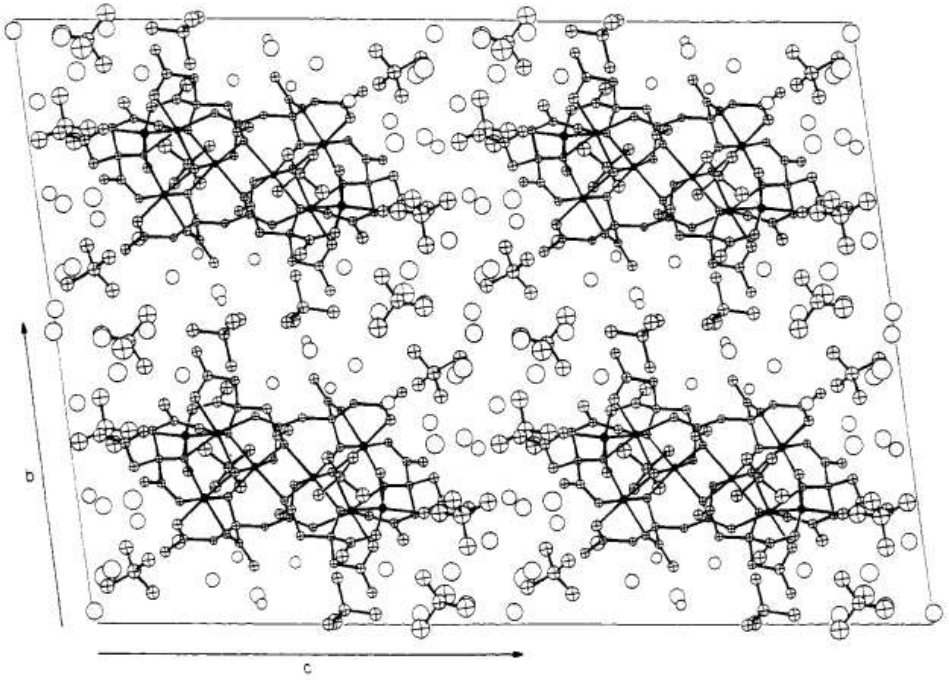

Figure 4. Packing diagram of $[[N(CH_3)_4]_5[Ni(II)_4(C_6H_4O_7)_3(OH)(H_2O)]\cdot 18H_2O]_2$ viewed down the *a* axis. The open circles represent the oxygen atoms of the noncoordinated water molecules.

- Nickel triionized citrate was used to obtain a crystallographic structure
- Obtain ideas and precedents for metal complexes with aconitase

Strouse, J. et al. *J. Am. Chem. Soc.* **1977**, *99*, 562-572

References

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