

# **Acetyl-CoA Synthase/ Carbon Monoxide Dehydrogenase (ACS/CODH)**

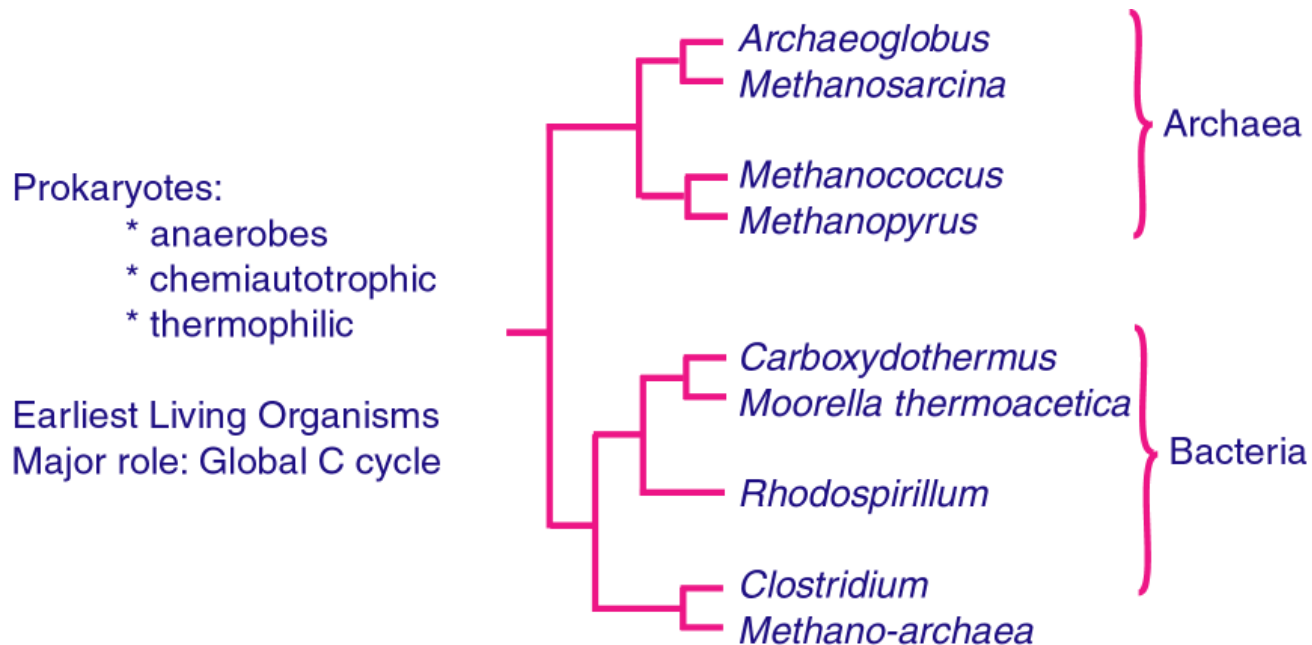
**A Ni-Fe-S-containing Bifunctional Enzyme  
with a Bio-organometallic Reaction Mechanism**

Paul A. Lindahl

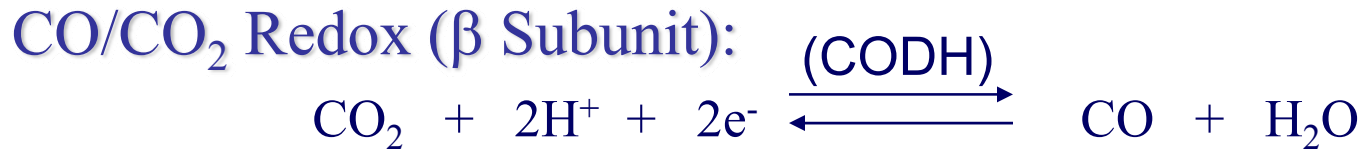
Departments of Chemistry and Biochemistry/Biophysics  
Texas A&M University, College Station, TX

# Where are ACS/CODH's found?

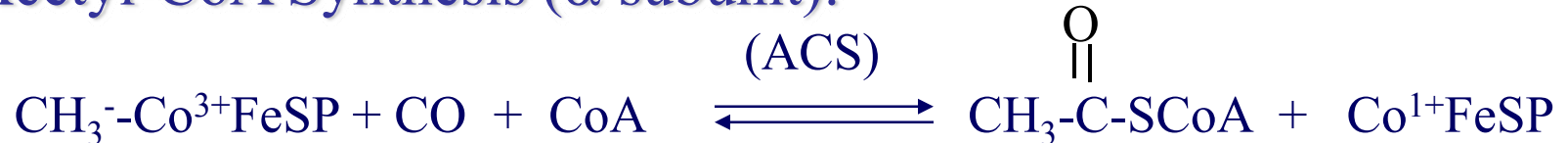
- Anaerobic Bacteria and Archaea
- Chemoautotrophic (grows on CO<sub>2</sub>/H<sub>2</sub> or CO)
- Evolutionarily primitive (thermophiles)
- Major role in global C<sub>1</sub> cycle
- Three major classes
  - $\alpha_2\beta_2 + \text{CoFeSP}$  ( $\gamma\delta$ ) *Moorella thermoacetica*
  - $(\alpha\beta\gamma\delta\varepsilon)_2$  *Methanosarcina thermophila*
  - $\beta_2$  *Rhodospirillum rubrum*



Reactions catalyzed by the 310 kDa  $\alpha_2\beta_2$  tetramer  
From *Moorella thermoacetica*:

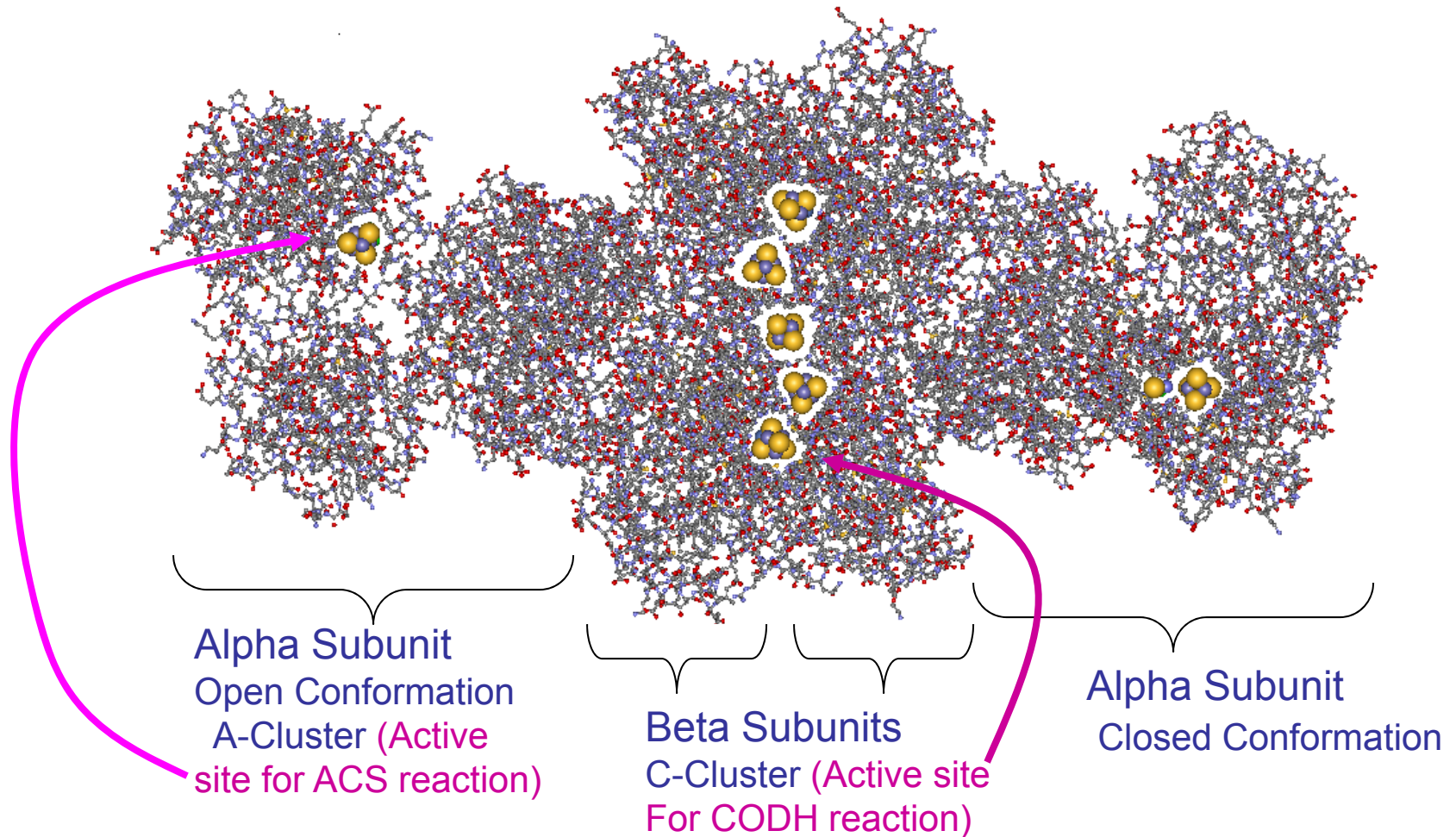


Acetyl-CoA Synthesis ( $\alpha$  subunit):



Recombinant  $\alpha$  subunit also catalytic if incubate apo- $\alpha$  with Ni

# Structure of ACS/CODH

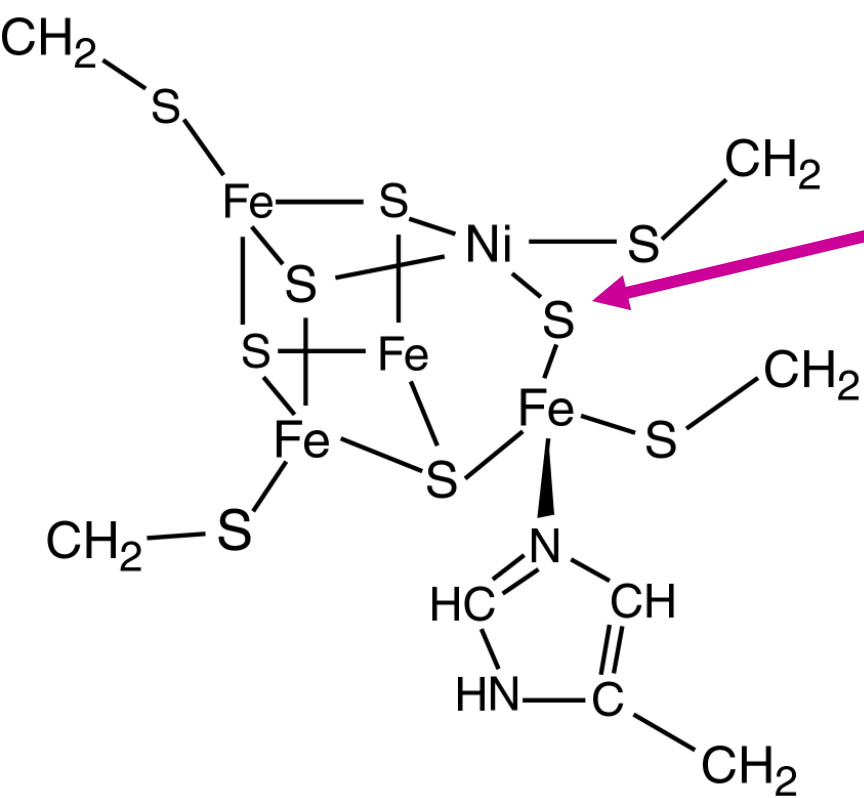


*Doukov et al., Science 2002*

*Darnault et al, Nature Structural Biology, 2003*

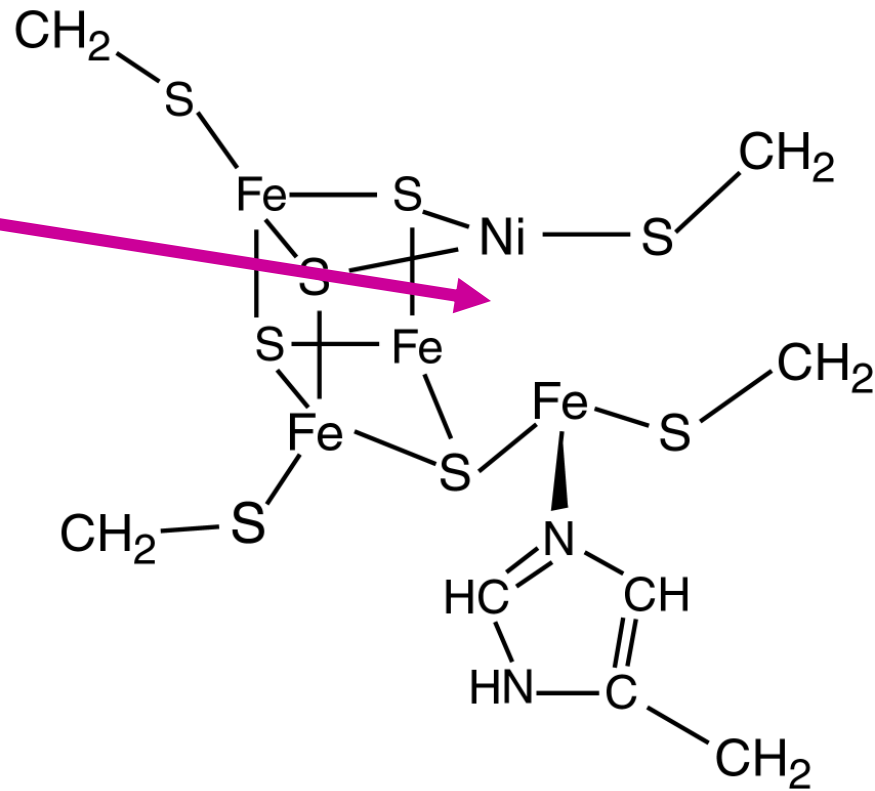
# Mechanism of the CODH Reaction

# Controversy: Role of Bridging Sulfide in C-cluster



*Carboxythermus hydrogenoformans*

Dobbek, Svetlitchnyi, Gremer, Huber, Meyer  
(2001) *Science* 293, 1281-1285.



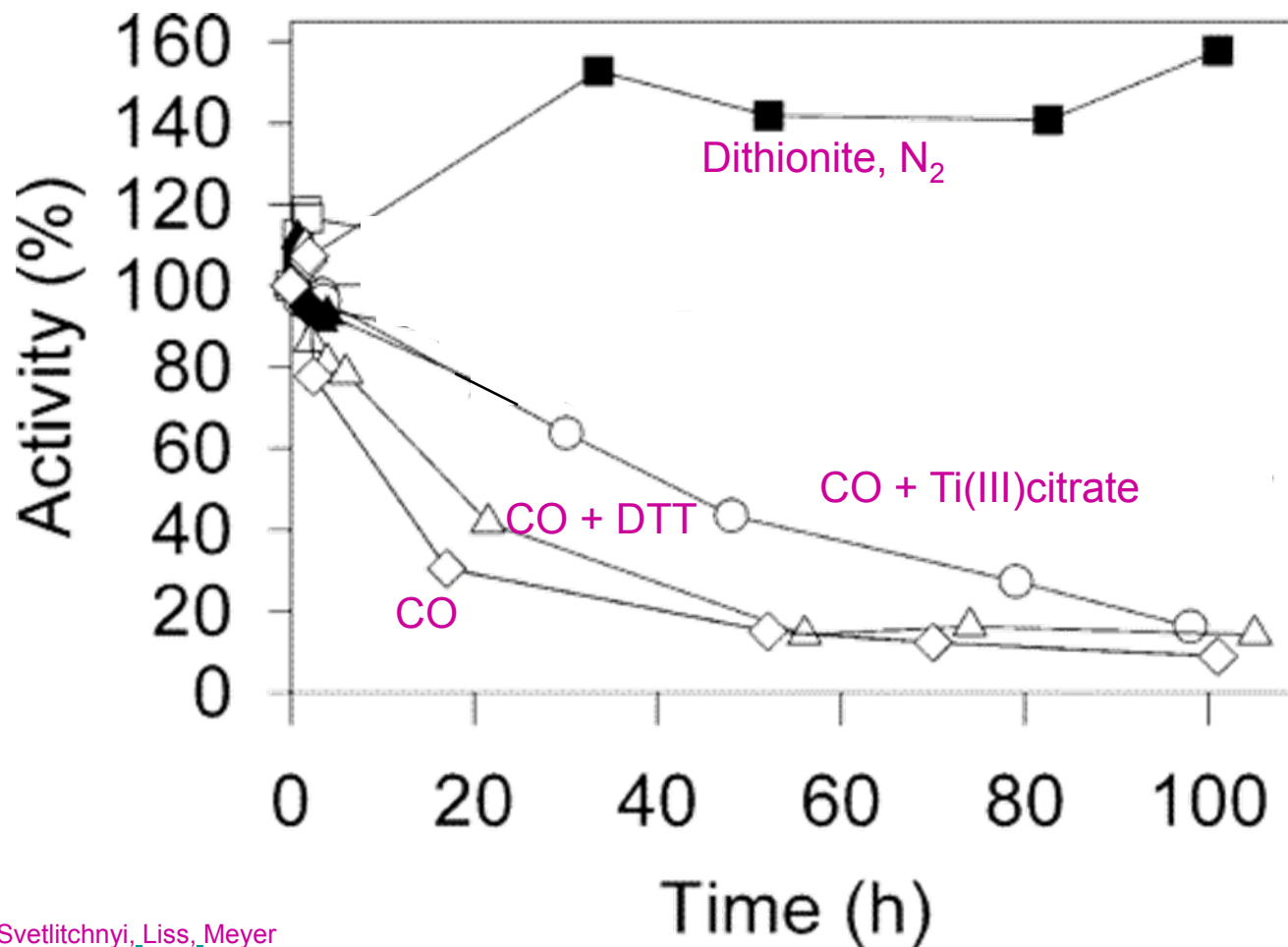
*Moorella thermoacetica*

Doukov, Iverson, Saravalli, Ragsdale, Drennan,  
(2002) *Science* 298, 567-572.

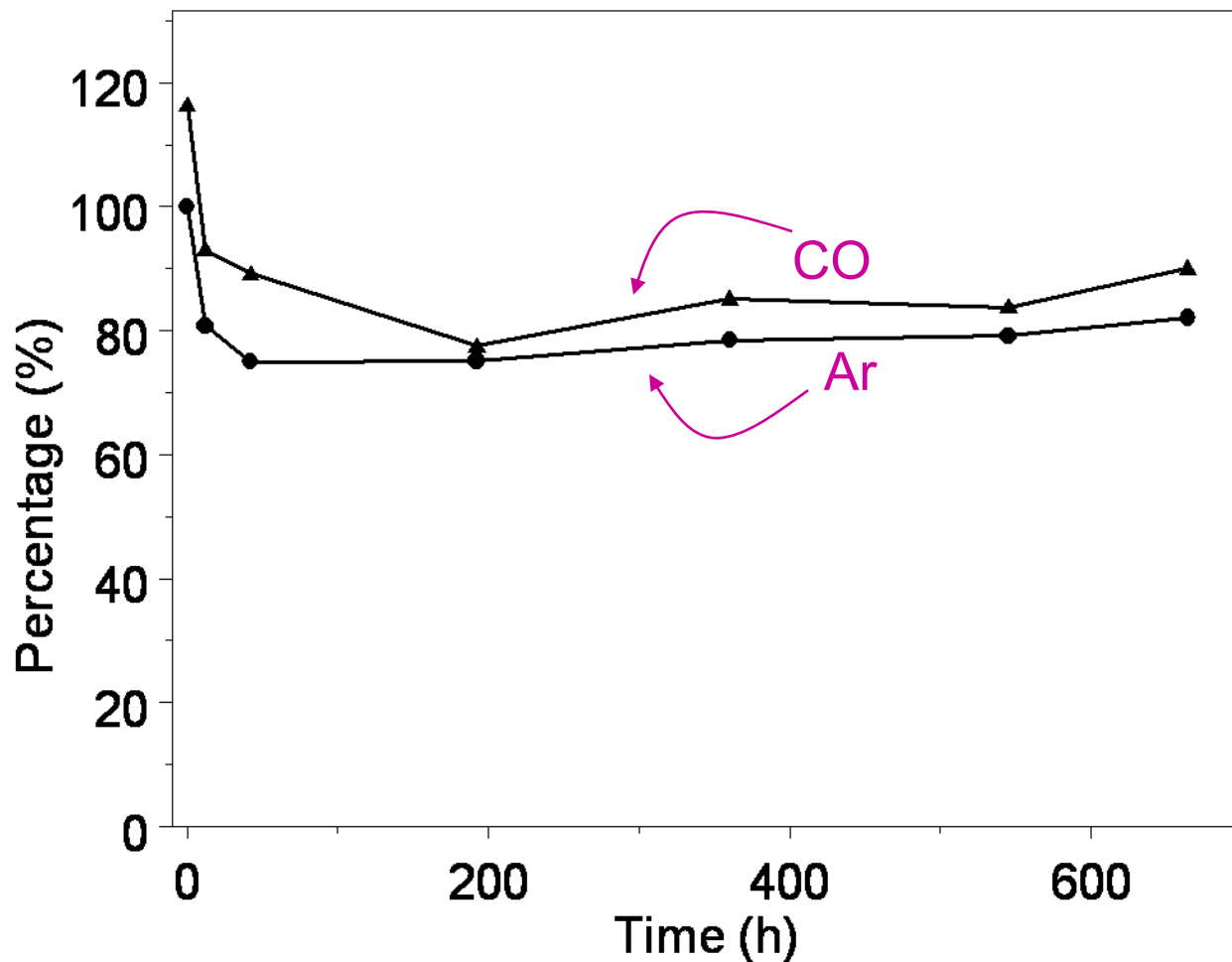
Darnault, Volbeda, Kim, Legrand, Vernede, Lindahl, Fontecilla-Camps  
(2003) *Nature Structural Biology* 10, 271-279.

# Proposal of Dobbek, Svetlitchnyi, Liss and Meyer (JACS 2004):

- Bridging Sulfide is Required for Activity
- Incubation in CO abstracts S, forming COS and inactivating CODH



# We tried the same experiment...

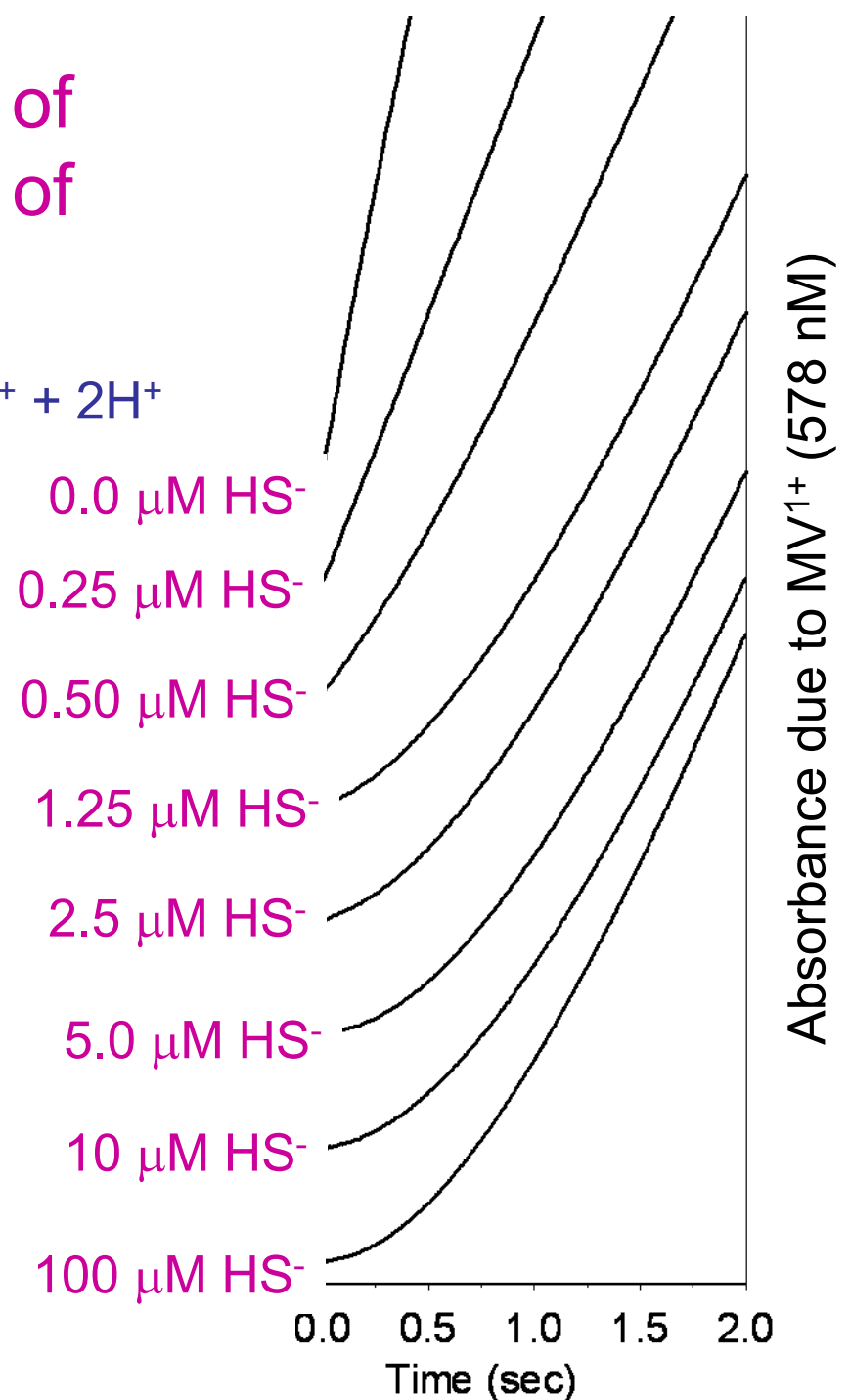




# CO Oxidation Activity of CODH<sub>Rr</sub> in the Presence of Sodium Sulfide

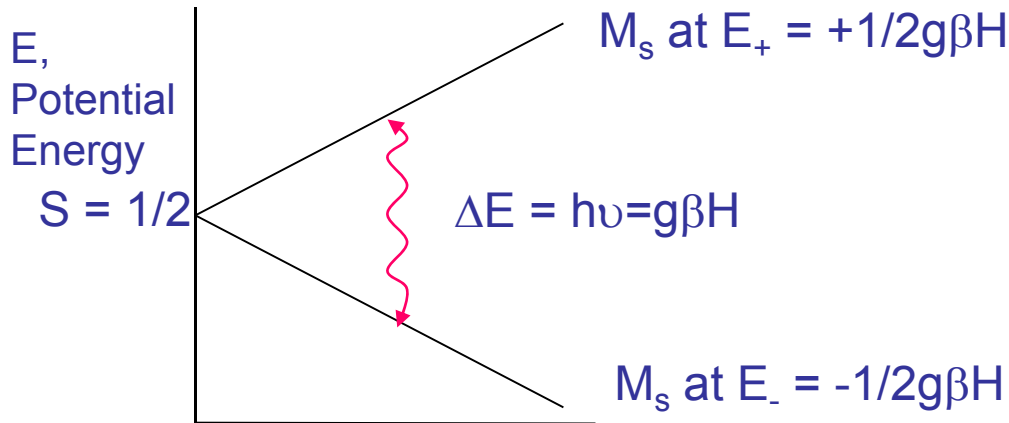


- CODH<sub>Rr</sub> incubated in HS<sup>-</sup> then assayed
- HS<sup>-</sup> inhibited catalysis
- Inhibition was partial
- Lag phase evident
- HS<sup>-</sup> bound CODH prior to catalysis, Yielding an inhibited state
- HS<sup>-</sup> is expelled, and rebinds during turnover



# EPR = Electron Paramagnetic Resonance

Can observe systems with  $S = 1/2, 3/2, 5/2, \text{etc}$



H, Magnetic Field

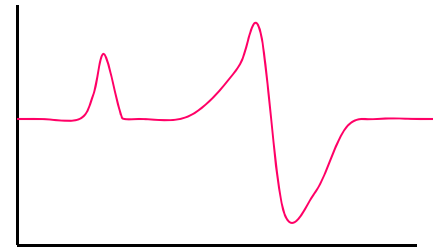
Abs

$\frac{dAbs}{dH}$

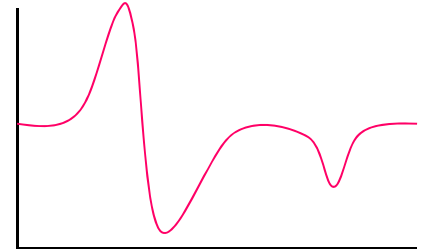
Isotropic Symmetry

H

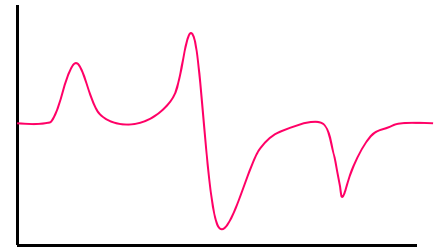
Principal g-value  
 $g = h\nu/\beta H$  ( $g = 2.0023$  for  $e^-$ )



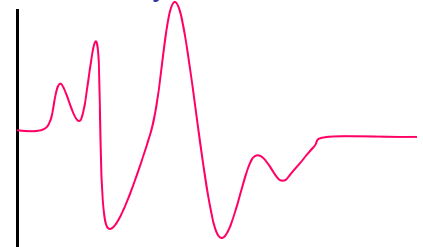
Axial Symmetry ( $g_{\parallel} > g_{\perp}$ )



Axial Symmetry ( $g_{\perp} > g_{\parallel}$ )



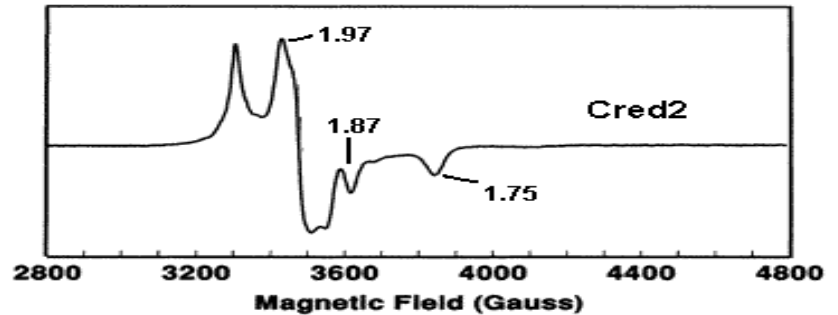
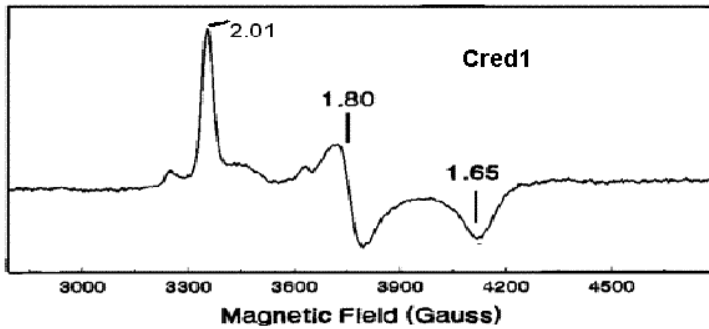
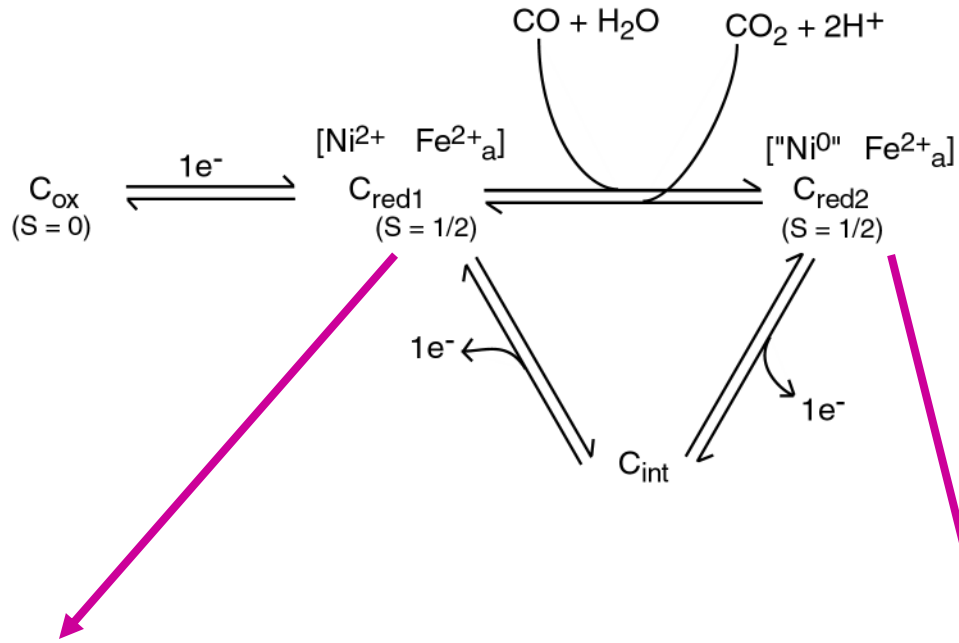
Rhombic Symmetry  
 $(g_x \neq g_y \neq g_z)$



Mixture of Signals

Quantified by Integration  
 and comparison to  
 Cu(II)EDTA standard

# Redox, EPR of C-Cluster

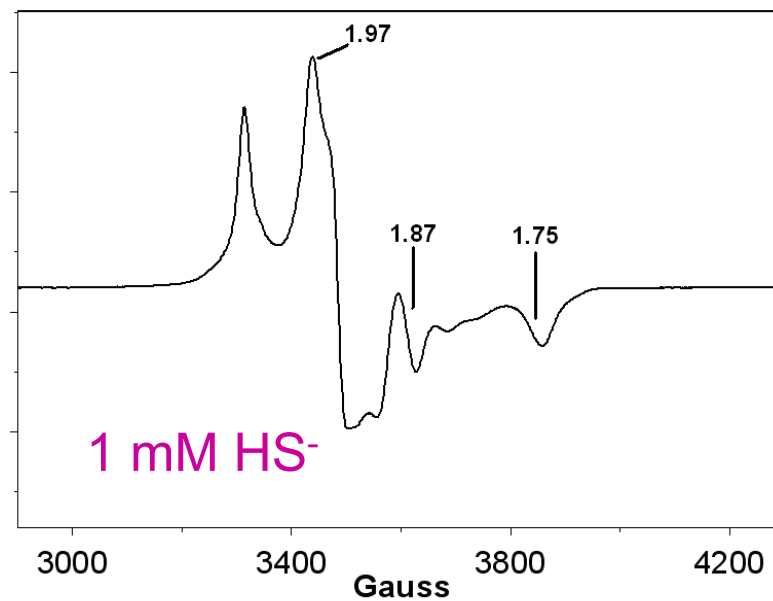
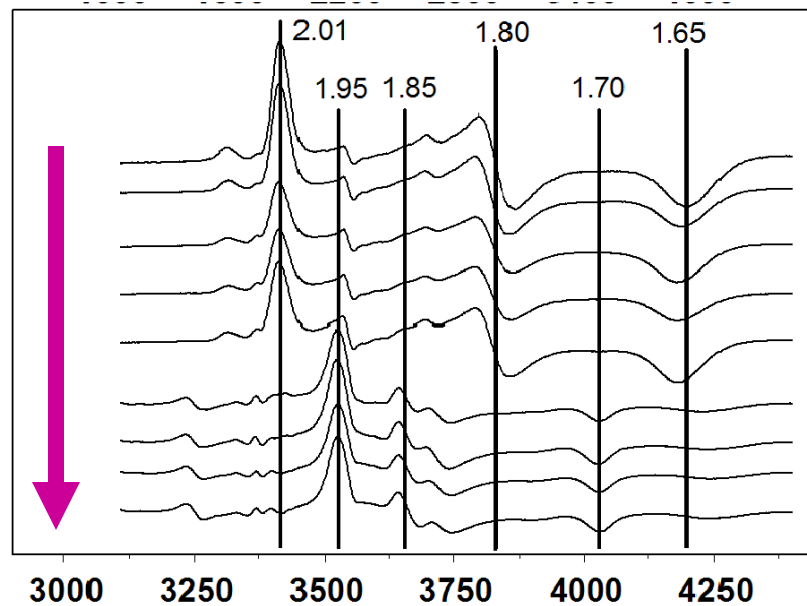


# Effect of Sodium Sulfide on $C_{red1}$ EPR Spectra

Increasing concentrations of sulfide

$C_{red1}$  signal shifts to  $g = 1.95, 1.85, 1.70$

$C_{red2}$  signal does not shift



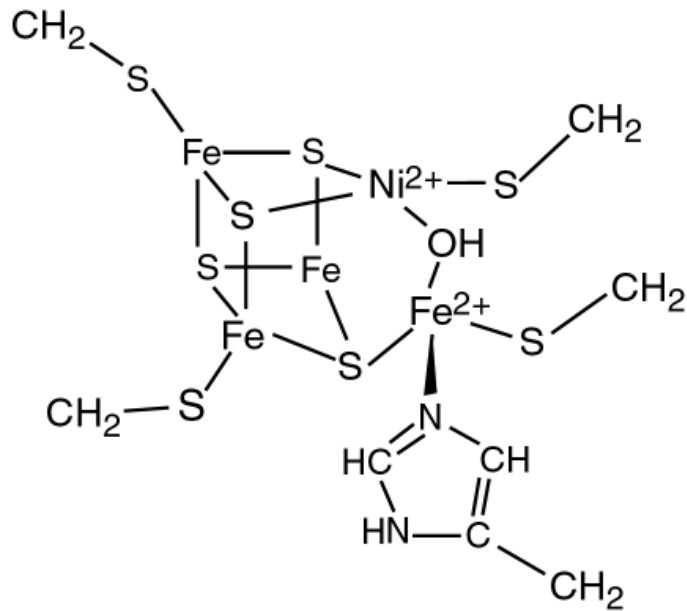
# Implication for Substrate Binding to C-cluster

Effect of HS<sup>-</sup> on activity and EPR similar to those of CN<sup>-</sup>

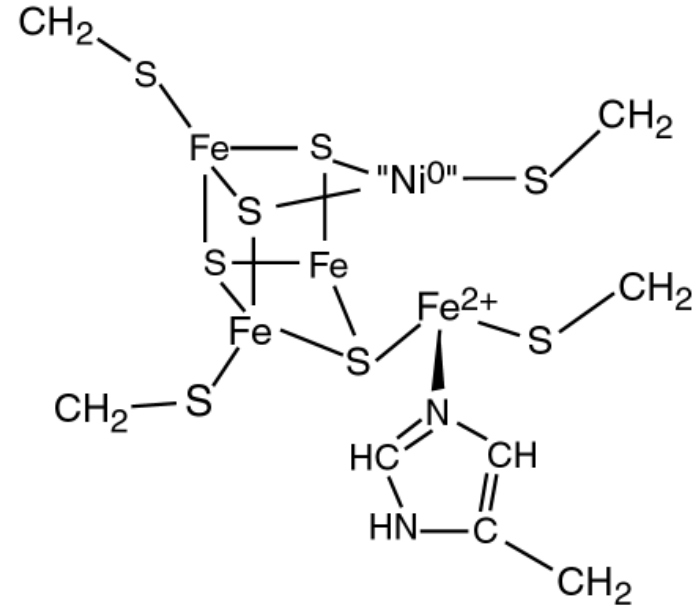
- Partial Inhibition
- Binds C<sub>red1</sub> not C<sub>red2</sub>
- CO incubation reactivates

Similarity to substrate HO<sup>-</sup> (ENDOR shows binding to C<sub>red1</sub> not C<sub>red2</sub>)

Proposal: Substrate HO<sup>-</sup> binds like HS<sup>-</sup> --- bridging between [Ni and Fe<sub>a</sub>] in C<sub>red1</sub> only

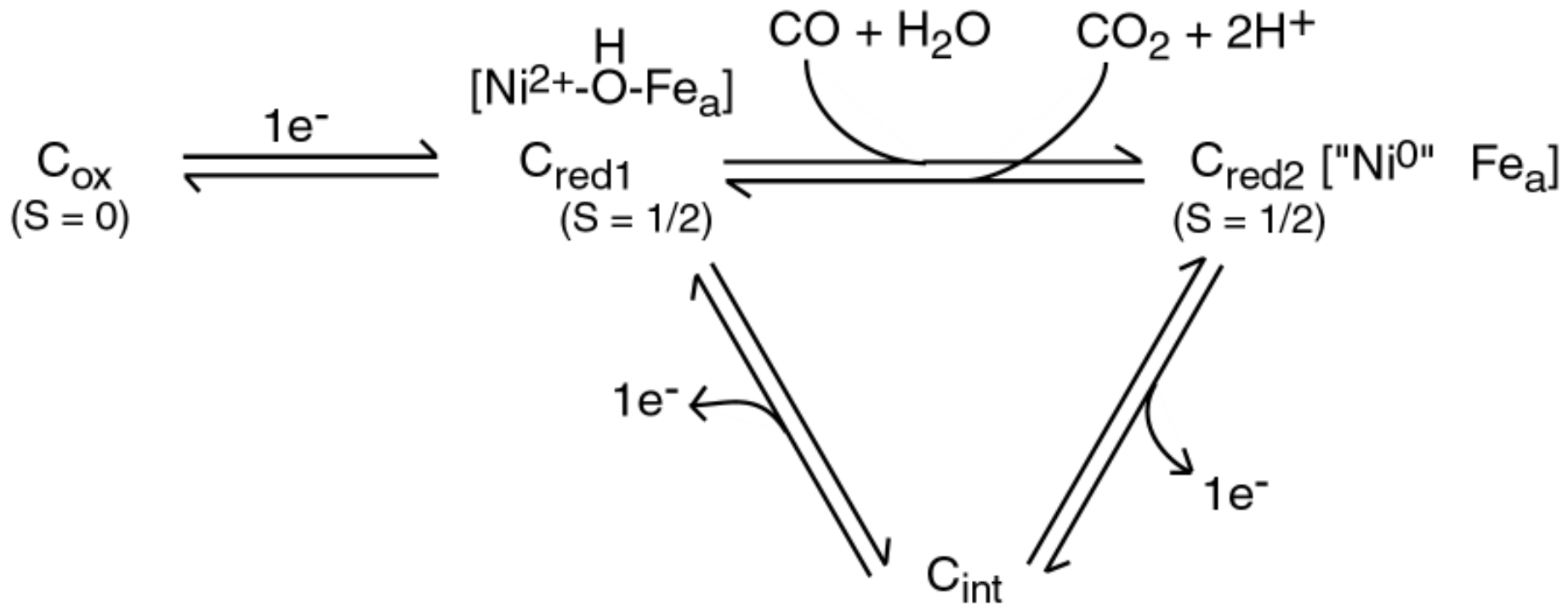


C<sub>red1</sub>



C<sub>red2</sub>

# Model of Sulfide Inhibition

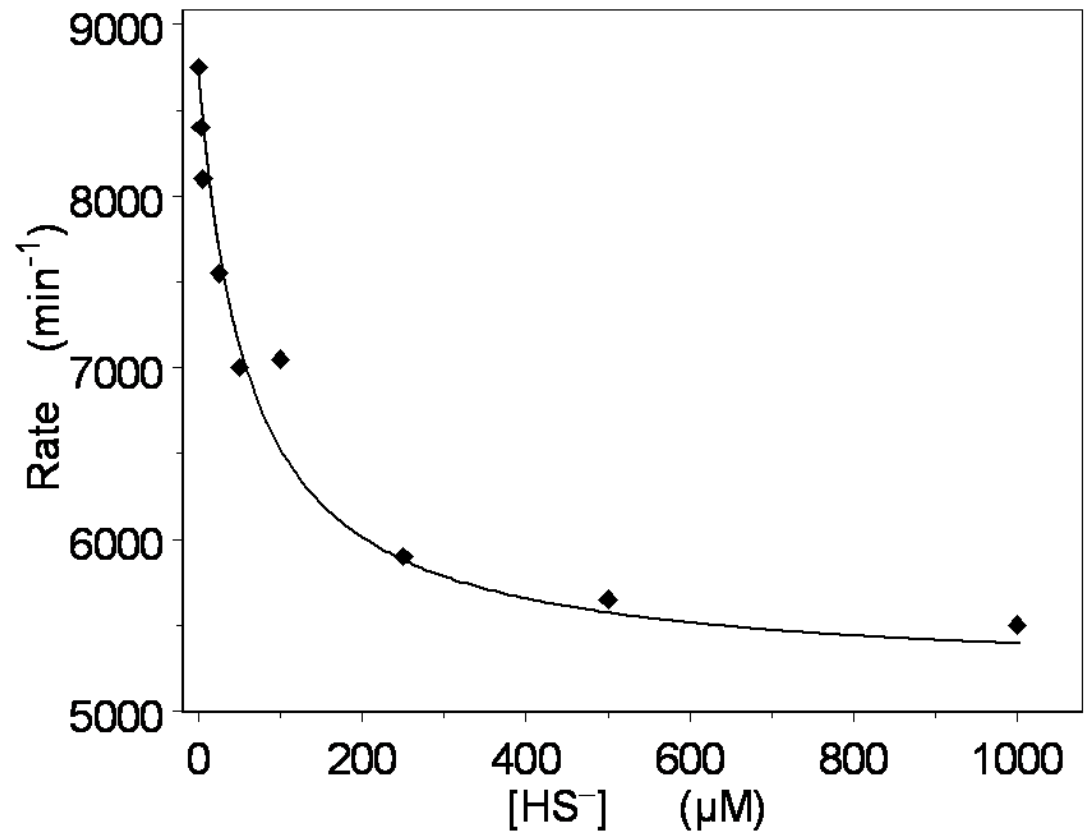


# CO Oxidation Activity of CODH<sub>Mt</sub> with HS<sup>-</sup>

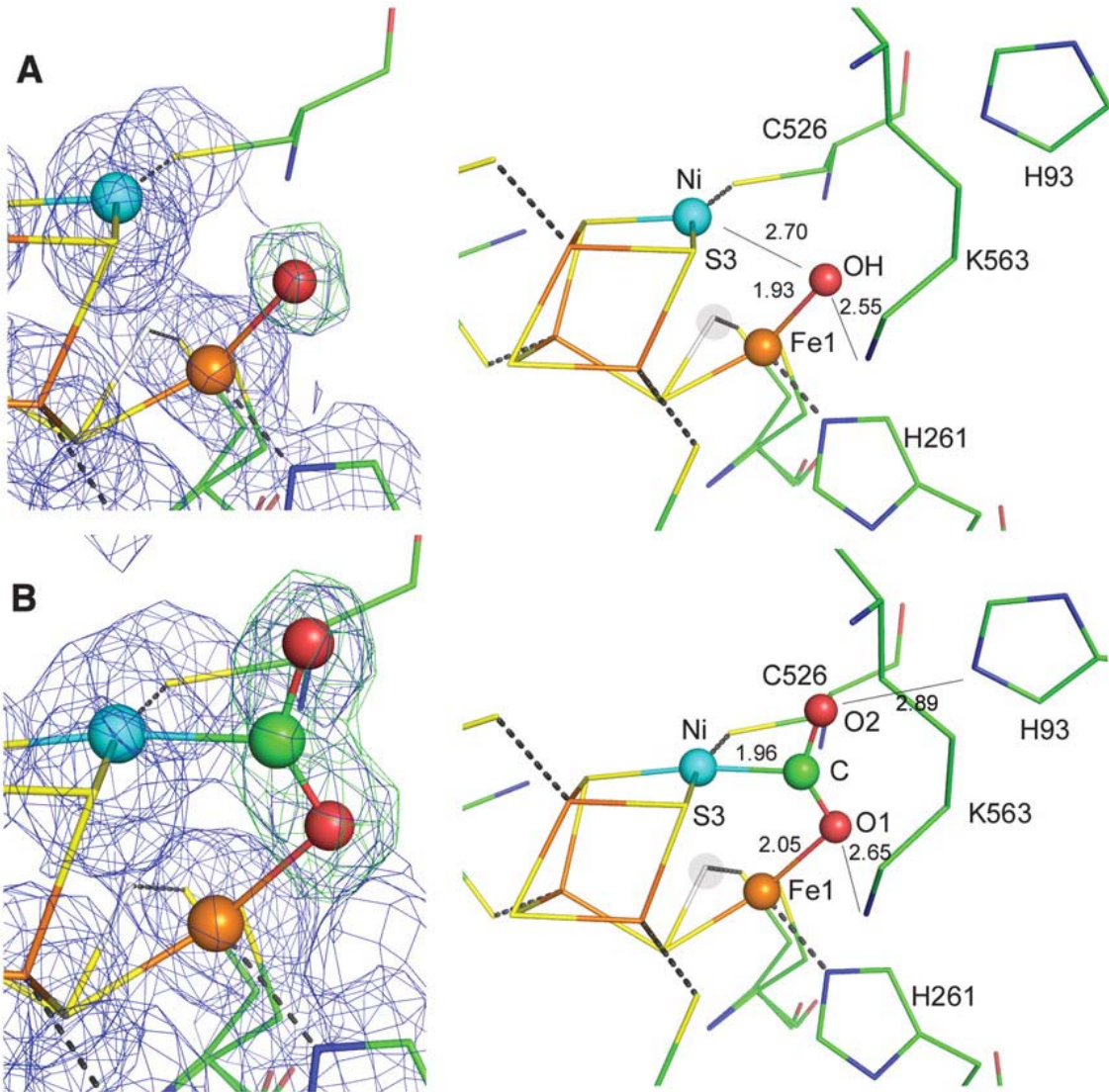
From the model we can derive the rate equation...

$$v = \frac{8750 K_I + 5190[HS^-]}{K_I + [HS^-]}$$

Using  $K_I = 60 \mu\text{M}$ ...



# Jeoung and Dobbek; *Science* 2007: Structure of the CO<sub>2</sub>-bound intermediate



Observed:

- Bridging OH in C<sub>red1</sub> state
- CO<sub>2</sub> Intermediate
- No bridging Sulfide!

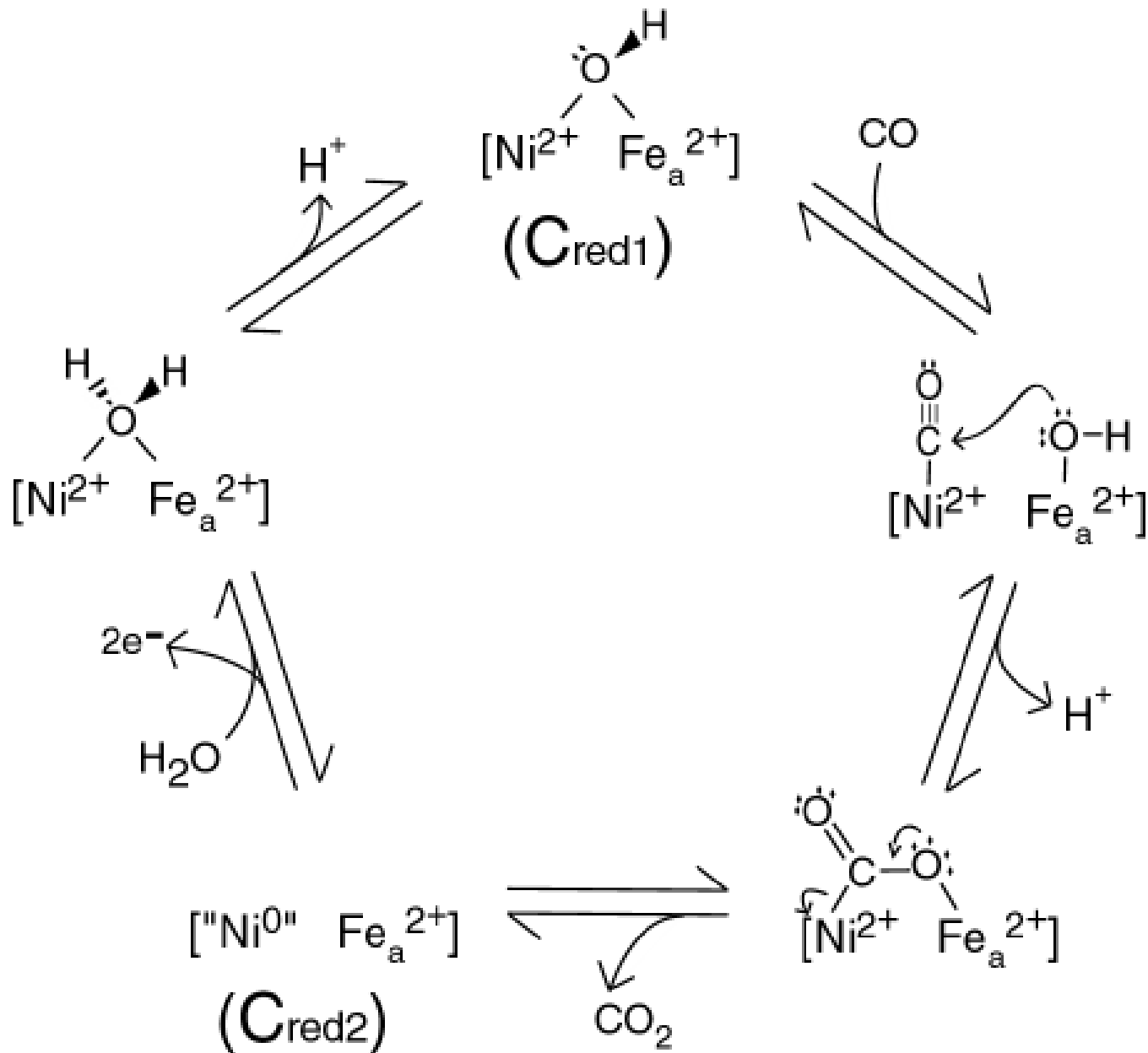
They Conclude...

“...the S2 ligand between Ni and Fe1 is absent in catalytically competent enzyme”

“The structure-based mechanism outlined agrees in all central aspects with the bimetallic mechanism proposed on the basis of EPR, ENDOR, and Mössbauer spectroscopy.”



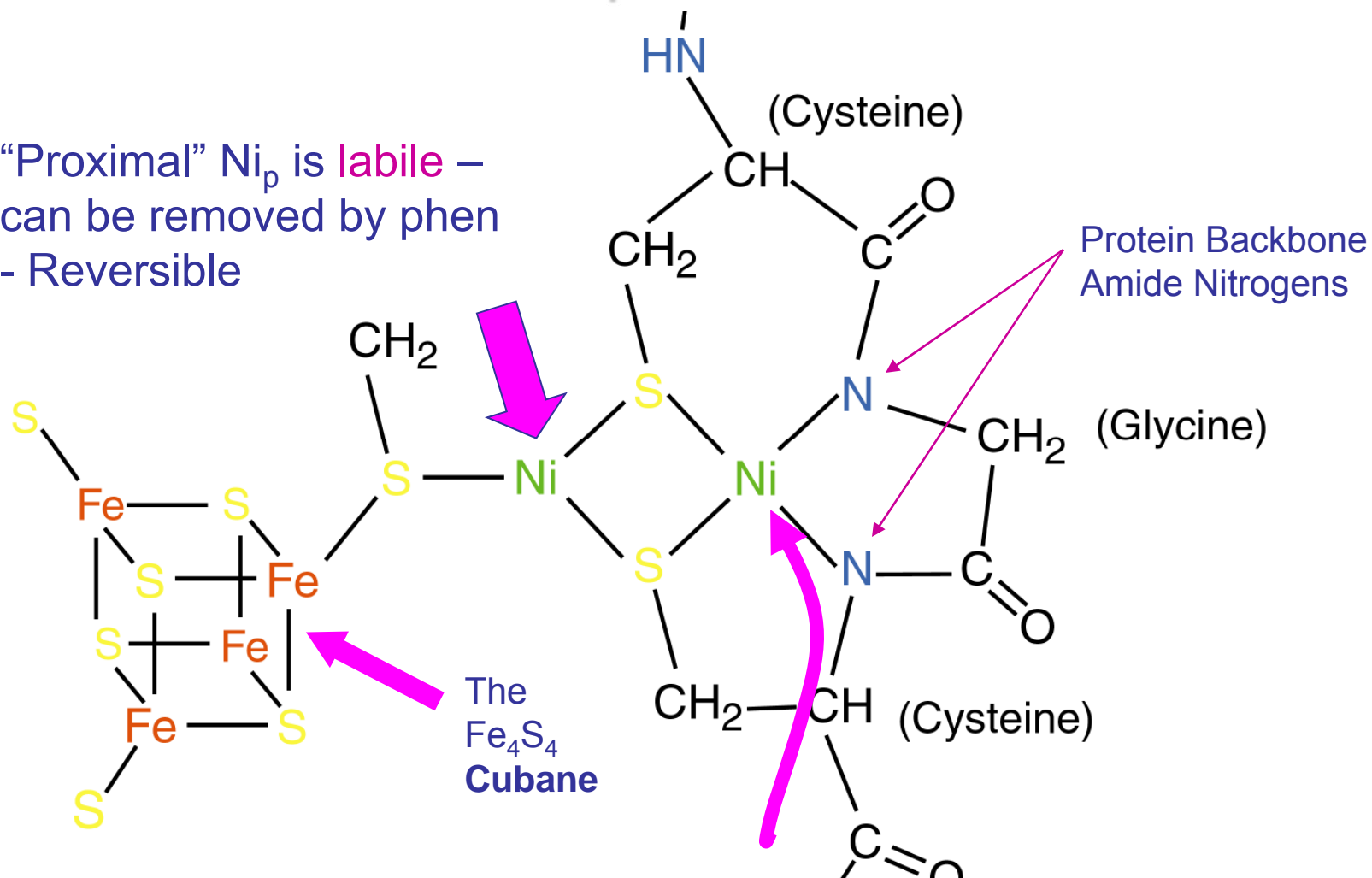
# CODH Catalytic Mechanism



# Mechanism of the ACS Reaction: Role of the Tunnel

# A-Cluster in Open Conformation

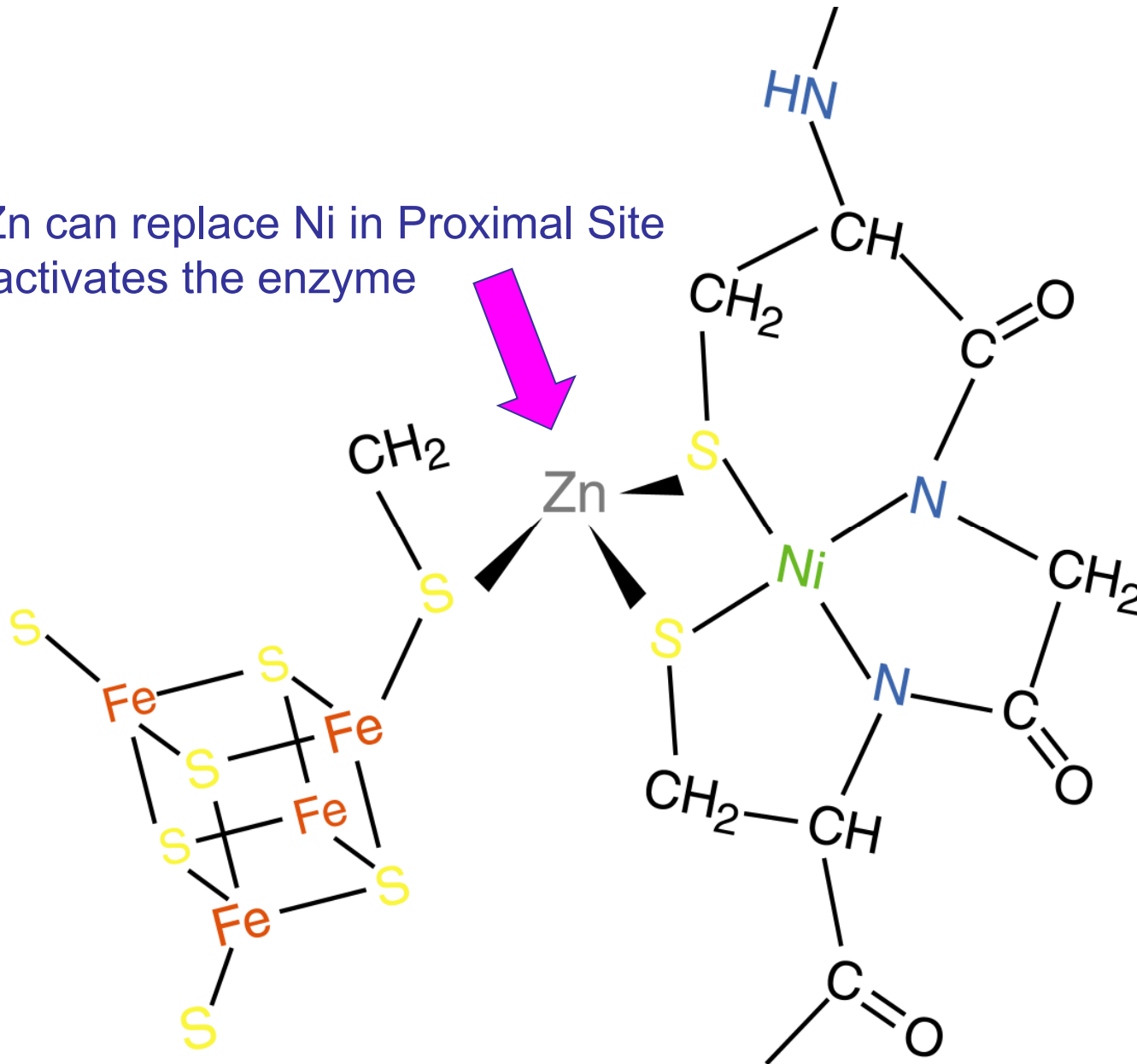
“Proximal”  $Ni_p$  is labile –  
can be removed by phen  
- Reversible



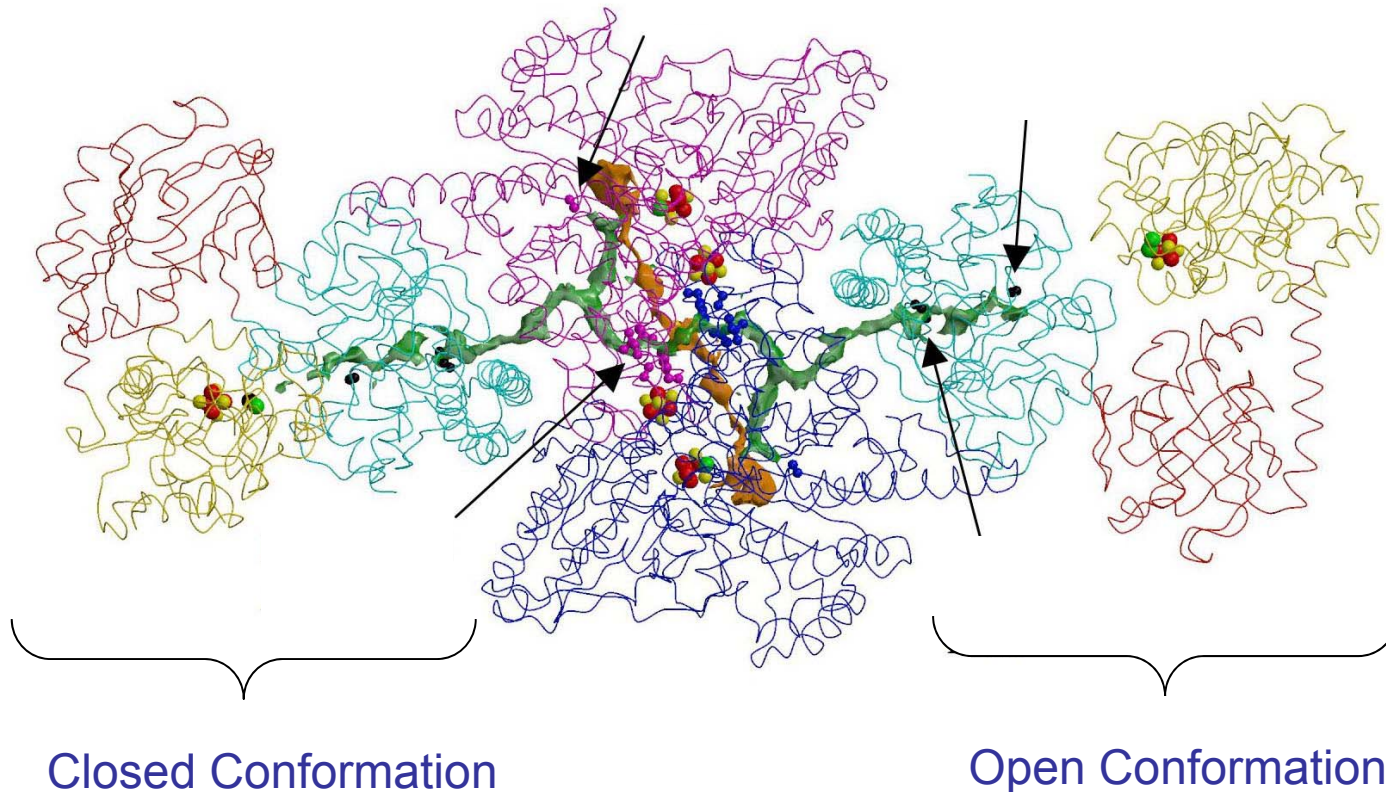
Distal Site,  
square planar – remains  $Ni^{2+}$

# A-cluster in Closed Conformation

Cu or Zn can replace Ni in Proximal Site  
This inactivates the enzyme



# Tunnel Network in ACS/CODH

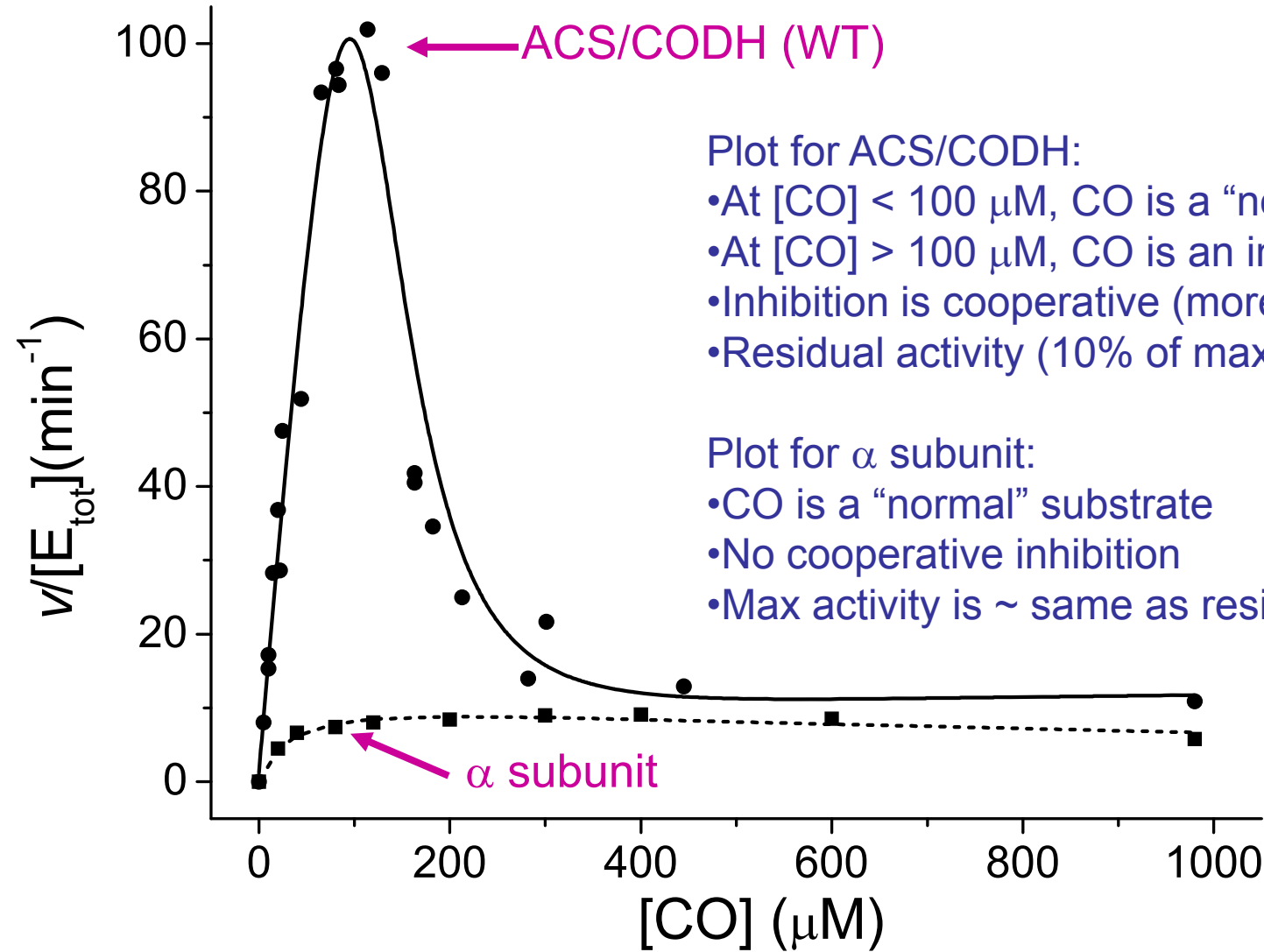


Hydrophobic Tunnel connects A- and C-clusters as well as the two C-clusters

In open conformation, tunnel to A-cluster is blocked

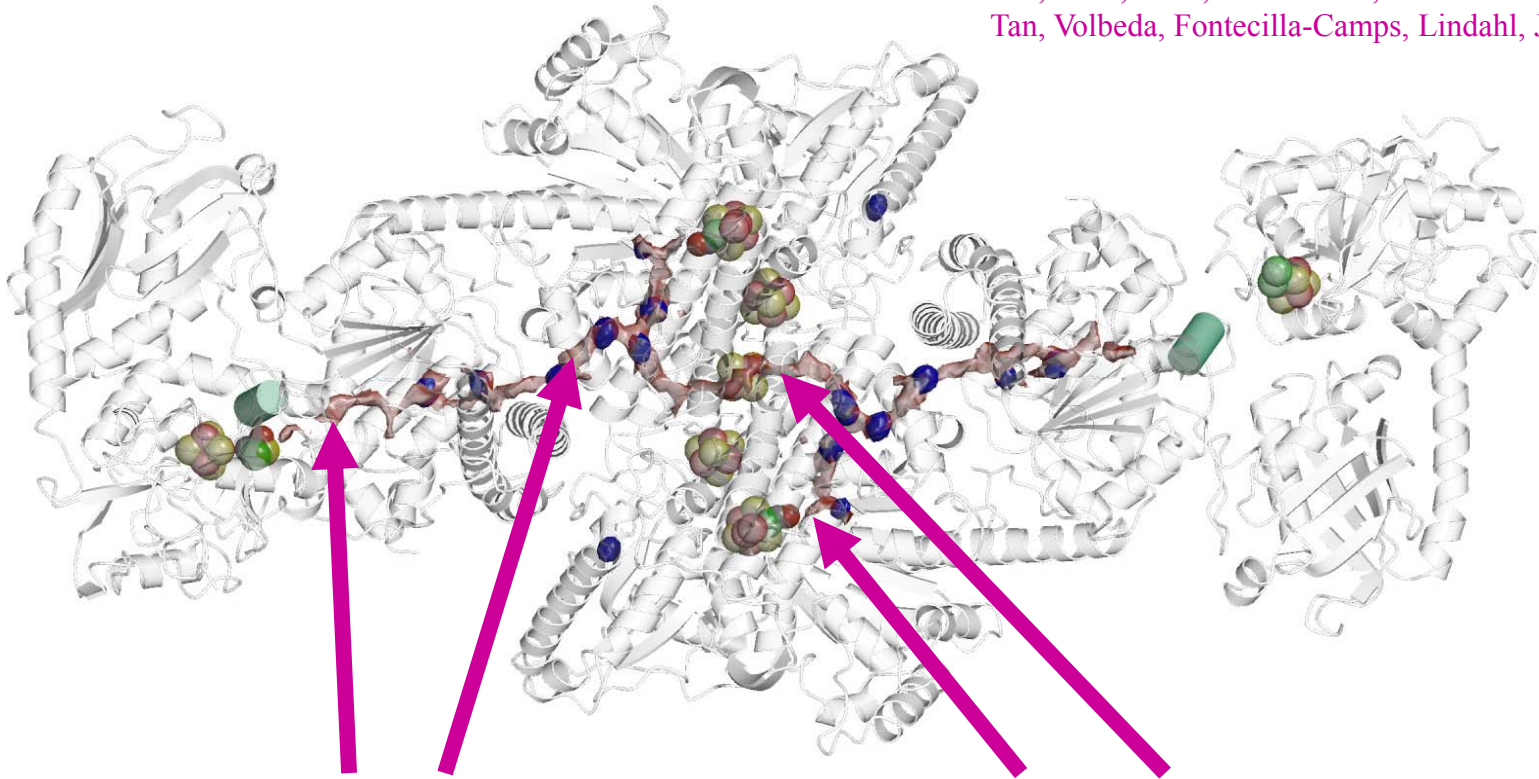
Tunnel controls delivery of CO to A-cluster

# ACS Activity vs [CO]



# Effect of Blocking Tunnel

Tan, Loke, Fitch, and Lindahl, *J. Am. Chem. Soc.* 2005  
Tan, Volbeda, Fontecilla-Camps, Lindahl, *JBIC* 2006



Blockage between A and C-clusters:  
CODH activity unaffected  
ACS activity (using CO<sub>2</sub>) ~ 0  
ACS activity using CO – residual only  
No CO-cooperative inhibition

Blockage between C and C-clusters:  
Same as AC mutants but  
CODH activity ~ 5% of WT  
CO/CO<sub>2</sub> may enter at beta:beta  
interface



# Two Migration Pathways for CO used in ACS Catalysis

## Tunnel Pathway:

**CO** enters at the  $\beta\beta$  interface and migrates to the A-cluster

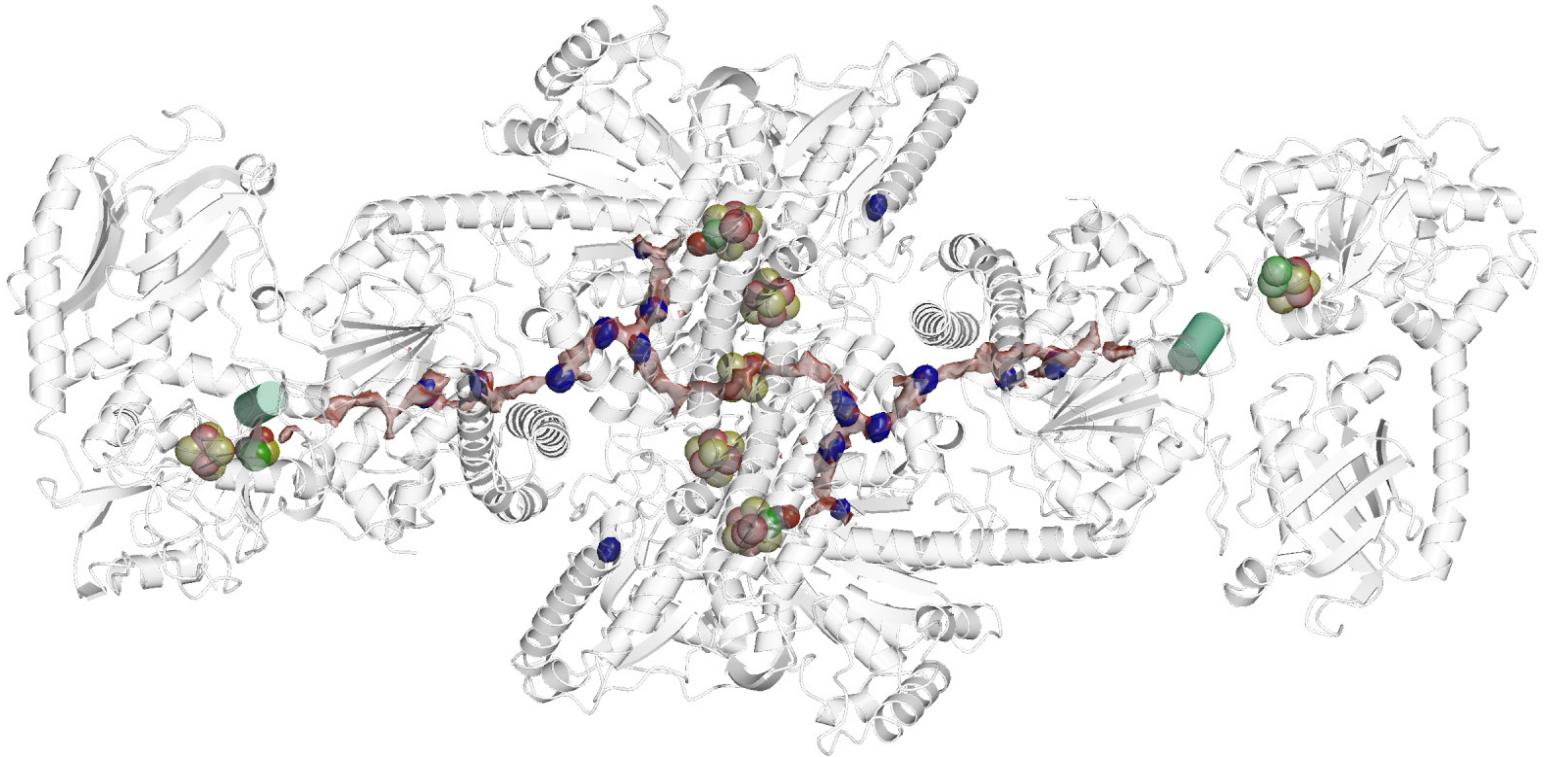
Responsible for majority activity and CO cooperative inhibition

## Direct-binding Pathway:

**CO** from solvent binds  $Ni_p$  of the A-cluster directly.

Responsible for the residual activity

Not associated with CO cooperative inhibition





# Conclusions

- The tunnel delivers CO/CO<sub>2</sub> to the active sites; delivery is regulated by protein conformational change
- The tunnel region between A- and C-clusters is exclusively used for ACS reaction, not CODH reaction
- The tunnel region between the two C-clusters participates in the CODH reaction (and indirectly in the ACS reaction)
- CO/CO<sub>2</sub> may enter/exit the enzyme at the β-β interface
- The tunnel is involved in the cooperative inhibition by CO
- CO used in “majority” activity approaches Ni<sub>p</sub> via tunnel
- CO used in residual activity approaches Ni<sub>p</sub> via solvent

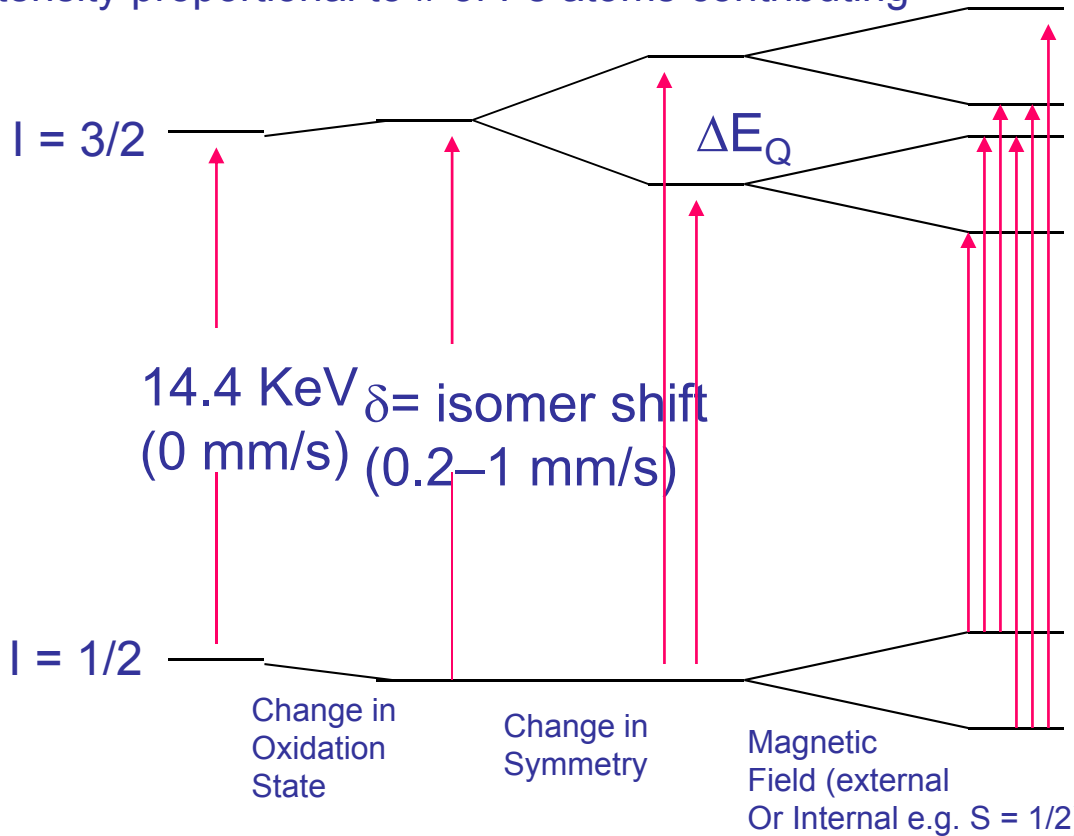
# Mechanism of the ACS Reaction: Electronic Configuration of the A-cluster

# Mössbauer Spectroscopy (Nuclear $\gamma$ -Ray Resonance)

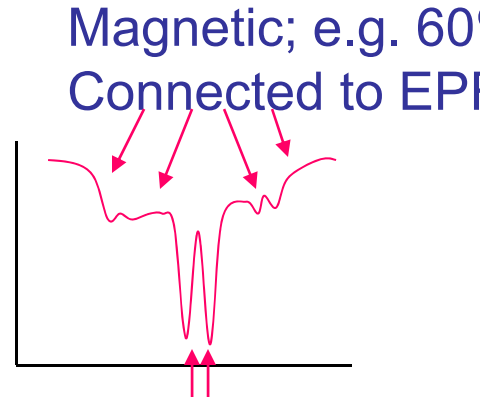
Useful for  $^{57}\text{Fe}$  ( $I = 1/2$ ) Systems

All electronic and magnetic states observed (no "Mössbauer-Silent Fe")

Intensity proportional to # of Fe atoms contributing

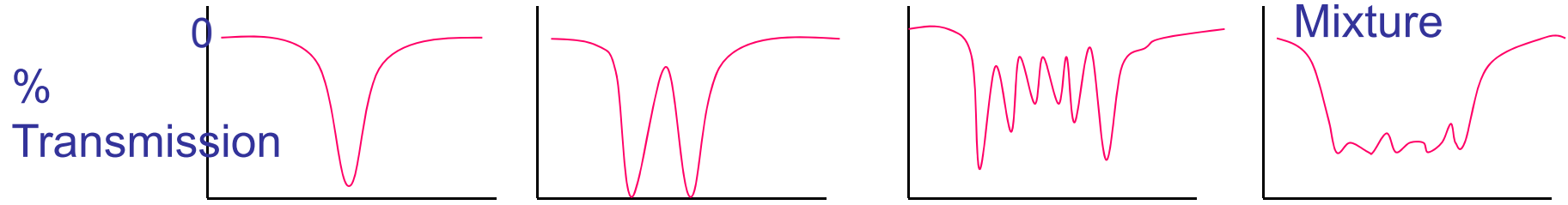


14.4 KeV  $\delta =$  isomer shift  
(0 mm/s) (0.2–1 mm/s)



40% Quadrupole Doublet

Rhombic symme  
Or Magnetic  
Mixture



Energy (mm/s) "Quadrupole Doublet" "Magnetic"

# Redox States of the A-cluster

## $A_{ox}$ State

Obtained when potential  $> -500$  mV vs. NHE

No EPR Signals (i.e. not  $Ni^{1+}$  or  $Ni^{3+}$ );  $Ni_p$  is  $\underline{2+}$

Mössbauer shows quadrupole doublet typical of  $S = 0$   $[Fe_4S_4]^{2+}$

Electronic Assignment:  $A_{ox} = \{[Fe_4S_4]^{2+} Ni_p^{2+}\}$   
 $S_{system} = 0$

## $CH_3-A_{ox}$ : Methylated State

Mössbauer shows  $S = 0$   $[Fe_4S_4]^{2+}$

EPR silent (i.e. not  $Ni^{1+}$  or  $Ni^{3+}$ ) i.e.  $Ni_p^{2+}$

Many examples of  $Ni^{2+}-CH_3$  model complexes

$Ni_p$  is required for methyl transfer; methylation blocks  $Ni_p$  removal

Electronic Assignment:  $CH_3-A_{ox} = \{[Fe_4S_4]^{2+} Ni_p^{2+}-CH_3\}$   
 $S_{system} = 0$

# $A_{\text{red}}\text{-CO}$ State



NiFeC EPR signal ( $g_{\perp} = 2.08$ ,  $g_{\parallel} = 2.03$ )

Mössbauer shows magnetic  $[\text{Fe}_4\text{S}_4]^{2+}$

Electronic Assignment:  $A_{\text{red}}\text{-CO} = \{[\text{Fe}_4\text{S}_4]^{2+} \text{Ni}_p^{1+}\text{-CO}\}$

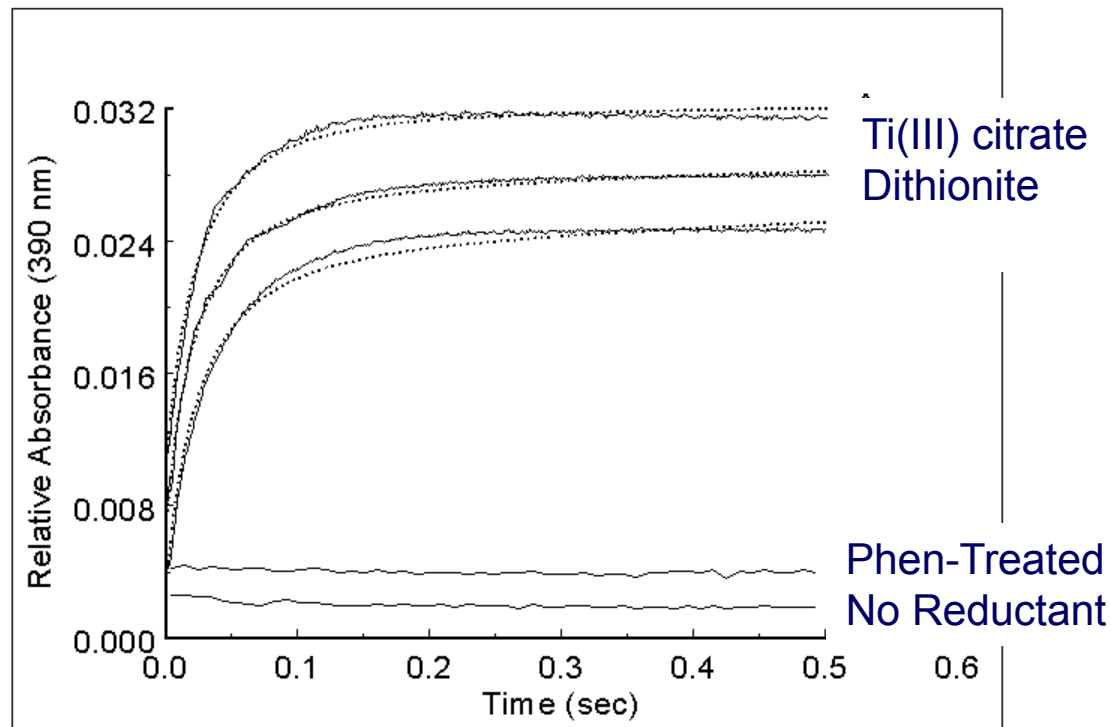
Catalytic Intermediate or Inhibitor of catalysis?



# $A_{\text{red-Act}}$ : The Reductively Activated State

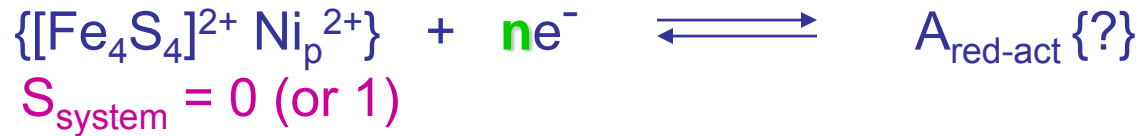


But in presence of low-potential reductant (e.g. dithionite,  $\text{Ti}^{3+}$  citrate)...

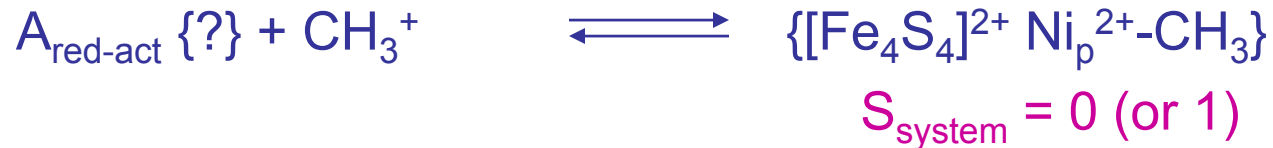


# Mechanistic Implications

Step 1: Reductive Activation:



Step 2: Methylation:



Implications:

$S_{\text{system}}$  for  $\text{A}_{\text{red-act}} = 0$  (or 1, 2 etc)

and

$n = 2$

Steps 3 and 4: CO Insertion, CoASH attack:



# The Heterogeneity Puzzle:

For all labs, enzymes, preps...

Spin concentration of NiFeC EPR signal 0.2 - 0.3 spin/ $\alpha$

Quantification of methyl group transfer: 0.3 – 0.5 Me/ $\alpha$

Quantification of labile Ni removed and inserted: ~ 0.2 Ni/ $\alpha$

Mössbauer of  $A_{\text{red}}\text{-CO}$ :

~30% is  $S = \frac{1}{2} [\text{Fe}_4\text{S}_4]^{2+}$ ; ~70% is  $S = 0 [\text{Fe}_4\text{S}_4]^{2+}$

## What does this mean?

~ 30% functional  $\alpha$  subunits

- Catalytically active

- Labile Ni

- capable of NiFeC EPR

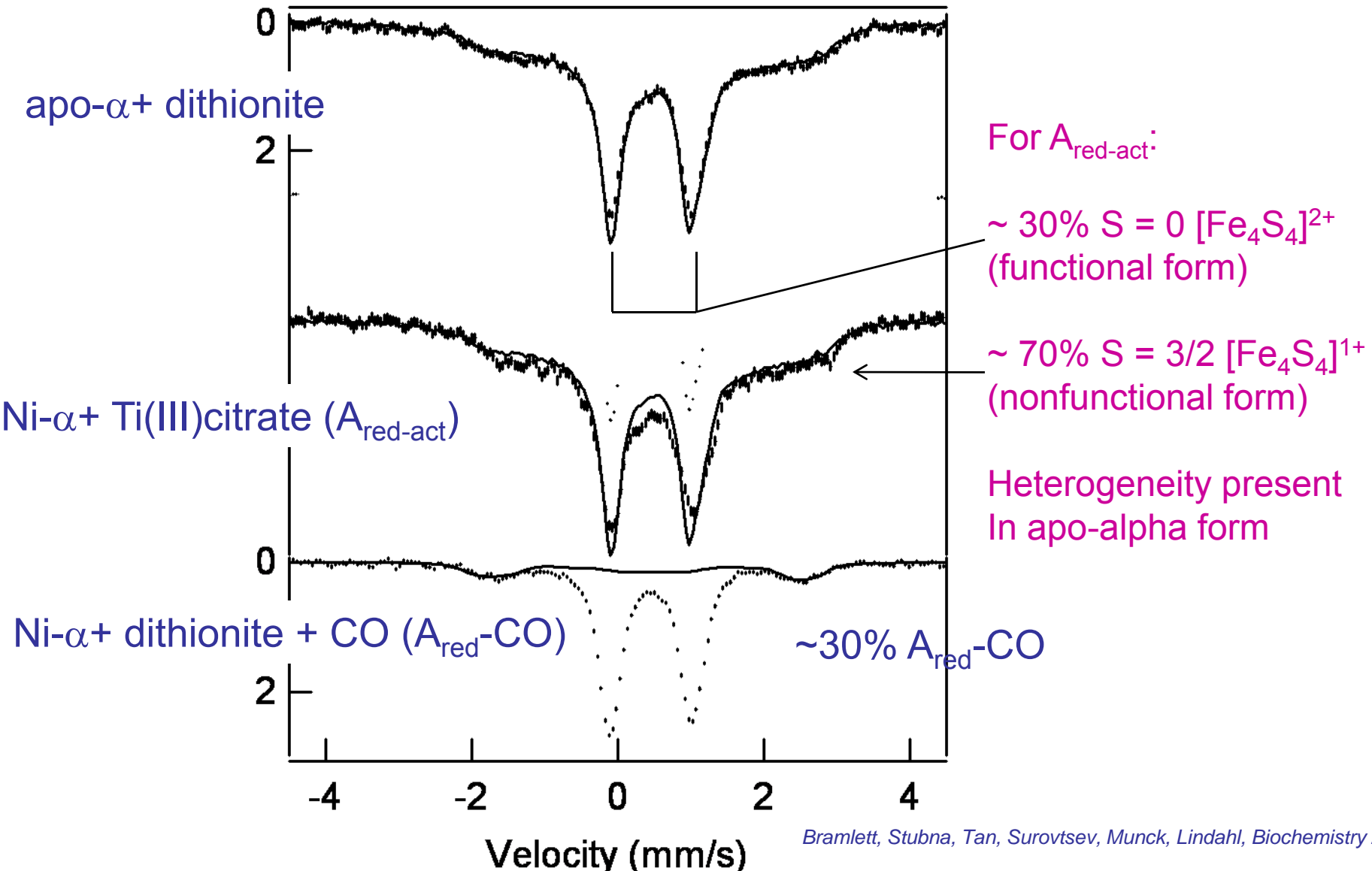
~70% nonfunctional  $\alpha$  subunits

- \* inactive

- \* no labile Ni (Zn? Cu?)



# 4.2 K Mössbauer Spectra of $\alpha$ ; ca. 2006



# Conclusions Regarding $A_{\text{red-act}}$

70% Component represents nonfunctional A-clusters



30% Component represents functional A-clusters



## Objections to a $\text{Ni}_p^0$ State

Ligands are inconsistent (not phosphines)

But bridging thiolates might mimic phosphines...

Shouldn't a zero-valent Ni reduce  $[\text{Fe}_4\text{S}_4]^{2+}$ ?

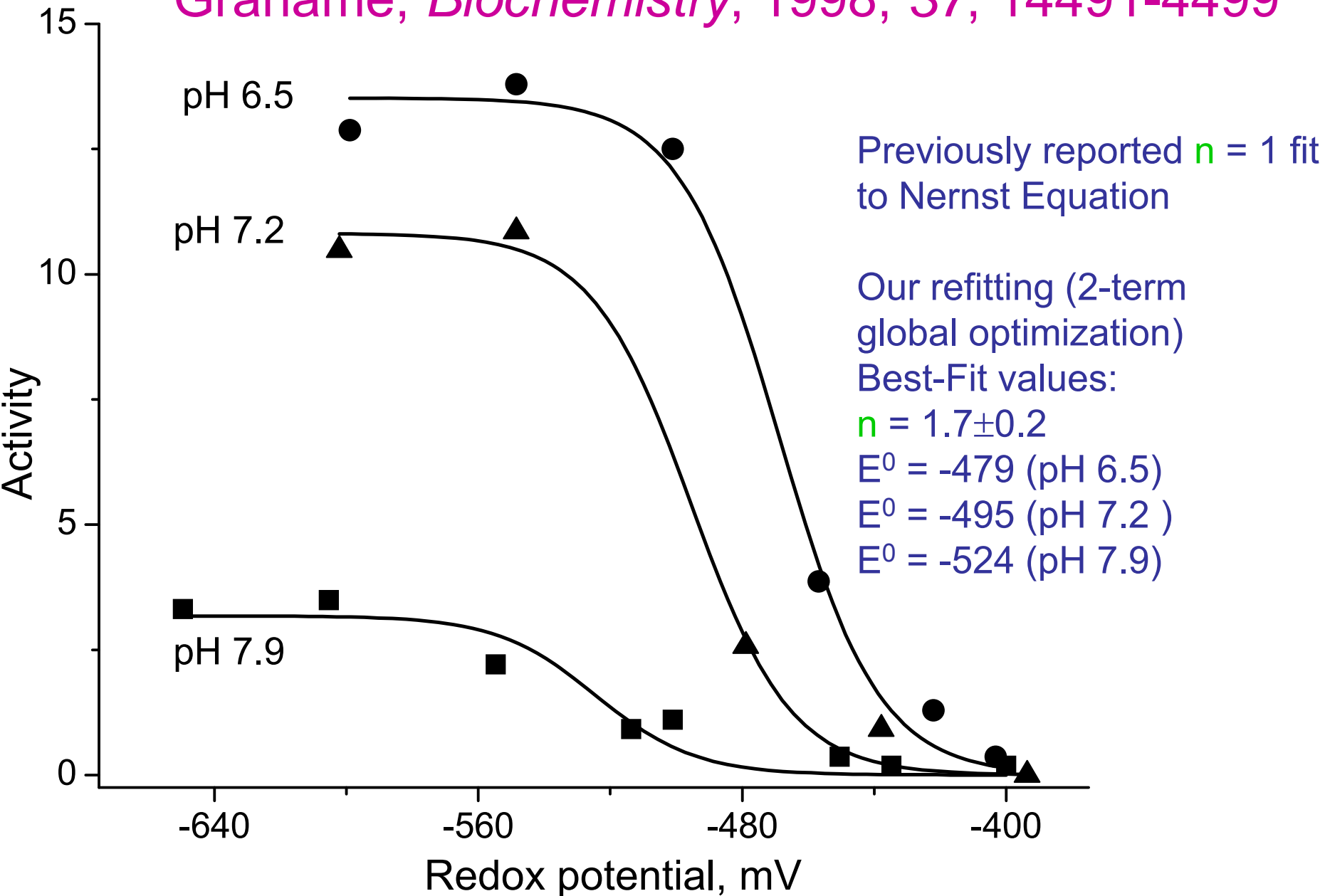
But Mössbauer study suggests  $E^0_{\text{cube}^{2+/1+}} < -800 \text{ mV}$

Two DFT computational studies disfavor  $\{[\text{Fe}_4\text{S}_4]^{2+} \text{Ni}_p^0\}$  state.

But  $E^0$  is very sensitive to environment  $\therefore$  difficult to model accurately

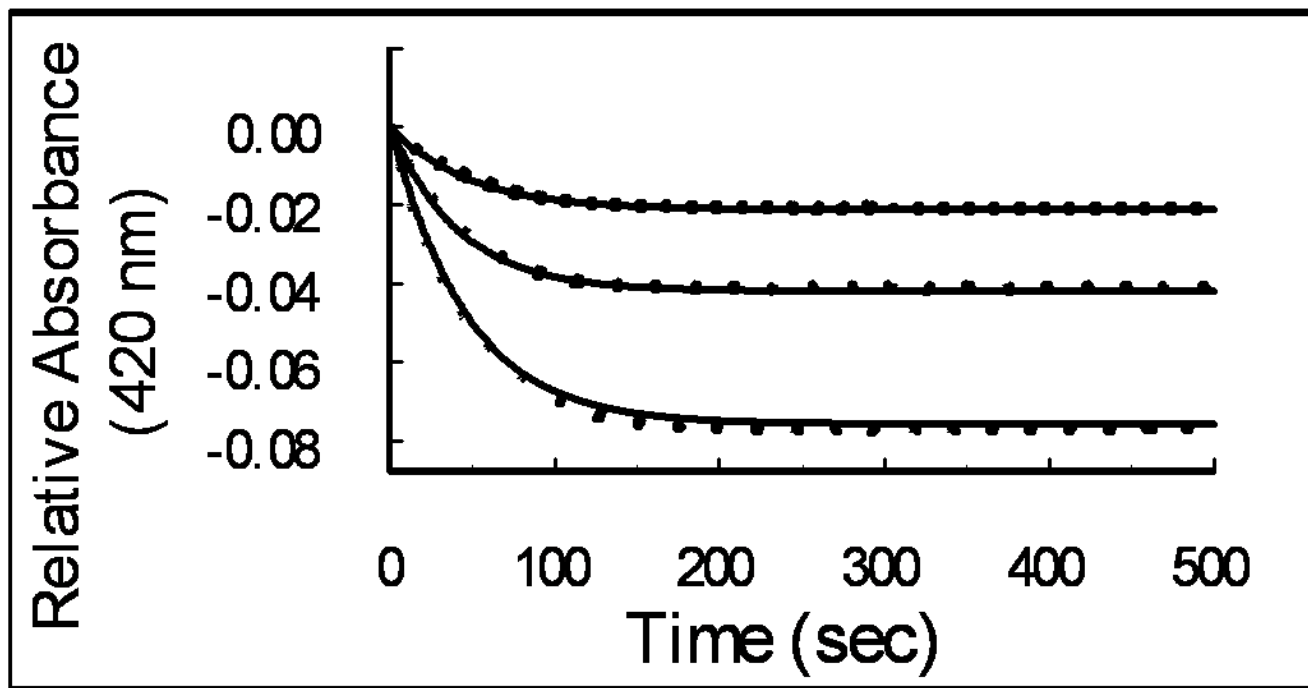
Two redox titrations suggest  $n = 1$  (not  $n = 2$ ) for reductive activation

# Refitting of Data of Bhaskar, DeMoll, and Grahame, *Biochemistry*, 1998, 37, 14491-4499



Shouldn't the  $\text{Fe}_4\text{S}_4$  be reduced in  $A_{\text{red-act}}$ ??

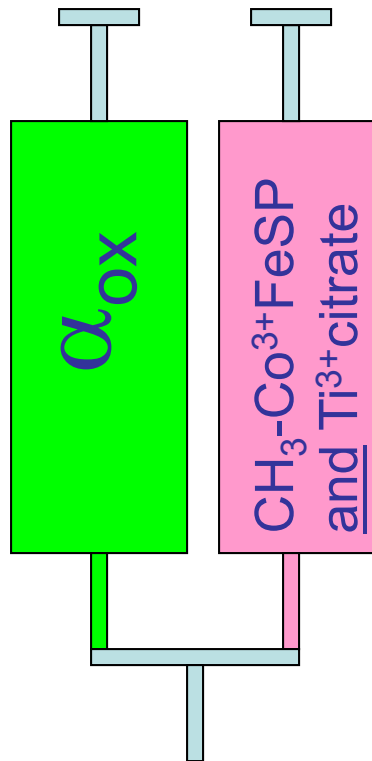
Let's monitor reduction of  $[\text{Fe}_4\text{S}_4]^{2+}$  cubane by  $\text{Ti}^{3+}$  citrate...



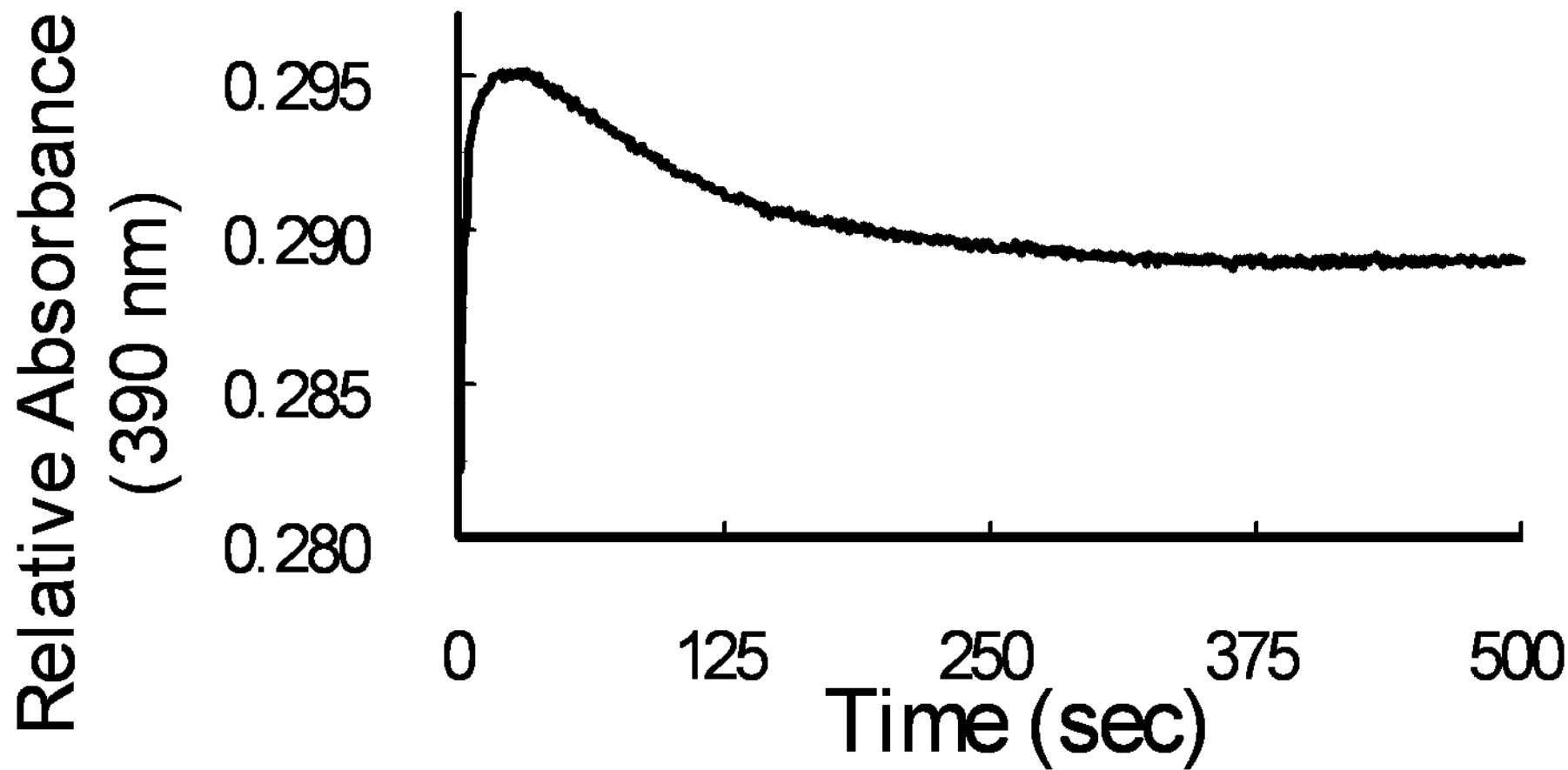
Reduction is slow relative to methylation rate  
(Nonfunctional form is probably becoming reduced)

# Competition Experiment:

(Add reductant and  $\text{CH}_3\text{-Co}^{3+}\text{FeSP}$  Simultaneously to  $\alpha_{\text{ox}}$ )



Monitor at 390 nm (sensitive to both  $[\text{Fe}_4\text{S}_4]^{2+}$  reduction and  $\text{Co}^{1+}$ )

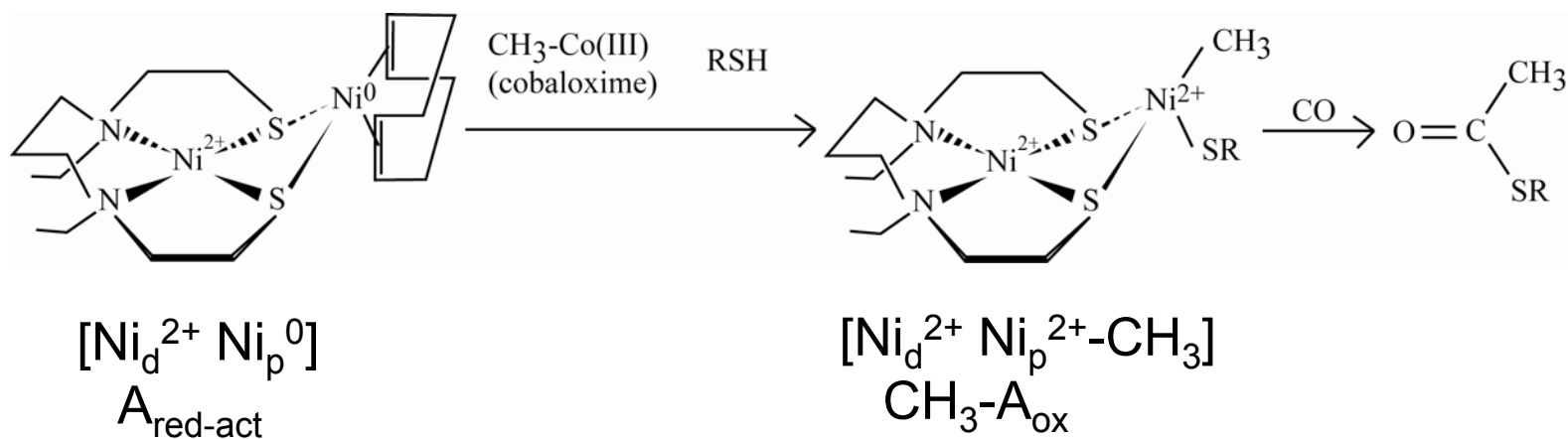


CH<sub>3</sub> transfer (and reductive activation) > 100X faster than cubane reduction

Cubane NOT reduced fast enough to be the site of reductive activation  
But heterogeneity could complicate interpretation...

# A-cluster model complexes support $n = 2$ , $Ni_p^0$ –based Mechanism

Ito, Kotera, Matsumoto, Tatsumi (PNAS, 2009)

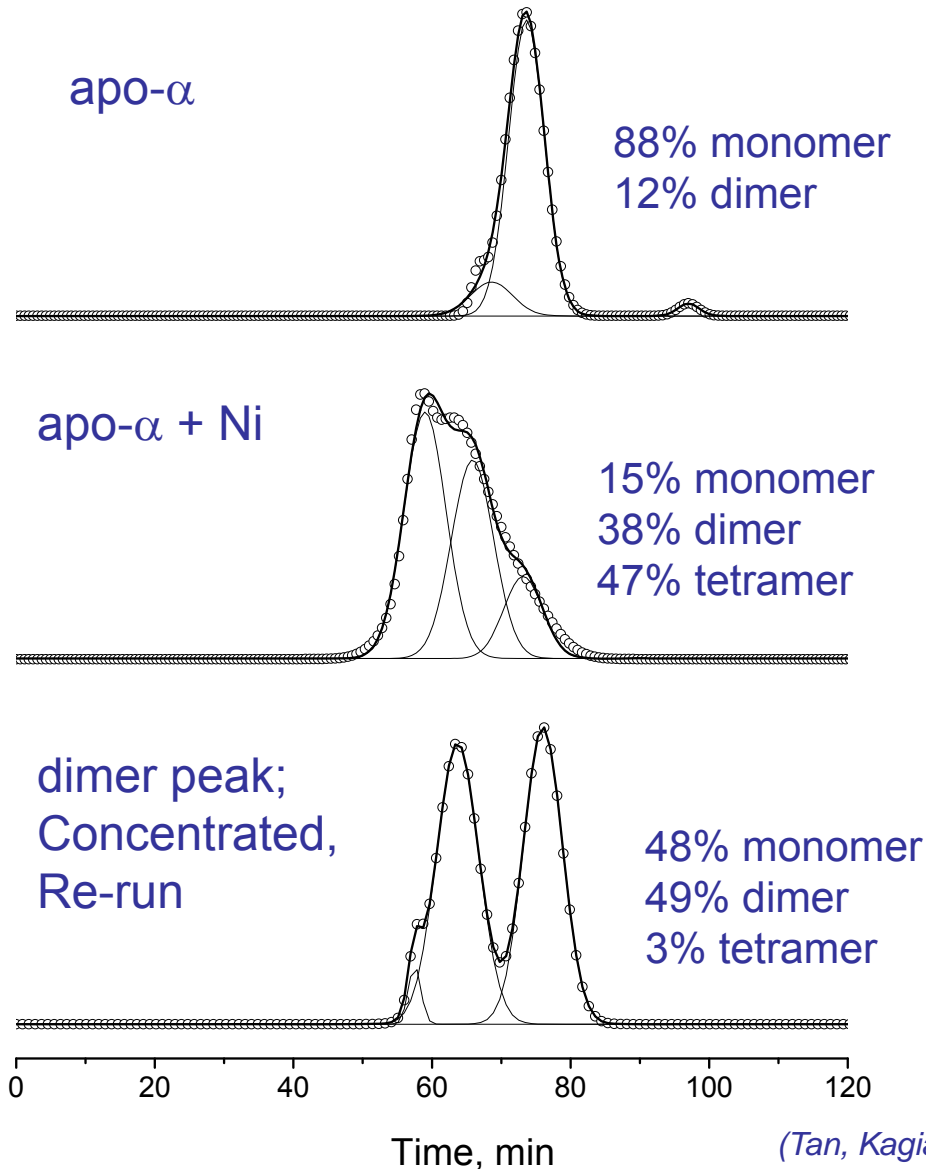


Also... Riordan, Rauchfuss, Holm, Darensbourg, Mascharak

Structurally Relevant Model Complexes display same essential chemistry!

# In 2007, we stopped adding Ni to E coli and started running $\alpha$ on FPLC

(FPLC; Superdex gel filtration)



Dimer and tetramer have Activity; not monomer

Dimer:

0.5 Me/ $\alpha$  transferred

0.4 spin/  $\alpha$  NiFeC spin intensity

Dimer is heterogeneous (one functional subunit; one nonfunctional subunit)



# Properties of Ni-activated Alpha Subunit Dimers

Methyl Group Acceptor Ability:  
Dimer accepts  $\sim 0.5 \text{ Me}/\alpha$   
Monomer  $\sim$  inactive

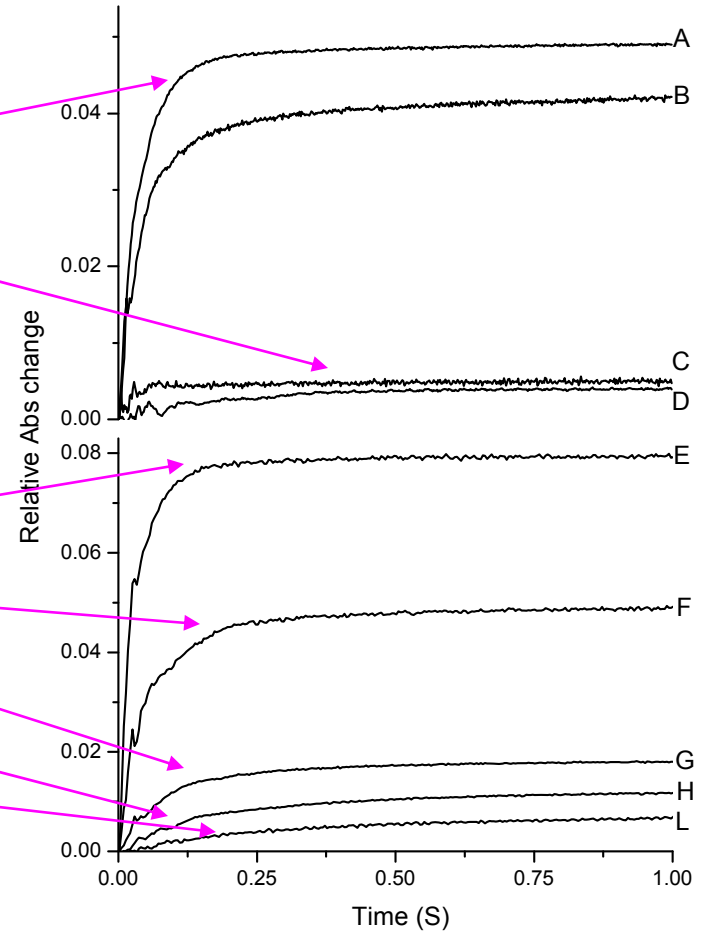
Why only  $0.5 \text{ Me}/\alpha$ ?  
Incomplete Reaction?

Change  $[\alpha]$  and see if  $\text{Me}/\alpha$  changes

- 20  $\mu\text{M}$
- 10  $\mu\text{M}$
- 5  $\mu\text{M}$
- 2.5  $\mu\text{M}$
- 1.5  $\mu\text{M}$

$\sim 0.5 \text{ Me}/\alpha$  at all  $[\alpha]$

What does this mean?



# Asymmetric Subunits in Ni-activated Dimer

EPR of reduced/CO dimer: NiFeC signal had 0.4 spin/ $\alpha$   
(EPR of monomer apo- $\alpha$  showed only residual signal)

Mössbauer Spectra of reduced/CO dimer:

40% associated with  $S = \frac{1}{2}$   $A_{\text{red}}\text{-CO}$  state

60% associated with  $S = 0$   $[\text{Fe}_4\text{S}_4]^{2+}$  inactive clusters

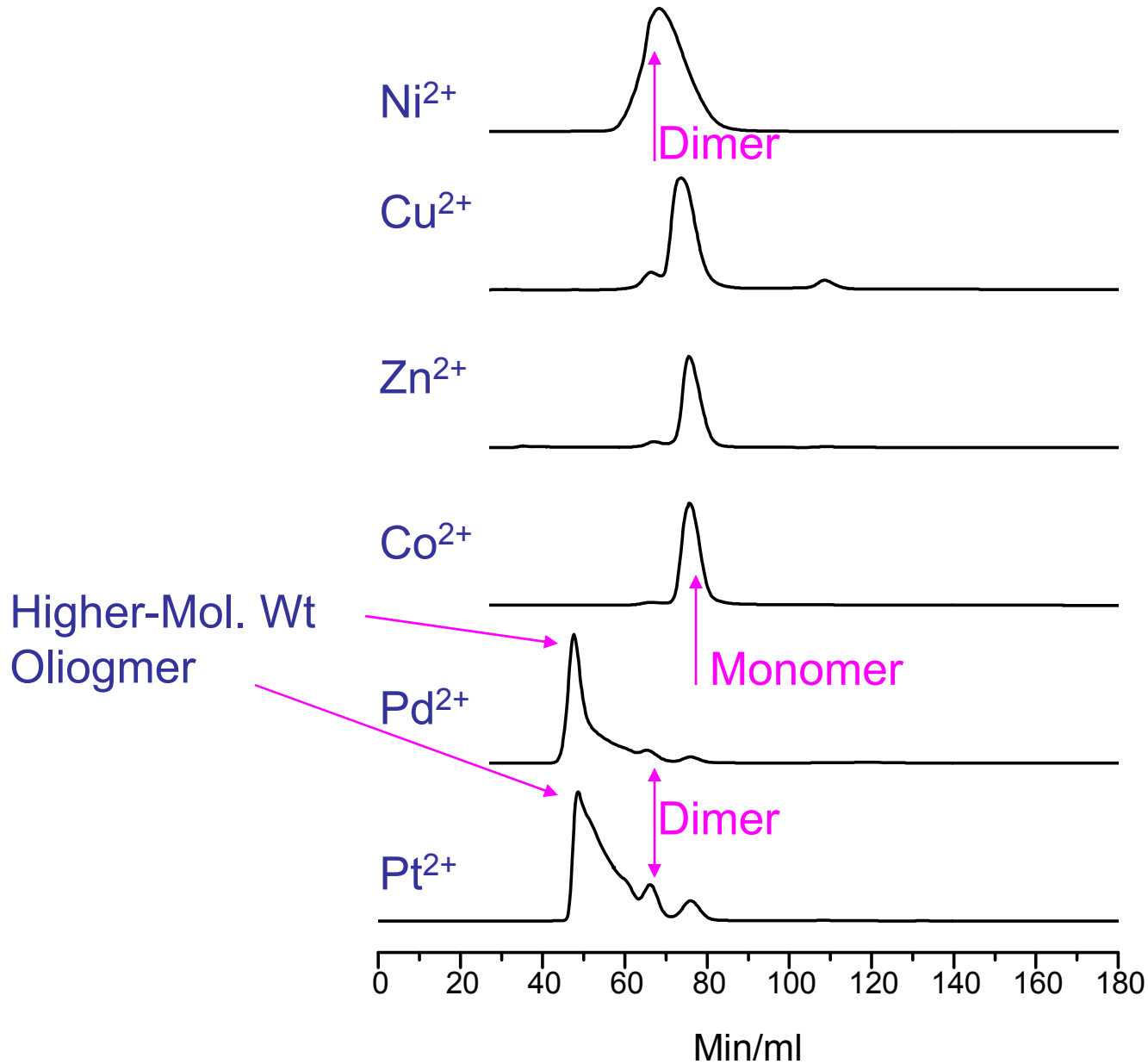
When considered with methyl group transfer quantification, (0.5 Me/ $\alpha$ )

Indicates that alpha dimers consist of **asymmetric subunits**

- \* One “catalytic” subunit (accepts Me group; exhibits NiFeC)
- \* One “structural” subunit

Could heterogeneity be functionally required??

# Is Dimerization Specific for Ni?



# Tentative Conclusions Regarding Dimerization

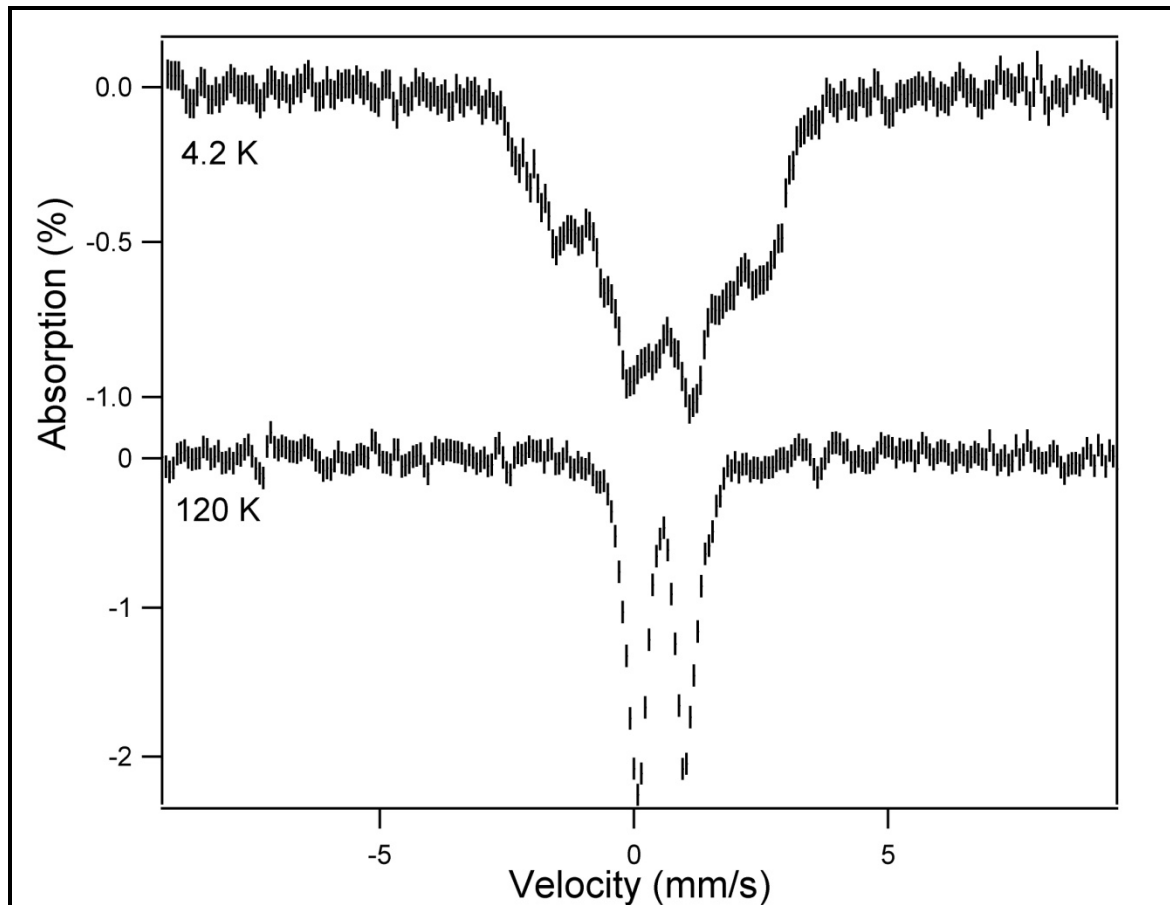
Alpha subunits dimerize when Ni binds to the proximal site of the A-cluster

Dimerization (Oligomerization) is specific for  $M^{2+}$  ions that prefer **square-planar** geometries (Ni, Pd, Pd)

This geometry at the proximal site enforces a particular subunit conformation (i.e. open) that is conducive to dimerization

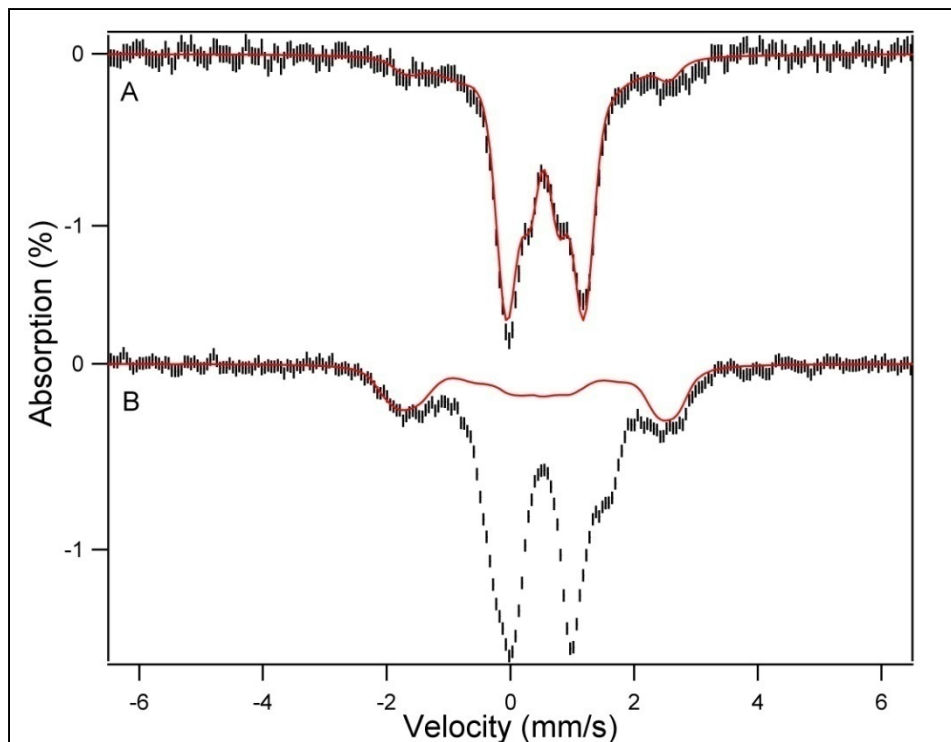
One subunit of the dimer is catalytic and in the open conformation while the other is structural and in the closed conformation

# Apo- $\alpha$ + Ti(III) citrate ca. 2008



~ 100% is  $S = 1/2$   $[\text{Fe}_4\text{S}_4]^{1+}$  cluster – no redox heterogeneity

## 4.2 K Mössbauer of $[\alpha(\text{Ni})]_2 + \text{Ti(III) Citrate}$

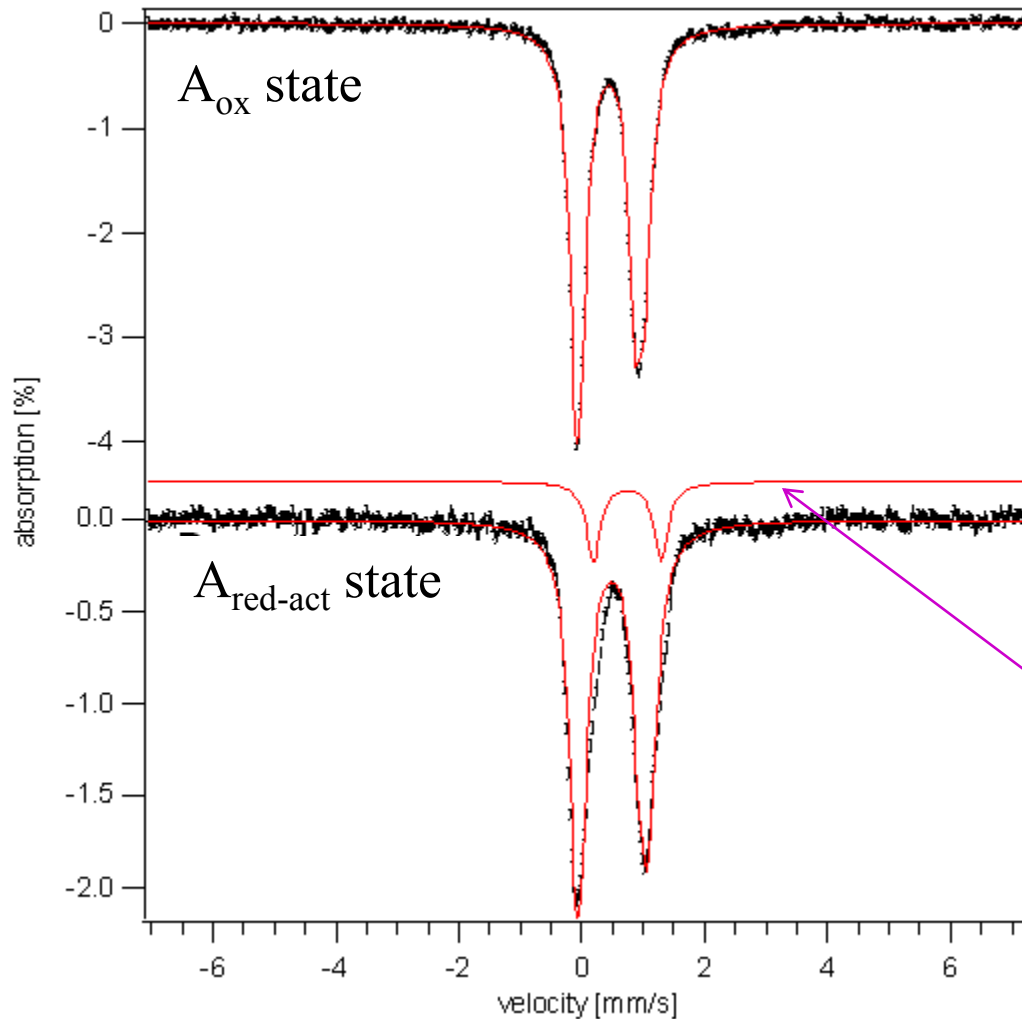


$\delta = 0.56 \text{ mm/s}$   $\Delta E_Q = 1.25 \text{ mm/s}$

$\delta = 0.55 \text{ mm/s}$   $\Delta E_Q = 0.47 \text{ mm/s}$

Coupled state proposed by Brunold and Field, based on DFT

# 4.5 K 0.05T Mössbauer of A-Cluster containing subunit from *Methanosarcina thermophila*



~100%  $S = 0$   $[\text{Fe}_4\text{S}_4]^{2+}$   
( $\delta = 0.46$  mm/s;  $\Delta E_Q = 1.1$  mm/s)

~80%  $S = 0$   $\{[\text{Fe}_4\text{S}_4]^{2+}\text{Ni}_p^{2+/0}\}$   
( $\delta = 0.46$  mm/s;  $\Delta E_Q = 1.1$  mm/s)

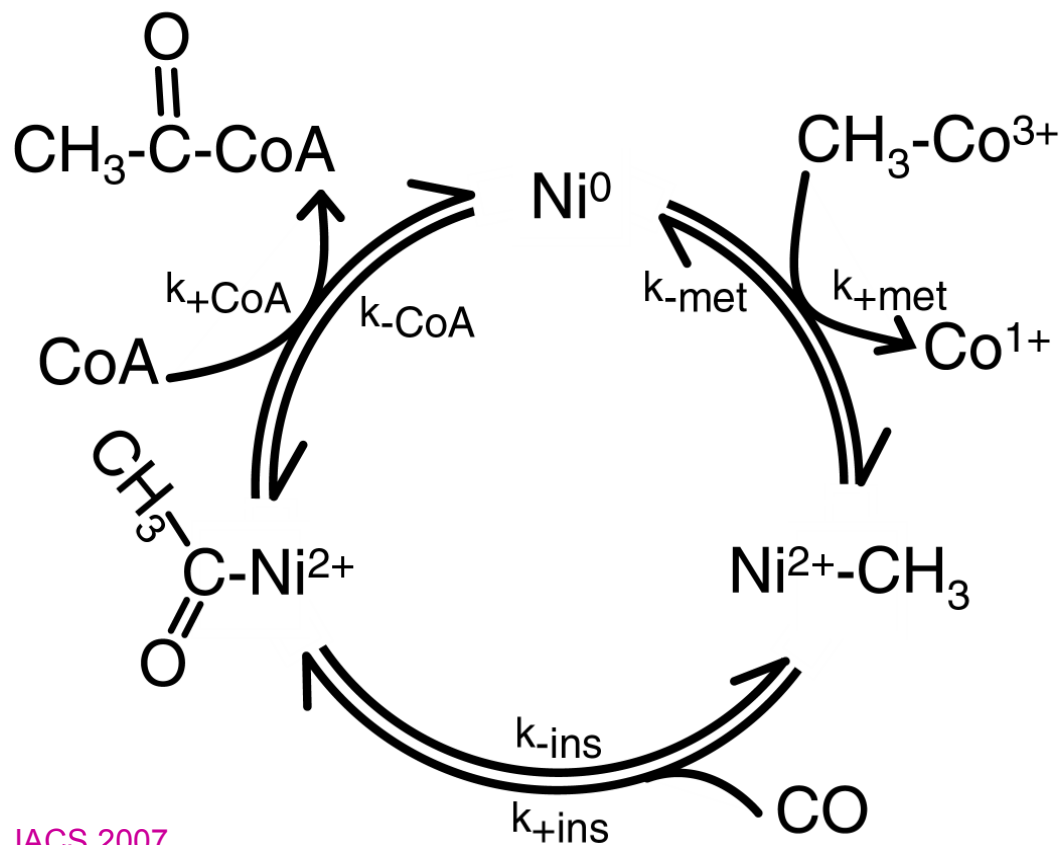
~20%  $S = 0$   $\{[\text{Fe}_4\text{S}_4]^{1+}\text{Ni}_p^{1+}\}$   
( $\delta = 0.53$  mm/s;  $\Delta E_Q = 1.1$  mm/s)

Sample was ~ 100% active  
in accepting methyl group

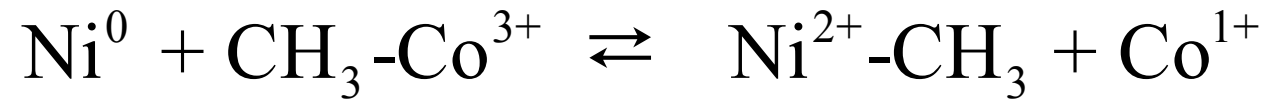
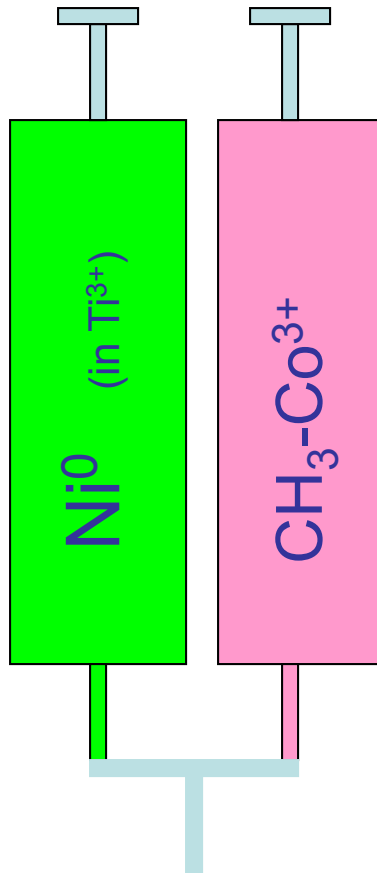
# Kinetic Model for the ACS Reaction:



# Kinetic Modeling of the Acetyl-CoA Synthesis Mechanism



# Monitoring the Kinetics of Methyl Group Transfer – Relatively Easy

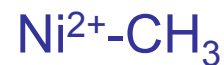
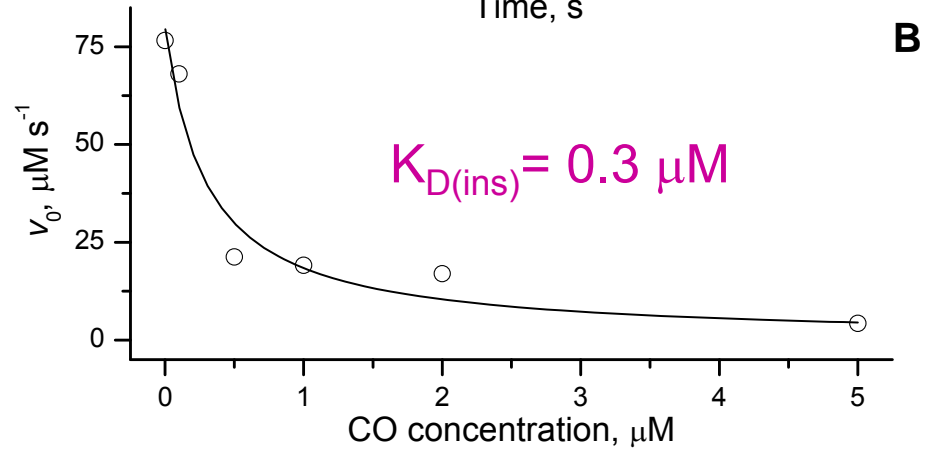
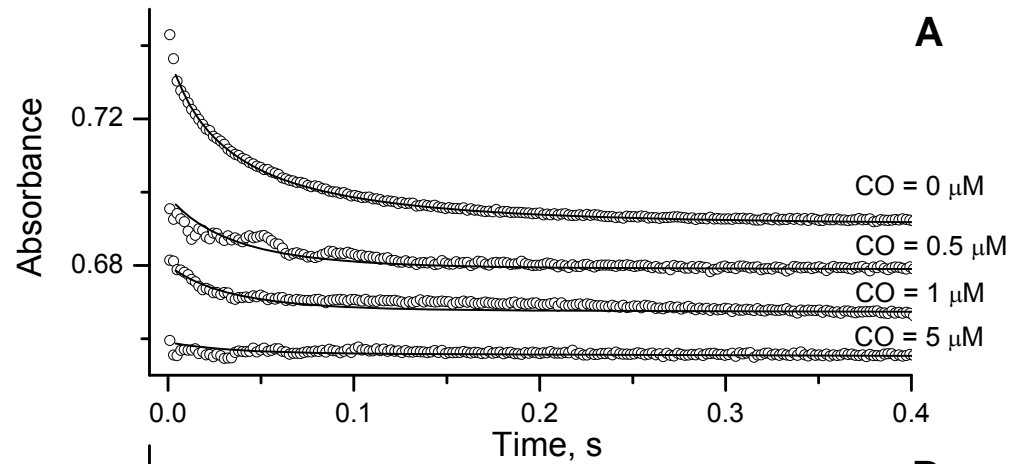
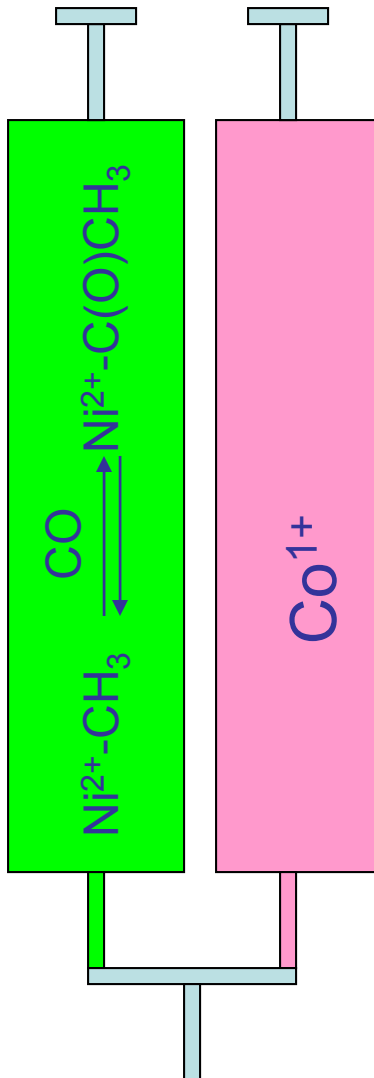


$$k_{+met} = 15 \mu\text{M}^{-1}\text{s}^{-1}$$

$$k_{-met} < 0.05 \mu\text{M}^{-1}\text{s}^{-1}$$

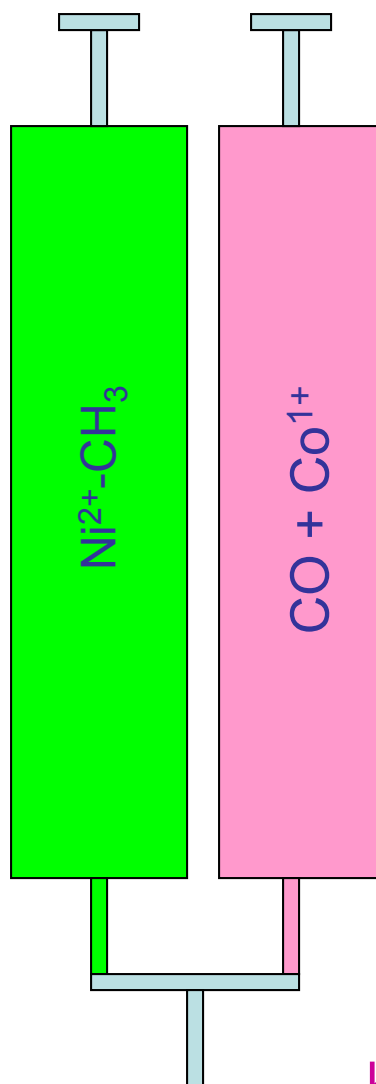
$$K_{met} > 300$$

# Monitoring the Thermodynamics of CO Insertion - *not so easy*



# Competition Reaction to Monitor Kinetics of CO Insertion

- *More difficult*



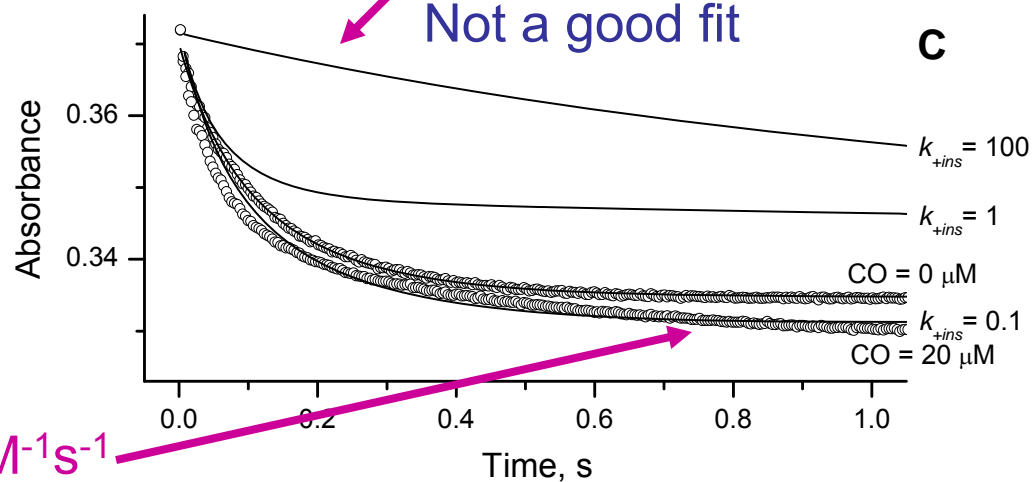
+

CO

$\uparrow \downarrow k_{+ins}$

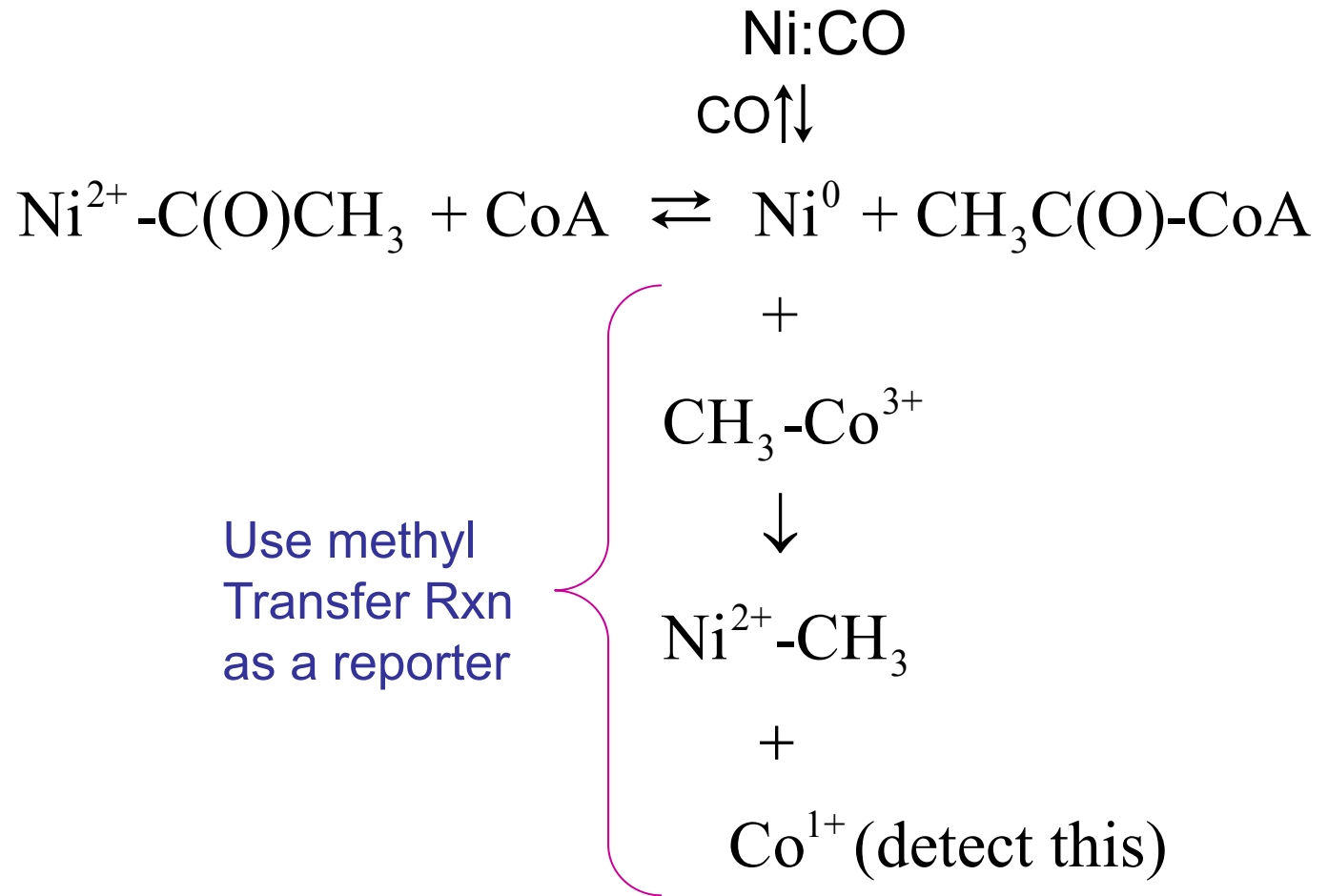
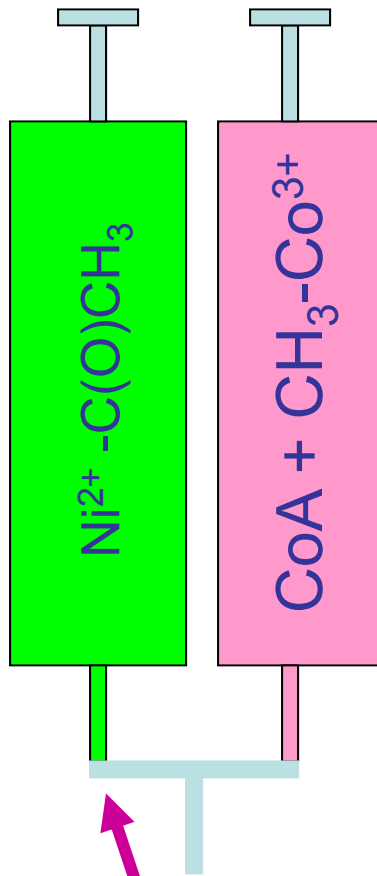


When  $k_{+ins} = 100 \mu\text{M}^{-1}\text{s}^{-1}$   
Not a good fit



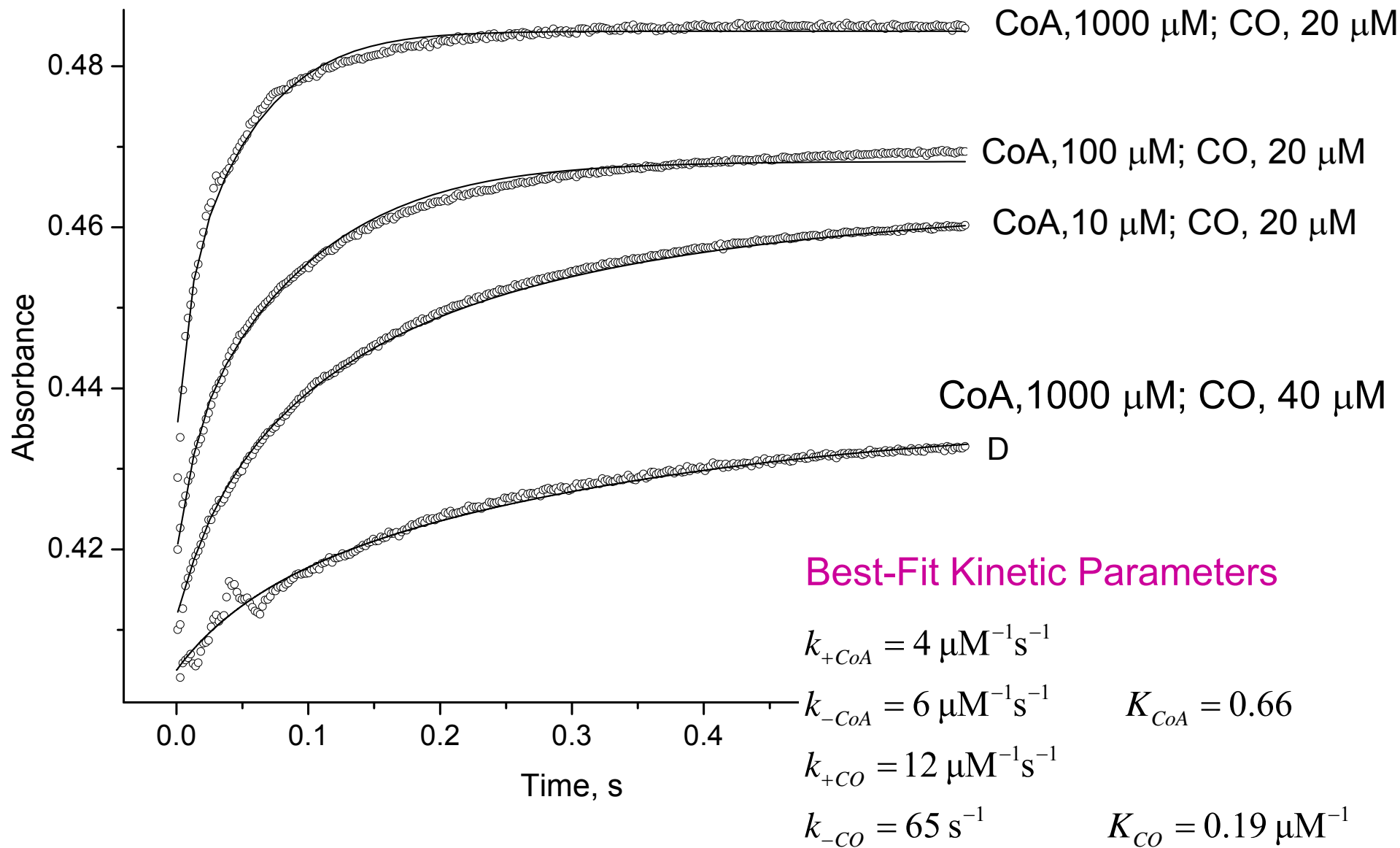
$k_{+ins} = 0.1 \mu\text{M}^{-1}\text{s}^{-1}$

# Monitoring Acetyl Group Transfer - Difficult

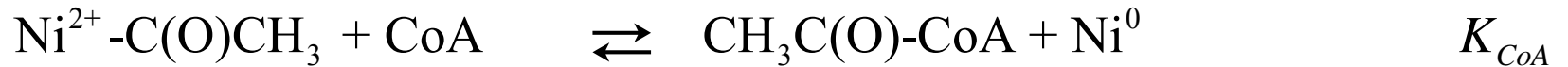
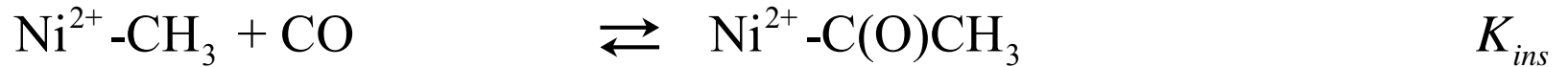
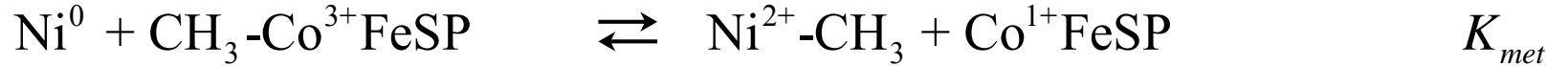


Incubate  $\text{Ni}^{2+}-\text{CH}_3 + \text{CO}$

# Works – but simulations must include CO inhibition



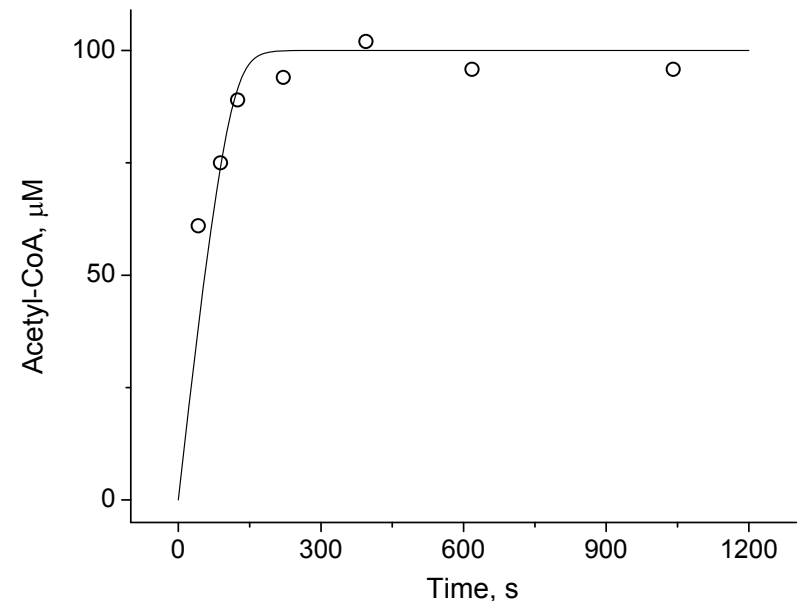
# Predictions of Model...



$$K_{met} \cdot K_{ins} \cdot K_{CoA} = K_{ACS}$$

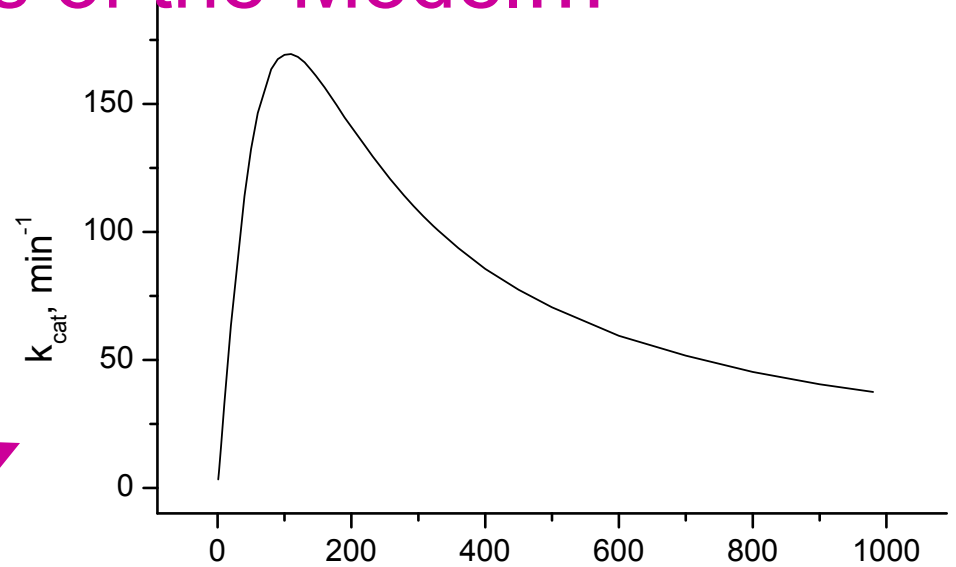
$$> 300 \cdot 3.3 \mu\text{M}^{-1} \cdot 0.66 = > 660 \mu\text{M}^{-1}$$

Actual lower limit  $K_{ACS} > 0.03 \mu\text{M}^{-1}$



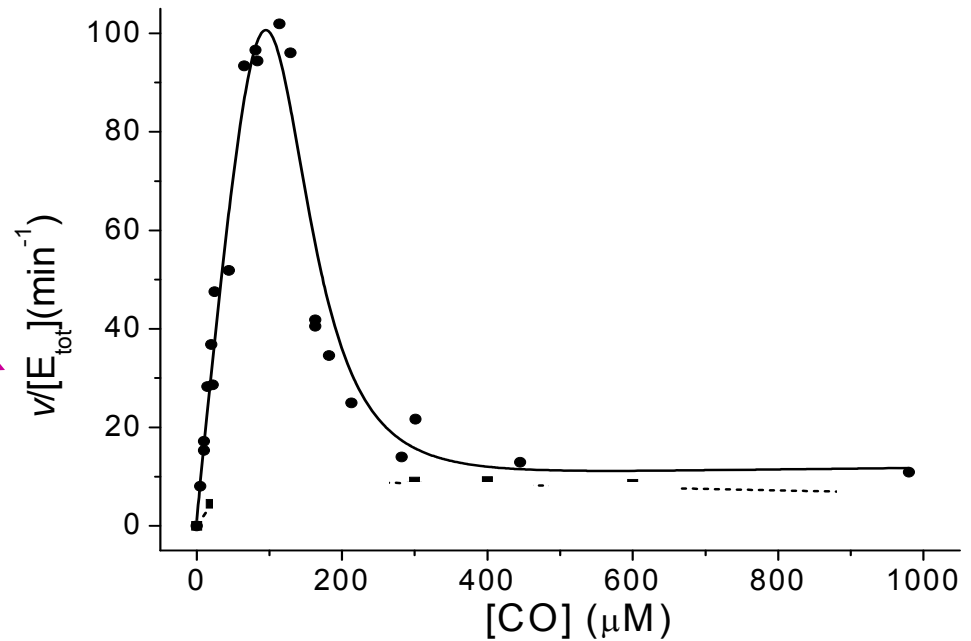
# Predictions of the Model...

Simulated effect of CO shows inhibition effect similar to that observed experimentally



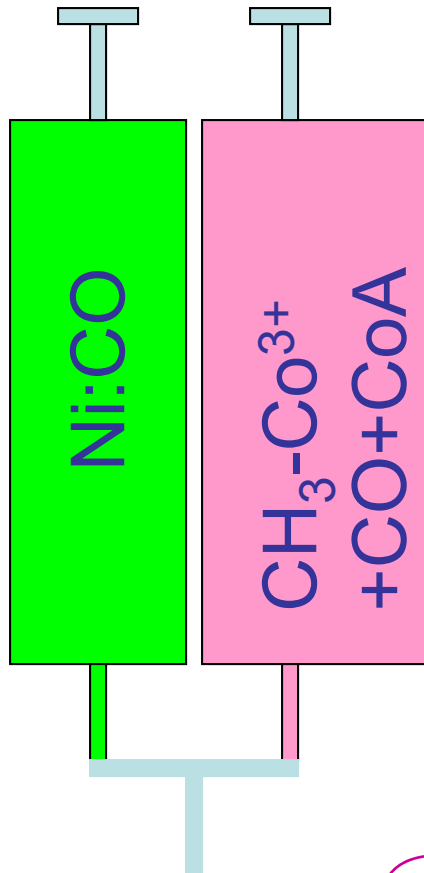
Simulation

Data

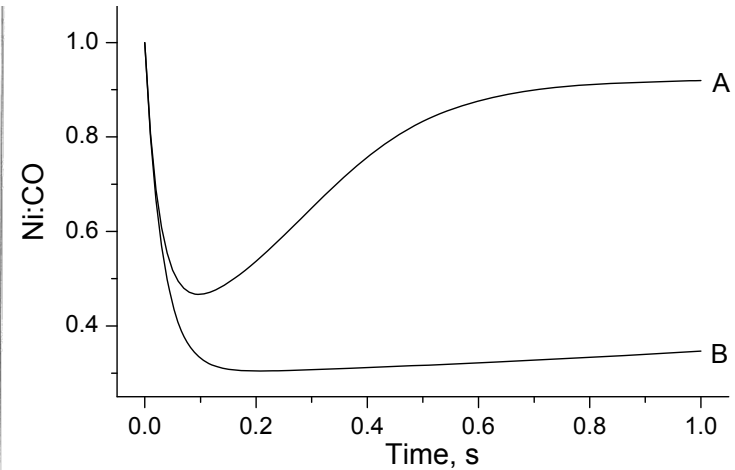
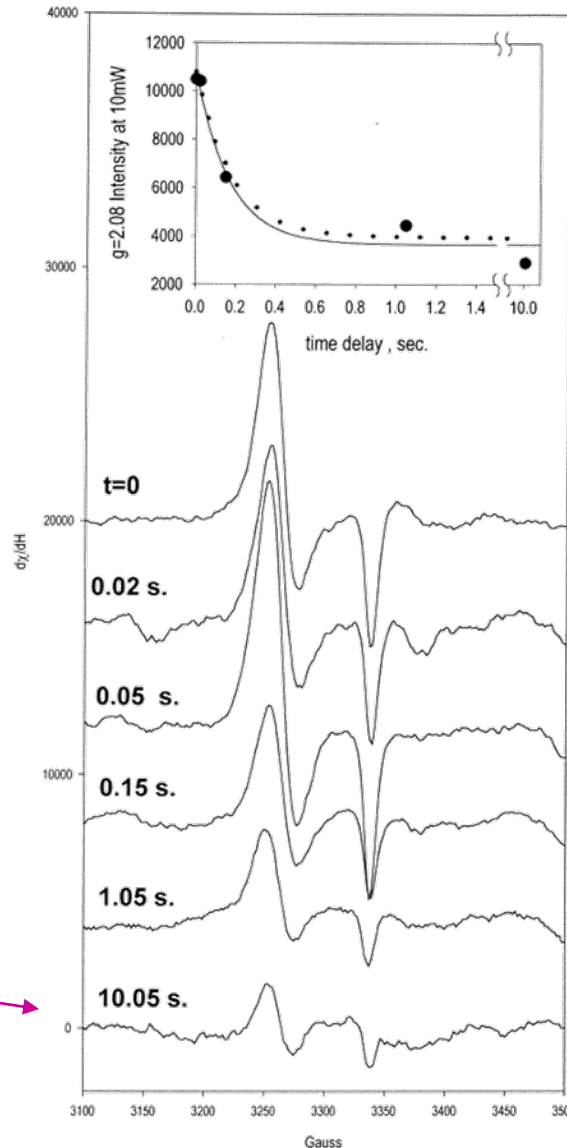




Seravalli, Kumar, Ragsdale  
*Biochemistry* 2002



Nonzero steady-state  
said to “prove”  
Intermediacy of Ni:CO state



Simulation shows decay of  
inhibitory Ni:CO state  
reaching a non-zero  
steady-state upon reacting  
with  $\text{CH}_3\text{-Co}^{3+}$ , CO and CoA  
as observed experimentally

# Predictions of the Model...

## Distribution of Enzyme States During Steady-State Catalysis

Fixed conditions as commonly used  
Calculated % in each intermediate state

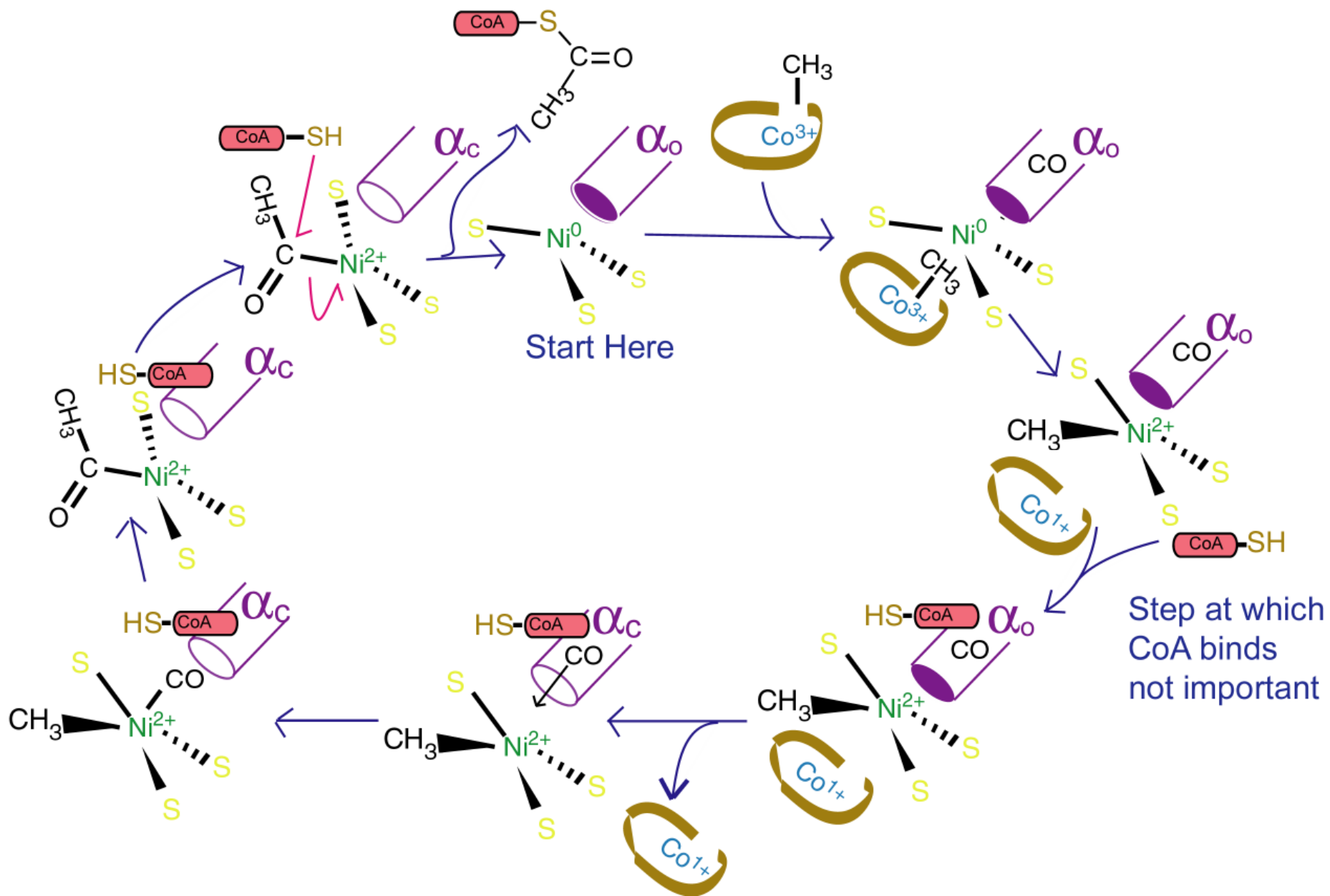
Found:	Ni <sup>0</sup> ,	0.2%
	Ni <sup>2+</sup> -CH <sub>3</sub>	95%
	Ni <sup>2+</sup> -C(O)CH <sub>3</sub>	0.3%
	Ni:CO	4.4%

According to these computations,  
CO Insertion is Rate Limiting Step

## Sensitivity Analysis

Rate Coefficient $k$	$\frac{\Delta v_0 / v_0}{\Delta k / k}$
$k_{+met}$	0.339
$k_{-met}$	-0.002
$k_{+ins}$	0.657
$k_{-ins}$	-0.0004
$k_{+CoA}$	0.002
$k_{-CoA}$	0.001
$k_{+CO}$	-0.332
$k_{-CO}$	0.317

# Acetyl-CoA Synthesis Mechanism (*circa* May 2006)



# Conclusions

ACS/CODH catalyzes the synthesis of Acetyl-CoA from CO, CoA  
And a methyl group donated by a corrin protein

Active site A-cluster is a novel  $\{[\text{Fe}_4\text{S}_4]\text{-Ni}_p \text{Ni}_d\}$  cluster.



Heterogeneity and batch-to-batch variations cause confusion  
Ni-dependent oligomerization

Chemical kinetic study:

- reductive activation
- Methyl group transfer
- CO insertion (slow step, probably involves protein conf. change)
- CoA attack, forming product

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