Nuclear Resonance Vibrational Spectroscopy (NRVS)
Nuclear Resonance Vibrational Spectroscopy (NRVS)

- Synchrotron-based vibrational technique
- Developed in the 1990’s
  - First reported in 1970’s, but proper equipment was not developed until later
- Combination of nuclear excitation and molecular vibrations
  - Uses the Mössbauer effect to excite the nucleus
  - Measures inelastic scattering of the system
  - Provides a complete set of bands that involve motion of the probed nucleus
Mössbauer Spectroscopy: Energy Source

- Mössbauer dominated by internal conversion
- Source of gamma rays is a radioactive isotope of an element which decays into an excited state of the isotope under study
- Returns to the ground state by the emission of a gamma ray or electron
  - Mössbauer active isotopes must have a meta-stable excited state
- The relaxation to the ground state produces the gamma rays used in experiment
Mössbauer Spectroscopy

- By moving the gamma ray source a change in energy of the emitted photons is achieved using the Doppler effect.
- When the energy of the modulated beam matches the difference in energy between the ground and first excited state of the absorber then the gamma rays are resonantly absorbed (note: $1 \text{ mm/s} \sim 4 \times 10^{-4} \text{ cm}^{-1}$!)
- Measures transmittance so peaks appear as a decrease in counts in the spectrum.
Theory of NRVS

- NRVS measures inelastic scattering of the gamma rays in resonance with a nuclear transition
  → nuclear resonance Raman

- Selective for vibrations involving displacement of Mössbauer active nuclei
  - No optical selection rules apply
  - Yielding the complete set of motions involving probed nucleus

- The peaks seen are recoil-free resonance energies corresponding to vibrational quanta

- Like Raman, NRVS is a very inefficient process, so an intense gamma ray source is needed
Nuclear Resonance Vibrational Spectroscopy (NRVS)

**Experimental Setup**

Undulator – synchrotron

- Only three 3\textsuperscript{rd} generation synchrotron’s in use
  - France, USA, Japan and Germany
Nuclear Resonance Vibrational Spectroscopy (NRVS)

NRVS Beamline

Resolution = 7-8 cm$^{-1}$

undulator slits

heat-load monochromator C(111)

high resolution monochromator Si(975)

14.413 keV

avalanche photodiode detector

sample

0 1 eV

0 0.85 meV

‘Raman’ spectroscopy (inelastic scattering) on the Mössbauer line

Refining the Incident Beam

- Heat-load Monochromator
  - Composed of 2 crystals
    - Silicon (France, Japan)
    - Diamond (USA)
  - Has to be well cooled
  - Beam is reduced to a few eV

- High Resolution Monochromator
  - Requires a separate crystal for each nuclei
  - Reduces the beam width to around 1 meV

Collection of Data

- Beam grazes sample at only 6°
- Detector is located 90° from sample
  - Avoids the large amount of elastic scattering that comes off 180° from sample
- Measures the amount of counts to hit the detector

Example of Raw Data

- Software converts from raw intensity (photon count) to Vibrational Density of States (VDOS; see later)
- VDOS data can be used to calculate sample temperature, analogous to Stokes/Antistokes ratio in Raman spectroscopy

Case Study 01: Ferrous Heme Nitrosyls

- NO binding to deoxy Mb
  - Effect of axial (proximal) ligand?
  - Effect of the distal hydrogen bond?

- Compare to model complex

\[
\text{[Fe(TPP)(MI)(NO)]}
\]
Ferrous Heme-Nitrosyls

- Vibrational Spectroscopy: isotope labeling
- Important vibrations:
  - N-O stretching
  - Fe-NO stretching
  - Fe-N-O bending (because of mode mixing)

Information about bond strengths, oxidation states, etc.

NRVS on [Fe(TPP)(MI)(NO)]

Vibrational Density of States (VDOS)

- Subtraction of recoilless line
- Transform raw intensity into VDOS, defined as:
  \[ D(\tilde{\nu}) = \sum_{\alpha=1}^{3N-6} e_{Fe,\alpha}^2 \cdot \Gamma(\tilde{\nu} - \tilde{\nu}_\alpha) \quad \text{(total VDOS)} \]
  \[ D_k(\tilde{\nu}) = \sum_{\alpha=1}^{3N-6} \left( k \cdot \tilde{e}_{Fe,\alpha} \right)^2 \cdot \Gamma(\tilde{\nu} - \tilde{\nu}_\alpha) \quad \text{(VDOS in direction } k; \ k = x, y, z) \]
- Factors \( e_{Fe}^2 \) : amount of iron motion in a normal mode \( \rightarrow \) specific property of a vibration:
  \[ e_{Fe}^2 = \frac{m_{Fe} r_{Fe}^2}{\sum_i m_i r_i^2} \quad \text{Also: } \sum_{\alpha=1}^{3N} e_{Fe,\alpha}^2 = 3 \quad \sum_{j=1}^{N} e_{j,\alpha}^2 = 1 \]

Vibrational Density of States (VDOS)

- Integrated VDOS intensity equals amplitude factor $e_{Fe}^2$, proportional to the amount of iron motion in a normal mode.
- Can be simulated using normal coordinate analysis!

EXP: $\frac{e_{Fe}^2[563]}{e_{Fe}^2[437]} \approx 0.48$

Mixed polarizations!

NRVS and NCA

- Definition of VDOS:
  \[ D(\vec{v}) = \sum_{\alpha=1}^{3N-6} e_{\text{Fe},\alpha}^2 \cdot \Gamma(\vec{v} - \vec{v}_\alpha) \]  
  (total VDOS)

- Factors \( e_{\text{Fe}}^2 \): amount of iron motion in a normal mode \( \rightarrow \) specific property of a vibration:
  \[ e_{\text{Fe}}^2 = \frac{m_{\text{Fe}} r_{\text{Fe}}^2}{\sum_i m_i r_i^2} \]

- \( r_i \) is the absolute length of the mass-weighted atomic displacement vector for atom \( i \) for a given normal mode \( \alpha \) (available from DFT and NCA!)
NRVS and DFT

- Experimental versus DFT-calculated VDOS

Vibrational Analysis (NCA)

- Simulation of Data using Normal Coordinate Analysis (with some help from DFT: QCC-NCA method)

QCC-NCA on full $[\text{Fe(TPP)(MI)(NO)}]$ 

- Quantum-chemistry centered Normal Coordinate Analysis (QCC-NCA) simulations reproduce experimental polarizations well!

Assignments

Lehnert, Sage, Silvernail, Scheidt, Alp, Sturhahn & Zhao, Inorg. Chem. 2010, 49, 7197
Or a bit more dynamic…

- The Fe-N-O bending mode
  (563 cm\(^{-1}\))

- The Fe-NO stretching mode
  (437 cm\(^{-1}\))
Effect of the proximal His?

Vibrational Spectroscopy

<table>
<thead>
<tr>
<th>Complex</th>
<th>Vibrational Frequency [cm(^{-1})]</th>
<th>Force Constant [mdyn/Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\nu(\text{N-O})) (\nu(\text{Fe-NO})) (\delta(\text{Fe-N-O}))</td>
<td>(f(\text{N-O})) (f(\text{Fe-NO}))</td>
</tr>
<tr>
<td>([\text{Fe(TPP)(NO)}]) 5C</td>
<td>1697 \ 532 \ 371</td>
<td>12.53 \ 2.98</td>
</tr>
<tr>
<td>([\text{Fe(TPP)(MI)(NO)}]) 6C</td>
<td>1630 \ 437 \ 563</td>
<td>11.55 \ 2.57</td>
</tr>
</tbody>
</table>

Direct correlation of N-O and Fe-NO bond strengths!

Weakening of Fe-NO \(\sigma\) bond

Electronic Structure

S = 1/2

[Fe(II)-porphyrin]  NO

Enemark-Feltham: low-spin \{FeNO\}^7

General Electronic Structure

$S = 1/2$

$\sigma$-bond forms

$dx^2$, $dy^2$, $dxy$, $dz^2$

[Fe(II)-porphyrin] NO

Inorganic Chemistry
Farmer on Nitric Oxide

The *Trans* Effect of NO

- NO is a signaling molecule that controls blood pressure, and is involved in nerve signal transduction (brain).
- NO activates soluble guanylate cyclase via its thermodynamic *trans* effect!

Effect of Hydrogen Bonds?

- \([\text{Fe(PP)}(\text{MI})(\text{NO})]\) models hydrogen-bond free Mb mutants!

- Similar Fe-NO stretching frequencies in wt Mb(II)-NO (443 cm\(^{-1}\))** and [Fe(PP)(MI)(NO)] (437 cm\(^{-1}\)) indicates weak effect of H-bond on Fe-NO bond!

- Shift in N-O stretching frequency is due to polarization of the \(\pi/\pi^*\) orbitals of NO


### Case Study 02: DNICS

Dinitrosyl Iron Complexes (DNICS) exist in two oxidation states: \(\{\text{Fe(NO)}_2\}^9\) and \(\{\text{Fe(NO)}_2\}^{10}\)

\[
\begin{align*}
[\text{Fe(NO)}_2(\text{CO})_2] & \quad \xrightarrow{2\text{ L} \ 2\text{ CO}} \quad [\text{Fe(L)}_2(\text{NO})_2] \\
\{\text{Fe(NO)}_2\}^{10} & \quad \xrightarrow{[\text{ox}]} \quad [\text{Fe(L)}_2(\text{NO})_2]^+ \\
\{\text{Fe(NO)}_2\}^9 & \quad \text{Here: } L = \text{dmp}
\end{align*}
\]

Mössbauer Spectroscopy

| Redox Level | \(\delta\) (mm/s) | \(|\Delta E_0|\) (mm/s) |
|-------------|-------------------|-------------------------|
| \([\text{Fe(dmp)}(\text{NO})_2]^+ (1)\) | \(\{\text{Fe(NO)}_2\}^9\) | 0.37 | 1.77 |
| \([\text{Fe(dmp)}(\text{NO})_2] (2)\) | \(\{\text{Fe(NO)}_2\}^{10}\) | 0.29 | 0.83 |
| \([\text{Fe(ar-nacnac)}(\text{NO})_2]\) | \(\{\text{Fe(NO)}_2\}^9\) | 0.19 | 0.79 |
| \([\text{Fe(ar-nacnac)}(\text{NO})_2]^-\) | \(\{\text{Fe(NO)}_2\}^{10}\) | 0.22 | 1.31 |


NRVS shows characteristic differences between the two DNIC oxidation states!

QCC-NCA on NRVS Data

{\text{Fe(NO)}_2}^9

\begin{align*}
\text{VDOS (cm)} & \quad 300 \quad 400 \quad 500 \quad 600 \quad 700 \\
\text{Energy (cm}^{-1}) & \quad 0.00 \quad 0.01 \quad 0.02 \quad 0.03
\end{align*}

\{\text{Fe(NO)}_2\}^9 \text{ 1-NO} \\
\text{Expt} \quad \text{QCC-NCA fit}

{\text{Fe(NO)}_2}^{10}

\begin{align*}
\text{VDOS (cm)} & \quad 450 \quad 500 \quad 550 \quad 600 \quad 650 \quad 700 \\
\text{Energy (cm}^{-1}) & \quad 0.00 \quad 0.01 \quad 0.02 \quad 0.03
\end{align*}

\{\text{Fe(NO)}_2\}^{10} \text{ 2-NO} \\
\text{Expt} \quad \text{QCC-NCA fit}

{\text{Fe(}^{15}\text{N}^{18}\text{O)}_2}^9 \text{ 1-}^{15}\text{N}^{18}\text{O}

{\text{Fe(}^{15}\text{N}^{18}\text{O)}_2}^{10} \text{ 2-}^{15}\text{N}^{18}\text{O}
QCC-NCA on NRVS Data

\[
\{\text{Fe(NO)}_2\}_9
\]

<table>
<thead>
<tr>
<th>Band Assignment</th>
<th>Experimental</th>
<th>QCC-NCA</th>
<th>(e^2_{\text{Fe}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu(\text{N-O})_{\text{as}})</td>
<td>1748 (1673)</td>
<td>1748 (1674)</td>
<td>--</td>
</tr>
<tr>
<td>(\nu(\text{N-O})_{\text{s}})</td>
<td>1836 (1759)</td>
<td>1835 (1757)</td>
<td>--</td>
</tr>
<tr>
<td>(\nu(\text{Fe-NO})_{\text{s}})</td>
<td>534 (518)</td>
<td>534 (518)</td>
<td>0.263 (0.242)</td>
</tr>
<tr>
<td>(\nu(\text{Fe-NO})_{\text{as}})</td>
<td>581 (565)</td>
<td>580 (564)</td>
<td>0.415 (0.439)</td>
</tr>
<tr>
<td>(\delta(\text{Fe-N-O})_{\text{s, ip}})</td>
<td>581 (565)</td>
<td>594 (576)</td>
<td>0.138 (0.169)</td>
</tr>
<tr>
<td>(\delta(\text{Fe-N-O})_{\text{s, oop}})</td>
<td>355 (347)</td>
<td>357 (346)</td>
<td>0.127 (0.152)</td>
</tr>
</tbody>
</table>

\[
\{\text{Fe(NO)}_2\}_{10}
\]

<table>
<thead>
<tr>
<th>Band Assignment</th>
<th>Experimental</th>
<th>QCC-NCA</th>
<th>(e^2_{\text{Fe}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu(\text{N-O})_{\text{as}})</td>
<td>1688 (1617)</td>
<td>1689 (1616)</td>
<td>--</td>
</tr>
<tr>
<td>(\nu(\text{N-O})_{\text{s}})</td>
<td>1621 (1559)</td>
<td>1621 (1553)</td>
<td>--</td>
</tr>
<tr>
<td>(\nu(\text{Fe-NO})_{\text{s}})</td>
<td>559 (538)</td>
<td>558 (541)</td>
<td>0.233 (0.239)</td>
</tr>
<tr>
<td>(\nu(\text{Fe-NO})_{\text{as}})</td>
<td>616 (600)</td>
<td>615 (599)</td>
<td>0.440 (0.462)</td>
</tr>
<tr>
<td>(\delta(\text{Fe-N-O})_{\text{s, ip}})</td>
<td>646 (631)</td>
<td>647(629)</td>
<td>0.163 (0.201)</td>
</tr>
<tr>
<td>(\delta(\text{Fe-N-O})_{\text{s, oop}})</td>
<td>545 (538)</td>
<td>545 (534)</td>
<td>0.208 (0.208)</td>
</tr>
</tbody>
</table>

Dramatic stiffening of the FeN₄ core!
Electronic Structure

\[ \{\text{Fe(NO)}_2\}_9 \rightarrow \{\text{Fe(NO)}_2\}_{10} : \]

- **Metal-centered reduction**: \( \text{Fe}^{III}(\text{NO}^-)_2 \rightarrow \text{Fe}^{II}(\text{NO}^-)_2 \)
  

- **Mössbauer spectroscopy**: isomer shift decreases upon reduction
  → the iron center becomes more oxidized upon reduction?

- **NRVS**: increase in Fe-NO multiple bond character
  and Fe-NO bond strength (stretching frequency)
  decrease in N-O bond strength (stretching frequency)
  → dramatic increase in Fe-NO backbonding upon reduction
  → direct, experimental evidence from vibrational spectroscopy!

Supported by DFT

Speelman, Zhang, Silakov, Skodje, Alp, Zhao, Hu, Kim, Krebs & Lehnert,
*Inorg. Chem.* 2016, 55, asap
Electronic Structure

Speelman, Zhang, Silakov, Skodje, Alp, Zhao, Hu, Kim, Krebs & Lehnert,
Inorg. Chem. 2016, 55, asap

Case Study 03: Cytochrome c

- NRVS data of ferric *Hydrogenobacter thermophilus*
  Cytochrome c

Cyt c (green) : Cytochrome c peroxidase (blue)
complex from *S. cerevisiae* (PDB: 2PCB)

*Hydrogenobacter thermophilus*
Cytochrome c
Case Study 03: Cytochrome c

- NRVS data of ferric *Hydrogenobacter thermophilus* Cytochrome c

Heme vibrations are coupled into the polypeptide chain!

NRVS on Cytochrome c

- Spectral Simulations
NRVS on Cytochrome c

- Heme vibrations are strongly coupled to the vibrations of the protein environment, especially the CysXXCysHis loop!
- CXXCH loop is exposed to the protein surface, in the binding region for redox partners
- Modulation of heme vibrations in the protein-protein (ET) complex to minimize reorganization energy or condition the heme for ET?

Acknowledgement

Current Coworkers
Dr. Subhra Samanta
Max Bilodeau
Andrew Hunt
Jacques Kumutima
Molly MacInnes
Ashley McQuarters
Corey White
Matt Wolf
Diamond Thomas
David Vargas
Richard Wan

http://www.umich.edu/~lehnert/

Thank you for your attention!