Ultrafast 2D-IR Spectroscopy
Method and Biomolecular Applications

Neil T. Hunt

Dept. of Physics, University of Strathclyde, Glasgow, UK

Outline

2D-IR – an introduction

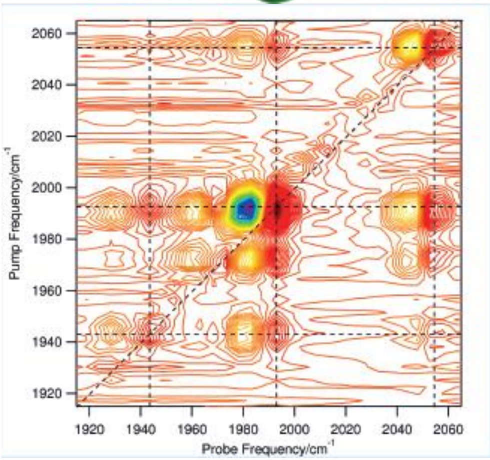
- Method
- Contributions to spectra

2D-IR measurements of protein structural dynamics

- Catalase-NO and the role of bound water
- Ferric myoglobin-NO
- The effects of mutation on Mb dynamics
- How do structural dynamics relate to function?

Future perspective

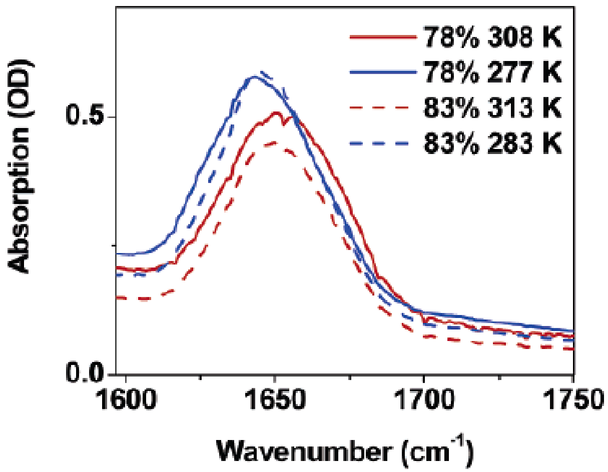
- TRMPS and reaction-following…



2D-IR Introduction – why 2D?

1D or (FT-IR) is a widely-used technique in chemistry and biology

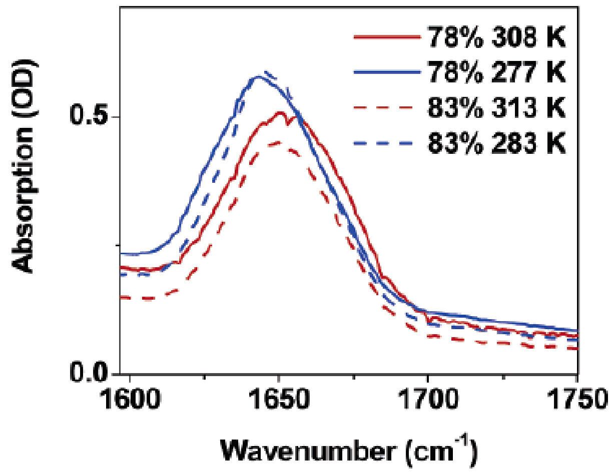
- Molecular Structure
- Vibrational energy transfer
- Solvent Interactions
- Ultrafast fluctuations

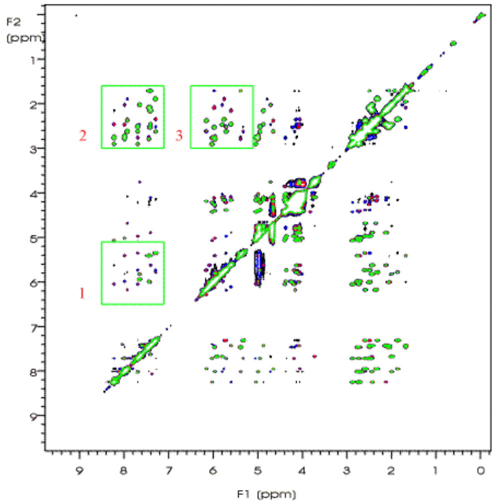


2D-IR Introduction – why 2D?

1D or (FT-IR) is a widely-used technique in chemistry and biology

- Molecular Structure
- Vibrational energy transfer
- Solvent Interactions
- Ultrafast fluctuations





But 1D methods do not reveal all of this - we need a technique to extract this in an efficient manner

Use analogous approach to 2D-NMR and spread our information over two frequency axes

Measuring 2D-IR

Correlation of excitation frequency with detection frequency

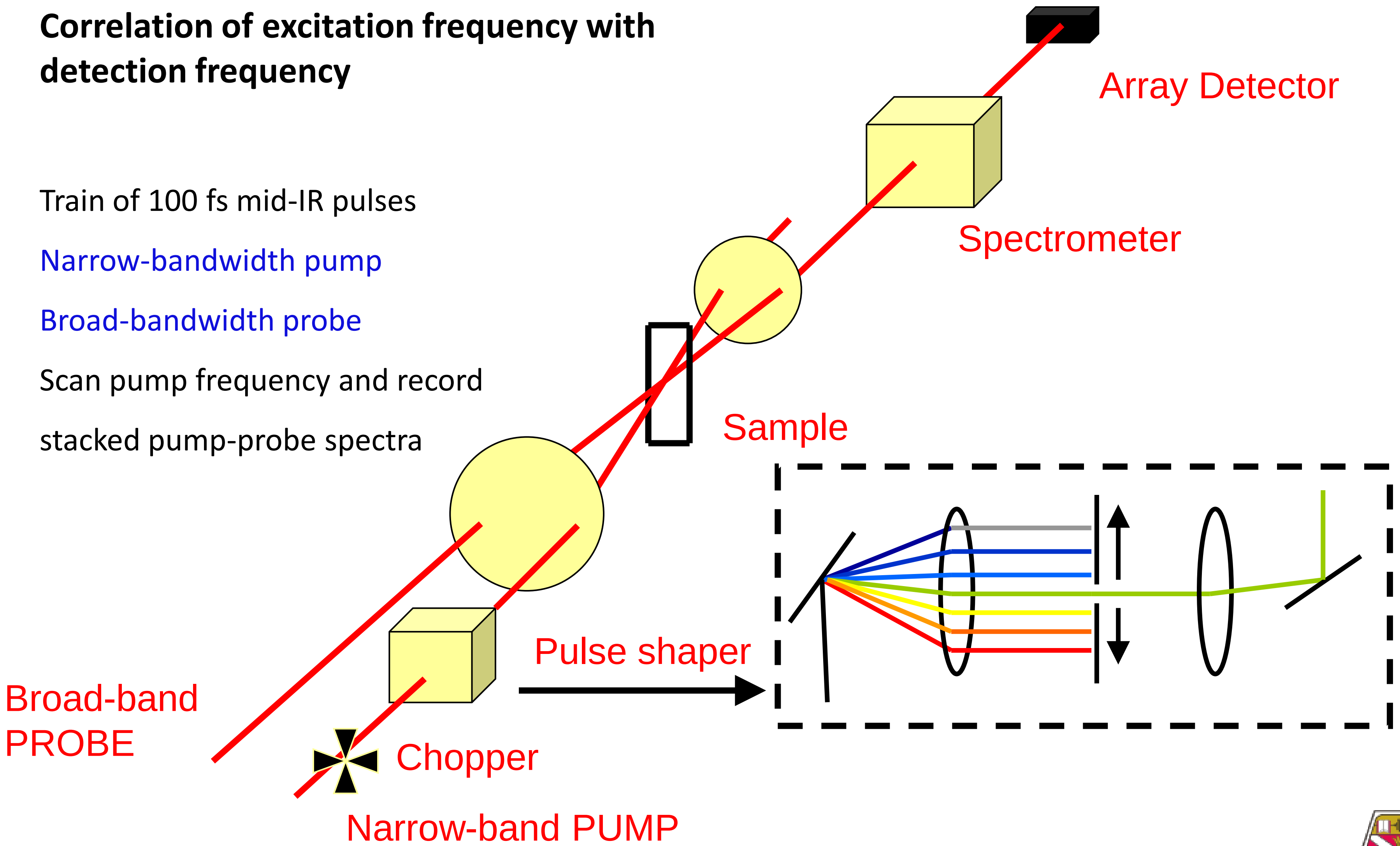
Train of 100 fs mid-IR pulses

Narrow-bandwidth pump

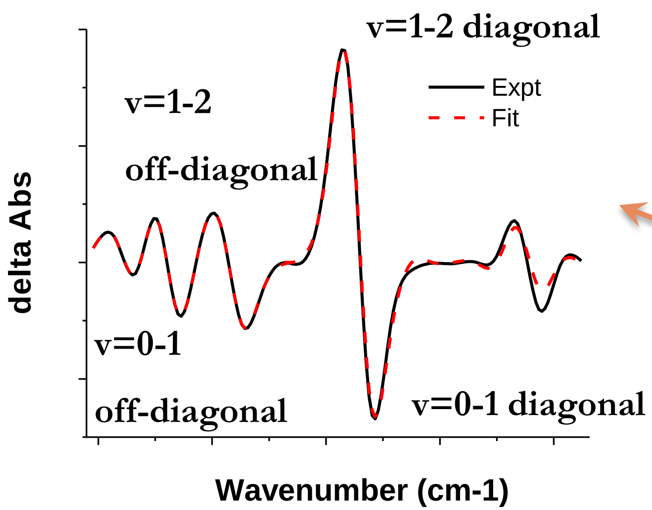
Broad-bandwidth probe

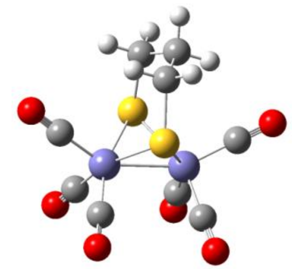
Scan pump frequency and record

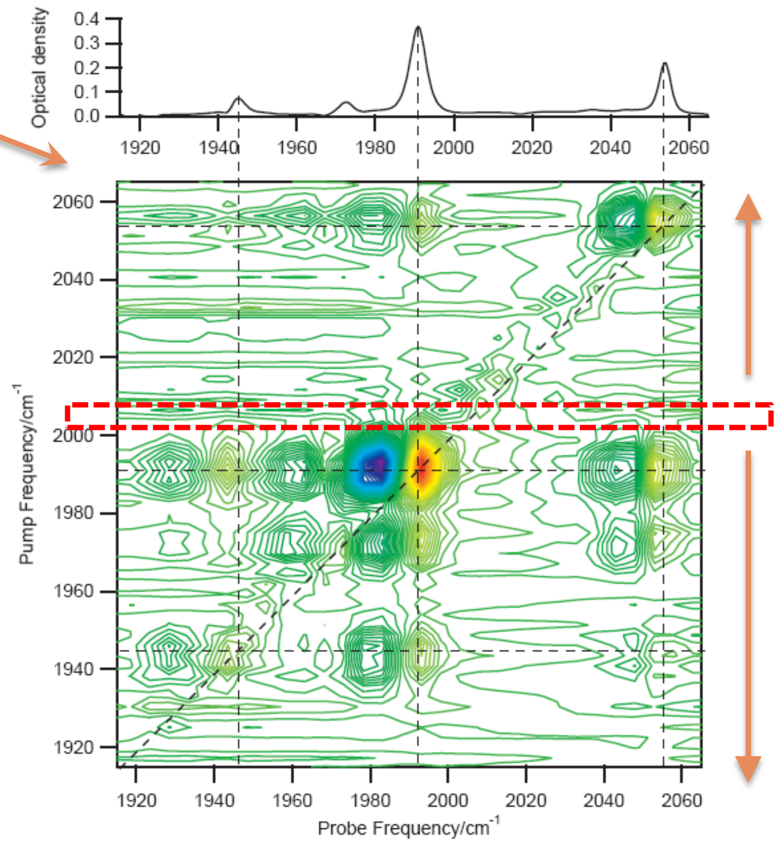
stacked pump-probe spectra



Double Resonance 2D-IR

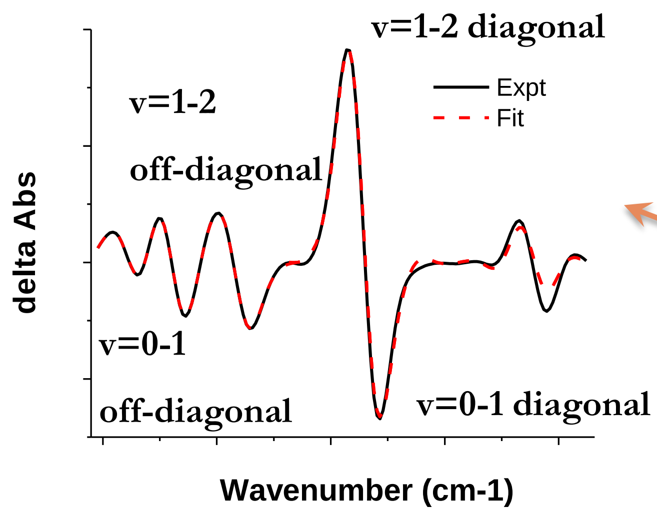


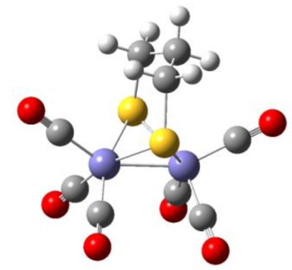


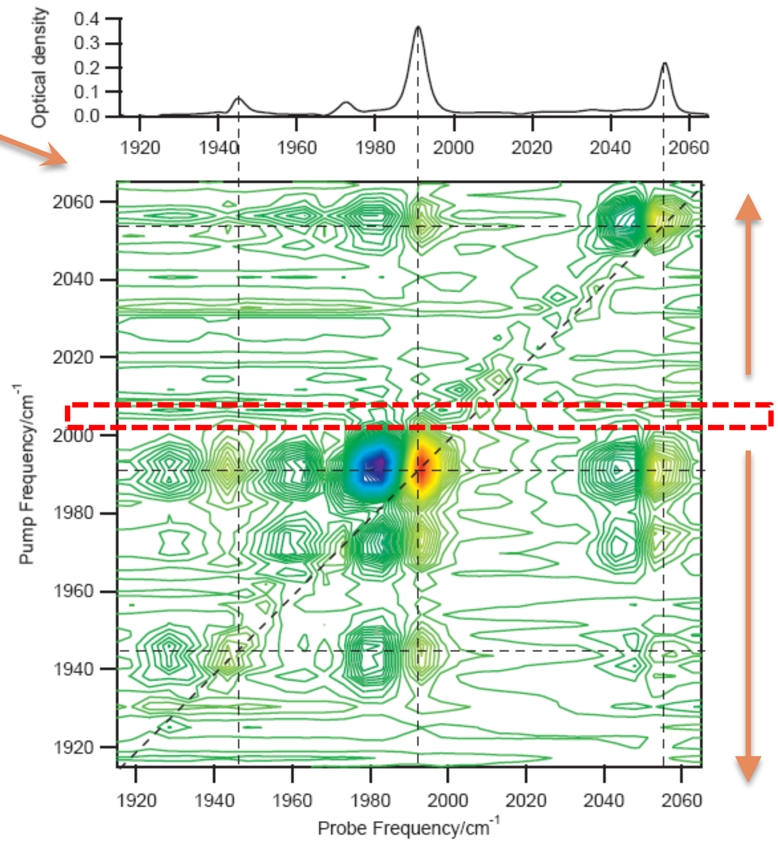


Scanning pump pulse frequency enables us to build up a stepwise picture of the 2D-IR plot by investigating interactions and energy transfer pathways one mode at a time

Double Resonance 2D-IR



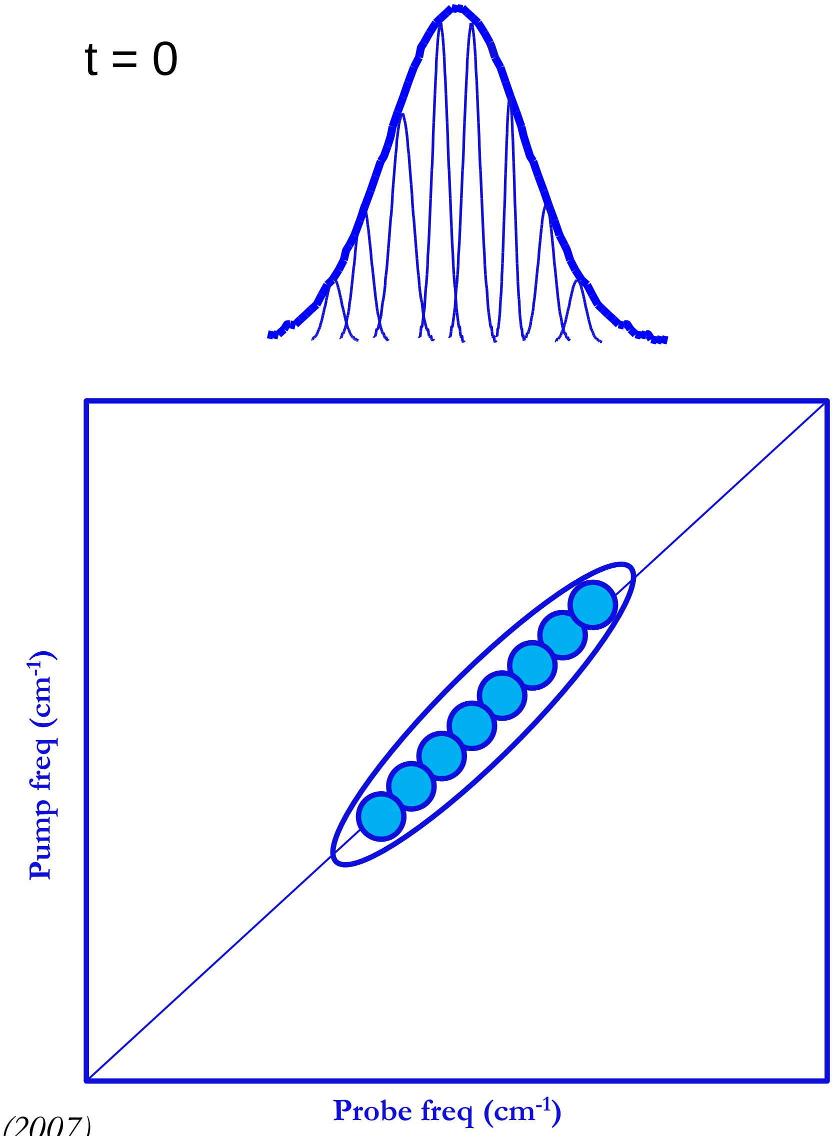




Off-diagonal peaks reveal:

- Spectral assignments
- Vibrational relaxation
- Chemical exchange
- With 100 fs resolution – bridges gap between NMR and MD

Structural Dynamics



Spectral Diffusion

- Inhomogeneous broadening due to fluctuations on timescales slower than experimental time delays give rise to diagonal elongation and 2D lineshape evolution

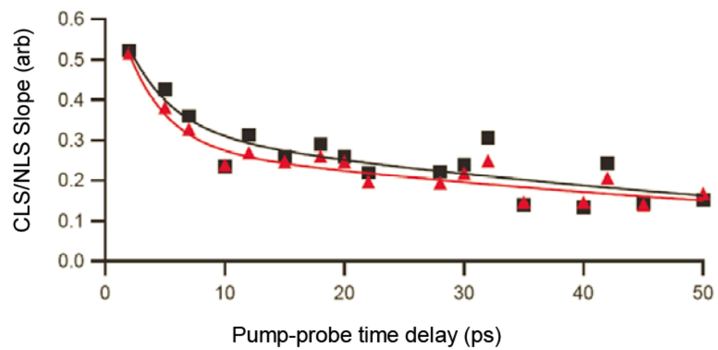
- 2D fitting provides frequency-frequency correlation function (FFCF) via ellipticity, CLS or NLS methods

Faraday Discussions **145**, 429 (2010)
Ishikawa *et al Proc Nat Acad Sci,* **104**, 16116-16121 *(2007)*
Roberts *et al J Chem Phys,* **125**, 084502 (2006)

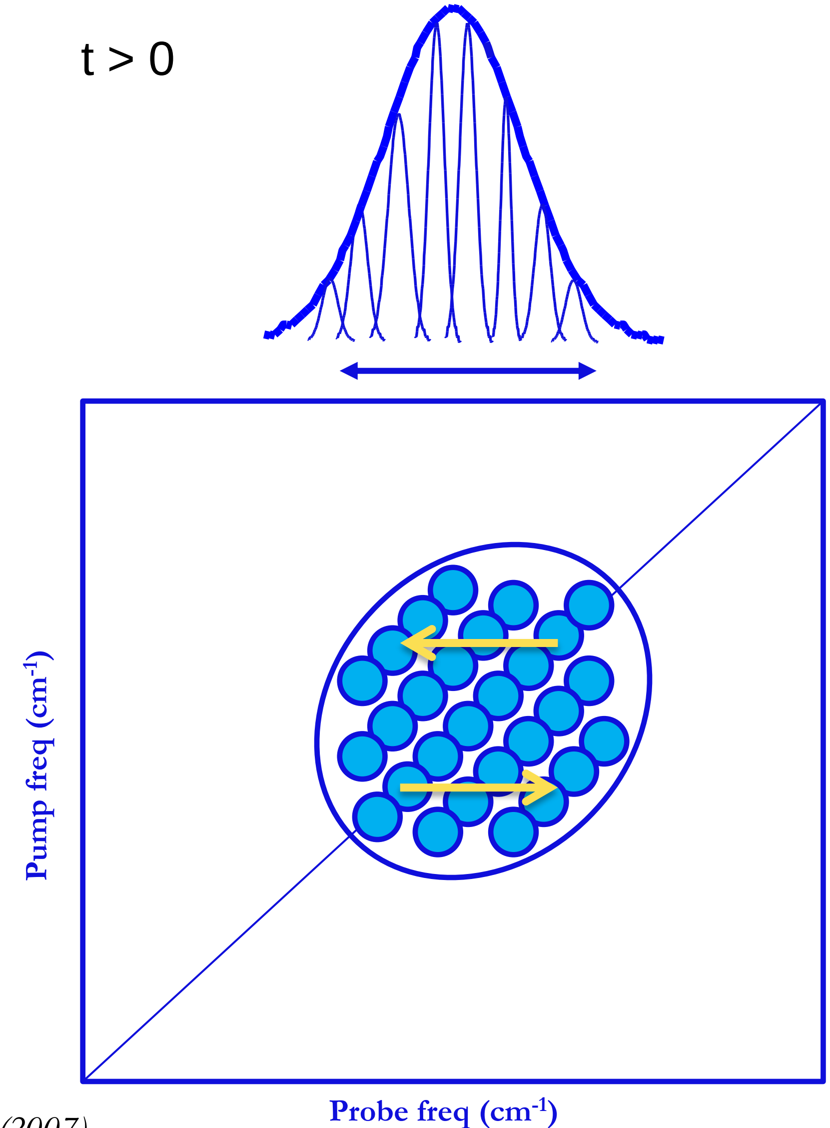
Structural Dynamics

Spectral Diffusion

Exponential decays from quantifying lineshape evolution report solvation or structural dynamics



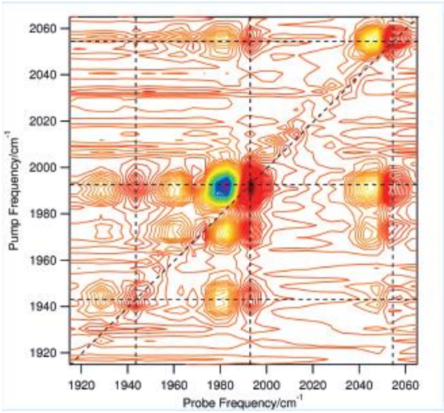
Faraday Discussions **145**, 429 (2010)
Ishikawa *et al Proc Nat Acad Sci,* **104**, 16116-16121 *(2007)*
Roberts *et al J Chem Phys,* **125**, 084502 (2006)



Catalase and Haem Proteins

Role of Structural Dynamics in Protein Function

- Do ultrafast fluctuations play a direct role in function?



Catalase and NO

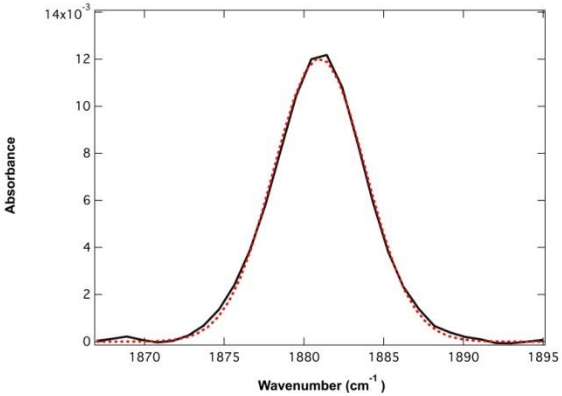
- Haem protein – catalyses $2H_2O_2$ -> $2H_2O + O_2$
- Inhibition by NO – use as probe
- Conserved structure features distal His

Structural dynamics and functionality

- 2D-IR spectroscopy of catalase-NO
- Comparisons with ligand transport proteins (Mb) which also feature distal His
- Does the local ligand chemical environment differ between Mb and Cat?
- X-ray crystal structure of catalase-NO

Catalase-NO 2D-IR

Corynebacterium glutamicum catalase

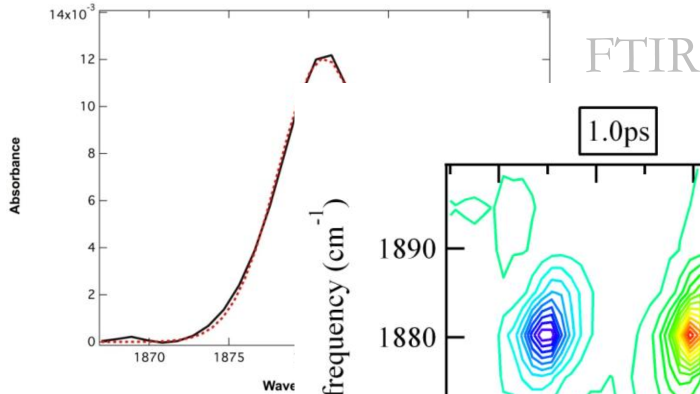


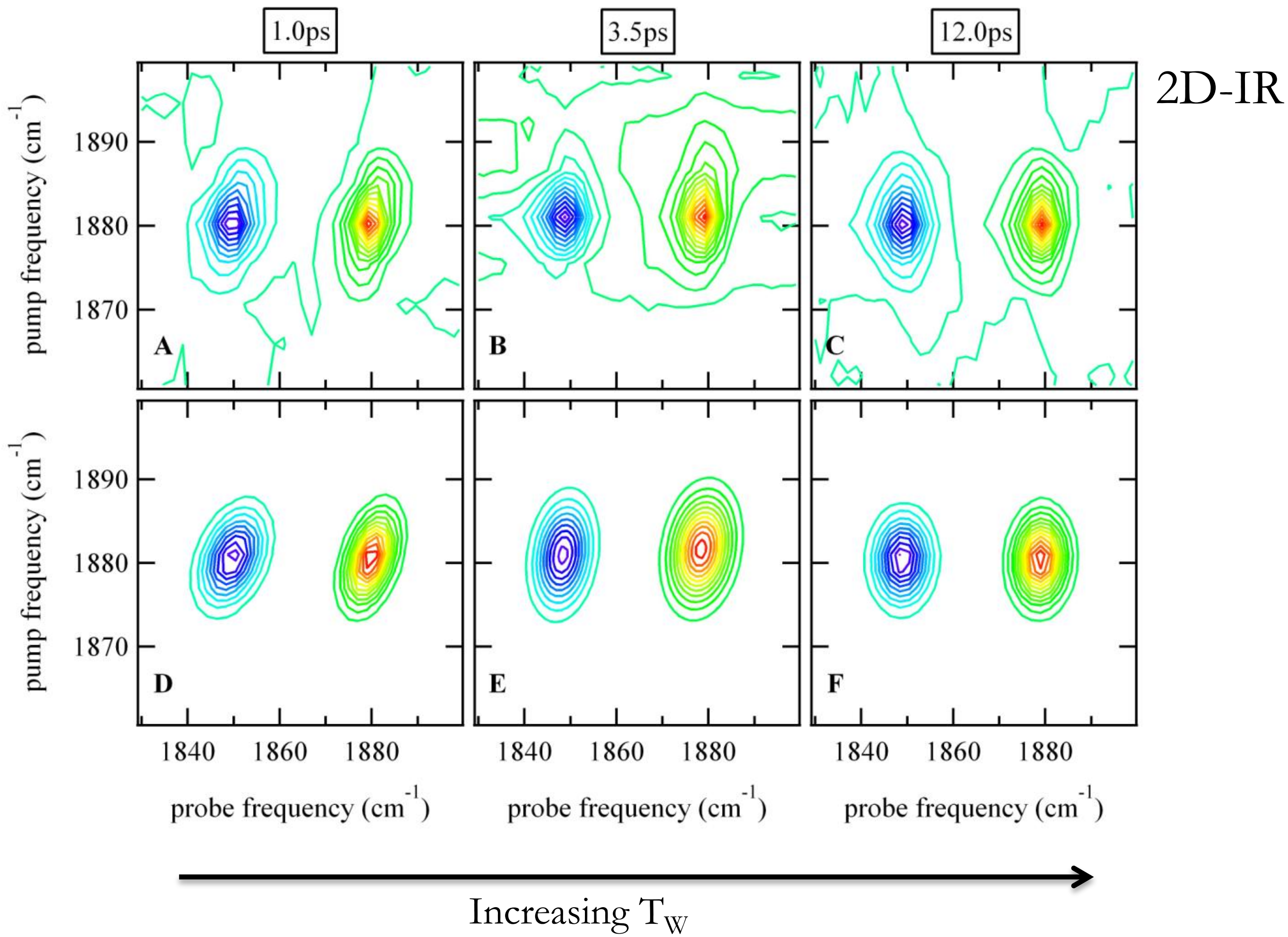
FTIR

Organic and Biomolecular Chemistry **11**, 7778 (2013)

Catalase-NO 2D-IR

Corynebacterium glutamicum catalase

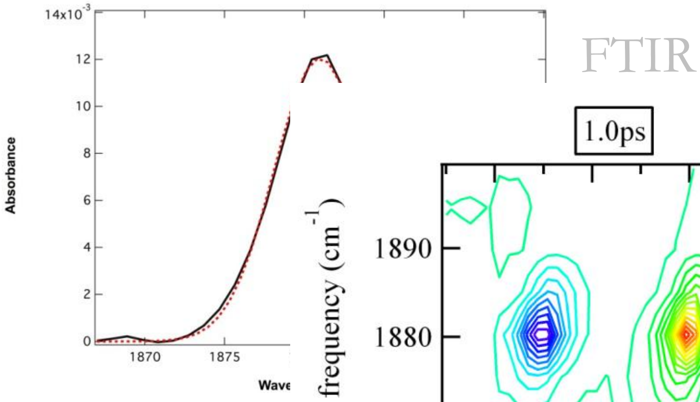


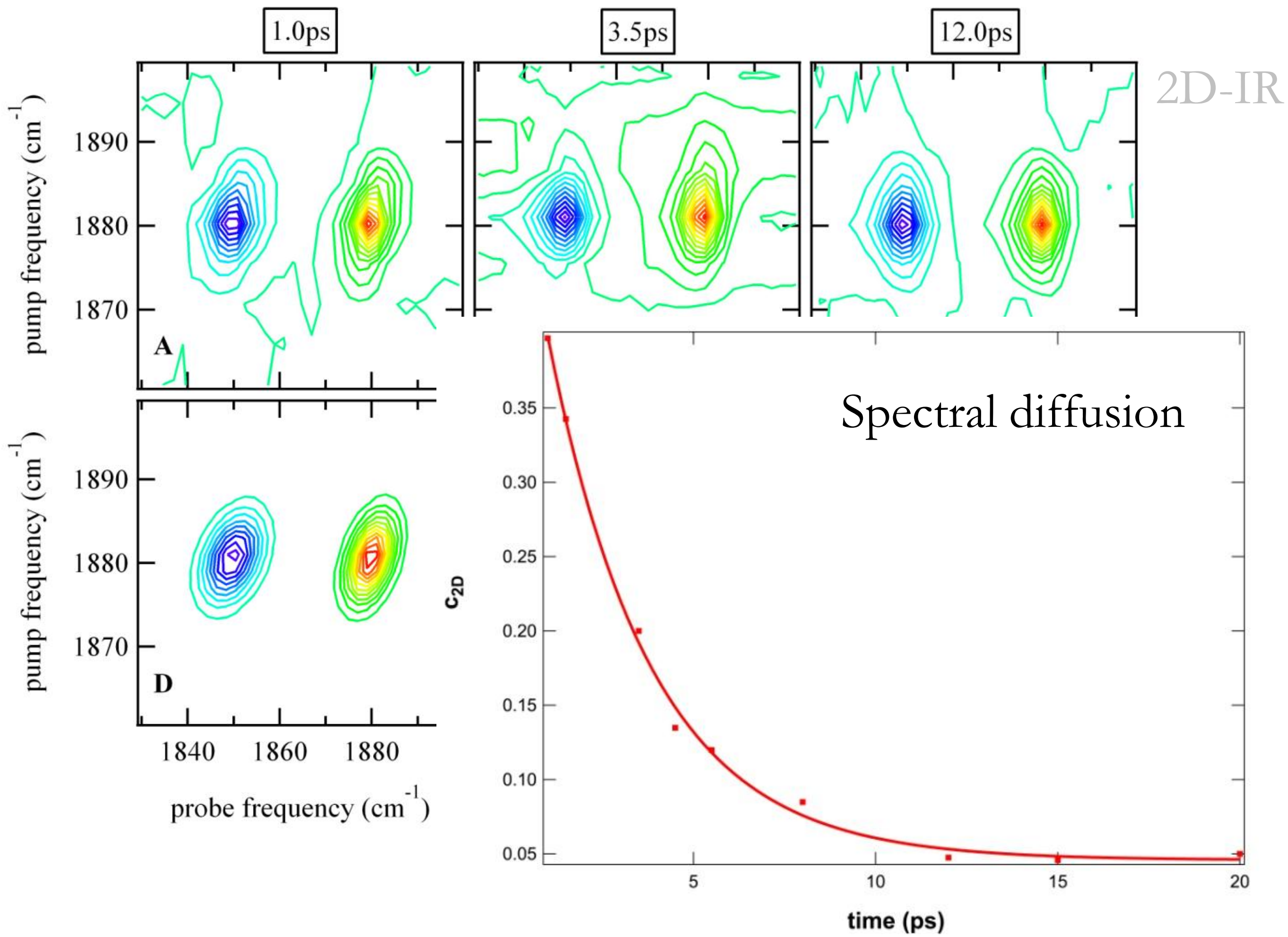


Organic and Biomolecular Chemistry **11**, 7778 (2013)

Catalase-NO 2D-IR

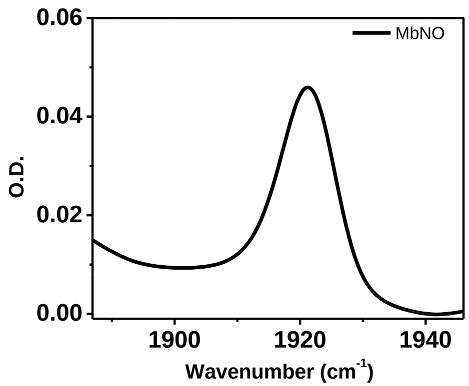
Corynebacterium glutamicum catalase





Organic and Biomolecular Chemistry **11**, 7778 (2013)

Mb Fe^{III}-NO 2D-IR

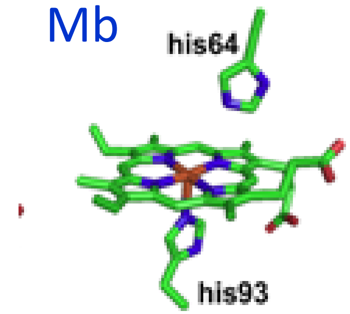


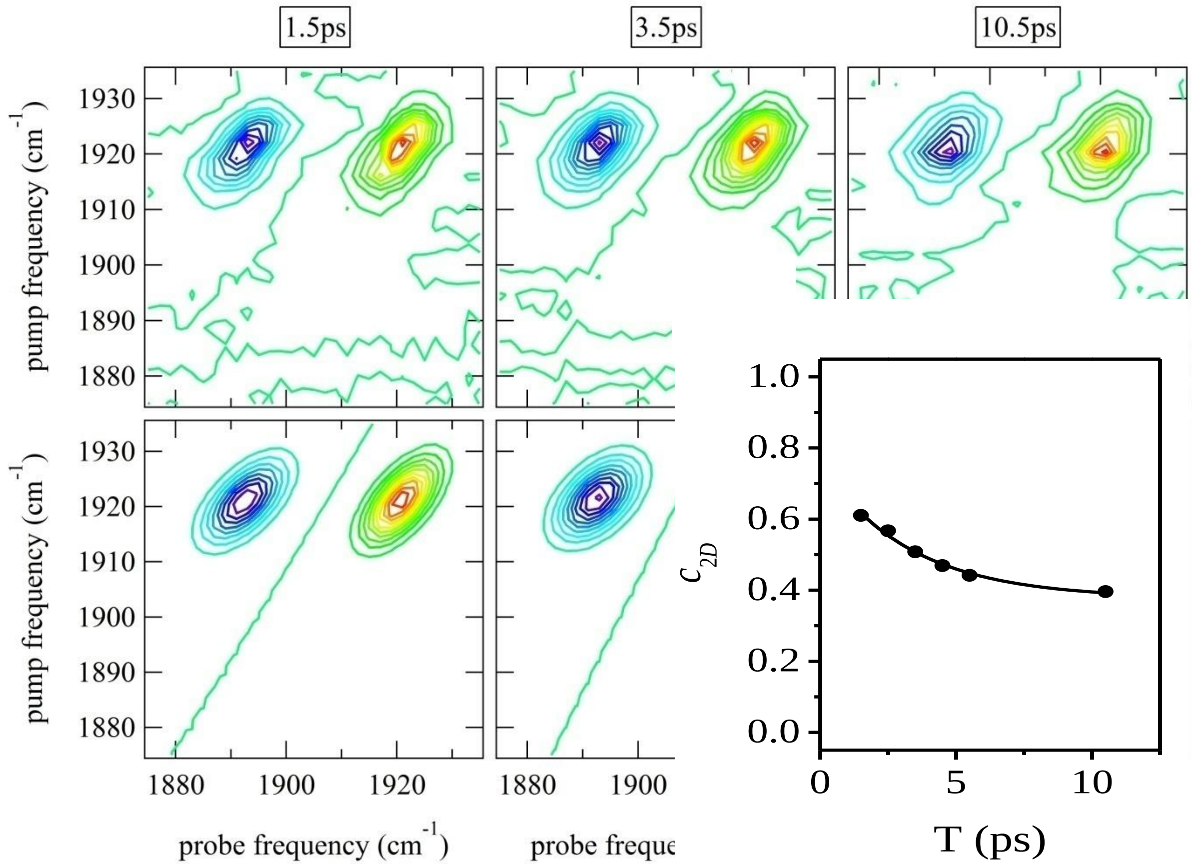
Mb-NO FTIR data implies single conformational state

Peak is inhomogeneously broadened and undergoes spectral diffusion

Spectral diffusion dynamics show a 3 ps decay and a substantial static offset

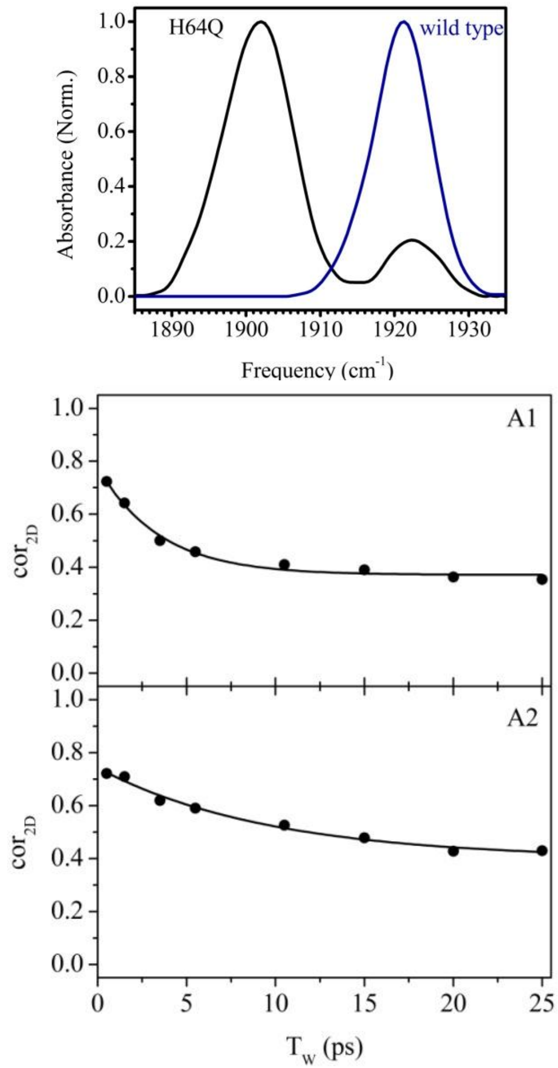
Mb

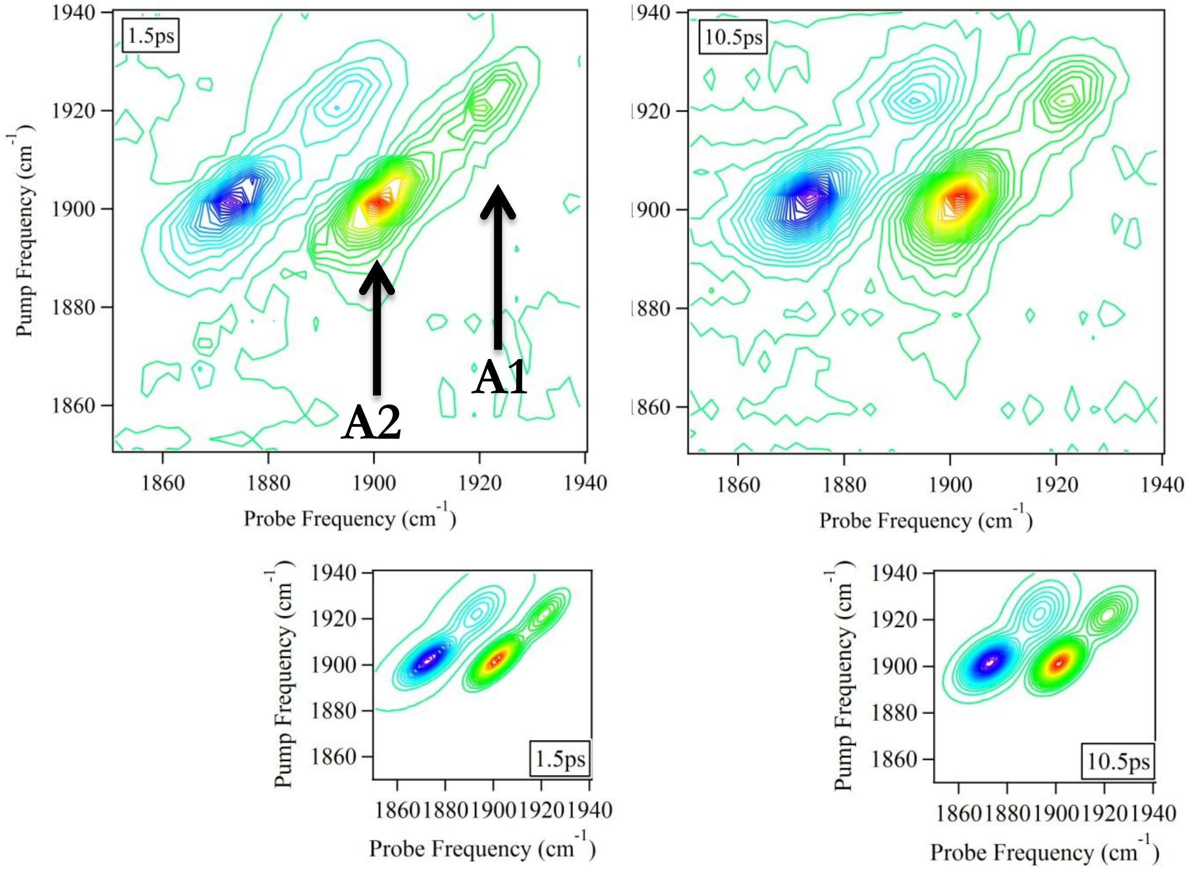




PCCP **14**, 7411 (2012)

H64Q Mb Fe^{III}-NO

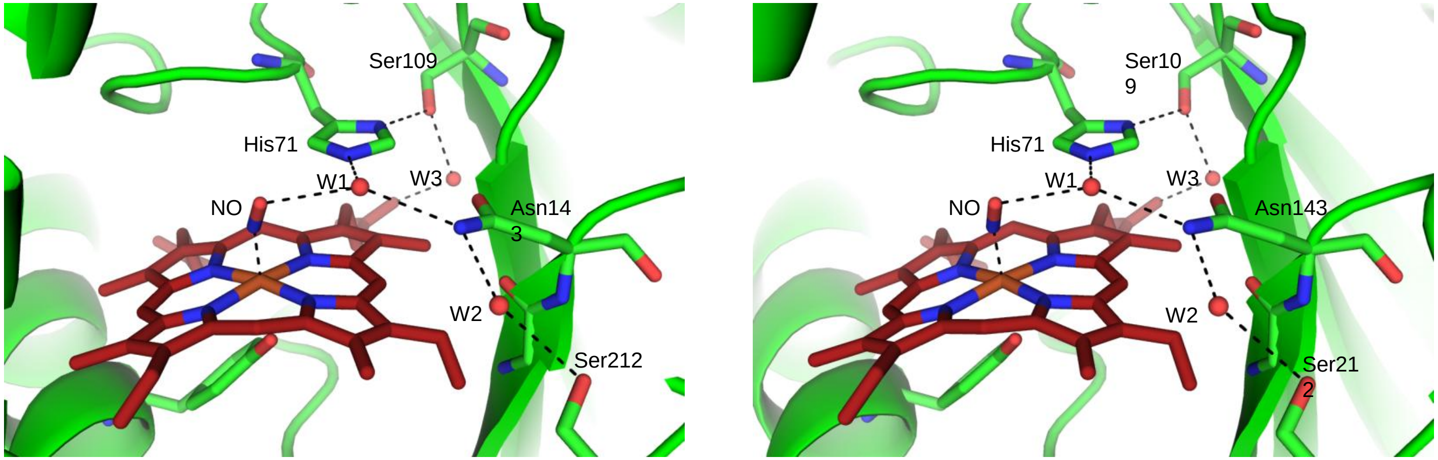




Mutating distal His residue to a Gln leads to markedly different dynamics – effect of interactions between NO probe and side chains?

PCCP **14**, 7411, (2012)

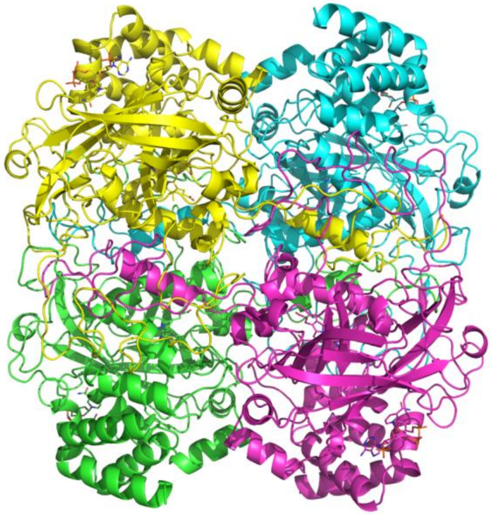
Catalase X-ray diffraction



Catalase dynamics are similar to wt-Mb and **consistent with a distal His** residue but lack of static component is a significant contrast

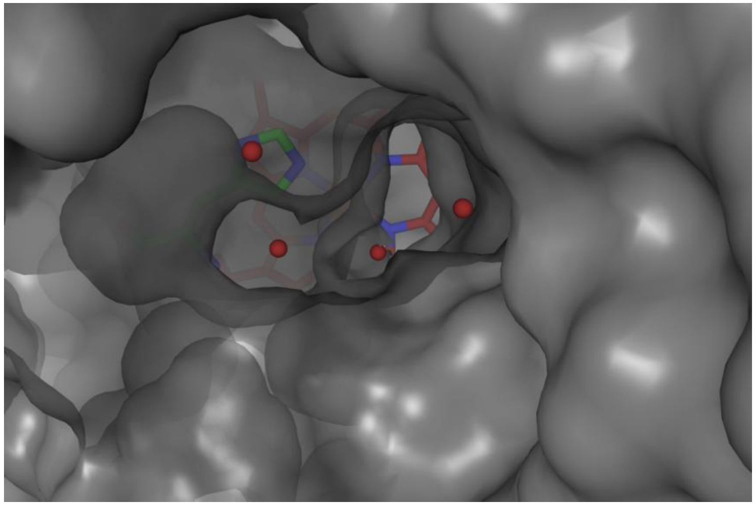
Crystal structure shows chain of **'bound' water molecules** that are conserved in bacterial and bovine catalases and geometry indicates shorter distance and preferential interaction angle between water and NO than His

Similar water does not exist in Mb structure



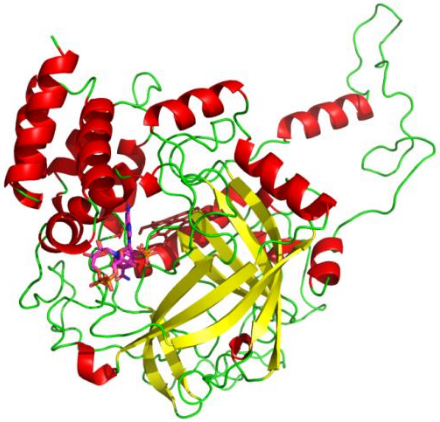
Conclusions/Summary

Despite similar structural features *near the ligand,* Catalase-NO and Mb-NO show some differences in spectral diffusion dynamics.



Does the lack of a static component indicate a substantial change in the protein structural dynamics related to inhibition? Does the bound water 'chain' contribute to this?

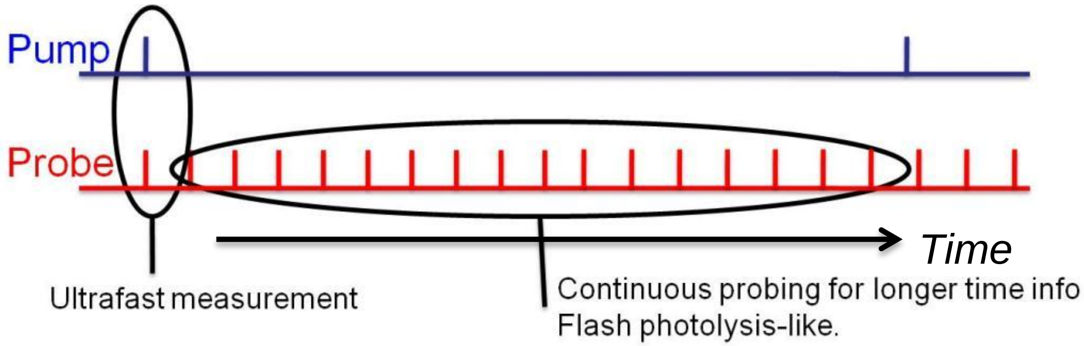
Mutation of Mb suggests a role for the distal residue side chain in determining short timescale dynamics. Though water may contribute to these in Catalase.



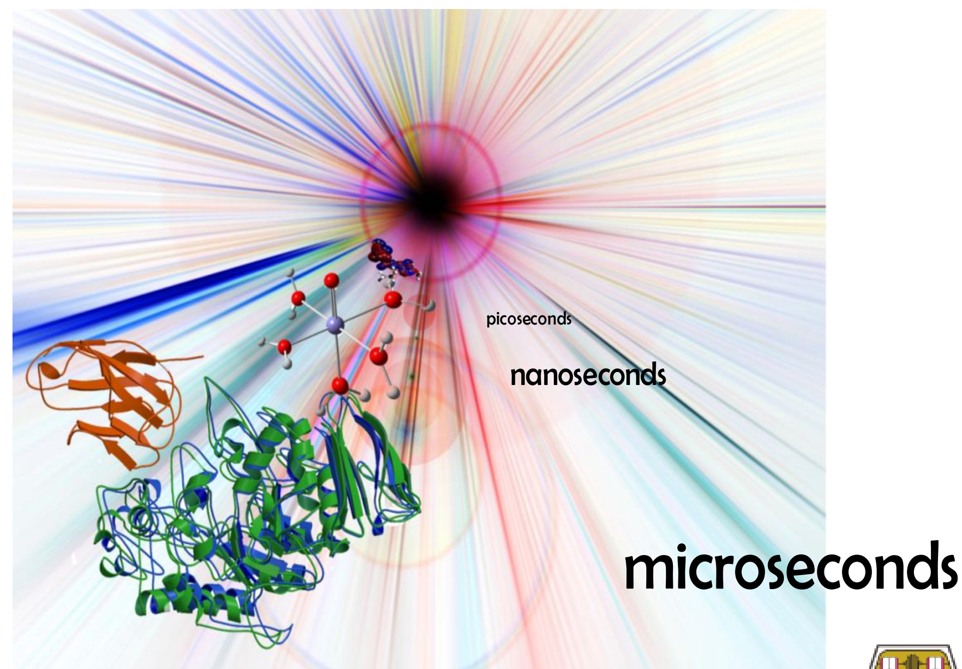
Does the water play any role in the ligand binding or biochemically-observed inhibition of Cat by NO?

The future: TR^{M}PS

PI: Mike Towrie



- Time-Resolved Multiple Probe Spectroscopy combines different repetition rate lasers in a pump – probe – probe… configuration.
- Currently we measure femtosecond to microscecond dynamics in a single pump measurement.
- Covering > 10 orders of timescales accesses a wide range of nature's timescales.

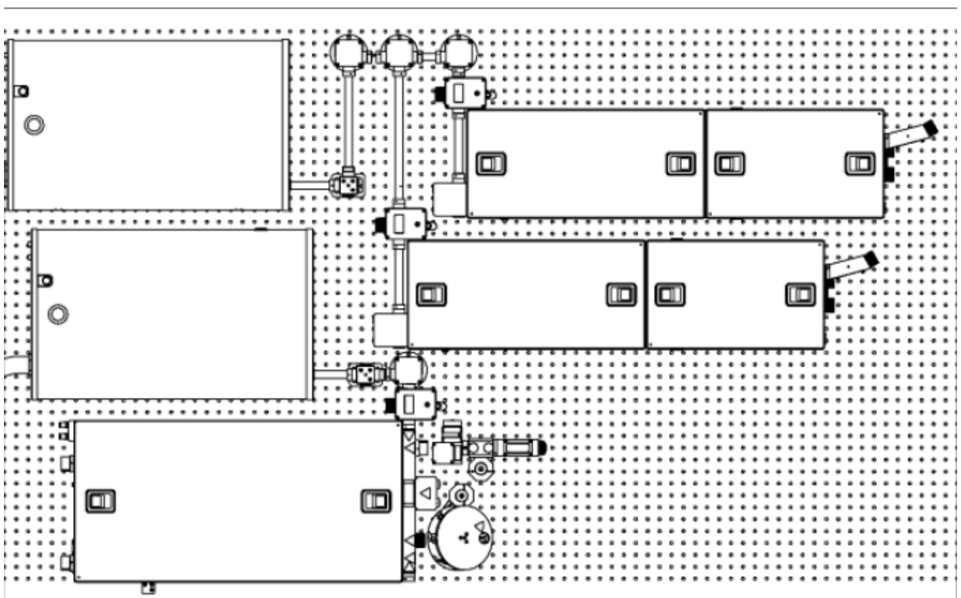


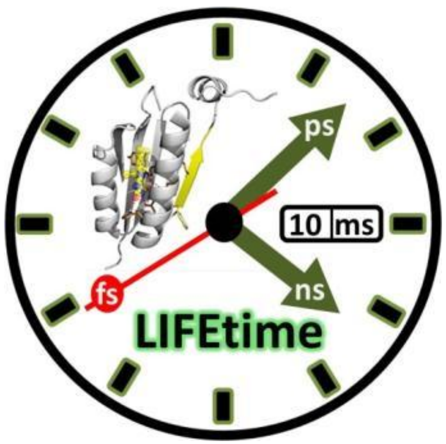
LIFEtime

BBSRC bioscience for the future

PI: Mike Towrie

- LIFEtime is a new BBSRC-funded 100 kHz laser and detection system currently being installed at the Research Complex at Harwell
- Capability to measure molecular changes with higher sensitivity or TR^{M}PS measurements probing 10x more time points per pump pulse.





Acknowledgements

EPSRC Engineering and Physical Sciences Research Council

Science & Technology Facilities Council

European Research Council erc

Niall Simpson

Strathclyde SIPBS
Nick Tucker
Paul Hoskisson
Kirsty Robb
Cesar Bellota-Anton

Diamond Light Source: MX I04-1
Martin Walsh,
Andrea Gumiero

Lasers for Science Facility: ULTRA
Tony Parker,
Greg Greetham
Michael Towrie