Life Sciences at ISOLDE Using PAC-spectroscopy

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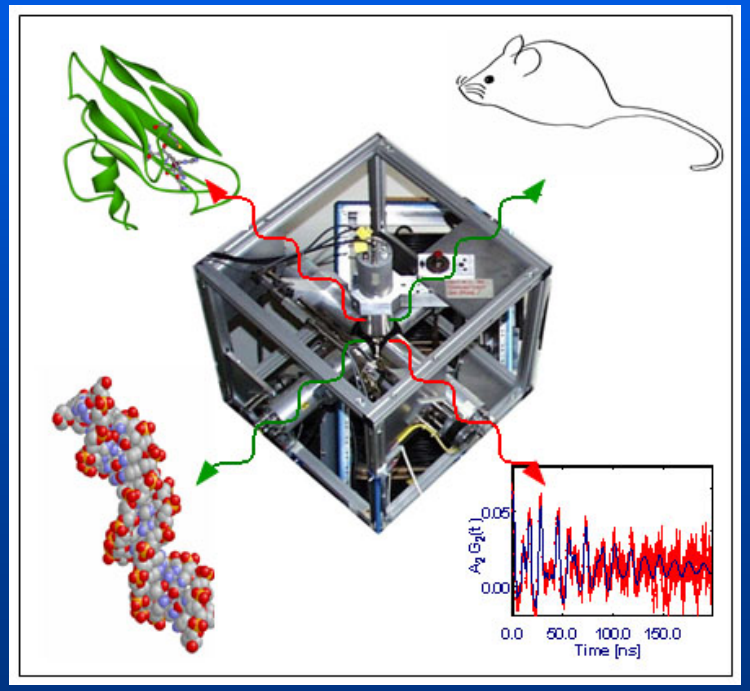
Outline

- Why Spectroscopy?
- Perturbed angular correlation (PAC) of γ-rays
- Why ISOLDE?
- Selected examples
- Advantages and limitations



Why Hyperfine Spectroscopy on Biomolecules?

Structural information is usually obtained by single crystal diffraction

What to do if the biomolecules do not crystallize?

What to do if only very small amounts of material are available?

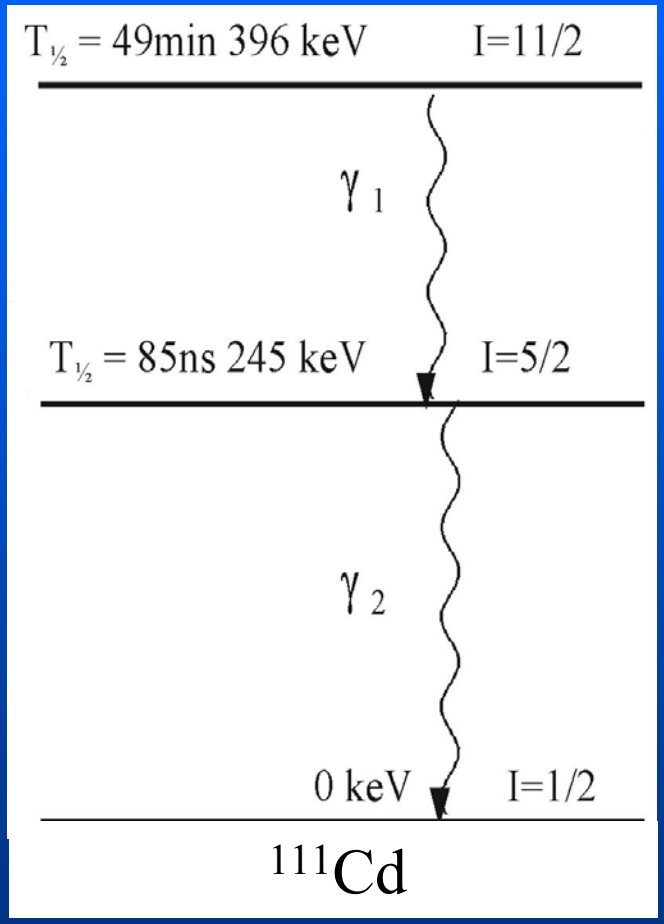
Sometimes internal dynamics renders entire domains „invisible“ by diffraction, e.g. in trypsinogen. Often internal dynamics is crucial for the function.

Solution:

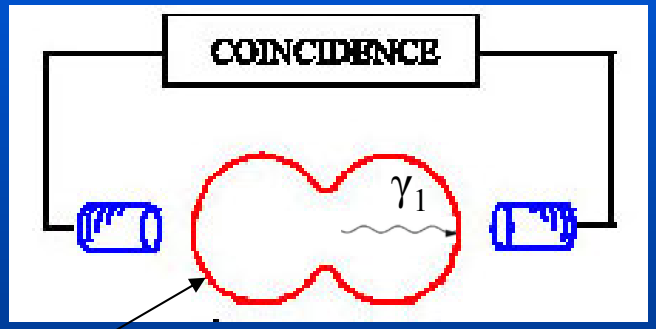
Spectroscopy like EPR, Mössbauer, Perturbed Angular Correlation

Requires isomorphous replacements with suitable isotopes

Angular correlation of γ-rays:

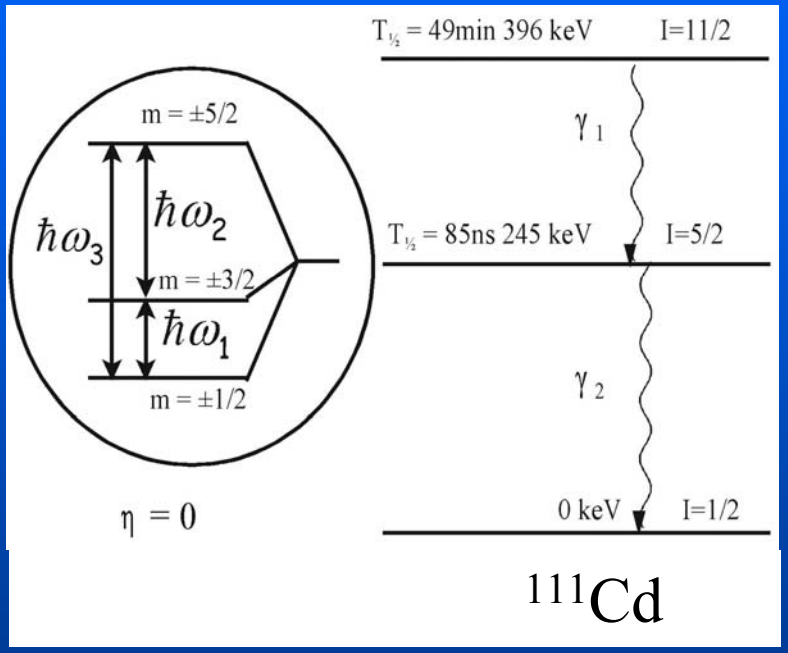


Angular correlation of γ-rays is a property of the nuclear decay:



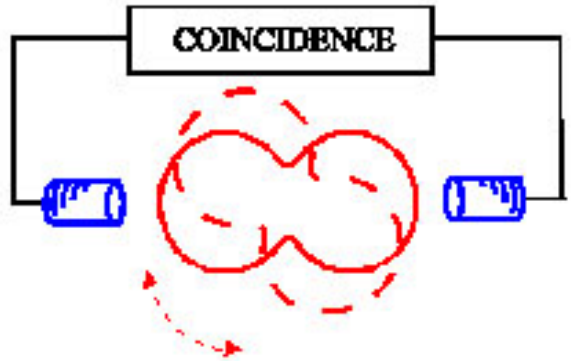
The distance from the center to the curve gives the probability of emission of γ_2 in that direction

Perturbed angular correlation of γ-rays: The influence of extra-nuclear fields

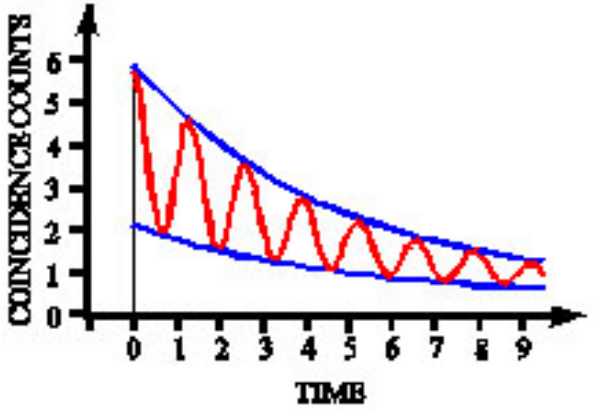


^{111}Cd

Hamilton *Phys. Rev.*, **1940**, 58:122, Brady and Deutsch *Phys. Rev.* **1947**, 72:870, Goertzel *Phys. Rev.*, **1946**, 70:897, Aeppli et al. *Phys. Rev.*, **1951**, 82, 550, Leipert et al. *Nature*, **1968**, 220:907

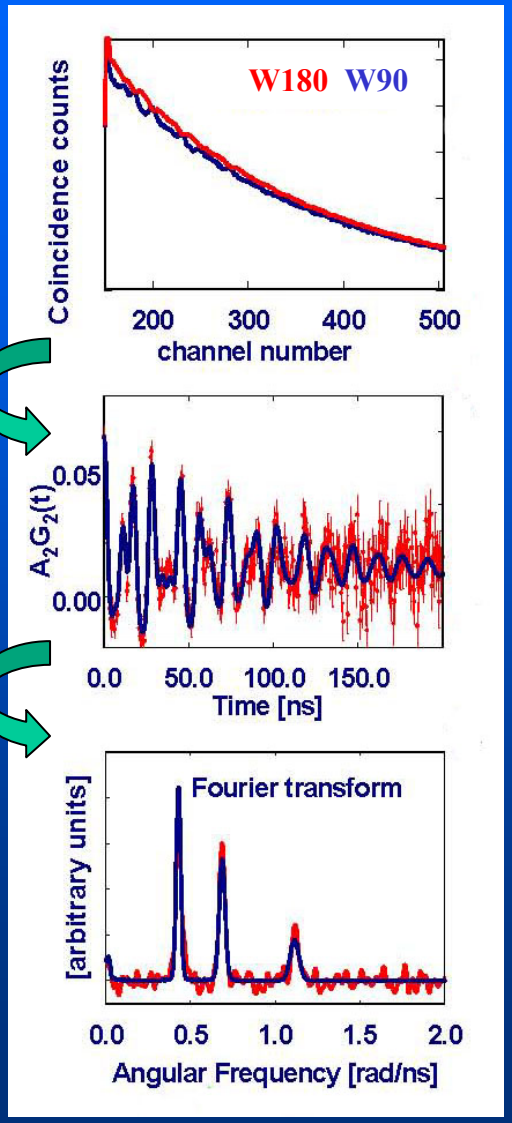


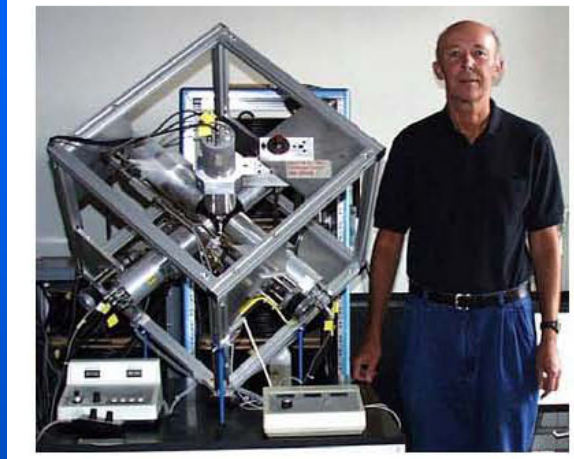
Oscillations in the angular correlation give ω_1, ω_2, and ω_3 – a finger print of the local charge distribution

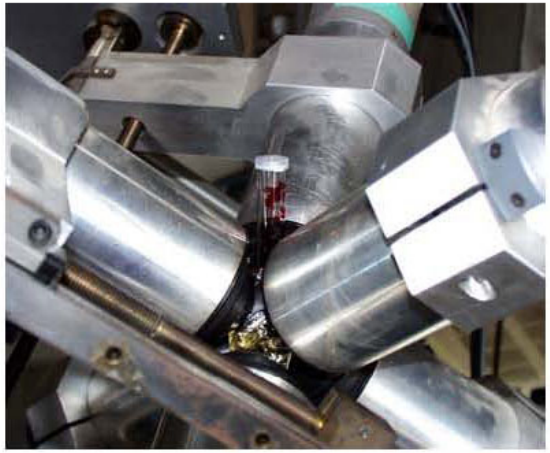


Butz *Z. Naturforsch.* **1996**, 51A:396

PAC in a nutshell

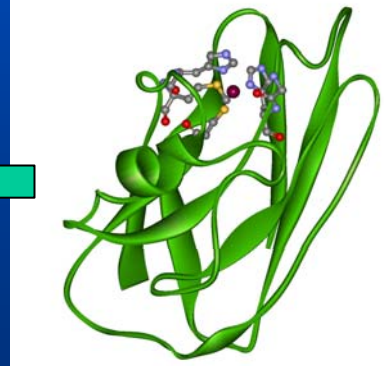






Compare

Calculate spectroscopic data based on putative structure



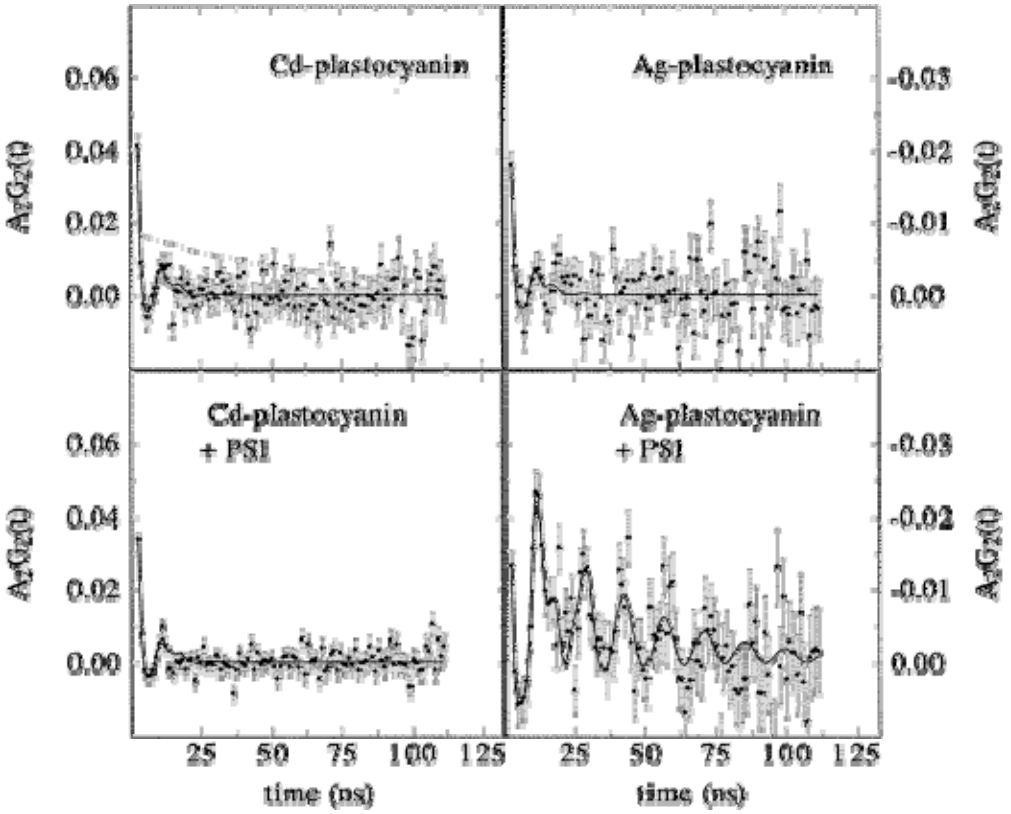
L. Hemmingson, Copenhagen

Why ISOLDE?

- Isomorphous replacement should be performed with isotopes which have isomeric states; otherwise, chemical transmutation can cause after-effects.
- The additional requirement of a cascade with a sufficiently longlived intermediate state narrows down the number of suitable radioisotopes.
- Suitable PAC-isotopes are e.g. ^{111m}Cd, ^{199m}Hg, ^{204m}Pb; all happen to be shortlived (about 1 hour) and have to be produced on-line.
- Because of the large molecular weight of the biomolecules no-carrier added activity is required.
- ISOLDE meets all these requirements !

Protein-protein interactions

Plastocyanin binding to photosystem 1



Cu-plastocyanin (wildtype) binds to PS1
Ag-plastocyanin binds to PS1, but Cd-plastocyanin does not.
(^{111}Ag decays to ^{111}Cd)

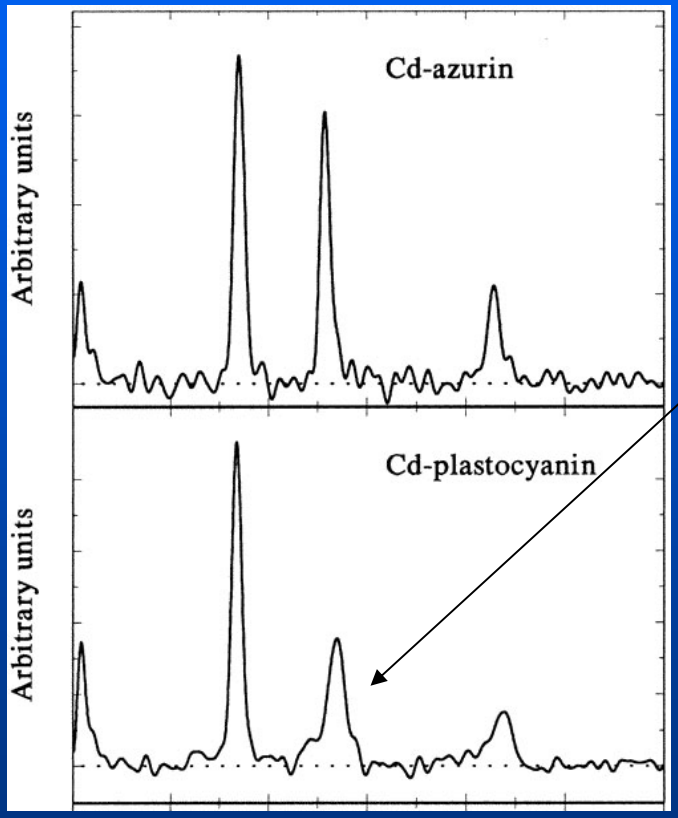
^{111}Ag mimicks Cu^{+}
^{111}Cd mimicks Cu^{++}

Note: The experiments were carried out without added sucrose.

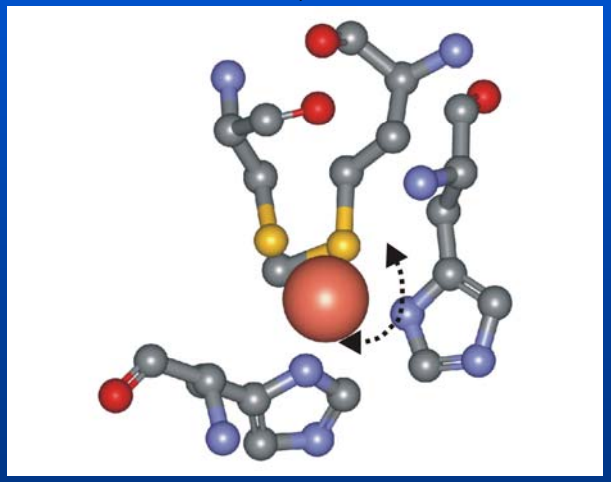
Danielsen et al. *Biochemistry*, **1999**, 38:11531

Metal ion binding site structure and dynamics

Azurin (*P. aeruginosa)* and plastocyanin (spinach)

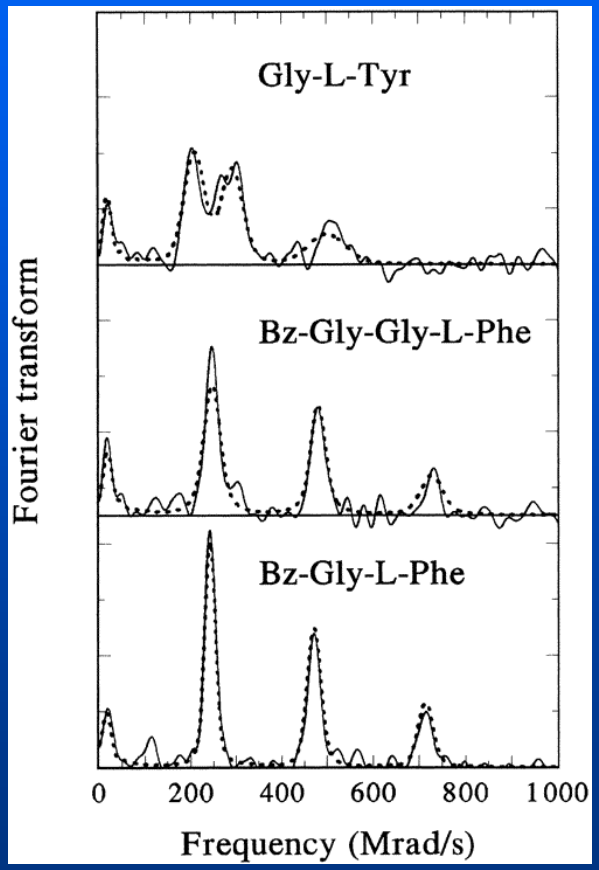


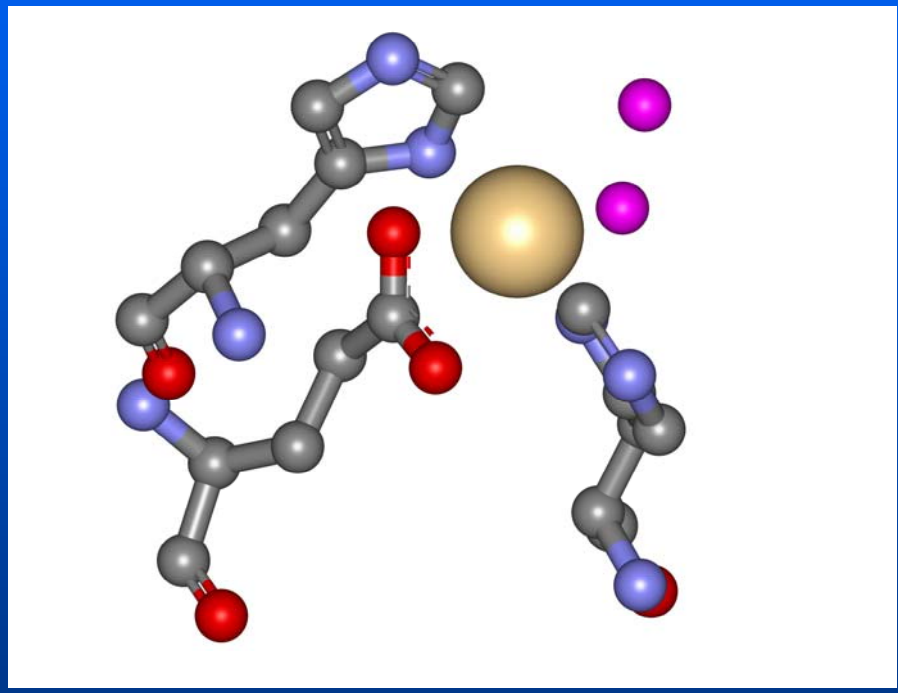
Linewidth reveals internal motion in plastocyanin



Bauer et al. *JACS*, **1997**, 119:157, Tröger et al. *Z. Naturforsch.*, **1996**, 51A:431, Danielsen et al. *Biochemistry*, **1999**, 38:11531,

Metal ion binding site structure during catalysis (bovine Carboxypeptidase A)



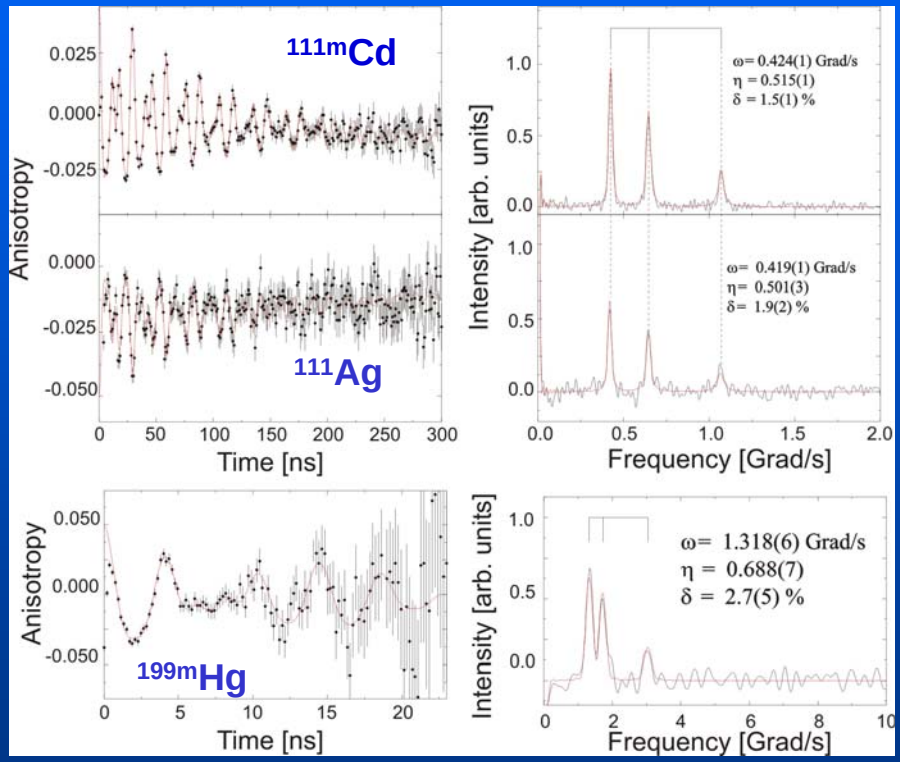


Bauer et al. *Biochemistry*,**1997**, 36:11514

Results on Azurin with ^{111m}Cd and ^{199m}Hg

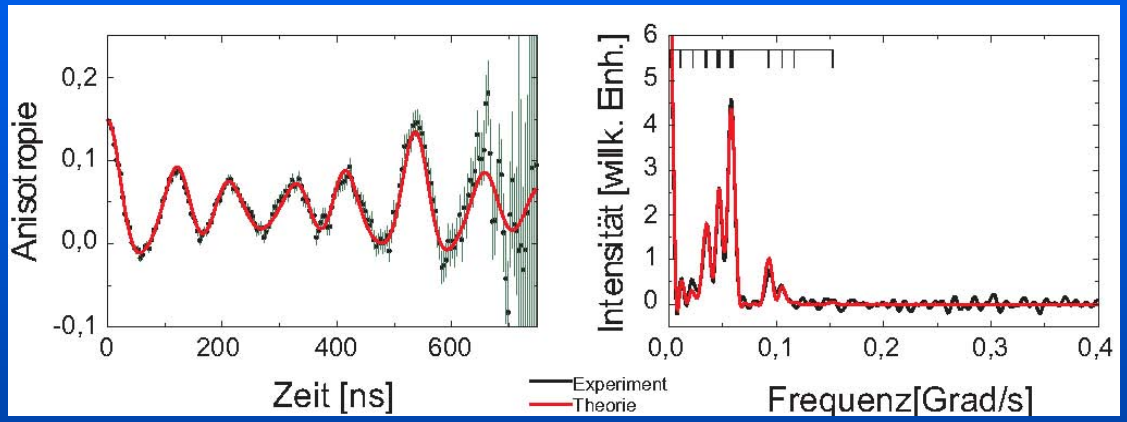
Experiments performed at ISOLDE

Azurin
a small electron transfer protein



W. Tröger and T. Butz, Hyp. Int. **129** (2001) 511

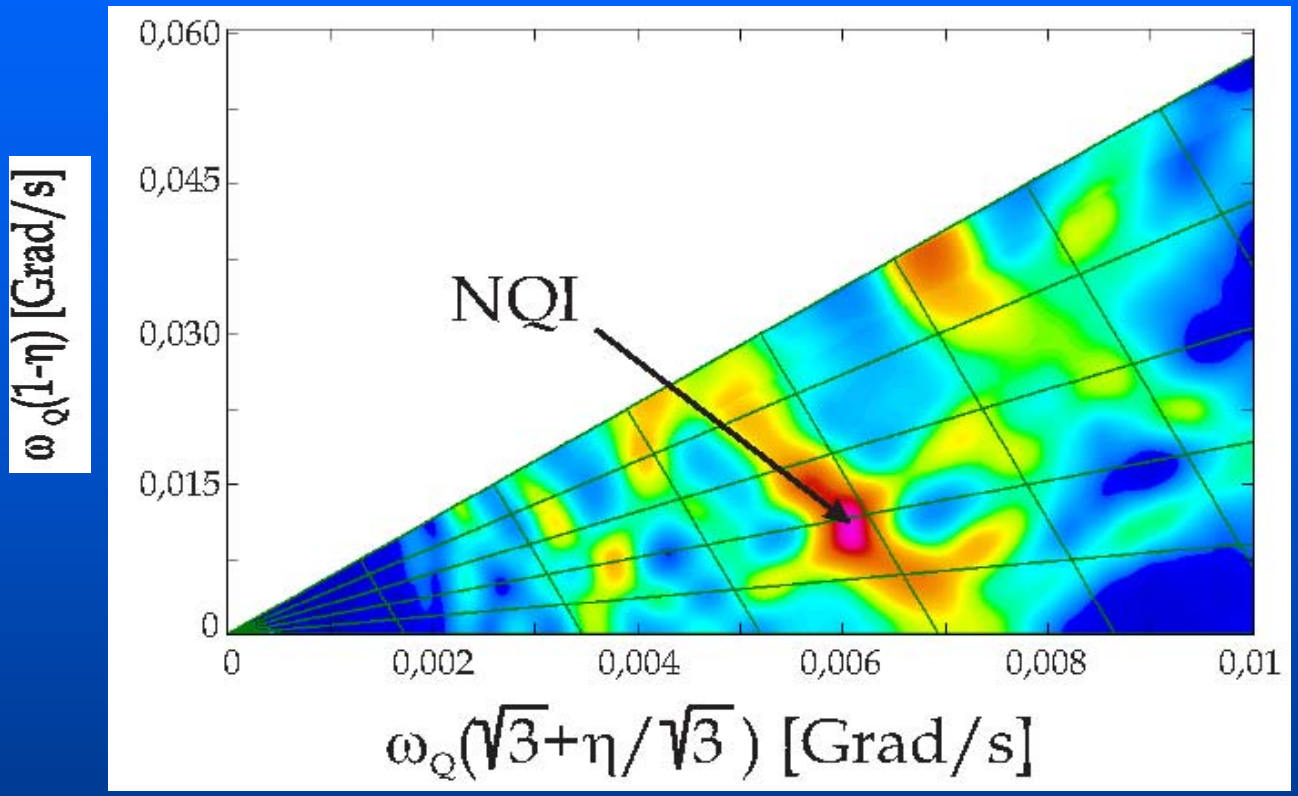
First results with $^{204m}PbBr_2$ at ISOLDE



Complication: I = 4 → many spectral lines

Sven Friedemann, Diploma Thesis, **2004**, U. Leipzig
Frank Heinrich, PhD thesis **2005**, U. Leipzig

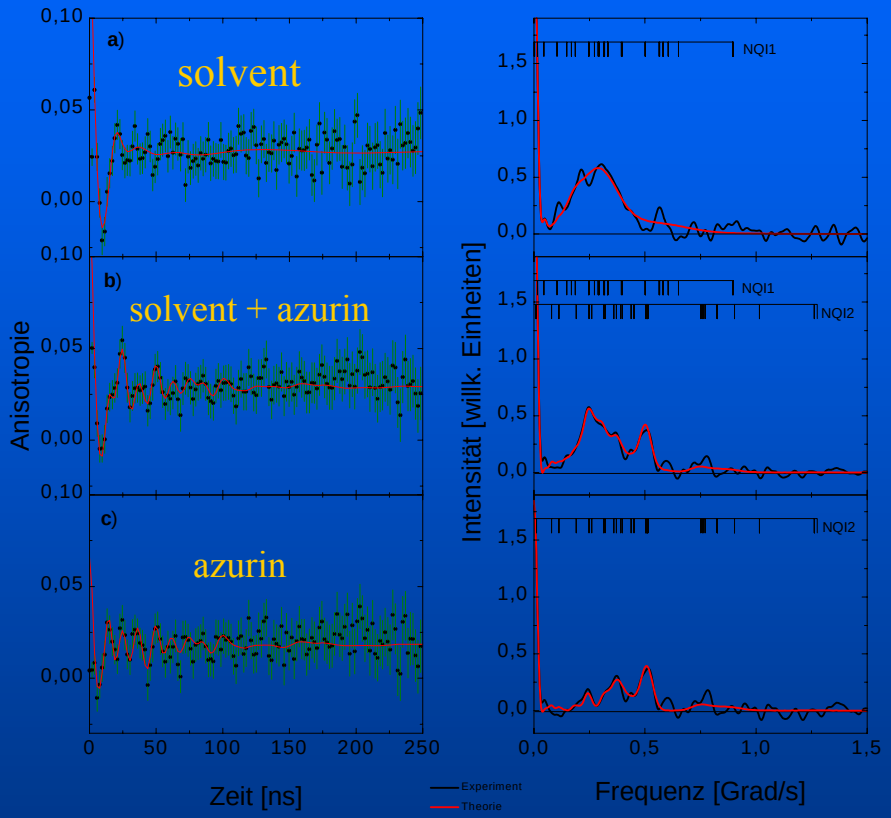
First results with ^{204m}Pb



Solution: Cross-Correlation compresses information into a spot

Sven Friedemann. Diploma Thesis, **2004**, U. Leipzig

First results with ^{204m}Pb in Azurin



probe	V_{zz} (a.u.)	η
^{111m}Cd	1.84	0.52
^{199m}Hg	6.21	0.70
^{204m}Pb	4.26	0.50

Similar η means rigid metal coordination

Result: incomplete isomorphous replacement

Frank Heinrich, PhD Thesis, **2005**, U. Leipzig

Metal ion containing proteins studied by PAC-spectroscopy

- Carbonic anhydrase B and C
- Carboxypeptidase A
- Superoxide dismutase
- Angiotensin converting enzyme
- β-lactamases
- Alcohol dehydrogenase
- Insulin
- Metallothionein
- Laccase
- Ascorbate oxidase
- Azurin and mutants
- Plastocyanin
- Stellacyanin
- Hemocyanin
- Serum transferrin
- Ovotransferrin
- Lactoferrin
- Rubredoxin
- Nitrogenase FeMo cofactor
- Molybdenum storage protein
- Mercury reductase
- De novo designed peptides
- Peptides (Cys, His, Tyr containing)

Related studies:

- Binding of cadmium to DNA
- Porphyrin and phtalocyanine
- DTPA labelled proteins
- In vivo studies of mice

Hemmingsen et al. *Chem. Rev.*, **2004**, accepted

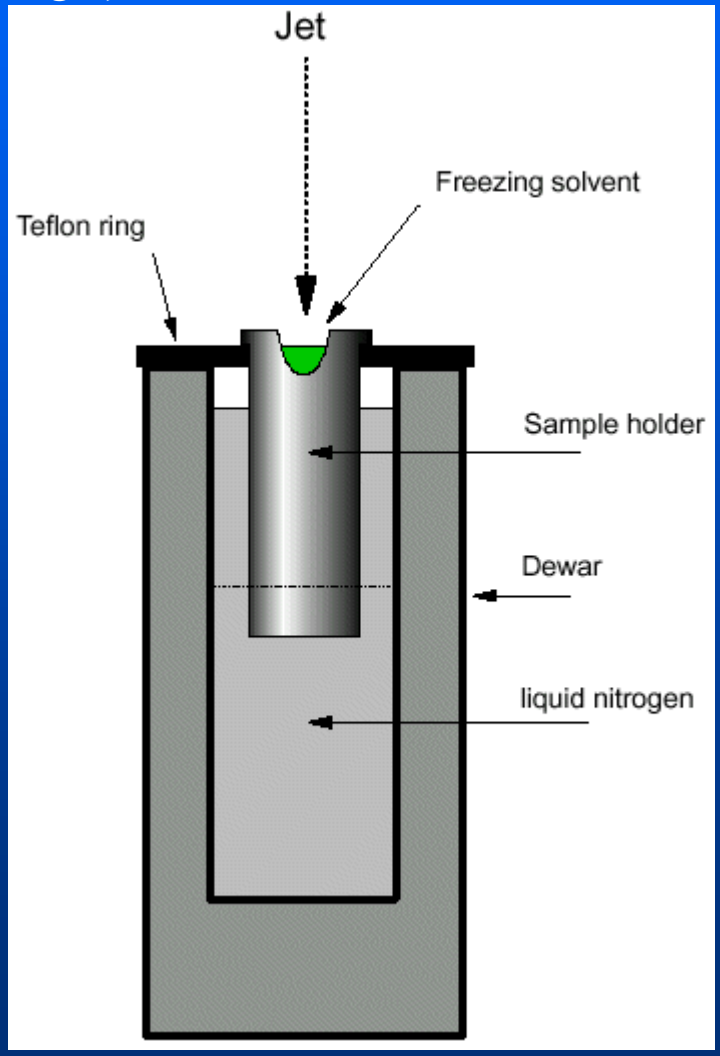
Outlook: Freeze quench PAC spectroscopy

Snap shots of structures evolving during enzyme catalyzed reactions

(Future research planned by L. Hemmingsen, Copenhagen)

Enzyme

Substrate



Advantages and limitations of PAC-spectroscopy

Advantages:

- Metal ion binding site structure and dynamics can be measured
- Protein-protein and protein-membrane interactions can be measured
- High sensitivity to structural changes
- Small amounts of sample needed (in principle 1 pmol, but 100 nM for ^{111m}Cd substituted proteins)
- Different physical states (crystals, solutions, in vivo...)
- Mechanically stable, allowing for stirring, flow, ...

Limitations:

- PAC isotope must bind strongly to the molecule of interest
- Spectral parameters do not uniquely determine structure
- After effects can cause problems (in particular for EC)
- Production of PAC-isotopes

Researchers involved

- Copenhagen, Denmark
  - Rogert Bauer († 2004)
  - Lars Hemmingsen
  - Mikael Jensen
  - Lars Olsen
  - Eva Danielsen, Klara N. Sas
  - Morten J. Bjerrum
  - Jens Ulstrup & group
  - Henrik V. Scheller
- University of Leipzig, Germany
  - Tilman Butz
  - Wolfgang Tröger
  - Frank Heinrich
  - Sven Friedemann
- Saarland University, Gemany
  - Hans Werner Adolph
  - Michael Zeppezauer & group
- Lund University, Sweden
  - Eila Cedergren-Zeppezauer
  - Ulf Ryde
- University of Michigan, USA
  - Vincent L. Pecoraro & group
- Göteborg University, Sweden
  - Örjan Hansson
- Harvard Medical School, USA
  - David S. Auld