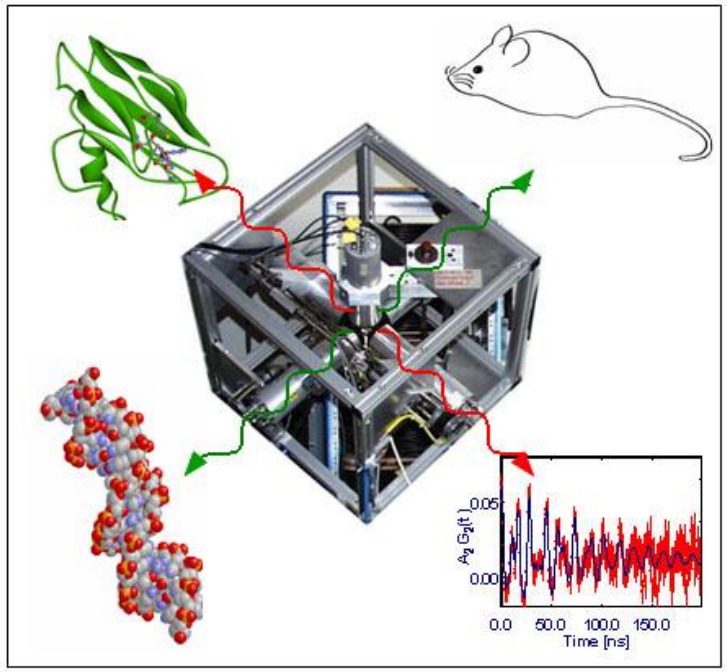
Department of Basic Sciences and Environment - IGM
Faculty of Life Sciences

Biochemical applications of perturbed angular correlation of γ-rays (PAC) spectroscopy

Lars Hemmingsen (lhe@life.ku.dk), HFI/NQI 2010

Outline

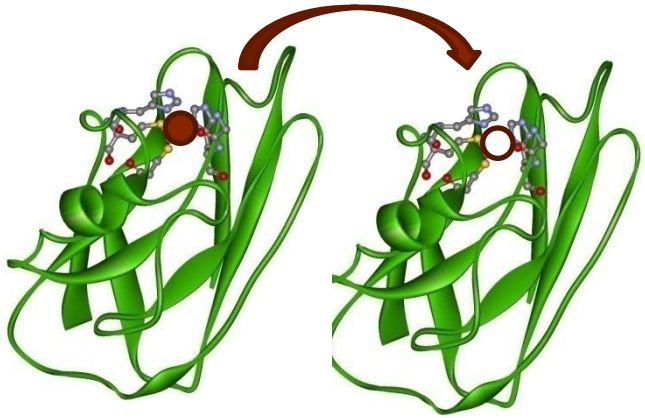
- Bio PAC introduction
- Selected examples:
  - *De novo* design of metal ion binding sites in proteins
  - Protein folding and misfolding
  - Catalysis – enzymes
  - Protein-protein interactions
  - *In vivo* experiments



Bio PAC sample preparation

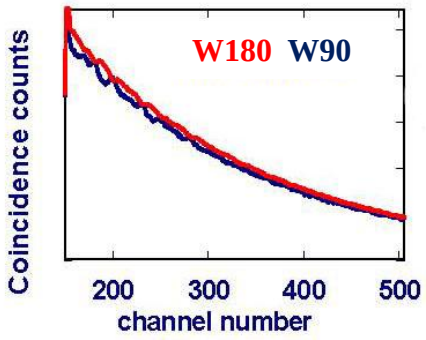
ISOLDE/CERN Copenhagen

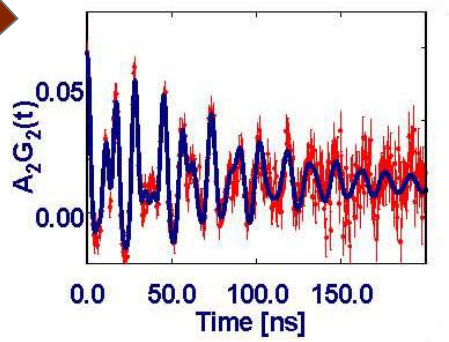
Metal ion replacement

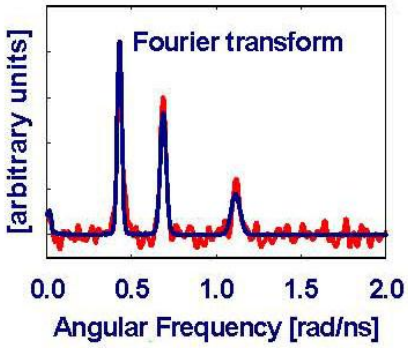


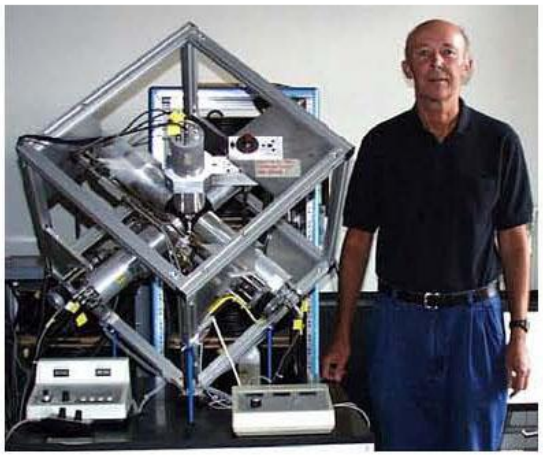
Biomolecule in buffered solution
+ PAC probe (+carrier)
& immobilisation (sucrose, flash freeze, precipitation)

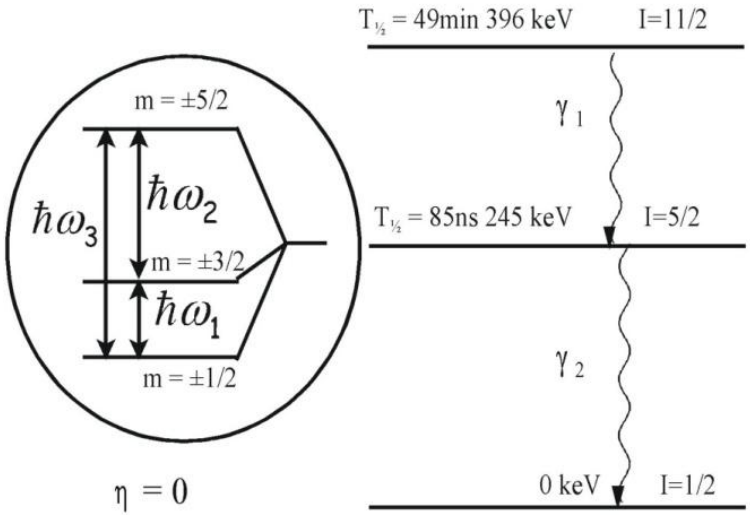
PAC in a nutshell







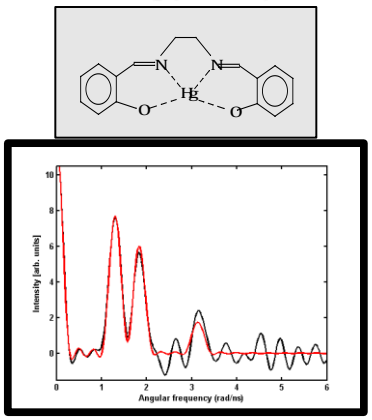




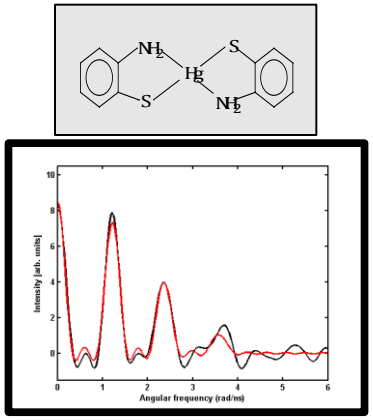
^{111m}Cd

Interpretation of data: Model complexes and first principle calculations

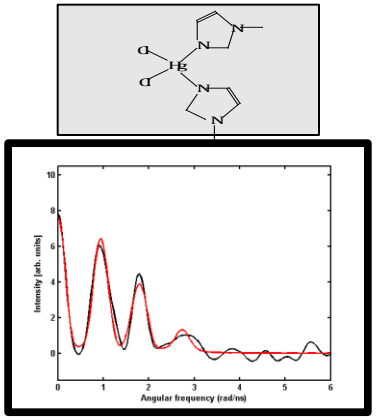
Hg(salen)



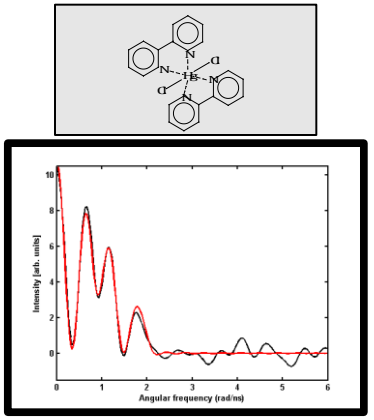
$Hg(2\text{-mercaptoanilin})_2$



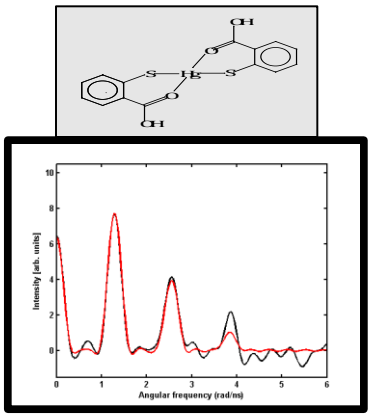
$Hg(1\text{-methylimidazole})_2Cl_2$



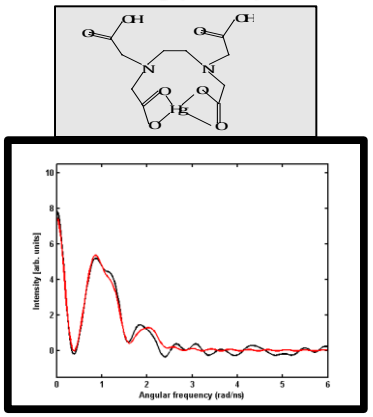
$Hg(2,2'\text{-bipy})_2Cl_2$



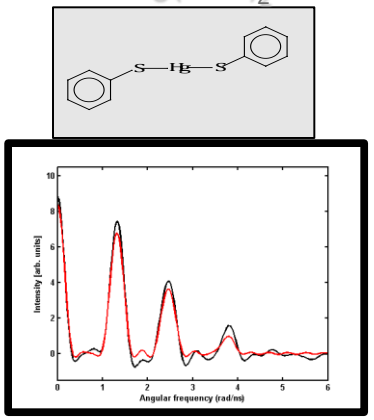
$Hg(thiosalicylate)_2$



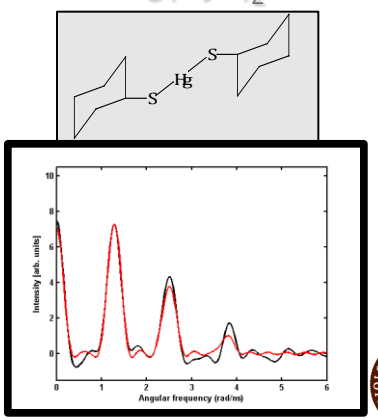
Hg(edta)



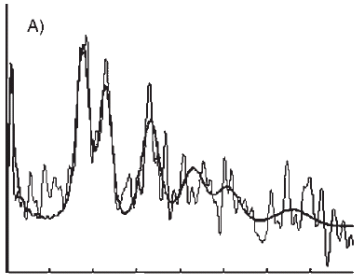
$Hg(PhS)_2$



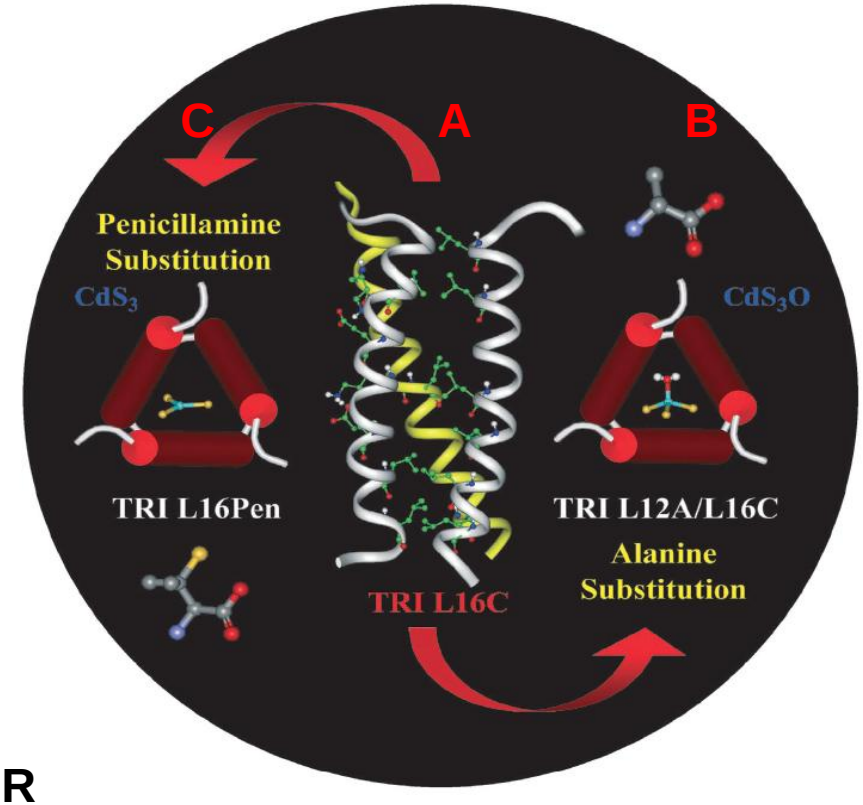
$Hg(CyS)_2$

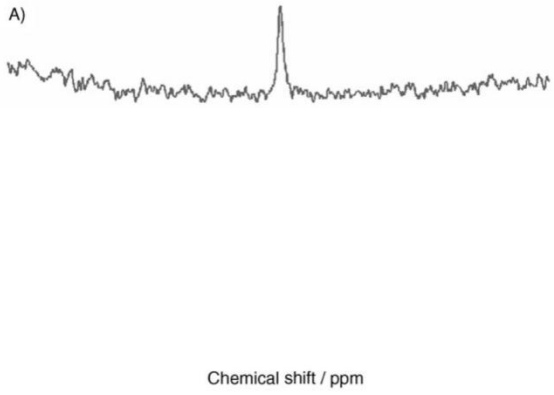


De novo designed heavy metal ion binding proteins



^{111m}Cd-PAC

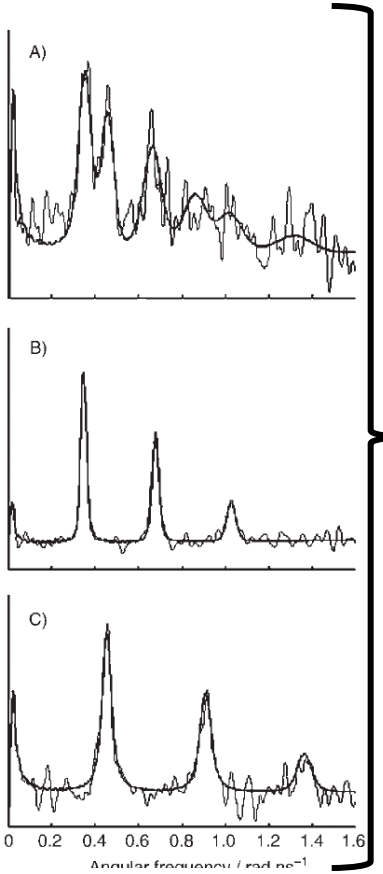




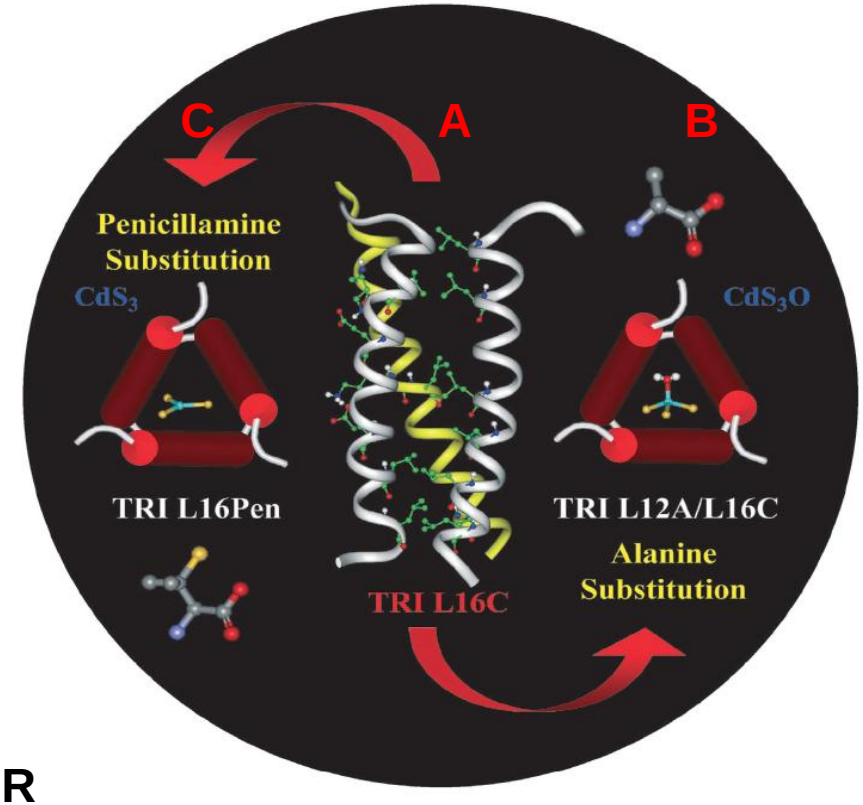
^{113}Cd-NMR

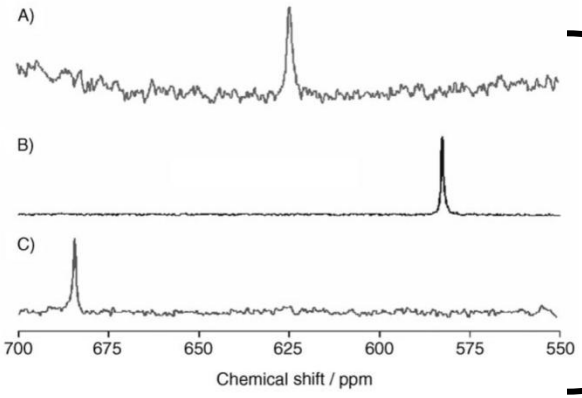
Matzapetakis *et al*. J. Am. Chem. Soc. **2002**, 124: 8042; Lee *et al*. Angew. Chem., **2006**, 45: 2864; Peacock *et al.* Proc. Nat. Acad. Sci. **2008**, 105: 16566

De novo designed heavy metal ion binding proteins



^{111m}Cd-PAC

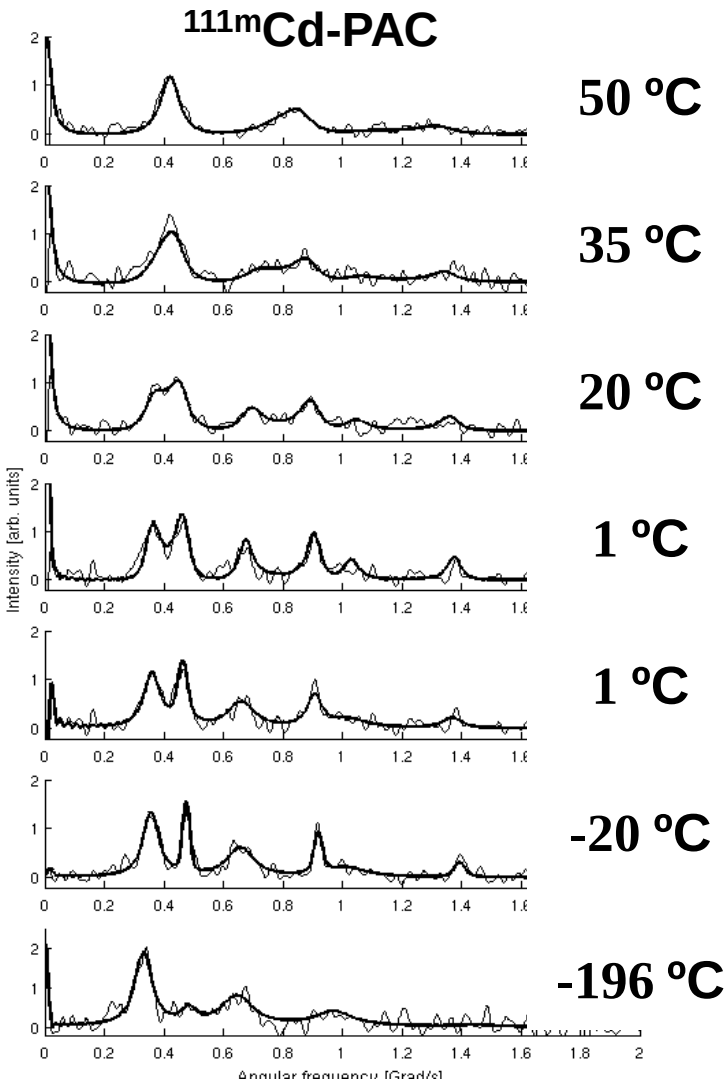


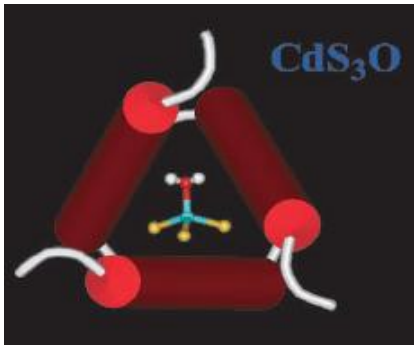


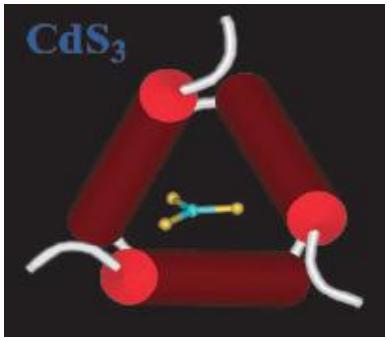
^{113}Cd-NMR

Matzapetakis *et al*. J. Am. Chem. Soc. **2002**, 124: 8042; Lee *et al*. Angew. Chem., **2006**, 45: 2864; Peacock *et al.* Proc. Nat. Acad. Sci. **2008**, 105: 16566

De novo designed heavy metal Ion binding proteins: ns dynamics





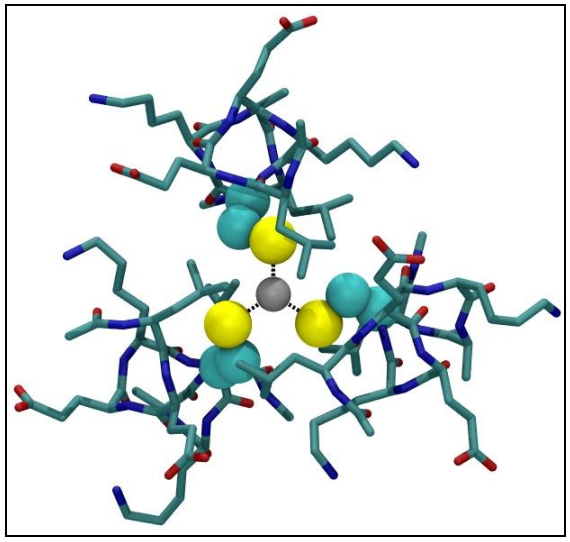


Temp [ºC]	τ_1 [ns]	τ_{-1} [ns]
1	52	48
20	42	36
35	28	20
50	19	12

Stachura et al. Manuscript in preparation

Quantum mechanical structure optimization and property calculation

	ν_Q	η	$\delta_{average}$	Cd-$S_{average}$
	(MHz)		(ppm)	(Å)
41% CdS_3	466 465	0.16 0.20		
			647 624	2.48 2.49
59% CdS_3O	340 355	0.43 0.22		



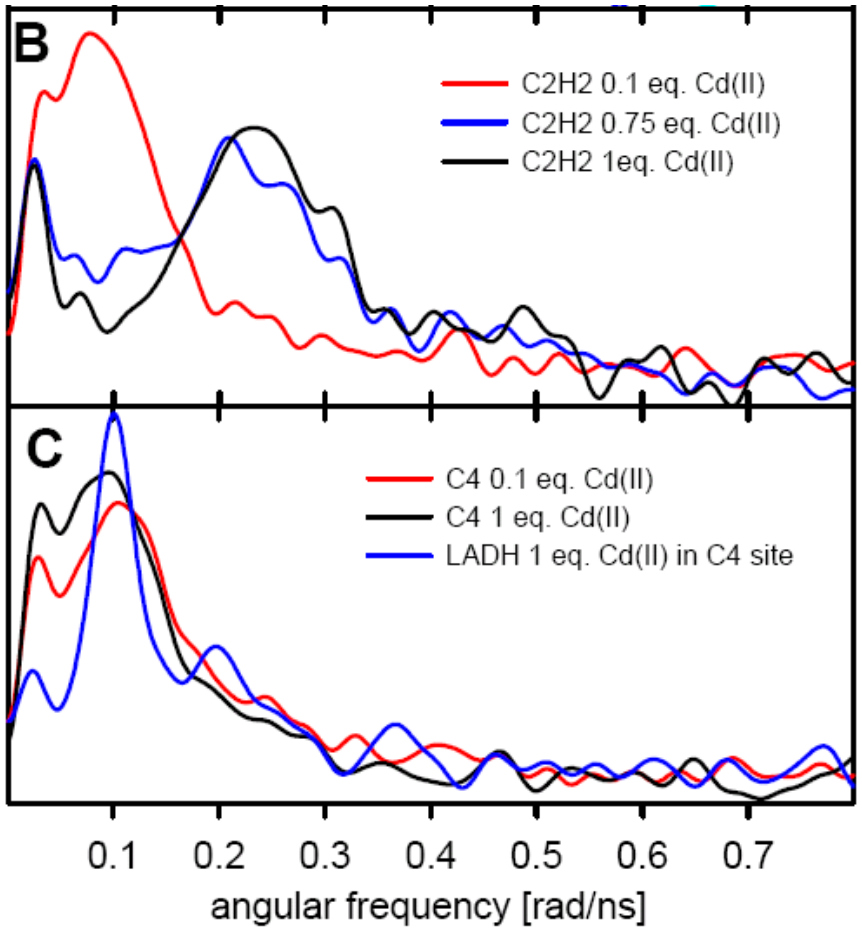
Experimental data:
^{111m}Cd-PAC, ^{113}Cd-NMR, EXAFS

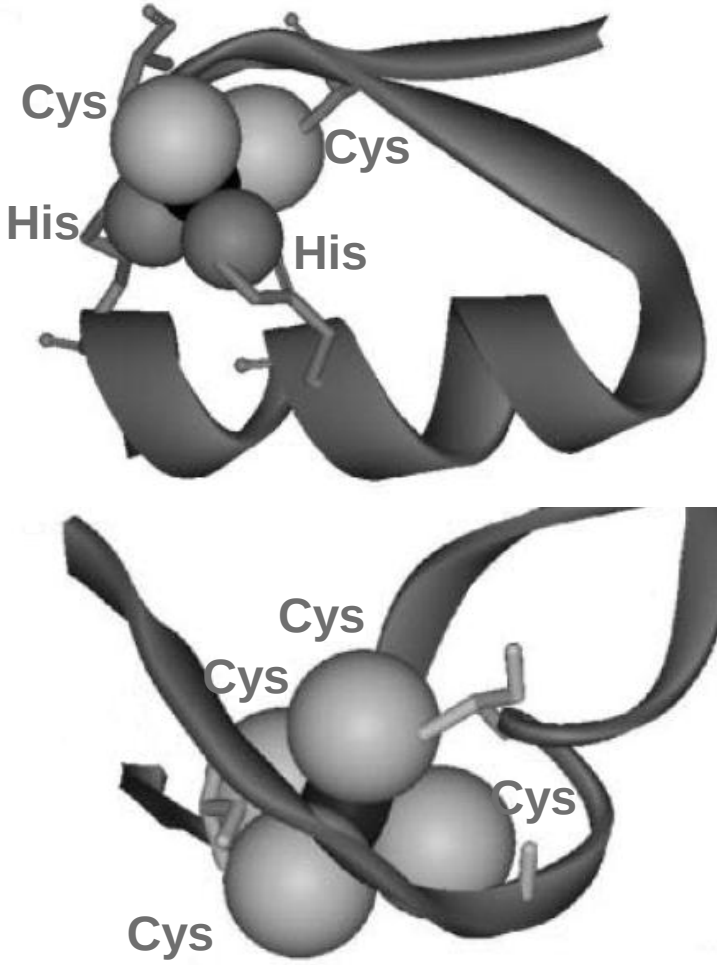
Calculations:
ONIOM (Gaussian03)
PBE1PBE/6-31G(d), LanLDZ and PM3MM for structures;
B3LYP/6-31G(d), Kellö & Sadlej for properties

Hemmingsen et al. J. Biol. Inorg. Chem**. 2004**, 9, 591; Antony et al. J. Phys. Chem. **2000**, 104, 6047; Hemmingsen and Ryde **1996**, J. Phys. Chem. 100, 4803, N.J. Christensen, Master thesis, **2005,** the Technical University of Denmark

Metal ion controlled protein folding and misfolding

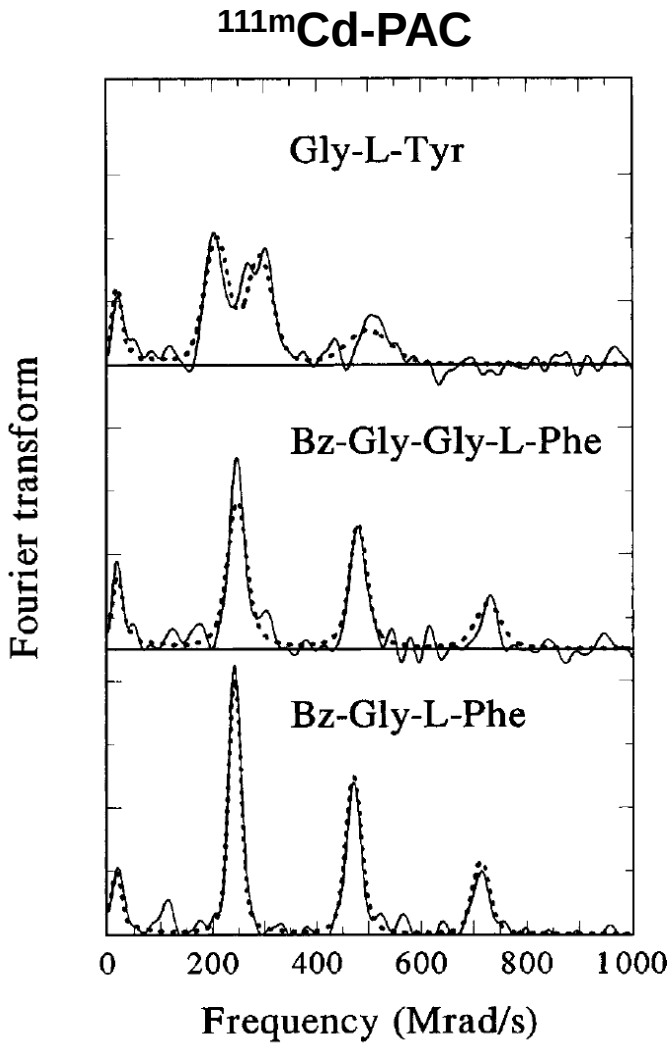
^{111m}Cd-PAC

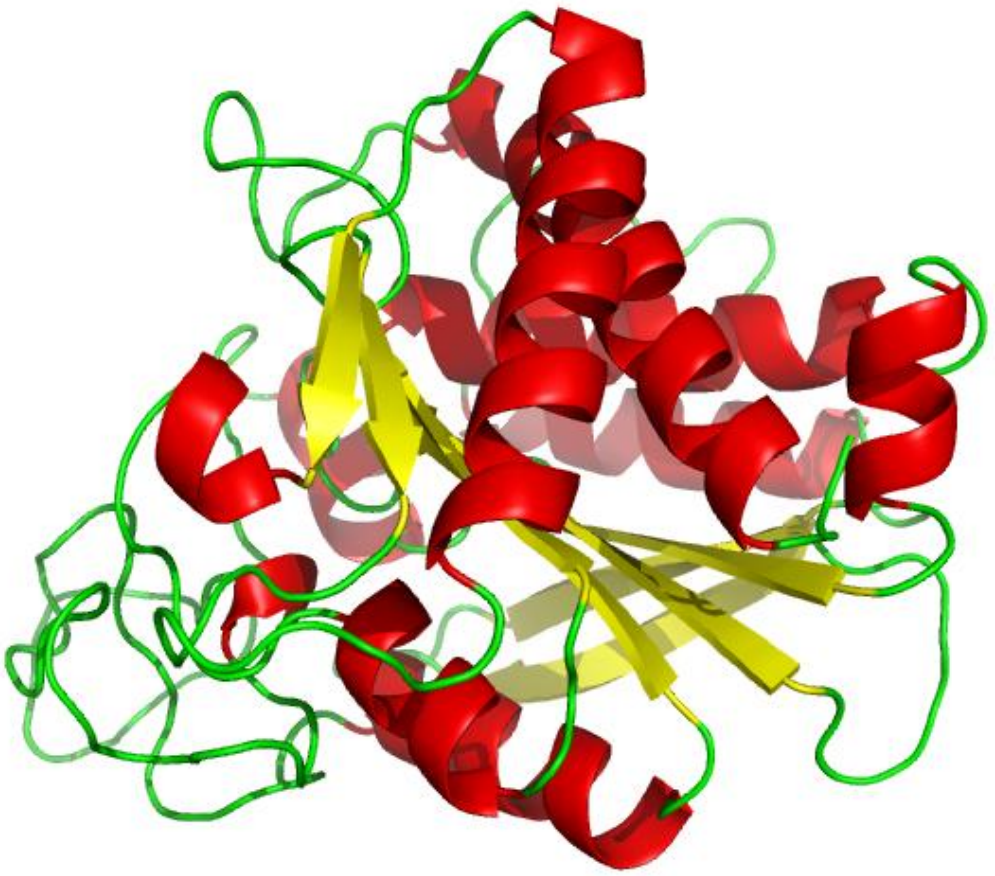




Heinz et al., J. Biol. Chem. **2005**, 280: 3197; Heinz et al., Chem. Eur. J. **2009**, 15:7350.

Metal ion binding site structure during steady state catalysis (carboxypeptidase A)



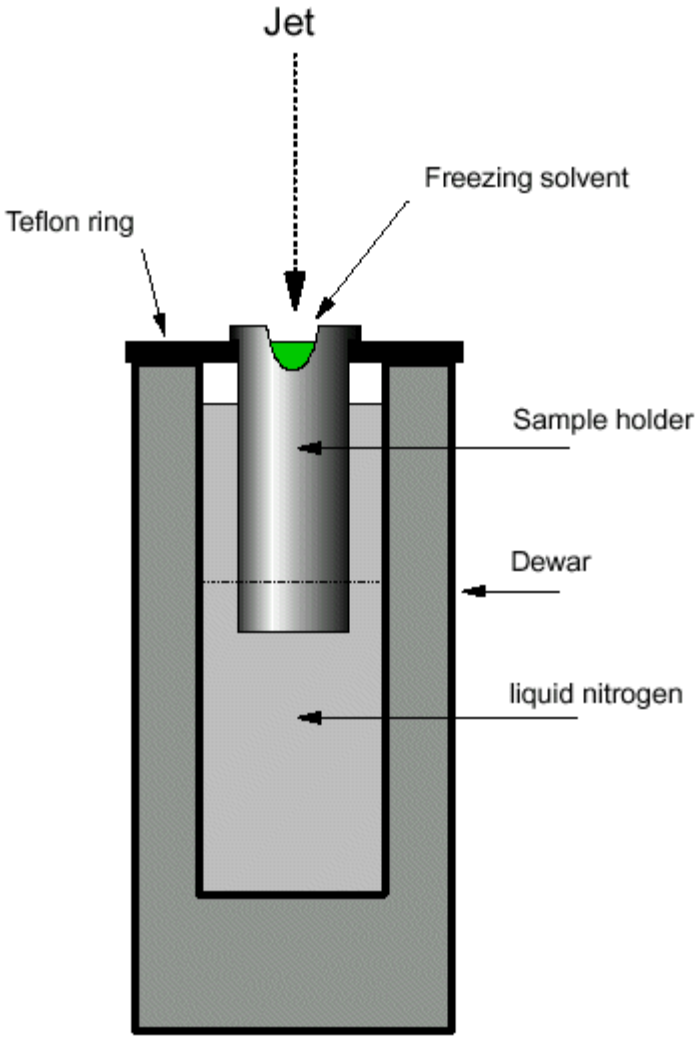


Bauer et al Biochemistry 1997, 36, 11514;
http://upload.wikimedia.org/wikipedia/commons/5/5b/Carboxypeptidase_A.png

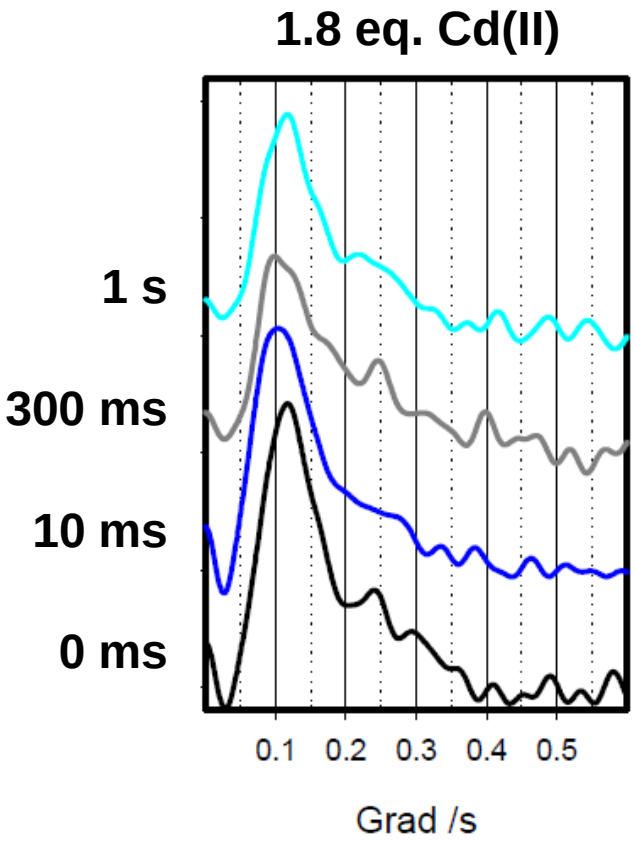
Freeze quench PAC spectroscopy:

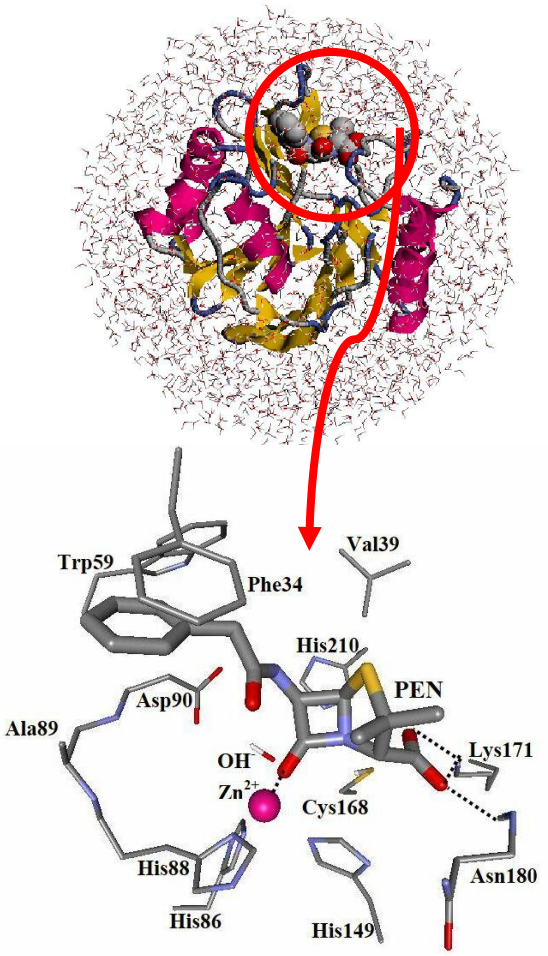
Snap shots of structures evolving during enzyme catalyzed reactions

Enzyme Substrate



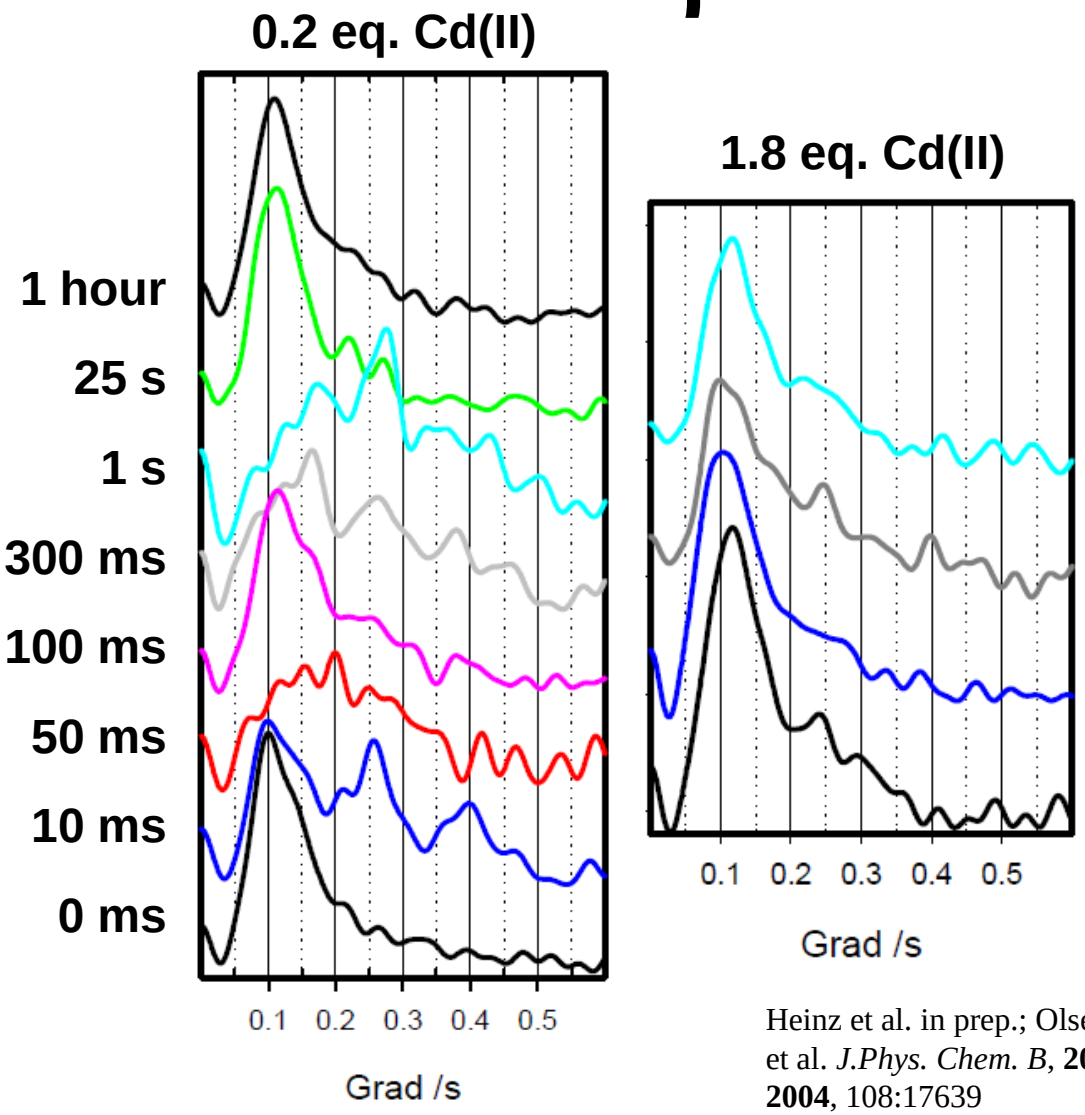
Bacterial resistance to antibiotics – β-lactamases

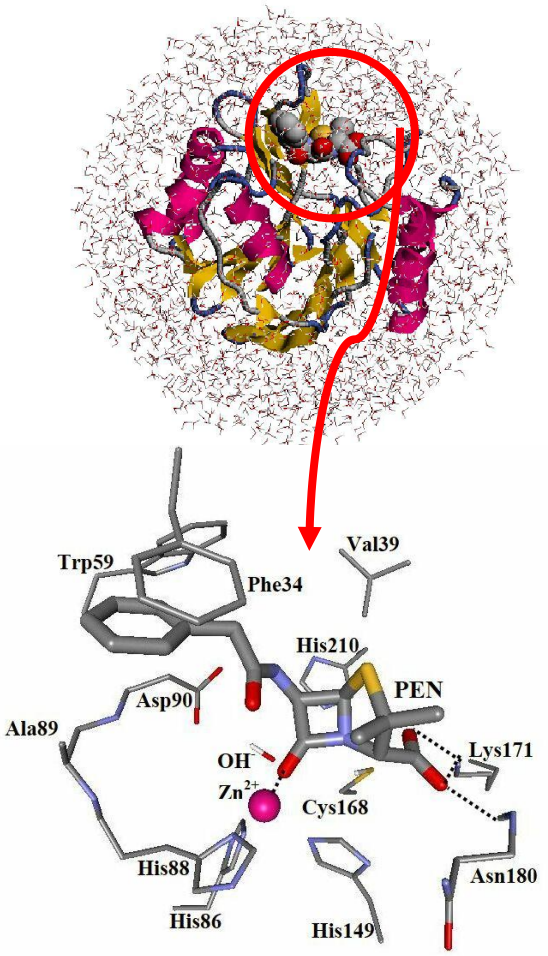




Heinz et al. in prep.; Olsen et al. *J.Phys. Chem. A*, **2002**, 106:1046; Olsen et al. *J.Phys. Chem. B*, **2003**, 107:2366; Olsen et al. *J.Phys. Chem. B*, **2004**, 108:17639

Bacterial resistance to antibiotics – β-lactamases

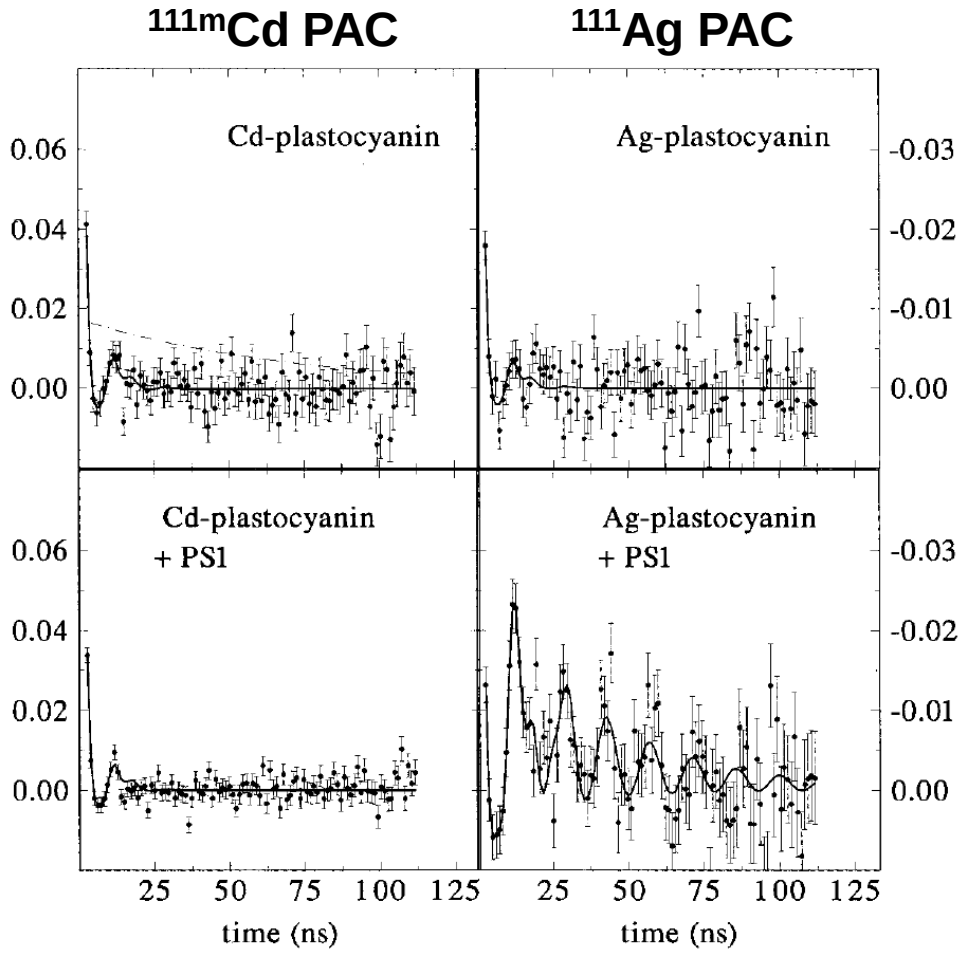


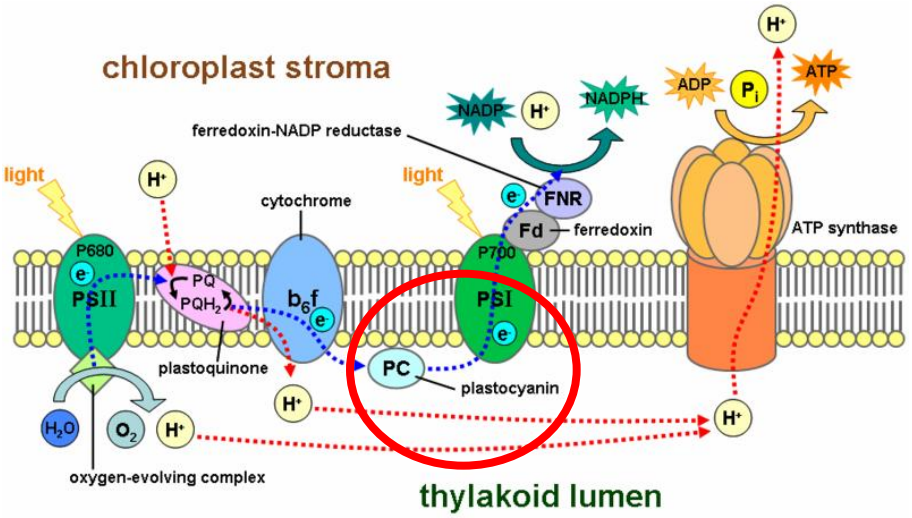


Heinz et al. in prep.; Olsen et al. *J.Phys. Chem. A*, **2002**, 106:1046; Olsen et al. *J.Phys. Chem. B*, **2003**, 107:2366; Olsen et al. *J.Phys. Chem. B*, **2004**, 108:17639

Protein-protein interactions:

Plastocyanin binding to photosystem 1

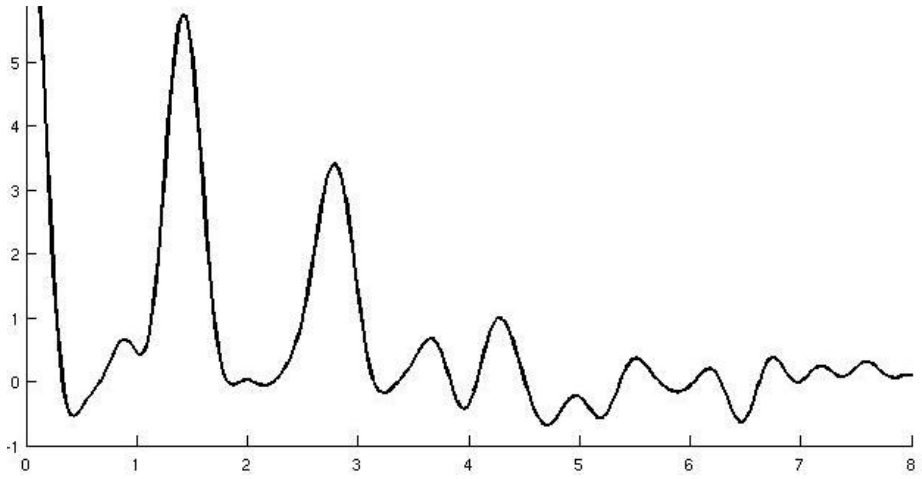




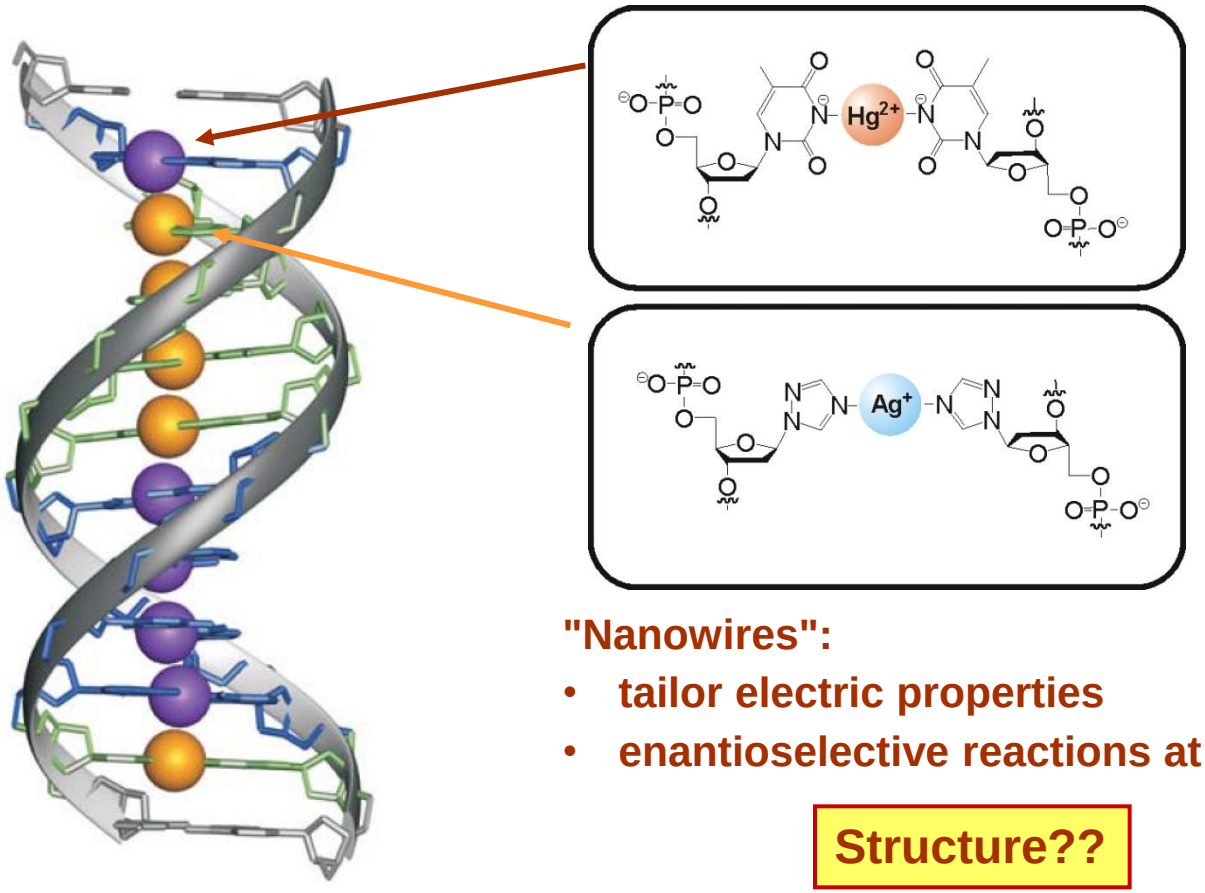
Danielsen et al. *Biochemistry*, **1999**, 38:11531;
http://en.wikipedia.org/wiki/Photosynthesis

In vivo PAC experiments: Hg(II) binding to barley

^{199m}Hg-PAC



Molecular wires based on metal-modified nucleic acids



"Nanowires":

- **tailor electric properties**
- **enantioselective reactions at the metal center**

Structure??

Johannsen, Paulus, Düpre, Müller, Sigel, J. Inorg. Biochem., 2008, *102*, 1141-1151; *Müller Eur. J. Inorg. Chem.*, 2008, 3749-3763

Advantages and limitations of PAC-spectroscopy

Advantages:

- Characterisation of structure and dynamics at the PAC probe site (including rotational correlation times)
- High sensitivity to structural changes, e.g. during enzyme catalysis
- Small amount of PAC probe needed (in principle about 1 pmol)
- Different physical states (crystals, surfaces, solutions, *in vivo*...)
- Mechanically stable, allowing for stirring, flow, ...

Limitations:

- Suitable PAC isotopes do not exist for all elements
- 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

Hemmingsen et al. *Chem. Rev.*, **2004**, 104: 4027; Hemmingsen and Butz, in "Application of Physical Methods to Inorganic and Bioinorganic Chemistry" **2007**, Ed. R.A. Scott, Wiley

Acknowledgements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- Universität Leipzig, Germany
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CERN

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Ministry of Science Technology and Innovation

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DCSC Danish Center for Scientific Computing

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& VELUX FONDEN