Observation of ultrafast conformational changes in MbCO by time resolved circular dichroism experiments

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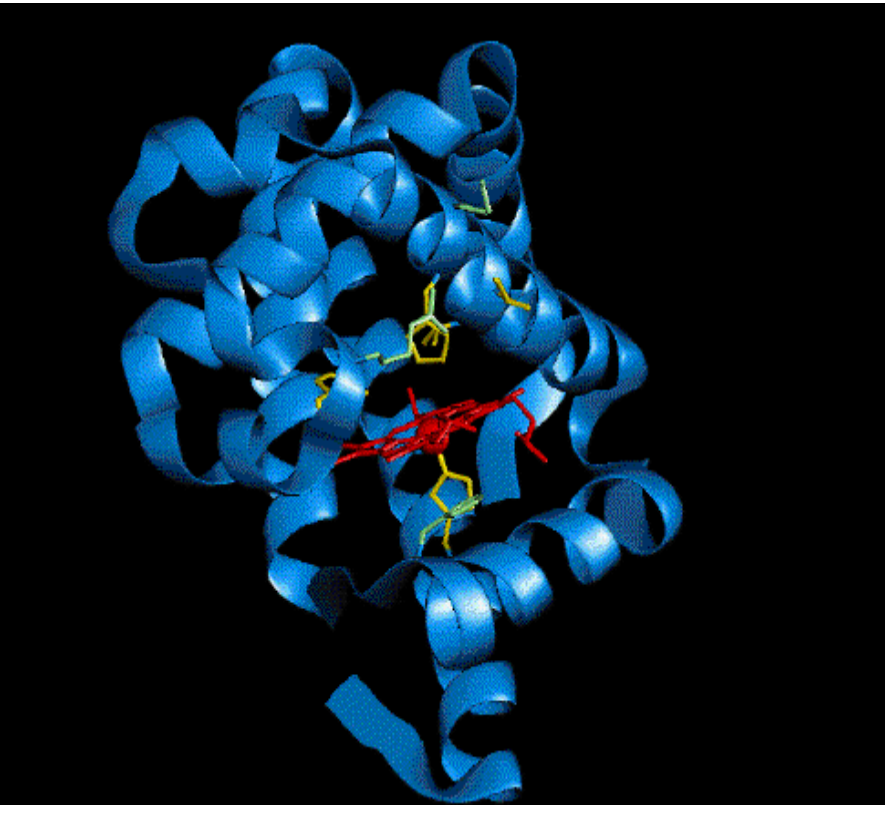
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

LOB POLYTECHNIQUE

Inserm Institut national

Photolysis of MbCO



Femto-chemistry
local pump-probe
spectoscopy

Need a
Global probe:
X-ray, CD

-Absorption
of a photon

-Charge trans

-Heme domin
Motion of th

-Histidine pr
is pushed

Conformation
Relaxation

Outline

- 1/Time resolved CD?
- 2/CD calculation in MbCO
- 3/ Experimental results and discussion

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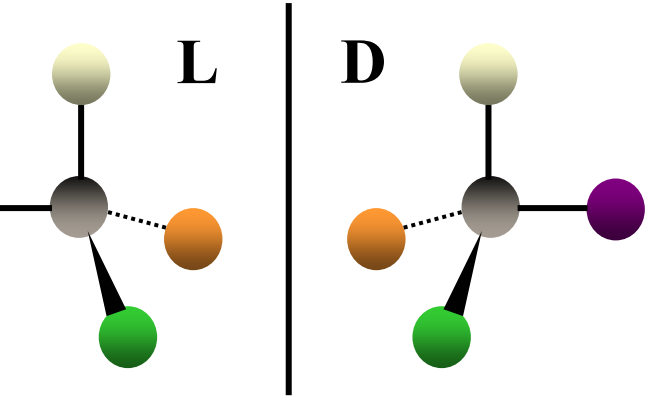
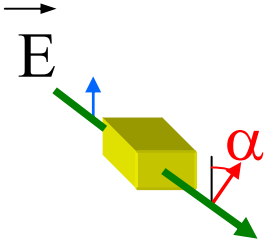
Chirality and Optical activity

chirality → *Optical activity*



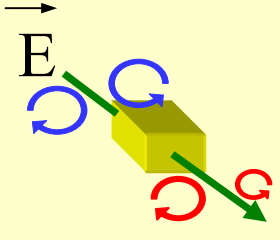
2 enantiomers

Rotation of polarization



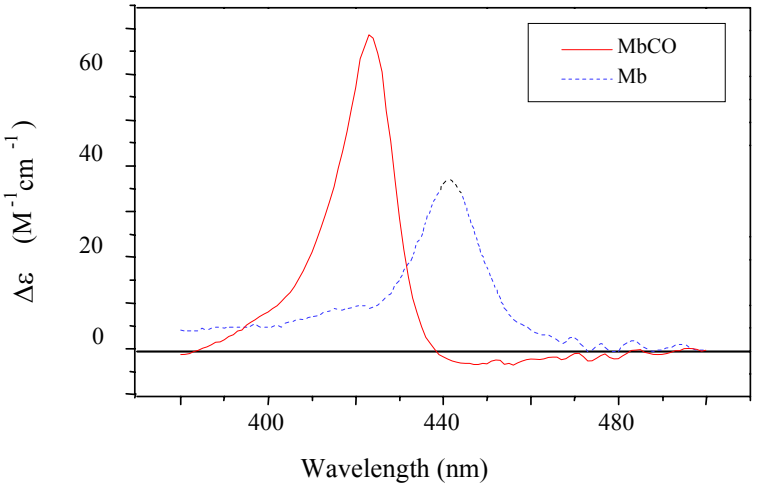
Circular dichroism

CD ~ 1/10000

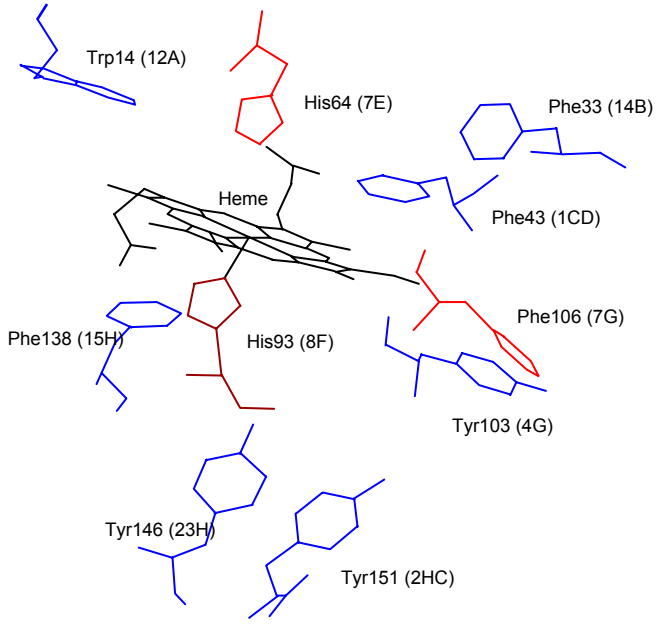


Circular dichroism in MbCO

CD spectra of MbCO and Mb



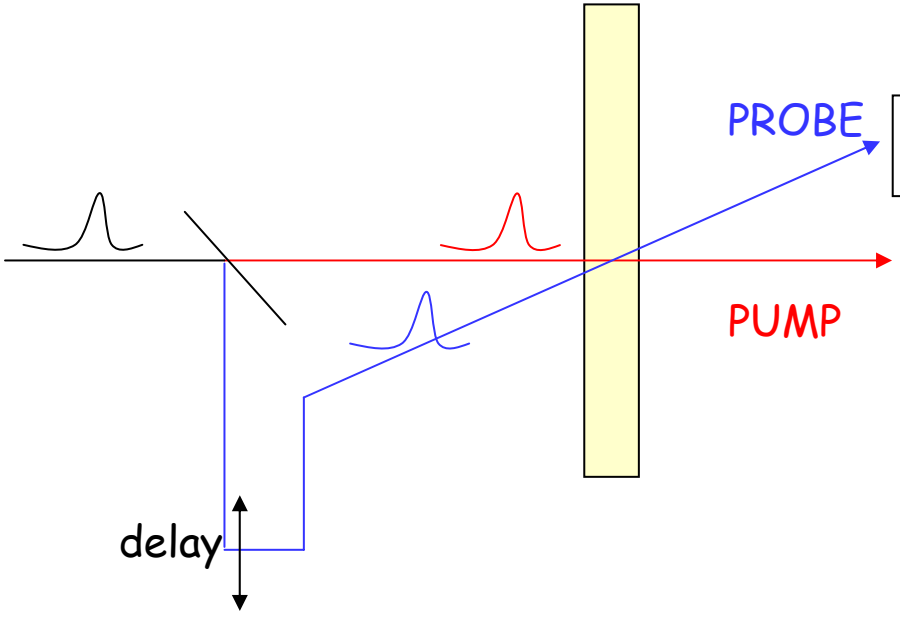
Origin of the CD : coupling of the heme with the surrounding aromatic aminoacids



*[Hsu and Woody, J. Am. Chem. Soc. **93**, 3515 (197*

Time resolved CD

Classical pump probe experiment

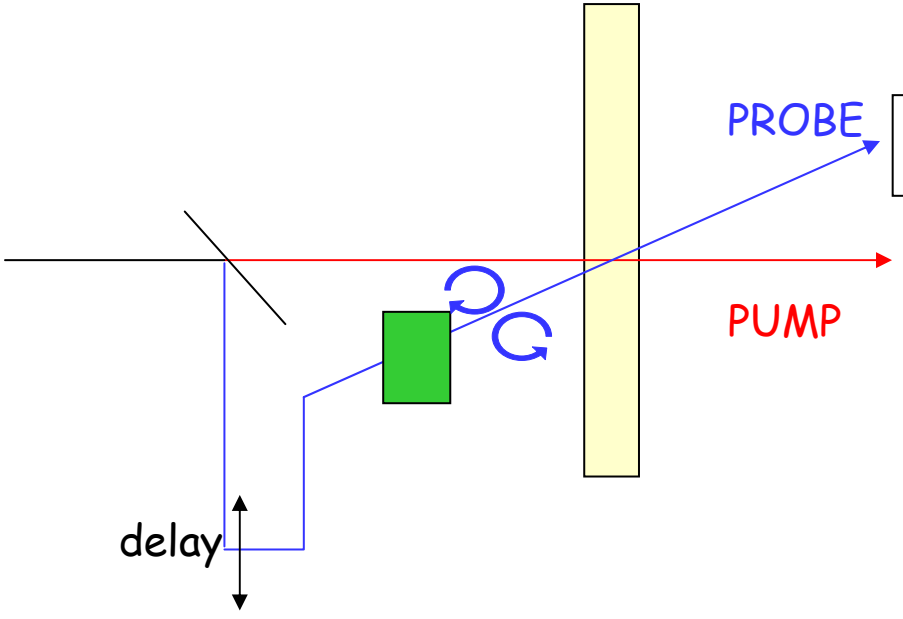


detector

measurement of the
probe transmission
as a function of the d

Time resolved CD

Time resolved CD experiment



detector

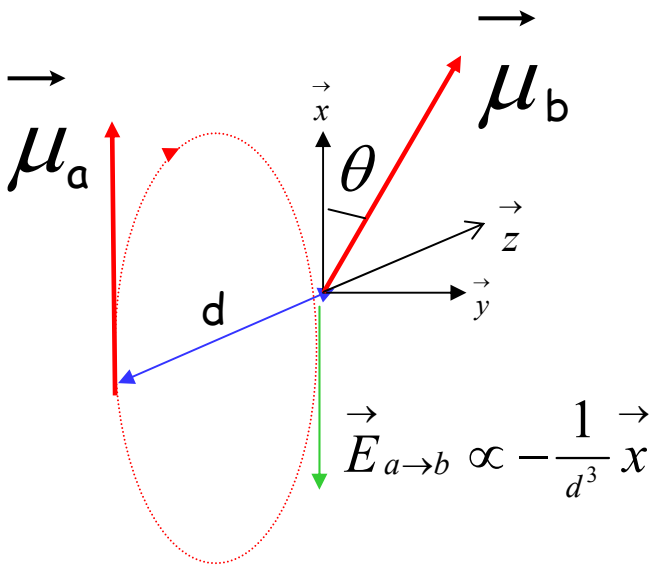
measurement of the
circular dichroism
as a function of the d

Following conformatic
change

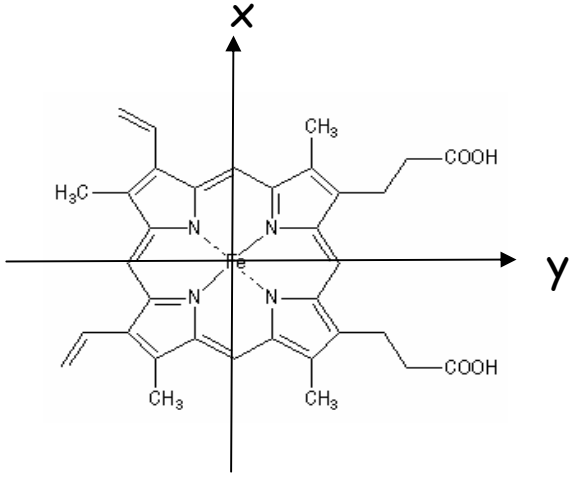
- 1/Time resolved CD?
- 2/CD calculation in MbCO
- 3/ Experimental results and discussion

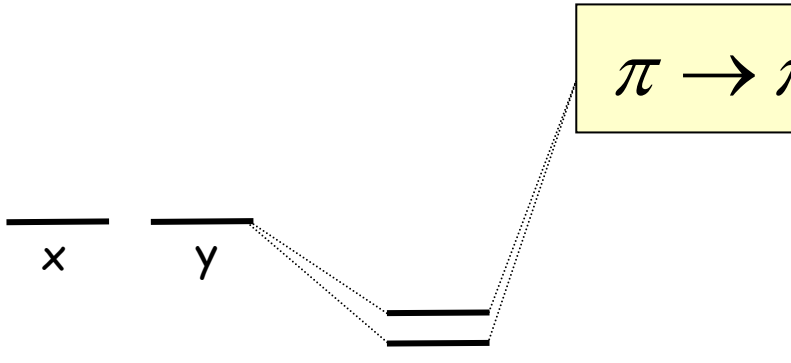
CD in a coupled oscillator system

oupling of two non-chiral chromophores



lift of the heme transition degeneracy



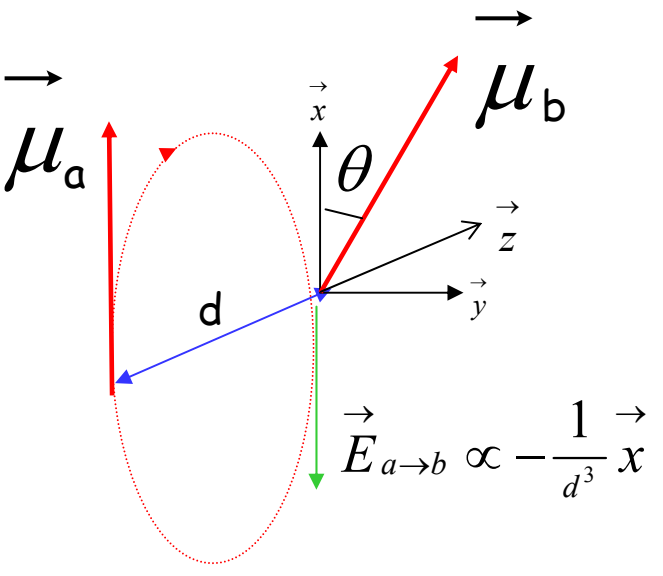


Absorption of the
Heme in the Soret band
(420 nm)

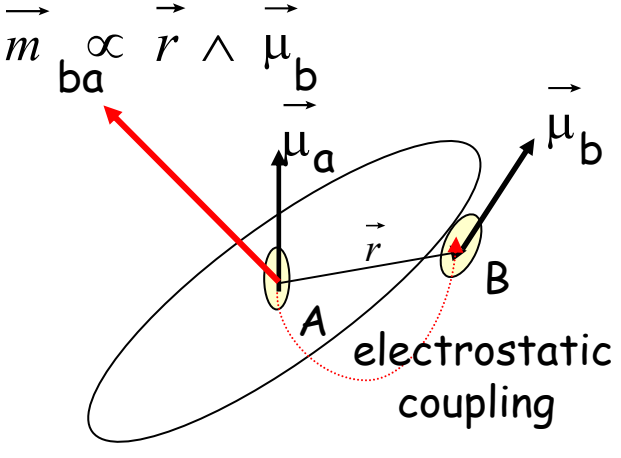
Near UV trans
of aromatic res

CD in a coupled oscillator system

oupling of two non-chiral chromophores



rotational strength $R \propto Im(\vec{\mu}.\vec{m})$



$$R \propto \vec{r}.\left\{\vec{\mu}_b \wedge \vec{\mu}_a\right\}$$

Polarization theory

There are several chromophores, subscript i

In each chromophore, there are several dipoles, subscript s

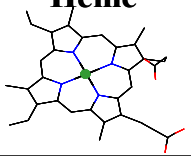
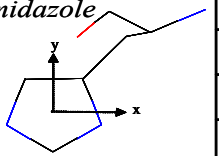
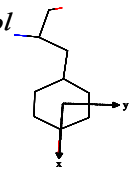
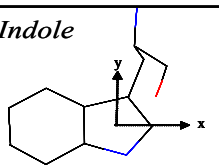
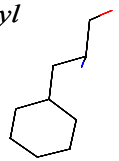
$$\left.\begin{aligned}\vec{\mu} &= \vec{\vec{\alpha}}\vec{E}_{loc} \\ \vec{E}_{loc} &= \vec{E} - \vec{\vec{T}}\vec{\mu}\end{aligned}\right\} \Rightarrow \begin{pmatrix} \\ E_{is} \\ \\ \end{pmatrix} = \begin{pmatrix} A_{11}^{-1} & & \\ & \ddots & \\ & & T_{is,jt} \\ & A_{is}^{-1} & \\ & & \ddots \end{pmatrix} \begin{pmatrix} \\ \mu_{is} \\ \\ \end{pmatrix}$$

oles *i.s* { Direction; Position; Transition frequency; Transition strength } ⟹ N normal modes $|n\rangle = \sum_{is} t_{is}^{\,n} |is\rangle$ { - Transi; frequen; - Osc. S; - Rot. St

J. Applequist, J. Chem. Phys. **58**, 4251-4259 (1973).

Parameters of the calculation

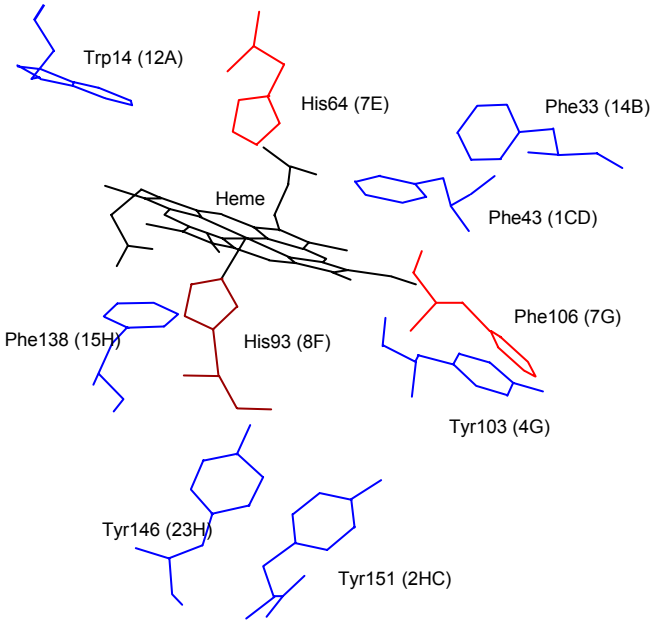
14 residues, 50 transitions

Heme	Protein	Energy (eV)	Osc. Str.	Polarization
	*MbCO*	2.96	1.3	x,y
	Mb	2.83	0.7	x,y
		3.02	0.3	x,y
The Heme parameters were adjusted to fit the experimental CD curves				
Amino-acid	**Residue**	**Energy (eV)**	**Osc. Str.**	**Polarization[a]**
Histidine[b]	*Imidazole* 	5.10	0.12	58.4
		6.02	0.03	-25.7
		7.56	0.41	50.9
		8.00	0.07	-22.5
Tyrosine[c]	*Phenol* 	4.51	0.02	x
		5.82	0.13	y
		6.66	1.1	y
		6.66	1.1	x
Tryptophan[c]	*Indole* 	4.37	0.05	28
		4.77	0.12	-41
		6.02	0.6	23
		6.35	0.5	-33
Phenylalanine[c]	*Phenyl* 	6.94	0.9	x,y

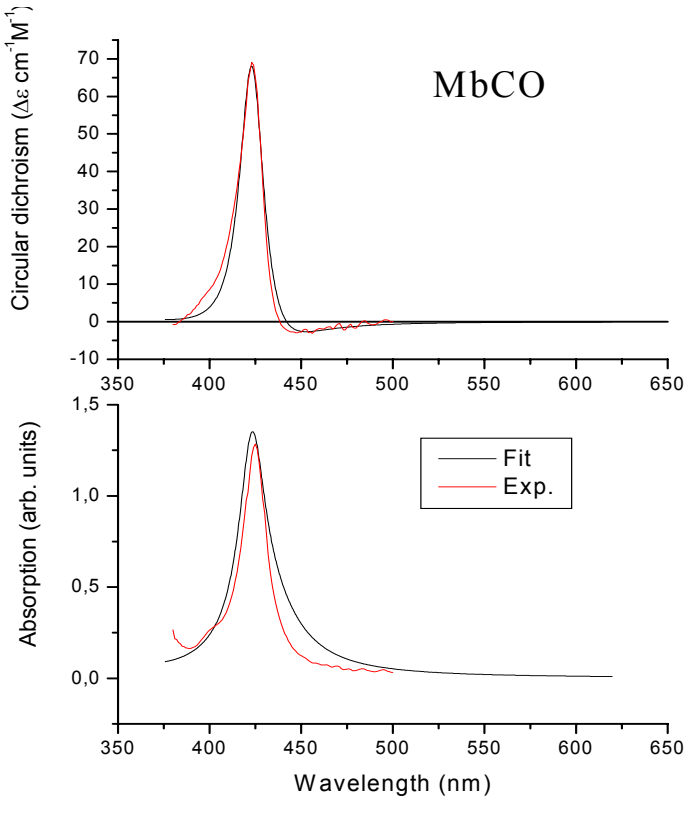
(a) Angle (in degrees) of the transition moment with respect to the x-axis
(b) M.C. Hsu and R.W. Woody, J. Am. Chem. Soc. **93**, 3515 (1971).
(c) D.M. Rogers and J.D. Hirst, J. Phys. Chem. **107**, 11191 (2003).

Origin of CD in myoglobin

coupled oscillators: the heme and aromatic residues



$$R_i^{(n)} \propto \mathbf{r}_i . \sum_{s,t} t_{is}^{(n)} t_{heme,t}^{(n)} \left[\mathbf{u}_{is} \times \mathbf{u}_{heme,t} \right]$$



Time resolved CD

- 1/Time resolved CD?
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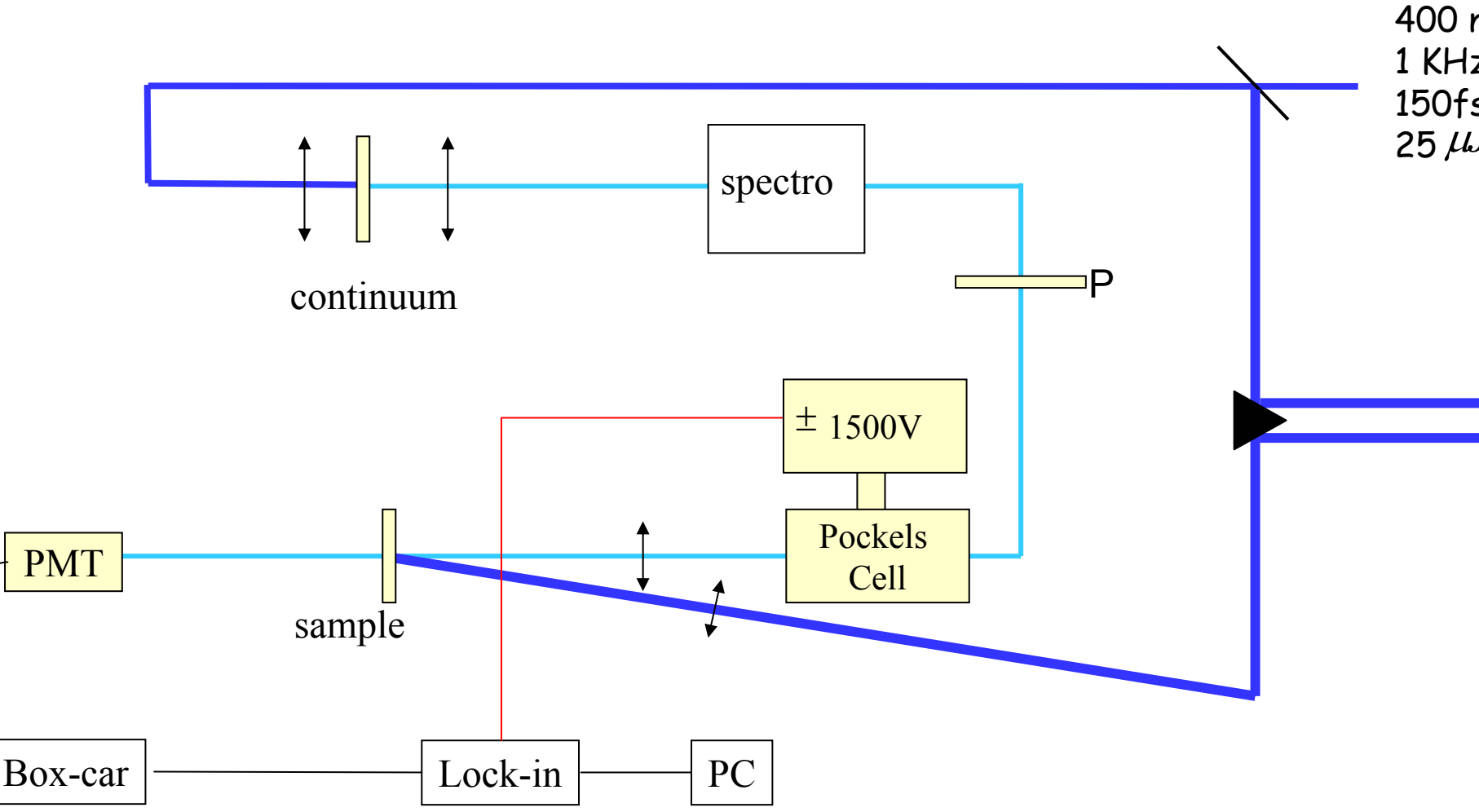
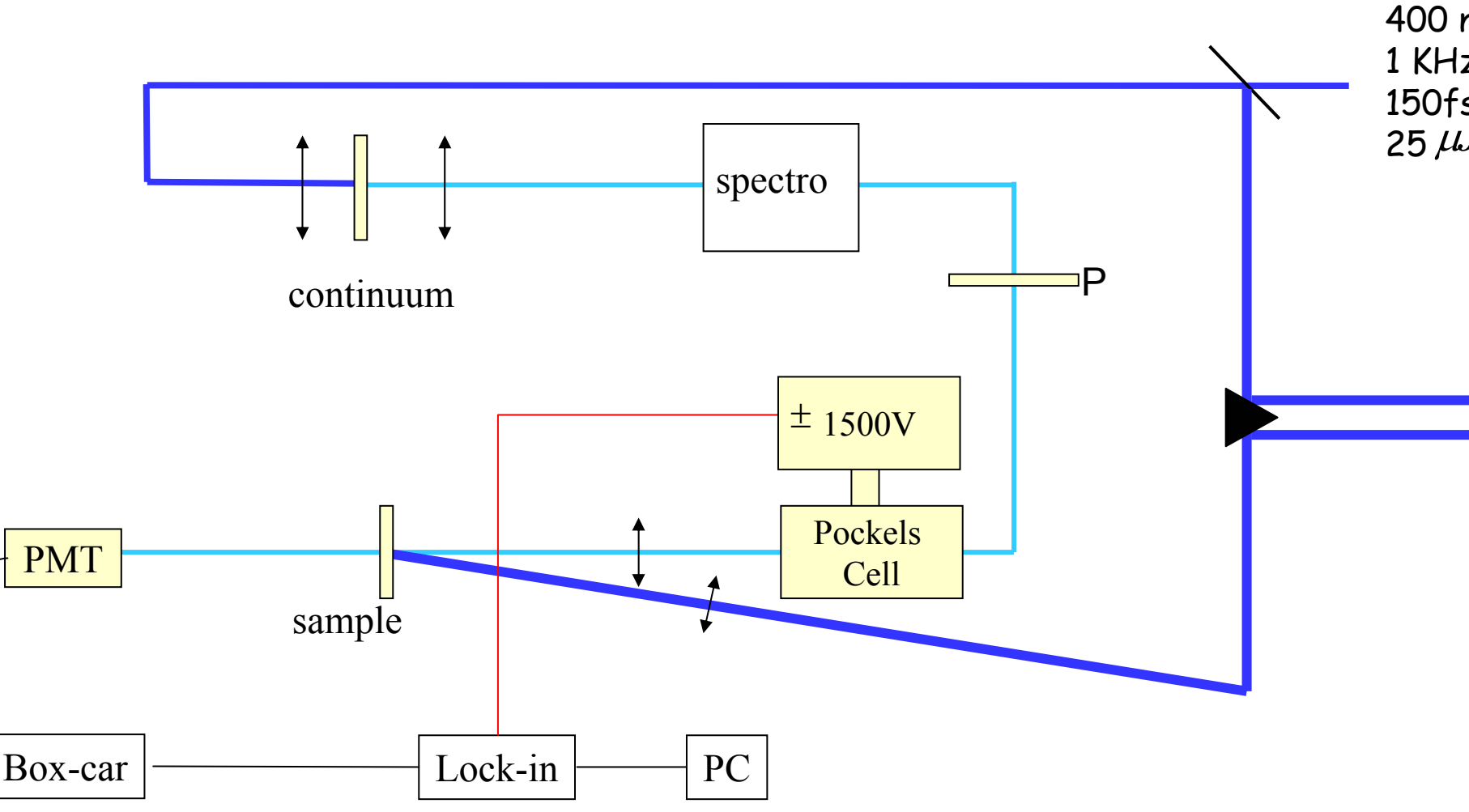
Experimental set-up



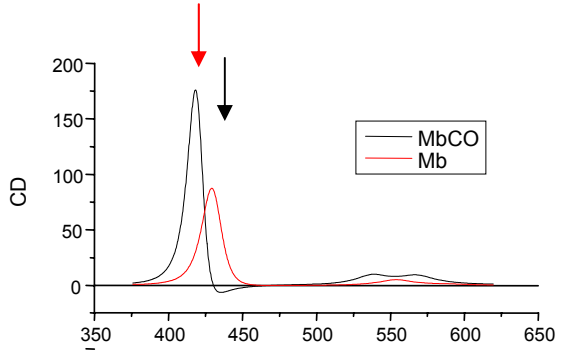
Simultaneous measurement of the Transmission and of the Circular Dichroism as a function of the pump-probe delay.

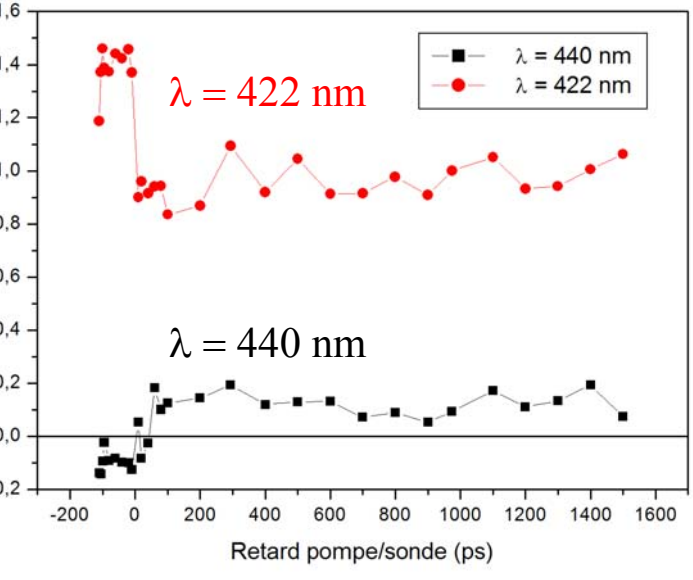
Experimental set-up



T. Dartigalongue and F. Hache, J. Opt. Soc. Am. B **20**, 1787 (2003).

Dissociated state CD spectra

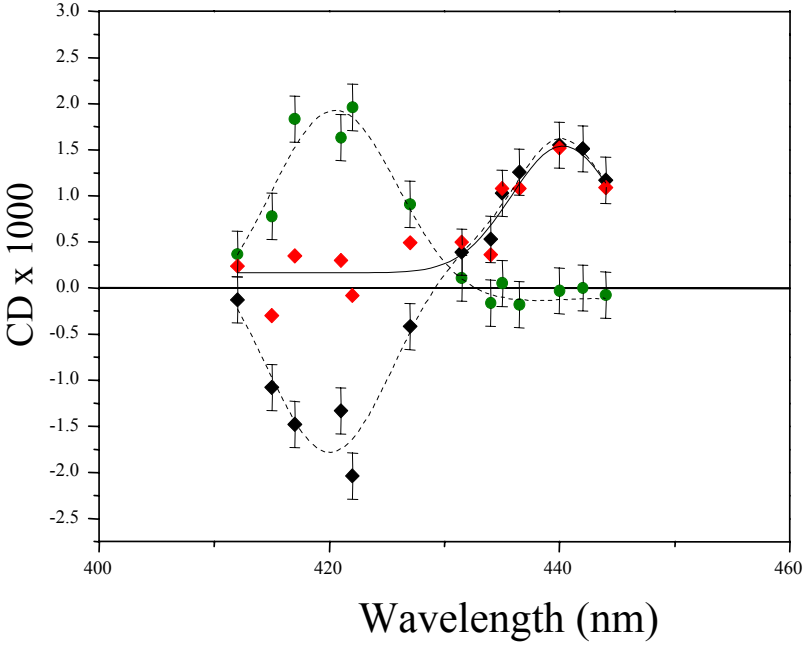




CD MbCO

ΔCD

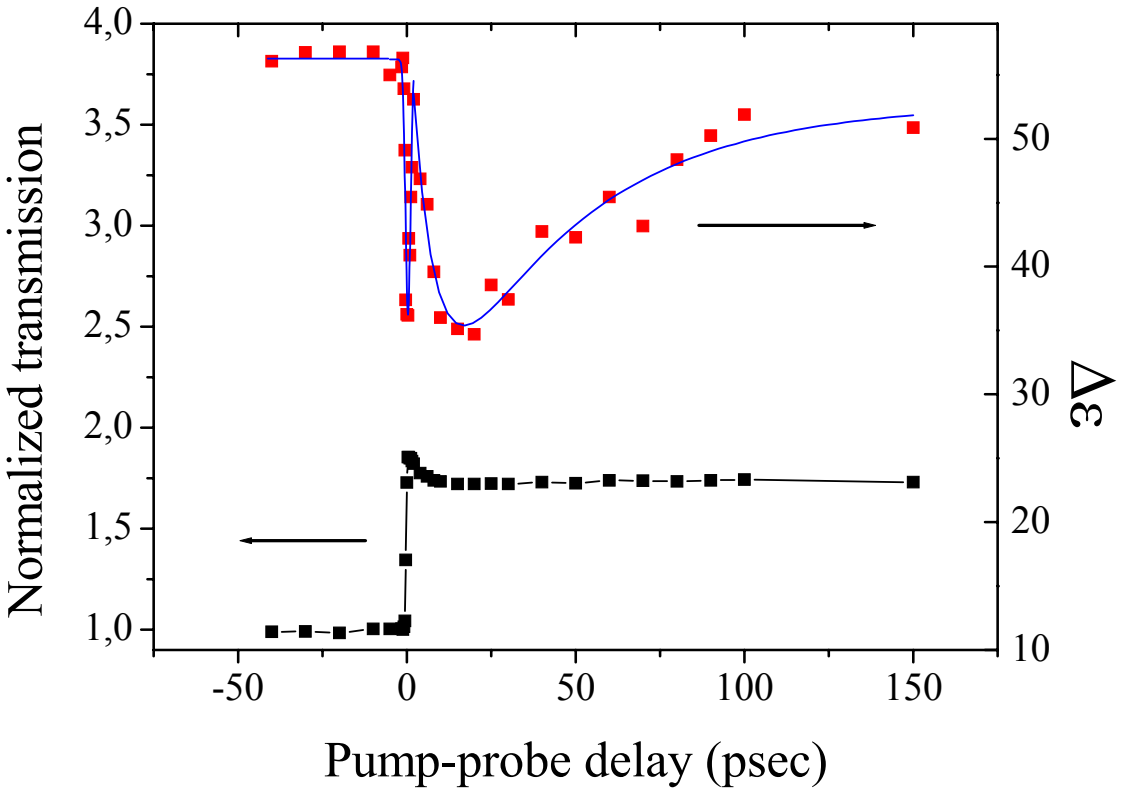
CD Mb*



- CD of the excited molecules = Mb spectrum
- No dynamics on a >100 ps timescale

Ultrashort timescale

MbCO
Probe = 418 nm

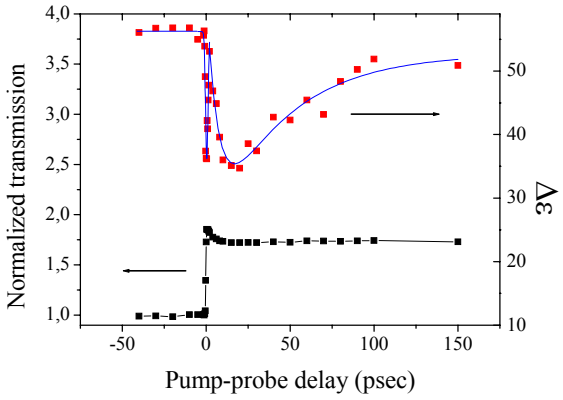


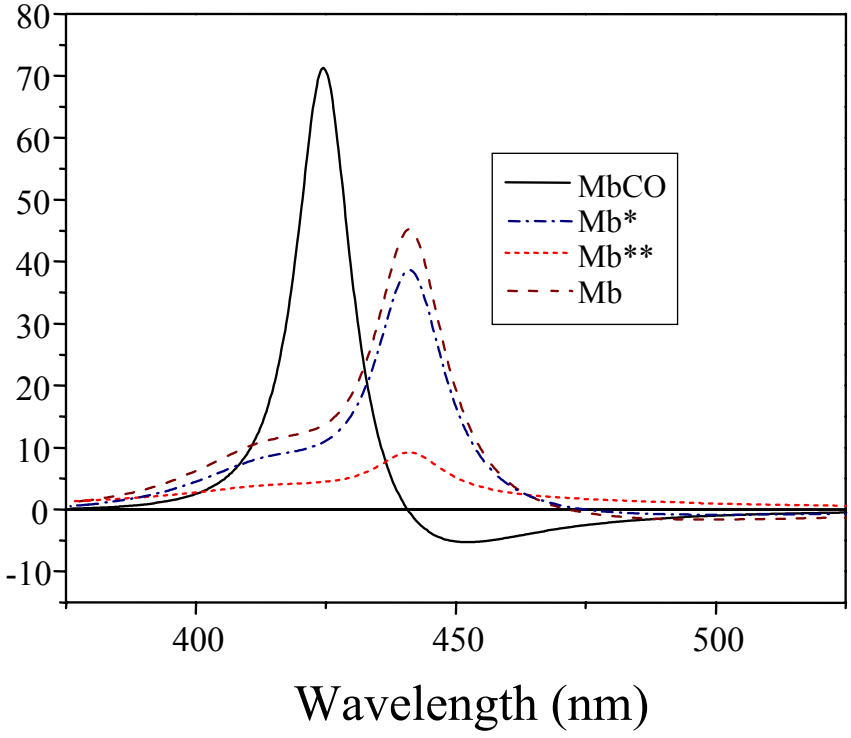
Signal ⇔ ultrafast conformational change

Discussion

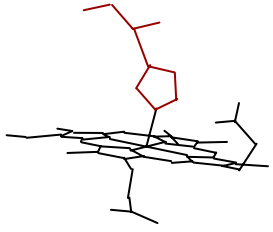
Simulation of intermediate configurations:

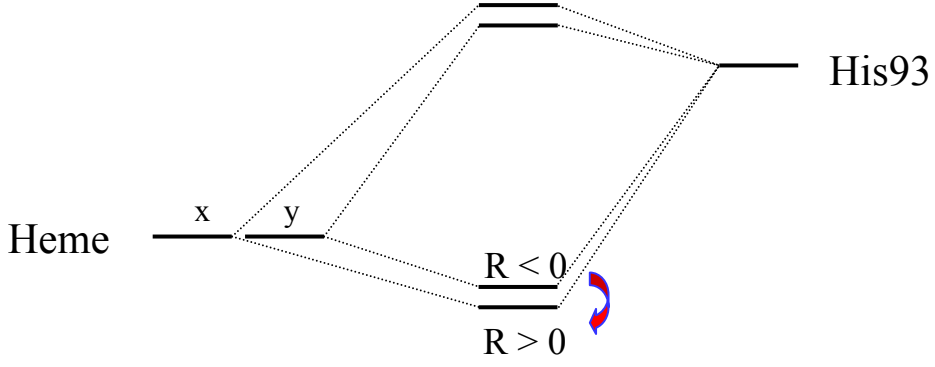
- MbCO
- Mb*
- Mb** = Mb* + rotation H93
- Mb





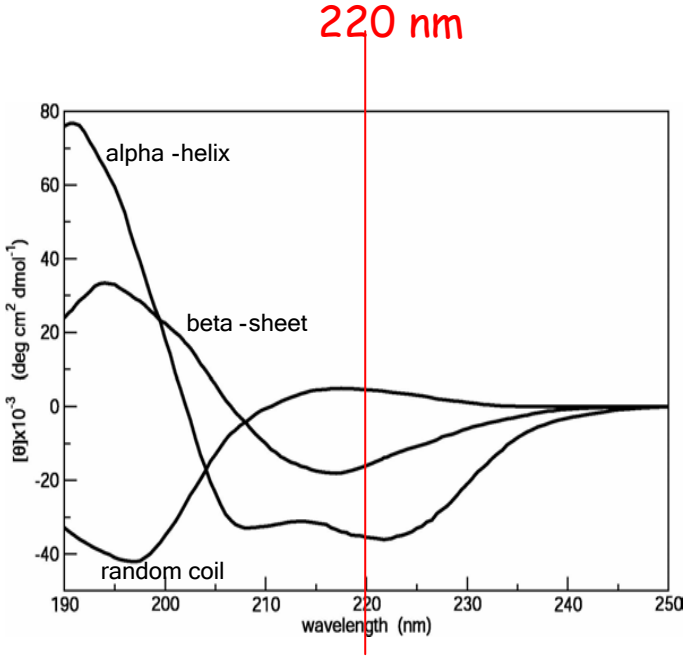
Rotation of the proximal Histidine





Conclusions and Perspectives

- First observation of 100 ps conformational change following MbCO photolysis
- Stress of the proximal histidine



Time-resolved CD in the **UV**

= investigation of the early events

in protein folding