UV-visible optical probes in biomimetic environments

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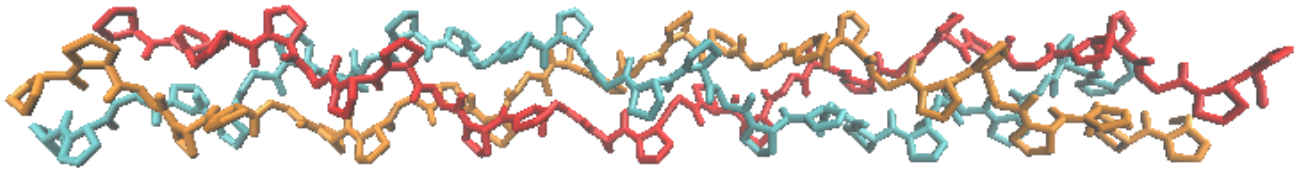
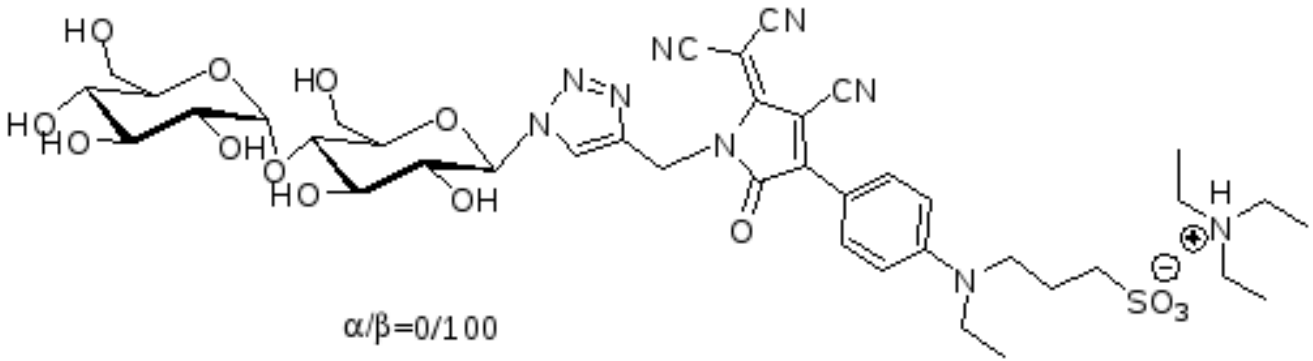
Outline

- Collagen-mimicking peptides



- Artificial probes grafted on sugars



How does the environment / conformations influence the optical properties ?

Collaborations

Laboratoire de Spectrométrie Ionique et Moléculaire

- Philippe Dugourd
- Rodolphe Antoine
- Amandine Racaud
- Pierre-Francois Brevet
- Julien Duboisset
- Daniel Simon

Inst. Sciences Analytiques

- Jérome Lemoine

Lab. Chemistry of ENSL

- Yann Bretonnière

Lab. Optics and Biosciences

- M.-C. Schanne-Klein
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Inst. Chimie et Biochimie Moléculaires et Supramoléculaires

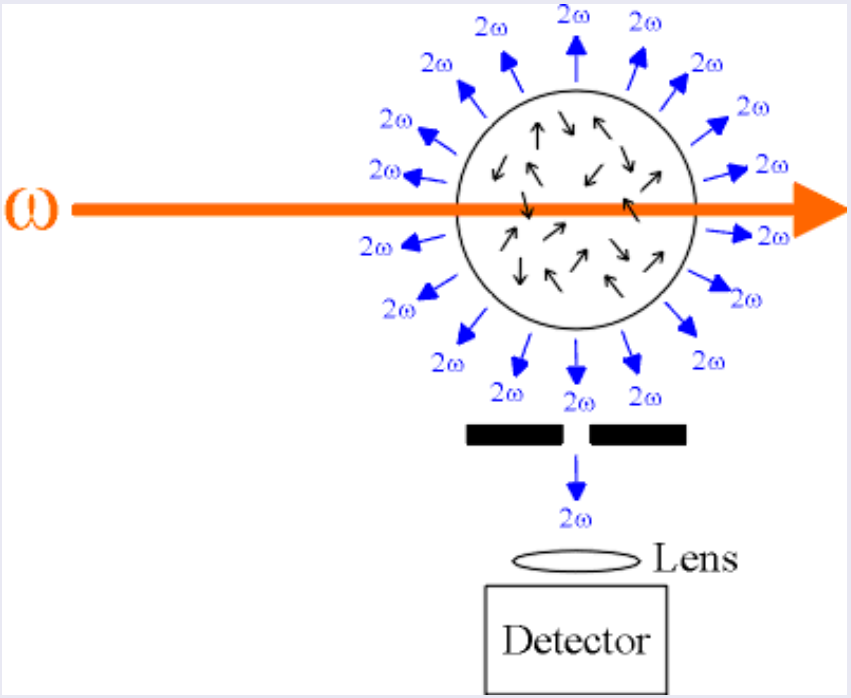
- Stéphane Chambert
- Francoise Besson

Outline

SHG imaging of biological tissues... and beyond

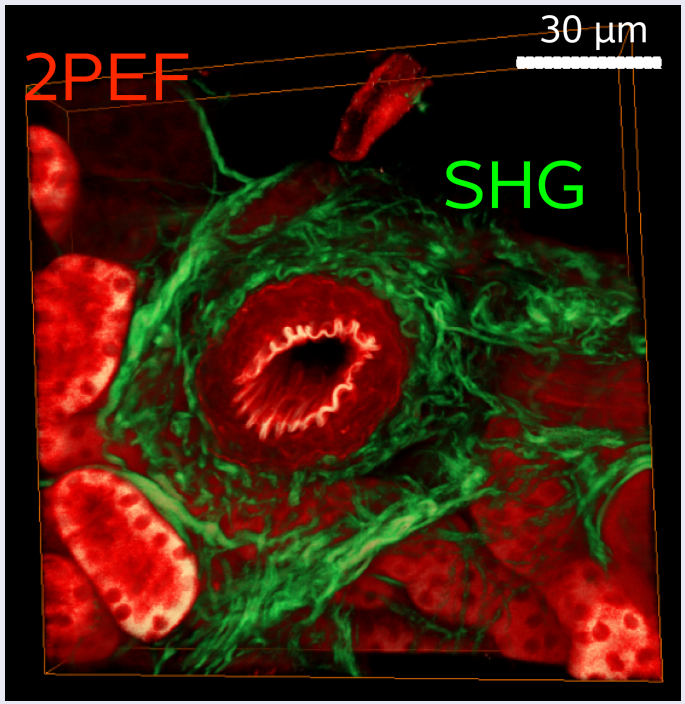
Second Harmonic Generation

2 photons $\hbar\omega \Rightarrow$ 1 photon $2\hbar\omega$



Endogeneous chromophores

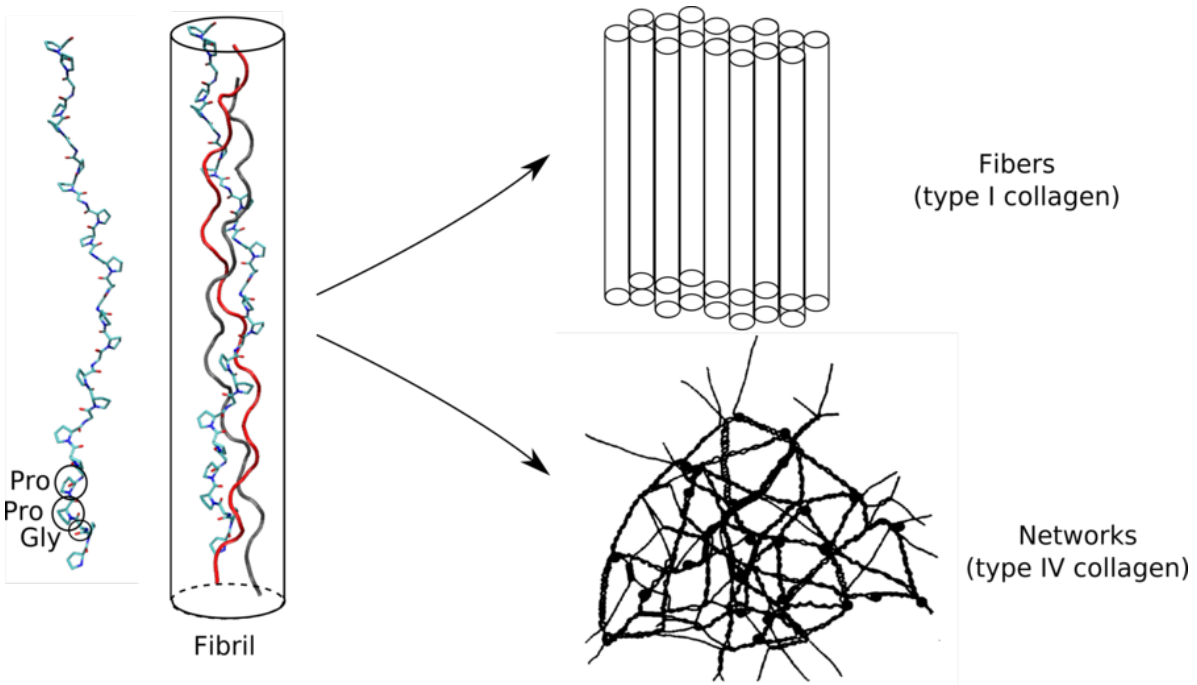
Arcuate artery in a fibrotic murine kidney



Strupler *et al. Opt. Express* (2007)

Questions

- SHG signal of collagen ... from peptide bonds ?
- Structure/reponse relationship ?
- Difference between native and denaturated states ?



Second Harmonic Generation of a small molecule

Non linear response : Hyperpolarizability β

Induced Dipole Moment $\vec{\mu}$ under a STRONG electric field $\vec{E}$

$$\mu_i = \mu_i^0 + \sum_i \alpha_{ij} E_j + \sum_{j,k} \beta_{ijk} E_j E_k$$

Two-states Model (Oudar 1977)

$$\beta_{ijk}(0) \propto \frac{\mu_i^{eg} \mu_j^{eg} \left(\mu_k^e - \mu_k^g\right)}{(E^e - E^g)^2}$$

$$\beta_{ijk}(\omega) = \frac{\beta_{ijk}(0)}{\left(1 - \frac{\omega^2}{\omega_0^2}\right)\left(1 - \frac{4\omega^2}{\omega_0^2}\right)}$$

Easy case

Conformations
PDB, classical MD

\+ Quantum Chemistry
LR-DFT, FF-CAS(PT2)

$\Rightarrow \langle$Optical properties$\rangle$

Additive model for the hyperpolarizability...

For a peptide of N amino-acids

$$\beta^{peptide} = \sum_{i}^{N-1} R_i \ \beta_{(i,i+1)}$$

$\beta_{(i,i+1)}$ for 1 peptide bond between a.a. i and $i+1$

R_i = Rotation matrix as a function of Euler angles α, β, γ

$$\begin{bmatrix} \cos\alpha & -\sin\alpha & 0 \\ \sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos\beta & -\sin\beta \\ 0 & \sin\beta & \cos\beta \end{bmatrix} \begin{bmatrix} \cos\gamma & -\sin\gamma & 0 \\ \sin\gamma & \cos\gamma & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

References : Sunberg J Chem Phys (1977)

Simplistic additive model

Approximations !

- Neglect polarisation effects : $\beta_{(i,i+1)} \sim \beta_0$
- Neglect charge transfer between amino-acids
- Neglect terminal chromophores
- Neglect destructive interferences at longer distances

Choice of the parameters β_0 ?

- fit on experimental data... underdetermined
- use theoretical data...

Typical results : N-methylamide

NV1 Excitations energies (eV)

TDDFT (SAOP/QZ4P)	7.03
CASPT2 (ANO-TZP/6 electrons, 10 orbitals)	6.88
exp. VUV abs. spectrum	∼6.8

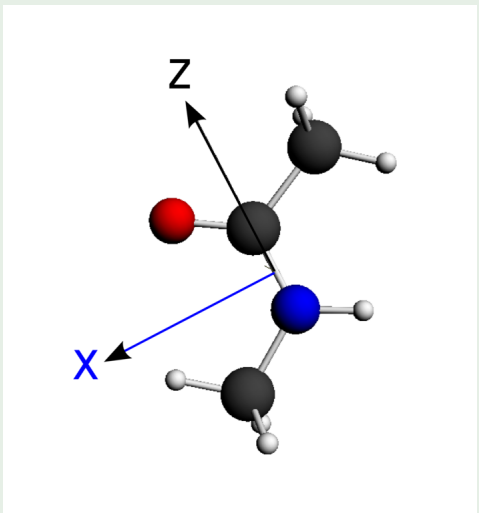
Hyperpolarizability at 800nm (TDDFT, SAOP/QZ4P)

$|\beta| = 0.5 \times 10^{-30}$esu (T conv.)

$$\beta_{xxx} = 100\%$$

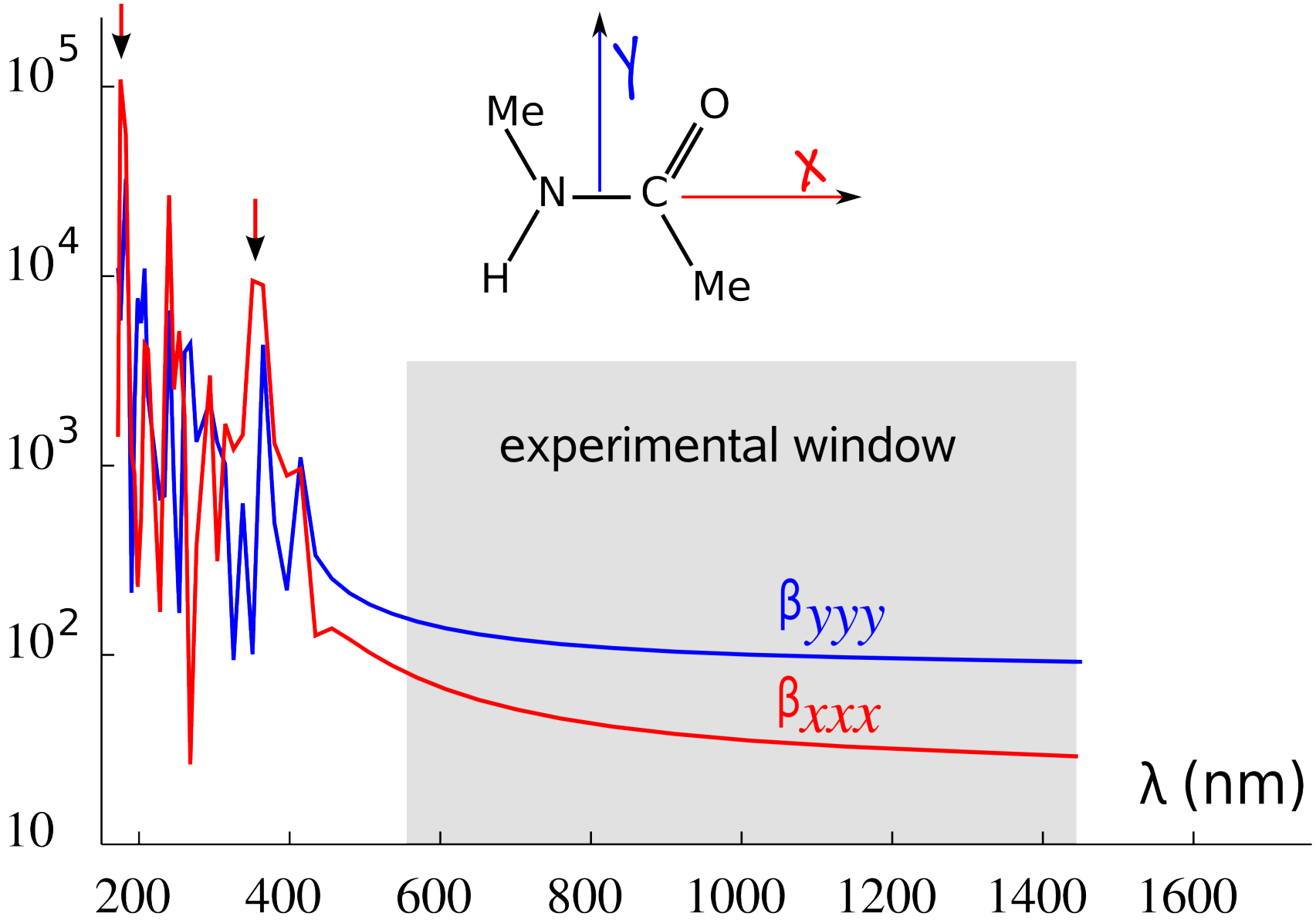
$$\beta_{xzz} \sim \beta_{zxz} \sim \beta_{zzx} \sim 30\%$$

$$\beta_{xxz} \sim \beta_{zxx} \sim \beta_{xzx} \sim 23\%$$



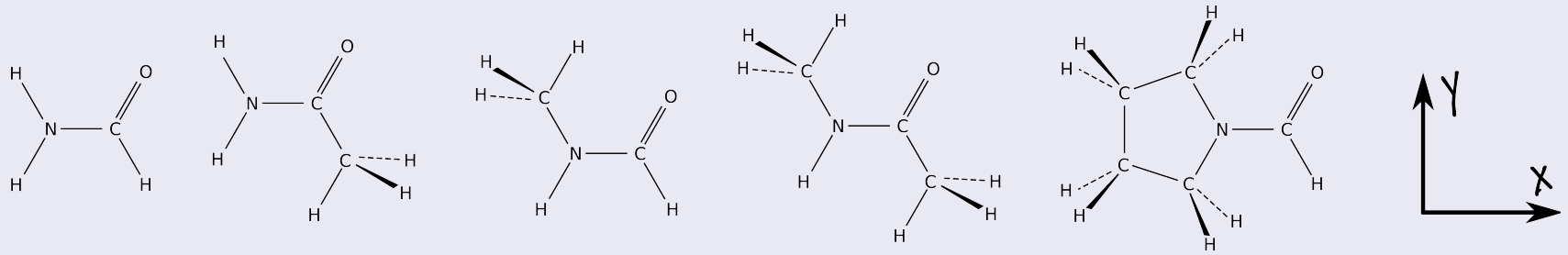
CH_3 orientation not important !

Importance of wavelength



Choice of the parameters ?

A reasonable guess ...amide molecules



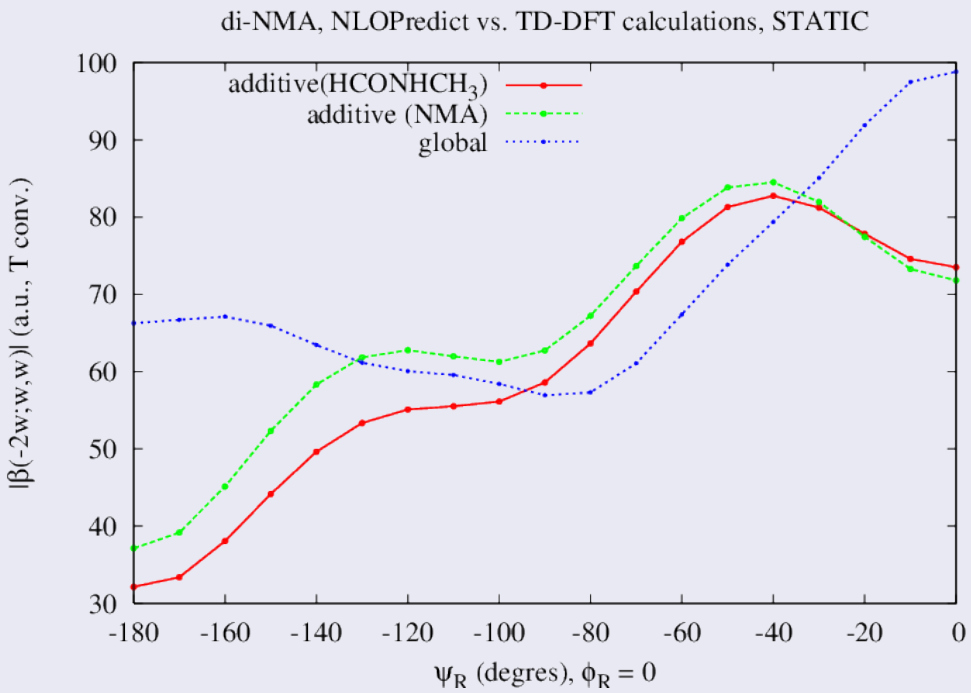
β_{SHG} at 800 nm (au)

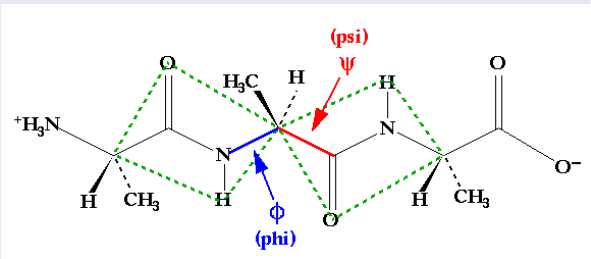
LR-DFT (SAOP/QZ4P) calculations (T conv.)

Mol.	β_{yyy}	β_{xxx}	β_{xyy}	β_{xzz}	β_{yxx}	β_{yzz}	$\lvert\beta\rvert$
1	50	27	18	6	8	6	50
2	39	8	7	2	7	9	32
3	55	8	11	15	9	-1	32
4	44	-13	15	7	12	2	23
5	50	1	20	20	16	4	43

How bad is that simplistic approach ?

Additive model vs. full calculations (di-peptide model)





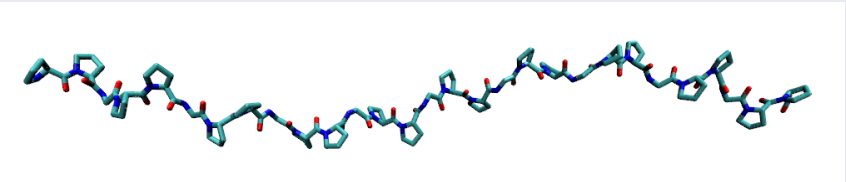
"Conjugated" / extended conformations are hopeless !

Native Conformation

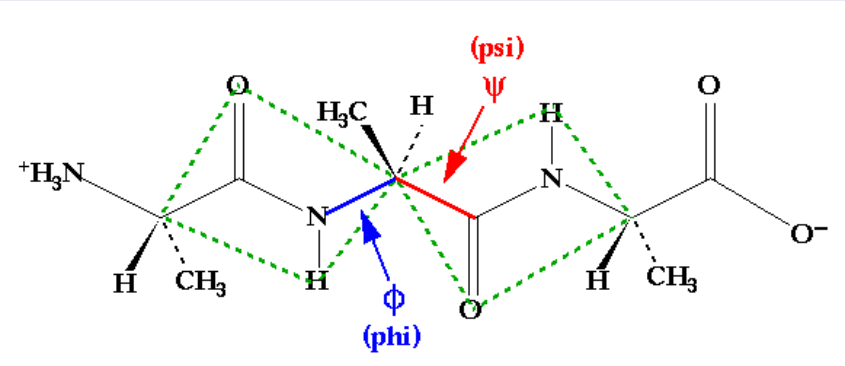
Collagen-like peptides in PDB

about 20 structures in the Protein Data Bank...

- 1ZWB = $(\text{Hyp-Hyp-Gly})_{10}$
- 1CAG = $(\text{Pro-Hyp-Gly})_{10}$
- 1K6F = $(\text{Pro-Pro-Gly})_{10}$
- ...



Ramachandran angles



$$\Phi = -70^\circ$$

$$\Psi = +170^\circ$$

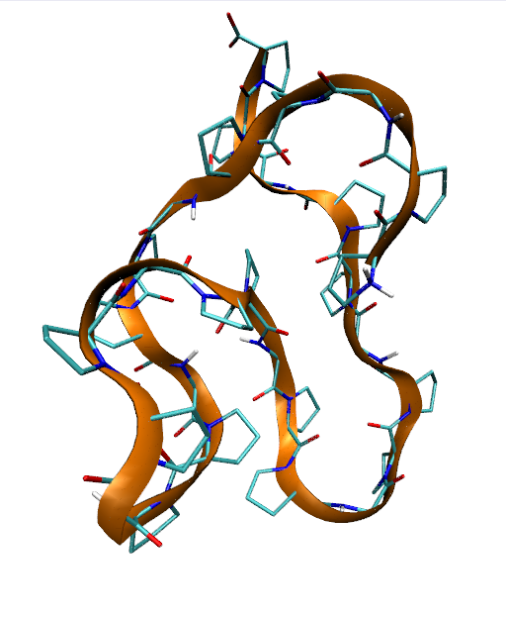
$\rightsquigarrow$ no dispersion
$\rightsquigarrow$ no length-dependency
$\rightsquigarrow$ no sequence-dependency

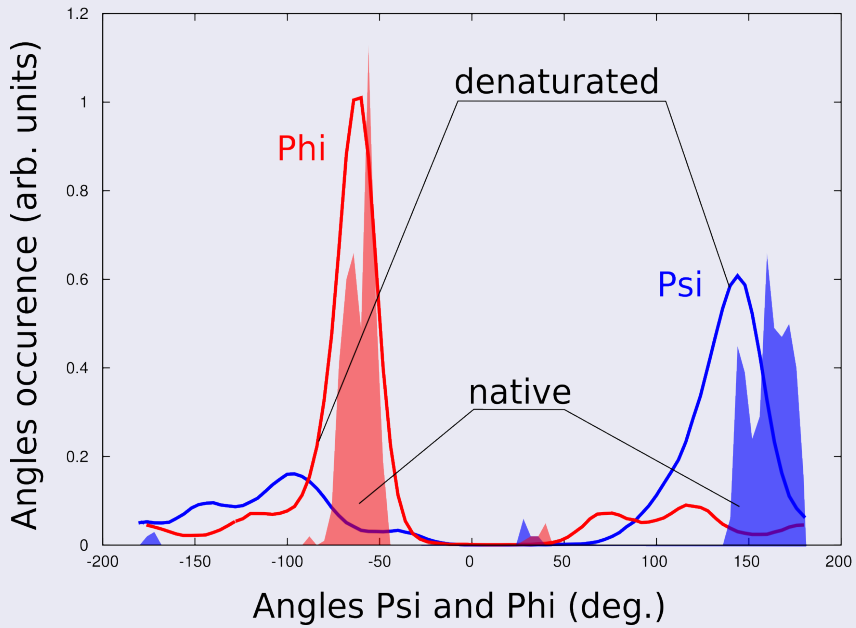
Denaturated Conformation

Numerical Simulations of $(Pro\text{-}Pro\text{-}Gly)_{10}$ in solution

- 7273 water molecules + $(PPG)_{10}$ + Na^+ + Cl^-
- NPT ensemble (1 atm, 300K)
- Standard molecular dynamics simulation (GROMACS)

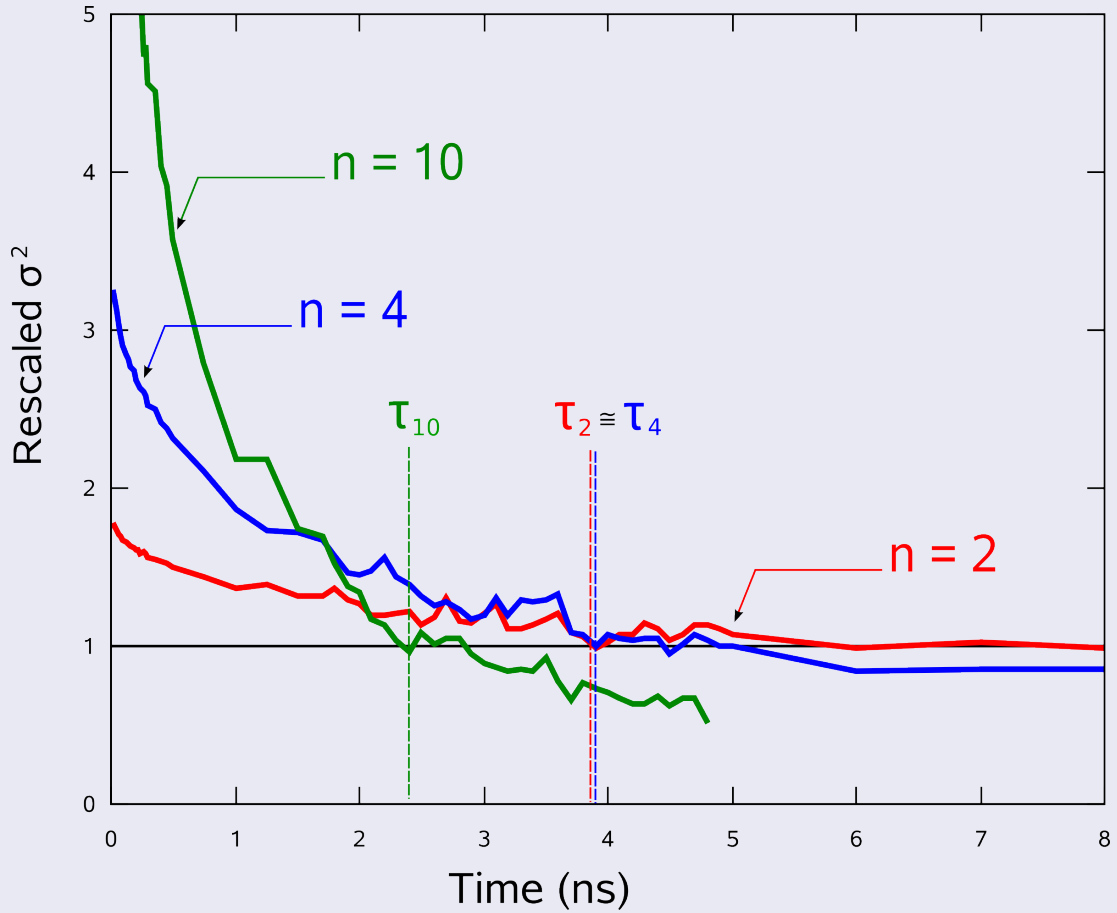
...15 independent conformations (70 ns simulation)





Denaturated Conformation

Analysis of decorrelation time $\rightsquigarrow \tau \simeq 5$ ns



Reference for the analysis method : E. Lyman et al. *J.Phys.Chem.B* (2007)

Results for a peptide model : $(Pro\text{-}Pro\text{-}Gly)_{10}$

Solutions of native and denaturated peptides.
Comparison for a single strand.

Observables

$$\frac{|\beta|_{denat}}{|\beta|_{nat}}$$

$$D = \frac{|\langle \beta^2_{XXX} \rangle|}{|\langle \beta^2_{XZZ} \rangle|}$$

$$u = \frac{\beta_{zxx}}{\beta_{zzz}}$$

Theoretical vs. exp results

		Native	Denaturated
theory	$\frac{\lvert\beta\rvert_{denat}}{\lvert\beta\rvert_{nat}}$	3.1	
	D	0.15	0.5 ± 0.1
	u	0.9	
exp.	$\frac{\lvert\beta\rvert_{denat}}{\lvert\beta\rvert_{nat}}$	≤ 3	
	D	0.11	NA
	u	0.2-0.7	

Deniset-Besseau *et al. J Phys Chem A* (2009)

C. Loison *et al. J Phys Chem A* (2010)

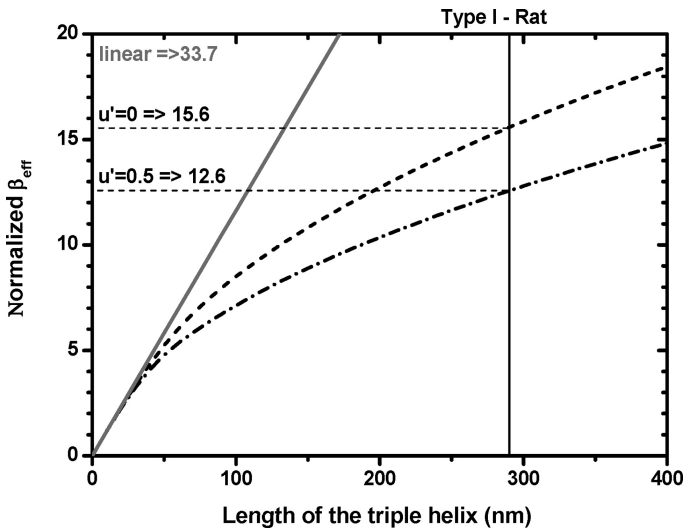
Refining the additive model.

- Polarization and charge transfer between 1st neighbors

$$\frac{\beta_{iiz}}{\beta_{zzz}} = 0.9 \Rightarrow 0.7$$

Tuer et al. *J Phys Chem B* (2011)

- Phase factor



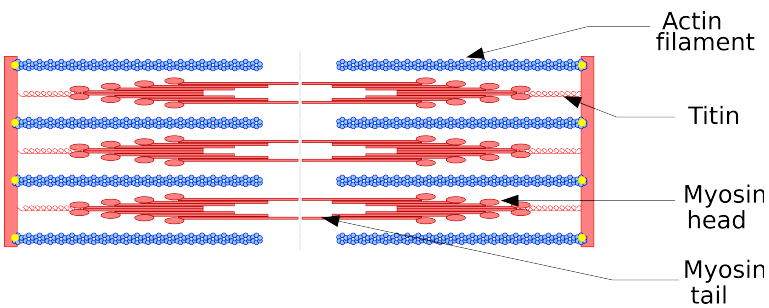
Deniset-Besseau *et al. J Phys Chem A* (2009)

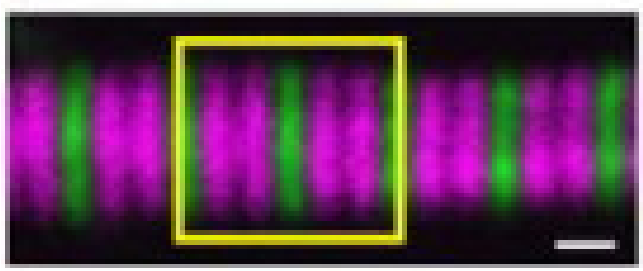
- Collective excitation modes Terenzian et al. *J Phys Chem B* (2008)

Conclusions

- Additive model seems to work for collagen
- No transferability for different wavelength !
- No transferability to arbitrary conformations (extended)
- β remains very small $\Rightarrow$ Fibrillar structure necessary !
- Fibrils of α-helices : also strong SHG ...
  no exp. data yet on small peptides.

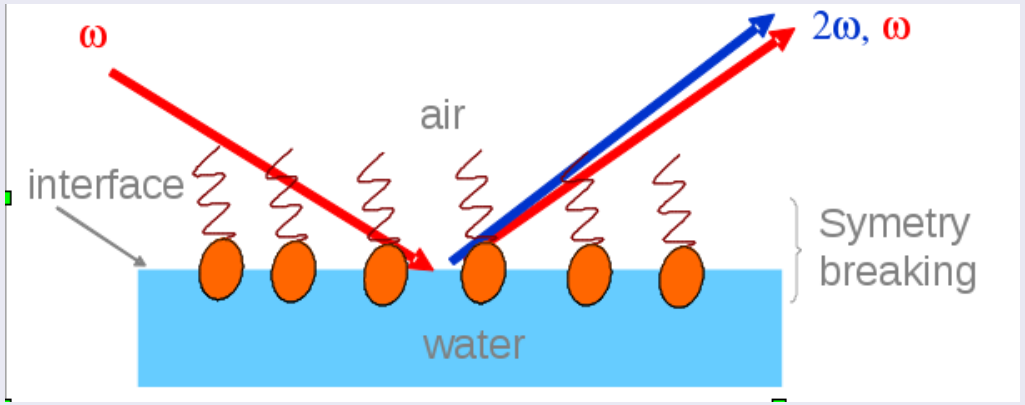
Sarcomer of a muscle fibril (SHG is violet)





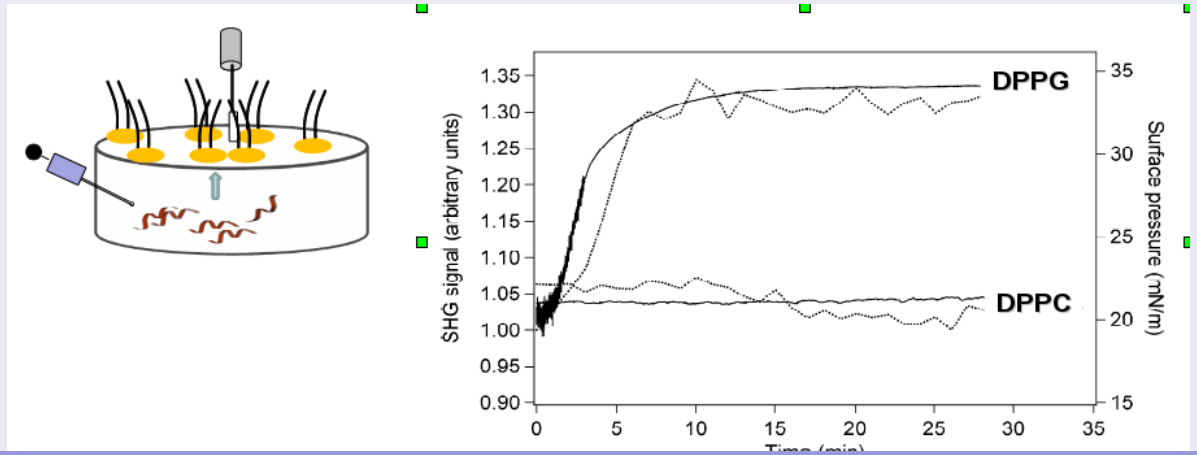
SHG of biomolecules at interfaces

SHG of Langmuir films of lipids



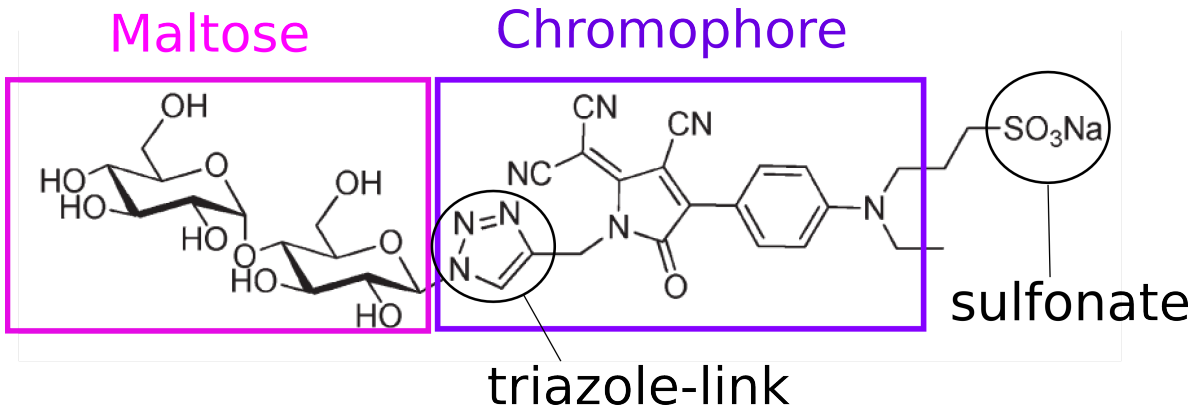
Mitchell et al. *J Chem Phys* (2005)

SHG following the adsorption of peptides

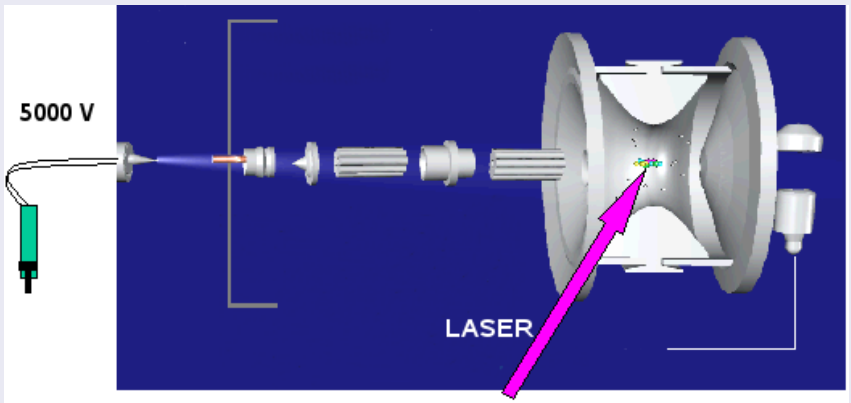


Outline

Chromophore grafted on sugars



schematic experimental setup

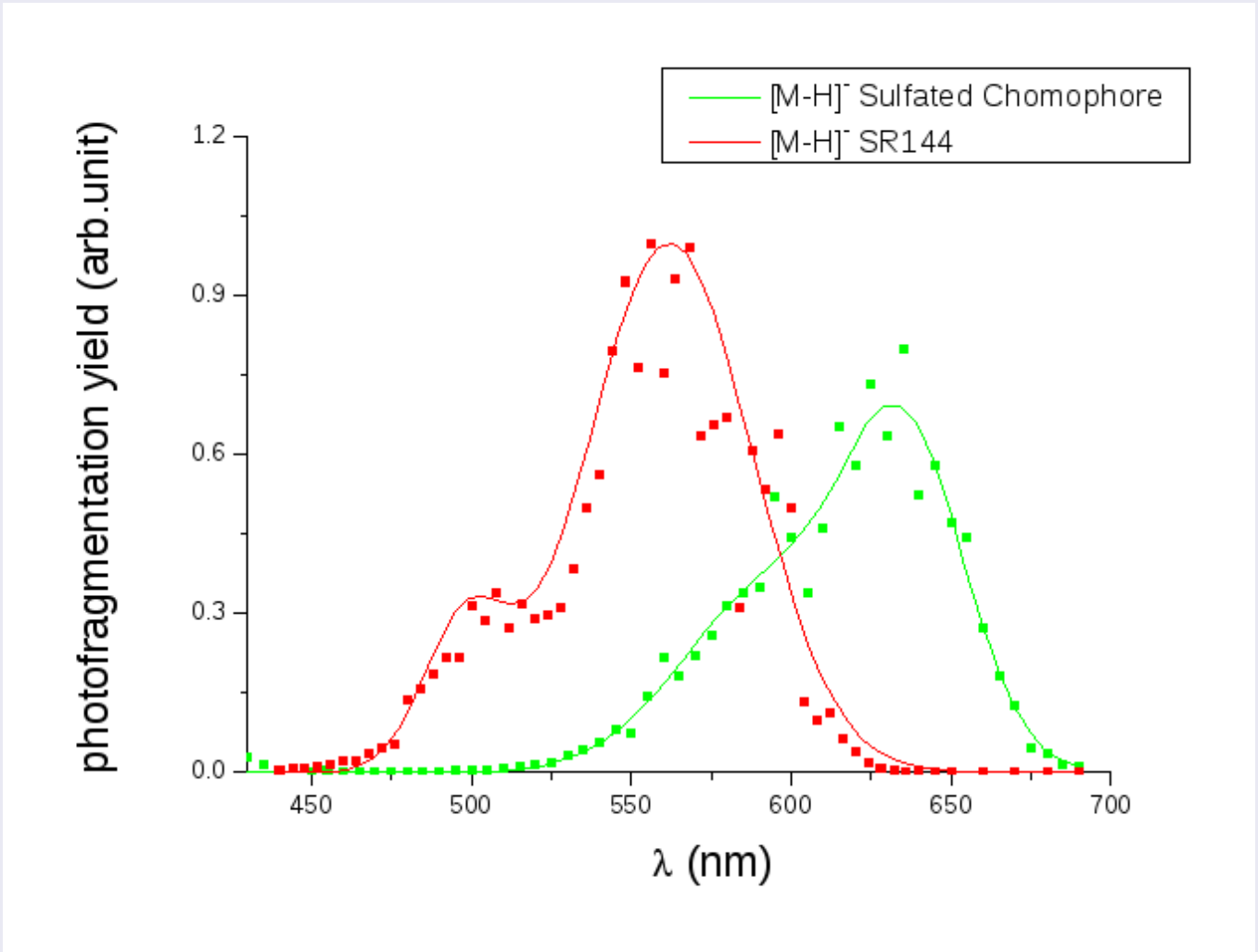


Hypothesis : fragmentation yield $\simeq$ absorption probability

A. Racaud et al. *J. Mol Struct. Theochem* (2010) and *J. Am, Soc. Mass Spectrom* (2009)

Absorption of dye and dye-grafted sugars

Influence of intermolecular interactions on the UV absorption !



Theoretical approach

Questions ...

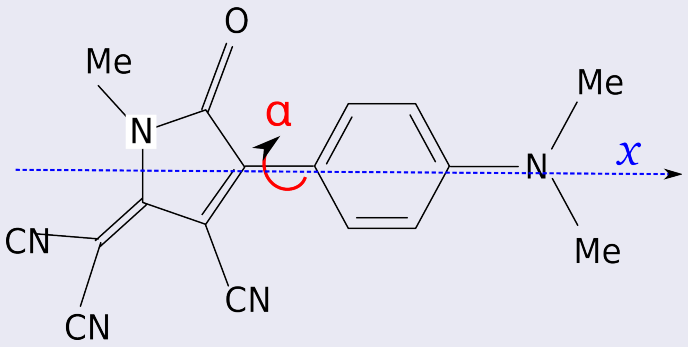
- Understand the shift due to the sugar ?
- Shift structure-dependant ?

Study the optical response of the probe

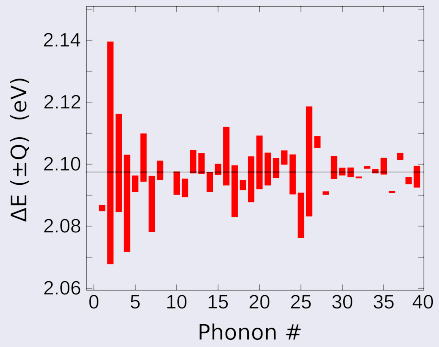
1. Optical response
   $\Rightarrow$ quantum chemical calculations (TD-DFT)
2. Structure of the sugar and probe
   $\Rightarrow$ classical MD calculations + DFT calculations

Probe Model : Structure/Response Relationship

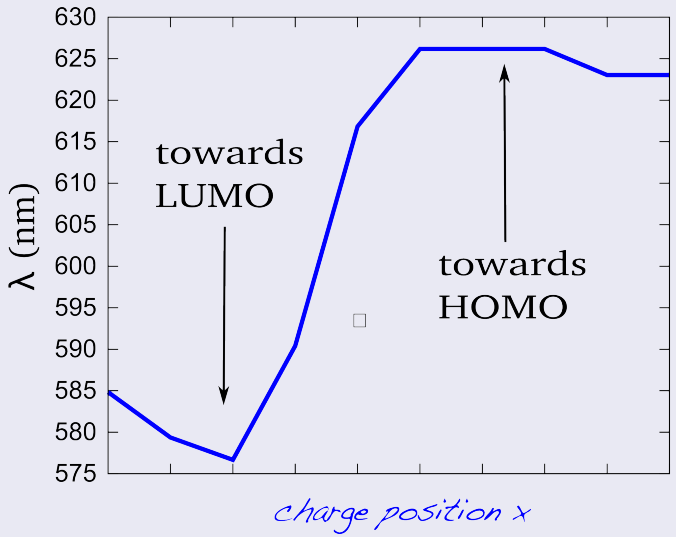
Model Molecule



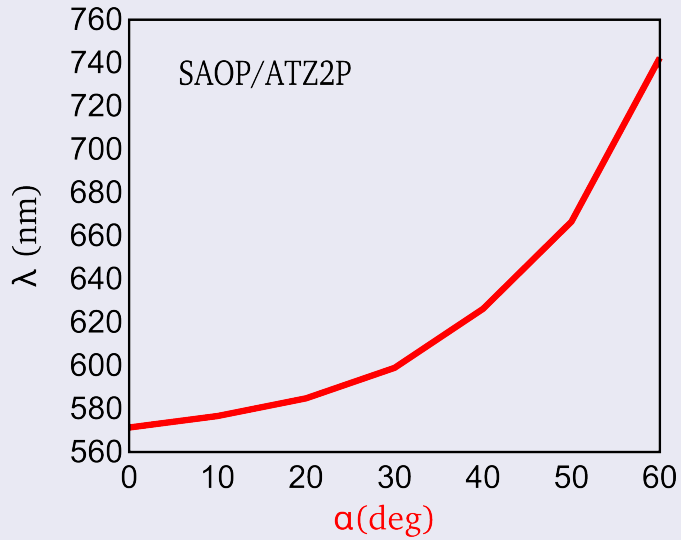
Influence of various phonons



Influence of a negative charge



Influence of α dihedral angle

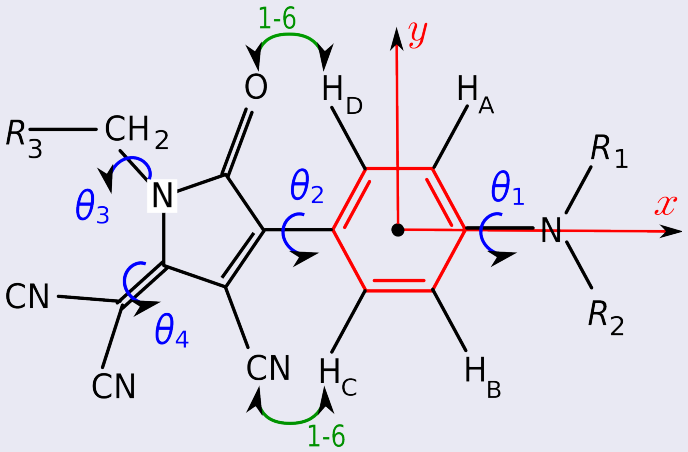


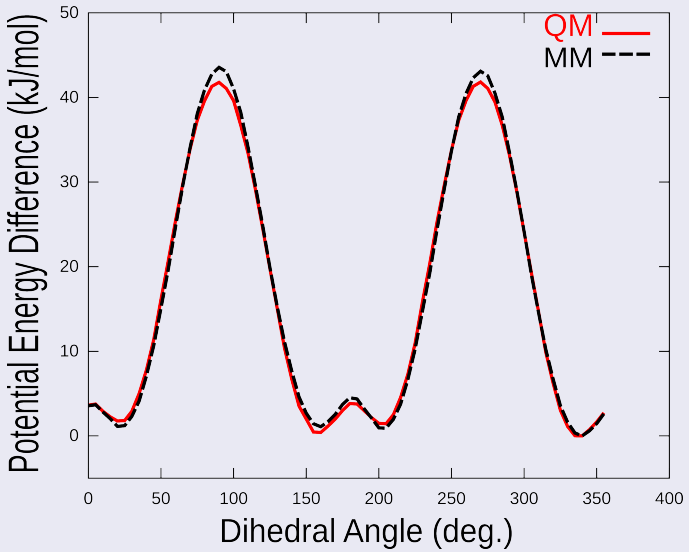
MD parametrisation of the chromophore

Model : classical MD

- OPLS-AA : sugars (2009), pyrrole and pyrrazole (1998)
- Charges from RESP from small molecules
- Angles from DFT total energy curves (M06-2X/6-311+G*)

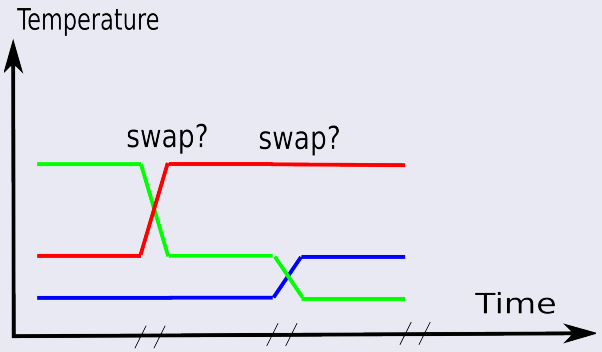
Match MM on QM curves for dihedral angles





Method : Replica-Exchange MD

Principle of REMD



Metropolis exchange

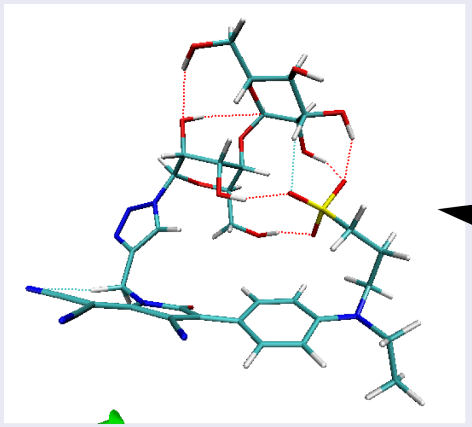
$$w(T \rightarrow T') = max\left\{e^{(\Delta\beta)(\Delta E^{pot})}, 1\right\}$$

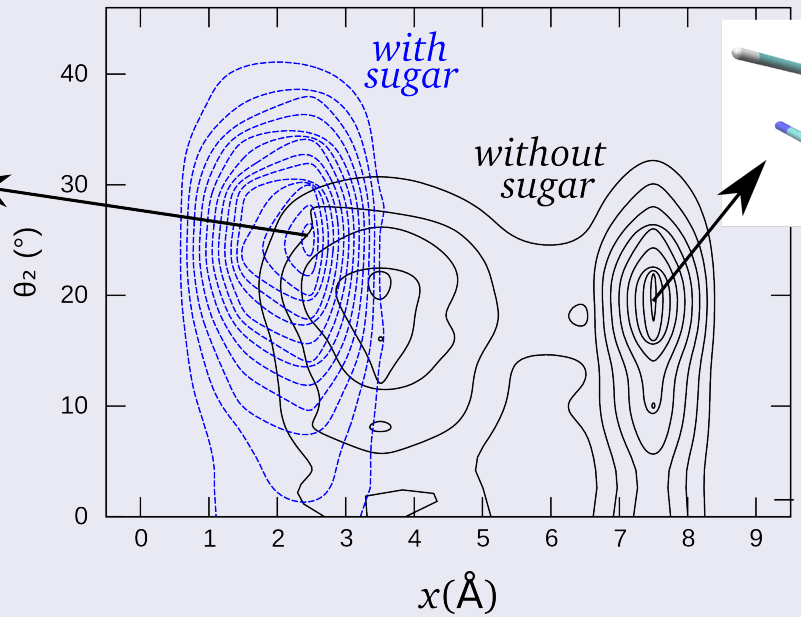
Sugita and Okamoto *Chem. Phys. Lett.* (1999)

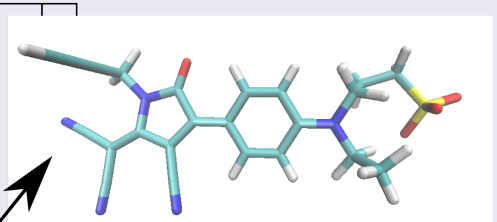
- Exchange every 100 fs
- 28 trajectories of 50 ns
- from 65 K to 2800K
- Berendsen thermostat
- high T necessary for the complex form!
- about 10^4 PM6 structure optimization
- selection $\Delta E \leq 3RT$ $\Rightarrow$ 45 geometries

Influence of sugar

Impact of sugar in the gas phase

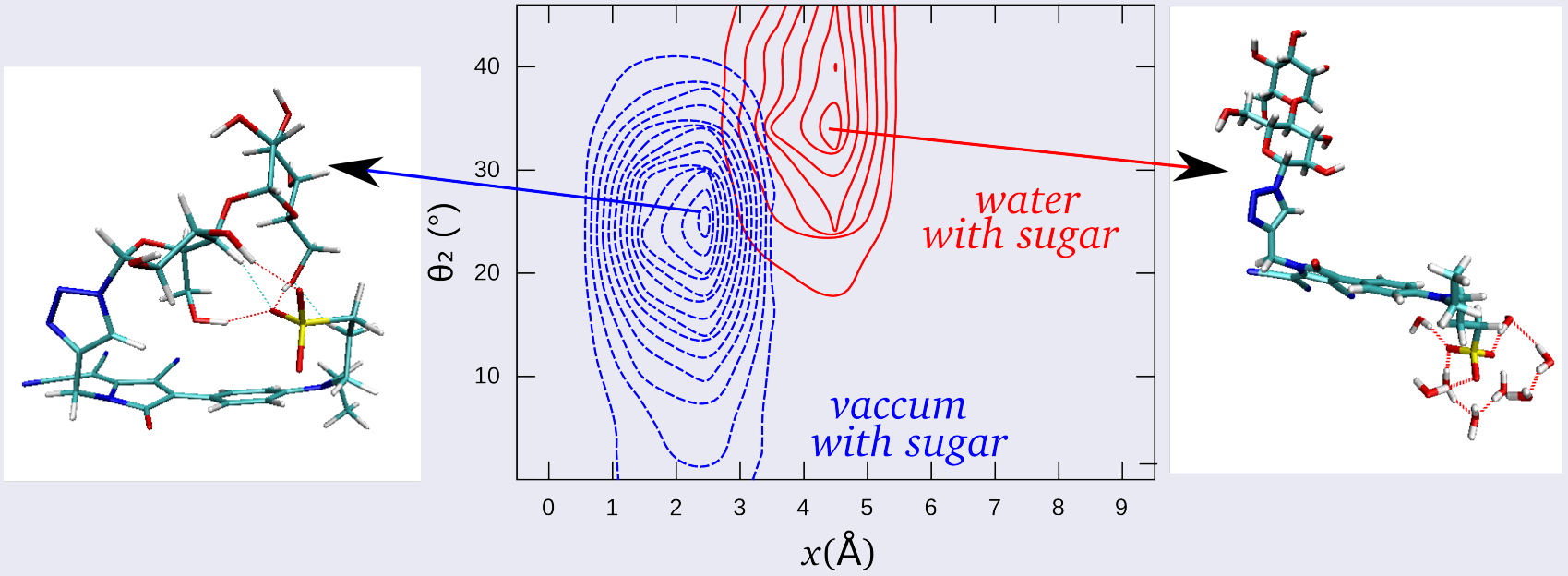






Influence of solvent on the structure

Impact of solvent on conformation



Influence of sugar on the spectra

From the gas phase calculations at T= 300K

- Dihedral angle $\langle|\theta_2|\rangle$
  - no sugar : $21 \pm 2°$
  - with sugar $24 \pm 7°$

$\Rightarrow$ no impact here

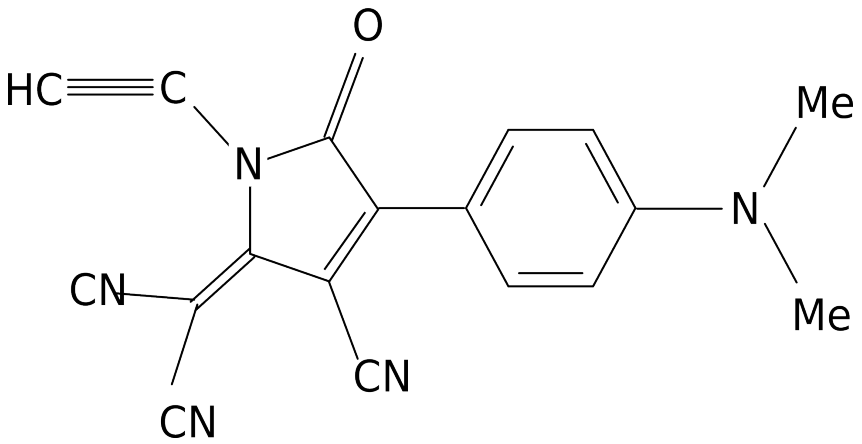
- Charge position $\langle x \rangle$
  - no sugar : towards amine (HOMO)
  - with sugar : towards sugars

$\Rightarrow$ can explain the blue shift

Points of view of a chemist on Coarse-Graining

- Back-mapping necessary and difficult
- Force-Fields for conjugated π systems are tricky
- Correlations between parameters (bonds-angles, polarization)
- Gas phase : not so simple !

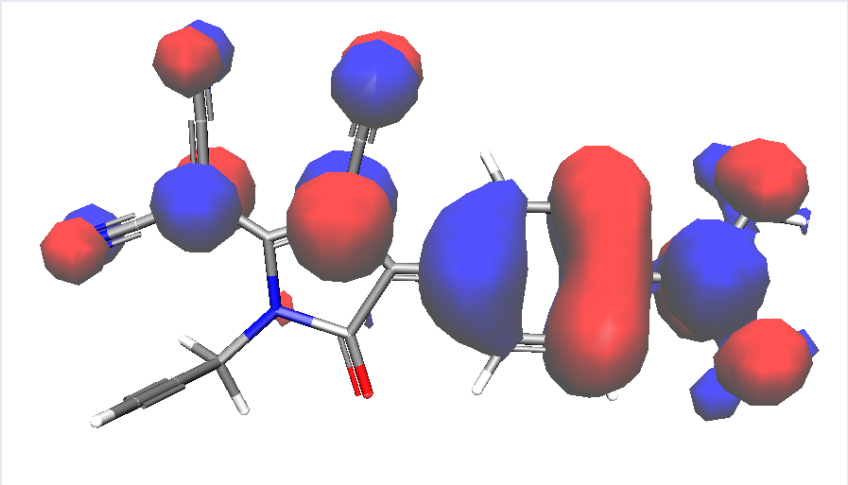
UV Optical response of a probe model



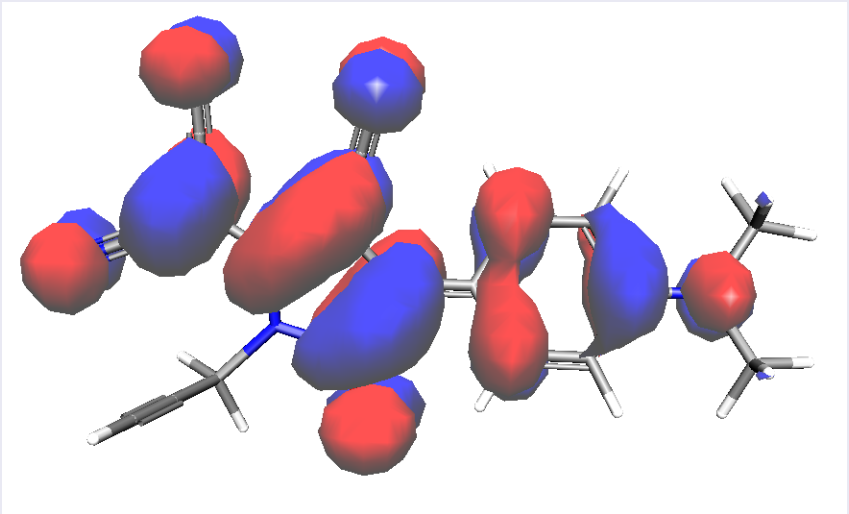
Model : no sulfonate, no sugars !

TD-DFT (SAOP/ATZ2P)
ΔE (HOMO-LUMO) $\sim$ 2.05 eV

HOMO (donor)



LUMO (acceptor)



Results for a collagen model : $(Pro\text{-}Pro\text{-}Gly)_{10}$

HRS data

$$\frac{|\beta|_{denat}}{|\beta|_{nat}} = 0.3$$

$$D = \frac{|\langle\beta^2_{XXX}\rangle|}{|\langle\beta^2_{XZZ}\rangle|}$$

$$D = 0.11$$

$$u =$$

$$u \in 0.2 - 0.7$$

Theoretical results for various input parameters

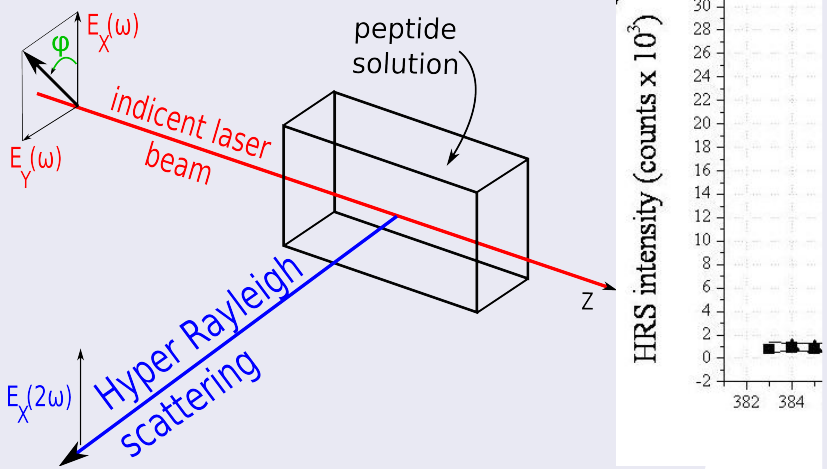
β_0		Native	Denaturated
1	$\|\beta\|$	2.7	0.9±0.2
	D	0.51	0.25±0.1
	u	-0.3	
4	$\|\beta\|$	3.5	1.1 ± 0.2
	D	0.15	0.5 ± 0.1
	u	0.9	
5	$\|\beta\|$	1.85	1.2±0.2
	D	0.46	0.45±0.2
	u	-1.3	

Native : X-ray structure (1K6F.pdb)

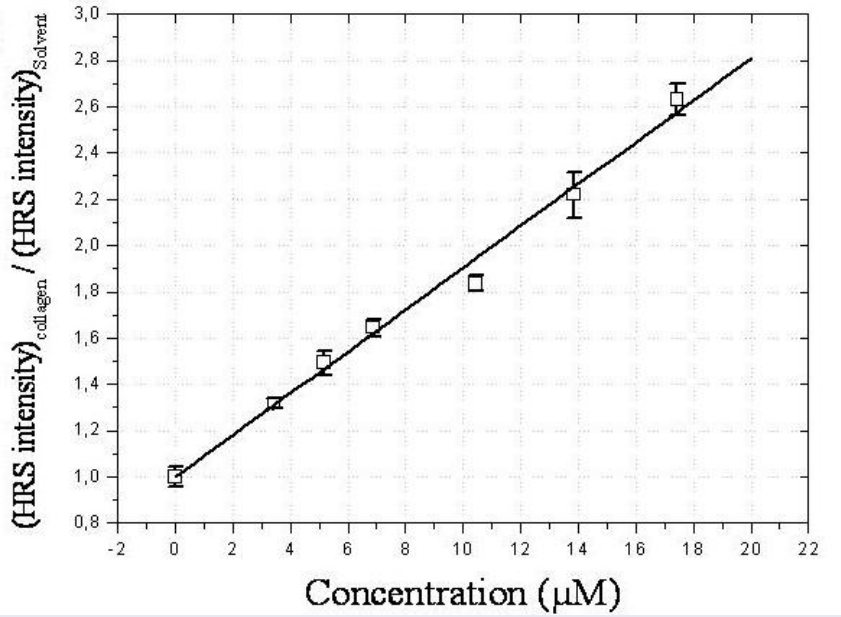
Denaturated : from classical MD in water

Hyper Rayleigh Scattering of $(Pro\text{-}Pro\text{-}Gly)_{10}$

Spectrum



Analysis



$$slope \propto \left(\frac{\beta_{peptide}}{\beta_{solvent}} \right)^2$$

A. Deniset-Besseau et al. , *J.Phys.Chem.B* (2009)

Theoretical results for $(PPG)_{10}$ - single strand

Conformation	native (exp.)	denaturated (exp.)
$\|\beta\|(800nm)$	**7.15** (20) (400)	**2.9 ± 0.8** (-) (160)
$D = \frac{\|\langle\beta^2_{xxx}\rangle\|}{\|\langle\beta^2_{xzz}\rangle\|}$	**0.13** (0.13) (0.11)	**0.47 ± 0.17** (-) (0.30)

Optical Properties of native $(PPG)_{10}$ triple helix

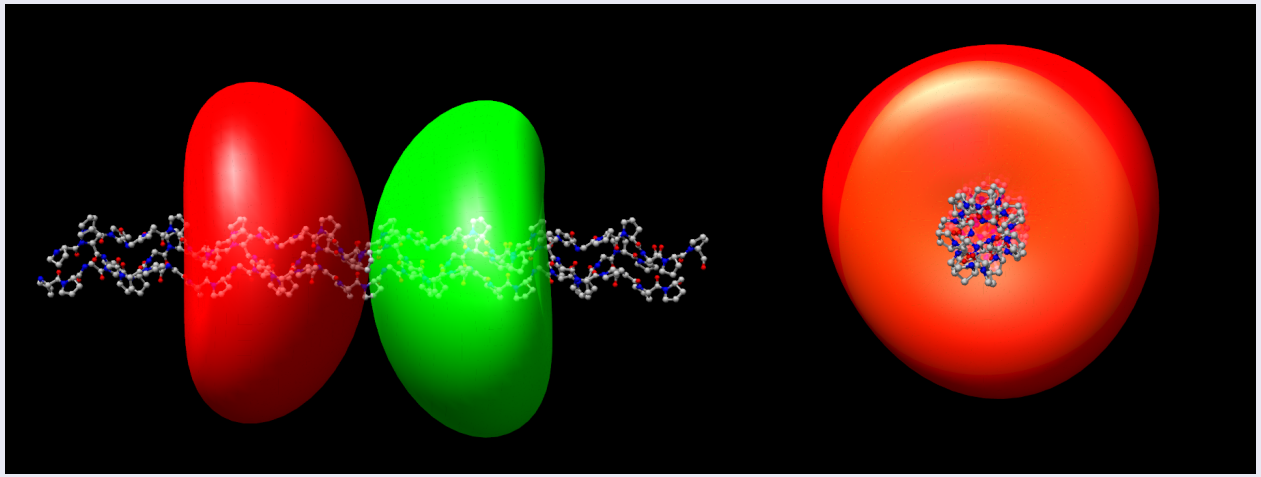
Major components of β (at 800 nm, 1K6F configuration)

$$\beta_{zzz} = 100\%$$

$$\beta_{xxz} = \beta_{xzx} = \beta_{zxx} \sim 87\%$$

$$\beta_{yyz} = \beta_{yzy} = \beta_{zyy} \sim 80\%$$

$$|\beta_{other}| \leq 10\%$$



$\hookrightarrow$ almost symmetry C_∞ with principal axis along the triple helix