How spectroscopic computations fit into the standard structural assignment protocols:

- To validate proposed assignments of spectral signals within a given structure (signal assignment)

- To evaluate the congruence of a structure to a set of experimental spectroscopic parameters (structural determination)

- To provide insight into the structural, electronic and environmental factors that influence the spectroscopic parameters

- To build structure-property relationships

Fully theoretical prediction of the ESR spectra

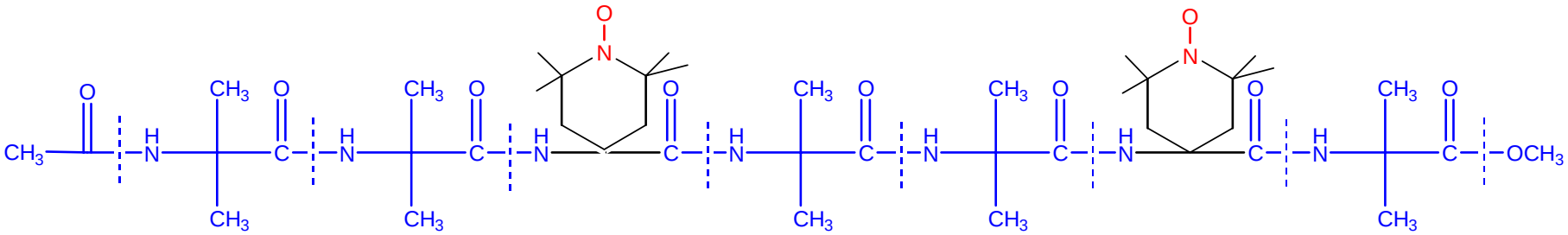
QM →
geometric and magnetic observables (A_N and g-tensors) of the radical in its enviroment (DFT methods)

+

Direct feeding of calculated molecular parameters in a stochastic dynamic model (Stochastic Liouville Equation, SLE, formalism)

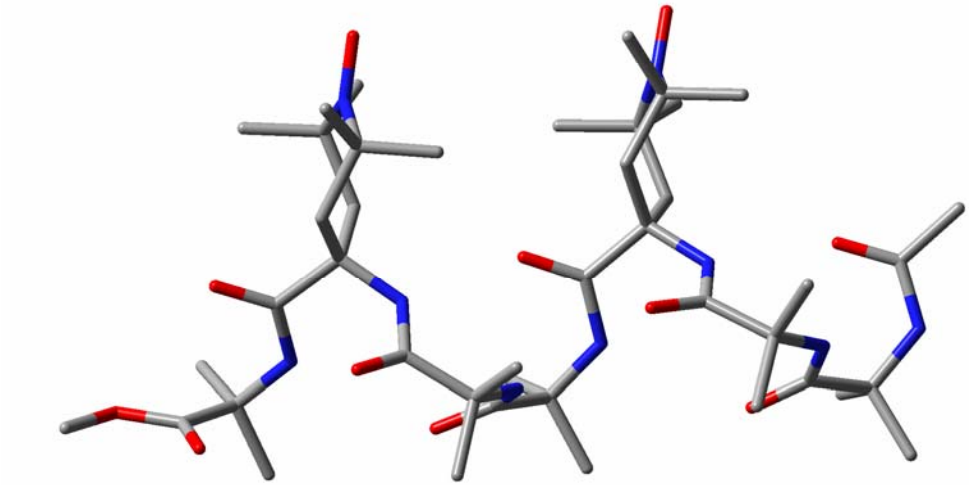
Integrated computational approach

Double spin labelled peptide:



Procedure:

1. Optimization (DFT) of the structure [PBE0/6-31G(d) in CH3CN] → 3_{10}-helical structure



2. Calculation DFT of the spectroscopic paramters (A and g tensors)

3. Evaluation of the diffusion tensor D and the dipolar interaction tensor T

4. ESR spectra simulation (Stochastic Liouville Equation)

Stochastic Liouville Equation (SLE):

$$\frac{\partial \rho(Q,t)}{\partial t} = -i[\hat{H}(Q), \rho(Q,t)] - \hat{\Gamma}(Q)\rho(Q,t) = -\left[i\hat{H}^{\times}(Q) + \hat{\Gamma}(Q) \right] \rho(Q,t) = -\hat{\mathcal{L}}(Q)\rho(Q,t)$$

the time evolution of the density matrix of the system, depending upon general stochastic coordinates Q, controlled by the stochastic operator Γ.

$$\hat{H}^{\times} = \sum_{\mu} \sum_{l=0,2} \sum_{m,m'=-l}^{l} D^{l}_{mm'}(\Omega) F^{(l,m')*}_{\mu,MF} \hat{A}^{(l,m)}_{\mu,LF} \qquad \hat{\Gamma} = D_x \hat{J}_x^2 + D_y \hat{J}_y^2 + D_z \hat{J}_z^2$$

The ESR spectrum is obtained as the Fourier-Laplace transform of the correlation function for the x-component of the magnetization:

$$|v\rangle = (2I+1)^{-1} \left(\left| \hat{S}_{x,1} \right\rangle + \left| \hat{S}_{x,2} \right\rangle \right)$$

I = nuclear spin

$$I(\omega - \omega_0) = \frac{1}{\pi} \Re \left\langle v \left| [i(\omega - \omega_0) + (i\hat{H}^{\times} + \hat{\Gamma})]^{-1} \right| v P_{eq} \right\rangle$$

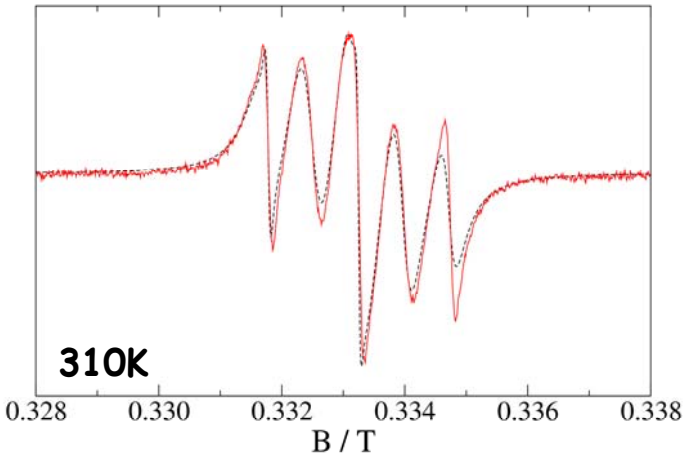
The diffusion tensor (D) was calculated for the rigid structure, considered as a set of spherical beads (i.e. extended atoms), was calculated via an "hydrodynamic approach".

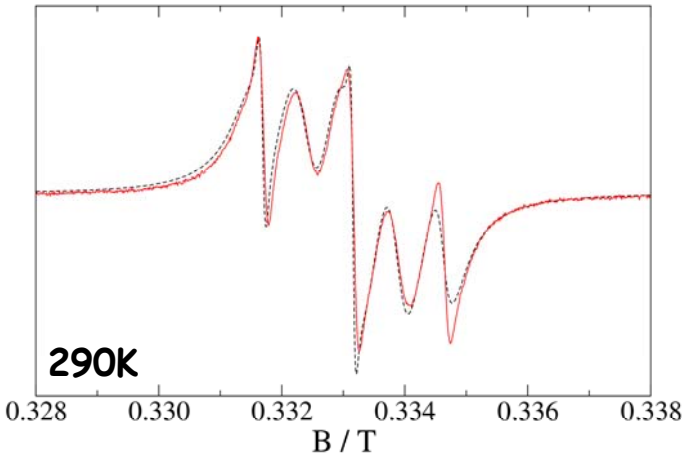
A friction tensor (T) is calculated via the comparison of the constrained and unconstrained molecular systems. The resulting tensor depends on the molecular geometry and solvent viscosity.

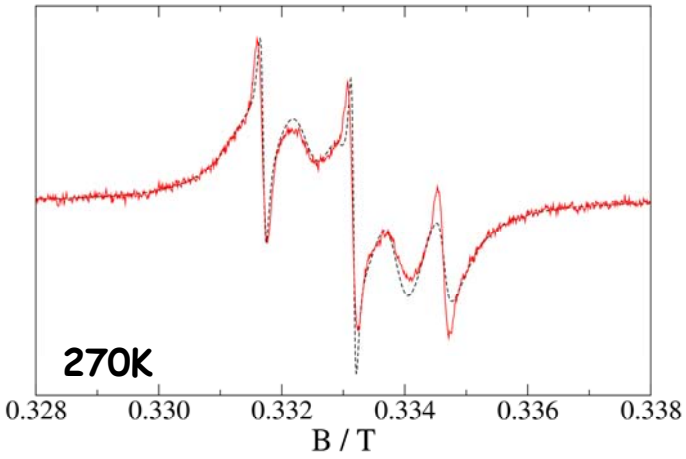
D is obtained as the inverse of T $\mathbf{D} = k_B T \boldsymbol{\xi}^{-1} = \begin{pmatrix} \mathbf{D}_{TT} & \mathbf{D}_{TR} \\ \mathbf{D}_{TR}^{\mathrm{tr}} & \mathbf{D}_{RR} \end{pmatrix}$

Parameter	Source
r_{12}	6.6 Å/QM calculations
J	300 Gauss/Literature
D	Hydrodynamic model
T	Dipolar Interaction model
g	2.009, 2.006, 2.002/QM calculations
A	3.089, 3.325, 29.901 Gauss/QM calculations

ESR spectra of the eptapeptide in acetonitrile



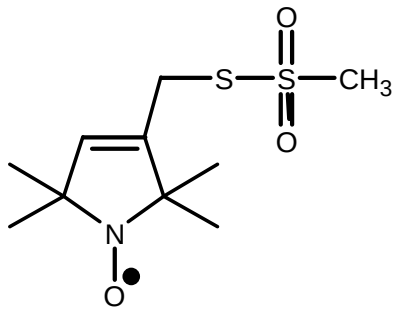




Red lines: experimental spectra
Black lines: simulated spectra

Computed values of g-tensor in gas-phase and in different protic solvents (water and methanol) and corresponding experimental data. Isotropic g value=(g_xx+g_yy+g_zz)/3

Exp.I‡	TEMPO		TEMPO+1S		TEMPO+2S	
	MTSSL	*Gas-phase*	*Solution*	*Gas-phase*	*Solution*	*Gas-phase*	*Solution*
Methanol	2.00574	2.00624	2.00608	2.00589	2.00578	2.00563	2.00555
Water	2.00551	2.00624	2.00607	2.00592	2.00580	2.00565	2.00559
	Δ=0.00023					Δ=0.00019	



(1-oxyl-2,2,5,5-tetramethyl pyrroline-3-methyl) methanesulfonate
(MTSSL)

g=g_e+g_RMC+g_DC+g_OZ/SOC

g_e=g value of the electron free (2.0023193)

g_RMC=relativistic mass correction (-0.29196557*10-3)

g_DC=diamagnetic correction to the g tensor

g_OZ/SOC= Orbital Zeeman and Spin Orbit coupling contribution to g tensor

‡Owenius R. *et al.*, J.Phys.Chem.A, 2001, 105, 10967-10977.

A combinated qualitative analysis of experimental and calculated data make it possible to interpret the parameter shifts as due to the changed e and/or the increased propensity for hydrogen bonding.

The integrated computational methods consisting of the most recent hybrid density functionals (DFT) and mixed discrete-continuum solvent models can be used to calculate shifts in A_N and g-tensor values due to changed dielectric (aprotic solvents) and hydrogen bonding properties (protic solvents), as well as the thermodinamic parameters.

CW-ESR Spectra of Fmoc-(Aib-Aib-TOAC)$_2$-Aib-OMe

"Experimentally by NMR (in solution) and X-Rays measurements" → Secondary/Tertiary Structures of proteins and peptide?

Limit?

Investigation of :

- ♦ conformational transitions
- ♦ protein folding in real-time
- ♦ detailed study of dynamic properties of proteins in solution

Promising approach

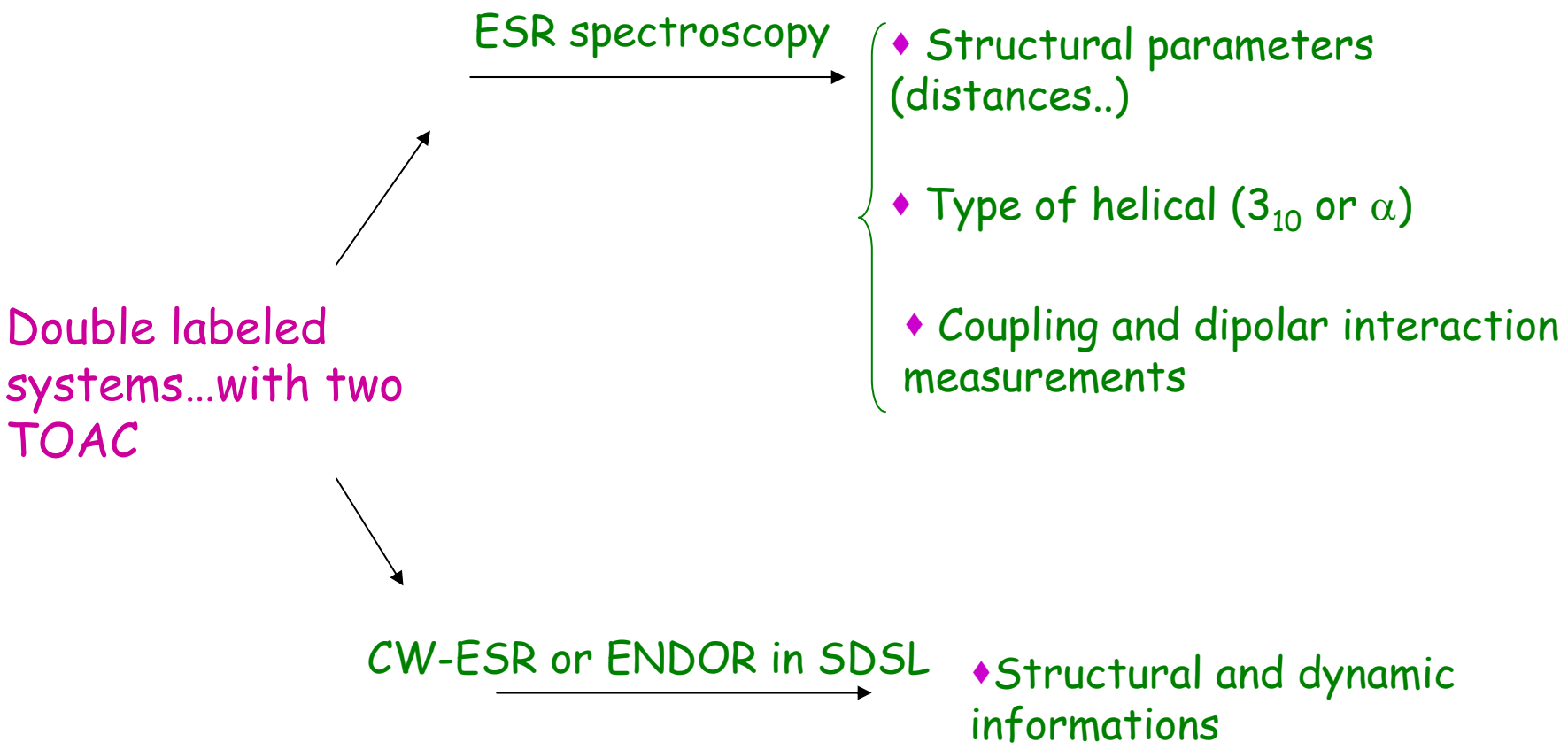
↓

SDLS
Site-Directed Spin Labelling

+

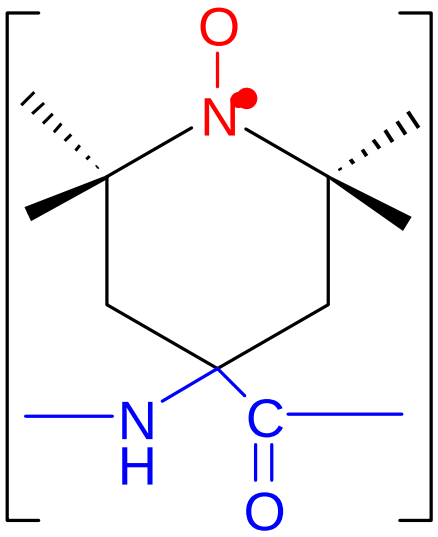
ESR
Electronic Spin Resonance

A combination of ESR spectroscopy


Double labeled systems…with two TOAC
ESR spectroscopy
♦ Structural parameters (distances..)
♦ Type of helical (3_{10} or α)
♦ Coupling and dipolar interaction measurements
CW-ESR or ENDOR in SDSL
♦Structural and dynamic informations

TOAC

"artificial α-aminoacid" which acts as a rigidly attached spin label in any chosen position of the peptide sequence



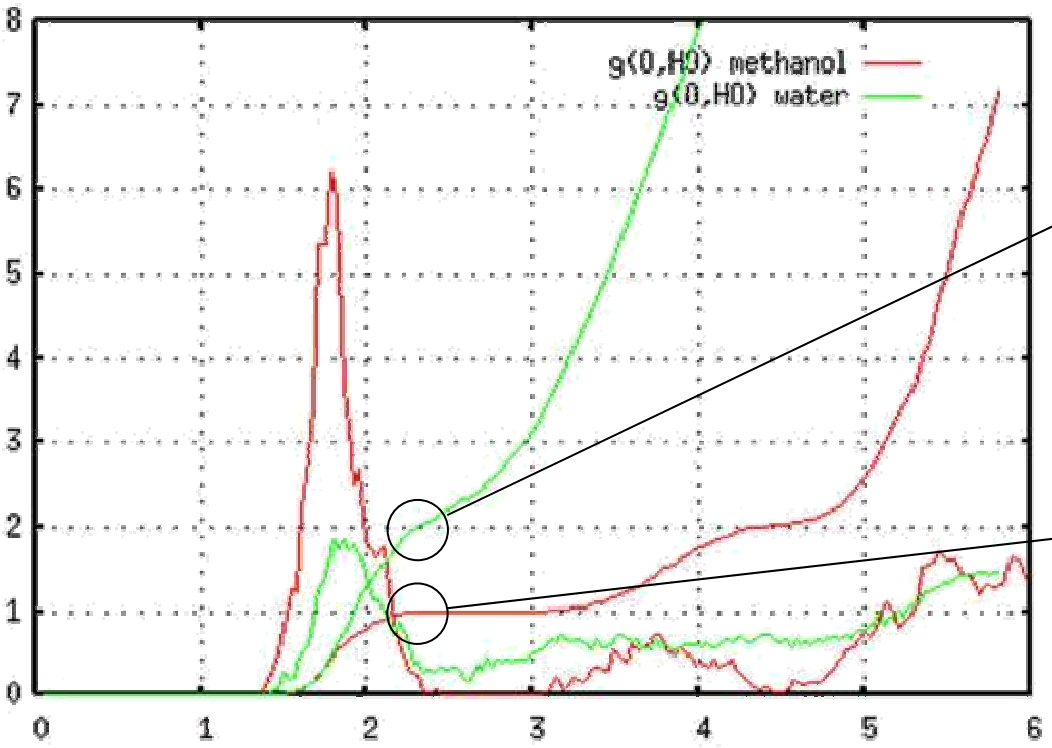
TOAC (4-ammino-2,2,6,6-tetramethylpiperidine-1-oxyl-4-carbossilic acid)

♦TOAC belongs to the family of conformationally constrained C^{α}-tetrasubstituted α-aminoacids.

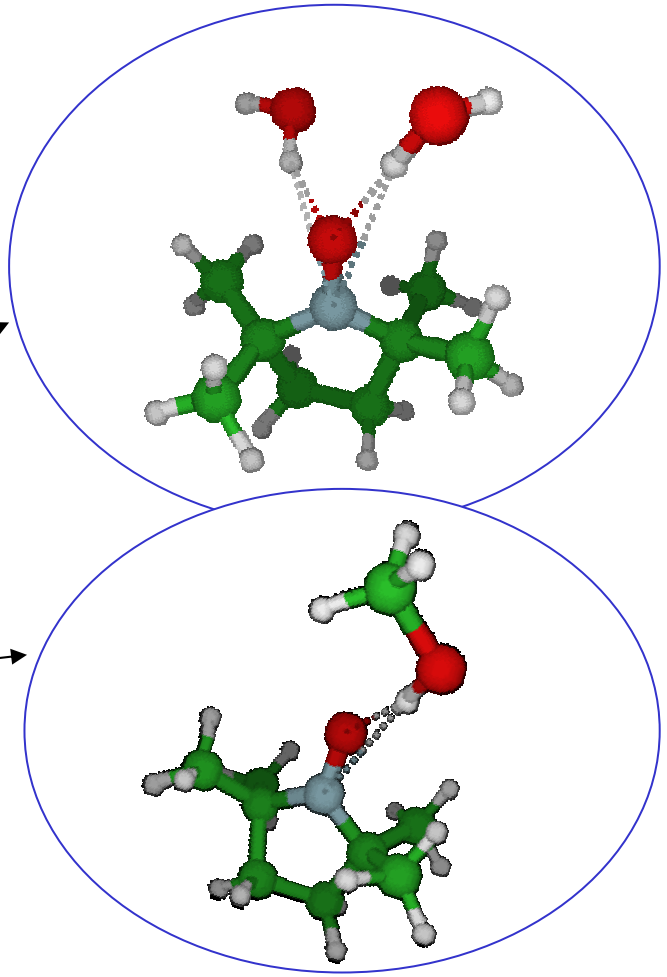
♦ TOAC is known to fold in a 3_{10}-helical structure because the ring is rigidly attached to the backbone α-carbon

Car-Parrinello Molecular Dynamics (Proxyl)

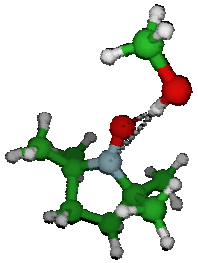
Radial Distribution Functions (RDFs)

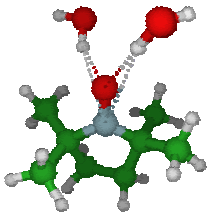


SOLVATION SPHERE



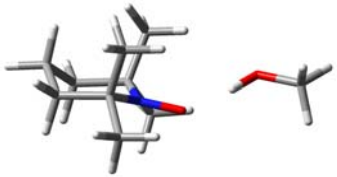
Interaction Energies (kcal/mol)

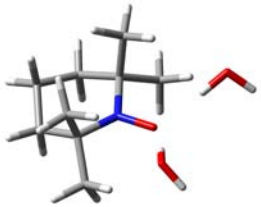




	Solvent-Solvent		PROXYL+1S		PROXYL+2S	
	Gas-phase	*Solution*	*Gas-phase*	*Solution*	*Gas-phase*	*Solution*
Methanol	-5.9	-1.4	-7.7	-0.3	-14.4	0.6
Water	-5.7	-2.9	-7.9	-1.9	-15.6	-2.8

⇒ The interaction energies have been calculated in gas-phase on geometries optimized at **PBE0/6-31+G (d,p)** level. In solution they have been computed as single points at **PCM/PBE0/6-31+G(d,p)** level by UAHF model.

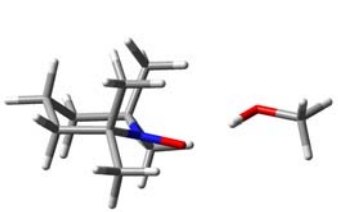




	Solvent-Solvent		TEMPO+1S		TEMPO+2S	
	Gas-phase	Solution	Gas-phase	Solution	Gas-phase	Solution
Methanol	-6.1	-1.7	-7.1	-0.7	-12.2	0.3
Water	-6.5	-3.5	-7.8	-2.5	-13.8	-3.8

⇒ The interaction energies have been calculated in gas-phase on geometries optimized at **PBE0/6-31G (d)** level. In solution they have been computed as single points at **PCM/PBE0/EPR-II** level by UAHF model.

Hyperfine Coupling Constants (A_N in Gauss) of TEMPO



	Exp.	TEMPO		TEMPO+1S		TEMPO+2S	
		Gas-phase	*Solution*	*Gas-phase*	*Solution*	*Gas-phase*	*Solution*
Methanol	16.15 ‡	**14.94**	**15.75** (methanol ε=32)	**15.51**	**16.14**	**16.06**	**-**
Water	16.91 ♯	**14.94**	**15.80** (water ε=78)	**15.50**	**16.10**	**16.03**	**16.50**

⇒ The change in A_N can separately be interpreted in terms of the dielectric properties of the enviroment and the degree of hydrogen bonding.

⇒ The higher A_N-value in water might be explained by a larger number of hydrogen bonds formed in this solvent, since the influence of dielectric constant is very small in this region.

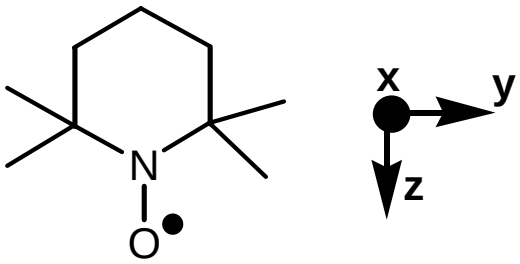
‡Aurich H.G.. *et al.*, Tetrahedron, 1977, 33, 969-975.
♯ Lim Y.Y. *et al*, JACS, 1971, 93:4, 891-894.

Calculated g-tensor of TEMPO in some solvents with different properties

Solvent	g_{iso} $(g_{xx}+ g_{yy}+ g_{zz})/3$	g_{xx}	g_{yy}	g_{zz}	Δg_{zz} (ppm)
Gas-phase	2.00624	2.00210	2.00638	2.01024	0.0
Cyclohexane	2.00621	2.00210	2.00637	2.01015	-91.7
Toluene	2.00620	2.00210	2.00636	2.01013	-110.2
Chloroform	2.00617	2.00210	2.00635	2.01007	-176.9
Isoquinoline	2.00615	2.00210	2.00634	2.01002	-224.0
Acetone	2.00614	2.00210	2.00634	2.01000	-246.9
Ethanol	2.00608	2.00210	2.00631	2.00983	-413.0
Methanol	2.00608	2.00210	2.00631	2.00984	-401.2
Water	2.00607	2.00210	2.00630	2.00982	-427.1

Polarity ↓

Decrease ↓

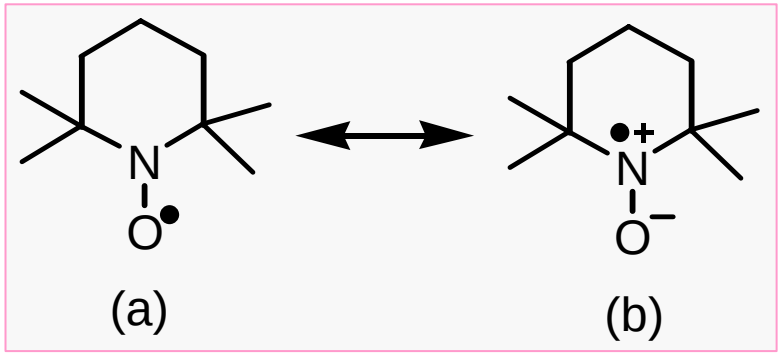


⇒ $\mathbf{g_{zz}}$ is identified as the dominant contribution to the solvent dependence and decreases with increasing ε of the solvent.

The g-tensor is calculated as a correction to the free electron value, g_e=2.002319 ⇒ **g**=ge**1**+Δ**g**

g_xx, g_yy, g_zz: diagonal elements of **g**.

Hyperfine Coupling Constants (A_N in Gauss)



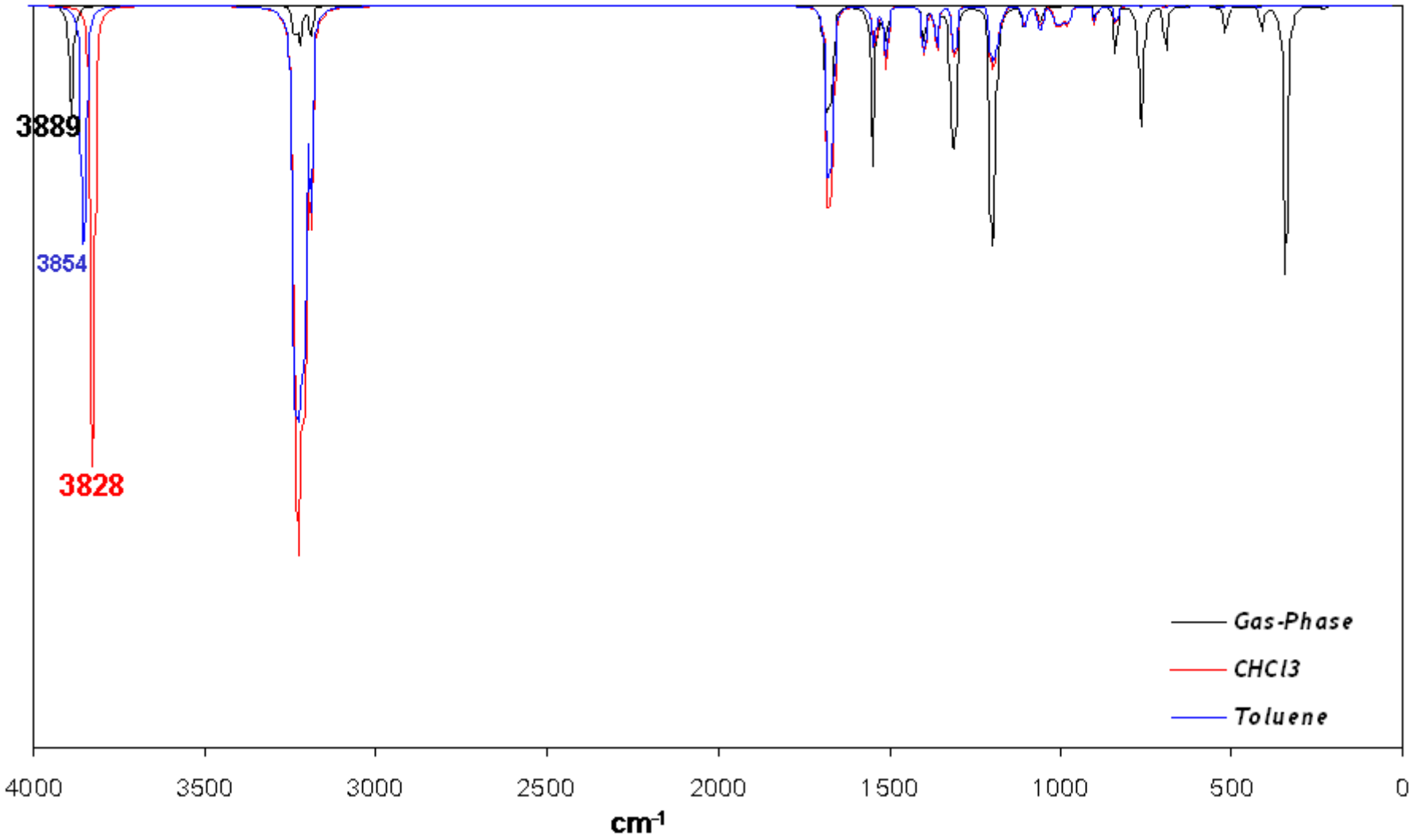
Polar solvent increase A_N through the relative stabilizatione of the "more polar" resonance structure

Bulk Effects + **Hydrogen Bond Effects** = **Overall Solvent Effects**

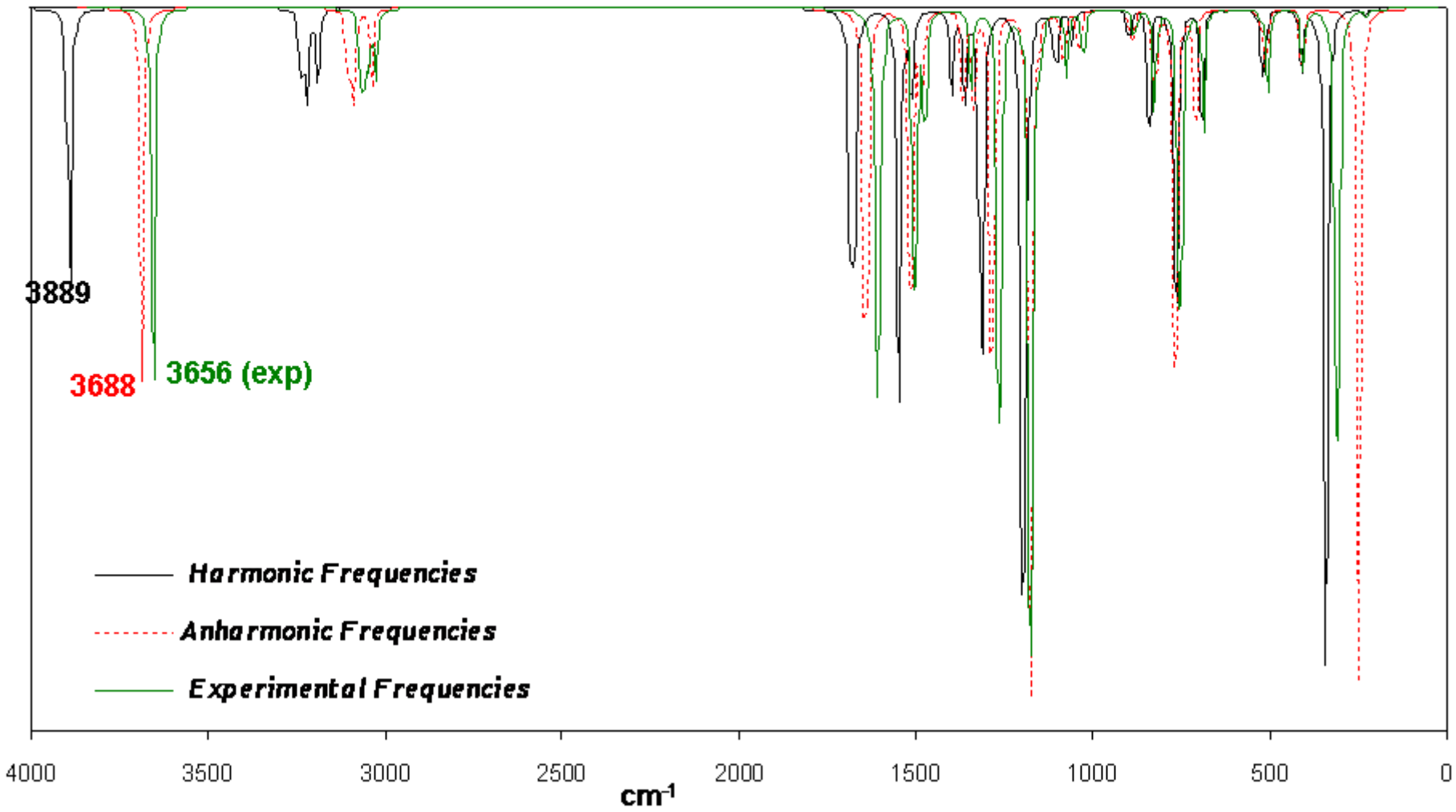
	Exp	**TEMPO**			**TEMPO+1S**		
		Gas-phase	*Solution*		*Gas-phase*	*Solution*	
Benzylic Alcohol	**15.91** (cyclohexane)	**14.94**	***15.12*** (cyclohexane ε=***2.0***)	***15.67*** (benzylic alcohol ε=***10***)	**15.46**	**15.57** (cyclohexane ε=2.0)	**15.89** (benzylic alcohol ε=10)
Phenol	**16.58** (toluene)	**14.94**	***15.16*** (toluene ε=***2.4***)	***15.70*** (phenol ε=***13***)	**15.85**	**16.01** (toluene ε=2.4)	**16.34** (phenol ε=13)

⇒ The A_N have been calculated in gas-phase as single points at **PBE0/EPR-II** on geometries optimized at **PBE0/6-31G (d)** level. In solution they have been computed as single points at **PCM/PBE0/EPR-II** level by UAHF model.

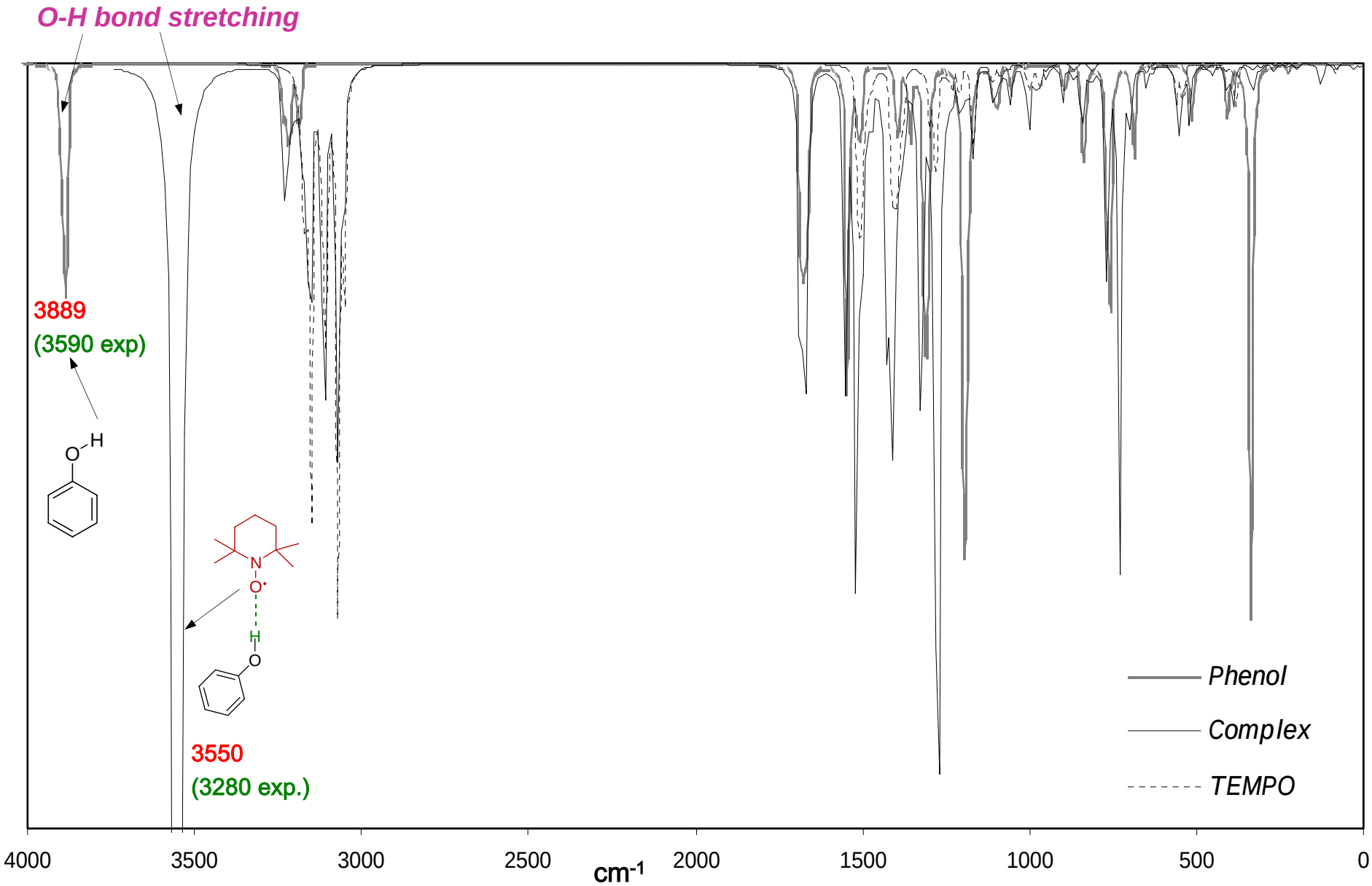
Computed FT-IR spectra for the free Phenol in gas-phase, $CHCl_3$ and Toluene at PBE0/631+G(d,p) level and PCM/ PBE0/631+G(d,p) with UAHF model.



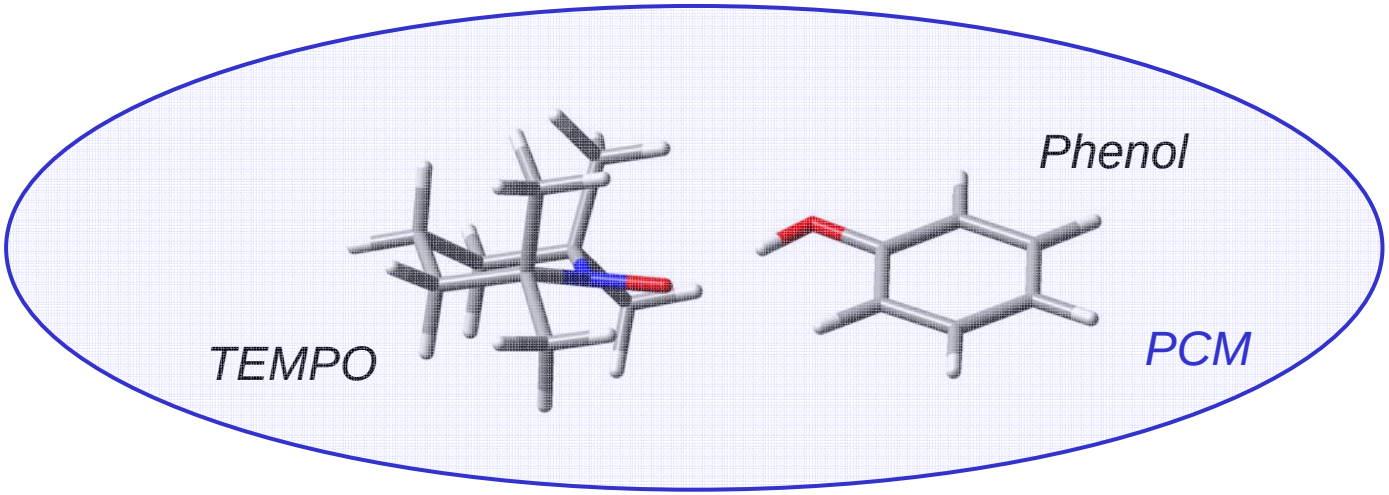
Experimental (gas-phase) and Computed FT-IR spectra for the free Phenol in gas-phase at PBE0/631+G(d,p) level.



Computed FT-IR spectra for the free phenol, TEMPO and hydrogen bond complex in gas-phase at PBE0/631+G(d,p) level.



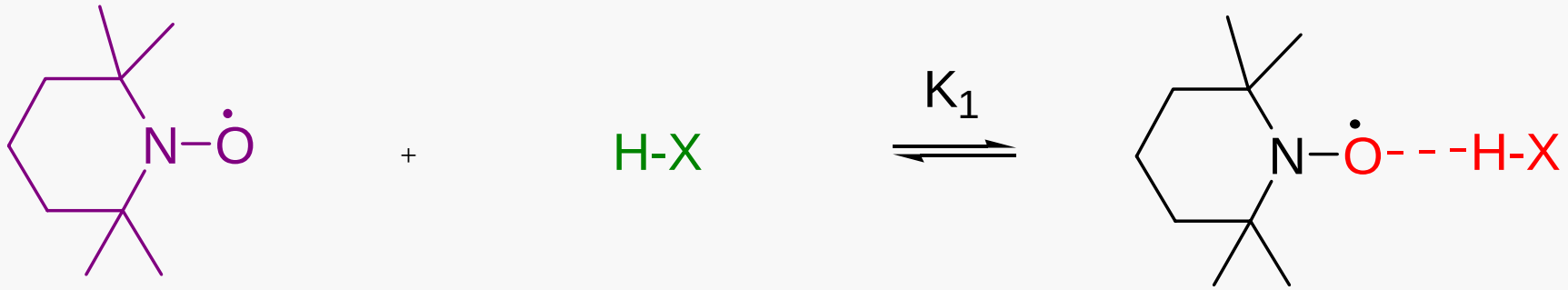
Thermodinamic parameters → ΔH



Computed vs Experimental

Gas-Phase	Solvent	Exp.
-6.03 kcal/mol	-4.91 kcal/mol	-4.69 kcal/mol

⇒ The formation energies have been calculated in gas-phase on geometries optimized at **PBE0/6-31+G (d,p)** level. and corrected for the basis set superposition error by the counterpoise method. In solution they have been computed as single points at **PCM/PBE0/6-31+G(d,p)** level by UAHF model.



Persistent radical

TEMPO
(2,2,6,6, tetramethylpiperidine-N-oxyl)

Hydrogen Bond Donor (HBD)

Hydrogen Bond Complex

↝ Experimentally in solution by FT-IR and EPR measurements.‡

Strength of the Hydrogen Bond

→ Thermodinamic parameters (ΔG, ΔH, ΔS)

→ Equilibrium constants (k_1)

→ Spectroscopic properties (Hyperfine Coupling Constant A_N).

‡Pedulli *et al.*, CHEMPHYSCHEM, 2002, 3, 789-793.

Study of Nitroxide Radicals in Condensed Phases

Embedding medium (solvent) influences → *Energies, structures and properties*

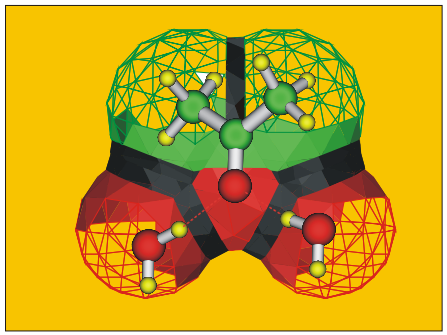
Solvent models

Bulk Effects ***Specific Effects***

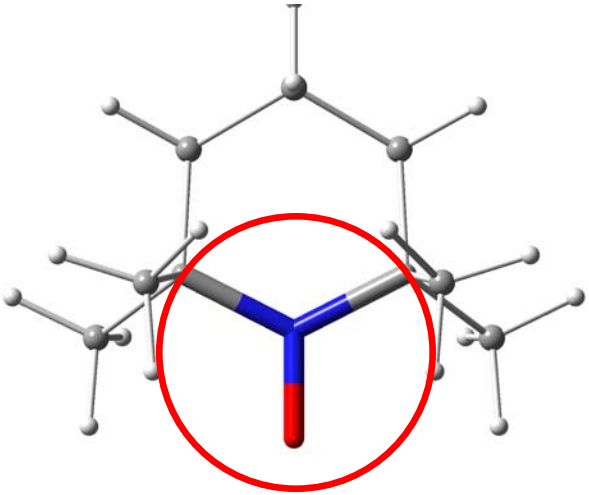
Polarizable Continuum

Cluster

A mixed discrete/continuum model



TEMPO



N-O = dimethyl nitroxide moiety
(unpaired electron)

↓

Once incorporated into the protein, spin label relaxation processes are dictated by molecular motion and local enviromental properties.

↓ ESR spectroscopic observables (A and g tensors)

Direct Information on solvent accessibility, topography of the polypeptide chain, electrostatic potential at any surface site, dynamic of the side chain, the distance from a second nitroxide and so on.

The Spin Hamiltonian

$H_s = \mu_B S\mathbf{g}\mathbf{B} + S\mathbf{A}I$ + small terms **EPR**

✓The **g**-tensor is calculated as a correction to the free electron value, g_e=2.002319 ⇒ **g**=g_e**1**+Δ**g**.

✓μ_B=Bohr magneton=eh/2m_ec

g, **A** (hyperfine coupling tensor) can be expressed as **2nd derivatives** of the **energy** with respect to **the external field (B)** and/or **electron (*S*)/nuclear spin (*I*)**

$$< X >= \frac{\partial^2 E}{\partial\lambda\partial\gamma}$$

For instance $$g = \frac{\partial^2 E}{\partial B \partial s}$$

➡ Find the terms of the electronic Hamiltonian that depends on external field and/or spin

Hyperfine Coupling Constant ($A=A_N 1+A_{dip}$)

Generally well reproduced by *ab-initio* methods for light atoms

- Fermi contact term

Non classical term: density at the nuclei

$$H_{iso} = -(2/3) g_e \gamma_e \gamma_N \mu_0 \delta(\mathbf{r}_N) \boldsymbol{SI}$$

Isotropic (A_N)

Purposely taylored basis sets: EPR-II, EPR-III but 6-31+G(d,p) for structures and thermodynamic properties

- Dipolar term

Classical dipole-dipole interaction

$$H_{dip} = [(g_e \gamma_\varepsilon \gamma_N \mu_0)/4\pi] [\boldsymbol{SI}/r_N^3 - 3(\boldsymbol{S}\mathbf{r}_N)(\mathbf{r}_N \boldsymbol{I})/\mathbf{r}_N^5]$$

Anisotropic (A_{dip})

In solvent with low viscosity (at ambient T that is true for all solvents in this study), the fast rotational motion of the spin label causes $\mathbf{A}_{dip}$ to average out ($\mathbf{A}_{dip}=0$).

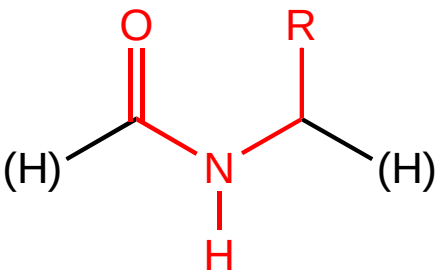
γ_e and γ_N=magnetogyric ratios for electron and nucleus; μ_0=vacuum permeability; $\delta(r_N)$=delta function which extracts the spin density at the nucleus; $\mathbf{r}_N$=electron-nucleus distance.

Three different partition schemes (1-2-3) are tested within a QM/MM hybrid framework

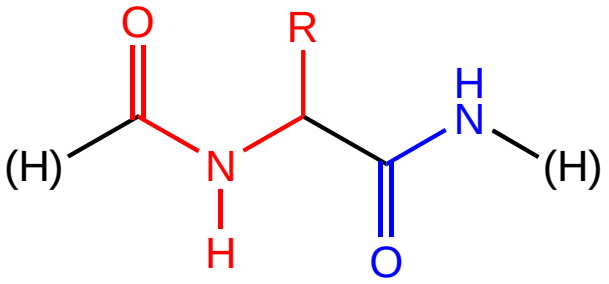
Inner → QM level

Atoms in the remaining part of the molecule → Point charges from AMBER parameters

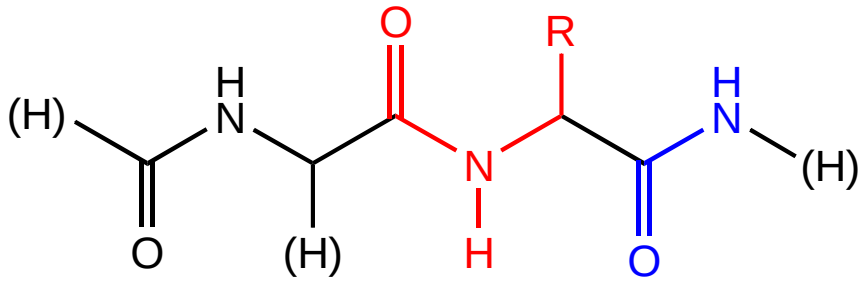
QM regions: A1-A2-A3



A1



A2



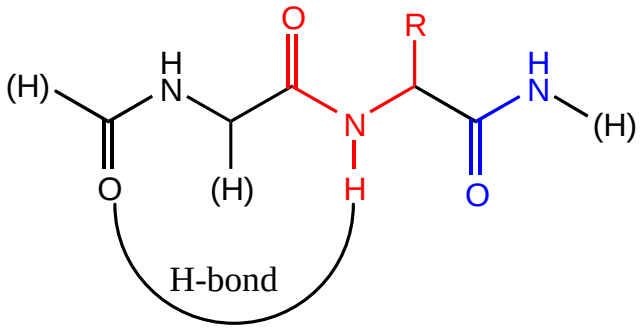
A3

Link atoms to saturate dangling bonds (to ensure that the model system has same elctronic structure as the real system): H atoms for the broken bonds and the original bond distance is scaled in order to resemble a C-H bond lenght (0.7).

$\Delta_{2\text{-}3}$= significant differences for Ala2 , Gly4 and Gly8

$\Delta_{1\text{-}2}$= constant

Residue	1	2	3	BCP2 PBE0/ 6-311+G(2d,p)
Glu1	28.4	27.5	27.2	25.9
Ala2	26.6	25.9	24.4	22.8
Gly4	27.4	27.4	26.6	25.0
Lys5	29.2	28.2	27.8	26.0
Ala6	28.4	27.3	27.0	25.9
Gly8	27.0	26.8	25.7	24.0
Gly9	29.1	28.4	27.6	26.0



A3

$$\sigma_{iso} = \sigma_{iso}(MM_{all}) + \sigma_{iso}(DFT_{A3}) - \sigma_{iso}(MM_{A3})$$

♦ QM region = fragment centered on the amide moiety of interest and including both peptidic bonds before and after the nucleus of interest.

The effect due to AMBER charges is significant only for those residues in which H^N is involved i hydrogen bonds (Ala2, Gly4 and Glu8), and for partition schemes 1 and 2, where the QM region is relatively small

♦ MM region = side chains have a minor influence on the chemical shift of amidic hydrogen atoms

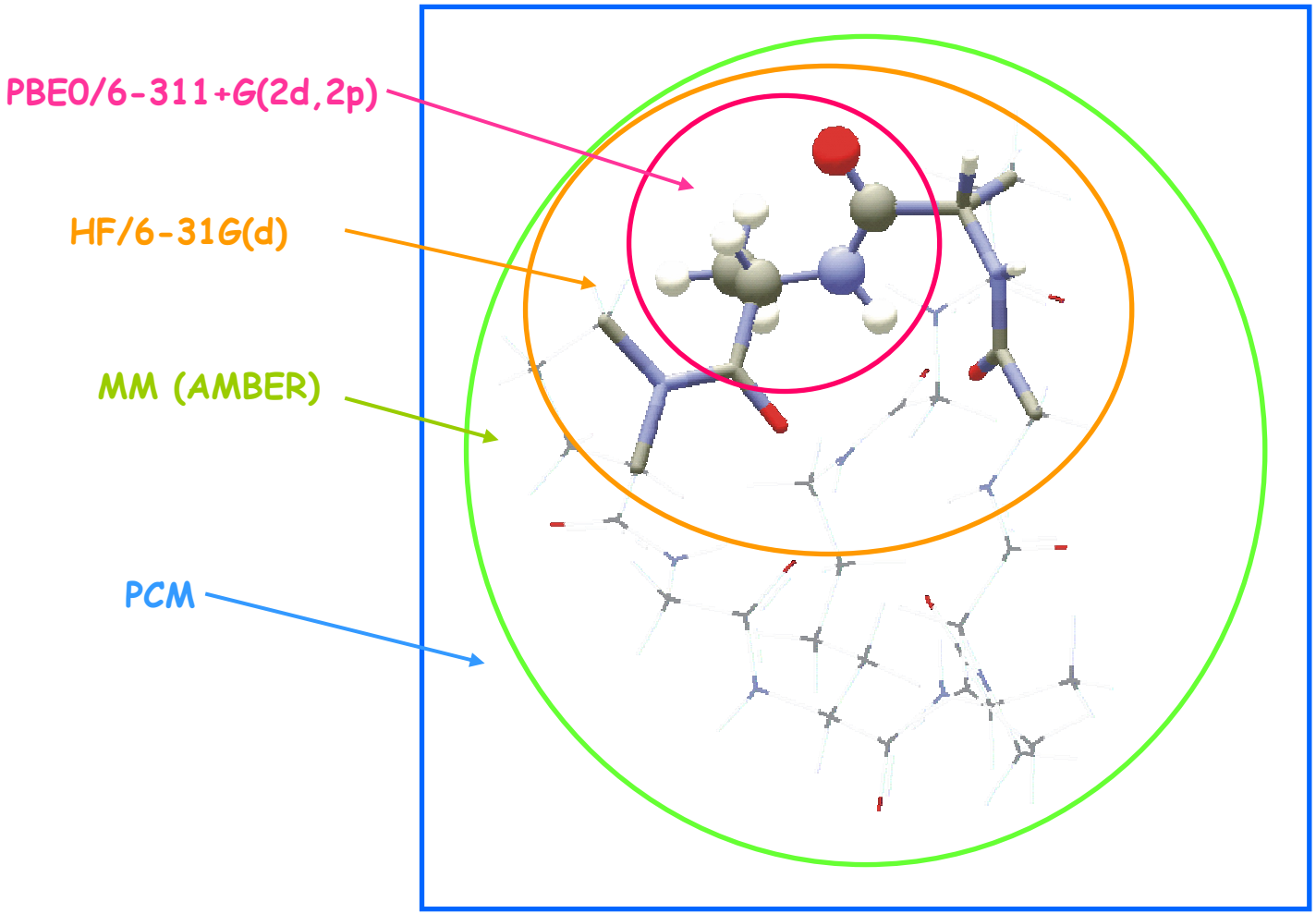
Good agreement with full QM results !!!!

Final three-layer QM/QM/MM hybrid method

Two different QM approaches are used in an ONIOM-like scheme

+

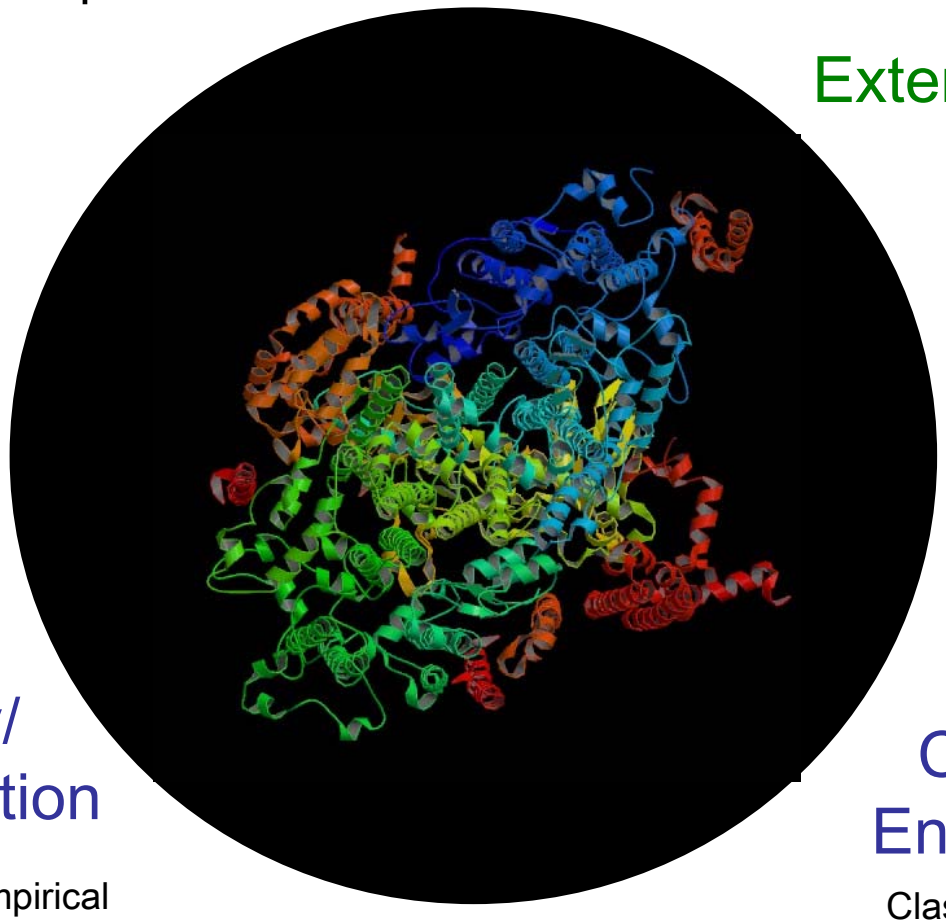
Point charges take into account polarization due to the remaining part of the molecule



This partition leads to a reduction in computer time of about one order of magnitude with respect to a two-layer (QM/MM) model and two order with respect to a full QM model

What do we want to compute?

A property that depends on:



Structure

Classical (MM, MD, ...)
Quantum (structural minimization, CPMD, BOMD, …)

External field

Relativistic effects

Spin density/ density distribution

Quantum: from semi-empirical to correlated methods

Chemical Environment

Classical or quantum: embedding, dynamics, ...

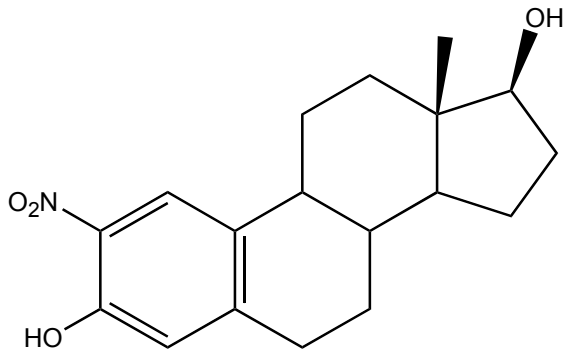
Main computational methods we use:

- DFT with hybrid functionals (PBE0): fast, amenable to treat large structures, or to explore significant portions of the conformational space (systematic conformational searches, grid searches etc.)

- For organic compounds, DFT works usually well for geometries and energies [6-31G(d), 6-31+G(d,p)], as well as for ^{1}H, ^{13}C and ^{15}N isotropic shifts [GIAO, 6-311+G(d,p)]

- PCM to model solvent medium; when specific interactions are involved (hydrogen-bonds), cluster-PCM approaches

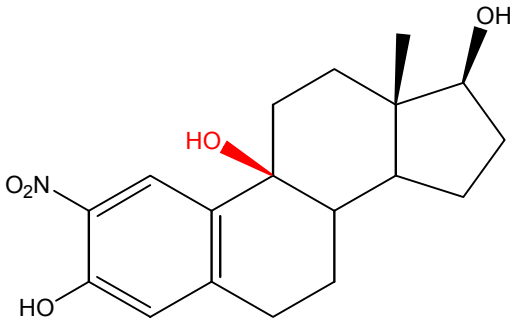
Calculation of ^{13}C chemical shifts of nitroestradiol oxidation products



2-nitro-17β-estradiolo

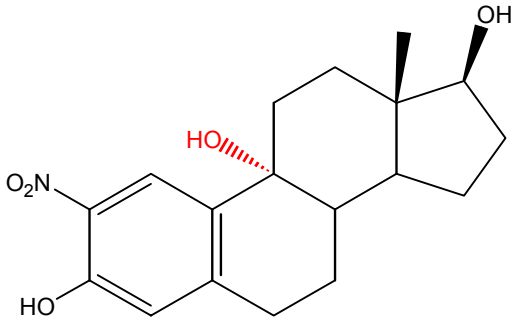
Precursor to compounds with anticancer activities and as probes for ligand-receptor interactions

Oxidation (peroxidase/H_2O_2)



A

(1:0.3)

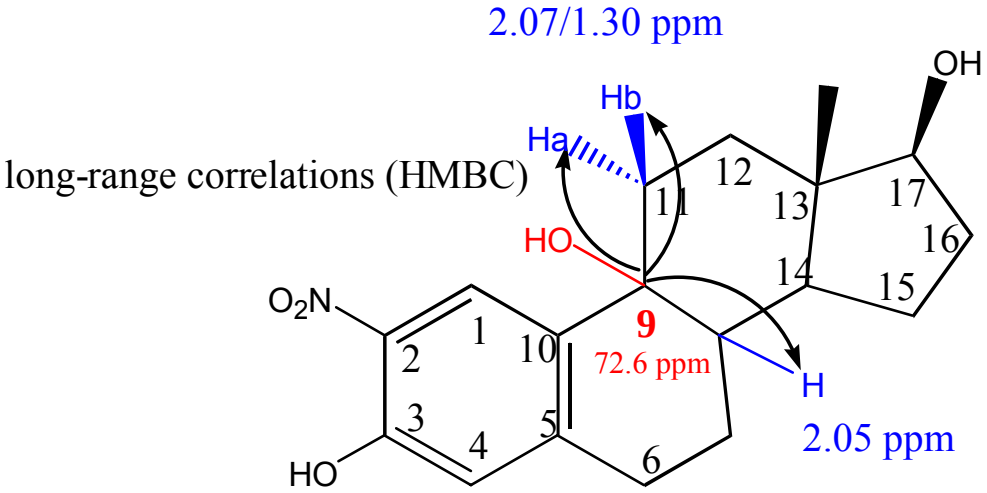


B

Very similar proton and carbon resonances

Isomers?

NMR experiments: ^{1}H, ^{13}C, ^{1}H COSY, ^{13}C HMQC, ^{1}H and ^{13}C HMBC
UV-IR, MS-ESI



A

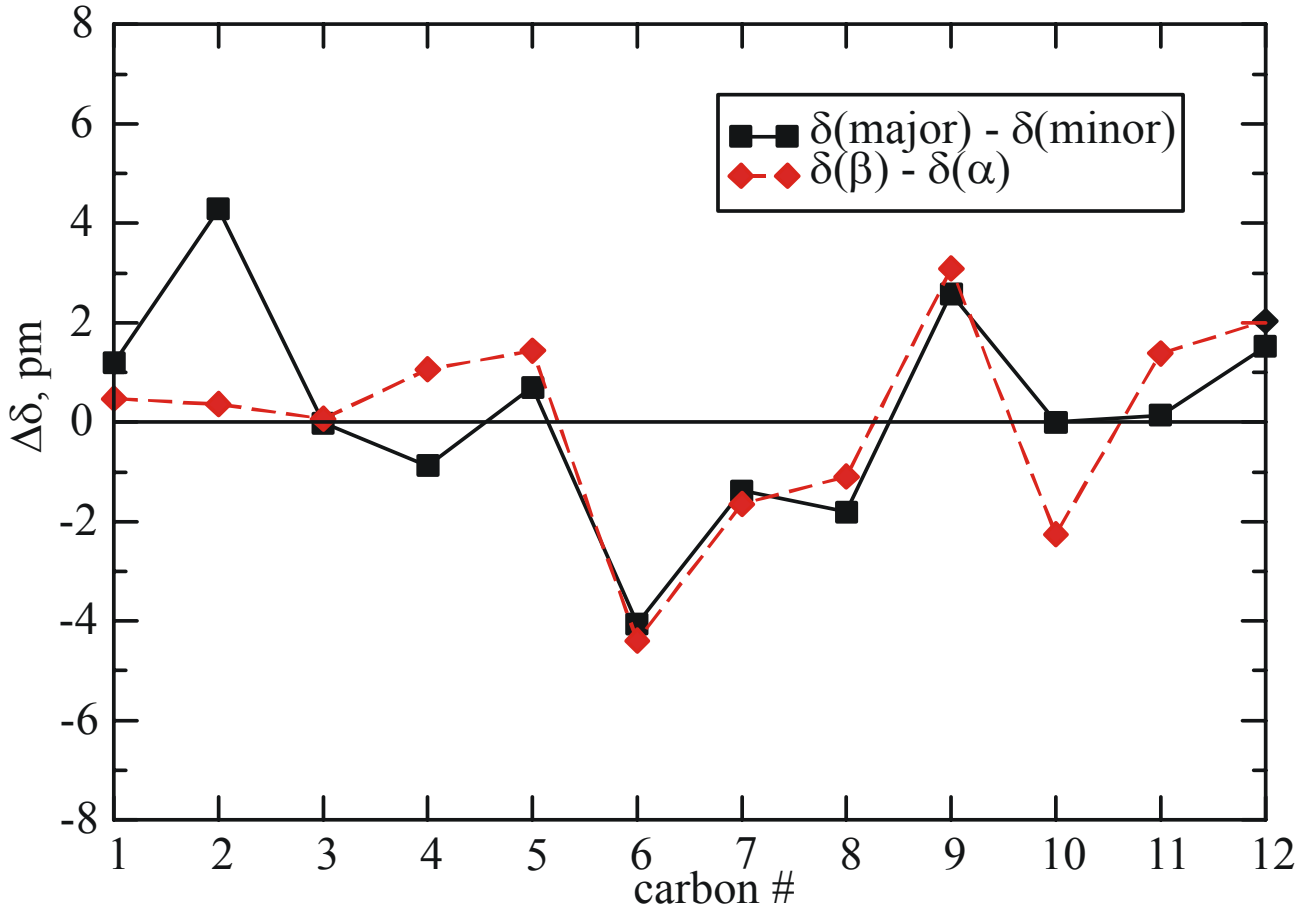
NMR analysis do not allow unambiguous stereochemical assignment to the distereoisomes

↓

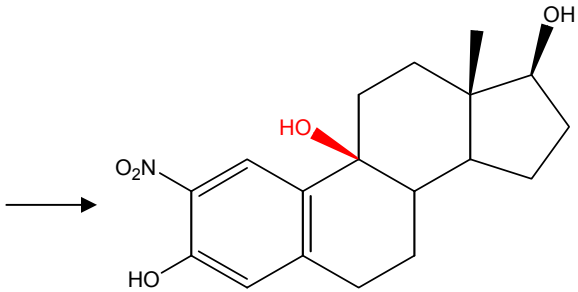
Quantum mechanical (QM) study on structures A and B (DFT)

Optimization PBE0/6-31G(d). NMR calculations PBE0/6-311+G(d,p).
Solvent: acetone (PCM). Reference: TMS

Comparison of ^{13}C chemical shift values calculated for the optimized structures of the 9α and 9β configurations with experimental data



Assignment of the β configuration to the most abundant isomer (A)



A

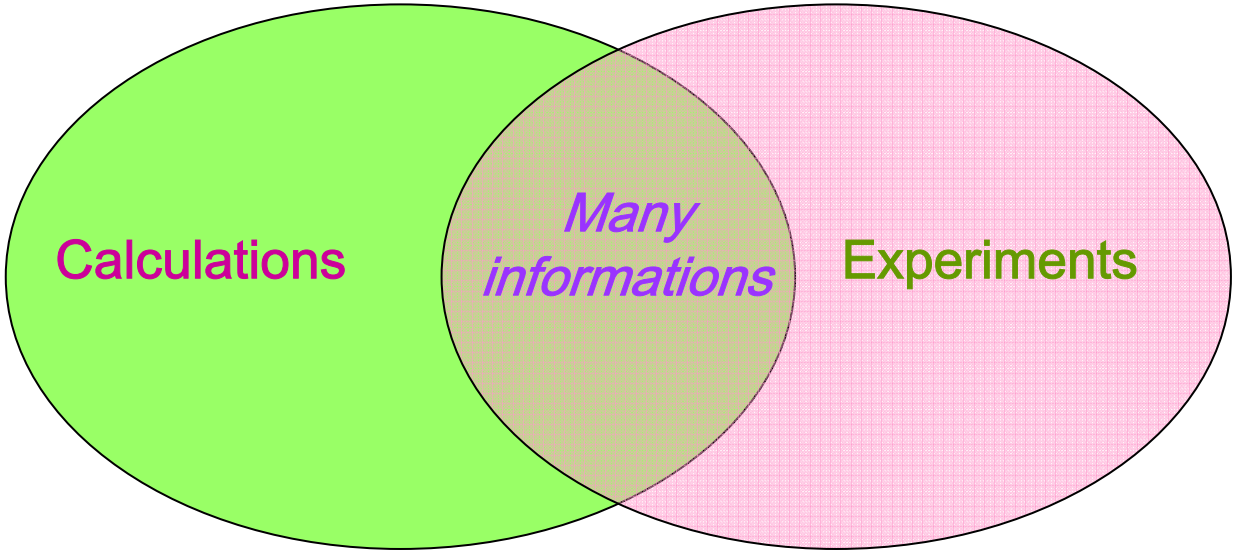
Calculation of amide proton chemical shift in a calcium binding protein

Chemical shift (δ) ⟷ NMR (Nuclear Magnetic Resonance)

sensitive probe of molecular conformation, composition and enviroment.

widely used approach for studying the structure of chemical and biological systems.

Which is the relationship between chemical shifts and molecular structure ?



In peptides and proteins amide protons (H^N) are susceptible to additional local structural effects with respect to aliphatic protons.

NMR database → Isotropic

- "Local"
- Secondary structure
- Hydrogen bonds.

Ab initio study → Anisotropic

- "Local" (weakly on of the secondary structure of the protein and strongly on the hydrogen bonds)
- "Non local" (aromatic ring currents, megnetic anisotropies, electrostatic and solvent effects, influence of different parts of the proteic system, presence of metal ions) effects.

Which is the accurate computational model?

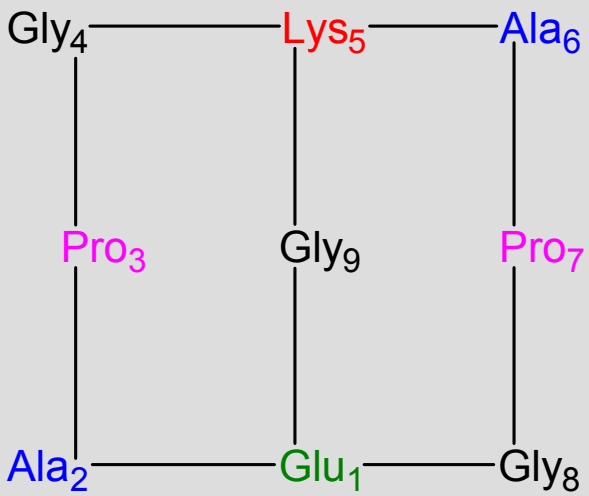
- A quite rigid peptide → local (structural) effects are of minor importance
- Right dimension of the system

↓

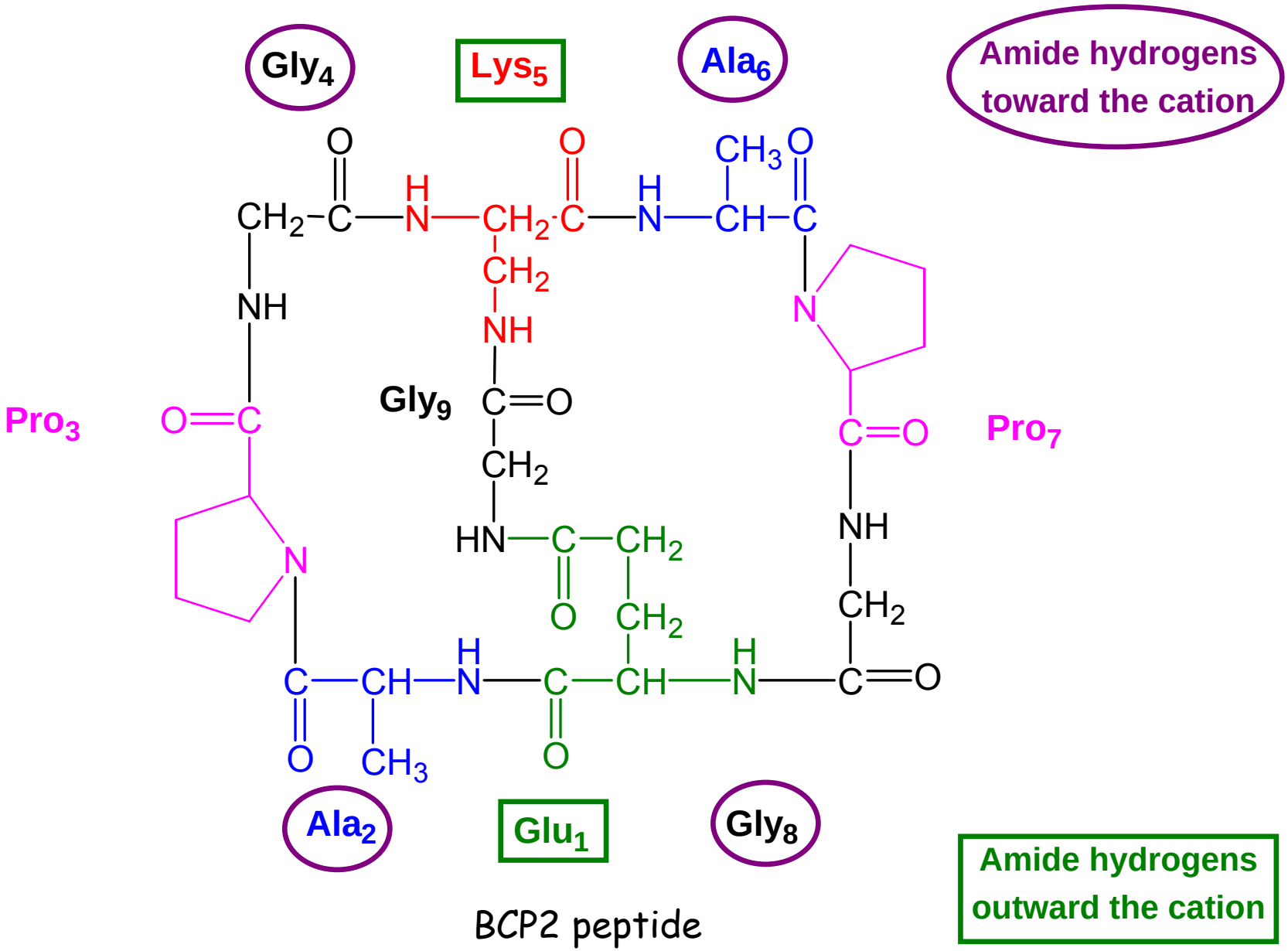
BCP2

(bicyclic homodetic nanopeptide)

cyclo-(Glu^1-Ala^2-Pro^3-Gly^4-Lys^5-Ala^6-Pro^7-Gly^8)-cyclo-(1γ→5ε)Gly^9



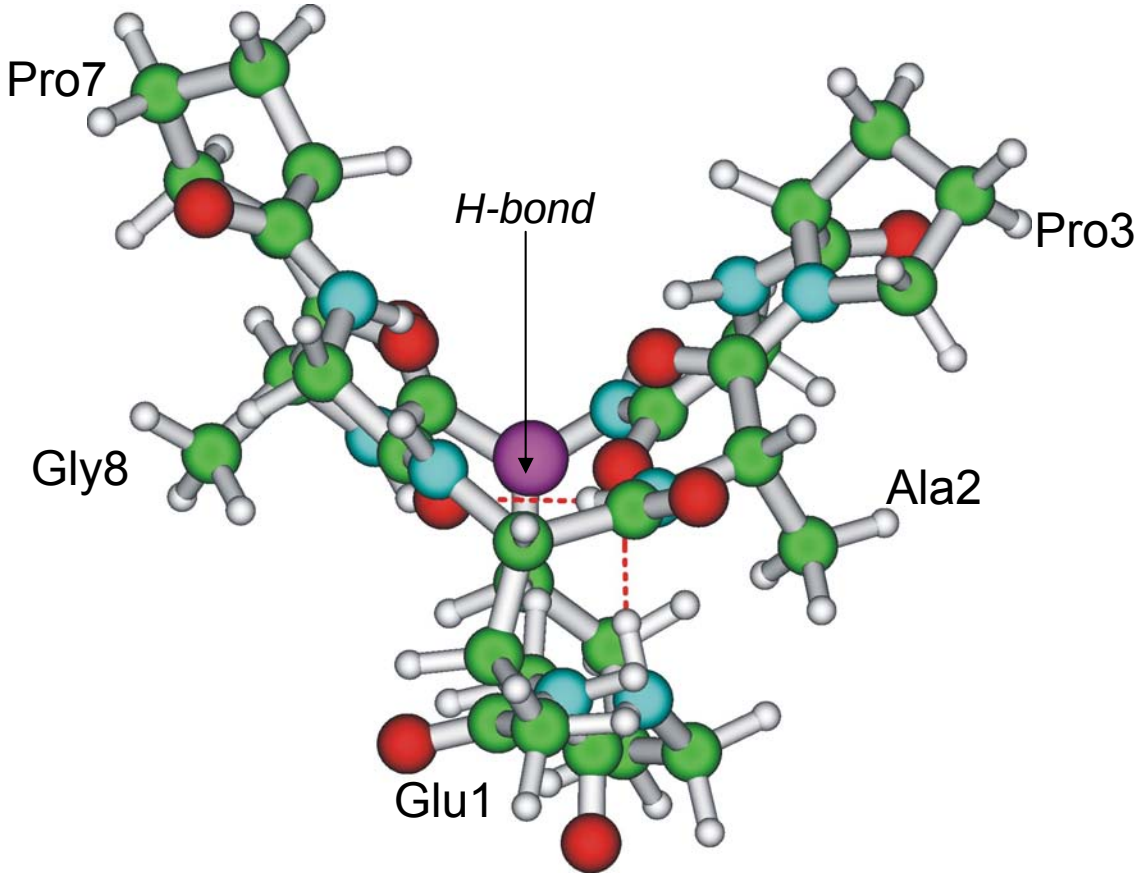
✓ A syntetic cyclic peptide: model for metal (calcium) binding polypeptides.



BCP2 peptide

…for the ab initio calculations

1. Optimization (DFT) of the threedimensional structure experimentally determined



Model of the 1:1 calcium-BCP2 complex

The geometrical structure of the the calcium-$BCP2^{+}$ complex has been determined from NMR data followed by restrained molecular dynamics (RMD) calculations.

2. Chemical shielding calculations for the free peptide and the calcium $BCP2^{+}$ complex

$$\sigma_{ij}^{N}=[\partial^2 E/\partial B_i \partial m N_i]_{B=0}$$

HF and DFT level
6-311+G(d,p) basis set

Chemical shift reference: acetamide

Chemical shift calculated + 6.40 ppm (i.e. the experimental proton shift of acetamide with respect TMS)

3. Calculations in solution (CH_3CN)

Test with PCM → negligible solvent effect ⟶ "isolated peptide"

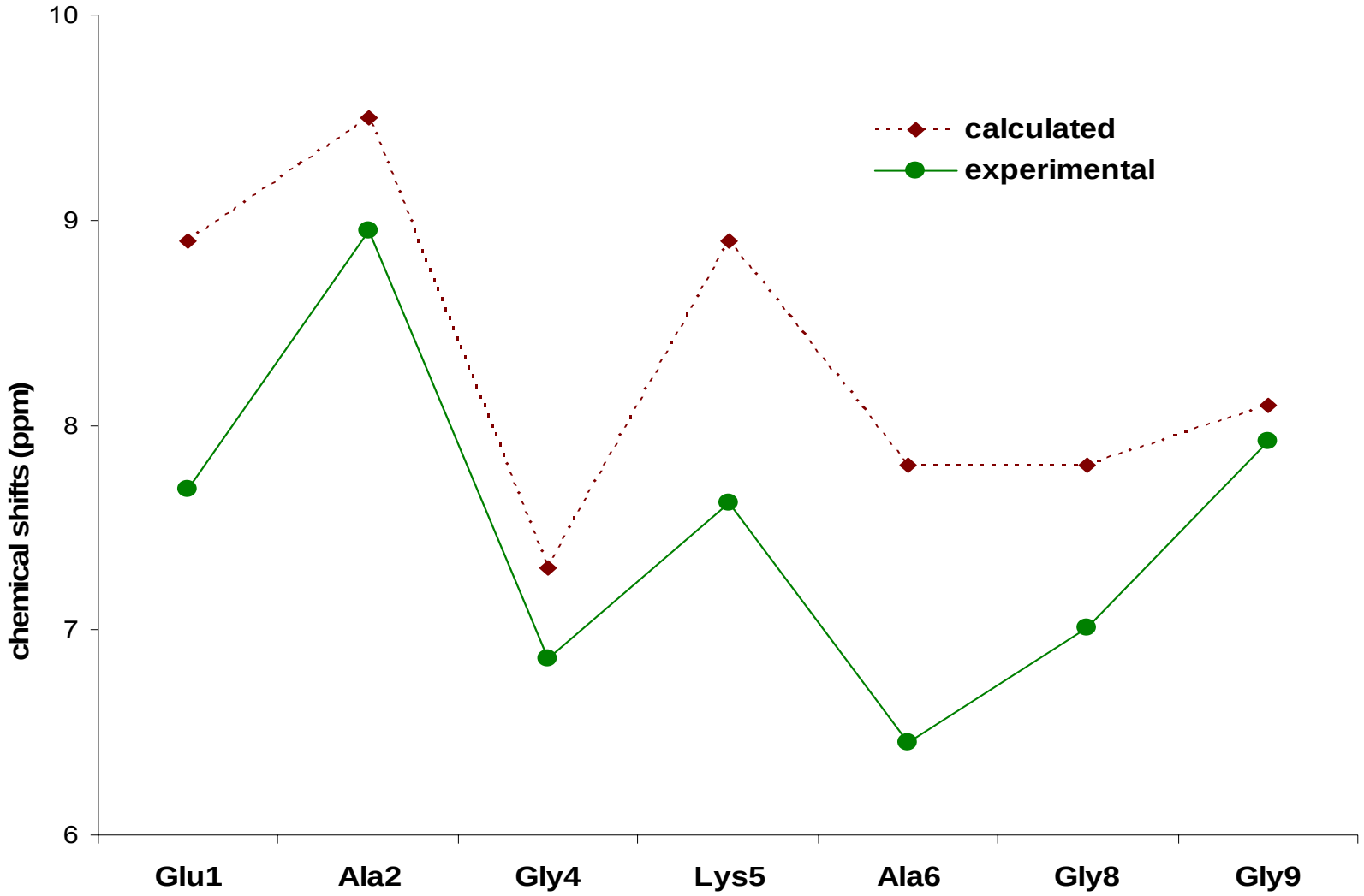
Ca^{2+}-BCP2 complex

	Glu1	Ala2	Gly4	Lys5	Ala6	Gly8	Gly9
DFT/6-311+G(2d,p)	8.9	9.5	7.3	8.9	7.8	7.8	8.1
HF/6-311+G(2d,p)	8.8	9.0	6.9	8.8	7.3	7.4	7.7
HF/6-31+G(d,p)	8.7	8.6	6.6	8.7	7.1	7.1	7.5
HF/6-31G(d)	8.8	8.5	6.6	8.9	7.1	7.0	7.7
HF/6-311+G(2d,p) for Ca2+ and 6-31G(d) for all atoms	8.8	8.6	6.6	8.9	7.1	7.0	7.7
HF/6-31+G(2d,p) for N, O and 6-31G(d) for all atoms	8.6	8.8	6.6	8.6	7.1	7.2	7.5
Experimental	***7.69***	***8.95***	***7.62***	***7.62***	***6.45***	***7.01***	***7.92***

Free BCP2

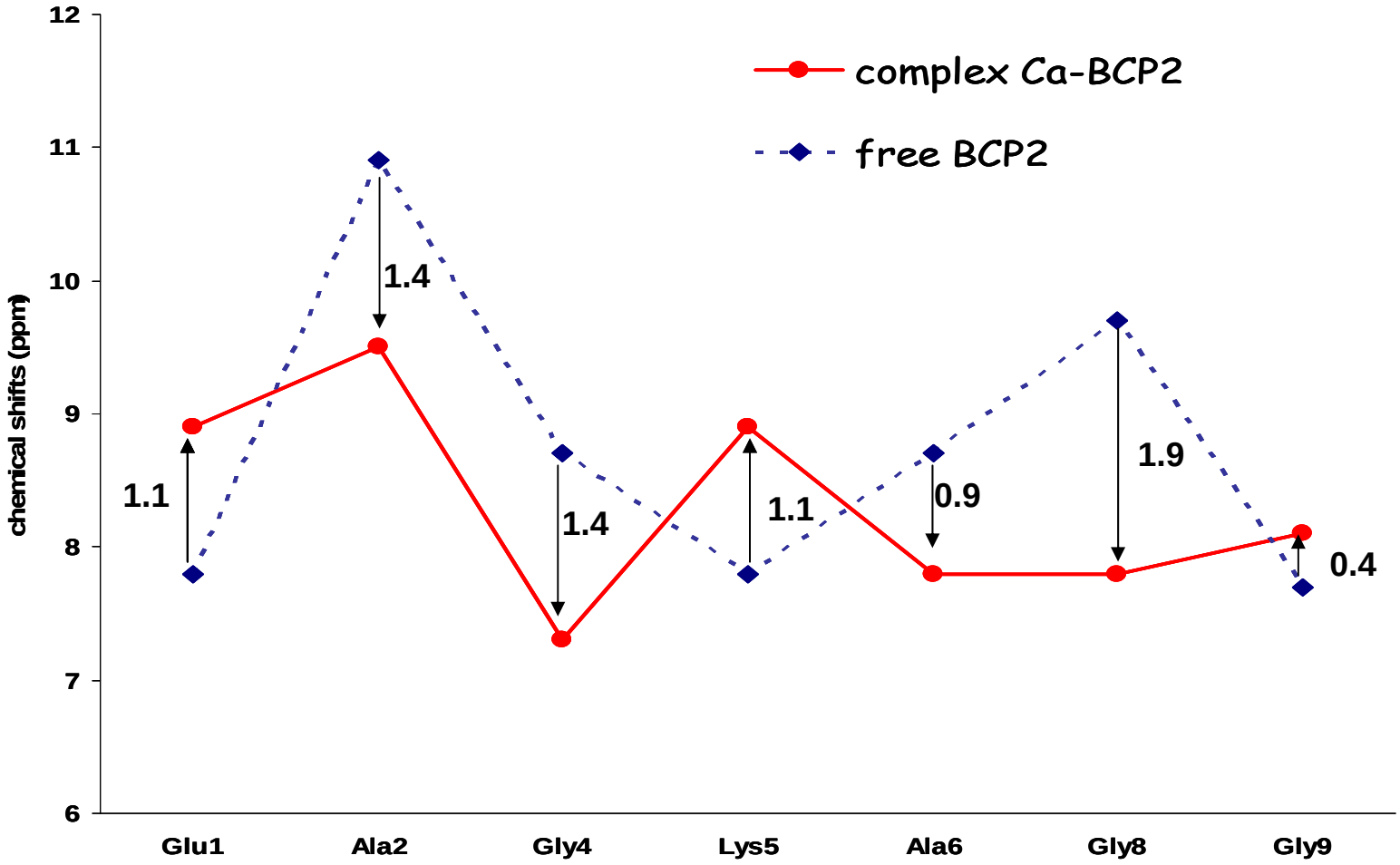
	Glu1	Ala2	Gly4	Lys5	Ala6	Gly8	Gly9
DFT/6-311+G(2d,p)	7.8	10.9	8.7	7.8	8.7	9.7	7.7
HF/6-311+G(2d,p)	7.4	10.7	8.5	7.4	8.3	9.6	7.3
HF/6-31G(d)	7.5	10.6	8.5	7.6	8.4	9.4	7.6

Comparison of calculated [PBE0/6-311+G(2d,2p)] and experimental data for the Ca-BCP2 complex



Comparison of calculated for the Ca-BCP2 complex and for the free peptide BCP2

[PBE0/6-311+G(2d,p)]



…focus on electronic effects due to the presence of the metal!!!!

First conclutions:

♦Trends with different basis sets are in excellent agreement

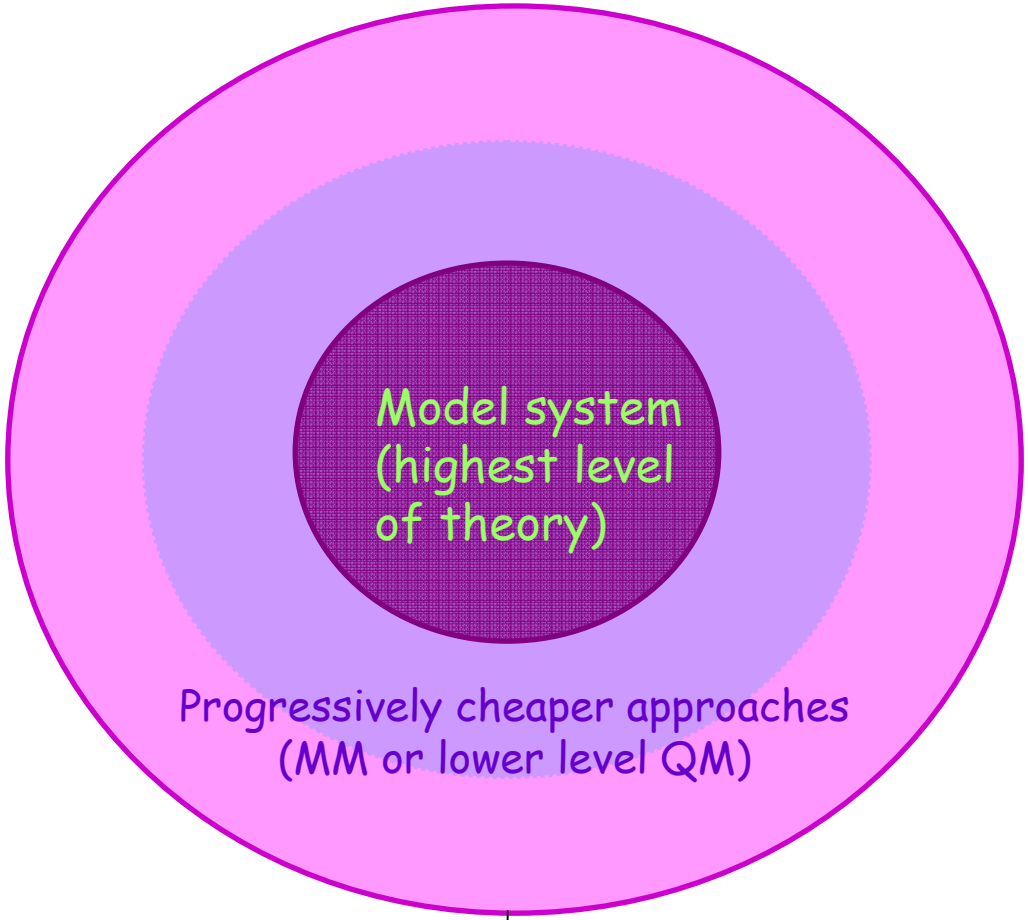
♦The use of an extended basis set limited to the metal cation is not sufficient for a good representation of metal-peptide interaction

♦Relative shieldings values for amide protons (involved in the metal-peptide interaction) are almost equal in all calculations.

“The effect of the cation is well reproducted”.

Hybrid Methods:

mixing QM and MM descrptions of different parts of the same molecule



MM region is described by atomic point charges.

1. Difficult parts of the molecule (those containing non parametrized atoms, uncommon interactions, or atoms directly partecipating in the transition state of reaction)

2. Parts of the molecule containing well parametrized atoms and simple interactions

3. Long range contributions on amide proton shieldings are mainly electrostatic

…hybrid model

What level (QM and MM) the ion and its ligands must be described?

✓ Test of different partition schemes ⇒ division of the system into QM and MM region trying to reproduce the full QM results

1. Interaction between the cation and the peptidic chain

♦ The metal has to be treated at the quantum level

♦ Extended basis set reproduce better the charge transfer →

Test: Calcium cation represented through different point charges, with charge +2.0 and +1.6 (chemical shifts calculated at HF/6-31G* level)

↓

+2.0 in accordance with QM HF/6-31G*
+1.6 in accordance with QM/6-311+G(2d,p)

2. Long-range interactions

♦ Electrostatic

3. Special attention to hydrogen bonds