Solvation of conformationally flexible molecules. Experiment and computer simulations.

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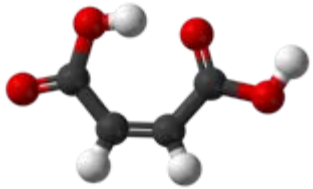
Motivations:

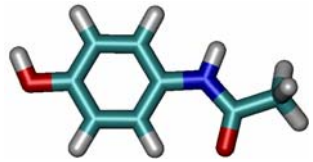
- Development and approve a new screening methodology of the drug-like compound polymorphs at ambient and supercritical conditions

"…every compound has different polymorphic forms and…the number of forms known for a given compound is proportional to the time and energy spent in research on that compound."

W. C. McCrone, in *Physics and Chemistry of the Organic Solid State*, Vol. 2, Wiley Interscience (1965).

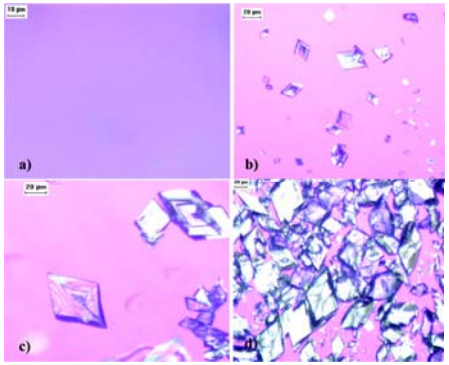


William Jones and colleagues obtained a second polymorph, of maleic acid in their Cambridge laboratory 124 years after the first crystal structure of this substance was reported.

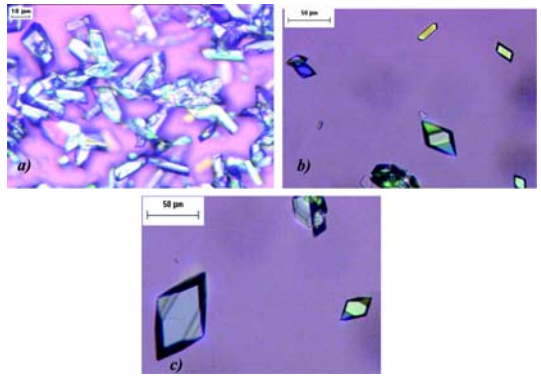


Paracetamol as an example

Form II



Form I



Published in: Stephanie C. Barthe; Martha A. Grover; Ronald W. Rousseau; *Crystal Growth & Design* **2008,** 8, 3316-3322.

DOI: 10.1021/cg800232x Copyright © 2008 American Chemical Society

Screening of the polymorphism

What kind of parameters should we observe in order to control of polymorph crystallization ?

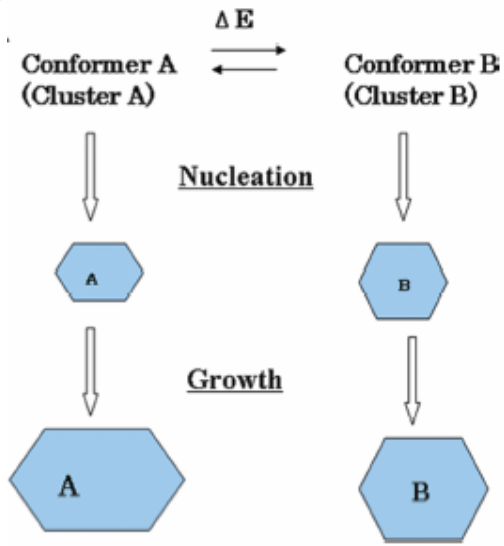
solvation → **nucleation of the polymorphs** → **growth of the crystal**

Nature of solvent, intermolecular interactions (solute – solvent, solute-solute), ***conformations***

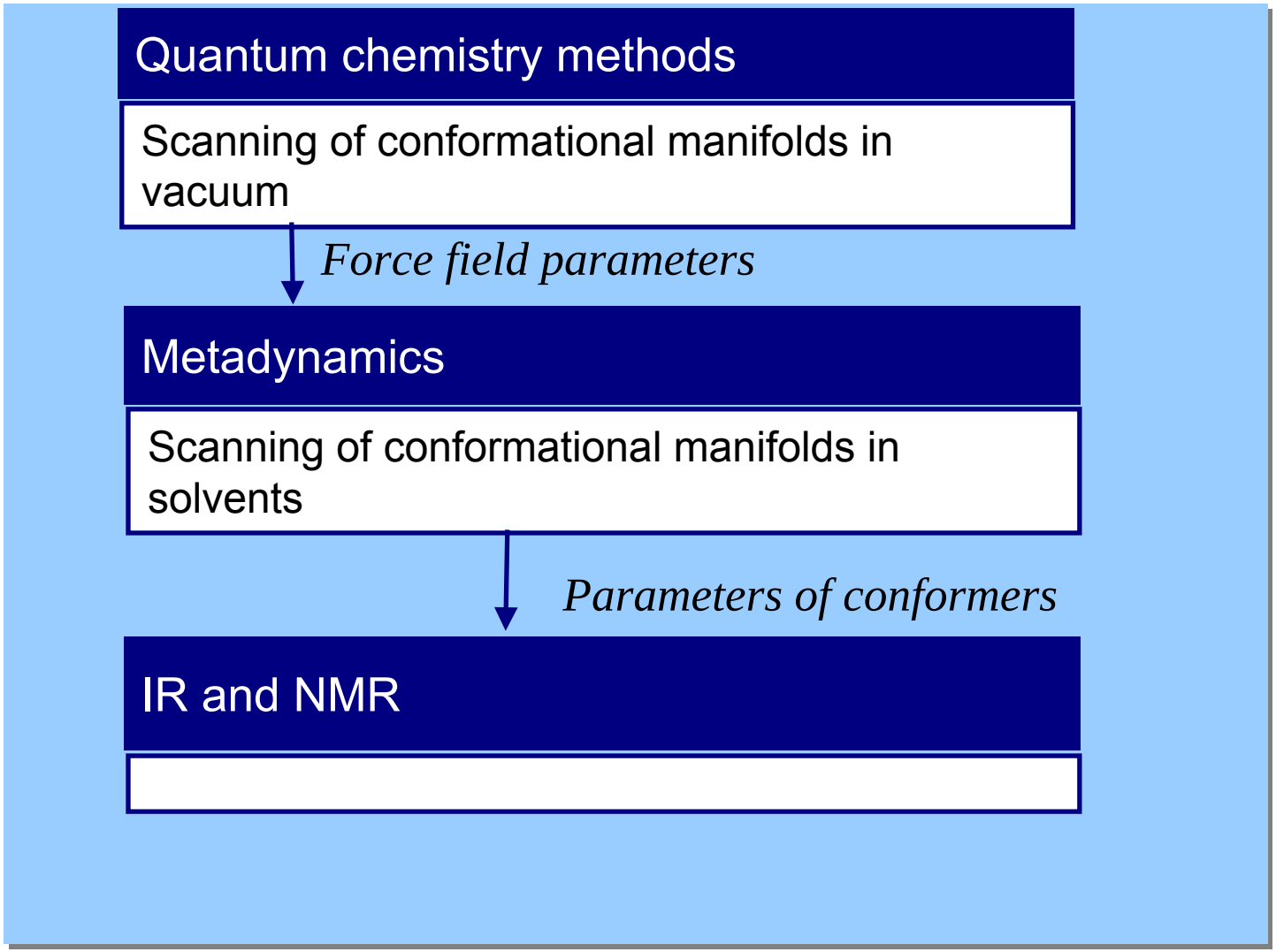
Solute-solute interactions, association parameters, thermodynamics parameters, ***conformations***

Lattice energy, entropy of the crystal, symmetry of the lattice, ***conformations***

The conformational manifolds is the candidate to be a coordinate of "reaction"

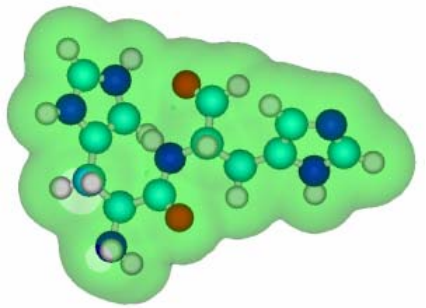


Methods of investigations



Description of conformational manifold by computer simulation methods

Quantum chemistry



$$H\Psi = E\Psi$$

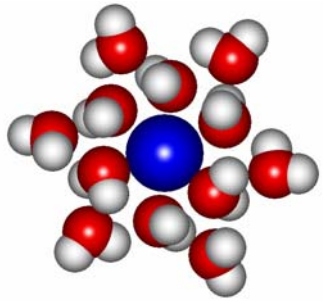
Advantages:

-Accurate description of the potential energy surface of the molecule "from the first principles" (no empirical parameters)

Disadvantages:

- As usual this approach doesn't take solvent into account
- It doesn't count the influence of the temperature

Classical mechanics



$$m_i \mathbf{r}_i = -\frac{\partial U(\mathbf{r})}{\partial \mathbf{r}_i}$$

Advantages:

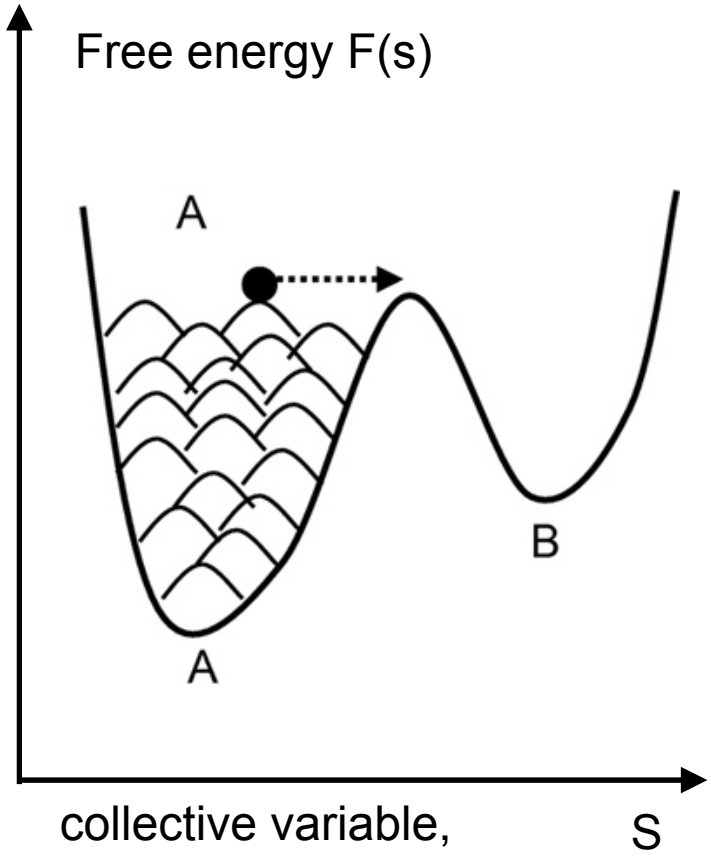
- Implicit model of the solvent

Disadvantages:

-Potential energy surface of the molecule is described by the effective potentials

=>We have to choose parameters that best reproduce the potential energy surface obtained from the quantum-mechanical calculations

Metadynamics

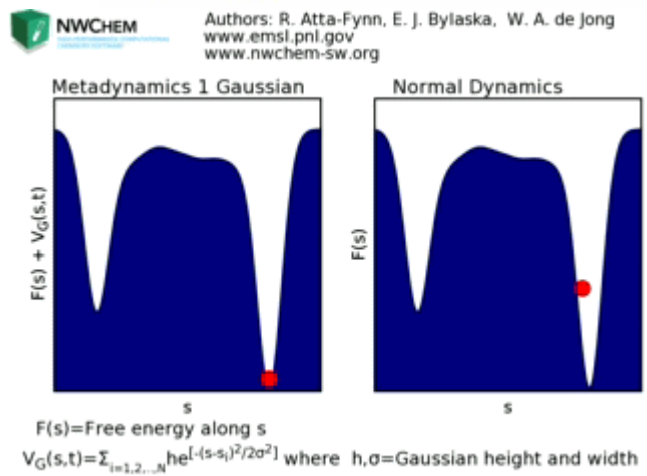


Additional potential:

$$V_{\mathrm{G}}(S(x),t) = w \sum_{\substack{t' = \tau_{\mathrm{G}}, 2\tau_{\mathrm{G}}, \ldots \\ t' < t}} \exp\left(-\frac{(S(x) - s(t'))^2}{2\delta s^2}\right)$$

Free energy:

$$\lim_{t\to\infty} V_{\mathrm{G}}(s,t) \sim -F(s).$$

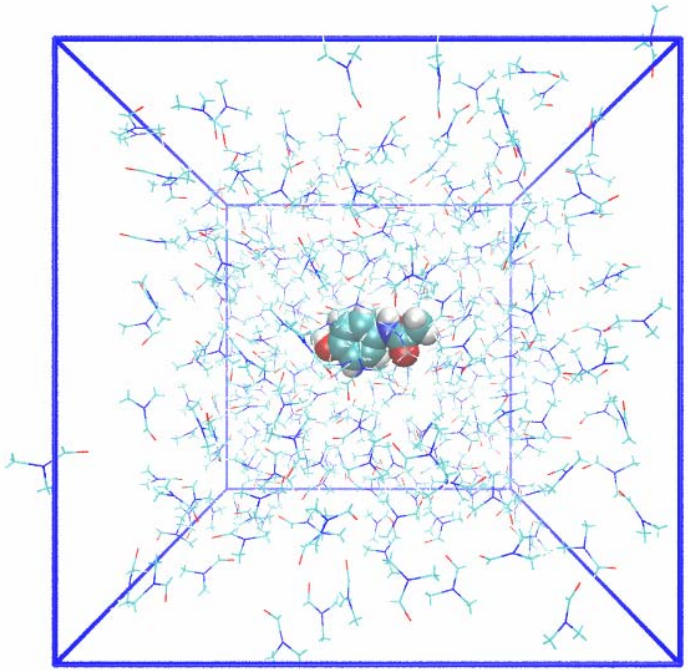


Alessandro Lai and Francesco L Gervasio, Rep. Prog. Phys. **71** (2008) 126601

Details of simulations

Time of simulation 35 нс
- Gromacs 4.5

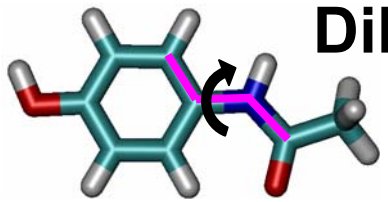
Simulation box



Collective coordinates

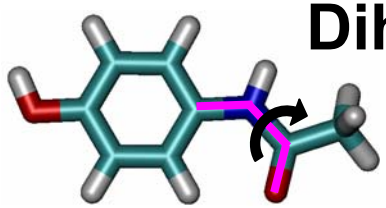
φ

Dih II



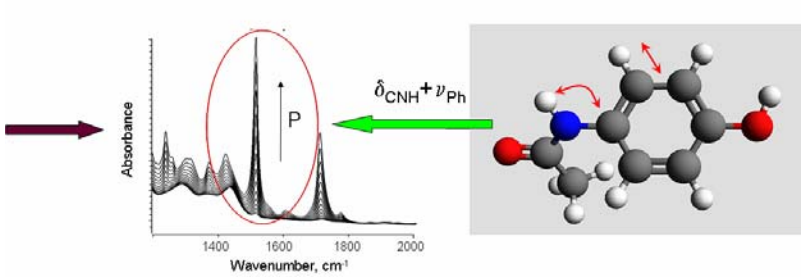
θ

Dih III

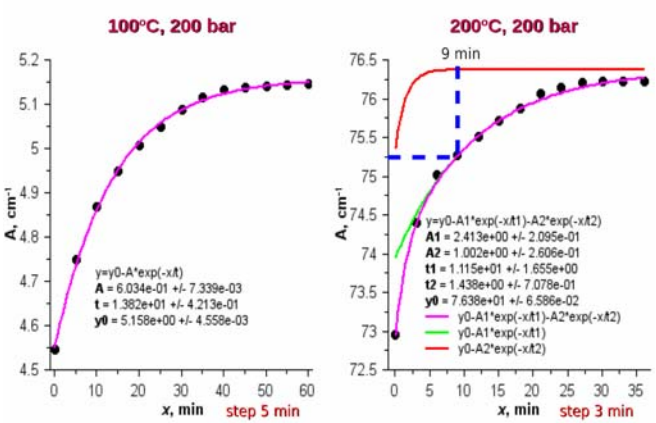


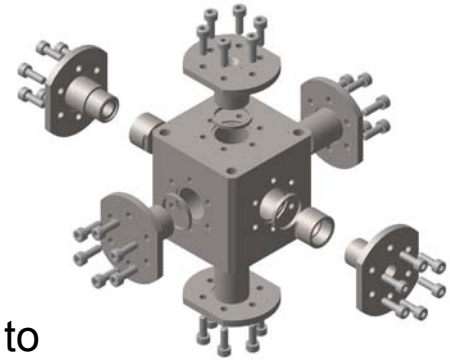
Vibrational spectroscopy

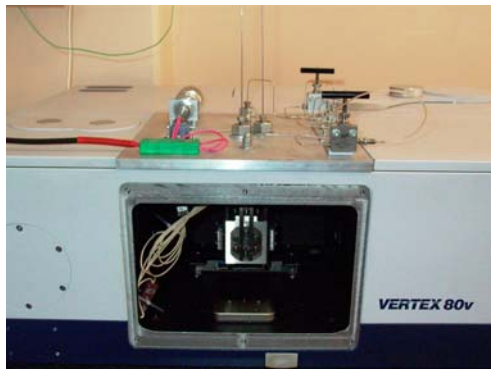
Highly accurate and rapid method for measuring the solubility based on the analysis of the characteristic bands of the IR spectra of these substances dissolved in the solvent.



The design of the installation, which includes a cell, may be used in combination with a variety of IR and Raman spectrometers.







The use of infrared spectroscopy allows to determine the extremely low solubility values (10^{-6} m.f.) and free from the use of photo-active markers used in spectroscopy of the visible spectrum.

For measurements of populations of the conformations and solubility we use high temperature and high pressure cell, which allow to perform study up to 500° C and 1000 bar.

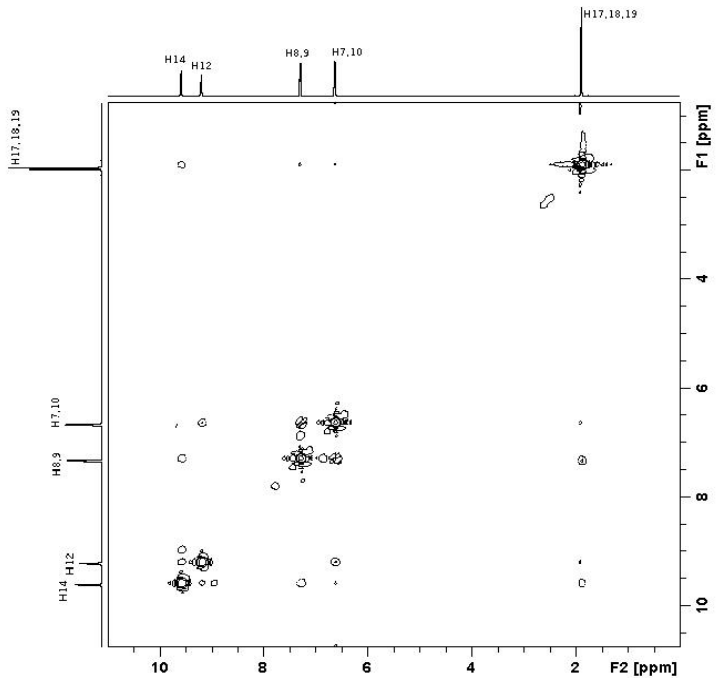
2D NMR spectroscopy for the populations of conformations

Determination of intramolecular distance based on NOESY and ROESY methods, which in combination with quantum chemical calculations provides an estimation of probability of conformations.

$$\sigma_{ij}^{noe} \sim \left(6J_{ij}^{2}(\omega) - J_{ij}^{0}(\omega)\right)$$

$$\sigma_{ij}^{roe} \sim \left(3J_{ij}^{1}(\omega) + 2J_{ij}^{0}(\omega)\right)$$

$$r_{ij} = r_0 \left(\frac{\sigma_0}{\sigma_{ij}}\right)^{\frac{1}{6}}$$



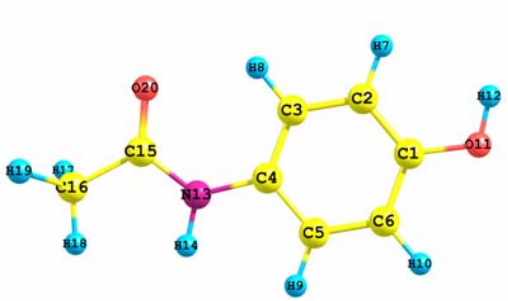
What is new?

We are taking into account the internal rotation of the CH3 groups and the time of the re-orientation obtained from MD simulations

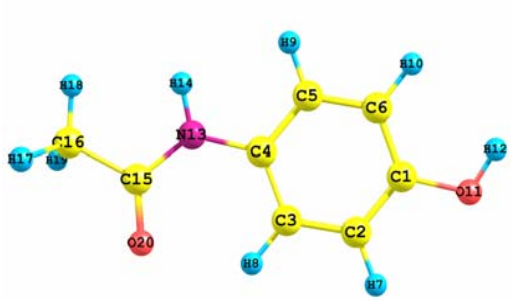
$$J_{ij}^{n}(\omega) = \frac{1}{4\pi r_{ij}^{6}}\left[\frac{\tau_c}{1 + n^2\omega^2{\tau_c}^2}\right]$$

$$J_{ij}^{n}(\omega) = \frac{1}{5}\frac{\tau_c}{\left(1 + n^2\omega^2{\tau_c}^2\right)}\sum_{m=-2}^{2}\left|\frac{1}{3}\sum_{n=1}^{3}\frac{Y_{2m}\left(\theta_{mol}^{i}\ \phi_{mol}^{i}\right)}{r_{ij}^{3}}\right|^2$$

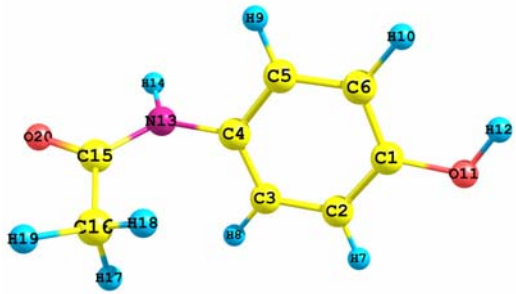
Quantum chemical calculations



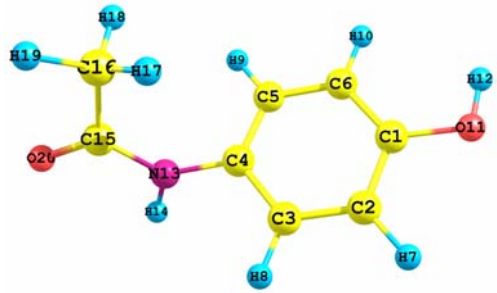
I



II



III

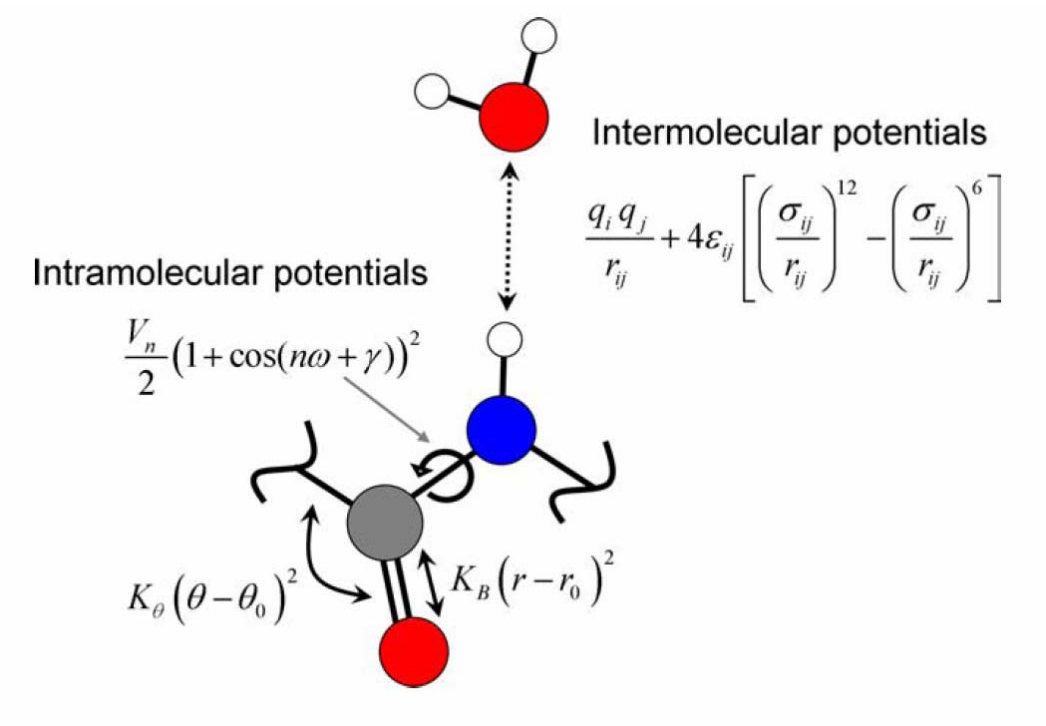


IV

	ΔE, kJ/mol	D, debye
I	0	2.31
II	1.724954	5.003
III	10.82985	4.5464
IV	10.72514	4.4575

Choice of the force field

Force Fields



Most famous force fields:

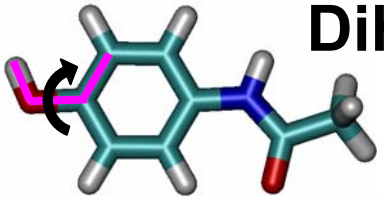
OPLS-AA (optimized molecular potential for liquid simulations – all atom) [1]
CGenFF (charmm general force field) [2]
GAFF (general amber force field) [3]

1. Jorgensen W.L., Maxwell D.S., Tirado-Rives J., *J. Am. Chem. Soc.* **1996**, 118 (45), 11225–11236
2. Vanommeslaeghe K. Et al., *J. Comput. Chem.* **2010**, 31(4), 671-90.
3. Wang J., Wolf R.M., Caldwell J.W., Kollman P.A., Case D.A. *J. Comput. Chem.* **2004**, 25(9), 1157-74.

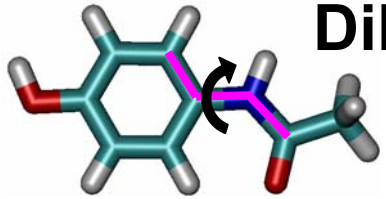
Comparison of energy profiles for QM, GAFF, OPLSAA, CGenFF

Single molecule of paracetamol in vacuum

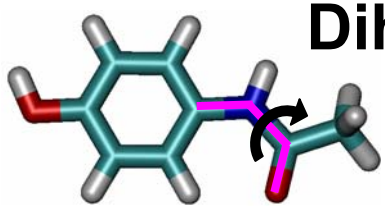
Energy minimization with fixed value of specified dihedral angle



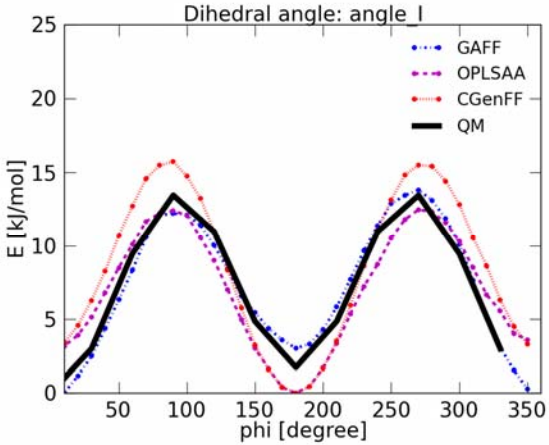
Dih I

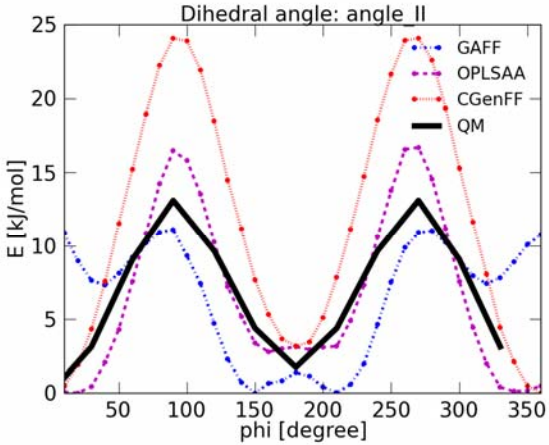


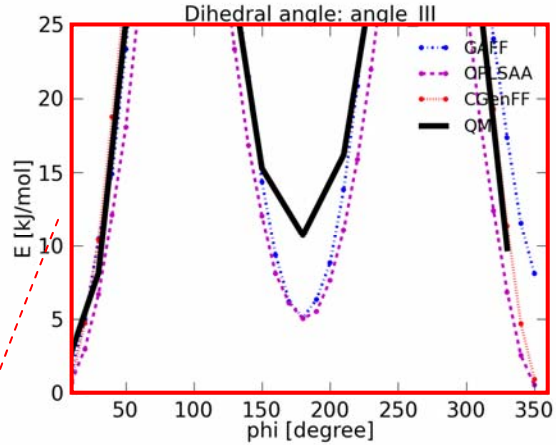
Dih II

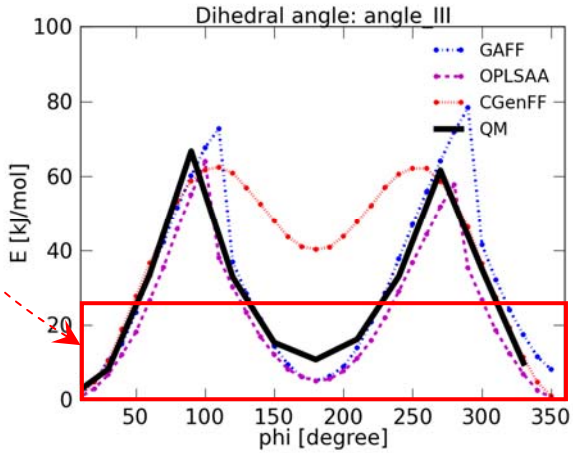


Dih III









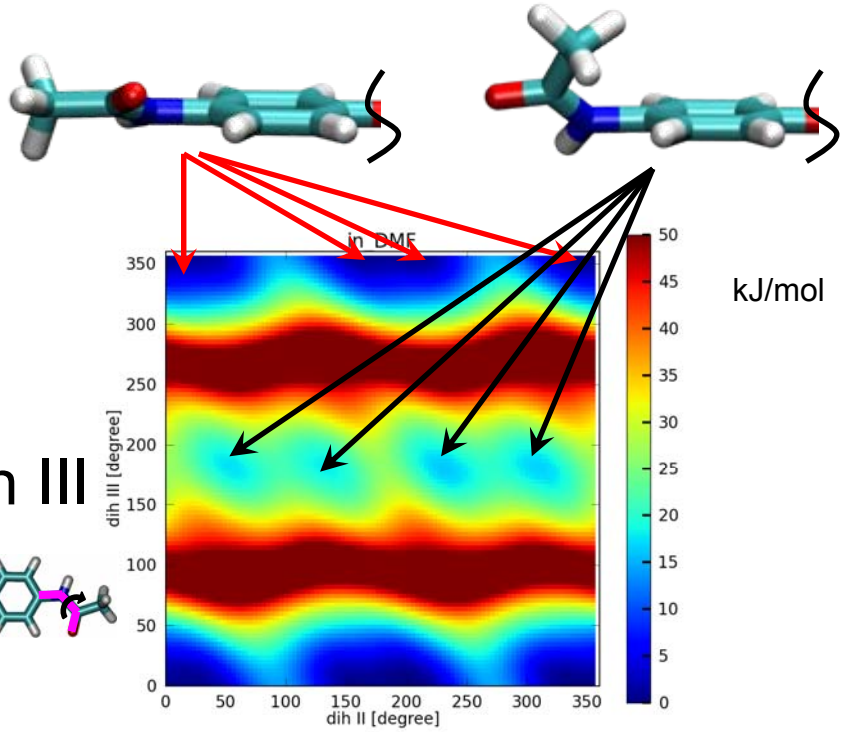
Conclusion:
OPLSAA force field gives qualitatively correct energy profiles for all three dihedral angles => we stick to OPLSAA

Metadynamics with OPLS-AA force field at 300K

In DMF

conf I + II

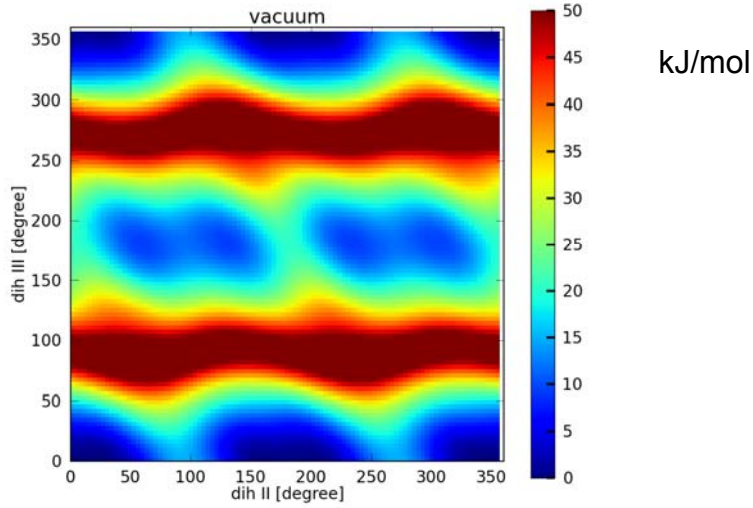
conf III + IV



Dih III

Dih II

In vacuum



2D free energy map shows that in fact there are two different conformations of paracetamol molecule (if we neglect position of OH group). The conformations are determined by rotation around dihedral III.

Conformer populations

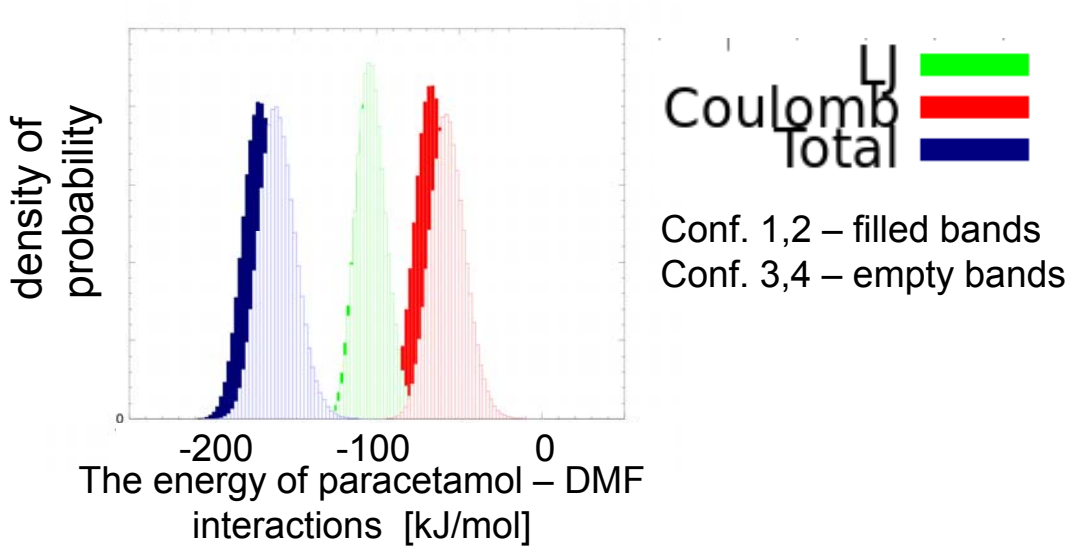
$$\mathrm{p}(\varphi,\theta) = \frac{\exp(-F(\varphi,\theta)/RT)}{\int d\varphi d\theta \exp(-F(\varphi,\theta)/RT)}$$ - Probability

$$P(conf\,3,4) = \int_{90}^{270} d\varphi \int_{0}^{360} d\theta \cdot \mathrm{p}(\varphi,\theta)$$ - Conformer population 1,2

	In DMF	In vacuum
Conf I+II	99.8%	95.6%
Conf III+IV	0.2%	4.4%

DMF stabilizes of conformers 1 and 2

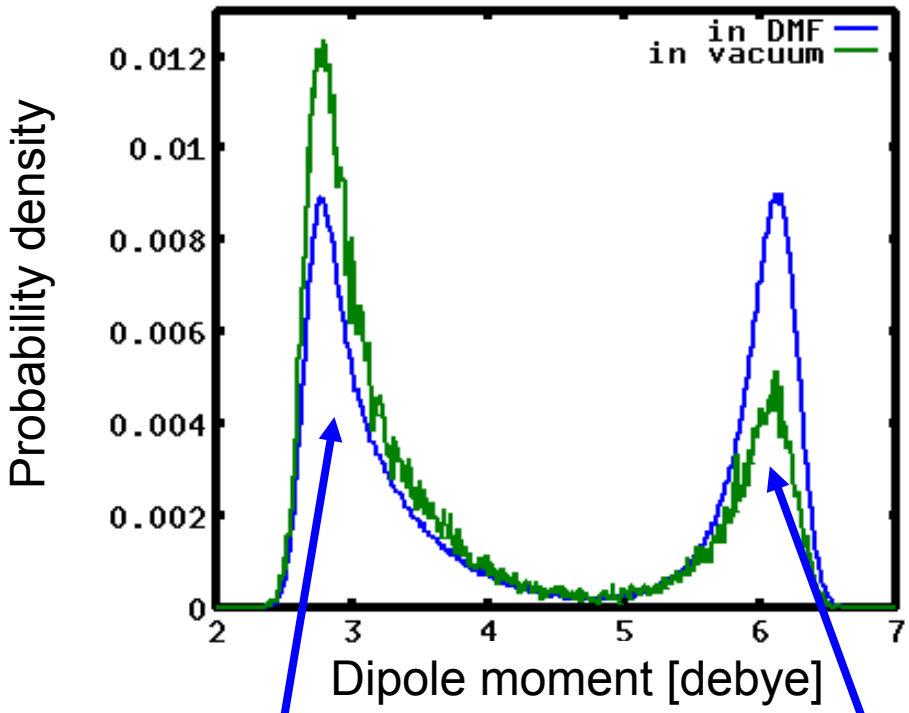
Differences between of conformers

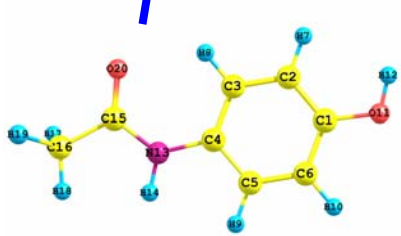


Dipole moments of conformers in vacuum:

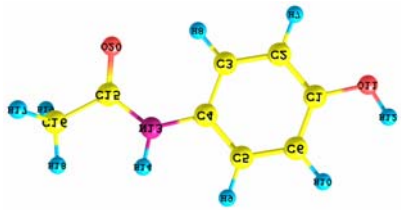
	D [debye]	
I	2.8	
II	6.1	
III	3.2	
IV	3.6	

Differences between of conformers 1 и 2





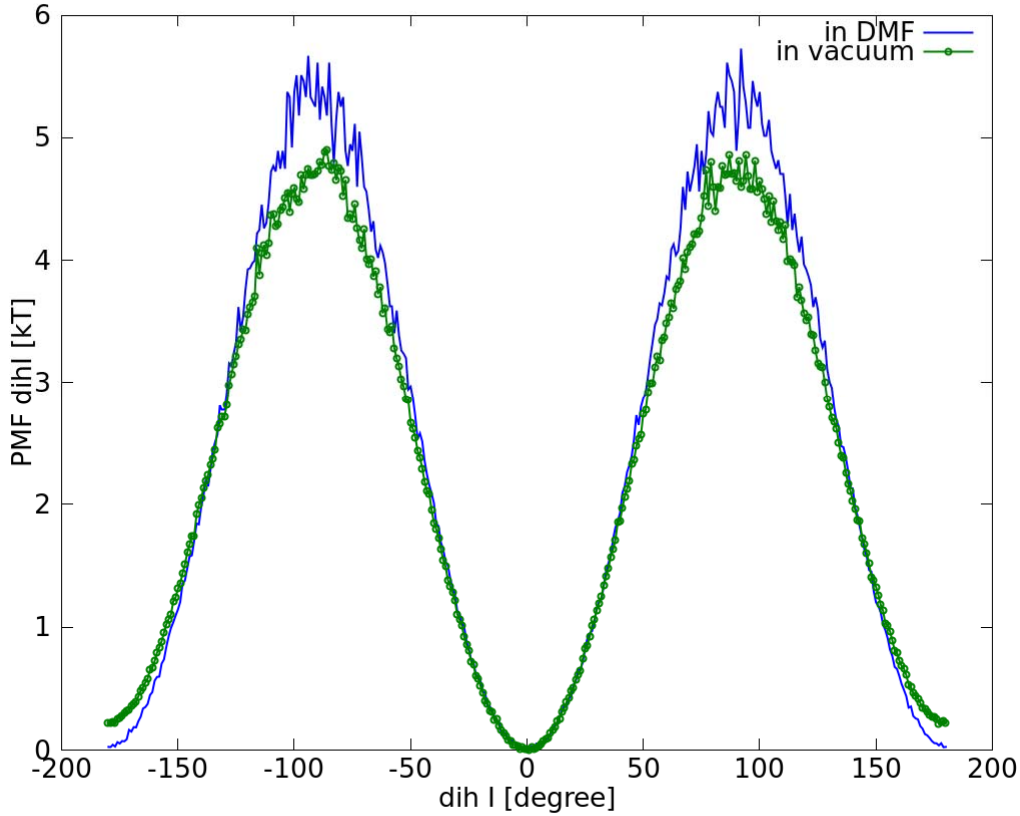
Conformer 1

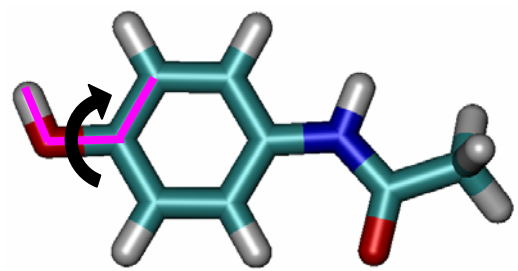


Conformer 2

Flip of the OH group

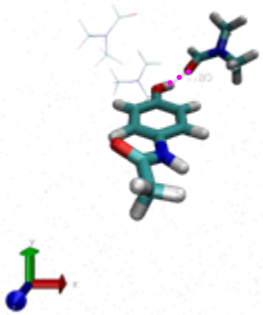
Potential of mean force as a function of dihedral I



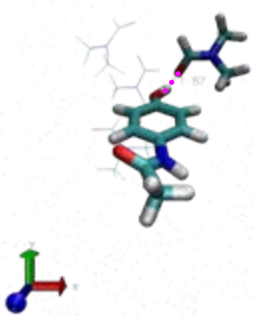


Example of the flip of OH group in paracetamol

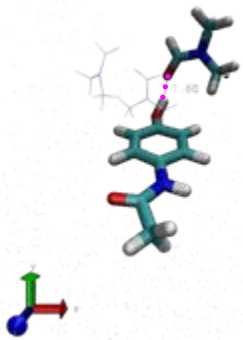
0.0 ps



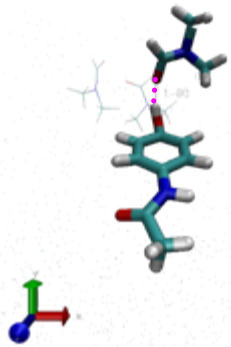
0.1 ps



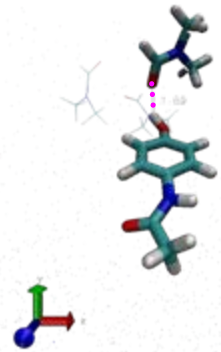
0.2 ps



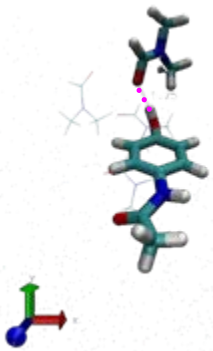
0.3 ps



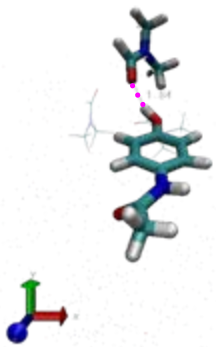
0.4 ps



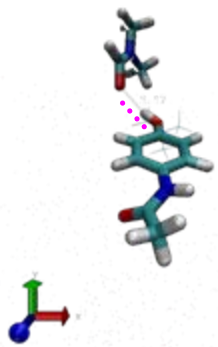
0.5 ps



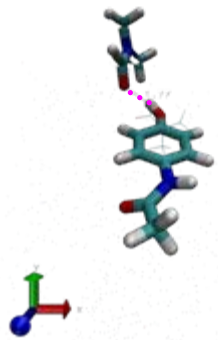
0.6 ps



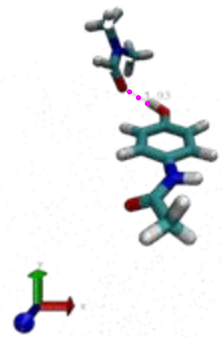
0.7 ps



0.8 ps

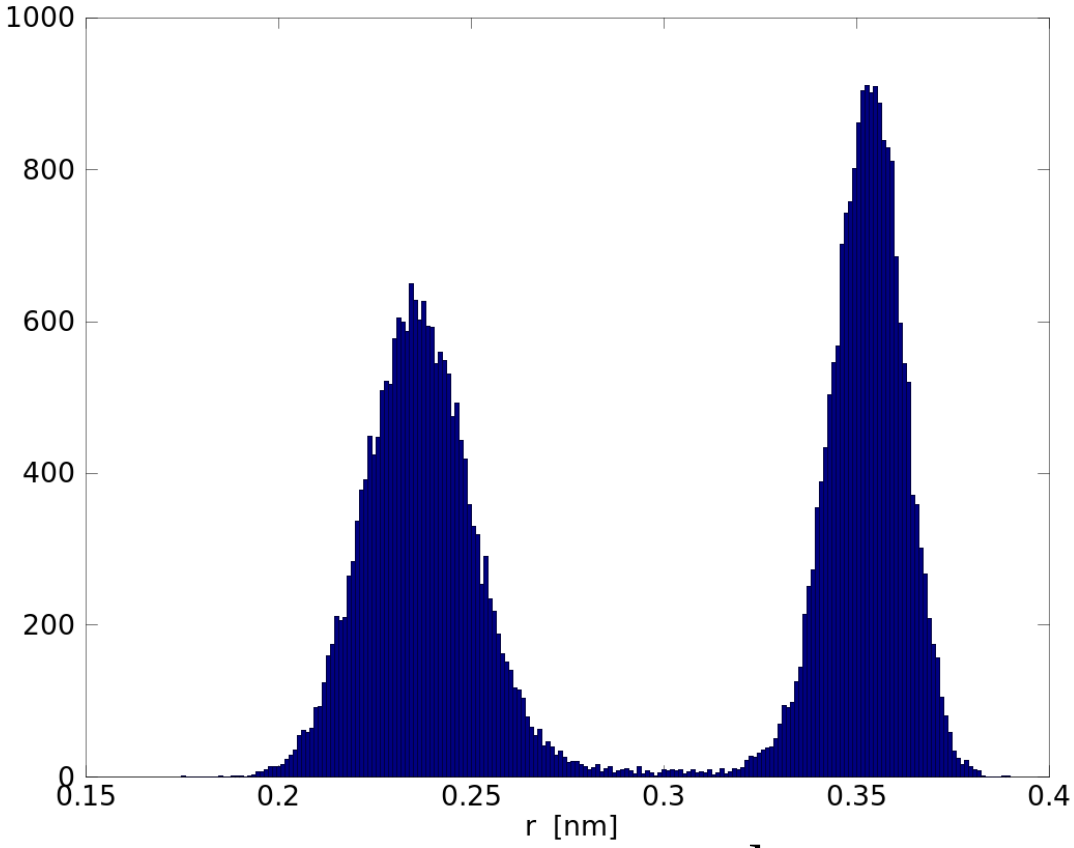


0.9 ps



Data for NMR

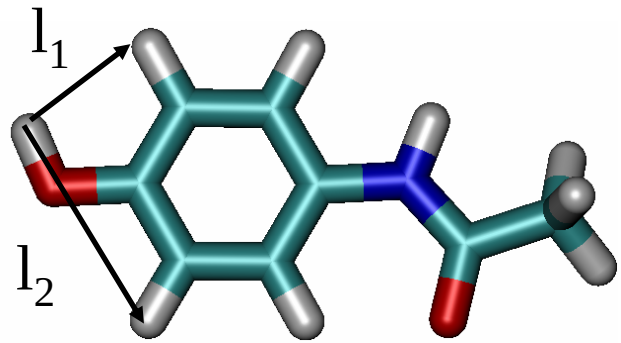
Distribution of the calibration length in simulation



Mean 0.295 nm

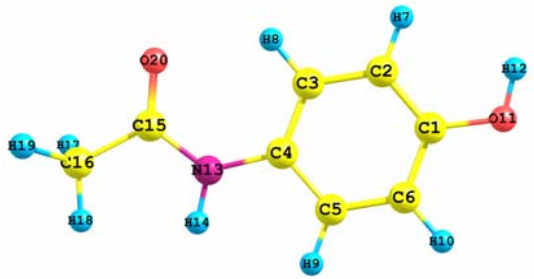
Max 0.390

Min 0.175

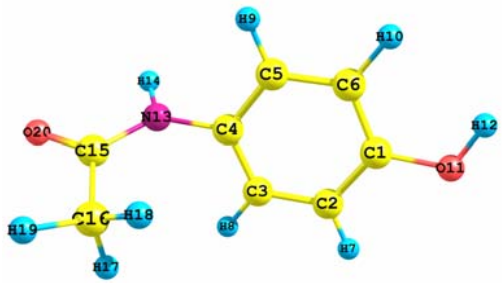


NMR results

Atomic groups	Experimental interproton distances	Calculated interproton distances											
		Conf 1			Conf 2			Conf 3			Conf 4		
OH-benB	calibration	2.58			2.58			2.58			2.58		
NH-BenA	2.62±0,05	2.53			2.53			2.97			2.97		
Nh-CH3	2.89±0,08	2.75	2.69	2.54	2.74	2.69	2.54	3.99	3.85	3.84	3.99	3.85	3.84
CH3-BenA	4.13±0,09	4.97	4.88	4.84	4.97	4.88	4.84	3.54	3.40	3.30	3.54	3.40	3.30



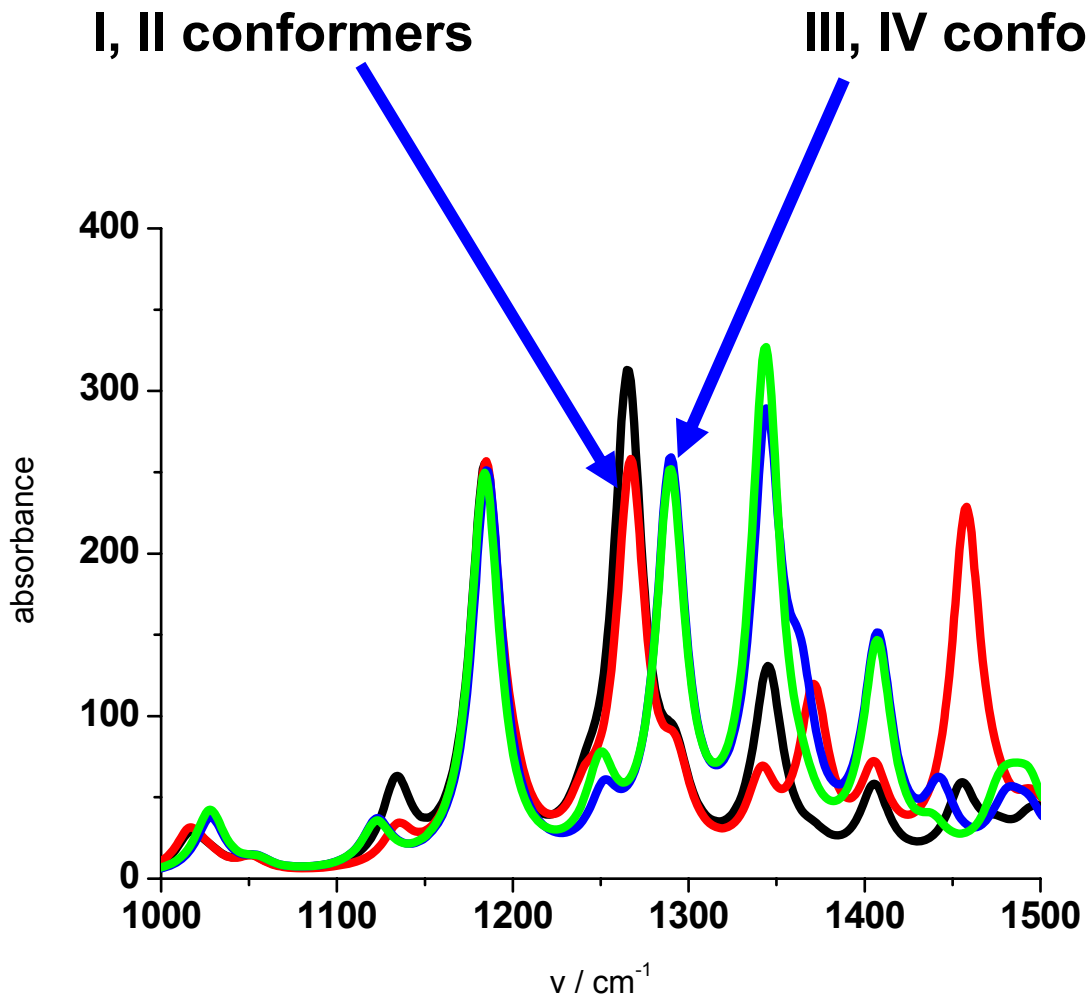
I-II conformations P1=0.7



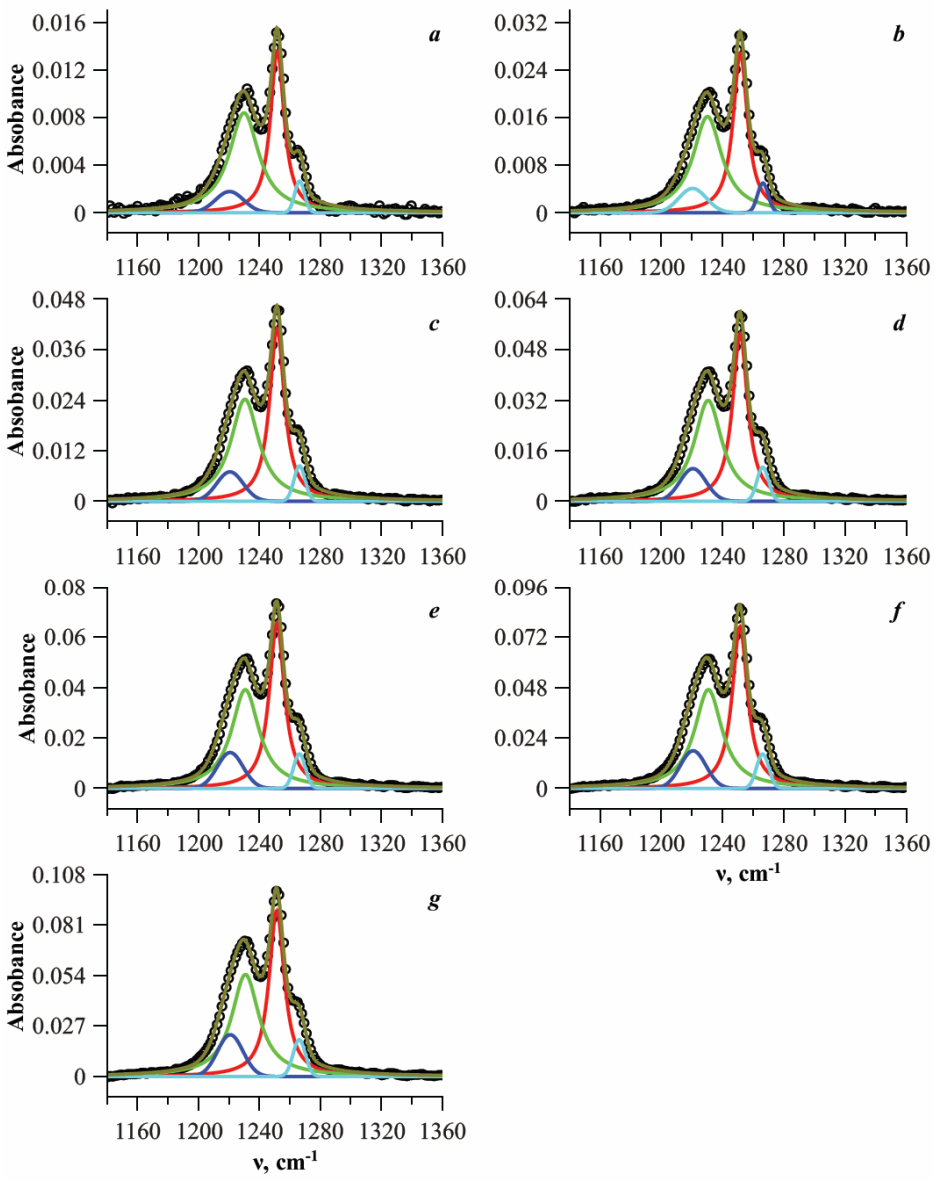
III-IV conformations P2=0.3

IR spectroscopy results

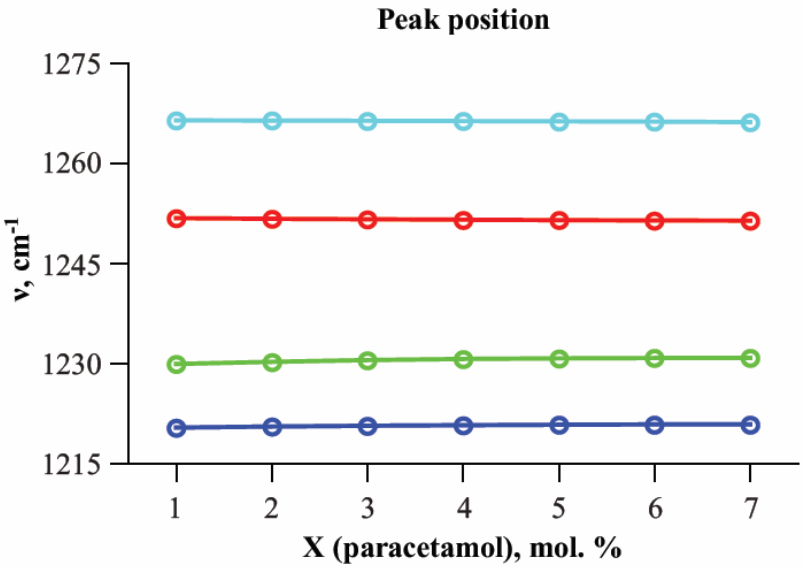
IR spectra from quantum chemistry calculations

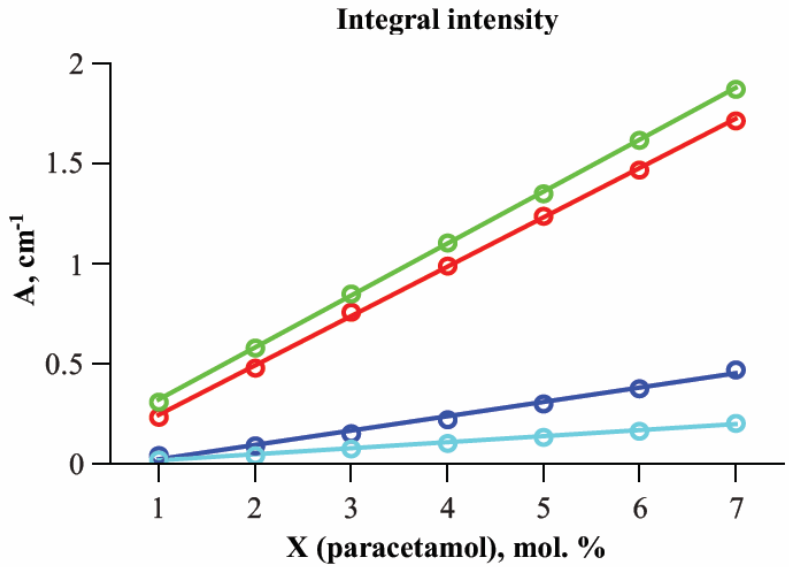


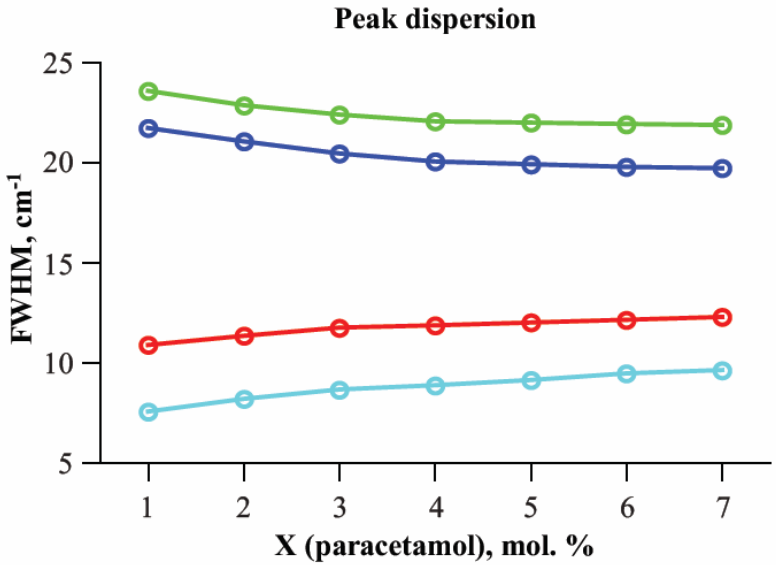
Deconvolution of the IR peaks on contributions from conformers

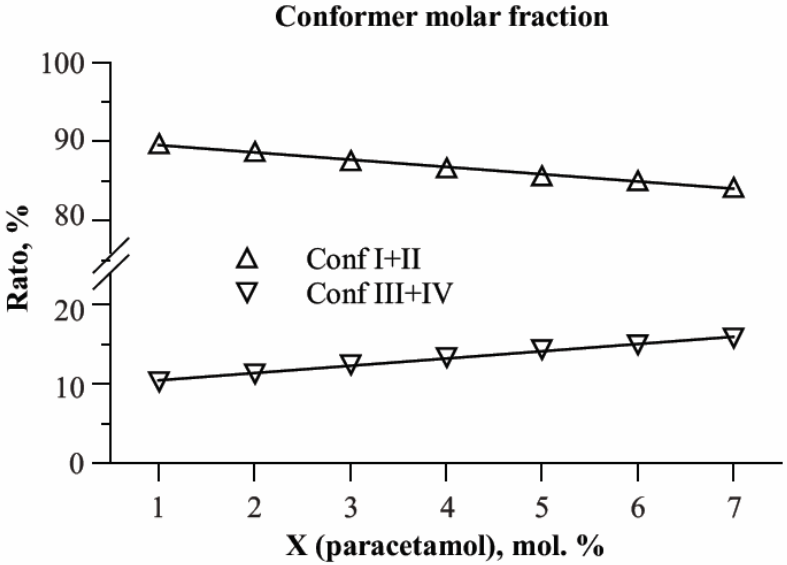


Conformers analysis from intensities of IR spectra





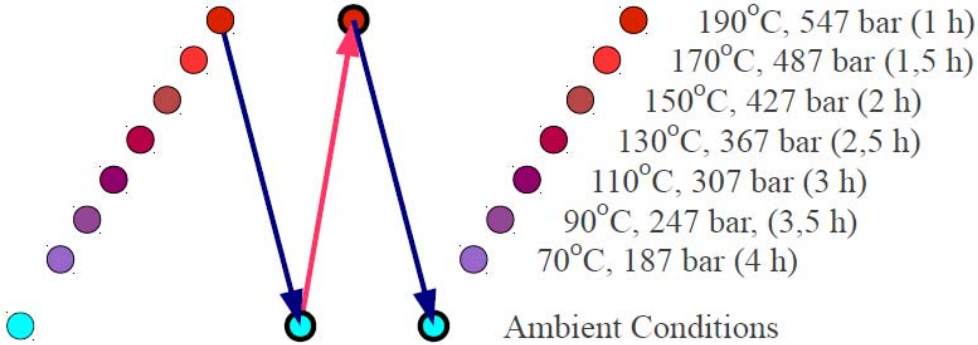




Polymorphism of Paracetamol in supercritical carbon dioxide

Recrystallization of Polymorph I to polymorph II as observed from IR spectra

Schematic presentation of experiment (isochoric conditions)

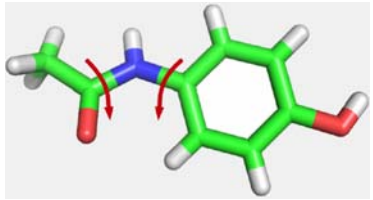


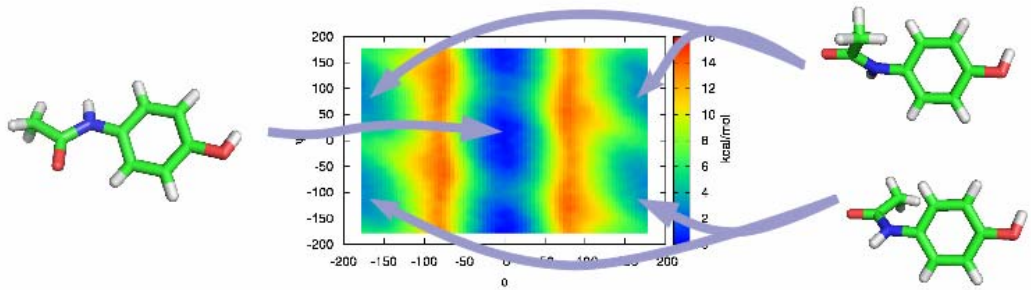
$\rho(CO_2) = const = 1{,}35\rho_C(CO_2) = 14.3436$ mol l^{-1}

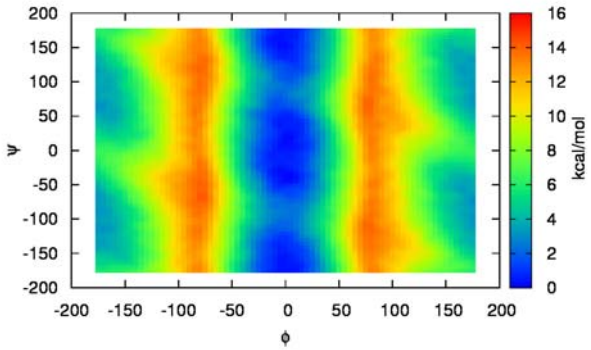
[$\rho_C(CO_2) = 10.6249$ mol l^{-1}]

- Spectra registration (the first)
- Cooling down during 4 hours
- Retention during 8 hours
- Heating up during 4 hours
- Retention during 2 hours
- Spectra registration (the second)
- Cooling down during 4 hours
- Retention during 8 hours
- Spectra registration (the third)

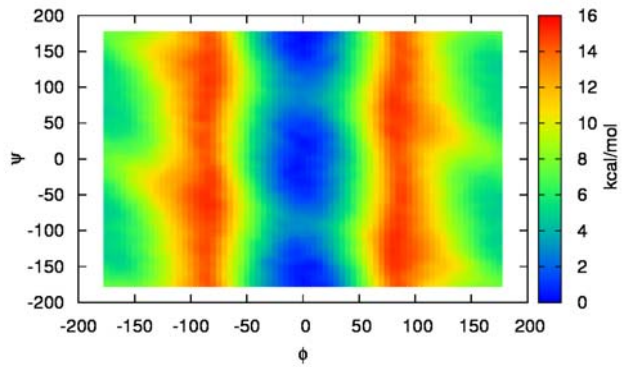
Results of the paracetamol polymorph screening as obtained from mixture with the supercritical carbon dioxide





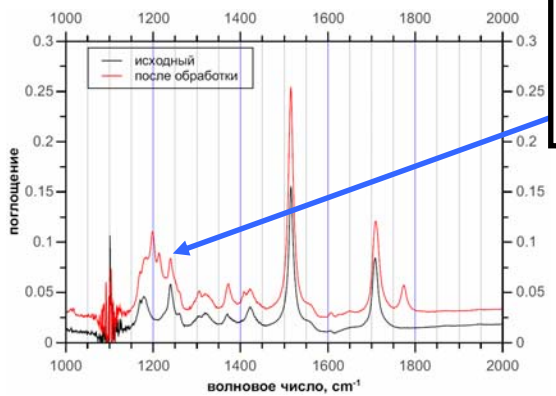


Metadynamics predicts an increase in the statistical weight of the III-IV conformations on the isobar 200 bar in the temperature range 110-150 C

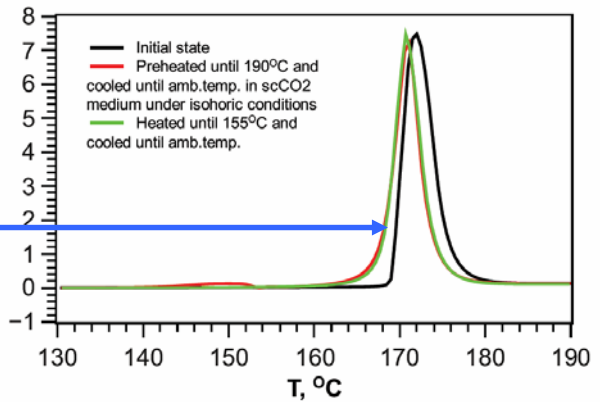


Results of IR in region of predicted P and T

III-IV conformation



DSC II polymorph



Conclusions

-A new approach to scan polymorphic bioactive compounds derived from supercritical fluids based on a combination of theoretical and experimental approaches.

-As a theoretical method developed by the authors used an effective sampling of conformational manifold, based on the calculation of the mean force potential.

-The main experimental methods are IR and NMR spectroscopy, allowing to study a population of conformations in the condensed phase

-The hysteresis of conformation of paracetamol in the supercritical carbon dioxide has been found on the basis of IR spectroscopic studies for the first time

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Dr. M. Nikiforov