

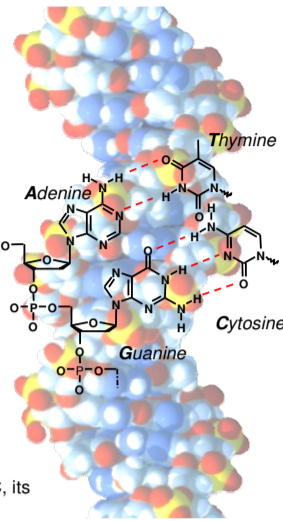
### Introduction

The DNA nucleobases exhibit an astonishingly high UV photostability, owing to an ultrafast deactivation of the photo-excited molecules on a femtosecond timescale. H-bonding is supposed to participate in the relaxation pathways [1].

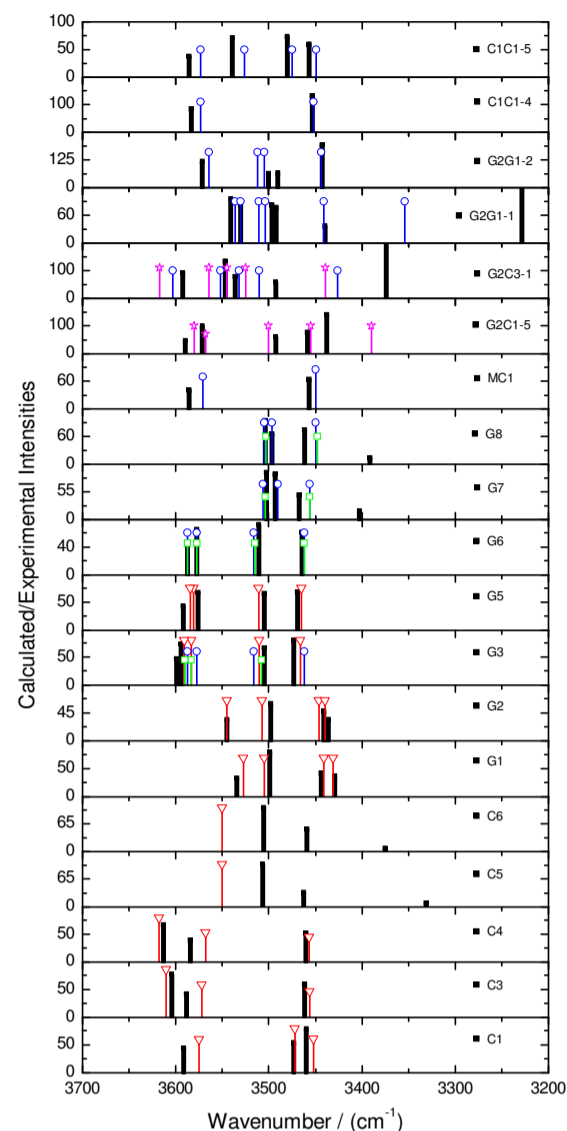
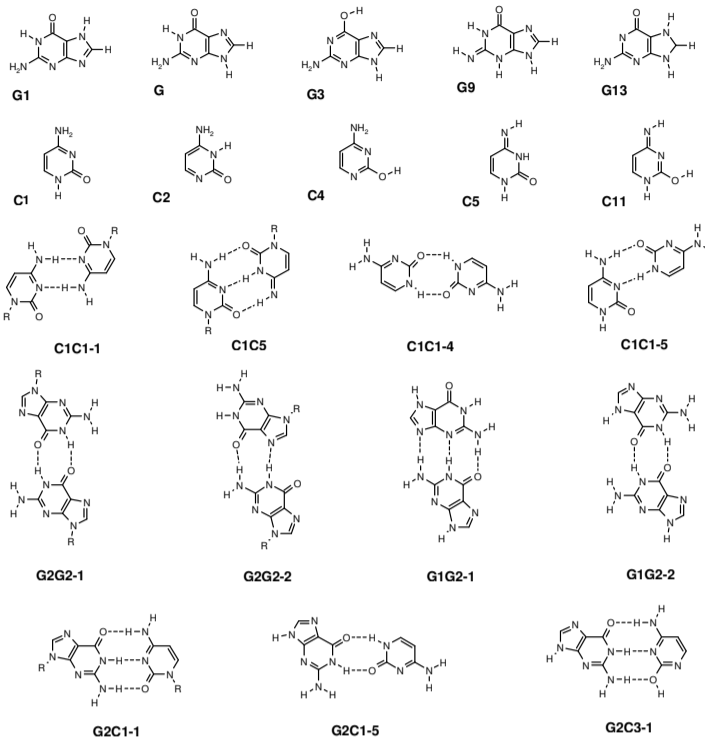
To explore this intriguing property, the tautomeric form of each single nucleobase, its homodimers and possible heterobase dimers, present in solution, have to be determined. Therefore, the following strategy was applied:

- quantum-mechanical calculations of energies & vibrational frequencies of relevant tautomers in solution.
- determination of association equilibria and structures via static FTIR measurements.

Here, the focus is set on the nucleobases G and C, its homodimers and G-C heterodimers.



### Nucleobase tautomerism



### Results in the gas phase

All monomers and dimers have been initially calculated in the gas phase and, if data were available, were compared to experimental values from the literature:

- ▽ IR in He nanodroplets from Choi [7,8]
- IR/UV hole burning in molecular beam from Nir [9,10,11]
- IR/UV hole burning in molecular beam from Mons [12]
- ★ IR/UV hole burning in molecular beam from Abo-Riziqs [13]
- PW91/6-311++G(d,p) calculations from this work
- TPSS/cc-pVTZ calculations from this work

The accordance between theory and experiment proved to be excellent. It encouraged us to perform similar comparisons for solution phase data.

### Experimental approach

#### FTIR spectroscopy:

- hydrogen bonded nucleobase complexes are formed in aprotic solvents.
- nonpolar TBDMSi protecting groups were used to achieve solubility in aprotic solvents and to suppress H-bonding through the sugar hydroxy groups.
- spectra have been recorded concentration and temperature dependent.

#### Quantum Chemical Calculations:

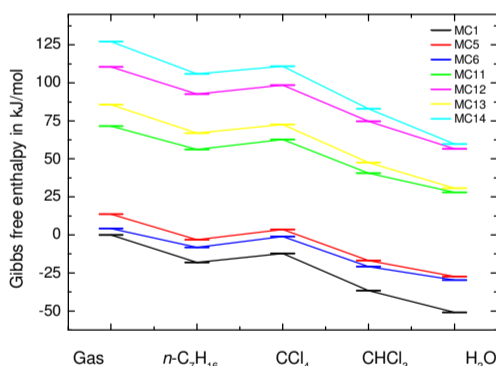
|             |                         |  |
|-------------|-------------------------|--|
| Stabilities | G3B3                    | accurate enthalpies of formation, computational error < 1 kcal/mol [2]   |
| Frequencies | DFT: PW91/6-311++G(d,p) | established as a highly reliable approach for H-bonded DNA model systems [3,4]                                     |
|             | DFT: TPSS/cc-p-VTZ      | state of the art functional, but computationally more intense – gives better results for the CO stretch region [5] |

The solvent was taken into account using the PCM method [6].

### Results in CHCl<sub>3</sub> – Cytidine

#### G3B3 calculations:

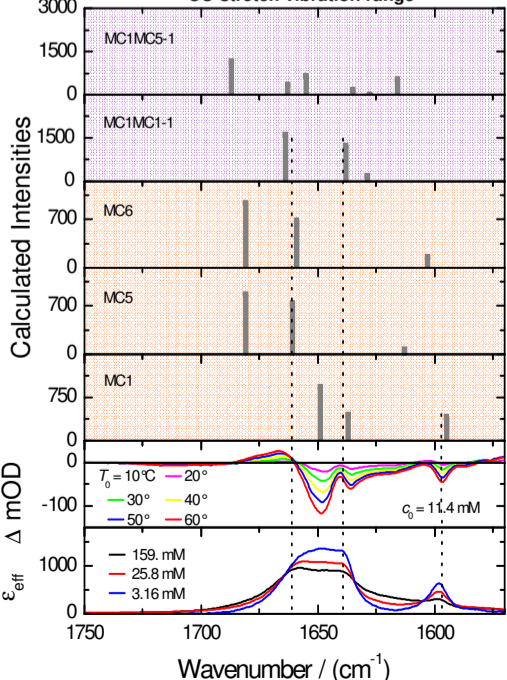
Stability sequence of various cytidine tautomers in different solvents



#### FTIR spectra and DFT calculations:

Frequencies of all monomer and dimer structures have been compared to the experimental FTIR spectra:

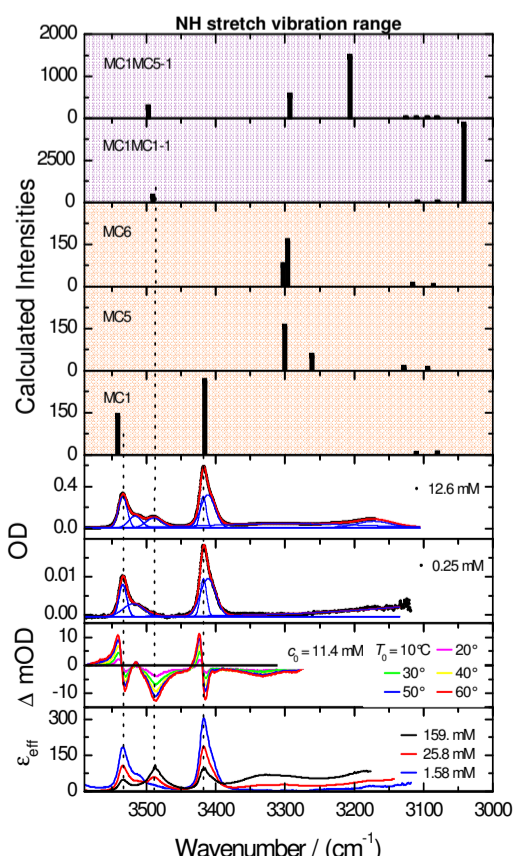
#### CO stretch vibration range



#### DFT calculations:

Bond energies of selected homodimers, which are combinations of the 3 most stable monomers.

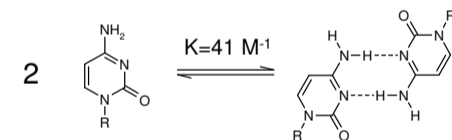
| Dimer    | Bond energy (kJ/mol) |
|----------|----------------------|
| MC1MC1-1 | 36.26                |
| MC1MC5   | 29.56                |
| MC1MC1-2 | 12.96                |
| MC5MC5   | 12.62                |
| MC5MC6   | 9.52                 |
| MC6MC6   | 7.00                 |



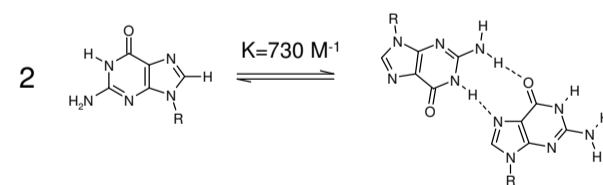
The calculated frequencies of the canonical monomer MC1 and its homodimer MC1MC1 fit to the experimentally observed bands. One isosbestic point was observed in the concentration dependent spectra, which supports the assumption of just one C-C homodimer present in solution. An association constant of  $41 \pm 3 \text{ M}^{-1}$  was determined.

### Discussion and Conclusion

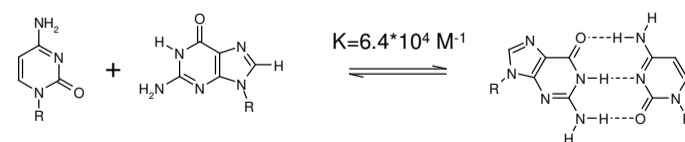
This work presents a comprehensive theoretical & experimental study on the different tautomeric forms of nucleoside monomers and dimers present in CHCl<sub>3</sub>. The applied methodology proved to be successful for determining the structure and association equilibria in solution. As shown for cytidine the following equilibrium was found:



Studies on guanosine have been performed in analogy to the procedure for cytidine. Only the canonical monomer MG2 could be found. It associates to an asymmetric dimer shown below. Another guanosine homodimer, which is symmetric, was predicted by the calculations to be present in CHCl<sub>3</sub>, but it could not be found in the FTIR spectra.



For the combination of guanosine and cytidine, only the Watson-Crick base pair, consisting of the canonical structures MC1 and MG2, was found. The bond energy proved to be 18 kJ/mol higher than for the homodimers, resulting in almost complete association for the investigated concentration range.



### Results in other solvents

A higher degree of association and the formation of higher-order aggregates was found in CCl<sub>4</sub> and n-C<sub>7</sub>H<sub>16</sub>. Even for low concentration ranges the spectra are dominated by broad and complex bands, which could be assigned by DFT calculations to trimers and tetramers.

### Literature

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