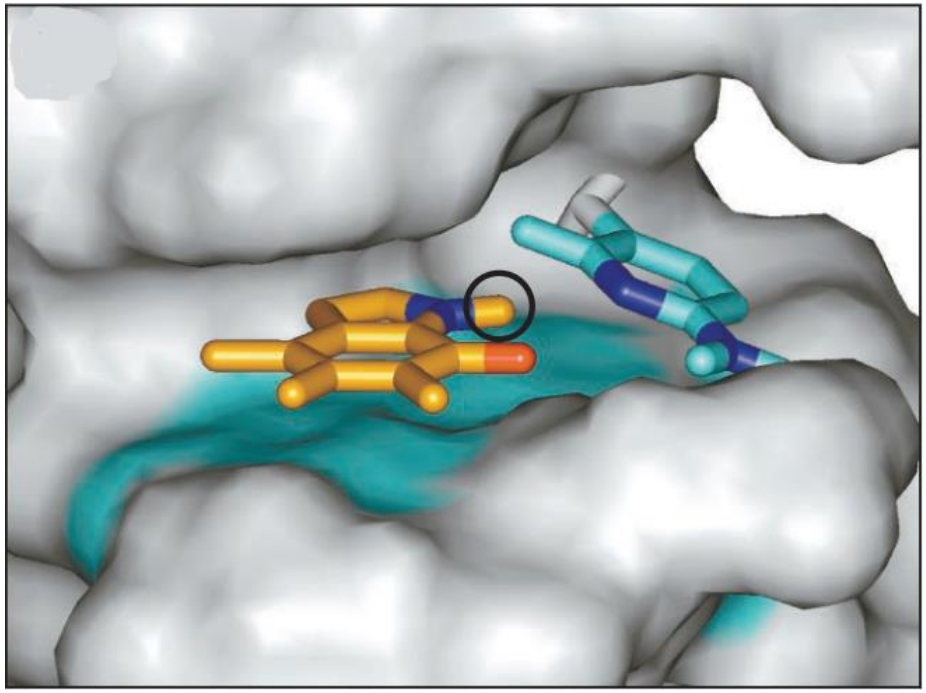
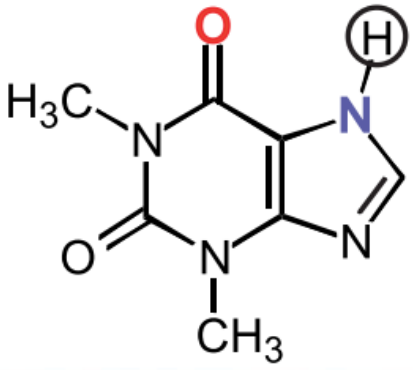
Group meeting 260619 – Electrochemical aptasensors - Molecular investigation of the interaction target-aptamer

Molecular investigation of the interaction target-aptamer: where does the binding event takes place ?



Stacking and hydrogen bond interaction !
RNA structural interactions in a ligand binding site proved by NMR spectroscopy

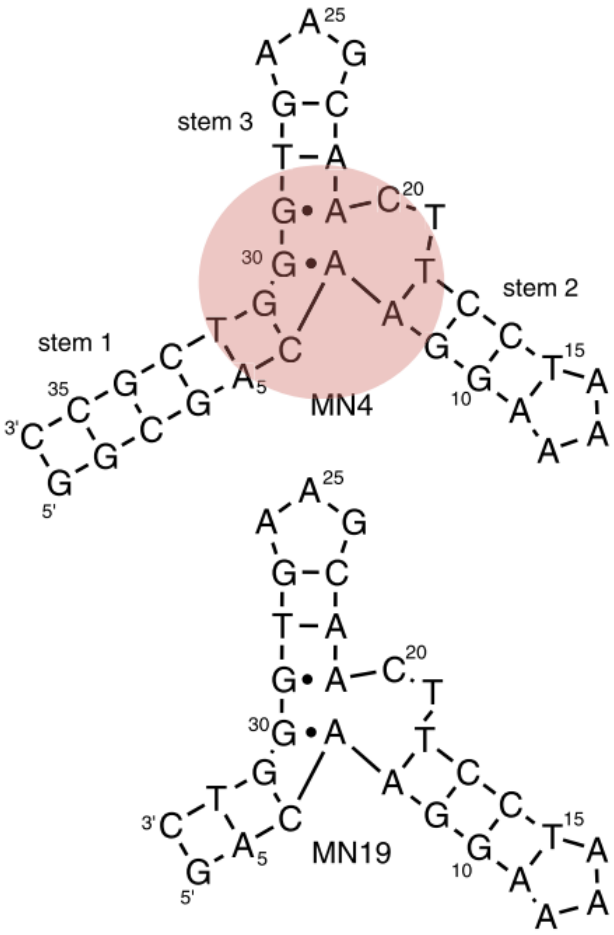


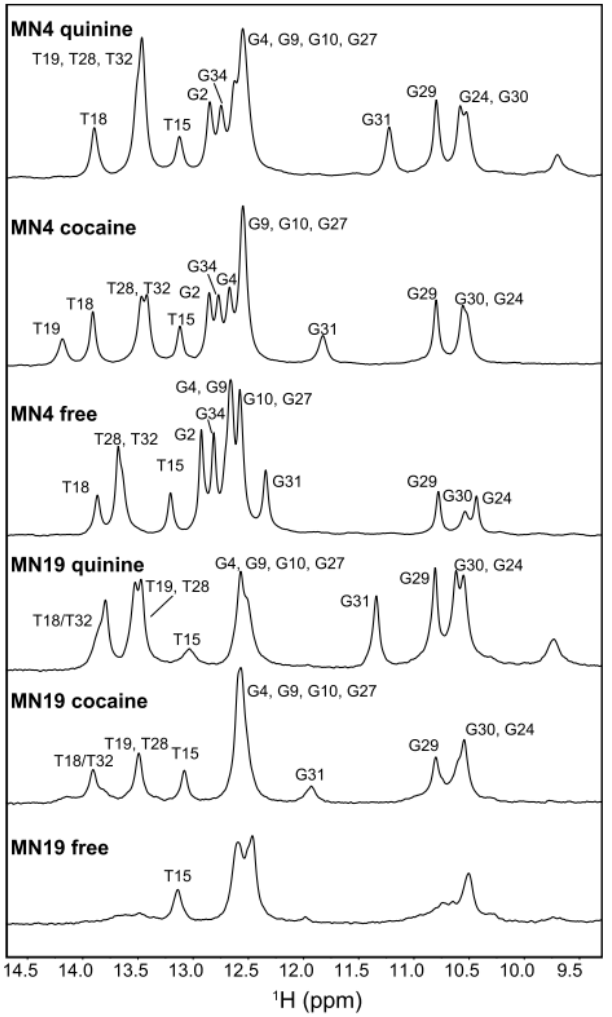
Caffeine differs by a methyl group (encircled H replaced by a CH_3)

Displays affinity for theophylline 10,000 times that of caffeine

Hermann, T. and D. J. Patel (2000). "Adaptive Recognition by Nucleic Acid Aptamers." Science **287**(5454): 820-825.
Zimmermann, G. R., et al. (1997). "Interlocking structural motifs mediate molecular discrimination by a theophylline-binding RNA." Nature Structural Biology **4**(8): 644-649.

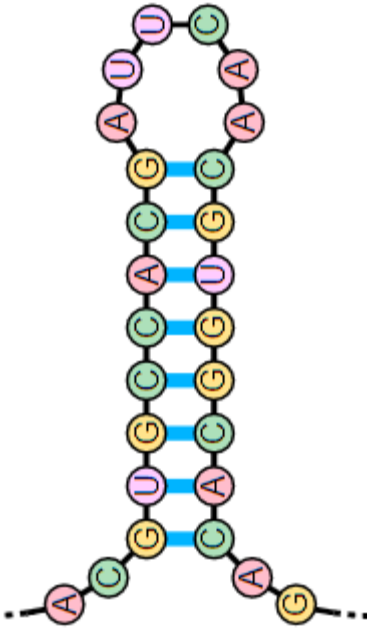
Molecular investigation of the interaction target-aptamer: need to assign nucleotides with NMR





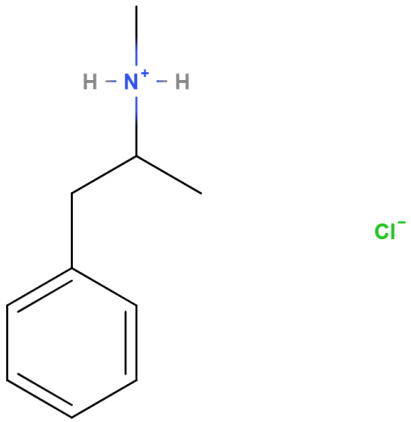
Churcher, Z. R., et al. (2017). "Comparison of the free and ligand-bound imino hydrogen exchange rates for the cocaine-binding aptamer." **68**(1): 33-39.

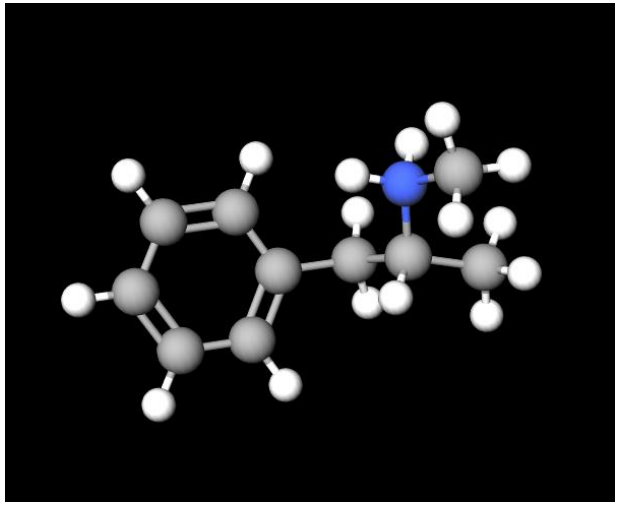
Molecular investigation of the interaction target-aptamer: What we did and what we'll do ?
NMR spectroscopy with our meth-aptamer



Possible interactions:

- **Hydrogen bonding**
- **Stacking interaction**





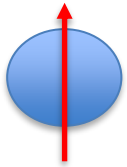
https://upload.wikimedia.org/wikipedia/commons/3/3f/Stem-loop.svg

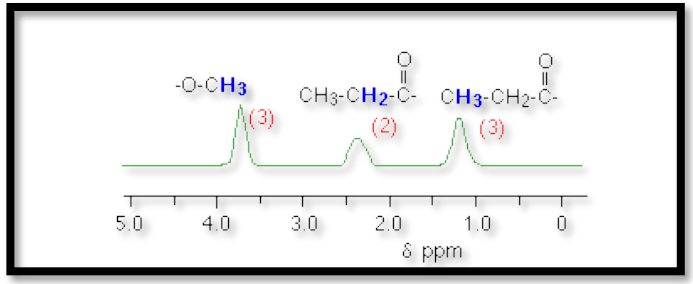
Molecular investigation of the interaction target-aptamer: general principle of NMR

Change of the orientation of the spin without molecular environment

Change of the orientation of the spin with molecular environment

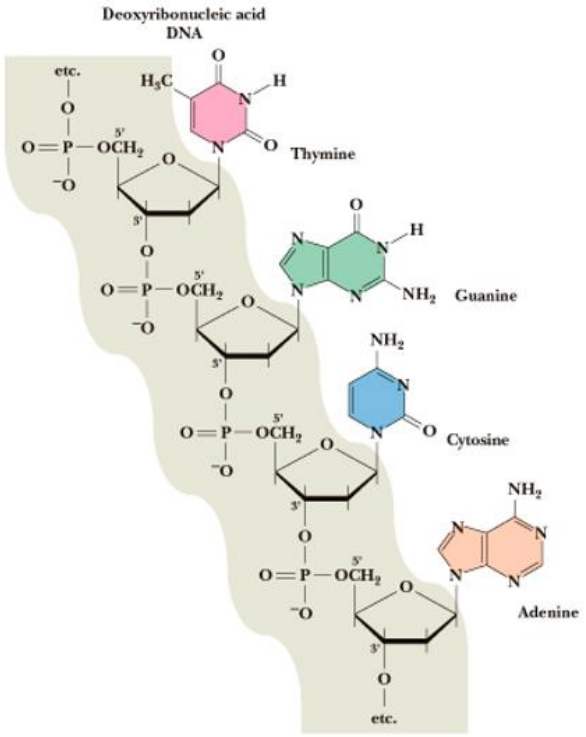
Coming back to initial magnetic field
"Relaxation"





Different relaxation with different molecular environment ➔ different chemical shift based on molecular environment of the proton

Molecular investigation of the interaction target-aptamer: what are the different hydrogen found into aptamer structure ?



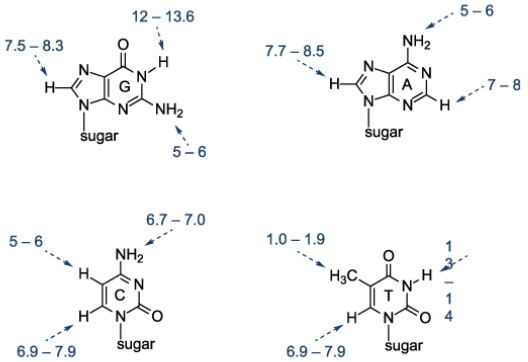


Figure 13 | NMR chemical shifts of the key residues observed in 1H NMR spectra of nucleic acid bases Data from Wijmenga and van Buuren, Progress in Nuclear Magnetic Resonance Spectroscopy 32, 287–387 (1998).

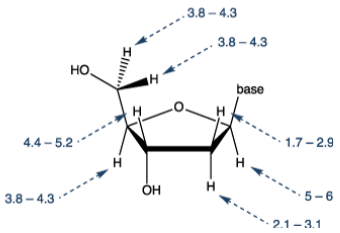
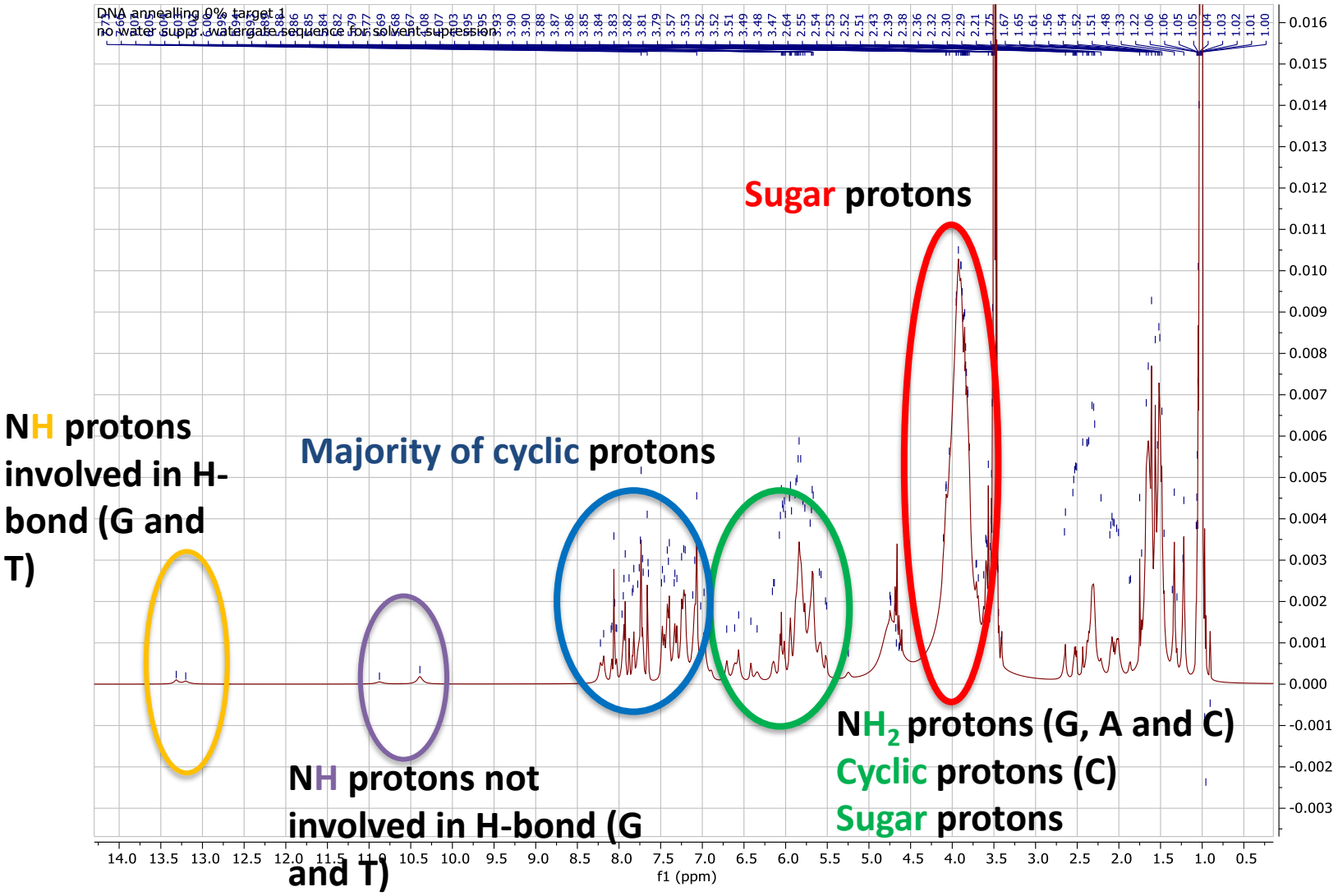


Figure 14 | NMR Chemical shifts of the key residues observed in 1H NMR spectra of the deoxyribose sugar in nucleic acids Data from Wijmenga and van Buuren, Progress in Nuclear Magnetic Resonance Spectroscopy 32, 287–387 (1998).

Possible to see different group of proton on the NMR proton spectra ➔ sugar proton, methyl proton, cyclic proton, imino proton (involved and non involved in Hydrogen bond)

Wijmenga, S. S. and B. N. M. van Buuren (1998). "The use of NMR methods for conformational studies of nucleic acids." Progress in Nuclear Magnetic Resonance Spectroscopy **32**(4): 287-387.

Molecular investigation of the interaction target-aptamer: preliminary results NMR proton ^{1}H

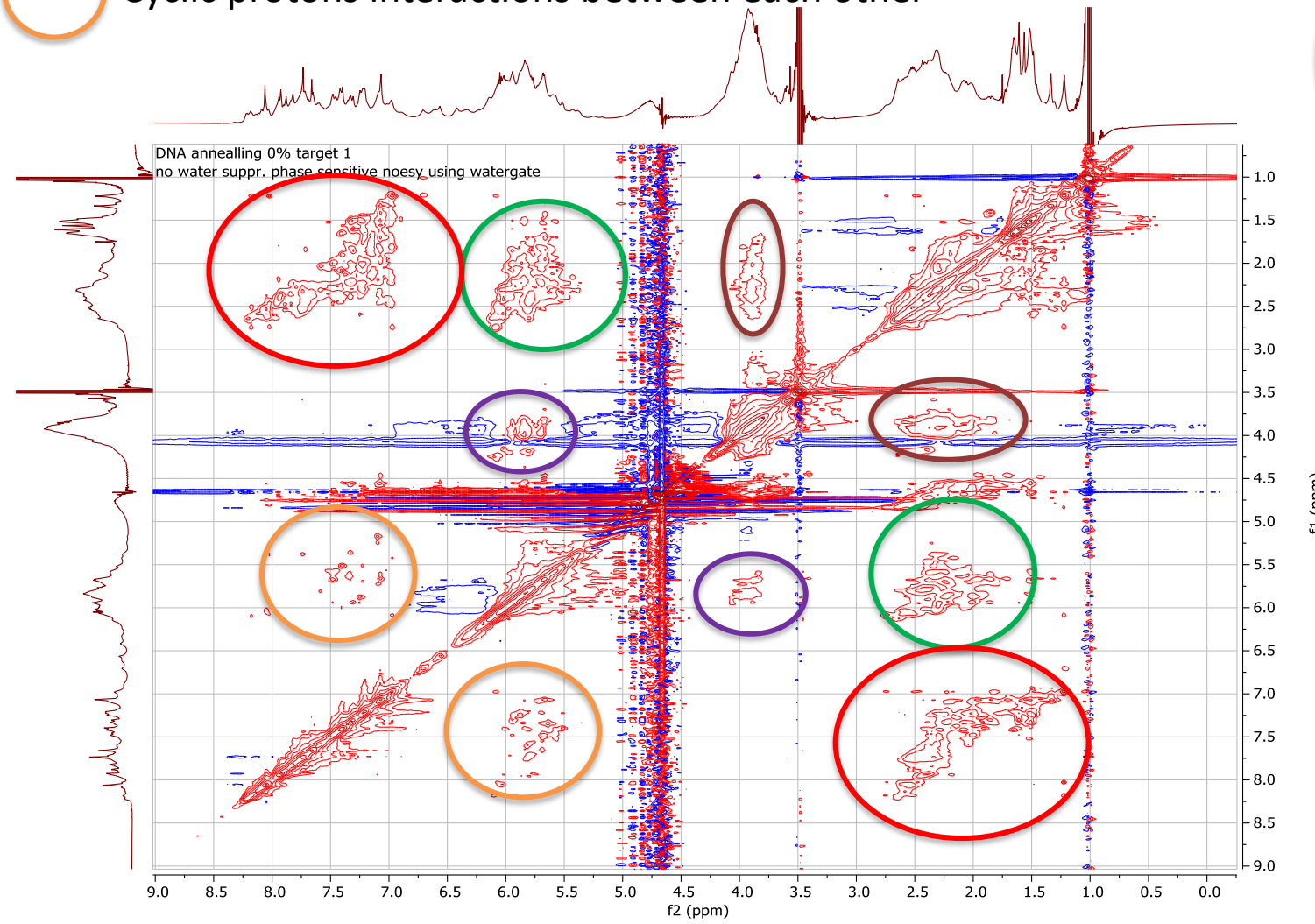


Molecular investigation of the interaction target-aptamer: NMR proton 2D

- Goal of those two techniques: create a 2 dimensional NMR proton spectra → to see interactions between protons
  - COSY → interaction through bond
  - NOESY → interaction through bond but also through space

Molecular investigation of the interaction target-aptamer: NOESY (longer distance)

Cyclic protons interactions between each other

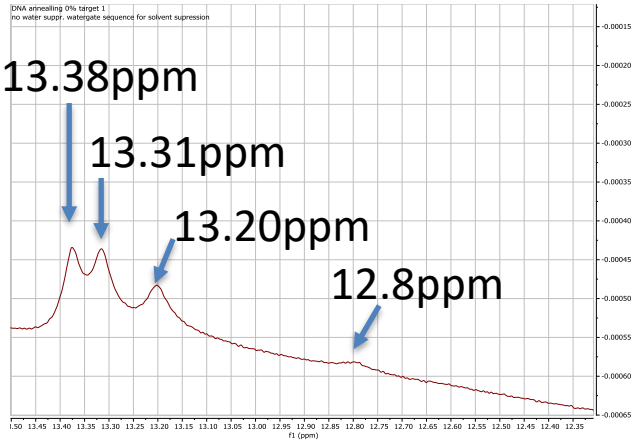


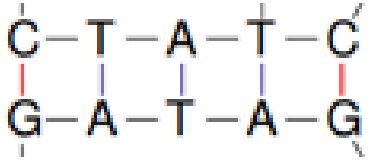
Sugar protons interactions **in space** with other protons from nucleotides or from sugars

Sugar protons and cyclic protons interactions **in space** with other protons from nucleotides or from sugars

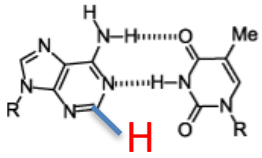
Molecular investigation of the interaction target-aptamer: Imino proton (in H-bond) investigation (above 12ppm)

- 1D spectra ➔ three distinct peaks



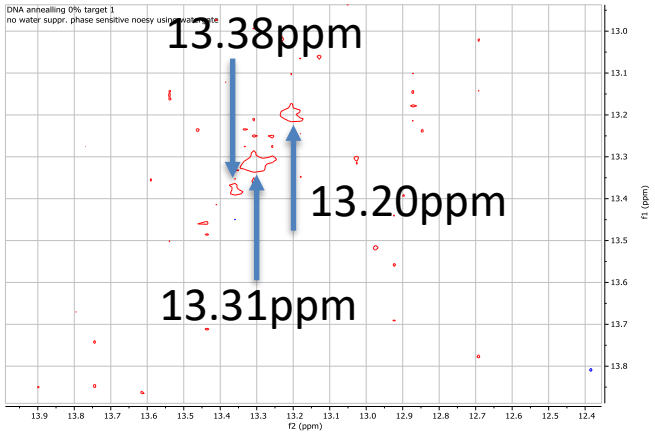


Only stem from our aptamer predicted by *mfold* software

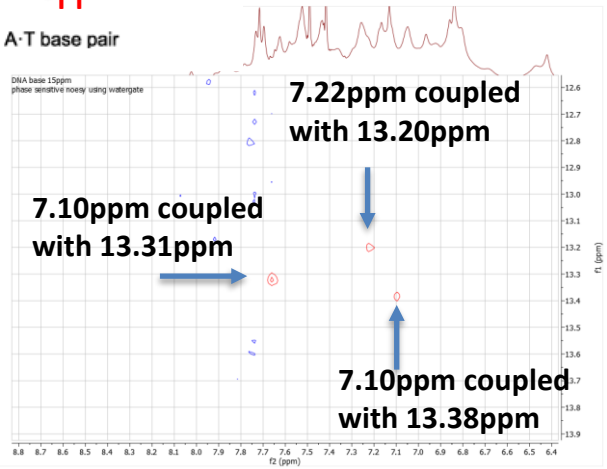


A·T base pair

- NOESY spectra zoomed ➔ three distinct peaks



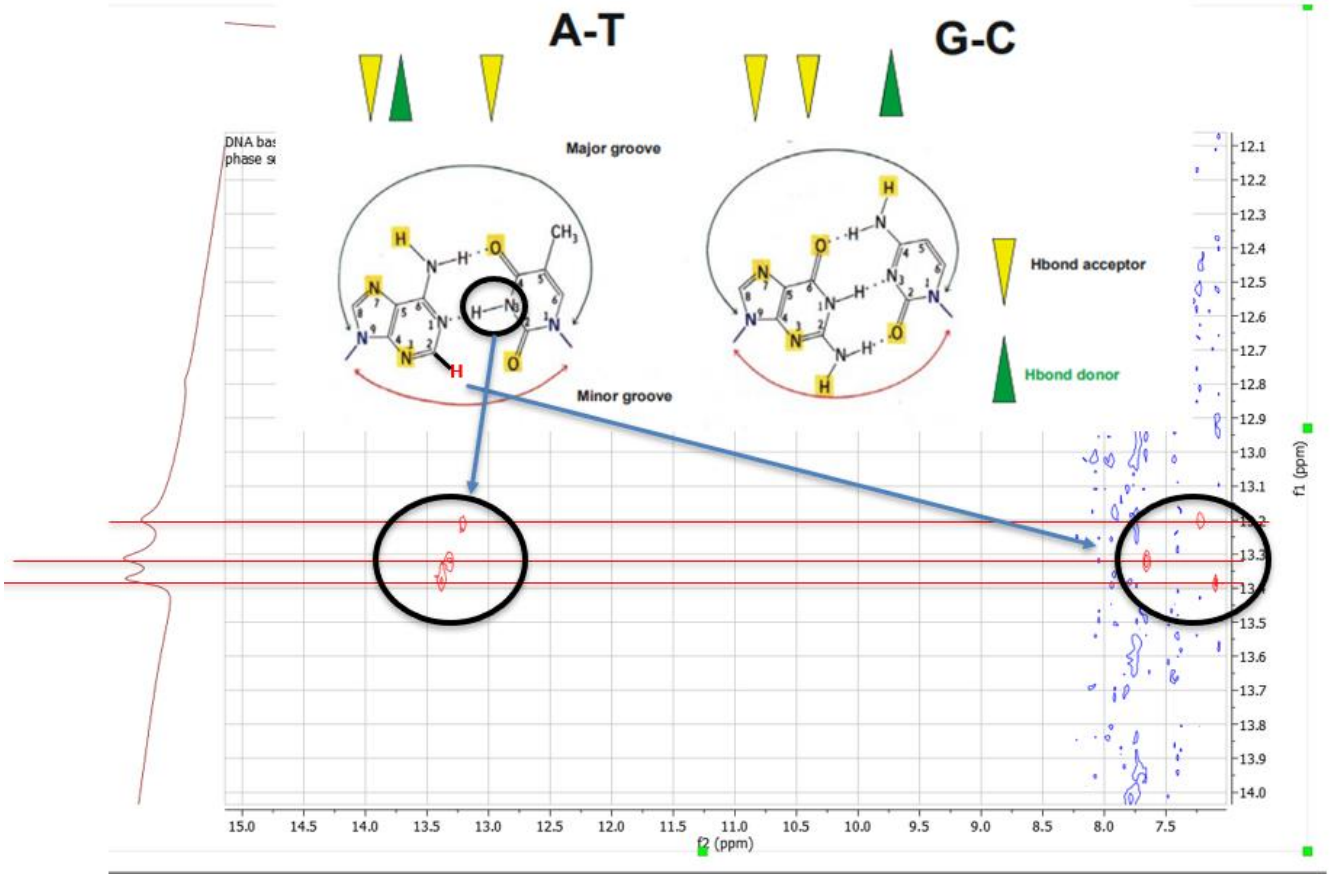
No conclusion just confirm three main peaks observed in 1D



3 special H (only found in A) clearly coupled with our 3 imino protons from T ➔ so 3 base pair AT ➔ confirm the stem and assignment of the nucleotides form the stem possible !!!!!!!

Molecular investigation of the interaction target-aptamer: SI concerning NOESY results

2D NOESY from 12 to 15ppm



Molecular investigation of the interaction target-aptamer: meth titration experiments

- After assignment, need to titrate methamphetamine with our aptamer.
- Decrease of intensity proportional to methamphetamine added