Wpływ pseudourydyny na strukturę i stabilność RNA: symulacje dynamiki molekularnej i obliczenia kwantowo-mechaniczne

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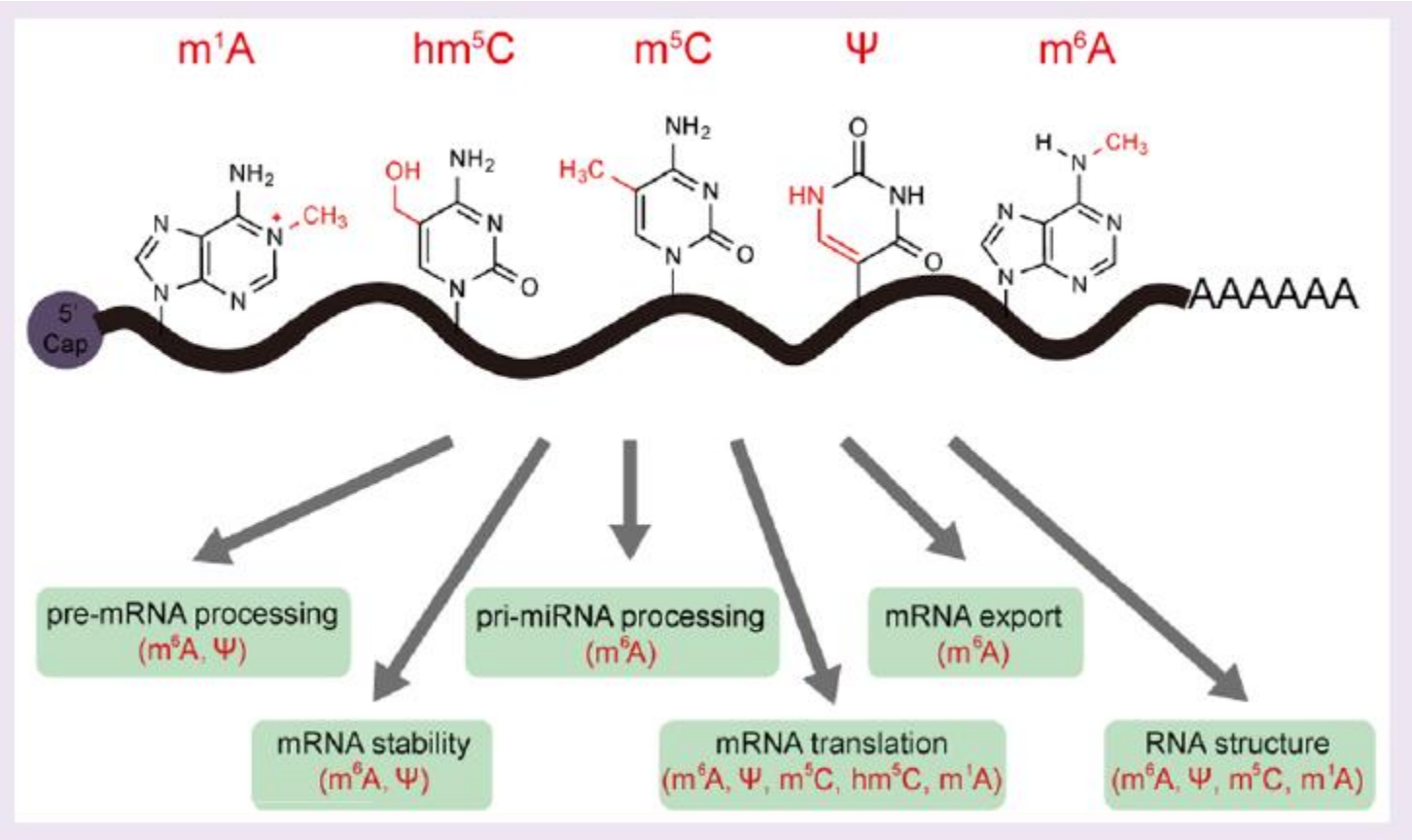
Motywacja

- Znanych jest ponad 100 różnych modyfikacji RNA

109 modified nucleosides in the RNA Modification Database (RNAMDB)
93 in tRNA, 31 in rRNA, 13 in mRNA and 14 in other RNA species (snRNA).

- Modyfikacje epigenetyczne

dotyczą mRNA, dynamiczne – dodawane i usuwane przez specyficzne enzymy, wpływają na ekspresję genów

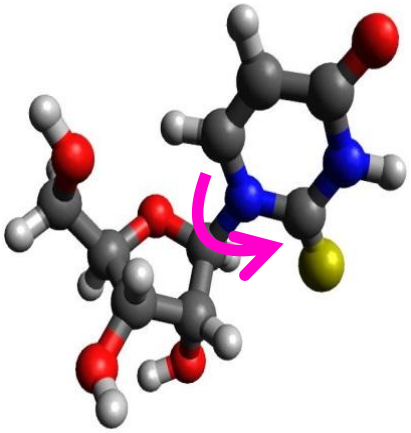


Song J, Yi C,. ACS Chem Biol. 2017, 12(2):316-325

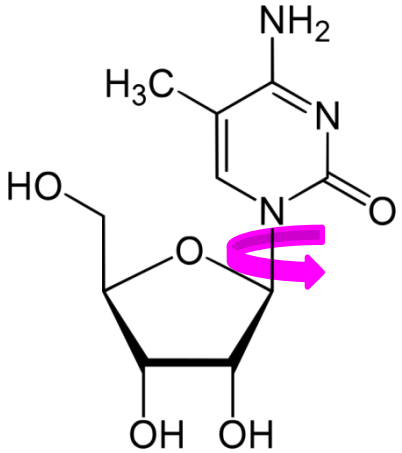
Development of accurate AMBER force field parameters for modified ribonucleosides

Objectives

- To reparameterize the glycosidic **Chi (χ) torsion** and other relevant torsion parameters at the individual **nucleoside level**
- To study the consequences of the reparameterization at the nucleoside and oligonucleotide level using MD and REXMD



2SU



5MC

NMR : Sugar pucker N:74%
FF_Aduri: Sugar pucker N:33%
FF_IDRP+bsc0: Sugar pucker **N:74%**

NMR : Sugar pucker N:59%
FF_Aduri: Sugar pucker N:24%

I Deb, R Pal, J Sarzynska, A Lahiri, Journal of Computational Chemistry, **2016**, 37, 1576–88
I Deb, J Sarzynska, L. Nilsson, A Lahiri, J Chem Inf Model, **2014** 54(4):1129-42

Flowchart of parameter development and validation strategy: reoptimization of torsion potential

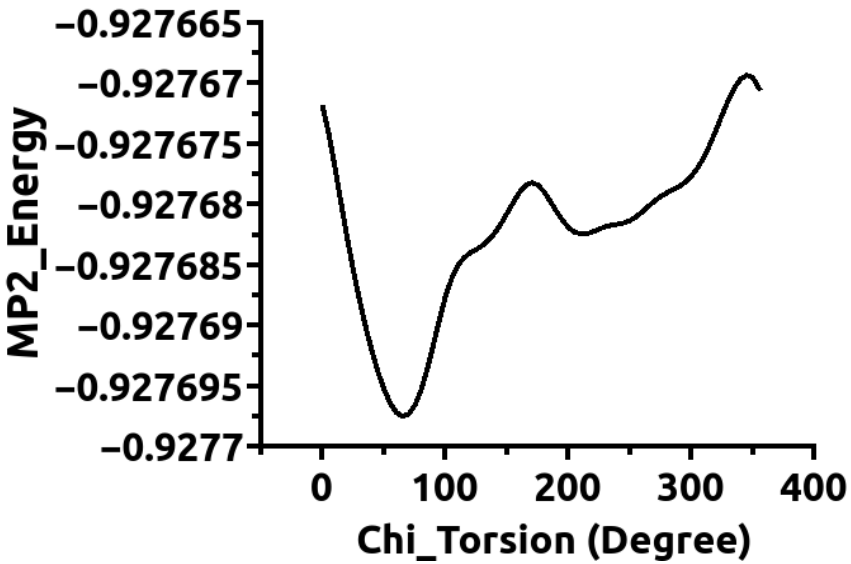
Obtain quantum mechanical energy (**E_{QM}**) profile around the torsion angle.

Obtain molecular mechanical energy (**E_{MM}**) profile around the same torsion angle.

Reproduce the QM energy profile by fitting the differences between E_{QM} and E_{MM} to the Fourier series

New set of torsion parameters.

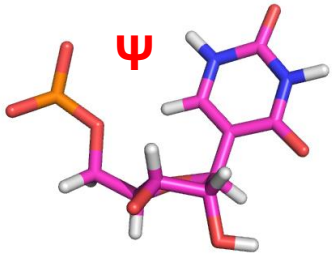
Extensive validation of the newly obtained parameter set using REMD simulation

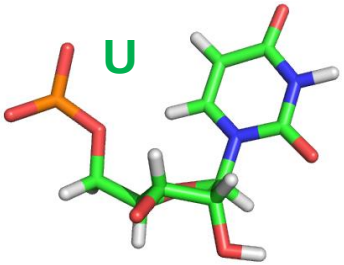


QM energy landscape for the Chi torsion for the 2'O-methyl cytidine

Obiekty badawcze

- **RNA duplexes with central Ψ-A base pair**



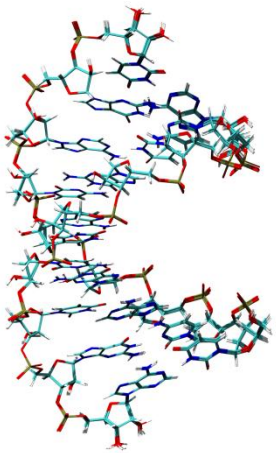


5’-UCAGΨCAGU-3’
3’-AGUCAGUCA-5’

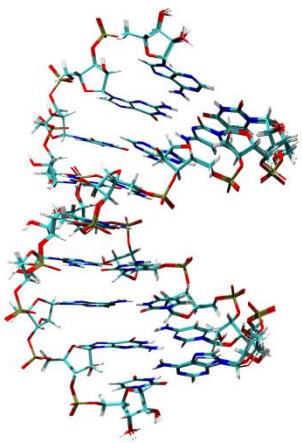
5’-UCACΨGAGU-3’
3’-AGUGACUCA-5’

5’-UCAAΨUAGU-3’
3’-AGUUAAUCA-5’

5’-UCAUΨAAGU-3’
3’-AGUAAUUCA-5’



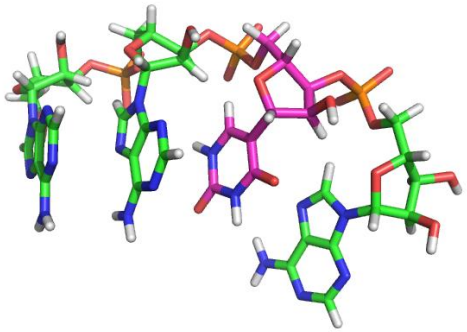
Seq-GΨC

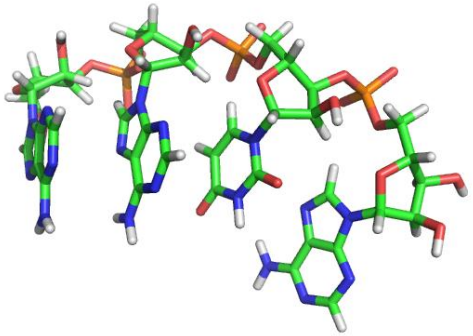


Seq-CΨG

Superposed structures of 10 models calculated by simulated annealing method.

- **RNA tetramers AAΨA, AAUA,**

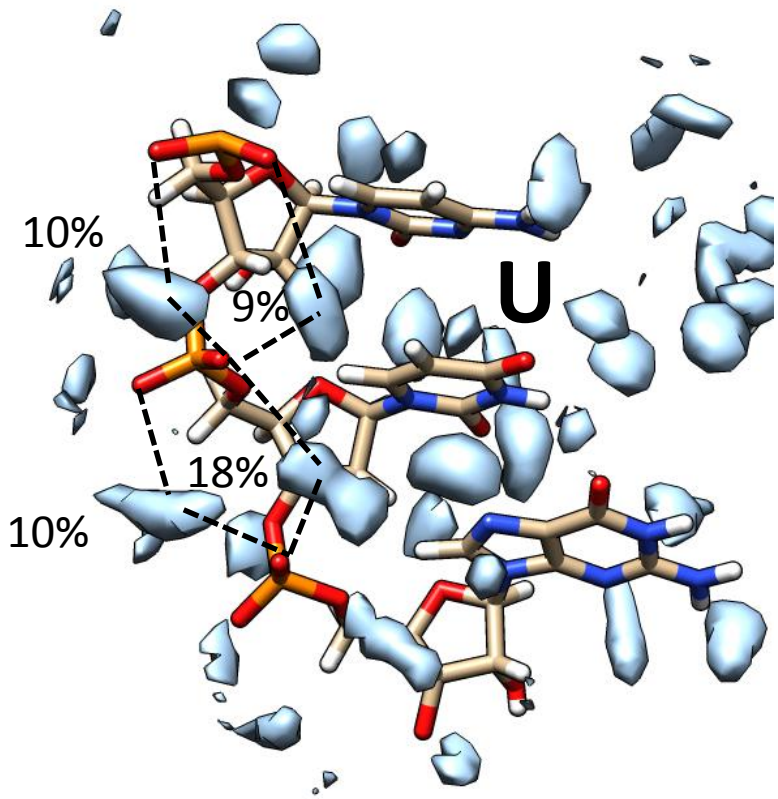




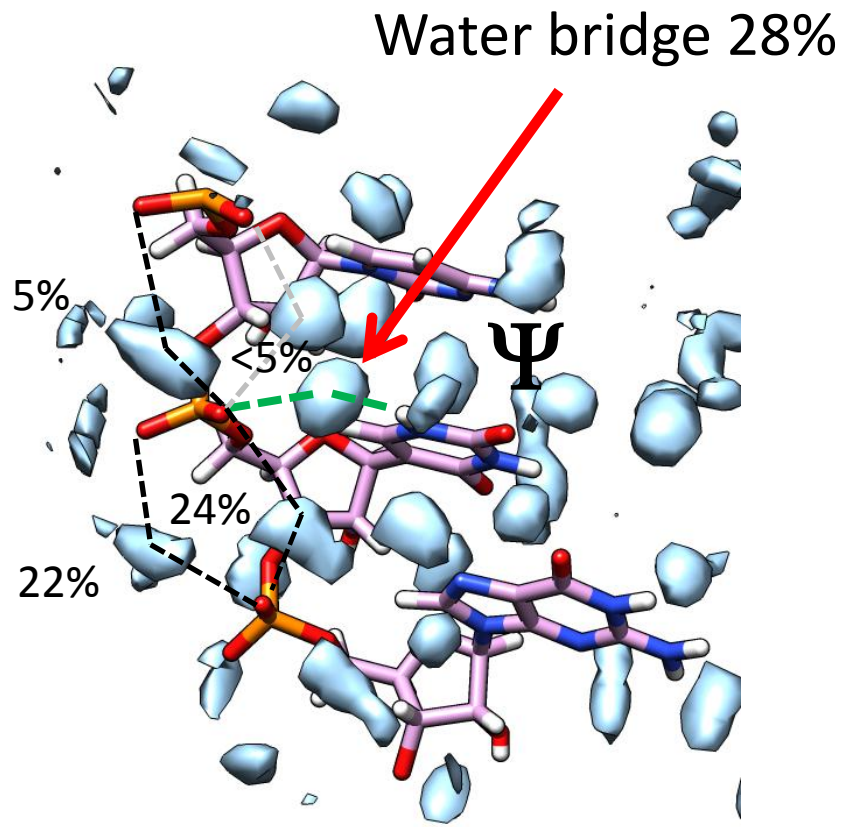
I Deb, R Pal, J Sarzynska, A Lahiri, Journal of Computational Chemistry, **2016**, 37, 1576–1588

Backbone hydration – stable water bridge made by Ψ

RNA duplexes with central Ψ-A base pair



Seq-CUG

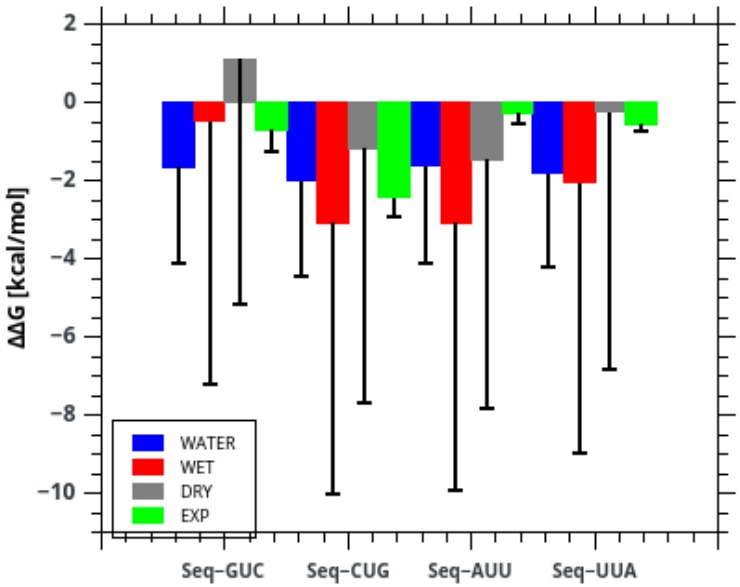


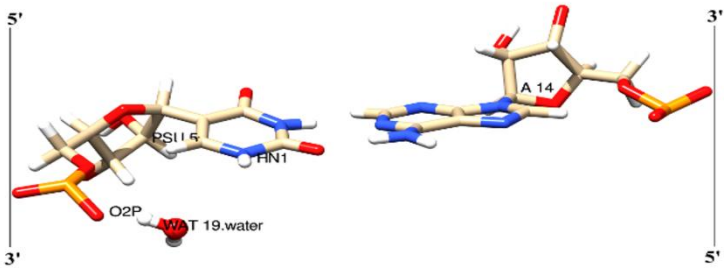
Seq-CΨG

500ns MD, ff10, Amber14, GPU inula

Thermodynamic parameters of duplex formation calculated by MM/PBSA method in presence/absence of one explicit water molecule

– using 8000 frames of last 400ns of the simulated trajectories



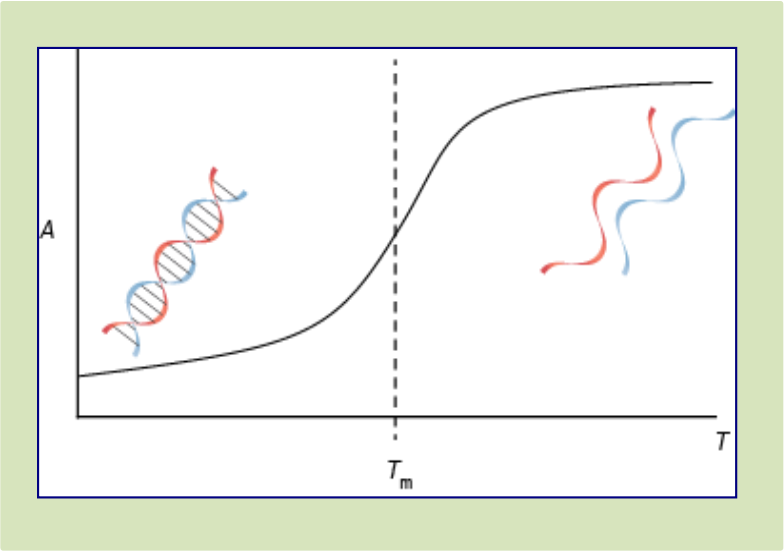


	$\Delta\Delta G_{SIM}$ (kcal/mol)	$\Delta\Delta G_{SIM}$ + **WAT** (kcal/mol)	$\Delta\Delta G_{Exp}$ (kcal/mol)
Seq-GΨC	+1.11 (±6.30)	-0.50 (±6.72)	-0.71 (±0.55)
Seq-CΨG	-1.17 (±6.54)	-3.08 (±6.94)	-2.43 (±0.49)
Seq-AΨU	-1.47(±6.37)	-3.09 (±6.83)	-0.27 (±0.27)
Seq-UΨA	-0.25(±6.58)	-3.08 (±6.92)	-0.55 (±0.19)

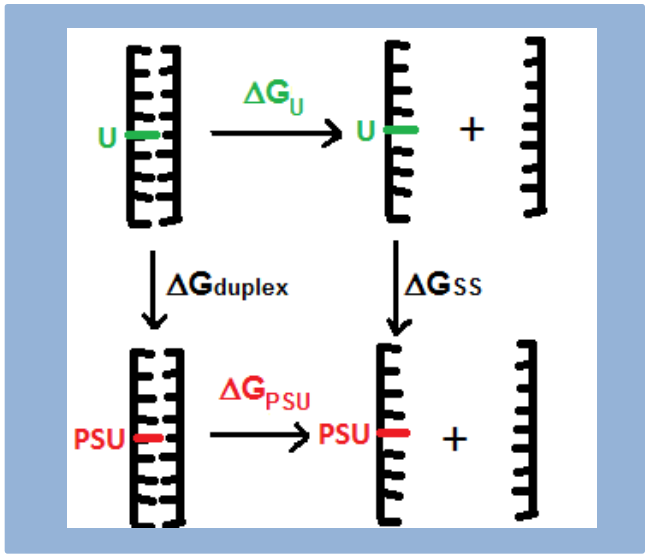
Kierzek E, Malgowska M, Lisowiec J, et al (2014) The contribution of pseudouridine to stabilities and structure of RNAs. Nucleic Acids Res 42:3492–501

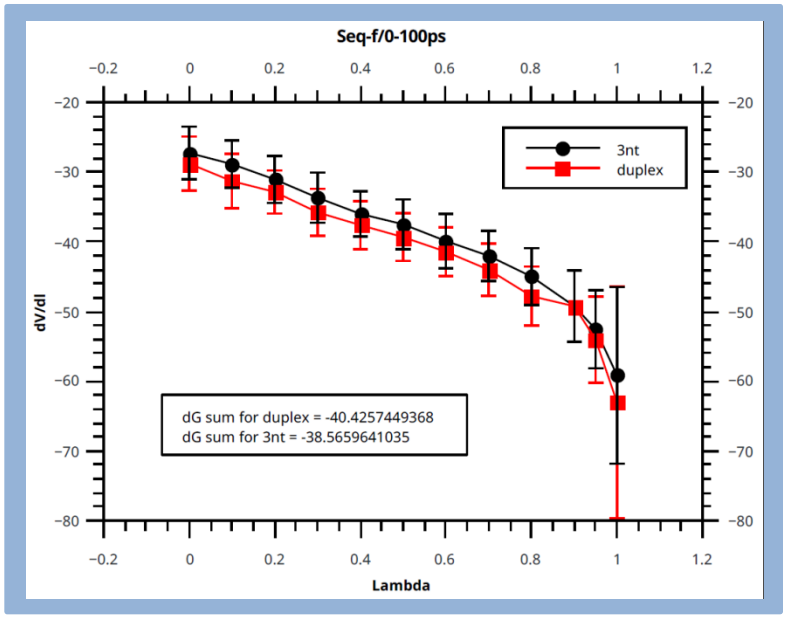
Free energy perturbation (Amber16, CPU eagle)

A typical UV melting curve



Termodynamic cycle used in FEP



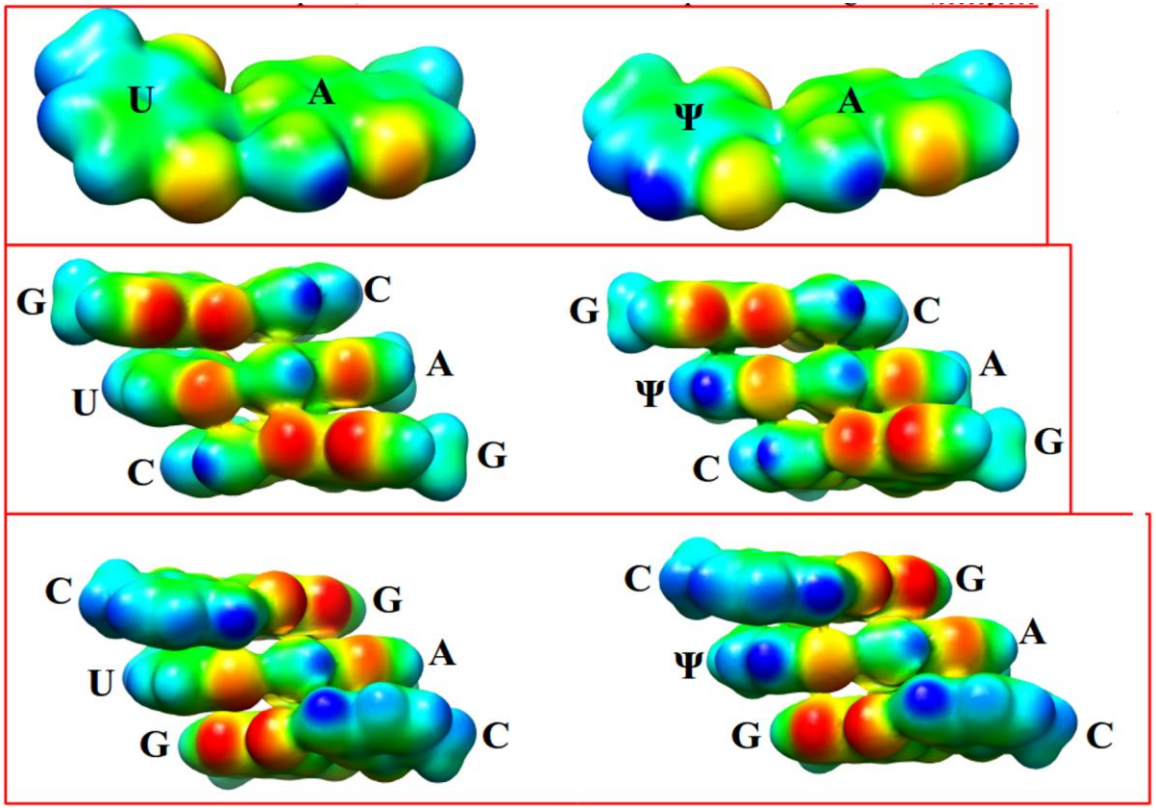


$$\Delta G_U + \Delta G_{ss} = \Delta G_{duplex} + \Delta G_{PSU}$$

$$\underbrace{\Delta G_{duplex} - \Delta G_{ss}}_{\text{Values from FEP/TI simulations}} = \underbrace{\Delta G_U - \Delta G_{PSU}}_{\text{Experimental values}}$$

Oddziaływania warstwowe (1)

Isodensity surface color-coded with electrostatic potential

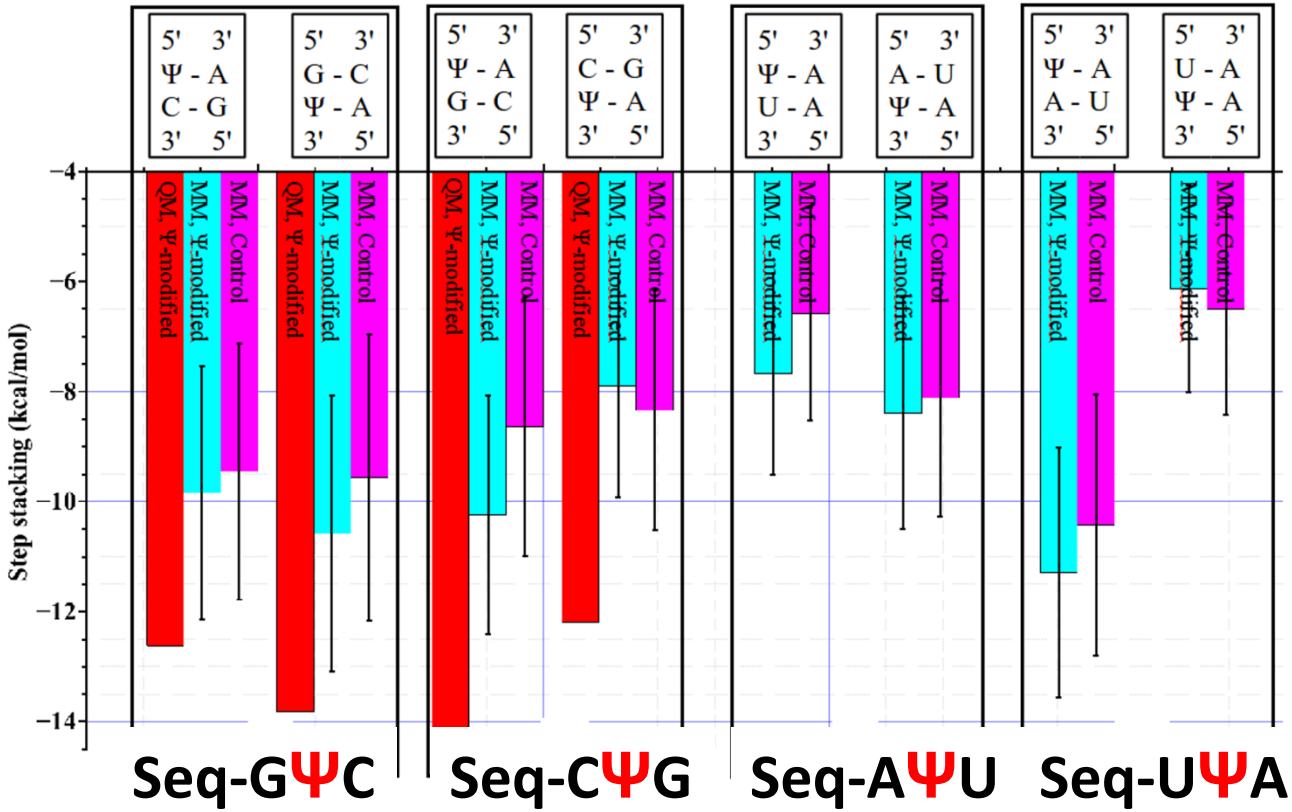


Seq-GΨC

Seq-CΨG

- geometries for the RNA duplexes were lowest energy structure calculated from simulated annealing or the average structure calculated from the highest populated cluster generated from the cluster analysis
- monomers of bases were optimized individually in the gas phase at the B97D/def2TZVPP level of theory
- N1/N9-C1' bond replaced by N1/N9-CH3 bond to better distinguish between uracil and psudouracil bases

Oddziaływania warstwowe (2)



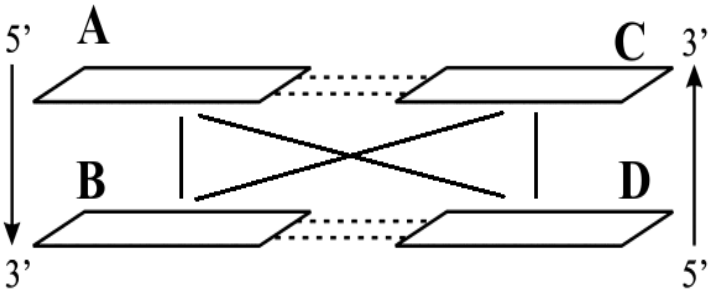
Base pair step stacking energies

- QM-based:

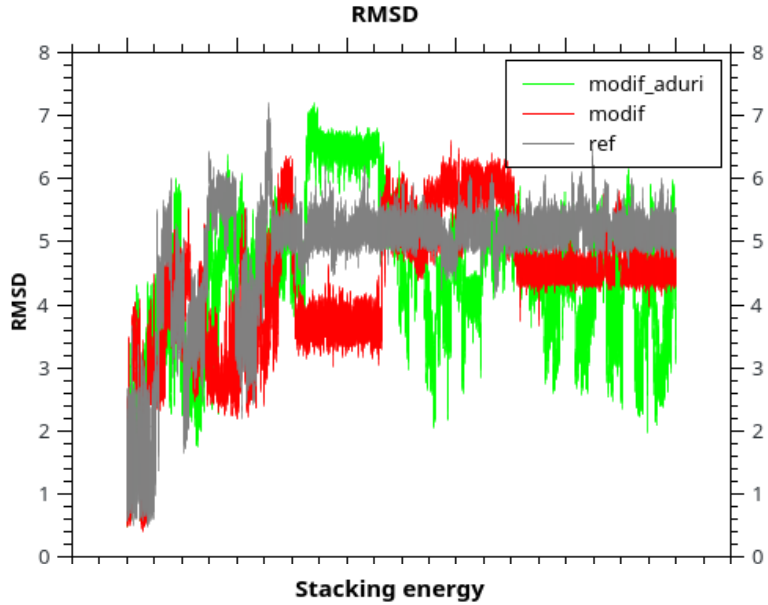
B97D/def2TZVPP level of theory

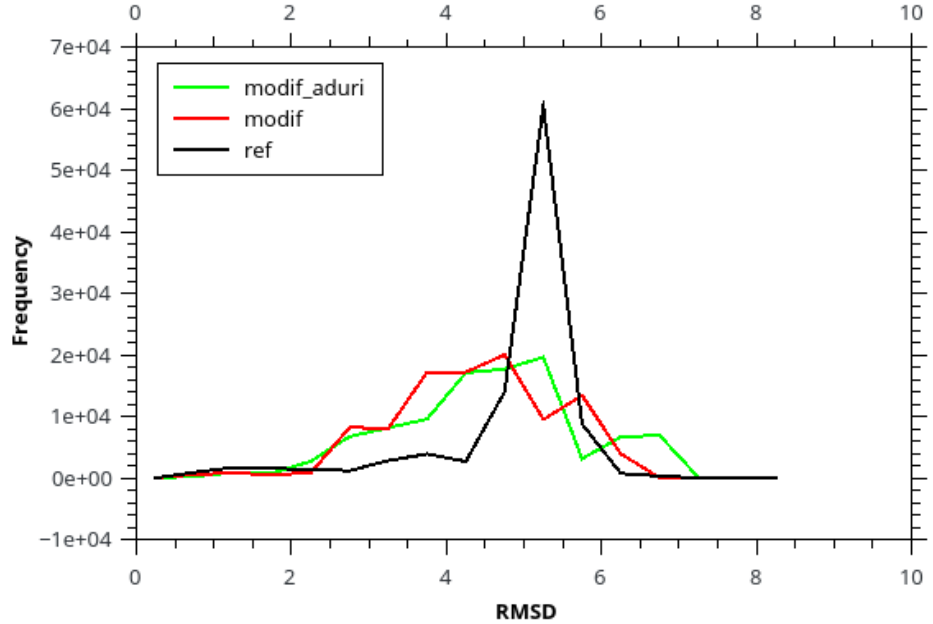
- molecular mechanics (MM)

vdW+Ele (*lie* utility in *cpptraj* program)

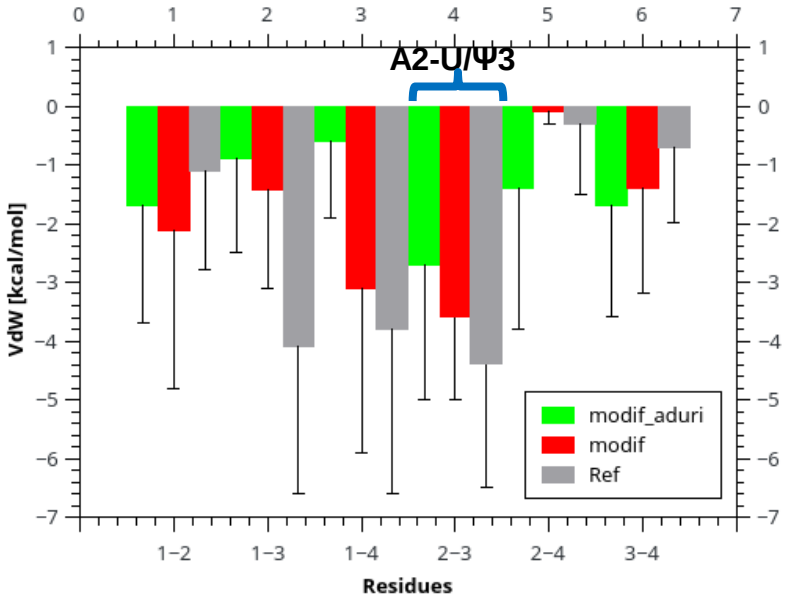


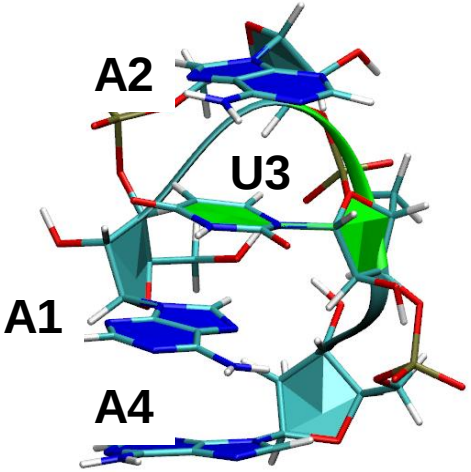
RNA tetramers AAΨA, AAUA – RMSD 500ns of MD





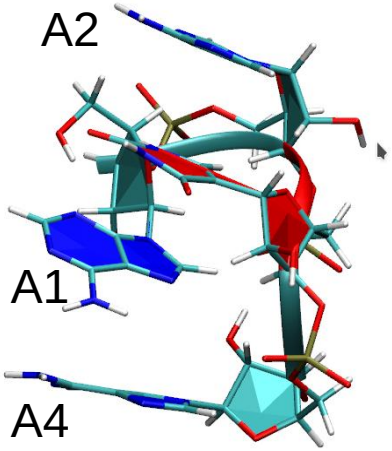
Base-base interaction Energy





I Deb, R Pal, J Sarzynska, A Lahiri, Journal of Computational Chemistry, **2016**, 37, 1576–1588

RNA tetramers AAΨA, AAUA - cluster analysis 500ns of MD



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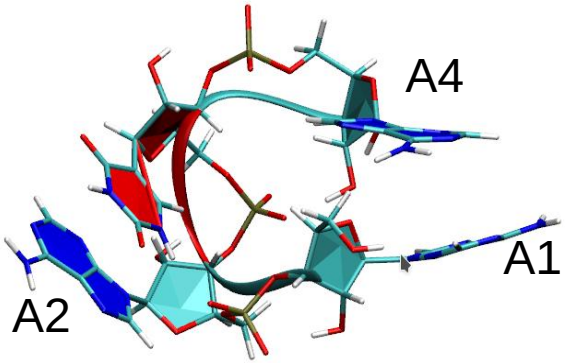
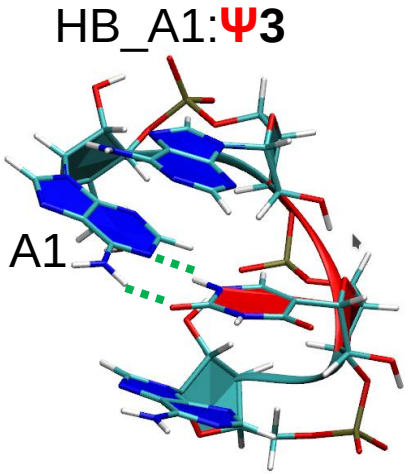


1_idrp 24%



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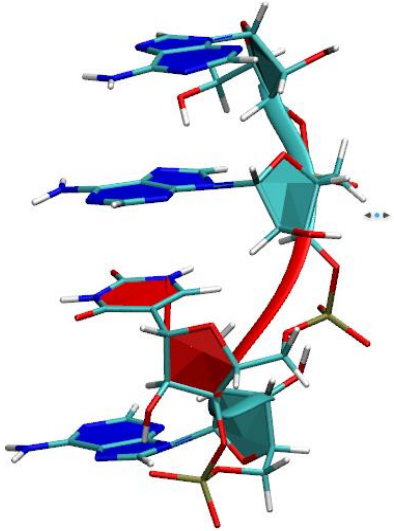

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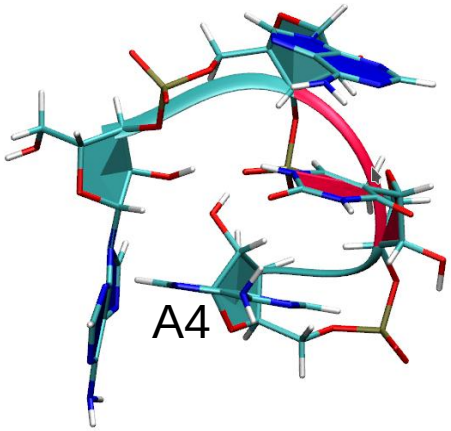

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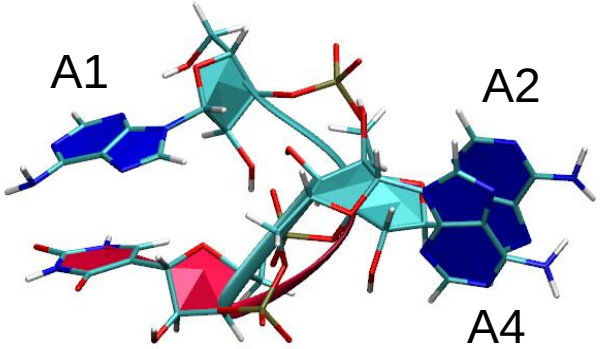
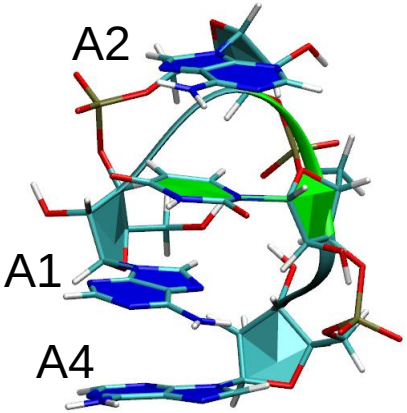


1_aduri 5%



0_Ref 66%

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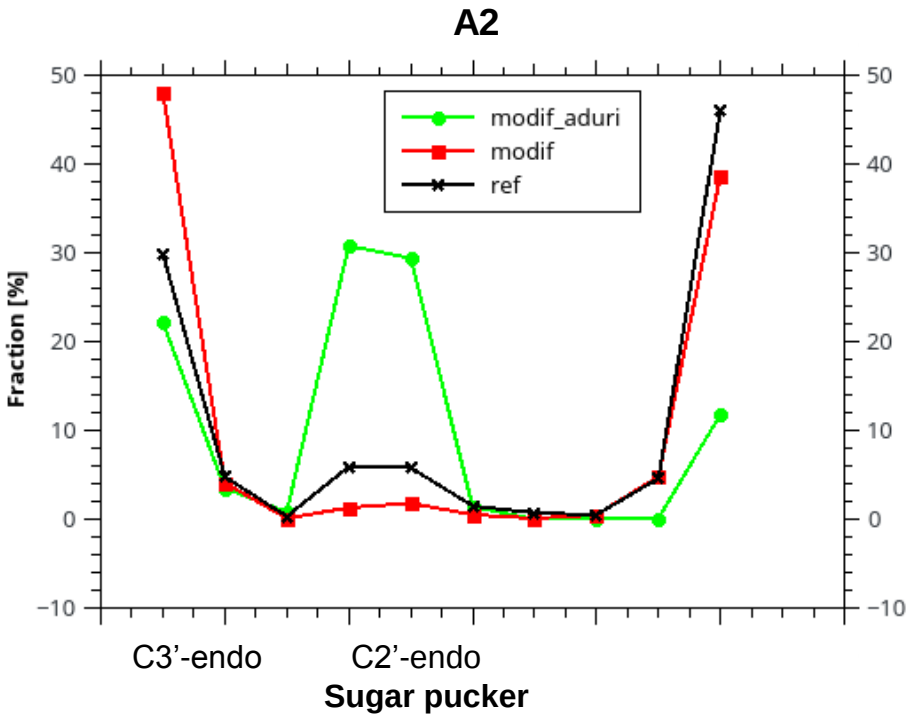
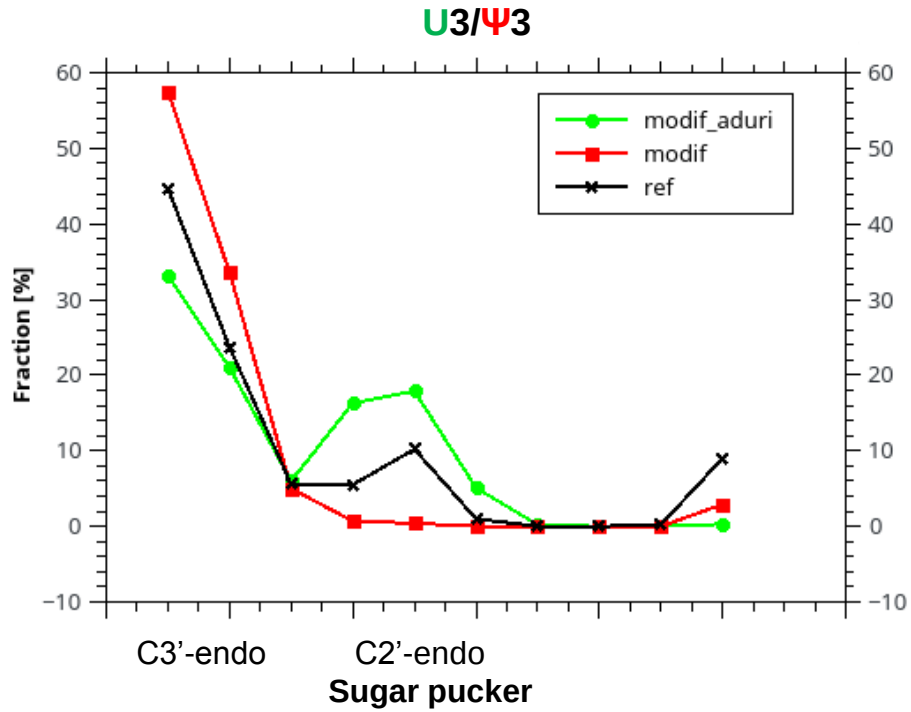
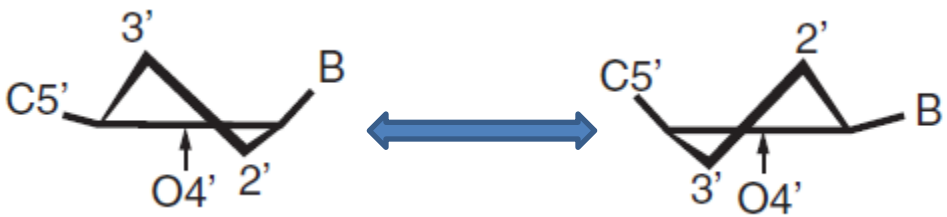
RNA tetramers AAΨA, AAUA – sugar pucker 500ns of MD





N
C3'-endo



S
C2'-endo

Experimental data

AAΨA: A2: 75%N, Ψ3:100%N

AAUA: A2: 57%N, U3:53%N

Davis,D.R. (1995) Stabilization of RNA stacking by pseudouridine. *Nucleic Acids Res.*, **23**, 5020–5026

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[2] Pracownia Biomolekularnego NMR, IBCh

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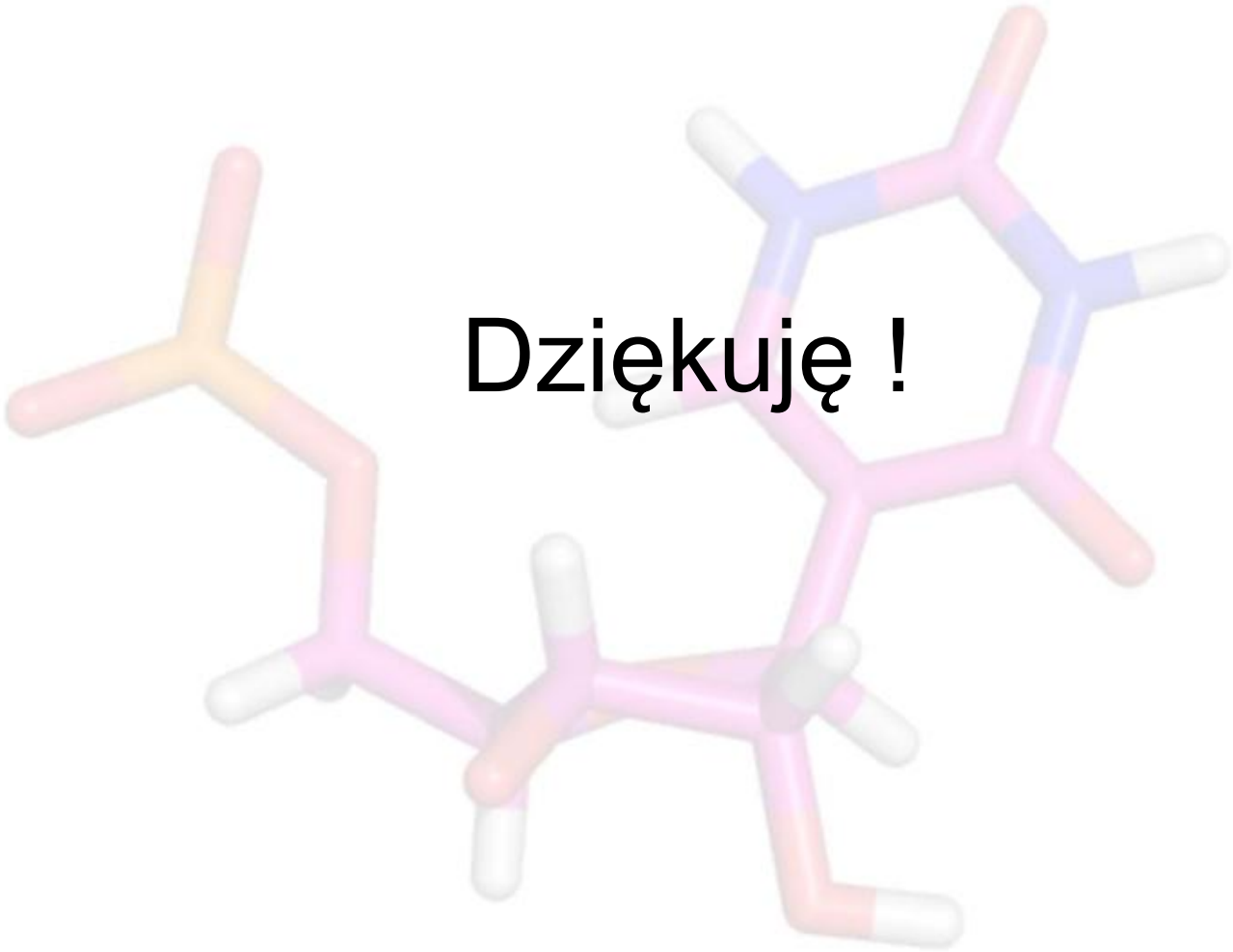
- European Center for Bioinformatics and Genomics (ECBiG), Poznan, Poland for computer lab support



Dziękuję !

"Mutations in human AID differentially affect its ability to deaminate cytidine and 5-methylcytidine in ssDNA substrates in vitro"

Lucyna Budzko, Paulina Jackowiak, Paulina Jackowiak, Karol Kamel, Joanna Sarzynska, Janusz M. Bujnicki and Marek Figlerowicz

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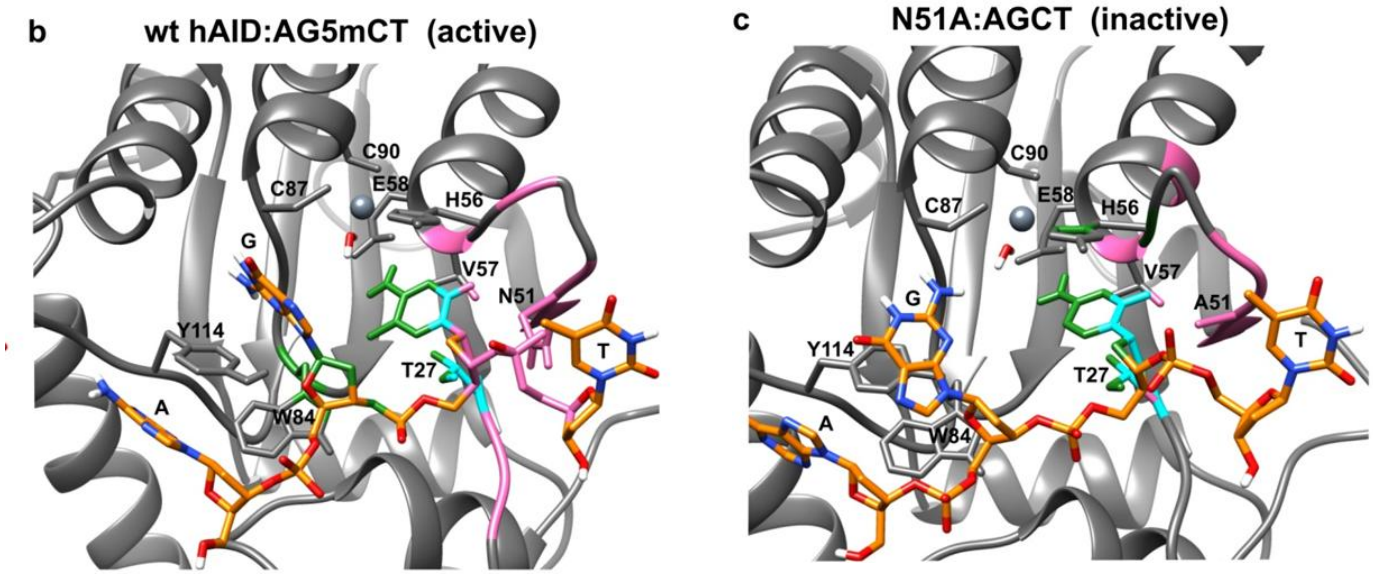
Pole siłowe Chrmm36, program NAMD



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