Supplementary material for
“Structural flexibility of DNA-like conformers of canonical 2'-deoxyribonucleosides”

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Table S1. Relaxed force constants (kcal·mole^{-1}·rad^{-2}) for endocyclic torsions of pyrimidine nitrogenous bases in free state and incorporated into canonical 2'-deoxyribonucleosides

Base	Torsion					
	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
Cyt (free) [a]	22.0	32.9	32.8	**43.4**	25.7	**15.9**
dC (AI)	22.8	35.6	35.2	**44.6**	31.1	**18.4**
dC (BI)	22.2	34.1	34.4	**43.2**	25.5	**16.4**
dC (ZI)	19.5	32.1	30.6	**42.2**	26.8	**15.0**
Thy (free) [a]	**17.0**	17.2	30.6	**41.1**	23.3	19.6
dT (AI)	17.0	**16.8**	30.7	**40.8**	29.3	24.5
dT (BI)	**16.1**	16.7	30.8	**39.3**	28.5	23.0

[a]: values from [s1]; extreme values are marked with **bold**.

Table S2. Vibrational RMS deviations (deg.) of endocyclic torsions of pyrimidine nitrogenous bases in free state and incorporated into canonical 2'-deoxyribonucleosides

Base	Torsion					
	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
T = 0 K						
Cyt (free) [a]	7.6	7.7	7.4	7.2	8.2	8.3
dC (AI)	7.5	7.6	7.2	7.2	7.6	7.6
dC (BI)	7.6	7.6	7.2	7.3	7.8	7.7
dC (ZI)	7.7	7.7	7.3	7.2	7.7	7.7
Thy (free) [a]	8.3	8.3	6.9	7.5	8.6	7.9
dT (AI)	8.4	8.3	6.8	7.5	8.1	7.3
dT (BI)	8.5	8.4	6.7	7.4	8.0	7.3
T = 298.15 K						
Cyt (free) [a]	10.4	9.1	9.0	8.0	10.0	12.0
dC (AI)	10.3	8.8	8.8	8.0	9.2	11.2
dC (BI)	10.4	9.0	8.9	8.2	10.0	11.8
dC (ZI)	11.0	9.2	9.3	8.2	9.8	12.2
Thy (free) [a]	11.7	11.6	9.0	8.4	10.5	11.0
dT (AI)	11.7	11.7	9.0	8.4	9.5	9.9
dT (BI)	12.0	11.8	9.0	8.5	9.6	10.2

[a]: values from [s1]

Table S3. Relaxed force constants (kcal·mole^{-1}·rad^{-2}) for endocyclic torsions of purine nitrogenous bases in free state and incorporated into canonical 2'-deoxyribonucleosides

Base	Torsion										
	$N_9C_4C_5N_7$	$C_4C_5N_7C_8$	$C_5N_7C_8N_9$	$N_7C_8N_9C_4$	$C_8N_9C_4C_5$	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
Ade (free)[a]	106.8	**121.6**	111.6	**89.2**	104.6	**51.9**	51.9	**40.7**	43.7	47.4	44.6
dA (AI)	108.0	**117.8**	111.8	**96.9**	113.2	**52.3**	50.4	**40.2**	44.0	49.1	47.4
dA (BI)	103.6	**117.1**	114.8	**97.4**	109.9	49.9	**52.2**	**41.5**	42.9	47.2	44.5
Gua (free)[a]	105.5	**118.0**	110.8	**91.1**	104.7	**47.9**	40.0	32.9	35.4	**22.9**	23.5
dG (AI)	97.2	112.1	**114.1**	**96.9**	103.7	**46.7**	37.6	32.0	36.0	**23.2**	23.6
dG (BI)	109.4	111.1	108.8	**103.1**	**122.0**	**50.4**	38.6	31.6	35.8	**23.3**	24.3
dG (ZI)	105.7	**116.2**	113.9	**100.6**	115.1	**47.4**	39.9	33.5	35.0	**22.0**	22.6

[a]: values from [s1]; extreme values are marked with **bold**.

Table S4. Vibrational RMS deviations (deg.) of endocyclic torsions of purine nitrogenous bases in free state and incorporated into canonical 2'-deoxyribonucleosides

Base	Torsion										
	$N_9C_4C_5N_7$	$C_4C_5N_7C_8$	$C_5N_7C_8N_9$	$N_7C_8N_9C_4$	$C_8N_9C_4C_5$	$N_1C_2N_3C_4$	$C_2N_3C_4C_5$	$N_3C_4C_5C_6$	$C_4C_5C_6N_1$	$C_5C_6N_1C_2$	$C_6N_1C_2N_3$
T = 0 K											
Ade (free)[a]	5.2	5.1	5.3	5.6	5.0	7.1	6.9	7.3	6.6	6.9	7.7
dA (AI)	5.2	5.2	5.3	5.5	5.0	7.1	7.0	7.3	6.6	6.8	7.5
dA (BI)	5.2	5.1	5.2	5.4	4.9	7.2	7.0	7.2	6.6	6.8	7.6
Gua (free)[a]	5.2	5.2	5.3	5.6	5.1	6.9	7.5	7.8	6.5	7.7	8.4
dG (AI)	5.2	5.1	5.3	5.4	4.9	6.9	7.6	7.8	6.4	7.6	8.4
dG (BI)	5.1	5.2	5.3	5.4	4.8	6.8	7.5	7.7	6.4	7.6	8.3
dG (ZI)	5.2	5.2	5.3	5.4	4.9	6.9	7.4	7.6	6.5	7.7	8.5
T = 298.15 K											
Ade (free)[a]	5.5	5.4	5.5	6.0	5.5	7.8	7.7	8.4	8.0	7.8	8.4
dA (AI)	5.6	5.4	5.5	5.8	5.3	7.7	7.8	8.5	7.9	7.7	8.2
dA (BI)	5.6	5.4	5.5	5.8	5.4	7.9	7.7	8.4	8.0	7.8	8.4
Gua (free)[a]	5.6	5.4	5.6	6.0	5.5	7.8	8.5	9.2	8.4	10.2	10.5
dG (AI)	5.7	5.5	5.5	5.8	5.5	7.9	8.7	9.3	8.4	10.2	10.4
dG (BI)	5.5	5.5	5.6	5.7	5.2	7.7	8.6	9.3	8.4	10.1	10.3
dG (ZI)	5.6	5.4	5.5	5.7	5.3	7.8	8.5	9.1	8.5	10.4	10.6

[a]: values from [s1]

Table S5. Flexibility parameters for exocyclic torsions describing nitrogenous bases (in free state and incorporated into canonical 2'-deoxyribonucleosides) side atomic groups positions

Base	Atomic group	Torsion	Equilibrium value, deg.	Relaxed force constant, kcal·mole^{-1}·rad^{-2}	VRMS deviation, deg.	
					T = 0 K	T = 298.15 K
Thy (free)[a]	–CH_3	$C_4C_5C_7H_{72}$	180.0	5.80	12.5	19.3
		$C_6C_5C_7H_{72}$	0.0	5.33	13.5	20.4
dT (AI)		$C_4C_5C_7H_{72}$	-179.8	5.63	12.6	19.6
		$C_6C_5C_7H_{72}$	0.7	5.30	13.4	20.4
dT (BI)		$C_4C_5C_7H_{72}$	179.8	5.94	12.4	19.1
		$C_6C_5C_7H_{72}$	0.4	5.46	13.3	20.1
Cyt (free)[a]	–NH_2	$N_3C_4N_4H_{41}$	-8.0	6.50	15.3	19.3
		$C_5C_4N_4H_{41}$	173.0	7.88	14.0	17.5
		$N_3C_4N_4H_{42}$	-168.4	3.35	18.1	25.4
		$C_5C_4N_4H_{42}$	12.6	2.85	19.7	27.6
dC (AI)		$N_3C_4N_4H_{41}$	-9.9	8.64	14.4	17.2
		$C_5C_4N_4H_{41}$	171.5	10.0	13.4	15.9
		$N_3C_4N_4H_{42}$	-166.1	4.66	16.7	22.0
		$C_5C_4N_4H_{42}$	15.3	4.01	18.2	23.8
dC (BI)		$N_3C_4N_4H_{41}$	-9.6	7.86	14.8	17.9
		$C_5C_4N_4H_{41}$	171.6	9.43	13.5	16.3
		$N_3C_4N_4H_{42}$	-166.6	4.38	16.9	22.5
		$C_5C_4N_4H_{42}$	14.6	3.73	18.4	24.5
dC (ZI)		$N_3C_4N_4H_{41}$	-9.7	8.66	14.4	17.1
		$C_5C_4N_4H_{41}$	171.7	10.2	13.3	15.8
		$N_3C_4N_4H_{42}$	-166.3	4.56	16.8	22.2
		$C_5C_4N_4H_{42}$	15.1	3.90	18.3	24.0
Ade (free)[a]	–NH_2	$N_1C_6N_6H_{61}$	-8.6	3.42	18.0	25.4
		$C_5C_6N_6H_{61}$	172.3	4.14	16.4	23.0
		$N_1C_6N_6H_{62}$	-171.9	3.73	17.0	24.2
		$C_5C_6N_6H_{62}$	9.1	3.05	18.9	26.8
dA (AI)		$N_1C_6N_6H_{61}$	11.6	6.21	15.9	19.7
		$C_5C_6N_6H_{61}$	-169.6	7.27	14.6	18.1
		$N_1C_6N_6H_{62}$	169.0	7.01	14.8	18.4
		$C_5C_6N_6H_{62}$	-12.2	5.82	16.4	20.3
dA (BI)		$N_1C_6N_6H_{61}$	11.6	6.40	15.7	19.4
		$C_5C_6N_6H_{61}$	-169.6	7.35	14.6	18.0
		$N_1C_6N_6H_{62}$	169.0	6.91	14.9	18.5
		$C_5C_6N_6H_{62}$	-12.2	5.78	16.4	20.3
Gua (free)[a]	–NH_2	$N_1C_2N_2H_{21}$	-31.7	8.12	14.9	17.6
		$N_3C_2N_2H_{21}$	150.6	8.72	14.1	16.8
		$N_1C_2N_2H_{22}$	-169.4	15.8	11.9	13.3
		$N_3C_2N_2H_{22}$	13.0	15.6	12.5	13.7
dG (AI)		$N_1C_2N_2H_{21}$	-33.2	8.25	14.7	17.4
		$N_3C_2N_2H_{21}$	149.3	8.85	14.0	16.7
		$N_1C_2N_2H_{22}$	-169.1	15.6	11.9	13.3
		$N_3C_2N_2H_{22}$	13.4	15.2	12.5	13.8
dG (BI)		$N_1C_2N_2H_{21}$	-32.9	8.53	14.6	17.2
		$N_3C_2N_2H_{21}$	149.5	8.99	13.9	16.6
		$N_1C_2N_2H_{22}$	-168.9	15.8	11.8	13.2
		$N_3C_2N_2H_{22}$	13.5	15.4	12.5	13.7

dG (ZI)		$N_1C_2N_2H_{21}$	31.5	8.22	14.7	17.5
		$N_3C_2N_2H_{21}$	-150.9	8.89	13.9	16.6
		$N_1C_2N_2H_{22}$	167.9	14.3	12.1	13.7
		$N_3C_2N_2H_{22}$	-14.4	14.3	12.7	14.1

[a]: values from [s1]

References

s1. *T. Yu. Nikolaienko, L. A. Bulavin and D. M. Hovorun.* How Flexible are DNA Constituents? The Quantum-Mechanical Study // *Journal of Biomolecular Structure & Dynamics*, 2011, **29**, Iss. 3, P. 563 – 575.

Table S6. Relative contributions of different normal vibration modes into vibrational root-mean-square deviations of conformational parameters of the 2'-deoxycytidine B-DNA-like conformation [a,b]

Normal vibration unscaled harmonic frequency, cm^{-1}	Conformational parameter				
	β	γ	ε	P	χ
20	0.00	0.00	0.00	0.01	**0.56**
46	0.00	0.00	0.00	**0.08** [c]	0.00
54	0.00	0.00	0.00	0.03	0.05
96	0.00	0.02	0.02	0.06	0.03
121	0.00	**0.29**	0.00	0.03	0.04
146	0.00	**0.15**	0.00	0.02	0.01
174	0.01	0.00	0.00	0.00	0.03
209	0.00	0.00	0.00	0.01	0.01
235	**0.45**	0.09	0.00	0.04	0.00
247	0.00	0.00	0.00	0.00	0.00
257	**0.27**	0.04	0.01	0.01	0.00
259	**0.21**	0.00	0.04	**0.08** [c]	0.01
288	0.01	0.00	**0.86**	0.02	0.00
334	0.04	0.07	0.01	0.03	0.05
354	0.00	0.00	0.01	0.00	0.01
369	0.00	0.00	0.00	0.01	0.00
387	0.00	0.03	0.02	0.00	0.00
427	0.00	0.00	0.00	0.01	0.01
465	0.00	0.02	0.00	0.00	0.02
527	0.00	0.00	0.00	0.03	0.01
535	0.00	0.00	0.00	0.01	0.00
589	0.00	0.00	0.00	0.01	0.00
612	0.00	0.00	0.00	0.03	0.00
646	0.00	0.00	0.00	0.01	0.02
661	0.00	0.00	0.00	0.03	0.01
728	0.00	0.00	0.00	0.00	0.00
752	0.00	0.03	0.00	0.02	0.00
772	0.00	0.00	0.00	0.01	0.00
779	0.00	0.00	0.00	0.00	0.01
801	0.00	0.00	0.00	0.00	0.09
841	0.00	0.01	0.00	0.05	0.00
882	0.00	0.00	0.00	0.05	0.00
886	0.00	0.00	0.00	0.02	0.00
922	0.00	0.00	0.00	0.00	0.00
959	0.00	0.00	0.00	0.01	0.00
968	0.00	0.00	0.00	0.01	0.00
997	0.00	0.00	0.00	0.00	0.00
1000	0.00	0.00	0.00	0.00	0.00
1016	0.00	0.05	0.00	0.01	0.00
1066	0.00	0.02	0.00	0.01	0.00
1083	0.00	0.01	0.00	0.00	0.00
1095	0.00	0.00	0.00	0.01	0.00
1109	0.00	0.09	0.00	0.00	0.00
1117	0.00	0.00	0.00	0.02	0.00

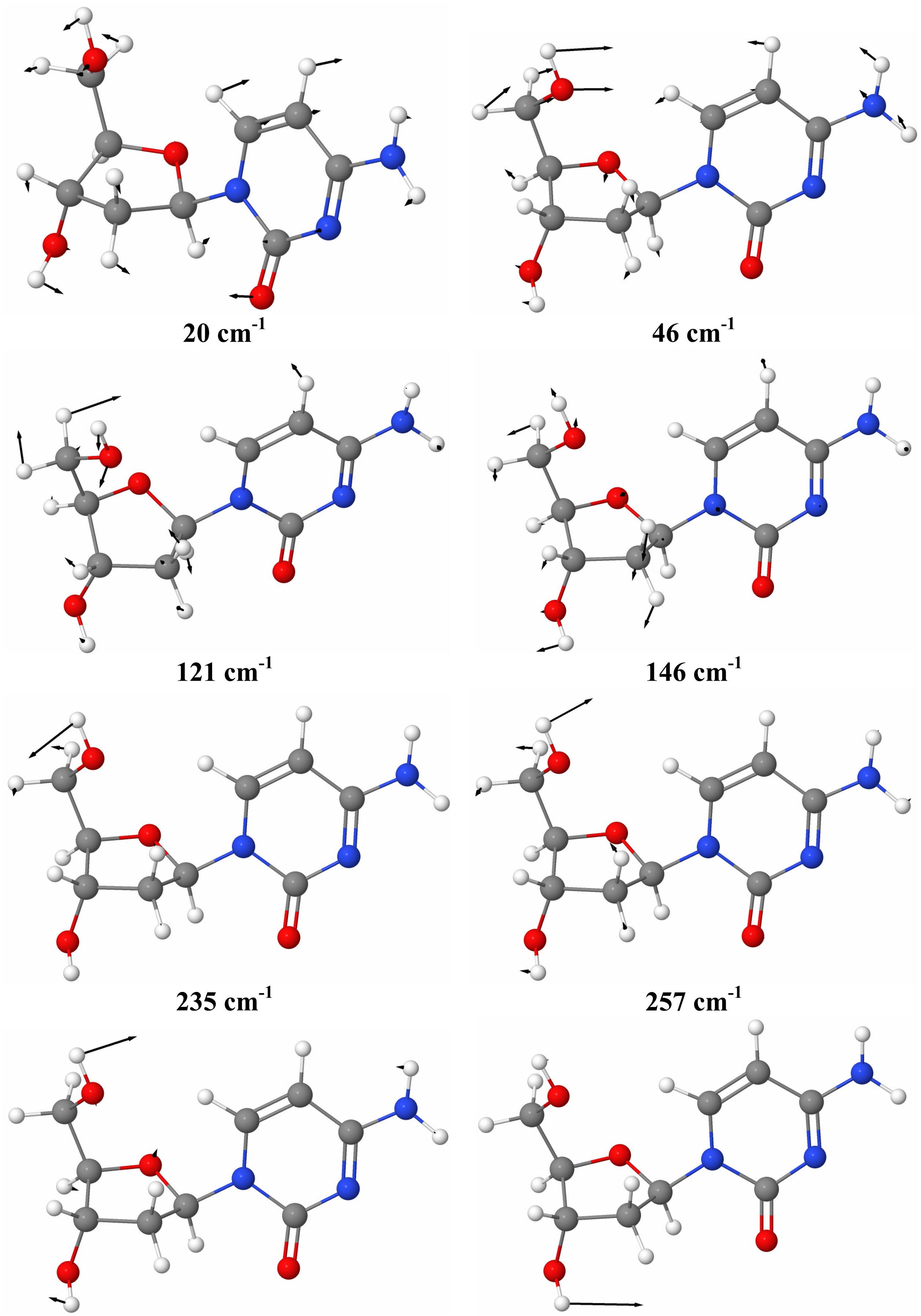
1127	0.00	0.00	0.00	0.01	0.00
1196	0.00	0.00	0.00	0.00	0.00
1211	0.00	0.00	0.00	0.01	0.00
1220	0.00	0.01	0.00	0.01	0.00
1228	0.00	0.00	0.00	0.02	0.00
1242	0.00	0.00	0.00	0.01	0.00
1267	0.00	0.00	0.00	0.01	0.00
1291	0.00	0.00	0.00	0.00	0.00
1310	0.00	0.00	0.00	0.01	0.00
1338	0.00	0.01	0.00	0.00	0.00
1345	0.00	0.00	0.00	0.01	0.00
1365	0.00	0.00	0.00	0.01	0.00
1378	0.00	0.01	0.00	0.02	0.00
1390	0.00	0.01	0.00	0.00	0.00
1424	0.00	0.00	0.00	0.02	0.00
1443	0.00	0.00	0.00	0.00	0.00
1447	0.00	0.00	0.00	0.01	0.00
1482	0.00	0.00	0.00	0.00	0.00
1508	0.00	0.00	0.00	0.00	0.00
1514	0.00	0.00	0.00	0.00	0.00
1560	0.00	0.00	0.00	0.00	0.00
1631	0.00	0.00	0.00	0.00	0.00
1683	0.00	0.00	0.00	0.00	0.00
1747	0.00	0.00	0.00	0.00	0.00
2992	0.00	0.00	0.00	0.00	0.00
3028	0.00	0.00	0.00	0.00	0.00
3033	0.00	0.00	0.00	0.00	0.00
3041	0.00	0.00	0.00	0.01	0.00
3065	0.00	0.00	0.00	0.00	0.00
3101	0.00	0.00	0.00	0.00	0.00
3118	0.00	0.00	0.00	0.00	0.00
3203	0.00	0.00	0.00	0.00	0.00
3217	0.00	0.00	0.00	0.00	0.00
3596	0.00	0.00	0.00	0.00	0.00
3726	0.00	0.00	0.00	0.00	0.00
3814	0.00	0.00	0.00	0.00	0.00
3837	0.00	0.00	0.00	0.00	0.00

Notes and definitions:

[a] The table contains the ratios $r_i = \frac{(\sigma^\tau{}_i)^2}{(\sigma^\tau)^2}$ where $\sigma^\tau{}_i = \frac{\hbar}{2\mu_i\omega_i}\cdot\left(c^\tau{}_i\right)^2$ is the *i*-th normal vibration contribution into the total vibrational mean-square $(\sigma^\tau)^2 = \sum_{i=1}^{M}(\sigma^\tau{}_i)^2$ deviation of the conformational parameter τ (at $T = 0\text{K}$); possible values of the ratios are $0 \le r_i \le 1$ with $r_i = 0$ meaning that *i*-th normal vibration doest not change the value of τ and $r_i = 1$ meaning that it is *i*-th normal vibration that is fully 'responsible' for vibrational 'noise' of the parameter τ.

[b] The values of r_i greater than 0.10 are underlined and marked with blue **bold**.

[c] Maximum values of r_i for pseudorotation phase *P* are marked with green **bold**.



$20\ \mathrm{cm^{-1}}$ $46\ \mathrm{cm^{-1}}$

$121\ \mathrm{cm^{-1}}$ $146\ \mathrm{cm^{-1}}$

$235\ \mathrm{cm^{-1}}$ $257\ \mathrm{cm^{-1}}$

$259\ \mathrm{cm^{-1}}$ $288\ \mathrm{cm^{-1}}$

Figure SF1. Displacement vectors for selected normal vibrations of the B-DNA-like conformation of 2'-deoxycytidine molecule

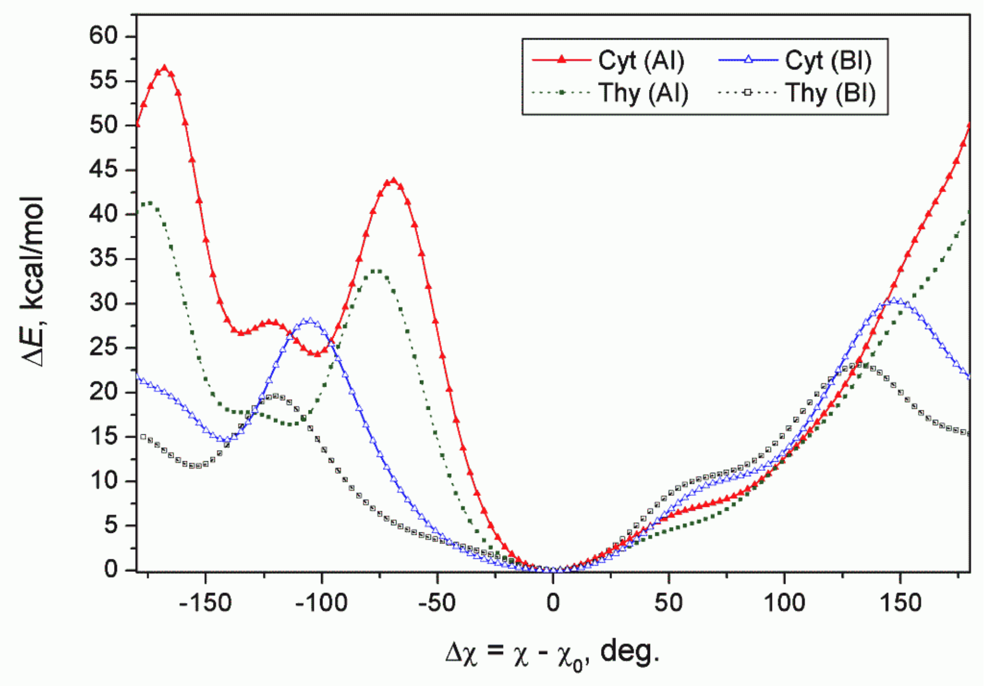


Figure SF2. 'Rigid' potential energy surface scan for A- and B-DNA-like conformations of 2'-deoxycytidine (dC) and thymidine (dT) obtained at the DFT B3LYP/6-31G(d,p) level of theory; $\Delta\chi$ stands for the deviation of the torsion angle χ from its equilibrium value reported in the paper.

Appendix A: Anharmonicity and the Relaxed Force Constants

In this section our aim is to show that the quadratic terms in the molecules electronic energy Taylor series expansion with respect to molecules normal coordinates are the only terms, which are essential for the Relaxed Force Constant (RFC) evaluation.

To show this consider the molecule's electronic energy expansion with the first anharmonic correction included

$$\Delta E = \sum_i \frac{k_i x_i^2}{2} + \varepsilon \sum_{i,j,l} \alpha_{ijl} x_i x_j x_l \, , \qquad (1)$$

where x_i are dimensionless normal coordinates of the molecule and k_i are corresponding dimensionless force constant, $\varepsilon \cdot \alpha_{ijl}$ are anharmonicity constants and/or normal vibrations coupling coefficients with ε being small parameter, i.e., we assume that $\alpha_{ijl} \sim k_i \sim 1$ while $|\varepsilon| << 1$; the summation over all indices goes from 1 to the total number of the molecules normal coordinates.

According to the general idea of the RFC evaluation algorithm as outlined in the paper, one has to minimize ΔE as the function of x_i under constraint $\sum_i C_i x_i = \Delta\tau$ where C_i are dimensionless permittivity coefficients for the structural parameter τ. The variational problem would be then

$$L = \Delta E - \lambda \cdot \left(\sum_i C_i x_i - \Delta\tau \right) = \min \, .$$

Forcing first derivatives of the Lagrange function L be zero, we obtain

$$0 = \frac{\partial L}{\partial x_n} = k_n x_n + \varepsilon \cdot \sum_{i,j,k} \alpha_{ijk} \left(\delta_{in} x_j x_k + x_i \delta_{jn} x_k + x_i x_j \delta_{kn} \right) - \lambda \cdot C_n$$

or, introducing $A_{nij} = \alpha_{nij} + \alpha_{inj} + \alpha_{ijn}$, after minor simplifications,

$$k_n x_n + \varepsilon \cdot \sum_{i,j} A_{nij} x_i x_j = \lambda \cdot C_n \, , \qquad (2)$$

and $0 = \dfrac{\partial L}{\partial \lambda} = \sum_i C_i x_i - \Delta\tau$ yielding

$$\sum_i C_i x_i = \Delta\tau \, . \qquad (3)$$

If there were no anharmonic terms in (1), i.e., when $\varepsilon = 0$, the solution of equations (2) and (3) would be (we denote this 'unperturbed' solution with the superscript $^{(0)}$)

$$x_i^{(0)} = \lambda \cdot C_i / k_i \, , \qquad (4)$$

$$\lambda^{(0)} = \frac{\Delta\tau}{\sum_i C_i^2 / k_i} \, . \qquad (5)$$

When ε is not zero, but is still small, we can use it as the small parameter to expand solution with, i.e., solve nonlinear equation (2) with the successive approximation method. To do it let us expand unknown quantities x_i and λ in series with respect to ε:

$$x_i = x_i^{(0)} + \varepsilon \cdot x_i^{(1)} + \varepsilon^2 \cdot x_i^{(2)} + \ldots , \qquad (6)$$

$$\lambda = \lambda^{(0)} + \varepsilon \cdot \lambda^{(1)} + \varepsilon^2 \cdot \lambda^{(2)} + \ldots . \qquad (7)$$

Surely, if such an expansion is substituted into (1), we find the molecules energy increment in the form of ε-expansion as well:

$$\Delta E = \Delta E^{(0)} + \varepsilon \cdot \Delta E^{(1)} + \varepsilon^2 \cdot \Delta E^{(2)} + \ldots ,$$

where, in particular,

$$\Delta E^{(0)} = \sum_n \frac{k_n \left(x_n^{(0)} \right)^2}{2} ,$$

$$\Delta E^{(1)} = \sum_n k_n x_n^{(0)} x_n^{(1)} + \sum_{i,j,l} \alpha_{ijl} x_i^{(0)} x_j^{(0)} x_l^{(0)} . \tag{8}$$

Since $x_i^{(0)}$ is proportional to the first power of $\Delta\tau$ (see Eqs. (4) and (5) as well as Eq. (10) below), $\Delta E^{(0)}$ is proportional to $(\Delta\tau)^2$ with the coefficient being half the RFC

$$K_\tau^{(0)} = \frac{1}{\sum_i C_i^2 / k_i} . \tag{9}$$

One may consider this expression as the 'zero-th order approximation' to the 'true' FRC

$$K_\tau \overset{def}{=} \left.\frac{\partial^2 \Delta E}{\partial(\Delta\tau)^2}\right|_{\Delta\tau=0} = \left.\frac{\partial^2 \Delta E^{(0)}}{\partial(\Delta\tau)^2}\right|_{\Delta\tau=0} + \varepsilon \cdot \left.\frac{\partial^2 \Delta E^{(1)}}{\partial(\Delta\tau)^2}\right|_{\Delta\tau=0} + \ldots = K_\tau^{(0)} + \varepsilon \cdot K_\tau^{(1)} + \ldots .$$

However, it can be easily shown that all 'higher order corrections' $K_\tau^{(1)}$, $K_\tau^{(2)}$, … are identically zero.

Indeed, this simply stems from the fact that $\Delta E^{(1)}$ is proportional to $(\Delta\tau)^3$, so its second derivative over $\Delta\tau$ goes to zero when evaluated at $\Delta\tau = 0$. Similarly, $\Delta E^{(2)}$ is proportional to $(\Delta\tau)^4$ and so forth. Let us prove this explicitly for the case of $\Delta E^{(1)}$.

According to (8), $\Delta E^{(1)}$ consists of two summands: $\sum_n k_n x_n^{(0)} x_n^{(1)}$ and $\sum_{i,j,l} \alpha_{ijl} x_i^{(0)} x_j^{(0)} x_l^{(0)}$, in which $x_n^{(0)}$ is proportional to $\Delta\tau$, so $\sum_{i,j,l} \alpha_{ijl} x_i^{(0)} x_j^{(0)} x_l^{(0)} \sim (\Delta\tau)^3$. Hence, to prove that $\Delta E^{(1)} \sim (\Delta\tau)^3$ it is sufficient to show that $x_n^{(1)} \sim (\Delta\tau)^2$.

To do it, let us just substitute expansions (6) and (7) into Eq. (2) and consider its terms at each power of ε independently:

$$\varepsilon^0: \qquad x_i^{(0)} = \lambda^{(0)} \cdot C_i / k_i , \tag{10}$$

which is exactly Eq. (4), written above. Next,

$$\varepsilon^1: \qquad k_n x_n^{(1)} + \sum_{i,j} A_{nij} x_i^{(0)} x_j^{(0)} = \lambda^{(1)} \cdot C_n . \tag{11}$$

Multiplying both sides of this equation on $\frac{C_n}{k_n}$ and summing over *n* we obtain

$$\sum_n C_n x_n^{(1)} + \sum_{i,j} \left(\sum_n \frac{C_n}{k_n} A_{nij} \right) x_i^{(0)} x_j^{(0)} = \lambda^{(1)} \cdot \sum_n \frac{C_n^{\ 2}}{k_n} .$$

But it follows form (3) that $\sum_n C_n x_n^{(1)}$ since right-hand side of Eq. (3) has no terms of the first order in ε. Hence,

$$\lambda^{(1)} = \frac{1}{\sum_n \frac{C_n^{\ 2}}{k_n}} \cdot \sum_{i,j} \left(\sum_n \frac{C_n}{k_n} A_{nij} \right) x_i^{(0)} x_j^{(0)} .$$

Here C_n and k_n do *not* depend on $\Delta\tau$, while $x_n^{(0)} \sim \Delta\tau$. Hence, $\lambda^{(1)}$ is proportional to $(\Delta\tau)^2$ and this implies by means of Eq. (11) that $x_n^{(1)} \sim (\Delta\tau)^2$ *which was to be proved.*

In the same way, considering the ε^2 terms of Eq. (2), one can find that $\lambda^{(2)} \sim (\Delta\tau)^3$, which implies that $x_n^{(2)} \sim (\Delta\tau)^3$ and

$$\Delta E^{(2)} = \sum_i \frac{k_i \left\{ (x_i^{(1)})^2 + 2 x_i^{(0)} \cdot x_i^{(2)} \right\}}{2} + \sum_{i,j,l} \alpha_{ijl} \left(x_i^{(0)} x_j^{(0)} x_l^{(1)} + x_i^{(1)} x_j^{(0)} x_l^{(0)} + x_i^{(1)} x_j^{(0)} x_l^{(0)} \right)$$

is proportional to $(\Delta\tau)^4$, so its second derivative w.r.t. $\Delta\tau$ is zero when evaluated at $\Delta\tau = 0$ and so on.

To summarize, we would like to stress that the RFC is, by its definition, the second-order property and just this fact makes it insensible to 3-rd, 4-th, … contributions to the energy. No anharmonic corrections should therefore be added to (9).