pH-Dependent Conformational Transient States Control Optical Properties in Cyan Fluorescent Protein

Elena N. Laricheva,[†] Garrett B. Goh,[†] Alex Dickson,[†] Charles L. Brooks III*[,†,‡]

[†]Department of Chemistry and [‡]Biophysics Program, University of Michigan, Ann Arbor, Michigan, 48109, United States

Supporting Information

S1. Calculating absorption properties of a synthetic chromophore: The calculations of the excitation energies and oscillator strength for the five lowest energy transitions in chromophore were performed using several scenarios, all providing the same qualitative picture: (i) gas-phase TDDFT on the gas-phase optimized geometries (denoted as "Gas-phase//gas-phase"; (ii) PCM TDDFT on the gas-phase optimized geometries ("PCM//gas-phase"); and (iii) PCM TDDFT on the PCM optimized geometries ("PCM//PCM").

Table S1. TDDFT//B3LYP/6-31G* vertical excitation energies and oscillator strength for the 5 lowest energy transitions in CRF

State	Transition	Gas-phase//gas-phase		PCM//gas-phase		PCM//PCM	
		Energy, eV	*f*	Energy, eV	*f*	Energy, eV	*f*
Neutral (proto-nated)	0→1	**3.31**	**0.60**	**3.17**	**0.70**	**3.15**	**0.71**
	0→2	3.74	<0.1	3.78	<0.1	3.77	<0.1
	0→3	3.86	<0.1	3.90	<0.1	3.85	<0.1
	0→4	4.05	<0.1	3.96	<0.1	3.95	<0.1
	0→5	4.24	<0.1	4.29	<0.1	4.27	<0.1
Charged	0→1	3.02	<0.1	**2.99**	**0.95**	**2.96**	**0.97**
	0→2	**3.10**	**0.87**	3.62	<0.1	3.62	<0.1
	0→3	3.57	<0.1	3.91	<0.1	3.91	<0.1
	0→4	3.67	<0.1	4.11	<0.1	4.11	<0.1
	0→5	3.74	<0.1	4.16	<0.1	4.16	<0.1

Those values that are highlighted refer to the strongest lowest energy transitions for both charged and neutral forms, with the values of bathochromic shift equal to 23-25 nm dependent on the level of theory used. All calculations were performed using Gaussian 03.[5] As noted in the paper, the computed vertical excitation energies for the lowest energy 0→1 transition in neutral form are in quantitative agreement with both experiment[6] and previous computational studies,[3,4] and,

thus, the origin of the underestimation in the bathochromic shift between the neutral and the charged forms originates from the uncertainties in the TDDFT-computed energies for anionic state.[7]

S2. Building input structures and calculating pK_a of a model compound:

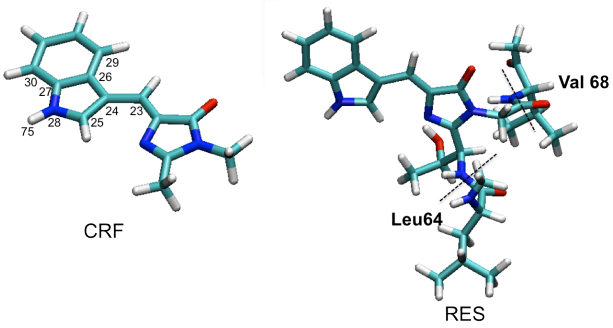

Figure S1. Structures of the CRF and RES chromophores. Numbers illustrate those atoms that constitute the titratable fragment. The rest of the atoms belong to the environment. Dashed lines show positions at which the chromophore is bound to the protein (at residues Leu64 and Val68).

Atoms included in the patch representing the charged (deprotonated) form of CRF were those that differed in charge between protonated and deprotonated forms of CRF, such as nitrogen of the indole ring (N28), all of its adjacent carbon atoms (C24, C25, C26, C27), two carbons from the six-membered ring (C29, C30), and a single carbon from the bridging CH_2– moiety (C23) connecting two heterocyclic rings. In order to distinguish the atoms of the patch, in the dual topology approach they were defined as N28m, C24m, C26m, C27m, N28m, C29m, and C30m.

S2a. Parameterizing the chromophore

CHARMM parameters for both CRF and RES were derived following the procedure previously employed for adenine and cytosine nucleobases, as reported in ref.[10]. In particular, partial charges for the neutral (protonated) and charged (deprotonated) forms were first generated using the MMFF94 force field.[15] To assign a CHARMM charge distribution for the charged form, the difference in the MMFF94 charges was then added to the partial charge parameters generated for the neutral form using the multipurpose atom typer MATCH.[16] The atoms differing in charge between the two forms, as well as the corresponding atom types, are shown in Figure S1 and Table S2. All bonded parameters (bond, angle, dihedral) for charged species were adapted from

those generated by MATCH for the neutral form. A complete list of parameters is provided at the end of the Supporting Information.

Table S2. Charges and atom types for neutral and charged states of titratable CRF and RES

Atom	Neutral		Charged	
	Atom type	Partial charge	Atom type	Partial charge
C23	C2D1	-0.0900	C2D1m	-0.1030
C24	C251	-0.1600	C251m	-0.1470
C25	C251	-0.0400	C251m	0.2616
C26	C2C0	0.0300	C2C0m	-0.0110
C27	C2C0	0.1500	C2C0m	0.4106
N28	N251	-0.5200	N251m	-1.7532
C29	C261	-0.1150	C261m	-0.0740
C30	C261	-0.1150	C261m	-0.0740
H75	HGP1	0.3700	—	—

S2b. Calculating the pK_a value of a model compound

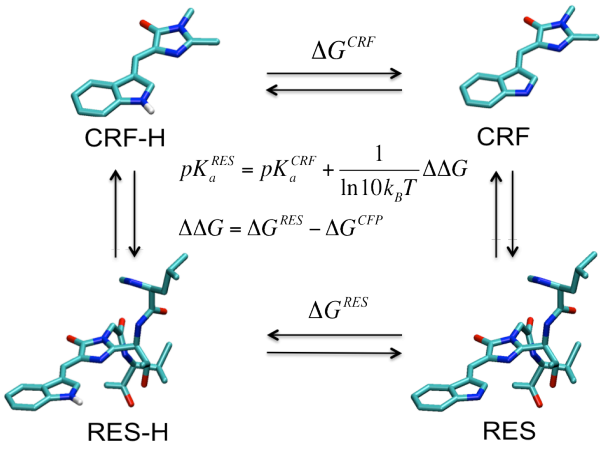

Figure S2. Thermodynamic cycle showing alchemical transformations of CRF to RES with two deprotonation reactions (CRF-H/CRF^- and RES-H/RES^-) considered in FEP/TI calculations.

S3. Details of the CPHMD$^{MS\lambda D}$ simulations

Table S3. Parameters for the model potential at pH=pK_a

Residue	F^{fixed} (kcal/mol)	F^{var} (kcal/mol)	Reference pK_a
RES	125.7	66.0	12.7

The transitions in λ-space are illustrated for RES in Figure S3:

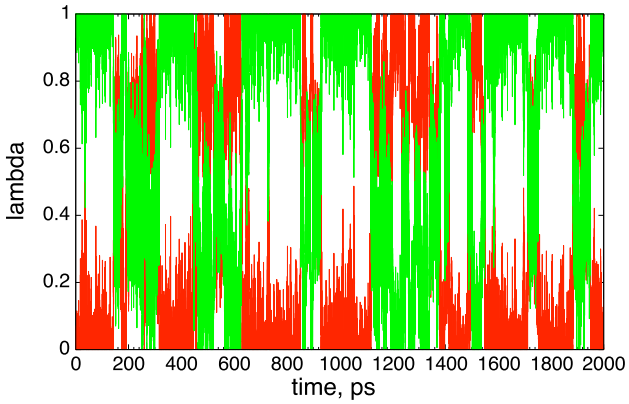

Figure S3. Transitions between the protonated and unprotonated states of RES during the 2 ns-long production run of the CPHMD$^{MS\lambda D}$ simulations with F^{fixed} =125.7 and F^{var} =66.0.

S4. Determining F^{open} from CPHMD^{MSLD} simulations

Two-state model

A simplified two-state model, considering an interconversion between one dominant open and one dominant closed state was recently applied to describe protonation equilibria in the buried Lys mutants of staphylococcal nuclease.[24] Below we provide the details on the derivation of the ratio of open to closed states (R_{oc}), which is defined as the total concentration of the former relative to the total concentration of the latter states.

Firstly, the equilibrium constant characterizing the deprotonation reaction for the open (K_{open}) and closed (K_{open}) states can be written as follows:

$$K_{open} = \frac{[\text{Open}][\text{H}^+]}{[\text{OpenH}]} \tag{1}$$

$$K_{closed} = \frac{[\text{Closed}][\text{H}^+]}{[\text{ClosedH}]} \tag{2}$$

Eq. (1) and (2) can than be rearranged into the following functional forms:

$$\frac{K_{open}}{[\text{H}^+]} + 1 = \frac{[\text{Open}] + [\text{OpenH}]}{[\text{OpenH}]} \tag{3}$$

$$\frac{K_{closed}}{[\text{H}^+]} + 1 = \frac{[\text{Closed}] + [\text{ClosedH}]}{[\text{ClosedH}]} \tag{4}$$

By dividing Eq. (3) by Eq. (4), we obtain:

$$\frac{[\text{Open}] + [\text{OpenH}]}{[\text{Closed}] + [\text{ClosedH}]} \frac{[\text{ClosedH}]}{[\text{OpenH}]} = \frac{\frac{K_{open}}{[\text{H}^+]} + 1}{\frac{K_{closed}}{[\text{H}^+]} + 1} \tag{5}$$

Using Eq. (5) we can now obtain the expression for R_{oc}:

$$\text{R}_{\text{oc}} = \frac{[\text{Open}] + [\text{OpenH}]}{[\text{Closed}] + [\text{ClosedH}]} = \frac{\frac{K_{open}}{[\text{H}^+]} + 1}{\frac{K_{closed}}{[\text{H}^+]} + 1} \frac{[\text{OpenH}]}{[\text{ClosedH}]} \tag{6}$$

The use of (pK_{open}) and closed (pK_{closed}) microscopic states will transform Eq. (6) into the following functional form representing the ratio of the open to closed states where both states are in the protonated form.:

$$\text{R}_{\text{oc}} = \frac{\frac{10^{-pK_{open}}}{10^{-pH}} + 1}{\frac{10^{-pK_{closed}}}{10^{-pH}} + 1} \frac{[\text{OpenH}]}{[\text{ClosedH}]} = \frac{10^{-pK_{open}} + 10^{-pH}}{10^{-pK_{closed}} + 10^{-pH}} \frac{[\text{OpenH}]}{[\text{ClosedH}]} \tag{7}$$

Here, the first term is pH-dependent (K^{pH} term) and the second term is pH-independent (K^0 term) and is related to the free energy difference between the two states:

$$K^0 = \frac{[\text{OpenH}]}{[\text{ClosedH}]} = \exp\left(-\frac{\Delta G}{k_B T}\right) \tag{8}$$

From previous studies,[24] the relationship between the microscopic pK_a of both closed and open states are the apparent (macroscopic) pK_a (pK_{app}) of the system can be obtained

$$pK_{app} = -\log\left(\frac{[\text{OpenH}]}{[\text{OpenH}]+[\text{ClosedH}]}K_{open} + \frac{[\text{ClosedH}]}{[\text{OpenH}]+[\text{ClosedH}]}K_{closed}\right) \tag{9}$$

A series of rearrangement will then lead to the ratio [OpenH]/[ClosedH]:

$$10^{-pK_{app}}([\text{OpenH}]+[\text{ClosedH}]) = [\text{OpenH}]K_{open} + [\text{ClosedH}]K_{closed} \tag{10}$$

$$[\text{OpenH}]\left(10^{-pK_{app}} - K_{open}\right) = [\text{ClosedH}]\left(K_{closed} - 10^{-pK_{app}}\right) \tag{11}$$

$$\frac{[\text{OpenH}]}{[\text{ClosedH}]} = \frac{\left(K_{closed} - 10^{-pK_{app}}\right)}{\left(10^{-pK_{app}} - K_{open}\right)} = -\frac{\left(10^{-pK_{closed}} - 10^{-pK_{app}}\right)}{\left(10^{-pK_{open}} - 10^{-pK_{app}}\right)} \tag{12}$$

Combining Eq. 7 and Eq. 12 we can now obtain the R_{OC} for the two-state model:

$$\mathrm{R_{OC}} = -\frac{\left(10^{-pK_{open}} + 10^{-pH}\right)}{\left(10^{-pK_{closed}} + 10^{-pH}\right)}\frac{\left(10^{-pK_{closed}} - 10^{-pK_{app}}\right)}{\left(10^{-pK_{open}} - 10^{-pK_{app}}\right)} \tag{13}$$

Estimation of apparent pK_a

Here, pK_{app} refers to the apparent (macroscopic) pK_a that we estimated to be 7.8— which is a pH value at which the fraction of the open state, determined from experiment (Table 2 of the manu-script), corresponds to 0.5 (considering pH range 6.1 to 8.1).

The values of R_{oc} at different pH can then be converted to the pH-dependent fraction of the open (deprotonated) state, considering the following expression:

$$F_{\text{open}} = \mathrm{R_{OC}}/(\mathrm{R_{OC}}+1) \qquad (2)$$

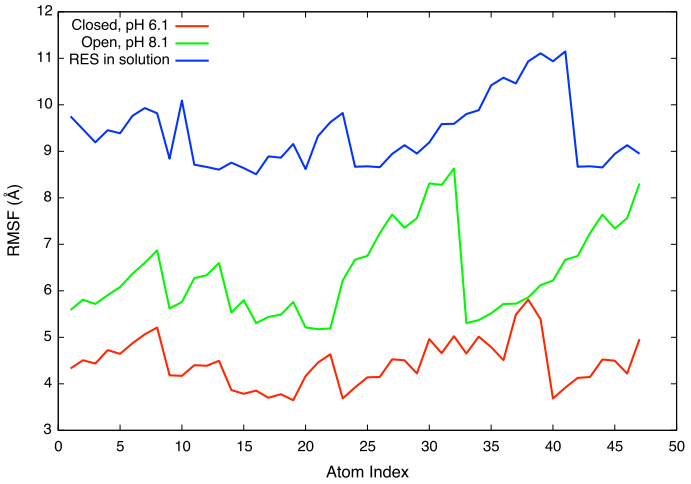

Figure S4. All-atom RMSF of the chromophore in the closed state (pH=6.1), open state (pH=8.1), and in solution shows its fluctuations broken down by atom.

REFERENCES

(1) Tomasi, J.; Mennucci, B.; Cammi, R. *Chem. Rev.* **2005**, *105*, 2999–3094.

(2) Nemukhin, A. V; Topol, I. A.; Burt, S. K. *J. Chem. Theory Comput.* **2005**, *2*, 292–299.

(3) Demachy, I.; Ridard, J.; Laguitton-Pasquier, H.; Durnerin, E.; Vallverdu, G.; Archirel, P.; Lévy, B. *J. Phys. Chem. B* **2005**, *109*, 24121–24133.

(4) Nifosí, R.; Amat, P.; Tozzini, V. *J. Comput. Chem.* **2007**, *28*, 2366–2377.

(5) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Pet, and J. A. P. Gaussian 03, Revision A.1, 2003.

(6) Sarkisyan, K. S.; Yampolsky, I. V; Solntsev, K. M.; Lukyanov, S. A.; Lukyanov, K. A.; Mishin, A. S. *Sci. Rep.* **2012**, *2*, 1–5.

(7) Wanko, M.; Hoffmann, M.; Strodel, P.; Koslowski, A.; Thiel, W.; Neese, F.; Frauenheim, T.; Elstner, M. *J. Phys. Chem. B* **2005**, *109*, 3606–3615.

(8) Marcus D Hanwell, Donald E Curtis, David C Lonie, Tim Vandermeersch, E. Z. and G. R. H. *J. Cheminform.* **2012**, *4*, 1–17.

(9) Goh, G. B.; Hulbert, B. S.; Zhou, H.; Brooks III, C. L.*Proteins: Struct., Funct., Bioinf.* **2014**, DOI: 10.1002/prot.24499.

(10) Goh, G. B.; Knight, J. L.; Brooks III, C. L.*J. Chem. Theory Comput.* **2011**, *8*, 36–46.

(11) Lelimousin, M.; Noirclerc-Savoye, M.; Lazareno-Saez, C.; Paetzold, B.; Le Vot, S.; Chazal, R.; Macheboeuf, P.; Field, M. J.; Bourgeois, D.; Royant, A. *Biochemistry* **2009**, *48*, 10038–10046.

(12) The PyMOL Molecular Graphics System, Version 1.2r3pre.

(13) Brooks, B. R.; Brooks, C. L.; Mackerell, A. D.; Nilsson, L.; Petrella, R. J.; Roux, B.; Won, Y.; Archontis, G.; Bartels, C.; Boresch, S.; Caflisch, A.; Caves, L.; Cui, Q.; Dinner, A. R.; Feig, M.; Fischer, S.; Gao, J.; Hodoscek, M.; Im, W.; Kuczera, K.; Lazaridis, T.; Ma, J.; Ovchinnikov, V.; Paci, E.; Pastor, R. W.; Post, C. B.; Pu, J. Z.; Schaefer, M.; Tidor, B.; Venable, R. M.; Woodcock, H. L.; Wu, X.; Yang, W.; York, D. M.; Karplus, M. *J. Comput. Chem.* **2009**, *30*, 1545–1614.

(14) Feig, M.; Karanicolas, J.; Brooks III, C. L. *J. Mol. Graph. Model.* **2004**, *22*, 377–395.

(15) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490–519.

(16) Yesselman, J. D.; Price, D. J.; Knight, J. L.; Brooks III, C. L.*J. Comput. Chem* **2012**, *33*, 189–202.

(17) Kong, X.; III, C. L. B. *J. Chem. Phys.* **1996**, *105*, 2414–2423.

(18) Van Gunsteren, W. F.; Berendsen, H. J. C. *Mol. Phys.* **1977**, *34*, 1311–1327.

(19) Knight, J. L.; Brooks III, C. L.*J. Chem. Theory Comput.* **2011**, *7*, 2728–2739.

(20) Huber, G. A.; Kim, S. *Biophys. J.* **1996**, *70*, 97–110.

(21) Dickson, A.; Warmflash, A.; Dinner, A. R. *J. Chem. Phys.* **2009**, *131*, -.

(22) Dickson, A.; Maienschein-Cline, M.; Tovo-Dwyer, A.; Hammond, J. R.; Dinner, A. R. *J. Chem. Theory Comput.* **2011**, *7*, 2710–2720.

(23) Dickson, A.; Brooks III, C. L. *J. Phys. Chem. B* **2014**, *118*, 3532–3542.

(24) Goh, G. B.; Laricheva, E. N.; Brooks III, C. L.*J. Am. Chem. Soc.* **2014**.

CHARMM Parameters of Model Compound

Geometry (xyz coordinates)

N	−2.915	2.737	−0.784
C	−3.420	1.569	−1.466
C3	−2.629	0.321	−1.128
O	−3.220	−0.748	−0.956
C5	−3.365	1.795	−2.950
C6	−4.289	2.876	−3.540
C7	−3.884	3.283	−4.925
C8	−5.762	2.424	−3.495
N9	−1.317	0.467	−1.016
C10	−0.417	−0.662	−0.609
C11	0.282	−1.179	−1.881
C12	0.911	−0.138	−2.803
O13	1.207	−2.197	−1.501
C14	0.455	−0.105	0.450
N15	1.669	0.409	0.282
N16	0.055	−0.075	1.755
C17	1.033	0.481	2.507
O18	0.991	0.661	3.751
C19	2.098	0.805	1.565
C20	−1.183	−0.541	2.275
C21	−1.413	−1.833	2.804
O22	−2.362	−2.050	3.572
C23	3.296	1.385	1.842
C24	4.296	1.791	0.775
C25	4.272	1.575	−0.535
C26	5.512	2.519	1.006
C27	6.119	2.674	−0.234
N28	5.340	2.080	−1.207
C29	6.101	3.035	2.179
C30	7.349	3.356	−0.349
C31	7.299	3.703	2.089
C32	7.903	3.855	0.845
N33	−0.579	−2.759	2.364
C34	−0.738	−4.170	2.723
C35	0.620	−4.696	3.183
O36	1.199	−5.625	2.629
C37	−1.386	−5.001	1.601
C38	−2.874	−4.595	1.435
C39	−0.645	−4.886	0.258
H	7.839	3.488	−1.302
H41	8.844	4.382	0.793
H42	7.767	4.106	2.975
H43	5.617	2.907	3.136
H44	3.497	1.050	−1.075
H45	3.554	1.570	2.895
H46	−0.470	−1.741	−2.473
H47	1.691	0.435	−2.257
H48	0.133	0.571	−3.158
H49	1.385	−0.641	−3.673
H50	−3.916	2.405	−5.606
H51	−2.848	3.685	−4.913
H52	−4.581	4.057	−5.311
H53	−4.262	3.770	−2.881
H54	−6.071	2.233	−2.445
H55	−5.888	1.487	−4.078

```
H56     -6.414   3.218  -3.918
H57     -3.618   0.834  -3.444
H58     -2.322   2.071  -3.207
H59     -4.479   1.412  -1.170
H60     -2.949  -3.517   1.175
H61     -3.422  -4.769   2.386
H62     -3.340  -5.190   0.620
H63     -1.322  -6.081   1.855
H64      0.402  -5.241   0.368
H65     -0.627  -3.826  -0.075
H66     -1.152  -5.510  -0.508
H67     -1.912  -0.434   1.449
H68     -1.443   0.156   3.095
C69      1.071  -4.250   3.997
H70     -1.480  -4.254   3.545
H71      0.307  -3.705   4.592
H72      1.840  -3.527   3.651
H73      1.540  -5.026   4.638
H74     -1.029  -1.465  -0.145
H75      5.519   2.029  -2.199
H76     -0.867   1.338  -1.198
H77     -2.603   3.527  -1.208
C78     -2.945   2.582   0.224
H79      0.192  -2.540   1.771
H80      1.985  -1.739  -1.176
H81     -2.074   1.967   0.534
H82     -3.878   2.044   0.494
H83     -2.903   3.556   0.756
```

Atom types and charges

```
GROUP
ATOM N    N311  -0.500000
ATOM C    C311  -0.020000
ATOM C3   C2O1   0.510000
ATOM O    O2D1  -0.510000
ATOM C5   C321  -0.180000
ATOM C6   C311  -0.090000
ATOM C7   C331  -0.270000
ATOM C8   C331  -0.270000
ATOM N9   N2S1  -0.310000
ATOM C78  C331  -0.200000
ATOM H50  HGA3   0.090000
ATOM H51  HGA3   0.090000
ATOM H52  HGA3   0.090000
ATOM H53  HGA1   0.090000
ATOM H54  HGA3   0.090000
ATOM H55  HGA3   0.090000
ATOM H56  HGA3   0.090000
ATOM H57  HGA2   0.090000
ATOM H58  HGA2   0.090000
ATOM H59  HGA1   0.090000
ATOM H76  HGP1   0.310000
ATOM H77  HPA1   0.360000
ATOM H81  HGA3   0.090000
ATOM H82  HGA3   0.090000
ATOM H83  HGA3   0.090000
GROUP
ATOM C10  C311  -0.090000
```

```
ATOM C11  C311   0.140000
ATOM C12  C331  -0.270000
ATOM O13  O311  -0.650000
ATOM H46  HGA1   0.090000
ATOM H47  HGA3   0.090000
ATOM H48  HGA3   0.090000
ATOM H49  HGA3   0.090000
ATOM H74  HGA1   0.090000
ATOM H80  HGP1   0.420000
GROUP
ATOM C14  C253   0.460000
ATOM N15  N250  -0.680000
ATOM N16  N251  -0.200000
ATOM C17  C253   0.620000
ATOM O18  O2D1  -0.490000
ATOM C19  C251   0.260000
ATOM C20  C321  -0.180000
ATOM C21  C2O1   0.510000
ATOM O22  O2D1  -0.510000
ATOM H67  HGA2   0.090000
ATOM H68  HGA2   0.090000
GROUP
ATOM C23  C2D1  -0.090000
ATOM C24  C251  -0.160000
ATOM C25  C251  -0.040000
ATOM C26  C2C0   0.030000
ATOM C27  C2C0   0.150000
ATOM N28  N251  -0.520000
ATOM C29  C261  -0.115000
ATOM C30  C261  -0.115000
ATOM C31  C261  -0.115000
ATOM C32  C261  -0.115000
ATOM H    HG61   0.115000
ATOM H41  HG61   0.115000
ATOM H42  HG61   0.115000
ATOM H43  HG61   0.115000
ATOM H44  HG52   0.140000
ATOM H45  HGA4   0.150000
ATOM H75  HGP1   0.370000
GROUP
ATOM N33  N2S1  -0.310000
ATOM C34  C311   0.010000
ATOM C35  C2O5   0.350000
ATOM O36  O2D1  -0.490000
ATOM C37  C311  -0.090000
ATOM C38  C331  -0.270000
ATOM C39  C331  -0.270000
ATOM H60  HGA3   0.090000
ATOM H61  HGA3   0.090000
ATOM H62  HGA3   0.090000
ATOM H63  HGA1   0.090000
ATOM H64  HGA3   0.090000
ATOM H65  HGA3   0.090000
ATOM H66  HGA3   0.090000
ATOM C69  C331  -0.230000
ATOM H70  HGA1   0.090000
ATOM H71  HGA3   0.090000
ATOM H72  HGA3   0.090000
ATOM H73  HGA3   0.090000
ATOM H79  HGP1   0.310000
```

List of bonded parameters

C311	N311	263.00	1.4740
HPA1	N311	447.80	1.0190
C331	N311	255.00	1.4630
C2O1	C311	250.00	1.4900
C311	C321	222.50	1.5380
C311	HGA1	309.00	1.1110
C2O1	O2D1	620.00	1.2300
C2O1	N2S1	370.00	1.3450
C321	HGA2	309.00	1.1110
C311	C331	222.50	1.5380
C331	HGA3	322.00	1.1110
C311	N2S1	320.00	1.4300
HGP1	N2S1	440.00	0.9970
C311	C311	222.50	1.5000
C253	C311	250.00	1.4900
C311	O311	428.00	1.4200
HGP1	O311	545.00	0.9600
C253	N250	400.00	1.3200
C253	N251	320.00	1.3740
C251	N250	400.00	1.3800
C321	N251	400.00	1.4580
C253	O2D1	570.00	1.2350
C251	C253	340.00	1.4050
C2D1	C251	340.00	1.4050
C2O1	C321	250.00	1.4900
C2D1	HGA4	360.50	1.1000
C251	C251	410.00	1.3600
C251	C2C0	350.00	1.4300
C251	N251	400.00	1.3800
C251	HG52	375.00	1.0830
C2C0	C2C0	360.00	1.3850
C261	C2C0	300.00	1.3600
C2C0	N251	300.00	1.3750
HGP1	N251	474.00	1.0100
C261	C261	305.00	1.3750
C261	HG61	340.00	1.0800
C2O5	C311	200.00	1.5220
C2O5	O2D1	570.00	1.2350
C2O5	C331	220.00	1.5150

List of angle parameters

2O1	C311	N311	50.00	107.00
C321	C311	N311	67.70	107.50
HGA1	C311	N311	32.40	109.50
C311	N311	HPA1	45.00	104.00
C311	N311	C331	58.35	113.50
HPA1	N311	C331	45.00	104.00
HGA3	C331	N311	30.50	109.70
O2D1	C2O1	C311	80.00	121.00
N2S1	C2O1	C311	80.00	116.50
C2O1	C311	C321	52.00	108.00
C2O1	C311	HGA1	50.00	109.50
HGA1	C311	C321	34.50	110.10
C311	C321	C311	58.35	113.50
C311	C321	HGA2	33.43	110.10
N2S1	C2O1	O2D1	80.00	122.50

```
C2O1   N2S1   C311   50.00      120.00
C2O1   N2S1   HGP1   34.00      123.00
C331   C311   C321   53.35      114.00
HGA2   C321   HGA2   35.50      109.00
C331   C311   C331   53.35      114.00
HGA1   C311   C331   34.50      110.10
C311   C331   HGA3   33.43      110.10
HGA3   C331   HGA3   35.50      108.40
C311   C311   N2S1   70.00      113.50
C253   C311   N2S1   50.00      107.00
HGA1   C311   N2S1   48.00      108.00
C311   N2S1   HGP1   35.00      117.00
C253   C311   C311   52.00      108.00
HGA1   C311   C311   34.50      110.10
C311   C311   C331   53.35      108.50
C311   C311   O311   75.70      110.10
N250   C253   C311   100.00     113.00
N251   C253   C311   20.00      112.50
C253   C311   HGA1   50.00      109.50
O311   C311   C331   75.70      110.10
HGA1   C311   O311   45.90      108.89
C311   O311   HGP1   50.00      106.00
N251   C253   N250   100.00     113.00
C253   N250   C251   130.00     103.50
C253   N251   C253   130.00     107.50
C253   N251   C321   70.00      126.90
C253   C251   N250   130.00     110.00
C2D1   C251   N250   130.00     110.00
O2D1   C253   N251   65.00      127.80
C251   C253   N251   130.00     106.00
C2O1   C321   N251   50.00      107.00
HGA2   C321   N251   33.43      110.10
C251   C253   O2D1   30.00      121.00
C2D1   C251   C253   48.00      123.50
C251   C2D1   C251   90.00      107.20
HGA4   C2D1   C251   52.00      119.50
O2D1   C2O1   C321   80.00      121.00
N2S1   C2O1   C321   80.00      116.50
C2O1   C321   HGA2   33.00      109.50
C2D1   C251   C251   70.00      106.90
C2D1   C251   C2C0   85.00      105.70
C2C0   C251   C251   85.00      105.70
C251   C251   N251   130.00     106.00
C251   C251   HG52   22.00      130.00
C251   C2C0   C2C0   85.00      108.00
C251   C2C0   C261   130.00     132.00
HG52   C251   N251   25.00      124.00
C251   N251   C2C0   85.00      110.00
C251   N251   HGP1   30.00      125.50
C261   C2C0   C2C0   50.00      120.00
C2C0   C2C0   N251   100.00     105.70
C261   C261   C2C0   50.00      120.00
HG61   C261   C2C0   30.00      120.00
C261   C2C0   N251   130.00     132.60
C2C0   N251   HGP1   28.00      126.00
HG61   C261   C261   30.00      120.00
C261   C261   C261   40.00      120.00
C2O5   C311   N2S1   50.00      107.00
O2D1   C2O5   C311   80.00      121.00
C331   C2O5   C311   30.00      114.00
```

```
C2O5   C311   C311   52.00      108.00
C2O5   C311   HGA1   50.00      109.50
C331   C2O5   O2D1   80.00      121.00
C2O5   C331   HGA3   33.00      109.50
```

List of dihedral parameters

```
O2D1   C2O1   C311   N311   0.0000     1      0.00
N2S1   C2O1   C311   N311   0.6000     1      0.00
C2O1   C311   N311   HPA1   0.0000     1      0.00
C2O1   C311   N311   C331   0.2000     3      0.00
C311   C321   C311   N311   0.3000     3      180.00
HGA2   C321   C311   N311   0.1500     3      180.00
C321   C311   N311   HPA1   0.0000     3      0.00
C321   C311   N311   C331   0.0500     3      0.00
HGA1   C311   N311   HPA1   0.0000     3      0.00
HGA1   C311   N311   C331   0.0500     3      0.00
C311   N311   C331   HGA3   0.1500     3      180.00
HPA1   N311   C331   HGA3   0.4200     3      0.00
O2D1   C2O1   C311   C321   1.4000     1      0.00
O2D1   C2O1   C311   HGA1   0.0000     1      0.00
C311   N2S1   C2O1   C311   2.5000     2      180.00
C311   N2S1   C2O1   C311   1.6000     1      0.00
HGP1   N2S1   C2O1   C311   2.5000     2      180.00
N2S1   C2O1   C311   C321   0.0000     1      0.00
N2S1   C2O1   C311   HGA1   0.0000     1      0.00
C2O1   C311   C321   C311   0.2000     3      0.00
C2O1   C311   C321   HGA2   0.2000     3      0.00
HGA1   C311   C321   C311   0.1950     3      0.00
HGA1   C311   C321   HGA2   0.1950     3      0.00
C311   C321   C311   C331   0.2000     3      0.00
C311   N2S1   C2O1   O2D1   2.5000     2      180.00
HGP1   N2S1   C2O1   O2D1   2.5000     2      180.00
C2O1   N2S1   C311   C311   1.8000     1      0.00
C2O1   N2S1   C311   C253   0.2000     1      180.00
C2O1   N2S1   C311   HGA1   0.0000     1      0.00
HGA3   C331   C311   C321   0.2000     3      0.00
C331   C311   C321   HGA2   0.2000     3      0.00
HGA3   C331   C311   C331   0.1950     3      0.00
HGA1   C311   C331   HGA3   0.1950     3      0.00
C331   C311   C311   N2S1   0.2000     3      0.00
O311   C311   C311   N2S1   0.2000     3      0.00
HGA1   C311   C311   N2S1   0.2000     3      0.00
C311   C311   N2S1   HGP1   0.0000     1      0.00
N250   C253   C311   N2S1   0.4000     1      0.00
N251   C253   C311   N2S1   0.4000     1      0.00
C253   C311   N2S1   HGP1   0.0000     1      0.00
HGA1   C311   N2S1   HGP1   0.0000     1      0.00
N250   C253   C311   C311   11.0000    2      180.00
N251   C253   C311   C311   0.0000     1      0.00
C253   C311   C311   C331   0.2000     3      0.00
C253   C311   C311   O311   0.2000     3      0.00
C253   C311   C311   HGA1   0.2000     3      0.00
HGA1   C311   C311   C331   0.1950     3      0.00
HGA1   C311   C311   O311   0.1950     3      0.00
HGA1   C311   C311   HGA1   0.1950     3      0.00
C311   C311   C331   HGA3   0.2000     3      0.00
C311   C311   O311   HGP1   0.1800     2      0.00
C311   C311   O311   HGP1   0.3200     3      0.00
C311   C311   O311   HGP1   1.3300     1      0.00
```

```
C251   N250   C253   C311   14.0000    2      180.00
N250   C253   C311   HGA1   0.0000     1      0.00
C253   N251   C253   C311   0.0000     3      180.00
C321   N251   C253   C311   2.5000     2      180.00
C321   N251   C253   C311   1.6000     1      0.00
N251   C253   C311   HGA1   0.0000     1      0.00
HGP1   O311   C311   C331   0.1800     2      0.00
HGP1   O311   C311   C331   0.3200     3      0.00
HGP1   O311   C311   C331   1.3300     1      0.00
O311   C311   C331   HGA3   0.2000     3      0.00
HGA1   C311   O311   HGP1   0.0000     3      0.00
C253   N251   C253   N250   14.0000    2      180.00
C321   N251   C253   N250   11.0000    2      180.00
N251   C253   N250   C251   14.0000    2      180.00
C253   N250   C251   C253   14.0000    2      180.00
C253   N250   C251   C2D1   14.0000    2      180.00
C253   N251   C253   O2D1   2.5900     2      180.00
C253   N251   C253   C251   14.0000    2      180.00
C253   N251   C321   C2O1   0.2000     1      180.00
C253   N251   C321   HGA2   0.0000     3      0.00
N251   C253   C251   N250   14.0000    2      180.00
O2D1   C253   C251   N250   0.0000     2      180.00
C251   C2D1   C251   N250   8.5000     2      180.00
HGA4   C2D1   C251   N250   2.7000     2      180.00
O2D1   C253   N251   C321   2.5900     2      180.00
C2D1   C251   C253   N251   4.0000     2      180.00
C251   C253   N251   C321   11.0000    2      180.00
O2D1   C2O1   C321   N251   0.0000     1      0.00
N2S1   C2O1   C321   N251   0.6000     1      0.00
C2D1   C251   C253   O2D1   1.0000     2      180.00
C251   C2D1   C251   C253   9.0000     2      180.00
HGA4   C2D1   C251   C253   1.0000     2      180.00
C251   C251   C2D1   C251   15.0000    2      180.00
C2C0   C251   C2D1   C251   2.0000     2      180.00
O2D1   C2O1   C321   HGA2   0.0000     3      180.00
C311   N2S1   C2O1   C321   2.5000     2      180.00
C311   N2S1   C2O1   C321   1.6000     1      0.00
HGP1   N2S1   C2O1   C321   2.5000     2      180.00
N2S1   C2O1   C321   HGA2   0.0000     3      0.00
C2O1   N2S1   C311   C2O5   0.2000     1      180.00
HGA4   C2D1   C251   C251   1.0000     2      180.00
HGA4   C2D1   C251   C2C0   1.0000     2      180.00
C2D1   C251   C251   N251   4.0000     2      180.00
C2D1   C251   C251   HG52   1.5000     2      180.00
C2D1   C251   C2C0   C2C0   4.0000     2      180.00
C2D1   C251   C2C0   C261   3.0000     2      180.00
C2C0   C2C0   C251   C251   4.0000     2      180.00
C261   C2C0   C251   C251   3.0000     2      180.00
C2C0   C251   C251   N251   4.0000     2      180.00
C2C0   C251   C251   HG52   2.8000     2      180.00
C251   C251   N251   C2C0   5.0000     2      180.00
C251   C251   N251   HGP1   1.0000     2      180.00
C251   C2C0   C2C0   N251   6.5000     2      180.00
C251   C2C0   C2C0   C261   1.5000     2      180.00
C251   C2C0   C261   C261   4.0000     2      180.00
C251   C2C0   C261   HG61   4.0000     2      180.00
HG52   C251   N251   C2C0   2.6000     2      180.00
HG52   C251   N251   HGP1   1.0000     2      180.00
C251   N251   C2C0   C2C0   1.5000     2      180.00
C251   N251   C2C0   C261   1.5000     2      180.00
```

```
C261   C261   C2C0   C2C0   3.0000     2      180.00
HG61   C261   C2C0   C2C0   3.0000     2      180.00
C261   C2C0   C2C0   N251   1.5000     2      180.00
C261   C2C0   C2C0   C261   3.0000     2      180.00
2C0   C2C0   N251   HGP1   0.8500      2      180.00
C261   C261   C261   C2C0   3.0000     2      180.00
HG61   C261   C261   C2C0   3.0000     2      180.00
C261   C261   C2C0   N251   3.0000     2      180.00
HG61   C261   C2C0   N251   3.0000     2      180.00
C261   C2C0   N251   HGP1   0.2000     2      180.00
HG61   C261   C261   C261   4.2000     2      180.00
HG61   C261   C261   HG61   2.4000     2      180.00
C261   C261   C261   C261   3.1000     2      180.00
O2D1   C2O5   C311   N2S1   0.0000     1      0.00
C331   C2O5   C311   N2S1   0.2000     3      0.00
C2O5   C311   N2S1   HGP1   0.0000     1      0.00
O2D1   C2O5   C311   C311   1.4000     1      0.00
O2D1   C2O5   C311   HGA1   0.0000     1      0.00
HGA3   C331   C2O5   C311   0.0300     3      0.00
C331   C2O5   C311   C311   0.1580     3      0.00
C331   C2O5   C311   HGA1   0.0000     1      180.00
C2O5   C311   C311   C331   0.2000     3      0.00
C2O5   C311   C311   HGA1   0.2000     3      0.00
HGA3   C331   C2O5   O2D1   0.0000     3      180.00
C331   C2O5   C311   C311   0.1580     3      0.00
C331   C2O5   C311   HGA1   0.0000     1      180.00
C2O5   C311   C311   C331   0.2000     3      0.00
C2O5   C311   C311   HGA1   0.2000     3      0.00
HGA3   C331   C2O5   O2D1   0.0000     3      180.00
```

List of improper dihedral parameters

```
C2O1   X      X      O2D1   120.0000   0      0.00
N2S1   X      X      HGP1   20.0000    0      0.00
C253   N250   N251   C311   96.0000    0      0.00
C2O1   C321   N2S1   O2D1   120.0000   0      0.00
C2O5   C311   C331   O2D1   90.0000    0      0.00
```

List of non-bonded parameters

```
C311   0.0000     -0.0320    2.0000
N311   0.0000     -0.4500    2.0000
HPA1   0.0000     -0.0090    0.8750
C331   0.0000     -0.0780    2.0500
C2O1   0.0000     -0.1100    2.0000
C321   0.0000     -0.0560    2.0100
HGA1   0.0000     -0.0450    1.3400
O2D1   0.0000     -0.1200    1.7000
N2S1   0.0000     -0.2000    1.8500
HGA2   0.0000     -0.0350    1.3400
HGA3   0.0000     -0.0240    1.3400
HGP1   0.0000     -0.0460    0.2245
C253   0.0000     -0.0500    1.8000
O311   0.0000     -0.1921    1.7650
N250   0.0000     -0.2000    1.8500
N251   0.0000     -0.2000    1.8500
C251   0.0000     -0.0730    1.9900
C2D1   0.0000     -0.0680    2.0900
HGA4   0.0000     -0.0310    1.2500
C2C0   0.0000     -0.0990    1.8600
```

```
HG52   0.0000     -0.0460    0.9000
C261   0.0000     -0.0700    1.9924
HG61   0.0000     -0.0300    1.3582
C2O5   0.0000     -0.2300    1.7000
```