Electronic Supplementary Information

Protonation state of $F_{420}H_2$ in the prodrug-activating deazaflavin dependent nitroreductase (Ddn) from *Mycobacterium tuberculosis*

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Methods

Experimental Materials and Methods

Purification and reduction of F_{420}. F_{420} was obtained from *M. smegmatis* 4517 cultures grown following the procedure in Bashiri et al.,[1] followed by extraction and purification as per the methods of Isabelle et al.[2] The enzymatic reduction of F_{420} to $F_{420}H_2$ was carried out using F_{420}-dependent glucose-6-phosphate dehydrogenase (Fgd) purified as previously published.[3] . Reactions were incubated for 15-30 min (until colourless) and typically contained 100-200 µM F_{420}, 2.5 mM glucose-6-phosphate (G6P) (Sigma) and 1 µM Fgd in 20 mM Tris pH 7.5. Fgd was then removed by centrifugation in a 1 mL 10 K MWCO spin filter (Millipore) and the $F_{420}H_2$ eluted was used immediately to minimise air oxidation.

Purification of Ddn and MSMEG_2027. MBP-tagged Ddn was expressed and purified similar to previous methods by Singh et al.[4] Briefly, the codon optimised gene for Ddn (Invitrogen) was cloned into the expression vector pMALc2X using the EcoRI and PstI restriction sites (Fermentas). The construct was transformed into the *Escherichia coli* strain BL21DE3 (Invitrogen) and a 5 mL starter culture was grown over night at 30 °C in LB media containing 100 µg/mL ampicillin (Astral) and 1% glucose. This was transferred to 1 L of LB media containing 100 µg/mL ampicillin that was initially grown at 37 °C until the optical density at 600 nm reached about 0.5. The culture was then induced with 0.4 mM IPTG (Astral), after which it was further incubated at room temperature for an additional 3 hr. Cells were harvested by centrifugation at 5000 × g for 15 min at 4 °C and resuspended in 30 mL of lysis buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4). The cell suspension was lysed by sonication using an Omni Sonicator Ruptor 400 (3 × 3 min at 60% power) and the soluble fraction was separated by further centrifugation at 13000 × g for 1 hr at 4 °C. The lysate was passed through 1.5 mL amylose resin (New England Biolabs) in a gravity column and washed with a further 30 mL of lysis buffer. Finally the MBP-tagged Ddn was eluted with elution buffer (20 mM Tris, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM maltose, 10% glycerol, pH 7.4), flash frozen and stored at -20 °C.

MSMEG_2027 was expressed and purified exactly as described in Ahmed et al [ref], where the His-tag used for the purification of the full-length protein by nickel affinity chromatography was removed, and the protein was stored in 50 mM Tris, 150 mM NaCl, pH 7.5 at 4 °C.

UV-visible spectroscopy. The UV-visible spectra of the samples were obtained using a Molecular Devices SpectraMax M2 Multi-mode Microplate reader and an Agilent Cary 60 UV-vis spectrophotometer. Initially, aliquots of 20 mM Tris were adjusted to the desired pH. After the addition of F_{420}, $F_{420}H_2$ or enzyme to the buffer, the pH of the solutions were checked again and adjusted if required before obtaining the final spectra. The enzymes were added in 2 × excess compared to the cofactor to maximise the presence of enzyme associated $F_{420}H_2$. Experiments were repeated to ensure reproducibility.

Computational Methods

For computational purposes a truncated version of the cofactor was used where the R group as seen in **Fig. 1** have been substituted for a methyl group with the chemically active chromophore kept intact. Simulated UV-Vis spectra were calculated using time-dependent density functional theory (TD-DFT) utilising the dispersion-corrected density functional, ωB97XD, with the 6-311+g(2d,p) basis set.[5] Solvation effects in both water and the low polarity solvent toluene were taken into account using the density based solvation model (SMD).[6] The calculations conducted in water allowed for a comparison with the aqueous experimental spectra meanwhile the low polarity solvent toluene provides a more accurate comparison with that of the enzyme active site environment. For this latter calculation the dielectric constant was adjusted to $\varepsilon = 12$ so as to better mimic the polarity within the enzyme active site. All calculations were performed using the GAUSSIAN09 suite of programs.[7]

References

1.	G. Bashiri, A. M. Rehan, D. R. Greenwood, J. M. Dickson and E. N. Baker, *PloS one*, 2010, **5**, e15803.
2.	D. Isabelle, D. R. Simpson and L. Daniels, *Appl. Environ. Microbiol.*, 2002, **68**, 5750-5755.
3.	G. Bashiri, C. J. Squire, N. J. Moreland and E. N. Baker, *J. Biol. Chem.*, 2008, **283**, 17531-17541.
4.	R. Singh, U. Manjunatha, H. I. Boshoff, Y. H. Ha, P. Niyomrattanakit, R. Ledwidge, C. S. Dowd, I. Y. Lee, P. Kim, L. Zhang, S. Kang, T. H. Keller, J. Jiricek and C. E. Barry, 3rd, *Science*, 2008, **322**, 1392-1395.
5.	J.-D. Chai and M. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 6615-6620.
6.	A. V. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378-6396.
7.	M. J. Frisch, *et al.*, *GAUSSIAN 09, Rev D.01*, Gaussian Inc., Wallingford CT, 2009.
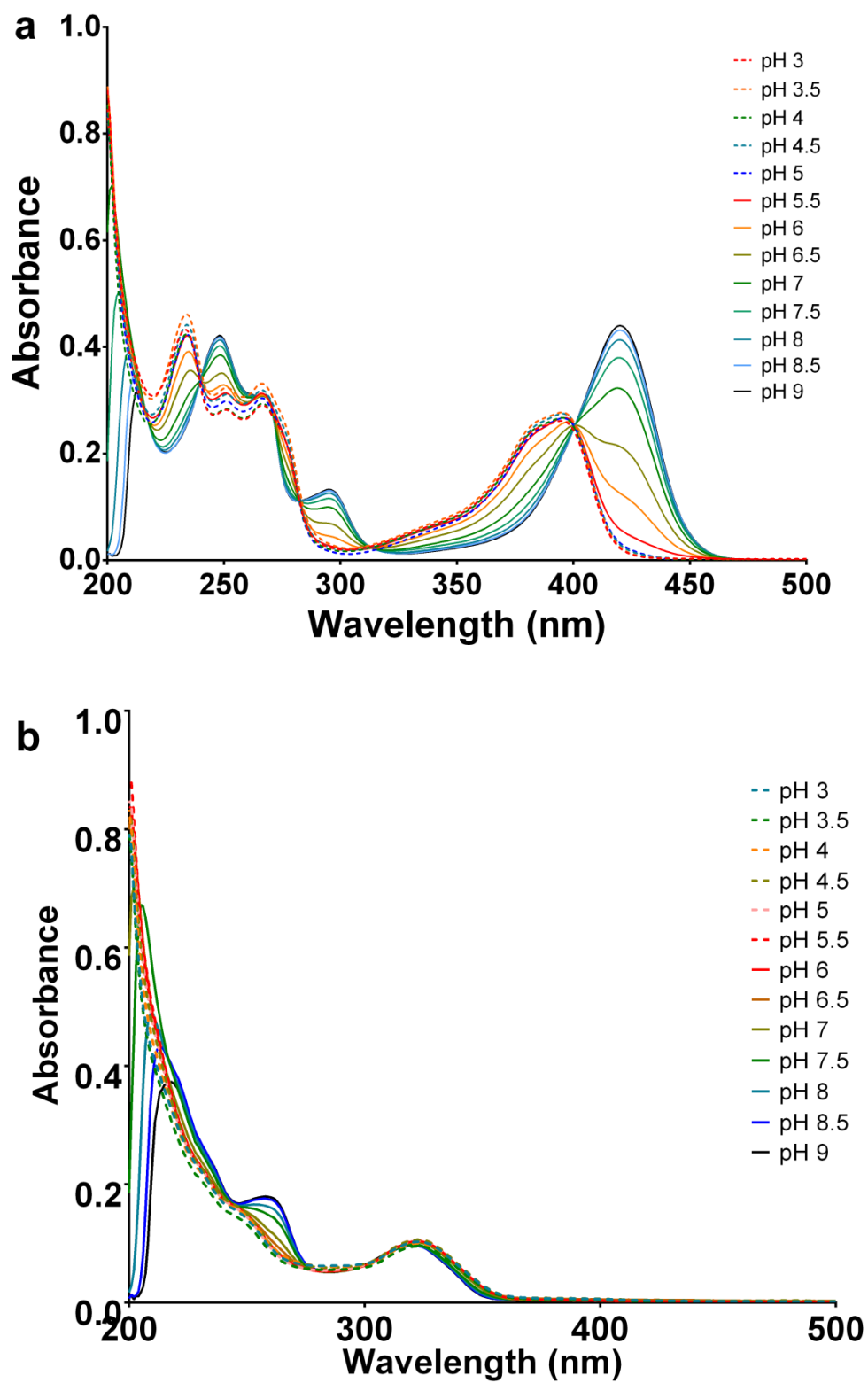
Figure S1. Full absorbance spectra of F_{420} (a) and $F_{420}H_2$ (b) from 200 to 500 nm for the pH range 3 to 9.

Table S1. Summary table of calculated vertical excitation energies for absorbance with the excited states and orbitals involved in the transition assigned. The values are compared with the experimental data in aqueous presented in bold.

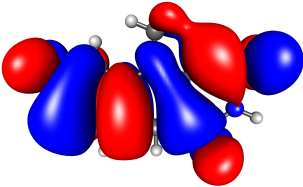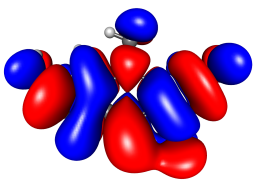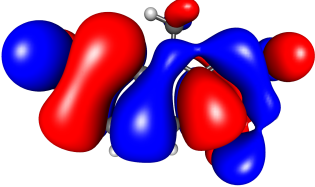| Cofactor Species	Transition	Solution Phase (Low Polarity ε = 12)			Solution Phase (Aqueous)		
		f	μ	ΔE (nm)	f	μ	ΔE (nm)
F_{420}	Main absorbance peak Excited State 1: 63 → 64	0.6258	6.9215	335.99	0.5773	6.3101	332.03
	Experimental	-	-	-	-	-	**400**
F_{420} (Deprotonated)	Main absorbance peak Excited State 1: 63 → 64	0.8197	9.9844	370.00	0.7523	8.9864	362.84
	Experimental	-	-	-	-	-	**420**
$F_{420}H_2$	Main absorbance peak Excited State 1: 64 → 65	0.2947	2.8501	293.74	0.2889	2.7275	286.79
	Experimental	-	-	-	-	-	**322**
	Secondary absorbance peak Excited State 3: 62 → 65*	0.2414	1.7857	224.73	0.1775	1.3035	223.13
	Experimental	-	-	-	-	-	**245**
$F_{420}H^-$ (Deprotonated)	Main absorbance peak Excited State 1: 64 → 65	0.2359	0.7791	287.31	0.2252	2.0687	278.98
	Experimental	-	-	-	-	-	**320**
	Secondary absorbance peak Excited State 3: 63 → 65*	0.2960	2.3341	239.53	0.2993	2.3188	235.31
	Experimental	-	-	-	-	-	**260**

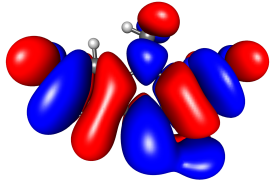*The absorbance transition is the result of a combination of multiple orbital transitions. The orbital transition listed here are the strongest contributors for each absorbance transition.
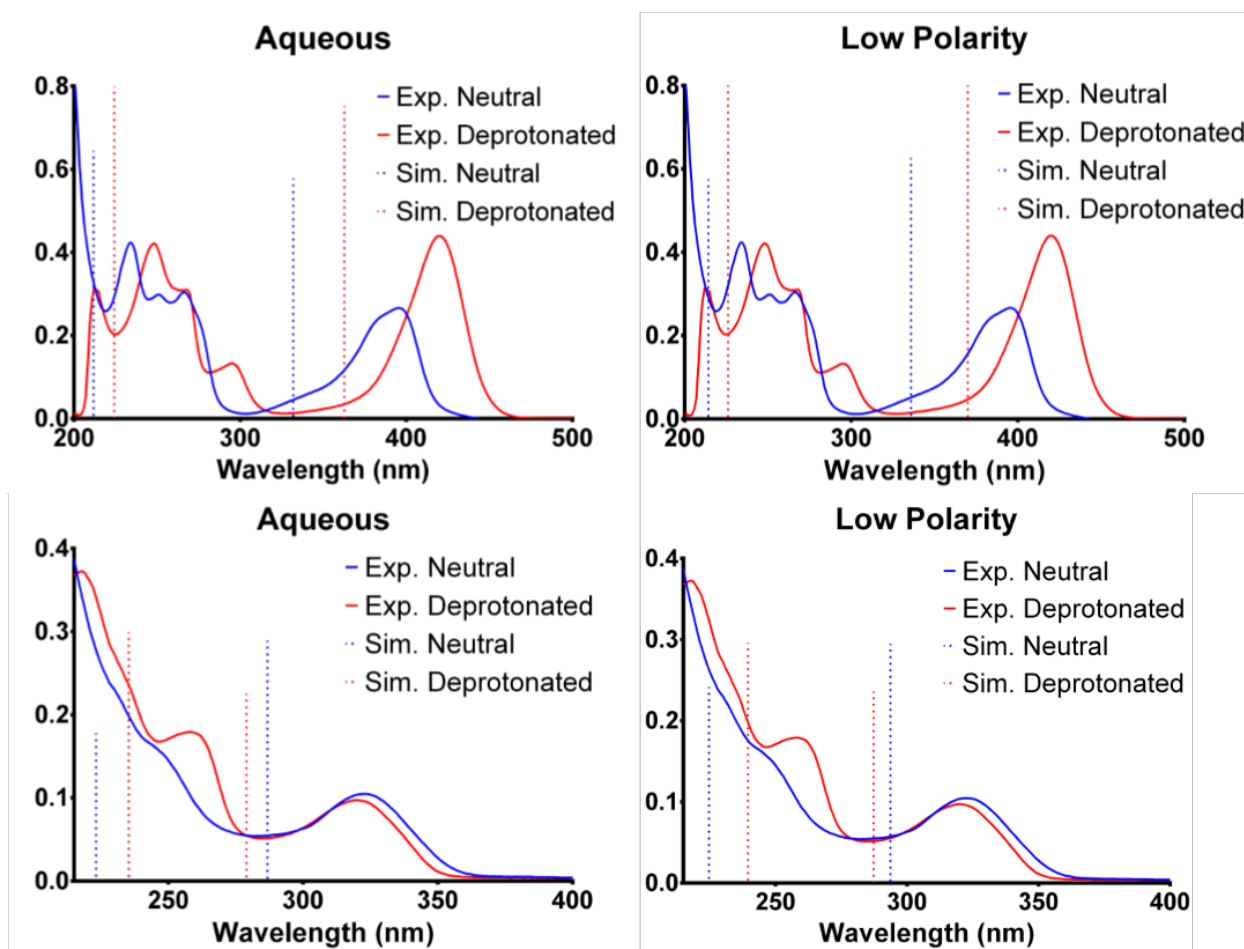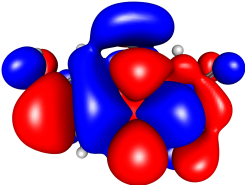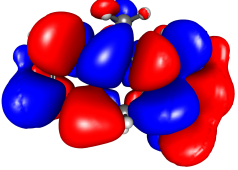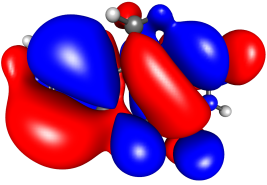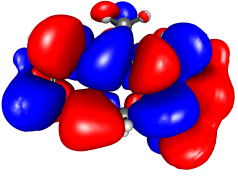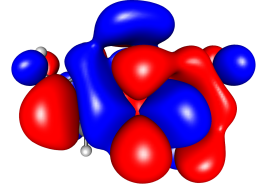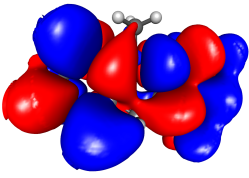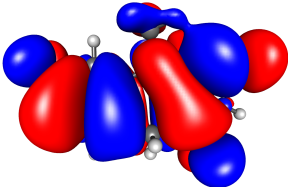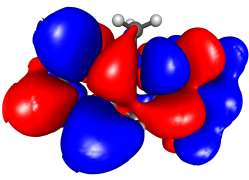
Figure S2. The calculated electronic transitions overlaid with experimental results (solid lines) for F_{420} (top) and $F_{420}H_2$ (bottom). The experimental results are those for pH 5 and pH 9 for neutral and deprotonated respectively. The electronic transitions (dashed lines) were calculated using ωB97XD/6-311+G(2d,p) level of theory in GAUSSIAN09. The absolute values of the calculated transitions are blue-shifted however the trends and relative shifts between the transitions emulate the experimental results with strong agreement.

Table S2. Assignments and visualization of the important electronic transitions and the orbitals involved for both protonated and deprotonated F_{420}. The results are shown for calculations done using ωB97XD/6-311+G(2d,p) under aqueous solvent conditions using the SMD solvent model.

Cofactor Species	Assignment	ΔE(nm)	f	From	To
F_{420}	Excited State 1 ^{1}A (63)→(64)	332.03	0.5773		
F_{420} (Deprotonated)	Excited State 1 ^{1}A (63)→(64)	362.84	0.7523		

Table S3. Assignments and visualization of the important electronic transitions and the orbitals involved for both protonated and deprotonated $F_{420}H_2$. The results are shown for calculations done using ωB97XD/6-311+G(2d,p) under aqueous solvent conditions using the SMD solvent model.

Cofactor Species	Assignment	ΔE(nm)	f	From	To
$F_{420}H_2$	Excited State 1 ^{1}A (64)→(65)	286.79	0.2889		
	Excited State 3 ^{1}A (62)→(65)	223.13	0.1775		
$F_{420}H^-$ (Deprotonated)	Excited State 1 ^{1}A (64)→(65)	278.98	0.2252		
	Excited State 3 ^{1}A (63)→(65)	235.31	0.2993		
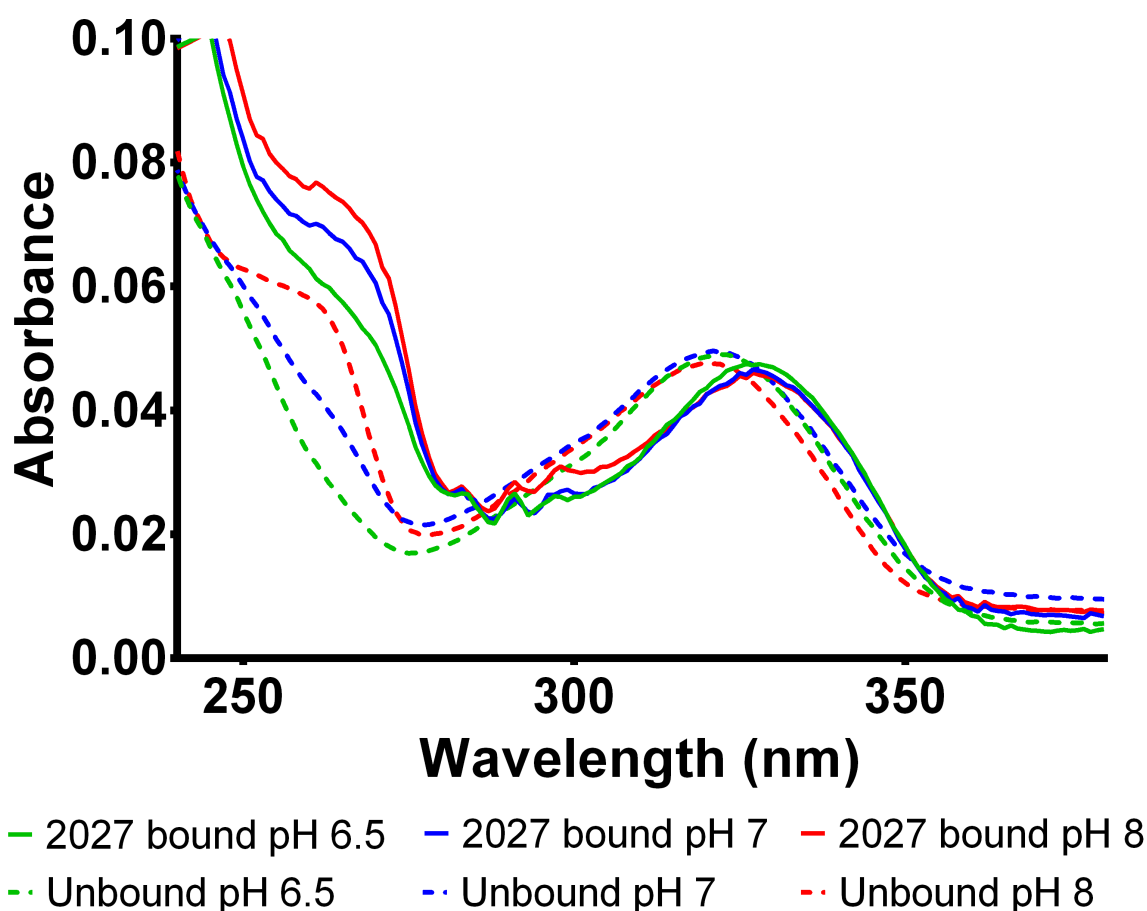
Fig S4. Comparison of the absorbance spectrum for $F_{420}H_2$ when bound to 2027 at three different pH values with their unbound counterparts. The same 5 − 10 nm red shift of all spectroscopic features observed in the Ddn bound spectrum (Fig. 5) is observed here as well. The spectrum of MSMEG_2027 itself is less noisy and clearer due to the lack of the Maltose Binding Protein (MBP) tag that was present in the Ddn sample for protein solubilisation. The 260 nm peak is distinctly present at all three pH values in the bound species whereas the unbound species rapidly loses the feature at pH 7.

Computational Geometries and Data

F_{420} Aqueous Optimisation

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1\1\GINC-R175\FOpt\RM06L\6-311+G(2d,p)\C12H9N3O3\ROOT\04-Dec-2014\0\\#
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8\\F1solv.m06l\\0,1\C,-3.4271941664,2.0774041477,0.000629506\C,-2.1825
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00892,0.0009331205\C,-3.5701069854,0.678828827,0.0005946172\N,0.246792
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F_{420} Low Polarity Optimisation

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F_{420} Deprotonated Aqueous Optimisation

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483648\\F2D1solv.m06.m06l\\-1,1\C,-3.4757895176,2.1120359675,0.0004414
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\\@

F$_{420}$ Deprotonated Low Polarity Optimisation

1\1\GINC-R228\FOpt\RM06L\6-311+G(2d,p)\C12H8N3O3(1-)\ROOT\19-Mar-2015\
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Ethanol,read) maxdisk=1610612736\\F2D1solvE.freq\\-1,1\C,-3.4834018886
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01 [X(C12H8N3O3)]\\@

F$_{420}$H$_2$ Aqueous Optimisation

1\1\GINC-R3398\FOpt\RwB97XD\6-311+G(2d,p)\C12H11N3O3\ROOT\29-Apr-2015\
0\\#wB97XD/6-311+g(2d,p) OPT SCRF=(SMD,Solvent=Water,Read) maxdisk=268
4354560\\F1H2solv.w\\0,1\C,-3.3357096141,2.1115050812,-0.3490105802\C,
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.116638755\C,1.2962173398,0.2659411716,0.1677436708\C,0.0192534624,-0.
3937909518,0.5909368657\N,2.5545966879,2.2509401511,-0.1903198578\C,3.
7132944138,1.5759886436,-0.4620595783\N,3.6144668423,0.2215889565,-0.4

034261362\C,2.4611467677,-0.4995843467,-0.0999850079\C,0.4200291098,3.8700791776,0.4867279213\O,4.7526394613,2.1616564982,-0.7430640201\O,2.5311034893,-1.7343457207,-0.0679507453\O,-4.4336865623,2.8815983113,-0.6279431751\H,-1.9768261693,3.7506856785,-0.2304381666\H,-2.5353994559,-1.1388142122,0.0282948871\H,-4.4871943205,0.3068434175,-0.470367029\H,-0.0736264611,-1.3701314123,0.1131634723\H,4.4590987602,-0.3052822522,-0.5854532336\H,-0.3933865319,4.2424108666,1.1051076674\H,0.4020542903,4.3811721991,-0.4790061849\H,1.3429310927,4.1080646484,1.0120446019\H,-4.1947543695,3.8149628588,-0.6136866826\H,0.0279577802,-0.5758980322,1.6733531739\H,2.6046545802,3.2524477423,-0.3116063595\\Version=ES64L-G09RevD.01\State=1-A\HF=-853.8914107\RMSD=7.594e-09\RMSF=2.711e-05\Dipole=-1.8212769,3.5828827,0.5992011\Quadrupole=-15.786752,10.7521698,5.0345822,0.0205398,2.323926,1.4911068\PG=C01 [X(C12H11N3O3)]\\@

$F_{420}H_2$ Low Polarity Optimisation

1\1\GINC-R531\FOpt\RM06L\6-311+G(2d,p)\C12H11N3O3\ROOT\03-May-2015\0\\#M06L/6-311+g(2d,p) OPT SCRF=(SMD,Solvent=Ethanol,Read) maxdisk=2684354560\\F1H2solvT.m06l\\0,1\C,-3.3625683783,2.120066324,-0.3048701752\C,-2.1027888786,2.6842813299,-0.1330598073\C,-1.0039842403,1.8640597881,0.123913764\C,-1.1565660053,0.4739787855,0.1957576316\C,-2.4253972378,-0.0551283081,-0.003623373\C,-3.5321797135,0.7427308532,-0.2444020754\N,0.2812296534,2.4340346732,0.2848585774\C,1.37398723,1.6337960169,0.0899317124\C,1.2997935992,0.2650604746,0.1403849703\C,0.0179139654,-0.4040209674,0.5095494989\N,2.5744450152,2.2525384997,-0.1680684686\C,3.7534395619,1.5803136189,-0.4062619916\N,3.6409563903,0.2233063178,-0.3427202704\C,2.4722948617,-0.5072407922,-0.0784838548\C,0.4207704963,3.8731726587,0.4271465024\O,4.7957472315,2.1698945464,-0.6556251574\O,2.5401419013,-1.7395866431,-0.0364782961\O,-4.4661734621,2.8814813209,-0.5524811315\H,-1.9939141972,3.7583331906,-0.2249027164\H,-2.5494715223,-1.1335213955,0.045932854\H,-4.5178353701,0.3142966473,-0.388511536\H,-0.0689052879,-1.3576335286,-0.0197428956\H,4.489707082,-0.3080125576,-0.4947585343\H,-0.3870605317,4.2507068856,1.0516455102\H,0.399700809,4.3967827675,-0.5343028521\H,1.3495395966,4.1071382836,0.945923813\H,-4.2229800073,3.8161491427,-0.5603522624\H,0.0196118973,-0.669999301,1.5783245761\H,2.6232055409,3.2524213683,-0.3063040121\\Version=ES64L-G09RevD.01\State=1-A\HF=-854.0557788\RMSD=6.977e-09\RMSF=1.665e-05\Dipole=-1.7625005,3.3487579,0.4386554\Quadrupole=-14.4945666,9.6095167,4.8850499,-0.8811483,2.0114442,1.166726\PG=C01 [X(C12H11N3O3)]\\@

$F_{420}H_2$ Deprotonated Aqueous Optimisation

1\1\GINC-R423\FOpt\RwB97XD\6-311+G(2d,p)\C12H10N3O3(1-)\ROOT\29-Apr-2015\0\\#wB97XD/6-311+g(2d,p) OPT SCRF=(SMD,Solvent=Water,Read) maxdisk=2684354560\\F2H2D1solv.w\\-1,1\C,-3.329846199,2.1079948102,-0.3503140021\C,-2.0801731757,2.6727234119,-0.1304105108\C,-0.9890161174,1.8558133565,0.1679056201\C,-1.1573008243,0.4676538261,0.2342798234\C,-2.4116463594,-0.0651026104,-0.0175378643\C,-3.5086382633,0.7345823511,-0.3031831412\N,0.2852312754,2.4125622688,0.3869630193\C,1.4056953773,1.6375106729,0.1131814419\C,1.2912597078,0.2545811737,0.1641794573\C,0.0156788447,-0.3925657869,0.6116838783\N,2.5331818185,2.3091857696,-0.1804795188\C,3.6361225417,1.600116417,-0.4529161301\N,3.564735246,0.2242069592,-0.4471097329\C,2.435725514,-0.5079048526,-0.130166114\C,0.409789846,3.8585767173,0.4515962768\O,4.7367600569,2.1321499944,-0.7310441069\O,2.5149199794,-1.7603552436,-0.1179702034\O,-4.4250584524,2.8852473066,-0.6346791511\H,-1.9757041729,3.7466702988,-0.2103676898\H,-2.5367589313,-1.1420778436,0.0251039534\H,-4.4865115199,0.3068007904,-0.4868155

268\H,-0.0874583334,-1.3830683325,0.1647188498\H,4.4122535459,-0.28896
19134,-0.6474421183\H,-0.362961212,4.2537710433,1.1099767687\H,0.32065
35015,4.3315703722,-0.5313396462\H,1.3759276805,4.1157871919,0.8716866
216\H,-4.1786847938,3.8165053284,-0.6178540758\H,0.0236334193,-0.54569
3477,1.7003838214\\Version=ES64L-G09RevD.01\State=1-A\HF=-853.422114\R
MSD=8.110e-09\RMSF=1.082e-05\Dipole=-5.8006138,1.9459168,0.8961631\Qua
drupole=-29.2469923,11.0232446,18.2237477,-6.5247616,4.8186671,1.87111
98\PG=C01 [X(C12H10N3O3)]\\@

F$_{420}$H$_2$ Deprotonated Low Polarity Optimisation

1\1\GINC-R595\FOpt\RM06L\6-311+G(2d,p)\C12H10N3O3(1-)\ROOT\03-May-2015
\0\\#M06L/6-311+g(2d,p) OPT SCRF=(SMD,Solvent=Ethanol,Read) maxdisk=26
84354560\\F2H2D1solvE.freq\\-1,1\C,3.7173424403,-0.2193706703,-0.29916
57095\C,2.5682742137,-0.975366326,-0.0963772185\C,1.340322384,-0.34429
37201,0.1358502099\C,1.2740286174,1.0593477441,0.1572133484\C,2.438248
3534,1.7794810426,-0.0701068575\C,3.664338974,1.1675572623,-0.29244272
84\N,0.1789123217,-1.102895563,0.3310362443\C,-1.0740233251,-0.5241585
867,0.1046074596\C,-1.1843866567,0.8613602788,0.1155583372\C,-0.026485
3692,1.7387800056,0.4608851715\N,-2.0803293962,-1.3890013454,-0.111398
4124\C,-3.3107882617,-0.8878906275,-0.333958258\N,-3.4643855626,0.4906
742169,-0.3581174321\C,-2.4580122851,1.4210614543,-0.1226953003\C,0.29
45743471,-2.54346022,0.4328053972\O,-4.3152928286,-1.6020756619,-0.528
1421909\O,-2.7405122306,2.6398080085,-0.1257751301\O,4.9320571849,-0.8
060179111,-0.5264439402\H,2.6413764814,-2.0554566219,-0.1427111866\H,2
.3837573951,2.8652808548,-0.0584645222\H,4.5664169449,1.7456880982,-0.
4602086898\H,-0.093943266,2.6869855347,-0.0833632031\H,-4.3971286854,0
.8509623025,-0.5156560658\H,1.0910277508,-2.7970208165,1.1344222428\H,
0.5115689315,-3.0185674239,-0.5314877646\H,-0.6405131752,-2.9488408716
,0.805111307\H,4.8339493788,-1.7663006805,-0.5017347745\H,-0.056794676
4,2.0251412428,1.5263806668\\Version=ES64L-G09RevD.01\State=1-A\HF=-85
3.5792612\RMSD=8.954e-09\RMSF=1.788e-05\Dipole=5.6122031,-0.9415771,0.
6351711\Quadrupole=-25.4007124,6.8030137,18.5976987,-12.4249406,-2.983
9231,-1.754964\PG=C01 [X(C12H10N3O3)]\\@

F$_{420}$ Aqueous TD-DFT

1\1\GINC-R38\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H9N3O3\ROOT\19-Nov-2015
\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solvent=Wa
ter) maxdisk=2684354560\\f420 in water\\0,1\C,0,-3.4318763492,2.076872
3471,0.0006281174\C,0,-2.1894817555,2.6881775133,0.0009940072\C,0,-1.0
278685728,1.903981555,0.0013434552\C,0,-1.1585237139,0.491228674,0.001
3122859\C,0,-2.4447511274,-0.0946937513,0.0009332327\C,0,-3.5707645254
,0.6727700342,0.0005944547\N,0,0.24376602,2.459368662,0.0017183373\C,0
,1.3743141704,1.6896056738,0.0020584754\C,0,1.2320239431,0.2686501562,
0.002023893\C,0,-0.0021530188,-0.3054503114,0.0016602436\N,0,2.5392079
123,2.3161658408,0.0024012515\C,0,3.680177598,1.5746257168,0.002744333
6\N,0,3.6031955893,0.183183494,0.0027289989\C,0,2.452359607,-0.5439682
184,0.0023909456\C,0,0.4565325382,3.9136524933,0.0017736825\O,0,4.7950
710828,2.0929867957,0.0030728375\O,0,2.4555187249,-1.7688406298,0.0023
985236\O,0,-4.5695753454,2.7866476832,0.0002861795\H,0,-2.1672715702,3
.776255372,0.0009947097\H,0,-2.5114359563,-1.1809949341,0.0009193041\H
,0,-4.5705471522,0.237791337,0.0002997063\H,0,-0.0954761542,-1.3914996
231,0.0016383975\H,0,4.5323684832,-0.3503565861,0.0030080138\H,0,-0.49
32773736,4.4383800841,0.0014885827\H,0,1.0294859015,4.1881727942,-0.88
75695278\H,0,1.0289572278,4.1881824372,0.8914542896\H,0,-4.3714528619,
3.7735733269,0.0003397062\\Version=ES64L-G09RevD.01\State=1-A'\HF=-852

.6754498\RMSD=4.654e-09\PG=CS [SG(C12H7N3O3),X(H2)]\\@

F₄₂₀ Low Polarity TD-DFT

1\1\GINC-R39\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H9N3O3\ROOT\19-Nov-2015
\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solvent=Et
hanol,read) maxdisk=2684354560\\f420 in d12\\0,1\C,0,-3.4318763492,2.0
768723471,0.0006281174\C,0,-2.1894817555,2.6881775133,0.0009940072\C,0
,-1.0278685728,1.903981555,0.0013434552\C,0,-1.1585237139,0.491228674,
0.0013122859\C,0,-2.4447511274,-0.0946937513,0.0009332327\C,0,-3.57076
45254,0.6727700342,0.0005944547\N,0,0.24376602,2.459368662,0.001718337
3\C,0,1.3743141704,1.6896056738,0.0020584754\C,0,1.2320239431,0.268650
1562,0.002023893\C,0,-0.0021530188,-0.3054503114,0.0016602436\N,0,2.53
92079123,2.3161658408,0.0024012515\C,0,3.680177598,1.5746257168,0.0027
443336\N,0,3.6031955893,0.183183494,0.0027289989\C,0,2.452359607,-0.54
39682184,0.0023909456\C,0,0.4565325382,3.9136524933,0.0017736825\O,0,4
.7950710828,2.0929867957,0.0030728375\O,0,2.4555187249,-1.7688406298,0
.0023985236\O,0,-4.5695753454,2.7866476832,0.0002861795\H,0,-2.1672715
702,3.776255372,0.0009947097\H,0,-2.5114359563,-1.1809949341,0.0009193
041\H,0,-4.5705471522,0.237791337,0.0002997063\H,0,-0.0954761542,-1.39
14996231,0.0016383975\H,0,4.5323684832,-0.3503565861,0.0030080138\H,0,
-0.4932773736,4.4383800841,0.0014885827\H,0,1.0294859015,4.1881727942,
-0.8875695278\H,0,1.0289572278,4.1881824372,0.8914542896\H,0,-4.371452
8619,3.7735733269,0.0003397062\\Version=ES64L-G09RevD.01\State=1-A'\HF
=-852.6766205\RMSD=3.714e-09\PG=CS [SG(C12H7N3O3),X(H2)]\\@

F₄₂₀ Deprotonated Aqueous TD-DFT

1\1\GINC-R38\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H8N3O3(1-)\ROOT\20-Nov-
2015\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solven
t=Water) maxdisk=2684354560\\deprotonated f420 in water\\-1,1\C,0,-3.4
999792008,2.1208174246,0.0005494885\C,0,-2.1728197183,2.6908251736,0.0
009239217\C,0,-1.0343478211,1.9182721608,0.0013801533\C,0,-1.151306768
4,0.4820706457,0.001447161\C,0,-2.4597828482,-0.0937917008,0.001091320
8\C,0,-3.5773318545,0.6614687961,0.0006752643\N,0,0.2611474768,2.45147
10336,0.0017743287\C,0,1.4010692579,1.6750961284,0.0020829017\C,0,1.24
96620693,0.2647641312,0.002106291\C,0,-0.0140695837,-0.2969155554,0.00
18076823\N,0,2.5540389564,2.3228578436,0.0023541055\C,0,3.7080082369,1
.5976610558,0.0027419486\N,0,3.6064966541,0.1930463754,0.0026935131\C,
0,2.4486704663,-0.552167117,0.0024347267\C,0,0.4894151222,3.8934942027
,0.0018797186\O,0,4.8255535927,2.0860535207,0.0028299136\O,0,2.4766476
32,-1.7752617437,0.0024542793\O,0,-4.5222277396,2.8220118972,0.0001318
136\H,0,-2.1594116274,3.7686213516,0.0007831428\H,0,-2.5205605248,-1.1
779496476,0.0011608939\H,0,-4.5680212788,0.2233873572,0.0004007518\H,0
,-0.0901524389,-1.3798614769,0.0018428826\H,0,4.4811864545,-0.31141064
67,0.0028961984\H,0,-0.453883043,4.4192219512,0.0020080675\H,0,1.07204
28332,4.1691045607,-0.8757440253\H,0,1.0721556952,4.1689122782,0.87948
35561\\Version=ES64L-G09RevD.01\State=1-A\HF=-852.2117468\RMSD=8.645e-
09\PG=C01 [X(C12H8N3O3)]\\@

F₄₂₀ Deprotonated Low Polarity TD-DFT

1\1\GINC-R39\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H8N3O3(1-)\ROOT\20-Nov-
2015\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solven
t=Ethanol,read) maxdisk=2684354560\\deprotonated f420 in d12\\-1,1\C,0
,-3.4999792008,2.1208174246,0.0005494885\C,0,-2.1728197183,2.690825173
6,0.0009239217\C,0,-1.0343478211,1.9182721608,0.0013801533\C,0,-1.1513

067684,0.4820706457,0.001447161\C,0,-2.4597828482,-0.0937917008,0.0010
913208\C,0,-3.5773318545,0.6614687961,0.0006752643\N,0,0.2611474768,2.
4514710336,0.0017743287\C,0,1.4010692579,1.6750961284,0.0020829017\C,0
,1.2496620693,0.2647641312,0.002106291\C,0,-0.0140695837,-0.2969155554
,0.0018076823\N,0,2.5540389564,2.3228578436,0.0023541055\C,0,3.7080082
369,1.5976610558,0.0027419486\N,0,3.6064966541,0.1930463754,0.00269351
31\C,0,2.4486704663,-0.552167117,0.0024347267\C,0,0.4894151222,3.89349
42027,0.0018797186\O,0,4.8255535927,2.0860535207,0.0028299136\O,0,2.47
6647632,-1.7752617437,0.0024542793\O,0,-4.5222277396,2.8220118972,0.00
01318136\H,0,-2.1594116274,3.7686213516,0.0007831428\H,0,-2.5205605248
,-1.1779496476,0.0011608939\H,0,-4.5680212788,0.2233873572,0.000400751
8\H,0,-0.0901524389,-1.3798614769,0.0018428826\H,0,4.4811864545,-0.311
4106467,0.0028961984\H,0,-0.453883043,4.4192219512,0.0020080675\H,0,1.
0720428332,4.1691045607,-0.8757440253\H,0,1.0721556952,4.1689122782,0.
8794835561\\Version=ES64L-G09RevD.01\State=1-A\HF=-852.2082774\RMSD=7.
980e-09\PG=C01 [X(C12H8N3O3)]\\@

$F_{420}H_2$ Low Polarity TD-DFT

1\1\GINC-R1555\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H11N3O3\ROOT\04-May-2
015\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solvent
=Ethanol,read) maxdisk=2684354560\\F1H2solvE.SMD.gsg_ese.w\\0,1\C,0,-3
.3625683783,2.120066324,-0.3048701752\C,0,-2.1027888786,2.6842813299,-
0.1330598073\C,0,-1.0039842403,1.8640597881,0.123913764\C,0,-1.1565660
053,0.4739787855,0.1957576316\C,0,-2.4253972378,-0.0551283081,-0.00362
3373\C,0,-3.5321797135,0.7427308532,-0.2444020754\N,0,0.2812296534,2.4
340346732,0.2848585774\C,0,1.37398723,1.6337960169,0.0899317124\C,0,1.
2997935992,0.2650604746,0.1403849703\C,0,0.0179139654,-0.4040209674,0.
5095494989\N,0,2.5744450152,2.2525384997,-0.1680684686\C,0,3.753439561
9,1.5803136189,-0.4062619916\N,0,3.6409563903,0.2233063178,-0.34272027
04\C,0,2.4722948617,-0.5072407922,-0.0784838548\C,0,0.4207704963,3.873
1726587,0.4271465024\O,0,4.7957472315,2.1698945464,-0.6556251574\O,0,2
.5401419013,-1.7395866431,-0.0364782961\O,0,-4.4661734621,2.8814813209
,-0.5524811315\H,0,-1.9939141972,3.7583331906,-0.2249027164\H,0,-2.549
4715223,-1.1335213955,0.045932854\H,0,-4.5178353701,0.3142966473,-0.38
8511536\H,0,-0.0689052879,-1.3576335286,-0.0197428956\H,0,4.489707082,
-0.3080125576,-0.4947585343\H,0,-0.3870605317,4.2507068856,1.051645510
2\H,0,0.399700809,4.3967827675,-0.5343028521\H,0,1.3495395966,4.107138
2836,0.945923813\H,0,-4.2229800073,3.8161491427,-0.5603522624\H,0,0.01
96118973,-0.669999301,1.5783245761\H,0,2.6232055409,3.2524213683,-0.30
63040121\\Version=ES64L-G09RevD.01\State=1-A\HF=-853.8939858\RMSD=6.62
2e-09\PG=C01 [X(C12H11N3O3)]\\@

$F_{420}H_2$ Aqueous TD-DFT

1\1\GINC-R730\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H11N3O3\ROOT\03-May-20
15\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solvent=
Water) maxdisk=2684354560\\F1H2solv.SMD.gsg_ese.w\\0,1\C,0,-3.33570961
41,2.1115050812,-0.3490105802\C,0,-2.0849967663,2.6777417754,-0.141376
199\C,0,-0.9987614785,1.8587572767,0.1535440278\C,0,-1.1551763713,0.47
32777649,0.2261097095\C,0,-2.4116402074,-0.0621249272,-0.0162360114\C,
0,-3.5087451749,0.7365423026,-0.2949318151\N,0,0.2846263402,2.42526991
73,0.3516160296\C,0,1.3684930652,1.631897416,0.116638755\C,0,1.2962173
398,0.2659411716,0.1677436708\C,0,0.0192534624,-0.3937909518,0.5909368
657\N,0,2.5545966879,2.2509401511,-0.1903198578\C,0,3.7132944138,1.575
9886436,-0.4620595783\N,0,3.6144668423,0.2215889565,-0.4034261362\C,0,
2.4611467677,-0.4995843467,-0.0999850079\C,0,0.4200291098,3.8700791776

,0.4867279213\O,0,4.7526394613,2.1616564982,-0.7430640201\O,0,2.531103
4893,-1.7343457207,-0.0679507453\O,0,-4.4336865623,2.8815983113,-0.627
9431751\H,0,-1.9768261693,3.7506856785,-0.2304381666\H,0,-2.5353994559
,-1.1388142122,0.0282948871\H,0,-4.4871943205,0.3068434175,-0.47036702
9\H,0,-0.0736264611,-1.3701314123,0.1131634723\H,0,4.4590987602,-0.305
2822522,-0.5854532336\H,0,-0.3933865319,4.2424108666,1.1051076674\H,0,
0.4020542903,4.3811721991,-0.4790061849\H,0,1.3429310927,4.1080646484,
1.0120446019\H,0,-4.1947543695,3.8149628588,-0.6136866826\H,0,0.027957
7802,-0.5758980322,1.6733531739\H,0,2.6046545802,3.2524477423,-0.31160
63595\\Version=ES64L-G09RevD.01\State=1-A\HF=-853.8914107\RMSD=5.674e-
09\PG=C01 [X(C12H11N3O3)]\\@

$F_{420}H_2$ Deprotonated Low Polarity TD-DFT

1\1\GINC-R3588\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H10N3O3(1-)\ROOT\04-M
ay-2015\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Sol
vent=Ethanol,read) maxdisk=2684354560\\F2H2D1solvE.SMD.gsg_ese.w\\-1,1
\C,0,3.7173424403,-0.2193706703,-0.2991657095\C,0,2.5682742137,-0.9753
66326,-0.0963772185\C,0,1.340322384,-0.3442937201,0.1358502099\C,0,1.2
740286174,1.0593477441,0.1572133484\C,0,2.4382483534,1.7794810426,-0.0
701068575\C,0,3.664338974,1.1675572623,-0.2924427284\N,0,0.1789123217,
-1.102895563,0.3310362443\C,0,-1.0740233251,-0.5241585867,0.1046074596
\C,0,-1.1843866567,0.8613602788,0.1155583372\C,0,-0.0264853692,1.73878
00056,0.4608851715\N,0,-2.0803293962,-1.3890013454,-0.1113984124\C,0,-
3.3107882617,-0.8878906275,-0.333958258\N,0,-3.4643855626,0.4906742169
,-0.3581174321\C,0,-2.4580122851,1.4210614543,-0.1226953003\C,0,0.2945
743471,-2.54346022,0.4328053972\O,0,-4.3152928286,-1.6020756619,-0.528
1421909\O,0,-2.7405122306,2.6398080085,-0.1257751301\O,0,4.9320571849,
-0.8060179111,-0.5264439402\H,0,2.6413764814,-2.0554566219,-0.14271118
66\H,0,2.3837573951,2.8652808548,-0.0584645222\H,0,4.5664169449,1.7456
880982,-0.4602086898\H,0,-0.093943266,2.6869855347,-0.0833632031\H,0,-
4.3971286854,0.8509623025,-0.5156560658\H,0,1.0910277508,-2.7970208165
,1.1344222428\H,0,0.5115689315,-3.0185674239,-0.5314877646\H,0,-0.6405
131752,-2.9488408716,0.805111307\H,0,4.8339493788,-1.7663006805,-0.501
7347745\H,0,-0.0567946764,2.0251412428,1.5263806668\\Version=ES64L-G09
RevD.01\State=1-A\HF=-853.4165125\RMSD=7.450e-09\PG=C01 [X(C12H10N3O3)
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$F_{420}H_2$ Deprotonated Aqueous TD-DFT

1\1\GINC-R512\SP\RwB97XD TD-FC\6-311+G(2d,p)\C12H10N3O3(1-)\ROOT\03-Ma
y-2015\0\\# wB97XD td=(singlet,nstates=9)/6-311+g(2d,p) SCRF=(SMD,Solv
ent=Water) maxdisk=2684354560\\F2H2D1solv.SMD.gsg_ese.w\\-1,1\C,0,-3.3
29846199,2.1079948102,-0.3503140021\C,0,-2.0801731757,2.6727234119,-0.
1304105108\C,0,-0.9890161174,1.8558133565,0.1679056201\C,0,-1.15730082
43,0.4676538261,0.2342798234\C,0,-2.4116463594,-0.0651026104,-0.017537
8643\C,0,-3.5086382633,0.7345823511,-0.3031831412\N,0,0.2852312754,2.4
125622688,0.3869630193\C,0,1.4056953773,1.6375106729,0.1131814419\C,0,
1.2912597078,0.2545811737,0.1641794573\C,0,0.0156788447,-0.3925657869,
0.6116838783\N,0,2.5331818185,2.3091857696,-0.1804795188\C,0,3.6361225
417,1.600116417,-0.4529161301\N,0,3.564735246,0.2242069592,-0.44710973
29\C,0,2.435725514,-0.5079048526,-0.130166114\C,0,0.409789846,3.858576
7173,0.4515962768\O,0,4.7367600569,2.1321499944,-0.7310441069\O,0,2.51
49199794,-1.7603552436,-0.1179702034\O,0,-4.4250584524,2.8852473066,-0
.6346791511\H,0,-1.9757041729,3.7466702988,-0.2103676898\H,0,-2.536758
9313,-1.1420778436,0.0251039534\H,0,-4.4865115199,0.3068007904,-0.4868
155268\H,0,-0.0874583334,-1.3830683325,0.1647188498\H,0,4.4122535459,-

0.2889619134,-0.6474421183\H,0,-0.362961212,4.2537710433,1.1099767687\
H,0,0.3206535015,4.3315703722,-0.5313396462\H,0,1.3759276805,4.1157871
919,0.8716866216\H,0,-4.1786847938,3.8165053284,-0.6178540758\H,0,0.02
36334193,-0.545693477,1.7003838214\\Version=ES64L-G09RevD.01\State=1-A
\HF=-853.422114\RMSD=7.822e-09\PG=C01 [X(C12H10N3O3)]\\@