Computational studies of guanine oxidation

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Prof. Smriti Anand
Dr. Jia Zhou
Prof. Richard Lord
Prof. Peng Tao
Prof. Barbara Munk
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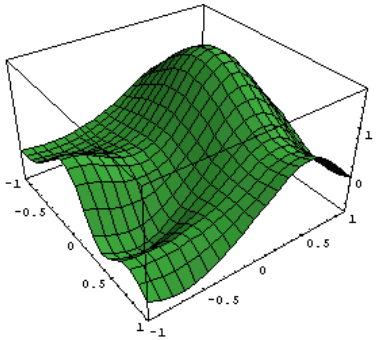
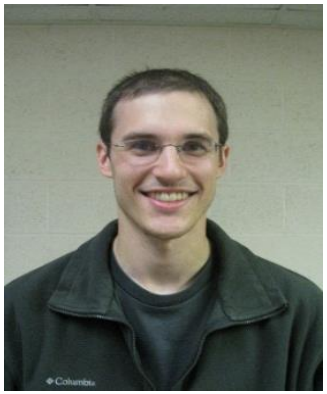


Computational studies of guanine oxidation

Bishnu Thapa



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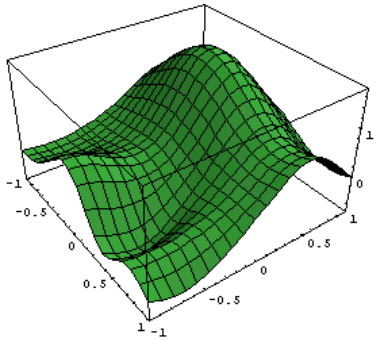
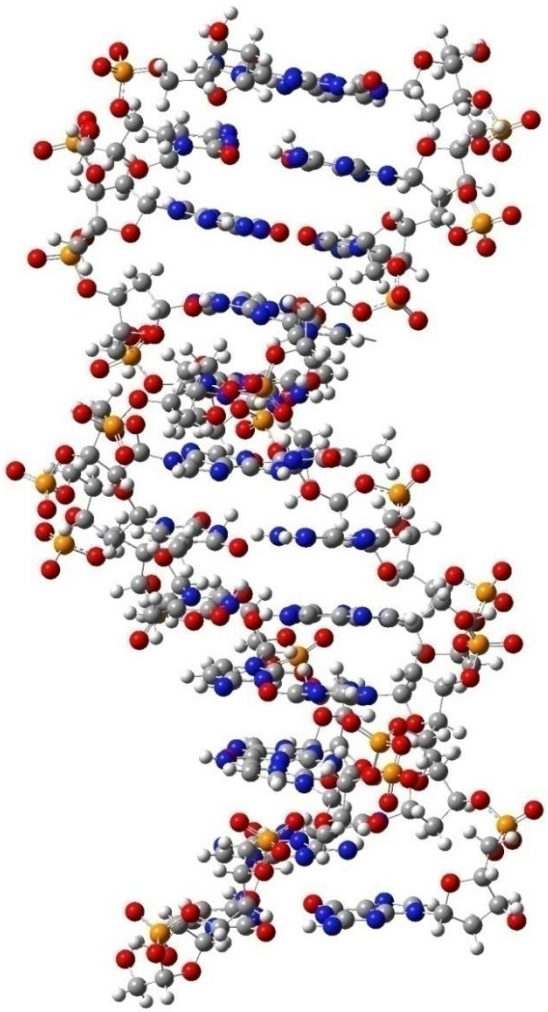
THE UNIVERSITY OF UTAH



Oxidative Damage to DNA

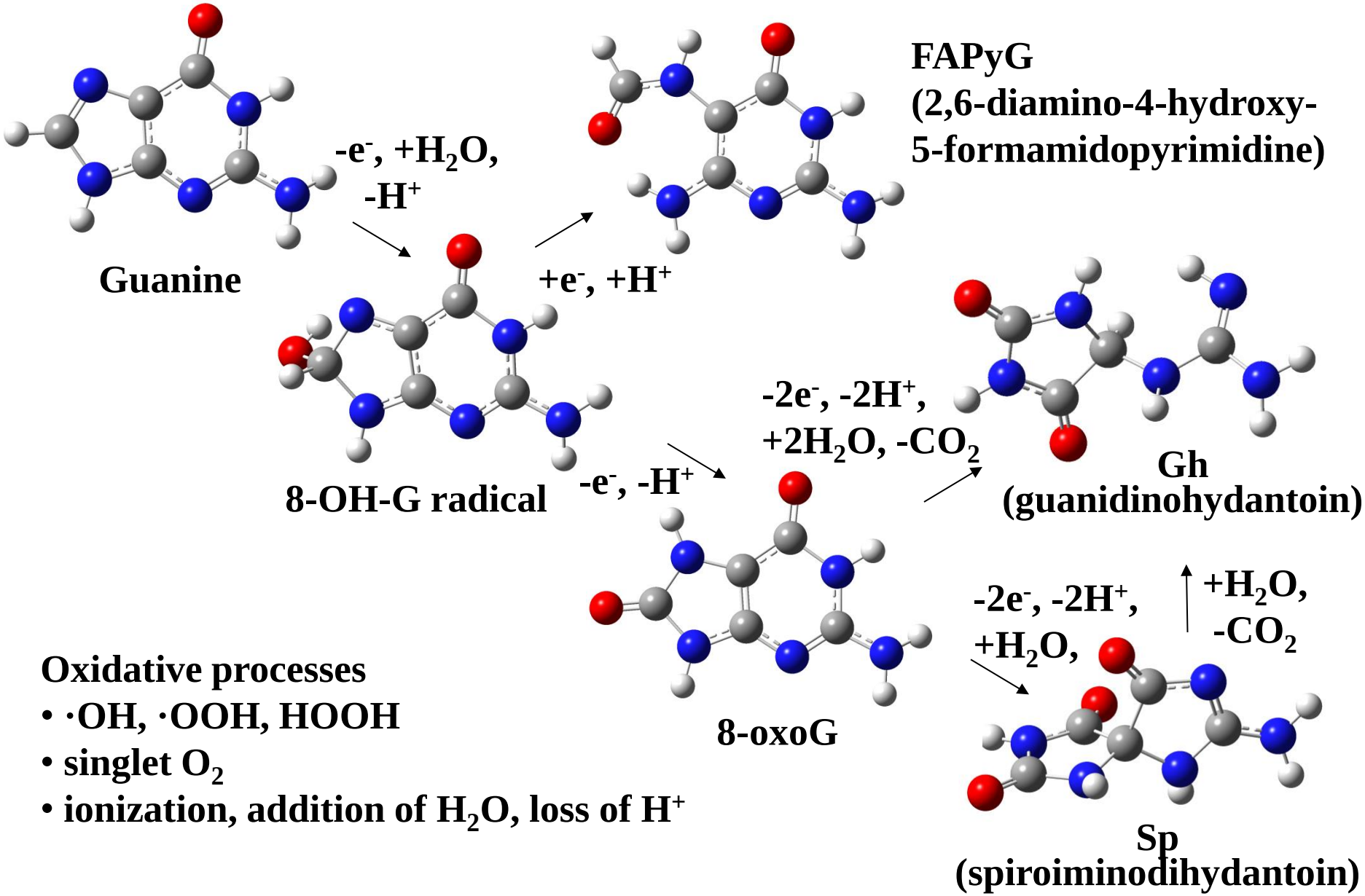


- Studied experimentally by a large number of research groups

  Burrows et al., *Chem. Rev.*, **1998**, *98*, 1109.
  Pratviel et al., *Chem.-Eur. J.*, **2006**, *12*, 6018.
  Gimisis et al., *Eur. J. Org. Chem.*, **2006**, 1351.
  Delaney et al., *Chem. Res. Toxicol.*, **2008**, *21*, 232.
  and numerous others

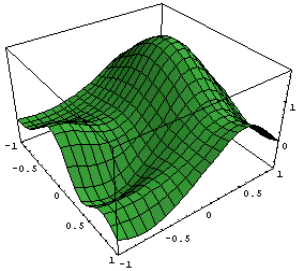
- Caused by a variety of reactive species
  - O_2^-, ONO_2^-, H_2O_2, ·OH, Cr(VI), etc.
- Can result in the formation of
  - DNA strand breaks
  - Protein-DNA crosslinks
  - Nucleobase mutations
  - Nucleobase deletions
- Guanine is the most easily oxidized of the nucleobases

Guanine Oxidation Products

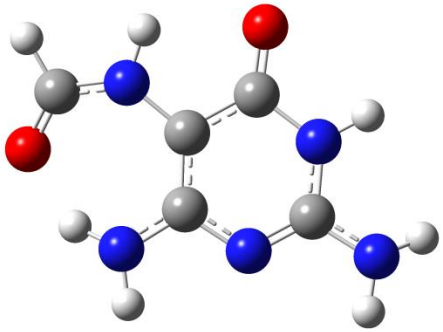


Oxidative processes

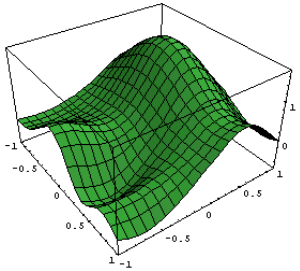
- ·OH, ·OOH, HOOH
- singlet O_2
- ionization, addition of H_2O, loss of H^+



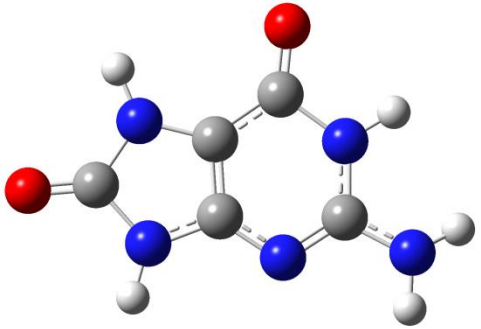
Formamidopyrimidines (FAPyG)



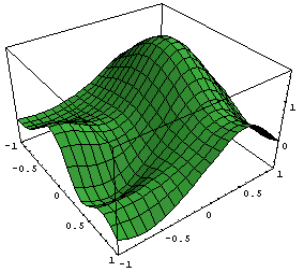
- Produced in vivo with both oxidizing (·OH) and alkylating agents (cyclophosphamide)
- Elevated levels observed in the cortical region of the brain of patients with dementia
- Elevated levels observed in patients with acute lymphoblastic leukaemia, breast cancer and skin cancer. Strong polymerase arresting capability vs 8-oxoG[1]
- In vitro
  - low level of G→T and G→C mutations[1]
  - repaired by *E. coli* DNA glycosylase MutM[2]



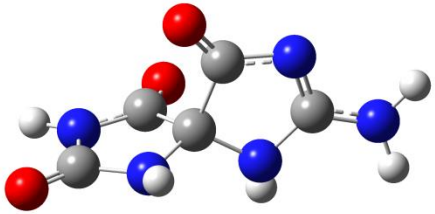
8-Oxoguanine (8-oxoG)



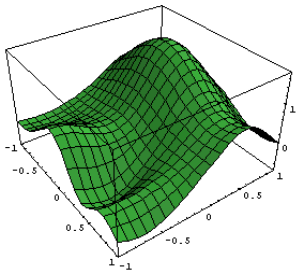
- Most common in vivo (3-4 lesions per 10^7 bases)[1]
- Generated at the rate of ca 2000 lesions per cell per day
- Elevated levels observed in patients with neurological disorders such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS)
- Elevated levels observed in patients with acute lymphoblastic leukemia, and a variety of cancers of the breast, colon, kidney, lung and skin.
- In vitro:
  - nucleobase bypass (88%) and G→T mutations (7%)[2]
  - repaired by *E coli* DNA glycosylases MutM (8-oxoG:C,T,G), Nei (8-oxoG:A) and MutY (8-oxoG:A) and human hOGG1[2,3]



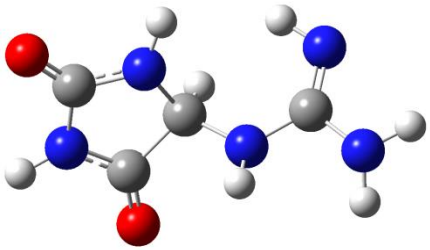
Spiroiminodihydantoin (Sp)



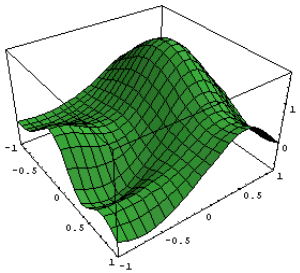
- Produced in vivo following chromate exposure, a known human lung carcinogen[1]
- In vitro
  - nucleobase bypass of 9%
  - higher mutation frequency than 8-oxoG
  - G→T (27-41%) and G→C (57-72%) mutations[2]
  - Strong block to DNA strand replication[2]
  - repaired by *E. coli* Nei (Sp:C,G,A), Nth (Sp:C,G) and MutM (Sp:C,G) [2]
  - Not repaired by human hOGG1[2]



Guanidinohydantoin (Gh)



- Has not yet been isolated from human tissue or fluids
- In vitro data
  - Nucleobase bypass of 75-80%
  - Higher mutation rate than 8-oxoG
  - G→T (1.4%) and G→C (98%) mutations[2]
  - Strong block to DNA strand replication[2]
  - Repaired by *E coli* Nei (Sp:C,G,A), Nth (Sp:C,G) and MutM (Sp:C,G)
  - Not repaired by human hOGG1 [2]



Methodology

Geometry Optimization:

(a) B3LYP/6-31+G(d,p)

(b) IEF-PCM//B3LYP/6-31+G(d,p) (for acyl migrations)

Single points in solution: IEF-PCM/B3LYP/aug-cc-pVTZ

Relative energies in solution:

ΔE_{gas}(B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p))

+ ΔZPE(B3LYP/6-31+G(d,p))

+$\Delta\Delta G_{sol}$(IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p)

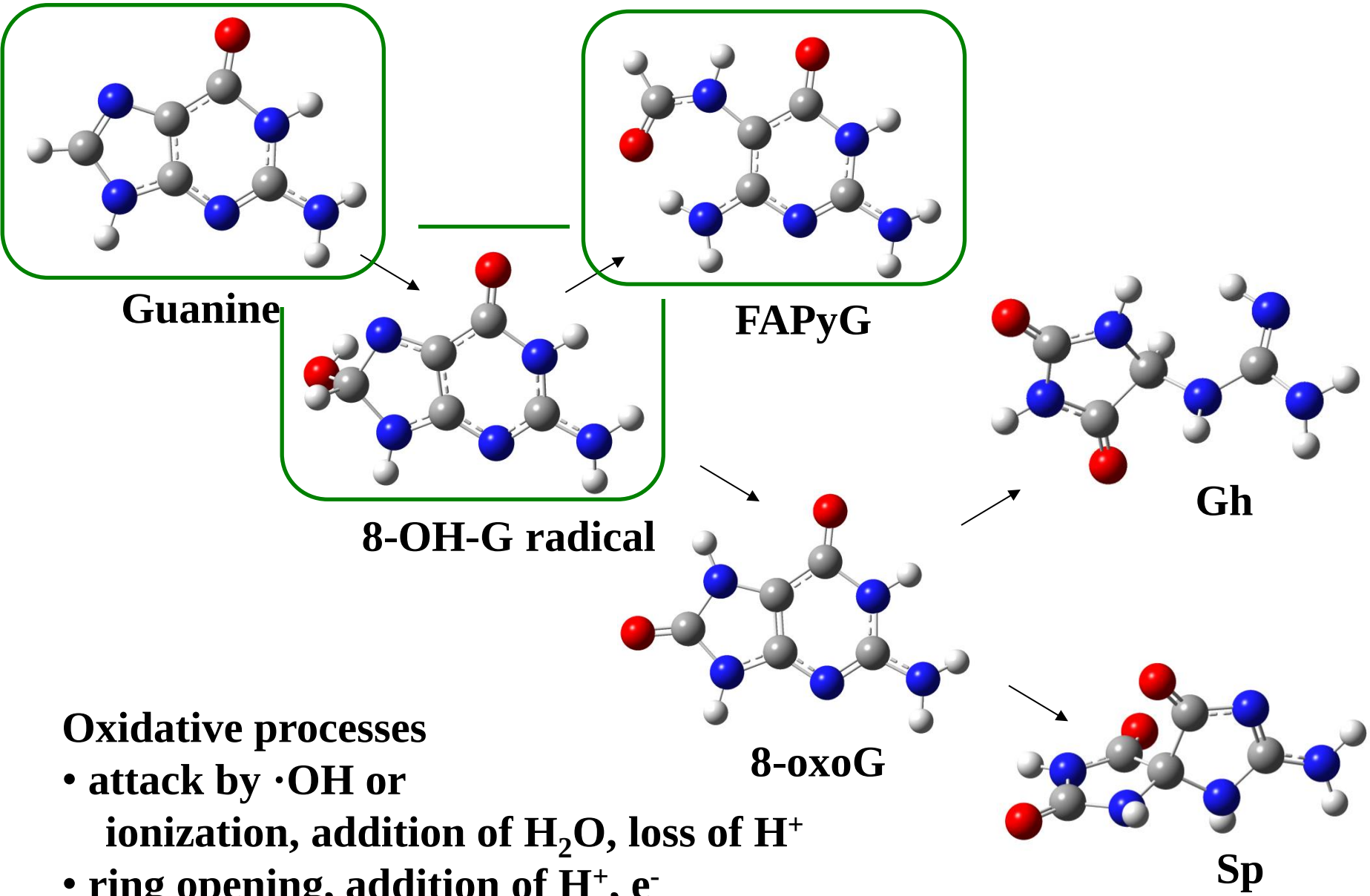
Tautomeriztions assisted by one or more waters

Cystosine/cytosine cation as proton donor / acceptor

Guanine/guanine radical cation as electron donor / acceptor

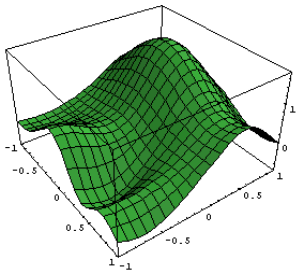
Munk, Burrows, Schlegel, *Chem. Res. Tox*. 2007, *20*, 432-444.
Munk, Burrows, Schlegel., *J. Am. Chem. Soc.* 2008, *130*, 5245.
Ye, Munk, Miller, Cogbill, Burrows, and Schlegel, *Chem. Res. Toxicol*. 2009, DOI:10.1021/tx800402y

Guanine to 8-OH-G and FAPyG



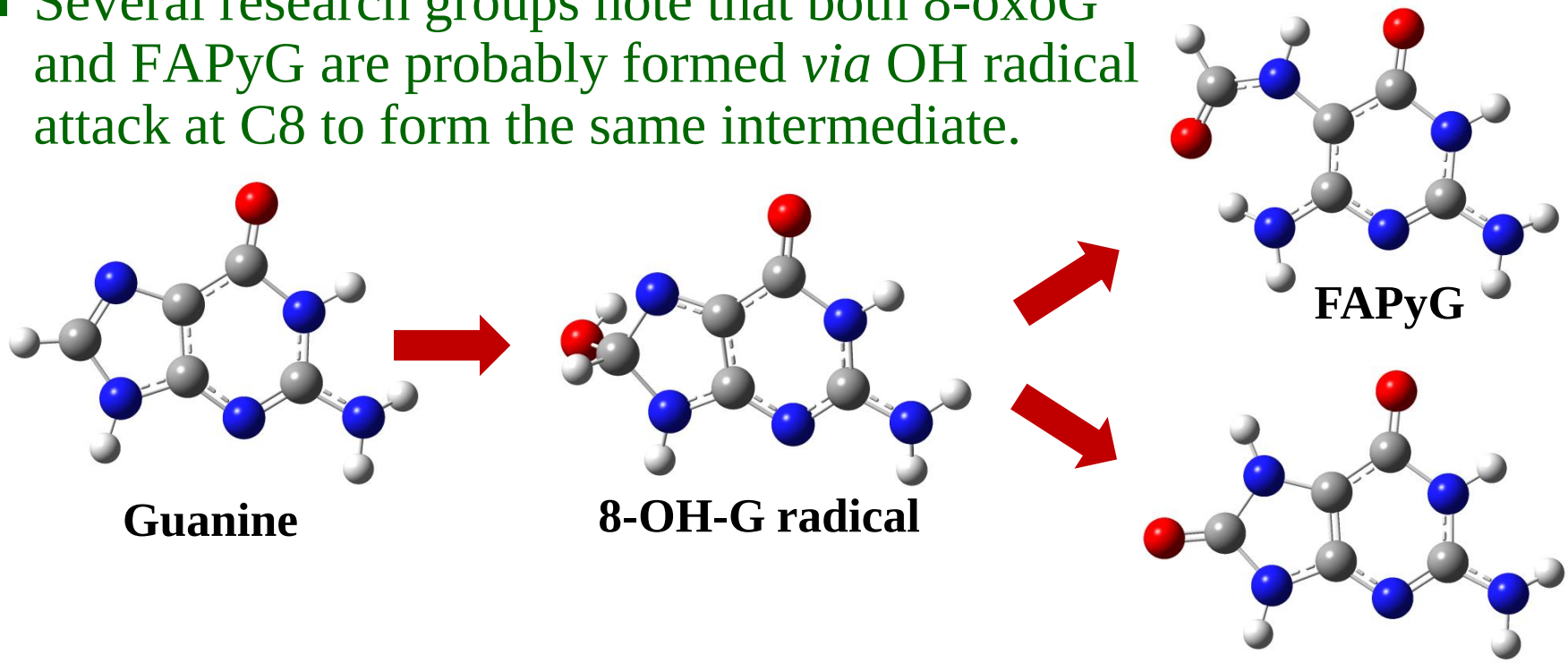
Oxidative processes

- **attack by ·OH or ionization, addition of H_2O, loss of H^+**
- **ring opening, addition of H^+, e^-**



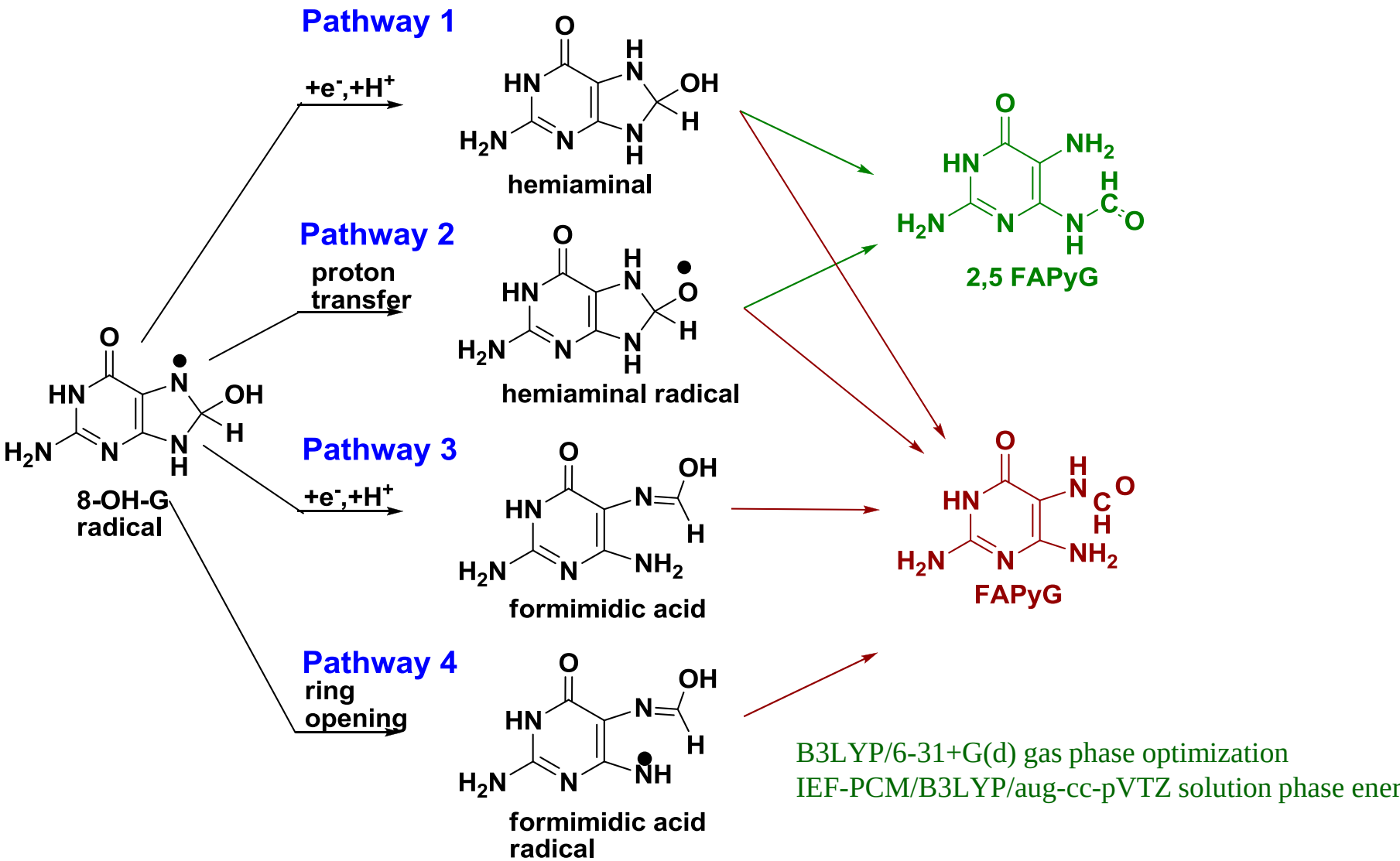
Experimental Studies of the Oxidation Mechanism

- Several research groups note that both 8-oxoG and FAPyG are probably formed *via* OH radical attack at C8 to form the same intermediate.



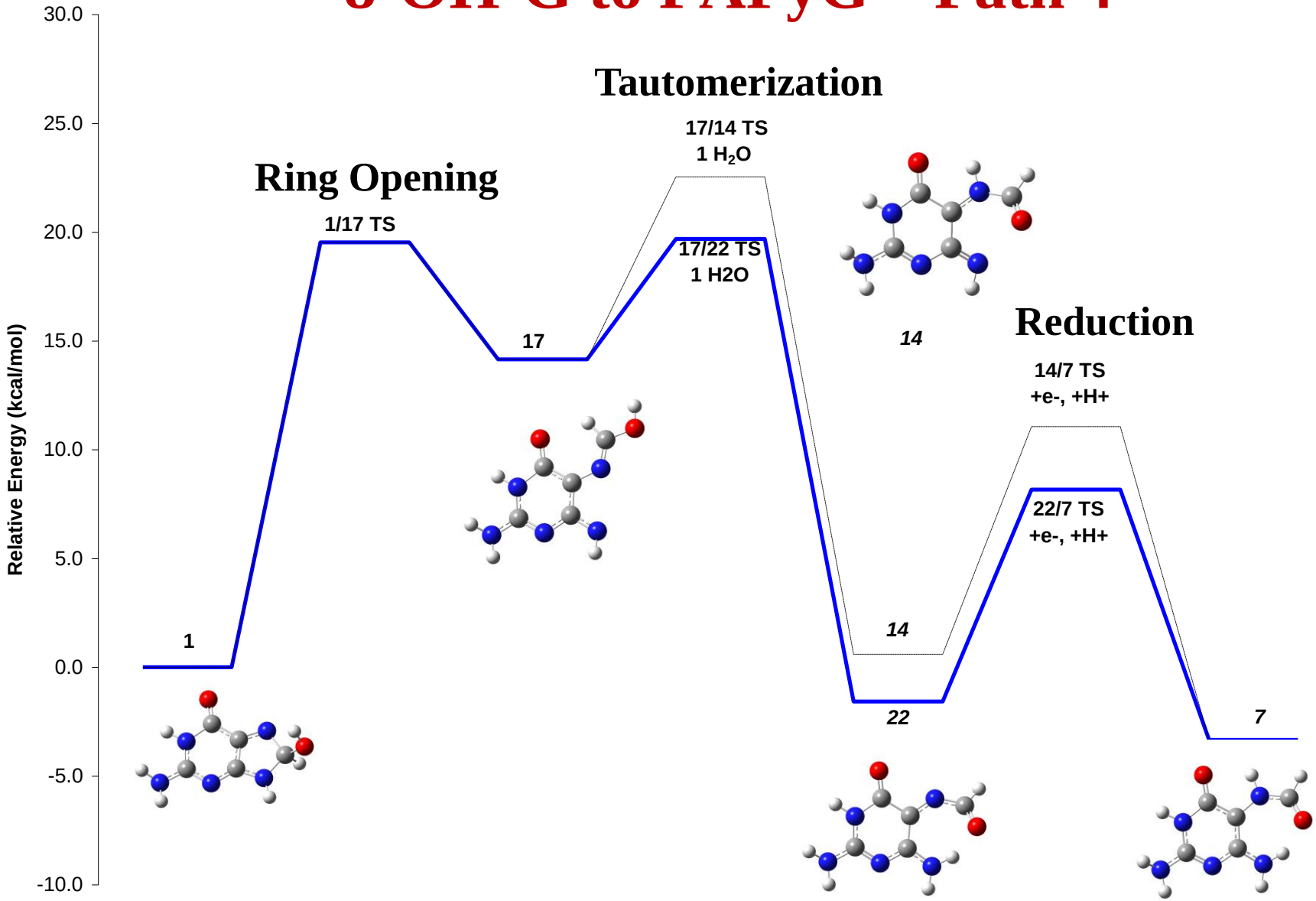
Steenken, S. *Chem. Rev.* 1989, *89*, 503. Breen et al. *Free Radical Biol. Med*. 1995, *18*
Burrows et al. *Chem. Rev*. 1998, *98*, 1109. Candeias et al. *Chem. Eur. J*. 2000, *6*, 475.
Evans et al. *Mutat. Res.-Rev. Mutat. Res. 2004*, 567, 1. Kalam et al. *Nucleic Acids Res*. 2006, *34*, 2305. Birincioglu et al. *J. Am. Chem. Soc*. 2003, *125*, 11607. Neeley et al. *Chem. Res. Toxicol*. 2006, *19*, 491.
Ober, M., et al.; J. Am. Chem. Soc. 2005, 127, 18143-18149. Jiang, Y.L., et al.; J. Org. Chem. 2005, 70, 141-149.

Formation of FAPyG from 8-OH-G•



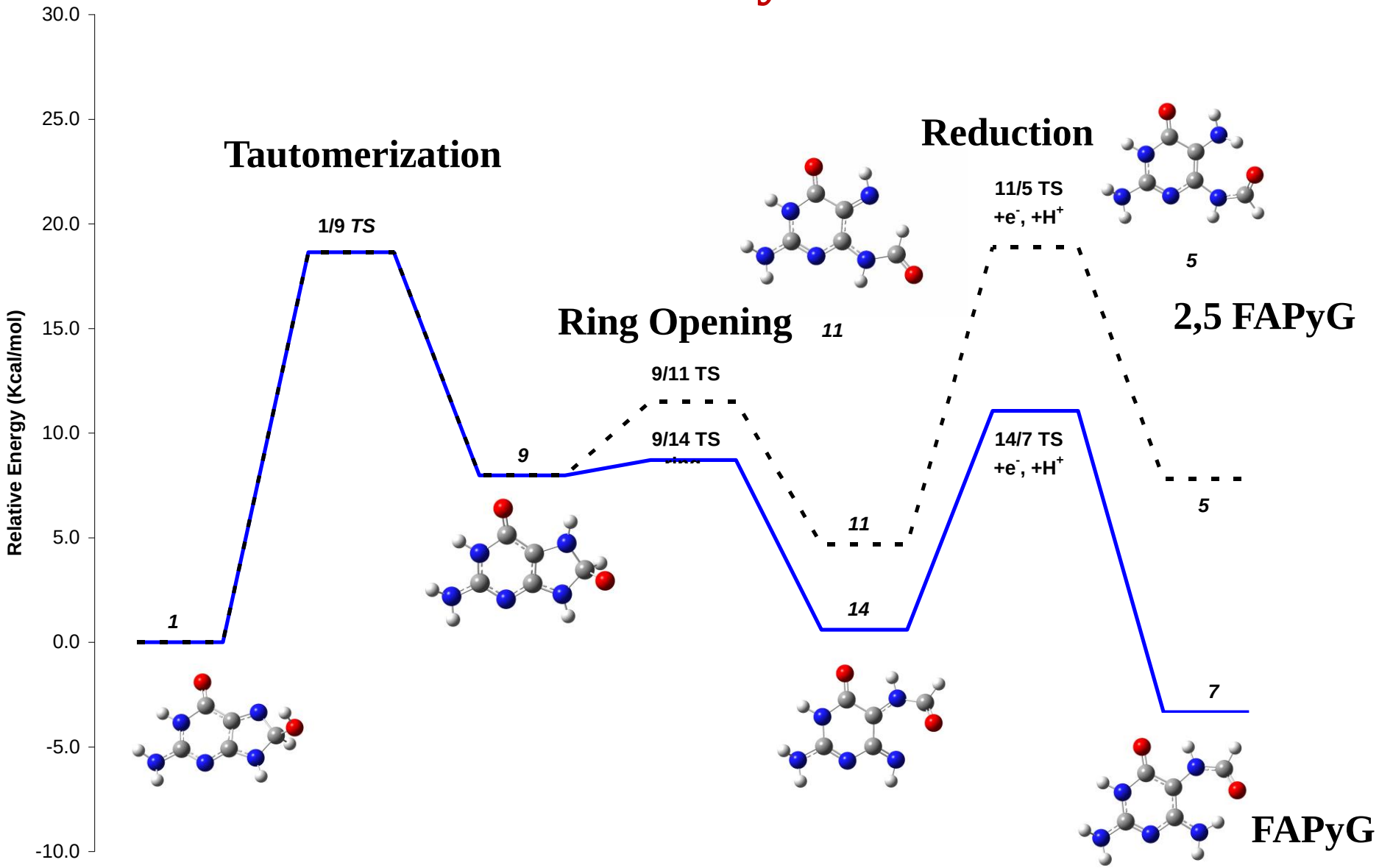
B. M. Munk, C. J. Burrows, H. B. Schlegel, *Chem. Res. Toxicol.* 2007, *20*, 432-444

8-OH-G to FAPyG – Path 4

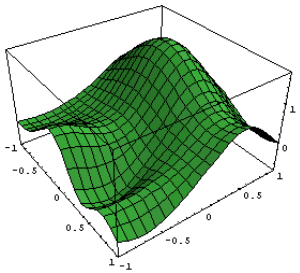


All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).

8-OH-G to FAPyG – Path 2



All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).

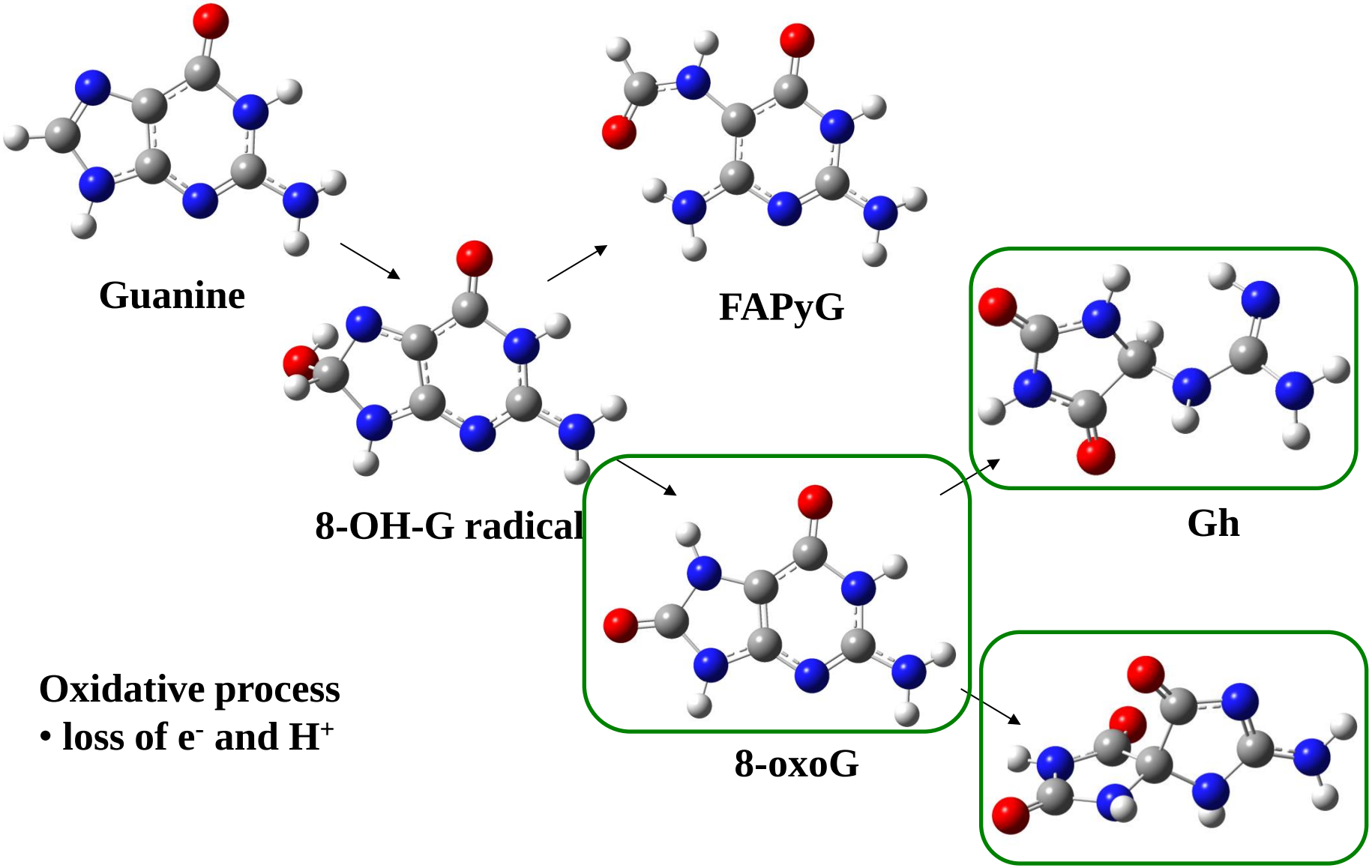


Results

***G to FAPyG*:**

- Of the four reaction pathways studied for formation of FAPyG, Pathway 2, via the 8-oxyguanine radical appears to be the kinetically favored pathway.
- 2,5 FAPyG, a thermodynamically less stable intermediate, may also be formed and IR and NMR calculations suggest that its presence should be differentiable experimentally.

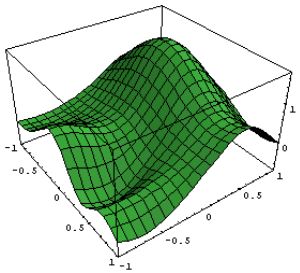
8-oxoG to Gh and Sp



Oxidative process

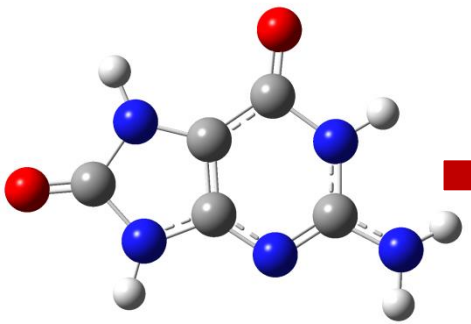
- **loss of e^- and H^+**

Munk, Burrows, Schlegel *J. Am. Chem. Soc.* 2008, *130*, 5245-56.

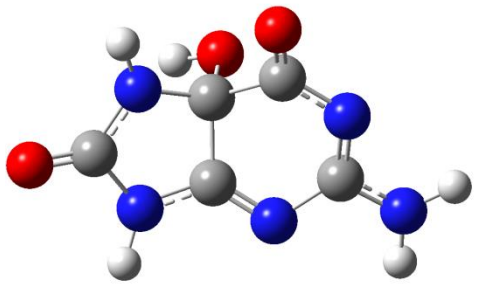


Experimental Studies of 8-Oxoguanine Oxidation

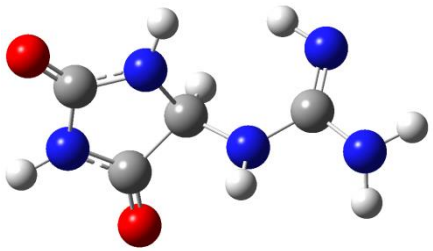
ds DNA
ss DNA, T<4°C
Nucleosides, pH<6



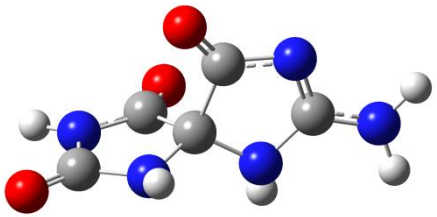
8-oxoG



5-OH-OG



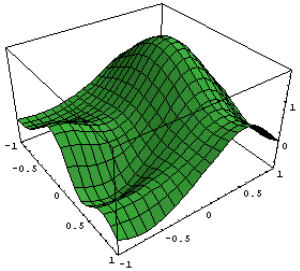
Gh



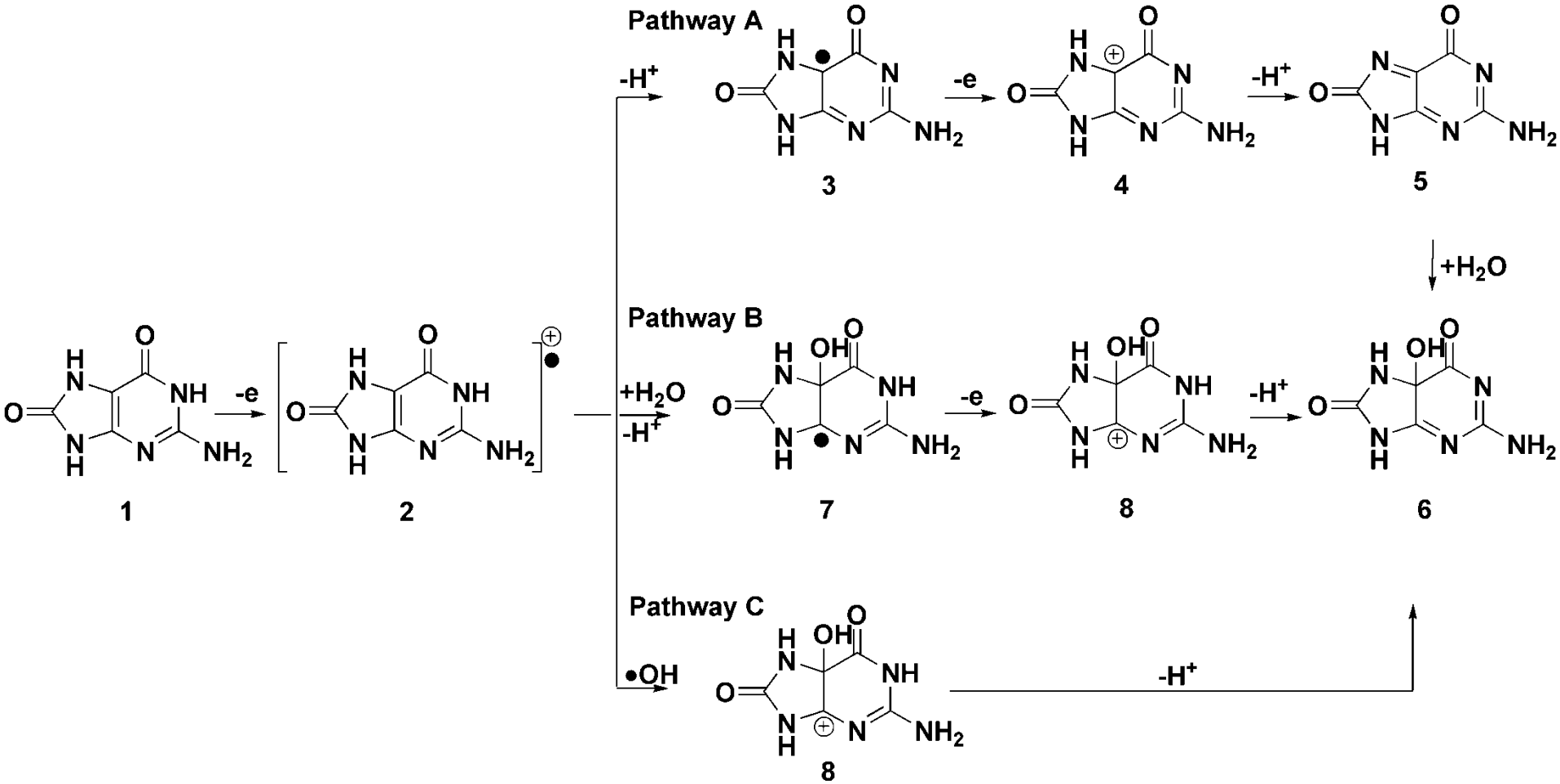
Sp

ss DNA, T>20°C
Nucleosides, pH>7

Luo et al., *Org. Lett.*, 2000, *2*, 613; *Org. Lett.*, 2001, *3*, 280; *Chem. Res. Toxicol.*, 2001, *14*, 927.
Kornyushyna et al., *Biochemistry*, 2002, *41*, 15304.
Ye et al., *J. Am. Chem. Soc.*, 2003, *125*, 13926.
McCallum et al., *J. Am. Chem. Soc.* 2004, *126*, 16777.
Jia et al., *Biochemistry*, 2005, *44*, 13342; *Biochemistry*, 2005, *44*, 6043.

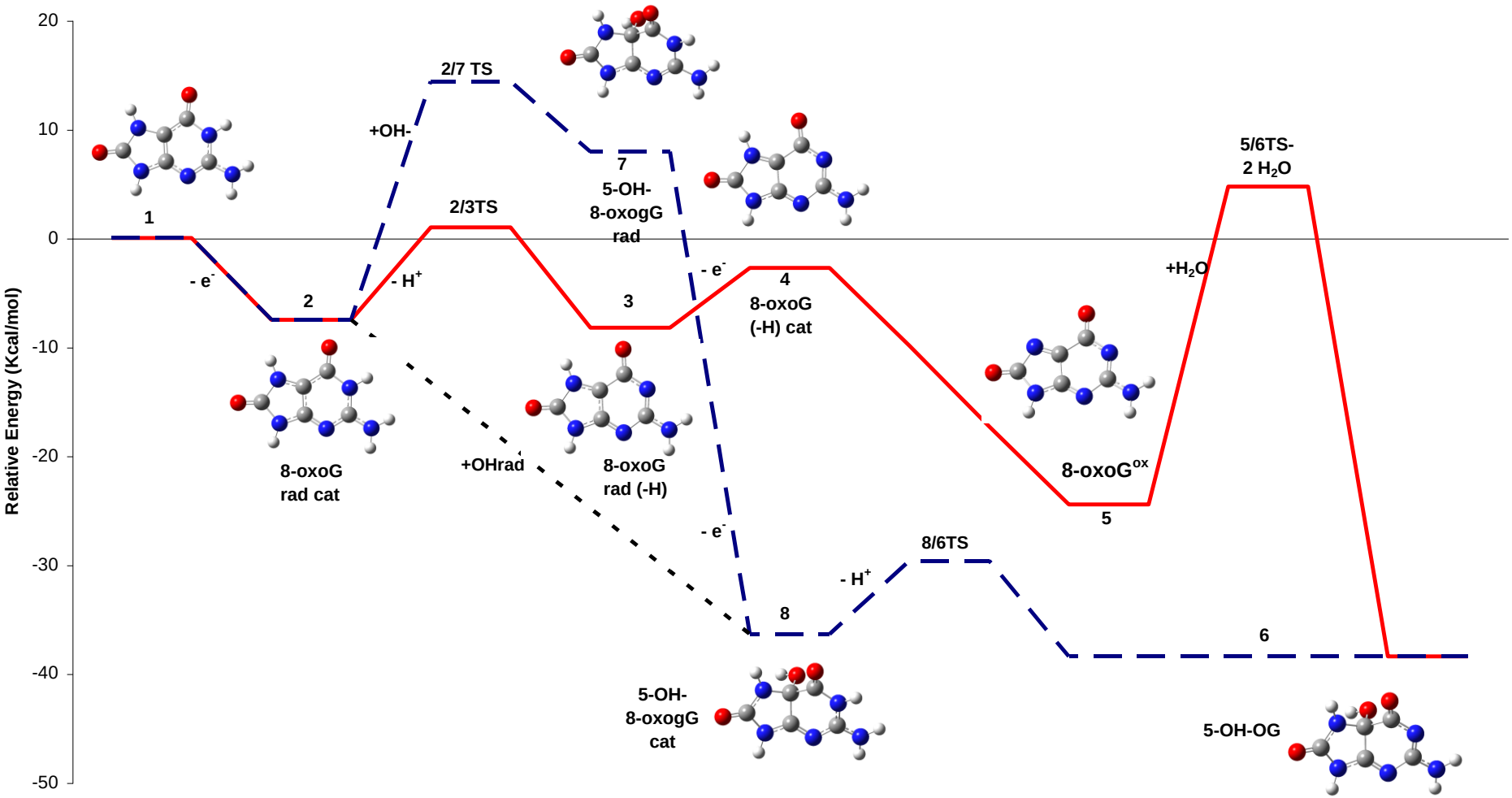


8-oxoG to 5-OH-OG Pathways



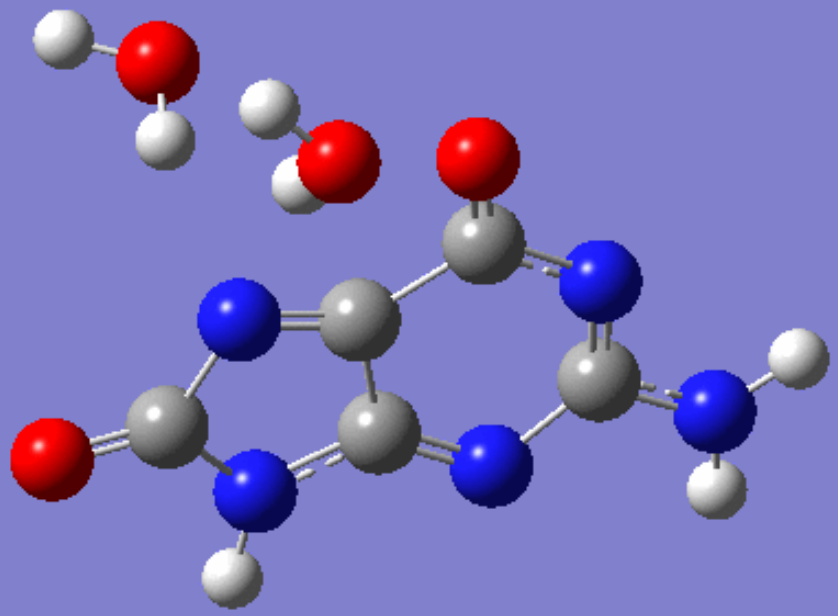
Munk, Burrows, Schlegel., *J. Am. Chem. Soc.* 2008, *130*, 5245-56.

8-oxoG to 5-OH-OG

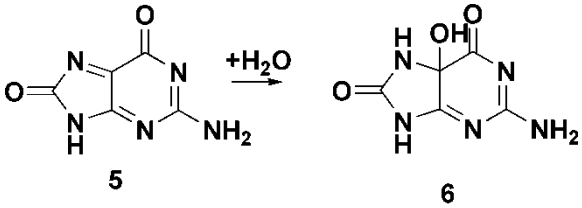


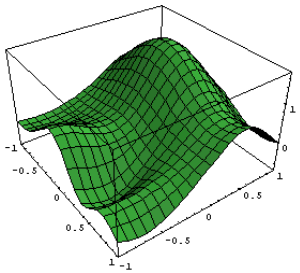
Pathway A —— **Pathway B** — — **Pathway C** ·····

All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).



Addition of water to imine



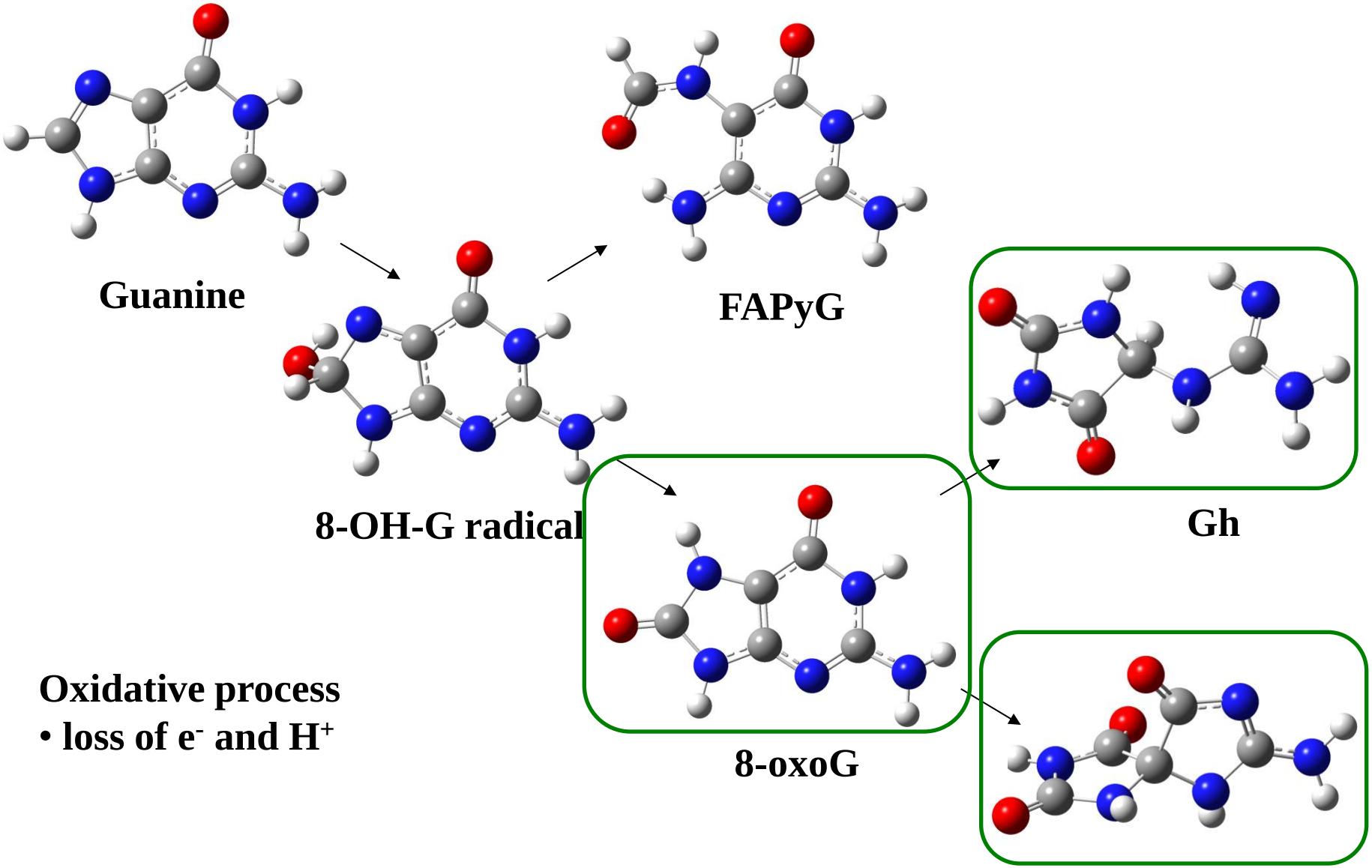


Results

8-oxoG to 5-OH-OG:

- All three pathways are energetically feasible mechanisms.
- Pathway A may be kinetically favored over Pathway B. Both Pathways A and B depend on the local concentration of oxidizing agent.
- Pathway C, addition of ·OH, has lower barriers than either A or B, but will be in competition with other biochemical reactions for the reactive oxygen species, ·OH, and may play a smaller role in the production of 5-OH-OG.

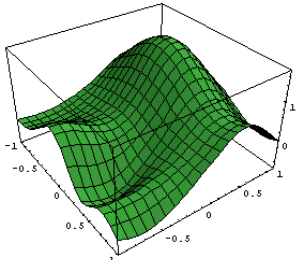
5-OH-OG to Gh and Sp



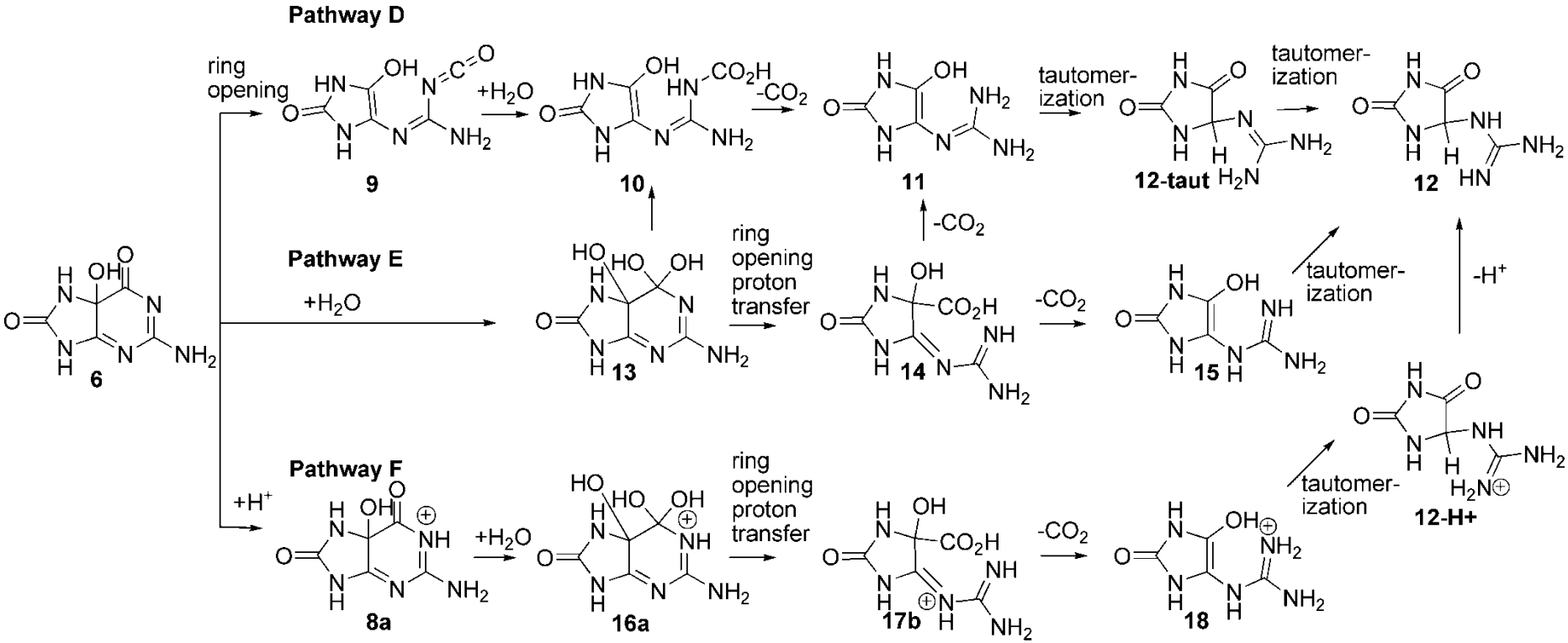
Oxidative process

- **loss of e^- and H^+**

Munk, Burrows, Schlegel *J. Am. Chem. Soc.* 2008, *130*, 5245-56.

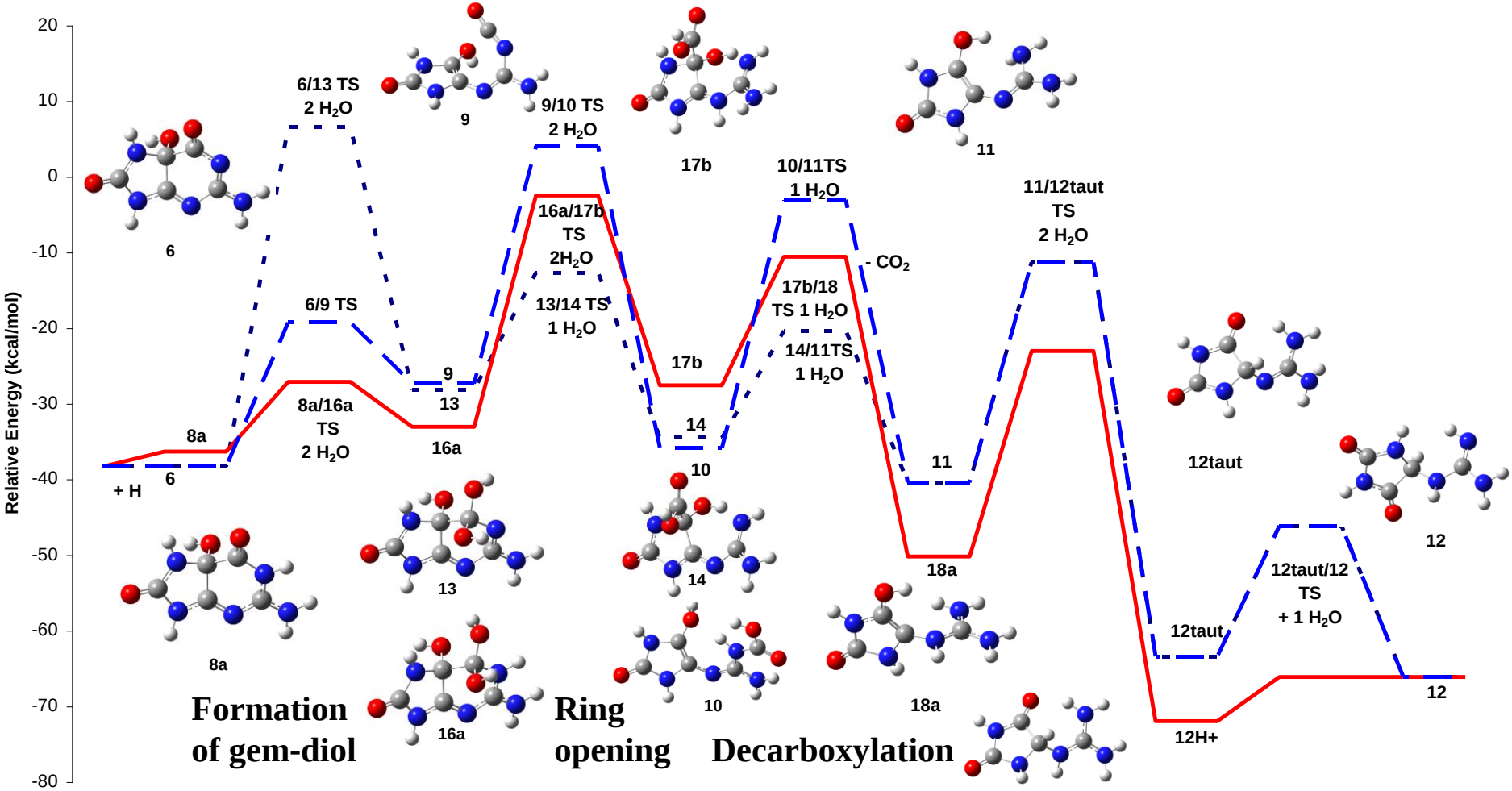


5-OH-OG to Gh Pathways



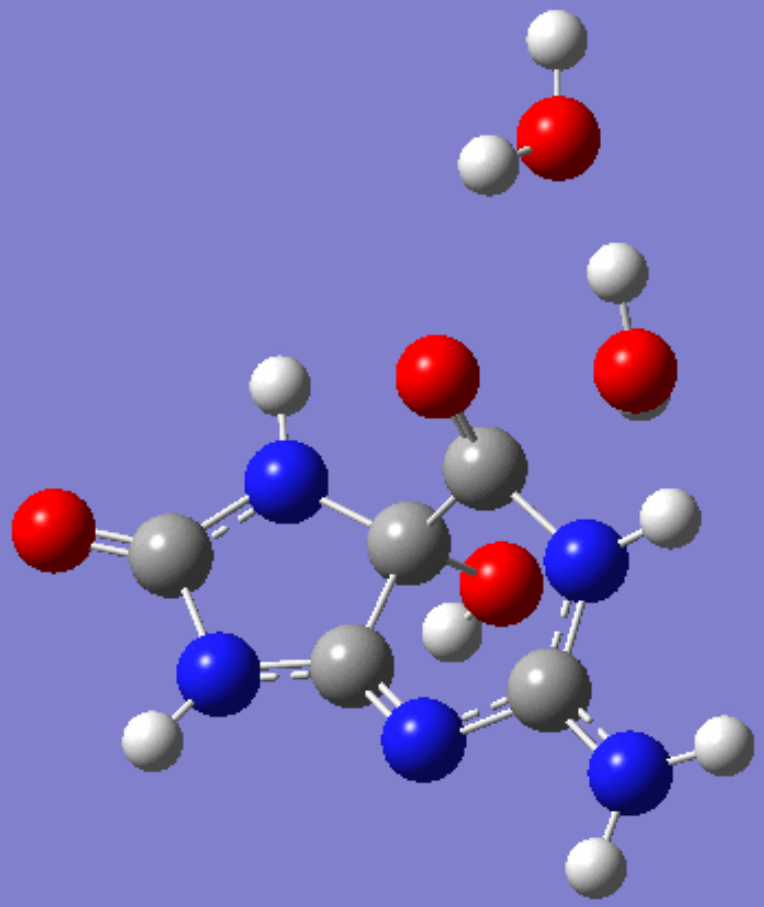
Munk, Burrows, Schlegel, *J. Am. Chem. Soc.* 2008, *130*, 5245-56.

5-OH-OG to Gh

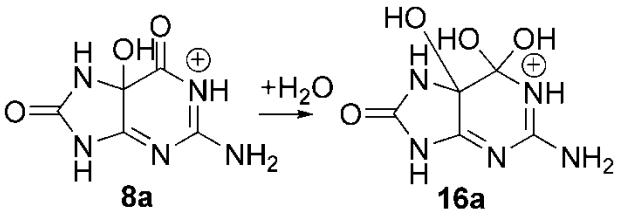


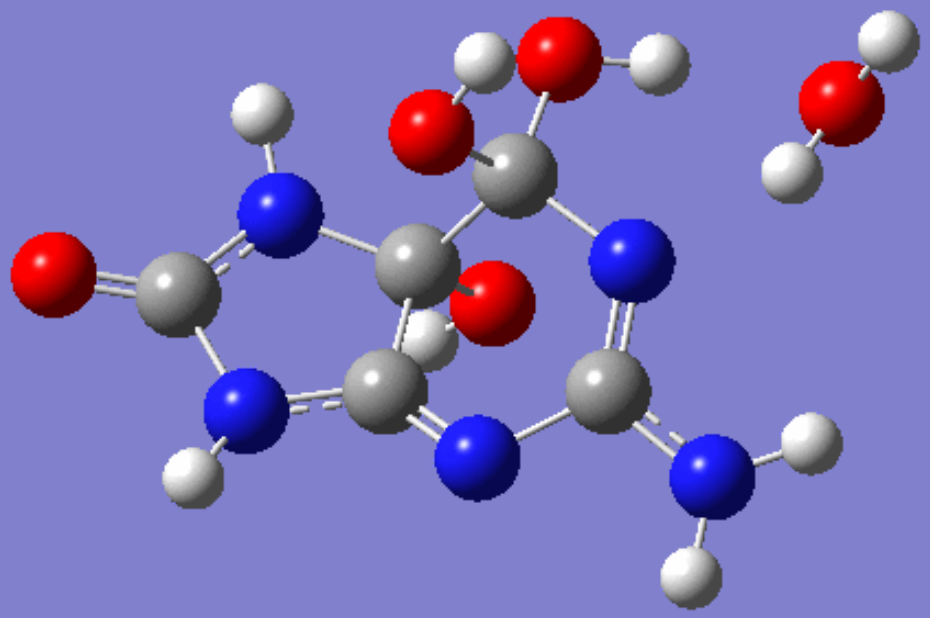
Pathway D —— —— Pathway E ····· Pathway F ————

All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).

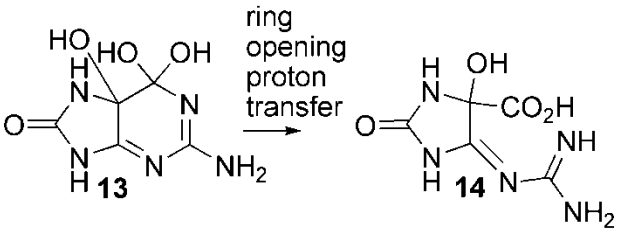


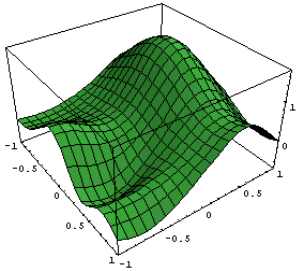
Formation of gem diol



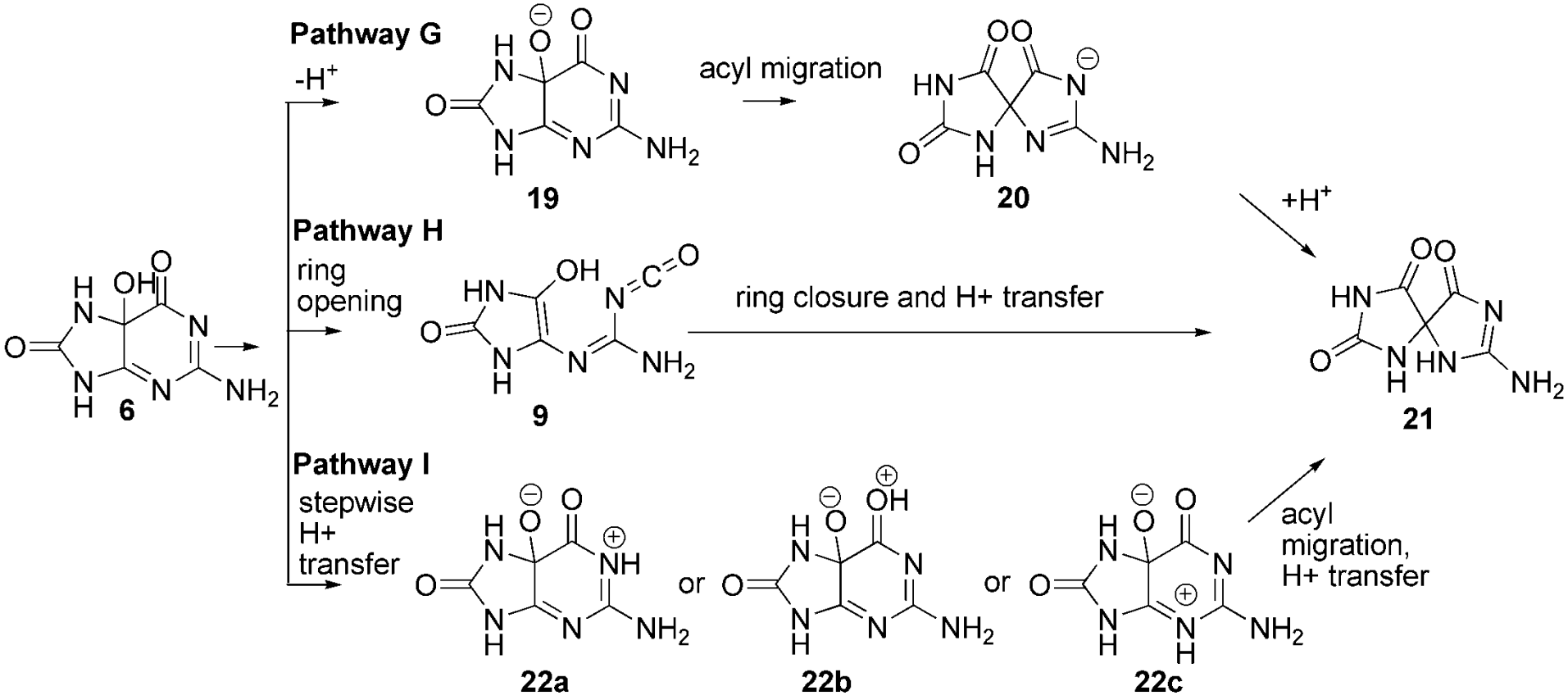


Cleavage of C6-N1 bond



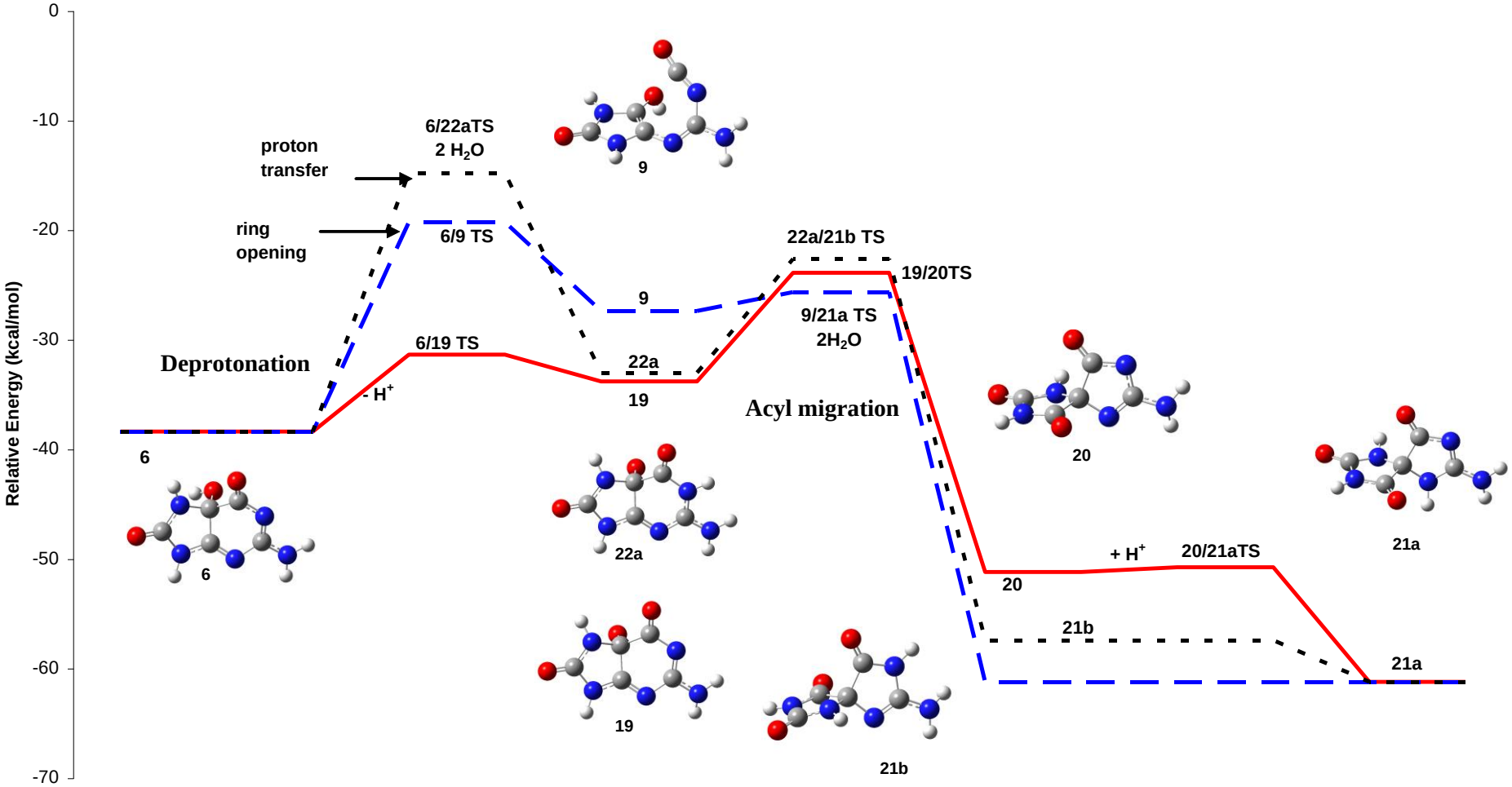


5-OH-OG to Sp Pathways



Munk, Burrows, Schlegel., *J. Am. Chem. Soc.* 2008, *130*, 5245-56.

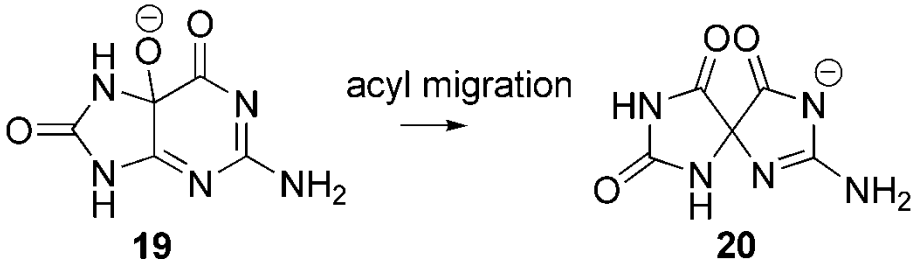
5-OH-OG to Sp

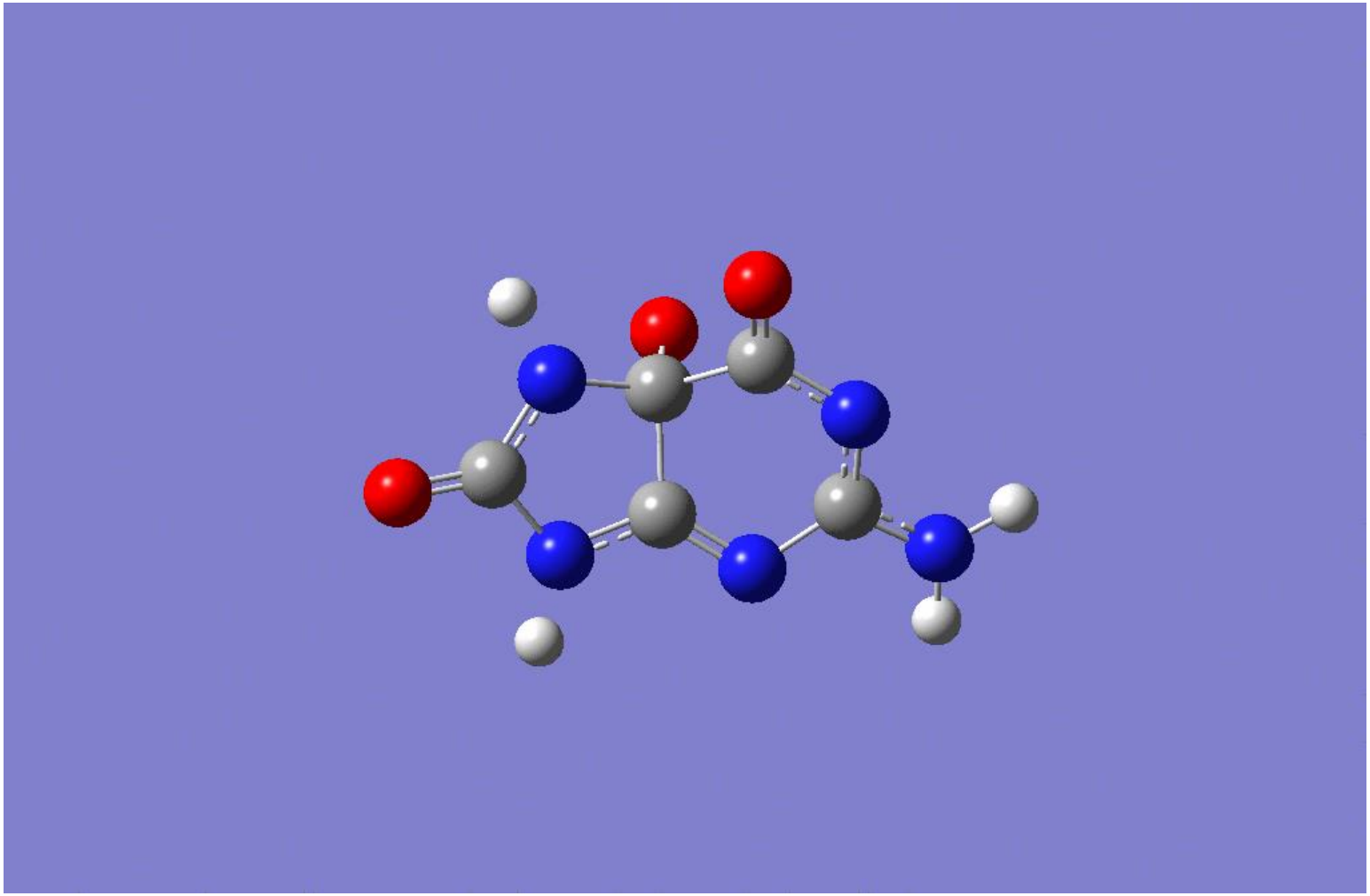


Pathway G ——— Pathway H — — Pathway I ·····

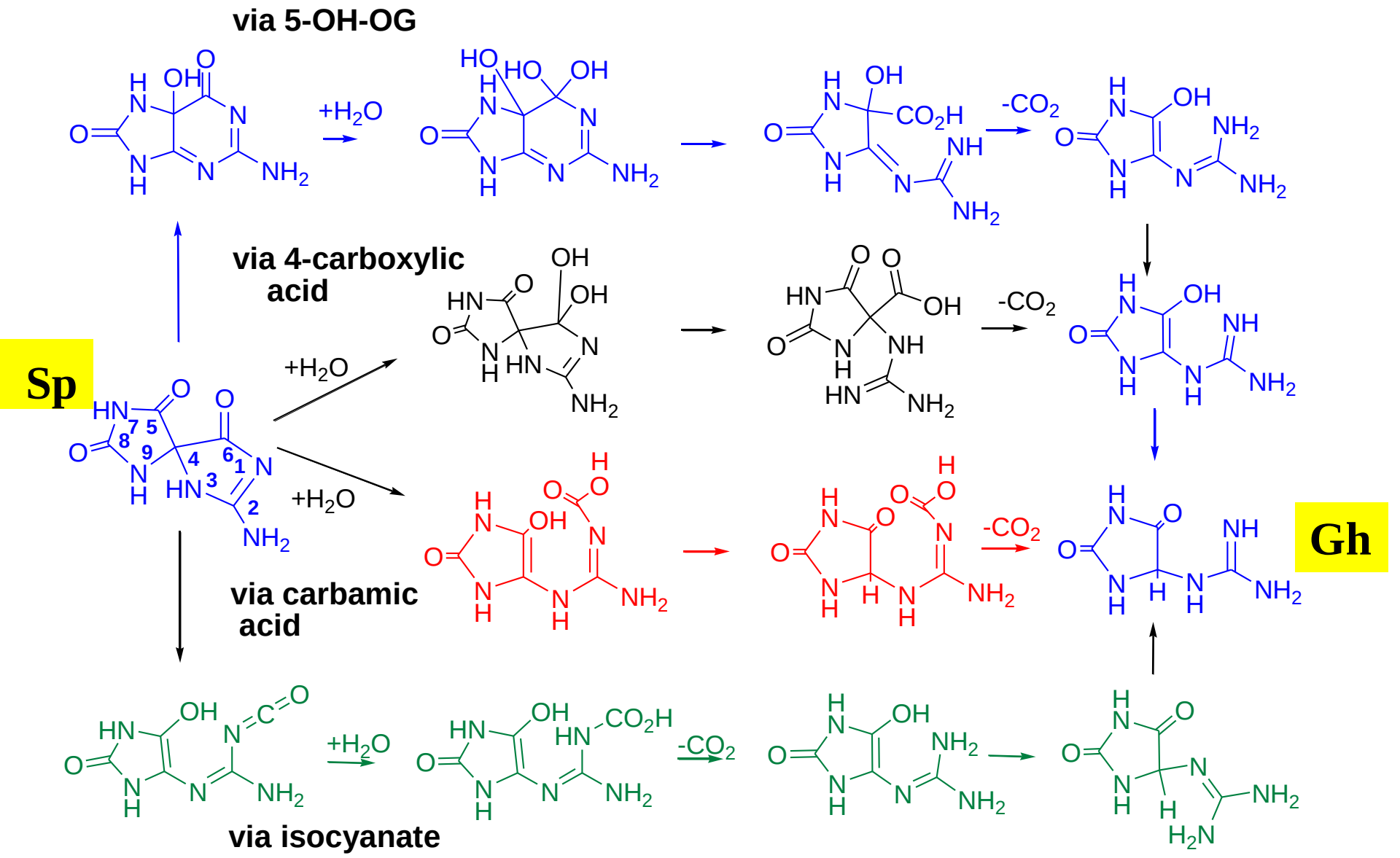
All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).

Acyl Migration

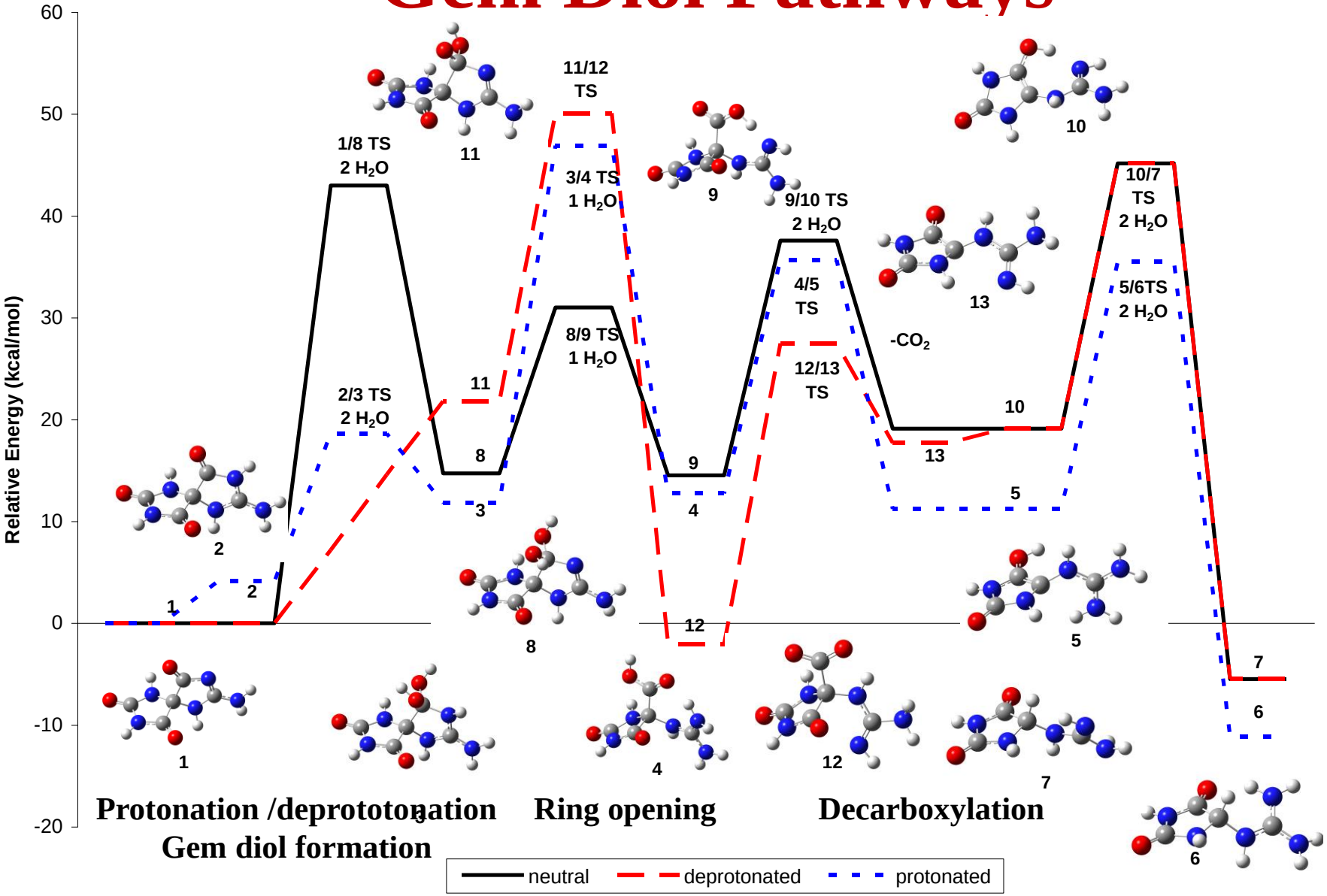




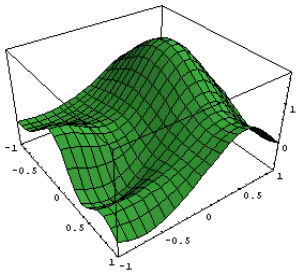
Sp to Gh Pathways



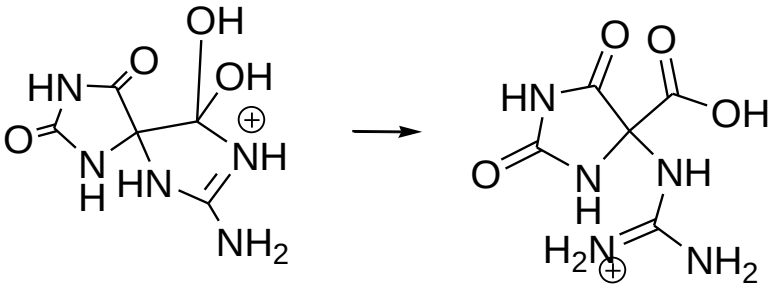
Gem Diol Pathways

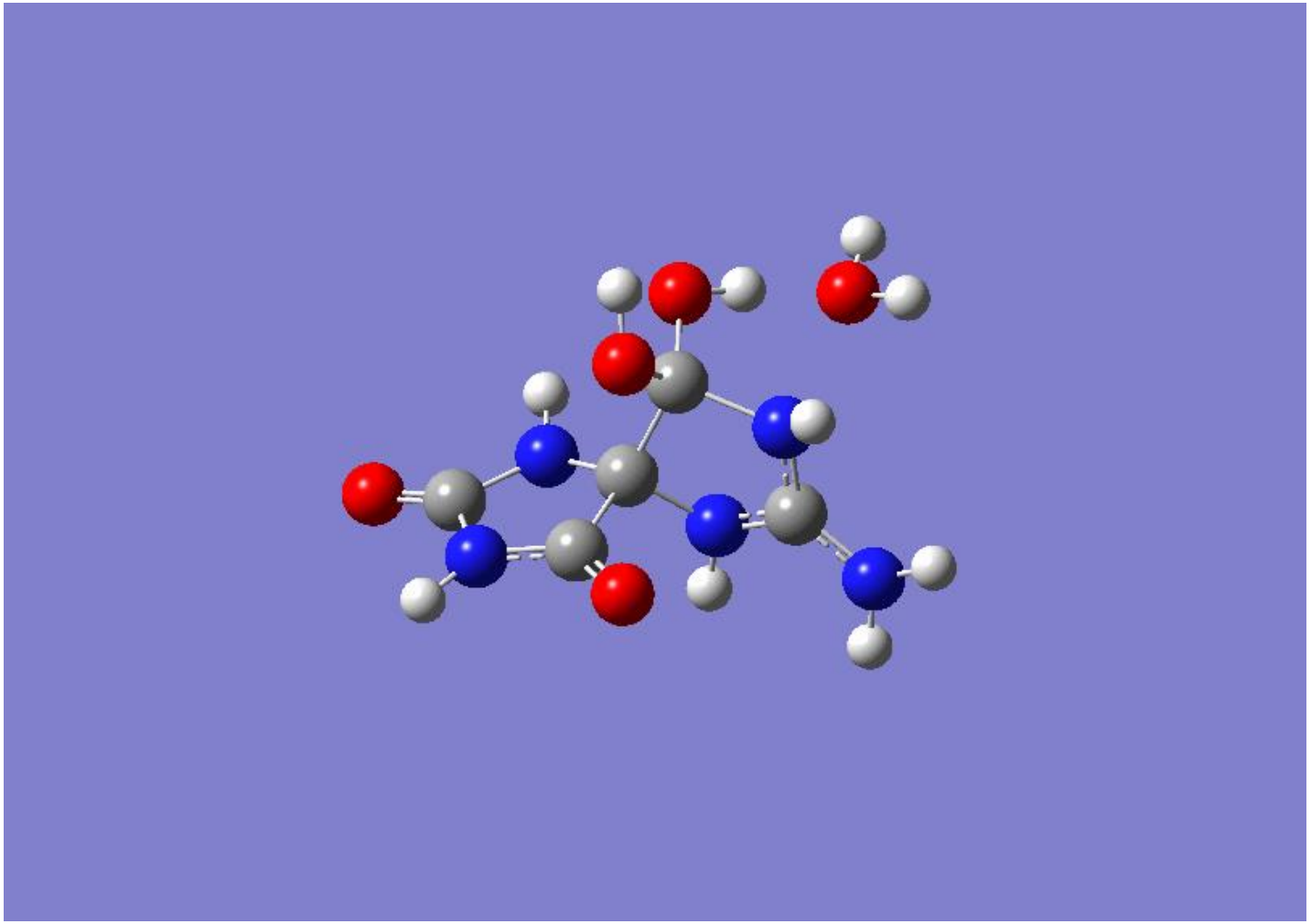


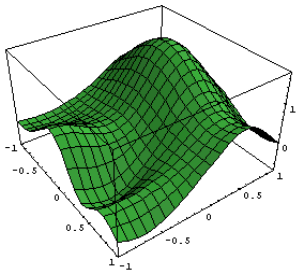
All relative energies were calculated at IEF-PCM/B3LYP/aug-cc-pVTZ//B3LYP/6-31+G(d,p).



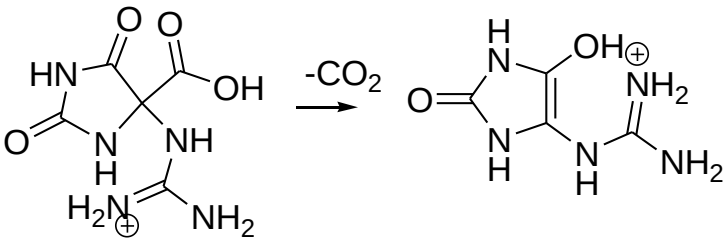
Ring Opening of Protonated Diol

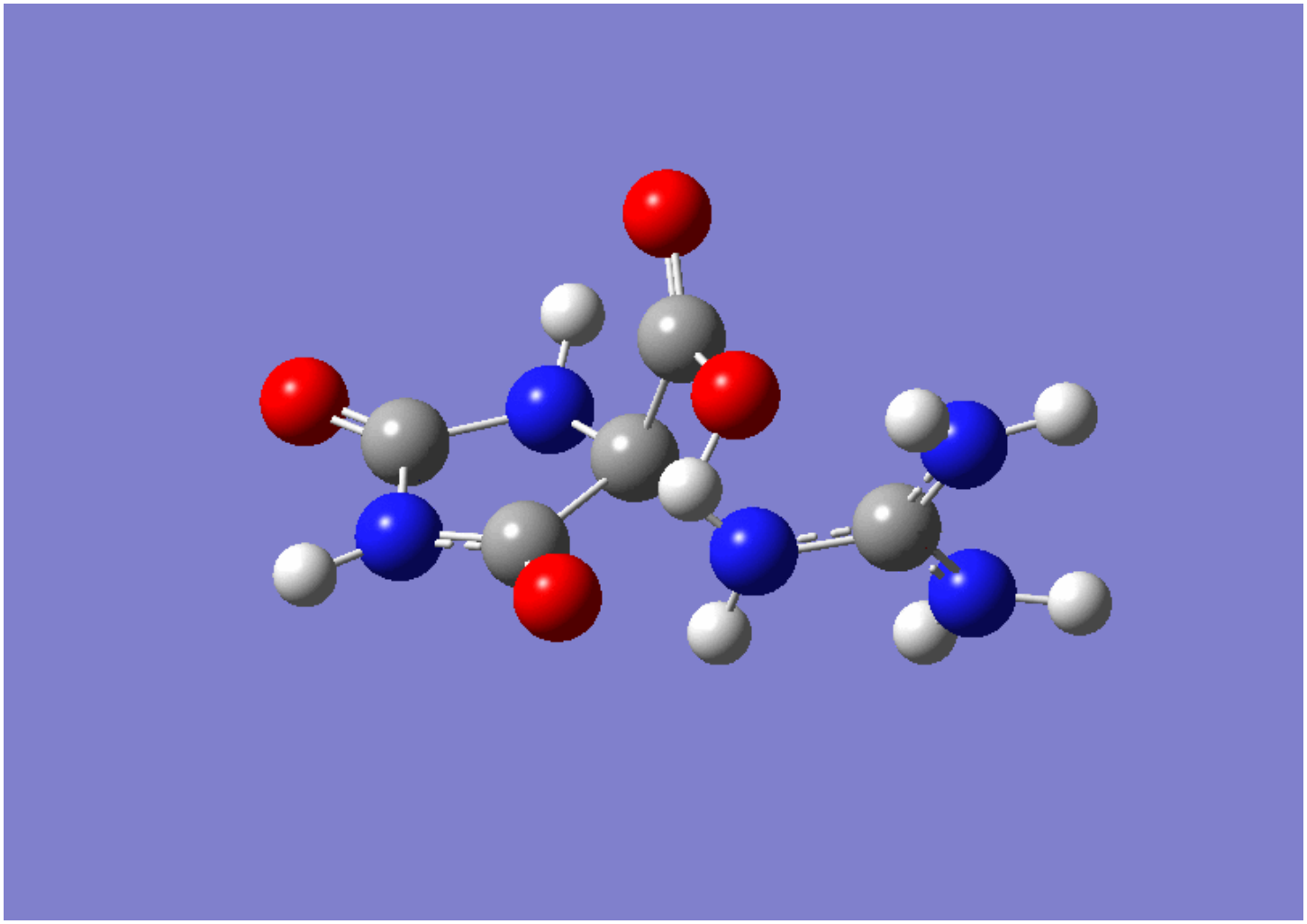


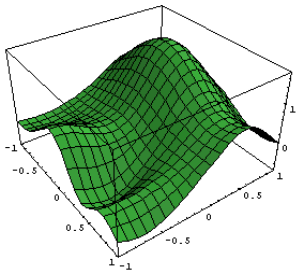




Decarboxylation to form Gh Enol



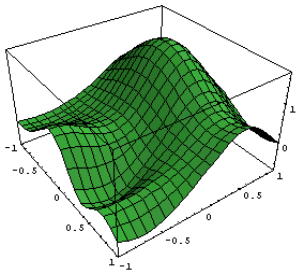




Results

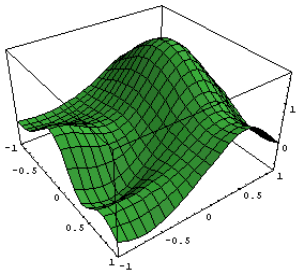
Sp to Gh:

- Most likely pathway is via a Sp C6 gem diol.
- Protonation of Sp at the N1 position is predicted to facilitate formation of the gem diol.
- Consistent with experiment, formation of Gh from Sp is predicted to be favored at low pH.



pK_a and Redox Potentials

- Oxidation of guanine occurs in water at physiological pH
- Reaction pathways and barrier heights depend on the protonation / deprotonation of intermediates
- Agents that oxidize guanine can also oxidize other intermediates
- Need a protocol to estimate the pK_a's and redox potentials along the guanine oxidation pathway



Calculation of pK_a's

$$pK_a = \frac{1}{2.303\,RT}\Delta G_{(aq)}$$

$$\begin{tikzcd}
HA_{(g)}(R) \arrow[r, "\Delta G^\circ_{(g)}"] \arrow[d, "\Delta E_{(dis)}(HA)"'] & A^-_{(g)}(R) \arrow[d, "\Delta E_{(dis)}(A^-)"] & + & H^+_{(g)} \arrow[ddd, "\Delta G^*_{(sol)}(H^+)"] \\
HA_{(g)}(R') \arrow[d, "\Delta G^*_{(sol)}(HA)"'] & A^-_{(g)}(R') \arrow[d, "\Delta G^*_{(sol)}(A^-)"] & & \\
HA_{(aq)}(R') \arrow[r, "\Delta G^*_{(aq)}"] & A^-_{(aq)}(R') & + & H^+_{(aq)}
\end{tikzcd}$$

$$\Delta G^\circ_{(g)} = G^\circ_{(g)}(A^-_R) + G^\circ_{(g)}(H^+) - G^\circ_{(g)}(HA_R)$$

$$\Delta G^*_{(aq)} = G^*_{(aq)}(A^-_{R'}) + G^*_{(aq)}(H^+) - G^*_{(aq)}(HA_{R'})$$

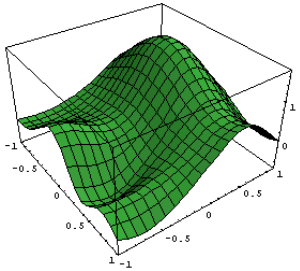
$$E_{dis}(A^-) = G^\circ_{(g)}(A^-_{R'}) - G^\circ_{(g)}(A^-_R)$$

$$E_{dis}(HA) = G^\circ_{(g)}(HA_{R'}) - G^\circ_{(g)}(HA_R)$$

$$\Delta G^*_{solv}(H^+) = G^*_{(aq)}(H^+) - G^*_{(g)}(H^+)$$

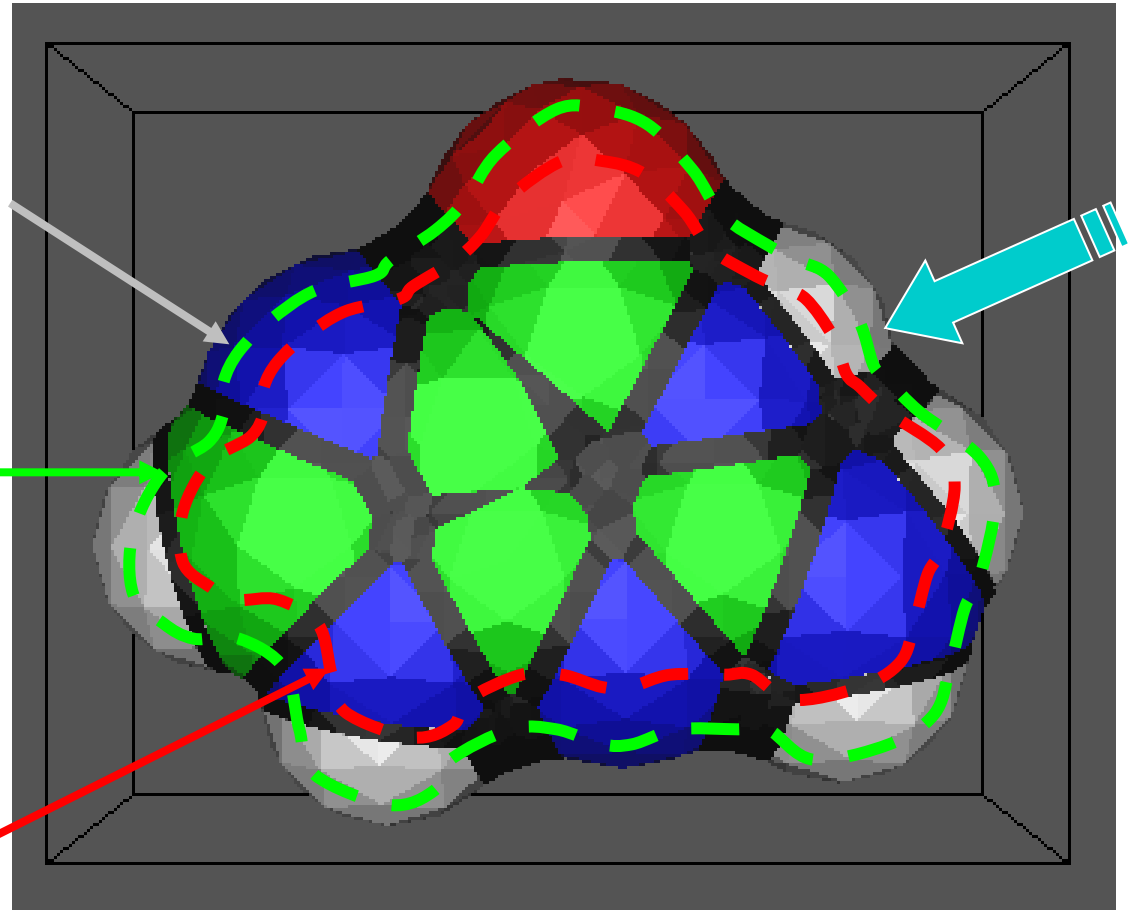
$$\Delta G^*_{solv}(A^-) = G^*_{(aq)}(A^-_{R'}) - G^*_{(g)}(A^-_{R'})$$

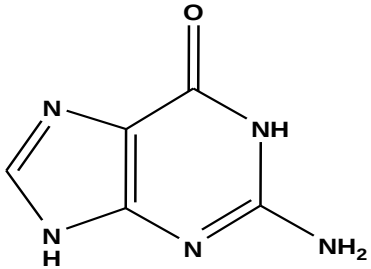
$$\Delta G^*_{solv}(HA) = G^*_{(aq)}(HA_{R'}) - G^*_{(g)}(HA_{R'})$$



Polarizable Continuum Model for Solvation

STANDARD CAVITY FOR NEUTRAL

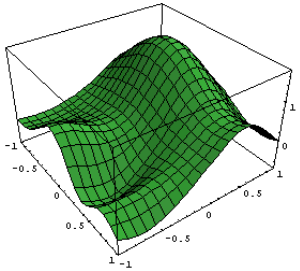




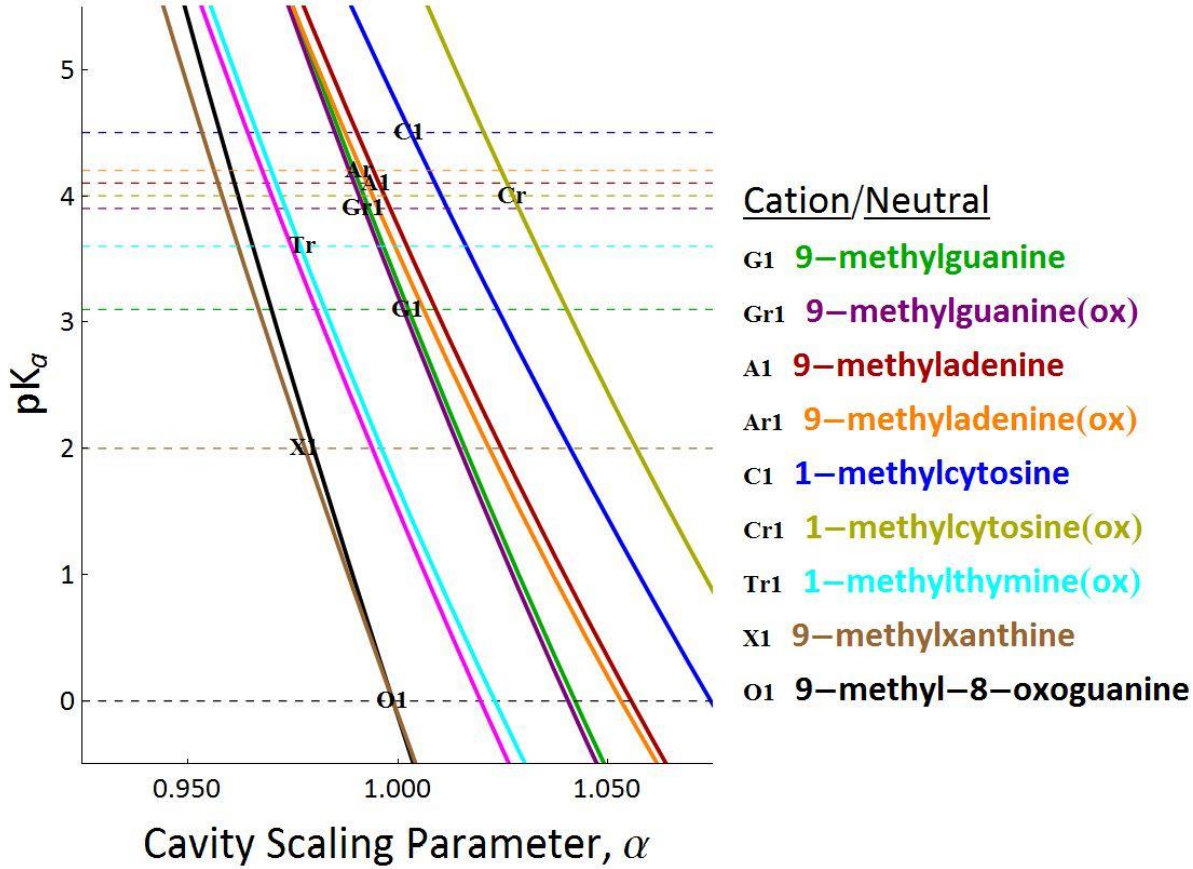
CATION

ANION

$$\Delta G_{(aq)} = \Delta G_{el} + \Delta G_{disp} + \Delta G_{rep} + \Delta G_{cav}$$



Cation Cavity Scaling



B3LYP

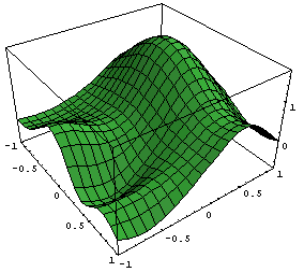
cation $\alpha = 1.000 \pm 0.015$

anion $\alpha = 0.900 \pm 0.050$

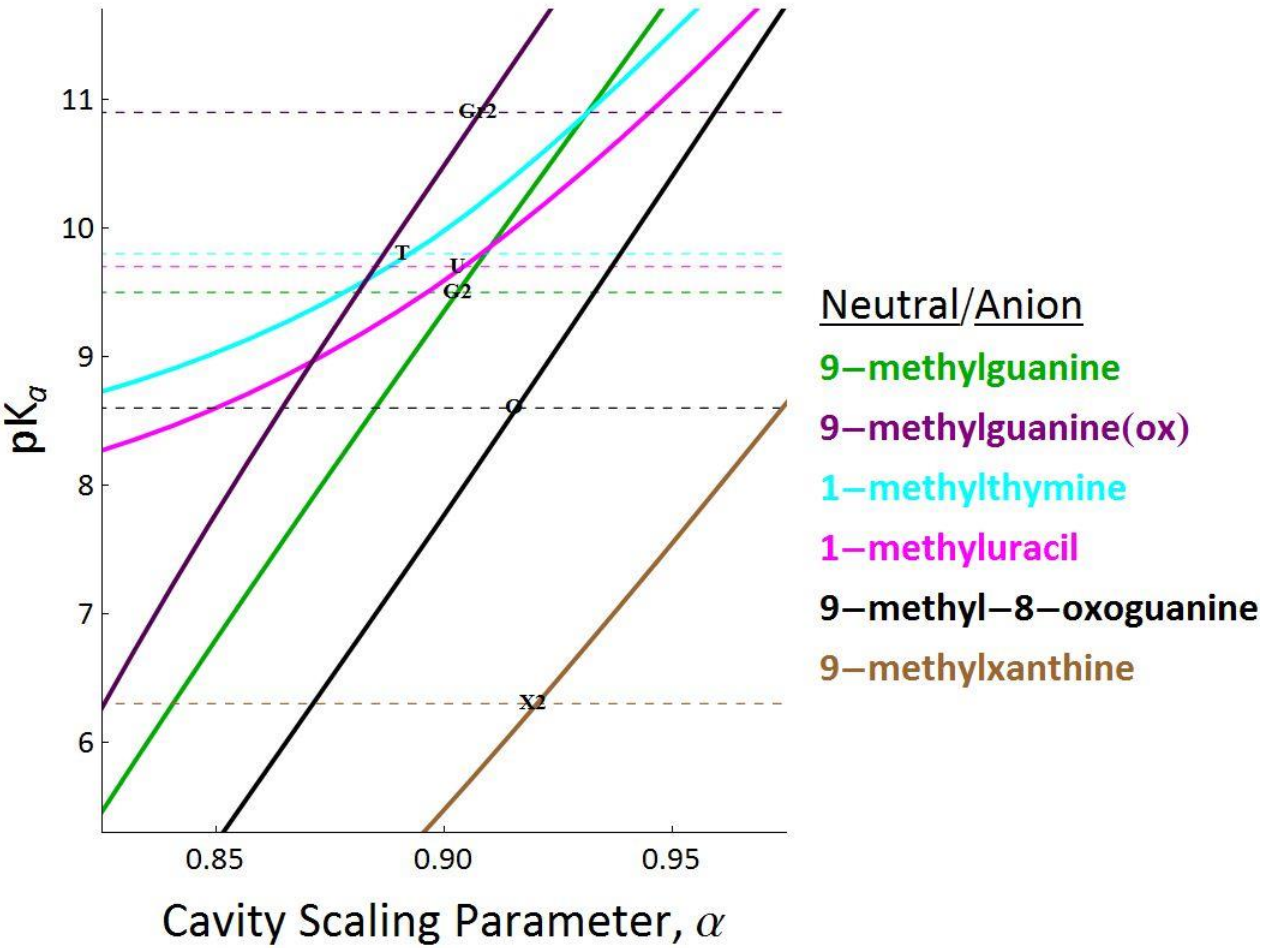
CBS-QB3

cation $\alpha = 0.975 \pm 0.014$

anion $\alpha = 0.925 \pm 0.058$

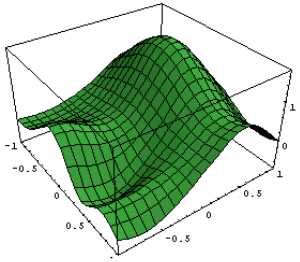


Anion Cavity Scaling

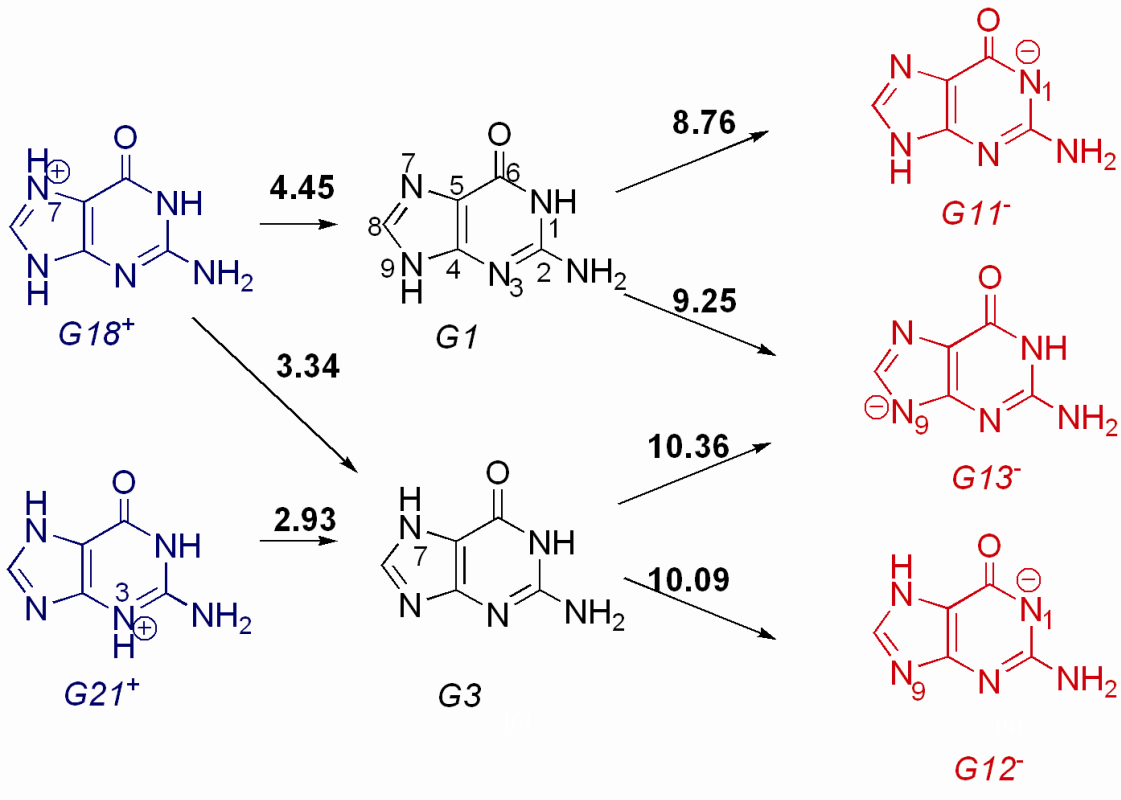


B3LYP $\alpha = 0.900 \pm 0.050$

CBS-QB3 $\alpha = 0.925 \pm 0.058$



Guanine pK_a's

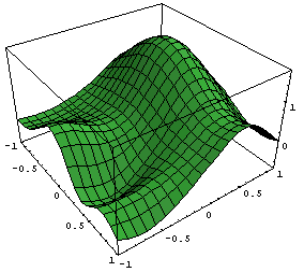


MOL	$\Delta G_{(g)}$ (kcal/mol)	$\Delta G_{(aq)}$ (kcal/mol)	ΔG_{solv} (kcal/mol)
$G18^+$	0.0	0.0	-64.8
$G21^+$	4.3	0.6	-68.9
G1	0.3	1.5	-18.5
G3	0.0	0.0	-19.0
$G11^-$	2.8	0.0	-75.4
$G12^-$	0.0	0.3	-72.0
$G13^-$	0.8	0.7	-73.1

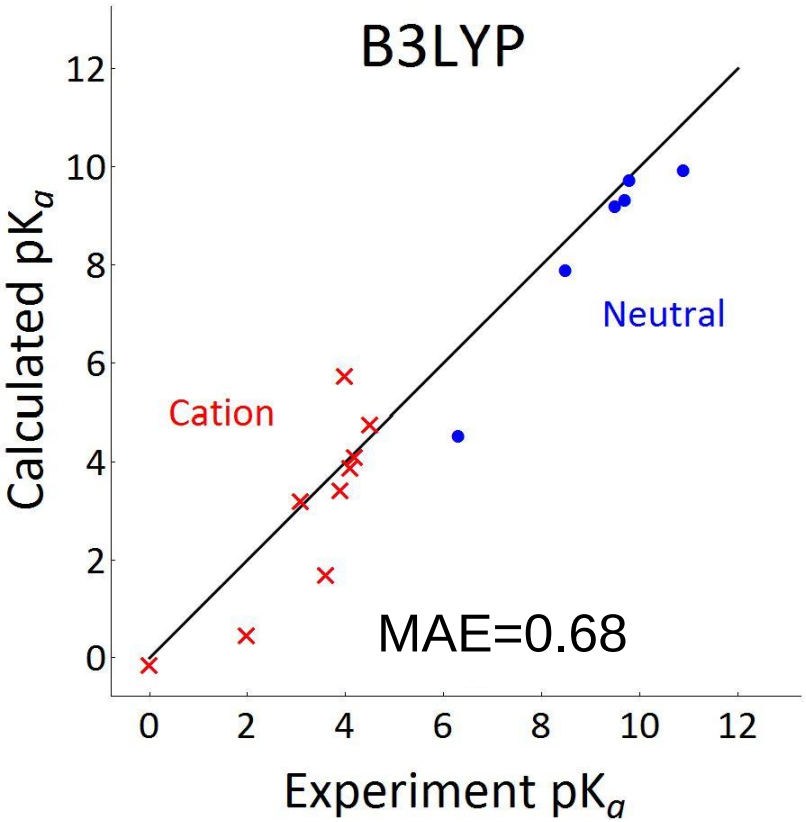
pK_{a1}(calc) = 3.45
pK_{a1}(exp) = 3.2-3.3

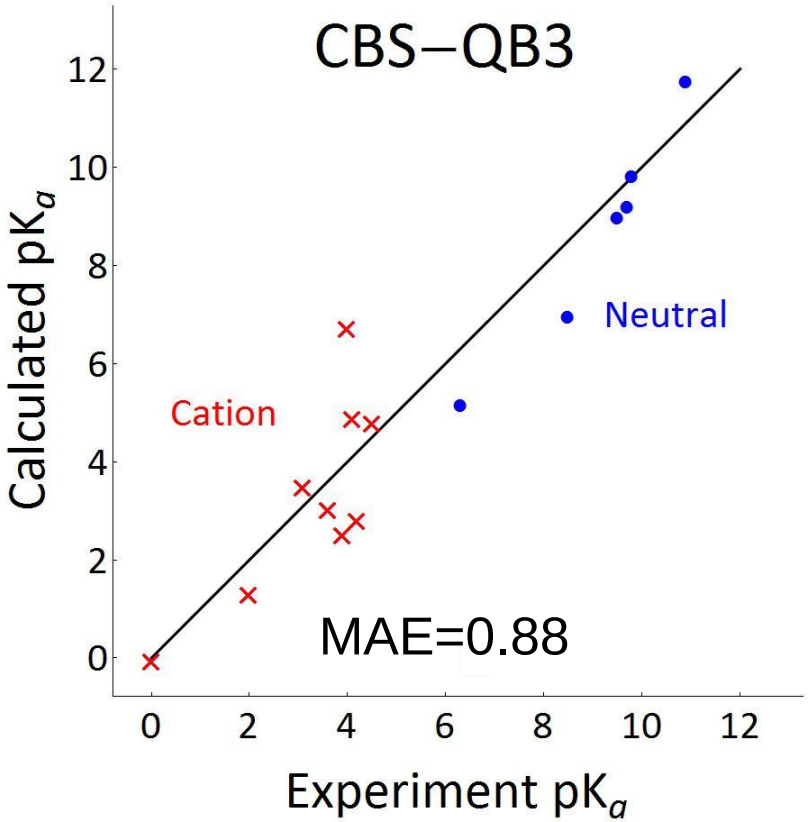
pK_{a2}(calc) = 9.6
pK_{a2}(exp) = 9.2-9.6

Verdolino, V.; Cammi, R.; Munk, B. H.; Schlegel, H. B.; *J. Phys. Chem. B* 2008, *112*, 16860–16873



Nucleobase pK_a's





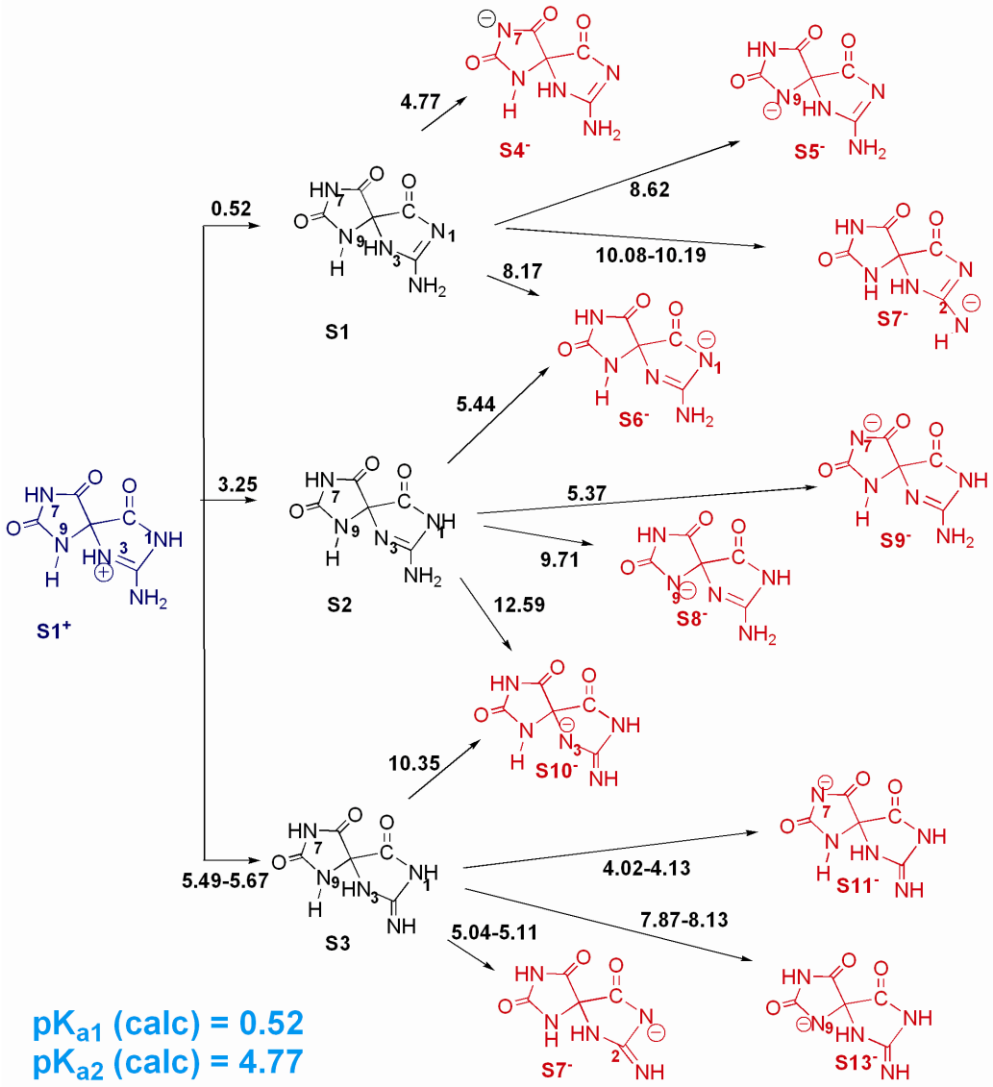
<u>Solvent cavity scaling factors</u>

B3LYP: 1.00 for cations, 0.90 for anions

CBS-QB3: 0.975 for cations, 0.925 for anions

1 pKa = 1.36 kcal/mol = 5.69 kJ/mol

Spiroiminodihydantoin (Sp)

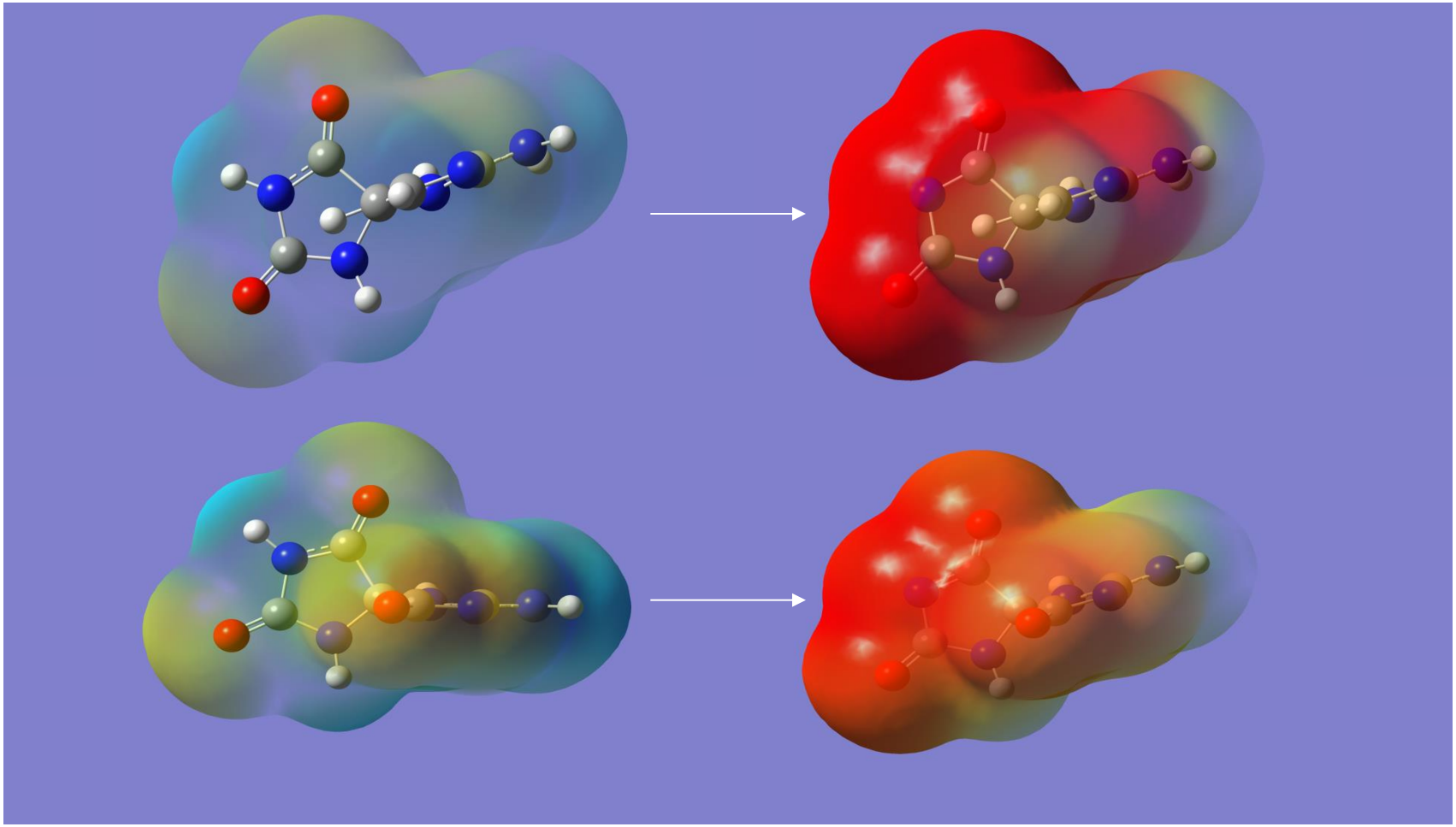


pK_{a1} (calc) = 0.52
pK_{a2} (calc) = 4.77

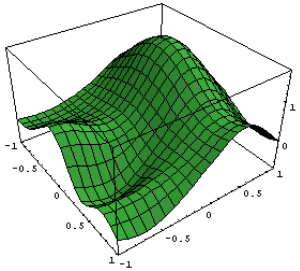
MOL	$\Delta G_{(g)}$ (kcal/mol)	$\Delta G_{(aq)}$ (kcal/mol)	ΔG_{solv} (kcal/mol)
$S1^+$	0.0	0.0	-79.2
S1	0.0	0.0	-26.2
S2	1.2	3.7	-22.6
S3	1.3, 2.0	6.8, 7.0	-19.4,-19.6
$S4^-$	5.2	0.0	-83.0
$S5^-$	11.8	5.3	-83.0
$S6^-$	0.9	4.6	-70.9
$S7^-$	4.0, 0.0	7.3, 7.4	-72.1,-67.4
$S8^-$	18.2	10.5	-83.8
$S9^-$	12.0	4.5	-84.3
$S10^-$	6.4	14.4	-66.3
$S11^-$	1.7, 3.3	5.9, 6.0	-71.2,-72.5
$S13^-$	6.9, 8.5	11.4, 11.3	-70.1,-71.6

A total of 1 cation, 21 neutral and 13 anionic tautomers were examined. These results are for the major tautomers in solution.

Anomalously low pK_a of Sp



	ΔG_g	$\Delta\Delta G_{solv}$	ΔE_{dis}	Calc pK_a
Isomer S1 with C6=CH2	328.26	-56.84	1.58	6.6
Isomer S1 with C6=O (Sp)	325.26	-56.9	2.11	4.8
ΔpKa contribution	+2.2	+0.0	-0.4	+1.8



Calculation of Redox Potentials

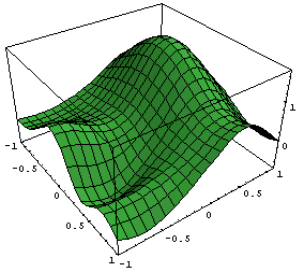
$$\begin{array}{ccccc}
XH^{+\bullet}_{(g)} & + & e^{-}_{(g)} & \xrightarrow{\Delta G^{\circ}_{(g)red}} & XH_{(g)} \\
\downarrow \Delta G_{solv} & & \downarrow \Delta G_{solv} & & \downarrow \Delta G_{solv} \\
XH^{+\bullet}_{(aq)} & + & e^{-}_{(aq)} & \xrightarrow{\Delta G^{*}_{(aq)red}} & XH_{(aq)}
\end{array}$$

$$\frac{1}{2}H_{2\ (aq)} \xrightarrow{\Delta G^{*}_{(aq)ox}} H^{+}_{(aq)} + e^{-}_{(aq)} \qquad E^{abs}_{(aq)}(\mathrm{SHE}) = 4.281\ \mathrm{V}$$

$$XH^{+\bullet}_{(aq)} + \frac{1}{2}H_{2\ (aq)} \rightarrow H^{+}_{(aq)} + XH_{(aq)}$$

$$E^{\circ}_{red}\left(XH^{+\bullet}/XH\right) = \frac{-\Delta G^{*}_{(aq)red}}{nF} - E^{abs}_{(aq)}(\mathrm{SHE})$$

Isse, A. A. and Gennaro, A.; *J. Phys. Chem. B* **2010**, *114*, 7894



Redox Potentials in Acetonitrile

$E^{\circ}\left(XH^{+\bullet} / XH\right)$

Seidel et al.

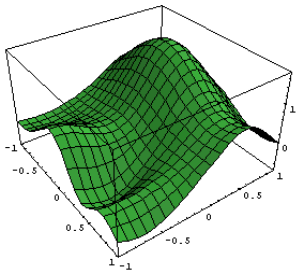
One-electron oxidation potentials for DNA nucleosides

Cyclic voltammetry measurements in acetonitrile solution against saturated calomel electrode (E_{abs} = 4.429 V)

	Seidel et al. Deoxyribose	*CBS-QB3 Methyl*	*B3LYP Methyl*	*B3LYP Ribose*
Guanine	1.25	1.14	0.96	1.04
Adenine	1.72	1.58	1.44	1.41
Cytosine	1.90	1.58	1.50	1.57
Thymine	1.87	1.63	1.51	1.60
Uracil	>2.15	1.91	1.81	1.88
MAE to Exp.		*0.21*	*0.33*	*0.28*

(in Volts)

Seidel, C. A. M.; Schulz, A. and Sauer, M. H. M.; *J. Phys. Chem.* **1996**, *100*, 5541



Relative $E^{\circ}\left(XH^{+\bullet} / XH\right)$ in Acetonitrile

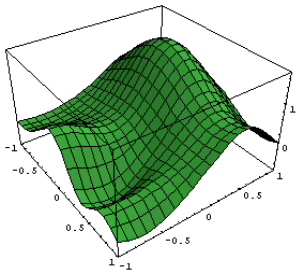
	Seidel et al.[1] *Deoxyribose*	*CBS-QB3* [2] *Methyl*	*B3LYP* [2] *Methyl*	*B3LYP* [2] *Ribose*
Guanine	-0.47	-0.44	-0.47	-0.37
Adenine	0.00	0.00	0.00	0.00
Cytosine	0.18	0.00	0.06	0.16
Thymine	0.15	0.05	0.07	0.19
Uracil	0.43	0.34	0.37	0.47
Xanthine	-	-0.11	0.03	0.07
8-Oxoguanine	-	-0.77	-0.79	-0.73

(in Volts)

Relative Mean Absolute Errors

0.10 V CBS-QB3 to Exp.
0.07 V B3LYP to Exp.
0.05 V CBS-QB3 to B3LYP

[1] Seidel, C. A. M.; Schulz, A. and Sauer, M. H. M.; *J. Phys. Chem.* **1996**, *100*, 5541
[2] Psciuk, B. T.; Lord, R. L.; Munk, B. H.; Schlegel, H. B.; *J. Chem. Theory Comput.* **2012**, *8*, 5107



Relative Potentials in Aqueous Solution

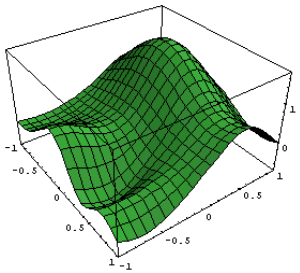
$$E_7\left(X^{\bullet}, H^{+} / XH\right)$$

	Steenken et al.	*Fukuzumi et al.*	*CBS-QB3*	*B3LYP*	*B3LYP*	*Li et al.*
	Ribose	*Nucleotide*	*Methyl*	*Methyl*	*Ribose*	*Base*
Guanine	-0.13	-0.11	-0.40	-0.42	-0.32	-0.28
Adenine	0.00	0.00	0.00	0.00	0.00	0.00
Cytosine	~ 0.18	0.19	0.31	0.29	0.42	0.38
Thymine	~ 0.28	0.14	0.17	0.07	0.26	0.04
Uracil	-	-	0.51	0.34	0.43	0.24
Xanthine	-	-	-0.35	-0.40	-	-
8-Oxoguanine	-0.68	-	-0.93	-0.98	-	-0.51

(in Volts)

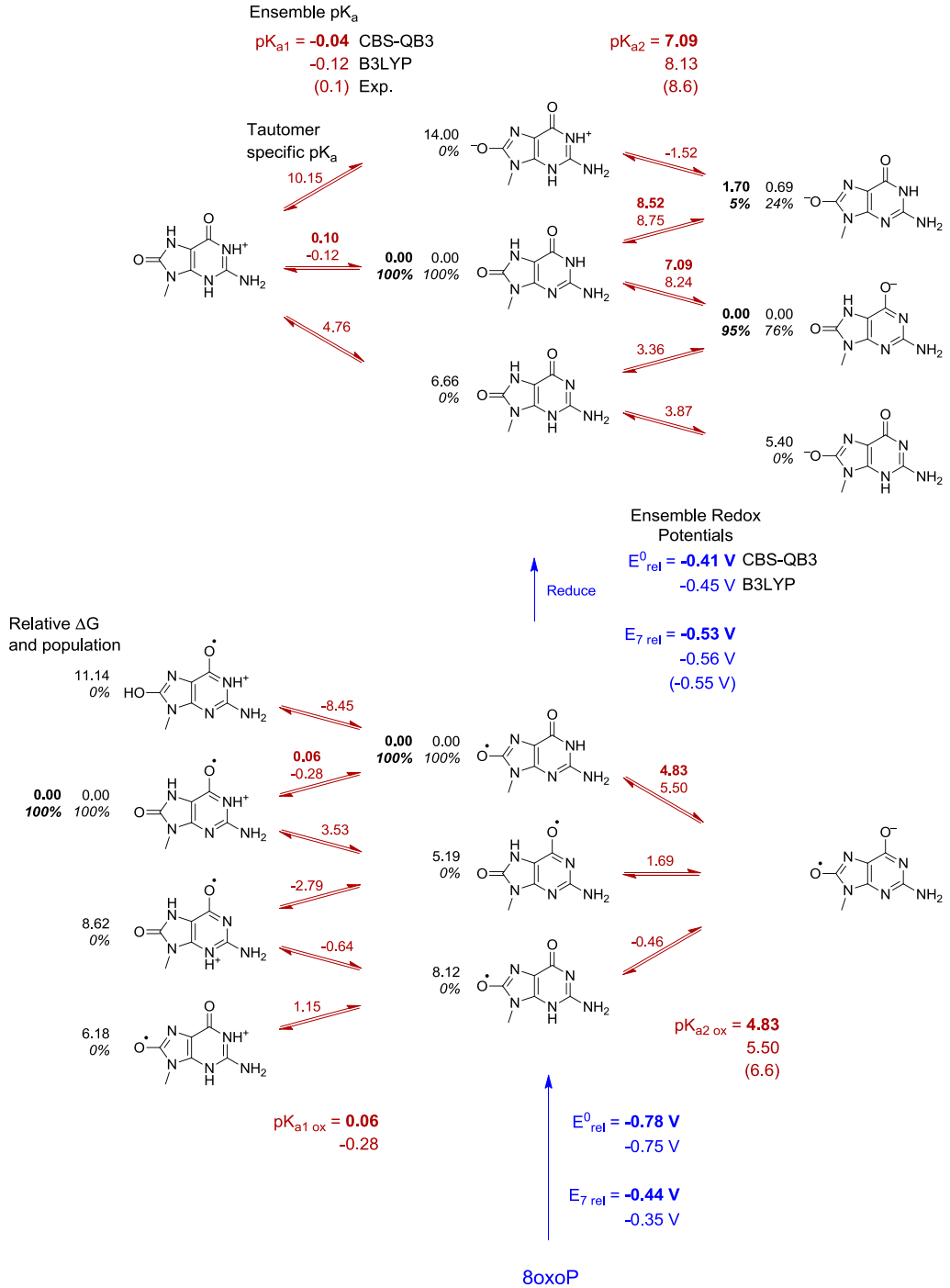
Li, M. J., Liu; W. X.; Peng, C. R. and Lu, W. C.; *Acta Phys.-Chim. Sin.* **2011,** *27*, 595-603

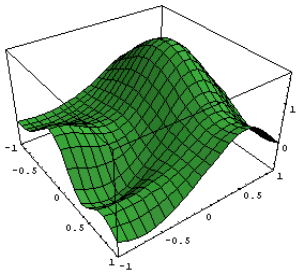
Fukuzumi, S.; Miyao, H.; Ohkubo, K. and Suenobu, T.; *J. Phys. Chem. A* **2005**, *109*, 3285



8oxoGuanine

pK_a's and redox potentials





8oxoPurine

pK_a's and redox potentials

Relative ΔG and population

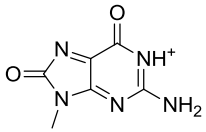
0.00 0.00
100% *100%*

17.36 15.17
0% *0%*

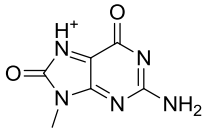
10.23 10.15
0% *0%*

Ensemble pK_a

pK_{a1} = **1.21** CBS-QB3
-0.04 B3LYP
Exp.

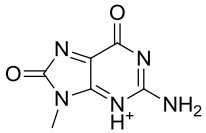


1.21
-0.04

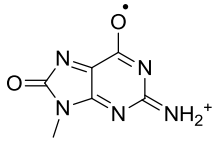


-11.52
-11.16

-6.29
-7.49

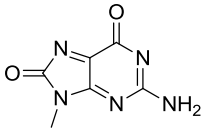


Tautomer specific pK_a

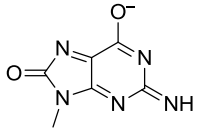


-8.59
-1.92

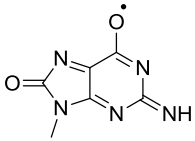
$pK_{a1\ ox}$ = **-8.59**
-1.92



8.25
7.52



pK_{a2} = **8.25**
7.52



Reduce

$8oxoG_{ox}$

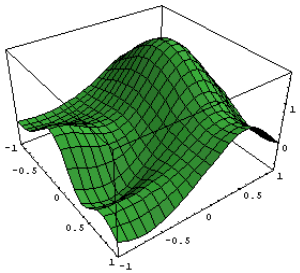
E^0_{rel} = **-0.78 V**
-0.75 V

$E_{7\ rel}$ = **-0.44 V**
-0.35 V

Ensemble Redox Potentials

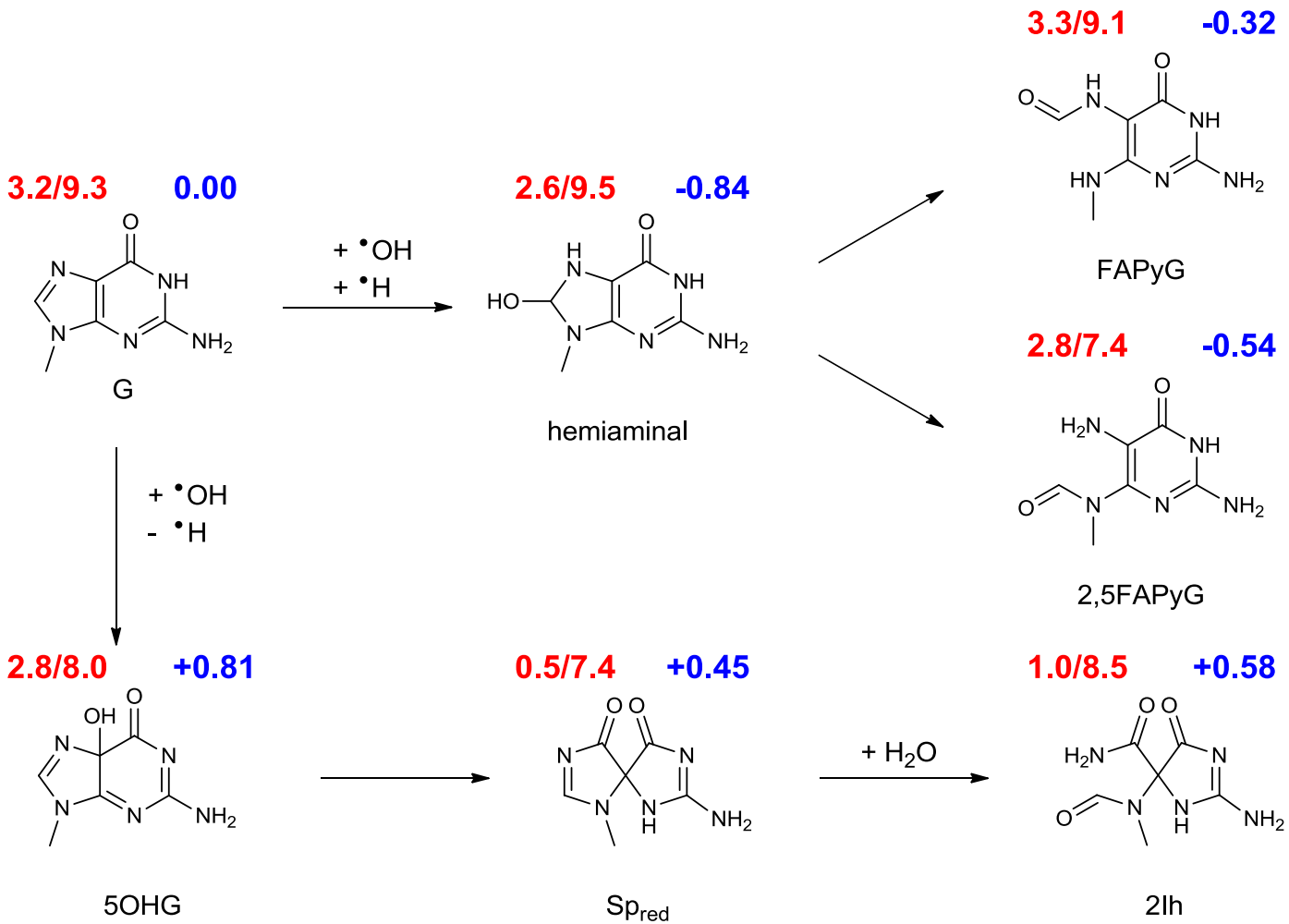
E^0_{rel} = **+1.07 V** CBS-QB3
+1.13 V B3LYP

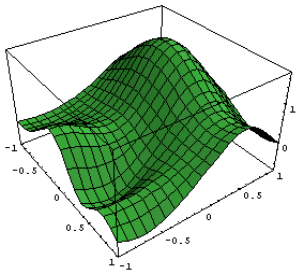
$E_{7\ rel}$ = **+1.07 V**
+1.13 V



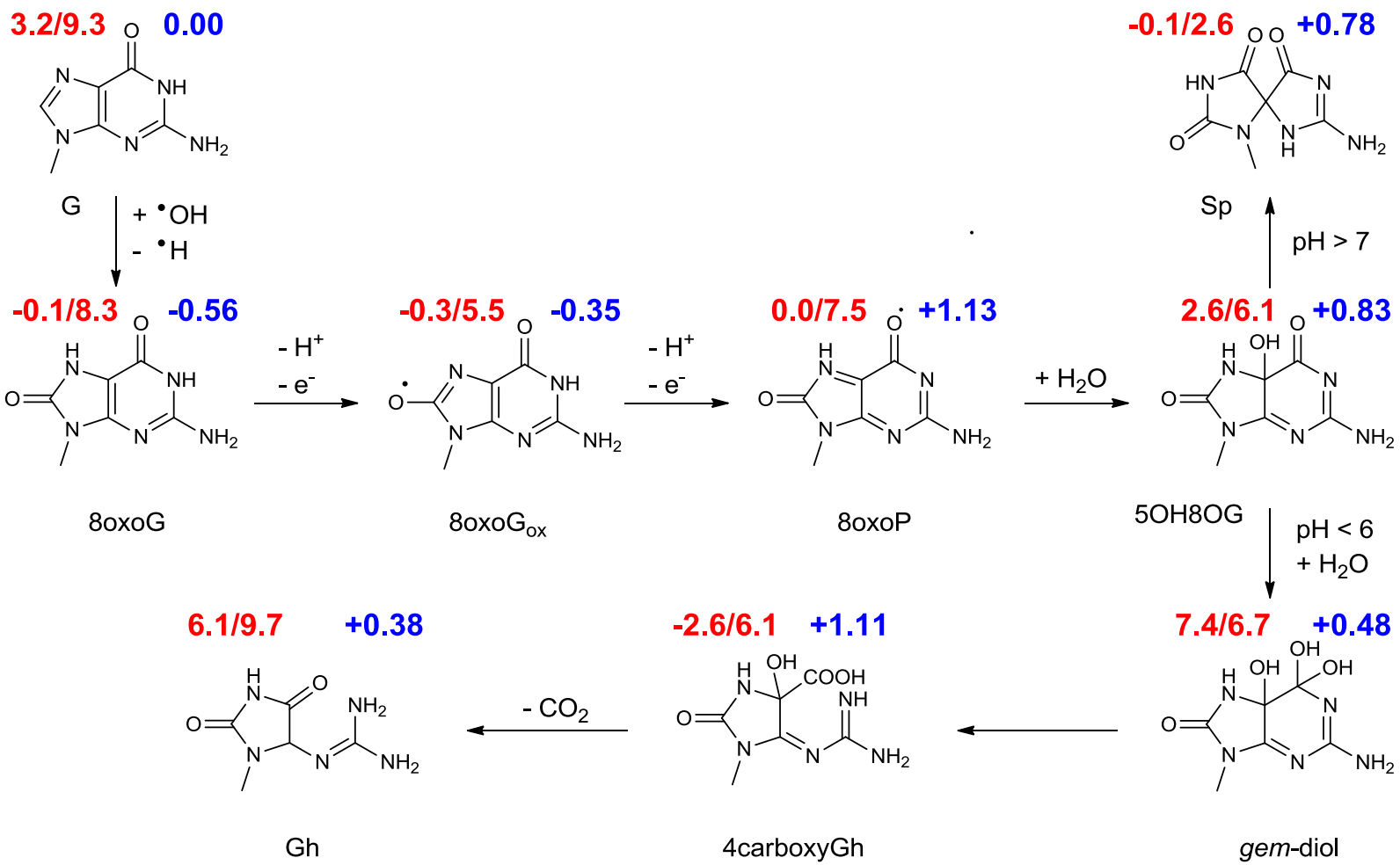
Guanine Oxidation to FAPyG and Ih

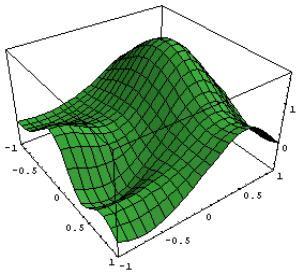
pK_{a1}/pK_{a2} and E_7 relative to G





Guanine Oxidation to Sp and Gh
pK_{a1}/pK_{a2} and E_7 relative to G





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