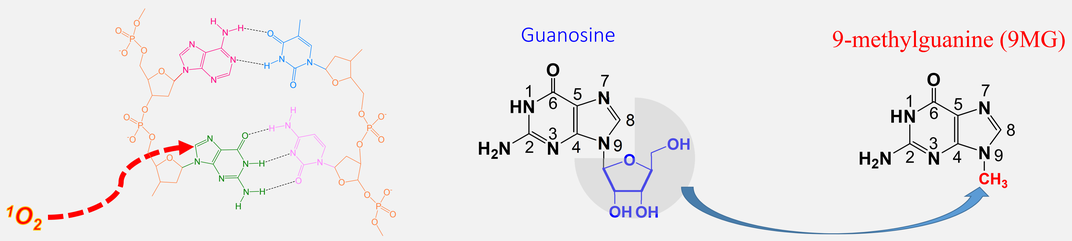
Monitoring the Singlet O_2 Oxidation Kinetics of Guanine and 9-Methylguanine by Real-Time Mass Spectrometry and Spectroscopy

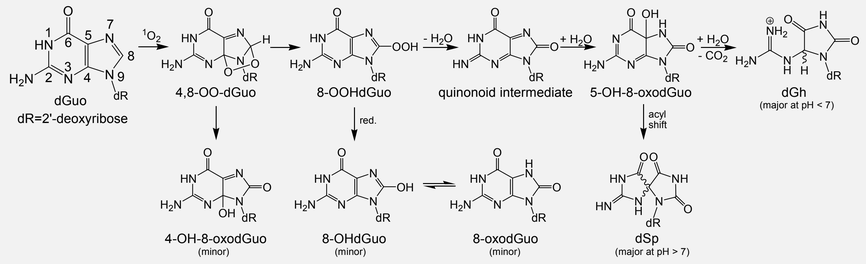
Wenchao Lu[#], Yan Sun, Jessie Zhou and Jianbo Liu*
Department of Chemistry and Biochemistry, Queens College and the Graduate Center of CUNY
65-30 Kissena Blvd., Queens, NY 11367, USA

Introduction

- $^1O_2$: one of the most common endogenous reactive oxygen species (ROS) in live organisms, which may cause mutation and cell death.
- Guanine: the exclusive target toward $^1O_2$ oxidation.
- 9-Methylguanine: a commonly used prototype substrate of guanosine.



- Literature proposed reaction mechanism of guanine + $^1O_2$:

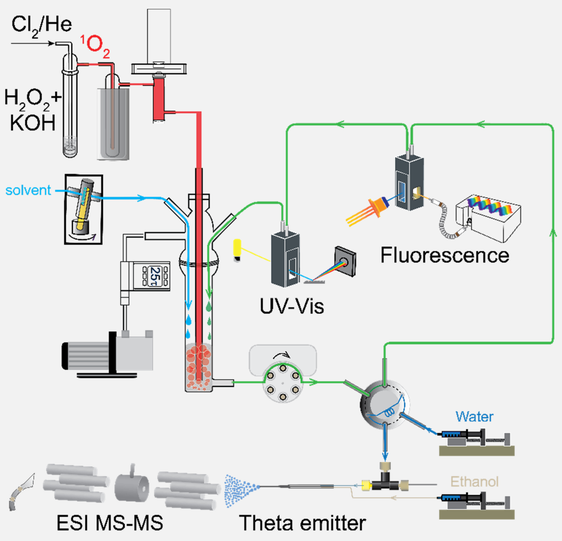


C. Sheu and C. S. Foote, *J. Am. Chem. Soc.*, 1993, **115**, 10446-10447.

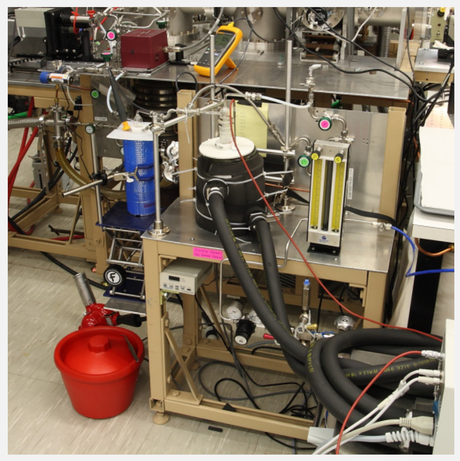
- Our previous gas-phase studies have demonstrated the early-stage oxidation mechanism.
  W. Lu and J. Li*, Chem. Eur. J., 2016, **22**, 3127-3138.
  W Lu, H Teng and J. Liu, *Phys. Chem. Chem. Phys*., 2016, **18**, 15223-15234.

Instrumentation and Methodology

- Solution-Phase Reaction System and $^1O_2$ Generator

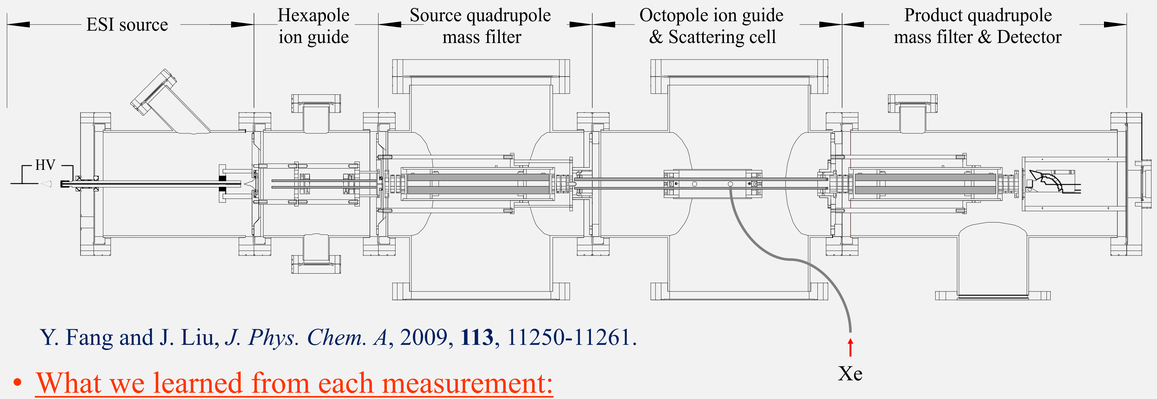


Chemical $^1O_2$ generator



$$2H_2O_2 + Cl_2 + 2KOH \xrightarrow{-18\,^\circ C} {}^1O_2/{}^3O_2 + 2KCl + 2H_2O$$

- Guided-Ion-Beam Tandem Mass Spectrometer

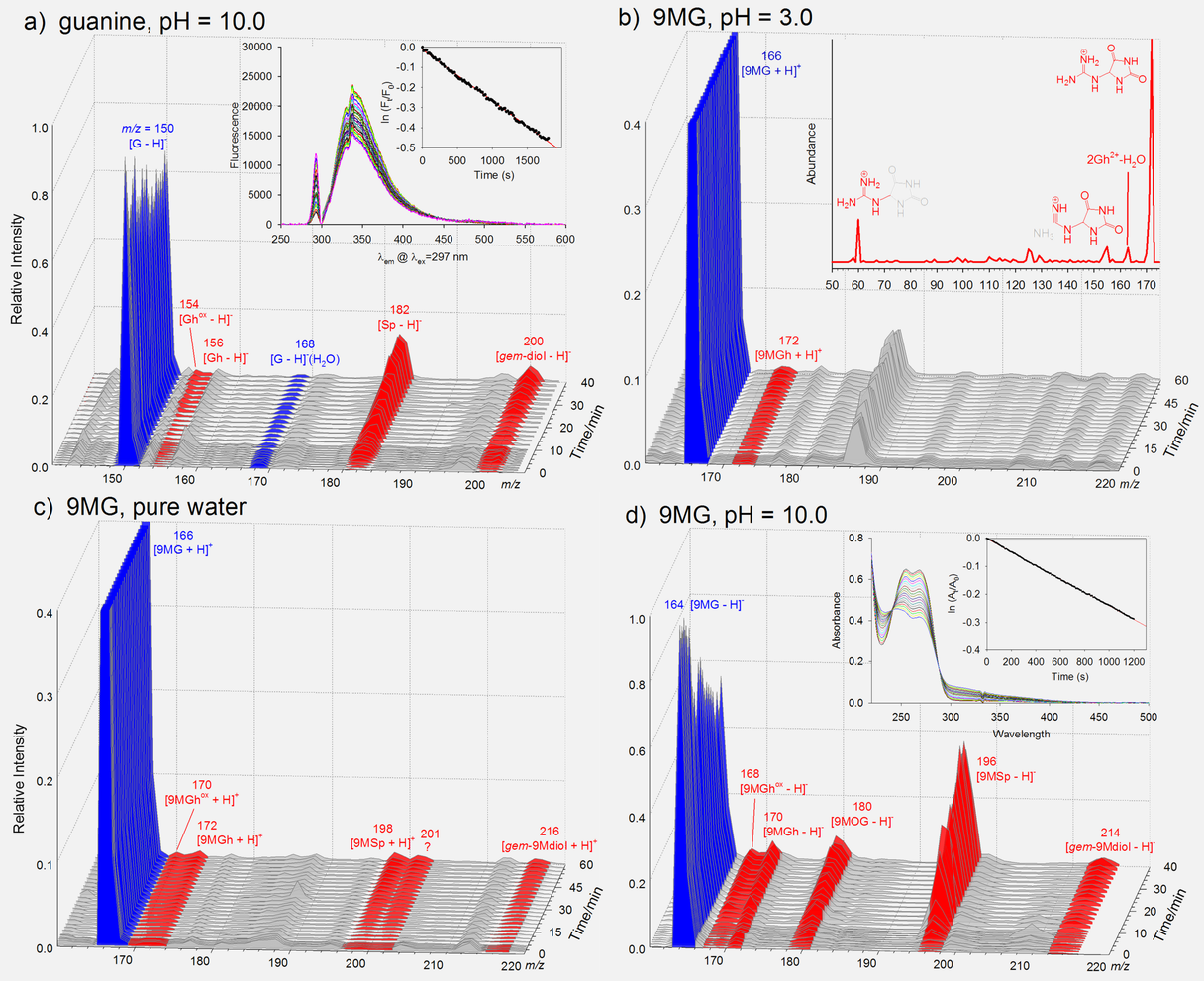


Y. Fang and J. Liu, *J. Phys. Chem. A*, 2009, **113**, 11250-11261.

- What we learned from each measurement:

On-line ESI tandem mass spectra and CID spectra	(1) Product distributions, intermediates involved, and reaction rate of individual product.
	(2) MS^2 characterization of products or intermediates.
UV/Fluorescence Spectra	Reaction kinetics.
DFT calculations	Theoretical support for mechanistic interpretation.

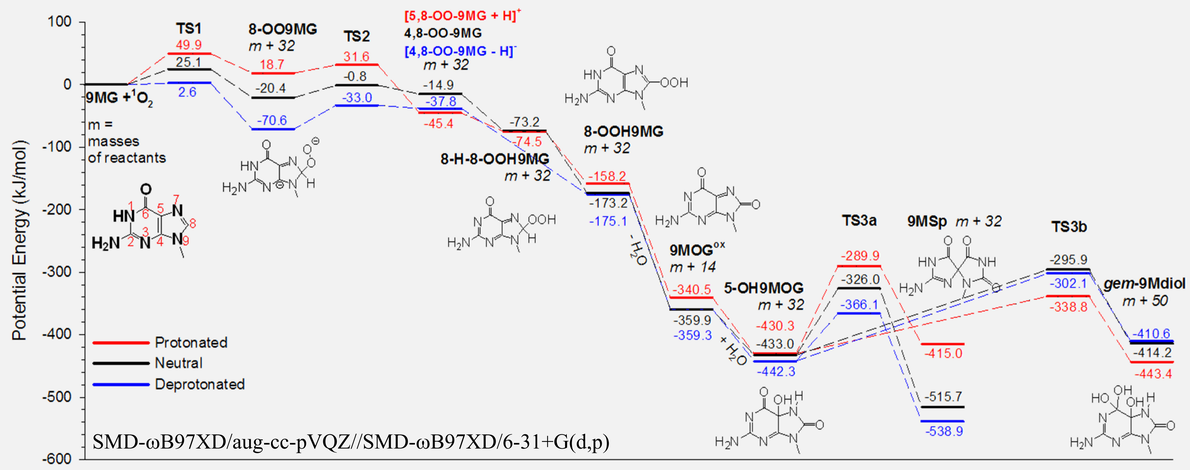
Results



- Oxidation followed first-order reaction kinetics toward guanine/9MG and $^1O_2$.

Guanine	Rate constant, k ($M^{-1}s^{-1}$)	Major products	9MG	Rate constant, k ($M^{-1}s^{-1}$)	Major products
pH = 3	No reaction.	insignificant (< 0.01 %)	pH = 3	—	—
Pure water	No reaction.	insignificant (< 0.01 %)	Pure water	1.2×10^6	9MSp (45%), *gem*-9Mdiol (33%)
pH = 10	$3.2 – 3.6 \times 10^7$	Sp (70%), *gem*-diol (23%)	pH = 10	$4.6 – 4.9 \times 10^7$	9MSp (72%), 9MOG (19%)

- DFT-calculated reaction PES:



- k_3 is rate-limiting.
- Calculated PES rationalizes that deprotonated guanine favors formation of Sp.
- For protonated guanine, the route leading to formation of Gh is blocked by early-stage barriers.

Conclusion

- We conducted a combined kinetic and mechanistic study of guanine/9MG + $^1O_2$ in aqueous solution using coupled mass spectrometry and UV absorption/emission spectroscopy. $^1O_2$ was generated using a chemical reaction separately to avoid interferences from oxygen radicals. The oxidation showed strong pH dependence, with basic solution being the most reactive with $^1O_2$. DFT calculations supported experimental results.

Acknowledgements
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