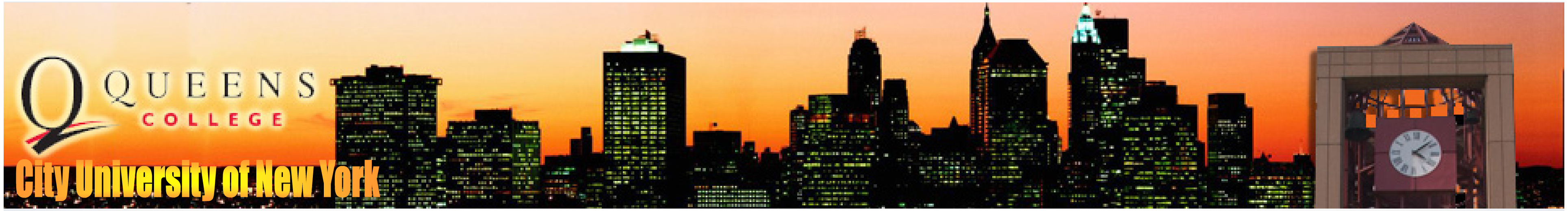

Mass Spectrometry Study of Histidine Oxidation by $^1O_2$:

from Gas-Phase Single Ions through Water Clusters to Aqueous Solution

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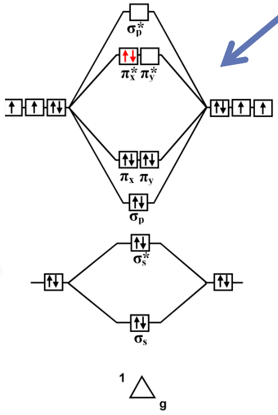
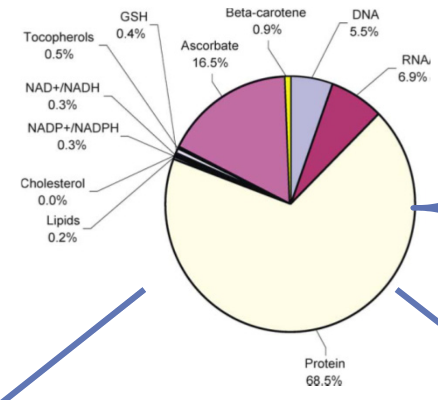
2014 Fall ACS Meeting, San Francisco
08/13/2014

Biosystems
- Enzymatic or nonenzymatic
- Radical termination
- Energy transfer from protein-bound chromophores

Sun Light

$^1O_2$





Tyrosine
Methionine
Cysteine
Tryptophan
Histidine

- Progression of cell death
- Aging and diseases
- Photodynamic therapy

- Loss of amino acids in atmospheric aerosols
- Chemical markers

Photooxidation of Amino Acids

IN SOLUTION

Sensitizer & **Light**
(type II photosensitization)

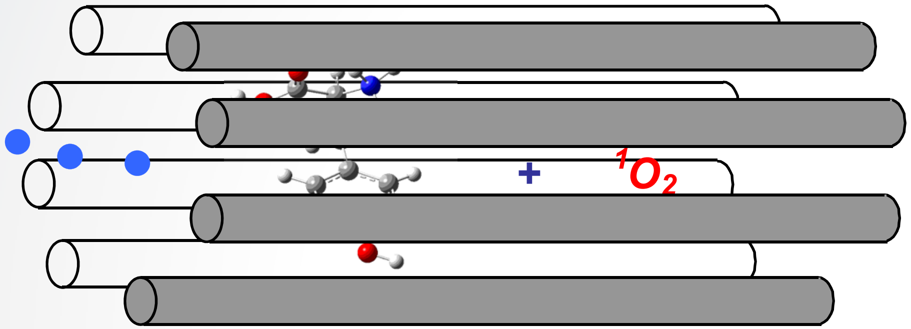
pH

solvent compositions → $^1O_2$ → competition with radical-mediated Reactions (type I photosensitization)

oxygen concentration

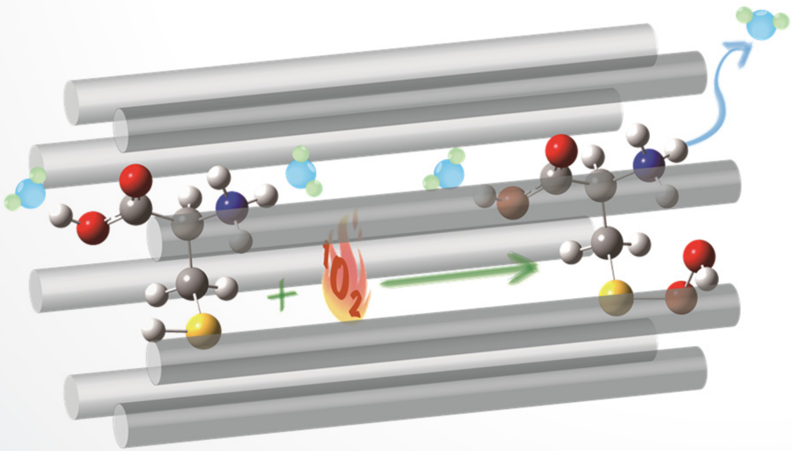
Our Approaches

1. GAS-PHASE REACTIONS of Amino Acid Ions with Clean $^1O_2$



- Distinguish intrinsic *vs.* external imposed properties of biomolecules
- Complemented by and compared with MD simulations

2. MICROSOLVATION of Amino Acid Reactant Ions in the Gas Phase



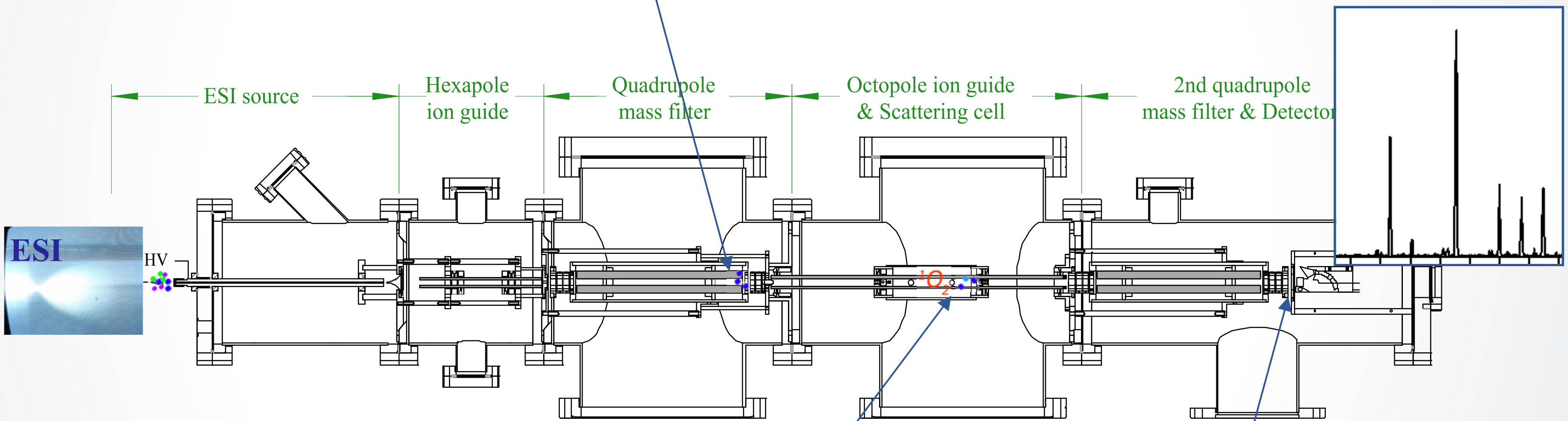
- Dynamical roles of hydrogen-bounded waters

How We Run Reactions of His Ions with $^1O_2$

1. Generation of His ions by ESI

2. Ions are passed into a quadrupole for mass selection

$$\sigma = k / v_{rel} = \frac{I_{product} k_B T}{I_{reactant} P_{cell} l_{cell}}$$



3. Mass-selected ions are guided into an octopole surrounded by a collision cell, and scattered from $^1O_2$.

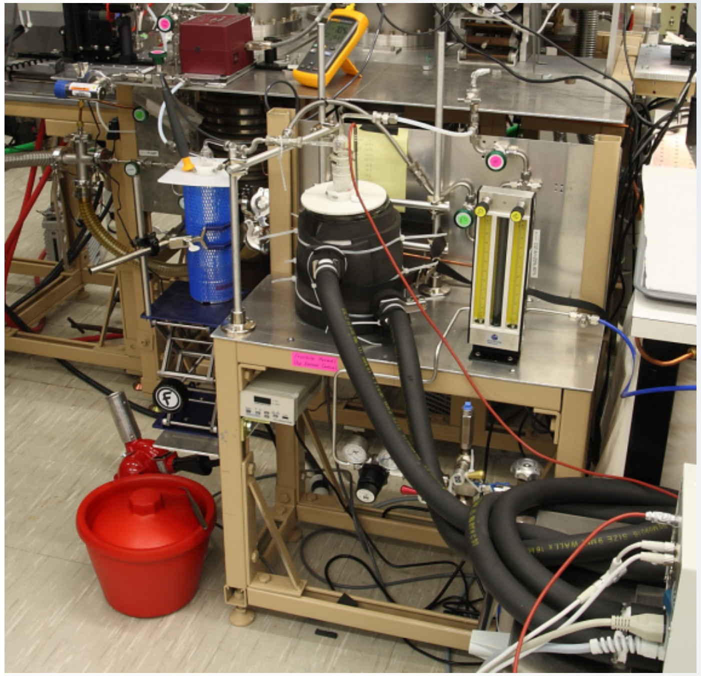
4. Product ions are mass analyzed & counted.

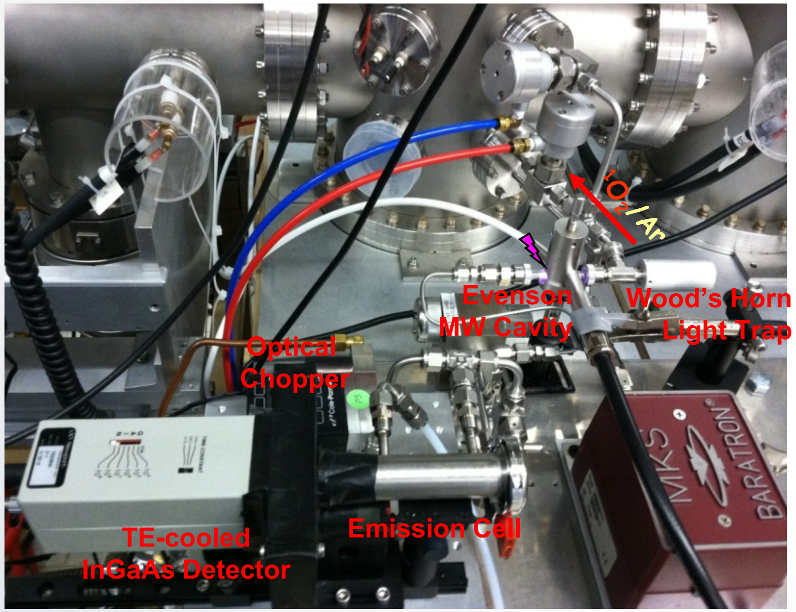
Guided-Ion-Beam Tandem Mass Spectrometer

Generation & Detection of $^1O_2$

- Chemical $^1O_2$ generator

$$2H_2O_2 + Cl_2 + 2KOH \xrightarrow{-21°C} {}^1O_2/{}^3O_2 + 2KCl + 2H_2O$$





Emission detection

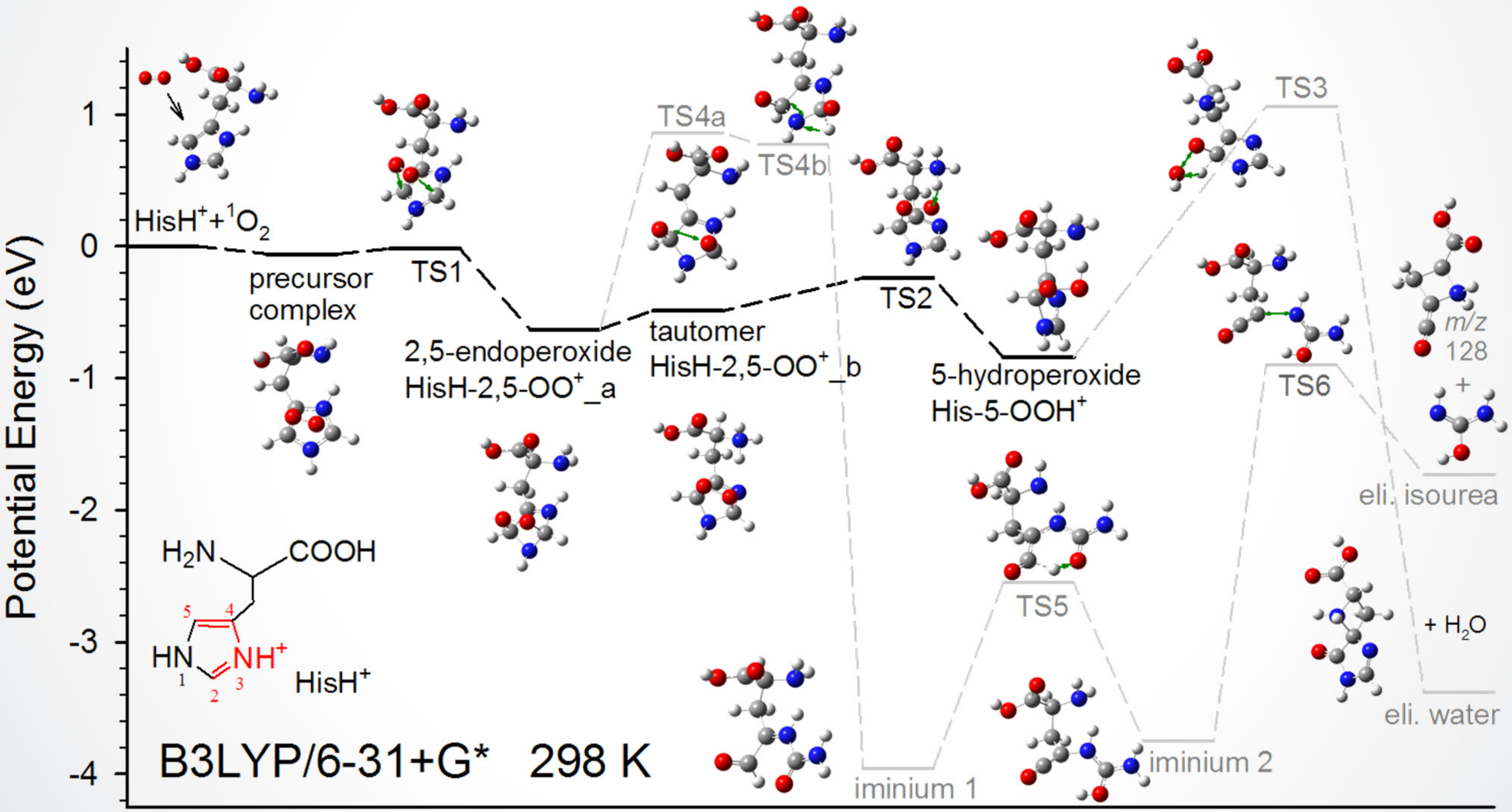
$$O_2(^1\Delta_g) \xrightarrow{Emission} O_2(^3\Sigma_g^-) + hv\,(1270\ nm)$$

Gas-Phase Exp 1.
$^1O_2$ Oxidation of Protonated and Deprotonated His in the Gas Phase

Gas-Phase Exp 1.
$^1O_2$ Oxidation of Protonated and Deprotonated His in the Gas Phase

No oxidation products were observed.
Gas-phase isolated His cannot be oxidized by $^1O_2$

Why Is Gas-Phase Isolated His Non-Reactive?

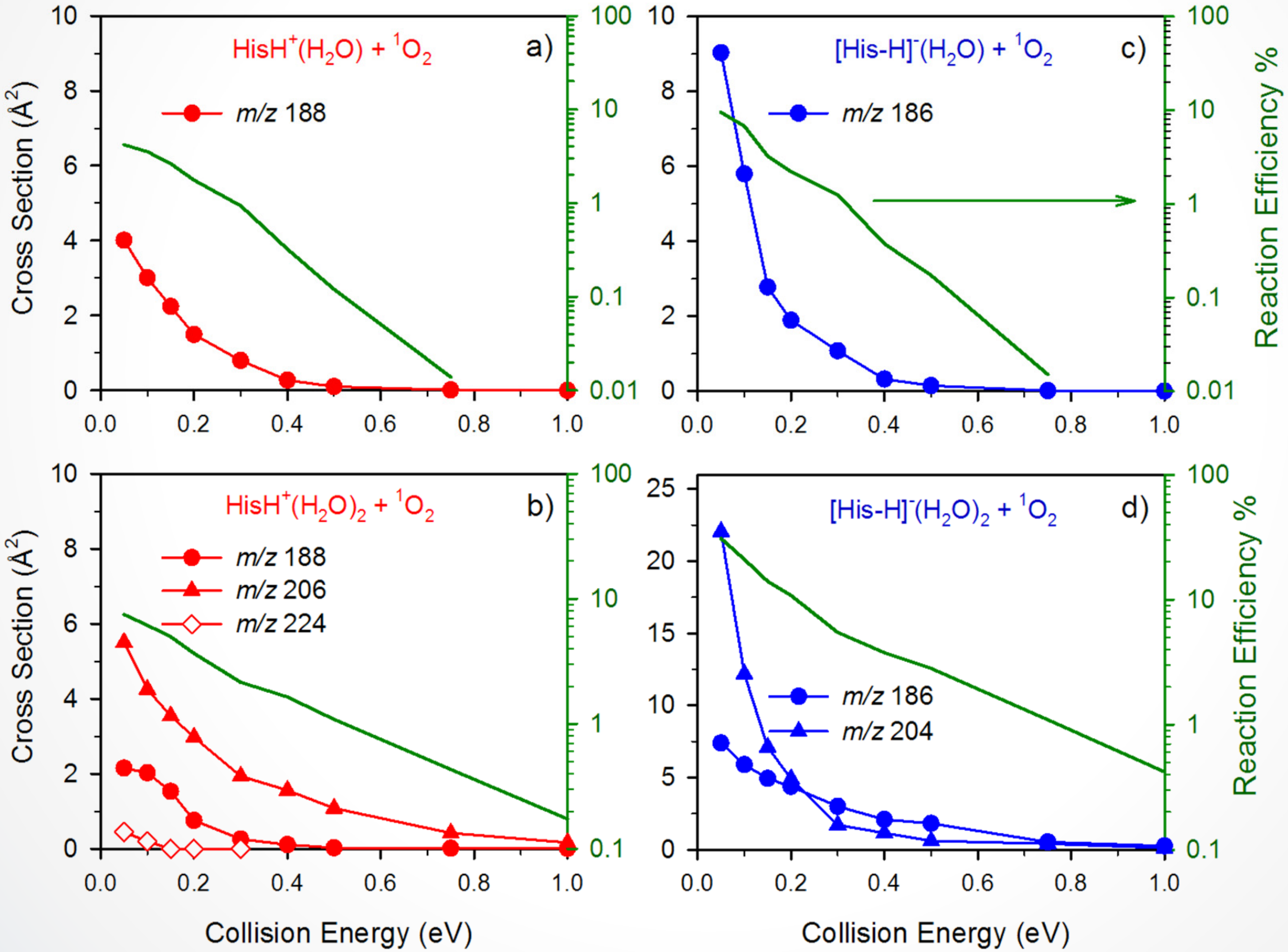


Make Gas-Phase Experiments More Biologically Relevant

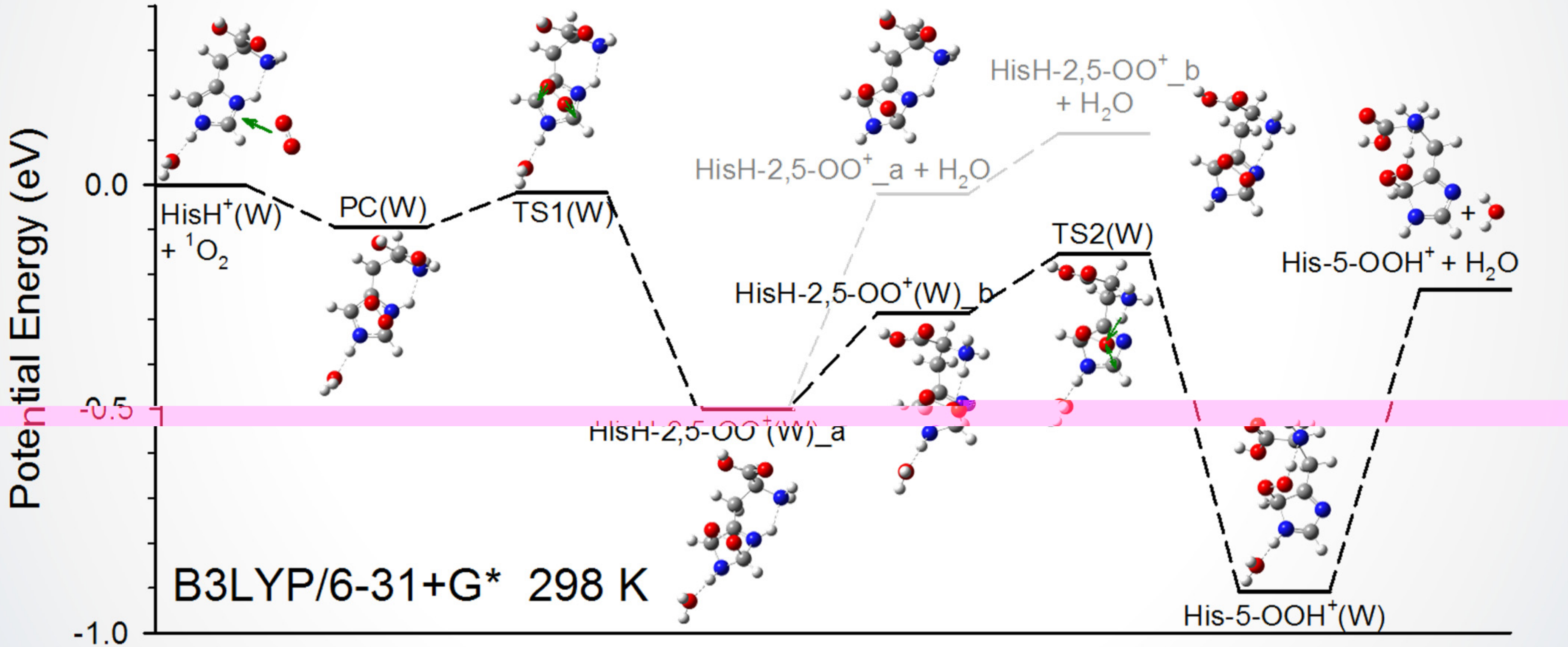
↓

Use Hydrated Clusters to Approach Solution-Phase Oxidation Behaviors

Gas-Phase Exp 2. Reactions of $^1O_2$ with Hydrated $HisH^+(H_2O)_n$ and $[His\text{-}H]^-(H_2O)_n$



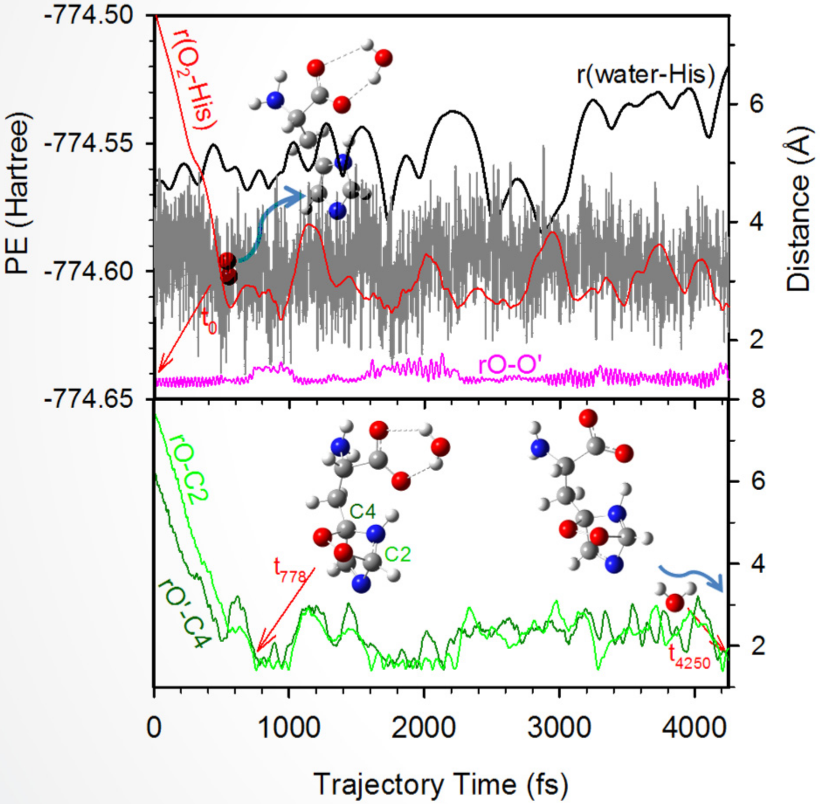
Hydration effect: Suppression of Dissociative Pathways of Peroxide Intermediates by Water Cluster Dissociation

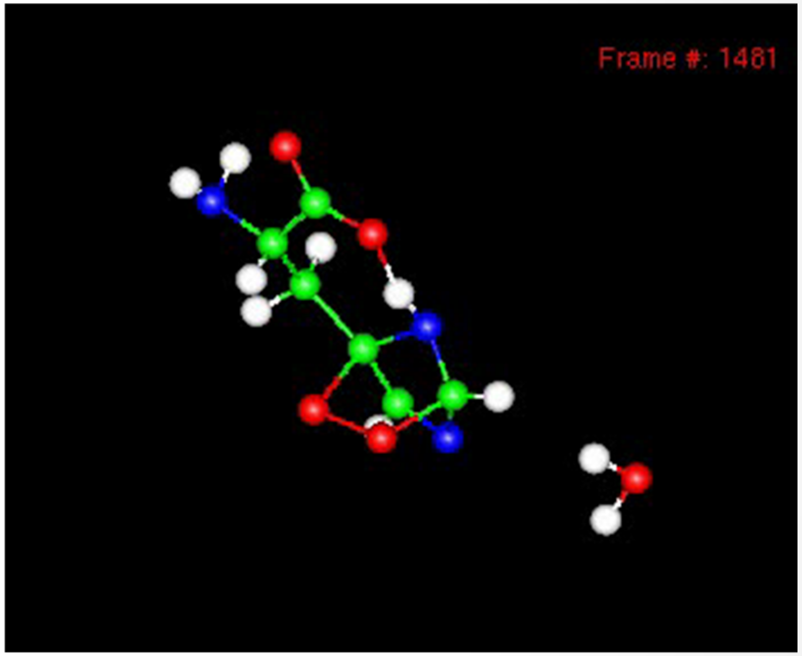


Dynamical Role of Water:

Direct Dynamics Trajectory of [His-H]$^-$(H_2O) + $^1O_2$ at E_{col} = 0.1 eV

Using Venus/Gaussian 09, w/ forces and Hessians calculated at B3LYP/4-31G*





Use Hydrated Clusters to Mimic pH-Dependence of Photooxidation

Photooxidation of His in solution

Reaction of hydrated His in the gas phase

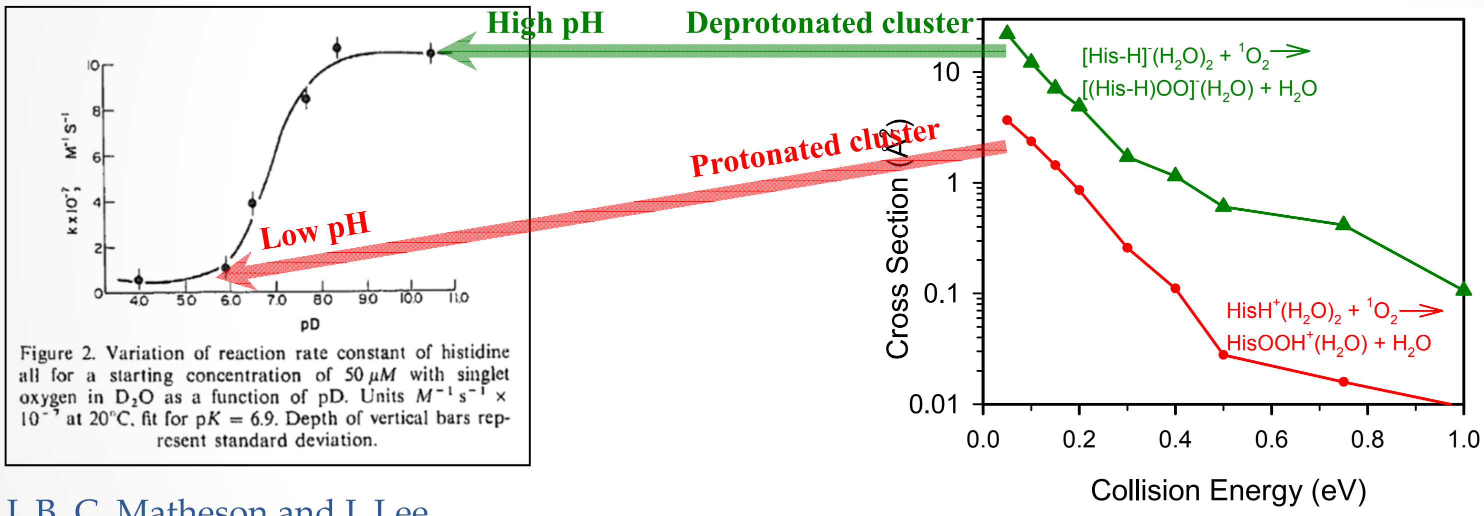
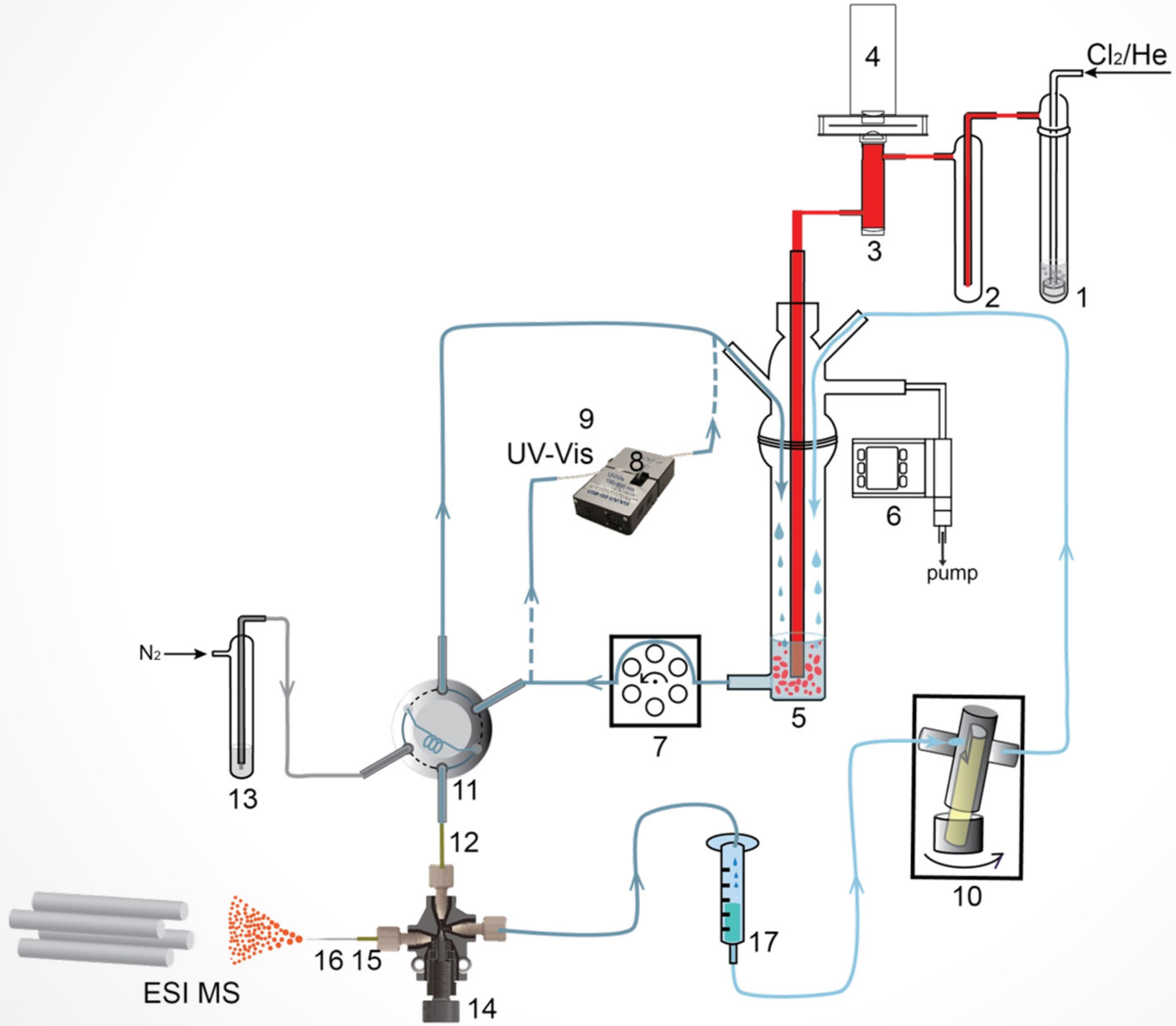


Figure 2. Variation of reaction rate constant of histidine all for a starting concentration of 50 μM with singlet oxygen in D_2O as a function of pD. Units $M^{-1} s^{-1} \times 10^{-7}$ at 20°C, fit for $pK = 6.9$. Depth of vertical bars represent standard deviation.

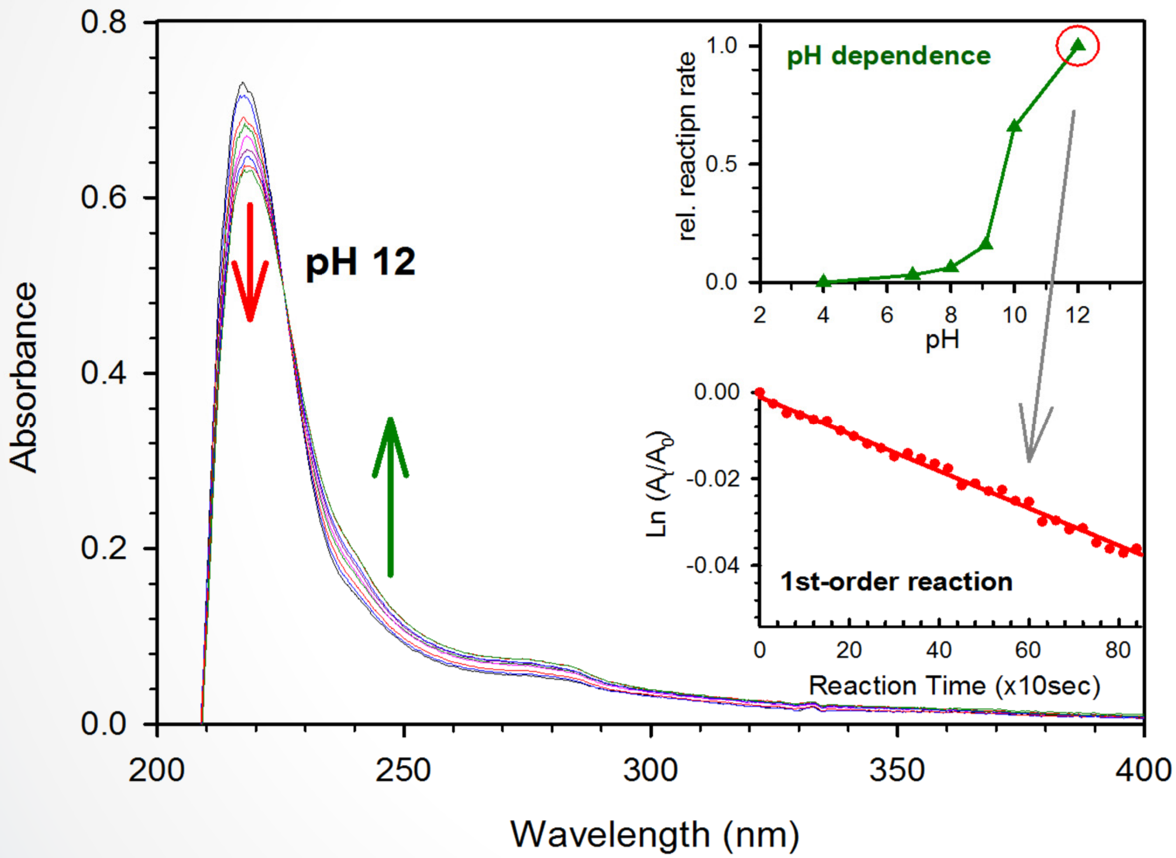
I. B. C. Matheson and J. Lee,
Photochem. Photobiol., 1979, **29**, 879.

Gas-phase solvated clusters provide a platform to elucidate intrinsic reactivity of biomolecules *in vacuo.*
Can these results can be extrapolated to condensed phase?

Exp 3: On-Line Reaction Monitoring of His + $^1O_2$ (w/o sensitizers) in Aqueous Solution

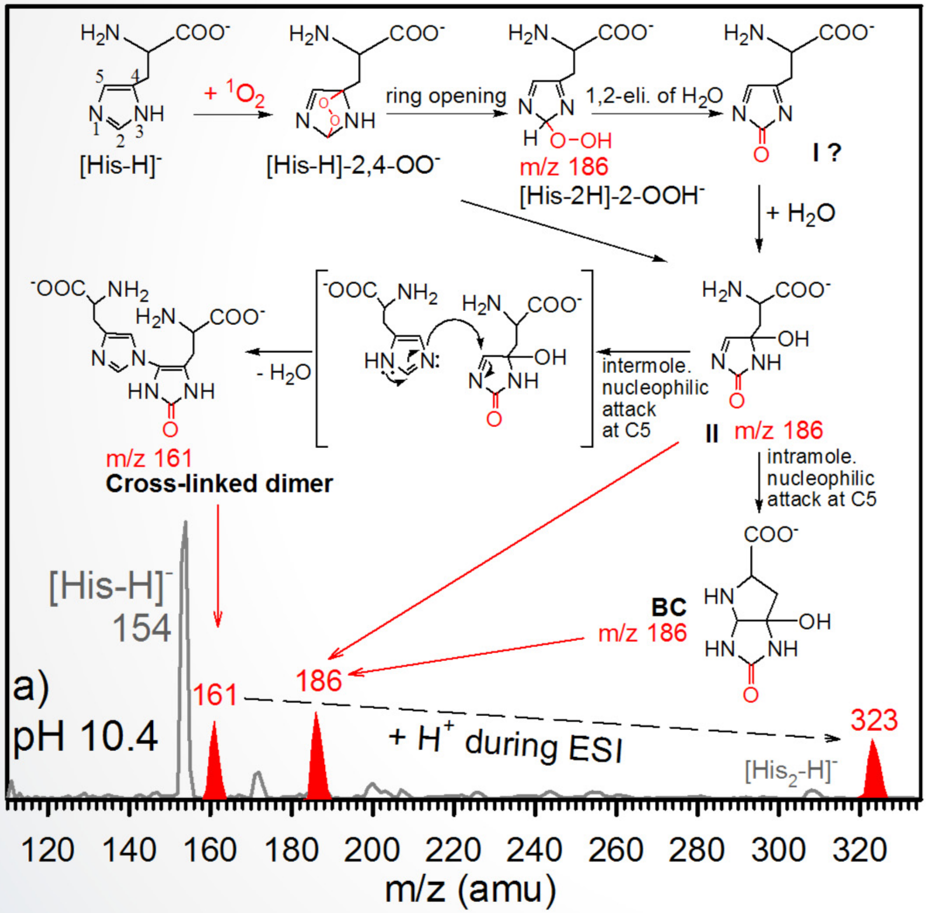


UV-Vis Kinetics Analysis of His + $^1O_2$ in Aqueous Solution

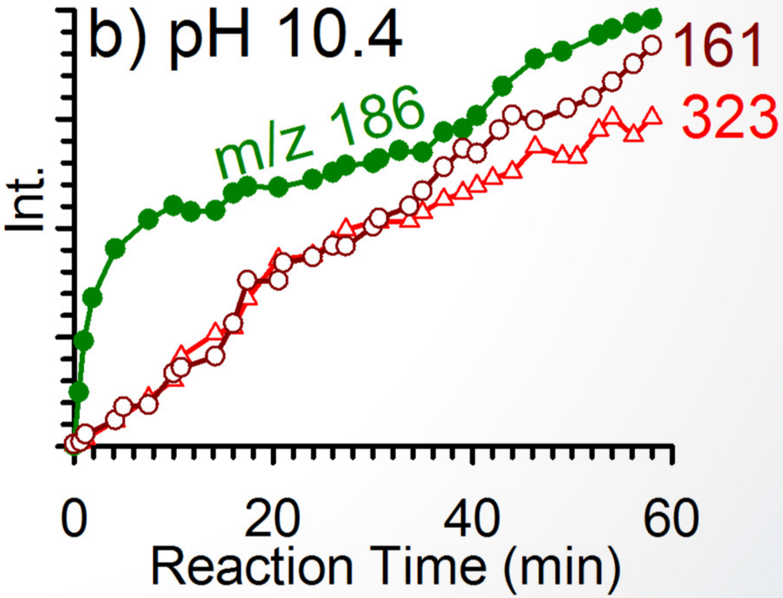


Real-time
UV-Vis Monitoring

On-Line ESI MS of His + $^1O_2$ in Aqueous solution



Time Profiles of Products



Conclusions: Non-Reactivity in the Gas Phase

Peroxides in Water Clusters

pH-Dependence in Solution

- A common process: endoperoxide via [4+2] cycloaddition, and rearrange to hydroperoxide.
- Hydration effect: suppression of intermediate dissociative pathways and production of stable peroxide products.
- Contrasting mechanisms of ***protonated*** vs. ***deprotonated*** His lead to pH dependence in solution

$HisH^+ + {}^1O_2 \xrightarrow{gas\ phase}$ 2,5-endoperoxide $\xrightarrow{ring-opening}$ 5-hydroperoxide $\xrightarrow{in\ solution}$ stable hydrated imidazole

vs.

$[His\text{-}H]^- + {}^1O_2 \xrightarrow{gas\ phase}$ 2,4-endoperoxide $\xrightarrow{ring-opening}$ 2-hydroperoxide $\xrightarrow{in\ solution}$ hydrated imidazolone

→ 6α-hydoxy-2-oxo-octahydro-pyrrolo[2,3-d] imidazole-5-carboxylate + His-His cross-linking.

- *Biological Implications*

pK_a (imidazole) 6.04, His exists in neutral/protonated/deprotonated forms at physiological pH

${}^1O_2$ oxidation of the guanine moiety of DNA

From Gas-Phase to Solution-Phase Dynamics

Acknowledgements

Ph.D. Students
Yigang Fang
Fangwei Liu
Wenchao Lu

Master's Students
Rifat Emre
Yun Chen

Undergraduate Students
Vicnent Yin

Collaborators
Bill Hase (Texas Tech Univ)
Al Viggiano (AFRL)