金属結合ペプチドの触媒反応機構についてのマルチスケール・シミュレーションを基盤とした理論的解析
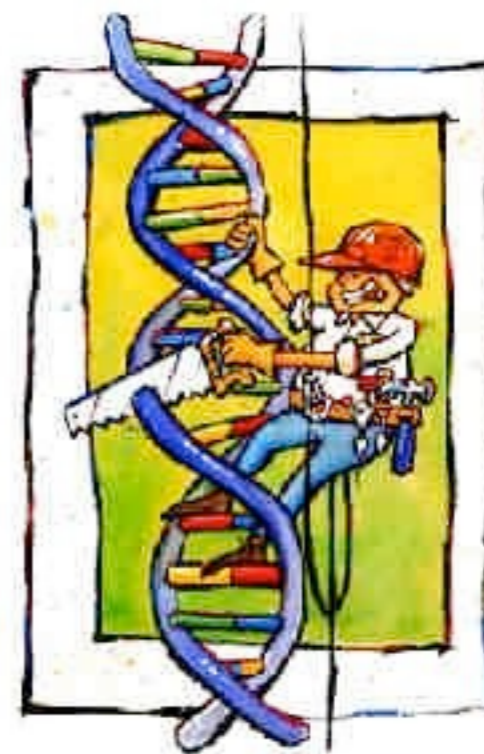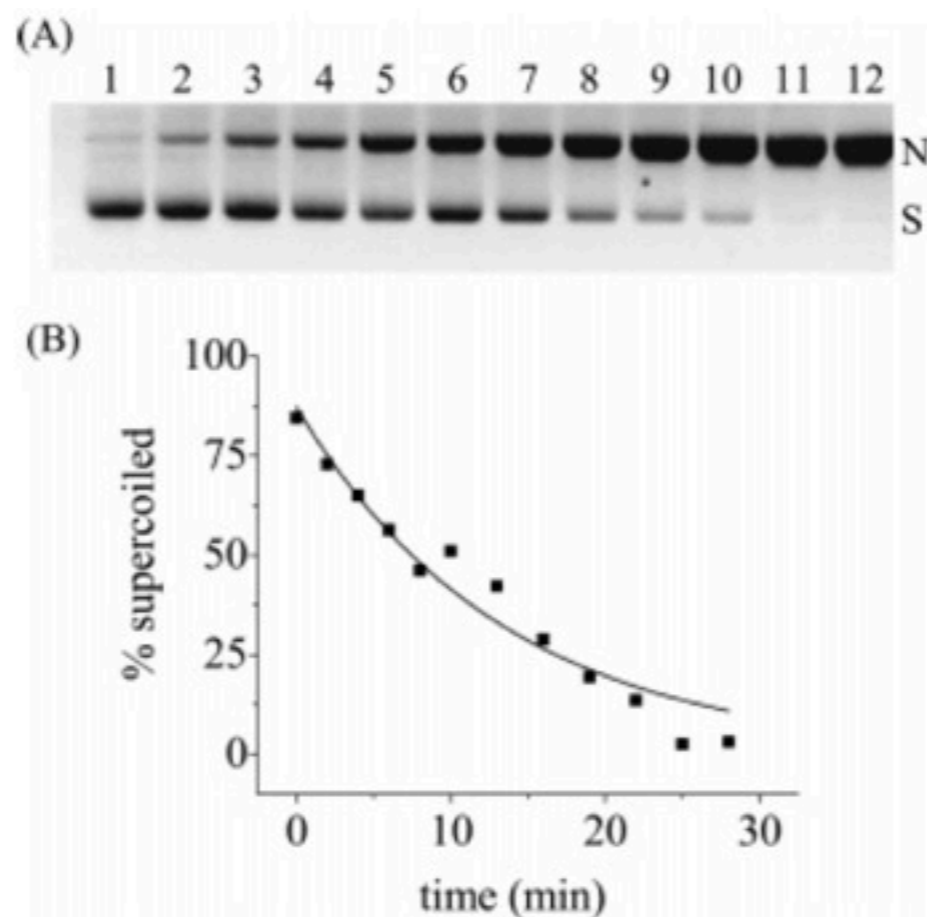
名古屋大学・大学院・情報科学研究科・古賀研究室

山本典史

Background

- **DNA Cleavage Catalyzed by ATCUN**

- **ATCUN Motif**

- **Fenton-type Prooxidation Reaction**

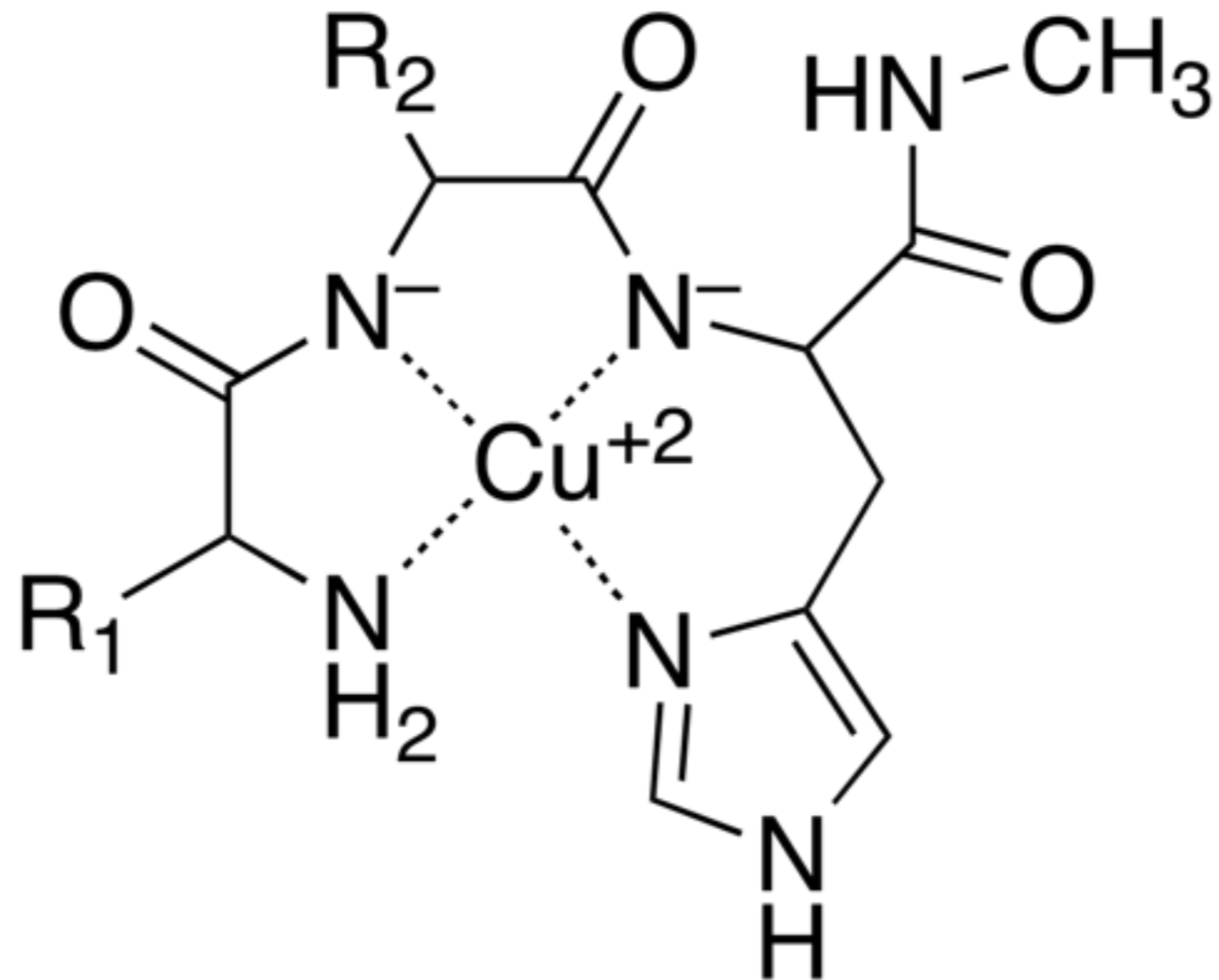- **Research Interests**

- **Annual Schedule 2010**

DNA Cleavage Catalyzed by ATCUN

Figure 1. Cleavage activity of [GGH−Cu]$^-$ monitored by 0.8% agarose gel electrophoresis, where [DNA] = 50 μM, [[GGH−Cu]$^-$] = 25 μM, and [ascorbate] = 250 μM. Time course measured in 10 mM Tris buffer, pH = 7.4, 37 °C, showing the disappearance of supercoiled DNA (S) at (1) 0 min, (2) 2 min, (3) 4 min, (4) 6 min, (5) 8 min, (6) 10 min, (7) 13 min, (8) 16 min, (9) 19 min, (10) 22 min, (11) 25 min, and (12) 28 min. (A) Gel image showing nicked (N) and supercoiled (S) DNA. (B) Reaction curve, showing a pseudo-first-order kinetic profile (R^2 = 0.952), k_{obs} ∼0.07 min^{-1}.
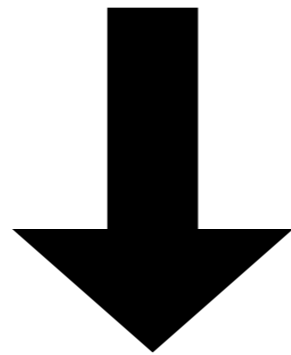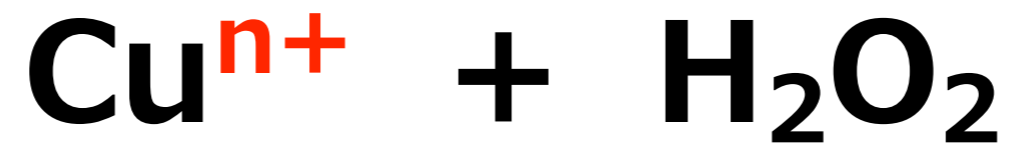
Jin Y. et al., JACS, 129, 8353 (2007)

ATCUN Motif

Amino **T**erminal **Cu** **N**i
(ATCUN) Motif

Fenton-type Prooxidation Reaction

$$Cu^{n+} + H_2O_2$$

$$\downarrow$$

$$Cu^{(n+1)+} + OH^{\bullet} + OH^{-}$$

Research Interests

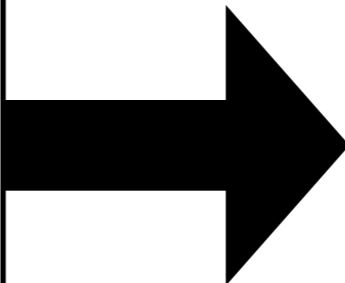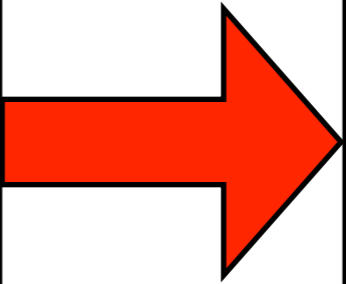■ **ATCUN の redox 触媒作用を決める要因**

➡ 研究方法：QM 計算

✤ 触媒作用を高める分子設計戦略
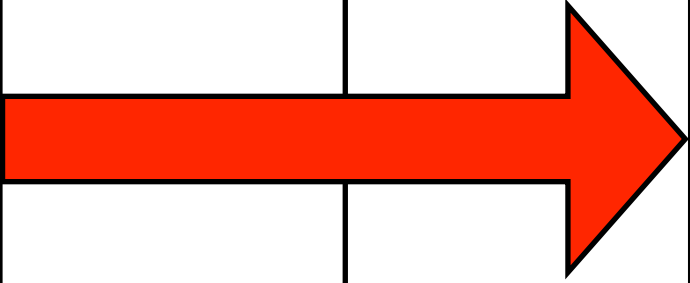
■ **Fenton-like 反応に伴う電荷移動機構**

➡ 研究方法：QM 及び QM/MM-MD

✤ 反応経路に沿ったエネルギー変化

✤ 溶媒和分子との動的・静的相関

Annual Schedule 2010

	7~9	10~12	1~3
QMによるATCUN触媒活性の解析： Lys 置換した ATCUN motif の酸化 還元活性を網羅的に計算し比較する	→		
QMによるFenton反応機構の解析： 金属結合ペプチド上で起こる H_2O_2 不均一解離過程を詳細に追跡する		→	
QMMM-MD法を用いた統合的解析： 溶媒分子を露わに含めた系でFenton 反応の動的機序を明らかにする		→	→

金属結合ペプチドの触媒反応機構：

酸化還元電位と化学反応性

Fenton-type Reaction on ATCUN

$$Cu^{II} \; + \; H_2O_2$$

$$\downarrow$$

$$Cu^{III} \; + \; OH^{\bullet} \; + \; OH^{-}$$
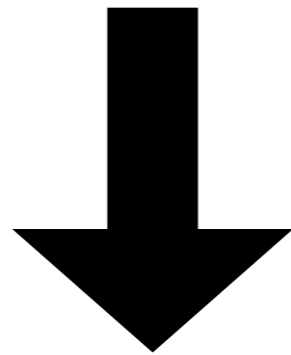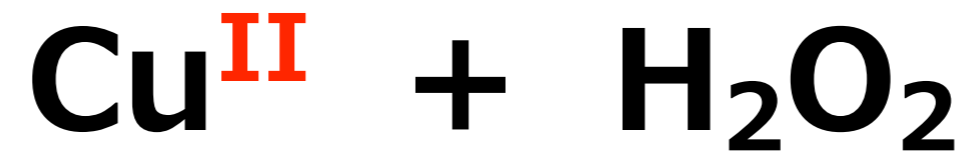
Redox Potential & Reactivity

Table 4. Influence of Lys Positioning and Stereochemistry on the Redox Activities and DNA Cleavage Reactivities of Cu(II)· and Ni(II)·ATCUN Metallopeptides[a]

peptide	$\Delta E°$ ($M^{3+/2+}$) (mV) relative to M(III/II)·Gly-Gly-His	
		D-Lys
Cu(II)·Gly-Lys-His	-11 ($k_2 = 46$)	-36 ($k_2 = 57$)
Cu(II)·Lys-Gly-His	-17 ($k_2 = 67$)	-35 ($k_2 = 77$)
Cu(II)·Lys-Lys-His	-51 ($k_2 = 116$)	-75 ($k_2 = 200$)
Cu(II)·Lys-Gly-His-Lys	-19 ($k_2 = 109$)	
Ni(II)·Gly-Lys-His	$+10$ ($k_2 = 19$)	$+70$ ($k_2 = 23$)
Ni(II)·Lys-Gly-His	$+10$ ($k_2 = 21$)	-20 ($k_2 = 26$)
Ni(II)·Lys-Lys-His	-10 ($k_2 = 35$)	-40 ($k_2 = 34$)
Ni(II)·Lys-Gly-His-Lys	-20 ($k_2 = 34$)	

Jin Y. et al., JACS, 129, 8353 (2007)

Redox Potential vs Reactivity
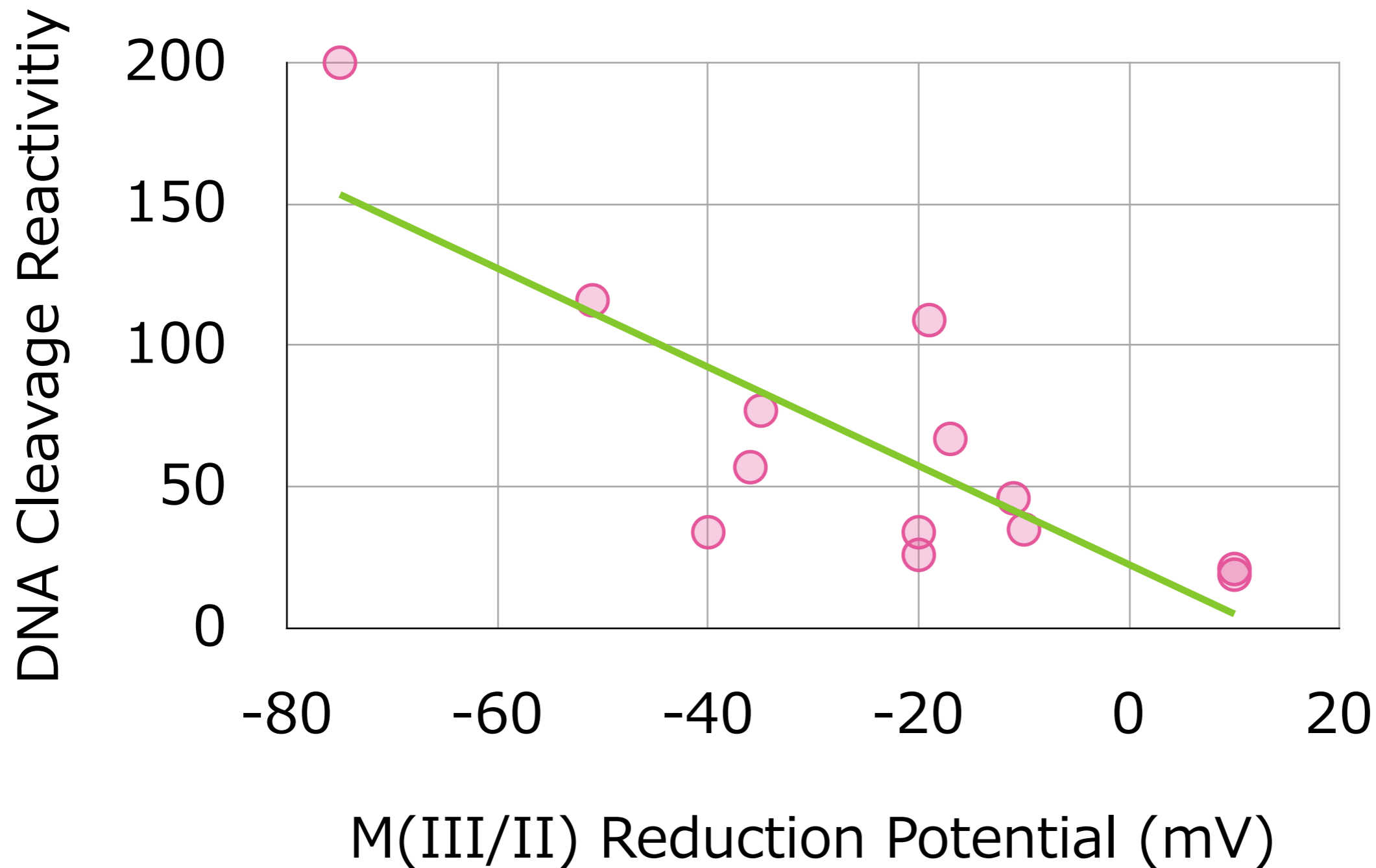
Jin Y. et al., JACS, 129, 8353 (2007)

Models & Methods

- **Cu-ATCUN Models**

- **DFT Calculations**

- **Redox Potentials**

- Electrophilicity Index

- Electronegativity & Hardness

- Local Electrophilicity Index

- General APT Charge

Cu-ATCUN Models

Cu$^{II/III}$ • Gly-Gly-His	
Cu$^{II/III}$ • Gly-L-Lys-His	Cu$^{II/III}$ • Gly-D-Lys-His
Cu$^{II/III}$ • L-Lys-Gly-His	Cu$^{II/III}$ • D-Lys-Gly-His
Cu$^{II/III}$ • L-Lys-L-Lys-His	Cu$^{II/III}$ • D-Lys-D-Lys-His

DFT Calculations

- Gaussian 03 E.01

- Geom. Opt.; Normal Mode Anal.

- Solvation Effect | SCRF=IEF-PCM (Radii=UFF)

- (U)B3LYP / Dunning's CC Basis Sets

  ➡ cc-pVDZ | H [2s1p], C [3s2p1d]

  ➡ aug-cc-pVDZ | N, O [4s3p2d]

  ➡ aug-cc-pVTZ | Cu [8s7p5d3f2g]

Redox Potential

$$E^\circ = \frac{1}{F}\left(G^\circ[\text{Red}] - G^\circ[\text{Ox}] - G^\circ[\text{H}^+] + \frac{1}{2}G^\circ[\text{H}_2] \right)$$

$$= E^\circ_{\text{abs}}[\text{Red}/\text{Ox}] \quad - \quad 4.28 \text{ V}$$

※ Standard Hydrogen Electrode (SHE) Potential

Results

- **Optimized Geometry**

  ➡ **Cu^{II}-Gly-Lys-His**

  ➡ **Cu^{II}-Lys-Gly-His**

  ➡ **Cu^{II}-Lys-Lys-His**

- **Redox Potential**

- **Local Electrophilicity Index**

Cu^II-Gly-Lys-His
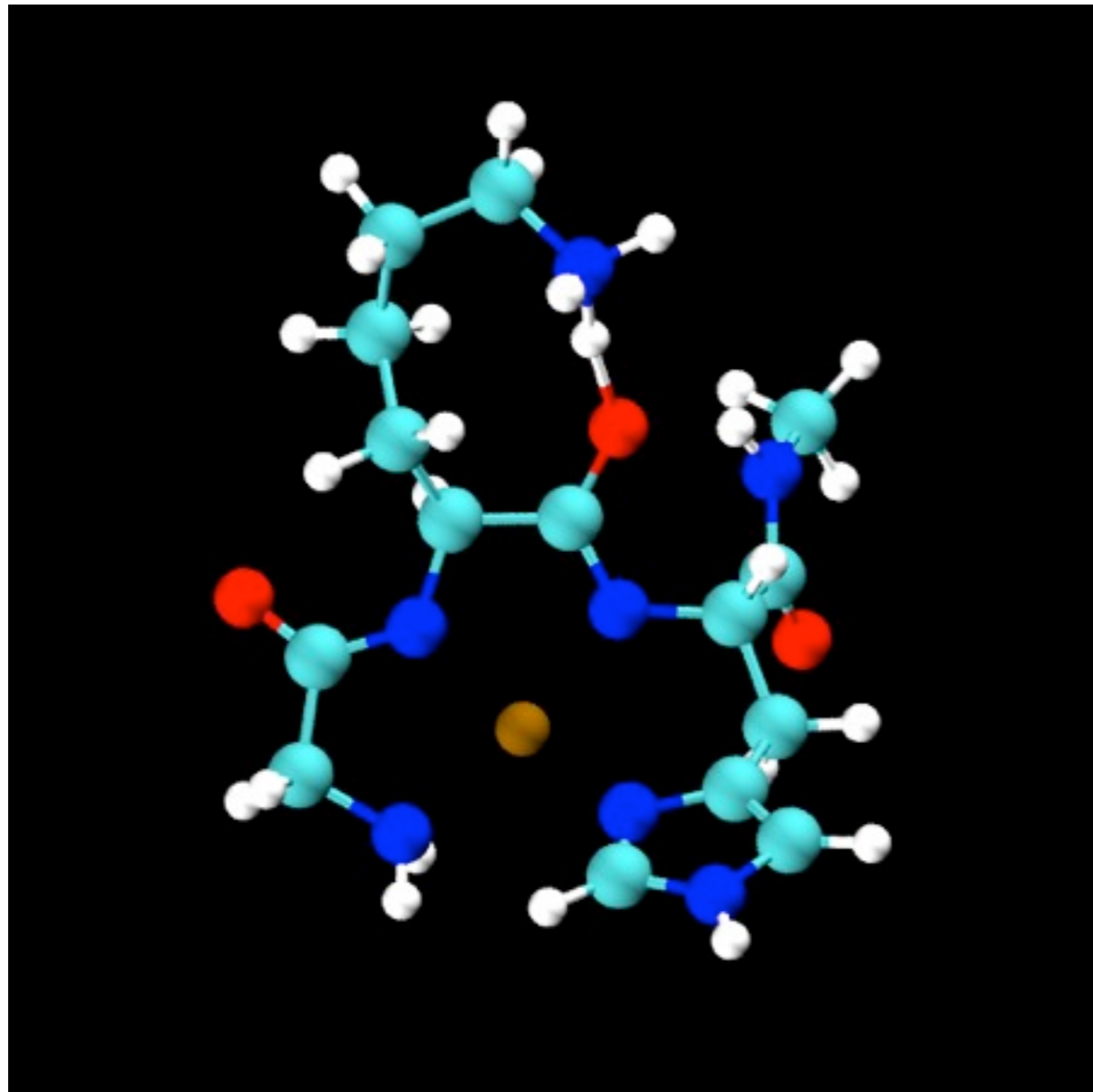
L-Form
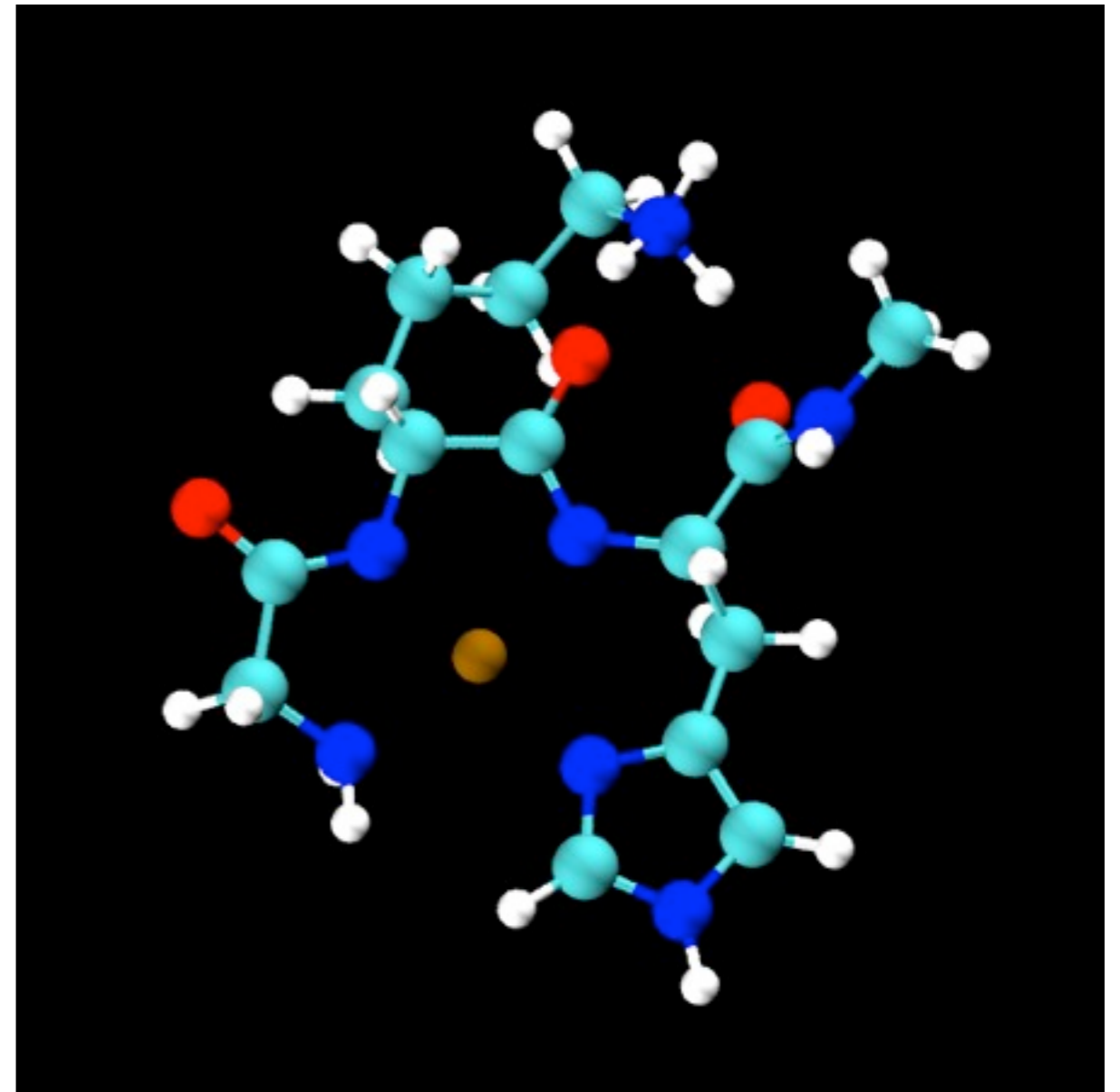
D-Form

Cu^{II}-Lys-Gly-His
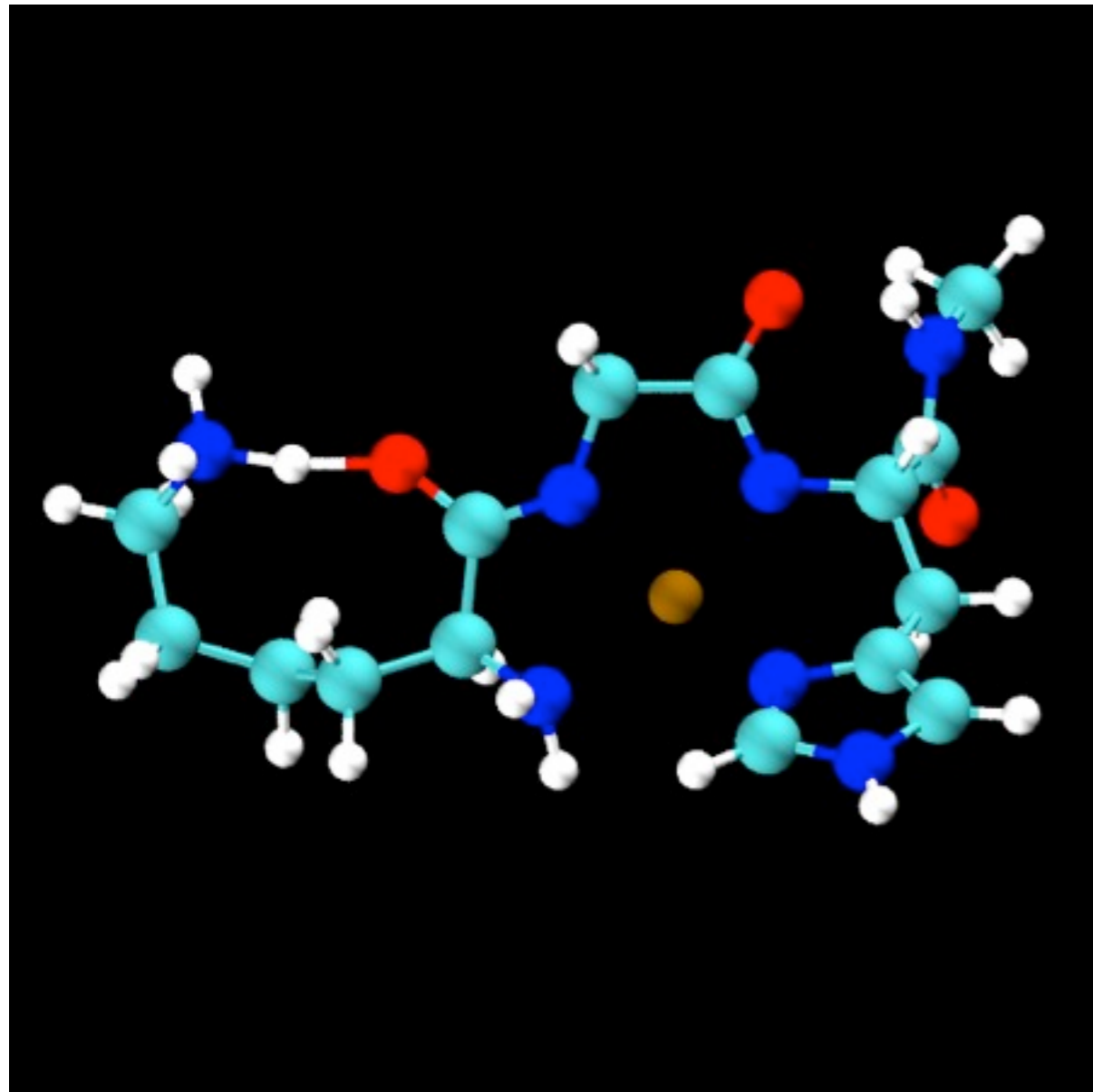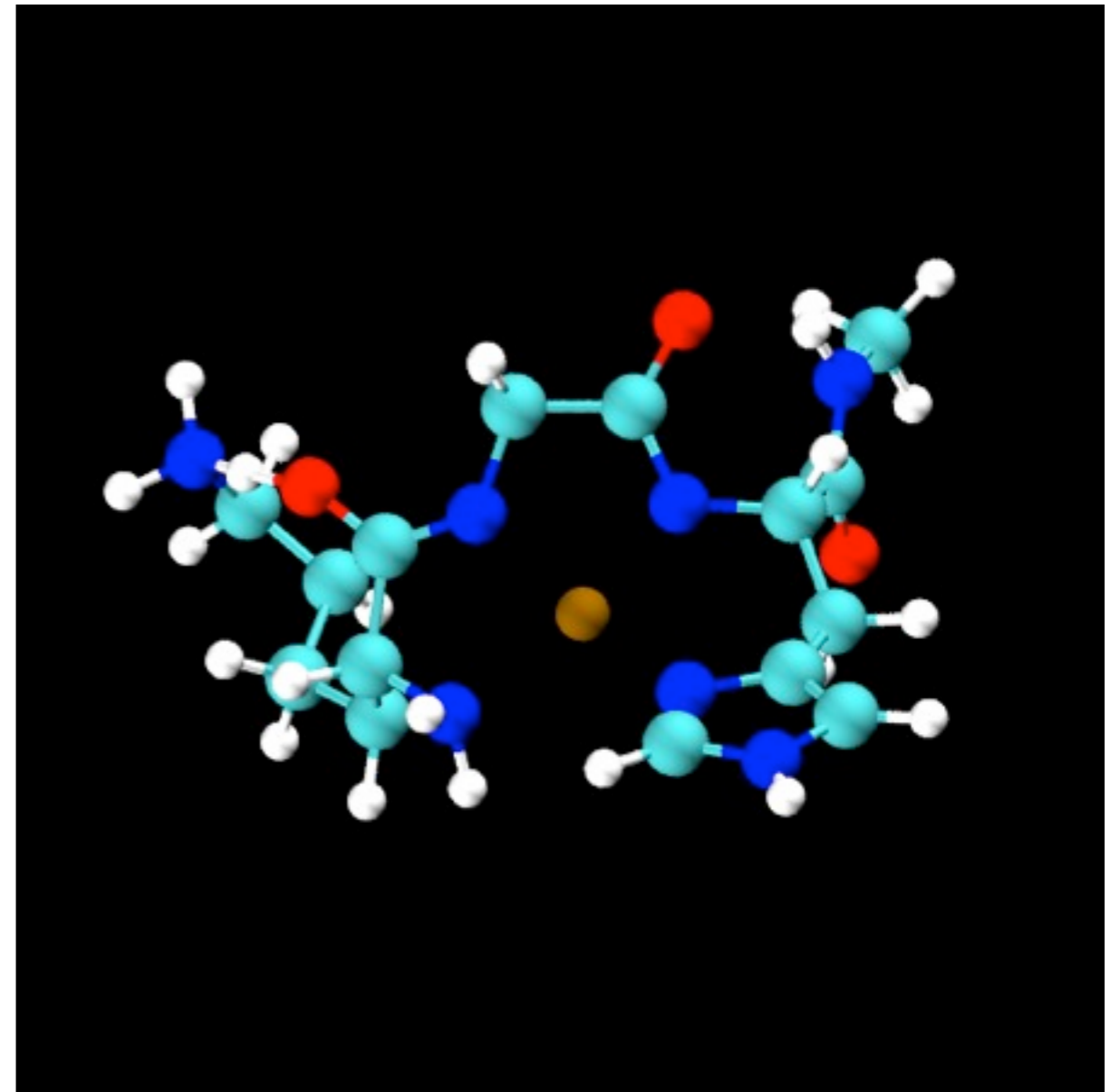
L-Form   D-Form

Cu^{II}-Lys-Lys-His
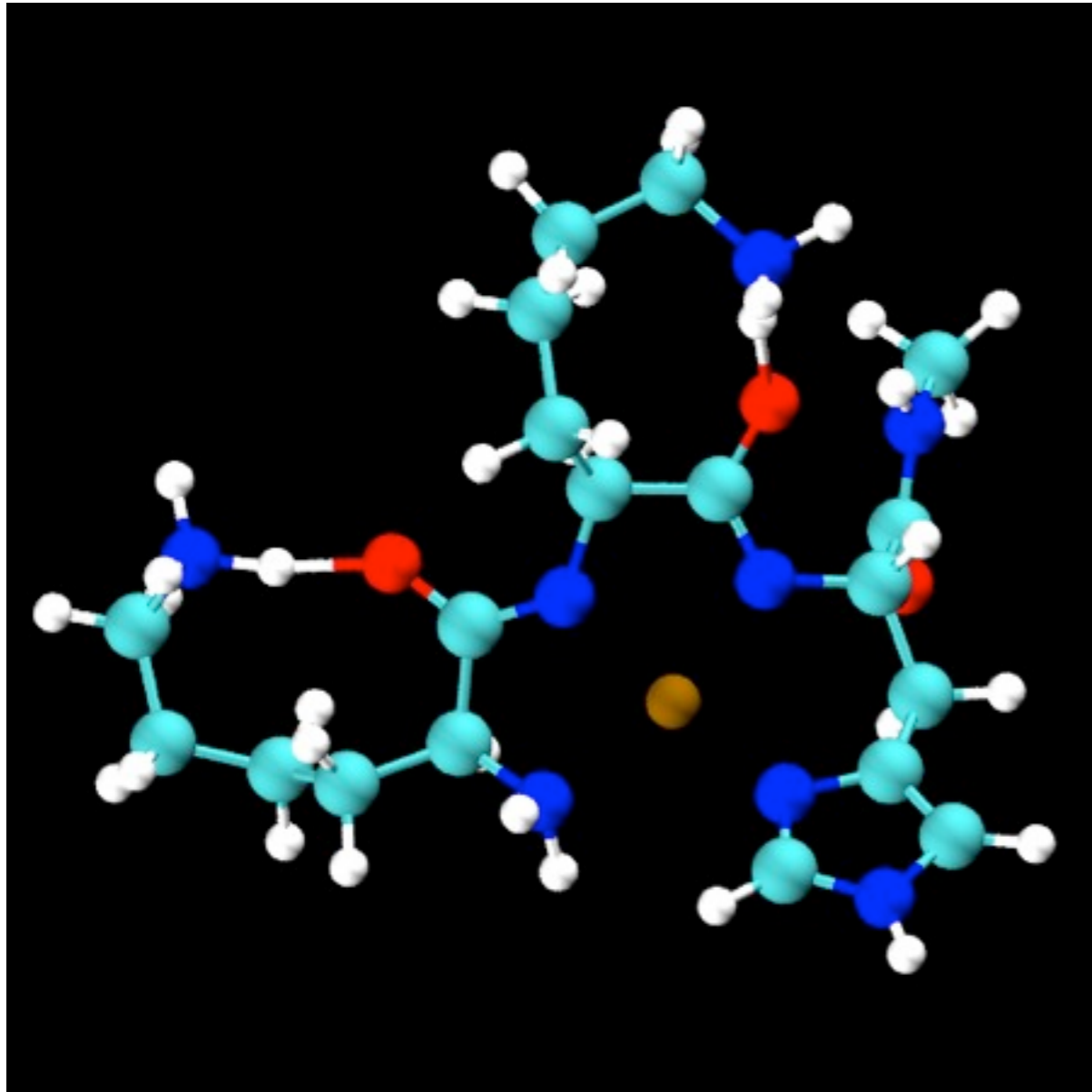
L-Form
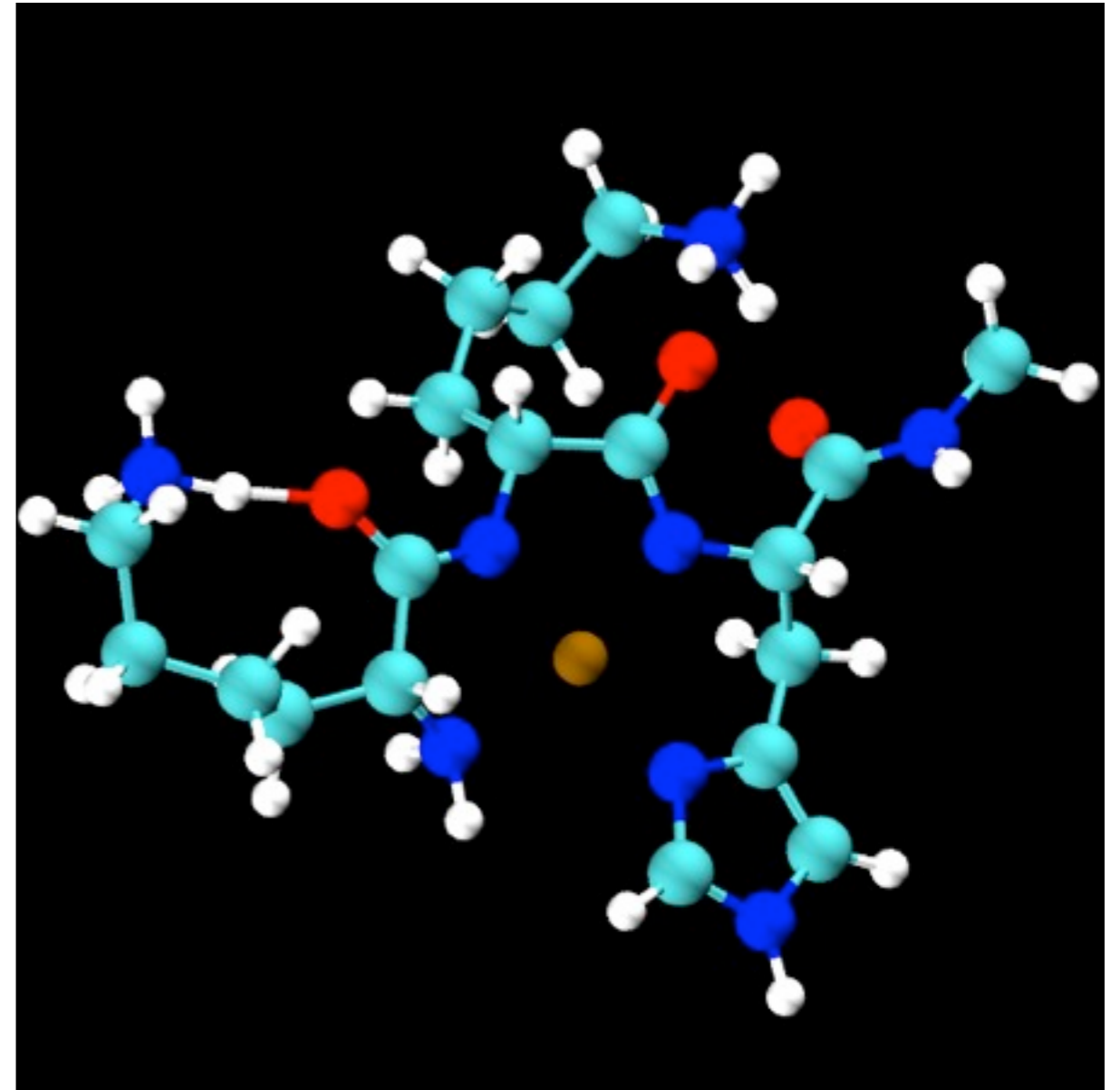
D-Form

Relative Redox Potential

[mV]	L-Form	D-Form
Gly-Gly-His	0	
Gly-Lys-His	-48	71
Lys-Gly-His	80	73
Lys-Lys-His	310	260

Discussion

- **Redox-coupled Proton Transfer**

- **Coexistence of Isomeric Forms**

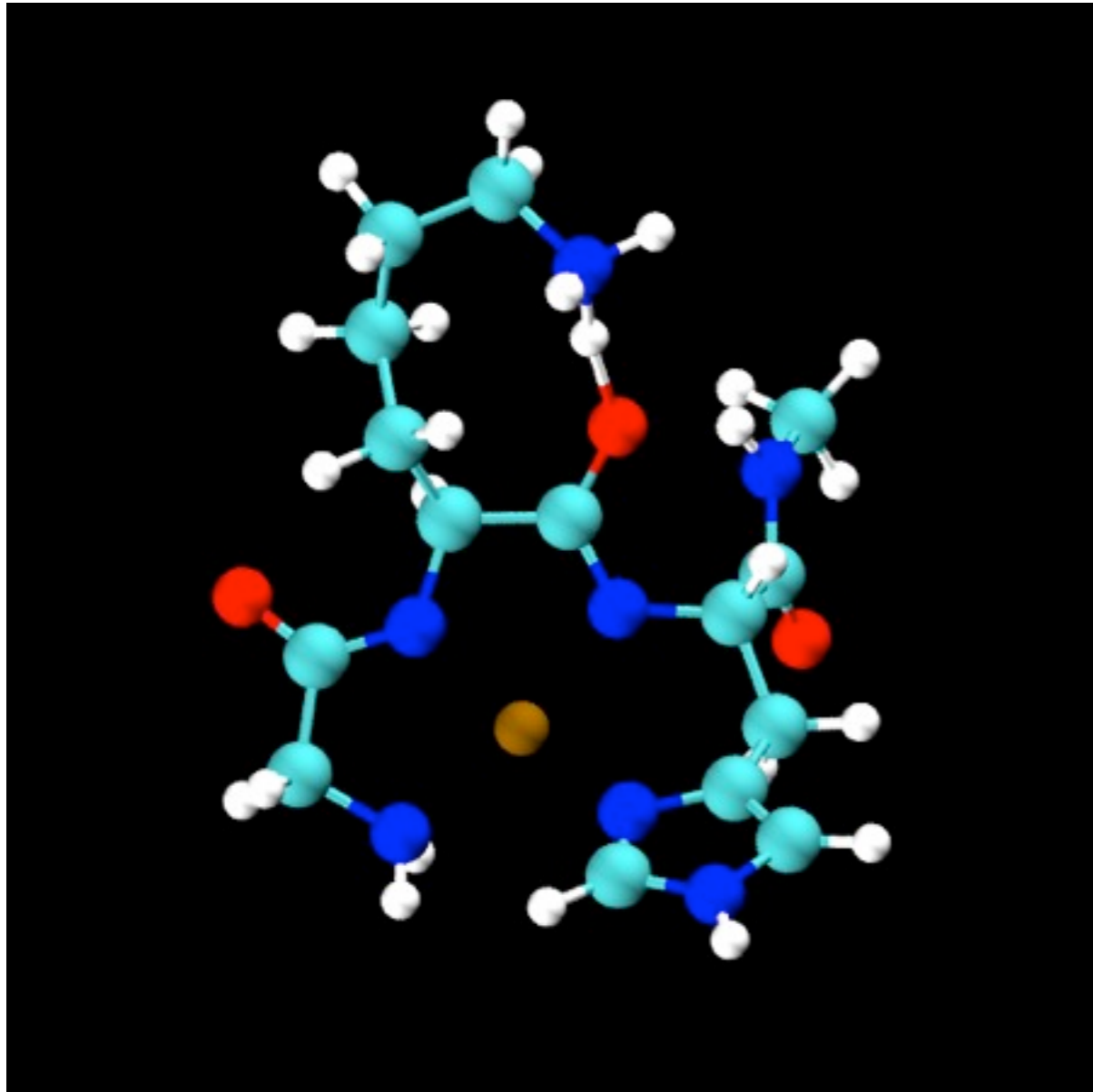- **Structural Transition of Side Chain**

Redox-coupled Proton Transfer
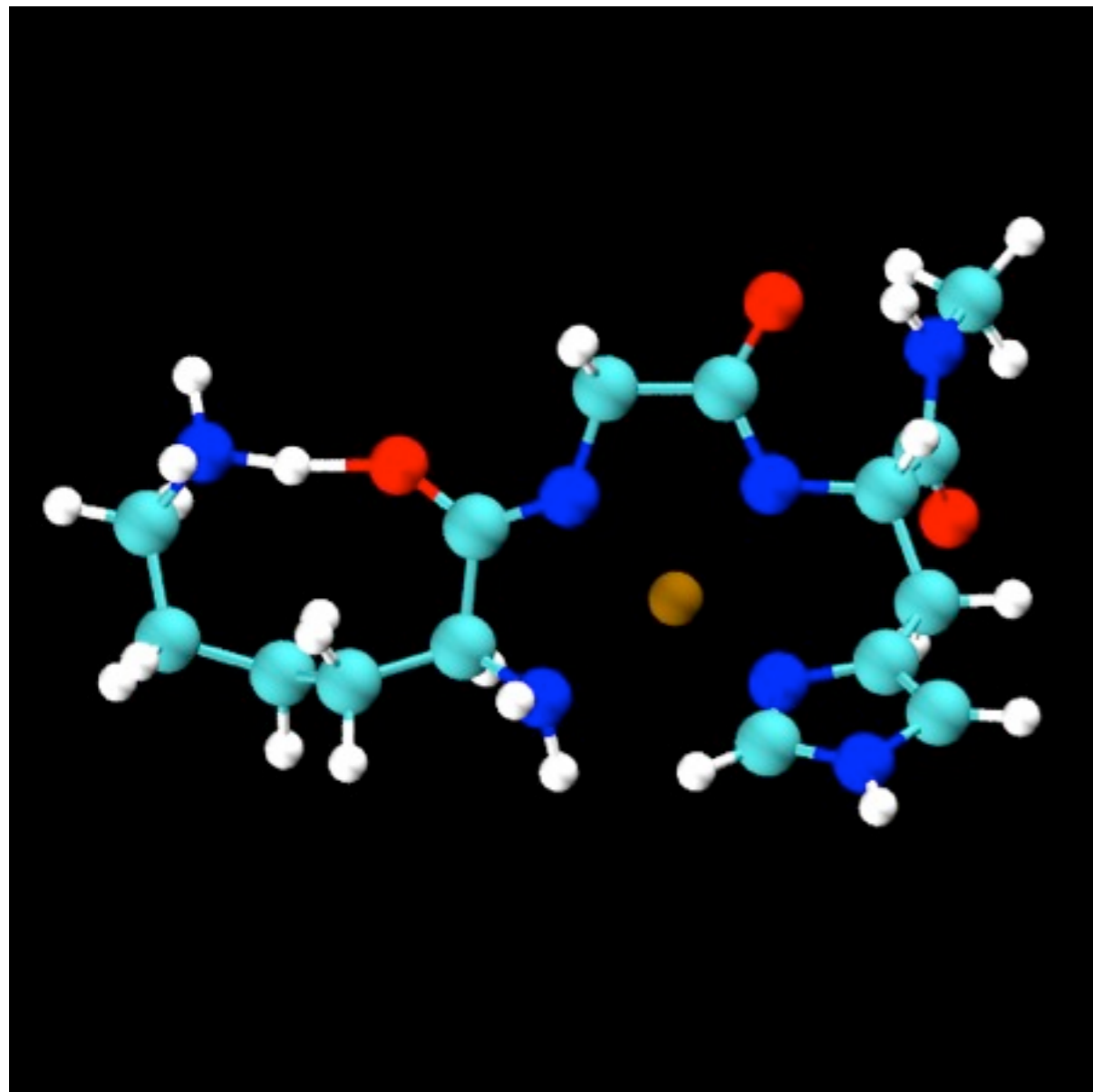
Cu^{II} Complex
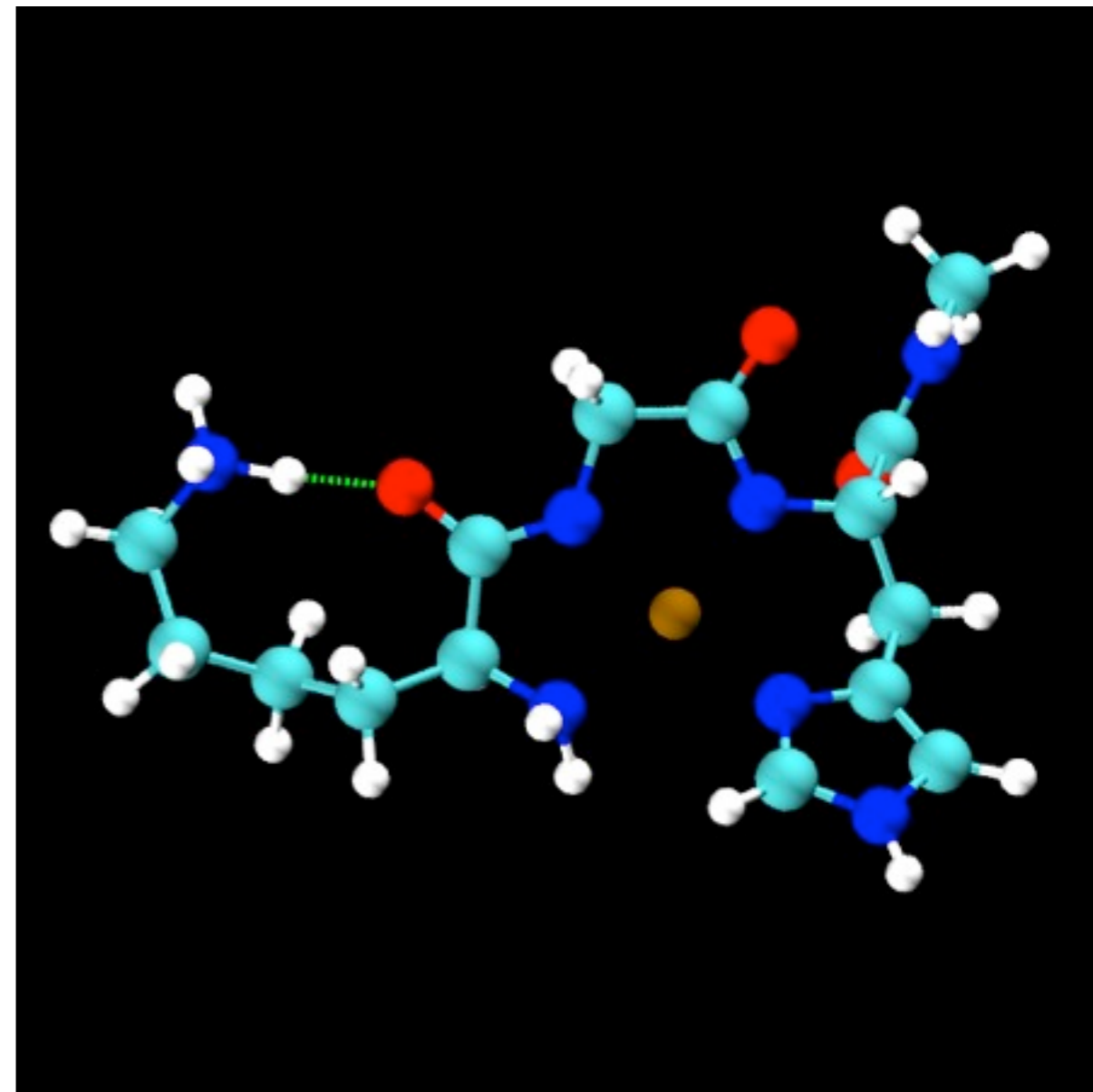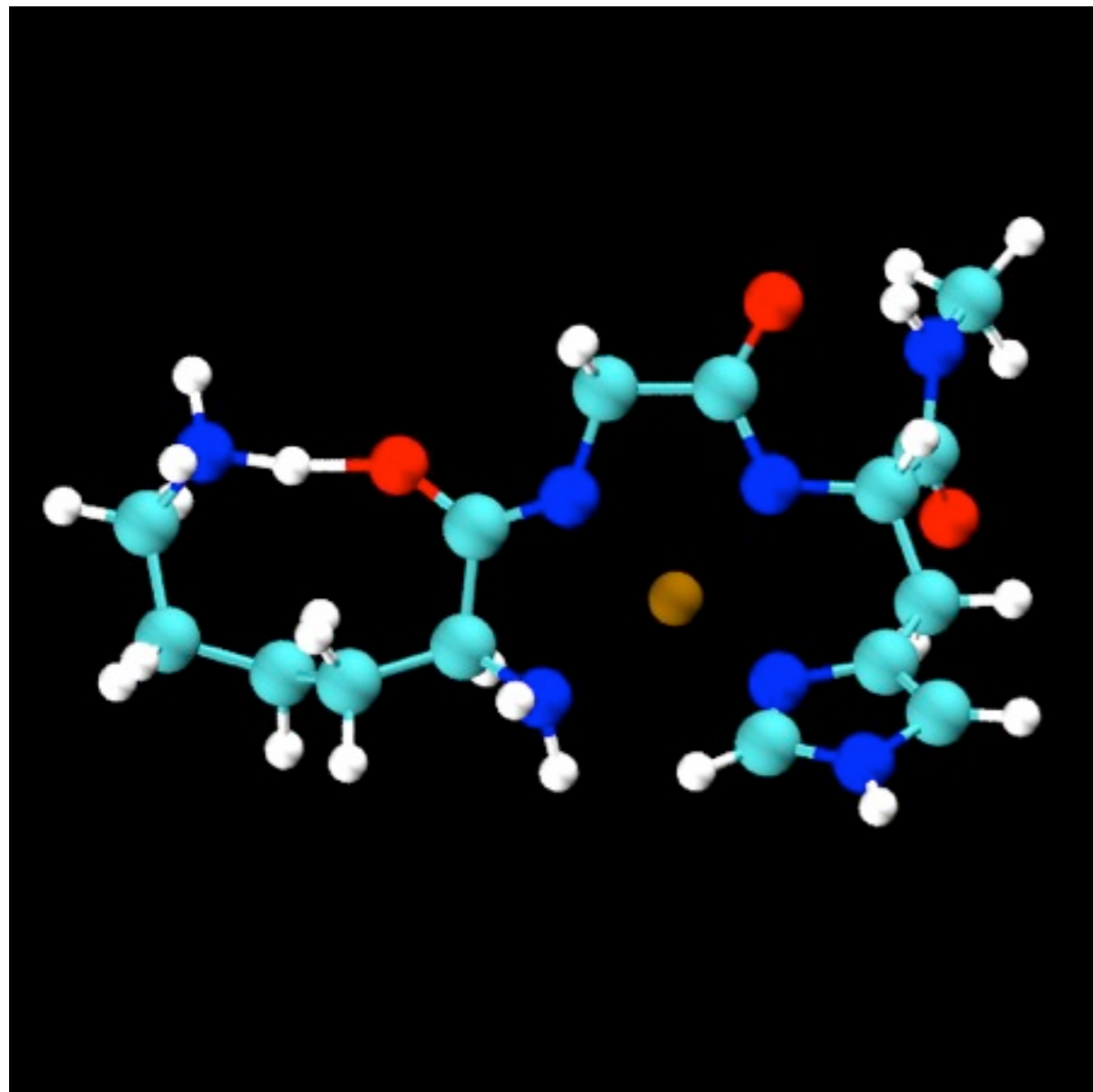
Cu^{III} Complex
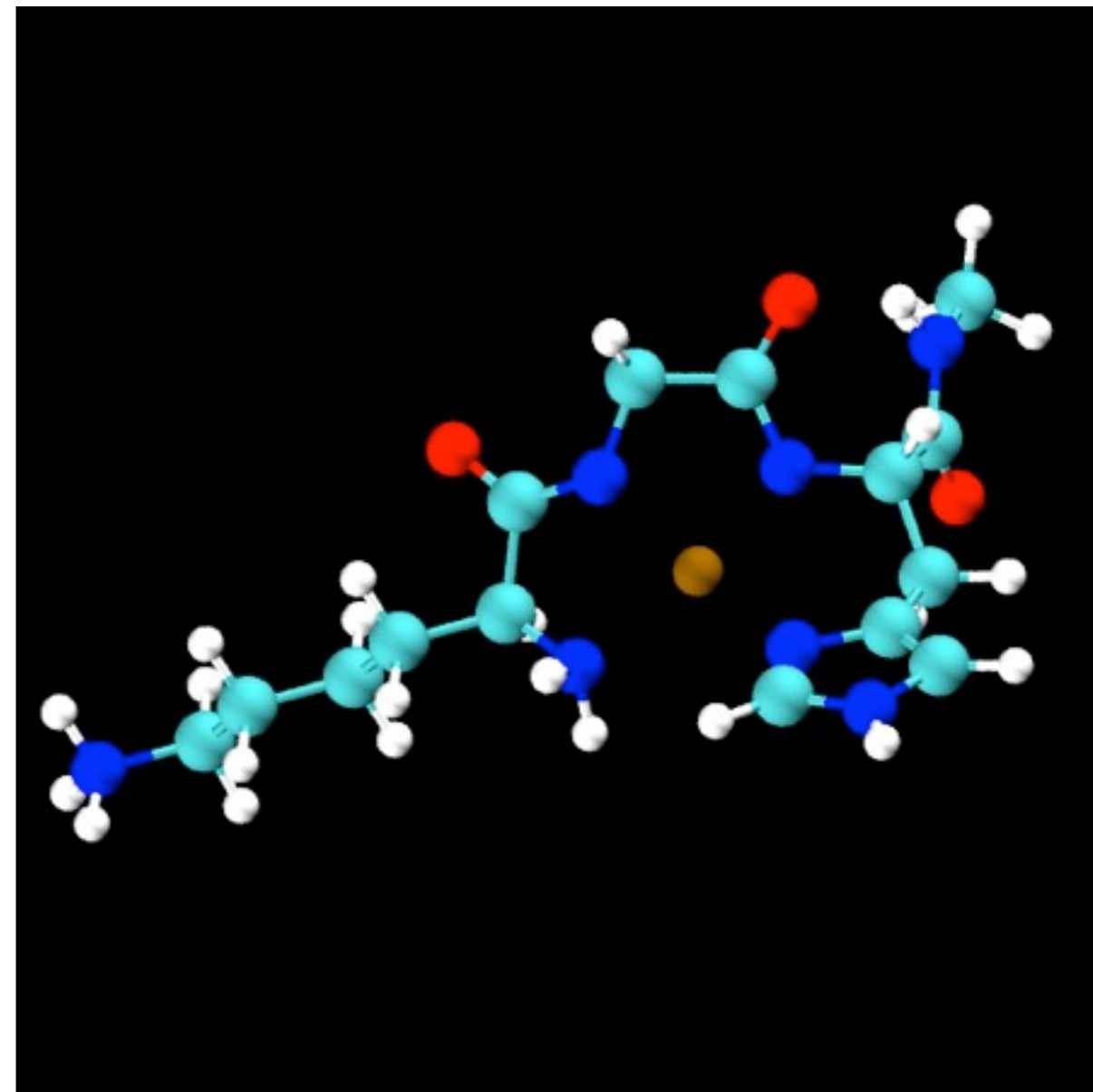
Coexistence of Isomeric Forms

Minimum
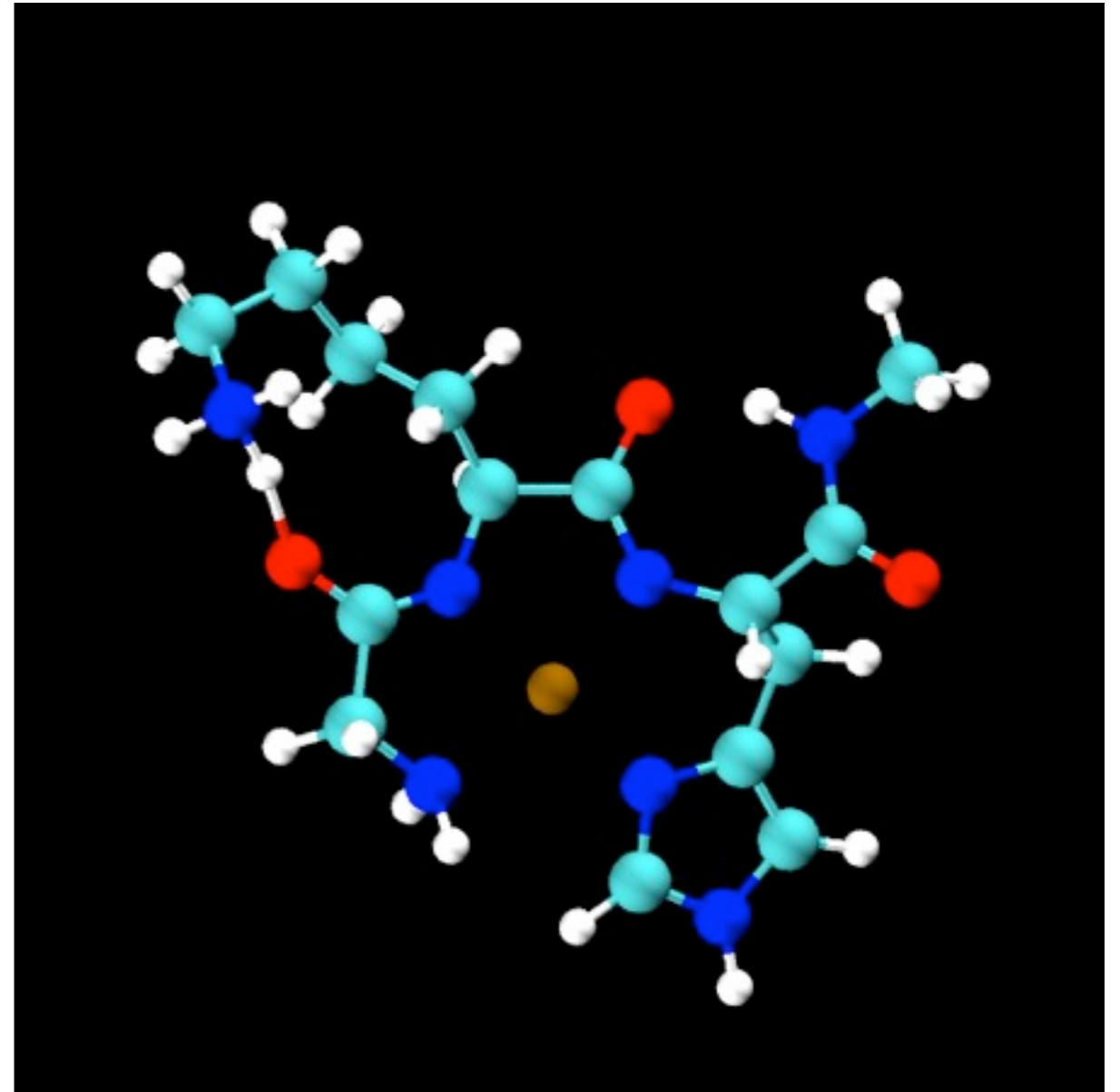
Isoform

Structural Transition of Side Chain

Folded

Extended
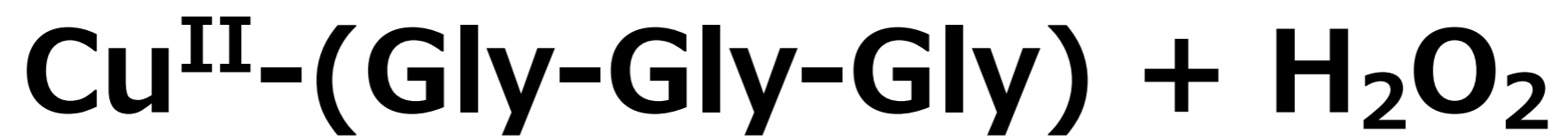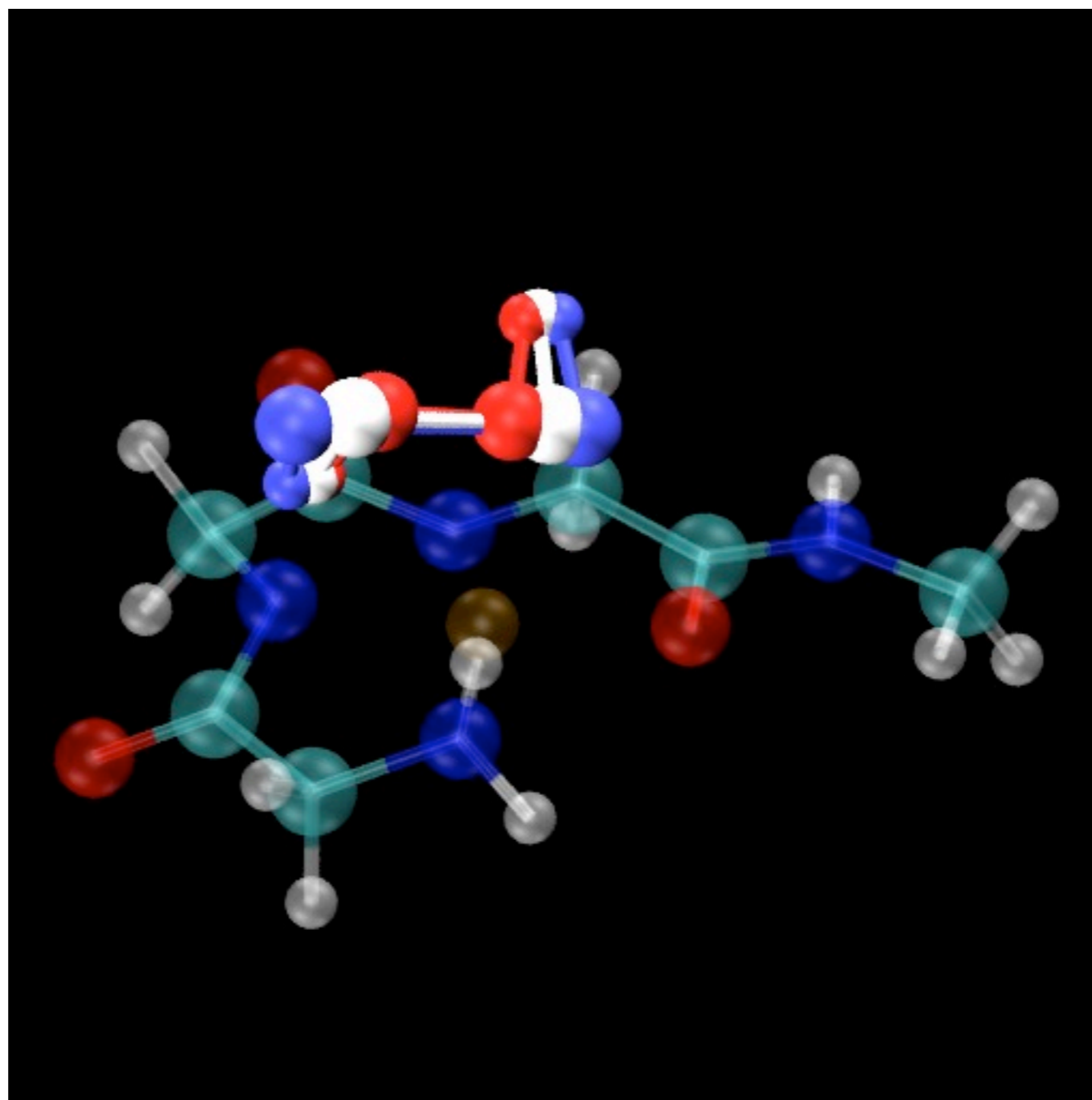
Todo

- Lys 置換が酸化還元電位およびDNA切断活性に及ぼす影響を明らかにする

    ➡ Extended 構造と H-bonded 構造の比較

- Lys 側鎖が形成する水素結合構造の状態分布および構造変化の動的挙動を明らかにする

    ➡ QM/MM-MD を使いたい。。。

- 金属中心部位で起こる触媒過程を定量化する局所的な反応指標を考える

金属結合ペプチドの触媒反応機構：
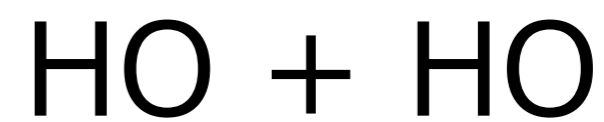# 過酸化水素の結合解離過程

Cu^{II}-(Gly-Gly-Gly) + H$_2$O$_2$
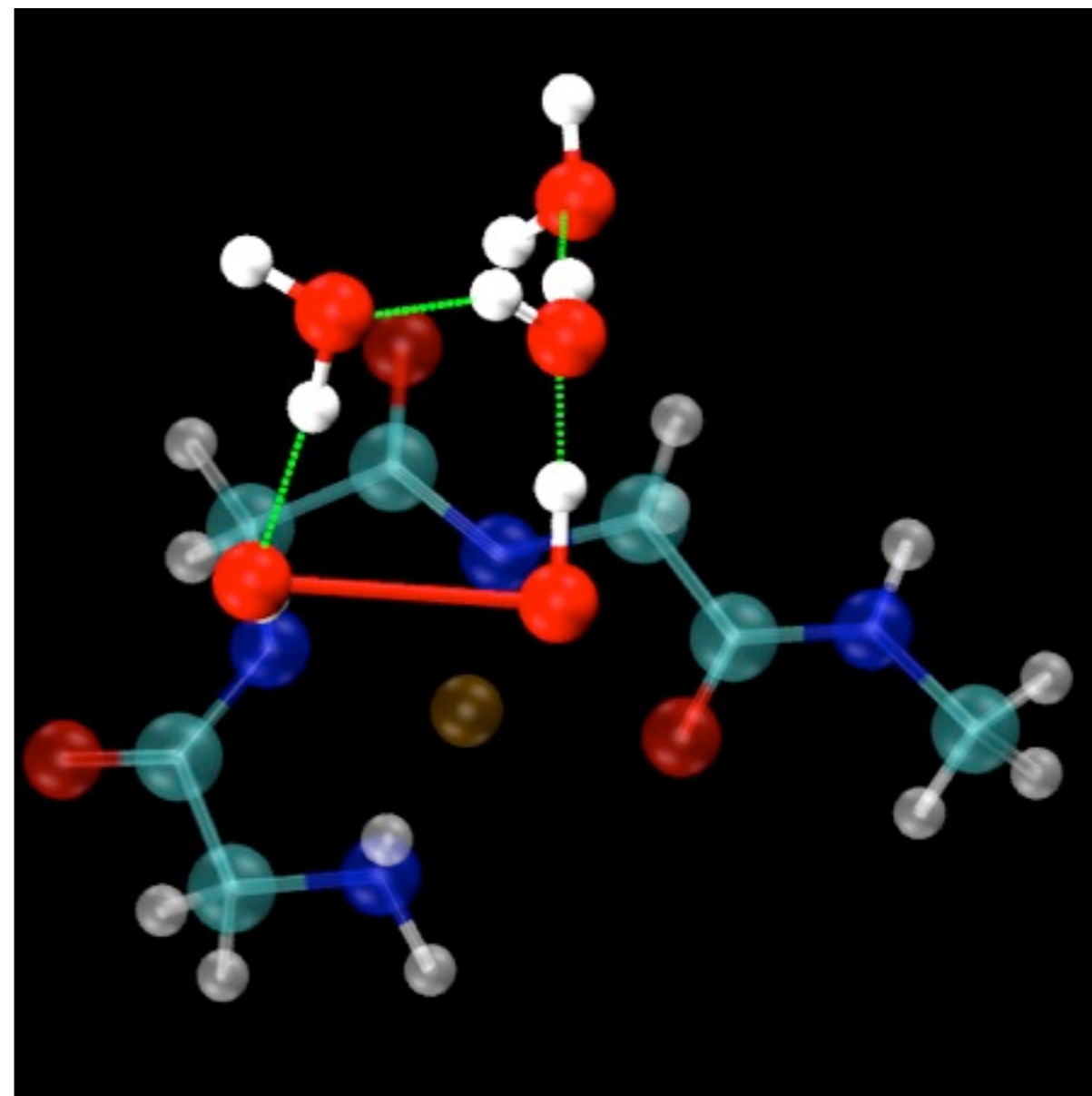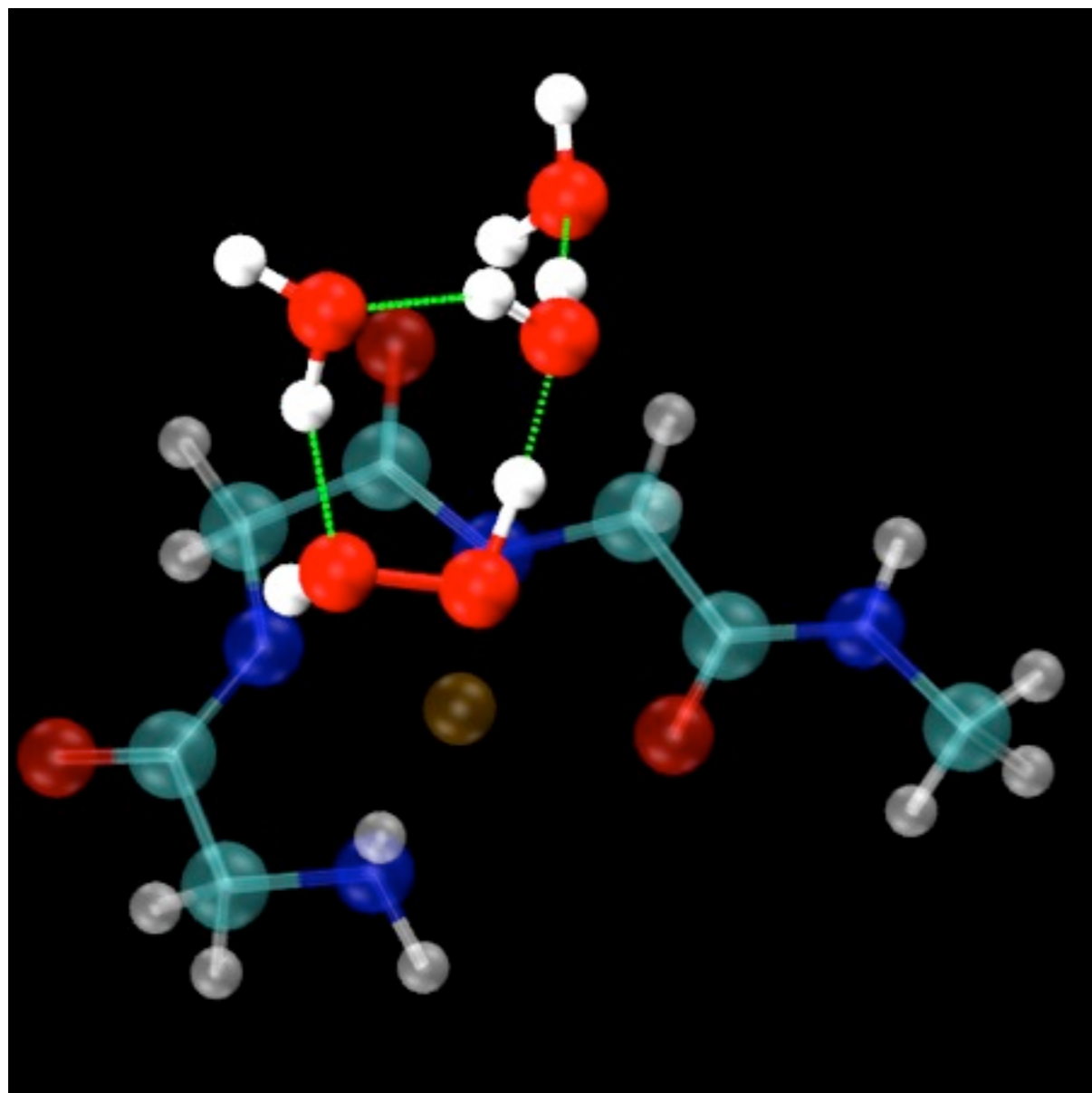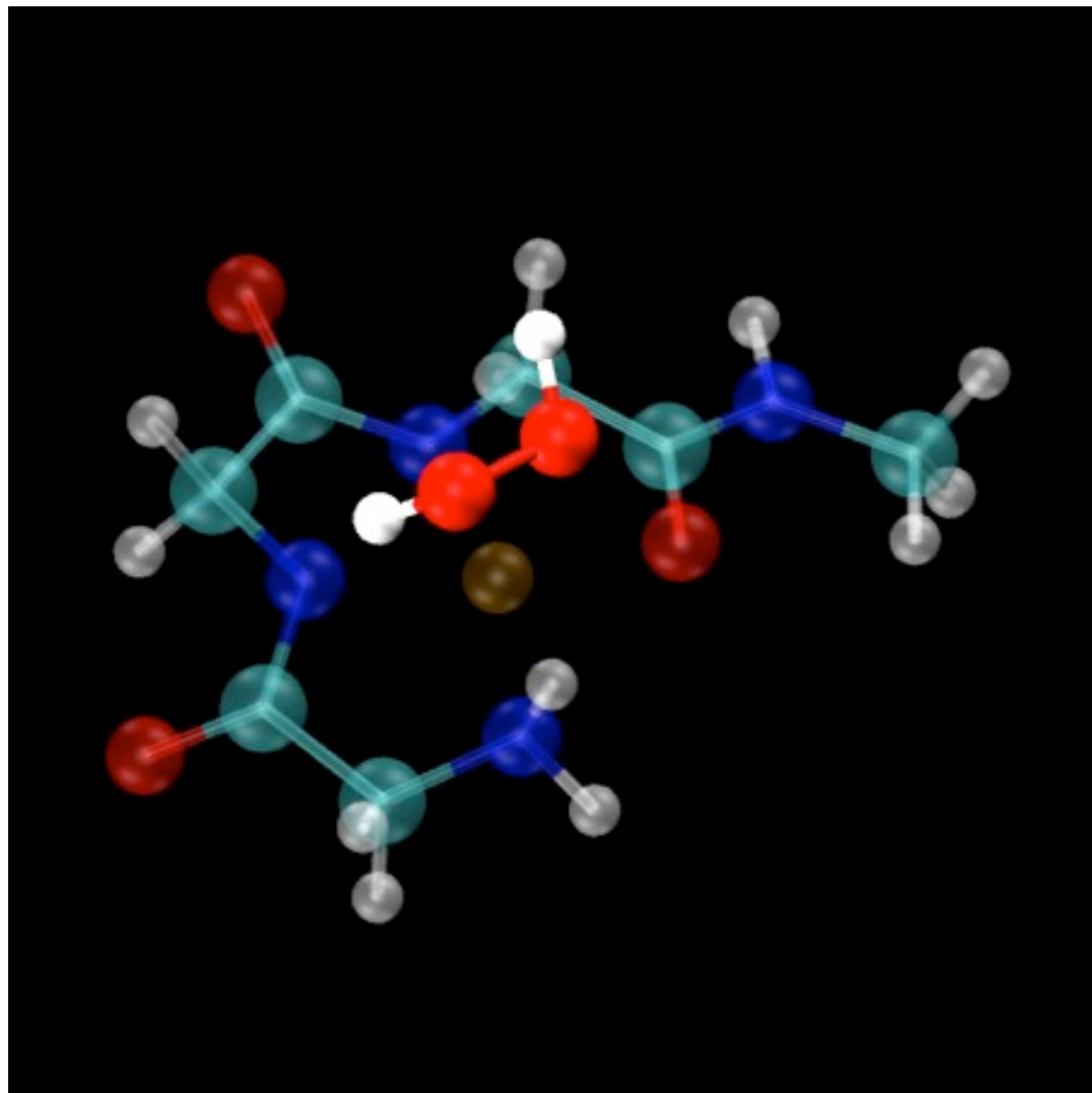
O-O Cleavage Reaction

DFT Calculations

- Gaussian 03 E.01

- Partial Geometry Optimization

  ➡ Freeze | Cu-ATCUN (& Solvated Waters)

  ➡ Optimize | H_2O_2

- UB3LYP / Huzinaga-Dunning & Hay-Wadt ECP

  ➡ D95V(d,p) | H [2s1p], C [3s2p1d]
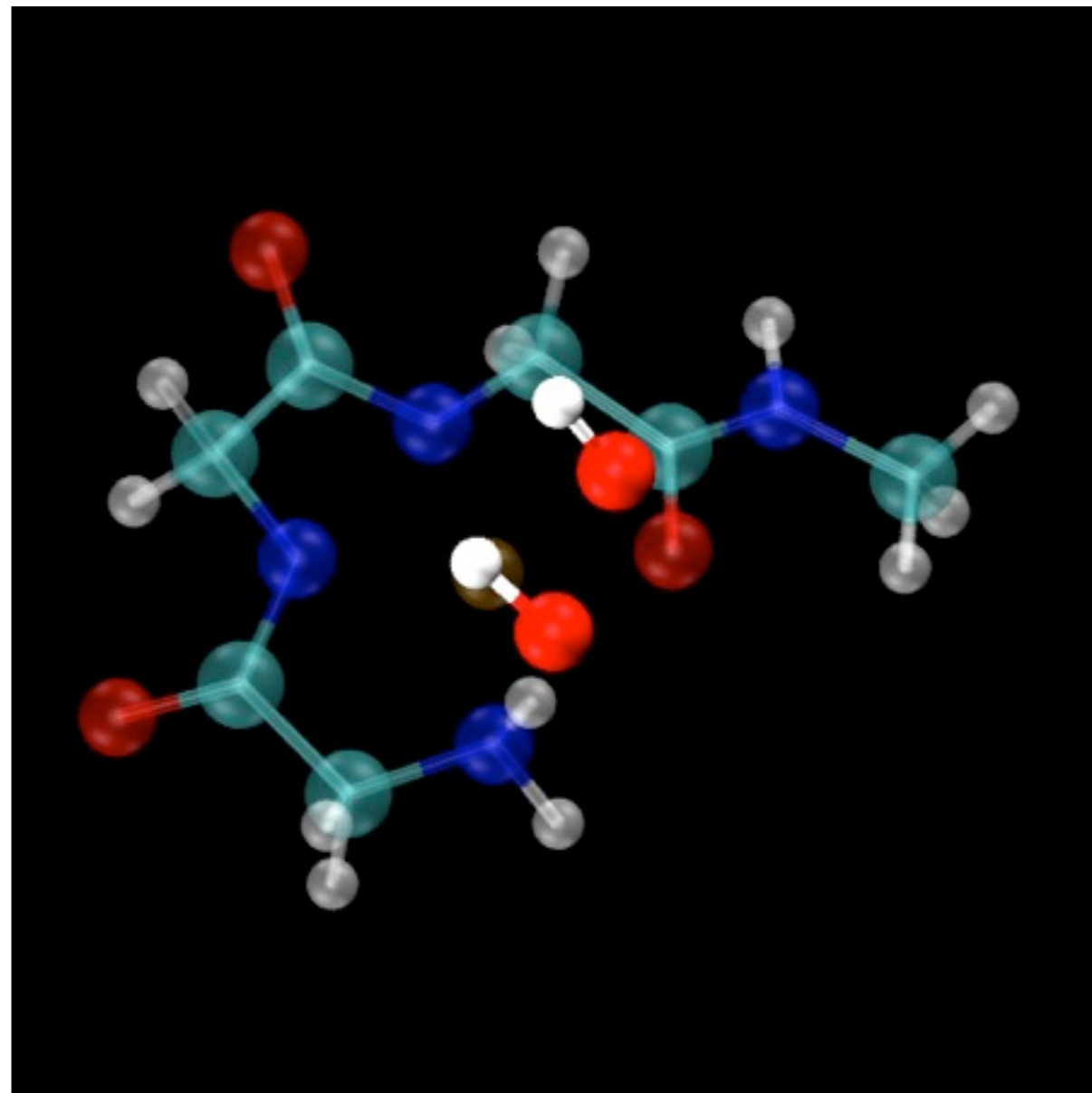
  ➡ D95V+(d) | O, N [3s3p1d]

  ➡ LanL2DZ | Cu [3s3p2d]

Reaction with Solvated Waters
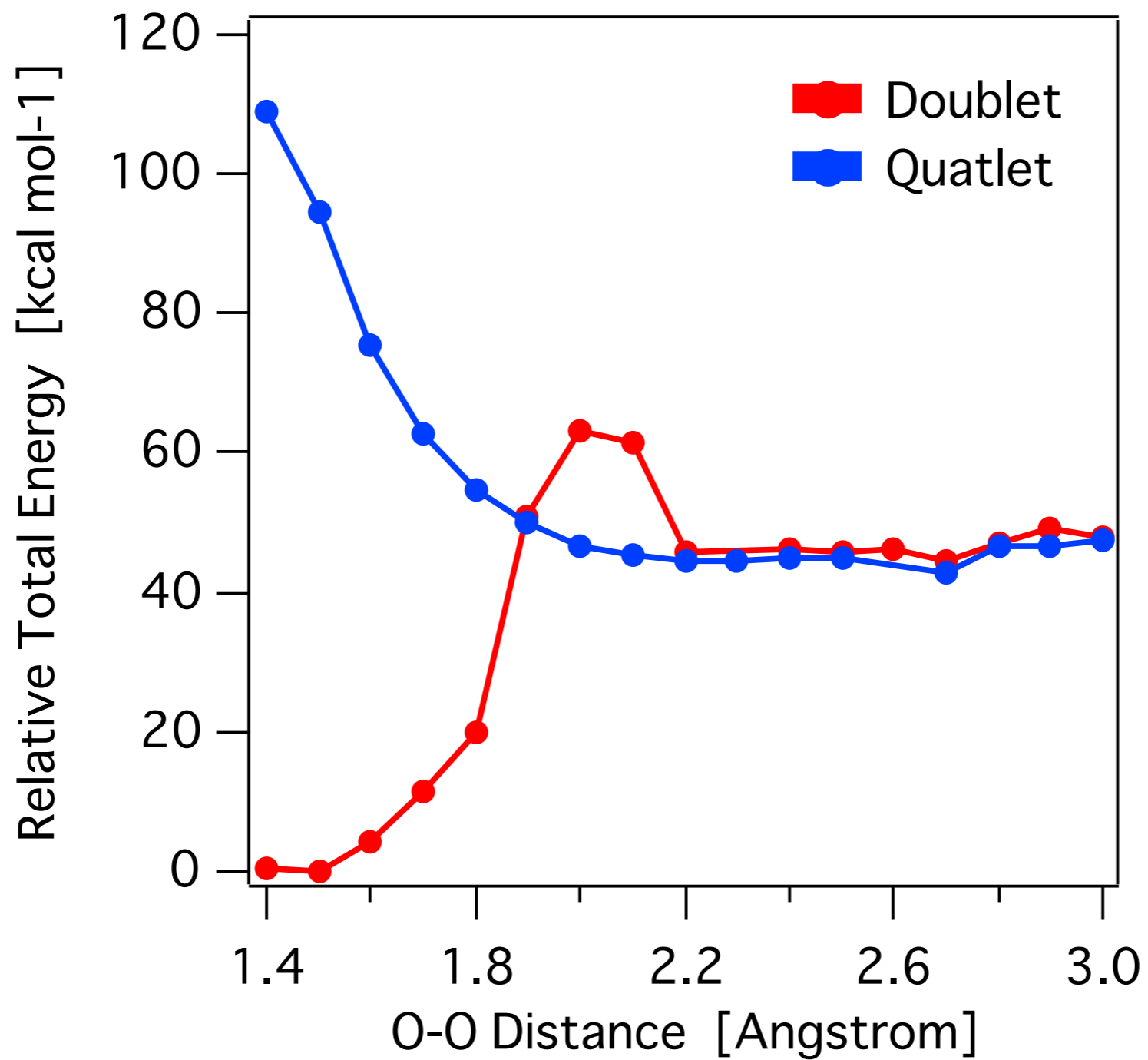

H_2O_2

$HO + HO$

cf.) Reaction in Gas Phase

H_2O_2

HO + HO

Potential Energy Surface

Relative Total Energy [kcal mol-1] vs O-O Distance [Angstrom]

Legend:
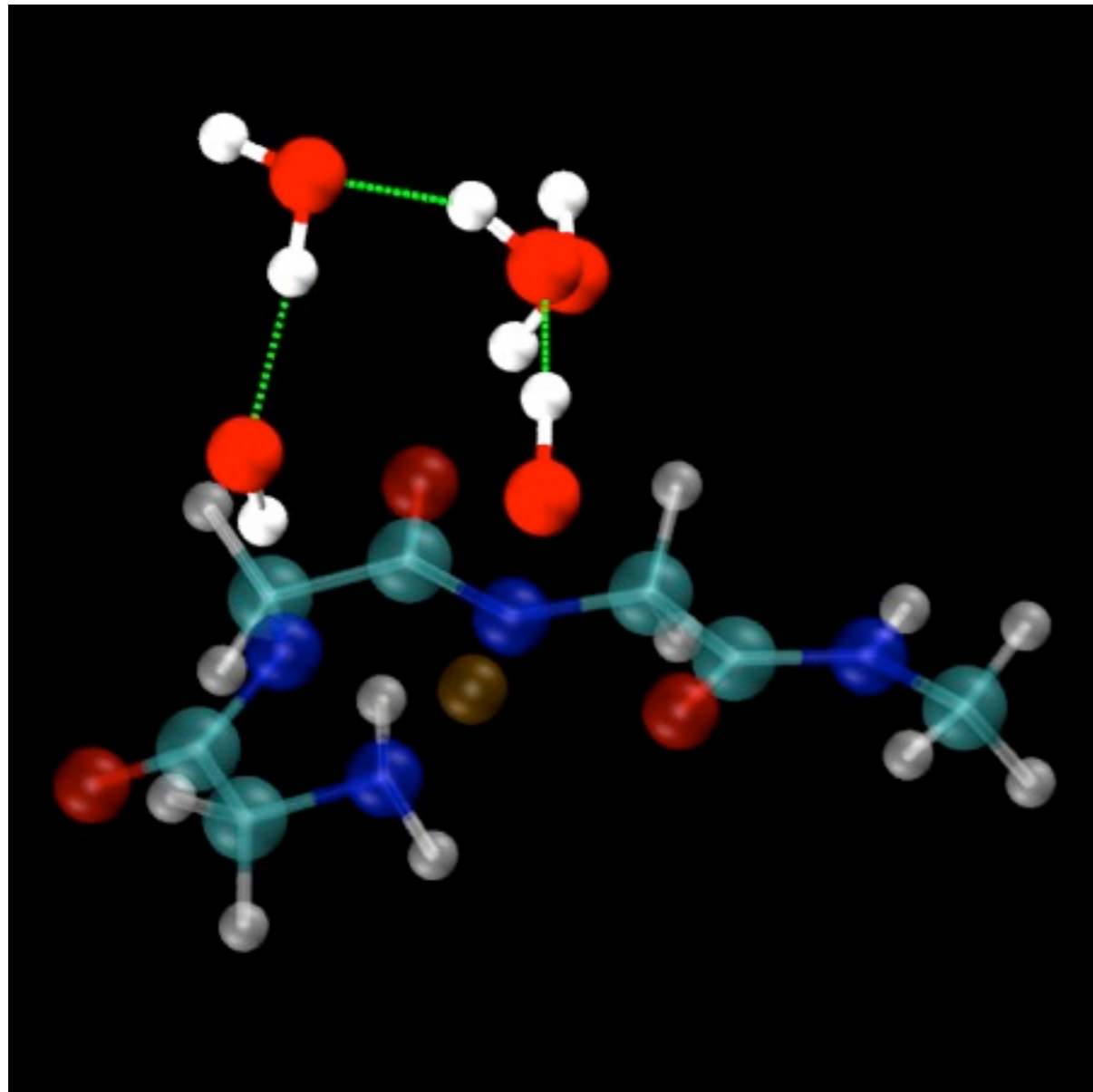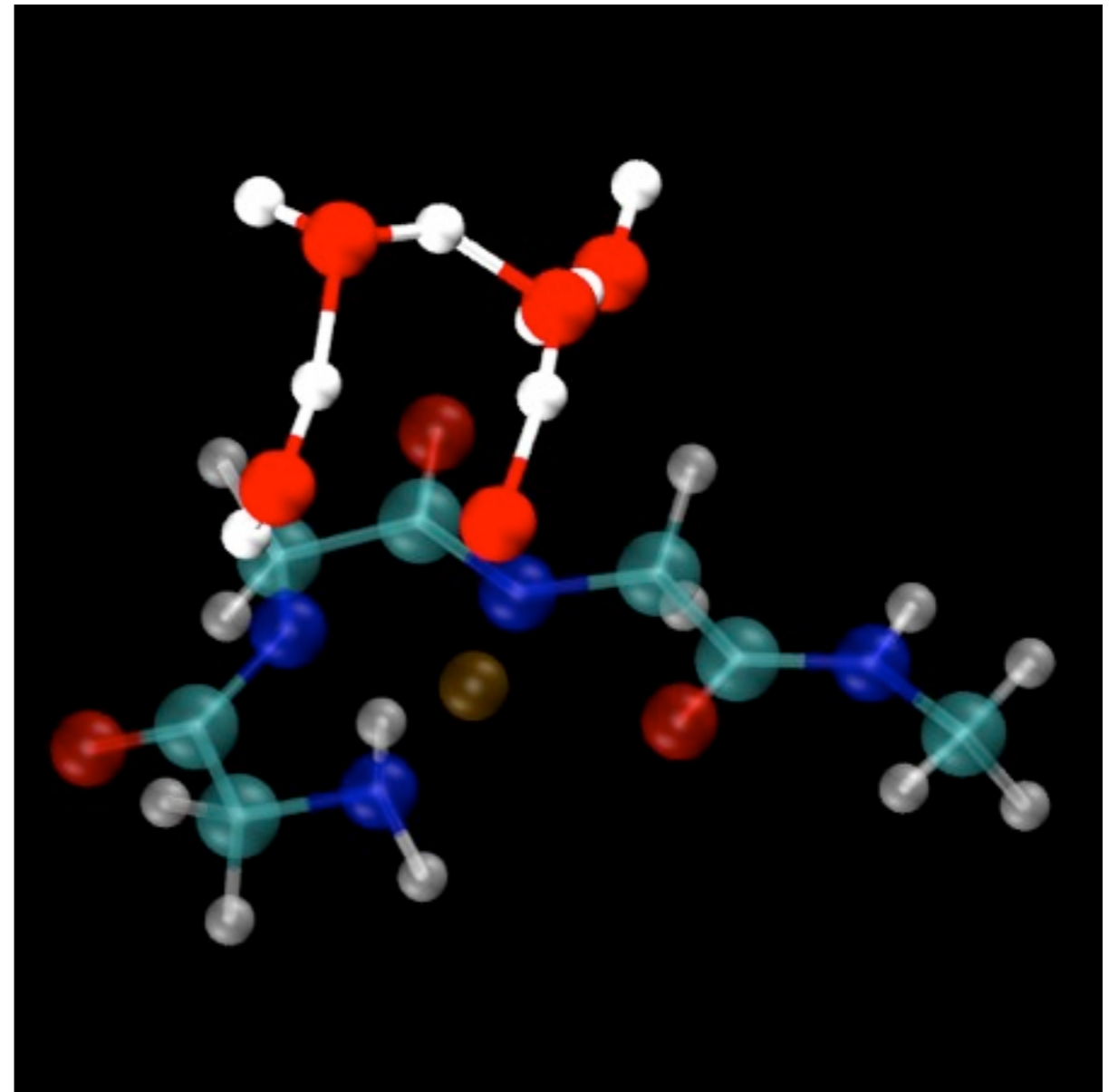- Doublet
- Quatlet

Todo / Plan

■ ポテンシャルエネルギー曲面の解析

   ➡ ROB3LYP (or CASSCF) で再計算

■ 自由エネルギー曲面の解析

   ➡ QM/MM; FEG-NEB を使える？

■ 反応に伴うプロトン移動過程の解析

   ➡ ab initio QM-MD を使う？

Proton Transfer

HO + HO
O + H$_2$O