QM/MM study of electron addition on protein disulfide bonds

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In the proteins, the redox systems Disulfides/Dithiols

are important for

- the regulation of the cell growth
- the development of human cancer

- the defence against oxidative stress
- the development of post-irradiation effects

Characteristics of disulfide bridges in proteins

- **Their number varies within proteins:**
  - ✓ **4 in Lysozyme (Lys)**
  - ✓ **3 in AcetylCholinEsterase (AChE)**
  - ✓ **1 in Thioredoxine (Trx)**

- **their environment (contraints or flexibility)**

- **the accessibility to the solvent**

Experimental studies of **monoelectronic reduction** of Lyzozyme (Lys), Thioredoxine (Trx)

by **γ and pulse radiolysis** in aqueous medium
Species characterized by **UV-visible spectra**

and of AcetylCholinEsterase (AChE) par **X ray**.

M. Weik, R. Ravelli

Laboratoire de Biophysique Moléculaire et EMBL Outstation de Grenoble

During the **redox** processes

P/SS$^{\bullet -}$ **or** P/**S**$^{\bullet}$ radical species are formed

(P/ represents the protein)

In vivo, electrons involved are produced by the

ionizing radiations, oxidative stress

and H^+ from neighbouring **residues**

Kinetic Scheme for the reduction of disulfide bridges

$(CO_2^{\bullet -}) + P/SS \rightarrow (CO_2) + P/SS^{\bullet -}$ (1)

$P/SS^{\bullet -} + H^+ \rightleftarrows P/SSH^{\bullet}$ (2)

$P/SS^{\bullet -} + H^+ \rightleftarrows P/(SH)S^{\bullet}$ (3)

$P/SSH^{\bullet} \rightarrow P/(SH)S^{\bullet}$ (4)

$2\ P/SS^{\bullet -} \rightarrow P/SS + P/(SH)_2$ (5)

$P/SS^{\bullet -} \rightarrow P^{\bullet}/SS$ (6)

$P^{\bullet}/SS \rightarrow$ products (7)

Methodological questions

- What is the **well-adapted quantic method** to study the radicals resulting from ionization and reduction?

  - depends on the physical or chemical property studied geometry, energy, wave length, coupling constant.

  - depends if the **electron is localized or not**

  on the atom → difficulties with bonds S-S, S-O, S-N **2centers-3electrons,** found in **cations** and **anions**

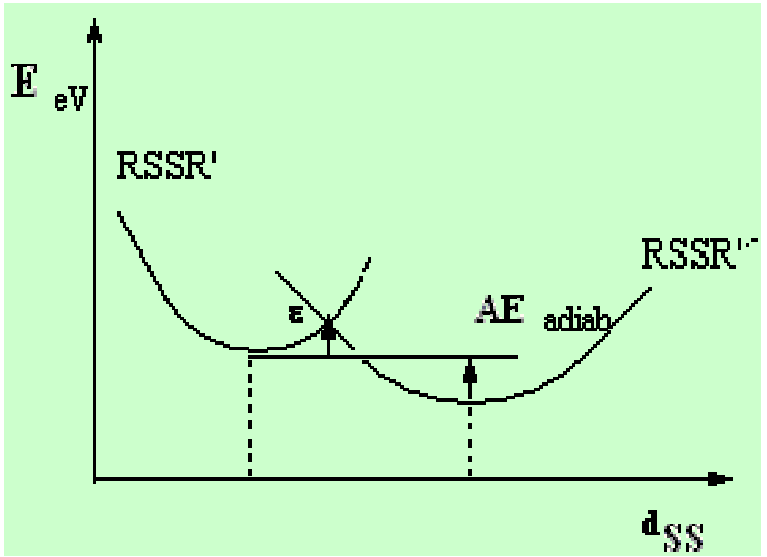
- How take into acount the **interactions with environment**: water, crystal, macromolecules...?

Calculations of the radical anions

- **Bond Dissociation Energies :**

$$BDE = E(RS^-) - E(R'S^\bullet) - E(RSSR'^{\bullet -})$$

- **Adiabatic Electronic Affinity, EA**



Model Molecules

The disulfide bridges were represented by: **RSSR′**

1) for Lyz: **R= R′=H or CH_3**
   then in **interaction with H^+**
   and **guanidinium ion (Arg)**

2) for AChE: **R= $(CH_2)_2COH$** and **R′=$(CH_2)_2NH_2$**

3) for Trx: as for AChE but **in interaction with a part of the protein; residues 32-39**

Calculation Methods

- **MP2** with 6-31G* et 6-31+G* bases sets
  for geometry optimisations with G94 and G98

- The **QM/MM** approach (ONIOM with G03) was tested with **HF, B3LYP and MP2** methods for the QM part (14-18 atoms) and **UFF** potential
  for the MM part (91 atoms)

Results for « simple » radicals

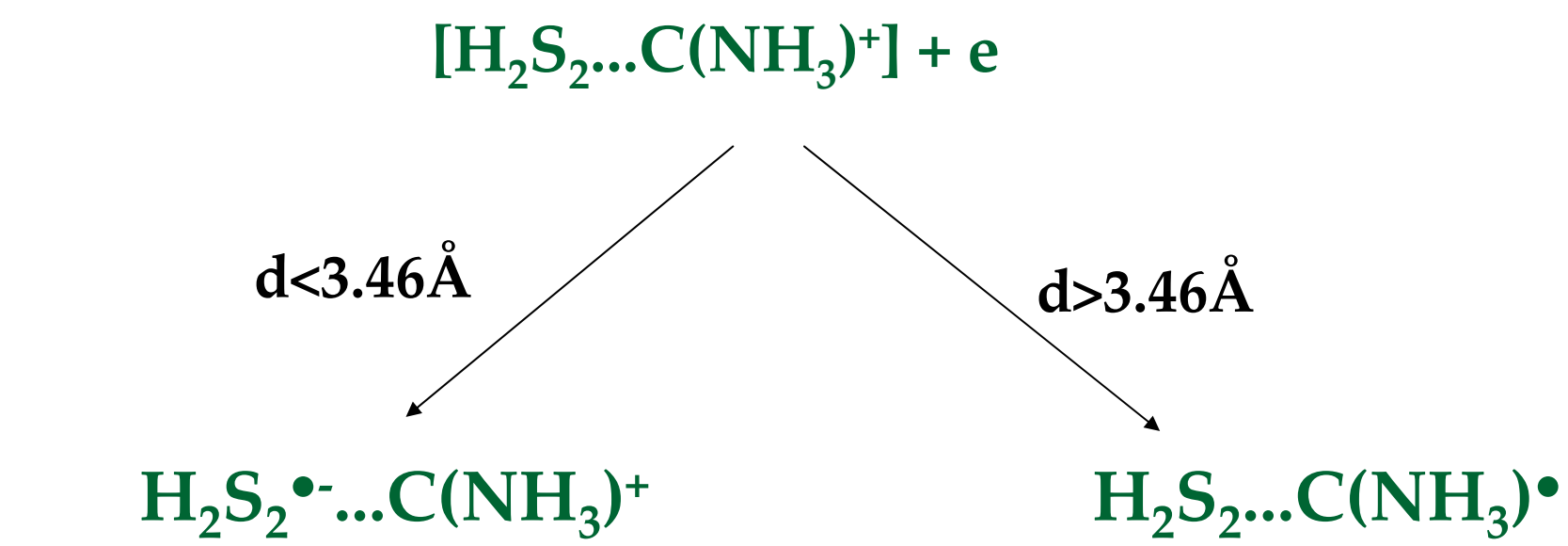
	r_{SS} (Å)	BDE_{SS} (kJ/mol)	EA (eV)
H_2S_2	2.072	224.0	
$H_2S_2^{\cdot -}$	2.810	96.6	0.55
$H_3S_2^{\cdot}$	3.535	7.9	
$(CH_3)_2S_2$	2.056	237.0	
$(CH_3)_2S_2^{-}$	2.788	98.6	-0.02
$(CH_3)_2S_2H$	3.744	11.3	

Disulfide bridge in the Lyzosyme: 1- Interaction with other residue

1 bridge more reactive for reduction

→ Cys6-Cys127 close to the charged Arginine

model complex $[H_2S_2...C(NH_3)^+]$ optimised



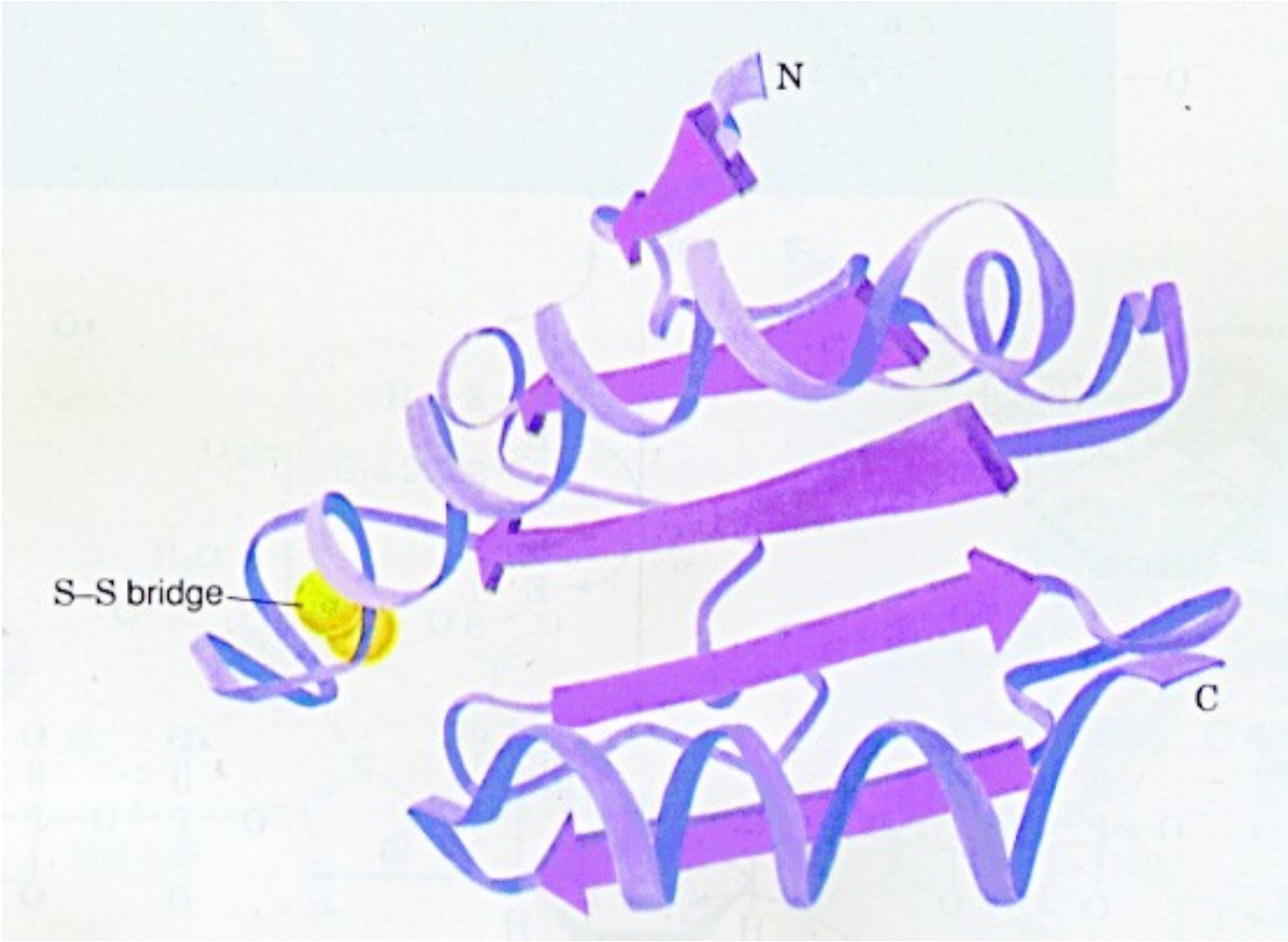
Disulfide bridge in the Lyzosyme: 2- Interaction with water

solvent was modelled by a continuum with a dielectric constant ε = 78 (option CPCM in gaussian)

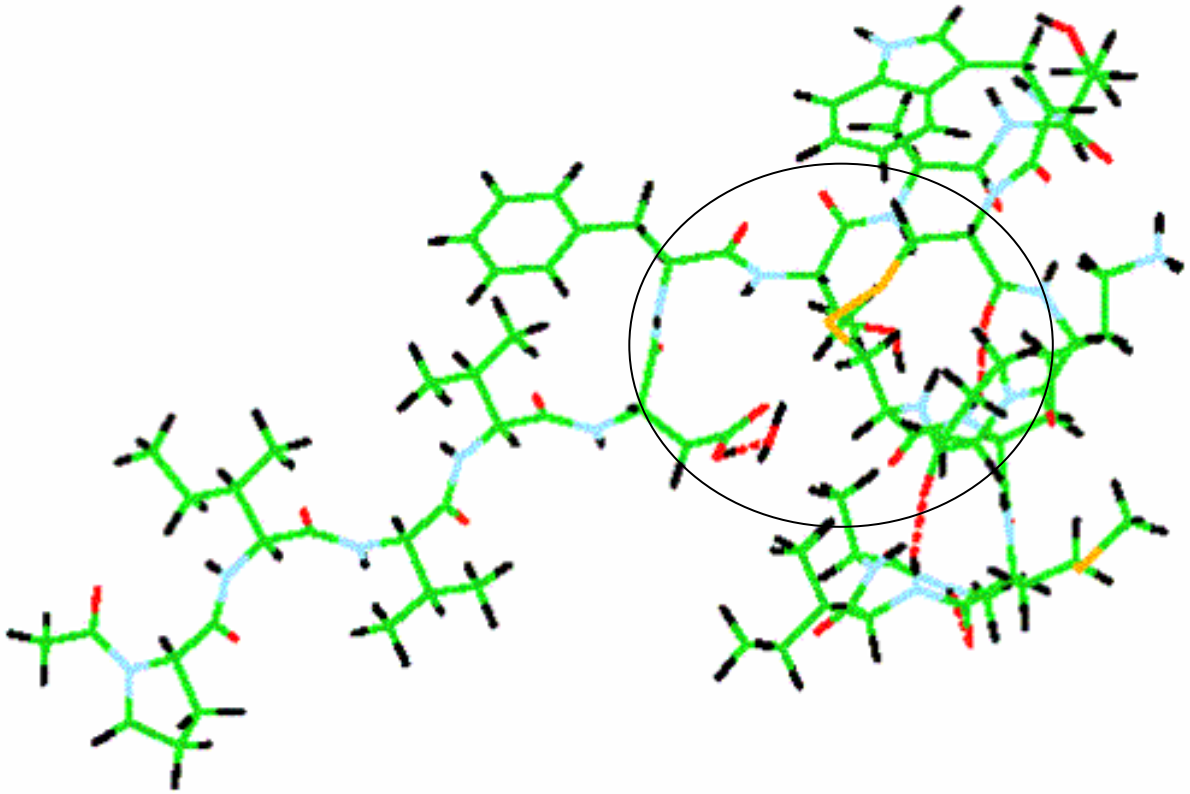
for Cys6-Cys127 solvent stabilizes the ZW complex

J. Bergès et al JPC (1997)

Thioredoxine Trx



- Close **Environment** of SS bond



- **Residues around SS bridge:**

  Cys32 and Cys35 ⇒

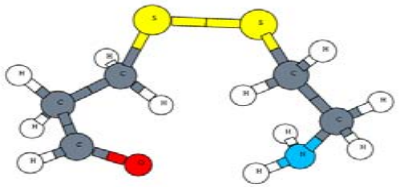
  R= $(CH_2)_2COH$; R′=$(CH_2)_2NH_2$

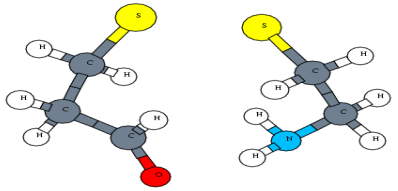
- **Residue close to SS bridge :**

  Asp30 ⇒ model: **CO_2H**

- **Water between Asp30 and SS bridge**

Disulfide modelling in Trx





Optimised Conformation close to the PDB one

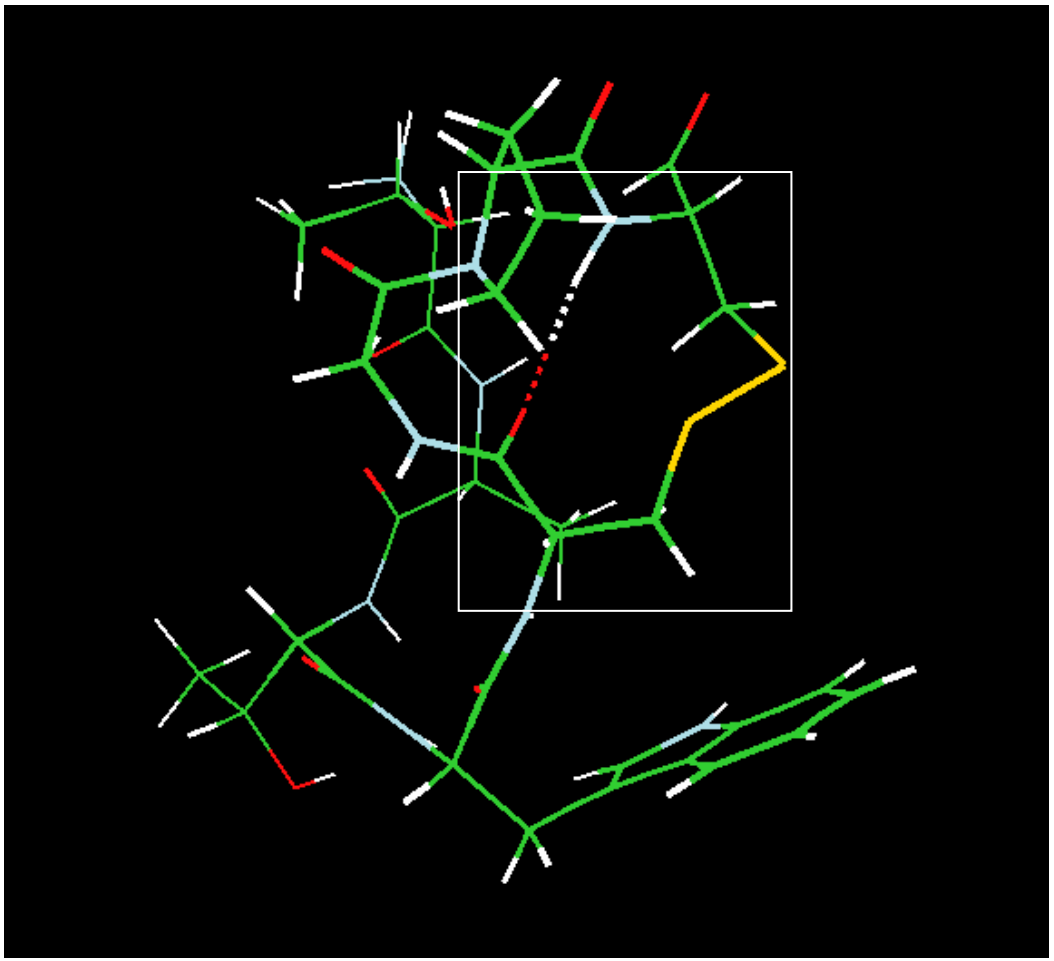
TrxSC **TrxSN**

	E (u.a.)	rSS (Å)	EA (eV)
Trx	-1121.31709	2.056	
$Trx^{\bullet -}$	-1121.33237	2.752	0.42

Breakage of the SS bond in Trx model

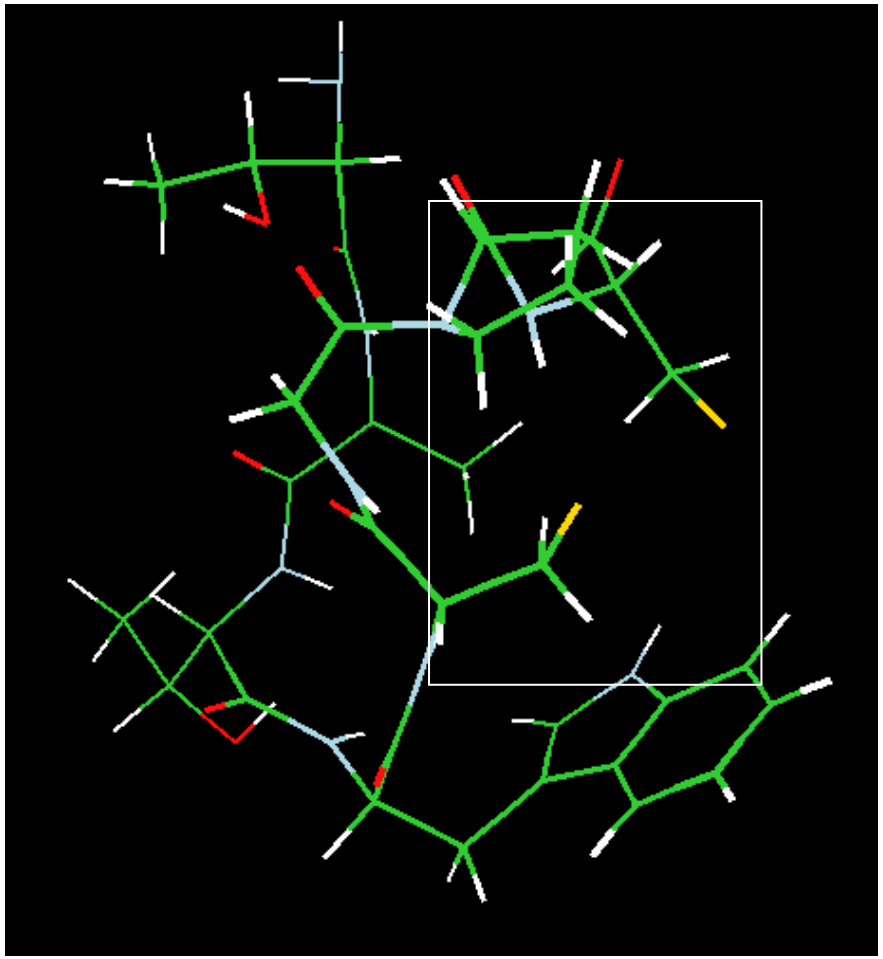
	BDE_{SS} (kJ/mol)
Trx	257.5
$TrxSC^{\bullet}$ + $TrxSN^{-}$	131.7
$TrxSC^{-}$ + $TrxSN^{\bullet}$	138.4

Disulfide RR'SS in Trx

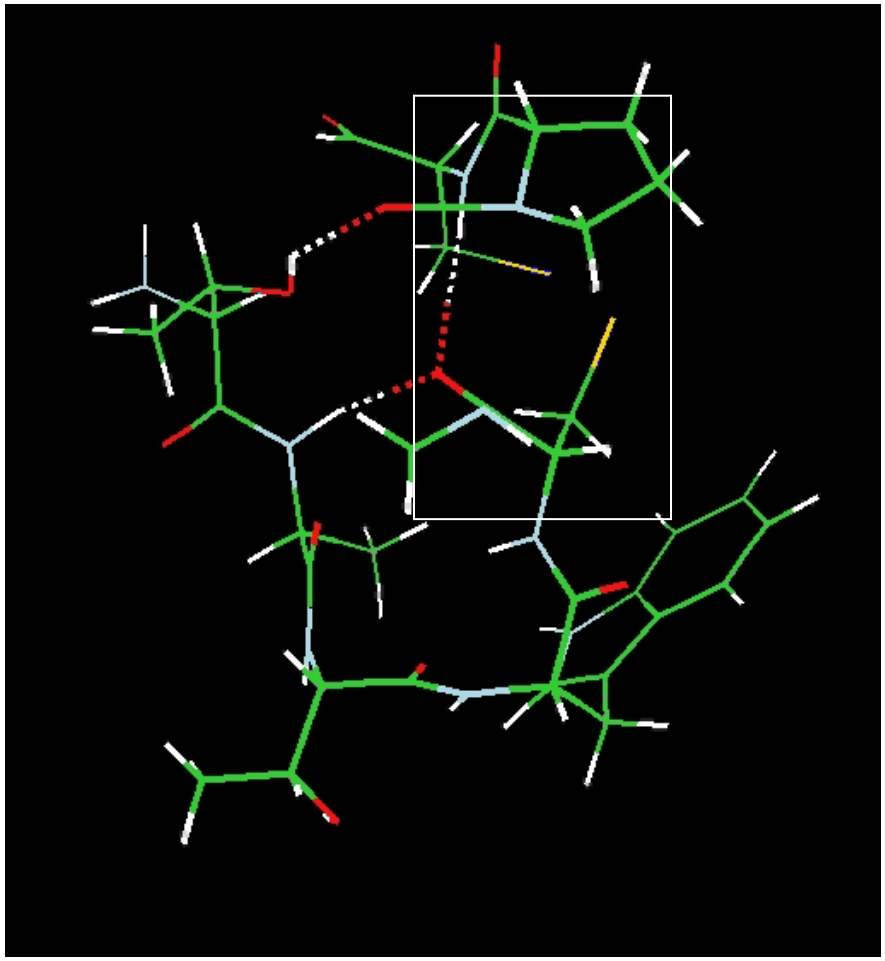


14 atoms QM (MP2/6-31+G*)+ 91 atoms MM (UFF)

Radical RR′SS$^{\bullet-}$ in Trx



Different structures around SS for TrxSS$^{\bullet-}$



Electronic Affinity of the SS bond
for different Trx models

	r_{S-S} (Å)	A.E.(eV)
(14QM)	2.752 2.856 (2.910)[(a)]	0.42 0.31 (0.39)[(a)]
(16QM+CO)	2.793	0.81
(16QM+NH)	2.766	0.31
(18QM)	2.798	0.85

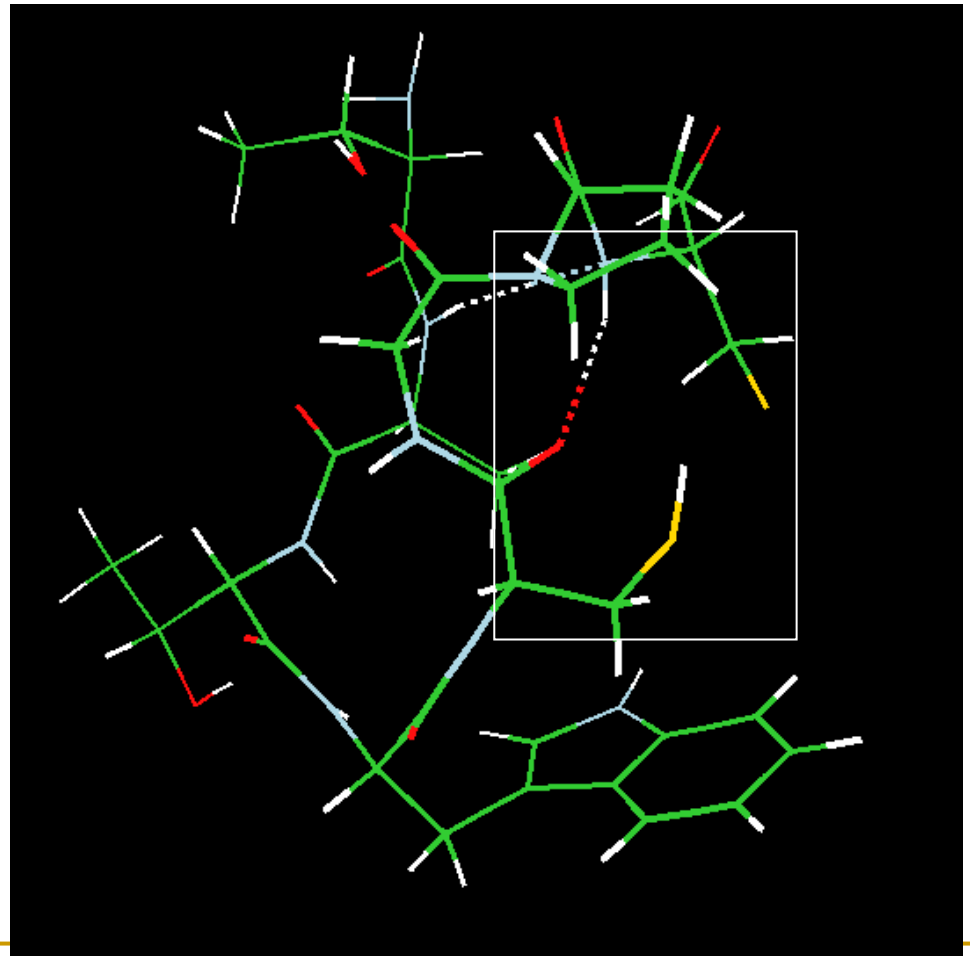
(a) HF/3-21+G* (6-31+G*)

Electronic Affinity of the SS bond
for different Trx in « proteins »

	r_{S-S} (Å)	A.E.(eV)
(14QM)	2.803 3.049 (6.7)[(a)]	0.55 0.13 (0.24) [(a)]
(16QM+CO)	2.810	0.68
(16QM+NH)	2.796	0.24
(18QM)	2.777	0.63

(a) HF/3-21+G* (6-31+G*)

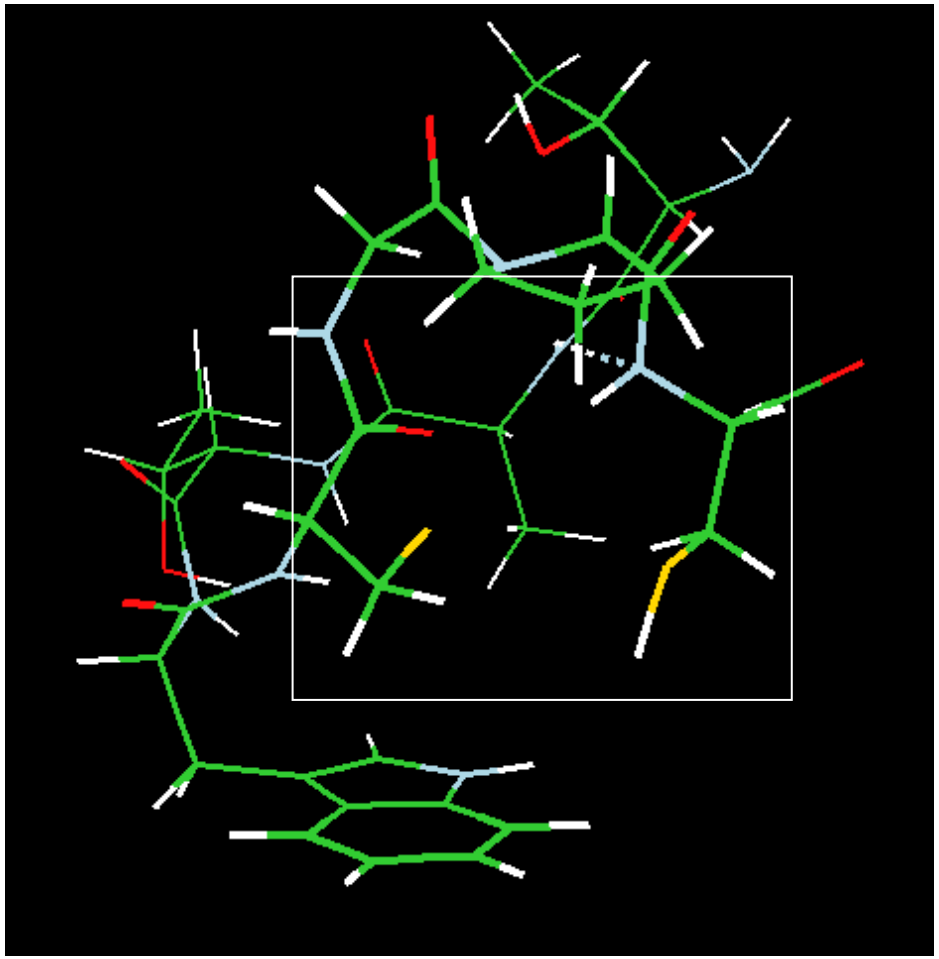
Addition of H^+ on TrxSC



ΔE = 102.4 kJ/mole

dS-S =3.498 Å

Addition of H^+ on trxN



ΔE = 138.4 kJ/mole

dS-S = 4.224 Å

Conclusions

From a chemical point of view:

- the SS bond **(2c-3e)** of the radical anion is **very elongated**, at least **0,7 Å**
- its **stability** strongly depends of **conformation** (AchE) and of the **environment**: important variations of Electronic Affinity
- For Trx, **protonation** is favoured on the **side terminal C**, in agreement with experimental hypotheses.

Conclusions

From a methodologic point of view :

- Choice of the size of the **QM fragment**
  - R =H better than alkyles
  - 14 QM close to 18 QM

- Influence of **part MM** on EA
  (**different structures**: H bonds…)

- disymetrical protonation more evident in QM/MM than for the QM part alone

Dynamical study of electronic localisation in the disulfide bridges

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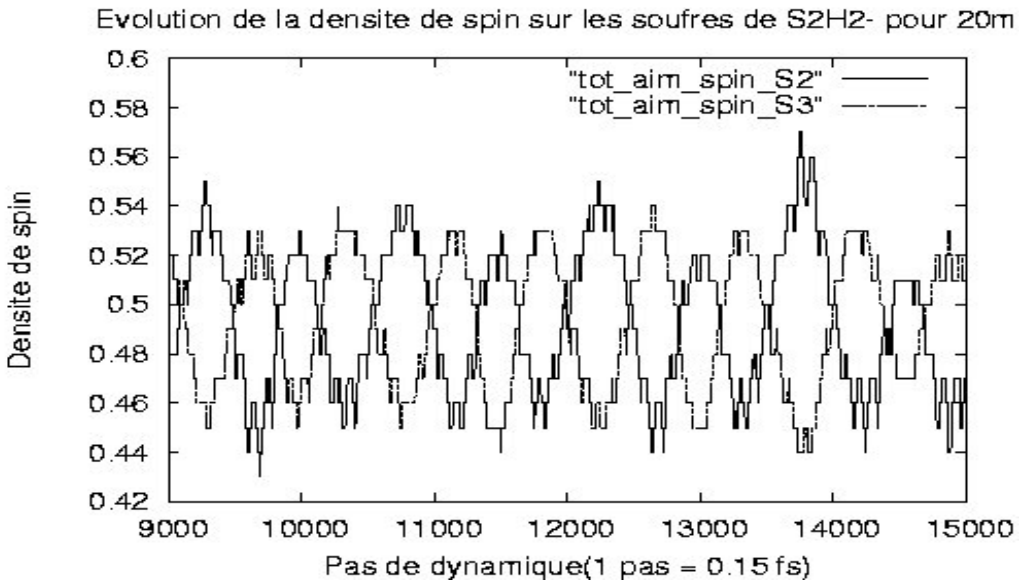
Method: *ab initio* Molecular Dynamics **ADMP**
(Atom centered Density Matrix Propagation)
option in G03 with **B3PW91/6-31+G****

Dissociations for too high kinetic energies

- $H_2S_2^- \rightarrow HS + HS^-$ $\Delta E = 1.27$ eV

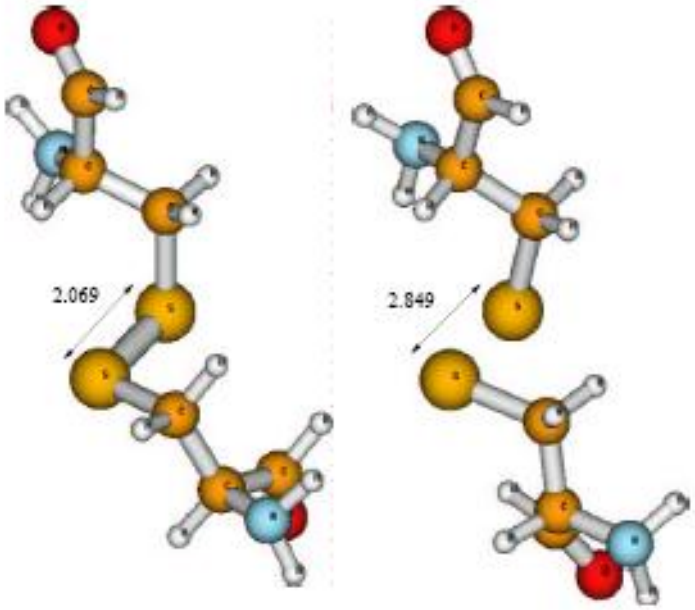
but **stability** with given energy of 0.54 eV

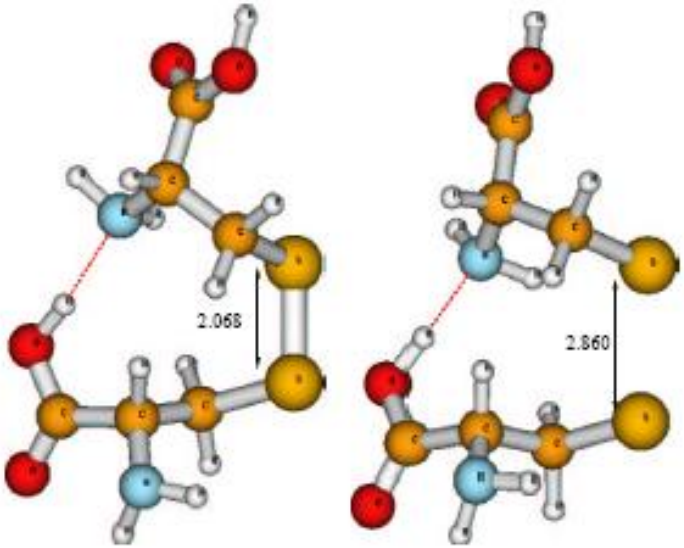
Trajectories of **3 ps** (20 000 steps of 0.15 fs)



Sulfur Spin Densities in the radical anion

Two models of **cystine** from proteins :





ADMP trial: 1 week for 1000 steps

Perspectives

- Increasing size of QM part including **residues without link** to the disulfide and **water** molecules
- Using **Molecular Dynamics** (instead of Molecular Mechanics) with Quantum Mechanics

Electron addition to asparagin and aspartic acid

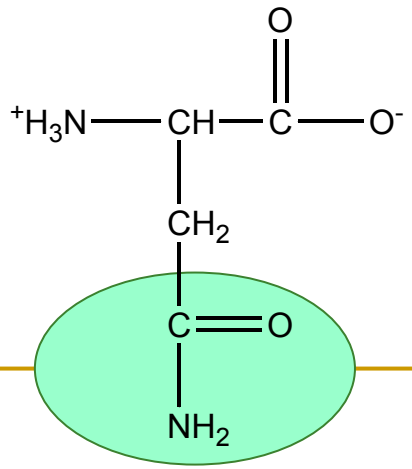
J. Bergès and C. Houée-Levin

Aim: to determine the consequences of electron addition on two amino-acids

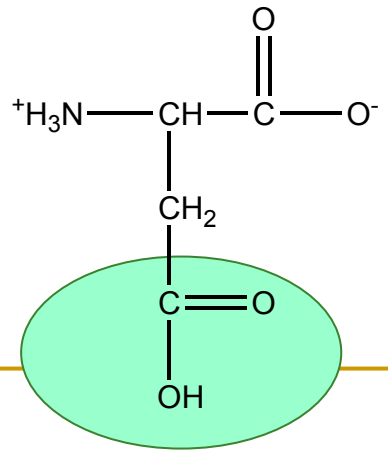
Theoretical study by DFT method

- to identify the stable radical species
- To get hypothesis of the chemical mechanism induced by electron addition.
- All geometries were fully optimized using B3LYP/6-31G* with Gaussian 03.

Asn

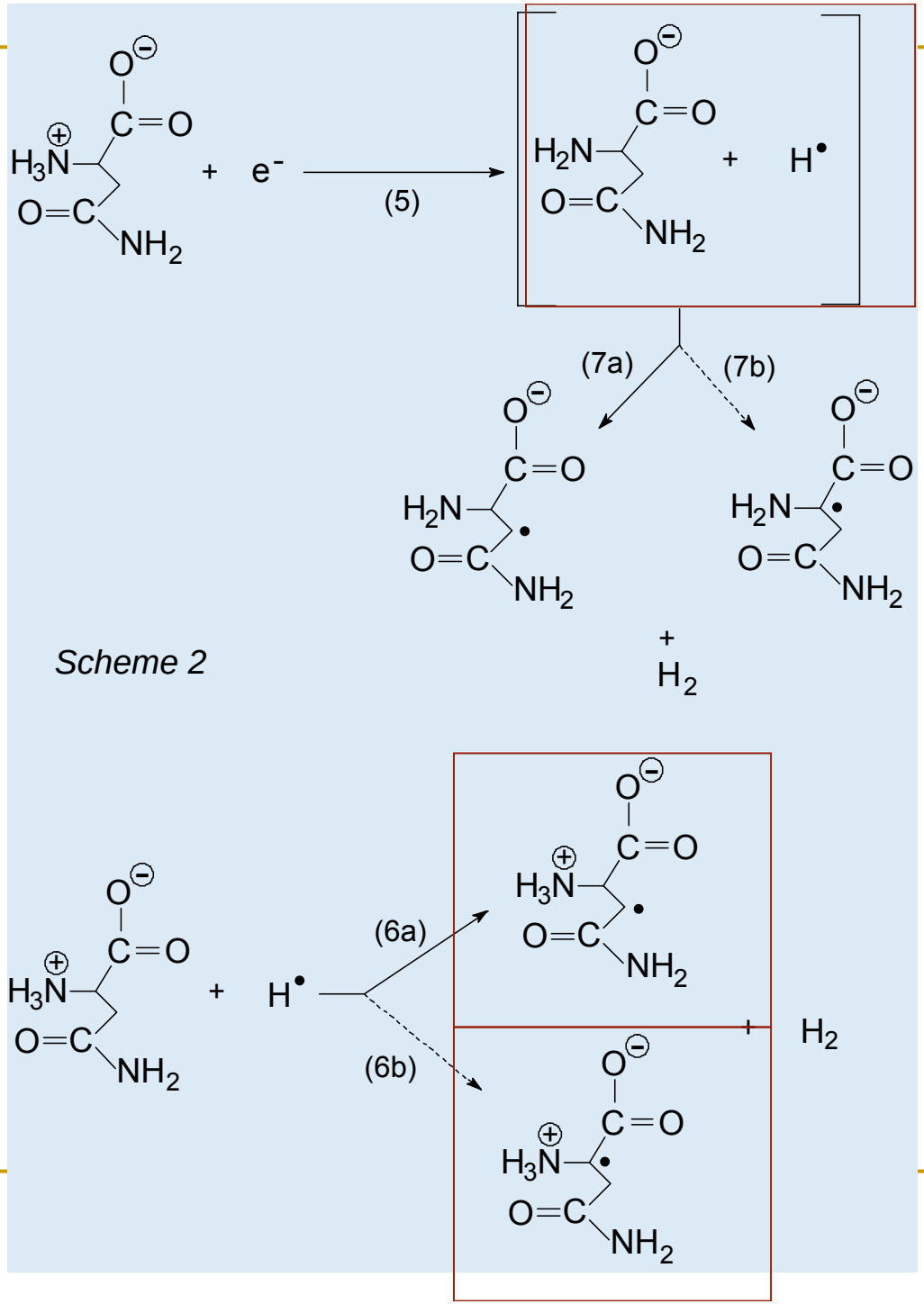


Asp



Asn

- The anion is unstable and loses H atom
- The H atom reacts with other molecule, abstract another H (to give H_2)
- Hydrogen abstraction can lead to two stable C-centred radicals
- Both processes are quasi-isoenergetic.



Scheme 2

Asp

- Conversely to Asn, the radical anion is stable, electron being localized on CO_2
- It can undergo decarboxylations
- Both processes lead to stable C-centred radicals.