La radiolyse, un outil pour l'étude des protéines

Serge Pin

UMR 3685
Nanosciences et Innovation pour les Matériaux, la Biomédecine et l'Energie

Radiolyse et biochimie

Le contexte historique

Etude par radiolyse
des réactions d'oxydation ou de réduction
des molécules biologiques ou d'intérêt et biologique
par des radicaux

Les macromolécules dont les protéines, lipides et l'ADN
et leurs constituants

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Applications of Pulse Radiolysis to Protein Chemistry

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INTRODUCTION

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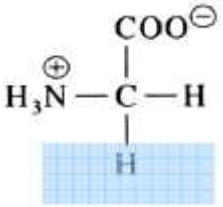
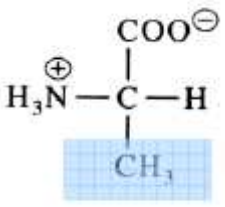
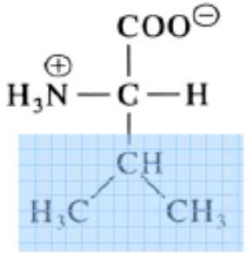
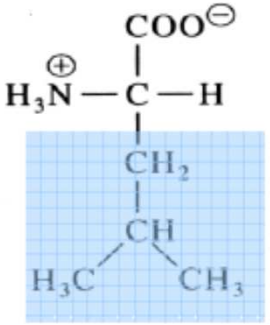
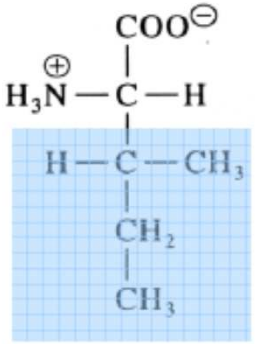
Rappel 1 : les acides aminés

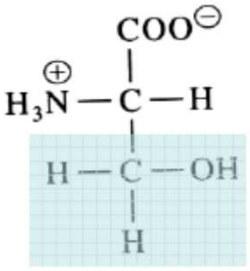

Glycine [G]
(Gly)

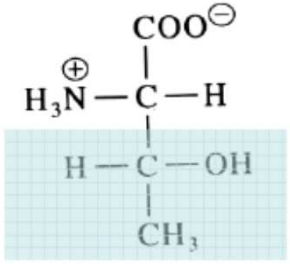

Alanine [A]
(Ala)

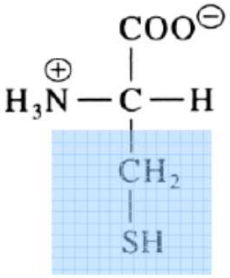

Valine [V]
(Val)

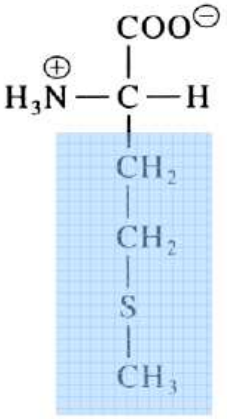

Leucine [L]
(Leu)


Isoleucine [I]
(Ile)


Serine [S]
(Ser)


Threonine [T]
(Thr)


Cysteine [C]
(Cys)


Methionine [M]
(Met)

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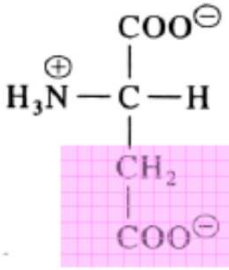
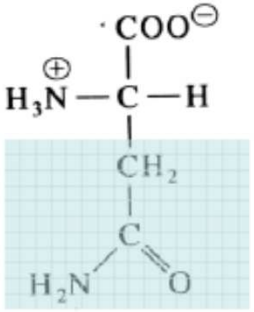
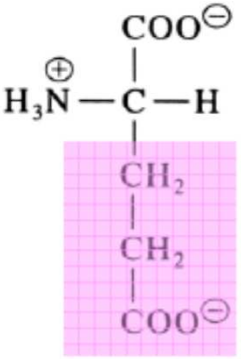


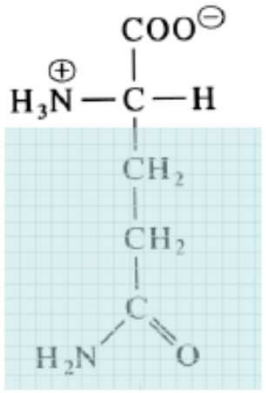
Aspartate [D]
(Asp)



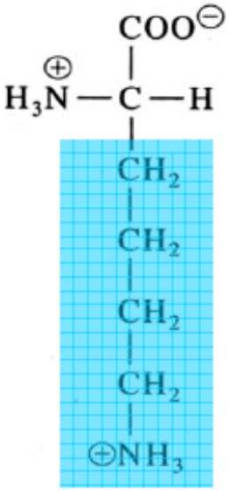
Asparagine [N]
(Asn)



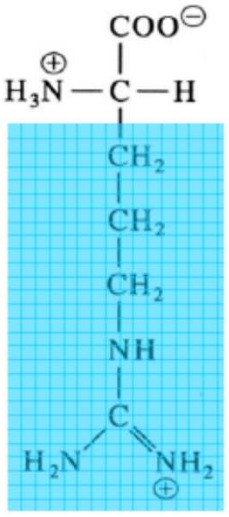
Glutamate [E]
(Glu)



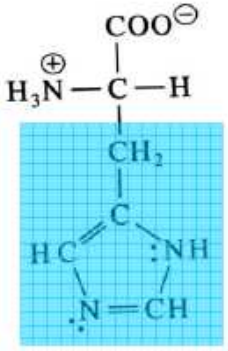
Glutamine [Q]
(Gln)



Lysine [K]
(Lys)

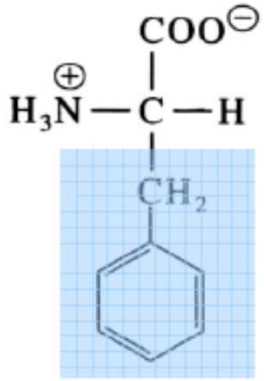


Arginine [R]
(Arg)

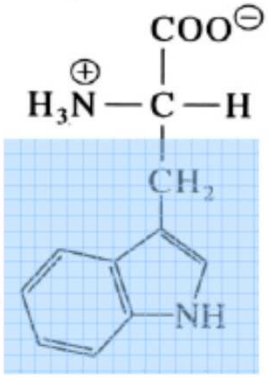


Histidine [H]
(His)

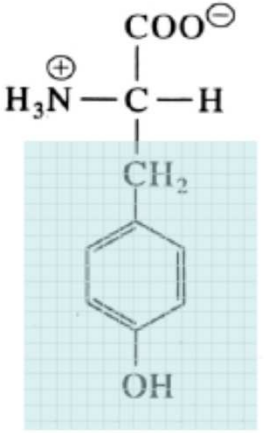
Rappel 1 : les acides aminés



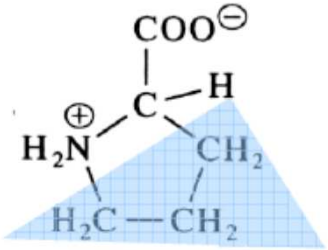
Phenylalanine [F]
(Phe)



Tryptophan [W]
(Trp)

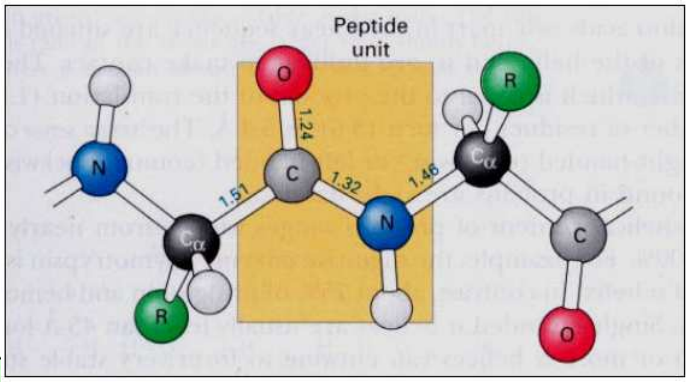


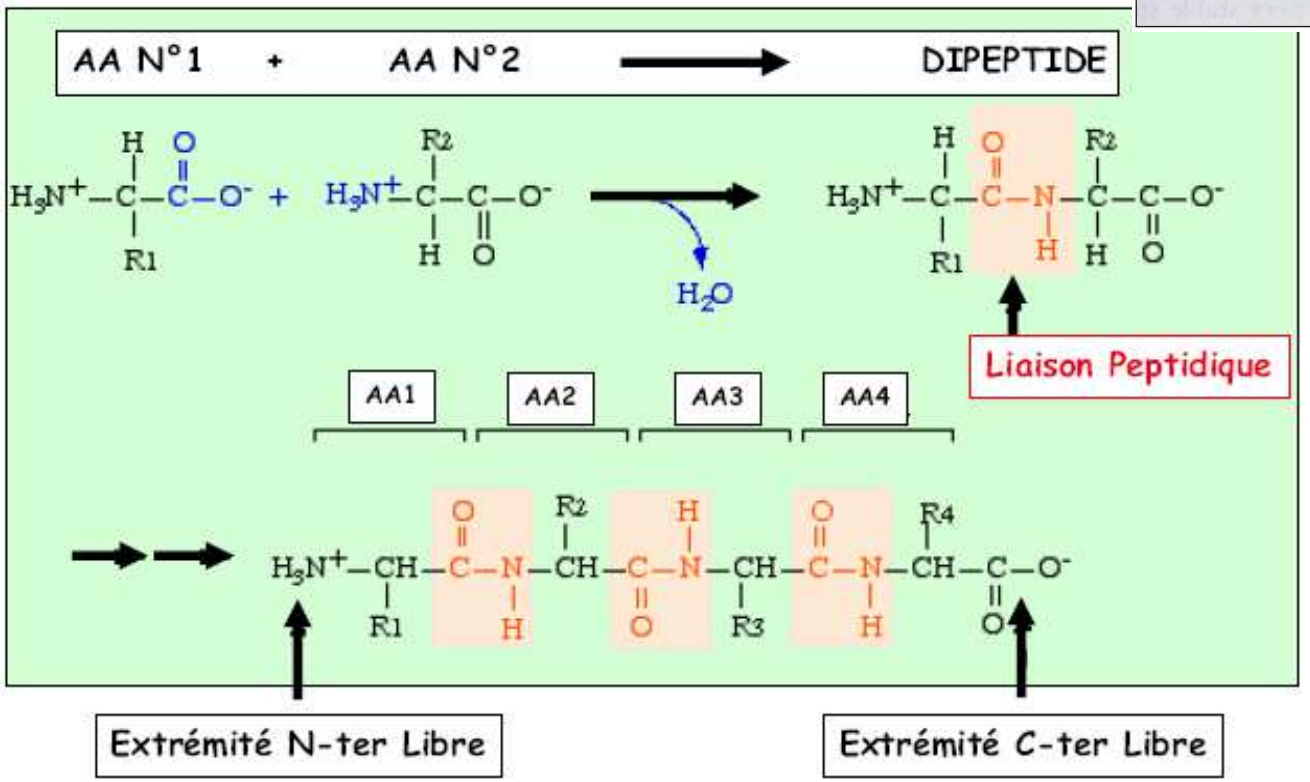
Tyrosine [Y]
(Tyr)



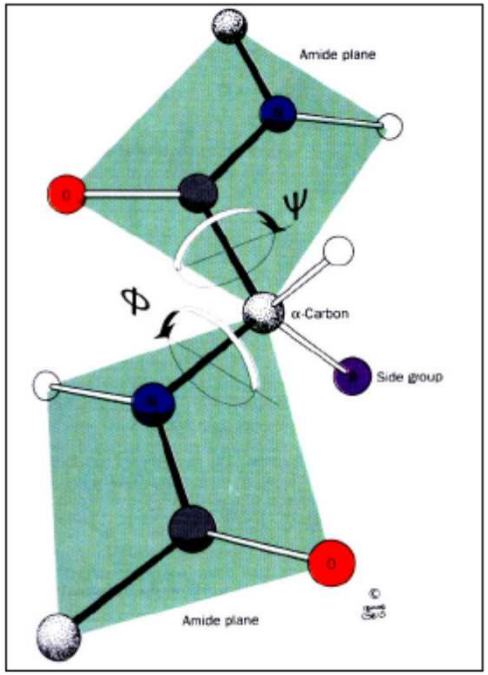
Proline [P]
(Pro)

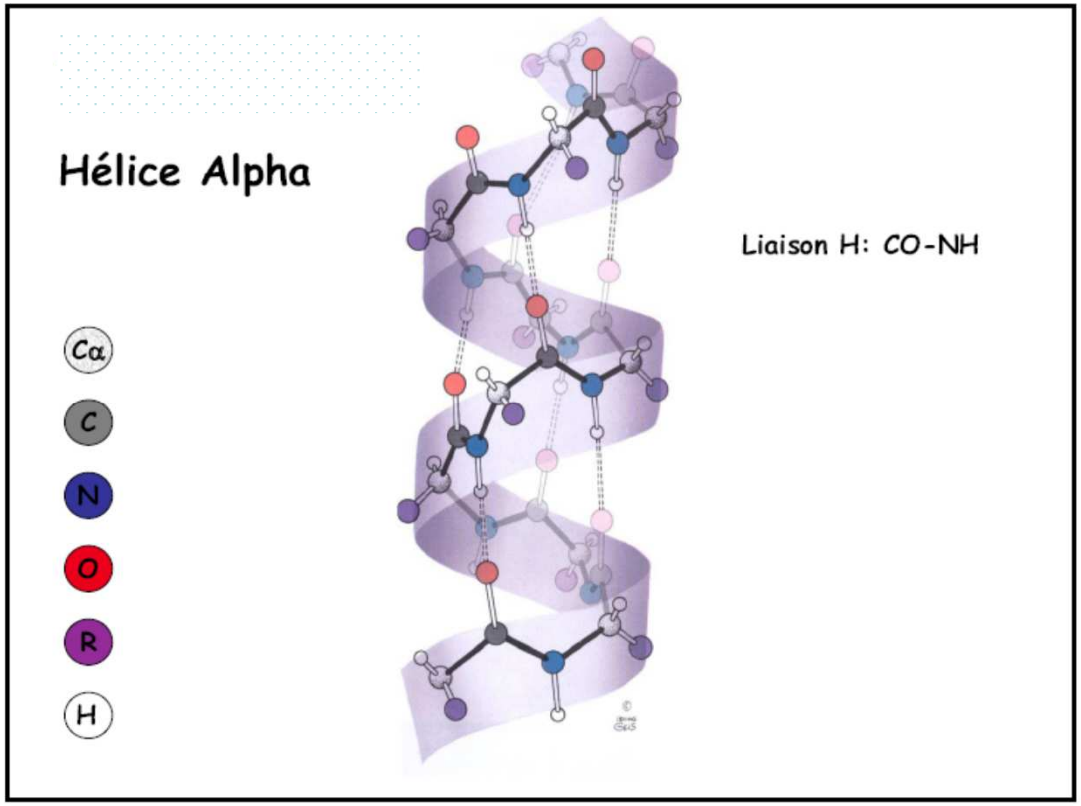
Rappel 2 : la structure I^{aire} des protéines





Rappel 2 : la structure II^{aire} des protéines





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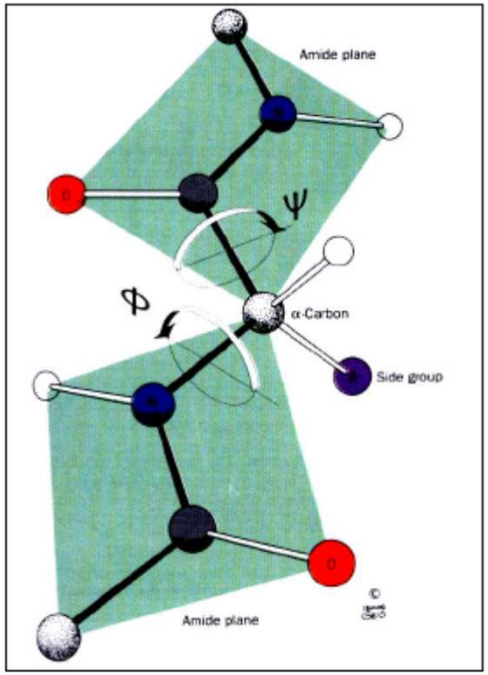
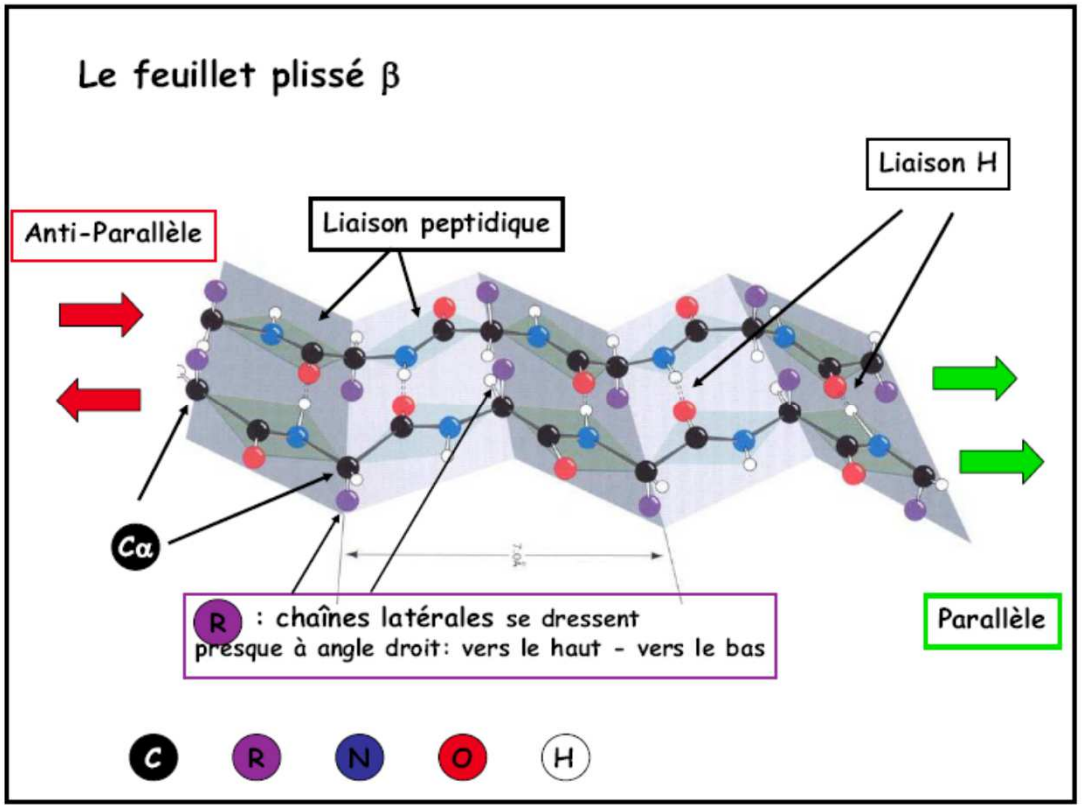
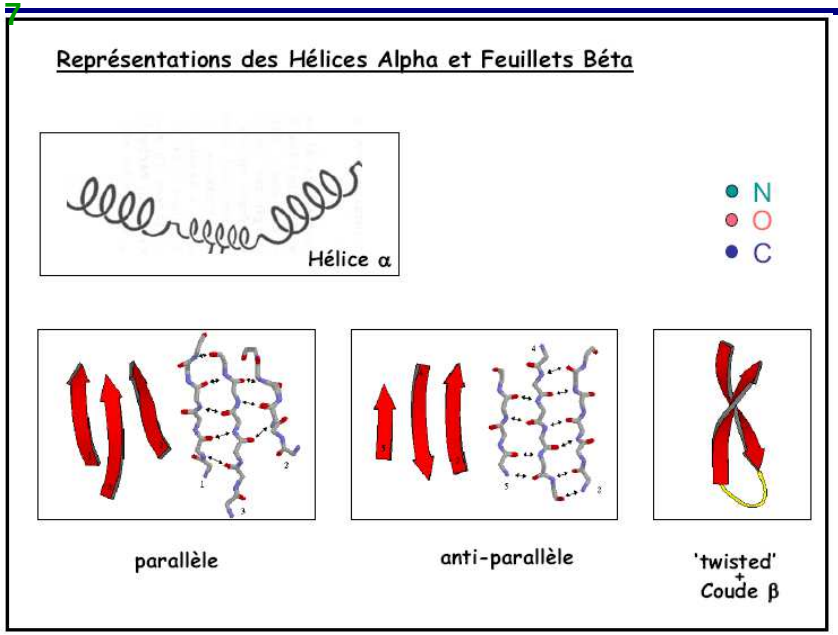
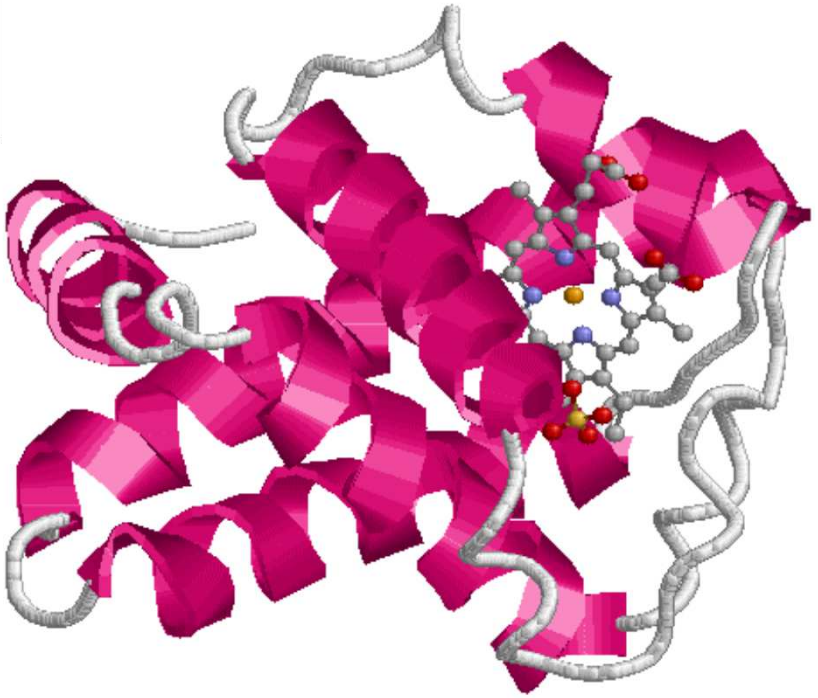




Rappel 2 : la structure III^{aire} des protéines



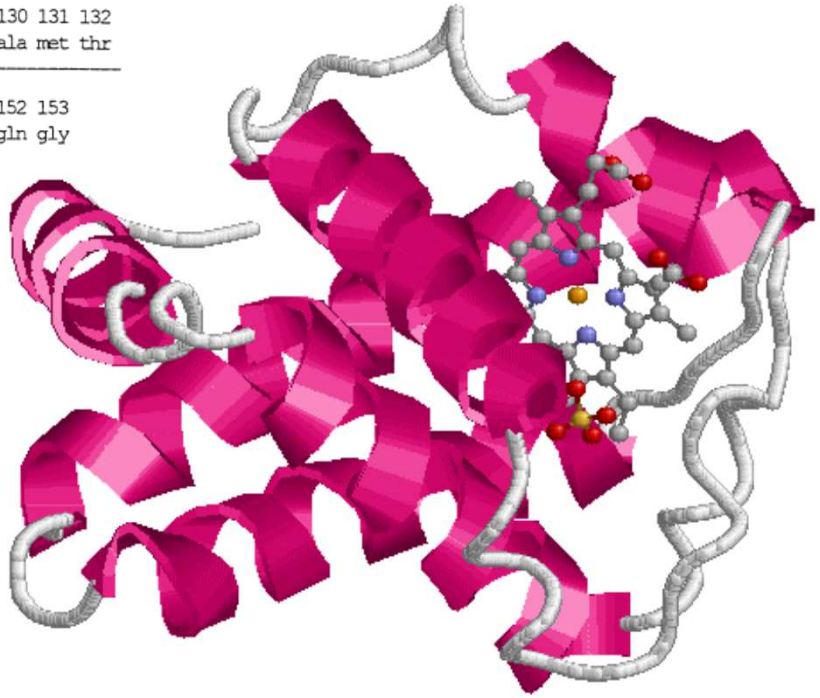


Exemple 1 : les structures I, II et III^{aire} de la myoglobine

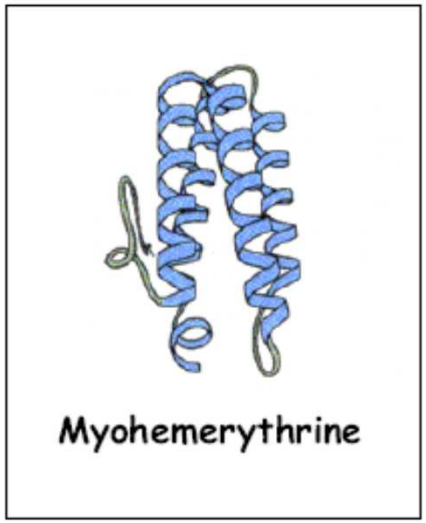

```
  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22
gly leu ser asp gly glu trp gln gln val leu asn val trp gly lys val glu ala asp ile ala
       └1──────────────────────── hélice A ──────────────────────18┘     └1─────────

 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
gly his gly gln glu val leu ile arg leu phe thr gly his pro glu thr leu glu lys phe asp
──────────── hélice B ──────────────────16┘ └1────── hélice C ──────7┘

 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66
lys phe lys his leu lys thr glu ala glu met lys ala ser glu asp leu lyslys his gly thr
                  └1─────── hélice D ──────7┘ └1──────────────────────────────

 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88
val val leu thr ala leu gly gly ile leu lys lys lys gly his his glu ala glu leu lys pro
 hélice E ──────────────────────────20┘              └1──────────────────────

 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110
leu ala gln ser his ala thr lys his lys ile pro ile lys tyr leu glu phe ile ser asp ala
- hélice F ──────────────────16┘       └1──────────────────────────── hélice G ──

111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132
ile ile his val leu his ser lys his pro gly asn phe gly ala asp ala gln gly ala met thr
──────────────────────────19┘                    └1──────────────────────────────

133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153
lys ala leu glu leu phe arg asn asp ile ala ala lys tyr lys glu leu gly phe gln gly
──────── hélice H──────────────────────────────────────────────────────27┘
```

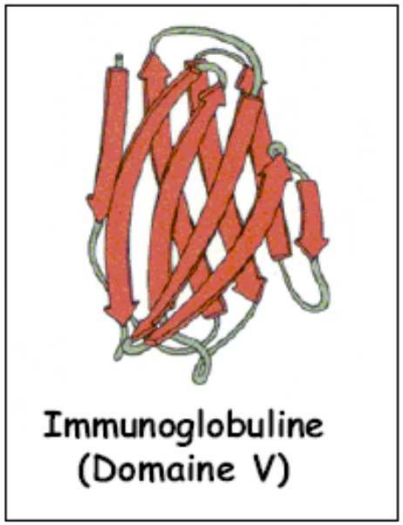
le groupe prosthétique
ou hème
contenant une porphyrine
et un atome de fer

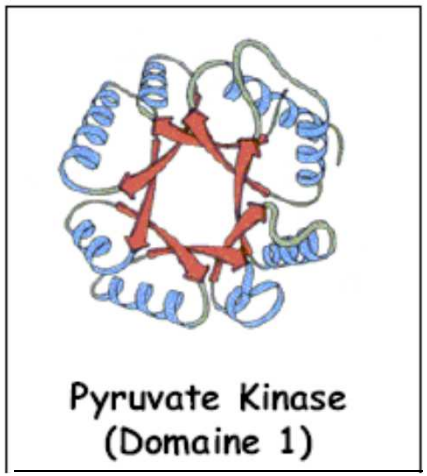
la chaîne polypeptidique
ou apomyoglobine
153 acides aminés
réparties en 8 hélices
nommées de A à H

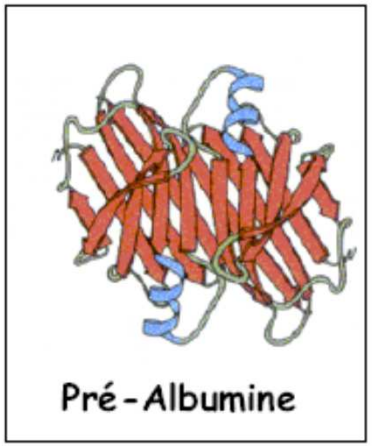


Exemples 2 : la structure III^{aire} de protéines

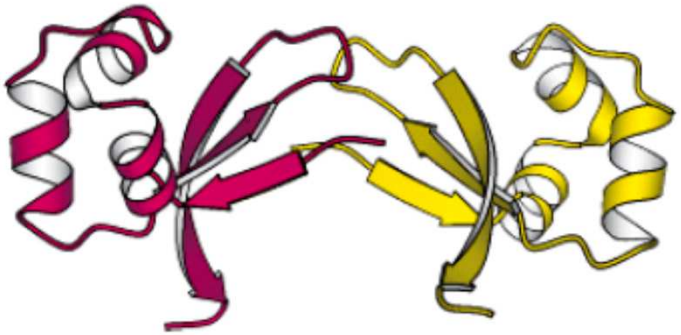






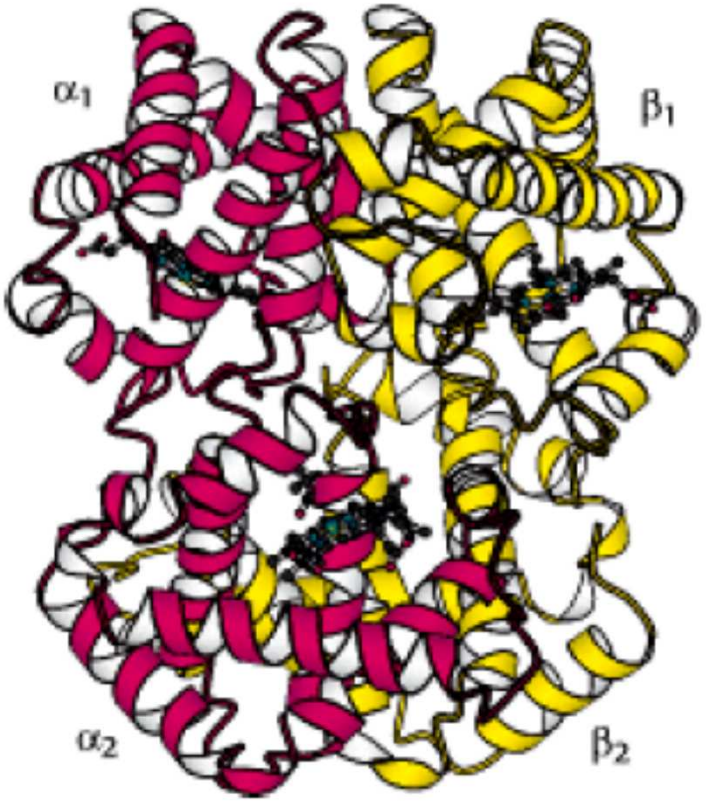


Rappel 3 : la structure IV^{aire} des protéines



le dimère de Cro

le tétramère d'hémoglobine



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Les protéines
et leurs constituants acides aminés et peptides

Les mécanismes :
les constantes de vitesse
et les intermédiaires réactionnels

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Réduction des acides aminés, peptides et protéines

TABLE 1. *Reactions of e^-_{aq} with compounds of biological interest*

	pH	k, $M^{-1} \cdot sec^{-1}$	Properties of the transient species: λ_{max}, nm	ϵ_{max}, $M^{-1} cm^{-1}$	pK_a	$2k$, $M^{-1} \cdot sec^{-1}$
ala-ala	6·2	$2{\cdot}7 \cdot 10^8$	395	—	—	—
ala-ala-ala	6·0	$4{\cdot}9 \cdot 10^8$	385	—	—	—
$(ala)_4$	6·1	$1{\cdot}2 \cdot 10^9$	375	—	—	—
$(ala)_5$	6·0	$1{\cdot}9 \cdot 10^9$	365	—	—	—
$(ala)_8$	6·0	$4{\cdot}0 \cdot 10^9$	—	—	—	—
$(ala)_{12}$	6·0	$6{\cdot}1 \cdot 10^9$	—	—	—	—
$(ala)_{20}$	6·0	$1{\cdot}2 \cdot 10^{10}$	—	—	—	—
N-acetylcysteine	7·1	$5{\cdot}6 \cdot 10^9$	—	—	—	—
albumin, egg white	11·5	$1{\cdot}3 \cdot 10^{10}$	—	—	—	—
albumin, human serum	9·0	$8{\cdot}2 \cdot 10^9$	—	—	—	—
aldolase	7·2	$4{\cdot}0 \cdot 10^{10}$	—	—	—	—
azurin	7·0	$3{\cdot}0 \cdot 10^{10}$	—	—	—	—
benzene	11·0	$7{\cdot}2 \cdot 10^8$	312	3500	—	$8{\cdot}7 \cdot 10^8$
carboxypeptidase A	7·8	$2{\cdot}5 \cdot 10^{10}$	—	—	—	—
chloroacetylglycine	10·2	$2{\cdot}1 \cdot 10^9$	430	1100	—	$1{\cdot}8 \cdot 10^9$
α-chymotrypsin	4·3	$2{\cdot}7 \cdot 10^{10}$	—	—	—	—
α-chymotrypsin	8·5	$1{\cdot}1 \cdot 10^{10}$	—	—	—	—
Cu(II)-alanylhystidine complex	11·0	$5{\cdot}6 \cdot 10^9$	—	—	—	—
Cu(II)-histidine complex	11·0	$6{\cdot}5 \cdot 10^8$	—	—	—	—
Cu(II) glycylhistidine complex	6·5	$6{\cdot}0 \cdot 10^9$	—	—	—	—
Cu(II)-glycylhistidine complex	11·0	$4{\cdot}6 \cdot 10^8$	—	—	—	—
Cu(II)-glyglyglycine complex	9	$4{\cdot}8 \cdot 10^9$	—	—	—	—
Cu(II)-glutathione(ox) complex	11	$8{\cdot}3 \cdot 10^9$	—	—	—	—
p-cresole	7·9	$4{\cdot}2 \cdot 10^7$	—	—	—	—
cyanocobalamine	—	$5{\cdot}2 \cdot 10^{10}$	—	—	—	—
cysteamine	5·5	$3{\cdot}0 \cdot 10^{10}$	—	—	—	—

Exemple de réduction d'une protéine

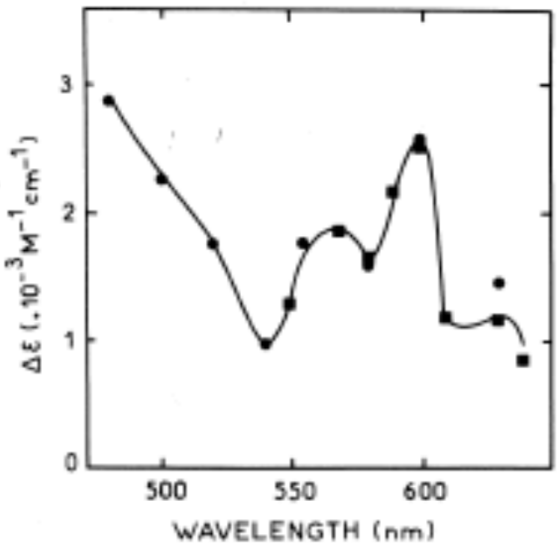

Fig. 4. Difference spectrum between the α(FeII) reduced species and deoxygenated α(FeII) in a 10 mM phosphate/100 mM tertiobutanol buffer (pH 7). The molar absorption at each wavelength has been normalized. ● mean dose of $3.2 \cdot 10^{18}$ eV/cm^3, ■ mean dose of $0.8 \cdot 10^{18}$ eV/cm^3.

$$\text{Apo-P–Fe(III)} + e_{aq}^- \rightarrow (\text{Apo-P})^- \text{–Fe(III)} \rightarrow \text{Apo-P–Fe(II)}$$

Oxydation des acides aminés, peptides et protéines

TABLE 2. *Oxidation reactions of OH radicals with compounds of biological interest*

Solute	pH	k, $M^{-1} \cdot sec^{-1}$	Properties of the transient species		
			λ_{max}, nm	ϵ_{max}, M^{-1} cm^{-1}	$2k$, $M^{-1} \cdot sec^{-1}$
acetamide	5·5	$1·9 \cdot 10^{8}$	<235, 400	>2000, —	$2·4 \cdot 10^{9}$
N,*N*-dimethylacetamide	—	$3·5 \cdot 10^{9}$	238, 340	7000, 2000	$1·6 \cdot 10^{9}$
aldolase	7·2	$1·9 \cdot 10^{11}$	—	—	—
carboxypeptidase	7·8	$6·9 \cdot 10^{10}$	—	—	—
cysteine	6·4	$1·8 \cdot 10^{10}$	330	320	$3·4 \cdot 10^{9}$
diethylsulfide	—	$1·4 \cdot 10^{10}$	420	1800	$4·6 \cdot 10^{9}$
ferricytochrome c	—	$1·7 \cdot 10^{10}$	—	—	—
ferrocytochrome c	—	$3·0 \cdot 10^{10}$	—	—	—
formamide	5·6	$\geq 5·0 \cdot 10^{8}$	<245, 320	>1500, 350	10^{9}–10^{10}
N,*N*-dimethylformamide	5·5	$1·7 \cdot 10^{9}$	248, 380	6900, 1600	$2·3 \cdot 10^{9}$
N-methylformamide	5·5	$1·2 \cdot 10^{9}$	235, 360	3500, 1000	$1·3 \cdot 10^{9}$
glycylphenylalanine	5·8	$6·2 \cdot 10^{9}$	318	—	—
glycyltyrosine	5·8	$6·0 \cdot 10^{9}$	330	—	—
lactate dehydrogenase	7·2	$2·1 \cdot 10^{11}$	—	—	—
lysozyme	7·0	$4·9 \cdot 10^{10}$	—	—	—
methemoglobin	6·2	$\approx 10^{10}$	—	—	—
dimethylsulfide	—	$1·9 \cdot 10^{10}$	370, 470	1800	—
papain	6·4	$4·7 \cdot 10^{10}$	—	—	—
phenylalanine	5·8	$6·6 \cdot 10^{9}$	318	—	—
dimethylpropanamide	5·5	$1·4 \cdot 10^{9}$	242, 350	>5200, >1800	$2·1 \cdot 10^{9}$
tryptophane	7·0	$1·25 \cdot 10^{10}$	320, 340, 420	—	—
tyrosine	6·0	$6·5 \cdot 10^{9}$	300, 330	—	—

Oxydation des acides aminés, peptides et protéines

TABLE 3. *Oxidation reactions of halide and pseudohalide radical ions with compounds of biological interest*

Solute	Oxidizing agent	pH	k (M^{-1} sec^{-1})	Properties of transient species: λ_{max}, nm	Properties of transient species: ϵ_{max}, M^{-1} cm^{-1}
albumin, bovine serum	$(CNS)_2^-$	6·0	$1·0·10^8$	—	—
albumin, human serum	$(CNS)_2^-$	6·5	$5·0·10^9$	—	—
aldolase	$(CNS)_2^-$	7·2	$5·0·10^8$	—	—
aldolase	$(CNS)_2^-$	11·2	$3·6·10^{10}$	—	—
Diaminoacid oxidase (holo enzyme)	$(CNS)_2^-$	7·0	$6·6·10^8$	—	—
α-chymotrypsin	Br_2^-	6·7	$1·6·10^8$	—	—
α-chymotrypsin	$(CNS)_2^-$	6·7	$9·10^7$	—	—
cysteine	Br_2^-	6·6	$1·8·10^7$	—	—
cysteine	$(CNS)_2^-$	6·6	$5·10^7$	—	—
cysteine	CO_3^-	11·2	$2·7·10^6$	—	—
cysteine	Cl_2^-	1·8	$8·5·10^8$	—	—
carboxypeptidase-A	Br_2^-	7·8	$9·0·10^8$	—	—
carboxypeptidase-A	Br_2^-	10	$2·1·10^9$	—	—
carboxypeptidase-A	$(CNS)_2^-$	7·8	$4·10^8$	—	—
carboxypeptidase-A	$(CNS)_2^-$	10	$9·10^8$	—	—
diethylsulfide	Br_2^-	—	$1·8·10^9$	420	1800
histidine	Br_2^-	7·6	$1·5·10^7$	400	—
histidine	$(CNS)_2^-$	7	10^6	400	—
histidine	CO_3^-	11·2	$7·10^6$	400	—
histidine	Cl_2^-	1·8	$1·4·10^7$	400	—
lactate dehydrogenase	Br_2^-	7·2	$5·5·10^9$	—	—
lactate dehydrogenase	$(CNS)_2^-$	7·2	$1·5·10^9$	—	—
lysozyme	$(CNS)_2^-$	7·0	$6·6·10^8$	—	—
methionine	Br_2^-	7·3	$1·1·10^7$	—	—
methionine	$(CNS)_2^-$	7	$2·0·10^6$	—	—

Radiolyse et biochimie des protéines

Le contexte historique

Etude par radiolyse
des réactions d'oxydation ou de réduction
des molécules biologiques ou d'intérêt et biologique
par des radicaux

Les protéines
et leurs constituants acides aminés et peptides

Les mécanismes :
les constantes de vitesse
et les intermédiaires réactionnels

L'effet du pH

Réduction des acides aminés en fonction du pH

RADIATION RESEARCH 27, 319–329 (1966)

Rate Constants of Hydrated Electron Reactions with Amino Acids

REINIER BRAAMS[1]

Chemistry Division, Argonne National Laboratory, Argonne, Illinois

INTRODUCTION

Most of the radiation chemistry of compounds of biological interest so far has been in terms of reactions with H and OH radicals only. It appeared, therefore, worth while to study the reactions between the hydrated electron and amino acids in order to evaluate the possible importance for protein radiation chemistry. In this paper the results of measurements of the rate constants of amino acids are presented. In a second paper, results of measurements on peptides and proteins will be reported and discussed.

EXPERIMENTAL PROCEDURE

The methods employed to measure the rate constant between the hydrated electron and the investigated compounds are the same as those reported by Gordon *et al.* (*1*, *2*) for measurements on organic and inorganic materials. After a single electron pulse of 0.4-μsec duration from a 13-Mev linear accelerator had delivered a dose of about 2 to 3 krads in a cell containing an aqueous solution, the resulting absorption at 720 mμ and its subsequent decrease were followed on the screen of an oscilloscope and photographed.

The light source was a constant-intensity d-c high-pressure mercury arc source. The transmitted light was detected with a phototube or a photomultiplier. The signal that caused the accelerator to fire one single pulse was also used to trigger the oscilloscope and allow the beam in the cathode-ray tube to make one sweep. Together with the time-dependent absorption, lines for 100% absorption were recorded.

When the bimolecular reaction between e^-_{aq} and the investigated compound is pseudo first order, the rate constant equals the reciprocal of the product of the experimentally found average lifetime of the hydrated electron and the reactant concentration. When the average lifetime of the electron in the reactant solution is

[1] On leave of absence from Utrecht University, the Netherlands. Present address: Physics Laboratory, University of Utrecht, the Netherlands.

TABLE I

RATE CONSTANTS FOR REACTIONS OF THE HYDRATED ELECTRON WITH COMMON AMINO ACIDS

Amino acid	*pH*	*Concentration (mM)*	$k(M^{-1}\ sec^{-1})$
Glycine[a]	6.4	5–50	8×10^6
dl-α-Alanine[b]	6.8	10	$\leqq 5 \times 10^6$
dl-Valine[b]	6.4	11	$\leqq 5 \times 10^6$
	9.5	11	$< 5 \times 10^6$
l-Leucine[b]	6.5	10	$< 10^7$
dl-Aspartic acid[b]	7.3	5	$< 10^7$
	10.5	5	$< 5 \times 10^6$
dl-Threonine[b]	6.2	10	$\leqq 10^7$
	9.5		$\leqq 5 \times 10^6$
l-Glutamic acid[a]	5.7	5	$\approx 2 \times 10^7$
l-Proline[a,c]	6.7	2–10	2×10^7
	10.1	5	$\approx 5 \times 10^6$
dl-Lysine HCl[a]	7.8	7	$\approx 2 \times 10^7$
dl-Serine[c]	6.1	5	$< 3 \times 10^7$
dl-Methionine[b]	6.0	10	3.5×10^7
l-Hydroxyproline[a]	7.0	5	4.5×10^7
	10.8	5	1.1×10^7
l-Phenylalanine[a]	7.0	2	1.5×10^8
dl-Phenylalanine[b]	6.28	4	1.1×10^8
	8.65	4	8.8×10^7
	11.20	4	1.7×10^7
l-Tyrosine[a]	5.8	2	1.6×10^8
l-Tryptophan[d]	6.9	2	4.6×10^8
dl-Tryptophan[e]	6.76	2	4.1×10^8
	8.92	2	3.1×10^8
	11.46	2	1.5×10^8
l-Asparagine[f]	7.3	7	1.5×10^8
	11.7	7	2.4×10^7
l-Arginine-HCl[b]	6.10	5	1.53×10^8
	11.5	5	6.3×10^7
dl-Histidine-2HCl[c]	5.96	0.2	3.87×10^9
	6.70	0.5	1.41×10^9
	8.58	0.5	4.5×10^7
	11.14	1	1.2×10^7
l-Cysteine-HCl[a]	6.3	0.1	8.7×10^9
	11.6	1	7.5×10^7
l-Cystine[a]	6.1	0.15	1.3×10^{10}
	10.7	0.5	2.5×10^9

Réduction des peptides et protéines en fonction du pH

RADIATION RESEARCH 31, 8–26 (1967)

Rate Constants of Hydrated Electron Reactions with Peptides and Proteins[1]

REINER BRAAMS[2]

Chemistry Division, Argonne National Laboratory, Argonne, Illinois

REINER BRAAMS, Rate Constants of Hydrated Electron Reactions with Peptides and Proteins. *Radiation Res.* **31**, 8–26 (1967).

Results of measurements of absolute rate constants for reactions between the hydrated electron and peptides or proteins are presented and analyzed. The reactivity of the protonated amino group is shown to depend on the pK_a of the group. Estimates of the rate constants for reactions involving the amino acid side chains are presented. From the estimated rate constants for the side chains an approximate rate constant for three different protein molecules has been calculated. In the linear gelatin molecule the calculated value agrees well with the experimental value. For ribonuclease and lysozyme the calculated values differ from the measured values. The differences are ascribed to the influence of the protein charge on the encounter frequency and to the effect of folding of the peptide chain on the effective collision radius of the molecule.

INTRODUCTION

In a previous paper (*1*) experiments to determine absolute rate constants for reactions between the hydrated electron and amino acids were described, and the results of a number of measurements were reported. It was found that cystine, cysteine, and histidine are very reactive toward the hydrated electron, and it could be shown that for these amino acids the reactive site is located in the side chain. These results were considered of importance for an understanding of the effects of ionizing radiation on aqueous solutions of proteins.

In a protein the amino acids are linked together through peptide bonds, and each protein molecule contains a large number of these bonds. Therefore it is of great importance to know if the presence of the peptide bond introduces a reactive site

[1] Based on work performed under the auspices of the U. S. Atomic Energy Commission.

[2] Present address: Physics Laboratory, University of Utrecht, The Netherlands.

TABLE I

RATE CONSTANTS FOR REACTIONS OF THE HYDRATED ELECTRON WITH SOME PEPTIDES

Peptide	*Abbreviation*	*pH*	*Concentration (mM)*	*k in 10^8($M^{-1}sec^{-1}$)*
glycyl-glycine	gly-gly	6.38	2	2.5
		11.75	2	0.5
glycyl-dl-alanine	gly-dl-al	6.22	2	2.9
dl-alanyl-glycine	dl-al-gly	6.22	2	2.1
glycyl-l-leucine	gly-l-leu	6.46	2	2.8
		8.74		0.7
		8.94		0.65
dl-leucyl-glycine	dl-leu-gly	6.09	2	1.1
glycyl-dl-valine	gly-dl-val	5.97	2	2.6
dl-alanyl-dl-leucine	dl-al-dl-leu	6.46	2	1.3
l-leucyl-l-alanine	l-leu-l-al	6.1	2	1.65
dl-alanyl-dl-alanine	dl-al-dl-al	6.27	2	1.3
l-leucyl-l-leucine	l-leu-l-leu	5.97	2	0.9
glycyl-l-asparagine	gly-l-asp	5.33	2	5.4
		11.41	2	0.8
glycyl-l-proline	gly-l-prol	6.66	1	11
glycyl-l-tyrosine	gly-l-tyr	6.13	2	4.1
glycyl-l-tryptophane	gly-l-tryp	6.37	1	4.5
l-phenylalanyl-l-phenylalanine	l-phe-l-phe	5.66	1	4.5
histidyl-histidine	his-his	7.3	2	13
glycyl-glycyl-glycine	gly-gly-gly	6	1	9
		11.1		0.9

Exemple de réduction d'un peptide en fonction du pH

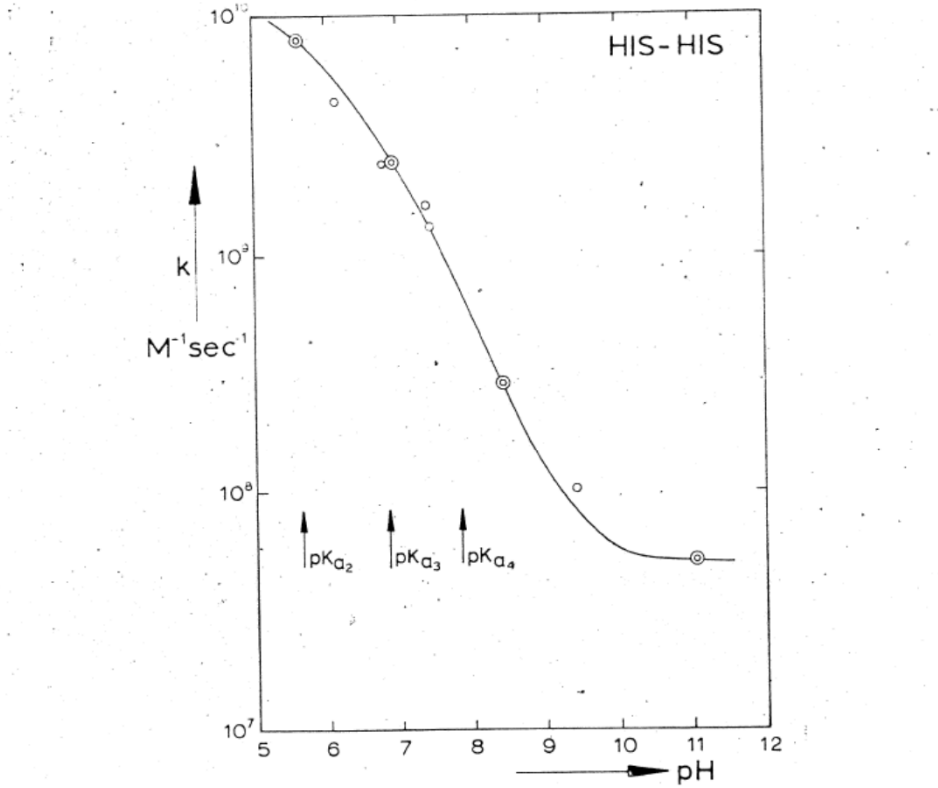

FIG. 2. Rate constants for the reaction between the hydrated electron and histidyl–histidine at different pH's. The curve represents the function given in equation 2, with values for the rate constants of the different ionic forms given in the text. These rate constants have been calculated from the four accurate measurements indicated with ◎ and given in Table I.

Exemple de réduction d'une protéine en fonction du pH

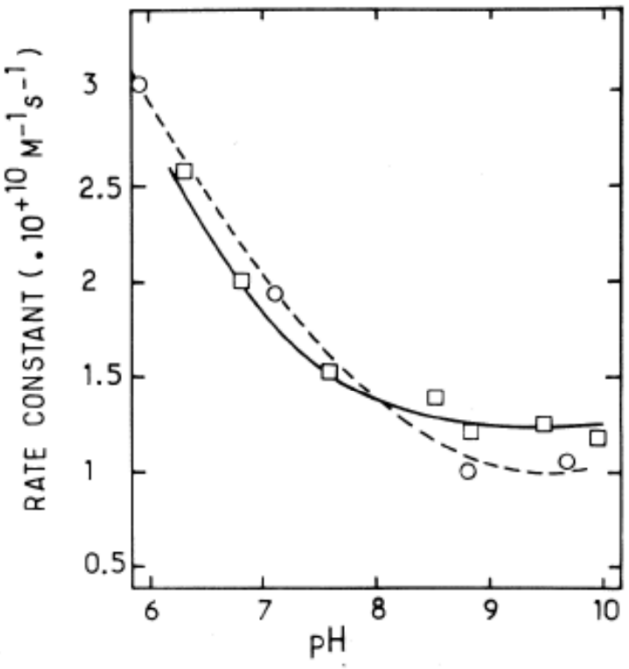

Fig. 5. Rate constants for the reduction of iron(III) myoglobin derivatives by hydrated electrons as a function of the pH: ○, mixture of $Mb(FeIII)H_2O$ and $Mb(FeIII)OH^-$; □, $Mb(FeIII)N_3^-$.

La radiolyse, un outil pour l'étude des protéines

Le contexte
La biochimie radicalaire du stress oxydant

Le contexte du stress oxydant

métabolisme normal

⇓

espèces activées de l'oxygène (EAO ou ROS)
radicaux et espèces moléculaires

$HO^{\bullet}$ $O_2^{-\bullet}$ $RO^{\bullet}$ $RO_2^{\bullet}$ $NO^{\bullet}$ H_2O_2

production

⇓

réponses adaptatives cellulaires
activation des systèmes de défense antioxydants
induction de protéines

homéostasie
équilibre oxydants/antioxydants

Le contexte du stress oxydant

métabolisme anormal
rayons UV, X ou γ
agents chimiques exogènes*

espèces activées de l'oxygène (EAO ou ROS)
radicaux et espèces moléculaires

$HO^{\bullet}$ $O_2^{-\bullet}$ $RO^{\bullet}$ $RO_2^{\bullet}$ $NO^{\bullet}$ H_2O_2

réponses adaptatives cellulaires
activation des systèmes de défense antioxydants
induction de protéines

*** alcool, tabac, métaux, amiante, pesticides, substances toxiques, médicaments, NPs**

Le contexte du stress oxydant

métabolisme anormal
rayons UV, X ou γ
agents chimiques exogènes

⇓

espèces activées de l'oxygène (EAO ou ROS)
radicaux et espèces moléculaires

$HO^{\bullet}$ $O_2^{-\bullet}$ $RO^{\bullet}$ $RO_2^{\bullet}$ $NO^{\bullet}$ H_2O_2

surproduction

⇓

réponses adaptatives cellulaires
activation des systèmes de défense antioxydants
induction de protéines

toxicité
dommages macromoléculaires
protéines, ADN, lipides

⇓

pathologies :
lésions cardiovasculaires, pulmonaires, cutanées, oculaires
inflammations, infections, fibroses, carcinogenèse

Le contexte du stress oxydant

métabolisme normal

métabolisme anormal
rayons UV, X ou γ
agents chimiques exogènes

⇓ ⇓

espèces activées de l'oxygène (EAO ou ROS)
radicaux et espèces moléculaires

$HO^{\bullet}$ $O_2^{-\bullet}$ $RO^{\bullet}$ $RO_2^{\bullet}$ $NO^{\bullet}$ H_2O_2

production

surproduction

⇓ ⇓

réponses adaptatives cellulaires
activation des systèmes de défense antioxydants
induction de protéines

homéostasie
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toxicité
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⇓

pathologies :
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La radiolyse, un outil pour l'étude des protéines

Le contexte
La biochimie radicalaire du stress oxydant

La toxicité du stress oxydant
La formation d'espèces radicalaires
La modification des protéines
Son implication dans nombre de pathologies et le vieillissement

La radiolyse, un outil pour l'étude des protéines

Le contexte
La biochimie radicalaire du stress oxydant

La toxicité du stress oxydant
La formation d'espèces radicalaires
La modification des protéines
Son implication dans nombre de pathologies et le vieillissement

Les échelles

- **l'échelle de temps**
  - 10^{-15}-10^{-12} sec → **radiolyse de l'eau et formation des radicaux**
  - 10^{-9}-10^{-6} sec → **réactions sur les protéines**
  - mn-h / j-an → **réponses cellulaires / organisme**
- **l'échelle d'espace**
  - atome et molécule → **domaine du physico-biochimiste**
  - cellule → **domaine du biologiste**
  - tissu et organisme → **domaine du biologiste et du médecin**

La radiolyse, un outil pour l'étude des protéines

L'étude des réactions des espèces radicalaires sur les molécules biologiques

La radiobiologie

Les échelles

- l'échelle de temps

- 10^{-15}-10^{-12} sec → radiolyse de l'eau et formation des radicaux
- 10^{-9}-10^{-6} sec → réactions sur les protéines
- mn-h / j-an → réponses cellulaires / organisme

- l'échelle d'espace

- atome et molécule → domaine du physico-biochimiste
- cellule → domaine du biologiste
- tissu et organisme → domaine du biologiste et du médecin

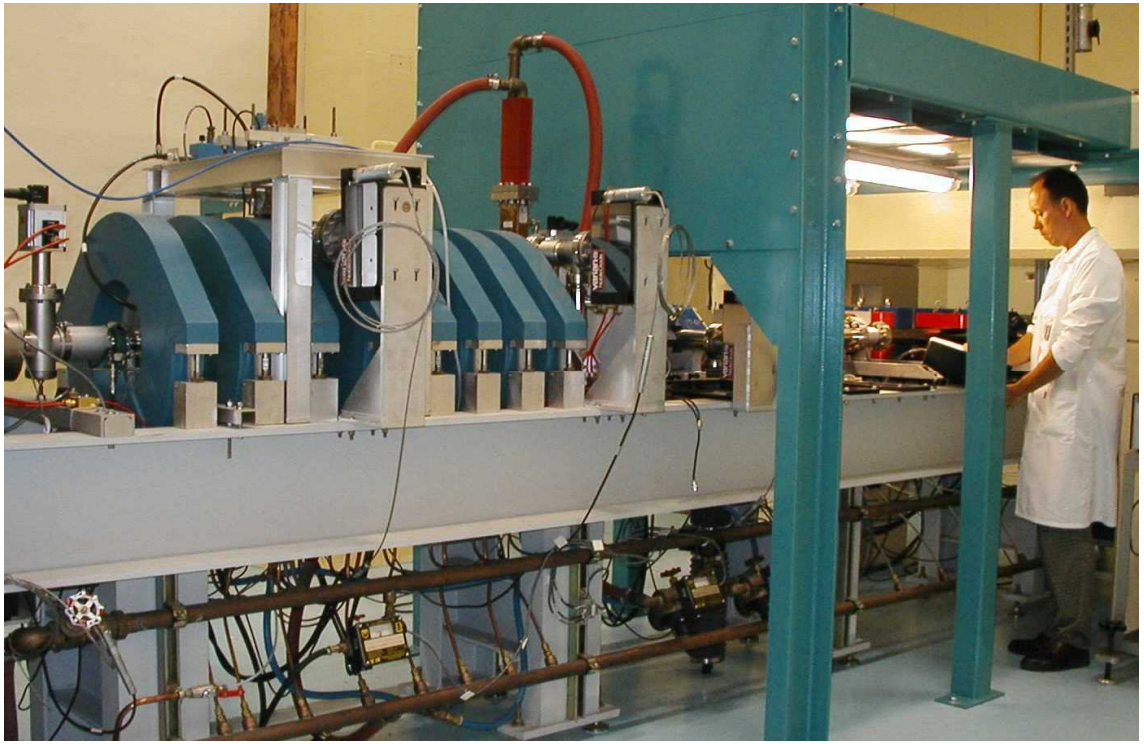
Radiolyse et biochimie des protéines

Un outil
pour étudier les effets moléculaires induits
par les ROS sur les protéines

La radiolyse de l'eau
permet de reproduire les conditions de toxicité du stress oxydant

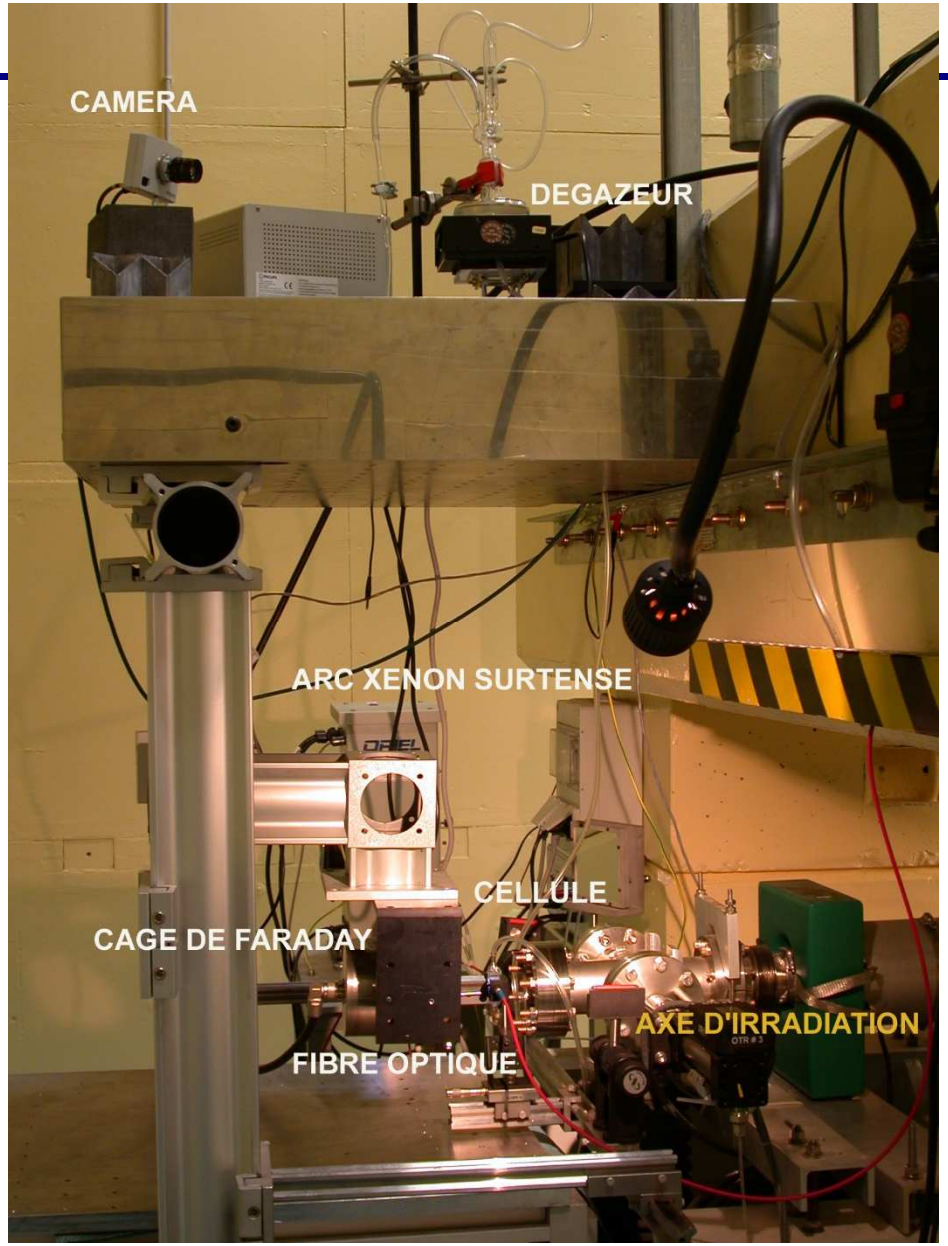
en
- générant de manière sélective et quantitative un seul radical
- simplifiant l'analyse des mécanismes de la ns à la mn
- permettant la caractérisation des produits finaux de réaction

1) La radiolyse pulsée : accélérateur d'électrons



Radioprotection

LINAC	**mode/charge/répétition**
spectroscopie d'absorption résolue en temps	10ns/50nC/100Hz 100ns/100nC/50Hz 1µs/200nC/25Hz 5µs/1000nC/5Hz
domaines UV-visible échelle de temps ns	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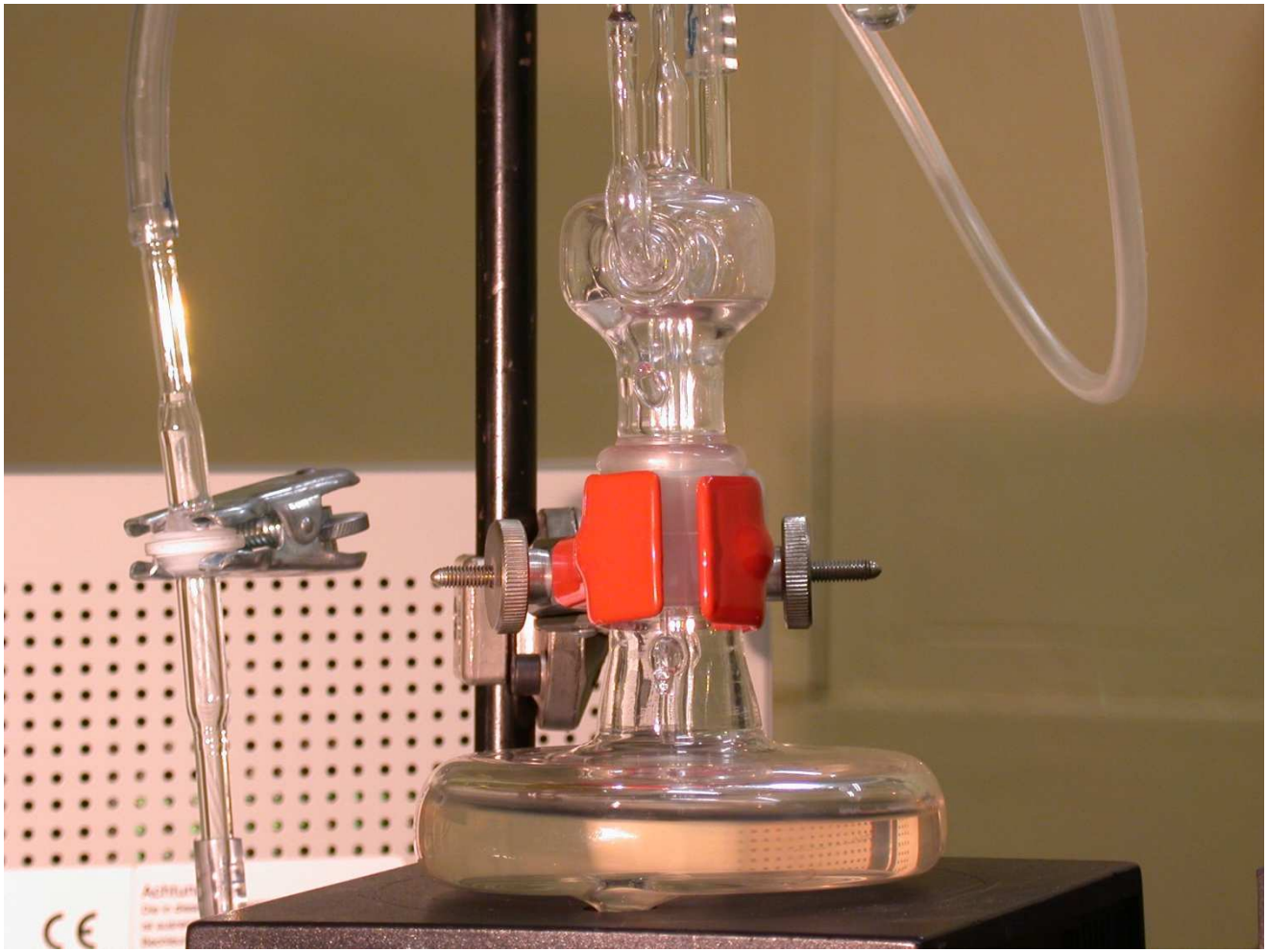
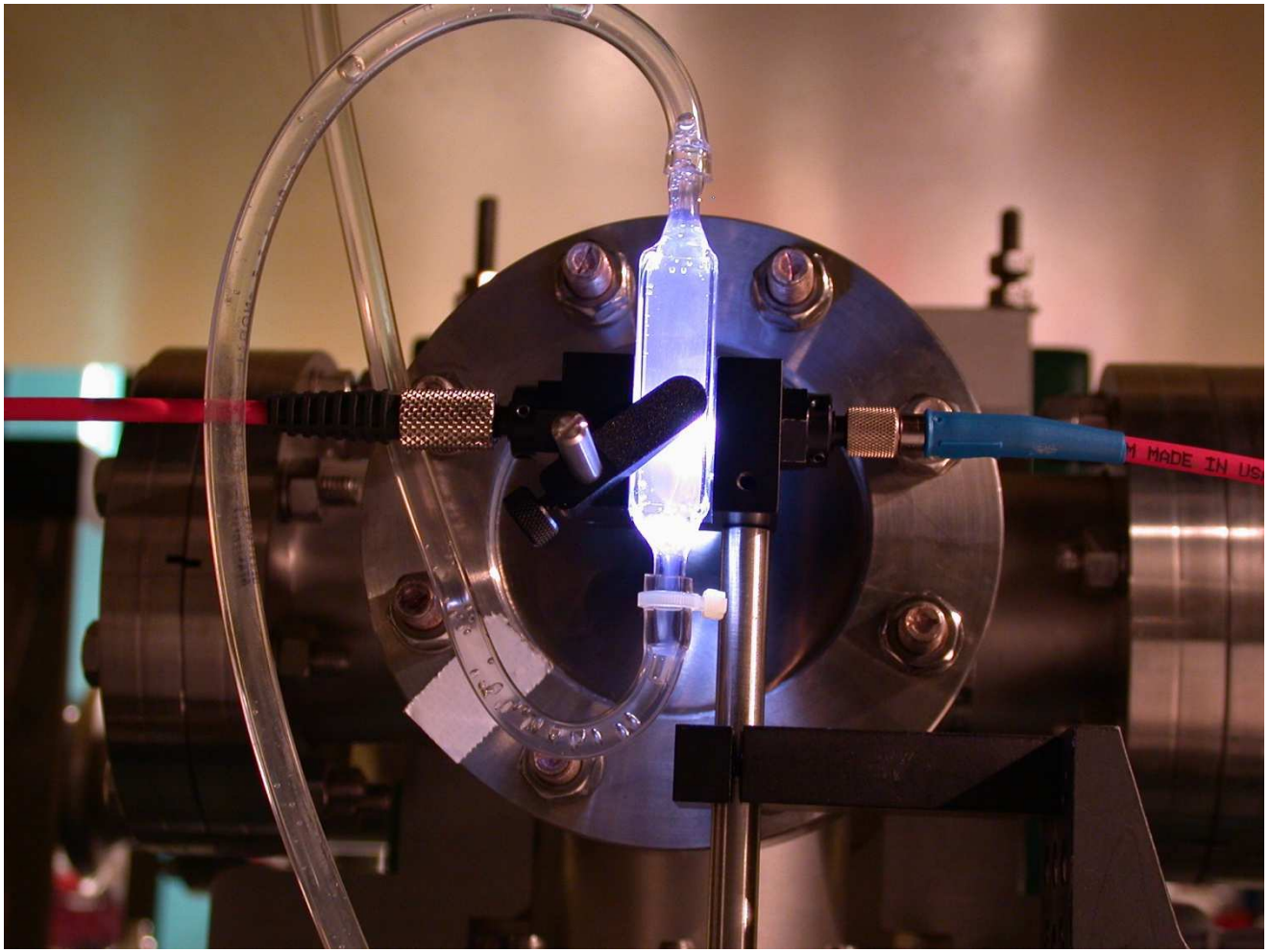
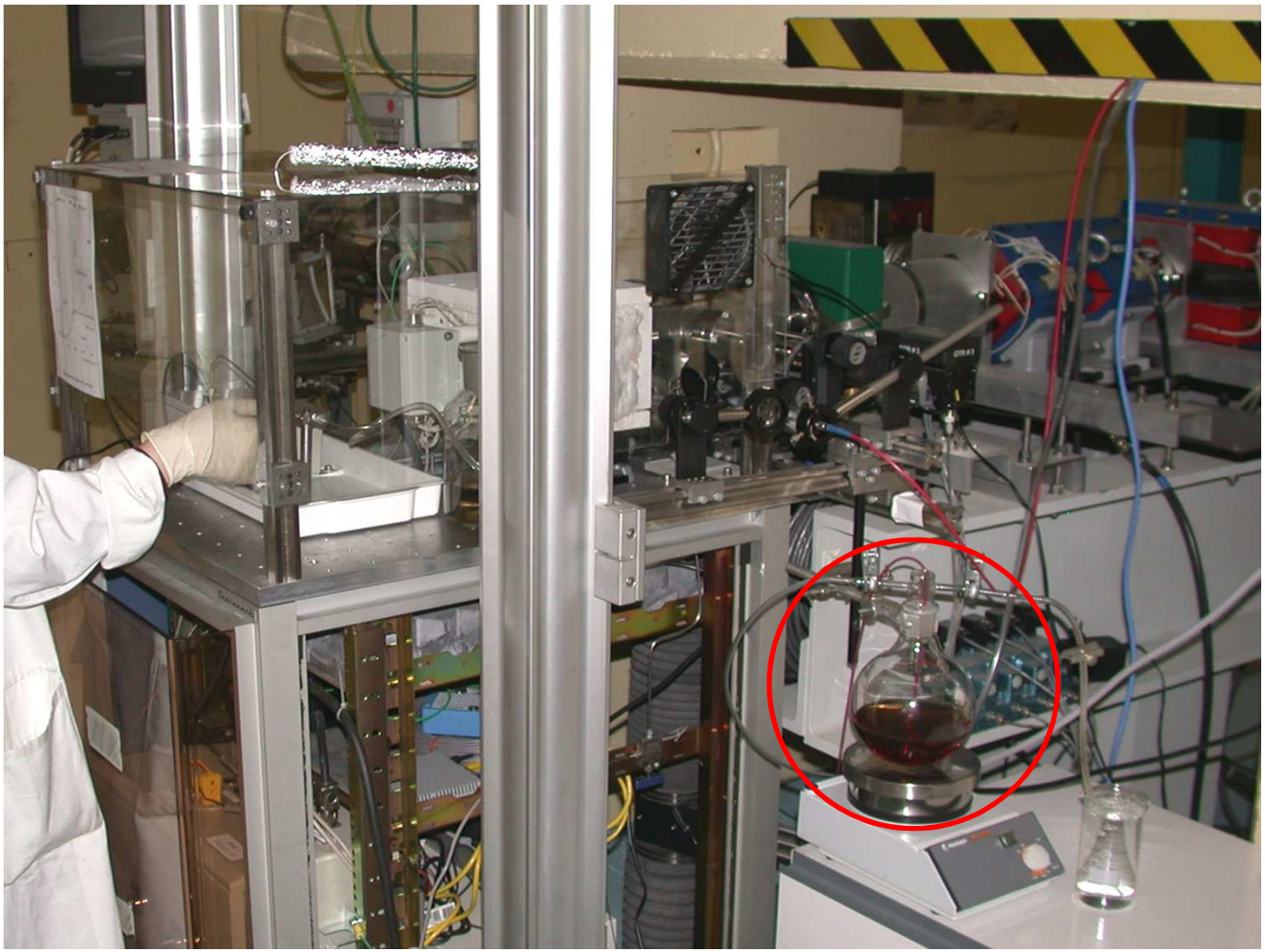
DEGAZEUR
ARC XENON SURTENSE
CELLULE
CAGE DE FARADAY
AXE D'IRRADIATION
FIBRE OPTIQUE




MADE IN US



POWER

2) La radiolyse continue : irradiateur gamma

Radiolyse et biochimie des protéines

Un outil
pour étudier les effets moléculaires induits
par les ROS sur les protéines

La radiolyse de l'eau
permet de reproduire les conditions de toxicité du stress oxydant

en
- générant de manière sélective et quantitative un seul radical
- simplifiant l'analyse des mécanismes de la ns à la mn
- permettant la caractérisation des produits finaux de réaction

Les objectifs se répartissent en 3 thèmes et leurs applications

Le stress oxydant

métabolisme normal

⇓

production

métabolisme anormal

⇓

surproduction

ROS

+

antioxydants

⇓

équilibre ROS/antioxydants

⇓

réactivité des macromolécules

Le stress oxydant : thème 1

métabolisme normal

⇓

production

métabolisme anormal

⇓

surproduction

ROS

+

antioxydants

⇓

équilibre ROS/antioxydants

⇓

réactivité des macromolécules

Le stress oxydant : thème 2

métabolisme normal

⇓

production

métabolisme anormal

⇓

surproduction

ROS

+

antioxydants

⇓

équilibre ROS/antioxydants

⇓

réactivité des macromolécules

Le stress oxydant : thème 3 et ses applications

métabolisme normal

⇓

production

métabolisme anormal

⇓

surproduction

ROS

+

antioxydants

⇓

équilibre ROS/antioxydants

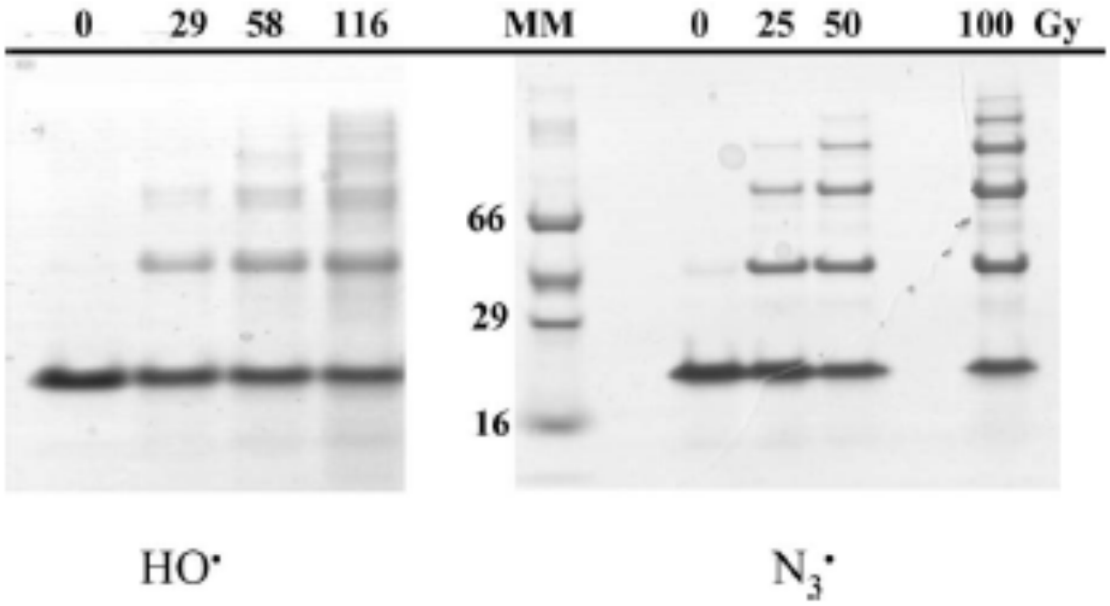
⇓

réactivité des macromolécules

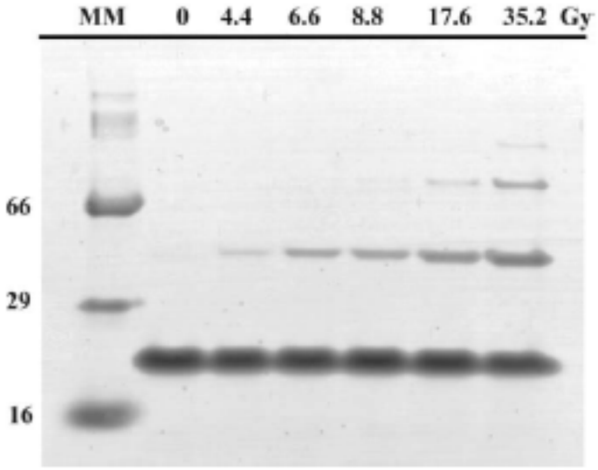
biochimie structurale

Réactivité des protéines

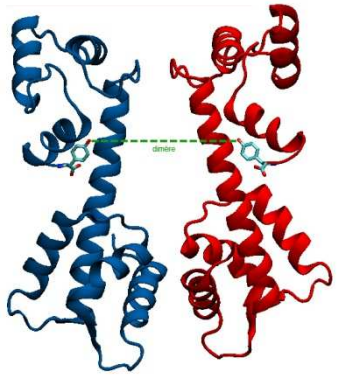
Cas de l'oxydation radiolytique de la centrine
20 kDa
172 aa, pas de C, W et 1 seul Y



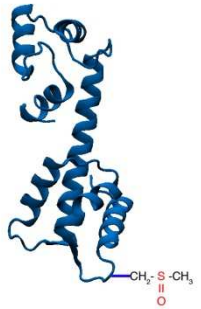
Analyse par SDS-PAGE



Réactivité des protéines



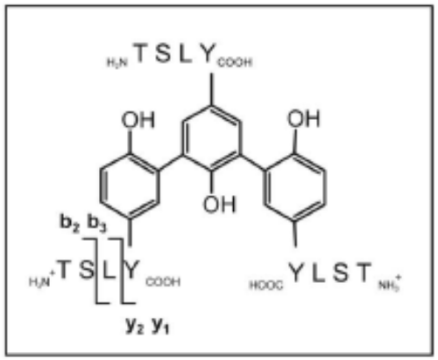
Protéine dimérisée



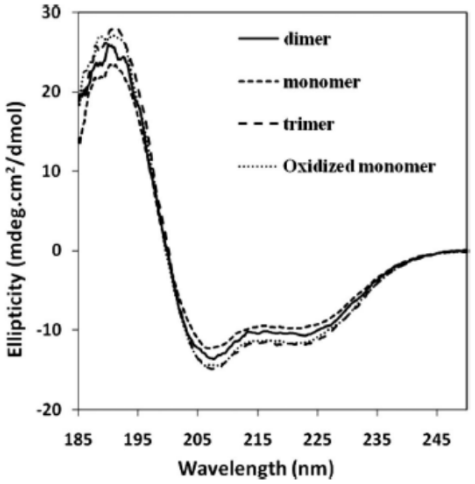
Protéine oxydée

Analyse par LC-MS

Experimental Mr (Da)	Theoretical Mr (Da)	Type of oligomer
	Total centrin 2	
19,608	19,608	Monomer
39,230	39,214	Dimer
58,808	58,820	Trimer
78,476	78,426	Tetramer

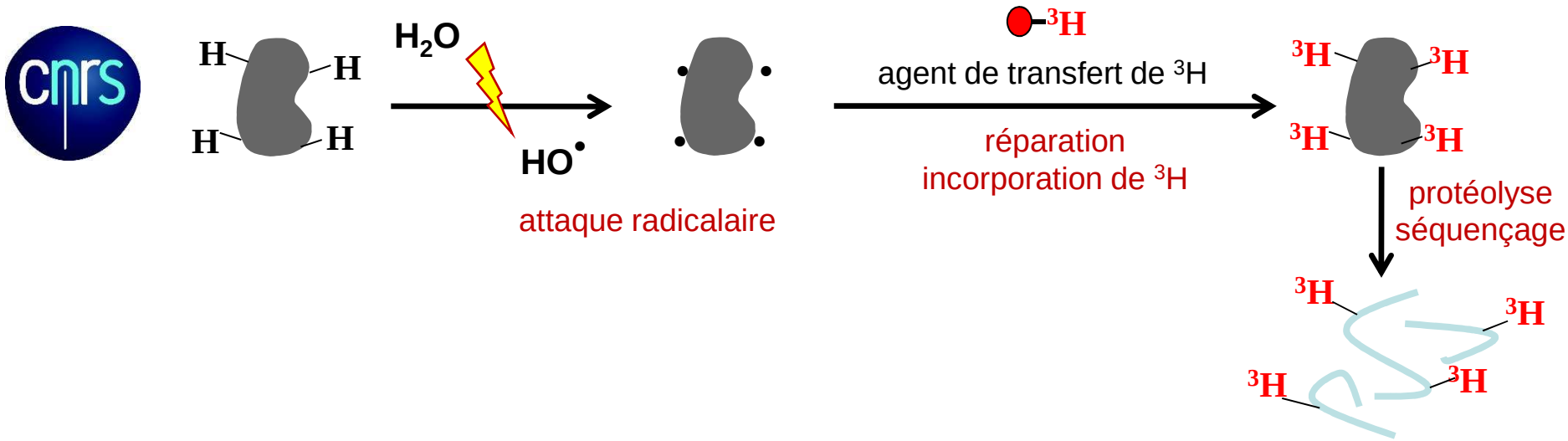


Analyse par DC

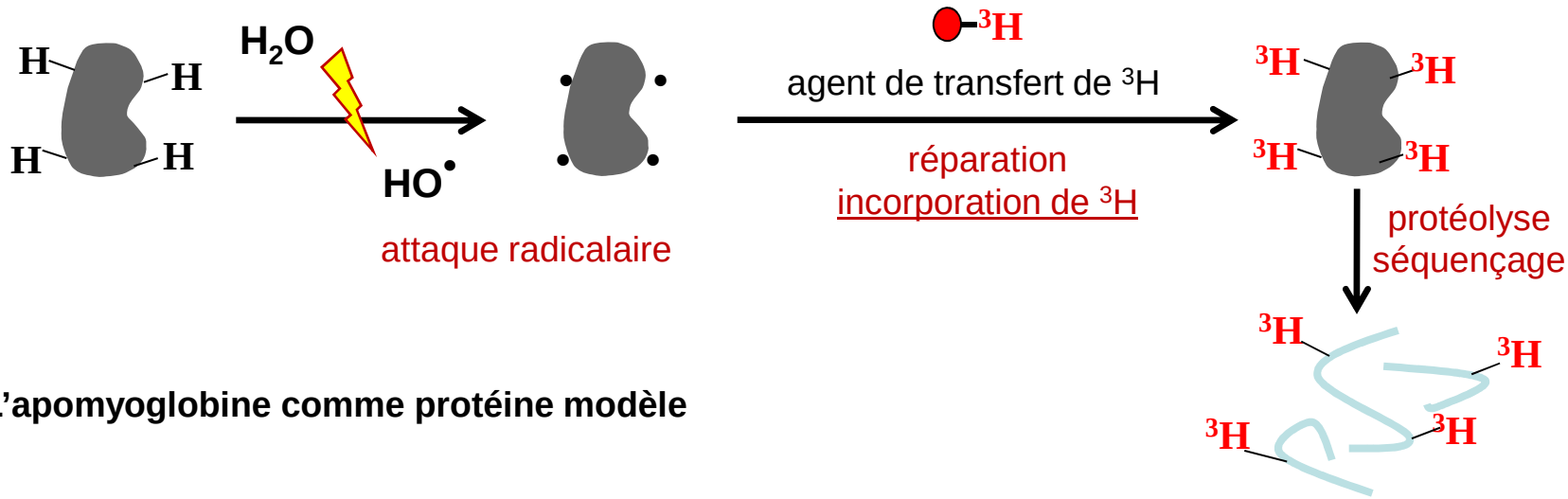


Illustrations copyright Cécile Sicard-Roselli

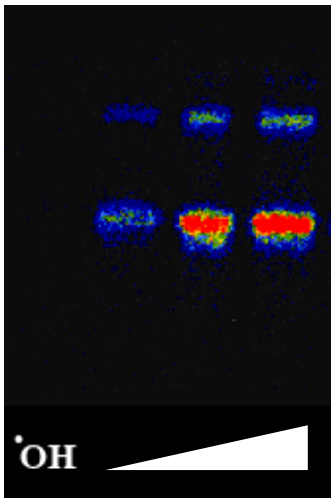
1) Etude des surfaces accessibles par marquage



1) Etude des surfaces accessibles par marquage

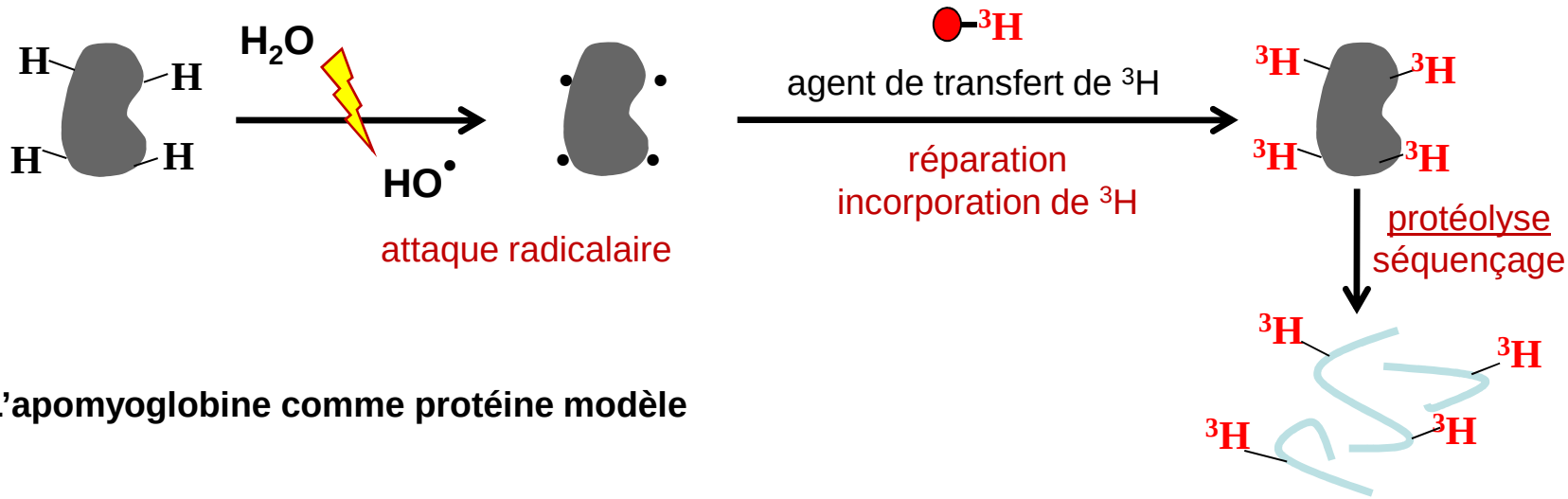


L'apomyoglobine comme protéine modèle

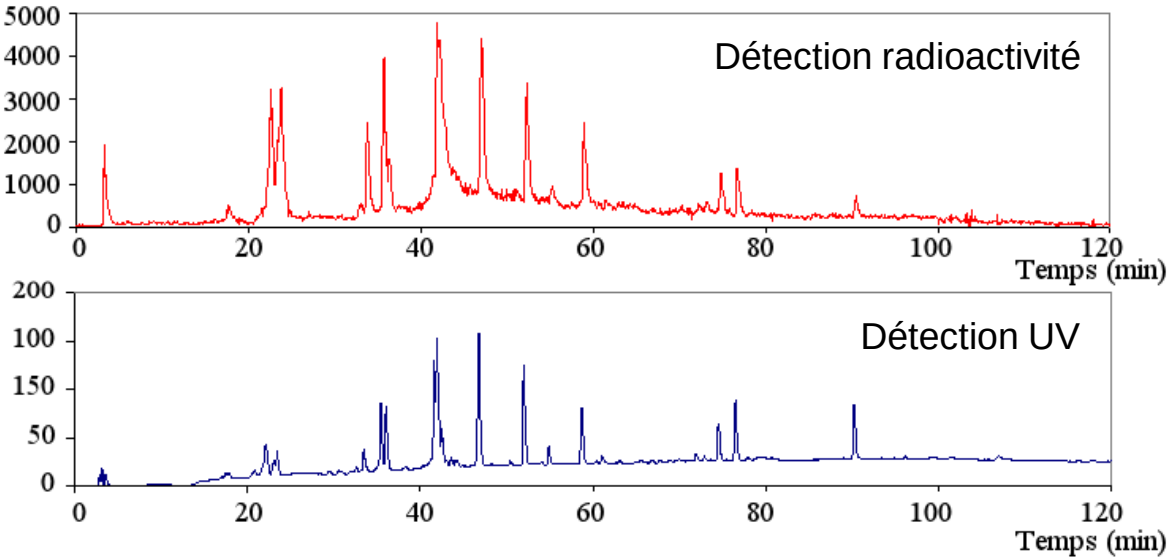


Gel d'électrophorèse après irradiation
Détection radioactive (β-imager)

1) Etude des surfaces accessibles par marquage

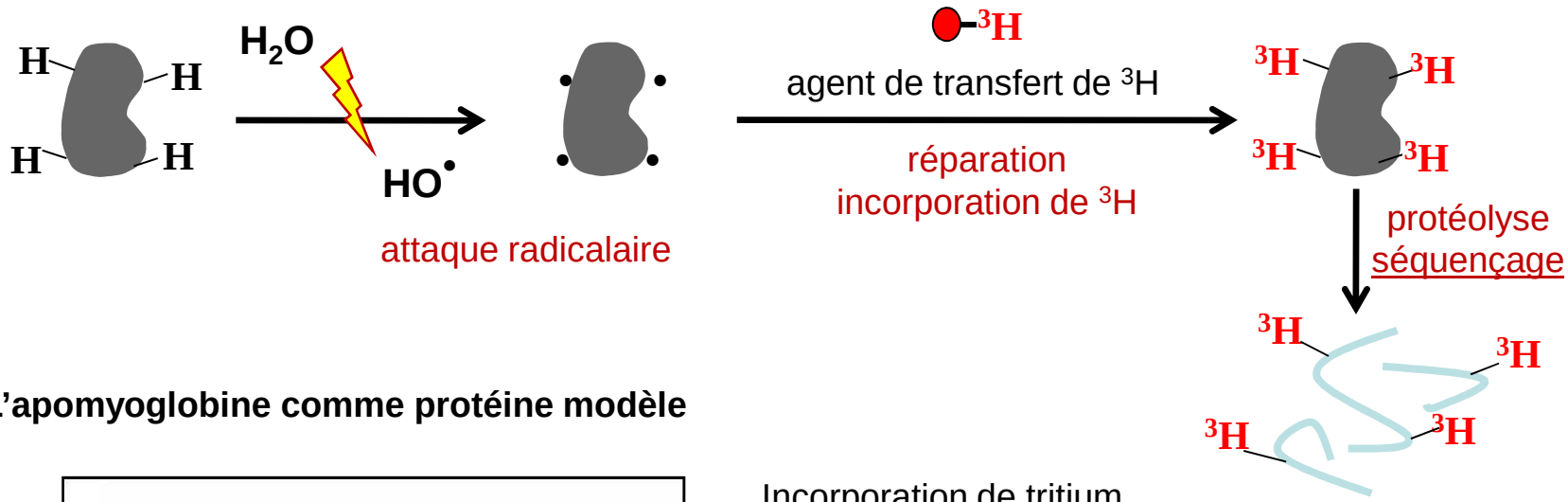


L'apomyoglobine comme protéine modèle

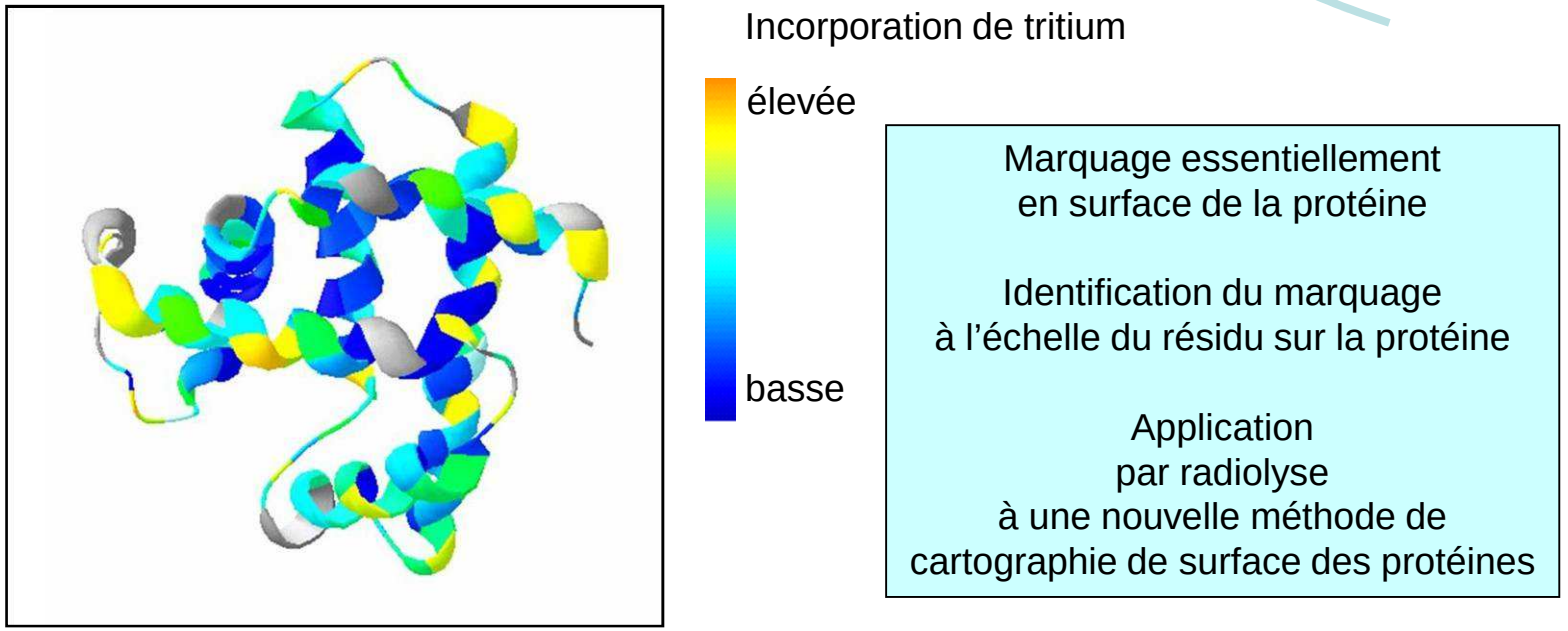


Analyse des peptides par HPLC

1) Etude des surfaces accessibles par marquage



L'apomyoglobine comme protéine modèle

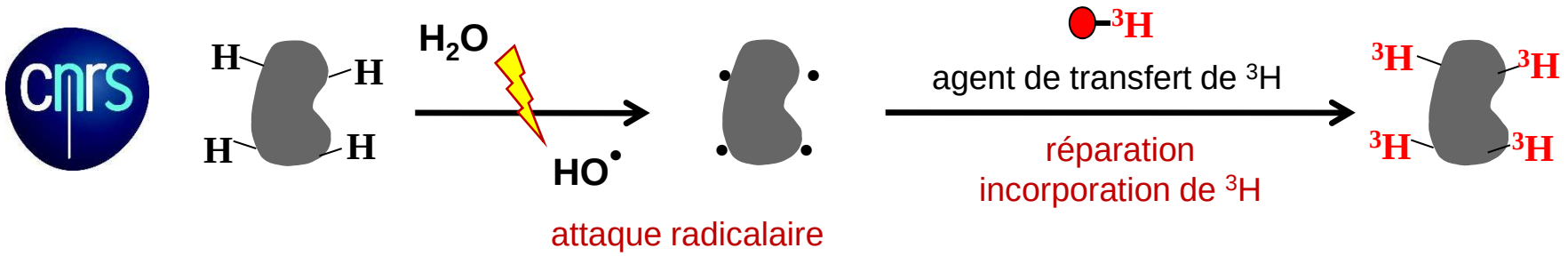


Marquage essentiellement
en surface de la protéine

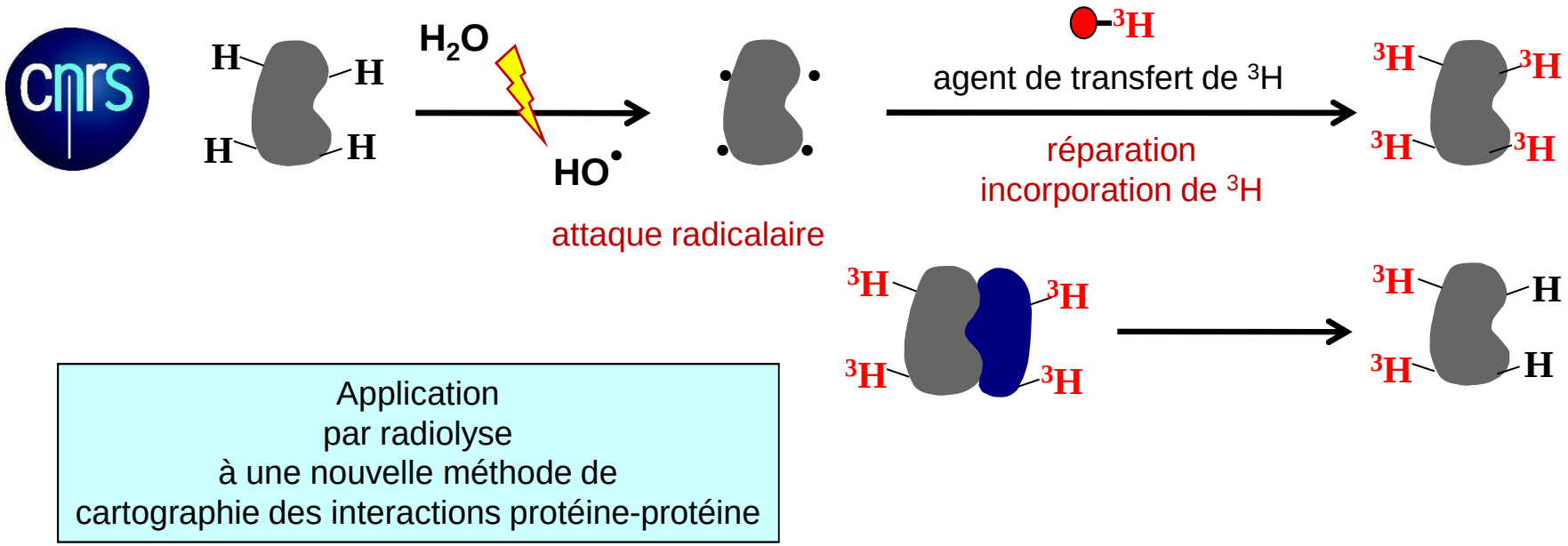
Identification du marquage
à l'échelle du résidu sur la protéine

Application
par radiolyse
à une nouvelle méthode de
cartographie de surface des protéines

2) Etude des interactions moléculaires par marquage

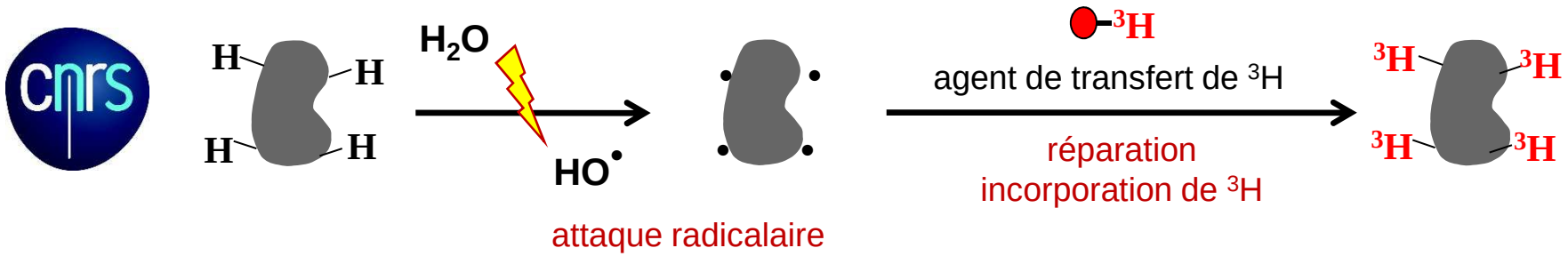


2) Etude des interactions moléculaires par marquage

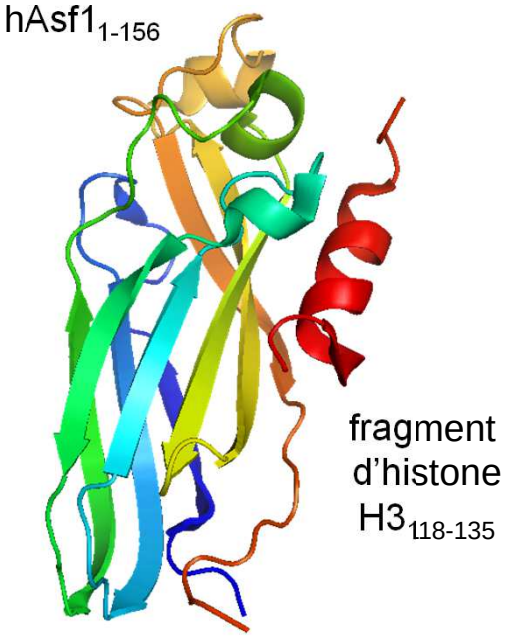


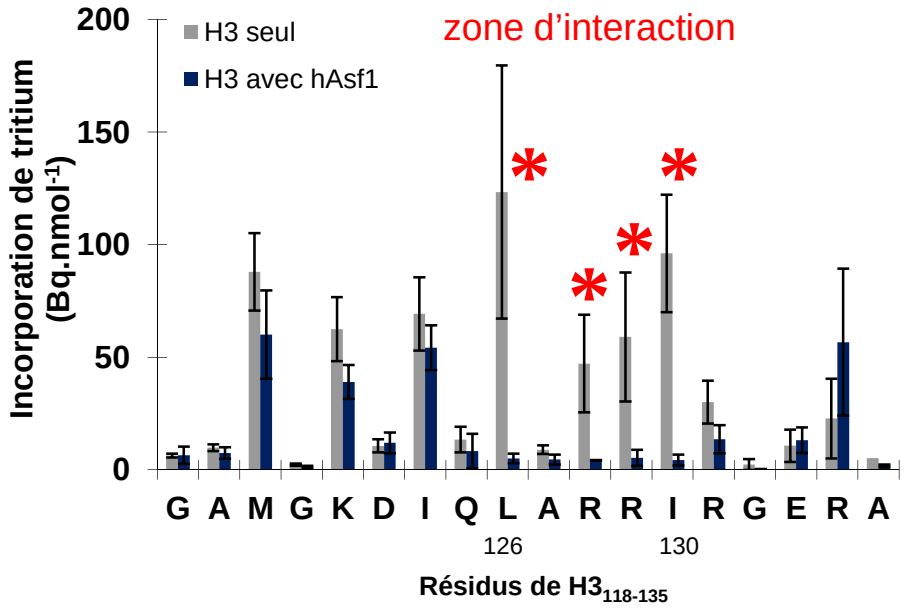
Application
par radiolyse
à une nouvelle méthode de
cartographie des interactions protéine-protéine

2) Etude des interactions moléculaires par marquage

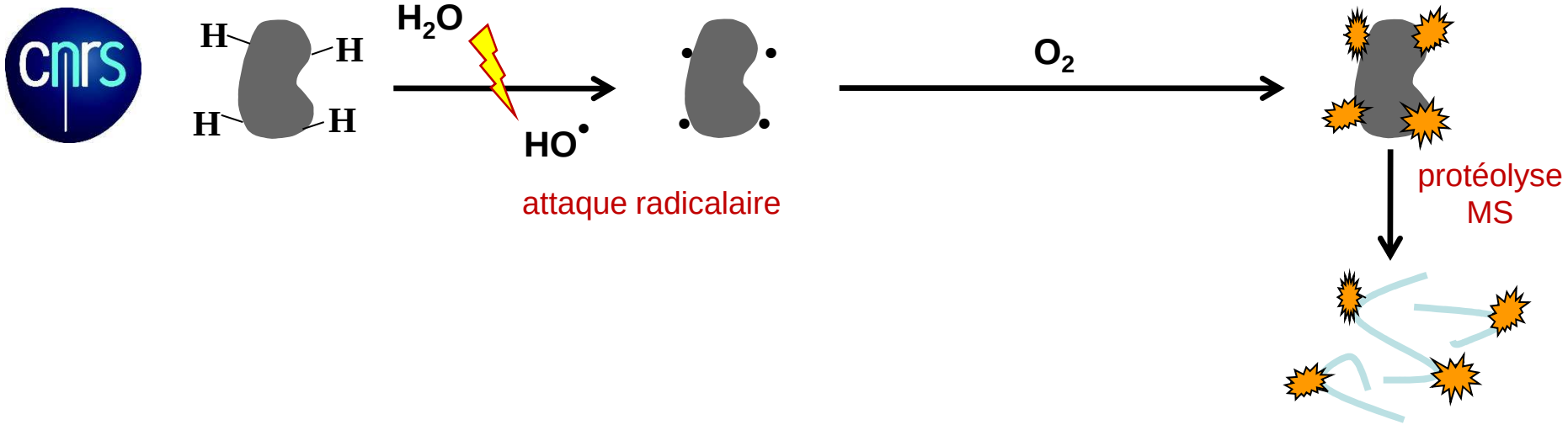


Asf1- H3 comme complexe modèle des interactions protéine-protéine

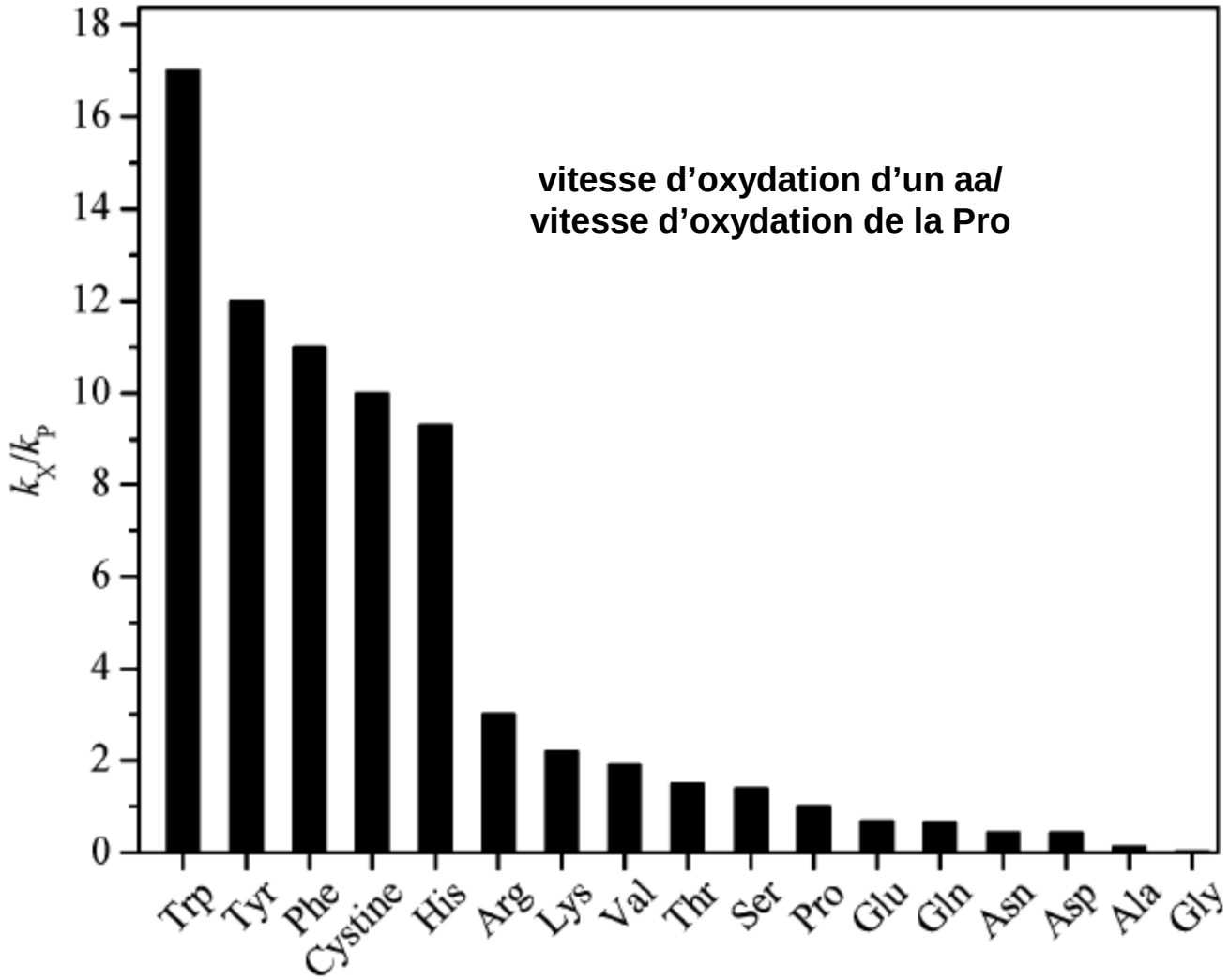




2) Etude des interactions moléculaires par oxydation

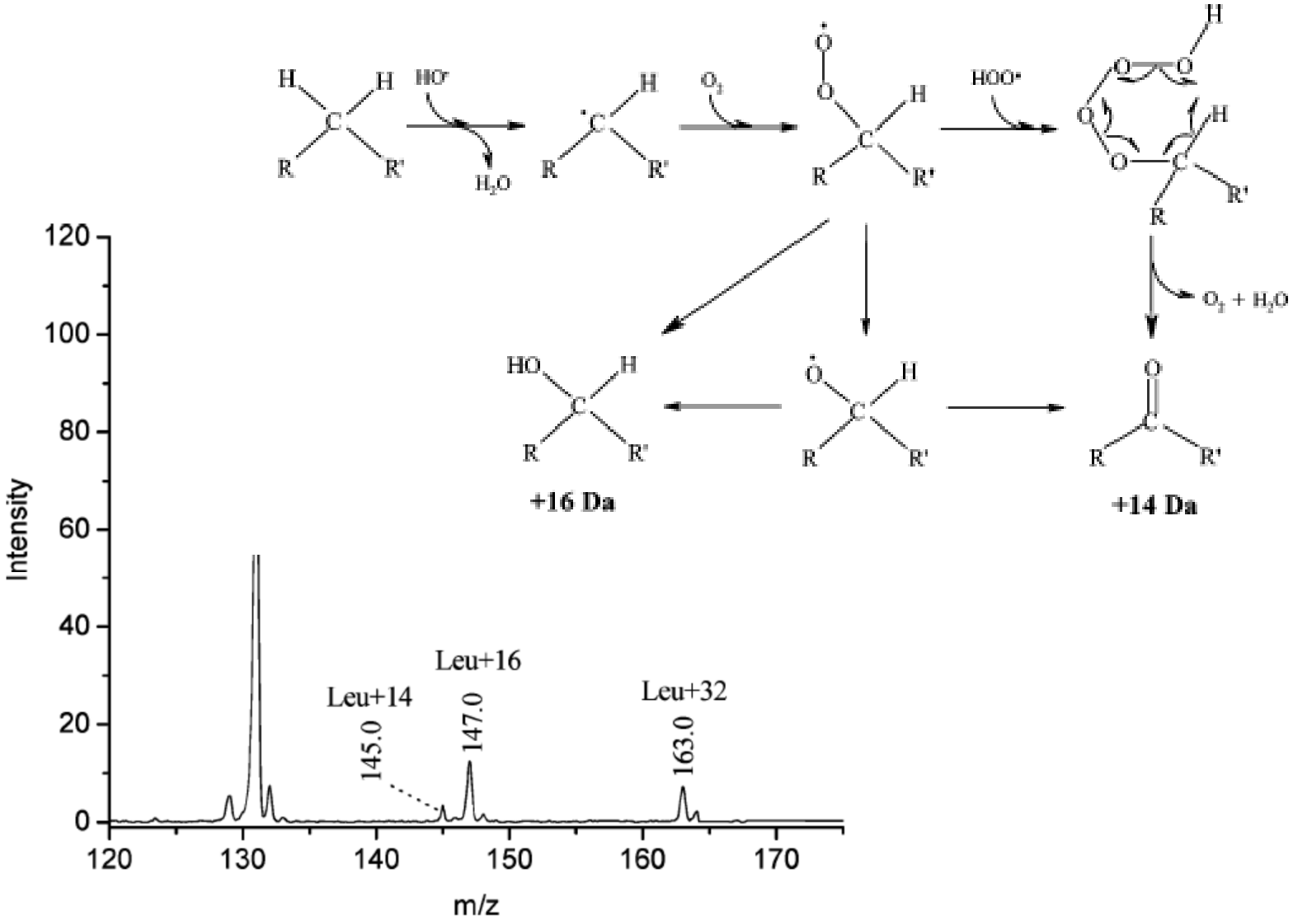


1) La vitesse d'oxydation des acides aminés par HO$^{\cdot}$



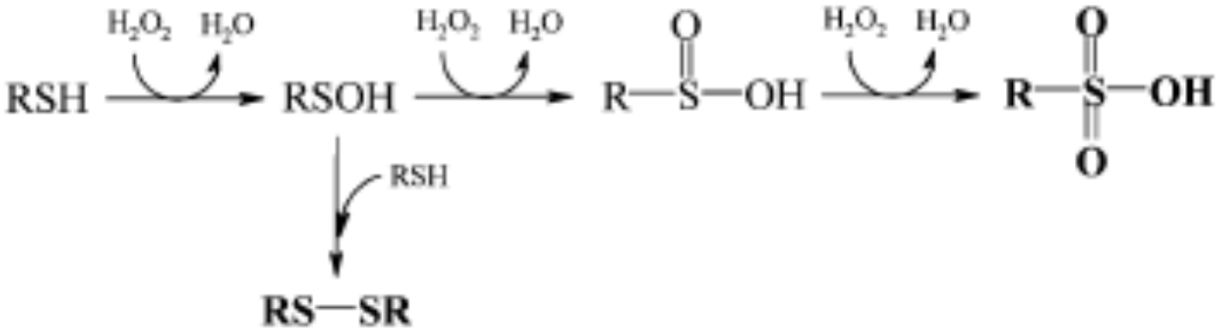
2) Les produits d'oxydation des acides aminés

Les chaînes alcanes



2) Les produits d'oxydation des acides aminés

La cystéine



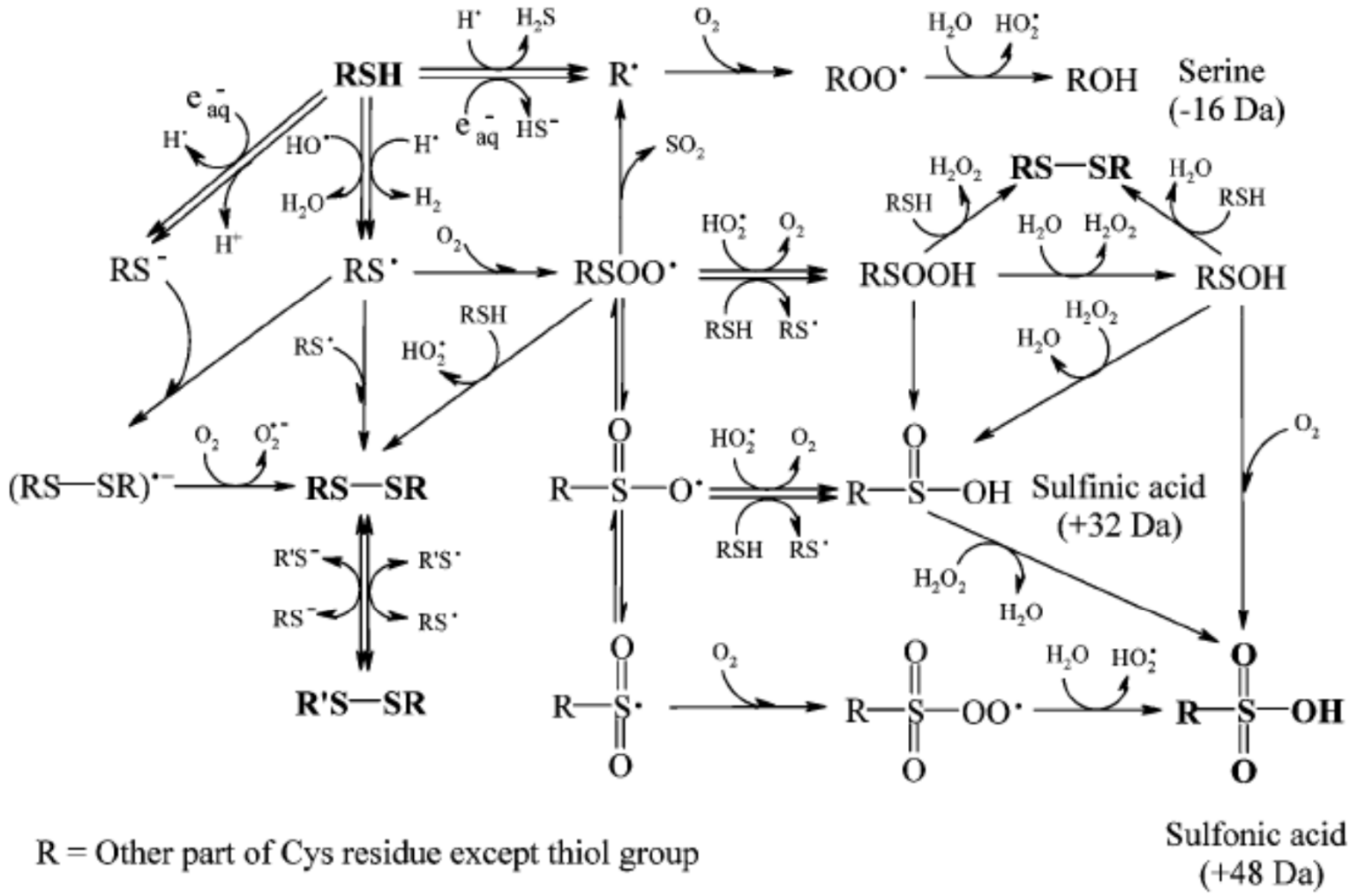
La cystéine est le seul aa sensible à l'eau oxygénée et au superoxyde :
action du rayonnement à temps long sur de grandes distances

La formation de pont disulfure est de plus en plus identifiée
comme un élément clef dans la réponse cellulaire au stress Redox

Les espèces radiolytiques peuvent agir directement
sur ces sites de régulation extrêmement sensibles
soit en déplaçant l'équilibre
soit en détruisant les cystéine

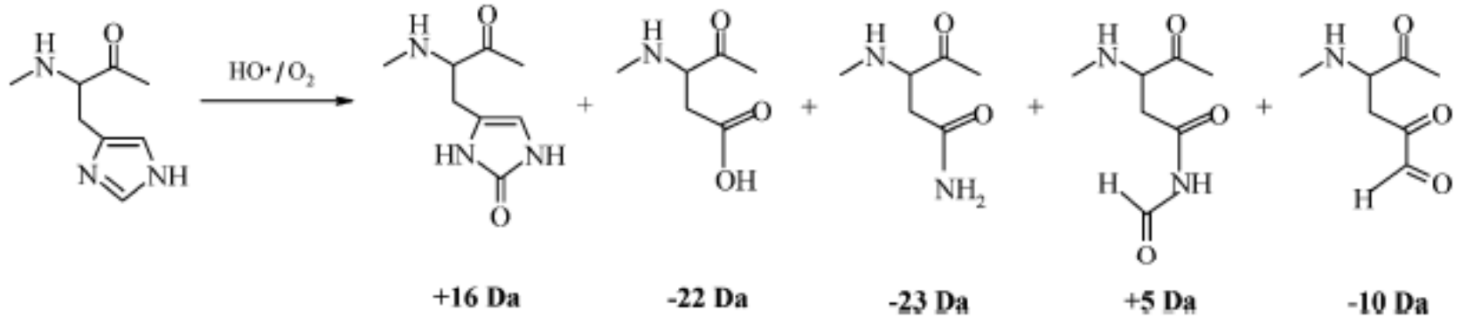
2) Les produits d'oxydation des acides aminés

La cystéine

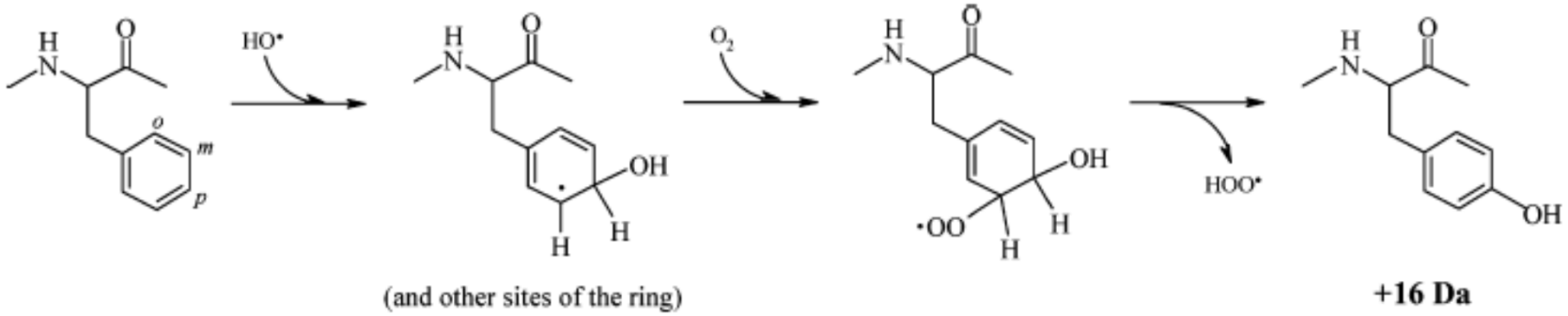


2) Les produits d'oxydation des acides aminés

L'histidine



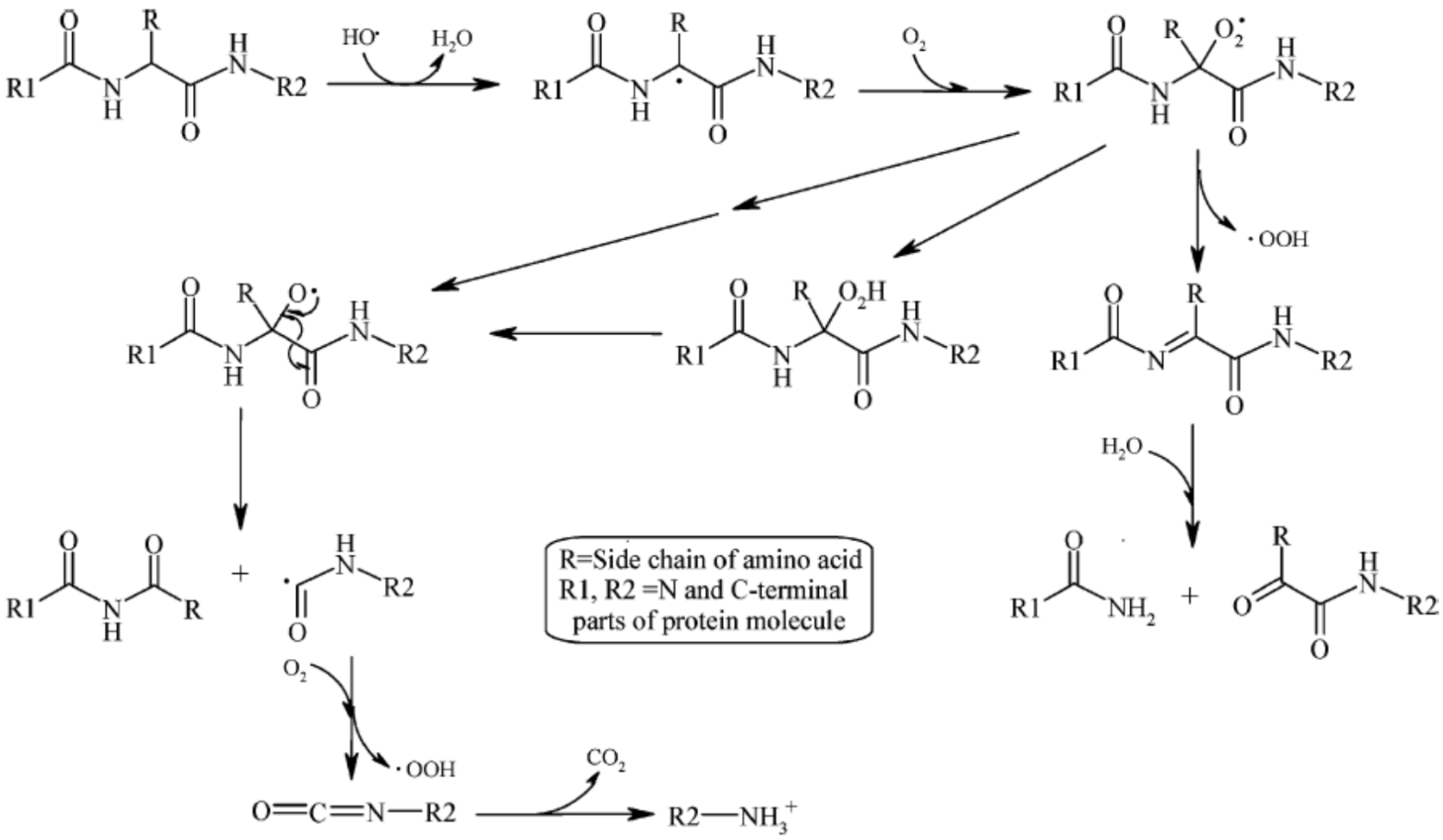
La phénylalanine et la tyrosine



2) Les produits d'oxydation des acides aminés

Cys	sulfonic acid, sulfinic acid, hydroxy
Met	sulfoxide, sulfone, aldehyde,
Trp	hydroxyl, ouverture du cycle
Tyr	hydroxy-
Phe	hydroxy-
His	oxo, ouverture du cycle
Leu	hydroxyl, carbonyl
Ile	hydroxyl, carbonyl
Val	hydroxyl, carbonyl
Pro	hydroxy-, carbonyl
Arg	deguanidination, hydroxy-, carbonyl
Lys	hydroxy- , carbonyl
Glu	decarboxylation , hydroxy- , carbonyl
Gln	hydroxy- , carbonyl
Asp	decarboxylation , hydroxy-
Asn	hydroxy-
Ser	hydroxy- , carbonyl
Thr	hydroxy- , carbonyl
Ala	hydroxy-

3) La fragmentation de la chaîne peptidique



A forte dose
fragmentations de la chaîne peptidique
moins accessible que les chaînes latérales

4) Le transfert de radicaux dans les protéines

Eur. J. Biochem. **270**, 3565–3571 (2003) © FEBS 2003 doi:10.1046/j.1432-1033.2003.03741.x

Re-evaluation of intramolecular long-range electron transfer between tyrosine and tryptophan in lysozymes

Evidence for the participation of other residues

Marilyne Stuart-Audette[1], Yves Blouquit[2], Moshe Faraggi[3], Cécile Sicard-Roselli[1], Chantal Houée-Levin[1] and Pierre Jollès[4]

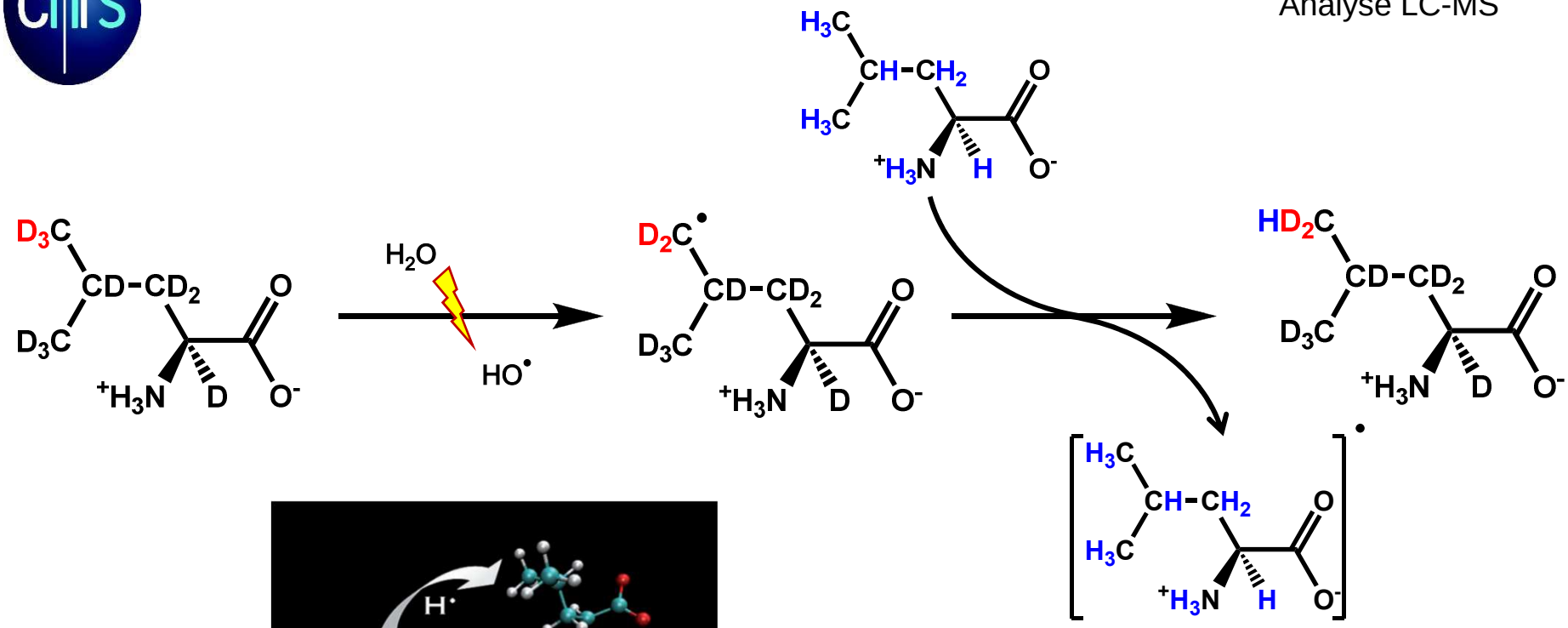
[1]*LCP, Centre Universitaire, Orsay Cedex, France;* [2]*Institut Curie-Section de Recherche, Centre Universitaire, Orsay Cedex, France;* [3]*Department of Nuclear Engineering, Ben Gurion University of the Negev, Israel;* [4]*Laboratoire de Chimie, Muséum National d'Histoire Naturelle, and LNCP, Université Paris 6, France*

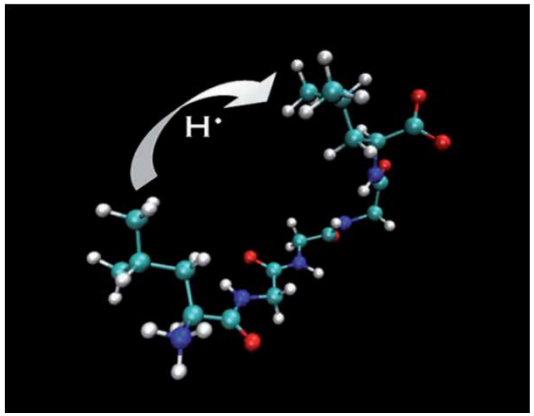
One-electron oxidation of six different c-type lysozymes from hen egg white, turkey egg white, human milk, horse milk, camel stomach and tortoise was studied by gamma- and pulse-radiolysis. In the first step, one tryptophan side chain is oxidized to indolyl free radical, which is produced quantitatively. As shown already, the indolyl radical subsequently oxidizes a tyrosine side chain to the phenoxy radical in an intramolecular reaction. However this reaction is not total and its stoichiometry depends on the protein. Rate constants also vary between proteins, from $120 \cdot s^{-1}$ to $1000 \cdot s^{-1}$ at pH 7.0 and room temperature [extremes are hen and turkey egg white ($120 \cdot s^{-1}$) and human milk ($1000 \cdot s^{-1}$)]. In hen and turkey egg white lysozymes we show that another reactive site is the Asn103–Gly104 peptidic bond, which gets broken radiolytically. Tryptic digestion followed by HPLC separation and identification of the peptides was performed for nonirradiated and irradiated hen lysozyme. Fluorescence spectra of the peptides indicate that Trp108 and/or 111 remain oxidized and that Tyr20 and 53 give bityrosine. Tyr23 appears not to be involved in the process. Thus new features of long-range intramolecular electron transfer in proteins appear: it is only partial and other groups are involved which are silent in pulse radiolysis.

Keywords: gamma and pulse radiolysis; intramolecular long-range electron transfer; lysozyme; one-electron oxidation.

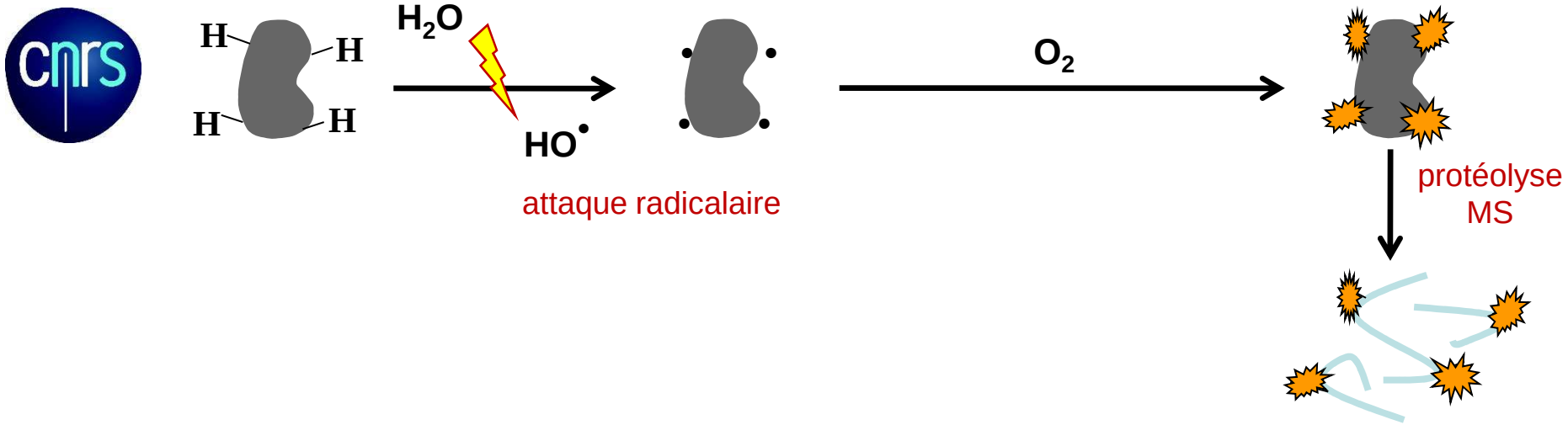
4) Le transfert de radicaux dans les protéines

Echange H-D
Radiolyse
Analyse LC-MS





2) Etude des interactions moléculaires par oxydation



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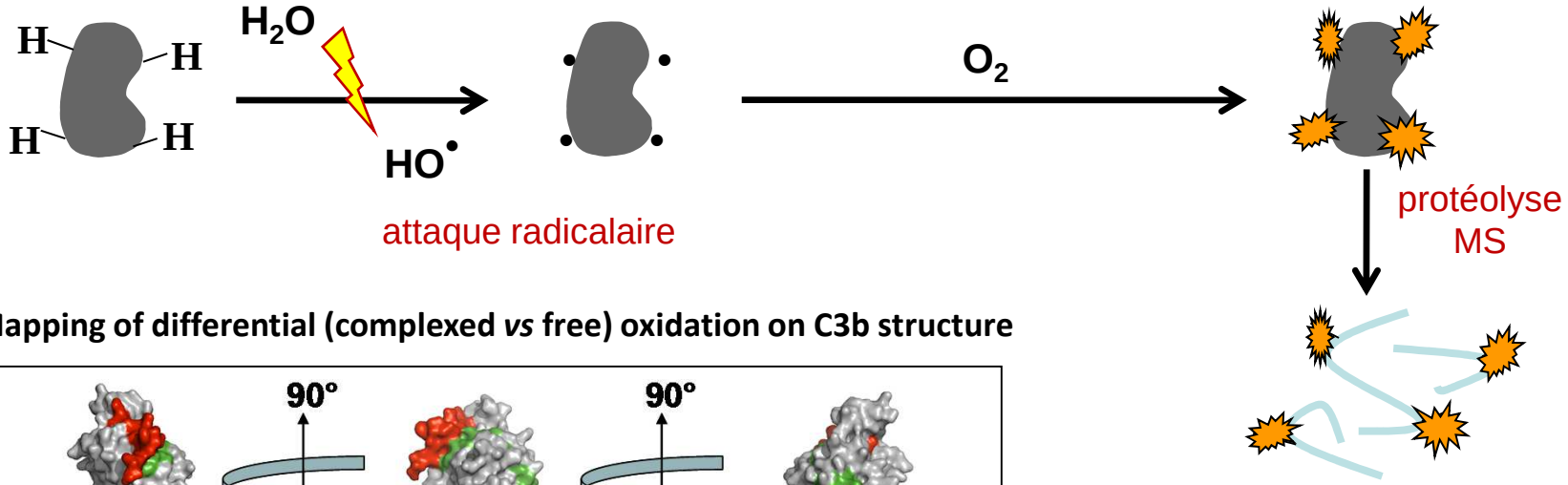
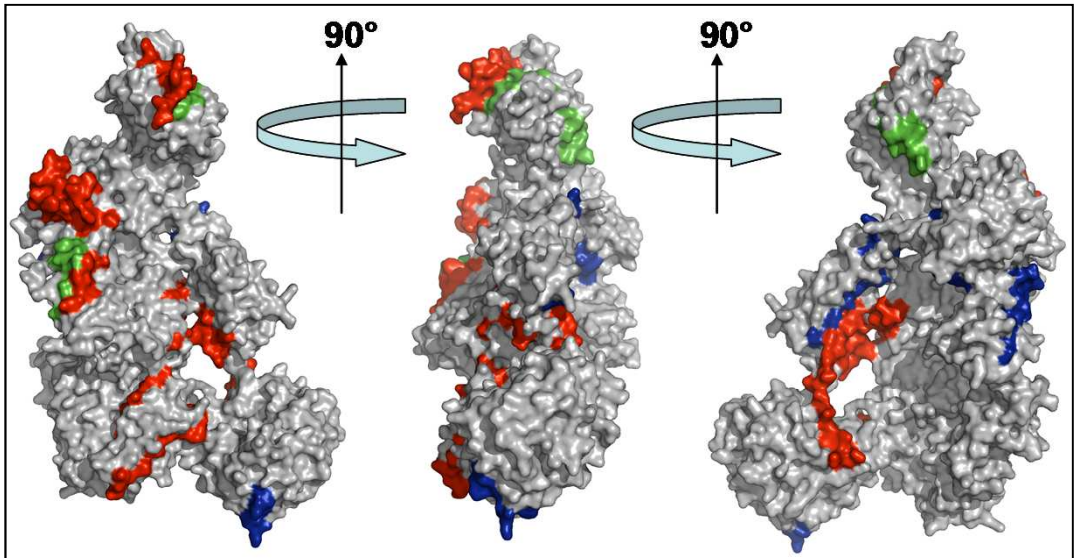
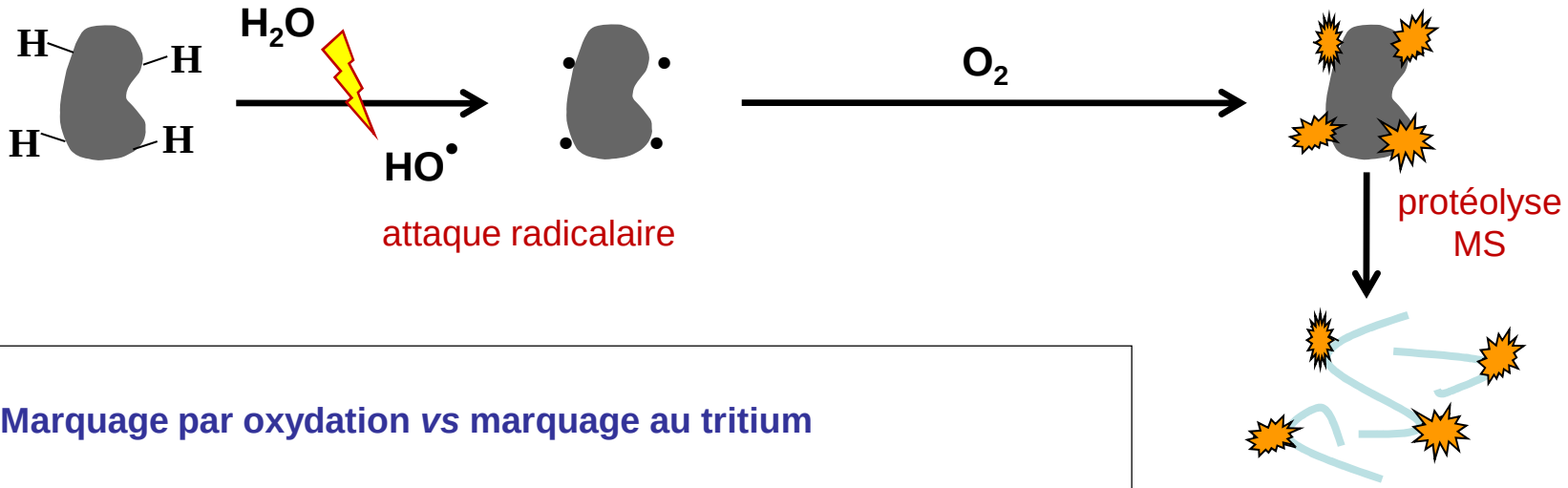


Mapping of differential (complexed *vs* free) oxidation on C3b structure



In red, peptides with decreased oxidation rate upon complexation
In green, peptide with increased oxidation rate
In blue, unmodified peptide

2) Etude des interactions moléculaires



Marquage par oxydation *vs* marquage au tritium

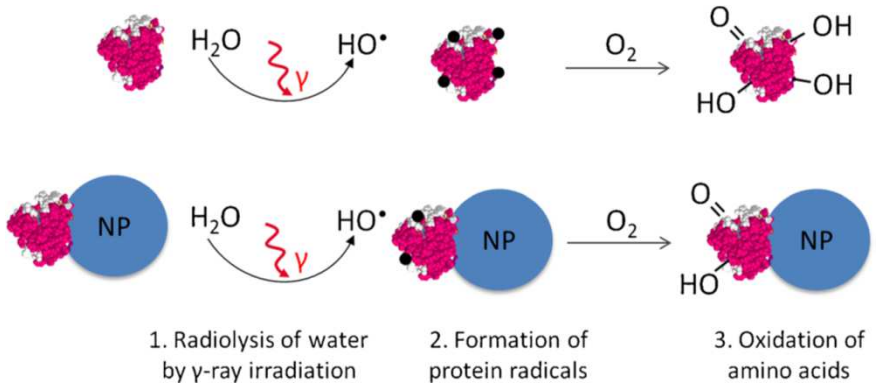
Pas de tritium
LC/MS-MS
Oxydations spécifiques : C, M, W, Y, F puis H, R, K...
Possibilités d'une résolution temporelles des structures

Intérêt du marquage par oxydation en biochimie structurale pour des études en

température
pression
milieu complexe

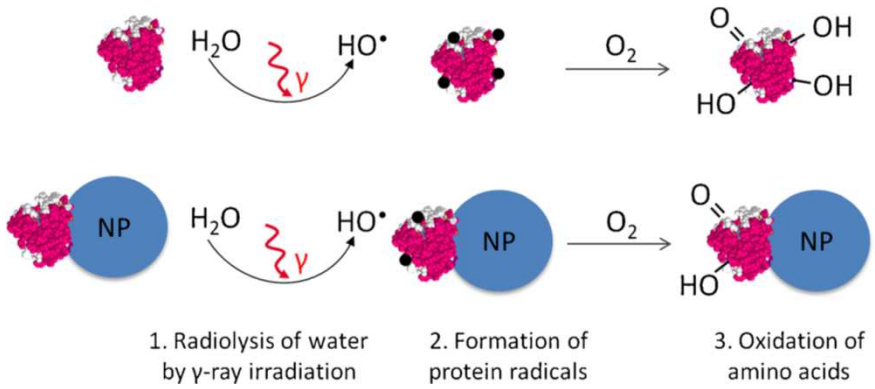
2) Etude des interactions moléculaires

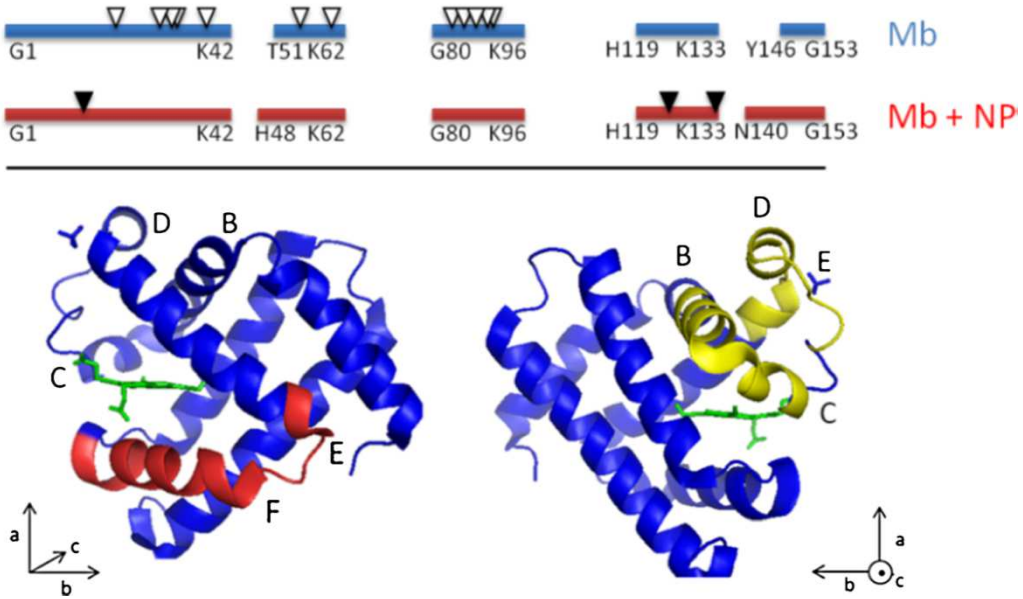
Cas des interactions protéines-NPs



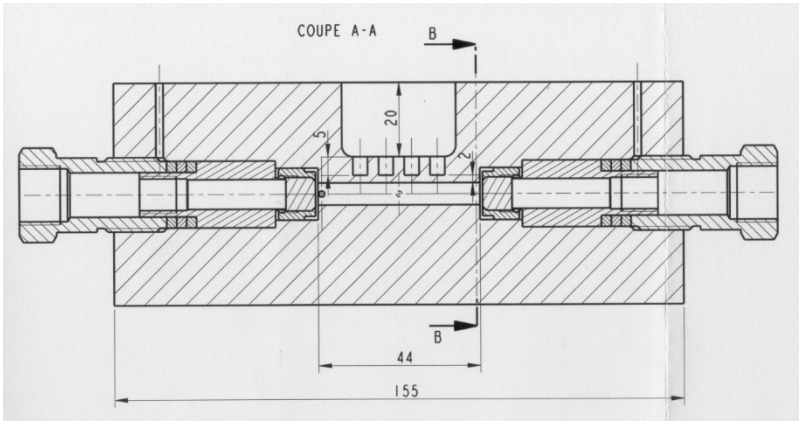
2) Etude des interactions moléculaires

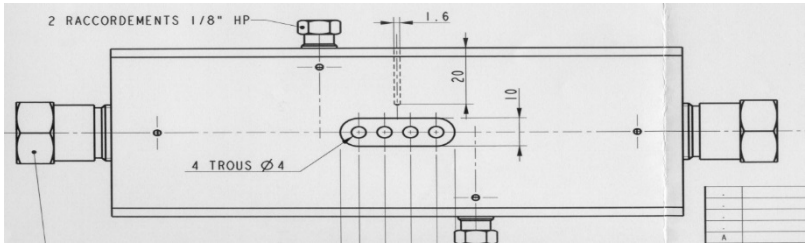
Cas des interactions protéines-NPs





Couplage LINAC-cellule de pression (2005)



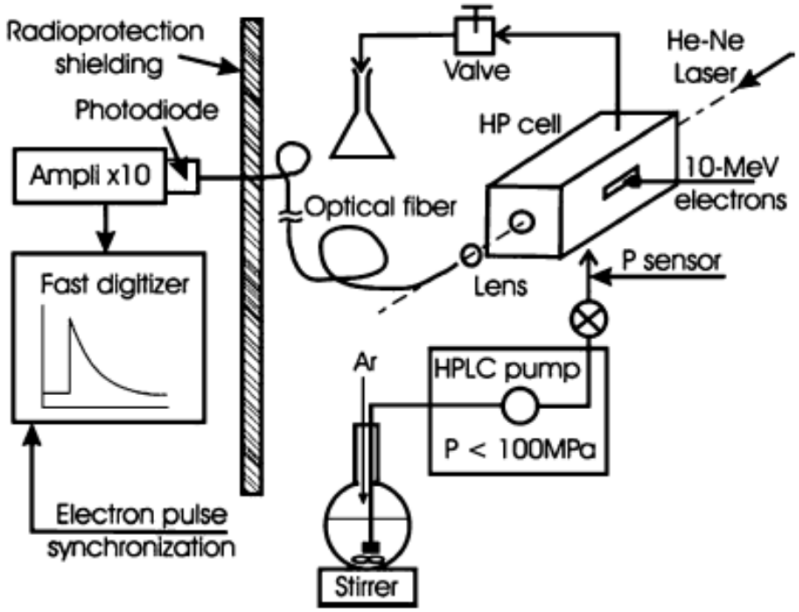


Cellule de T et P
500°C + 100 MPa

Cellule optique à circulation
Faisceau pulsé d'électrons
Mesures de transmission résolues en temps

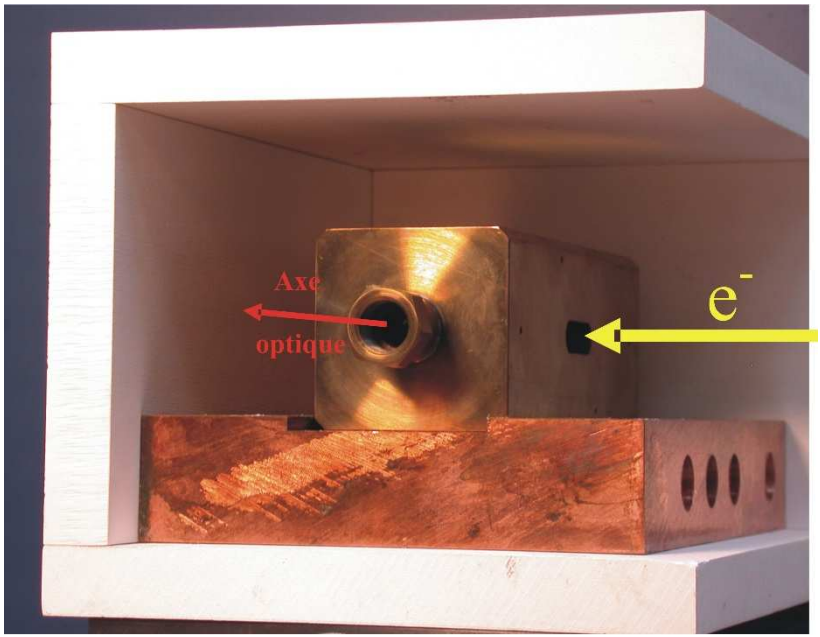
Cellule :
double cahier des charges

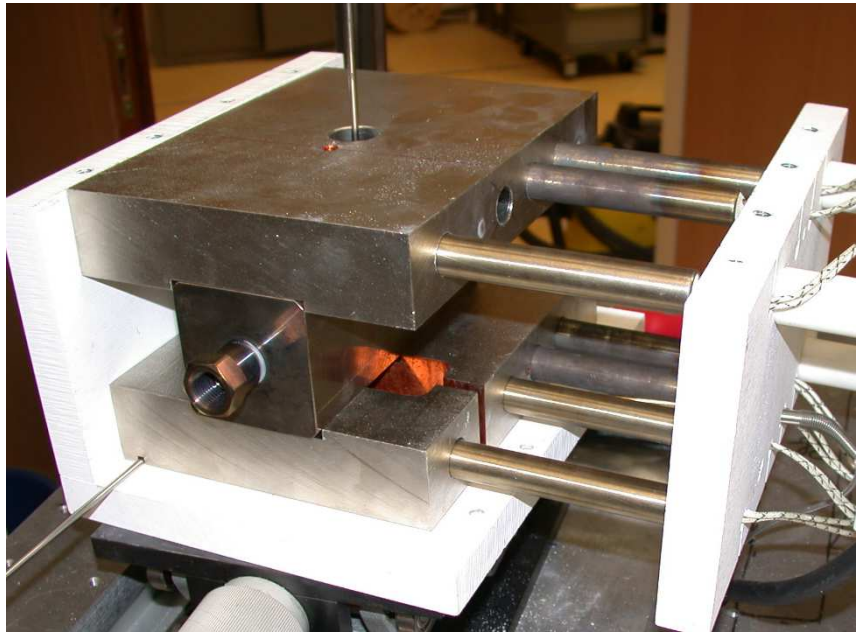
étude en eau supercritique
(374°C et 22 MPa)
+
étude des protéines



Couplage LINAC-cellule de pression (2005)

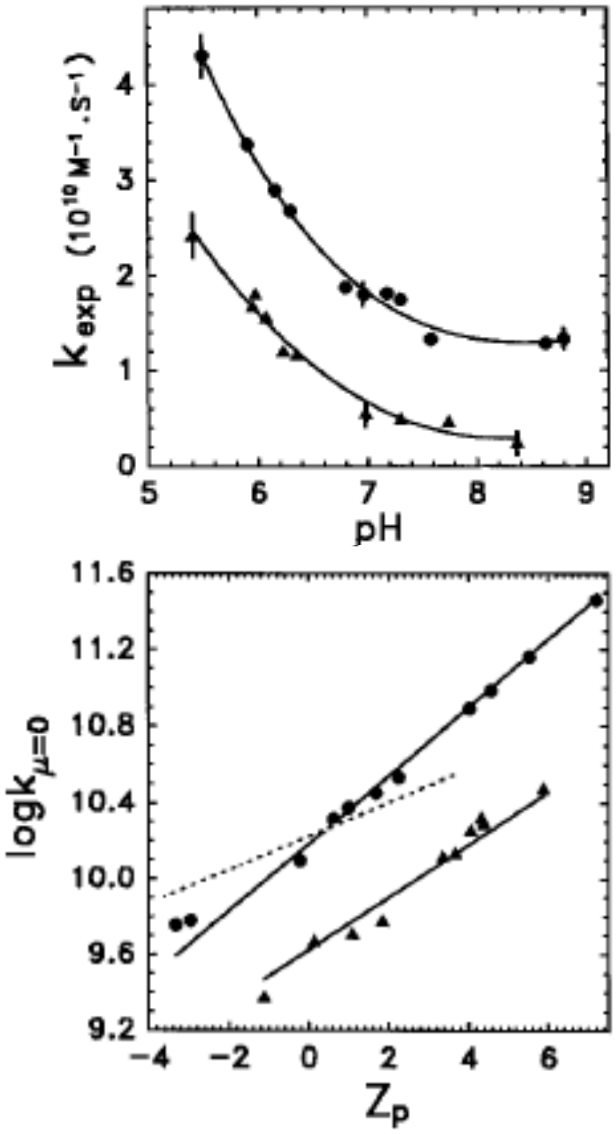
1 fenêtre pour les électrons de 10 MeV
épaisseur de 2 mm
2 fenêtres en saphir pour le trajet optique
épaisseur de 8 mm

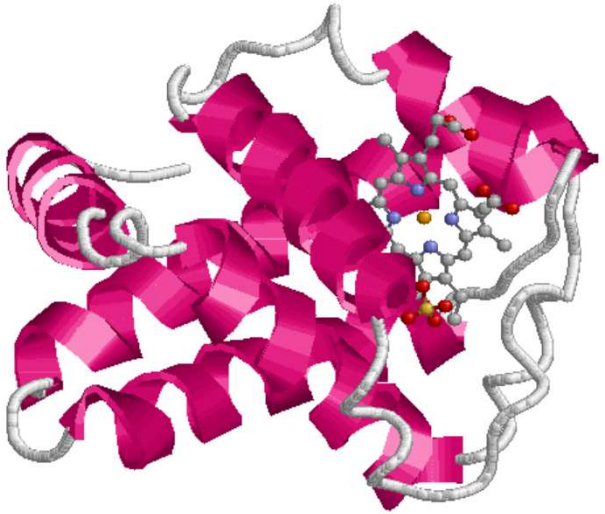




doigts chauffants

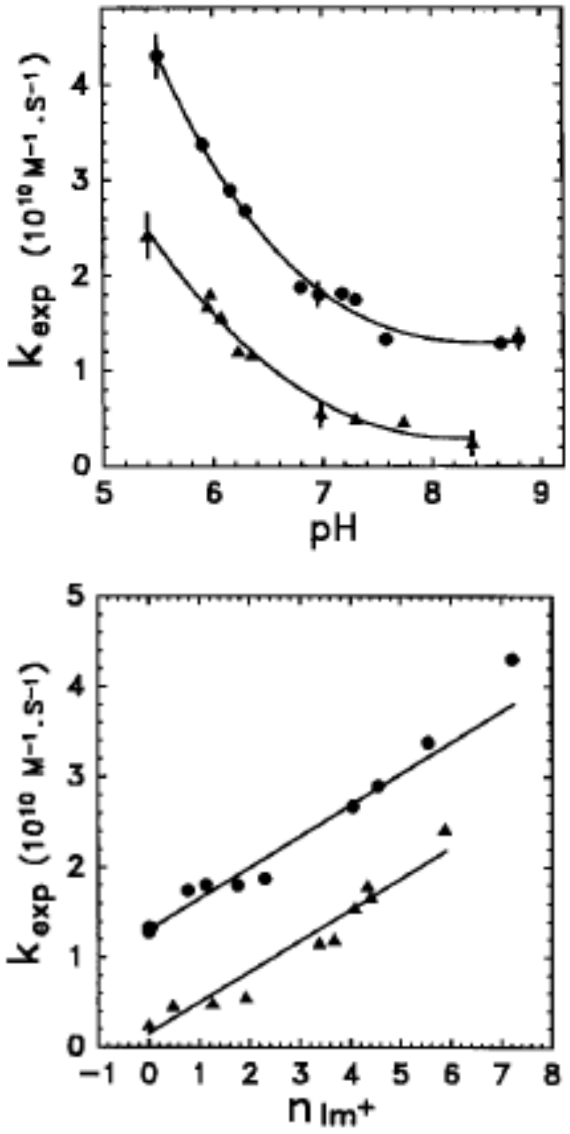
Réduction à P_{atm} de la myoglobine par e^-_{aq}

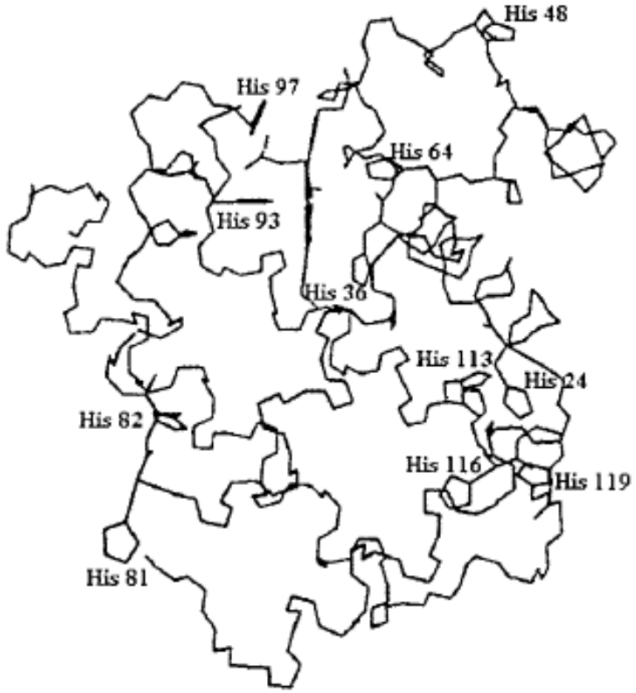




Réduction par e^-_{aq}
les sites réductibles de la myoglobine
les AA de la globine + la porphyrine + le fer

Réduction à P_{atm} de la myoglobine par e^-_{aq}





Réduction sous pression des tampons par e^-_{aq}

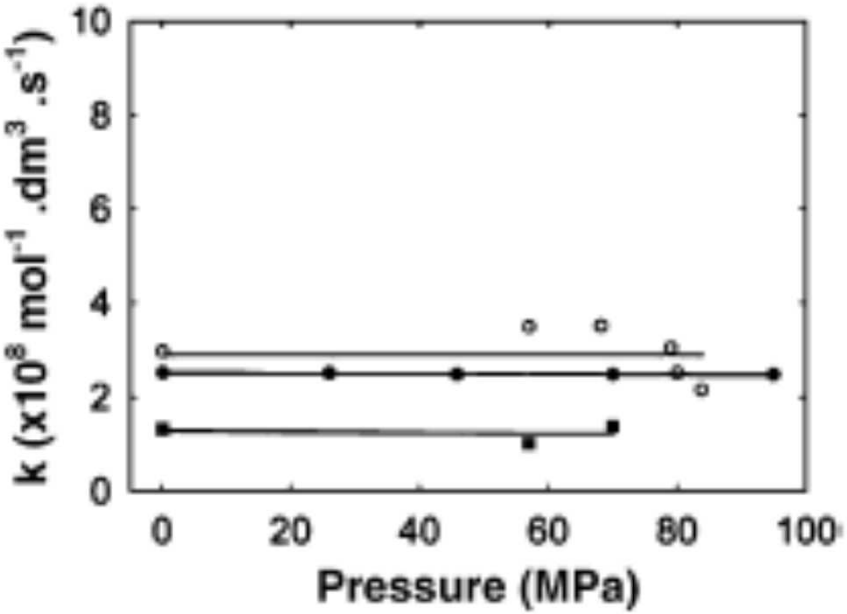


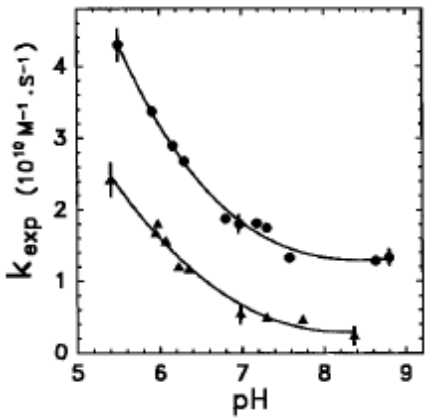
Fig. 4. Rate constants for the reduction of buffer solutions by e^-_{aq} as a function of the pressure: ●, 1×10^{-3} mol dm^{-3} bis-Tris pH 6; ○, 1×10^{-3} mol dm^{-3} bis-Tris pH 7; ■, 1×10^{-3} mol dm^{-3} Tris pH 8.

Méthode directe pour évaluer les variations de pH des tampons en fonction de la pression

Méthodes indirectes avec des indicateurs colorés en absorption ou fluorescence

Neuman, Kauzman et Zipp (1973)
Hayert, Perrier-Cornet et Gervais (1999)
Quinlan et Reinhart (2005)

Réduction sous pression de la myoglobine par e^-_{aq}



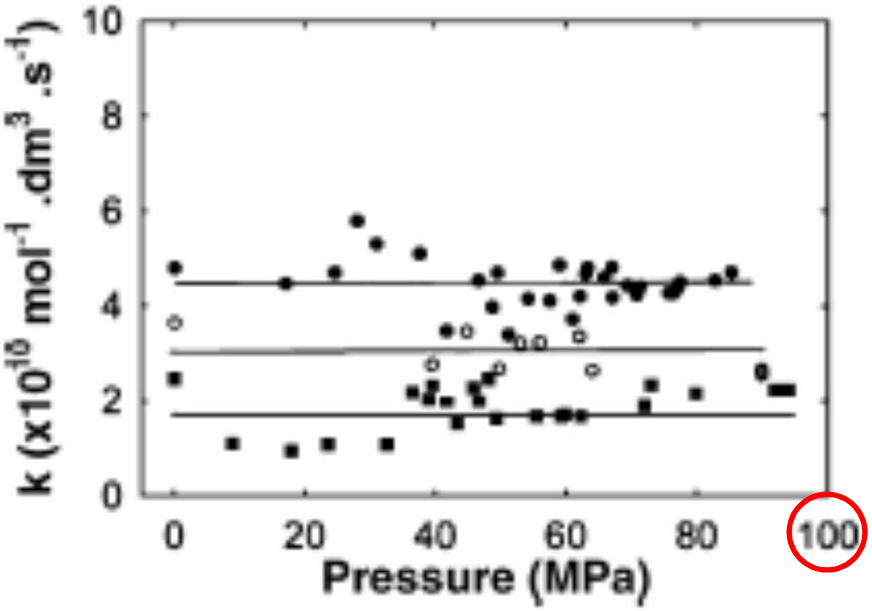
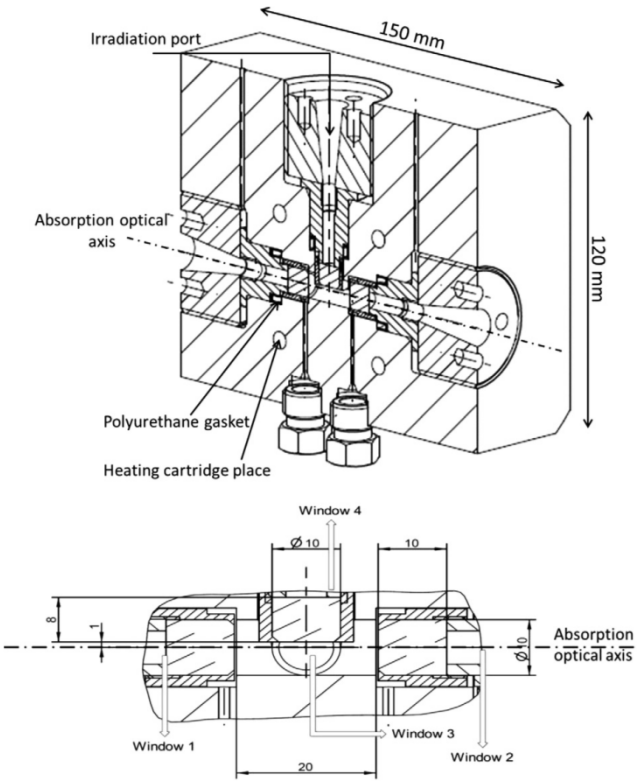


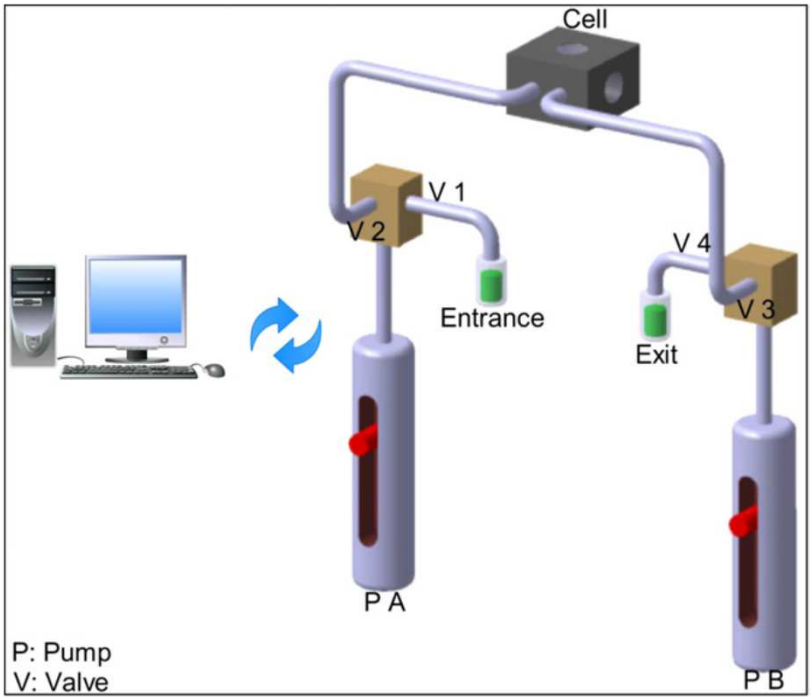
Fig. 5. Rate constants for the reduction of metmyoglobin solutions by e^-_{aq} as a function of the pressure: ●, in 1×10^{-3} mol dm^{-3} bis-Tris pH 6; ○, in 1×10^{-3} mol dm^{-3} bis-Tris pH 7; ■, in 1×10^{-3} mol dm^{-3} Tris pH 8. The Mb concentrations were $4-6\times10^{-5}$ mol dm^{-3}.

Couplage LINAC-cellule de pression (2013)

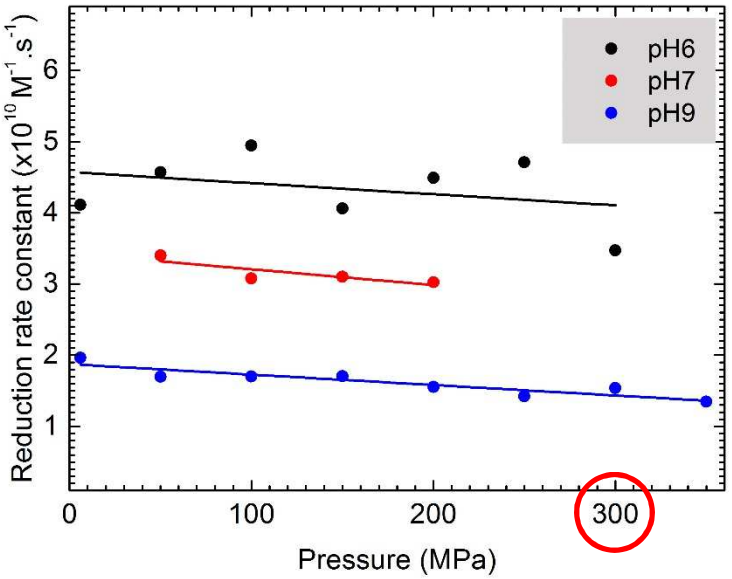


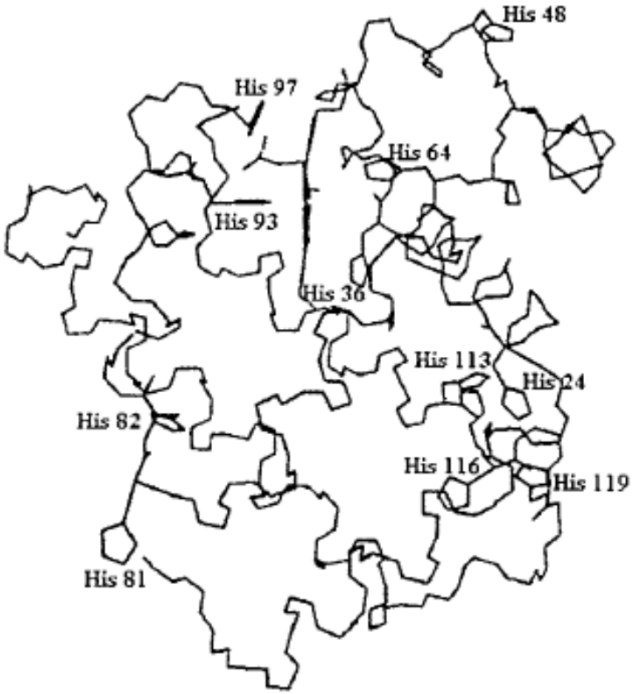
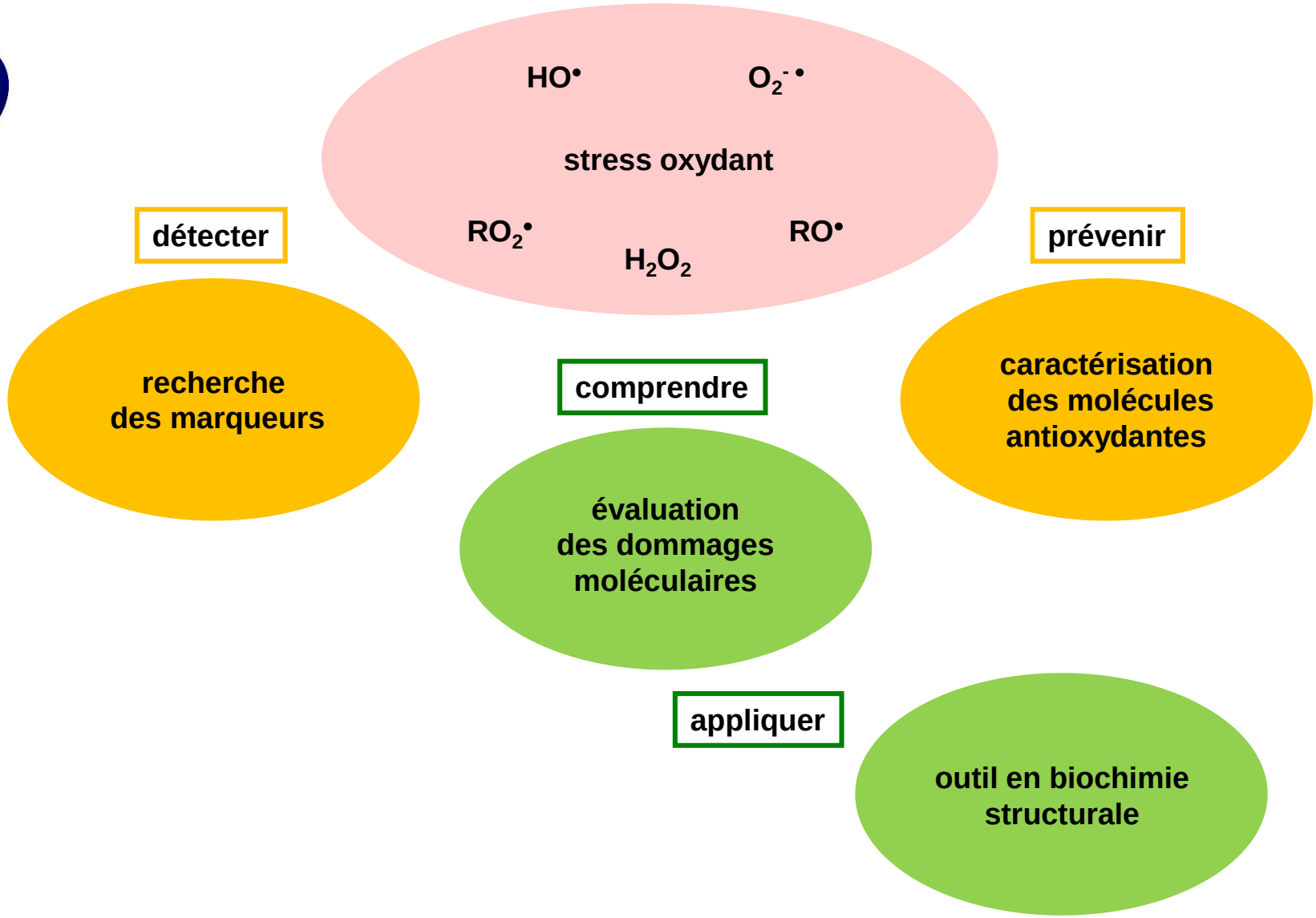
Cellule de P
400 MPa

Cellule optique à circulation
Faisceau pulsé d'électrons
Mesures de transmission résolues en temps



Réduction sous pression de la myoglobine par e^-_{aq}





La radiolyse, un outil pour l'étude des protéines