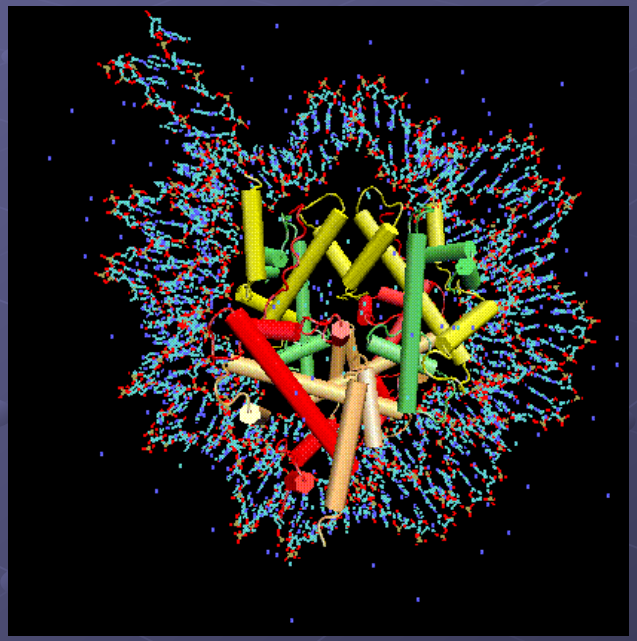
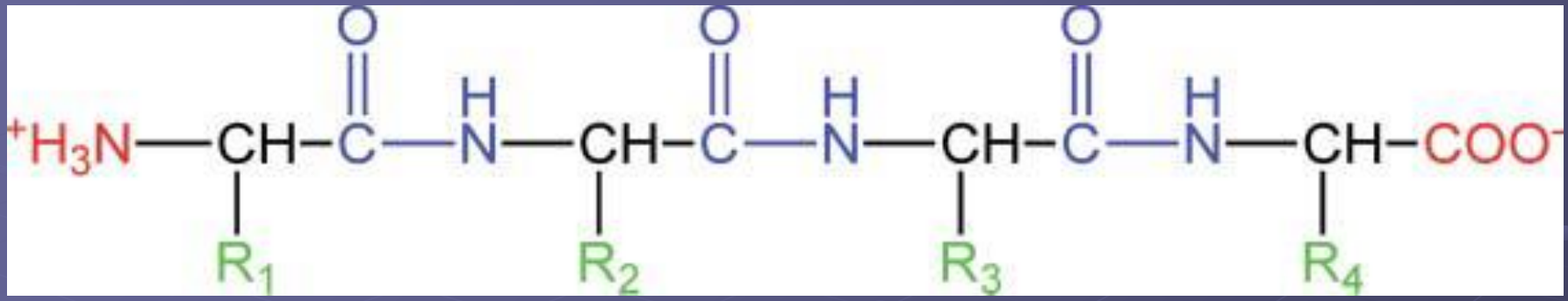
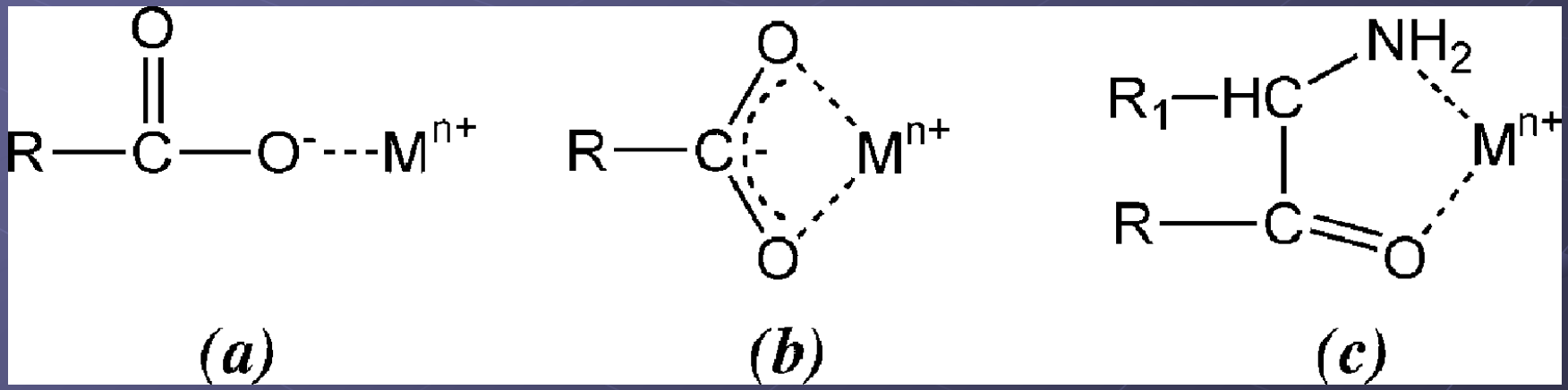
ΔΙΕΡΕΥΝΗΣΗ ΠΙΘΑΝΩΝ ΜΗΧΑΝΙΣΤΙΚΩΝ ΟΔΩΝ ΤΗΣ ΕΠΑΓΟΜΕΝΗΣ ΑΠΟ Cu(II) ΚΑΙ Ni(II) ΤΟΞΙΚΟΤΗΤΑΣ-ΚΑΡΚΙΝΟΓΕΝΝΕΣΗΣ ΜΕΛΕΤΩΝΤΑΣ ΤΙΣ ΑΛΛΗΛΕΠΙΔΡΑΣΕΙΣ ΤΟΥΣ ΜΕ ΠΕΠΤΙΔΙΚΑ ΜΟΝΤΕΛΑ ΙΣΤΟΝΩΝ



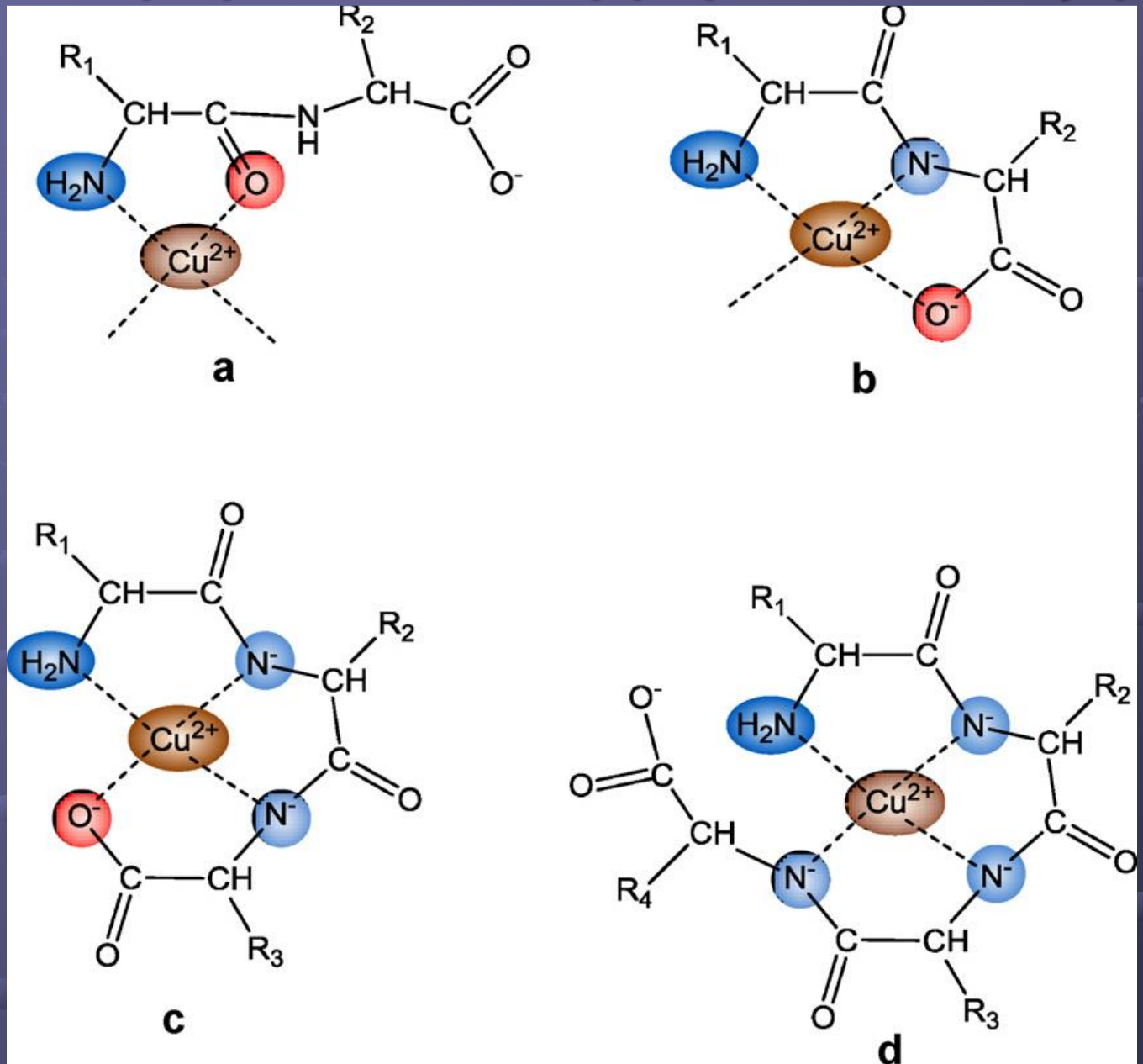
Αναπ. Καθ. Γερ. Μαλανδρίνος

Σύντομη επανάληψη Α’ διάλεξης....

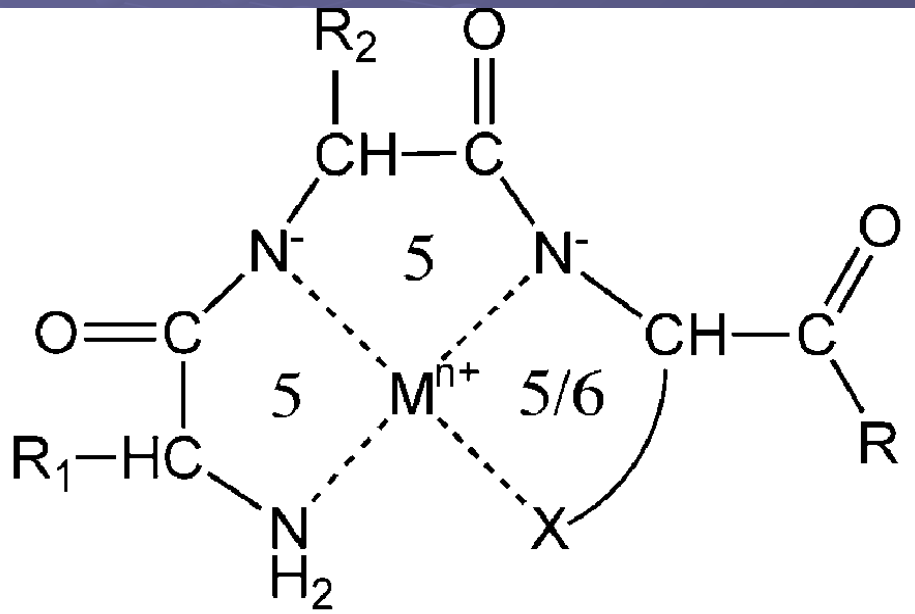




Σύντομη επανάληψη Α’ διάλεξης....



Σύντομη επανάληψη Α’ διάλεξης....



a) X = N_{Im} (His); M^{n+} = Cu(II), Ni(II), Pd(II), Au(III), Pt(II)

b) X = β-COO^- (Asp); M^{n+} = Cu(II), (Ni(II), Pd(II))

c) X = S^- (Cys); M^{n+} = Ni(II), Pd(II)

d) X = S-CH_3 (Met); M^{n+} = Pd(II), Pt(II)

Σύντομη επανάληψη Α’ διάλεξης....

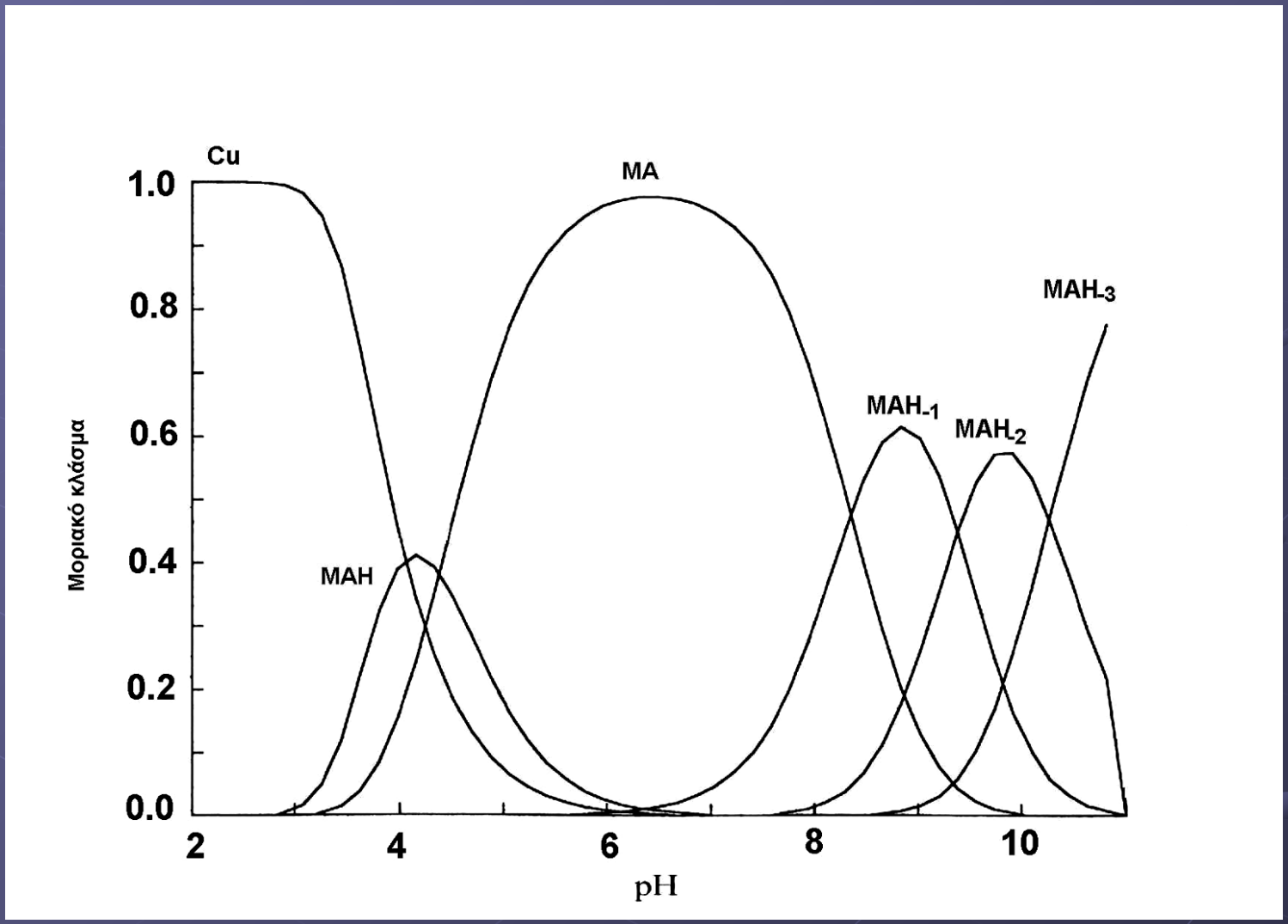
$$pM + qH + rL \leftrightarrow M_pH_qL_r$$

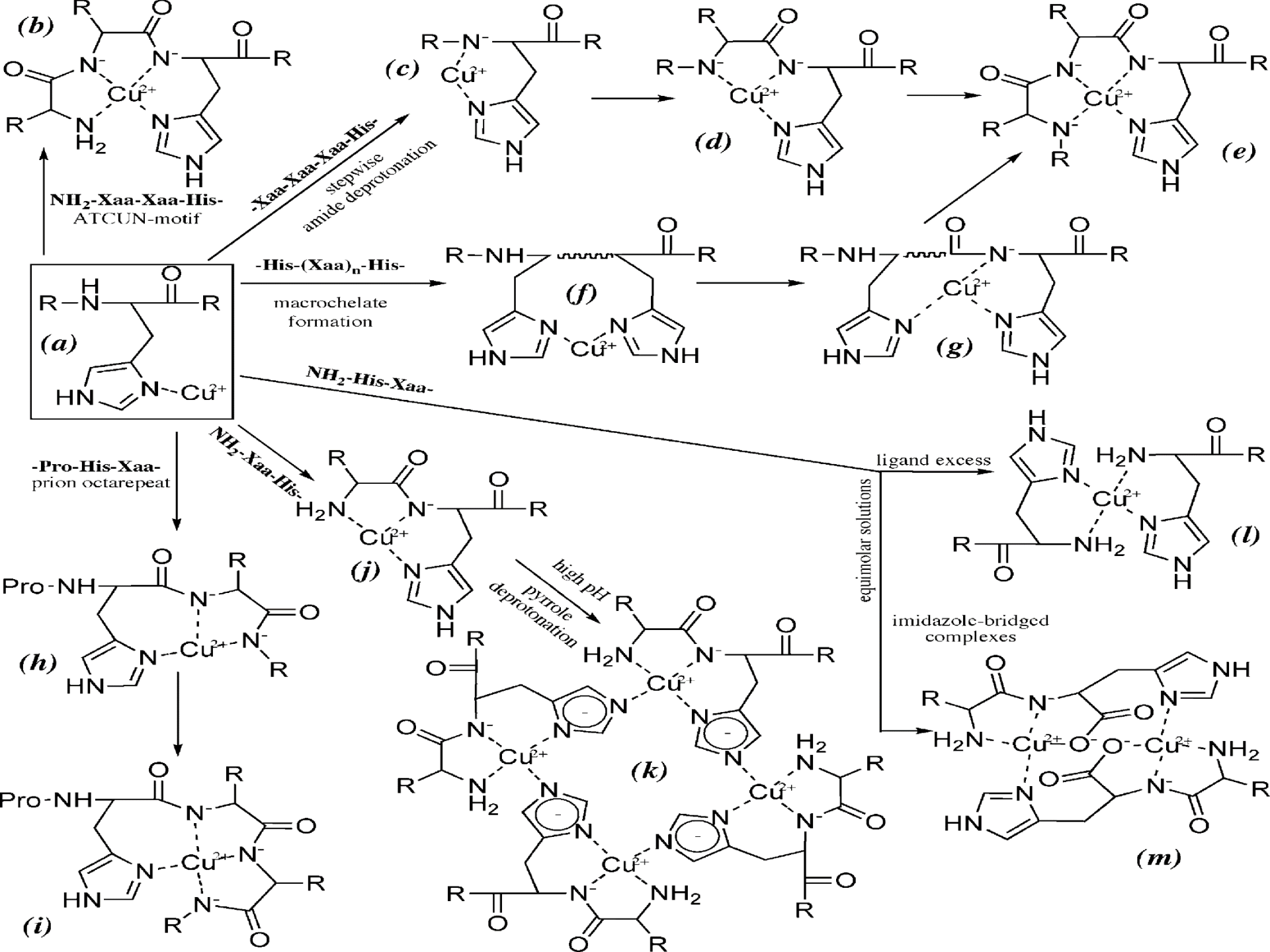
και

$$\beta_{p,q,r} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$$

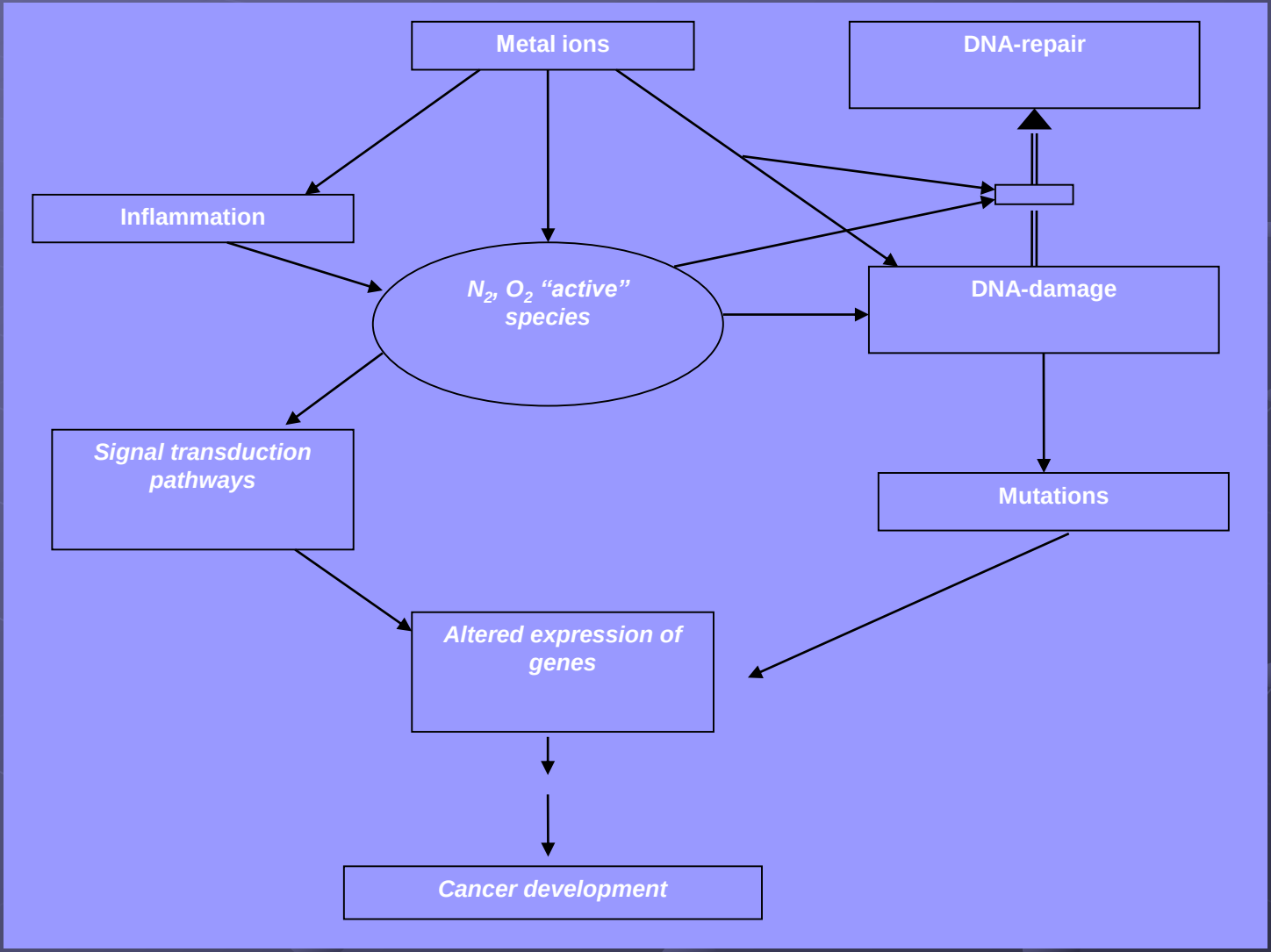
$$Pd^{2+}(2) > Cu^{2+}(4) > Ni^{2+}(8) > Co^{2+}(10)$$

Σύντομη επανάληψη Α’ διάλεξης....




(b)
NH2-Xaa-Xaa-His-
ATCUN-motif
-Xaa-Xaa-Xaa-His-
stepwise
amide deprotonation
(c)
(d)
(e)
(a)
-His-(Xaa)n-His-
macrochelate
formation
(f)
(g)
NH2-His-Xaa-
-Pro-His-Xaa-
prion octarepeat
NH2-Xaa-His-
(j)
high pH
pyrrole
deprotonation
(k)
ligand excess
equimolar solutions
(l)
imidazole-bridged
complexes
(m)
(h)
(i)

METAL IONS INDUCED TOXICITY-CARCINOGENESIS



(Galaris et al, Cr.Rev.Onc, 2002)

DNA DAMAGE CAUSED BY METAL IONS

NEOPLASTIC TRANSFORMATION OF CELLS RESULTS FROM AN ALTERATION IN THE GENETIC CODE

THUS ANY MOLECULE THAT CAN BIND WITH CONSTITUENTS OF THE CELL NUCLEI (DNA, PROTEINS) MAY AFFECT THE GENETIC CODE CAUSING CANCER

METAL IONS

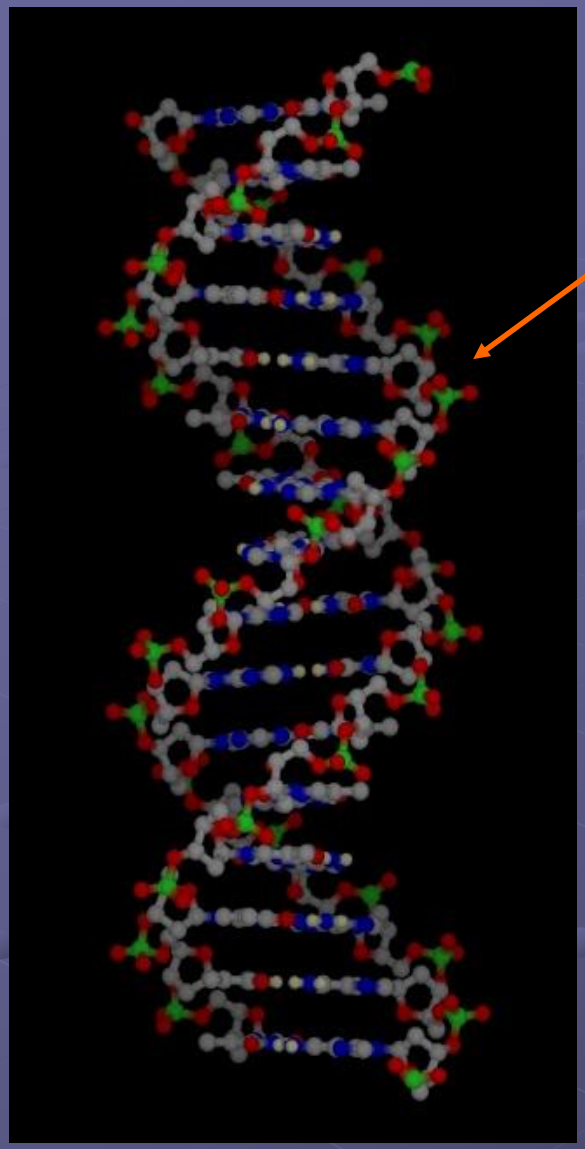
STRAND SCISSION

DEPURINATION

CROSS-LINKING

DNA BASE MODIFICATIONS

K. S. Kasprzak. *Oxidative DNA damage in metal-induced carcinogenesis. In: Toxicology of metals.* L. W. Chang., Ed. vol 18 (1996)



Cu(II)

Ni(II)

THE BINDING OF METAL IONS MAY GENERATE GENOTOXICITY

THE INITIALLY METAL-BINDING TO DNA MAY NOT BE EXCLUSIVELY RESPONSIBLE FOR THE ENTIRE WIDTH OF DAMAGE OBSERVED FOR DNA

SEVERAL METAL IONS ARE ABLE TO ACTIVATE O_2 OR H_2O_2 PRODUCING ACTIVE OXYGEN SPECIES WHICH MAY DAMAGE THE CONSTITUENTS OF THE CELL NUCLEI

THE MOST IMPORTANT MECHANISM OF OXYGEN ACTIVATION BY TRANSITION METALS INVOLVE

FENTON/HABER-WEISS REACTIONS

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + {}^{\bullet}OH$$

$$M^{(n+1)+} + O_2^{-\cdot} \rightarrow M^{n+} + O_2$$

K. S. Kasprzak. *Oxidative DNA damage in metal-induced carcinogenesis. In: Toxicology of metals*. L. W. Chang., Ed. vol 18 (1996)

Cu(II), Ni(II) toxicity

induces double strand breaks on DNA

- Cu(II)

  is highly redox active (Cu(II)/Cu(I))

  produces relatively low, but measurable levels of reactive oxygen species (ROS) in cells

  DNA single-strand scission

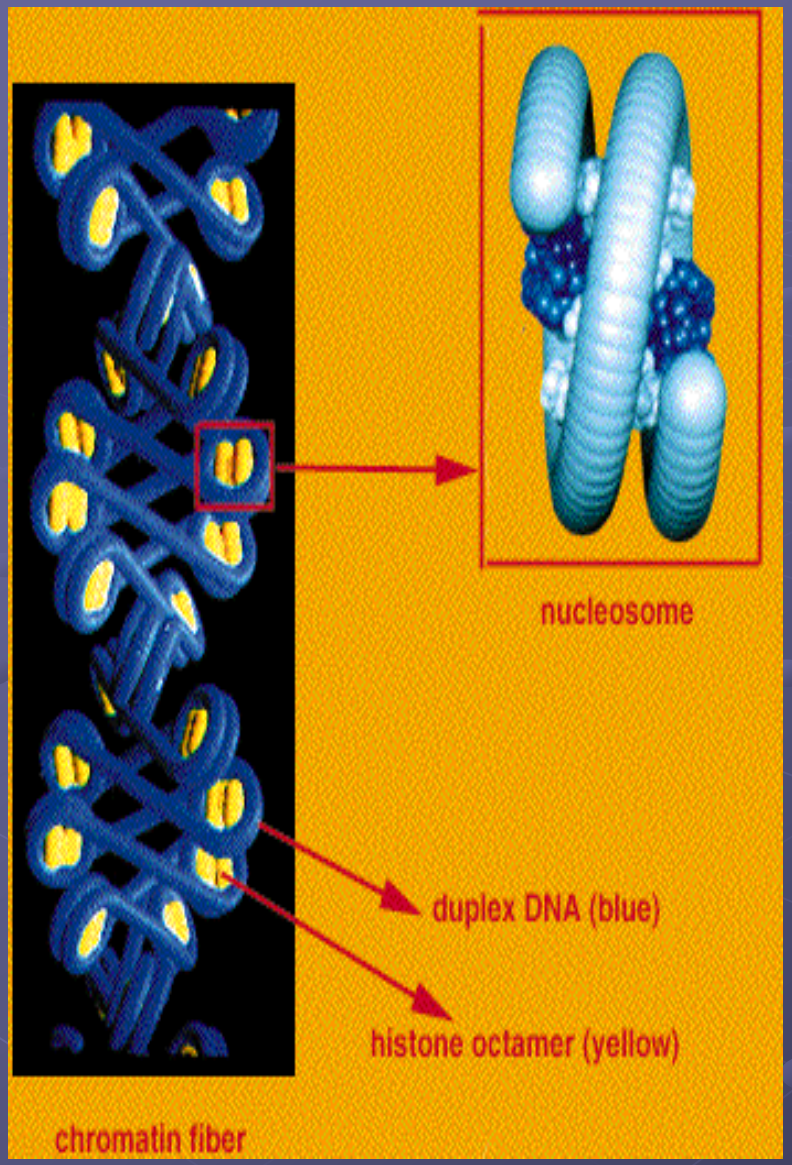
  binds weakly to DNA and requires the presence of proper chelation to become reactive

- Ni(II)

  the redox couple Ni(III)/Ni(II) is only possible when the metal ion is coordinated with some natural ligands, mostly peptides and proteins.

  In the presence of O_2 or H_2O_2 generate not only hydroxyl, but also other oxygen-, carbon-, and perhaps, sulfur-centered radicals originated from the ligand, all able to attack the ligand and other molecules

HISTONES



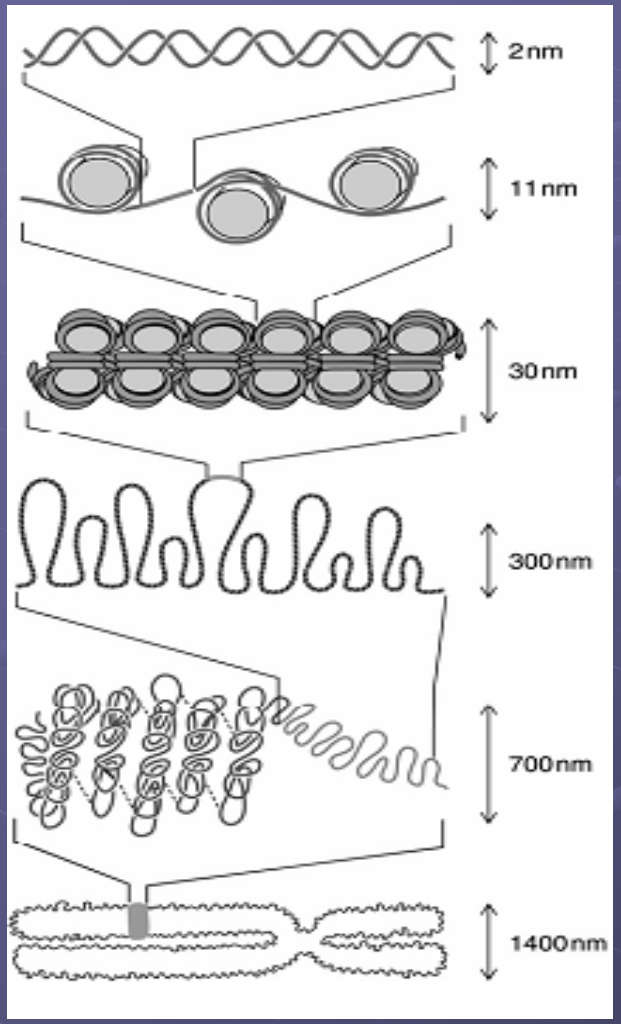
THESE ARE HIGHLY BASIC PROTEINS THAT PROVIDE SCAFFOLD FOR DNA DOUBLE HELIX IN THE CELL NUCLEUS

DNA IS WRAPPED AROUND THEM FORMING THE ***NUCLEOSOMES***

THE REPEATED ***NUCLEOSOMES*** ARE ORGANIZED IN HIGHER ORDER STUCTURES FORMING THE ***CHROMATIN***

R. D. Kornberg. *Science*. 184 (1974) 868, R. D. Kornberg. *Annu. Rev. Biochem*. 46 (1977) 931

Η Έννοια της Χρωματίνης



Το DNA περιτυλίγεται γύρω από τα οκταμερή ιστονών, σχηματίζοντας τις "χάντρες", γνωστές ως νουκλεοσώματα

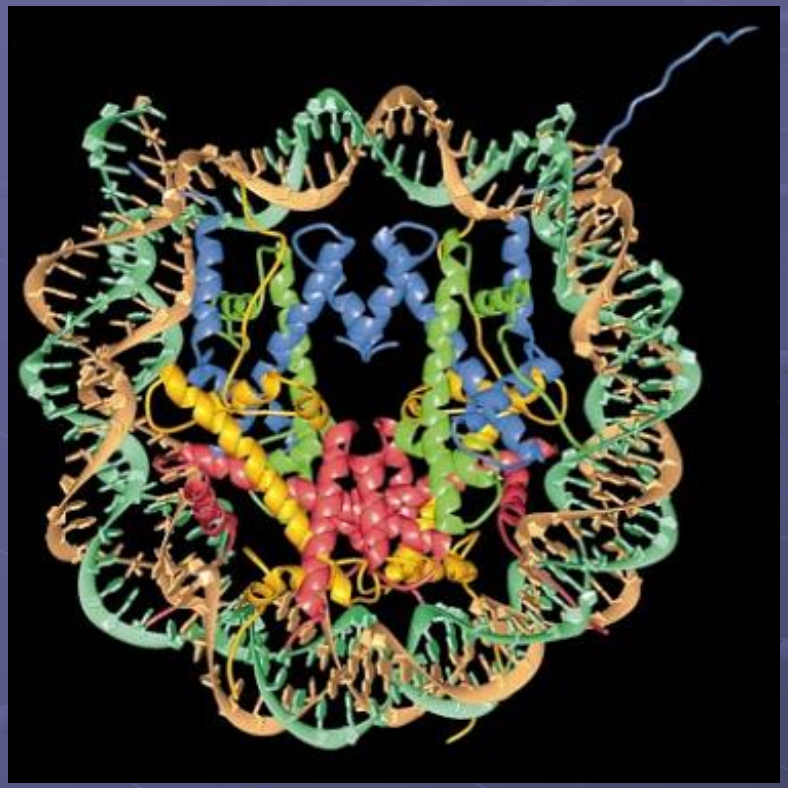
Τα νουκλεοσώματα πακετάρονται σχηματίζοντας τα ινίδια χρωματίνης

Τα ινίδια χρωματίνης αναδιπλώνονται για να σχηματίσουν θηλιές

Οι θηλιές αναδιπλώνονται για να σχηματίσουν το χρωμόσωμα

Μεταφασικό χρωμόσωμα

Νουκλεόσωμα



- 146 ζεύγη βάσεων DNA περιελίσσονται γύρω από τον πρωτεϊνικό πυρήνα
- Ο πρωτεϊνικός πυρήνας του νουκλεοσώματος είναι το ιστονικό οκταμερές και αποτελείται από 2 ζεύγη κάθε μιας από τις πυρηνικές ιστόνες
- Υπάρχουν 5 διαφορετικοί τύποι ιστονών : H1, H3, H4, H2A, H2B
- Η ιστόνη H1 αναφέρεται ως συνδετική ιστόνη, επειδή σχετίζεται με εκείνο το τμήμα του DNA που ενώνει τα νουκλεοσώματα
- Οι ιστόνες H3, H4, H2A και H2B αναφέρονται ως πυρηνικές ιστόνες

Δομικές Περιοχές Πυρηνικών Ιστονών

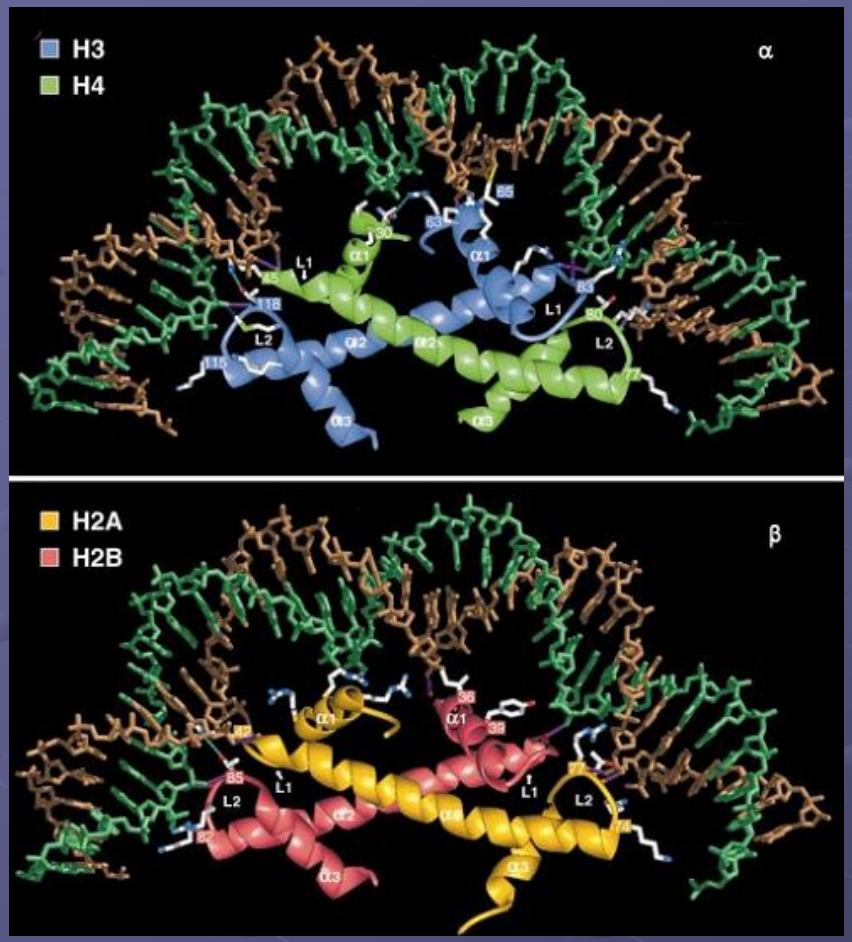
Οι πυρηνικές ιστόνες περιέχουν 3 διαφορετικούς τύπους δομικών περιοχών, ανάλογα με τα μοτίβα δευτεροταγούς δομής που υιοθετούν

- Μία κεντρική περιοχή περίπου 70 αμινοξέων, αποτελούμενη από 3 α-έλικες που συνδέονται με βραχείες θηλιές, γνωστή ως ιστονική πτύχωση (histone-fold domain).

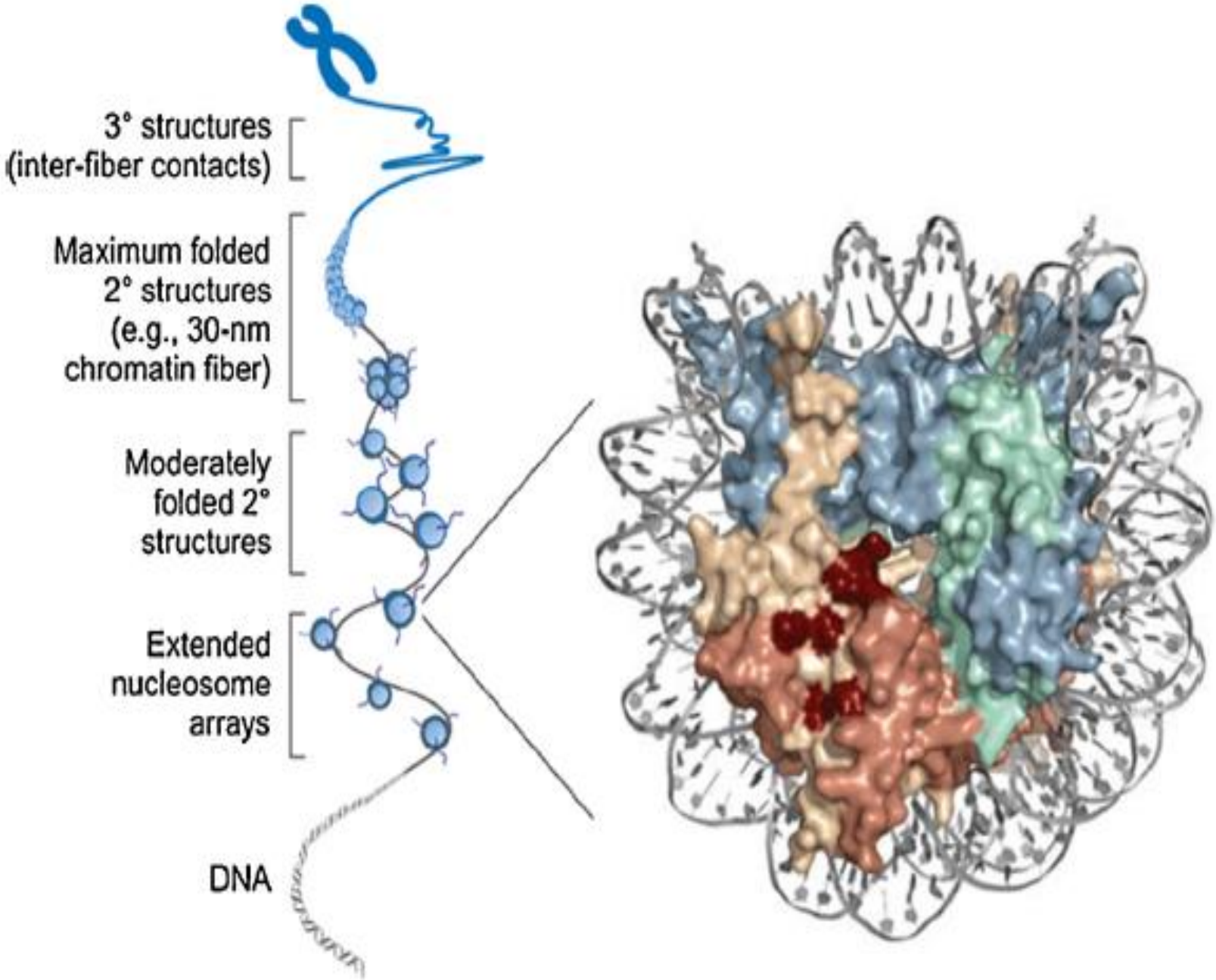
- Τις προεκτάσεις της ιστονικής πτύχωσης (histone-fold extensions). Αυτές είναι δομικές περιοχές, εξωτερικά της ιστονικής πτύχωσης και είναι υπεύθυνες για τις πρωτεϊνικές αλληλεπιδράσεις εντός του οκταμερούς, ενώ συνεισφέρουν και στη δέσμευση του DNA

- Τις «ουρές» (histone tails). Τα N-τελικά άκρα των πυρηνικών ιστονών είναι τυχαία περιελισσόμενες δομές (random coil), που χαρακτηρίζονται από μεγάλη ευκαμψία.

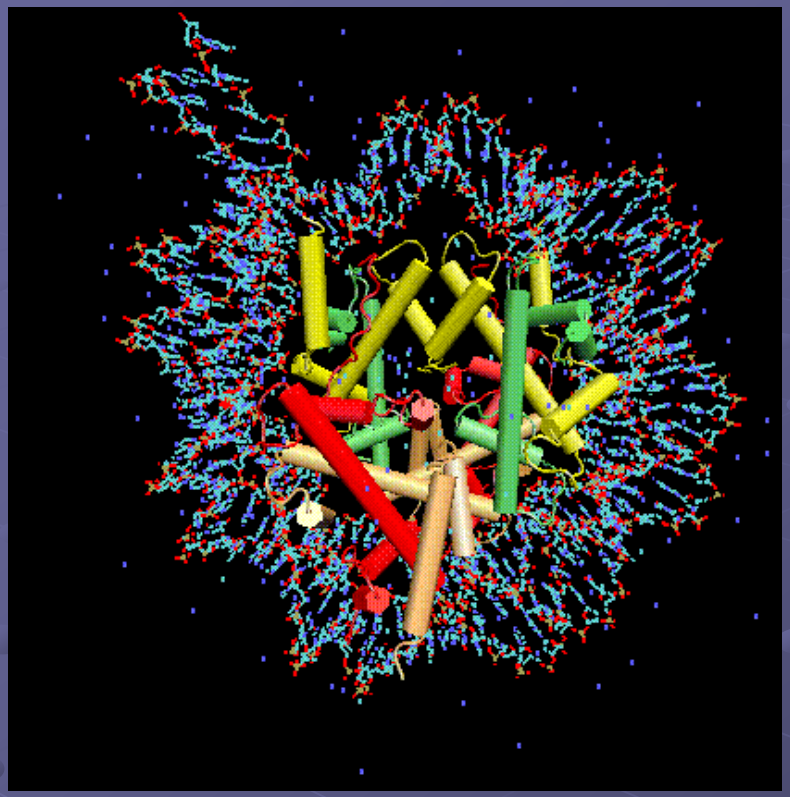
Ετεροδιμερή Ζεύγη Πυρηνικών Ιστονών



- Οι ιστονικές πτυχώσεις συνδυάζονται για να σχηματίσουν ετεροδιμερικά ζεύγη του τύπου : H2A/H2B και H3/H4
- Σε κάθε ζεύγος οι περιοχές α1-L1-α2-L2-α3 αλληλεπιδρούν με έναν αντιπαράλληλο προσανατολισμό, κυρίως μέσω υδροφοβικών επαφών των α1, α2 και α3 ελικών
- Η εκλεκτικότητα του διμερισμού είναι πολύ μεγάλη και συνδέεται με την ύπαρξη σημαντικών διαφορών στην διεπιφάνεια επαφής των πραγματικά σχηματιζόμενων ετεροδιμερών και άλλων εναλλακτικών μη υφιστάμενων ζευγών.


3° structures
(inter-fiber contacts)
Maximum folded
2° structures
(e.g., 30-nm
chromatin fiber)
Moderately
folded 2°
structures
Extended
nucleosome
arrays
DNA

MAJOR TYPES OF HISTONES

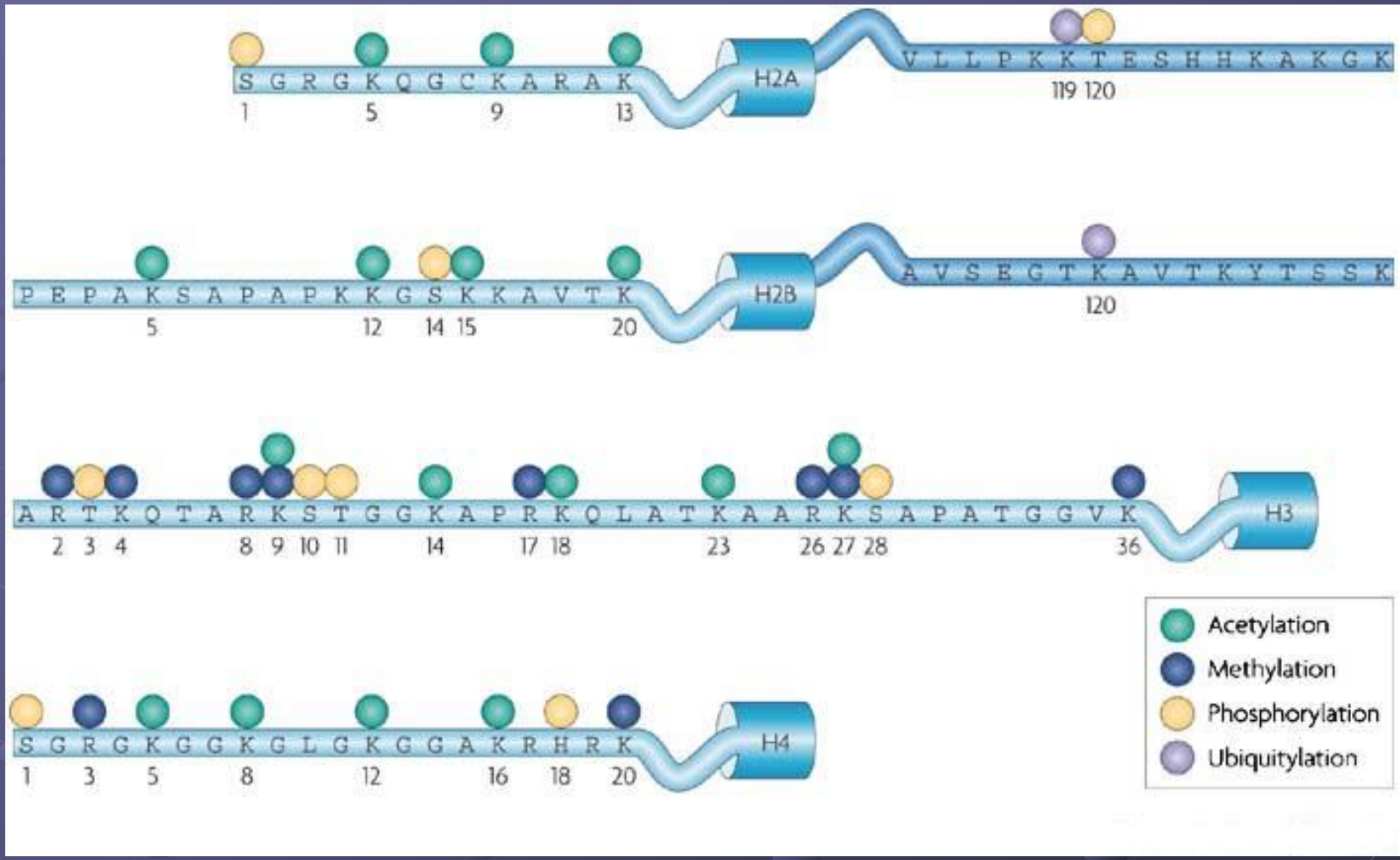


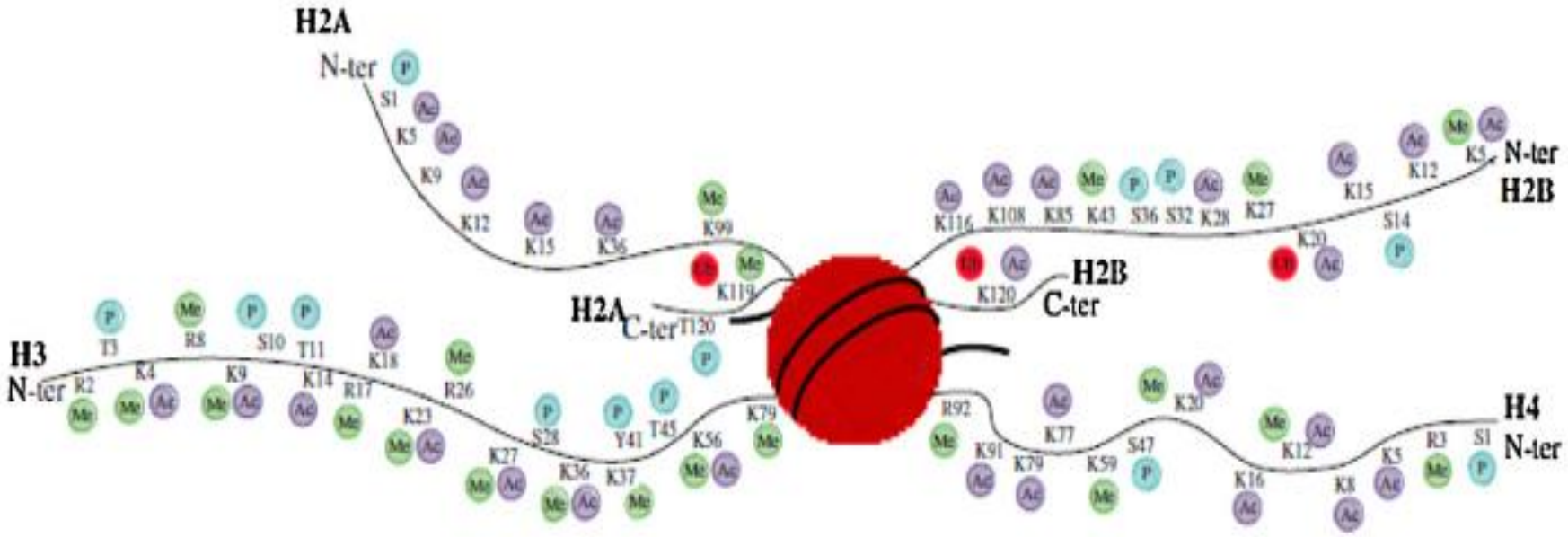
Histones	Amino acids	Mass	Location inside the nucleosome
H1	215	21.000	Linker DNA
H2A	129	14.500	Nuclei
H2B	125	13.700	Nuclei
H3	135	15.300	Nuclei
H4	102	11.300	Nuclei

THE C- AND N- TERMINAL TAILS OF HISTONES ARE THE MAJOR BUT NOT THE ONLY INTERACTION SITES WITH DNA

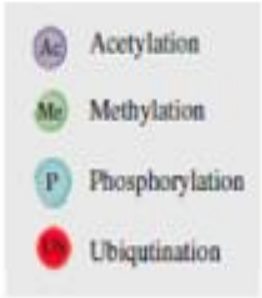
K. Luger, et al. *Nature*. 389 (1997) 251, A. L. Lehninger, et al. Cox. *Principles of Biochemistry.* (1998) 642

METAL IONS INDUCED TOXICITY-CARCINOGENESIS





Chromatin Modification	Residues modified	Function regulated
Acetylation	Lysine	Transcription, DNA repair, replication and condensation
Methylation (Lysine)	Lysine me1, me2, me3	Transcription, DNA repair
Methylation (Arginine)	Arginine-me1, Arginine-me2a Arginine-me2s	Transcription
Phosphorylation	Serine, Threonine, Tyrosine	Transcription, DNA repair and condensation
Ubiqutination	Lysine	Transcription, DNA repair
Sumoylation	Lysine	Transcription
ADP ribosylation	Glutamic	Transcription
Deimination	Arginine	Transcription
Proline isomerization	P-cis, P-trans	Transcription



Πεπτιδικά μοντέλα ιστονών

H4: Ac-$A_{15}K_{16}R_{17}H_{18}R_{19}K_{20}$-Am

Ac-S_1GRGKGGKGLGKGGAKRHRKVL_{22}-Am

Ac-AK(Ac)RHRK(Ac)V-Am

Ac-GK(Ac)GGAK(Ac)RHRK(Ac)V-Am

Ac-T_{71}YTEHA_{76}-Am

H2A: Ac-T_{120}ESHHK$_{125}$-Am (and variants)

H_2N-SHHK-Am and the variant H_2N-SAHK-Am

H2B: Ac-E_{102}LAKHA_{107}-Am

Ac-L_{80}AHYNK_{85}-Am

Ac-P_1EPAKSAPAPKKGSKKAVTKAQKKDGKKRKR$_{31}$-Am

Ac-S_{32}RKESYSVYVYKVLKQVHPDTGISSKAMGIM$_{62}$-Am

Ac-N_{63}SFVNDIFERIAGEASRLAHYNKRSTITSRE_{93}-Am

Ac-I_{94}QTAVRLLLPGELAKHAVSEGTKAVTKYTSSK$_{125}$-Am

PART A: Studies in small peptide histone models

C-terminal of histone H2A 121-124

Ac-TESHHK-am and variants

Histones H2B and H4 (fold domains, 71-76 and 80-85

Ac-TYTEHAK-am

Ac-LAHYNK-am

Ιστόνη H1

SETAPAAPAAPAPAEKTPVKKKARKSAGAAKRKASGPPVSELITKAVAASK
ERSGVSLAALKKALAAAGYDVEKNNSRIKLGLKSLVSKGTVLQTKGTGASS
FKLNKKAASGEAKPKAKKAGAAKAKKPAGAAKKPKKATGAATPKKSAKT
PKKAKKPAAAAGAKKAKSPKKAKAAKPKKAPKSPAKAKAVKPKAAKPK
TAKPKAAKPKKAAAKKK

Ιστόνη H2A (τύπος H2A.1)

[1]SGRGKQGGKAPAKAKTRSSRAGLQFPVGRVHRLLRKGNYSERVGAGAPV
[50]YLAAVLEYLTAEILELAGNAARDNKKTRIIPRHLQLAIRNDEELNKLLGRVTIA
[104]QGGVLPNIQAVLLPKKTESHHKAKGK

Ιστόνη H2B (τύπος H2B.1(a))

[1]PEPAKSAPAPKKGSKKAVTKAQKKDGKKRKRSRKESYSVYVYKVLKQVHP
[51]DTGISSKAMGIMNSFVNDIFERIAGEASRLAHYNKRSTITSREIQTAVRLLLP
[104]GELAKHAVSEGTKAVTKYTSSK

Ιστόνη H3 (κύριος τύπος)

[1]ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALRE
[51]IRRYQKSTELLIRKLPFQRLVREIAQDFKTDLRFQSSAVMALQEACEAYLVG
[103]LFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

Ιστόνη H4

[1]SGRGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLYE
[53]ETRGVLKVFLENVIRDAVTYTEHAKRKTVTAMDVVYALKRQGRTLYGFY
[102]Y

STUDIED SEQUENCE

THE SEQUENCE **-GluSerHisHis-** (-ESHH- AMINO ACIDS 121-124), OF THE C-TERMINAL TAIL OF **HISTONE H2A**

-TESHHK- $\xrightarrow{\text{Ni(II)}}$ **Ni(II)-SHHK-**

W. Bal, et al. *Chem. Res. Toxicol.* 11 (1998) 1014

SYNTHESIZED

-TESHHK-

-TASHHK-

-TEAHHK-

-TESAHK-

-TESHAK-

SHHK-

SAHK-

1. COMPARISON OF THE EFFECT OF Glu, Ser AND His SUBSTITUTION ON THE STABILITY OF THE COMPLEXES FORMED

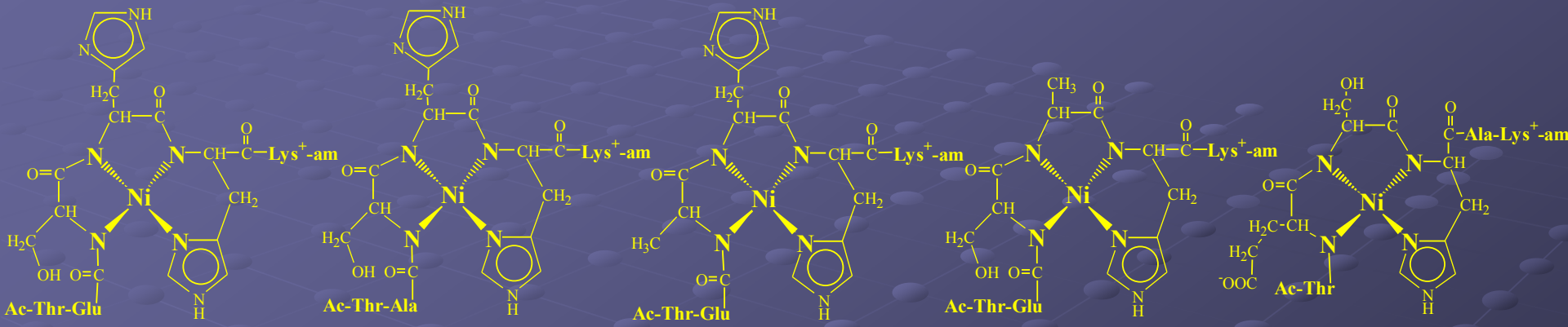
2. LOCATE THE AMINO ACID WHICH IS RESPONSIBLE FOR HYDROLYSIS

3. STUDY OF THE OXIDATIVE ABILITY OF Cu(II) / -TESHHK- COMPLEX AGAINST DNA BASES

4. BETTER CHARACTERIZATION OF THE HYDROLYSIS PRODUCTS AND UNDERSTANDING OF THE HYDROLYSIS MECHANISM

HYDROLYTIC CLEAVAGE OF THE HEXAPEPTIDES

-TESHHK- **-TASHHK-** **-TEAHHK-** **-TESAHK-** **-TESHAK-**



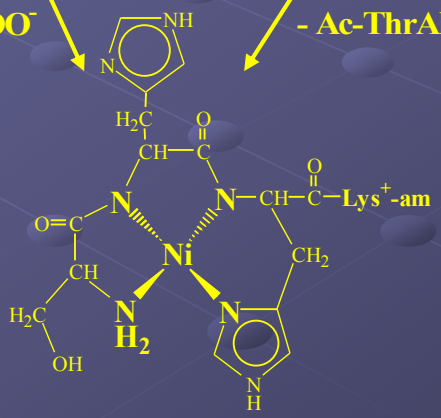
pH > 7.4 — - Ac-ThrGluCOO⁻

pH > 7.4 — - Ac-ThrAlaCOO⁻

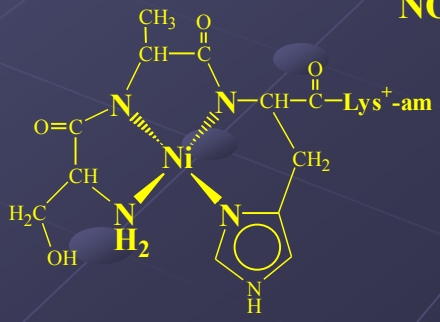
pH > 7.4 — NO CLEAVAGE

pH > 7.4 — - Ac-ThrGluCOO⁻

pH > 7.4 — NO CLEAVAGE



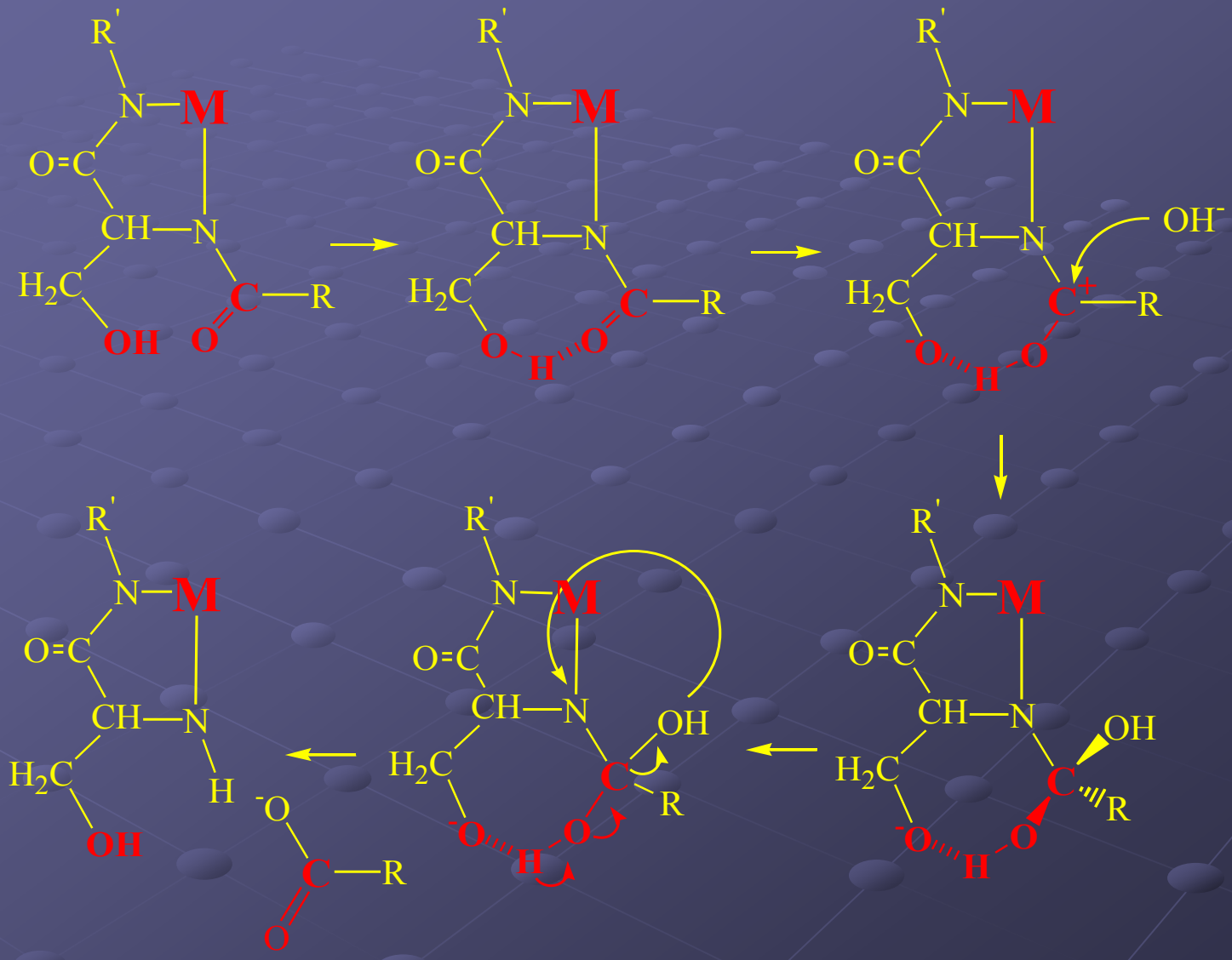
SHHK-



SAHK-

M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, et al.

PROPOSED MECHANISM OF THE HYDROLYSIS OF THE HEXAPEPTIDES IN THE PRESENCE OF Ni(II) OR Cu(II) IONS



M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, et al. *J. Chem. Soc., Dalton Trans*. (2002) 4296

OXIDATION PROPERTIES OF Cu(II) / -TESHHK- COMPLEX

1. dG WAS USED AS A TARGET / REPORTER MOLECULE OF OXIDATIVE DAMAGE

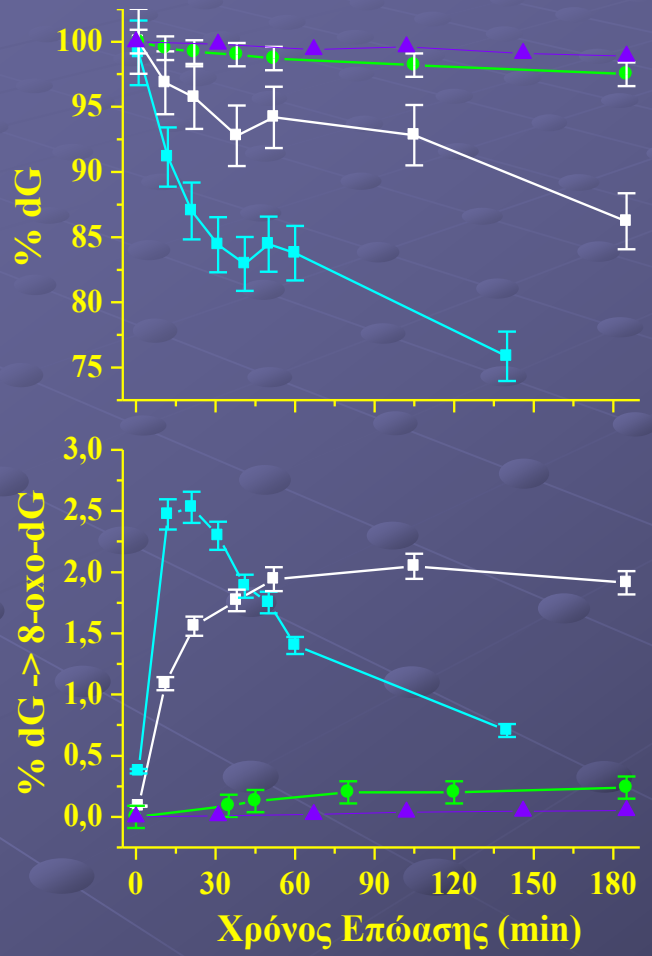
- In this system, unlike with DNA as a target, the yield of the oxidation product, 8-oxodG, is high and its quantification can be accomplished quickly and precisely by HPLC with a standard UVdetector

- The shortness of the hexapeptide model -TESHHK- would not allow the reproduction of the protein-DNA interaction occurring in nucleosome

2. H_2O_2 WAS USED AS OXIDATIVE FACTOR

M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, et al. *Chem. Res. Toxicol*. 14 (2001) 1177

THE COMPLEX $CuH_{-1}L$ WITH -TESHHK- EFFICIENTLY PROMOTED OXIDATION OF dG, WITH A TRANSIENT FORMATION OF SUBSTANTIAL AMOUNTS OF 8-oxo-dG IN PHYSIOLOGICAL CONDITIONS (pH = 7.4 and T = 37 ºC)



1. **ONLY** THE COMBINATION OF Cu(II) / -TESHHK- AND H_2O_2 RESULTED IN THE SUBSTANTIAL dG OXIDATION

2. NO OXIDATIVE DAMAGE WAS DETECTED IN THE ABSENCE OF H_2O_2

3. NARROW AMOUNTS OF 8-oxo-dG WAS GENERATED IN THE PRESENCE OF Cu(II) ALONE OR -TESHHK- ALONE

4. NO OXIDATIVE DAMAGE WAS DETECTED IN THE PRESENCE OF Cu(II) / SHHK- AND H_2O_2

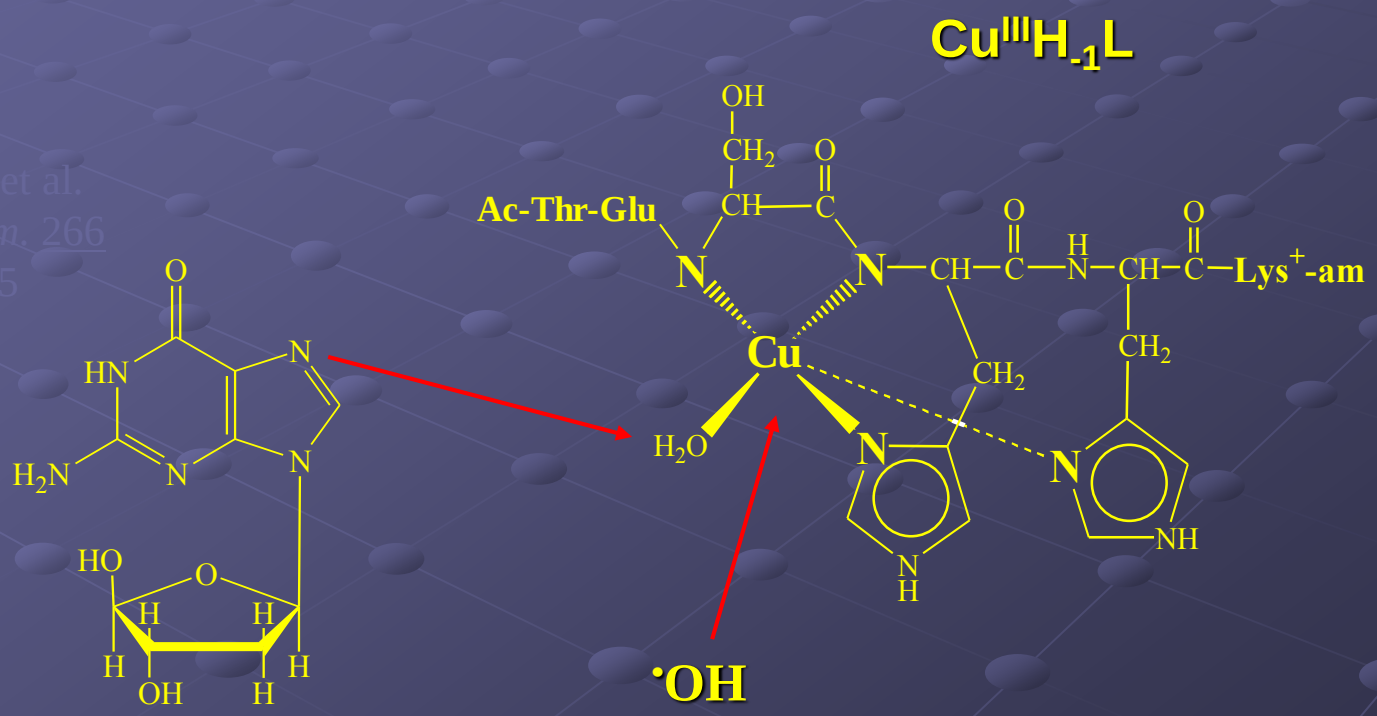
M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, et al. *Chem. Res. Toxicol.* 14 (2001) 1177

PROPOSED MECHANISM OF THE OXIDATIVE REACTION

$$Cu^{II}H_{-1}L + H_2O_2 \Leftrightarrow Cu^{III}H_{-1}L\text{-}^{\bullet}OH + OH^-$$

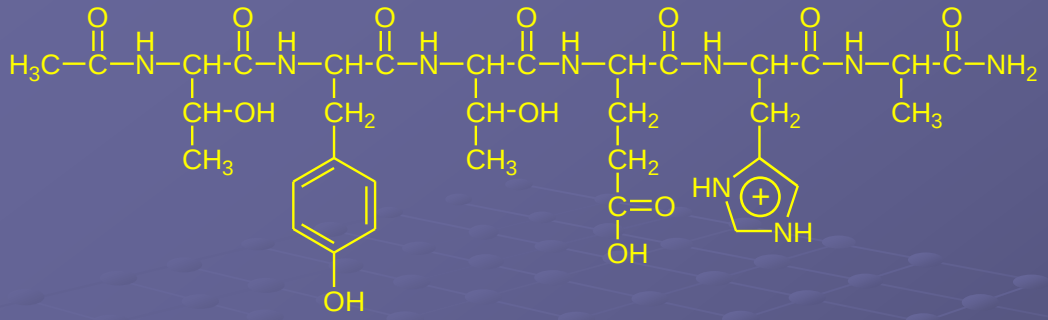
$$Cu^{III}H_{-1}L\text{-}OH + H_2O_2 \Leftrightarrow Cu^{II}H_{-1}L + O_2^{-\bullet} + H_2O + H^+$$

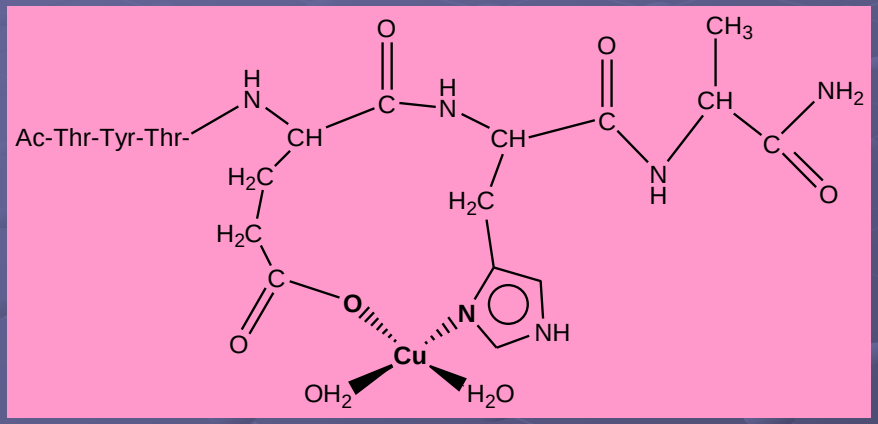
F. Kagawa, et al.
J. Biol. Chem. 266
(1991) 20175

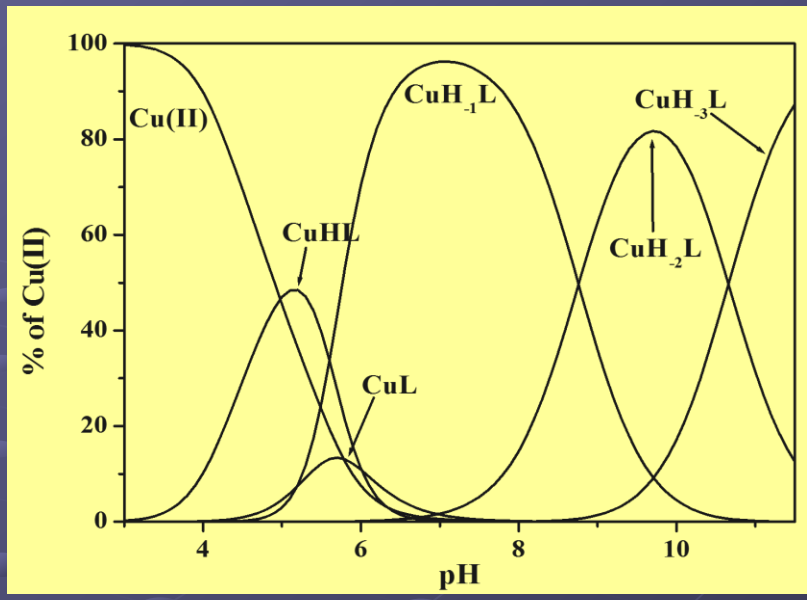


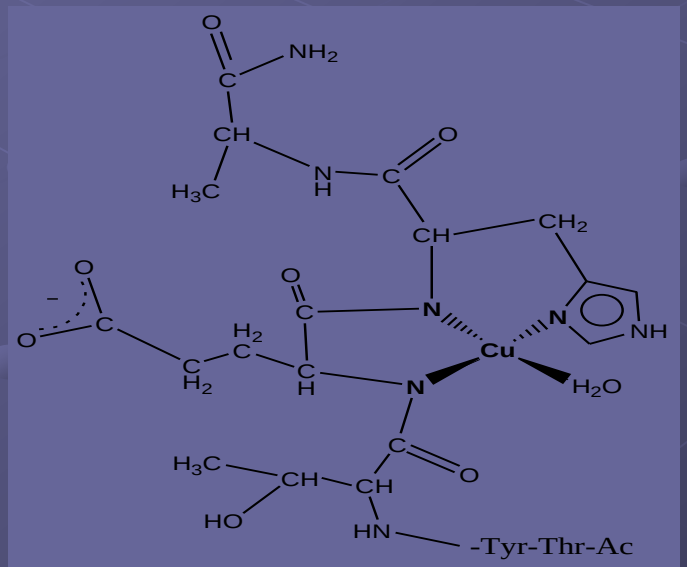
M. Mylonas, J. C. Plakatouras, N. Hadjiliadis, et al. *Chem. Res. Toxicol.* 14 (2001) 1177

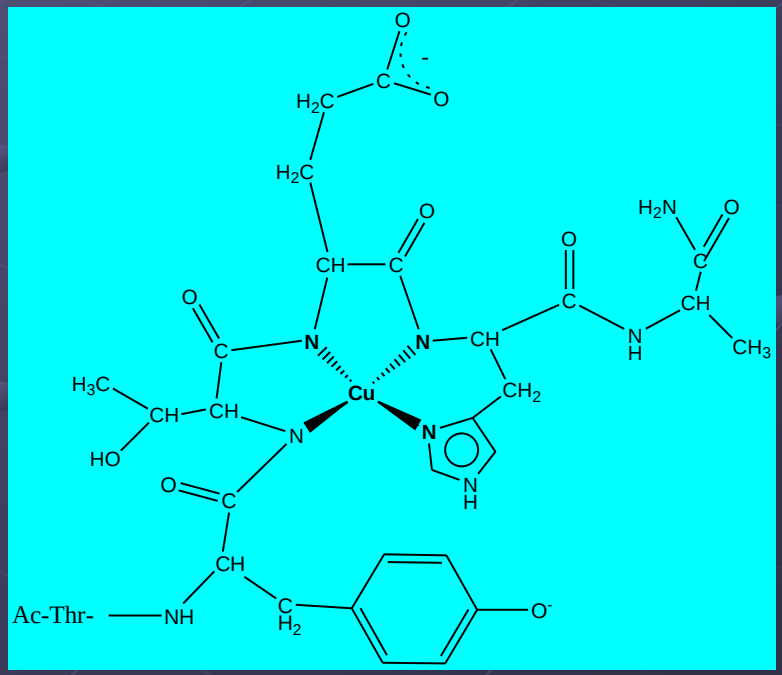
I. Ac-TYTEHA-am (Cu(II), Ni(II))

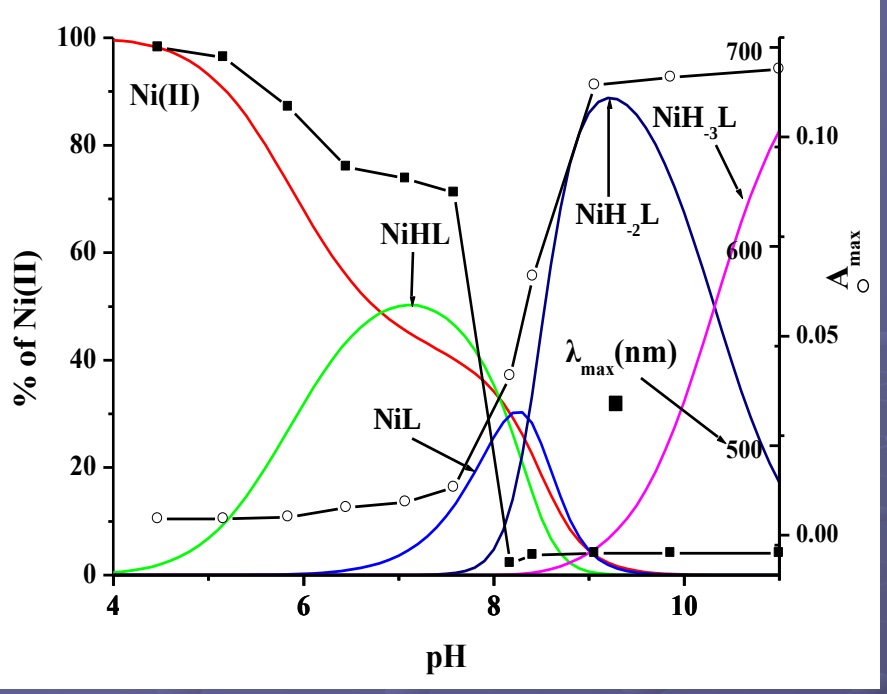


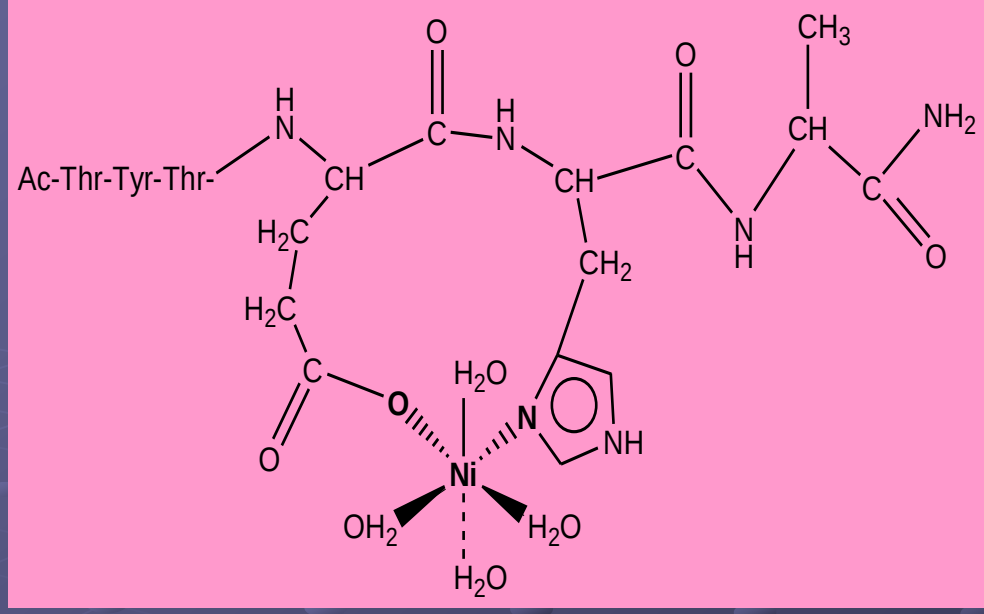


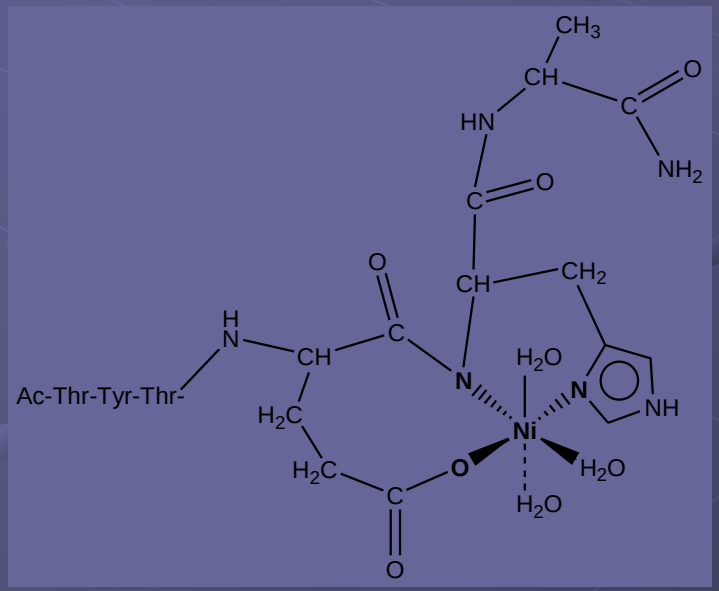


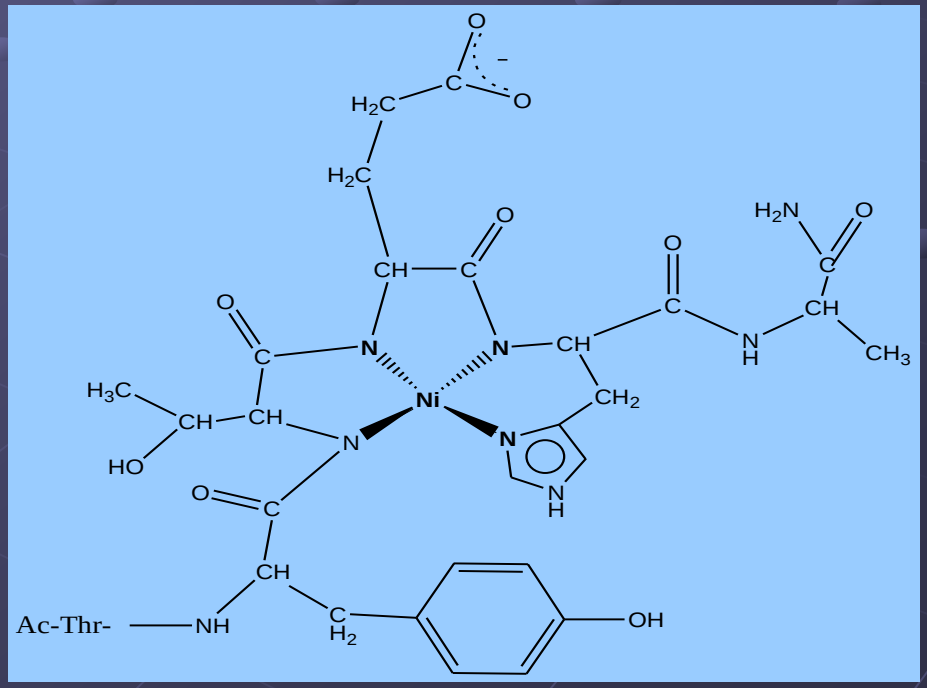




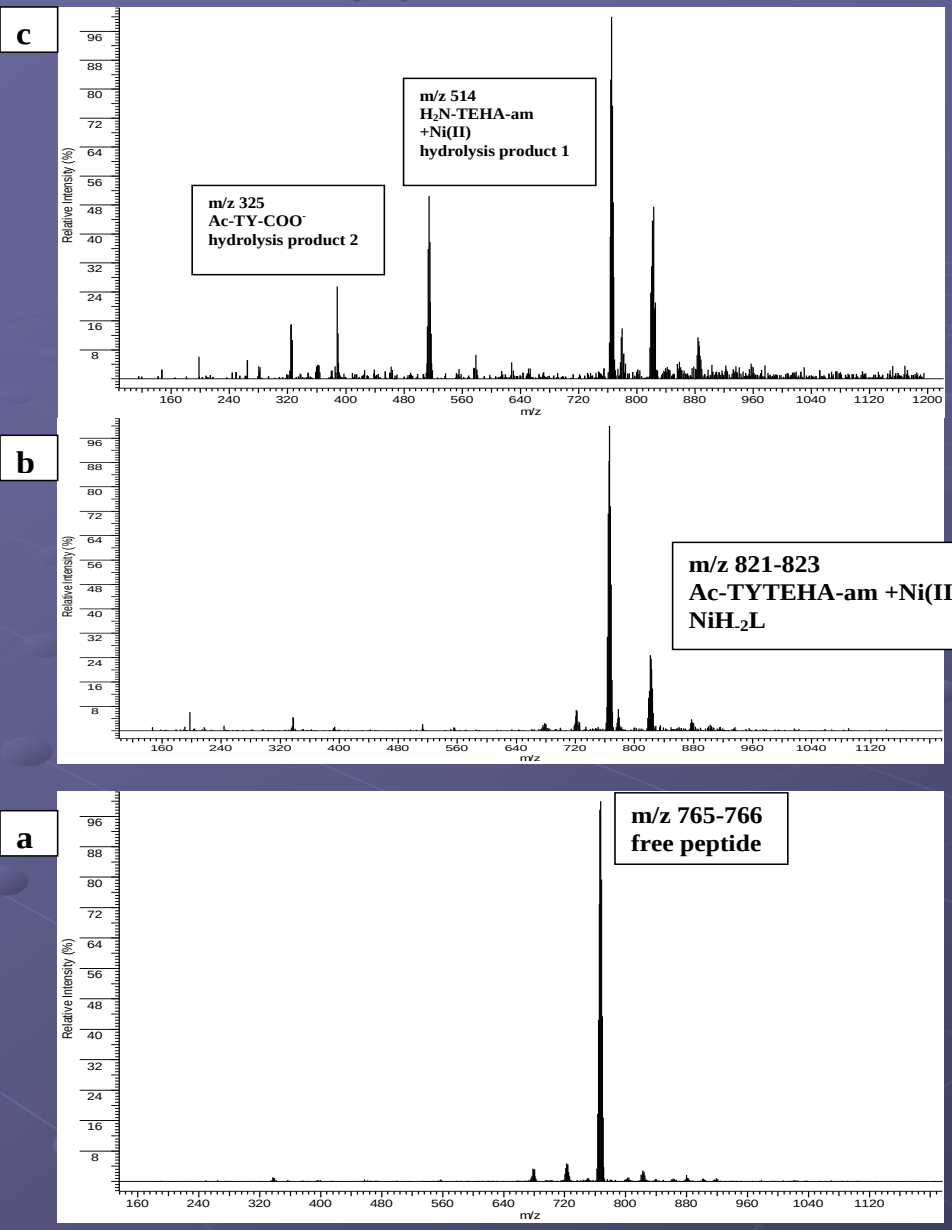

Ni(II)
NiHL
NiL
NiH_-2L
NiH_-3L
λmax(nm)
% of Ni(II)
A
pH
100
80
60
40
20
0
4
6
8
10
700
600
500
0.10
0.05
0.00

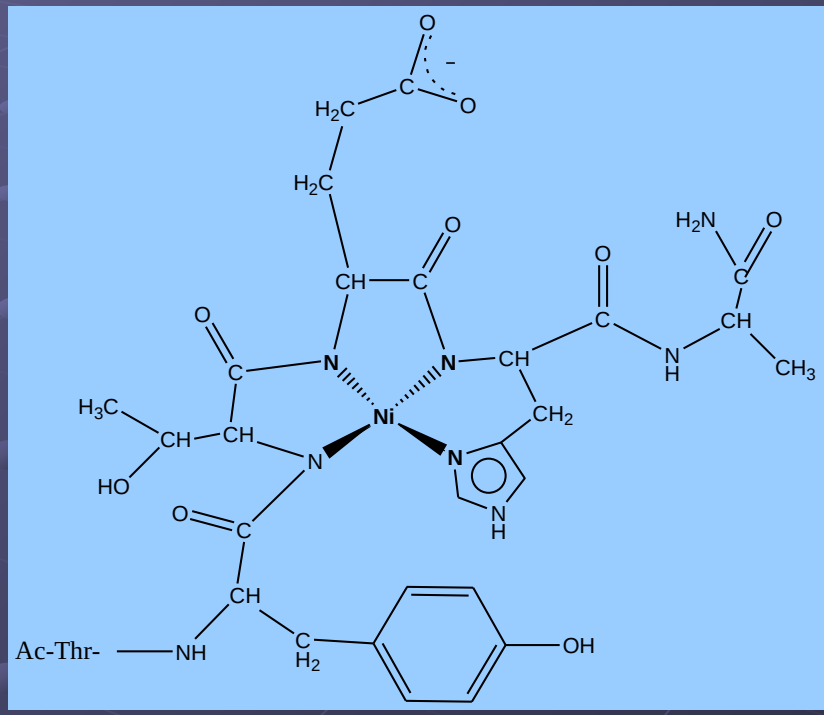

Ac-Thr-Tyr-Thr-
Ni
H2O
OH2
NH
NH2
CH3


Ac-Thr-Tyr-Thr-
Ni
H2O
NH
NH2
CH3


Ac-Thr-
Ni
NH
OH
H3C
HO
CH3
NH2

Ni(II)-TYTEHA interaction: Hydrolysis studies

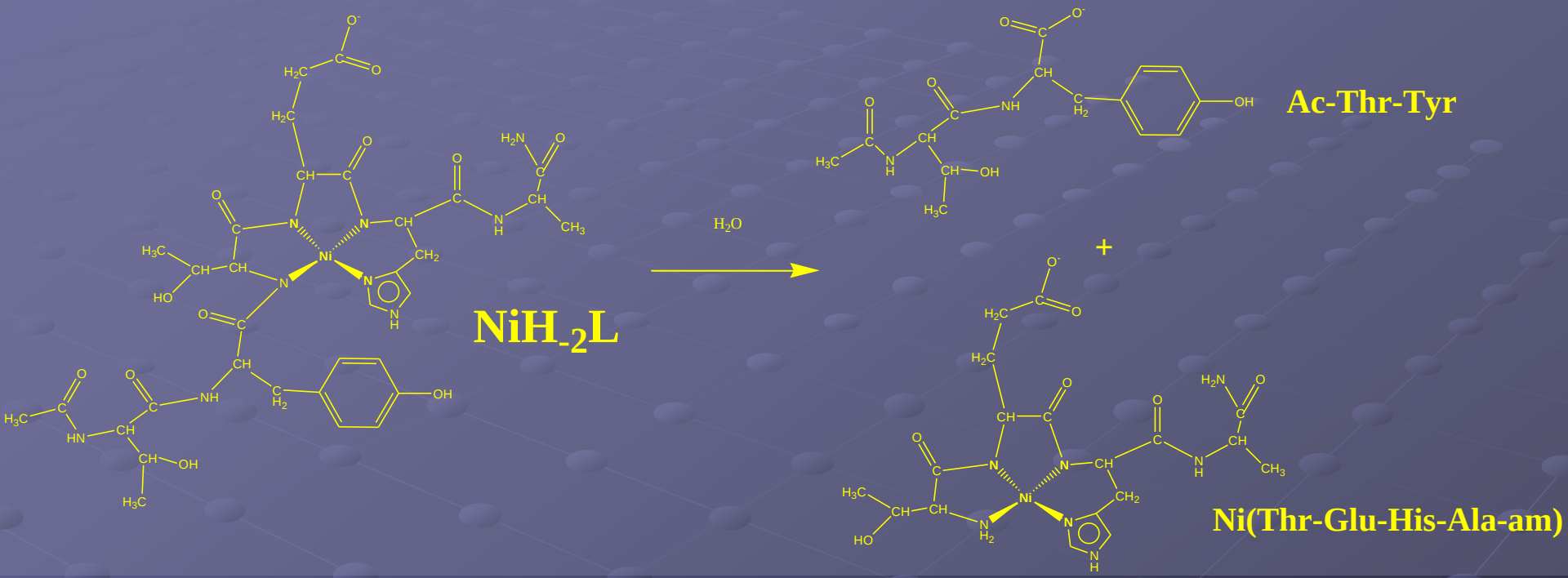




ESI-MS spectra series of (a) Ac-TYTEHA-am (b) Ac-TYTEHA-am + Ni(II) 1:1, 30 min of incubation at 37oC, (c) Ac-TYTEHA-am + Ni(II), 48h of incubation

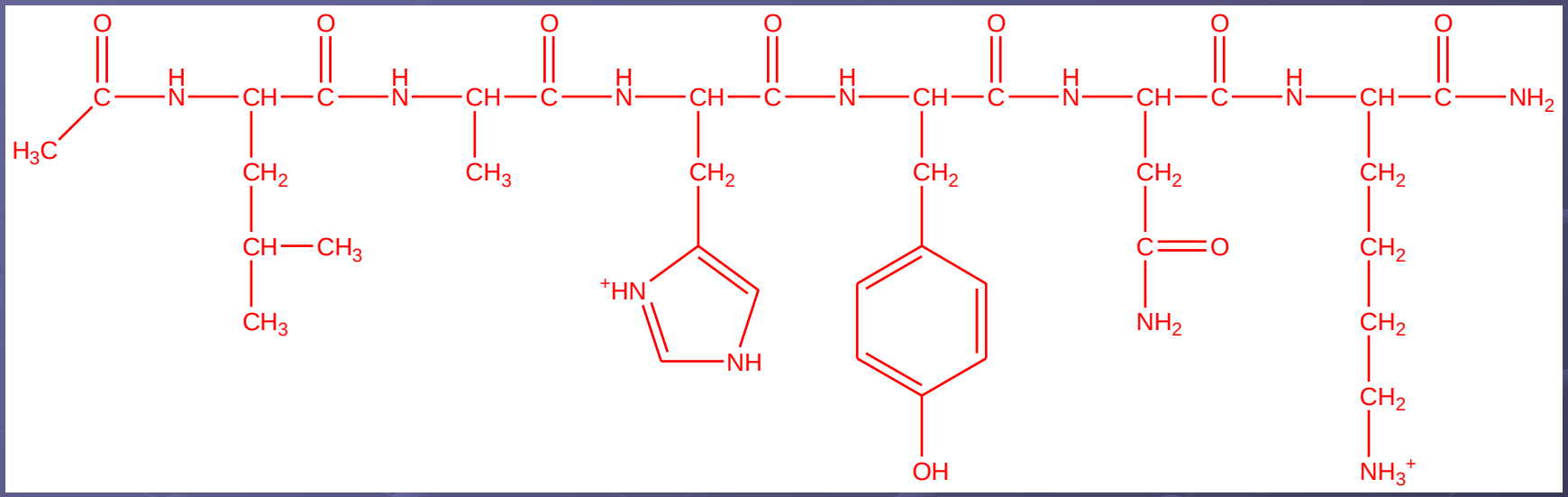
Ni(II)-TYTEHA interaction: Hydrolysis studies

- The hydrolysis reaction



The presence of Thr residue is important for hydrolytic cleavage!!

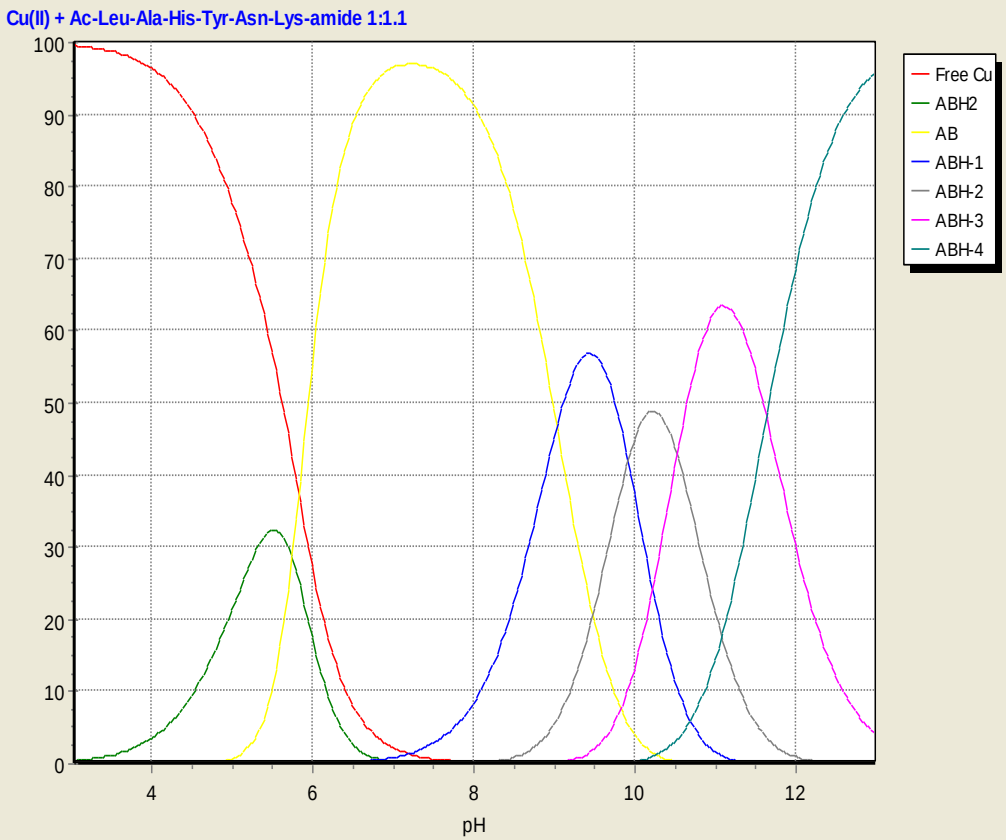
Cu(II)-LAHYNK- *interaction.*



Ionization constants of the peptide -LAHYNK-.

Species	***logβ***	***pK_a***	***Std.Dev***	***Group***
HL	10.53	10.53	±0.01	Lys
H_2L	20.05	9.52	±0.01	Tyr
H_3L	26.28	6.23	±0.01	His

Cu(II)-LAHYNK- *interaction.*



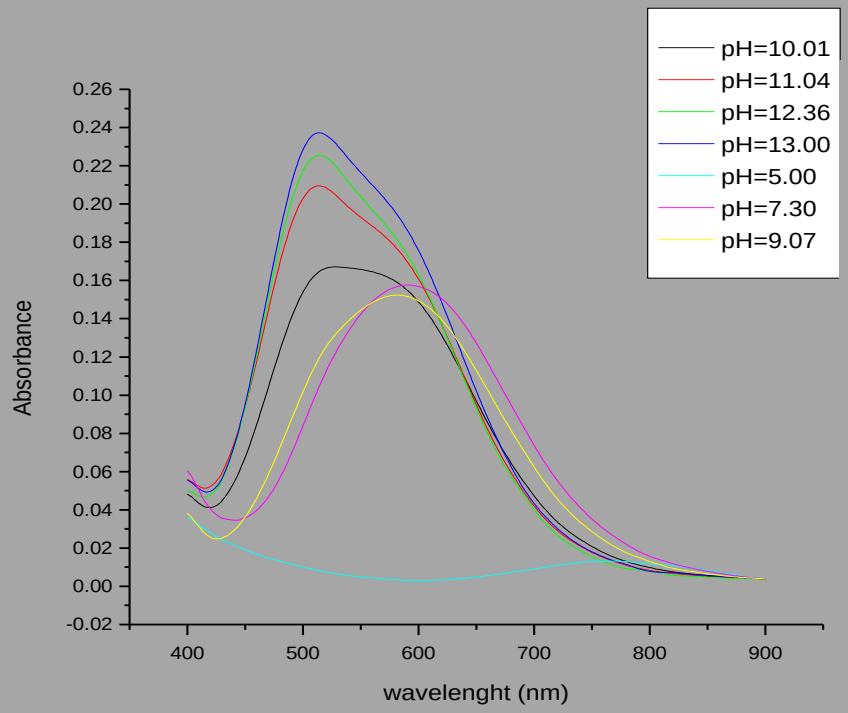
Species distribution diagram of the -LAHYNK- :Cu(II) system (1:1).

Stability constants of -LAHYNK- : *Cu(II) system (1:1).*

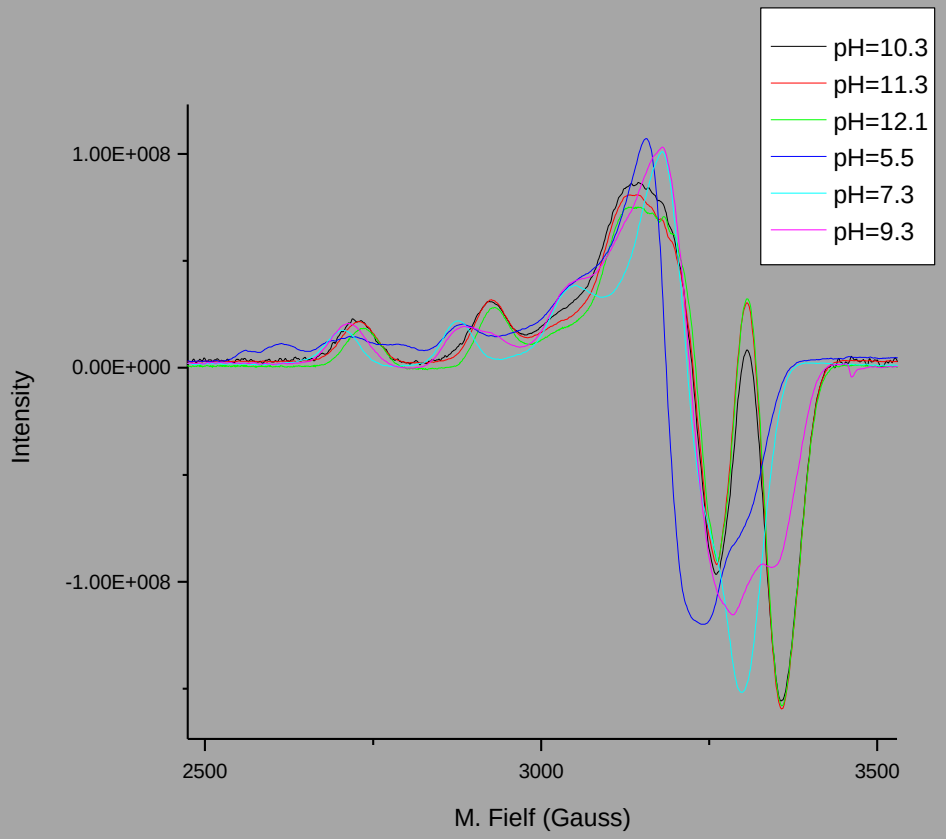
Species	***logβ***	***pK_a***
CuH_2L	23.55 (1)	
CuL	12.06 (1)	
$CuH_{-1}L$	3.02 (1)	9.04
$CuH_{-2}L$	-6.89 (1)	9.91
$CuH_{-3}L$	-17.42 (1)	10.53
$CuH_{-4}L$	-29.07 (2)	11.65

Spectroscopic parameters of the system -LAHYNK- :Cu(II)

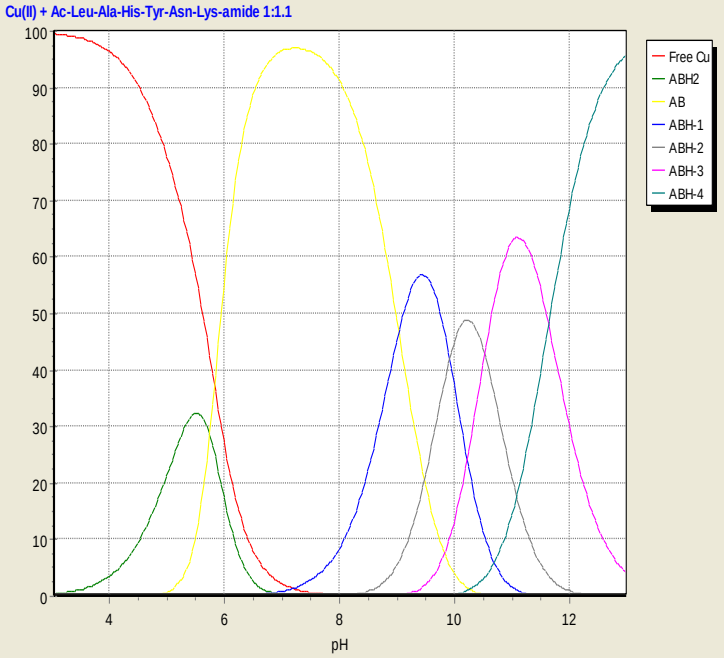
Species	UV/Vis λ_{max} (ε / M^{-1} cm^{-1})	EPR $A_{\|}$ (G)	$g_{\|}$
CuH_2L (1N)	*	*	*
CuL (3N)	591 (110)	170	2.230
$CuL_{-1}L$ (3N)	582 (94)	173	2.231
$CuL_{-2}L$ (4N)	528 (120)	196	2.190
$CuL_{-3}L$ (4N)	513(140)	196	2.180
$CuL_{-4}L$ (4N)	512(150)	196	2.180



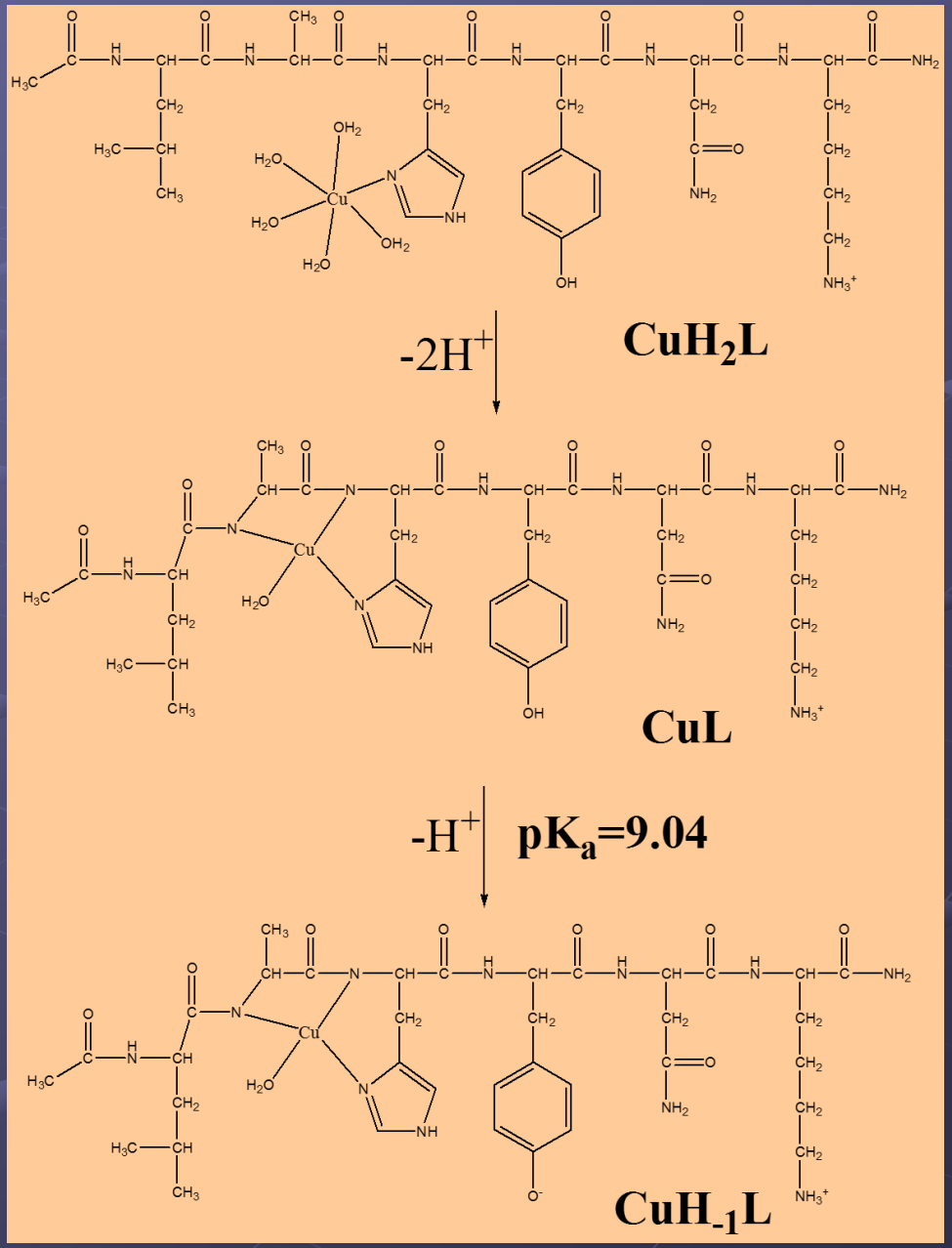
UV-Vis spectra of the system Cu(II): Ac-LAHYNK-amide recorded at various pH values .

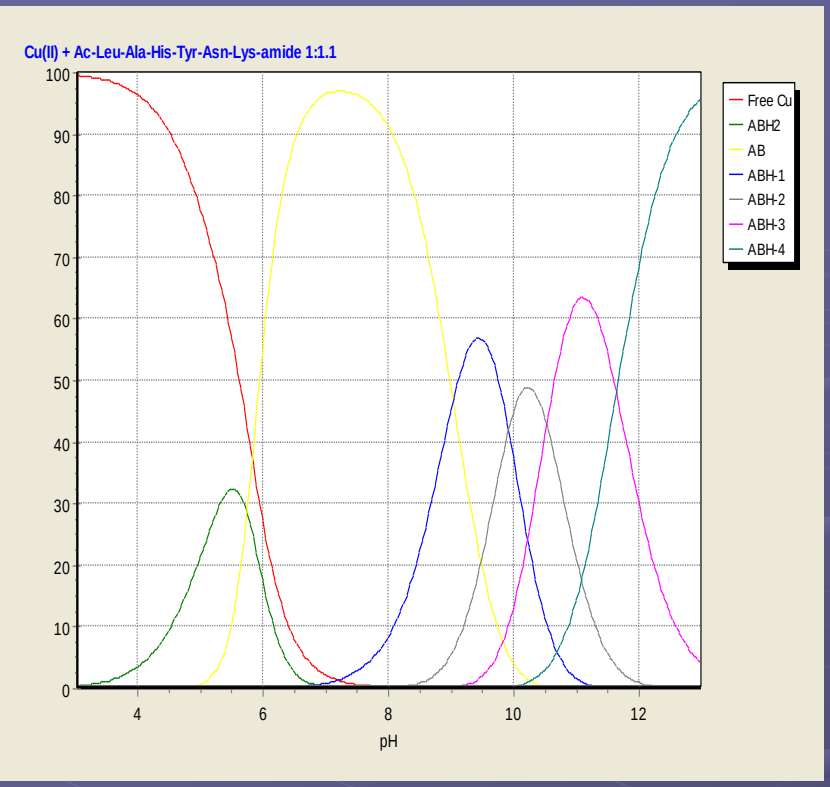


CW-EPR spectra of the system Cu(II): Ac-LAHYNK-amide recorded at various pH values.

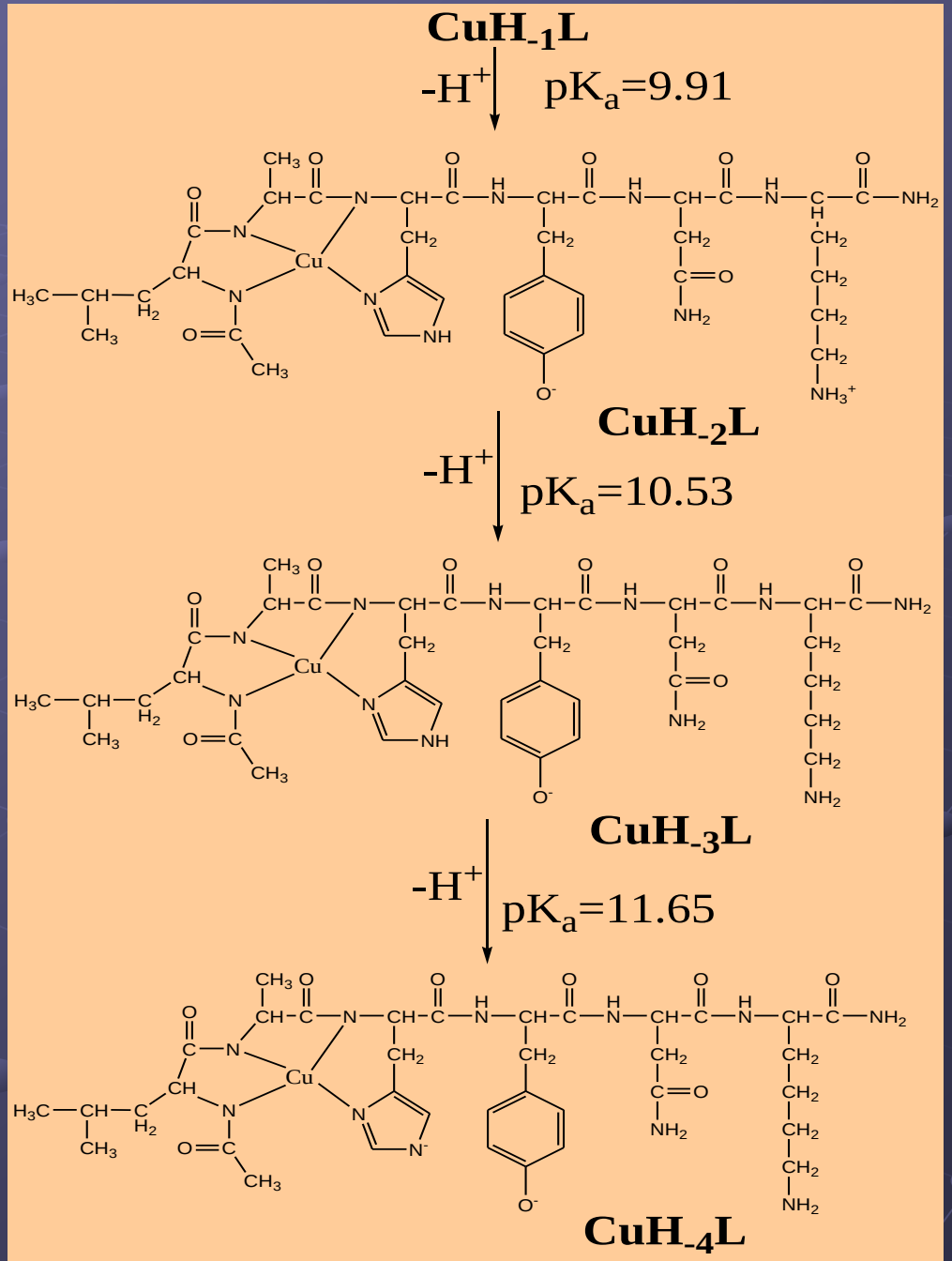


Species	UV/Vis λ_{max} (ε / M^{-1} cm^{-1})	EPR $A_{\parallel}$ (G) $g_{\parallel}$
CuH_2L (1N)	*	*
CuL (3N)	591 (110)	170 2.230
$CuH_{-1}L$ (3N)	582 (94)	173 2.231

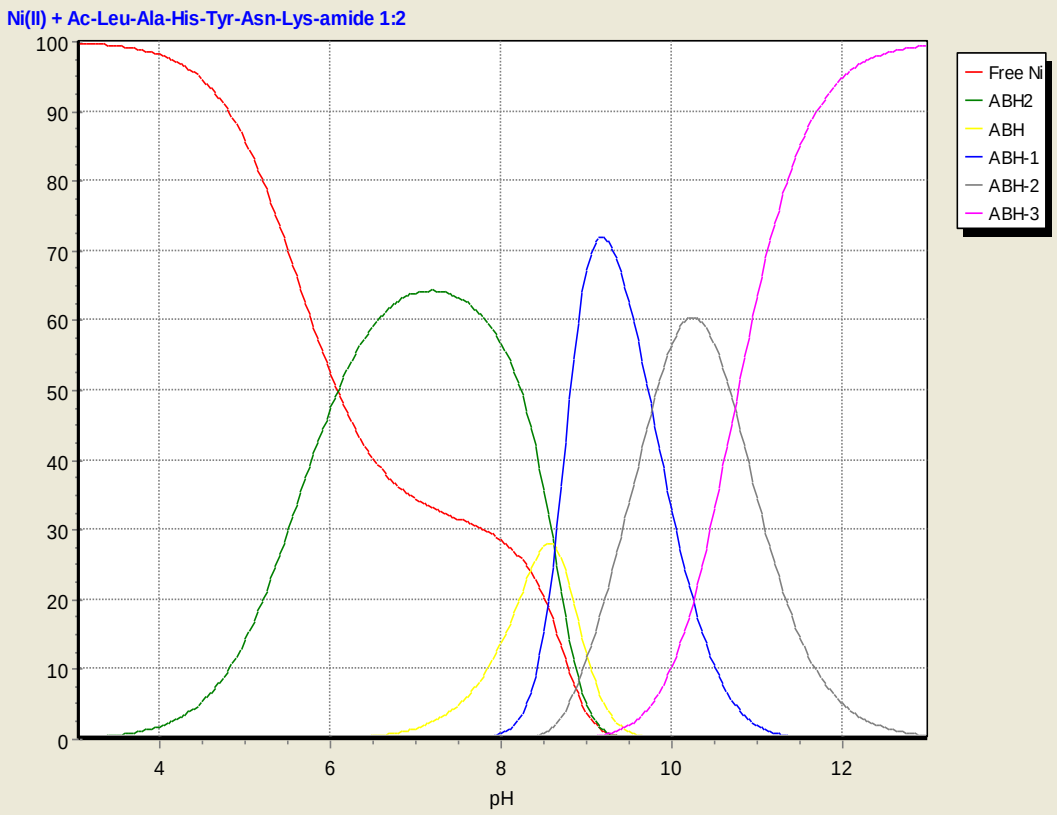




Species	UV/Vis λ_{max} (ε / M^{-1} cm^{-1})	EPR $A_{\|\|}$ (G) $g_{\|\|}$
$CuH_{-2}L$ (4N)	528 (120)	196 2.190
$CuH_{-3}L$ (4N)	513(140)	196 2.180
$CuH_{-4}L$ (4N)	512(150)	196 2.180



Ni(II)-LAHYNK- *interaction.*



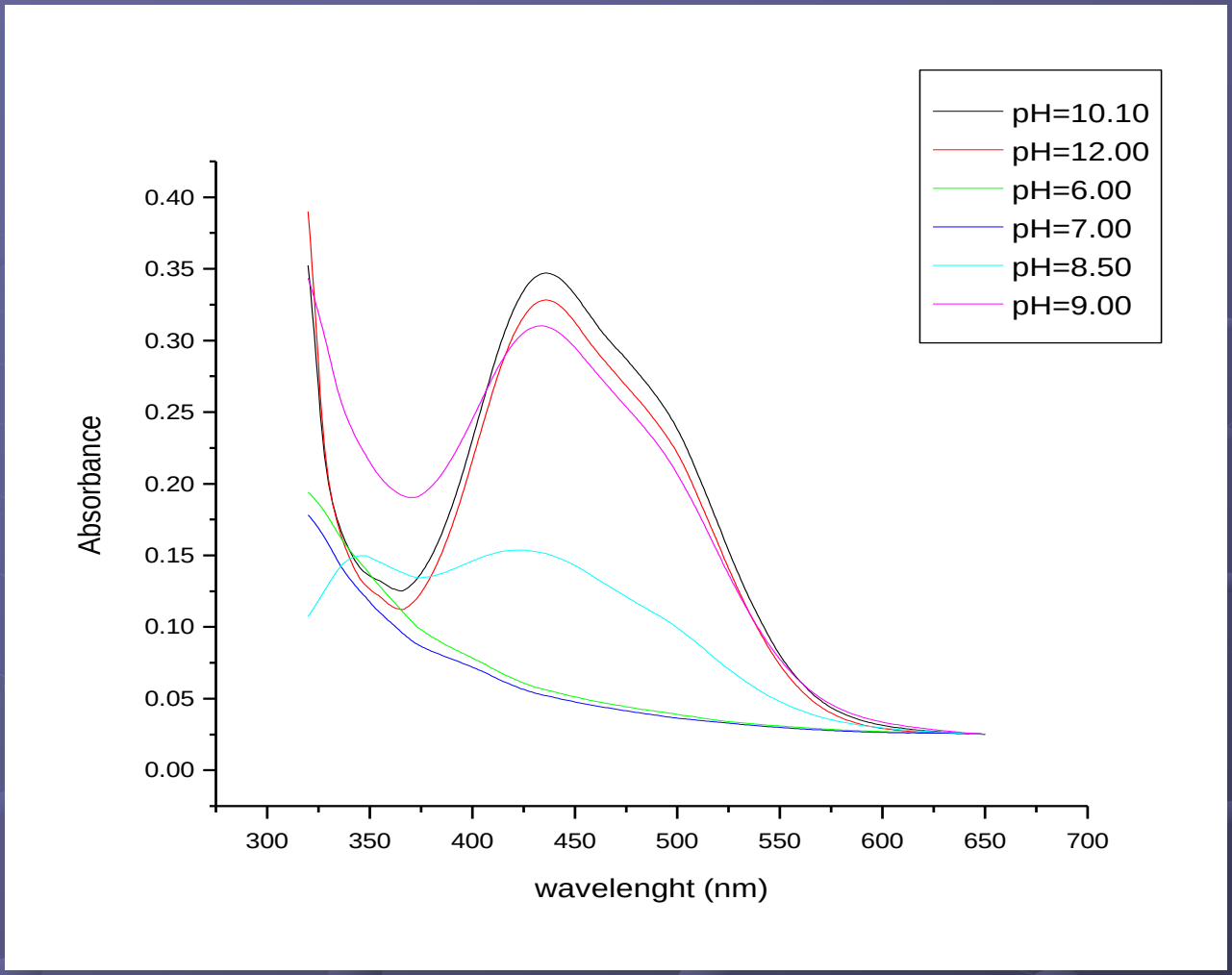
Species distribution diagram of the -LAHYNK- :Ni(II) system (2:1).

Stability constants of -LAHYNK- : ***Ni(II)*** *system (2:1).*

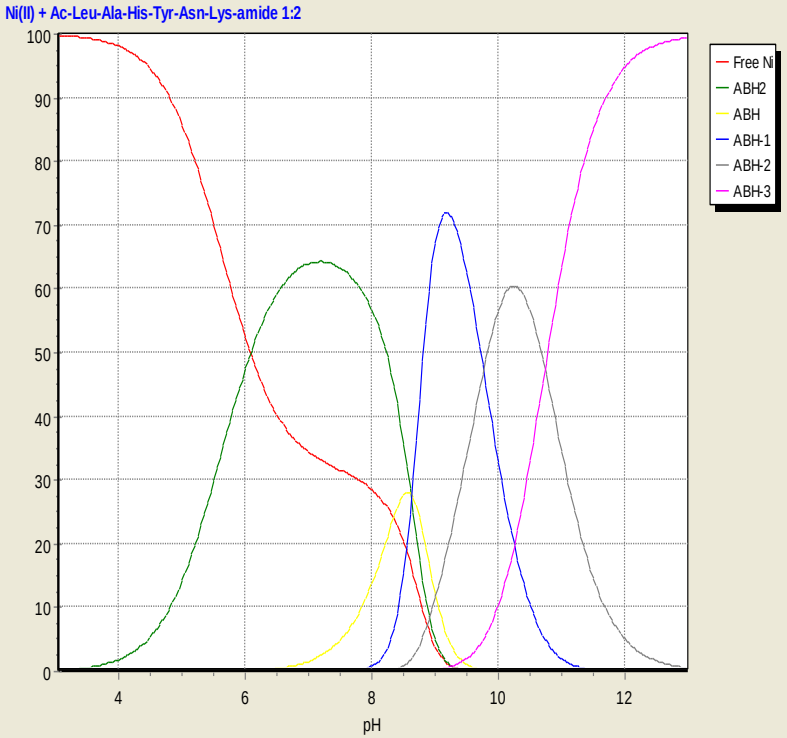
Species	***logβ***	***pK_a***
NiH_2L	23.26 (3)	
NiHL	14.65 (3)	8.61
$NiH_{-1}L$	-2.60 (2)	-
$NiH_{-2}L$	-12.63 (3)	10.03
$NiH_{-3}L$	-23.09 (4)	10.46

Spectroscopic parameters (UV-Vis) of the system -LAHYNK- :Ni(II)

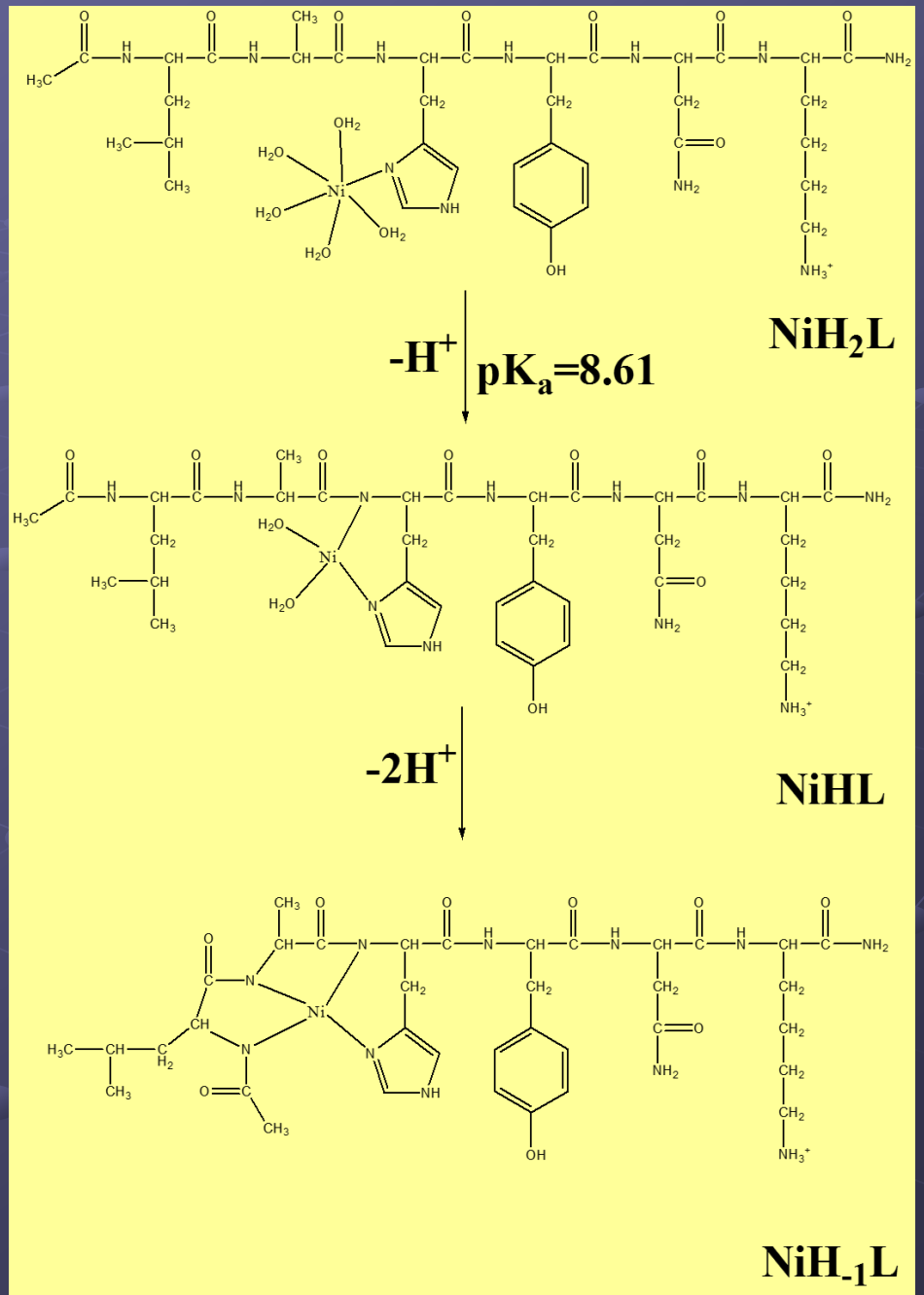
Species	**UV/Vis** **λ_{max} (ε / M^{-1} cm^{-1})**
NiH_2L (1N)	**-**
NiHL (2N)	*****
$NiL_{-1}L$ (4N)	**422 (84)**
$NiL_{-2}A$ (4N)	**434 (140)**
$NiL_{-3}A$ (4N)	**437 (130)**

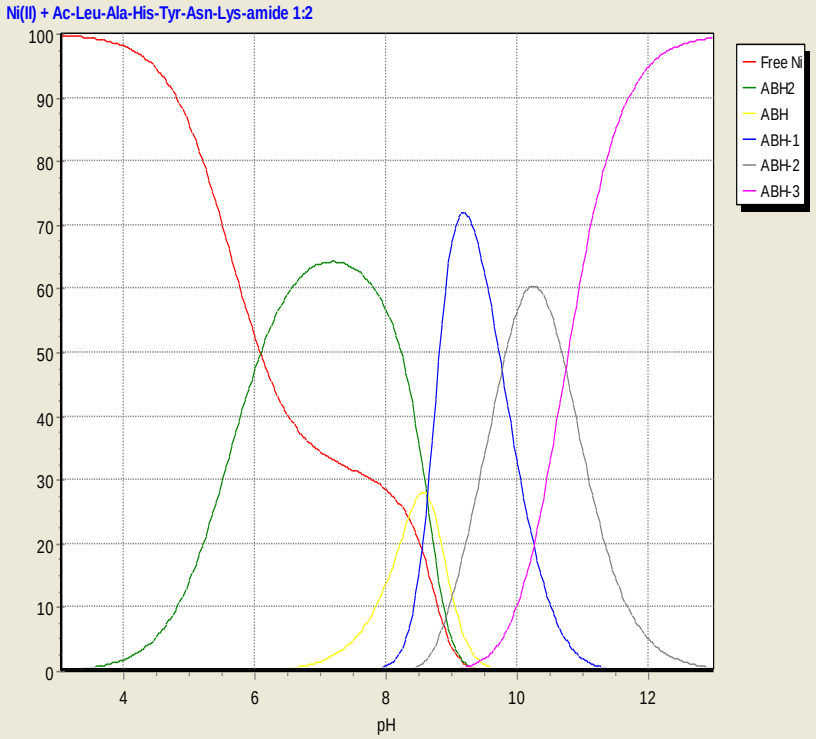


UV-Vis spectra of the system Ni(II): Ac-LAHYNK-amide 1:2 recorded at various pH values .



Species	UV/Vis λ_{max} (ε / M^{-1} cm^{-1})
NiH_2L (1N)	-
NiHL (2N)	-
$NiL_{-1}L$ (4N)	422 (84)

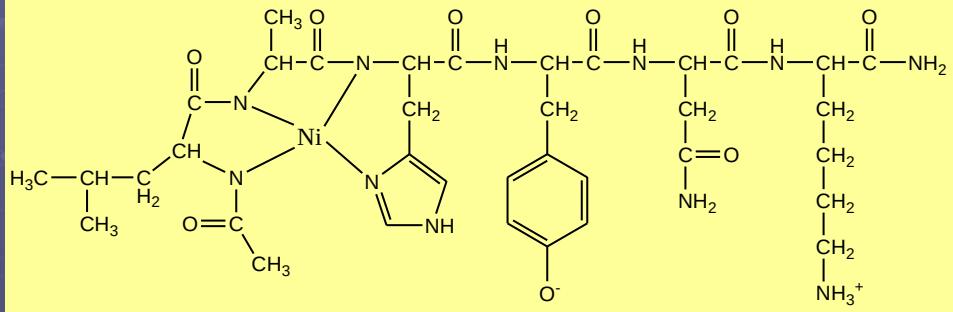




Species	UV/Vis λ_{max} (ε / M^{-1} cm^{-1})
$NiH_{-2}L$ (4N)	434 (140)
$NiL_{-3}L$ (4N)	437 (130)

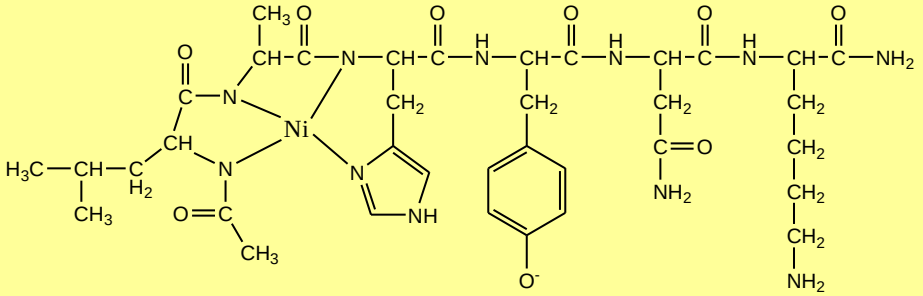
$NiH_{-1}L$

$-H^+$ pK_a=10.03



$NiH_{-2}L$

$-H^+$ pK_a=10.46



$NiH_{-3}L$

MAIN CONCLUSIONS- PART A

1. All peptide models interact strongly with the metal ions over the pH range 5-10.5. The imidazole side chain of His is the initial metal anchoring group while successive coordination of amide donors saturate the equatorial plane leading to 4N complexes above pH~9

2. peptides hydrolytic cleavage in the presence of metal ions was observed only when Ser or Thr residues are located near the coordination sites

3. THE COMPLEX $CuH_{-1}L$ WITH -TESHHK- EFFICIENTLY PROMOTED OXIDATION OF dG, WITH A TRANSIENT FORMATION OF SUBSTANTIAL AMOUNTS OF 8-oxo-dG IN PHYSIOLOGICAL CONDITIONS (pH = 7.4 and T = 37 °C)

PART B: Studies in larger peptide models of histone H2B

- **Synthesized sequences:**
- **Peptide (1):** (1-31)

Ac-PEPAKSAPAPKKGSKKAVTKAQKKDGKKRKR-am

- **peptide (2):** (32-62)

Ac-SRKQSYSVYVYKVLKQVHPDTGISSKAMGIM-am

- **peptide (3):** (63-93)

Ac-NSFVNDIFERIAGEASRLAHYNKRSTITSRE-am

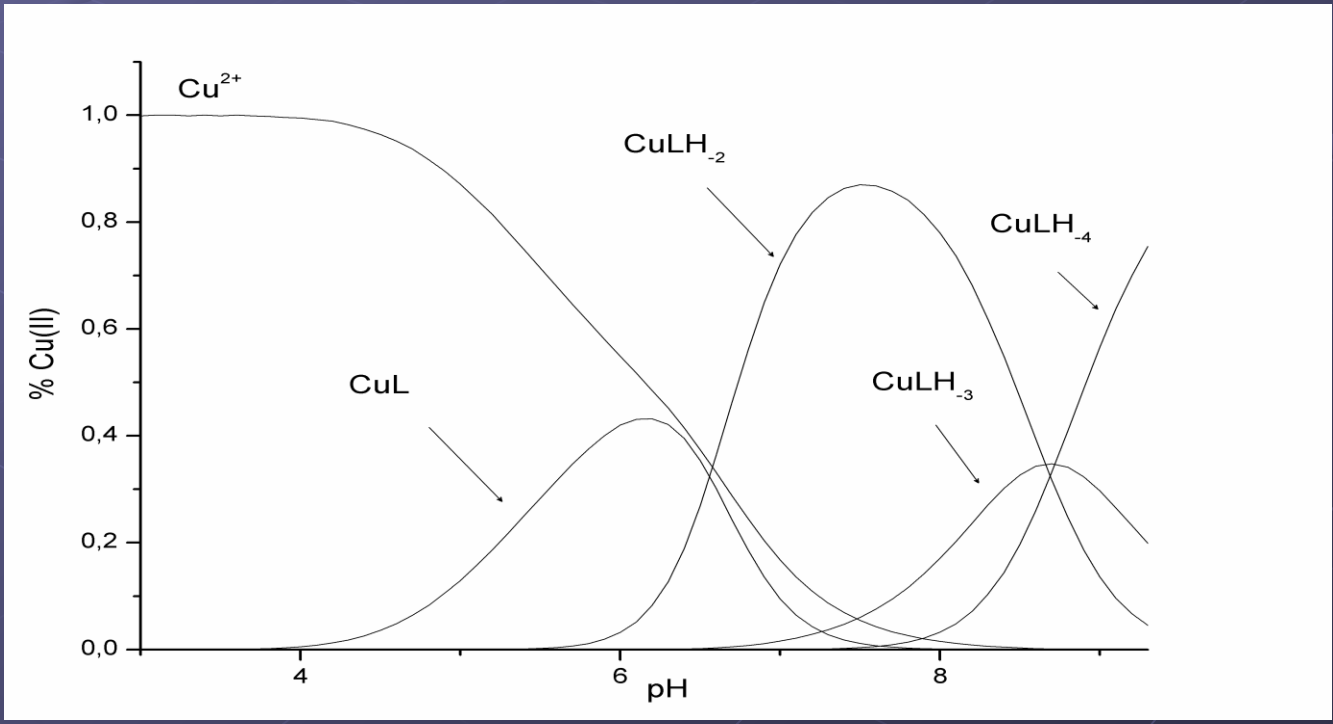
- **peptide (4):** (94-125)

Ac-IQTAVRLLLPGELAKHAVSEGTKAVTKYTSSK-am

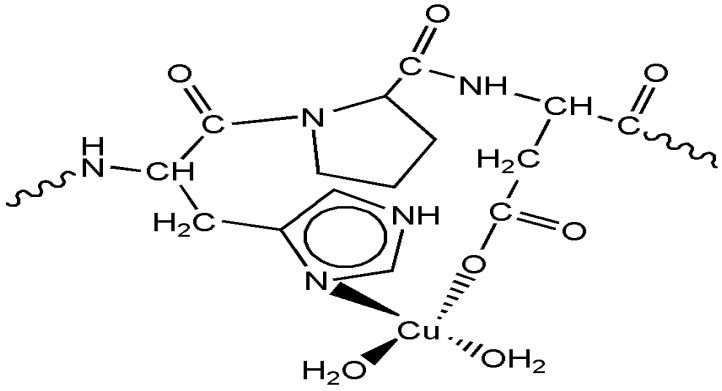
2. $H2B_{32-62}$ Cu(II), Ni(II) interaction

$H2B_{32-62}$: AcSRKQSYSVYVYKVLKQVHPDTGISSKAMGIM**NH_2**

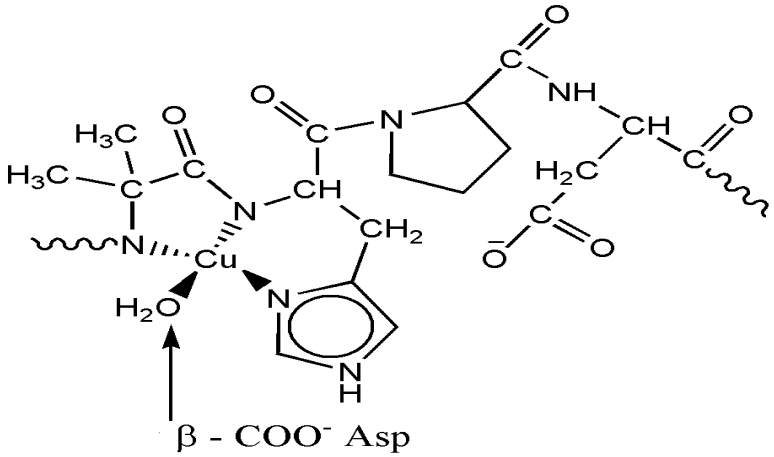
- Free peptide precipitates above pH ~ 7.8
- In the presence of Cu(II) above pH ~10.5
- Ni(II) ions didn't interact with the peptide



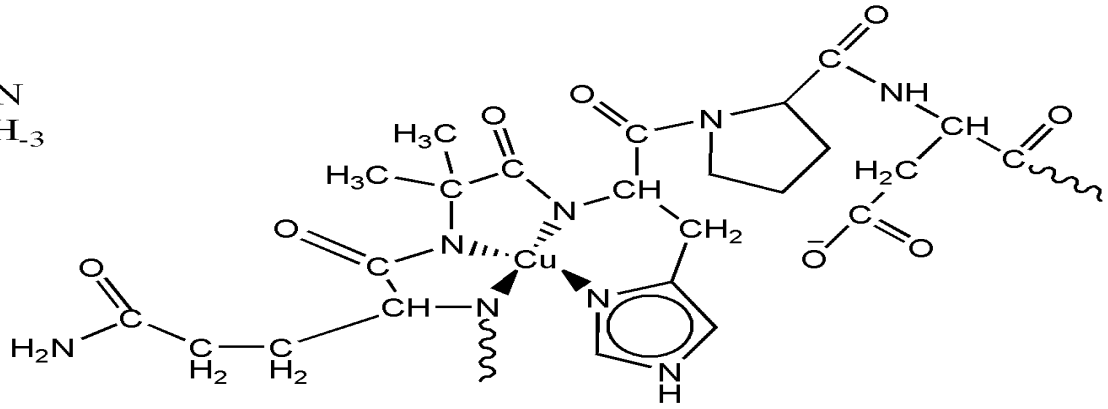
(a) 1N
CuL



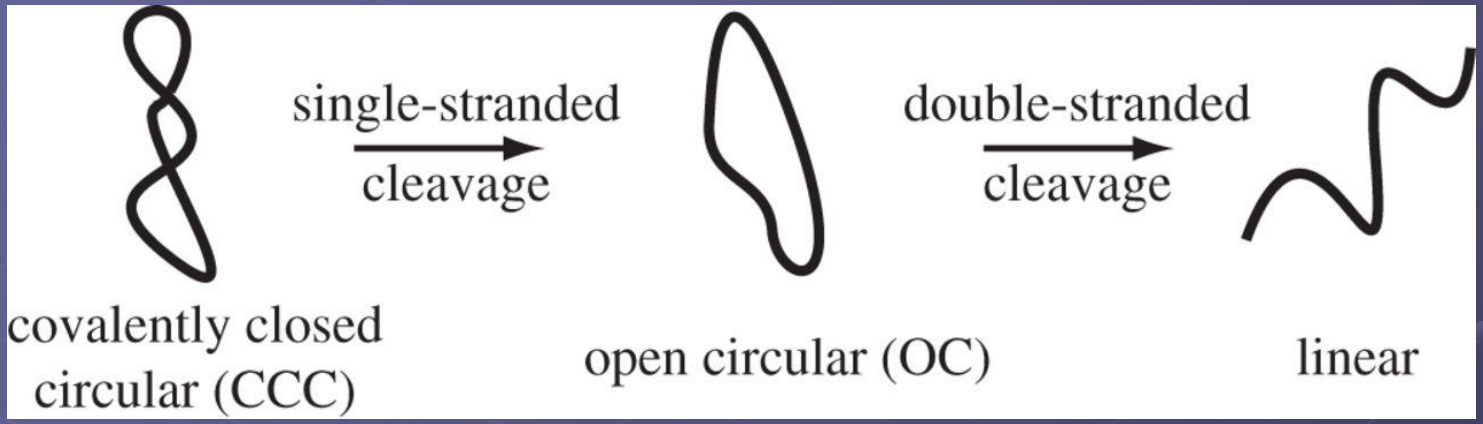
(b) 3N
$CuLH_{-2}$



(c) 4N
$CuLH_{-3}$



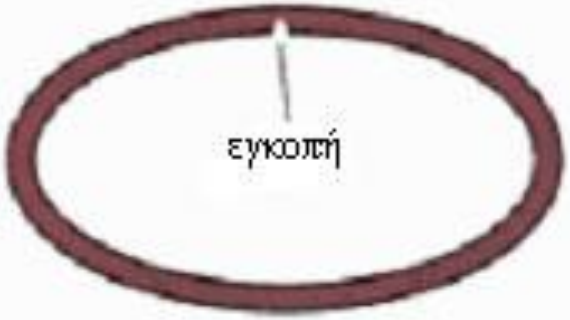
DNA strand breakage studies



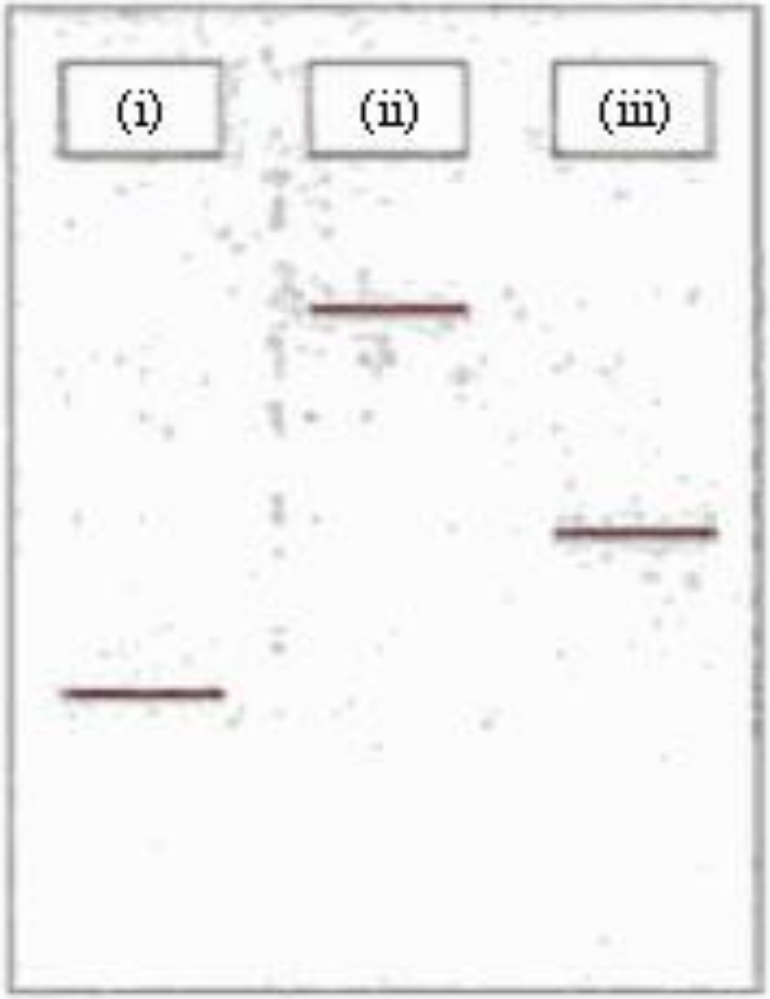
- pUC19 plasmid
- CCC form→high electrophoretic mobility
- OC form (single strand break)→reduced mobility
- Lin form (double strand breaks)→ intermediate mobility

(i) supercoiled μορφή πλασμιδίου
(ισχυρά συνεστραμμένο σαν ελαστικό καλώδιο)

(ii) open circular
(με μία εγκοπή στη μία αλυσίδα)



(iii) linear
(γραμμική μορφή)



DNA strand breakage studies

- single strand breaks → production of hydroxyl radicals in the bulk of the solution
- double strand scissions

→ single strand breaks in close parts of the DNA molecule

and/or

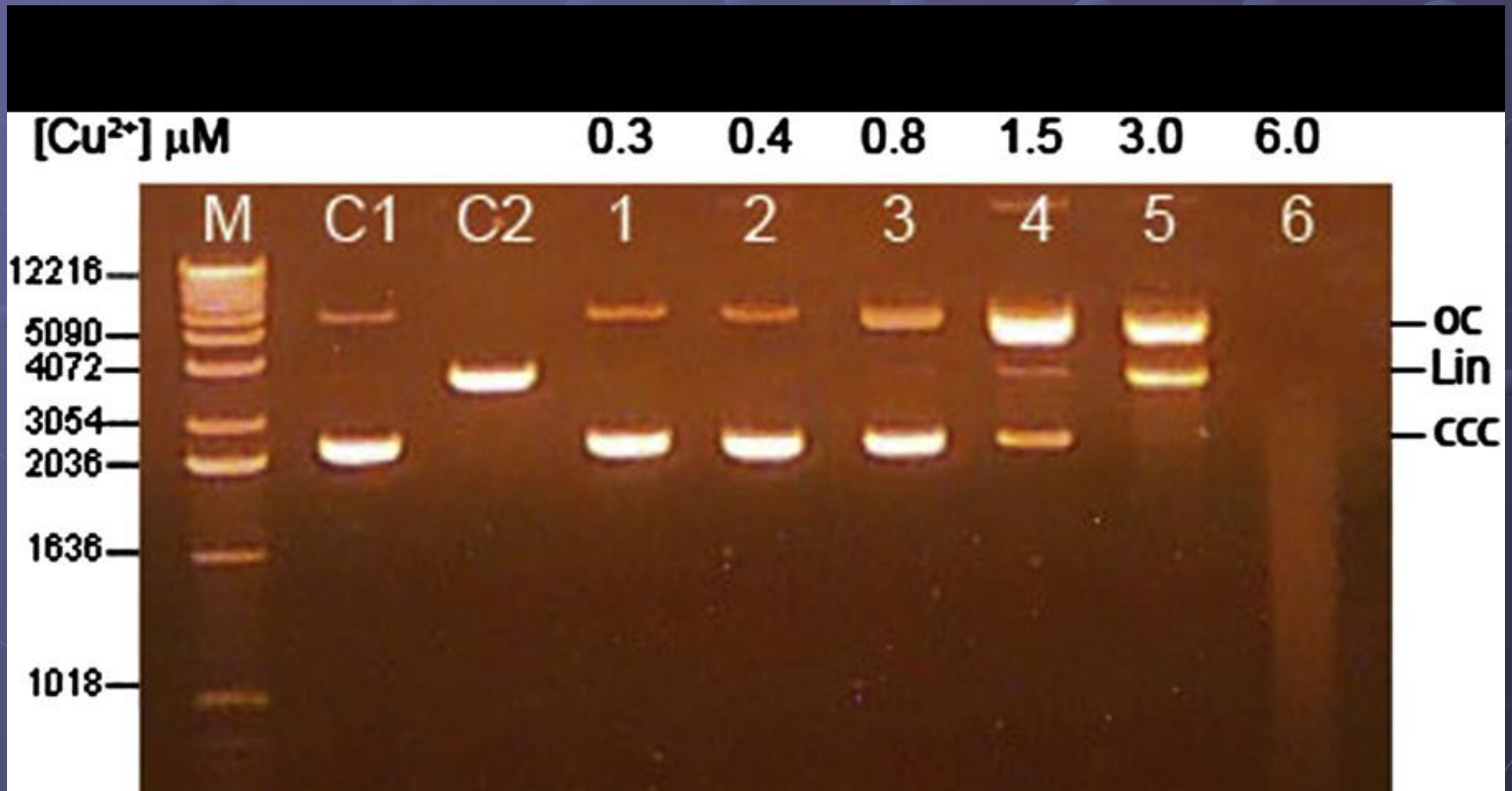
→ site-selective mechanism that requires binding of the metal ion with DNA right before the production of the hydroxyl radical.

DNA strand breakage studies

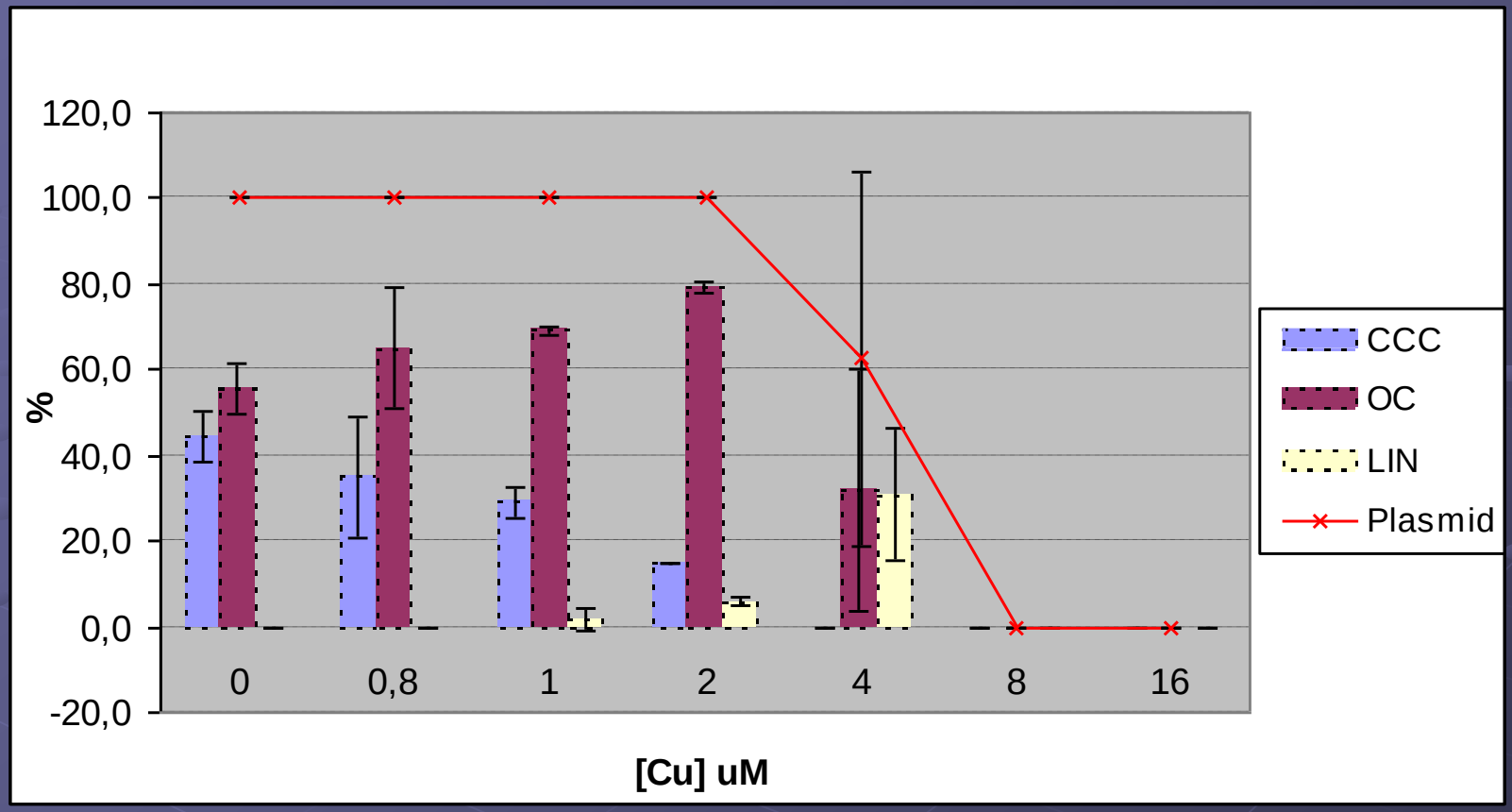
$H2B_{32-62}$: AcSRKQSYSVYVYKVLKQVHPDTGISSKAMGIM**NH_2**

histone fold domain model -strong interaction with DNA-

- I. Cu(II)/peptide/plasmid pUC19/H_2O_2

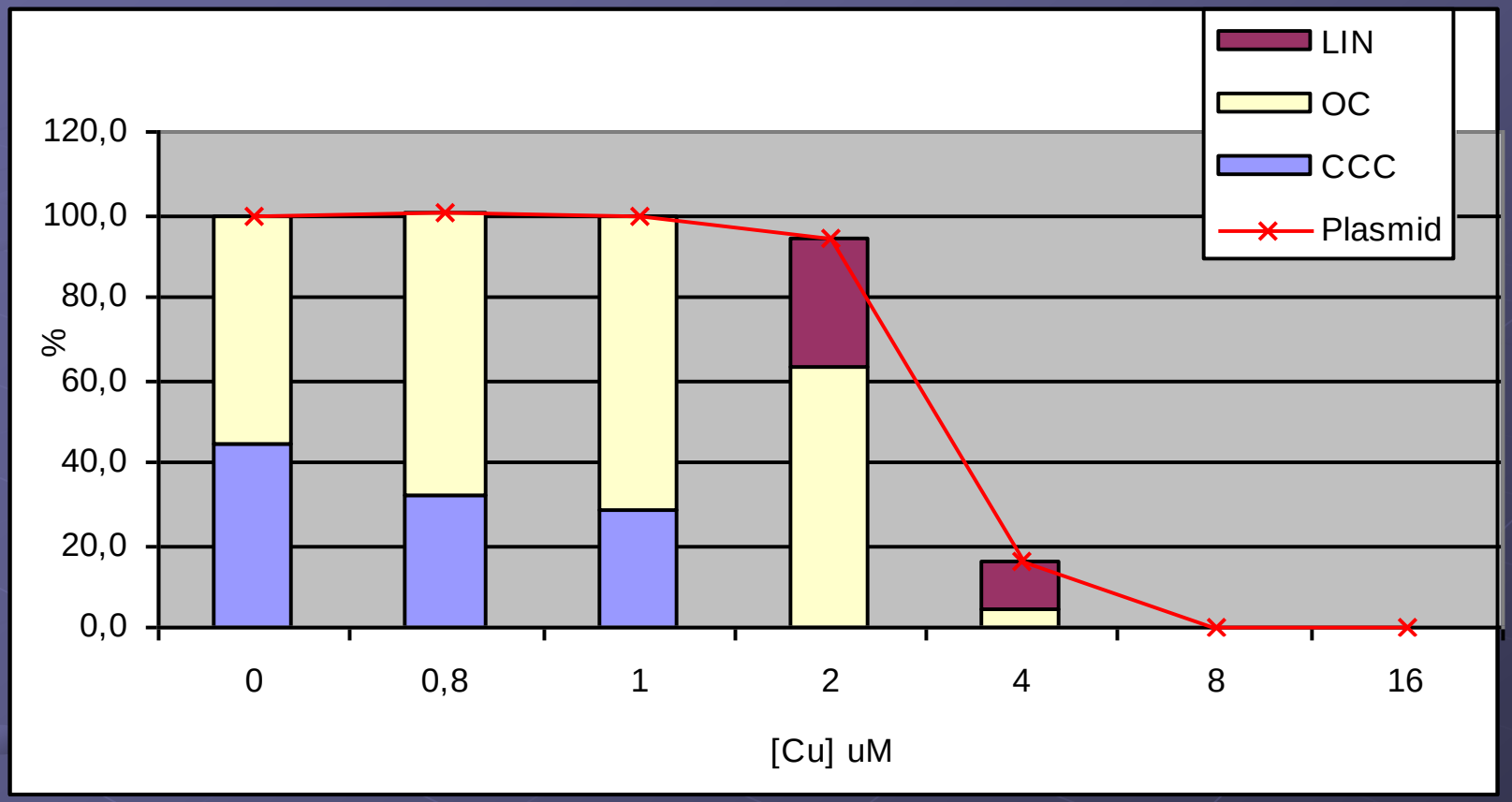


Επίδραση ιόντων Cu^{2+}στο πλασμιδιακό DNA

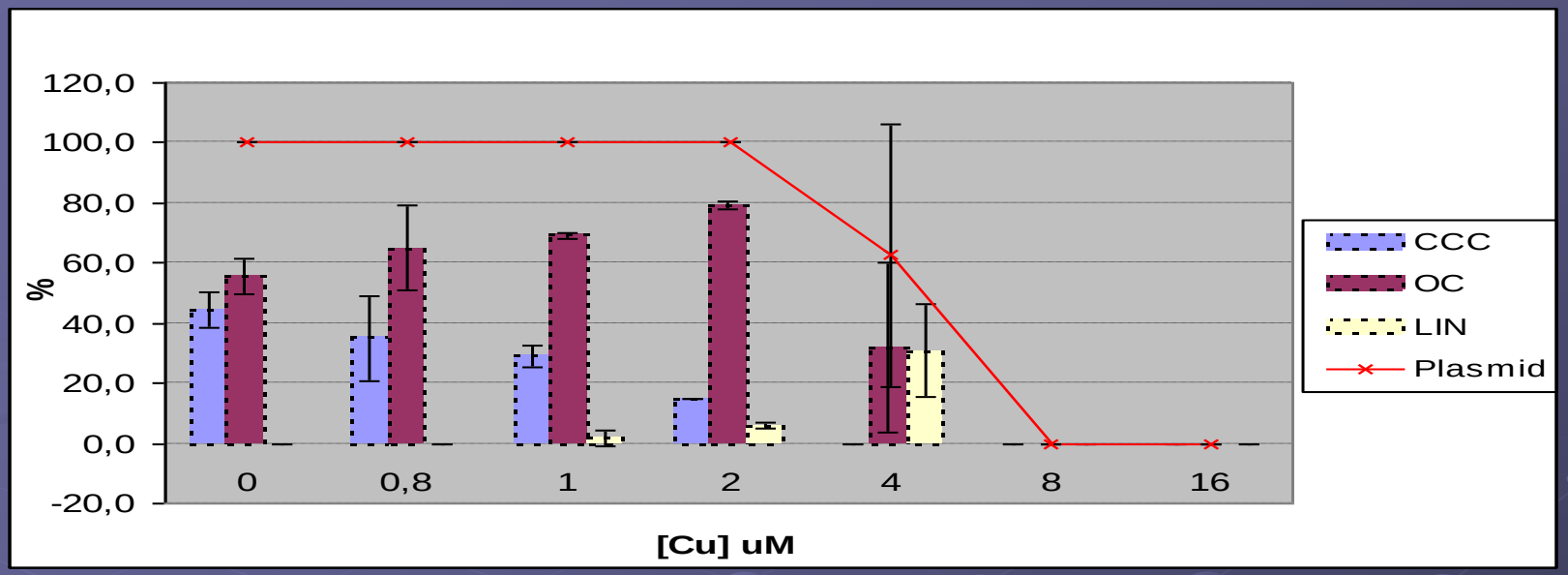


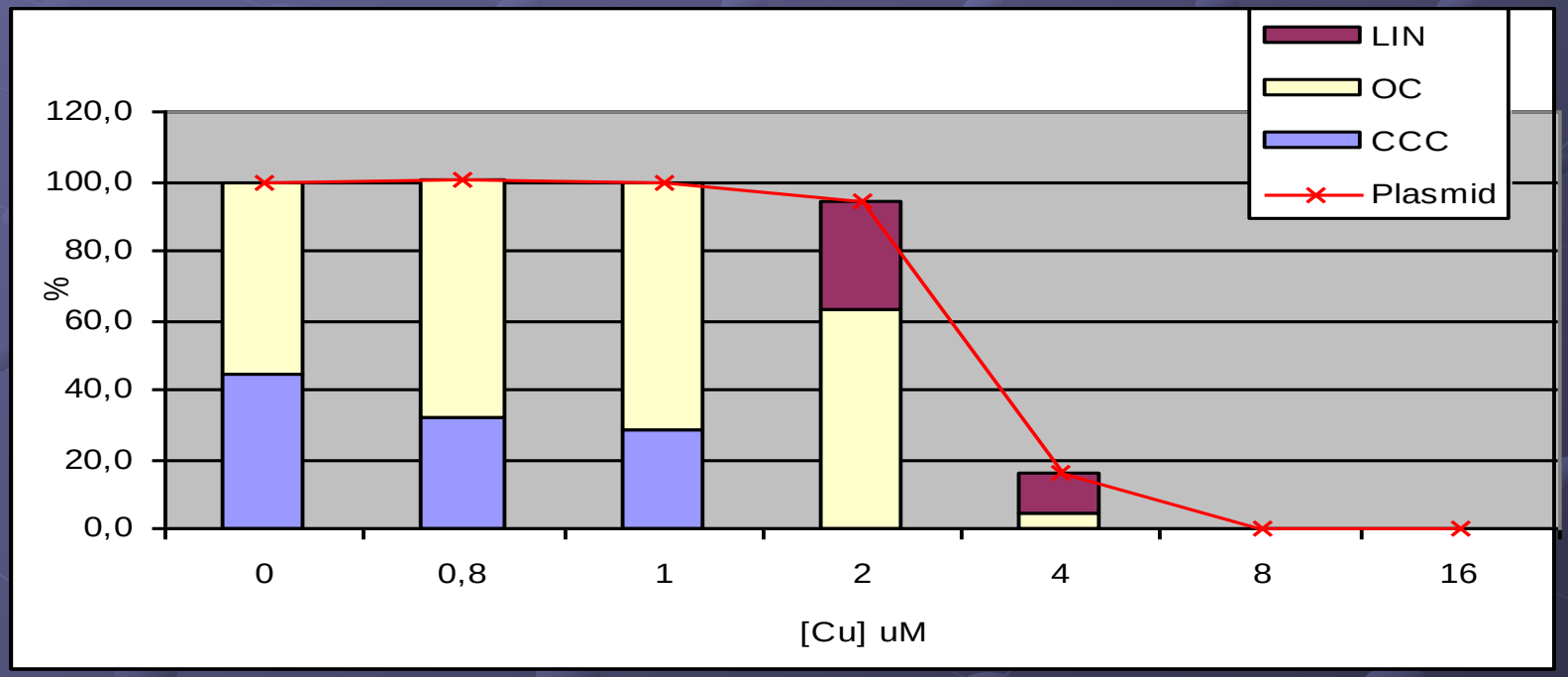
Ποσοστά supercoiled, open-circular και linear πλασμιδίου έπειτα από επώαση με διάφορες συγκεντρώσεις χαλκού (μέσος όρος 2 πειραμάτων) pUC19 (0.8 μg), H_2O_2 (1 mM)

Επίδραση Συμπλόκων Cu^{2+} - $H2B_{32-62}$ στο πλασμιδιακό DNA



- Ποσοστά supercoiled, open-circular και linear πλασμιδίου έπειτα από επώαση με διάφορες συγκεντρώσεις χαλκού, H_2O_2 (1 mM) και $H2B_{32-62}$ (8 μM) (μέσος όρος 2 πειραμάτων)


120,0
100,0
80,0
60,0
40,0
20,0
0,0
-20,0
%
0
0,8
1
2
4
8
16
[Cu] uM
CCC
OC
LIN
Plasmid


LIN
OC
CCC
Plasmid
120,0
100,0
80,0
60,0
40,0
20,0
0,0
%
0
0,8
1
2
4
8
16
[Cu] uM

Gels bands quantification results

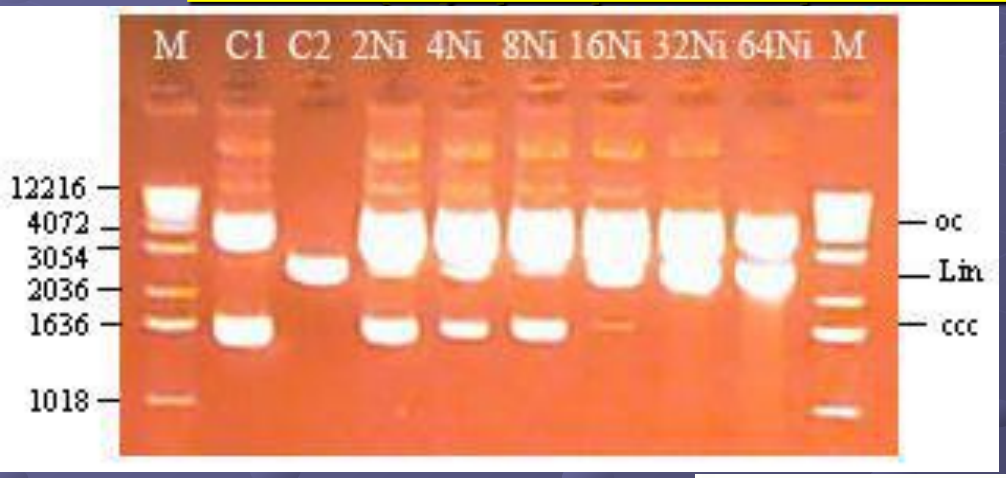
- The plasmid oxidative damage is bigger in presence of the peptide
- Double strand scission is enhanced in the presence of **$H2B_{32-62}$**
- **Due to the high DNA affinity of $H2B_{32-62}$ the 3N (N_{im}, $2N^-$) redox active Cu(II)-peptide complex at pH~7.4 may approach DNA molecule (double strand scission)**

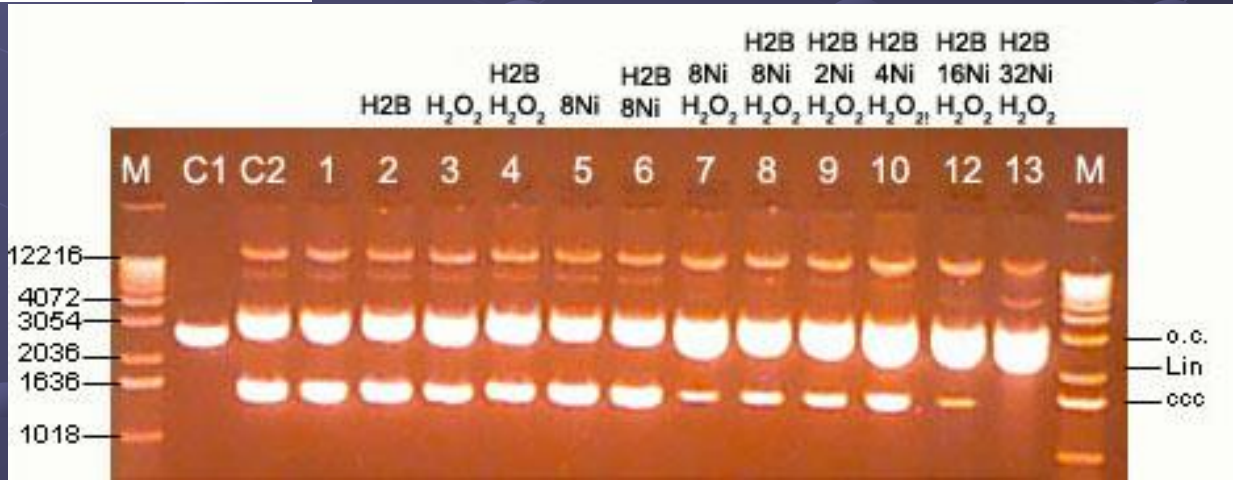
DNA strand breakage studies

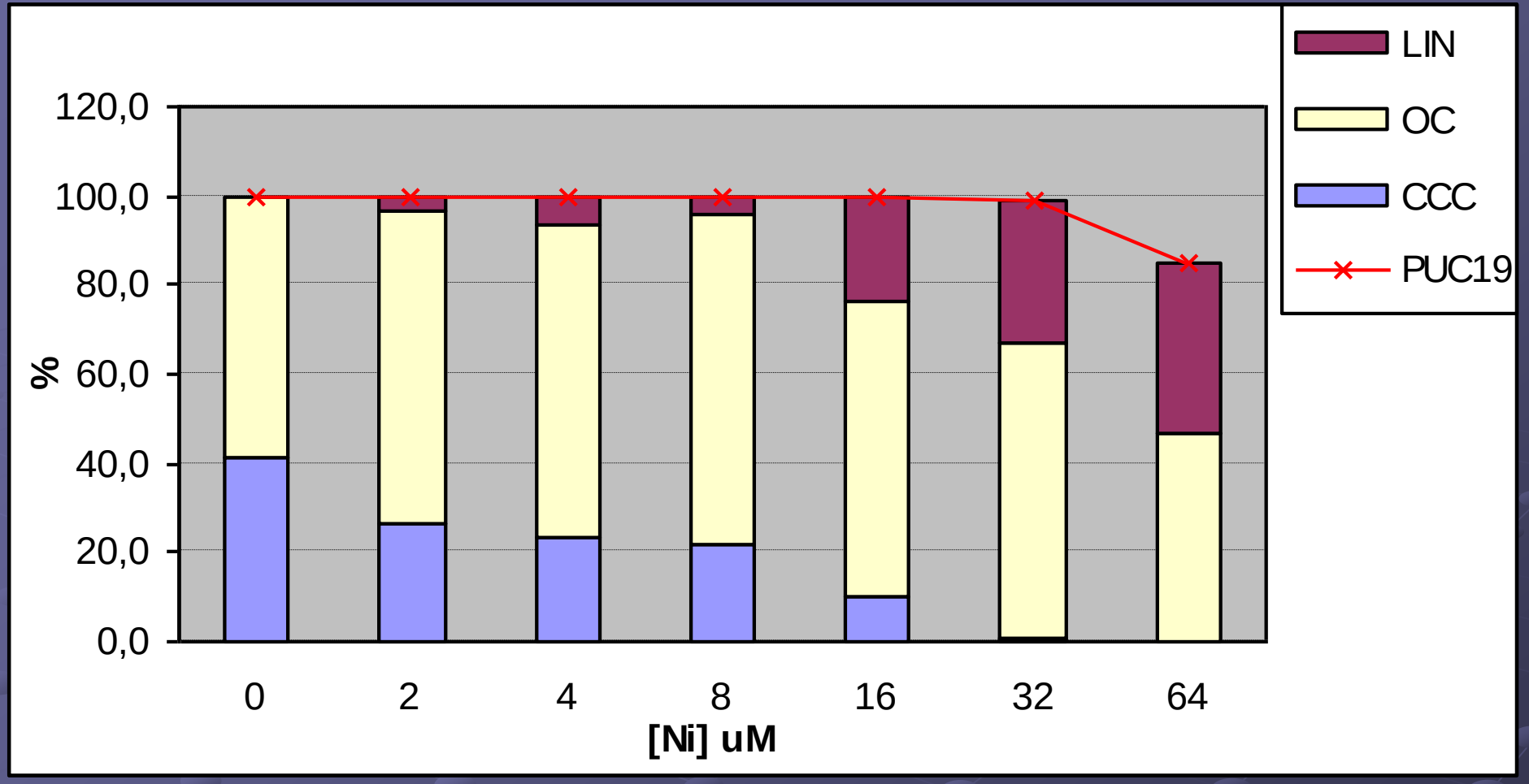
$H2B_{32-62}$: AcSRKQSYSVYVYKVLKQVHPDTGISSKAMGIM**NH_2**

histone fold domain model -strong interaction with DNA-

- II. Ni(II)/peptide/plasmid pUC19/H_2O_2







. Ποσοστά supercoiled, open-circular και linear πλασμιδίου έπειτα από επώαση με διάφορες συγκεντρώσεις νικελίου και H_2O_2 (1 mM) (μέσος όρος 2 πειραμάτων)

supercoiled → open-circular και open-circular → linear

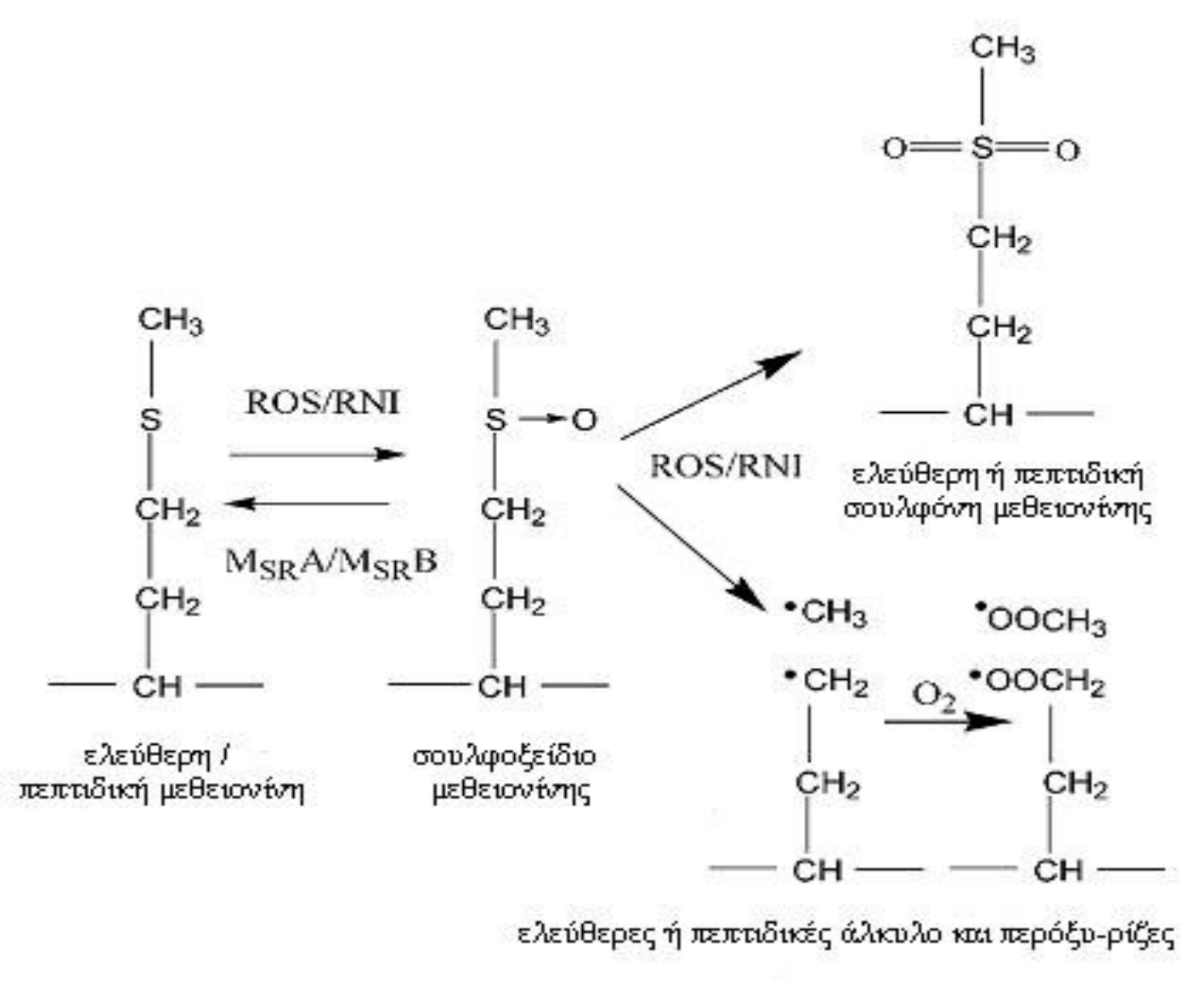
Gels bands quantification results

[Ni^{2+}] (μM)	(supercoiled) (%)		(open circular) (%)		(linear) (%)		total (%)	
	No pept		No pept		No pept			
0	41.7	45.0	58.3	55.0	0	0	100	100
2	26.7	18.3	70.0	78.3	3.3	3.3	100	100
4	23.3	14.2	70.0	71.7	6.7	14.2	100	100
8	21.7	14.0	74.2	65.2	4.2	20.8	100	100
16	10.0	3.3	66.7	61.7	23.3	35.0	100	100
32	0.8	0	65.8	65.0	32.5	35.0	99.2	100
64	0	0	46.7	50.8	38.3	42.5	85.0	93.3

Gels bands quantification results

- The plasmid decomposition in small fragments is less in the presence of the peptide (protective role)
- Double strand scission is enhanced in the presence of **$H2B_{32-62}$** **Why??**
- **Due to the high DNA affinity of $H2B_{32-62}$ the later may act as a protective layer to the plasmid and attacked first by oxygen radicals produced by Ni(II) complex in the bulk of the solution. Oxidation of Met residues may lead to active peroxy or alkoxy radicals mediating double strand scission !!**

Met residues oxidation

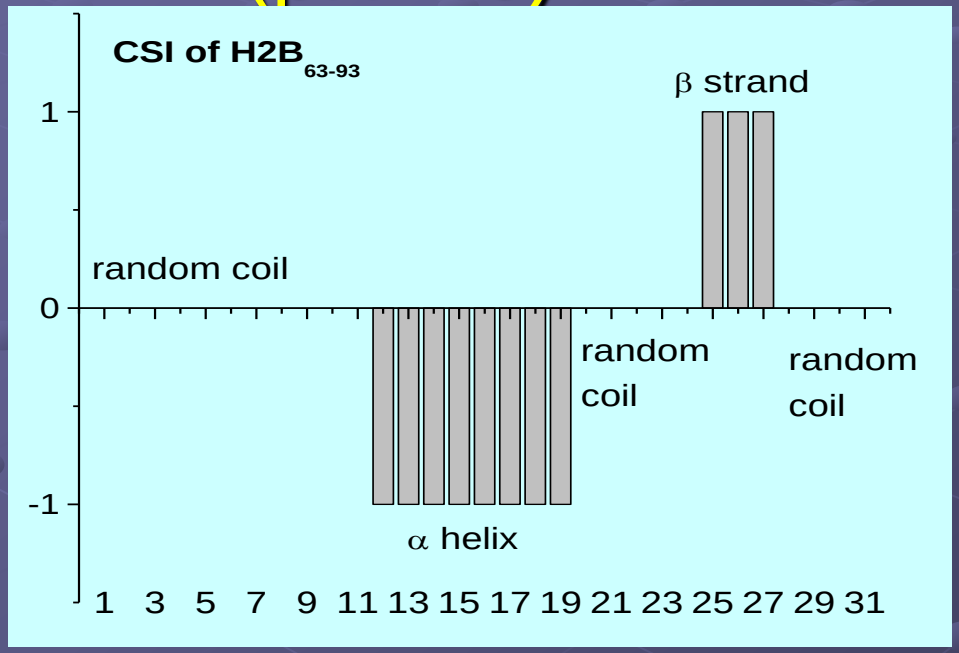


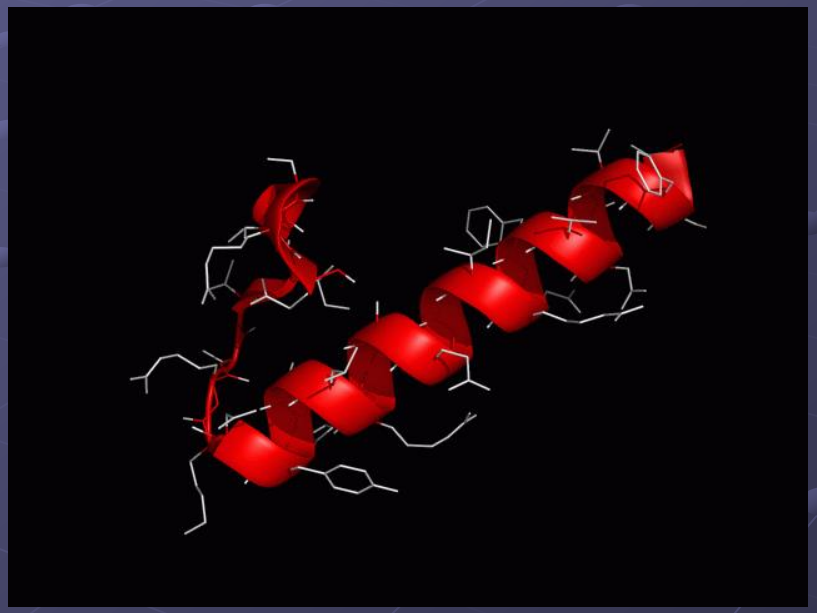
N.S Nakao et al, 2003

3. $H2B_{63-93}$ Cu(II), Ni(II) interaction

$H2B_{63-93}$: Ac-NSFVNDIFERIAGEASRLAH_{20}YNKRSTITSRE-NH_2

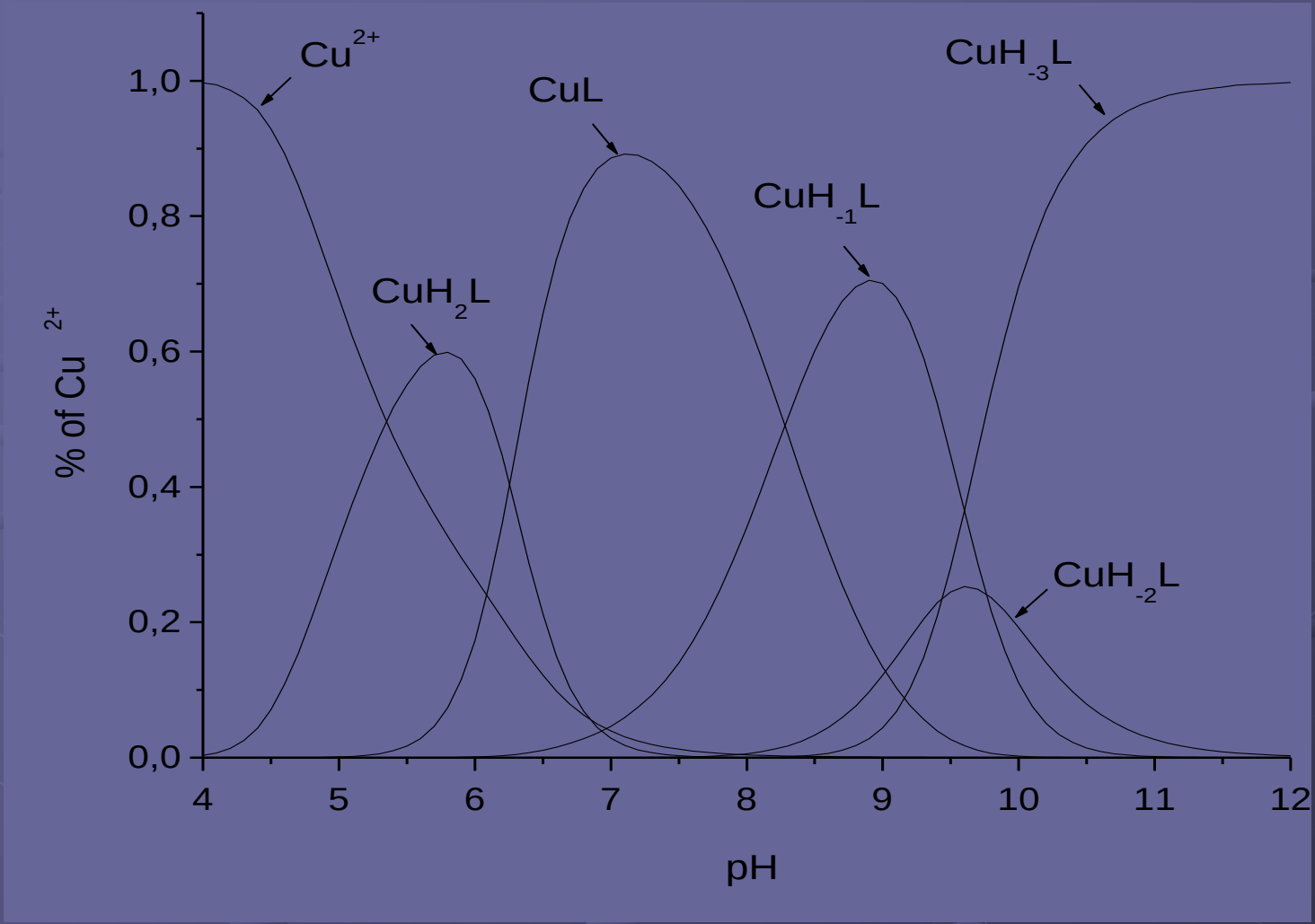
- Free peptide's structure in solution (pH~10)



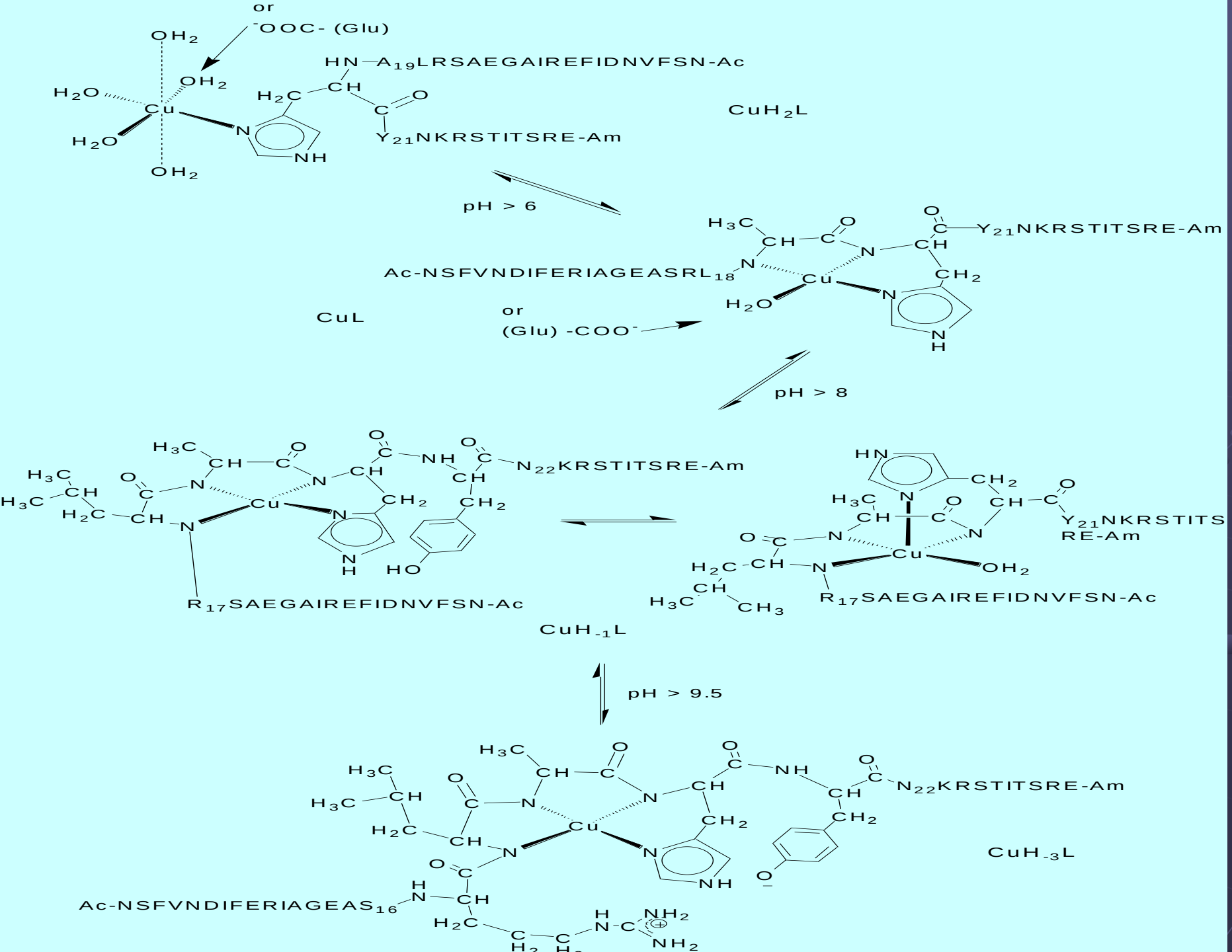


Reliable model of 63-93 residues of H2B histone-fold domain

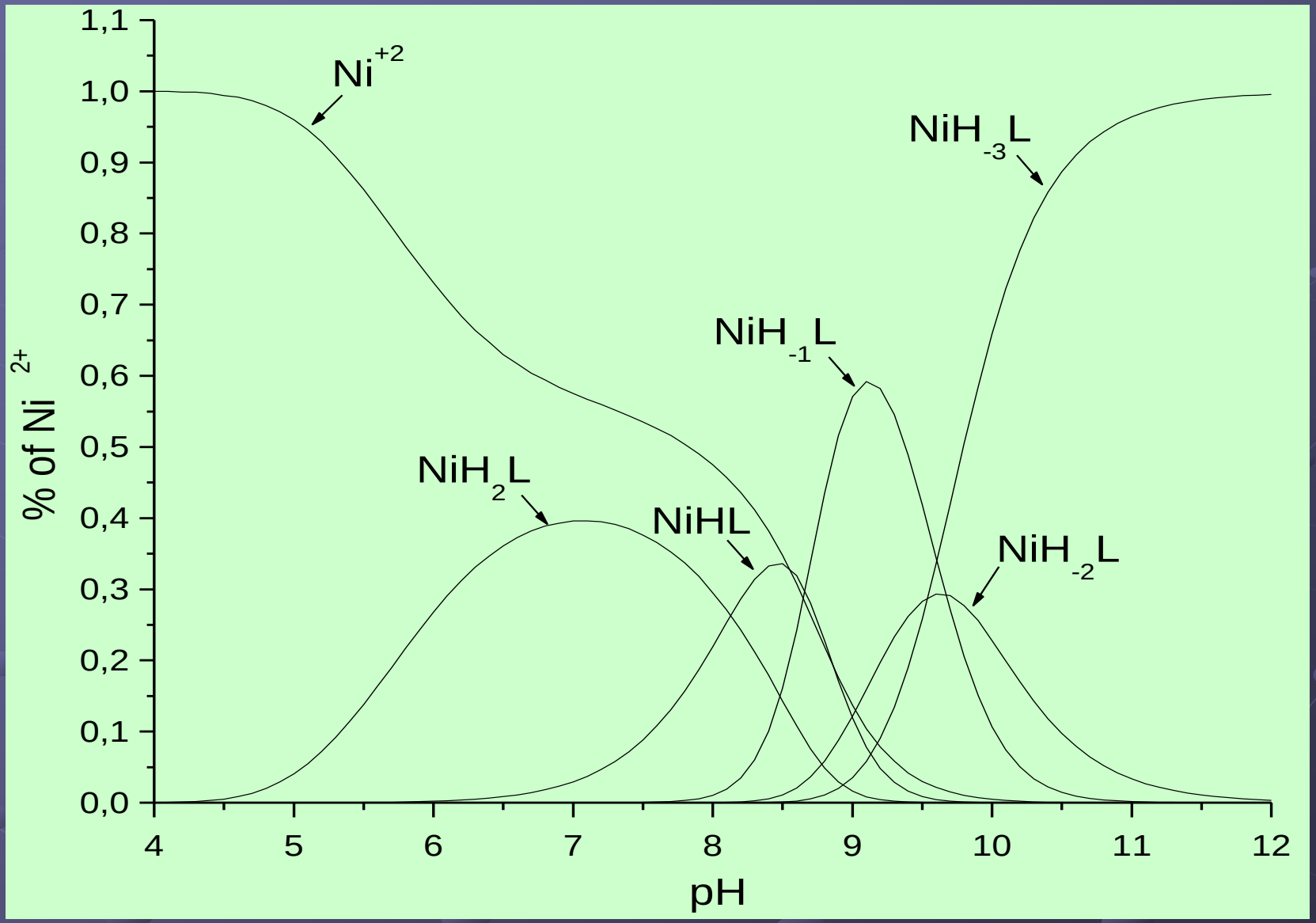
Cu(II) coordination towards $H2B_{63-93}$



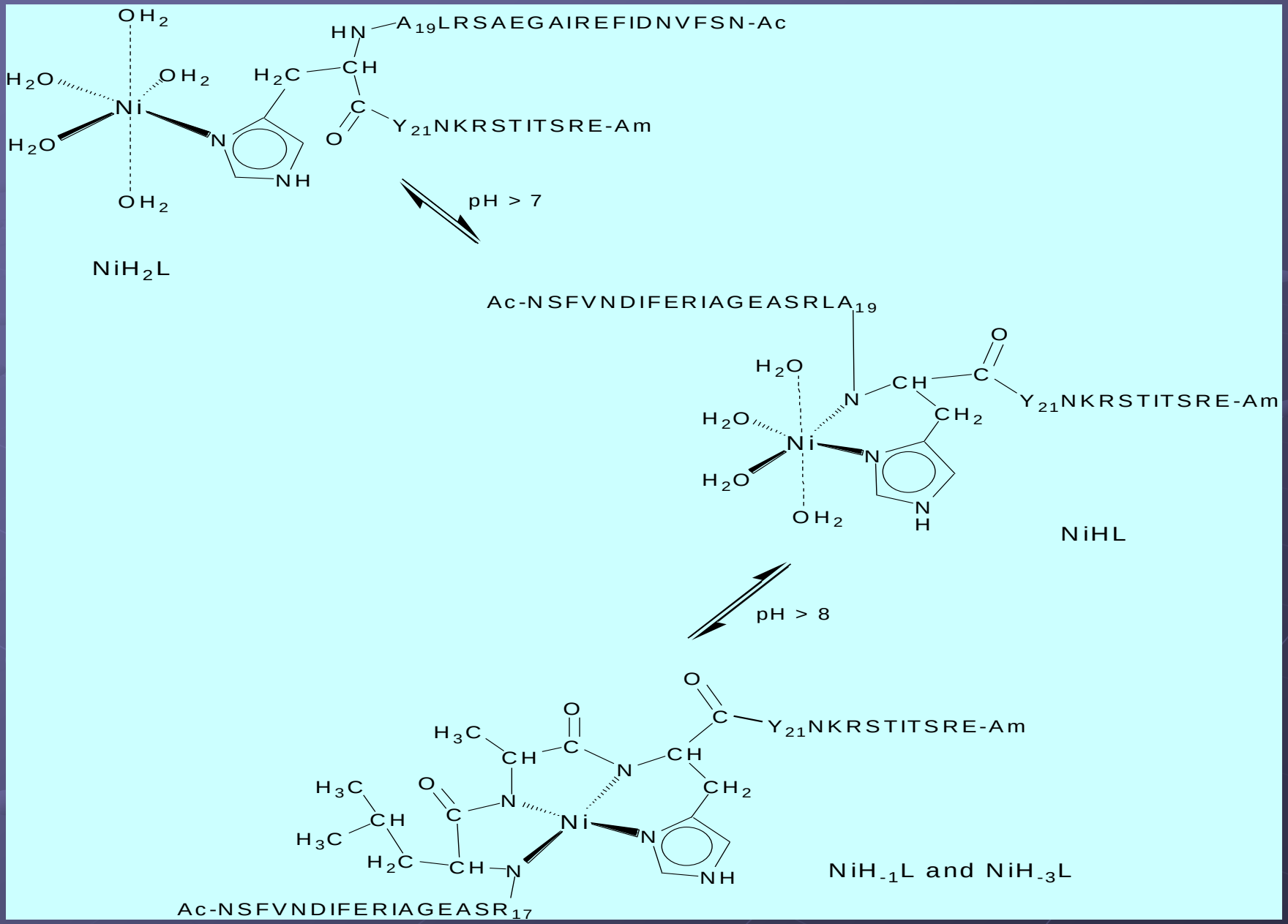
- Free peptide is considered as H_7L


or
-OOC- (Glu)
HN—A19LRSAEGAIREFIDNVFSN-Ac
Y21NKRSTITSRE-Am
CuH2L
pH > 6
Ac-NSFVNDIFERIAGEASRL18
CuL
or
(Glu) -COO-
Y21NKRSTITSRE-Am
pH > 8
N22KRSTITSRE-Am
R17SAEGAIREFIDNVFSN-Ac
CuH-1L
pH > 9.5
N22KRSTITSRE-Am
CuH-3L
Ac-NSFVNDIFERIAGEAS16

Ni(II) coordination towards $H2B_{63-93}$



Ni(II) coordination towards $H2B_{63-93}$



Ni(II) coordination towards $H2B_{63-93}$ NMR studies pH~10

- Structural information

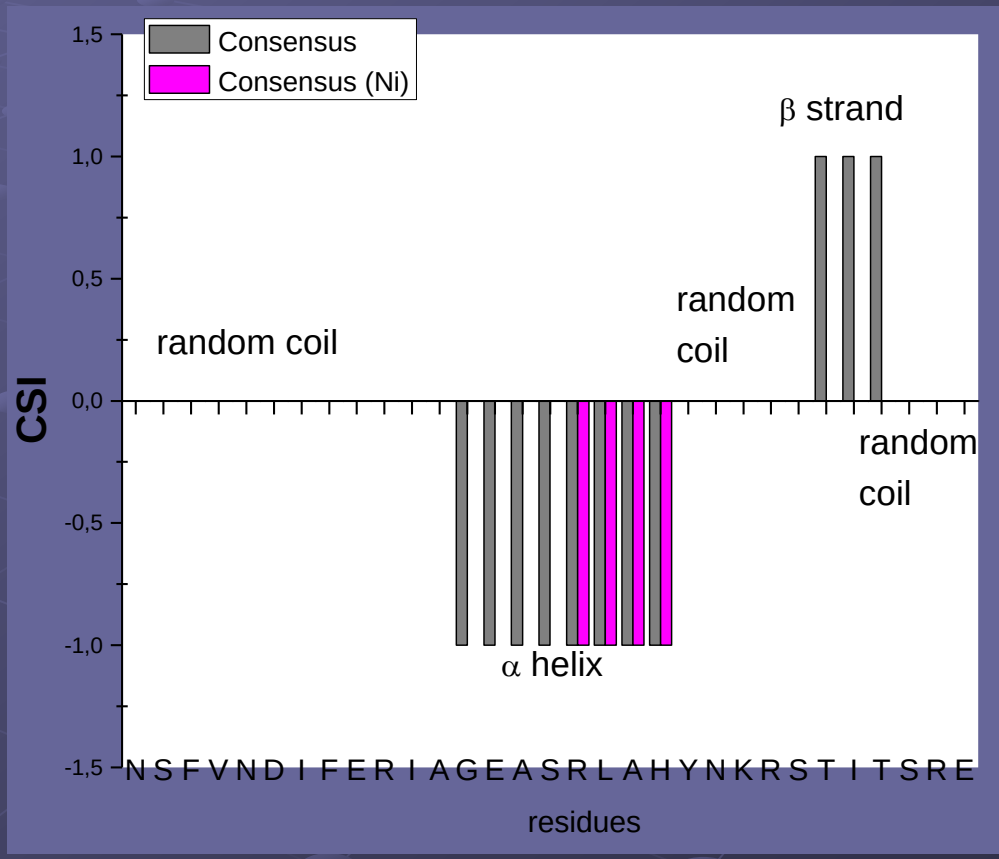
CSI confirms the big conformation change

↳ Transition from a helical and beta conformation from G13-T28 to a α-helix only in R17-L18-A19-H20

☟

Binding site!

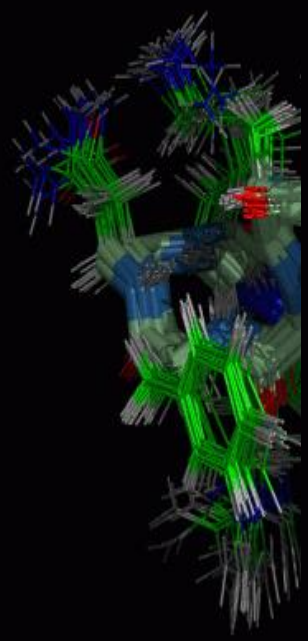
Adoption of a unique backbone geometry around the metal!



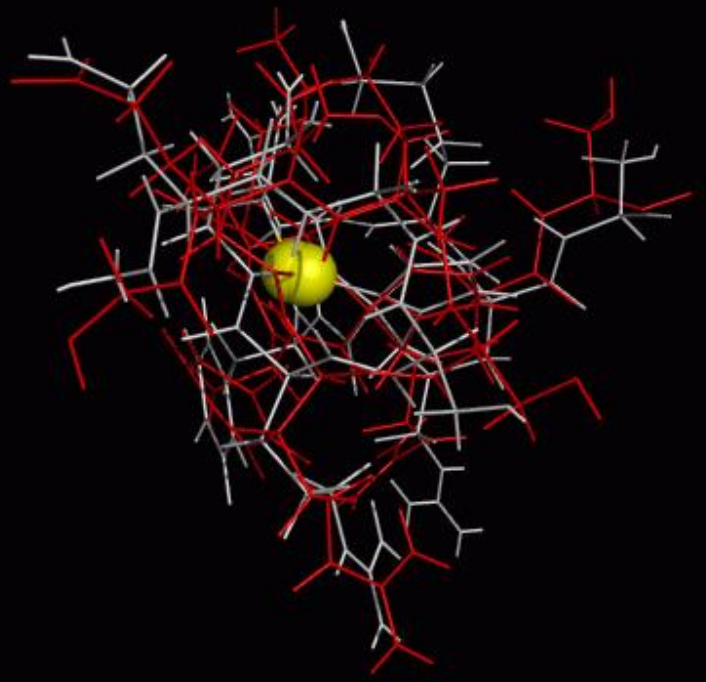
Ni(II) coordination towards $H2B_{63-93}$ NMR studies pH~10

- structure calculation of the 13 amino acid Ni(II)-bound $H2B_{75-87}$ (-G_{13}EASRLAH_{19}YNKRS$_{25}$-)

TF = 0.023 ± 0.009 Å^2
RMSD (3-11) $_{backbone}$= 0.10 ± 0.03
RMSD (3-11) $_{heavy}$= 0.37 ± 0.08 Å



Mean structure: E = 3630.09 kcal/mol
Optimized structure: E = 134.66 kcal/mol

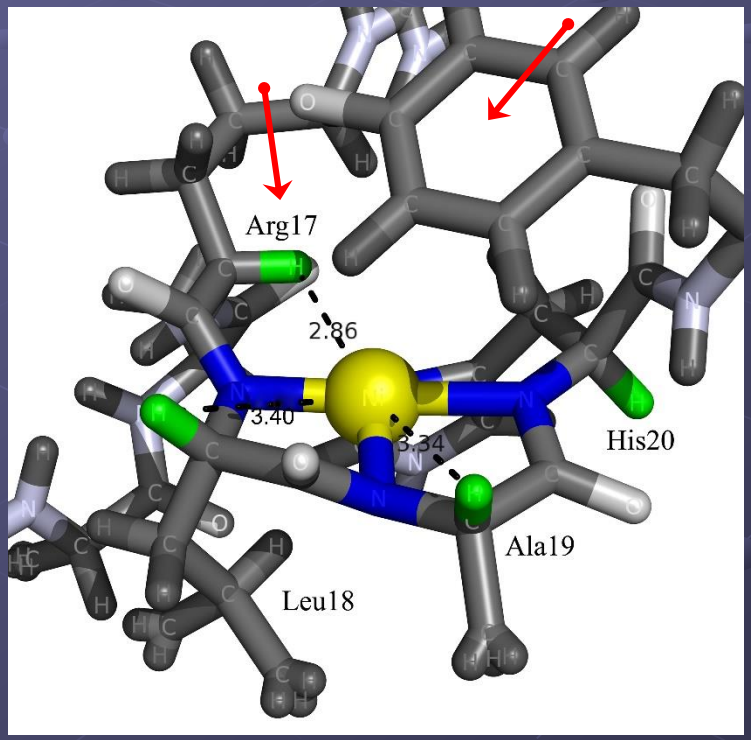


Mean
Optimized

Ni(II) coordination towards $H2B_{63-93}$ NMR studies pH~10

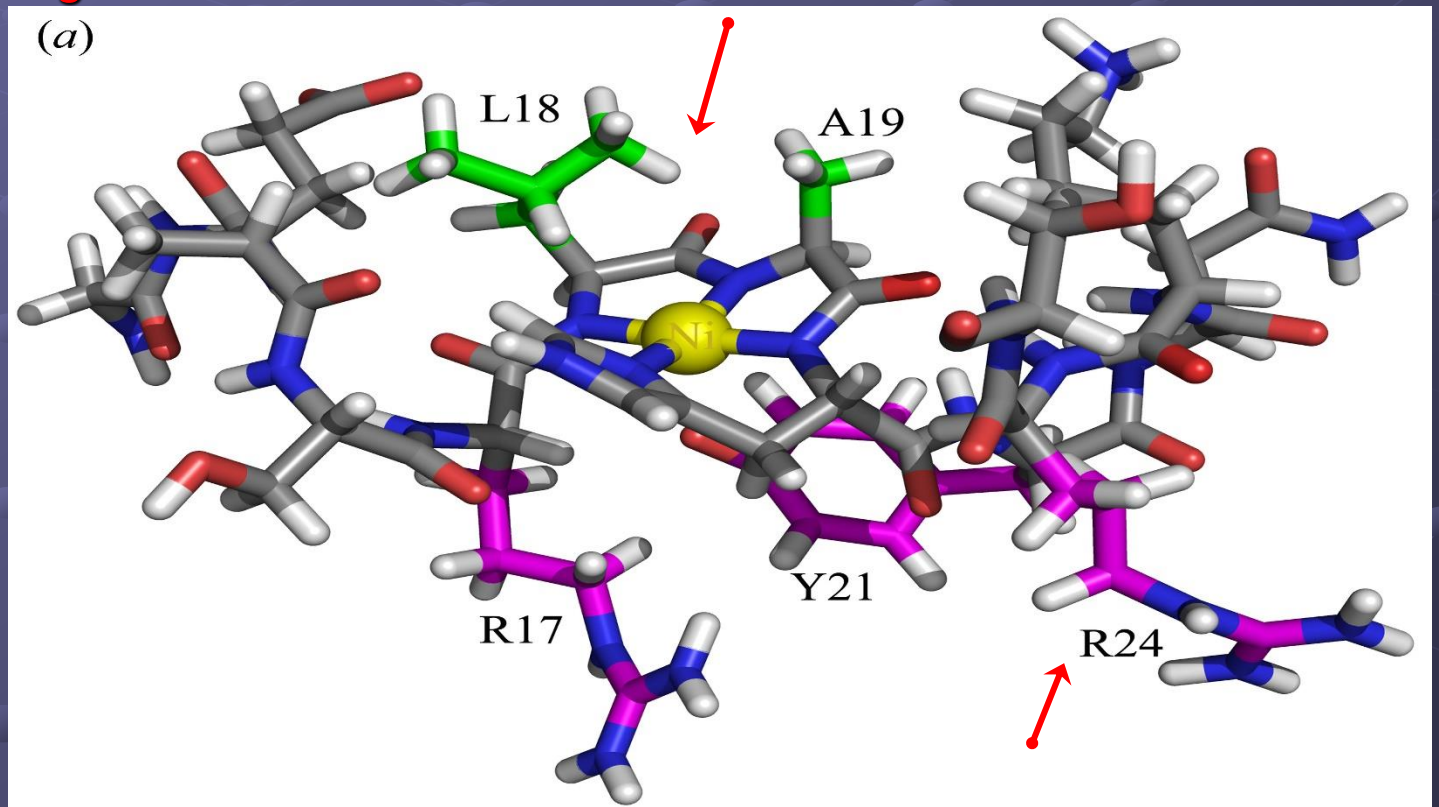
- Some interesting features of the structure

1. the location of the Tyr21 aromatic ring near the nickel coordination site indicating a possible interaction of the negative partial charge of the phenolic oxygen with the positive part of the electrostatic potential generated by the nickel complex
2. Close proximity of Arg17 to the metal centre



Ni(II) coordination towards $H2B_{63-93}$ NMR studies pH~10

3. the formation of an axial hydrophobic fence (side chains of Leu18 and Ala19, shielding one side of the coordination plane from the bulk of the solution
4. the arrangement of the Arg24 side chain near the Tyr21 ring in a tilted parallel equatorial position to the coordination plane and to the Arg17 side chain

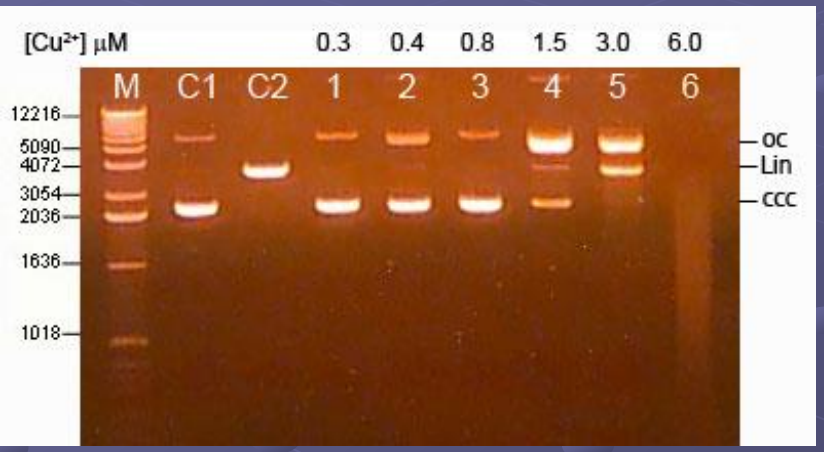


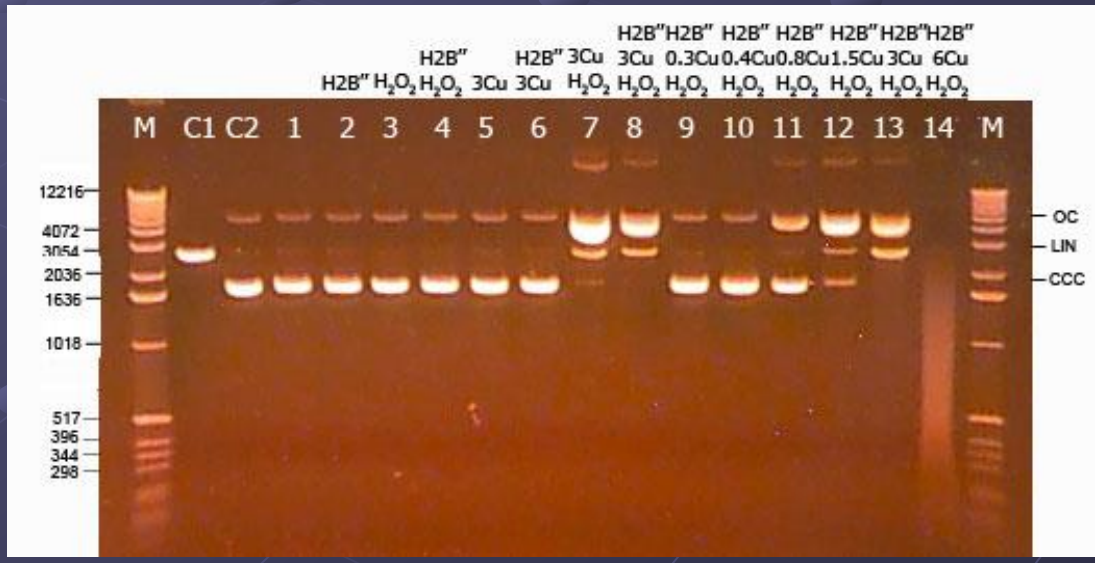
DNA strand breakage studies

$H2B_{63-93}$: Ac-NSFVNDIFERIAGEASRLAH_{20}YNKRSTITSRE-NH_2

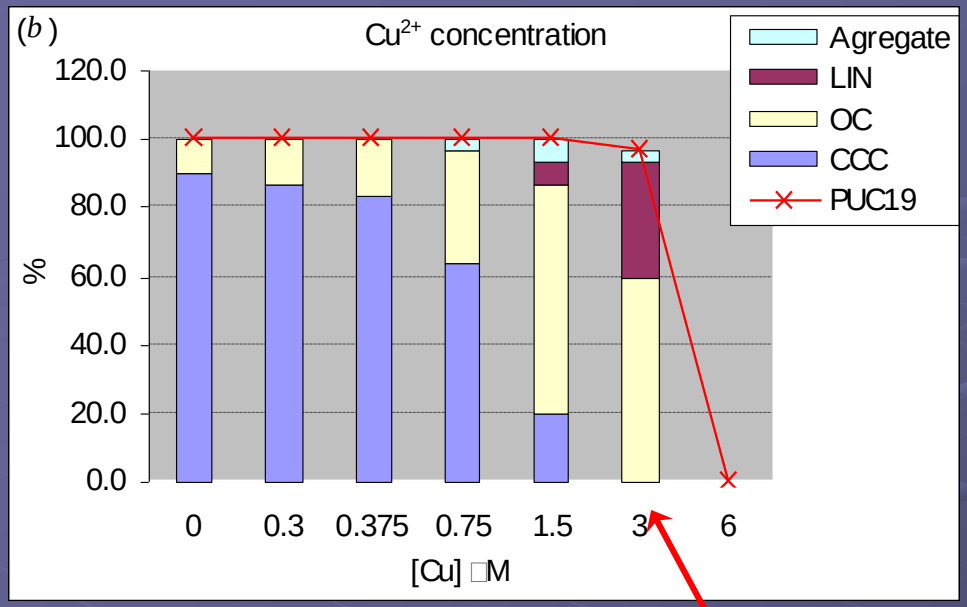
histone C-terminal fold domain model -interacts with DNA-

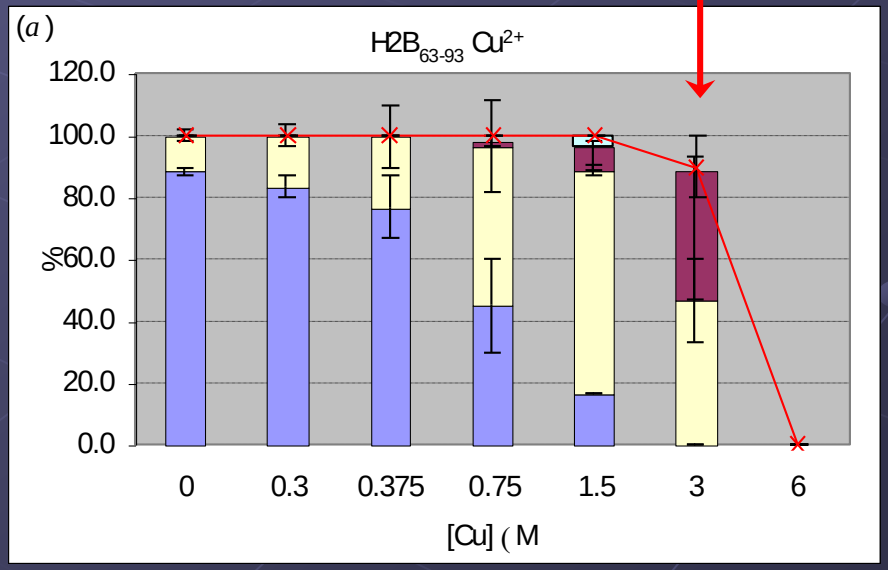
- **I. Cu(II)/peptide/plasmid pUC19/H_2O_2**





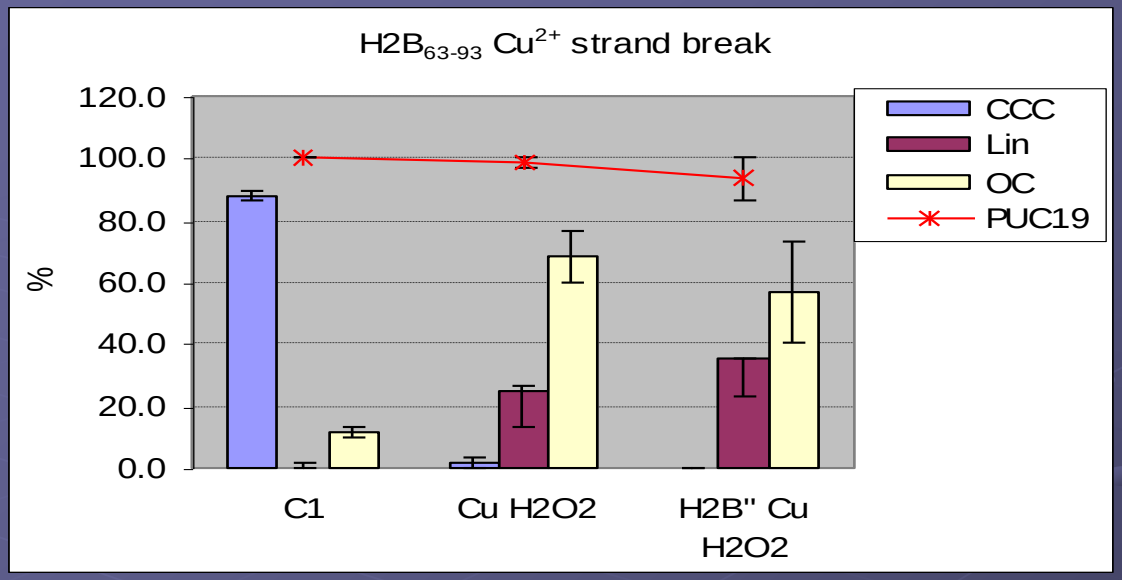
Gels bands quantification results





H_2B 3 uM

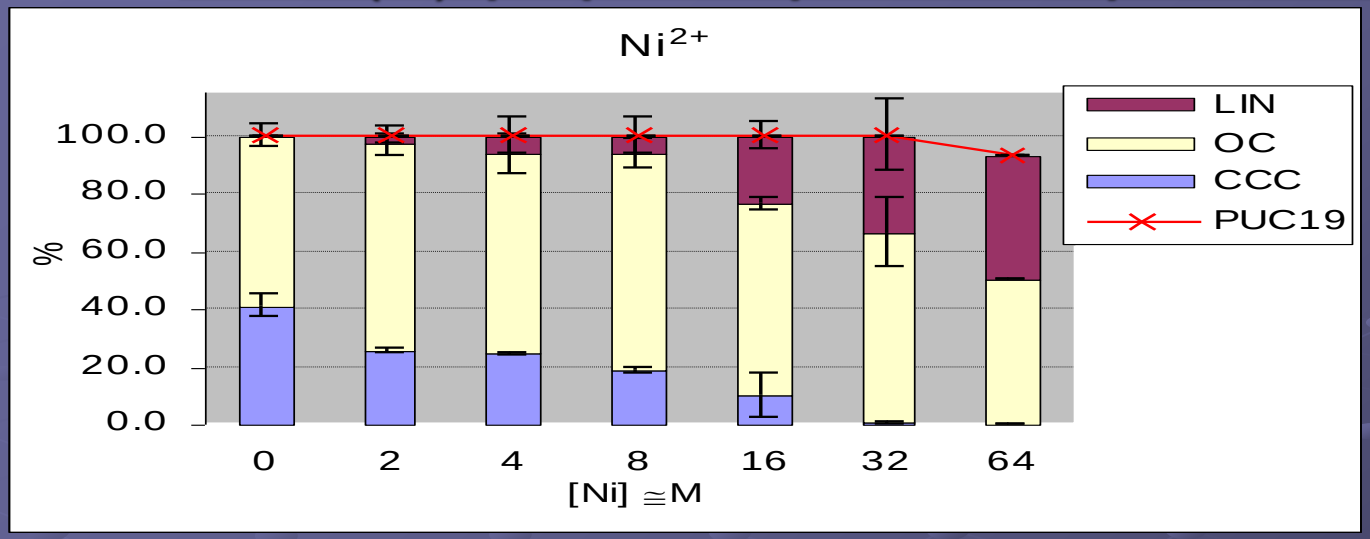
Gels bands quantification results

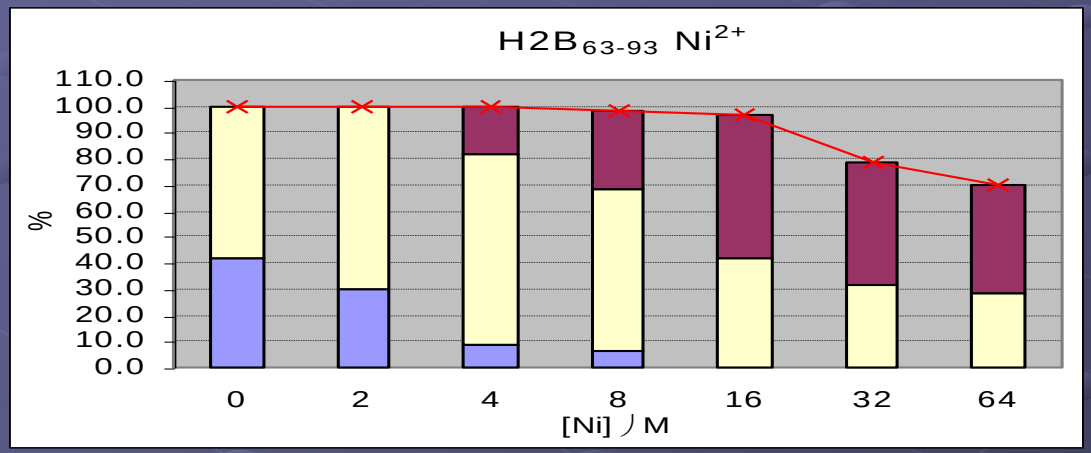


- DNA damage was observed only with Cu(II)/H_2O_2 and Cu(II)/H_2O_2/peptide combination
- The damage extent was more pronounced in the later case especially double strand scission

- neighbouring residues of the binding site (R17, K23 and R24) will in fact interact with the DNA bringing the redox active 3N complex closer to it → site specific generation of ROS →double strand scission

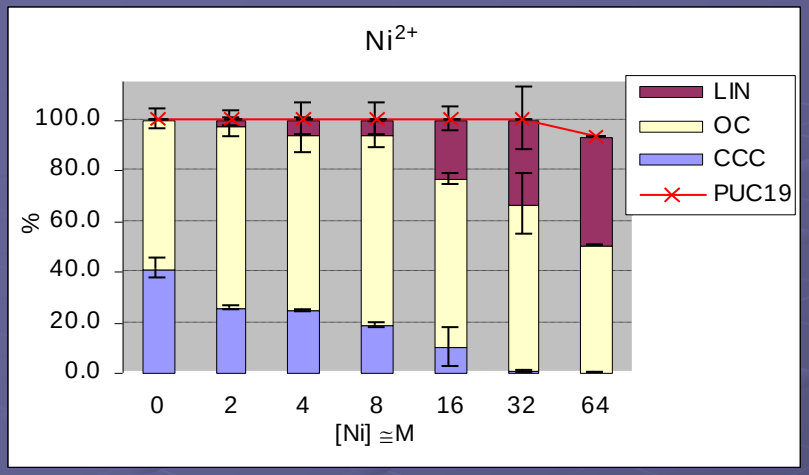
II. Ni(II)/peptide/plasmid pUC19/H_2O_2

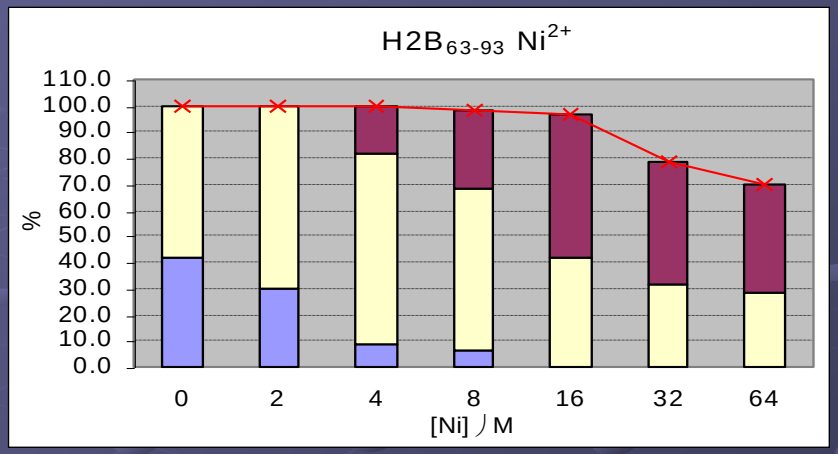




enhancement of DNA double strand scission and degradation in the presence of the peptide

Ni(II) concentration effect





- enhancement of DNA double strand scission and degradation in the presence of the peptide

The effectiveness of Ni(II) to induce DNA cleavage in the presence of H_2O_2 was rather surprising since Ni(II) is redox inactive under physiological conditions

Ni(II) is only possible to generate DNA single strand breaks, through the production of ROS in the bulk of the solution

Double strand scission may derive from single strand break in the open circular form of the peptide

supercoiled → open-circular και open-circular → linear

CONCLUSIONS

- **A. Coordination properties**

1. Cu(II) and Ni(II) ions can form stable complexes with the histone H2B peptides all over the pH range 3.5 – 11

2. At low pH values both ions interact with the imidazole N3 nitrogen atom

3. Very stable Cu(II) 3N complexes {$1N_{im}$, $2N^-$} are formed at physiological pH values (~7.4)

4. 4N {$1N_{im}$, $3N^-$} yellow coloured square planar diamagnetic Ni(II) species predominate over pH~8
5. Structural rearrangements upon coordination

CONCLUSIONS

- **B. Hydrolytic properties**

1. Hydrolytic cleavage was only observed in the case of peptides containing Ser or Thr residues near the coordination site

- **C. Oxidative properties**

1. In the presence of both long peptides, enhancement of both single/double DNA strand scission and degradation is observed, more pronounced in the case of Cu(II) ions

CONCLUSIONS

This work strongly supports the fact that histones may be the prime candidates for metal ion binding. Possible metal ion mediated hydrolysis or oxidation of either histones or DNA as indicated in this study could cause cleavage of the nucleosome core and/or DNA mutations leading to cancer.

Βιβλιογραφία

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