LABINC
Laboratório de Química Bioinorgânica e Cristalografia

"Bioinorganic Catalysis"

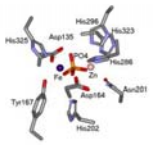
Prof. Dr. Ademir Neves

Departamento de Química – UFSC
LABINC – Laboratório de Bioinorgânica e Cristalografia
e-mail: ademir@qmc.ufsc.br
phone: (55)48 – 3721-6852 r-219
Home page:

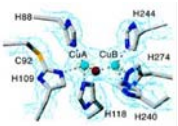
LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Research in our laboratories is concerned with the design of symmetrical and **unsymmetrical dinuclear small molecules** as structural and functional models for the active-site of metalloenzymes including **hydrolases (PAPs), proteases, and catechol oxidases.**

rkbPAP



Klabunde et al. ***J. Mol.Biol.*** 259 (1996) 737



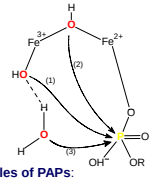
Sp Catox

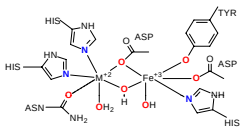
Krebs et al. ***Nature***, 5 (1998) 1084

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

PAPs – catalyze - ***hydrolysis*** - phosphorylated substrates - pH range 4-7.

Proposed Mechanisms





M II - Fe, Mn, Zn

Biological roles of PAPs:

Mammalian - Iron transport during pregnancy and macrophage-specific generation of ROS. Associated with metabolic bone diseases (osteoporosis, cancers with metastases).

Plant - May play a crucial role in immobilizing organic phosphate esters in the soil during germination.

PAP enzymes are targets for the design of drugs for a wide variety of disorders

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Catechol Oxidase

✓**Catechol oxidase** - plant enzyme - belongs to the oxidoreductase class.
✓**Active site** - Dinuclear Copper center .
✓**Catalyzes oxidation of *o*-diphenols** (like caffeic acid) to the corresponding *o*-quinones, with the reduction of molecular oxygen to water.
✓The highly reactive quinones auto-polymerize to form brown polyphenolic catechol melanins, a process thought to protect the damaged plant from pathogens or insects.
Reaction of great importance in the medical diagnosis of hormonal catechol amines (adrenalin, noradrenaline, and dopamine).

In spite of extensive studies on the mechanism of CatOx the coordination mode of the substrate remains unknown.

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Catalytic Promiscuity

Catalytic promiscuity means, in the broadest terms, the ability of a given active site to catalyze more then one chemical transformation and constitutes a very important property of many enzymes, having a natural role in evolution and, occasionally, in the biosynthesis of secondary metabolites. Important role in the diversification of enzymes by providing a duplicated gene a head start towards being captured by adaptive evolution.

R. J. Kazlauskas, ***Curr. Opin. Chem. Biol.*** 9 (2005)195-201.

Ex.

Acid phosphatase from plant uniquely exhibits chloroperoxidase activity with loss of phosphatase activity when orthovanadate is added to the apo form of the enzyme.

Carbonic anhydrase also catalyses the hydrolysis of triesters – **phosphotriesterase activity**.

Important: Very well known phenomena for a diversity of enzymes. However, very few examples are described for models.

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

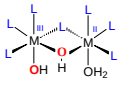
Strategy Utilized – Synthetic Hydrolase (metal ion at the catalytically active site)

Activate the substrate and/or stabilize the transition state

cis-orientated labile sites

Reduce pK_a of coordinated H_2O (provide nucleophiles at ≈ neutral pH)

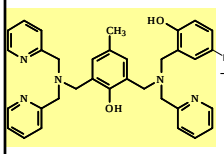
Liberate products at a reasonable rate



Use of unsymmetrical ligands – mimicking most natural metalloenzymes

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Synthesis of the unsymmetrical hetero-dinuclear FeZn and FeCu complexes:



H_2L-R and H_2py$_3$mfp

1 - $Zn(ClO_4)_2.6H_2O$
2 - $Fe(ClO_4)_3.9H_2O$
3 – NaOH/MeOH

→ $[L(OH_2)\ Fe^{III}(\mu\text{-}OH)Zn^{II}](ClO_4)_2$ **FeZnL-R**

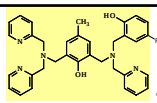
1 - $Cu(ClO_4)_2.6H2O$
2 - $Fe(ClO_4)_3.9H_2O$
3 – NaOH/MeOH

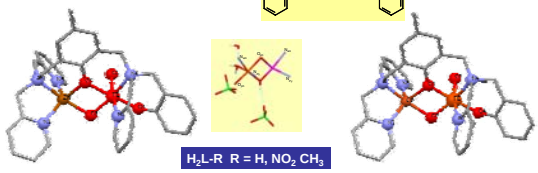
→ $[L(OH_2)Fe^{III}(\mu\text{-}OH)Cu^{II}](ClO_4)_2$ **FeCuL-R**

Schenk, Neves *et al* , *Chem. Rev.* **2006**, *106*, 3338-3363
Inorg. Chem. **2002**, *41*, 4624;
Inorg. Chem. **2002**, *41*, 5641;
Inorg. Chem. Commun. **2002**, *5*, 434 and **2003**, 6, 1161;
J. Biol. Inorg. Chem. **2005**,*10*, 319; *J. Biol. Inorg. Chem.* **2008**, *13*, 139; *J. Biol. Inorg. Chem.* **2007**, *12*, 1207

$Fe^{III}(OAc)_2M^{II}$ (M^{II} = Fe,Mn,Zn,Cu,Ni,Co)
$Ga^{III}(OAc)_2Zn^{II}$

LABINC
Laboratório de Química Bioinorgânica e Cristalografia





H_2L-R R = H, NO_2 CH_3

Zn···Fe 3.040(5)	Cu···Fe 3.020(5)
Zn-O(1)-Fe 96.2(3)	Cu-O(1)-Fe 96.2(3)
Zn-O(1)-Fe 102.4(3)	Cu-O(2)-Fe 102.9(3)

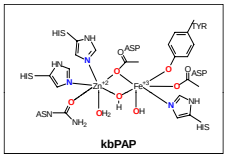
The structure is stabilized by H-contacts:
1) Bridging OH with oxygen of ClO_4^-
2) Terminal Fe-OH_2 with O1W;

$\tau = (\alpha - \beta)/60 = 0.71$ for Zn^{II} 0.70 for Cu^{II}
– Addison –
distorted trigonal bipyramidal

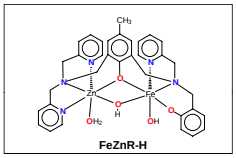
Neves et al, *JACS*, **2007**, *129*, 7486
First $[Fe^{III}(\mu\text{-}OH)Zn^{II}]$

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Comparison of the structures of FeZnL-H and kbPAP



kbPAP



FeZnR-H

Important: Both structures contain an $Fe^{III}(\mu\text{-}OH)Zn^{II}$ with Fe···Zn distances of 3.05 Å in the FeZnL-H complex and 3.2 Å in kbPAP – Krebs, Witzel *et al*., *J. Mol. Biol.* **1996**, *259*, 737.

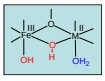
Both structures also contain terminal Fe^{III}-phenolate and Fe^{III}-OH bonds

Neves et al, *JACS*, **2007**, *129*, 7486

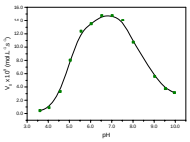
LABINC
Laboratório de Química Bioinorgânica e Cristalografia

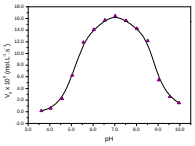
Hydrolytic Activity in the Hydrolysis of BDNPP

✓***pH dependence***



Active species





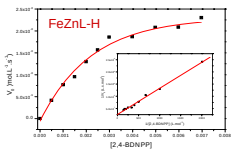
FeZnL-H – optimum at 6.5
pK_1 = 5.0 and pK_2 = 8.1

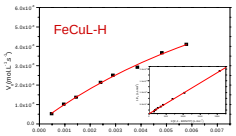
FeCuL-H – optimum at 7.0
pK_1 = 5.3 and pK_2 = 8.6

Active species under physiological conditions

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Dependence of the catalytic activity on [2,4-BDNPP] – pH optimum





186 turnovers in 24 h;
[FeZnL-H] = 5x10^{-7}M

Complex	K_{ass} (L.mol^{-1})	K_M (mol.L^{-1})	k_{cat} (s^{-1})	k_{cat}/k_{unc}
FeZnL-H p*K* = 4.66	238	4.20 x 10^{-3}	9.13 x 10^{-4}	**4830**
FeCuL-H p*K* = 5.23	117	8.52 x 10^{-3}	19.0 x 10^{-4}	**10100**

$v_0 = V_{máx}[S]/(K_m + [S])$

$1/v_0 = K_m/V_{max}.[S] + 1/V_{max}$

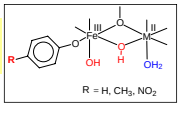
LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Kinetics parameters for FeZnL-R and FeCuL-H

Complex	pK_a	Kass (L.mol^{-1})	E= k_{cat}/K_M (mol^{-1}.L.s^{-1})	k_{cat} (s^{-1})	$E_{1/2}$ mV *vs* NHE (pH 6.0)	k_{cat}/k_{unc}
FeZnL-CH_3	4.88	192	19 x 10^{-2}	9.74 x 10^{-4}	-197	5.2 x 10^3
FeZnL-H	4.66	238	20 x 10^{-2}	9.13 x 10^{-4}	-180	4.8 x 10^3
FeZnL_Br	4.50	168	11 x 10^{-2}	6.55 x 10^{-4}	-160	3.5 x 10^3
FeZnL-NO_2	4.25	286	12 x 10^{-2}	4.20 x 10^{-4}	-100	2.2 x 10^3
FeCuL-H	5.23	117	85 x 10^{-2}	19.0 x 10^{-4}	-430	10.0 x 10^3
FeZnpy$_3$mfp	4.93	67	6 x 10^{-2}	9.53 x 10^{-4}	-252	5.1 x 10^3

Kinetic isotope effect k_H/k_D = 1.00 – 1.34

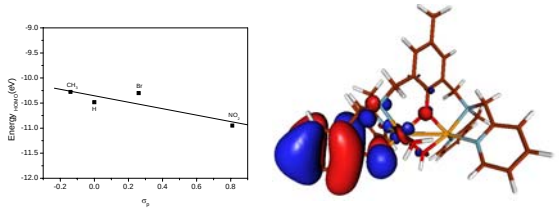
EXAFS studies - in CH_3CN/H_2O solution reveal a $Fe^{II}\cdots Zn^{II}$ bond distance of 3.043 Å for FeZnL-H in full agreement with the X-ray structure



R = H, CH_3, NO_2

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

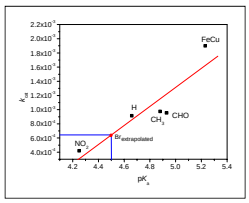
Free energy relationships – common mechanism for the hydrolysis of BDNPP - FeZnL-R complexes



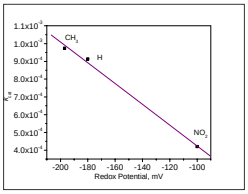
Linear correlation from calculated energies of the a-HOMO for the complexes **1-4** and the Hammett parameter (σ_p).

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Free energy relationships – common mechanism for the hydrolysis of BDNPP - FeZnL-R complexes



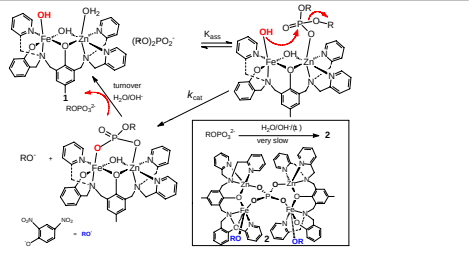
k_{cat} *vs* pK_a



k_{cat} *vs* $E_{1/2}$ (mV)

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Based on all the data presented above we propose the following mechanism:

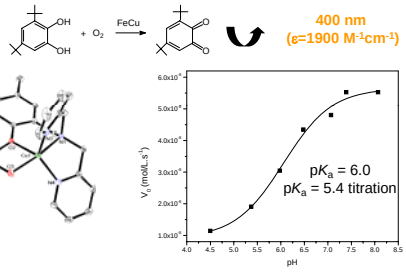


Neves et al, *JACS*, **2007**, *129*, 7486

180 turnovers, first ex., with a plausible mechanism for PAPs given the similarities of both structures

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Catecholase Activity of the **FeCuL-CH$_3$** Complex

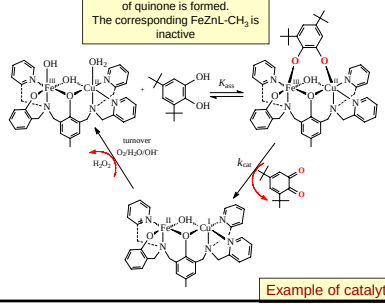


Conditions: [FeCuL-CH$_3$] = 2.4x10^{-5} mol.L^{-1}; [3,5-*DTBC*] = 5.0x10^{-3} mol.L^{-1}; MeOH/H_2O (30:1) at 25 ºC.

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Proposed Mechanism for the oxidation of **3,5-D*t*-BC by FeCuL-CH$_3$**

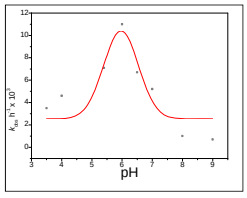
H_2O_2 is formed and in the absence of O_2 only one equivalent of quinone is formed. The corresponding FeZnL-CH$_3$ is inactive



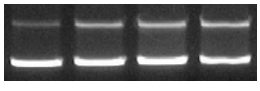
Example of catalytic promiscuity

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

pH dependence of DNA cleavage by [FeCuL-H]

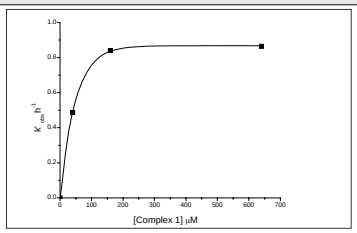


Conditions:
[Buffers] = 10 mM; *I*=0.1 M
[FeCuOH] = 40 μM
[pBSK II] = 34 μM bp
Reaction Time 0, 4, 8 and 11 h, 37ºC



LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Dependence of the DNA Cleavage on [FeCuL-H]



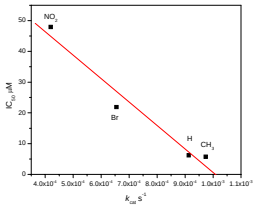
Under pseudo-Michaelis-Menten conditions the following parameters were obtained: k_{cat} = 0.98 h^{-1}; $t_{1/2}$ = 42 min; K_m = 3.9 x 10^{-5} M, Providing a rate enhancement of **2.7 x 10^7** over the uncatalyzed reaction thus being one of the most active described to date.

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Cytotoxicity Studies on cancer Cells

$Fe^{III}(\mu\text{-}OH)Zn^{II}$

Complex	IC_{50} (μM) K562
1 – CH_3	5.73±06
2 - H	6.2±05
3 - Br	21.9±2.0
4 – NO_2	47.9±4.0



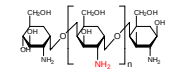
It is worth noting that cytotoxic activity increases in the same order as the hydrolysis of 2,4-bdnpp and DNA.

A. Neves, et al, ***J. Biol. Inorg. Chem.*** 2009 submitted.

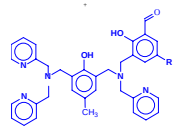
LABINC
Laboratório de Química Bioinorgânica e Cristalografia

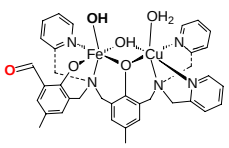
Innovation - New Projects Underway in the Group

Development of new bioinorganic-materials with chitosan as drug-delivery systems



Non-toxic natural material





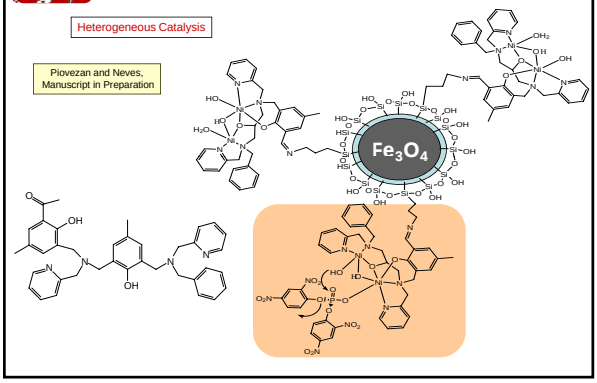
Complex already synthesized and fully characterized

Heterogeneous Catalysis

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Heterogeneous Catalysis

Piovezan and Neves, Manuscript in Preparation



LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Complex	V_0 (mol.L.s^{-1})	K_M (mol/L)	k_{cat} (s^{-1})	$^aK_{AS}$ (L/mol)	^bE (L.s/mol)	F (k_{cat}/k_{uncat})	Turn over (1h)
NiNibppamff	5.37x10^{-7}	1.57x10^{-3}	**5.4x10^{-2}**	**637**	34.2	298.300	**33.4**
Si3AP-NiNi	9.20x10^{-7}	7.30x10^{-4}	**3.5x10^{-2}**	**1370**	48.2	195.500	**78.5**
Si3APTS-NiNi	3.40x10^{-7}	6.9x10^{-4}	**4.6x10^{-2}**	**1450**	66	255.500	**32.7**
Simag-NiNi	4.19x10^{-7}	1.21x10^{-3}	**3.5x10^{-2}**	**826**	29	196.650	**23**

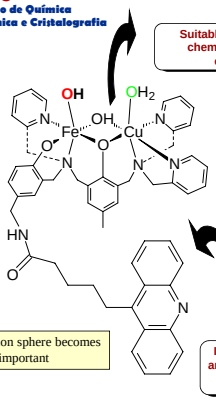
[a] $K_{AS} = 1/K_M$. [b] $E = k_{cat}/K_M$ (catalytic efficiency).

at pH 9.0
Si3AP – sílica 3-aminopropyl
Si3ATPS – sílica 3-aminopropyl trietoxisilano
Simag – magnetic particule recovered with silica

Piovezan and Neves, Manuscript in Preparation

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Suitable for Catalysis as chemical Nuclease or catecholase

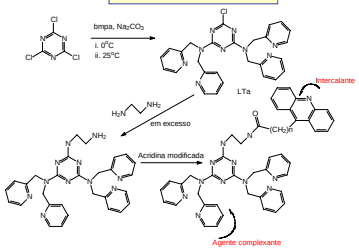


The second coordination sphere becomes much more important

Intercalates with DNA and shows Fluorescent properties

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Very recent results



Preliminar results reveal that the $Cu^{II}Cu^{II}$ complex of Lta catalyses the oxidation of 3,5-di-tert-butylcatechol with high efficienty and formation of H_2O as observed in the enzyme catechol oxidase. The complex also catalyses the hydrolysis of DNA and proteins (BSA)

Catalytic promiscuity and possibility to solve the mechanism of catechol oxidase

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

Facilities available in our Labs in Collaboration with other Groups

1) **X-ray structure determinations.** (UFSC - Florianópolis/Brazil – Profs. Ademir Neves & Adailton Bortoluzzi - New CCD available up from 2010).
2) **EXAFS – structure in solution.** (Australia – Prof. Dr. G. Schenk).
3) **Magnetic susceptibility and MCD** in the solid state and in solution between 4.2 e 350 K (Germany– Prof. Dr. W. Haase).
4) **HFEPR** measurements. (USA - Prof. Dr. Joshua Telser).
5) **DNA and protein interactions.** (UFSC - Florianópolis/Brazil – Profs. Hernan Terenzi & Ademir Neves).
6) **Cytotoxic Activity.** (UFMG – Prof. Dr. Elene Pereira Maia).

LABINC
Laboratório de Química Bioinorgânica e Cristalografia

O grupo unido, jamais será vencido

LABINC
Laboratório de Química Bioinorgânica e Cristalografia