
Metal complexes as hydrolytic catalysts of phosphate diester cleavage

2020/5/14

Literature Seminar

M1 Fujiyoshi

Contents

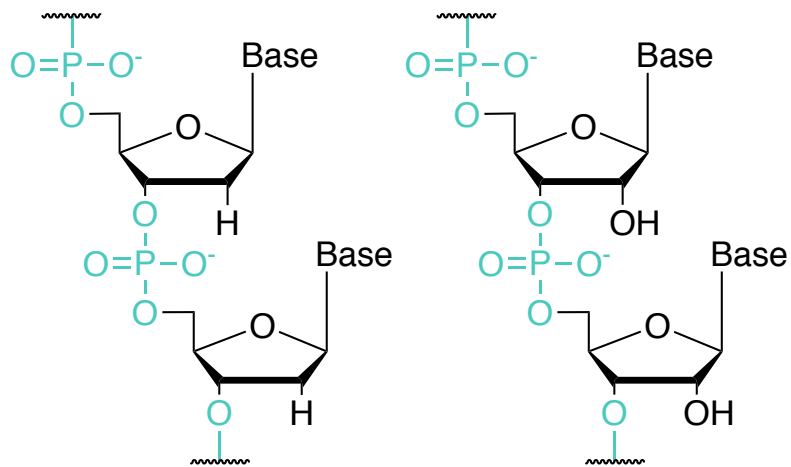
- Introduction
- Examples of artificial catalyst ligand
- Ligand with hydroxy group
- DNA plasmid cleavage
- Summary

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Phosphate diester in nature

Nucleic acids

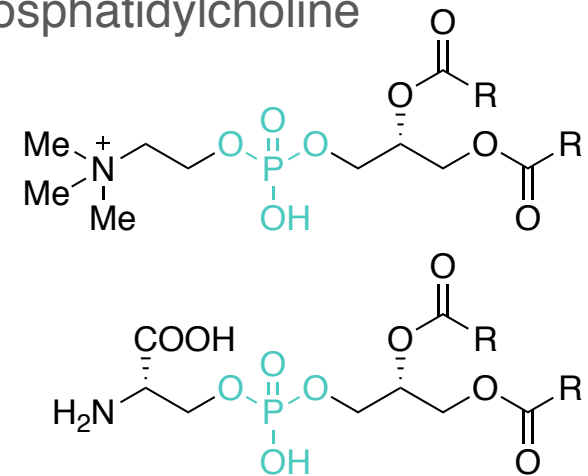


DNA

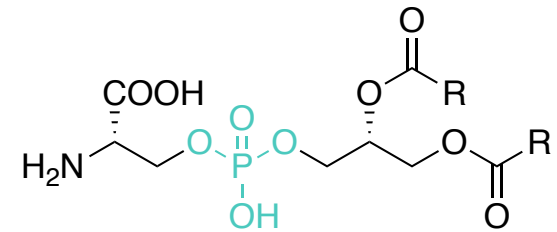
RNA

Phospholipids

Phosphatidylcholine



Phosphatidylserine



Enzymes cleave phosphate ester

Protein phosphatases

Remove phosphate group from Ser/Thr/Tyr

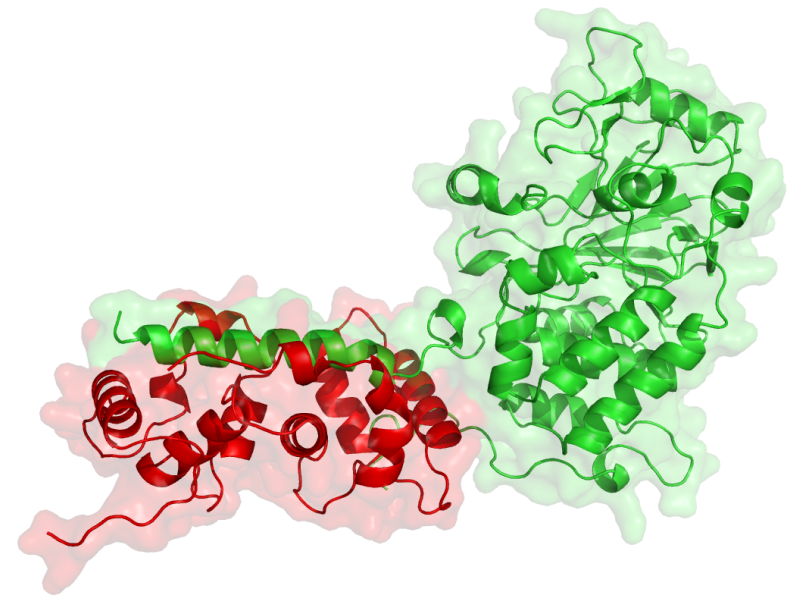
Nucleotidases

Nucleotide to nucleoside

DNA cleavage (DNase, Topoisomerase)

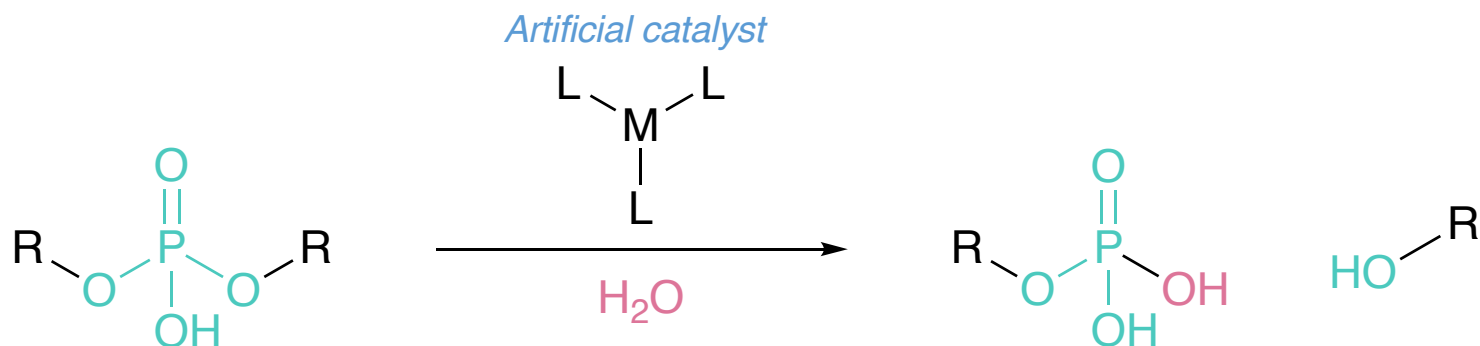
In gluconeogenesis

Remove phosphate group from six-carbon phosphate intermediate



Calcineurin (PP2B) is a protein phosphatase enzyme involved in immune system function.

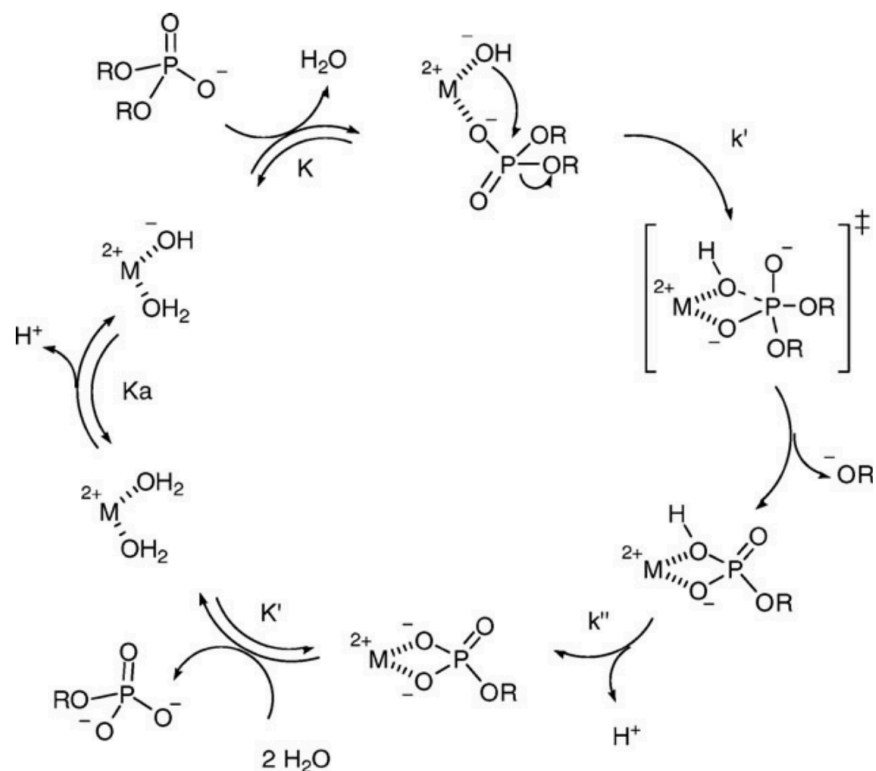
Artificial catalyst mimicking phosphatase



What is the motivation of studies in this field?

1. For a better understanding of the chemistry of the corresponding hydrolytic enzymes
2. For the detoxification of pesticides and chemical weapons (which often have phosphate ester-like structures)
3. For employments as artificial restriction enzymes for molecular biology

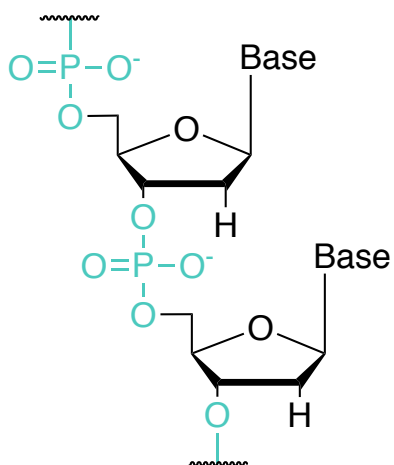
Hydrolysis catalyzed by a metal ion



1. Deprotonation of coordinated water
2. Ligand exchange between water and phosphate diester
3. Intramolecular nucleophilic attack of OH⁻
4. Pentavalent intermediate
5. Departure of leaving group
6. Deprotonation of phosphate monoester
7. Ligand exchange between water and phosphate monoester

DNA model substrate

DNA



DNA

Half-life time ($t_{1/2}$): 100,000~100,000,000 years

(in water, 25 °C, pH = 7)

DNA is tremendously resistant to hydrolysis due to its polyanionic nature.

Bis para-nitrophenyl phosphate (BNP)

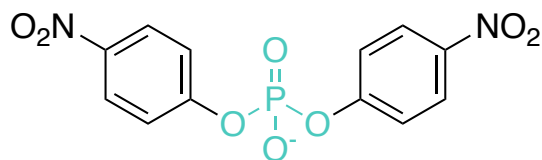
Rate of spontaneous hydrolysis: $1.6 \times 10^{-11} \text{ s}^{-1}$

$t_{1/2}$: 1300 years

(in water, 25 °C, pH = 7)

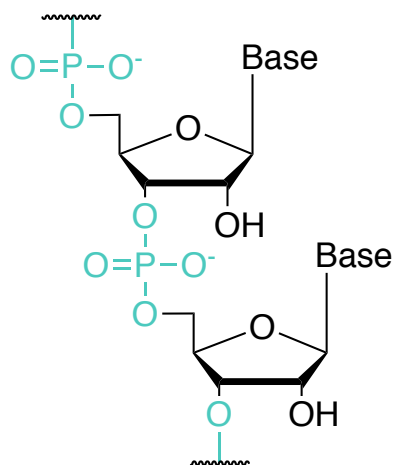
→ rate-limiting step is nucleophile attack (not leaving group departure)

BNP



RNA model substrate

RNA



RNA

Half-life time: **110 years** (in water, 25 °C, pH = 7)

RNA is less resistant to hydrolysis than DNA due to the presence of the 2'-hydroxyl group on the ribose ring, which acts as internal nucleophile in the cleavage reaction.

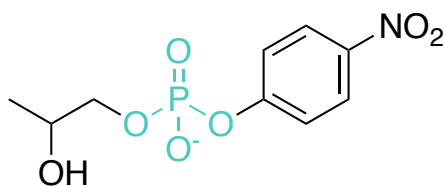
2-hydroxypropyl-para-nitrophenyl phosphate(**HPNP**)

The pseudo-first-order rate constant for the cleavage of HPNP:

$2.0 \times 10^{-8} \text{ s}^{-1}$ (in water, at 25 °C, pH = 7.0)

(Half-life time: about **1 year**)

HPNP

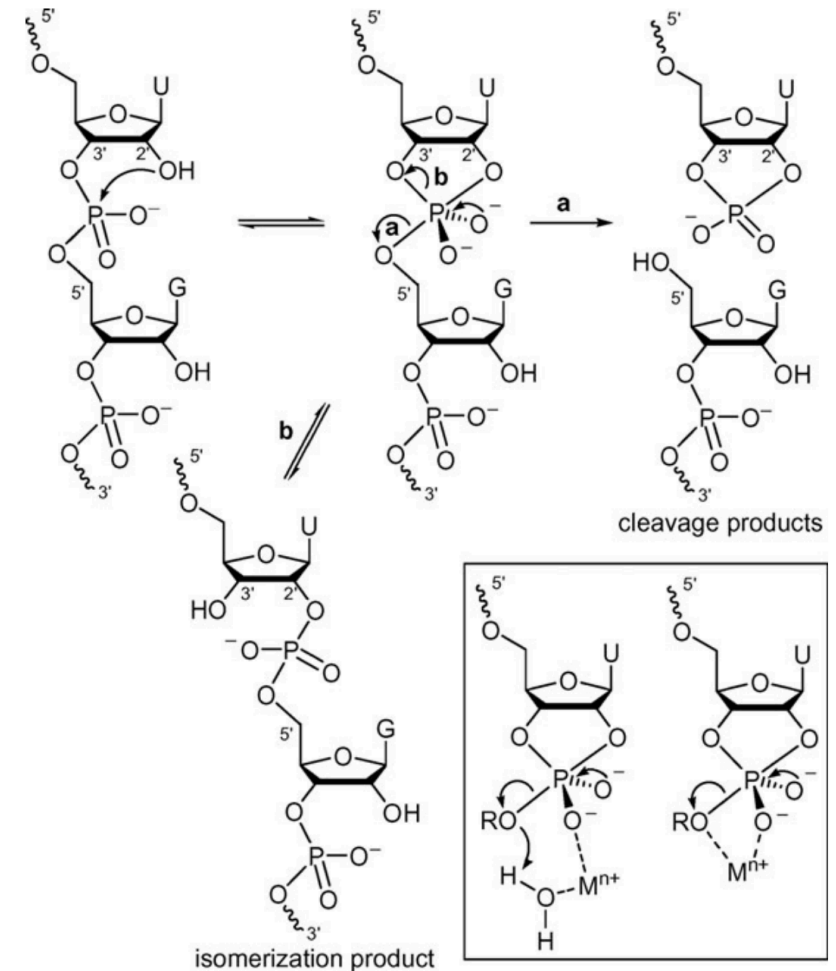
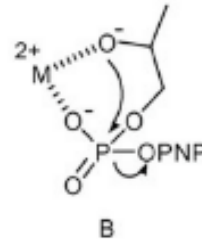
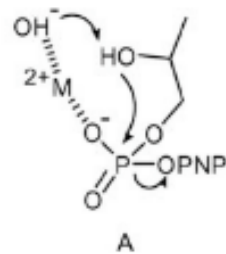
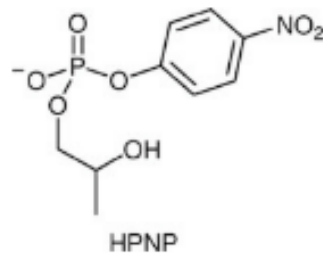


RNA cleavage reaction mechanism

Hydrolysis of RNA

Different mechanism from hydrolysis of DNA

→ Intermolecular nucleophilic attack by hydroxyl group



What metal ion is suitable for this catalyst?

In Nature

Mg^{2+} , Zn^{2+} , Ca^{2+} , Fe^{2+}

In Artificial system

Zn^{2+} , Cu^{2+} , Co^{3+} , Fe^{3+} and lanthanide ions (Eu^{3+} , Ce^{4+})

better Lewis acid and better reactivity

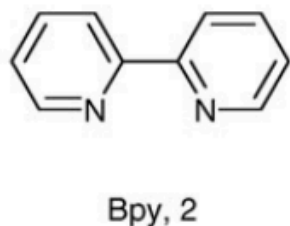
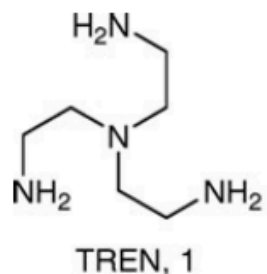
Zn^{2+} is the only metal frequently encountered in both natural and artificial agents.

- A good Lewis acid
- Exchange ligands rapidly
- Not toxic
- Not redox active
- No ligand field stabilization energy

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The first study of Zn complex catalyst for hydrolysis of BNP



TREN (1): $k_{\text{BNP}} = 5.1 \times 10^{-8} \text{ s}^{-1}$
($\times 2$ over background reaction)

Bpy (2): $k_{\text{BNP}} = 1.4 \times 10^{-6} \text{ s}^{-1}$ ($\times 53$)

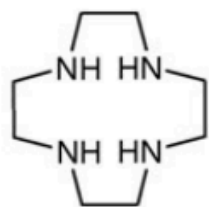
pH = 7.0, 75 °C, 1 mM catalyst, 0.1 M NaNO₃

M. A. De Rosch and W. C. Trogler, *Inorg. Chem.*, 1990, 29, 2409–2416.

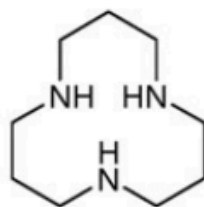
The different activity of the two ligands

→ The availability of free binding sites in the complexes

The number of ligand bindings to a metal ion



[12]aneN₄, 3



[12]aneN₃, 4

[12]aneN₄ (3): $k_{\text{BNP}} = 2.8 \times 10^{-9} \text{ s}^{-1}$ (×46)

[12]aneN₃ (4): $k_{\text{BNP}} = 3.3 \times 10^{-8} \text{ s}^{-1}$ (×550)

pH = 7, 35 °C, 1 mM catalyst, 0.2 M NaClO₄

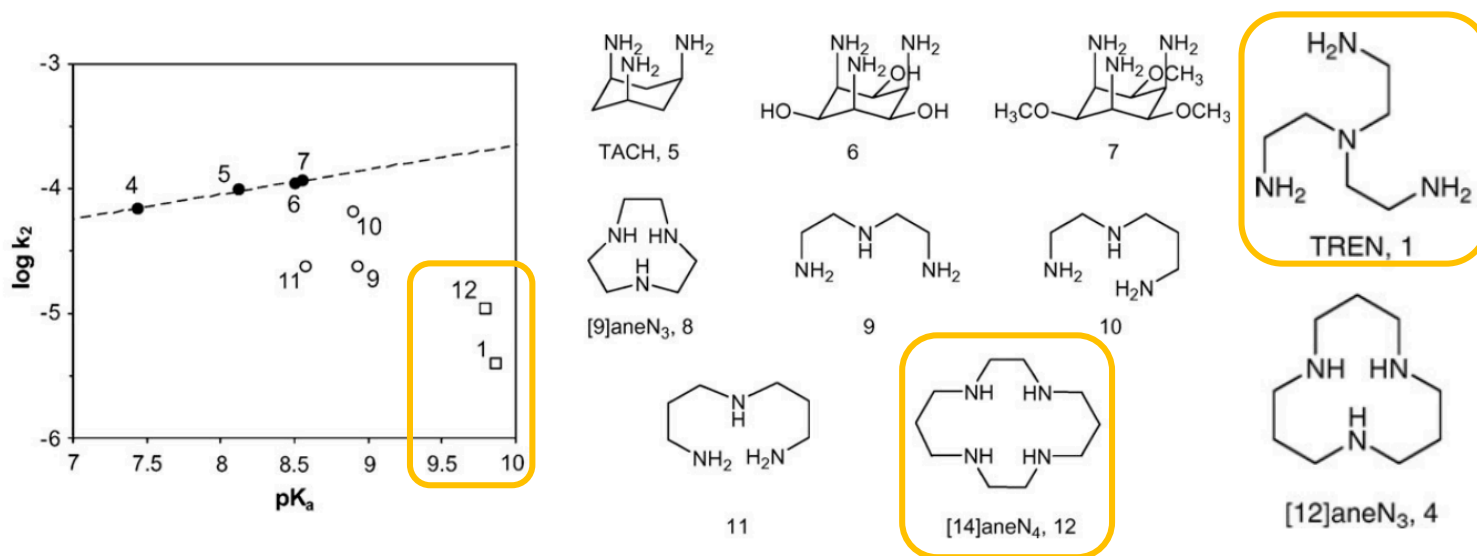
T. Koike and E. Kimura, *J. Am. Chem. Soc.*, 1991, 113, 8935–8941.

Tridendate ligand > Tetradendate ligand

→ The occupation of binding sites on the metal ion

→ The acidity of the metal-bound water molecule

Tetradentate ligand



L. Bonfa', M. Gatos,
F. Mancin, P. Tecilla
and U. Tonellato,
Inorg. Chem., 2003,
42, 3943–3949.

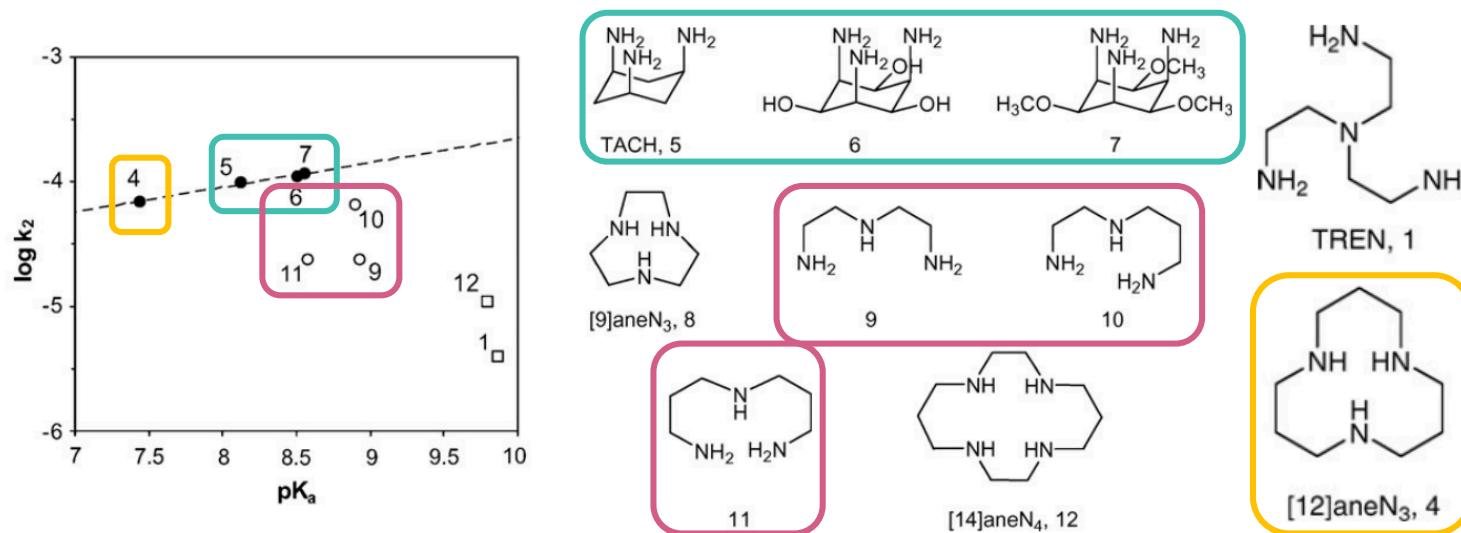
[14]aneN₄ (12) > TREN (1) (3-fold)

Common: Tetradentate and pK_a of water

TREN (1) wraps around the metal in a trigonal bipyramidal geometry

Less space for additional ligands than the facial coordination mode of [14]aneN₄ (12)

Tridentate ligand



L. Bonfa', M. Gatos,
F. Mancin, P. Tecilla
and U. Tonellato,
Inorg. Chem., 2003,
42, 3943–3949.

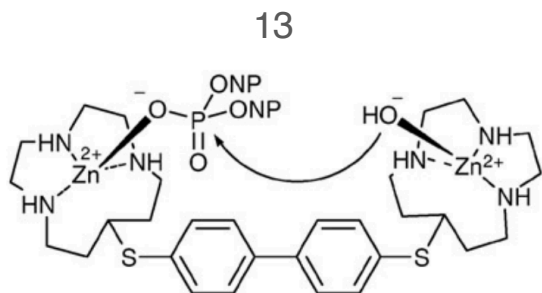
Tripodal ligand (5, 6, 7) > Linear ligand (9, 10, 11)

A linear correlation with a slope value (β_{nuc}) of 0.20 (5, 6, 7)

1. A strongly acidic metal ion activates the substrate toward nucleophilic attack
2. The correlated increase in acidity of the metal-bound water decreases its nucleophilicity

[12]aneN₃ (4): the most reactive Zn-based monometallic agent at pH 7

Bimetallic complex for BNP hydrolysis

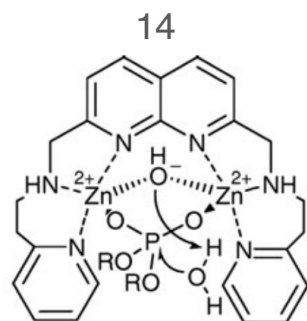


(13): $k_{\text{BNP}} = 6.4 \times 10^{-6} \text{ s}^{-1}$, ($\times 2000$)
 ($\times 5$ over monometallic system)

in water 20 % DMSO, 55 °C, pH = 8.36

W. H. Chapman, Jr and R. Breslow, *J. Am. Chem. Soc.*, 1995, 117, 5462–5469

substrate binds to one Zn^{2+} , OH^- to the other Zn^{2+}



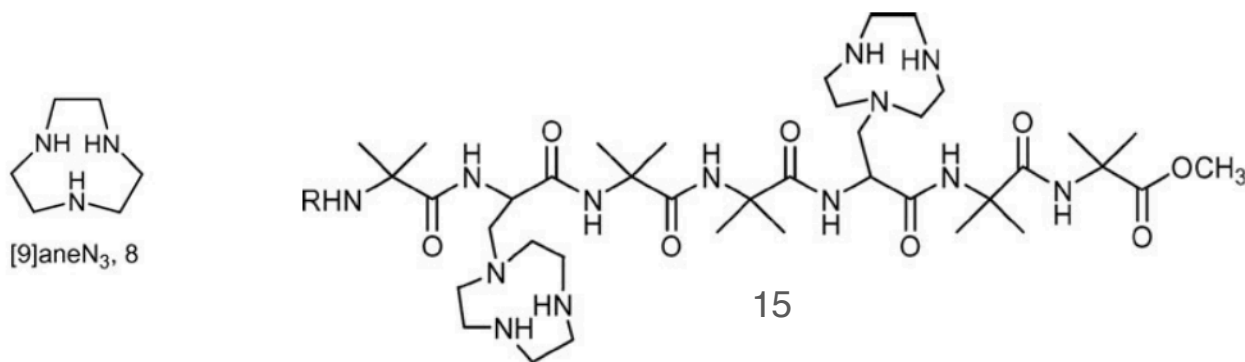
BPAN (14): $k_{\text{BNP}} = 3.7 \times 10^{-7} \text{ s}^{-1}$ ($\times 9200$)
 ($\times 1.8$ over monometallic system)

in water 10 % DMSO, pH 7, 40 °C

N. V. Kaminskaia, C. He and S. J. Lippard, *Inorg. Chem.*, 2000, 39, 3365–3373.

μ -hydroxo bridge \rightarrow general base catalyst

Plasmid DNA cleaving bimetallic system



(15): cleavage of pBR322 DNA (12 μ M bp)

$k = 1.0 \times 10^{-5} \text{ s}^{-1}$ ($\times 20$ over monometallic system)

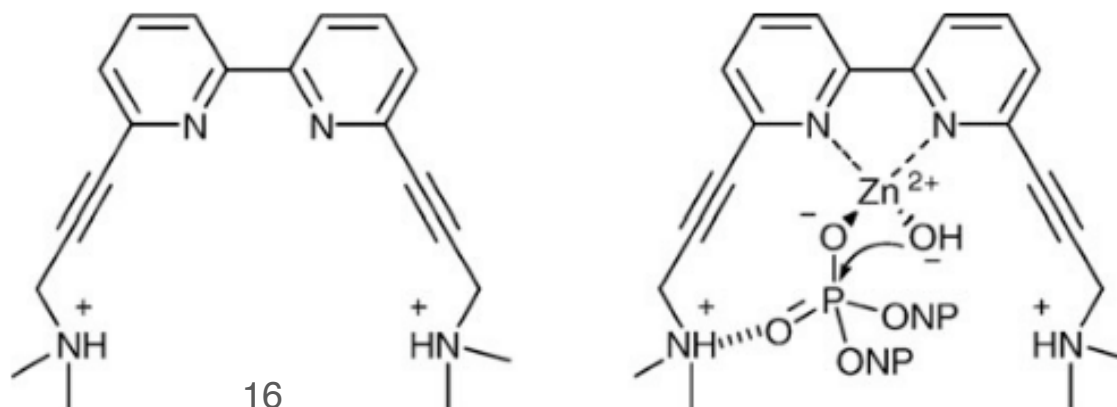
in 20 mM HEPES, pH 7.0, 37°C, 3.6 μ M (complex)

3₁₀-helical conformation

→ bind to DNA by inserting the two macrocycles within three adjacent phosphate groups

C. Sissi, P. Rossi, F. Felluga, F. Formaggio, M. Palumbo, P. Tecilla, C. Toniolo and P. Scrimin, *J. Am. Chem. Soc.*, **2001**, 123, 3169–3170.

Cooperation between Zn^{2+} ions and organic functional groups



(16): Zn^{2+} complex ($\times 5$ over Bpy) (in MeCN/water 19 : 1)

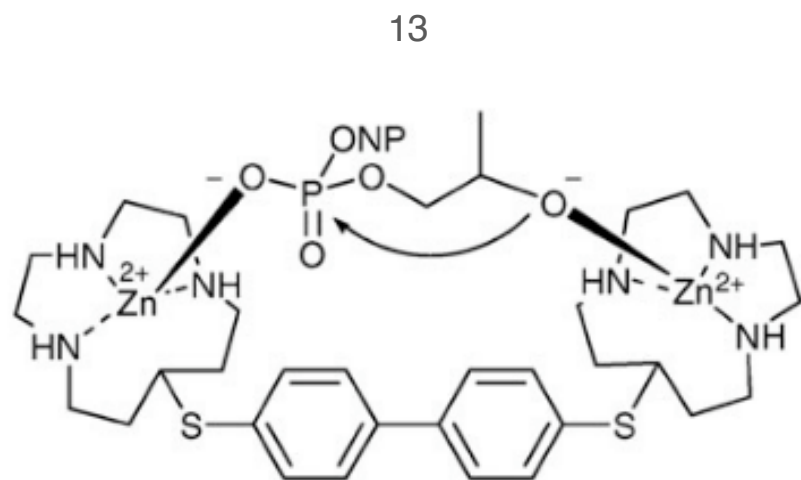
E. Kovari and R. Krämer, *Chem. Ber.*, **1994**, 127, 2151–2157

Cu^{2+} complex is more reactive ($\times 4 \times 10^7$ over the background reaction) pH = 7, 20 °C

→ The square planar coordination geometry of this metal placed the substrate in a better position to interact with the ammonium groups.

E. Kovari and R. Krämer, *J. Am. Chem. Soc.*, **1996**, 118, 12704–12709.

Bimetallic complex for HPNP



(13): ×1072 for HPNP

×39 for UpU

×5 over the monometallic complex

One metal ion binds the phosphate oxygen

→ Lewis acid activation

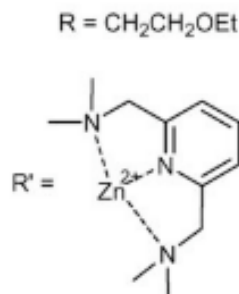
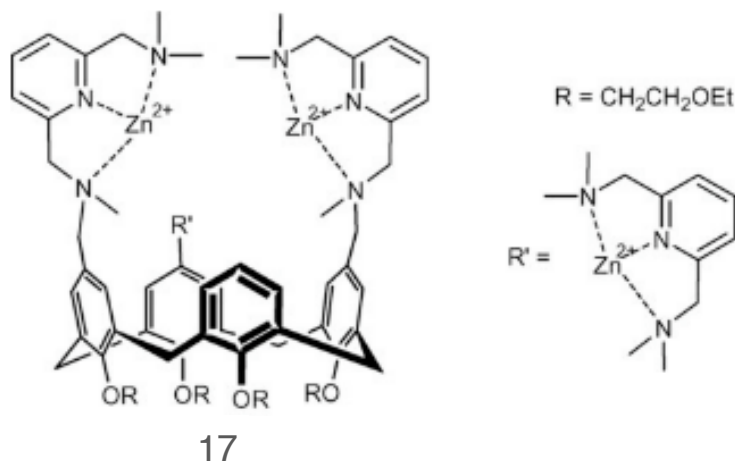
The second coordinates to the substrate hydroxyl group

→ Promote deprotonation and subsequent nucleophilic attack

π -stacking between ligand and substrate

W. H. Chapman, Jr and R. Breslow, *J. Am. Chem. Soc.*, **1995**, 117, 5462–5469

Calixarene-based trimetallic complex



Calix[4]arene (17)

HPNP transesterification **×32000**

×35 over monometallic system

in 50% water/CH₃CN, pH 7, 25 °C

Hydrophobic pocket → the binding of the substrate

Flexibility → adjustment of the intermetallic distance

Not active for simple phosphate ester

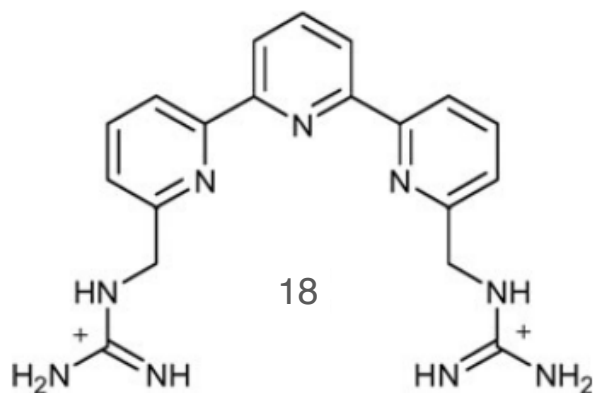
→ One Zn²⁺ activates phosphate and second promotes deprotonation of hydroxyl group.

RNA UpU: **×8600**, UpU and GpG > ApA

→ Third Zn²⁺ ion binds acidic amide of U or G

- P. Molenveld, et al., *Chem. Soc. Rev.*, **2000**, 29, 75–86;
P. Molenveld, et al., *J. Org. Chem.*, **1999**, 64, 3896–3906;
P. Molenveld, et al., *Angew. Chem., Int. Ed.*, **1999**, 38, 3189–3191;
P. Molenveld, et al., *Eur. J. Org. Chem.*, **1999**, 3269–3275;
P. Molenveld, et al. *J. Am. Chem. Soc.*, **1997**, 119, 2948–2949.
R. Cacciapaglia, et al., *J. Am. Chem. Soc.*, **2006**, 128, 12322–12330.
R. Cacciapaglia, et al., *J. Org. Chem.*, **2005**, 70, 624–630

Guanidinium group promotes the reaction



The terpyridine-like ligand bearing two guanidinium groups (18)
Hydrolysis of RNA ApA

×6 magnitude (pH = 7.4, 37 °C)

×3300 over complex without guanidium group

×9 over complex substituted for ammonium group

- ① Hydrogen bonding
- ② Electrostatic interactions
- ③ Acid catalysis by the two guanidinium groups

→ additional substrate activation

→ transition state stabilization

→ possibly leaving group assistance

H. Ait-Haddou, J. Sumaoka, S. L. Wiskur, J. F. Folmer-Andersen and E. V. Anslyn, *Angew. Chem., Int. Ed.*, **2002**, 41, 4014–4016.

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- **Ligand with hydroxy group**
- DNA plasmid cleavage
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Serine at the active site of alkaline phosphatase

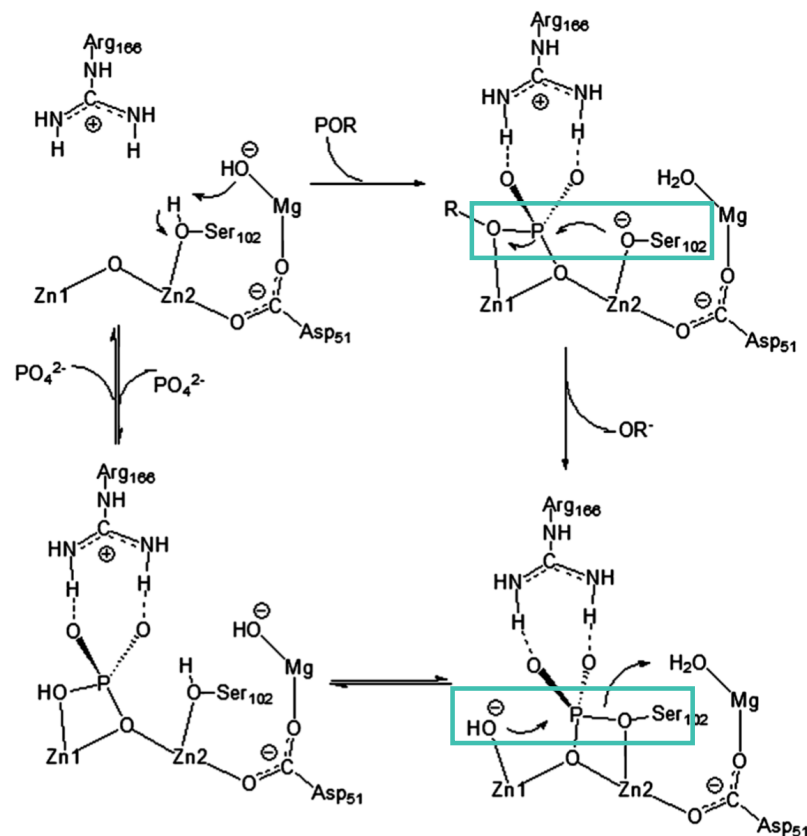
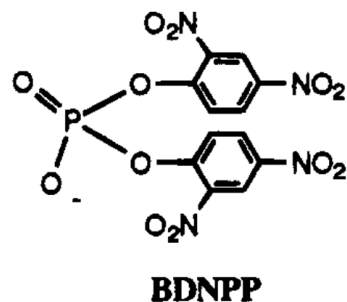
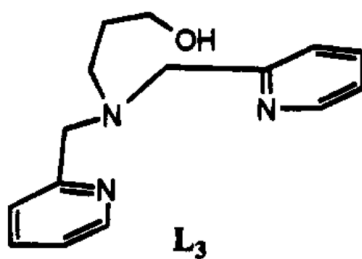
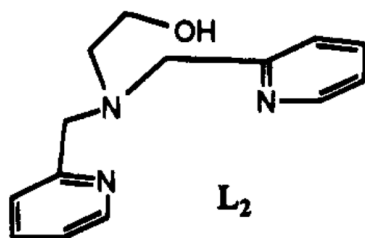
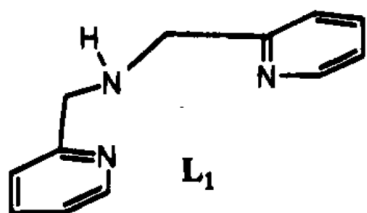


Fig. 1 Proposed mechanism of three-metal ion catalysis in the hydrolysis of phosphate monoesters by AP. Most of the ligands of Zn1, Zn2 and Mg are not shown

Cleavage of phosphodiester catalysed by Cu complex with –OH group



Reactivity

CuL₃ **×100** over CuL₂
 ×30 over CuL₁

CuL₁: direct hydrolysis

CuL₂: mainly hydrolysis

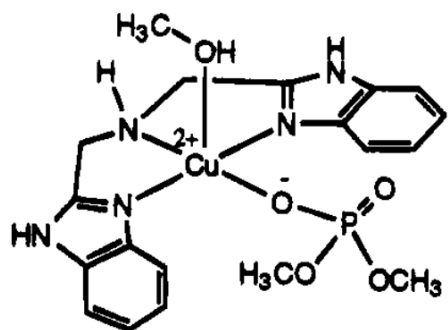
& some transesterification

CuL₃: mainly transesterification

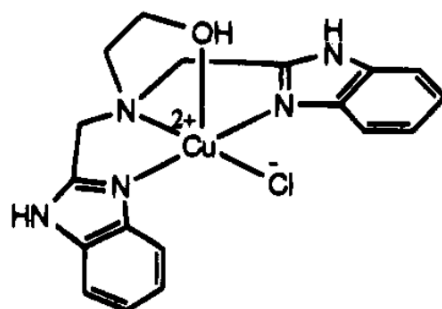
→ similar pK_a (~ 8.8)

→ The difference between CuL₃ and CuL₂ is derived from the structure.

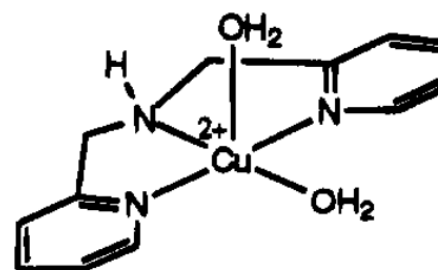
Structural insight of Cu complex with –OH group



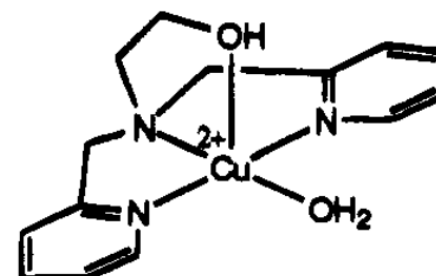
$\text{CuL}_1'(\text{P})$



CuL_2'



CuL_1



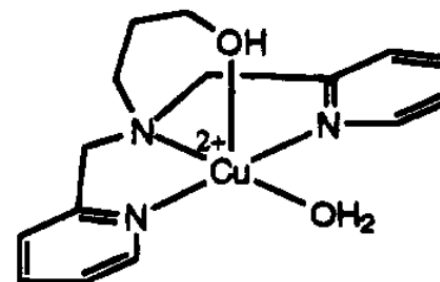
CuL_2

Bond angle

$\text{CuL}_1'(\text{P})$: $\text{O}-\text{Cu}-\text{O}$ 95.70° , $\text{N}-\text{Cu}-\text{O}$ 99.34°

CuL_2' : $\text{O}-\text{Cu}-\text{Cl}$ 102.75° , $\text{N}-\text{Cu}-\text{O}$ 79.20°

$\text{CuL}_3(\text{predict})$: $\text{O}-\text{Cu}-\text{O} < 95.70^\circ$, $\text{N}-\text{Cu}-\text{O} > 99.34^\circ$



CuL_3

The distance between phosphate and alkoxide or hydroxide
 $\text{CuL}_3 > \text{CuL}_2 > \text{CuL}_1$ (= reactivity)

Pyridine amino group promote hydrolysis

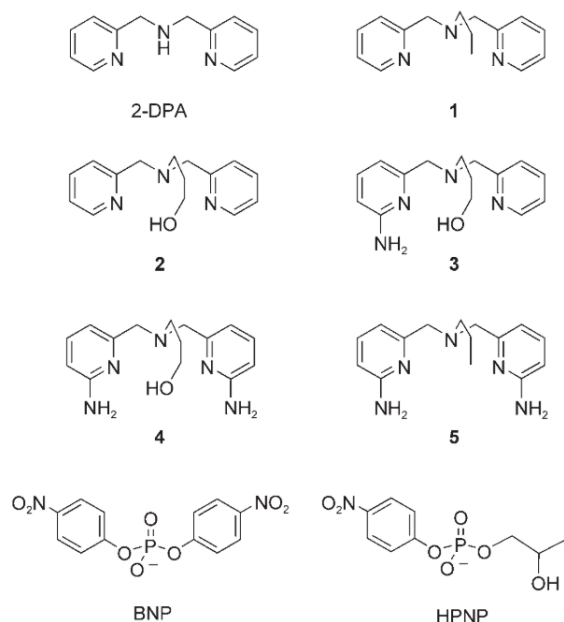


Table 3. Kinetic acid dissociation constants for the coordinated species (pK_a^n) and second-order rate constants (k_2) for BNP and HPNP cleavage by using Zn^{II} complexes of ligands **1**–**5** in water at 25 °C.^[a]

Ligand	BNP			HPNP		
	pK_a^1	pK_a^2	k_2 [$M^{-1}s^{-1}$]	pK_a^1	pK_a^2	k_2 [$M^{-1}s^{-1}$]
1	— ^[b]	— ^[b]	— ^[b]	9.1	10.9	3.9×10^{-2}
2	8.3 ^[d]	10.9 ^[d]	$4.2 \times 10^{-4[d]}$	8.8	10.9	2.1×10^{-2}
3	8.6	11.0	3.8×10^{-3}	— ^[c]	— ^[c]	— ^[c]
4	7.9 ^[d]	10.2 ^[d]	$9.7 \times 10^{-2[d]}$	7.2	10.4	2.8×10^{-1}
5	8.1 ^[d]	10.3 ^[d]	$5.6 \times 10^{-3[d]}$	7.7	10.5	2.8×10^{-1}

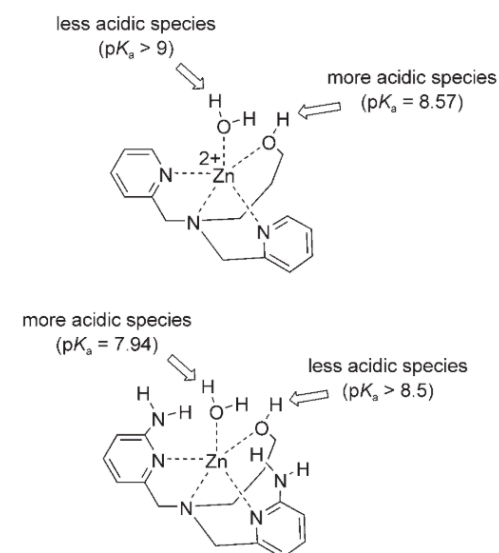
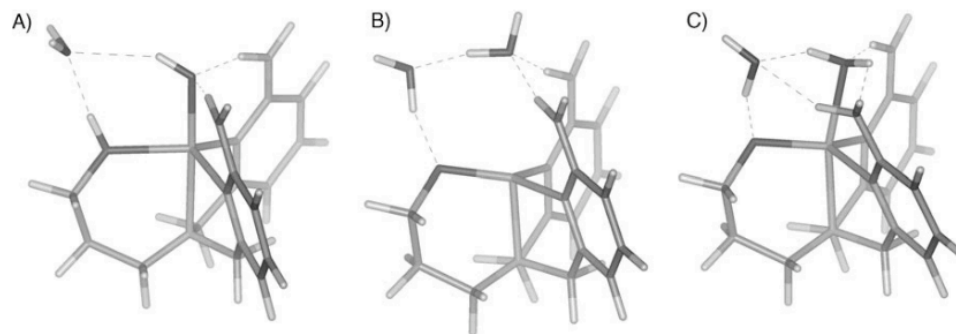
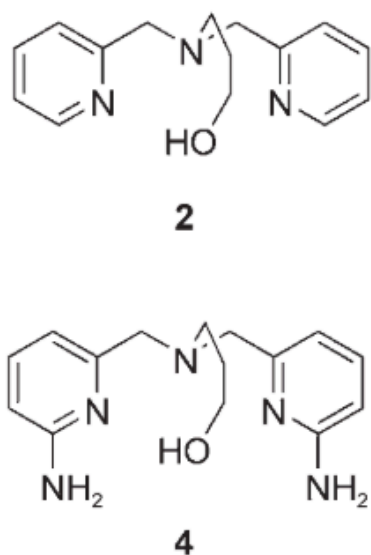
[a] [Buffer] = 5.0×10^{-2} M, estimated errors in the values of these kinetic constants are within 10 %. [b] No reactivity observed. [c] Not determined. [d] See ref. [13].

BNP cleavage: **4** > **5** ~ **3** > **2**

Pyridine amino group promotes hydrolysis of phosphate diester.

→ Increase of the acidity of metal bound water

Structures of hydroxide (A) and alkoxide (B, C) complexes



Scheme 1. Tentative identification of the microscopic pK_a values of the metal-bound species in Zn^{II} -**2** and Zn^{II} -**4**.

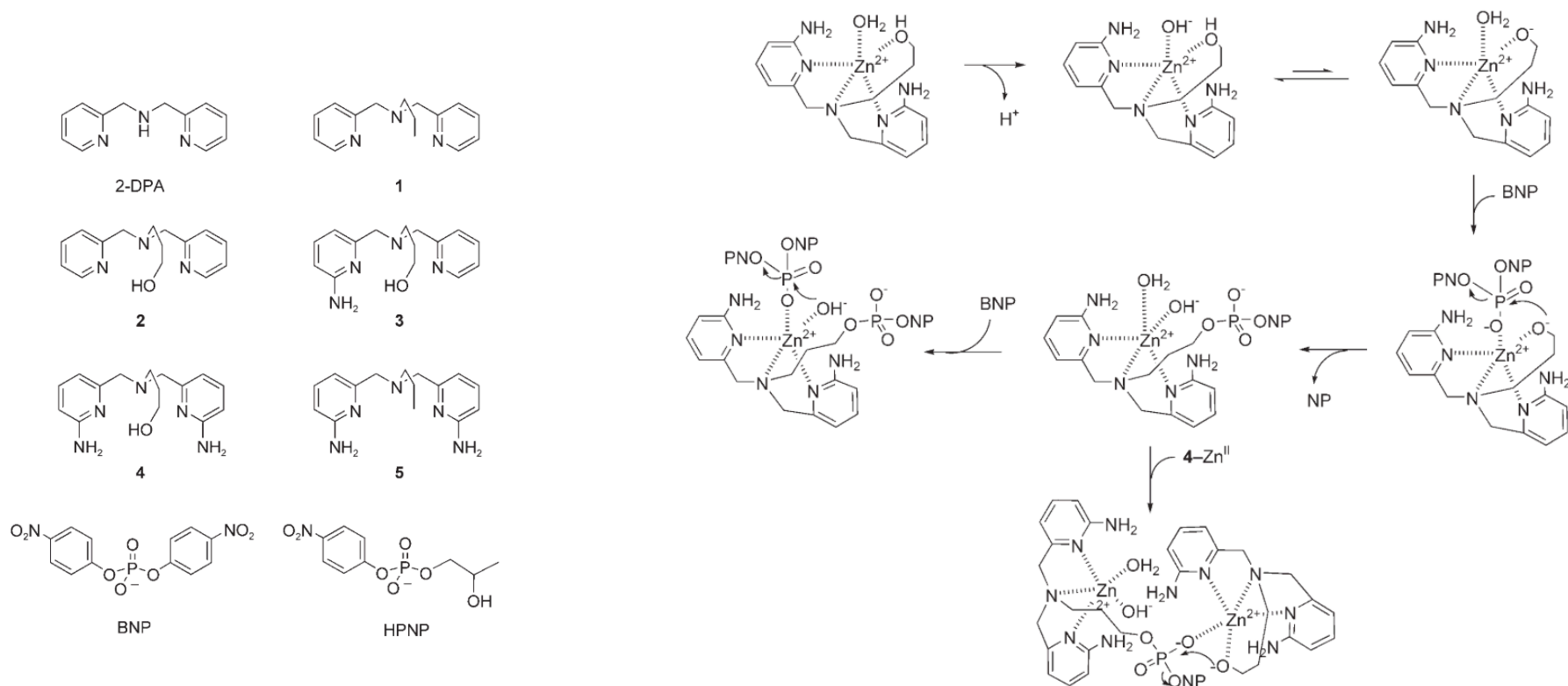
Trigonal-bipyramid geometry

Equatorial: the two pyridine nitrogen atoms and the hydroxyl group

Apical: the tertiary amino group and the water molecule

The energy of the hydroxide species is about 5–6 kcal mol⁻¹ lower than that of the alkoxide.

Proposed mechanism for BNP cleavage by using Zn^{2+} -4 complex.

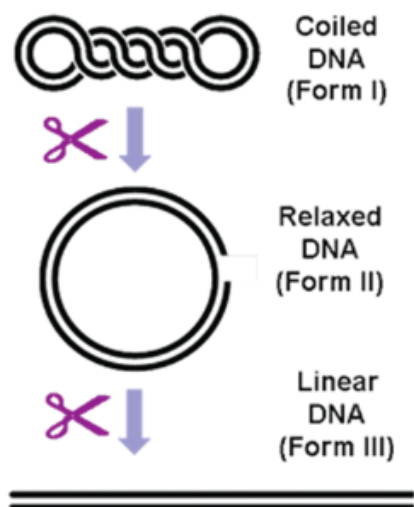


Scheme 2. Proposed mechanism for BNP cleavage by using Zn^{II} -4 complexes.

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DNA cleavage by Cu complex catalyst: pBR 322 plasmid DNA



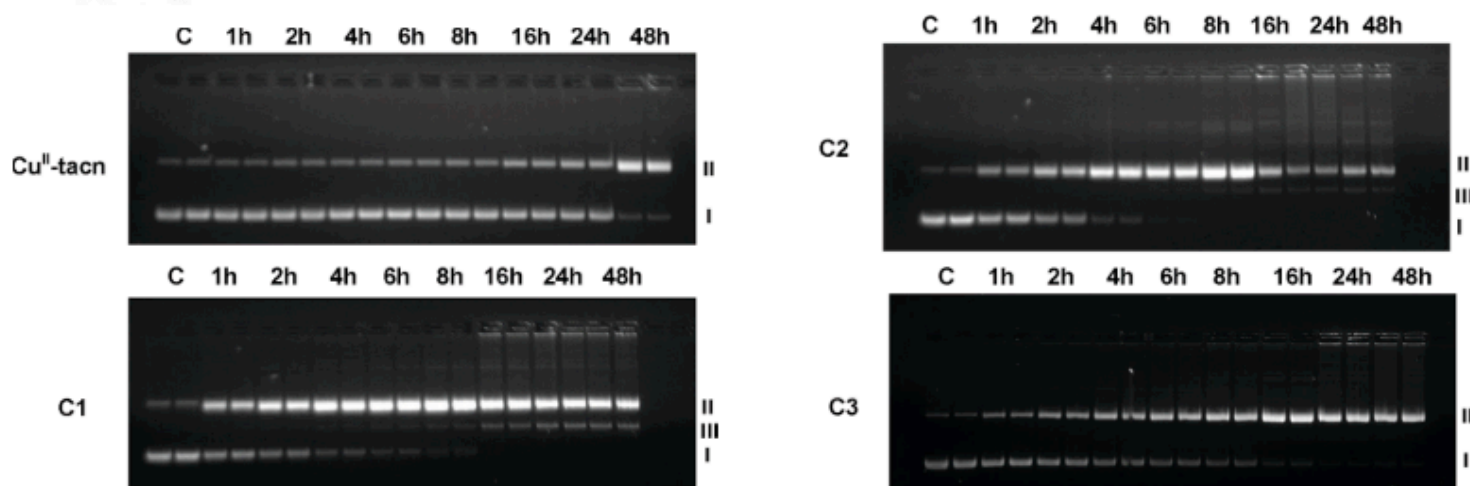
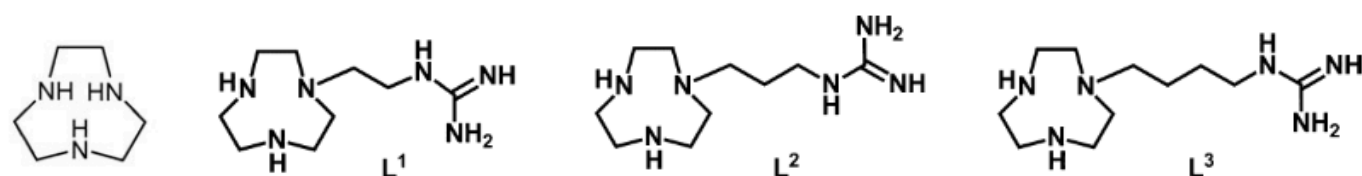
k_{obs} (s^{-1})

Tacn: 1.3×10^{-5}

C1: 1.3×10^{-4}

C2: 1.9×10^{-4}

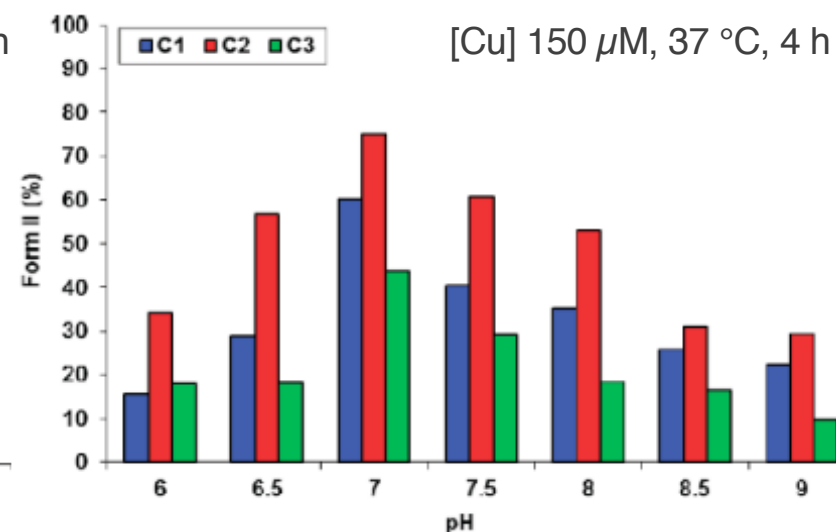
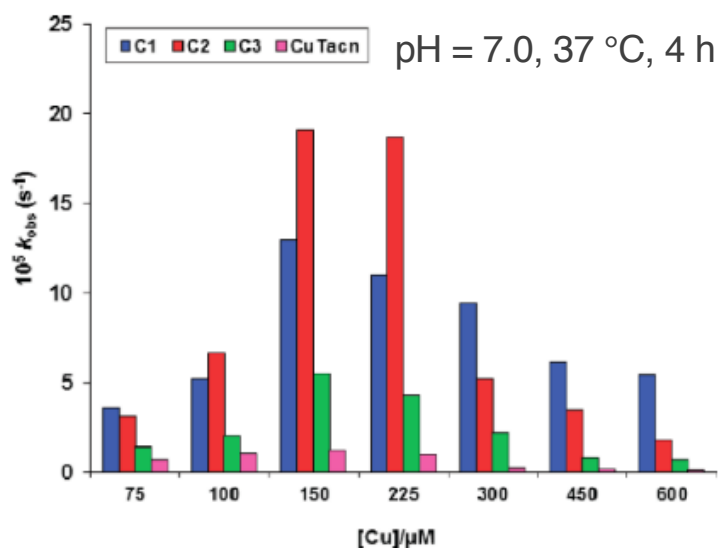
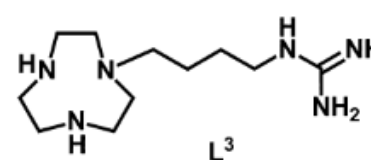
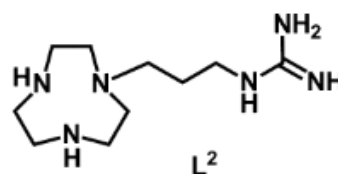
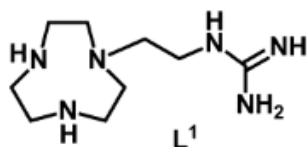
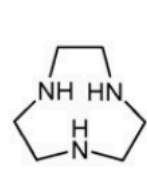
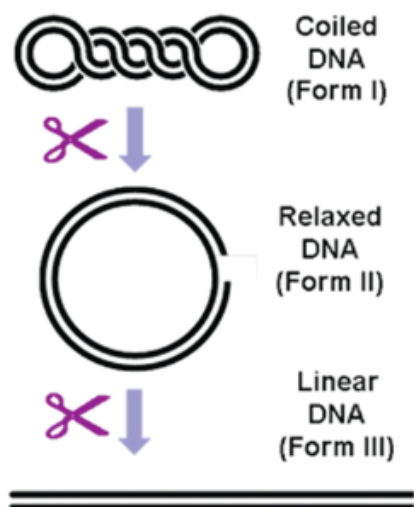
C3: 5.5×10^{-5}



pBR 322 plasmid DNA (38 μM bp), $[\text{Cu}(\text{tacn})(\text{OH}_2)_2]^{2+}$ and C1–C3 (150 μM), in 40 mM HEPES buffer, pH 7.2, 37 $^\circ\text{C}$

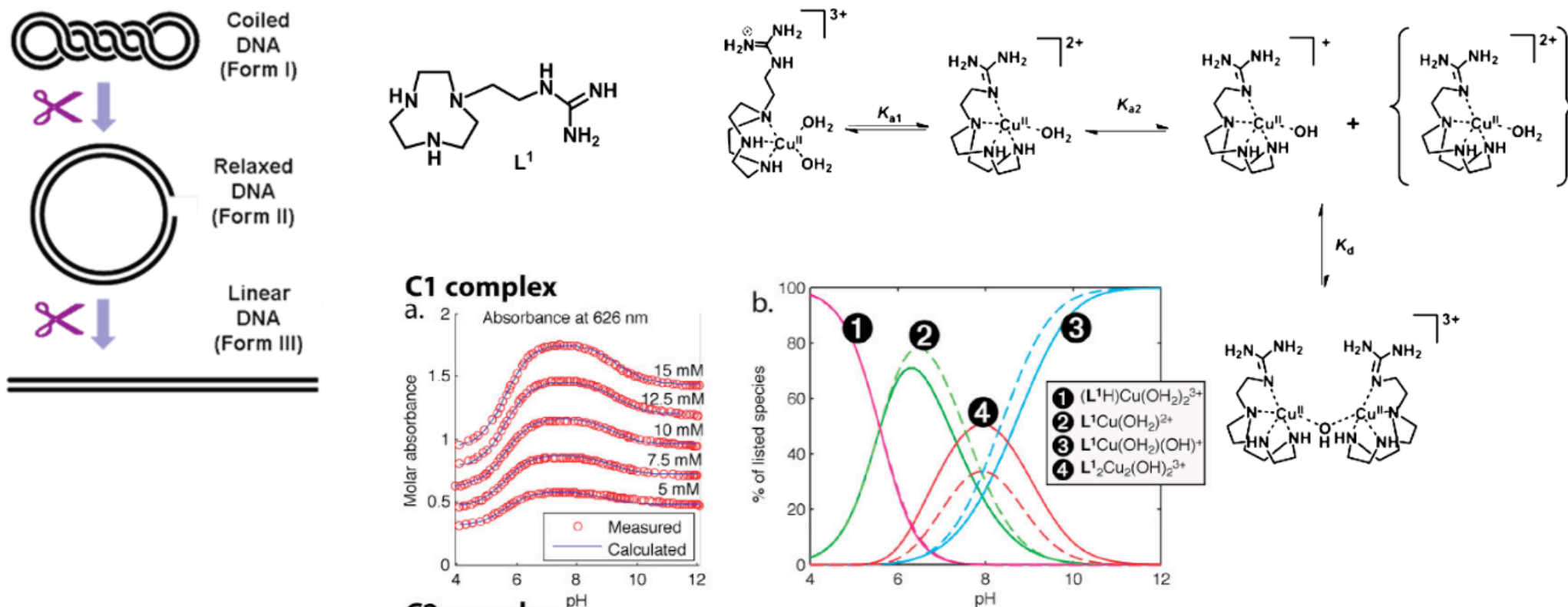
Linda Tjioe, Tanmaya Joshi, Craig M. Forsyth, Boujemaa Moubaraki, Keith S. Murray, Joël Brugger, Bim Graham, and Leone Spiccia, *Inorganic Chemistry*, **2012** 51 (2), 939-953

DNA cleavage by Cu complex catalyst: Concentration and pH



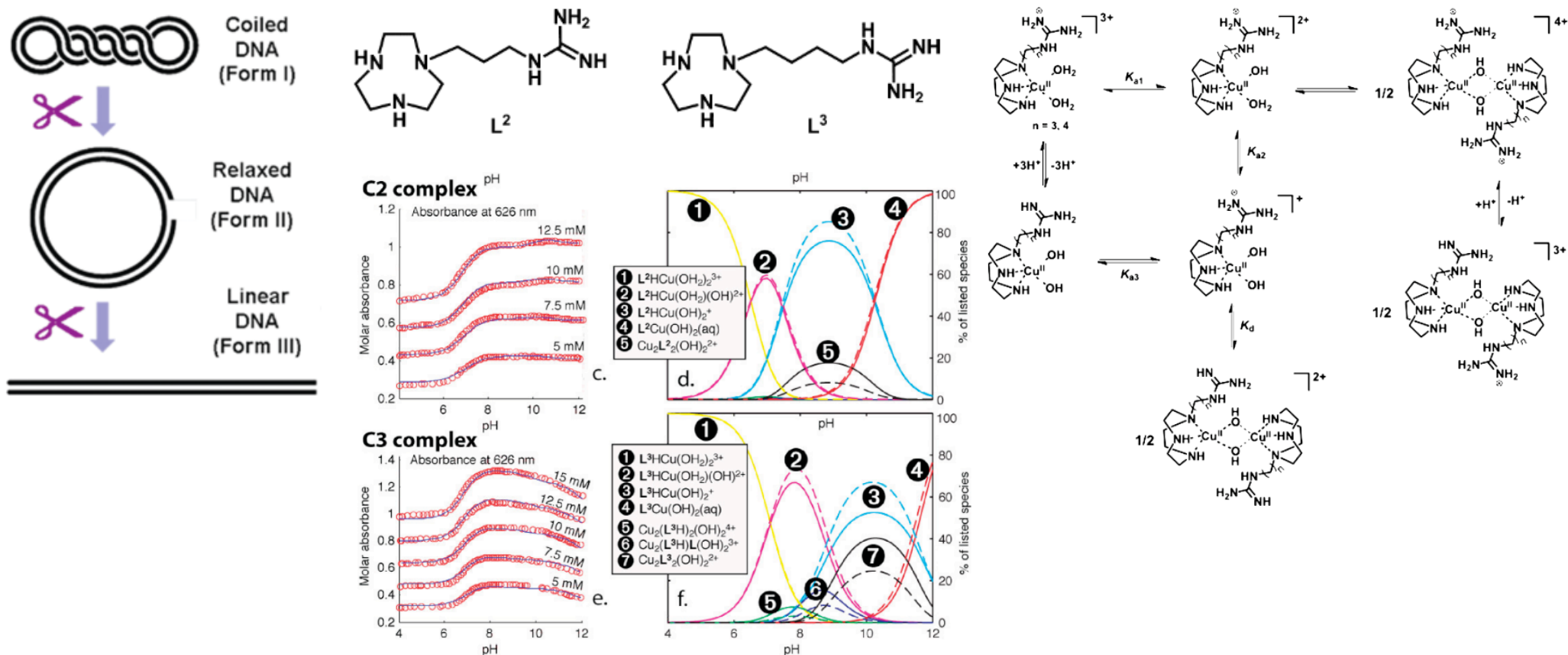
Linda Tjioe, Tanmaya Joshi, Craig M. Forsyth, Boujemaa Moubaraki, Keith S. Murray, Joël Brugger, Bim Graham, and Leone Spiccia, *Inorganic Chemistry*, **2012** 51 (2), 939-953

DNA cleavage by Cu complex catalyst: L₁



Linda Tjioe, Tanmaya Joshi, Craig M. Forsyth, Boujemaa Moubaraki, Keith S. Murray, Joël Brugger, Bim Graham, and Leone Spiccia, *Inorganic Chemistry*, **2012** 51 (2), 939-953

DNA cleavage by Cu complex catalyst: L_2 and L_3



Linda Tjioe, Tanmaya Joshi, Craig M. Forsyth, Boujemaa Moubaraki, Keith S. Murray, Joël Brugger, Bim Graham, and Leone Spiccia, *Inorganic Chemistry*, **2012** 51 (2), 939-953

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Summary

- This system is one of “nanozymes” and has been developed since 1990’s.
- Zn^{2+} ion is one of the suitable metal ion for artificial catalyst of phosphate diester hydrolysis.
- Zn^{2+} complex reactivity depends not only on Lewis acidity but also on structure.
- Functional groups increase the reactivity of metal complexes.
- Cu-tacn ligand with guanidinium group can cleavage the phosphate diester of plasmid DNA.