Techniques used to Study MetalloDrugs-DNA interactions

Maribel Navarro
mnavarro@ivic.gob.ve
IVIC-Venezuela
Brasil-Florianópolis
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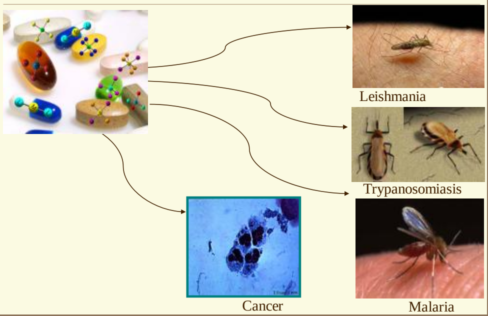
Techniques for studying Metal-Drug- DNA interactions

- Study of the interaction between the metal complexes with DNA by different techniques :

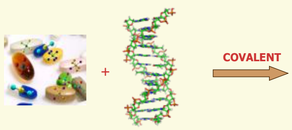
  Covalent Binding
  No Covalent : Spectrophotometric Titration by UV-vis

Metal Complexes against Different Diseases

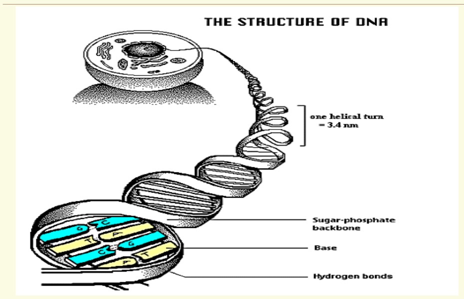


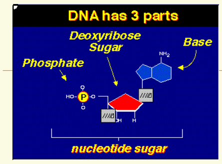
Mini-Reviews in Medicinal Chemistry, 2004, 4, 23-30

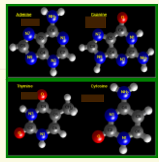
DNA : important target for metal complexes

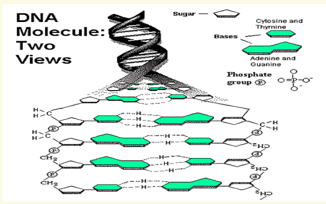


This kind of interaction involves covalent bond formation of the metal complex to either the phosphate or the nucleic acid bases





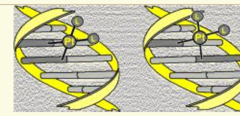




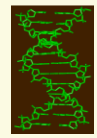
Covalent inner-sphere binding

- This kind of interaction involves covalent bond formation of the metal complex to either the phosphate or the nucleic acid bases.
- Where more than one metal - DNA bond is formed, crosslink results which can be either *intra*- or *inter*-strand.

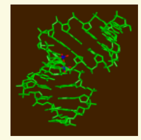
Covalent binding of the cisplatin to the ADN



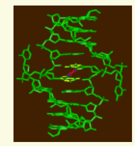
1,2-Intrastrand 1,2-Interstrand



ADN



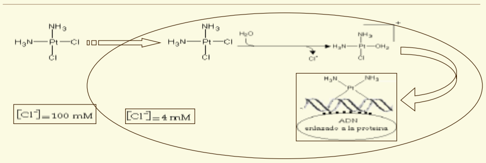
1,2-Intrastrand

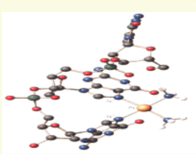


1,2-Interstrand

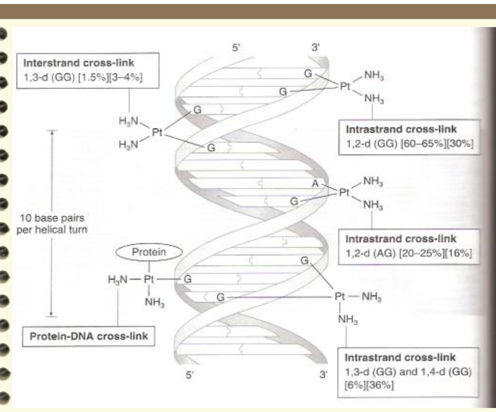
Coor. Chem. Rev. **216-217**, 383,(2001)

Covalent binding of the cisplatin to the ADN

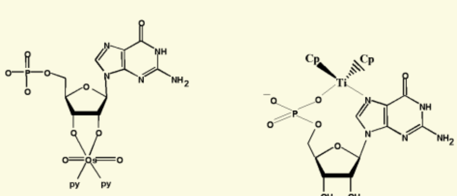




Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *Science* 1985, *230*, 412−417.
Sherman, S. E.; Gibson, D.; Wang, A. H.-J.; Lippard, S. J. *J. Am. Chem. Soc.* **1988**, *110*, 7368−7381.

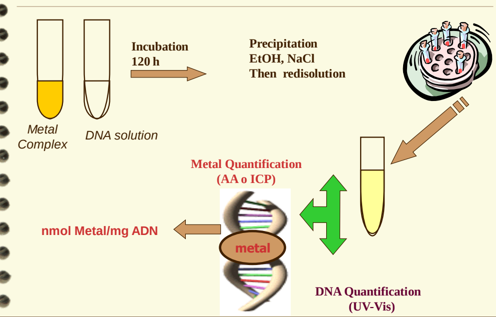


Covalent binding of the metal complexes to the ADN

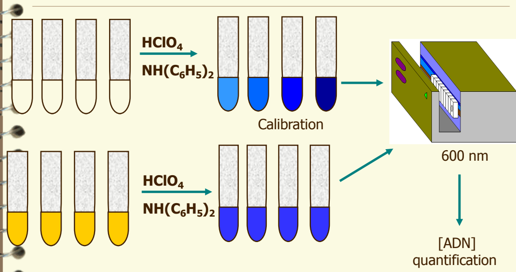


In the Laboratory

Covalent binding



Burton Assay



K. Burton, *Biochem. J.* ,62, 315 (1956)

Covalent binding

Complejos	$[Metal]_0$ nmol/mL Promedio	$[ADN]_0$ µg/mL Promedio	[Metal]/[ADN] nmol/µg	$[ADN]_0$ mg/mL Promedio
$Pt(CQDF)_2(I)_2$	43.278	235.967	0.183	0.236
$Pt(CQDF)_2(Cl)_2$	38.485	224.111	0.172	0.224
Transplatina	243.656	214.585	1.135	0.215
$Pt(CQ)_2(Cl)_2$	137.456	190.516	0.721	0.191
Cisplatino	264.464	232.697	1.137	0.233
$Pd(CQ)_2(Cl)_2$	366.670	269.767	1.359	0.270
$Pd(CQDF)_2(I)_2$	660.523	255.853	2.582	0.256
Au(CQ)(Cl)	704.394	194.544	3.621	0.195
Au(TgTa)(CQ)	151.874	285.734	0.532	0.286
Au(CQDF)(PPh3)	3.384	310.114	0.011	0.310

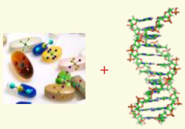
Complejo	nmol metal/mg ADNtt	Base Pair /metal (P.B./A.M.)
(1) $Pt(CQDF)_2(I)_2$,	183,41	8,39
(2) $Pt(CQDF)_2(Cl)_2$	171,72	8,96
(3) $Pt(CQ)_2(Cl)_2$	721,49	2,13
(4) $Pd(CQDF)_2(I)_2$	125,07	12,32
(5) $Pd(CQ)_2(Cl)_2$	1359,21	1,13
(6) Au(CQ)(Cl)	1420,75	1,03
(7) Au(CQ)(TgTa)	531,52	2,89
(8) $[Au(CQDF)(PPh_3)]PF_6$	10,91	140,98
cis-$Pt(NH_3)_2(Cl)_2$	1136,52	1,45
trans-$Pt(NH_3)_2(Cl)_2$	1135,47	1,46

Techniques for studying Metal-Drug- DNA interactions

- **Study of the interaction between the metal complexes with DNA by different techniques :**

  Covalent Interaction
  Spectrophotometric Titration by UV-vis

DNA : important target for metal complexes



NO COVALENT

Hydrogen bonding

Electrostatic interactions

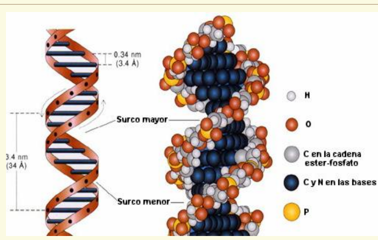
Minor or Major Groove

Intercalation.

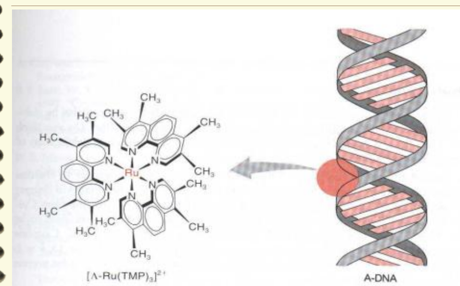
Non-covalent outer sphere binding

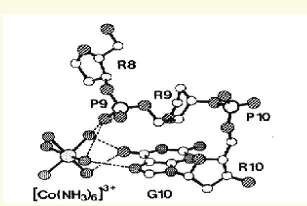
- Negatively charged backbone of DNA interacts with positively charged molecules through electrostatic interactions or phosphate - oxygen binding.
- Exocyclic groups on the purines/pirimidines can be involved trough hydrogen bonding to suitable ligand atoms.
- Depends primary, on the nature and concentration of the metal. measured by **T_m** and CD.

No covalent interaction

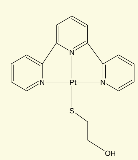


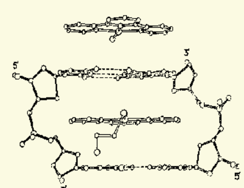
Mutat Res. **623**, 3, (2007)



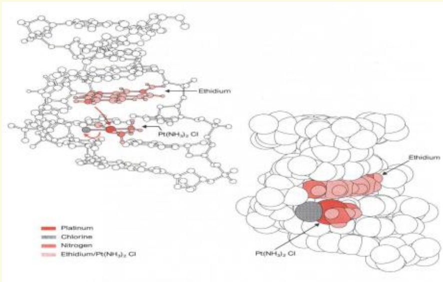


Interacción no covalente





Mutat Res. **623**, 3, (2007)



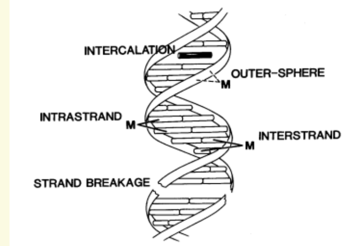
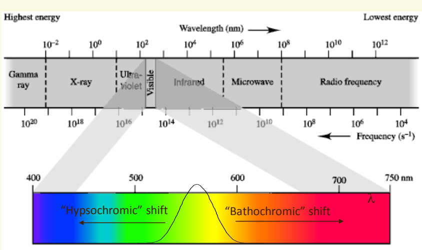
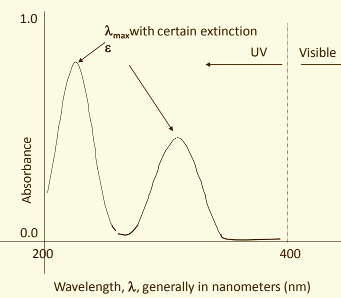


Fig. 1.4. Schematic presentation of the modes of interaction of metal complexes with DNA.

Electronic Excitation by UV/Vis Spectroscopy :



An Electronic Spectrum



Make solution of concentration low enough that A≤ 1

(Ensures Linear Beer's law behavior)

Even though a dual beam goes through a solvent blank, choose solvents that are UV transparent.

Can extract the **ε** value if conc. (M) and b (cm) are known

UV bands are much broader than the photonic transition event. This is because ***vibration levels*** are superimposed on UV.

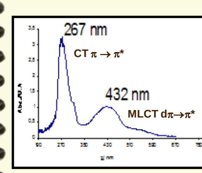
UV-vis

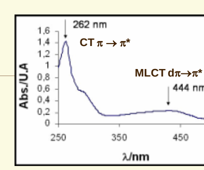
σ → σ* and σ → π* transitions: high-energy, accessible in vacuum UV (λmax <150 nm). Not usually observed in molecular UV-Vis.

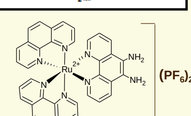
n → σ* and π → σ* transitions: non-bonding electrons (lone pairs), wavelength (λmax) in the 150-250 nm region.

n → π* and π → π* transitions: most common transitions observed in organic molecular UV-Vis, observed in compounds with lone pairs and multiple bonds with λmax = 200-600 nm.

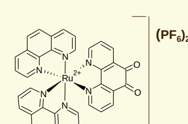
Metal Ligand Charge Transitions (MLCT) or Metal Ligand Charge Transitions (LMCT)







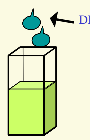
Buffer Tris-HCl(5mM), NaCl(50mM) pH 7.2

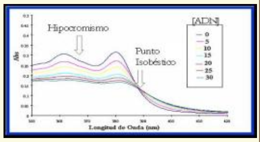


Buffer Tris-HCl(5mM), NaCl(50mM) pH 7.2

Studies of no covalent interaction

Spectroscopic Titrations

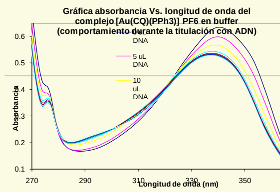




Neighbor exclusion equation.

[ADN]/(εa-εf) = [ADN]/(εa-εb) + 1/[Kb(εa-εb)]

%H= (Ai-Af/Ai)100



[DNA]/(ε a- ε f) = [DNA]/(εa- εb) + 1/[Kb(εa-εb)]

The intrinsic binding constant Kb was determined from the plot of [DNA]/(εa - εf) vs [DNA],

where [DNA] is the concentration of DNA in base pairs

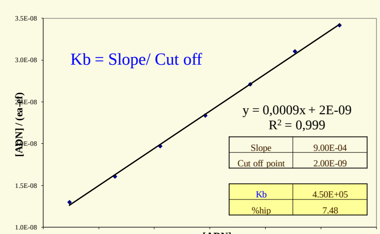
the apparent absorption coefficients, εa, εf, εb correspond to Aobs/[M], the extinction coefficient for the free metal complex and the extinction coefficient of the metal complex in the totally bound form.

The slope equal to 1/(εb - εf) and the intercept equal to 1/[Kb(εb - εf)] and Kb was obtained adjusting the data to the corresponding curve

[ADN]/(εa-εf) = [ADN]/(εa-εb) + 1/[Kb(εa-εb)]

V ADN	Vfinal, uL	[ADN]	[M]	ea	ef	[ADN] / (ef-ea)
0	2500	0	7.98E-05	7.95E+03	7.95E+03	---
5	2505	4.14E-06	7.97E-05	7.51E+03	7.95E+03	9.41E-09
10	2510	8.26E-06	7.95E-05	7.11E+03	7.95E+03	9.92E-09
15	2515	1.24E-05	7.94E-05	6.81E+03	7.95E+03	1.09E-08
20	2520	1.65E-05	7.92E-05	6.73E+03	7.95E+03	1.35E-08
25	2525	2.05E-05	7.90E-05	6.71E+03	7.95E+03	1.66E-08
30	2530	2.46E-05	7.89E-05	6.70E+03	7.95E+03	1.97E-08
35	2535	2.86E-05	7.87E-05	6.69E+03	7.95E+03	2.28E-08
40	2540	3.26E-05	7.86E-05	6.70E+03	7.95E+03	2.62E-08
45	2545	3.67E-05	7.84E-05	6.67E+03	7.95E+03	2.88E-08

[ADN]/(εa-εf) = [ADN]/(εa-εb) + 1/[Kb(εa-εb)]

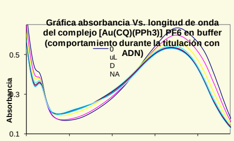


•Spectroscopic titration

Complex metal

$$\frac{\bar{\upsilon}}{[L]} = K_f(n - \bar{\upsilon})$$

Scatchard's Equation

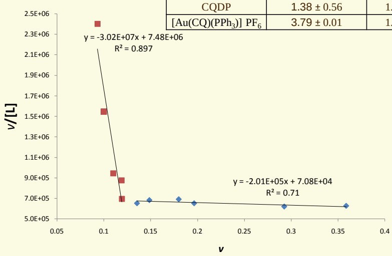


$$\frac{\bar{\upsilon}}{[L]} = K_f(n - \bar{\upsilon})$$

Scatchard's Equation

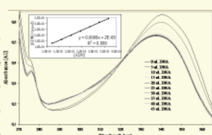
Abs 340	V DNA	V final	[ADN]	[M]	Aa	A0 (f)	Amax (b)	A0 - Aa	A0 - Ab	alfa	[L]	L	v	v/[L]
0.86866522	0	2000	0	2.59E-06	0.868665	0.868665	0.482765	0	0.3859	0	2.59E-06	0	#DIV/0!	#DIV/0!
0.82639933	10	2010	3.52E-08	2.58E-06	0.826399	0.868665	0.482765	0.042266	0.3859	0.109525	2.29E-06	2.82E-07	8.022691	3496224
0.78579235	30	2030	1.04E-07	2.55E-06	0.785792	0.868665	0.482765	0.082873	0.3859	0.214752	2E-06	5.48E-07	5.243499	2617068
0.75061035	50	2050	1.72E-07	2.53E-06	0.75061	0.868665	0.482765	0.118055	0.3859	0.30592	1.75E-06	7.73E-07	4.481712	2555602
0.71615648	70	2070	2.39E-07	2.5E-06	0.716156	0.868665	0.482765	0.152509	0.3859	0.395202	1.51E-06	9.89E-07	4.135488	2732698
0.68548632	90	2090	3.04E-07	2.48E-06	0.685486	0.868665	0.482765	0.183179	0.3859	0.474679	1.3E-06	1.18E-06	3.86334	2967490
0.6581316	110	2110	3.69E-07	2.45E-06	0.658132	0.868665	0.482765	0.210534	0.3859	0.545565	1.12E-06	1.34E-06	3.632945	3256670
0.63063192	130	2130	4.32E-07	2.43E-06	0.630632	0.868665	0.482765	0.238033	0.3859	0.616826	9.32E-07	1.5E-06	3.475557	3730029
0.61130571	150	2150	4.93E-07	2.41E-06	0.611306	0.868665	0.482765	0.25736	0.3859	0.666906	8.02E-07	1.61E-06	3.256709	4058409
0.59261942	170	2170	5.54E-07	2.39E-06	0.592619	0.868665	0.482765	0.276046	0.3859	0.715329	6.79E-07	1.71E-06	3.08221	4536108
0.57020998	190	2190	6.13E-07	2.37E-06	0.57021	0.868665	0.482765	0.298455	0.3859	0.773399	5.36E-07	1.83E-06	2.981643	5563442
0.55199194	210	2210	6.72E-07	2.34E-06	0.551992	0.868665	0.482765	0.316673	0.3859	0.820609	4.2E-07	1.92E-06	2.862346	6807970
0.52308559	230	2230	7.29E-07	2.32E-06	0.523086	0.868665	0.482765	0.34558	0.3859	0.895515	2.43E-07	2.08E-06	2.852005	1175182
0.51378202	250	2250	7.86E-07	2.3E-06	0.513782	0.868665	0.482765	0.354883	0.3859	0.919624	1.85E-07	2.12E-06	2.694483	1456243
0.49011469	270	2270	8.41E-07	2.28E-06	0.490115	0.868665	0.482765	0.378551	0.3859	0.980954	4.35E-08	2.24E-06	2.661277	6123653
0.48799133	280	2280	8.68E-07	2.27E-06	0.487991	0.868665	0.482765	0.380674	0.3859	0.986456	3.08E-08	2.24E-06	2.580626	8387252
0.48749733	290	2290	8.95E-07	2.26E-06	0.487497	0.868665	0.482765	0.381168	0.3859	0.987736	2.77E-08	2.23E-06	2.494872	8994215
0.48298502	300	2300	9.22E-07	2.25E-06	0.482985	0.868665	0.482765	0.38568	0.3859	0.999429	1.29E-09	2.25E-06	2.44026	1.9E+09
0.48276472	310	2310	9.49E-07	2.24E-06	0.482765	0.868665	0.482765	0.3859	0.3859	1	0	2.24E-06	2.362891	#DIV/0!

Compounds	K_{b1}(x10^7 M^{-1})	K_{b1}(x10^5 M^{-1})
CQDP	1.38 ± 0.56	1.02 ± 0.15
[Au(CQ)(PPh$_3$)] PF$_6$	3.79 ± 0.01	1.84 ± 0.89

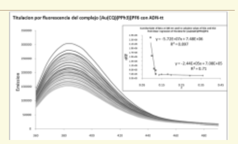


Data from UV-vis experiment

Complex	% Hypochromism	Bathochromism (Δnm)	Constant binding		
			Neighbor exclusion	Scatchard	
			Kb(x10^5 M^{-1})	Kb2(x10^5 M^{-1})	Kb1(x10^7 M^{-1})
Au(CQ)(Cl)	21%	4	2,68 ± 0,09	2.37 ± 0.76	4.03 ± 0.87
[Au(CQ)(PPh3)]PF6	7.5%	3	4.50 ± 0.25	3.79± 0.01	1.84 ± 0.25
[Au(CQDF)(PPh3)]PF6	31%	5	0,79 ± 0.62	0.51 ± 0.25	0.92 ± 0.61
CQDF	24%	2	1,02 ± 0,15	0.93 ± 0.21	1.38 ± 0.55



Spectrofotometric Titration of [Au(CQ)(PPh$_3$)]PF$_6$ with DNA.
[Complejo] = 9,98x10$^{-4\ M}$ and [DNA] = 0–50 μM

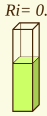


Fluorimetríc Titration [Au(CQ)(PPh$_3$)]PF$_6$ with DNA.
[Complejo] = 7,32x10$^{-4\ M}$ and [DNA] = 0–10 μM

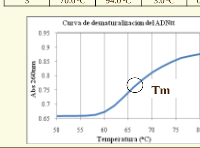
Compounds	UV-Vis Titration		Fluorescence Titration	
	K_{b1}(x10^7 M^{-1})	K_{b1}(x10^5 M^{-1})	K_{b1}(x10^7 M^{-1})	K_{b2}(x10^5 M^{-1})
CQDP	1.38 ± 0.56	1.02 ± 0,15	3.24 ± 1.21	3.26 ± 1.01
[Au(CQ)(PPh$_3$)] PF$_6$	3.79 ± 0.01	1.84 ± 0.89	5.27 ± 1.82	2.44 ± 1.69

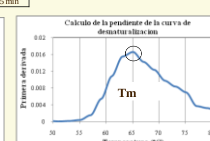
Thermal denaturation of DNA

Metal Complex/DNA
Ri= 0.2



Number	Start	Stop	Increment	Hold time
1	40.0 ºC	55.0 ºC	3.0 ºC	0.5 min
2	55.0 ºC	70.0 ºC	1.0 ºC	0.5 min
3	70.0 ºC	94.0 ºC	3.0 ºC	0.5 min





DNA Melting Temperature after interaction with metal complex

Complex	Tm	Δ Tm
Au(CQ)(Cl)	69,6 ± 0,6	4,7
[Au(CQ)(PPh$_3$)] PF$_6$	80,6 ± 0,4	15,7
[Au(CQDF)(PPh3)]PF6	85,4 ± 1,5	20,5
DNA	64,9 ± 0,1	---
CQDP	87,6 ± 0.9	22,7