"Basic and translational oncology" [Italian-French Erasmus Intensive Course in Oncology organized in collaboration with European Master of Genetics - University Paris7-Paris5], Florence, jan 22, 2020

Metal based drugs for cancer treatment: the case of gold compounds"

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Outline

1. A general introduction to Bioinorganic Chemistry and Metal based drugs

2. Cisplatin and Platinum Based drugs

3. Gold as an opportunity

4. Mechanistic studies of gold compounds

5. Omics technologies

1. General Introduction

BIOINORGANIC CHEMISTRY is the branch of Inorganic Chemistry which investigates the role of Metals in Biological Systems.

"METALS IN MEDICINE" is a part of Bioinorganic Chemistry that specifically considers the medical implications of the role of metals in biological systems.

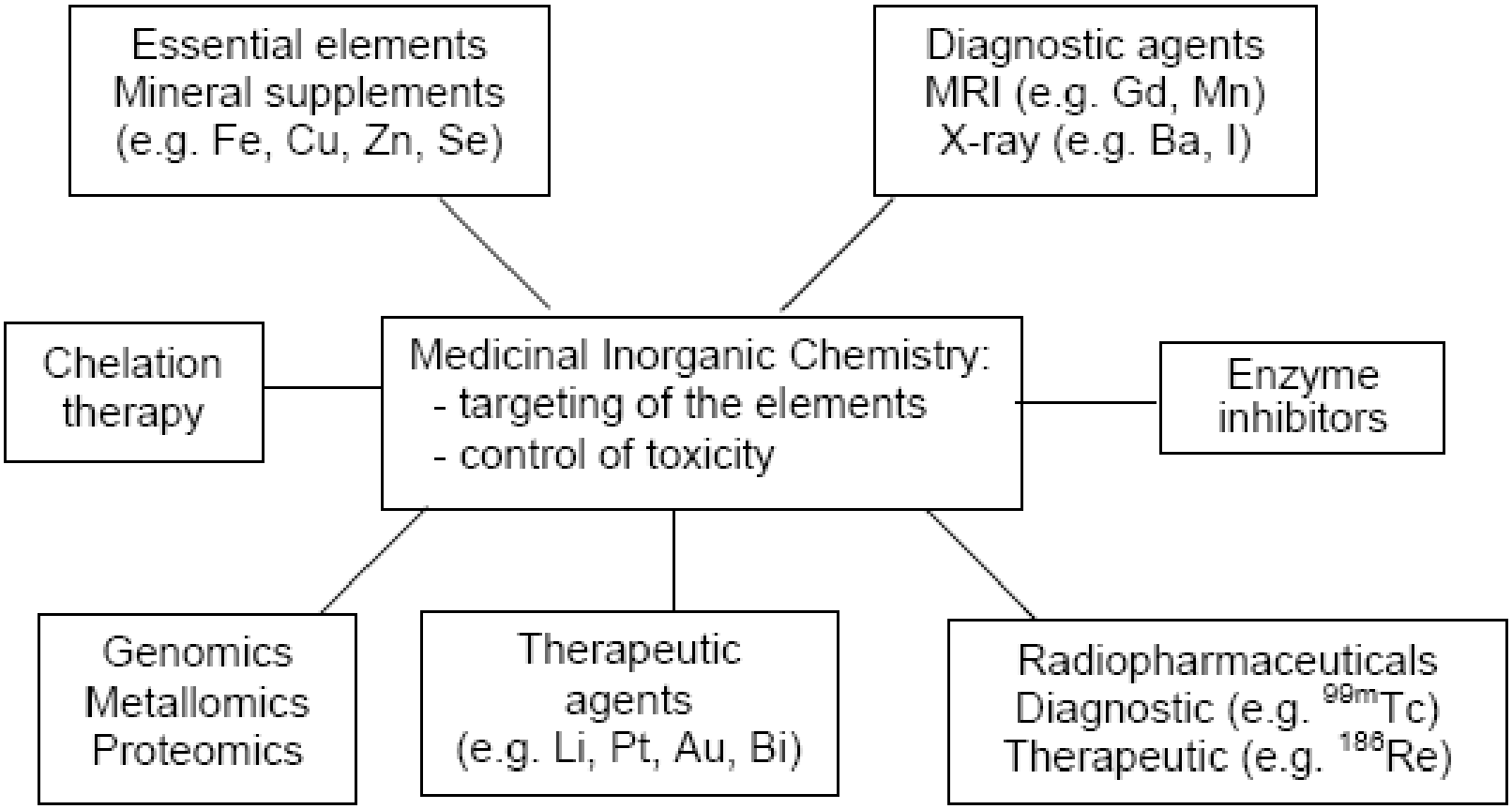
Ad es Metal disregulation

Metal toxicity

Metal centers as targets for drugs

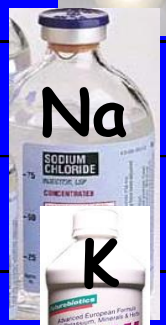
Metallodrugs for diagnosis and therapy

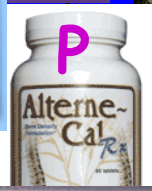
Medicinal Inorganic Chemistry (Inorganic Pharmacology)

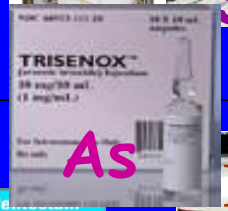


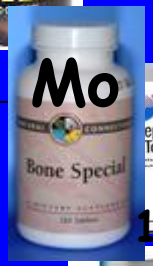
A Periodic Table of Medicines

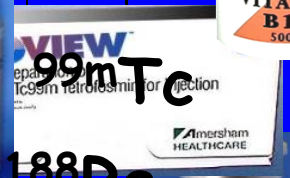
Some Inorganic Drugs











from Peter Sadler

Metal Compounds as Anticancer Agents

- It is the most important branch of metals in medicine which grew up dramatically after the discovery of the anticancer properties of cisplatin in the 70's.

Metal compounds as Drugs to fight Cancer

a) Metal Compounds

b) Cancer

c) Drug

a) Metal compounds

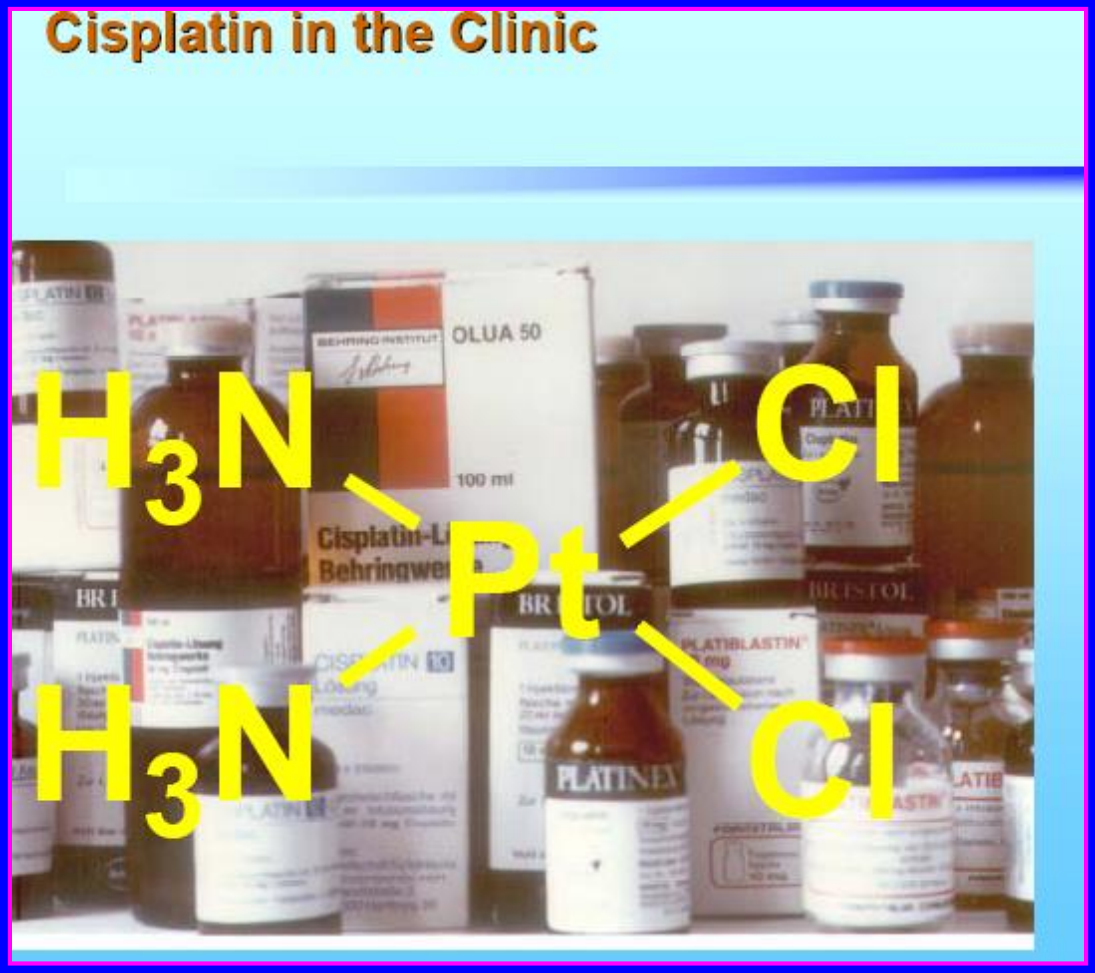
Mainly transition metal complexes
Low molecular weight compounds

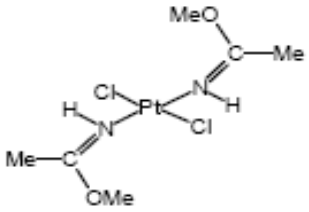
Design, synthesis and characterisation.

Structural and solution chemistry

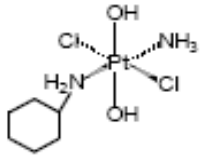
Reactivity.

Cisplatin as an example



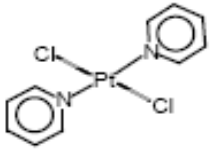


Trans-EE complex with iminoether ligands (more active than *cis* isomer)

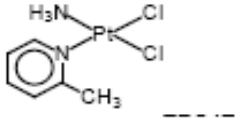


JM3355

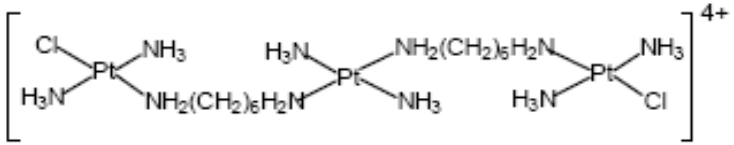
The Pt(II) analogue of this Pt(IV) complex, without axial OH^- ligands, is inactive



Trans complex with pyridine ligands

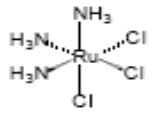


Two *cis*-N groups, but only one has NH group

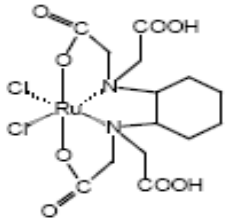


BBR3464

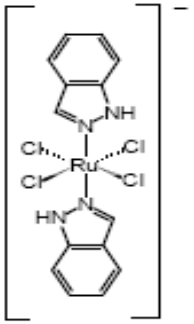
High positive charge, only one leaving group on each terminal Pt



fac-[$RuCl_3(NH_3)_3$]

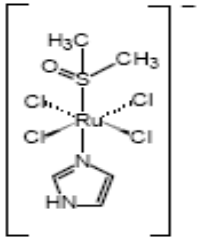


Ru(IV) cdta complex



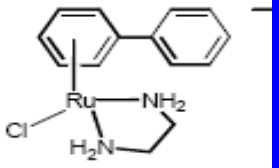
Ru(III) indazole complex (KP1019)

(indazolium salt)



NAMI-A

(imidazolium salt)



Ru(II) arene (biphenyl) complex

Chart 2.6

Concepts related to the "chemistry" of metal based drugs

- **Metal based drugs as Prodrugs**
- **Design and tuning of specific properties of the metal complex**
- **Definition of SAR relationships**
- **Libraries of metal compounds capable of exploring the "chemical space"**

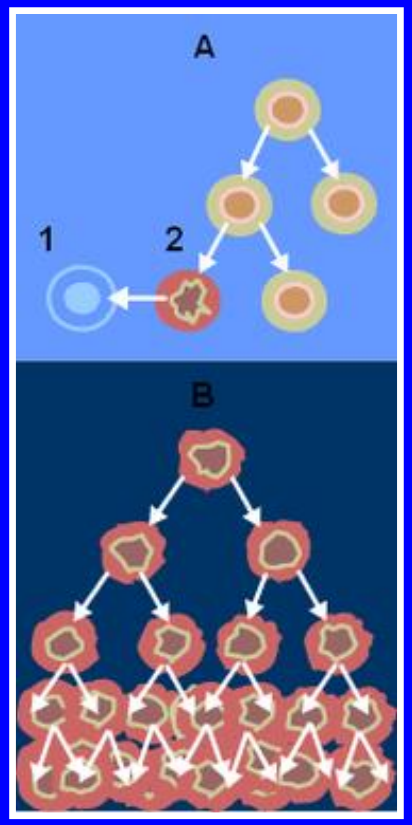
b) Cancer

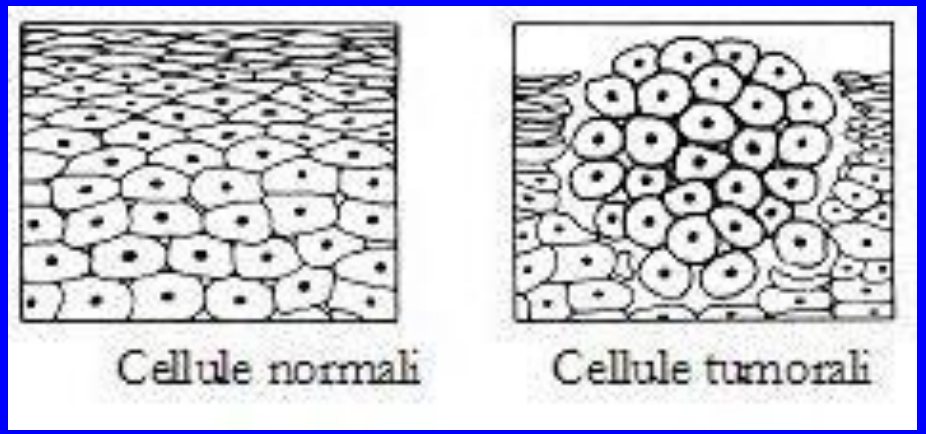
It is a collective term to designate a large number of diseases that may be even very different one another.

Anyway, the different types of cancer invariantly manifest a few common traits.

"Biology of tumors"

Cancer may be considered as a "disease of genes"; gene alterations accumulate and eventually lead to uncontrolled tissue growth. '





c) Drug

A substance producing a therapeutic effect "an improvement" when treating a disease.

"magic bullet";

"therapeutic window"

Cancer is a very severe disease. Various treatment appraoches were developed:

Surgery

Radiotherapy

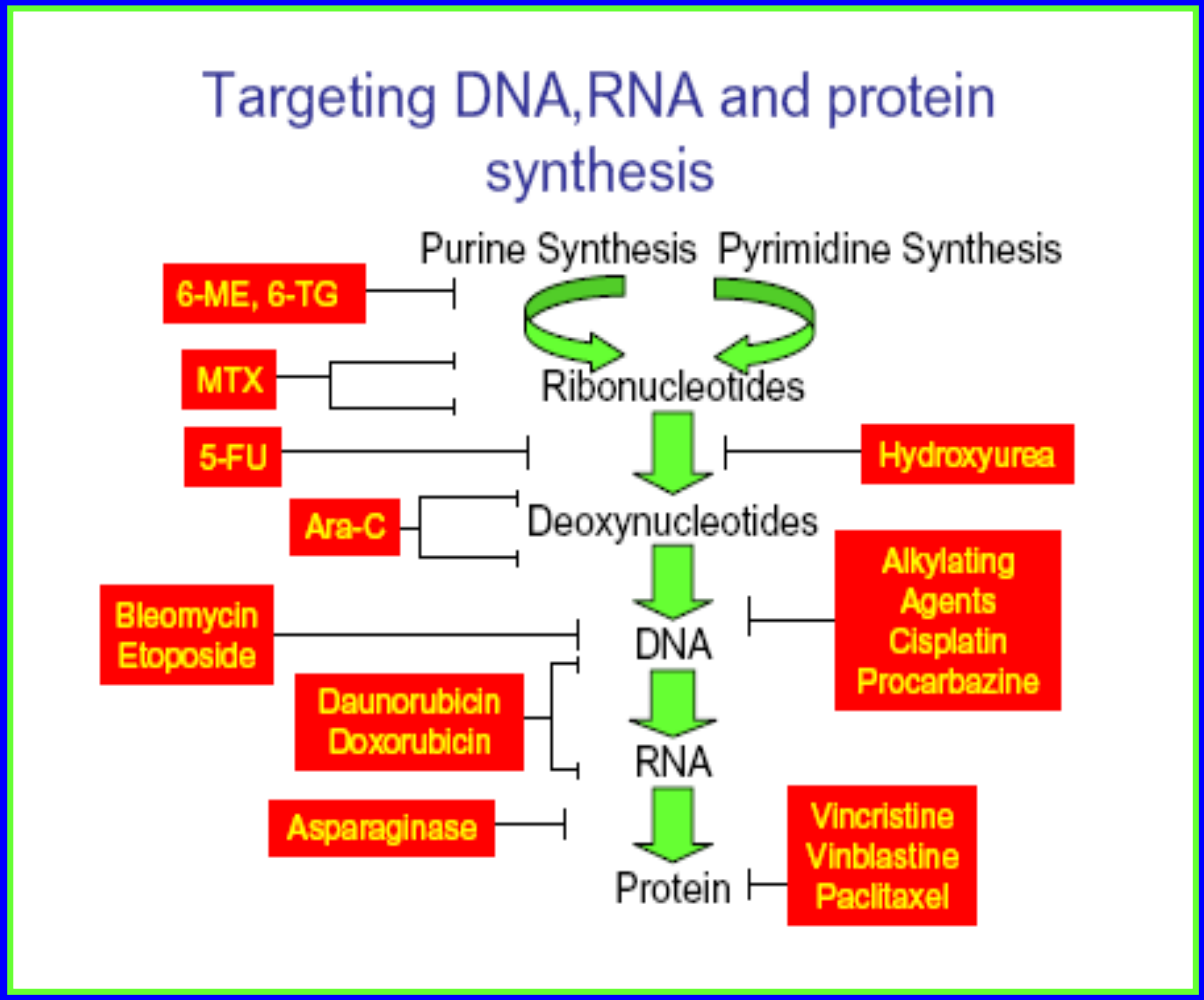
Chemotherapy

Immunotherapy

- Today a rather wide arsenal of drugs exists to treat different kinds of cancer "Cytotoxic drugs"

- Yet there is a strong need to find new and better anticancer drugs.

Classical anticancer drugs (cytotoxic drugs)



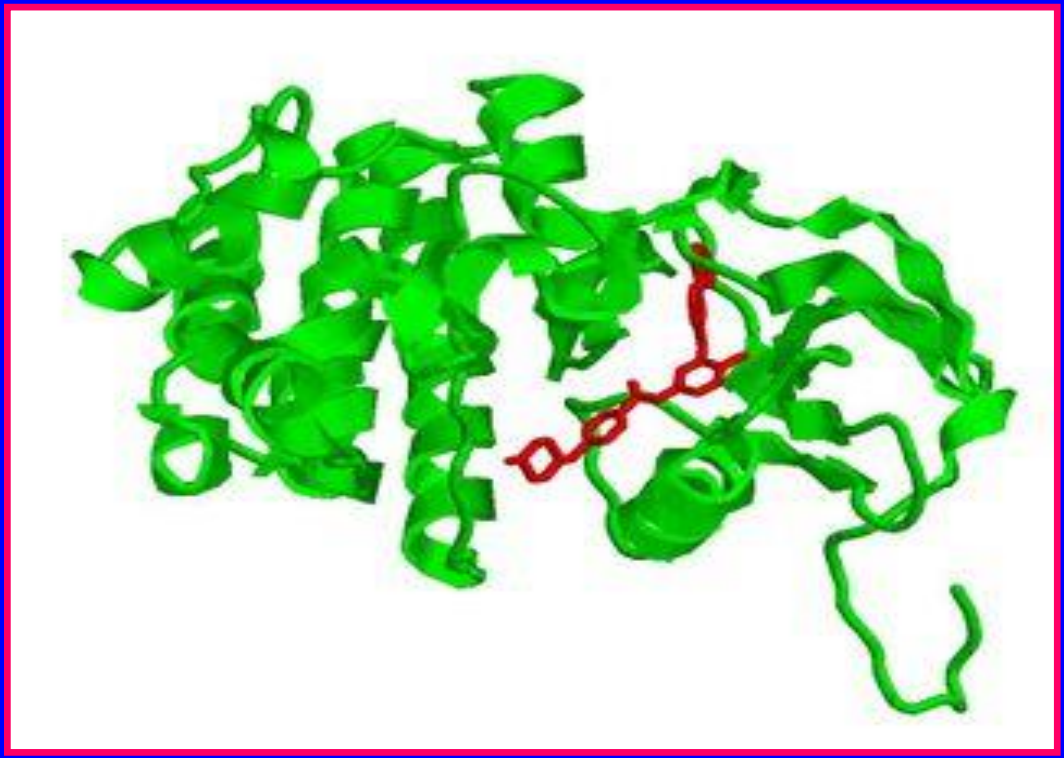
Anticancer drugs target proliferating cells - I

- Anticancer drugs are NOT magic bullets. Ideally they should target only the cancer cells. However, they target proliferating cells whether normal or neoplastic.
- Not all cancer cells are rapidly proliferating. Of the four major types of tumors, the faster growing hematological (nonsolid) types (leukemias and lymphomas) are more responsive to treatment than the slower growing solid types (carcinomas and sarcomas).

Anticancer drugs target proliferating cells - II

- Normal cells of the hair follicles, bone marrow and intestinal epithelium are rapidly dividing and are especially sensitive to inhibition by anti-neoplastic drugs. This results in the toxic side effects common to most anticancer drugs.

Targeted drugs



Imatinib

Discovery and development of new anticancer metal based drugs.

NCI screening protocol

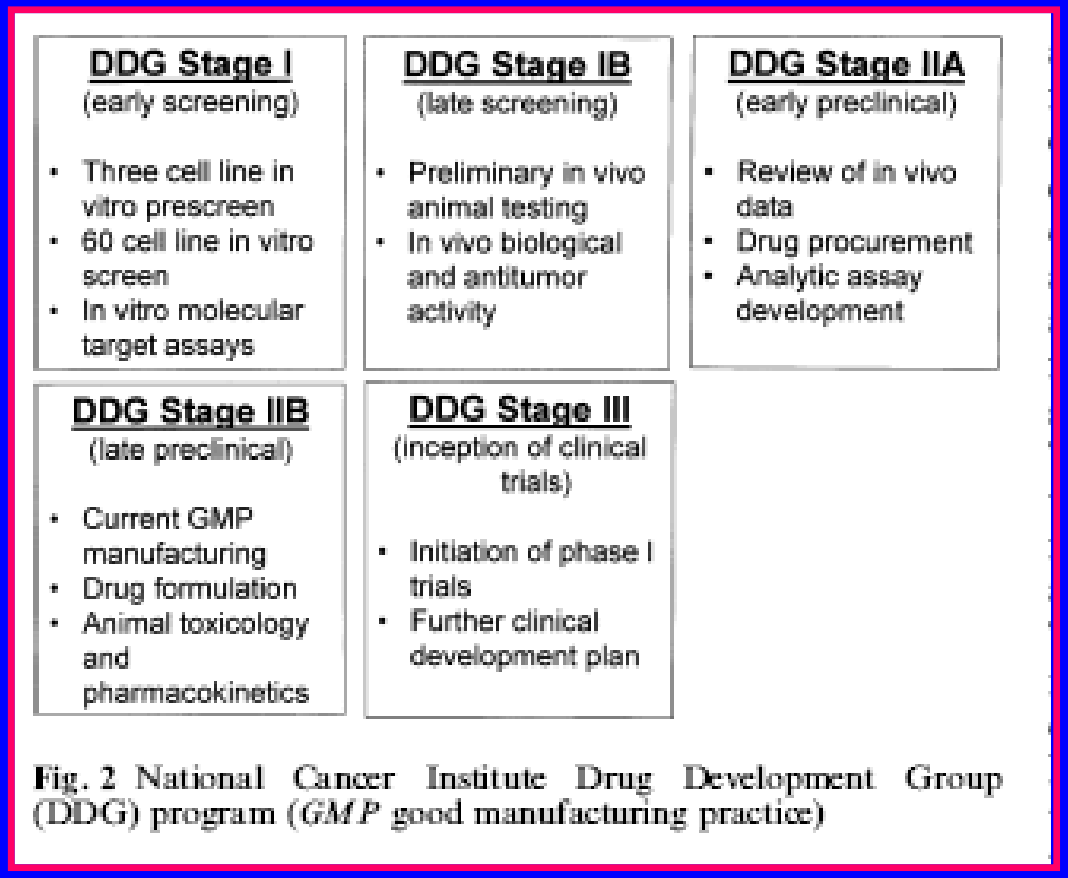
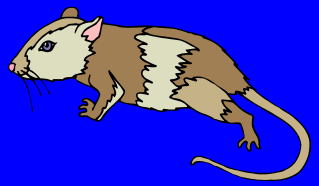


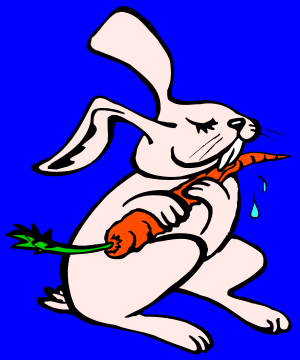
Fig. 2 National Cancer Institute Drug Development Group (DDG) program (*GMP* good manufacturing practice)

Animal models

mouse

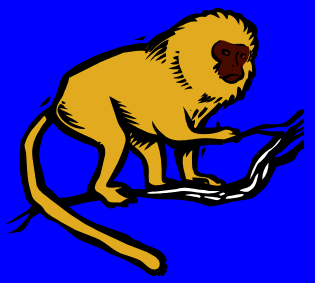


rat



rabbit

dog



primates

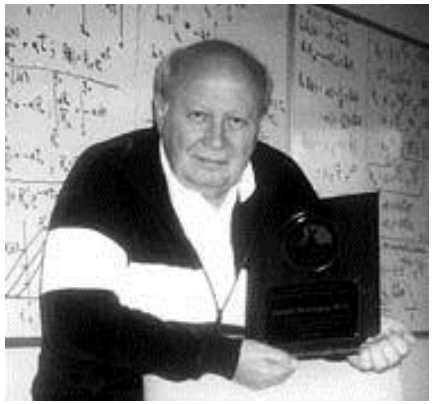
2. Cisplatin and Platinum based drugs

Some general remarks on cisplatin and platinum drugs

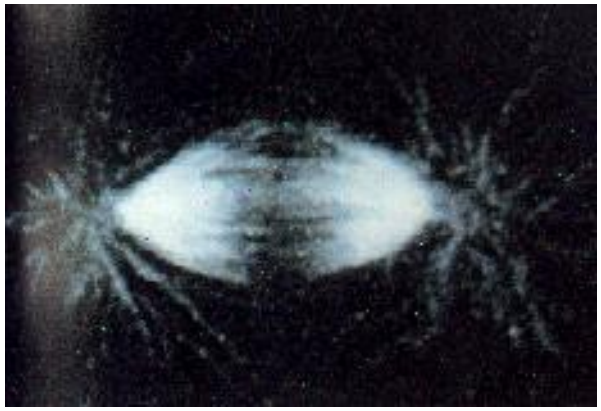
A serendipitous discovery

The discovery of cisplatin

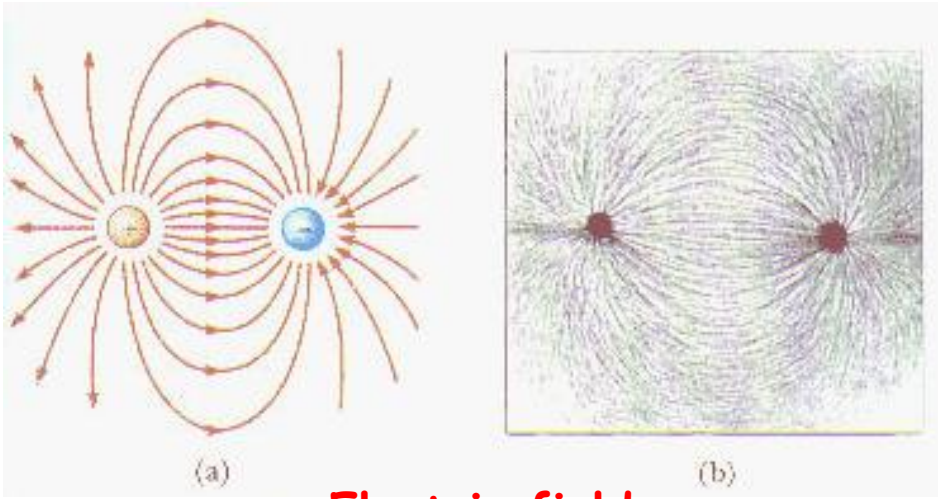
1961 – Michigan State University



Barnett Rosenberg a physicist was fascinated by the similarity between the mitotic spindle and the field lines of an electric field



mitosis

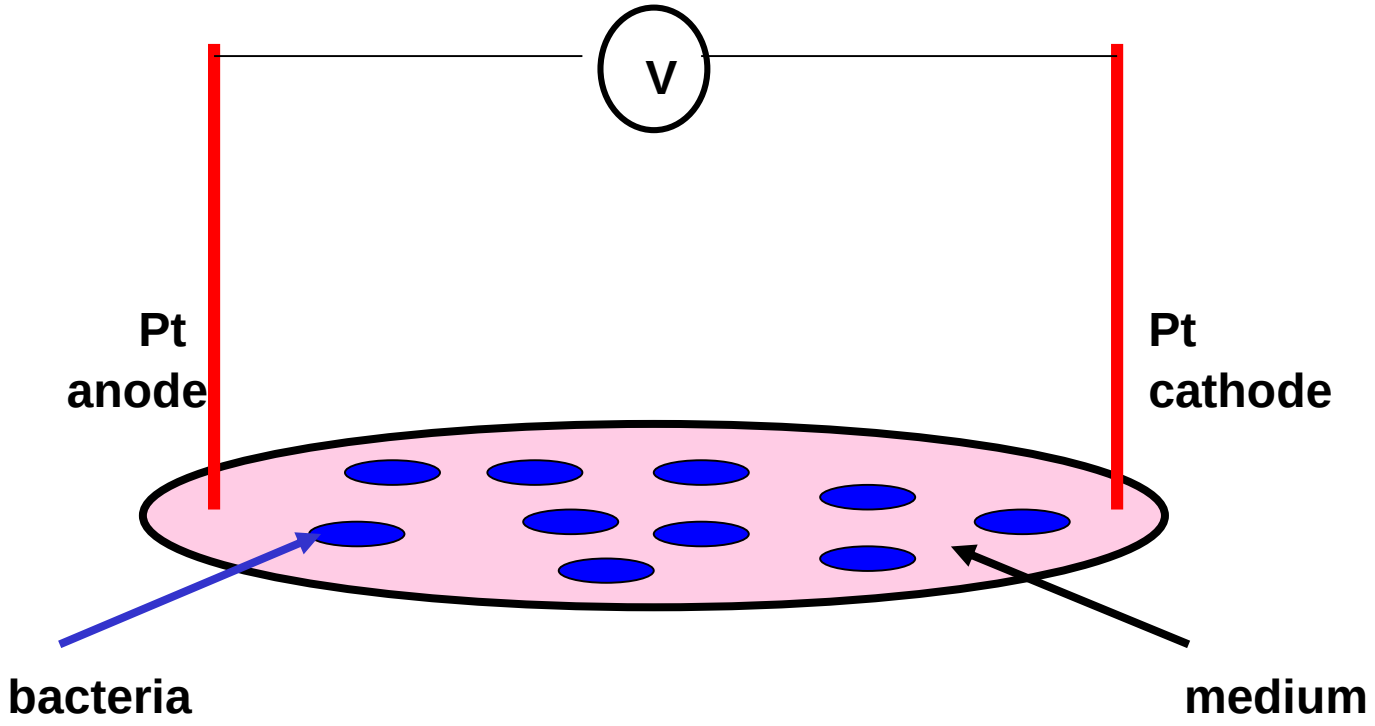


Electric field

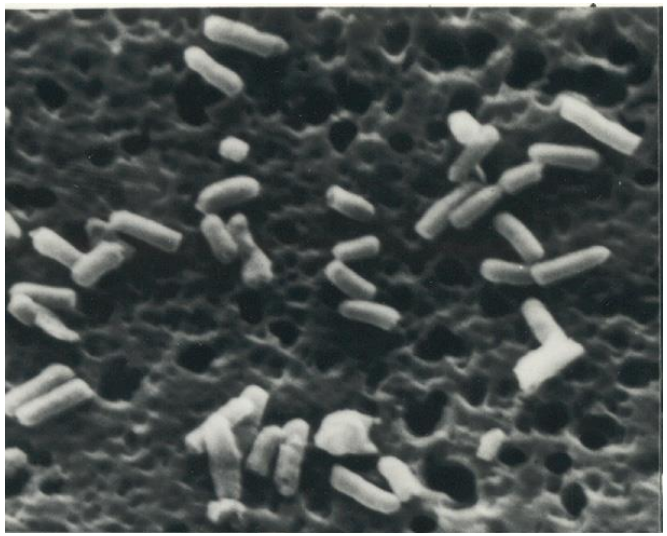
The discovery of cisplatin

Rosenberg thought that applying an electromagnetic field during mitosis at the same frequency of "mitosis" would establish resonance an allow energy transfer to the cells.

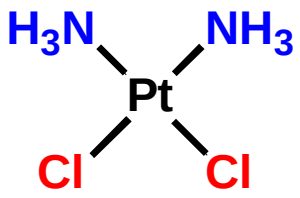
Rosenberg's electrolysis experiment



Rosenberg's electrolysis experiment

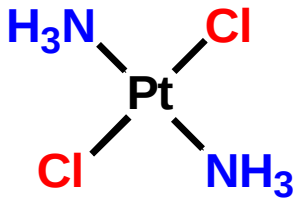


Cisplatin and transplatin



cis-Pt(NH$_3$)$_2$Cl$_2$

Cisplatin, cis-DDP



cis-Pt(NH$_3$)$_2$Cl$_2$

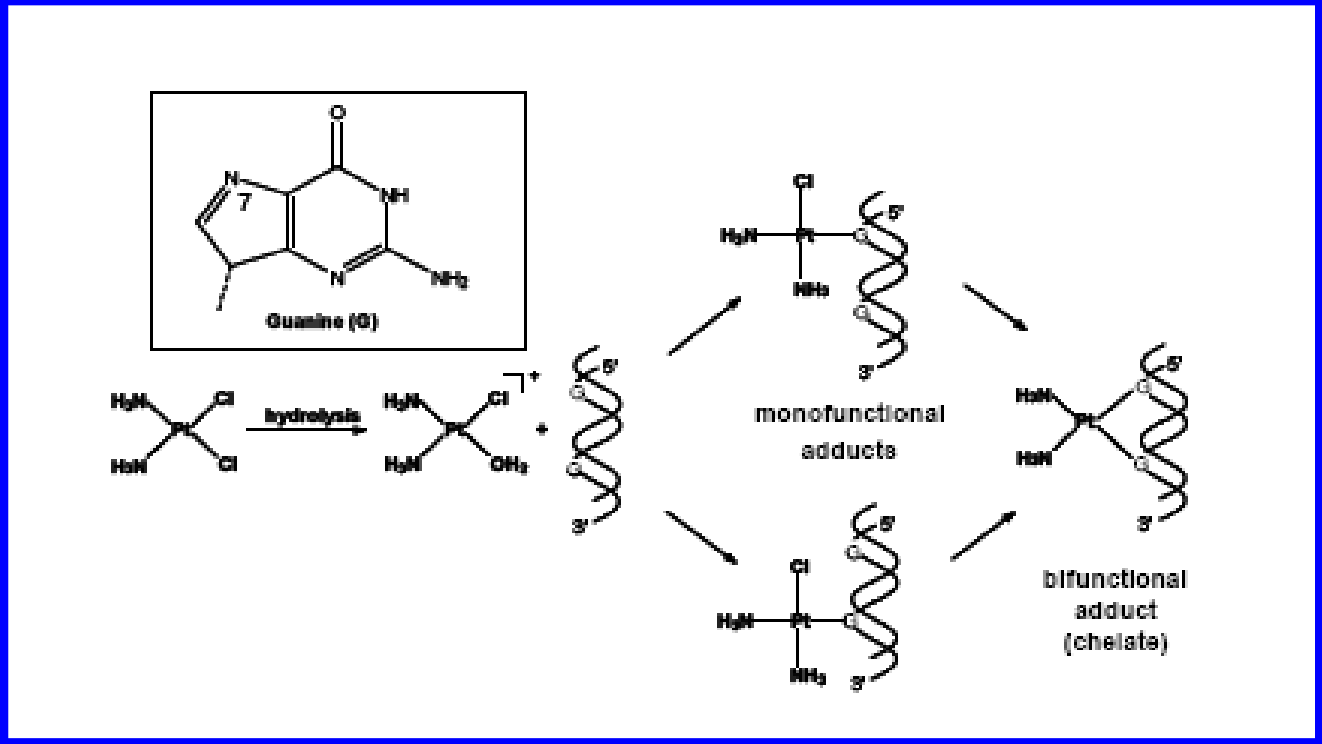
transplatin, trans-DDP

Very effective in the treatment of testicular, ovarian and head and neck cancers

NO anticancer activity

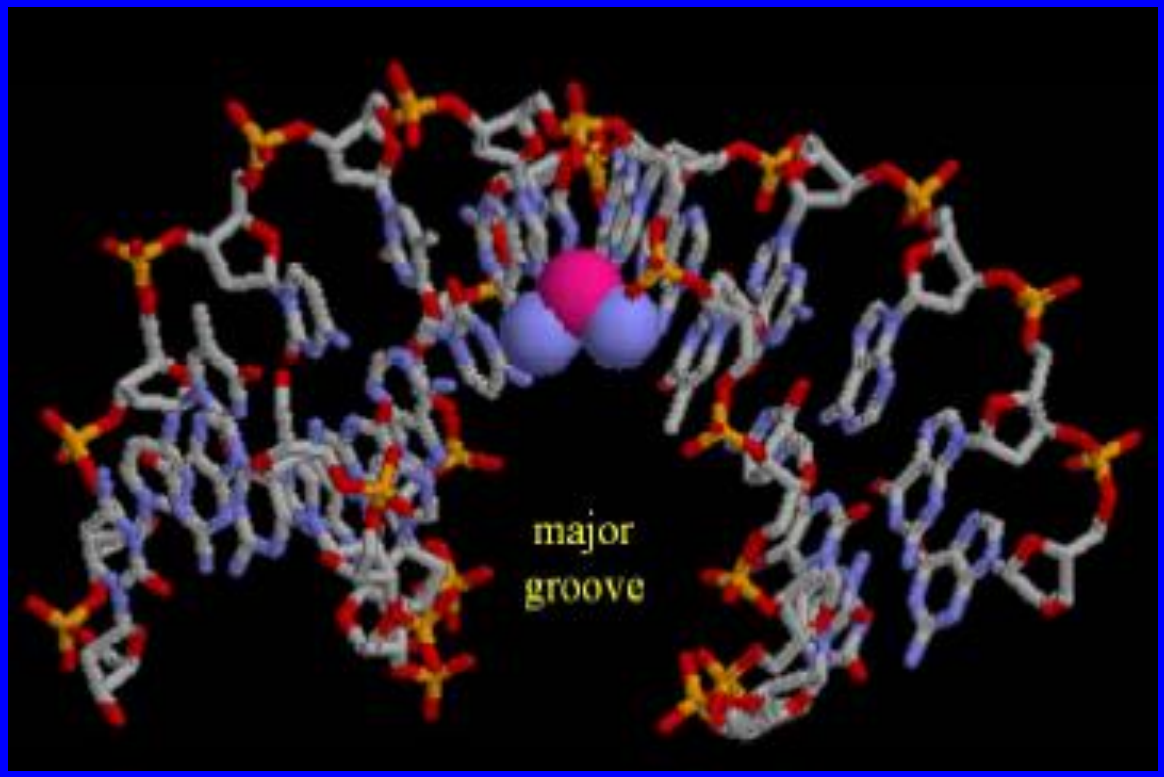
The Mechanism of Action of cisplatin: the "DNA paradigm".

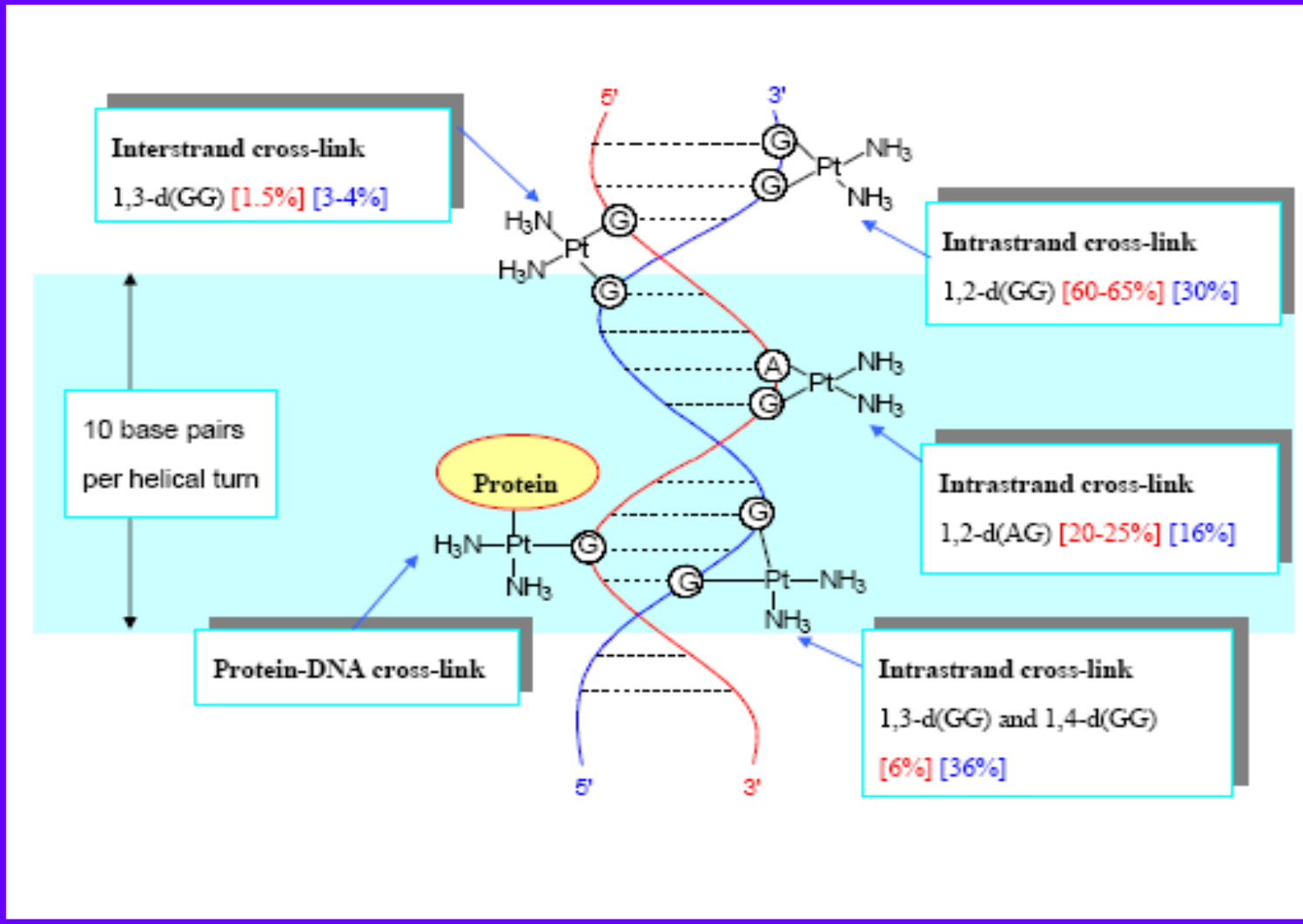
PLATINATION OF DNA BY CISPLATIN



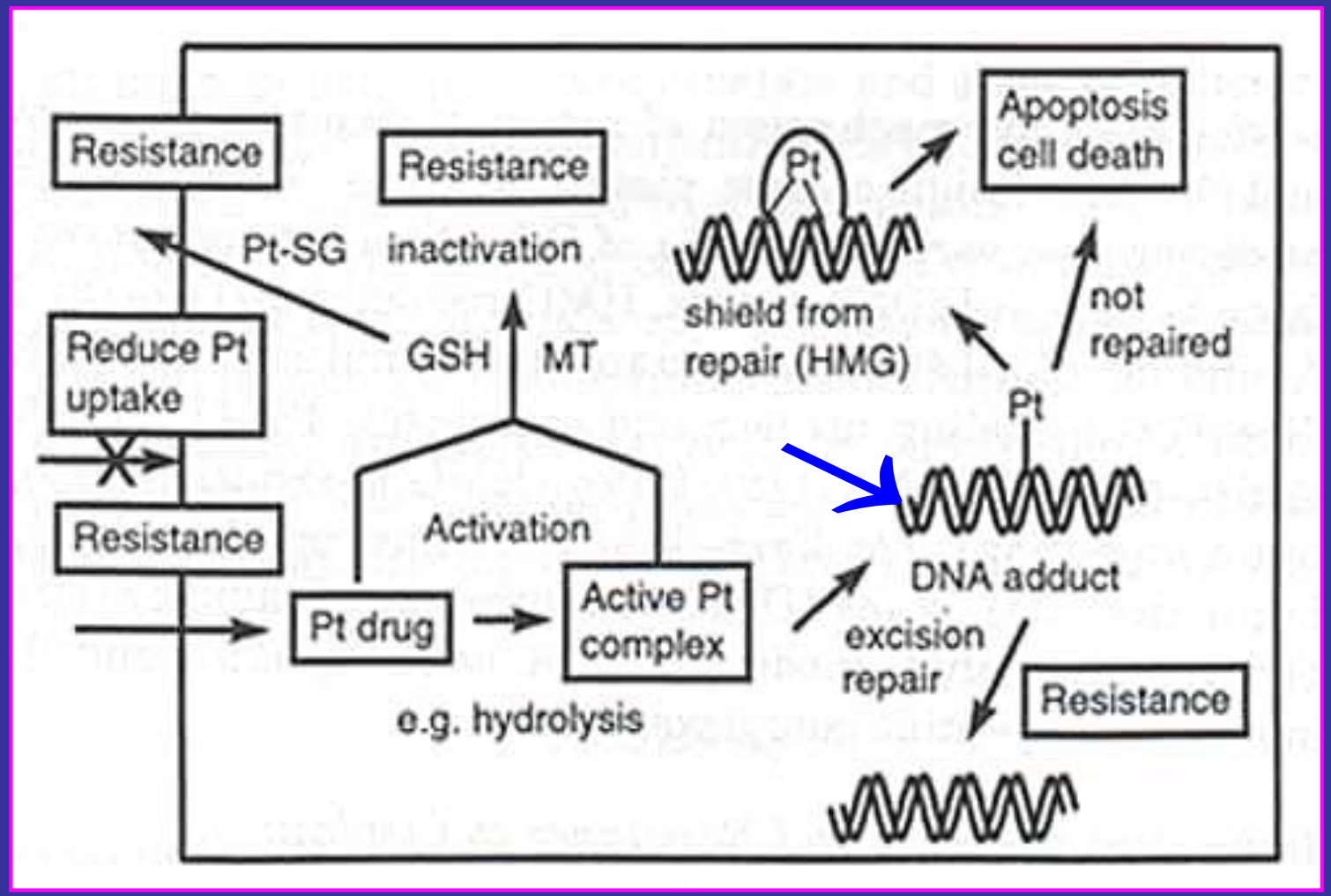
Nuclear DNA is believed to be the critical pharmacological of Pt drugs

The drug binds "to the DNA and distorts it –




Interstrand cross-link
1,3-d(GG) [1.5%] [3-4%]
Intrastrand cross-link
1,2-d(GG) [60-65%] [30%]
Intrastrand cross-link
1,2-d(AG) [20-25%] [16%]
Intrastrand cross-link
1,3-d(GG) and 1,4-d(GG)
[6%] [36%]
Protein-DNA cross-link
10 base pairs
per helical turn
Protein
5'
3'
5'
3'
G
A
Pt
NH3
H3N

The "enlarged" mechanism of action of cisplatin: a role also for proteins within the "DNA paradigm" of platinum metallodrugs.



cisplatin

Is used as the major anticancer agent in the treatment of testicular, ovarian and head and neck cancers.

"testicular cancer went from a disease that normally killed about 80% of the patients, to one which is close to 95% curable."

Cisplatin has been a commercial success as well and has been among the leading anticancer drugs in terms of sales and revenues.

Lance Armstrong

At age 25 was diagnosed with testicular cancer

Was treated with cisplatin

Was cured and proceeded to win the Tour de France 7 times

Drawbacks of cisplatin

1. Lack of activity against major forms of cancer such as colon cancer and breast cancer

2. Becomes ineffective due to development of acquired resistance

3. Severe side effects - nephrotoxicity, emetogenicity, neurotoxicity etc.

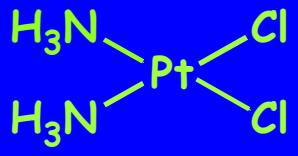
THE SEARCH FOR NEW METALLODRUGS

*The **LIMITS** of cisplatin are the **REASON** for the intensive search of new metal based anticancer agents*

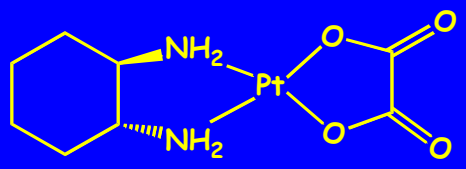
- ***Beyond cisplatin** (but still within platinum!):*
  Thousands of platinum compounds; SAR; unconventional Pt's

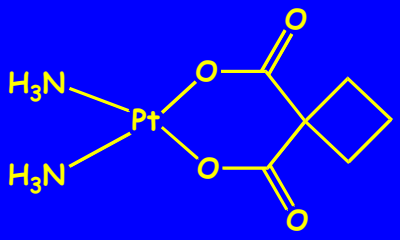
- ***Beyond platinum**:*
  Thousands of non platinum metal complexes
  (Sn, Ti, Ru, Rh, Cu, …)

CISPLATIN



Discovered in 1969,
and in the clinics since 1978,
is the "reference" compound.

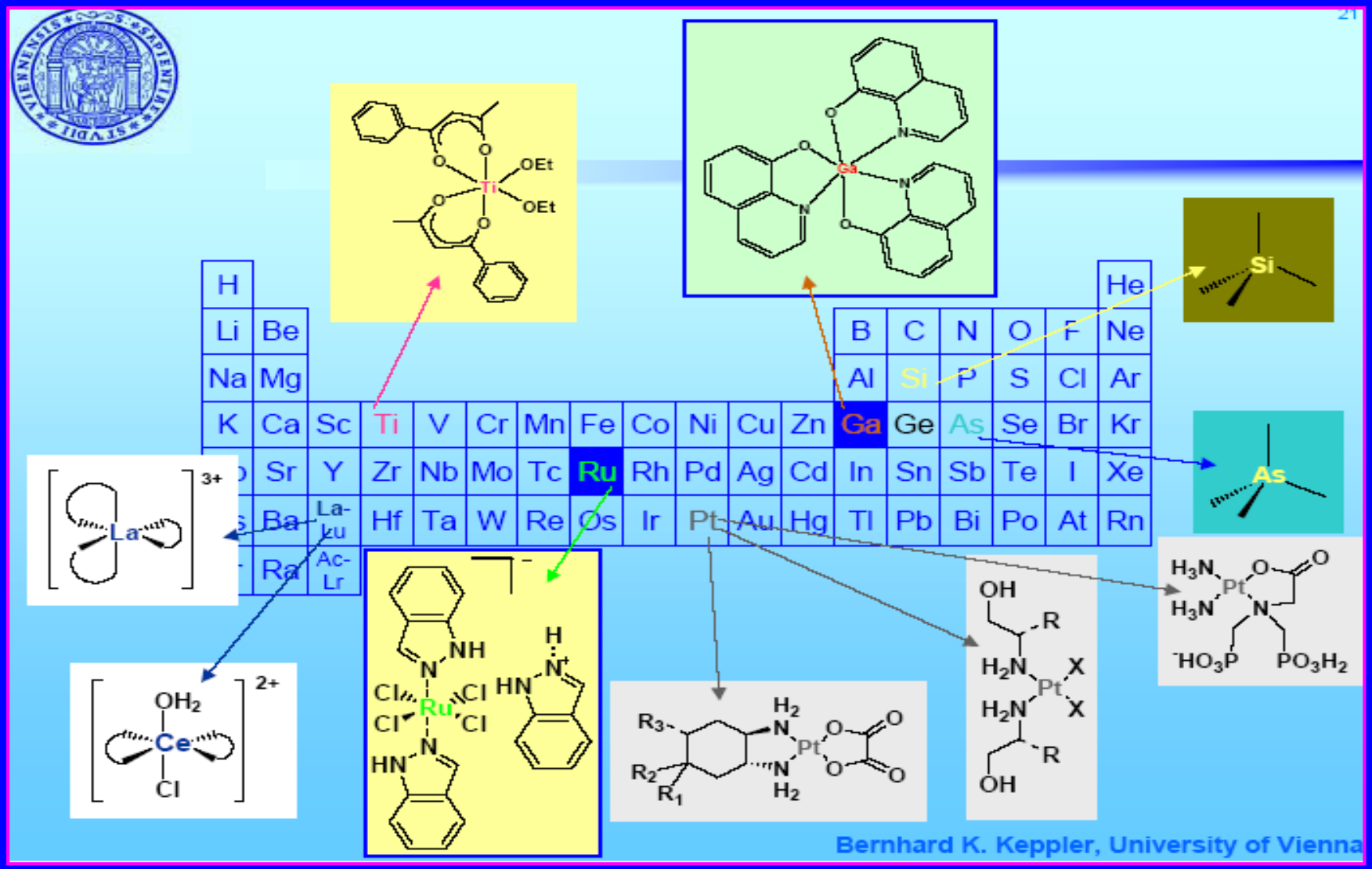




CARBOPLATIN

OXALIPLATIN

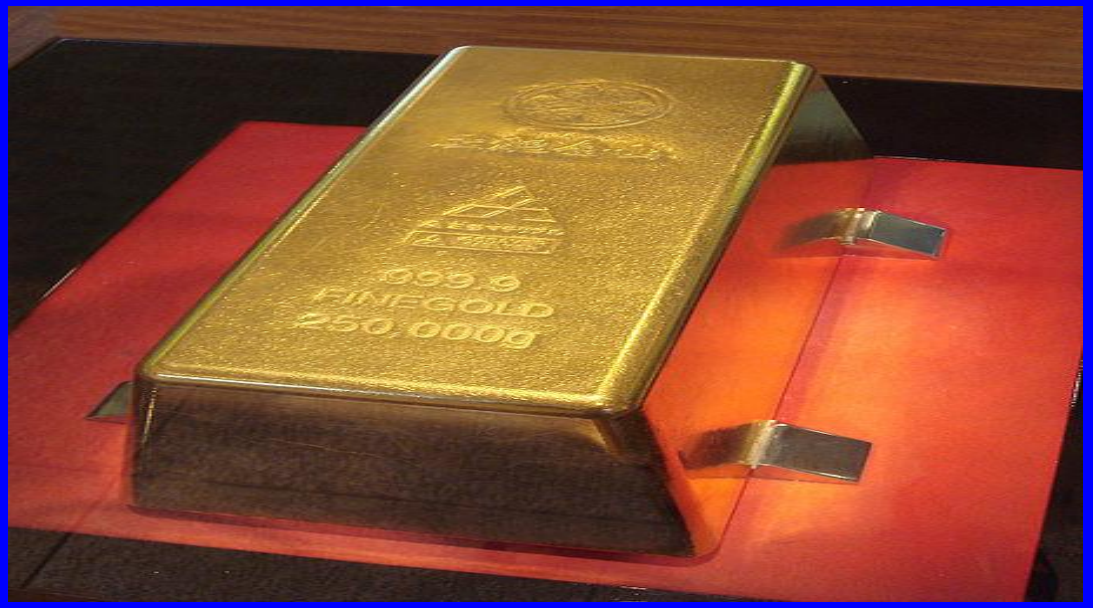
Travelling through the periodic table in the search of new opportunities



WHAT IS THE RATIONALE ?

To exploit the extreme chemical variety and versatility of the different metal centers and to incorporate them into pharmacologically useful substances through the fine tuning of the ligands (the "organic" portion).

3. GOLD AS AN OPPORTUNITY.



Why Gold?

- ***Gold has a rich chemistry***
  Three stable oxidation states: Au(0), Au(I), Au(III)
  Two main coordination geometries (linear and square planar)
  Very interesting coordination and redox properties
  "Soft" character of Au(I); Au(III) to Pt(II) analogy;

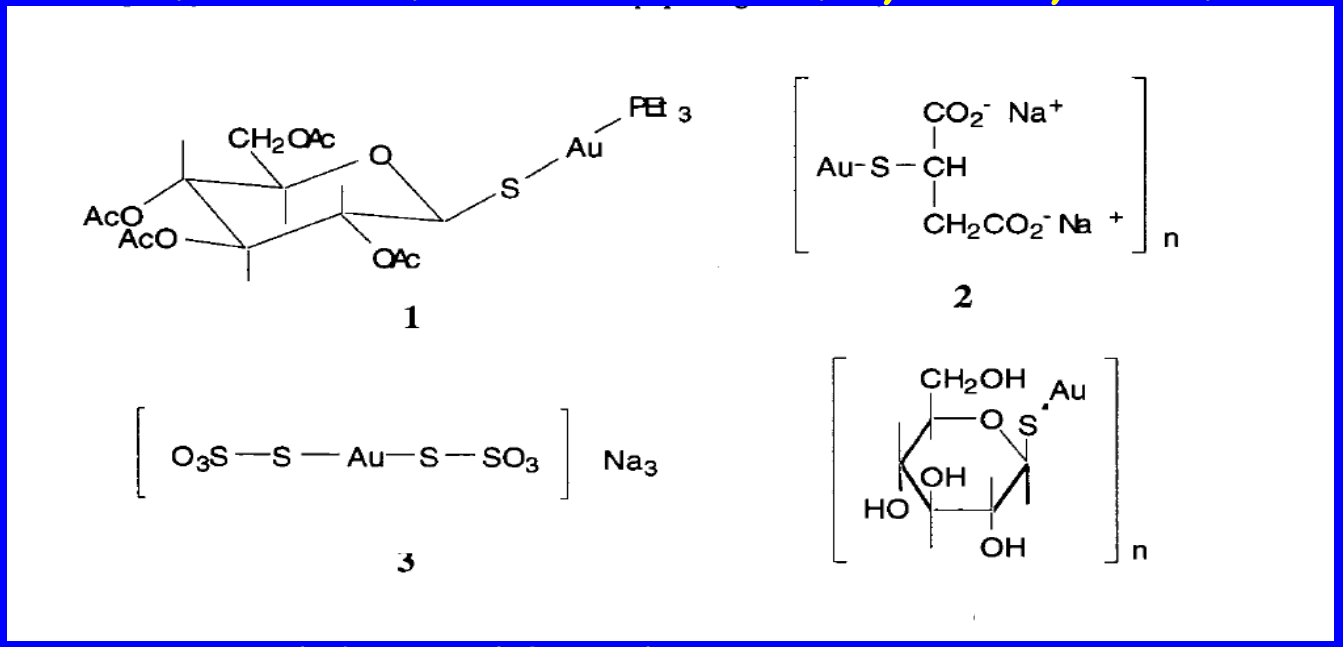
- ***Gold (Au) has been used medicinally for centuries***

- ***The mechanism of action of gold drugs is poorly understood.***

Clinically established gold(I) drugs for chrysotherapy of rheumatoid arthritis

Auranofin
(Ridaura)

Sodium Aurothiomalate
(Myochrysine)



Auro-bis(thiosulfate)
(Sanochrysine)

Aurothioglucose
(Solganol)

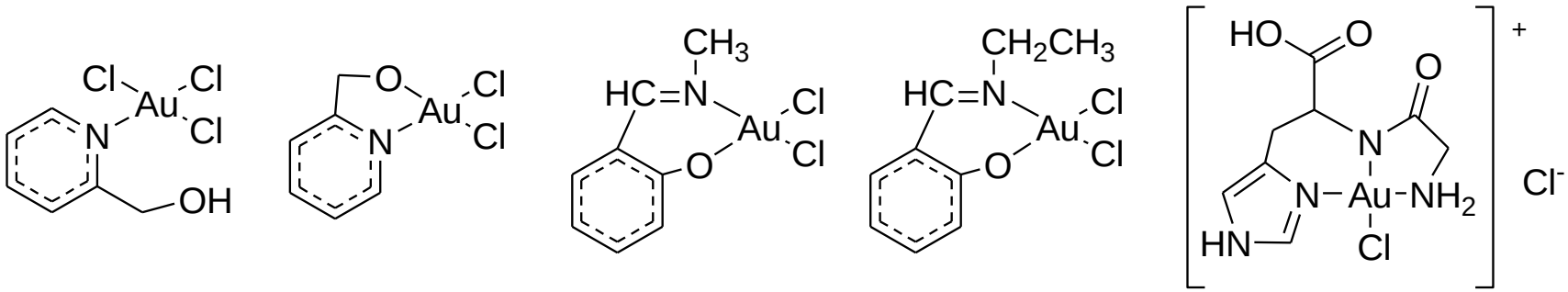
NEW GOLD(III) ANTICANCER COMPOUNDS

A great enthusiasm for gold(III) compounds soon after the discovery of cisplatin owing to strict chemical analogy, followed by a long and profound disappointment.

A renaissance of interest for gold(III) compounds as potential anticancer agents starting from the early 90's owing to the appearance of new promising compounds.

A Gallery of Examples

The first active gold(III) complexes developed and tested in our laboratory



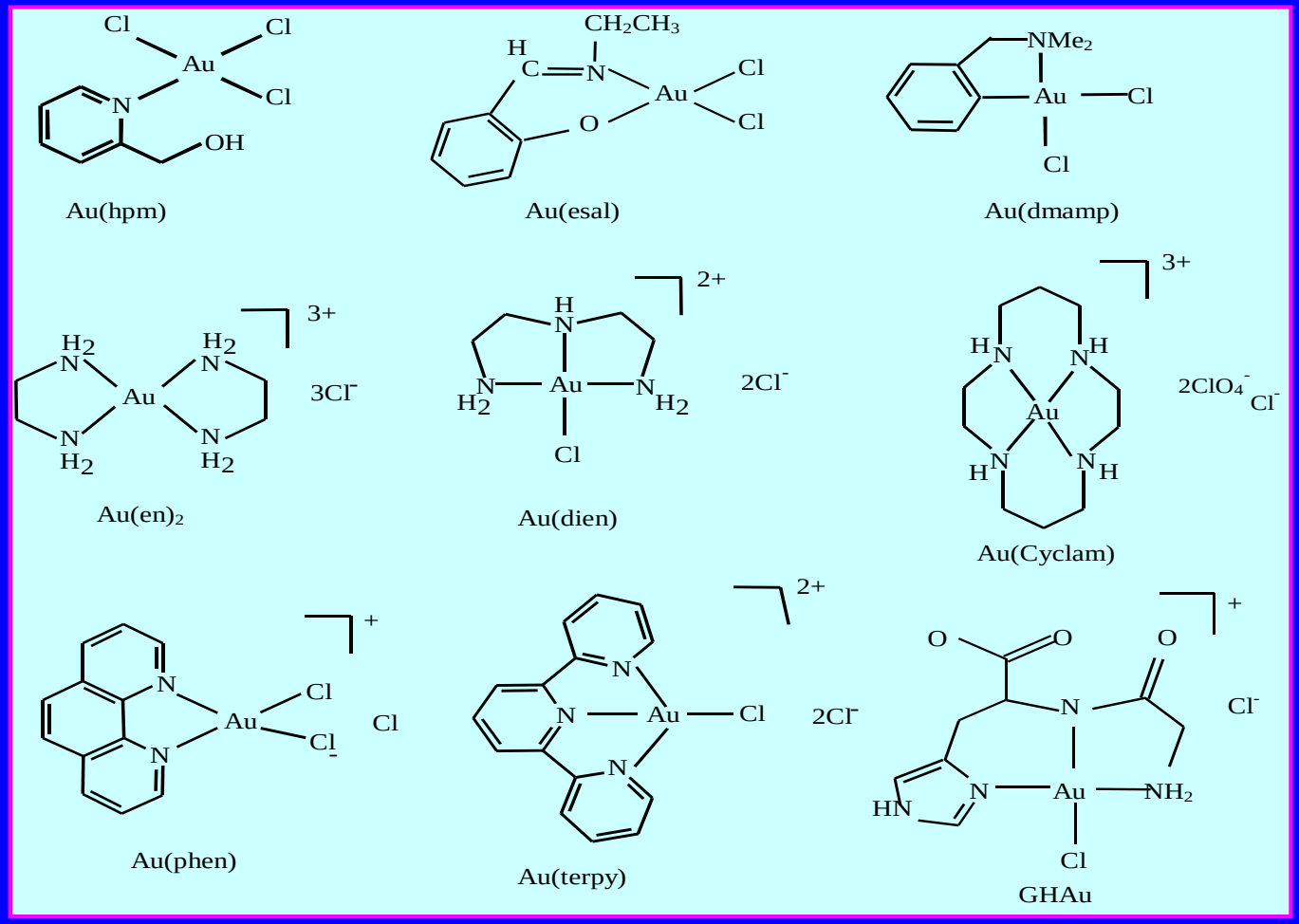
(**a**) (**b**) (**c**) (**d**) (**e**)

Messori, Mini, Mazzei, Orioli et al, 1996-1999

- Novel gold(III) complexes **stabilised** through the selection of appropriate ligands and thus suitable for *in vitro* and *in vivo* pharmacological testing.

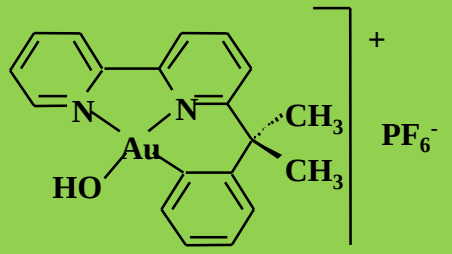
- A few representative examples from our experience and from other research groups worldwide

Classical gold(III) complexes

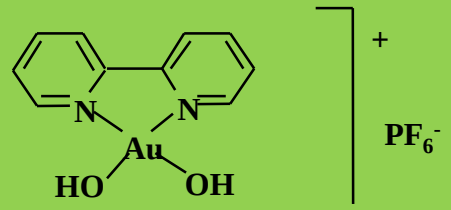


Messori, Marcon et al., JMedChem 2000

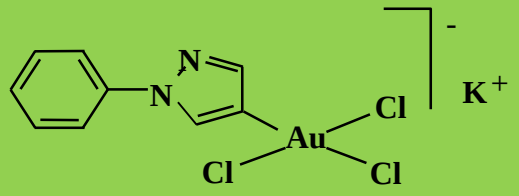
ORGANOGOLD(III) COMPOUNDS WITH BIPYRIDYL LIGANDS: HIGHLY CYTOTOXIC AGENTS



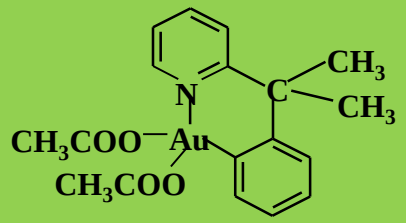
[Au(bipy^c-H)(OH)](PF$_6$)



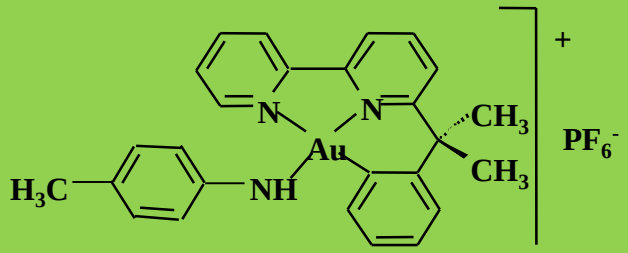
[Au(bipy)(OH)$_2$](PF$_6$)



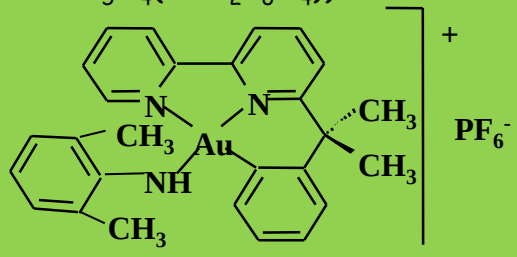
[Au(pz$^\Phi$-H)Cl$_3$]K (pz$^\Phi$= phenil-pirazolo)



[Au(py^c)(CH$_3$COO)$_2$] (py^c= NC$_5$H$_4$(CMe$_2$C$_6$H$_4$))



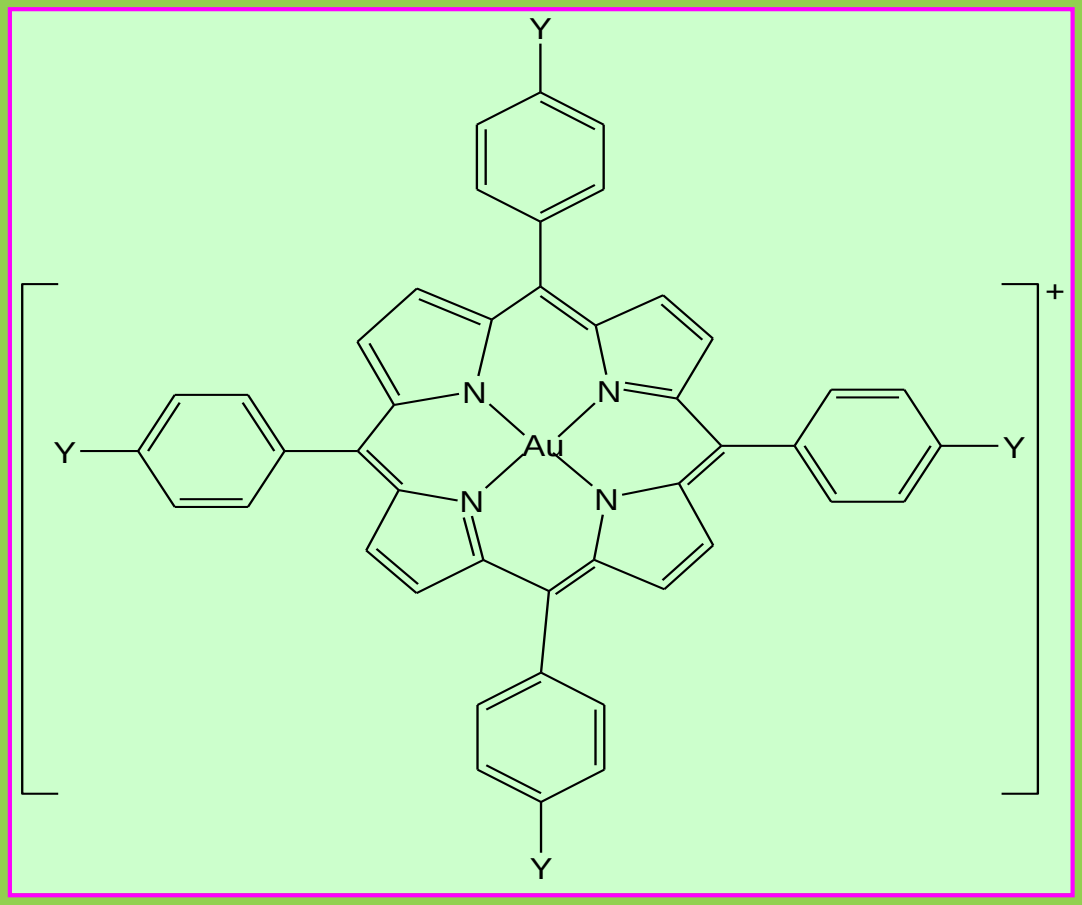
[Au(bipy^c-H)(4-metil-p-toluidina)](PF$_6$)



[Au(bipy^c-H)(2,6-dimetil-o-xilidina)](PF$_6$)

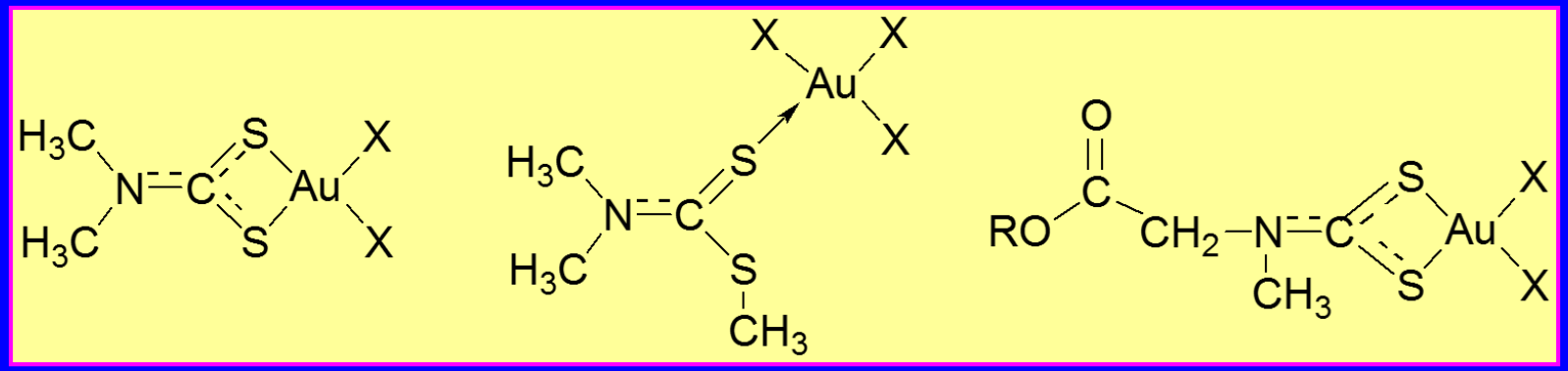
Cinellu, Mini, et al. J.Med.Chem 2002; Messori, Cinellu, et al. BMC 2004

Gold(III) Porphyrins



Gold(III) meso-tetraarylporphyrins complexes. Y = H , Me, OMe, Br, Cl.
From Chi Ming Che et al., HK *(2003-2008)*

Dithiocarbamate gold(III) complexes: inhibitors of the proteasome?

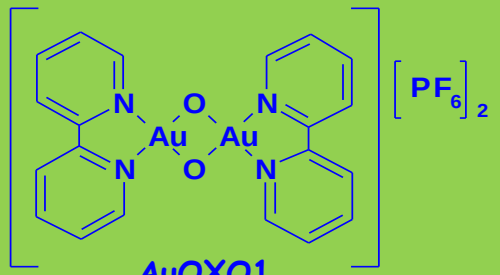


(X = Cl, Br; R = H, CH_3, CH_3CH_2).

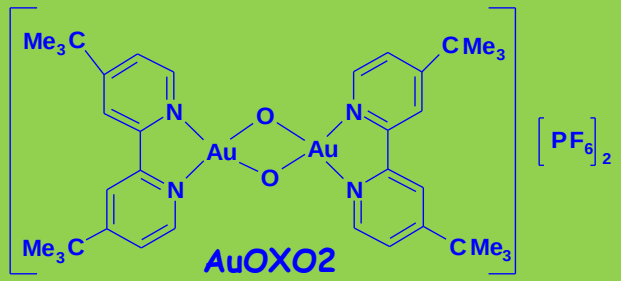
N,N-dimethyldithiocarbamate (a), *S*-methyl-*N,N*-dimethyldithiocarbamate (b), sarcosinedithiocarbamate (R = H), methylsarcosinedithiocarbamate (R = CH_3) and ethylsarcosinedithiocarbamate (R = CH_3CH_2) (c).

Fregona et al. Cancer Res. 2006

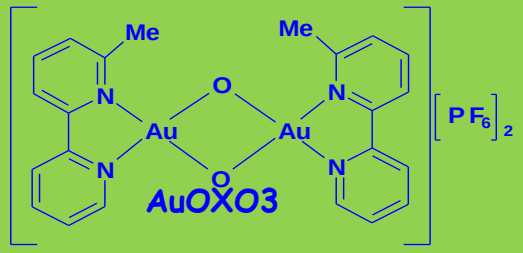
DINUCLEAR GOLD(III) COMPOUNDS



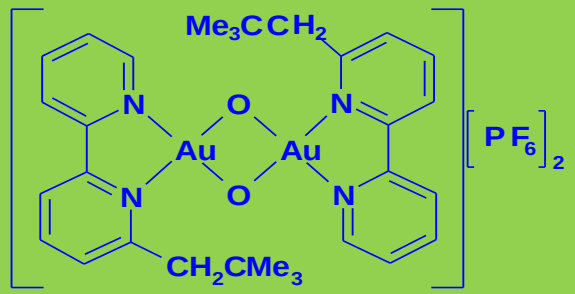
AuOXO1



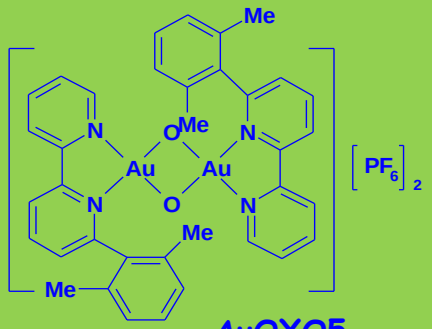
AuOXO2



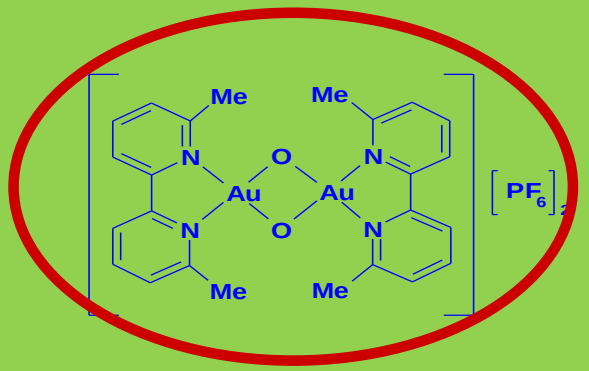
AuOXO3



AuOXO4



AuOXO5



AuOXO6

- ✓These compounds turned out to be sufficiently stable under physiological-like conditions;
- ✓the stability of the various compounds toward biologically occurring reductants was further evaluated;
- ✓Their antiproliferative properties were measured *in vitro* toward the reference ovarian carcinoma cell line A2780;
- ✓The interactions of the dinuclear gold(III) compounds with human serum albumin, ubiquitin cytochrome c and calf thymus DNA were investigated in detail.

Casini, Messori, Mini, Coronnello et al JMedChem 2006; Inorg. Chem. 2008

Cytotoxicity as a first screening criterion

Specific and relevant antiproliferative effects were demonstrated on some selected human tumor cell lines

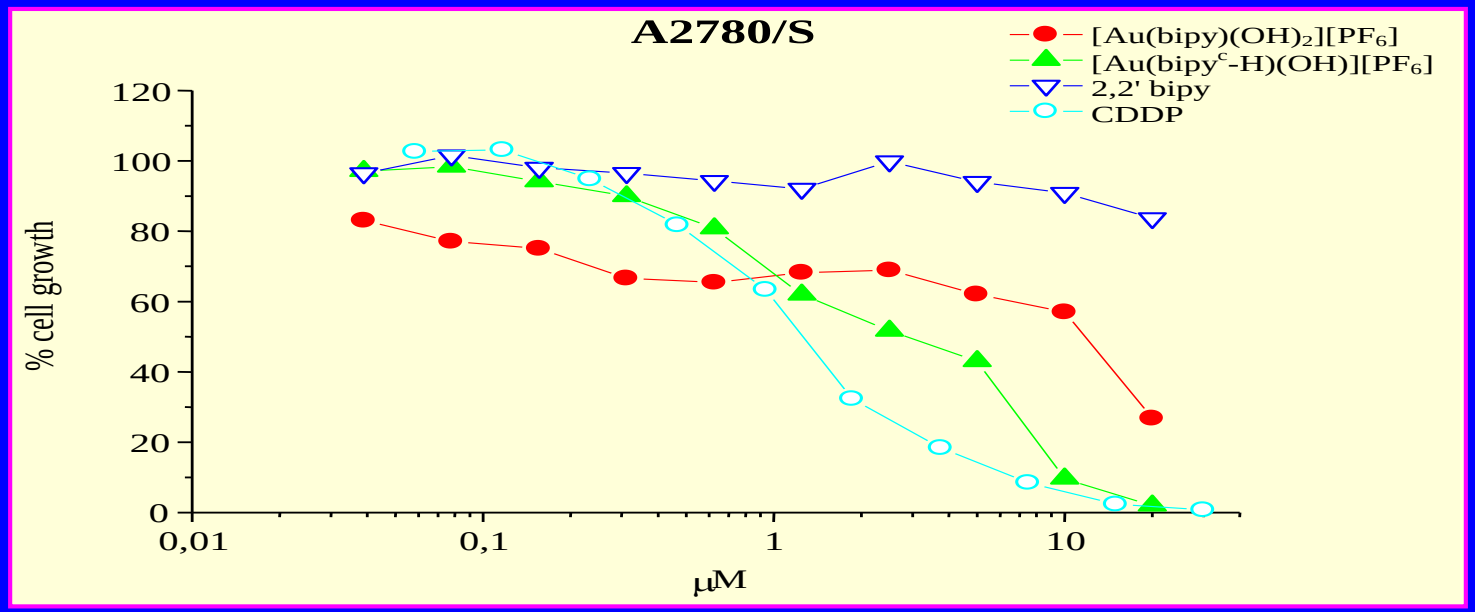
→ A2780S

→ A2780R

Messori, Mini et al. ***J.Med.Chem.2000***

THE COMMON SIGNATURE FOR ALL THESE COMPOUNDS IS AN ACCEPTABLE STABILITY IN AQUEOUS MEDIA ACCOMPANIED BY STRONG ANTIPROLIFERATIVE EFFECTS IN VITRO

- **All the described compounds manifest outstanding in vitro growth inhibitory effects toward human tumor cell lines.**
- **This is the main screening criterion, according to NCI phylosophy, based on large panels of human tumor cell lines.**
- **<u>This is (only!) the starting point to assess effective antitumor properties</u>.**



Cytotoxic properties of the gold(III) compounds studied in Florence

Table 1

Cytotoxicity (IC_{50} μM) of the gold compounds studied in Florence during the last years towards different tumour cell lines. Cisplatin is reported as reference compound. Data were collected after 72 h exposure to drug

Compounds	A2780/S	A2780/R	CCRF-CEM/S	CCRF-CEM/R	SK-OV-3	MCF7	HT29	A549
cisplatin	1.2±0.43	14±2.72	0.7±0.1	20.1±7.2	5.2	5.30±0.87	6.30±0.23	-
$[Au(en)_2]Cl3$	8.36±0.77	17.0±4.24	-	-	-	-	-	-
$[Au(dien)Cl]Cl_2$	8.2±0.93	18.7±2.16	12.6±2.0	32.7±6.6	-	-	-	-
$[Au(cyclam)]ClO_4)_2Cl$	99.0	>120.0	-	-	-	-	-	-
$[Au(Terpy)Cl]Cl_2$	0.2	0.37±0.032	-	-	-	-	-	-
$[Au(Phen)Cl_2]Cl$	3.8±1.1	3.49±0.91	2.3	6	-	-	-	-
GHAu	5.2±1.63	8.5±2.3	-	-	-	-	-	-
$[Au(bipy)(OH)_2][PF_6]$	8.8±3.9	24.1±8.7	52.9±11.6	58.6±0.9	34.4±4.7			
$[Au(bipy^c\text{-}H)(OH)][PF_6]$	3.3±1.4	8.2±1.5	11.9±2.1	51.2±5.6	13.3±1.6	35.30±8.8	24.60	>50
$Au(bipy^{dmb}\text{-}H)$ (2.6-xylidine-H)][PF_6]	2.50±0.43	5.7±0.3	-	-	-	5.20±0.40	~25	~35
$Au(py^{dmb}\text{-}H)(AcO)_2]$	2.90±0.34	6.40±1.0	-	-	-	17.70±0.44	8.60	~49
Auoxo1	22.8±1.53	23.3±0.35	-	-	-	-	-	-
Auoxo2	12.1±1.5	13.5±1.8	-	-	-	-	-	-
Auoxo3	25.4±2.47	29.8±3.1	-	-	-	-	-	-
Auoxo4	12.7±1.06	19.8±1.8	-	-	-	-	-	-
Auoxo5	11.0±1.5	13.2±1.2	-	-	-	-	-	-
Auoxo6	1.79±0.17	4.81±0.5	-	-	-	-	-	-

GOLD COMPOUNDS ARE GOOD CANDIDATES FOR CANCER TREATMENT:

WHAT NEXT?

Two possible approaches

- **"Massive" Drug Development and In vivo testing.**
  - Synthesis & characterization of new gold agents
  - Testing the efficacy of the new agents on appropriate "BIOLOGICAL" models i.e. *cell lines or animal models*
  - comparison to other compounds
  - chemical modification for optimization

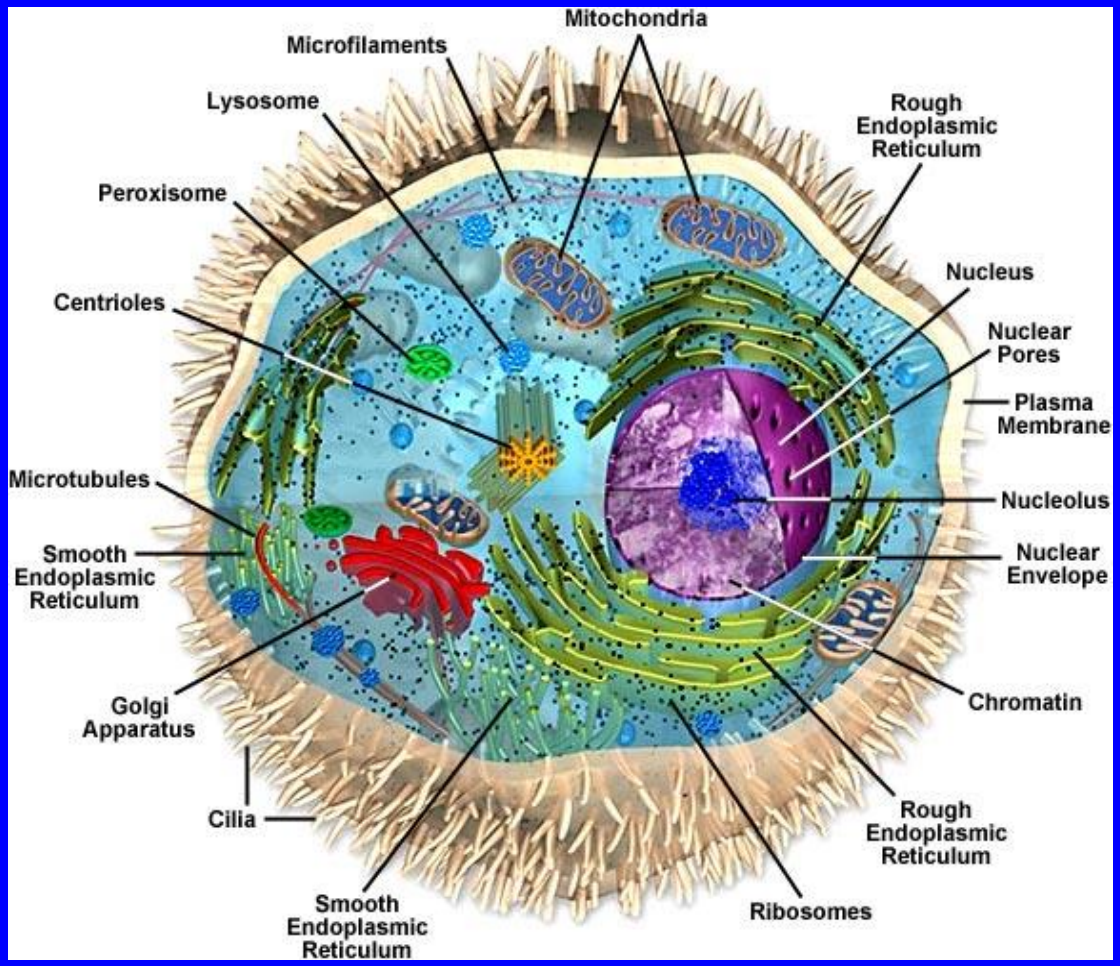
- **Mechanistic Studies (both at the cellular and the biochemical level).**
  - how do these new (*or even old*) agents work?
  - develop insight to aid in new discovery- "mechanism-" or "target- oriented" drug discovery";

4. MECHANISTIC STUDIES

A DETAILED DESCRIPTION
OF THE **CELLULAR EFFECTS**
AND OF THE
MOLECULAR MECHANISMS

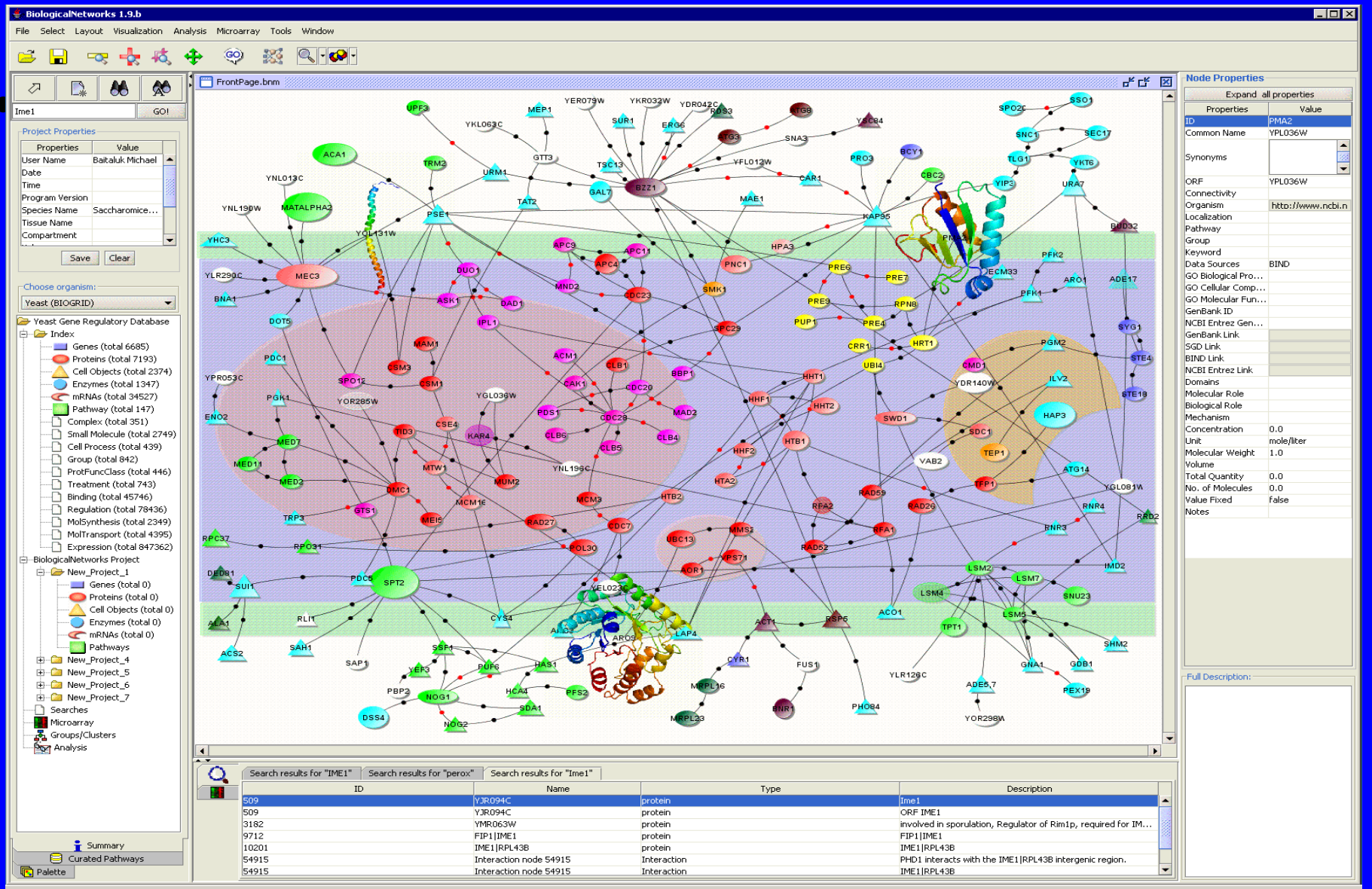
To what in the cell does gold bind?

Cells are extremely complex entitities.



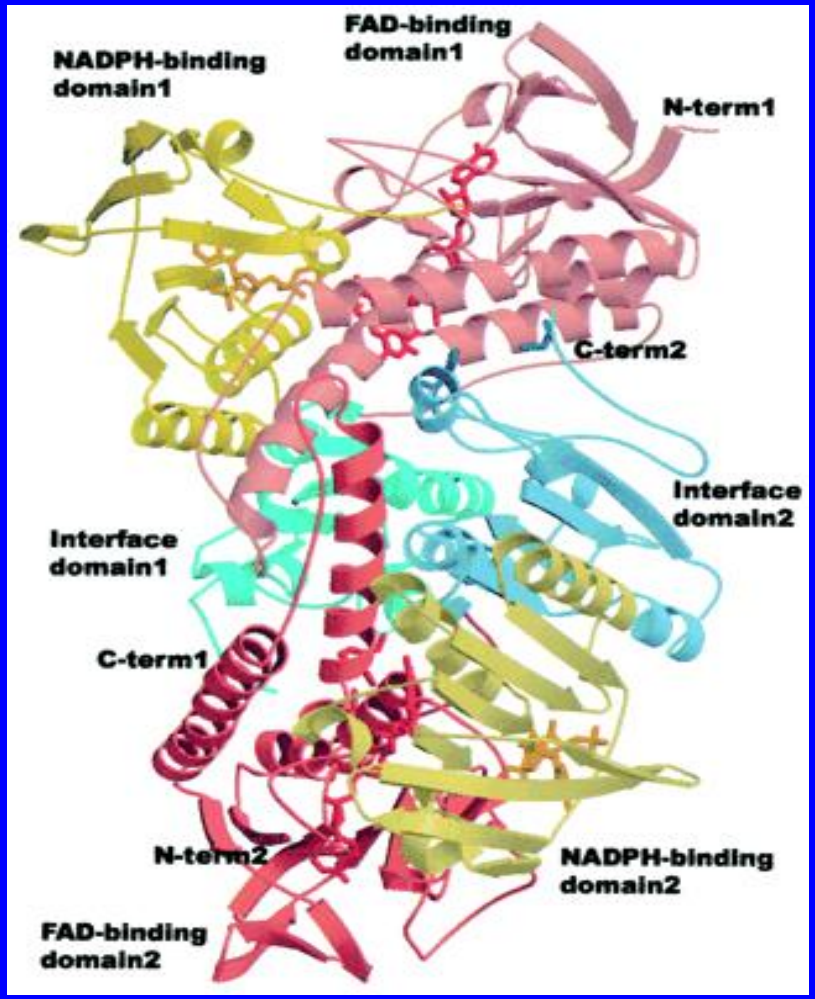
Information on gold uptake and intracellular distribution and speciation would be required

The challenge of complexity in biological systems…

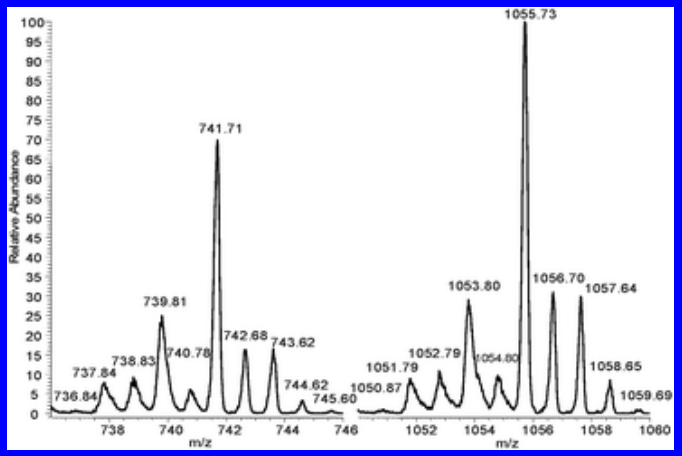


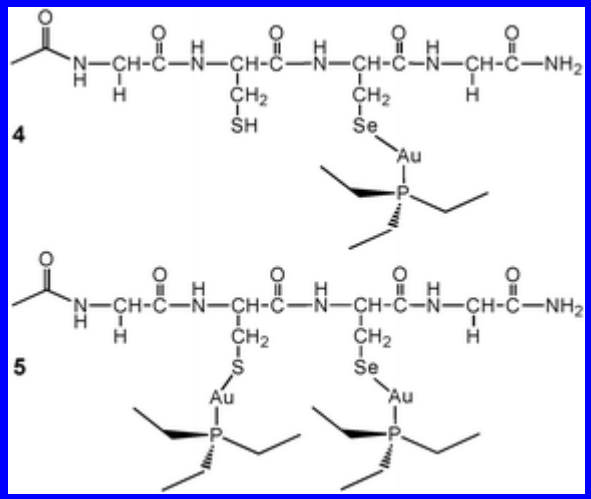
Proposed molecular targets for cytotoxic gold compounds

THIOREDOXIN REDUCTASE: A HOMODIMERIC SELENOENZYME INVOLVED IN REDOX BALANCE, Bindoli, Messori et al. CCR 2009



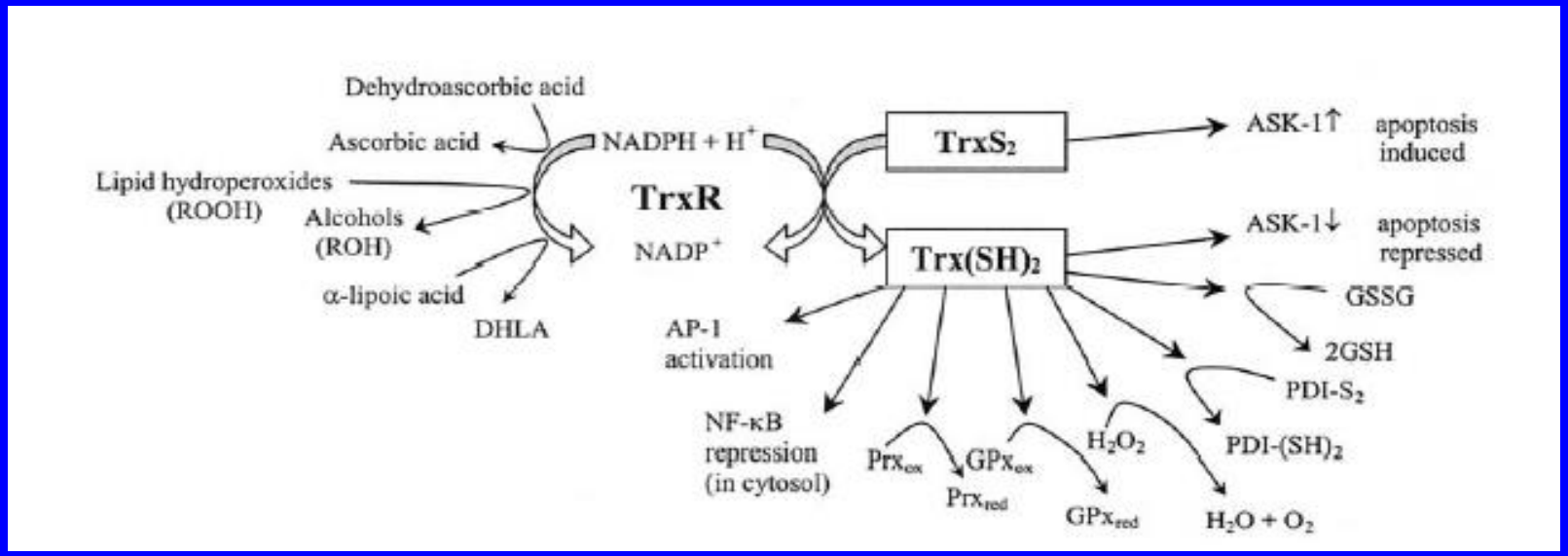
Reactions of medicinally relevant gold compounds with the C-terminal motif of thioredoxin reductase elucidated by MS analysis





Pratesi, Messori et al. ChemComm 2010

Thioredoxin reductase (TrxR)



Mammalian enzymes differ greatly from that in lower organisms; all contain selenium.

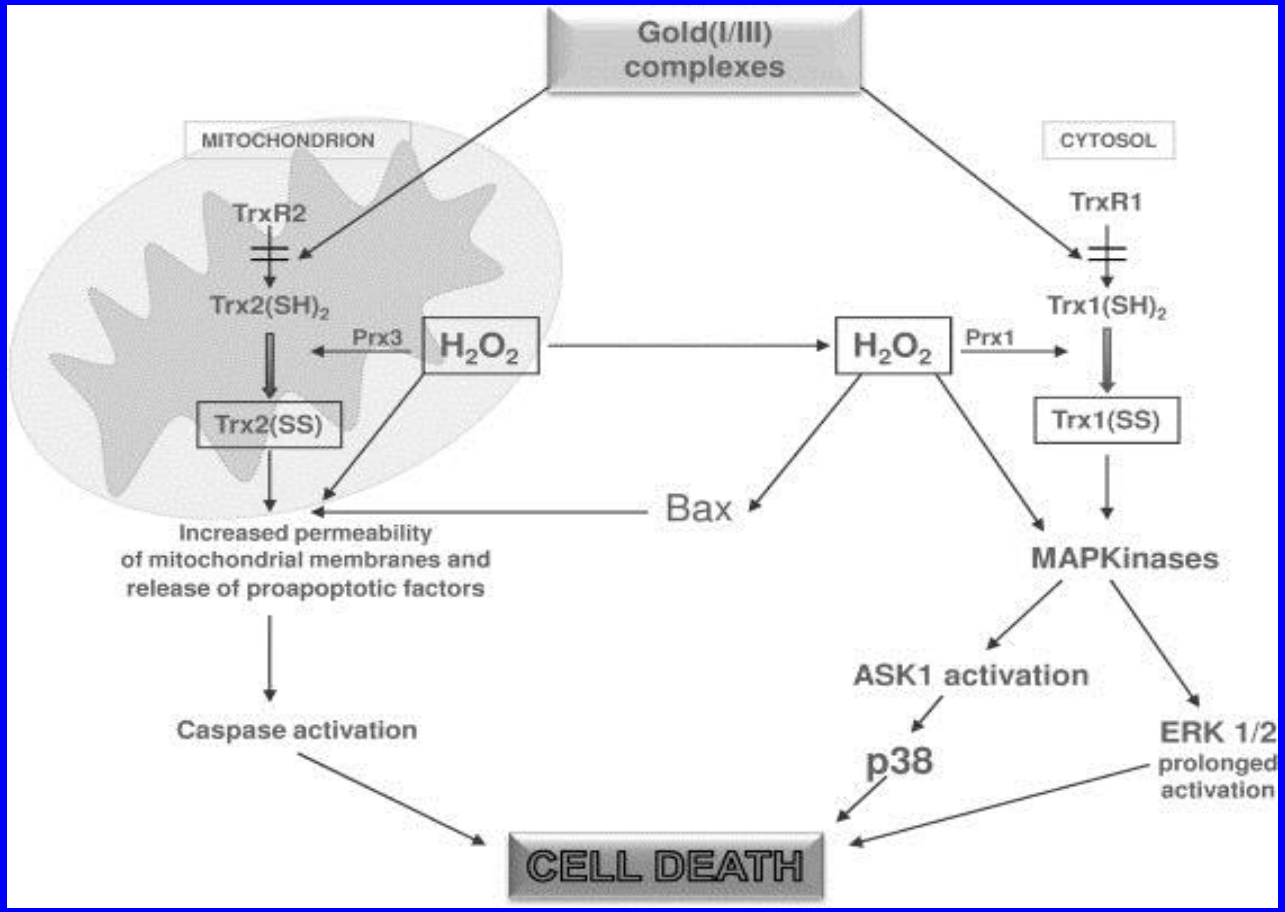
Quinones, retinoic acid, nitrosureas, (BCNU,cisplatin) inhibit TrxR

Gold compounds as TrxR inhibitors

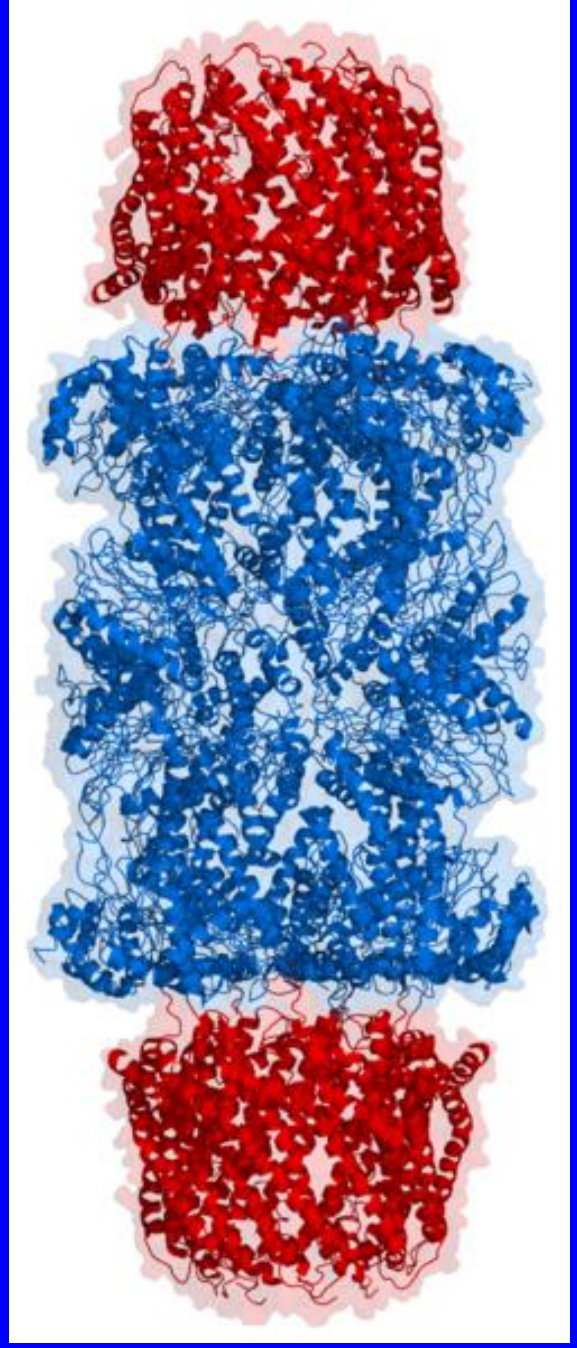
Table 2
Inhibitory effect (IC_{50}) of some gold (I/III) compounds on different thioredoxin reductase isoforms.

Compound	Human cytosolic TrxR1	Rat cytosolic TrxR1	Rat mitochondrial TrxR2
Au(I)			
Auranofin	0.0200[#]	0.0007	0.0020[#]
Au(triethylphosphine)Cl		0.0012	0.0058[‡]
Aurothiomalate		0.0050	0.0280[‡]
Aurothioglucose	0.0650[#]		
Aurothiosulfate		0.0500[§]	
Au(triphenylphosphine)Cl		0.1000[§]	
(Dimethylsulfide)AuCl			0.5840[¥]
Au phenyl(di(2-pyridyl) phosphole)Cl	0.0008[&]		
Au(III)			
Tetrachloroaurate	0.0058[*]	0.0120	0.1000
$Au(OAc)_3$		4.000[§]	
$[Au(2,2'\text{-diethylendiamine})]Cl_2$	0.2000[*]		
$[Au(2,2'\text{-diethylentriamine})Cl]Cl_2$		0.0028	0.4200[#]
$(Au(py^{dmb}\text{-}H)(OAc)_2$		0.0147	1.4200[#]
$[Au(bipy^{dmb}\text{-}H))(OH)](PF_6)$		0.0043	0.2800[#]
$[Au(bipy^{dmb}\text{-}H)(2,6\text{-xylidine})](PF_6)$		0.0041	0.2100[#]
$Au(2,2'\text{-bipyridine})Cl_2$	0.0120[*]		
$Au(2\text{-phenylpyridine})Cl_3$	0.0300[*]		
$Au(2\text{-phenylpyridine})Cl_2$	0.0360[*]		
$Au(damp)Cl_2$	0.1800[*]		
$Au(damp)(OAc)_2$	0.0300[*]		
Au(damp)(phenyl)Cl	0.0022[*]		
Au(dimethyl)(damp)	1.8000[*]		
Au(trimethyl)(triphenylphosphine)	0.6800[*]		
$Au(DMDT)Cl_2$		0.0057[◦]	0.0247[◦]
$Au(DMDT)Br_2$		0.0077[◦]	0.0284[◦]
$Au(ESDT)Cl_2$		0.0170[◦]	0.0346[◦]
$Au(ESDT)Br_2$		0.0139[◦]	0.0359[◦]

A model for the mechanism



Bindoli, Messori et al. CCR 2009



THE PROTEASOME

Cartoon representation of a proteasome. Its active sites are sheltered inside the tube (blue). The caps (red; in this case, 11S regulatory particles) on the ends regulate entry into the destruction chamber, where the protein is degraded

A target for gold compounds?

The main catalytic activities of the proteasome -CTL, TL and CL- were monitored in the presence of various gold compounds. A strong inhibition was documented. In collaboration with Nicola Micale and Tanja Schirmeister

5. Omics Technologies and the Mode of Action of Cytotoxic Gold Compounds

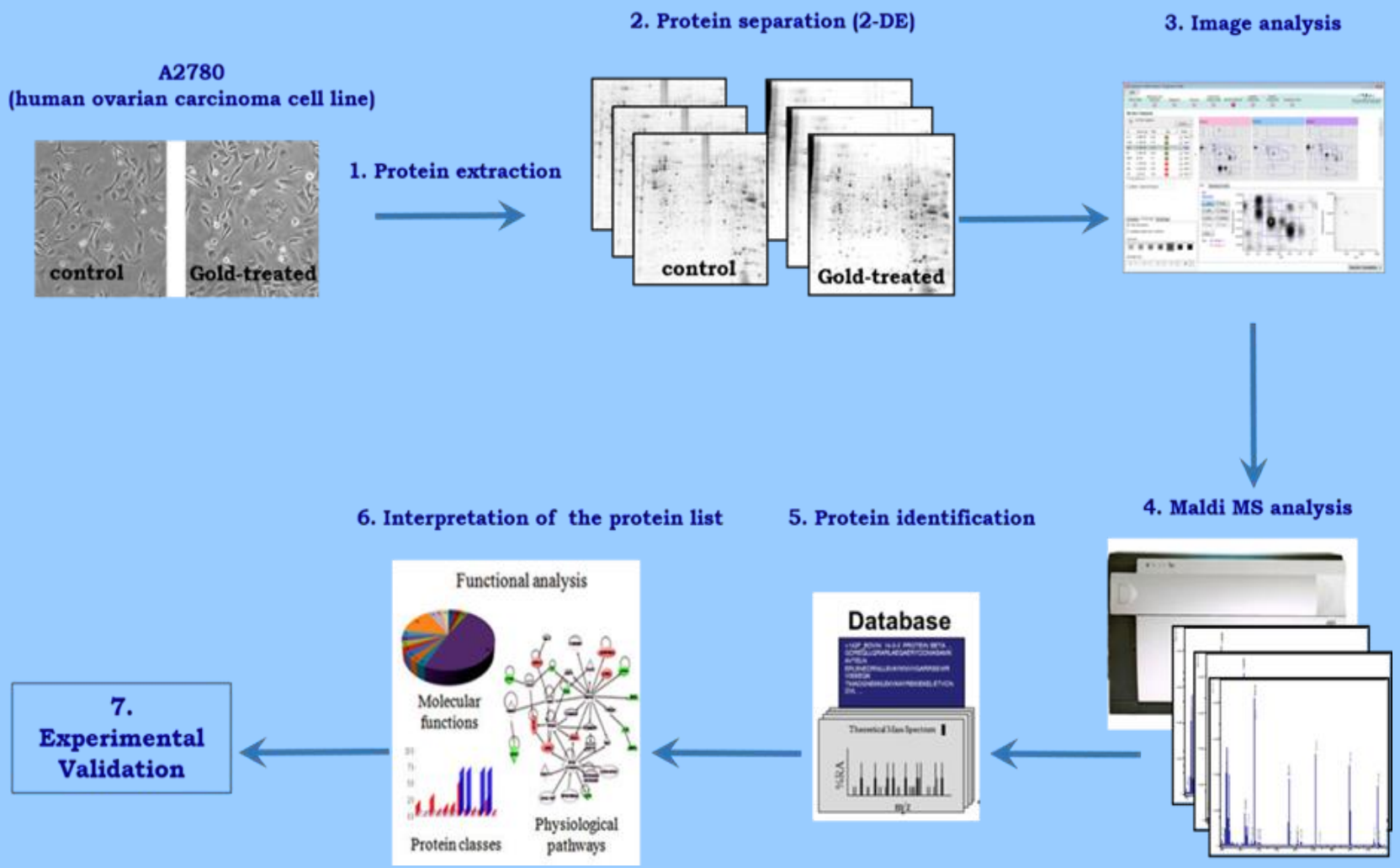
Transcriptomics…
Proteomics…
Metabolomics…
Metallomics..

PROTEOMICS

Proteomics is the study of the proteome
and of its quantitative and qualitative changes.

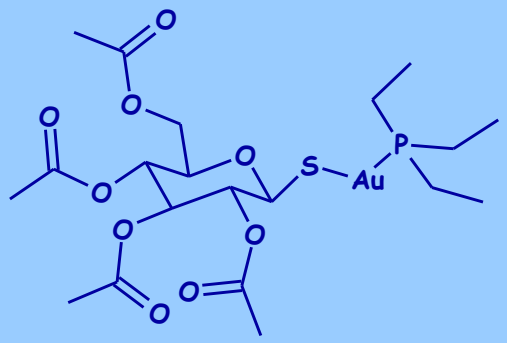
It provides a "portrait" of the functional state of the cell.

Thus, we have analysed the Proteomic alterations induced by cytotoxic gold drugs in comparison to controls



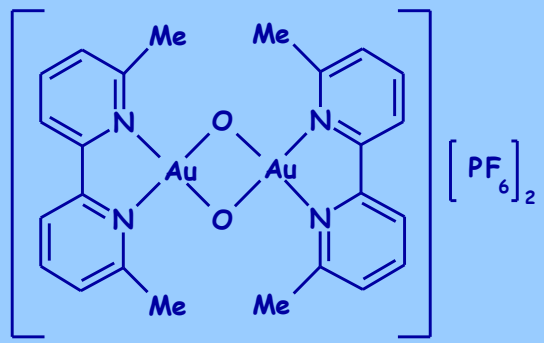
The classical proteomic workflow

Selected gold compounds…



I

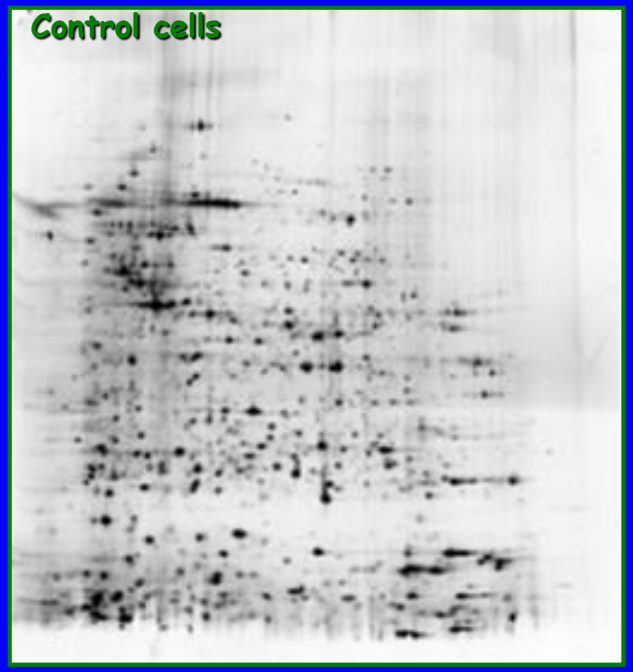
Auranofin

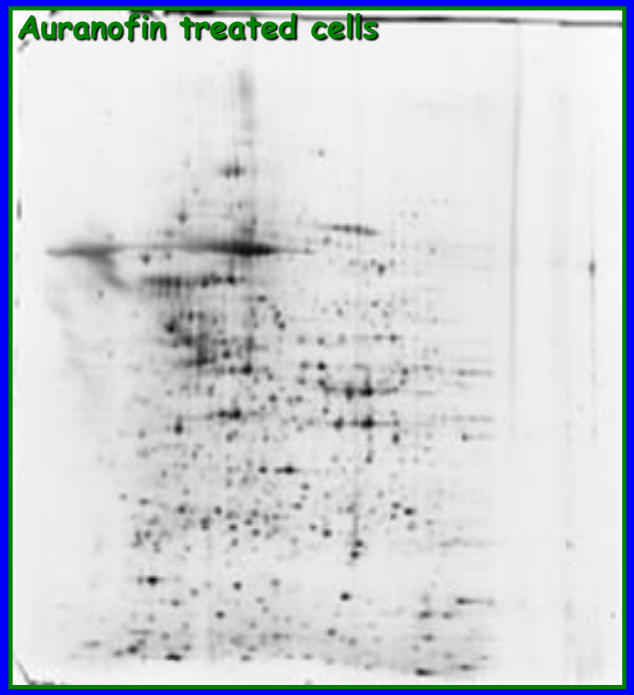


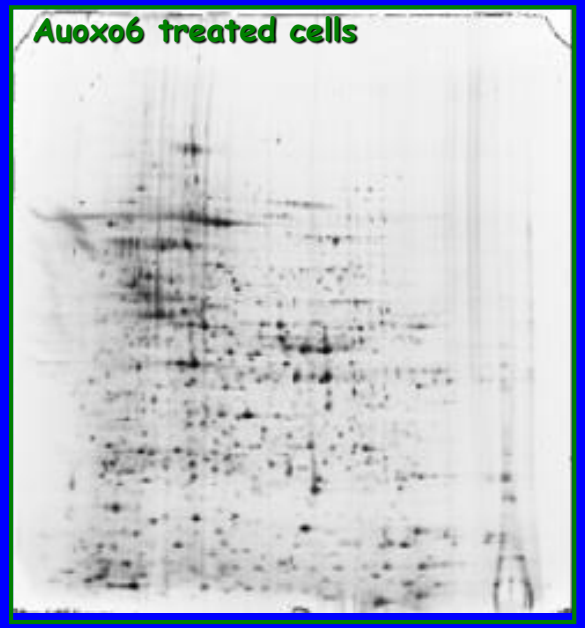
II

Auox06

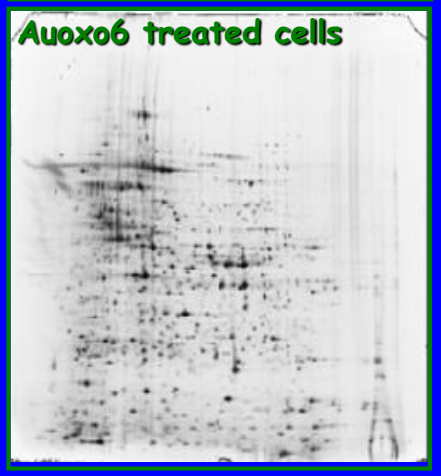
2D gel images

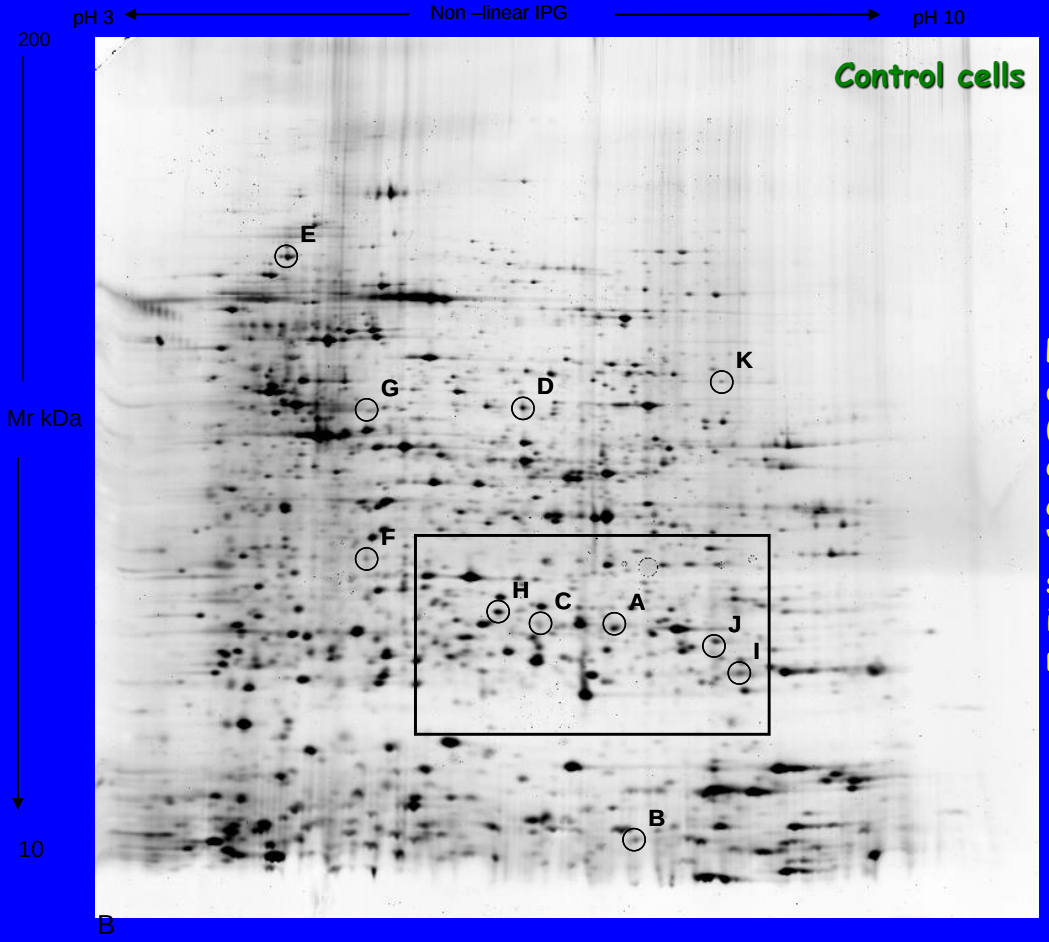






2D gel images





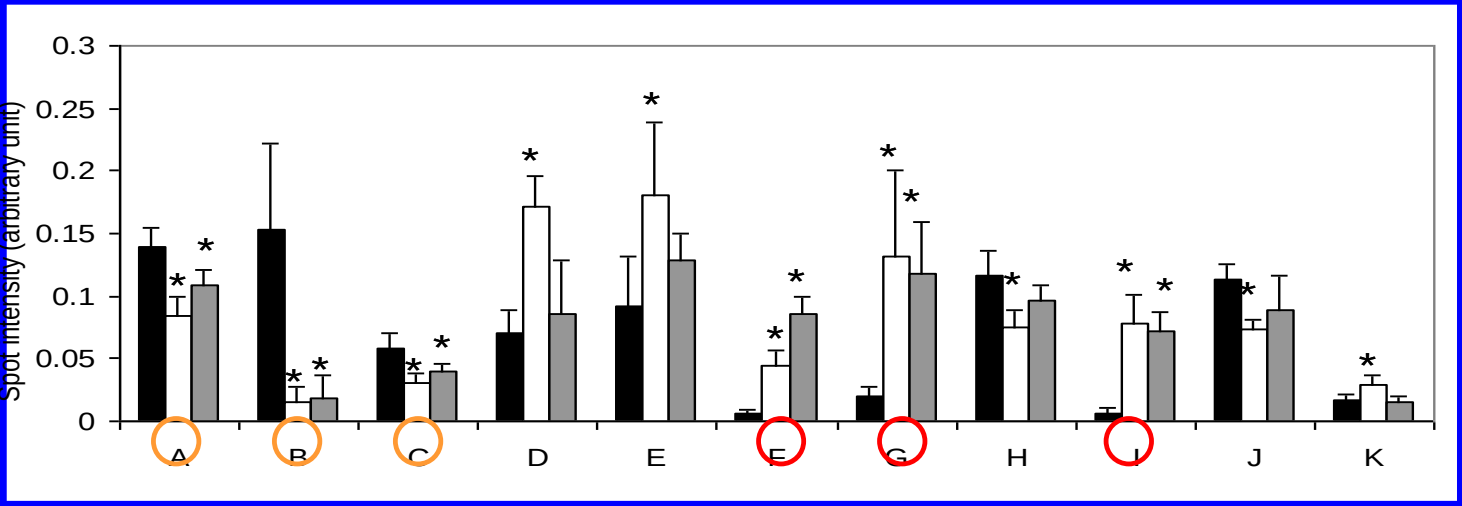
Proteins with at least a value of 1.5-fold ($p<0.05$) change in expression level were considered as "*changed*" and were selected for further identification by mass spectrometry.

Notably treatment of A2780 cells with a cytotoxic amount of these two compounds induced relative moderate changes in protein expression.

Most of the altered proteins were in common between the two tested gold compounds implying a substantial similarity in their mechanisms.

Only a very limited number of proteins, out of the more than 1300 monitored, did show appreciable down or up regulation.

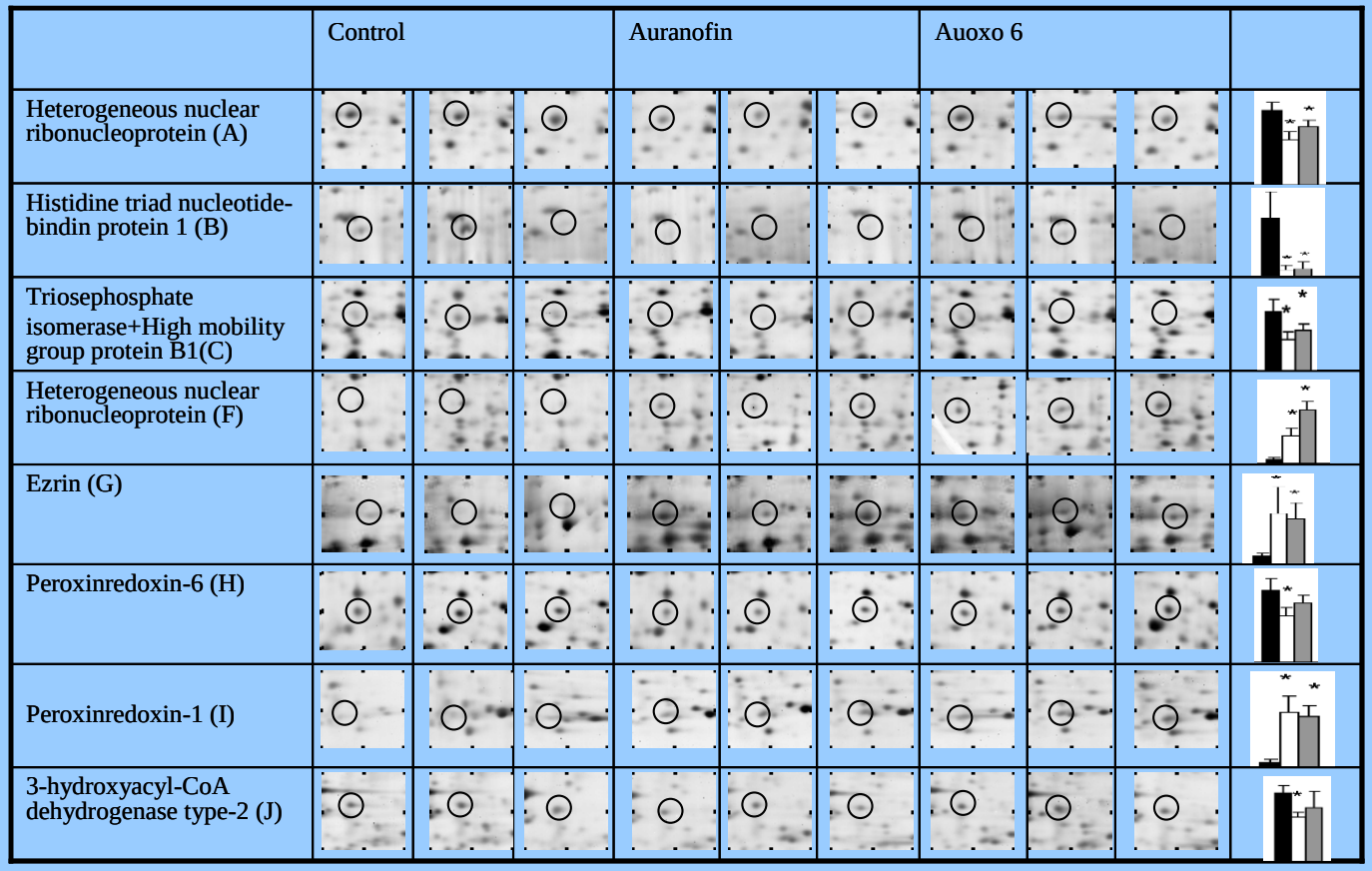
Relative protein expression changes of *auranofin* (white bars) and *Auoxo6* (grey bars) treated cells *vs.* control cells (black). Bars represent the mean ± SD of spots volume percentage from three different experiments."*" indicates that the difference is statistically significant P<0.05. %V is calculated as: V single spot/V total spots (V = integration of OD over the spot area).



Comparative computer analysis highlighted a total of 11 differentially expressed protein spots detected in both cell treatments.

Three spots (spots F, G and I) show a pronounced up-regulation in both treated cells in comparison to the control; on the contrary three spots (spots A, B and C) show a down-expression in both drug treatments. Finally four spots (D E H and J) present a significant variation only when cells were treated with *auranofin*.

Magnified regions of triplicate 2D gel images of spots corresponding to identified proteins.



The histograms illustrate the variation of protein expression for both drug treatments (Auranofin, white bars, and Auoxo6, grey bars) in comparison with untreated cells (black).

Eight altered proteins were identified by mass spectrometry: among them, notably, Ezrin, a protein associated to the cytoskeleton and involved in apoptosis. Interestingly, two altered proteins, i.e. peroxiredoxin 1 and 6, play crucial roles in the cell redox metabolism. Increased cleavage of heterogeneous ribonucleoprotein H was also evidenced consistent with caspase 3 activation.

Main results of this proteomic investigaiton

- The reported proteomic approach turned out to be very informative
- Selective alterations in the proteome were evidenced upon metallodrug treatment.
- A roughly similar mechanism may be hypothesised for *auranofin* and *Auoxo6* owing to the strict analogies in the observed proteome alterations.
- A significant perturbation of the redox metabolism is documented.

Classical Proteomics and Metallomics

Anti-Cancer Agents in Medicinal Chemistry, 2010, *10*, 324-337

Proteomic and Metallomic Strategies for Understanding the Mode of Action of Anti-cancer Metallodrugs

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Abstract: Since the discovery of cisplatin and its introduction in the clinics, metal compounds have been intensely investigated in view of their possible application in cancer therapy. In this frame, a deeper understanding of their mode of action, still rather obscure, might turn crucial for the design and the obtainment of new and better anticancer agents. Due to the extreme complexity of the biological systems, it is now widely accepted that innovative and information-rich methods are absolutely needed to afford such a goal. Recently, both proteomic and metallomic strategies were successfully implemented for the elucidation of specific mechanistic features of anticancer metallodrugs within an innovative "Systems Biology" perspective. Particular attention was paid to the following issues: i) proteomic studies of the molecular basis of platinum resistance; ii) proteomic analysis of cellular responses to cytotoxic metallodrugs; iii) metallomic studies of the transformation and fate of metallodrugs in cellular systems. Notably, those pioneering studies, that are reviewed here, allowed a significant progress in the understanding of the molecular mechanisms of metal based drugs at the cellular level. A further extension of those studies and a closer integration of proteomic and metallomic strategies and technologies might realistically lead to rapid and significant advancements in the mechanistic knowledge of anticancer metallodrugs.

METALLOMICS

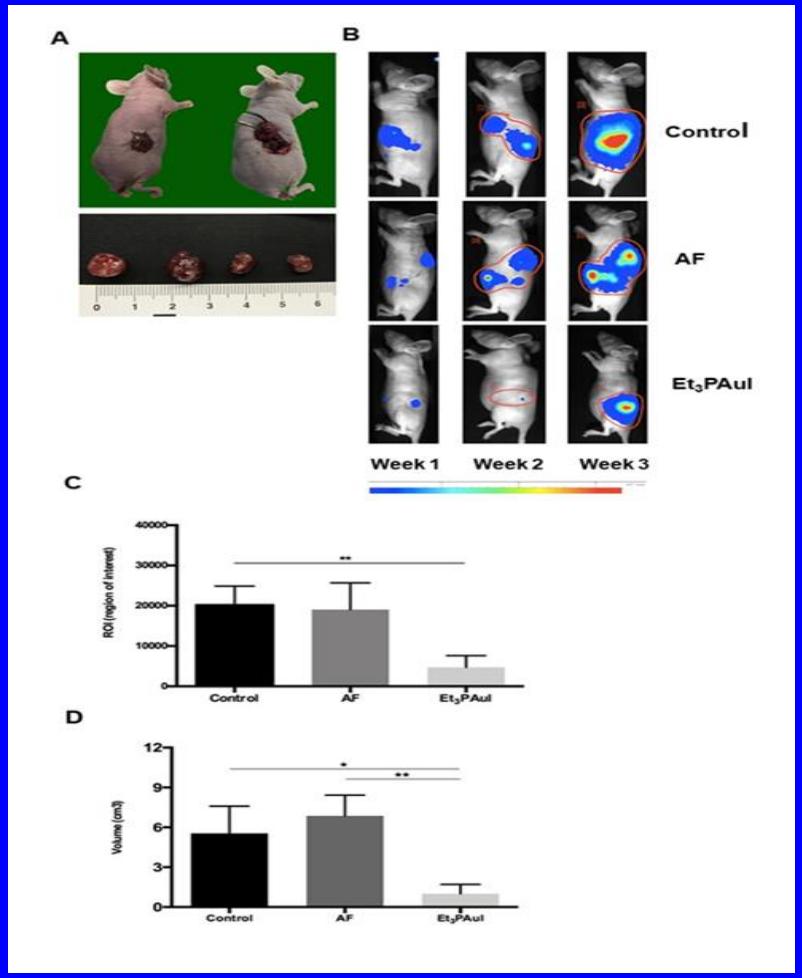
- The term **metallome** has been introduced by R.J.P. Williams by analogy with proteome as distribution of free metal ions in every one of cellular compartments.
- Subsequently, the term **metallomics** has been coined as the study of metallome. Szpunar (2005) defined metallomics as "comprehensive analysis of the entirety of metal and metalloid species within a cell or tissue type".
- Therefore, metallomics can be considered a branch of metabolomics, even though the metals are not typically considered as metabolites
- Election methods for metallomics are ICP MS and XAS spectroscopy

The "target identification and validation" problem remains a major issue in the mechanistic studies of anticancer metallodrugs

GENERAL CONCLUSIONS AND PERSPECTIVES

- **Gold compounds are today a real opportunity for anticancer drug development**.
- A variety of gold compounds with well defined chemical properties and pronounced cytotoxic properties are now available.
- Some aspects of their reactivity in vitro have been elucidated.
- A lot of work is still needed both in terms of animal (in vivo) and mechanistic studies (cellular and biochemical studies). However auranofin is in clinical trials fro cancer through repurposing.
- Hypotheses on the biological mechanism have been put forward and **a few protein targets identified**.
- The peculiar chemistry of the gold center may be eventually exploited for other pharmacological applications (even not cancer!).

In vivo results for iodauranofin in an orthotopic model of ovarian cancer

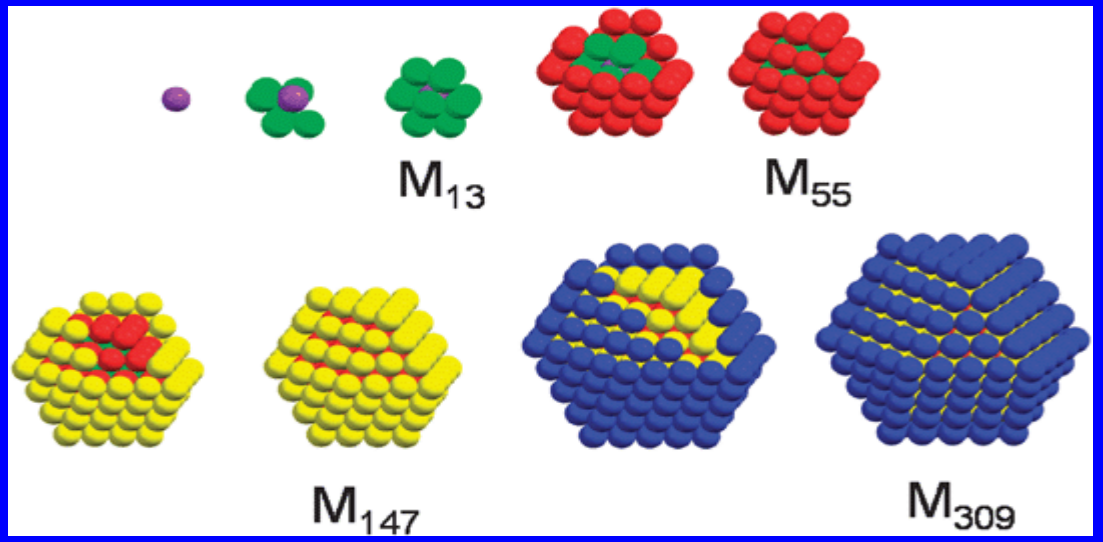


ACS Med Chem Lett, 2019

Metallic Gold and Cancer

GOLD NANOPARTICLES AS ANTICANCER AGENTS

From Schmid , Chem Soc. Rev. 2008



"nanobiotechnology"

A truly new chapter of gold in medicine

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