PROJECT PROPOSAL

1 - Research project title

DNA metalation: a combined experimental/computational approach

2 - Key words

DNA metalation, anticancer drugs, metal-based drugs

3 - Research project abstract

Metal-based therapy remains a highly utilized and effective option in the treatment of many types of cancer. However, due to the persistence of severe side effects and the increase of resistance events, new metal-based chemotherapeutics that could overcome these limitations are required. In this context, the investigation of the mode of action of these metal compounds and their interaction with nucleic acids or proteins is necessary and could benefit the design of new powerful antitumor drugs. However, despite the amount of new antitumor metal complexes increases day-by-day, detailed structural information on their interaction with DNA is still lacking. The aim of this project is to fill this gap by studying in solution and in the solid state the interaction of different classes of metal compounds with DNA oligonucleotides. The effect of the binding of the metallodrugs on these structural motifs will be investigated also by computational methods. The results will be compared with analogue studies obtained in protein metalation studies.

4 - State of the art

Since the discovery of cisplatin, metal complexes have attracted a lot of interest due to their therapeutic potential in cancer therapy. Indeed, despite the clinical use of Pt compounds is associated with undesired side effects, such as general toxicity and drug-resistance, there is a growing demand for metal-based compounds in cancer therapy, due to the increasing plague of cancer and the level of cytotoxic effects displayed by these compounds.

Although it is widely demonstrated that the cytotoxic activity of these compounds is due to their interaction with various targets (DNA, metabolites, proteins), a deeper understanding of the molecular bases of the interaction of the metallodrugs with these molecules is required. In particular, it is reported that cisplatin interferes with DNA replication and transcription, kinking the DNA duplex structure by covalently binding two adjacent guanine N7 positions in the major groove. Conversely, much remains to be investigated regarding the interaction of cisplatin derivatives and novel non-Pt-based drugs with the duplex DNA and other structural organizations (cruciform, four-way junction, G-quadruplex) with specific functional role. Cruciform DNA, a secondary structure formed by a four-way junction and two closed hairpin-shaped points, plays an important role in a wide range of biological processes (replication, regulation of gene expression, and recombination). G-quadruplexes are four-stranded nucleic acid secondary structures, which form in guanine-rich sequences. These structures are formed, under physiological conditions, in regions of biological significance, such as human telomeres, oncogene promoter regions, replication initiation sites, and 5’ and 3’-untranslated (UTR) regions. All these non-canonical structural motifs (cruciform DNA and G-quadruplex) are found to be implicated in the evolution and development of cancer.

5 - Objectives and results that the project aims to achieve

The principal aim of this project is to analyse the interaction of metal-based anticancer drugs (including cisplatin derivatives and novel chemotherapeutic drugs containing Pt or non-Pt metals) with double helix and other DNA structural organizations (cruciform, four-way junction, G-quadruplex). The effect of the binding of the metallodrugs on these structural motifs will be investigated by spectroscopic, crystallographic, and computational methods. Different DNA systems will be used. For example:

- the sequence 5'-CGCGAATTCGCG-3' or others that form a self-complementary B-form duplex structure (Figure 1A);
- the sequence 5'-CCGGGCCCGG-3' or others that fold in a self-complementary A-form duplex structure (Figure 1B);
- the sequence 5'-TCGGTACCGA-3' or others that adopt a four-way junction architecture (Figure 1C);
- sequences of variable chain length derived by the human telomere repeat TTAGGG, forming G-quadruplex structures (Figure 1D).

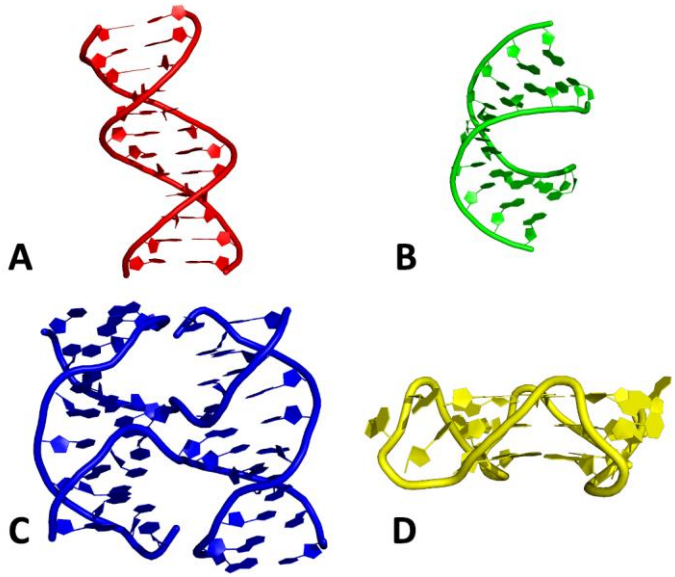

Figure 1. Cartoon representations of some of the DNA structures that will be studied in this project: A) duplex B-form (PDB code: 289D), B) duplex A-form (PDB code: 240D), C) four-way junction (PDB code: 1NVY), and D) G-quadruplex (PDB code: 3T5E).

On the other side, various classes of metal compounds will be considered:

- cisplatin derivatives, including iodoplatin, carboplatin, oxaliplatin
- Ru-, Au- and Rh-based drugs.

The structural analyses will allow to identify the effects that different classes of metal compounds have on different DNA structures. Indeed, a deep comparison between the interactions that the same metal-compounds make with the different structural motifs of DNA will allow us to point out the molecular bases of their mechanism of action. The results will be compared with those obtained in protein metalation studies. Altogether these studies will provide insights into the molecular bases of the cytotoxic activity of different metal-based drugs.

Statistical analyses on different databases (Protein Data Bank, Cambridge structural database…) will be also carried out to support the new results and compare them with those already reported in the literature and to delineate the common features shown by drugs containing the same metal.
When divulged in scientific journals, the results of this project will allow to the scientific community interested in this field to design new metallodrugs with a potential as chemotherapeutics. In particular, the findings could help to synthetize new chemotherapeutics with potentially increased selectivity towards a specific DNA structural motif. Indeed, an increased selectivity is one of the main goals pursued by researchers as it will allow to better modulate the action of these drugs, whose biggest limitation remains the general toxicity.

6 - Scientific and/or technological and/or social and/or economic impact of the project

Cancer kills an increasing number of people every year. Metal compounds are used in chemotherapy to inhibit the uncontrolled growth and proliferation of cancer cells. Chemotherapy is associated with a range of adverse effects, and some agents increase the risk of secondary neoplasm development. DNA, metabolites, peptides, and proteins have a central role in the recognition, transport, and mechanism of action of metal-based antitumoral compounds. A deeper understanding of the molecular bases of the interaction of metallodrugs with these molecules is essential to help the scientific community to design novel antitumoral drugs with improved performances.
Moreover, due to the differential expression of the telomerase in normal and cancer cells, the enzyme inhibition via stabilization of human telomeric G-quadruplex sequences by small compounds has emerged as a novel anticancer strategy. The relatively low cost of this approach when compared to traditional industry-led ones may hopefully lead to a reduced cost for any licensed medicines. Our multidisciplinary approach will also help the search for new metal compounds able to increase the telomeric G-quadruplex antitumoral activity.

7 - Methodologies that are planned to be used (Max. 1000 characters)

Different tasks will be carried out to achieve the objectives of the project, in a multidisciplinary approach.

Experimental tasks:

Task E1. Selection of metal compounds based on their chemical properties and cytotoxic activity.
Task E2. Spectroscopic characterization of target-ligand complexes/adducts.
Task E3. Co-crystallization and/or soaking experiments of complexes/adducts.
Task E4. Structure determination and analysis of complexes/adducts.

Computational tasks:

Task C1. Analysis and comparison of the structures of target-ligands complexes/adducts present in Protein Data Bank.
Task C2. Docking of target-ligands complexes/adducts that hardly crystallize.
Task C3. Molecular dynamics simulations of target-ligands complexes/adducts.

Equipment to be used:

- Spectrophotometers, spectrofluorimeters, spectropolarimeter equipped with Peltier temperature control systems, Raman and IR microscopes.
- Crystallization laboratories at three different temperatures (20 °C, 10 °C, and 4 °C).
- Molecular and cellular biology equipment.
- X-ray diffraction data collection facilities.
- Computer facilities.

8- *PI*

First and last name: Alessandro Vergara (Full Professor of Physical Chemistry, CHIM-02)
PI bibliography:
H-index =26
Number published papers in the last 5 years: 28
I.F. of best publications in the last 5 y: 21,286; 12,221; 9,405; 5,16; 4,85

L. Vitagliano, A. Merlino, L. Mazzarella, A. Vergara* "A fine sampling of the R-T quaternary structure transition of a tetrameric hemoglobin" **Chem. Eur. J**. (2017), 23, 605 – 613 (IF **5.16**)

L. Sirleto, A. Vergara, A. Ferrara "Advances in Stimulated Raman scattering in nanostructures" **Advances in Optics and Photonics** (2017) 9 (1), 169-217 (IF **21.286**)

M. Caterino, M. Herrmann, A. Merlino, C. Riccardi, D. Montesarchio, M. A. Mroginski, D. Musumeci, F. Ruffo, L. Paduano, P. Hildebrandt, J. Kozuch, A. Vergara* "On the pH-Modulated Ru-Based Prodrug Activation Mechanism" **Inorg. Chem.** (2019), 58, 1216−1223 (IF **4.850**)

S. Esposito, B. Silvestri, V. Russo, B. Bonelli, M. Manzoli, F. A. Deorsola, A. Vergara, A. Aronne, M. Di Serio "Self-Activating Catalyst for Glucose Hydrogenation in the Aqueous Phase under Mild Conditions" **ACS Catalysis** (2019) 9, 3426−3436 (IF **12.221**)

N. J. Clayden, C. Imparato, R. Avolio, G. Ferraro, M. E. Errico, A. Vergara, A. Aronne, B. Silvestri, "A Chloride-Free Hydrolytic Sol-Gel Synthesis of Nb-P-Si Oxides: a Green Approach to Solid Acid Materials" **Green Chemistry** (2020), 22(20), pp. 7140–7151 (IF **9,405**).

PI's publications most relevant to the project (biomolecules metalation)

A. Vergara, G. D'Errico, D. Montesarchio, G. Mangiapia, L. Paduano, A. Merlino "Interaction of anticancer Ruthenium compounds with proteins: High resolution X-ray structures and Raman microscopy studies of the adduct between hen egg white lysozyme and AziRu." **Inorg. Chem**. (2013) 52(8), 4157-4159 (IF. 4,601).

L. Messori, F. Scaletti, L. Massai, M. A. Cinellu, C. Gabbiani, A. Vergara, A. Merlino "The mode of action of anticancer gold-based drugs: a structural perspective" **Chem. Comm**. (2013) 49(86), 10100-10102. DOI: 10.1039/c3cc46400h (IF 6.378)

A. Vergara, I. Russo Krauss, D. Montesarchio, L. Paduano, A. Merlino, "Investigating the ruthenium metallation of proteins: X-ray structure and Raman microspectroscopy of the complex

between RNase A and AziRu" **Inorg. Chem**. (2013) 52 (19), 10714–10716 doi.org/10.1021/ic401494v (IF 4.601)

L. Messori, F. Scaletti, L. Massaia, M. A. Cinellu, I. Russo Krauss, G. di Martino, A. Vergara, L. Paduano, A. Merlino, "Interactions of Gold-based drugs with proteins: crystal structure of the adduct formed between Ribonuclease A and a cytotoxic gold(III) compound" **Metallomics** (2014), 6, 233-236. doi 10.1039/C3MT00265A (IF. 4.099).

A. A. Petruk, A. Vergara, D. Marasco, D. Bikiel, F. Doctorovich, D. A. Estrin, A. Merlino, "Interaction between proteins and Ir based CO releasing molecules: mechanism of adduct formation and CO release" **Inorg. Chem**. (2014), 53(19), 10456–10462 (IF. 4,762).

A. Merlino, M. Caterino, I. Russo-Krauss, A. Vergara* "Missing gold atoms in lysozyme crystals used to grow gold nanoparticles" **Nature Nanotechnology** (2015), 10(4), 285 (IF 34,048).

A. Di Fiore, A. Vergara, M. Caterino, V. Alterio, S. M. Monti, J. Ombouma, P. Dumy, D. Vullo, C.T. Supuran, J.-Y. Winum, G. De Simone "Hydroxylamine-O-sulfonamide is a versatile lead compound for the development of Carbonic Anhydrase Inhibitors" **Chem. Comm.** (2015), 51(57) 11519-11522 (IF 6,834).

M. Caterino, A. A. Petruk, A. Vergara, G. Ferraro, F. Doctorovich, D. A. Estrin, A. Merlino "Mapping the protein-binding sites for iridium(III)-based CO-releasing molecules" **Dalton Transactions**, (2016) 45(30), 12206-12214 (IF 4.177).

A. Vergara, M. Caterino, A. Merlino "Raman-markers of X-ray radiation damage of proteins" **Int. J. Biol. Macromol.** (2018) 34(1) 150-159 (IF 3,908)

M. Caterino, M. Herrmann, A. Merlino, C. Riccardi, D. Montesarchio, M. A. Mroginski, D. Musumeci, F. Ruffo, L. Paduano, P. Hildebrandt, J. Kozuch, A. Vergara* "On the pH-Modulated Ru-Based Prodrug Activation Mechanism" **Inorg. Chem.** (2019), 58, 1216−1223 (IF 4.850)

9 - Technical and economic sustainability of the project (Max. 1000 characters)

A. Vergara is expert in Raman and IR microscopies that are useful to study the coordination and oxidation state of the metal in the complexes (Task E2). The project is in collaboration with other researchers of CF-S group (http://www.scienzechimiche.unina.it/ricerca/cfs) at the Department of Chemical Sciences at the University of Naples Federico II. They will combine their expertise to ensure that the candidate will achieves the objectives of this project. In particular, F. Sica has a long experience in the DNA and DNA-protein structural characterization by spectroscopic techniques and X-ray crystallography (Tasks E2, E3, E4). A. Merlino has a deep expertise in protein metalation studied by means of spectroscopic and crystallographic methods (Tasks E2, E3, E4). Moreover, he has multi-year collaborations with groups that synthetize antitumoral metal compounds or perform cellular experiments to reveal their cytotoxic effects (Task E1). Both F. Sica and A. Merlino have experience with the computational methods necessary to perform MD simulations of biological macromolecules (Tasks C1, C2, C3). In particular, F. Sica has recently studied via MD simulations DNA-protein complexes (Task C3). G. Sciortino of the Institute of Chemical Research of Catalonia (ICIQ) (Spain) will contribute to the project with his expertise in computational analysis of dual and

multimetallic compounds able to interact with biological macromolecules (Tasks C2, C3). The researchers involved in the project have already worked together successfully. They possess the resources to perform the project: crystallization facilities, access to data collection facilities and HPC resources, spectrophotometers, and computer facilities.

10 - Other possible participants
A. Merlino (Dept. Chem. Sci.-UNINA)
F. Sica (Dept. Chem. Sci.-UNINA)
G. Sciortino (University of Terragona, Spain)

11 - Scientific publications, relevant to the project, of PI and of other possible participants (20 most representative in the last five years)

G. Sciortino, J. Rodríguez-Guerra Pedregal, A. Lledós, E. Garribba, J-D Maréchal "Prediction of the interaction of metallic moieties with proteins: An update for protein-ligand docking techniques" **J. Comp. Chem.** (2022) 39(1), 42-51.

M. Aureliano, N. I Gumerova, G. Sciortino, E. Garribba, C. C McLauchlan, A. Rompel, D. C Crans "Polyoxidovanadates' interactions with proteins: An overview" **Coordination Chemistry Reviews**, (2022) 454, 214344.

J. Costa Pessoa, M. FA Santos, I. Correia, D. Sanna, G. Sciortino, E. Garribba "Binding of vanadium ions and complexes to proteins and enzymes in aqueous solution" **Coordination Chemistry Reviews**, (2021), 449, 214192.

L. Roldán-Martín, F. Peccati, G. Sciortino, M. Sodupe, J.D. Maréchal "Impact of Cu (ii) and Al (iii) on the conformational landscape of amyloidβ 1-42", **Phys. Chem. Chem. Phys.** (2021), 23, 13023.

A. Vergara, M. Caterino, A. Merlino "Raman-markers of X-ray radiation damage of proteins" **Int. J. Biol. Macromol.** (2018) 34(1) 150-159

M. Caterino, M. Herrmann, A. Merlino, C. Riccardi, D. Montesarchio, M. A. Mroginski, D. Musumeci, F. Ruffo, L. Paduano, P. Hildebrandt, J. Kozuch, A. Vergara* "On the pH-Modulated Ru-Based Prodrug Activation Mechanism" **Inorg. Chem.** (2019), 58, 1216−1223

D. Loreto, A. Merlino "The interaction of rhodium compounds with proteins: A structural overview", **Coordination Chemistry Reviews**, (2021), 449, 214192.

A. Merlino, "Recent advances in protein metalation: structural studies", **Chem. Comm.** (2021), 57, 1295-1307.

A. Giorgio, A. Merlino, "Gold metalation of proteins: structural studies", **Coordination Chemistry Reviews** (2020) 407, 213175

Đ Miodragović, A. Merlino, E. P Swindell, A. Bogachkov, R. W Ahn, Sara Abuhadba, G. Ferraro, T. Marzo, A. P Mazar, L. Messori, T. V O'Halloran "", **J. Am. Chem. Soc**., (2019), 141, 6453-6457

G. Ferraro, A. Pratesi, D. Cirri, P. Imbimbo, D.M. Monti, L. Messori, A. Merlino "Arsenoplatin-Ferritin nanocage: Structure and cytotoxicity" **Int. J. Mol. Sci.** (2021), 22, 1874.

M. De Fenza, E. Eremeeva, R. Troisi, H. Yang, A. Esposito, F. Sica, P. Herdewijn, D. D'Alonzo, A. Guaragna "Structure–Activity Relationship Study of a Potent α-Thrombin Binding Aptamer Incorporating Hexitol Nucleotides", **Chem. Eur. J.** (2020), 26, 9589-9597.

R Troisi, V Napolitano, V Spiridonova, I Russo Krauss, F Sica , "Several structural motifs cooperate in determining the highly effective anti-thrombin activity of NU172 aptamer", **Nucleic acids research** (2018) 46 (22), 12177-12185

A. Pica, I. Russo Krauss, V. Parente, H. Tateishi-Karimata, S. Nagatoishi, K. Tsumoto, N. Sugimoto, F. Sica "Through-bond effects in the ternary complexes of thrombin sandwiched by two DNA aptamers", **Nucleic acids research**, (2017) 45, 461-469