DAYS

Chemistry Towards Biology: Molecules
Venerdì 6 Giugno 2014, ore 14, aula D1

14:15-14:30 Sviluppo di complessi di vanadile con leganti fluorescenti e/o target-specifici con proprietà citotossiche come potenziali agenti antitumorali.

Elena Ghibaudi (10 min di discussione)

14:40-14:55 Synthetic Strategies for the Preparation of Lipophilic MRI/GdBNCT Agents: a new approach towards multimodal theranostic agents
Annamaria Deagostino (10 min di discussione).

15:05-15:20 Fluorescent Bioactive Molecules for *in vivo* Investigation
Cristina Prandi (*10 min discussione*)

15:30-15:40 Commissione Spokes

15:40-16:10 Coffee break (discussion)

16:10-16:25 Protein-Protein and Protein-Ligand Interaction Studies.
Viscardi Guido (10 min discussione)

16:35-16:50 Ambient Mass Spectrometry for Diagnostic Imaging in Cancer Research, for Embryological Studies on Reproduction Biology, and for Immediate Screening Protocols in Bioanalysis
Valentina Pirro (*10 min* discussione)

17:00-17:15 Pharmaceutical Crystal Forms. From the Crystal Design to the Improving of Pharmaceutical Properties of an Api
Michele Chierotti (*10 min discussione*)

17.30 chiusura

Sviluppo di complessi di vanadile con leganti fluorescenti e/o target-specifici con proprietà citotossiche come potenziali agenti antitumorali

Elena Ghibaudi

I complessi di vanadile sono impiegati da tempo in campo terapeutico come antidiabetici, ma solo recentemente ne sono state evidenziate le potenzialità antitumorali, che associano una buona efficacia terapeutica alla bassa tossicità sistemica del vanadio. Malgrado ciò, sono disponibili poche informazioni sul meccanismo d'azione di questi composti e sui loro target cellulari. Questo progetto è nato dall'idea di porre in collaborazione competenze disponibili nel nostro Dip.to e in Dip.ti vicini (sintesi organica e inorganica, risoluzione di strutture cristallografiche, caratterizzazione spettroscopica dei complessi allo stato solido e in soluzione mediante UV-Vis e fluorescenza, IR e Raman, EPR, test biochimici e farmacologici) per sviluppare complessi di vanadile con leganti fluorescenti e/o altamente specifici. L'obiettivo è consentire la tracciabilità dei complessi mediante microscopia confocale e/o veicolarli selettivamente su target cellulari. Ad oggi sono stati prodotti e caratterizzati 6 composti e si sta lavorando su altri 4. I risultati sono stati pubblicati sul J. Inorg. Biochemistry. Obiettivi per il futuro: identificare molecole carrier capaci di veicolare i complessi su target altamente specifici; potenziare la parte farmacologica; tentare studi *in vivo*.

Sgarbossa S., Diana E., Marabello D., Deagostino A., Cadamuro S., Barge A., Laurenti E., Gallicchio M., Boscaro V., Ghibaudi E. (**2013**) Synthesis, characterization and cell viability test of six vanadyl complexes with acetylacetonate derivatives, **J. Inorg. Biochem**. 128, 26–37

Synthetic Strategies for the Preparation of Lipophilic MRI/GdBNCT Agents: a new approach towards multimodal theranostic agents

A. Deagostino, C. Prandi, P. Venturello

BNCT (boron neutron capture therapy) is a binary radiation therapy for the treatment of cancer, based on the capture of thermal neutrons by ^{10}B nuclei that have been selectively delivered to tumour cells.[1] The neutron capture event results in the formation of excited ^{11}B nuclei that undergo fission to yield highly energetic $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions. Cell death is triggered by the release of these charged particles which create ionisation tracks along their trajectories, resulting in cellular damage. It has been estimated that approximately 10–30 μg of boron per gram of tumour mass is needed to attain an acceptable therapeutic advantage. Several functionalised carboranes have been employed to construct boron delivery vehicles for BNCT, because of their high content of boron and their stability *in vivo*. In recent years our research group has been working on the preparation of dual agents for BNCT/MRI

applications. In these systems a carborane cage is linked to a lipophilic unit, in order to exploit LDLs as biological vectors, and a MRI probe. *In vivo* MR image acquisition showed that the amount of B taken up in the tumour region was above the threshold for successful NCT treatment.[2] With the goal of improving the efficacy as theranostic agents (therapy + diagnostic), new compounds have been prepared and tested. In one case, a triazole unit was as a linker between the carborane cage, the MRI probe and the lipophilic unit (figure 1).[3] The ability of the Gd complex of the synthesised ligand to form stable adduct with LDLs was evaluated and then MRI has been performed on tumour melanoma cells incubated in the presence of a Gd-complex/LDL imaging probe. In was demonstrated that the high amount of intracellular boron necessary to perform BNCT can be reached even in the presence of a relatively low-boron-containing LDL concentration. In order to exploit liposomes as biological vectors, a cholesterol moiety has also been introduced (Gd-B-AC01).[4] An in vitro test on IGROV-1-cells demonstrated that this Gd-B-AC01 loaded liposomes are efficient carriers for the delivery of the MRI/BNCT probed to the tumour cells. The BNCT treatment of IGROV cells showed that the number of surviving cells was markedly smaller when the cells were irradiated after internalisation of the folate-targeted Gd-B-AC01/liposomes.

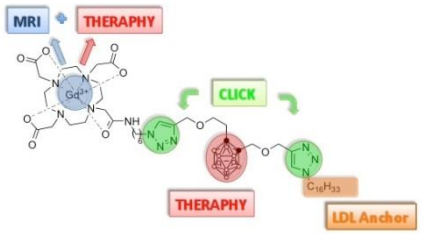


Fig. 1. Example of MRI/BNCT dual agent where the MRI probe and the lipophilic unit are linked to the carborane cage via a triazole unit.

In order to reduce the synthetic steps, a new strategy, based on the hydroboration reaction, has been elaborated. In this way the lipophilic unit will be linked to the carborane by a B-C bond instead of C-C, allowing the desired dual agent in only four passages to be obtained.

References

[1] R. F. Barth; J. A. Coderre,; M. G. H. Vicente,; T. E. Blue, *Clin. Canc. Res.* 11, (2005) 3987. M. F. Hawthorne, M. W. Lee, *J. Neurooncol.* 62, (2003), 33.

[2] S. Geninatti-Crich, D. Alberti, I. Szabo, A. Deagostino, A. Toppino, A. Barge, F. Ballarini, S. Bortolussi, P. Bruschi, N. Protti, S. Stella, S. Altieri, P. Venturello, S. Aime, *Chemistry* 17, (2011), 8479.

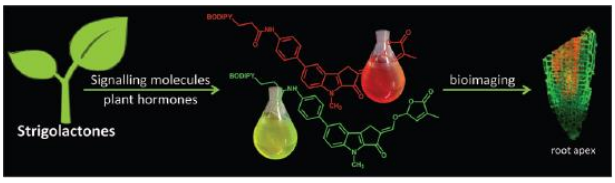
[3] A. Toppino, M. E. Bova, S. Geninatti- Crich, D. Alberti, E. Diana, A. Barge, S. Aime, P. Venturello, A. Deagostino, *Chemistry* 9, (2013), *1*720.

[4] D. Alberti, A. Toppino, S. Geninatti- Crich, C. Meraldi, C. Prandi, N. Protti, S. Bortolussi, S. Altieri, S. Aime, A. Deagostino *Org. Biomol. Chem.* 12, (2014), 2457.

Fluorescent bioactive molecules for *in vivo* investigation

Cristina Prandi, Annamaria Deagostino

Strigolactones (SLs) are a new class of plant hormones whose role has been recently defined in shoot branching, root development and architecture, and nodulation. They are also active in the rhizosphere as signalling molecules in the communication between plants, AMF (arbuscular mycorrhizal fungi) and parasitic weeds. They have been recently claimed to act as potent antitumoral agents. In spite of the crucial and multifaceted biological role of SLs, the current knowledge on the SL biosynthetic pathway and the perception/transduction mechanism is still incomplete. Both genetic and molecular approaches are required to understand the molecular mechanism by which SLs regulate plant development. Our contribution to this topic is the design and synthesis of fluorescent labelled SL analogues to be used as probes for the detection in vivo of the receptor(s). Knowledge of the putative receptor structure will boost the research on analogues of the natural substrates as required for agricultural applications.



Ambient Mass Spectrometry For Diagnostic Imaging In Cancer Research, For Embryological Studies On Reproduction Biology, And For Immediate Screening Protocols In Bioanalysis

Department Of Chemistry, University Of Turin
Fablab Group
Valentina Pirro, Marco Vincenti

Ambient MS methods generate ions outside the vacuum of the mass spectrometer and require little or no sample preparation before analysis. Therefore, they allow intact samples to be analyzed in their native environment, which means great potentialities for imaging experiments, *in situ* and *in vivo* investigations. Since the first technique (desorption electrospray ionization-mass spectrometry, DESI-MS) was presented in 2004, quite a number of other techniques was developed and they were largely adopted in diverse bioanalytical applications. Aim of the presentation is to give an overview on applications of DESI and the derived “touch spray” technique in clinical diagnostics and forensic toxicology, focusing on results achieved, current challenges and future perspectives. The applications range from cancer diagnostics, to lipid characterization of single cells, bacteria and drugs identification on biological samples. The research work is in collaboration with the University of Genova,

Purdue University (USA), and the Friedrich-Loeffler Institute of Farm Animal Genetics (Germany).

Protein-protein and protein-ligand interaction studies.

Guido Viscardi, Pierluigi Quagliotto, Claudia Barolo, Nadia Barbero, Sonja Visentin**.**

In the post-genomic era, the importance of protein-protein interaction has become even more apparent. There is evidence that most, if not all, catalytic and regulatory pathways operate as networks, with frequent and extensive input from signalling pathways, feedback and cross-talk.

Quantitative determinations of the dissociation constants of biomolecular interactions, in particular protein-protein interactions, are essential for a detailed understanding of the molecular basis of their specificities. Affinity constants are numeric representations of the strength with which two molecules interact and can provide insight into the mechanism of interactions and, when coupled with biological experiments, can aid in predicting if and when specific interactions function in cell. Fluorescence spectroscopy is particularly well suited for such studies.

The aim of this work is to provide an understanding of affinity and kinetic constants and how to experimentally measure them for the following biomolecular interactions:

- ✓ protein-protein
- ✓ protein-ligand
- ✓ protein-drug
- ✓ protein-nanomaterials
- ✓ DNA-PNA

PHARMACEUTICAL CRYSTAL FORMS

From the crystal design to the improving of pharmaceutical properties of an API

Michele Chierotti

The crystal engineering field is concerned with design, quest for and characterization of new crystal forms with desired properties. The main challenge is that of being able to assemble new crystalline materials and to pre-determine the resulting physico-chemical properties by selecting building blocks and supramolecular interactions adequately.

Co-crystallization with a different entity is used to modify the final property of a target molecule. This is particularly relevant in the pharmaceutical field and several examples of co-crystals of active pharmaceutical ingredient (API) have been recently exploited for improving properties such as thermal stability, solubility, processability and also for patent protection/extention.

Co-crystals can be obtained in solution, but mechanochemical processes can be a successful alternative route. The main drawback is product (microcrystalline powders) characterization

which can be achieved by recrystallization via seeding or by combining X-ray powder diffraction, SSNMR and calculations.