Dottorandi Ciclo XXV
Progetti di Ricerca

Dottorando: **Micoli Alessandra**
Relatore: Prof. Maurizio Prato (DSF)
Carbon nanotubes (CNTs) are very attractive materials for their utilization in a wide range of biological application; nevertheless their manipulation is still evolving, especially for their interaction with biological systems. In particular CNTs have attracted our attention as potential scaffolds for restablishing intricate connections between neurons. In fact, hybrid neuronal/CNT networks might be useful for promoting tissue repairing in damaged spinal cord.
For the integration of CNTs into biocompatible functional nanostructures, the inclusion of non-covalent bond is a powerful synthetic tool. Such non-covalent interactions include electrostatic, hydrophobic, hydrogen bonding, phase segregation, metal-ligant chemistry and π-π interactions. Of these forces, the self-complementary Watson-Crick hydrogen bonding interactions dictates specific base-pairing that are crucial for establishing the fidelity required for efficient storage, replication, and transcription of genetic information. Utilization of nucleobases adenine, thymine, guanine, and cytosine in supramolecular chemistry offers the possibility of exploiting different bonding characteristic. The covalent integration of nucleobases as supramolecular motifs on CNTs might lead to development of metal binding, the preparation of selective carriers for various biologically active nucleobases, electron and energy transfer systems and the creation of elegant architectures. A limiting factor lies in the fact that in polar solvent, in which nucleobases derived systems and CNTs are most soluble, the energies of the complementary are small. In nature this problem have been solved by the combination of different non-covalent interaction; in fact the substituents attached to the nucleobase can play a role in controlling the nature of the supramolecular assembly that is formed. For example, the conformation of the sugar with respect to the base-pair can lead to variant base pairing modes; moreover the nucleobases attached to the CNTs, including the sugars group may increase their solubility. With this proposal we plan to functionalized CNTs with nucleobases, by changing the substituents attached to them, we intent to create different molecular architectures for diverse applications:

- Metal binding architectures: the presence of accessible nitrogen and oxygen lone pairs in the nucleobases opens up the possibility of these molecules to acting as metal ligands. CNTs architectures including metal ions have been used as targeted drug-delivery systems and markers for dynamical tracking. So incorporating CNT with nucleobases for metal coordination might produce new devices for these kind of biological applications.
- Molecular architectures: the self-recognition of guanine-cytosine, and adenine-thymine/uracil attached as supramolecular motifs on CNTs as scaffold might produce elegant architectures.

All the synthetized products will be characterize by meand of termogravimetric analysis, transmission electron microscopy and atomic force microscopy. Moreover, we will investigate the property of nanotubes in solution using UV-Vis-NIR.

Dottorando: **Meduri Angelo**
Relatore: Dr.ssa Barbara Milani (DSCH)
Il progetto di ricerca riguarderà lo sviluppo di catalizzatori omogenei per la reazione di oligo- e polimerizzazione di alcheni terminali con monomeri polari con l'obbiettivo di ottenere oligomeri e/o polimeri con una distribuzione controllata dei diversi monomeri in catena.
I dati di letteratura evidenziano che i principali problemi riguardanti questa reazione consistono nella formazione di intermedi chiave che costituiscono il resting state del ciclo catalitico. Sembra che la natura di questi intermedi sia legata alla natura del legante ancillare presente sul centro metallico: con leganti chelanti azotati il resting state è un metallaciclo che si forma dopo l'inserzione dell'acrilato nella catena polimerica in crescita e che implica un legame σ tra il metallo

e l'ossigeno carbonilico dell'acrilato; con leganti chelanti ibridi fosforo-ossigeno il metallaciclo non si forma, e il resting state è il complesso contenente un legame metallo-alchile, di un alchile recante in α un gruppo carbonilico, e il monomero coordinato. La strategia su cui si basa il progetto di ricerca consiste nello sviluppare dei leganti che sfavoriscano la formazione di questi resting state.
Inizialmente verranno studiati composti di coordinazione del palladio con tre classi di leganti: *i.* leganti bidentati azotati non simmetrici appartenenti alla famiglia delle α-diimine a scheletro acenaftenico recanti gruppi arilici diversi sui due atomi di azoto donatori; *ii.* leganti bidentati ibridi fosforo-azoto; *iii.* leganti tridentati N-N-O in cui l'atomo di ossigeno potrebbe comportarsi da donatore emilabile.
I substrati modello scelti per le reazioni target sono lo stirene e l'etilene, come alcheni terminali, e acrilati variamente sostituiti come monomeri polari. In aggiunta, per sfavorire la formazione del metallaciclo su menzionato si intendono saggiare degli acrilati il cui gruppo carbossilico sia già coordinato ad un centro metallico.
La metodologia dell'attività di ricerca consisterà nella sintesi dei composti di coordinazione, in alcuni casi potrebbe anche essere necessaria la sintesi dei leganti, nella loro caratterizzazione, sia allo stato solido che in soluzione (con particolare riferimento all'utilizzo della spettroscopia NMR avanzata), nello studio del loro comportamento catalitico nelle reazioni target, nella caratterizzazione dei polimeri sintetizzati via NMR e con la tecnica MALDI-TOF.
Infine, una volta individuata una classe di complessi particolarmente attiva verranno condotti degli studi meccanicistici con lo scopo di caratterizzare gli intermedi del ciclo catalitico.

Dottorando: **Turco Antonio**
Relatore: Prof. Maurizio Prato (DSF)
The need to reconnect damaged nervous system has pushed the study and development of new techniques to produce and characterize biocompatible materials. In the last decades, by means of nanotechnology the problem has been differently faced. One of the most used materials in this field is carbon nanotubes (CNTs). Since their discovery, CNTs have offered new perspectives and possibilities towards reconnecting damaged neuronal tissue. Noteworthy, it has been demonstrated that some properties of neurons can be enhanced if they were grown on CNT substrates. Indeed, CNTs can elicit the triggered activity of neurons and the capability to integrate action potential, and in the presence of CNTs the number of synaptic connection is augmented and the synaptic transmission enhanced.
On this basis, during my first year of PhD, the work will focus on the fabrication of a "CNT bridge" to connect two slices of spinal cord and allow their communication. Our idea is to employ a thin film of CNTs, obtained by layer-by-layer deposition, that will provide the electrical contact between two portion of the spinal cord. To pursue this final goal, we need to morphologically and electrically characterize the film and, afterwards, to perform electrophysiological measurements in order to investigate the properties of the cells grown on the CNTs substrates. The electrophysiological part will be held in collaboration with the laboratory of Prof. Laura Ballerini.
In detail, the work on the formation and characterization of the film will include the purification, chemical modification and characterization of CNTs used in the experiments. As characterization techniques we will use thermogravimetric analysis, transmission electron microscopy and atomic force microscopy. Subsequently the films will be morphologically characterized by means of scanning electron microscopy, and the resistivity will be measured to define the electrical properties. This information will be fundamental to understand electrophysiological results and optimize the cellular response of the system.

Dottorando: **de Ceglie Pasquale**
Relatore: Dr. Pierluigi Barbieri (DSCH)
Il progetto di ricerca ha lo scopo di fornire un contributo alla comprensione dei processi di formazione del particolato atmosferico secondario, con particolare riferimento alla sua componente

organica, al fine di identificare strumenti per valutarne la rilevanza e fornire indicazioni su misure per limitare le emissioni di precursori.
Oltre al particellato emesso direttamente da fonti antropiche domestiche, industriali e dai trasporti, vi è crescente attenzione per la quota di particolato che si forma a partire da precursori gassosi. La messa a punto di strumenti sperimentali e l'acquisizione di informazioni per calibrare modelli su scala regionale risultano importanti per la messa a punto di strumenti di gestione della qualità dell'aria a fini regolatori e per valutare la tossicità dell'aerosol.
Lo studio si articolerà nella:
1) caratterizzazione della componente organica e inorganica in filtri di particolato atmosferico (PM_2.5 , PM_10), con identificazione di specie generate da reazioni con precursori gassosi, significative come marcatori di sorgente o per la rilevanza tossicologica;
2) messa a punto di sistemi di campionamento di precursori biogenici (es. pineni ed altri terpeni) ed industriali (es. idrocarburi da tankfarm), ai fini della valutazione dei contributi al budget dell'aerosol secondario;
3) implementazione di un modulo per la modellizzazione dell'aerosol organico secondario nel sistema modellistico WRF-CHIMERE;
Sono previste durante il primo anno collaborazioni attive con l'Università "Parthenope" di Napoli (Dr. Angelo Riccio), con Sincrotrone Trieste e con l'Università di Bari (Dr. Gianluigi De Gennaro).

Dottorando: **Pavan Silvia**
Relatore: Dr. Federico Berti (DSCH)
Titolo: Short peptides as biosensor transducers
This PhD project will focus on the design of transducers to be used in sensing systems; the model medical targets will be a number of existing drugs widely used for the HIV "Highly Active Anti-Retroviral Therapy" (HAART). Drug monitoring of HAART is a major clinical problem calling for systems allowing a rapid measure of pharmacokinetic parameters on each patient. Patients are required to assume very high doses of anti-viral drugs, under a very strict, life-long regimen. This often leads to poor compliance, which decreases the efficiency of the therapy and favours the appearance of resistant mutant strains. In this project Efavirenz, Tenofovir and Amprenavir, the most frequent drugs used in the associations of HIV therapy, will be studied.
Any target class will require a different peptide design; peptide receptors for small molecule need two parts: a scaffold, constant region to ensure spatial organisation, and a binder region to be randomised. Target molecules will be synthesised, when not commonly available, and carefully quantified in order to obtain reference samples. Modified targets will also be prepared, with linkers at one or more positions to allow immobilization and bioconjugation. At the same time, also molecular biology activities will start and combinatorial libraries will be generated by phage display technology or by methods such as the bacterial two-hybrid system. Phage libraries will be selected first against immobilized targets by ELISA techniques. A set of about ten binders are expected to be identified for any given target. Aminoacid sequence of binders will be deducted from the relevant DNA coding sequence; best peptide binders will then be produced in quantity by soluble expression systems, such as maltose-binding protein. A first evaluation of the affinity constant for the targets will be carried out at this stage by ELISA; peptides confirming high affinity will be chemically synthesised on a 10 mg scale. Full characterization of the selected binder peptides (by CD, calorimetry, light scattering, X-ray crystallography,..) will be also performed during the project.
In the first year Efavirenz (EFV) will be paid particular attention to. EFV is a potent non nucleoside reverse transcriptase (RT) inhibitor and belongs to the recent generation of NNRTIs characterized by noncompetitive binding to an allosteric site. One part of this year will be performed at the Synthetic Chemistry group of Queen Mary University of London to widen the molecular imprinting. This approach provides a unique opportunity for the creation of three-dimensional cavities with tailored recognition properties. It is a process where functional and cross-linking monomers are copolymerized in the presence of the target analyte, which act as a molecular

template. EFV could be an imprint molecule and the short peptide or the single aminoacids, important in the interaction of EFV with non-nucleoside binding pocket, could be prepared as functional monomers. In this way it could be possible to recreate synthetically the interactions and the orientation of the functional groups that play a significant role in determing the specificity and the efficiency of the inhibition of RT by EFV.
In the other part modified analogues of EFV will be synthesized with different linkers that will be introduced in the most convenient positions to allow immobilization and selective recognition. These linkers will be designed with different lengths and polarities. The linking strategy will vary and techniques such as click chemistry, biotinylation, carboxylation, amination and thiolation, involving both partial modification of the target and full synthesis of target derivatives will be addressed. After purification and full spectroscopic characterization, the analogues will be conjugated with carrier proteins, to be immobilized on a solid surface, with enzymes, to act as competitors in immunoenzyme assays, and with fluorescent labels, to be used as marker in binding and affinity analyses.

Dottorando: **Barreras Alvaro**
Relatore: Prof. Aurelia Tubaro (DMRN)
Correlatore: Prof.ssa Gago-Martinez Ana (Università di Vigo. Spagna)
Palytoxin (PTX), Yessotoxin (YTX) and their analogues are toxins produced by marine dinoflagellates that can contaminate seafood. PLTX is a highly toxic compound associated to seafood intoxications in tropical and subtropical areas, but recently it has been detected also in microalgae and shellfish from Mediterranean Sea. On the other hand, no human intoxications due to YTX have been reported so far, but experimental studies in mice showed ultrastructural alterations in cardiac cells after acute and repeated oral administration.
Since in the last years PLTX and YTX were detected in Mediterranean Sea and a possible co-exposure to both toxin can occur through contaminated seafood consumption The *in vivo* toxic effects of repeated oral co-administration of these toxins will be studied in mice, in comparison with the effects of each toxin alone. By this way, it will be tentatively defined the maximum dose *without* harmful response (No Observed Adverse Effect Level, NOAEL) or the lowest dose causing adverse effects (Lowest Observed Adverse Effect Level, LOAEL), useful for the toxicological risk assessment purposes. Particular attention will be also given to the main target organs of these toxins, i.e. the heart.
To complete the research Analytical Chemistry Assays based on antibody affinity toward the toxins will be set up in order to develop other methods for PLTXs analysis and compare their results to those obtained by HPLC, LC-MS or GC-MS. This part of research will be carried out at Analytical Chemistry Department of the Universidade de Vigo (Spain).

Dottorando: **Corvaglia Valentina**
Relatore: Dr. Davide Bonifazi (DSF)
Titolo: PNA-assisted cellular migration on smart surfaces.
PNA is a structural DNA mimic obtained by polymerization of monomers of *N*-(2-aminoethyl) glycine that replace the ribose-phosphate backbone characteristic of natural nucleic acids. In PNA, the nucleobases (adenine, cytosine, guanine, or thymine) are connected by methylenecarbonyl linkages to the polyamide structure being an achiral, uncharged, and relatively rigid biopolymer of high biological and chemical stability, therefore it exhibits unique physicochemical properties. As a consequence of these structural features, PNA can bind complementary ssDNA strands with higher affinity than the corresponding DNA sequences; for this reason several examples of surface modification with PNAs have nowadays been reported in literature. In this research project we are interested in the use of PNA to functionalize a gold surface and assist cellular migration.
To achieve this objective we want to use and characterize self-assembled monolayers (SAMs) of thiol-derivatized PNA chains adsorbed on gold surface. In the literature there are some examples

concerning the synthesis of thiolated PNA and for this proposal different approaches have been analysed as starting point. These examples of PNA-SH suggest that there are three different portions to engineer:

- the ssPNA sequence, which synthesis is based on standard SPPS protocols;
- the spacer group length and physicochemical properties, to separate the hybridization portion from the surface;
- the thiol-group bearing portion, which react with the gold surface.

Once a suitable PNA-SH molecule will be obtained, several parameters have to be considered when analyzing the resulting SAMs:

- ssPNA/ssPNA interactions (PNA-SH density) ;
- ssPNA/Au surface interactions (spacer physicochemical properties);
- dsPNA/Au surface interactions (spacer length and degrees of freedom from the conformational modes);
- ordering of the layers.

Different analytical techniques can be adopted for such characterization, like Reflection absorption infrared spectroscopy (RAIRS), X-ray photoemission spectroscopy (XPS) and Atomic force microscopy (AFM).

We are also interested in the formation of PNA-DNA hetero-duplexes to promote cellular migration along the engineered surface, through the presence of a chemotactic function on the DNA molecule. After cellular migration along the surface it is necessary to unwind the PNA-DNA complex and promote the hybridization of another ssPNA oligomer with a complementary ssDNA sequence for obtaining the reverse cellular migration along the surface. It is known that PNA-DNA dissociation can be induced by means of different techniques:

- physical techniques, such as temperature or terahertz radiation (THz radiation);
- chemical techniques, in particular using denaturing agents, like urea, formamide, nitrosamine, sodium nitrite and sodium nitrate, which they are able to intercalate inside the DNA double helix;
- enzymatic techniques which involve specific enzymes like helicases and topoisomerases.

The hybridization step can be followed both in solution, through AFM measurements, and in solid state by XPS and RAIRS analysis. At last, other techniques like fluorescence, ion mass spectrometry (TOF-SIMS) and surface plasmon resonance (SPR) could be used.

Dottorando: **Buzzi Debora**
Relatore: Prof. Cynthia Ebert (DSF)
Titolo: Studio di fenomeni di riconoscimento molecolare in fase solida

Lo scopo del progetto è studiare dei sistemi modello di riconoscimento molecolare in fase solida, allo scopo di sviluppare o ottimizzare su base razionale, strategie cromatografiche e diagnostiche.
Sulla base di una conoscenza maggiormente dettagliata dei fenomeni di riconoscimento molecolare e della chimica dei polimeri (fase solida) si intende quindi progettare sistemi di riconoscimento antigene-anticorpo, enzima-ligando chirale, ed eventualmente oligonucleotide-RNA. Il progetto prevede la combinazione di competenze relative ai seguenti campi:
a) modifica ed ingegnerizzazione di proteine,
b) sintesi organica in fase solida e sviluppo di supporti solidi innovativi,
c) studio e modellazione delle interazioni proteina ligando e proteina-fase solida.
Lo studio si avvarrà sia di metodi sperimentali che computazionali (per es. modellistica molecolare, statistica multivariata).

Dottorando: **Hasa Dritan**
Relatore: Prof. Dario Voinovich (DSF)
Correlatore: Prof. Mario Grassi (DMRN)
Titolo: Innovative systems for drug delivery: pharmacokinetik and technological aspects.

An increasing demand for innovative oral formulation, still the most required from the market, is shaping the pharmaceutical landscape. This need comes not only from patent motivations, but especially from the fact that most of the drugs are suffering for reduced oral bioavailability, and from the fact that existing innovative formulations are frequently very sophisticated and not easy to be marketed.
The aim of this research project is to accomplish at the same time the biopharmaceutical issue and the technological requirements of an easy production.
These two aims will be carry out following two strategies.
From the one hand the poorly bioavailable drugs will be processed by means of a single-step technology (such as mechanochemical activation and or incorporation in microemulsions) to change their physical status thereby improving their solubility/permeability and consequently their absorption.
From the other hand, innovative solvent-free processes, such as melt agglomeration (in a extruder or in a high shear mixer), will be developed to realise in a single step the final dosage form having the suitable drug release by appropriate selection of the low-melting binder mixture.
After the production, the drug delivery systems will be extensively characterised from a physicochemical, technological, biopharmaceutical and pharmacokinetic point of view. Then, mathematical models will be adopted to verify the correctness of the mechanisms proposed to describe drug release, absorption, distribution and elimination and thus reduce the number of expensive and time consuming experiments that have to be performed.
During the first year drug models suitable for the both strategies will be selected. In particular, *Vinca minor L.* alkaloids, notoriously poorly water soluble compounds, will be processed with common inert excipients by mechanochemical activation in optimized conditions. In the second strategy, widely used water soluble molecules, such as theophylline or paracetamol, will be incorporated (and suitably shaped) in lipidic matrices, constituted of an appropriate mixture of low melting binders. These formulations will be extensively characterised as above mentioned, ending the studies with *in vitro* dissolution tests, preliminary *in vivo* studies and *in vitro-in vivo* correlations.

Dottorando: **Garziera Marica**
Relatore: Prof. Silvano Geremia (DSCH)
Correlatore: Dott.ssa Valli De Re (Centro di Riferimento Oncologico di Aviano)
Il programma di ricerca prevede lo sviluppo e la produzione di proteine miniaturizzate mimanti la regione idiotipica di anticorpi associati a malattie linfoproliferative per la produzione a scopo diagnostico e terapeutico di anticorpi anti-idiotipo.
Già da alcuni anni è attiva una collaborazione tra il Centro di Eccellenza in Biocristallografia (CEB) dell'Università di Trieste ed il Centro di Riferimento Oncologico di Aviano (CRO), volta a comprendere le origini molecolari di patologie croniche di tipo autoimmunitario come la crioglobulinemia mista di tipo II (CM) collegata all'infezione da virus HCV. La CM è una patologia caratterizzata da proliferazioni oligoclonali di linfociti B che in un significativo numero di casi evolve a linfoma di tipo non Hodgkin (NHL) conclamato. Il ruolo eziopatologico del virus dell'epatite C (HCV) nell'insorgenza della CM e nella degenerazione a NHL è avvalorato da un'ampia casistica medica. Recentemente il CEB ha iniziato uno studio di modellizzazione della struttura tridimensionale delle immunoglobuline di tipo M (IgM) con attività di fattore reumatoide presenti nel crioprecipitato di pazienti affetti da CM causata da infezione da HCV.
Per comprendere le origini molecolari della risposta autoimmune conseguente all'infezione da HCV è essenziale conoscere il modello strutturale del sistema antigene-anticorpo. Tra le diverse tecniche utilizzabili per validare i modelli biologici proposti per la produzione di autoanticorpi, la biocristallografia può fornire direttamente una rappresentazione tridimensionale degli immunocomplessi ed i dettagli dei siti specifici di interazione tra il recettore e l'antigene/i. La produzione in vitro di proteine ricombinanti omologhe alla regione ipervariabile delle

immunoglobuline di superficie (mIg) presenti nei linfomi CM e HCV-positivi è fondamentale per gli studi biologici, biochimici e strutturali atti ad analizzare le interazioni esistenti fra il recettore delle IgM e gli antigeni espressi su proteine di HCV e/o dell'autoantigene IgG, patognomici della MC. Sulla base dei modelli strutturali degli anticorpi ricostruiti per omologia si è disegnata una proteina miniaturizzata chiamata "MALOT" (acronimo per Miniaturized Antibody Light chain CDR One and Three).

La sequenza amminoacidica della proteina MALOT desunta da studi cristallografici, mima quindi una regione idiotipica VL opportunamente modificata e in precedenza individuata come prototipo comune di soggetti con linfoproliferazione B CM e HCV-positivi. Essa è riconducibile alle regioni ipervariabili CDR1 e CDR3 esposte sulla superficie dell'immunoglobulina contenenti sequenze linkers idonee a ricostruire una conformazione simile alla sequenza VL nativa. L'obiettivo del presente progetto di ricerca è di sintetizzare la proteina MALOT come proteina ricombinante che mima la regione idiotipica di anticorpi associati alla CM, caratterizzare strutturalmente questa proteina miniaturizzata al fine di comprendere le basi molecolari della bispecificità degli anticorpi IgM associati alla CM, ed utilizzarla successivamente per produrre anticorpi anti-idiotipo altamente specifici da utilizzare a scopo diagnostico ed eventualmente terapeutico.