# DOCTORAL PROGRAM IN INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING

Chair:
**Prof. Tiziano Faravelli**

The Doctoral Program in Industrial Chemistry and Chemical Engineering (***CII***) is designed for students aiming to get a deep expertise on chemical processes and on material processing either as the inherent *research* or in the *process design and development* activities.

The program is the ideal extension of the *Laurea Magistrale* degrees in *Chemical Engineering*, *Safety and Prevention Engineering, Materials Science*, *Material Engineering*, *Industrial Chemistry* and *Chemistry*, but it is also open to graduated in other scientific disciplines.

The general topic for the doctoral program in Industrial Chemistry and Chemical Engineering is the application of the chemical and physical knowledge to all the activities related to the synthesis, design, production and transformation of chemical substances and materials (like advanced inorganic, polymers and biomaterials). These studies involve not only the synthesis processes but also the related plants, here analyzed starting from the laboratory tests and the pilot plant experiments up to the industrial size ones, always including the exam of the related energetic, safety and environmental issues.

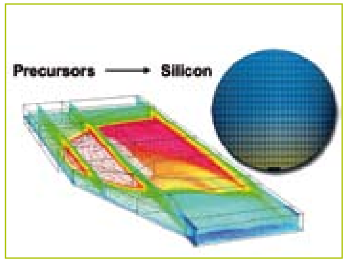
The final goal is to provide to the Research Doctor the tools and the skills: (1) to design and manage industrial processes at any size scale; (2) to develop new technical applications and (3) to create and to characterize new products and services.

The ***CII*** program covers three years for an overall amounts of 180 credits. The responsibility of the organization and of the contents of the doctoral program is attributed to the Professor Committee (PC). At the beginning of the Doctoral program, a tutor and a thesis advisor is assigned to each student. The tutor has a supervisor function during the whole doctoral program, whereas the thesis advisor is responsible for the thesis work.

The PC defines a specific curriculum for each student, on the basis of the tutor suggestions. The curriculum has to be designed in order to both cover deficiencies in the student scientific formation and to give a high-quality technical and scientific preparation.

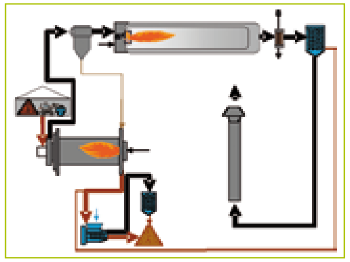
Since 2001 (XVI cycle) the ***CII*** program graduates 88 students, being the 80% now working in industry and the remaining in the university or government research centers. This point demonstrate the great link existing between the performed researches and the industrial environment. In fact, more than 70% of the research topics were directly supported by industrial companies, like ENEL, LPE Epitaxial Technology, Bracco, Flamma, Mapei Solvay Solexis.

**1. Structure simulation of Nitrogenase enzyme for N2 conversion to NH3**

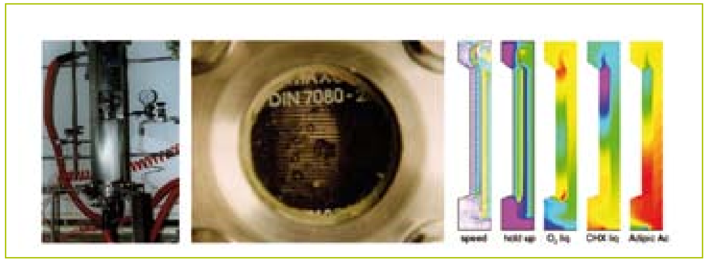


**2. Hazardous gas dispersion simulation from an industrial accident**

**3. Simulation of a chemical vapor deposition reaction for silicon films deposition**



**4. Contaminated soil remediation process**



**5. Lab-scale air-lift gas-liquid reactor and simulation of flow field, gas hold up and concentrations of main reactants (cyclohexane oxidation to adipic acid)**

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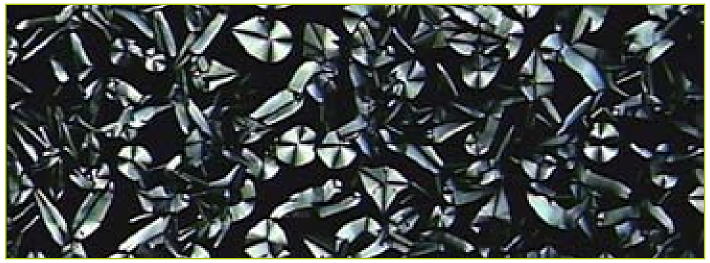
# MOLECULAR NANOSTRUCTURE MATERIALS FOR ADVANCED APPLICATIONS

**Antonio Abate**

In the last years, experimental research in the field of chemistry and materials science is increasingly focusing on molecular and nanometer scale self organized systems.
In this work, self-assembly and recognition processes have been considered as basic principles for different applications to show how the 'nanoscale' is affecting the technology.
Mechanisms of self-assembly, driven by weak interactions, have been exploited to generate ordered structures in liquid crystalline phases. The structural anisotropy of these systems is reflected in the properties of the material; in particular, the dynamics of charge transport through ionic conduction in redox electrolytes were investigated. Although the single crystal conductivity can be high, the polycrystalline nature of molecular materials creates grain boundaries which lower the overall conductivity. Self organization by the formation of liquid crystalline phases is a key strategy to control the long range order. The material may be annealed in the mesophase and the high degree of uniform molecular order/orientation is locked in by subsequent solidification. The key advantage of liquid crystallinity is the ability to form monodomain samples without defects caused by grain barriers between neighbouring crystalline domains.
We reported on the liquid-crystalline layered assembly consisting of alkyl-fluoroalkyl substituted imidazolium salts, which form nano-level phase segregated structures. Discontinuity achieved in temperature dependence of ion diffusion, switching from the ordered phase to isotropic one, suggests a more efficient diffusion mechanism in the ordered phase.
Although silicon solar cells play the leading role for practical use nowadays, organic photovoltaic devices have attractive features in terms of production cost. Low light-to-electricity conversion efficiency and durability are the most serious problems that preclude their practical application. The control of morphology at the interface of organic – inorganic semiconductors allows to improve the kinetics of charge transfer and then the efficiency of device. Ionic liquids were introduced as solvent in electrolytes for organic photovoltaic applications thanks to their low volatility, high thermal stability, and large ranges of temperatures over which they are liquid, coupled with excellent chemical stability. We have demonstrated that the introduction of polyfluorinated ionic liquids results in an improved light conversion efficiency. A mechanism based on alkyl- fluoroalkyl nano-level phase segregation was proposed as a general strategy for the control of the interfacial charge transfer.
Although the conversion efficiencies of quantum dots sensitized solar cell are in this moment far away from the most commonly used organic photovoltaic devices, in the last 2 years the maximum reported efficiencies have increased in a factor 2–3, highlighting the enormous potentiality of this kind of solar device. We focused on the role of the linker molecule used to attach colloidal quantum dots to $TiO_2$ semiconductor surface, showing that not only the linker but also the treatment carried out to attach these molecules influences the final performance. The photoelectrochemical behaviours of the tested devices suggest that a large amount of cysteine on the surface of the $TiO_2$ could reduce the electron recombination at the interface. Moreover the amino groups of cysteine, interacting with the slightly acidic surface of $TiO_2$, reduce the distance between quantum dot and electrode, which increases the performance of device.
Processes of molecules recognition by halogen bond have been exploited to create molecular sensors for phosphate groups, which are involved in many important biological processes such as biosynthesis, molecular transport, and muscle contraction. Flexible podand-type receptors, among others, have been demonstrated to function as effective anion-binding agents. The majority of tripodal receptors utilize substituted benzene cores to which functionalized arms are attached. As far as the anion coordination capability of these receptors is concerned, most frequently a combination of electrostatics and hydrogen bonds is used in order to increase the binding constants thanks to the synergistic effect of ionic bond strength and the directionality of hydrogen bond. We have reported the synthesis of receptors that have been designed for the specific coordination of phosphate anions. The strategy for the design of the receptor has relied on the combination of halogen bond directionality and ionic bond strength in a tripodal scaffold, to take advantage of chelate cooperativity. Our compound is the first example of halogen bond based receptor for the binding of polyoxyanions in water solution. The high binding constant measured demonstrates that our strategy based on the exploitation of halogen bond is very promising for the design of new anion receptors.


1. Mesophase texture of a fluorinated ionic liquid crystal under polarized light

# HYBRID LNT/SCR SYSTEMS FOR NOX REMOVAL UNDER LEAN CONDITIONS

**Rossella Bonzi**

The growing demand for diesel-equipped vehicles has raised new environmental concerns, since these engines have been claimed to be one of the primary source of $NO_x$ and particulate (soot) emission. For this reason, the next-coming Euro VI rules in 2014 will provide a severe decrease in the $NO_x$ emissions, down to 0.08 g/km.

Different solutions have been proposed for the abatement of $NO_x$ under lean conditions: the Selective Catalytic Reduction (SCR) and the $NO_x$ Storage Reduction (NSR) or Lean $NO_x$ Trap (LNT) techniques. These technologies are the top contenders for reducing $NO_x$ concentrations in the exhausts of diesel and lean burn gasoline engines.

Due to its technical complexity, the urea-SCR system is preferably applicable to trucks and heavy-duty vehicles, although applications for passenger cars have also been proposed. On the other hand the NSR technique is more suitable for small engines.

To achieve the reduction of $NO_x$ in fuel-lean, i.e., oxygen-rich atmosphere, NSR utilizes unsteady state operation by switching between fuel-lean and fuel-rich conditions.

Standard LNT catalysts consist of precious metal and alkaline/alkaline-earth metal, typically platinum and barium components finely dispersed on metal oxides, e.g. $\gamma$-alumina. During fuel-lean periods (typical length >1 min), nitric oxide present in the flue gases reacts with oxygen to produce nitrogen dioxide on Pt particles, and the so formed $NO_2$ is captured by Ba components as nitrites and nitrates. In parallel with the above mechanism, direct NO storage on BaO assisted by $O_2$ and Pt can also occur. The stored $NO_x$ are then reduced to $N_2$ during a short fuel-rich period (usually few seconds).

The chemistry and mechanisms involved in the reduction of the stored $NO_x$ are still a matter of debate. Recently, the involvement of ammonia as intermediate species in the reduction of the stored nitrates by $H_2$ has been considered, and a mechanism which is able to account for both the high initial selectivity of the reaction and the temporal sequence of the products during the rich operation (with $NH_3$ evolution which follows that of $N_2$) has been proposed. In particular, it has been shown that an $H_2$ front develops in the trap during the rich phase, which travels along the reactor axis. Clear evidences have been provided on the fact that $N_2$ formation involves the occurrence of an in-series two step molecular process involving at first the formation of $NH_3$ upon reaction of $H_2$ with the stored nitrates, followed by the reaction of the so-formed ammonia with nitrates located downstream the $H_2$ front. This reaction leads selectively to $N_2$. Accordingly the regeneration of the trap proceeds both in the part of the reactor in which nitrates are reduced by $H_2$ to give ammonia and in the zone in which the ammonia formed upstream reacts with nitrates leading to $N_2$.

Notably, $NH_3$ formation has been found to be much faster than nitrogen formation via reaction of nitrates with $NH_3$. Accordingly, if regeneration of the trap is carried out at low temperatures the reaction of $NH_3$ with the stored nitrates does not take place and ammonia leaves the catalyst unreacted, leading to significant $NH_3$ emissions ($NH_3$ slip).

In principle, the ammonia slip can be avoided by placing a $NH_3$-SCR catalyst layer downstream the NSR catalyst. One of the most suitable SCR catalysts is FeZSM-5 because of its high activity and durability. In this case the $NH_3$ released from the NSR catalyst during the rich phase is adsorbed on the SCR bed; the stored ammonia then reacts with gaseous NO according to the $NH_3$-SCR reaction during the subsequent lean cycle.

The study of hybrid NSR-SCR catalytic systems has been the object of this Doctorate work.

In particular the reactivity in the $NO_x$ storage and reduction has been investigated over both single NSR and SCR catalyst layers, and in the case of combined catalyst configurations as well (i.e. a double bed configuration with the NSR catalyst layer placed upstream the SCR catalyst and a physical mixture of the two catalyst sample in one layer). For this purpose model NSR ($PtBa/Al_2O_3$) and SCR (FeZSM-5) catalyst samples have been used, and their reactivity in the different configuration previously depicted has been analyzed under controlled isothermal conditions to avoid spurious effects due to temperature upon lean/rich cycling. The study has been accomplished using limited concentration of $H_2$, in the presence and in the absence of $CO_2$ and $H_2O$ and in a wide temperature range (150-350°C) to operate under nearly isothermal conditions and to address the effect of temperature and of $CO_2$ and $H_2O$ present in the exhausts.

It has been demonstrated that in the dual bed configuration the storage of $NO_x$ occurs primarily onto $Pt\text{-}Ba/Al_2O_3$ although a limited contribution of FeZSM-5 is possible. In the presence of $CO_2$ and $H_2O$ the storage of $NO_x$ is lower because $BaCO_3$ formation inhibits $NO_x$ adsorption.

The trapping (during the rich phase) and the reaction (during the lean phase) of ammonia over the zeolite SCR catalyst explains the very low ammonia slip (and the higher $N_2$ selectivity) which is observed in the case of the double-bed configuration.

In the case of the LNT+SCR physical mixture, the trapping of $NH_3$ is most favoured because $NH_3$ is formed as intermediate in the reduction of nitrates, and the particles of $Pt\text{-}Ba/Al_2O_3$ and of FeZSM-5 are evenly distributed in the reactor. Accordingly the storage of $NH_3$ onto the SCR catalysts prevents reaction of $NH_3$ with $NO_x$ stored downstream onto $Pt\text{-}Ba/Al_2O_3$, following the 2-steps reduction pathway of stored $NO_x$ and the integral behaviour of the reactor. This explains the huge $N_2$ production during the lean phase in the case of the LNT+SCR physical mixture.

In conclusion, $NH_3$ trapped onto the FeZSM-5 particles during the rich phase reacts during the subsequent lean phase with $NO_x$ from the gas phase, which have not been trapped onto the $Pt\text{-}Ba/Al_2O_3$ particles, to give $N_2$ according to the fast SCR reaction, and possibly to the standard SCR reaction as well. This results in both higher $NO_x$ removal efficiency and increased $N_2$ selectivity over the LNT+SCR dual bed and the LNT+SCR physical mixture, particularly at low temperatures.

# MATHEMATICAL AND TOPOLOGICAL CRITERIA TO SAFELY OPTIMIZE POTENTIALLY RUNAWAY SYSTEMS

**Sabrina Copelli**

Fast and strongly exothermic chemical processes are particularly difficult to optimize and scale-up from laboratory to full plant scale because of the possible triggering of reactor temperature loss of control, occurring whenever the rate of heat evolution becomes greater than the rate of heat removal provided by the installed cooling equipment.
Such an unwanted phenomenon, called “runaway”, can take place into every type of reactor because of a great number of operating and human factors.
In fine chemical and pharmaceutical industries, which produce small amounts of a wide range of chemical compounds, it is not possible to investigate in detail the microkinetics of all the reactions involved in a process. As a consequence, such enterprises call for optimization and scale-up procedures that must be not only simple, fast and low-cost but also reliable.
Aim of this work has been to investigate the possibility of developing such procedures, paying particular attention to potentially runaway semibatch processes operated in the isoperibolic mode.
First of all, single reaction systems have been analyzed through graphical tools already presented in the literature (inherently safe conditions, ISC, and temperature rise curve, TRC, diagrams) and a simple and reliable combined optimization – scale-up procedure has been developed. This procedure is scale-independent and it allows for keeping safe operating conditions also maximizing reactor productivity. Particularly, TRC diagrams are employed to identify the maximum tolerable process exothermicity accounting for the reacting mixture thermal stability.
Contextually, a minimum dosing time can be calculated providing a value of the system reactivity that has to be checked with respect to excessive co-reactant accumulation conditions through a suitable ISC diagram.
A sensitivity analysis of the developed procedure with respect to the experimental parameters subjected to uncertainty has been performed to demonstrate that the selected optimum operating conditions are inherently safe. Particularly, easy check correlations have been provided for single slow reactions occurring in the continuous phase.
In order to validate experimentally such an optimization – scale-up procedure, the nitration of 4-chlorobenzotrifluoride to 4-chloro,3-nitrobenzotrifluoride has been analyzed through calorimetric techniques.
After this preliminary work, it has been tried to extend such a simple approach to complex reacting systems (consecutive reactions with autocatalytic behavior) but the excessive complexity of the process made such an approach not useful in practice so that more sophisticated mathematical tools has been searched for in order to attain the same goal achieved for single reaction systems.
It has been found that a general optimization procedure, able to handle both simple and extremely complex reacting systems, could be developed by the means of topology.
Topology is a major area of mathematics concerning with spatial properties of objects, called manifolds or varieties, that are preserved under continuous deformations.
Varieties of this typology are, as an example, all phase portraits (PP) generated by reporting the solution of a non-linear ordinary differential equations system onto its corresponding phase space while one of the system constitutive parameters or initial conditions is varying.
As it can be easily observed, mass and energy balance equations needed to describe whatever complex reacting system fit in this category.
In order to observe whatever notable topological property exhibited by the analyzed variety it is necessary to project it onto suitable viewable spaces.
Concerning the problem of the thermal behavior characterization of potentially runaway semibatch processes, the simplest solution is to employ projections of the corresponding variety onto bidimensional spaces, called 2-D charts, whose coordinated axes are couples of the N dependent variables generating the manifold that fulfill the following specific criteria:
1) only dependent variables that are involved into the energy balance equation can be employed to characterize the observed system thermal behavior; 2) temperature is the most relevant system dependent variable so that it must be compulsory involved in whatever possible couple generating 2-D charts.
According to these features, suitable 2-D charts can be built to search for the system thermal behavior boundaries and the optimum set of operating conditions fulfilling process safety and productivity constraints.
In order to achieve these goals, first, it must be defined the product X with respect to the optimum operating conditions (that is, QFS conditions) are searched for and, then, the maxima of the trajectories generating the analyzed PP must be projected onto a suitable 2-D chart, referred to as X-space, whose coordinated axes are constituted by the conversion with respect to the desired product X and the reactor temperature divided to the coolant one. The result is a curve, referred to as topological curve, whose inversion points represent transitions between different system thermal behavior regions.
Particularly, it has been demonstrated that whenever the topological curve exhibits an inversion whose concavity is towards left one of the following three system thermal behavior boundaries is encountered: transition, runaway or starving; on the contrary, whenever an inversion with a concavity towards right is observed a QFS boundary is detected.
It is possible to observe that the different left concavities appear only as singlets or doublets in an X-space. Therefore, by knowing only the generating parameter of the topological curve and the reaction scheme, it is relatively simple to define which ones among them may be encountered and, in the presence of doublets, the sequence of their appearance.
Particularly, when dosing time $t_{dos}$ is the generating parameter and one exothermic reaction occurs only the transition singlet or the transition/starving doublet may be observed (as a function of the system constitutive parameters values).
It has been demonstrated that, in the presence of the transition/starving doublet, the transition inversion is always the first encountered when $t_{dos}$ is increased.
The optimization procedure based on the analysis of the topological curve uses the QFS inversion as a boundary beyond which the optimum operating conditions (that is, dosing time and coolant temperature) can be searched for accounting for reacting mixture thermal stability and desired productivity constraints.
It has been demonstrated that such a topological procedure can be applied both theoretically (though the knowledge of the system micro and macrokinetics) and experimentally (without any explicit knowledge of the system kinetics) in order to detect the optimum operating conditions for a fast and strongly exothermic semibatch process.
Particularly, the relevant case studies of the 4-chlorobenzotrifluoride nitration by the means of mixed acids, the free radical emulsion homopolymerization of vinyl acetate and the solution homopolymerization of butyl acrylate have been analyzed experimentally at the RC1 scale to validate this topological approach. Moreover, the nitric acid oxidation of 2-octanol to 2-octanone (and further carboxylic acids) and the acid esterification between propionic anhydride and 2-butanol have been studied using literature experimental data for the sake of completeness.

# QUANTUM CHEMISTRY STUDY OF FREE-RADICAL POLYMERIZATION KINETICS

**Marco Dossi**

In the last decades, remarkable efforts and considerable progresses have been made in polymer reaction engineering to obtain accurate kinetic information by different experimental methods.
With reference to the free radical polymerizations, thanks to the assessment of sophisticated experimental techniques such as Pulsed Laser Polymerization combined with Size Exclusion Chromatography (PLP-SEC), thermodynamic and kinetic parameters can be directly and accurately evaluated.
However, the experimental determination of rate coefficients is often time consuming or complicated to achieve, mainly due to the complexity of the kinetic mechanism and considering the numerous radical species present in the system.
Moreover these difficulties are increased moving to the study of copolymer systems.
Keeping this scenario on the background, theoretical predictions of kinetic properties based on Quantum Mechanics (QM) methods could represent a powerful tool to support and enrich the experimental efforts.
In this work mechanisms and reaction paths involved in free radical polymerizations of widely used monomers have been studied through a computational approach based on Density Functional Theory (DFT). The final aim of the work is to elucidate the capabilities of the proposed method to reproduce and predict thermodynamic and kinetic parameters.
As a first step, a validation of the computational method has been performed studying systematically the reactivity between different carbon-centered radicals and a large number of alkenes, representative of monomers used in polymer synthesis.
Once an efficient method has been defined, the analysis has been focused on the initiation kinetics, i.e., the reaction of the first monomer unit with the initiator fragment. Different monomers of industrial relevance such as methyl acrylate (MA), methyl methacrylate (MMA), acrylonitrile (AN) and styrene (ST) have been studied in free-radical polymerization initiated by five initiators, exhibiting different behaviors in terms of water solubility and dissociation mechanism: azobisisobutyronitrile (AIBN), di-tert-butyl peroxide (DTBP), potassium persulfate (KPS), 2,2-dimethoxy-2-phenyl acetophenone (DMPA) and dibenzoyl peroxide (DBP).
The obtained results have shown that the initiators largely affect the kinetic rate of the first reaction step in free radical polymerization. Depending upon the kind of employed initiator, the rate constant values of the reaction between the initiator fragment and the first monomer unit are increased from one to eight orders of magnitude with respect to the propagation rate constant of the same monomer with long active chains.
Moreover, after this first kinetic step, the initiator effect on the homo-polymerization kinetic constant decreases very quickly and after no more than three reaction steps the rate constant approaches the value reported in the literature for long growing chains. In all the investigated systems the initiator reactivity decreases moving from DBP to AIBN while DTBP, KPS and DMPA exhibit intermediate reactivity.
Afterwards, according to the most conventional kinetic scheme of free radical polymerization, propagation kinetic has been investigated.
In particular, the propagation kinetic rate constants for ST, AN, vinyl acetate (VAc) and different acrylates and methacrylates have been calculated and compared with experimental data reported in the literature. A general good agreement between computational and experimental results has been reached.
This agreement is particularly satisfactory in terms of activation energy values: as a matter of fact computational method reproduces the literature values with an absolute error smaller than 2 $kcal \cdot mol^{-1}$. Moreover computational propagation rate coefficient differs by two or three times maximum from the experimental one.
The third application of the computational method proposed has been focused on the so-called secondary reactions. Their relevance as events responsible of the mid-chain radicals (MCRs) formation, has been recently proved. Two important classes of secondary reactions, backbiting and beta-scission, have been investigated.
With reference to the free radical homo-polymerization of vinyl chloride (VC) and AN, the backbiting 1:3, 1:5, 1:7 reactions have been investigated and it has been found that the 1:5 backbiting reactions are the most favorite. For all the MCRs produced the right and left beta-scission reactions have been also studied. It has been found that both the right and left beta-scission reactions require the same activation energy, while the activation energy seems to be more affected by the position of the mid chain radical.
Finally, the most relevant and innovative topic explored in the research activity has been the application of the computational methodology to the study of copolymer. The fundamental challenge in the free-radical copolymerization reactions is to control the kinetic to make useful well-designed materials. All the strains of the scientific research in this field have been oriented in the direction to determine the reactivity of different systems and to propose a model able to predict the composition of copolymers.
In the work the analysis has been finalized to show the capabilities of the computational approach to reproduce composition data and copolymer propagation kinetics. In particular the differences between terminal and penultimate models are analyzed examining different cases. Promising and important results have been obtained.
At first the computational technique adopted is sensitive enough to capture differences in monomer addition rates experimentally found in ST/methacrylate (xMA) copolymerization when changing the methacrylate ester group.
Afterwards a complete kinetic and composition analysis has been done for the VAc/MMA system which exhibits a weak penultimate unit effect. In this work an experimental PLP-SEC study has been combined with the computational analysis.
New copolymer composition data have been well represented by the terminal model using monomer reactivity ratios obtained by use of ab initio method. VAc/MMA composition-averaged copolymer propagation rate ($k_{p,cop}$) have been measured for the first time using the PLP-SEC technique. The addition of only 10 mol % MMA to VAc reduces $k_{p,cop}$ by more than a factor of 40 from the literature VAc homopolymer propagation rate. This kinetic behavior is described reasonably well by both terminal and penultimate chain-growth models, using the monomer and radical reactivity ratios determined computationally.
Successively, the ST/MMA system, which is characterized by a strong penultimate unit effect, has been investigated.
Composition data and copolymer propagation kinetics have been well reproduced even for this copolymer. In particular the curves obtained using the reactivity ratios computationally determined prove clearly the superior performance of the implicit penultimate model to describe this system.
Good results in terms of copolymer composition data have been obtained even in the study of 2-hydroxyethyl acrylate (HEA)/ST, especially considering all the difficulties related to the experimental analysis of this system. The prediction by the terminal copolymerization model, obtained using computational parameters, well described the composition trend showed in literature, confirming the potentiality of the computational approach proposed.
To conclude, the computational method has been able to reproduce with good accuracy the experimental data in all the reactions investigated.
This agreement demonstrates that the technique represents a powerful tool to study the reactivity in free radical homo- and co-polymerization.
Therefore, the proposed approach can be considered an effective semi-quantitative tool to predict kinetic parameters and relative reactivity, reducing the amount of experimentation required in polymeric systems.

# FORMULATED HYDROGELS FOR CELL HOUSING AND DRUG DELIVERY

**Filippo Rossi**

The present PhD work thesis regards the discovery and development of a new three-dimensional polymeric system able to release drugs or cells for tissue engineering applications. The experimental work was divided as follow.

The chemical-physical characterization was studied at the Department of Chemistry, Materials and Chemical Engineering "Giulio Natta" of Politecnico di Milano (CFA Lab) and at the Royal School of Mines of the Imperial College London (Prof. Boccaccini's group) while the biological experiments at the Mario Negri Institute for Pharmacological Research (Dr. Forloni's group).

Introducing the research topic is important to know that when treating many pathologies, which lead to the degeneration of specific organs, the only possible therapeutic alternative is often represented by the organ transplantation. Where possible, however, it is associated to a high grade of risk for the patient, since complications are likely to occur, such as the transmission of pathologies from donor to recipient or the rejection of the organ itself, along with the chronic shortage of available organs. Moreover, such an approach cannot be applied to all the body zones susceptible to degenerative pathologies. Actually, at present, transplant therapies may not be applied to many degenerative pathologies, such as, in some cases, those related to some zones of central nervous system (CNS).

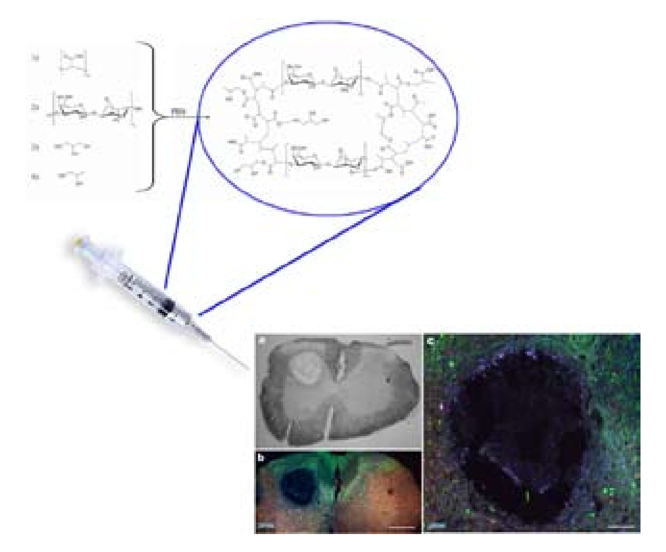
With the complete understanding that cell or drug therapies alone are not sufficient for guarantee the success of tissue engineering in the repair of damaged tissues, in the last decades engineered polymeric matrices (scaffold) gained a lot of interests both from researchers and companies. In particular, in spinal cord injury pathology, the presence of a cavity that should be replaced suggests the necessity of a biomaterial able to deliver in loco cells and additional factors (drugs, peptides or antibodies).

Agar-Carbomer (AC) hydrogels, discovered and developed in this PhD thesis work, seem to be a very promising tool for this application. As three-dimensional matrices they allow good survival of cells included (glia and mesenchymal stem cells) within their polymeric network and optimal functionality of them once extracted. After hydrogel inclusion there are no differences, in terms of biological properties, between them and classic *in vitro* cultured cells. Moreover, their biocompatibility was tested *in vivo* in spinal cord of mice (control and injured) and the result neutrality underlines their promising use as smart delivery system.

Nowadays no therapies for spinal cord repair are available, just few trials in preliminary testing and methylprednisolone for neuroprotection in the first hours after injury. So, due to the absence of a proved therapy, it is necessary to design and optimize a very tunable smart system able to cover and satisfy different medical needs. In this way the AC material library allows a very high adaptability to many needs. In order to be suitable for *in vivo* applications, we performed many studies in order to be able to control and tune the sol/gel transition with a final polymerization *in situ* and consequent reduction of risks due to surgery procedures (Figure 1). Once verified matrix properties as cell carrier, we focused our attention on the hydrogel ability to deliver one or more drugs.

On one side we delved into delivery through hydrogels transport phenomenon, a very controversial matter and, on the other, its ability to cover with release all the damaged area.

Our investigation started with methylprednisolone (neuroprotective drug) and drug mimetic with similar molecular weight and steric hindrance performing, as said, *in vitro* and *in vivo* studies that underline complete release within the first 24 hours, extremely compatible with medical needs, for neuroprotective agents. The area of delivery resulted to be bigger than the injured one, extremely favored toward caudal direction due to the presence of CSF flow. Moreover higher molecular weight agents, like antibodies, were released with a slower kinetic. This could be a key point in order to study a multiple drug delivery approach or a combined drug/cell therapy.

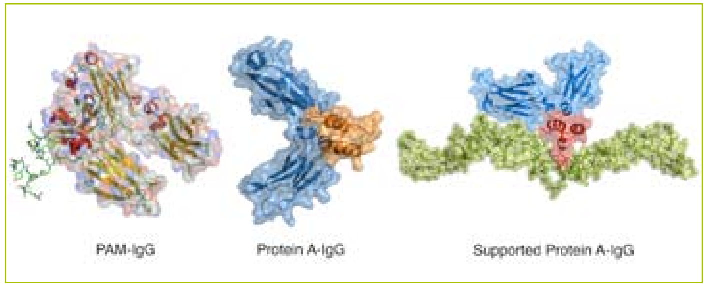


1. Schematic view of the work: synthesis of a new three-dimensional polymeric network that can be injected into the target tissue. Inside the body it is able to retain and release drugs and cells.

# MOLECULAR INVESTIGATION OF PROTEIN-LIGAND COMPLEXES IN SOLUTION AND SUPPORTED

**Matteo Salvalaglio**

In recent times the increasing opportunities offered by proteins in many branches of the sciences, medicine, and engineering ranging from the production of bioactive materials to the development of protein based therapeutic agents have raised a whole new set theoretical challenges with a direct influence on technological issues. Some of the theoretical and practical tools that are nowadays commonly employed to treat well-known processes involving small chemicals need to be retuned in order correctly describe processes that involve large complex macromolecules such as proteins. The interaction of proteins with the surrounding environment and the association processes that lead to the formation of complexes both in solution and at the solid/liquid interface represent a wide field of interest in which technical issues and problems need to be tackled without neglecting the fundamental chemical physical bases. Protein complexes formation, in fact, represents a physical chemical phenomenon that characterizes a wide range of science and technology fields, ranging from molecular biology to material science passing through pharmaceutical research. In this work the formation of such complexes was investigated from a molecular standpoint, employing both a modelistic and an experimental approach.
The investigation of protein complexes was performed focusing the attention to systems that constitute the core of a technological problem in which the research of molecular-based rational criteria could have a positive impact.
In all the faced cases the comprehension of fundamental-level information about ligand-receptor systems plays in fact a primary role for the profound understanding of the macroscopic functioning of a process; the investigated complexes span from those formed by perfluoro surfactants and Human Serum Albumin (HSA), to the pseudo complexes formed by a globular protein adsorbed on the surface of a charged solid.
The description at the molecular level of structures, dynamics and energies is nowadays possible in force of a continuous development of the calculation tools. The growth of the computational power and storage capabilities that characterized the last decade allowed tackling the simulation of large and complex systems thus extending the employment of molecular modeling methods to technologically relevant systems.
The first problem assessed was the complexation of Human Serum Albumin (HSA) by means of perfluoro surfactants such as perfluoro octanoate (PFOA) and perfluoro octane sulfonate (PFOS). The molecular modeling investigation of these complexes was aimed at the understanding of the molecular features that determine the long time persistence of these compounds in living organisms. The information obtained from this study was employed for developing rational guidelines for the identification of potential substitutes of these compounds with lower bio persistence potential.
A second group of systems investigated through molecular modeling regards the affinity complexes representing the technological core of the vast majority of the state-of-the-art monoclonal antibodies (MAbs) purification processes.
The growing discovery, patenting and approval by public authorities of therapeutic proteins such as MAbs represent one of the major issues of the contemporary biotechnological and pharmaceutical industry. This evolution of the pharmaceutical compartment has driven the downstream processing industry to face the problem of the bio-macromolecules purification with an emphasis on the reduction of processing costs.
In this framework the development of innovative materials and process schemes plays a key role. The protein A complex with a constant portion of the IgG was modeled both in solution and supported on an agarose surface with the aim of capturing the molecular features responsible for its high selectivity in binding MAbs. Also a synthetic dendrimeric ligand (Protein A Mimetic, PAM) was modeled in solution with the aim of providing useful information for a rational development of a new class of synthetic dendrimeric ligands with the potential to compete with protein A at the industrial scale.
The adsorption of proteins on surfaces in systems dominated by electrostatic interactions was then investigated employing both modeling and experimental approaches. The protein-surface interactions are in fact responsible for many technologically relevant phenomena that range from biomedical sciences to industrial practical needs.
The model-based estimation of the interaction energy of globular model proteins with charged surfaces was performed with the aim of providing an estimation of retention factors in the framework of protein purification by ion exchange and assessing the physical consistency of coarse grained approximated methods developed in this field. Experimental measure of chromatographic retention factors was carried out in the Morbibelli Group (ETH Zurich, Switzerland).
The estimation of thermodynamic parameters characterizing the protein-surface interaction was complemented by an experimental investigation of kinetics of adsorption of polyelectrolytes and proteins in presence of an applied potential. The aims of this study were the development of an impedance-based approach for the detection of the continuous adsorption regime and the assessment of the role played by counter ions in the kinetics of this process.
This experimental investigation was performed during a visiting period in the group of prof. Paul Van Tassel (Yale University, US)

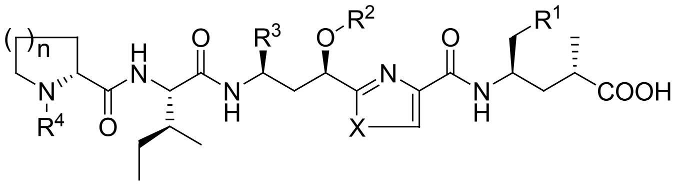


**1. Complexes investigated in the framework of MABs affinity chromatography purification. PAM (protein A mimetic) is a promising dendrimeric synthetic ligand while protein A represent the state-of-the-art technology in this field. In all the complexes a portion of the constant Fc domain of the Immunoglobulin G was employed as receptor**

# DEVELOPMENT OF TUBULYSIN ANALOGUES – PEPTIDOMIMETICS TARGETING CANCER AND ANGIOGENESIS THROUGH IMMUNOCONJUGATES

**Sreejith Shankar. P.**

Cancer, characterised by uncontrolled growth of cells, has been one of the most studied malignancies and hence anti-cancer research is undoubtedly one of the most advanced as well as demanding fields of modern day medical research. Cancer chemotherapy relies on the expectation that highly toxic and potent anti-cancer drugs (or toxins) will preferentially kill cancerous cells or make them commit suicide, while rendering themselves inactive to normal cells. Thus, the development of more selective anti-cancer drugs with an improved ability to discriminate between tumour cells and normal cells is possibly the most important goal of modern anti-cancer research. Most of the anti-cancer drugs currently available are based on the inhibition of microtubule dynamics by the inhibition of cell proliferation and apoptosis. Natural products are being used as lead compounds in medicinal chemistry for the development of large libraries of compounds targeting the cytoskeleton of the cells of higher organisms and are generally obtained from plants or microorganism through secondary metabolism. The chemical synthesis of such compounds provides better opportunities for scale up, improved supply and mechanistic studies directed to their biological action. Tubulysin, a natural peptide isolated from the strain of Myxobacterium, at picomolar concentrations, depolymerises microtubules, and induces mitotic arrest and apoptosis in cancer cells, and to a lesser extent, in normal cells. Tubulysin is a tetrapeptide, composed of three unnatural amino acid fragments (Tubuvaline [Tuv], Tubuphenylalanine [Tup] and *N*-methyl pipecolic acid [Mep]) and the proteinogenic amino acid, L-Isoleucine [Ile]. Chemical modification strategies are badly needed for the site-specific coupling of tubulysins to macromolecular carriers, which are compatible with the cleavage and liberation of cytotoxic moieties either in intracellular compartments or in the tumour stroma, possibly by proteolytic activation.



n = 1, 2 X = S, O

$R^1$ = Ph, PMP $R^2$ = H, Ac

$R^3$ = *i*Pr, Ph, PMP, *c*-hex $R^4$ = Me, Ac

We have developed general synthetic routes towards tubulysins, with a focus on the control of individual stereocentres and their selective derivatisation and these studies have led to the identification of synthetic derivatives with improved biological activity. We have also developed efficient methodologies for the synthesis of the two unnatural fragments, Tuv and Tup, in their stereopure form. The structure-activity relationship studies on the synthesised analogues gave valuable insights for the future development of compounds with an improved pharmacological profile. Replacing the *i*Pr group with other alkyl and aryl groups rendered the molecule biologically inactive. From initial biological experiments, the *N*-methyl proline analogue exhibited almost one-fourth of the activity of natural Tubulysin U, which shows that changing the ring size of the N-terminal residue (Mep) also affects the activity adversly. To our astonishment, initial screening of the oxazole analogue produced better results compared to Tubulysin U. This may be ascribed to the polarity difference between thiazole and oxazole as well as the capability of the oxazole moiety to withstand demanding physiological environments. Moreover, the oxazole ring is capable of exerting better H-bonding interactions within the system as compared to the natural thiazole moiety. We do not expect much variation in activity for the *N*-acetyl pipecolic acid analogue (Acp), but experimental verification is underway and the results shall be published in due course. Different strategies were explored for the stereoselective generation of Tuv fragment – (a) Use of a cyclic intermediate involved nucleophilic ring opening strategy, (b) Organocatalysed asymmetric Mannich type reactions, (c) Transition metal complex catalysed aza-Michael additions, (d) Asymmetric aza-Michael reactions involving metallated oxazolidinones. The first three methodologies gave no promising results even in the initial stages of the study. The aza-Michael reaction of the $\alpha,\beta$-unsaturated acceptor obtained from the 2-enoyl thiazole and oxazole with metallated oxazolidinones afforded the corresponding adduct with good to excellent diastereoselectivity under optimised conditions. Experimental factors such as temperature, base, solvent and quenching procedure were screened and the use of KO*t*Bu as base in THF at -78° C afforded the best results, when quenched by pouring abruptly into a stirred excess of aq. $NH_4Cl$ solution. Attempts to cleave the chiral auxiliary resulted in either no reaction or partial/complete destruction of the substrate. The same reaction conditions when applied to phenyl acceptors (phenyl group in place of thiazole/oxazole), gave no products, with complete retrieval of the starting materials. We have also been involved in the biological studies of the synthesised tubulysin analogues. Immunoconjugates are generally bifunctional molecules that consist of a "targeting" domain, which are capable of localising in tumours, coupled to a therapeutic moiety and in the broadest definition, may utilize MAb, MAb fragments, hormones, peptides or growth factors to selectively localise cytotoxic drugs, plant and bacterial toxins, enzymes, radionuclides, photosensitisers, or cytokines to antigens expressed on tumour cells or on cells of the tumour neovasculature. Spacer reagents (linkers) were synthesised for carrying out conjugation studies involving tubulysin analogues and monoclonal antibodies (MAb) for targeted drug delivery, and quite a few Tub-MAb immunoconjugates were proposed. Radiolabelling of these MAbs were also carried out in collaboration with Prof Dr. Guus van Dongen at Vrije Universiteit Medisch Centrum, Amsterdam.

# DETAILED KINETICS OF PYROLYSIS OF SOLID FUELS

**Samuele Sommariva**

The growing energy demand of developing countries finds the answer always in fossil fuels. The larger availability and lower cost of coal, in respect to other fossil fuels, make it the leading energy resource for the electric production in the world.

On the other hand, coal is a source of environmental concern, not only for its greenhouse impact in terms to $CO_2$. For these reasons, clean coal technologies are becoming nowadays attractive and remarkable, aiming at a higher efficiency and a careful emission control.

In contrast, biomasses, because of to the negligible impact on greenhouse effect, are an interesting answer to the growing demand of renewable energy. Researchers have carried out characterizations of biomass pyrolysis, gasification, and combustion in order to design efficient and environmentally sustainable units.

Solid properties can significantly influence both heat transfer and reaction rates such that the optimal operating conditions are highly variable. Therefore, times, yields, and emissions depend on the original source.

The key to understanding the complex phenomena occurring inside the process units thus lies in the characterization of the initial solid and in describing the primary devolatilization phase, the released products, the gasification phase, and the secondary or successive gas phase reactions.

This renewed scientific and technological interest pushes the development of reliable models to support the design and optimization of both combustors and gasifiers.

Development of devolatilization units requires significant efforts in the mathematical modelling that is to create powerful tools for designing and simulating reactor performances, understanding pollutants evolution, analyzing process transients, and examining strategies for effective control.

Thermo-chemical conversion of biomasses in practical systems results from a strong interaction between chemical and physical processes at the levels of both the single particle and the surrounding environment.

Such a problem is multiscale and multiphase phenomenon: characteristic length of the solid fuel is of the order of few centimetres, while the entire units is of the order of some meters, while all the presence at the particle scale of solids, liquids and gases, makes the model multiphase. Mathematical models, in order to be predictive, need an appropriate description of both the chemical kinetics and the transport phenomena. This works deals with all the facet of this problem.

One of the main effort in modelling solid fuels, is the scarcity of available data.

For biomasses experiments of reference compounds (cellulose, emicellulose and lignin) makes this work easier, while for coals, whose structure is not well described or understood and varies even for a fixed coal, reliable experimental data are scarcer.

First of all, the reduction of a detailed kinetic scheme for biomasses have been addressed.

The obtained scheme is detailed enough to predict selectivity of the different products at different temperatures, but small enough to be coupled with other model as described later. Biomass characterization is considered a straightforward combination of three reference components: cellulose, hemicellulose, and lignins. Lignins, whose composition may vary because of the different degree of metoxylation of its monomers, are described using three different reference compounds in order to guarantee the closure of balances in term of carbon, hydrogen and oxygen.

Kinetic models of the different steps of solid fuel volatilization and secondary gas phase reactions were developed and validated through comparison with experimental data. Despite their simple expressions, these models allow the characterization of the degradation steps, their characteristic times, and the prediction of product distributions. These aspects make the proposed mechanisms a useful tool in the design and optimization of industrial gasifiers, where the presence of tars needs to be either enhanced or lowered.

In a similar way, a multi-step kinetic model of coal devolatilization was developed and model predictions were compared with a very large set of experimental measurements relating to thermogravimetric experiments, as well as pyroprobe, Curie point and drop-tube reactors. First step was the definition of few reference compounds, which have been chosen in order to build a model as flexible as possible. Once the elemental composition in terms of C/H/O is known, the lumped composition is strictly defined and no tuning activity on the parameters is needed.

The general agreement is satisfactory and confirms the reliability of this model, able to predict details of the released gas and tar products during the devolatilization process.

The novelty of this kinetic model lies in its predictive approach. The resulting model is small enough to be coupled with detailed fluidynamic models of combustion and gasification processes at the particle scale, retaining a general validity and giving a detailed description of the main volatile products. For the sake of completeness also preliminary models to predict nitrogen and sulphur fate during coal pyrolysis have been developed: even if they are able to predict the yield and selectivity of the main products, they are not yet completely integrated with the core pyrolysis model.

Both coals and biomasses pyrolysis models are a reasonable compromise between accuracy and detail of the obtained results and computational efforts, in terms of number of reference species and lumped reactions.

Finally, these models have been used in a mathematical model of a fixed bed gasifier: such a model gives particular emphasis to the kinetics of devolatilization and gas phase reactions.

This model constitutes a prototype towards the characterization of complex multiphase and multiscale problem, where solid fuel devolatilization together with a detailed gas phase kinetic scheme interact at the particle and the reactor scale. At the particle scale, an internal description of the solid fuel particle, in order to account for intraparticle heat and mass resistances, is achieved through a proper discretization.

This feature becomes fundamental for the analysis of the gasification of thermally thick solid fuel particles.

The reactor is considered as a series of elemental units (solid particle and surrounding gas) which exchange mass and heat to each others.

The single unit accounts for gas-solid interactions with particular attention to the inter-phase resistances, while the gases are modelled as a perfectly stirred reactor in dynamic conditions.

Some applications of the model have been extensively discussed both at the level of a single stage as well as at the scale of the gasifier unit. Future works require to validate the proposed model at the reactor scale and the main difficulty lies in the scarcity of reliable experimental data.

# REACTIONS WITH FLUORINATED “REACTIVE GASES”

## Direct Fluorination in microreactors. Maskless micromachining of microfluidic devices with F.L.I.C.E. Single step trifluoro-methoxylation of aromatics with F.T.M.

**Francesco Venturini**

The work herein presented, developed in collaboration with Solvay Solexis, is centered on “Fluorine Chemistry”.

This scientific branch of chemistry was formally born in the 1940’s with the goal of aiding the development of atomic warfare. Nowadays fluorinated molecules and processes involving them are widely used up to the point that without fluorine many of the common things we are used to would be a dream.

Amongst all the molecules that belongs to the fluorinated family we’ve focused on the trifluoro-methoxylated ($CF_3O$-) moieties since this group is, perhaps, the fluorinated substituent in circulation less studied and because it is also finding more and more uses in bioactive molecules. For the synthesis of this fluorinated molecules we’ve used one of the simplest trifluoro-methoxylating agent: perfluoro-methyl-hypofluorite ($CF_3OF$) since producing fluorinated molecules by direct fluorination, with elemental fluorine, of the homologous hydrogenated ones is not an effective method. Direct fluorination experiments are generally characterized by large heat release, formation of undefined products and often led to uncontrollable reactions.

Instead for the synthesis of the vast majority of fluorinated compounds multi-step processes, involving several intermediates, are employed.

The reactivity of $CF_3OF$ (FTM) has been widely studied especially in halogenated olefinic systems. Up to now few and scattered data regarding its use as electrophilic fluorinating agent of variously substituted aromatic compounds are present in the literature.

Also its use as a direct trifluoro-methoxylating agent of aromatic substrates has been reported once. This lack of information mainly comes from the fact that FTM is not a commercial product due to its toxicity and intrinsic instability. Also from the atomistic efficiency point of view the aromatic mono-fluorination with FTM is not convenient because only one of the four fluorine atoms of the hypofluorite is transferred onto the substrate.

In this work the reactivity of $CF_3OF$ with electron rich and electron poor aromatics has been investigated, in particular the possibility to bind the fluorine atom (via electrophilic addition) or the trifluoro-methoxy group (via radical mechanism) varying the reaction conditions has been explored.

Direct trifluoro-methoxylation via FTM addition on simple aromatic has been studied. In the standard conditions the reaction between $CF_3OF$ and our test molecules mainly resulted in the production of the mono-fluorinated moiety while the controlled introduction of a small amount of perfluoro-methyl-vinyl-ether ($CF_3OCF{=}CF_2$) resulted in a mechanism switch with the consequent production of the trifluoro-methoxylated aromatic. The direct aromatic trifluoro-methoxylation with FTM provides a unique, very fast and efficient way to obtain highly valuable trifluoro-ethers in a single reaction step.

Also the trifluoro-methoxylation using FTM is favored at low temperatures and this characteristic makes it suitable for particular reactions.

We’ve also combined the results of the trifluoro-methoxylation trials with some simple DFT simulations to formulate a prediction on the behavior of untested aromatics.

In this work the synthesis of FTM and the other precursor, carbonyl-difluoride, has been optimized in a continuous plant built in collaboration with Solvay Solexis. This plant, during the past five years, undergone several revampings up to the point that it has reached a high flexibility that allowed us to safely synthesize many fluorinated reactive intermediates.

FTM has been made by feeding a stream of fluorine and carbonyl-difluoride on a solid catalyst while the continuous synthesis of carbonyl-difluoride, by controlled fluorination of carbon monoxide, posed several problems and thus it undergone a more in depth study. Carbonyl-difluoride is produced via direct fluorination of carbon monoxide.

This reaction is recognized as gas phase, radical, fast and exothermic reaction. In the absence of proper temperature control a thermal run-away, with the consequent production of many byproducts, is possible.

Two different approaches to perform the direct fluorination of carbon monoxide are present in the literature: a “flame-less” approach based on the use of a CSTR filled with a fluorine resistant solvent and a “boiler approach” based on the use of a conventional burner coupled with a heat exchanger.

The problem of the temperature control of direct fluorinations has been addressed by developing, in collaboration with the Klavs F. Jensen group at MIT, a new microfluidic reactor.

With this device it was possible to precisely control the direct fluorination reaction avoiding unsafe conditions, product decomposition and runaways. On the lab scale the microreactors used have proven to be safe, reliable and they generally showed a better performance and controllability compared to the “boiler” and “flame-less” approaches.

During our research on microfluidic devices, conducted in collaboration with the Physics Department of Politecnico di Milano, we have found that the channels in many commercial devices are currently engraved into silicon using technologies borrowed from semiconductor processing. Glass is the preferred material for hosting microfluidic devices due to its low cost, transparency and good corrosion resistance, but nowadays it is relegated mainly as a top seal for silicon chips. The use of glass as the only substrate for microfluidic devices is relegated to small applications because, on the opposite of silicon, glass is isotropically etched and the absence of a preferential etching direction doesn’t allow to fabricate complex micro-devices inside it. It has been discovered recently that by coupling a femtosecond laser-irradiation with a etching technique it is possible to produce three dimensional micro-channels, chambers and complex structures inside transparent solid materials.

This is possible because the laser irradiated volume acquires an increased reactivity to acids such as hydrofluoridric acid. In the most implemented method, a preparation of 3D structures consists of two steps: first, a photomodification is executed by irradiating a train of pulses of a focused femtosecond laser beam into the transparent materials placed on a piezoelectric or motorized stage. Second, the irradiated material is immersed in a aqueous hydrofluoridric acid for a specified period of time.

We have identified several flaws in the etching procedures reported in literature that prevents to quickly obtain high aspect ratio micro-channels.

To overcome this limits we have studied the first implementation of the femtosecond laser assisted micro-machining (FLICE) technique using gaseous hydrogen fluoride (HF) as etchant. This procedure allowed us to address the main technical limitation of FLICE which lead to short conical channels. Using HF gas-phase etching we have realized double-side etched channels with an unprecedented length for HF etching of fused silica.

The advantage of this technique is that it does not require a “clean room” while having the potential to replace, in some applications, the photo-lithographic approach and the use of photo-sensitive glasses like FOTURAN.