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Book of Abstracts

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Invited Speakers

Quantum criticality in biomolecules

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Why life persists at the edge of chaos is a question at the very heart of evolution. Here we show that molecules taking part in biochemical processes from small molecules to proteins are critical quantum mechanically. Electronic Hamiltonians of biomolecules are tuned exactly to the critical point of the metal-insulator transition separating the Anderson localized insulator phase from the conducting disordered metal phase. Using tools from Random Matrix Theory we confirm that the energy level statistics of these biomolecules show the universal transitional distribution of the metal-insulator critical point and the wave functions are multifractals in accordance with the theory of Anderson transitions. The findings point to the existence of a universal mechanism of charge transport in living matter. The revealed bio-conductor material is neither a metal nor an insulator but a new quantum critical material which can exist only in highly evolved systems and has unique material properties.

Mathematical modeling in systems biology

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We consider the role of mathematical modeling in systems biology in the light of our experiences in cancer research and other biological disciplines in the realm of big data. We examine the methodologies of machine learning, observing the differences between the modeling approach and the black box approach. Next, we consider the role of mathematical models in natural sciences, observing three simultaneous goals: prediction, knowledge accumulation, and communication. Finally, we consider the differences of the pathway model and the attractor model in describing genetic networks, and explore the long-standing criticality hypothesis, speculating about its generality, validity, and predictive value.

Computational challenges in large-scale metagenomics

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The human microbiome includes the multitude of bacteria, archaea, viruses, and microbial Eukaryotes that populate our body outnumbering our own cells. The recent technological revolution of high-throughput DNA sequencing has enabled the study of such microbial ecosystems with a new field of research called metagenomics. However, understanding which organisms are present in the microbiome, which functions they carry out, and how they interact between themselves and with our body is a challenging problem which involves the analysis of massive datasets of short sequencing reads. In my talk I will present some of these challenges and illustrate the computational methods we are developing for improving the resolution and computational efficiency of metagenomic data analysis.

The chromatin organization of an eukaryotic genome: sequence specific + statistical=combinatorial

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Motivation: Thanks to research spanning nearly 30 years, two major models have emerged that account for nucleosome organization in chromatin: statistical and sequence specific. The first is based on elegant, easy to compute, closed-form mathematical formulas that make no assumptions of the physical and chemical properties of the underlying DNA sequence. Moreover, they need no training on the data for their computation. The latter is based on some sequence regularities but, as opposed to the statistical model, it lacks the same type of closed-form formulas that, in this case, should be based on the DNA sequence only.

Results: We contribute to close this important methodological gap between the two models by providing three very simple formulas for the sequence specific one. They are all based on well-known formulas in Computer Science and Bioinformatics, and they give different quantifications of how complex a sequence is. In view of how remarkably well they perform, it is very surprising that measures of sequence complexity have not even been considered as candidates to close the mentioned gap. We provide experimental evidence that the intrinsic level of combinatorial organization and information-theoretic content of subsequences within a genome are strongly correlated to the level of DNA encoded nucleosome organization discovered by Kaplan et al. Our results establish an important connection between the intrinsic complexity of subsequences in a genome and the intrinsic, i.e. DNA encoded, nucleosome organization of eukaryotic genomes. It is a first step towards a mathematical characterization of this latter 'encoding'. Moreover, we also identify k-mer epigenomic dictionaries, shedding light on how sequence composition influences *in vivo* nucleosome postioning.

Joint work with **Davide Corona**[1] ,**Valeria Di Benedetto**[2], **Simona Ester Rombo**[2] and **Filippo Utro**[3]

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A Strategy to Face Complexity: The Development of Chemical Artificial Intelligence

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Nowadays, science is spurred to win the Complexity Challenges. There are challenges regarding Natural Complexity. But there are also challenges regarding Computational Complexity. A strategy to face both of them consists in developing Chemical Artificial Intelligence. Its development requires an analysis of the Human Nervous System and Human Intelligence at three levels; at the (i) Computational, (ii) Algorithmic, and (iii) Implementation levels, respectively. The effectiveness of this approach is demonstrated with an example: the extension of human ability to distinguish colors from the visible to the ultraviolet region of the electromagnetic spectrum.

Hyaluronic acid in drug delivery; clear and not-so-clear facts in CD44 targeting

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The presentation will first provide an introduction to the use of hyaluronic acid (HA) in biomedicine, focusing then on nanoparticles used for the delivery of nucleic acids. Finally, the issue of targeting through the interactions between HA and its most important receptor (CD44) will be focus of the last part of the talk..

Building functional 3D human tissue in vitro: impact on well being and aging population

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In designing scaffolds for tissue engineering, the principal objective is to recreate the extracellular matrix (ECM) function in a temporally coordinated and spatially organized structure. Therefore, future generation of biomaterials with extended functionality and bioactivity requires an increased integration with cell and molecular biology, to identify novel design parameters and novel bio-inspired design approaches. Mastering the interaction between cells and extracellular environment is a fundamental prerequisite in order to engineer functional biomaterial interfaces able to instruct cells with specific commands. Such advanced biomaterials might find relevant application in prosthesis design, tissue engineering, diagnostics and stem cell biology. Because of the highly complex, dynamic, and multifaceted context, a thorough understanding of the cell-material crosstalk has not been achieved yet; however, a variety of material features including biological cues, topography, and mechanical properties have been proved to impact the strength and the nature of the cell-material interaction, eventually affecting cell fate and functions. Although the nature of these three signals may appear very different, they are equated by their participation in the same material-cytoskeleton crosstalk pathway as they regulate cell adhesion events. Attempts to guide and control biological tissues growth in vitro pursued by applying these novel concepts of material design have proved that the enhancement of control in biological signals presentation is favourable for complex tissue morphogenesis. By tightly controlling the presentation of relevant matricellular cues and by tuning their spatial distribution is possible to control cell function, differentiation, and morphogenesis. In this lecture I will present recent and relevant findings on the material-induced cell responses, with a particular emphasis on how the presentation of biochemical/biophysical signals modulates cell behavior. Emphasis will be given to the experimental assessment of the optimal signal presentation via robust and reliable models and their role in unraveling the biochemical mechanisms that underlie morphogenetic potency. Finally, a strategy based on a bottom up approach that aims at integrating this important basic information in the design and manufacturing of a complex human tissue in vitro will be presented. The approach enables the production of viable even heterotypic biological tissue in vitro to be used in tissue-on-chip applications.

Graphene in 3-dimensions

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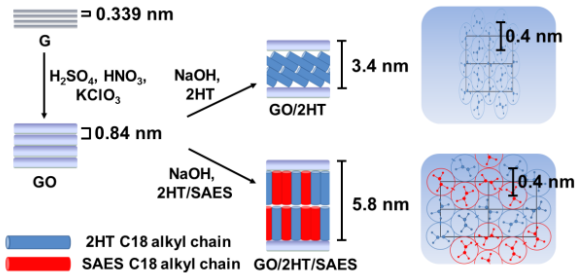
The first part of the conference will describe preparation procedures and structural characterizations of graphite oxide, chemically reduced graphite oxide, exfoliated graphite oxide (often referred as graphene oxide, GO), which are the generally used precursors for bulk production of graphene materials. The main emphasis will be given to the crystalline order along different crystal directions [1].

Preparation and characterization of Graphite Oxide Intercalation Compounds (GOIC) will be also discussed [2,3]. When small ions, mainly inorganic, are intercalated, the observed crystalline structures generally exhibit a 3D long-range order, with relatively small distance between graphene sheets (< 0.6 nm), along the stacking direction. These 3-dimensional (3D) ordered GOIC generally also present order in the organization of the guest species in the interlayer space. For the intercalation of bulky ions, mainly organic, or of polar polymers, on the contrary, the crystalline order is generally limited to the distance between the graphite layers [3], which can largely increase (also up to 5 nm). By using suitable organic cations, the formation of new GOICs exhibiting a long-range order in the organization of the guest species in the interlayer space as well as a large distance between graphene sheets, can be achieved (Scheme 1) [2]. An analogous behaviour is observed for clay intercalates [4].



Scheme 1. Progressive large increase of the interlayer spacing going from G, GO, GO/2HT to GO/2HT-SAES [2].

Graphene and graphene oxide are not only useful nanomaterials but also highly efficient catalysts of many organic reactions [5-10]. These catalysts are eco-friendly because metal-free and able to operate in solvent free conditions. The occurrence of some reactions, which are catalyzed not only by graphene oxide (being acidic and rich of functional groups) but also by unfunctionalized graphitic layers, has been described and rationalized [7]. The catalytic activity of graphene-based nanomaterials is also relevant for their applications as filler in polymer composites.

In fact, a technologically relevant catalytic activity of graphene nanofillers on the curing (crosslinking) reactions of thermoset epoxy resins has been recently described [11,12]. Catalytic activity of GO for some polymerisation reactions leading to low-molecular-mass unsaturated polyesters, being precursor of polyester thermosets, will be also described. It is worth noting that, for these applications in thermoset polymers, graphene-based nanomaterials have a *dual role*: as catalyst and as reinforcing filler.

As for polymer nanocomposites, the relevant influence of edge-carboxylation of graphite based materials on mechanical and electrical properties of polymer composites will be also described [13].

Part of the lecture will be devoted to other kinds of 3D graphene: flexible paper and aerogels based on graphene oxide and graphene. As for graphene paper, possible routes for preparation of flexible and solvent resistant films is discussed [14]. As for aerogels, the presentation will be concentrated on *polymeric aerogels* with large amount of graphene, which can be used as catalysts or masterbatches for polymer processing [15].

In the final part of the talk, some recent unpublished results relative to the activity of functionalized carbon nanofillers in thermal stabilization and crystalline phase nucleation for biodegradable polyesters will be possibly presented.

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Oral Presentations

A GPU-based library for searching relevant sets of variables in complex systems

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Motivation. The behavior of a complex system can be described by identifying emergent dynamical structures within it, i.e., subsets of variables whose members tightly interact with (depend on) one another, as well as, hierarchically, by identifying higher-level interactions that occur between such sets.

Villani et al. [1] introduced a method to identify such structures in complex systems. Based on a dataset including samples of the system's status at different times, one can compute, for each possible subset of variables, the Dynamical Cluster Index (DCI). The T_c index is a normalization of the DCI which quantifies how much a subset deviates from the behavior of a reference (homogeneous) system in which the variables have, individually, the same distribution as in the dataset, but are uncorrelated. Therefore, the higher its T_c, the higher the degree of correlation/interaction between the variables in a subset. The subsets characterized by high T_c values are referred to as Candidate Relevant Sets, the properly called Relevant Subsets (RSs) being candidates that do not include (or are not included in) other candidate sets with higher T_c values.

For a complete description of the dynamical system, the T_c index must be computed for each possible set, which becomes infeasible as the dimension of the system increases. Subsets of variables describing high-dimensional systems can therefore be identified by using a metaheuristic which smartly explores the search space. Even in this case, the number of computations of the DCI index is huge. An efficient implementation of such a function is therefore definitely necessary. Considering that the computation of T_c for each candidate RS is independent of the others, using GPU-based parallel code seems to be the most efficient way of computing such an index.

We have developed a library in CUDA C [2] which includes a set of CUDA kernels that provide a fine-grained parallel implementation of the main building blocks needed to compute the T_c index, upon which smart and efficient search algorithms can be designed.

Applications. Our library has been used to accomplish three different goals:

1. Speeding up an exhaustive sequential search by computing the T_c values of several candidate RSs in parallel.

2. Using the parallel code that computes the T_c as the objective function of a heuristic that allows one to analyze large systems for which an exhaustive search is infeasible.
3. Detecting hierarchical dependencies between RSs.

Results. We tested our library on a number of data sets which contain samples of the status of complex systems of different nature and dimensions: the status of a chemical reaction, synthetic data generated by a leader-follower model, and data about the participation of a number of representatives in meetings about the environmental policies of a region. The number of variables in the first two systems (26, 36) allowed us to perform also a full exhaustive search. The third system was considered as a whole (130 variables) or in a reduced version which include the 56 representatives who participated in more than one meeting.

With regard to the first goal, we computed the speedup that could be obtained running an exhaustive search in parallel with respect to a sequential Python implementation of the same search, used as reference. We achieved a speedup of about two orders of magnitude, which is very satisfactory, even considering that the reference sequential code was not fully optimized.

Regarding the second goal, we developed HyReSS (Hybrid Relevant Set Search), a hybrid metaheuristic which consists of a genetic algorithm followed by a number of local searches, each of which is driven by the results obtained previously, according to different criteria. Such an algorithm produces virtually the same results as an exhaustive search, up to system dimensions which make the comparison possible, in times that are at least one order of magnitude shorter. The results of the experiments on larger systems were evaluated by domain experts who confirmed their correctness.

Finally, we are obtaining encouraging results applying our analysis iteratively, either as an exhaustive search or using HyReSS, to identify groups of RSs that compose higher-level organization forms.

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New paths for the application of DCI in social sciences: theoretical issues regarding an empirical analysis

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Extended Abstract

Dynamic Cluster Index analysis (DCI) takes its origin from the neurological studies of Giulio Tononi in the 90's. Tononi supposed that neurons with similar functions show high level of coordination in their behaviors over time, independently from being, or not, situated in the same brain region[1]. Tononi introduced the notion of 'functional cluster', defining it as "a set of elements that are much more strongly interactive among themselves than with the rest of the system, whether or not the underlying anatomical connectivity is continuous" (Tononi 1997). Functional clusters should have had an internal exchange of information (among neurons belonging to the same group) stronger than the exchange of information that the same neurons have with the rest of the system. Taking advantage of two information theory concepts (integration and mutual information), Tononi introduced a new concept: the 'cluster index' (CI) (Tononi *et al*. 1994). The study of CI demonstrated that neurons with integrated profiles of activity over time (i) have similar functions and (ii) have a location that is independent from anatomical proximity.

Following this pioneering contribution, Villani *et al*. (2013a, 2013b) developed an algorithm for the detection of subsets of agents introducing the comparison between the CI of an observed subset and the CI of an homogeneous system[2]. Thanks also to the introduction of an heuristic procedure (Villani et al. 2015), the established algorithm, named by

[1] In the field of neurological activity, two theories have always been opposed: the first, a localizationist theory sustains that the brain is divided into separate areas characterized by specific functions, while the second sustains the presence of a holistic scheme of the brain activity. Neither of these formulations were compatible with the hypothesis of the presence of groups of neurons that, regardless of their position, have specific and common functions.

[2] An homogeneous subset is a subset characterized by having has the same size (in terms of number

the authors 'Dynamic Cluster Index' (DCI), is able to produce a final ranking of all possible subsets that can be considered in any initial set. Without considering any information about the topology of the network, the pattern of the behaviors of the agents is the only information that is used in the process of subset detection. DCI up to now has been tested in research areas of artificial network models, of catalytic reaction networks and of biological gene regulatory systems (Villani *et al*. 2013a, Villani *et al*. 2013b, Filisetti *et al*. 2011), giving an important and recognized contribution to the problem of identifying emergent meso-level structures (Villani *et al*. 2013a).

The creation and the implementation of the DCI algorithm opens new paths for addressing socio-economic problems regarding the analysis of group of agents. Up to now the detection and the analysis of communities typically have been performed through the consideration of similar characteristics of agents, or through the analysis of the observed network structure. Indeed, DCI methodology makes possible to shift the attention into a new dimension of organizations of agents: the presence of a common function characterizing their actions. In this paper we discuss the implications of the use of this methodology in the domain of social sciences, with specific reference to the application to an empirical analysis.

In Sec. 1 we propose an overview of the theoretical elements of the CI proposed by Tononi *et al*. (1994, 1996, 1997, 1998) and of the DCI as proposed by Villani *et al*. (2013a, 2013b, 2015). In Sec. 2 we present the specific case study, regarding network innovation policies that Tuscany Region (Italy) implemented in the programming period 2000-2006. In Sec. 3 we describe the advantages of applying DCI in a context where the application of Complex Network modeling of community detection come up against the absence of stepwise processes of formation/dissolution of relational structures. In Sec. 4 we describe theoretical considerations regarding the application of DCI in a socio-economic context of analysis. In Sec. 5 we discuss the problem of how to define agents' activity. In Sec. 6 we underline the potentiality of DCI analysis to investigate unobserved relations. Conclusions summarize the investigation of functional communities (group of agents that share a common function) in the landscape of community detection techniques, and highlight the potentialities of the application of DCI in socio-economic analyses aimed at detecting emerging functional communities.

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Management Science for Complex Networks and Smart Water Grids: a case study in Italy

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As the effects of climate change unfold and become more visible, infrastructures, especially those related to the distribution of water are the most exposed to the deep changes expected in the next years. Water is fundamental for people, and for infrastructures like energy, waste, and food production. Water sustainability is then a fundamental aspect to address by an efficient use of the resources and the maintenance of quality standards adopting a management science perspective. Therefore, water industry and infrastructure need a deep transformation, and we claim that this transformation is the result of a synergy between different fields or research. Our paper presents a managerial framework based on a complex systems used to reshape and optimize in different meanings the performance of the water infrastructure through the development of a case study in Italy. Our framework, called Acque 2.0 (Water 2.0) is based on these pillars: 1. The current and future scenarios for water management 2. Management science and water 3. Digitalisation of water infrastructure 4. Increase the network resiliency and quality of service using complex networks 5. Use of predictive maintenance methods based on network simulations and big data 6. Involve utilities, regulators, policy makers, and citizens 7. Remarks and conclusion. The case study will be developed in the municipality of Viareggio, characterised by old infrastructures, seasonal variation of population, and water scarcity

Reducing dimensionality of molecular systems: a Bayesian non-parametric approach

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In several scientific research fields, we observe an increasing number of really imposing datasets; imposing in size, for the huge number of measurements provided by technological advances; in dimensions, for the very large number of variables that investigators wish to consider in developing their research; and in complexity, for the high level of connectivity among attributes in these large dimensional data spaces. These increases in the size, dimensionality and complexity of datasets pose a challenging methodological/inferential problem. The development of new statistical tools proposed to analyze these data is therefore crucial in contemporary research development. Many proposals are available in literature with the main aim of reducing the dimensionality and selecting the most relevant variables for the problem under study [3,2]. In the research field of drug discovery, lead molecule optimization is a process that identifies new molecules with specified properties. The set of variables that affect these properties represents a high dimensional space that needs to be explored. Exploration entails experimentation that requires high investments of resources. From a statistical perspective, lead molecule optimization can be framed as the task of detecting a relevant set of variables able to predict the desired properties of molecules. In this paper, we propose a strategy to reduce the dimensionality of the experimental space and achieve the essential informative elements affecting the response of the experiments. We developed this strategy by addressing a drug discovery problem [4,1] where the experimental space consists of 2500 molecules described by 22272 binary variables (fragments) indicating presence/absence of fragments in each molecule. The proposed strategy hinges on the idea to reduce the dimensionality by grouping fragments into non-overlapping homogeneous clusters. The resulting clusters are then proposed as a new small set of variables considered in modeling the experimental responses. Clustering of fragments is performed by Bayesian non-parametric methods that provide flexible and computationally efficient tools. For our purposes we focus on the proposal by [5], which is a Bayesian non-parametric approach developed for binary high-dimensional data. The approach assumes that the distribution of clusters arises from a Dirichlet process and variables are generated by a mixture of Bernoulli distributions whose parameters follow a Beta distribution. The methodology allows the Beta distribution associated to each variable to have its own set of parameters that can

be updated from data. Furthermore, this approach, in analogy with Bayesian clustering approaches, and in contrast to routinely used clustering methods, dispenses the user from having to choose the number of clusters in advance. Preliminary results with our data led to the identification of 41 clusters. The results suggest that the proposed method can identify different conglomerates of fragments that form similar patterns in the molecule structure. The relative number of fragments present in each cluster is given in figure 1.

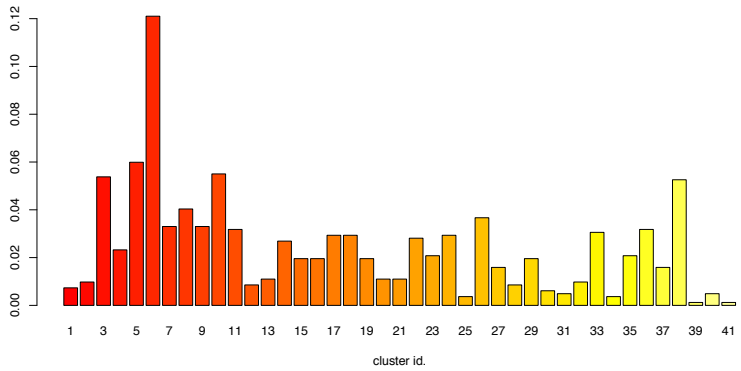

Fig. 1: Relative size of clusters

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Modeling and exploiting molecular docking: a gamification approach

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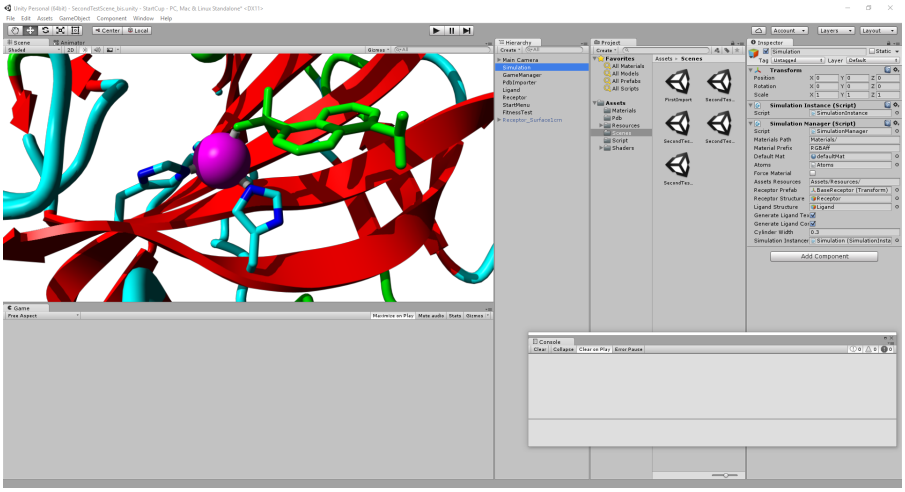
In the searching of new active compounds, molecular docking still remain the primary in silico analysis tool for two of the main problems of medicinal chemistry: looking for new molecules active on a protein target and estimating their activity.
Up to now, a universally valid procedure to perform accurate molecular docking is not available because of the low accuracy of available force fields and the dimension of energy hypersurface. For these reasons, most of the modern algorithms are based on randomized approach and on scoring functions that are an abstraction of chemical and physical rules: modern approaches try to guess where a ligand should land and its interaction energy estimated by the scoring functions.
The main goal of this project is to develop a novel approach to molecular docking through an interactive 3D game, which models a system of inter-atom forces. Nowadays, video games represent the driving factor for growth in several sectors. A video game, as mentioned by James Paul Gee [1], is an instrument to orient the player throughout a problem-solving place, providing challenging paths to master through entertainment and pleasure.
Here we present a reimplementation of the docking idea of Yada [2], a novel and more accurate tool for molecular docking, in a videogame. The main idea is to exploit the pattern finding ability of human mind to solve real molecular docking problems. Not being limited to a single gamer, the docking game will also permit collaborative search in a crowd-sourcing environment.
This game will be developed using Unity 3D [3], a cross-platform game engine. It is extensible and offers many features to simplify development of a game.
This game will be played using different control schemes, depending on the player input controller: on a desktop the player can interact with the game using first person style commands, on a mobile device the movement sensors (accelerometer, gyroscope) will be used to control the environment.
For the first time, state-of-the-art medicinal chemistry research is made available to a broad audience of experience gamers as well as newbies.



The docking game will be available on different platform such us computer desktop and mobile. Furthermore, this docking game will provide information about the ligand and receptor involved in each game level, in order to help the players to increase their knowledge about docking problem, to complete level or beat the opponents.

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Scale-free networks out of multifractal chaos.

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In the last decades, network theory has rapidly emerged as a transversal interpretative framework for understanding many complex systems and phenomena, ranging from financial crises to pandemic spreading [1]. Though this approach may appear as a drastic simplification of the specific features of a system constituents, it is able to disentangle the intrinsic topology of their interactions and to address it to a general class.

In the realm of dynamical systems, network statistical techniques have been applied to analyse nonlinear time series, with a particular focus on characterizing chaotic dynamics. The main idea of this methodology is to transform the information of a time series from the temporal domain onto the topology of a network. In this context the key point resides in the way one defines nodes and links. So far, several transformation approaches have been proposed [2] and a bench of network tools have been adapted to the analysis of nonlinear time series. However much less effort has been devoted to investigate how time series could, in turn, represent a source for growing complex network with non-trivial connectivity patterns.

Most of real-world networks are inhomogeneous, in the sense that they show scale-free properties described by a power-law degree distribution $P(k) \sim k^{\gamma}$, where k is the number of connections of a node (degree). This feature has been successfully explained through preferential attachment mechanisms [3] that, following stochastic rules, lead to the formation of a small number of highly connected nodes in spite of a broad spectrum of moderately and scarcely connected nodes. Nevertheless, it has been recently pointed out how an intrinsic aspect of this hierarchical connectivity is the presence of fractal and self-similar properties embedded in the network topology. Stimulated by the seminal paper by Song et al. [4], fractal properties of scale-free networks have been revealed and measured by applying suitable partitions of the network structure into sub-graphs or clusters with characteristic diameters (in the sense of network distance) centered on target nodes. Following this kind of a posteriori partition strategies, the possibility for multifractality in power-law networks has been also analytically demonstrated by Furuya and Yakubo [5] and attributed to the large fluctuations of local node density in scale-free structures.

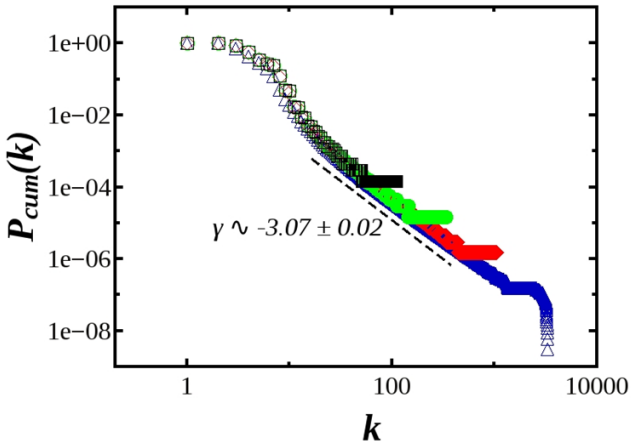

Fig. 1: Power-law cumulative degree distribution characterizing networks of different sizes arising from the Logistic map, x(n+1) = r x(n) [x(n) – 1], in the chaotic regime r=3.7.

In this context, an open question is whether (and which) deterministic multifractal processes could be considered a priori as alternative evolution mechanisms for growing scale-free networks that preserve the multifractality of the original source in the ultimate structure.

In this paper we present a model for developing scale-free networks starting from a multifractal chaotic generator of numbers [6]. We derive analytically the relation that ties the power-law connectivity of these networks to the generalized dimension of the chaotic source. Finally, we discuss this relation as a robust tool for predicting the multifractal spectrum [8] of a time series from the analysis of the network connectivity.

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Photogrammetric Meshes and 3D Points Cloud Reconstruction: A Genetic Algorithm optimization procedure

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1 Abstract

Cultural heritage safekeeping and preservation is a topic of great relevance in all those countries with long history. Time elapsing, together with both unpredictable weather events and devaluation policies of the cultural heritage value by the local governments, led to the need of preservation and requalification of such assets. This necessity unveiled many different approaches for the protection of cultural heritage, without reducing the availability of cultural sites, thus not affecting the local tourism sector. Virtual reconstruction of heritage is one of the most interesting and innovative tool for preservation and keeping of historical, architectural and artistic memory of many sites that are in danger of disappearing [1].

In order to preserve heritage for future generations it is necessary to maintain and requalify the existing assets. In the last decades, ICT technologies have constantly risen up involving different sectors; for example, if applied in the field of cultural heritage these technologies might help in requalification and improvement of cultural sites. Using the Augmented Reality (AR) technologies it is possible to improve the user experience on the site overlaying the augmented content, such as information or 3D models, directly on the real objects. In this way, making users interactive on site, the fruition of a cultural content is more interesting and engaging [2]. Furthermore, thanks to Virtual Reality (VR) it is possible to show fictional or past environments, as well as monuments or cities, as they were in past ages; moreover this technology allows the comparison between the current and the past state, highlighting the changes caused by aging. The reconstruction of the real object in VR is useful also when there are broken relics: Virtual Reality allows their visualization as not only mere reproduction, but also reconstructing the missing parts for a complete fruition of the finds.

There are several techniques for the 3D model creation starting from real objects and formerly the authors addressed the VR reproduction of a little square located in Matera

(Italy). A first approach for the 3D reconstruction of this environment was attempted using photogrammetry [3]. This technique allows the definition of position, shape and dimensions of objects extracting information coming from photographic images appropriately captured. However, another technique for 3D models reconstruction regards laser scanners [4]. Laser scanning of 3D surfaces allows capturing huge points cloud datasets that can be used in a Computer Aided Design (CAD) environment to build accurate 3D models of several meaningful objects in the reconstructed scene. The process is time consuming because after a preliminary data cleaning and registration phase, a digital representation of the original surface has to be computed through a process of surface reconstruction that generates polygonal meshes.

The study conducted showed that photogrammetry is a good technique mostly in open spaces, as it can be a square. However, we noticed that for small and detailed objects post processing takes several steps to get a reasonable result. Fig.1 shows the result of 3D model reconstruction using photogrammetry.

Fig. 1. A detail of square at Vico Giumella, Matera (Italy) in VR

Our purpose is to minimize the rendering computational cost for the photogrammetric 3D reconstructed object avoiding quality loss of the reconstruction. The quality is inversely proportional to the error, which is intended as the distance between the vertices of mesh from the laser scanner reconstructed object and the related ones from the photogrammetric object. In this way, less error implies that reconstructed object points are closer to real object points. In order to reach the goal, we decide to revisit the GA used in [4] on a points cloud obtained by photogrammetry.

In conclusion, a trade-off between quality and computational cost for rendering a virtual object is necessary for its adaption to the available presentation platform. Cultural heritage safekeeping and preservation using AR and VR techniques is strictly correlated to the choice of right presentation platform based on the application context; so the proposed optimization technique is a good approach to the absolution of this aim.

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Constraint-based Modeling and Simulation of Cell Populations

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Metabolomics aims at concurrently identifying and quantifying the full set of metabolites that are present within a given cell or tissue type at a given time, thus providing a snapshot of the cell phenotype [1–3]. Information and knowledge can be extracted from these large collections of data only by rationalizing and integrating them into computational predictive models. In this regard, constraint-based modeling has been by far the most applied technique to study metabolism and, in particular, Flux Balance Analysis (FBA), which identifies the distribution of the metabolic flux that optimizes a given metabolic objective, has extensively been applied to cancer research [4–7]. However, classic FBA is limited to the simulation of a single (or average) cell that is representative of the metabolism of the entire population this cell belongs to. This is a major drawback if we consider that a cell population is not necessarily homogeneous and that multiple sources of intratumor heterogeneity exist and may generate phenotypic differences among cells belonging to the same population, drastically reducing the efficacy of anti-cancer treatments [8–10]. Single-cell metabolomics techniques are now under development to unravel metabolic heterogeneity among cells belonging to the same tumor. However, these kind of experiments are still at an early stage and numerous technical limitations remain to be solved [11, 12]. To address the issue, we here propose an extension of constraint-based modeling approach in order to simulate metabolism of cell populations with the aim of providing a more complete characterization of these systems and investigating the relationships among their components. As a proof of concept, we tested our methodology by using a generic and non-compartmentalized toy-model of cancer metabolism (referred to as "single entity model") that has been reconstructed based on the current knowledge on the main metabolic pathways involved in cancer metabolic rewiring. This toy-model is used as building block for constructing a "population model" characterized by multiple interacting components, which

are assumed to be single cells that are representative of the metabolism of distinct subpopulations this cells belong to, all having the same topology and stoichiometry, and sharing the same nutrients supply. We therefore investigated the potentialities of the constraint-based approach in the simulation of both the single entity and the population models in order to understand if this approach is able to highlight some differences between the two models in terms of their resulting flux distributions. The two models under investigation share the same objective function (which in this case corresponds to the maximization of the overall ATP production), exchange (sink and demand) reactions and boundaries on nutrients uptake.
As first result, we obtained that FBA on individual metabolic networks well approximates the average cell of an optimal population, since the net flux distribution of the different cells perfectly mirrors the flux distribution obtained as a solution of the single FBA model. Then, by shifting the focus toward a more in-depth study of how the flux distribution identified in the single entity model distributes among multiple cells within the population model, we observed that cancer cells cooperate with each other to reach the optimal value of the objective function, but without necessarily having the same metabolic traits. In fact, the flux distribution analysis showed that two identified subpopulations do not contribute in an independent manner to the achievement of the common goal, but they interact with each other showing a very different ATP production rate and and a difference in their energy generating pathways.
Through our approach, we observed that the entire cancer population can be represented, at a first level, through a single entity model which provides a snapshot of the average behavior of the cell population, and at a second level, through a network of metabolic networks, each of them representing the individual subpopulations. The advantage of performing FBA simulations on a population model compared to that on single entity model is the possibility of investigating the tumor population on several levels of detail, elucidating the ways in which the average behavior of the system can be achieved, how the individual cells may differ in their metabolism, how different subpopulations of cells having different phenotypes, but coexisting within the same system, may interact with each other to attain the common goal, and the possibility of better understanding the heterogeneity degree within a cancer population. Overall, our methodology allowed us to explore another level of complexity owned by cancer disease: the heterogeneity emerging from the population model emerges from the mismatch between the objective of the entire population and the objectives of its individual entities.

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Improving Biological Data Analysis Capabilities by Exploiting Distributed Computing

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In the last 30 years the *Information Technology* has driven the development of devices and sensors able to acquire large amounts of data from different operating contexts. One relevant application scenario is the Bioinformatics. Here, *Next-Generation DNA Sequencing* (NGS) machines generate a huge amount of genomic data at a very fast rate and at a relatively low cost [7,8], placing unprecedented demands on traditional single-processor read mapping [19]. Consequently, bioinformatics researchers have to deal with new issues like: how to store and how to process a huge amount of data that is produced at a very fast rate [16]. This phenomenon has led to the spreading of Big Data in the computational biology field [15]. For instance, many biomedical researchers changed their approach considering that the treatment of many diseases has become increasingly "Big Data-driven".

Efficient and timely processing of Big Data requires more and more the resorting to *distributed systems*. In computer science, this term refers to a collection of independent computers (also called *nodes*) that communicate over a network (see [21] for details). Although some bioinformatics projects already exploit distributed systems to get results faster (see, e.g., [3,6,12,14,17,20]), as outlined in [2,11,22] there are still many bioinformatics applications designed to run only on a single machine, as parallel applications executed on multi-thread shared-memory systems (e.g., [1,4,5,9,10,13,18,23]) or as sequential applications (i.e., single-thread), independently of the size of the problems to be solved. These applications may take too long to be executed on a single machine when used to process very large datasets. This may happen independently of the number of computing cores available with that machine. As a matter of fact, due to a number of architectural bottlenecks mainly related to the need of multiple cores to share the same memory bus, multi-core shared-memory architectures do not allow to efficiently use more than a rather limited number of cores. For this reason the performance of a parallel algorithm developed for these architectures may not scale well with the number of cores. In most of these cases, the same

datasets could be processed in a reasonable running time using a properly-sized distributed system.

The advantage of this approach is that if the problem under investigation allows for the division of the input in a number of independent parts, one can add more nodes to a distributed system without compromising its scalability (i.e., *scale out* or scale horizontally). This is possible since each computer (node) can access local resources without incurring in racing conditions with other nodes. This can radically improves the architecture scalability supporting large number of nodes, and, therefore, reducing the execution time of an application. If whenever more computing units are added the execution times are correspondingly reduced, then the application is considered *scalable*.

On the other side, the adoption of a distributed approach often requires more specific and complex skills to design and develop a solution to a given problem, while mapping this against the target distributed architecture. To make it easier the transition toward distributed systems, many new distributed architectures and paradigms have been proposed in the field of *High Performance Computing* (HPC) in the recent years. Among these, the MapReduce paradigm is becoming a standard *de facto* for Big Data computing.

Here we present a set of guidelines to help the bioinformatics researchers to fully exploit the computational power of a distributed system to process virtually unbounded input datasets. Starting from the specific problem characteristics (input size, complexity, etc.), we provide a set of criteria that could be applied in order to develop the most efficient solution for the underlying hardware resources. With this goal in mind, we show the state of the art of the contributions existing in bioinformatics area that are based on a distributed approach. In particular, we focus on the MapReduce paradigm and its most popular implementation, i.e., Apache Hadoop, specifically designed to process Big Data. We also investigate the advantages of *Explode Point* approach in some bioinformatics problems like Virtual Screening, Folding, Molecular Dynamics and Docking. In addition, we present two other emerging frameworks, namely Apache Spark and Apache Storm, that offer two more flexible computational models. We also show some application scenarios that seem to be well-suited for these frameworks. Our contribution has a dual purpose: on the one hand, to promote among bioinformatics researchers the need for fast, efficient and accurate analysis tools and, on the other hand, to show how to develop distributed and scalable solutions running on a virtually unbounded number of computing units and, therefore, able to process huge input data in a limited time amount.

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Automatic design of boolean networks for cell differentiation

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Cell differentiation is the process whereby a cell undergoes a *cell type* change, typically from less specialised to more specialised types. This process is at the roots of several crucial biological phenomena, such as morphogenesis, and models of cell differentiation may also help understand the dynamics of severe diseases, like e.g. cancer. Cell differentiation processes are characterised by highly complex dynamics, being the result of the interactions among genes and possibly other molecular agents. Recently, a cell differentiation model based on boolean networks [1,2] subject to noise has been proposed [3,4]. This model reproduces the main abstract properties of cell differentiation, which are:

- attainment of different degrees of differentiation;
- differentiation may take place both deterministically (i.e. as a response to a specific chemical signal) and stochastically;
- (limited) reversibility;
- induced pluripotency and cell type change.

In a nutshell, the model considers the asymptotic behaviours of a boolean network (BN) as cell types; BNs are subject to noise, i.e. their update may be perturbed by node flips: the higher this noise, the higher the probability to move across BN attractors. High level of noise corresponds to pluripotent cell states, where the BN trajectory can wander freely among the attractors; conversely, low level of noise induces low probabilities to jump between attractors, thus representing the case of specialised cells. A fundamental role in the model is played by *threshold ergodic sets* (TES_θ) which are sets of attractors in which the dynamics of the network remains trapped, under the hypothesis that attractor transitions with probability less than threshold θ are not feasible. The transitions between attractors and their probabilities are summarised in the *attractor transition matrix* (ATM).

The generic abstract properties of the model have been already shown to match those of the real biological phenomenon. A direct comparison with specific cell differentiation processes would require to design a BN (i.e. topology and node transition functions) such that its dynamics gives origin to a differentiation tree matching the properties of the real case at hand. The BN differentiation tree is

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characterised by the attractor set of the BN and the transitions between them, as well as their probabilities. Not surprisingly, attaining such a complex dynamics by designing a BN by hand is not possible and an approach based on brute force is definitely impractical; indeed, the number of N nodes networks with exactly k inputs per node is $(2^{2^k})^N$. Notably, each candidate solution, i.e. a BN, is evaluated by computing its ATM, which is a highly demanding computational operation. Therefore, an automatic design method able to efficiently explore the search space is required. To the best of our knowledge, the only current method for attempting to attack this problem is a random generate and test procedure [5], which draws BNs at random until either an acceptable solution is found or the time limit is reached.

In this work we present an automatic design method for this purpose, based on metaheuristic algorithms [6]. This approach maps the BN design into an optimisation problem, where functions and topology of the BN are considered as decision variables and a measure of the matching between the BN differentiation tree generated by its ATM and a target differentiation tree is used as objective function. The objective function we defined for our algorithms is a combination of two tree distance measures: the *edit distance*, E, and the *histogram distance*, H. E is computed as the minimum cost sequence of node edit operations (node deletion, node insertion, node rename) that transforms one tree into the other, while H compares two trees in terms of the number of nodes with $k \in \{1, 2, 3, \ldots\}$ children for each level. Several combinations of the two distances have been tested; the one leading to the best results is $F = E + (E \times H)$, which was used for the final experiments.

We devised two variants of the method, each based on a different metaheuristic algorithm; a simpler one, namely M_{aw}, based on *adaptive walk*, designed mainly for test purposes and a more advanced one, M_{vns}, implemented with *variable neighbourhood search*, which is capable of efficiently exploring the search space and escaping from local minima. It is important to stress that a BN whose ATM can be used to obtain a given target differentiation tree just represents one possible model for the real system to be matched. For this reason, randomised techniques are of great help as they make it possible to explore different solutions and provide an ensemble of hypothesis. To this aim, metaheuristic methods are indeed particularly effective.

Experiments were run on 10 nodes BNs with $k = 2$ and with $k = 3$. The topology was set randomly and kept constant during the search process, which instead could change the boolean functions by modifying node function truth table entries. As experimentally verified in previous work [7], a fixed topology is typically not a limitation for the dynamical properties of BNs. The differentiation trees chosen as targets for the design process were defined on the basis of common differentiation tree features, such as the hematopoietic lineage. Typical tree structures are depicted in Figure 1. The threshold values at which the TESs where split have been chosen so as to be evenly distributed in the interval $[0, 1]$.

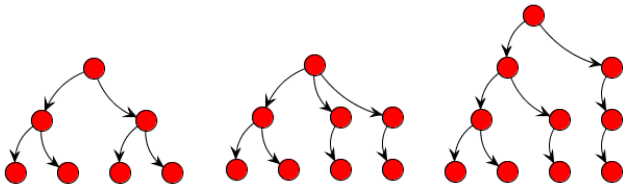

Fig. 1. Examples of differentiation tree structures used as target for the search process.

Results, although preliminary, show that the technique is far more efficient than both random search and complete enumeration and it is able to find solutions to instances which were not solved by those techniques.

Currently, we are improving the algorithms so as to design BNs with a greater number of nodes and be able to find suitable BNs matching real biological data.

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Synchronization in Near-membrane Reaction Models of protocells

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Extended abstract

The term "protocell" is used in a loose sense to denote a class of (still hypothetical) entities that are similar to, but much simpler than present-day cells. They should be able to grow and to divide, giving birth to offsprings that are similar but not necessarily identical to their parents. Different individuals should reproduce at possibly different rates, thereby allowing selection to occur. The protocells discussed here should not be confused with the "minimal cells" that have been synthesized by simplifying the genome of existing microorganisms, since they should be built starting from non-living components. While the relevance of this research for the problem of the origin of life is apparent, in this paper we will be concerned with the behaviour of possibly synthetic protocells, making no reference to plausible scenarios for abiogenesis.

While a number of alternative protocell architectures have been proposed, most of them are based on a lipid container (e.g. a vesicle), with an internal aqueous phase and a membrane formed by a double layer of amphiphilic molecules. Moreover, it is assumed that there is a set of collectively self-replicating molecules, that can be referred to as "replicators" or "genetic molecules". Let us call for brevity "key reactions" those that are involved either in the growth of the container or in the duplication of the genetic material [Villani et al. 2016]. In general, one can distinguish models where the key reactions take place inside the membrane (*Surface Reaction Models* or SRMs) [Serra et al. 2007] from those models where they take place in the in the homogeneous internal aqueous phase (*Internal Reaction Models*, shortly IRMs) [Filisetti et al. 2010]. Simplifying assumptions about the concentration profiles inside the aqueous phase are often considered, the simplest one being that of homogeneous concentrations.

However, there is a problem with IRMs that is often overlooked: suppose indeed that the vesicle is large enough so that composition fluctuations are small in a volume of the same size as that of the internal phase. If the protocells are generated by some spontaneous process taking place in a homogeneous environment, then the internal composition of all the protocells will be very similar to each other, and to the external environment. So

essentially the same reactions take place in each protocell, and in the environment – and there is really no need to have a closed compartment. Since all life forms are based upon cells, these must instead be very important, probably from the very beginnings of life. A possible solution to this problem is that of assuming that the initial protocells were so small that the fluctuations were large in their very small volumes, so their compositions are different and selection can take place [Serra et al. 2014].

However, there is an alternative possibility, which would hold also in the case where the initial vesicles were quite large: it is possible that the key reactions take place inside the vesicle, but only very close to its membrane, which is supposed to provide direct catalytic activity or to give rise to a local environment that favours those reactions. We will refer to these architectures as *Near-Membrane Reaction Models*, shortly NMRMs (see fig. 1).

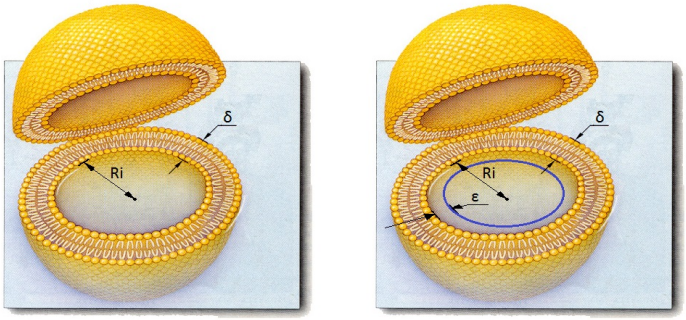

Figure 1: schematic representation of an IRM (left) and of a NMRM (right)

While they resemble IRMs, where the internal phase is supposed to be homogeneous, the main difference is that in this case the production of new self-replicators takes place in a narrow spherical shell close to the membrane. One might argue that the same catalytic activity takes place on the outer side of the membrane, but if the external volume is much larger than the internal one the reactions products will be quickly diluted on the outside – therefore the internal composition will be different form the external one.

In this paper we will introduce a (strongly simplified) abstract Near-membrane Reaction Model and we will then address the important problem of synchronization.

In a series of papers, we [Serra et al. 2007] [Carletti et al. 2008] [Filisetti et al. 2008] [Filisetti et al. 2010] [Villani et al. 2014] and others [Munteanu et al. 2007] have drawn attention on the importance of synchronization between the replication rates of the "container" and of its genetic material, that is obviously a necessary condition for sustained growth of a protocell population. Using fairly abstract models, it had been possible to prove that, under a wide set of hypotheses, such synchronization spontaneously emerges,

generation after generation both in Internal Reaction Models and in Surface Reaction Models, under a broad set of different hypotheses.

In the case of NMRMs, the replication of the genetic molecules takes place only in a fraction of the internal volume, so the self-replicators can then undergo dilution during the growth of the protocell - and this might affect synchronization. However, we show here that synchronization is achieved also in this case under a broad range of assumptions concerning the type of equations and the sets of parameter values that describe: i) the interactions among the replicators and ii) the interactions of some replicators with the lipid container.

We will then compare the behaviour of IRMs and NMRMs using simplified deterministic dynamical models, and assuming that the concentrations of the self-replicators are the same in every point of the internal aqueous phase for IRMs and NMRMs, while only a part of the self-replicators in the NMRM case participate to the reaction processes (that is, the part in the spherical shell close to the membrane). Diffusion in the internal phase is supposed to be instantaneous, while transmembrane diffusion of the precursors of the genetic molecules and of the amphiphiles can be either instantaneous or ruled by a finite diffusion coefficient. In the first case, some results have been obtained using analytical methods while in the second case all the results are based upon simulations.

It turns out that the behaviour of the Near-membrane Reaction Models are qualitatively similar to those of the IRMs, although there are some values of the kinetic parameters that lead to extinction (i.e. extreme dilution) in the former case but not in the second. In general, with instantaneous diffusion, one observes coexistence of molecular species that have different replication rates when the kinetics of the self-replicators is sublinear, while in the linear case the fastest one (i.e. the "fittest") prevails. Note that while the replicator equations may be linear, the whole model is definitely nonlinear. If the replicators kinetics are superlinear, one observes that the molecular types with a higher initial concentration have an edge with respect to the other species (the “survival of the first”).

Note also that the above remarks refer essentially to molecular species that self-replicate individually. When replication involves cooperation between two or more species, they together determine the overall rate of replication - they can survive and synchronize (see fig. 2)

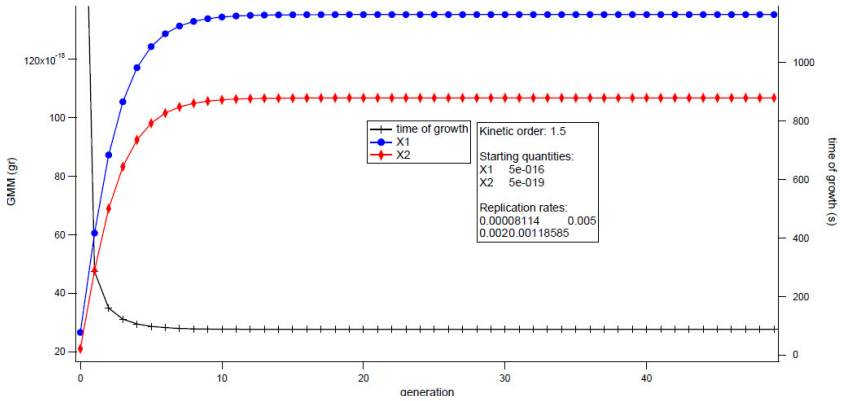

Figure 2: quantities of two self-replicators that mutually catalyse each other's formation, plotted vs. time. In black, a third species that is not self reproducing: its concentration drops generation after generation

The presence of several different sets of self-replicating polymers, with different rates, is a desirable feature of protocells, as it might allow them to become more complex and to perform a wider set of functions. It is shown in the paper that the coexistence of different sets of this type, even when the kinetics are linear or superlinear, can be achieved in both IRM and NMRM organisations (the IMS organisation being the more flexible one) when the diffusion rate across the membrane is finite – provided that the replication rates of the genetic molecules are not too different from each other.

Moreover, a limited analysis is performed concerning the evolvability of the protocell, by assuming that a new molecular species enters the scene when the system has already reached a steady state, and by defining "evolvable" a case when the newcomers can survive together with the pre-existing molecules. It is shown that the introduction of a finite diffusion rate allows the protocell population to evolve, in the sense defined above, also when the replication kinetics is linear or superlinear.

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Model-based lead molecule design

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In the research field of drug discovery, a key problem is the design of functional molecules that affect proteins associated with diseases. This issue is usually referred to as drug design, and the use of computer-aided methods can help in the difficult process of the discovery and the optimization of the lead molecules, by eliminating compounds with undesirable properties (poor activity and/or poor Absorption, Distribution, Metabolism, Excretion and Toxicity, ADMET) and selecting the most promising candidate solutions. Several approaches have been proposed over the last few years mostly based upon computational search and stochastic optimization methods: in particular, nature-inspired algorithms such as genetic algorithms, artificial neural networks or evolutionary programming have been quite successful in this regard [4,3]. Addressing this problem with an evolutionary approach, we recently developed an experimental design where the evolution is driven by predictive statistical models, [1,2,5]. This model-based evolutionary approach leads to a drug design that is sequential, adaptive and self-organizing. The approach, named EDO (Evolutionary Design for Optimization), selects an initial very small set of candidate compounds, tests them and achieves a first set of experimental responses. These data are then used to estimate predictive statistical models that yield information on the most promising candidates or hypotheses that suggest new experiment tests. The process continues in a sequential way estimating different models at each generation. The model-based design is evolutionary and adaptive, since it can be constructed using different classes of models in each generation depending on the data resulting from experimentation. This approach has shown very good performance in reaching the target of the system in an effective and efficient way, but of course the choice of the particular class of statistical models embedded in the procedure can affect the evolutionary process. In the work reported here we propose a new drug design by deriving a model-based evolutionary procedure that takes into account the information achieved by several competitive statistical models and combines the relevant information extracted by these models, without an a-priori choice on the model. At each generation of experiments, the proposed approach, named Combined Model-based Evolutionary Design for Optimization [6], derives the set of experimental points by evaluating the predictive results of different statistical models estimated and evaluated for the same set of data (under the assumption that different data generating processes can have originated the data). More specifically, each model predicts the responses of the whole experimental space,

i.e. all the possible candidate solutions, and these solutions are ranked according to their estimated responses. The procedure then selects the subset of candidate experimental points with the best predicted response values from each of the statistical models. This new combined set of experimental points composed of the solutions achieved from different models becomes the next population of the evolutionary procedure, and it is evaluated in the laboratory, producing the new set of response data. The process is iteratively repeated, generation after generation, maintaining the same size in each population of experimental points and ends when the optimum value is achieved, or the maximum total number of experimental points is reached. This design does not require an a-priori choice of the statistical model directing the evolutionary path, and it provides more reliable and robust solutions not depending on the assumptions or hypotheses of a specific model. The design can in fact be constructed using a wide class of statistical models ranging from linear to non-linear and complex structures. To show the efficiency and the effectiveness of the procedure, we evaluate this new design on the lead optimization problem of MMP-12 inhibitors developed by Pickett et al [4]. The data consist of a library of 2500 molecules, identified by 175 fragments representing the presence/absence of particular chemical compositions, and the corresponding experimental response, namely the activity value of each molecule. The results are analyzed and compared both to genetic algorithm optimization and to model-based evolutionary procedures with the a-priori choice of the single model used across the procedure. The very good performance of the approach is shown by its capacity to uncover the optimum value using a very limited set of experimental points with respect to the alternative methods, avoiding unnecessary experimentation.

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Linking alterations in metabolic fluxes with shifts in metabolite levels by means of kinetic modeling

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Metabolic profiling provides a readout of the biochemistry and physiological status of an individual or population, resulting from genetic factors and environmental exposure, that can be exploited in personalized medicine and public healthcare [1]. Analyzing the full complement of metabolites in body fluids such as urine and plasma using various spectroscopic methods allows indeed to link human metabolic variations (biomarkers) to disease risk factors. For instance, metabolite profiling has identified a key role for glycine in rapid cancer cell proliferation [2].

A major limit to the informative power of newly discovered metabolic biomarkers is posed by the impossibility to ascribe variations in metabolite concentrations to modifications in either their production or consumption pathways. Knowledge about the deregulation of the involved pathways, which might be effectively targeted for treatment of the disease, requires information about alterations in the metabolic fluxes (i.e. the rate at which a substance is transformed into another through a given reaction or pathway). Although isotopic labeling and metabolic flux analysis allow to indirectly derive such information through ad hoc laborious experiments, there is a quest for systematic high-throughput techniques.

Accordingly, metabolic network modeling is increasingly being exploited as a way to understand shifts in metabolism at a genome-wide level. As knowledge of kinetic parameter on a large scale is currently impracticable, constraint-based modeling is by far preferred to dynamic modeling [3]. The former models exploit a reasonable steady state assumption for internal metabolites and deal with fluxes only, while disregarding quantification of metabolites. Hence, the simulation outcomes can be difficulty compared against the growingly rich availability of metabolomics data, with metabolic models mainly lacking validation, pushing forward the urge for a strategy capable of linking variations in concentrations with variations in fluxes, and viceversa.

We propose to seek for recurrent patterns or "rules" of association between the changes in fluxes and metabolites observed in the steady states possibly ob-

tained when simulating the response to a given perturbation (e.g. change in a metabolite level) of a fixed metabolic network stoichiometry for a large ensemble of randomly generated kinetic parameters. As a proof of concept, we computed the difference in the fluxes (δv_i, for each flux v_i), and metabolites (δx_j, for each metabolite x_j), observed when increasing the concentration of extracellular glucose in a core model of yeast metabolism [4], for 10.000 steady states obtained filtering out trajectories simulated by means of ODEs whose kinetic constants are randomly generated according to our Monte Carlo approach. We then computed, as a first step, a Pearson correlation coefficient between δv_i and δx_j for any pair i, j, which are reported in the HeatMap in Figure 1.
Notably, several pairs show a significative correlation, suggesting that, at steady state, alterations in metabolites are somehow constrained by alterations in fluxes, or viceversa. Intriguingly, covariations may be not obvious at all: for istance the flux from Pyruvate and OAA to AccoA shows a stronger positive correlation with a metabolite that is further in the network (isocitrate) than with the reaction product; whereas, the flux from SuCoA to Succ surprisingly shows a negative correlation with the reaction product. These results on the one hand emphasize the inefficacy of naive predictions of fluxes variations from metabolites alterations and viceversa, on the other hand they support the hypothesis that strong complex relationships exist which, if disentangled, may make such attempt less daring.

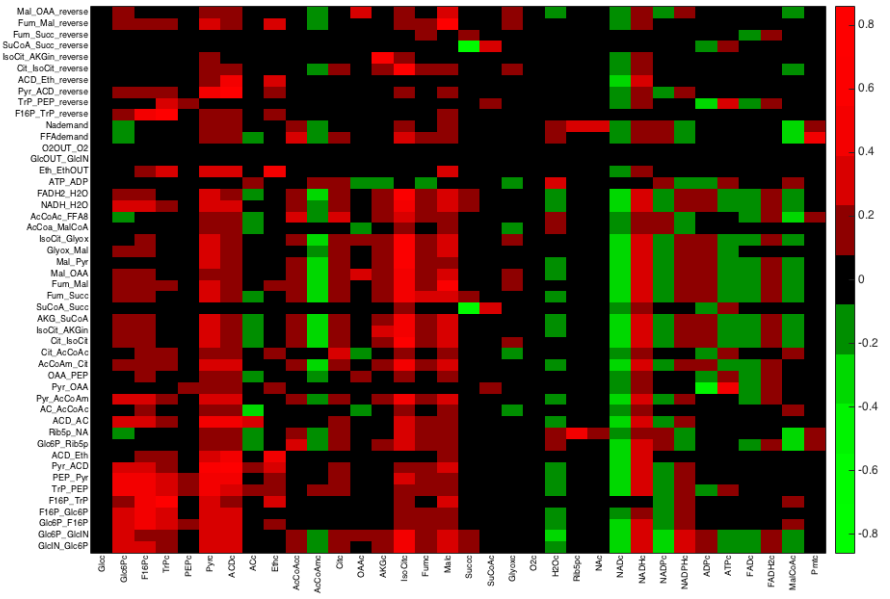

Fig. 1. Rows: fluxes; columns: metabolites; color: correlation coefficient.

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Multi-objective Stochastic Local Search for the Optimal Web Services Composition

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This paper deals with the problem of web service composition where the aim is to find the combination of services executing a set of given tasks and offering the best quality of services. The web services composition is one of the most important difficulties in the development of services-oriented architectures. It is an active research area and development endeavors for application integration and interoperation.

The problem of optimizing the composition of Web services can be considered as an NP-hard problem. It is to be considered in the optimization several QoS attributes when composing simple elementary services for a complex service. Thus, we will model this problem as a multi-objective optimization problem. the objective functions are said to be conflicting, there exists a number of Pareto optimal solutions. A solution is called non-dominated or Pareto optimal.
Several studies have focused on the web services composition: [1], [2], [3]...etc. Claro et al. [1] discussed the advantages when using Multi-Objective Genetic Algorithms (MOGA) in Web service selection and a popular multi-objective algorithm NSGA-II. Moustapha et al. [3] proposed a multiple QoS objectives based approach with multi-constraints by basing on Reinforcement Learning algorithm (MORL). In [2], authors propose a dynamic selection of Web services, a decision needs to be made on which services should be selected such that the user's end-to-end QoS requirements are satisfied. The web service composition is a challenge that needs further efforts to achieve a highest level of optimization.

In our work we search for an execution plan that indicates for each task the assigned service. We propose a quality of service (QoS) based model that includes non-functional properties and uses a multi-objective stochastic local search based method (SLS) to find a good combination of services. We deal with four objective functions of QoS model which are given as :
The first one is the cost minimization given as: $Min \sum_{i=1}^{n} \sum_{i=1}^{m} c_{ij} p_{ij} x_{ij}$
The second objective is the time minimization given as :$Min \sum_{i=1}^{n} \sum_{i=1}^{m} t_{ij} p_{ij} x_{ij}$
The third objective is the availability to be maximized:$Max \sum_{i=1}^{n} \sum_{i=1}^{m} a_{ij} p_{ij} x_{ij}$
The fourth objective is the value of the composite service to be maximized: $Max \sum_{i=1}^{n} \sum_{i=1}^{m} r_{ij} p_{ij} x_{ij}$

The proposed method SLS returns a set of nondominated solutions, these are optimal distinct solutions. The SLS approach includes three principal steps:

1. The generation of the initial solution: in this step, we create a vector that represents the initial solution. Based on this solution, we will create a collection of initial candidate solutions.
2. Neighborhood search and evaluation: in each iteration, we generate the neighborhood of the current solution that will be the next collection of candidate solutions. We select the best solution of this collection. Then, we will replace the current solution by the best one solution found in the current iteration if it is better than the current one.
3. Selection of the best solutions : after the execution of last iteration, the algorithm us give the optimal solution found, its non-dominate solution.

The proposed approach SLS is evaluated on two datasets, the first dataset generated randomly and on the second dataset is cited in [4] [5], the objective is to select the best fit services in terms of maximum or minimum aggregated end-to-end QoS parameters. The numerical results are encouraging and demonstrate the benefit of the proposed approach.

Using the first dataset generated randomly, in Figure 1, we show the evolution of our model based on the number of distinct Pareto optimal solutions found for four instances.

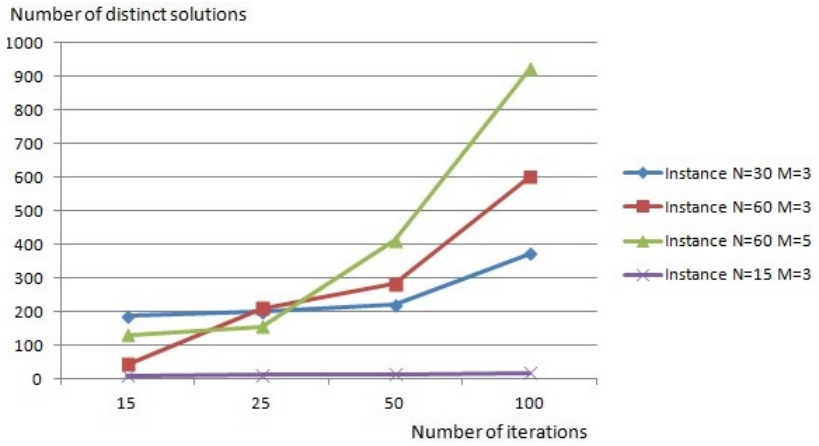

Fig. 1. Non-dominated set solutions - Instances

We use 15 services for 3 tasks. We can see in Figure 1 that 12 solutions are found in the 15^{th} iterations in 140 milliseconds. We see also that 18 solutions are found in the 100^{th} iterations in 490 milliseconds.

We can see it the results by using the second dataset in Figure 2.
In Figure 2, we show the results of 4 instances of the model evolution based on the number of distinct Pareto optimal solutions.

For example, in the instance of N=30 and M=3 (30 services and 3 tasks), on 15 iterations we have found 195 distinct solutions in 2046 milliseconds. The tradeoff solutions respect all the considered constraints. The proposed method gives for 100 iteration 375 distinct non-dominated solutions in approximately 3 seconds.

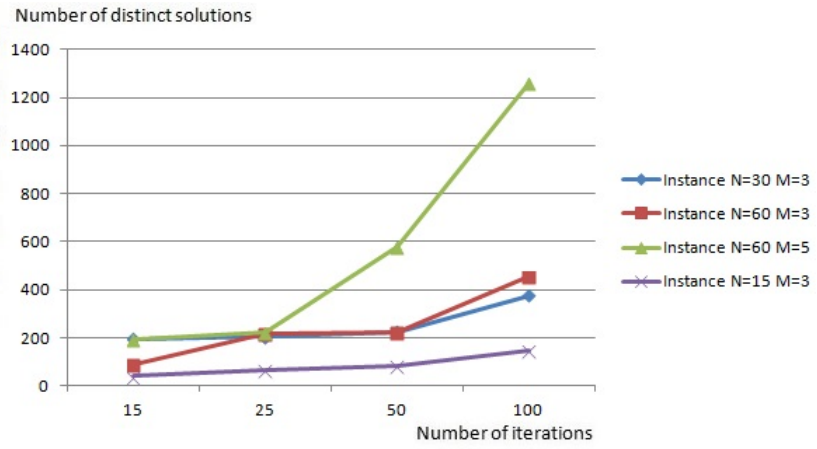

Fig. 2. Non-dominated set solutions by instance

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Collective Behaviour of Enzyme-loaded microbeads

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Individual components of a biological system expressing simple behaviours often show coordinated activity through chemical communication. Here we study the emergence of group behaviour in clusters of enzyme-loaded microbeads represented as hexagonally-close-packed cells coupled via diffusion (Fig 1a).

The catalytic hydrolysis of urea by the enzyme urease produces ammonia and carbon dioxide. Due to the basic character of ammonia the pH typically increases during the course of reaction in non-buffered conditions. Moreover, as a result of the bell- shaped pHrate dependency of enzymatic processes, base-driven positive feedback may arise in the ureaurease reaction. Produced by many plants, funghi and micro- organisms, urease is a virulence factor for bacteria including Helicobacter pylori which forms biofilms in a wide range of living and man-made environments.

In our two-variable, cellular particle model simplified from an 8variable model previously used for studying bistability [1] and front propagation [2] in the urea urease system, urea and acid diffuses from the surrounding solution with fixed substrate and acid concentrations into the microbead clusters. Cells in an unreacted, low pH state switch first to excitable, then oscillatory and eventually to high pH states as the group size is increased (Fig 1e). Excitatory and oscillatory pH spikes typically initiate at the middle of the clusters and rapidly expand outward, followed by an initially slow but steadily accelerating collapse of the high pH phase (Fig 1b-c). In some cases, near the high pH boundary, the collapse is not complete and the centre of the cluster remains in the high pH state before spreading out again (Fig 1d). Furthermore, mixed oscillatory-pulsating behaviour was also observed, where consecutive high pH phases shrink and collapse alternatingly.

Bistability in millimetre-sized single beads has been previously demonstrated experimentally [3]. Oscillations, however, despite predicted by models [4], are still yet to be seen. A promising attempt towards their experimental realization, involving first the successful encapsulation of urease in vesicles [5], is currently underway. Understanding these transitions may provide useful information about the emergence of collective behaviour within bacterial colonies, such as quorum sensing in ureolytic biofilms, as well as applications in biotechnology.

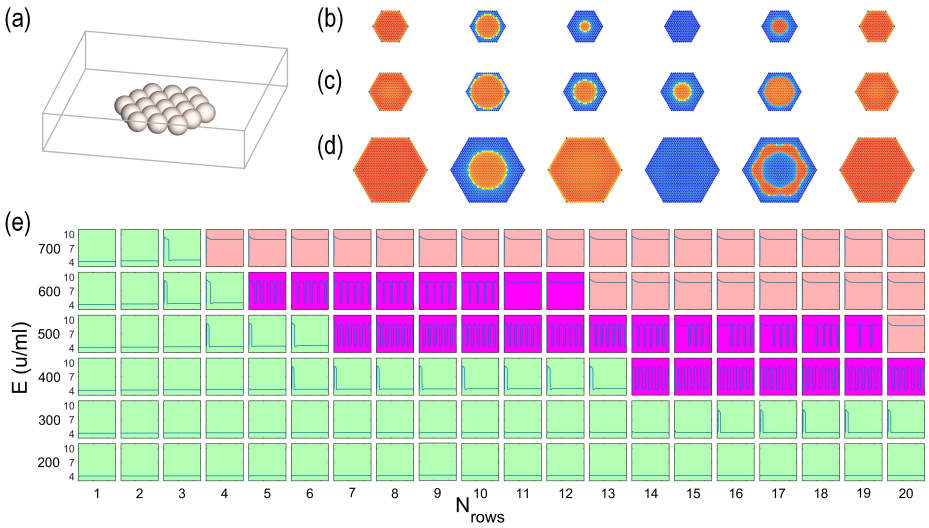

Fig. 1. (a) Close-packed cluster of microbeads on a surface surrounded by acidic substrate solution. Spatial dynamics of microbead arrays: excitability (b), oscillations (c), pulsating (d); High pH regions (orange) expand out from the centre into the low pH domain (blue); The black area represents the solution surrounding the cluster. (e) Dynamic behaviour of arrays of microbeads as a function of enzyme concentration. pH traces taken at the middle cell (blue curves). $[S]_0 = 0.3$ mM, $pH_0 = 4$. In a few cases (Number of rows: 11,12;19; E: 600; 500) beads at the centre display small amplitude oscillations only in every second or all cycles.

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Ich bin ein phototropes Bakterium

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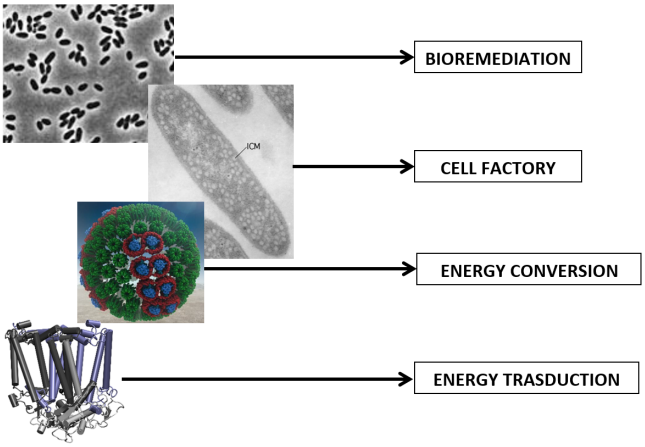
If aliens from outside the planet visit the Earth, they would probably miss the ecologic niche where phototrophic bacteria live and prosper, and would probably take off without bringing with them a sample of these fantastic microorganisms.

It would be a real pity. Phototrophic bacteria are amongst the oldest inhabitants of the planet we live in, and represent a model that humankind has learned to appreciate in several useful fields, from environmental applications to artificial photosynthesis to cell factory.

A short journey through the live of these phototrophic bacteria will be presented, focussing on their on-going or potential applications deriving of the exploitation of their metabolic and enzymatic activities. In other words, taking advantages of processes of different scales of complexity that these microorganism are able to perform depending on the size and degree of completeness of the bacterial structures involved.



Examples of bacterial structures from the purple photosynthetic bacterium *Rhodobacter sphaeroides*

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Giant Lipid Vesicles designed for Light Energy Transduction

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The photosynthetic reaction center (RC) is a transmembrane pigment-protein complex that plays a major role in the photochemical conversion of light into chemical energy in plants, algae and photosynthetic bacteria[1, 2]. It couples light-induced electron transfer to the generation of a proton concentration gradient across a lipid membrane, via reactions involving a quinone molecule that binds two electrons and two protons at its active site. The so-obtained electrochemical gradient can be harnessed to synthesize ATP[2].

In a previous work[3] it has been shown that the reconstitution of functional, but randomly oriented, RC is possible in conventional (diameter 50-100 nm) lipid vesicles typically obtained by the detergent depletion method[4]. In this contribution, we show that following a bottom-up approach and by using the droplet transfer method for giant lipid vesicles (1-100 μm) preparation, synthetic protocells, embedding uniformly and physiologically oriented reaction centers, are capable of generating a photo-induced proton gradient across the membrane.

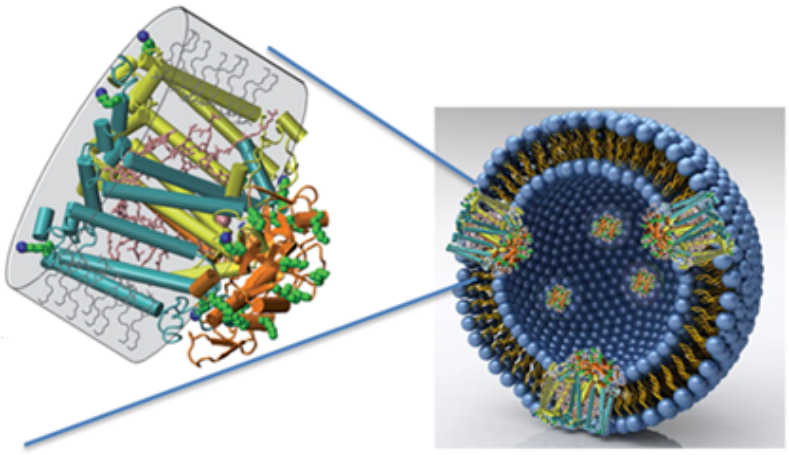

Fig. 1. Physiologically oriented photosynthetic RCs within the GV membrane.

Under constant illumination, protocells generate 0.061 ± 0.004 pH gradient units in one minute, contributing to a proton motive force of 3.4 mV per minute determined by following the fluorescence increase of pyranine encapsulated inside giant vesicles.

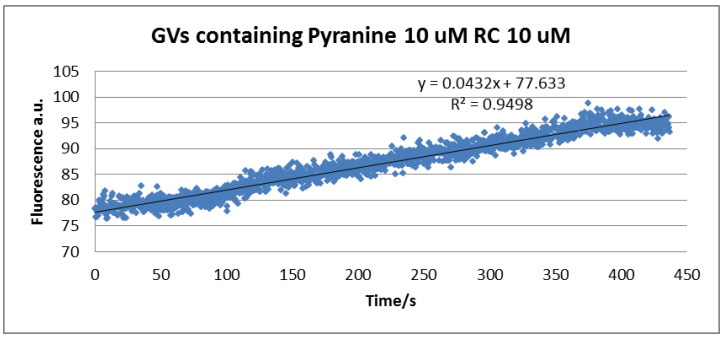

Fig. 2: Increase of the pyranine fluoresce of a vesicle suspension followed by spectrophotometer under constant illumination.

Remarkably, the facile assembly of the sophisticated reaction center into the synthetic lipid membrane, as obtained by the droplet transfer method [5], paves the way to the construction of novel and more functional protocells for synthetic biology.

Different theoretical approaches in simulating Giant Lipid Vesicles as protocell models

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Giant Lipid Vesicles (GVS) have been extensively used in recent years as simplified *in vitro* cell models both in studies on the origins of Life on Earth and in modern synthetic biology [1-7]. In fact, these lines of research are aimed at designing, constructing, and characterizing micro-compartmentalized structures of minimal complexity (*protocells*) that share with primitive cells or with modern living cells their peculiar static and dynamic organization. In this framework, GLVs can be designed as micro-sized enzymatic chemical reactors fed by a flux of substrates from the outside and indirectly monitored by means of traditional spectrophotometer analysis, getting the average time behavior of the entire population, or followed directly by confocal light microscopy obtaining the time course of single compartments.

In this contribution, two different theoretical approaches will be discussed and compared: the 0D and the 3D approach respectively, that differ in the description level and in the modelling purposes.

The 0D modelling aims to describe the time evolution of a population of GLVs taking into account the size dispersion and the solute concentration distribution. This model allows the investigation of extrinsic stochasticity [8] on the time behavior of the vesicle suspension. The theoretical outcomes of this approach can be contrasted with flow cytom-

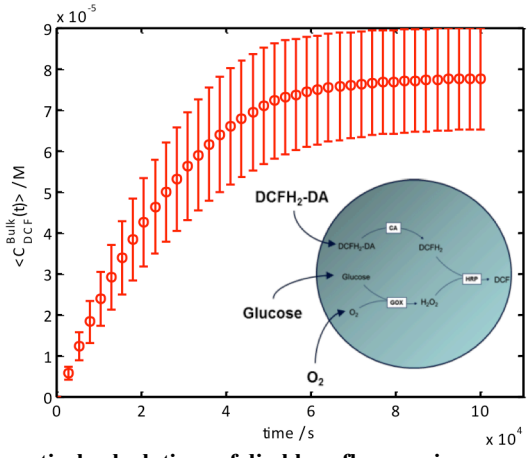

Fig. 3. 0D theoretical calculations of di-chloro fluorescein average production in a population of poly-dispersed GVs.

eter or spectrophotometer experimental analysis and can give hints on the vesicle preparation procedure.

On the other hand, the 3D approach describes single GVs giving also morphological 3d-space details and allows to take into account explicitly the diffusion of substrates, through the external solution and in the internal vesicle water core, along with molecular transport across the lipid membrane. The theoretical outcomes can be contrasted with confocal microscopy analysis and can be useful in designing communication experiments among GVs [9].

Both these two approaches have been successfully applied [8-9] in order to improve the implementation and to elucidate time evolution of protocells.

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Supercritical Antisolvent Process: PVP/Nimesulide Coprecipitates

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Nimesulide (4-nitro-2-phenoxymethanesulfonamide) (NIM) is a non-steroidal anti-inflammatory drug (NSAID), widely used in the treatment of acute pain associated with different diseases, such as back pain, toothache, postoperative pain and inflammation, headache and migraine. A major limitation in its usage is due to its reduced solubility in water (< 0.01 mg/mL); therefore, large doses are required to reach the therapeutic level, with consequent undesired effects, like heartburn, nausea, diarrhea, vomiting, peptic ulcer and hepatic damages. Considering that, for poorly soluble orally administered drugs, the rate of absorption is often controlled by the rate of dissolution, the attainment of a fast solubilization for those drugs indicated for various pains is required. In order to improve the NIM dissolution rate, and correspondingly reduce its dose, a possible solution is represented by their particle size reduction at micrometric diameters. Traditional micronization techniques show several drawbacks: lack of control over the particle morphology and particle size distribution, difficulty in elimination of the solvent and use of high temperatures.

An alternative to conventional techniques is represented by supercritical carbon dioxide ($scCO_2$) based processes, characterized by fast mass transfer, high solvent power, high density, near zero surface tension, low viscosity and high diffusivity, that can be tuned varying pressure and temperature. It was demonstrated that it is possible to accurately control particles size and particle size distribution. In particular, nanoparticles, microparticles and expanded microparticles of different kind of materials were successfully obtained by supercritical antisolvent (SAS) precipitation. However, when processed using SAS, nimesulide precipitated in form of large crystals or it is completely extracted by the mixture formed by the organic solvent and $scCO_2$, as a result of the partial solubility of this drug in the mixture solvent/antisolvent. A solution to this problem can be the production of composite microspheres drug-polymer, using a water soluble polymer in which the drug is entrapped. The fast solubilization of the polymer should release the drug in nanometric sub-microparticles obtaining an improvement of their bioavailability.

Therefore, in this work, to overcome SAS limitation in micronizing NIM, its coprecipitation with Polyvinylpyrrolidone (PVP) is proposed, trying to take advantage of PVP ability to retard crystal growth. The effects of the main process parameters, such as poly-

mer/drug ratio, overall concentration, operating pressure and temperature are investigated to identify successful operating conditions for SAS coprecipitation. Microparticles with a mean diameter ranging between 1.7 and 4 μm (calculated in number of particles) were successfully produced; they were characterized using different analytical techniques, to demonstrate the occurred coprecipitation. Precipitation yield was found to be about 100 % with respect to the amount of solute dissolved in the starting solution. Drug release analyses revealed that NIM dissolution rate from PVP/NIM microparticles in a phosphate buffered saline solution (PBS) was 2.5 times faster with respect to unprocessed drug. The possible precipitation mechanisms involved in the process were discussed.

PLA-based nanobiocomposites with modulated biodegradation rate

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1. Introduction

The disposal of polymeric waste is increasingly becoming an issue of international concern. The use of biodegradable polymers is a possible strategy to face most of the problems related to the disposal of the durable (non-biodegradable) polymers. Among biodegradable polymers, PLA (obtained from renewable sources) is a very attractive one, due to its good processability, biocompatibility, interesting physical properties. Hydrolysis is the major depolymerisation mechanism and the rate-controlling step of PLA biodegradation in compost. The propensity to degradation in the presence of water significantly limits specific industrial applications such as automotive, biomedical, electronic and electrical appliances, agriculture. Therefore the control of biodegradation rate is somewhat even more important than the characteristic of biodegradability itself.

2. Materials and methods

In this work the PLA 4032D produced by Natureworks was used. This PLA grade contains about 2% of D-lactide with a maximum degree of crystallinity of about 45% and with molecular weights M_n = 119 kg/mol, M_w = 207 kg/mol [1,2]. Fumaric acid was supplied by Sigma Aldrich. LDHs were obtained by co-precipitation according to the procedure described in the literature [3]. This filler was used both in nitrate form (LDH in the following), and intercalated with fumaric acid (LDH+fumaric acid).

The pellets of PLA and all the fillers were dried for 24 h under vacuum at a temperature of 60°C. The materials were melt compounded in a twin-screw mini-extruder, with a homogeneous temperature of 170 °C and at 100 rpm, with a cycle time of 5 min. Several materials were obtained: pure PLA 4032D extruded; 4032D+3% (LDH); 4032D+3% (LDH+fumaric acid); 4032D+1% (fumaric acid). Percentage reported above are wt/wt; 1% fumaric acid corresponds about to the same quantity of organic acid intercalated in 3% (LDH+fumaric acid).

From all the extruded materials several amorphous films were obtained by compression molding (T=170 °C) with an average thickness of 200-300 μm.

3. Results

In order to assess the significance of hydrolysis in the molten state, time sweep tests were performed. The equipment used to carry out the rheological measures was a rotary rheometer Haake Mars Thermo Scientific. Rheological tests were performed at 200 °C, with a stress set to 500 Pa and a strain rate of 1 rad/s, maintaining all values constant for 3 hr. The materials were not dried before the test.

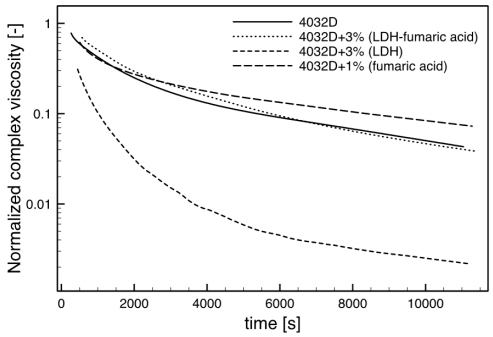

Fig.1. Time evolution of viscosity during rheological tests at 1rad/s. The values were normalized with respect to starting values for each material

As shown in Fig. 1, all the materials present a decrease of viscosity during time, due to hydrolysis and thermal degradations. The presence of LDH enhances significantly the degradation, whereas fumaric acid alone seems to reduce the phenomenon. The intercalation of LDH with fumaric acid largely reduces the degradation, so that the compound with 3% (LDH+fumaric acid) behaves similary to 4032D alone. Similar results are found by TGA analysis.

Hydrolysis tests were conducted in distilled water at 58 °C whose pH was measured to be 6-7. This temperature was selected because it is the temperature adopted for biodegradation tests according to ASTM and ISO standards. Several films (having a thickness of about 250 μm, weighing about 50 mg and dimensions 1.5x1.5 cm^2) of each of the samples were placed in a glass vessel containing water. The results of hydrolysis in the solid state are substantially different from what reported above concerning the degradation in the molten state. PLA containing acid alone is the less resistant to hydrolysis process. The

presence LDH intercalated with the same amount of acid instead improves significantly the stability of the material.

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Zein/luteolin coprecipitated particles production using a supercritical fluid assisted process

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Antioxidants are extremely fragile compounds that can easily degrade in presence of light, heat and oxygen with a consequent reduction of the antioxidant power. Moreover, they often suffer of low bioavailability. Therefore, technologies that minimize their decomposition during the production and storage of the product and improve their bioavailability are required. One possible strategy is the encapsulation of antioxidants in biocompatible polymers. Recently the use of vegetable proteins as polymeric carriers has been proposed. Indeed, they are a natural and renewable sources. Particularly, zein is a vegetable protein extracted from corn which is often used to maintain the integrity of food and pharmaceutical products. For these reasons, in this work, a new supercritical based process, named Supercritical Assisted Injection in a Liquid Antisolvent (SAILA), has been proposed for the encapsulation of an antioxidant, luteolin, in zein microparticles in order to improve the bioavailability of luteolin and protect its antioxidant properties. First feasibility tests for the micronization of zein proteins using the SAILA process were conducted. Zein submicro and micrometric particles, with an average size between 0.26 and 2 μm, were obtained from ethanol-water mixtures with composition ranging from 70/30 to 90/10 in volume. Particle morphology is spherical and not aggregated. Then, the coprecipitation of zein/luteolin was performed. With the SAILA process, microparticles of zein/luteolin with good encapsulation efficiencies, up to 82%, were obtained. The coprecipitates were characterized by homogeneous antioxidant dispersion in the polymer matrix. The antioxidant activity of the coprecipitate particles was maintained high as the luteolin in the native state. Moreover, the coprecipitates showed accelerated dissolution rate (2/3 h) with respect to the physical mixture (20 h) zein/luteolin, with a consequent increase in the bioavailability of luteolin. Future developments will concern the combined coprecipitation of luteolin and another antioxidant capable to promote and optimize the action of luteolin.

Current Directions in Synthetic Cell Research

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Synthetic Cells (SCs) can be defined as those cell-like molecular systems constructed in the laboratory by inserting biological or synthetic molecules inside and on the surface of lipid vesicles (liposomes). Ideally, SCs mimic biological cells with respect to structure, functions, interactions, and any other possible property (including the ultimate and long-term goal of being alive). SCs are systems whose complexity is purposely kept at the minimum.

Historically, SCs have been first explored to shed light on some origin of life questions, but it became clear that they can be created and adapted for investigating biological mechanism or developing biotechnological tools.

SCs are relevant in origin of life studies [1] because they are useful models of primitive cells (protocells), especially when they are built with allegedly primitive membrane-forming compounds (e.g., fatty acids), and host compounds and reactive systems which are relevant in an origin-of-life perspective (ribozymes, simple peptides, self-replicating molecules, etc.). One of the goal is to show that simple living cells, maybe imperfect and still "limping", can emerge from self-organization of non-living molecules.

On the other hand, SCs can be use to reconstruct, in realistic cell-like architecture, those biological processes that can be advantageously studied in a simplified platform. In fact, by reconstructing a certain process in a SC, its understanding will result easier because the interfering "noise" of background cellular processes, as unwanted interactions with other components, are purposely removed. The process of interest can be finely tuned owing to the control of SC composition – whereas such control is impossible in living cells.

Finally, SCs can be used as tools for biotechnological applications. Their design and construction is developed under the *synthetic biology* paradigm, which includes the use of standard parts, the concepts of modularity, orthogonality, and fully programmability (at least in principle). Such bioengineering perspective has led to sketch the possible use of SCs in nanomedicine [2]. Note that synthetic biology follows, by definition, the "constructive" approach.

Starting from the 1990s, several research groups have been involved in synthetic cell research, contributing to setting up the stage for actual and future developments (for a review, see [3]). The construction of SCs with minimal com-

plexity is today one of the most attractive and challenging goals in synthetic biology.

In this contribution, we will first illustrate and comment the state-of-the-art in synthetic cell research; next we will present some current trends and scenarios, especially those that could lead to a qualitative jump in the next years. In particular, we will focus on some aspects that we consider of particular interest and that will foster significant advancements. These are:

- the construction and exploitation of multi-compartment vesicles (vesosomes or MVVs);
- the achieving of new functions via the reconstitution membrane-bound proteins;
- the shift from the isolated cell- to the cell population- (or cell community-) perspective;
- the development of chemical signalling among SCs or between SCs and natural cells;
- the integration of stochastic mathematical models with experimental approaches.

Moreover, the ambitious goal of approaching embodied and minimal cognition from this experimental perspective will be shortly mentioned.

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Scanning Probe Microscopy Investigation of ZnO and Co-doped ZnO thin films in dark and UV-light conditions

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Abstract. Zinc oxide has widely attracted the interest of scientific community as multi-purpose material. It exhibits unique nano-structured, semiconducting, optical, and piezoelectric properties hence has been investigated for a wide variety of applications. Moreover, zinc oxide is broadly used in medicine and cosmetology for anti-sore cream, soap, beauty-mask and sun lotions, as consequence of its absorbance in the UV range. Moreover, UV radiation strongly affects the ZnO electronic transport, making such a material widely considered for opto-electronic devices. Here, we present a scanning probe microscopy investigation of undoped and Co-doped zinc oxide thin films grown by Pulsed Laser Ablation on Ag coated Si(p-type) substrate, giving rise to a ZnO/Ag/Si heterostructures.

In particular we used Conductive-AFM to investigate the photoconduction, Kelvin Probe Force Microscopy to measure variation in the surface potential and Piezoforce microscopy to characterize the ferroelectric behavior. The experiments were performed on both as grown and post-growth hydrogen irradiated sample surfaces. The ferro-electricity and the local electric properties were studied under both dark and UV light conditions, at room temperature and pressure. Transport and structural properties of hydrogenated Co-doped ZnO have been further investigated by Hall effect, micro-Raman, and X-ray absorption spectroscopy.

We observe that the Co doping enhances the ferro-eletricity but at same time causes a decrease of the electrical conduction as well as of the UV photoresponse.

Poster Presntations

On the employ of time series in the numerical treatment of differential equations modelling oscillatory phenomena

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We present a numerical scheme to integrate systems of differential equations modelling phenomena characterized by an oscillatory dynamics. In particular, we numerically treat problems for which time series of experimental data are available in order to exploit all the deriving a-priori known information.
We focus on what is now considered the prototype oscillator, the Belousov-Zhabotinsky reaction, which can be modelled by a system of kinetics equations for the concentrations of the key elements. We numerically integrate this system of differential equations (commonly known as Oregonator) following the strategy of exponential fitting [3]. Indeed, classical methods could require a very small step-size to accurately reproduce the oscillatory behaviour of the exact solution because they are developed in order to be exact (within round-off error) on polynomials up to a certain degree. We rather propose a method that is constructed in order to be exact on functions other than polynomials. These basis functions are supposed to belong to a finite dimensional space (the so-called fitting space) and are properly chosen according to the behaviour of the exact solution. As a consequence, the coefficients of the resulting adapted method are no longer constant as in the classical case, but rely on a parameter linked to exact solution, whose value is clearly unknown. Therefore, we need to choose a proper fitting space and estimate the above-mentioned parameter. We show how dealing with these aspects by taking into account the existing theoretical studies on the problem and extracting useful information from the time series of experimental data. Indeed, we select a trigonometrical fitting space because of the a-priori known oscillatory dynamics occurring in BZ reaction. In this case, the basis functions depend on a parameter representing the frequency of the oscillations of the exact solution. We propose estimating this parameter by minimizing the leading term of the local truncation error [2]. However, since the time series of experimental data are available [5], we can select the frequency of the observed oscillations as an approximation of the parameter, thus avoiding expensive procedures involving the resolution of non-linear systems of equations as in [1]. Numerical experiments will be provided to show the effectiveness of the presented approach.
To summarize, trigonometrically fitted methods may guarantee a better balance between accuracy and efficiency than classical ones in case of problems having an oscillatory dynamics. However, they require the computation of the param-

eter which can be very expensive. This limit is overtaken when time series of experimental data are given because the parameter can be estimated without any increase in the computational cost.

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Environmental Application of Extra-Framework Oxygen Anions in the Nano-cages of Mayenite

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Mayenite, often labeled as C12A7, is a nano-structured calcium aluminate ($Ca_{12}Al_{14}O_{33}$) with extra-framework oxygen anions with a characteristic crystalline structure [1]. The framework of mayenite is composed of interconnected cages with a positively electric charge per unit cell that includes two molecules, $[Ca_{24}Al_{28}O_{64}]^{4+}$, and the re-maining two oxide ions O_2^-, often labelled as *free oxygen*, were trapped in the cages defined by the framework [2]. The ability of storing O_2^- ions in the cages is a prominent property of mayenite and it was exploited in catalysis because oxygen ions can migrate between the surface and the bulk showing a unique ionic conductivity [3].
In this work, we report on the use of undoped mayenite as catalyst for the total oxidation of gaseous trichloroethylene (TCE). TCE is a halogenated aliphatic organic compound that, when released in the environment, tends to penetrate into the soil and stratify at the bottom of the groundwater, often forming a permanent source of pollution [4].
Experiments were carried out at atmospheric pressure in a quartz fixed bed reactor, schematically shown in Figure 1. To assess the efficiency of mayenite in

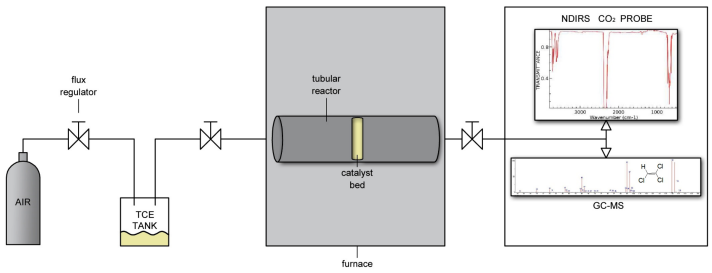

Fig. 1. Experimental scheme for gaseous TCE oxidation

degrading trichloroethylene, we tested the catalytic material at different temperatures, ranging in the interval 20–500 °C, and monitoring TCE by means of a GC-MS apparatus. Figure 2 shows the degradation yield expressed as the percentage of TCE converted during the catalytic reaction. The conversion yield

was found to increase drastically with the temperature, becoming quantitative above 450 °C. Catalyst recyclability experiments were also performed at 450

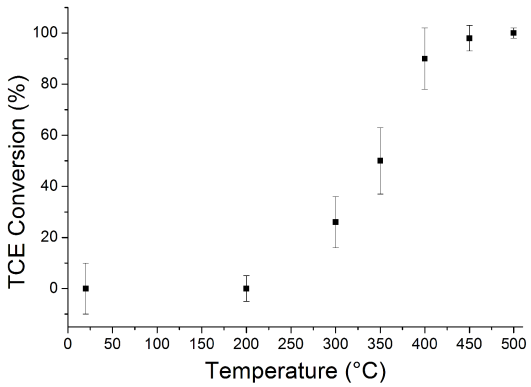

Fig. 2. TCE Conversion over mayenite catalyst

°C under the optimized reaction conditions for the complete oxidation of TCE. Mayenite showed a high recyclability and could be used for ten consecutive reaction cycles (10 h) without any loss in activity and selectivity.
In conclusion, our results showed that mayenite was an effective catalyst for the TCE oxidation. TCE was totally converted in CO_2 and the released Cl^- was incorporated in the mayenite structure [5]. The high performances of the catalyst were due to the presence of O_2^- and O_2^{2-} anions, which favoured the total oxidation of TCE and avoided the formation of coke.

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Modelling Approach to Enzymatic pH Oscillators in Giant Lipid Vesicles

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Giant lipid vesicles (GVs) are intensively studied in different areas of biomimetic chemistry, biomembrane physics and in the field of artificial cell synthesis due to their cell mimicking characteristics. GVs and natural cells share physicochemical properties, such as stability, permeability, self-reproduction and the capability to act as hosts of chemical reactions: indeed, giant vesicles can be easily filled with various compounds, even macromolecules, to allow chemical reactions inside [1,2]. In this field, one of the most promising applications is the use of liposomes as micro-reactors for nonlinear chemical reactions in order to understand how different compartments communicate with each other and exchange information emitting chemical signals. In this context, preliminary results about the encapsulation of the oscillating reaction of Belousov-Zhabotinsky in liposomes were presented from our group in a previous WIVACE [3].
Recently [4], in collaboration with the Dr. Taylor's group, we replaced the harsh acidic conditions of the Belousov-Zhabotinsky reaction with a more biomimetic system, the urea-urease reaction [5,6]. This reaction occurs in numerous cellular systems and is used, for example, by bacteria H.pylori in order to raise the local pH. The production of ammonia leads to a decrease in the acid concentration, followed by a rate acceleration of the enzymatic reaction. In the proper conditions, this reaction could give rise to bistability, high steady state, low steady state and oscillations. In order to simulate the dynamical behaviour of the urea-urease reaction confined in a cell-like system, a two-variable model was initially derived in which acid and substrate, urea, were supplied at rates k_H and k_S from an external medium to an enzyme-containing compartment. Oscillations were found in a limited region provided that the condition $k_H > k_S$ was satisfied [7]. This two-variable model derives from the assumption that only acid and substrate can diffuse through the membrane; however, experimental investigations carried out for the urea-urease system showed that giant vesicles act as open compartments: the reactants enter the vesicles, while the products leak out, both the fluxes are supposed to occur through a passive diffusion mechanism. Thus, the two-variable model has been modified: the inclusion of the ammonia leakage and the hydrolysis of the pH fluorescent probe used in the experiments (pyranine) allows for a better match with the experimental conditions.
In this contribution, we will discuss the results obtained with the modified model, with a focus on the model response to the concentration changes of the substrate,

of the enzyme and of the acid. The effect of these species on the induction period for the autocatalytic reaction and the influence on the oscillatory parameters will be investigated. Figure 1 reports a preliminary phase-diagram obtained by changing k_{H} *vs* k_{S} at a fixed value of k_{N} (the rate of diffusion of ammonia).

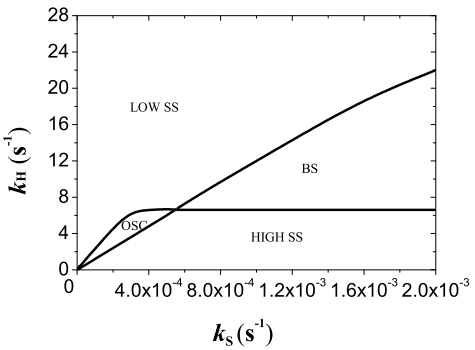

Fig. 1. Phase diagram for [urease]=500 U/mL, k_{N}= 0.1 s^{-1}, $[\mathrm{urea}]_{\mathrm{out}}$ = 0.6 M

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Green synthesis of gold nanoparticles from extracts of *Cucurbita pepo* L. leaves: insights on the role plaid by plant growth

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Environment-friendly and cost effective methods to obtain metal nanoparticles represent a major issue in modern material science.[1] In particular, synthetic routes relying on green chemistry appear to be promising for large scale production. In this work we have prepared and characterized gold nanoparticles (AuNPs) from extracts of *Cucurbita pepo* L. leaves, which constitute an agricultural byproduct of large diffusion and abundant biomass. The investigation was carried out as a function of plant ageing from 30 to 120 days and the production of nanoparticles (in term of size, shape and relative yield) was correlated with the concentration of chlorophylls and carotenoids in the fresh leaves. These two classes of compounds can potentially act both as reducing and capping agents. The synthesis was carried out by using purely aqueous extracts at relatively low temperature (70 °C) to comply with the requirements of environmental sustainability. Diluted solutions of $HAuCl_4$ (from 5 10^{-5} M to 10^{-3}M in the final solution) were used to provide for the gold precursor, according to well-established synthetic methods.[2]

Figure 1 shows the typical micrograpfs obtained from samples at different growth stage, treated with the same Au (III) concentration.

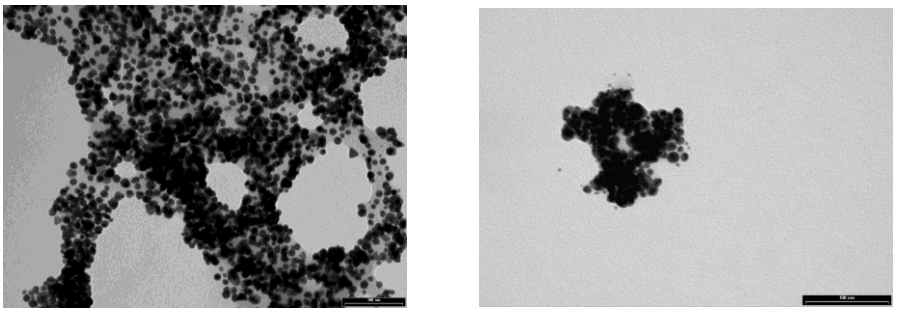

Figure 1. AuNPs obtained from extracts of *Cucurbita pepo* L. leaves grown for 30 days (left panel) and 120 days (right panel). In both cases the starting Au(III) concentration was 6.3 10^{-4} M, while the extract was obtained from 0.3 g of fresh leaves and 1 mL of water.

The concentration of chlorophylls and carotenoids in the fresh leaves of these samples (expressed as mg/g) is reported in table 1. The obtained values suggest that a higher amount of chlorophylls and carotenoids in the leaves from which extracts are obtained generates larger, more polydisperse and aggregated particles.

Table 1

	Chlor a	Chlor b	Total Carot.
30 days	3.27	3.40	0.055
120 days	11.40	8.15	0.082

Different extract/Au ratios were also tested, to study the role played by nucleation and growth processes,[3] which are supposed to gain importance at high and low extract amount, respectively. TEM microscopy showed that the interplay of these mechanisms can lead to a huge production of fairly monodisperse AuNPs with size in the range of 10-15 nm, as in the samples in figure 1. TEM also evidenced that lower Au(III) concentration promtes deviation from the spherical shape and formation of anisotropic particles, such as nanorods. This constitute an interesting outcome for applications in many technological fields, from nanocatalysis to biomedicine.

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Molecular dynamics simulations to increase the accuracy of cross-docking

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1 Introduction

Crystallographic data show that different ligands may stabilize different receptor conformations. Consequently, the 3D structure represents only a successful binding event of a specific protein conformation and a particular ligand. It is clear that in the docking studies a single representative structure for the receptor is not enough. Since the structures of the same receptor can be rather different, the cross-docking analyses are typically very poor [1]. Flexibility in molecular docking studies is particularly important when the binding pocket is buried inside the protein and the ligand binding induces the backbone deformation of the receptor to accommodate the ligand.

2 Material and methods

The flexible model was used for cross-docking tests of 10 ligands and 10 different conformations of the androgen receptor. To address the receptor flexibility we collected 5000 conformations for each receptor from the molecular dynamics simulations (MD) at regular time interval. An energy minimization was run for side-chain optimization, removal of atomic clashes, and optimization of electrostatic energy. The molecular docking simulations were performed using a new docking software (YADA) in which the docking analysis is correlated to the receptor sequence conservation. In addition YADA performs an extensive genetic algorithm study to improve the pose ranking [2]. To validate the procedure, the predicted pose with the highest binding energy was superposed on the crystallographic complex conformation to calculate the root mean square deviation (RMSD).

3 Results

To use a single representative structure for the receptor can radically affect the outcome and alter the cross-docking results. This is because in that conformation the internal cavity of the buried binding pocket does not have space enough to accommodate all ligands. In these cases the most favorite positions for the docked ligands are only on the receptor molecular surface. MD was an inexpensive method to generate a series of target structures to use as starting point for docking analysis. In figure 1 are shown the changes in receptor internal cavity during the molecular dynamics simulations of the androgen receptor at time 0 ns (a) and 30 ns (b).

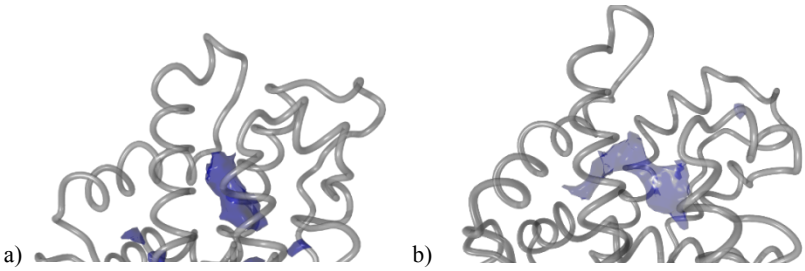

Fig. 1 Snapshots during MD of androgen receptor (PDB 2AX9)

MD deformed the shape of the cavity of the buried binding pocket that may now accommodate the ligand in the docking studies. Based on the docking results we suggest to use the MD to provide various conformations with different internal cavities that can host ligands of different geometry.

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Alginate/gelatin modified Aerogels obtained by Supercritical Drying

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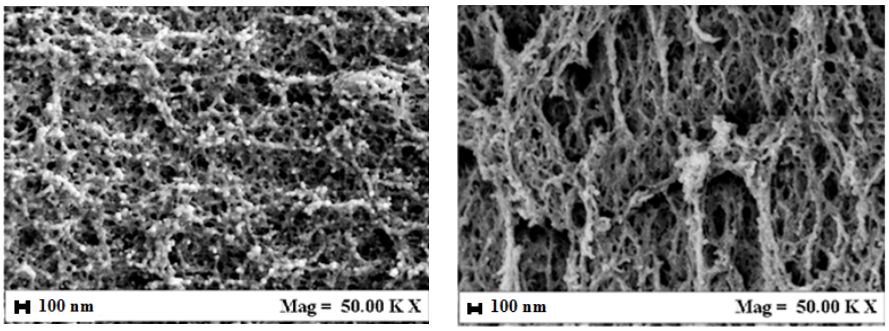
Natural polymers, such as alginate, can be used to produce scaffolds for tissue engineering applications; but their mechanical and biochemical performance should be improved. A possible solution to obtain this result is the generation of multi-component scaffolds, by blending two or more polymers. One way to realize it is the formation of an interpenetrating polymer network (IPN), or the functionalization of the polymer with peptide RGD sequences, in order to promote cell recognition [1].

Alginate is largely used in biomedical field, due to its biodegradability, biocompatibility, hydrophilicity and low toxicity [2]. Nevertheless, its negative charge inhibits protein adsorption and reduces cellular adhesion. For this reason, bioactive molecules such as arginine-glycine-aspartic acid (RGD) and fibronectin were proposed for the immobilization within the hydrogel, to induce cells adhesion. Gelatin is formed by denatured collagen; it has relatively low antigenicity compared to its precursor and maintains signals that may promote cell adhesion, differentiation and proliferation, such as RGD sequence of collagen [3].

In this work, the RGD-coupled alginate and interpenetrated alginate/gelatin hydrogels have been successfully obtained and preserved by SC-CO_2 drying, performed at 200 bar and 35 °C. The process allowed modulation of morphology and mechanical properties of these blends. The overall result was made possible by the supercritical drying process that, working at zero surface tension, allows preserving the hydrogels nanostructure in the corresponding aerogels.

In Figure 1 (a,b), examples of FESEM images of A/G 20/80% v/v and 80/20% v/v are shown.



a b

A/G aerogels morphology changes with the relative proportion of the two polymers. In particular, it evolves from nanofibrous to nanoporous by increasing alginate percentage in the starting gel, a hybrid between that of the two polymers when equal percentages are used. As a result, it is possible to select the scaffold structure by changing the polymers relative proportions. The supercritical process does not modify gel organization and the avoidance of gel collapse during drying.

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Computational approach to design new antimicrobial peptides

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In the last years, the overuse of conventional antibiotics has caused high rates of microbial resistance. We focused our attention on antimicrobial peptides (AMP), highly variable in composition, generally shorter than 50 amino acids, mostly positively charged and produced by multicellular organisms that have important functions in the innate immunity [1]. They are also able to kill or inhibit a variety of organisms.

Genetic Algorithm, Artificial Neural Network and Support Vector Machine are often used in bioinformatics analyses. Successful models can be obtained for sets of similar peptides, but AMP show a large variability in sequences and 3D structures. This probably explains the failure of previous computational analysis performed on AMP. The poor predictive abilities of many models is also due to the lack of accurate microbiological data. Furthermore, because of the killing action of AMP is correlated with their chemical-physics characteristics, it is also important to extend the pool of data (molecular descriptors) about antimicrobial peptides.

We focused on particular aspects such as their chemical-physical properties (QSAR analysis and molecular docking) and in the search of recurrent motifs (Protcomp).

The element of novelty of this work is the creation of homogenous sets of AMP for QSAR analysis. Our hypothesis was that similar peptides act through the same mechanism. We have developed a database of antimicrobial peptides, Yadamp [2], to facilitate the access to important information on AMPs (microbiologic, chemical, physical and biological data, 3D structures and energy values). We have clustered AMP with common chemical-physical properties and have performed QSAR analysis by means of genetic algorithms (GA) and artificial neural networks (ANN). GA shed light on the AMP mechanism of action. ANN verify the correlation between the input data (molecular descriptors and the experimental data of antimicrobial activity). We obtained some predictive models of activities that were also appropriately validated through a statistical method.

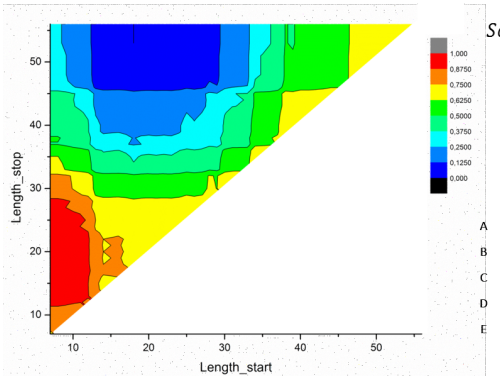
Here, we present the results of an analysis performed on peptides active on S.*aureus*, shorter than 30 residues and with a Boman index between 1 and 2 kcal/mol (92 peptides).

The QSAR analysis has generated an equation with R^2 of 0.81 and LOF of 876.01. However, a good R^2 cannot capture the quality of an activity model because the intrinsic experimental error in microbiological tests, due to serial dilutions, is not considered. It is more correct to talk about activity classes and the goodness of a QSAR model must be judged in terms of its ability to discriminate among very active, active and non-active peptides. For that reason, we clustered AMP in five classes, having roughly the same population (Table 1).

Table 1. AMP activity is clustered on their MIC values (μM), where A, B, C, D represent active peptides and E inactive peptides.

A	B	C	D	E
0 ≤ MIC ≤ 2	2 < **MIC** ≤ 5	5 < **MIC** ≤ 10	10 < **MIC** ≤ 30	**MIC** > 30

In this way, the reliability of the model can be better evaluated using a scoring matrix. The score is calculated comparing the predicted class of activity with the experimental one (Figure 1). We evaluated the domain of applicability (IDA region).



$$Score = \sum_{i=1}^{n} [Class\ observed - Class\ predicted]$$

	A	B	C	D	E
A	2	1	0	-1	-2
B	1	2	1	0	-1
C	0	1	1	0	-1
D	-1	0	0	1	0
E	-2	-1	-1	0	1

Fig. 1. On the x axis there is the initial length of the considered interval and on the y axis there is the value of the final length of the interval. The score is shown with a colorimetric scale.

The results are not improved in comparison to the previous studies on AMP. From here the need to increase the number of data on AMP and to optimize the analyses.

We must also consider the amino acidic sequences of the AMP.

The traditional sequence alignment (APD[3], AntiBP2[4]) do not allow to identify any recurring motif in AMP. Therefore, we have developed a new tool for alignment-free analysis of sequences (http://yadamp.unisa.it/protcomp). This tool can extract essential

information on patterns in proteomes or in subset of proteomes using compression algorithms to build up dictionaries of substrings. The dictionaries of different organisms can be then compared by means of ad hoc metrics. It is possible to determine how many motifs have in common the two organisms compared. The idea is that two organisms closely related shared some common features in the respective dictionaries. The distances calculated can be used to generate phylogenetic relationships and, furthermore, can serve as molecular descriptor for future QSAR Analysis.

We have also created a new docking software, YADA [5], for the prediction of free energy of binding between protein-ligand complexes. Our idea is to evaluate this tool in the protein-protein docking process to understand the mechanism of interaction of AMP when they touch and fit in the target membranes (Figure 2).

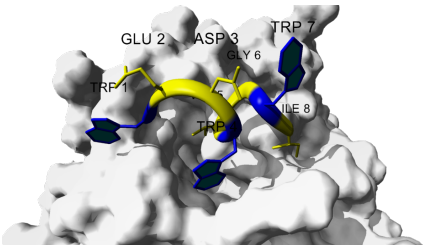


Fig.2. Interaction between an AMP and a micelle

In this way, YADA permits to calculate useful molecular descriptors for further QSAR analysis.

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Functional coatings based on TiO_2-Fe nanoparticles and its incorporation in a polymeric matrix

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Abstract. In the present study, the development of functional coatings by the use of TiO_2 and Fe-doped TiO_2 nanoparticles was carried out. TiO_2 has very interesting chemical and physical properties for applications such as functional coatings, photo-catalysis, solar cells, and so on. TiO_2 and TiO_2-Fe nanoparticles were prepared by a novel oil-in-water microemulsion method. The nanoparticles were functionalized by chemical solution in order to be incorporated into a polymeric matrix of polyethylene oxide and develop the nanocomposite. Nanocomposite films (onto glass) were prepared by spin coating technique. TiO_2 and TiO_2-Fe nanoparticles were characterized by SEM, EDS, XRD and XPS; the nanocomposite films were characterized by SEM, XRD, contact angle and mechanical properties.

1 Introduction

The problem of waste water is an increasingly important issue. Photocatalytic treatment plays an important role in environmental and energy applications, including purification and recycling of wastewater as well as drinking water and degradation of medicines (antibiotics), pesticides, herbicides, dyes and other volatile organic compounds [1].

In this work the development of functional coatings by the use of TiO_2 and Fe-doped TiO_2 nanoparticles was carried out. TiO_2 has very interesting chemical and physical properties for applications such as functional coatings, photo-catalysis, solar cells, and so on [2]. Photocatalytic properties of TiO_2, e.g. activity under visible light can be tuned by doping this material with other transition metal elements, such as Fe[3].

2 Materials and method

2.1 Materials

Synperonic 91/5 was purchased from Croda. Titanium (IV) 2-ethylhexanoate and Iron (III) 2ethylhexanoate were purchased from Alfa Aesar. Isooctane was purchased from Aldrich. Ammonia 29% was purchased from CTR.

2.2 Method

TiO_2 and TiO_2-Fe nanoparticles were prepared by the novel oil-in-water microemulsion reaction method under mild conditions [4]. The microemulsion system was prepared by mixing the corresponding amount of surfactant, oily phase and deionized water. The mixture was stirred at 26.5°C until a homogeneous, transparent and fluid isotropic phase was obtained. Then, pH of the microemulsion was measure and an amount of ammonia was added under vigorous stirring at 26.5°C to take the pH of the microemulsion to 11. Microemulsion was kept under stirring at 26.5°C overnight. Later the nanoparticles obtained were washed with deionized water to neutral pH. The obtained material was dried by the use of a nano-spray dryer equipment B-90 from B.U.C.H.I. The morphology was observed by scanning electron microscopy (SEM), the equipment employed for this was a Nova NanoSEM 200 from FEI. The chemical composition was analyzed by energy-dispersive X-ray spectroscopy (EDS) for this purpose a system of microanalysis INCA X-Sight from OXFORD was used and X-ray photoelectron spectroscopy (XPS), analyses were carried out by means of a Thermo Scientific Escalab 250 Xi instrument. The crystalline structure was determined by X Ray Diffraction technique (XRD) the equipment used for the technique was a Panalytical Empyream.

The materials were functionalized by chemical solution and incorporated into a polymeric matrix of polyethylene oxide in order to develop the nanocomposite. Films of the nanocomposites onto glass were prepared by spin coating technique.

3 Results

The microemulsion reaction method allowed the formation of TiO_2 and Fe-doped TiO_2 nanoparticles with a tetragonal anatase crystalline structure after calcination at 500°C; this was confirmed by XRD technique. The size of the crystallite was 10 and 7nm respectively; it was estimated with the Debye Scherrer equation.

The morphology was observed by scanning electron microscopy (SEM); it was observed that the material dried using the spray dryer equipment resulted in well-defined

spheres and donut-like assemblies of small nanoparticles. The approximately sizes were of 1-2μ and 10-20nm respectively.

The chemical composition was determined by XPS and EDS. TiO_2 and Fe were observed, obtaining an approximately molar percentage of 13.7 of iron in TiO_2 by both techniques.

The obtained materials and coatings may possess interesting functional properties which could be useful for its applications in superhydrophilicity, self-cleaning and the photo-degradation of contaminants under solar-light radiation.

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Nanoliposomes production by a protocol based on a simil-microfluidic approach

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Abstract. In this work a protocol based on the microfluidic principles has been developed and applied to produce nanoliposomes. The protocol basically consists in the realization of a contact between two flows, lipids/ethanol and water solutions, inside a tubular device where interdiffusion phenomena allow the formation of lipid vesicles. Effects of solutions flow rates and lipids concentrations on size and size distribution have been investigated. Moreover ultrasonic energy was used to enhance homogenization of the hydroalcoholic final solutions and to promote the vesicles size reduction. By this protocol a massive output has been achieved; increasing the ratio between the water volumetric flow rate to the lipids-ethanol volumetric flow rate the liposomes dimension decreases; at equal flow rates, when the lipids concentration increases also the liposomes size has been observed increasing.

Lipid-based drug delivery systems are biocompatible**,** safe and efficacious carriers even more investigated by the scientific world for their ability in encapsulating and releasing, in a controlled manner, degradable active ingredients to be used for pharmaceutical and nutraceutical purposes. In particular liposomes have attracted a lot of attention for their biodegradability, high drug loading, low intrinsic toxicity, accumulation in pathological areas, reduced size, membrane mimetic behavior, prolonged half-life in the bloodstream, low cost and easiness of preparation [1]. In particular, size and size distribution are key parameters determining liposomes performance as carrier systems in both biomedical applications (i.e. influencing liposomes time of circulation in the blood stream and/or their permeability through membrane fenestration in tumour blood vessels [2]) and nutraceutical applications (i.e. improving taste, flavor, stability, absorption and bioavailability of nutraceuticals [3-4]). Nowadays there is a wide set of possibilities to produce lipid-based drug delivery systems through the use of conventional or more recently discovered techniques [5-7]. However, despite the leaps and bounds made with the novel technologies in

the last few years, the majority of these methods are characterized by high energy request, long times of process together with a low productivity.

To overcome these limitations, in this study microfluidics based methods, which are expensive for special devices needed and microfabrication costs, have been transposed to a millimeter scale, drastically reducing the production costs and increasing the yields. With the aim to have a control on flow, typically chaotic in a bulk phase which is insted laminar, and thus controllable, in a microfluidic system, starting from a work of Pradhan and collaborators [8], in which a syringe pump driven microfluidic device was used to produce liposomes, the design and the developed a new semicontinuous bench scale apparatus for a massive nanoliposomes production, overcoming the limits imposed by the syringe volumes, has been done. The preparative protocol pointed out basically consists in the realization of a contact between two flows, lipids/ethanol and water solutions, inside a tubular device where interdiffusion phenomena allow the formation of lipid vesicles. Ultrasonic energy was also used as intensification tool for liposomes production, size reduction and homogenization [9]. Furthermore in this work, similarly as done by Jahn and collaborators for a microfluidic hydrodynamic focusing (MHF) platform [10], a size and size distribution control of the produced nanoliposomes was demonstrated by tuning not only the flow rates, as done by Jahn research group, but also the lipids concentration.

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Production of nanostructured microspheres biopolymer-active principle-magnetic nanoparticles by Supercritical Assisted Atomization

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Nanoparticles with Magnetic Properties (NMPs) can have several biomedical applications; they can be used as contrast agent in magnetic resonance imaging (MRI) [1], as hyperthermia agents and as vectors for the targeted delivery of drugs; in this case they will be coprecipitated with the drug and driven to the target tissue [2].

In this work Supercritical Assisted Atomization (SAA) has been studied for the production of nanostructured microspheres biopolymer-ampicillin-NMPs for biomedical applications. The aim is to entrap the drug and the NMPs in a carrier that has the role to protect the active principle, to control its release and to drive it to the site of interest. The encapsulation in a polymeric coating makes the MNPs biocompatible; moreover, polymeric coating provide a steric barrier to prevent nanoparticle agglomeration and avoid opsonization.

Chitosan (CH) is biopolymer of great interest as a carrier because of its natural origin; ampicillin tri-hydrate (AMP) is an antibiotic and has been chosen as model drug; Fe_3O_4 nanoparticles have been used as MNPs.

SAA working principle is based on the formation of organic solvent+solid solutions that are contacted with SC-CO_2 to form an expanded liquid of reduced viscosity and surface tension. These conditions produce an improved atomization [3, 4]. The atomized solution produces controlled micro and submicro droplets that, upon drying, produce the corresponding polymer+drug coprecipitated microparticles [5-8]. In a previous work, the formation of polymer microparticles loaded with NMPs using SAA technique has been studied [9]. In this work is proposed the formation of composite microparticles by SAA, starting from a suspension in which NMPs have been finely dispersed in a solution containing chitosan and ampicillin.

The following SAA process parameters have been selected: static mixer temperature and pressure at 90°C and 100 bar, respectively; precipitator temperature set at 110°C with a gas to liquid ratio (GLR) of 1.8 and a concentration of chitosan of 10 mg/mL. 40% wt of NMPs have been suspended in the starting solution and AMP/CH ratios of 1/2,1/4, 1/6 have been studied.

In all the experiments the particles obtained have a spherical morphology, as shown in the Fig. 1, that reports, as an example, the SEM (Scanning Electron Microscope) photomicrograph of coprecipitates with AMP/CH ratio 1/2. In the figure also the particle size distributions in terms of cumulative volumetric percentage are reported, showing that the mean diameter ranges between 0.5 and 0.7 μm and the maximum observed particle size is 1.4 μm. There is not a clear influence of drug/polymer ratio on the particle size and distribution, probably because the same concentration of chitosan in all the starting solutions is used and, as a consequence, similar viscosity of the solution is obtained.

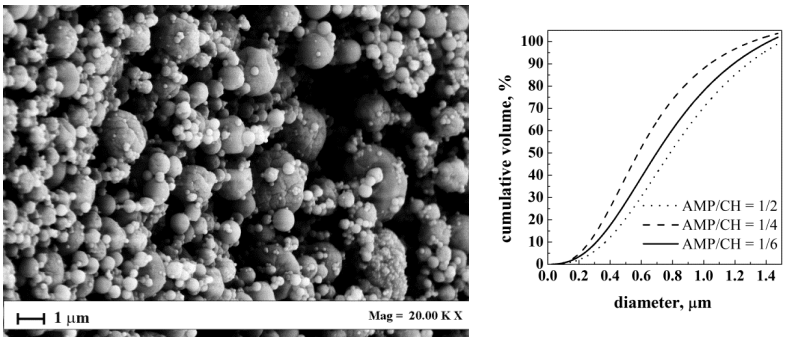

Fig. 1. SEM photomicrographs of microparticles (left) and particle size distribution in terms of cumulative volumetric percentage (right)

The obtained microspheres have been characterized by several analytical techniques to attest the efficacy of SAA micronization process.

EDX (Energy-dispersive X-ray spectroscopy) microanalysis allows to identify the elemental composition of the particles in a SEM photomicrograph. Oxygen (present in all the compounds), Sulphur (present in the ampicillin) and Iron (present in the MNPs) were verified and the presence and the distribution of these three compounds demonstrated that composite polymer-drug-NMPs microparticles have been obtained. The atoms are uniformly distributed in all the microparticles present in the SEM photomicrographs.

To obtain also a quantitative information about the NMPs present in the microparticles, TGA (Thermo Gravimetric Analysis) analysis has been performed on the coprecipitates obtained by SAA: the increase of temperature in oxidizing environment leads to the combustion only of the organic material. Chitosan and ampicillin, that are the organic part in the coprecipitiates, are destroyed by the combustion, while the inorganic residue, that represents the quantity of Fe3O4 present in the coprecipitates, remains. NMPs loaded in the microparticles varies between 31 and 37%, as reported in Table 1.

UV-vis spectrometric analyses attested in all the microspheres a loading efficiency of the active principle in the order of 63-99%, as reported in the table. AMP loading effi-

ciency increases with the decrease of AMP/CH ratio, this is because the increase of the relative amount of CH improves the entrapment of the active principle.

Table 1. Loading efficiency of ampicillin and NMPs in SAA coprecipitates

CH concentration, mg/mL	AMP/CH, wt/wt	AMP loading efficiency, %	Fe_3O_4 loading efficiency, %
10	1/2	63	37
10	1/4	66	31
10	1/6	99	31

The X-ray powder diffraction analyses of the coprecipitates showed the presence of the characteristic halo typical of an amorphous material and the Fe_3O_4 peaks typical of crystals. This attests that the microspheres are amorphous, the drug is intimately mixed with the polymer and that, during the precipitation, the NMPs do not modify their characteristic crystalline structure and are simply entrapped in the polymeric matrix.

The composite microparticles chitosan-ampicillin loaded with Fe_3O_4 nanoparticles successfully produced by SAA can be used for the production of pharmaceutical formulation for the targeted release of the active principle in specific sites. Drug release studies are required to attest that the system produced is effective for the controlled release of the active principle.

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Encapsulation of hydrophilic and lipophilic compounds in nanosomes produced with a supercritical based process

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Liposomes are created when phospholipids self-assemble in an aqueous medium creating spherical closed structures. These vesicles can be loaded with hydrophilic active principles (PA) into the aqueous inner core or with lipophilic compounds in the lipidic double layer. Due to their similarity to living cell membranes, liposomes are used as non-toxic and biocompatible drug carriers for proteins, vitamins, antibiotics, markers, anticancer and genetic material. The simplest method for the production of liposomes is the thin film hydration. However, this process suffers of some drawbacks; for example, it is a batch process with low reproducibility and low encapsulation efficiency (EE) of hydrophilic compounds. Moreover, micrometric vesicles are created, with difficulties in the elimination of the organic solvent. To overcome these problems linked to conventional processes, several supercritical methods have been developed, obtaining stable and homogeneous liposomes suspensions. Nevertheless, many of them still present some drawbacks as low EE of hydrophilic PA. Post-processing is often required to obtain nanometric-sized liposomes such as ultrasound and extrusion, with a high risk of disruption of lipidic membranes. In this work a new supercritical based process for the one-step continuous production of nanosomes is proposed for the encapsulation of hydrophilic and lipophilic compounds. This process is called Supercritical Assisted Liposome Formation (SuperLip). The innovation of this process consists in the inversion of the traditional phases of production of liposomes: water droplets are created by a spray atomization in a high pressure vessel, and then a double layer of phospholipids fast surrounds them. A systematic study on liposome size, morphology, encapsulation efficiency and release study has been performed for several different hydrophilic PA (Ampicillin, Ofloxacin, Bovine Serum Albumin, Fluorescein and Theophylline). Some operative parameters were also optimized to achieve the production of nanometric liposomes with high encapsulation efficiencies. Operating in this way nanometric and monodispersed liposome suspensions were produced with EE up to 98%. To complete the study, cholesterol was encapsulated in the double lipidic layer, obtaining high EE also in this case, up to 65%. Drug release studies were also performed to characterize produced liposomes.

Biocompatible Composite Aerogel Production by a Supercritical Process

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A crucial step of the replacement of human tissues is the scaffold fabrication. The temporary substitution of different biological materials requires different structural characteristics; but, all tissues share a series of common characteristics that have to be simultaneously fulfilled: a highly regular and reproducible 3-D structure similar to the tissue to be substituted; a very high porosity (exceeding 90%); the scaffold should present nanostructural surface characteristics that allow cell adhesion, the proper identification of the extracellular matrix (ECM), proliferation, migration and differentiation; mechanical properties to maintain the predesigned tissue structure and support the specific loadings applied to the original tissue; biocompatibility and a proper degradation rate, to match the rate of the neo-tissue formation.

In this work, Polyvinylidene fluoride/Hydroxyapatite (PVDF)/(HA) aerogels were produced by supercritical gel drying. HA was selected since it is chemically similar to the mineral component of bones and hard tissues in mammals. It is one of few materials that are classed as bioactive, meaning that it will support bone ingrowth and osseointegration. PVDF/HA aerogels showed an homogeneous, nanoporous morphology, with HA nanoparticles deposited on it. The high surface area (≈270 m^2/g) coupled with enhanced mechanical properties of the composite aerogels with respect to the starting single polymer (Young modulus up to 2.5 MPa) make these aerogels suitable for bone tissue engineering.

Graphene coated FeCo Nanoparticles for PAHs Extraction

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1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are a class of several hundred individual compounds consisting of two or more fused aromatic rings. Since PAHs are very difficult to degrade due to their high stability and complex molecular structures, they are considered as very significant environmental pollutants. The structural features of PAHs decide that they are carcinogenic, mutagenic, and teratogenic [1]. Consequently, there is an increasing interest in the detection of PAHs in environmen- tal water sources for the protection of health and environment. Magnetic Solid-Phase Extraction (MSPE), a novel form of the most common Solid-Phase Extraction (SPE), based on the use of magnetic nanoparticles (NPs) has gained more attention in trace analysis [2]. An appropriate surface functionalization of magnetic NPs is crucial for the extraction efficiency, as well as a quickly extraction time in order to obtain a ready recovering by a magnet. Carbon materials, including activated carbon, graphi- tized carbon black, porous graphitic carbon and graphene, possess strong adsorption ability [3].

In this work, we reported on a new promising nanosorbents constituted by core-shell few layer graphene coated metal nanoparticles (G-FeCo), that combine the superparamagnetism of the FeCo alloy and the strong adsorption ability of the carbon materi- als, in order to obtain an excellent absorption efficiency and rapid separation. To ex- amine the feasibility of this method, we selected two representative PAHs as model compounds: fluoranthene and anthracene.

2. Experimental section

2.1 Preparation of G-FeCo

The stable core-shell G-FeCo NPs were synthesized by catalytic chemical vapor deposition (CCVD) of methane at atmospheric pressure using a catalyst prepared by wet

impregnation of gibbsite (γ-$Al(OH)_3$) powder with cobalt acetate and iron acetate solutions [4,5].

2.2 Characterization techniques

Raman Spectroscopy, Transmission Electron Microscopy (TEM), Thermogravimetric analysis (TG-DTG) and X-ray diffraction (XRD) were employed for characterization. Magnetic measurements have been performed using a Quantum Design PPMS 9T instrument. Determination of PAHs was carried out by gas chromatography-mass spectroscopy (GC-MS).

2.3 Extraction procedure

For the extraction procedure a certain amount of the nanosorbents (G-FeCo) was dispersed into a 200 mL water sample. The mixture was sonicated for 5 min to make the sorbents dispersed uniformly in the solution. Successively, G-FeCo NPs were depos- ited at the bottom of the beaker under a magnetic field. The supernatant solution was discarded and PAHs were eluted from the sorbents. The eluent was collected and evaporated to about 100 mL under a nitrogen flow at 30 °C. Finally, a volume of 1 μL of the eluting solution was used for GC–MS analysis.

3 Results and discussion

TEM characterization showed very small G-FeCo NPs with a mean diameter of 4.1 nm and covered by 1–2 layers of graphene. G-FeCo NPs exhibit a typical superpara- magnetic behavior. A very high-saturation magnetization value of 238 e.m.u./g was found for our NPs [5].

Two PAHs as model analytes, fluoranthene and anthracene, were determined by the combination of MSPE and GC-MS analysis.

Limits of detection of PAHs was found in the linear range of 2-200 ng L-1. The accuracy of the method was evaluated by the recoveries of spiked samples. Very good recoveries was achieved with G-FeCo.

Our results proved that the present approach is sensitive and efficient for the extrac-

tion of trace PAHs. This method provided a very high extraction efficiency and short analysis times.

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Benchmarking Spark Data Management Objects for Sequence Analysis

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The introduction of next-generation sequencing technology, about a decade ago, have changed the landscape of biology [1, 2], thanks to the possibility of sequencing DNA at a much faster speed than the one achievable with traditional Sanger sequencing approach[3]. This advancement poses now new problems, mostly about the proper approach to adopt for managing and processing timely the vast amount of data that is produced thanks to this technology. A solution that is gaining popularity in the bioinformatics field is to resort to the technologies that have been developed for dealing with Big Data. By this term, we refer to the problem of storing and/or processing data that may be big with respect to several dimensions like size, diversity or generation rate.

A very popular approach to Big Data processing, allowing the tractability of enormous datasets, is the one based on the MapReduce paradigm[4]. It works by splitting a computation in two steps. In the first step, a *map* function is used to process, filter and/or transform input data. In the second step, a *reduce* function is used to aggregate the output of the map functions. Map and reduce functions are executed as tasks on the nodes of a distributed system. The most used implementation of this paradigm is Hadoop[5].

One of the great advantages of MapReduce and of its Hadoop implementation is the ability to move the computation close to data. This is possible because the distributed system used to run a computation acts also as a distributed file system. This allows to run a computation on all the pieces of data being stored by a node of the system, without having to send the input data back and forth across the network. Another advantage of this solution is the abstraction it provides, leaving the programmer only the task of implementing map and reduce functions, while hiding the remaining (parallel) programming issues.

Despite providing the most advanced implementation of the MapReduce paradigm, Hadoop is often criticized for a number of issues, first being its disappointing performance. A competing framework is gaining a lot of attention in the very recent years: Spark[6]. It is a sort of evolution of Hadoop, but with some important differences allowing it to outperform Hadoop in many application scenarios [7]. First of all, wherever there is enough RAM, Spark is able to perform computations in-memory, without having to write intermediate data on disk, as required by Hadoop. In addition, it goes far beyond Hadoop, by providing a rich set of distributed operations rather than just supporting the MapReduce ones.

The growing success of Spark is also witnessed by the increasing number of Bioinformatics solutions developed using this framework. Sparkseq [8] is a fast and scalable tool for genomic data analysis with nucleotide precision, it allows interactive computation and may run in cloud. ADAM [9] is a genomics analysis

platform released under apache2 license. It allows Big Data Genomics computation supporting a large variety of standard format for storing and managing genomics related data. SparkScore [10], instead, is a set of distributed algorithm implemented in Spark that exploit parallel nature of genomic resampling interference to be applied to genomics analysis.

Indeed, one of the aspect that has the deepest impact on the performance of a distributed application is the way distributed data structures are managed. This is especially the case of Bioinformatics application, where a single genomic sequence may be even several gigabytes long. In such a context, a poor or inefficient data distribution scheme may prevent even a good algorithm to exploit the parallelism of a distributed system.

In Spark, data objects encapsulate, together with semantic information, the operation they support and the way for accessing them. Data objects in the former Spark version are based on RDD (Resilient Distributed Dataset). As the name suggests, they provide a distributed data structure to be used for storing the data process, where the real location of data is completely transparent to the programmer. This data, in turn, can be processed using several operations including map and reduce functions.

The fast development of Spark introduced two other types of distributed data structures, mainly aimed at overcoming one weak point of RDD, that is, the needs for marshalling and unmarshalling of complete objects and their associated metadata. The advancement developed to overcome these weaknesses are the DataFrame, and the Dataset distributed data structures. The former avoid the objects management costs by relying on a schema definition representative of data, at the cost of a less intuitive programming style. The latter tries to keep the best of the two former approaches, by allowing a lightweight data management.

The goal of this paper is to investigate the complexity and the performance of the different distributed data structures offered by Spark, with the aim of providing useful hints to the biology community about which is the best option to choose. This has been done by analyzing the three different solutions when used for the implementation of a typical algorithmic pattern found in Bioinformatics application. That is, the extraction and the count of the distinct k-mers found in an input sequence of characters. The three outcoming implementations have been tested on two different datasets: the first consisting of a few number of very long sequences, the second consisting of a large number of very short sequences.

The results of the experimentation show that the different strategies employed by the Spark distributed data structures for managing input data have a deep impact on the performance of the algorithm using them. As expected, the experimental results show that the RDD approach is the one exhibiting the worst behaviour, especially when dealing with a large number of small objects, as its performance are compromised by the overhead to be paid for managing the metadata associated with each object. On the contrary, DataFrame and Dataset exhibit better performance, also proving to be in someway resilient to the structure of the data input pattern.

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Probing antimicrobial activity of star-like polymers by AFM based experiments

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Abstract. This experimental work is focused on the analysis of antimicrobial activity of novel star-like $A(BC)_n$ copolymers, based on the m-PEG (block A), the methylmethacrylate (MMA) and the nonquaternized 2-(dimethylamino)ethyl methacrylate (DMAEMA) (blocks BC). The final goal is to inspect the use of such armed copolymers as substrates to prevent bacterial biofilm contamination. As shown by microbiological assays, copolymer films with comparable amounts of DMAEMA have antimicrobial properties strongly depending on the topological structure (i.e., the number of arms). In particular $A(BC)_2$ showed a higher antimicrobial activity with respect to $A(BC)_4$ and linear copolymers A(BC)[1].
Here, copolymers surface properties and antimicrobial activity are evaluated by AFM based experiments. The copolymer samples were made in the form of thin films (20-200nm thick) by drop casting methods on glass substrates. The effect on roughness and stiffness of a prolonged soak in water was followed in time, revealing a gradually increase of roughness together with a reduction of stiffness for all the studied polymers. Unexpectedly a reentrance of the Young modulus for the $A(BC)_4$ sample was observed after permanence in water for more than 24 h.

The antimicrobial activity was tested on Escherichia Coli by Fluorescence-Atomic and Force Spectroscopy combined experiments. First, the in fluid-AFM experiments were performed on healthy bacteria in order to get insight into their usual morphology and stiffness. Consequently bacteria were kept in water suspension together with 1 cm^2 of polymeric films, for different incubation time, and later measured by AFM to unveil possible membrane weakness effects. The viability of bacterial populations as a function of the membrane integrity of the cell was checked by means of fluorescence microscopy by using syto 9 and propidium iodide fluorophores, helping us to discern between dead or live bacteria before analyzing them by AFM. Results indicates that the interaction with $A(BC)_2$ copolymers causes a reduction of the external membranes stiffness.

As a different experimental AFM based approach, single cell force spectroscopy was also employed to measure the bacterium-surface adhesion properties. In this case bacterial cells were immobilized at the free end of a tipless cantilever and force-distance curves were performed against either glass surface or polymer surface. By using this technique specific cell-surface interactions were detected and change in adhesion was followed in time.

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Air assisted production of alginate beads using focusing flow microfluidic devices: numerical modeling of geometrical effects on beads formation

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Alginate micro-bead production represents an interesting technological application in many fields such as pharmaceutical, food, and cosmetics. Usually the studies of micro droplet or micro-bead creation in micro channels formed in different geometries and different techniques (mostly T channel or flow-focusing) have been the subject of many research studies using pure, well characterized solutions and do not take into account the behavior and interaction of food grade and natural products . The possibility of using air as focusing flow (*Bong et al., 2010*) in microfluidic devices to produce sodium alginate micro-bead introduce some advantages; for example, the utilization of different focusing fluids like oil frequently requires complicate production processes, introducing a barrier to the interaction of alginate solution with the calcium ions during gelification phase and requiring a posteriori filtering and washing procedure.
Moreover, direct immersion of liquid alginate drops in a calcium chloride bath to induce gelification usually happens at relatively high speed, inducing a bead shape deformation due to inertial effects.
As in microfluidics details really matter, the geometry of the device represents an important issue: amsll changes in geometrical configuration, like coaxial misalignment could results in major changes in the dynamics of droplet formation.
In this work such effects are investigated for different focusing and co-focusing microfluidic devices in order to control jet instabilities (Vega et al, 2010) , showing that small changes could trigger different dynamics in beads formation.

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