MICROFLUIDIC SYSTEMS TO STUDY ACTIVE MOLECULES

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Over last years, the study of molecules has broadened their potential utilizations in many fields, ranging from the agricultural to industrial and biomedical sectors [1].
In particular, the study of active molecules (*e.g.* proteins, DNA and RNA strands) has caught the attention of pharmaceutical industries to discover new products that have become or potentially could be top sellers. The understanding of molecules behaviour is of huge importance for many reasons, including discovering of their potential applications (*e.g.* therapeutic) and interpretation of phenomena or diseases occurring in human body. A complete comprehension of molecules function and behaviour is not possible to achieve if they are not properly involved in well-defined microenvironmental conditions. In other words, a same molecule may exhibit several behaviours depending on the surrounding. As matter of fact, it is well known that both physical and chemical stimulations like mechanical stress, light conditions, temperature changes, pH level and tonicity of surrounding solution (ions and co-solutes concentration) strongly affect molecules structures and properties, thus compromising their stability [2,3].
Currently, typical strategies to study active molecules stability both at the research and at the industrial level rely on *in batch* static protocols. Poor control and scarce tuneability of fluid dynamic parameters, as well as inefficient controllability of thermodynamic and chemical variables, are the main limitations of the widely diffused *in batch* strategies. In addition, they are time and reagent consuming, since every time an active molecule is wanted to be tested at different conditions, a new experimental setting is needed, thus involving the use of fresh reagents.

The miniaturization of analytical systems can provide several advantages including the use of small amount of sample to be analysed, high precision detection and analysis, shorter time for a higher number of analyses, portability of analytical devices. The potentialities of miniaturizing, along with the demand of high controllability of experimental parameters, time and costs, lead to consider the use of the *microfluidics* (*i.e.* the dynamic manipulation of small volumes of reagents in a network of microchannels). Microfluidics has gained the attention of many pharmaceutical industries since it represents a great tool to provide a *disruptive innovation* and a *paradigm shift* in the study of active molecules. In fact, the microfluidics offers a *superb control of the transport phenomena* occurring throughout microchannels, giving the opportunity of a fine tuneability of fluid dynamics (high control on viscous and inertial forces and their interplay), mass transport (diffusive and convective contributions) and thermodynamic conditions.

The PhD project aims to study, through the microfluidics, the instability phenomena induced by specific stimulations of biological molecules such as protein, antibodies, oligonucleotides etc. The study finds several application fields, ranging from the nanotechnologies to the precision medicine, also including the comprehension of metabolic and thermodynamic phenomena as well as the development of industrial processes.

Precisely, it is intended to design and realize a smart and versatile microfluidic platform able to provide *physicochemical stimulations* and with the capability of *inline real-time monitoring* the molecules conditions. In other words, in the very same platform it is intended not only subjecting active molecules to different stimuli, but also to directly monitor their current physicochemical properties while they are in flow, with the final goal of investigating on their properties.
More in detail, the microfluidic platform is thought to present two main compartments: the stimulating and the analytical one. The former is intendent to provide thermal, light, mechanical and chemical stimuli with a fine control of the process parameters and automation of fluid manipulation. The latter, based on some analytical techniques such as dynamic light scattering, turbidimetry, fluorescence etc., wants to exploit the emerging technologies of the artificial intelligence (*e.g. machine learning*) to help data collection and inline analysis to readily get results about molecules conditions as soon as they experience the stimulations.
The PhD project wants to bring *technological innovation*, since for the first time a complex and versatile microfluidic platform for active molecule stimulation and analysis would be realized. Beside this, also *scientific progress* is intended

to be introduced, as a paradigm shift in the exploration of biological molecules instability under specific stimulations along with the possibility to get an improved comprehension of involved metabolic and thermodynamic phenomena, can be reached with reduced time and costs.

References:

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