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# Moving Forward in Biosensing Technology to Decode the Human Pathology Interactome and to Identify New Drugs

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Apart from few monogenic pathologies, human diseases are the outcome of the concurrent contribution of many processes, as it occurs in cardiovascular diseases and cancer, which can be started by infections that activate inflammation that, in turn, induces neovascularization from which depend both atherosclerosis and tumor growth and metastatization. At a molecular level, all these processes are governed by an intricate network of extracellular interactions (the so called "extracellular interactome") occurring among microbial proteins, inflammatory cytokines, growth factors and extracellular matrix components that engage cellular receptors. In turn, ligand/receptor engagement transduces a signal that, inside the cell, rapidly recruits plenty of second messengers, transactivating factors and nucleic acids that interact among themselves generating what can be defined the "intracellular interactome", whose complexity mirrors that of the extracellular one.

In this scenario, what becomes mandatory is a throughout comprehension of the intra- or extracellular interactome(s) functional to draw 'connectivity maps' that could lead to the identification of "hub-interactions", whose inhibition could affect multiple signaling pathways implicated simultaneously in more than one of the above mentioned processes, more likely causing the overall failure of the disease.

Once a therapeutically relevant "hub-interaction" is identified, a whole new demanding work must start, consisting in the identification of new drugs or antibodies able to bind to one of the proteins participating to the chosen interaction. Relevant to the point, this aim is more often pursued by screening libraries consisting of hundreds, if not thousands of molecules. Unfortunately, the most common procedures for macromolecular interaction analysis are rather lengthy, complex and demanding, being affected by limitations related to the use of large amounts of highly purified proteins (i.e micro-calorimetry), of expensive equipments (i.e. surface plasmon resonance, SPR), or involving the labeling of the interactants (i.e radioisotope or fluorochrome tags), or using secondary antibodies or enzymes to generate a signal (i.e ELISA assay) [1, 2] . From this scenario it emerges the need for simple, reliable and economic detection devices to speed up the analysis of the interactome(s) and/or to lower the cost of the screening of pro-drugs libraries. At this regard, two very promising, easy-access and label-free biosensing technologies have been recently described: Contact Angle Molecular Recognition (CONAMORE) [2-4] and Reflective Phantom Interface (RPI) [1].

CONAMORE emerges as one of the most inexpensive nanomechanical biosensors, from both the point of view of equipment and volume of reagents, which may go down to few hundreds of nanoliter of ligand solution per run. It exploits the fact that part of the energy released by a ligand-receptor interaction confined at a solid-liquid interface is translated into a variation of the surface wettability (viz. of the solid-solution interfacial tension), that mirrors with high fidelity the work performed to "accommodate" the ligand onto the surface and to drive the related nanoscale phenomena [5]. This wettability variation can be easily tracked by a *ad hoc* variant of the classic sessile drop contact angle technique (Fig.1).

CONAMORE displays comparable performance in terms of reliability and sensitivity to conventional label and label-free assays, but at lower cost and with the peculiarity of probing both ligand-receptor surface interaction and nanomachinery [5]. On the other hand, its architecture does not allow using it for high throughput and real time analysis. These features suggest it as important complement to mass/optical based biosensors, such as SPR biosensors.

For example, a CONAMORE assay was assessed to investigate tumor angiogenic ligand–receptor protein interactions [4]. Subsequently it was put in action to study the role of nanomechanics in activation of the vascular endothelial growth factor receptor-2 (VEGFR2) by its prototypic ligand VEGF-A [3], two molecules that acts as main pro-angiogenic factors involved in both tumor growth and metastatization and atherosclerosis. Results from CONAMORE combined with those from SPR spectroscopy and in-vivo assays allowed to widen the description of ligand-VEGFR2 interactions, pointing to in-plane pN intermolecular attractions between activated receptors as a key modulator of the intracellular signaling cascade.

A completely different approach lies at the basis of the RPI method. The detection principle (covered by patent) relies on the measurement of the light reflected by a particular perfluorinated plastic material, which becomes substantially invisible when immersed in water, because of the matching of the refractive indices of the two media. The surface of this material is treated in order to present dozens of spots with size of a tenth of a millimeter, containing different antibodies toward specific molecular targets, which, adhering on the surface even in small amounts, yields a pronounced increase of the reflected light (Fig.2) [1]. The surface reflectivity is then imaged using simple equipment, basically consisting of LED illumination and CCD camera detection.

Despite the simplicity of the approach, the RPI method enables a direct (label-free) and real-time quantification of the amount of interacting target molecules through the basic formulas of thin films optical reflection. The resulting sensitivity is comparable to other commercially available solutions for optical label-free biosensing. In

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particular, the general performance is similar to that of widely used SPR instruments, albeit the RPI system is much less complex. Using a measuring cell consisting of a common 1-cm cuvette containing a magnetic stirring bar to mix the solution and a prism of perfluorinated plastic, it was shown that diagnostically relevant concentrations (few ng/ml) of Hepatitis B and HIV markers (Fig. 2) can be detected in few minutes without the need of adding fluorescent or colorimetric labeling agents [1].

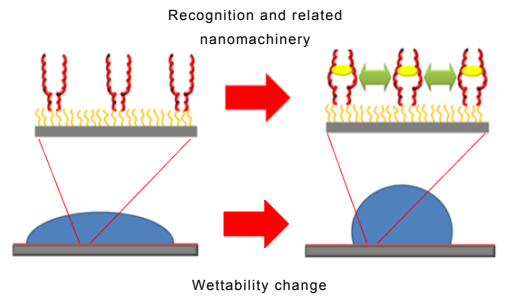


**Figure1: CONAMORE working principle.** A 300-500 nL droplet of ligand solution is deposited on a chip whose surface has been activated with the receptor. The nanomachinery occurring at the solution-chip interface upon ligand-receptor interaction triggers a variation of the surface wettability, that is mirrored by a change of the shape and contact angle of the solution droplet. Adapted from Ref. [3].

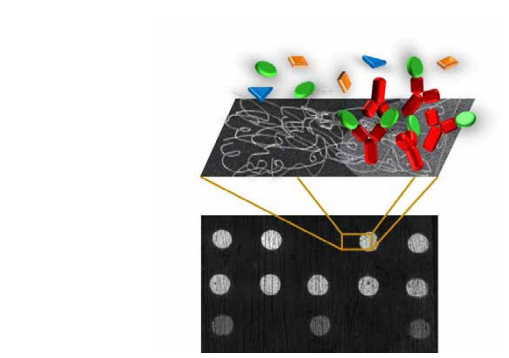

**Figure 2: Image acquired with the RPI method.** The surface of a prism of perfluorinated material isorefractive with water was coated with a functional copolymer enabling the immobilization of spot (200 m diameter) of different antibodies toward p24 capsid protein (upper and lower rows) and hepatitis B surface antigen (middle row). The amount of target molecules binding on the spots is directly obtained from the local brightness of the image, representing the intensity of light reflected by the surface. Adapted from Ref. [1].

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Real-time monitoring of the binding signal provides an intrinsic time-to-result optimization, since an unknown target concentration in solution can be estimated from the initial slope of the measured binding curve, hence well before reaching the equilibrium of the interaction. On the other hand, if the sample concentration of target molecule is known, the equilibrium and kinetic parameters fully characterizing the interaction with the immobilized probes are obtained from the analysis of the binding curves measured at increasing target concentrations, from fractions of ng/ml to hundreds of μg/ml.

Beside the undoubted contribution that CONAMORE and RPI will bring to the unraveling of the human pathology interactome, they will also provide unique opportunities not achievable with the available biosensing technologies. In effect, the physical principles on which is based CONAMORE will make easier the study of phenomena such as conformational changes, collective protein-protein interactions, clustering and recruiting of coreceptors, that are common themes of cell signaling. Important to note, this nanomachinery is often ignored or underestimated in in-vitro studies, probably because of the difficulty of their probing. Also, these kinds of analysis are hardly feasible with SPR that, to date, represents the golden standard of biosensing technology. On the other hand, the simplicity of the instrumental setup of RPI and the possibility to easily shape the sensing plastic substrate by moulding, pressing or mechanical machining, imply that RPI-based devices can be constructed using low-cost components, thus providing unprecedented flexibility in the design of biosensors for the analysis of macromolecular interaction.

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### References

1. Giavazzi F, Salina M, Cerbino R, Bassi M, Prosperi D, et al. (2013) Multispot, label-free biodetection at a phantom plastic-water interface. Proc Natl Acad Sci U S A 110: 9350-9355.
2. Bergese P, Oliviero G, Colombo I, Depero LE (2009) Molecular recognition by contact angle: proof of concept with DNA hybridization. Langmuir 25: 4271-4273.
3. Maiolo D, Mitola S, Leali D, Oliviero G, Ravelli C, et al. (2012) Role of nanomechanics in canonical and noncanonical pro-angiogenic ligand/VEGF receptor-2 activation. J Am Chem Soc 134: 14573-14579.
4. Olivero G, Maiolo D, Leali D, Federici S, Depero LE, et al. (2010) Nanoliter contact angle probes tumor angiogenic ligand-receptor protein interactions. Biosens Bioelectron 26: 1571-1575.
5. Federici S, Oliviero G, Maiolo D, Depero LE, Colombo I, et al. (2012) On the thermodynamics of biomolecule surface transformations. J Colloid Interface Sci 375: 1-11.