Final Publishable JRP Summary for HLT04 BioSurf
Metrology for the characterisation of Biomolecular Interfaces for Diagnostic Devices

Overview

This project developed methods to reliably and consistently measure the performance of biochemical interfaces used in in-vitro diagnostic devices (IVDs). We created the first reference biomolecular interfaces for IVDs, and developed techniques to more accurately characterise the properties of probe and target molecules at these interfaces. These developments will allow IVD manufacturers to develop increasingly accurate and reliable devices for a broader range of health conditions. Resulting in faster diagnoses at the point of patient care, ultimately helping to more effectively treat disease and drive down healthcare costs in Europe.

Need for the project

Healthcare costs are rising rapidly throughout Europe as populations' age and rates of chronic disease increase. The World Bank estimates that public expenditure on healthcare in the EU could rise as high as 14% of GDP by 2030 (up from 8% in 2000), continuing to rise thereafter. IVDs are handheld devices that have the potential to reduce healthcare costs through rapid diagnosis at the point of care (at home or in the field), lowering the costs of diagnosis by replacing expensive laboratory techniques and equipment, and by easing the demand for hospital services.

Although IVDs have great potential, only a small number are currently produced for a limited number of medical conditions, as it is challenging to manufacture devices that satisfy the performance requirements of the European IVD directive (98/79/EC). IVDs work by detecting target molecules in patient samples that are indicative of disease or other adverse health conditions. Target molecules are identified by using a probe molecule, which binds specifically with the target molecule. These probe molecules are attached to an interface, a surface that makes contact with the patient sample. The probe molecules on the interface ensure that the target molecules are held, and all other molecules in the sample rejected. The IVD detects changes at the interface, and needs to be sensitive enough to detect the small number of bound targets. The number of probe molecules present at the interface, and their ability to function, is the key determinant of IVD performance. However, there are no standard, industry-wide techniques to measure the properties and performance of interfaces, and lack of consistency in interface chemistry is the chief cause of accuracy and reproducibility issues in IVDs. Standard, accurate techniques are needed to understand the properties of biomolecular interfaces, in order to manufacture high-quality, reliable devices.

Specifically, techniques are required to quantify the amount of probe present at the interface, and the efficiency by which targets bind with probes, in order to accurately identify levels of target molecule in a sample. The development of innovative techniques to characterise the distribution, orientation and structure of probes would also further enhance diagnostic accuracy. As would the ability to detect multiple different targets with one interface, as health conditions can produce a range of target molecules, and their combined measurement would provide more detailed diagnoses. IVDs predominantly use flat interfaces, but interfaces based on nanoparticles are attractive, as they can indicate target binding events simply and clearly. But despite a number of proof-of-principle demonstrations, few nanoparticle-based IVDs are used in clinical practice. Measurement techniques need to be validated for both a reference flat interface, and a nanoparticle interface, to support the current use of flat interfaces and encourage the development of nanoparticle-based devices.

Scientific and technical objectives

The following objectives were set to achieve the overall goal of providing techniques to better characterise the properties and performance of biomolecular interfaces:

1. Produce reference flat and nanoparticle interfaces: Establish accurate, traceable and comparable methods to determine the amount of biomolecular probe immobilised on each interface. An inter-laboratory comparison will validate these methods using the reference interfaces produced.

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2. Use innovative approaches to determine the orientation and structure of biomolecules at an interface, and develop useful measurement approaches for the research, development and quality control of biomolecular interfaces in diagnostic devices.
3. Develop novel approaches for the measurement of interface structure that can be correlated with activity and binding efficiency. Develop methods to measure and predict the activity of immobilised probes, by measuring the activity of diagnostic surfaces, quantifying and modelling the interaction between probes and targets.
4. Assess and evaluate the capabilities of new, emerging techniques and approaches to biomolecular sensing which enable multiplexed, label-free identification and quantification of bound targets.

Results

1. Produce reference flat and nanoparticle interfaces: Establish accurate, traceable and comparable methods to determine the amount of biomolecular probe immobilised on each interface:

Versions of the same IVD with different amounts of probe will capture varying proportions of target molecules from the same sample, and may give different diagnoses. Therefore it is vital to understand how much probe is present at an interface. As interface chemistry varies between different IVDs, in order to develop accurate and traceable benchmark measurement methods, standardised reference surfaces must first be developed.

We achieved this by:

- Developing the first ever flat reference interface, based on biotin molecules (a commonly used probe) attached to a gold surface, and developing the first reference nanoparticle interfaces, also based on gold.
- Probe density on these interfaces could be accurately measured, and could be adjusted from 100% to 0.1% coverage, a ten-fold increase in precision over previous methods.

A major project highlight was the achievement of the first successful inter-laboratory study of nanoparticle interface chemistry, in which consistent results were achieved across each laboratory. The collaboration demonstrated that reproducible interface measurements could be achieved in different locations, using the reference interfaces, if standardised preparation and data analysis procedures were followed.

2. Use innovative approaches to determine the orientation and structure of biomolecules at an interface:

The amount of probe is not always the only guide to the functional performance of interfaces, as interfaces with poorly distributed (clumped) probes capture less target than surfaces with more evenly distributed probes. Probe molecules have active sites where they bind with target molecules, if probes are in the wrong orientation, or have lost the structural shape of their active site, they will not be able to bind with their targets. Techniques that provide information on probe distribution, orientation and structure will further enhance diagnostic performance.

We developed proof-of-concept techniques for these measurements by testing two methods previously identified in the literature as possible approaches:

- We demonstrated the first use of soft X-ray spectroscopy to measure properties of the interface, and developed a novel measurement device to analyse probe molecule distribution, orientation and structure.
- Also for the first time, we used Secondary Ion Mass Spectrometry (SIMS) to measure both the distribution and structure of probes on flat interfaces.

The techniques developed represent a vital first step in the measurement of these additional probe properties, and present a direction for further research to incorporate the techniques into commercial devices.

3. Develop novel approaches for the measurement of interface structure that can be correlated with activity and binding efficiency:

In addition to understanding the properties of probe molecules at the interface, it is also vital to understand the efficiency by which the probes are binding with their target, to accurately assess the level of target molecules in a sample.

We studied the binding of avidin target molecules with the biotin reference surface, as avidin selectively and strongly binds with biotin, and is used routinely in IVDs. Two techniques were used. Ellipsometry measures changes in the speed of light at the interface caused by target binding. The quartz crystal microbalance (QCM) method measures changes in mass and stiffness at the interface caused by target binding.

- Simultaneous measurements with ellipsometry and QCM demonstrated that the sensitivity of the QCM method dropped as target density increased. Therefore accurate QCM measurements can only be performed if the probe molecules are separated by a specific minimum distance. This result demonstrates the importance of the reference surfaces in establishing the response of detection systems.
- The two methods revealed a novel approach to determine the type of binding between biotin and avidin targets – avidin bound by two biotin probes was significantly stiffer than avidin bound by one biotin probe. This binding was easily detected using QCM.

To study target binding of avidin with the reference nanoparticle surface, optical spectroscopy was used to detect changes in colour of nanoparticles as they bound with targets. This approach is potentially very accurate, but can be made uncertain if targets bind with multiple nanoparticles (agglomeration). To ensure measurement accuracy, agglomeration must be accounted for.

- We developed a method for measuring the number of target molecules attached to each nanoparticle by combining the optical spectroscopy with particle size measurement.
- We used high resolution sedimentation techniques to demonstrate that genetically modified avidin molecules with a single binding site did not produce agglomerates, but normal avidin molecules did form agglomerates.

4. Assess and evaluate the capabilities of new, emerging techniques and approaches to biomolecular sensing which enable multiplexed, label-free identification and quantification of bound targets:

Health conditions may produce multiple target molecules, and measuring more than one can provide a more accurate diagnosis. For example, in the treatment of heart attacks, three target molecules are measured to determine the time since the heart attack, and to treat the patient appropriately. In most situations, it is necessary to 'tag' target molecules with 'label' molecules that can be more easily detected. However, sometimes this is not possible, either because the target has not previously been identified, or the presence of the label changes the behavior of the target. In these cases a multiplexed method is preferable, which detects multiple targets without using label molecules.

- A novel optical waveguide device was developed and demonstrated, capable of ultra-sensitive detection of molecules on surfaces without using labels. The waveguide device uses light to cause target molecules bound by probes to fluoresce, so that they can be detected directly. This device offers major advantages over conventional methods in terms of ease of use, the adaptability to different illumination wavelengths, a larger field of view, and the ability to detect unlabelled targets directly.
- Additionally, the ability of secondary ion mass spectrometry to detect multiple target molecules using unique labels was demonstrated for the first time. And label-free detection of targets on a diagnostic surface using ambient surface mass spectrometry was demonstrated.

Multiple techniques were developed for multiplexed, label-free target identification, providing avenues for further research to refine the techniques for commercial exploitation.

Actual and potential impact

Dissemination of results

To promote the uptake of the outputs of this project, results have been shared through the publication of 15 papers in international journals (listed in the next section), and over 60 presentations delivered at international conferences and workshops, including five invited talks. The number of invited talks is testament to the fact that our project consortium is seen to be leading the European effort to provide measurement methods for biological interfaces. Stakeholder engagement was greatly enhanced by the consortium bringing together world-leading experts at two international workshops: the 2012 65th IUVSTA workshop on "Measuring molecular Adsorption at the Solid-liquid Interface", and the 2015 "Nanoparticle concentration, chemistry and interfaces" workshop. Two press releases highlighted the work of the

consortium, and resulted in an interview with the journal "The Analytical Scientist". In addition, five training courses were organised during the project to share best practices amongst the consortium members and external stakeholders. BioSurf consortium members have also been consulted by several research groups, including the University of Washington, the Technical University of Vienna and the University of Manchester, on the best approaches to understand and measure nanoparticle surface chemistry.

Impact on standardisation

This project will have a fundamental impact on standards through enabling the initiation of the first ever European standardisation activities for measuring the properties of interfaces. During the project, consortium partners participated in the Versailles Project on Advanced Materials and Standards (VAMAS) and ISO meetings: VAMAS TWA 2 "Surface Chemical Analysis", ISO TC 201 "Surface Chemical Analysis" and ISO TC 229 "Nanotechnology". Resulting in a request for an ISO TC 201 SC4 New Work Item on "Surface chemical analysis - Characterization of Glass substrates for biosensing applications", arising from the work by BAM. As a result of an ISO TC 201 request, an inter-laboratory comparison was launched to use the reference gold nanoparticle interfaces to develop an ISO standard on the preparation of nanomaterials for surface chemical analysis.

Early impact on industry

Through participating in the VAMAS study, we shared our improved methods for measuring nanoparticle interface properties with 14 research organisations, 6 national laboratories (including the NMIs of Brazil, China and Korea) and 4 instrument manufacturers. These institutes perform measurements for the developers of IVD systems, and more accurate measurements will support the production of new and improved IVDs.

Two patent applications have been prepared by two academic partners. A researcher based at Technische Universität Berlin has filed an application for their experimental cell for soft X-ray fluid interface analysis. A researcher based at Chalmers Tekniska Högskola AB has filed an application for their novel optical waveguide device, which has gained interest from both the public and private sector, including pharmaceutical company AstraZeneca.

Potential future impact on industry

The reference interfaces and traceable methods developed in this project are expected to have a direct impact on the manufacture of IVDs in Europe. Almost all European manufacturers of IVDs, and private test laboratories offering clinical diagnosis services, are SMEs that lack the financial and technical infrastructure required to develop standardised measurement techniques for biomolecular interfaces. In contrast to Europe, National Measurement Institutes (NMIs) in North America and Asia already support manufacturers by providing such standardisation activities. With this project we have initiated a new area of activity for European NMIs, creating the foundational infrastructure from which high-performance IVDs can be developed and tested. The results of this project will allow European IVD manufacturers to produce devices that consistently meet the requirements of the EC IVD directive, and which will be competitive in European and international markets.

Specific, high-potential innovations we developed include:

- The novel waveguide technique opens up new opportunities for carrying out measurements with both improved sensitivity and increased ease. The potential commercialization of the technique will likely have significant social impact as it will facilitate a more sensitive, simpler, faster and more precise detection of disease biomarkers, considerably improving diagnoses and the selection of successful treatments. To demonstrate this, we successfully detected Amyloid-beta molecules, a biomarker for Alzheimer´s disease, using a gold nanoparticle interface.
- The possibility of monitoring scattered light from surface immobilized nanoparticles has raised an interest in using the waveguide device for nanoparticle size determination, which could have a considerable impact within the nanotechnology community because of the proposed EU definition of nanomaterials and their potential regulation. The waveguide device has also gained interest from the private sector,

particularly from the pharmaceutical company AstraZeneca, which sees the waveguide as a possible tool for carrying out screening of drug candidates with extraordinarily high sensitivity.

- There has also been a considerable interest within the research community to use the waveguide device for studying the interaction of virus and bacteria with interfaces. Collaborations have been initiated with BOKU University in Vienna and the University of Göttingen, who want to use the device to study bacteria and the kinetics of platelet activation.

We anticipate the results of this project will be used by manufacturers to develop more accurate and reliable IVDs for a broader range of medical conditions. This will lead to a more competitive European IVD industry, will aid in the treatment of disease, and will ultimately provide a much needed means to help tackle the rise in public healthcare expenditure throughout Europe.

List of publications

- Quantitation of IgG protein adsorption to gold nanoparticles using particle size measurement. N. C. Bell, C. Minelli, A. G. Shard. Analytical Methods, 2013, 5, 4591-4601. (Front Cover Image)
- Recent advances in vacuum sciences and applications, M. Mozetič, P. Dietrich, W. Unger, et al. Journal of Physics D: Applied Physics 2014, 47, 153001.
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- Cation-mediated electrostatic interaction in collagen–integrin complex, P. Ansalone, O. Bottauscio, A. Manzin. Surface and Interface Analysis 2014, 46, 693-697.
- Liposome binding for multiplexed biomolecule detection and imaging using ToF-SIMS. P. Sjövall, B. Agnarsson, L. Carlred, A. Gunnarsson and F. Höök. Surface and Interface Analysis 2014, 46, 707–711.
- Simultaneous Imaging of Amyloid-β and Lipids in Brain Tissue using Antibody-coupled Liposomes and Time-of-Flight Secondary Ion Mass Spectrometry, Louise Carlred, Anders Gunnarsson, Santiago Solé Domènech, Björn Johansson, Vladana Vukojevic, Lars Terenius, Alina Codita, Bengt Winblad, Martin Schalling, Fredrik Höök, Peter Sjövall, Journal of the American Chemical Society, 2014, 136, 9973–9981.
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- Nanoparticle characterization by continuous contrast variation in small-angle X-ray scattering with a solvent density gradient, R. Garcia-Diez, C. Gollwitzer, M. Krumrey. Journal of Applied Crystallography: 48 (2015), 1, 20 – 28.
- En route to traceable reference standards for surface group quantifications by XPS, NMR and fluorescence spectroscopy, A. Hennig, P. M. Dietrich, F. Hemmann, T. Thiele, H. Borcherding, A. Hoffmann, U. Schedler, C. Jäger, U. Resch-Genger and W. E. S. Unger. Analyst 2015, 10, 1039.

JRP start date and duration:	
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The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union