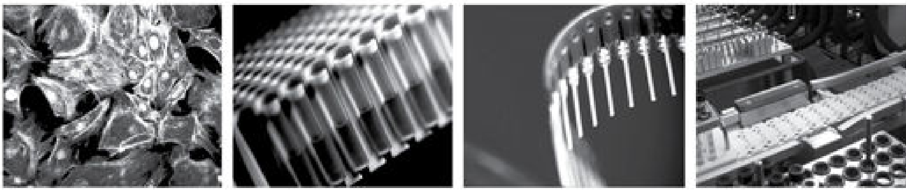

TTP LABTECH

Biomolecular interaction analysis in undiluted human serum at physiological temperature using label-free resonant acoustic profiling (RAP)

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Introduction

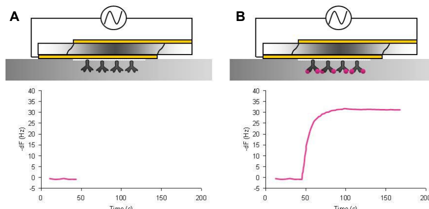
The detection and knowledge of the molecular basis of protein-protein recognition is an essential element in understanding protein function because their ability to form specific complexes with other proteins underlies most cellular processes.

The use of labels has limitations, such as changes to the binding kinetics due to the alterations in structure and function that occur with label addition, the difficulty in detecting biochemical activities, and the need for additional steps in assay development. These issues have driven the development of label-free formats for identifying the full range of biochemical activities.

Although optical-based systems dominate the biosensor market, piezoelectric and acoustic devices represent similar but significantly less expensive alternatives. Acoustic biosensors have been employed in the label-free detection of an incredibly broad range of analytes: from interfacial chemistries and lipid membranes to small molecules and whole cells. Resonant Acoustic Profiling (RAP) technology offers label-free, real-time analysis of biomolecular interactions. The analytical capabilities of a RAP-based biosensor are ideally suited to the development of biotherapeutics, a rapidly expanding area of drug research. In particular, direct measurement in crude and complex samples, such as cell culture media or periplasmic extracts, eliminates expensive time-consuming purification of often limited material while delivering high content information.

Biological and biotechnology-derived proteins are increasingly used as therapeutic agents. These products may induce an unwanted immune response in treated patients, which can be influenced by various factors, including patient- or disease-related factors and product-related factors. The consequences of such immune reactions to a biotherapeutic range from transient appearance of antibodies without any clinical significance to severe life threatening conditions. Therefore, it is essential to adopt an appropriate strategy for the development of adequate screening and confirmatory assays to measure an immune response against a therapeutic protein. Protein-protein interactions are affected by the environments that they are studied in, thus the ability to study these effects at physiological temperature and in a physiological milieu, such as serum, enables the generation of more biologically relevant data than has previously been permitted using biosensors.

1. RAP Biosensor Technology



(A) A quartz resonator coated with target receptor is integrated with a liquid system for sample delivery. The target is attached to the sensor surface through direct linkage or capture. With buffer passing over the sensor surface the resonant frequency is measured in real time and displayed on a sensorgram (baseline). (B) Samples containing potential binding partners are then applied to the sensor surface. Analyte binding results in a change in the resonance profile of the resonator. The change in frequency (-dF, Hz) is recorded on the sensorgram, providing information about the specificity, affinity, kinetics and concentration of molecular binding interactions in real-time.

2. Reference Control Subtraction

A reference surface and blank buffer injections were applied for all experiments to improve the quality of the binding data by correcting for artefacts such as bulk changes, matrix effects, nonspecific binding, injection noise and baseline drift. RAP technology was successfully used to detect the binding of recombinant human TNF-α to a monoclonal human TNF-α antibody (TNF-α mAb) in 100% human serum. A bulk shift was observed in the reference channel which was reference subtracted from the raw data collected in the active channel.

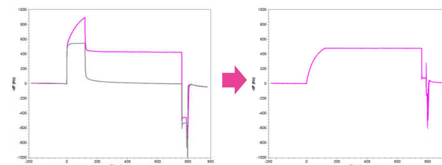


Fig 1. Reference control subtraction of a bulk shift caused TNF-α mAb by differences in viscosity and density between running buffer and sample. A rabbit anti-mouse antibody was immobilized as control surface. Human TNF-α was injected over the active channel (TNF-α mAb) (pink) and the control channel (grey) in parallel.

3. Kinetic analysis of a specific mAb binding to human TNF-α in HEPES buffer and undiluted human serum at 25°C and 37°C

Binding kinetics were determined using RAP technique by flowing several concentrations of human TNF-α (17.1 kDa) over the immobilized antibody. After double reference subtraction, a 1:1 Langmuir binding model was fitted to the concentration data and kinetic parameters were determined (Table 1). The binding interaction of the antibody with human TNF-α were measured in HEPES buffer at 25°C (Fig 2A) and at 37°C (Fig 2B), in 100% human serum at 25°C (Fig 2C) and at 37°C (Fig 2D). The dissociation constant (K_D) of ~1.8 x 10^{-10} M, determined in buffer as well as undiluted human serum, correlates very well with previously reported data of the commercially available therapeutic antibody adalimumab. Both groups carried out the kinetic analysis using SPR technology and measured a K_D of ~1.0 x 10^{-10} M† and ~1.8 x 10^{-10} M‡, respectively.

Table 1: Kinetic binding parameters are listed for the interaction of monoclonal antibody to human TNF-α with recombinant human TNF-α. All parameters were determined by RAP biosensor analysis. The respective dissociation constant (K_D) is represented as mean ± SD from at least two independent sets of experiments on two sets of sensors.

Sample	k_{on} (x 10^5 M^{-1} s^{-1})	k_{off} (x 10^{-5} s^{-1})	K_D (x 10^{-10} M)
HEPES buffer 25°C	1.0 1.3 1.4	1.9 2.4 2.4	1.9 1.8 1.7
			1.8 ± 0.10
HEPES buffer 37°C	3.8 3.0	7.0 5.8	1.8 1.9
			1.8 ± 0.07
100% human serum 25°C	1.0 1.4 1.2	1.7 2.4 2.4	1.7 1.7 2.0
			1.8 ± 0.17
100% human serum 37°C	1.0 1.5 1.7	5.4 6.9 5.6	5.4 4.6 3.3
			4.4 ± 1.06

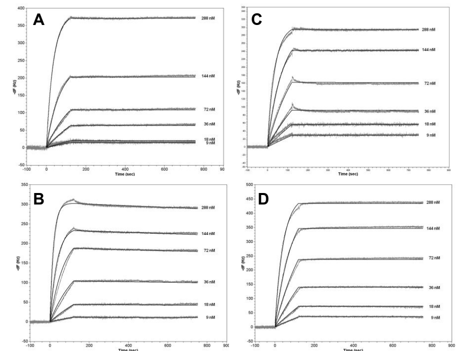


Fig 2. Representative sensorgrams for the kinetic binding interactions obtained from injections of human TNF-α at 9, 18, 36, 72, 144 and 288 nM over an anti-human TNF-α antibody immobilized surface.
A mouse mAb against human TNF-α with a high picomolar binding affinity was characterized in buffer at 25°C (A) and 37°C (B) as well as in undiluted human serum at both 25°C (C) and 37°C (D), respectively. To our knowledge, this is the first time a biosensor has been used to characterize a biomolecular interaction in 100% serum and at physiological temperature.

The RAP based biosensor provides an equivalent technology to alternative biosensor technologies; in addition, the insensitivity to complex media ensures that samples can be processed with minimal purification or separation. This makes it an efficient technology for the generation of kinetic, affinity and concentration data in both buffer and complex matrices.

RAP Biosensors – A sound alternative to optical biosensors

Resonant Acoustic Profiling (RAP) technology offers label-free, real-time analysis of biomolecular interactions. RAP only measures physical binding events and is insensitive to refractive index and color changes. It enables high quality kinetic analysis and concentration measurements over a 3-log dynamic range in complex samples such as cell culture media or periplasmic extracts.

Direct measurement in undiluted crude and complex samples that may contain impurities such as organic solvents, serum or growth media simplifies experimental design, and eliminates expensive time-consuming purification of often limited material while delivering high content information.

Benefits of label-free RAP biosensor analysis include:

- Improved BIA assay performance in real-time
- Processing undiluted minimal purified samples
- Biologically relevant data
- Flexible assay design and analysis of a wide range of molecular interactions
- 3-log dynamic range for concentration determination

References:

† Santora et al., 2001. Anal. Biochem. 299,119-129.
‡ Song et al., 2008. Exp. Mol. Med. 40, 35-42.

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