

Particle interaction monitoring

Identification and analysis of particle-particle, particle-biomolecule, functionalisation and aggregation interactions.

- Quantifying dynamics
- Real-time
- *In situ*
- Determining aggregation kinetics

Introduction

Scanning Ion Occlusion Sensing (SIOS) can be used to detect, analyse and monitor particle interactions such as:

- Particle-Particle (aggregation)
- Disaggregation or Fragmentation
- Protein-Protein
- Biotin-Avidin
- DNA hybridisation assays
- Antibody – Antigen
- Receptors – Ligands

Quantifying the yield and extent of these binding interactions enables bioassay, identification and diagnostic regimes to be used and developed.

The beginning, middle and end of interactions can be effectively mapped by analysing subtle changes in particle size, surface charge and concentration simultaneously and on a particle-by-particle basis, allowing interaction dynamics and yields to be determined.

This can be applied to many different situations:

- Confirmation of binding interactions
- Comparison of sample properties prior to and post interaction
- Detection of molecules that may otherwise be below the detection limit of the instrument
- Real time monitoring of binding interaction events, particle-by-particle, by combining reactants in the top fluid cell

Quantifying interactions

Physical interactions between two particle sets can exhibit a change in particle size, surface charge and concentration. Monitoring these physical characteristics simultaneously can be used to quantify the extent of the interaction. Furthermore, this can be determined as a function of induced conditions, such as pH, ionic strength, amount of added reagents and temperature.

The example below shows the interaction of positively charged avidin (~ 3 x 4 x 6.5 nm), with negatively charged carboxylate groups on the surface of functionalised polystyrene particles (mean diameter 184 nm). Blockade duration is significantly affected after the reaction of avidin with carboxylate groups on the particles, reducing the net negative particle charge.

DETECTION OF CHEMICAL REACTIONS

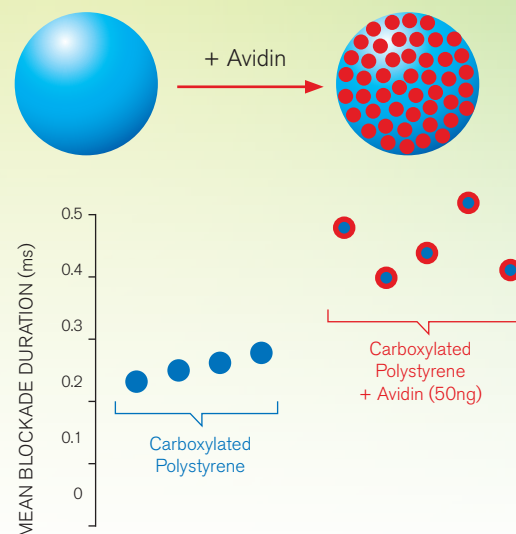


FIGURE 1: Interaction of Avidin (~ 3 x 4 x 6.5 nm) + carboxylated polystyrene nanoparticles (184 nm ± 8.5)

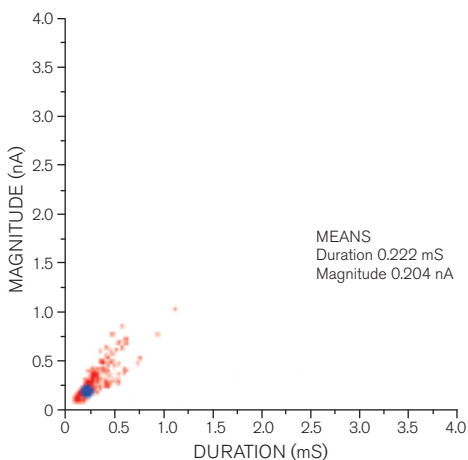
SIOS is a particle-by-particle detection method allowing individual particle data to be displayed in a 2D scatter plot of size vs duration. Particles with similar size and charge will cluster in one area on the 2D plot, exhibiting similar resistive peak signals. Thus, assessing deviations and movement of the population clusters before, during and after reaction with avidin offers a quantitative approach to determining reaction yields and particle dynamics.

Sample comparison

One area of application is monitoring the signal magnitude to detect and analyse the presence of aggregated particles in a sample following interaction. In the following example note the population of large magnitude blockade events in a sample of carboxylated

polystyrene to which 50ng/ μ L of avidin have been added. The avidin molecules have bound together some of the polystyrene particles in the sample, resulting in large aggregates.

CARBOXYLATED POLYSTYRENE ONLY



CARBOXYLATED POLYSTYRENE + AVIDIN

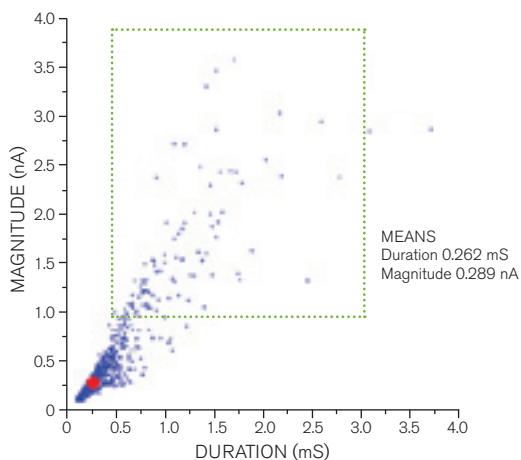


FIGURE 2: The presence of a large amount of avidin causes the polystyrene particles to aggregate. The aggregated particles have a larger signal magnitude and duration, clearly demonstrated in the figure on the right and in the calculated mean values.

Real-time monitoring of interactions *in situ*

Particle-particle interactions can be monitored *in situ* in real-time through changes in SIOS measurement parameters. In the example below 10ng of avidin was added to 184nm carboxylated polystyrene

particles *in situ* in the upper fluid cell. A clear increase in the measured mean blockade duration can be seen following introduction of the avidin.

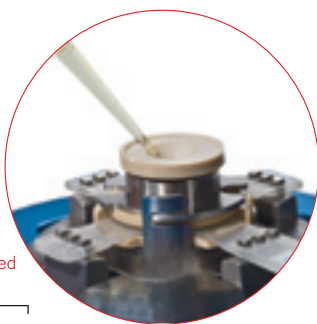
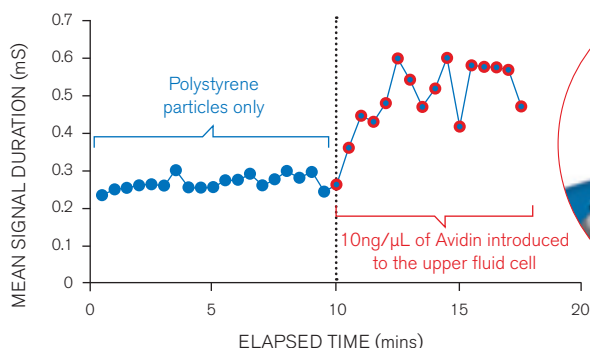


FIGURE 3: A binding interaction event (in this case avidin and polystyrene) is monitored in real-time after the reactants are combined in the top fluid cell of the instrument.

Small molecule detection and diagnostics

Molecules that otherwise may be too small to be detected by the SIOS technology at present may be detected indirectly by monitoring key signal parameters. The examples in this sheet demonstrate the use of carboxylated polystyrene to detect the presence of avidin through interaction monitoring. Changes in measured signal frequency (Interaction confirmation), signal duration (interaction

confirmation and real-time monitoring) and signal magnitude (sample comparison) can all be assessed.

This principle may be used as a diagnostic tool using specific functionalisation of nanoparticles to detect molecules of interest in a sample through changes in signal parameters caused by specific binding of the target molecules to the nanoparticle surface.

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