Label-Free SoPRano™ Gold Nano-Rod (GNR) assays on a spectrometer-based microplate reader

E.J. Dell[1], Meike Roskamp[2] and Frederik Van de Velde[2]
[1]BMG LABTECH [2]PharmaDiagnostics

- **The SoPRano™ label-free assay requires full spectral analysis**
- **Easily measure the SoPRano™ label-free LSPR signal with a BMG LABTECH spectrometer-based microplate reader**
- **BMG LABTECH luminescence reader used to monitor dose-dependent responses**

Introduction

Label-Free SoPRano™ Gold Nano-Rod (GNR) kits enable users to design and run label-free, microplate-based homogenous assays for high quality protein-protein interaction analysis based on Localized Surface Plasmon Resonance (LSPR). The interaction of a ligand with surface immobilized protein causes a concentration dependent and highly reproducible redshift of the LSPR peak of the GNR. Using spectrometer based microplate readers from BMG LABTECH, full spectra were captured and the specificity of the signal is demonstrated here. Human Serum Albumin (hSA) and Bovine Serum Albumin (bSA) were separately conjugated to the SoPRano™ GNRs and their respective monoclonal antibodies were bound at various concentrations.

Assay Principle

The SoPRano™ platform enables the monitoring of binding events (for example between two proteins) based on the detection of refractive index changes. This LSPR (localized surface plasmon resonance) based technology is an adaptation of the widely-used SPR technology.

How it works:

- Conjugate your protein to the gold nanorods (GNRs).
- Capture the full absorbance spectrum of the GNR-protein conjugate to determine the λmax, since each GNR-protein conjugate will be different.
- Add the protein ligand to the GNR-protein conjugate and measure a full spectrum. A dose-dependent red shift in λmax will occur upon specific interaction of the protein ligand with the conjugated protein.

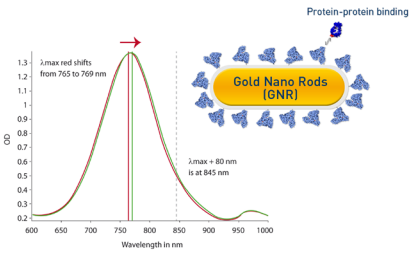

Fig. 1: Gold Nano Rods have an LSPR signal that can be detected with full spectral absorbance. The LSPR peak red-shifts upon ligand binding to the GNR surface, which is instantly captured by the spectrometer.

- This change in the spectrum upon interaction is due to a change in local refractive index at the GNR surface and is very reproducible.
- For kinetic measurements, full spectrum were taken every ten seconds for 3000 seconds. For slow kinetics, measurements may need to be taken for a longer time period; and for fast kinetics, measurements may need to be taken more frequently.

Materials & Methods

- bSA, hSA and their corresponding monoclonal antibodies were purchased from Abcam (United Kingdom) SoPRano™ Kit was from Pharma Diagnostics
- SoPRano™ Kit was from Pharma Diagnostics (Belgium)
- Non-binding, 384-well, black microplates with clear bottoms from Greiner (Germany)

bSA and hSA were separately conjugated to Gold Nano Rods (GNRs) via the proteins' free amine using EDC and sulfo-NHS (see Figure 2).

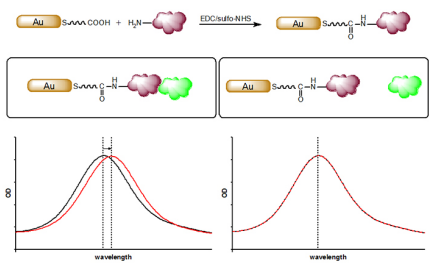

Fig. 2: Proteins are conjugated to Gold Nano Rods via the free amine of the protein using EDC and sulfo-NHS. The graphs show how the λmax red-shifts upon conjugation.

Increasing amounts of the antibodies (5 µL of a 16x concentration) were incubated with the conjugated GNRs (40 µL) for 50 minutes (35 µL of MES buffer was used to bring final volume to 80 µL), followed by full spectrum measurements. Using the MARS data analysis software and calculation templates, ratiometric analysis was easily done (ΔRu = λmax +80 nm/ λmax) and Kds were determined that correspond to other methods of analysis.

Results & Discussion

Using a one-site binding model, dose response curves were produced for hSA-GNRs and its antibody (Figure 3) and for bSAGNRs and its antibody (Figure 4).

Rev. 12/2014
Keywords: **Binding studies, Gold Nano Rods, Protein-protein interaction, Soprano, Spectral analysis**

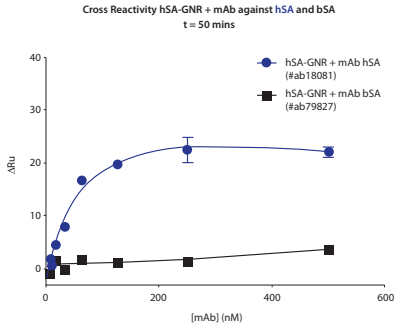

Fig. 3: Dose-response curve shows that hSA's antibody specifically binds to hSA conjugated GNRs, whereas the bSA antibody does not.

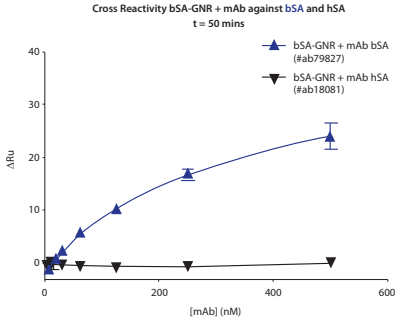

Fig. 4: Dose-response curve shows that bSA's antibody specifically binds to bSA conjugated GNRs, whereas the hSA antibody does not.

Using the opposite antibody for that system, cross-reactivity was shown not to occur. Signal to noise ratios (S:N) of more than 24 and Z'-factors greater than 0.80 prove this to be a robust and reproducible system. Furthermore, calculated Kds correspond with the expected results.

In figure 5, kinetic curves of hSA-GNRs binding to different concentrations of an hSA antibody were created from full spectral measurements. Using a one-site hyperbola binding fit and the last kinetic cycle when equilibrium is reached, a dose response curve can be created which gives the binding constant KD (not shown). The kinetic measurements were used to produce association (ka) and dissociation (kd) rates via a binding kinetic equation in the MARS Data Analysis software.

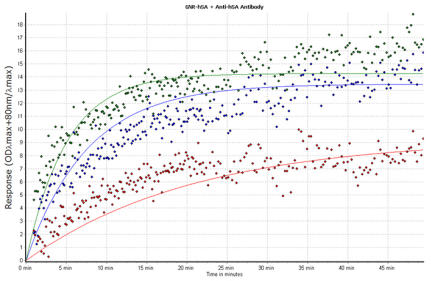

Fig. 5: Human serum albumin was coupled to the SoPRano™ gold nanorods. An antihuman albumin antibody was tested for binding. MARS Data Analysis software was used to produce binding kinetics from the spectral kinetic data.

Conclusion

This label-free SoPRano™ platform from Pharma Diagnostics enable high-throughput, plate-based, homogeneous LSPR assays to be performed for the determination of protein-protein interactions. The spectrometer-based microplate readers from BMG LABTECH capture a full spectra in less than one second per well. Full spectra are needed for the SoPRano™ assay to determine the shift in the λmax upon binding. Subsequently, the MARS data analysis software easily allows for the ratiometric analysis that is needed for this assay.