

High Sensitivity FT-SPR Label-Free Analysis of Nucleic Acid and Protein Interactions

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Introduction

The Surface Plasmon Resonance (SPR) effect arises when light is reflected under certain conditions from a conducting film at the interface between two media of different refractive indices. Usually the media is the sample on one side of a gold film layer and the glass of a sensor chip on the other side of the film. In order to generate conditions for SPR, the photons must be coupled into oscillating modes in the metal, and this is best achieved using a glass prism or a grating device.¹ A typical SPR prism/sensor configuration is shown in Figure 1. SPR causes a reduction in the intensity of reflected light over a narrow range of angles of incidence.

SPR is conventionally performed by measuring reflectivity over a series of angles of incident light of fixed wavelength.

A minimum in reflectivity occurs at the “SPR angle,” corresponding to the maximal SPR response. The SPR angle varies with the refractive index of the material close to the surface on the side opposite from the reflected light.

SPR reflectivity measurements are surface-sensitive spectroscopic methods that can be used to characterize thickness and refractive

index of chemical and biomolecular films on metal surfaces. SPR can be also used to quantitatively characterize many different types of biomolecular interactions including those involving DNA, RNA, protein, and carbohydrates, in real-time, without the use of molecular labels.

FT-SPR allows SPR to be measured on an FT-IR platform, where FT-IR multiplexing and wavelength precision features are expected to result in key technical advantages over conventional angle-shift based SPR. FT-SPR measurement is performed at a fixed angle of incident light, and reflectivity is measured over a range of wavelengths in the near-infrared.² In FT-SPR, the minimum in reflectivity occurs at the “SPR wavenumber.” Since FT-IR spectrometers typically have high wavenumber resolution and a wide range of reflection angles are available in an SPR™ 100 module from Thermo Electron Corporation, FT-SPR is a highly sensitive method with remarkable dynamic range for surface analysis.

This application note is based on the use of the innovative FT-SPR detection technique that couples the SPR 100 module to a FT-IR spectrometer. To demonstrate the broad utility of FT-SPR analysis, this note details how FT-SPR systems are used to monitor: 1) nucleic acid binding to surface-immobilized oligonucleotides; and 2) antibody binding to surface-immobilized peptides.

Experimental

Data Collection and Process

The FT-SPR experiment was performed by using a Nicolet™ 8700 spectrometer with a SPR 100 module. The Nicolet 8700 FT-IR spectrometer was controlled via OMNIC™ software to collect SPR data in reflectance mode. The FT-IR is equipped with a near IR tungsten-halogen light source. The incident angle of light on the prism in the SPR 100 module was then adjusted (to approximately 53°) until the FT-SPR spectral minimum was near 9000 cm⁻¹. A series of SPR spectra was then collected to establish a baseline from 11000 – 6000 cm⁻¹ and then each of the spectra was fit with a center-of-gravity algorithm applied to the bottom 25% of data points, using Thermo’s TQ Analyst™ chemometric software package. This curve-fitting routine determined the locations of the minimum for each SPR spectrum. The locations of these FT-SPR minima were then plotted versus time to show the wavenumber shift obtained for analyte binding. Binding of analytes to probe molecules was thus monitored by pumping the analytes into the flow cell in the SPR 100 module, and comparing reflectivity spectra taken before, during and after analyte binding.

Probe and Sample Preparation

A gold-coated glass chip from GWC Technologies Inc. (Madison, WI) was coated with a surface attachment layer by soaking in an organic solution of 1 mM alkanethiol with terminal amine groups for at least 2 hours. Chips were later rinsed copiously in ethanol and then dried under a stream of nitrogen. Immediately prior to probe attachment, the chip surface was modified with the coupling agent 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SSMCC). Five microliters of synthetic peptide probe with terminal cysteine residue³, or 5'-Thiolated oligonucleotide probe from the University of Wisconsin Biotechnology Center (Madison, WI) was then spotted on the chip, and a cover slip placed on top to cover the entire chip surface with probe solution. After incubating the probe for 30 minutes, the chips were washed with buffer, rinsed copiously in ethanol, then dried under a stream of nitrogen. Chips were then assembled into the flow cell and mounted in the SPR 100 module. Aqueous buffer was pumped through to fill the cell prior to collecting data. Probes on the SPR chips were then exposed to analyte by pumping analyte solution into the flow cell to replace the buffer, then

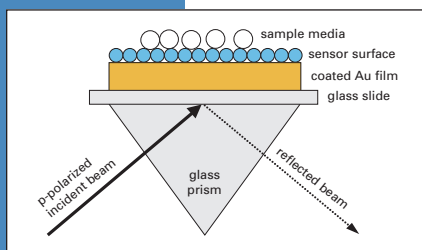


Figure 1: Configuration of FT-SPR prism/chip sensor

Key Words

- FT-IR
- FT-SPR
- Label-Free Detection
- Molecular Binding
- Protein Interactions
- SPR

incubating for 15 – 30 minutes or until equilibrium binding was reached, whichever was longer. For antibody-antigen binding analysis, FLAG antibody was obtained from Sigma-Aldrich (St Louis, MO). For DNA hybridization experiments, oligonucleotide analytes were obtained from the University of Wisconsin Biotechnology Center (Madison, WI).

Results and Discussions

DNA Hybridization

When oligonucleotide probe 5'-HS-YYYY GTG TTA GCC TCA AGT G-3' was exposed to 500 nM non-complementary analyte 5'-AGA CTC TGA CTC AGT G-3', no significant shift in the FT-SPR spectra was observed. As shown in Figure 2, when this probe was next exposed to complementary analyte 5'-CAC TTG AGG CTA ACA C-3', a shift of approximately 34.5 cm⁻¹ in the FT-SPR wavenumber was observed, which was fully reversible by washing in 8 M urea (not shown). This experiment demonstrates the surface specificity of the biosensor in this FT-SPR device.

The FT-SPR wavenumber shift of 34.5 cm⁻¹ obtained by adsorption of this ~5,300 MW analyte suggests that much smaller analytes will be detectable by this method. The FT-IR spectrometer is capable of distinguishing shifts of a fraction of a wavenumber, so the limiting factors for sensitivity in most biomolecular FT-SPR experiments will be the biosensor design and any system noise introduced by the SPR 100 module.

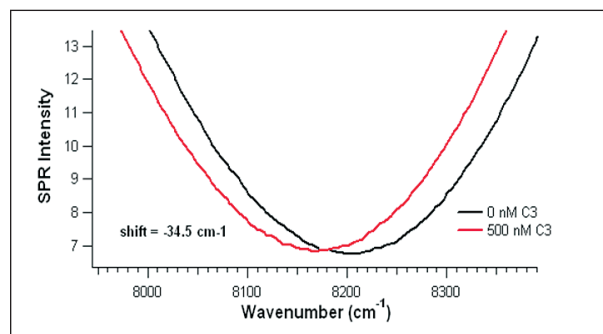


Figure 2: FT-SPR spectra before (right) and after (left) binding of complementary oligonucleotide to surface-immobilized oligonucleotide probe.

Antibody-Peptide Binding

A series of increasing concentrations of FLAG antibody was exposed to FLAG peptide immobilized on the SPR chip surface and FT-SPR spectra were collected for each concentration, as illustrated in Figure 3. FT-SPR spectra are shifted to lower wavenumbers as each higher concentration of antibody is exposed to the chip.

The SPR wavenumber for each concentration was obtained by curve fitting, then this value was converted to relative surface coverage, assuming the maximum shift corresponds to 100% coverage. All the SPR wavenumbers were then plotted versus analyte concentration and fit to a Langmuir isotherm, as shown in Figure 4, in order to estimate the affinity of the antibody-antigen interaction.

The estimated adsorption coefficient (K_{ads}) for this antibody-peptide interaction is $9.0 \times 10^7 \text{ M}^{-1}$, close to the value of $1.5 \times 10^8 \text{ M}^{-1}$ for the affinity of the same interaction as measured by solution experiments and by SPR imaging.³

This experiment confirms that the FT-SPR method may be used to measure affinities of biomolecular interactions. The high dynamic range of this system in combination with the ability to detect multiple distinct species of molecules (e.g. DNA, RNA protein) will be very valuable for monitoring multiple incremental binding species such as transcriptional complexes involved in quaternary structures.

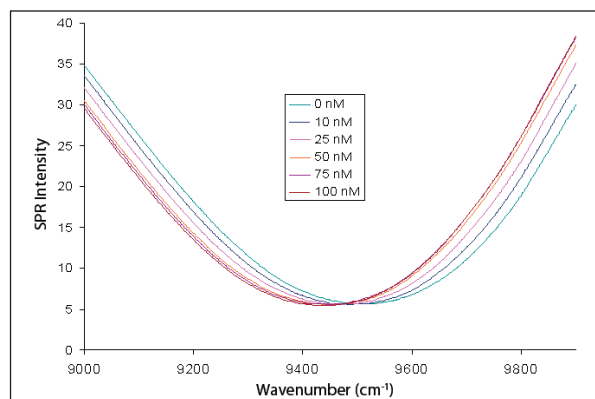


Figure 3: FT-SPR spectra for binding of increasingly concentrated FLAG antibody analyte to FLAG peptide probes.

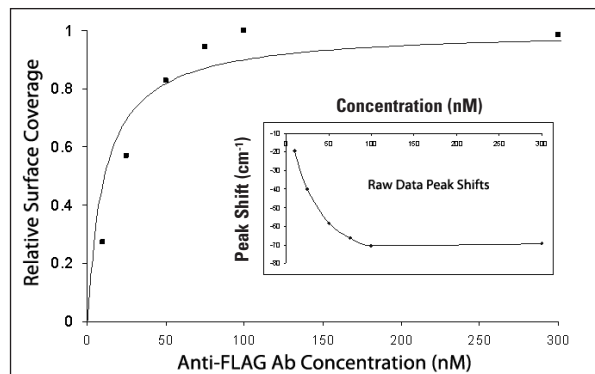


Figure 4: FT-SPR wavenumber shifts (inset) and relative surface coverage (main graph) for FLAG antibody analyte binding to FLAG peptide.

Summary

FT-SPR is a high sensitivity probe for label-free analysis of nucleic acid and protein interactions. This note reviews the basic principle of FT-SPR and details how FT-SPR is used to monitor nucleic acid binding to surface-immobilized oligonucleotides, and antibody binding to surface-immobilized peptides with high sensitivity. The SPR 100 module is the ideal instrument for this analysis.

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