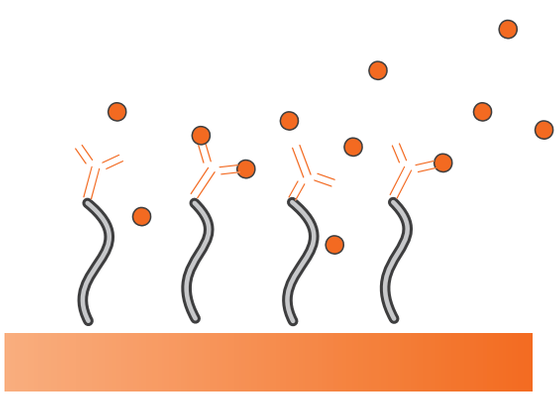
Application Note # 172

BioNavis Dextran like (BND) sensors for label-free affinity and kinetic measurements

This Application Note introduces the BioNavis Dextran like (BND) sensor for biomolecular interaction measurements. The sensors allow covalent immobilization of ligand on the sensor surface and analyte binding affinity and kinetics to be measured real-time and label-free. The BND sensor allows higher ligand binding capacity and lower non-specific binding compared to planar Carboxymethyl Dextran (CMD-2D) sensors. Sensor slides are compatible with all MP-SPR Navi™ instruments.

Introduction

Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments provides high precision and versatility for biomolecular interaction studies. Ligand molecules are immobilized on a sensor surface and analyte molecules are introduced in a flow to measure not only binding affinity but also kinetics of the interaction without labels. Various chemistries are exploited including covalent coupling and affinity capture. Also diverse experimental setups such as direct measurement or competitive assays are utilized.



Carboxymethyl dextran (CMD) was introduced as an immobilization matrix for biosensor applications in 1990 . Presently, CMD is widely used for conducting SPR based bio-interaction assays. Typically, ligand is covalently attached to CMD sensor surface using amine coupling chemistry. Presently, CMD sensors can be obtained in a great variety of types, optimized for biomolecules of various size ranges and it is mostly used in a relatively thick hydrogel-like form (“3D” BioNavis product no: SPR102-CMD-3DM) where the effective hydrated layer can be up to 700 nm. In its thinnest form it is applied as a monolayer (“planar” or “2D” BioNavis product no: SPR102-CMD-2D) . Like any chemical reagent, however, CMD has disadvantages depending on the specific context of its use. BioNavis has introduced BND sensors, which allow higher immobilization capacity and lower non-specific binding compared to CMD 2D sensors. The BND sensors have very low non-specific binding characteristics, which is especially useful when the standard CMD matrix has specific or non-specific binding to some of the components used in an assay.

Materials and methods

BND sensors are prepared with carboxyl groups and hydroxyl groups in the molar ratio of 1:5 with a long non-specific binding linker directly to gold. This yields a relatively high density of carboxylic acid groups compared to CMD-2D. The BND sensors allow similar chemistry and protocols to be used as CMD sensors.

Assay steps of chromatically purified antibodies against bovine IgG.

- Running buffer: HBS-EP
- Surface cleaning: NaCl + NaOH (2 M/10 mM)
- Activation: EDC + NHS (200 mM/ 50 mM (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-Hydroxysuccinimide)
- Antibody: anti-bovine-IgG 100µg/ml, Figure 1 reference channel treated with Human Serum Albumin (HSA)
- Deactivation: ethanolamine 1 M pH=8.5
- Binding of a broad concentration range of bovine IgG (Figure 2) against immobilized antibody. The concentration range 3.8 pM to 1000 nM (10 concentrations).
- Surface regeneration between concentrations using a mild regeneration reagent (10 mM glycine/HCl, pH = 2,1)

Measurement was performed using 4 channel fully automated MP-SPR Navi™ 420A ILVES instrument. The immobilization step of the anti-bovine IgG is shown in Figure 1. Flow channels 1, 2, and 3 were loaded with antibody, while flow channel 4 was loaded with human serum albumin (HSA) as a reference channel.

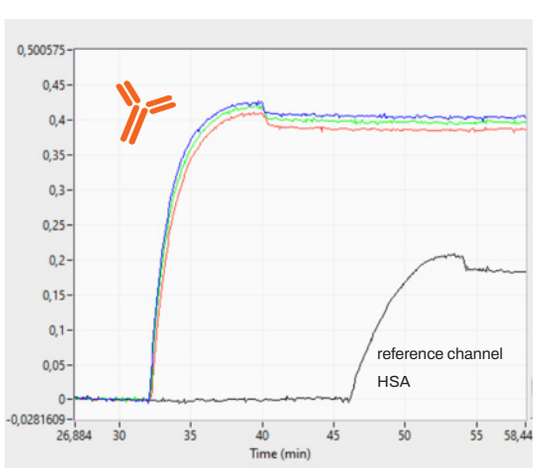


Figure 1. Immobilization step for anti-bovine IgG. Results are shown for the first 3 channels coated with ligand antibody (blue, green and red trace) and the 4th channel with Human Serum Albumin (HSA, black trace).

AN_172.b

BioNavis

email: info@bionavis.com
www.bionavis.com

Analyte binding steps to immobilized ligand are shown in Figure 2 from lowest to highest concentration. Kinetic fitting is shown in Figure 3.

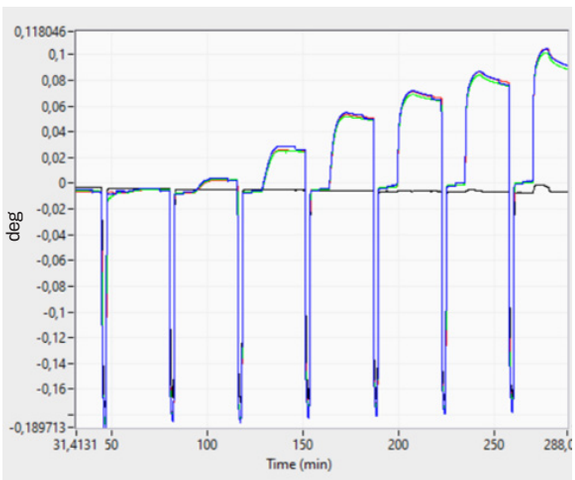


Figure 2. Binding of Bovine IgG in the concentration range 3.8 pM to 1000 nM with intermediate regeneration with 10 mM Glycine/HCl pH=2.0.

MP-SPR Navi™ instruments KineticTitration function allows sequential injection of concentrations without dissociation time and surface regeneration between the concentrations allowing faster measurement. More information in the Application Note #155.

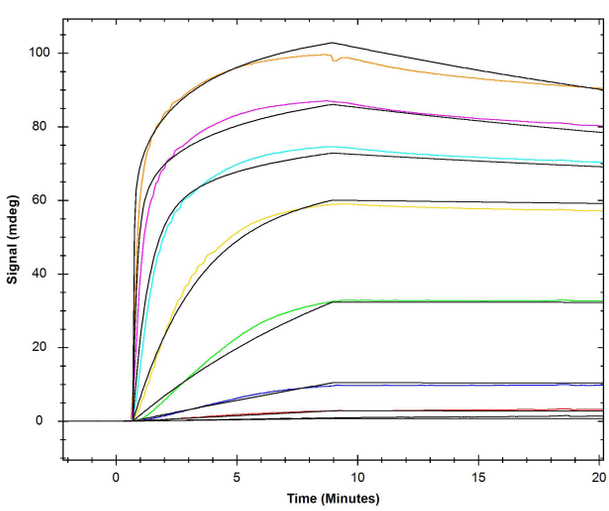


Figure 3. The measured data was analyzed using TraceDrawer for MP-SPR Navi™ software. The kinetic constants of bovine IgG binding to anti-bovine IgG were found to fit best with the 'bivalent' model, achieving maximum binding level B_{max}=127 mdeg and rate constants k_a1=1.27x10^5 M^{-1}s^{-1}, k_d1=1.46x10^{-4} s^{-1}, k_a2=8.71x10^{-2} M^{-1}s^{-1}, k_d2= 2.56x10^{-2} s^{-1}. The dissociation constant (K_D1) being 1.16x10^{-9} M (Average of three measurement channels).

Binding levels of the BND sensors are reported in Table 1, comparing the values with those obtained under similar conditions with the 2D-CMD and under typical conditions for the regenerable avidin kit sensor surface available from BioNavis for biotinylated ligands (SPR102-AVI-2).

	R_{max} (mdeg)	R_{max} (RU)
CMD-2D	110	880
BND	350	2800
Regenerable Avidin kit	225	1800

Table 1. Typical immobilization level of IgG, comparison of three sensor types (n=3).

Results and discussion

Working with a monolayer of ligands is desirable as it limits possible diffusion effects occurring in a thicker dextran substrates. To achieve increased sensitivity more neatly packed surface of the biosensor is needed, and the ability to concentrate more ligand molecules on the surface. This is of particular importance with low molecular weight analytes. Additionally, lower non-specific binding will also result in higher sensitivity when probe molecules are more tightly packed. The surface also avoids much of the problems involved with electrostatic preconcentration, which is very pH sensitive, and the BND sensors show less pH dependence.

Conclusions

BioNavis MP-SPR instrument are precise tools for biomolecular interaction studies such as small molecular and protein interaction studies with immobilized ligands. Affinity and kinetics of the biomolecular interaction can be measured. BioNavis BND sensors provide a new alternative for established CMD sensors introducing higher ligand density and lower non-specific binding. These sensors can be recommended for systems in which the analyte is smaller in size than the ligand.

See also how MP-SPR instruments unique PureKinetics™ feature works and advance biomolecular interaction measurements, Application Note #147.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI, 410A KAURIS or 420A ILVES

Sensor surface: BioNavis Dextran like sensor surface: SPR102-BND-2D

Software: MP-SPR Navi™ Controller, DataViewer and TraceDrawer™ for MP-SPR Navi™

BioNavis

email: info@bionavis.com
www.bionavis.com