Application Note # 138

Antibody and antigen interaction measured with MP-SPR

Human serum albumin antibody (anti-HSA) interaction with immobilized human serum albumin (HSA) was measured using Multi-Parametric Surface Plasmon Resonance (MP-SPR). As assumed, commercial anti-HSA binds strongly to the HSA and dissociates extremely slowly from the HSA. Steady state binding as well as kinetic of the binding was calculated for the interaction.

Introduction

Protein - antibody interactions are extremely important research area for pharmaceutical industry and protein research. Antibody-based biotechnology drugs have been shown to be effective treatments to some obstinate diseases, like Bevacizumab for cancer therapy and Adalimumab for rheumatoid arthritis.

Human serum albumin (HSA) is most abundant protein in human plasma [1]. It main functions is to transport fatty acids and maintain blood colloid osmotic pressure. Different immobilization protocols can be utilized to bind protein on a sensor surface in order to create a biosensor. In this case amine coupling to carboxymethyl dextran surface was used for HSA anti-HSA binding experiments.

SPR phenomenon is based on free electrons resonating at a metal surface, which are excited with visible or near infrared light. There is an absorption maximum as a function of the angle of the incident light, and the SPR phenomenon is highly dependent on the dielectric constant near the metal surface. Any changes near the surface, such as antibody binding, change the angle of the absorption maximum, and reflect the amount of molecules close to surface. Using the SPR phenomenon, the Multi-Parametric Surface Plasmon Resonance (MP-SPR) is sensitive tool to determine molecule interactions. Real time and label free measurements provide information also on the kinetics of the studied system making MP-SPR valuable tool for interaction experiments.

MP-SPR measures wide angular range and whole SPR curve is monitored which enables observing not only SPR peak minimum position but also other parameters as peak minimum intensity and total internal reflection (TIR). TIR region is sensitive to the optical properties of the media outside the evanescent field (bulk), whereas the main SPR peak intensity is giving information possible light absorption.

Materials and methods

Measurement was done in 21˚C using flow rate 30µl/min with SPR Navi™ 220A.

Immobilization

Carboxymethyl dextran sensor slide (CMD-P) and 5mM MES (2-(N-morpholino)ethanesulfonic acid) pH 5.0 buffer was used for immobilization. Surface was cleaned with NaCL 2M and NaOH 0.01M mixture. The CMD carboxylic acids were activated to reactive succinimide esters using NHS 0.05M and EDC 0.2M activation (Fig.1) (NHS= N-hydroxysuccinimide, EDC= N(-3-dimethylaminopropyl) -N-ethylcarbodiimide Hydrochlorid). HSA was injected immediately after activation in one of the two channels and protein was attached from primary amine to the hydrogel (Fig.1). Channel without protein was used as reference channel. The sensor was then deactivated with ethanolamine (pH8.0). All injections were seven minutes long in the immobilization process and flow rate was 30 µL/min.

Interaction

Anti-HSA binding experiment was done in phosphate buffered saline (PBS) buffer pH7.4. Four anti-HSA samples (3.25nM, 6,5nM, 13nM and 26nM) interaction with the immobilized HSA were measured. HSA surface was regenerated in order to remove anti-HSA using one minute injection of 50mM NaOH between each anti-HSA samples. Repetition of the largest anti-HSA sample was done to ensure that the sensor activity did not degrade with the regeneration. Binding constants for the interaction was calculated with TraceDrawer™ for SPR Navi™.

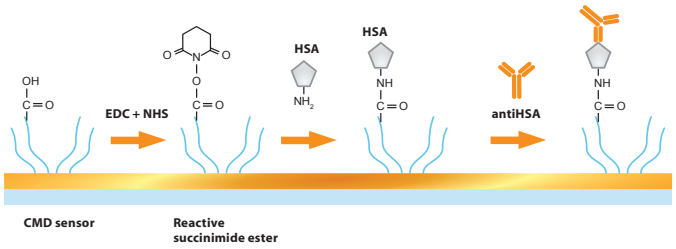

Figure 1. Human serum albumin (HSA) was immobilized on a carboxymethyl dextran (CMD) sensor surface with amine coupling chemistry, AntiHSA antibody interaction with immobilized HSA was measured. (EDC and NHS are activation compounds for amine coupling)

Excellence in Surface Plasmon Resonance
BioNavis

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 10 271 5030
e-mail : info@bionavis.com
www.bionavis.com

Results and discussion

Different immobilization protocols can be utilized for binding molecule to the sensor surface which enables that different kind of molecules interactions can be examined with MP-SPR technology. In the case of HSA, the simple CMD-amine coupling chemistry can be used with high efficiency.

Measurement was done in Angular scan mode so whole SPR peak is monitored during interaction. Anti-HSA binding to the HSA cause peak shift to the right (Fig.2). Reference channel is without protein and anti-HSA is not changing SPR peak position (Fig.2).

As expected Anti-HSA binds very strongly to the HSA and the complex dissociation rate is extremely slow, which is typical for commercial antibodies (Fig.3). Also the association rate was rather slow for interaction, also a typical property of high affinity antibodies. Bivalent interaction model was used for calculation which is typical for antibody protein interaction because an antibody contains two equal binding sites. Binding constants for the interaction were: steady state affinity (K_D) $8.1*10^{10}$ M, dissociation rate constant (k_d) $5.83*10^{-5}$ 1/s and association rate constant (ka) $7.18*10^{4}$ 1/(M*s). Anti-HSA was successfully removed from the HSA with short NaOH injection and HSA activity remained after regeneration based on repetition sample.

Conclusions

MP-SPR is excellent instrument to determine molecule binding event for example between antibody and antigen. Real time MP-SPR measurement enables determination not only steady state binding but also kinetics of the binding reaction. MP-SPR is powerful tool for wide range of research areas like protein research and pharmaceutical industry.

References:

[1] Peters T, All about albumin, 1995

Recommended instrumentation for reference assay experiments
SPR Navi™ 210A or 220A
Sensor surfaces: Planar Carboxymethyl dextran hydrogel (CMD)
Software: SPR Navi™ Control, SPR Navi™ DataViewer, TraceDrawer™ for SPR Navi™

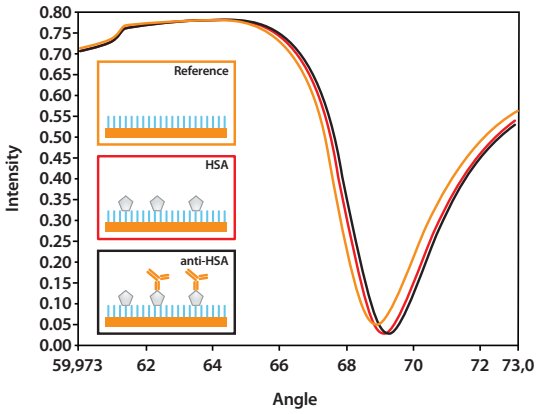

Figure 2. SPR curve before and after 26nM anti-HSA injection. Orange curve is reference channel without albumin and anti-HSA doesn´t cause any changes. HSA is immobilized in red channel and anti-HSA binding change curve angular position to the right (black curve).

KD (M)	kd (1/s)	ka (1/(M*s))
8,1E-10	5,83E-5	7,18E4

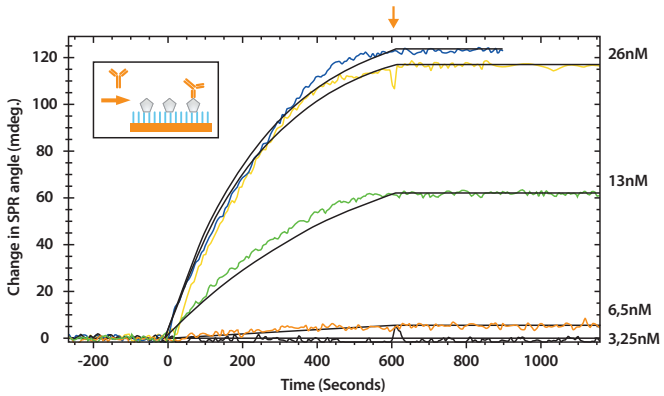

Figure 3. Anti-HSA binding to the immobilized HSA with four different concentration. Injection is started at time point zero and arrow is showing ending point. Calculated binding constants for the interaction is presented in the table.

Excellence in Surface Plasmon Resonance
BioNavis

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 10 271 5030
e-mail : info@bionavis.com
www.bionavis.com