Application Note # 148

Repeatability and Reproducibility of Drug-Protein Interaction Experiments Using MP-SPR

A challenging molecular pair was selected for testing the repeatability and reproducibility of the MP-SPR Navi™ instruments: the small molecule weight drug Indomethacin (357.8 g/mol) interacting with human serum albumin (HSA). The intra-laboratory measurements were repeated four times in the same laboratory using the same instrument (repeatability test), and inter-laboratory testing was done in four separate laboratories using different MP-SPR Navi™ systems (reproducibility test). Reproducibility experiments gave slightly higher average affinity K_D 22.6 µM and higher standard deviation of 27%. However, considering the variation in the quality of the chemicals, the experiment shows good reproducibility and credibility. Calculated affinity and kinetic parameters showed an excellent repeatability of the experiment, giving average affinity K_D 27.1µM, while the standard deviation was only 3.6%.

Introduction

Drug - protein interaction studies are a key research area for *in vitro* drug development and protein research. Human serum albumin (HSA) is the most important protein in blood plasma, due to its high abundance and its ability to bind various compounds which makes it an important protein for drug discovery.

Multi-Parametric Surface Plasmon Resonance (MP-SPR), which utilizes the Surface Plasmon Resonance phenomenon, is a highly sensitive tool to determine molecular interactions. SPR instruments provide real-time and label-free measurements of affinity and interaction kinetics, which has made it a popular tool for drug discovery. The unique optical setup of MP-SPR Navi™ instruments enables cross-correlation of the parameters provided by the instrument and allows a straightforward in line characterization of interfering bulk signal using the PureKinetics™ feature.

Materials and methods

HSA was attached with amine coupling chemistry to a carboxymethyl dextran (CMD-3D) coated sensor slide (Figure 1). The immobilization was performed *in situ* at 21 °C using 5 mM MES buffer (2-[N-morpholino] ethanesulfonic acid, pH=5) for protein attachment. The surface was activated with 0.2 M EDC/0.05 M NHS before protein injection and deactivated with 1 M ethanolamine pH=8 after protein adsorption. A channel without protein was used as a reference to monitor any non-specific binding to the substrate. In the reproducibility experiments, different HSA batches and quality of chemicals were used, which caused variation in the experimental conditions.

The interaction of small molecular weight drug Indomethacin (357.8 g/mol) with HSA surfaces was measured using MP-SPR Navi™ instruments. The repeatability testing was performed on one location using the 220A NAALI instrument, whereas in the reproducibility testing, four different 220A instruments, on different locations and with different operators were employed. Experiments were performed using PBS buffer (phosphate buffered saline, pH=7.4) as running buffer at 21°C. At least five and at maximum eight Indomethacin samples (0.1-50 µM) were measured. Samples contained 0.5% or 3% of DMSO (dimethylsulfoxide) to improve drug solubility. The kinetics and affinity of the interaction were analyzed using TraceDrawer™ for MP-SPR Navi™ (Figure 2).

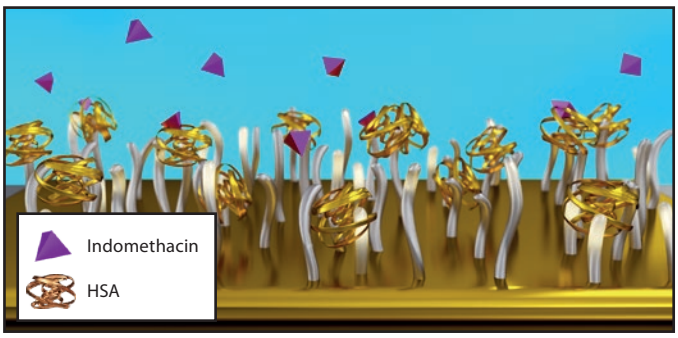

Figure 1. Schematic view of the covalent attachment of HSA onto a dextran surface, and interaction of indomethacin.

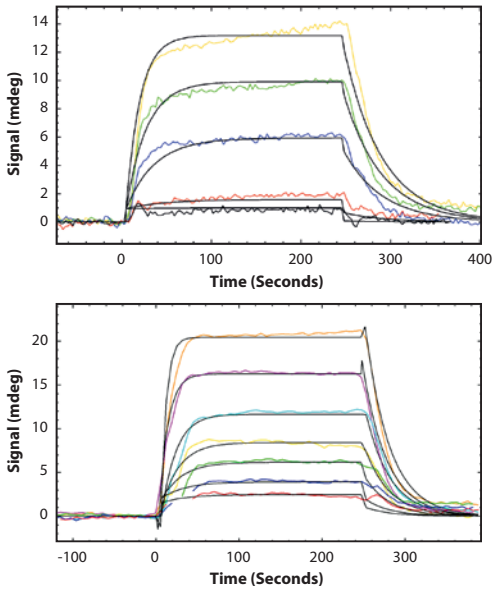

Figure 2. Binding of indomethacin to HSA was measured using different concentrations. Colored curves are measurements and black curves are fits to the measurements.

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Results and discussion

During the inter-laboratory experiments, HSA immobilizations were successfully performed in different laboratories, using the MP-SPR Navi™ instruments. However, the protein immobilization reached variable levels, due to differences in protein concentration and purity of chemicals used for immobilization.

Indomethacin binding constants (affinity and kinetic rates) were calculated from each measurement using TraceDrawer™ for MP-SPR Navi™. Excellent repeatability was achieved as the standard deviation was only 3.6% for affinity values (Figure 3). Calculated kinetic constants were also in good agreement, showing 8% and 9% standard deviations for k_d and k_a values.

Reproducibility tests revealed greater standard deviations for affinity, namely 27% (Figure 3). However, considering different users, instruments, time, immobilization level, buffer composition and quality of chemicals, values show good reproducibility of the experiment. A couple of measurements were performed using the systems that had been extensively used already for a couple of years, proving the instrument's great credibility. It is also notable that run 7 is a clear outlier: it gives higher affinity compared the other three measurements (Figure 3). If test 7 is excluded, the K_D standard deviation is only 9.1%.

The time between HSA immobilization and the interaction measurement varied between experiments. However, variation in the sample DMSO concentrations and loss of protein activity caused by time have been found to have only a minor effect on the calculated binding constants towards HSA [Rich et al. 2001]. The variation in the binding constants was similar in this experiment as has been reported in the literature. Not all proteins are as stable as HSA, and variation in the conditions might have greater effect on the calculated values in other experiments.

Conclusions

The measurement of interactions of a small molecular weight drug with a protein showed excellent repeatability and reproducibility of the MP-SPR Navi™ instruments. MP-SPR is a reliable, label-free tool for molecule – molecule interaction characterization, providing accurate affinity and kinetic values. MP-SPR can be used not only for biomolecular interaction analysis, but it is also an outstanding tool for biomaterial characterization and drug delivery studies.

See how MP-SPR PureKinetics™ feature is used in interaction studies in Application Note #147 or review how MP-SPR is used in nanoparticle characterization in Application Note #140.

References

Rich et al. Anal. Biochem. (2001) 296, 197-207

Recommended instrumentation for reference assay experiments
MP-SPR Navi™ 420A ILVES or 220A NAALI
Sensor surfaces: CMD-3D
Software: MP-SPR Navi™ Control, DataViewer and TraceDrawer™ for MP-SPR Navi™

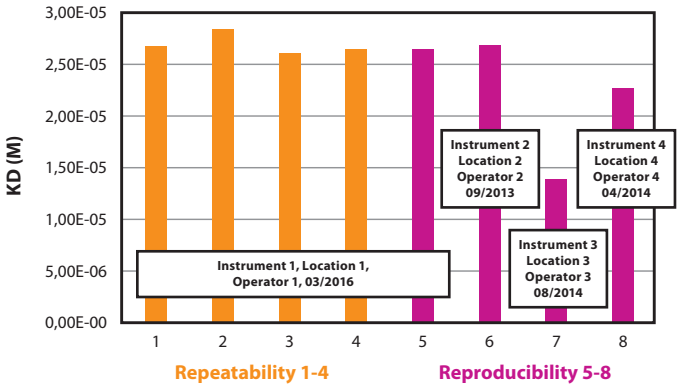

Figure 3. Calculated K_D values showed excellent repeatability and reproducibility of the experiment.

Repeatability

Instrument 1 Location 1 Operator 1	K_D µM	k_d (1/s)	k_a (1/M*s)
Run 1	26.9	1.99E-2	7.40E2
Run 2	28.5	2.32E-2	8.16E2
Run 3	26.3	2.27E-2	8.63E2
Run 4	26.6	2.41E-2	9.08E2
AVG	27.1	2.25E-2	8.32E2
STD	±0.98	±1.81E-3	±7.18E1
STD %	3.6%	8%	9%

Reproducibility

	K_D µM	k_d (1/s)	k_a (1/M*s)
Instrument 1 Location 1 Operator 1 03/2016	26.6	2.41E-2	9.08E2
Instrument 2 Location 2 Operator 2 09/2013	27.0	4.36E-2	1.61E3
Instrument 3 Location 3 Operator 3 08/2014	13.9	3.40E-2	2.45E3
Instrument 4 Location 4 Operator 4 04/2014	22.8	5.67E-2	2.48E3
AVG	22.6	3.96E-2	1.86E3
STD	±6.09	±1.39E-2	7.53±E2
STD %	27.0%	35.1%	40.4%

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