Application Note # 129

Effect of hydrodynamic flow in biomolecular interaction and targeted drug delivery studies

MP-SPR can be used to study the effect of hydrodynamic flow on the interaction kinetics, making it a valuable tool in biomolecular interaction and targeted drug delivery studies.

Introduction

Blood stream hydrodynamics is known to be an important pharmacokinetic factor that varies in different vessels and organs throughout the body. Liposomes are used in drug delivery systems to carry drug molecules to the effective site with some products already on the market. Liposomes are also widely studied as drug delivery systems for new drug targeting applications.

Multi Parametric Surface Plasmon Resonance (MP-SPR) is an *in vitro* label free method capable of providing information about interfacial adsorption events and molecular interaction kinetics. The SPR Navi 200 instrument´s open design allows easy customization and control of the hydrodynamic conditions inside the measurement chamber. The Quartz Crystal Microbalance technique (QCM) senses the amount of water dynamically coupled to biomolecules, which is sometimes a useful additional information, but which can also be a complicating factor in assays. The difference in the hydrodynamic flow conditions for SPR and QCM measurement chambers is often disregarded when SPR and QCM data is used in combination. This causes non-definable differences between the data from these two sources.

Materials and methods

The hydrodynamic flow conditions for SPR Navi 200 and QCM-Z500 (KSV Instruments Ltd) instruments were calculated and equalized in terms of shear stress at the bottom of the measurement chambers. A calibration constant for both instruments was obtained with computational fluid dynamics modeling (CFD). Flow behavior inside the measurement chambers was modeled with Navier-Stokes equations and the multi-physics software Elmer. The geometries of the measurement chambers were constructed and meshed using the pre-processing software Gambit. More information about equations used in the calculations can be found from the original publication [1].

Biomolecular and liposome interactions were studied with the flow synchronized SPR Navi 200 and QCM-Z500 instruments. The flow rates used for the studies, based on CFD modeling, were 10 and 100 µl/min for SPR and 73 and 733 µl/min for QCM. Interaction parameters were calculated using the Lanqmuir adsorption equation. Further details can be found from the original publication [1].

In biomolecular interaction studies a self-assembled monolayer of biotin was immobilized on the gold surface. Streptavidin interaction with biotin was measured using streptavidin concentrations of 1.25, 2.5, 5, 10 and 20 mM (Fig. 1).

Biotinylated liposomes prepared by extrusion technique were used as a model drug delivery system. PEGylated phospholipids and cholesterol were used to reinforce the liposome structure and to prevent deformation of the liposomes. The interaction of liposomes with a streptavidin functionalized sensor surface was studied by using lipid concentrations of 14.7, 28.3, 56.7, 113.3 and 226.7 µM (Fig. 1).

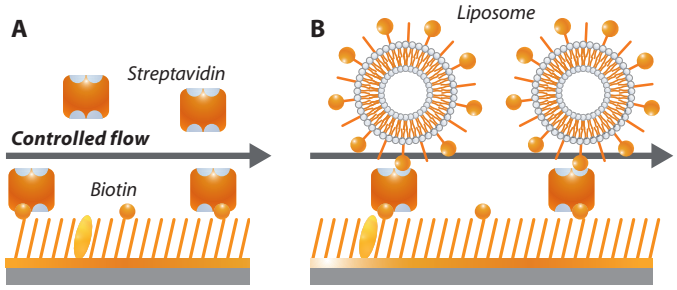

Figure 1. Illustration of the research frame of the two interaction systems used in this study: A) streptavidin biotin, B) liposome streptavidin

Results and discussion

Based on the CFD modeling, the flow conditions in the SPR Navi 200 and QCM–Z500 instruments could be synchronized through equation [1], which takes into account the height of the measurement chamber (*h*) and the used flow rate (*f*).

$$f_{QCM} = 2.64 f_{SPR} \left(\frac{h_{QCM}}{h_{SPR}}\right)^2 \qquad [1]$$

In order to synchronize the hydrodynamic flow conditions on the surface it was necessary to use flow rates of 10 and 100 µl/min in SPR Navi 200 and 73.3 and 733 µl/min in QCM–Z500, respectively.

Results from interaction studies with streptavidin and immobilized biotin shows that the apparent equilibrium constants (K) obtained from SPR Navi 200 and QCM-Z500 measurements were equal when CFD –modeled flow rates were used (Table 1). The maximum adsorbed amount $(\Delta M/A)_{max}$ differs due to the fact that QCM also senses the water incorporated in the sample layer. The QCM signal response (flow rate 73.3 and 733 µl/min) was normalized in order to remove contributions of coupled water, resulting

Excellence in Surface Plasmon Resonance
BioNavis

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 44 5872001
e-mail : info@bionavis.com
www.bionavis.com

		Streptavidin interaction with immobilized biotin			Biotinylated liposomes interaction with streptavidin surface	
	Flow rate (µl/min)	K (±SD) nM	$(\Delta M/A)_{max}$ (±SD) ng/cm²	ϕ (m_{QCM}/m_{SPR} - 1)	K (±SD) nM	$(\Delta M/A)_{max}$ (±SD) ng/cm²
SPR	10	16,2 (± 2,5)	196,5 (± 11,8)	1,33	87 (± 30)	1805 (± 272)
QCM	73,3	15,9 (± 3,1)	458,4 (± 50,5)		76 (± 28)	9266 (± 1431)
SPR	100	4,1 (± 0,8)	169,8 (± 13,6)	1,07	35 (± 11)	1019 (± 107)
QCM	733	4,3 (± 1,8)	350,2 (± 53,9)		35 (± 9)	6745 (± 561)

Table 1. Lagmuir fit parameters and the amount of coupled water (ϕ) from streptavidin biotin and liposome streptavidin interaction studies.

in equal responses compared to the SPR signal response (10 and 100 µl/min) (data not shown). From these results the coupled water mass per streptavidin mass (ϕ) was calculated. The amount of coupled water in the sample layer was lower at the higher flow rate compared to the lower flow rate, which indicates that streptavidin was able to form a more compact layer with less water entrapped in the case of higher flow. The amount of entrapped water is probably affected by the higher shear stress at higher flow rates. Streptavidin adsorption was strongly mass transport controlled as seen from the flow rate dependency of the signal responses.

The apparent equilibrium constants (K) and normalized signal responses of streptavidin and liposomes were also equal in SPR Navi 200 and QCM-Z500 measurements when CFD-modeled flow rates were used (Table 1, Fig.2A). Again, QCM results obtained higher maximum adsorbed amount than SPR due to the coupled water. Mass of bound liposomes was estimated assuming an ideal monolayer of liposomes on the surface. The liposome interaction study shows that the mass of bound liposomes was higher for the highest lipid concentrations at the lower flow rates (Fig.2 B and C). A possible explanation for this behavior was the increasing shear stress at higher flow rates, which might cause the rupture of some of the adsorbed liposomes into lipid bilayers.

Conclusions

The MP-SPR is a valuable tool to study biomolecular interactions and for investigating targeted drug delivery systems, such as targeted liposomes. The MP-SPR can be used to study the effect of flow hydrodynamics on the interaction kinetics, because it is easy to customize and precisely control the flow conditions in the measurement chamber. This will in the future allow to better take into account e.g. the blood stream hydrodynamic effects during drug development. The MP-SPR hydrodynamic flow conditions can also be correlated with other flow channel based measuring techniques by using CFD-modeling. Thus, this would allow additional and more accurate information to be obtained in multiple method-combining interaction studies.

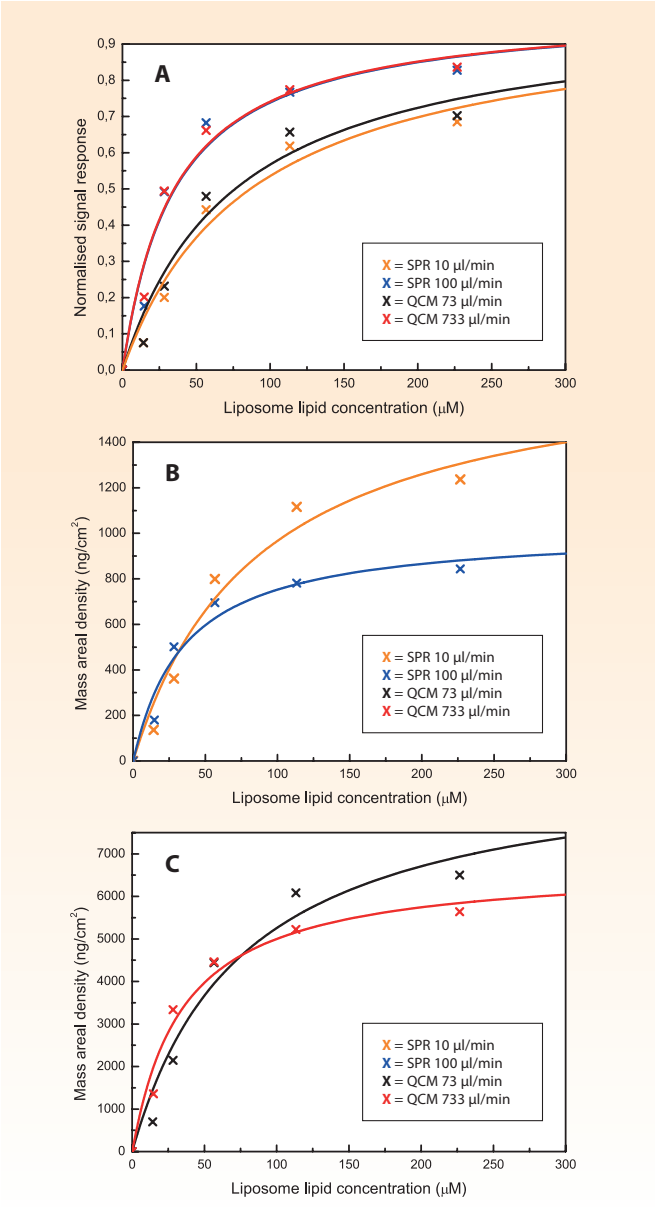

Figure 2. Liposomes interaction with streptavidin functionalized sensor surface at different flow rates. A) Normalized signal responses from both SPR and QCM measurements B) Signal responses from SPR measurement C) Signal responses from QCM experiment

Acknowledgements

We thank Dr. Tapani Viitala and co-workers from University of Helsinki, Faculty of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics for their co-operation.

[1] Viitala et al. Journal of colloid and interface science 2012, 378,251-259

Oy BioNavis Ltd.
Elopellontie 3 C
33470 Ylöjärvi
Finland

Tel : +358 44 5872001
e-mail : info@bionavis.com
www.bionavis.com