GRATING-COUPLED INTERFEROMETRY ANALYSIS OF AGONISTS BINDING TO A MEMBRANE PROTEIN

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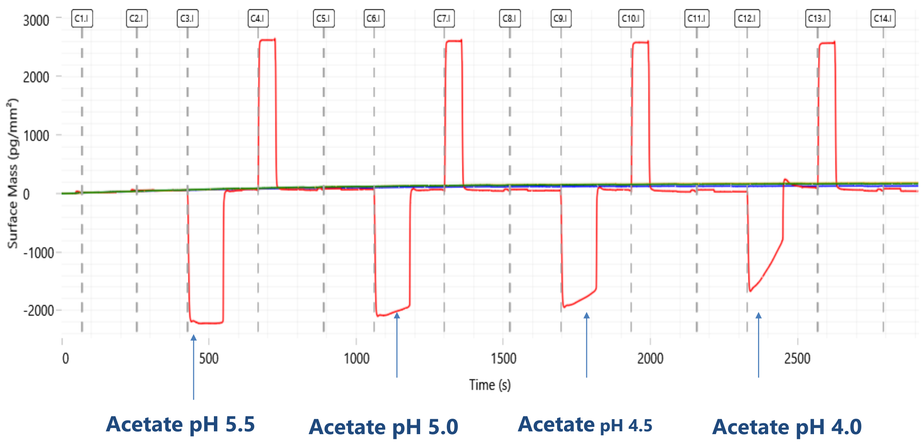
Introduction

Membrane proteins are notoriously challenging to study in cell-free systems, especially so in biophysical surface capture methods such as Surface Plasmon Resonance (SPR) and Grating-Coupled Interferometry (GCI). This is because of the inherent difficulties in capturing membrane proteins on chip surfaces while retaining their native conformation.

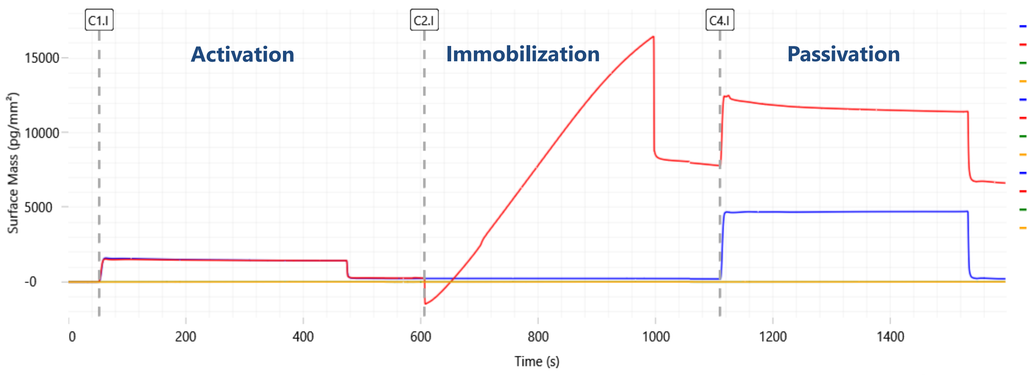
Domainex has invested in the Creoptix® wave Delta instrument. Here we show that the new waveRAPID technology facilitated the analysis of compound binding to a purified, detergent stabilized, membrane protein. The target protein is a membrane located ion channel with an approximate mass of 180 kDa. A protein of this size can be difficult to use as there will be a large ligand:analyte MW ratio. Crystal structures of this protein in the presence and absence of bound tool compounds 1 and 2 have been elucidated and show an open and closed conformation of the channel.

pH Buffer Scouting

Finding the right pH at which to immobilize is important for surfaced-based biophysical techniques. If the pH is too high the protein will not be ionized, and attracted to the chip surface, too low and the protein may not be stable for long. The isoelectric point of this membrane protein was determined to be 6.5. From this a good starting point for the pH of the immobilization buffer would be 5.5. At this pH we would expect 90% of the protein to be ionized. However, when the buffer scouting was performed the rate of immobilization was only suitable at pH 4.

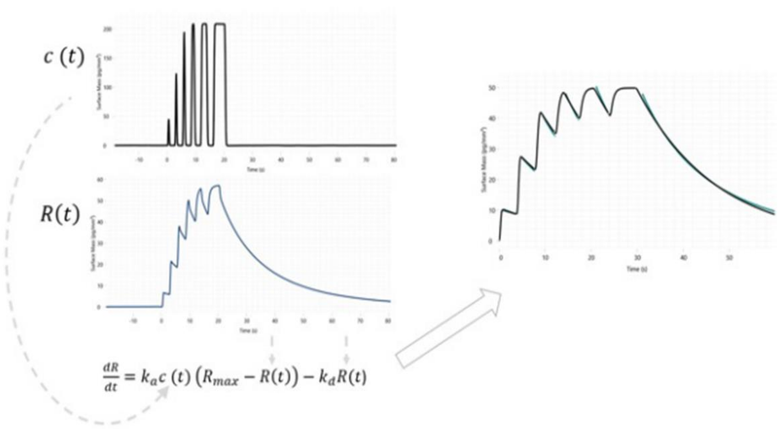


Immobilization of Membrane Protein



After finding the correct pH, the protein was immobilized to a Creoptix® wave DHX chip which has a functionalized carboxymethyl-dextran surface. The surface was first activated with a mixture of NHS (N-hydroxysuccinimide) and EDC (N-ethyl-N'-(dimethylaminopropyl) carbodiimide). The protein was diluted to 10 µg/mL in acetate buffer, pH 4, and then flowed over the chip. The surface was passivated using ethanolamine. Due to the large molecular weight of this protein, we aimed for a high level of protein immobilization on the surface of the chip. This way the compounds expected Rmax's would be large enough to measure successfully. The protein was immobilized to approximately 8000 pg/mm² (RU).

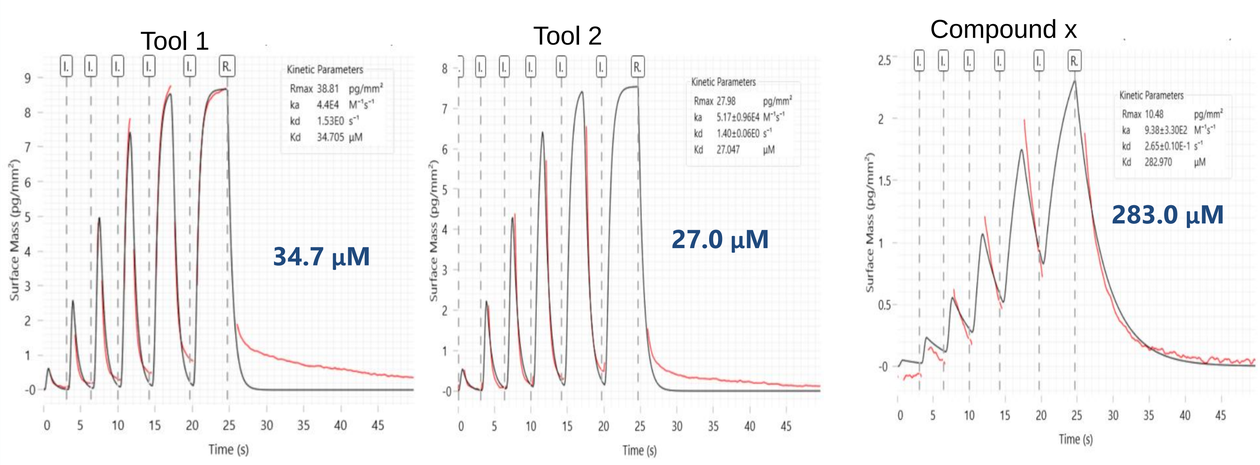
Creoptix RAPID mode



The Creoptix® wave can utilize the proprietary WaveRAPID analysis mode. In the WaveRAPID mode one analyte concentration is pulsed repeatedly with increasing duration. The time-dependent concentration function is shown at the top left, and the sensorgram response is shown at the lower left. As with a traditional kinetics approach, the association and dissociation constants can be obtained by applying c(t) and R(t) values to the equation at bottom. The observed binding curve is a response to the time-dependent concentration input of the injected analyte. RAPID allows screening to be run 10x faster than traditional kinetic methods, saving time on both preparation and actual run time.

1:1 Kinetic Binding Fit

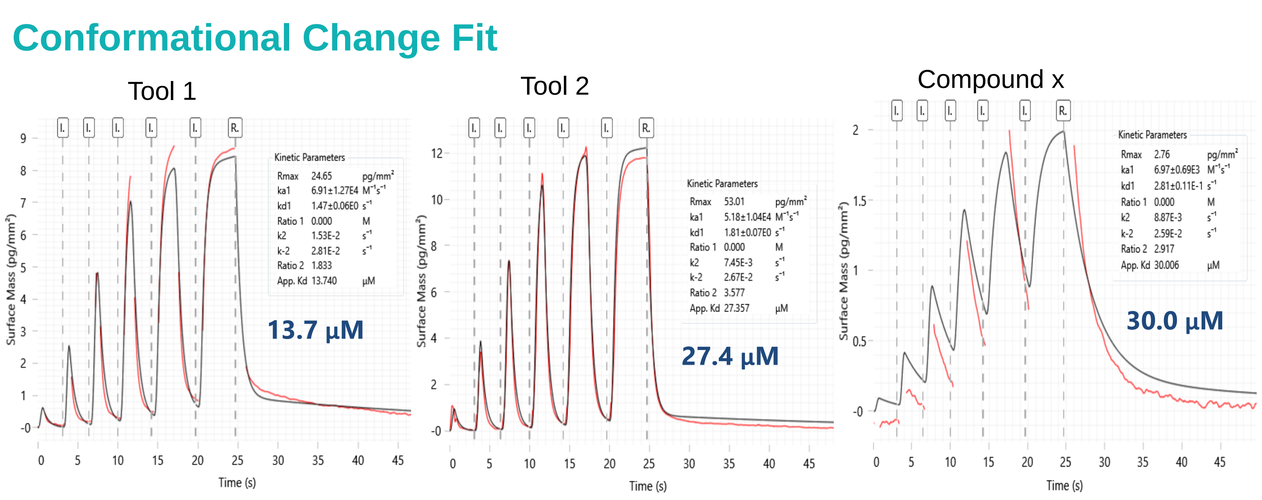
Three compounds were tested for binding against the membrane protein. The running buffer used was 100 mM HEPES pH 7.5, 150 mM NaCl, 0.03% LMNG, 0.003% CHS and 1% DMSO. Flow rate was set to 100 µL/min with injections of 25 s and a 300 s dissociation.



The 1:1 kinetic binding fit is the default fitting on the software and whilst the fit looks possible, the errors for Tool 1 and Tool 2 are close to 30%. Over a n=3 for each compound the k_a, k_d and K_D values are similar, and the data is very reproducible.

	Compound	n	Average k_a (M^{-1} s^{-1})	Average k_d (s^{-1})	Average K_D (µM)
1:1 Binding Kinetics	**Tool 1**	n=3	3.29E+04 ± 0.8	1.15E+00 ± 0.3	34.7 ± 1.6
	Tool 2	n=3	3.12E+04 ± 1.5	1.41E+00 ± 0.1	53.6 ± 19
	Compound x	n=3	1.06E+03 ± 0.3	2.87E-01 ± 0.1	289.0 ± 72

Conformational Change Fit



Based on the literature it is possible that the binding of Tool 1 and Tool 2 cause a conformational change to the ion channel when they bind. The Creoptix® wave can fit a model that simulates conformational change of the immobilized protein. The compounds were re-analyzed using the conformational change model. For Tool 1 and Tool 2, the conformational change model showed an improved fit and a reduced fitting error. For compound x the fit appeared to be worse, and the fitting error grew larger. This is a clear sign that Tool 1 and Tool 2 both cause a conformational change when binding to the protein but Compound x does not.

	Compound	n	Average k_a (M^{-1} s^{-1})	Average k_d (s^{-1})	Average K_D (µM)
Conformational Change	**Tool 1**	n=3	6.76E+04 ± 0.9	9.25E-01 ± 5.6	13.4 ± 1.5
	Tool 2	n=3	4.51E+04 ± 0.6	1.63E+00 ± 0.2	25.9 ± 2.3
	Compound x	n=3	3.45E+03 ± 2.6	2.88E-01 ± 0.01	45.0 ± 12

The k_a, k_d and K_D values of each compound across the two different fitting models are not significantly different. Small variations in the model produce slightly lower K_D values when the data is fitted using the conformational change model.

Conclusion

The Creoptix® wave system was able to successfully immobilize a large detergent stabilized membrane protein to a sufficiently high level to see binding of small molecule agonists. The data shows that the two tool compounds, previously known to cause a conformational change, can be seen to bind and cause this same conformational change. The quality of the data fit is exceptionally good when using a conformational change model giving very small residuals. Compound x is also shown to bind, but the fit of the 1:1 binding model is preferred to the conformational change, showing that the binding of this molecule alone is not enough to cause the conformational change from the closed to the open conformation.

Overall, the Creoptix® wave deals well with both the fact that this is a membrane protein but also one of a significant size. The excellent sensitivity of the Creoptix® wave enables easy detection of small molecule binders.

Services/Contact

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