Biacore experiments

18.11.12

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Purpose: Test inhibitors from in silico screening.

Test of liposomes

20.11.12 Liposome immobilization HBS-N buffer with 5% DMSO.

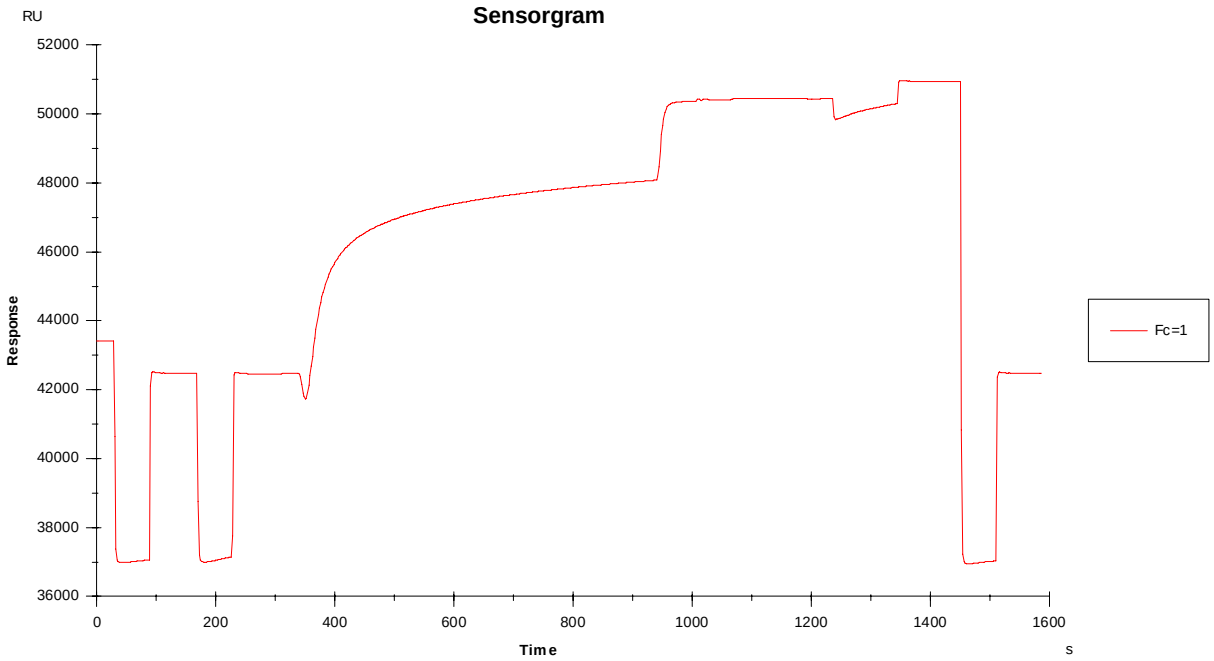
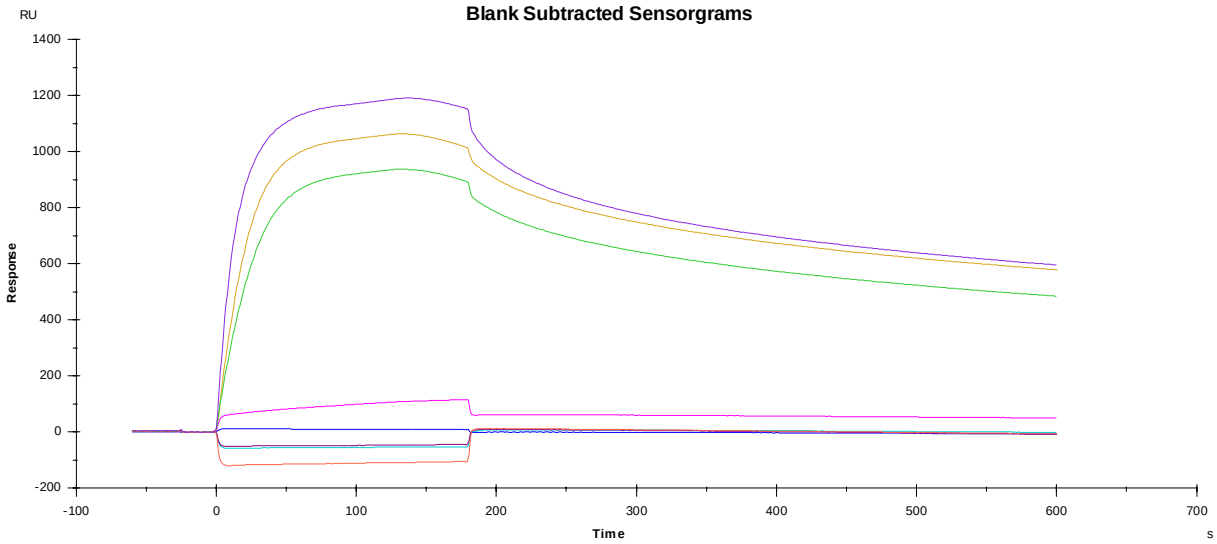


Figure 1: Fc1 42 467 RU (t=322 s) and 50 437 RU (t=1023 s)

20.11. 12 Test of PR3 binding in HBS-N with 5% DMSO

Startup		2	90,7
	0	3	75,1
blank 1		4	84,7
250 nM		5	190,6
blank 2		6	22,4
500 nM		7	1098,2
blank 2		8	31,2
500 nM		9	977,6
blamk 3		10	-30
1000 nM		11	1236,3

PR3 is injected over immobilized POPC at 3 different concentrations (250, 500, 1000 RU).



21.11.12 Test of inhibitors

Here I tested 3 inhibitors at approximately 100 uM concentration. Blanks are used to test the possible binding of the inhibitors to the chip and access artifacts while measuring the PR3 binding.

21.11.12 PR3 in HBS-N 5% DMSO
with inhibitors

startup		2	102,2
	0	3	87,7
blank 1		4	123,5
500 nm inhi1		5	821,5
blank2		6	113,8
500 nm inhi2		7	779,4
blank3		8	132,4
500 nm inhi3		9	718,7

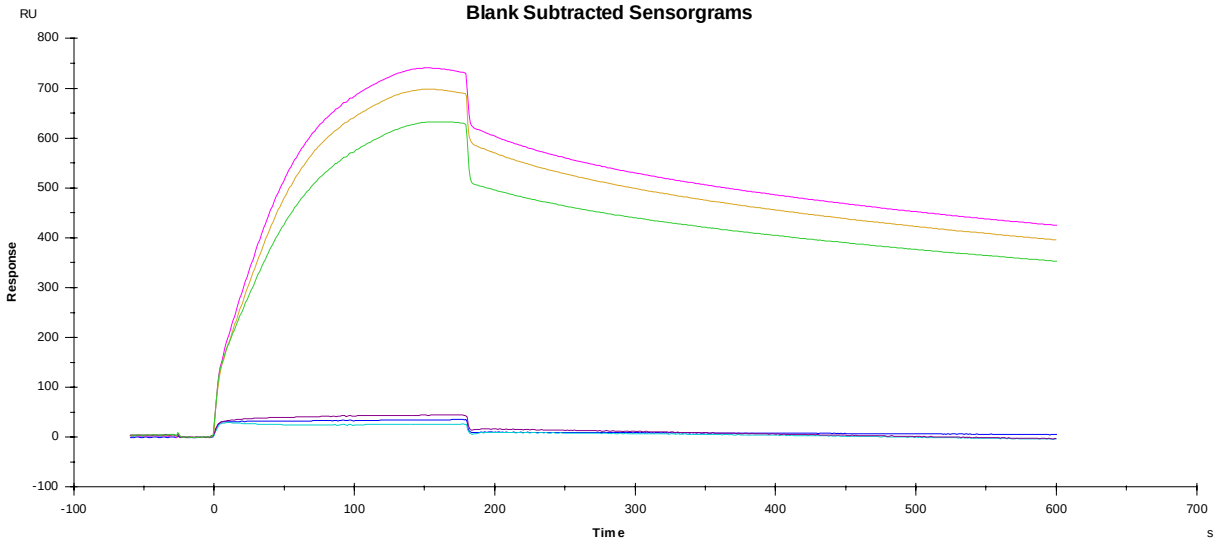


Figure 2: PR3 injected at 500 nM and pre-mixed with 3 different inhibitors

Inhibitors do not bind to the chip. RUs vary from 821 to 719 (+- 100 RUs) which might just be due to buffer.

22.11.2012 Test of inhibitors diluted in pure DMSO

Here I tested a new method to prepare PR3 + inhbitors solutions. The inhibitors were rehydrated in pure DMSO at high concentrations and re-diluted to obtain approximately 100 uM of inhibitor and 5 % DMSO.

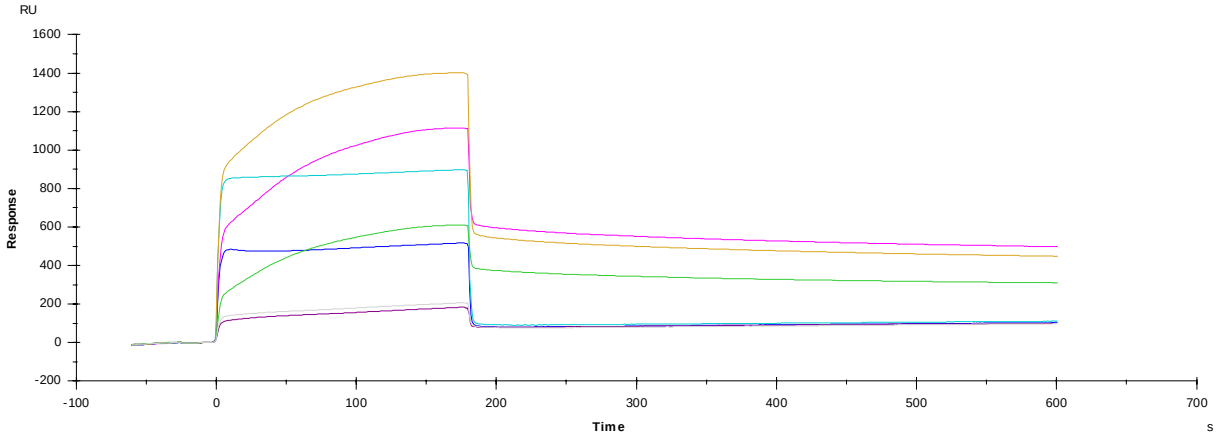


Figure 3: PR3 control (500 nM without inhibitor): (Purple + Red). Inhibitor1 (Dark blue + Pink), Inhibitor 2 (Yellow+green)