Inhibition of protein-membrane interactions by small compound inhibitors and screening by surface plasmon resonance spectroscopy

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November-December 2014

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Project description

The project is aimed at testing potent inhibitors of Proteinase 3 (PR3) and POPC liposome interactions by surface plasmon resonance spectroscopy. As known from a former bioinformatic study, a druggable cavity in proximity to the PR3 membrane-binding site was identified on using the PocketID program of the Sybyl8.0 software (cf. Figure 1). The cavity notably comprises the following residues: R186A, R186B and K187 known to mediate interaction with the membrane. The additional residues F166, F167 and L223 are located at the edge of the cavity, the later having a site chain pointing towards the cavity, and are participating to the membrane binding. The pharmacophore model used to target the cavity contains two hydrogen bound donors, two hydrogen bound acceptors, a hydrophobic, an aromatic and an anionic feature (Broemstrup, 2010). We selected the best 50 compounds of this study for experimental screening. These are low molecular weight compounds (LMWC) (between 150 and 300 Da) and were purchased from Chembridge (HitToLead), Chemdiv and Asinex (cf. Annexe 1 for the list of purchased compounds). The experimental setup used to monitor PR3 and POPC interactions is based on a pre-established SPR protocol (Schillinger, Grauffel, Khan, Halskau, & Reuter, 2014). This project aims at optimizing a protocol to test the LMWC and screening the inhibition of PR3-POPC interaction by those compounds.

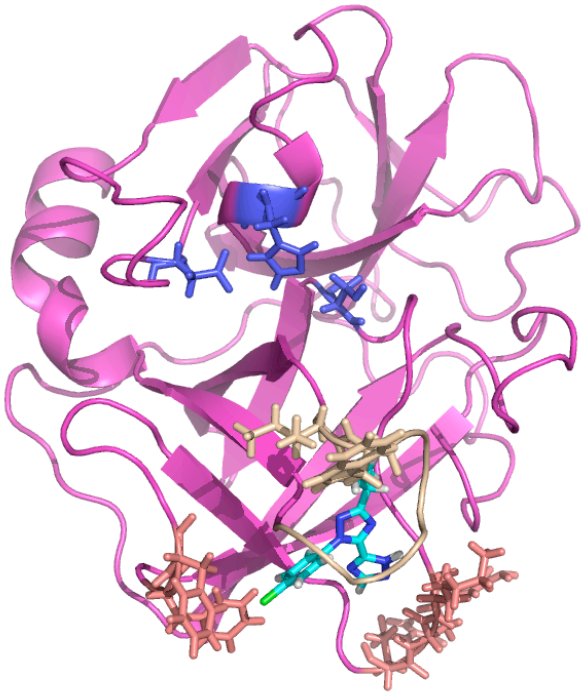

Figure 1: Representation of PR3 (purple) with an inhibitor (cyan) docked in the cavity (main residues in salmon red: R186A, R186B, K187, F166, F167 and L223) near the membrane-binding site. Residues from the β11-β12 loop are highlighted in wheat color due to their ability to form interaction with both substrate and membrane. The catalytic triad is shown in blue.

Methodological considerations and sample preparations

The LMWCs we have require the use of DMSO for adequate solvation. DMSO is a high refractive-index additive and requires special precautions and corrections for SPR studies. Because of low solubility, these compounds must be dissolved in 100% DMSO at high concentration. The concentration we choose for this shall be adapted for use with the SPR protocol to minimize buffer mismatch. The later could be in theory corrected with a procedure that shall also be optimized during this project (cf. next paragraph). Selected references for this work are the following (Christopeit et al., 2011; Rich, Day, Morton, & Myszka, 2001; Segers et al., 2007).

Data processing and analysis

The data analysis should ideally be processed using a double referencing procedure. As we cannot use a reference channel in our setup (Schillinger et al., 2014), this may have to be done manually. We suppose here that the small MW compounds do not bind the ligand (i.e. the immobilized POPC liposome surface). In general, very low binding is expected from these types of compounds (10-50 RU). Because of the mismatch of sample and running buffer, bulk shifts in the response signal may occur. This can be corrected using data from DMSO concentration series and correct for excluded volume effect. (Frostell-Karlsson et al., 2000; Rich et al., 2001). However, as we do not use a reference channel in our original setup, this procedure has to be done manually (if possible at all). Detailed procedure can be found in the Biacore handbook Appendix B "Solvent correction principles and practice" (GE Healthcare Life Science, 2012). Figure 1 illustrates the principle of the solvent correction.

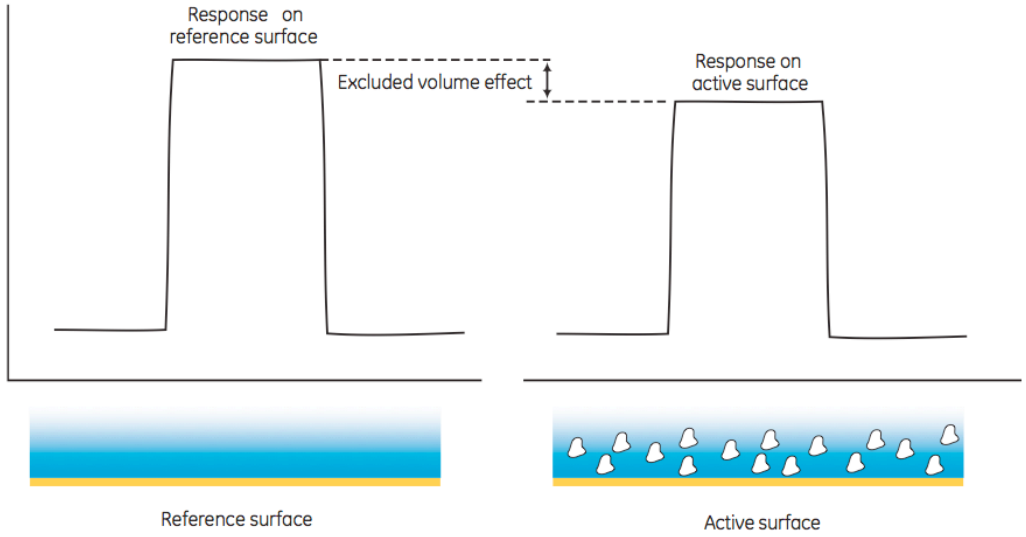

Figure B-1. Bulk solution is excluded from the volume occupied by ligand molecules on the active surface, so the contribution of bulk solution to the relative response is smaller than on the reference surface.

Figure 2 Solvent correction principle. From GE Healthcare Life Science, 2012

In general, changes in refractive index can be eliminated mostly by substracting the signal to the reference surface (Myszka, 1999). The percentage of inhibition could in theory be calculated as well according to Rich et al (Rich et al., 2001).

Assay development and main results

This paragraph provides a summary of the results based on the Biacore laboratory report (Biacore_laboratory_report.doc).

Setup

DMSO concentration was chosen to be 3% in all the assays. The strategy chosen to run the experiments was to investigate the inhibitory effect of the LMWCs using a mix of compounds and protein rather than observing the displacement by the inhibitor of the protein already bound to the phospholipid bilayer. This would allow the compounds to bind to the protein and perhaps prevent the binding to the lipids. The percentage of inhibition shall be calculated from these experiments when a proper protocol is validated.

Optimization of inhibitor preparation

All the compounds were dissolved in 2 mL DMSO. The experiments are run in accordance with the Biacore control software requirement with all details available in the method files. The first experiment (20.11.2014) displays a fairly minimal noise from the inhibitor preparation within 200 RU of variation from the baseline, supporting that a lower percentage of DMSO might decrease buffer artifacts. However the results were not sufficiently reproducible (21.11.2014). Solutions are usually prepared to match the volume requirement from the biacore control software in order to minimize sample waste. The mode of preparation of the inhibitor and DMSO calibration solution is therefore changed to prevent buffer artifacts from the use of small volume. A calibration curve around 3% DMSO using 2 mL final volume was used. The inhibitor or DMSO solutions are diluted is the following manner: 60µl in 1940µl HBS-N with no DMSO for the inhibitor; or appropriate volume of DMSO, i.e. 54, 56, 58, 60, 62, 64, 66µl DMSO respectively, to built a calibration curve around 3% DMSO). The objectives were to (1) test the compounds preparation procedure and (2) provide calibration data to correct for artifact. Results from 24.11.2014 from the calibration data show a concentration dependency that was not observed from previous calibration data using smaller volume (21.11.2014). However there is still poor reproducibility in the RU (24.11.2014 bis). We therefore cannot use the calibration curve to correct the data. The method is still used to prepare the inhibitors since a concentration dependency was observed in the calibration data, therefore improving the quality of the measured data.

Solvent correction

Solvent correction can be typically used when working with LMWC with SPR. A simple substraction from a reference surface may not be sufficient to correct for bulk effect. In order to improve the quality of the data, a solvent correction using running

buffer (HBSN with 3% DMSO) before and after sample injection was added to the original method (25.11.14). This however did not particularly improve the quality of the data. It was proven difficult to get reliable data with or without solvent correction (26.11.14). Data collected on PR3 without inhibitor are however very stable: 861.9, 843.3, 845.6 RU on three different cycles of injections of PR3 (27.11.2014).

Final setup

A new strategy to test the inhibitors was used for the final stage of this project. Here we immobilized the liposomes first and then inject PR3. As PR3 remains bound in sufficient amount to the liposomes after the injection stops (around 400 RU), we use this property of our systems to test the inhibitors. The inhibitors are thus injected after PR3. Potent displacement of PR3 from the liposomes by the inhibitor is monitored (from 03.12.2014). Inhibitors from Chembridge, Chemdiv and Asinex (references of the inhibitors are in the Biacore Laboratory report and Annex 1) were tested. No inhibition was observed for the molecules tested.

Key results

- ✓ 3% DMSO was used in all experiments.
- ✓ 2 ml volume for inhibitor preparation improves to quality of the results
- ✓ Solvent correction is insufficient to correct bulk effect
- ✓ A setup using successive immobilization of liposomes, then PR3 followed by the inhibitor seems to be the most appropriate for our system.

Summary and further work

The use of SPR can be challenging in some context and in particular when DMSO must be used. While DMSO has a high refractive index, extraordinary precautions must be taken to ensure that no interferences are coming from the use of such solvent. Our setup do not allow for the direct use of a reference channel, which brings an additional constraint. Other techniques may circumvent these issues. Further reading on methodological advancements to test bi-molecular interactions using back scattering interferometry could be considered (Morcos, Kussrow, Enders, & Bornhop, 2010). No attempt to use ITC has been done during this project due to high sample consumption.

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Annex 1: List on compounds tested

Company	Reference	Numbering of tubes in storage
Chembridge (HitToLead)	73851232	1
	49130108	2
	98864604	3
	91491149	4
	46251294	5
	42860873	6
	81769419	7
	5366442	8
	6126085	9
	5577150	10
	7515600	11
	70489111	12
	91438032	13
	5877763	14
	5741007	15
	13450319	16
	19904430	17
	18777612	18
	5774808	19
	5871258	20
	5848442	21
	54310288	22
	99187539	23
Chemdiv	D150-00170	24
	D361-2594	25
	F421-0120	26
	G262-060	27
	F454-0037	28
	G008-1125	29
	F652-0169	30
Asinex	ASN 18546438	31
	BAS 02791359	32
	BAS 12290470	33
	BAS 02988876	34
	BAS 01318520	35
	BAS 07211477	36
	BAS 00505574	37
	BAS 02982208	38
	BAS 02169322	39
	BAS 02303532	40
	BAS 02975554	41