How to Perform Excluded Volume Correction on the ProteOn™ XPR36 Protein Interaction System

In experiments where analytes are dissolved in a cosolvent with a high refractive index, such as DMSO, the reference surface produces a larger bulk solvent response than the ligand surface. Normally this bulk effect can be cancelled out after reference subtraction. However, the bulk effect is not equal on the interaction and reference spots. The reference surface produces a larger bulk shift/effect because of the larger concentration of cosolvent near the chip surface, caused by the exclusion of cosolvent from the chip surface of the interaction spot by the ligand. This is known as the excluded volume (EV) effect (Figure 1).

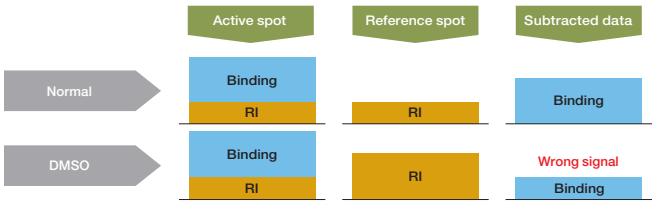

Fig. 1. Explanation of the EV effect when using cosolvents with high refractive index, such as DMSO. Normally the bulk effect will be cancelled out after reference subtraction. However, the bulk effect is not equal on both the active and reference spots due to DMSO exclusion from the surface on the active channel by the ligand.

Small differences in the concentration of DMSO in the analyte and running buffers also lead to large changes in response. This bulk effect is ~100 RU for every 0.1% difference in DMSO concentration.

Performing an EV correction step can cancel out these differences and lead to more reliable binding results.

To Run an Experiment With a Highly Refractive Cosolvent (DMSO)

1. Flush the instrument with ligand immobilization buffer in buffer position B. The ligand immobilization buffer usually does not contain cosolvent unless it is known not to interfere with immobilization.
2. Immobilize the ligand onto the sensor chip.
3. Determine the cosolvent concentration to be used in the experiment (e.g. DMSO 5%) that will keep the analyte soluble. DMSO concentrations up to 10% are acceptable.
4. Prepare an analyte stock solution, EV Calibration standards, and running buffer. These solutions should be prepared similarly to make the EV correction (EVC) the most accurate.

For example, prepare 4%–6% DMSO EVC standards, 5% DMSO running buffer, and 5% DMSO stock analyte from 10x PBS buffer as shown below. Dilute the stock analyte solution using the fresh 5% DMSO interaction running buffer (Figure 2).

Table 1. Preparation of DMSO solutions. Analyte and DMSO running buffer concentrations are given as an example and may change according to individual experimental needs.

	DMSO, ml 4%	DMSO, ml 6%	DMSO, ml 5%	Analyte, ml 20 µM
10x PBS	1.0	1.0	200	0.2
DMSO	0.4	0.6	100	0.08
Water	8.6	8.4	1,700	1.7
Analyte (2 mM, 100% DMSO)	—	—	—	0.02
Final Volume	10	10	2,000	2.0

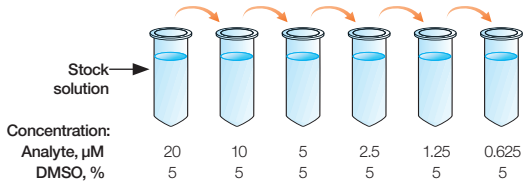

Fig. 2. Analyte preparation. Dilute the stock analyte solution with the highest concentration of analyte using the freshly prepared DMSO running buffer. The analyte concentration will be reduced but the DMSO concentration will stay the same (e.g., DMSO 5%/PBS).

5. Flush the instrument twice with the interaction analysis buffer containing the cosolvent at the preferred concentration in buffer position A.
6. Prepare six different concentrations of DMSO in fresh running buffer (Figure 3).

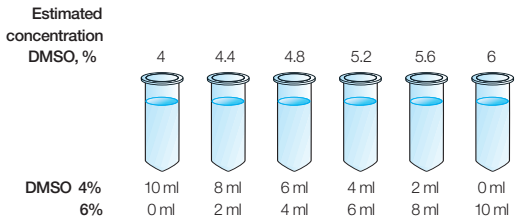

Fig. 3. Preparation of DMSO dilutions for EV calibration. Prepare two dilutions of DMSO in fresh running buffer, one above and one below the concentration used for the DMSO running buffer. In this example the running buffer contains 5% DMSO. Mix the two dilutions at the ratios described in the diagram to create a concentration series that has concentrations that cross over the DMSO concentration in the running buffer.

7. In the Protocol tab, after creating your protocol, click and drag the EV correction step group to the end of your protocol. The EV correction step group contains six injections by default (Figure 4).

Fig. 4. EVC calibration button.
Note: Blank injections that are used for double referencing must be made from the running buffer with the cosolvent.

8. Place the six DMSO dilutions into the instrument at the positions shown in the sample layout.

Processing and Applying EV Correction Data

When processing SPR data collected using buffer that include high refractive index cosolvents, its bulk reference (primary reference) must first be corrected for excluded volume effects. The data should be processed as follows:

1. Use the controls in the Data screen to select and group the analyte data for processing.
2. Select Channel Reference and choose EVC Calibration (Figure 5).

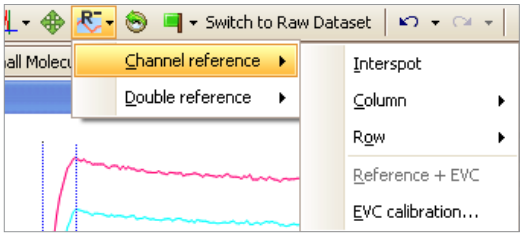

Fig. 5. Opening the EVC calibration wizard.

3. A wizard opens at the bottom of the Data screen. Select a row, column, or interspot reference. If you are using a Column or Row reference, use the associated dropdown menu to identify which channel the reference data are in.
4. In the step list, select a minimum of three EVC injections, if they are not already selected (Figure 6). The wizard displays EVC calibration data as thumbnail plots that show a best-fit line. These plots are accompanied by a table that lists the R^2 values for the best-fit lines.

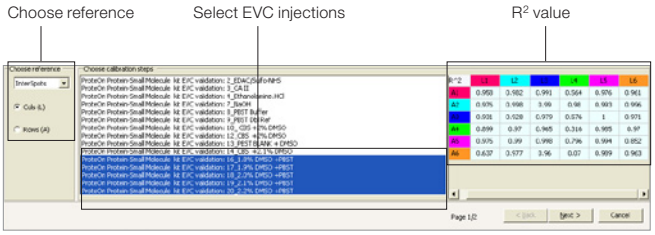

Fig. 6. Choosing reference and EVC injections, and viewing the R^2 value of the fit.

5. Double click the thumbnail plots with low R^2 values and then click on the bad data point to remove it. The excluded data point is represented by an empty circle and will not be included in the analysis. At least three solid data points must be selected from each calibration plot (Figure 7).

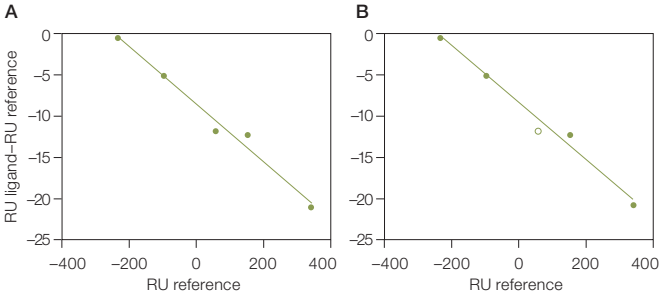

Fig. 7. Viewing the quality of the data for calibration. A, original data showing all five data points included in the calibration plot; **B,** modified calibration plot after removal of a data point.

6. In the second wizard step, select all the analyte steps for which you want to apply the EV correction. Click Finish to apply the reference and display the corrected data (Figure 8).

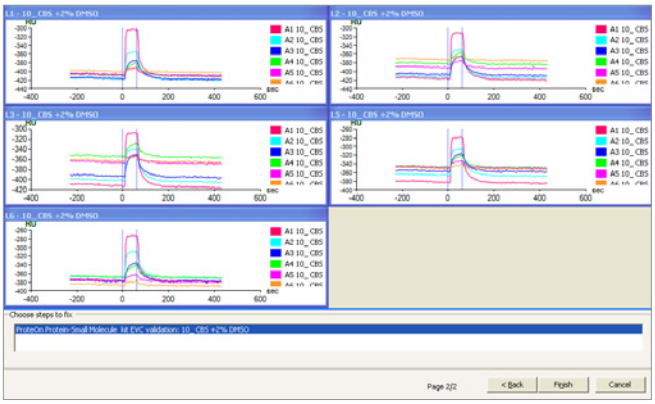

Fig. 8. Choosing the steps for applying EV calibration.

7. Apply a double reference, if desired.
8. Autoprocess the data.
9. Save the processed dataset using the Create dataset option.

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