Supporting Information

Partitioning of Catechol Derivatives in Lipid Membranes: Implications for Substrate Specificity to Catechol-O-methyltransferase

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1 EQUATIONS FOR THE CALCULATIONS OF THE SPR BINDING DATA

Jung model (1) states that the ‘SPR response’ can be written as being dependent on two structural parameters, optical layer thickness \( d \) and refractive index \( n \), and two sensor parameters, sensitivity constant \( S \) and decay length of the evanescent electric field above the sensor \( \delta \):

\[
\Delta \theta_{\text{SPR}} = R = S(n - n_b)(1 - e^{-d/\delta}) \approx \frac{S}{\delta}(n - n_b)d,
\]

(S1)

where \( n_b \) is the refractive index of the bulk liquid and the last approximation is made under assumption of a thin layer \( (d \ll \delta) \). Resolving both \( n \) and \( d \) is difficult, and Fresnel layer analysis of the full SPR angular spectrum by using more than one wavelength is usually employed. Even then, the knowledge of the exact dispersion relation of the bulk liquid, \( n_b \) as a function of the wavelength \( \lambda \), is needed (2). Therefore, it is often more convenient to write Eq. S1 in terms of the surface-mass density of the layer, \( \Gamma \):

\[
\Gamma = \frac{R\delta}{S(dn/dC)},
\]

(S2)

where \( (dn/dC) \) is the refractive index increment of the layer. An estimated value of 0.155 cm\(^3\)/g for a lipid bilayer at 670 nm was established previously (2), and for different soluble analytes, the value can be calculated by measuring the change in \( n_b \) as a function of increasing analyte concentration in the bulk. The surface-mass density change induced by the bound analyte can be written as

\[
\Gamma_a = \frac{R_a}{R_l}(dn/dC)_l \Gamma_l,
\]

(S3)

where subscripts ‘l’ and ‘a’ refer to the lipid and analyte, respectively.

The difference of measurement volumes (bilayer versus the whole measurement channel) provides a challenge in the formalism of the binding kinetics. However, certain approximations can be made. Using the mass balance equation for the analyte inside the flow channel, the concentration of the bound analyte \( C_a \) in the measurement volume is written as a function of the free analyte concentration \( C_f \):

\[
C_a = \frac{C_{a_{max}}}{1 + 1/KC_f} = \frac{\gamma nC_l}{1 + 1/KC_f},
\]

(S4)

which is the traditional Langmuir model taking into account the saturation of the lipid bilayer. Parameter \( C_{a_{max}} \) is the equilibrium saturation concentration, \( C_l \) is the concentration of lipid, \( K \) is the equilibrium mole ratio distribution coefficient, \( \gamma = 0.5 \) is an asymmetry factor taking into account the availability of lipids for binding (3, 4), \( n \) is the number of binding sites per lipid molecule. For SPR, which is a surface-based technique, this expression is not particularly useful since the lipid and bound analyte concentrations refer to the volume of the flow cell. Also, surface-mass densities are usually used instead of concentrations. When the analyte can be considered to be contained in the same volume as the lipids available for binding, Eq. S4 can be rewritten as

\[
\Gamma_a = \frac{\gamma n\Gamma_l (M_a/M_l)}{1 + 1/KC_f},
\]

(S5)

where \( \Gamma_{a_{max}} = \gamma n\Gamma_l (M_a/M_l) \) is the surface-mass density of the analyte at saturation and \( M_i \) \((i = a, l)\) are the molecular weights of analyte and lipid. From now on, uppercase (‘C’) refers to the concentrations in the bulk phase and lowercase (‘c’) refers to the concentrations in relation to the lipid phase. Coefficients of membrane partitioning can have multiple definitions (5, 6) but in this work, it is assumed as the ratio of concentrations of the bound analyte in the lipid phase and free analyte in the bulk phase:

\[
D_m = \frac{c_a}{C_f} = \frac{\Gamma_a}{C_f M_a d_l},
\]

(S6)

where \( d_l \) is the thickness of the bilayer. Relating the Langmuir model (Eq. S4) to the linear distribution coefficient in experiments is not always straightforward (7, 8). However, in the case where the equilibrium saturation concentration can be reached, distribution coefficient can be related to the parameters using series expansion of Eq. S5:

\[
D_m \approx \frac{\gamma n\Gamma_l M_l d_l}{K} = \gamma n c_l K.
\]

(S7)
However, it is $\Gamma^\text{max}_a$ and $K$ which are obtained from experiments. Therefore we write Eq. S6 in the final form (kinetic model):

$$D_m = \frac{\Gamma^\text{max}_a}{M_a d_l} K,$$

(S8)

In the case the equilibrium saturation concentration cannot be determined from the data, linear equation for the distribution coefficient should be used instead (linear model):

$$D_m = \left( \frac{\Delta \Gamma_a}{\Delta C_f} \right) \frac{1}{M_a d_l}.$$

(S9)

Figueira et al. (9) arrived at a combination of Eqs. S9 and S3 which does not require to know the surface-mass density of the lipid layer. Instead, only the concentration of lipid in the layer is needed ($c_l$). This is a more robust approach for traditional SPR instruments for the calculation of partition parameters since no sensor-related parameters (i.e. $S$ and $\delta$) are needed for the analysis. However, here we have also calculated the surface-mass densities of both lipid and analyte, and used $d_l = 3.8$ nm (PC, PC-PS and PC-PE-PS) and 4.6 nm (PC-Sm-Chol) for the lipid bilayers in the calculation of $D_m$ (2).

To find the values for $K$ and $\Gamma^\text{max}_a$, fitting of the adsorption kinetics using one-site kinetic binding model was performed in TraceDrawer software (v. 1.7, Ridgeview Instruments AB, Vänge, Sweden), except for L-dopa which showed barely detectable binding. For L-dopa, Eq. S9 with $C_f = 10$ mM was used instead to estimate the distribution coefficients. The linear fitting of Eq. S9 was also performed for the other compounds using the first two concentrations. Per the analysis of the previous section, the value of $\Gamma^\text{max}_a$ depends on the conversion from SPR response to surface-mass density. In the final calculations for the obtained kinetic fit parameters, $\Gamma^\text{max}_a$ was calculated by multiplying the corresponding response value with the ratio of the surface-mass density and response at the highest analyte concentration. The average surface-mass densities for each lipid composition were used. For dopamine with the PC-PE-PS membrane, the highest concentration (80 mM) was omitted in the analysis. Also, only the wavelength of 670 nm was used in the final mass calculations.

2 CALCULATION OF THE DECAY LENGTH PARAMETERS

For the SPR sensor slides used in the experiments, angular spectra of the sensors were recorded in air before the first use. After all the experiments were performed, SiO$_2$ layers of the sensors were cleaved by immersing the sensors in boiling solution of H$_2$O:NH$_3$:H$_2$O$_2$ in molar ratio of 5:1:1. The angular spectra of the cleaved sensors were measured in air and then analyzed using SPR Navi LayerSolver™ software. Fresnel layer formalism for the individual layers (prism, chromium, gold, SiO$_2$, air/buffer) was used in the software to calculate the layer parameters (thickness and refractive index) using two-medium method, as described elsewhere (10). Initial sensitivity constants ($S_{670\,nm}$ and $S_{785\,nm}$) were determined by sequential injections of 0.5, 1.0, 1.5 and 2.0 % DMSO as done by Emilsson et al. (11), and these values were used to obtain decay lengths ($\delta_{670\,nm}$ and $\delta_{785\,nm}$) using Eq. S3. The results of the modeling are presented in Table S1.

| Sensor | $d_{670\,nm}$ | $n_{670\,nm}$ | $\delta_{670\,nm}$ | $S_{670\,nm}$ | $\Gamma^\text{670\,nm}$ | $d_{785\,nm}$ | $n_{785\,nm}$ | $\delta_{785\,nm}$ | $S_{785\,nm}$ | $\Gamma^\text{785\,nm}$ |
|--------|---------------|---------------|----------------|-------------|----------------|---------------|---------------|----------------|-------------|----------------|
| Sensor 1 | CH1 | 1.57 | 1.68631 | 112.8 | 113.9 | 394 | 1.60 | 1.68212 | 186.7 | 97.3 | 427 |
| | CH2 | 1.45 | 1.73273 | 121.2 | 112.3 | 416 | 1.52 | 1.69719 | 187.5 | 96.7 | 447 |
| | CH3 | 1.53 | 1.66438 | 114.7 | 116.35 | 500 | 1.58 | 1.64696 | 184.2 | 98.1 | 550 |
| | CH4 | 2.34 | 1.51575 | 101.5 | 112.35 | 471 | 2.17 | 1.52319 | 163.5 | 95.2 | 524 |
| Sensor 2 | CH1 | 4.95 | 1.53794 | 125.2 | 107.4 | 424 | 5.31 | 1.52421 | 185.0 | 93.1 | 429 |
| | CH2 | 6.34 | 1.53109 | 127.3 | 104.0 | 452 | 6.78 | 1.51528 | 186.4 | 91.5 | 454 |
| | CH3 | 5.42 | 1.52476 | 108.4 | 114.8 | 421 | 5.66 | 1.52029 | 167.4 | 97.1 | 456 |
| | CH4 | 5.02 | 1.49503 | 107.6 | 122.2 | 461 | 5.07 | 1.49638 | 167.2 | 102.4 | 505 |

Initially calculated decay length values for the two sensors were used to calculate initial surface-mass densities of SLBs from the baseline-corrected responses of the deposited SLBs using Eq. S3. However, the correction procedure for the decay lengths had to be performed in order to account for the gradual changes in the sensor properties when the same sensor slide was used for multiple experiments. Using the initial values for decay lengths
in all experiments resulted in surface-mass densities of $469 \pm 97$ (PC), $559 \pm 313$ (PC-PS), $529 \pm 144$ (PC-PE-PS) and $618 \pm 140$ (PC-Sm-Chol) ng/cm$^2$ using the 670 nm wavelength. The initial values of different parameters calculated from the first experiment of each sensor are presented in Table S1. It can be seen that the average surface-mass densities over all experiments differ a lot from the values of the initial experiments. Therefore, we estimated what the decay lengths in different experiments would be if the surface-mass densities from the initial experiment would remain fixed. In Fig. S1, the estimated decay length is plotted against the difference between the initial SPR peak minimum angle and the angle obtained before every experiment. It seems that there is a nearly linear correlation between these two parameters. Therefore, for the corrected decay length values, we used the values indicated by the linear best fit to the data shown in Fig. S1. This correction resulted in a more realistic variation in SLB surface-mass densities: $394 \pm 10$ (PC), $393 \pm 15$ (PC-PS), $421 \pm 23$ (PC-PE-PS) and $510 \pm 19$ (PC-Sm-Chol) ng/cm$^2$. The presented data are calculated with standard deviations.

![Graph](image-url)

Figure S1: Estimated decay lengths versus the differences in initial SPR peak minimum angle ($\theta_{SPR}$) of the first experiment and subsequent experiments. The data is plotted for the wavelength 670 nm (blue dots) and 785 nm (red crosses) separately.

### 3 CALCULATION OF THE SENSITIVITY PARAMETERS

The dependence of the change in TIR angle on the change in bulk refractive index can be described as

$$B (\degree) = \frac{\Delta \theta_b}{\Delta n_b} = \frac{360\degree / 2\pi}{n_p \cos \theta_{b,0}},$$

where $\theta_{b,0}$ is the TIR angle before the change in bulk refractive index and $n_p$ is the refractive index of the prism. A similar parameter, sensitivity constant $S$, relates the change of SPR peak angle minimum to the change in bulk refractive index:

$$S (\degree) = \frac{\Delta R_b}{\Delta n_b}.$$

For a single thin layer, linear correction for the effect of the change in bulk refractive index on the measured response can be written as

$$R = R_m - G \Delta \theta_p = R_m - \frac{S}{B} \left(1 - \frac{d}{\delta}\right) \Delta \theta_p,$$

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where $R_m$ is the measured response and $G$ is referred to as gain. Now if we consider a second layer of adsorbant with layer parameters $d_a$ and $n_a$ on top of the bilayer with parameters $d_l$ and $n_l$, then the same equations are valid but in this case the bilayer will contribute to the response from the adlayer:

\[
R_a = R_{m,a} - \frac{S}{B} \left[ 1 - \frac{d_a}{\delta} \left( 1 - \frac{d_l}{\delta} \right) \right] \Delta \theta_b
\]

When comparing Eqs. S12 and S13 it is evident that the correction made for the partitioning event must be smaller in the case where adsorbant is distributed inside the lipid bilayer. For a typical bilayer with thickness of ∼4 nm, however, there would be a relative error of ∼3-4% to the gain values when omitting the thickness-dependent terms from the equations. Therefore, the terms in the equations were approximated as $d_a/\delta \approx 0$ and $d_l/\delta \approx 0$.

Correction of the bulk signal affecting the change in SPR peak minimum angle was done by finding the values of sensitivity constants in Eqs. S1 and S2 which minimized the difference upon a change in the running buffer. For dopamine, L-dopa, and 3-MT, values of $S$ were calculated using the first water injection in the SLB deposition phase, except for PC-PE-PS for which the average obtained from three other depositions was used instead. For tolcapone and entacapone, the correction was performed entirely using the shift in the signal resulting from the change to the buffer containing DMSO. The dependence of the change in TIR angle on the change in bulk refractive index, $B$, was calculated using the sequential injections of 0.5, 1.0, 1.5 and 2.0 % (vol/vol) DMSO for each sensor. Values were kept constant for each sensor used in the study, and the gain values ($G$) ultimately used for the correction were calculated as the ratios of $S$ and $B$ according to Eq. S12.

Using the equations above, the refractive index increment of the analyte can be calculated using the linear relationship

\[
\frac{dn}{dC}_a \approx \frac{\Delta n_p}{\Delta C_a} = \frac{\Delta \theta_b}{B \Delta C_f}
\]

where $C_f$ is the free analyte concentration inside the SPR flow channel, equal to the prepared concentration of the analyte. The measured average values for $\frac{dn}{dC}_a$ were 0.248 cm$^3$/g (dopamine), 0.230 cm$^3$/g (3-MT), 0.206 cm$^3$/g (L-dopa), 0.361 cm$^3$/g (entacapone), and 0.371 cm$^3$/g (tolcapone).

### 4 DETAILS OF THE QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP MODELING

Quantitative structure-activity relationship model (QSAR) was trained for the compounds analyzed by Osanai et al. (3) and the catechol compounds by using linear regression in statsmodels Python package (OLS function). The initial feature parameters were the physiological charge, number of hydrogen bond acceptors (#HA) and donors (#HD), the sum of bond acceptors and donors (#HA+#HD), polar surface area, predicted log $D_{oct/w}$ and log $P_{oct/w}$, provided by ChemSpider website (data generated using the ACD/Labs Percepta Platform). The lowest $p$-values for the null-hypothesis that the parameter coefficient is non-zero were 0.150 for #HD and 0.275 for log $D_{oct/w}$, #HA+#HD and log $D_{oct/w}$ were selected for the subsequent model, since the selection of #HA+#HD resulted in higher $R$-squared for the catechol compounds than using the number of hydrogen bond donors only. The $R$-squared for all compounds was 0.856 while the prediction for the five catechol compounds only (obtained in this study) resulted in the value of 0.717. When log $D_{oct/w}$ was replaced by log $P_{oct/w}$, the corresponding statistics were 0.800 and 0.509. It should be noted that the five catechol compounds were used in the training of the model. Coefficients of the model using #HA+#HD and log $D_{oct/w}$ are given as:

\[
3.5552 - 0.1403 \times (#HA + #HD) + 0.4175 \times \log D_{oct/w} = \log D_{m,\text{pred}}
\]
Table S2: The parameters used in the QSAR modeling, measured log $D_m$, predicted log $D_{m,\text{pred}}$ and the ratio of $D_{m,\text{pred}}$ and $D_m$.

| Compound             | #HA/#HD | log $D_{\text{oct/w}}$ | log $P_{\text{oct/w}}$ | log $D_m$ | log $D_{m,\text{pred}}$ | $D_{m,\text{pred}}/D_m$ |
|----------------------|---------|-------------------------|-------------------------|-----------|-------------------------|--------------------------|
| Amitriptyline        | 1       | 2.96                    | 4.92                    | 4.48      | 4.65                    | 1.48                     |
| Nortriptyline        | 2       | 2.28                    | 5.65                    | 4.34      | 4.23                    | 0.77                     |
| Chlorpromazine       | 2       | 3.41                    | 5.20                    | 4.74      | 4.70                    | 0.91                     |
| Imipramine           | 2       | 2.68                    | 4.80                    | 4.14      | 4.39                    | 1.79                     |
| Promethazine         | 2       | 3.10                    | 4.78                    | 4.34      | 4.57                    | 1.69                     |
| Propranolol          | 5       | 1.15                    | 3.10                    | 3.81      | 3.33                    | 0.33                     |
| Diclofenac           | 5       | 1.37                    | 4.06                    | 4.02      | 3.43                    | 0.25                     |
| Miconazole           | 3       | 6.13                    | 5.93                    | 4.74      | 5.69                    | 8.99                     |
| Indomethacin         | 6       | 0.75                    | 3.11                    | 3.86      | 3.03                    | 0.15                     |
| Nifedipine           | 9       | 3.45                    | 2.97                    | 4.38      | 3.73                    | 0.23                     |
| Desipramine          | 3       | 1.58                    | 4.13                    | 3.76      | 3.79                    | 1.08                     |
| Diflunisal           | 5       | 1.16                    | 4.44                    | 3.81      | 3.34                    | 0.34                     |
| Dopamine             | 7       | -2.18                   | 0.12                    | 1.26      | 1.66                    | 2.53                     |
| 3-methoxytyramine    | 6       | -1.84                   | 0.43                    | 2.18      | 1.94                    | 0.58                     |
| L-dopa               | 10      | -2.70                   | -0.22                   | 0.14      | 1.02                    | 7.66                     |
| Entacapone           | 10      | -0.04                   | 2.38                    | 1.83      | 2.14                    | 2.02                     |
| Tolcapone            | 8       | 1.22                    | 4.07                    | 2.76      | 2.94                    | 1.52                     |
5 EFFECT OF CALCIUM ON THE ANALYTE BINDING

Figure S2: The change in the SPR peak minimum angle for dopamine interacting with the PC-PS lipid bilayer with and without treatment with 5 mM EDTA.
6 UNPROCESSED SURFACE PLASMON RESONANCE DATA
Figure S3: The change in the SPR peak minimum angle (solid lines) and angle of total internal reflection (dashed lines) for the deposition of SLBs. Repeat experiments are spaced horizontally.
Figure S4: The change in the SPR peak minimum angle for the binding events. Repeat experiments are spaced horizontally.
Figure S5: The change in the angle of total internal reflection for the binding events. Repeat experiments are spaced horizontally.
UNPROCESSED QUARTZ CRYSTAL MICROBALANCE DATA
Figure S6: The changes in the normalized frequency and dissipation of the third overtone for the deposition of SLBs.
Figure S7: The changes in the normalized frequency and dissipation of the third overtone for the binding events. Black dashed line shows the signal without SLBs.

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