S2 Table. FEP calculations and equilibrium binding constants for singly- and doubly-occupied sites $j$ of the open channel structure.\textsuperscript{*}

| Site | $k_0(1)$ | $W_j'(1) \pm \varepsilon$ | $K_0(0,...,1,...,0)$ | $\Delta G_j'(0,...,1,...,0)$ | $k_0(2)$ | $W_j'(2) \pm \varepsilon$ | $K_0(0,...,2,...,0)$ | $\Delta G_j'(0,...,2,...,0)$ |
|------|----------|----------------|-----------------|-----------------|----------|----------------|-----------------|-----------------|
| CL   | 0.238    | -4.1 ± 0.2    | 3.17E-02        | -2.0            | 0.238    | -5.6 ± 0.2    | -9.7 ± 0.2    | 6.31E-03        | -5.2            |
| 2    | 0.243    | -4.5 ± 0.2    | 6.02E-02        | -2.4            | 0.155    | -6.7 ± 0.2    | -11.2 ± 0.4   | 1.46E-01        | -7.1            |
| 3    | 0.283    | -4.7 ± 0.2    | 6.74E-02        | -2.5            | 0.144    | -4.8 ± 0.2    | -9.5 ± 0.4    | 7.41E-03        | -5.3            |
| 4    | 0.253    | -5.7 ± 0.2    | 4.29E-01        | -3.6            | 0.142    | -6.3 ± 0.2    | -12 ± 0.4     | 6.01E-01        | -7.9            |
| 5    | 0.108    | -3.8 ± 0.3    | 6.27E-02        | -2.5            | 0.140    | -5.1 ± 0.3    | -8.9 ± 0.6    | 1.19E-02        | -5.6            |
| 6    | 0.045    | -6.4 ± 0.2    | 1.84E+01        | -5.8            | 0.105    | -8.1 ± 0.2    | -14.5 ± 0.4   | 8.49E+02        | -12.2           |
| 7    | 0.127    | -7.8 ± 0.3    | 4.17E+01        | -6.3            | 0.158    | -7.3 ± 0.2    | -15.1 ± 0.5   | 2.69E+02        | -11.5           |
| 8    | 0.050    | -6.9 ± 0.2    | 3.70E+01        | -6.2            | 0.109    | -6.1 ± 0.2    | -13.0 ± 0.4   | 5.55E+01        | -10.6           |
| 9    | 0.033    | -6.7 ± 0.3    | 4.86E+01        | -6.4            | 0.072    | -5.2 ± 0.2    | -11.9 ± 0.5   | 2.94E+01        | -10.2           |
| VSD  | 0.070    | -3.5 ± 0.3    | 7.28E-02        | -2.5            | 0.371    | -2.7 ± 0.2    | -6.2 ± 0.5    | 5.59E-05        | -2.4            |
| 11   | 0.112    | -1.4 ± 0.4    | 1.04E-03        | 0.0             | 0.117    | -1.3 ± 0.4    | -2.7 ± 0.8    | 4.25E-07        | 0.5             |
| 12   | 0.139    | -3.3 ± 0.3    | 1.85E-02        | -1.7            | 0.076    | -4.7 ± 0.4    | -8 ± 0.7      | 4.50E-03        | -5.0            |
| 13   | 0.111    | -4.8 ± 0.3    | 3.25E-01        | -3.4            | 0.098    | -4.0 ± 0.4    | -8.8 ± 0.7    | 1.65E-02        | -5.8            |
| 14   | 0.137    | -6.1 ± 0.2    | 2.11E+00        | -4.5            | 0.074    | -9.8 ± 0.3    | -15.9 ± 0.5   | 2.92E+03        | -12.9           |
| 15   | 0.118    | -2.2 ± 0.3    | 3.68E-03        | -0.8            | 0.711    | -0.5 ± 0.4    | -2.7 ± 0.7    | 2.61E-08        | 2.2             |
| 16   | 0.143    | -7.9 ± 0.2    | 4.15E+01        | -6.3            | -        | -            | -             | -               | -               |
| 17   | 0.113    | -5.2 ± 0.2    | 6.22E-01        | -3.8            | 0.303    | -5.2 ± 0.3    | -10.4 ± 0.5   | 4.39E-02        | -6.3            |
| Est  | 0.530    | 1.5 ± 0.4     | 7.57E-07        | 4.3             | -        | -            | -             | -               | -               |
| 19   | 0.453    | -0.8 ± 0.4    | 4.63E-05        | 1.8             | -        | -            | -             | -               | -               |
| 20   | 0.283    | -0.8 ± 0.4    | 9.36E-05        | 1.4             | -        | -            | -             | -               | -               |
| 21   | 0.332    | -4.2 ± 0.5    | 2.28E-02        | -1.9            | -        | -            | -             | -               | -               |

\textsuperscript{*}For singly occupied sites, units for $k_0(1)$, $W_j'(1)\varepsilon$, $K_0(0,...,1,...,0)$ and $\Delta G_j'(0,...,1,...,0)$ are kcal/mol/Å$^2$, kcal/mol, mM and kcal/mol, respectively. For doubly occupied sites, units for $k_0(2)$, $W_j'(2)\varepsilon$, $K_0(0,...,2,...,0)$ and $\Delta G_j'(0,...,2,...,0)$ are kcal/mol/Å$^2$, kcal/mol, mM$^2$ and kcal/mol, respectively. FEP estimates, $W_j'(1)$, and $W_j'(2)$, and statistical errors $\varepsilon$ were determined using the simple overlap sampling (SOS) formula (Li et al., 2004) based on at least two independent FEP runs. $W_j'(2)$ was computed as a two-step process $W_j'(2)=W_j'(1)+W_j'(2)$ involving ligand coupling to a vacant site $W_j'(1)$ followed by binding of a second ligand at the preoccupied site $W_j'(2)$. Binding constants, $K_0(0,...,1,...,0)$ and $K_0(0,...,2,...,0)$, and the related binding free energies, $\Delta G_j'(0,...,1,...,0)$ and $\Delta G_j'(0,...,2,...,0)$, were quantified relative to a homogeneous and diluted aqueous solution occupied by ligands at an excess chemical potential of $\mu=0.10\pm0.09$ kcal/mol$^{-1}$.