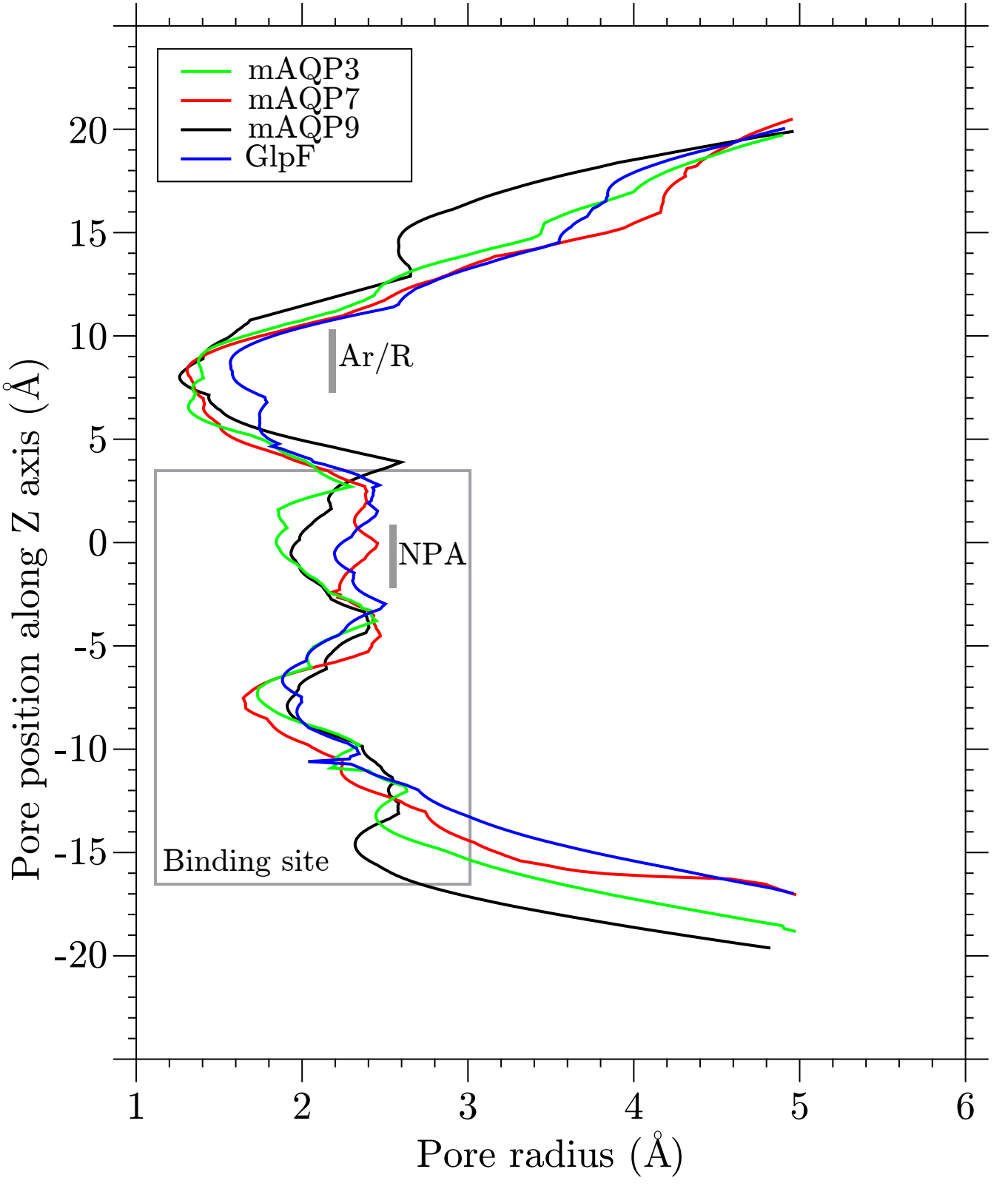
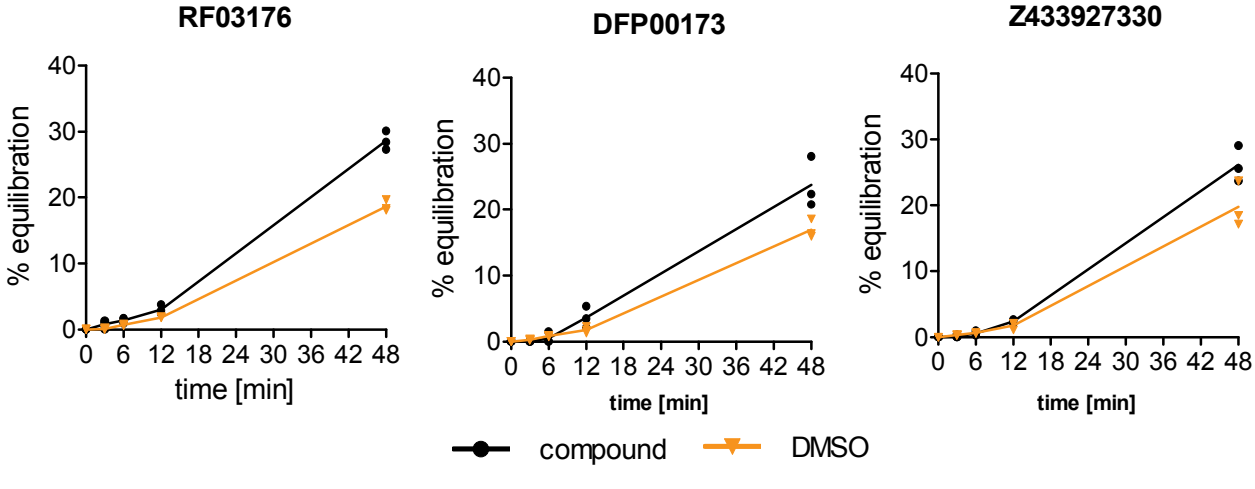

mAQP3
mAQP7
mAQP9
GlpF
Ar/R
NPA
Binding site
Pore position along Z axis (Å)
Pore radius (Å)
20
15
10
5
0
-5
-10
-15
-20
1
2
3
4
5
6


RF03176
DFP00173
Z433927330
% equilibration
time [min]
0
6
12
18
24
30
36
42
48
10
20
30
40
compound
DMSO

Supporting Figure 1: Pore diameter of aquaporins 3, 7 and 9, as well as *E. coli* GlpF, calculated using the HOLE software. Locations of the Aromatic/Arginine region (Ar/R) and the canonical NPA box are indicated with arrows. The binding site for the four docked ligands is indicated with a square.

Supporting Figure 2: mpkCCD epithelial barrier permeability assays. AQP inhibitors and DMSO readily crossed the epithelial barrier, while cell-impermeable TO-PRO-3 remained undetectable at the apical side. Permeability is expressed as % equilibration at the apical side.
Cells were seeded on top of 8 µM PE Millicell hanging cell culture inserts (Merck Millipore) and grown for two days, before TEER reached 388 (+/-83 SD) mΩ/cm2. Filters were washed and transferred into wells of a 12-well plate containing 1.5 ml Ringer buffer, 1.1% DMSO, 0.1% TO-PRO-3 (ThermoFisher Scientific), as well as one of the following: RF03174 (50 µM), DFP00173 (25 µM), and Z433927330 (25 µM). Assays were then started immediately, by adding 0.5 ml of Ringer buffer to the top of the filter inserts, and 20 µl aliquots were removed before the start, as well as from the top side at 3, 6, 12, and 48 minutes after the start. Test substance diffusion to the apical side was quantified as % equilibration, where 100% equilibration indicates equal distribution of test substances in 2 ml Ringer buffer. DMSO (200 nm) and AQP inhibitors (260 nm) were quantified by UV absorbance and peak integration after reversed phase HPLC, utilizing a 250 × 4.6 mm C18 column (YMC ODS-AQ12S03-2546WT, YMC, Kyoto, Japan) with 75% Acetonitrile and 25% water as the mobile phase. TO-PRO-3 was quantified after dilution in four volumes of calve thymus DNA dissolved in water (500 ng/µl). A Probe Drum instrument (Probation labs, Lund, Sweden) was utilized as a fluorimeter, with a 638 nm laser for illumination and quantifying emission at 661 nm.