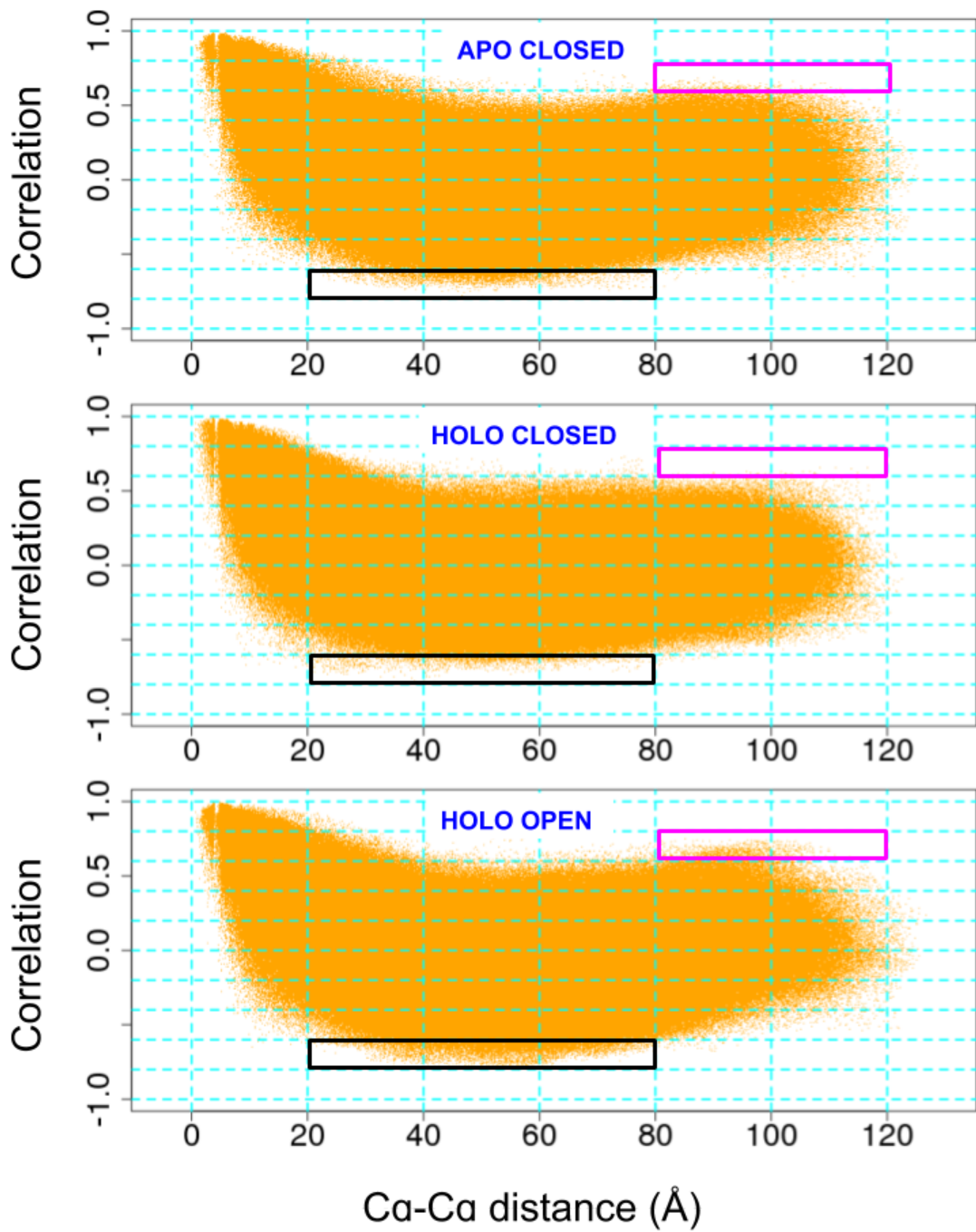
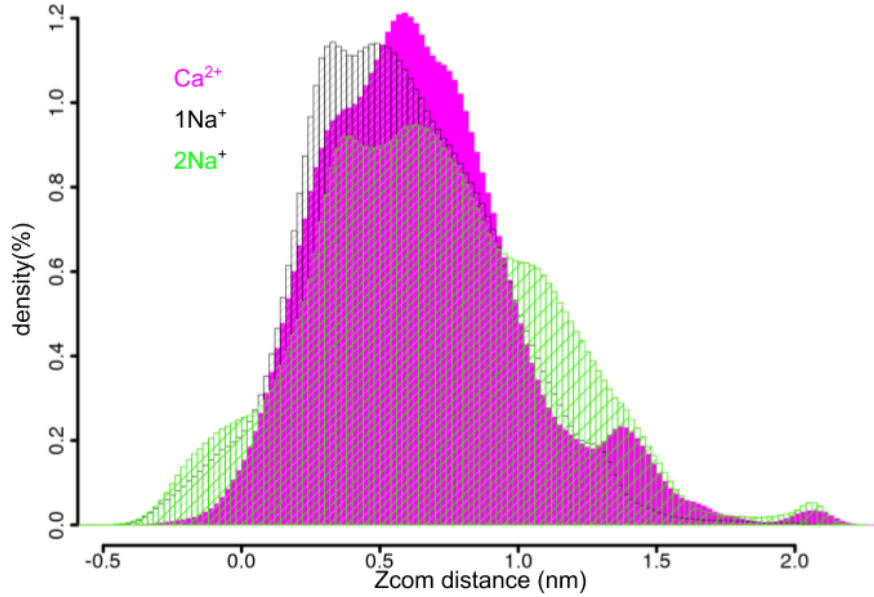


SI 1. The molecular graphics of significant (<0.6) Ca-Ca cross-correlation in three systems holoO, apoC, and holoC shown for each independent trajectory.



SI 2. The Ca-Ca cross-correlation in three systems apoC, holoC and holoO plotted against corresponding Ca-Ca average distances. The regions of interest are highlighted with black and magenta rectangles.



SI 3. Histograms of the Z-component of the center of mass distance between the ion(s) kept inside the cavity during the MTD simulations: MTD_Ca²⁺(magenta), MTD_Na¹⁺(black-shaded bars) and MTD_2Na¹⁺(green-shaded bars).

1. Free energy decomposition in occupancy contributions.

We consider a channel that can be occupied by more than one substrate, e.g. ions. In a NVT ensemble containing $N + 1$ ions, we consider one specific ion (ion “0”) and limit its motion by a repulsive cylinder of radius R and length L oriented along the channel (z) and centered at the COM of the channel. All the other particles do not see (do not interact with) the cylinder.

We consider the probability, q_i , of $i(= 0, 1, 2)$ occupation number (occupancy) of the pore central region Ω by any ion, and the conditional probability density, $P(z|i)$, for the ion “0” to be in position z at the condition of the $i(= 0, 1, 2)$ specific ions are in Ω .

The occupancies, q_i , can be calculated in the plain MD simulations by counting the events in which the cavity has a certain occupation number and dividing it by the total length of the simulation. The conditional probabilities, $P(z|i)$, require much longer trajectories in plain MD. These can be more readily calculated by using an enhanced sampling method, e.g. the metadynamics, where ion “0” is biased. We consider three free energy profiles for ion “0”: $G_0(z)$ - when no other ion is allowed to enter the pore central region Ω ; $G_1(z)$ - when another specific ion (ion “1”) is always kept in region Ω ; $G_2(z)$ - when the two specific ions (ions “1” and “2”) are kept in region Ω . Then, one obtains,

$$P(z|i) = \frac{\exp(-G_i(z)/kT)}{\int \exp(-G_i(x)/kT) dx}. \quad (1)$$

The “reconstructed” unrestricted probability density, $p(z)$, and the corresponding free energy profile, $G(z)$, was obtained as follows,

$$p(z) \propto \exp(-G(z)/kT) = A \sum_i \frac{\exp(-G_i(z)/kT)}{\int \exp(-G_i(x)/kT) dx} \frac{q_i}{g_i} \quad (2)$$

where g_i is the statistical combinatorial factor giving the number of options to select i ($= 0, 1, 2$) ions out of N fixed in region Ω ,

$$g_0 = 1; \quad g_1 = N; \quad g_2 = \frac{N(N-1)}{2}. \quad (3)$$

The normalization constants, A , was selected to set the free energy to zero outside the channel.

2. Error calculations for metadynamics free energy surfaces

We calculated the error for each free energy surface as the difference between the free energy obtained summing up the accumulated hills ($F(s,T)$) and the free energy obtained directly from the sampled values of the collective variable s accumulated during metadynamics simulations, up to time T ($F_N(s,T)$). While hills are added with a frequency of 5 ps, the variable s is stored for each walker with a higher frequency, 1 ps. The accumulated histogram calculated from the walker trajectories, weighted with the relative bias factor, represents a free-energy estimator, as discussed in Ref. 1 and 2.

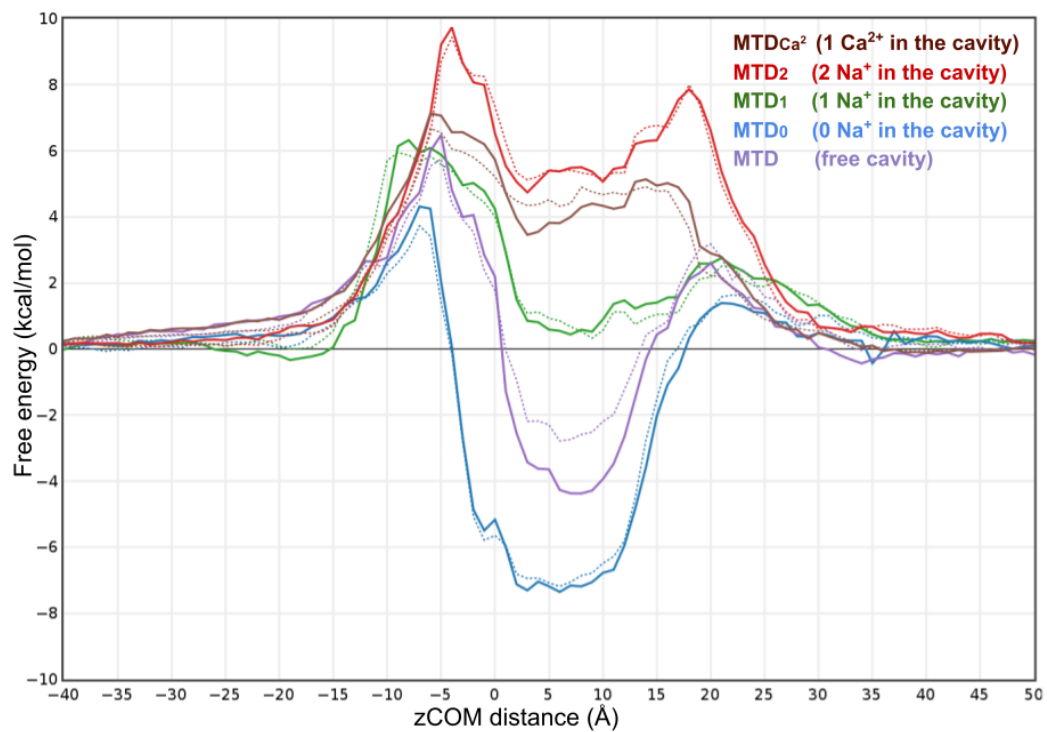
In figure SI4 we reported for each metadynamics simulation the $F(s,T)$ and the $F_N(s,T)$, the former with straight lines and the latter with dotted lines. As we can see, though there are some differences, the use of the free-energy estimator does not change qualitatively our results.

The average error among the two curves is obtained with the formula (22) of Ref. 1, without excluding any points.

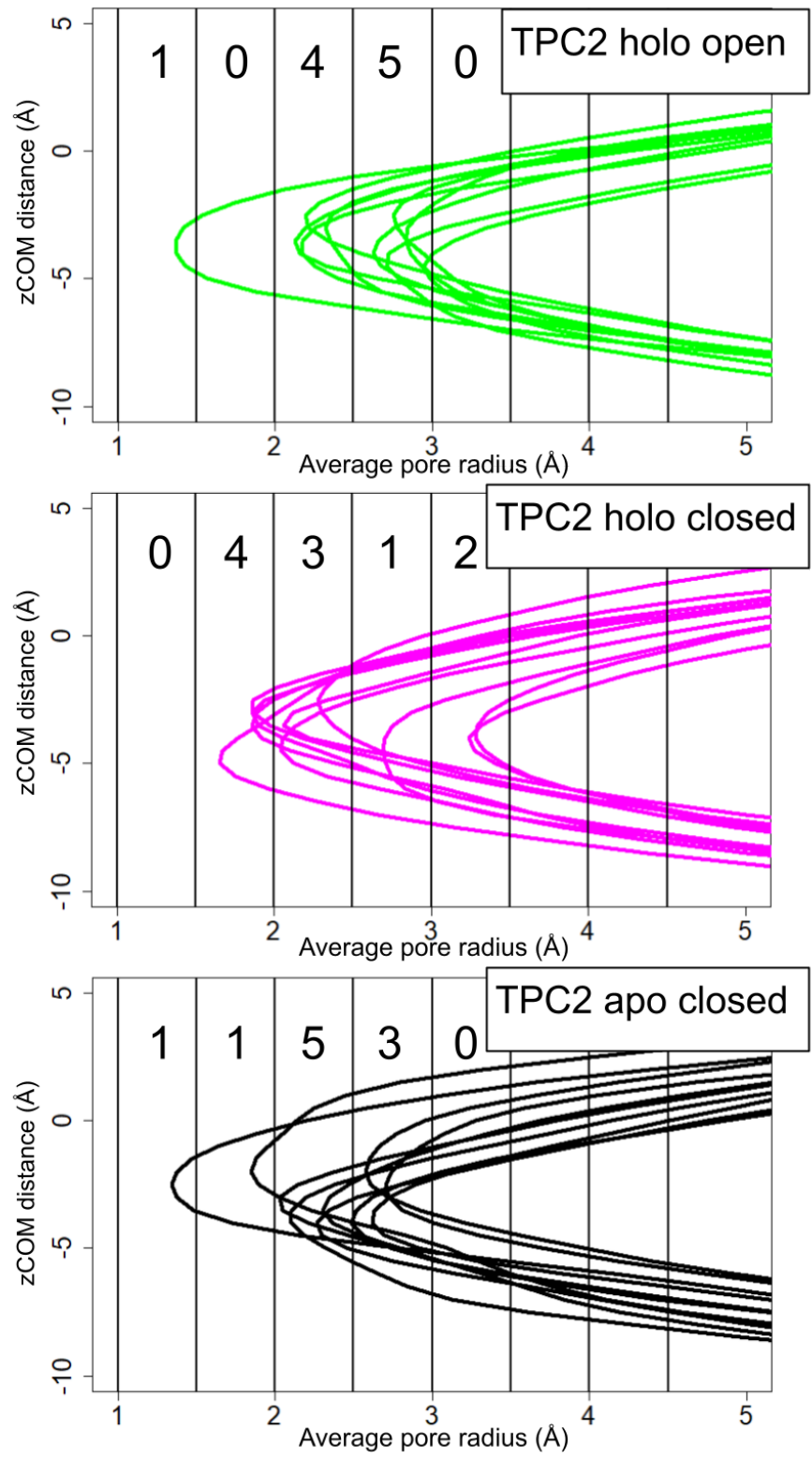
$$\varepsilon = \left[\frac{\int_s [\overline{F_r}(s) - \overline{F}(s)]^2 \theta(v - F_r(s)) ds}{\int_s \theta(v - F_r(s)) ds} \right]^{1/2}$$

1. Branduardi D., Bussi, G., Parrinello, M. 2012. Metadynamics with adaptive Gaussians. *Journal of Chemical Theory and Computation* 8, 2247–2254 and
2. Barducci, A.; Bussi, G.; Parrinello, M. *Phys. Rev. Lett.* 2008, 100,020603.

SI 4:



SI4. The free energy profiles of various MTD simulations (solid lines) accompanied by their F_N counterparts (dashed lines), as described above.



SI 5. Average pore radius profiles for different simulations. The counts of their minimal values are given for 0.5 angstrom bin at the top of each panel.

#x300 ns	Ion dynamics with the time of the entry, time of the exit and the life time inside the cavity.												
1	$t_1(\text{in})$ =0	$t_1(\text{out})$ =34.6	τ_1 =35	$t_2(\text{in})$ =35.4	$t_2(\text{out})$ =38.9	τ_2 =3.4	$t_3(\text{in})$ =38.3	$t_3(\text{out})$ =100.2	τ_3 =62	$t_4(\text{in})$ =96	$t_4(\text{out})$ =168.1	τ_4 =72	$t_5(\text{in})$ =160.1
2	$t_5(\text{out})$ =440	τ_5 =280	$t_6(\text{in})$ =467.1										
3	$t_6(\text{out})$ =667	τ_6 =200											
4	$t_7(\text{in})$ =981												
5	$t_7(\text{out})$ =1400	τ_7 =419	$t_8(\text{in})$ =1394	$t_8(\text{out})$ =1414	τ_8 =20	$t_9(\text{in})$ =1446							
6	$t_9(\text{out})$ =1523	τ_9 =77	$t_{10}(\text{in})$ =1527										
7	$t_{10}(\text{out})$ =2099	τ_{10} =570											
8	$t_{11}(\text{in})$ =2147												
9													
10													

SI TABLE 1. The timeline of ion passages in the long 3 μ s simulation. Each ion is described with three fields, time of entry, time of exit and the time spent inside the cavity. The exception is the last ion which doesn't leave the cavity for the time of the simulation.

Cavity state	Zero ions	Single ion	Two ions
holoO MD	38%	60%	2%
holoO -200mV	10%	85%	5%
holoO MTD	23%	75%	2%

SI TABLE 2. The ion occupancy for three different types of holoO simulations.