

# Supporting Information

Computational microscopy of the role of protonable surface residues in  
nanoprecipitation oscillations

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## HPO<sub>4</sub><sup>-2</sup> model

Atomic coordinates and topology file for HPO<sub>4</sub><sup>-2</sup> ion are provided below. Atom types comply with the CHARMM force field (A. MacKerell Jr., *et al.*, *J. Phys. Chem. B*, 1998, **102**, 3586-3616).

### HPO<sub>4</sub><sup>-2</sup> atomic coordinates

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REMARK	Atomic coordinates in Å						
ATOM	1	H1	HP2	-0.83	1.00	1.67	H
ATOM	2	O1	HP2	-0.74	0.06	1.39	O
ATOM	3	P1	HP2	0.00	0.00	0.00	P
ATOM	4	O2	HP2	-0.80	-0.63	-1.08	O
ATOM	5	O3	HP2	1.37	-0.54	-0.22	O
ATOM	6	O4	HP2	0.18	1.64	-0.41	O

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### HPO<sub>4</sub><sup>-2</sup> topology

\* < topology file for HPO<sub>4</sub><sup>-2</sup> >

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MASS	1	HN4	1.008	H	! nonpolar H
MASS	2	ON3	15.999	O	! oxygen
MASS	3	ON4	15.999	O	! oxygen
MASS	4	P	30.974	P	! phosphorus

### AUTO ANGLES DIHE

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RESI	HP2	-2.0	! Phosphate ion, dianionic		
GROUP			!	H1	
ATOM	H1	HN4	0.3	!	
ATOM	O1	ON4	-0.7	!	O1
ATOM	P1	P	1.1	!	
ATOM	O2	ON3	-0.9	!	(-) O4==P1==O3 (-)
ATOM	O3	ON3	-0.9	!	
ATOM	O4	ON3	-0.9	!	O2
BOND	P1	O1	P1	O2	
BOND	P1	O3	P1	O4	
BOND	O1	H1			

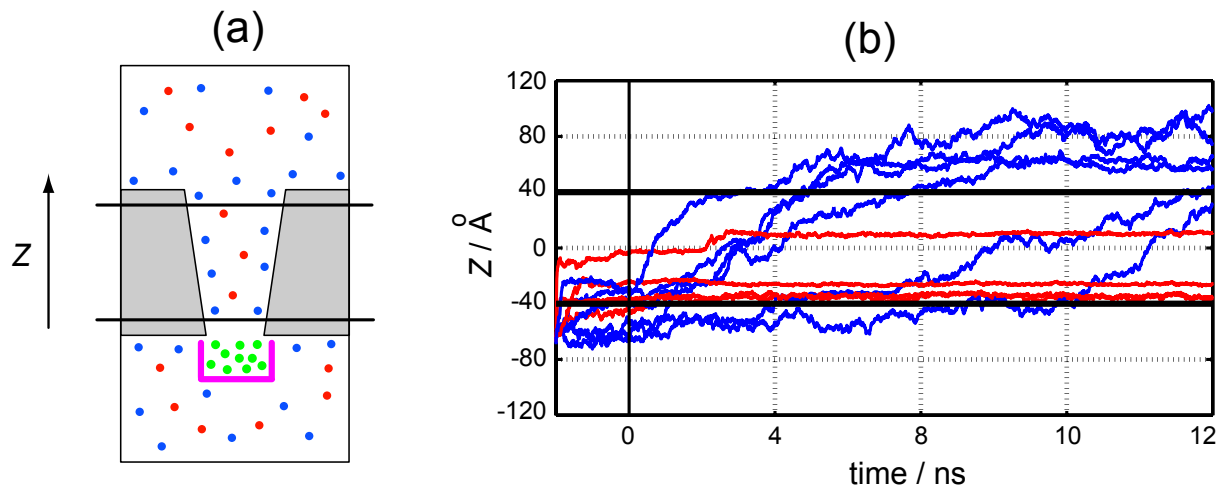
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## Calcium saturation of PET nanopores

The procedure for placing  $\text{Ca}^{2+}$  ions has been briefly described in a previous publication (E. R. Cruz-Chu *et al.*, *Faraday Discuss.*, 2009, 143, 47-62). The number of  $\text{Ca}^{2+}$  ions was chosen to minimize the electrostatic energy of the system. Therefore, we added 145  $\text{Ca}^{2+}$  ions to balance the 291 carboxyl groups exposed at the surface. For the initial location of  $\text{Ca}^{2+}$  ions, we used the program *cionize* (J. E. Stone *et al.*, *J. Comput. Chem.*, 2007, 28, 2618-2640), that locates the ions based on the electrostatic potential of the nanopore, near the exposed PET carboxyl groups, the optimal location for adsorption.

However, there is still a question whether this arrangement is possible in reality; in other words, whether solvated  $\text{Ca}^{2+}$  can freely translocate and saturate the PET carboxyl groups, outcompeting the more abundant  $\text{K}^+$  ions. To answer this question, we performed two simulations, containing 10 and 100  $\text{Ca}^{2+}$  ions. The purpose of the new simulations was to observe  $\text{Ca}^{2+}$  saturation inside the nanopore. Here, we used a setup that should mimic free diffusion of  $\text{Ca}^{2+}$  ions inside the nanopore. First, all exposed PET carboxyl groups were deprotonated and saturated with  $\text{K}^+$  ions. Then,  $\text{Ca}^{2+}$  ions were located in the lower solvent compartment and constrained to diffuse into the nanopore using phantom surfaces and a +1 V bias.

In the first simulation, 10  $\text{Ca}^{2+}$  ions were located at the lower solvent compartment, in a disc region of 3 nm height and 1.2 nm radius near the bottom pore opening. The movement of  $\text{Ca}^{2+}$  ions was limited to a disc region using a phantom surface. Figure S1.a shows a schematic representation of the system. The system was first equilibrated following the protocol described in the Methods section in the main text. Then, the system was simulated for 12 ns.



**Figure S1.** Calcium adsorption in PET nanopores. Panel (a) shows a schematic figure of the initial system.  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Cl}^-$  ions are colored in green, blue and red. Ten calcium ions were located within a cylindrical volume located at the bottom pore opening. When a calcium ion crossed the bottom or lateral walls of the cylinder, it experiences an elastic collision normal to the surface. Panel (b) shows the  $Z$ -position of the calcium ions. Red lines denote  $\text{Ca}^{2+}$  ions that are eventually adsorbed, blue lines show  $\text{Ca}^{2+}$  ions that translocate the pore without being adsorbed. The horizontal black lines in panels (a) and (b) are reference lines to indicate the boundaries of the nanopore.

Figure S2.b shows the displacement of the ions along the Z-axis. A constant horizontal line implies that the calcium ion does not move anymore, *i.e.*, it has been adsorbed by a carboxyl group. Four out of ten ions were adsorbed while moving through the pore (red lines), while 6 were able to cross the pore (blue lines). Figure S2 shows that the adsorption takes place at discrete spots, where the carboxyl groups are located.

To further test  $\text{Ca}^{2+}$  adsorption, we performed a second simulation containing 100  $\text{Ca}^{2+}$  ions. The  $\text{Ca}^{2+}$  ions were randomly located in the lower solvent compartment, in a region between two horizontal planes located 3 nm (plane 1) and 5 nm (plane 2) below the bottom nanopore surface.  $\text{Ca}^{2+}$  ions crossing plane 2 experience elastic collisions in the upward direction. After equilibration, the system was simulated for 22 ns under +1 V bias. We were able to observe  $\text{Ca}^{2+}$  ions adsorbed in the nanopore region, and in the upper and lower surfaces of the nanopore. Table S1 presents the number of  $\text{Ca}^{2+}$  ions adsorbed and the number of carboxyl groups at each region. About half of the  $\text{Ca}^{2+}$  ions were adsorbed (55 out of 100), *i.e.*, over a short period of 22 ns, about 19% of the carboxyl groups became saturated. It is reasonable to expect that, in an experimental situation, over a longer time scale and with more  $\text{Ca}^{2+}$  ions flowing from both solvent compartments, the entire PET surface will be saturated with  $\text{Ca}^{2+}$  ions.

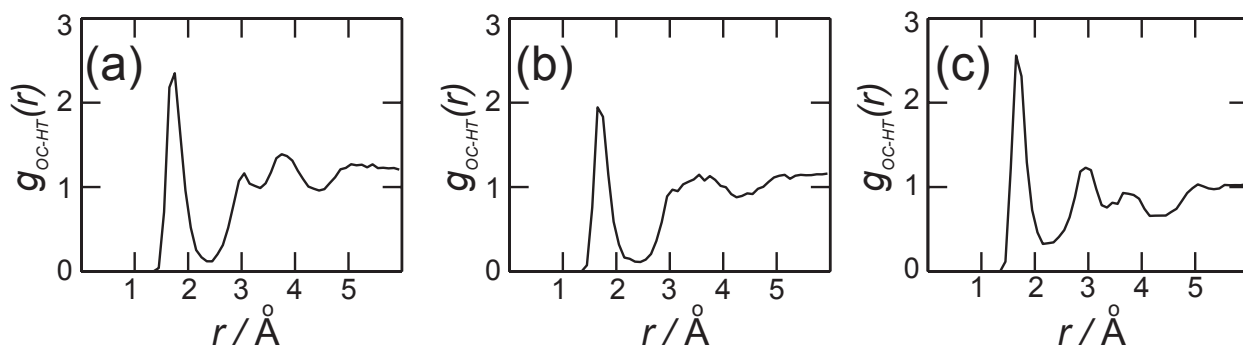
Region	# PET carboxyl groups	# adsorbed $\text{Ca}^{2+}$
Upper surface	102	5
Nanopore surface	106	21
Lower surface	83	29

**Table S1.** Number of adsorbed  $\text{Ca}^{2+}$  ions at different regions in the nanopore.

## Water accessibility for PET carboxyl groups

A prerequisite for reprotonation is that the PET carboxyl group attached to the nanoprecipitate are accessible to H-bond donors from the water molecules. In this regard, we inspected simulation 15, which was probed for the reprotonation mechanism, and observed that the three carboxyl groups holding the precipitate are indeed accessible to water.

Figure S2 shows  $g(r)$  for the carboxyl groups in contact with the precipitate in simulation 15. Panels (a), (b) and (c) present  $g(r)$  for each of the three carboxyl groups attached to the precipitate.  $g(r)$  was computed for atom pairs OC-HT, where OC is the atom name for the oxygens of the PET carboxyl groups and HT is the atom name for hydrogens of water molecules. For the three vcarboxyl groups, one observes sharp peaks within a 2 Å distance that reveal contact between hydrogens from water and oxygens from PET carboxyl groups.



**Figure S2.** Radial distribution function,  $g(r)$ , for carboxyl groups in contact with nanoprecipitate. Panels (a), (b) and (c) refer to the three different carboxyl groups attached to the precipitate in simulation 15 (see Table 3 and Figure 8b in the manuscript).

## Movies

The movies listed below show simulations 3 and 7 (Sim0307.mpg); 10 (Sim10.mpg), 11 (Sim11.mpg), 12 (Sim12.mpg), 13 (Sim13.mpg), 14 (Sim14.mpg), 19 (Sim10.mpg), 20 (Sim10.mpg); and 21, 22 and 23 (Sim212223.mpg). Detailed descriptions of those simulations are presented in the Results and Discussion section in the main text. All movies were made with VMD (W. Humphrey, *et al.*, *J. Mol. Graphics*, 1996, **14**, 33-38).

### **Sim0307.mpg**

This movie shows two MD simulations of calcium phosphate precipitation in solution. Simulation 3 is shown on the left, simulation 7 is shown on the right.  $\text{Ca}^{2+}$ ,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  ions that are not part of a precipitate are colored in green, red and blue, respectively. When an ion becomes part of a precipitate, its color changes to gray. The criteria for an ion to be a part of a precipitate are described in the Methods section in the main text. Black lines indicate the periodic boundaries of the simulation boxes.

### **Sim10.mpg**

This movie shows calcium phosphate precipitation inside polymeric nanopores.  $\text{Ca}^{2+}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{K}^+$  and  $\text{Cl}^-$  ions are colored in opaque green, opaque red, transparent blue, and transparent orange, respectively. Deprotonated carboxyl groups are shown as purple lines.

### **Sim11.mpg, Sim12.mpg, Sim13.mpg and Sim14.mpg**

These movies show calcium phosphate precipitation in nanopores assuming different phosphate protonation states.  $\text{Ca}^{2+}$ ,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  ions are colored in green, red and blue, respectively. Deprotonated carboxyl groups are shown as purple lines.  $\text{K}^+$  and  $\text{Cl}^-$  ions are not shown.

### **Sim19.mpg and Sim20.mpg**

These movies present the MD simulations probing total reprotonation.  $\text{Ca}^{2+}$  and  $\text{H}_2\text{PO}_4^-$  ions are colored in green and blue, respectively. Reprotonated carboxyl groups are shown as cyan lines.  $\text{K}^+$  and  $\text{Cl}^-$  ions are not shown.

### **Sim212223.mpg**

This movie shows the MD simulation probing partial reprotonation.  $\text{Ca}^{2+}$  and  $\text{H}_2\text{PO}_4^-$  ions are colored in green and blue, respectively. Deprotonated carboxyl groups are shown as purple lines. Reprotonated carboxyl groups for simulations 21, 22 and 23 are shown in blue, cyan and yellow, respectively.  $\text{K}^+$  and  $\text{Cl}^-$  ions are not shown.