Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information for: Detection of Posner's clusters during Calcium Phosphate Nucleation: a Molecular Dynamics Study

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In this document, we label the oxygen atom belonging to a phosphate group as O_{ph} and the oxygen atom of water as O_{wt}

Choice of the Water Model

Two different models for liquid water were tested; the three-particle model TIP3P and a four particle model, in which the polarisation effects are taken into account through the use of a shell. The former model is less computationally demanding, by the contrary the shell-model for water requires more than twice the time.

```
WATER
nummols
atoms 4
O_wt      15.8000    1.25      1    0
O_wt_s     0.2000   -2.05      1    0
H_wt       1.0000    0.40      1    0
H_wt       1.0000    0.40      1    0
shell 1
1   2    209.45
bonds 8
mors    2    3    6.203713    0.92376    2.22003
mors    2    4    6.203713    0.92376    2.22003
mors    3    4    0.000000    1.50000    2.840499
coul    1    3    1.0
coul    1    4    1.0
coul    2    3    0.5
coul    2    4    0.5
coul    3    4    0.5
angles 1
harm    3    2    4    4.19978    108.693195
FINISH
```

```
WATER
nummols
ATOMS          3
O_wt    15.8000      -0.834
H_wt     1.0080       0.417
H_wt     1.0080       0.417
BONDS          2
harm 1    2    23.9907488   0.9572
harm 1    3    23.9907488   0.9572
ANGLES         1
harm 2    1    3    4.3382909   104.52
FINISH
```

Figure S1: Water model force fields in *DL_POLY* FIELD format. Left: shell model.[S1,S2] Right: TIP3P model.[S3]

First we run a simulation of a 50 × 50 × 50 Å test box in a NPT ensemble for 1 ns for each water model. The pressure in the system was equilibrated at 1 bar and the temperature at 310 K. The density for each model was calculated and used for building new water boxes with the correct starting density.

Table S1: Density of the water obtained after equilibration at 1 bar and 310 K.

Model	Final density $[Kg/dm^3]$ at 1 bar 310 K
Shell-model	1.263
TIP3P	1.026
Experiment[S4]	0.993

We equilibrated the 50 × 50 × 50 Å boxes with the correct density for 25 ps in a NVE ensemble and for other 25 ps in a NVT ensemble. The rdf of water were then collected between 0.75 and 1 ns in a NPT ensemble.

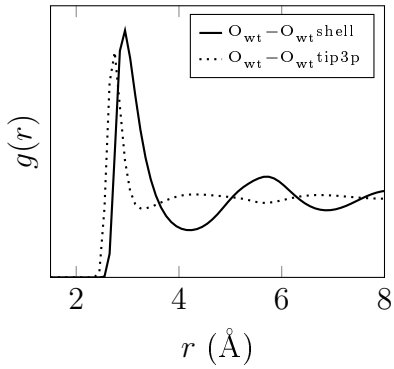


Figure S2: rdf $O_{wt}-O_{wt}$

Table S2: $O_{wt}-O_{wt}$ distances for first and second coordination sphere: comparing the two water model tested with the experiment.[S5]

Water model	I peak (Å)	I minimum (Å)	II peak (Å)	II minimum (Å)
Shell-model	2.95	4.25	5.75	6.85
TIP3P	2.75	3.35	4.25	5.75
Experiment[S5]	2.73	3.41	4.44	5.51

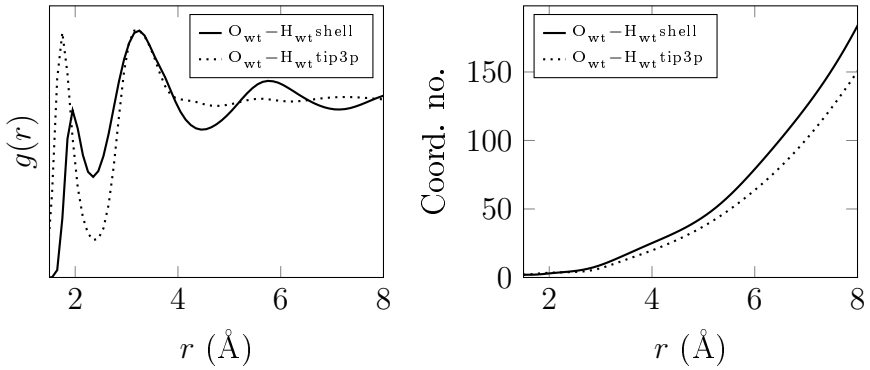


Figure S3: rdf and integration number for the pairing $O_{wt}-H_{wt}$

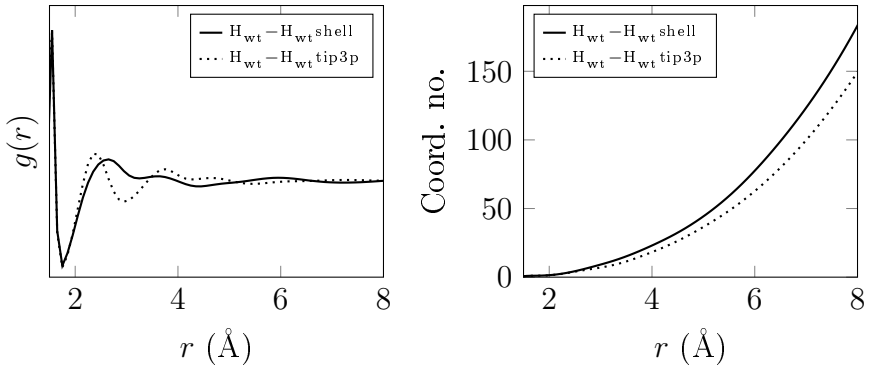


Figure S4: rdf and integration number for the pairing $H_{wt}-H_{wt}$

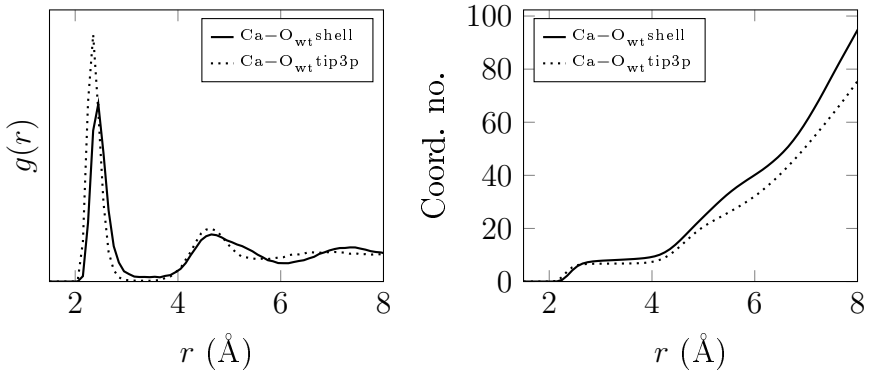


Figure S5: rdf and integration number for the pairing $Ca-O_{wt}$

Table S3: $Ca-O_{wt}$ distances for first and second coordination sphere: comparing the two water model tested with the experiment.[S6,S7]

Water model	I peak (Å)	I min (Å)	II peak (Å)	II min (Å)	I coord. no.
Shell-model	2.45	3.45	4.65	6.05	7.3
TIP3P	2.35	3.25	4.65	5.65	6.7
Experiment	2.4	3.1			7.1

Considering the position of the I peak, there is good agreement with the experimental work of Kulik *et al.*,[S7] whereas the first minimum is shifted towards higher values in the shell model, which gives broader I and II peaks with respect to the not polarized model. However the shell water reproduces better the coordination number of the calcium ion.

Building of the Solutions

Table S4: Phosphoric acid speciation: pK_a at 25 °C, values from ref.[S8]

Equilibrium	pK_a 25 °C
$H_3PO_4 + H_2O \rightleftharpoons (H_2PO_4)^- + H_3O^+$	2.16
$(H_2PO_4)^- + H_2O \rightleftharpoons (HPO_4)^{2-} + H_3O^+$	7.21
$(HPO_4)^{2-} + H_2O \rightleftharpoons (PO_4)^{3-} + H_3O^+$	12.32

Validation of the Force Field

The parameter of the force field of Ainsworth *et al.*[S9] are presented in Table S5.

Table S5: Force field parameters used in this work

Potential parameters				
Atom		Mass (u)		Charge (e)
Ca		40.078		+2.000
Na		22.9898		+1.000
P		30.9738		+5.000
O_c		15.8000		+0.84819
O_s		0.2000		-2.84819
Oh_c		15.8000		+0.9000
Oh_s		0.2000		-2.3000
H		1.01000		+0.4000
Core-Shell Spring $\frac{1}{2}k_{c-s}r^2$				
				k_{c-s} $(\frac{eV}{A^2})$
O_c	O_s			74.92038
Oh_c	Oh_s			74.92038
Buckingham $Ae^{-r/\rho} - Cr^{-6}$				
		A (eV)	ρ(Å)	C (eV/A^6)
O_s	O_s	22764.3000	0.149	27.88
O_s	Oh_s	22764.0000	0.149	13.94
P	O_s	1020.0000	0.343220	0.030
P	Oh_s	814.2	0.343220	0.03
Ca	O_s	2152.3566	0.309227	0.09944
Ca	Oh_s	1250.000	0.3437	0
Na	O_s	56465.3453	0.193931	0
Na	Oh_s	858.79	0.3065	0

We present the radial distribution functions obtained during the simulation of a single Posner's cluster in water in Figure S6. The cluster was simulated by means of Classical MD

with the force field of Ainsworth *et al*,[S9] with the protocol described in the Methods section and *ab initio* MD, code CP2K. The *ab initio* MD was carried out employing PBE functional, 60 ps of equilibration in a NPT ensemble were followed by 95 ps in a NVT ensemble. The rdf were collected in the NVT ensemble. The cubic box was measuring only 15.276 × 15.276 × 15.276, in order to make the simulation feasible *ab initio*.

The experimental data reported by Betts and Posner[S10] are also reported in the Table S6 for comparison purposes.

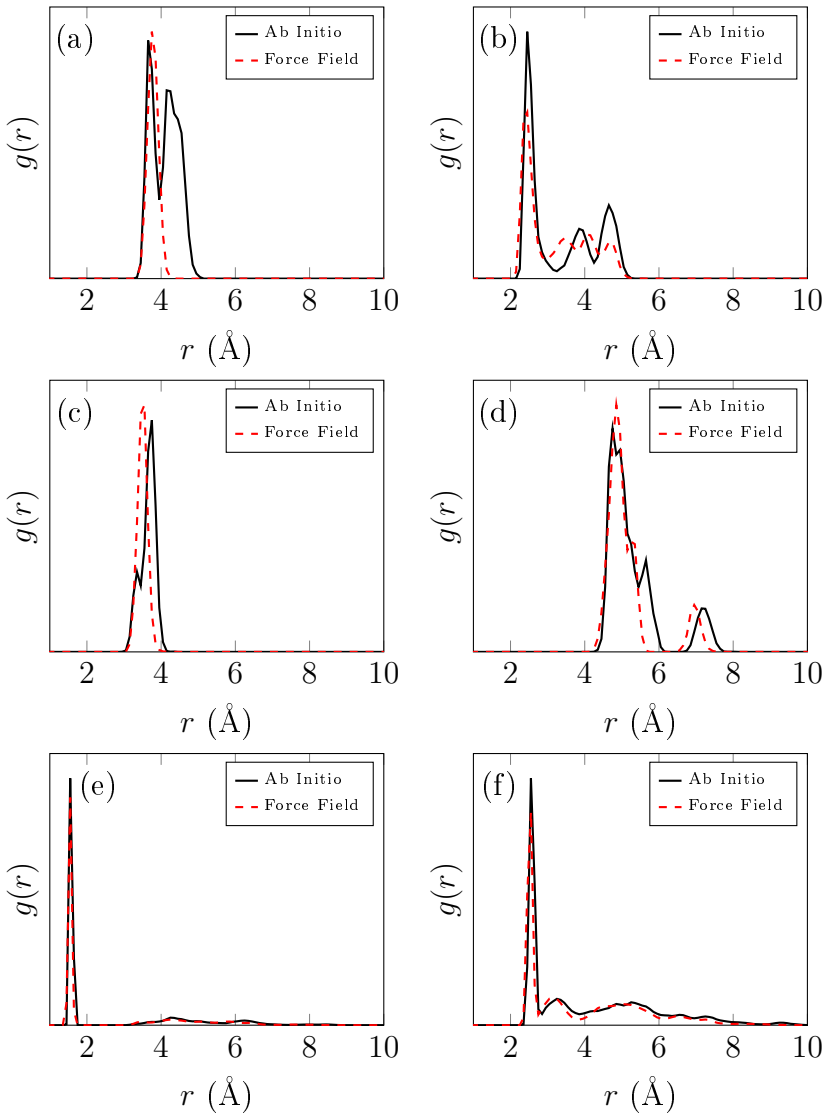


Figure S6: rdf for the atom pairs (a) $Ca_c - Ca$, (b) $Ca_c - O_{ph}$, (c) $Ca_c - P$, (d) $P - P$, (e) $P - O_{ph}$ and (f) $O_{ph} - O_{ph}$.

Table S6: Comparison of the atom pair average distances obtained with *ab initio* MD, Classical MD and the experimental values of Betts and Posner.[S10]

	$Ca_c - Ca$ (Å)	$Ca_c - O_{ph}$ (Å)	$Ca_c - P$ (Å)	$P - P$ (Å)	$P - O_{ph}$ (Å)	$O_{ph} - O_{ph}$ (Å)
Ab Initio	3.65-4.15	2.45	3.75	4.75-5.65	1.55	2.55
Force Field	3.75	2.45	3.55	4.85-5.25	1.55	2.55
Exp. (ACP)	–	2.3	3-6	3-6	1.5	2.3

Table S7: Comparison of the coordination numbers obtained with *ab initio* MD, Classical MD and the values proper of crystalline HA.[S11]

	$Ca_c - Ca$ (Å)	$Ca_c - O_{ph}$ (Å)	$Ca_c - P$ (Å)	$P - P$ (Å)	$P - O_{ph}$ (Å)	$O_{ph} - O_{ph}$ (Å)
Ab Initio	3.95 - 8.00	4.75	5.55	3.80	4.00	1.95
Force Field	8.00	3.75	5.55	3.75	4.00	2.70
HA	8	6	3-6	4	4	0

Table S8: Comparison of the Posner's cluster radius in *ab initio* MD in water, Classical MD in water and DFT in gas phase performed by Yin *et al.*[S12]

Method	Radius (Å)
Ab Initio MD in water	5.25[a]
Classical MD in water	5.25[a]
DFT in gas phase	4.35

[a] This value refers to the first minimum in the Ca–O_{wt} rdf.

Analysis of the symmetry of Posner's cluster in HA

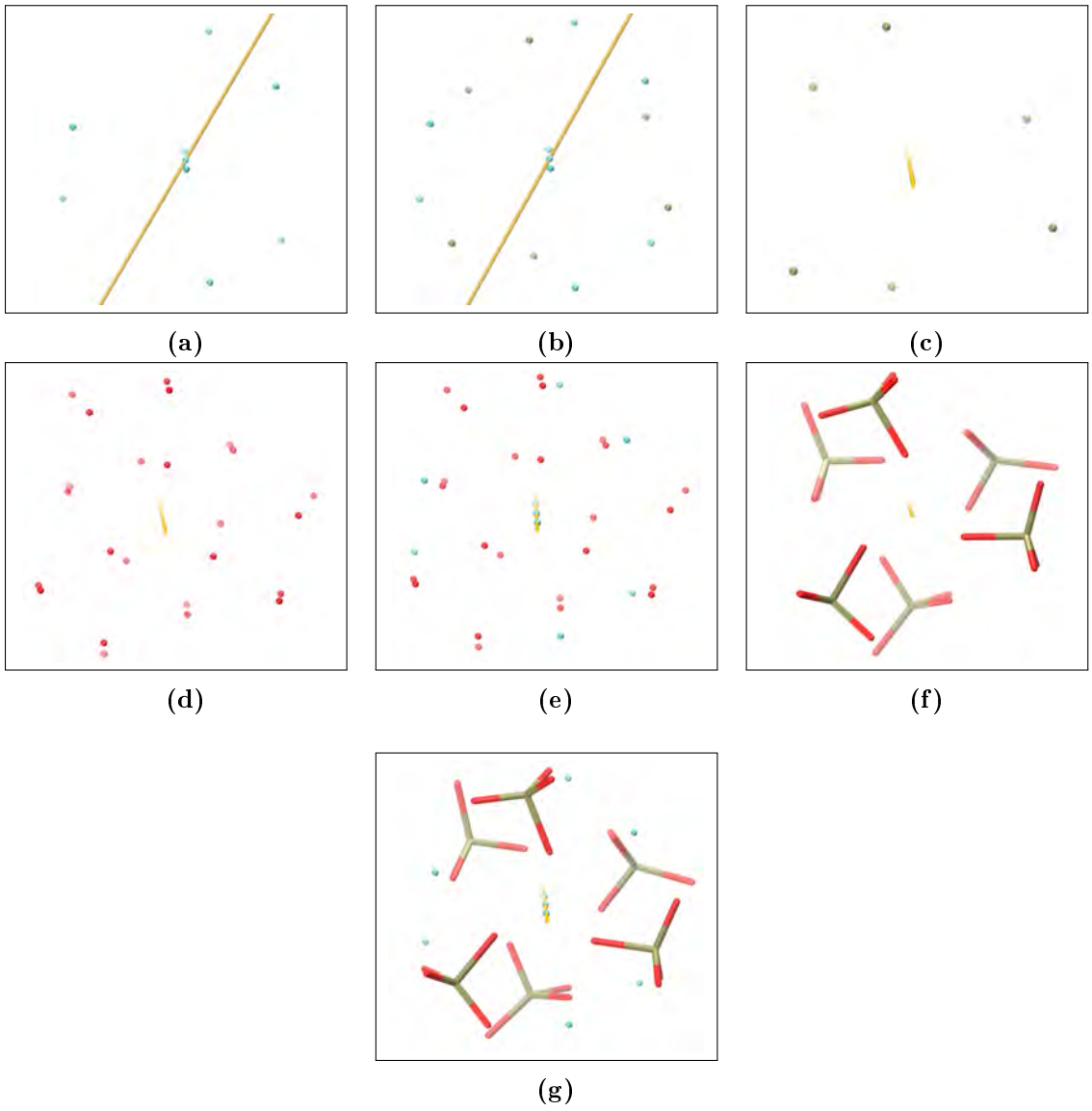
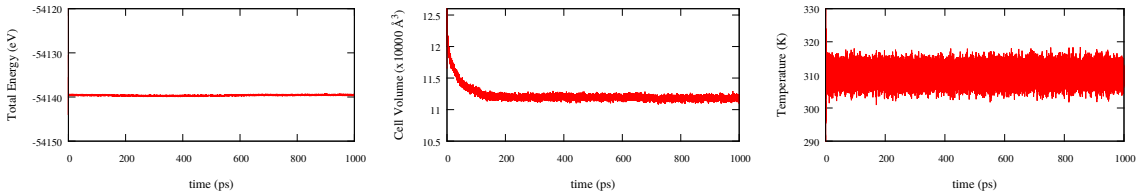


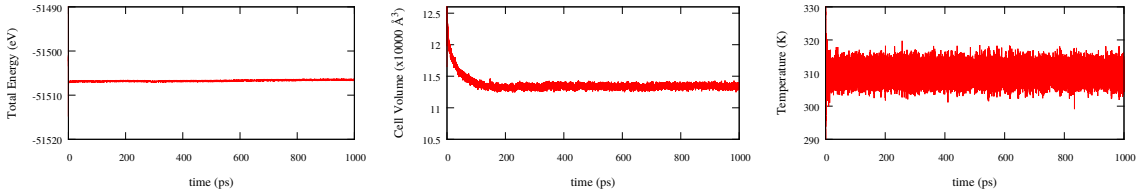
Figure S7: (a) Posner's cluster in hydroxyapatite, only Ca atoms give C2 symmetry; (b) Posner's cluster in hydroxyapatite, Ca and P atoms give C2 symmetry; (c) Posner's cluster in hydroxyapatite, only P atoms give C3 symmetry; (d) Posner's cluster in hydroxyapatite, only O atoms give C3 symmetry; (e) Posner's cluster in hydroxyapatite, Ca and O atoms give C3 symmetry; (f) Posner's cluster in hydroxyapatite, P and O atoms give C3 symmetry; (g) Posner's cluster in hydroxyapatite, Ca, P and O atoms give C3 symmetry. Color key Symmetry axis:yellow, Ca:light blue, P:green, O:red.

Proof that the collected data refer to an equilibrated system



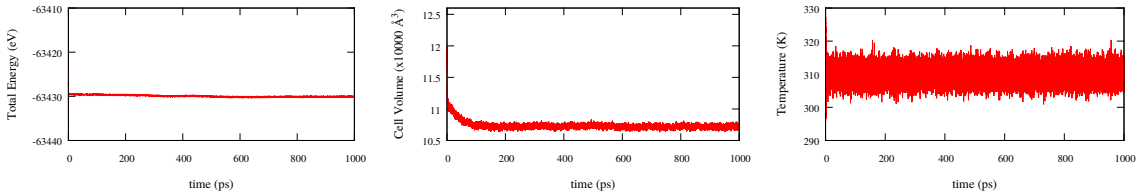
(a) **(b)** **(c)**

Figure S8: Solution I: (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.



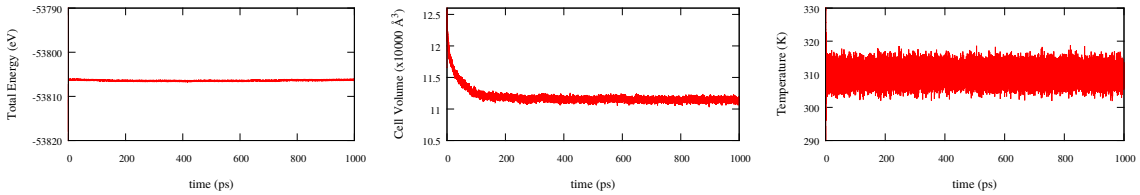
(a) **(b)** **(c)**

Figure S9: Solution II: (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.



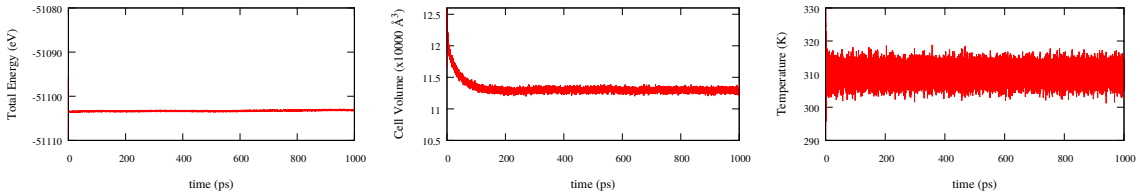
(a) **(b)** **(c)**

Figure S10: Solution III: (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.



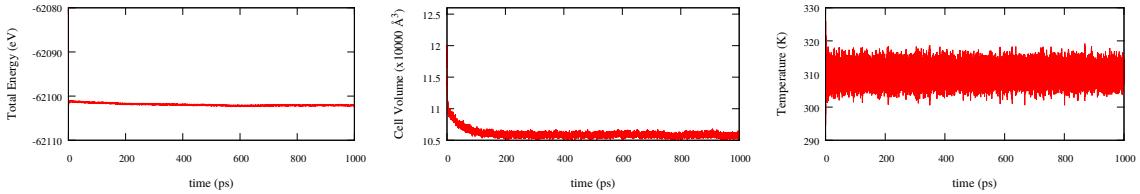
(a) **(b)** **(c)**

Figure S11: Solution I(a): (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.



(a) **(b)** **(c)**

Figure S12: Solution II(a): (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.



(a) **(b)** **(c)**

Figure S13: Solution III(a): (a) Total energy vs simulation time, (b) cell volume vs simulation time and (c) temperature vs simulation time during the first ns of production run in an NPT ensemble.

Analysis of Ca coordination

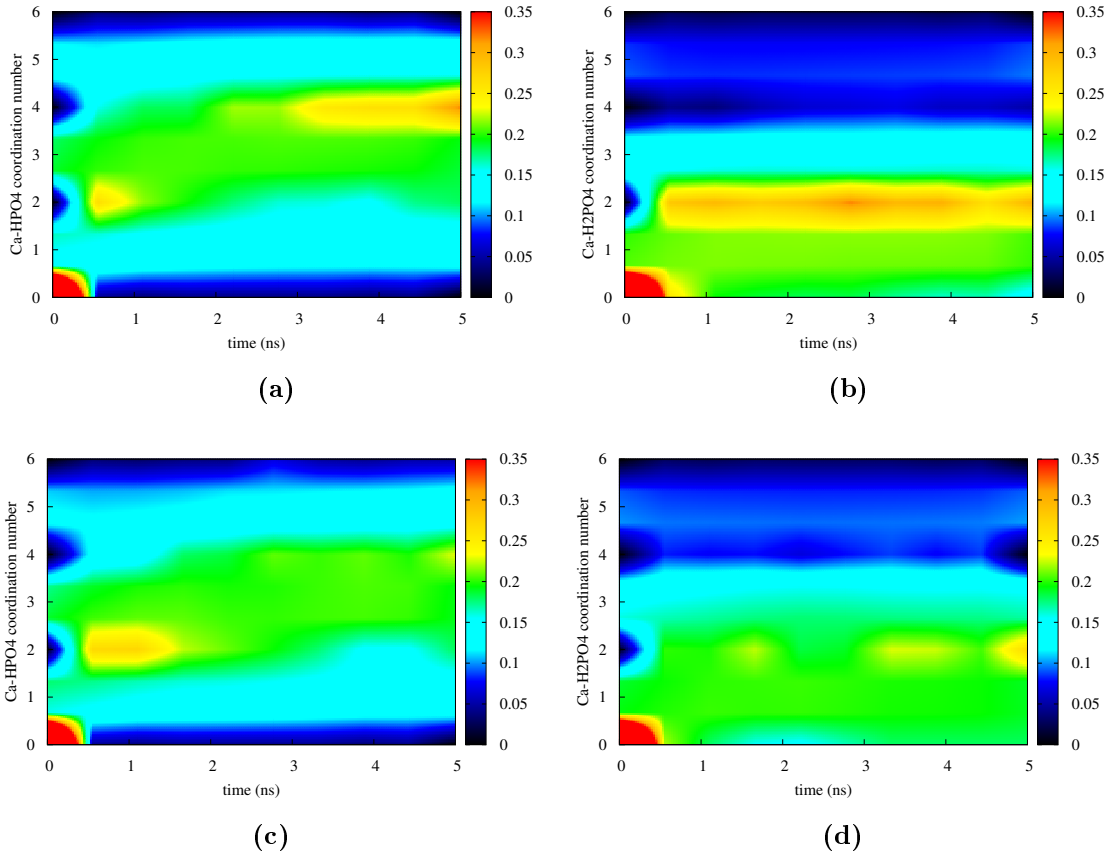


Figure S14: 2D plot for Ca-P coordination during 5 ns, Comparison between the Ca coordination of H_2PO_4 and HPO_4 ions at pH 7.4.

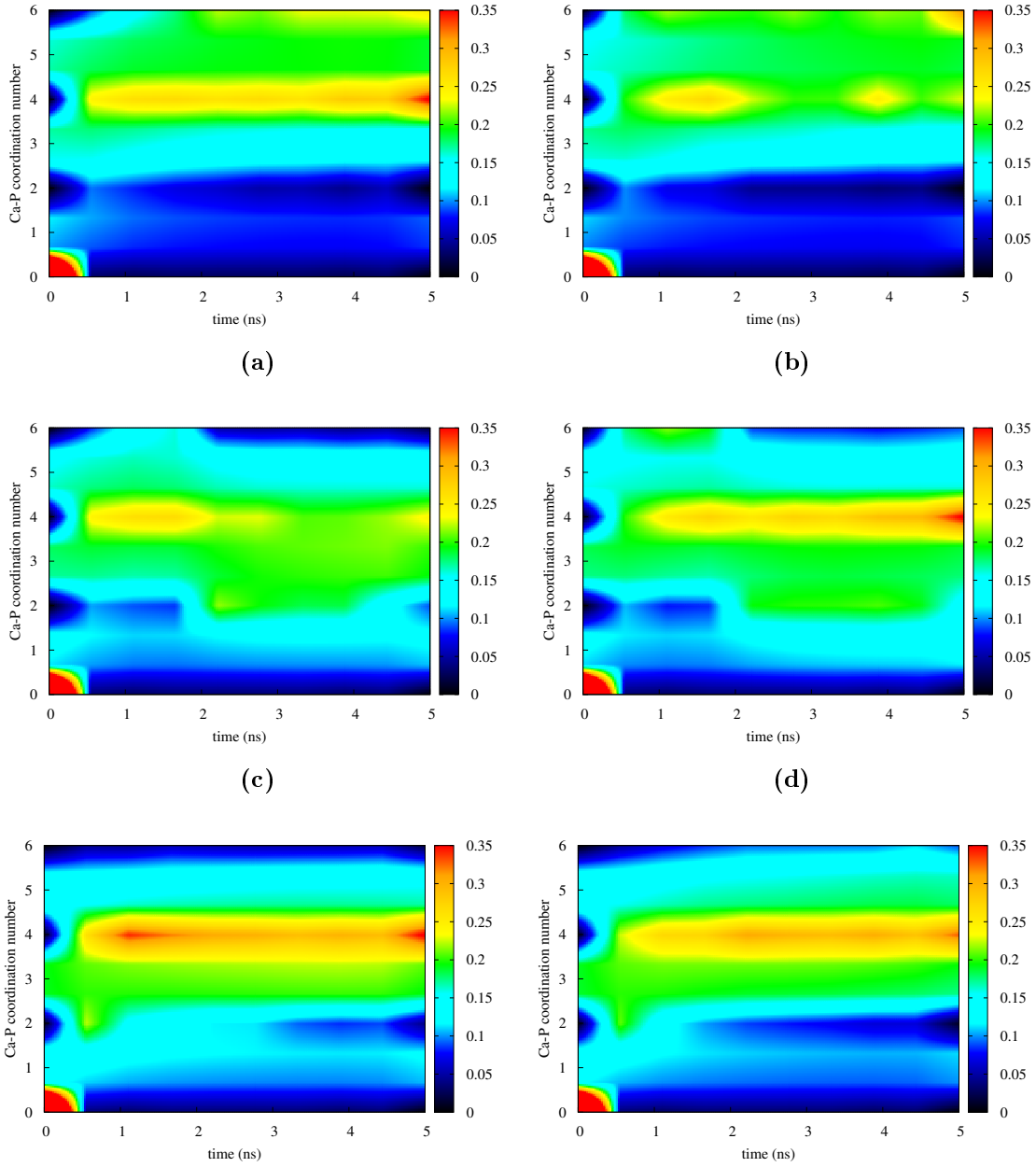


Figure S15: 2D plot for Ca-P coordination during 5 ns. (a) pH 7.4, [Ca^{2+}]=0.6 mol/l, [Na^{+}]=0 mol/l; (b) pH 7.4, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (c) pH 14, [Ca^{2+}]=0.6 mol/l, [Na^{+}]=0 mol/l; (d) pH 14, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (e) pH 14, [Ca^{2+}]=2.0 mol/l, [Na^{+}]=0 mol/l; (f) pH 14, [Ca^{2+}]=2.0 mol/l, Na:Ca=2:1

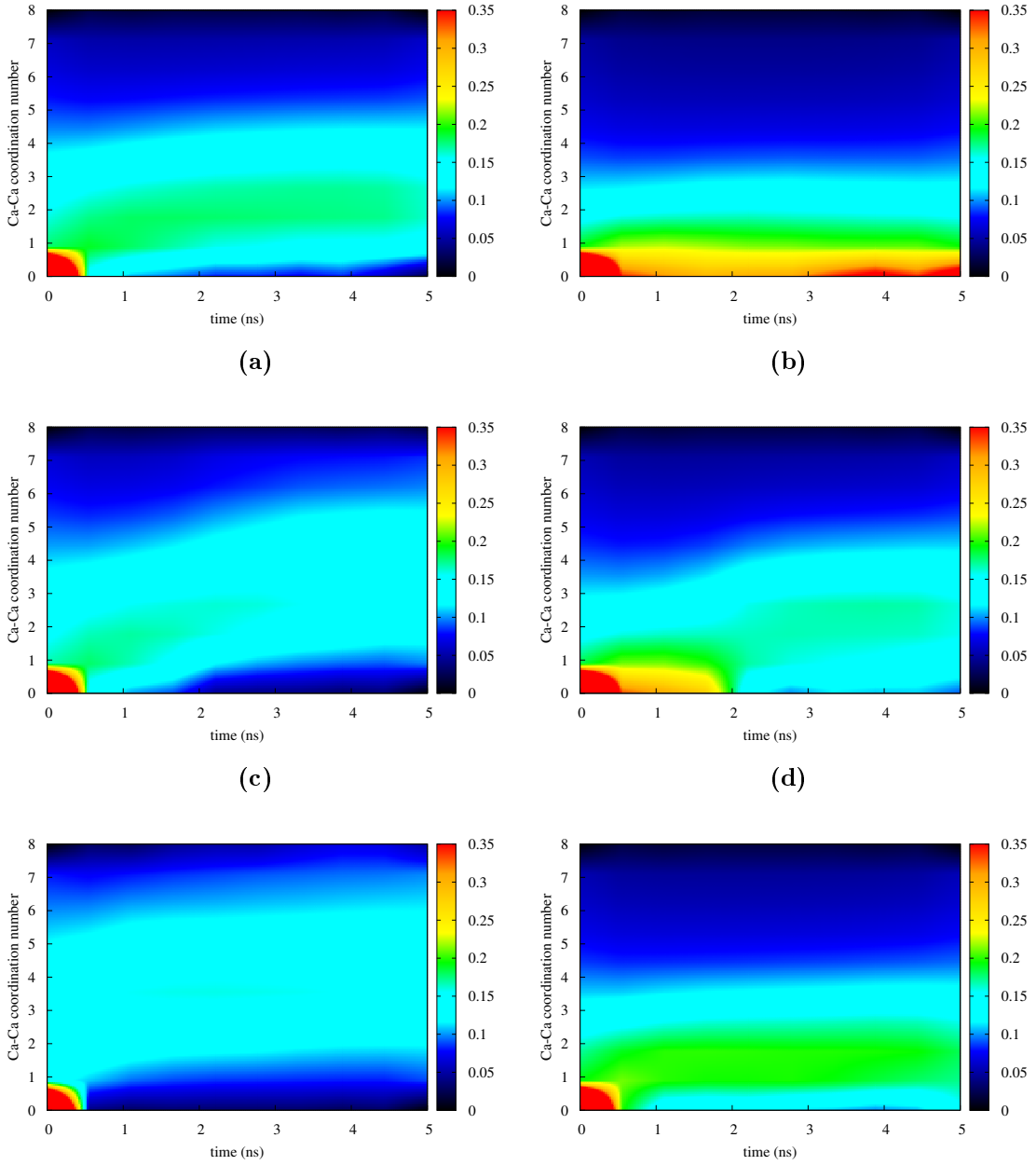
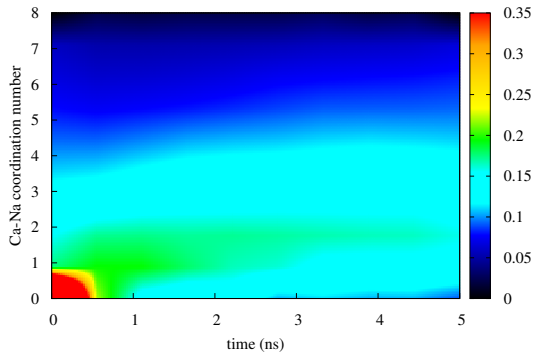
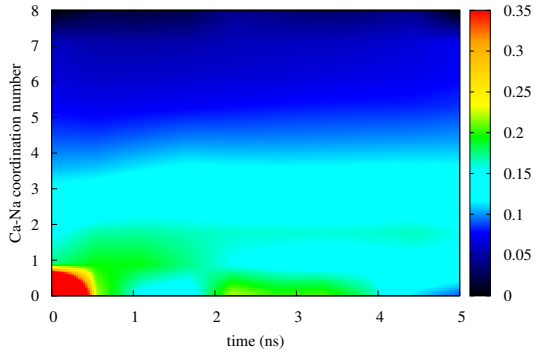


Figure S16: 2D plot for Ca-Ca coordination during 5 ns. (a) pH 7.4, [Ca^{2+}]=0.6 mol/l, [Na^{+}]=0 mol/l; (b) pH 7.4, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (c) pH 14, [Ca^{2+}]=0.6 mol/l, [Na^{+}]=0 mol/l; (d) pH 14, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (e) pH 14, [Ca^{2+}]=2.0 mol/l, [Na^{+}]=0 mol/l; (f) pH 14, [Ca^{2+}]=2.0 mol/l, Na:Ca=2:1

(a)



(b)



(c)

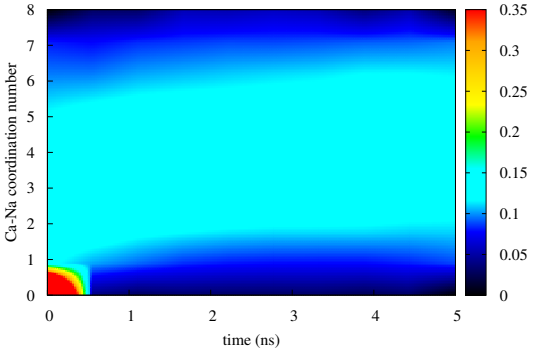


Figure S17: 2D plot for Ca-Na coordination during 5 ns. (a) pH 7.4, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (b) pH 14, [Ca^{2+}]=0.6 mol/l, Na:Ca=2:1; (c) pH 14, [Ca^{2+}]=2.0 mol/l, Na:Ca=2:1

Comparison of Radial Distribution Functions of Posner-like clusters and single Posner cluster in water

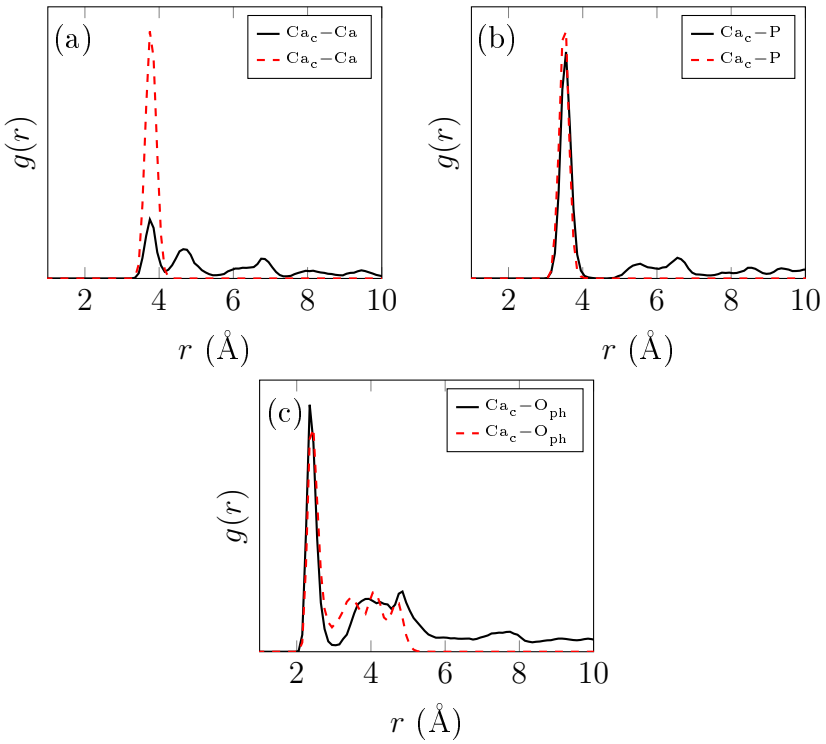


Figure S18: Solution I: rdf of (a) Ca_c-Ca, (b) Ca_c-P, (c) Ca_c-O_{ph}. In red are reported the rdf of the single Posner's cluster in water for comparison.

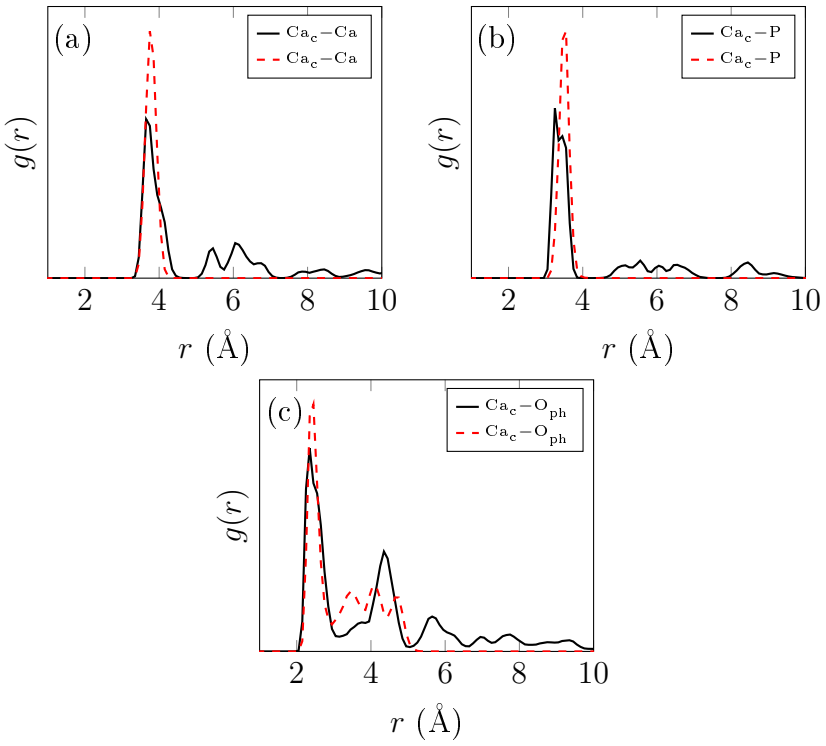


Figure S19: Solution II: rdf of (a) Ca_c-Ca, (b) Ca_c-P, (c) Ca_c-O_{ph}. In red are reported the rdf of the single Posner's cluster in water for comparison.

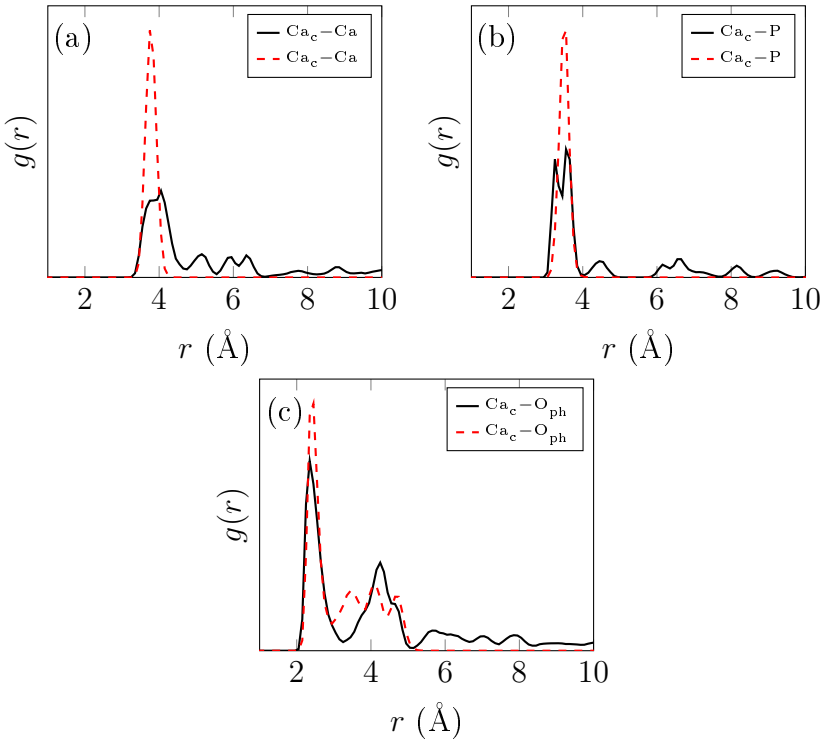


Figure S20: Solution III: rdf of (a) Ca_c-Ca, (b) Ca_c-P, (c) Ca_c-O_{ph}. In red are reported the rdf of the single Posner's cluster in water for comparison.

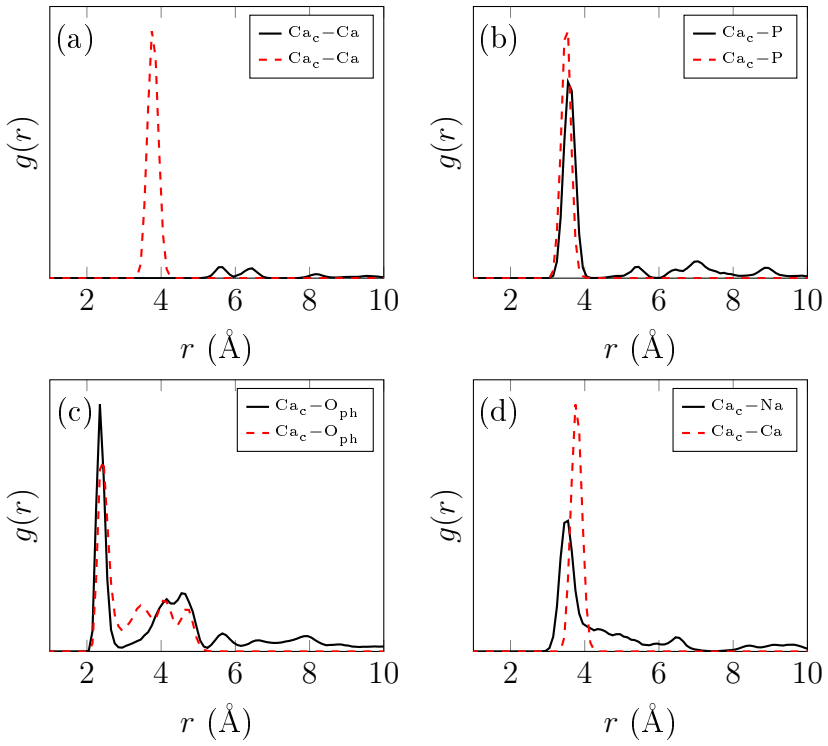


Figure S21: Solution Ia: rdf of (a) Ca_c–Ca, (b) Ca_c–P, (c) Ca_c–O_{ph}, (d) Ca_c–Na. In red are reported the rdf of the single Posner's cluster in water for comparison.

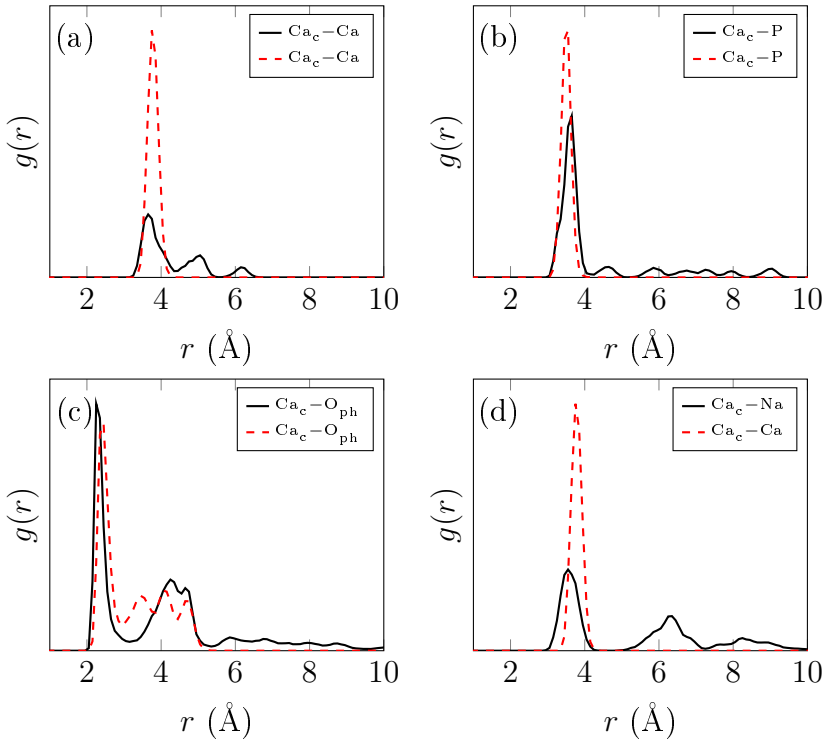


Figure S22: Solution IIa: rdf of (a) Ca_c–Ca, (b) Ca_c–P, (c) Ca_c–O_{ph}, (d) Ca_c–Na. In red are reported the rdf of the single Posner's cluster in water for comparison.

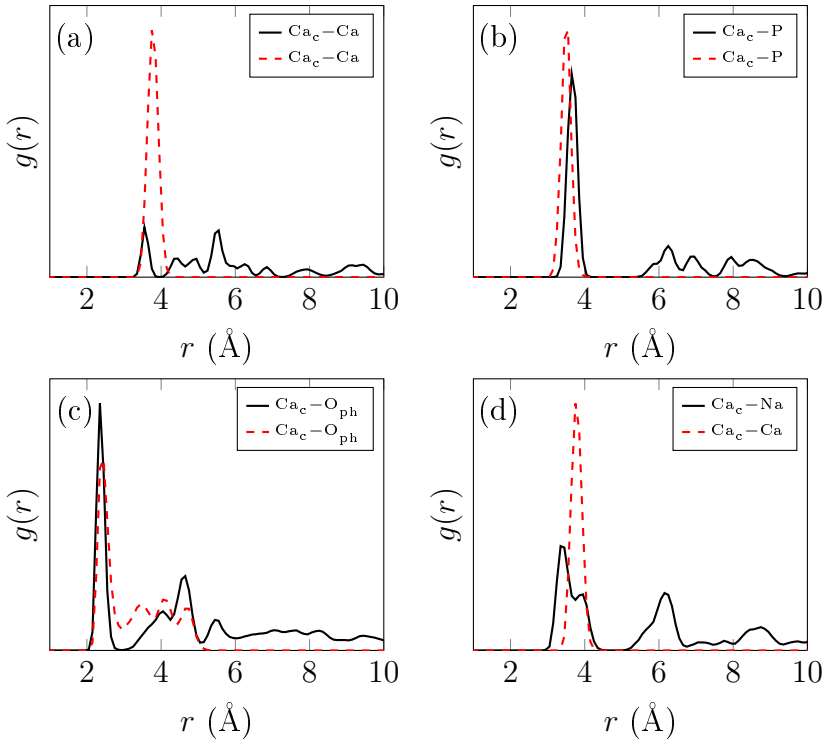


Figure S23: Solution IIIa: rdf of (a) Ca_c-Ca, (b) Ca_c-P, (c) Ca_c-O_{ph}, (d) Ca_c-Na. In red are reported the rdf of the single Posner's cluster in water for comparison.

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