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ELECTRONIC SUPPLEMENTARY INFORMATION

Mechanism of water/ion exchange at a protein surface: a weakly bound chloride in Helicobacter pylori apoflavodoxin.

Juan J. Galano[a,b], M. Carmen Morón[c,d] and Javier Sancho[a,b]

[a] Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain.

[b] Biocomputation and Complex Systems Physics Institute (BIFI). Joint Unit BIFI-IQFR (CSIC). Edificio I+D, Mariano Esquillor, 50018, Zaragoza, Spain.

[c] Instituto de Ciencia de Materiales de Aragón (ICMA), Consejo Superior de Investigaciones Científicas-Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain.

[d] Departamento de Física de la Materia Condensada, Facultad de Ciencias, Universidad de Zaragoza, Pedro Cerbuna 12, 50009, Zaragoza, Spain.

Correspondence to: Javier Sancho; e-mail: jsancho@unizar.es.

Table S1. Statistics of the main events concerning the chloride release process in the ten high-resolution simulations. Different frequencies of occurrences of PARs in the replicas illustrate how diverse, in terms of dynamic behaviour, the chloride unbinding process is. The big standard deviations associated with these frequencies reveal the very different gaps of time there is between FIPARs within the simulations (see the different spacing between vertical red lines in charts on the left in Fig. S4). On the other hand, the very different WEs frequencies calculated before and after chloride unbinding confirm the different residence times showed by water molecules in these two environments (see figure S4). Data concerning the number of simultaneous occurrences of events, such as, water entry (WEs), polar atom release (PARs), the increase of the total number of H-bonds of the chloride (+THB), and the increase of the total number of chloride/water H-bonds (+WHB), is also included. Notoriously, out of the 11.5 PAR events registered per simulation (on average), only in 0.5 of them WEs were simultaneously detected (FIPAR and FIWE moments within a margin of $\pm$ 1 frame between them). These simultaneous occurrences, lead in every case to an increase in the number of chloride/water H-bond (+WHB). Nevertheless, in none of them the total number of chloride H-bonds was increased (+THB).

Table S1.

	r1	**r2**	**r3**	**r4**	**r5**	**r6**	**r7**	**r8**	**r9**	**r10**	**Mean**
No. of frames (1frame=50 fs)	20000	20000	7000	20000	20000	20000	20000	20000	7000	20000	17400
No. of frames Cl^- remaining bound	10690	14862	4126	18571	11720	17839	10690	8269	2145	16545	11546
PAR events[a]	19	9	6	18	23	6	14	11	3	6	11.5
Frequency of PARs (in ps)	45.5±90.0	65.8±115.6	13.7±27.7	46.7±68.9	25.6±37.9	53.9±87.3	66.4±109.1	22.8±36.3	*Very few events*	160.0±364.7	47.0±101.3
WE events[b]	263	341	126	278	217	172	304	377	171	262	251.1
Frequency of WEs (in ps)	3.8±6.0	2.9±4.3	2.6±4.2	3.6±6.5	4.6±6.6	5.7±10.0	3.3±4.7	2.6±4.3	2.0±3.1	3.8±5.7	3.4±5.7
WEs in Cl-bound trajectory section	88	224	44	258	90	144	100	114	20	159	124.1
Frequency of WEs in Cl-bound section (in ps)	6.1±9.0	3.3±4.9	4.3±6.4	3.6±6.7	6.4±8.9	6.1±10.7	5.4±7.0	3.6±6.3	5.4±7.1	5.2±7.0	4.6±7.4
Total H-bond increase[c] in Cl-bound section: +THb	395	490	171	490	384	455	425	500	157	508	397.5
Water H-bond increase in Cl-bound section: +WHb	167	309	69	352	185	286	207	158	35	287	205.5
WE & PAR[d]	0	0	1	1	0	1	1	0	0	1	0.5
WE & PAR & +WHb[d]	0	0	1	1	0	1	1	0	0	1	0.5
WE & PAR & +WHb & +THb[d]	0	0	0	0	0	0	0	0	0	0	0

[a]: The registration time for these events was FIPAR (see section IIc). [b]: Events registered in FIWE instants (see section IIc). [c]: Total H-bond refers to the sum of chloride/protein plus chloride/water H-bonds (see Fig. 5a). [d]: Events were accounted as simultaneous within a margin of ± 1 frame between them.

Supplementary FIGURES and VIDEOS

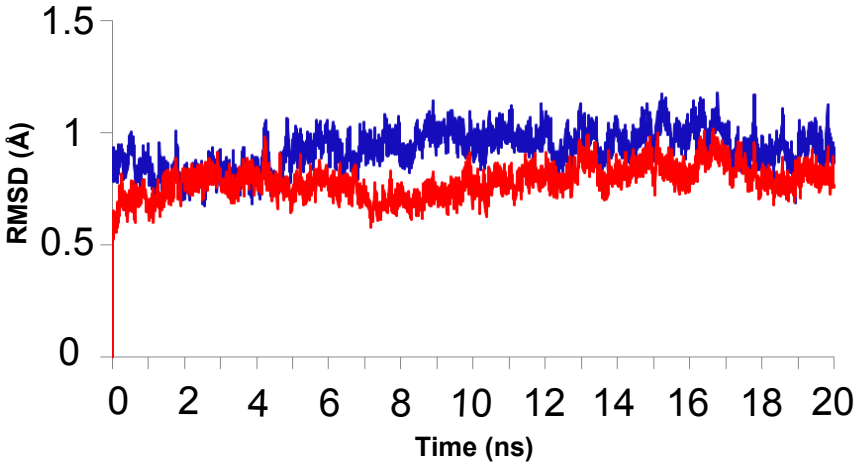

Fig. S1. RMSD analysis. Root Mean Square Deviation (RMSD) plots for one representative simulation out of the fifty replicas initially performed. Red line represents RMSD values respect to the first frame of the trajectory, whilst blue line shows RMSD values respect to the apo *Hp*-Fld crystal structure.

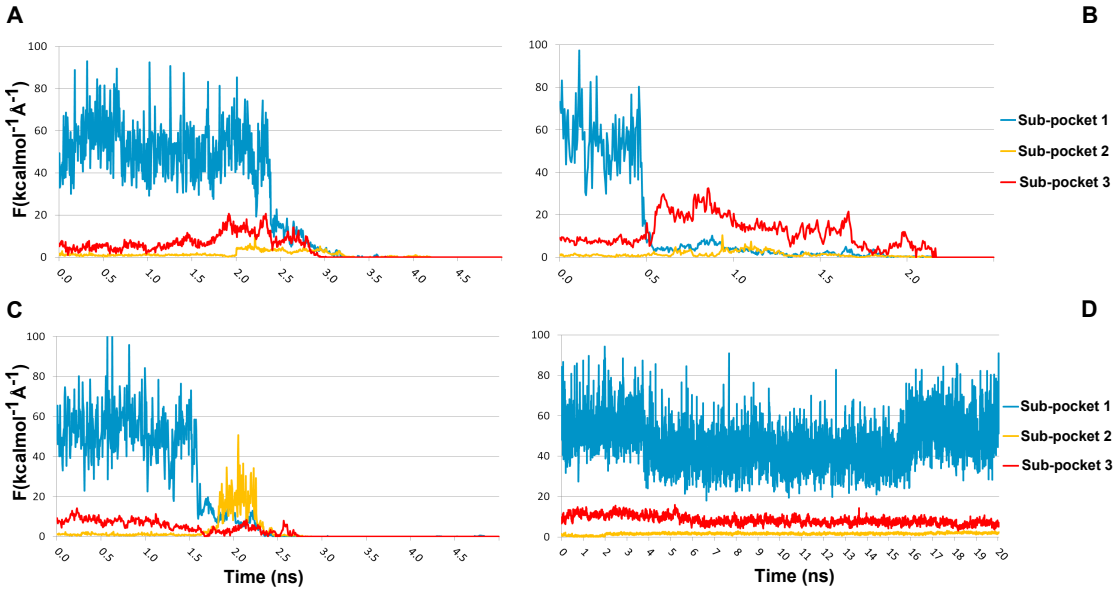

Fig. S2. Chloride/sub-pockets interaction forces. Plots of interaction forces (van der Waals + electrostatic) between the chloride and each of the three sub-pockets that constitute the anion binding site. This type of graphs allows to identify and to follow the exit route of the chloride into the bulk. Some examples are presented here: A) the chloride is directly released into the bulk water; B) the chloride first unbinds from sub-pocket 1, and then remains interacting with sub-pocket 3 for a while before being definitively released into the bulk; C) the chloride is released from sub-pocket 1, and afterwards remains some time bound to sub-pocket 2 before heading into the bulk, and D) the chloride is not released from sub-pocket 1. Sub-pockets 1, 2 and 3 refer to the ones described in Fig. 4 of the manuscript.

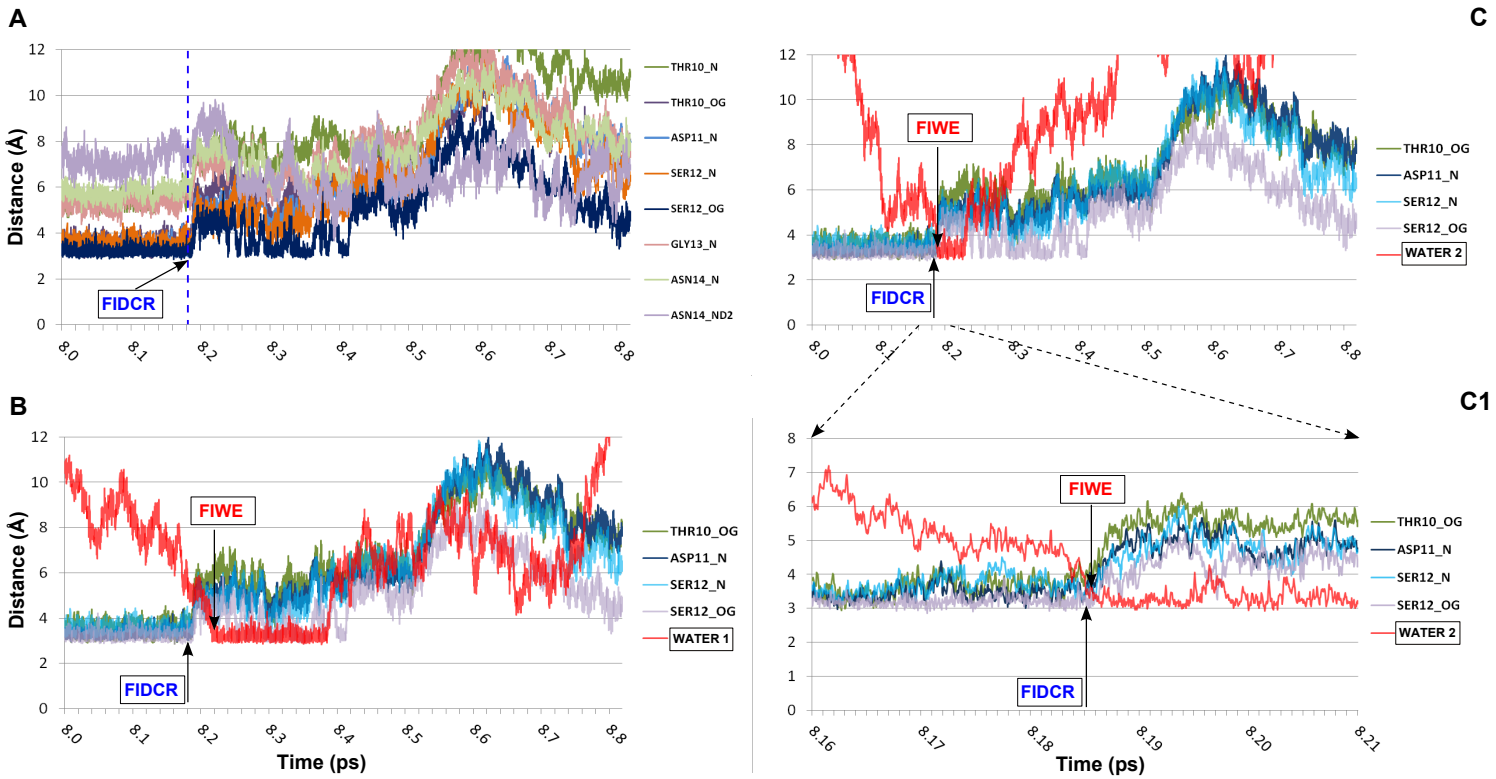

Fig. S3. Distance analysis. Joint plots of the chloride/water and chloride/protein H-bond distances obtained for one simulation in which it is difficult to draw conclusions about the role of water in chloride unbinding. A) Plots of the distances between every polar atom belonging to the chloride binding pocket and the anion. The calculated FIDCR are indicated. B, C, D and E) are plots just showing the last polar atoms of the protein that are released from the chloride (blue lines), as well as one water molecule (red lines) whose entrance into the first solvation shell of the chloride may be related to the anion release. C1, D1 and E1) are zoomed plots of a reduced window time very close to the FIDCR moment. FIDCR and FIWE instants are indicated. Graphs D/D1 and E/E1 show that it is not trivial to determine whether water entries into the first solvation shell of the chloride occur with implication on the chloride unbinding event or not.

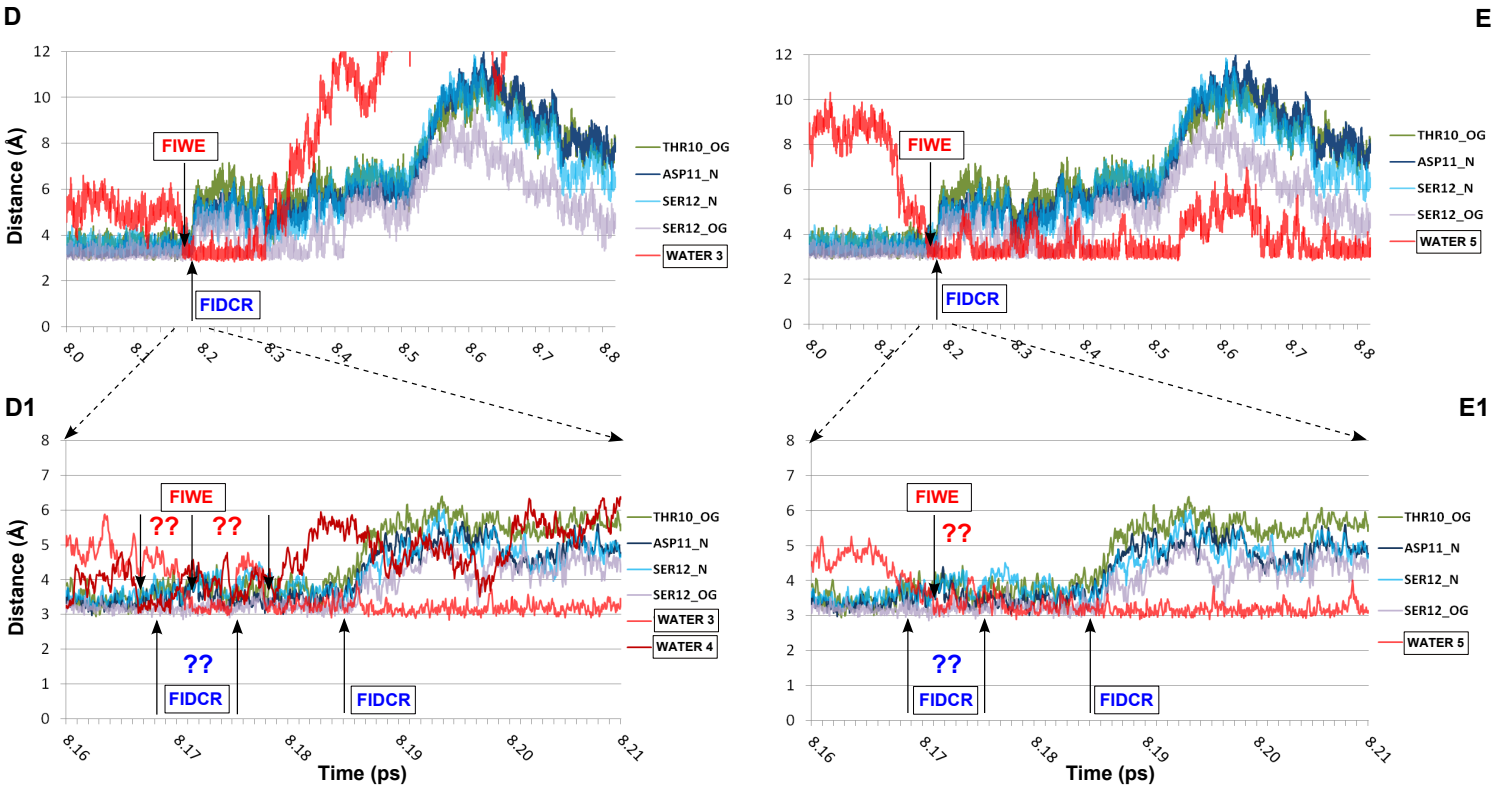

Fig. S3. (continued ...)

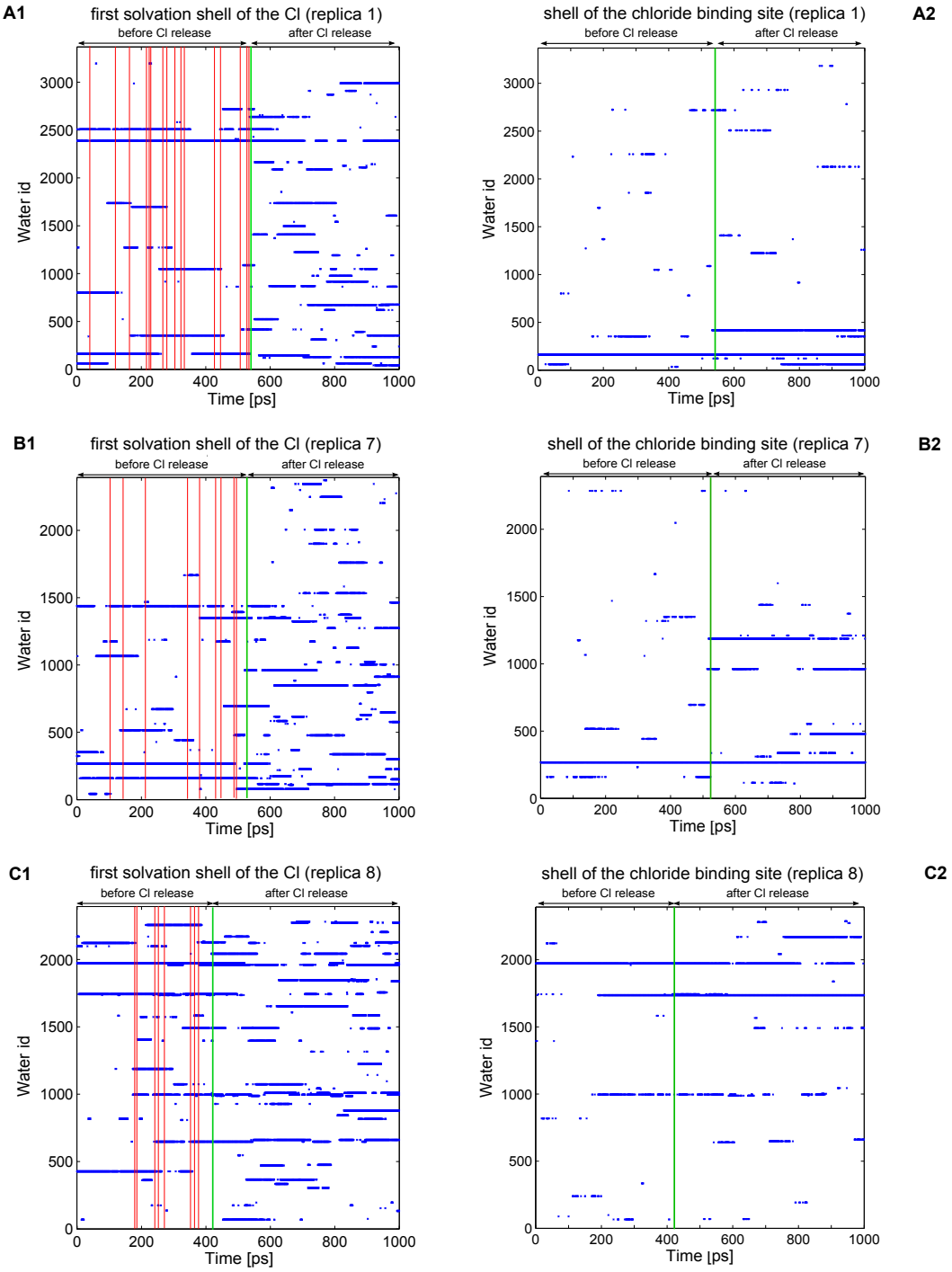

Fig. S4. Water residence times. Binary charts representing residence times of water molecules in two different environments along three out of the ten high-resolution simulations. Series 1 (A1, B1, C1) shows residence times for waters in the first solvation shell of the chloride (< 3.7 Å, see section IId), whilst series 2 (A2, B2, C2) does the same for waters in the first solvation shell of the chloride binding site (< 4.5 Å, see section IId). Asterisks in blue indicate the presence of water molecules inside the established cutoffs in the corresponding times. Vertical red lines in series 1 (graphs on the left) indicate the presence of FIPARs along the simulations.

Videos S1, S2 and S3. Short videos showing different chloride exit processes from its binding site in *Hp*-Fld. Videos S1 and S2 show in blue spheres the coordinated residues to the chloride anion (yellow sphere), and the water molecules coordinating the anion during the chloride release process (the remaining protein in cartoon). In video S1 anion release is produced in a more direct way into the bulk solvent whilst in video S2 the chloride first unbounds from the polar atoms in the centre of the binding pocket but afterward it remains some time bound by just one polar atom in one side part of this pocket before going into the bulk. Video S3 is an example showing the apoprotein surface and the chloride being released directly into the solvent.