Electronic Supplementary Information

Multi-scale modeling of folic acid-functionalized TiO_2 nanoparticles for active targeting of tumor cells

Edoardo Donadoni[a], Paulo Siani[a], Giulia Frigerio[a], Cristiana Di Valentin[a,b,*]

[a]Dipartimento di Scienza dei Materiali, Università di Milano-Bicocca
via R. Cozzi 55, 20125 Milano Italy
[b]BioNanoMedicine Center NANOMIB, University of Milano-Bicocca, Italy

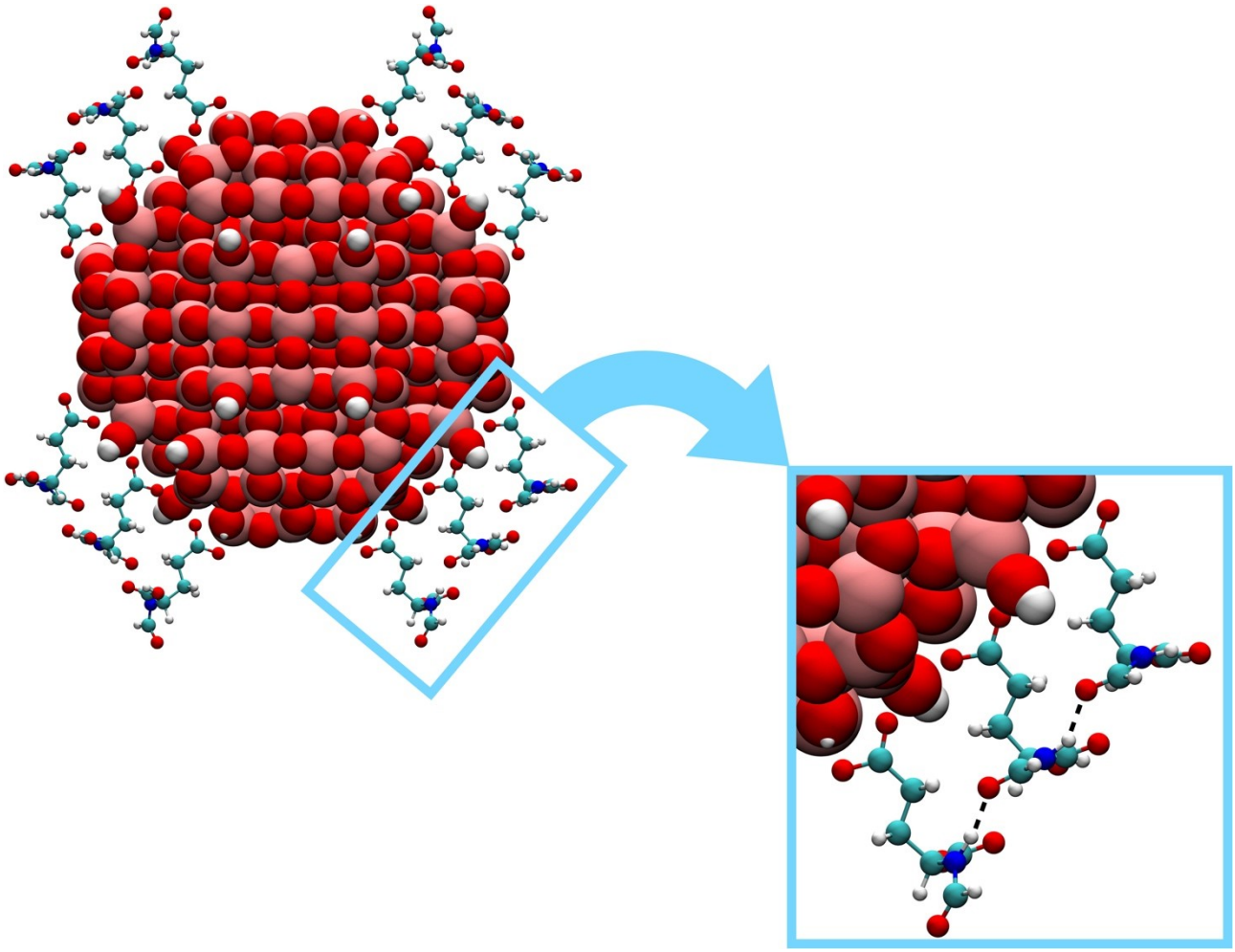

Fig. S1. Graphical representation of the TiO_2/8-FGA-γ system and insight on the equatorial H-bonds network. Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white.

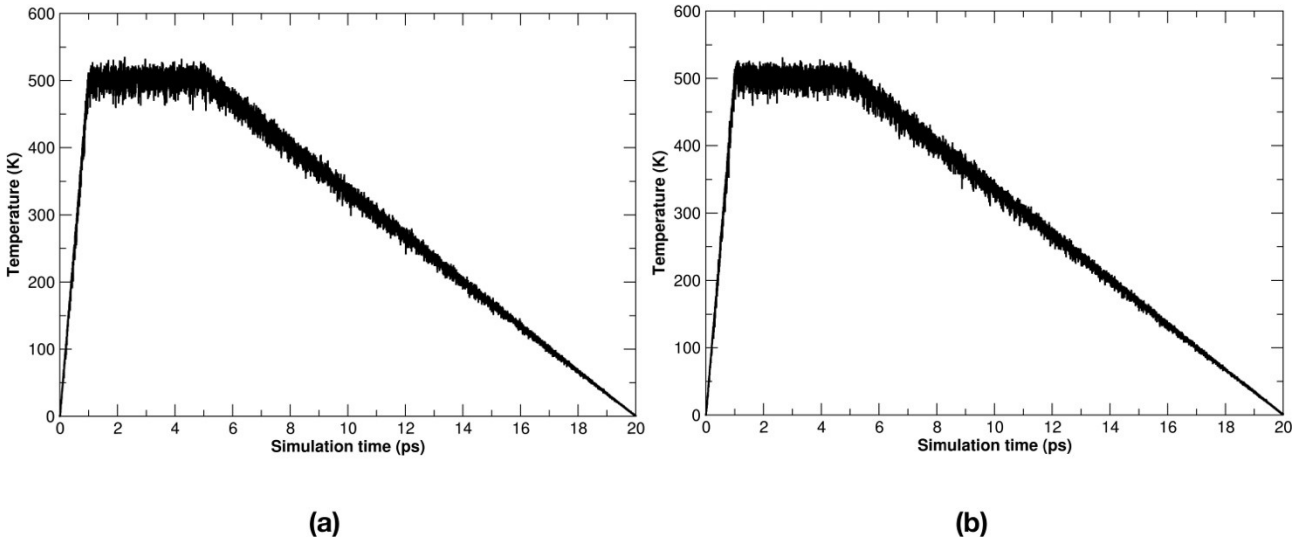

Fig. S2. Temperature profiles adopted for the DFTB-simulated annealing calculations for TiO_2/52-FGA-α (a) and TiO_2/48-FGA-γ (b) systems.

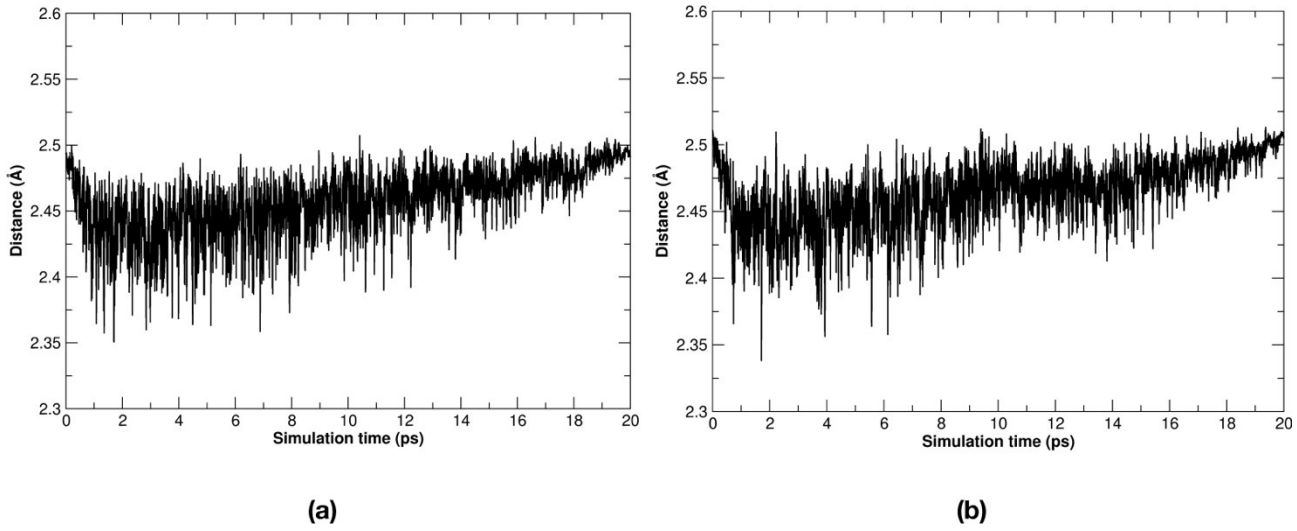

Fig. S3. Time evolution of the average distance between the C atom of FGA carboxylic groups anchoring the NP and the coordinated Ti atom of the NP, along the DFTB-simulated annealing calculations, for TiO_2/52-FGA-α (a) and TiO_2/48-FGA-γ (b) systems.

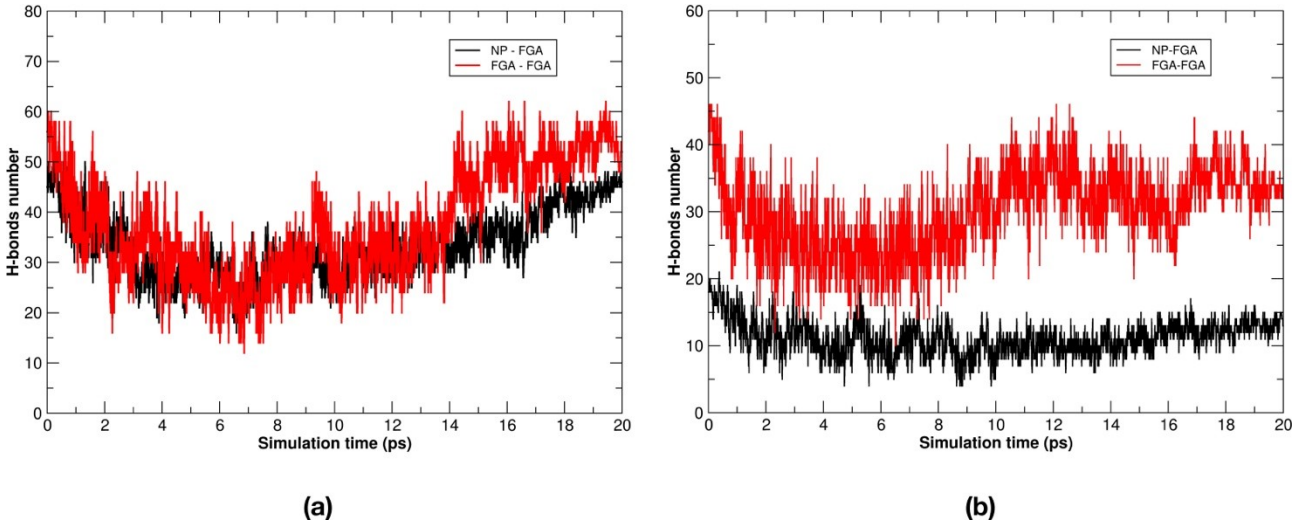

Fig. S4. Time evolution of the NP-FGA and FGA-FGA hydrogen bonds number, along the DFTB-simulated annealing calculations, for TiO_2/52-FGA-α (a) and TiO_2/48-FGA-γ (b) systems.

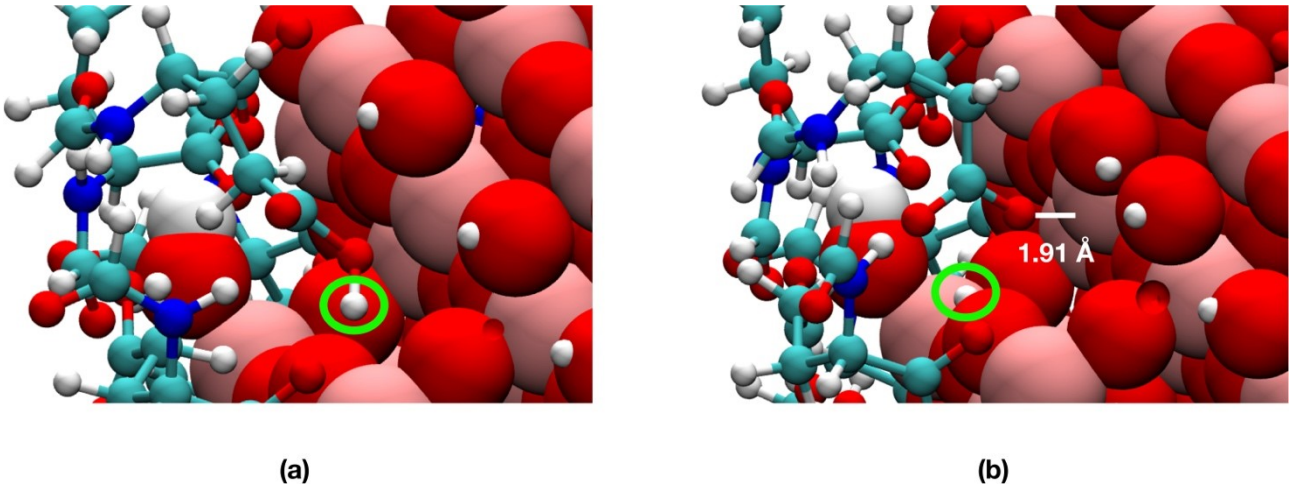

Fig. S5. Proton transfer from the free carboxylic group of one FGA molecule (a) to a near O_{2c} atom of the NP (b), with the formation of one NP-FGA Ti-O bond in a monodentate configuration, during the DFTB-simulated annealing for the TiO_2/52-FGA-α system. Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white.

Table S1. Average distances, hydrogen bonds number and non-bonding (vdW and electrostatic) interaction energies for the 100 ns production simulations of the TiO_2/1-FA-α and TiO_2/1-FA-γ in vacuum and in water.

Indicator	**TiO_2/1-FA-α vacuum**	**TiO_2/1-FA-γ vacuum**	**TiO_2/1-FA-α water**	**TiO_2/1-FA-γ water**
	Distances (Å)			
d N^{FA}-NP^{center}	15.5 (± 0.1)	13.5 (± 0.1)	18 (± 4)	16 (± 1)
d com^{FA}-$NP^{surface}$	5.3 (± 0.1)	5.5 (± 0.1)	6 (± 1)	6 (± 1)
	Hydrogen bonds number			
NP-FA	0	0.6 (± 0.7)	0.1 (± 0,3)	0.04 (± 0.21)
FA-wat	-	-	9 (± 2)	9 (± 2)
NP-wat	-	-	39 (± 4)	39 (± 5)
	Non-bonding interaction energies (kcal/mol)			
vdW NP-FA	-22 (± 1)	-27 (± 5)	-19 (± 6)	-21 (± 5)
ele NP-FA	-38 (± 2)	-19 (± 3)	-5 (± 4)	-8 (± 4)
vdW FA-wat	-	-	-18 (± 5)	-17 (± 5)
ele FA-wat	-	-	-95 (± 10)	-97 (± 10)
vdW NP-wat	-	-	-443 (± 13)	-456 (± 12)
ele NP-wat	-	-	-523 (± 21)	-527 (± 22)

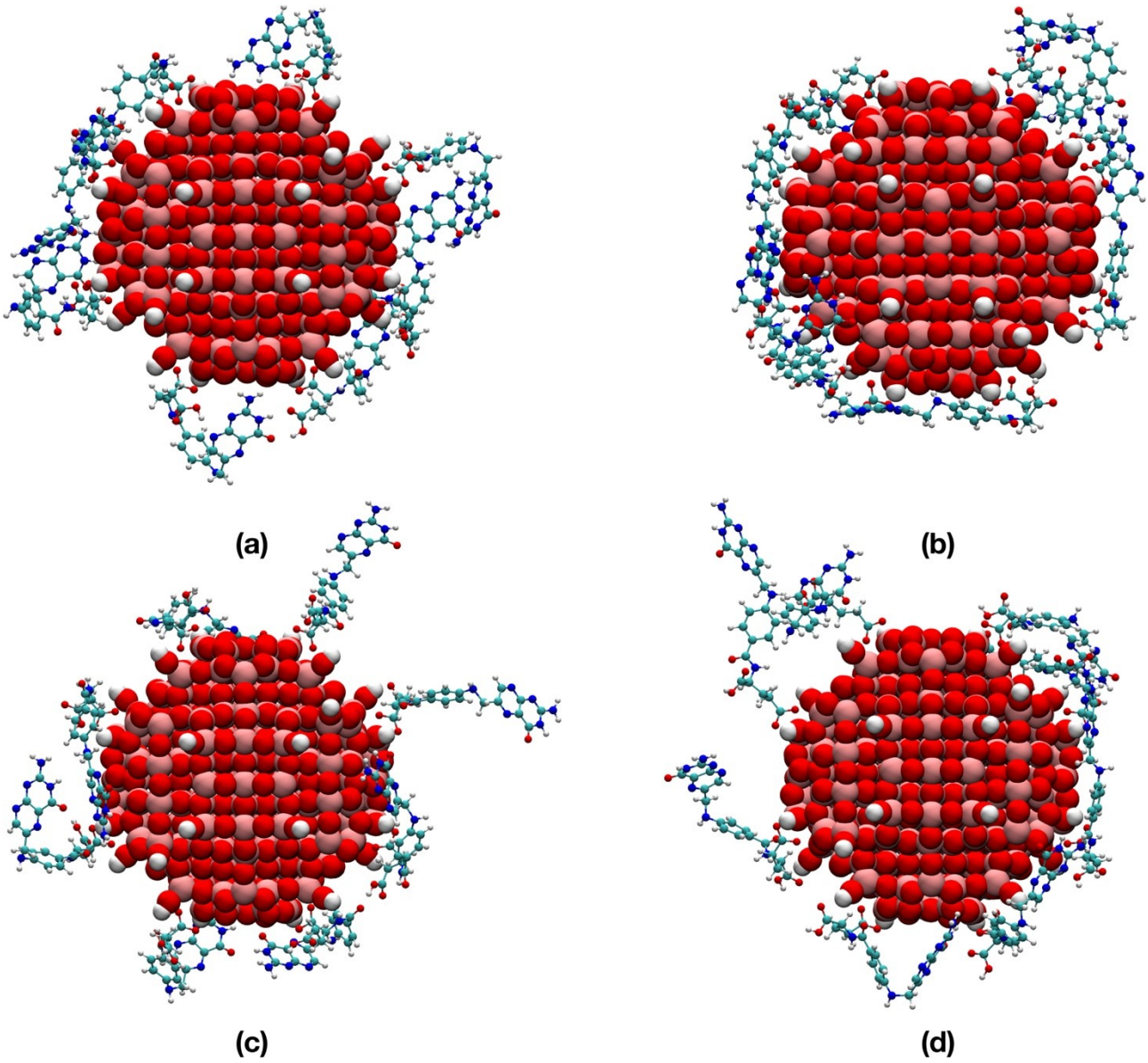

Fig. S6. Last snapshots from the 100 ns production simulations of TiO_2/8-FA-α in vacuum (a) and in water (c) and TiO_2/8-FA-γ in vacuum (b) and in water (d). Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white. The water molecules are not shown for clarity.

Table S2. Average distances, hydrogen bonds number and non-bonding (vdW and electrostatic) interaction energies for the 100 ns production simulations of the TiO_2/8-FA-α and TiO_2/8-FA-γ in vacuum and in water.

Indicator	**TiO_2/8-FA-α vacuum**	**TiO_2/8-FA-γ vacuum**	**TiO_2/8-FA-α water**	**TiO_2/8-FA-γ water**
	Distances (Å)			
d N^{FA}-NP^{center}	16 (± 2)	16 (± 2)	20 (± 8)	20 (± 11)
d com^{FA}-$NP^{surface}$	6 (± 1)	6 (± 1)	7 (± 1)	8 (± 4)
	Hydrogen bonds number			
NP-FA	4 (± 1)	3 (± 1)	0,2 (± 0,4)	0,9 (± 0,9)
FA-FA	5 (± 3)	5 (± 2)	0,3 (± 0,8)	0,9 (± 1,2)
FA-wat	-	-	72 (± 5)	69 (± 6)
NP-wat	-	-	32 (± 4)	34 (± 4)
	Non-bonding interaction energies (kcal/mol)			
vdW NP-FA	-185 (± 9)	-209 (± 5)	-176 (± 11)	-115 (± 24)
ele NP-FA	-107 (± 9)	-110 (± 8)	-28 (± 7)	-31 (± 11)
vdW FA-FA	-16 (± 3)	-24 (± 4)	-3 (± 2)	-24 (± 5)
ele FA-FA	-78 (± 10)	-61 (± 7)	-3 (± 5)	-11 (± 9)
vdW FA-wat	-	-	-157 (± 13)	-149 (± 14)
ele FA-wat	-	-	-766 (± 28)	-756 (± 33)
vdW NP-wat	-	-	-420 (± 13)	-456 (± 18)
ele NP-wat	-	-	-515 (± 23)	-541 (± 27)

Table S3. Average distances, hydrogen bonds number and non-bonding (vdW and electrostatic) interaction energies for the 100 ns production simulations of the TiO_2/52-FA-α and TiO_2/48-FA-γ systems in vacuum and in water.

Indicator	**TiO_2/52-FA-α vacuum**	**TiO_2/48-FA-γ vacuum**	**TiO_2/52-FA-α water**	**TiO_2/48-FA-γ water**
	Distances (Å)			
d N^{FA}-NP^{center}	21 (± 3)	20 (± 3)	24 (± 2)	24 (± 3)
d com^{FA}-$NP^{surface}$	8 (± 3)	8 (± 2)	8 (± 1)	9 (± 2)
	Hydrogen bonds number			
NP-FA	5 (± 1)	5 (± 1)	4 (± 1)	2 (± 1)
FA-FA	86 (± 9)	73 (± 9)	23 (± 6)	18 (± 6)
FA-wat	-	-	392 (± 13)	376 (± 14)
NP-wat	-	-	11 (± 3)	22 (± 3)
	Non-bonding interaction energies (kcal/mol)			
vdW NP-FA	-612 (± 10)	-535 (± 15)	-610 (± 9)	-424 (± 19)
ele NP-FA	-167 (± 14)	-180 (± 13)	-128 (± 12)	-86 (± 12)
vdW FA-FA	-536 (± 17)	-464 (± 16)	-494 (± 19)	-443 (± 19)
ele FA-FA	-1308 (± 37)	-1241 (± 38)	-276 (± 50)	-256 (± 45)
vdW FA-wat	-	-	-643 (± 34)	-652 (± 34)
ele FA-wat	-	-	-4308 (± 97)	-4110 (± 106)
vdW NP-wat	-	-	-130 (± 9)	-246 (± 16)
ele NP-wat	-	-	-333 (± 19)	-541 (± 24)

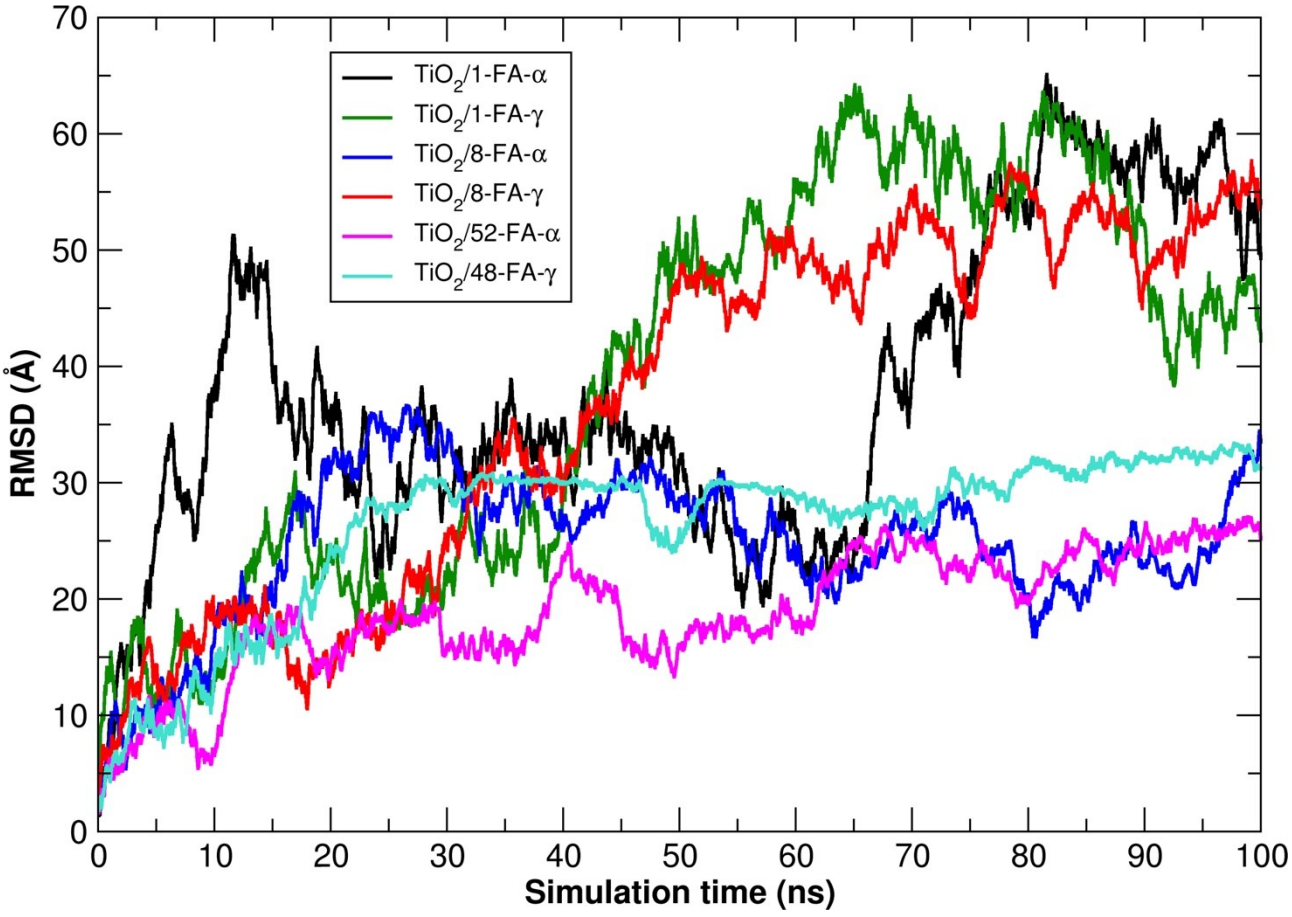

Fig. S7. Root-mean-square deviation of the NP+FA atomic positions along the 100 ns MD simulations, with respect to the 0 ns-reference atomic positions, for all the TiO_2/n-FA systems in water.

Table S4. Estimated NP+FA diffusion coefficients for the 100 ns production simulations of all the TiO_2/n-FA-α systems in water.

System	**D/10^{-10} (m^2/s)**
TiO_2/1-FA-α	7.7 (± 0.3)
TiO_2/8-FA-α	2.06 (± 0.06)
TiO_2/52-FA-α	1.70 (± 0.07)

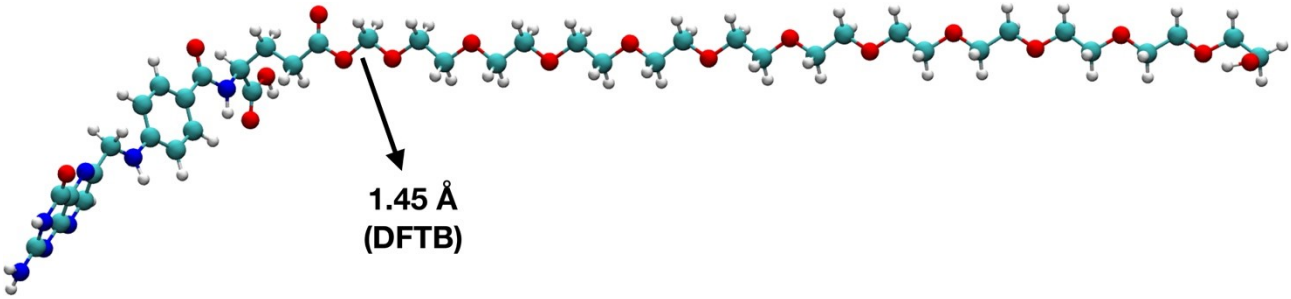

Fig. S8. DFTB-optimized geometry of a PEG-FA chain. Oxygen is shown in pink, carbon in cyan, nitrogen in blue and hydrogen in white.

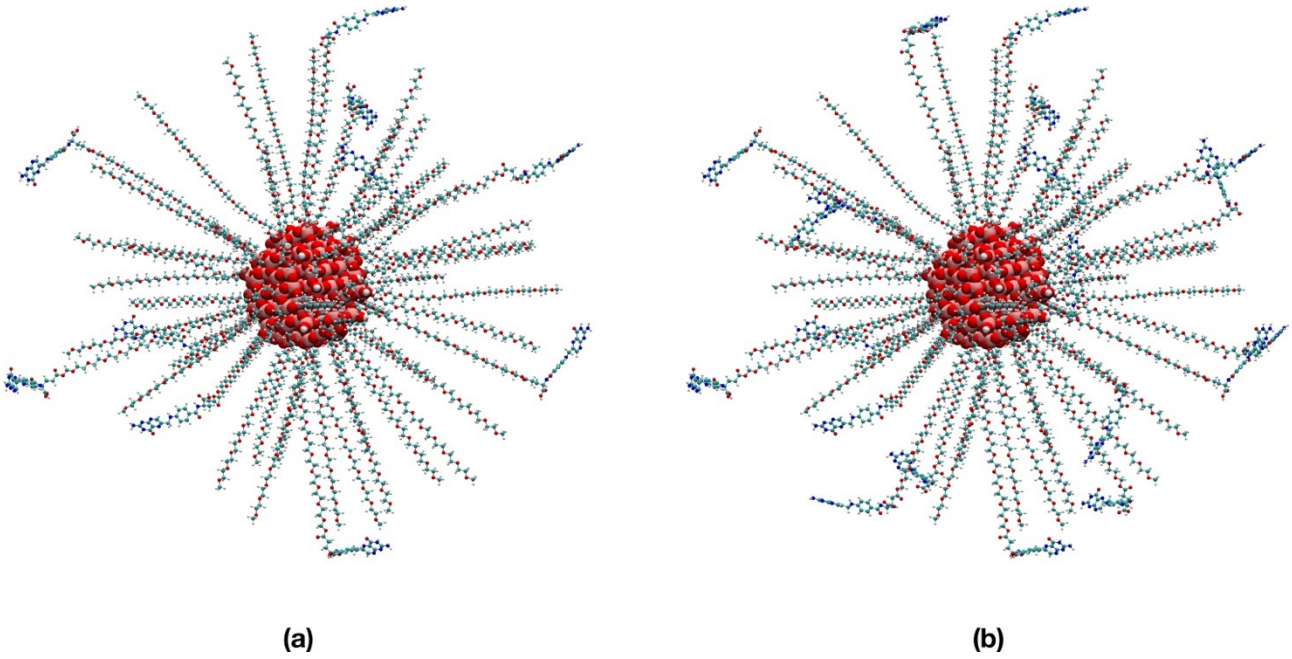

Fig. S9. DFTB-optimized geometries of the TiO_2/PEG/10-FA (a) and the TiO_2/PEG/20-FA (b) systems. Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white.

Table S5. Average distances, hydrogen bonds number and non-bonding (vdW and electrostatic) interaction energies for the 100 ns production simulations of the TiO_2/PEG/10-FA and TiO_2/PEG/20-FA systems, in vacuum and in water.

Indicator	**TiO_2/PEG/10-FA vacuum**	**TiO_2/PEG/10-FA water**	**TiO_2/PEG/20-FA vacuum**	**TiO_2/PEG/20-FA water**
	Distances (Å)			
d N^{FA}-NP^{center}	21 (± 2)	31 (± 5)	22 (± 3)	32 (± 3)
d com^{FA}-$NP^{surface}$	12 (± 3)	17 (± 2)	13 (± 3)	17 (± 2)
$<h^2>^{½}$ PEG	13 (± 4)	18 (± 1)	13 (± 3)	18 (± 1)
$<h^2>^{½}$ PEG-FA	11 (± 4)	16 (± 2)	12 (± 3)	17 (± 1)
$MDFS^{PEG}$	9 (± 3)	14 (± 1)	9 (± 2)	14 (± 1)
$MDFS^{PEG-FA}$	11 (± 3)	13 (± 2)	9 (± 3)	13 (± 1)
R_g^{PEG}	5.3 (± 0.1)	6.7 (± 0.1)	5.3 (± 0.1)	6.8 (± 0.1)
R_g^{PEG-FA}	5.7 (± 0.1)	7.6 (± 0.1)	6.8 (± 0.1)	8.8 (± 0.1)
	Hydrogen bonds number			
NP-FA	0	0.7 (± 0.5)	0.5 (± 0.5)	0.3 (± 0.6)
NP-PEG	0	0	0	0
PEG-PEG	3 (± 2)	2 (± 1)	1 (± 1)	2 (± 1)
PEG-FA	18 (± 3)	2 (± 1)	32 (± 4)	5 (± 2)
FA-FA	6 (± 3)	1 (± 1)	16 (± 4)	2 (± 2)
FA-wat	-	90 (± 6)	-	178 (± 9)
PEG-wat	-	308 (± 13)	-	298 (± 13)
NP-wat	-	59 (± 4)	-	58 (± 3)
	Non-bonding interaction energies (kcal/mol)			
vdW NP-FA	-89 (± 7)	-12 (± 3)	-17 (± 1)	-5 (± 5)
ele NP-FA	-53 (± 5)	-18 (± 3)	-13 (± 3)	-6 (± 8)
vdW NP-PEG	-478 (± 13)	-274 (± 20)	-525 (±12)	-276 (± 18)
ele NP-PEG	-269 (± 10)	-99 (± 10)	-303 (± 11)	-114 (± 11)
vdW PEG-PEG	-820 (± 17)	-382 (± 22)	-808 (± 17)	-396 (± 24)
ele PEG-PEG	-517 (± 27)	10 (± 13)	-169 (± 19)	5 (± 15)
vdW PEG-FA	-335 (± 12)	-94 (± 20)	-420 (± 18)	-180 (± 33)

ele PEG-FA	-93 (± 17)	-46 (± 21)	-558 (± 31)	-98 (± 29)
vdW FA-FA	-6 (± 2)	-10 (± 9)	-60 (± 7)	-47 (± 16)
ele FA-FA	0.4 (± 1.6)	-6 (± 6)	-205 (± 14)	-39 (± 18)
vdW FA-wat	-	-151 (± 24)	-	-298 (± 32)
ele FA-wat	-	-1041 (± 50)	-	-2059 (± 71)
vdW PEG-wat	-	-1490 (± 45)	-	-1421 (± 47)
ele PEG-wat	-	-3906 (± 88)	-	-3755 (± 93)
vdW NP-wat	-	-94 (± 19)	-	-98 (± 17)
ele NP-wat	-	-978 (± 37)	-	-950 (± 37)

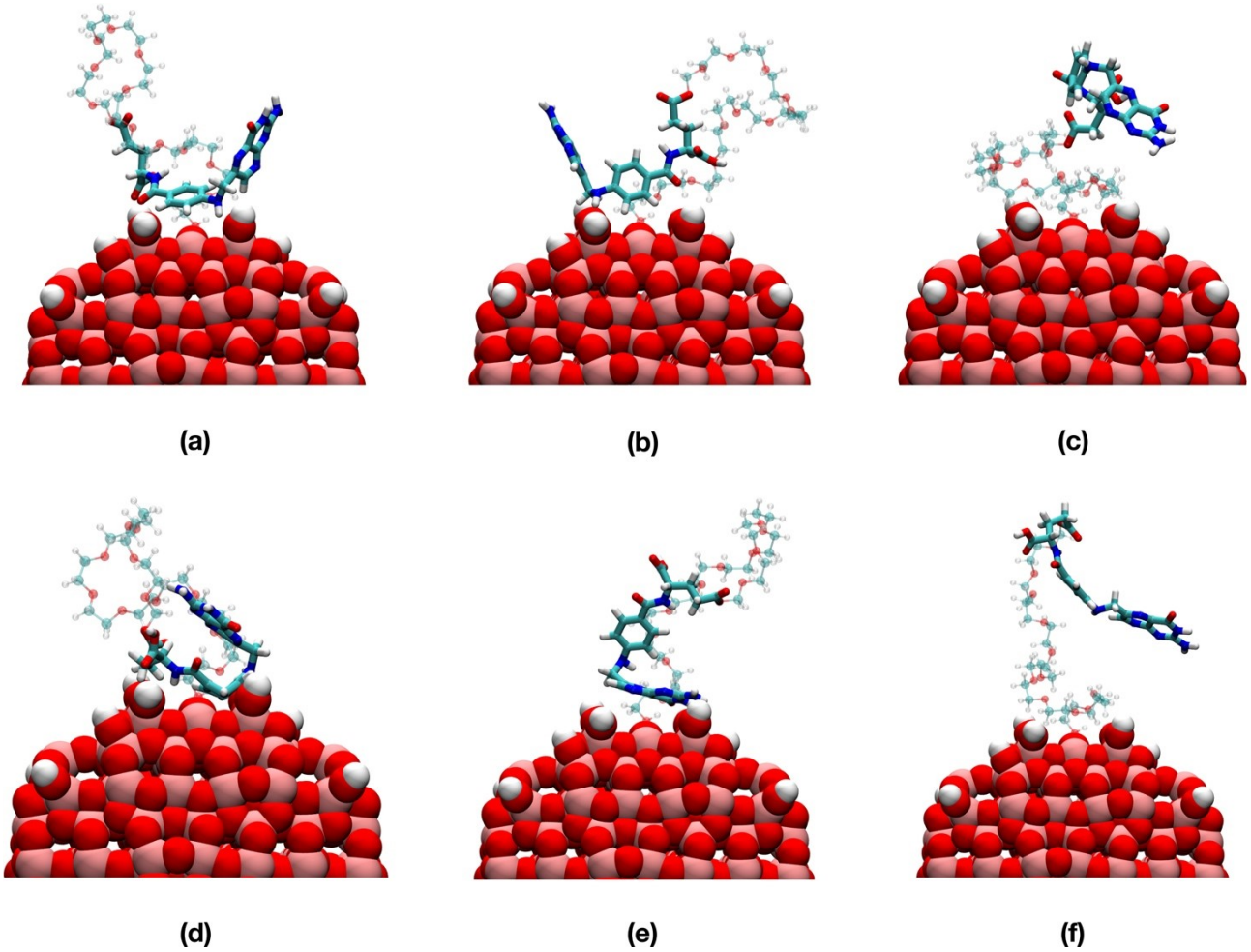

Fig. S10. Last snapshots from the 150 ns production ABF simulations of TiO_2/PEG/10-FA in water with a FA molecule (opaque) restrained respectively between 10-15 Å (a), 15-20 Å (b) and 20-25 Å (c) from the NP center with its relative PEG chain (transparent) and the ABF simulations with the same FA molecule uncharged and restrained respectively between 10-15 Å (d), 15-20 Å (e) and 20-25 Å (f) from the NP center. Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white. The other PEG and FA molecules and the water molecules are not shown for clarity.

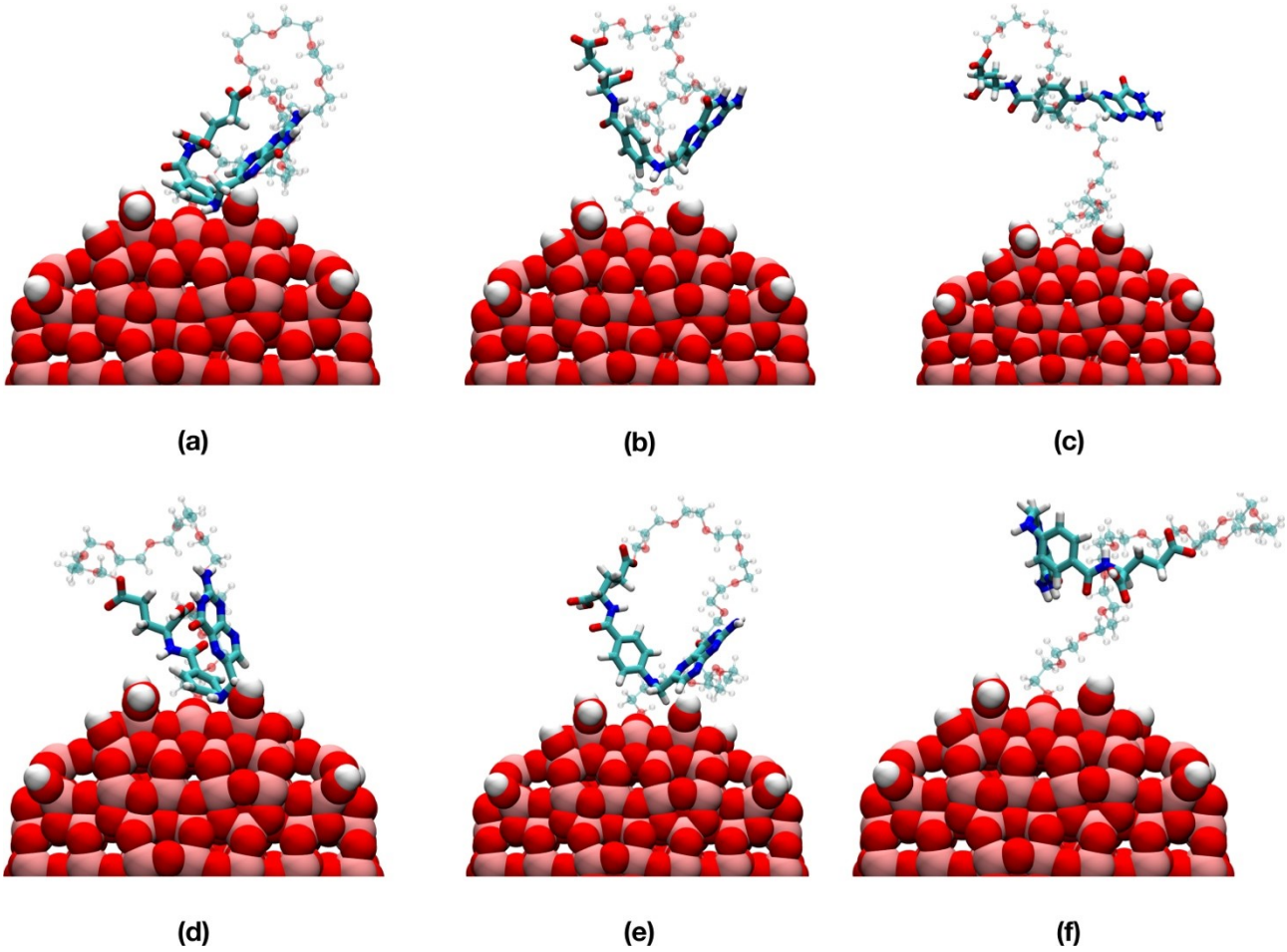

Fig. S11. Last snapshots from the 150 ns production ABF simulations of TiO_2/PEG/20-FA in water with a FA molecule (opaque) restrained respectively between 10-15 Å (a), 15-20 Å (b) and 20-25 Å (c) from the NP center with its relative PEG chain (transparent) and the ABF simulations with the same FA molecule uncharged and restrained respectively between 10-15 Å (d), 15-20 Å (e) and 20-25 Å (f) from the NP center. Titanium is shown in pink, oxygen in red, carbon in cyan, nitrogen in blue and hydrogen in white. The other PEG and FA molecules and the water molecules are not shown for clarity.

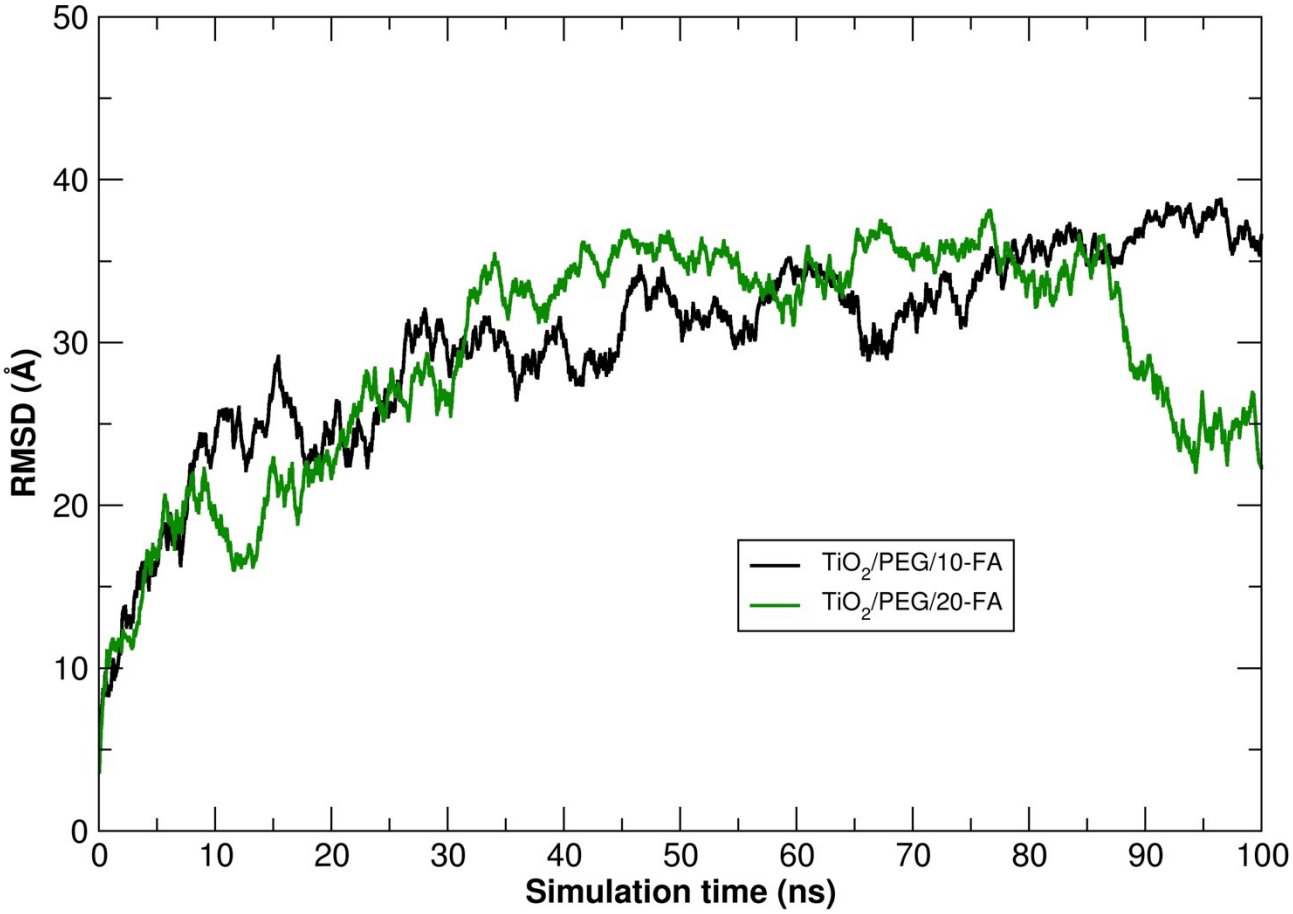

Fig. S12. Root-mean-square deviation of the NP+PEG+FA atomic positions along the 100 ns MD simulations, with respect to the 0 ns-reference atomic positions, for the TiO_2/PEG/10-FA and TiO_2/PEG/20-FA systems in water.

Table S6. Estimated NP+PEG+FA diffusion coefficients for the 100 ns production simulations of the TiO_2/PEG/10-FA and TiO_2/PEG/20-FA systems in water.

System	**D/10^{-10} (m^2/s)**
TiO_2/PEG/10-FA	2.5 (± 0.4)
TiO_2/PEG/20-FA	2.32 (± 0.09)

Table S7. Average distances, number of hydrogen bonds and non-bonding (vdW and electrostatic) interaction energies for the last 100 ns of MD production phase of the TiO_2/52-FA-α, TiO_2/48-FA-γ, TiO_2/PEG/10-FA and TiO_2/PEG/20-FA systems in physiological environment.

Indicator	**TiO_2/52-FA-α**	**TiO_2/48-FA-γ**	**TiO_2/PEG/10-FA**	**TiO_2/PEG/20-FA**
	Distances (Å)			
d N^{FA}-NP^{center}	24 (± 2)	24 (± 3)	30 (± 3)	32 (± 2)
d com^{FA}-$NP^{surface}$	8 (± 1)	9 (± 2)	17 (± 3)	18 (± 2)
$<h^2>^{½\ PEG}$	-	-	16 (± 2)	17 (± 2)
$<h^2>^{½\ PEG\text{-}FA}$	-	-	16 (± 3)	16 (± 2)
$MDFS^{PEG}$	-	-	13 (± 1)	13 (± 1)
$MDFS^{PEG\text{-}FA}$	-	-	13 (± 2)	13 (± 2)
R_g^{PEG}	-	-	6.4 (± 0.1)	6.6 (± 0.1)
$R_g^{PEG\text{-}FA}$	-	-	8.4 (± 0.1)	8.1 (± 0.1)
	Hydrogen bonds number			
NP-FA	6.4 (± 0.9)	2.1 (± 0.9)	0	0
NP-PEG	-	-	0	0
PEG-PEG	-	-	1 (± 1)	1 (± 1)
PEG-FA	-	-	2 (± 1)	3 (± 2)
FA-FA	24 (± 6)	16 (± 5)	1 (± 1)	4 (± 3)
FA-wat	420 (± 12)	422 (± 13)	101 (± 7)	198 (± 9)
PEG-wat	-	-	348 (± 17)	358 (± 13)
NP-wat	24 (± 3)	45 (± 4)	76 (± 4)	77 (± 4)
	Non-bonding interaction energies (kcal/mol)			
vdW NP-FA	-588 (± 10)	-348 (± 10)	-0.7 (± 0.7)	-0.2 (± 0.6)
ele NP-FA	-229 (± 11)	-99 (± 6)	0.0 (± 0.3)	-0.3 (± 0.8)
vdW NP-PEG	-	-	-203 (± 11)	-178 (± 11)
ele NP-PEG	-	-	-84 (± 5)	-78 (± 4)
vdW PEG-PEG	-	-	-372 (± 23)	-364 (± 21)
ele PEG-PEG	-	-	12 (± 14)	9 (± 13)
vdW PEG-FA	-	-	-79 (± 17)	-140 (± 26)
ele PEG-FA	-	-	-40 (± 14)	-65 (± 22)

vdW FA-FA	-503 (± 23)	-421 (± 23)	-15 (± 7)	-75 (± 14)
ele FA-FA	-305 (± 33)	-245 (± 37)	-13 (± 13)	-63 (± 27)
vdW FA-wat	-684 (± 36)	-726 (± 37)	-177 (± 22)	-327 (± 36)
ele FA-wat	-4416 (± 127)	-4356 (± 124)	-1090 (± 57)	-2131 (± 94)
vdW PEG-wat	-	-	-1752 (± 54)	-1742 (± 56)
ele PEG-wat	-	-	-4200 (± 207)	-4287 (± 128)
vdW NP-wat	-113 (± 10)	-232 (± 16)	-150 (± 16)	-175 (± 15)
ele NP-wat	-577 (± 27)	-1038 (± 32)	-1155 (± 36)	-1172 (± 33)
vdW FA-solution	-682 (± 36)	-724 (± 37)	-176 (± 22)	-325 (± 35)
ele FA-solution	-4489 (± 124)	-4429 (± 120)	-1134 (± 52)	-2192 (± 94)
vdW PEG-solution	-	-	-1739 (± 58)	-1735 (± 57)
ele PEG-solution	-	-	-4703 (± 125)	-4588 (± 127)
vdW NP-solution	-113 (± 10)	-232 (± 16)	-150 (±16)	-175 (± 15)
ele NP-solution	-577 (± 27)	-1037 (± 32)	-1156 (± 36)	-1173 (± 33)

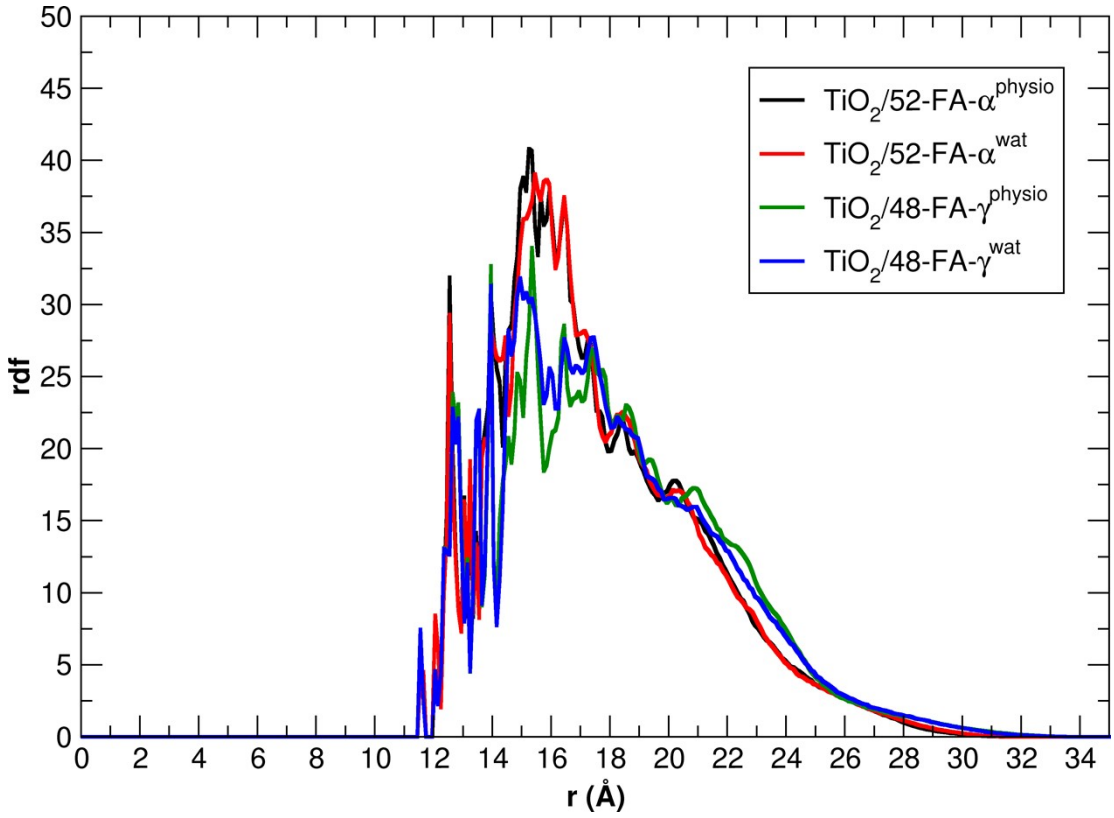

Fig. S13. Comparison of the radial distribution function (rdf) of the FA center of mass with respect to the six-fold coordinated Ti atom at the center of the NP, for the TiO_2/52-FA-α and TiO_2/48-FA-γ systems, in pure water and under physiological conditions, averaged over the last 100 ns of MD production phase.

Table S8. Estimated NP+FA or NP+PEG+FA diffusion coefficients for the last 100 ns of MD production phase of the TiO_2/52-FA-α, TiO_2/48-FA-γ, TiO_2/PEG/10-FA and TiO_2/PEG/20-FA systems in physiological environment.

System	**D/10^{-10} (m^2/s)**
TiO_2/52-FA-α	1.7 (± 0.1)
TiO_2/48-FA-γ	2.2 (± 0.2)
TiO_2/PEG/10-FA	1.9 (± 0.2)
TiO_2/PEG/20-FA	1.8 (± 0.2)