Supporting Information

Studies of dynamic binding of amino acids to TiO_2 nanoparticle surfaces by Solution NMR and Molecular Dynamics Simulations

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Well Tempered Metadynamics

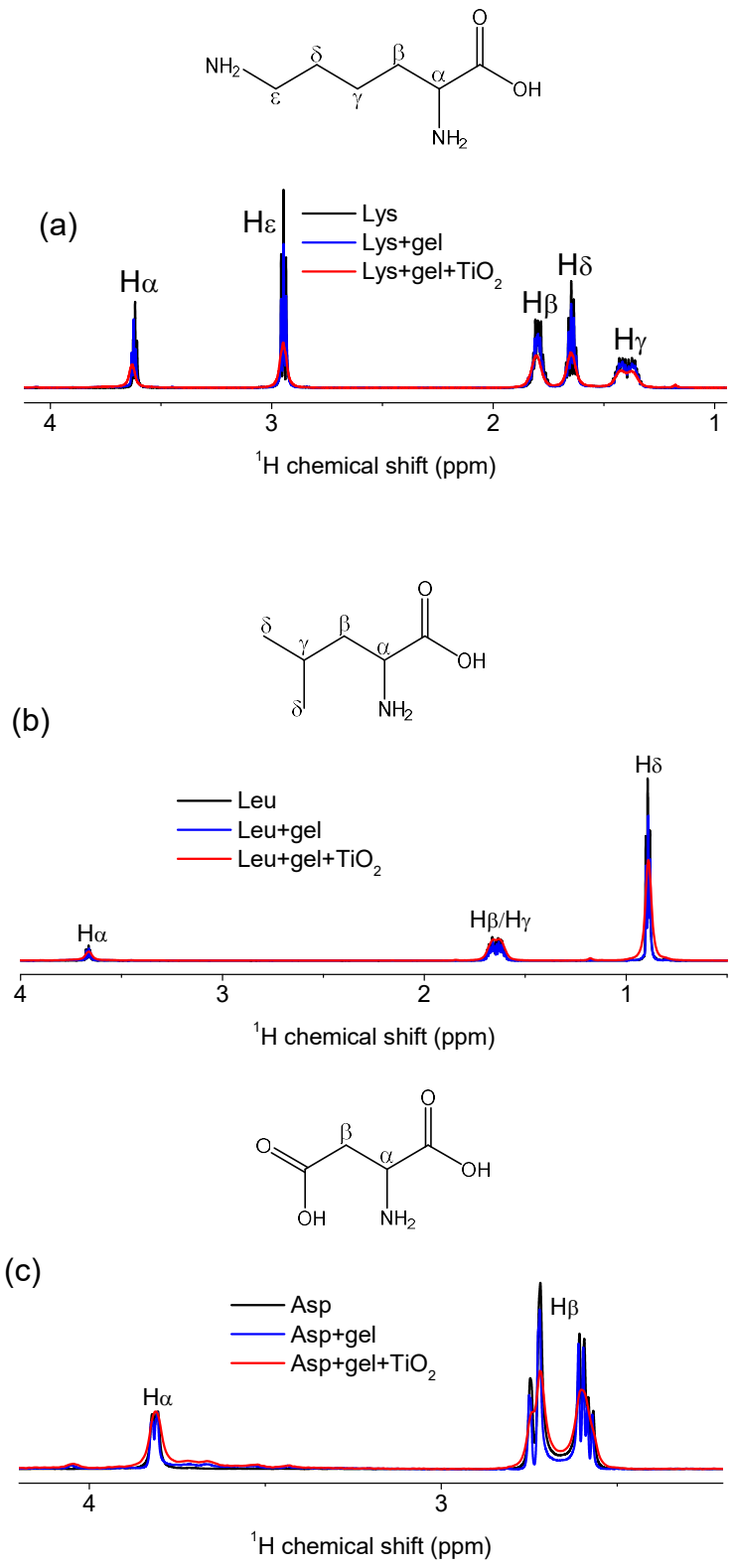
At the pH considered, arginine (pK=12.5) and lysine (pK=10.5) bear a positive charge, and aspartate (pK=3.7) bears a negative charge. We study the binding of uncapped arginine, lysine, and aspartate on TiO_2. These amino acids were chosen based on the fact that they are the three primary residues of TBP that are shown to interact with the TiO_2 surface. As detailed in the main manuscript, NPT equilibration is first performed during which the compressibility was set to 4.5e-15/bar in the x and y directions, and 4.5e-5/bar in the z direction, so that the box did not undergo significant changes along the x and y dimensions, preserving the structure of the surface. The last frame from this step was used as the input for the subsequent NVT equilibration runs. The binding free energy is calculated using the relation

$$\Delta G_{binding} = \Delta G_{so} \ - \Delta G_{ads}$$

where ΔG_{ads} is the Boltzmann averaged free energy of adsorption and ΔG_{sol} is the Boltzmann averaged free energy of solvation.

A timestep of 2 fs was employed in all simulations. PME algorithm with a cutoff of 1.0 nm, and a short-ranged cutoff of 1.0 nm was employed to calculate electrostatic and LJ interactions, respectively. The LINCS algorithm was used to restrain the positions of the hydrogen atoms in the amino acids. The steepest descent algorithm was used during energy minimization to remove unfavorable contacts.

Amino acid binding was calculated using well-tempered metadynamics (WTM), and the parameters used for each system are listed Table S1. A harmonic restraint was placed 3 nm from the surface to limit sampling to just the top face of the surface. A single replica was used for each system.


(a)
Hε
Hα
Hβ
Hδ
Hγ
Lys
Lys+gel
Lys+gel+TiO2
4
3
2
1
1H chemical shift (ppm)
(b)
Hδ
Leu
Leu+gel
Leu+gel+TiO2
Hα
Hβ/Hγ
4
3
2
1
1H chemical shift (ppm)
(c)
Hβ
Asp
Asp+gel
Asp+gel+TiO2
Hα
4
3
1H chemical shift (ppm)

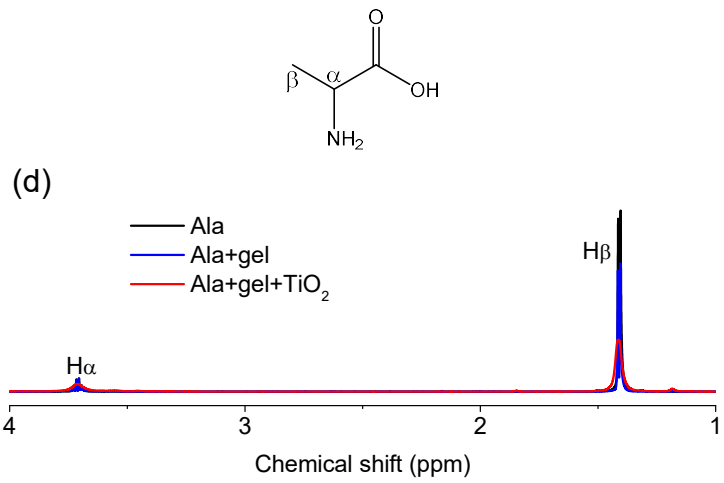


Figure S1. ^{1}H NMR spectra of 10 mM amino acids Lysine (a), Leucine (b), Aspartic acid (c), and (d) Alanine, in the absence of gel (black), in the presence of gel (blue), and in the presence of gel and 1 wt % TiO_2 (red).

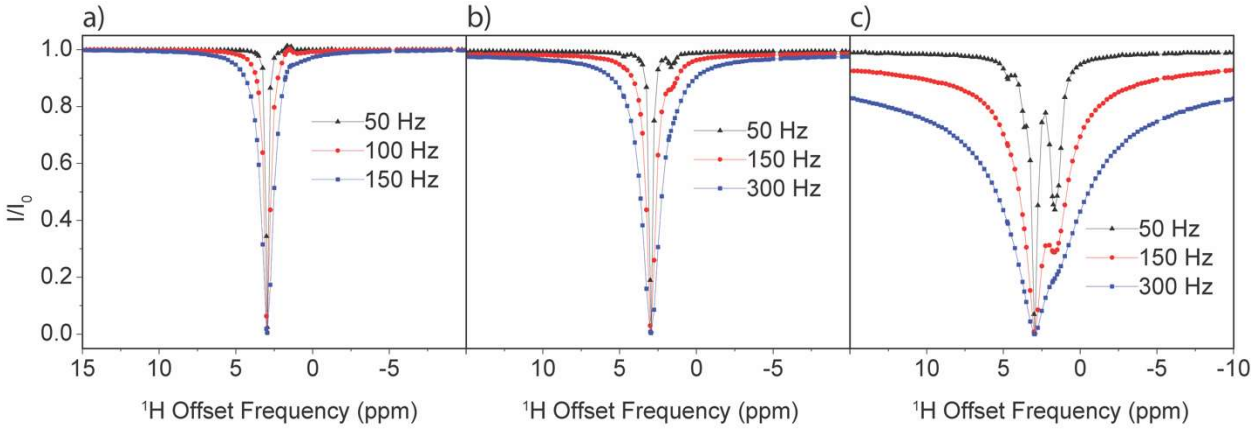


Figure S2. ^{1}H DEST profiles for Hε of 10 mM Lysine in the absence of gel (a), in the presence of gel (b), and in the presence of gel and 1 wt % TiO_2 (c).

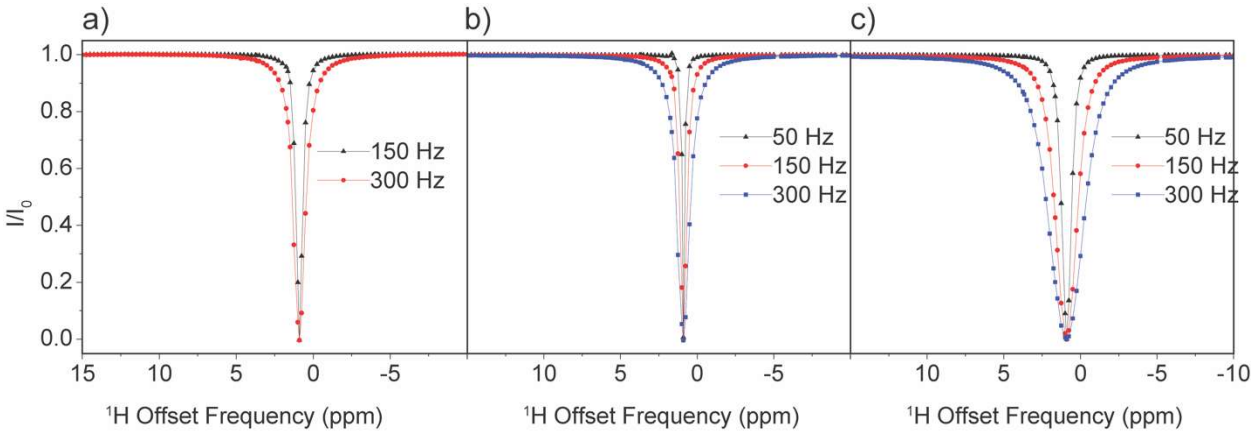


Figure S3. ^{1}H DEST profiles for H_{δ} of 10 mM Leucine in the absence of gel (a), in the presence of gel (b), and in the presence of gel and 1 wt % TiO_2 (c).

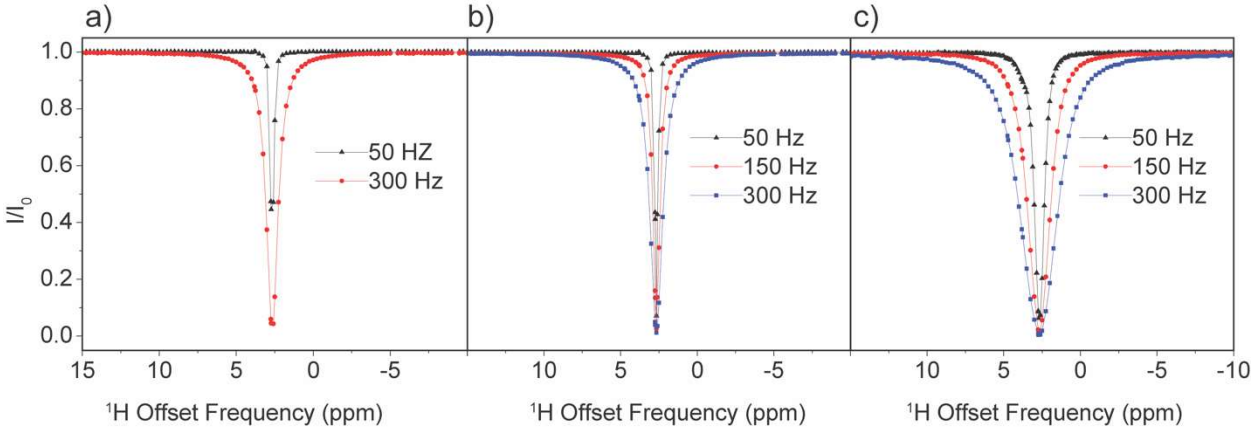


Figure S4. ^{1}H DEST profiles for H_{β} of 10 mM Asp in the absence of gel (a), in the presence of gel (b), and in the presence of gel and 1 wt % TiO_2 (c).

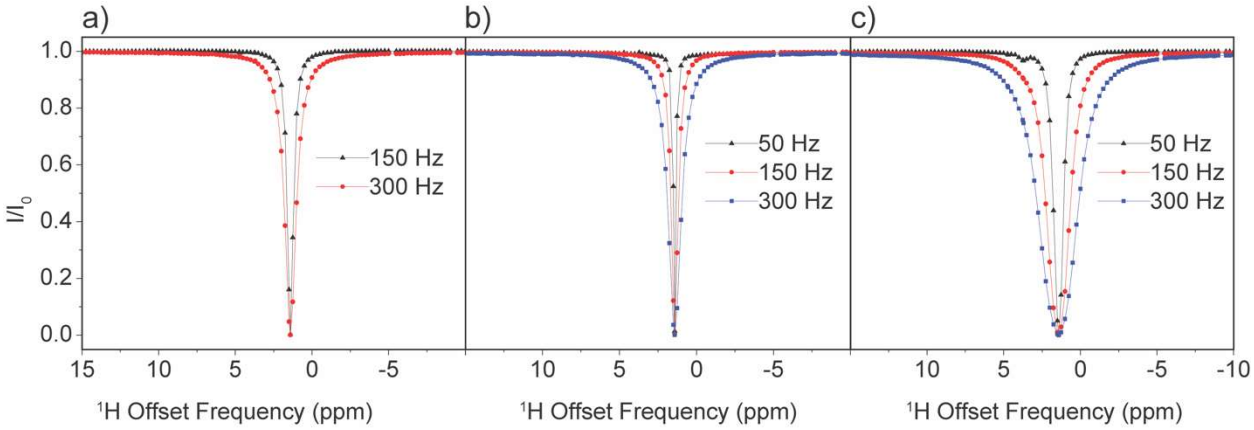


Figure S5. ^{1}H DEST profiles for H_{β} of 10 mM Alanine in the absence of gel (a), in the presence of gel (b), and in the presence of gel and 1 wt % TiO_2 (c).

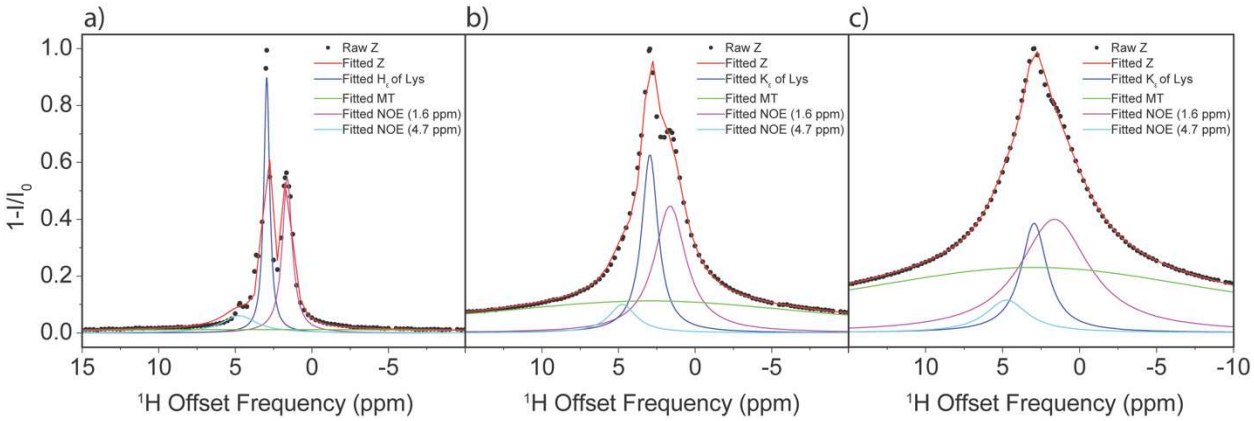


Figure S6. Deconvoluted ^{1}H DEST profiles for H_{ε} of 10 mM Lys at 50 Hz (a), 150 Hz (b), and 300 Hz (c). Data was acquired on 10 mM Lys sample in presence of 1 wt % TiO_2 and 1 wt % agarose on 700 spectrometer. Z-spectrums were fitted as the sum of multiple Lorentzian functions

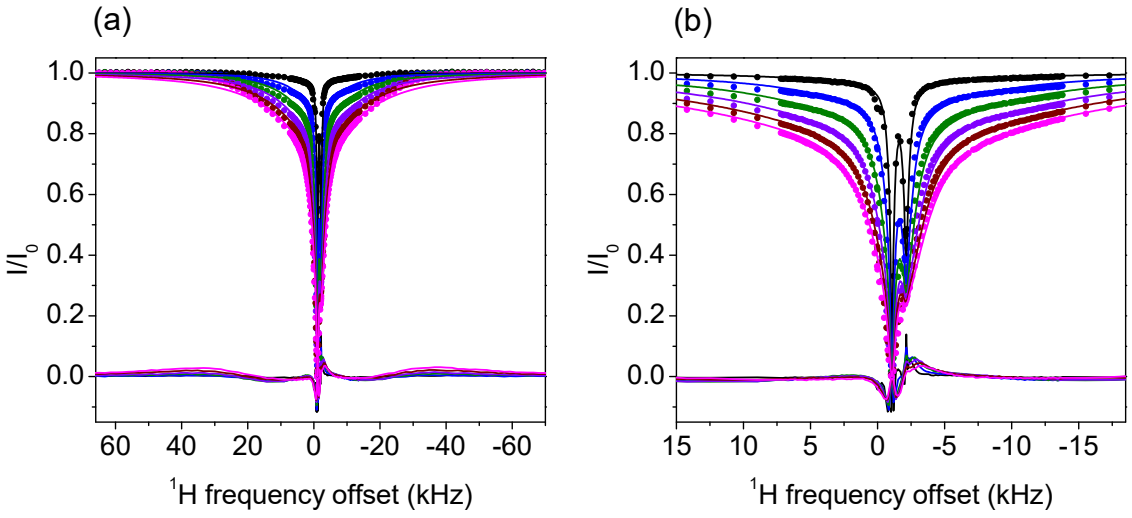


Figure S7. ^{1}H DEST profiles zoomed out (a) and zoomed in (b) for for H_δ of 10 mM Arg in presence of 1 wt % TiO_2 and 1 wt % agarose recorded on a 700 MHz spectrometer with different B_1 saturation fields (50 Hz, 100 Hz, 150 Hz, 200 Hz, 250 Hz, and 300 Hz), and global fitting with a homogenous form of McConnell equations using DESTfit, where a single spin in exchange between an observable free state A with low R_2 and two bound states (B and C) with larger R_2 values (A <==> the mixture of B and C). The cross-relaxation rate σ_A between H_δ (the observed signal) and H_γ (coupled with H_δ by cross relaxation) in free Arginine is assumed to be -0.5 s^{-1}, and cross-relaxation rate σ_B between H_δ and H_γ of Arginine bound on particle is assumed to be -500 s^{-1}, the output of global fitting: R_2 (strong binding) = 38785±119 s^{-1}, R_2 (weak binding) = 784±2s^{-1} with population weights of 0.296 and 0.704, respectively, k_{off} = 36.9±0.1 s^{-1}, k_{on} = 2.9±0.0 s^{-1}, total population of binding state = 0.073, population of free state=0.927, population of strong binding state=0.022, population of weak binding state=0.051.

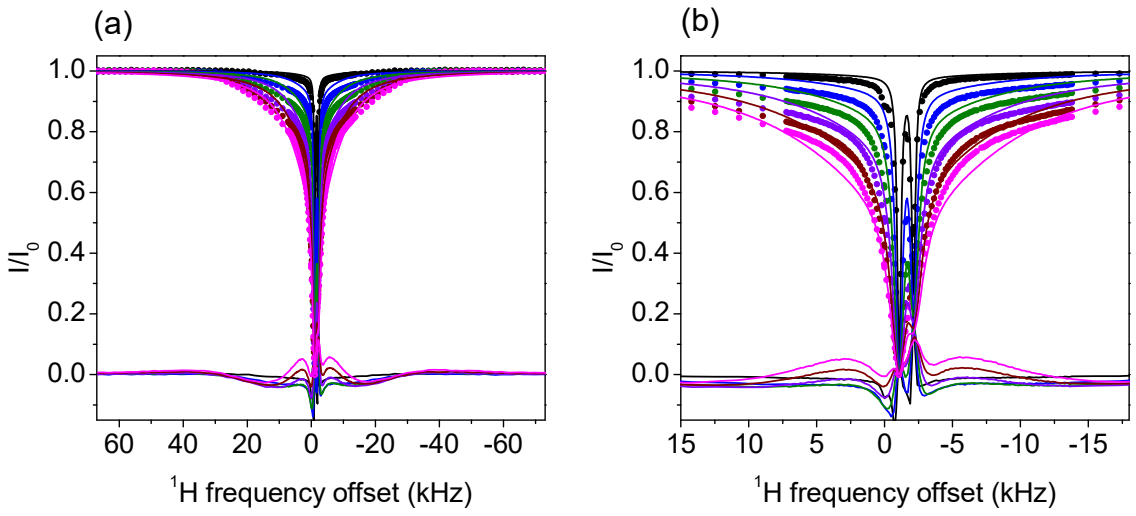


Figure S8. ^{1}H DEST profiles zoomed out (a) and zoomed in (b) for for H_δ of 10 mM Arg in presence of 1 wt % TiO_2 and 1 wt % agarose recorded on a 700 MHz spectrometer with different B_1 saturation fields (50 Hz, 100 Hz, 150 Hz, 200 Hz, 250 Hz, and 300 Hz), and global fitting with a homogenous form of McConnell equations using DESTfit, where a single spin in exchange between an observable free state A with low R_2 and one bound state with larger R_2 value (A <==> B). The cross-relaxation rate σ_A between H_δ (the observed signal) and H_γ (coupled with H_δ by cross relaxation) in free Arginine is assumed to be -0.5 s^{-1}, and cross-relaxation rate σ_B between H_δ and H_γ of Arginine bound on particle is assumed to be -500 s^{-1}, the output of global fitting: R_2 (binding) = 41168±98 s^{-1}, k_{off} = 1817 s^{-1}, k_{on} = 19.3±0.0 s^{-1}, population of binding state = 0.011, population of free state=0.989.

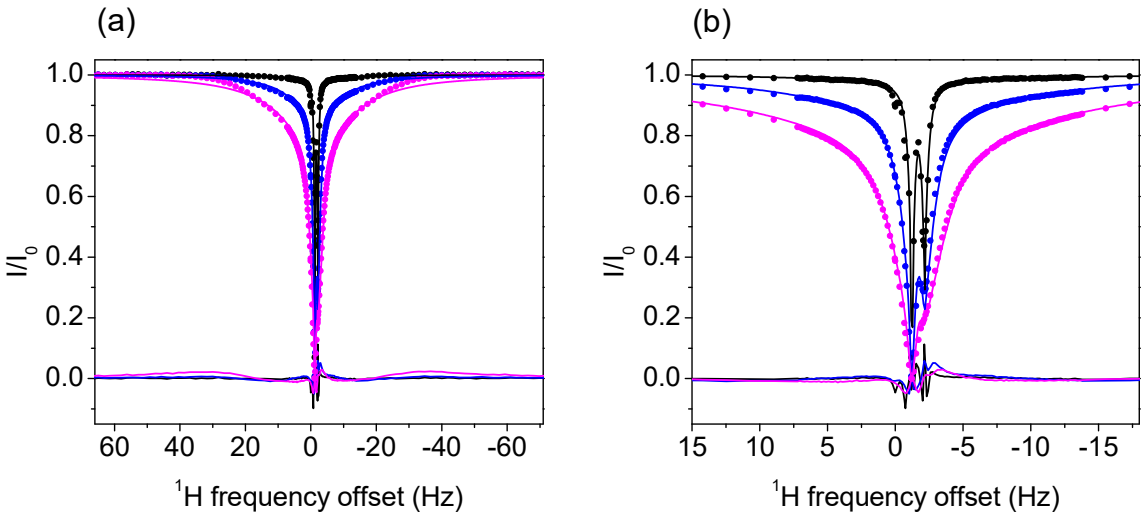


Figure S9. [1] H DEST profiles zoomed out (a) and zoomed in (b) for H_ε of 10 mM Lys in presence of 1 wt % TiO_2 and 1 wt % agarose recorded on a 700 MHz spectrometer with different B_1 saturation fields (50 Hz, 150 Hz, and 300 Hz), and global fitting with a homogenous form of McConnell equations using DESTfit, where a single spin is modeled in a two-site between an observable free state A with low R_2 and two bound states (B and C) with larger R_2 values (A <==> the mixture of B and C). The cross-relaxation rate σ_A between H_ε (the observed signal) and H_δ (coupled with H_ε by cross relaxation) of free Lys is assumed to be -0.5 s^{-1}, and cross-relaxation rate σ_B between H_δ and H_ε of Arginine bound on particle is assumed to be -300 s^{-1}. The output of global fitting: R_2 (strong binding) = 40255±155 s^{-1}, R_2 (weak binding) = 996±3s^{-1} with population weights of 0.255 and 0.745, respectively, k_{off} = 51.5±0.2 s^{-1}, k_{on} = 2.7±0.0 s^{-1}, total population of binding state = 0.049, population of free state=0.951, population of strong binding state=0.013, population of weak binding state=0.037.

Table S1. List of the fitted values of the amplitudes, frequency offsets and linewidths of the four components contributing to Z-spectra shown in Figure 3A. Arg+gel+TiO_2 at B1=50Hz. $\chi^2=1.486\times10^{-4}$.

Center (ppm)	FWHM	Peak Height	Peak Area	Peak Area (%)
1.68	0.65	0.50	0.52	18.42
3.16	0.57	0.89	0.81	28.82
4.73	2.17	0.05	0.18	6.28
3.16	31.63	0.03	1.30	46.48

Table S2. List of the fitted values of the amplitudes, frequency offsets and linewidths of the four components contributing to Z-spectra shown in Figure 3B. Arg+gel+TiO_2 at B1=150Hz. $\chi^2=1.50\times10^{-4}$.

Center (ppm)	FWHM	Peak Height	Peak Area	Peak Area (%)
1.68	2.19	0.39	1.32	13.83
3.16	1.26	0.67	1.33	13.85
4.73	2.03	0.11	0.35	3.64
3.16	31.43	0.15	6.57	68.68

Table S3. List of the fitted values of the amplitudes, frequency offsets and linewidths of the four components contributing to Z-spectra shown in Figure 3C. Arg+gel+TiO_2 at B1=300Hz. $\chi^2=1.13\times10^{-4}$.

Center (ppm)	FWHM	Peak Height	Peak Area	Peak Area (%)
1.68	4.98	0.35	2.68	15.35
3.16	1.80	0.41	1.14	6.55
4.73	3.07	0.15	0.70	4.02
3.16	35.44	0.26	12.93	74.07

Table S4. Summary of kinetic parameters for the binding of 10 mM Arg and Lys with TiO_2 extracted from the McConnell equations model using DESTfit.

	*p*F (free state)	*p*B (total bound state)	*p*B (strong bound)	*p*B (weak bound)	R_2 (strong bound, s^{-1})	R_2 (week bound, s^{-1})	k_{on} (s^{-1})	k_{off} (s^{-1})
Arg+gel +TiO_2	0.927	0.073	0.022	0.051	38785	784	2.9	36.9
Lys+gel +TiO_2	0.951	0.049	0.013	0.037	40255	996	2.7	51.5

Table S5: Simulation setup for the arginine (Arg), lysine (Lys), and aspartate (Asp) on the four titania surfaces, NNH (Neutral Non Hydroxylated), NH (Neutral Hydroxylated), NeNH (Negative Non Hydroxylated), NeH (Negative Hydroxylated). HH – Hill Height (kJ/mol), BF (Bias Factor), CV (Collective Variable), σ (kJ/mol). CV1: amino acid – surface distance.

Amino Acid	# of Replicas	Time Per Replica (ns)	HH	BF	CV1	σ	Pace (/ns)
Arg NeNH	1	700	0.2	10	AA.dz	0.2	1
Arg NeH	1	800	0.2	10	AA.dz	0.2	1
Lys NeNH	1	700	0.2	10	AA.dz	0.2	1
Lys NeH	1	600	0.2	10	AA.dz	0.2	1
Asp NeNH	1	800	0.2	10	AA.dz	0.2	1
Asp NeH	1	600	0.2	10	AA.dz	0.2	1