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Supporting Information

Effects of Immobilization Site on the Orientation and Activity of Surface-Tethered Enzymes

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SFG Characterization of Alkyne Terminated SAM and Maleimide Terminated SAM Surfaces

Background SFG spectra were collected from the SAM surfaces in the absence of immobilized proteins. The supporting substrate used in this study for SAM deposition is a CaF_2 right-angle prism with a layer of 100 nm SiO_2 coating (to facilitate silane chemistry). SFG spectra were collected in the amide I frequency region (1500 cm^{-1}-1800 cm^{-1}) from clean CaF_2 prism with the SiO_2 coating, alkyne terminated SAM on the prism, as well as the alkyne terminated SAM reacted with the maleimide linker. As shown in Figure S1a, no noticeable SFG signals were generated from the clean prism before the SAM deposition in the amide I frequency range. Once the alkyne-silane SAM was immobilized onto the CaF_2 prism, a small peak at ~1710 cm^{-1}, originated from the C=O stretching in the alkyne silane molecule, was observed in the ppp SFG spectrum (Figure 1b).

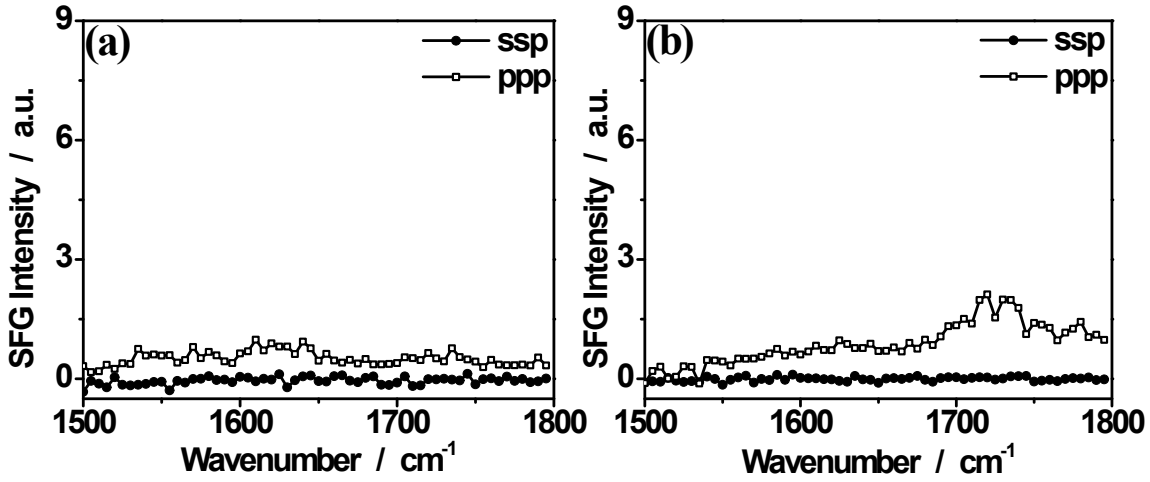

Figure S1. SFG ssp and ppp spectra collected from (a) clean CaF_2 prism with a layer of 100 nm SiO_2 coating and (b) alkyne silane functionalized prism surface in contact with phosphate buffer.

After the characterization of the alkyne-terminated SAM surface, a maleimide terminated surface after coupling the maleimide linker to the alkyne terminated SAM was also examined using SFG. The azido-PEG_3-Maleimide linker (Figure 1 in the main text) was attached to the alkyne derivatized surface by "click" chemistry. The SFG spectra were collected from the maleimide terminated SAM surface in phosphate buffer (Figure S2). As seen in Figure S2, a peak at ~1710 cm^{-1} was observed from the maleimide-terminated SAM surface in both the ssp and ppp spectra. The measured intensity of this peak in the ppp spectrum was higher than that detected from the alkyne-terminated SAM surface (before the linker coupling, Figure S1b). This is likely due to the increase of the number of C=O bonds in the system from the azido-PEG_3-Maleimide linkers. This result was also confirmed by angular-resolved XPS, shown in Figure S3. After fitting the SFG spectra shown in Figure S2 (fitting parameters shown in Table S1), the peak center was measured to be 1711 cm^{-1}. We also studied the mixed SAM surface (1:10 maleimide:hydroxyl group terminated SAMs prepared by coupling the maleimide and hydroxyl linkers with a ratio of 1:10) using SFG (data not shown). The results are similar to that from the pure maleimide terminated SAM surface. Nevertheless, no SFG signal was detected from all the above background surfaces between 1600 and 1700 cm^{-1}, where the protein amide I signal would be detected.

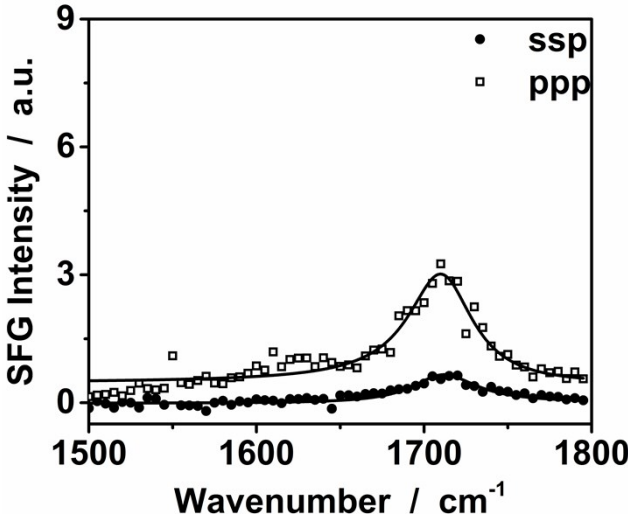

Figure S2. SFG ssp and ppp spectra collected from maleimide functionalized surface in phosphate buffer.

Further Introduction of SFG Orientation Studies

Polarized SFG spectra have been used to determine orientation of molecules and functional groups on surfaces/at interfaces, including peptides and proteins.[1-5] SFG amide I signals collected using different polarization combinations of the input and signal beams such as ssp (s-polarized signal, s-polarized input visible beam, and p-polarized input IR beam) and ppp have been used to study orientations of α-helical, 3-10 helical, and β-sheet structures.[1,2] SFG has also been developed into a powerful tool to study orientations of proteins at interface.[3-5] To study protein orientations at interface, a computer software package was constructed to read the protein crystal structure, find all the α-helical structures, and calculate the overall SFG signal strength (or second order nonlinear susceptibility χ terms such as χ_{zzz} and χ_{yyz} terms) contributed by all the α-helices as a function of protein orientation.[3] For experimentally collected ssp and ppp spectra, after spectral fitting, χ_{ssp} and χ_{ppp} could be obtained. Our experimentally measured χ_{ssp} and χ_{ppp} terms can be compared to the calculated χ_{zzz} and χ_{yyz} terms directly. This is because using the near total reflection experimental geometry, $\chi_{ssp} = F_{yyz}\chi_{yyz}$, and $\chi_{ppp} = F_{zzz}\chi_{zzz}$.[1,3.4]. F_{yyz} and F_{zzz} are Fresnel coefficients and can be calculated using the refractive indices of the interface and the two contacting media, as well as the input and output angles of the laser beams in the SFG experiment. Details about how to calculate F_{yyz} and F_{zzz} have been published extensively.[1,6] In this research, the following parameters were used to calculate the Fresnel coefficients:

Parameters		Values
Refractive indices of CaF_2 prism, n_1	VIS	1.435
	IR	1.385
	SFG	1.437
Refractive indices of buffer, n_2	VIS	1.335
	IR	1.297
	SFG	1.337
Refractive indices of the interface	Average of refractive index of CaF_2 prism and refractive index of buffer at each wavelength	
Input beam angles (outside of prism)	VIS	57
	IR	55
Output beam angles (reflected from the interface inside prism)	SFG	67.5

The calculated F_{yyz} and F_{zzz} are 2.99 and 2.76 respectively.

In this research, we used MD simulations to calculate the orientations of the proteins at interfaces (we will discuss more details about the "orientation" below), then use such orientations to calculate the average χ_{zzz} and χ_{yyz} (in arbitrary unit) as well as the ratio χ_{zzz}/χ_{yyz} (As discussed, the detailed method for calculating χ terms can be found in ref.3). From the experiments, we collected SFG ssp and ppp spectra from the enzymes at interfaces. We then fitted the spectra and calculated the Fresnel coefficients and deduced χ_{zzz}, χ_{yyz} (in arbitrary unit) as well as the ratio χ_{zzz}/χ_{yyz}.

It is worth noting that to determine the orientation of a protein, it is necessary to specify two angles of the protein, a tilt angle and a twist angle of the protein relative to a reference orientation, or (0, 0) orientation.[3] For this study, the (0, 0) orientation is plotted in Figure 5c in the main text. The definitions of the tilt and twist angles vs. the (0,0) orientation were presented in detail before.[3]

XPS Studies

With angular-resolved XPS, more accurate results could be collected from thin SAM surfaces, minimizing the signal contribution from silicon wafer substrates. However, the ratio calculation of different chemical bonds based on the XPS intensity is still not reliable due to the small thickness of the SAM.

XPS results were shown below in Figure S3. Figure S3a was collected from silicon wafer used for SAM deposition. The signals collected were contributed from the air contaminants on wafer surfaces. After the surface was functionalized using alkyne terminated SAM, XPS result was shown in Figure S3b. Signals of C-C/C-H, C-O, and C=O increased and such bonds could all be found in the alkyne silane molecular structure (shown in Figure 1a in the main text). Then the surface was functionalized either with pure maleimide terminated SAM, or a mixture of maleimide and hydroxyl terminated SAMs. XPS were collected from such surfaces, as shown in Figure S3c and d. In both cases, a large increase of the C-O signal was observed. As seen in Figure 1b and 1c in the main text, large amounts of C-O bonds were present in both azido-PEG_3-maleimide and azido-PEG_3-alcohol linkers. XPS results demonstrated the successful functionalization of the substrates with SAMs.

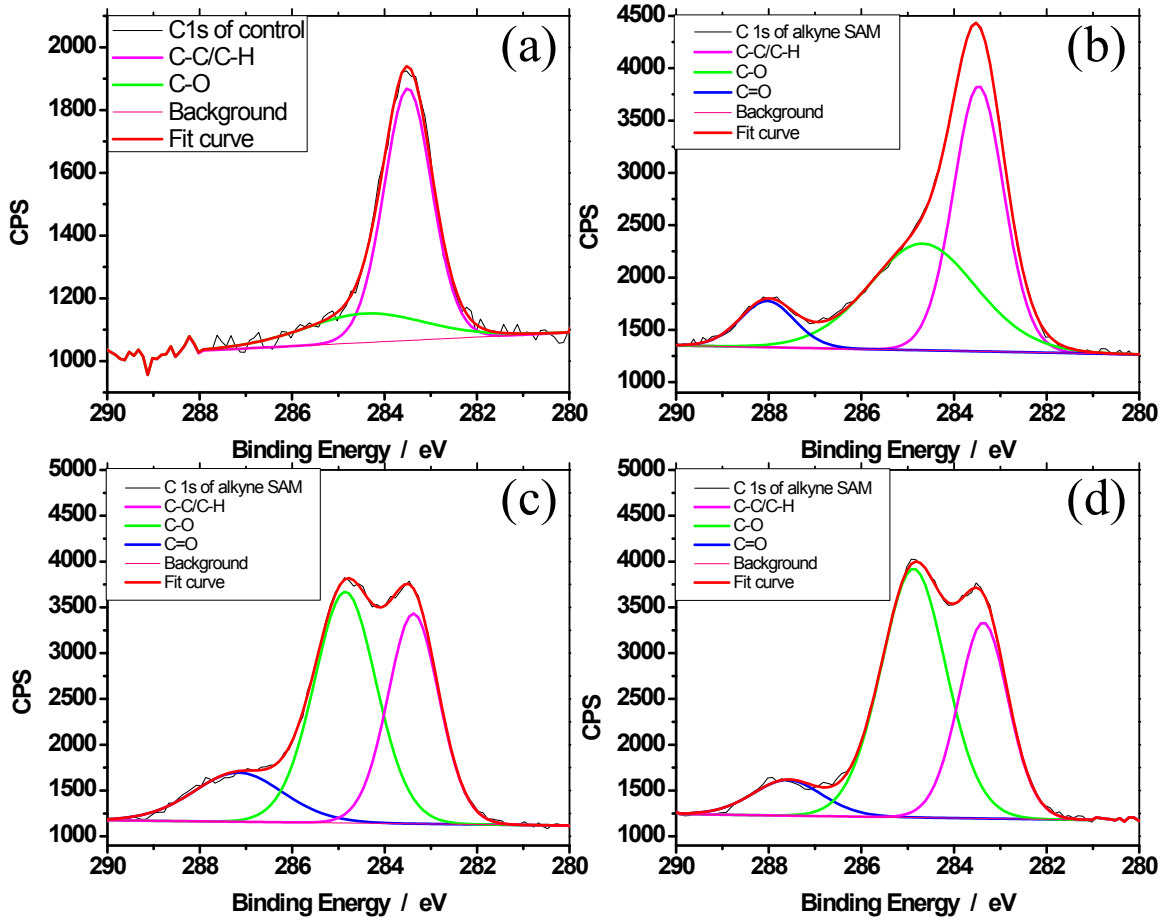

Figure S3. Angular-resolved XPS spectra collected from (a) silicon wafer with 100 nm silica (control); (b) alkyne terminated SAM; (c) maleimide terminated SAM; (d) 1:10 (maleimide: hydroxyl group) mixed SAM.

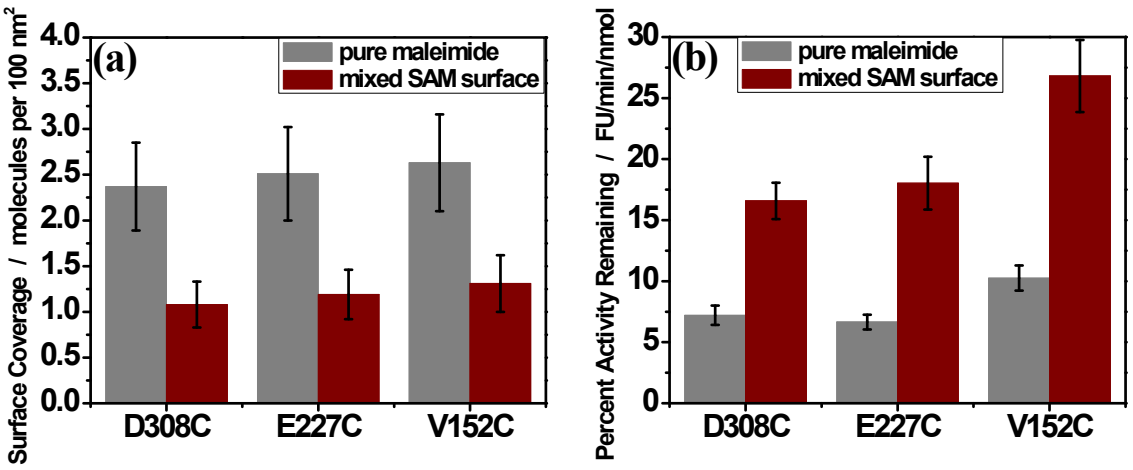

Figure S4. (a) Surface coverage and (b) activity of surface immobilized β-gal-D308C, E227C, and V152C. Both surface coverage and enzymatic activity figures summarized results obtained from all three mutants, D308C, E227C, and V152C. Both D308C and E227C were discussed in this paper. Interestingly, a similar effect was also observed on mutant V152C, which was studied before.[5] Surface coverage of V152C is lower after immobilization on a mixed SAM surface compared to that on a maleimide SAM surface. Moreover, enzymatic activity was found to increase when the enzyme was immobilized on mixed SAM surfaces for all three cases.

Table S1. Fitting parameters for SFG spectrum shown in Figure S2.

	χ_{NR}	A	ω (cm^{-1})	Γ (cm^{-1})
ppp	0.45	37.6 (0.8)	1711	23.5
ssp	-0.01	19.2 (0.8)	1711	23.5

Table S2. Fitting parameters for SFG spectrum shown in Figure 2b.

	χ_{NR}	A	ω (cm^{-1})	Γ (cm^{-1})
ppp	-0.57	58.4 (1.5)	1648	25
		21.8 (2.9)	1711	23.5
ssp	-0.25	33.4 (0.6)	1650	25
		20.3 (1.9)	1711	23.5

Table S3. Fitting parameters for SFG spectrum shown in Figure 3b.

	χ_{NR}	A	ω (cm^{-1})	Γ (cm^{-1})
ppp	-0.68	26.9 (0.8)	1648	25
		32.3 (1.7)	1711	23.5
ssp	-0.39	15.7 (1.9)	1648	25
		16.0 (1.7)	1711	23.5

Simulation Details

The coarse-grained simulation model was used to measure the protein structure. A coarse grained potential for the protein-surface interaction was recently developed based on the KB Go-like protein model:

$$V_{surface} = \sum_{i}^{N}\left\{\pi\rho\sigma_i^3\epsilon_i\left[\theta_1\left(\frac{\sigma_i}{z_{is}}\right)^9 - \theta_2\left(\frac{\sigma_i}{z_{is}}\right)^7 + \theta_3\left(\frac{\sigma_i}{z_{is}}\right)^3 - (\theta_s(\chi_s - 4.5) + \theta_p\chi_p)\left(\frac{\sigma_i}{z_{is}}\right)^3\right]\right\} \quad (1)$$

where N is the number of residues in the peptide/protein, z_{is} is the distance between residue i and the surface, σ_i and ϵ_i are residue specific van der Waals parameters. The parameters (shown in Table S4) used in this work were determined in a previous study.[7]

Table S4: Parameters for the surface model

θ_1	θ_2	θ_3	θ_s	θ_p
0.2340	0.4936	0.1333	0.0067	0.0333

The last two third power terms were parameterized to specifically account for hydrophobic effects of different SAM surfaces and different residues in a protein or peptide by using the hydrophobic index of the surface χ_s and amino acid χ_p. This potential model was used in the current study.

For different surfaces, we used different values of χ_s (Maleimide: 1.5) to account for their hydrophobic properties. For the mixed SAM surface used in this study, the total effect of the two types of surface functional groups was described by the sum of the two surface energy functions weighted by the corresponding surface concentration ρ (0.91 for the hydroxyl and 0.09 for the

maleimide group) and using two specific $\chi_s$values (−1.0 for the hydroxyl and 1.5 for the maleimide group).

The tethering bond from the terminal cysteine residue to the maleimide group is modeled by a harmonic restraint with an interaction potential:

$$U_{restraint} = \frac{1}{2} k_r (r - r_{eq})^2 \qquad (2)$$

where $k_r = 10\ kcal/mol$ is the parameter describing the strength of the restraint and r is the distance of the restrained site from its original position of $(0, 0, 0)$ Å. The equilibrium length is $r_{eq} = 5.8$ Å for the maleimide and mixed maleimide/OH SAM surfaces, which approximately represents the distance between the surface and C_α of the cysteine residue at the tethering site.

The β-gal protein structure was obtained from the protein data bank (www.pdb.org) with pdb ID 2PGB. The all-atom structure was then submitted to the Go model builder on the MMTSB website (www.mmtsb.org) to generate an input file for the coarse grained simulation. For each simulation, three independent MD simulations were performed within the canonical ensemble (NVT). Each simulation was performed with 10 million steps of equilibrium and 30 million steps of production with a time step of 5 fs.

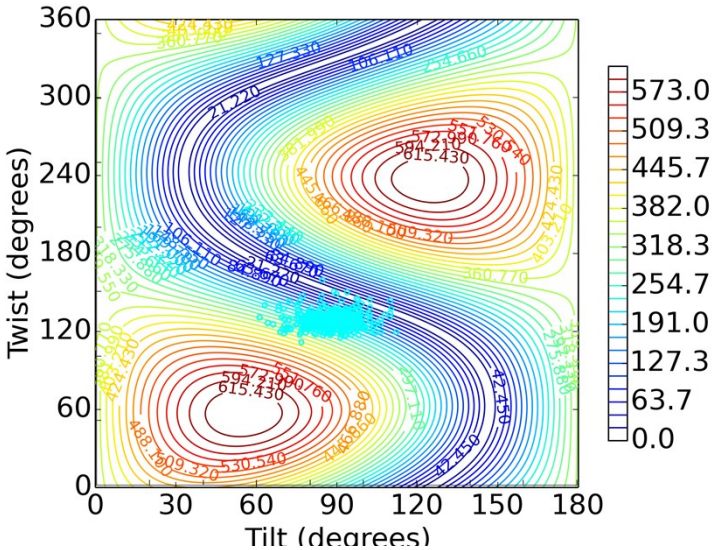

Figure S5 The calculated orientation dependent map of SFG χ_{zzz} of β-gal. The orientational angle regions of β-gal-E227C on mixed SAM surface from our MD simulations are denoted by the blue dots in the map.

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