Supporting Information for

Surface Hydration for Antifouling and Bio-adhesion

Chelsey A. Del Grosso, Chuan Leng, Kexin Zhang, Hsiang-Chieh Hung, Shaoyi Jiang, Zhan Chen, and Jonathan J. Wilker

Methods

Methods

Statistical Analysis of Adhesion Data

Force, area, and adhesion measurements were all averaged per testing round. Data from each round was combined to give an overall mean force, area, and adhesion measurement for each surface. A nested ANOVA was performed to examine any differences in mean values of mussel adhesion between rounds (round 1, 2 and 3) and animals within each round. Dunnett's test was performed as a post hoc comparison of the mean adhesion values on coatings to aluminum controls. A confidence level of 99% ($P < 0.01$) was used for rejecting the null hypothesis (i.e., adhesion strength is not different among animals or testing rounds). Data were analyzed with SAS 9.4.

Statistical results from the nested ANOVA are provided in Supplementary Table S4. Results showed statistical significant differences ($P < 0.01$) between animals on all surfaces with the exception of fused silica and OEGMA. When comparing rounds among the same coatings there were no significant differences found. Although there is variation among animals, there was not a significant difference among rounds of adhesion testing, therefore averaging adhesion measurements of all animals on a given coating was statistically acceptable. All surfaces, with the exception of fused silica, were statistically different from aluminum controls (Dunnett's: $P < 0.01$).

Animal Health Test

To examine the toxicity of these surfaces, mussel health was assessed. Animals were in contact with test plates in 10 gallons of water with aeration for 3 days. Often, healthy mussels exhibit valve gape when in water and will close their valves when tapped. All animals on surfaces passed this tap test. In addition, the condition index, which relates the mass of animal tissue to the

shell weight, was measured for each animal (Table S2). Relative to aluminum controls, if animals are ill, wasting of tissue will occur thus showing a decrease in condition indices. Animals were removed from test plates and aquaria and stored in polyethylene bags at -80 °C for 1-3 days prior to analysis. For each surface, a total of 10 animals were tested and 60 animals were examined on aluminum controls. Frozen samples were boiled in 1 L of water for ~1.5 minutes. The soft tissue was removed from the shell and both were dried at 60 °C to a constant weight. All samples of dried tissue and shell were massed to the nearest 0.001 gram and the condition index (CI)[1] calculated using the following equation:[2]

$$\mathrm{CI} = \left(\frac{\text{dry meat weight}}{\text{dry shell weight}} \right) \mathrm{X}\ 100$$

SFG Data Analysis

The SFG spectra collected from buried mussel adhesive plaque interfaces were fitted using the following equations:

$$I(\omega) \propto \left| \chi^{(2)}_{eff,ssp} \right|^2 I_1(\omega_1) I_2(\omega_2)$$

$$\chi^{(2)}_{eff,ssp} = \chi_{NR} + \sum_q \frac{A_q}{\omega_2 - \omega_q + i\Gamma_q}$$

where $I(\omega)$ is the observed SFG signal intensity, $I_1(\omega_1)$ and $I_2(\omega_2)$ are intensities of the input IR and visible beams, respectively. The χ_{NR} is nonresonant background, A_q is signal strength, ω_2 is IR wavenumber, ω_q is the peak center of the vibrational mode q, and Γ_q is the damping factor (or width). Comparison of signal strengths from various interfaces was performed using A_q/Γ_q.

Fitted SFG spectra collected from the buried interfaces between mussel adhesive plaques and various surfaces are displayed in Figure S2. The fitting parameters are shown in Tables S5 and S6. As discussed in the main text, SFG spectra collected from the interfaces between mussel adhesive plaques and fused silica, PMMA or polystyrene could be fitted well with a single N-H stretching peak (along with a C-H stretching peak for polystyrene), without the need to include O-H signals. Along with the D_2O exposure experiments, the results indicate that dehydration occurred at these buried interfaces. To confirm the results obtained from the PMMA/mussel adhesive interface and the polystyrene/mussel adhesive interface, experiments were repeated using deuterated PMMA and deuterated polystyrene. It was found that the results using deuterated polymers matched well with those obtained from the experiments with hydrogenated polymers (Figure S2).

It is interesting to see that at the PMMA/water interface (Figure 3d in the main text), the PMMA ester methyl group SFG signal was observed, showing that the methyls stand up at the interface. While at the PMMA/mussel adhesive interface, no signal from the PMMA ester methyl signal was observed, indicating that methyls lay down when the glue was present. Figure S3 shows a schematic of the structural changes of PMMA at various interfaces. Differently, no C-H signals were collected from the polystyrene/water interface, although an aromatic C-H stretching signal was observed from the polystyrene/mussel adhesive plaque interface. At the deuterated polystyrene/mussel adhesive interface, no C-H signal was detected. The above observations indicated that the aromatic C-H stretching signal observed from the polystyrene/mussel adhesive interface came from the polystyrene phenyl C-H groups, not the mussel adhesive proteins. In water, the polystyrene surface phenyl groups were lying down on the surface. At the

polystyrene/mussel adhesive interface, such phenyl groups could stand up or tilt (Figure S3). It was difficult to fit signals from the PDMS/mussel adhesive plaque interface owing to such weak intensity observed. Most likely, this interface was disordered.

The SFG spectra collected from the interfaces between mussel adhesive plaques and SBMA or PEG (Figure S2) were fit with two O-H stretching peaks centered at 3200 cm^{-1} and 3450 cm^{-1}. We could obtain good fitting results without considering the N-H stretching signals. The fitting results are shown in Table S6. We also attempted to fit such spectra with two O-H stretching peaks along with an N-H stretching signal. The fitting results show that the N-H signal is very weak with quite large error bars (Table S6). Consequently, spectra collected from the interfaces between mussel adhesive plaques and SBMA or PEG appear to be dominated by contributions from O-H stretching signals from interfacial water molecules.

References

(1) Baird, R. H. "Measurement of Condition in Mussels and Oysters" *J. Conseil. Perm. Explor. Mer.* **1957**, *23*, 249-257.

(2) Davenport, J.; Chen, X. "A Comparison of Methods for the Assessment of Condition in the Mussel (*Mytilus edulis* L.)" *J. Molluscan Stud.* **1987**, *53*, 293-297.

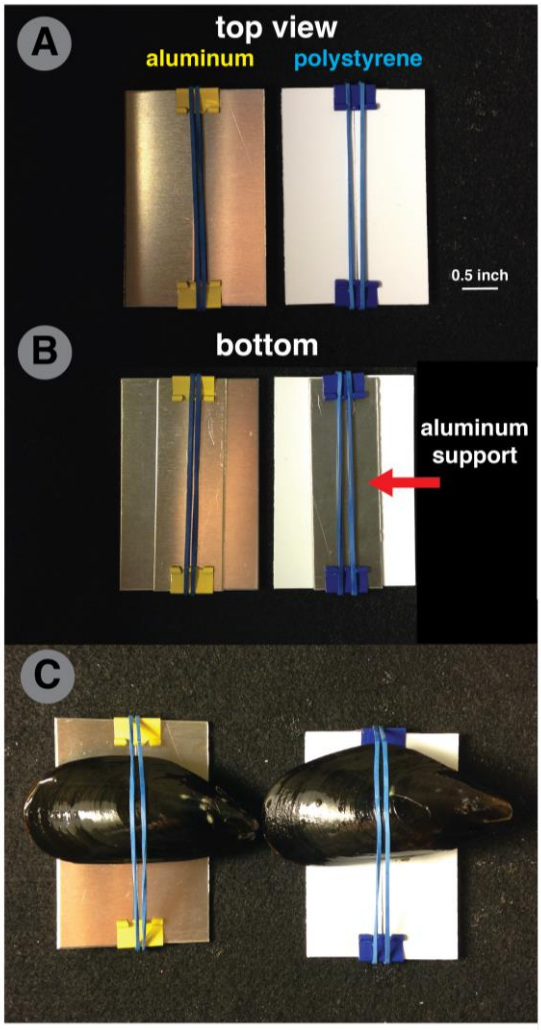

Figure S1. Photographs of animals on aluminum and polystyrene surfaces. The top (A) and bottom (B) views of substrates fixed with binder clips. (C) Two mussels secured with rubber bands to an aluminum control and a polystyrene coating.

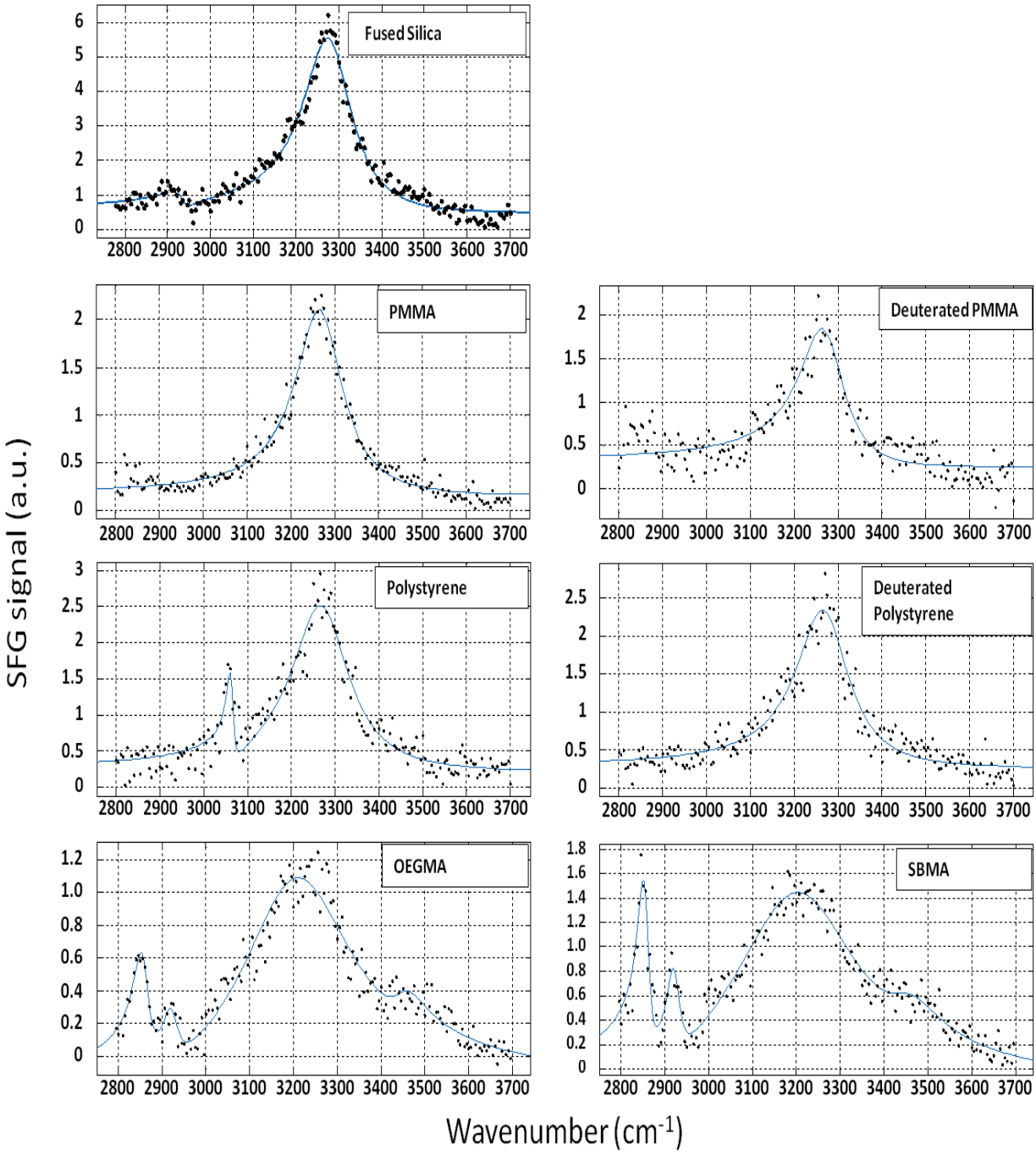

Figure S2. Fitting results of SFG spectra collected between mussel adhesive plaques and several surfaces. Black dots are from the raw data and the blue lines are fits. Summaries of fitting parameters are provided in Tables S5 and S6.

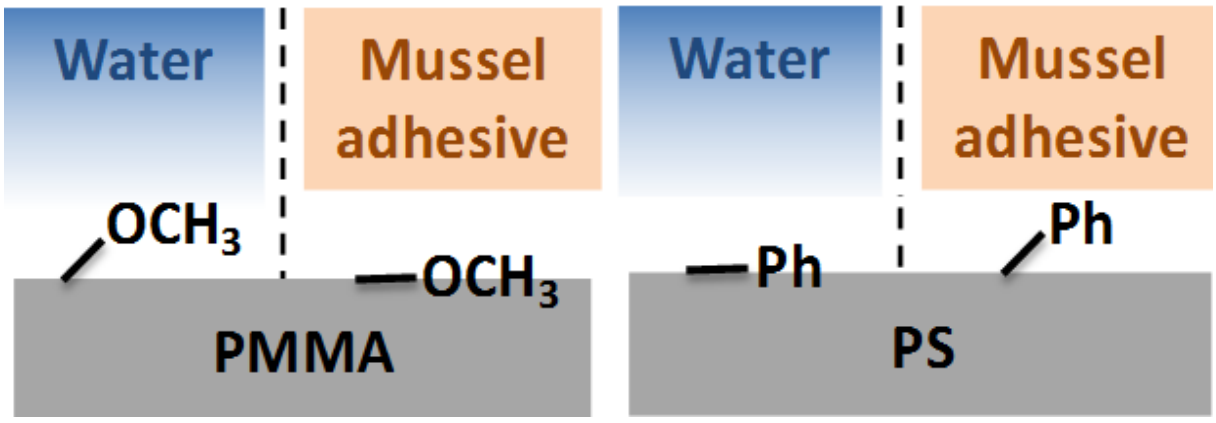

Figure S3. Schematics showing different interfacial structures of PMMA (left) and polystyrene (right)

Table S1. Adhesive production and analysis for mussels on coatings.

	number of animals	total plaques deposited	plaques/ animal	averaged plaque area x 10^{-7} (m^2)	average plaque removal force (N)	average adhesion (kPa)
aluminum (control)	75	225	3.0	44 ± 2	0.57 ± 0.04	133 ± 9
fused silica	10	60	6.0	45 ± 4	0.49 ± 0.06	115 ± 17
SBMA	15	26	1.7	52 ± 6	0.50 ± 0.11	99 ± 23
OEGMA	15	31	2.1	56 ± 8	0.47 ± 0.08	90 ± 21
PMMA	15	26	1.7	60 ± 7	0.31 ± 0.07	52 ± 11
PS	10	37	3.7	55 ± 5	0.40 ± 0.06	75 ± 14

Average adhesion was calculated by dividing the removal force by the plaque area ($Pa = N/m^2$). Errors provided are 99% confidence intervals.

Table S2. Health studies for mussels exposed to surfaces for 3 days in seawater.

coating	condition index
aluminum (control)	17 ± 5
fused silica	20 ± 6
SBMA	16 ± 6
OEGMA	16 ± 3
PMMA	20 ± 4
PS	19 ± 4

The provided condition indices in (grams of dry meat weight/grams of dry shell weight) x 100 are averaged from ten animals on each coating and sixty on aluminum controls**.** Errors provided are one standard deviation.

Table S3. Failure mode analysis of adhesives produced by mussels on surfaces.

	number of animals	total plaques deposited	adhesive failure*	cohesive failure†	thread break‡	thread-plaque break§
aluminum (control)	75	209	49%	35%	6%	10%
fused silica	10	54	33%	28%	13%	26%
SBMA	15	25	76%	12%	0%	12%
OEGMA	15	26	31%	19%	8%	42%
PMMA	15	24	84%	4%	8%	4%
PS	10	34	100%	0%	0%	0%

Average adhesion was calculated by dividing the removal force by the plaque area ($Pa = N/m^2$)

* Adhesive failure was when a plaque pulled off the coating intact.
† Cohesive failure was when a plaque tore leaving behind some plaque on both the coating and thread.
‡ Thread break was when the thread broke leaving a plaque intact on the coating.
§ Thread-plaque break was when the thread detached at the plaque interface leaving behind a complete plaque on the coating

Table S4. Statistical analysis of mussel adhesion (Nested ANOVA) on surfaces.

surface	source	P value
aluminum (control)	all 15 rounds	0.3544
	all animals	0.0008**
fused silica	round 1 vs. 2	0.1450
	all animals	0.2592
SBMA	round 1 vs. 2 vs. 3	0.9587
	all animals	0.0017**
OEGMA	round 1 vs. 2 vs. 3	0.8163
	all animals	0.0296
PMMA	round 1 vs. 2 vs. 3	0.4620
	all animals	0.0005**
PS	round 1 vs. 2	0.5125
	all animals	0.0072**

Mussel adhesion on coatings with animals nested within rounds. Statistically significant differences ($P < 0.01$) are designated by asterisks (**).

Table S5. Fitting parameters (in the O-H/N-H stretching frequency region) for the SFG spectra collected from interfaces between mussel adhesive plaques and fused silica, PMMA, deuterated PMMA, PS, or deuterated PS.

	fused silica	PMMA	d-PMMA	PS	d-PS
A/w	2.2 ± 0.2	1.4 ± 0.1	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.2
width	70 ± 4	66 ± 4	64 ± 8	80 ± 8	74 ± 7
center	3280 ± 4	3268 ± 4	3273 ± 7	3270 ± 7	3270 ± 6

Table S6. Fitting parameters (in the O-H/N-H stretching frequency region) for SFG spectra collected from mussel adhesive plaques and OEGMA or SBMA. Results with and without N-H signal are both shown.

	OEGMA	SBMA	OEGMA	SBMA
peak 1: A/w	1.1 ± 0.1	1.2 ± 0.2	0.9 ± 0.4	1.2 ± 0.2
width	159 ± 14	180 ± 15	153 ± 28	182 ± 19
center	3200	3200	3200	3200
peak 2: A/w	0.15 (large error)	0.18 (large error)	0.29 (large error)	0.22 (large error)
width	46 ± 34	68 ± 50	99 ± 77	81 ± 60
center	3450	3450	3450	3450
Peak 3: A/w	N/A	N/A	0.23 (large error)	0.07 (large error)
width	N/A	N/A	54 ± 36	36 ± 74
center	N/A	N/A	3262 ± 16	3250 ± 29

Video S1. Animal behavior on different surfaces. Two tanks of mussels in a refrigerator. On the left are mussels on SBMA zwitterionic substrates. The right tank holds mussels on aluminum. Note how some of the animals on the zwitterionic coating managed to escape the rubber bands and surfaces. For aluminum, all mussels remained attached, although one fell off the plastic pipe stand that was used to prevent adhesion to the aquarium bottom. This animal did remain bound to the aluminum substrate. This video was taken over 50 hours and sped up 200,000 times for easier viewing. The red light was used to provide some darkness at night, but to also allow the filming.