Electronic Supplementary Information

Room Temperature Freezing and Orientation Control of Surface Immobilized Peptide in Air

Yaoxin Li[1], Xiaoxian Zhang[1], John Myers[1], Nicholas L. Abbott[2] and Zhan Chen[1,]*

[1]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States

[2]Department of Chemical and Biological Engineering, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA

*Corresponding author E-mail: zhanc@umich.edu

Experimental Section

Materials

All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification unless otherwise stated. Azide terminated MSI-78, with the amino acid sequence: KGIGKFLKKAKKFGKAFVKILKK-NH_2 was synthesized using solid phase Fmoc method with one azido-lysine at N-terminus. Alkyne terminated silane was purchased from Creative PEG Works.

Surface Functionalization

All SFG spectra were collected from CaF_2 prisms. Right-angle CaF_2 prisms were purchased from Altos Photonics, Inc. (Bozeman, MT). The CaF_2 prisms were soaked in toluene for 24 h and then sonicated in 1% Contrex AP solution from Decon Laboratories (King of Prussia, PA, USA) for 10 min. After that, the prisms were thoroughly rinsed with ethanol, deionized water (18.2 MΩ cm) and dried under N_2 and then treated with O_2 plasma in a plasma cleaner (Glen 1000P) for 30 s immediately before being coated with SiO_2. A layer of 100 nm of SiO_2 was deposited onto the cleaned CaF_2 prism by an electron-beam deposition process using an SJ-26 evaporator system at a pressure below 10^{-5} Torr with a deposition rate of 5 Å/s. The SiO_2 coated CaF_2 prisms were cleaned under O_2 plasma (PE-25-JW) for 3 min and then were immediately placed into the freshly prepared 1.0 mM alkyne-EG4-silane (Figure S1a) in anhydrous toluene for 24 h at room temperature. The functionalized prisms were then rinsed with copious toluene and methanol, and were then dried under nitrogen.

The alkyne functionalized prisms were placed into a phosphate buffer solution (pH 8.0, ionic strength 5.0 mM) containing Nterminus azido MSI-78 (nMSI-78) (9.5 μM), sodium ascorbate (0.2 M), and copper sulfate (0.5 mM), and reacted overnight. The prisms were first rinsed with phosphate buffer containing EDTA to remove any residue copper ion. Then they were rinsed

with phosphate buffer and 1.0 mM sodium dodecyl sulfate (SDS) to wash away physically adsorbed peptides, followed by several additional buffer washes.

CD spectra were collected from quartz slides. Same surface preparation methods were used as mentioned above.

Sugar Surface Preparation

All sucrose solutions were prepared in water.

Spin Coating: several drops of sucrose solution were placed on top of the surface immobilized peptides and spin coated at 2000 rpm for 40 s. Then the surface was dried under a nitrogen stream for several minutes to remove the residual water left from the spin coating process.

Solvent Casting Fast Drying: several drops of sucrose solution were added to the surface with immobilized peptides and after 30 s, the surface was blown under a nitrogen stream for fast drying.

Solvent Casting Slow Drying: several drops of the sucrose solution were placed on the surface with immobilized nMSI-78. After 30s, the solution in contact with the surface was removed and the surface dried slowly in air (without blowing using N_2 stream).

SFG Measurement

SFG is a process with two input beams at frequencies ω_1 and ω_2 mixing on a surface and generating a third beam at the sum frequency $\omega = \omega_1 + \omega_2$. It probes the second order nonlinear optical susceptibility $\chi^{(2)}$ of a material. The selection rule of SFG makes it surface/interface sensitive when the centrosymmetric symmetry is broken. So it can be used to study the structure of molecules on surface/interface. SFG vibrational spectroscopy is a second order nonlinear optical spectroscopic technique which can provide vibrational spectra of surfaces and interfaces.

The SFG setup used in this study was purchased from EKSPLA. In this experimental setup, two laser beams (one 532 nm visible laser beam and one frequency tunable IR beam) pass through

one surface of a right angle CaF_2 prism and then overlap spatially and temporally at the other surface (shown in Figure S1b). The incident angles of VIS (ω_1)and IR (ω_2) beams are 57° and 55° relative to the surface normal before going through the prism. SFG spectra with different polarization combinations of the input and generated signal beams including ssp (s-polarized output SFG signal, s-polarized input visible beam and p-polarized input IR beam) and ppp were collected.

The vibrational spectra of peptides/proteins have been observed and widely studied previously using IR absorption and Raman scattering. [1-6] Extensive studies have focused on the amide I spectral region, which encodes the secondary structure of a peptide/protein. Amide I signals generated from different secondary structures have varied peak centers. For α-helical structure, it has been widely shown that its amide I signal is around 1650 cm^{-1}.

CD Measurement

CD measurements were performed on a J-815 CD spectrometer (Jasco Inc., Japan) using a continuous scanning mode at room temperature. Scans were made from 190 nm to 240 nm at a 0.5 nm resolution, 20 nm min^{-1} scan rate and averaged by five successive scans for each sample.

Ellipsometry Measurement

Sucrose with different concentrations were spin coated on silicon wafer (100 nm silica coating) with peptide immobilized. Sugar film thickness was measured with a multi-wavelength imaging null-ellipsometry (EP3 Nanofilm, Germany). The measured delta and psi values were used to determine the film thicknesses.

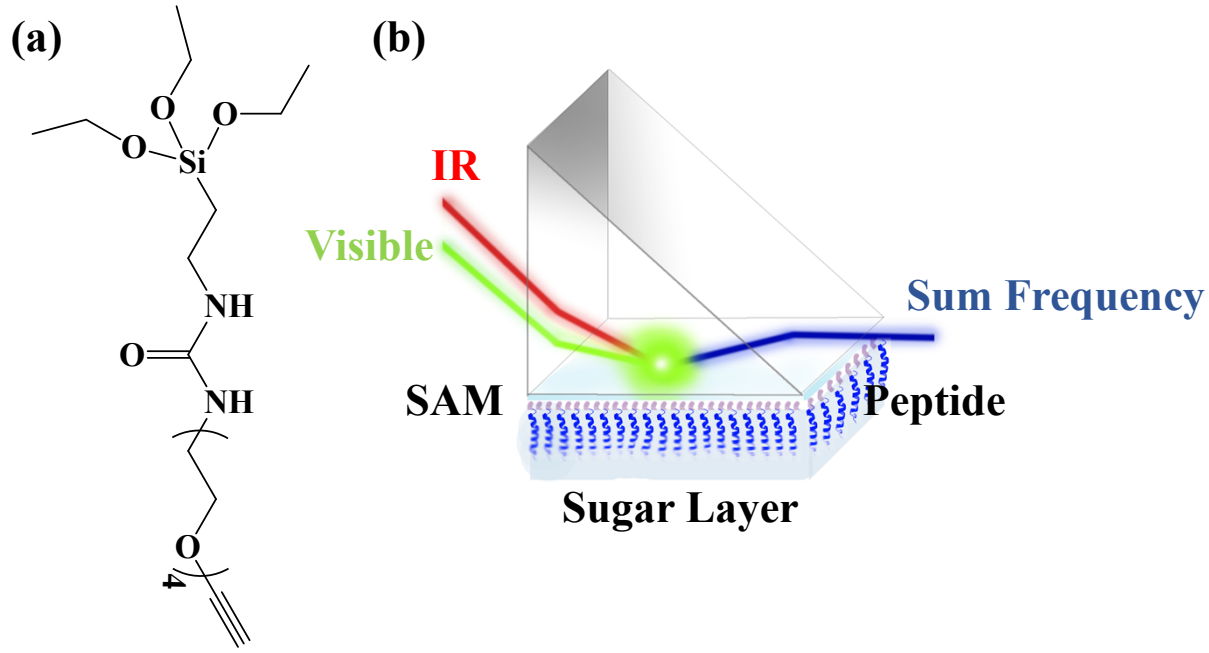

Figure S1. a) Molecular formula of the alkyne terminated silane used to prepare the self-assembled monolayer for peptide immobilization; b) SFG experimental geometry (near-total-reflection geometry) to study immobilized peptide on a right angle CaF_2 prism under a sugar layer. Different components in the figure were not drawn to scale.

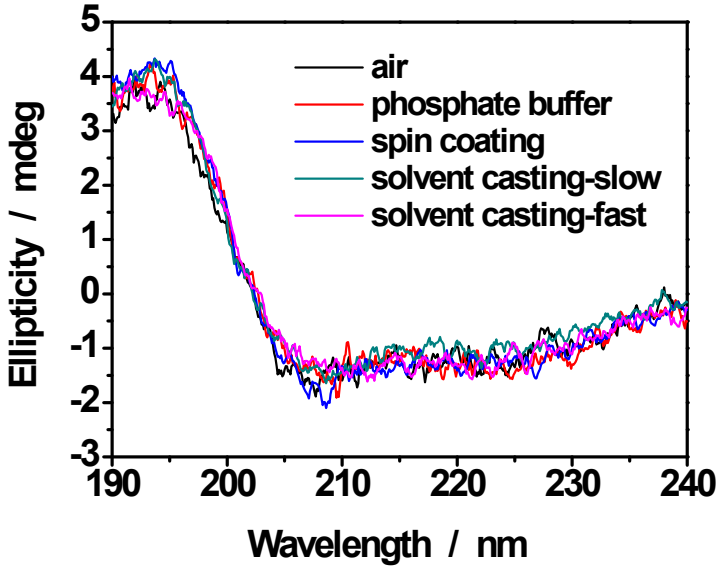

Figure S2. CD spectra of surface immobilized nMSI-78 in air, in phosphate buffer, with sucrose layer on top prepared using spin coating, solvent casting with the slow drying method, and solvent casting with the fast drying method.

Table S1. Refractive indices

	PB	Sugar Layer
VIS (532 nm)	1.335	1.501
IR (1064 um)	1.297	1.494
SFG (489 nm)	1.337	1.503

Refractive indices of sucrose layer were measured using ellipsometry.

Table S2. Fitted amide I χ_{ppp}/χ_{ssp} ratio values of the immobilized peptide in air with a sugar coating prepared with sugar solutions with different concentrations and when exposed to sugar solutions with different sugar concentrations.

Sucrose Concentration (mM)	Measured χ_{ppp}/χ_{ssp} Ratio in air (with a spin-coated sucrose layer)	Measured χ_{ppp}/χ_{ssp} Ratio (exposed to sucrose solution)
25	0.62	1.79
50	0.65	1.75
75	0.74	1.84
100	0.84	1.84
125	0.98	1.81
150	1.08	1.81
200	1.35	1.86
225	1.65	1.80
250	1.85	1.84
275	2.20	1.75
300	2.45	1.75
325	2.77	1.80

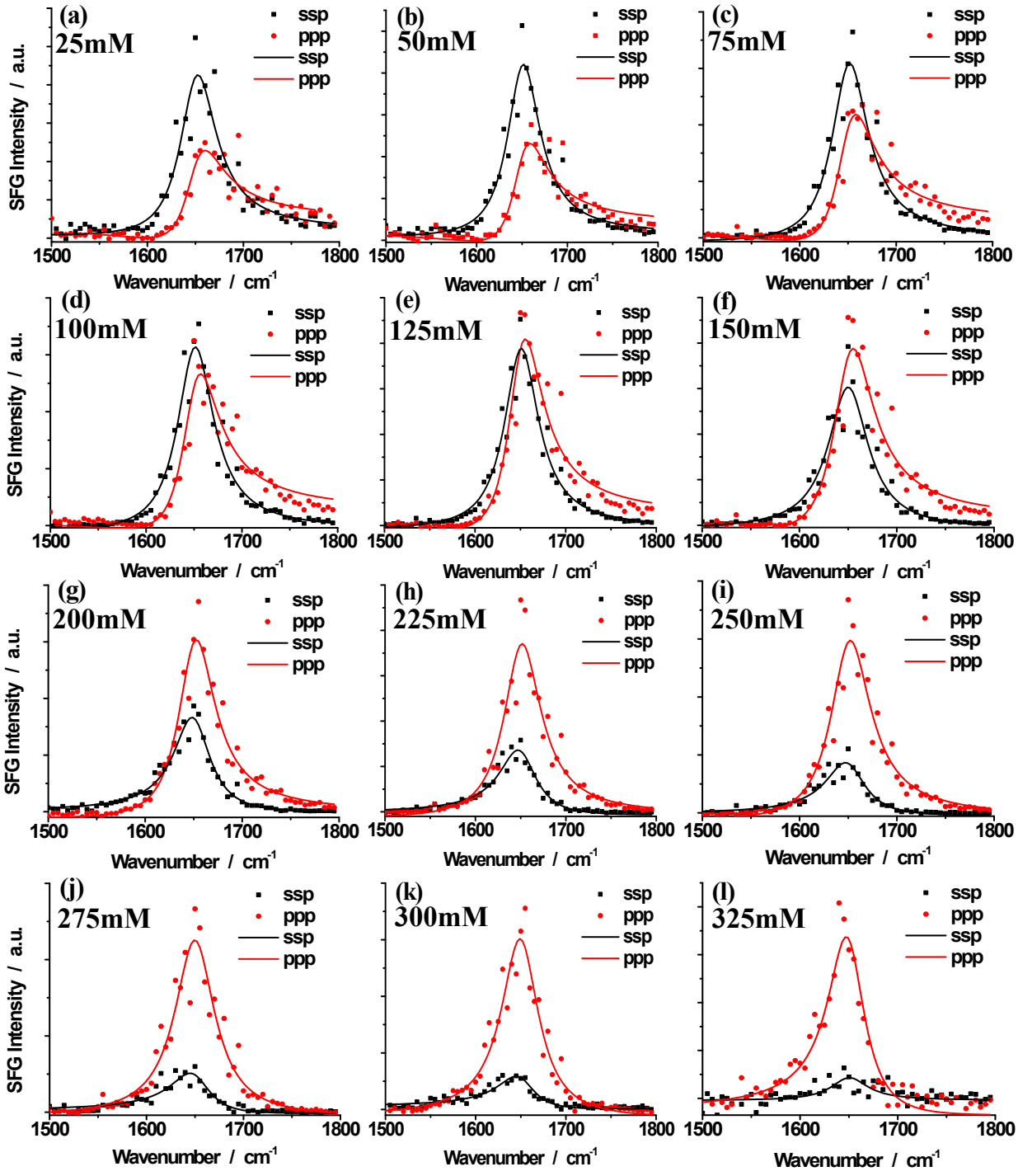

Figure S3. SFG ssp (■, black line) and ppp (●, red line) spectra collected from nMSI-78 immobilized on SAM surface with spin coated sucrose using sugar solutions with different concentrations: a) 25.0 mM; b) 50.0 mM; c) 75.0 mM; d) 100.0 mM; e) 125.0 mM; f) 150.0 mM; g) 200.0 mM; h) 225.0 mM; i) 250.0 mM; j) 275.0 mM; k) 300.0 mM; l) 325.0 mM.

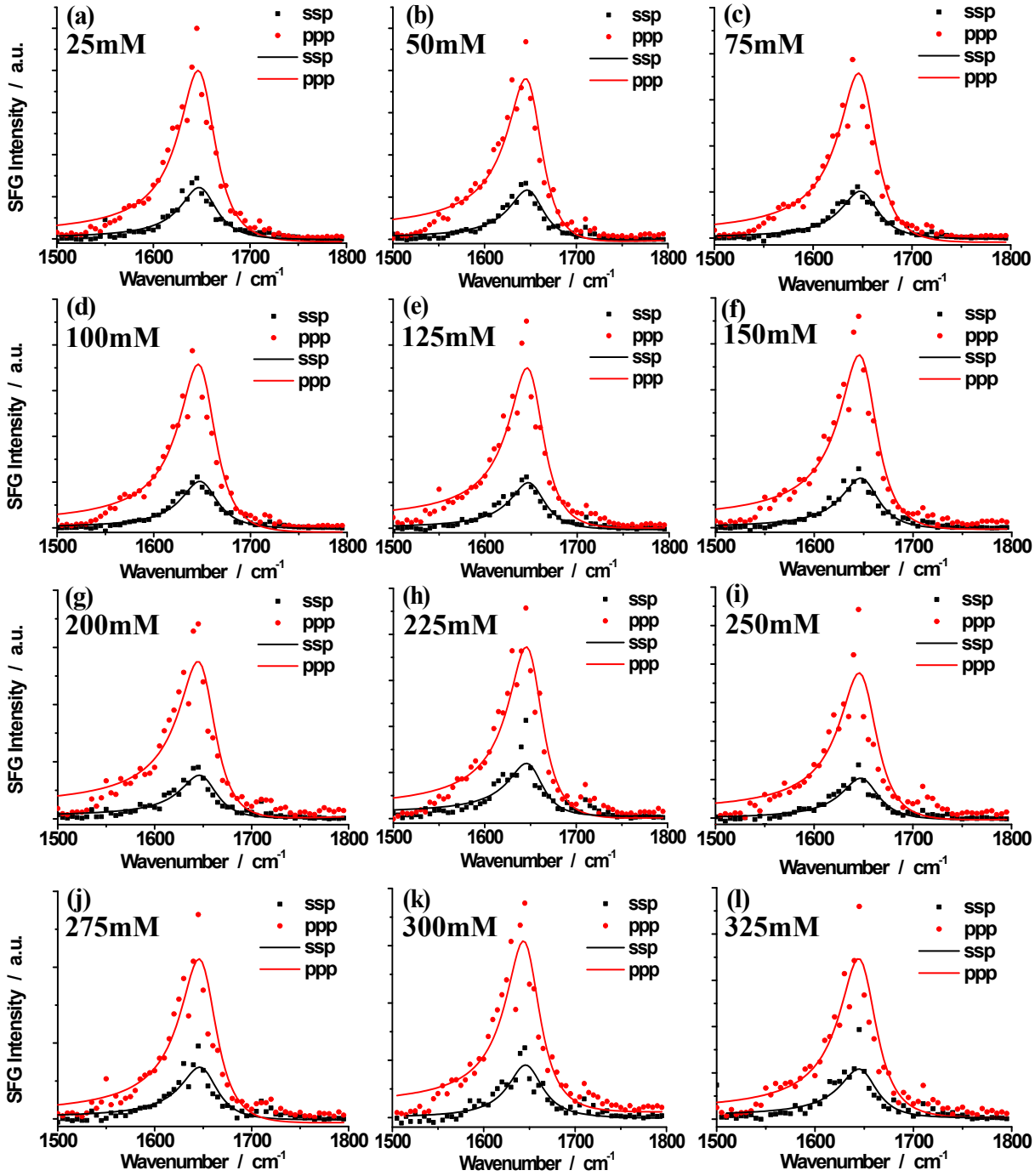

Figure S4. SFG ssp (■, black line) and ppp (●, red line) spectra collected from nMSI-78 immobilized on SAM surface exposed to sucrose solutions at different concentrations a) 25.0 mM; b) 50.0 mM; c) 75.0 mM; d) 100.0 mM; e) 125.0 mM; f) 150.0 mM; g) 200.0 mM; h) 225.0 mM; i) 250.0 mM; j) 275.0 mM; k) 300.0 mM; l) 325.0 mM.

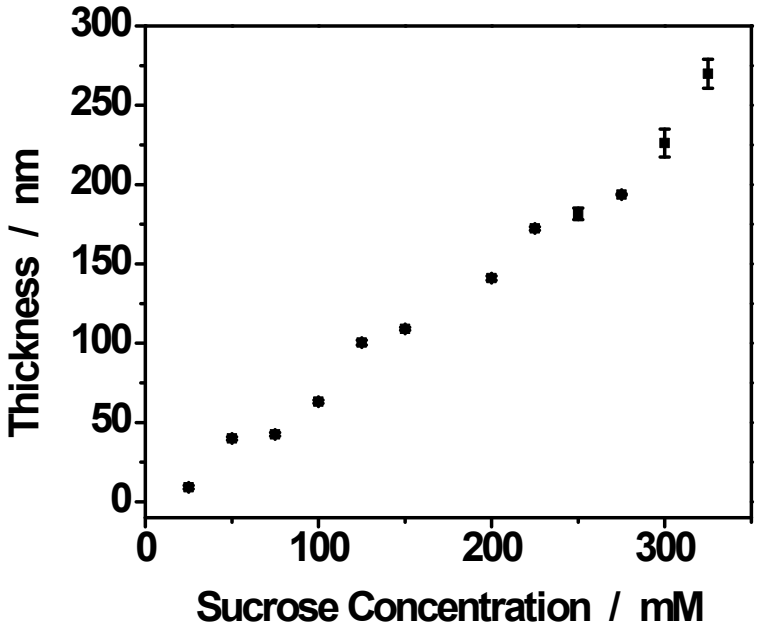

Figure S5. Measured sucrose thickness of the spin coated sugar layer as a function of the sucrose solution concentration used for spin coating.

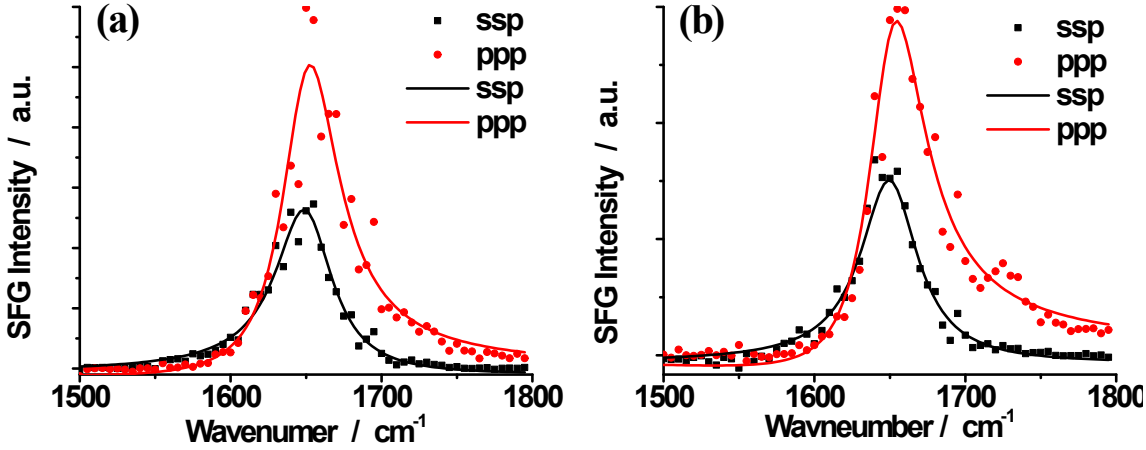

Figure S6. SFG ssp and ppp spectra collected from nMSI-78 immobilized on a SAM surface with sucrose spin coated: a) freshly prepared; b) stored at 75°C for two hours and tested at RT.

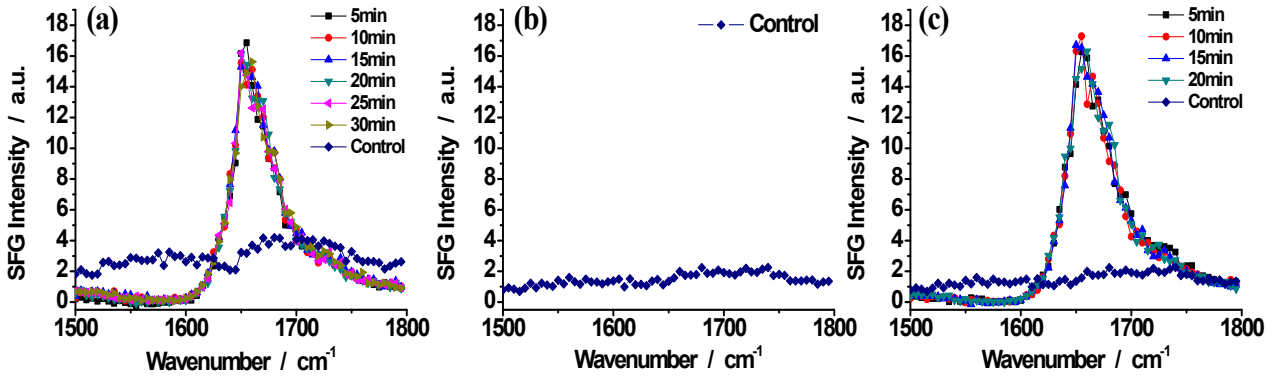

Figure S7. SFG ppp spectra collected from nMSI-78 immobilized on SAM surface a) with spin-coated sucrose layer on top in air; b) in air with no sugar; c) with sucrose spin coated again on top of the immobilized peptides in air.

References

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