An SFG Study of Interfacial Amino Acids at the Hydrophilic SiO_2 and Hydrophobic Deuterated Polystyrene Surfaces

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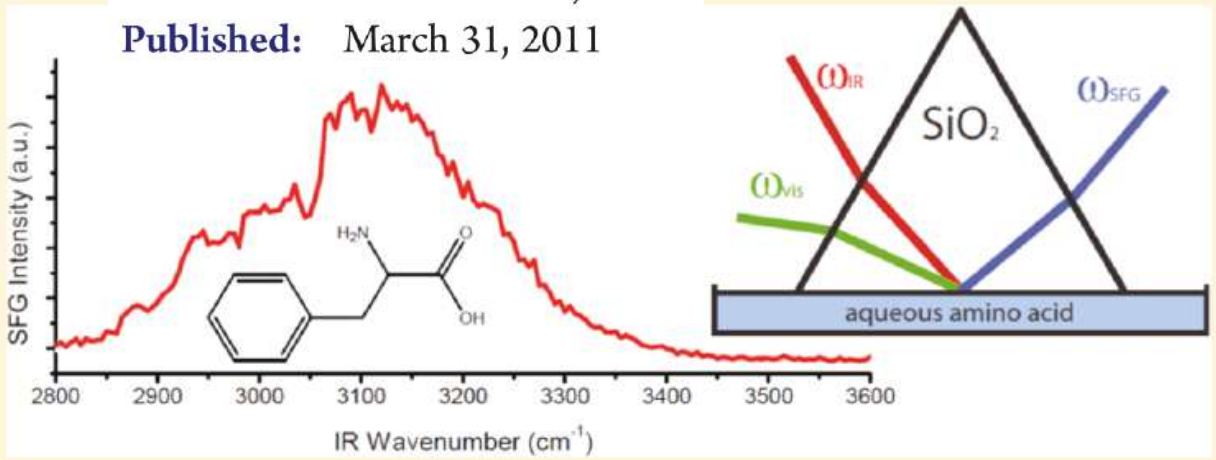
ABSTRACT: Sum frequency generation (SFG) vibrational spectroscopy was employed to characterize the interfacial structure of eight individual amino acids—L-phenylalanine, L-leucine, glycine, L-lysine, L-arginine, L-cysteine, L-alanine, and L-proline—in aqueous solution adsorbed at model hydrophilic and hydrophobic surfaces. Specifically, SFG vibrational spectra were obtained for the amino acids at the solid−liquid interface between both hydrophobic d_8-polystyrene (d_8-PS) and SiO_2 model surfaces and phosphate buffered saline (PBS) at pH 7.4. At the hydrophobic d_8-PS surface, seven of the amino acids solutions investigated showed clear and identifiable C−H vibrational modes, with the exception being L-alanine. In the SFG spectra obtained at the hydrophilic SiO_2 surface, no C−H vibrational modes were observed from any of the amino acids studied. However, it was confirmed by quartz crystal microbalance that amino acids do adsorb to the SiO_2 interface, and the amino acid solutions were found to have a detectable and widely varying influence on the magnitude of SFG signal from water at the SiO_2/PBS interface. This study provides the first known SFG spectra of several individual amino acids in aqueous solution at the solid−liquid interface and under physiological conditions.

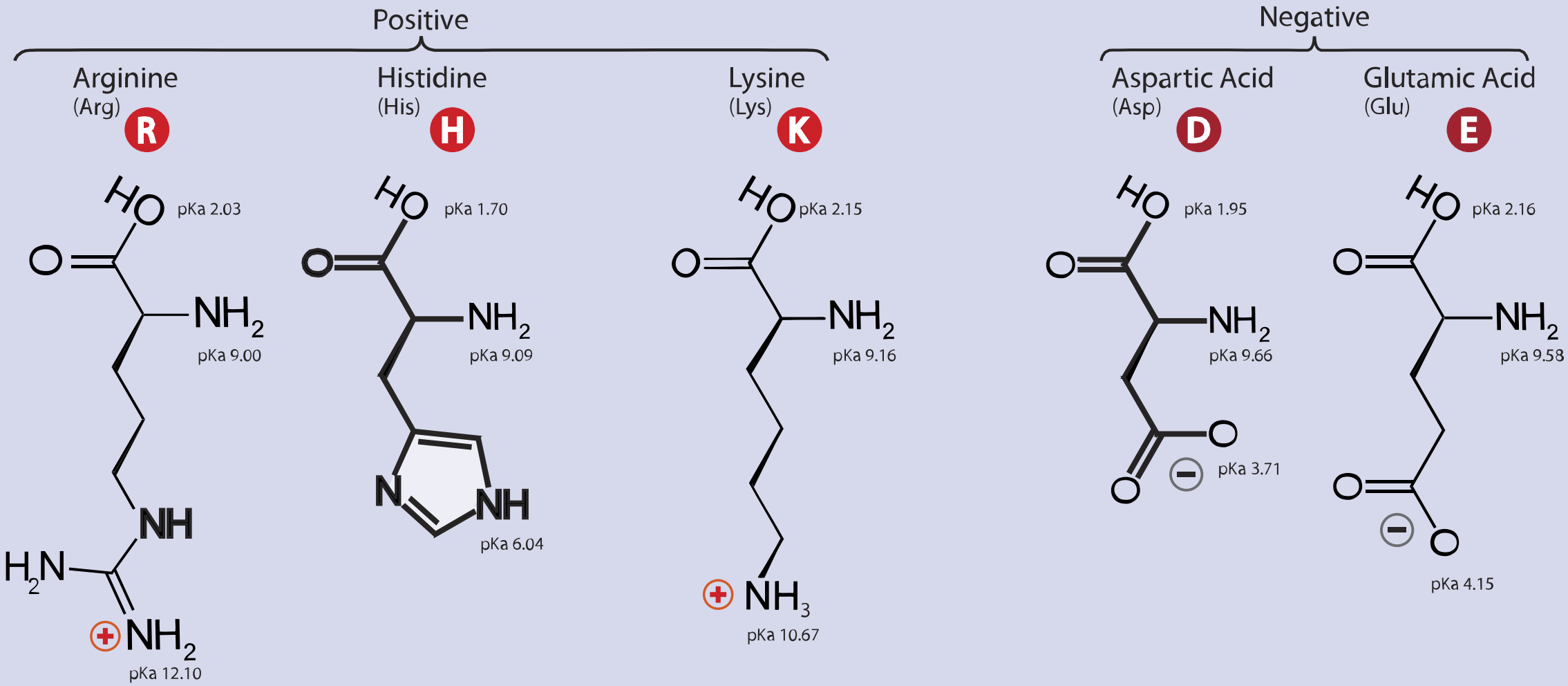


Twenty-One Amino Acids

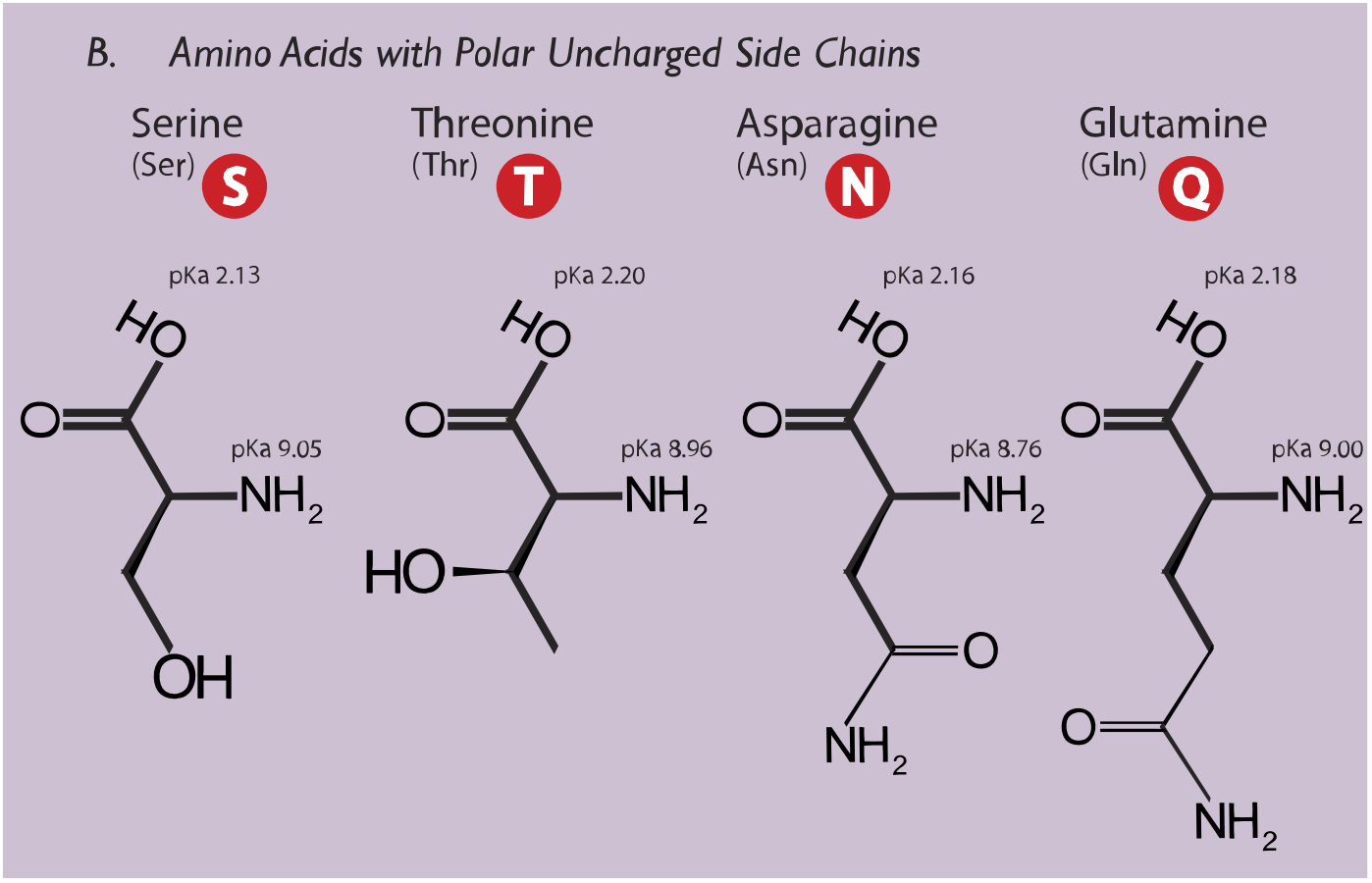
Positive Negative

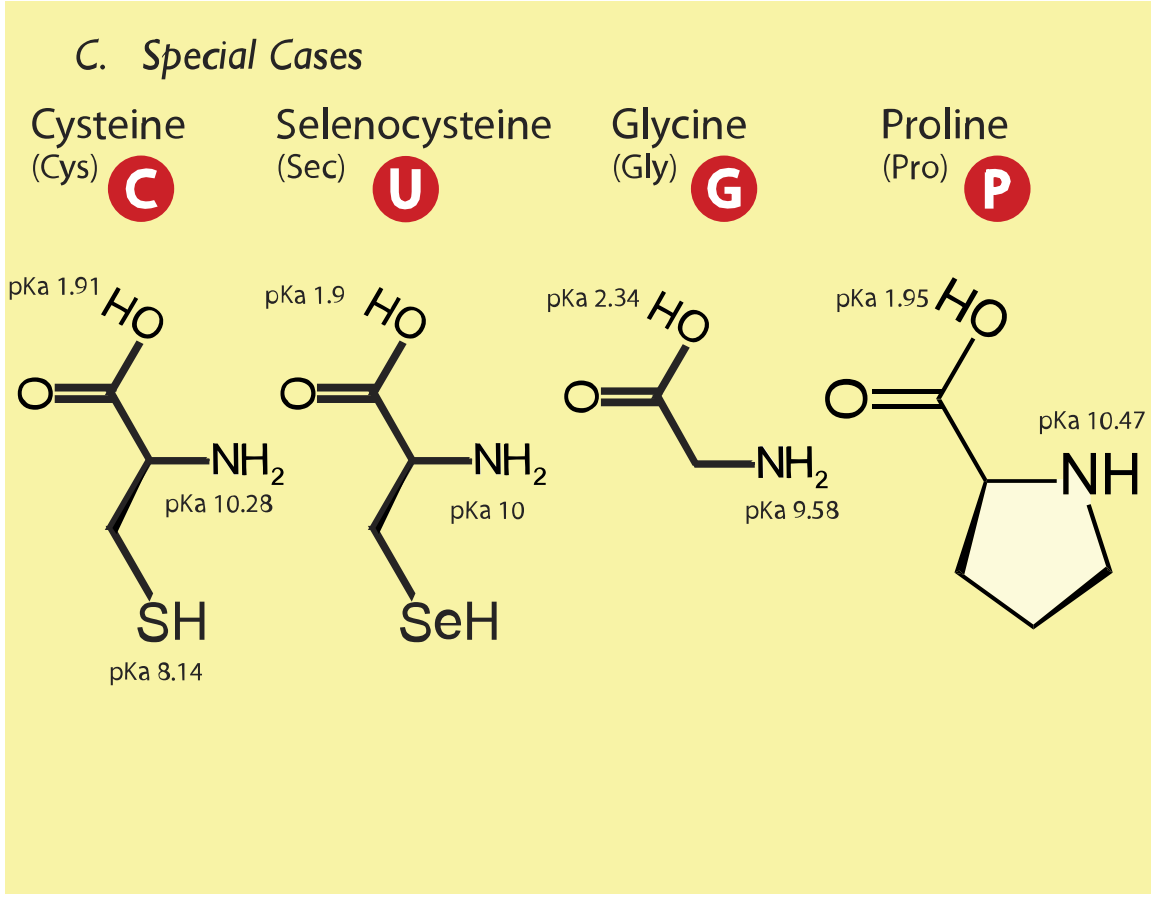
• Side chain charge at physiological **pH 7.4**

A. Amino Acids with Electrically Charged Side Chains



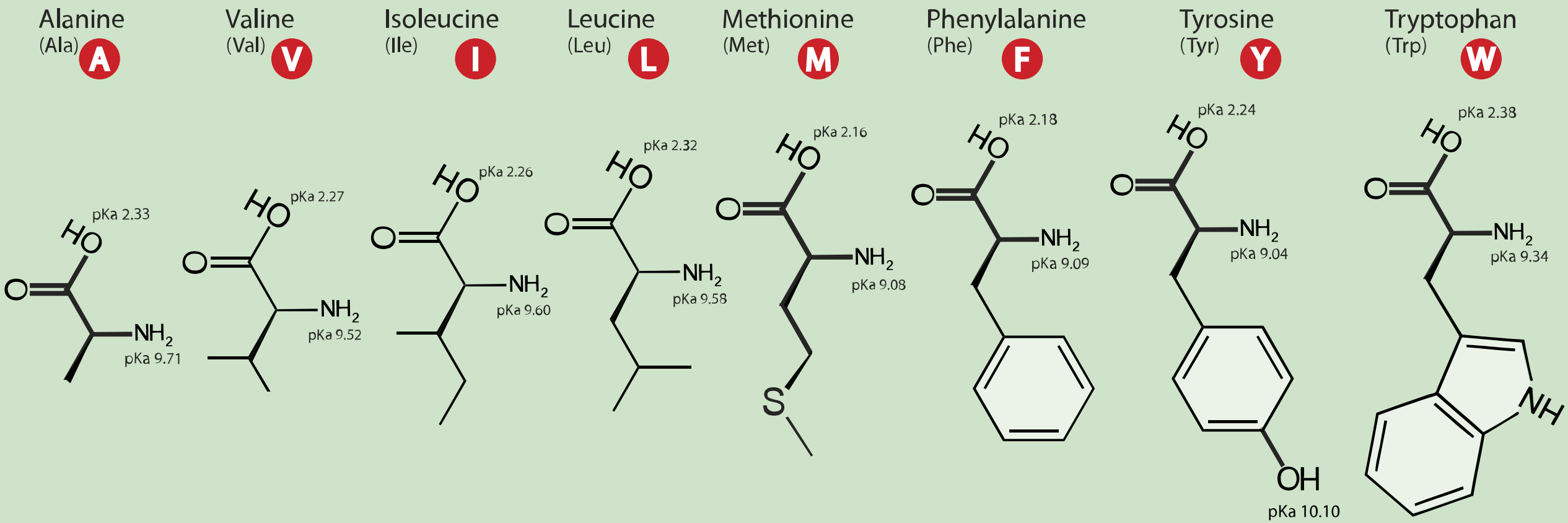
https://en.wikipedia.org/wiki/Amino_acid





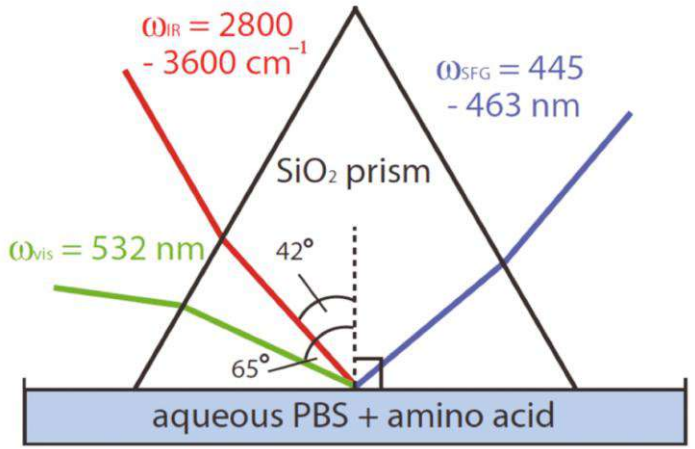
https://en.wikipedia.org/wiki/Amino_acid3

D. Amino Acids with Hydrophobic Side Chain



https://en.wikipedia.org/wiki/Amino_acid4

Tool: Sum frequency (Solid/Liquid Interface)



$$I_{ssp}^{SFG} = \frac{32\pi^3\omega_s^2}{c^3}\frac{\mathrm{Re}[\varepsilon_1^{1/2}(\omega_s)]}{|\varepsilon_1(\omega_s)|\mathrm{Re}[\varepsilon_1^{1/2}(\omega_{Vis})\varepsilon_1^{1/2}(\omega_{IR})]}\times$$
$$\left|\chi^{(2)}_{yyz}(\omega_s)\sec\beta\sin\beta_{IR}L^S L_T^{Vis} L_T^{IR}\frac{\varepsilon_1^{1/2}(\omega_{IR})\varepsilon_2^{1/2}(\omega_{IR})}{\varepsilon_M(\omega_{IR})}\right|^2 I^{Vis} I^{IR} \quad (1)$$

where

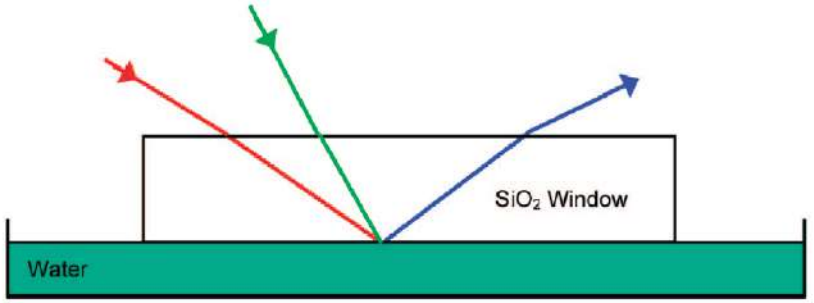
$$L^S = \frac{2\varepsilon_1^{1/2}(\omega_s)\cos\beta}{\varepsilon_2^{1/2}(\omega_s)\cos\gamma + \varepsilon_2^{1/2}(\omega_s)\cos\beta} \quad (2)$$

$$L_T^{Vis} = \frac{2\varepsilon_2^{1/2}(\omega_{Vis})\cos\beta_{Vis}}{\varepsilon_2^{1/2}(\omega_{Vis})\cos\gamma_{Vis} + \varepsilon_1^{1/2}(\omega_{Vis})\cos\beta_{Vis}} \quad (3)$$

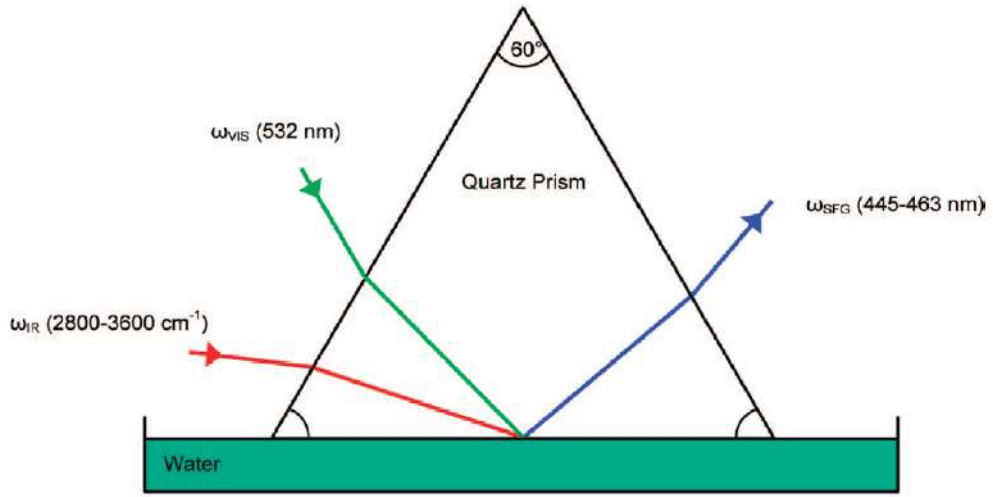
$$L_T^{IR} = \frac{2\varepsilon_1^{1/2}(\omega_{IR})\cos\beta_{IR}}{\varepsilon_2^{1/2}(\omega_{IR})\cos\beta_{IR} + \varepsilon_1^{1/2}(\omega_{IR})\cos\gamma_{IR}} \quad (4)$$

Chosen angles:

- maximize the collection of SFG signal
- While minimizing wavelength dependent



(a)



(b)

Figure 2. Scheme of the (a) "slab" geometry and (b) "prism" geometry used in this work. See Table 1 for the respective angles of incidence.

J. Phys. Chem. A ***2009,*** *113, 2768–2774*

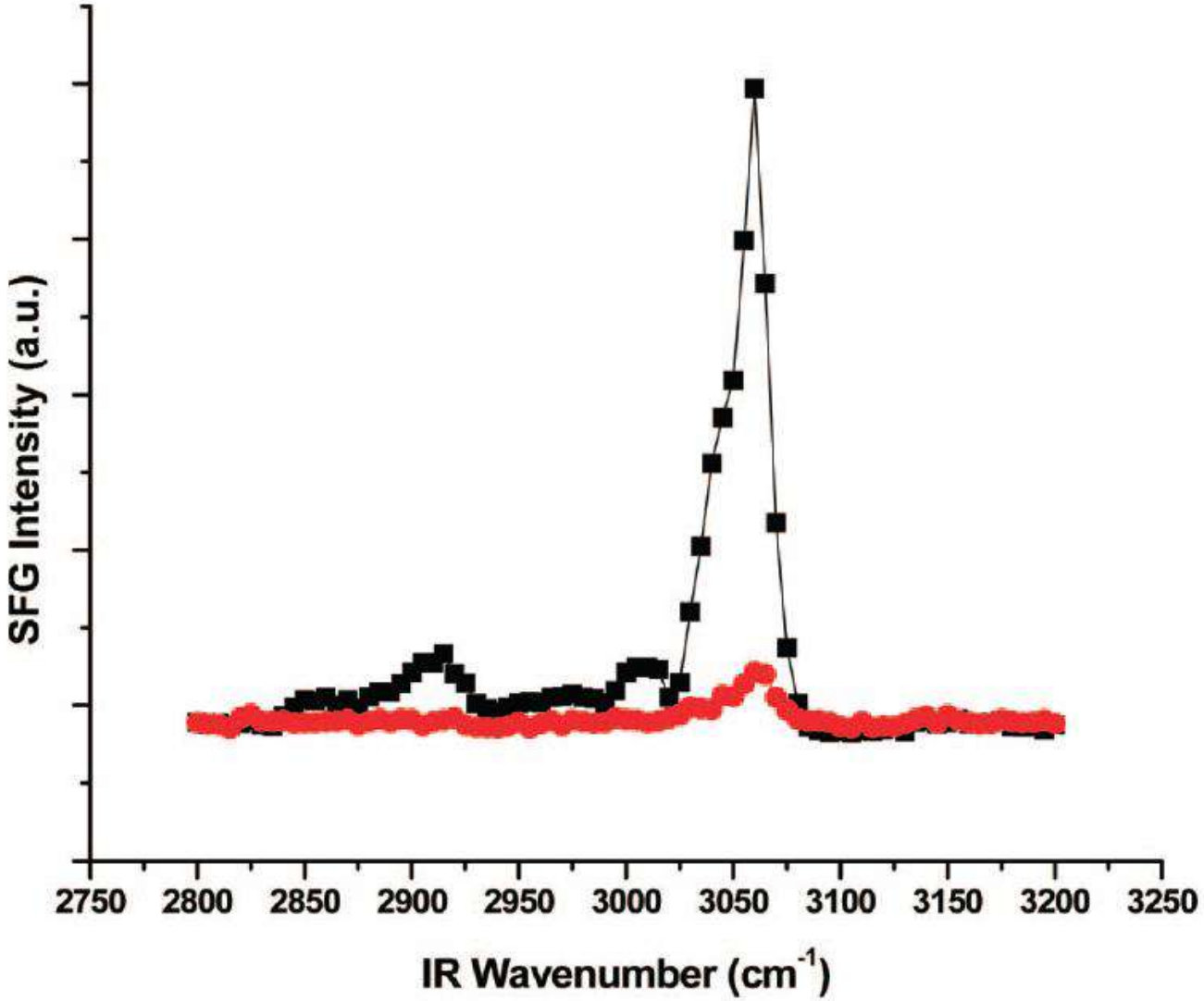


Figure 5. SFG spectra of the polystyrene/air interface in the slab (red circle) and prism (black squares) geometries. Note the increase in the SFG signal in the prism geometry. Additionally, vibrational modes not clearly seen in the slab geometry (due to the low signal-to-noise ratio) are clearly present in the prism geometry (e.g., the mode around 2915 cm^{-1}).

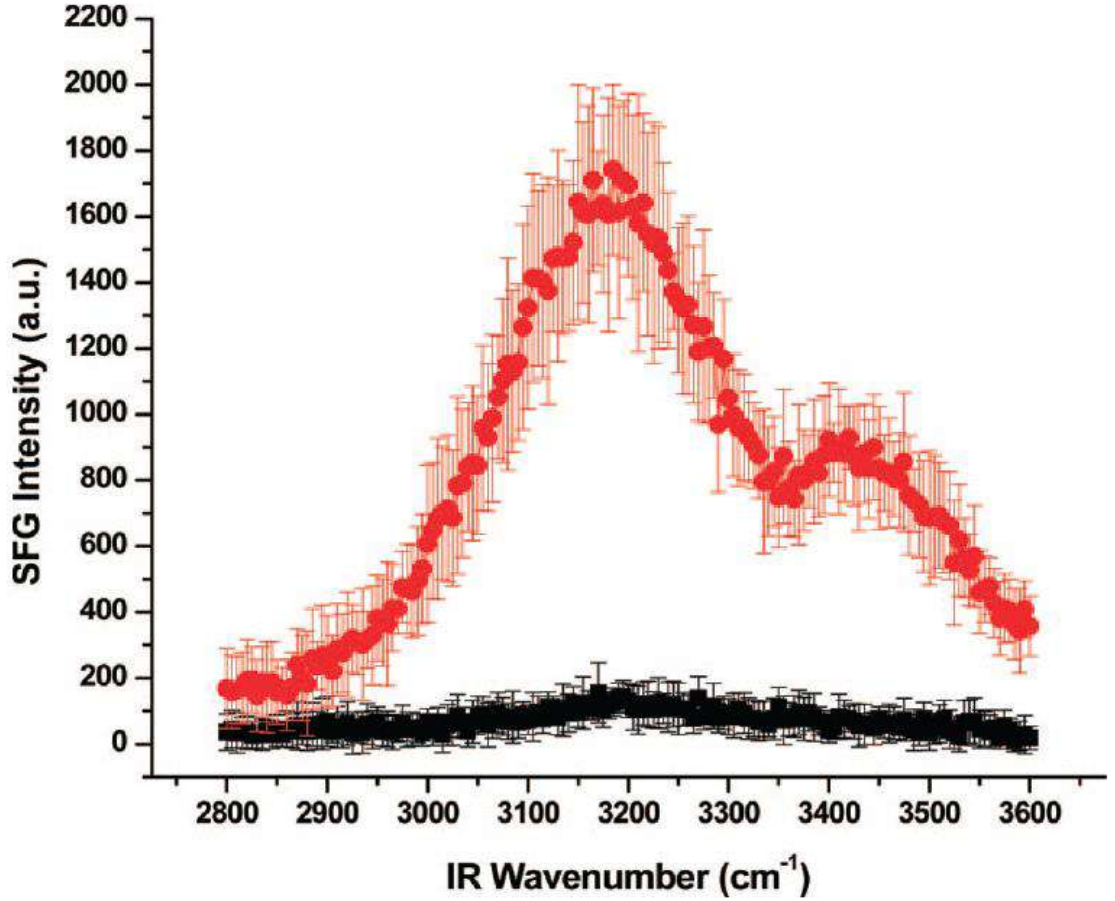


Figure 6. SFG spectra of the pure water/silica interface for both the prism (red circles) and the slab (black squares). Error bars come from the average of 5 scans of 200 shots per data point (for the prism) and 8 scans of 200 shots per data point (for the slab).

J. Phys. Chem. A ***2009,*** *113, 2768–2774*

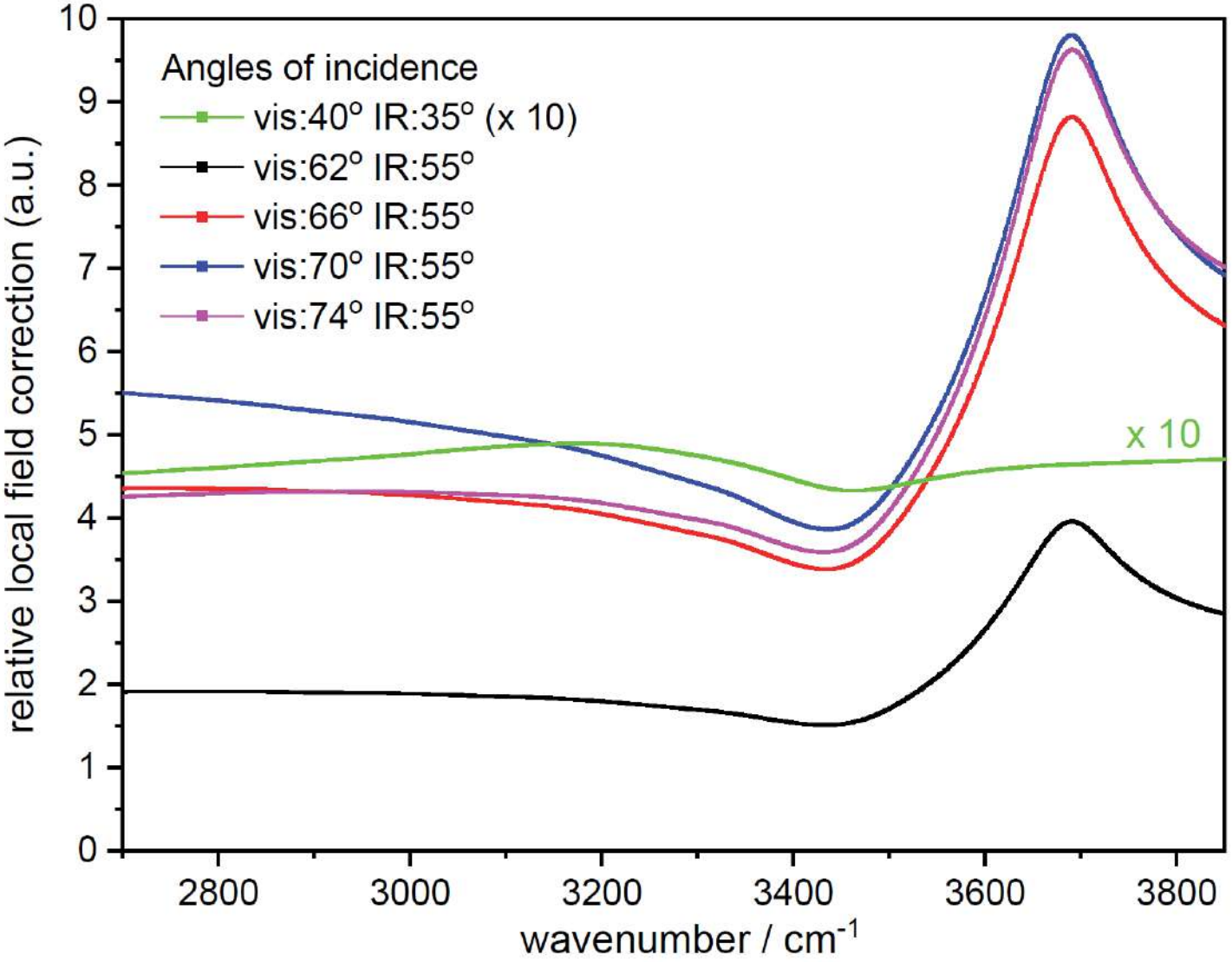


Fig. S5. IR dependence of the local field corrections for each of the experimental geoemetries studied. Note that the values for 40° visible AOI and 35° IR AOI experimental geometry, were multiplied by 10 for ease of comparison. The maximum enhancement in the overall spectra intensity is attained close to the critical angles.

D. Hore, J. Phys. Chem. C 2019, 123, 16911−16920

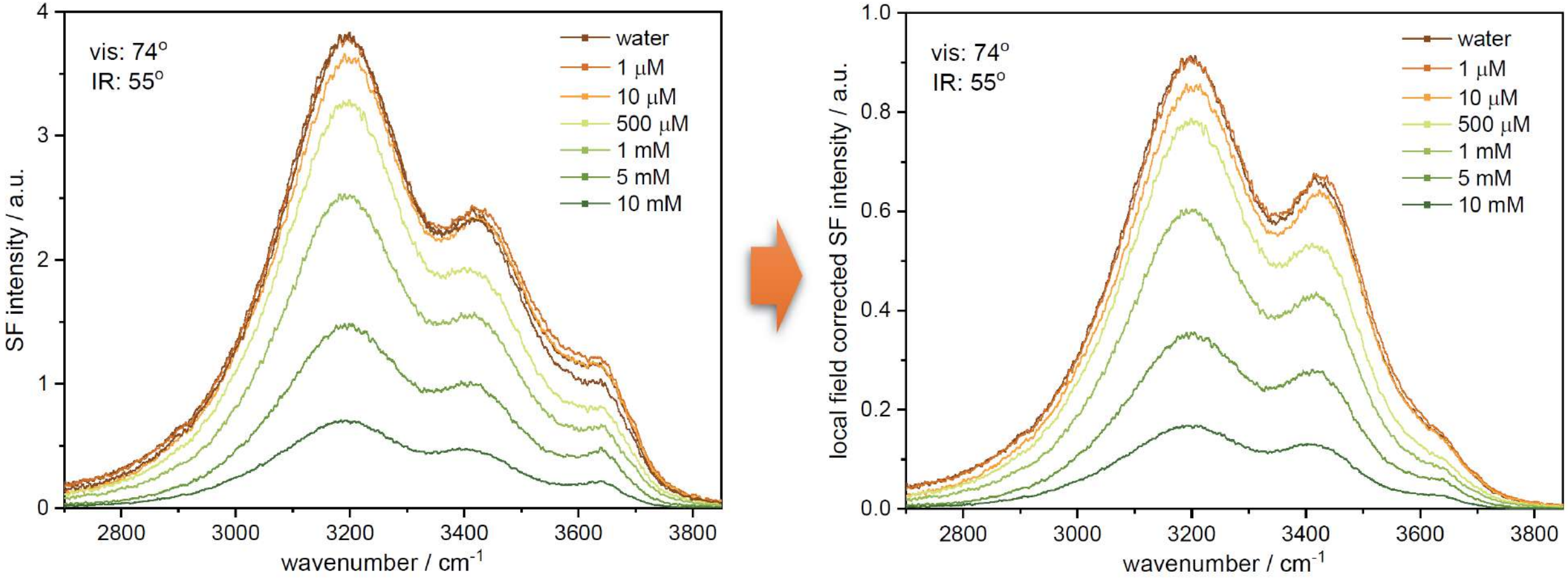


Fig. S6. (left) SF spectra collected in the ssp polarization combination as a function of NaCl for an IR angle of incidence of 55° and visible angle of 74°. (right) Local field corrected spectra at the same experimental geometry.

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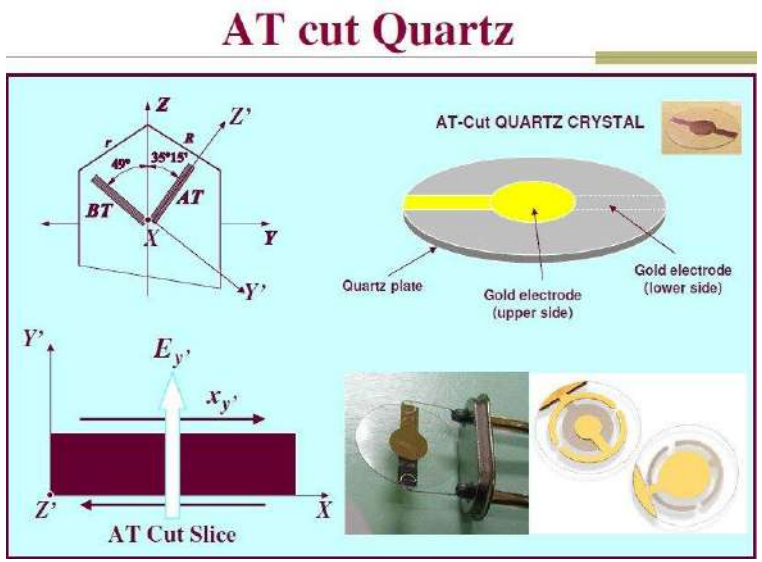
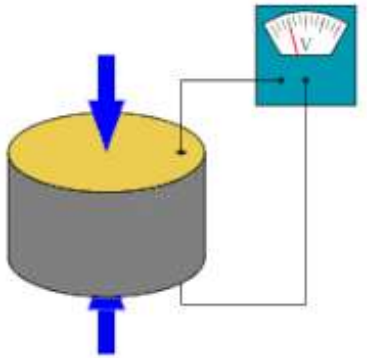
Complementary Tool:
Quartz Crystal Microbalance (QCM)

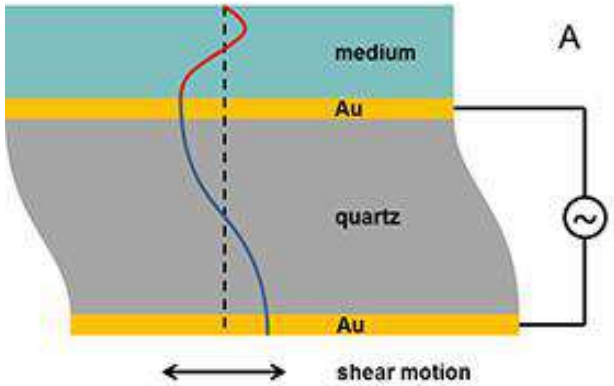


https://www.tradekorea.com/product/detail/P416979/Electrochemical-research-Biosensor-crystal-.html

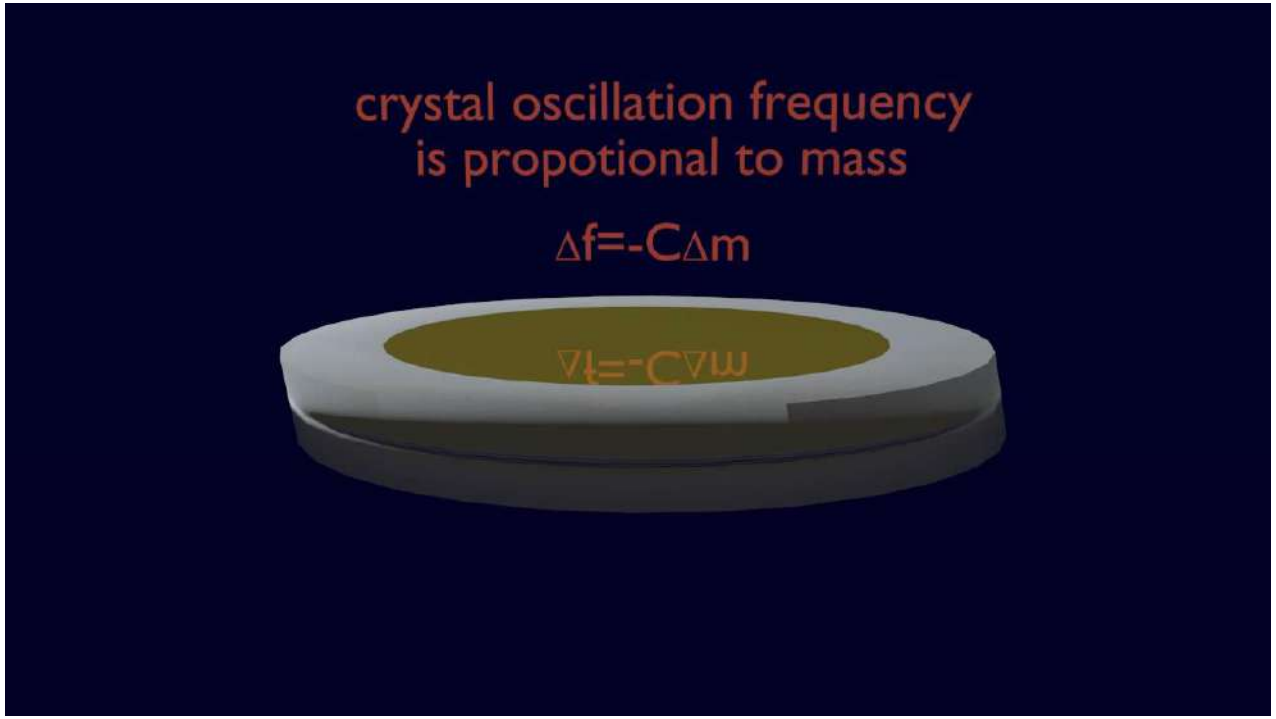
Piezoelectric



Wikipedia



https://www.3t-analytik.de/technologies/qcm-d/what-qcm-d



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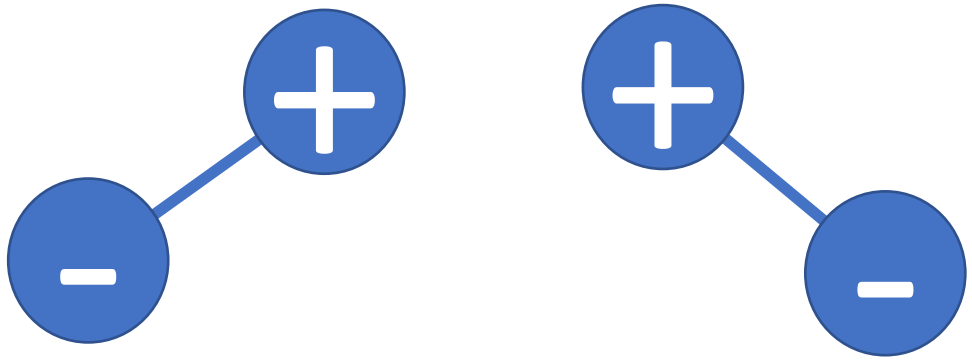
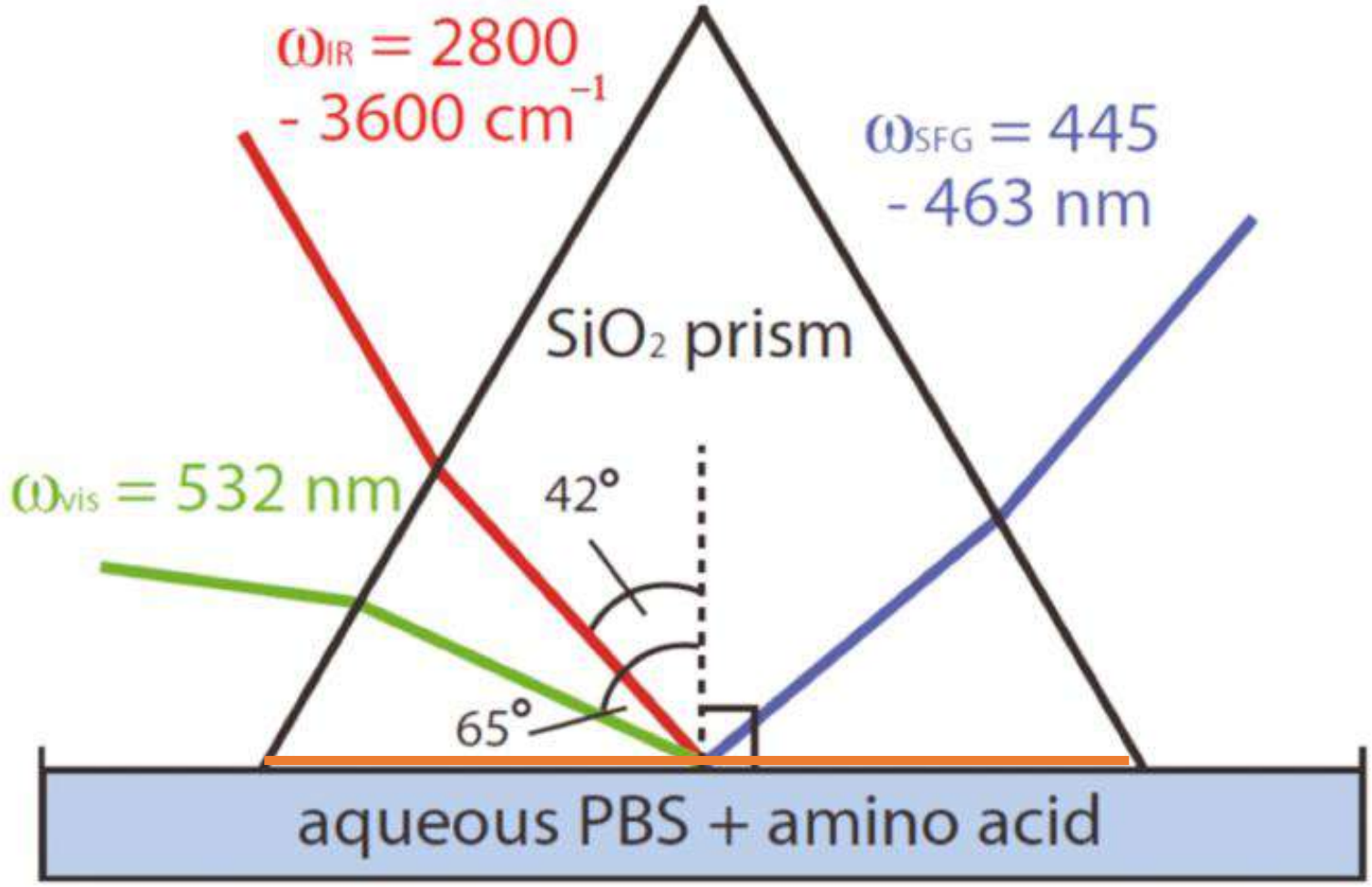


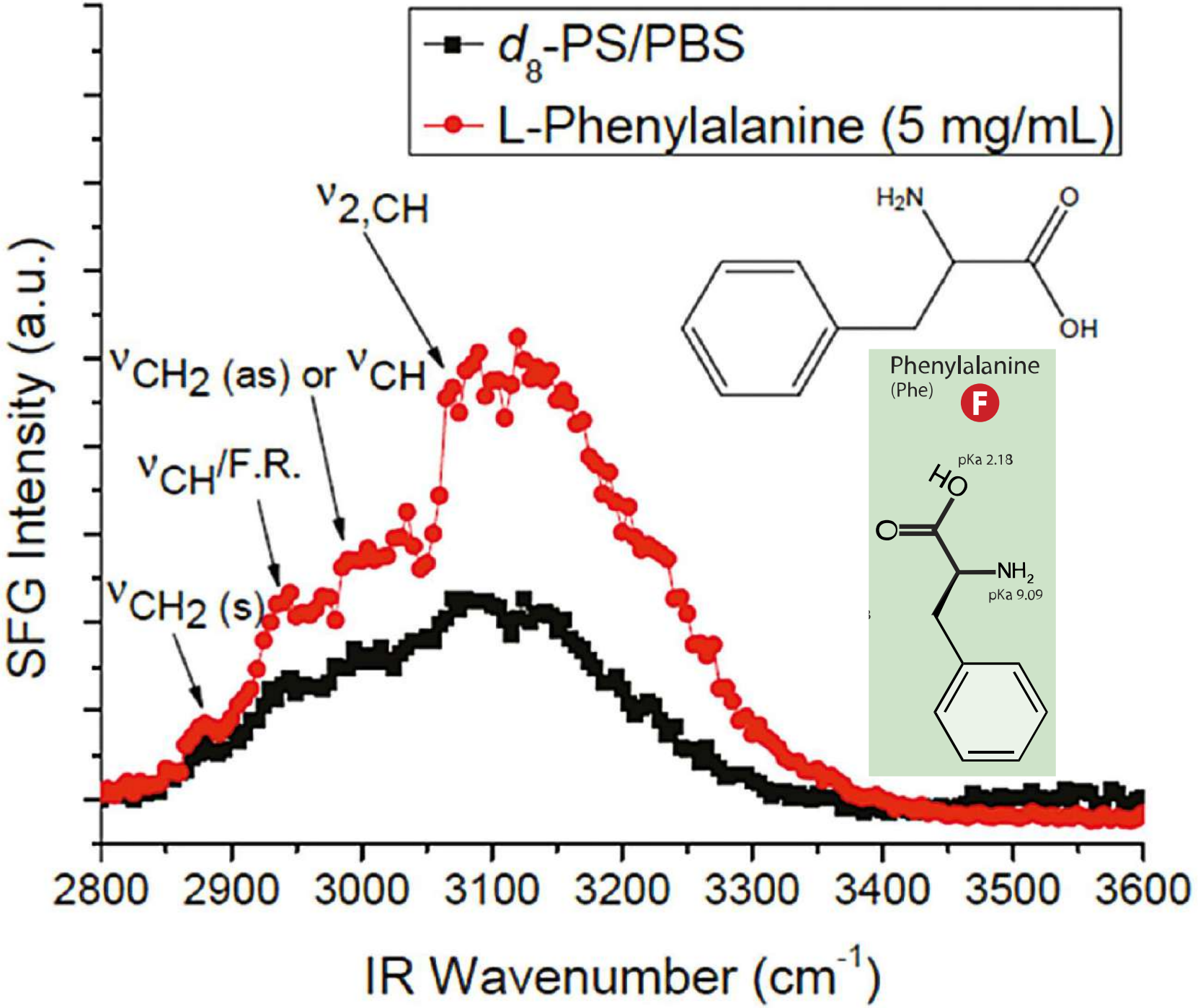
QCM results

Table 1. Adsorbed Mass at the Aqueous Liquid−Solid Interface Determined by QCM

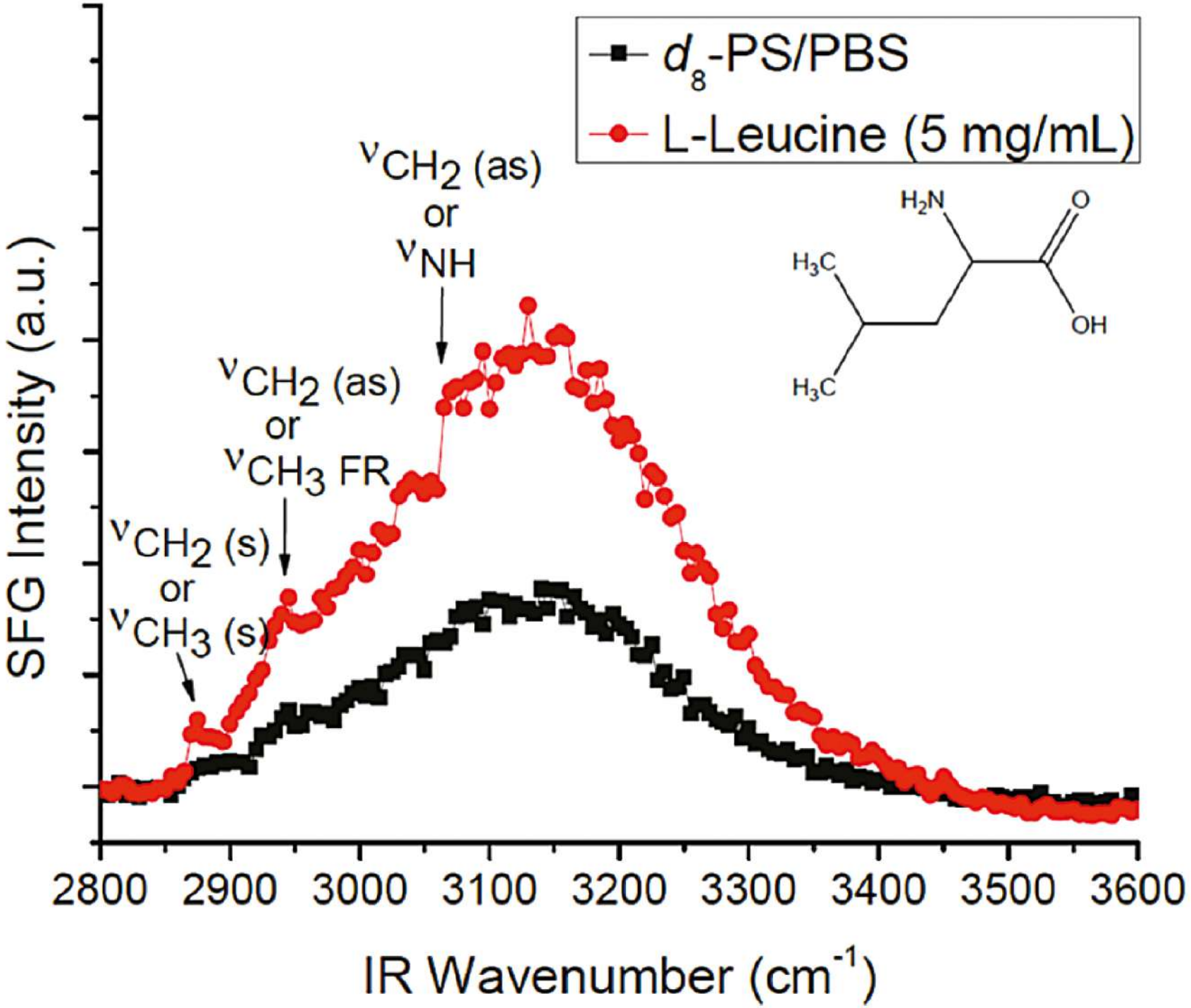
amino acid	concentration (mg/mL)	interface	adsorbed mass (ng/cm^2)	95% confidence interval (ng/cm^2)
L-phenylalanine	5	PS	57	4
L-phenylalanine	5	SiO_2	63	4
L-arginine	15	SiO_2	196	20
L-alanine	40	PS	469	33
L-alanine	40	SiO_2	467	20
L-cysteine	25	SiO_2	245	22

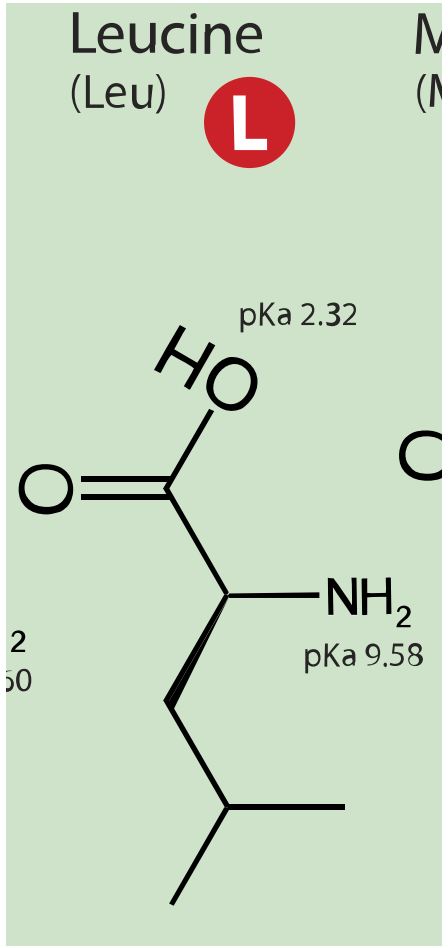
SFG results
d-PS coated

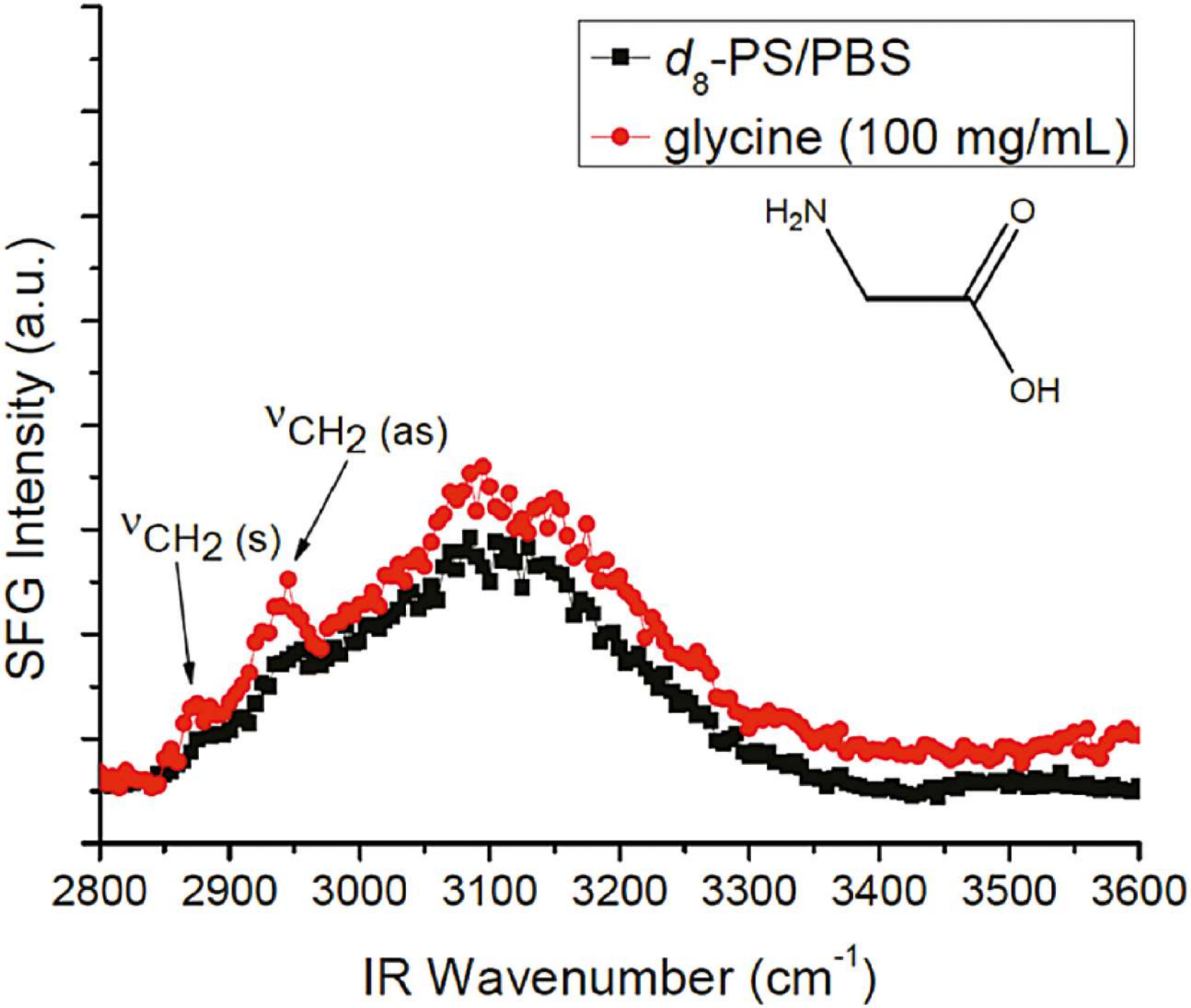


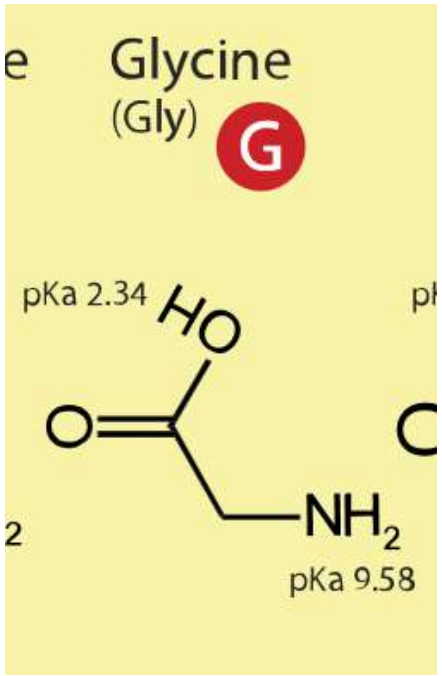


- Fig2: L-Phenylalanine
- 1points=500 laser shots.
- Larger OH due to adsorption of L-PA
- 2875/cm $CH_{2(S)}$
- 2939/cm CH and FR, CH connecting phenyl and amino acid backbone
- 2991/cm C-H combination band or $CH_{2(as)}$
- 3049/cm Aromatic C-H v_2 stretch from phenyl
- QCM: adsorbed mass 57 ng/cm^2= 48Å^2 per molecule

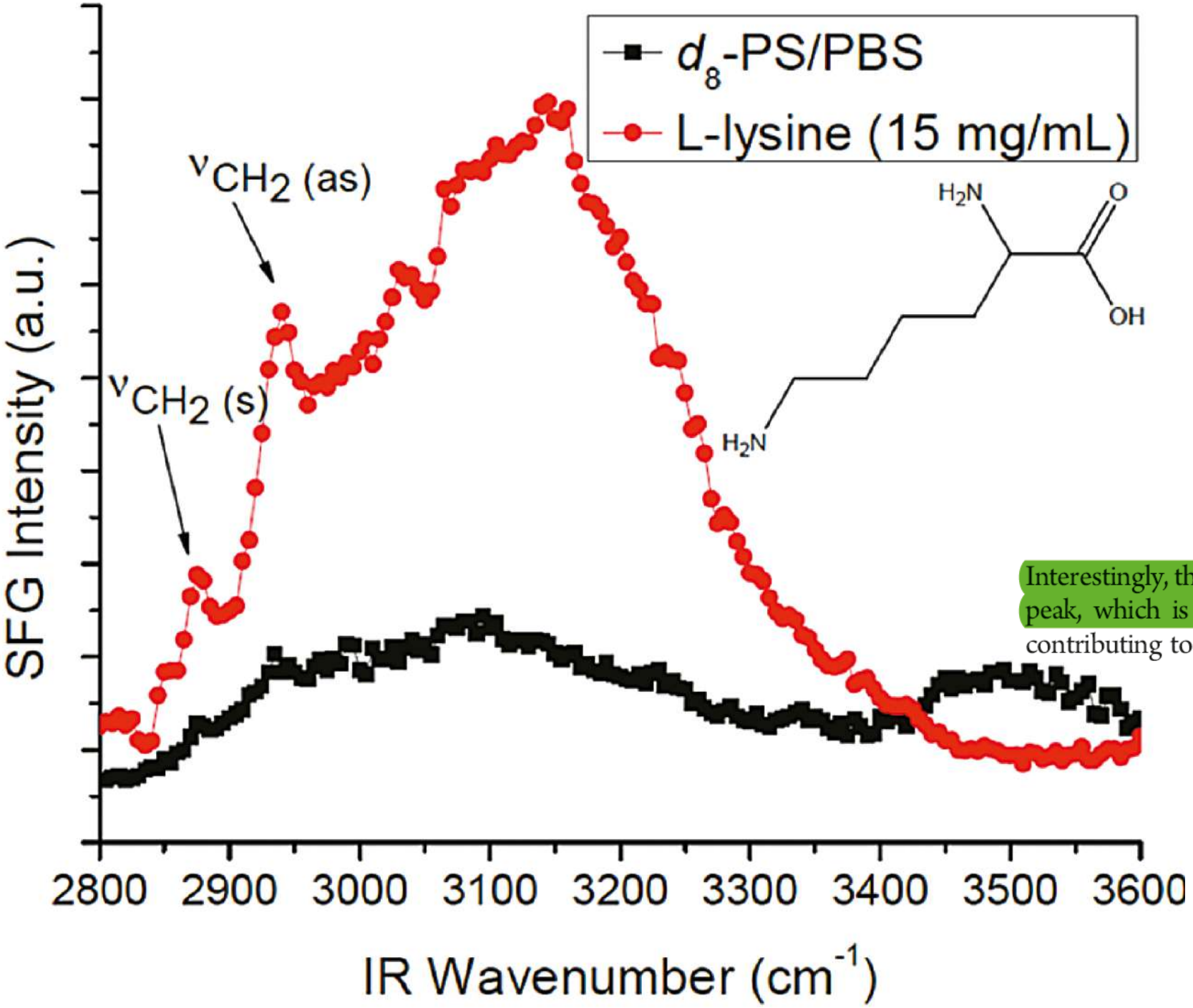

d_8-PS/PBS
L-Leucine (5 mg/mL)
νCH_2 (as)
or
νNH
νCH_2 (as)
or
νCH_3 FR
νCH_2 (s)
or
νCH_3 (s)
H_2N
O
H_3C
OH
H_3C
SFG Intensity (a.u.)
2800
2900
3000
3100
3200
3300
3400
3500
3600
IR Wavenumber (cm^{-1})

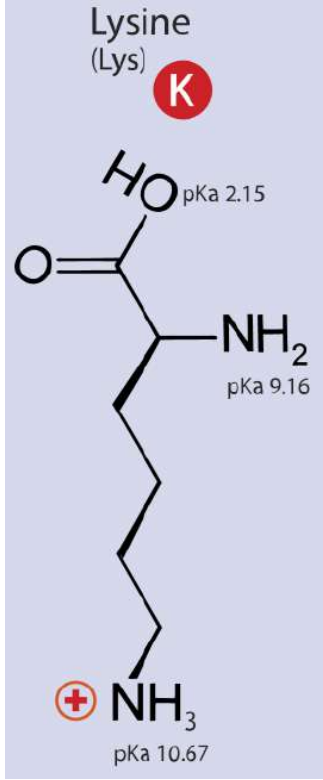

Leucine
(Leu)
L
pKa 2.32
O
HO
NH_2
pKa 9.58



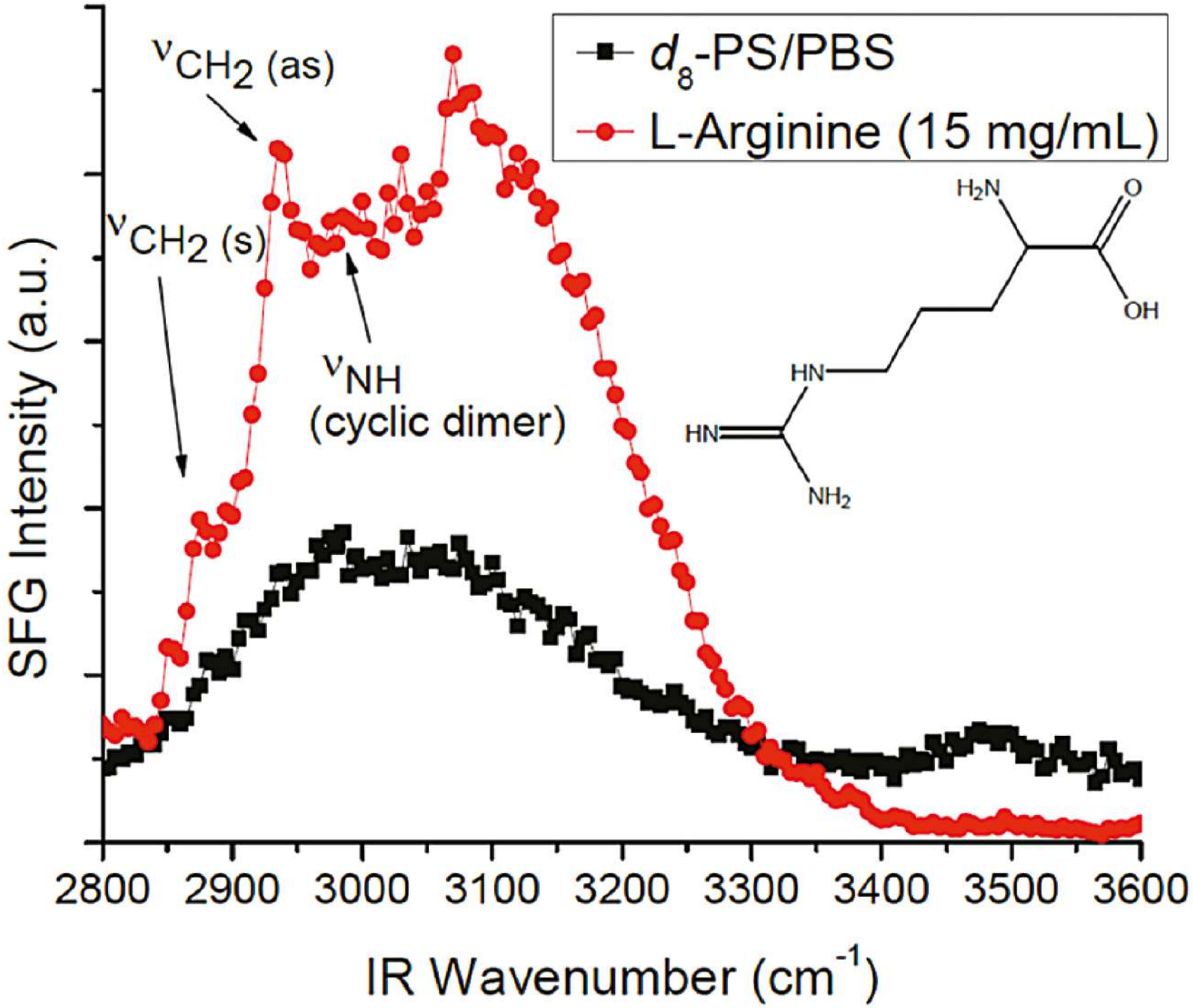


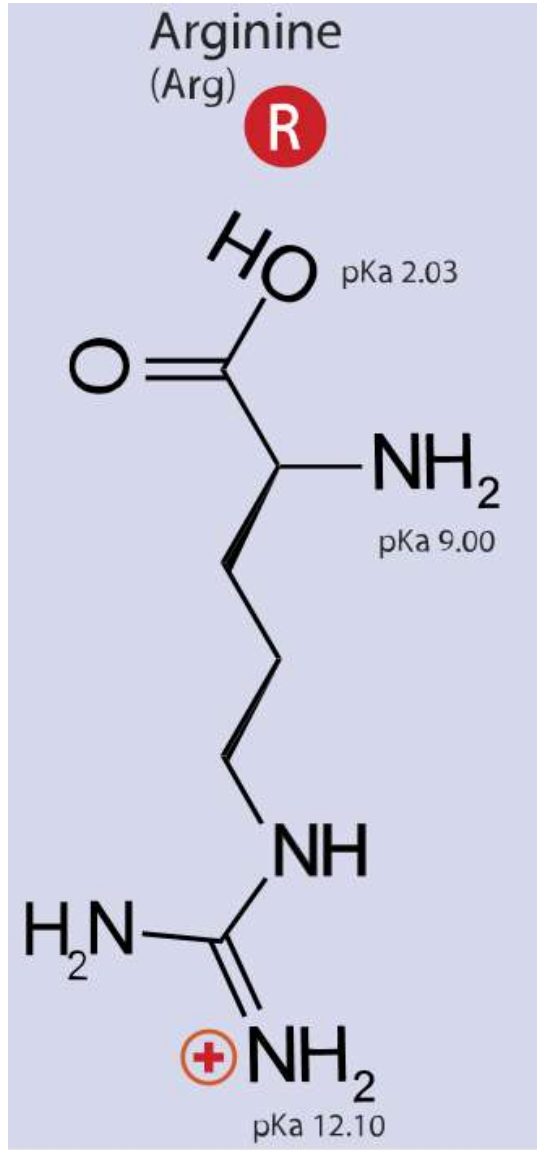
Similar OH due to higher concentration of amino acid.

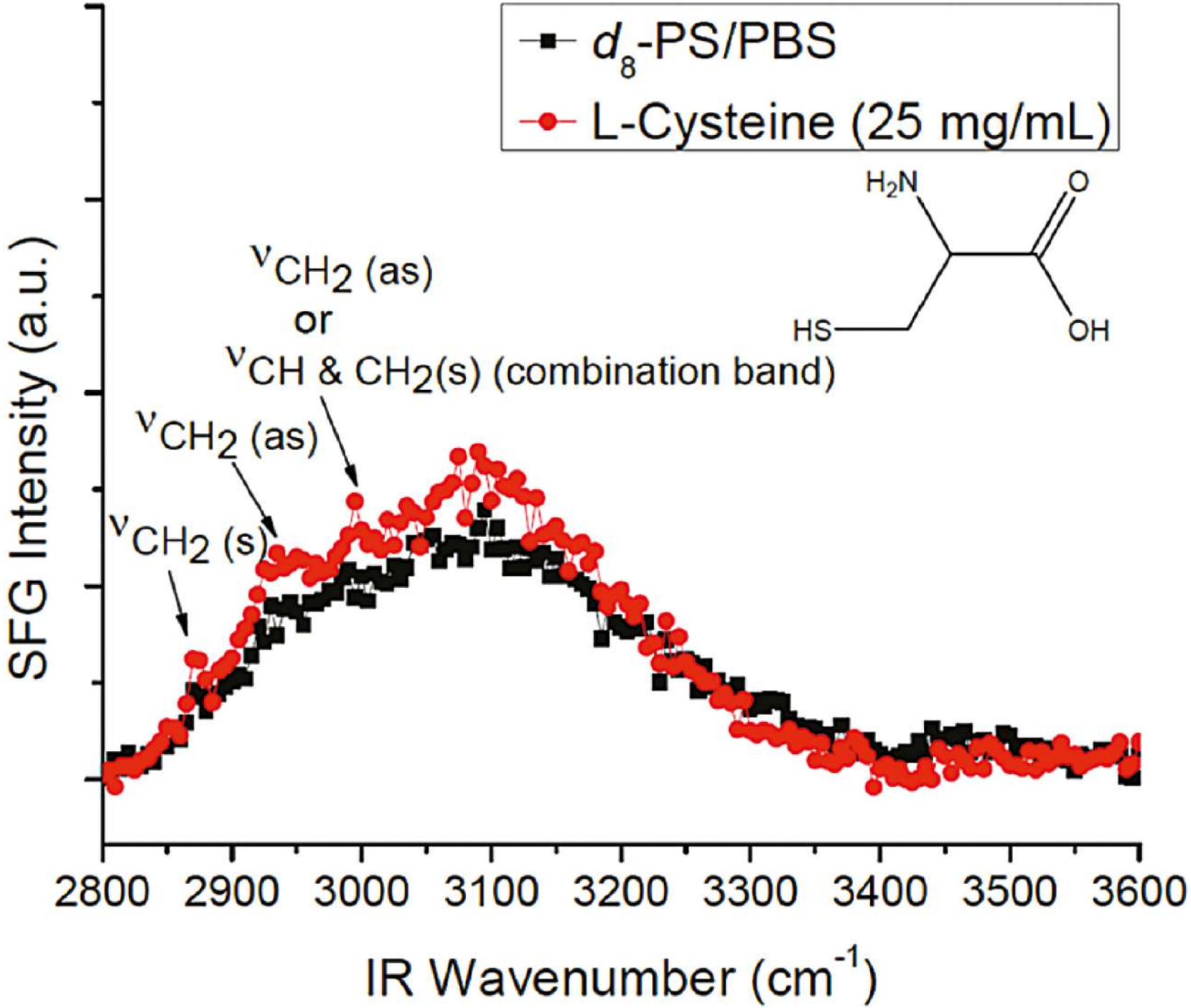


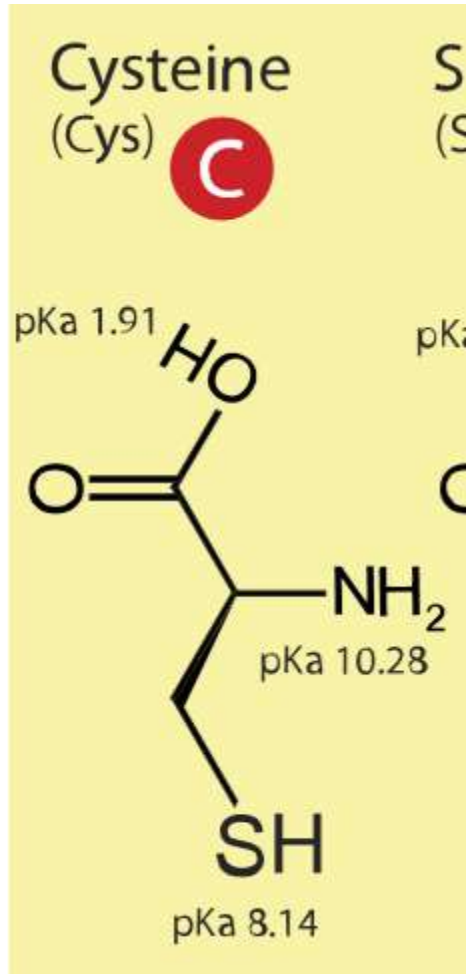


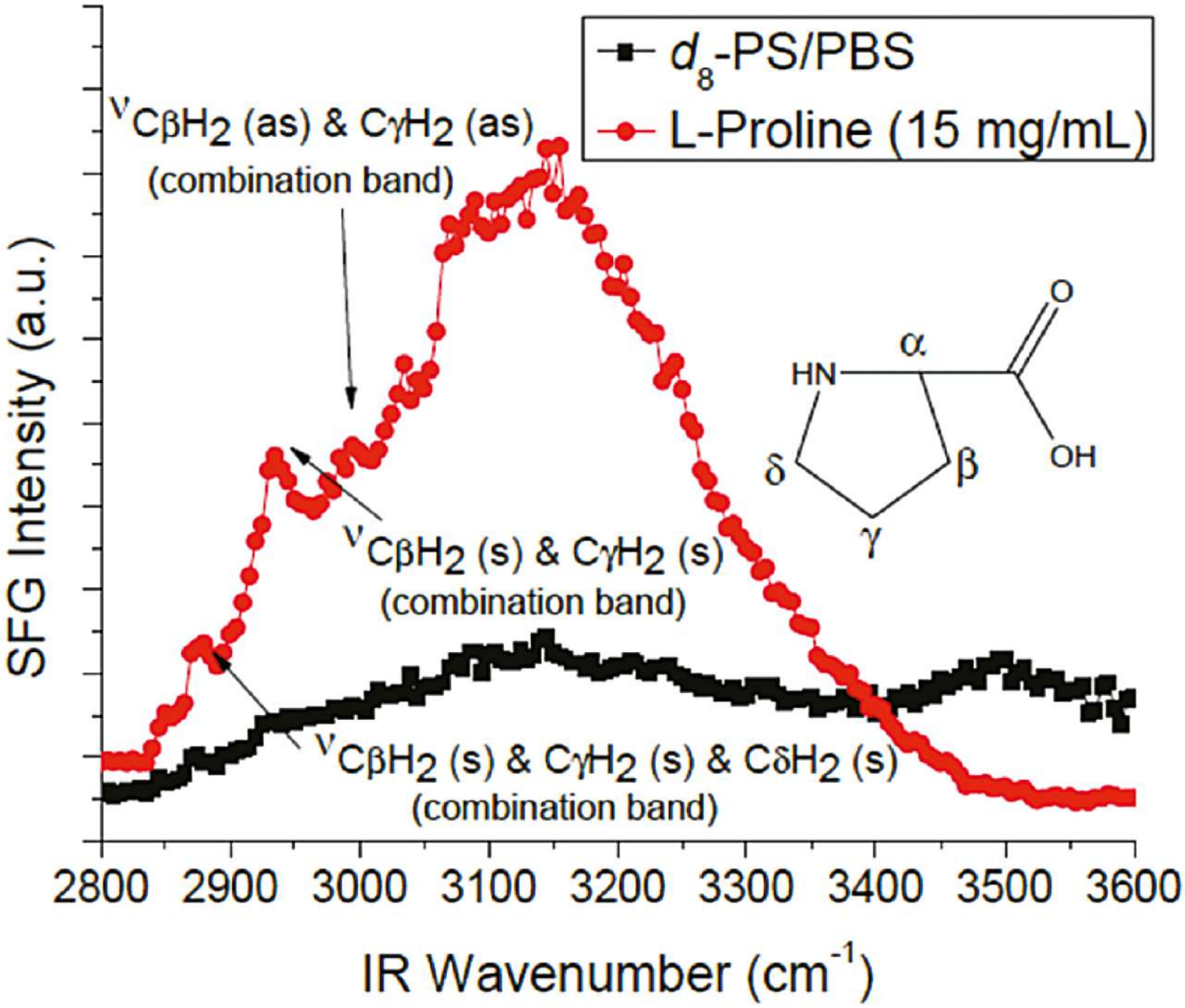
Interestingly, there is an apparent blue-shift in the broad O—H bond peak, which is consistent with the presence of an N—H stretch contributing to the shoulder of the O—H peak.[88,89]

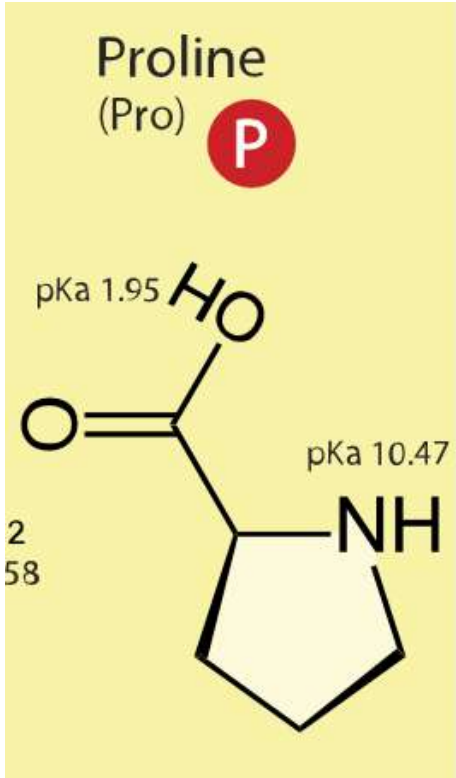

νCH_2 (as)
νCH_2 (s)
νNH (cyclic dimer)
d_8-PS/PBS
L-Arginine (15 mg/mL)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm^{-1})


Arginine
(Arg)
R
pKa 2.03
pKa 9.00
pKa 12.10


d_8-PS/PBS
L-Cysteine (25 mg/mL)
ν_{CH_2} (as)
or
ν_{CH} & CH_2(s) (combination band)
ν_{CH_2} (as)
ν_{CH_2} (s)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm^{-1})

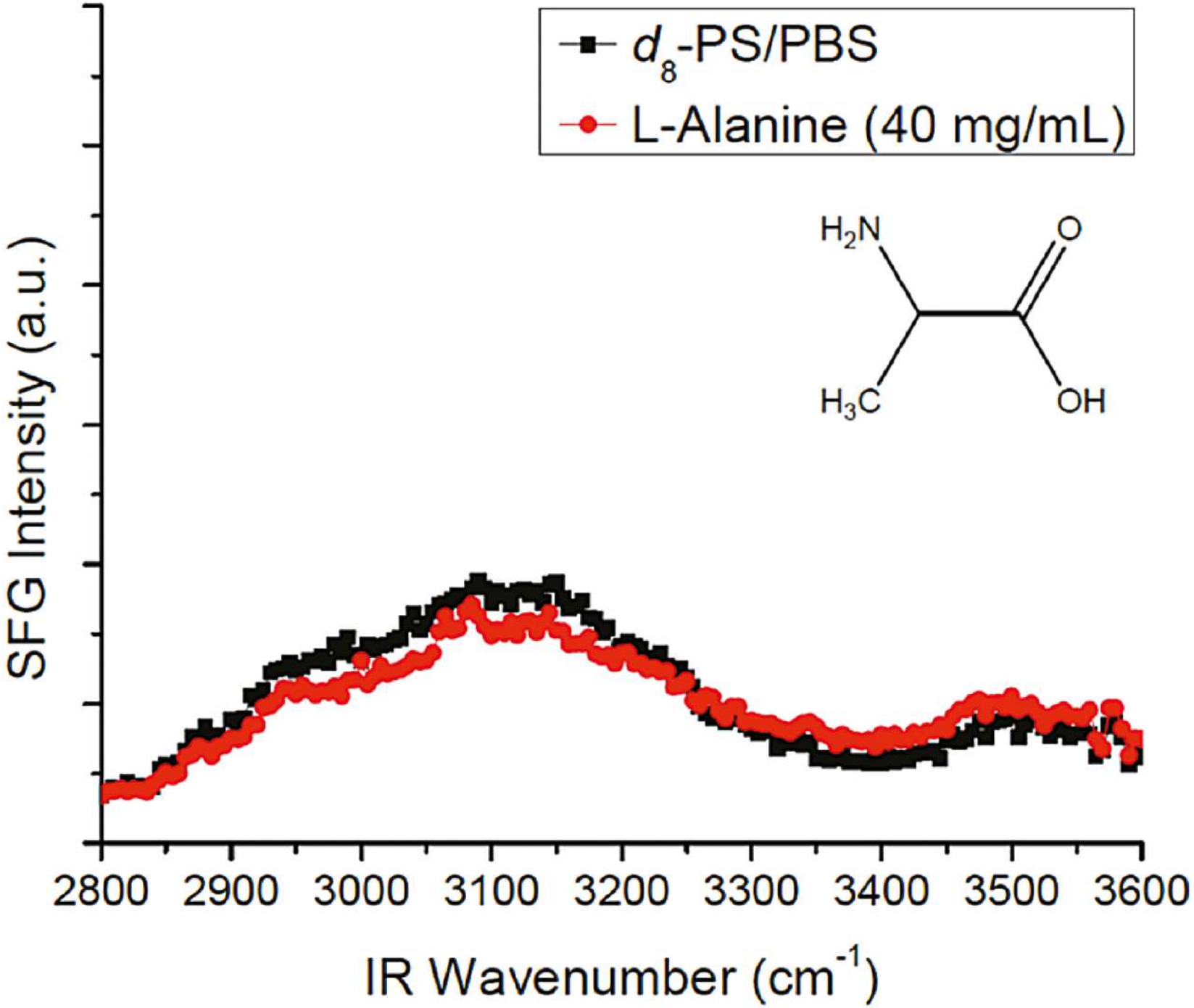

Cysteine
(Cys)
C
pKa 1.91
HO
O
NH_2
pKa 10.28
SH
pKa 8.14

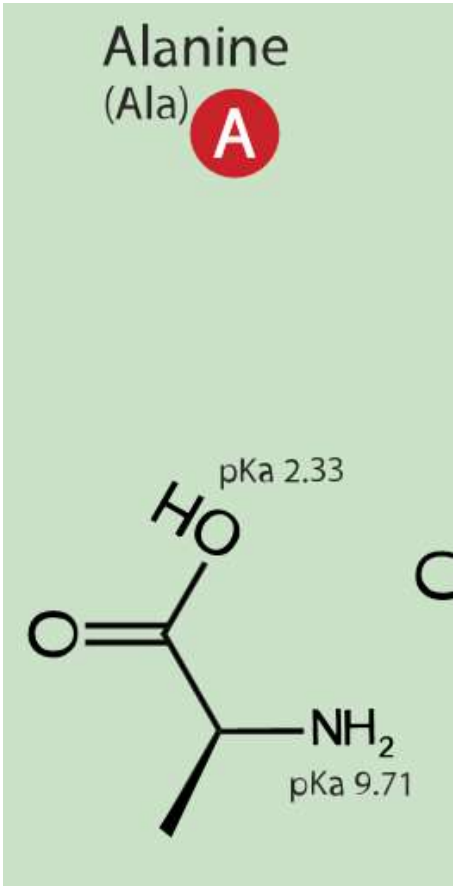




3000-3400/cm region

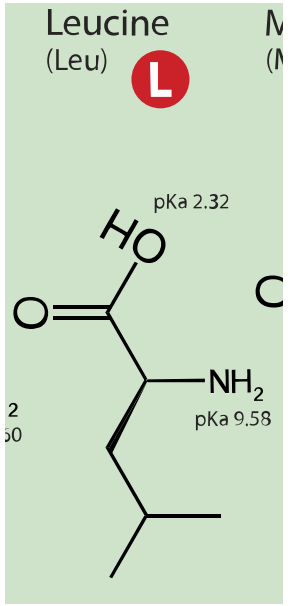
OH and NH contribution

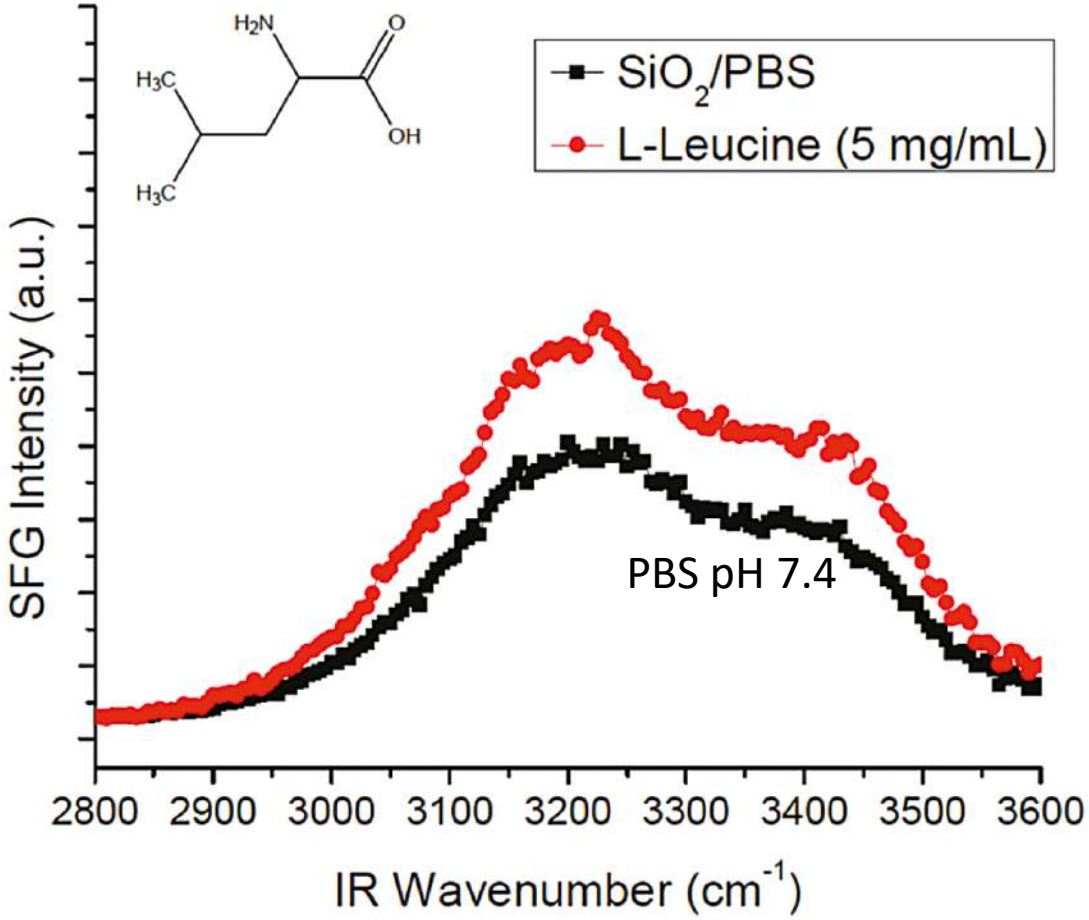




- No CH band due to molecular arrangement, not to absence.
- QCM: 469 ng/cm^2

SiO_2/PBS: Black spectrum





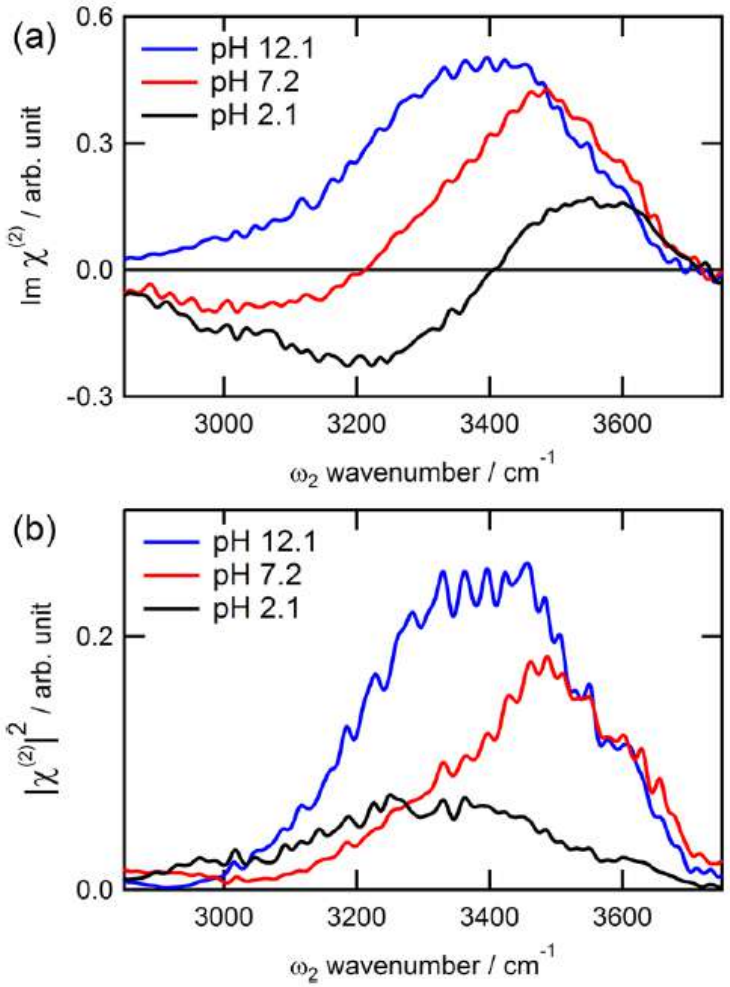
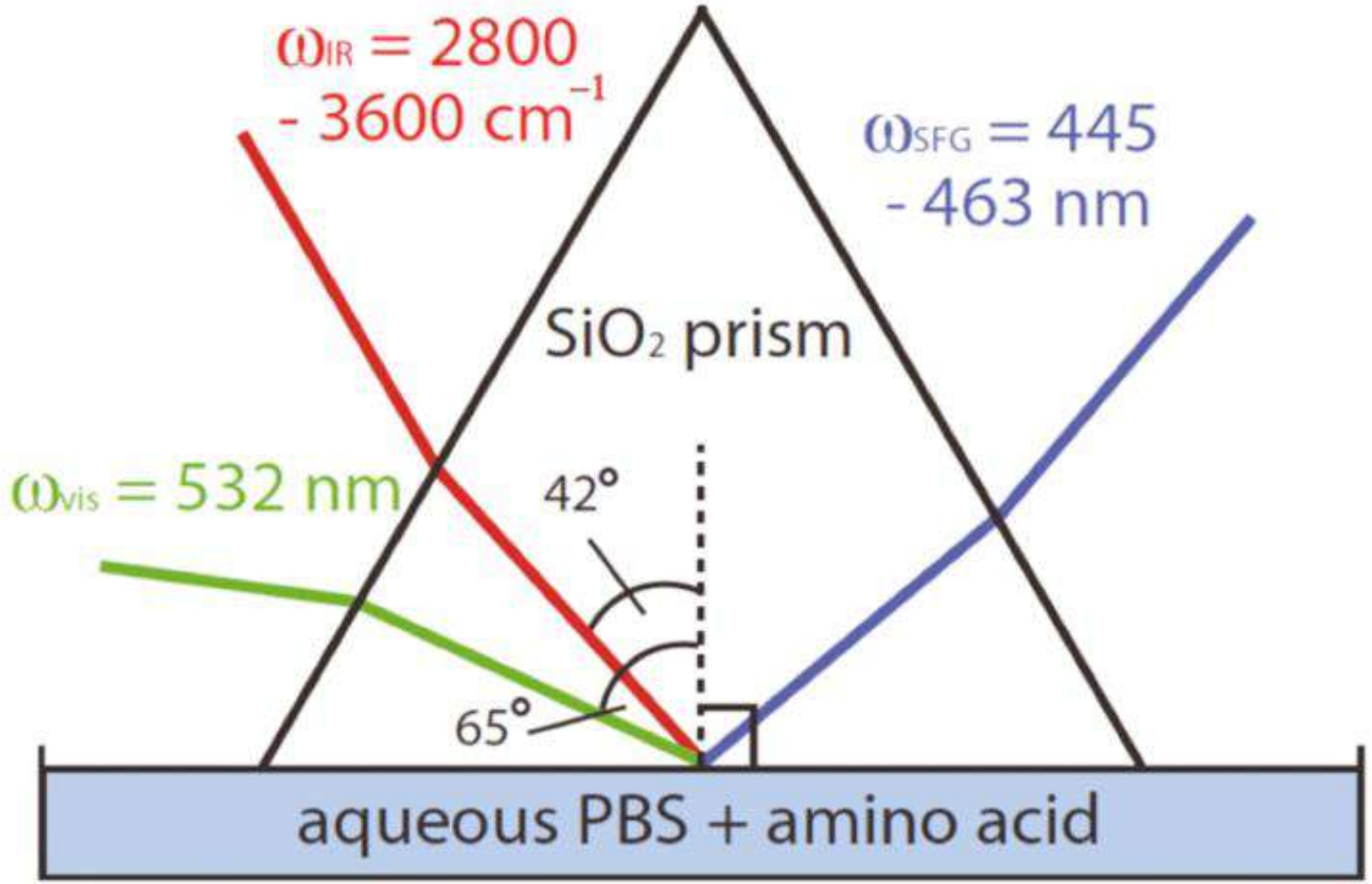


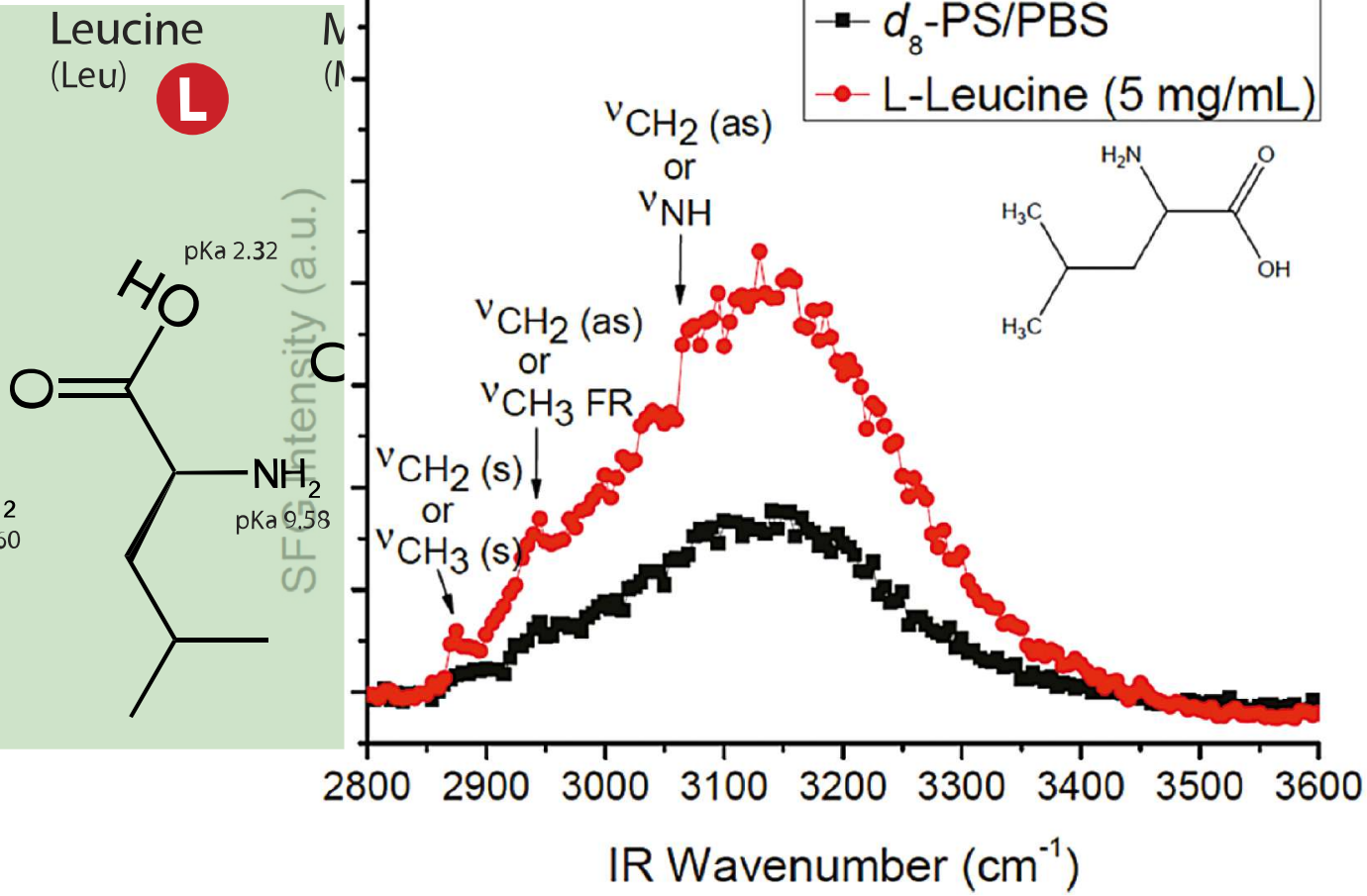
Figure 3. (a) Imaginary part of the $\chi^{(2)}$ spectra of the buried silica/HOD-D_2O interface for different pH in phosphate buffer (I = 10 mM, ratio of H_2O:HOD:D_2O = 1:8:16). (b) Corresponding magnitude square of the $\chi^{(2)}$ spectra ($|\chi^{(2)}|^2$ spectra) of the buried silica/HOD-D_2O interface for different pH in phosphate buffer. The pH of the solution for each spectrum is indicated in the Figure.

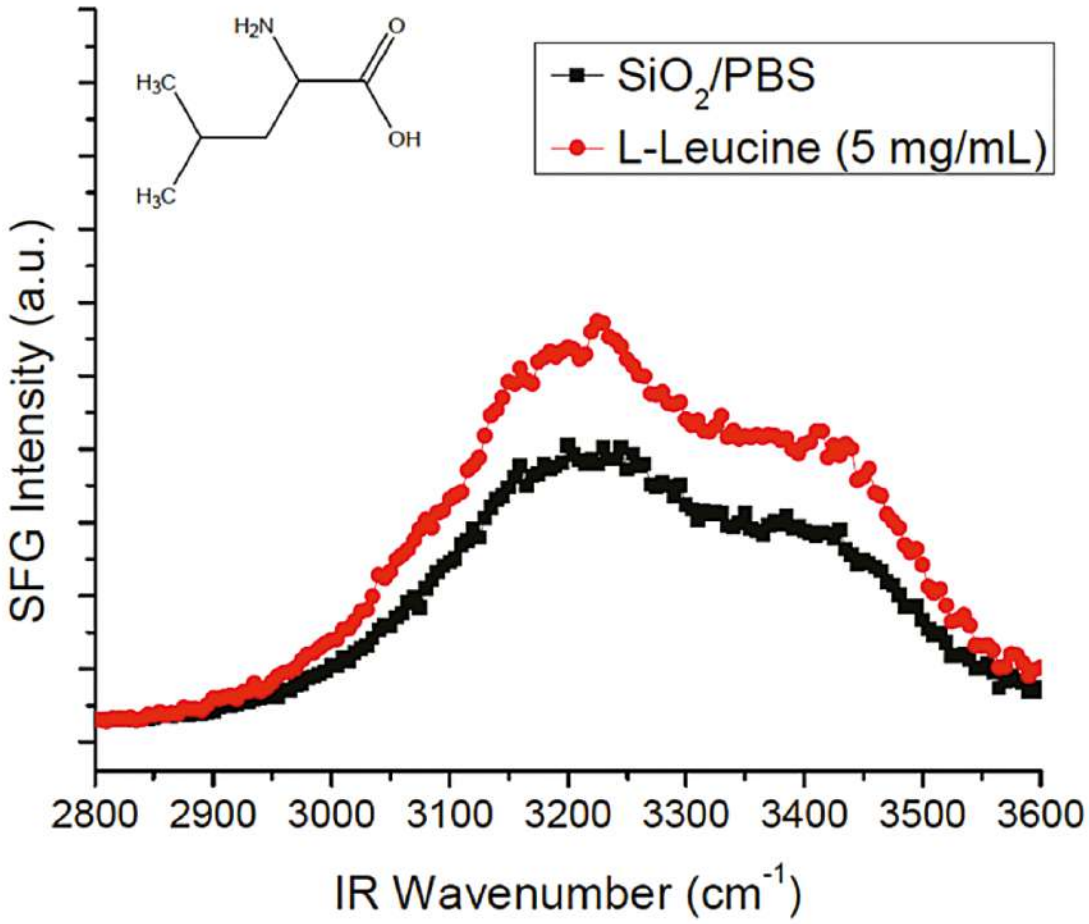
J. Phys. Chem. C 2016, 120, 9357−9363

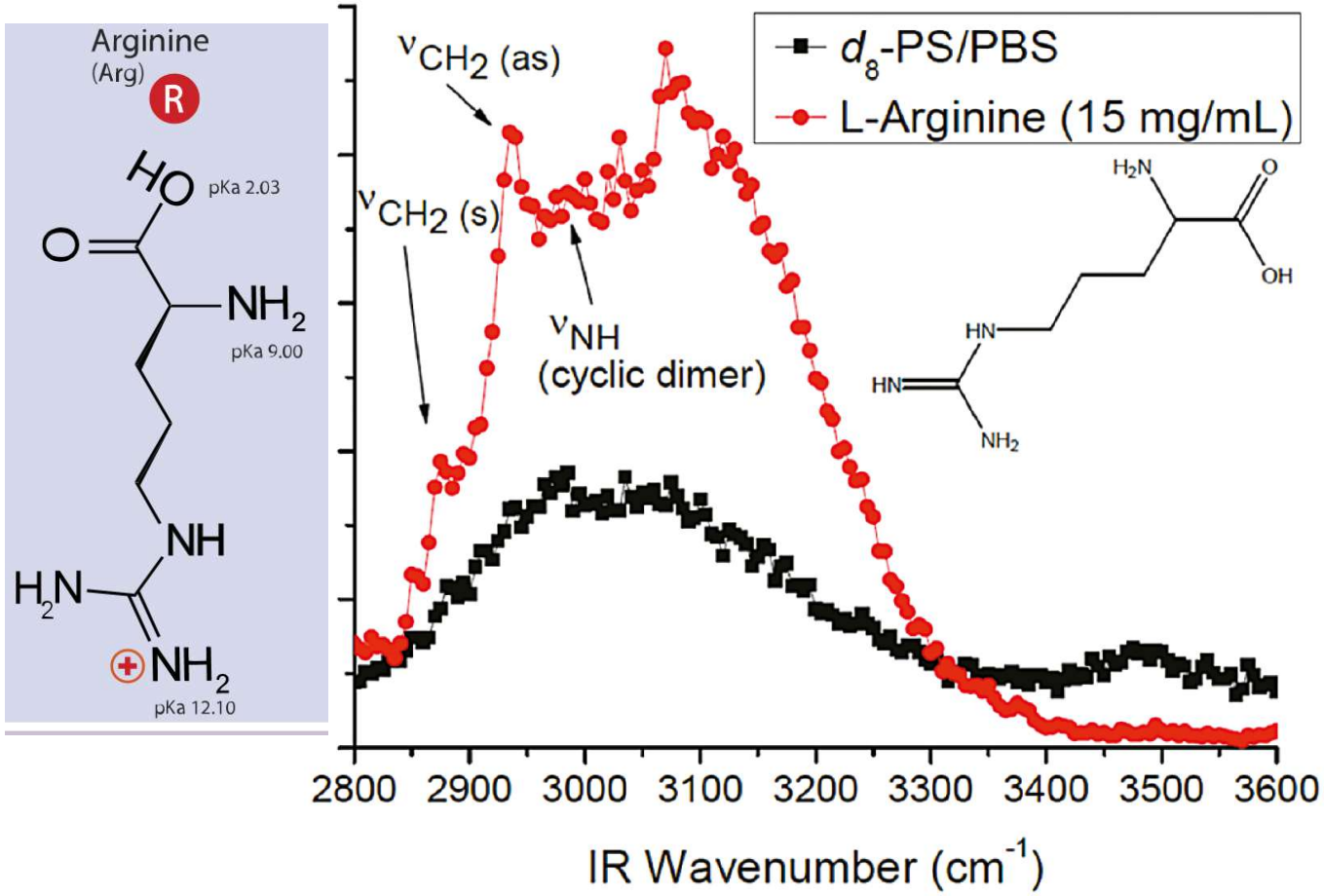
SFG results
No d-PS coated

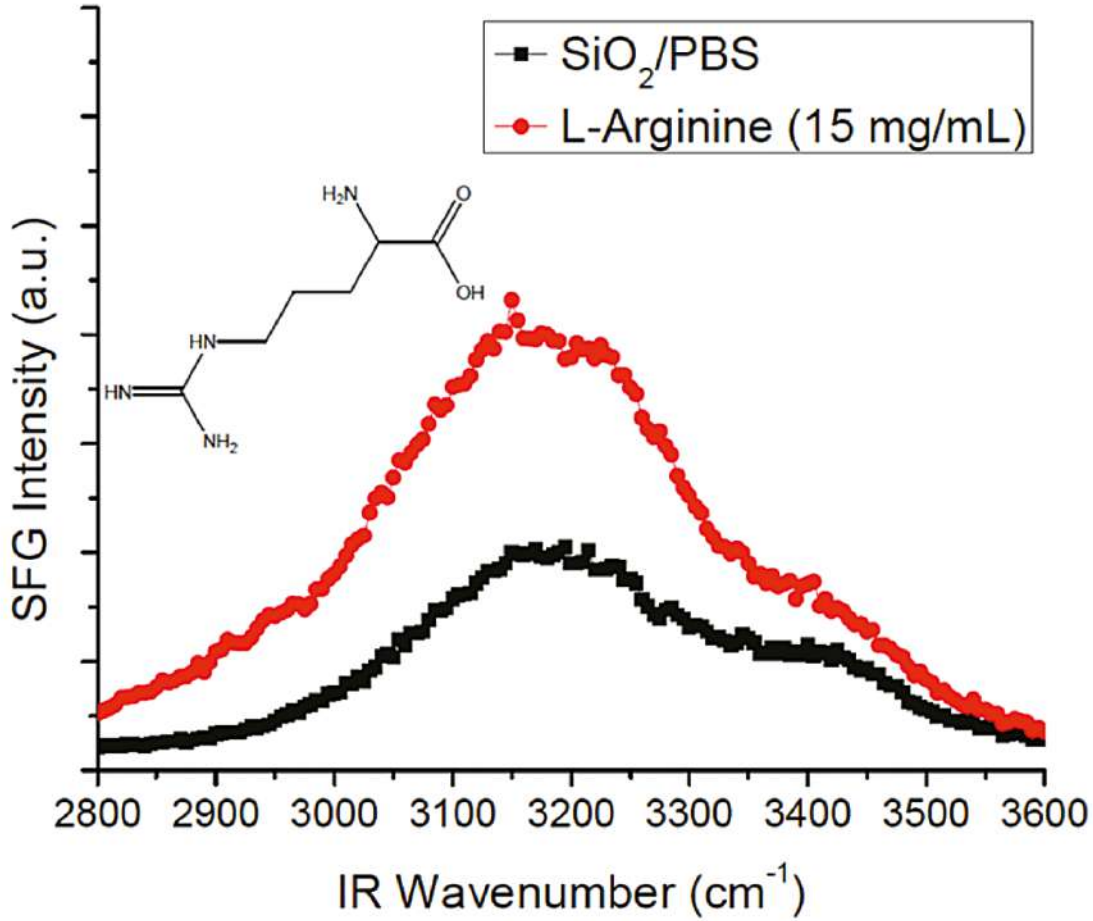


L-Leucine, L-Arginine, and L-Lysine

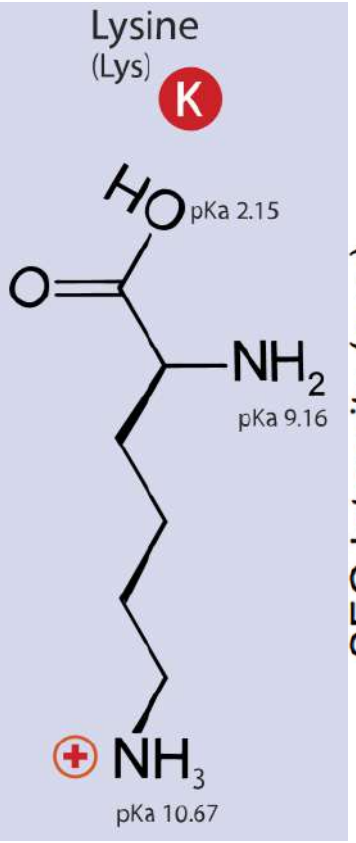


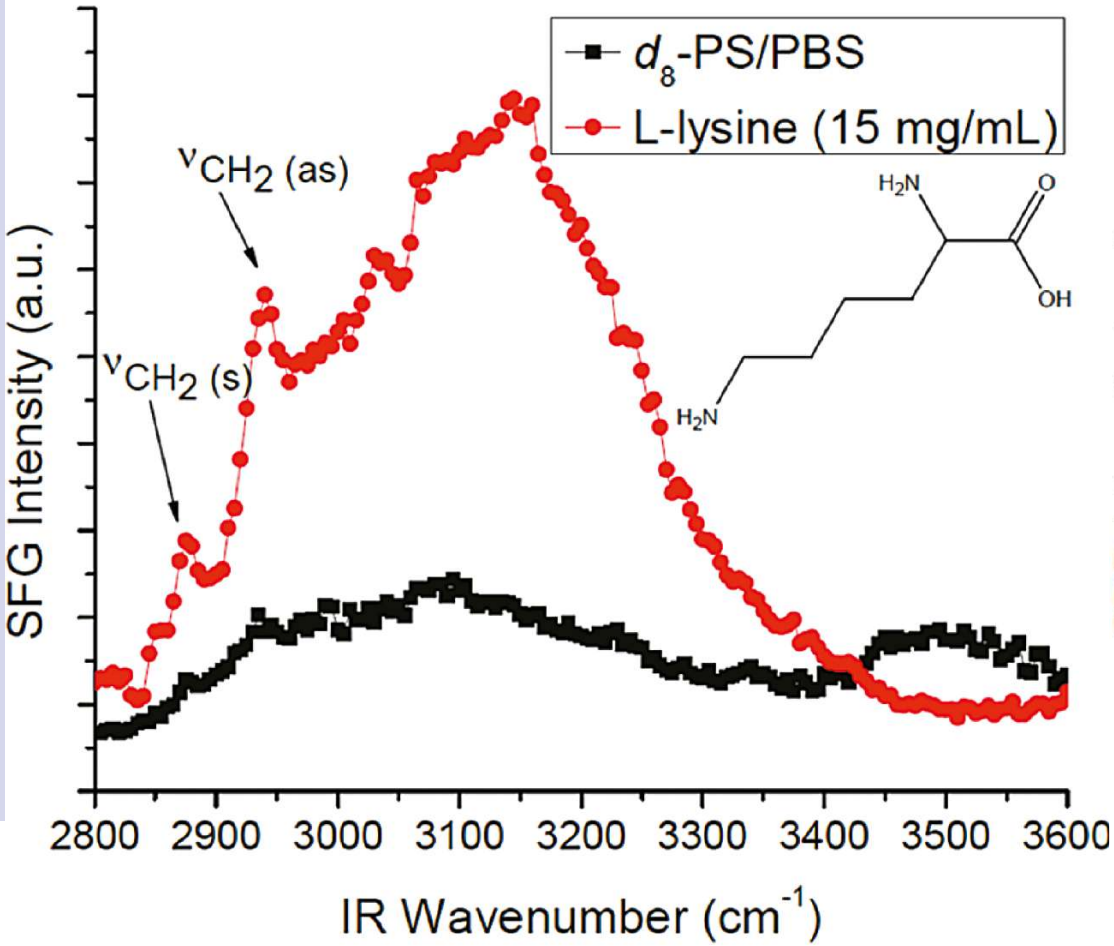


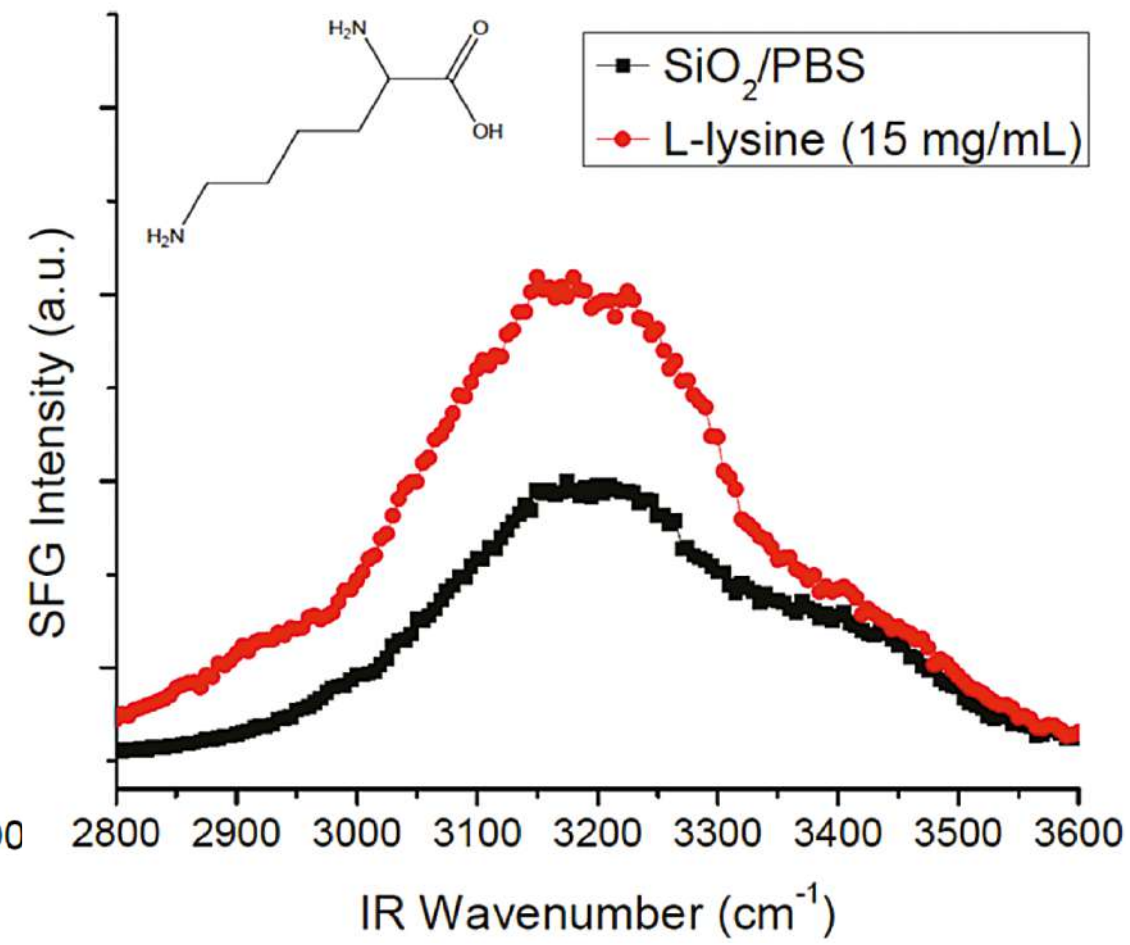


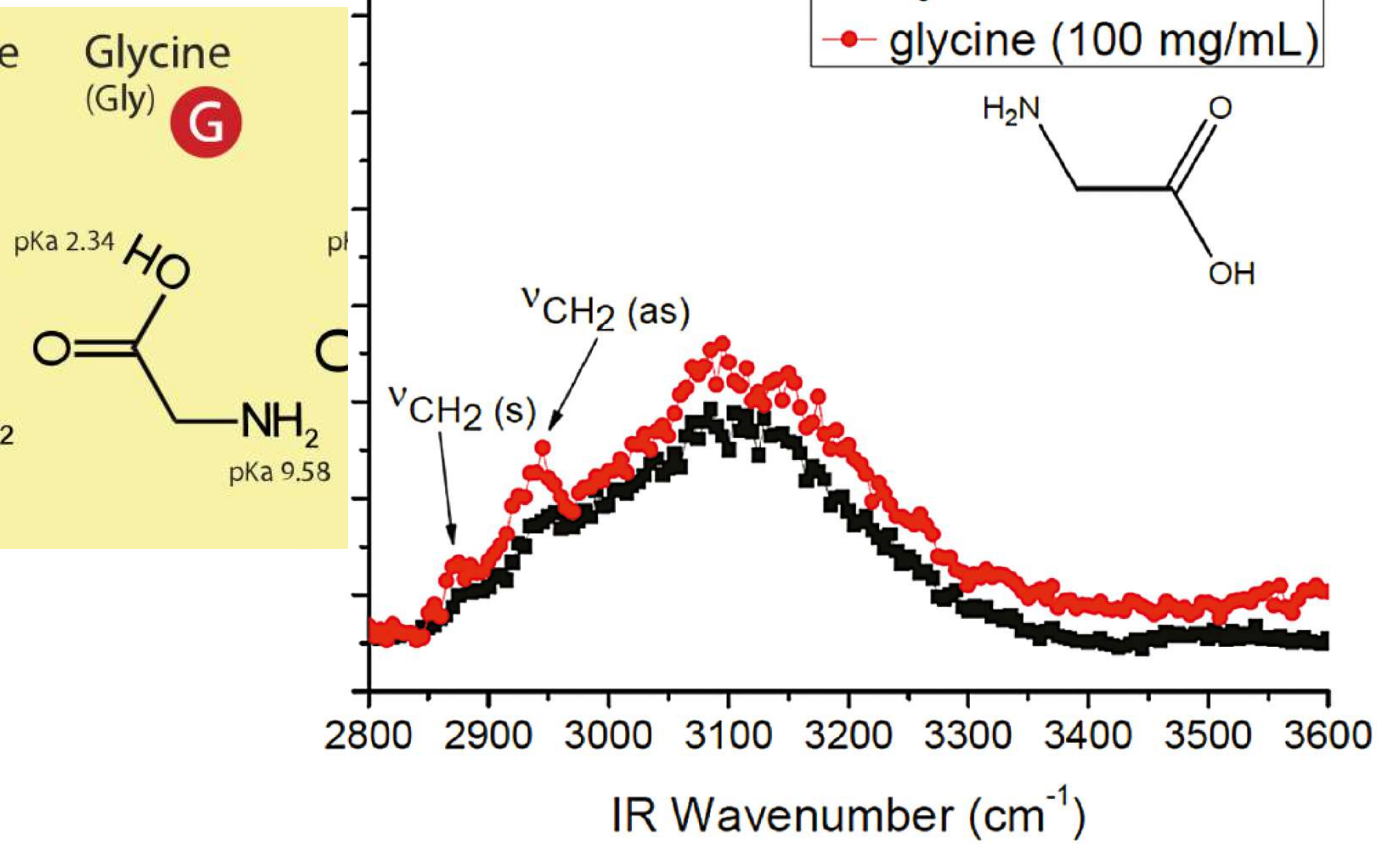


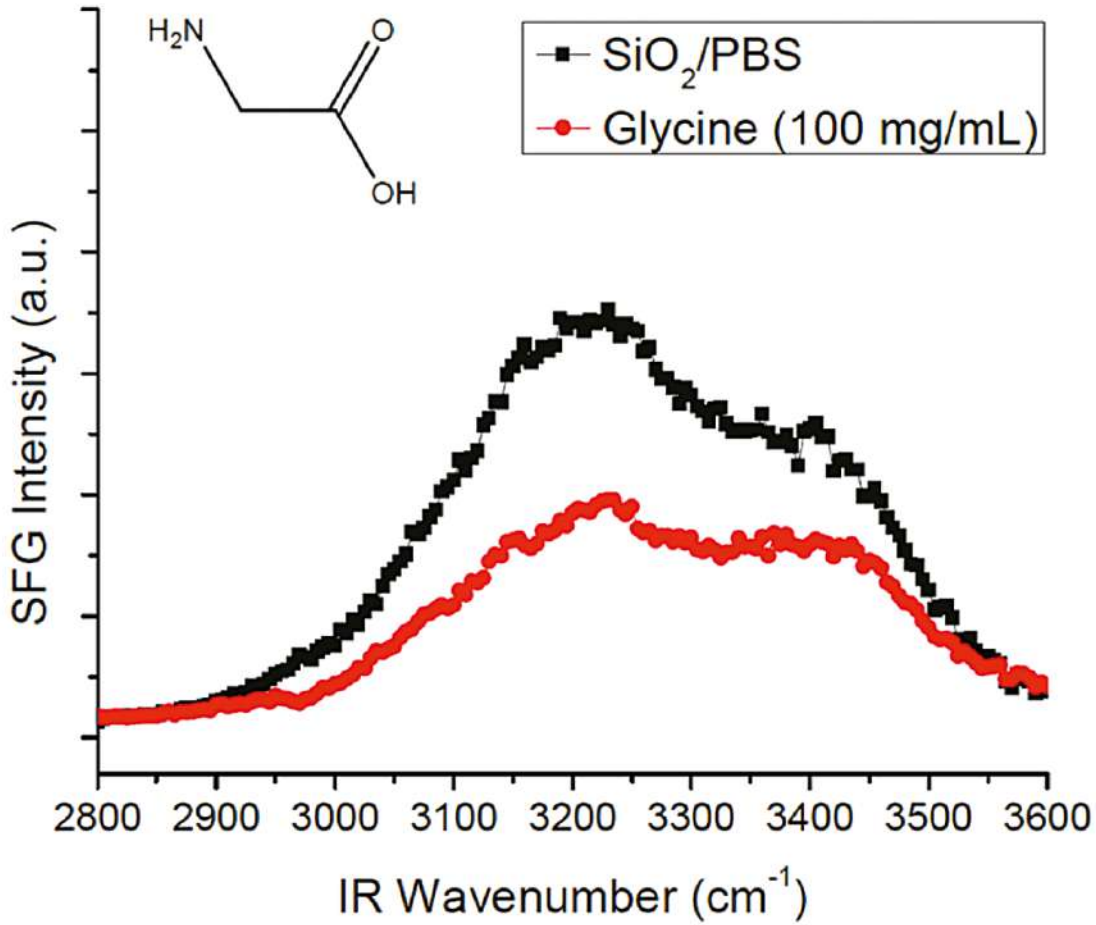
Band Broadening due to NH


Lysine
(Lys)
K
pKa 2.15
NH2
pKa 9.16
NH3
pKa 10.67

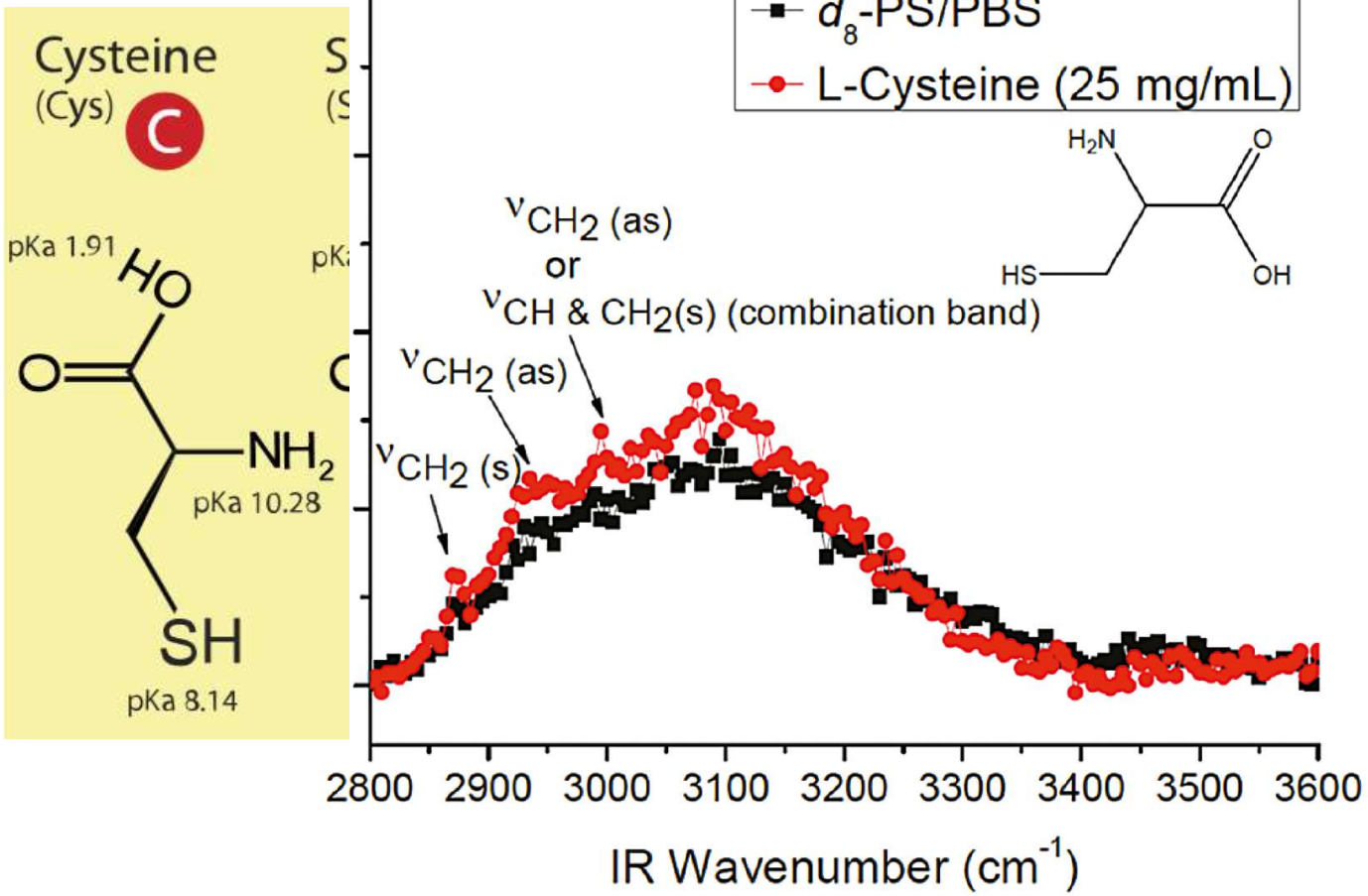

d8-PS/PBS
L-lysine (15 mg/mL)
νCH2 (as)
νCH2 (s)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)


SiO2/PBS
L-lysine (15 mg/mL)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)

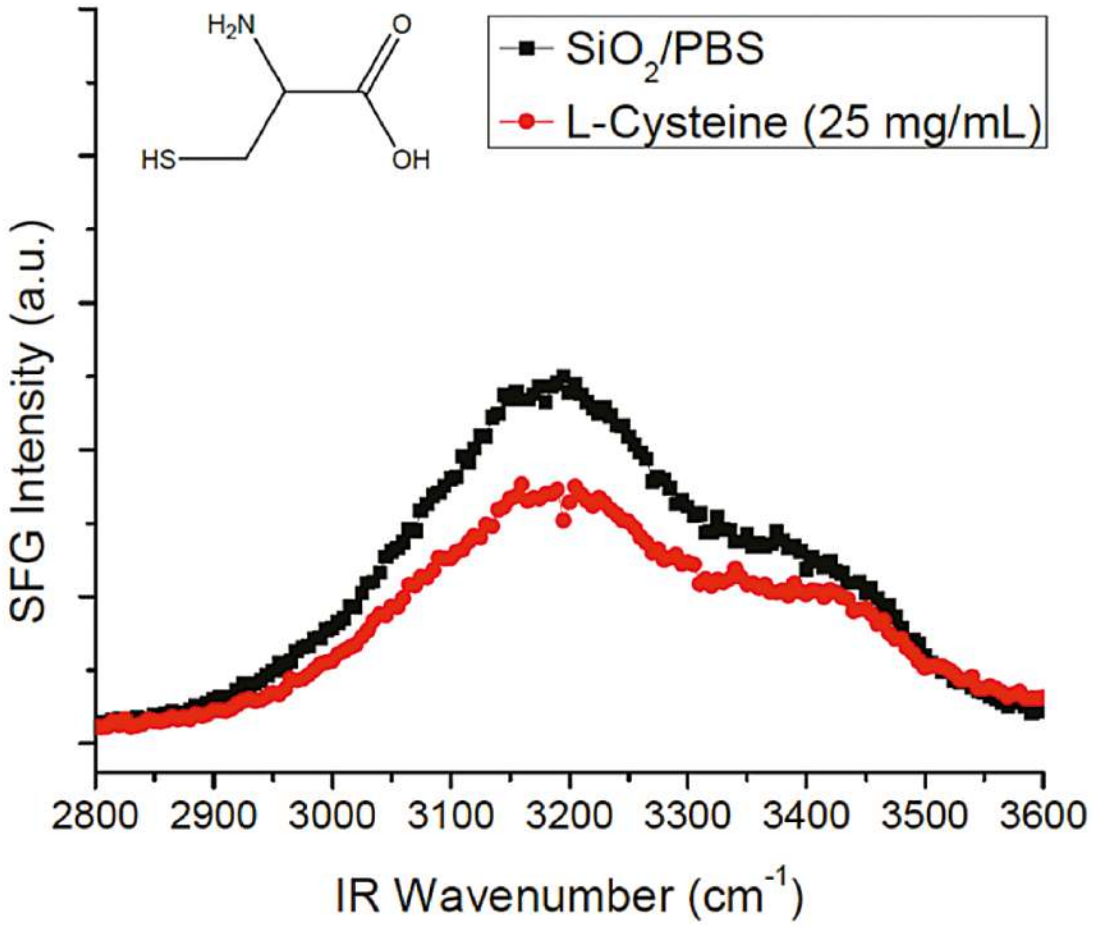


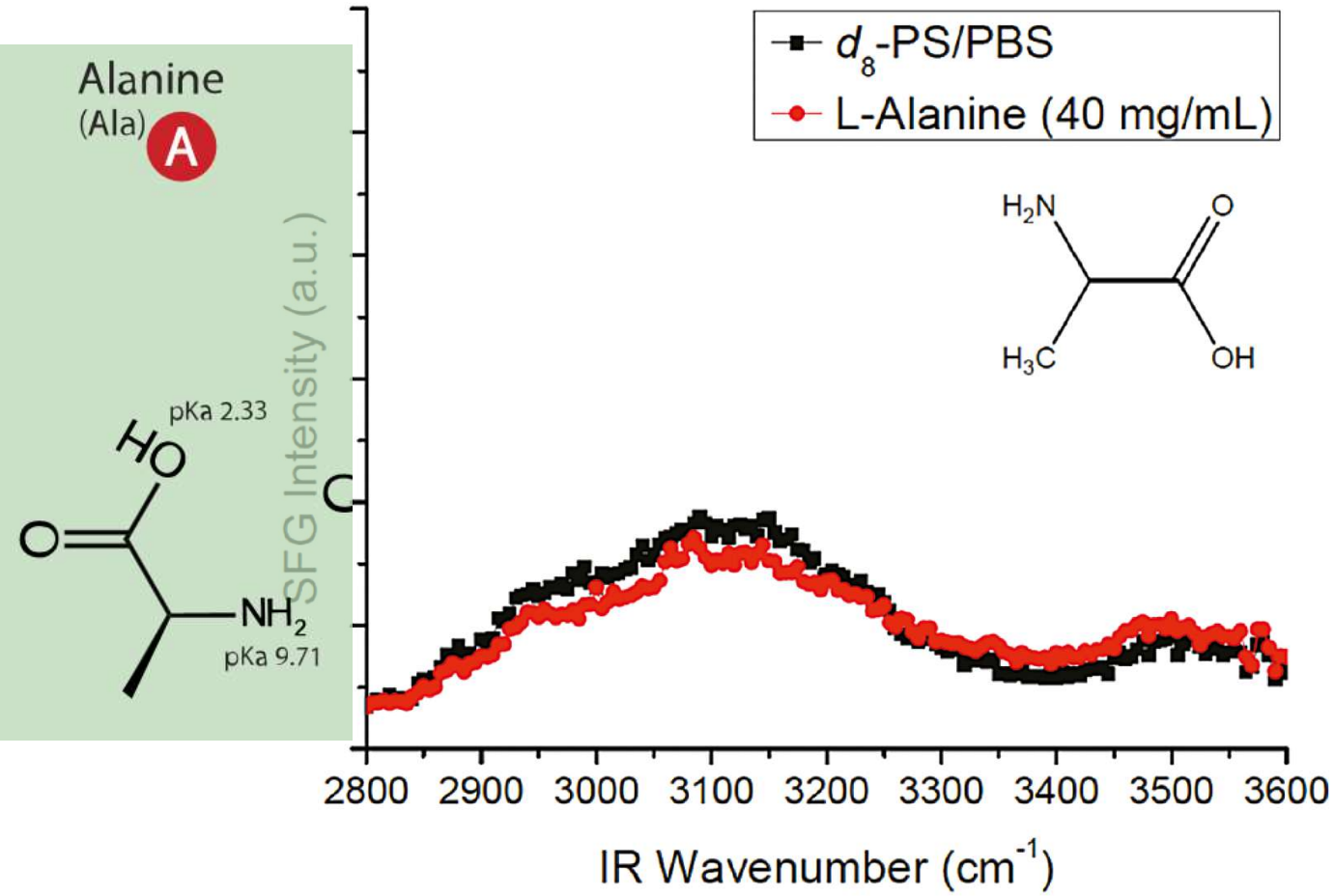


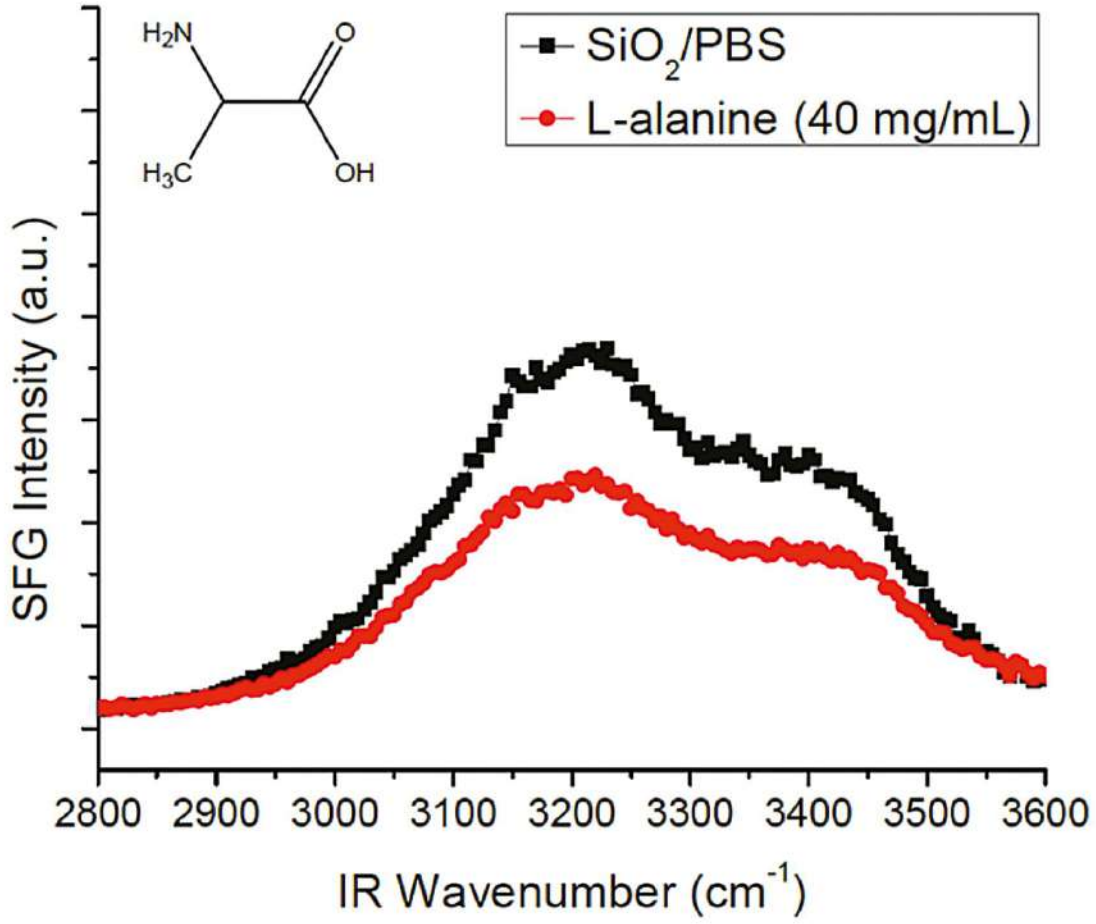
Weaker OH due to
higher solution concentration
displacement of interfacial water molecule.


Cysteine
(Cys)
C
pKa 1.91
HO
O
NH2
pKa 10.28
SH
pKa 8.14

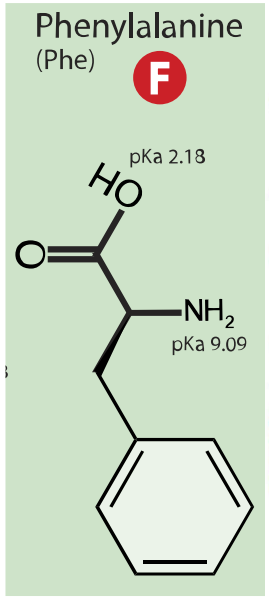
d8-PS/PBS
L-Cysteine (25 mg/mL)
H2N
O
HS
OH
νCH2 (as)
or
νCH & CH2(s) (combination band)
νCH2 (as)
νCH2 (s)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)

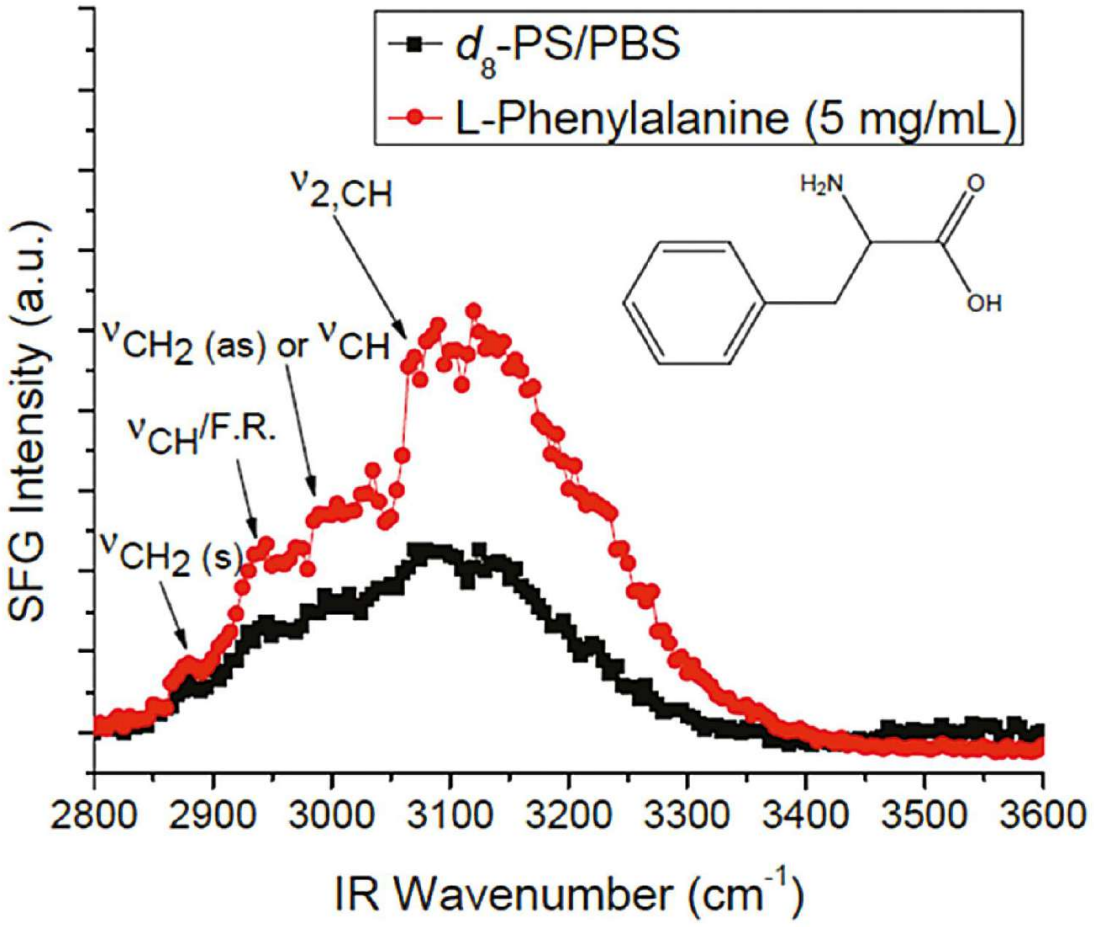

H2N
O
HS
OH
SiO2/PBS
L-Cysteine (25 mg/mL)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)

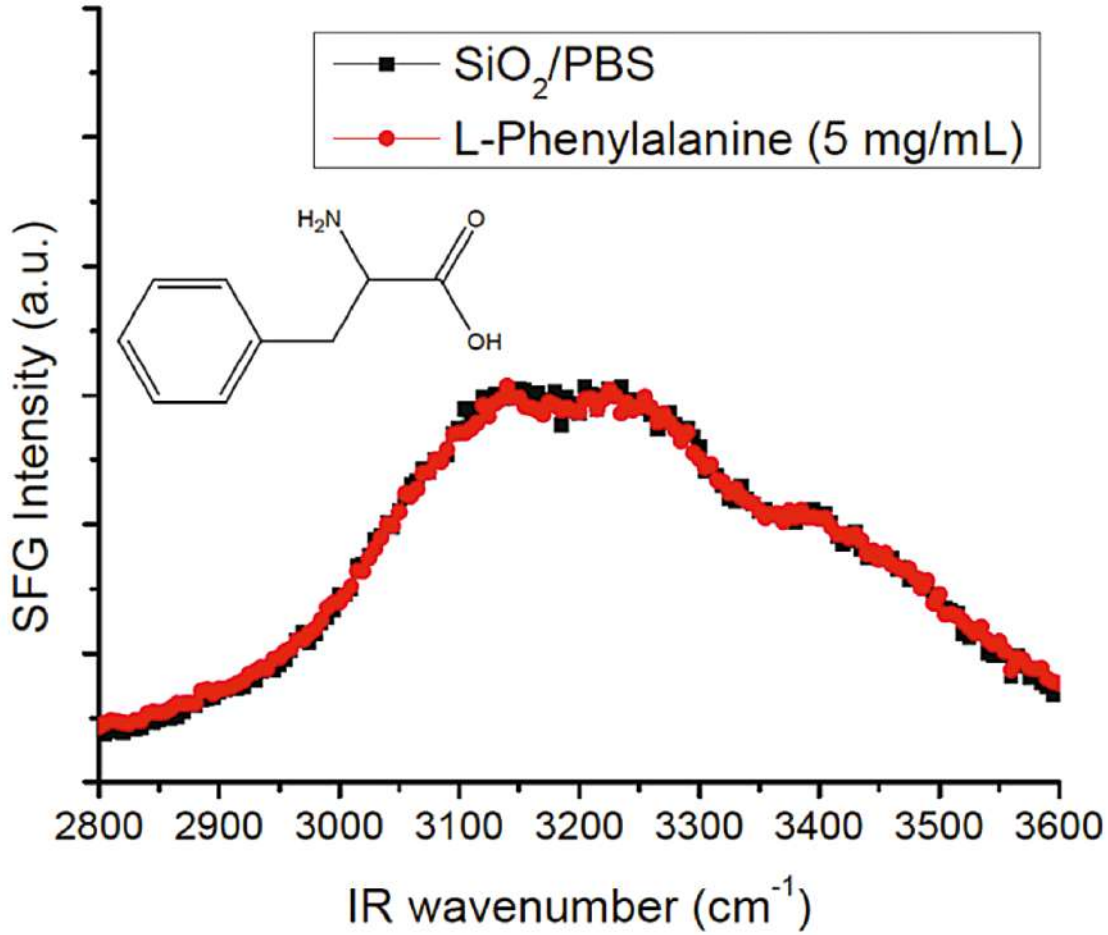

Alanine
(Ala)
A
pKa 2.33
pKa 9.71
SFG Intensity (a.u.)
d_8-PS/PBS
L-Alanine (40 mg/mL)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm^{-1})


SiO_2/PBS
L-alanine (40 mg/mL)
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm^{-1})

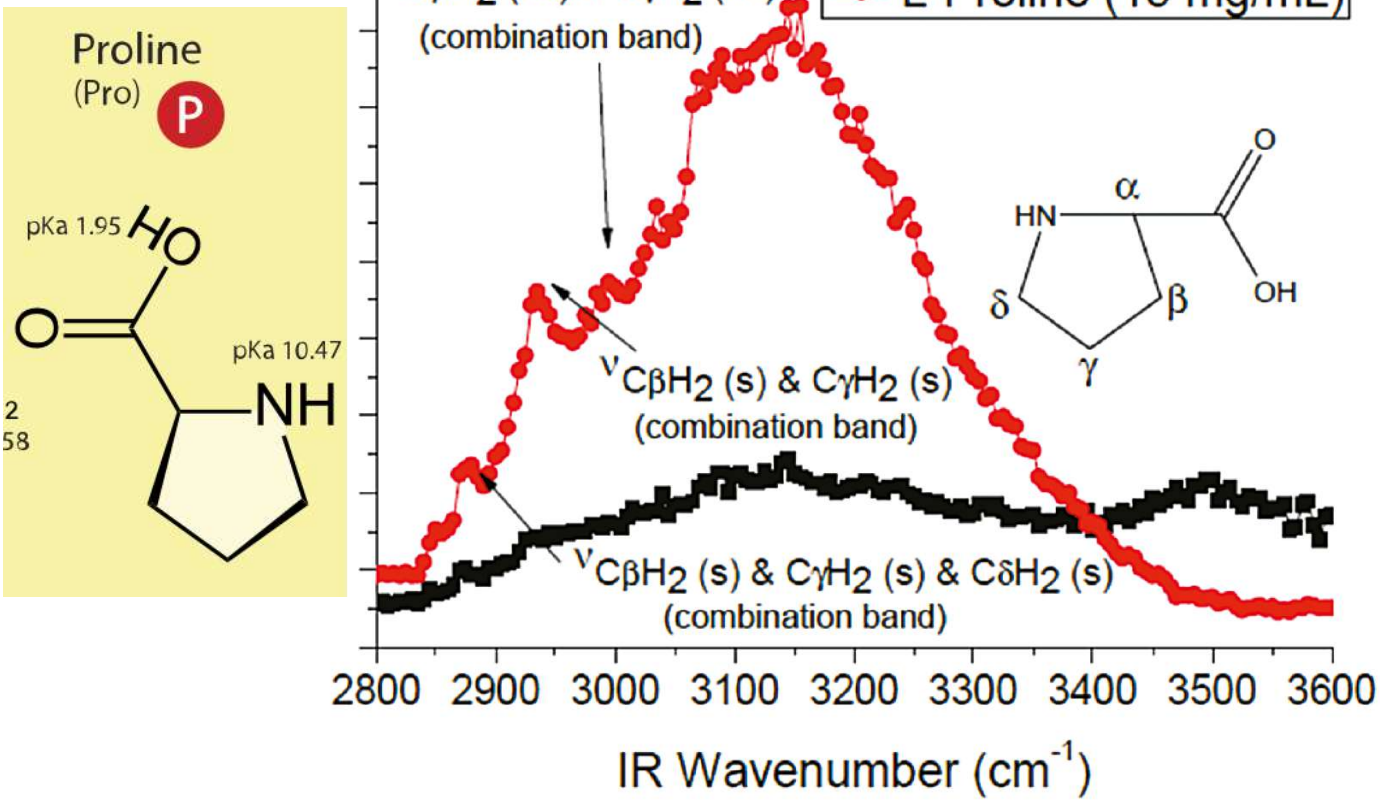
L-Phenylalanine and L-Proline.



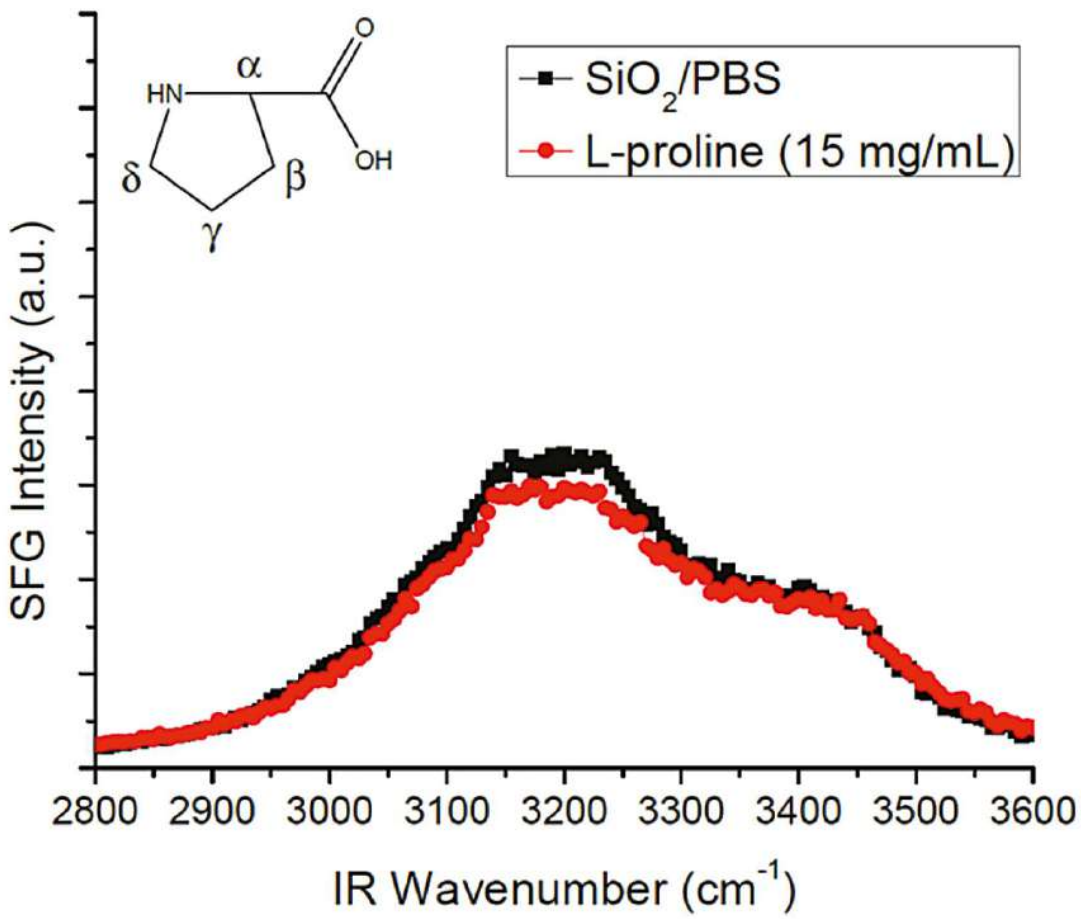




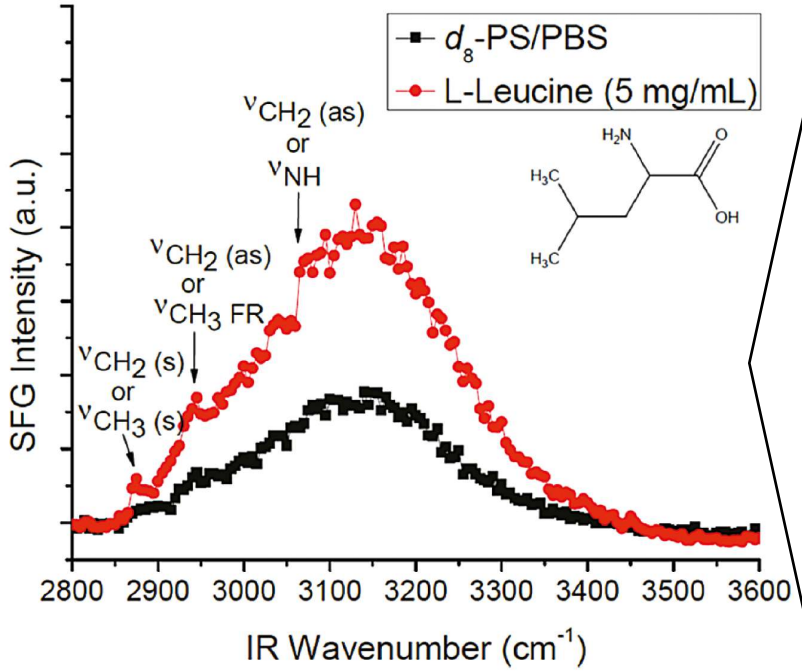
QCM: 63 ng/cm$_2$ on SiO_2 surface
Same spectrum: due to surface organization, not to an absence


Proline
(Pro)
P
pKa 1.95
HO
O
pKa 10.47
NH
2
58

d_8-PS/PBS
L-Proline (15 mg/mL)
νCβH2 (as) & CγH2 (as)
(combination band)
νCβH2 (s) & CγH2 (s)
(combination band)
νCβH2 (s) & CγH2 (s) & CδH2 (s)
(combination band)
HN
α
O
OH
δ
β
γ
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)

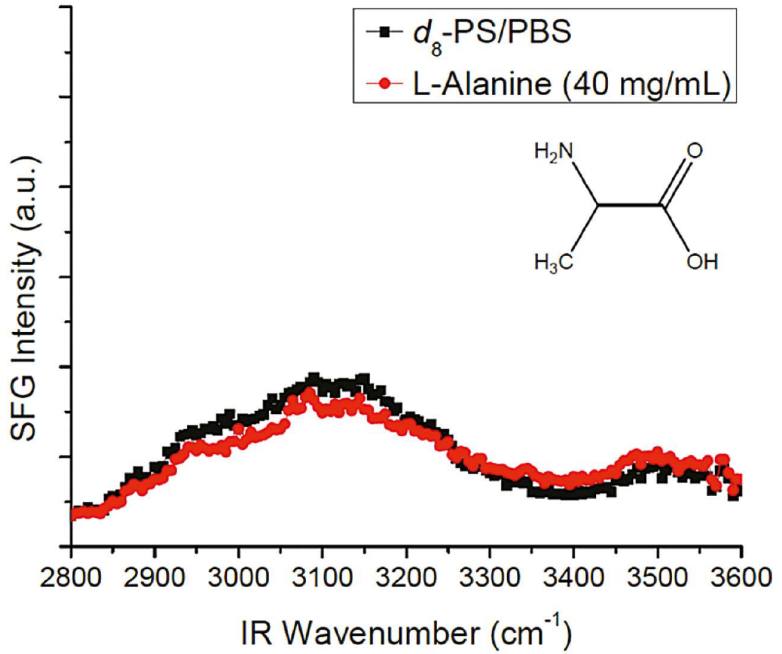

SiO2/PBS
L-proline (15 mg/mL)
HN
α
O
OH
δ
β
γ
SFG Intensity (a.u.)
2800 2900 3000 3100 3200 3300 3400 3500 3600
IR Wavenumber (cm-1)

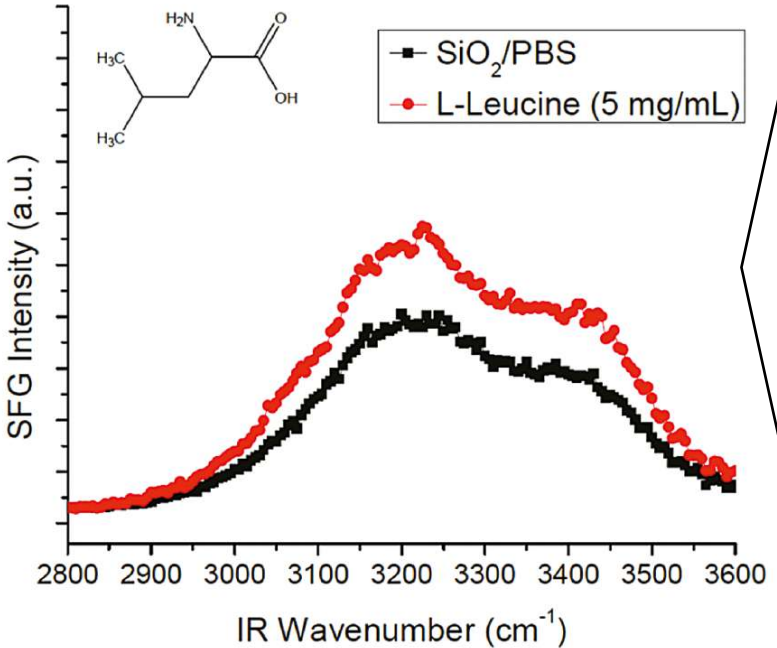
Conclusion



7 over 8 show CH except L-Alanine

(due to configuration of molecule at surface)





No CH=not well ordered as in d-PS case

OH: concentration, frequency of interaction