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Sum frequency generation spectroscopy probing of formation of self-assembled monolayers from thiols with terminal phenylalanine ring and intrachain amide groups

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Formation of a self-assembled monolayer onto the polycrystalline gold substrate from thiols containing a terminal phenylalanine (Phe) ring and intrachain amide groups ($HS-(CH_2)_7-CONH-(CH_2)_2-C_6H_5$) was characterized by vibrational sum frequency generation (SFG) spectroscopy. The temporal evolution of the characteristic Phe ring stretching vibration ν(=C–H) near 3069 cm^{-1} and the Amide A mode were monitored by nonlinear vibrational spectroscopy. The SFG data revealed the complete ordering of the terminal Phe rings after about 60 min of adsorption. Formation of a strong hydrogen bonding between the adjacent chains of adsorbed molecules was evident by appearance of the Amide A mode at the relative low frequency (3280–3308 cm^{-1}). The well-ordered and strongly hydrogen bonded SAM with the terminal Phe ring functionality is a promising platform for the analysis of interactions and the function of aromatic rings in biomolecular processes.

Keywords: SAM, phenylalanine, amide, SFG, Raman

INTRODUCTION

Self-assembled monolayers (SAMs) of bifunctional thiol compounds on noble metal surfaces provide a useful platform for a detailed analysis of the interaction of functional groups with solution components and adsorbed molecules, studies of electron transfer, construction of (bio)sensors, electrocatalytical studies, and development of bioelectronics devices [1–4]. The structure of the monolayer controls the stability and function of the terminal group. The important interactions responsible for the architecture of the monolayer are (i) covalent bonding between the sulfur atom and the metal, (ii) interactions between the hydrocarbon chains, and (iii) properties of the terminal functional group. Introduction of amide functionality in the hydrocarbon chain of adsorbing molecules considerably increases the stability of the formed SAMs due to the formation of hydrogen bonds between the adjacent molecular chains in the monolayer [5–8]. A terminal phenylalanine (Phe) ring group containing monolayers provide a possibility to study various interactions of the ring with solution components and adjacent thiols in the monolayer. Phe possesses a delocalized π-electron cloud ring which participates mainly in the π-π interaction with other aromatic residues and the cation-π interaction with the positively charged metal ions (Na^+, K^+) [9–12].

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Such interactions stabilize the tertiary structure of proteins [12]. Bifunctional monolayers on metal surfaces may provide a promising platform to get insights into various interactions of aromatic moieties important for the biological function of peptides and proteins.

To prepare stable and functional monolayers with controllable properties, detailed molecular level information on the structure and interactions between the functional groups in the monolayer is required. Vibrational spectroscopy is able to provide such knowledge. In particular, sum frequency generation (SFG) spectroscopy is both a surface and molecule specific tool able to provide rich atomistic information on bonding, interactions, and orientation of particular molecular groups [13–19]. Within the electric dipole approximation the SFG signal arises from the overlapped visible and IR beams at the interface where the inversion symmetry is broken. The signal generation is forbidden in the centrosymmetric media or in the case of randomly oriented molecules. Because at the interfaces the inversion symmetry is broken, SFG is an intrinsically surface specific technique. Molecular sensitivity comes from the capacity to record the vibrational spectrum. To be active in the SFG spectrum the particular vibrational mode must be active in both the Raman and infrared spectra.

In this work, SFG spectroscopy was applied to determine structural transformations during the formation of a self-assembled monolayer from the thiols containing a terminal phenylalanine ring and an intrachain amide group.

EXPERIMENTAL

Millipore-purified water (18 MΩ cm) was used for the preparation of monolayer formation solutions. The monolayer forming compound (N-(2-phenylethyl)-8-sulphanyloctanamide) (MOPHE) (Fig. 1) was synthesized in our laboratory. The synthesis details will be published elsewhere. 8-Bromooctanoic acid (97%) and 2-phenylethylamine (99%) were purchased from Sigma-Aldrich Chemie GmbH (Germany) and were used without any additional purification. N-(2-phenylethyl)-8-sulphanyloctanamide was obtained through the amidation of 8-bromooctanoic acid with phenylethylamine and further by replacing bromine to thiol. The used inorganic salts were of ACS grade and were purchased from Sigma-Aldrich Chemie GmbH (Germany).

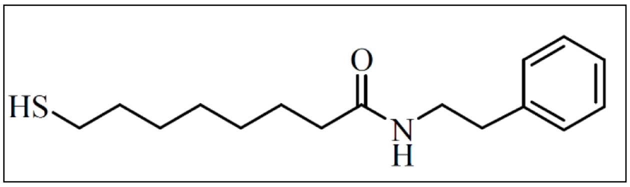

Fig. 1. The molecular structure of N-(2-phenylethyl)-8-sulphanyloctanamide (MOPHE)

Gold substrates were prepared by magnetron sputtering of the Cr sublayer (~3 nm) and subsequently the 200 nm layer of Au on the cleaned glass substrates. These substrates were immersed into a 10 mM MOPHE ethanol solution for various adsorption times. Subsequently the substrates were rinsed with ethanol (≥99.8%) and dried under N_2 flow.

Sum frequency spectra were collected on an EKSPLA (Vilnius, Lithuania) picosecond SFG spectrometer PG501/DFG + PL2251A + SFG046. The spectrometer is based on a mode-locked EKSPLA PL2251A Nd:YAG laser generating 30 ps pulses at 1064 nm with the 50 Hz repetition rate. The second harmonic radiation from this laser was used as a visible beam (ω_{VIS}). The tunable infrared pulses (ω_{IR}) in the 1000–3800 cm^{-1} frequency range were produced in a parametric generator EKSPLA PG501/DFG. The bandwidth of infrared radiation was <6 cm^{-1}. To produce SFG spectra, the ω_{IR} and ω_{VIS} beams were incident at angles of 55 and 60°, respectively, and overlapped at the sample within an area of 0.04 mm^2. The sum frequency (ω_{SF}) radiation was filtered by a holographic notch filter and monochromator and detected by a photomultiplier tube (PMT) and gated registration system. Typically, the signal was averaged over 200 pulses. The SFG spectra were normalized according to the intensity of the infrared beam to take into account the changes in ω_{IR} energy with the wavelength. The frequencies were calibrated by the IR spectrum of the polystyrene film placed in the path of the infrared beam.

Raman spectra were recorded with an Echelle spectrometer RamanFlex 400 (PerkinElmer, Inc.) by using the 785 nm radiation of the diode laser as an excitation source. The spectra were registered with a CCD camera thermoelectrically cooled down to −50°C. A fiber-optic cable served for excitation and collection of the Raman spectra. The laser beam (100 mW power) was focused to a 200 μm diameter spot on the sample. The integration time was 300 s. The relative intensities of Raman spectra (instrument response function) were calibrated by using the luminescence of the NIST Intensity Standard SRM 2241 [20]. The Raman frequency axis was calibrated by the spectrum of the polystyrene standard ASTM E 1840.

RESULTS AND DISCUSSION

Figure 2 compares the ppp-polarized (p-SFG, p-532 nm and p-IR beams) SFG spectra of MOPHE adsorption layers at the gold substrate obtained at different immersion times in the adsorption solution. The SFG resonances from the MOPHE monolayer appear as the negative-going features because of the interference of molecular signals with the gold background [13]. The well-defined resonance at 3069 cm^{-1} is associated with the characteristic Phe ring stretching vibration, ν(=C–H) (F2) [21]. Frequency of this mode is sensitive to the environment and interactions between the Phe rings. Previously, we have demonstrated that for the monolayers with a pyridinium ring terminal group the ν(=C–H) mode can be employed as the solvent dielectric constant marker [22]; the wavenumber downshifts with increasing the dielectric constant. Figure 3 compares the Raman spectra of the MOPHE compound in the solid state and dissolved in methanol. One can clearly see the downshift of the F2 mode frequency by as much as 7 cm^{-1} going from the methanol solution (0.1 M) to the solid state Raman spectrum. In the SFG spectrum the frequency of F2 resonance is upshifted by 4–5 cm^{-1} compared with the Raman spectrum of MOPHE dissolved in methanol. Interestingly, the increase in adsorption time results in a slight upshift of the ν(=C–H) frequency (Fig. 2).

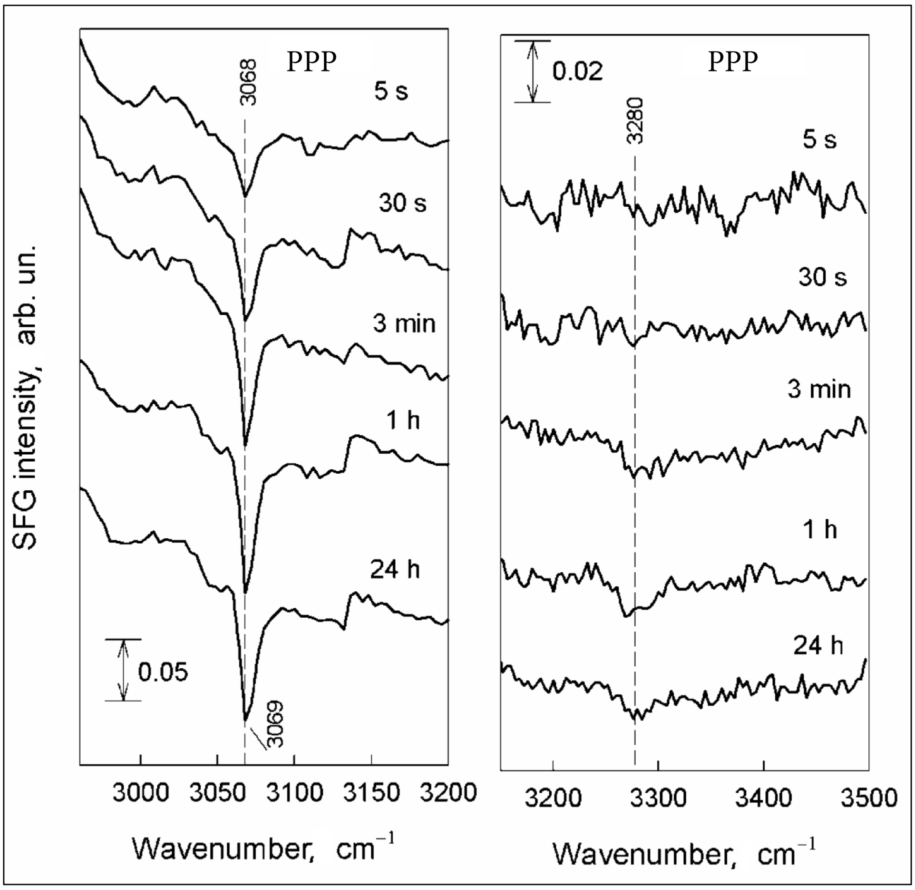

Fig. 2. Vibrational sum-frequency generation (SFG) spectra of MOPHE on the gold substrate at different immersion times in the adsorption solution in 2960–3200 cm^{-1} (left panel) and 3160–3500 cm^{-1} (right panel) spectral regions taken with ppp (p-SFG, p-532 nm, and p-IR) polarization combinations

The intensity of the SFG signal clearly increases for the samples kept in the adsorption solution for 5–180 s. The right panel of Fig. 2 shows the ppp-polarized SFG spectra in the frequency region where vibrational modes associated with the amide group ν(N–H) stretching vibration (Amide A) are expected to be observed. Indeed, the low intensity SFG resonance is visible near 3280 cm^{-1} for the SAM formed longer than for 3 min in the adsorbtion solution. In the Raman spectrum of the solid state MOPHE compound the ν(N–H) band appears at 3311 cm^{-1} (Fig. 3). The relative low frequency value indicates the strong hydrogen bonding between the adjacent amide groups [6]. It was demonstrated that for the long chain thiols with the intrachain amide group dissolved in CCl_4 the Amide A mode can be found at 3453 cm^{-1} [6]. A relatively high frequency value corresponds to an insignificant hydrogen bonding interaction of the amide groups in the CCl_4 solvent. One can see from Fig. 2 that the intensity of Amide A resonance is very weak in the ppp-polarized SFG spectra. Important information on the orientation of molecular groups with respect to the surface might be extracted from the analysis of differently polarized SFG spectra [13–17]. In the case of the gold substrate only the ppp- and ssp-polarized SFG spectra are expected to be observed, because an infrared beam must have a z component (parallel to the surface normal) [13]. The ssp-polarization combination probes the susceptibility component, $\chi^{(2)}_{yyz}$, while two components, $\chi^{(2)}_{zzz}$ and $\chi^{(2)}_{xxz}$, are responsible for the SFG signal in the ppp-polarized spectrum [13]. Figure 4 compares the ssp-polarized (s-SFG, s-532 nm and p-IR beams) SFG spectra of MOPHE adsorption layers at the gold substrate obtained at different immersion times in the adsorption solution. Again, an increase in the intensity of the Phe ring ν(=C–H) resonance near 3064–3067 cm^{-1} with increasing the adsorption time up to 1 h is clearly visible; however, the intensity of the SFG signal is considerably lower. It should be noted that the frequency shift of the ν(=C–H) mode to higher wavenumbers at

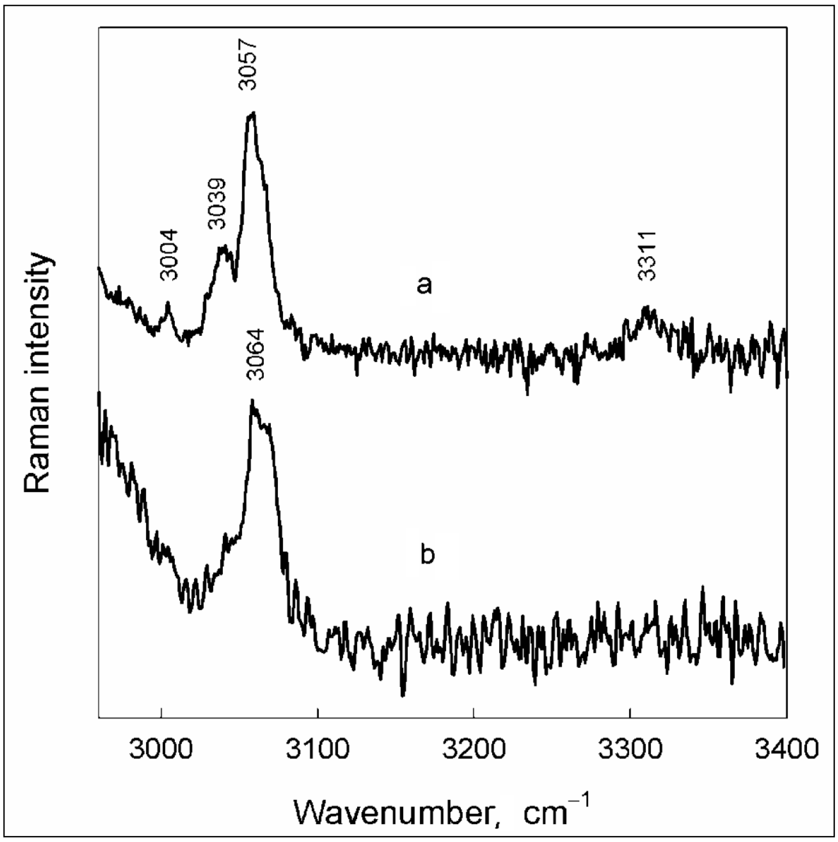

Fig. 3. Raman spectra of (a) the MOPHE compound in the solid state and (b) the 0.1 M MOPHE in methanol. The Raman spectrum of the solvent was subtracted. The excitation wavelength is 785 nm (100 mW)

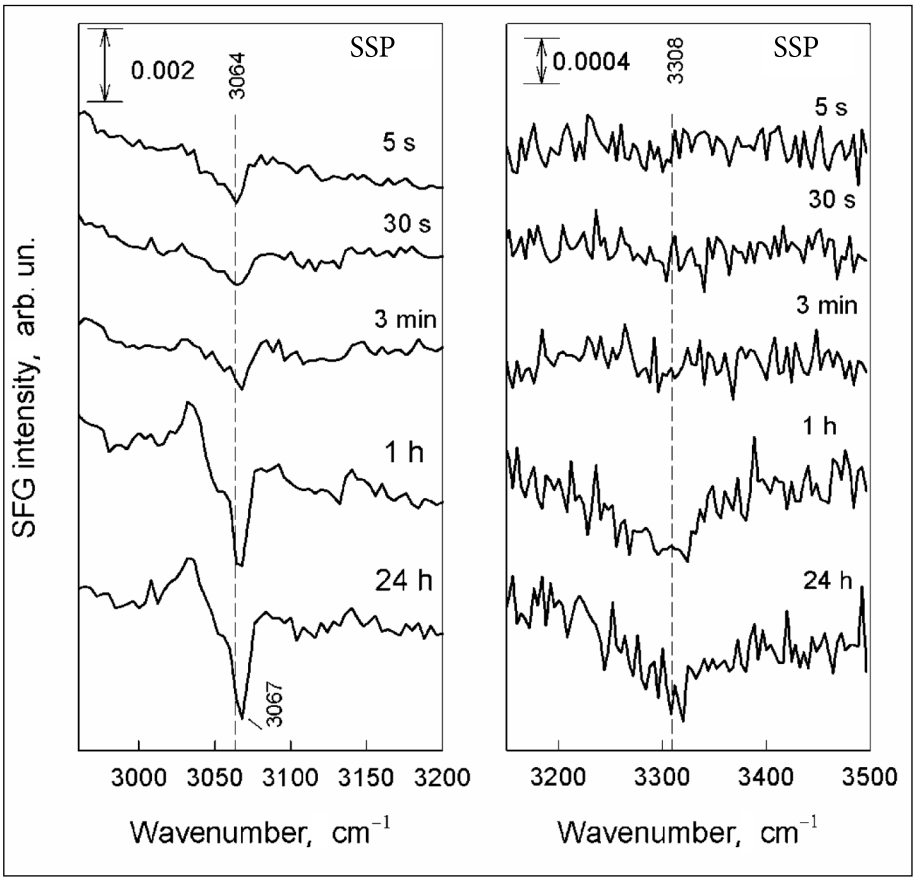

Fig. 4. Vibrational sum-frequency generation (SFG) spectra of MOPHE on the gold substrate at different immersion times in the adsorption solution in 2960–3200 cm^{-1} (left panel) and 3160–3500 cm^{-1} (right panel) spectral regions taken with ssp (s-SFG, s-532 nm, and p-IR) polarization combinations

longer incubation times is evident. In the Amide A mode frequency region a broad negative-going feature is visible near 3308 cm^{-1} for a longer time incubated in the adsorption solution samples. The presence of SFG resonances indicates ordering in the monolayer; no SFG signal is expected to be observed from the randomly oriented molecular groups at the interface.

Figure 5 shows the changes in the ppp-polarized SFG resonance intensity of the Phe ring mode ν(=C–H) as a function of the gold substrate immersion time in the ethanol solution containing 0.01 M MOPHE. The experimental data shown in Fig. 5 were fitted to the expression

$$I_{SEG} = I_0 + I_{\max} (1 - e^{-kt}), \qquad (1)$$

where I_0 is the initial SFG intensity, $I_{\max}$ is the maximal band intensity, and k is the constant reflecting the rate of changes in the studied process. The intensity of SFG resonance increases rapidly with increasing the immersion time in the adsorption solution and reaches the maximal value after about a 15 min period. Similar time is required for the formation of strong hydrogen bonds between the adjacent amide groups in the monolayer. The constant k was found to be $(9.7 \pm 2.1) \times 10^{-3}$ s^{-1}. The previous detailed SFG study of the self-assembly of docosanethiol ($CH_3(CH_2)_{21}SH$) monolayers on a polycrystalline gold substrate from an ethanol solution revealed three different steps associated with (i) formation of the Au–S bond, (ii) straightening of the alkane chains, and (iii) reorientation of the terminal methyl groups [23]. The first step was found to be a very fast process; it was completed within the first 5 min of adsorption. The second step was considerably slower (3–4 times) and was essentially completed after ~20 min of adsorption. The reorganization of methyl groups was the slowest process. Based on the SFG analysis it might be suggested that the monolayer was structurally completed after about 2 h of adsorption. It should be noted that the kinetics of monolayer

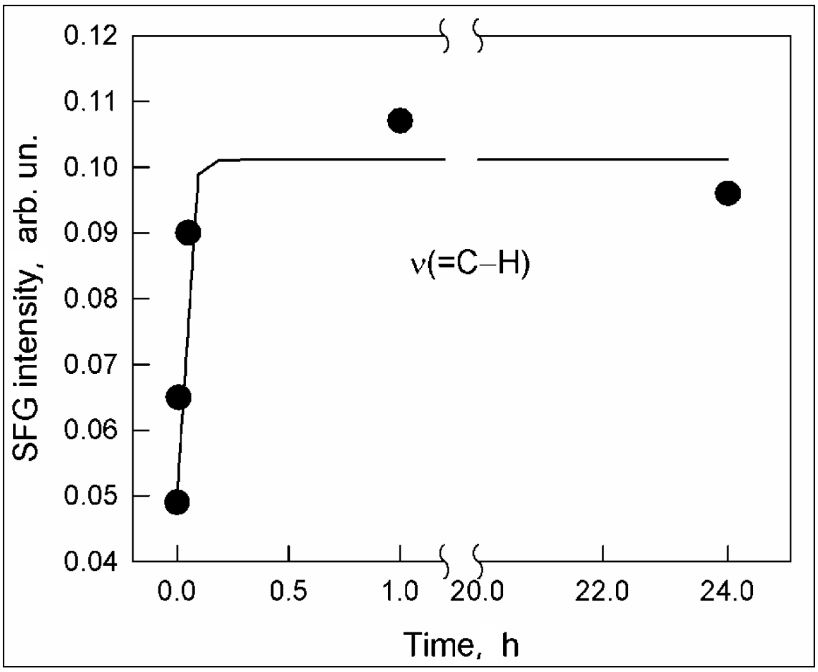

Fig. 5. Dependence of the SFG resonance intensity of the Phe ring mode ν(=C–H) on the immersion time in the ethanol solution containing 0.01 M MOPHE. The polarization combination was ppp. The solid line is the best fit to Eq. (1)

formation depends strongly on the molecule structure (the number of methylene groups, the origin of a terminal functional group and the presence of a specific intrachain group) and the solvent used. In the case of the MOPHE compound, the SFG data of this work revealed the complete ordering of the terminal Phe ring after about 60 min of adsorption. Similar time is required for development of ordered and strongly hydrogen bonded amide groups.

CONCLUSIONS

Nonlinear vibrational spectroscopy was applied for the analysis of self-assembled monolayer (SAM) formation from thiols containing a terminal phenylalanine ring and intrachain amide groups ($HS\text{-}(CH_2)_7\text{-}CONH\text{-}(CH_2)_2\text{-}C_6H_5$, MOPHE) at the magnetron sputtered polycrystalline gold substrate. The sum frequency generation data show the formation of well-ordered structures strongly hydrogen bonded through the amide group after the immersion of the gold substrate for ~1 h in the ethanol solution containing the 0.01 M MOPHE compound.

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SAVITVARKIŲ MONOSLUOKSNIŲ FORMAVIMOSI IŠ TIOLIŲ SU GALINE FENILALANINO ŽIEDO FUNKCINE IR AMIDO GRUPĖMIS GRANDINĖJE TYRIMAS SUMINIO DAŽNIO GENERACIJOS SPEKTROSKOPIJOS METODU

Santrauka

Savitvarkių monosluoksnių formavimasis ant polikristalinio aukso paviršiaus iš tiolių, turinčių galinę fenilalanino (Phe) žiedo ir amido grupes grandinėje (HS-$(CH_2)_7$-CONH-$(CH_2)_2$-C_6H_5), buvo tiriamas virpesinės suminio dažnio generacijos (SFG) spektroskopijos metodu. Charakteringo Phe žiedo valentinio virpesio ν(=C–H) ties 3069 cm^{-1} ir amido A virpesio parametrų kitimas bėgant laikui buvo atliekamas panaudojant netiesinę virpesinę spektroskopiją. SFG duomenys parodė, kad galiniai Phe žiedai susiformuoja į tvarkingą struktūrą išlaikius aukso plokštelę adsorbciniame tirpale maždaug 60 min. Amido A juosta SFG spektruose buvo stebima ties santykinai žemais dažniais (3280–3308 cm^{-1}). Tai rodo, kad susiformuoja stiprus vandenilinis ryšys tarp šalia esančių adsorbuotų molekulių grandinių. Tvarkingos struktūros, vandeniliniais ryšiais susietas savitvarkis monosluoksnis su galine Phe žiedo funkcine grupe gali būti naudojamas kaip perspektyvi modelinė sistema tiriant aromatinių amino rūgščių sąveikas ir funkcionalumą biomolekuliniuose procesuose.