Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics
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Supplementary Information

for

Myoglobin-directed assemblies of binary monolayers functionalized with iminodiacetic acid ligands at the air–water interface through metal coordination for multivalent protein binding

Xiaoyu Wang, Xuan Huang, Yanyan Xin and Xuezhong Du*

Key Laboratory of Mesoscopic Chemistry (Ministry of Education), State Key Laboratory of Coordination Chemistry, and School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, People's Republic of China

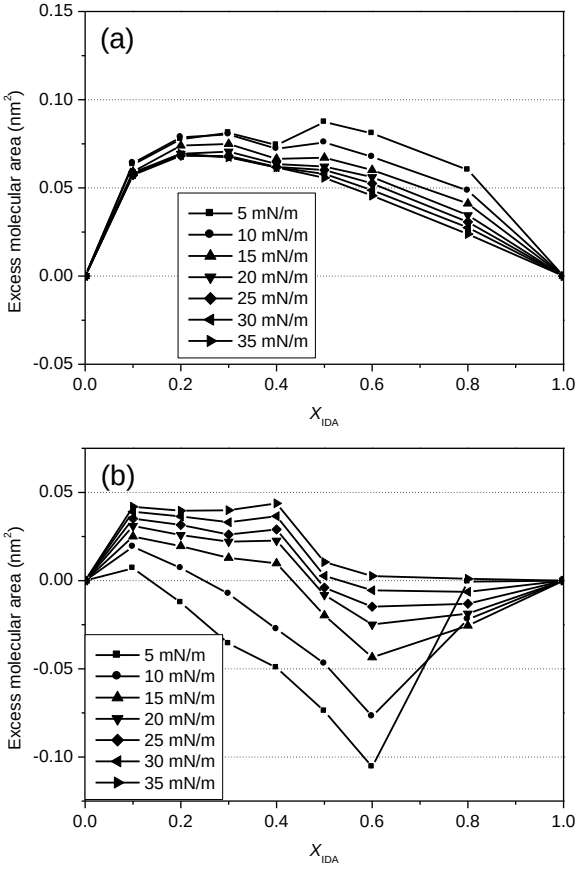

Fig. S1 Excess molecular area of the binary monolayers of C_{16}IDA and C_{16}OH on HEPES buffer solutions (pH 7.5) as a function of mole fraction of C_{16}IDA at different surface pressures: (a) in the absence of Cu^{2+}; (b) in the presence of Cu^{2+}.

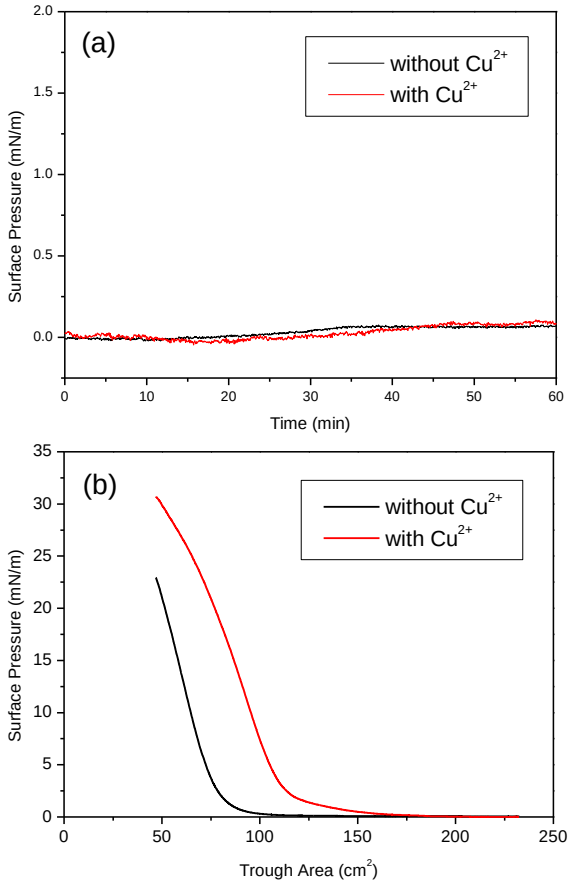

Fig. S2 (a) Time-dependent surface pressure curves of the HEPES buffer solutions (pH 7.5) in the absence and presence of Cu^{2+} (0.1 mM) upon injection of myoglobin into the buffer solutions[S1] with the final concentration of 1.5 μg/mL. (b) Surface pressure–area isotherms of myoglobin at the air–water interface in the absence and presence of Cu^{2+} (0.1 mM). Myoglobin was introduced by injecting into the HEPES buffer solutions[S1] (pH 7.5) with the final concentration of 1.5 μg/mL for 1 h.

Reference

S1 W. R. Glomm, S. Volden, Ø. Halskau, Jr. and M.-H. G. Ese, *Anal. Chem.*, 2009, **81**, 3042–3050.

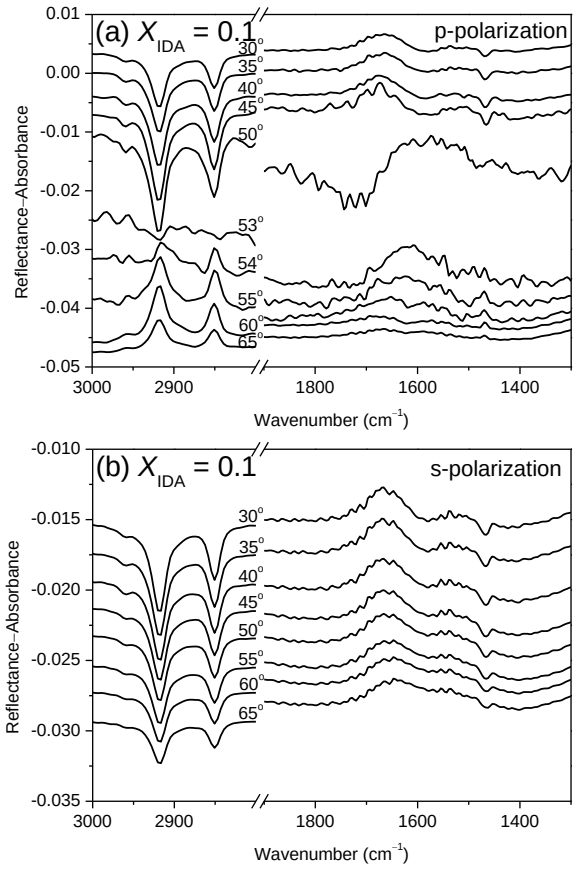

Fig. S3 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.1 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m before myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization.

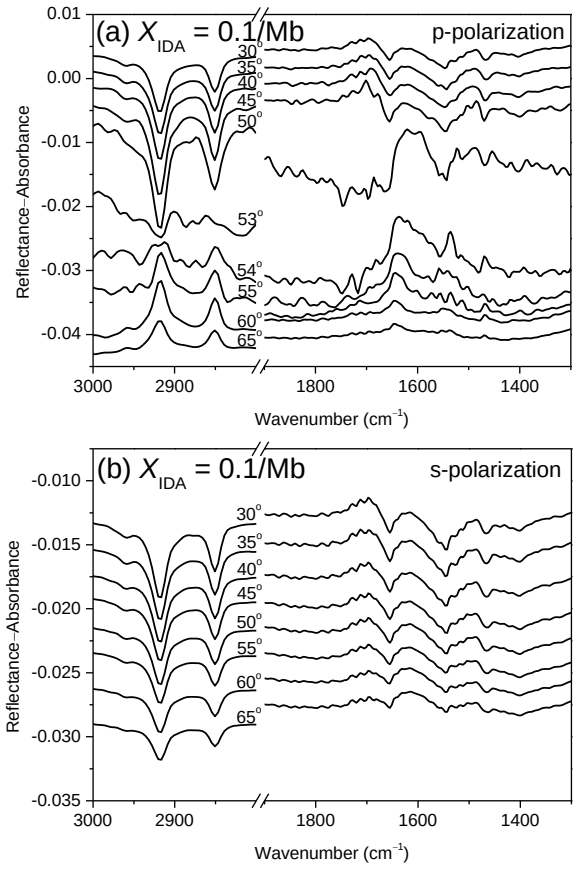

Fig. S4 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.1 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization.

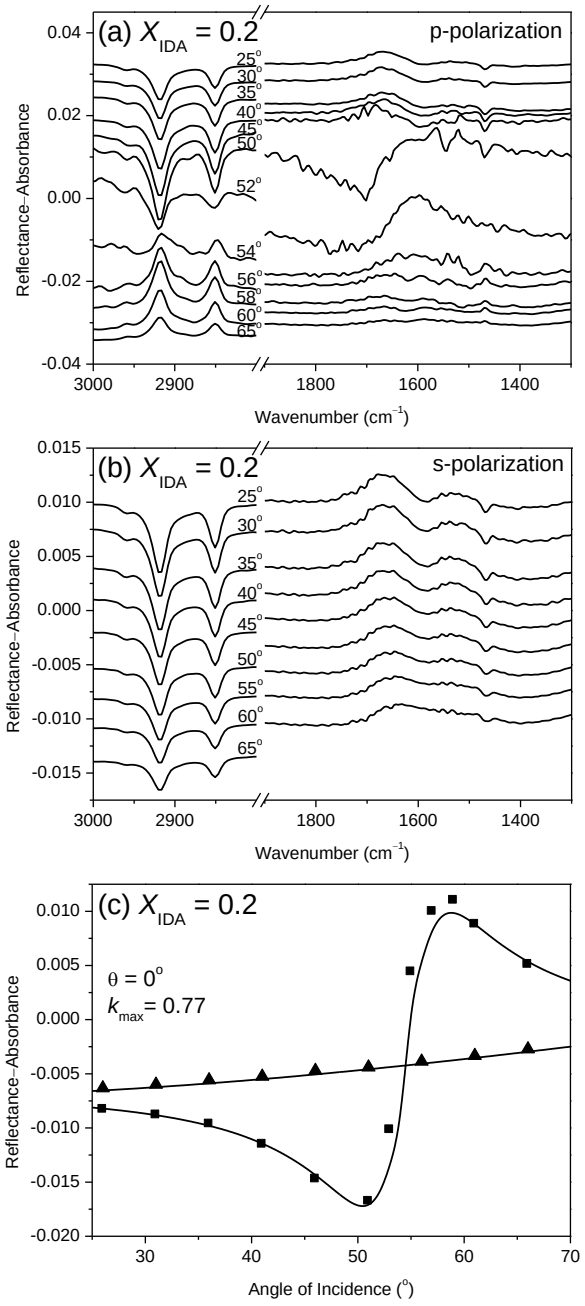

Fig. S5 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.2 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m before myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

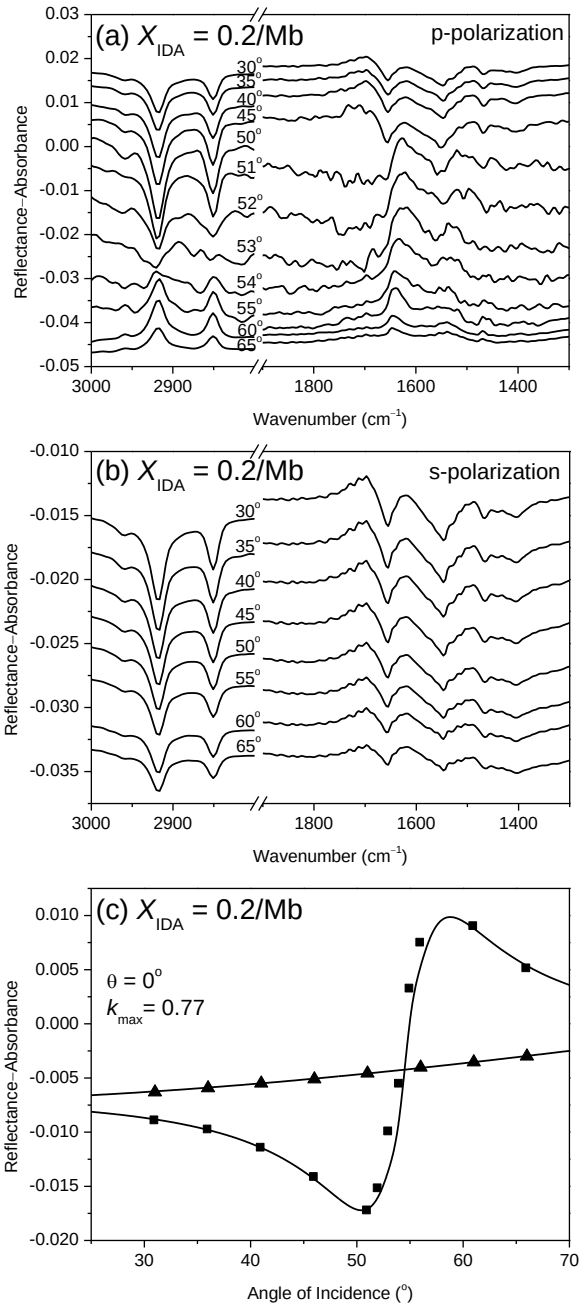

Fig. S6 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.2 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

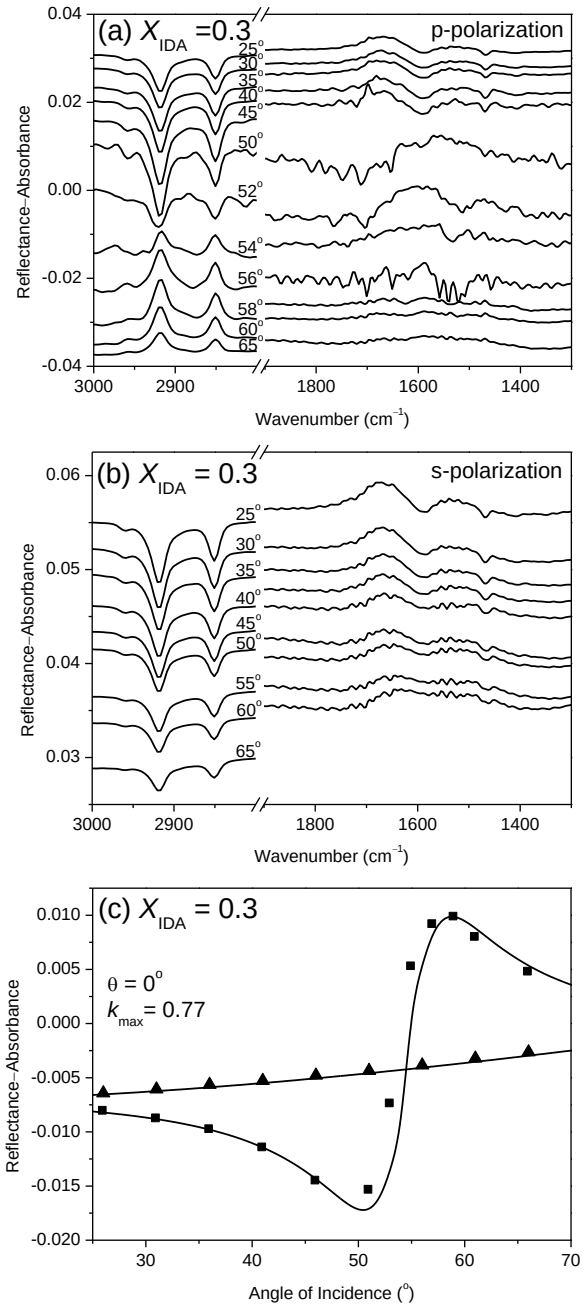

Fig. S7 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.3 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m before myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

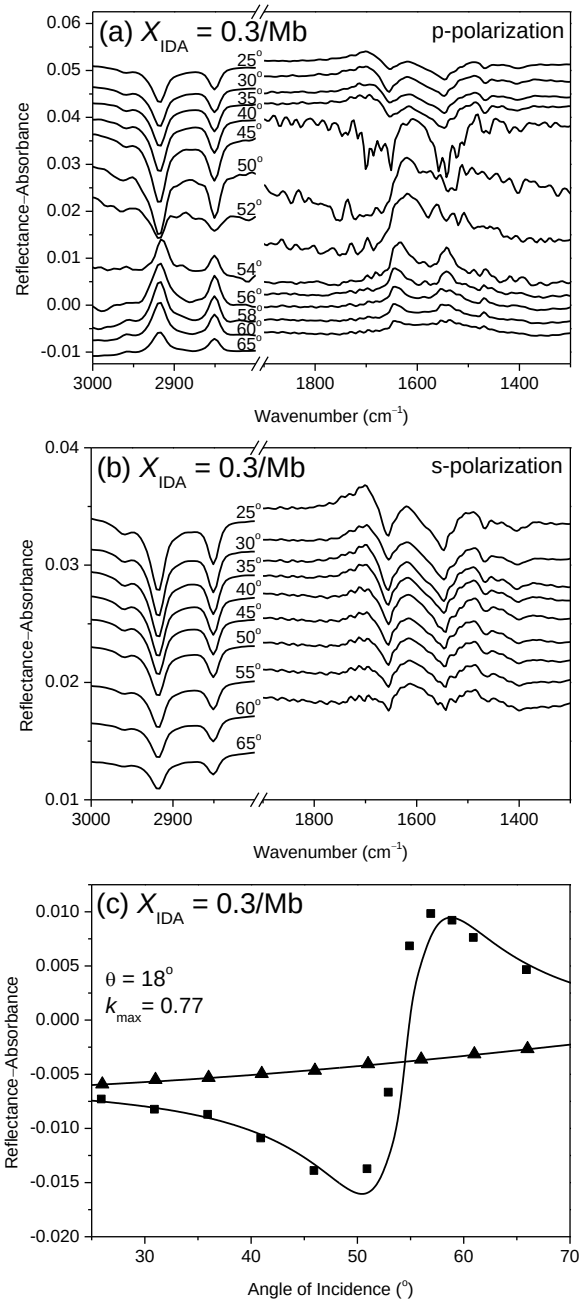

Fig. S8 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.3 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin binding with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

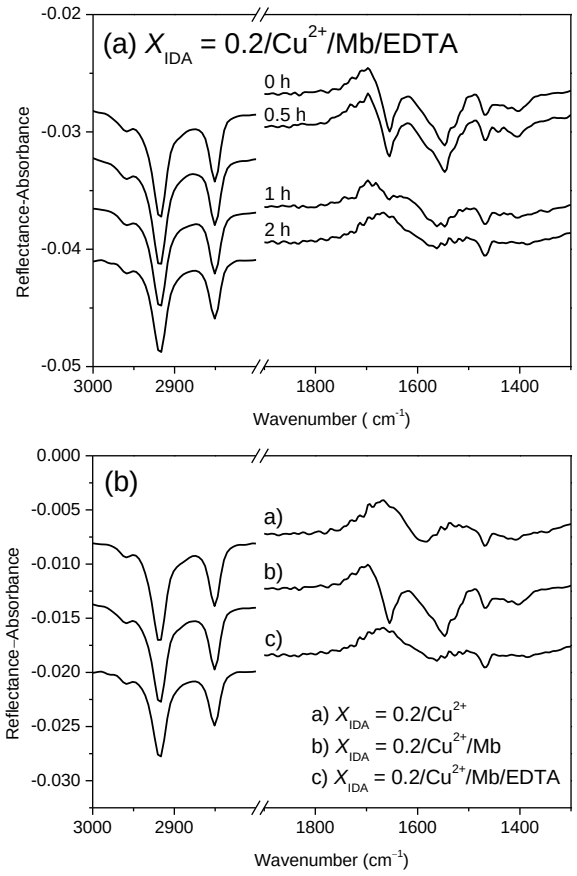

Fig. S9 (a) Time-dependent p-polarized IRRAS spectra of the myoglobin-bound binary monolayers of C_{16}IDA and C_{16}OH at X_{IDA} = 0.2 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m at an incidence angle of 30° upon introduction of EDTA (a final concentration of 0.3 mM) and (b) comparison of p-polarized IRRAS spectra of the binary monolayers of C_{16}IDA and C_{16}OH at X_{IDA} = 0.2 before and after myoglobin binding and after protein desorption.

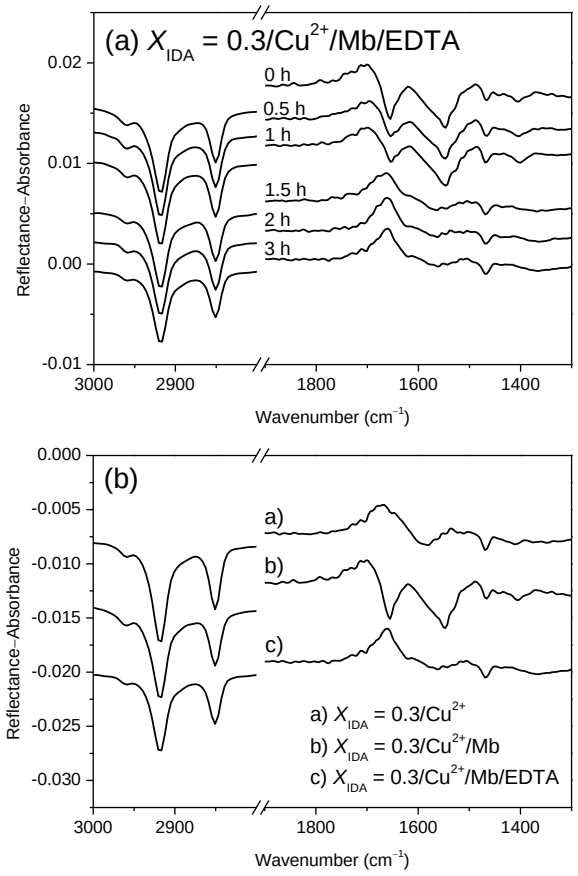

Fig. S10 (a) Time-dependent p-polarized IRRAS spectra of the myoglobin-bound binary monolayers of C_{16}IDA and C_{16}OH at X_{IDA} = 0.3 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m at an incidence angle of 30° upon introduction of EDTA (a final concentration of 0.3 mM) and (b) comparison of p-polarized IRRAS spectra of the binary monolayers of C_{16}IDA and C_{16}OH at X_{IDA} = 0.3 before and after myoglobin binding and after protein desorption.

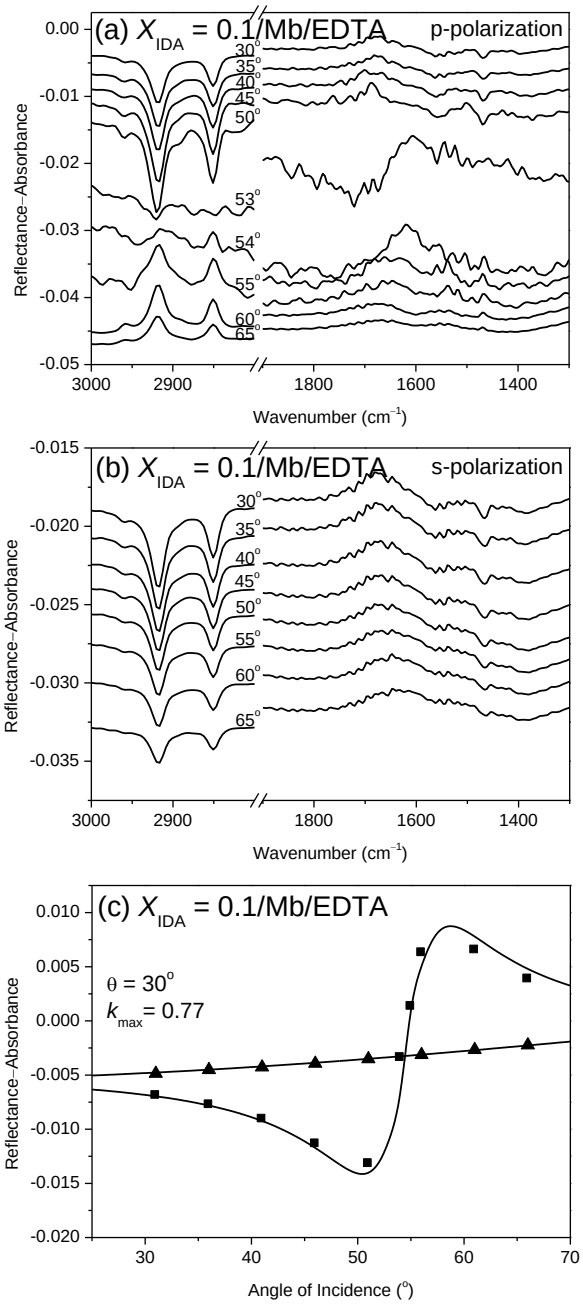

Fig. S11 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.1 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin desorption with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

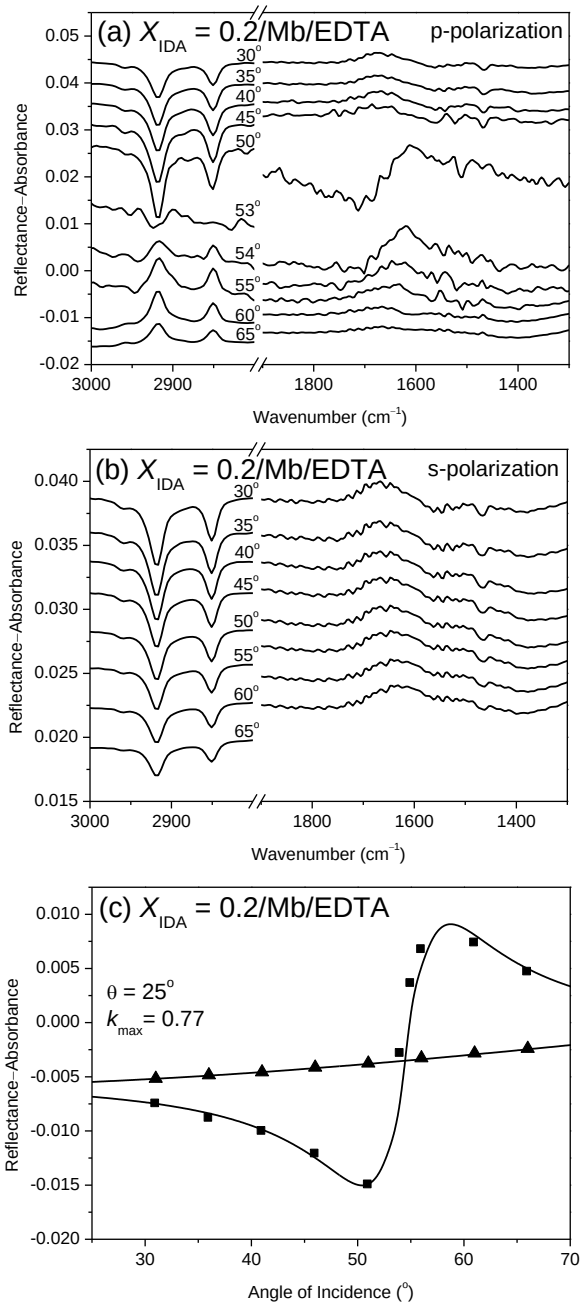

Fig. S12 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.2 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin desorption with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.

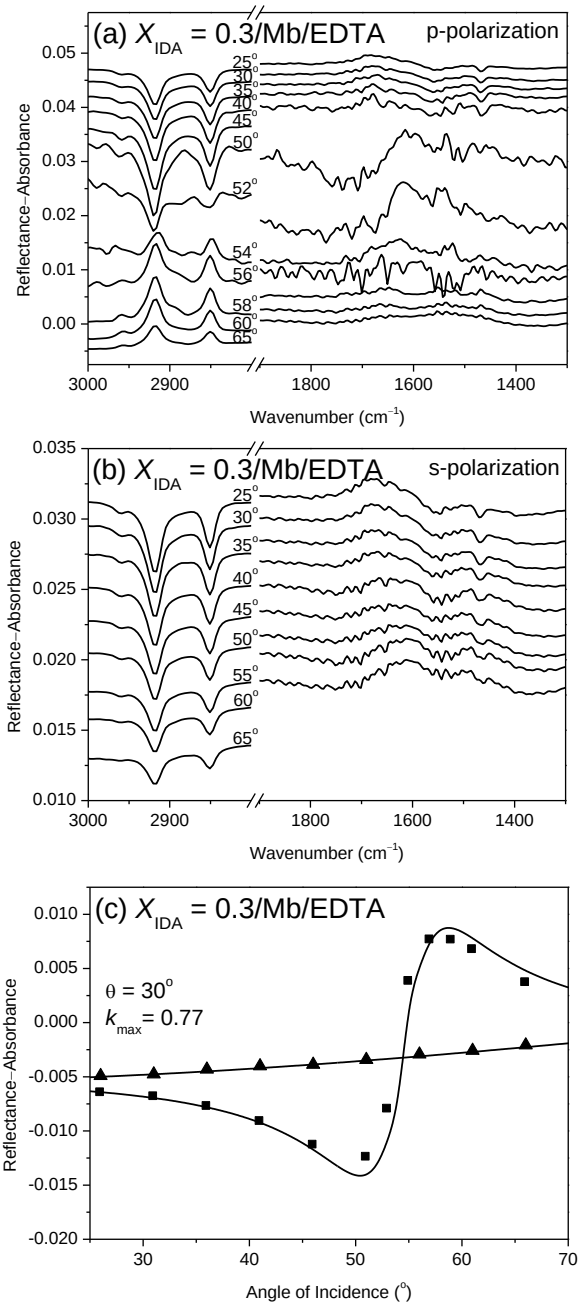

Fig. S13 IRRAS spectra of the binary monolayer of C_{16}IDA and C_{16}OH at X_{IDA} = 0.3 on HEPES buffer solutions (pH 7.5) in the presence of Cu^{2+} (0.1 mM) at the surface pressure of 30 mN/m after myoglobin desorption with different incidence angles: (a) p-polarization; (s) s-polarization. (c) Orientation angle of the alkyl chains by the best fit of the simulated (lines) and measured (symbols) RA values of $\nu_a(CH_2)$ bands.