Gold Nanoparticle Self-Assembly in Two-Component Lipid Langmuir Monolayers†

Alina Mogilevsky and Raz Jelinek*

Ilse Katz Institute for Nanotechnology and Department of Chemistry, Ben Gurion University, Beer Sheva 84105, Israel

Received September 16, 2010. Revised Manuscript Received October 17, 2010

Self-assembly processes are considered to be fundamental factors in supramolecular chemistry. Langmuir monolayers of surfactants or lipids have been shown to constitute effective 2D “templates” for self-assembled nanoparticles and colloids. Here we show that alkyl-coated gold nanoparticles (Au NPs) adopt distinct configurations when incorporated within Langmuir monolayers comprising two lipid components at different mole ratios. Thermodynamic and microscopy analyses reveal that the organization of the Au NP aggregates is governed by both lipid components. In particular, we show that the configurations of the NP assemblies were significantly affected by the extent of molecular interactions between the two lipid components within the monolayer and the monolayer phases formed by each individual lipid. This study demonstrates that multicomponent Langmuir monolayers significantly modulate the self-assembly properties of embedded Au NPs and that parameters such as the monolayer composition, surface pressure, and temperature significantly affect the 2D nanoparticle organization.

Introduction

Langmuir monolayers have traditionally been employed to study the behavior of amphiphilic molecules, such as lipids and fatty acids, on water surfaces. 1 In particular, the 2D organization of monolayers makes such systems amenable to comprehensive physicochemical investigation using varied surface-characterization techniques. 1 Numerous reports have illuminated the thermodynamic profiles, phase transitions, and structural features of Langmuir monolayers and films of surfactant molecules using varied experimental methods.

Langmuir monolayers constitute promising platforms for bottom-up self-assembly processes, particularly those involving nanoparticles and colloids. 2–5 Monolayers have been employed as versatile templates for the in situ growth of crystalline particulate films because their favorable properties allow for the occurrence of diverse self-assembly processes. Specifically, numerous natural and synthetic surfactants have been shown to form Langmuir monolayers, and their 2D phase behavior and structures are well understood. 6 Hydrophobic colloids also adopt monolayer organization at the air/water interface. 7 In particular, the structure, morphology, orientation, and total film thickness can be readily controlled via the selection of the monolayer and experimental conditions such as temperature, concentration, mole ratio, surface pressure, and subphase composition. 8–12 Additionally important from a practical/applyed standpoint is the fact that monolayer films can be conveniently transferred to solid substrates without disrupting the film structure.

Recent studies have demonstrated that Langmuir monolayers of surfactants and lipids could form “templates” for the deposition of varied types of nanoparticles (NPs). 13–16 Gold nanoparticles (Au NPs) coated with hydrophobic substances allow the NPs to “float” upon the aqueous subphase. 17 Recent studies have shown that Langmuir monolayers of amphiphilic molecules and lipids constrain hydrophobically coated Au NPs into diverse 2D patterns. 4 We have demonstrated that a careful selection of surfactant constituents and external parameters, such as temperature and surface pressure, could produce varied Au NP configurations at the air/water interface, including nanowires, nanoislets, and nanorods. 18,19

Identification and optimization of the factors affecting the organization of gold (and other types of) NPs at the air/water interfaces are critical to attaining a better understanding of their 2D assembly properties and for potential utilization of the film assembly approach for practical purposes. The types and properties of surfactants generally constitute primary factors affecting the NP film patterns. 20–22 The capping agents of the NPs additionally exert significant effects upon NP organization because...

1 Part of the Supramolecular Chemistry at Interfaces special issue.
2 Corresponding author. Tel: +972-8-6461747. Fax: +972-8-6472943.
E-mail: raz@bgu.ac.il.
these molecules modulate the interactions between both the NPs and the surrounding surfactants and among the NPs themselves in the monolayer environment.\(^\text{13,23,24}\)

Multicomponent Langmuir monolayers offer intriguing possibilities for modulating nanoparticle self-assembly because the interactions between the monolayer constituents add another parameter that could significantly modulate a film’s physical properties.\(^\text{25–29}\). In particular, the distinct sensitivity of monolayer phases to molecular composition\(^\text{30,31}\) might affect self-assemble phenomena involving embedded nanoparticles. Here we present an investigation of Au NP organization within two-component lipid Langmuir monolayers. Our analysis reveals the distinct dependence of Au NP self-assembly upon the interactions between the two lipid components. The experimental results point to the intimate relationship between nanoparticle film configurations at the air/water interface and compositions of multicomponent lipid monolayers.

**Materials and Methods**

**Materials.** HAuCl\(_4\), tetradecylammonium bromide, sodium borohydride, and dodecanethiol were purchased from Sigma-Aldrich and used as provided. Lipids including 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-diacylhexadecyl-sn-glycero-3-phosphocholine (DAPC), and C\(_{18}\) ceramide (brain) were provided in powder form from Avanti Polar Lipids (Alabaster, AL) and used as provided. Chloroform (CH\(_3\)OH) were HPLC grade (Frutarom Ltd., Haifa, Israel).

**Synthesis of Dodecanethiol-Capped Au NPs.** Synthesis of the dodecanethiol-capped Au NPs was carried out as previously described.\(^\text{32}\) Briefly, we used a slightly modified Brust\(^\text{33}\) procedure based on a two-phase (toluene/water) reduction of HAuCl\(_4\) in the described.\(^\text{32}\) Briefly, we used a slightly modified Brust\(^\text{33}\) procedure (CH\(_3\)OH) were HPLC grade (Frutarom Ltd., Haifa, Israel).

**Transmission Electron Microscopy (TEM).** Samples for microscopy analyses were extracted at a surface pressure of 10 mN/m using horizontal transfer onto 400 mesh copper Formvar/carbon grids (Electron Microscopy Sciences, Hatfield, PA). TEM images were recorded on a Jeol JEM-1230 transmission electron microscope (JEOL LTD, Tokyo, Japan) operating at 120 kV.

**Results**

**Au NP Organization within Single-Lipid Monolayers.** This study examines the assembly properties of spherical Au NPs within two-component lipid monolayers specifically comprising ceramide/dimyristoylphosphatidylcholine (DMPC) and ceramide/diacylphosphatidylcholine (DAPC). Such mixed monolayers exhibit distinct thermodynamic properties that are dependent upon the lipid molecules and the interactions between them. Figure 1 depicts the surface-pressure/area isotherms of the three lipids studied. DMPC exhibits a liquid-expanded monolayer phase over almost the entire pressure range at the temperatures examined (solid isotherms, Figure 1).\(^\text{34}\) DAPC, however, forms a liquid-condensed monolayer instantly upon deposition on the water subphase (short-dashed-line isotherms, Figure 1).\(^\text{35}\) Liquid-condensed/liquid-condensed phase transitions are observed at around 20 mN/m (Figure 1). Ceramide, the N-acylated derivative of sphingosine, also adopts a condensed monolayer phase upon deposition at the air/water interface at both temperatures examined (long-dashed-line isotherms, Figure 1).\(^\text{36}\)

To examine the organization of the Au NPs in binary lipid monolayers and the impact of mixing the two lipids, we first examined the gold assemblies within single-component lipid monolayers (Figure 2). Figure 2 depicts transmission electron microscopy (TEM) images of Au NP lipid films compressed to a surface pressure of 10 mN/m at subphase temperatures of 18 and 35 °C, respectively. The TEM images in Figure 2 demonstrate significant differences in Au NP organization within monolayers of the three lipids. In addition, a temperature effect upon NP self-assembly is apparent.

Specifically, Figure 2A shows that the Au NPs formed irregularly shaped thick stripes in DMPC monolayers.\(^\text{32}\) The thick stripes, which comprised partially fused individual Au NPs (thereby losing their spherical shape), were similar at both temperatures examined. The formation of randomly shaped Au NP aggregates in DMPC monolayers has been ascribed to the fluidity of the monolayer.\(^\text{34}\) Indeed, Figure 2B shows that Au NPs embedded within DAPC monolayers adopted more ordered configurations.
most likely because of the liquid-condensed phase of the DAPC monolayer at both 18 and 35 °C. In particular, Figure 2B features thin columns of individual Au NPs assembled around condensed DAPC domains at 18 °C whereas somewhat thicker Au assemblies accumulated around the circularly shaped condensed domains at 35 °C (Figure 2B). Different nanoparticle configurations were observed in Au NP/ceramide monolayers (Figure 2C). These monolayers yielded isolated ring-shaped Au NP islets at 18 °C whereas merging of the isolated gold assemblies into long branched stripes was apparent at 35 °C (Figure 2C).

Au NPs/Ceramide/DMPC Monolayers. Whereas Figure 2 underscores the close relationship between the Au NP self-assembled structures and lipid environments at the air/water interface, we further aimed to assess whether and to which extent the modulation of monolayer properties in two-component lipid mixtures affects the organization of embedded nanoparticles. Figures 3–6 present TEM results obtained from mixed monolayers comprising Au NPs, ceramide, and DMPC (Figures 3 and 4) or Au NPs, ceramide, and DAPC (Figures 5 and 6). Figures 3–6 also display graphs correlating monolayer compositions with the average molecular areas of the two-component monolayers, calculated from the respective isotherms at 10 mN/m. These graphs were designed to assess the deviations of the two-lipid monolayers from ideal mixtures, thus evaluating the interactions between the lipid molecules comprising the monolayers and the impact of such interactions upon the Au NP assemblies.

Figure 3A depicts a graph correlating the average molecular areas of Au NP/ceramide/DMPC monolayers as a function of ceramide mole fraction, extracted from the isotherms acquired in 18 °C at a surface pressure of 10 mN/m. The straight broken line in Figure 3A corresponds to values calculated according to the additivity rule, which essentially predicts physical parameters in ideal mixtures. According to the additivity rule, in a monolayer comprising an ideal mixture of noninteracting components (ceramide and phospholipids in this study) the mean molecular area \( A_{\text{mean}} \) at a given surface pressure will be essentially equal

\[
A_{\text{mean}} = X_{\text{ceramide}} A_{\text{ceramide}} + (1 - X_{\text{ceramide}}) A_{\text{phospholipid}}
\]

in which \( X_{\text{ceramide}} \) and \( A_{\text{ceramide}} \) correspond to the mole fraction and molecular area of ceramide, respectively, \( (1 - X_{\text{ceramide}}) \) and \( A_{\text{phospholipid}} \) similarly correspond to the phospholipid mole fraction and molecular area, respectively, in a pure phospholipid monolayer. An important point that needs to be emphasized is that the straight line in Figure 3A (and other additivity rule analysis graphs in this article) was calculated for monolayers that comprised Au NPs in addition to lipids. The concentration of Au NPs in all monolayers containing different ceramide/phospholipid ratios, however, was identical, and the only variable in the experiments was the ratio between the lipid components. Indeed, a comparison between the additivity rule results with and without Au NPs (Supporting Information) indicates that the Au NPs had a minimal effect upon the interactions between the two lipid components.

Figure 3A demonstrates that in all ceramide mole ratios above 0.2 the average molecular areas were lower than the values calculated according to the additivity rule. This result indicates a predominance of attractive forces between ceramide and DMPC at 18 °C, leading to the reduction of the average molecular areas as shown in Figure 3A. Importantly, the graph in Figure 3A was qualitatively similar to the results obtained for a ceramide/DMPC monolayer without Au NPs (Supporting Information), suggesting that the incorporation of Au NPs in the monolayers did not alter the physical properties of the monolayers and the interactions between the lipid components.

The TEM data in Figure 3B,C attest to the changes in Au NP organization induced by the two distinct monolayer compositions examined. Figure 3B shows the formation of elongated Au NP “necklaces” within a ceramide/DMPC (3:7 mol ratio) monolayer. The Au NP features in Figure 3B appear to emanate from merging, or superimposition, of the long Au NP stripes recorded in the monolayer of pure DMPC (Figure 2A) and the ring-shape Au NP assemblies observed in the monolayer of pure ceramide (Figure 2C). This superimposition is consistent with the ideal-mixture formation of the monolayer at a ceramide mole ratio of 0.3 (left arrow, Figure 3A).

In contrast to the organized Au NPs in the ceramide/DMPC monolayer comprising a 3:7 mole ratio (Figure 3B), no ordering of the Au NPs occurred when the ratio between ceramide and DMPC was inverted (ceramide/DMPC 7:3 mol ratio, Figure 3C). The TEM image acquired at this composition showed long, thick aggregates of Au NPs and no evidence of ring-shape film.

Figure 1. Surface pressure/area isotherms of the lipids examined. The isotherms were recorded at the two temperatures indicated. (-) DMPC, (---) DAPC, and (------) ceramide.

\[18^\circ\] 

\[35^\circ\]
structures that were abundant in the ceramide/DMPC (3:7 ratio) film (Figure 3B). Indeed, an examination of the average molecular area graph in Figure 3A reveals that at a 7:3 ceramide/DMPC ratio the mixed monolayer exhibited significant deviation from an ideal mixture, specifically, a lower molecular area resulting from the apparent attraction between the ceramide and DMPC molecules (right arrow, Figure 3A). The significant interactions between ceramide and DMPC could explain the reorganization of the Au NPs in the monolayer, which leads to a distinct configuration that was significantly different than the monolayers of the pure lipids (Figure 2) or the Au NP/ceramide/DMPC monolayer at the 3:7 ceramide/DMPC ratio (Figure 3B).

Figure 2. Au NP organization in monolayers of the pure lipids. TEM images of (A) Au-NP/DMPC, (B) Au-NP/DAPC, and (C) Au NP/ceramide films. All films were transferred from the air/water interface at a surface pressure of 10 mN/m. The scale bars in the images correspond to 100 nm. (The scale bars in the two insets in (18\(^{\circ}\)C) corresponds to 25 nm.)
Figure 4 features the thermodynamic and TEM analysis of Au NPs incorporated within ceramide/DMPC monolayers at 35 °C. The graph depicting the average molecular area versus monolayer composition in Figure 4A indicates different lipid interactions compared to the data acquired at 18 °C (Figure 3A). In particular, slight repulsion between the two lipid components appeared to dominate in monolayers comprising a small percentage of ceramide (left arrow in Figure 4A). Importantly, attraction forces were clearly absent in the high-mole-ratio ceramide samples (Figure 4A), in contrast to the scenario of the mixed monolayer at 18 °C (Figure 3A).

The TEM results in Figure 4B,C show representative nanoparticle assemblies within Au NPs/ceramide/DMPC monolayers at 35 °C. Specifically, the images reveal necklace assemblies of the Au NPs at both a 3:7 mol ratio between ceramide and DMPC (Figure 4B) and at a 7:3 ceramide/DMPC ratio (Figure 4C). However, Au NPs in the 3:7 ceramide/DMPC monolayer featured a narrower necklace configuration (Figure 4B) compared to the corresponding arrangement within the 7:3 ceramide/DMPC monolayer (Figure 4C). Although the necklace structures can be ascribed to the superimposed strip structure of Au NPs in DMPC at 35 °C (Figure 2A) and Au NPs in ceramide (Figure 2C), the much broader ring configurations in Figure 4C cannot be easily explained according to the additivity rule results. We speculate that the fluidity of DMPC allows the aggregation of Au NPs in larger monolayer areas, resulting in the thicker ring structures in monolayers of the 7:3 ceramide/DMPC ratio. Indeed, it was previously reported that ceramide displaying long, unsaturated N-acyl chains was very miscible within DMPC monolayers.39

Au NP/Ceramide/DAPC Monolayers. A similar interplay between Au NP configurations and interactions among the lipid molecules within the binary monolayers was apparent in Au NP/ceramide/DAPC monolayers (Figures 5 and 6). Figure 5 presents thermodynamic and TEM analyses of Au NP/ceramide/DAPC monolayers at 18 °C. The graph in Figure 5A depicting the relationship between the mean molecular areas and ceramide mole fraction demonstrates that in the entire composition range the molecular area values were localized in close proximity to the straight line calculated according to the additivity rule (e.g., ideal mixtures). This result

indicates that ceramide and DAPC exhibit minimal interactions within the mixed monolayers.

The TEM data in Figure 5B,C highlight the Au NP distribution within the ceramide/DAPC monolayers at 18 °C at two different mole ratios. In a monolayer comprising ceramide/DAPC in a 3:7 mole ratio (Figure 5B), the Au NPs produced thick rings around circular spaces, most likely corresponding to condensed lipid domains. Similar to the analysis of the ceramide/DMPC monolayers above, this configuration probably arises from a superimposition of the Au NP columns around the abundant condensed lipid domains in monolayers of pure DAPC (Figure 2B) and the ring-shaped Au NP aggregates observed in monolayers of pure ceramide (Figure 2C). This description is consistent with the apparent ideal mixture behavior of the ceramide/DAPC monolayer inferred from Figure 5A (left arrow). The apparent retention of the spherical shape of the individual Au NPs (inset in Figure 5B) is particularly noteworthy. This result stands in sharp contrast to the pronounced fusion of Au NPs into irregularly shaped particles in the ceramide/DMPC monolayer (insets in Figure 3B) and is evidence of the influence of the condensed DAPC monolayer phase.32

The TEM image in Figure 5C of a Au NP/ceramide/DAPC monolayer (ceramide/DAPC 7:3 mol ratio) depicts long strips of Au NP aggregates, within which gold-free circular domains appear. This Au NP configuration seems different from the structures formed in the monolayers of the single-lipid components (Figure 2) and cannot be easily explained according to the mean molecular area result in Figure 5A (which pointed to an almost ideal mixture at this mole ratio, right arrow). However, the long strips in Figure 5C could be affected by the DAPC component within the mixed monolayer because Au NPs in pure DAPC monolayers indeed formed interconnected networks (Figure 2B). Such gold stripes could be possibly modulated further by the broader ring-shaped Au NP aggregates observed in pure ceramide films (Figure 2C). The higher abundance of ceramide in the sample examined in Figure 5C consequently leads to a spreading out of the gold-free domain structure, which is depicted in the TEM image.

Figure 6 presents the thermodynamic and TEM analyses of Au NPs/ceramide/DAPC monolayers examined at 35 °C. In contrast to the seemingly ideal mixture of the two lipid components at 18 °C (mean molecular area graph in Figure 5A), Figure 6A
indicates the existence of significant attractive forces between ceramide and DAPC at 35 °C, giving rise to the lower average molecular areas recorded in monolayers comprising between 20 and 70% ceramide. The TEM results in Figure 6B,C reflect the impact of the lipid interactions upon the Au NP organization within the two-component monolayers at 35 °C. At a ceramide/DAPC ratio of 3:7 (Figure 6B), the TEM image reveals elongated, irregularly shaped strips of Au NPs. Importantly, no encircled gold-free domains were observed in the TEM image in Figure 6B, in contrast to the Au NP configurations within pure DAPC (Figure 2B) or pure ceramide (Figure 2C) monolayers and also different from the Au NPs/ceramide/DAPC monolayer at 18 °C (Figure 5B). The distinct, seemingly disordered Au NP assembly shown in Figure 6B is most likely due to the significant interactions between ceramide and DAPC (left arrow in Figure 6A), leading to a monolayer exhibiting different physical properties compared to an ideal mixture within Langmuir monolayers of the two lipids.

Similar to the TEM result in Figure 6B, the image in Figure 6C corresponding to Au NPs assembled in ceramide/DAPC monolayers in a 7:3 mole ratio shows elongated Au NP aggregates that do not exhibit specific shapes. However, unlike the ceramide/DAPC monolayer in the 3:7 mol ratio (Figure 6B), Figure 6C shows that some encircled domains did appear within the monolayer. This observation might be related to the fact that the deviation from an ideal mixture of the Au NP/ceramide/DAPC monolayer at the ceramide/DAPC 7:3 mol ratio (right arrow in Figure 6A) was less pronounced than for the corresponding monolayer in a 3:7 ratio (Figure 6A). Accordingly, the Au NP aggregates in the Au NP/ceramide/DAPC monolayer (7:3 ratio between ceramide and DAPC) retain the gold-free domain structure, as recorded in the TEM experiment in Figure 6C.

**Discussion**

Self-assembly phenomena involving hydrophobically coated nanoparticles at the air/water interface have attracted interest as possible platforms for bottom-up structural techniques. Varied studies have shown that surfactant and lipid monolayers constitute effective templates for the 2D organization of Au NPs. In particular, it has been shown that Au NPs preferentially aggregate at interfaces between different monolayer phases, particularly the interface between liquid-expanded and liquid-condensed...
surfactant phases. Here we show that the nanoparticle templating approach can be expanded through the incorporation of hydrophobically coated Au NPs in multicomponent lipid monolayers. In particular, we demonstrate that the extent of mixing and concomitant molecular interactions in binary lipid monolayers exert significant effects upon the aggregation and configurations of nanoparticle assemblies.

The thermodynamic and TEM experiments carried out clearly show that Au NP organization within two-component lipid monolayers is dependent upon the mole ratios between the lipids in the mixed monolayers. Specifically, the additivity rule analyses, in conjunction with TEM images extracted at specific ratios between the two lipid components, indicate that the deviations from ideal mixtures observed in specific ceramide/phospholipids ratios were a primary factor affecting the Au NP configurations. In particular, the TEM results demonstrated that, in the majority of monolayers examined, when the monolayers appeared to contain noninteracting lipid components, the Au NP assemblies could be characterized as weight-averaged superimpositions between the respective structures formed within the monolayers of individual lipids. This interpretation explains, for example, the remarkable “necklace” structures obtained in the Au NPs/ceramide/DMPC monolayers at 18 °C (Figure 3) and 35 °C (Figure 4) most likely corresponding to superimpositions between the ring-shaped Au NP aggregates in monolayers of pure ceramide (Figure 2C) and the elongated Au NP “stripes” assembled in DMPC monolayers (Figure 2A).

Beside the degree of mixing and molecular interactions between the lipid constituents forming the films, the data indicate that the phases of the specific lipids in the Langmuir monolayer environment exert substantial effects upon film configurations. Indeed, the degree in which one or both lipids display a liquid-expanded phase in the pure monolayer appeared to be an important parameter for promoting superimposition between the Au NP configurations recorded in monolayers of the individual lipids. Thus, superimposed Au NP assemblies appeared more abundant and visually distinctive in monolayers comprising ceramide and DMPC (Figures 3 and 4) ascribed to the liquid-expanded monolayer phase of DMPC. In comparison, in the binary monolayers containing ceramide and DAPC, which both exhibit liquid-condensed phases, the Au NP structures were less ordered and exhibited lesser uniformity. These results suggest that the contributions of fluid lipid components within binary monolayers aid the reorganization of the nanoparticles into the distinct configurations shaped by the second lipid component that exhibits a condensed phase.

Figure 6. Au NP organization in mixed ceramide/DAPC monolayers at 35 °C. (A) Graph showing the mean molecular areas recorded at 10 mN/m for different monolayer compositions. The broken line corresponds to values calculated according to the additivity rule. The two arrows indicate the compositions at which the TEM images were obtained. (B) TEM images of a film comprising Au NPs and ceramide/DAPC in a 3:7 mole ratio. (C) TEM image of a film comprising Au NPs and ceramide/DAPC in a 7:3 mole ratio. The scale bars in the images correspond to 100 nm. (The scale bar in the inset corresponds to 50 nm.)
Although the experimental data demonstrate the distinct variations of Au NPs organization within two-component lipid monolayers, this study also underscores the fact that Au NPs essentially constitute a sensitive measure of the extent and impact of intermolecular forces in mixed monolayers. Essentially, the TEM analysis reveals that the configurations of the Au NP aggregates, in particular the extent of superimposition between the single-component lipid monolayers, compared to the Au NP structures observed in the binary mixtures, were dependent upon deviations of the films from ideal mixtures. This observation could open the way to using hydrophobically coated Au NPs (or other monolayer-forming colloidal systems) as a vehicle for investigating multicomponent, complex monolayers.

Conclusions

This study presents an intriguing new approach to modulating the organization of supramolecular Au NP assemblies at the air/water interface, namely, through exploiting bimolecular interactions between different lipid molecules in two-component monolayers. The experimental data presented in this work highlight the dramatic effects of the molecular interactions between the lipid molecules upon the Au NP organization. In particular, the distinct gold configurations were dependent upon the mixing of the two lipid molecules within the monolayers, and the degrees of their thermodynamic properties were affected through the mixing. Overall, this work constitutes an interesting contribution to the diverse “toolbox” for constructing colloidal patterns at the air/water interface.

Acknowledgment. We are grateful to R. Jeger for help with the TEM experiments.

Supporting Information Available: Isotherms of the Au NP/individual lipids, additivity rule graphs of the mixed-lipid monolayers without Au NPs, Brewster angle microscopy (BAM) images of representative Au NP/lipids monolayers, and atomic force microscopy (AFM) images of representative Au NP/lipids films transferred to solid substrates. This material is available free of charge via the Internet at http://pubs.acs.org.