



**HAL**  
open science

# Surfactant Uptake Dynamics in Mammalian Cells Elucidated with Quantitative Coherent Anti-Stokes Raman Scattering Microspectroscopy

Masanari Okuno, Hideaki Kano, Fujii Kenkichi, Kotatsu Bito, Naito Satoru,  
Philippe Leproux, Vincent Couderc, Hiro-O Hamaguchi

► **To cite this version:**

Masanari Okuno, Hideaki Kano, Fujii Kenkichi, Kotatsu Bito, Naito Satoru, et al.. Surfactant Uptake Dynamics in Mammalian Cells Elucidated with Quantitative Coherent Anti-Stokes Raman Scattering Microspectroscopy. PLoS ONE, 2014, 9 (4), pp.e93401. 10.1371/journal.pone.0093401 . hal-01321000

**HAL Id: hal-01321000**

**<https://hal.science/hal-01321000v1>**

Submitted on 1 Oct 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Surfactant Uptake Dynamics in Mammalian Cells Elucidated with Quantitative Coherent Anti-Stokes Raman Scattering Microspectroscopy

Masanari Okuno<sup>1</sup>, Hideaki Kano<sup>1</sup>, Kenkichi Fujii<sup>2</sup>, Kotatsu Bito<sup>3</sup>, Satoru Naito<sup>3</sup>, Philippe Leproux<sup>4</sup>, Vincent Couderc<sup>4</sup>, Hiro-o Hamaguchi<sup>1,5\*</sup>

**1** Department of Chemistry, School of Science, The University of Tokyo, Bunkyo-ku, Tokyo, Japan, **2** Safety Science Research Laboratories, Kao Corporation, Haga-Gun, Tochigi, Japan, **3** Analytical Science Research Laboratories, Kao Corporation, Haga-Gun, Tochigi, Japan, **4** Institut de Recherche XLIM, UMR CNRS, Limoges, France, **5** Institute of Molecular Science and Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan

## Abstract

The mechanism of surfactant-induced cell lysis has been studied with quantitative coherent anti-Stokes Raman scattering (CARS) microspectroscopy. The dynamics of surfactant molecules as well as intracellular biomolecules in living Chinese Hamster Lung (CHL) cells has been examined for a low surfactant concentration (0.01 w%). By using an isotope labeled surfactant having CD bonds, surfactant uptake dynamics in living cells has been traced in detail. The simultaneous CARS imaging of the cell itself and the internalized surfactant has shown that the surfactant molecules is first accumulated inside a CHL cell followed by a sudden leak of cytosolic components such as proteins to the outside of the cell. This finding indicates that surfactant uptake occurs prior to the cell lysis, contrary to what has been believed: surface adsorption of surfactant molecules has been thought to occur first with subsequent disruption of cell membranes. Quantitative CARS microspectroscopy enables us to determine the molecular concentration of the surfactant molecules accumulated in a cell. We have also investigated the effect of a drug, nocodazole, on the surfactant uptake dynamics. As a result of the inhibition of tubulin polymerization by nocodazole, the surfactant uptake rate is significantly lowered. This fact suggests that intracellular membrane trafficking contributes to the surfactant uptake mechanism.

**Citation:** Okuno M, Kano H, Fujii K, Bito K, Naito S, et al. (2014) Surfactant Uptake Dynamics in Mammalian Cells Elucidated with Quantitative Coherent Anti-Stokes Raman Scattering Microspectroscopy. PLoS ONE 9(4): e93401. doi:10.1371/journal.pone.0093401

**Editor:** Maria A. Deli, Biological Research Centre of the Hungarian Academy of Sciences, Hungary

**Received:** September 5, 2013; **Accepted:** March 4, 2014; **Published:** April 7, 2014

**Copyright:** © 2014 Okuno et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work is supported by the SENTAN project (Program-S) of the Japan Science and Technology Agency (JST). H. Kano gratefully acknowledges financial support by Grand-Aid for Scientific Research on Priority "Molecular Science for Supra Functional Systems" [477] from MEXT, and the Global COE Program for "Chemistry Innovation." The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** J. Ukon, UKON CRAFT SCIENCE, Ltd. assisted with a fruitful collaboration between Japanese and French labs. There are no patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

\* E-mail: hhama@nctu.edu.tw

## Introduction

Interactions of surfactants with living cells are of considerable interest with regard to their biological functions including cellular toxicity [1]. Understanding their toxicological mode of action is highly important in order to assess and control their safety on human exposure [2–4]. Previous studies have shown that microorganisms solubilization by surfactants occurs with cell lysis, in which the cell membrane is degraded by surfactants with eventual breakdown of the whole cell [5–8]. However, the dynamical process of surfactant action in single living cells is still unexplored because of the lack of the mean to visualize surfactant molecules *in vivo* and *in situ*. In the present study, we use a recently-emerging new tool, CARS microspectroscopy [9–13], which is powerful for studying lipid molecules in living cells. We also use an isotope labeled surfactant ( $d_{25}$ -sodium dodecyl sulfate (SDS)) and visualize the dynamics of surfactant molecules in the cell lysis process. Deuterium substitution enables us to selectively trace the SDS molecules among a number of unlabeled biomolecules [10,14–16].  $d_{25}$ -SDS gives CD stretch bands in the 2000–2200  $\text{cm}^{-1}$  spectral region, which is a "window" of Raman

spectra of unlabeled biomolecules, facilitating its selective detection.

Although fluorescence labeling is a powerful technique for tracing the dynamics of lipid molecules in a living cell [17–19], introduction of fluorophores may well perturb the physical and chemical properties of the surfactant, such as charge, hydrophobicity, and hydrophilicity. Isotope labeling in vibrational spectroscopy is well established as a unique method for distinguishing the labeled molecule from the others. A great advantage of isotope substitution is the same chemical properties between the labeled and unlabeled species. Recently, we have developed quantitative CARS microspectroscopy [20], which combines multiplex CARS microspectroscopy with the maximum entropy method (MEM) [21–23]. The spectral coverage in this method is broad enough ( $>3000 \text{ cm}^{-1}$ ) to observe all the fundamental vibrational modes including not only the C-H, C-D stretch regions but also the fingerprint region. Thus, quantitative CARS microspectroscopy with deuterium substitution is ideally suited for real-time spectral tracing of cells and the surfactant molecules during the lysis process.

**Table 1.** The concentration dependence of  $d_{25}$ -SDS cell lysis efficiency; ++ corresponds to clear cell lysis, +: moderate cell lysis, -: remaining stable.

SDS concentration [w%]	1	0.1	0.01	0.001	0.0001
CHL lysis efficiency	++	++	+	-	-

doi:10.1371/journal.pone.0093401.t001

## Materials and Methods

### Quantitative CARS microspectroscopy

We use a CARS microspectrometer developed in our laboratory. The details of the CARS system are described in File S1 [20].

### Sample

Chinese Hamster Lung (CHL) cells [24], which are routinely used for toxic evaluation, were used as a sample in the present study. CHL cells were incubated at 37°C under 5% CO<sub>2</sub>. The culture medium were D-MEM (Dulbecco's modified essential medium, Gibco) supplemented with 10% fetal bovine serum (FBS).

### Chemicals

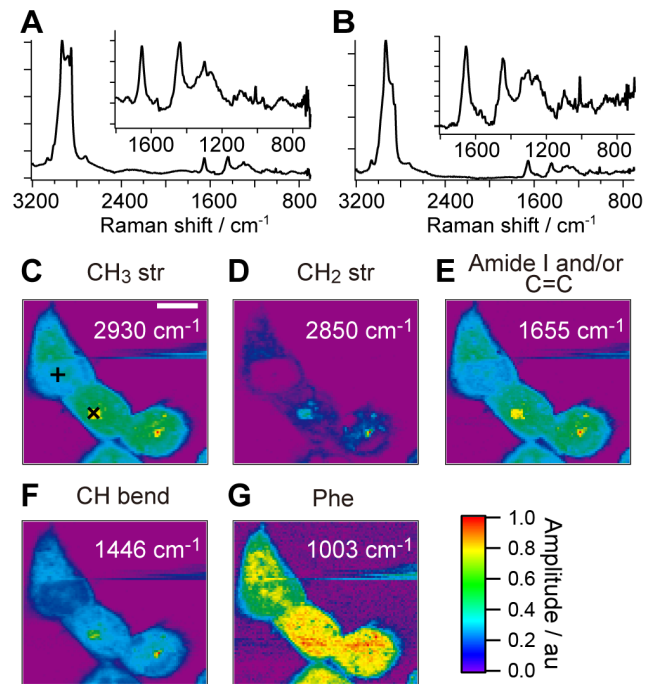
<sup>2</sup>H-substituted sodium dodecyl sulfate ( $d_{25}$ -SDS) was used as a surfactant. The culturing media was suspended with  $d_{25}$ -SDS solution (0.1 wt% SDS in PBS buffer) so that the final

concentration of  $d_{25}$ -SDS was approximately 0.01 w%, 0.3 mM. This concentration is too low to be detected by the CARS microspectroscopic system. We found no spectroscopic signature of the CD stretch from the suspended medium. Nocodazole was used as an inhibitor of intracellular membrane trafficking in CHL cells [19]. It inhibits the polymerization of tubulin and subsequent formation of microtubules. Since nocodazole is not soluble in water, it was solved in dimethylsulfoxide. This solution was added to the medium with the final nocodazole concentration of 25 μM. Cells were incubated for 30 min after the addition of nocodazole.

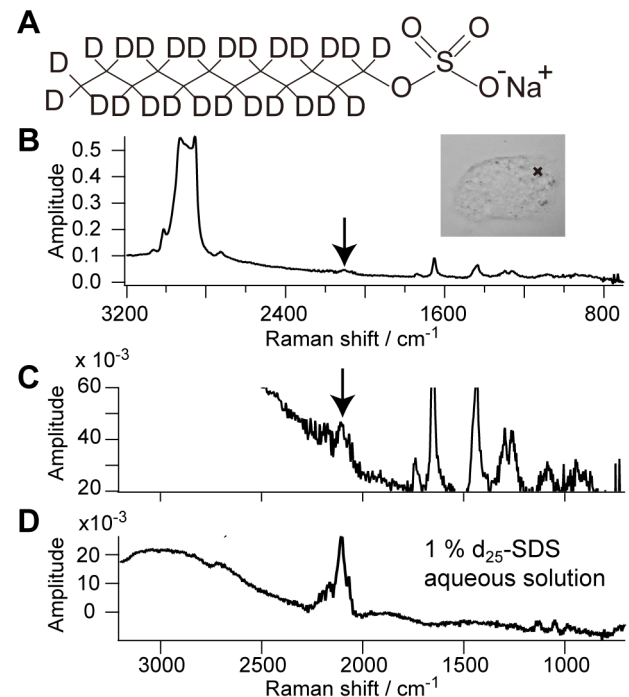
## Results and Discussion

### Cell lysis efficiency

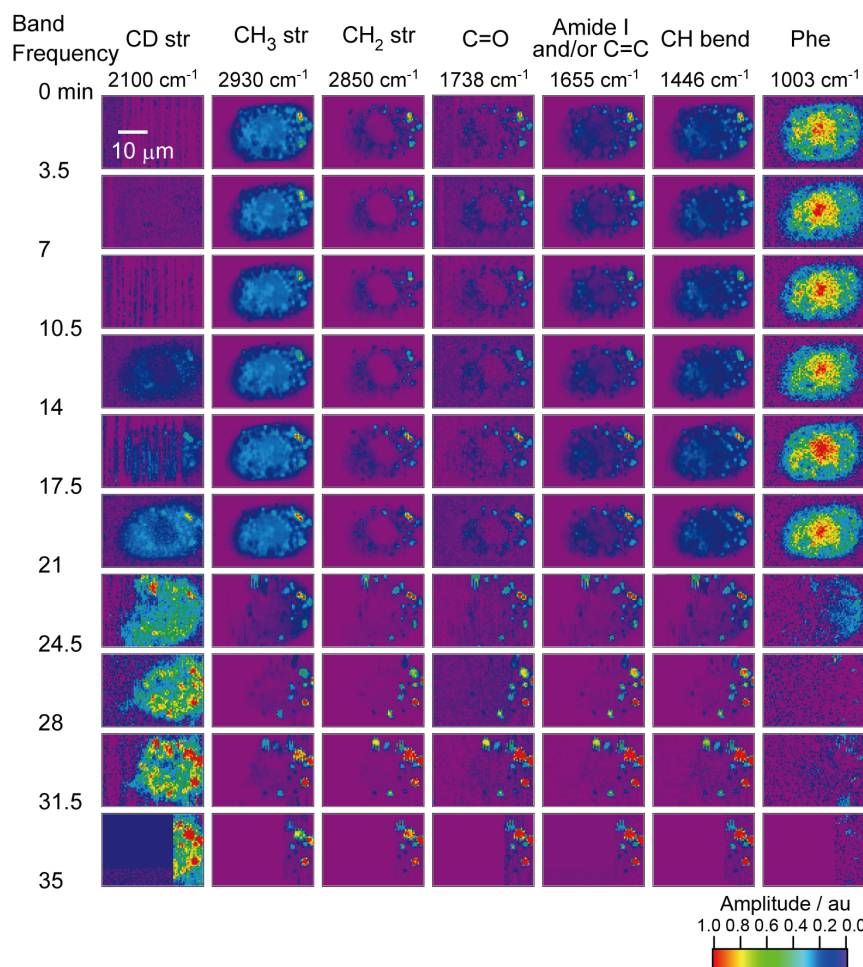
We first analyzed the cell lysis efficiency of  $d_{25}$ -SDS as a model surfactant. Cultured CHL cells were scrape-harvested to microcentrifuge tubes and spin-downed. Then, the supernatants were removed from the solution. Cell pellets were then resuspended to SDS solutions at each concentration of 1, 0.1, 0.01, 0.001 and 0.0001 w% by vortex for 1 min. The suspensions were spin-downed and we checked the degree of cell lysis. High concentration of 1~0.1 w% of SDS solution apparently lyses the CHL cells. On the other hand, below 0.001 w% of SDS solution, the cells remain stable as a pellet (Table 1). These results suggest that the '0.01 w% concentration of SDS solution' is approximately a threshold of CHL cell lysis and denaturation. Thus, we determined the concentration of SDS (0.01 w%, 0.3 mM) for tracing the lysis process of CHL cells with CARS microspectroscopy.



**Figure 1.**  $\text{Im}[\chi^{(3)}]$  spectra and images from a CHL cell.  $\text{Im}[\chi^{(3)}]$  spectra from the two points of the CHL cell. **A** and **B** are obtained from the points indicated as  $\times$  and  $+$  in **C**, respectively. The inset of each spectrum is the expanded spectrum in the fingerprint region. The exposure time is 50 msec.  $\text{Im}[\chi^{(3)}]$  images at 2930  $\text{cm}^{-1}$  (**C**), 2850  $\text{cm}^{-1}$  (**D**), 2655  $\text{cm}^{-1}$  (**E**), 2446  $\text{cm}^{-1}$  (**F**) and 1003  $\text{cm}^{-1}$  (**G**), respectively. The scale bar in the image is 10 μm. The image consists of 91×81 pixels and the exposure time for each pixel is 50 msec. Each image is normalized at the intensity maximal of each band.  
doi:10.1371/journal.pone.0093401.g001



**Figure 2.** SDS molecules are condensed in a CHL cell. **A**. The molecular structure of  $d_{25}$ -SDS. **B**.  $\text{Im}[\chi^{(3)}]$  spectrum obtained from one point of a CHL cell indicated as the cross in the inset several minutes after the addition of  $d_{25}$ -SDS. **C**. The expanded spectrum of **B**. **D**.  $\text{Im}[\chi^{(3)}]$  spectrum of 1%  $d_{25}$ -SDS aqueous solution. The exposure time for **B**–**D** is 50 msec and **B**–**D** are measured under the same experimental condition.  
doi:10.1371/journal.pone.0093401.g002



**Figure 3. Time-resolved  $\text{Im}[\chi^{(3)}]$  images of the CHL cell with the surfactant.** The scale bar in the image is 10  $\mu\text{m}$ . The image consists of  $71 \times 51$  pixels and the exposure time for each pixel is 50 msec. Each row of the CARS images is measured every 3.5 min. Each column is normalized at the intensity maximal of each band. doi:10.1371/journal.pone.0093401.g003

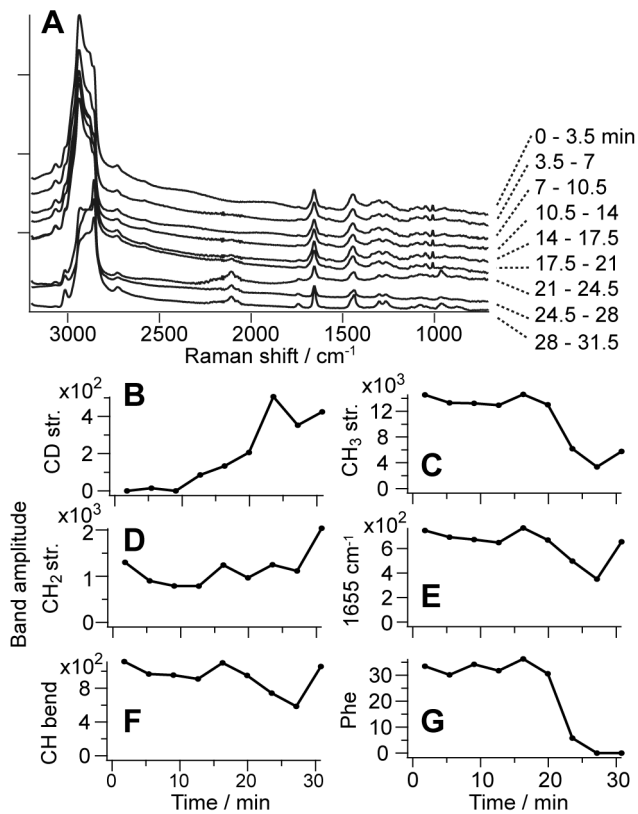
### CARS measurement of a living CHL cell without any treatment

Figures 1A and 1B show typical CARS spectra ( $\text{Im}[\chi^{(3)}]$ ) obtained from two different points in a CHL cell. Using the MEM and a singular value decomposition analysis [25], we obtain  $\text{Im}[\chi^{(3)}]$  spectra with a high signal-to-noise ratio ( $\sim 10^3$  in the CH stretching region and  $\sim 10^2$  in the fingerprint region) from observed multiplex CARS spectra. Compared with the reported Raman spectra of known biomolecules [26], it is clear that Figures 1A and 1B show characteristic spectral features of lipids and proteins, respectively. Based on the  $\text{Im}[\chi^{(3)}]$  spectra obtained from a cell, we construct  $\text{Im}[\chi^{(3)}]$  images, whose amplitude is proportional to the molecular concentration. Figures 1C–H show the  $\text{Im}[\chi^{(3)}]$  images of CHL cells at  $2930\text{ cm}^{-1}$ ,  $2850\text{ cm}^{-1}$ ,  $1655\text{ cm}^{-1}$ ,  $1446\text{ cm}^{-1}$  and  $1003\text{ cm}^{-1}$ . They are assigned to the  $\text{CH}_3$  stretch, the  $\text{CH}_2$  stretch, the superposition of the  $\text{C}=\text{C}$  stretch of lipid chains and the amide I of proteins, the CH bend and the phenylalanine residues, respectively. The image of the  $\text{CH}_2$  stretch mode shows localized and intense signals inside the cell. This image mainly reflects the distribution of lipid molecules such as lipid droplets. On the other hand, the image of phenylalanine is homogeneous in the cell, reflecting the homogeneous distribution of proteins. The images of  $\text{CH}_3$  stretch,  $\text{C}=\text{C}$

and/or amide I and CH bend modes seem to be the sum of the former two groups. The relatively strong phenylalanine signal and no  $\text{CH}_2$  stretch signal are observed around the center of the cells. These areas correspond to the nuclei of the cells. It has been confirmed that the CARS images do not show any appreciable change for an hour, which indicates that the laser irradiation causes no serious photo or thermal damage to the CHL cells on this time scale (the time-resolved CARS images are shown in Figure S1 in File S1).

### CARS measurement of a living CHL cell with surfactant

First, we focus on a CHL cell with the addition of the surfactant SDS (Fig. 2A). Figures 2B and 2C are  $\text{Im}[\chi^{(3)}]$  spectra obtained from one particular position (indicated in the inset of Fig. 2B) of the CHL cell with the addition of  $d_{25}$ -SDS. Surprisingly, the CD stretch band at  $2100\text{ cm}^{-1}$  is found in the cell only in several minutes after the addition of  $d_{25}$ -SDS. The concentration of  $d_{25}$ -SDS inside the cell is estimated to be approximately  $\sim 10\text{ mM}$  by comparing the signal intensity with that of 1 w% (32 mM)  $d_{25}$ -SDS aqueous solution in Fig. 2C. This relatively high concentration indicates that the observed CD stretch intensity does not originate from cell membranes, which are too thin to accumulate a large number of CD carrying molecules to make a high



**Figure 4. Accumulation of SDS in a CHL cell and subsequent cellular death.** **A.** Time-resolved  $\text{Im}[\chi^{(3)}]$  spectra obtained with the summation over all the spectra in the cell shown in Fig. 3. Time-profiles of band amplitudes at  $2100\text{ cm}^{-1}$  (**B**),  $2930\text{ cm}^{-1}$  (**C**),  $2850\text{ cm}^{-1}$  (**D**),  $1655\text{ cm}^{-1}$  (**E**),  $1446\text{ cm}^{-1}$  (**F**) and  $1003\text{ cm}^{-1}$  (**G**). doi:10.1371/journal.pone.0093401.g004

concentration within the laser focal volume, but is more likely to come from the bulk cytoplasm of the cell. Then, the SDS molecules must be taken into the cell efficiently through cell membranes. The concentration of  $10\text{ mM}$  is 30 times higher than the original concentration of  $d_{25}$ -SDS in the medium, namely,  $0.3\text{ mM}$ . It should be noted, however, that the observed CD stretch band may not originate solely from  $d_{25}$ -SDS. Living cells can modify the molecular structure of the surfactant through metabolism, while these molecules are unlikely to be completely metabolized in this time range [27]. We need to confirm that what we observe is not the surfactant molecule itself but the CD stretch Raman signal.

Next, the surfactant uptake dynamics is traced in the time-resolved CARS mapping study. Figure 3 shows the time-resolved CARS mapping images of a  $d_{25}$ -SDS treated CHL cell over a time span of 35 min. The CARS measurement of the CHL cell was started immediately after the addition of  $d_{25}$ -SDS.

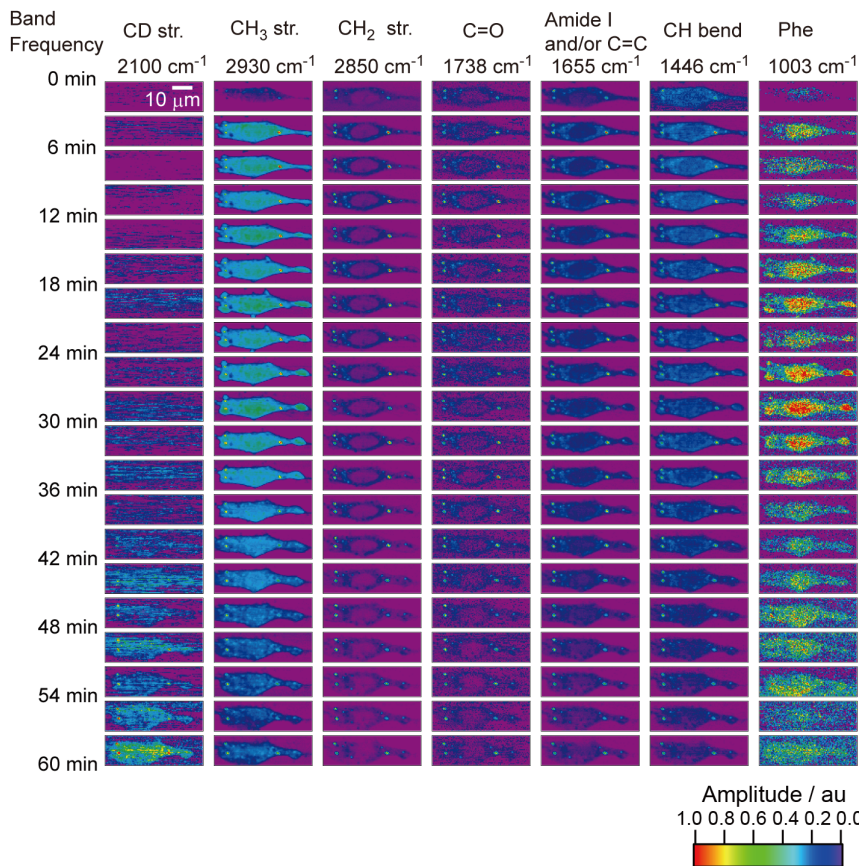
The dynamics of the surfactant uptake and cell lysis is clearly visualized in this experiment. The CD stretch signal increases with time from 0 to 21 min. The signal intensities of all the bands, in particular that of the  $1003\text{ cm}^{-1}$  band, drastically change around 23 min. The Raman band of phenylalanine residues almost disappears at 24.5 min, while the strong Raman signal of lipid is still observed between 24.5 to 35 min. The image of the CD stretch is similar to that of the CH bend mode, and is totally different from that of phenylalanine residues (proteins). Taking into account of the assignment of these bands as discussed above,

this fact suggests that the surfactant molecules are widely spread over the cell cytoplasm. There are several possible pathways of surfactant transport through cellular membranes; protein-mediated transport, flip-flop, diffusion, and endocytosis. Protein-mediated transportation such as ion channels and transporters plays an important role to transport molecules through cellular membranes. Since SDS is relatively large and amphipathic, it should not be transported through ion channels. Furthermore, no transporter for SDS has been known. Protein-mediated transportation is thus unlikely taking place in the present system. Second, trans-membrane lipid translocation (so called flip-flop) could introduce SDS into intracellular membrane. However, the flip-flop mechanism cannot explain accumulation of SDS in cytoplasm. Third, the diffusion process could contribute to the SDS accumulation in the cytoplasm, but it will be shown in the next subsection that it is not the main pathway of the SDS accumulation. Therefore, we exclude three possibilities, protein-mediated transport, flip-flop, and diffusion in the surfactant uptake process.

Based on the above discussion and our CARS results, the overall dynamic behavior inside the cell is summarized in the following way. First, the surfactant molecules are diffused in the medium, and inserted into the cell membranes. Then, they are internalized into the cell possibly through the endocytosis [28,29]. In the present study, it was difficult to identify each endosome in cytoplasm through endocytosis. Taking into account of the final concentration of the surfactant inside the cell ( $\sim 10\text{ mM}$ ), it is highly likely that the cell actively uptakes the surfactant into itself.

The SDS molecules inserted in the endosome membrane are ongoingly accumulated, and condensed inside the cell in  $\sim 20$  min. Judging from the CARS images in 20 min, the strong CD stretch signal is observed from the points where the  $\text{CH}_2$  stretch signal is strong, indicating that the SDS molecules are accumulated in the lipid droplets. It is an intriguing clue to the elucidation of lipotoxicity [30]. Finally, the morphology of the cell drastically changes due to the disruption of the cell membranes, indicating that the surfactant lyses the cell after all. The time-resolved images of phenylalanine residues (proteins) suggest that the concentration of proteins is dramatically lowered at 24.5 min due to the leak of proteins from the cell. Some part of the cell, most probably reflecting the lipid droplets, still remains at the same positions as cell debris even after the disruption of cell membranes.

For the purpose of quantitative analysis, we sum up the spectral profiles of the  $\text{Im}[\chi^{(3)}]$  obtained in the whole cell at each time. Figure 4A shows the time-resolved  $\text{Im}[\chi^{(3)}]$  spectra from the whole cell. The CD stretch signal at  $2100\text{ cm}^{-1}$  gradually increases after the addition of the surfactant. The decreases of the  $\text{CH}_3$  stretch at  $2930\text{ cm}^{-1}$  and phenylalanine residues at  $1003\text{ cm}^{-1}$  at 21 min indicate the leak of proteins from the cell. We fitted each  $\text{Im}[\chi^{(3)}]$  spectrum by the sum of Lorentzian functions shown in Fig. 4A, and obtain the time profiles of the  $2100\text{ cm}^{-1}$ ,  $2930\text{ cm}^{-1}$ ,  $2850\text{ cm}^{-1}$ ,  $1655\text{ cm}^{-1}$ ,  $1446\text{ cm}^{-1}$  and  $1003\text{ cm}^{-1}$  Raman bands as shown in Fig. 4B–G. In the CH stretching region, we fitted the spectra with the sum of three Lorentzian functions whose band positions are  $2930$ ,  $2880$  and  $2850\text{ cm}^{-1}$ . The band amplitude is plotted against the time delay from the addition of the surfactant in each time-profile. Figure 4B strongly suggests that the surfactant is gradually taken into the cell and Fig. 4G indicates that the proteins leak suddenly from the cell. On the other hand, the  $\text{CH}_2$  signal intensity does not change with time (Fig. 4D). This indicates that the total amount of lipid molecules remains almost the same in the region of interest during the measurement. The sharp rise of the ( $2100\text{ cm}^{-1}$ ),  $2930\text{ cm}^{-1}$ ,  $2850\text{ cm}^{-1}$ ,



**Figure 5. Time-resolved  $\text{Im}[\chi^{(3)}]$  images of the CHL cell with the surfactant and inhibitor of intracellular membrane trafficking.** The scale bar in the image is 10  $\mu\text{m}$ . The image consists of  $101 \times 31$  pixels and the exposure time for each pixel is 50 msec. Each row of the CARS images is measured every 3 min. Each column is normalized at the intensity maximal of each band. doi:10.1371/journal.pone.0093401.g005

$1655 \text{ cm}^{-1}$  and  $1446 \text{ cm}^{-1}$  around 30 min can be ascribed to the laser trapping effect of lipid droplets.

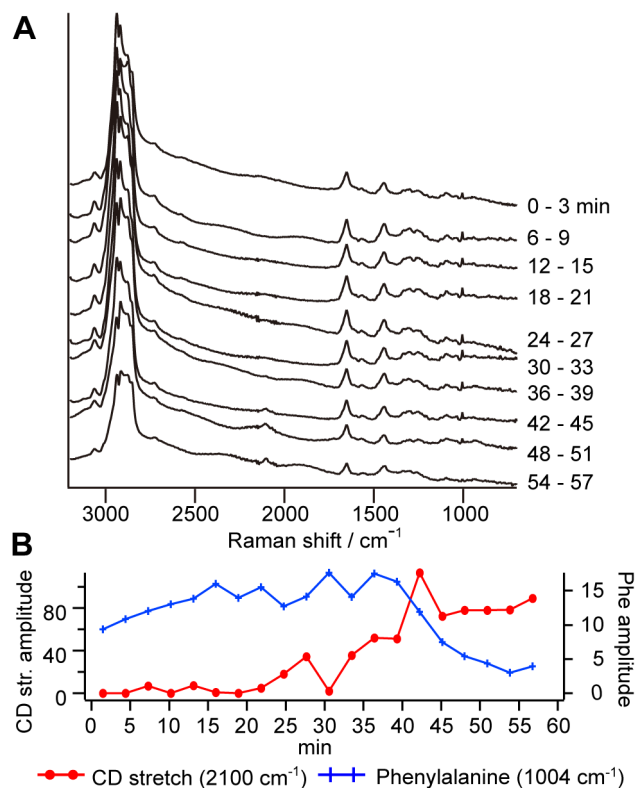
In order to confirm the reproducibility of the observed phenomenon we have measured nine cells. The time-resolved profiles of the  $1003 \text{ cm}^{-1}$  and  $2100 \text{ cm}^{-1}$  Raman bands of the nine cells are summarized in Figure S2 in File S1. Even though they show different temporal profiles, it is certain that the CD stretch signal increases gradually and the phenylalanine signal decreases suddenly (15–30 min). This suggests that cells are lysed by the surfactant on the similar time scale through the same mechanism. Interestingly, cell lysis is initiated after the  $2100 \text{ cm}^{-1}/1003 \text{ cm}^{-1}$  amplitude ratio (CD stretch/Phe) reaches a certain level (3 to 10). Although it is difficult to discuss this finding quantitatively, because the sizes and conditions of the cells are different from one another, there seems to exist a threshold of SDS concentration for cell lysis to occur. The  $2100 \text{ cm}^{-1}/1003 \text{ cm}^{-1}$  amplitude ratio could thus be used as a cell-lysis progress indicator.

#### CARS measurement of a living CHL cell with surfactant and inhibitor

The time-resolved CARS measurements shown in Figs. 3 and 4 indicate that CHL cells actively internalize the surfactant molecules. Next, we investigate the effect of an inhibitor of intracellular membrane trafficking, nocodazole, upon the surfactant uptake. Nocodazole is known as an inhibitor of tubulin

polymerization and the subsequent formation of microtubules [31,32].

Figure 5 shows the results of the time-resolved CARS imaging experiment on a nocodazole treated CHL cell (incubated with nocodazole) with  $d_{25}$ -SDS over a time span of 60 min. Highly localized distributions of lipid bands are clearly observed, while the image of proteins is homogeneously distributed. In addition, the gradual increase of the CD stretch ascribed to the surfactant uptake is also observed. Using the same procedure as the experiment without nocodazole, we sum up all the spectral profiles obtained from the whole cell at each time. Figure 6A shows the time-resolved  $\text{Im}[\chi^{(3)}]$  spectra from the whole cell. The strong background signal from dimethylsulfoxide (solvent of nocodazole) is already subtracted from the observed spectra. The spectral profiles in the CH stretch region ( $2700 \sim 3000 \text{ cm}^{-1}$ ) are slightly distorted by this background subtraction. Figure 6B shows the time-profiles of the  $2100 \text{ cm}^{-1}$  (the CD stretch mode; circle, left axis) and  $1003 \text{ cm}^{-1}$  (the phenylalanine residues; cross, right axis) Raman band amplitudes obtained from Fig. 6A. The gradual increase of the CD stretch signal is observed in this case as well as in the experiments without nocodazole. On the other hand, the decrease of the  $1003 \text{ cm}^{-1}$  band is not as fast as that without nocodazole. Moreover, it takes approximately an hour for the cell to lyse, while several tens of minutes in the experiments without nocodazole. This experimental result indicates that nocodazole, an inhibitor of polymerization of tubulin, slows down the surfactant uptake dynamics. We have measured six cells to check the



**Figure 6. Nocodazole lowers the surfactant uptake rate of a CHL cell.** **A.** Time-resolved  $\text{Im}[\chi^{(3)}]$  spectra obtained with the summation over all the spectra in the cell shown in Fig. 5. **B.** Time-profiles of band amplitudes at  $2100\text{ cm}^{-1}$  (circle, left axis) and  $1003\text{ cm}^{-1}$  (cross, right axis). doi:10.1371/journal.pone.0093401.g006

reproducibility (see Figure S3 in File S1). We ascribe this change to the inhibition of the membrane trafficking, which plays a key role to transport of the surfactant to organelles. Now, we can discuss the main contribution of the surfactant uptake we observed. While the inhibitor does not affect diffusion, it is ensured that nocodazole slows down/stops endocytosis. The fact that nocodazole slows down the rate of the surfactant uptake strongly suggests that the surfactant uptake is controlled mainly by endocytosis.

The increase of the CD stretch band amplitude and the decrease of phenylalanine residues seem to take longer time (30~60 min) compared with the experiments without nocodazole (15~30 min). The decrease of phenylalanine residues and the increase of the CD stretch band are almost synchronized. The final amount of the surfactant inside the cell is similar for the experiments with and without nocodazole. However, the total amount of phenylalanine inside the cells decreases gradually with nocodazole, while it suddenly occurs without nocodazole due to

## References

- Moore PN, Shiloach A, Puvvada S, Blankschtein D (2003) Challenging the surfactant monomer skin penetration model: Penetration of sodium dodecyl sulfate micelles into the epidermis. *Journal of Cosmetic Science* 54: 29–46.
- Agner T, Serup J (1990) Sodium Lauryl Sulfate for Irritant Patch Testing - a Dose-Response Study Using Bioengineering Methods for Determination of Skin Irritation. *Journal of Investigative Dermatology* 95: 543–547.
- diNardo A, Sugino K, Wertz P, Ademola J, Maibach HI (1996) Sodium lauryl sulfate (SLS) induced irritant contact dermatitis: A correlation study between ceramides and in vivo parameters of irritation. *Contact Dermatitis* 35: 86–91.
- Tupker RA, Willis C, Berardesca E, Lee CH, Fartasch M, et al. (1997) Guidelines on sodium lauryl sulfate (SLS) exposure tests - A report from the

the cell lysis. This fact indicates that cell lysis with SDS occurs in different ways for CHL cells with and without nocodazole. Nocodazole seems to change the surfactant uptake and cell lysis mechanism profoundly.

## Conclusion

Multiplex CARS microspectroscopy has been used to study the surfactant uptake dynamics in living CHL cells. The dynamics of surfactant accumulation inside the cells and subsequent cell lysis is clearly observed with several-minutes temporal resolution. Given that the observed CD stretch signal exclusively originates from the surfactant, the concentration of the surfactant in a CHL cell is estimated to be several 10 mM. The surfactant is found to accumulate in the overall cell cytoplasm. There seems to be a threshold of the surfactant concentration for cell lysis to occur. By adding nocodazole as an inhibitor of active transport, the surfactant uptake speed significantly decreases. We have thus proved that the surfactant uptake and following cell lysis are closely related with intracellular membrane trafficking in living cells. This result indicates that living cells retain the surfactant not only in the plasma membrane but also in the whole intracellular membrane system and that the intracellular metabolism is related to detoxification of surfactant. The findings in the present study will contribute not only to the fundamental understanding of surfactant/cell interactions but also to the improvement of surfactant toxicity in our daily life.

## Supporting Information

**File S1** Experimental setup. Figure S1, Time-resolved  $\text{Im}[\chi^{(3)}]$  images of CHL cells without any treatment. Figure S2, Time-profiles of the  $\text{Im}[\chi^{(3)}]$  amplitudes of the  $2100\text{ cm}^{-1}$  (CD stretch; red circle, left axis) and  $1004\text{ cm}^{-1}$  (phenylalanine; blue cross, right axis) obtained from nine CHL cell without the addition of nocodazole. Figure S3, Time-profiles of the  $\text{Im}[\chi^{(3)}]$  amplitudes of the  $2100\text{ cm}^{-1}$  (CD stretch; red circle, left axis) and  $1004\text{ cm}^{-1}$  (phenylalanine; blue cross, right axis) obtained from six CHL cell with the addition of nocodazole. (DOCX)

## Acknowledgments

J. Ukon, UKON CRAFT SCIENCE, Ltd. assisted with a fruitful collaboration between Japanese and French labs. There are no patents, products in development or marketed products to declare. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials.

## Author Contributions

Conceived and designed the experiments: KF SN HH. Performed the experiments: MO KF KB. Analyzed the data: MO KF KB. Contributed reagents/materials/analysis tools: PL VC. Wrote the paper: MO HK KF KB HH.

Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 37: 53–69.

- Salton MRJ (1951) The Adsorption of Cetyltrimethylammonium Bromide by Bacteria, Its Action in Releasing Cellular Constituents and Its Bactericidal Effects. *Journal of General Microbiology* 5: 391–404.
- Partearroyo MA, Ostolaza H, Goni FM, Barbera-Guillem E (1990) Surfactant-induced cell toxicity and cell lysis a study using B16 melanoma cells. *Biochemical Pharmacology* 40: 1323–1328.
- Vieira DB, Carmona-Ribeiro AM (2006) Cationic lipids and surfactants as antifungal agents: mode of action. *Journal of Antimicrobial Chemotherapy* 58: 760–767.

8. Smith ARW, Lambert PA (2008) Antimicrobial action of N-(n-dodecyl)diethanolamine on *Escherichia coli*: effects on enzymes and growing cultures. *Journal of Applied Microbiology* 105: 2161–2168.
9. Chen JX, Volkmer A, Book LD, Xie XS (2002) Multiplex coherent anti-stokes Raman scattering microspectroscopy and study of lipid vesicles. *Journal of Physical Chemistry B* 106: 8493–8498.
10. Kee TW, Cicerone MT (2004) Simple approach to one-laser, broadband coherent anti-Stokes Raman scattering microscopy. *Optics Letters* 29: 2701–2703.
11. Kano H, Hamaguchi H (2007) Supercontinuum dynamically visualizes a dividing single cell. *Analytical Chemistry* 79: 8967–8973.
12. Petrov GI, Arora R, Yakovlev VV, Wang X, Sokolov AV, et al. (2007) Comparison of coherent and spontaneous Raman microspectroscopies for noninvasive detection of single bacterial endospores. *Proceedings of the National Academy of Sciences of the United States of America* 104: 7776–7779.
13. Bonn M, Muller M, Rinia HA, Burger KNJ (2009) Imaging of chemical and physical state of individual cellular lipid droplets using multiplex CARS microscopy. *Journal of Raman Spectroscopy* 40: 763–769.
14. van Manen HJ, Lenferink A, Otto C (2008) Noninvasive Imaging of Protein Metabolic Labeling in Single Human Cells Using Stable Isotopes and Raman Microscopy. *Analytical Chemistry* 80: 9576–9582.
15. Ganikhanov F, Evans CL, Saar BG, Xie XS (2006) High-sensitivity vibrational imaging with frequency modulation coherent anti-Stokes Raman scattering (FM CARS) microscopy. *Optics Letters* 31: 1872–1874.
16. Potma EO, Xie XS (2005) Direct visualization of lipid phase segregation in single lipid bilayers with coherent anti-stokes Raman scattering microscopy. *Chemphyschem* 6: 77–79.
17. Koval M, Pagano RE (1989) Lipid Recycling between the Plasma-Membrane and Intracellular Compartments - Transport and Metabolism of Fluorescent Sphingomyelin Analogs in Cultured Fibroblasts. *Journal of Cell Biology* 108: 2169–2181.
18. Mayor S, Presley JF, Maxfield FR (1993) Sorting of Membrane-Components from Endosomes and Subsequent Recycling to the Cell-Surface Occurs by a Bulk Flow Process. *Journal of Cell Biology* 121: 1257–1269.
19. Castillo C, Hernandez Y, Roychowdhury S, Das S (2009) Cytoskeleton-Based Lipid Transport in a Parasitic Protozoan, *Giardia lamblia*. In: Ortega-Pierres MG, Caccio S, Fayer R, Mank T, Smith H, et al., editors. *Giardia and Cryptosporidium*: CABI.
20. Okuno M, Kano H, Leproux P, Couderc V, Day JPR, et al. (2010) Quantitative CARS Molecular Fingerprinting of Single Living Cells with the Use of the Maximum Entropy Method. *Angewandte Chemie-International Edition* 49: 6773–6777.
21. Rinia HA, Bonn M, Muller M, Vartiainen EM (2007) Quantitative CARS spectroscopy using the maximum entropy method: The main lipid phase transition. *ChemPhysChem* 8: 279–287.
22. Day JPR, Domke KF, Rago G, Kano H, Hamaguchi H, et al. (2011) Quantitative Coherent Anti-Stokes Raman Scattering (CARS) Microscopy. *Journal of Physical Chemistry B* 115: 7713–7725.
23. Day JPR, Rago G, Domke KF, Velikov KP, Bonn M (2010) Label-Free Imaging of Lipophilic Bioactive Molecules during Lipid Digestion by Multiplex Coherent Anti-Stokes Raman Scattering Microspectroscopy. *Journal of the American Chemical Society* 132: 8433–8439.
24. Kokyama H, Utakoji T, Ono T (1970) A new cell line derived from newborn Chinese hamster lung tissue. *Gann* 61: 161–167.
25. van Manen HJ, Kraan YM, Roos D, Otto C (2004) Intracellular chemical imaging of heme-containing enzymes involved in innate immunity using resonance Raman microscopy. *Journal of Physical Chemistry B* 108: 18762–18771.
26. Parker FD (1983) *Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry*: Springer.
27. Decourcelles DD, Leyen JE, Declercq F, Vanbelle H, Janssen PAJ (1985) Evidence That Phospholipid Turnover Is the Signal Transducing System Coupled to Serotonin-S2 Receptor-Sites. *Journal of Biological Chemistry* 260: 7603–7608.
28. Mukherjee S, Ghosh RN, Maxfield FR (1997) Endocytosis. *Physiological Reviews* 77: 759–803.
29. Nichols BJ, Lippincott-Schwartz J (2001) Endocytosis without clathrin coats. *Trends in Cell Biology* 11: 406–412.
30. Listenberger LL, Han XL, Lewis SE, Cases S, Farese RV, et al. (2003) Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* 100: 3077–3082.
31. Samson F, Donoso JA, Hellerbettinger I, Watson D, Himes RH (1979) Nocodazole Action on Tubulin Assembly, Axonal Ultrastructure and Fast Axoplasmic-Transport. *Journal of Pharmacology and Experimental Therapeutics* 208: 411–417.
32. Vasquez RJ, Howell B, Yvon AMC, Wadsworth P, Cassimeris L (1997) Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. *Molecular Biology of the Cell* 8: 973–985.