

## DFT calculations for NEXAFS analysis measured for organic molecules

Shohei Asakura<sup>a</sup>, Hashimoto Genki<sup>b</sup>, Ohura Yuri<sup>a</sup>, Shogo Tendo<sup>a</sup>,  
Kakuto Yoshioka<sup>a</sup>, Akinobu Niozu<sup>c</sup>, and Wada Shin-ichi<sup>a,b,d</sup>

<sup>a</sup> Faculty of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan

<sup>b</sup> Graduate School of Science and Engineering, Hiroshima University, Higashi-Hiroshima 739-8526, Japan

<sup>c</sup> Graduate School of Humanities and Social Sciences, Hiroshima University,  
Higashi-Hiroshima 739-8526, Japan

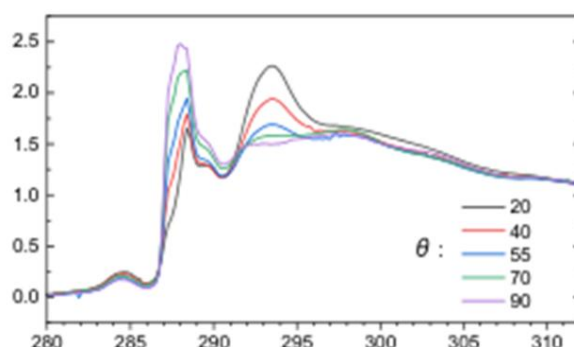
<sup>d</sup> Research Institute for Synchrotron Radiation Science, Hiroshima University 739-0046, Japan

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Density functional theory (DFT) is a powerful technique that allows theoretical predictions of electronic states and is also effective in interpreting X-ray absorption spectra (NEXAFS). However, proper implementation of DFT calculations requires setting parameters and mastering analysis techniques according to the characteristics of the target molecules. Gaining insights into the structure and order of molecular membranes, which are aggregates, is particularly challenging. Therefore, we focused on phospholipid molecules as the molecules forming the molecular membrane and performed DFT calculations on these.

Phospholipids play a vital role in living organisms, especially in forming the basic structure of cell membranes. These membranes are not just physical barriers but also function as sites for information transmission and protein function regulation, deeply involved in fundamental biological activities. Therefore, understanding the structure and properties of cell membranes is crucial for elucidating biological processes. Recent research has advanced by forming biomimetic membranes supported on metal substrates to study their structure and properties. In this context, understanding the electronic structure of lipid membranes is essential, and DFT calculations have gained attention as one method for this analysis.

Additionally, the hydrocarbon chains of lipid molecules are desirable systems for the introduction of DFT calculations. Hydrocarbon chains in lipid molecules are generally composed mostly of saturated chains without carbon-carbon double bonds. This characteristic provides ideal conditions for implementing DFT calculations and is suitable for verifying whether the analysis technique is appropriate through comparison with experimental results. Therefore, in this study, we focused on the hydrocarbon chains of lipid molecules and reproduced NEXAFS spectra using DFT calculations to examine their effectiveness by comparing with experimental results.

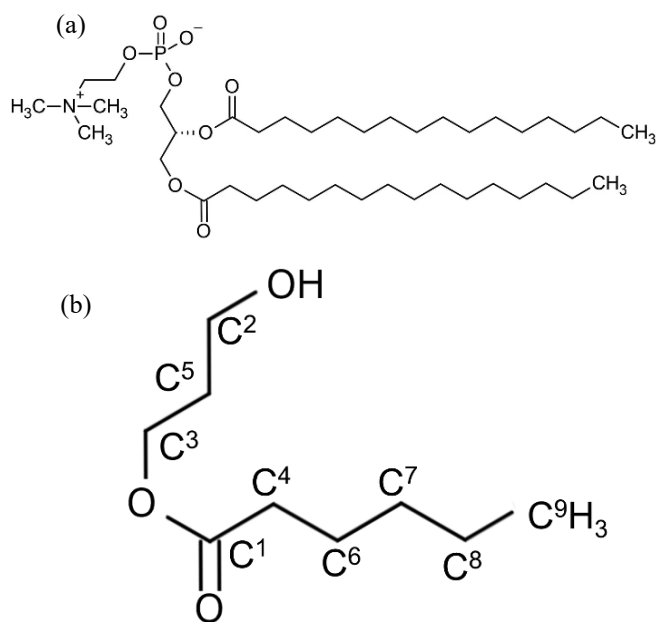


**FIGURE 1.** The carbon K-edge NEXAFS spectra of phospholipid membranes supported on metal substrates obtained from experiments.  $\theta$  represents the angle between the soft X-ray and the substrate. The intensity of the spectra changes with  $\theta$ , indicating orientation dependence.

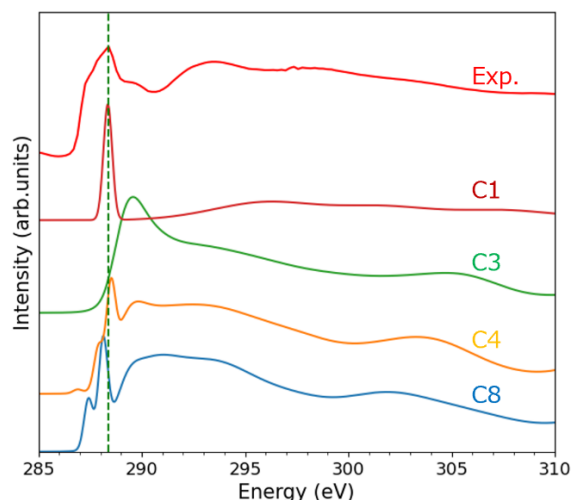
The carbon K-edge NEXAFS spectra of phospholipid membranes supported on metal substrates obtained from previous studies are shown in Fig. 1. Strong polarization dependence at 288 eV and 293 eV can be observed in these spectra. Previous studies attributed this polarization dependence to the hydrocarbon chains that significantly contribute to the order of the molecular membrane and discussed the orientation and order of the phospholipid membranes on metal substrates. In this study, to verify the transitions attributed in previous studies, we focused on the hydrocarbon chains of DPPC as shown in Fig. 2(a) and assumed a model molecule as shown in Fig. 2(b). Calculations were performed on this molecule, with the superscript letters of each carbon atom representing the labeling used in the spectrum calculation. This model molecule includes carbons thought to be related to the orientation of the lipid membrane. The calculation results of this model molecule allow the decomposition of the soft X-ray absorption spectra of phospholipids into each carbon atom. This information is expected to provide detailed insights into the spectrum.

In the actual calculations, we performed structural optimization of the model molecule using DFT calculations, followed by reproducing the NEXAFS spectra using the TP method, which calculates the energy difference between the ground state and the excited state of the obtained structure. Gaussian16 was used for structural optimization with DFT calculations. The functional used was B3LYP, and the basis set was 6-31G. StoBe/Demon was used for TP method calculations.

The green dashed line in Fig. 3(b) indicates the calibrated position where the first peak of the experimental data matches the  $\pi(C=O)$  peak from the calculation result of C1. At this point, it was found that the low-energy peak of the experimental data is composed of three peaks originating from C-H and a peak originating from  $\pi(C=O)$ . The first peak originating from C-H on the higher energy side of the green dashed line is composed of contributions from carbon atoms adjacent to electronegative oxygen or carbonyl carbons, such as C2, C3, and C4. The remaining peaks originating from C-H\* are composed of contributions from C6, C7, and C8 in the hydrocarbon chain. This reveals which positions in the molecule contribute to the three peaks originating from C-H\* and the peak originating from  $\pi^*(C=O)$  that constitute the first peak of the experimental data.



**FIGURE 2.** (a) Structural formula of DPPC. (b) Structural formula of the model molecule used for theoretical calculations. The superscript letters of each carbon atom represent the labeling used in the calculations.



**FIGURE 3.** Comparison between the NEXAFS spectra obtained from calculations and experimental results.