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^1H - ^{31}P home-built solid-state NMR probe with a scroll coil for 400-MHz NB magnet for biological lossy sample

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Abstract

Phosphorus is one of the most important constituents of human biofilms, in particular lipid bilayer packing, phase transition (gel phase, physiological liquid crystal phase, ripple phase, non-biphasic), lipid head group orientation/mechanics, and pure lipid bilayers. Phosphorus observations in biofilms play an important role in the study of the interaction of many types of proteins and biofilms in the human body. The design and construction of a ^1H - ^{31}P double resonance probe with a scroll coil for solid-state NMR experiment are introduced. For good efficiency at the relatively high frequency, minimal RF sample heating during the experiment with a lossy sample, and a wide range of tuning frequency, this probe adapted the low inductance of a scroll coil. The ^{31}P NMR spectra obtained on the biological lossy sample were used to demonstrate the possibility for monitoring the protein dynamics on biomembrane mimetic system and phase change of phospholipid.

Keywords: Home-built solid-state NMR probe, Biological lossy sample, ^1H - ^{31}P double resonance, Scroll coil, Membranes

Introduction

Phosphorus is one of the most abundant minerals in the human body and is an essential element for life. In particular, phosphorus is a component of phospholipids, one of the components of human biological membrane, and phospholipids form a bilayer in vivo (Marcus 2013). Membrane proteins are present in biological membrane consisting of phospholipid bilayers, and they have a variety of structures and perform various functions essential for survival through the role of ion channels, receptors, and transporters (Cooper 2000). Most complex interactions occur outside the cell membrane, and structural and morphological modifications of the cell membrane can be important indicators of how well the protein-cell interactions work. Therefore, observing and analyzing the phosphorus of various phospholipids in the cell membrane is very important for pathological studies on

the correlations of disease occurrence when homeostasis is broken down by external factors (Watson 2015). In addition, since phosphorus is widely used in various materials such as plastics, glass, various organic and inorganic synthetic materials, synthetic catalysts, and pharmaceuticals as well as biological membranes, analysis of a substance containing phosphorus is important (Marry and Delbert 1995; Hodnett 1985). ^{31}P NMR spectroscopy is a very suitable analytical technique for observing compounds containing phosphorus (Sklenar and Miyashiro 1986). ^{31}P NMR does not require isotopic labeling and has the advantage of being able to quantify the proportion of lipids containing phosphorus (Argyropoulos 1994). We experiment ^{31}P NMR in bicelle with an environment similar to human biological membrane (Triba and Warschawski 2005; Dubinnyi and Lesovoy 2006). We designed and tested a ^1H - ^{31}P double resonance solid-state nuclear magnetic resonance probe with a scroll coil for narrow-bore (NB) 400-MHz magnet using a Cross-Waugh circuit for the analysis of a variety

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of materials and biofilms including the phosphorus mentioned above (Fig. 1). In this paper, we introduce the ^1H - ^{31}P home-built double resonance solid-state NMR probe with a scroll coil for 400-MHz NB magnet for lossy biological samples. While the B_1 homogeneity of conventional solenoid coil degrades as the number of turns in a solenoid is decreased (Stringer et al. 2005), the scroll coil has better advantages in B_1 homogeneity, ^1H field strength, and has minimal perturbations of tuning by a wide range of samples than solenoid coil (Grant et al. 2007). Since the scroll coil is compatible with a lumped element circuit that is a well-matched transmitter and receiver system at the proper frequency, this resonator is good for a multi-channel SSNMR probe.

Experimental methods

Probe design

The all probe compositions were made of non-magnetic materials such as aluminum, brass, glass, and polytetrafluoroethylene (PTFE) (Choi et al, 2012, Jeong et al, 2013, Park et al, 2010). The aluminum pipe of 6061 was used for probe body and refined to 39.5-mm outer diameter (OD) and 39.1-mm inner diameter (ID). If aluminum is used as

it is, it can be easily deteriorated and corroded, which can damage its appearance and lose its non-conducting function. To make up these drawbacks, the aluminum pipe was anodized to increase its strength, abrasion resistance, corrosion resistance, and electrical insulation. Many solid-state NMR probes with double resonances have been made using Cross-Waugh type of circuit because it had good isolation of the frequencies. This type of circuit adapted transmission lines of variable length for tuning elements on both sides of the coil (Cross et al. 1976; Doty et al. 1981; Jiang et al. 1987).

The schematic circuit diagram for the 400-MHz narrow-bore ^1H - ^{31}P double resonance probe is shown in Fig. 2. A set of “C” indicated the fixed capacitors (American Technical Ceramics, USA) or variable capacitors (Polyflon, USA). Appropriate fixed and variable capacitors were optimized and adapted with a given scroll coil for the best circuit efficiency. Capacitors C1 and C2 were variable capacitors that were used for tuning and matching the high-frequency ^1H side of 400.13 MHz. C3 and C4 were variable capacitors that were used for tuning and matching the low-frequency ^{31}P channel of 161.97 MHz. All variable capacitors have a changeable

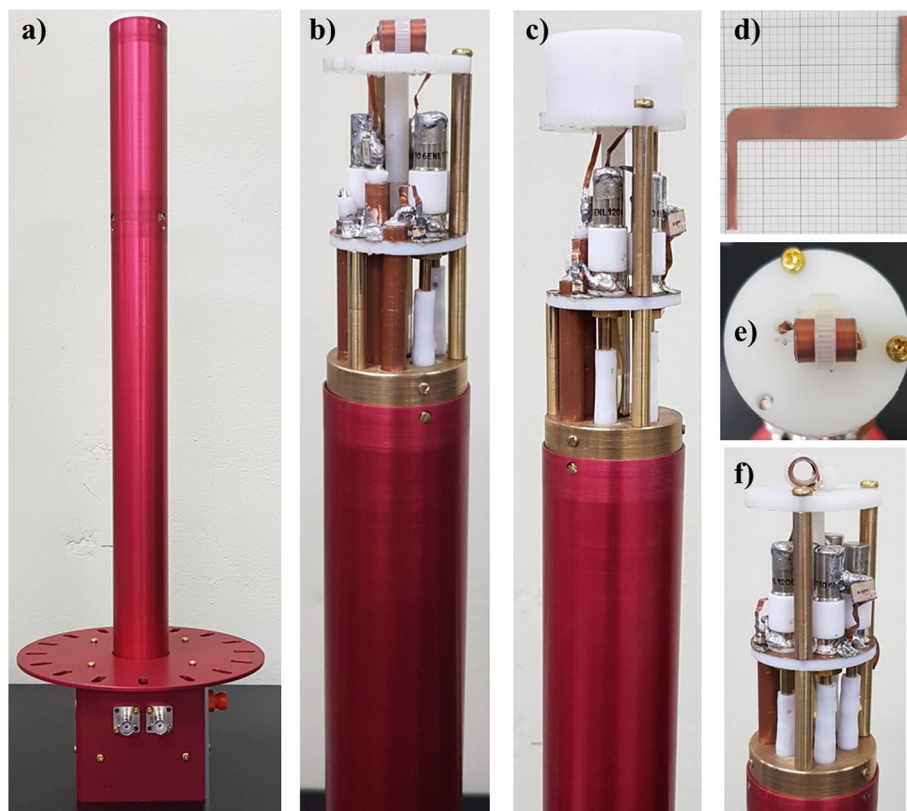
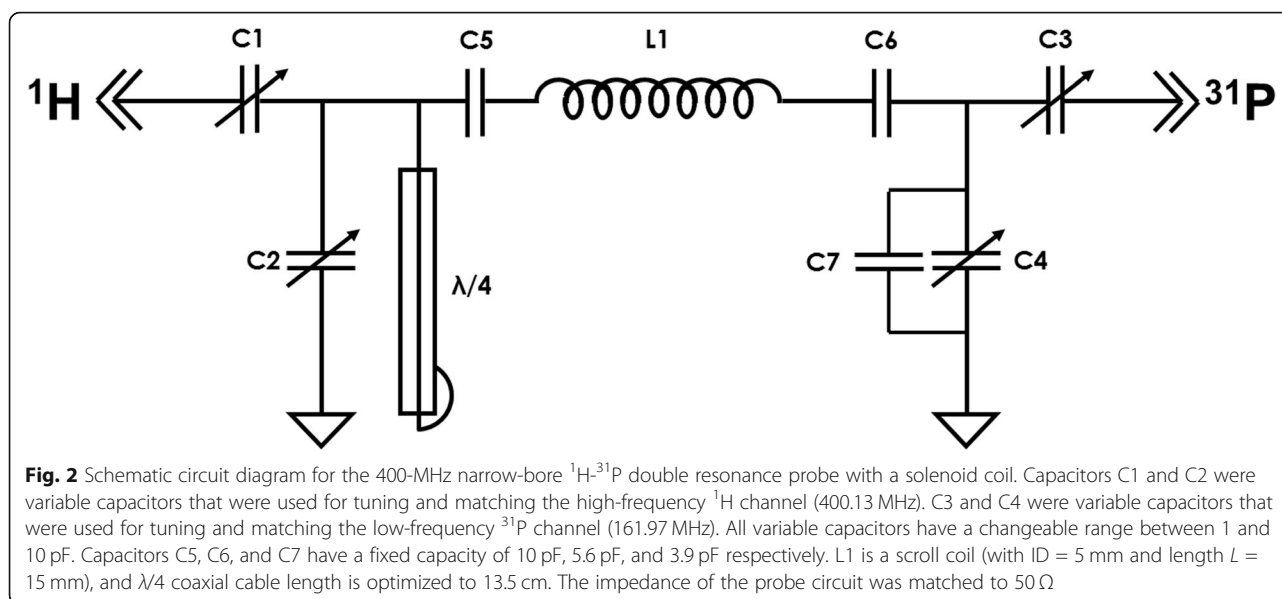


Fig. 1 The pictures of the 400-MHz NB home-built ^1H - ^{31}P double resonance solenoid probe. **a** The appearance of the probe. **b** The completed assembly without the probe cap. **c** The probe head with a cap which fully covers the scroll coil and minimizes the heat loss. **d** A copper blank for scroll coil coated with a Teflon tape. **e** The 5-mm inner diameter scroll coil tied up with the cable tie. **f** A side view of the scroll coil. The cable tie provided mechanical stability of the scroll coil with 5-mm inner diameter



range between 1 and 10 pF. Capacitors C5, C6, and C7 have a fixed capacity of 10 pF, 5.6 pF, and 3.9 pF respectively. L1 is a scroll coil with ID = 5 mm and length $L = 15$ mm, and $\lambda/4$ coaxial cable length is optimized to 13.5 cm. The impedance of the probe circuit was matched to 50 Ω . The 3-turn rolled-up scroll coil of ID = 5 mm length $L = 15$ mm was made of copper sheet (Zhang et al. 1998; Stoll et al. 1977). The initial shape of the scroll coil is shown in Fig. 1d. The PTFE, non-conducting and dielectrical material, tape that prevented the occurring electrical shortening at the edges was adhered to one side of the copper. The cable tie helped the resonator to be stable mechanically. C7 was an optional fixed capacitor on low-frequency channel reducing interference from the high frequency and voltage to the low side channel. For the isolation between the high side channel of ^1H and the low side channel of ^{31}P , the grounded coaxial $\lambda/4$ line was used. The coaxial $\lambda/4$ line length was calculated considering the ^1H resonance frequency of 400.13 MHz following equation.

$$\begin{aligned} \text{Length of } \lambda/4 \text{ cable} &= \frac{c \cdot k}{4 \cdot \nu} \\ &= \frac{3 \times 10^{10} (\text{cm} \cdot \text{s}^{-1}) \times 0.69}{4 \times 400.13 \times 10^6 (\text{s}^{-1})} \\ &= 13.0 (\text{cm}) \end{aligned}$$

c indicates the speed of light, ν is the resonance frequency of the proton channel in 9.4-T magnet, and k is the shorten factor of the coaxial cable filled with PTFE materials. In order to improve the isolation efficiency from ^{31}P low-frequency channel to ^1H high-frequency channel, the low channel trap of C7 was used along with

C6. It was confirmed that the resulting circuit with a scroll coil was electrically and mechanically stable when the radio frequency flew through the probe circuit by using a network analyzer (Hewlett Packard 85046A, USA). And the network analyzer was used for monitoring the transmission between the 400.13 MHz of ^1H high side and 161.97 MHz of low side while the value of lead length between C6 and C7 was changing. Additional temperature control unit consisted of the probe heater, thermocouple sensor, and dewar which can help the monitoring of the phase transition of biological lossy samples as a variation of temperature.

Solid-state NMR experiments

The ^{31}P solid-state NMR spectra were obtained using a Bruker Avance III HD spectrometer that consisted of a 9.4-T AscendTM narrow-bore magnet equipped with our home-built scroll coil probe. The chemical shifts of one-dimensional ^{31}P NMR experiments were referenced with single resonance peak on 0 ppm of 85% H_3PO_4 in a 5-mm flat bottom round NMR tube (New Era enterprises). ^{31}P NMR experiment of 85% H_3PO_4 was obtained with the $\pi/2$ pulse length of 4 μs , RF power of 250 W, a 2048 complex point, 16 scans, and 5-s recycle delay. In order to ensure the possibility of using this home-built probe for trashing a change of phospholipid phase, we monitored ^{31}P resonances of bicelle consisted of [14-O-PC/6-O-PC] or [14-O-PC/DMPG/6-O-PC] along varying temperatures in the 5-mm flat bottom round NMR tube. Bicelles consist of long-chain and short-chain phospholipids, which form a bilayer like biological membrane in aqueous medium and align spontaneously in a high magnetic field (De Angelis and Opella 2007). The intrinsic structure and function of membrane proteins can be studied using solid-state NMR

spectroscopy in the presence of bicelles. Long-chain (1,2-di-O-tetradecyl-sn-glycero-3-phosphocholine; 14-O-PC) and short-chain (1,2-di-O-hexyl-sn-glycero-3-phosphocholine; 6-O-PC) have been used to obtain long-term bicelle stability (Schiller and Muller 2007), and DMPG (dimyristoyl phosphatidylglycerol) have been used to simulate the anionic bacterial membrane (Marcotte and Bélanger 2006; Sanders and Hare 1994). All lipids dissolved in chloroform were evaporated in the solvent with N_2 gas and lyophilized to completely remove the solvent. The lyophilized 6-O-PC were solubilized in ddH_2O to construct micelle and co-solubilized with hydrated mixture of 14-O-PC and DMPG. Repeat the freeze-and-thaw cycles with vortexing and sonicating until bicelle solution has completely become transparent. The lipid molar ratios q of long chain/short chain is 3.2, and the total volume of the bicelle solution was 200 μ l. ^{31}P NMR experiment of phospholipid bicelle was obtained with the $\pi/2$ pulse length of 6 μ s, RF power of 260 W, a 2048 complex point, 16 scans, and 5-s recycle delay. All the bicelle samples were equilibrated in the magnetic field at each targeted temperature at least 30 min before ^{31}P NMR experiments.

Results and discussion

The photograph of the complete probe assembly is shown in Fig. 1. ^{31}P NMR experimental results obtained by using the 400-MHz 1H - ^{31}P double resonance home-built probe are shown in Figs. 3 and 4. Probe tuning was accomplished with a wide range of

frequency from lossy to non-lossy samples. The frequency tuning and matching of probe circuit containing 85% H_3PO_4 sample were performed well with Bruker Avance III HD system, and the resulted single distinct ^{31}P resonance from 85% H_3PO_4 appeared at 0 ppm in Fig. 3. The characteristic ^{31}P NMR spectra from phospholipid bicelles are shown in Fig. 4. The bicelle was adapted fast-tumbling isotropic phase at 25 $^{\circ}C$ with or without DMPG. But the phase of bicelle was changed at rising temperature (Luchette and Tatiana 2001). The resonance at about -16 ppm was from 14-O-PC and about -8 ppm was from 6-O-PC. Well-resolved clear two peaks demonstrated that the large bicelle ($q = 3.2$) is magnetically aligned and adapted liquid crystalline phase at 40 $^{\circ}C$ in Fig. 4a. The resonance at about -14 ppm was from 14-O-PC, about -9 ppm was from DMPG, and about -5 ppm was from 6-O-PC. Well-resolved clear three peaks demonstrated that the large bicelle ($q = 3.2$) is magnetically aligned and adapted liquid crystalline phase at 40 $^{\circ}C$ in Fig. 4b.

Conclusions

A 1H - ^{31}P double resonance solid-state NMR probe with a scroll coil sample inductor was successfully built for the study of biological lossy samples such as phospholipid membrane. The scroll coil has the advantage of reducing sample heating by RF power deposition because the low electric field component

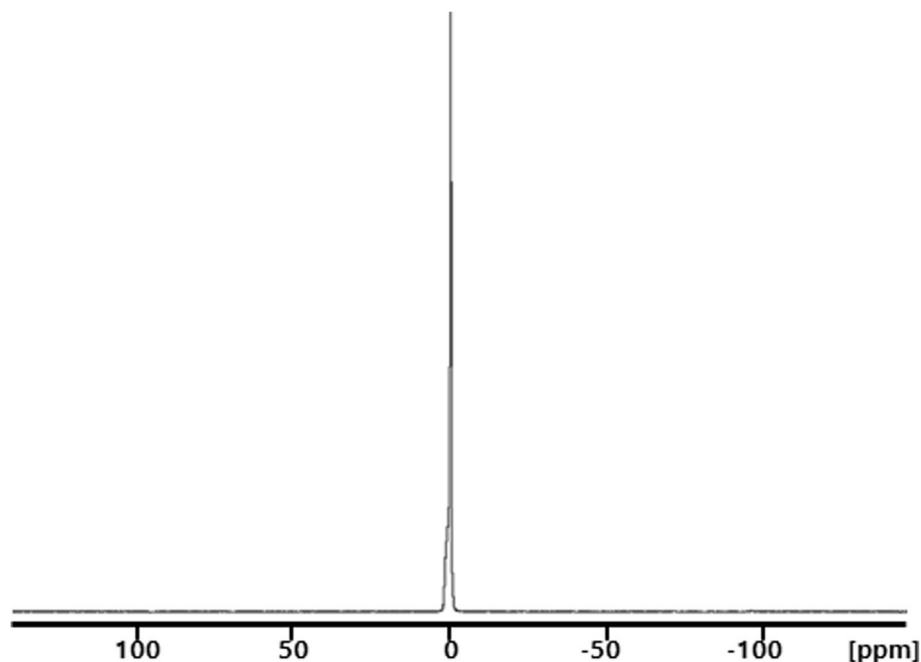


Fig. 3 The ^{31}P NMR spectrum of 85% H_3PO_4 in a 5-mm flat bottom glass tube at room temperature. The only single resonance appeared at 0 ppm

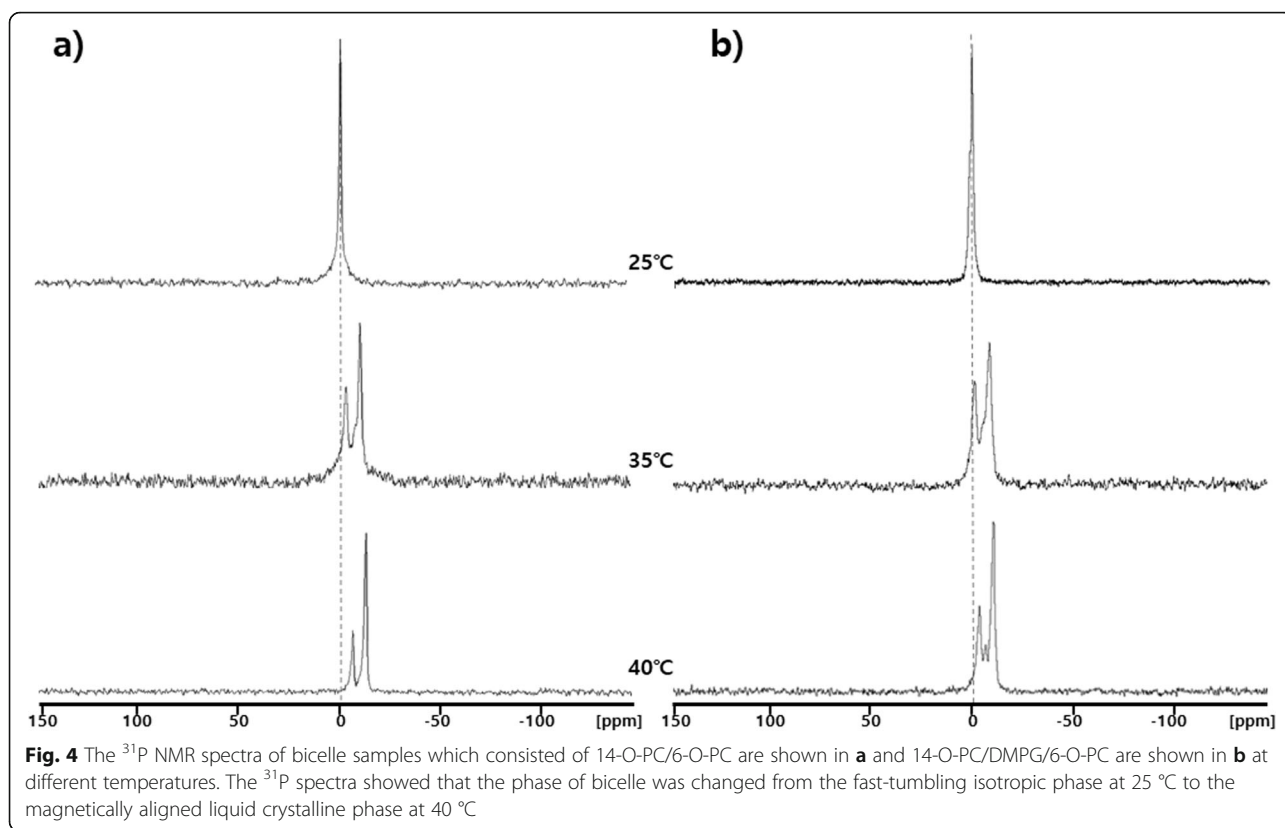


Fig. 4 The ^{31}P NMR spectra of bicelle samples which consisted of 14-O-PC/6-O-PC are shown in **a** and 14-O-PC/DMPG/6-O-PC are shown in **b** at different temperatures. The ^{31}P spectra showed that the phase of bicelle was changed from the fast-tumbling isotropic phase at 25 °C to the magnetically aligned liquid crystalline phase at 40 °C

increased within the coil during the experiments and the scroll coil was electrically stable when it is exposed even in high field strength (Grant et al. 2007; Houtl and Richards 1976). One side of the copper sheet was covered with the dielectric Teflon tape to prevent electrical shorting at the edges, and this assembly was wrapped around a cylindrical with a cable tie tensioning the inductor thus improving mechanical stability of scroll coil. The impedance of the ^1H - ^{31}P home-built solid-state NMR probe was matched to the standard 50- Ω level. The final tuning ranges of the high side channel and the low side channel are 398 to 402 MHz and 168 to 171 MHz respectively. The standard reference ^{31}P NMR spectrum of H_3PO_4 was obtained with the Bruker Avance III HD system. And the phase transition of bicelles which depends on various temperatures was successfully monitored regardless of the bicelle composition using this scroll coil probe.

Preliminary results about in this paper appeared that this ^1H - ^{31}P solid-state NMR probe had a wide range of tuning frequency. So, it could be possible to accommodate both lossy and non-lossy biological samples. Also, this home-built solid-state NMR probe has high efficiency and suggested the possibilities of dynamic behavior study of protein as the monitoring phospholipid head group regardless of the bicelle composition.

Abbreviations

RF: Radio frequency; NMR: Nuclear magnetic resonance; NB: Narrow bore; SSNMR: Solid-state nuclear magnetic resonance; PTFE: Polytetrafluoroethylene; OD: Outer diameter; ID: Inner diameter; 14-O-PC: 1,2-Di-O-tetradecyl-sn-glycero-3-phosphocholine; 6-O-PC: 1,2-Di-O-hexyl-sn-glycero-3-phosphocholine; DMPG: Dimyristoyl phosphatidylglycerol; pF: Pico Faraday; ppm: Parts per million

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Authors' contributions

The experimental work was performed by JJ, JJ, MK, and JS wrote the draft of the manuscript. MK and JS revised it critically. The authors read and approved the final manuscript.

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Availability of data and materials

All the research data have been provided in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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