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Sandwich-ELISE NMR: Reducing the sample volume of NMR samples

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ABSTRACT

We present Sandwich-ELISE, a concatenated version of our previously proposed Experimental LIquid SEaling (ELISE) protocol, in which an aqueous sample is effectively sealed by the addition of a small layer of mineral oil, or, alternatively, a chloroform sample was sealed by a water layer. With Sandwich-ELISE, a triple layered geometry composed of deuterated chloroform/aqueous buffer/mineral oil can be used to limit the sample to the active coil volume, effectively replacing the popular Shigemi tubes. Importantly, this procedure is readily applicable to smaller diameter tubes, for which no Shigemi tubes are available. We further present spectra of a 1 μl protein sample sandwiched between the chloroform and Nujol phases in a 1 mm tube, demonstrating thereby that the volume of the aqueous phase of interest can be reduced even further.

1. Introduction

NMR spectroscopy inherently suffers from a poor sensitivity, providing thereby an important impetus to concentrate the sample of interest into the active volume of the receiver coil. Protein samples, for example, are often analyzed in Shigemi tubes, where the active volume is confined by water susceptibility matched glass elements. Besides the higher cost and sometimes problematic sample preparation, these tubes do suffer from the appearance of air bubbles between the plunger and aqueous phase, especially when one wants to work at higher temperatures. Because these air bubbles tend to destroy the field homogeneity, we previously developed the ELISE technique, where we seal the aqueous phase by a small layer of Nujol mineral oil (Applied Biosystems—CAS No. 8012-95-1) before introducing the plunger, or where we replace the plunger by a larger layer of the same oil [1]. Simultaneously, we discovered that the same principle was applicable to seal samples dissolved in chloroform, whereby the seal was composed of a small layer of water. Here, we propose to concatenate both sealing procedures, and demonstrate that a geometry in which the aqueous sample is sandwiched between deuterated chloroform and mineral oil not only can replace the classical Shigemi tube, but can equally be used for smaller tube diameters such as 3 mm or 1 mm NMR tubes. In the latter case, restriction of the protein sample volume to a mere 1 μl still allowed to obtain a ^{1}H–^{15}N HSQC spectrum on as little as 8 μg of a 20 kDa protein.

2. Comparison with a regular 5 mm Shigemi tube

In order to evaluate the Sandwich-ELISE procedure, we prepared three different samples of the same 400 μM Cyclophilin B protein solution in deuterated Tris buffer, pH 6.8. The first sample contained 270 μl in a regular Shigemi tube, the second one 270 μl in a Shigemi tube without the plunger but sealed with 200 μl of Nujol mineral oil, and the last one 100 μl of deuterated chloroform, 270 μl of protein solution and 200 μl of Nujol oil in a standard 5 mm tube. Water is immiscible with both hydrophobic phases, and the density differences lead to a distinct localization of the sample of interest. The same samples prepared after colouring the aqueous phase with methylene blue clearly illustrate the different geometries, and visually show the immiscibility of the phases (Fig. 1). Even after shaking, the phases separate readily under gentle centrifugation of the NMR tube.

Magnetic susceptibility differences between the oil phase, water or chloroform are minimal, eliminating nearly the effects of the seal on the resolution of the obtained spectra. To illustrate this, we recorded 1D spectra on a 10 mM sucrose solution in water at 298 K, for the three geometries shown in Fig. 1. The standard proton 1D spectra of the three samples differ by the presence of the additional signal of the aliphatic Nujol protons, but the region of the anomeric proton is nearly identical, with only a minimal loss in the line shape due to the different liquid phases (Fig. 2). When we recorded ^{1}H, ^{15}N HSQC spectra on a Cyclophilin B solution in the three geometries, the resulting a spectra were indistinguishable, as the signals of protons not bound to a ^{15}N nucleus are effectively filtered out.

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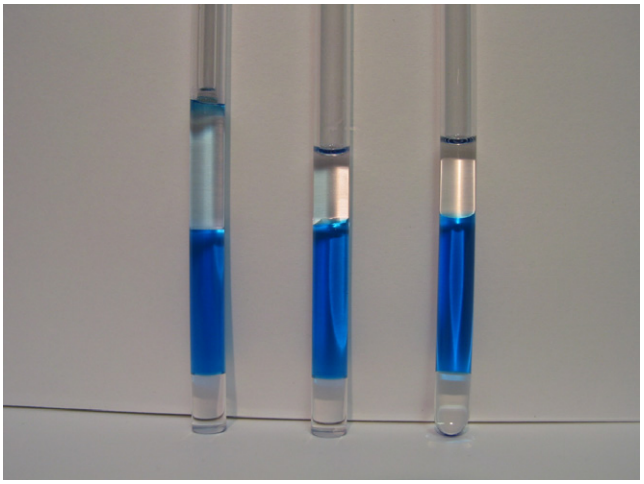

Fig. 1. Different sample geometries tested with a regular 5 mm probe. The standard Shigemi tube on the left, the tube with the plunger replaced by 70 μl of mineral oil in the middle, and the sandwich geometry in a regular 5 mm tube on the right.

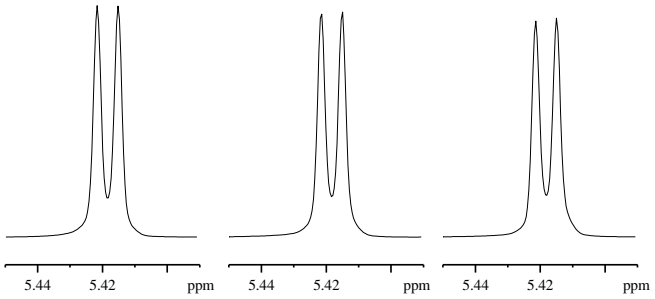

Fig. 2. Sucrose water suppression spectra of 10 mM sucrose in the tubes described in Fig. 1.

One potential pitfall of our method, that we already mentioned while describing the ELISE method [1], is the possible deleterious effect of the chloroform/water or water/mineral oil interface on the protein integrity. We therefore kept our sample for several months, and periodically recorded a 1D HSQC spectrum. After 3 months, a diffuse white film indeed had formed at the interface of the chloroform/water phase, and NMR indicated a 10% loss of soluble protein. A similar stability was obtained with the WW domain of the prolyl cis/trans isomerase Pin1. However, this phenomenon is very slow and protein dependent, as our initial protein sample containing the OpgG protein [2] still does not show any deterioration after 2 years.

3. Application to a 3 mm tube

The manufacturer recommends a standard volume of 120 μl for the 3 mm tube, of which roughly two thirds are in the NMR active volume determined by the receiver coil. We replaced the inactive volumes below and above the coil by 35 μl of chloroform and 35 μl of Nujol oil, respectively. Both 1D proton spectra and 1D $^{1}H–^{15}N$ HSQC traces of both samples were indistinguishable (Fig. 3, top and middle), validating our Sandwich-ELISE procedure for the smaller diameter tubes. When we halved the protein volume to 60 μl, with 50 μl of deuterated chloroform and Nujol oil below and above the protein solution so as to center the latter with respect to the coil, the residual signal from the Nujol oil, now inside the coil, forced us to lower the receiver gain by a factor of four in both types of spectra (Fig. 3, bottom).

We further recorded a $^{1}H–^{15}N$ HSQC and a homonuclear NOESY 2D spectrum. As long as the protein sample covered the active volume, spectra were indistinguishable, and we obtained identical spectra for the 120 μl and 80 μl CypB samples. As for the 5 mm case above, a good quality HSQC spectrum was obtained even for the 60 μl CypB sample. However, the residual Nujol signal in this minimal volume sample led to serious distortions in the aliphatic region of the NOESY spectrum (Fig. 4). After a suitable baseline

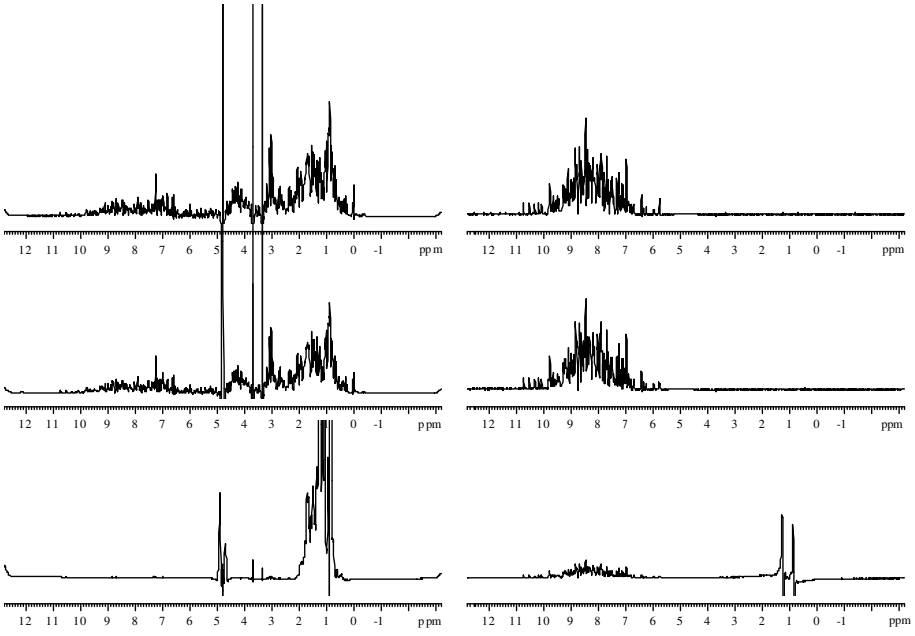

Fig. 3. Proton 1D spectra (left) and $^{1}H–^{15}N$ HSQC 1D spectra (right) of 120 μl CypB solution (top), 35 μl chloroform-d/80 μl CypB/35 μl Nujol (middle) and 50 μl chloroform-d/60 μl CypB/50 μl Nujol (bottom). Samples were in a regular 3 mm NMR tube, in a 3 mm BBI probe at 800 MHz. The receiver gain for the last sample was divided by a factor of four.

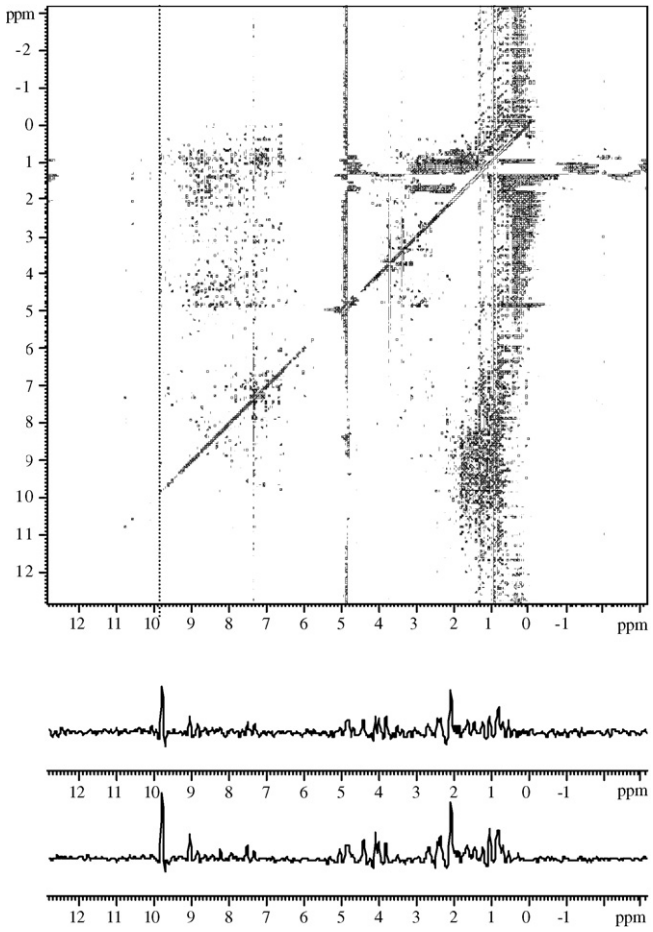

Fig. 4. Homonuclear NOESY spectrum (with 200 ms mixing time) of the 50 μl chloroform-d/60 μl CypB/50 μl Nujol sample recorded with a 3 mm BBI probe at 800 MHz. The aliphatic region is dominated by the strong residual Nujol signals around 1 ppm. A vertical trace through this spectrum taken at 9.81 ppm is shown in the middle, and compared with the same trace (bottom) of the 120 μl CypB sample recorded with a 4-fold higher receiver gain.

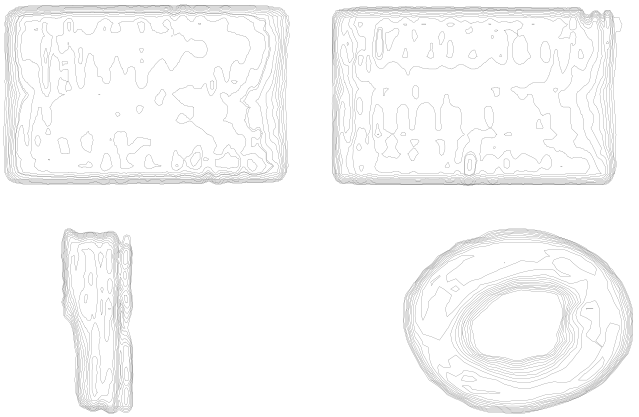

Fig. 5. Longitudinal proton images of a 1 mm tube filled with 15 μl of protein solution (top, left), of 1.5 μl chloroform-d/5 μl protein solution/6.5 μl Nujol mineral oil (top, right). The longitudinal and horizontal sections of the 1 μl protein solution sandwiched between 3.5 μl chloroform-d and 6.5 μl Nujol are shown in the bottom left and right panels, respectively. Images were acquired on a 700 MHz Bruker spectrometer equipped with a 1 mm TXI probe.

correction, however, the fingerprint region of this NOESY spectrum could still be exploited. For comparison, we show the traces of the NOESY spectra at 9.81 ppm for the 60 and 120 μl CypB solutions in the 3 mm tube (Fig. 4).

4. Application to a 1 mm probe

Beyond its general interest in analysis of mass-limited natural product or organic synthesis molecules [3,4], the possibility to analyze mass-limited protein samples in a 1 mm probe has equally been exploited in the framework of structural genomics initiatives [5]. Very recently, a complete protein structure determination was performed on the basis of spectra acquired on such a 1 mm triple resonance probe [6], with as little as 70 μg of protein dissolved in 6 μl of buffer. Therefore, we investigated the possibility to apply our Sandwich-ELISE approach using the 1 mm sample tube set up. Because the 1 mm tubes do not come with a gauge indicating the active coil position and height, we used the pulsed field gradients of the probe to obtain an image of the sample and thereby correctly position the protein solution (Fig. 5). A sample with 1.5 μl of deuterated chloroform, 5 μl of CypB and 5 μl of Nujol oil adequately covered the active coil volume, and led to the same NMR spectra as for a 15 μl protein sample. We tried to further reduce the protein volume to 1 μl, sandwiched between 3.5 μl of chloroform and 5 μl of Nujol. The image of this sample shows a small torus-like geometry for the aqueous phase, with only a very narrow film in the middle (Fig. 5). Our initial protein solution was at 400 μM, with a molecular weight of 20 kDa for CypB. Therefore this setup represents a total amount of only 8.0 μg of protein. The resulting HSQC spectrum recorded with 512 scans per increment is shown in Fig. 6. Despite the extreme proximity of both organic phases, CypB remains folded, and gives a decent spectrum when accumulating data during 20 h.

In conclusion, we have shown that the two ELISE approaches that we previously proposed to seal aqueous or chloroform samples can be successfully concatenated into one Sandwich-ELISE geometry, whereby the protein solution is positioned into the active volume of the coil by deuterated chloroform and Nujol mineral oil layers below and above the aqueous phase. Whereas this geometry can replace advantageously the traditional Shigemi tube, it is particularly useful for smaller tube diameters, where the latter are not available. For the 1 mm probe, we show that one single μl can be confined in the center of the coil, and

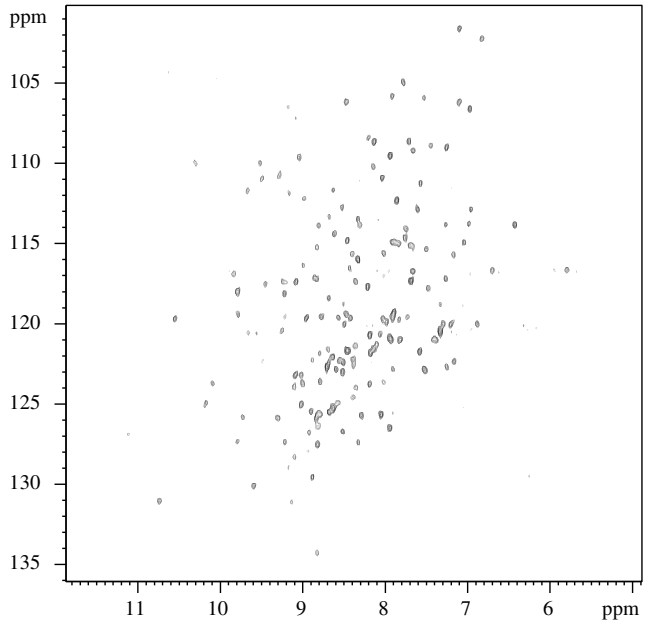

Fig. 6. $^{1}H,^{15}N$ HSQC on the 1 μl protein solution sandwiched between 3.5 μl chloroform and 6.5 μl Nujol, recorded with a 1 mm TXI probe at 700 MHz. The total measurement time was 20 h.

can allow protein spectra on less than 10 μg of protein. The use of cryogenic 1 mm probes, as recently proposed for a 600 MHz spectrometer [7], together with our sandwich-ELISE protocol, should allow NMR analysis of samples containing less than 1 μg of protein.

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