J Biomol NMR (2012) 54:123–127
DOI 10.1007/s10858-012-9657-y

COMMUNICATION

On the use of ultracentrifugal devices for sedimented solute NMR

Ivano Bertini · Frank Engelke · Leonardo Gonnelli · Benno Knott · Claudio Luchinat · David Osen · Enrico Ravera

Received: 25 May 2012 / Accepted: 26 July 2012 / Published online: 8 August 2012

Abstract We have recently proposed sedimented solute NMR (SedNMR) as a solid-state method to access biomolecules without the need of crystallization or other sample manipulation. The drawback of SedNMR is that samples are intrinsically diluted and this is detrimental for the signal intensity. Ultracentrifugal devices can be used to increase the amount of sample inside the rotor, overcoming the intrinsic sensitivity limitation of the method. We designed two different devices and we here report the directions for using such devices and the relevant equations for determining the parameters for sedimentation.

Keywords Ultracentrifuge · Sedimentation · Solid state NMR · Large biological macromolecules · Aggregates

Prof. Dr. Ivano Bertini passed away on July 7th, 2012.

CAD drawings for the production of device 2 are available upon request. If appropriate the authors provide service for filling rotors.

Electronic supplementary material The online version of this article (doi:10.1007/s10858-012-9657-y) contains supplementary material, which is available to authorized users.

I. Bertini · L. Gonnelli · C. Luchinat (✉) · E. Ravera
Center for Magnetic Resonance (CERM), University of Florence, Via L. Sacconi 6, 50019 Sesto Fiorentino, FI, Italy
e-mail: luchinat@cerm.unifi.it

I. Bertini
e-mail: ivanobertini@cerm.unifi.it

I. Bertini · C. Luchinat
Fondazione Farmacogenomica FiorGen onlus, Via L. Sacconi 6, 50019 Sesto Fiorentino, FI, Italy

I. Bertini
Giotto Biotech, Via Madonna del Piano 6, 50019 Sesto Fiorentino, FI, Italy

F. Engelke · B. Knott · D. Osen
Bruker Biospin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany

C. Luchinat · E. Ravera
Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, FI, Italy

Communication

Solution NMR is a crucial tool for structural biology, allowing for atomic-level determination of structure and dynamics of biomolecules. The recent advances in theoretical background (Hu et al. 2011; Loening et al. 2012; Bjerring et al. 2012; Nielsen et al. 2012) and in the sample preparation (Lewandowski et al. 2011a; Akbey et al. 2012) have brought solid state NMR to compete with state-of-the-art solution NMR for the determination of structure (Bertini et al. 2010a; Knight et al. 2011; Luchinat et al. 2012; Huber et al. 2012) and dynamics (Lewandowski et al. 2010, 2011b) for micro-to nanocrystalline systems, and even in the case of systems lacking long-range order like fibrils (Petkova et al. 2002; Tycko and Ishii 2003; Debelouchina et al. 2010; Bertini et al. 2011a; Lewandowski et al. 2011c; Bayro et al. 2011; Parthasarathy et al. 2011) or insoluble aggregates (Sun et al. 2009; Loquet et al. 2010).

Anyway, there is a wealth of systems that result inaccessible to these techniques for a number of reasons that are summarized in the following paragraphs.

Very large biomolecular assemblies have long rotational correlation times, resulting in broadening of the solution NMR lines beyond detection (Wider 2005; Fernández and Wider 2003; Riek et al. 1999; Fiaux et al. 2002; Guo et al. 2008; Tugarinov et al. 2006, 2005a, b; Bermel et al. 2007, 2008, 2010). To solve this problem, specific sample

preparation and labelling schemes were proposed (Tugarinov et al. 2006; Matzapetakis et al. 2007). In many cases, solid state NMR requires the sample to be crystalline, with defined levels of hydration to achieve good resolution (Martin and Zilm 2003). The crystal contacts can perturb the native structure of the protein (Barbato et al. 1992; Fischer et al. 1999; Skrynnikov et al. 2000; Chou et al. 2001; Poon et al. 2007; Bertini et al. 2008, 2009). Finally, the larger the system, the more difficult the crystallization becomes.

Manipulation of the sample (i.e. precipitation, freezing or lyophilisation) is known to deteriorate the quality of the spectra (Martin and Zilm 2003; Linden et al. 2011; Pauli et al. 2000).

In this scenario, sedimented solute NMR (SedNMR) (Bertini et al. 2011b, 2012; Polenova 2011) can be regarded as a simple way to select the best of both worlds: the sample can be kept in the buffer that is used for the solution studies and the possible addition of interaction partners can be followed like in a common NMR titration; the system is always hydrated enough and its size turns from foe to friend.

Anyway the method suffers from the intrinsic sensitivity limitation that comes from having a solution (although concentrated) sealed in the volume (2–50 µl) of a solid state NMR rotor. The concentration can optimistically arrive to 60 % of the corresponding crystal in highly soluble proteins such as BSA (Lundh 1980, 1985; Andersson and Hovmoller 2000).

In addition to the volume limitation, the 1.3 rotor, that can be used to achieve high resolution spectra (Bertini et al. 2010b; Knight et al. 2011; Webber et al. 2012) and site-specific dynamics information (Lewandowski et al. 2010; 2011b), suffers from its smaller internal radius that requires higher molecular weights for sedimentation as compared to the other rotors (Bertini et al. 2012).

We thus proposed (Bertini et al. 2012) the use of ultracentrifugal devices like the one described by Böckmann et al. (2009) to increase the amount of sample in the rotor. This approach was later used by Gardiennet et al. (2012) for sedimenting a 59 kDa dodecameric helicase (total molecular weight 708 kDa).

The scheme for the design of devices of this type is the following:

- A reservoir for increasing the amount of solution, thus allowing for more diluted solutions to be used;
- A funnel to convey the pellet into the rotor;
- A tight junction based on o-ring for sealing;
- A jacket made in delrin® or vespel® to prevent rotor shattering during the ultracentrifugation;
- A housing for the rotor that is as close to the bottom of the tube as possible.

The position of the bottom of the rotor (b_4 in Fig. 2) and of the top of the solution (b_0 in Fig. 2) will determine the effective clearing factor of the ultracentrifugation, i.e. the time efficiency of the process, according to Eq. 4).

We here report the design of two centrifugal devices with different geometries (Fig. 1a, b). Both of them can be used in SW32Ti rotor for Optima type floor preparative ultracentrifuge (Beckman Coulter) at the maximum speed (32,000 rpm).

Device 1 (Bruker Karlsruhe) has a light polycarbonate funnel fitted into an outer aluminium funnel. This device has an internal volume of 20 ml, allowing for the use of less concentrated solutions. The polycarbonate funnel must be cut to the proper level in order to avoid crushing during the centrifugation.

Device 2 (Experimental Physics Workshop, University of Florence) is made in delrin® acetal resin. The device does not have a soft funnel so it does not require any manipulation, and delrin is resistant to corrosion as opposed to aluminium, so that the device can be cleaned through acids and bases, as required for some toxic proteins. Anyway, the internal volume is 1.38 ml, thus the device can only be used for concentrated solutions.

The amount of the macromolecule that will get into the pellet is given by the Eq. 1 (Bertini et al. 2012):

$$c(h) = \frac{c_l}{Ae^{-kh^2} + 1} \quad (1)$$

where h is the distance from the rotation axis, c_l the limiting concentration of the macromolecule [experimentally found to be approximately 700 mg/ml for proteins (Lundh 1980, 1985)], k is given by:

$$k = \frac{M(1 - \rho_{solvent}/\rho_{biomolecule})\omega^2}{2RT} \quad (2)$$

and A is an integration constant that needs to be determined according to the law of concentration of mass:

Fig. 1 **a** View of the components of device 1; **b** view of the components of device 2

$$\pi r_1^2 \int_{b_0}^{b_1} c(h)dh + \pi \int_{b_1}^{b_2} \left(\frac{h_p - h + b_1}{h_p} r_1\right)^2 c(h)dh + \pi r_2^2 \int_{b_2}^{b_3} c(h)dh + \pi r_3^2 \int_{b_3}^{b_4} c(h)dh = c_0 V_{device} \quad (3)$$

where the meaning of the parameters r_1, r_2, r_3 and h_p and of the integration limits is shown in Fig. 2.

The integrals in Eq. 3 cannot be evaluated analytically for the geometry of the device and therefore they must be evaluated numerically. This task can be accomplished through the annotated Mathematica® notebook provided as supporting material.

Following this approach we can estimate that almost any protein above 20 kDa can pellet from a solution at concentration $c_0 = 2$ mmol/dm^{-3} at a centrifugation rate of 32,000 rpm.

On the basis of these numbers we can clearly understand that any protein can be sedimented. What makes then the real difference is the time required for completing the sedimentation. To calculate it, one should only know the sedimentation coefficient s of the biomolecule (easily attainable by analytical ultracentrifugation and usually quite well documented in the literature) and resort to the integrated Svedberg equation:

$$t = 2.533 \times 10^{11} \frac{\ln(b_4/b_0)}{sf^2} \quad (4)$$

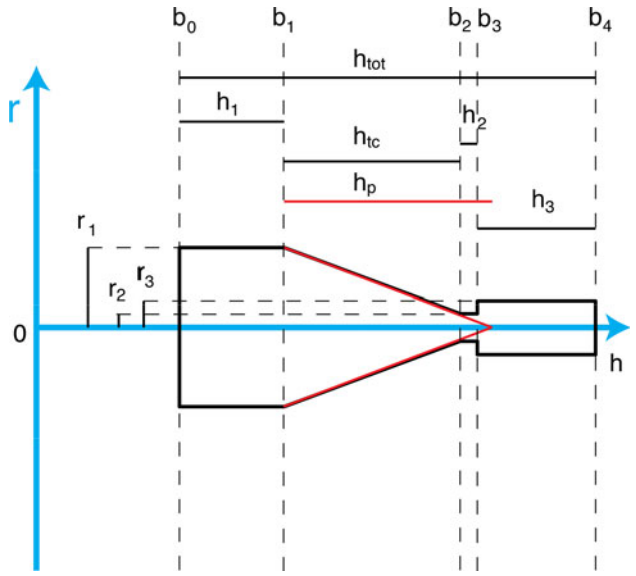

Fig. 2 Schematics of the relevant parameters for the device geometry

where f is the rotation rate in rpm, as it is commonly done in the preparative ultracentrifugation.

It is important to remark that selective sedimentation of the desired solute from a mixture of solutes with different molecular weights can be attained by the classical differential centrifugation approach.

Overall, sample preparation for an experiment of sedimented solute NMR by means of an ultracentrifugal device can be summarized as follows:

- Choose the concentration and volume that will allow for sedimentation (Eqs. 1–3) and will provide enough material to fill the rotor;
- Choose the experimental time according to Eq. 4;
- Add the solution to the ultracentrifugal device and, if needed, cut the funnel to avoid crushing;
- Run the ultracentrifugation;
- Seal the rotor through CRAMPS insert (4 mm), silicon plugs (3.2 mm) or marker ink on the caps (1.3 mm). This is necessary to avoid spilling of the solution and possible probe damage.

Acknowledgments This work has been supported by the EC contracts East-NMR no. 228461 and Bio-NMR no. 261863, INSTRUCT (European FP7 e-Infrastructure grant, contract no. 211252, http://www.instruct-fp7.eu/), and Ente Cassa Risparmio Firenze. Fruitful discussion with Anja Böckmann (IBCP, Lyon) is acknowledged. We are grateful to Alberto Catelani, Massimo Falorsi, Massimo Merciai and Nicola Pasqualetti (Department of Physics and Astronomy, University of Florence, Italy) for the production of device 2.

References

Akbey Ü, van Rossum B-J, Oschkinat H (2012) Practical aspects of high-sensitivity multidimensional ^{13}C MAS NMR spectroscopy of perdeuterated proteins. J Magn Reson 217:77–85

Andersson KM, Hovmoller S (2000) The protein content in crystals and packing coefficients in different space groups. Acta Crystallogr D Biol Crystallogr 56:789–790

Barbato G, Ikura M, Kay LE, Pastor RW, Bax A (1992) Backbone dynamics of calmodulin studied by ^{15}N relaxation using inverse detected two-dimensional NMR spectroscopy; the central helix is flexible. Biochemistry 31:5269–5278

Bayro MJ, Debelouchina GT, Eddy MT, Birkett NR, MacPhee CE, Rosay MM, Maas W, Dobson CM and Griffin RG (2011) Intermolecular structure determination of amyloid fibrils with magic-angle spinning and dynamic nuclear polarization NMR. J Am Chem Soc 133:13967–13974

Bermel W, Felli IC, Matzapetakis M, Pierattelli R, Theil EC, Turano P (2007) A method for C^{α} direct-detection in protonless NMR. J Magn Reson 188:301–310

Bermel W, Felli IC, Kümmerle R, Pierattelli R (2008) ^{13}C direct-detection biomolecular NMR. Concepts Magn Reson 32A:183–200

Bermel W, Bertini I, Felli IC, Peruzzini R, Pierattelli R (2010) Exclusively heteronuclear NMR experiments to obtain structural and dynamic information on proteins. Chem Phys Chem 11: 689–695

Bertini I, Calderone V, Fragai M, Jaiswal R, Luchinat C, Melikian M, Mylonas E, Svergun D (2008) Evidence of reciprocal reorientation of the catalytic and hemopexin-like domains of full-length MMP-12. J Am Chem Soc 130:7011–7021

Bertini I, Kursula P, Luchinat C, Parigi G, Vahokoski J, Willmans M, Yuan J (2009) Accurate solution structures of proteins from X-ray data and minimal set of NMR data: calmodulin peptide complexes as examples. J Am Chem Soc 131:5134–5144

Bertini I, Bhaumik A, De Paepe G, Griffin RG, Lelli M, Lewandowski JR, Luchinat C (2010a) High-resolution solid-state NMR structure of a 17.6 kDa protein. J Am Chem Soc 132:1032–1040

Bertini I, Emsley L, Lelli M, Luchinat C, Mao J, Pintacuda G (2010b) Ultrafast MAS solid-state NMR permits extensive 13C and 1H detection in paramagnetic metalloproteins. J Am Chem Soc 132:5558–5559

Bertini I, Gonnelli L, Luchinat C, Mao J, Nesi A (2011a) A new structural model of Aβ40 fibrils. J Am Chem Soc 133:16013–16022

Bertini I, Luchinat C, Parigi G, Ravera E, Reif B, Turano P (2011b) Solid-state NMR of proteins sedimented by ultracentrifugation. Proc Natl Acad Sci USA 108:10396–10399

Bertini I, Engelke F, Luchinat C, Parigi G, Ravera E, Rosa C, Turano P (2012) NMR properties of sedimented solutes. Phys Chem Chem Phys 14:439–447

Bjerring M, Paaske B, Oschkinat H, Akbey Ü, Nielsen NC (2012) Rapid solid-state NMR of deuterated proteins by interleaved cross-polarization from ^{1}H and ^{2}H nuclei. J Magn Reson 214: 324–328

Böckmann A, Gardiennet C, Verel R, Hunkeler A, Loquet A, Pintacuda G, Emsley L, Meier BH, Lesage A (2009) Characterization of different water pools in solid-state NMR protein samples. J Biomol NMR 45:319–327

Chou JJ, Li S, Klee CB, Bax A (2001) Solution structure of Ca^{2+} calmodulin reveals flexible hand-like properties of its domains. Nat Struct Biol 8:990–997

Debelouchina GT, Platt GW, Bayro MJ, Radford SE, Griffin RG (2010) Magic angle spinning NMR analysis of beta (2)-microglobulin amyloid fibrils in two distinct morphologies. J Am Chem Soc 132:10414–10423

Fernández C, Wider G (2003) TROSY in NMR studies of the structure and function of large biological macromolecules. Curr Opin Struct Biol 13:570–580

Fiaux J, Bertelsen EB, Horwich AL, Wüthrich K (2002) NMR analysis of a 900 KDa GroEL GROES complex. Nature 418:207–211

Fischer MW, Losonczi JA, Weaver JL, Prestegard JH (1999) Domain orientation and dynamics in multidomain proteins from residual dipolar couplings. Biochemistry 38:9013–9022

Gardiennet C, Schütz AK, Hunkeler A, Kunert B, Terradot L, Böckmann A and Meier BH (2012) A sedimented sample of a 59 kDa dodecameric helicase yields high-resolution solid-state NMR spectra. Angew Chem Int Ed 51:7855–7858

Guo C, Zhang D, Tugarinov V (2008) An NMR experiment for simultaneous TROSY-based detection of amide and methyl groups in large proteins. J Am Chem Soc 130:10872–10873

Hu B, Lafon OTJ, Chen Q, Amoureux J-P (2011) Broad-band homonuclear correlations assisted by ^{1}H irradiation for biomolecules in very high magnetic field at fast and ultra-fast MAS frequencies. J Magn Reson 212:320–329

Huber M, Böckmann A, Hiller S, Meier BH (2012) 4D solid-state NMR for protein structure determination. Phys Chem Chem Phys 14:5239–5246

Knight MJ, Webber AL, Pell AJ, Guerry P, Barbet-Massin E, Bertini I, Felli IC, Gonnelli L, Pierattelli R, Emsley L, Lesage A, Hermann T, Pintacuda G (2011) Fast resonance assignment and fold determination of human superoxide dismutase by high-resolution proton-detected solid state MAS NMR spectroscopy. Angew Chem Int Ed 50:11697–11701

Lewandowski JR, Sein J, Sass HJ, Grzesiek S, Blackledge M, Emsley L (2010) Measurement of site-specific ^{13}C spin-lattice relaxation in a crystalline protein. J Am Chem Soc 132:8252–8254

Lewandowski JR, Dumez JN, Akbey Ü, Franks WT, Emsley L, Oschkinat H (2011a) Enhanced resolution and coherence lifetimes in the solid-state NMR Spectroscopy of perdeuterated proteins under ultrafast magic-angle spinning. J Phys Chem Lett 2:2205–2211

Lewandowski JR, Sass HJ, Grzesiek S, Blackledge M, Emsley L (2011b) Site-specific measurement of slow motions in proteins. J Am Chem Soc 133:16762–16765

Lewandowski JR, Van der Wel PCA, Rigney M, Grigorieff N, Griffin RG (2011c) Structural complexity of a composite amyloid fibril. J Am Chem Soc 133:14686–14698

Linden AH, Franks WT, Akbey Ü, Lange S, van Rossum B-J, Oschkinat H (2011) Cryogenic temperature effects and resolution upon slow cooling of protein preparations in solid state NMR. J Biomol NMR 51:283–292

Loening NM, Bjerring M, Nielsen NC, Oschkinat H (2012) A comparison of NCO and NCA transfer methods for biological solid-state NMR spectroscopy. J Magn Reson 214:81–90

Loquet A, Giller K, Becker S, Lange A (2010) Supramolecular interactions probed by (13)C–(13)C solid-state NMR spectroscopy. J Am Chem Soc 132:15164–15166

Luchinat C, Parigi G, Ravera E, Rinaldelli M (2012) Solid state NMR crystallography through paramagnetic restraints. J Am Chem Soc 134:5006–5009

Lundh S (1980) Concentrated protein solutions in the analytical ultracentrifuge. J Polym Sci Polym Phys Edn 18:1963–1978

Lundh S (1985) Ultracentrifugation of concentrated biopolymer solutions and effect of ascorbate. Arch Biochem Biophys 241: 265–274

Martin RW, Zilm KW (2003) Preparation of protein nanocrystals and their characterization by solid state NMR. J Magn Reson 165: 162–174

Matzapetakis M, Turano P, Theil EC, Bertini I (2007) 13C–13C NOESY spectra of a 480 kDa protein: solution NMR of ferritin. J Biomol NMR 38:237–242

Nielsen AB, Székely K, Gath J, Ernst M, Nielsen NC and Meier BH (2012) Simultaneous acquisition of PAR and PAIN spectra. J Biomol NMR 52:283–288

Parthasarathy S, Long F, Miller Y, Xiao Y, McElheny D, Thurber K, Ma B, Nussinov R, Ishii Y (2011) Molecular-level examination of $Cu2^{+}$ binding structure for amyloid fibrils of 40-residue Alzheimer's β by solid-state NMR spectroscopy. J Am Chem Soc 133:3390–3400

Pauli J, van Rossum B, Forster H, de Groot HJ, Oschkinat H (2000) Sample optimization and identification of signal patterns of amino acid side chains in 2D RFDR spectra of the alpha-spectrin SH3 domain. J Magn Reson 143:411–416

Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F, Tycko R (2002) A structural model for Alzheimer's beta-amyloid fibrils based on experimental constraints from solid state NMR. Proc Natl Acad Sci USA 99:16742–16747

Polenova T (2011) Spinning into focus. Nat Chem 3:759–760

Poon DKY, Withers SG, McIntosh LP (2007) Direct demonstration of the flexibility of the glycosylated proline-threonine linker in the Cellulomonas fimi xylanase Cex through NMR spectroscopic analysis. J Biol Chem 282:2091–2100

Riek R, Wider G, Pervushin K, Wüthrich K (1999) Polarization transfer by cross-correlated relaxation in solution NMR with very large molecules. Proc Natl Acad Sci USA 96:4918–4923

Skrynnikov NR, Goto NK, Yang D, Choy W-Y, Tolman JR, Mueller GA, Kay LE (2000) Orienting domains in proteins using dipolar couplings measured by liquid-state NMR: differences in solution

and crystal forms of maltodextrin binding protein loaded with β-cyclodextrin. J Mol Biol 295:1265–1273

Sun SJ, Siglin A, Williams JC, Polenova T (2009) Solid-state and solution nmr studies of the CAP-Gly domain of mammalian dynactin and its interaction with microtubules. J Am Chem Soc 131:10113–10126

Tugarinov V, Choy WY, Orekhov VY, Kay LE (2005a) Solution NMR-derived global fold of a monomeric 82-kDa enzyme. Proc Natl Acad Sci USA 102:622–627

Tugarinov V, Kay LE, Ibraghimov I, Orekhov VY (2005b) High-resolution four-dimensional 1H–13C NOE spectroscopy using methyl-TROSY, sparse data acquisition, and multidimensional decomposition. J Am Chem Soc 127:2767–2775

Tugarinov V, Kanelis V, Kay LE (2006) Isotope labeling strategies for the study of high-molecular-weight proteins by solution NMR spectroscopy. Nat Protoc 1:749–754

Tycko R, Ishii Y (2003) Constraints on supramolecular structure in amyloid fibrils from two-dimensional solid-state NMR spectroscopy with uniform isotopic labeling. J Am Chem Soc 125:6606–6607

Webber AL, Pell AJ, Barbet-Massin E, Knight MJ, Bertini I, Felli IC, Pierattelli R, Emsley L, Lesage A and Pintacuda G (2012) Combination of DQ and ZQ coherences for sensitive through-bond NMR correlation experiments in biosolids under ultra-fast MAS. Chem Phys Chem 13:2405–2411

Wider G (2005) NMR techniques used with very large biological macromolecules in solution. Methods Enzymol 394:382–398