Chapter 29

NMR Screening for Rapid Protein Characterization in Structural Proteomics

Justine M. Hill

In the age of structural proteomics when protein structures are targeted on a genome-wide scale, the identification of proteins that are amenable to analysis using x-ray crystallography or NMR spectroscopy is the key to high throughput structure determination. NMR screening is a beneficial part of a structural proteomics pipeline because of its ability to provide detailed biophysical information about the protein targets under investigation at an early stage of the structure determination process. This chapter describes efficient methods for the production of uniformly ^{15}N-labeled proteins for NMR screening using both conventional IPTG induction and autoinduction approaches in *E. coli*. Details of sample preparation for NMR and the acquisition of 1D ^{1}H NMR and 2D ^{1}H-^{15}N HSQC spectra to assess the structural characteristics and suitability of proteins for further structural studies are also provided.

1. Introduction

The efficient identification of proteins that are amenable to structure determination using x-ray crystallography or NMR spectroscopy is a key issue in structural proteomics and structural biology in general. NMR is increasingly used as a screening tool to identify folded proteins that are promising targets for three-dimensional structure elucidation *(1–9)*. Some structural proteomics projects primarily use 1D ^{1}H NMR spectra of unlabeled proteins that are used for initial crystallization trials *(1)*. However, 2D ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled proteins are more routinely used *(2–9)* due to their improved resolution and higher information content. In the ^{1}H-^{15}N HSQC spectrum, one peak is expected for each backbone and side chain NH group in a protein, providing a diagnostic fingerprint. The dispersion pattern, intensity, and number of observed cross peaks reports directly on the folded state and overall sample quality. As the number of peaks in the HSQC spectrum corresponds approximately to the number of residues in the protein under investigation, conformational heterogeneity can easily be detected by a surplus of peaks.

Samples for NMR screening are most commonly expressed in *E. coli* grown in modified M9 medium containing $^{15}NH_4Cl$ as the sole nitrogen source *(3–6)* or using cell-free expression systems *(7,10,11)*. Recent advances such as the

From: *Methods in Molecular Biology, Vol. 426: Structural Proteomics: High-throughput Methods*
Edited by: B. Kobe, M. Guss and T. Huber

development of autoinduction medium *(12)* have facilitated high throughput protein production in *E. coli*. Autoinduction medium takes advantage of the *lac* operon in *E. coli* that inhibits lactose induction in the presence of glucose. These media are an optimized blend of carbon sources (glucose, glycerol, and lactose) that promote culture growth to high cell densities, followed by lactose-induced protein expression (*12*). Autoinduction is more convenient than IPTG induction because the expression strain is simply inoculated into autoinducing medium and grown to saturation without the need to monitor culture growth and add inducer at the appropriate time. Furthermore, the cell mass and target protein yield are often increased severalfold compared with conventional protocols using IPTG induction *(12)*. This methodology has been widely adopted in structural proteomics initiatives and is readily modified for the production of isotopically labeled proteins for NMR analysis *(13)*.

This chapter focuses on the application of NMR as a diagnostic tool for the rapid identification of well-folded proteins for structure determination. Methods for the efficient production of uniformly ^{15}N-labeled proteins in *E. coli* by both conventional IPTG induction and autoinduction are described. Details of the preparation of samples for NMR analysis, and their screening using 1D ^{1}H NMR and 2D ^{1}H-^{15}N HSQC spectra are also provided. These simple NMR experiments provide a fast and reliable assessment of the amenability of the protein to further structural analysis.

2. Materials

2.1. M9 Medium for Uniform ^{15}N-Labeling

1. M9 salts (5×): Dissolve 34 g anhydrous Na_2HPO_4, 15 g anhydrous KH_2PO_4 and 2.5 g NaCl in water to a final volume of 1 L (see Note 1). Sterilize by autoclaving and store at room temperature for up to 6 months.
2. Glucose (50% w/v): Dissolve 125 g of D-glucose in water to a final volume of 250 ml. Glucose is slow to dissolve and requires stirring for several hours at room temperature. Sterilize the solution by passing it through a 0.2 μm filter and store at room temperature.
3. $MgSO_4$ (1 M): Dissolve 24.7 g of $MgSO_4 \cdot 7H_2O$ in water to a final volume of 100 ml. Sterilize by autoclaving and store at room temperature.
4. Ammonium chloride (^{15}N, 99%) can be purchased from a number of suppliers, including Cambridge Isotope Labs, Sigma-Aldrich, Silantes, and Spectra Stable Isotopes.
5. Vitamin solution (5 mg/ml): Dissolve 0.5 g thiamine hydrochloride and 0.5 g nicotinic acid in 100 ml water. Filter sterilize and store at 4°C.
6. Trace element solution (1,000×): 40.8 mM $CaC1_2 \cdot 2H_2O$, 21.6 mM $FeSO_4 \cdot 7H_2O$, 6.1 mM $MnCI_2 \cdot 4H_2O$, 3.4 mM $CoC1_2 \cdot 6H_2O$, 2.4 mM $ZnSO_4 \cdot 7H_2O$, 1.8 mM $CuC1_2 \cdot 2H_2O$, 0.3 mM boric acid, and 0.2 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ *(14)* (see Note 2). To 100 ml of water in a 500-ml Erlenmeyer flask, add the salts in the order listed in Table 29.1 and cover with a perforated parafilm top. Fully dissolve each salt by stirring before adding the next. Filter sterilize and store in the dark at room temperature.
7. Antibiotics for plasmid selection (1,000×): Ampicillin (100 mg/ml in water) or kanamycin (35 mg/ml in water) is selective for most of the T7-based expression vectors that are commonly used. Filter sterilize and store at 4°C.
8. IPTG (isopropyl-β-D-thiogalactopyranoside)

Table 29.1 Recipe for 1,000× trace element solution

	per 100 ml
$CaCl_2 \cdot 2H_2O$	0.60 g
$FeSO_4 \cdot 7H_2O$	0.60 g
$MnCl_2 \cdot 4H_2O$	0.12 g
$CoCl_2 \cdot 6H_2O$	0.08 g
$ZnSO_4 \cdot 7H_2O$	0.07 g
$CuCl_2 \cdot 2H_2O$	0.03 g
Boric acid	2 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	25 mg
EDTA	0.50 g

2.2. Autoinduction Medium for Uniform ^{15}N-Labeling

The stock solutions required for autoinduction medium were originally defined by Studier *(12)*. Here the same naming convention is used, where "N" identifies a variant of the media used for ^{15}N-labeling.

1. 20× N: 1 M Na_2HPO_4, 1 M KH_2PO_4 and 0.1 M Na_2SO_4. Dissolve 14.2 g Na_2SO_4, 136 g KH_2PO_4 and 142 g Na_2HPO_4 in water to a final volume of 1 L. Add in sequence to a beaker and stir until all dissolved. Sterilize by autoclaving and store at room temperature.
2. 50× 5052 (5052 = 0.5% glycerol, 0.05% glucose, 0.2% α-lactose): 250 g glycerol (weigh into a beaker), 730 ml water, 25 g D-glucose, and 100 g α-lactose. Add in sequence in a beaker and stir until all dissolved. Lactose is slow to dissolve and may take two hours or more at room temperature. Sterilize by autoclaving and store at room temperature.
3. The other components of autoinduction medium including 1 M $MgSO_4$, $^{15}NH_4Cl$, vitamins, trace element solution (1,000×), and antibiotics (1,000×) are prepared as described in Section 2.1.

3. Methods

3.1. Uniform ^{15}N-Labeling in M9 Medium

This protocol describes the preparation of uniformly ^{15}N-labeled protein in M9 minimal medium on a 1 L scale (see Note 3).

1. Inoculate 5 ml of LB medium containing the appropriate antibiotic with a single colony from a freshly transformed plate of *E. coli* BL21(DE3) cells containing plasmid for overexpression of the target protein.
2. Grow at 37°C overnight with shaking at approximately 200 rpm.
3. In a sterile 250-ml Erlenmeyer flask, prepare 50 ml of M9 minimal medium from sterile concentrated stock solutions as described in Table 29.2 (see Note 4).
4. To adapt the cells to minimal medium, transfer 20 µl of the overnight culture in rich growth medium to 2 ml of M9 minimal medium. Grow at 37°C for 6–8 hours until the culture is visibly turbid.

Table 29.2 Recipe for M9 minimal medium

	per 50 ml	per 1 L
Sterile water	40 ml	780 ml
5× M9 salts	10 ml	200 ml
Vitamins	300 µl	6 ml
Glucose (50% w/v)	600 µl	12 ml
Antibiotic (1,000×)	50 µl	1 ml
Trace elements (1,000×)	50 µl	1 ml
1 M $MgSO_4$	150 µl	3 ml
$^{15}NH_4Cl$	50 mg	1 g

5. Transfer the entire 2 ml minimal culture to the remaining 48 ml minimal medium and continue growth at 37°C overnight. This will provide a starter culture for large-scale expression the following day (see Note 5).
6. Prepare 1 L of M9 minimal medium as described in Table 29.2, and divide 500-ml aliquots into sterile 2-L Erlenmeyer flasks.
7. Add 25 ml of the starter culture to each flask and grow at 37°C until the optical density $(OD)_{600}$ is approximately 1.0. This will take 2–3 hours.
8. Induce protein expression by the addition of IPTG. The authors typically use a 1 mM final concentration of IPTG, with overexpression carried out at either 37°C for 5 hours or at 20°C overnight (see Note 6).
9. Harvest the cells by centrifugation at 5,000*g*, 4°C for 15 minutes.
10. Purify the target protein using the appropriate method.
11. Prepare a sample for NMR analysis as described in Section 3.3.

3.2. Uniform ^{15}N-Labeling in Autoinduction Medium

This protocol describes the preparation of uniformly ^{15}N-labeled protein in autoinduction medium on a 1 L scale (see Notes 7 and 8).

1. Inoculate 5 ml of LB medium containing the appropriate antibiotic with a single colony from a freshly transformed plate of *E. coli* BL21(DE3) cells containing plasmid for overexpression of the target protein (see Note 9).
2. Grow at 37°C overnight with shaking at approximately 200 rpm.
3. In a sterile 250-ml Erlenmeyer flask, prepare 50 ml of NG minimal medium from sterile concentrated stock solutions as described in Table 29.3 (see Note 10).
4. Transfer 20 µl of the overnight culture in rich growth medium to 2 ml of NG minimal medium. Grow at 37°C for 6–8 hours until the culture is visibly turbid.
5. Transfer the entire 2 ml minimal culture to the remaining 48 ml NG medium and continue growth at 37°C overnight. This provides a starter culture for large-scale expression the following day (see Note 5).
6. Prepare 1 L of N-5052 autoinduction medium from sterile concentrated stock solutions as described in Table 29.4, and divide 500-ml aliquots into sterile 2-L Erlenmeyer flasks.

Table 29.3 NG defined medium for growth to saturation

	per 50 ml
Sterile water	47 ml
Vitamins	300 µl
1 M $MgSO_4$	50 µl
Trace elements (1,000×)	50 µl
Glucose (50% w/v)	500 µl
20× N	2.5 ml
Antibiotic (1,000×)	50 µl
$^{15}NH_4Cl$	125 mg

Table 29.4 N-5052 defined medium for autoinduction

	per 1 L
Sterile water	922 ml
Vitamins	6 ml
1 M $MgSO_4$	1 ml
Trace elements (1,000×)	1 ml
50× 5052	20 ml
20× N	50 ml
Antibiotic (1,000×)	1 ml
$^{15}NH_4Cl$	2.5 g

7. Add 25 ml of the starter culture to each flask and grow in a refrigerated incubator at 30°C with shaking at 200 rpm for 24 hours (see Notes 11 and 12).
8. Harvest the cells by centrifugation at 5,000*g*, 4°C for 15 minutes.
9. Purify target protein using the appropriate method.
10. Prepare a sample for NMR analysis as described in Section 3.3.

3.3. NMR Sample Preparation

1. Dialyze the purified protein against a suitable buffer for NMR. The authors typically use 50 mM sodium phosphate or 10 mM sodium acetate with a pH of 5, 6, or 7 (depending on the pI of the target protein), 150 mM NaCl, 1 mM DTT, and 50 µM NaN_3 (see Note 13). If sufficient protein is available, it is advantageous to screen the protein in several buffer conditions.
2. Concentrate the dialyzed protein to 0.2–1.0 mM in a final volume of 500 µl using a centrifugal concentrator such as Amicon Ultra-4 (Millipore, Billerica, MA) with an appropriate molecular weight cutoff.
3. Add 50 µl of D_2O for deuterium lock of the NMR spectrometer and transfer the entire sample into a 5 mm NMR tube (535PP; Wilmad, Buena, NJ). If the amount of protein is limited, the sample can be further concentrated and placed in a 300 µl Shigemi tube. Store at 4°C prior to NMR data collection.

3.4. NMR Screening

1. Record 1D ^{1}H NMR and 2D ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectra of the target protein. The authors routinely perform NMR measurements at 25°C on a 500- or 600-MHz spectrometer equipped with a z-shielded gradient triple-resonance probe. Typical 2D data sets are recorded as 1,024×256 complex points with 8–32 scans per increment depending on the sample concentration. Quadrature detection in the indirect dimension is achieved by the States-TPPI method, and a binomial 3–9–19 sequence with water flip-back is used for water suppression. The ^{1}H dimension is referenced to H_2O at 25°C, and the carrier frequency of the ^{15}N dimension is set to 118 ppm. 1D ^{1}H NMR spectra can be recorded in a time frame of a few minutes, and measurement time for the HSQC is typically 30–60 minutes.
2. Process the NMR data using TopSpin (Bruker, Billerica, MA), NMRPipe/NMRDraw *(15)* or other suitable software package.
3. Examine the spectra to evaluate the protein's suitability for structure determination. NMR spectral quality is assessed based primarily on spectral dispersion, line widths, and number of resolved peaks observed compared with the number expected from the amino acid sequence.
4. To optimize sample conditions, pH titrations or titrations with cofactors as well as variation of temperature may be performed and monitored using NMR. Following the appearance of the spectra over time can also be used to assess protein stability under the screening conditions.
5. After NMR measurements, the samples can be recovered and used for other biophysical or functional studies.

Representative examples of 1D ^{1}H NMR and 2D ^{1}H-^{15}N HSQC spectra are shown in Fig. 29.1. In 1D ^{1}H NMR spectra, the dispersion of signals in the amide proton (6–10 ppm), α-proton (3.5–6 ppm) and methyl proton (–0.5 to 1.5 ppm) regions, provides the main indicators of folded globular proteins (see Fig. 29.1A,B). In contrast, an unfolded protein shows a smaller dispersion of backbone amide chemical shifts. The appearance of intense peaks near 8.3 ppm is an indicator of disorder in a protein (see Fig. 29.1C), as this region is characteristic of backbone amides in a random-coil configuration.

The positions of peaks in 2D ^{1}H-^{15}N HSQC spectra are indicative of structured or disordered proteins in a similar way to that described for the one-dimensional spectrum. In the spectrum of an unfolded protein, all signals cluster in a characteristic "blob" around 8.3 ppm with little dispersion in either dimension, and side chain NH peaks are generally degenerate. For a well-folded protein, the HSQC spectrum should show well-dispersed peaks of equal intensity, with the number of peaks corresponding to that calculated from the protein sequence (see Fig. 29.1A,B). These spectra indicated that the proteins were amenable to structure determination using NMR methods *(16,17)*. For poorly to moderately folded proteins, HSQC spectra often show a lack of dispersion of peaks with varying intensities and peak numbers inconsistent with the protein sequence (see Fig. 29.1C). Furthermore, line width is highly dependent on the tumbling rate of a molecule in solution and therefore on its molecular weight. Thus, a protein exhibiting a poor HSQC could suggest that it is forming higher-order oligomers or nonspecific aggregates under

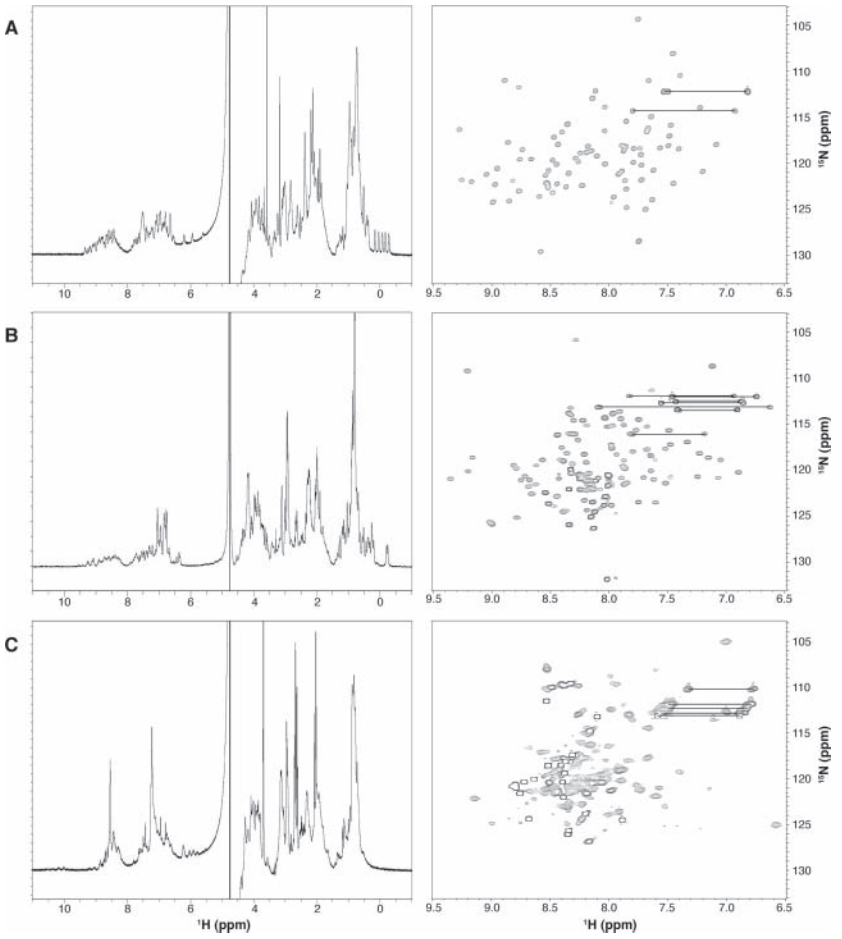

Fig. 29.1 Representative examples of 1D ^{1}H NMR *(left)* and 2D ^{1}H-^{15}N HSQC spectra *(right)* of ^{15}N-labeled proteins. The proteins are: **(A)** ASC2, an 11-kDa pyrin domain-only protein *(17)*, **(B)** PEA-15, 15-kDa *(16)*, and **(C)** a 15-kDa target protein from the macrophage structural proteomics initiative at the University of Queensland (see Meng et al: "Overview of the Pipeline for Structural and Functional Characterization of Macrophage Proteins at the University of Queensland" in this volume). These spectra were recorded at 25°C on a Bruker Avance 600 MHz spectrometer. Peaks corresponding to side chain NH_2 groups are connected by horizontal lines.

the NMR screening conditions. This category is not readily amenable to NMR structural analysis without further optimization of the protein construct and/or buffer conditions.

4. Notes

1. Unless otherwise stated, all solutions should be prepared using distilled and deionized water that has a resistivity of 18.2 MΩ·cm. Bacterial growth reagents, antibiotics, and routine laboratory chemicals were obtained from Sigma-Aldrich (St. Louis, MO) or other major distributors.

2. Trace metals are required for maximal growth in fully defined media. Addition of the trace element solution ensures that the large number of metal ion containing enzymes in *E. coli* can function optimally.
3. This protocol can readily be adapted for uniform $^{13}C/^{15}N$-labeling of proteins and for use with D_2O for the production of deuterated proteins *(18)*. To prepare minimal medium for production of $^{13}C/^{15}N$-labeled proteins, substitute the 50% w/v glucose solution (Section 2.1.) with ^{13}C-glucose added at 2 g per L.
4. Media should be used within 24 hours of assembly from sterile stock solutions.
5. The volume of the starter culture can be scaled according to the desired volume of the large-scale growth. In the author's experience, good results are obtained from using a starter culture that is approximately 5% of the final volume.
6. Conditions for optimal growth should be determined in small-scale cultures prior to isotopic labeling. Several different parameters can critically influence the total and soluble protein expression including choice of expression vector and expression strain, temperature, OD at induction, concentration of inducer, and the induction time.
7. This procedure is very efficient for well expressed proteins, requiring only a 250-ml culture to produce sufficient material for ^{1}H-^{15}N HSQC screening in several buffer conditions.
8. $^{13}C/^{15}N$-labeled proteins can also be produced using autoinduction medium with ^{13}C-glycerol as the carbon source *(12,13)*. Glucose cannot be used because it prevents autoinduction. Although the cost of ^{13}C-labeled precursors with this approach is higher than for conventional methods, this is compensated by the ease and reproducibility of the autoinduction medium.
9. If the expression vector uses a T7*lac* promoter, an *E. coli* host strain without a pLysS plasmid is recommended. The combination of T7 lysozyme (expressed by the pLysS plasmid) and the *lac* repressor causes significantly reduced protein expression in autoinduction medium.
10. NG is a defined minimal medium for growth to saturation with little or no induction of target protein expression. This is a variant of PG (also previously known as P-0.5G) *(12)*. Overnight cultures in NG can be used to make freezer stocks that remain viable indefinitely and generate cultures that produce high levels of target protein.
11. Cells should be grown to saturation when using autoinduction. Higher saturation densities mean that cultures may be quite dense after overnight incubation but not yet induced, so care must be taken not to harvest low-temperature cultures before they have saturated *(12)*. Saturation is usually reached in 8–10 hours if the cultures are incubated at 37°C. When lower temperatures are used, saturation may only be reached by incubation for 24 hours or more.
12. Aeration, particularly during the induction phase, is an important parameter for efficient expression in shake flasks. Yields can be improved by the use of baffled flasks, and the volume of the culture should not exceed 25% of the flask volume.
13. The protein's theoretically calculated isoelectric point (pI) should be considered when choosing a buffer for NMR analysis, as the solubility of

a protein tends to decrease in solutions with a pH near its pI. Buffers with a slightly acidic pH are preferred to reduce the chemical exchange rate of the amide protons with water. If the protein contains a large number of cysteine residues, the concentration of DTT can be increased to 10 mM. Sodium azide (NaN_3), added to inhibit bacterial growth, is highly toxic and gloves should always be worn during sample preparation.

Acknowledgments

The author's research was supported by an RD Wright Biomedical Career Development Award (401748) and project grant (351503) from the Australian National Health and Medical Research Council (NHMRC).

References

1. Page, R., Peti, W., Wilson, I. A., Stevens, R. C., and Wüthrich, K. (2005) NMR screening and crystal quality of bacterially expressed prokaryotic and eukaryotic proteins in a structural genomics pipeline. *Proc. Natl. Acad. Sci. USA* **102,** 1901–1905.
2. Rehm, T., Huber, R., and Holak, T. A. (2002) Application of NMR in structural proteomics: screening for proteins amenable to structural analysis. *Structure* **10,** 1613–1618.
3. Yee, A., Chang, X., Pineda-Lucena, A., Wu, B., Semesi, A., Le, B., Ramelot, T., Lee, G. M., Bhattacharyya, S., Gutierrez, P., Denisov, A., Lee, C., Cort, J. R., Kozlov, G., Liao, J., Finak, G., Chen, L., Wishart, D., Lee, W., McIntosh, L. P., Gehring, K., Kennedy, M. A., Edwards, A. M., and Arrowsmith, C. H. (2002) An NMR approach to structural proteomics. *Proc. Natl. Acad. Sci. USA* **99,** 1825–1830.
4. Peti, W., Etezady-Esfarjani, T., Herrmann, T., Klock, H. E., Lesley, S. A., and Wüthrich, K. (2004) NMR for structural proteomics of Thermotoga maritima: screening and structure determination. *J. Struct. Funct. Genomics* **5,** 205–215.
5. Yee, A. A., Savachenko, A., Ignachenko, A., Lukin, J., Xu, X., Skarina, T., Evdokimova, E., Liu, C. S., Semesi, A., Guido, V., Edwards, A. M., and Arrowsmith, C. H. (2005) NMR and x-ray crystallography, complementary tools in structural proteomics of small proteins. *J. Am. Chem. Soc.* **127,** 16512–16517.
6. Snyder, D. A., Chen, Y., Denissova, N. G., Acton, T., Aramini, J. M., Ciano, M., Karlin, R., Liu, J., Manor, P., Rajan, P. A., Rossi, P., Swapna, G. V. T., Xiao, R., Rost, B., Hunt, J., and Montelione, G. T. (2005) Comparisons of NMR spectral quality and success in crystallization demonstrate that NMR and x-ray crystallography are complementary methods for small protein structure determination. *J. Am. Chem. Soc.* **127,** 16505–16511.
7. Tyler, R. C., Aceti, D. J., Bingman, C. A., Cornilescu, C. C., Fox, B. G., Frederick, R. O., Jeon, W. B., Lee, M. S., Newman, C. S., Peterson, F. C., Phillips, G. N., Shahan, M. N., Singh, S., Song, J., Sreenath, H. K., Tyler, E. M., Ulrich, E. L., Vinarov, D. A., Vojtik, F. C., Volkman, B. F., Wrobel, R. L., Zhao, Q., and Markley, J. L. (2005) Comparison of cell-based and cell-free protocols for producing target proteins from the *Arabidopsis thaliana* genome for structural studies. *Proteins* **59,** 633–643.
8. Ab, E., Atkinson, A. R., Banci, L., Bertini, I., Ciofi-Baffoni, S., Brunner, K., Diercks, T., Dötsch, V., Engelke, F., Folkers, G. E., Griesinger, C., Gronwald, W., Günther, U., Habeck, M., de Jong, R. N., Kalbitzer, H. R., Kieffer, B., Leeflang, B. R., Loss, S., Luchinat, C., Marquardsen, T., Moskau, D., Neidig, K.-P., Nilges, M., Piccioli, M., Pierattelli, R., Rieping, W., Schippmann, T., Schwalbe, H., Travé, G., Trenner, J., Wöhnert, J., Zweckstetter, M., and Kaptein, R. (2006) NMR in the SPINE structural proteomics project. *Acta Cryst.* **62,** 1150–1161.

9. Hondoh, T., Kato, A., Yokoyama, S., and Kuroda, Y. (2006) Computer-aided NMR assay for detecting natively folded structural domains. *Protein Sci*. **15,** 871–883.
10. Kigawa, T., Yabuki, T., Matsuda, N., Matsuda, T., Nakajima, R., Tanaka, A., and Yokoyama, S. (2004) Preparation of *Escherichia coli* cell extract for highly productive cell-free protein expression. *J. Struct. Funct. Genomics* **5,** 63–68.
11. Endo, Y., and Sawasaki, T. (2003) High throughput, genome-scale protein production method based on the wheat germ cell-free expression system. *Biotechnol. Adv*. **21,** 695–713.
12. Studier, F. W. (2005) Protein production by autoinduction in high-density shaking cultures. *Protein Expr. Purif*. **41,** 207–234.
13. Tyler, R. C., Sreenath, H. K., Singh, S., Aceti, D. J., Bingman, C. A., Markley, J. L., and Fox, B. G. (2005) Autoinduction medium for the production of [U-^{15}N]- and [U-^{13}C, U-^{15}N]-labeled proteins for NMR screening and structure determination. *Protein Expr. Purif*. **40,** 268–278.
14. Cai, M., Huang, Y., Sakaguchi, K., Clore, G. M., Gronenborn, A. M., and Craigie, R. (1998) An efficient and cost-effective isotope labeling protocol for proteins expressed in *Escherichia coli. J. Biomol. NMR* **11,** 97–102.
15. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J. and Bax, A. (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes *J. Biomol. NMR* **6,** 227–293.
16. Hill, J. M., Vaidyanathan, H., Ramos, J. W., Ginsberg, M. H., and Werner, M. H. (2002) Recognition of ERK MAP kinase by PEA-15 reveals a common docking site within the death domain and death effector domain. *EMBO J*. **21,** 6494–6504.
17. Natarajan, A., Ghose, R., and Hill, J. M. (2006) Structure and dynamics of ASC2, a pyrin domain-only protein that regulates inflammatory signalling. *J. Biol. Chem*. **281,** 31863–31875.
18. Tugarinov, V., Kanelis, V., and Kay, L. E. (2006) Isotope labeling strategies for the study of high-molecular-weight proteins by solution NMR spectroscopy. *Nature Protocols* **1**, 749–754.