^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the micelle immersed FAT C-terminal (FATC) domains of the human protein kinases ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) fused to the B1 domain of *Streptococcal* protein G (GB1)

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Abstract

FAT C-terminal (FATC) is a circa 33 residue-long domain. It controls the kinase functionality in phosphatidylinositol-3 kinase-related kinases (PIKKs). Recent NMR- and CD- monitored interaction studies indicated that the FATC domains of all PIKKs can interact with membrane mimetics albeit with different preferences for membrane properties such as surface charge and curvature. Thus they may generally act as membrane anchoring unit. Here, we present the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the DPC micelle immersed FATC domains of the human PIKKs ataxia-telangiectasia mutated (ATM, residues 3024–3056) and DNA protein kinase catalytic subunit (DNA-PKcs, residues 4096–4128), both fused to the 56 residue long B1 domain of *Streptococcal* protein G (GB1). Each fusion protein is 100 amino acids long and contains in the linking region between the GB1-tag and the FATC region a thrombin (LVPRGS) and an enterokinase (DDDDK) protease site. The assignments pave the route for the detailed structural characterization of the membrane mimetic bound states, which will help to better understand the role of the proper cellular localization at membranes for the function and regulation of PIKKs. The chemical shift assignment of the GB1-tag is useful for NMR spectroscopists developing new experiments or using GB1 otherwise for case studies in the field of in-cell NMR spectroscopy or protein folding. Moreover it is often used as purification tag. Earlier we showed already that GB1 does not interact with membrane mimetics and thus does not disturb the NMR monitoring of membrane mimetic interactions of attached proteins.

Keywords

Ataxia telangiectasia mutated (ATM), DNA-dependent kinase catalytic subunit (DNA-PKcs); FATC; phosphatidylinositol-3 kinase-related kinases (PIKKs); B1 domain of *Streptococcal* protein G (GB1); chemical shift assignment

Biological context

Ataxia-telangiectasia mutated (ATM) and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) are members of the family of phosphatidylinositol-3 kinase-related kinases (PIKKs), which phosphorylate serine/threonine residues of various substrates (Lempiäinen and Halazonetis 2009). PIKKs govern numerous cellular pathways regulating cell growth and metabolism and generally the response to different types of cellular stress thereby controlling processes such as the DNA damage response, mRNA decay, transcription and other (Lempiäinen and Halazonetis 2009; Lovejoy and Cortez 2009; Morita et al. 2007; Wullschleger et al.). ATM and DNA-PKcs are long known to regulate the DNA damage response (Shiloh 2003). More recently it has been shown that they are further involved in the control of metabolic processes and the oxidative stress response (Cheng and Patel 2004) (Chen et al. 2012; Kong et al. 2011; Kruger and Ralser 2011; Shiloh and Ziv 2013). They are large multidomain proteins consisting of circa 2500 to 4500 amino acids residues and share a similar domain organization (De Cicco et al. 2015; Lempiäinen and Halazonetis 2009; Lovejoy and Cortez 2009). All contain besides the catalytic kinase domain (KD), the FAT and the FAT C-terminal (FATC) domains. In addition, they harbor other domains that are as the FAT domain often composed of helical repeat units that typically mediate protein-protein interactions (Bosotti et al. 2000; Perry and Kleckner 2003). Mutagenesis studies indicated that the short but highly evolutionary conserved FATC domain plays an important role for the control of the PIKK kinase functionality (Hoke et al. 2010; Jiang et al. 2006; Mordes et al. 2008; Morita et al. 2007). Based on NMR- and CD-monitored interaction studies with different membrane mimetics, the FATC domains of all PIKKs can interact with membrane mimetics albeit showing differences regarding the preferences for specific membrane properties such as surface charge and curvature and the lipid packing density (Dames 2010; Sommer and Dames 2014; Sommer et al. 2014; Sommer et al. 2013). This suggested that the FATC domains of PIKKs act further as one component of a network of protein-lipid and protein-protein interactions mediating the observed localization at different cellular membrane regions (De Cicco et al. 2015; Sommer et al. 2013). Whereas the FATC domain of yeast TOR1 can be cleaved off the GB1-purification-tag (Dames et al. 2005), the respective yields for the FATC domains of ATM and DNA-PKcs were only low. Thus the GB1-tagged fusion proteins had been used for NMR monitored interaction studies with membrane mimetic micelles, bicelles, and liposomes of the small unilamellar vesicle (SUV) type (Sommer et al. 2013). It has been shown before that the presence of the GB1-tag does not

disturb the monitoring of membrane mimetic interactions of fused target proteins (Sommer et al. 2012). The GB1-tag is not only known to improve the protein expression level, solubility and folding efficiency (Cheng and Patel 2004; Huth et al. 1997), but allows for small and easy to refold target proteins to employ a heat purification approach (Koenig et al. 2003).

In order to better understand the role of PIKK membrane localization for the specific signaling output, a detailed characterization of their interactions with membrane mimetics and how they influence their structures and dynamics would be beneficial. Here, we present the assignment of the resonances of the GB1-tagged FATC domains of human ATM and human DNA-PKcs immersed in membrane mimetic micelles composed of dodecylphosphocholine (DPC). The assignment of the resonances provides the basis for the structural and dynamic characterization of the membrane mimetic immersed states by multidimensional, heteronuclear NMR spectroscopy. The GB1-tag does not interact with membrane mimetics (Sommer et al. 2012). Thus the additionally provided assignments of the GB1 part correspond to the free, buffer dissolved state. These are useful for scientists using GB1-tagged proteins (Huth et al. 1997) for membrane mimetic (Sommer et al. 2012) and other interaction studies and to characterize the effect of the GB1-tag on the solubility and stability of the fused target protein (Huang et al. 2010). GB1 is further used as test protein for newly developed NMR methods (Clore et al. 1998; Frueh et al. 2005) and in-cell NMR experiments (Luchinat and Banci 2016; Selenko et al. 2006), the characterization of specific NMR phenomena (Walsh et al. 2010), and protein folding studies (Byeon et al. 2004; Ding et al. 2004).

Methods and experimental

Plasmid cloning, expression and purification

Residues 3024-3056 of human ATM (Uniprot-ID Q13315) and residues 4096-4128 of human DNA-PKcs (Uniprot-ID P78527) corresponding to the highly conserved FATC domains were cloned into the expression vector pGEV2 (Huth et al. 1997) and overexpressed in *Escherichia coli* strain BL21 (DE3) as described previously (Sommer et al. 2013). The expressed 100-residue long fusion proteins (Fig. 1) comprise the B1 domain of *Streptococcal* protein G (GB1-tag), a linker region with a thrombin (LVPRGS) and an enterokinase (DDDDK) protease recognition site and either the 33-residue long FATC domain of human ATM (= hATMfatc-gb1ent) or that of human DNA-PKcs (= hDNAPKfatc-gb1ent). Cells were grown in M9 minimal media containing $^{15}NH_4Cl$ and/or ^{13}C-glucose as the sole nitrogen

and carbon sources for the fully isotope labeled samples or 10 % ^{13}C- and 90 % ^{12}C-glucose for the sample used for the stereo-specific assignment of valine and leucine methyl groups (Neri et al. 1989) at 37 °C until the optical density at 600 nm (OD_{600}) reached a value of 0.7-0.9 and then induced with 1 mM IPTG for three hours. Cells were harvested by centrifugation for 30 min at 6000 x g at 4 °C. The supernatant was discarded. Cells were lysed and the GB1-tagged protein extracted and initially purified based on a published heating procedure (Koenig et al. 2003). For this the cell pellet was resuspended in 15 ml 50 mM Tris, 100 mM NaCl, 2 mM EDTA and 2 mM benzamidine, pH 7.6 per 0.5 l culture and incubated for 5 minutes at 80 °C in a water bath, followed by 10 minutes incubation on ice. Most of the *E. coli* proteins precipitate at this temperature leaving rather pure GB1-tagged fusion protein in the soluble fraction if the attached target protein is a peptide (Koenig et al. 2003) or a rather small protein. The fusion proteins hATMfatc-gb1ent and hDNAPKfatc-gb1ent were further purified using IgG sepharose affinity chromatography following the protocol from the manufacturer (GE Healthcare). Fractions containing based on SDS-PAGE analysis pure fusion protein were pooled, concentrated, washed three to six times with 50 mM Tris, 100 mM NaCl, pH 6.5 and finally concentrated using a centrifugal filter device (Amicon Ultra, Millipore Merck, MWCO 3000). The so purified GB1-tagged proteins were directly used for the NMR measurements in the presence of membrane mimetic DPC micelles because the GB1-tag does not disturb the detection of the NMR signals arising from the FATC parts (Sommer et al. 2012).

Preparation of membrane mimetics and NMR samples

Deuterated DPC (d_{38}-DPC) was purchased from Cambridge Isotopes. Protein samples in the presence of DPC were prepared as follows and as described previously (Sommer et al. 2013). A defined amount of DPC from a concentrated stock in chloroform (usually 0.5 M) was placed in a glass vial and dried under a stream of nitrogen gas. The dried DPC film was then dissolved by a protein sample. The samples used to record NMR data for the chemical shift assignment and the structure determination contained circa 0.4 mM protein in 50 mM Tris, 100 mM NaCl, 150 mM d_{38}-DPC, 0.02 % NaN_3, pH 6.5 (95 % / 5 % H_2O / D_2O).

NMR spectroscopy

The NMR data for hATMfatc-gb1ent were acquired at 298 K on Bruker Avance 500, 600, and 900 MHz spectrometers, the 500 and 900 MHz ones equipped with cryogenic probes and that for hDNAPKfatc-gb1ent at 298 K on Bruker Avance 500 and 750 MHz spectrometers, the 500 MHz one equipped with a cryogenic probe. Assignments for ^{13}C, ^{15}N,

and ^{1}H nuclei were based on two-dimensional ^{1}H-^{15}N- and ^{1}H-^{13}C-HSQC spectra (for the 10 % ^{13}C-labeled sample used for the stereo-specific assignments of valine and leucine methyl groups without decoupling (Neri et al. 1989)) three-dimensional HNCA (Grzesiek and Bax 1992), HNCACB, HCCH-TOCSY (Bax et al. 1990; Olejniczak et al. 1992), CCONH-TOCSY (Grzesiek et al. 1993; Lyons and Montelione 1993; Montelione et al. 1992), HNHA (Vuister and Bax 1993; Vuister and Bax 1994), HNHB (Archer et al. 1991), and ^{15}N- and ^{13}C-edited NOESY spectra. Data were processed with NMRPipe (Delaglio et al. 1995) and analyzed using NMRView (Johnson 2004).

Assignments and data deposition

Figure 1 depicts the amino acid sequences of the each 100-residue long hATMfatc-gb1ent and hDNAPKfatc-gb1ent fusion proteins as well as that of GB1 followed by a thrombin and a factor Xa protease recognition site (= gb1xa). Figures 2 and 3 show superpositions of the ^{1}H-^{15}N HSQC spectra of hATMfatc-gb1ent and hDNAPKfatc-gb1ent in the presence of DPC micelles (150 mM d38-DPC) and of gb1xa in buffer. Due to the interaction of the FATC domains with the circa 20 kDa micelles their signals were generally broader and thus appeared less intense than those of the GB1-tag that does not interact with membrane mimetic micelles (Sommer et al. 2012).

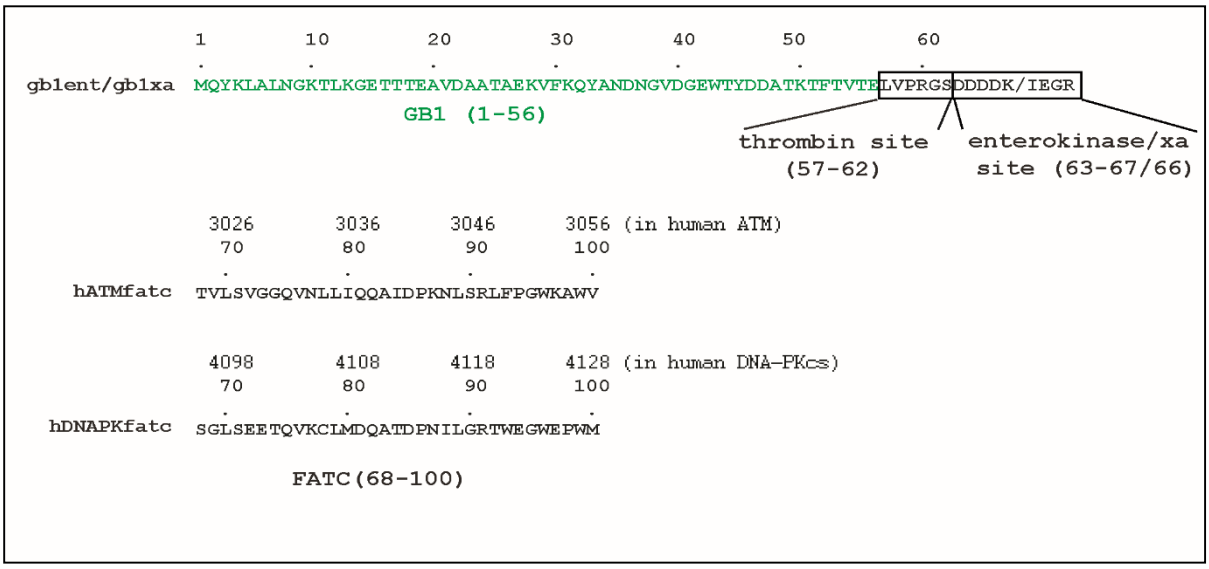

Figure 1

Amino acid sequences of the fusion proteins hATMfatc-gb1ent and hDNAPKfatc-gb1ent consisting of GB1 (as expressed from pGEV2 (Huth et al. 1997)) followed by a thrombin and an enterokinase site (= gb1ent) and the coding sequence for either the human ATM FATC (hATMfatc, Uniprot-ID Q13315) or the human DNA-PKcs FATC (hDNAPKfatc, Uniprot-ID

P78527) domain as well as that of GB1 followed by a thrombin and a factor Xa instead of a enterokinase site (= gb1xa).

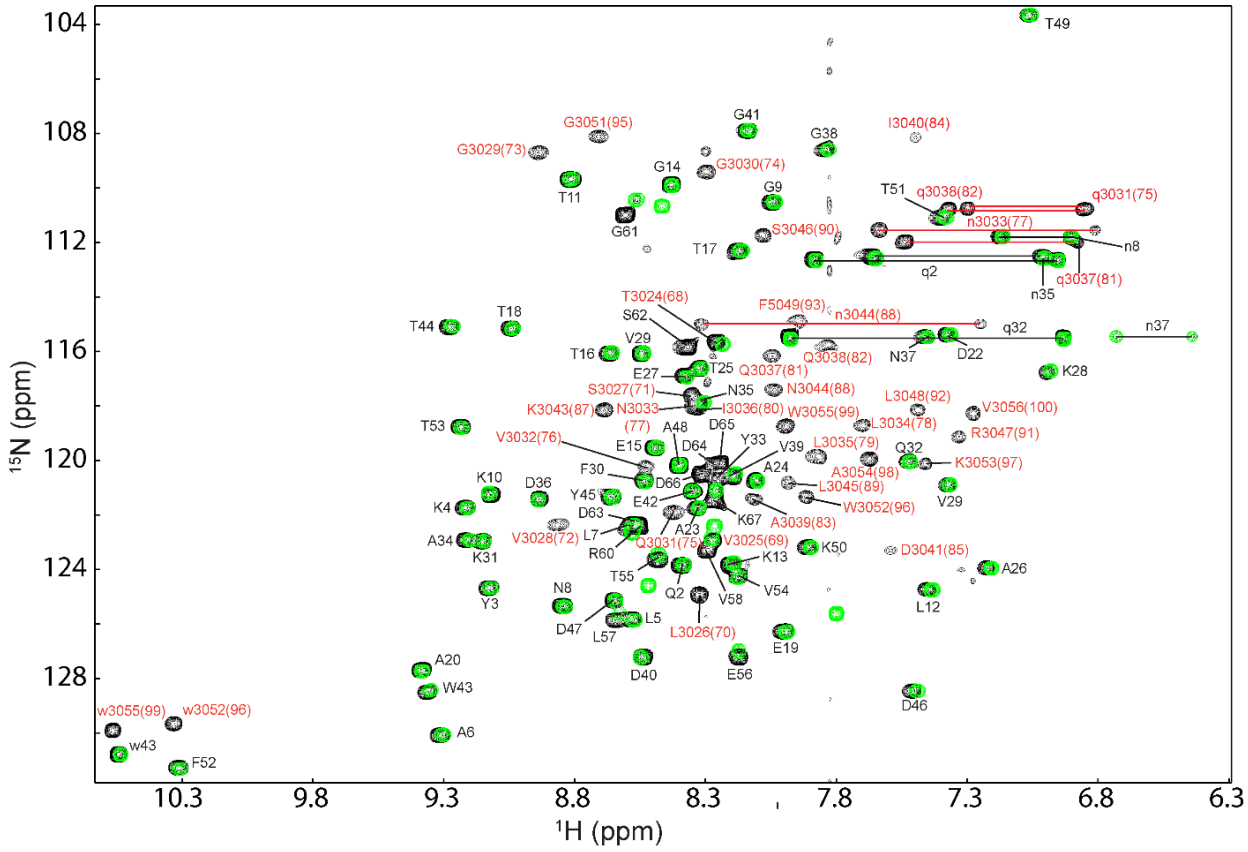

Figure 2

Superposition of the ^{1}H-^{15}N HSQC spectra of micelle immersed hATMfatc-gb1ent (black) and gb1xa in micelle-free buffer (green). The sequence-specific assignments are indicated by the one-letter amino acid code (capital letters for backbone and small letters for side chain signals) and the sequence position in the 100-residue long hATMfatc-gb1ent fusionprotein (for the FATC part – numbers in brackets). For the FATC domain also the sequence position in full-length human ATM (Uniprot ID Q13315) is provided.

The backbone ^{1}H and ^{15}N nuclei were assigned for 96 % of the residues (100 – 3 prolines and the N-terminal methionine) and the backbone ^{13}Cα for all residues. Fig. 2 and 3 show further the assignment of the side chain -NH_2 signals of glutamines and asparagines. Whereas the side chains -NH signals of tryptophans could be assigned for the GB1 part and the FATC domain of ATM, this was not possible for the three tryptophans of the FATC domain of DNA-PKcs due to partial signal overlap and only weak signals in the NOESY data.

The aliphatic ^{1}H and ^{13}C side chain resonances of both FATC domains and those of the GB1-tag were successfully assigned based on 2D ^{1}H-^{13}C constant time HSQC, 3D CCONH and HCCH-TOCSY data in combination with 3D HNHB, and ^{15}N- and ^{13}C-edited NOESY data. In addition, stereospecific assignments for the leucine δ-methyl and valine γ-methyl groups of the GB1 part and of the FATC of human ATM, except for V3025 (69), could be obtained using a 10 % ^{13}C-labeled sample (Neri et al. 1989). Despite overall weak signal intensity in the 2D aromatic ^{1}H-^{13}C HSQC and 3D ^{13}C-edited aromatic NOESY data, the aromatic ^{1}H and ^{13}C nuclei of the phenylalanine and the two tryptophans of the ATM FATC domain could be assigned.

The chemical shift assignments of the each 100-residue long fusion proteins hATMfatc-gb1ent and hDNAPKfatc-gb1ent in the presence of DPC micelles have been deposited at the BioMagResBank under the BMRB accession numbers 27167 and 27168, respectively. Note the assignments for the GB1-tag correspond to the free, buffer dissolved state since it does not interact with membrane mimetics (Sommer et al. 2012).

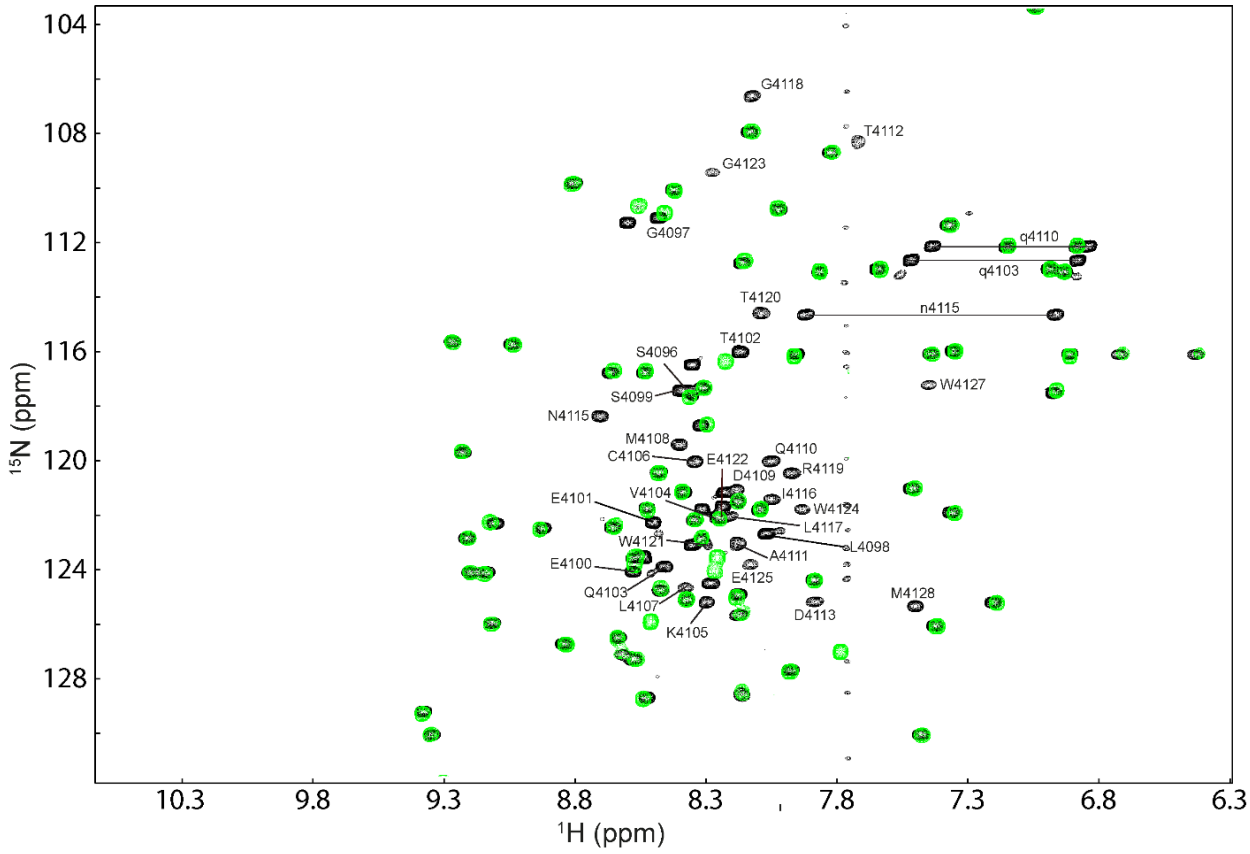

Figure 3

Superposition of the ^{1}H-^{15}N HSQC spectra of micelle immersed hDNAPKfatc-gb1ent (black) and gb1xa in micelle-free buffer (green). The sequence-specific assignments are indicated by

the one-letter amino acid code (capital letters for backbone and small letters for side chain signals) and the sequence position in full-length human DNA-PKcs (Uniprot ID P78527).

References

Archer SJ, Ikura M, Torchia DA, Bax A (1991) An alternative 3D NMR technique for correlating backbone 15N with side chain Hβ resonances in larger proteins. Journal of Magnetic Resonance (1969) 95:636-641. doi:http://dx.doi.org/10.1016/0022-2364(91)90182-S

Bax A, Clore GM, Gronenborn AM (1990) 1H瑇1H correlation via isotropic mixing of 13C magnetization, a new three-dimensional approach for assigning 1H and 13C spectra of 13C-enriched proteins. Journal of Magnetic Resonance (1969) 88:425-431. doi:http://dx.doi.org/10.1016/0022-2364(90)90202-K

Bosotti R, Isacchi A, Sonnhammer EL (2000) FAT: a novel domain in PIK-related kinases. Trends in biochemical sciences 25:225-227

Byeon IJ, Louis JM, Gronenborn AM (2004) A captured folding intermediate involved in dimerization and domain-swapping of GB1. J Mol Biol 340:615-625. doi:10.1016/j.jmb.2004.04.069

Chen BP, Li M, Asaithamby A (2012) New insights into the roles of ATM and DNA-PKcs in the cellular response to oxidative stress. Cancer letters 327:103-110. doi:10.1016/j.canlet.2011.12.004

Cheng Y, Patel DJ (2004) An efficient system for small protein expression and refolding. Biochemical and biophysical research communications 317:401-405. doi:10.1016/j.bbrc.2004.03.068

Clore GM, Gronenborn AM, Bax A (1998) A robust method for determining the magnitude of the fully asymmetric alignment tensor of oriented macromolecules in the absence of structural information. Journal of magnetic resonance (San Diego, Calif : 1997) 133:216-221. doi:10.1006/jmre.1998.1419

Dames SA (2010) Structural Basis for the Association of the Redox-sensitive Target of Rapamycin FATC Domain with Membrane-mimetic Micelles. Journal of Biological Chemistry 285:7766-7775. doi:10.1074/jbc.M109.058404

Dames SA, Mulet JM, Rathgeb-Szabo K, Hall MN, Grzesiek S (2005) The Solution Structure of the FATC Domain of the Protein Kinase Target of Rapamycin Suggests a Role for Redox-dependent Structural and Cellular Stability. Journal of Biological Chemistry 280:20558-20564. doi:10.1074/jbc.M501116200

De Cicco M, Rahim MS, Dames SA (2015) Regulation of the Target of Rapamycin and Other Phosphatidylinositol-3 kinase-related Kinases by Membrane Targeting. Membranes 5:553-575. doi:10.3390/membranes5040553

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277-293

Ding K, Louis JM, Gronenborn AM (2004) Insights into conformation and dynamics of protein GB1 during folding and unfolding by NMR. J Mol Biol 335:1299-1307

Frueh DP, Arthanari H, Wagner G (2005) Unambiguous assignment of NMR protein backbone signals with a time-shared triple-resonance experiment. Journal of biomolecular NMR 33:187-196. doi:10.1007/s10858-005-3204-z

Grzesiek S, Anglister J, Bax A (1993) Correlation of Backbone Amide and Aliphatic Side-Chain Resonances in 13C/15N-Enriched Proteins by Isotropic Mixing of 13C

Magnetization. Journal of Magnetic Resonance, Series B 101:114-119. doi:http://dx.doi.org/10.1006/jmrb.1993.1019
Grzesiek S, Bax A (1992) Improved 3D triple-resonance NMR techniques applied to a 31 kDa protein. Journal of Magnetic Resonance (1969) 96:432-440. doi:http://dx.doi.org/10.1016/0022-2364(92)90099-S
Hoke SM et al. (2010) Mutational analysis of the C-terminal FATC domain of Saccharomyces cerevisiae Tra1. Curr Genet 56:447-465
Huang A, de Jong RN, Folkers GE, Boelens R (2010) NMR characterization of foldedness for the production of E3 RING domains. J Struct Biol 172:120-127. doi:10.1016/j.jsb.2010.07.014
Huth JR, Bewley CA, Clore GM, Gronenborn AM, Jackson BM, Hinnebusch AG (1997) Design of an expression system for detecting folded protein domains and mapping macromolecular interactions by NMR. Protein Science 6:2359-2364. doi:10.1002/pro.5560061109
Jiang X, Sun Y, Chen S, Roy K, Price BD (2006) The FATC domains of PIKK proteins are functionally equivalent and participate in the Tip60-dependent activation of DNA-PKcs and ATM. J Biol Chem 281:15741-15746. doi:10.1074/jbc.M513172200
Johnson BA (2004) Using NMRView to visualize and analyze the NMR spectra of macromolecules. Methods in molecular biology 278:313-352. doi:10.1385/1-59259-809-9:313
Koenig BW, Rogowski M, Louis JM (2003) A rapid method to attain isotope labeled small soluble peptides for NMR studies. J Biomol NMR 26:193-202
Kong X, Shen Y, Jiang N, Fei X, Mi J (2011) Emerging roles of DNA-PK besides DNA repair. Cellular Signalling 23:1273-1280. doi:http://dx.doi.org/10.1016/j.cellsig.2011.04.005
Kruger A, Ralser M (2011) ATM is a redox sensor linking genome stability and carbon metabolism. Science signaling 4:pe17. doi:10.1126/scisignal.2001959
Lempiäinen H, Halazonetis TD (2009) Emerging common themes in regulation of PIKKs and PI3Ks. The EMBO journal 28:3067-3073. doi:10.1038/emboj.2009.281
Lovejoy CA, Cortez D (2009) Common mechanisms of PIKK regulation. DNA repair 8:1004-1008. doi:10.1016/j.dnarep.2009.04.006
Luchinat E, Banci L (2016) A Unique Tool for Cellular Structural Biology: In-cell NMR. The Journal of biological chemistry 291:3776-3784. doi:10.1074/jbc.R115.643247
Lyons BA, Montelione GT (1993) An HCCNH Triple-Resonance Experiment Using Carbon-13 Isotropic Mixing for Correlating Backbone Amide and Side-Chain Aliphatic Resonances in Isotopically Enriched Proteins. Journal of Magnetic Resonance, Series B 101:206-209. doi:http://dx.doi.org/10.1006/jmrb.1993.1034
Montelione GT, Lyons BA, Emerson SD, Tashiro M (1992) An efficient triple resonance experiment using carbon-13 isotropic mixing for determining sequence-specific resonance assignments of isotopically-enriched proteins. Journal of the American Chemical Society 114:10974-10975. doi:10.1021/ja00053a051
Mordes DA, Glick GG, Zhao R, Cortez D (2008) TopBP1 activates ATR through ATRIP and a PIKK regulatory domain. Genes Dev 22:1478-1489. doi:10.1101/gad.1666208
Morita T, Yamashita A, Kashima I, Ogata K, Ishiura S, Ohno S (2007) Distant N- and C-terminal domains are required for intrinsic kinase activity of SMG-1, a critical component of nonsense-mediated mRNA decay. The Journal of biological chemistry 282:7799-7808. doi:10.1074/jbc.M610159200
Neri D, Szyperski T, Otting G, Senn H, Wuethrich K (1989) Stereospecific nuclear magnetic resonance assignments of the methyl groups of valine and leucine in the DNA-binding

domain of the 434 repressor by biosynthetically directed fractional carbon-13 labeling. Biochemistry 28:7510-7516. doi:10.1021/bi00445a003
Olejniczak ET, Xu RX, Fesik SW (1992) A 4D HCCH-TOCSY experiment for assigning the side chain1H and13C resonances of proteins. Journal of biomolecular NMR 2:655-659. doi:10.1007/bf02192854
Perry J, Kleckner N (2003) The ATRs, ATMs, and TORs are giant HEAT repeat proteins. Cell 112:151-155
Selenko P, Serber Z, Gadea B, Ruderman J, Wagner G (2006) Quantitative NMR analysis of the protein G B1 domain in Xenopus laevis egg extracts and intact oocytes. Proceedings of the National Academy of Sciences of the United States of America 103:11904-11909. doi:10.1073/pnas.0604667103
Shiloh Y (2003) ATM and related protein kinases: safeguarding genome integrity. Nature reviews Cancer 3:155-168. doi:10.1038/nrc1011
Shiloh Y, Ziv Y (2013) The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol 14:197-210
Sommer LAM, Dames SA (2014) Characterization of residue-dependent differences in the peripheral membrane association of the FATC domain of the kinase 'target of rapamycin' by NMR and CD spectroscopy. FEBS Letters 588:1755-1766. doi:http://dx.doi.org/10.1016/j.febslet.2014.03.031
Sommer LAM, Janke JJ, Bennett WFD, Bürck J, Ulrich AS, Tieleman DP, Dames SA (2014) Characterization of the Immersion Properties of the Peripheral Membrane Anchor of the FATC Domain of the Kinase "Target of Rapamycin" by NMR, Oriented CD Spectroscopy, and MD Simulations. The Journal of Physical Chemistry B 118:4817-4831. doi:10.1021/jp501533d
Sommer LAM, Meier MA, Dames SA (2012) A fast and simple method for probing the interaction of peptides and proteins with lipids and membrane-mimetics using GB1 fusion proteins and NMR spectroscopy. Protein Science : A Publication of the Protein Society 21:1566-1570. doi:10.1002/pro.2127
Sommer LAM, Schaad M, Dames SA (2013) NMR- and Circular Dichroism-monitored Lipid Binding Studies Suggest a General Role for the FATC Domain as Membrane Anchor of Phosphatidylinositol-3 kinase-related Kinases (PIKK). Journal of Biological Chemistry 288:20046-20063. doi:10.1074/jbc.M113.467233
Vuister GW, Bax A (1993) Quantitative J correlation: a new approach for measuring homonuclear three-bond J(HNH.alpha.) coupling constants in 15N-enriched proteins. Journal of the American Chemical Society 115:7772-7777. doi:10.1021/ja00070a024
Vuister GW, Bax A (1994) Measurement of four-bond HN-Hα J-couplings in staphylococcal nuclease. Journal of biomolecular NMR 4:193-200. doi:10.1007/BF00175247
Walsh JD, Meier K, Ishima R, Gronenborn AM (2010) NMR studies on domain diffusion and alignment in modular GB1 repeats. Biophys J 99:2636-2646
Wullschleger S, Loewith R, Hall MN TOR Signaling in Growth and Metabolism. Cell 124:471-484. doi:10.1016/j.cell.2006.01.016

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