# Letter to the Editor: $^1$H, $^{13}$C and $^{15}$N resonance assignments of the catalytic domain of human MAPK phosphatase, PAC-1

Gaurav Chaturvedi, Amjad Farooq, Lei Zeng & Ming-Ming Zhou*
*Structural Biology Program, Department of Physiology and Biophysics, Mount Sinai School of Medicine, New York University, New York, NY 10029-6574, U.S.A.*

## Biological context

Mitogen-activated protein kinases (MAPKs) play a pivotal role in numerous cellular processes, including neuronal differentiation, mitogenesis, oncogenic transformation and apoptopic cell death (Cobb and Goldsmith, 1995). The biological importance of MAPK regulation is manifested by the tight control of their activity through dual-phosphorylation of threonine and tyrosine in the conserved **TXY** motif within the activation loop. Recently, a group of dual-specificity MAPK phosphatases (MKPs) have been shown to exhibit distinct substrate specificity towards MAPKs (Keyse, 1994). All MKPs consist of two functional domains – an N-terminal kinase-binding domain and a C-terminal phosphatase domain. The high specificity of MKPs is achieved not only by the phosphatase domain recognition of the dual-phosphorylation loop in MAPKs, but also through direct binding of their kinase-binding domains to selective MAPKs (Camps et al., 1998; Slack et al., 2001). Strikingly, this direct enzyme-substrate interaction results in catalytic activation of MKPs (Camps et al., 1998; Farooq et al., 2001), which otherwise exhibit very low phosphatase activity in the absence of substrates. In efforts to determine the structural basis of the mechanism of MAKP-induced MKP enzymatic activation, here we report the nearly complete sequence-specific backbone and side-chain $^1$H, $^{13}$C and $^{15}$N resonance assignments of the C-terminal phosphatase domain of the prototypical MKP, PAC-1.

## Methods and results

The C-terminal phosphatase domain (C257S mutant) from human PAC-1 (residues 170–314) was sub-

cloned into a pET15b vector and expressed in *E. coli* BL21(DE3) cells. The enzymatic nucleophile cysteine to serine mutant was important for enzyme-substrate binding study by NMR. Uniformly $^{15}$N-labeled or $^{13}$C/$^{15}$N-labeled protein samples were prepared by growing the bacteria in minimal media containing $^{15}$NH$_4$Cl with or without $^{13}$C$_6$-glucose. Uniformly $^{13}$C/$^{15}$N-labeled and fractionally deuterated proteins were prepared in 75% $^2$H$_2$O. The phosphatase domain was purified by affinity chromatography on a nickel-IDA column (Invitrogen), and its N-terminal histidine tag was removed by thrombin cleavage. The cleaved protein was further purified by ion-exchange chromatography. NMR samples of the protein ($\sim$0.5 mM) were prepared in a 5 mM sodium phosphate buffer of pH 6.5 containing 5 mM DTT-d$_{10}$ in H$_2$O/$^2$H$_2$O (9/1) or $^2$H$_2$O. All NMR experiments were carried out at 25 °C on Bruker DRX500 and DRX600 spectrometers equipped with four RF channels and a triple-resonance probe with triple-axis pulsed field gradients. The NMR spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed by using NMRView (Johnson and Blevins, 1994). The deuterium-decoupled 3D triple-resonance spectra of HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB with sensitivity-enhancement (Sattler et al., 1999) were used to obtain backbone resonance assignments. The backbone assignments were confirmed through sequential NH-NH and NH-Hα NOEs identified in the $^{15}$N-edited 3D NOESY-HSQC spectrum collected with a mixing time of 100 ms. The side chain $^{13}$C atoms were assigned using a 3D (H)C(CO)NH-TOCSY (Sattler et al., 1999) spectrum recorded on the $^2$H(75%)/$^{13}$C/$^{15}$N-labeled sample. Side chain $^1$H resonances were assigned using a 3D HCCH-TOCSY spectrum (mixing time = 18 ms) (Clore and Gro-

*To whom correspondence should be addressed. E-mail: zhoum@inka.mssm.edu
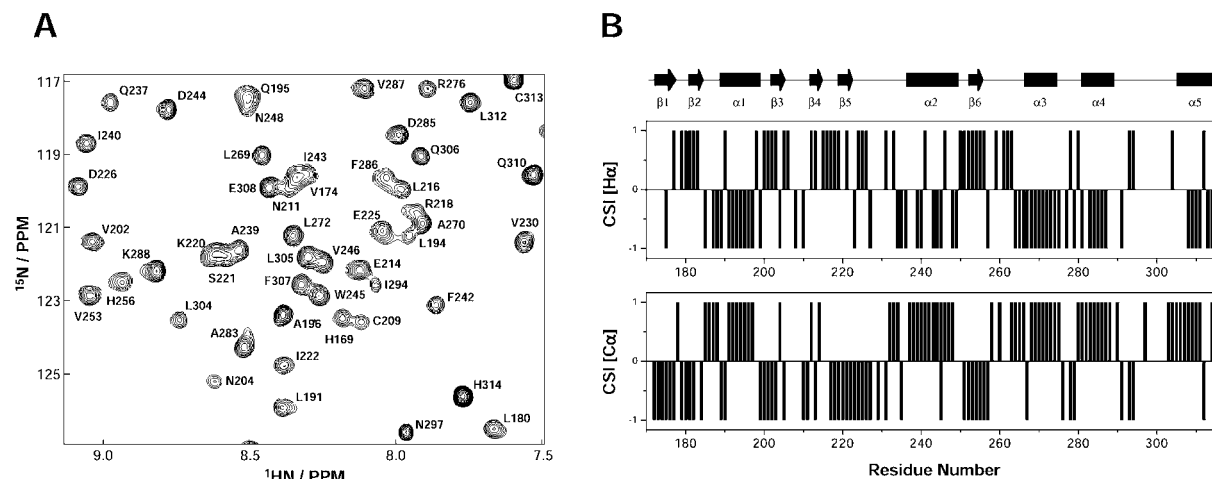
**A**



**B**



*Figure 1.* NMR spectral analysis of the phosphatase domain of human PAC-1. (A) A central region of 2D $^1$H-$^{15}$N HSQC spectrum of the protein collected at pH 6.5 and 25 °C. The assignments are annotated by the resonance peaks. (B) The chemical shift index of backbone $C_\alpha$ and $H_\alpha$ atoms of the protein residues. The secondary structural elements of the protein determined by a collection of the NMR data, including chemical shifts of $H_\alpha$ and $C_\alpha$ atoms, backbone amide hydrogen exchange rate, and sequential NOE patterns.

nenborn, 1994) and confirmed with a 3D $^{15}$N-edited TOCSY-HSQC experiment (mixing time = 80 ms). The side chain $^1$H and $^{13}$C resonances for aromatic residues were assigned using a combination of 2D $^1$H NOESY and TOCSY in addition to $^{13}$C HSQC and 3D HCCH-TOCSY recorded in the aromatic carbon region.

**Extent of assignment and data deposition**

The high quality of spectra from the 3D triple-resonance experiments allowed us to obtain nearly complete backbone assignments of $^1$H$^N$, $^{15}$N, $^{13}$C$_\alpha$ and $^{13}$C$_\beta$ atoms for the entire protein except residues Q289, S295, and F298-G302 that are located in a long and flexible loop between α4 and α5. Figure 1A displays the 2D $^1$H-$^{15}$N HSQC spectrum for the phosphatase domain of PAC-1. The side chain $^1$H and $^{13}$C resonance assignments were obtained for over 90% of the residues. A total of 55 slowly exchanging amide protons have been identified with a series of $^{15}$N-HSQC spectra recorded on a uniformly $^{15}$N-labeled sample after the H$_2$O buffer was changed to $^2$H$_2$O buffer. A total of 30 $^3J_{NH-H\alpha}$ coupling constants were obtained with a 3D HNHA spectrum (Vuister and Bax, 1993). Deviations of the $^{13}$C$_\alpha$ and $^1$H$_\alpha$ chemical shifts from random coil values (Wishart et al., 1995), characteristic sequential and medium range NOEs and $^3J_{NH-H\alpha}$ coupling constants indicate that the phosphatase domain of PAC-1 consists mainly of alternating β-strands and α-helices as

shown in Figure 1B. A table of the $^1$H, $^{15}$N and $^{13}$C chemical shift assignments of the PAC-1 phosphatase domain has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5000.

**References**

Camps, M., Nichols, A., Gillieron, C., Antonsson, B., Muda, M., Chabert, C., Boschert, U. and Arkinstall, S. (1998) *Science*, **280**, 1262–1265.

Clore, G.M. and Gronenborn, A.M. (1994) *Meth. Enzymol.*, **239**, 249–363.

Cobb, M.H. and Goldsmith, E. (1995) *J. Biol. Chem.*, **270**, 14843–14846.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Farooq, A., Chaturvedi, G., Mujtaba, S., Plotnikova, O., Zeng, L., Dhalluin, C., Ashton, R. and Zhou, M.-M. (2001) *Mol. Cell*, **7**, 387–399.

Johnson, B.A. and Blevins, R.A. (1994) *J. Biomol. NMR*, **4**, 603–614.

Keyse, S.M. (1994) *Biochim. Biophys. Acta*, **1265**, 152–160.

Sattler, M., Schleucher, J. and Griesinger, C. (1999) *Prog. NMR Spectrosc.*, **34**, 93–158.

Slack, D.N., Seternes, O.M., Gabrielsen, M. and Keyse, S.M. (2001) *J. Biol. Chem.*, **276**, 16491–16500.

Vuister, G. and Bax, A. (1993) *J. Am. Chem. Soc.*, **115**, 7772–7777.