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Letter to the Editor

^{1}H, ^{15}N, ^{13}C assignments for the activated form of the small Rho-GTPase Rac1

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In cell signaling events, small GTPases are classical on/off switches depending on the nucleotide, GTP or GDP, that is bound; Rac1 and Cdc42 are two of the best studied examples of the Rho-family of proteins which are known to regulate the actin cytoskeleton and are involved with cell motility. While Rac1.GDP assignments are published (Thapar et al., 2003), no NMR assignments have been reported for the active form of Rac1. Rac1, uniformly labeled with ^{13}C and ^{15}N, was loaded with a GTP analogue by nucleotide exchange in presence of excess GMPPNP and alkaline phosphatase. Following gel filtration, heteronuclear NMR experiments were carried out on this active protein at 0.8 mM and 25°C. A mutant C178S, K184Stop was used in physiological buffer (Kremer et al., 2001), with 4 mM DTT and 4 mM $MgCl_2$, at pH 6.8 to improve the quality of spectra. Partially deuterated protein was used in some experiments. Cross peaks for 150 out of 168 possible resonances are observed in ^{1}H–^{15}N HSQC spectra. Backbone assignment was completed for 141 resonances; side-chain carbon assignments for the corresponding residues are nearly complete, e.g. $>95\%$ for Cβ. We observe excellent agreement of the secondary structure predicted from the NMR data (based on CSI) with that found in the crystal structure of Rac1.GMPPNP (pdb 1 MH1). Additional materials are given in the online supplements. BMRB deposit number 6970.

References: Thapar et al. (2003) *J. Biomol. NMR*, **27**, 87–88; Kremer et al. (2001) *Methods Enzymol*, **339**, 3–19.

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