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Letters to the Editor

Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignment of the 46 kDa dimeric GAF A domain of Phosphodiesterase 5

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PDE5, one of 11 families of cyclic nucleotide phosphodiesterases, plays a key role in regulating cGMP levels by hydrolyzing 3′-5′-cyclic GMP (cGMP) to 5′-GMP. PDE5 consists of an N-terminal regulatory domain, comprised of a phosphorylation site and two GAF domains, and a C-terminal catalytic domain. GAF domains are ligand-binding domains found in proteins from bacteria through humans. PDE5 binds cGMP at one of the GAF domains with high affinity and specificity, preferring cGMP over cAMP by a factor $>10^3$. cGMP binding to the regulatory domain of PDE5 has not been investigated structurally. A detailed picture of the structural elements involved in cGMP binding of PDE5 is necessary to understand the mechanism of allostery on the molecular level.

Here we present a multidimensional NMR study of residues 125-320 (23 kDa), which constitute the N-terminal (GAF A) domain of PDE5 (PDE5 GAF A). Backbone NMR resonance assignments have been obtained for the PDE5 GAF A dimer (46 kDa) using specific amino acid labeling, perdeuteration, and TROSY-based double- and triple-resonance 2D and 3D NMR experiments. This represents the first NMR assignment of a GAF domain. The chemical shift assignments have been deposited (BMRB accession number 6602.)

Monica R. Sekharan[a], Ponni Rajagopal[a] & Rachel E. Klevit[a,*]
[a]*Department of Biochemistry, The University of Washington, Seattle, 98195-7742, Washington U.S.A*
*To whom correspondence should be addressed. E-mail: klevit@u.washington.edu
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NMR assignment of the DNA binding domain A of RPA from *S. cerevisiae*

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DNA binding domain A in the large subunit of human RPA (hRPA70A) is responsible for the ssDNA binding and protein partner interactions. Although *Saccharomyces cerevisiae* RPA70A (scRPA70A) shares high sequence homology with hRPA70A, the two are not functionally equivalent (Braun et al., 1997). To elucidate the similarities and differences between these two homologous proteins, we determined the solution structure of scRPA70A by NMR spectroscopy. For the assignments we used 2D and 3D heteronuclear NMR experiments with uniformly ^{13}C, ^{15}N-labeled scRPA70A (181–294). The assignments for ^{1}H, ^{13}C, and ^{15}N of scRPA70A (181–294) are essentially complete, the exceptions being the carbonyl carbons, the aromatic carbons, and nitrogen atoms of N214 and D240. BMRB deposits with accession numbers 6606.

References: Braun et al. (1997) *Biochemistry*, **36**, 8443–8454.

Chin-Ju Park[a], Joon-Hwa Lee[a,b] & Byong-Seok Choi[a,*]
[a]*Department of Chemistry and National Creative Research Initiative Center, Korea Advanced Institute of Science and Technology, 373-1, Guseong-dong, Yuseong-gu, Daejon, 305-701, Korea;* [b]*Department of Chemistry and Biochemistry, University of Colorado at Boulder, Boulder, CO, 80309, USA*
*To whom correspondence should be addressed. E-mail: byongseok.choi@kaist.ac.kr
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