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# Structural Basis for the Nrf2 interaction with the Keap1 repressor

Balasundaram Padmanabhan[1*], Kit I.Tong[2], Yoshihiro Nakamura[1], Akira Kobayashi[2], Masayuki Yamamoto[2] and Shigeyuki Yokoyama[1,3,4 *]

[1]RIKEN Genomic Sciences Center, Yokohama 230-0045, [2]Graduate School of Comprehensive Human Sciences, TARA and JST-ERATO Environmental Response Project, University of Tsukuba, Tsukuba 305-8577, [3]Cellular Signaling Laboratory and Structurome Research Group, RIKEN Harima Institute at SPring-8, 1-1-1 Kouto, Mikazuki-cho, Sayo, Hyogo 679-5148, and [4]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan

## Introduction

Oxidative and electrophilic stresses provoke physiological responses that induce the expression of various cytoprotective genes. Recently, the transcription factor Nrf2 was identified as the major regulator of the cytoprotective genes encoding phase 2 detoxication and antioxidant enzymes. Nrf2, which is normally localized in the cytoplasm, tranlocates into nuclei when cells are exposed to electrophiles. Under normal conditions, the cytoplasmic Keap1 protein directly interacts with Nrf2 and negatively regulates the Nrf2 function, and promoting its rapid degradation by the ubiquitin-dependent proteolytic pathway. Keap1 contains an N-terminal BTB domain, a conserved linker domain, and a C-terminal Kelch domain. The Kelch domain of Keap1 binds to the Neh2 domain of Nrf2, and enables Keap1 to sequester Nrf2 in the cytoplasm. To understand the functional importance of the Keap1 interaction for Nrf2, we have determined and analyzed the crystal structure of the Kelch domain of Keap1 (Keap1-DC) as well as that of the Keap1-DC complex with a Nrf2 interacting peptide (Padmanabhan et al, 2005, 2006).

## Materials and Methods

Mouse Keap1-DC was expressed in *Escherichia coli* and purified, and crystallized as described elsewhere (Padmanabhan et al., 2005). For co-crystallization of the protein with the Neh2 peptide (LDEETGEFL), the peptide was purchased from Promega K.K. The peptide and protein solutions were mixed and incubated at 4°C for about 3 hours before setting up the crystallization experiment. The diffraction data for native and heavy-atom derivatives were collected at the beamline BL26B of SPring-8, and for the complex at BL6A of Photon Factory, Japan. The structure was determined by the MIR method and refined to 1.60 Å with data from a native crystal. The current refined model at a resolution of 1.60 Å consists of 290 residues, 8 sulfate ions, and

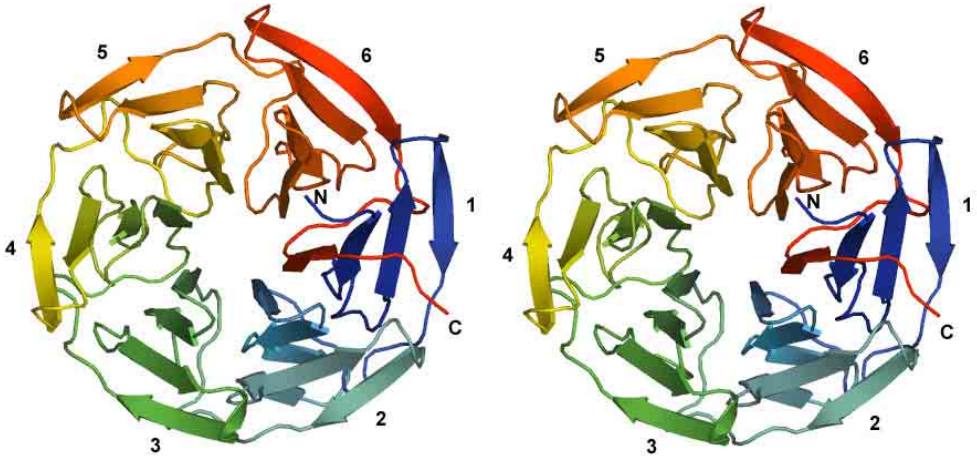


Figure1. The overall tertiary structure of mKeap1-DC. Stereo ribbon representation of the mKeap1-DC β-propeller domain is shown in “top view”. Each β-propeller blade is numbered from 1 to 6.

346 water molecules, with a final R-factor of 16.8% and an $R_{free}$ of 20.0%. The electron density for 15 residues at the N-terminus and 11 residues at the C-terminus was absent. For the protein-peptide complex, the initial orientation of the protein structure obtained from the native crystal was optimized by rigid-body refinement in the respective crystal form. After maximum likelihood refinement with CNS (Brunger et al., 1998), the 2mFo-DFc and mFo-DFc maps were computed to identify and build the peptide model.

## Results and Discussion

The overall tertiary structure of mKeap1-DC is approximately 49 Å x 36 Å, with a small cylindrical/disc shaped structure, and consists of a tandem of six twisted four-stranded (β1–β4) antiparallel β-sheets resembling a 6-bladed β-propeller structure with pseudo six-fold symmetry (Fig.1). In each kelch module, the outermost fourth strand runs almost perpendicular to the innermost first strand, which lies nearly parallel to the central axis of the central cavity of the β-propeller. Like other β-propeller proteins, β-strands of both termini meet and, the disc-like conformation is tightened by a "3+1" arrangement of β-strands. The peptide containing the ETGE motif binds to bottom surface of the Keap1 DGR domain which possesses highly basic surface-charged patch (Fig. 2). The peptide possesses a tight four-residue β-hairpin conformation comprised of the residues Asp-77, Glu-78, Glu-79, Thr-80, Gly-81, and Glu-82 (Fig. 2A). Our mutational and affinity studies using isothermal calorimetry (ITC) also demonstrated that the arginines, such as Arg-415, at the rim of the central cavity on the bottom side of Keap1-DC and the ETGE motif in Neh2 are important for the inter-molecular interactions. These results thus uncover the structural basis of the binding interface between Keap1-DC and the Neh2 domain of Nrf2.

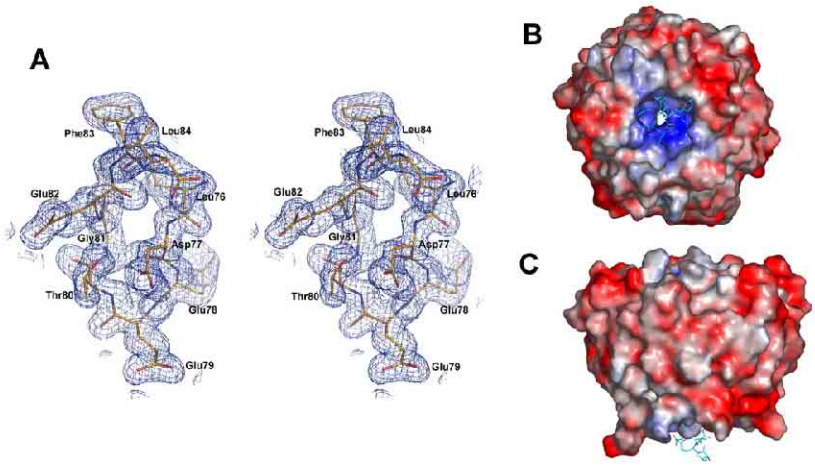


Figure2. The structure of mKeap1-DC complexed with a Neh2 peptide (Leu-76 to Leu-84). (A) Stereo view of the refined peptide in the protein bound form. The final electron density 2mFo-DFc map is contoured at 1.0 σ. (B) Representation of the bottom view of the surface of mKeap1-DC in complex with Neh2. Acidic, basic, and neutral residues are shown in red, blue, and white, respectively. The Neh2 peptide, shown as yellow sticks, binds to a basic patch at the entrance of the central cavity on the bottom side of mKeap1-DC. (C) Side view of (B).

## Reference

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*paddy@gsc.riken.jp or yokoyama@biochem.s.u-tokyo.ac.jp