

Crystal Structure of a vFLIP - IKK γ Complex: Insights into Viral Activation of the IKK Signalosome

The following commentary was written by Dr Clare Sansom. The original article was published in the June 6, 2008 issue of Molecular Cell [1]: Bagn ris, C., Ageichik, A.V., Cronin, N., Wallace, B., Collins, M., Boshoff, C., Waksman, G. & Barrett, T., Molecular Cell (2008) 30, 620-631

The transcription factor NF- κ B initiates gene expression in response to a variety of cell stimuli and is essential for animal life, although its over-expression and deregulation is closely linked to cancer development. In non-dividing cells it is “off” and associated with inhibitory proteins; it is activated by the degradation of these. The first step in initiating degradation is phosphorylation of the inhibitory proteins by a protein complex termed the IKK complex, or signalosome. Some viruses contain proteins known as vFLIPs that interact with, and activate, the signalosome, thus triggering the degradation of the inhibitory proteins, the activation of NF- κ B, and cell division. Tracey Barrett of the School of Crystallography, Birkbeck College, London, and a core member of the Institute of Structural and Molecular Biology, and her colleagues have now solved the X-ray crystal structure of vFLIP from the Kaposi’s sarcoma herpes virus (KSHV) bound to its host target IKK γ , shedding light on the mechanisms through which this viral protein activates the signalosome.

Kaposi’s sarcoma herpes virus or human herpesvirus 8 is best known as the causative agent of Kaposi’s sarcoma [2], a skin cancer which is rare in people with intact immune systems but is commonly associated with HIV infection and is one of the infectious diseases that the World Health Organisation defines as

indicative of full-blown AIDS¹. This virus, and others that similarly cause proliferative diseases, hijacks the signalling pathways that control division in normal cells through activation of NF- κ B. The clumsily named viral Fas-associated death domain interleukin-1 β -converting enzyme inhibitor protein (ks-vFLIP) is homologous to, and has a similar function to, proteins in animal cells that turn this pathway on through interactions with the IKK complex, or signalosome: with the crucial difference that vFLIP is present in, and sends proliferative signals to, all infected cells.

The signalosome is a dynamic, multi-protein assembly in which one subunit, known as IKK γ , regulates the first kinase to be activated, IKK β . IKK γ may therefore be thought of as a “molecular switch”; it has been associated with many other proteins including DNA repair proteins and phosphatases, and mutations that reduce its activity have been implicated in a range of genetic disorders. Previous studies have indicated that ks-vFLIP interacts directly with IKK γ [3, 4] but, although the structure of a homologous vFLIP protein from a pox virus is available [5], no

1

http://en.wikipedia.org/wiki/AIDS#WHO_disease_staging_system

structures of these two proteins in association have been previously known.

Barrett and her co-workers therefore set out to solve the structure of ks-vFLIP bound to a fragment of IKK γ . Although several complexes formed crystals, only one gave reproducible X-ray diffraction: a complex of a C-terminally truncated form of ks-vFLIP with a fragment of about 120 amino acids from the centre of IKK γ . Most of the ks-vFLIP sequence is formed from two copies of the death effector or DED domain (Figure 1a), and the construct crystallised contained the whole of both these domains. IKK γ has a complex domain structure (Figure 1b) with the fragment crystallised, between residues 150 and 272, consisting of parts of two coiled-coil domains spanning an alpha-helical domain.



Figure 1a: Domain structure of IKK γ showing two death effector (DED) domains.

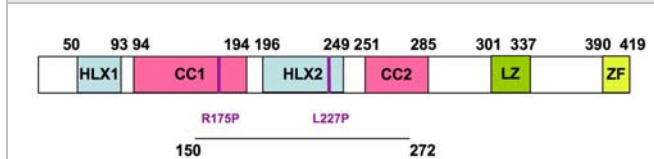


Figure 1b: Domain structure of vFLIP. HLX1 and HLX2 are helical domains; CC1 and CC2 coiled coil domains; ZF is a zinc finger.

The structure of the complex, solved to 3.2 Å resolution, forms a dimer with the two extended IKK γ fragments in the centre. It can be said to resemble an insect head, with the two vFLIP monomers representing the eyes and the IKK γ molecules the antennae (Figure 1c). In spite of the absence of canonical coiled-coil motifs from the centre of the IKK γ fragment crystallised, these molecules pack together to form a parallel, intermolecular

coiled coil. This is consistent with the theory that IKK γ undergoes a significant conformational change on activation, from a compact to an extended form [6].

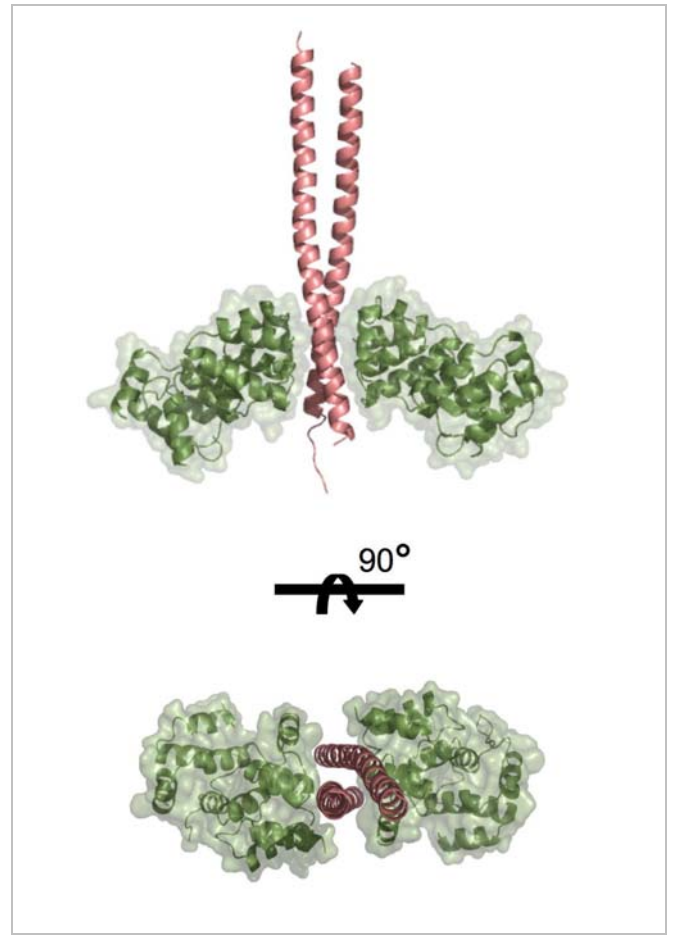


Figure 1c: Two views of the complex between vFLIP and a fragment of IKK γ , showing two helical IKK γ molecules (“insect antennae”) between two molecules of vFLIP (forming an “insect head”).

The interface between IKK γ and vFLIP is large and mainly hydrophobic, encompassing almost the whole of one face of the N-terminal DED domain of vFLIP. Two almost vertical clefts in this DED domain, termed cleft1 and cleft2, lie either side of the IKK γ monomer, with the majority of the protein-protein interactions between IKK γ and cleft1 (Figure 2). This cleft can be divided into two pockets, with the “upper” pocket the more

hydrophobic. Site directed mutagenesis of three residues in cleft1 confirmed the importance of this cleft in binding IKK γ ; the most disruptive mutations were to Ala57, which lines the upper pocket and binds a large phenylalanine residue; increasing the size of this residue will block the pocket, preventing this residue from binding.

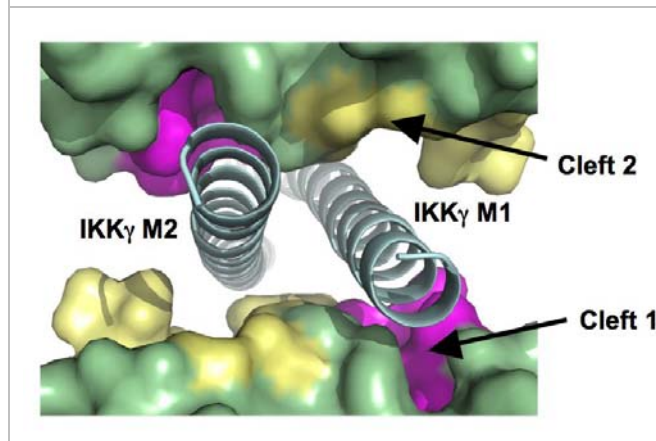
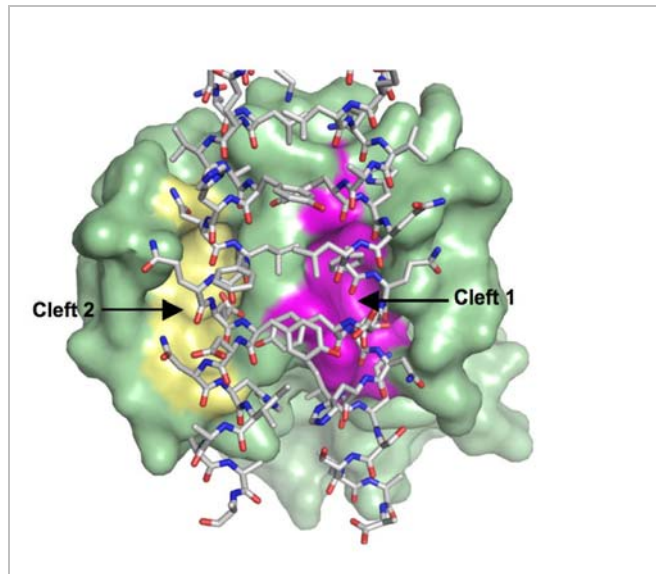


Figure 2: Two close-up views of the interaction between vFLIP and IKK γ . In both views, vFLIP is shown as a solvent-accessible surface with the two clefts colour-coded. In the top view, the vertical IKK γ helices are in an all-atom representation; in the bottom one, the helices are shown as ribbons. The close interaction between the IKK γ molecules and vFLIP cleft 1 is clearly seen.

Mutations in IKK γ can cause a serious genetic disorder, ectodermal dysplasia with immunodeficiency (EDA-ID) which is characterised by recurrent, severe and often life-threatening infections resulting from defective signalosome-mediated NF- κ B activation [7]. Point mutations associated with this disease have been found throughout the molecule; one of these, L227P, lies within the fragment of IKK γ observed in this structure. When this mutation was introduced into the complex, the resulting construct was disordered, susceptible to proteolysis and could not be purified. This result, consistent with the expected consequence of introducing a proline into a largely alpha-helical protein region, illustrates that this mutated IKK γ is unlikely to form part of a stable signalosome.

It is clear that the fragment of IKK γ that is stable in an extended conformation when bound to the hydrophobic cleft of vFLIP will not be stable in a similar conformation in solution. Several groups have suggested that the inactive solution conformation of this molecule will be a helical bundle, with key residues buried and so inaccessible to protein-protein interactions ([6]; Figure 3a).

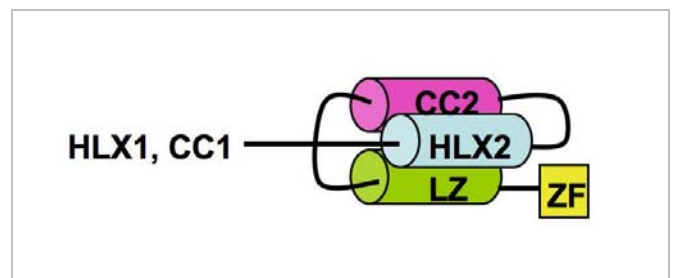


Figure 3a: Schematic domain representation of the isolated, inactive conformation of vFLIP as a helical bundle. Domains are labeled as in Figure 1.

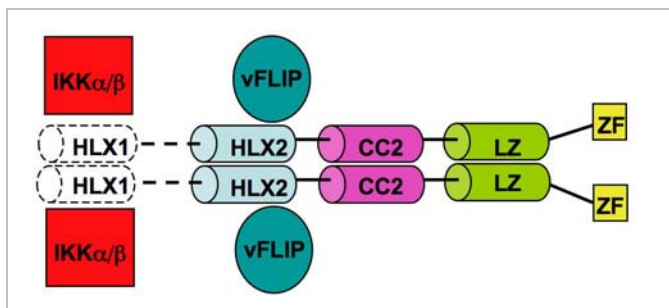


Figure 3b: Schematic domain representation of the extended, active form of vFLIP as a dimer, showing two IKK γ molecules bound to the helix 2 domain and a putative interaction site for IKK α/β .

Barrett and co-workers propose that IKK γ unfolds into its active form only when stimulated by the presence of a cellular or viral FLIP protein (Figure 3b), and that other elements of the complex are only then recruited to form the active signalosome. Designing molecules that disrupt vFLIP-IKK γ complex formation, and thus constitutive activation of the IKK complex, may present a novel therapeutic strategy against Kaposi's sarcoma and some other virally associated forms of cancer.

References

- [1] Bagneris, C., Ageichik, A.V., Cronin, N., Wallace, B., Collins, M., Boshoff, C., Waksman, G. & Barrett, T., *Molecular Cell* (2008) 30, 620-631
- [2] Wang, H.W., Trotter, M.W., Lagos, D., Bourboulia, D., Henderson, S., Makinen, T., Elliman, S., Flanagan, A.M., Alitalo, K. & Boshoff, C. *Nature Genetics* (2004) 36, 687-693
- [3] Field, N., Low, W., Daniels, M., Howell, S., Daviet, L., Boshoff, C. & Collins, M. *J. Cell Sci.* (2003) 116, 3721-3728
- [4] Guasparri, I., Keller, S.A. & Cesarman, E. *J. Exp. Med.* (2004) 199, 993-1003
- [5] Yang, J.K., Wang, L., Zheng, L., Wan, F., Ahmed, M., Lenardo, M.J. & Wu, H. *Mol. Cell* (2005) 20, 939-949
- [6] Hong, S., Wang, L.C., Gao, X., Kuo, Y.L., Liu, B., Merling, R., Kung, H.J., Shih, H.M. & Giam, C.Z. *J. Biol. Chem.* (2007) 282, 12119-12126.
- [7] Courtois, G., Smahi, A. & Israel, A. *Trends Molecular Med.* (2001) 7, 427-430