



# FEBS Letters

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## Hypothesis

# Hinge sequences as signaling agents?

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### ARTICLE INFO

#### Article history:

Received 13 April 2012

Accepted 18 April 2012

Available online 14 May 2012

Edited by Gianni Cesareni

#### Keywords:

Fas receptor

Calmodulin

Signaling

Hinge location

H-predictor

### ABSTRACT

**We report an unexpected finding of common structural principles in two unrelated signaling systems: the FAS death domain transformation that initializes the extrinsic apoptotic pathway and signaling by calmodulin bending. The location and design of the hinge is postulated to be a general principle for creating potential signaling event. We suggest that already existing tool can predict the existence of such a hinge and formulate the hypothesis that the internal instabilities designed into the hinge sequences are necessary devices in effective signaling events.**

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FAS receptor initiates signaling of an extrinsic apoptotic response pathway that culminates with the formation of the death inducing signaling complex (DISC) and the subsequent cell death in Type 1 cells. In our recent letter to Nature [1] we reported an X-ray crystal model for the interaction between the death domain of the FAS receptor and death effector domain (DED) of the FADD protein. This observation shed new light onto important apoptotic signaling events and enhanced our understanding of how the individual pieces of the apoptotic machinery work. However, the main question regarding a protein switch (conformational change) was not addressed in the paper. This communication is designed to fill this gap.

In a subsequent study, the conformational changes of all proteins in the PDB were reviewed [2]. Our analysis showed that, despite no sequence similarity, the FAS death domain shares a significant structural similarity with calmodulin [3]. Fig. 1 shows the helical fragments of both molecules that superpose particularly well, with RMSD  $\sim 2.3$  Å along 33 residues. As a result, a significant similarity is found between FAS–FADD and calmodulin based on the common conformational transition.

Calmodulin has been thoroughly investigated with particular emphasis on the mobility of the protein [4,5]. In calmodulin, a long, stem helix bends during a signaling event and brings two globular helical domains together, closing on a target [3]. The FAS death domain undergoes a transition from the closed to the open state [1] in a similar manner as calmodulin. The significant difference between

these systems is which conformation is adopted in the resting state. While the resting conformation of calmodulin is an open state, the resting conformation of FAS death domain is closed. What is worth stressing is that the design of the switch in both proteins has significant similarities. In FAS as well as in calmodulin the hinge is located in the middle of a long helix and its positioning is virtually identical in reference to the globular domain.

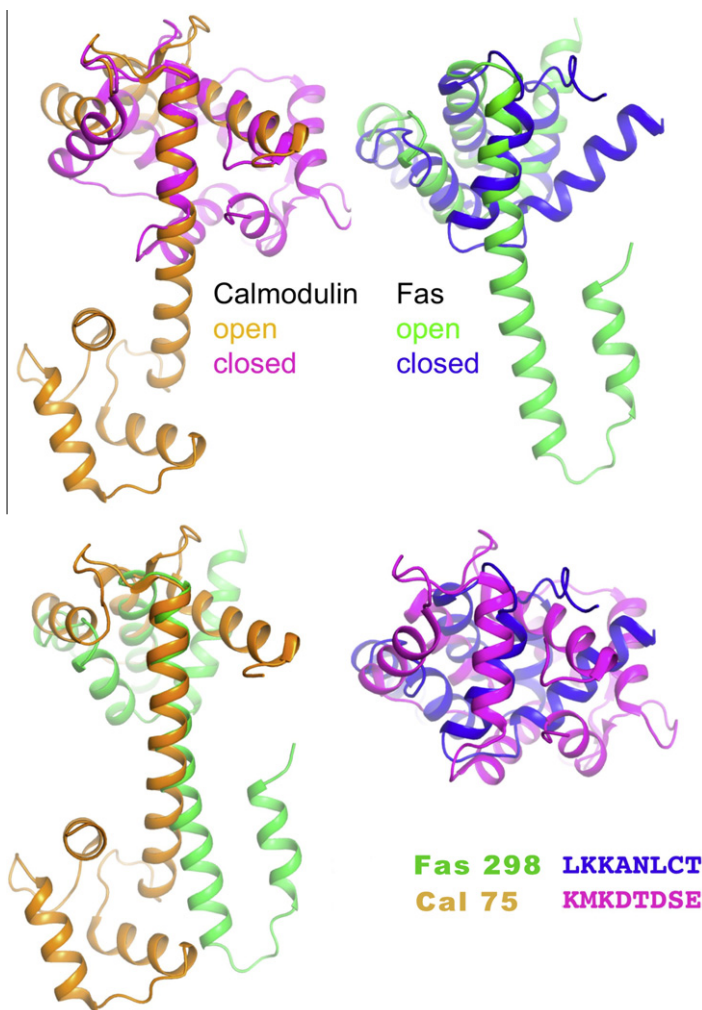
The rearrangement of a helical bundle has become a prominent motif in protein–protein signaling events. Such transformations have been observed in many cytoskeletal interactions, for example with vinculin, talin,  $\beta$ -catenin, integrins, etc. [6]. The structural design of FAS–DD and calmodulin encompasses helical bundles that open or close upon a signaling event. The mobility embedded in both sequences is very similar but serves opposite purposes. The hinge in the central helix is associated with the presence of a sequence (Fig. 1) that is ambivalent in preference to the secondary structure.

In recent years the presence of such sequences has been proposed as an evolutionary vehicle for emergence of new function [5,7]. The idea of designed mobility (or instability) transcends the boundaries of kingdoms of life and can be summarized by a novel principle that we named “differential velcro” effect. This molecular effect is defined by a capability of the intermolecular interactions to dominate in a complex and disrupt the original structure. This disruption causes the structural transformation that results in a signaling event that is frequently associated with a hinge motion.

Recent software tools using protein stability as a differentiating factor have tried to define such hinges. Servers such as Best/Corex [8], HingeMaster [9], H-predictor [10], Dfprot [11], HingeProt [12] and many others are capable of localizing sequences amenable to

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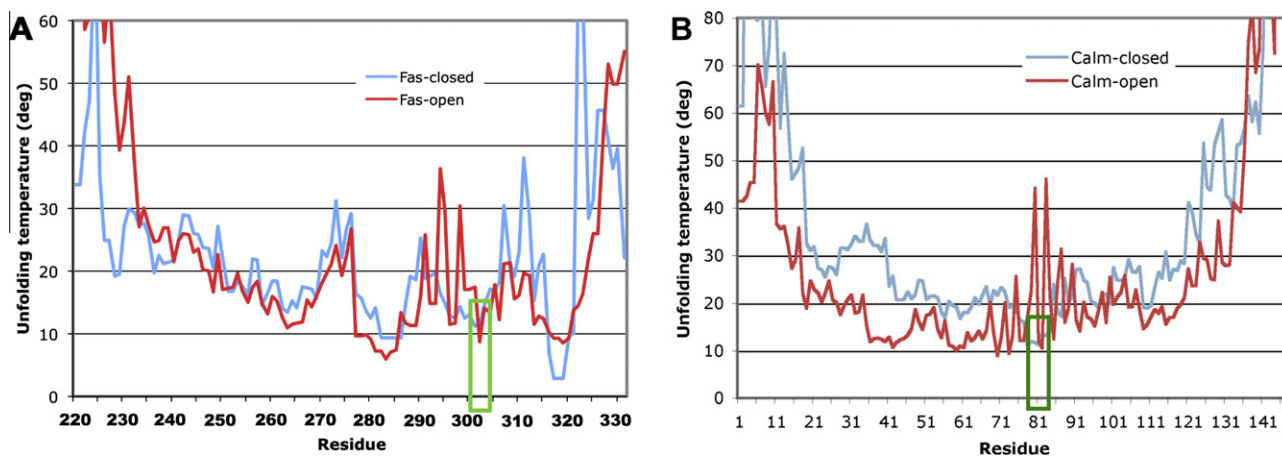
E-mail address: [bstec@sanfordburnham.org](mailto:bstec@sanfordburnham.org)



**Fig. 1.** Upper panel shows an open and closed state of FAS and calmodulin. The lower panel shows the superposition of the open and closed states of FAS and calmodulin. The sequence around the hinge is shown with color codes corresponding to both states.

conformational transformation. The FAS case provides a stringent probe to test such methods. The programs were generally unable to predict the hinge in FAS when presented with open or closed conformations. The only interpretable result was obtained using H-predictor [10] that was able to locate the hinge (Fig. 2) in both

conformers, but not as the most prominent dynamical feature. Despite that fact, limiting the target areas to only three choices for conducting the mutational studies to confirm the mode of action offers a significant advantage. Similar result was obtained for calmodulin (Fig. 2A and B). There is a need for more accurate software



**Fig. 2.** Stability plots produced by H-predictor for open and closed model of both proteins. The blue curve represents the closed state model while the red one represents the open state model. The green box marks the hinge region observed by crystallography. (A) The stability plots for FAS, death domain (B) The calmodulin stability curves look remarkably similar despite differences in amino acid composition.

tools to differentiate the intrinsic disorder from a specific instability constituting a hinge.

We can postulate that the precise knowledge of the location of the switch would allow for predicting and studying such transformations experimentally for systems where the structural information is available only for a single structural state. Additionally, the identification and localization of such sequences may provide real progress in designing of artificial molecular switches [13,14]. The hypothesis described in this communication provides an additional insight into how the mobility factors are designed into all protein structures; proteins that define solid–liquid, dual-nature units of life [15].

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