

"Solution structure of calcium-free Calmodulin" Kuboniwa *et al.*

A little background:

Calmodulin is a protein which is involved in a variety of biological functions, and binds to a variety of protein targets in a calcium-regulated manner. It consists of two domains, each of which is composed of two "EF-hand" calcium binding motifs. Calcium binding modulates the structure of each domain, causing exposure of patches of methionine residues which are then involved in target binding. Calcium also modulates the structure and flexibility of the linker between the two domains. This level of regulation is likely to be important for the binding of proteins which make interactions with both domains. NMR and X-ray structures have given different answers regarding both the structure of the linker and the structure within each domain. To get a visual idea of what is going in calmodulin, as well as a little more background info, **download the review by Chin and Means** from the course website, and look at Figures 2 and 5. It will also be useful (I hope) to first look at the powerpoint lecture "Solving NMR Structures II" and Teng, Chapter 7.

What I want you to get out of this paper:

I don't expect you to understand this entire paper. For example, the authors employ a number of unusual techniques, including the use of ROESY as an alternative to NOESY for generating distance restraints, and a "reverse labelling" technique for analyzing NOEs from Phe residues. I don't want you to focus on these sections. I want you to focus on two things: the overall quality of the structures, and the structure of the linker between the two domains. Below are a number of questions I want you to be prepared to answer in class on **Thursday, Feb 21**:

Quality of N- and C-terminal domain structures

The paper presents separate structural statistics for the N- and C-terminal domains of apo calmodulin.

Why is this done?

For which of the two domains is the structure more precise?

How many long distance ROE/NOE restraints are there per residue for each domain?

For which one are there more residues in disallowed regions of Ramachandran space?

Why are the NOE pseudoenergies and RMSDs from the NOE restraints so bad for the C-terminal domain?

Do the reported atomic RMSDs include the flexible linker region?

What program and type of structure calculation method was used to generate the structures?

How many trial structures were calculated?

How many of these were chosen for the ensemble, and on what basis were they chosen?

Structural nature of the interdomain linker

What is the structural nature of the linker between the N- and C-terminal domains in apo-calmodulin?

What pieces of NMR evidence are offered to demonstrate this?

How is this different from the NMR evidence previously found for the interdomain linker in the Ca(2+) bound form?

How do both contrast with the apparent structure of the linker in the X-ray crystal structure?