

"Solution structure of Ca²⁺-calmodulin reveals flexible hand-like properties of its domains" Chou, Li and Bax *Nat Struct Biol* **8**, 990 (2001).

In order to understand this paper, it will be useful to examine the lecture slides on **residual dipolar couplings** (which will be covered Tuesday, Feb 26) and on **the relationship between scalar couplings and conformation** (Solving NMR Structures I lecture, covered earlier). In addition, I have provided a supplemental optional reading (Tjandra *et al.*, PDF file) which gives a more thorough treatment of the ideas behind residual dipolar couplings than does Chou.

What I want you to get out of this paper:

I want you to focus on two things here: first, the use of residual dipolar couplings in structure calculation and the strengths and limitations of this approach; second, the characterization of the conformations of side chains involved in target binding, especially methionines. Below are a number of questions I want you to try to be prepared to answer in class on **Tuesday, February 26** (but probably won't get to until **Thursday, February 28**):

Overall features of the structure, methods etc.

- >>What is unusual about the way the NMR structure of Ca²⁺-calmodulin is solved?
- >>What structures are used as starting models for the calculations? Why aren't random starting structures used?
- >>What major differences exist between the structures calculated here and the X-ray structure?
- >>Could traditional NOE-based NMR methods have identified these differences?
- >>What do the structural differences seem to indicate about the Ca²⁺ bound state?
- >>How is the issue of *crossvalidation* addressed in this study? In other words, how do the authors independently verify the correctness of the final structure? Crossvalidation is very commonly used in X-ray crystallography (as you'll see in Dr. Montfort's section) but less commonly used in NMR.

Conformation of methionine residues involved in target recognition

- >>What experimental evidence is used to identify χ_1 rotamers?
- >>How would one identify the presence of rotameric averaging (multiple conformations in equilibrium)?