

Smirnov Group Research

My group investigates the domain-domain and linker-domain interactions in modular proteins. Recent advances in genomics (*Homo Sapiens* and other mammals) and structural biology indicate that the majority of human proteins are structurally modular. For understanding the structural, dynamic and functional properties of such multi-domain proteins we are using the solution-state NMR spectroscopy in combination with other biochemical and biophysical techniques. NMR is a uniquely powerful tool for the atomic-resolution structural characterization of proteins, DNA and other biological macromolecules, site-specific study of their local dynamics and probing the wide range of intra- and inter-molecular interactions under physiological conditions. Currently, we are investigating the solution structure and backbone dynamics of two modular proteins participating in regulation of the cytoskeleton, villin and supervillin (Figure 1).

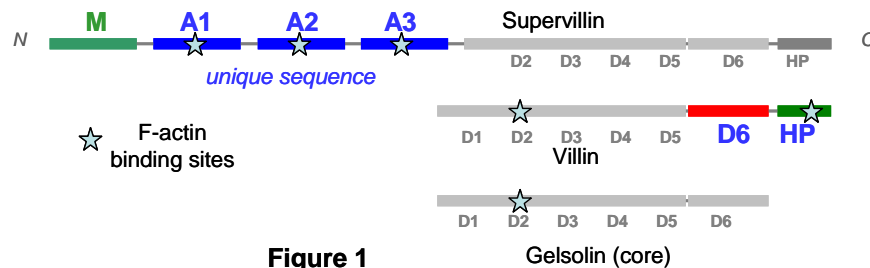


Figure 1

Villin and its Domains

Villin is a protein implicated in the invasion of the harmful microbe *Shigella flexneri* into human via the gut. Restricted to the microvilli of absorptive epithelial cells, villin functions as a Ca^{2+} -controlled, F-actin regulatory protein. In low calcium, villin is an F-actin bundling agent supporting the specialized brush border membrane of the absorptive epithelium and is an F-actin severing agent at higher calcium levels. This calcium-controlled functionality is achieved through repositioning of villin domains. The amino acid sequence of villin has seven proteolytic domains (Figure 1). The first six domains (D1-D2-D3-D4-D5-D6) form the gelsolin-like “core” which has an actin-binding site associated with the D1-D2-D3 part. The last domain, the C-terminal headpiece (HP), is crucial for F-actin bundling by villin and homologous only to other headpiece domains. The solution structure of villin headpiece domain (HP) in isolation has been determined by NMR spectroscopy. We investigated the actin-binding and backbone dynamics of D6-HP, a modular fragment of villin where domain six (D6) is linked to the headpiece (HP), by Electron Microscopy and NMR. Our study revealed that: a) domains D6 and HP are folded and connected via a flexible, unusually long,

40-residue, linker and b) unexpectedly, D6-HP bundles F-actin and thus has two actin binding sites with one associated with HP and the other with D6 or the linker. Currently, we are determining by NMR the solution structure of D6 and D6-HP fragments of villin in order to facilitate determination of the F-actin bundling mechanism of this fragment (Figure 2).

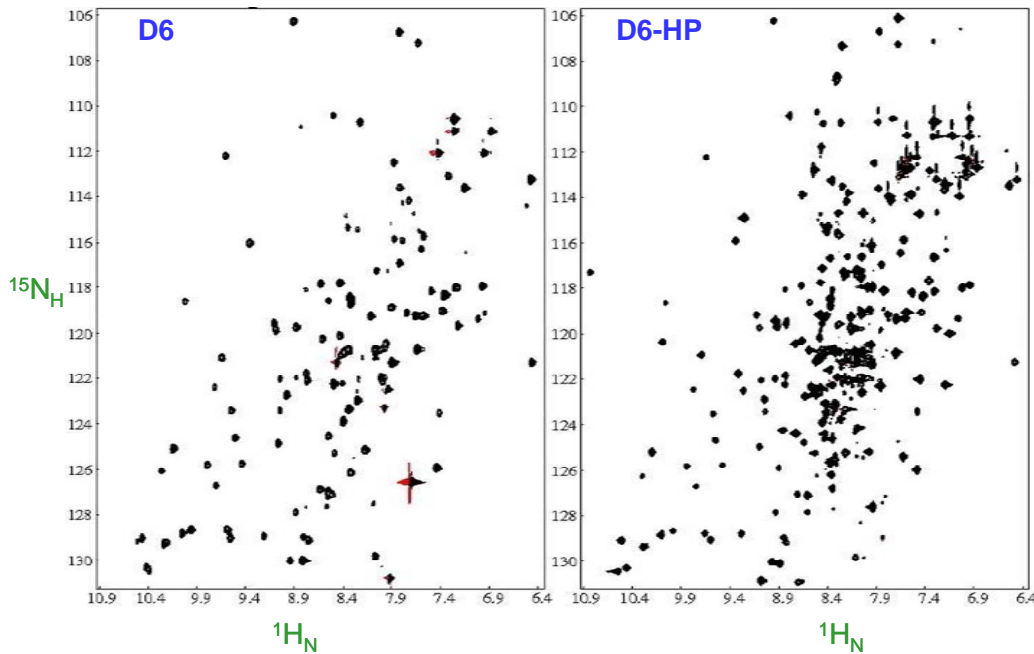


Figure 2
 $^{15}\text{NHSQ}$ NMR spectra of villin fragments D6 and D6-HP

Supervillin and its Fragments

Our other principal subject is supervillin (Figure 1), a modular protein involved in regulation of F-actin and myosin II. Due to the strong homology of the C-terminus of supervillin to villin (hence the name), it was proposed that supervillin binds F-actin with its C-terminal sequence. Surprisingly, it was reported that only three N-terminal supervillin fragments, A1, A2, A3, interact with F-actin. Additionally, N-terminal fragment M of supervillin binds Myosin II. These N-terminal supervillin fragments have unique sequence and therefore may present novel folds and modes of interaction with the cytoskeleton components F-actin and myosin II. Currently, our group is performing the structural NMR investigation of these unique N-terminal fragments of supervillin.

A number of other protein structural projects are also underway in my group.