

## Mapping the Functional Surface of Domain 2 in the Gelsolin Superfamily and Other Members of the Family

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Abstract No. puiu0478

Beamline(s): **X9B**

The crystal structure of the F-actin binding domain 2 of severin, the gelsolin homologue from *Dictyostelium discoideum*, has been determined by multiple isomorphous replacement and refined to 1.75 Å resolution. The structure reveals an alpha-helix-beta-sheet sandwich similar to the domains of gelsolin and villin, and contains two cation-binding sites, as observed in other domain 1 and domain 2 homologues. Comparison of the structures of several gelsolin family domains has identified residues that may mediate F-actin binding in gelsolin domain 2 homologues. To assess the involvement of these residues in F-actin binding, three mutants of human gelsolin domain 2 were assayed for F-actin binding activity and thermodynamic stability. Two of the mutants, RRV168AAA and RLK210AAA, demonstrated a lowered affinity for F-actin, indicating a role for those residues in filament binding. Using both structural and biochemical data, we have constructed a model of the gelsolin domain 1-domain 2-F-actin complex. This model highlights a number of interactions that may serve as positive and negative determinants of filament end- and side-binding.

In collaboration with Fred Southwick, we are pursuing the structure of CapG, a 3 domain family member that caps barbed ends with high affinity. Due to the weak diffraction, X9B has been essential to characterize these crystals. MIR and MAD studies are currently underway.