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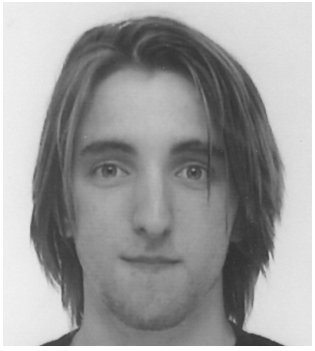
Insights into the structure and dynamics of the pathogen secreted effectors TARP and AVR3a11 through the use of NMR spectroscopy.



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The lifecycles of obligate pathogenic and parasitic microorganisms depend on a myriad of interactions with their hosts at the molecular level. The class of microbial proteins directly responsible for these inter-organism interactions have been termed “effector proteins” and can function in either an extracellular or, once secreted into the host cell, an intracellular environment. Primarily through the use of nuclear magnetic resonance spectroscopy (NMR), we have investigated the biophysical properties of two such pathogen effector proteins.

TARP (translocated actin recruiting protein) is a largely disordered 100 kDa effector, common to all chlamydial species, which functions to remodel the host actin cytoskeleton to facilitate the internalisation of the chlamydial cell. Using constructs of TARP comprising an expected actin binding domain, we have shown through NMR chemical shift indexing and ^{15}N relaxation that although the unbound domain is intrinsically disordered, a short region, which aligns to other helical actin binding domains, maintains some helical propensity. Furthermore, these residues map to chemical shift variations in the bound state and the K_d for the interaction has also been determined using isothermal titration calorimetry.

AVR3a11 is an 8 kDa effector from the pepper pathogen *Phytophthora capsici* that has been shown to inhibit plant programmed cell death. Using a combination of 2D and 3D NMR experiments we have assigned the majority of the backbone and side-chain resonances from the structured regions of AVR3a11. Through the acquisition and analysis of ^{13}C and ^{15}N edited HSQC-NOESY spectra we have also calculated a water refined structural ensemble for AVR3a11. Additionally, analysis of the slow (H:D exchange) and fast (T_1, T_2 and heteronuclear NOE) dynamic regimes describes AVR3a11 as a relatively tightly folded helical bundle which also exhibits a significant degree of conformational exchange.

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