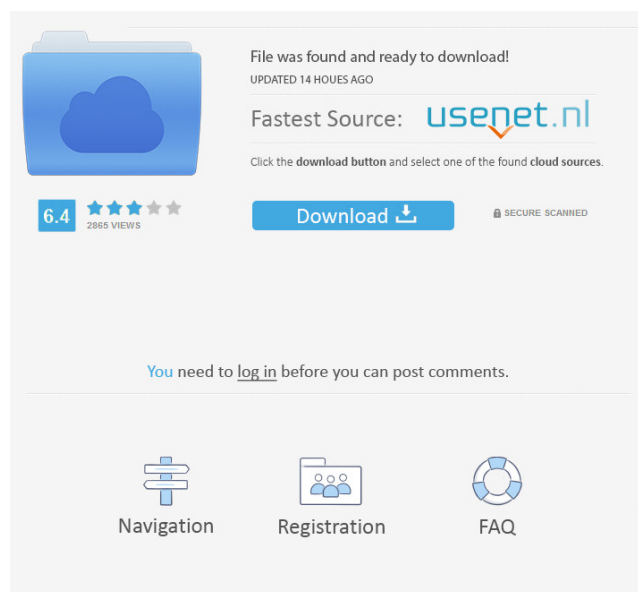

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A: I'm sure you're not actually looking for this but an example of what you're looking for is this: (Scroll down to "Buy e-books") The middle and inner (MIDAS) loop of the tobacco-metallothionein protein is crucial for metal binding and protein function. Two of the toxic heavy metals cadmium and mercury bind to metallothioneins (MTs) at the putative metal-binding site (MBD) within the MIDAS loop. This work investigates the contribution of this loop to metal binding and protein function. The residues involved in metal binding were mapped using substituted cysteine mutagenesis and by monitoring metal accessibility with cysteine-specific reagents. To investigate the importance of the MIDAS loop, conserved basic and acidic residues were substituted with alanine. The MIDAS residues are not accessible to Cd^{2+} and Hg^{2+} as determined by NMR measurements of $\text{Cd}(2+)$ -binding and $\text{Hg}(2+)$ -binding curves in the absence and presence of MIDAS mutations. $\text{Cd}(2+)$ -binding to the WT-protein was completely abolished by the MIDAS mutants K31A, E32A, and D34A. Mercury binding to the WT protein was approximately 70% reduced

by the three mutations. Detailed NMR analysis of Cd(2+)-binding to the mutant E32A protein shows that the MIDAS residues K31 and D34 are not accessible to the metal. We conclude that the MIDAS residues are crucial for metal binding, and they are likely to interact with Cd²⁺ and Hg²⁺ via their carboxylate groups. No significant effect of MIDAS mutations on protein folding and stability was observed. However, inhibition of ATP hydrolysis and enzyme activity was observed. Substitution of MIDAS residues with alanine alters the overall architecture of the protein and introduces strain at the protein-protein interface. We propose that the MIDAS residues are required for maintaining the structure of the protein to allow for conformational freedom in the domain.

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