Metallothioneins, which have been discovered in a wide range of organisms, from humans to worms, to crabs, to seaweeds and fungi, are cysteine-rich, metal binding proteins known to provide protection against, amongst other metals, cadmium toxicity. A key property is the metallation status in which clustered metal-thiolate binding sites are found with the divalent metals, Zn(II), Cd(II), and Hg(II), and the monovalent Cu(I) and Ag(I). As(III) binding has been reported although no structural data are currently available. Our focus is on the mechanisms of initial folding with metal uptake and subsequent metal exchange in natural and synthetic proteins [1-9]. Recent studies on the possible folding and early metallation mechanisms of the metal-free, apoprotein provide a new view of the possible functions of the protein based on its redox properties. Studies of artificial multiple domain proteins provide insight into the role of the two different domains found in the mammalian proteins and provide a route to exploitation of the metal cluster properties as surface modifiers. Metal exchange or metal replacement in MTs is a critically important process that requires mechanistic consideration; however, details of this process are completely lacking. Mechanistic information during metallation has been obtained from Cd-NMR, ESI-MS, CD techniques and by exploiting the specific emission of Ag(I) and Cu(I) bound to the cysteiny1 sulfurs. Both the emission intensity and the band maximum wavelength change as a function of the binding site environment – significantly, the coordination status of the Cu(I) (digonal, trigonal or tetrahedral) is indicated by the emission band maximum. In India and Bangladesh As-contamination of drinking water has reached a point of crisis. Our studies of As(III) binding to MT has allowed determination, for the first time, of the complete set of kinetic parameters that describe metallation on a metal by metal basis. We have studied the metal-binding properties of an algal MT from the brown seaweed Fucus vesiculosus, which grows in waters with high concentrations of As, and the group 11 and 12 metals. The binding properties suggest a two-domain protein with a β domain-like 9-cysteine-3-metals and a novel domain structure, named γ, involving 7 cysteines and 3 divalent metals [8]. The mechanism is controlled by the structure of the metal-thiolate clusters that form and the influence of the peptide chain that surrounds the binding site on the stability of the metal clusters [7]. Molecular modeling is shown to be an effective and successful tool for predicting metalloprotein structure when the geometry can be coupled directly to observed spectral data using the prediction of X-ray absorption spectroscopy [7].

Acknowledgements: We thank the Natural Sciences and Engineering Council of Canada for funding.