Approaches to unique S100 target protein interactions

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The S100 proteins are dimeric, calcium-binding molecules with 25 members in humans. Most members of this family undergo a calcium-induced conformational change allowing the S100 protein to interact with range of possible biological targets. Although a wealth of data exists from *in vitro* experimentation, the calcium-sensitivity for most of the S100 proteins makes traditional *in vivo* identification of target proteins and their interactions with S100 proteins quite difficult to address. In contrast, S100A10 does not bind calcium, and retains a calcium-like conformation having a structure nearly identical to other calcium-bound S100 proteins such as S100A11. In the absence of calcium S100A10 responds well to two-hybrid, co-immunoprecipitation and other *in vivo* methods to identify targets such as annexin A2, AHNAK and several membrane channel proteins. Using S100A10 as a template, our lab has initiated efforts to design a calcium-insensitive S100 protein to use for *in vivo* experiments. The initial results show that minor changes in the S100 sequence can have dramatic changes in the overall hydrophobicity of the protein. We have also engineered hybrid S100A10 and S100B proteins comprised of the S100 protein and a target protein arranged in tandem. These proteins effectively mimick an S100-target complex and are being used to identify the interactions and structures of multiprotein S100 complexes that include the annexins. The design of these proteins and initial experiments will be discussed.