A novel technique allows for structural characterization of high-affinity S100-peptide interactions

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S100 proteins belong to the EF-hand calcium-binding family of proteins. Other than S100A10 that is unable to bind calcium, the rest of the members accommodate one calcium ion per EF-hand. Calcium-binding to the C-terminal EF-hand causes a significant conformational change in S100s, such as S100B, leading to the exposure of hydrophobic residues for target-protein interactions. To determine the precise binding surface of the target proteins on the S100s, and to provide a template for larger S100 complex assemblies, a method for the generation of hybrid S100-target complexes was developed. In this method, peptides of annexin A2 (residues 1-14) and TRTK-12 (residues 1-12 of actin-capping protein) were expressed as S100 hybrid molecules. A linker between the S100 protein such as S100A10 and the binding peptide, annexin A2 was determined using molecular modeling. The linker residues were selected so that they were flexible enough to tolerate the interaction between the peptide and the S100 protein. A PreScission protease site was also engineered near the N-terminus of the peptide segment to facilitate its cleavage and purification. Cloning and high expression levels with no degradation of the S100A10-annexin A2 and S100B-TRTK-12 hybrid proteins are completed. NMR spectroscopy of the apo and Ca^{2+}-bound form of both complexes show that the structure and position of annexin A2 and TRTK-12 peptides in the hybrid proteins are very similar to those obtained for individual proteins. This work will allow these hybrid S100 proteins to be used to test *in vivo* and *in vitro* assembly of larger S100 complexes.