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Biophysical characterization of nanobody:protein interactions relevant in Alzheimer's disease

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S100B protein is one of the most abundant pro-inflammatory proteins which is chronically up-regulated in Alzheimer's disease (AD) and is found within senile plaques [1]. Moreover, S100B is engaged in several processes linked with AD: regulates APP levels and generation of Aβ peptides, interferes with tau protein phosphorylation and is involved in several AD-related signaling pathways. Recently we found that S100B binds to amyloid-β peptide (Aβ42) monomers and fibrils and acts as a suppressor of amyloid-β peptide aggregation and toxicity [2]. Our finding raised the need to develop molecules that target S100B as a novel approach to ameliorate AD neurodegeneration. To fill this gap, we developed and purified a library of 24 single domain antibodies (nanobodies) targeting S100B protein in order to develop biological tools to stabilize the S100B:Aβ42 complex. In most cases, the nanobody:S100B interaction occurs spontaneously, being favored by enthalpic forces. As determined by microscale thermophoresis (MST) and isothermal titration calorimetry (ITC), the binding affinity of nanobody:S100B interactions varies from high to low affinity (K_d=6-3000 nM) and the stoichiometry varies from 1 to 2 nanobodies per S100B protein. We found one nanobody that potentiates the inhibitory effect of S100B over Aβ42 aggregation, promoting a strong suppression of Aβ42 fibrillation, probably by stabilizing the S100B:Aβ42 interaction. These newly developed nanobodies open new avenues to study other protein-protein interactions involving S100B on relevant disease processes.

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