67th Biophysical Society Annual Meeting – late abstract
February 18-22, 2023

Analysis of V_HH dynamics

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Abstract

Protein conformational flexibility is crucial for its structural stability and function. The concerted displacements of residues in an antigen-antibody complex facilitate and determine their interactions' strength, making their study essential to modulating their function. Members of the family *Camelidae* express a unique subset of Immunoglobulin Gamma called the Heavy Chain only Antibody (HCAbs), consisting of one Variable domain (V_HH) at the N-terminus of each heavy chain. Each V_HH domain comprises two types of amino acid regions varying in sequence identity arranged alternatingly called the Framework Regions (FRs) and Complementarity Determining Regions (CDRs). Even when expressed independently *in-vitro*, V_HH domains exhibit excellent solubility and thermostability compared to the V_H-V_L complexes, so they present a valuable opportunity to exploit their biophysical and biochemical properties to generate the next generation of therapeutic and diagnostic molecules. Recent studies have reported sequence and structural features of V_HH domains contributing to these abilities in comparison to classical V_H-V_L complexes. In this study, we performed large-scale classical molecular dynamics simulations for a dataset of unrelated V_HH structures to understand the local and global differences in their dynamics. We used classical metrics such as the Normalised B-factors of Cα atoms, RMSF of Cα atoms and an innovative method called the Protein Blocks (PBs) to investigate flexibility in V_HH domains and trajectories. We have classified the trajectories based on Cα Root Mean Squared Fluctuation, which revealed four main clusters of the V_HH trajectories. We observed various local changes in CDRs but within different ranges in trajectories within the same cluster as well as from other clusters. The FR-CDR boundary regions showed distinct local backbone conformational diversity when assessed using PBs. This study sheds light on region-wise changes in flexibility during dynamics which could aid in improving the design and function of V_HH domains.