ANALYSES OF V_HH DOMAIN DYNAMICS

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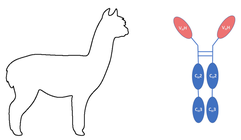
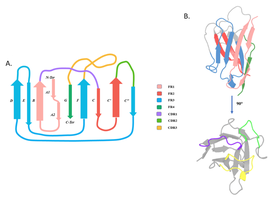
Abstract: Protein conformational flexibility is crucial for its structural stability and function. The concerted displacements of residues in an antigen-antibody complex facilitate and determine their interactions' strength, making their study indispensable to modulating their function. Members of the 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 in-house 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.

Introduction

Heavy Chain only antibodies from Camelids and their Variable domains (V_HH).



2D and 3D organisation of a V_HH domain



- Are the FRs as conformationally similar as they have been previously observed?
- Are the CDRs as conformationally diverse as they have been previously observed?
- Can we have other perspectives on flexibility in the V_HH domains?

Methods:

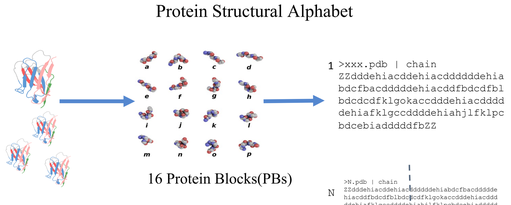
We used three different flexibility metrics to dissect conformational changes in VHH

1: Normalised B-factors

2: Cα RMSF

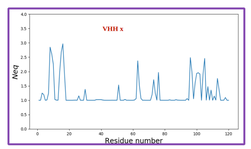
3: Protein Structural alphabet called as Protein Blocks(PBs) (de Brevern et al. 2000). PB assignment for a given protein using PBxplore(https://github.com/pierrepo/PBxplore).

Protein Structural Alphabet

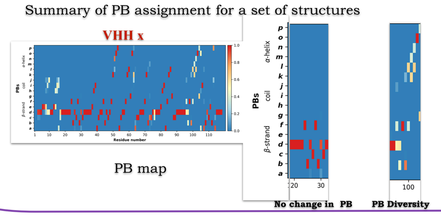


Protein Block entropy and PB frequency change

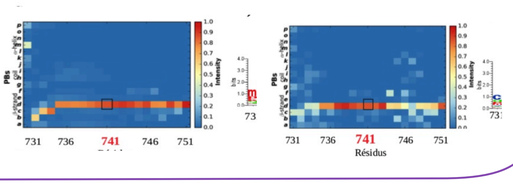
$$Neq = exp(-\sum_{x=1}^{16} f_x \ln f_x)$$



Summary of PB assignment for a set of structures



$$\Delta PB = \sum_{x}^{16} \left| (f_x^1 - f_x^2) \right|$$



Dataset

Sequence and structural features of 88 non redundant V_HH domains.

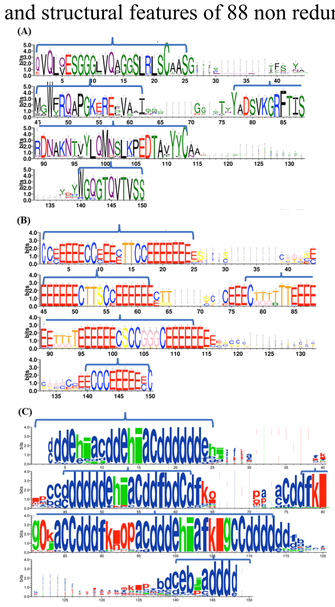


Figure 1: Sequence and Structure characteristics in V_HH *dataset*. Conservation of (A) Amino acid residues, (B) Secondary structures, and (C) Protein Blocks. The four Framework regions are delineated in each figure

Results:

- Flexibility in 88 V_HH PDB structures and corresponding 1μs trajectories.

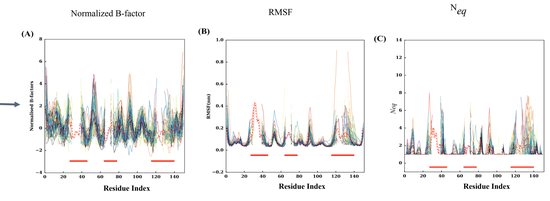
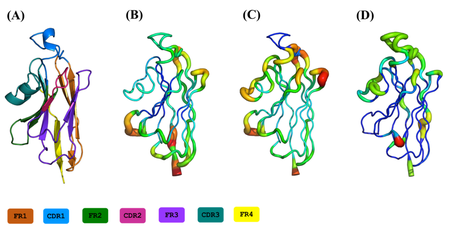


Figure 2: *Flexibility metrics at each residue position.* (A) Representation of normalised B-factors of Cα atoms, the x-axis is the residue position in the MSA and the y-axis the Normalised B-factor values, (B) Representation of all RMSF values Cα atoms, (C) Representation of N_{eq} values. The three CDR regions are highlighted using three red-coloured regions at the bottom of the plots. The average values of each metric are shown in dotted red lines.



- Flexibility metrics are not always correlated.
- Loops other than CDRs also show high RMSF.

Figure 3: *:Representation of mean values of flexibility metrics onto a 3D structure of a V_HH.* (A) FRs and CDRs coloured

- Classification of Flexibility in CDRs

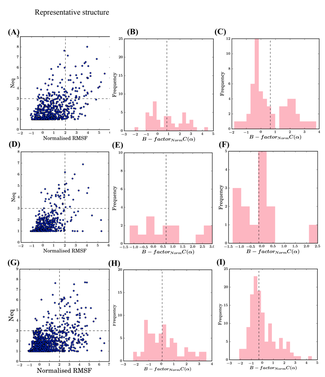


Figure 4: *CDR flexibility.* (A) to (C) is CDR1, (D) to F is CDR2 and (G) to (I) is CDR3. (A), (D) and (G) show the normalised RMSF vs N_{eq} while (B), (E) and (H) are the normalized B-factors of flexible quadrant (nRMSF > 2 and N_{eq} > 3), and (C), (F) and (I) for mobile quadrant (nRMSF > 2 and N_{eq} < 3). The dotted line in each B-factor distribution represents the mean value.

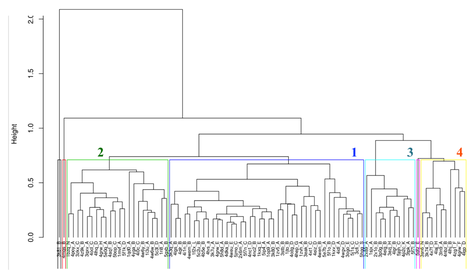


Figure 5: Hierarchical clustering of V_HH *trajectories using RMSF.* The different RMSF clusters are demarcated using coloured boxes, and their cluster number is marked accordingly.

- Hierarchical clustering of 88 1μs trajectories Distance between two RMSF vectors were calculated using .

$$d(v,w) = \sqrt{\frac{1}{n-m}\sum_{i=1}^{n}(v(i) - w(i))}$$

Four dense clusters and 3 outliers were obtained from the clustering.

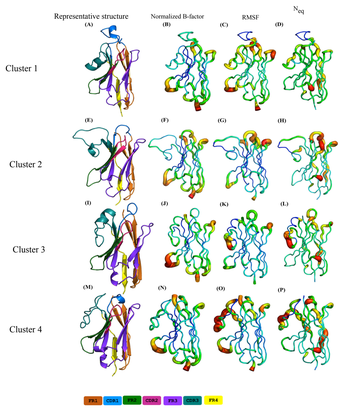


Figure 6: *:Representation of mean values of flexibility metrics onto a 3D structure of a V_HH from each cluster.*

- Mean flexibility metrics represented for each cluster.
- Not all the residue positions of CDRs are diverse.
- FRs show relatively less diversity when compared to CDRs except in the C-terminal loop regions and the 4th N-terminal loop.

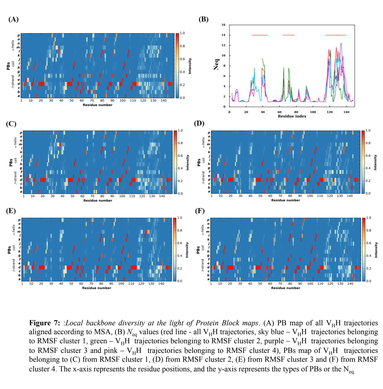


Figure 7: *:Local backbone diversity at the light of Protein Block maps.* (A) PB map of all V_HH trajectories aligned according to MSA, (B) N_{eq} values (red line – all V_HH trajectories, sky blue – V_HH trajectories belonging to RMSF cluster 1, green – V_HH trajectories belonging to RMSF cluster 2, purple – V_HH trajectories belonging to RMSF cluster 3 and pink – V_HH trajectories belonging to RMSF cluster 4), PBs map of V_HH trajectories belonging to (C) from RMSF cluster 1, (D) from RMSF cluster 2, (E) from RMSF cluster 3 and (F) from RMSF cluster 4. The x-axis represents the residue positions, and the y-axis represents the types of PBs or the N_{eq}

- Total conformational diversity observed in various V_HH trajectory groups quantified using Protein Blocks.
- The **β**-core is well conserved in the FRs represented using the PB 'd', except in some regions of FR2 and FR3.
- CDR1 also exhibits higher conformational diversity in terms of PBs.

Conclusions:

- Protein Blocks offer a unique perspective which is both qualitative and quantitative to characterize backbone diversity in structures and trajectories of V_HH domains.
- Higher RMSF values need not be always considered as flexible residues.
- There are few residue positions in CDR3 that do not show much diversity.
- The 4th N-terminal loop exhibits unexpected backbone diversity.

Acknowledgements

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