



HAL
open science

Analysis of VHH dynamics

Akhila Melarkode Vattekatte, Julien Diharce, Catherine Etchebest, Alexandre de Brevern

► **To cite this version:**

Akhila Melarkode Vattekatte, Julien Diharce, Catherine Etchebest, Alexandre de Brevern. Analysis of VHH dynamics. 67th Biophysical Society Annual Meeting, Feb 2023, San Diego (California), United States. inserm-04072706

HAL Id: inserm-04072706

<https://inserm.hal.science/inserm-04072706>

Submitted on 18 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - ShareAlike 4.0 International License

Akhila Melarkode Vattekatte¹, Julien Diharce², Catherine Etchebest³, Alexandre G. de Brevern⁴.

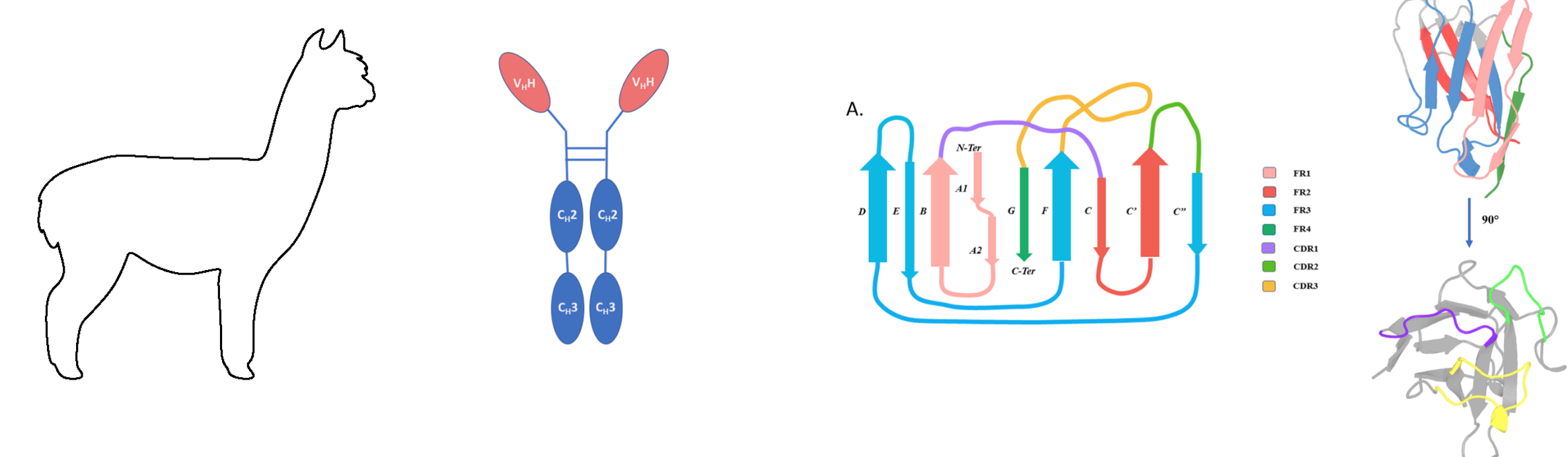
¹DSIMB Laboratory, INSERM UMRS_1134, Biochemistry, University of Reunion Island, Saint Denis, France, ²DSIMB, INSERM UMRS_1134, Univ Paris Cité, Paris, France, ³DSIMB, INSERM UMRS_1134 Paris-Cité, Paris, France, ⁴DSIMB, INSERM UMRS_1134 Univ Paris Cité, Paris, France.

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 alternately 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 C α atoms, RMSF of C α 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 C α 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?

Results:

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

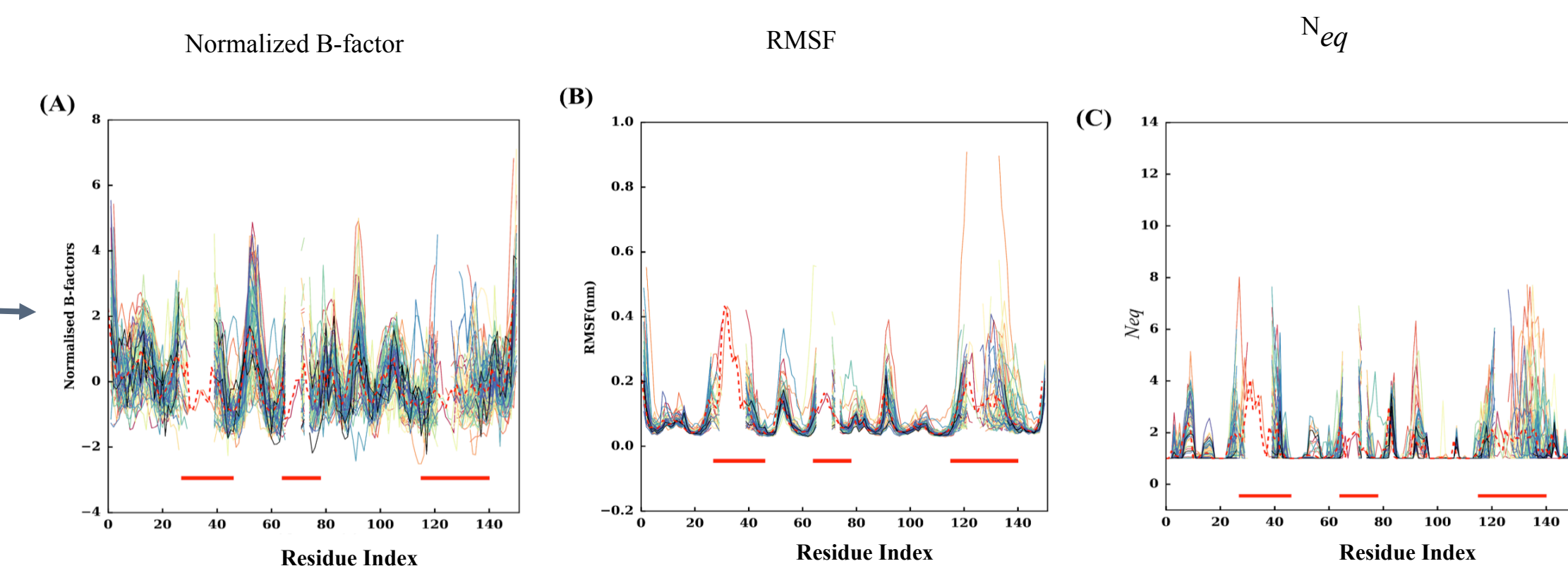


Figure 2: Flexibility metrics at each residue position. (A) Representation of normalized B-factors of C α atoms, the x-axis is the residue position in the MSA and the y-axis the Normalised B-factor values, (B) Representation of all RMSF values C α atoms, (C) Representation of N_{eq} values. The three 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.

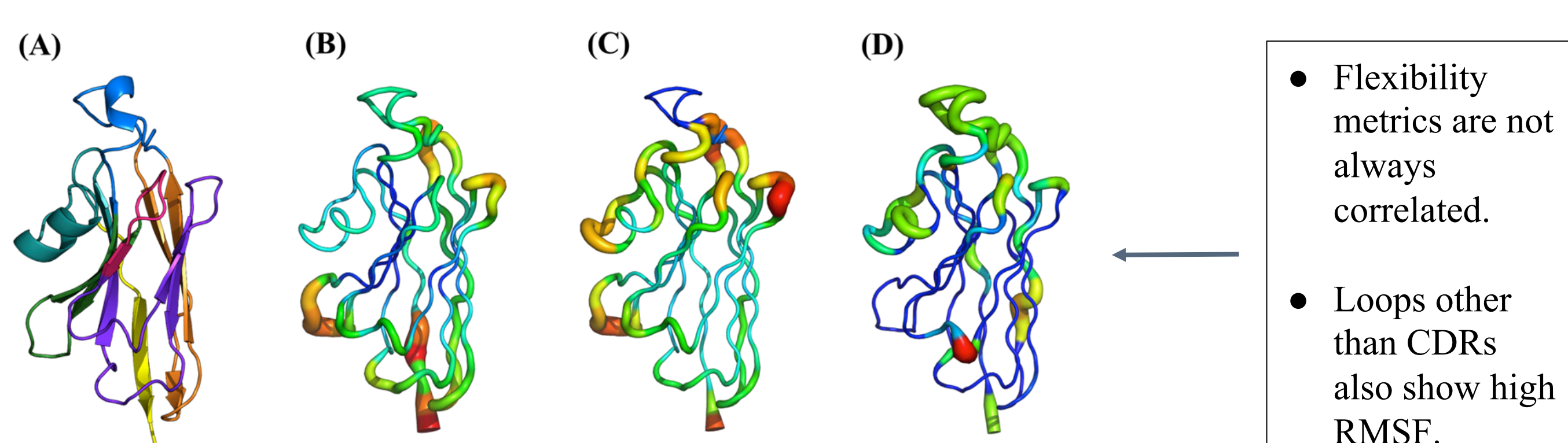


Figure 3: Representation of mean values of flexibility metrics onto a 3D structure of a V_HH. (A) FRs and CDRs coloured

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

- Classification of Flexibility in CDRs

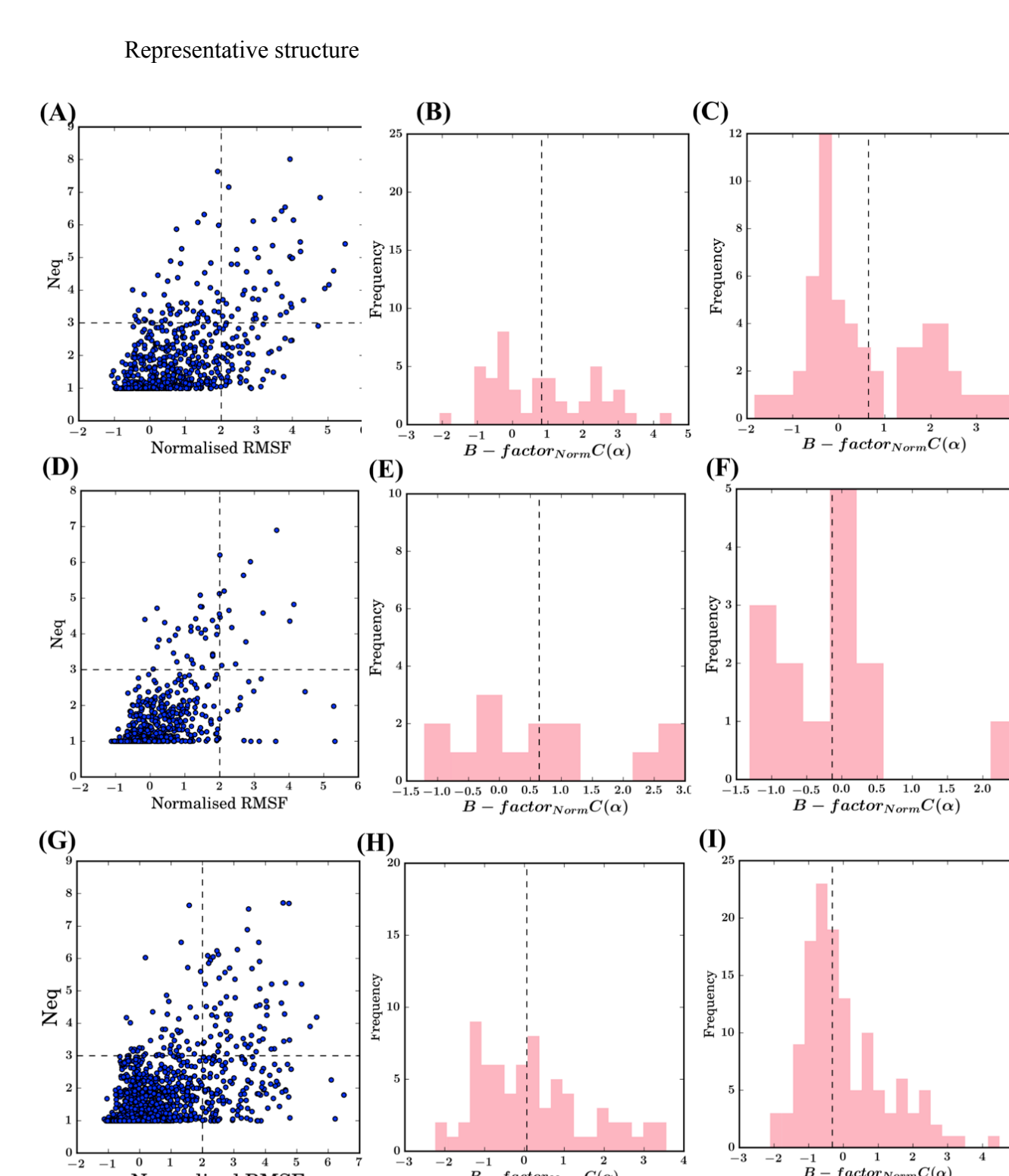


Figure 4: CDR flexibility. (A) to (C) is CDR1, (D) to (F) is CDR2 and (G) to (I) is CDR3. (A), (D) and (G) show the normalized RMSF vs N_{eq} while (B), (E) and (H) are the normalized B-factors of flexible quadrant (RMSF > 2 and N_{eq} > 3), and (C), (F) and (I) for mobile quadrant (RMSF > 2 and N_{eq}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

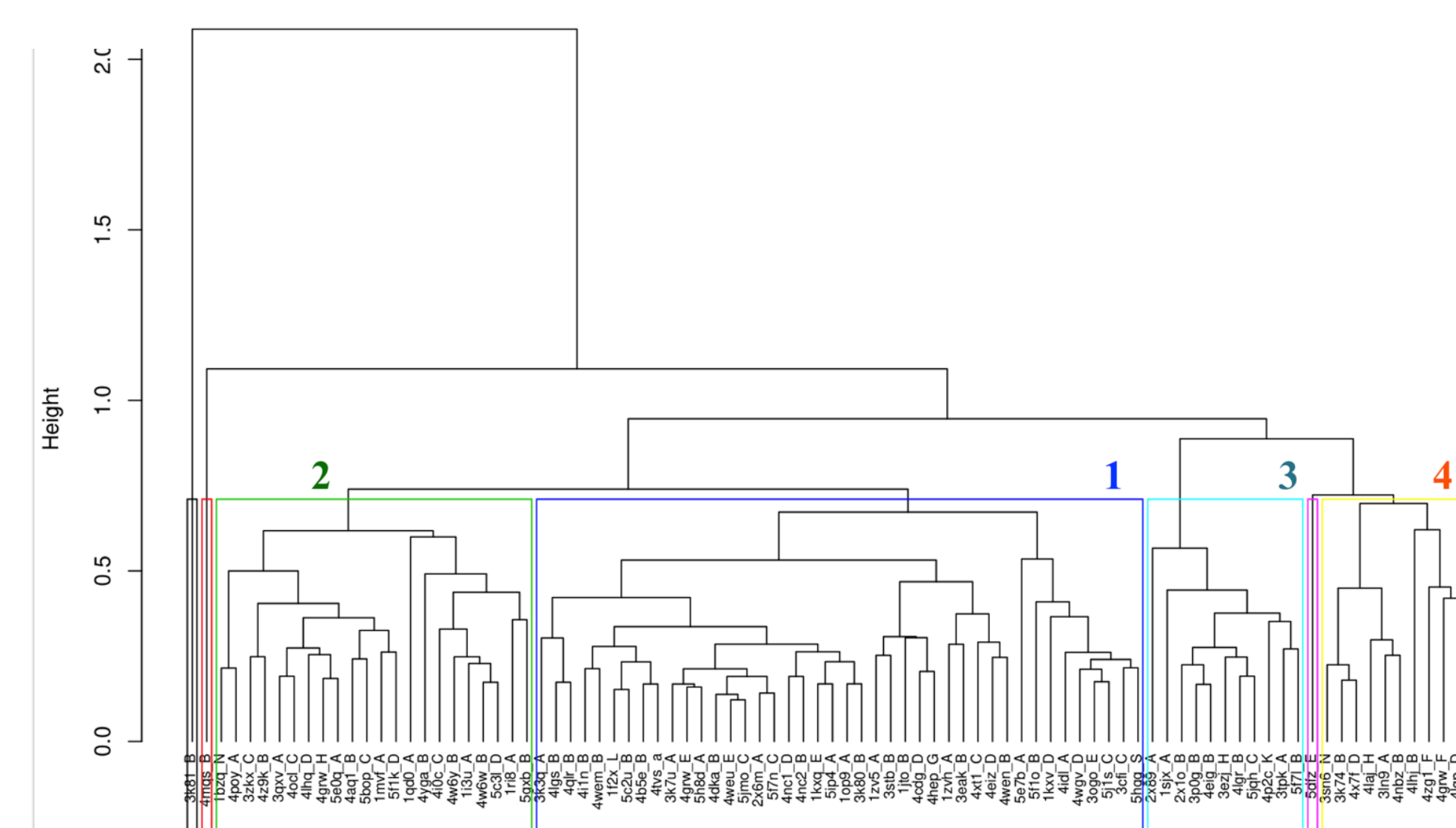


Figure 5: Hierarchical clustering of 88 1 μ s trajectories using RMSF. The different RMSF clusters are demarcated using coloured boxes, and their cluster number is marked accordingly.

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

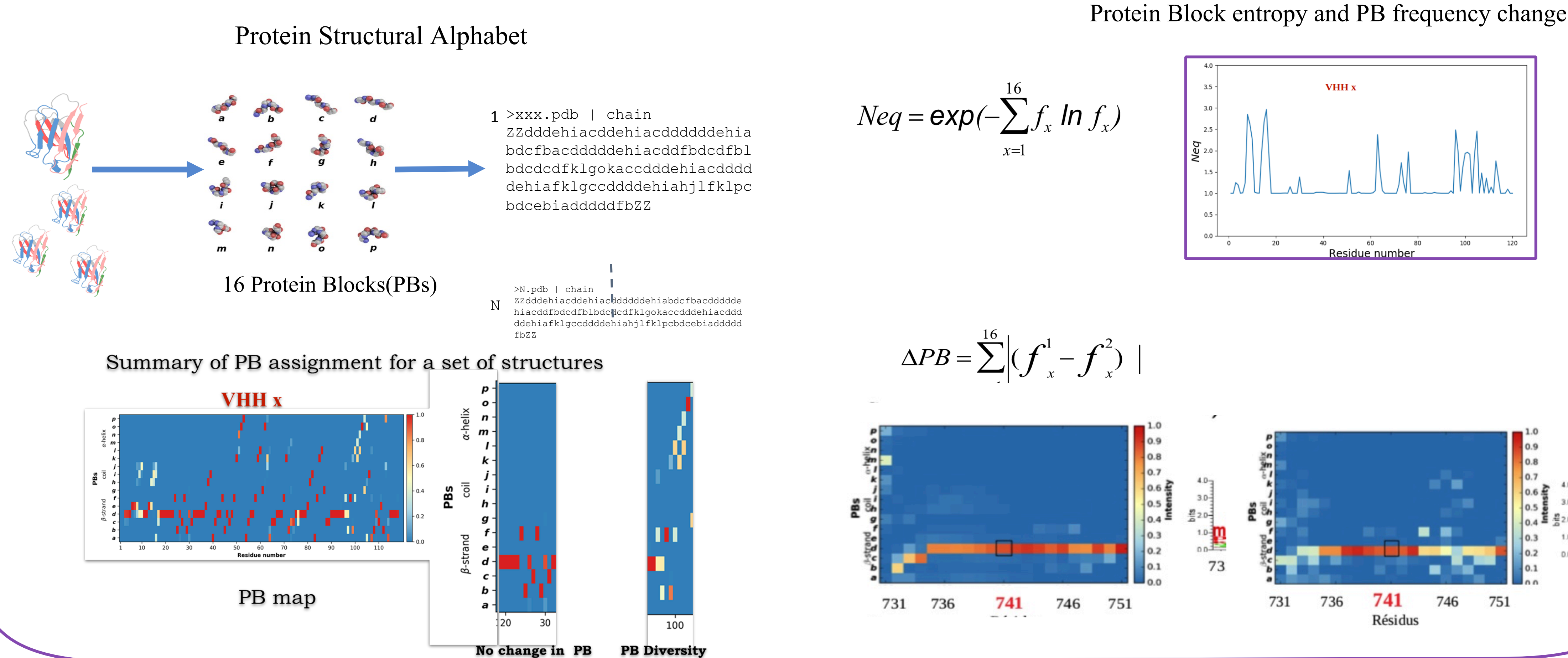
$$d(v, w) = \sqrt{\frac{1}{n-m} \sum_{i=1}^m (v(i) - w(i))^2}$$

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

Methods:

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

- 1: Normalised B-factors
- 2: C α RMSF
- 3: Protein Structural alphabet called as Protein Blocks (PBs) (de Brevern et al. 2000). PB assignment for a given protein using PBxplorer (<https://github.com/pierrepo/PBxplorer>).



Summary of PB assignment for a set of structures

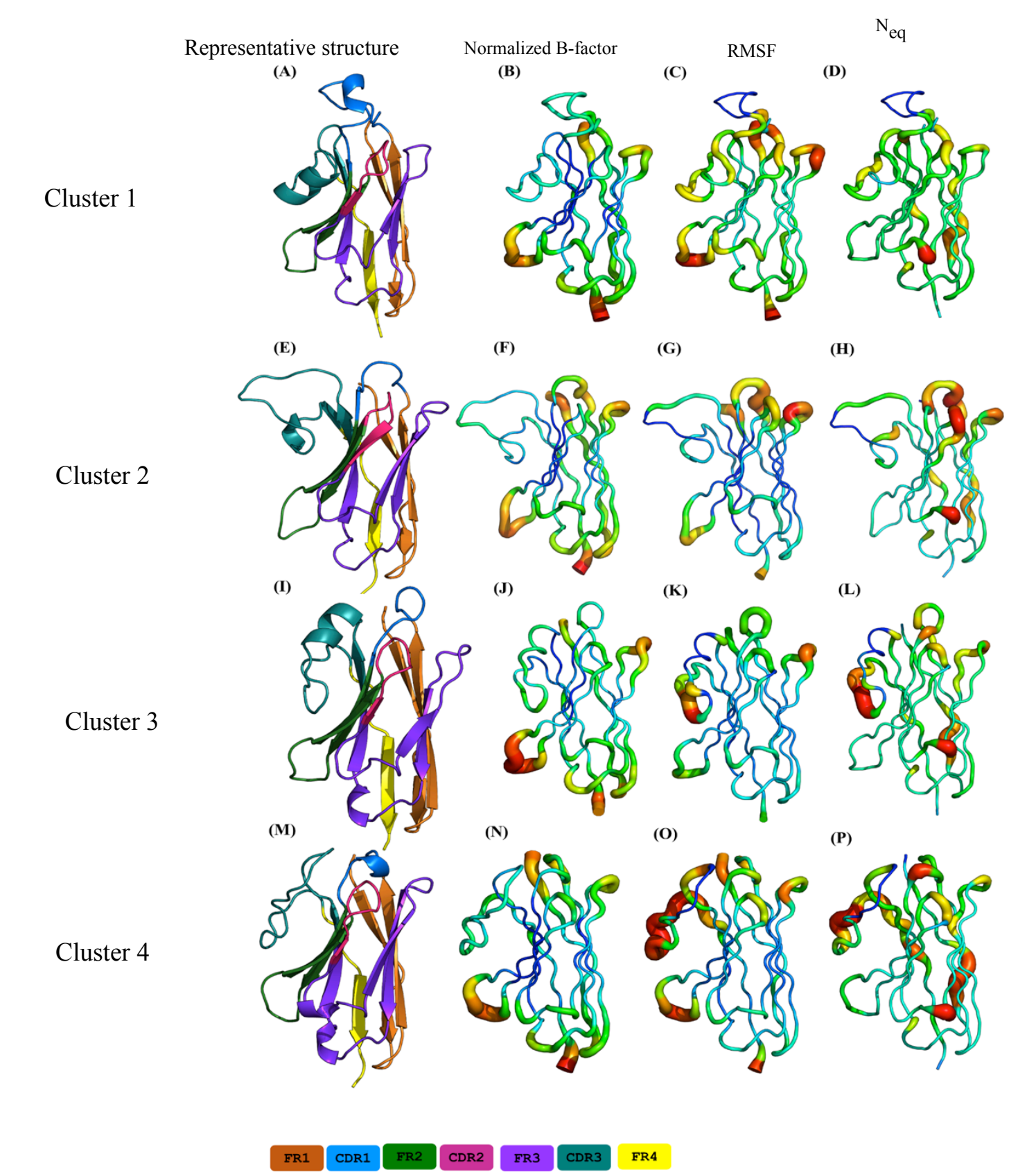
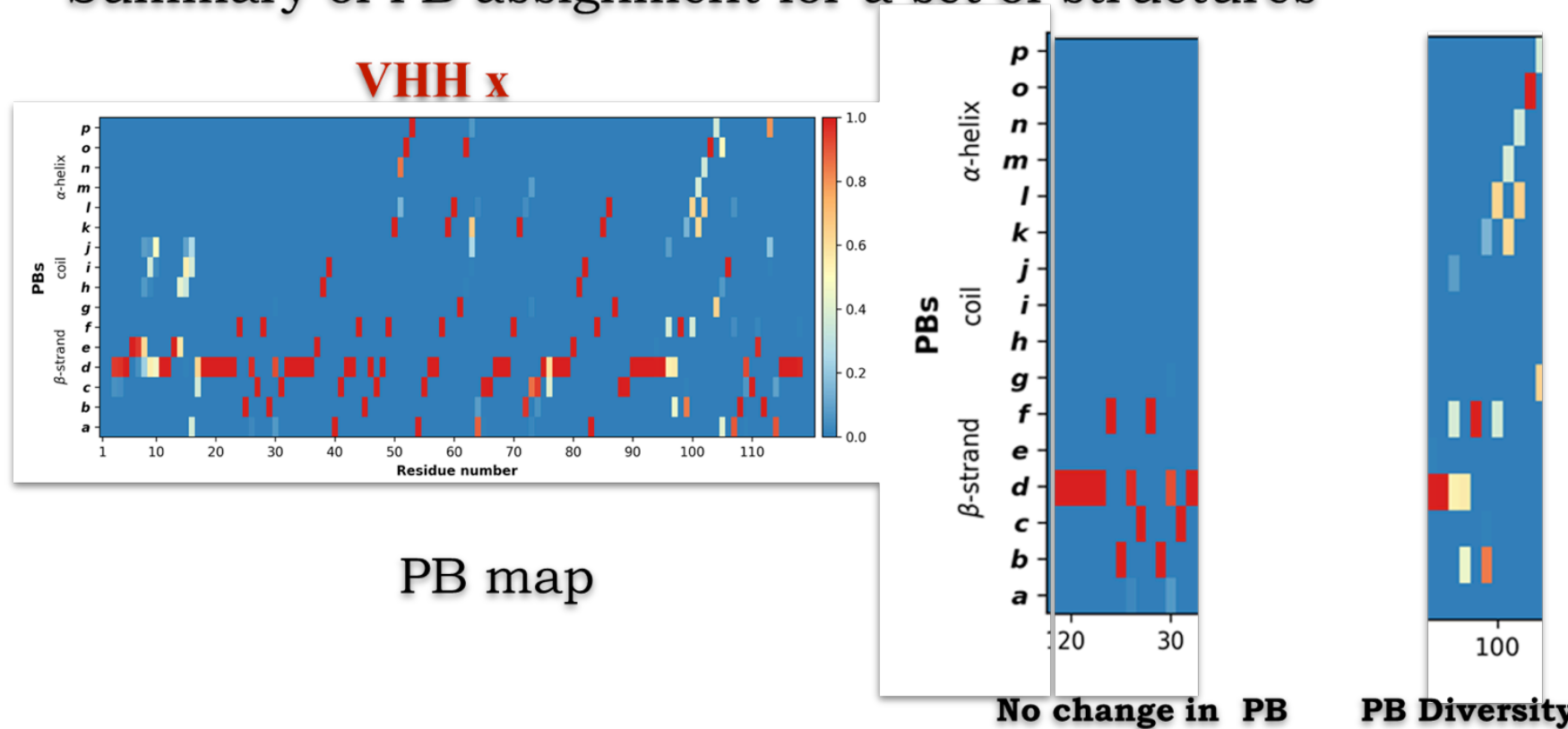


Figure 6: Representation of mean values of flexibility metrics onto a 3D structure of a V_HH from each cluster.

- 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.

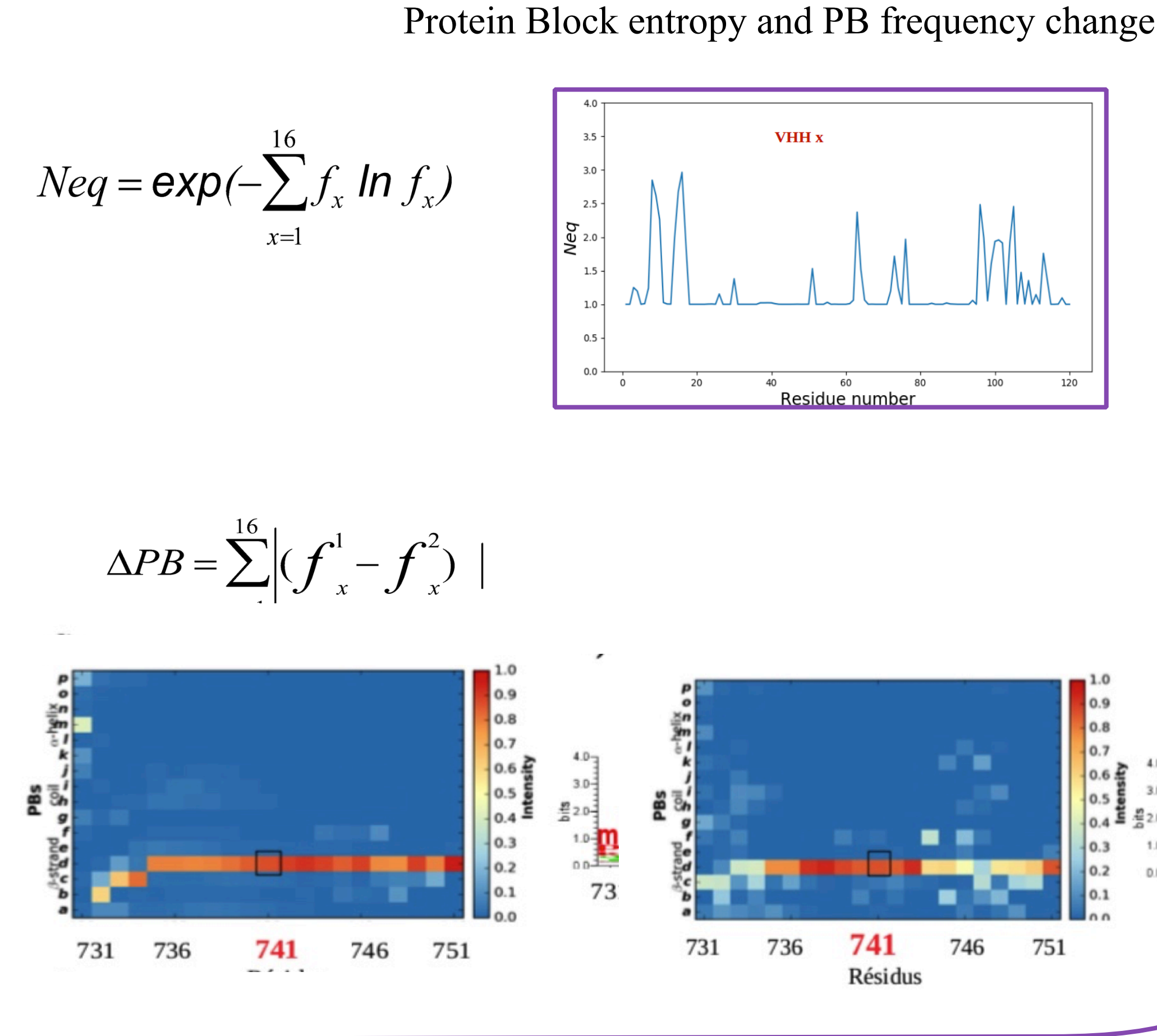


Figure 7: Local backbone diversity at the light of Protein Block maps. (A) PB map of all V_HH trajectories aligned according to MSA. (B) N_{eq} values (red line) for all V_HH trajectories, sky blue - V_HH trajectories belonging to RMSF cluster 1, green - V_HH trajectories belonging to RMSF cluster 2, purple - V_HH trajectories belonging to RMSF cluster 3 and pink - V_HH trajectories belonging to RMSF cluster 4. (C) PB 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 represent 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.

Dataset

Sequence and structural features of 88 non redundant V_HH domains.

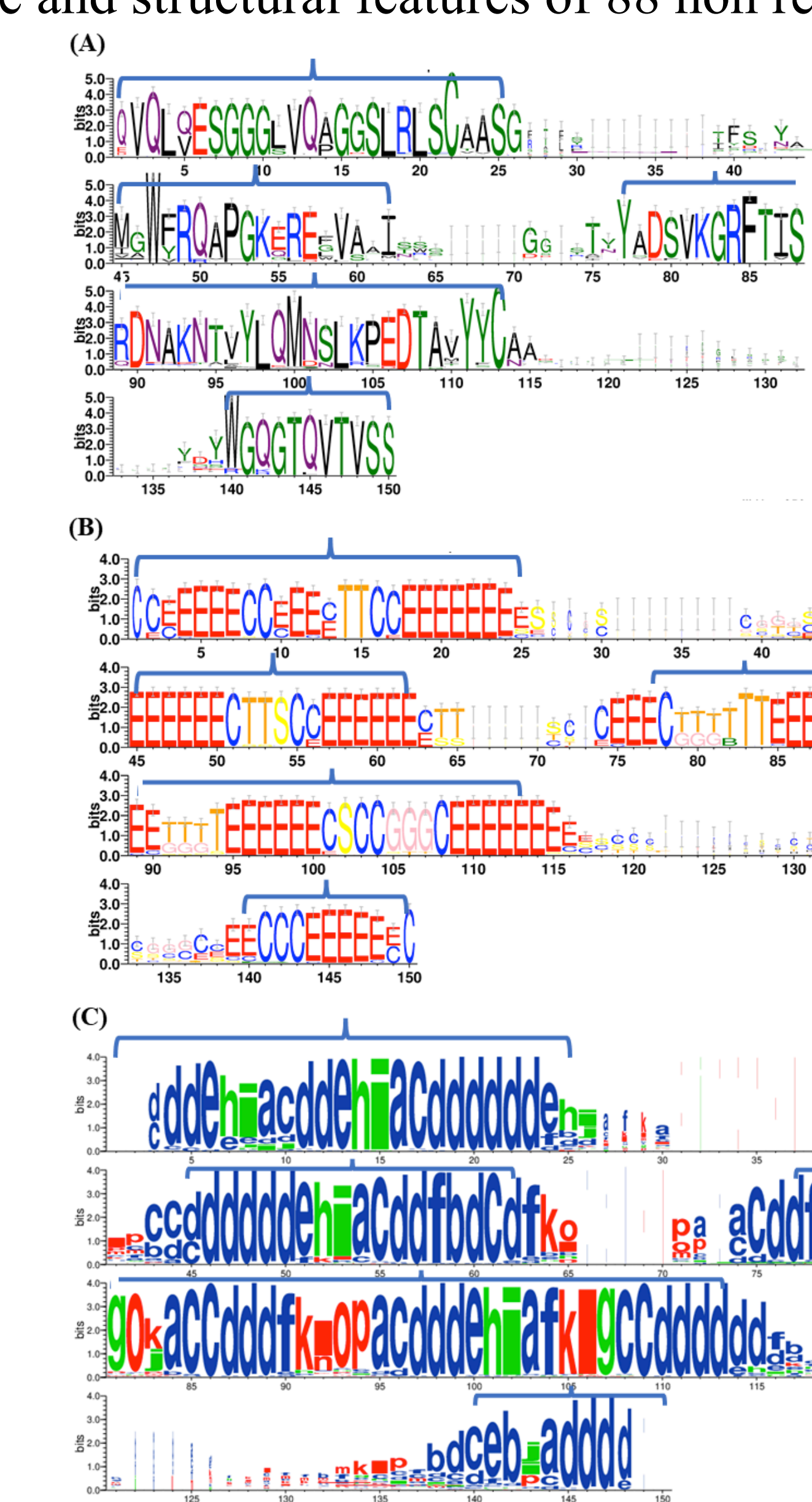


Figure 8: Sequence and structural characteristics in V_HH dataset. Construction of (A) Amino acid residues, (B) Secondary structures, and (C) Protein Blocks. The four Framework regions are delineated in each figure.

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

- This work is supported by the POE FEDER 2014-20 of the Conseil Régional de La Réunion (S3D VHH program, N^o SYNERGIE RE0022962), EU-H2020 and Université de la Réunion.
- A.M.V. acknowledges access granted to high performance computing (HPC) resources at the French National Computing Centre CINES under grants no. A0040710426 and A0110713032 funded by the GENCI (Grand Equipement National de Calcul Intensif).