entropy 2021

Entropy 2021: The Scientific Tool of the 21st Century
3-5 May 2021, Online

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 758588).

Investigating the *structure-dynamics-function* relationship in antibodies

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INTRODUCTION

*Molecular Dynamics (MD) simulations can help to understand the **complex behaviours** observed in proteins. The **characterization of the large-scale dynamics** through MD and the **correlation** between **dynamical properties** and **structural features** is of fundamental importance for the **elucidation** of **biological mechanisms**.*
*The all-atom dynamics also serves as a guide in the **identification** of those **structural patterns** whose preservation is necessary in the construction of simplified, that is **coarse-grained**, models.*
*This study is meant as a starting point for the **application of multi-scale methods** to biologically relevant macromolecules.*

*We simulate with plain MD (4μs) the therapeutic antibody Pembrolizumab [1], an immunoglobulin of the IgG4 family, in either **apo** or **holo** form.*
Here we compare show the results for both systems.

The Antibody

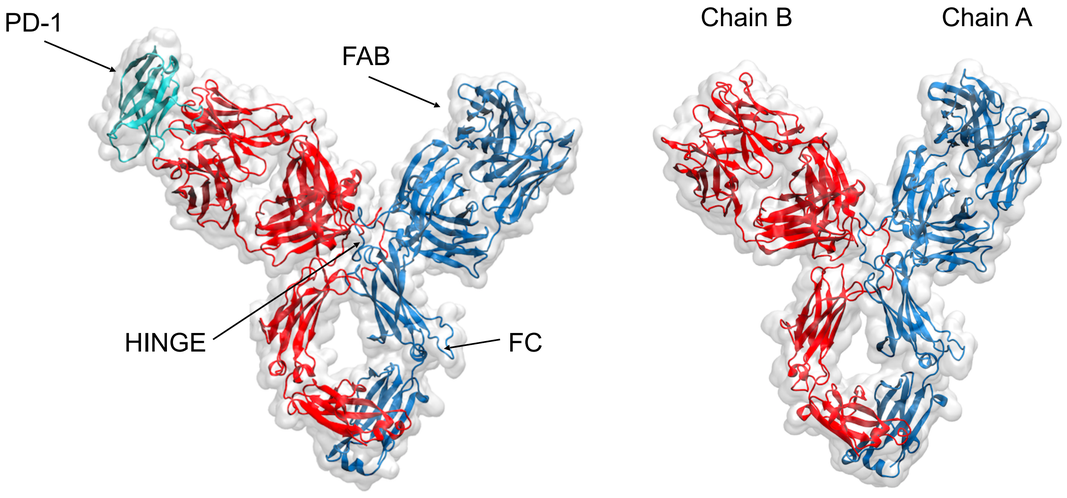


Figure 1 Ribbon representations of the therapeutic antibody Pembrolizumab and its antigen PD-1 (PDB code: 5DK3, 5GGS) and of the apo form.

IgG antibodies are composed by **2 heavy** and **2 light** chains, each of them has constant domains and variable domains which represent the binding region of the antigens.The whole structure can be divided in **Fab**, **Fc** and **hinge regions**.

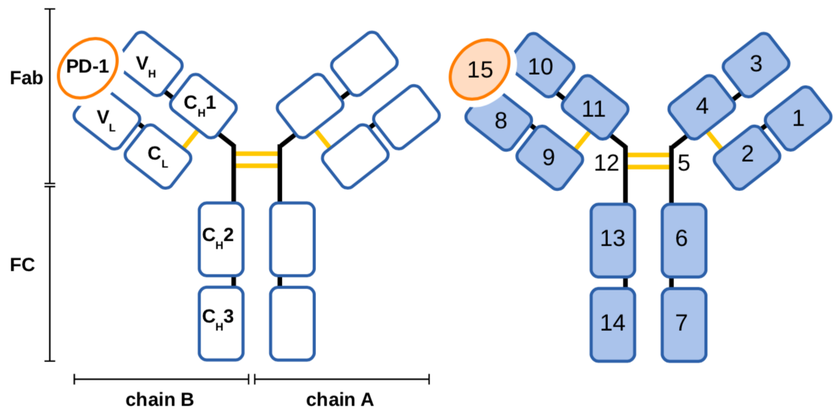
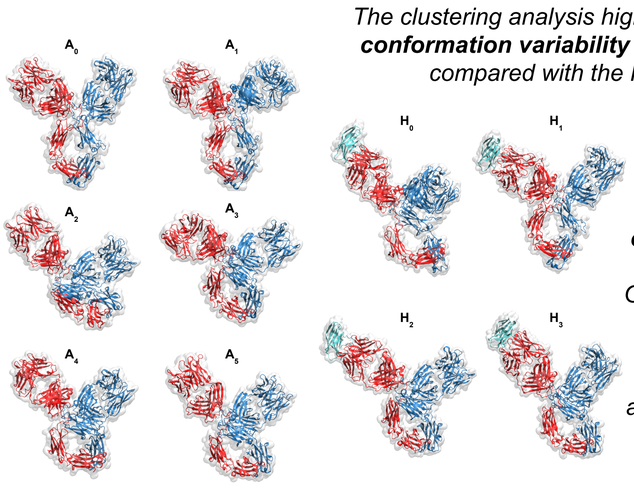


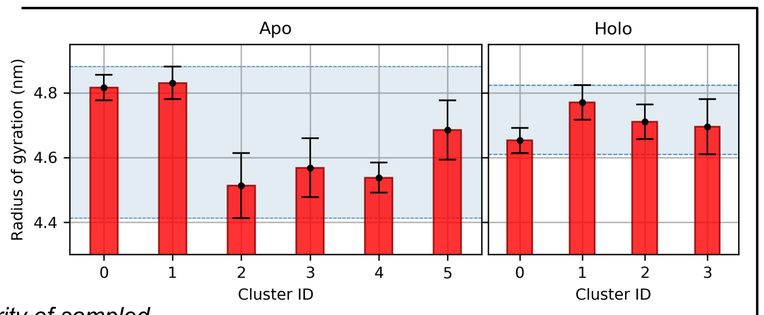
Figure 2 Schematic representation of the antibody structure, in yellow the disulfide bonds are depicted.

The role of monoclonal antibodies (**mAbs**) for therapeutic applications dramatically increased in recent years.
mAabs have been developed to treat a large variety of conditions, including **cancer**, autoimmune diseases and, very recently, COVID-19
Engineering of the antibody sequence is habitually performed to **optimize its therapeutic efficacy.**
MD is used as a valuable tool to assess the effects of these modifications on the antibody structure and to predict the **stability** and the **conformation variability** of the molecule.

Apo and Holo forms display different conformation variability

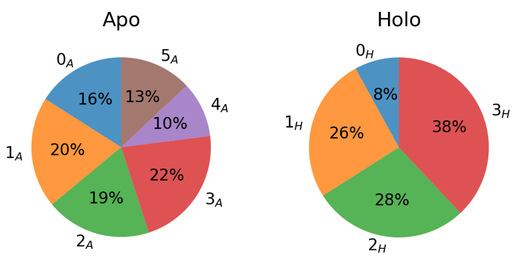
*The clustering analysis highlights a **greater conformation variability for Apo** system compared with the Holo one.*





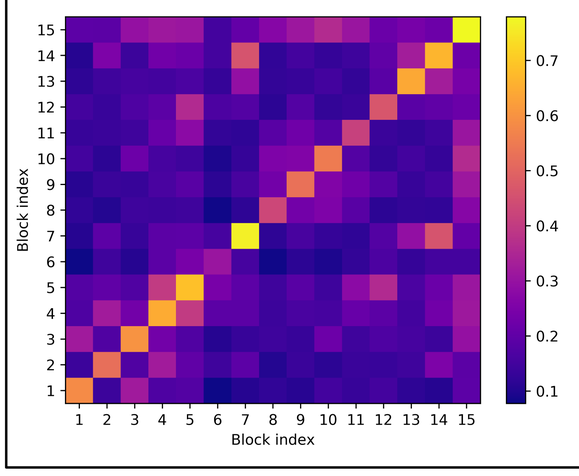
***Apo**: The majority of sampled conformations are represented by **compact structures** where interactions between Fab1 and Fc1 are present. Only two conformations, 4_A and 5_A display an open, **Y shaped structure**.*

***Holo**: Cluster 0_H presents a peculiar arrangement with the Fab1 perpendicular to the plane of Fab2 and Fcs.*



Mutual Information

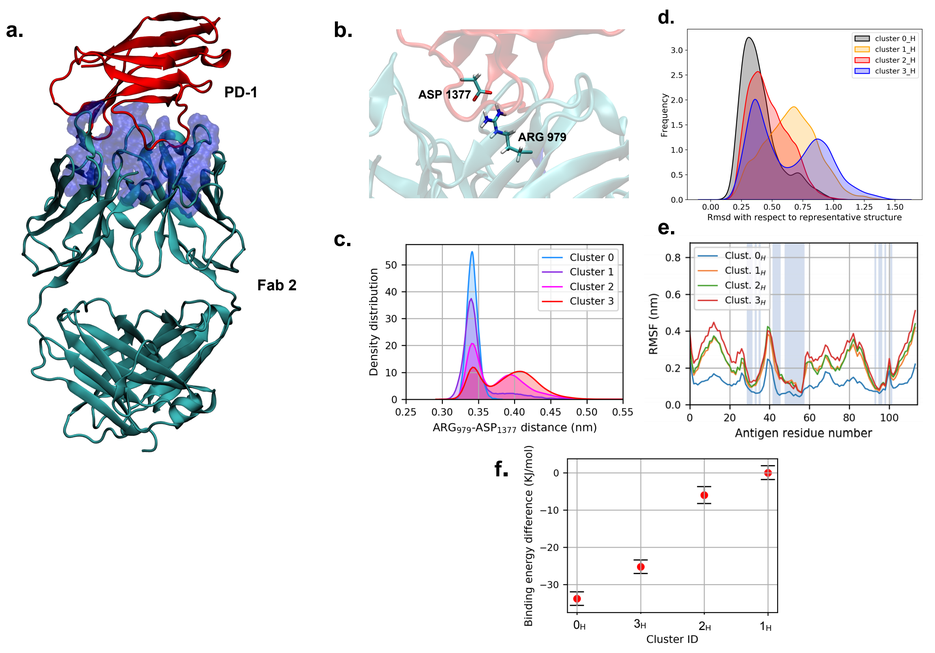
$$I_{i,j} = \iint p(x_i, x_j) \log\left(\frac{p(x_i, x_j)}{p(x_i)\,p(x_j)}\right) dx_i dx_j$$



The Mutual Information is calculated for each pair of C_α atoms.

Each block represents the average over the structural domain.

Binding Modes



***The binding is strengthened when the antibody is in cluster 0_H** resulting in large and stable values of the contact surface area between pembrolizumab and PD-1 (a. b. c.)*

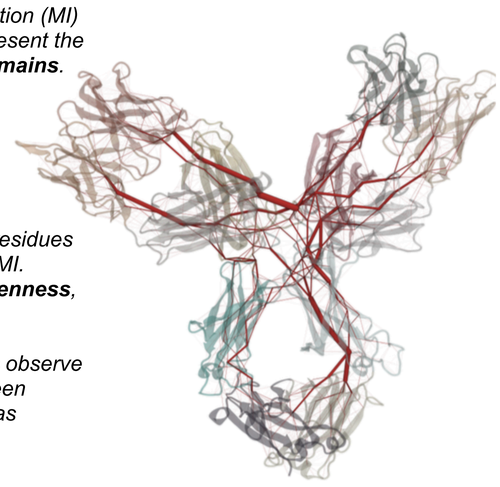
*The RMSF of the antigen molecule reflects the strength of the interactions, showing **the lowest values for cluster 0_H** (e.)*

*The calculation of RMSD in each cluster, with respect to the respective representative structure reveals a **sharper distribution in cluster 0_H** compared to the others, corroborating the hypothesis that the latter is the most stable conformation (d.).*

In the bottom figure (f.) the values of binding enthalpies, obtained with the MM/PBSA approach, are plotted [3].

Network Analysis

*On the basis of Mutual Information (MI) we built a **network** [2] that represent the **connection between the domains**.*



The network is composed by residues and it is weighted by the MI.
*We computed the edge **betweenness**, represented in figure.*

*From this graph it is possible to observe the communication between **Fab1** and **Fab2** as well as between **Fab1** and **Fc**.*

Conclusions

- *We observe a large conformational variability, especially in the apo form. The holo system exhibits a more uniform behaviour than the apo one, suggesting a correlation with the antigen binding.*
- *The network analysis based on information theory gives information about the communication between the protein domains.*
- *Cluster 0_H shows the highest structural uniformity, therefore the most stable binding mode.*

Perspectives

- *Simulation of the glycosylated antibody to investigate the differences, in terms of dynamical properties, with respect to the unglycosylated systems.*

References
[1] Scapin G., Yang X., Prosise W. W., McCoy M., Reichert P., Johnston J. M., Kashi R. S. & Strickland C. (2015). Structure of full-length human anti-PD1 therapeutic IgG4 antibody pembrolizumab. Nat Struct Mol Biol, 22(12):953-8.
[2] M. C. Melo, R. C. Bernardi, C. De La Fuente-nunez, and Z. Luthey-Schulten, "Gen- eralized correlation-based dynamical network analysis: a new high-performance approach for identifying allosteric communications in molecular dynamics trajecto- ries," The Journal of Chemical Physics, vol. 153, no. 13, p. 134104, 2020.
[3] R. Kumari, R. Kumar, O. S. D. D. Consortium, and A. Lynn, "g_mmpbsa: A gromacs tool for high-throughput mm-pbsa calculations," Journal of chemical in- formation and modeling, vol. 54, no. 7, pp. 1951–1962, 2014.