

High-Throughput Characterization of Antibody-Antigen Interactions Under Mechanical Stress

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Abstract (10 lines)*

B-cell recognition of pathogens relies on interactions between surface-expressed antibodies and antigens, often studied at equilibrium but shaped in vivo by mechanical stresses, especially during antibody evolution. Understanding these interactions is challenging due to antibody diversity and the need for specialized methods. We will develop a platform combining high-throughput yeast-display with automated fluidic assays to measure interactions under varying mechanical stress, benchmarked against our existing single-molecule antibody datasets. Coupled with sequencing, this enables parallel affinity measurements across thousands of antibodies. Experiments will be complemented by modeling antibody evolution and using AI-based structural embeddings to build predictive models. The goal is to generate datasets and computational tools to advance our understanding of antibody-antigen recognition and evolution, with applications in vaccine design and therapeutic antibody screening.

Keywords*

Antibody, fluidic assays, yeast display, antigens, affinity maturation, evolution, single-molecule

Scientific question and Objectives (10 lines)*

B cells recognize antigens on dendritic cells via a mechanical "tugging" process, absent from traditional in vitro assays. Preliminary evidence suggests these forces strongly influence affinity maturation, where antibodies improve their antigen-binding properties post-infection. Yet, no platform allows high-throughput quantification of these interactions. This project asks: **How do mechanical forces govern antibody-antigen binding dynamics and evolutionary trajectories during affinity maturation?**

We will develop a high-throughput platform combining yeast-display libraries and automated fluidic assays to measure antibody-antigen interactions under controlled mechanical stress. This platform will generate a dataset of force-dependent binding parameters, validated against single-molecule force spectroscopy. We will integrate these data into computational models of antibody evolution to explore how mechanical constraints shape them. Finally, AI-driven structural embeddings will enhance our predictive understanding.

*: Mandatory



Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

We will develop a **high-throughput platform** to apply controlled mechanical forces to antibody **yeast-display libraries**, enabling the quantification of force-dependent antibody-antigen interactions. Yeast displaying antibodies will be **flowed over antigen-coated slides under varying fluidic forces**, and those remaining bound, indicating stronger affinity, will be collected and sequenced. By iterating this process across a range of forces, we will map the binding properties of thousands of antibodies. This microfluidic approach was previously used with antibody-coated beads, the objective is to scale it up with yeast display and sequencing. We will begin with existing libraries, already displayed on yeast, including a SARS-CoV-2 antibody panel and a library of influenza broadly neutralizing antibodies. These libraries will demonstrate and test the platform's capabilities, but the objective is to build dedicated libraries, particularly of antibody with complex evolutionary patterns (antibodies targeting self-antigens, antibodies with cross-reactivities).

To complement these experiments, we will **simulate affinity maturation using population dynamics models**, such as the Wright-Fisher model with selection. These simulations will help us determine what is the influence of mechanical forces on antibody evolution. We will further enhance our analysis by integrating **AI-based structural embeddings**, such as AlphaFold3 and ESM, to be able to predict these mechanical properties of antibody-antigen interactions, at least for specific antigens.

The platform will be built and benchmarked in the first year. Different antibody libraries will be generated and measured in the second year as initial modeling start. The final year will focus on completing the modeling and writing the PhD thesis. This project builds on our teams expertise in yeast display, computational immunology, and microfluidics.

Interdisciplinarity and Implication of the two labs (15 lines)*

(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

The laboratory of Thomas Dupic at CIML will lead the yeast-display and computational modeling aspects of the project. Dupic has developed high-throughput yeast-display methods for measuring antibody-antigen interactions at equilibrium and now focuses on how antibody repertoires shape immune responses. His background in statistical physics and machine learning for protein modeling will drive the design of antibody libraries, sequencing data analysis, and integration of AI-based structural embeddings to interpret force-dependent binding.

The laboratory of Philippe Robert at LAI will develop the microfluidic assays, leveraging their expertise in fluidic platforms for studying antibody-antigen interactions. Robert's lab has previously used antibody-coated beads under mechanical stress, providing the technical foundation to adapt these methods for yeast-displayed antibodies.

This partnership is essential: Dupic's team brings tools for large-scale antibody dataset generation and analysis, while Robert's lab provides the microfluidic infrastructure to apply and quantify mechanical forces. Together, they bridge biology, biophysics, and computational immunology, aligning with CENTURI's interdisciplinary mission.

*: Mandatory



Specify with whom the person recruited will collaborate and on what aspects *

The recruited PhD student will work closely with Thomas Dupic's team on the design and construction of yeast-display antibody libraries, high-throughput sequencing, and development of computational pipelines for data analysis. Thomas Dupic will also supervise the computational modeling and machine learning aspects.

In parallel, they will collaborate with Philippe Robert's group to optimize the microfluidic assays, including experimental setup, force calibration, and physical interpretation of binding dynamics on antigen-coated surfaces.

PhD student's expected profile*

We are looking for a PhD student with a background in biology and experience in running biological experiments, ideally with some exposure to biophysics. Or alternatively a physics student with a strong interest in biology. The student should be interested in developing computational skills, but the project offers flexibility in the computational direction, whether focusing on physics-based modeling of antibody evolution or machine-learning approaches for structural and binding predictions, depending on the student's interests and skills. A willingness to learn and combine computational methods and experimental work is key.

Is this project the continuation of an existing project or an entirely new one?

In the case of an existing project, please explain the links between the two projects (5 lines)*

The project is an entirely new one.

Two to five references related to the project*

1. Phillips, A.M., Lawrence, K.R., Moulana, A., Dupic, T., Chang, J., Johnson, M.S., Cvijovic, I., Mora, T., Walczak, A.M., Desai, M.M., 2021. Binding affinity landscapes constrain the evolution of broadly neutralizing anti-influenza antibodies. *eLife* 10, e71393. <https://doi.org/10.7554/eLife.71393>
2. Sanicas, M., Torro, R., Limozin, L., Chames, P., 2024. Antigen density and applied force control enrichment of nanobody-expressing yeast cells in microfluidics. *Lab Chip* 24, 2944–2957. <https://doi.org/10.1039/d4lc00011k>
3. Spillane, K.M., Tolar, P., 2017. B cell antigen extraction is regulated by physical properties of antigen-presenting cells. *J Cell Biol* 216, 217–230. <https://doi.org/10.1083/jcb.201607064>
4. Jiang, H., Wang, S., 2023. Immune cells use active tugging forces to distinguish affinity and accelerate evolution. *Proceedings of the National Academy of Sciences* 120, e2213067120. <https://doi.org/10.1073/pnas.2213067120>

Two main publications from each PI over the last 5 years*

1. **Dupic, T.**, Bensouda Koraichi, M., Minervina, A.A., Pogorelyy, M.V., Mora, T., Walczak, A.M., 2021. Immune fingerprinting through repertoire similarity. *PLoS Genet* 17, e1009301. <https://doi.org/10.1371/journal.pgen.1009301>
2. Moulana, A., **Dupic, T.**, Phillips, A.M., Chang, J., Roffler, A.A., Greaney, A.J., Starr, T.N., Bloom, J.D., Desai,



M.M., 2023. The landscape of antibody binding affinity in SARS-CoV-2 Omicron BA.1 evolution. *eLife* 12, e83442. <https://doi.org/10.7554/eLife.83442>

3. **Robert, P.**, Biarnes-Pelicot, M., Garcia-Seyda, N., Hatoum, P., Touchard, D., Brustlein, S., Nicolas, P., Malissen, B., Valignat, M.-P., Theodoly, O., 2021. Functional Mapping of Adhesiveness on Live Cells Reveals How Guidance Phenotypes Can Emerge From Complex Spatiotemporal Integrin Regulation. *Front. Bioeng. Biotechnol.* 9. <https://doi.org/10.3389/fbioe.2021.625366>

4. Pettmann, J., Awada, L., Różycki, B., Huhn, A., Faour, S., Kutuzov, M., Limozin, L., Weikl, T.R., van der Merwe, P.A., **Robert, P.***, Dushek, O.*, 2023. Mechanical forces impair antigen discrimination by reducing differences in T-cell receptor/peptide-MHC off-rates. *EMBO J* 42, e111841. <https://doi.org/10.15252/emboj.2022111841> (* co-last authors)

Project's illustrating image

