Topographical structure, nanomechanics and nanosurgical manipulation of biological supramolecular systems

PhD thesis booklet

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1. Introduction

1.1. Supramolecular assemblies

Biomolecular systems are constructed from a surprisingly small number of low-atomic-number elements. Yet, via their combination into polymers, a myriad of different molecules is built which can further associate into supramolecular systems of remarkable elegance, beauty and abilities. With the advent of special biophysical tools, such as the atomic force microscope (*AFM*), we have the ability to investigate not merely the structure, but also the internal associations and the mechanical features of these supramolecular biological systems. During my doctoral studies I had the unique opportunity to explore various biomolecular and supramolecular systems and gain special insights that allowed us to develop novel theoretical understanding.

1.2. Muscle biophysics

Muscle serves the locomotion of the different parts of an organism. The basic functional and structural working unit of muscle is the sarcomere (3). Sarcomeres have to provide controlled force generation and structural integrity of the muscle. This is achieved by the multiple, sequentially positioned supramolecular assemblies. The most prominent of these are the myosin thick filaments, the actin thin filaments (4). To fix these filaments and keep them functional, many other assemblies and proteins are present such as the Z-band at the ends of each sarcomere, the M-band in the middle of the sarcomere and giant proteins like titin and nebulin. During my PhD, I conducted research and experiments mainly on myosin, titin and the M-band (**Figure 1**).

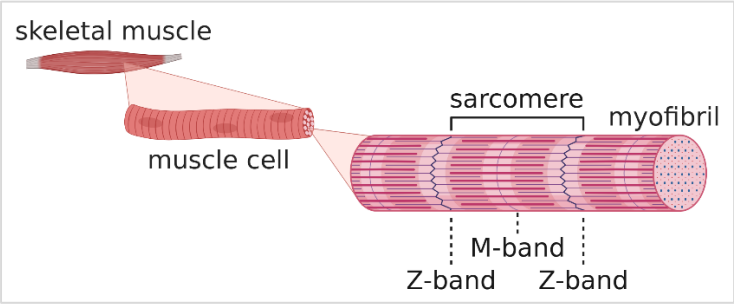

Figure 1. *Structural hierarchy of the muscle and the sarcomere. (Edited with BioRender.com)*

1.3. Coronavirus biophysics

Viruses are high level supramolecular assemblies, as they contain proteins, lipids and genetic material in a very compact structural unit, called the virion (5). Coronaviruses are single-stranded RNA viruses with a genome size ranging between 25 and 32 kilobases, encoding structural and non-structural proteins. The beta-coronavirus genus includes severe

pathogens such as SARS-CoV, MERS-CoV, and SARS-CoV-2, which have caused significant epidemic and pandemic outbreaks in humans. During my research, I studied SARS-CoV-2.

From a biological point of view, the role of a coronavirus particle is to protect and carry the viral genome, escape from the host cell and then enter another host cell (6). Every component of the virion serves this lifecycle. The proteins of the virion are encoded by the viral genome. We distinguish two types of proteins: structural and nonstructural proteins (nsp). The structural proteins are the spike, the envelope (E), the membrane (M) and the nucleocapsid (N) proteins (**Figure 2a**). Nonstructural proteins are encoded in ORFs of the coronavirus genome, and are responsible for tasks like RNA replication, cell membrane alteration and disruption of the host cell (**Figure 2b**).

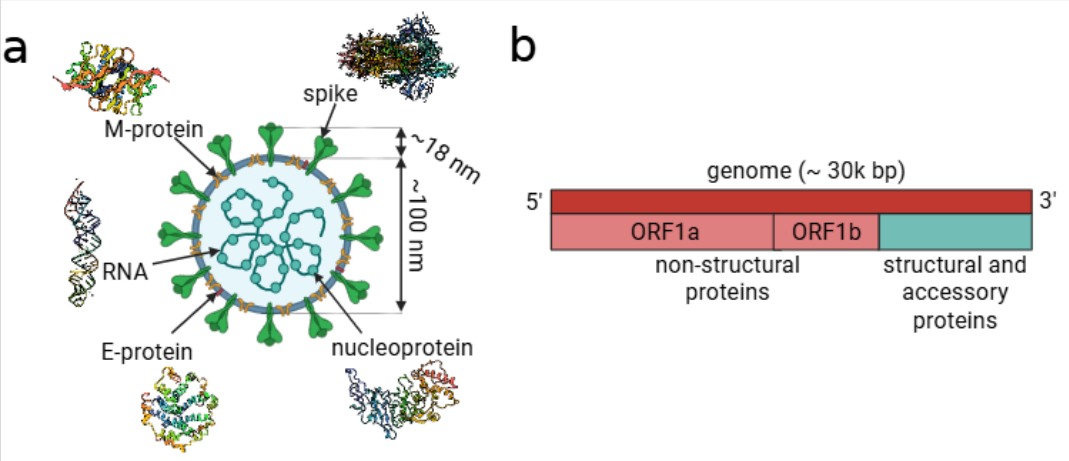

Figure 2. *Schematics of SARS-CoV-2.* ***(a)*** *An illustration of the basic form of a coronavirus particle, with the structural proteins and genetic material highlighted. The approximate scales are indicated.* ***(b)*** *The genome of SARS-CoV-2. (Edited with BioRender.com)*

SARS-CoV-2 research has mainly focused on the S protein and its interaction with host receptors, given its major role in viral fitness. However, mutations in other structural and nonstructural proteins (nsp) also affect fitness, although they are less studied. These components may act synergistically, amplifying each other's effects.

While S–ACE2 interactions are easily studied through assays and simulations, such a narrow focus misses the full picture. The nanomechanical properties of the virion arise from the combined contributions of all proteins and the lipid envelope. It is known that structural differences of virion membranes affect the infection. Understanding these mechanics could reveal deeper insights into viral behavior and differences between variants.

1.4. Atomic Force Microscopy

AFM is a powerful tool for studying biological samples at the molecular level. It uses a cantilever with a sharp tip to image structures at high resolution, revealing protein

conformations, and the surfaces of cells, bacteria, viruses, and other biomolecular assemblies (7) (**Figure 5a**). Beyond imaging, AFM measures nanomechanical properties like elasticity and adhesion force, offering insight into mechanical stability and interactions at the piconewton scale. With the right modality and device, it can also track dynamic processes—such as protein folding or molecular binding—in real time under near-physiological conditions (**Figure 3a**).

We named our method *nanosurgery* to reflect its mechanical mode of action (**Figure 3b**). With the AFM tip we disrupt and unfold protein structures directly on the surface, allowing us to probe the mechanical properties of complexes in a way that better reflects in vivo conditions than traditional single-molecule studies. *Nanosurgery* serves as a lateral-force counterpart to classical single-molecule force spectroscopy.

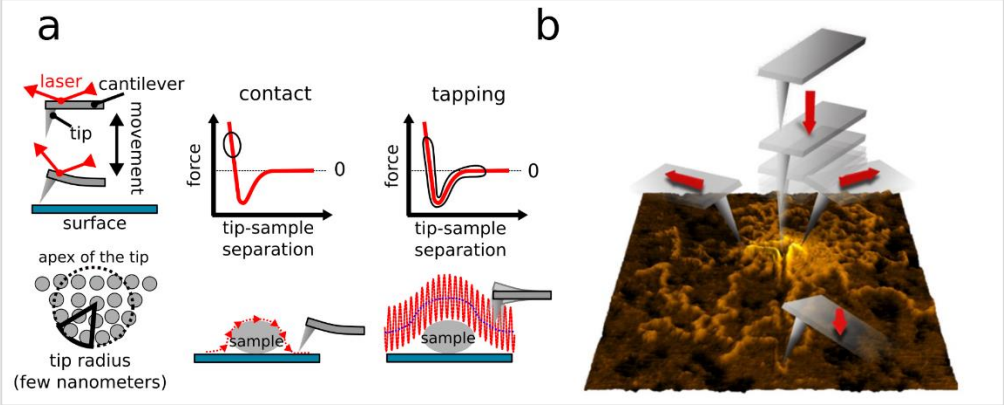

Figure 3. *Imaging and nanosurgery with atomic force microscopy (AFM).* ***(a)*** *AFM cantilever, its function and imaging modalities.* ***(b)*** *Nanosurgical manipulation of sample.*

The method involves sequential steps: first, the AFM tip is lowered into the center of the surface-bound complex with minimal force to avoid indenting the surface and create debris. This pN-scale force is not sufficient to damage the mica due to its high (~GPa) Young's modulus. The tip is then moved laterally under with parameters such as speed and direction varied to explore the mechanical response.

2. Objectives

Generally, my research was the structural study and characterization of different nanomolecular structures. The experimental methodology that these projects shared was AFM. The specific goals were:

1. To explore the topographical structure of the synthetic *myosin II* thick filament and its interaction with *titin* and the M-band derived *M-complex*.
2. To investigate the topological structure of the sarcomeric *M-complex*
3. To dissect the *M-complex* with nanosurgical methods.
4. To uncover the topographical properties of *SARS-CoV-2* variants.
5. To model the elastic behavior of the *SARS-CoV-2* virion.

3. Methods

To acquire the molecular samples for the experiments, we conducted several isolation/purification protocols for both muscle proteins and coronavirus. Here, I provide an overview of the general steps for sample preparation.

3.1. Titin and M-complex

We isolated the proteins from rabbit back muscle, then homogeneized it mechanically with a high concentration of protease inhibitors (1). Through multiple centrifugation/dilution and incubation steps, we finally put the sample on a high aspect-ratio column chromatography. This resulted in multiple fractions of the sample, from which we could select the fractions that contained specific molecular species (**Figure 4**).

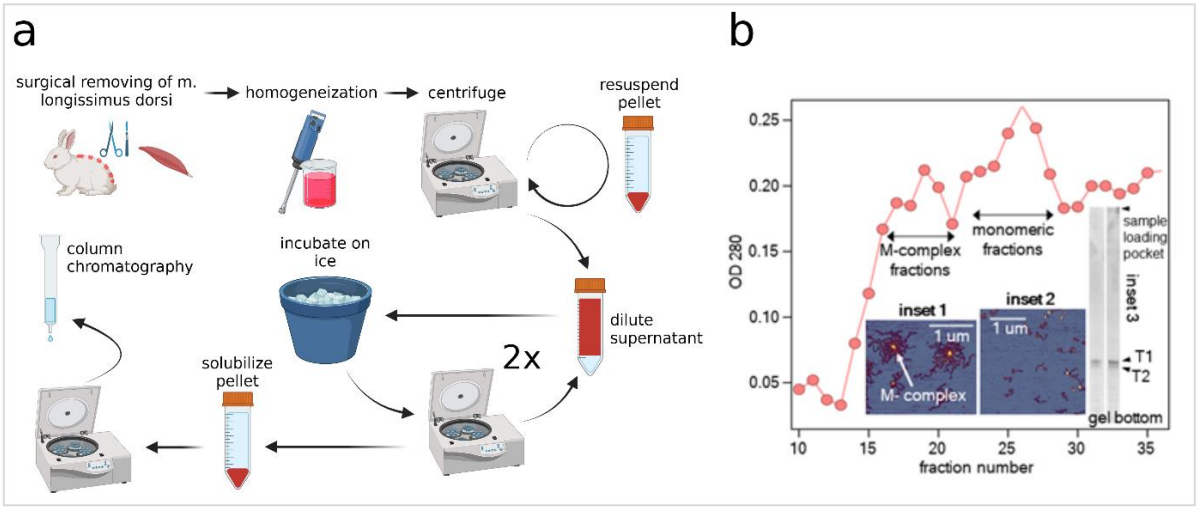

Figure 4. *Isolation of titin and the sarcomeric M-complex.* ***(a)*** *Schematic representation of the isolation steps.* ***(b)*** *The OD280 values of fractions during sample preparation, the two characteristic fractions are shown in inset 1 and 2. (**Inset 3**.) shows the SDS agarose (0.8%) gel profiles of purified titin samples. Left and right lanes are representative for M-complex and monomeric titin fractions, respectively. (Edited with BioRender.com, (b) is adapted from (1))*

3.2. Myosin II

Myosin II was purified from rabbit m. longissimus dorsi following established protocols using cycles of precipitation and dissolution at low and high ionic strengths (2) (**Figure 5**).

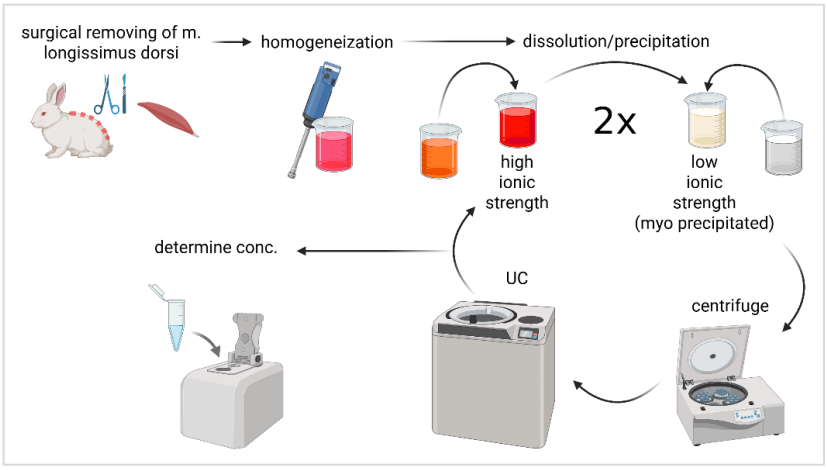

Figure 5. (Edited with BioRender.com)

3.3. SARS-CoV-2

All virus preparation steps were performed under biosafety level 3 (BSL-3) conditions at the National Biosafety Laboratory, National Public Health Center, Budapest, Hungary (8). SARS-Cov-2 variants used in this study were: *wild type*, *alpha* variant and *delta* variant obtained from the Hungarian National Collection of Highly Pathogenic Viruses. The variants were isolated from oropharyngeal swab of confirmed COVID-19 patients identified by RT-PCR.

4. Results

4.1 M-complex characterization from it's topography.

We characterized the M-band derived M-complex topography on the surface (1). We hypothesized how this structure remains after protein isolation and how it might carry some information from the original M-band molecular layout (**Figure 6**).

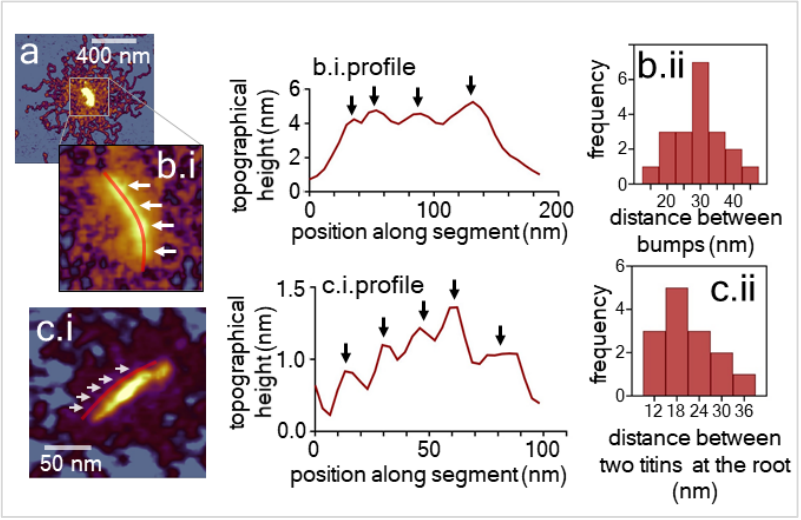

Figure 6. ***(a)*** *A single M-complex.* ***(b.i)*** *The finer structure reveals closely spaced bumps; a section of topography is showed on* ***b.profile****.* ***(b.ii)*** *The distribution shows the inter-bump distances.* ***(c.i)*** *The follow-up of titin molecules allows to measure the inter-titin distances at their roots.* ***(c.i.profile)*** *A section of such a topography, and the* ***(c.ii)*** *presents the inter-titin distance distribution. (Adapted from (1))*

4.2. Nanosurgery of titin.

The nanosurgery of titin molecules allowed us to explore the possibilities of the technique and guided us on how to interpret results (1). The resulting displacement and possible protein unfolding definitely depends on the manipulation speed. Moreover, we optimized a useful displacement model for analyzing the displacement of the filaments on the surface (**Figure 7**).

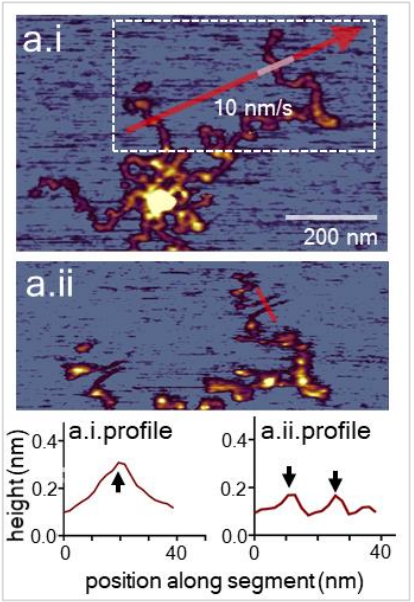

Figure 7. *Nanosurgery of titin molecules.* ***(a.i)*** *and* ***(a.ii)*** *Cutting through single titin molecules perpendicularly. The topography of the red segments is shown on* ***a.i.profile*** *and* ***a.ii.profile****, before and after respectively. (Adapted from (1))*

4.3. Myosin interaction with titin and M-complex.

Under conditions allowing configurational relaxation, titin oligomers appeared to bind the surface of preformed thick filaments, forming a mesh-like layer (2). Our observations suggest that titin does not serve as a geometric template but may regulate thick filament length indirectly, by forming a structural wrapping (scaffold) that restricts filament growth and stabilizes filament ends (**Figure 8**).

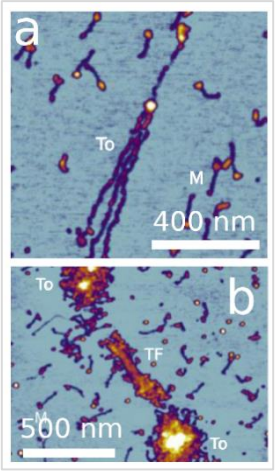

Figure 8. *Topography of the interaction between myosin and the titin M-complex.* ***(a)*** *Appearance of stretched M-complex mixed with single myosin II molecules (To = M-complex, M = myosin).* ***(b)*** *Possible interaction between M-complexes and myosin thick filaments (To = M-complex, TF = Thick filament). (Adapted from (2))*

4.4. SARS-CoV-2 variant comparison

The vesicle model, together with the corona layer provided interesting insights into the geometrical traits for each variants (8). Variants are definitely smaller in size compared to the *wild type* coronavirus. Our defined geometrical parameters and ratios emphasize the structural optimization of the virus focusing on the virus-host interaction (**Figure 9**).

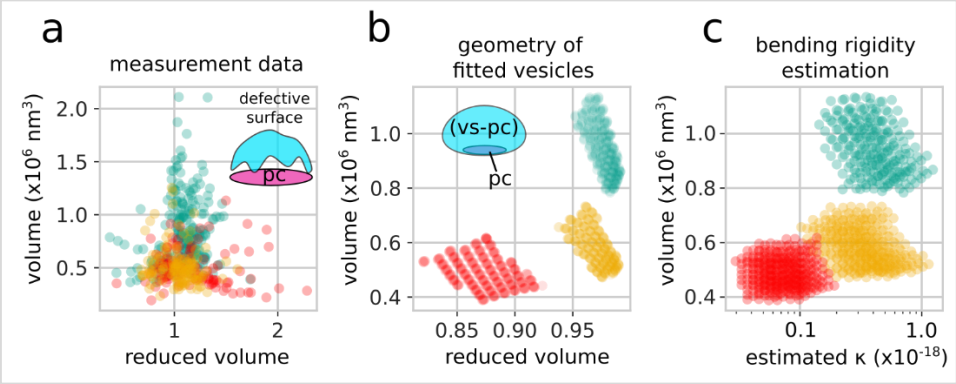

Figure 9*. Overview of the study and numerical results. Reduced volume is a dimensionless quantity expressing how closely a 3D object resembles a perfect sphere. The smaller the, the more deflated the vesicle is. Its maximal value is 1.* ***(a)*** *Illustration of how unreliable the original reduced volume values are, since the measured surface and projected areas are too noisy and can even miss surfaces (due to mask cutoff) on the sides of the virions* ***(inset)****.* ***(b)*** *After fitting the model on means per variant, the geometrical data becomes meaningful; furthermore, it can be used to estimate the bending rigidities* ***(c)****. Estimation results for each variants (color coding is teal=wild, red=alpha, orange=delta). (Adapted from (8))*

5. Conclusions

The thesis explored a wide spectrum of AFM biological experiments on the mesoscale, from single molecules to more complex multimolecular structures, exploring the self-, intermolecular- and surface interactions of these molecular species. One part of the thesis concentrated on striated muscle specific myosin II molecules and titins, as well as on the larger, sarcomere derived M-complex. The other part of the thesis focused on the structure of SARS-CoV-2, an enveloped virus responsible for the recent pandemic. The common characteristic of the observed multimolecular specimens is how the different components produce a generative effect when they assemble into complexes. Symmetry, stability and function emerges from individual, non-symmetric elements. Moreover, this emergent structure is incorporated in a dynamic biological and physical landscape of immense scale. As opposed to a pure reductionist approach, my research tried to grasp these molecular systems as a whole. The work reflects the advantages and disadvantages of such an approach (e.g. observing continuum mechanical phenomena emerging from a molecular complex). Our approach proved that the system-scale experimentation allows to uncover physical properties that remain hidden when only the components are observed.

We showed that the M-complex is a stable, titin-based structural hub, consistent with M-band organization. Despite the absence of myosin, the measured spacing and lattice patterns resemble physiological M-band arrangements. Our nanosurgery approach enabled controlled mechanical manipulation, revealing domain-level behavior and filament dynamics. Manipulated titin filaments exhibited structural deformation consistent with unfolding and showed velocity-dependent mechanical responses. The shape and displacement of these filaments were accurately described using a continuum mechanical model.

AFM nanosurgery of the M-complex demonstrated both plastic and elastic responses and revealed a compact filament reservoir capable of releasing extensible strands. The M-complex appears to store elastic energy, likely due to surface-induced deformation and kinetic trapping. Observed necking and post-manipulation fixation suggest ductile-like behavior at the mesoscale. Shortened titin extensions and spatial constraints indicate restricted equilibrium in the complex interior. Theoretical comparisons with Bell model and in-plane force loading help explain rupture patterns near the pulling tip.

We observed how the environmental conditions (buffer concentration) oblige single myosin-II molecules to form rudimentary thick filaments. These thick filaments resembled the in-vivo forms, but without spatial restrictions which led to myosin assemblies different from the assumed sarcomeric layout. We concluded that, for proper thick filament formation, more

components are required. Based on the in-vivo topological proximity and interconnection of titin and M-complex with the thick filaments, we explored how M-complex interacts with the Myosin-II molecules.

Notably, titin and myosin did not co-assemble under standard conditions, but titin oligomers bound thick filaments after relaxation. These findings suggest titin's role is more regulatory than templating, contributing to filament length control through structural confinement.

Methodological limitations, particularly in lateral force calibration and tip-sample geometry, highlight the need for more refined tools. Addressing these challenges could improve force quantification and dynamic modeling. Future work should integrate molecular dynamics simulations to resolve local interactions and structural transitions. Coarse-grained and atomic models could provide bottom-up insight into nanosurgical manipulation.

Nanosurgery offers a powerful platform for probing protein mechanics and spatial interactions at single-molecule resolution. As molecular-level precision becomes increasingly relevant in nanotechnology and biomedical research, techniques like this will be vital. Our results underline the M-complex's mechanical complexity and support its role in sarcomeric force transmission and structural maintenance.

Our AFM-based analysis revealed distinct structural and mechanical differences among SARS-CoV-2 variants. The alpha variant showed the lowest height and volume, while the wild type exhibited the largest values across all measured geometric parameters, with delta consistently in between. These differences suggest that virion morphology varies meaningfully between variants, possibly influencing infectivity or environmental stability.

Geometric modeling using a vesicle-plus-corona approach allowed detailed comparisons of surface area usage. Normalized ratios provided insight into how effectively each variant may engage with host cells, with alpha and delta showing more infection-optimized geometries than the wild type.

Finally, by fitting vesicle models to the virion shapes, we estimated bending rigidity (κ), finding the wild type to have the highest membrane stiffness, followed by delta and alpha. These nanomechanical differences may reflect underlying structural or compositional adaptations in each variant, contributing to their distinct biological behaviors.

6. References

1. Sziklai D, Sallai J, Papp Z, Kellermayer D, Mártonfalvi Z, Pires RH, et al. Nanosurgical Manipulation of Titin and Its M-Complex. Nanomaterials (Basel). 2022;12(2):178.

2. Kellermayer M, Sziklai D, Papp Z, Decker B, Lakatos E, Mártonfalvi Z. Topology of interaction between titin and myosin thick filaments. J Struct Biol. 2018;203(1):46-53.

3. Hsin J, Strümpfer J, Lee EH, Schulten K. Molecular origin of the hierarchical elasticity of titin: simulation, experiment, and theory. Annu Rev Biophys. 2011;40:187-203.

4. Agarkova I, Ehler E, Lange S, Schoenauer R, Perriard JC. M-band: a safeguard for sarcomere stability? J Muscle Res Cell Motil. 2003;24(2-3):191-203.

5. Erkihun M, Ayele B, Asmare Z, Endalamaw K. Current Updates on Variants of SARS-CoV- 2: Systematic Review. Health Sci Rep. 2024;7(11):e70166.

6. Ziebuhr J. Coronaviruses. Advances in Virus Research. London: Elsevier Science & Technology; 2016. p. 1-40.

7. Eaton P, West P. Atomic Force Microscopy. Oxford: Oxford University Press; 2010. p. 9-81.

8. Sziklai D, Budavári B, Kiss B, Herényi L, Kiss Z, Pályi B, et al. Unveiling the Structural and Mechanical Diversity of SARS-CoV-2 Variants Using Atomic Force Microscopy. In Preparation. 2025.

7. Bibliography

7.1. Publications related to the thesis

1. **Dominik Sziklai**, Judit Sallai, Zsombor Papp, Dalma Kellermayer, Zsolt Mártonfalvi, Pires R. H. & Miklós Kellermayer, *Nanosurgical Manipulation of Titin and Its M-Complex*, Nanomaterials, 2022, 12(2), 178.

2. Miklós Kellermayer, **Dominik Sziklai**, Zsombor Papp, Brennan Decker, Eszter Lakatos & Zsolt Mártonfalvi, *Topology of interaction between titin and myosin thick filaments*, J. Struct. Biol., 2018, Volume 203, Issue 1,

3. **Dominik Sziklai**, Bálint Budavári, Bálint Kiss, Levente Herényi, Zoltán Kiss, Bernadett Pályi & Miklós Kellermayer, *Unveiling the Structural and Mechanical Diversity of SARS-CoV-2 Variants Using Atomic Force Microscopy*, In Preparation, 2025

7.2. Unrelated publications

1. Csilla Csányi, **Dominik Sziklai**, Tímea Feller, Jolán Hársfalvi & Miklós Kellermayer, *Cryptic Extensibility in von Willebrand Factor Revealed by Molecular Nanodissection*. Int J Mol Sci. 2024 Jul 2;25(13):7296.