Journal of Mosaic of Autoimmunity
https://www.sciltp.com/journals/jmai

Short Communication

The Ro60-Ro52 Complex as a New Player in Intracellular Humoral Immunity

Jesus Vicente de Julián-Ortiz [1,*], Federico V. Pallardó [2,3], Pilar González-Cabo [2,3], Salvador Blasco [4] and David Gimenez-Romero [5,*]

[1] Department of Physical Chemistry, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés 0, 46100 Valencia, Spain
[2] Department of Physiology, Faculty of Medicine and Dentistry, University of Valencia-INCLIVA, 46010 Valencia, Spain
[3] CIBER Rare Diseases (CIBERER), 46010 Valencia, Spain
[4] Department of Inorganic Chemistry, Faculty of Chemistry, University of Valencia, C/Doctor Moliner 50, 46100 Burjassot, Spain
[5] Department of Physical Chemistry, Faculty of Chemistry, University of Valencia, C/Doctor Moliner 50, 46100 Burjassot, Spain
* Correspondence: jesus.julian@uv.es (J.V.d.J.-O.); david.gimenez-romero@uv.es (D.G.-R.)

How To Cite: de Julián-Ortiz, J.V.; Pallardó, F.V.; González-Cabo, P.; et al. The Ro60-Ro52 Complex as a New Player in Intracellular Humoral Immunity. *Journal of Mosaic of Autoimmunity* **2025**, *1*, 2

Received: 10 December 2024
Revised: 25 December 2024
Accepted: 14 January 2025
Published: 16 January 2025

Abstract: The Ro/SSA antigen complex contains Ro60 (TROVE2), Ro52 (TRIM21), and Y-RNA molecules that have recently emerged as the cornerstone of intracellular immunity and are thought to be the main target of autoantibodies in systemic autoimmune diseases. For decades, the precise nature of the Ro60-Ro52 interaction has been a matter of controversy. We have recently shown that the Ro60-Ro52 complex is transient and dynamic under physiological conditions. These results not only improve our understanding of Ro antigen biology but also highlight the possibility of targeted modulation approaches for autoimmune diseases. This communication explores a fascinating and largely unexplored aspect of intracellular humoral immunity through the Ro60-Ro52 complex, aiming to deepen our understanding of this pivotal interaction and its implications for cellular processes and disease.

Keywords: intracellular humoral immunity; Ro60-Ro52 complex; Ro52/TRIM21; Ro60/TROVE2; proteasomal degradation; RNA quality control; ubiquitin E3 ligase; systemic autoimmune diseases

Antibodies are proteins secreted by B cells to bind pathogens, neutralize toxins, and stimulate immune responses. While antibody-mediated neutralization is typically considered an extracellular process, as antibodies are excluded from the cell interior due to membrane compartmentalization, some viruses and bacteria can penetrate the cell membrane and enter the cytosol, even when opsonized by non-neutralizing antibodies [1], which, without directly inhibiting their infectivity, often mark them for immune clearance through other mechanisms. The antibody-opsonized non-enveloped viruses are quickly targeted for degradation by the proteasome, triggering an innate immune response.

In this context, Ro52 (also known as "TRIpartite Motif-containing 21" or TRIM21) acts as a bridge between the cellular self-defense system and adaptive immunity by employing the antibody repertoire to recognize and respond to antigens. Antibodies engage this protein to activate a secondary immune defense [2], inducing a synchronized effector and signaling response.

Ro52 functions as a cytosolic Fc receptor, with a particularly strong affinity for the fragment crystallizable region (Fc region) of antibodies. Structurally, Ro52 comprises four domains: an N-terminal RING domain, a type 2 B-box, a coiled-coil domain, and a C-terminal PRY-SPRY domain responsible for substrate binding [3]. In solution, Ro52 forms a homodimer, with its PRY-SPRY domains binding to the Fc region of antibodies [2]. Through its RING domain, Ro52 mediates E3 ligase activity, allowing it to degrade virus-antibody complexes within the cytosol while simultaneously activating immune signaling, thus driving a dual-response mechanism.

As a ubiquitin E3 ligase, it plays a crucial role in degrading virus-antibody complexes within the cytosol [1,4]. Upon recognizing an antibody-bound virus, Ro52 becomes activated and begins to form ubiquitin chains. These chains serve two key functions: they trigger proteasomal degradation of the virus and activate immune signaling. This enables Ro52 to act as both a sensor and an effector, providing an immediate antiviral response while also establishing a long-term immunity. This dual response enables non-neutralizing antibodies to mediate post-entry inhibition of viral replication.

The Ro52 protein is a component of the Ro-ribonucleoprotein complex (Ro RNP), which interacts with small cytoplasmic RNAs (hY-RNA) [5]. This complex is believed to consist of two main components: Ro52 (52 kDa) and Ro60 (60 kDa) [6–11]. Ro52 was identified as part of the Ro antigen as early as 1988 [6]. Ro60 (also known as "TeloRubisco, RO, Vault and Eukaryotic family member 2" or TROVE2) is the biggest protein chain present in Ro RNPs isolated from human HeLa cells [7]. Old studies supported the association between Ro60 and Ro52, although this fact could not be demonstrated in vivo [12–14]. Understanding the importance of Ro60 invites a closer look at its structure and interactions.

Ro60 plays a key role in RNA quality control by binding the single-stranded ends of misfolded noncoding RNAs and facilitating their degradation [15]. Bacterial Y RNA binds to Rsr, the Ro60 ortholog from the bacterium *Deinococcus radiodurans*, and recruits the 3′ to 5′ exoribonuclease polynucleotide phosphorylase (PNPase), forming a RNA degradation complex called RYPER (Rsr/Y RNA/PNPase Exoribonuclease RNP). PNPase, a homotrimeric ring, degrades single-stranded RNA, and the Y RNA-mediated binding of Ro60 enhances PNPase's efficiency in degrading structured RNAs [16]. Ro60 forms a toroid-shaped ring composed of antiparallel α-helical repeats and a von Willebrand factor A domain [8]. This domain, known for its involvement in various multiprotein complexes, enables protein-protein interactions through a metal ion-dependent adhesion site. However, the specific contribution of the adhesion site to Ro60's function remains unclear. Ro60 also exhibits genetic interactions that are enhanced during growth under temperature extremes, oxidative stress, and recovery from UV irradiation [17], suggesting that the RYPER complex may play a role in various stress responses.

The Ro RNP is primarily composed of Ro60 and noncoding Y RNAs [8], the number of which varies between species, but Ro60 is consistently found complexed with at least one Y RNA. Initially, the Ro52 and Ro60 proteins were believed to be part of the same Ro RNP, but subsequent studies revealed that they do not directly interact with one another [9,10]. Recently, we clarified this apparent contradiction by demonstrating that Ro52 and Ro60 form a weak transient complex in the cytoplasm. While initially believed to be stable components of the same Ro RNP, our findings revealed that their interaction is both weak and transient. This was achieved through the application of advanced techniques, including proximity ligation assay (PLA), indirect immunofluorescence (IIF), and quartz crystal microbalance (QCM) [11]. Figure 1 shows the result of the IFF assay for Ro60, for Ro52 and for the two proteins simultaneously. In the latter case, each protein is stained in a different color. When the molecules are close enough, the resulting color is the combination of the two. Figure 2 shows the PLA result where the red fluorescent dots gave the position of the complexes that remained bound for a sufficient time, indicating that the complex is actually produced and is not the result of casual approaches.

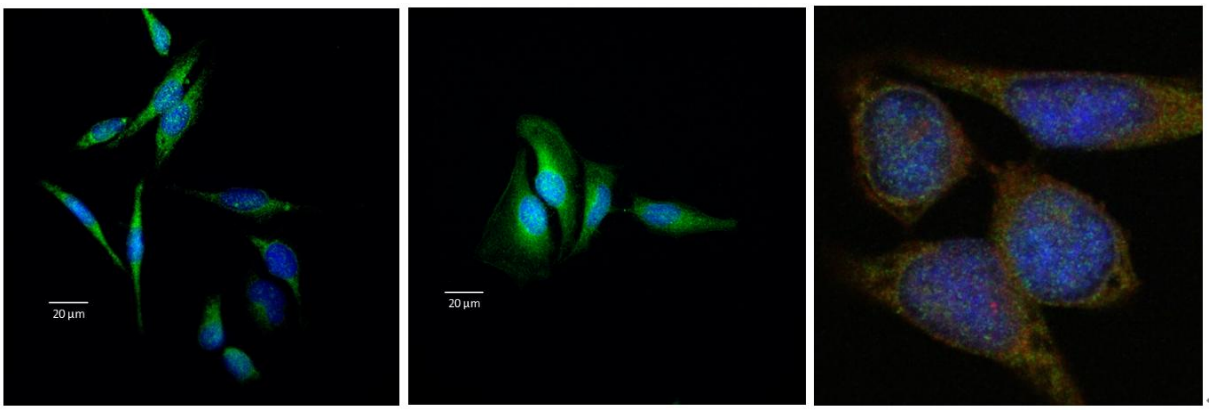

Figure 1. IIF assays results of Ro60 and Ro52 in HeLa cells [11]. It shows predominant cytoplasmic expression of Ro60 (**left**) and Ro52 (**center**) proteins. Colocalized proteins appear in yellow in the image on the **right**.

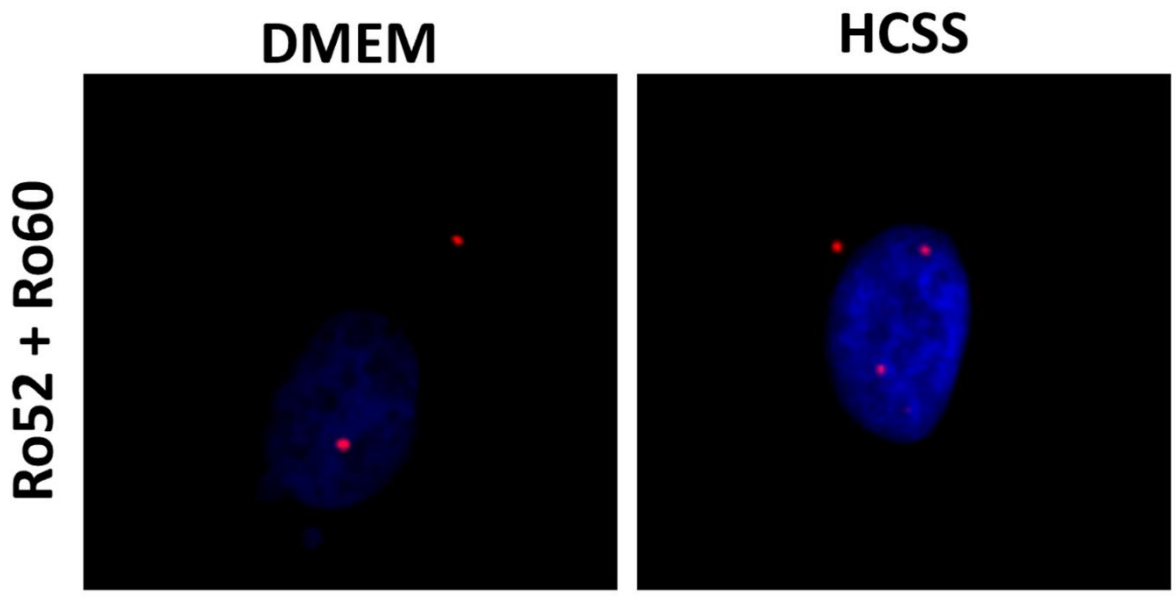

Figure 2. PLA in living cells incubated in two different media [11]. In the presence of two protein molecules close enough (less than 40 nm), red fluorescent spots appear.

The stability of the Ro60-Ro52 complex was analyzed using molecular dynamics and umbrella sampling, with the Weighted Histogram Analysis Method revealing a $\Delta G = +359.65$ kJ/mol. This indicated that complex formation is thermodynamically spontaneous under approximate physiological conditions (1 atm, 300 K, 0.1 M NaCl). Experimental details and references are given in the Supplementary Materials. In this complex, Ro60 aligns horizontally within the PRY-SPRY domains of Ro52's homodimer, which are crucial for detecting intracellular antibodies, a key component of the cellular self-defense system. When non-neutralizing antibodies are present in the cytoplasm, they prompt the dissociation of this transient complex, allowing Ro60 to participate in the assembly of the RYPER complex.

The function of Ro52 has been specifically demonstrated in the context of adenovirus infection [18]. Hypothetically, the Ro60-Ro52 complex, whose precise role remains unclear, may help stabilize the cytoplasmic presence of Ro60. When non-neutralizing antibodies bound to adenoviruses enter the cell, Ro52 interacts with their Fc regions, leading to the release of Ro60. This interaction not only triggers the proteasomal degradation of the virus but also activates immune signaling pathways via the Ro52 protein. At the same time, the released Ro60 may play a crucial role in regulating the upregulation of long noncoding RNAs during viral infection, which are involved in the later stages of adenovirus replication. One possible function of Ro60 could be to recruit exonucleases that degrade these RNAs. Alternatively, Ro binding may sequester them, preventing their incorporation into the ribosomal subunit.

The discovery that Ro52 and Ro60 form a weak transient complex presents an intriguing opportunity to investigate their potential collaborative functions. Gaining insight into the role of this complex in regulating biochemical pathways could deepen our understanding of the interplay between intracellular humoral self-defense mechanisms and noncoding RNA degradation processes. These functions extend across critical cellular processes, including DNA replication, RNA quality control, and responses to cellular stress. Such insights could pave the way for the development of targeted therapies. Specifically, modulating the formation of the Ro60-Ro52 complex may emerge as a promising therapeutic strategy for systemic autoimmune diseases, especially given the involvement of anti-SSA/Ro autoantibodies in these conditions.

Modulation of complex formation could be achieved using different strategies. In practice, we propose modulating the formation of the Ro60-Ro52 complex by altering the cations bound to the MIDAS domain of Ro60, as we hypothesize this domain plays a critical role in the complex's formation. This approach could enable us to either promote or inhibit complex formation as needed, thereby controlling its currently unknown function. Such control could be achieved by utilizing molecular gates, which allow for the controlled and selective release of drugs, to directly deliver the desired ionophores to the targeted altered cells, minimizing off-target effects. Ligands could be designed to selectively block or stabilize the interaction between Ro60 and Ro52. These ligand molecules would have to be specific and not affect other cellular interactions. They should be optimized to be specific and minimize off-target effects. Therapeutic monoclonal antibodies could be developed to neutralize autoantibodies targeting Ro60 or Ro52. This indirect approach has precedent in other

autoimmune diseases, such as the use of rituximab, which neutralizes B lymphocytes in lupus. Since Ro60 binds to Y-RNAs, interference with antisense RNA could influence the stability of the Ro60-Ro52 complex. Regarding risks associated with the alteration of the Ro60-Ro52 complex, this could affect other cellular processes. Interference with RNA quality control would be particularly delicate. Personalization of treatments is desirable given that patient symptoms are highly heterogeneous in autoimmune diseases. Personalized therapies based on the presence of distinct biomarkers could help identify patients who benefit from modulating Ro60-Ro52 complex formation. Precision delivery systems, such as nanoparticle carriers or cell-specific promoters, could minimize effects on healthy cells while targeting diseased cells. To do so, the structural and functional dynamics of the Ro60-Ro52 complex should be investigated and the feasibility of therapeutic modulation should be tested using disease-specific models. All of these treatments should balance without suppressing the immune response to avoid susceptibility to infections or cancer.

Future directions we are considering include: Evaluating the formation and dissociation of the Ro60-Ro52 complex throughout the cellular life cycle; Identifying specific phases of the cell cycle where the interaction is most relevant; Elucidating the specific conditions under which this complex interaction is promoted, whether it is due to cellular signals or a response to some stress condition; and Developing therapeutic strategies targeting the Ro60-Ro52 complex.

The battle between intracellular pathogens and the immune system is a dynamic and ongoing conflict. The discovery of the transient Ro60-Ro52 complex suggests it may play a pivotal role in this relationship. Such interplay could be involved in self-defense mechanisms, DNA replication, RNA processing, and stress responses, offering significant therapeutic and diagnostic potential (Figure 3).

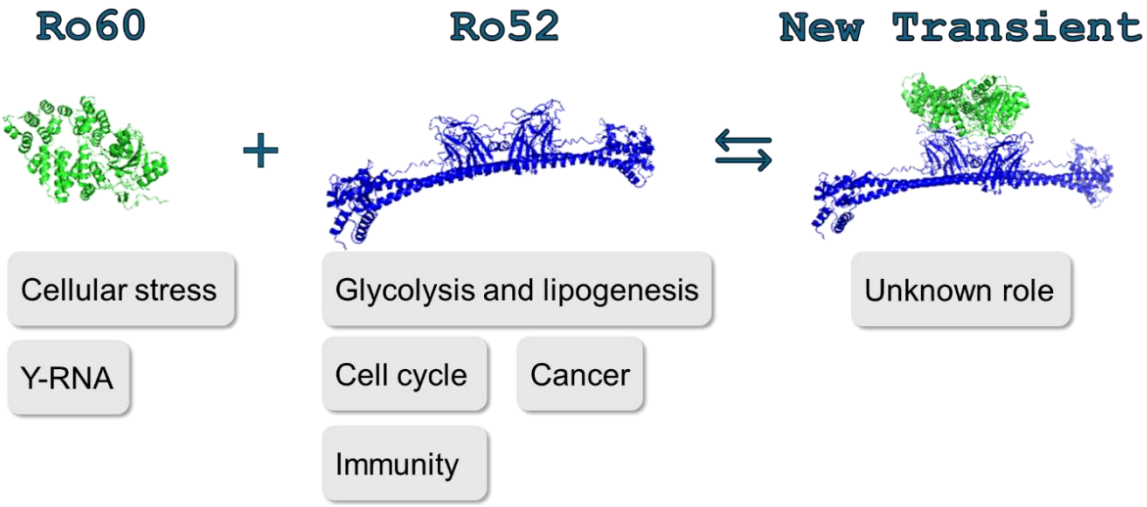

Figure 3. Ro60 is involved in Y-RNA management and cellular stress, while Ro52 has been linked to intracellular proteolysis and other fields. But what is the actual function of the complex they form in the cell? Reprinted with permission from Ref. [11].

We present this hypothesis not as an established truth, but as a framework to inspire deeper inquiry. Studying the complexities of intracellular humoral immunity via the Ro60-Ro52 complex is undoubtedly a challenging task, yet it holds the potential for transformative breakthroughs. We encourage readers to engage with this challenge and contribute to unraveling the mysteries of this compelling and impactful area of immunology. As we continue to explore the intricacies of this immune response, we gain valuable insights into the immune system's functioning and how we can harness its power to combat infectious diseases.

Supplementary Materials

The additional data and information can be downloaded at: https://www.sciltp.com/journals/jmai/2025/1/649/s1 References [19–27] are cited in the supplementary materials.

Author Contributions

J.V.d.J.-O., F.V.P., P.G.-C.: conceptualization, methodology, investigation, writing—reviewing and editing; S.B.: methodology, investigation, writing—original draft preparation; D.G.-R.: conceptualization, supervision, writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Data Availability Statement

Data are available upon request to the corresponding authors.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Foss, S.; Bottermann, M.; Jonsson, A.; et al. TRIM21-From Intracellular Immunity to Therapy. *Front. Immunol.* **2019**, *10*, 3049.
2. Mallery, D.; McEwan, W.; Bidgood, S.; et al. Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19985–19990.
3. Ozato, K.; Shin, D.; Chang, T.; et al. TRIM family proteins and their emerging roles in innate immunity. *Nat. Rev. Immunol.* **2008**, *8*, 849–860.
4. Fletcher, A.; Mallery, D.; Watkinson, R.; et al. Sequential ubiquitination and deubiquitination enzymes synchronize the dual sensor and effector functions of TRIM21. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 10014–10019.
5. Lerner, M.R.; Boyle, J.A.; Hardin, J.A.; et al. Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **1981**, *211*, 400–402.
6. Benchetrit, E.; Chan, E.K.; Sullivan, K.F.; et al. A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. *J. Exp. Med.* **1988**, *167*, 1560–1571.
7. Wolin, S.; Steitz, J. The Ro small cytoplasmic ribonucleoproteins: Identification of the antigenic protein and its binding site on the Ro RNAs. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 1996–2000.
8. Boccitto, M.; Wolin, S. Ro60 and Y RNAs: Structure, functions, and roles in autoimmunity. *Crit. Rev. Biochem. Mol. Biol.* **2019**, *54*, 133–152.
9. Boire, G.; Gendron, M.; Monast, N.; et al. Purification of antigenically intact Ro ribonucleoproteins; biochemical and immunological evidence that the 52-kD protein is not a Ro protein. *Clin. Exp. Immunol.* **1995**, *100*, 489–498.
10. Kelekar, A.; Saitta, M.; Keene, J. Molecular composition of Ro small ribonucleoprotein complexes in human cells. Intracellular localization of the 60-and 52-kD proteins. *J. Clin. Investig.* **1994**, *93*, 1637–1644.
11. Rodríguez, L.; de Julián-Ortiz, J.V.; de la Rúa, F.; et al. Unveiling the Ro60-Ro52 Complex. *EXCLI J.* **2024**, *23*, 888–903.
12. Slobbe, R.L.; Pluk, W.; van Venrooij, W.J.; et al. Ro ribonucleoprotein assembly in vitro: Identification of RNA-protein and protein-protein interactions. *J. Mol. Biol.* **1992**, *227*, 361–366.
13. Keech, C.L.; Gordon, T.P.; McCluskey, J. The immune response to 52-KDA ro and 60-KDA RO is linked in experimental autoimmunity. *J. Immunol.* **1996**, *157*, 3694–3699.
14. Tseng, C.E.; Chan, E.K.; Miranda, E.; et al. The 52-KD protein as a target of intermolecular spreading of the immune response to components of the SS-A/ro-ss-b/la complex. *Arthritis Rheum.* **1997**, *40*, 5.
15. Sim, S.; Wolin, S. Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. *Wiley Interdiscip. Rev. RNA* **2011**, *2*, 686–699.
16. Chen, X.; Taylor, D.; Fowler, C.; et al. An RNA Degradation Machine Sculpted by Ro Autoantigen and Noncoding RNA. *Cell* **2013**, *153*, 166–177.
17. Chen, X.; Wurtmann, E.; Van Batavia, J.; et al. An ortholog of the Ro autoantigen functions in 23S rRNA maturation in *D. radiodurans*. *Genes Dev.* **2007**, *21*, 1328–1339.
18. Jones, E.L.; Laidlaw, S.M.; Dustin, L.B. TRIM21/Ro52–Roles in Innate Immunity and Autoimmune Disease. *Front. Immunol.* **2021**, *12*, 738473.
19. Abraham, M.J.; Murtola, T.; Schulz, R.; et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **2015**, *1*, 19–25.
20. Bjelkmar, P.; Larsson, P.; Cuendet, M.A.; et al. Implementation of the CHARMM force field in GROMACS: Analysis of protein stability effects from correction maps, virtual interaction sites, and water models. *J. Chem. Theory Comput.* **2010**, *6*, 459–466.
21. Hess, B.; Bekker, H.; Berendsen, H.J.C.; et al. LINCS: A Linear Constraint Solver for molecular simulations. *J. Comput. Chem.* **1997**, *18*, 1463–1472.

22. Miyamoto, S.; Kollman, P.A. SETTLE: An Analytical Version of the SHAKE and RATTLE Algorithms for Rigid Water Models. *J. Comput. Chem.* **1992**, *13*, 952–962.
23. Essmann, U.; Perera, L.; Berkowitz, M.L.; et al. A smooth particle mesh Ewald method. *J. Chem. Phys.* **1995**, *103*, 8577–8592.
24. Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **2007**, *126*, 014101.
25. Bernetti, M.; Bussi, G. Pressure control using stochastic cell rescaling. *J. Chem. Phys.* **2020**, *153*, 114107.
26. Hub, J.S.; de Groot, B.L.; van der Spoel, D. g_wham-A free weighted histogram analysis implementation including robust error and autocorrelation estimates. *J. Chem. Theory Comput.* **2010**, *6*, 3713–3720.
27. Available online: https://www.uv.es/jejuor/SLE/Umbrella%20Sampling%20Pull.mp4 (accessed on 14 January 2025).