

The Structural Behavior and Molecular Interaction Between 4H11-scFv and MUC16^{ecto} Across Various Temperatures for Diagnostics

Research Question

How does the environment's temperature affect the 4H11-scFv's structure and its binding affinity with MUC16^{ecto}?

Understanding the effect of environmental conditions, particularly temperature, on the binding interaction between the 4H11-scFv and MUC16 is critical for developing reliable diagnostic tools. Antibody-antigen binding is a highly specific interaction that can be influenced by external factors, such as temperature. By studying how temperature affects 4H11-scFv's structure and affinity for MUC16, this research could provide insights into the stability of the complex under varying environmental conditions. These insights are highly relevant for designing effective assays in non-optimal or resource-limited settings.

Background Understanding

Despite being a natural physiological process, menstruation continues to be a subject of embarrassment and discrimination in many parts of the world ("Period Stigma"). Contemporary females arduously navigate complex cultural, social, and economic landscapes, where menstruation is often associated with shame and shrouded through euphemisms and discrete behavior (Yi 3). In Japan, a country ranked 120th out of 156 nations in the World Economic Forum's 2021 Gender Gap Index, people rarely discuss menstruation, even though most women must endure it for over 40 years (Akiyama et al., Crotti et al. 10). Identically, in China, the checker would wrap sanitary goods in an opaque, black paper bag when they are purchased at a convenience shop (Zhang). Even in the United Kingdom, 85% of adolescents have attempted to conceal their menstrual status ("Breaking Period").

Being on their period for women in more economically developed countries (MEDCs) is a mild inconvenience: just a few hours of cramps, a bit of moodiness, and the art of stealthily hiding a sanitary pad in their pocket on their way to the bathroom during school or work. Nevertheless, seeking safe and accessible menstrual products in less economically developed countries (LEDCs) is an endeavor for many women (Kottasová). Menstrual products in MEDCs are prohibitively expensive for many women, compelling them to rely on lower-quality alternatives. The women also lack safe, hygienic locations for washing and changing these products, further compromising their health and dignity.

Although more countries are leaving the traditional, constrained mindset and delving into inclusivity, the mainstream patriarchal society still restricts and limits females' actions, characteristics, and identity upon menstrual stigmas. These pervasive stigmas not only affect women's self-esteem and daily lives but also prohibit women from accessing necessary health information and facilities at critical times.

Women worldwide do not receive the number of preventive health examinations (PHE) and preventive gynecological examinations (PGE) as recommended. This is a substantial factor for late diagnosis. For instance, data from the United States shows that only 44.4 million or 20.9% of adults receive PHEs, and 19.4 million or 17.7% of females receive PGEs annually. In other words, 79.1% and 82.3% do not receive PHEs and PGEs as suggested (Mehrotra 1). Moreover, even when they attended these visits, less than half of preventive services were provided during PHEs, and while PGEs are more comprehensive, the coverage of preventive services is still limited. There is a similar trend in Austria. 58.2% of women had never attended a preventive health check-up within the last three years (Brunner-Ziegler et al. 1-3). Education and income level were universal determinants affecting the examination frequency (Mehrotra 1, Brunner-Ziegler et al. 1-3, Larson and Correa-de-Araujo 1-4).

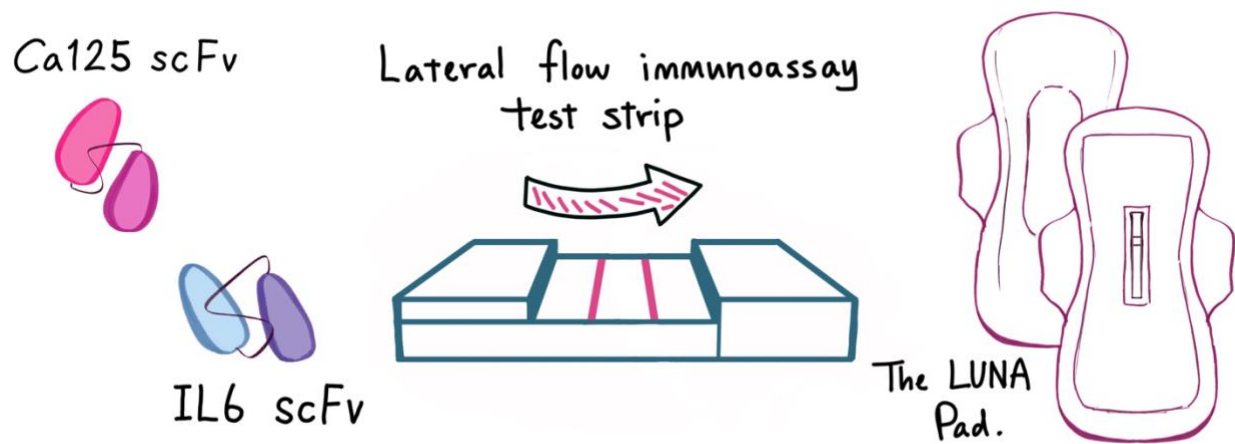
Residential regions may imply the financial status and level of education of individuals. Women from counties with less than or equal to 10,000 residents and not adjacent to a metropolitan country were less likely to report preventive health examinations (Larson and Correa-de-Araujo 1). Indeed, a third of suburban and rural residents in China exceed the suggested time interval for physical examination. 21.4% of those never actively sought examination. Thus, while many women receive PGEs, a majority do not undergo regular exams as frequently as recommended, and preventive services are underutilized. This indicates a gap between the need for regular preventive care and the actual utilization of such services. More importantly, the data suggests a lack of awareness of gynecological diseases, meaning some women are not aware of the necessity of screenings or even the existence of such diseases, again, due to the prevalent menstrual stigma.

Physical examinations are among the most accurate and comprehensive diagnostic tools for gynecological diseases. These include laparoscopy, transvaginal ultrasound, and pap smear. They allow doctors to directly examine the abdomen or pelvis via tiny incisions, pelvic organs, and abnormal cells in the female reproductive organs. However, they are time-consuming, invasive, and expensive. The pap smear test, for example, requires a 15–20-minute appointment to complete the swab. The doctor first swabs for abnormal discharge. Then, they would insert a speculum to expose the cervix carefully. Finally, they would apply a swab to collect cell samples from the ectocervix. However, the time for a pathology laboratory to process the sample and offer the results to one's doctor can range from 1-3 weeks (Smith). Across all facilities, the average cost for a pap smear is \$143 ("PAP Smear").

Lab tests are alternative approaches. Home kits enable women to collect samples and send them to the laboratory for PCR to evaluate further. Though this method can be less invasive, it still requires active examination, most occurring only after existing symptoms. Furthermore, each lab test is disease-specific, meaning women must classify their symptoms and identify the corresponding disease themselves, which can be a significant barrier to early detection. In fact, for many, the lack of distinct symptoms makes it difficult to recognize when testing is necessary.

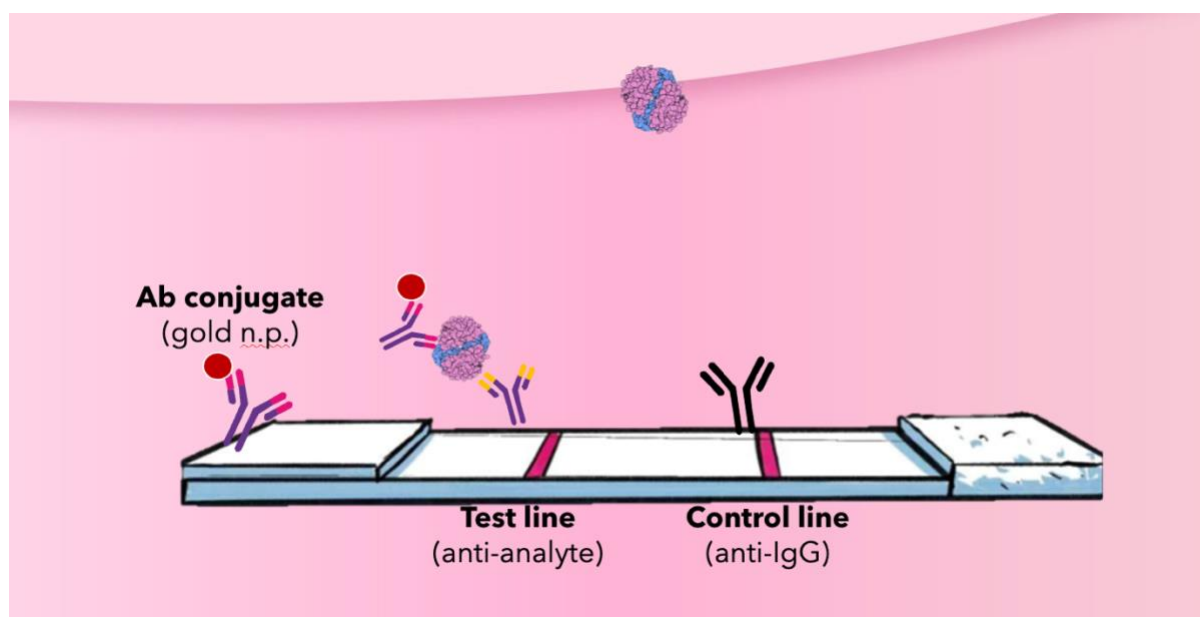
Together, the stigma prevents women from accessing necessary health information and help, and the inconvenience of current screening methods further fuels the late diagnosis of these gynecological diseases. Late diagnosis is a significant factor contributing to high mortality rates in gynecological diseases, such as ovarian and cervical cancer (Laitner). They often exhibit ambiguous symptoms mistaken for common ailments, delaying accurate diagnosis. For instance, over three-quarters of ovarian cancer cases are detected at advanced stages, where the mortality is high, while there is a 5-year relative survival rate of 93% at a local stage (Torre et al. 1-5). This demonstrates the pivotal role of early detection in significantly increasing survival rates. It highlights the need for improved screening and diagnostic methods to identify these cancers early, potentially improving patient outcomes and reducing mortality rates.

Considering all these factors and requirements, we, a group of high school students from various schools, propose our solution: Luna Pad. Our project addresses these issues by integrating a lateral flow immunoassay (LFIA) test strip as a point-of-care diagnostic tool into a sanitary napkin. It screens for gynecological diseases via menstrual blood, enabling women to monitor their health regularly without the constant need for invasive procedures or costly lab tests. We aspire to combat the silenced reality of menstrual shame and the lack of affordable and reliable products through the Luna Pad.



A simple diagram of the Luna pad and its mechanism ("Project Description")

The Luna Pad's screening mechanism is based on three main components. First, it functions based on the antibody-antigen binding principle. Many gynecological diseases produce antigens that are symbolic biomarkers. Although menstrual blood is whole blood, these antigens are present within it (Dosnon et al. 2). They can be detected when they bind to their corresponding antibodies immobilized on the LFIA test strip within the sanitary napkin. The second aspect is the application of single-chain variable fragments (scFv) instead of entire antibodies for the LFIA. Producing full-length antibodies is often challenging and typically relies on mammalian cell systems, which are both costly and inefficient. We selected scFvs, minimal antigen-binding fragments with intact paratopes, to address this. Due to their simplified structure, scFv can be expressed and folded correctly in prokaryotic systems like *E. coli*. This approach not only simplifies production but also significantly reduces costs, making mass production of the diagnostic tool more feasible and accessible, potentially lowering the expense required for these menstrual products (Lebozec et al. 33). Finally, the third aspect is the LFIA test strip. The test strip functions similarly to the COVID-19 antigen test, where a line of specific scFv antibodies will be immobilized, and the binding between the antigen and scFv will be reflected visually via LFIA's colorimetric mechanisms. With all the components combined, we envision an accessible, efficient, and affordable diagnostic tool for gynecological disease screening.



An illustration of the Luna pad's proposed LFIA system ("Project Description")

One of the chosen biomarkers for the LFIA is Cancer Antigen 125 (CA125), a glycoprotein that is encoded by MUC16's tandem repeat region. Specifically, CA125 is derived from MUC16's heavily glycosylated extracellular domain. Overexpression is associated with tumor progression and immune evasion in a range of gynecological diseases, particularly ovarian cancer, cervical cancer, and endometriosis (Dosnon et al. 1-4, Lee et al. 1-2).

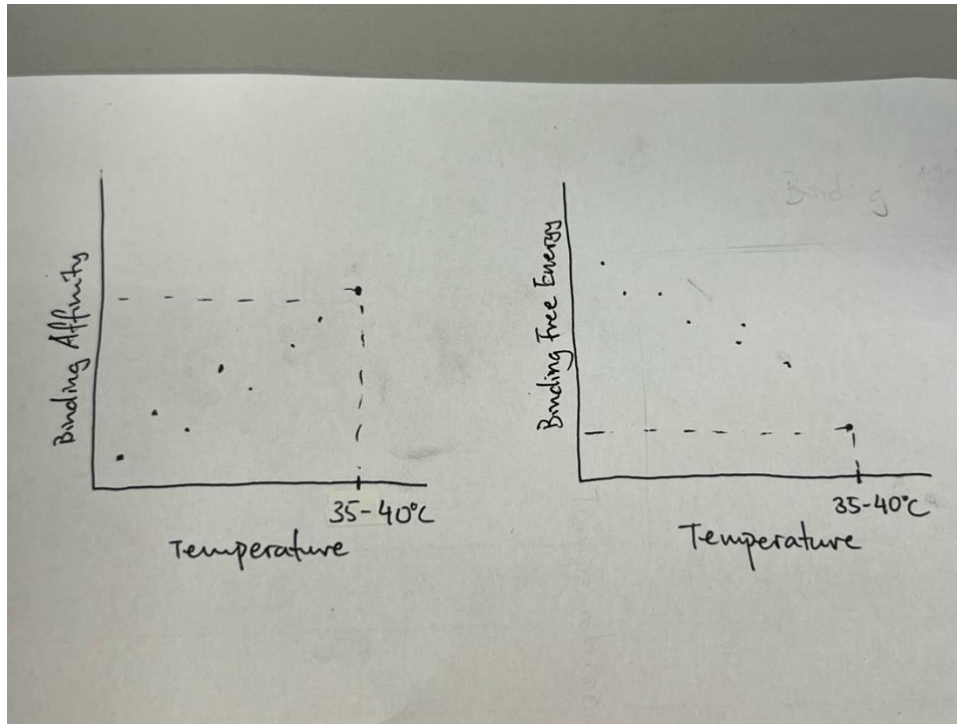
The selected scFv corresponding to MUC16 for the LFIA is derived from the humanized 4H11 antibody (h4H11) (Lee et al. 2). The h4H11 has demonstrated a high, consistent binding affinity with MUC16^{ecto} (Lee et al. 3). Via plasmid construction, expression, and protein purification, the target scFv of h4H11 has been extracted successfully in our experiments ("Project Description"). Its binding ability has also been validated. However, in real-world applications, various environmental factors, such as temperature, may influence the binding affinity between MUC16^{ecto} and the h4H11-scFv. Temperature can influence the structural integrity and binding affinity of antibodies and antigens, as antibodies are proteins that rely on precise folding and non-covalent interactions, including hydrogen bonds and hydrophobic forces, to maintain functional epitopes and paratopes. pH and temperature fluctuations destabilize such protein structures, potentially reducing the specificity in antigen recognition, thus elevating the chances of false-negative.

This study aims to identify the optimal temperature range for the interaction between h4H11-scFv and MUC16 using insilico applications. The findings can be leveraged to optimize the lateral flow immunoassay (LFIA) system within the wearable in-pad diagnostic platform, enhancing its reliability in diverse environmental conditions.

Hypothesis with Scientific Reasoning

The optimal temperature condition for antibody-antigen interaction between the scFv of 4H11 and the proximal MUC16 ectodomain is approximately between 35-40°C and at sub-zero temperatures, structural changes or slower binding kinetics may reduce binding affinity because of the hydrogen bond's nature. At this range, the hydrogen bonds formed between 4H11's epitope and MUC16's β -turns and β -hairpin structures stabilize without becoming too rigid or disrupted. This temperature range enhances the conformational flexibility required for high-affinity binding, allowing the scFv to maintain its structure and resist denaturation, which is crucial for strong, specific interaction (Lee et al.). Additionally, a study that evaluated temperature's effect on the binding kinetics of monoclonal antibodies in living cancer cells recognized that the antibody-antigen reaction achieved the equilibrium faster in cells incubated at 37 °C than at either 15 or 21°C (Encarnação et al. 404-405). Specifically, the K_a for 14.8 to 16.4°C and 20.2-21.3°C are $1.37 \times 10^5 \pm 0.01 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ and $1.16 \times 10^5 \pm 0.03 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ respectively, while the interaction occurred at 36.9 to 37.8°C has a K_a value of $3.33 \times 10^5 \pm 0.09 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, reflecting a higher binding rate at a higher temperature. The K_D for 14.8 to 16.4°C and 20.2-21.3°C are $5.12 \times 10^{-11} \pm 1.16 \times 10^{-11}$ and $8.44 \times 10^{-11} \pm 0.27 \times 10^{-11}$. Contrarily, K_D is merely $2.37 \times 10^{-11} \pm 0.87 \times 10^{-11}$ within the temperature range of 36.9 to 37.8°C, indicating higher binding affinity than any other tested temperature ranges (Encarnação et al. 403). Hence, as the 4H11-scFv is a fragment of the 4H11 monoclonal antibody, its optimal binding temperature should be around the range of 36-38°C, where the highest affinity and binding stability are observed.

A sketched graph based on the hypothesis (average binding affinity/binding free energy):



Variables

Independent Variable

The independent variable of this study is the temperature of the interacting environment. Temperature is crucial because it affects molecules' kinetic energy, influencing the antibody-antigen complex's stability and interaction dynamics. Understanding the temperature range in which the antibody-antigen complex remains intact and stable is essential for suggesting an optimal usage environment and helping users determine conditions that might affect the reliability of the results. By identifying temperatures where the complex's stability decreases, users can be informed about scenarios requiring greater caution or alternative measures, ensuring accurate and dependable screening outcomes. Furthermore, if the temperature range that allows the complex to maintain its integrity is incapacious, this would also be insightful for further research to optimize the 4H11-scFv that can adapt to a broader range of climates.

To account for real-world conditions, particularly in regions where temperatures can drop below freezing or rise significantly, the antibody-antigen interaction will be tested across a broader range of temperatures: 5°C, 15°C, 25°C, 35°C, and 45°C. These increments are selected to reflect the temperature changes of menstrual blood once it exists human body and is absorbed by the pad due to exposure to the surrounding environment. Menstrual blood is initially at body temperature (~37°C), but upon exposure to ambient air and the absorbent material of the pad, its temperature rapidly equilibrates with the surrounding environment. This range of temperatures simulates various climates in which the product may be used, including cool and mild environment temperatures (5°C to 15°C), moderate room and body temperatures (25°C to 35°C), and high temperatures (35°C to 45°C).

Testing across this range ensures the antibody-antigen complex is assessed under conditions that closely mimic its real-world use. The complex's stability and functionality will be evaluated at each temperature increment, with specific attention to factors such as free binding energy, structural integrity, and the consistency of results over time. This will provide critical insights into the current system's advantages and vulnerabilities.

Dependent Variable

The dependent variable in this study is the structural conformation and stability of the antibody-antigen complex formed between 4H11-scFv and MUC16. This variable will be quantified through the change in Gibbs free energy (ΔG), which represents the difference in free binding energy between the initial and final state of a reaction process. It offers critical insights into the spontaneity and thermodynamic feasibility of the reaction. This metric is critical for understanding how temperature variations influence the molecular dynamics of the antibody-antigen complex, which ultimately affects the reliability of the screening system.

Molecular dynamics simulations will be conducted using AMBER to measure these parameters. This tool allows for a detailed analysis of the complex's structural behavior under different temperatures and the stability and interaction dynamics of the 4H11-scFv and MUC16 complex. Additionally, PyMOL will be utilized to visualize changes in the 3D conformation of the antibody-antigen complex, enabling the identification of any structural disruptions or loss of integrity at specific temperature ranges. Origin/Excel can be employed to visualize trends in free binding energy, quantifying the stability of the complex.

Controlled Variables

Controlled Variable	How the variable is controlled & Why it needs to be controlled
pH	The pH of this simulation would be constant at 7, resembling the neutral-alkaline menstrual blood condition that elevates the overall pH level of the vaginal environment ("Vaginal Health"). Maintaining a constant pH is essential to isolate the effects of temperature on binding affinity, as pH can also significantly affect protein structure and function. Changes in pH can disrupt hydrogen bonds critical in stabilizing the secondary and tertiary structures of 4H11-scFv and MUC16, respectively, and the hydrogen bonds formed in the binding interface. Values outside of the optimal range can lead to the unfolding of the protein structures. Thus, controlling it ensures that any observed changes in binding affinity are due to temperature variations alone.
Pressure	Pressure can affect the volume and density of the simulation environment, which may denature the proteins and influence molecular interactions. This variable can be manipulated via a "barostat" throughout the simulations to ensure that any observed effects are not due to pressure fluctuations. In this case, the pressure of the simulation environment will remain constant at 1013.25 millibars or 1 atm to simulate the global average atmospheric pressure ("What Is Air Pressure?").
Ions	The number of ions in an environment can significantly alter the three-dimensional forces by the electrostatic interactions between residues in proteins. However, since observing how temperature fluctuations impact the proteins and complex is the objective of this study, this variable must be maintained the same throughout all simulations. Hence, the ionic strength would be kept at 0.15 M to mimic physiological conditions and an overall neutral charge for the protein complex (Hong et al.). To achieve this, two chlorine molecules will be added to counteract the existing two positive charges of the 4H11-scFv-MUC16 ^{ecto} complex.

Materials

- Laptop/computer with Linux operating system and PyMOL, AMBER, and Origin/Excel installed, search engine (e.g. Chrome)

Method

Step	Instruction
1	Gather necessary sequences

	- Retrieve the amino acid sequences for the 4H11-scFv-MUC16 complex from RCSB based on the information provided by the Structural basis for antibody recognition of the proximal MUC16 ectodomain (PDB ID 8VRS) - Download the file in PDB format - Ensure that these are correct sequences, including any necessary post-translational modifications (e.g. the 4H11 is humanized)
2	Delete the repeated region of the dimer - Open PyMol and click “S” to view the sequence (the primary protein structure) - Since the MUC16 is a dimer, delete half of the repeating region to simplify further steps and increase the accuracy of the simulation by clicking “Chains” from “Selecting Chains” and selecting half of the protein - Find “A” on the right-hand side and click “remove atoms
3	Remove excess H2O atoms (marked by the crosses) Find “A” on the right-hand side and click “remove waters”
4	Change selecting to residues and change the colors of the different structures - Select the MUC16^{ecto}’s residues (LQNFTLDRSSVLDGYSPN) - Find “C” and click on “by element” to change the color of the selected structure to yellow, in order to observe the structures more clearly - Select the two chains of 4H11-scFv and change the color to green - Select the Maltose Binding Protein (MBP), change the color to gray
5	Delete the excess MBP - Find “Selecting” then click “Residues” - Choose the S370 on the top band for residues, drag the scroll wheel to the far left, hold down Shift, and click M1 (the first amino acid residue) to select the entire gray structure - Delete this excess MBP, as this protein is used as a fusion partner for protein purification and characterization, which is unnecessary in this case Add TER to avoid misbinding two chains that were not supposed to
6	Export the processed MUC16ecto and 4H11-scFv protein complex - Click “File” in the upper left corner, “Export Molecule,” and “Save...” - At the pop-up window, name the file MUC16ecto.pdb and select a location to save
7	Prevent errors in the simulation - Use a text software, such as Terminal on macOS, to open the processed PDB file - Find the line “ANISOU 1024 NH2 ARG C 261...N,” click enter, and type TER, which marks the ends of a chain of residues, separating the ARG (residue 261 from MUC16) from the next, GLU (residue 262 from 4H11-scFv). Without this record, the simulation software might incorrectly interpret the residues as being covalently connected, leading to unrealistic bonds/errors in energy calculations
8	Add hydrogen ions (Because hydrogen ions are naturally a part of proteins, but in the PDB file provided, they are not there. Thus, they have to be manually added) - Create an account on newbiophysics H++ - Click “Process a structure” on the left and import the MUC16ecto.pdb and press “Process File” - Set pH as 7 for neutral and check the box below - Click on “View Results” to retain the possible download formats and download it in “AMBER compatible PDB structure” - Name the file MUC16ecto_H.pdb
9	Further processing and simulation (Using a laptop/computer with the Linux operating system) Place the MUC16ecto_H.pdb next to the leap.in file from AMBER

	• Right click empty space and select “Open in Terminal.” • Here, the program itself adds two chlorine ions to the complex, ensuring tha the overall charge of the complex is neutral • Enter “tleap -s -f leap.in” and click enter to operate
10	Manipulate the simulation temperature • Modify the heat.in, eqnpt.in, npt1.in, npt2.in, npt3.in npt4.in, and prod1.in files and change the tempi to 278.0 and temp0 to 278.0
11	Running Simulation • Place the heat.in, mdrun.sh, com.prmtop. and cominpcrd within the same folder too • Right click empty space and select “Open in Terminal” • Enter “nohup sh mdrun.sh &” and click enter to operate
12	Simulation monitoring (takes about 4 hours per simulation) • Open the terminal and enter “nvidia-smi” to check the percentage completed for the simulation (if a simulation is running, the last column will display a percentage above 95%. When it is completed, it’ll become 0%)
13	Free binding energy calculation: • Add the cpptraj.in file to the previous folder • Open the terminal, enter “cpptraj -I cpptraj.in” and click enter to operate. A file named nowater.nc, a trajectory file, will be created • Place com.prmtop and nowater.nc under a folder named mmpbsa • Open the terminal, enter “bash make-prmtop.sh” and press enter to operate • Enter “bash cal.sh” and press enter to operate, takes about 20 minutes
14	Retrieve the calculation • Enter “bash grep.sh” and press enter to retrieve energy.dat
15	Repeat step 10-14, with a change in the tempi to 288.0 and temp0 to 288.0
16	Repeat step 10-14, with a change in the tempi to 298.0 and temp0 to 298.0
17	Repeat step 10-14, with a change in the tempi to 308.0 and temp0 to 308.0
18	Repeat step 10-14, with a change in the tempi to 318.0 and temp0 to 318.0

* There is no specific concern with this experiment, as it is completed via a computer simulation. Its in-silico nature ensures minimal physical concerns.*

Results

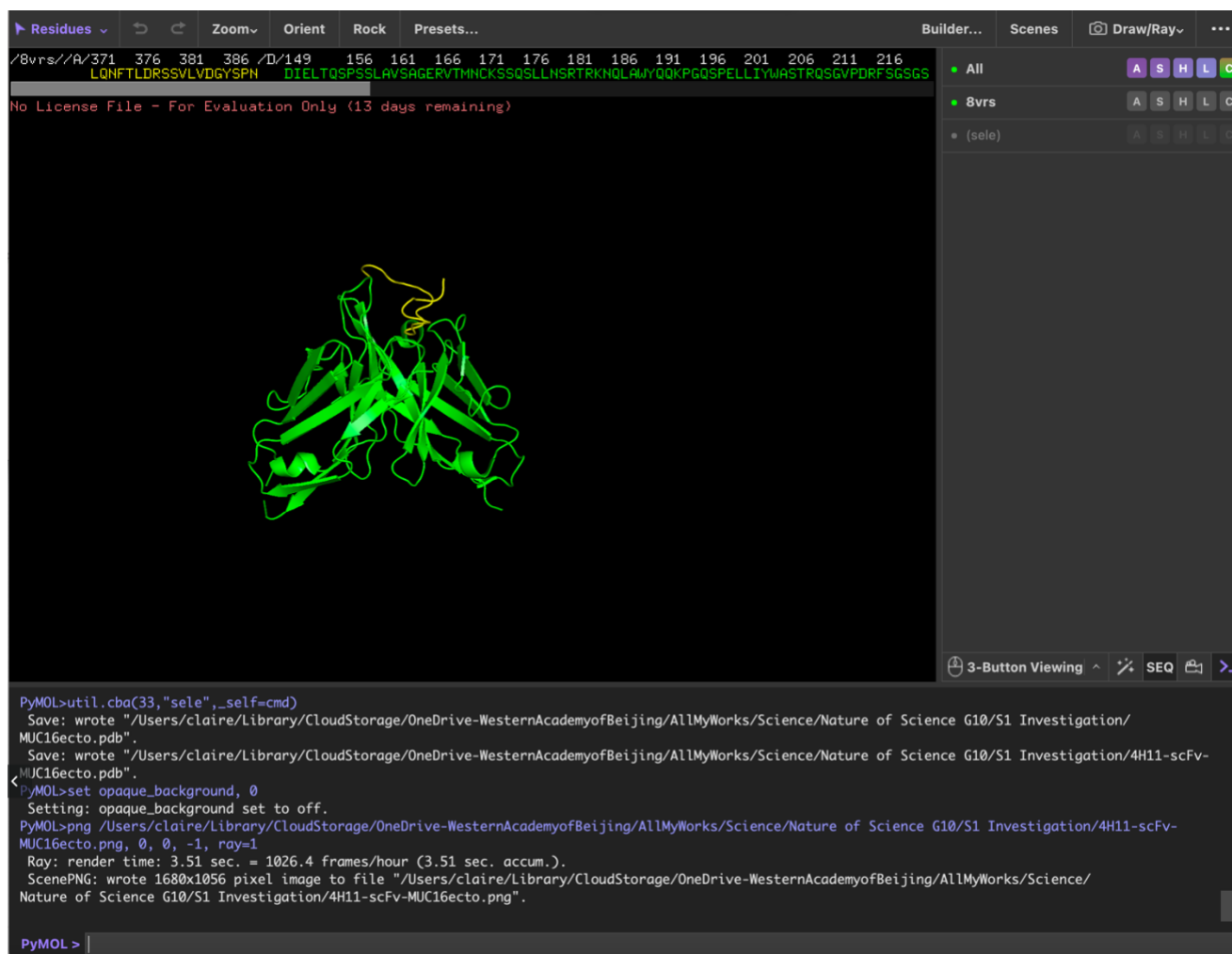


Figure 1: Processed 4H11-scFv-MUC16^{ecto} complex on PyMOL with repeated dimer region and MBP removed. MUC16^{ecto} is coded yellow by element. The chains of 4H11-scFv are coded green by element

Table 1: The effect of 5°C simulation temperature on the binding free energy (ΔG) of 4H11-scFv and MUC16^{ecto} in 50ns

Binding time (ns) ± 3	Trial 1 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 2 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 3 Binding Free Energy (ΔG) (kcal/mol) ± 1	Statistical Uncertainty (ΔG) (kcal/mol) ± 1 (rounded to 4 decimal places)
0.2	-97.4835	-94.1557	-79.9882	8.7477
0.4	-98.9013	-86.7958	-87.4049	6.0528
0.6	-96.6275	-86.9910	-78.4372	9.0952
0.8	-92.1114	-91.4782	-92.7807	0.6512
1.0	-98.2215	-94.1027	-82.7537	7.7339
1.2	-102.3612	-83.5175	-78.5621	11.8996
1.4	-99.2300	-89.0810	-81.1818	9.0241
1.6	-97.2201	-86.9742	-87.2338	5.1230
1.8	-90.8449	-88.5201	-87.3165	1.7642
2.0	-98.9889	-87.4964	-77.5901	10.6994
2.2	-94.7762	-91.7548	-81.5797	6.5983
2.4	-96.4095	-87.8398	-89.1167	4.2849
2.6	-97.7306	-84.6708	-88.3444	6.5299
2.8	-96.5767	-90.6984	-85.9442	5.3163
3.0	-93.2421	-89.2532	-80.2553	6.4934
3.2	-89.9450	-85.9934	-80.0192	4.9629
3.4	-94.3390	-84.8897	-89.5404	4.7247
3.6	-86.7061	-89.2001	-80.7289	4.2356
3.8	-95.5555	-87.2348	-75.7290	9.9133
4.0	-90.5822	-81.0164	-88.2723	4.7829
4.2	-92.9580	-93.8339	-87.3519	3.2410
4.4	-92.5939	-87.6231	-82.6739	4.9600
4.6	-95.0837	-79.8365	-83.5706	7.6236
4.8	-93.5971	-84.7627	-87.5932	4.4172
5.0	-87.6794	-81.3223	-77.1834	5.2480
5.2	-87.0972	-79.5728	-85.0871	3.7622
5.4	-93.5321	-80.1440	-82.5585	6.6941
5.6	-91.5690	-74.6428	-87.2033	8.4631
5.8	-94.3680	-93.6790	-91.9035	1.2323
6.0	-89.1628	-88.5817	-88.6074	0.2906
6.2	-85.5077	-91.8474	-82.7746	4.5364
6.4	-97.7224	-76.8893	-86.3310	10.4166
6.6	-91.0705	-78.5197	-87.0752	6.2754
6.8	-91.7124	-78.0254	-80.7767	6.8435
7.0	-94.0422	-88.1962	-91.6959	2.9230
7.2	-86.5118	-89.9431	-77.7264	6.1084
7.4	-89.6397	-86.4575	-87.3563	1.5911
7.6	-94.8205	-85.5004	-88.2060	4.6601
7.8	-89.6312	-91.9881	-88.1903	1.8989
8.0	-94.8353	-84.7145	-88.2097	5.0604

8.2	-97.4447	-80.6892	-94.3885	8.3778
8.4	-97.8635	-78.0253	-89.2411	9.9191
8.6	-98.0649	-80.4292	-80.2475	8.9087
8.8	-100.0118	-84.4143	-87.0322	7.7988
9.0	-98.6587	-85.9828	-87.0686	6.3380
9.2	-93.9878	-79.9201	-85.3392	7.0338
9.4	-95.6794	-87.5223	-85.6124	5.0335
9.6	-97.2858	-79.5680	-88.0996	8.8589
9.8	-99.9852	-83.4005	-92.4318	8.2924
10.0	-95.6106	-85.4974	-91.5764	5.0566
10.2	-100.3339	-88.9425	-89.2691	5.6957
10.4	-99.5296	-87.9136	-91.2443	5.8080
10.6	-94.5928	-84.3946	-90.6566	5.0991
10.8	-84.3736	-77.8579	-98.3013	10.2217
11.0	-82.0851	-87.0947	-92.6014	5.2582
11.2	-97.6275	-80.4350	-82.9643	8.5963
11.4	-101.3364	-82.6790	-87.2431	9.3287
11.6	-100.3478	-82.6512	-88.6955	8.8483
11.8	-93.5945	-87.2359	-79.7615	6.9165
12.0	-91.4940	-83.1297	-85.2912	4.1822
12.2	-99.4334	-85.4923	-88.1167	6.9706
12.4	-96.6960	-89.7975	-90.1008	3.4493
12.6	-89.8243	-77.3321	-89.2242	6.2461
12.8	-96.3915	-78.6290	-95.9167	8.8812
13.0	-89.9432	-82.5104	-88.0269	3.7164
13.2	-93.5772	-83.4013	-95.9277	6.2632
13.4	-91.5535	-82.6237	-91.7931	4.5847
13.6	-95.0733	-89.7914	-87.8895	3.5919
13.8	-94.5994	-79.1240	-99.0442	9.9601
14.0	-99.7764	-81.2913	-92.5510	9.2425
14.2	-104.9144	-83.2636	-90.8359	10.8254
14.4	-97.1036	-83.2831	-94.9648	6.9103
14.6	-97.9248	-92.0120	-85.6974	6.1137
14.8	-96.1851	-83.0059	-93.4114	6.5896
15.0	-89.6379	-88.2123	-92.2683	2.0280
15.2	-93.0692	-79.3468	-93.6300	7.1416
15.4	-96.2225	-84.1428	-89.1115	6.0399
15.6	-100.9268	-80.0888	-89.4705	10.4190
15.8	-91.6789	-85.5004	-96.7818	5.6407
16.0	-104.4402	-82.2877	-91.6025	11.0763
16.2	-101.2714	-84.6284	-94.5122	8.3215
16.4	-94.9127	-86.1552	-94.0907	4.3788
16.6	-97.7671	-85.7665	-90.2348	6.0003
16.8	-95.6489	-77.9313	-87.6352	8.8588
17.0	-87.0128	-84.4159	-91.9695	3.7768
17.2	-89.5854	-90.2568	-90.2514	0.3357
17.4	-96.4720	-79.3331	-84.5364	8.5695

17.6	-101.2201	-84.6716	-92.3224	8.2743
17.8	-88.4859	-89.2215	-87.7690	0.7263
18.0	-89.0932	-81.5761	-83.7911	3.7586
18.2	-92.2790	-79.3472	-87.0148	6.4659
18.4	-91.6455	-81.1281	-86.2983	5.2587
18.6	-88.2693	-85.4001	-75.1782	6.5456
18.8	-88.1672	-91.2443	-79.4489	5.8977
19.0	-90.4302	-76.7981	-78.4766	6.8161
19.2	-84.5735	-88.2530	-86.9502	1.8398
19.4	-88.3318	-87.7262	-82.5686	2.8816
19.6	-85.2139	-87.9099	-87.2780	1.3480
19.8	-88.0729	-81.6380	-86.0334	3.2175
20.0	-94.4530	-89.9760	-92.8329	2.2385
20.2	-95.2090	-81.2693	-94.8912	6.9699
20.4	-89.7692	-88.1039	-86.3504	1.7094
20.6	-99.1345	-85.4253	-93.9934	6.8546
20.8	-100.5649	-83.6679	-87.4334	8.4485
21.0	-89.7132	-86.2901	-89.1625	1.7116
21.2	-89.6678	-79.1889	-79.5925	5.2395
21.4	-97.0452	-81.8249	-80.7724	8.1364
21.6	-88.9765	-85.7420	-82.1203	3.4281
21.8	-102.0024	-79.8548	-80.0061	11.0738
22.0	-103.1746	-88.5593	-84.1499	9.5124
22.2	-94.9156	-80.0375	-88.5926	7.4391
22.4	-88.1654	-92.1963	-84.6371	3.7796
22.6	-88.5282	-84.5539	-96.6396	6.0429
22.8	-93.8262	-86.1335	-78.9676	7.4293
23.0	-100.7443	-79.8311	-94.7421	10.4566
23.2	-93.1315	-77.4139	-91.9418	7.8588
23.4	-94.4880	-92.1332	-84.3167	5.0857
23.6	-83.7026	-83.0529	-87.8349	2.3910
23.8	-87.6988	-83.8188	-90.1790	3.1801
24.0	-93.0620	-83.1358	-83.1380	4.9631
24.2	-84.6343	-91.7670	-88.4162	3.5664
24.4	-81.9105	-80.4037	-82.4369	1.0166
24.6	-87.7778	-90.6986	-93.0947	2.6585
24.8	-96.3374	-85.6126	-90.2805	5.3624
25.0	-92.2106	-89.3327	-84.9406	3.6350
25.2	-82.7731	-84.3177	-92.2992	4.7631
25.4	-87.3640	-91.8963	-90.8449	2.2662
25.6	-86.3052	-90.9589	-92.3669	3.0309
25.8	-90.9543	-83.5335	-94.0720	5.2693
26.0	-84.4269	-83.2438	-99.7685	8.2624
26.2	-87.9318	-95.6892	-90.7778	3.8787
26.4	-81.4500	-94.4959	-88.7481	6.5230
26.6	-78.8516	-84.3005	-93.6534	7.4009
26.8	-86.7481	-87.9958	-93.0760	3.1640

27.0	-89.2911	-86.9399	-96.3077	4.6839
27.2	-83.7361	-79.6951	-95.4685	7.8867
27.4	-85.6422	-84.5843	-89.2332	2.3245
27.6	-89.0923	-85.0485	-94.7346	4.8431
27.8	-85.3233	-88.9792	-95.2289	4.9528
28.0	-84.0690	-75.7291	-92.0185	8.1447
28.2	-84.5646	-79.8906	-94.6032	7.3563
28.4	-83.5542	-80.5652	-95.4145	7.4247
28.6	-84.9425	-79.4838	-94.2563	7.3863
28.8	-78.0230	-90.3128	-89.9162	6.1449
29.0	-91.5643	-83.4486	-92.4352	4.4933
29.2	-92.9491	-86.8373	-84.9939	3.9776
29.4	-89.7946	-84.4123	-83.5692	3.1127
29.6	-83.7307	-83.7050	-92.6625	4.4788
29.8	-87.3274	-83.7884	-90.8905	3.5511
30.0	-92.2607	-86.9627	-92.8789	2.9581
30.2	-93.1112	-82.5207	-87.7146	5.2953
30.4	-86.3618	-80.4430	-92.7481	6.1526
30.6	-88.8228	-77.9867	-95.1362	8.5748
30.8	-89.9029	-85.1056	-89.9965	2.4455
31.0	-89.9053	-88.5872	-92.9384	2.1756
31.2	-85.8268	-85.6000	-92.0499	3.2250
31.4	-82.8783	-79.3257	-94.0183	7.3463
31.6	-94.5052	-80.2843	-88.7115	7.1105
31.8	-95.2540	-84.4217	-100.9262	8.2523
32.0	-98.3721	-83.7037	-91.7091	7.3342
32.2	-97.3784	-88.2764	-90.2671	4.5510
32.4	-88.0710	-84.3312	-86.7312	1.8699
32.6	-104.3678	-89.9762	-88.8478	7.7600
32.8	-96.9919	-80.2262	-78.1716	9.4102
33.0	-91.5359	-82.9925	-89.4439	4.2717
33.2	-77.3466	-80.6097	-89.5842	6.1188
33.4	-93.2918	-86.0274	-83.4160	4.9379
33.6	-90.8114	-82.8741	-85.8701	3.9687
33.8	-91.6668	-84.3682	-86.6322	3.6493
34.0	-97.9943	-81.4794	-78.2149	9.8897
34.2	-94.5738	-90.3677	-81.6185	6.4777
34.4	-95.2223	-90.8925	-86.4975	4.3624
34.6	-92.2506	-85.0579	-85.4810	3.5964
34.8	-100.9622	-88.2804	-87.5667	6.6978
35.0	-90.6450	-78.3518	-85.6170	6.1466
35.2	-92.6266	-84.4308	-88.1027	4.0979
35.4	-89.6708	-89.4663	-86.0869	1.7920
35.6	-79.6333	-73.2245	-90.3896	8.5826
35.8	-89.1024	-82.9820	-87.0139	3.0602
36.0	-80.3047	-93.7887	-93.2613	6.7420
36.2	-86.4924	-88.9343	-86.9689	1.2209

36.4	-81.8187	-89.4193	-92.5958	5.3886
36.6	-86.4319	-86.4887	-90.0235	1.7958
36.8	-87.3268	-78.5118	-78.6110	4.4075
37.0	-82.1816	-78.5847	-87.6981	4.5567
37.2	-91.2802	-75.3842	-89.5959	7.9480
37.4	-79.0583	-76.8950	-85.1188	4.1119
37.6	-88.8893	-81.4392	-90.4598	4.5103
37.8	-81.9401	-76.7407	-90.2947	6.7770
38.0	-76.3814	-78.4149	-86.9976	5.3081
38.2	-83.2977	-83.2458	-86.9329	1.8436
38.4	-89.4621	-74.4282	-87.0001	7.5170
38.6	-87.2241	-78.3433	-85.7550	4.4404
38.8	-80.6877	-84.3189	-86.0716	2.6920
39.0	-83.3832	-78.3595	-86.1660	3.9033
39.2	-91.8399	-92.3256	-89.5761	1.3748
39.4	-84.5835	-81.6815	-85.5108	1.9147
39.6	-88.2074	-85.9872	-91.7431	2.8780
39.8	-85.5668	-81.8147	-81.9843	1.8761
40.0	-81.8290	-83.6629	-80.1494	1.7568
40.2	-87.5113	-80.8707	-78.0139	4.7487
40.4	-84.5151	-82.7451	-76.5262	3.9945
40.6	-90.7783	-83.3939	-81.5870	4.5957
40.8	-85.8985	-84.9609	-89.5090	2.2741
41.0	-82.1393	-82.9562	-87.2082	2.5345
41.2	-83.9720	-85.8075	-79.8728	2.9674
41.4	-85.2464	-84.9064	-86.6447	0.8691
41.6	-79.0096	-84.7702	-90.8902	5.9403
41.8	-86.4439	-75.9323	-85.6331	5.2558
42.0	-80.2628	-80.0016	-89.0460	4.5222
42.2	-76.9225	-79.6119	-86.4960	4.7868
42.4	-82.4399	-85.1291	-84.2939	1.3446
42.6	-94.1439	-78.7470	-86.4337	7.6985
42.8	-87.9730	-90.5480	-82.1927	4.1777
43.0	-83.7901	-83.1552	-94.2337	5.5393
43.2	-91.0027	-78.6050	-87.0530	6.1989
43.4	-82.0110	-69.6541	-84.1917	7.2688
43.6	-77.5150	-74.8547	-84.4839	4.8146
43.8	-81.1148	-77.3413	-90.2289	6.4438
44.0	-78.8337	-75.8636	-88.6724	6.4044
44.2	-68.8946	-83.2229	-79.5678	7.1642
44.4	-81.1749	-84.4960	-89.5473	4.1862
44.6	-86.1304	-73.1074	-82.8711	6.5115
44.8	-82.2598	-83.4816	-86.8460	2.2931
45.0	-88.6889	-86.0437	-82.3447	3.1721
45.2	-78.2861	-75.4863	-89.6554	7.0846
45.4	-86.3987	-87.8411	-84.1536	1.8438
45.6	-85.3470	-89.3469	-90.4121	2.5326

45.8	-77.3687	-89.0990	-91.1614	6.8964
46.0	-83.3446	-93.1088	-88.1202	4.8821
46.2	-82.7581	-87.6858	-97.7573	7.4996
46.4	-70.7506	-86.6046	-90.2488	9.7491
46.6	-77.4510	-85.4587	-91.0433	6.7962
46.8	-80.8608	-74.9800	-85.6847	5.3524
47.0	-80.6257	-79.6474	-89.4544	4.9035
47.2	-88.4184	-88.7655	-91.2549	1.4183
47.4	-68.6972	-86.6761	-87.6834	9.4931
47.6	-84.1518	-88.3961	-94.9069	5.3776
47.8	-86.9486	-84.4376	-94.2133	4.8879
48.0	-76.7145	-84.9213	-94.4404	8.8630
48.2	-77.8043	-77.2802	-85.5673	4.1436
48.4	-78.7929	-75.2882	-79.9960	2.3539
48.6	-79.6835	-88.1943	-85.8579	4.2554
48.8	-83.8500	-81.9996	-89.7886	3.8945
49.0	-71.2568	-85.8574	-86.4753	7.6093
49.2	-78.5363	-73.9747	-89.5984	7.8119
49.4	-85.0557	-84.5138	-91.9158	3.7010
49.6	-79.4974	-84.6052	-92.6147	6.5587
49.8	-80.4053	-87.9962	-90.1118	4.8533
50.0	-70.2192	-90.0677	-79.0248	9.9243

Table 2: The effect of 15°C simulation temperature on the binding free energy (ΔG) of 4H11-scFv and MUC16^{ecto} in 50ns

Binding time (ns) ± 3	Trial 1 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 2 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 3 Binding Free Energy (ΔG) (kcal/mol) ± 1	Statistical Uncertainty (ΔG) (kcal/mol) ± 1 (rounded to 4 decimal places)
0.2	-75.0620	-85.4520	-84.3254	5.1950
0.4	-73.1778	-88.5961	-83.6873	7.7092
0.6	-75.5383	-76.6908	-78.6170	1.5394
0.8	-76.5152	-82.5441	-83.4567	3.4708
1.0	-82.0054	-86.3592	-83.1289	2.1769
1.2	-72.3708	-79.4937	-92.6961	10.1627
1.4	-72.8879	-80.3543	-84.5505	5.8313
1.6	-72.5588	-78.9469	-82.4019	4.9216
1.8	-70.2966	-81.3970	-83.1684	6.4359
2.0	-71.1106	-86.8355	-91.3492	10.1193
2.2	-75.0664	-80.9285	-88.0104	6.4720
2.4	-81.4038	-78.1842	-81.0840	1.6098
2.6	-75.1142	-82.5634	-90.0792	7.4825
2.8	-72.1900	-82.1390	-91.1726	9.4913
3.0	-64.8631	-85.9138	-86.9595	11.0482
3.2	-69.9370	-85.5473	-82.1271	7.8052

3.4	-70.0577	-86.3492	-80.7454	8.1458
3.6	-70.3291	-84.2069	-80.2686	6.9389
3.8	-76.1593	-83.7924	-90.2575	7.0491
4.0	-78.6896	-86.9736	-89.2601	5.2853
4.2	-81.9024	-80.2176	-93.9119	6.8472
4.4	-78.0218	-83.3031	-80.4077	2.6407
4.6	-81.6067	-86.8988	-95.0830	6.7382
4.8	-75.9340	-84.5704	-79.8132	4.3182
5.0	-81.1159	-87.5367	-88.8997	3.8919
5.2	-82.9016	-77.8520	-92.1065	7.1273
5.4	-85.2482	-84.4321	-90.7457	3.1568
5.6	-75.2100	-79.9979	-88.1455	6.4678
5.8	-78.1584	-83.8959	-78.6776	2.8688
6.0	-72.9214	-80.6133	-92.8348	9.9567
6.2	-80.5311	-84.4932	-80.2775	2.1079
6.4	-65.8592	-83.1894	-87.1175	10.6292
6.6	-72.2424	-81.7632	-93.0865	10.4221
6.8	-74.3863	-80.0155	-86.8138	6.2138
7.0	-81.2610	-77.3311	-80.7699	1.9649
7.2	-71.6313	-80.4451	-90.7712	9.5700
7.4	-66.9686	-84.4746	-84.4816	8.7565
7.6	-72.3634	-87.4857	-81.3212	7.5612
7.8	-78.8298	-83.0396	-76.3021	3.3688
8.0	-77.7268	-78.6799	-84.3896	3.3314
8.2	-75.3073	-78.0400	-77.6939	1.3664
8.4	-82.5457	-82.9636	-76.1313	3.4162
8.6	-74.6034	-84.7501	-76.6005	5.0734
8.8	-81.8039	-75.4521	-84.3610	4.4545
9.0	-81.6847	-76.0056	-89.8010	6.8977
9.2	-81.0492	-84.3915	-82.0053	1.6712
9.4	-78.3618	-93.1257	-81.6726	7.3820
9.6	-73.8313	-81.0417	-85.1889	5.6788
9.8	-78.6776	-82.1100	-81.2967	1.7162
10.0	-75.2015	-84.5140	-89.3615	7.0800
10.2	-73.1439	-84.3367	-88.5231	7.6896
10.4	-69.0450	-84.7654	-82.7787	7.8602
10.6	-74.7566	-75.0152	-91.1578	8.2006
10.8	-72.8464	-78.9461	-84.4735	5.8136
11.0	-73.0545	-86.2512	-79.7206	6.5984
11.2	-76.8944	-81.4218	-75.3521	3.0349
11.4	-73.2630	-89.2228	-87.0993	7.9799
11.6	-69.4530	-88.6904	-83.3772	9.6187
11.8	-71.3074	-86.2082	-78.9032	7.4504
12.0	-81.1499	-85.5530	-90.4602	4.6552
12.2	-76.1163	-89.1430	-82.9854	6.5134
12.4	-80.7670	-83.0707	-86.4637	2.8484
12.6	-71.8909	-83.6154	-79.3730	5.8623

12.8	-78.5253	-77.0108	-71.7430	3.3912
13.0	-82.9915	-83.5754	-72.7408	5.4173
13.2	-80.7873	-77.7416	-67.8496	6.4689
13.4	-87.1900	-84.0885	-75.6030	5.7935
13.6	-78.7739	-82.1066	-70.4339	5.8364
13.8	-86.2872	-89.4848	-74.1952	7.6448
14.0	-76.7925	-81.5907	-76.4252	2.5828
14.2	-84.6640	-88.7599	-74.5352	7.1124
14.4	-79.2940	-82.1336	-76.1738	2.9799
14.6	-77.7645	-85.3417	-68.6454	8.3482
14.8	-79.7479	-84.2042	-83.1212	2.2282
15.0	-80.6541	-90.1583	-83.1831	4.7521
15.2	-70.6597	-79.3130	-68.2994	5.5068
15.4	-85.5886	-79.4680	-89.0473	4.7897
15.6	-86.7103	-81.8841	-84.6544	2.4131
15.8	-88.5180	-76.1120	-85.8844	6.2030
16.0	-83.6837	-81.1859	-87.5027	3.1584
16.2	-75.5924	-75.0214	-83.2611	4.1199
16.4	-81.8576	-75.9754	-73.0729	4.3924
16.6	-85.2559	-81.9271	-89.8710	3.9720
16.8	-75.7898	-85.4313	-88.9292	6.5697
17.0	-83.9467	-86.8884	-84.8952	1.4709
17.2	-82.9277	-81.5278	-84.5997	1.5360
17.4	-70.4810	-85.2063	-81.3014	7.3627
17.6	-76.1024	-83.8043	-81.6439	3.8510
17.8	-68.3098	-78.6710	-86.2216	8.9559
18.0	-82.1251	-79.0423	-77.1168	2.5042
18.2	-82.3205	-78.8465	-87.0643	4.1089
18.4	-76.9726	-81.5369	-80.2487	2.2822
18.6	-84.4974	-78.9947	-89.1588	5.0821
18.8	-82.1799	-84.0464	-90.5621	4.1911
19.0	-83.5187	-81.7782	-82.4814	0.8702
19.2	-78.3771	-81.3644	-89.6566	5.6398
19.4	-81.8837	-80.3221	-77.1278	2.3780
19.6	-79.9822	-72.7851	-84.8751	6.0450
19.8	-90.4499	-79.2866	-87.1869	5.5817
20.0	-79.9778	-78.5341	-76.3536	1.8121
20.2	-77.8579	-77.6826	-83.9091	3.1133
20.4	-88.4220	-91.3084	-82.2595	4.5245
20.6	-84.3178	-78.8602	-87.7381	4.4390
20.8	-91.8704	-77.9795	-93.2460	7.6333
21.0	-91.4044	-77.9569	-85.0778	6.7238
21.2	-85.3281	-75.4545	-74.0250	5.6516
21.4	-92.4641	-79.5913	-88.7605	6.4364
21.6	-85.9180	-83.1743	-74.7725	5.5728
21.8	-82.3954	-84.5267	-84.1417	1.0657
22.0	-85.7536	-83.0374	-85.5627	1.3581

22.2	-88.1277	-86.7226	-82.6194	2.7542
22.4	-80.1290	-76.8572	-87.1381	5.1404
22.6	-76.9088	-80.4907	-84.4127	3.7520
22.8	-70.8385	-83.1193	-75.1024	6.1404
23.0	-67.0326	-79.2209	-75.2496	6.0942
23.2	-83.3996	-87.3458	-83.7007	1.9731
23.4	-74.5747	-81.4189	-82.2663	3.8458
23.6	-75.6758	-84.3782	-79.4901	4.3512
23.8	-92.3095	-86.2606	-79.4469	6.4313
24.0	-80.4004	-86.0292	-88.3340	3.9668
24.2	-72.5614	-88.5874	-84.6033	8.0130
24.4	-67.3665	-80.7672	-83.4199	8.0267
24.6	-75.6047	-76.9150	-82.5904	3.4929
24.8	-74.5033	-86.8116	-87.3641	6.4304
25.0	-74.9657	-81.1727	-82.9752	4.0048
25.2	-90.7690	-82.3595	-78.1075	6.3308
25.4	-66.4701	-75.4345	-89.4531	11.4915
25.6	-86.4845	-78.7984	-88.5837	4.8927
25.8	-74.1689	-69.1266	-85.6575	8.2655
26.0	-83.5240	-67.4683	-82.2475	8.0279
26.2	-87.6455	-78.4181	-90.2010	5.8915
26.4	-89.4631	-84.7397	-81.4876	3.9878
26.6	-89.4430	-71.4409	-81.4838	9.0011
26.8	-89.7980	-78.5710	-85.6382	5.6135
27.0	-92.0655	-74.9964	-90.0389	8.5346
27.2	-93.4807	-81.5775	-86.2442	5.9516
27.4	-90.7497	-76.5347	-88.9855	7.1075
27.6	-82.3243	-82.6389	-95.0580	6.3669
27.8	-79.3234	-79.0826	-82.0317	1.4746
28.0	-85.3031	-82.6850	-85.2074	1.3091
28.2	-81.7806	-78.5708	-89.0272	5.2282
28.4	-88.5861	-85.6542	-94.5535	4.4497
28.6	-82.2009	-89.3029	-83.4930	3.5510
28.8	-87.2354	-80.5335	-83.6363	3.3510
29.0	-87.2737	-78.4687	-88.7447	5.1380
29.2	-82.8721	-66.4828	-95.3769	14.4471
29.4	-87.2294	-79.6186	-89.7961	5.0888
29.6	-84.6661	-75.2638	-90.4528	7.5945
29.8	-91.0556	-84.4247	-90.4037	3.3155
30.0	-82.2113	-78.5878	-82.2564	1.8343
30.2	-80.6489	-77.3872	-80.0650	1.6309
30.4	-84.9063	-76.0495	-82.2735	4.4284
30.6	-88.0987	-72.6116	-89.1455	8.2670
30.8	-84.4947	-81.9331	-98.3232	8.1951
31.0	-73.4806	-72.7514	-82.2619	4.7553
31.2	-81.3840	-76.5577	-92.2404	7.8414
31.4	-75.2268	-72.5170	-85.8633	6.6732

31.6	-83.1744	-68.1590	-94.9668	13.4039
31.8	-82.9852	-75.4211	-85.1615	4.8702
32.0	-79.3049	-82.7957	-80.8917	1.7454
32.2	-76.8523	-77.3018	-81.2642	2.2060
32.4	-91.6894	-75.3186	-87.2678	8.1854
32.6	-85.5220	-74.8511	-72.2008	6.6606
32.8	-77.4632	-75.8705	-79.1647	1.6471
33.0	-86.7788	-74.3972	-85.8710	6.1908
33.2	-82.3725	-85.8602	-91.1395	4.3835
33.4	-89.0988	-74.0250	-94.7453	10.3602
33.6	-86.0646	-76.9695	-89.3808	6.2057
33.8	-80.8387	-75.6431	-84.7020	4.5295
34.0	-82.8394	-76.7489	-82.3874	3.0453
34.2	-84.8188	-79.0862	-87.9022	4.4080
34.4	-85.3138	-77.1774	-93.6907	8.2567
34.6	-82.5698	-79.6280	-89.0462	4.7091
34.8	-95.5989	-84.5150	-78.0293	8.7848
35.0	-87.0938	-76.3111	-88.4146	6.0518
35.2	-77.8699	-78.3298	-89.4480	5.7891
35.4	-88.7031	-81.9101	-86.8057	3.3965
35.6	-85.6317	-77.5483	-90.7899	6.6208
35.8	-83.4456	-83.4756	-75.9979	3.7389
36.0	-78.5778	-81.6017	-77.4331	2.0843
36.2	-80.4992	-86.6173	-88.8287	4.1648
36.4	-92.1184	-77.2512	-85.5701	7.4336
36.6	-87.3148	-88.3853	-77.7465	5.3194
36.8	-94.0605	-81.6243	-84.0783	6.2181
37.0	-88.4629	-85.3742	-90.1947	2.4103
37.2	-92.2249	-77.5626	-88.9043	7.3312
37.4	-93.9685	-80.8549	-87.9377	6.5568
37.6	-89.1699	-79.4490	-92.4587	6.5049
37.8	-88.9254	-82.0600	-79.8220	4.5517
38.0	-84.7877	-81.3732	-79.7409	2.5234
38.2	-76.3645	-80.0833	-72.8065	3.6384
38.4	-80.2532	-85.9617	-82.5508	2.8542
38.6	-88.9629	-80.8523	-74.9072	7.0279
38.8	-83.7570	-74.9307	-75.0764	4.4132
39.0	-92.0884	-69.3032	-81.7949	11.3926
39.2	-78.5225	-79.0036	-84.9626	3.2201
39.4	-89.0041	-78.0946	-82.8032	5.4548
39.6	-88.2484	-75.9393	-80.5298	6.1546
39.8	-79.4824	-82.7054	-89.2350	4.8763
40.0	-89.8058	-85.9744	-87.2809	1.9157
40.2	-86.4979	-80.9529	-82.8012	2.7725
40.4	-84.8777	-82.6587	-89.7820	3.5617
40.6	-92.2648	-84.8009	-84.7948	3.7350
40.8	-93.6184	-83.5791	-81.6795	5.9694

41.0	-91.3568	-72.6501	-85.5033	9.3534
41.2	-76.4168	-74.9913	-84.5935	4.8011
41.4	-87.0342	-82.3810	-83.8821	2.3266
41.6	-79.4751	-75.4698	-90.5266	7.5284
41.8	-86.1982	-76.4552	-86.9017	5.2233
42.0	-83.2992	-78.7460	-84.7005	2.9773
42.2	-80.2185	-81.5490	-83.0682	1.4249
42.4	-83.6099	-80.2589	-83.4563	1.6755
42.6	-81.1174	-79.2808	-87.1495	3.9344
42.8	-89.7080	-82.4212	-91.2898	4.4343
43.0	-84.2714	-76.6412	-87.0284	5.1936
43.2	-78.3485	-73.3609	-83.8694	5.2543
43.4	-81.4854	-76.2787	-84.8582	4.2898
43.6	-84.3296	-75.0924	-80.8264	4.6186
43.8	-81.0469	-78.4242	-93.9978	7.7868
44.0	-90.2165	-83.9018	-92.0465	4.0724
44.2	-82.5339	-73.7868	-86.5124	6.3628
44.4	-77.9329	-83.7957	-84.1514	3.1093
44.6	-85.0034	-74.7586	-87.3881	6.3148
44.8	-89.5901	-76.4950	-95.7776	9.6413
45.0	-87.2928	-86.3466	-93.4855	3.5695
45.2	-89.9760	-82.6637	-89.8202	3.6562
45.4	-92.9610	-81.4234	-89.3787	5.7688
45.6	-86.5692	-69.4114	-92.4246	11.5066
45.8	-79.2056	-78.5481	-86.4985	3.9752
46.0	-82.9985	-84.0233	-89.3804	3.1909
46.2	-82.5143	-74.3200	-83.9180	4.7990
46.4	-88.1318	-77.8408	-82.5131	5.1455
46.6	-81.3949	-80.6336	-90.2513	4.8089
46.8	-90.8223	-78.6256	-87.4348	6.0984
47.0	-86.2006	-85.3861	-75.1611	5.5197
47.2	-89.5345	-79.2939	-75.3388	7.0978
47.4	-85.7382	-79.2605	-84.4371	3.2389
47.6	-89.3260	-79.9261	-81.0783	4.6999
47.8	-85.4532	-74.6029	-87.0620	6.2296
48.0	-75.1904	-81.3502	-94.1607	9.4852
48.2	-80.7907	-83.5335	-93.8770	6.5432
48.4	-80.1441	-78.5388	-82.8834	2.1723
48.6	-86.5315	-80.9133	-91.3622	5.2245
48.8	-90.9858	-75.6399	-84.2792	7.6730
49.0	-83.1722	-78.1630	-90.1469	5.9920
49.2	-87.2446	-84.9702	-73.8801	6.6823
49.4	-87.6308	-72.4811	-82.4999	7.5749
49.6	-86.0873	-79.1895	-86.5997	3.7051
49.8	-78.5888	-82.4335	-81.6629	1.9223
50.0	-84.0240	-77.9775	-80.6879	3.0233

Table 3: The effect of 25°C simulation temperature on the binding free energy (ΔG) of 4H11-scFv and MUC16^{ecto} in 50ns

Binding time (ns) ± 3	Trial 1 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 2 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 3 Binding Free Energy (ΔG) (kcal/mol) ± 1	Statistical Uncertainty (ΔG) (kcal/mol) ± 1 (rounded to 4 decimal places)
0.2	-74.6318	-85.6190	-81.9986	5.4936
0.4	-84.7177	-93.4180	-86.5045	4.3502
0.6	-84.7659	-89.2630	-93.2947	4.2644
0.8	-91.3838	-90.0487	-81.3776	5.0031
1.0	-85.9838	-92.9607	-80.1239	6.4184
1.2	-92.6840	-81.5868	-88.7683	5.5486
1.4	-85.5543	-88.9476	-70.5282	9.2097
1.6	-80.9594	-77.8148	-84.3074	3.2463
1.8	-71.1877	-86.5125	-76.0168	7.6624
2.0	-83.8061	-80.3047	-87.0854	3.3904
2.2	-79.0655	-84.2216	-88.5391	4.7368
2.4	-83.8723	-87.9110	-93.2061	4.6669
2.6	-82.7188	-86.0497	-94.7071	5.9942
2.8	-82.1002	-85.1334	-95.8092	6.8545
3.0	-74.5219	-91.7743	-91.0346	8.6262
3.2	-80.2573	-83.8336	-88.9256	4.3342
3.4	-81.6027	-89.7309	-84.7649	4.0641
3.6	-82.9160	-90.9216	-89.5451	4.0028
3.8	-71.1811	-84.4018	-83.9617	6.6104
4.0	-80.6985	-85.9606	-90.4417	4.8716
4.2	-84.6094	-87.0105	-90.7258	3.0582
4.4	-78.8578	-87.0362	-79.7751	4.0892
4.6	-82.8711	-84.2266	-90.0827	3.6058
4.8	-75.4807	-91.3018	-89.3748	7.9106
5.0	-80.9400	-85.2211	-90.2528	4.6564
5.2	-88.1709	-84.6531	-88.5254	1.9362
5.4	-83.1895	-82.8957	-90.9667	4.0355
5.6	-81.4345	-91.4747	-96.1523	7.3589
5.8	-92.8140	-86.4858	-91.1054	3.1641
6.0	-84.5850	-84.9878	-92.0176	3.7163
6.2	-82.9300	-97.0144	-81.8689	7.5728
6.4	-85.6060	-80.8628	-84.2252	2.3716
6.6	-88.4730	-93.9172	-86.6224	3.6474
6.8	-83.5326	-94.0851	-91.9285	5.2763
7.0	-90.5408	-94.6589	-82.3534	6.1528
7.2	-85.3457	-91.4595	-89.1466	3.0569
7.4	-79.0677	-87.1811	-92.4514	6.6919
7.6	-86.1651	-93.5394	-90.1448	3.6872
7.8	-95.6276	-87.8973	-74.6936	10.4670

8.0	-96.8085	-96.2238	-88.3358	4.2363
8.2	-84.5573	-93.6522	-94.8116	5.1272
8.4	-81.7854	-95.2525	-84.4846	6.7336
8.6	-82.8558	-90.5491	-85.3806	3.8467
8.8	-76.6562	-88.2871	-86.6030	5.8155
9.0	-83.7246	-89.0066	-93.0308	4.6531
9.2	-83.8751	-91.4701	-86.8510	3.7975
9.4	-89.9662	-99.1632	-97.7162	4.5985
9.6	-83.7659	-95.6171	-88.2863	5.9256
9.8	-90.5138	-95.8016	-72.6556	11.5730
10.0	-89.7974	-95.0777	-87.1061	3.9858
10.2	-86.0261	-93.2427	-92.0625	3.6083
10.4	-88.2394	-90.2718	-88.9986	1.0162
10.6	-86.1233	-96.4197	-88.2361	5.1482
10.8	-84.2446	-92.7846	-85.7600	4.2700
11.0	-89.3540	-95.3569	-85.862	4.7475
11.2	-80.6254	-92.6268	-92.9363	6.1555
11.4	-76.7313	-95.1909	-88.7623	9.2298
11.6	-86.8107	-92.4603	-88.0172	2.8248
11.8	-89.4979	-97.9937	-89.2116	4.3911
12.0	-84.9978	-95.3167	-87.1342	5.1595
12.2	-89.7681	-96.3801	-91.9098	3.3060
12.4	-79.5500	-92.4414	-88.9274	6.4457
12.6	-81.8843	-95.8528	-87.4262	6.9843
12.8	-86.1014	-94.0345	-85.2883	4.3731
13.0	-86.4359	-95.6223	-85.1471	5.2376
13.2	-84.4644	-91.2901	-89.4428	3.4129
13.4	-79.6352	-98.6191	-85.7739	9.4920
13.6	-85.0363	-96.4286	-79.0053	8.7117
13.8	-85.6641	-82.0244	-82.229	1.8199
14.0	-88.9310	-96.1471	-79.8574	8.1449
14.2	-82.3225	-81.1556	-79.0716	1.6255
14.4	-77.4365	-95.1419	-79.6157	8.8527
14.6	-82.9589	-92.2794	-84.258	4.6603
14.8	-83.9364	-89.7259	-81.0324	4.3468
15.0	-82.6205	-85.5318	-82.7407	1.4557
15.2	-89.2825	-89.0061	-91.1430	1.0685
15.4	-82.3517	-90.7864	-85.5751	4.2174
15.6	-82.5337	-92.8921	-76.8212	8.0355
15.8	-84.5008	-88.3185	-78.2007	5.0589
16.0	-82.7203	-85.7099	-86.6924	1.9861
16.2	-74.8378	-97.0227	-91.3503	11.0925
16.4	-78.0587	-77.7296	-84.7571	3.5137
16.6	-85.9751	-94.5363	-85.879	4.3287
16.8	-84.6881	-91.4188	-86.9862	3.3654
17.0	-90.2447	-87.5642	-88.3281	1.3403
17.2	-85.2359	-83.4960	-94.8563	5.6802

17.4	-81.5294	-88.9910	-79.3129	4.8391
17.6	-82.7757	-85.8395	-75.0815	5.3790
17.8	-91.4527	-94.1035	-84.2270	4.9383
18.0	-86.6536	-89.8576	-87.1719	1.6020
18.2	-80.8633	-96.9639	-77.9472	9.5084
18.4	-77.1984	-94.887	-87.4200	8.8443
18.6	-79.5128	-95.3403	-83.5326	7.9138
18.8	-84.8939	-86.6323	-83.2064	1.7130
19.0	-93.4469	-86.7720	-84.2598	4.5936
19.2	-88.6441	-95.3890	-79.1719	8.1086
19.4	-87.3014	-93.6783	-79.9207	6.8788
19.6	-86.8212	-98.3299	-81.9596	8.1852
19.8	-94.1411	-92.6789	-85.2615	4.4398
20.0	-93.6851	-95.2353	-78.2982	8.4686
20.2	-89.6803	-95.1539	-80.2752	7.4394
20.4	-89.0666	-99.2606	-81.6478	8.8064
20.6	-83.0161	-95.2045	-77.0887	9.0579
20.8	-90.0946	-99.8569	-81.9904	8.9333
21.0	-80.3054	-86.5340	-74.4695	6.0323
21.2	-84.0240	-94.0027	-79.4970	7.2529
21.4	-79.1191	-93.0254	-71.8734	10.5760
21.6	-80.2585	-98.2173	-91.0218	8.9794
21.8	-82.8133	-104.362	-84.8490	10.7744
22.0	-80.6613	-89.4923	-83.1091	4.4155
22.2	-90.3406	-94.8228	-76.2663	9.2783
22.4	-82.3220	-86.5868	-82.9568	2.1324
22.6	-79.6462	-90.3975	-83.9435	5.3757
22.8	-88.9442	-94.9043	-87.7591	3.5726
23.0	-75.6467	-91.6275	-88.5107	7.9904
23.2	-89.8495	-93.7937	-79.7168	7.0385
23.4	-86.8366	-94.5849	-87.1075	3.8742
23.6	-90.7112	-91.0665	-75.0018	8.0324
23.8	-82.9250	-92.6592	-79.3946	6.6323
24.0	-83.6265	-107.7133	-84.9634	12.0434
24.2	-87.7510	-87.7907	-87.4758	0.1574
24.4	-82.1200	-92.0518	-80.7276	5.6621
24.6	-78.7947	-97.6834	-85.2936	9.4444
24.8	-80.5045	-90.4310	-88.7149	4.9633
25.0	-88.3498	-95.5695	-82.1073	6.7311
25.2	-83.4349	-102.1779	-82.0020	10.0880
25.4	-85.7971	-92.7717	-81.9892	5.3913
25.6	-83.1874	-89.9843	-82.0666	3.9589
25.8	-84.8828	-95.5214	-84.0091	5.7562
26.0	-83.0992	-92.6363	-87.5627	4.7686
26.2	-87.9813	-97.1065	-83.5612	6.7727
26.4	-78.0366	-100.1837	-84.3937	11.0736
26.6	-77.0061	-100.9721	-80.2090	11.9830

26.8	-83.3507	-101.071	-75.2046	12.9332
27.0	-83.9516	-88.9898	-78.1673	5.4113
27.2	-87.4790	-88.9783	-87.0422	0.9681
27.4	-87.9611	-97.9983	-89.2964	5.0186
27.6	-79.8791	-90.9000	-83.7343	5.5105
27.8	-90.4497	-96.9766	-85.3205	5.8281
28.0	-78.9271	-97.4008	-81.4198	9.2369
28.2	-82.0354	-99.088	-85.1762	8.5263
28.4	-91.5676	-102.919	-90.6554	6.1318
28.6	-88.4550	-99.2002	-85.3379	6.9312
28.8	-88.2012	-94.4714	-89.1210	3.1351
29.0	-86.6272	-94.6224	-75.8722	9.3751
29.2	-88.7744	-91.9493	-74.4007	8.7743
29.4	-88.8764	-88.5287	-78.4562	5.2101
29.6	-86.0632	-92.1760	-85.0105	3.5828
29.8	-89.0829	-89.0302	-82.1421	3.4704
30.0	-91.1833	-100.9033	-80.8550	10.0242
30.2	-83.8333	-92.3489	-79.1666	6.5912
30.4	-90.1954	-100.5791	-78.7157	10.9317
30.6	-83.3282	-92.8784	-90.5131	4.7751
30.8	-82.1771	-92.4698	-96.7301	7.2765
31.0	-87.2332	-92.7963	-86.3505	3.2229
31.2	-77.0474	-96.6782	-85.2307	9.8154
31.4	-87.9089	-94.0347	-80.7878	6.6235
31.6	-80.9928	-91.3342	-88.0625	5.1707
31.8	-82.1550	-95.2181	-77.6374	8.7904
32.0	-75.5714	-95.3613	-75.2480	10.0567
32.2	-81.2956	-93.4733	-87.1255	6.0889
32.4	-80.3785	-93.8844	-77.4511	8.2167
32.6	-78.2710	-95.0950	-83.7418	8.4120
32.8	-88.0235	-96.4553	-80.2410	8.1072
33.0	-89.0639	-91.3760	-83.3316	4.0222
33.2	-85.2759	-88.8659	-91.5338	3.1290
33.4	-81.7470	-95.2589	-90.1080	6.7560
33.6	-86.9423	-94.2094	-92.2682	3.6336
33.8	-88.1372	-98.5574	-92.6389	5.2101
34.0	-88.2566	-85.6888	-91.5766	2.9439
34.2	-83.4721	-89.5786	-90.1122	3.3201
34.4	-81.9797	-100.4759	-83.5843	9.2481
34.6	-82.3524	-96.8458	-98.1934	7.9205
34.8	-77.6395	-91.7254	-87.4491	7.0430
35.0	-83.5978	-99.0108	-84.5717	7.7065
35.2	-96.2423	-90.8332	-87.4562	4.3931
35.4	-87.8066	-86.6835	-87.6916	0.5616
35.6	-78.1841	-88.3496	-78.1218	5.1139
35.8	-80.9380	-92.1986	-89.4088	5.6303
36.0	-87.4964	-94.2588	-89.3409	3.3812

36.2	-84.3668	-99.1772	-96.1233	7.4052
36.4	-80.5501	-90.6365	-92.2053	5.8276
36.6	-81.2866	-85.7644	-89.1026	3.9080
36.8	-85.5808	-91.8081	-87.1166	3.1137
37.0	-86.5811	-99.9956	-79.6686	10.1635
37.2	-83.5405	-94.6142	-82.8567	5.8788
37.4	-86.2237	-87.6611	-94.4355	4.1059
37.6	-86.2537	-103.0439	-84.1174	9.4633
37.8	-87.1895	-94.7640	-83.5775	5.5933
38.0	-79.4681	-94.4892	-85.0769	7.5105
38.2	-88.2455	-101.973	-86.2228	1.0114
38.4	-86.0255	-88.1128	-85.1437	1.4846
38.6	-91.5813	-93.6631	-77.3166	8.1733
38.8	-81.1768	-96.7085	-84.4788	7.7659
39.0	-84.4083	-102.1856	-93.3212	8.8887
39.2	-88.2528	-99.3674	-90.519	5.5573
39.4	-91.5366	-94.0693	-93.6943	1.2664
39.6	-79.7350	-91.3023	-88.3098	5.7837
39.8	-75.5208	-86.4302	-85.4303	5.4547
40.0	-81.3243	-92.9309	-86.0494	5.8033
40.2	-91.3752	-92.4689	-89.1370	1.6660
40.4	-92.7344	-91.5928	-87.7869	2.4738
40.6	-82.8483	-86.0014	-92.3846	4.7682
40.8	-86.6943	-94.3381	-91.7178	3.8219
41.0	-86.4349	-94.8038	-87.2102	4.1845
41.2	-90.3299	-102.3322	-86.7529	7.7897
41.4	-85.5471	-92.9401	-86.2695	3.6965
41.6	-89.8322	-94.565	-92.1539	2.3664
41.8	-85.4033	-87.7579	-84.4332	1.6624
42.0	-88.1103	-87.2063	-101.6170	7.2054
42.2	-90.6167	-89.1710	-88.3529	1.1319
42.4	-88.6313	-92.5771	-93.5673	2.4680
42.6	-95.5546	-92.9038	-93.1093	1.3254
42.8	-88.8826	-93.6391	-97.4359	4.2767
43.0	-85.4793	-94.5688	-92.5160	4.5448
43.2	-88.2260	-94.3522	-89.0371	3.0631
43.4	-86.8169	-89.3100	-98.5513	5.8672
43.6	-85.9240	-100.7162	-86.2064	7.3961
43.8	-94.5607	-87.1723	-94.1621	3.6942
44.0	-88.5764	-90.1529	-95.9828	3.7032
44.2	-97.2693	-96.4410	-83.4158	6.9268
44.4	-88.2641	-101.6182	-92.7068	6.6771
44.6	-88.0056	-97.5607	-90.5005	4.7776
44.8	-90.3601	-99.2774	-86.9188	6.1793
45.0	-87.8453	-95.7277	-93.9038	3.9412
45.2	-78.8854	-94.0991	-91.9461	7.6069
45.4	-91.5557	-86.7221	-94.1017	3.6898

45.6	-94.1727	-96.4536	-85.0252	5.7142
45.8	-90.2476	-86.2100	-84.7889	2.7294
46.0	-84.2461	-87.1531	-92.6324	4.1932
46.2	-86.2597	-103.2167	-85.2073	9.0047
46.4	-84.0782	-94.0541	-86.8119	4.9880
46.6	-82.9921	-92.7964	-96.1230	6.5655
46.8	-90.2162	-101.1705	-87.6781	6.7462
47.0	-83.4811	-93.2915	-90.922	4.9052
47.2	-78.5565	-93.5233	-86.4529	7.4834
47.4	-80.0554	-94.6662	-90.7711	7.3054
47.6	-77.0761	-99.5965	-83.7507	11.2602
47.8	-78.7512	-91.6698	-89.1777	6.4593
48.0	-77.7953	-91.5531	-96.9530	9.5789
48.2	-89.4866	-90.6983	-87.2995	1.6994
48.4	-85.6140	-87.3839	-85.9051	0.8849
48.6	-83.9551	-92.3953	-85.9289	4.2201
48.8	-84.3158	-89.9527	-90.3425	3.0134
49.0	-84.5523	-94.6392	-86.1529	5.0435
49.2	-80.9935	-97.4414	-85.7333	8.2240
49.4	-86.4713	-111.5497	-87.1067	12.5392
49.6	-85.1232	-94.1280	-86.3478	4.5024
49.8	-84.8356	-94.8651	-87.8704	5.0148
50.0	-89.2605	-96.8120	-75.0365	10.8878

Table 4: The effect of 35°C simulation temperature on the binding free energy (ΔG) of 4H11-scFv and MUC16^{ecto} in 50ns

Binding time (ns) ± 3	Trial 1 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 2 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 3 Binding Free Energy (ΔG) (kcal/mol) ± 1	Statistical Uncertainty (ΔG) (kcal/mol) ± 1 (rounded to 4 decimal places)
0.2	-73.2239	-83.1528	-104.6278	15.7020
0.4	-80.5414	-84.9247	-97.5903	8.5245
0.6	-76.3494	-85.5545	-88.7861	6.2184
0.8	-71.1271	-86.1234	-87.0312	7.9521
1.0	-73.0235	-86.7511	-85.1447	6.8638
1.2	-67.594	-80.6884	-79.5111	6.5472
1.4	-78.2448	-85.9227	-86.1637	3.9595
1.6	-71.3093	-84.0237	-81.7798	6.3572
1.8	-70.0697	-83.2662	-97.1694	13.5499
2.0	-70.9126	-81.8672	-96.5931	12.8403
2.2	-82.1193	-85.7324	-98.2373	8.0590
2.4	-80.08	-89.3011	-77.8185	5.7413
2.6	-75.7853	-71.2636	-95.0301	11.8833
2.8	-77.8815	-83.0364	-83.1869	2.6527
3.0	-78.8411	-77.1237	-88.9259	5.9011

3.2	-77.5293	-81.7942	-98.4029	10.4368
3.4	-73.2471	-81.2547	-85.0933	5.9231
3.6	-72.1374	-81.4314	-88.2239	8.0433
3.8	-79.6162	-80.6711	-100.5579	10.4709
4.0	-78.247	-83.5561	-92.2848	7.0189
4.2	-66.6946	-79.8649	-91.8660	12.5857
4.4	-80.9441	-86.9914	-91.9221	5.4890
4.6	-84.9685	-84.0582	-90.2427	3.0923
4.8	-83.6904	-87.0009	-92.3935	4.3516
5.0	-78.601	-86.6041	-97.0489	9.2240
5.2	-87.4429	-79.7150	-97.1607	8.7229
5.4	-79.2221	-86.1280	-86.0550	3.4530
5.6	-80.8722	-90.1621	-97.3934	8.2606
5.8	-70.8926	-92.8931	-83.0600	11.0003
6.0	-69.3641	-86.7846	-85.2031	8.7103
6.2	-80.1713	-82.3935	-86.7253	3.2770
6.4	-81.1448	-85.1429	-83.6218	1.9991
6.6	-68.0932	-89.4521	-77.2504	10.6795
6.8	-79.3062	-81.9671	-79.1930	1.3871
7.0	-71.3252	-86.1319	-87.4927	8.0838
7.2	-73.818	-89.8556	-81.6180	8.0188
7.4	-84.6519	-86.3857	-81.6853	2.3502
7.6	-72.5976	-94.6520	-85.9299	11.0272
7.8	-76.0775	-82.0115	-87.5091	5.7158
8.0	-77.1384	-87.8719	-87.7956	5.3668
8.2	-67.134	-91.5795	-84.1758	12.2228
8.4	-75.8007	-84.6290	-97.3069	10.7531
8.6	-78.4867	-92.2293	-96.1297	8.8215
8.8	-72.4244	-81.1271	-90.6068	9.0912
9.0	-81.0821	-82.7396	-90.8418	4.8799
9.2	-88.2579	-87.2401	-85.6038	1.3271
9.4	-77.6002	-78.7945	-85.4444	3.9221
9.6	-85.4093	-85.2857	-94.6820	4.6982
9.8	-77.0513	-80.1271	-82.0517	2.5002
10.0	-74.3768	-85.9785	-94.3826	10.0029
10.2	-73.7215	-87.8061	-93.0897	9.6841
10.4	-77.9906	-80.1700	-88.9672	5.4883
10.6	-75.0341	-81.2729	-89.7917	7.3788
10.8	-68.5045	-80.9260	-87.6345	9.5650
11.0	-67.3771	-83.9225	-95.3036	13.9633
11.2	-69.4457	-100.5199	-94.5307	15.5371
11.4	-64.7876	-95.7249	-96.6400	15.9262
11.6	-75.3572	-89.0127	-94.6733	9.6581
11.8	-72.3343	-83.3962	-80.1380	5.5310
12.0	-73.7711	-87.3832	-92.9102	9.5696
12.2	-77.2052	-81.6265	-92.9113	7.8531
12.4	-89.5728	-82.2747	-94.8136	6.2695

12.6	-79.8612	-74.7322	-87.6878	6.4778
12.8	-79.2834	-88.7297	-83.4773	4.7232
13.0	-81.3976	-80.0759	-84.1600	2.0421
13.2	-69.3707	-81.9056	-86.8029	8.7161
13.4	-63.5148	-88.8886	-75.0499	12.6869
13.6	-69.7551	-83.5619	-88.4131	9.3290
13.8	-71.9762	-84.1297	-90.4197	9.2218
14.0	-80.614	-86.8859	-97.6084	8.4972
14.2	-83.1484	-75.3307	-87.8463	6.2578
14.4	-80.8101	-81.9459	-75.4874	3.2293
14.6	-76.4286	-72.6502	-97.2310	12.2904
14.8	-82.696	-77.3087	-87.9128	5.3021
15.0	-77.996	-82.9949	-87.4817	4.7429
15.2	-83.8801	-78.0281	-92.1882	7.0801
15.4	-76.4905	-87.7106	-84.4146	5.6101
15.6	-84.3152	-87.1718	-90.9592	3.3220
15.8	-69.9945	-84.9446	-89.6947	9.8501
16.0	-75.427	-83.8694	-86.2019	5.3874
16.2	-71.6094	-97.3427	-80.5072	12.8667
16.4	-78.5594	-93.6148	-77.8361	7.8894
16.6	-71.1124	-85.7645	-96.0574	12.4725
16.8	-75.972	-82.9013	-87.9880	6.0080
17.0	-85.3542	-83.7496	-86.9742	1.6123
17.2	-82.7449	-89.8037	-90.6693	3.9622
17.4	-79.8046	-86.1627	-96.7627	8.4791
17.6	-84.3411	-86.3037	-94.2693	4.9641
17.8	-74.098	-93.4927	-104.2016	15.0518
18.0	-77.3862	-76.5383	-97.9679	10.7148
18.2	-85.6702	-88.2401	-91.9206	3.1252
18.4	-79.0218	-77.7007	-102.0452	12.1723
18.6	-88.2426	-83.8793	-93.1739	4.6473
18.8	-80.5203	-95.6709	-100.4555	9.9676
19.0	-84.049	-83.6248	-101.7069	9.0411
19.2	-86.5045	-84.6239	-95.2481	5.3121
19.4	-87.7261	-88.8546	-100.2916	6.2828
19.6	-86.0548	-76.0336	-93.7053	8.8358
19.8	-88.4985	-76.9509	-102.4110	12.7301
20.0	-82.5052	-73.9265	-96.4908	11.2822
20.2	-85.6191	-74.6186	-89.9024	7.6419
20.4	-83.5778	-84.0157	-86.7966	1.6094
20.6	-89.1167	-83.0153	-94.8266	5.9057
20.8	-81.9745	-74.1032	-91.9498	8.9233
21.0	-84.2387	-82.9885	-102.7110	9.8613
21.2	-90.1836	-83.1712	-102.2214	9.5251
21.4	-80.2712	-88.1410	-100.7577	10.2433
21.6	-80.7738	-81.0048	-94.9289	7.0776
21.8	-79.9743	-85.0509	-93.7012	6.8635

22.0	-88.6681	-85.8234	-98.6530	6.4148
22.2	-86.176	-82.0372	-100.2568	9.1098
22.4	-84.3683	-87.5001	-95.6747	5.6532
22.6	-80.5743	-83.6812	-100.5663	9.9960
22.8	-79.3261	-90.2775	-97.8005	9.2372
23.0	-85.4643	-84.1537	-86.1433	0.9948
23.2	-94.4341	-80.4223	-88.0178	7.0059
23.4	-77.8194	-82.2804	-93.0243	7.6025
23.6	-88.7028	-81.7454	-90.8161	4.5354
23.8	-83.175	-77.3148	-100.0199	11.3526
24.0	-78.2501	-79.6265	-90.1027	5.9263
24.2	-85.706	-84.2014	-88.1949	1.9968
24.4	-75.3942	-86.8143	-87.7723	6.1891
24.6	-90.3539	-89.9155	-89.2233	0.5653
24.8	-78.6767	-70.4889	-85.1148	7.3130
25.0	-70.9671	-84.6064	-82.4841	6.8197
25.2	-77.0365	-87.1004	-88.0700	5.5167
25.4	-78.9151	-87.0610	-89.7736	5.4293
25.6	-82.3947	-76.4481	-86.2942	4.9231
25.8	-81.7486	-79.4839	-85.7757	3.1459
26.0	-84.0681	-76.9344	-86.7596	4.9126
26.2	-80.8527	-81.9967	-88.0894	3.6184
26.4	-87.5166	-72.8425	-82.0229	7.3371
26.6	-82.567	-87.5048	-85.2401	2.4689
26.8	-85.3191	-81.4018	-89.1970	3.8976
27.0	-87.6508	-90.3306	-78.0484	6.1411
27.2	-83.861	-80.6483	-80.8307	1.6064
27.4	-78.9244	-79.6507	-81.5619	1.3187
27.6	-82.0181	-75.3781	-89.6495	7.1357
27.8	-71.6188	-83.3415	-88.5820	8.4816
28.0	-82.8164	-80.6642	-97.7210	8.5284
28.2	-89.6453	-87.2777	-95.4690	4.0957
28.4	-82.3737	-91.2037	-101.7165	9.6714
28.6	-76.1038	-85.9069	-91.6043	7.7502
28.8	-83.9318	-81.9944	-91.4018	4.7037
29.0	-77.8676	-81.4730	-84.8097	3.4711
29.2	-80.6294	-85.5758	-89.2470	4.3088
29.4	-89.0682	-85.3237	-94.9179	4.7971
29.6	-78.6332	-87.2673	-92.5427	6.9548
29.8	-72.0745	-90.7149	-96.5820	12.2538
30.0	-83.3313	-81.0195	-96.5386	7.7596
30.2	-78.2703	-85.0382	-82.6603	3.3840
30.4	-78.2966	-76.7052	-87.4388	5.3668
30.6	-78.7585	-85.4186	-85.8415	3.5415
30.8	-79.4009	-82.6283	-89.4046	5.0019
31.0	-81.1784	-85.6197	-91.4660	5.1438
31.2	-75.9416	-78.2151	-93.0710	8.5647

31.4	-75.4237	-79.9796	-86.7429	5.6596
31.6	-79.5678	-84.7935	-94.2019	7.3170
31.8	-81.9665	-70.3941	-85.2172	7.4116
32.0	-84.3536	-81.7127	-79.7800	2.2868
32.2	-83.9704	-84.8599	-85.6114	0.8205
32.4	-80.6595	-79.0036	-90.9397	5.9681
32.6	-80.905	-80.7738	-91.6451	5.4357
32.8	-81.0803	-81.9098	-94.1559	6.5378
33.0	-82.9313	-88.6347	-89.2658	3.1673
33.2	-85.9232	-88.8906	-82.9376	2.9765
33.4	-91.2798	-78.7148	-88.7366	6.2825
33.6	-81.6508	-78.1985	-89.9171	5.8593
33.8	-92.058	-85.6828	-92.3859	3.3516
34.0	-83.5569	-83.5317	-95.4487	5.9585
34.2	-84.5226	-84.8101	-87.3650	1.4212
34.4	-87.8502	-87.6406	-92.3699	2.3647
34.6	-92.2301	-77.1083	-92.1438	7.5609
34.8	-91.0501	-77.6497	-76.3991	7.3255
35.0	-73.6637	-76.1612	-84.9521	5.6442
35.2	-67.8494	-75.7849	-88.4033	10.2770
35.4	-85.9474	-87.4119	-85.9722	0.7323
35.6	-77.7762	-86.5863	-91.7571	6.9905
35.8	-79.0017	-83.6514	-77.6082	3.0216
36.0	-78.5156	-75.7476	-88.8108	6.5316
36.2	-66.7049	-91.4868	-92.4880	12.8916
36.4	-83.5735	-77.6805	-87.9515	5.1355
36.6	-81.4911	-73.0077	-79.3408	4.2417
36.8	-77.7266	-80.6877	-88.5309	5.4022
37.0	-85.8232	-78.4618	-82.5559	3.6807
37.2	-89.2039	-78.8974	-91.8250	6.4638
37.4	-80.9443	-93.1758	-84.0258	6.1158
37.6	-80.064	-76.0066	-81.8332	2.9133
37.8	-78.6027	-81.3787	-90.9191	6.1582
38.0	-83.6929	-87.9931	-85.8748	2.1501
38.2	-83.7282	-81.4895	-83.0504	1.1194
38.4	-84.3978	-75.6933	-90.0771	7.1919
38.6	-87.9811	-78.3965	-82.8476	4.7923
38.8	-83.1362	-80.6047	-76.6988	3.2187
39.0	-86.0301	-84.7193	-77.1003	4.4649
39.2	-84.1092	-85.7045	-88.0939	1.9924
39.4	-93.1758	-83.4051	-89.6983	4.8854
39.6	-78.3577	-90.8106	-87.8279	6.2265
39.8	-79.7446	-82.6654	-85.9983	3.1269
40.0	-80.72	-75.1846	-81.5862	3.2008
40.2	-85.912	-84.4630	-71.1057	7.4032
40.4	-80.2409	-84.9499	-88.0383	3.8987
40.6	-75.9242	-80.5837	-83.4043	3.7401

40.8	-82.5526	-76.2726	-85.9606	4.8440
41.0	-73.2075	-78.1161	-93.1813	9.9869
41.2	-79.0025	-79.3669	-89.3975	5.1975
41.4	-76.7258	-79.8101	-87.0933	5.1838
41.6	-86.1899	-84.2127	-89.8440	2.8157
41.8	-90.4344	-83.2767	-87.0019	3.5789
42.0	-74.3743	-79.5004	-89.7550	7.6904
42.2	-77.0638	-82.5419	-85.6244	4.2803
42.4	-83.3821	-89.6661	-84.1983	3.1420
42.6	-85.5026	-92.3686	-95.6707	5.0841
42.8	-81.2206	-84.0392	-83.5258	1.4093
43.0	-84.0051	-96.1680	-97.5542	6.7746
43.2	-81.4874	-88.5859	-89.4998	4.0062
43.4	-76.2807	-86.8191	-90.6891	7.2042
43.6	-80.5401	-92.4200	-89.3952	5.9400
43.8	-78.266	-93.8389	-89.7661	7.7864
44.0	-82.5719	-95.2771	-93.9253	6.3526
44.2	-66.9541	-79.3488	-83.0519	8.0489
44.4	-81.6686	-82.0661	-86.2077	2.2696
44.6	-78.1306	-80.0012	-90.5039	6.1867
44.8	-76.9909	-88.2397	-96.1675	9.5883
45.0	-82.3626	-72.3845	-91.0728	9.3442
45.2	-84.1365	-87.2556	-82.6801	2.2878
45.4	-77.7734	-80.8599	-93.9843	8.1055
45.6	-86.3159	-75.4899	-93.2875	8.8988
45.8	-81.2079	-89.0170	-93.5584	6.1753
46.0	-80.9697	-80.2872	-91.8249	5.7689
46.2	-78.3202	-72.6007	-85.7298	6.5646
46.4	-85.8059	-80.5579	-101.1786	10.3104
46.6	-72.7234	-75.8289	-87.3062	7.2914
46.8	-84.4454	-85.0883	-85.6295	0.5920
47.0	-77.1643	-75.5749	-90.0190	7.2221
47.2	-79.607	-82.6726	-87.3992	3.8961
47.4	-78.9244	-81.0147	-99.7754	10.4255
47.6	-75.3002	-79.8484	-96.8921	10.7960
47.8	-80.3197	-79.3301	-100.5452	10.6076
48.0	-77.1674	-74.5004	-87.8074	6.6535
48.2	-68.0486	-87.4830	-85.1128	9.7172
48.4	-85.3988	-79.7367	-97.7004	8.9819
48.6	-83.2488	-93.2132	-90.7721	4.9822
48.8	-75.2496	-98.3933	-85.2002	11.5719
49.0	-72.5349	-90.7625	-89.2447	9.1138
49.2	-73.2601	-78.9836	-89.6163	8.1781
49.4	-75.8426	-80.1884	-94.2134	9.1854
49.6	-76.89	-84.3741	-96.4721	9.7911
49.8	-73.8633	-94.1776	-89.6725	10.1572
50.0	-76.5942	-85.4928	-98.4014	10.9036

Table 5: The effect of 45°C simulation temperature on the binding free energy (ΔG) of 4H11-scFv and MUC16^{ecto} in 50ns

Binding time (ns) ± 3	Trial 1 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 2 Binding Free Energy (ΔG) (kcal/mol) ± 1	Trial 3 Binding Free Energy (ΔG) (kcal/mol) ± 1	Statistical Uncertainty (ΔG) (kcal/mol) ± 1 (rounded to 4 decimal places)
0.2	-83.7321	-85.4819	-95.5953	5.9316
0.4	-97.5767	-94.3906	-94.7044	1.5931
0.6	-90.5307	-91.4824	-97.2645	3.3669
0.8	-96.8310	-84.6337	-98.0199	6.6931
1.0	-87.4955	-88.4504	-98.7135	5.6090
1.2	-95.3767	-88.1105	-90.0659	3.6331
1.4	-99.1727	-90.4470	-93.0323	4.3629
1.6	-93.3981	-89.8003	-91.8992	1.7989
1.8	-81.1144	-84.3175	-87.7228	3.3042
2.0	-95.2162	-84.5524	-83.7591	5.7286
2.2	-97.2451	-89.6822	-92.9038	3.7815
2.4	-91.0286	-88.8687	-82.2697	4.3795
2.6	-98.3164	-92.2892	-87.0039	5.6563
2.8	-100.0409	-98.5459	-86.1094	6.9658
3.0	-94.0823	-97.3839	-91.8043	2.7898
3.2	-102.6991	-91.3665	-99.5362	5.6663
3.4	-89.6241	-96.3692	-84.8215	5.7739
3.6	-101.7159	-93.6769	-91.8722	4.9219
3.8	-95.8614	-91.1762	-97.2708	3.0473
4.0	-94.9655	-87.4559	-83.4462	5.7597
4.2	-104.1011	-84.3626	-87.8823	9.8693
4.4	-101.4572	-100.1499	-82.1355	9.6609
4.6	-105.9096	-100.8236	-88.6446	8.6325
4.8	-99.8140	-90.5550	-89.4163	5.1988
5.0	-90.5231	-84.6431	-94.1041	4.7305
5.2	-92.5259	-95.9233	-91.4326	2.2454
5.4	-95.5344	-92.3925	-86.4687	4.5329
5.6	-100.1829	-89.3122	-84.0349	8.0740
5.8	-95.1116	-84.1854	-83.5312	5.7902
6.0	-92.1098	-90.2080	-83.8892	4.1103
6.2	-95.5713	-89.1788	-81.8635	6.8539
6.4	-89.9729	-76.9328	-98.9086	10.9879
6.6	-107.2640	-89.8100	-80.1135	13.5753
6.8	-102.1436	-95.7918	-82.0708	10.0364
7.0	-116.0461	-88.8589	-81.1642	17.4410
7.2	-91.7644	-103.6763	-76.5148	13.5808
7.4	-110.8222	-96.8701	-82.6021	14.1101
7.6	-97.3688	-93.8876	-82.6818	7.3435

7.8	-92.4126	-85.0902	-89.9516	3.6612
8.0	-90.4149	-95.5901	-87.1287	4.2307
8.2	-90.4171	-95.4665	-89.8106	2.8280
8.4	-95.9588	-96.1708	-77.1100	9.5304
8.6	-103.2608	-97.6809	-80.7144	11.2732
8.8	-100.5103	-102.4279	-82.1622	10.1329
9.0	-103.4475	-100.9237	-91.4365	6.0055
9.2	-101.5602	-96.2915	-88.5128	6.5237
9.4	-98.2041	-88.9765	-81.6831	8.2605
9.6	-100.8923	-91.0661	-77.2744	11.8090
9.8	-103.6745	-101.2874	-75.0387	14.3179
10.0	-88.4732	-104.3423	-80.5552	11.8936
10.2	-95.6684	-97.0402	-90.0398	3.5002
10.4	-98.0103	-99.2894	-79.3380	9.9757
10.6	-94.4601	-94.0981	-80.8401	6.8100
10.8	-104.0120	-87.7197	-79.4663	12.2729
11.0	-88.6513	-86.7694	-78.3194	5.1660
11.2	-87.4464	-85.8173	-78.9832	4.2316
11.4	-98.9116	-92.6912	-79.9554	9.4781
11.6	-94.7196	-97.1293	-91.5081	2.8106
11.8	-90.5972	-99.3420	-89.5563	4.8929
12.0	-92.0013	-89.2113	-95.1427	2.9657
12.2	-97.9096	-92.2660	-99.9005	3.8172
12.4	-103.7357	-94.3443	-98.4343	4.6957
12.6	-97.1204	-82.1584	-90.1303	7.4810
12.8	-98.3412	-102.5690	-93.9294	4.3198
13.0	-101.4597	-95.9535	-95.1237	3.1680
13.2	-92.7781	-94.0396	-86.3948	3.8224
13.4	-87.6745	-94.8348	-95.4819	3.9037
13.6	-95.9239	-92.2714	-87.0027	4.4606
13.8	-90.3284	-88.5742	-85.2029	2.5628
14.0	-90.3411	-92.7090	-85.9836	3.3627
14.2	-89.2928	-87.1947	-88.6564	1.0491
14.4	-94.7841	-90.7880	-81.9542	6.4150
14.6	-103.0348	-93.1299	-92.2752	5.3798
14.8	-96.3769	-94.5036	-93.1294	1.6238
15.0	-90.4919	-94.7816	-96.9944	3.2513
15.2	-94.2388	-82.4997	-97.8719	7.6861
15.4	-93.6787	-96.1619	-84.5672	5.7974
15.6	-99.8094	-96.2802	-87.4932	6.1581
15.8	-105.9989	-91.5094	-90.2118	7.8936
16.0	-103.8863	-92.5220	-79.7427	12.0718
16.2	-94.1222	-105.4596	-83.6120	10.9238
16.4	-99.0316	-96.5325	-90.0547	4.4885
16.6	-90.3101	-101.5959	-84.2713	8.6623
16.8	-94.8559	-96.7701	-83.2602	6.7550
17.0	-88.9221	-92.2343	-82.9901	4.6221

17.2	-87.2371	-92.1948	-84.4663	3.8643
17.4	-98.8704	-96.6899	-88.6256	5.1224
17.6	-95.6116	-92.0570	-86.3306	4.6405
17.8	-97.8722	-96.9632	-91.2481	3.3121
18.0	-97.3639	-99.0951	-86.1165	6.4893
18.2	-95.6452	-94.4005	-88.6184	3.5134
18.4	-95.4843	-86.9847	-80.9928	7.2458
18.6	-83.9201	-93.2532	-80.1132	6.5700
18.8	-93.6291	-93.4285	-87.1642	3.2325
19.0	-101.2438	-89.2887	-88.0927	6.5756
19.2	-90.7995	-90.8079	-90.2115	0.2982
19.4	-83.3937	-93.6612	-83.8985	5.1338
19.6	-93.2384	-93.9049	-86.1271	3.8889
19.8	-93.8555	-87.8676	-86.9102	3.4727
20.0	-95.2135	-102.0918	-85.0077	8.5421
20.2	-84.8892	-103.3843	-87.2662	9.2476
20.4	-86.8669	-95.3673	-87.3118	4.2502
20.6	-97.4384	-97.9569	-93.4030	2.2770
20.8	-89.0141	-95.2486	-87.6443	3.8022
21.0	-100.3739	-88.4525	-85.0220	7.6760
21.2	-108.7768	-91.4627	-81.3863	13.6953
21.4	-96.7158	-88.9188	-90.5568	3.8985
21.6	-105.2096	-98.5621	-87.9384	8.6356
21.8	-105.6267	-98.8329	-84.0842	10.7713
22.0	-102.9140	-104.1495	-88.9949	7.5773
22.2	-101.2812	-90.8448	-79.4253	10.9280
22.4	-109.1675	-88.5446	-88.9235	10.3115
22.6	-102.2971	-91.1032	-93.5449	5.5970
22.8	-109.9235	-91.8117	-89.0398	10.4419
23.0	-105.9632	-90.3540	-83.4630	11.2501
23.2	-100.7597	-87.9319	-75.0126	12.8736
23.4	-101.3532	-97.1159	-78.5847	11.3843
23.6	-94.2403	-92.9036	-74.3754	9.9325
23.8	-100.0869	-83.8626	-83.5235	8.2817
24.0	-105.4452	-88.8676	-90.5686	8.2888
24.2	-108.0160	-93.6744	-82.9302	12.5429
24.4	-98.2173	-86.2811	-90.0645	5.9681
24.6	-103.2683	-97.6684	-94.7426	4.2629
24.8	-101.6509	-89.4742	-92.4144	6.0884
25.0	-94.4594	-96.9867	-88.8285	4.0791
25.2	-104.7610	-90.0003	-85.1846	9.7882
25.4	-97.8002	-95.7579	-77.8744	9.9629
25.6	-101.4790	-100.4084	-82.8956	9.2917
25.8	-105.1280	-98.0984	-97.9224	3.6028
26.0	-103.5657	-96.6261	-84.3287	9.6185
26.2	-97.7925	-90.4645	-80.5569	8.6178
26.4	-93.4242	-93.4145	-89.2189	2.1027

26.6	-101.0558	-85.9720	-81.3577	9.8491
26.8	-94.2836	-83.7943	-88.7765	5.2447
27.0	-104.0636	-96.9555	-91.1206	6.4715
27.2	-95.8379	-86.0750	-83.5733	6.1323
27.4	-103.0479	-105.7858	-88.3043	8.7408
27.6	-101.6311	-90.4625	-84.5540	8.5386
27.8	-94.3713	-96.0472	-97.0071	1.3179
28.0	-107.7756	-89.4859	-86.1823	10.7967
28.2	-97.1895	-101.8241	-92.0452	4.8895
28.4	-100.2535	-93.1370	-81.6609	9.2963
28.6	-93.3721	-98.2296	-82.4876	7.8710
28.8	-101.4421	-103.4765	-89.9820	6.7473
29.0	-85.8465	-89.7329	-82.2125	3.7602
29.2	-103.8778	-90.3235	-86.4137	8.7320
29.4	-103.4747	-98.5971	-83.9791	9.7478
29.6	-87.8941	-94.2917	-84.0254	5.1332
29.8	-107.0620	-93.0957	-83.1200	11.9710
30.0	-101.6569	-100.7212	-78.1913	11.7328
30.2	-101.6767	-95.8062	-91.6867	4.9950
30.4	-108.6931	-99.8726	-77.2240	15.7346
30.6	-104.1485	-87.1523	-96.1043	8.4981
30.8	-101.4516	-97.0384	-83.8188	8.8164
31.0	-102.6038	-86.6037	-91.4667	8.0001
31.2	-105.5732	-91.6323	-84.3763	10.5985
31.4	-95.2922	-89.7326	-78.8013	8.2455
31.6	-105.8208	-89.4350	-86.4674	9.6767
31.8	-110.2317	-96.2656	-86.0687	12.0815
32.0	-101.3956	-105.2370	-86.6648	9.2861
32.2	-104.9759	-98.6071	-80.8636	12.0562
32.4	-106.8862	-97.4500	-79.9794	13.4534
32.6	-102.6321	-91.7985	-84.9370	8.8476
32.8	-112.9422	-91.0523	-87.5143	12.7140
33.0	-98.1771	-89.3290	-71.2547	13.4612
33.2	-108.9740	-91.3426	-77.5697	15.7022
33.4	-105.6676	-91.8239	-81.4592	12.1042
33.6	-103.5749	-90.0880	-81.4436	11.0657
33.8	-96.3827	-92.9627	-82.8229	6.7799
34.0	-87.3688	-89.0682	-78.9858	5.0412
34.2	-105.9007	-96.5228	-73.4182	16.2413
34.4	-110.7644	-90.3098	-81.3748	14.6948
34.6	-96.3152	-95.8636	-82.9303	6.6925
34.8	-86.9634	-92.2190	-84.0056	4.1067
35.0	-94.0013	-94.3223	-82.3623	5.9800
35.2	-99.9235	-100.9328	-80.1922	10.3703
35.4	-107.5059	-83.8676	-82.3136	12.5962
35.6	-104.8294	-94.5666	-78.5191	13.1552
35.8	-98.6316	-89.4098	-77.7938	10.4189

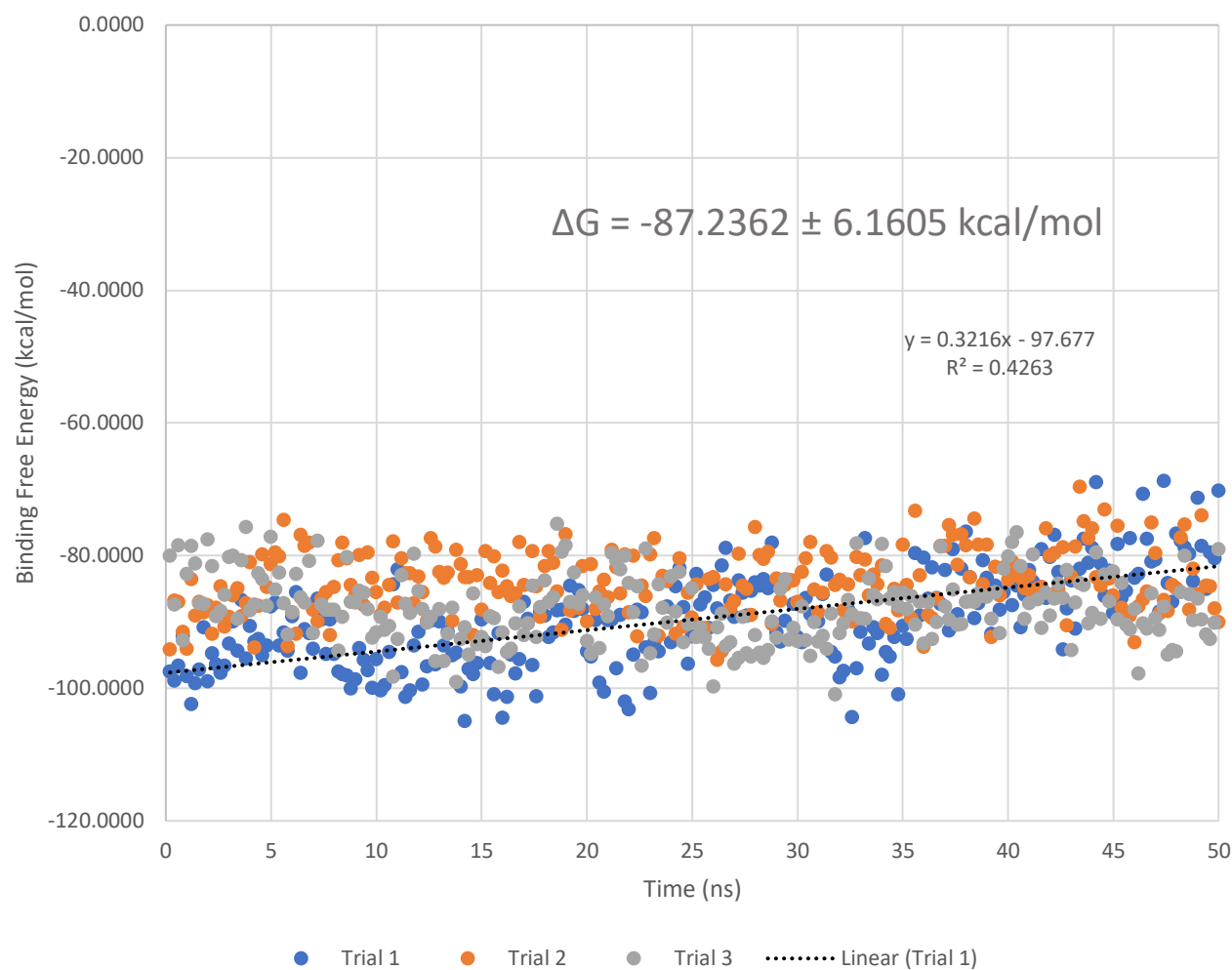
36.0	-102.7080	-99.4769	-83.6855	9.5113
36.2	-112.7522	-97.9283	-85.7863	13.4830
36.4	-104.0334	-94.2652	-87.6506	8.1914
36.6	-99.5796	-91.5233	-90.4646	4.5575
36.8	-90.0838	-91.2558	-80.8813	5.1873
37.0	-102.8634	-92.0587	-76.4068	13.2283
37.2	-106.2810	-94.3840	-78.1016	14.0897
37.4	-111.2165	-88.6990	-77.0726	17.0720
37.6	-99.0963	-86.2794	-82.0068	8.5448
37.8	-97.9057	-94.8209	-79.4107	9.2475
38.0	-101.3315	-91.8781	-93.1738	4.7267
38.2	-105.3614	-95.8983	-83.1907	11.0854
38.4	-103.5898	-92.3354	-83.5925	9.9987
38.6	-91.2858	-84.4279	-75.6756	7.8051
38.8	-94.3620	-93.8700	-69.6224	12.3698
39.0	-96.4823	-88.1702	-84.7550	5.8637
39.2	-100.2317	-90.2454	-74.6030	12.8144
39.4	-97.7405	-95.1225	-81.0333	8.3536
39.6	-100.6103	-97.0387	-76.6644	11.9730
39.8	-95.6148	-81.5736	-82.0214	7.0206
40.0	-102.6902	-101.0648	-96.1645	3.2629
40.2	-91.2708	-80.4456	-80.4085	5.4312
40.4	-103.4894	-84.7087	-82.3579	10.5658
40.6	-102.8849	-84.6355	-89.5462	9.1247
40.8	-108.4627	-89.0638	-80.5337	13.9645
41.0	-100.9454	-97.9989	-82.1305	9.4075
41.2	-103.5015	-96.6321	-75.0801	14.2107
41.4	-101.8311	-89.2385	-81.3730	10.2291
41.6	-101.8392	-87.6900	-84.4501	8.6946
41.8	-96.1077	-92.3913	-83.4312	6.3383
42.0	-97.3745	-85.0724	-82.1519	7.6113
42.2	-100.7674	-80.4759	-86.0615	10.1458
42.4	-101.2083	-101.8852	-76.9670	12.4591
42.6	-92.7845	-90.6628	-74.6085	9.0880
42.8	-102.7791	-99.6257	-73.9461	14.4165
43.0	-105.1845	-91.4617	-80.1622	12.5112
43.2	-105.3692	-92.3577	-82.8358	11.2667
43.4	-103.1308	-85.7538	-77.2825	12.9242
43.6	-102.4987	-84.9222	-90.2992	8.7883
43.8	-102.9060	-90.8664	-79.8215	11.5423
44.0	-102.5100	-98.0620	-82.9067	9.8017
44.2	-98.1587	-91.3159	-85.4556	6.3516
44.4	-106.6320	-96.4408	-77.5128	14.5596
44.6	-100.4958	-95.8269	-88.5645	5.9657
44.8	-100.2730	-90.2226	-90.7507	5.0252
45.0	-104.0882	-104.6502	-84.1405	10.2549
45.2	-96.5634	-94.1048	-79.2392	8.6621

45.4	-93.0895	-89.0813	-88.9947	2.0474
45.6	-93.6925	-95.3144	-88.7317	3.2914
45.8	-109.6758	-96.9734	-78.2823	15.6968
46.0	-101.0231	-94.5858	-82.8745	9.0743
46.2	-92.7355	-95.5683	-93.0306	1.4164
46.4	-106.1787	-94.3021	-90.0429	8.0679
46.6	-100.4182	-94.7093	-91.0049	4.7067
46.8	-106.0519	-88.8313	-79.2500	13.4010
47.0	-104.5470	-91.8637	-88.6629	7.9421
47.2	-99.9970	-84.2844	-82.6002	8.6984
47.4	-95.3606	-86.2484	-80.5305	7.4151
47.6	-87.0655	-87.5041	-80.3970	3.5535
47.8	-84.8818	-88.2213	-84.7049	1.7582
48.0	-82.4411	-86.3675	-93.7762	5.6676
48.2	-96.1616	-83.5328	-87.8981	6.3144
48.4	-88.9095	-82.4049	-79.6386	4.6355
48.6	-96.0839	-94.8719	-85.4976	5.2932
48.8	-96.4704	-90.6046	-88.2754	4.0975
49.0	-86.9620	-90.4512	-86.8933	1.7790
49.2	-96.4756	-96.2483	-84.7586	5.8585
49.4	-100.7157	-96.5200	-83.1563	8.7797
49.6	-109.9690	-90.6175	-84.6632	12.6529
49.8	-101.2778	-90.3545	-88.2442	6.5168
50.0	-105.4857	-85.0808	-87.6581	10.2025

Sample calculation for the statistical uncertainty (SU):

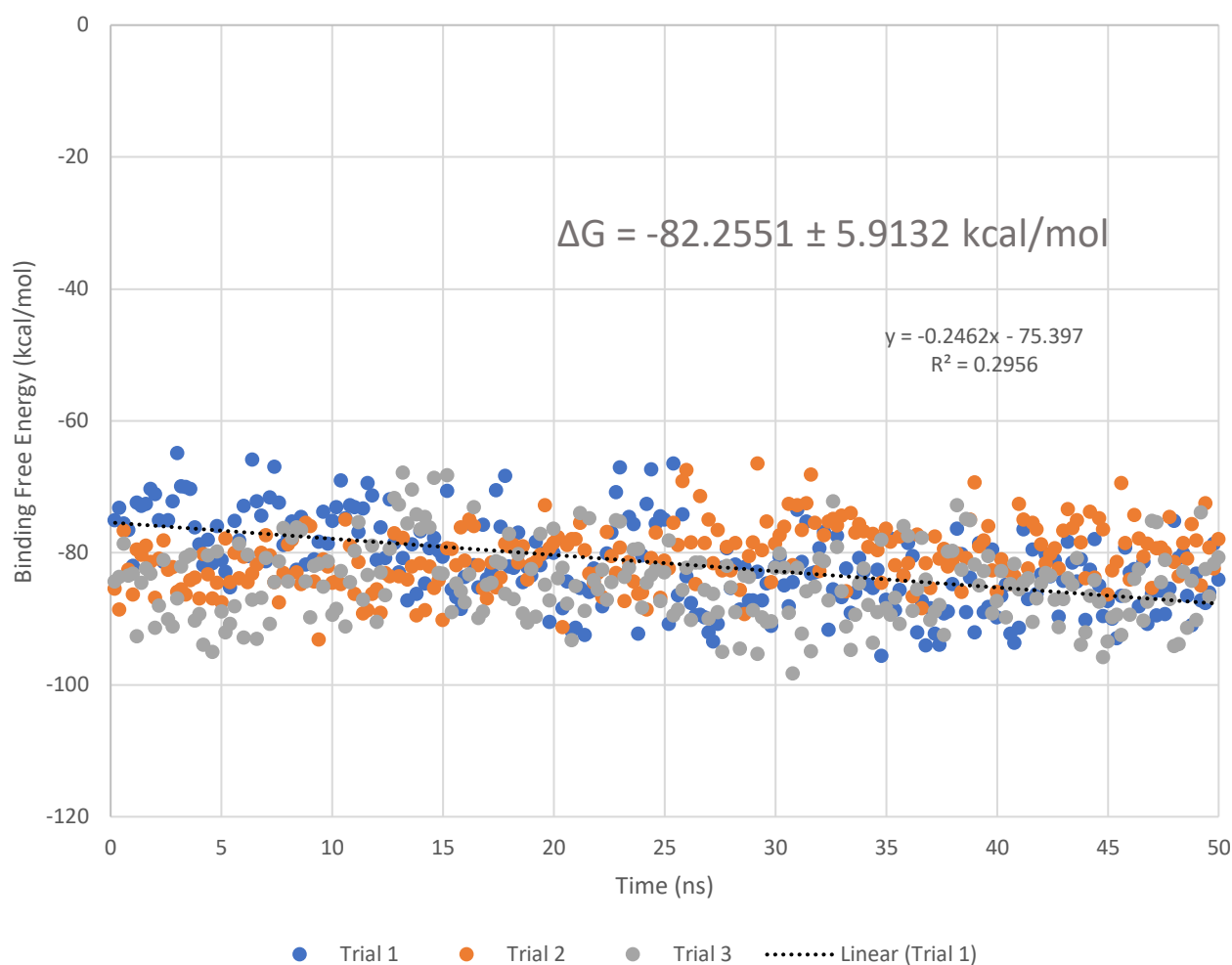
- $SU = \pm (|\text{Largest number in range}| - |\text{smallest number in range}|)/2$
- Formula used in Excel: $=(\text{MAX}(B1:D1) - \text{MIN}(B1:D1)) / 2$
 - $= \pm (97.4835 - 79.9882)/2$
 - $= \pm (85.4520 - 75.0620)/2$
 - $= \pm (85.6190 - 74.6318)/2$
 - $= \pm (104.6278 - 73.2239)/2$
 - $= \pm (95.5953 - 83.7321)/2$

The Effect of 5°C Simulation Temperature on 4H11-scFv-MUC16ecto Complex's Binding Affinity



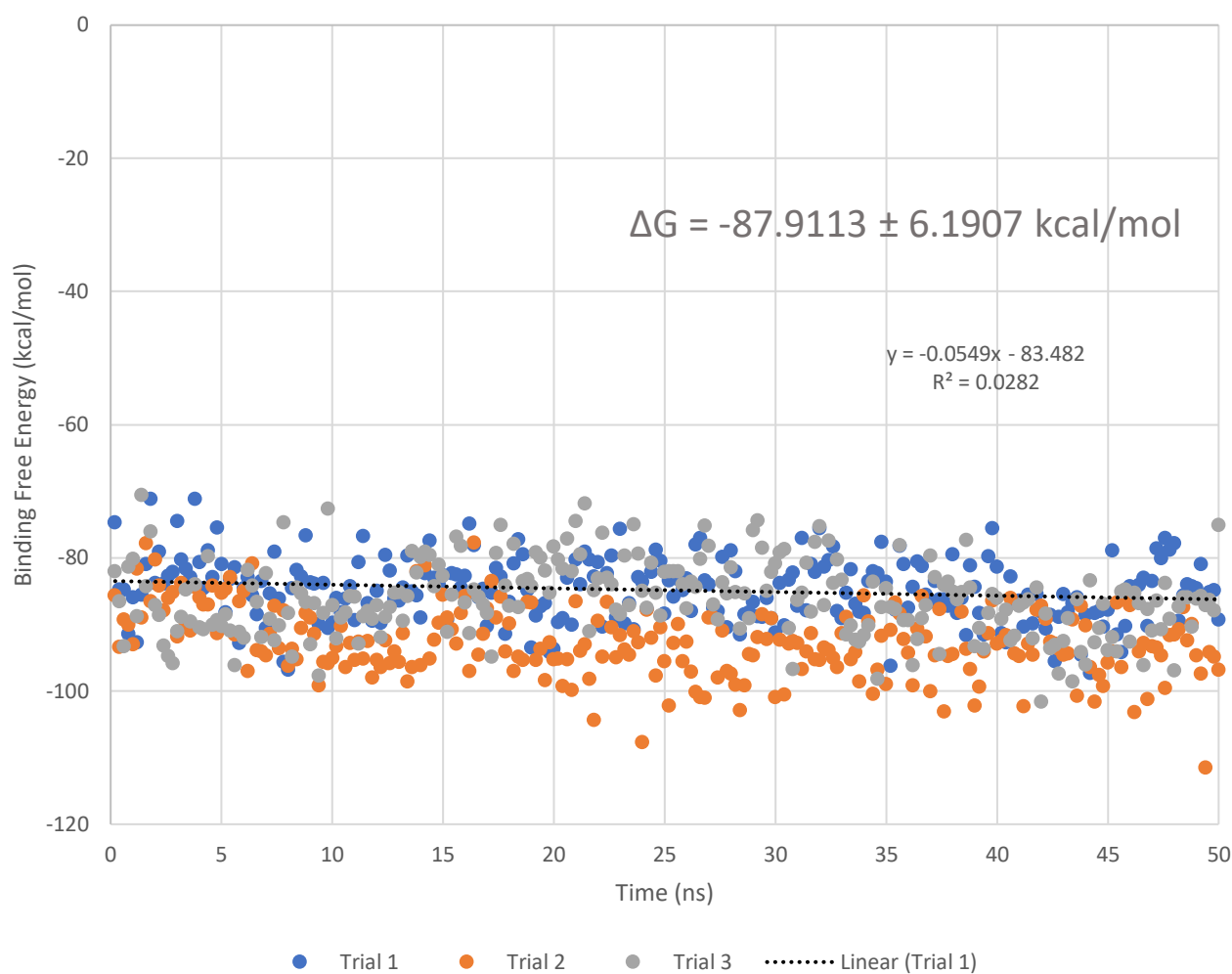
Graph 1: The effect of 5°C simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's binding affinity marked by ΔG over three trials

The Effect of 15°C Simulation Temperature on 4H11-scFv-MUC16ecto Complex's Binding Affinity



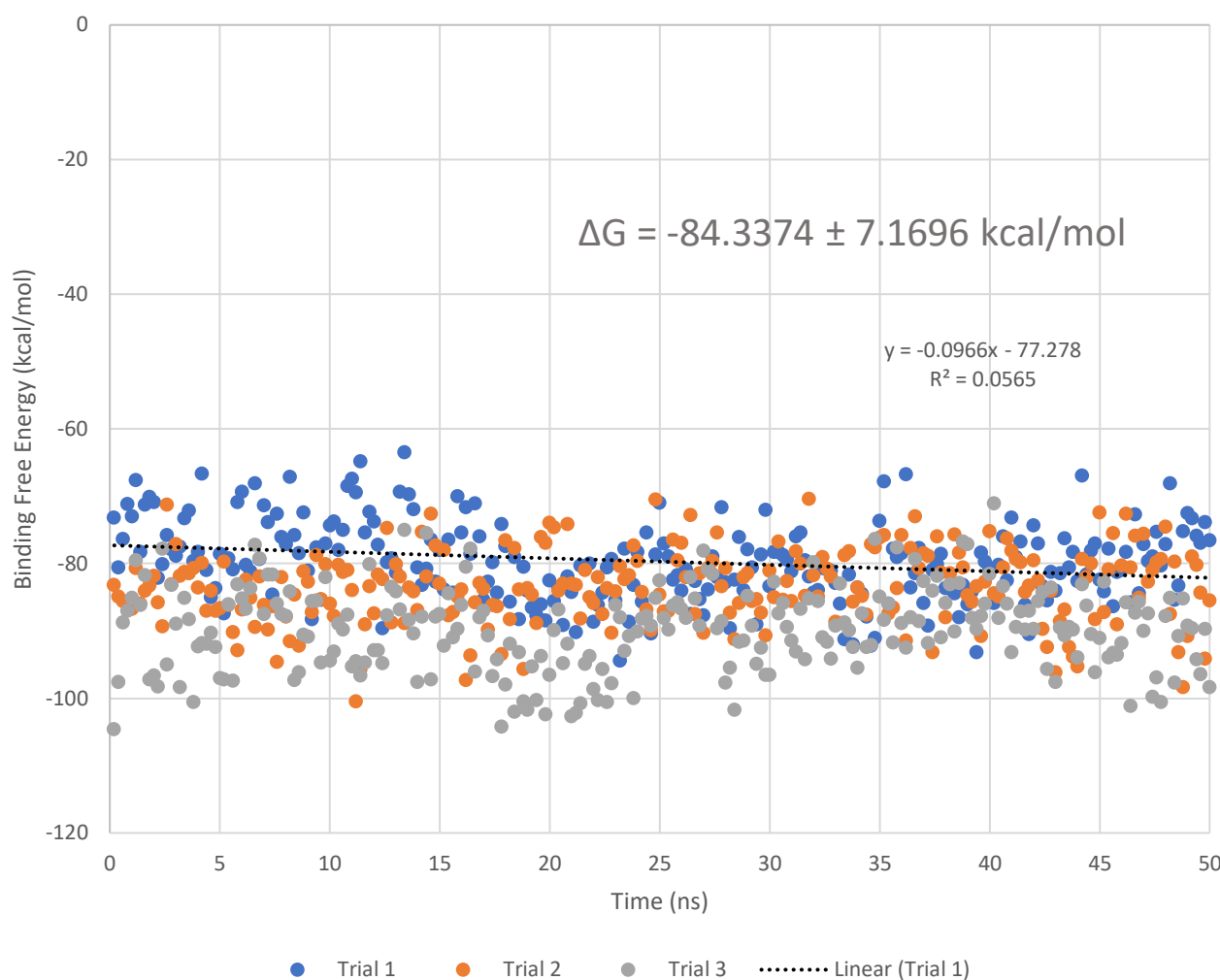
Graph 2: The effect of 15°C simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's binding affinity marked by ΔG over three trials

The Effect of 25°C Simulation Temperature on 4H11-scFv-MUC16ecto Complex's Binding Affinity



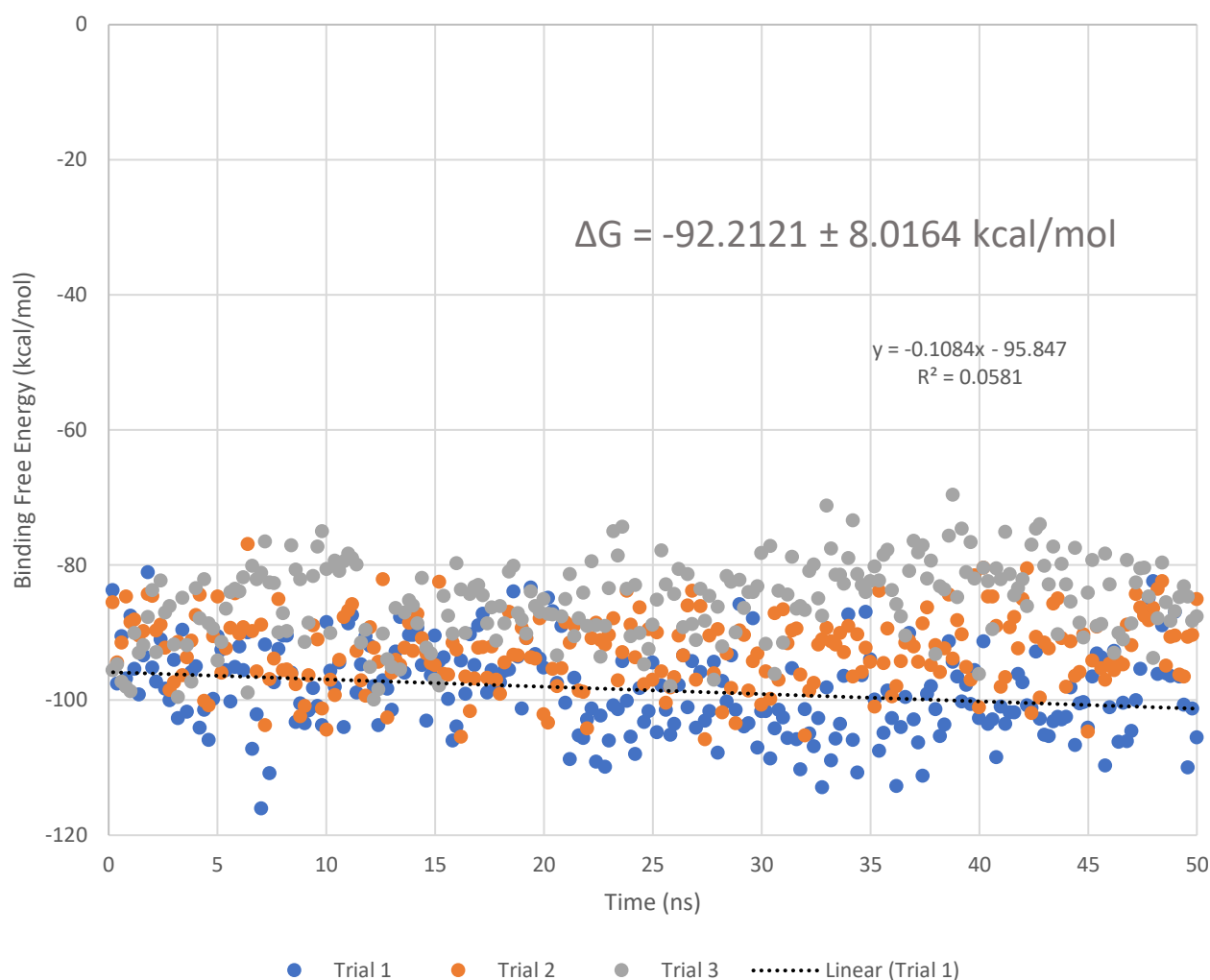
Graph 3: The effect of 25°C simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's binding affinity marked by ΔG over three trials

The Effect of 35°C Simulation Temperature on 4H11-scFv-MUC16ecto Complex's Binding Affinity

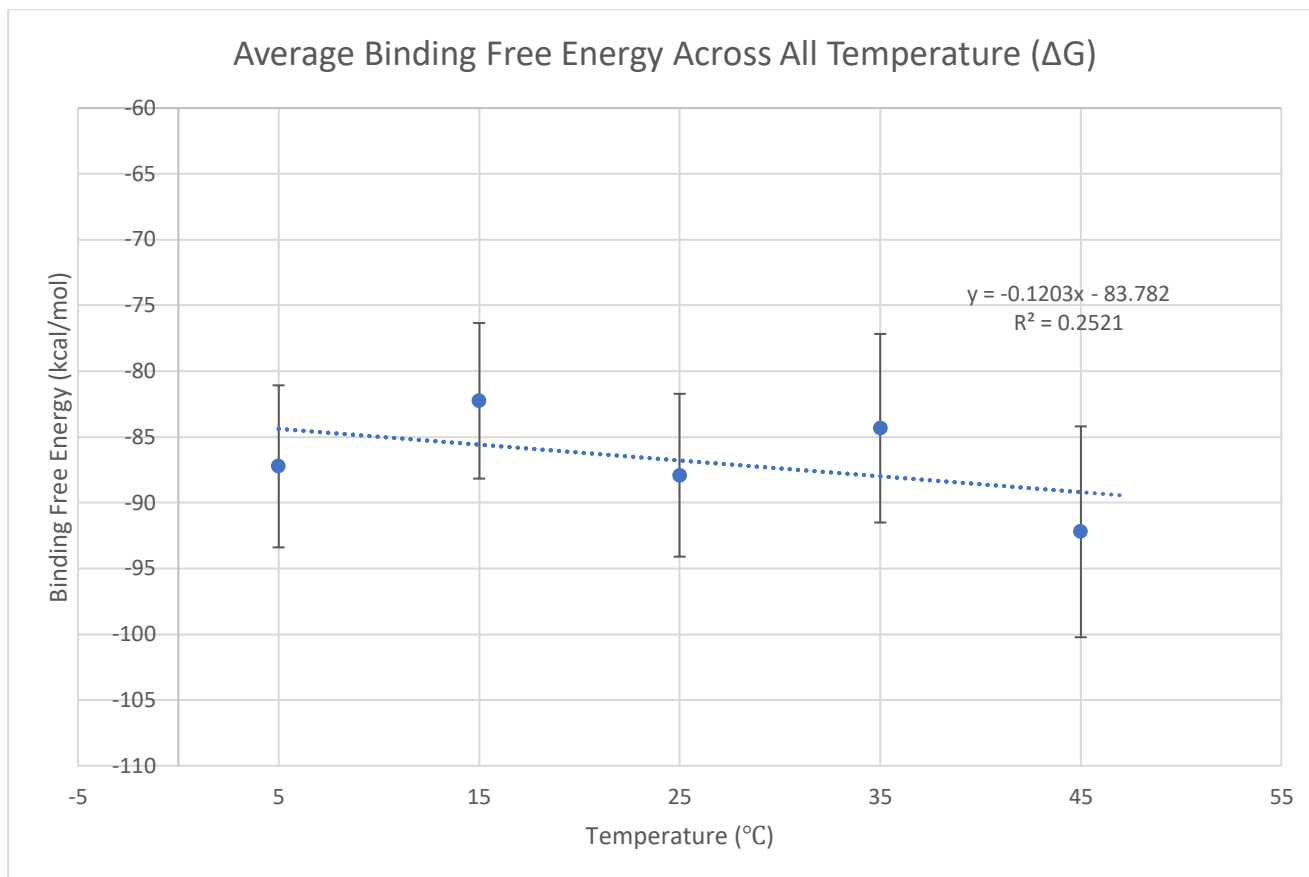


Graph 4: The effect of 35°C simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's binding affinity marked by ΔG over three trials

The Effect of 45°C Simulation Temperature on 4H11-scFv-MUC16ecto Complex's Binding Affinity



Graph 5: The effect of 45°C simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's binding affinity marked by ΔG over three trials



Graph 6: The effect of simulation temperature on the 4H11-scFv-MUC16^{ecto} complex's average binding affinity marked by ΔG for 5°C, 15°C, 25°C, 35°C, and 45°C

Range of the average binding free energy across 5°C, 15°C, 25°C, 35°C, and 45°C = $(-82.2551) - (-92.2121) = 9.957$ kcal/mol

Conclusion

The results of the simulation indicate that the 4H11-scFv-MUC16^{ecto} complex maintains a credible stability across a wide range of temperatures (5°C to 45°C), with the change in binding free energy (ΔG) values consistently reflecting high-affinity interactions. The average ΔG for all tested temperatures demonstrate that the binding reaction of 4H11-scFv and MUC16^{ecto} is carried out in the forward direction, indicating that it can occur without the input of external energy, for all $\Delta G < 0$. It also suggests that the complex is more stable than the reactants under the given conditions. The stability of this complex across a wide temperature range (5°C to 45°C) indicates that mechanism of the LFIA can theoretically function effectively in diverse environmental conditions, increasing the potential suitability for use in a range of geographical locations.

However, the degree of stability and binding strength varied with temperature. The strongest binding affinity occurred at 45°C, with an average ΔG of -92.2121 kcal/mol across three trials (Graph 5). The line of best fit for this temperature also appears to be negative ($m = -0.1084$), indicating a descending trend between binding free energy and reaction time of the 4H11-scFv-MUC16^{ecto} complex: if the simulation carried on, it is likely for the complex to continue display lower binding free energy values. This implies higher temperatures enhance the interaction's thermodynamic stability, likely improving conformational flexibility and hydrogen bonding. However, the highest variability ($SD = 8.0164$ kcal/mol) was also observed at 45°C, indicating potential instability of the structure under prolonged high-temperature exposure. This variability suggests that while binding strength improves at elevated temperatures, there may be structural disruptions or inconsistencies in the complex.

At 15°C, the complex exhibited the weakest binding affinity, with an average ΔG of -82.2551 kcal/mol, and appears to be the most consistent among the three trials (SD=5.9132) (Graph 2). This may be attributed to reduced molecular motion at lower temperatures, which could hinder the conformational flexibility required for optimal binding interactions.

Nevertheless, the average binding free energy for the complex at 5°C is -87.2362 kcal/mol, which appears contradictory to the implications of the results at 15°C. Interestingly, even though the binding affinity seems to be higher at 5°C than 15°C in the 50 ns, the positive linear trend, exquisite to the ΔG at 5°C, may infer otherwise ($m=0.3216$). Specifically, the binding free energy tends to rise in the later stage of the simulation. If the simulation operated for a prolonged time, it may display a more evident elevation in binding free energy. This demonstrates an unstable trend at a relatively low temperature. Hence, the last frame of the first trial of the protein structure under the 50ns simulation trajectory at 5°C was intercepted to analyze its interactions. The number of hydrogen bonds and salt bridge interactions was reduced from 16 reported in the literature (Lee et al.) to 14, whereas yellow represents the MUC16^{ecto} and green represents the 4H11-scFv (Figure 3). Particularly, the two pairs of hydrogen bonds formed between GLN30th and ASN103, and LEU26th and SER53 disappeared. This underscores potential amino acid sites for modification to improve the complex's binding affinity at a low temperature, thereby improving the structural integrity and stability for further medical applications. This essential modification would ensure consistent performance and decrease the false-negative rate of the LFIA screening system across various climates.

Finally, there is a general trend of increasing binding affinity temperature increased from 5°C to 45°C, with ΔG values becoming more negative on the plotted data for ΔG across all temperatures (Graph 6). The best line of fit for the data indicates a linear relationship between temperature and binding free energy, with a steeper slope at higher temperatures ($m=-0.1203$). However, temperature only explains approximately 25.21% of the variance in the binding free energy ($R^2=0.2521$). Moreover, the range of average ΔG values across all temperatures was 9.957 kcal/mol. Considering the R^2 value and the range, it suggests that the 4H11-scFv-MUC16^{ecto} complex remains relatively stable and spontaneous across different environmental conditions. Though, the influence of temperature alone may not fully account for the observed changes in binding free energy, pointing to the need for extended simulations or experimental studies to investigate molecular flexibility and stability over time to ensure optimal performance of the screening system under more diverse and unpredictable real-life settings.

Visualized structural changes using PyMOL revealed that the 4H11-scFv-MUC16^{ecto} complex maintained its overall integrity across all temperatures. However, as mentioned, minor structural disruptions were observed at 5°C, where reduced flexibility may have hindered optimal binding (Figure 3).

Evaluation of the Hypothesis

The hypothesis stated that the optimal temperature for the 4H11-scFv-MUC16^{ecto} interaction would lie between 35-40°C, where the binding affinity would be strongest due to optimal hydrogen bonding and molecular flexibility. The hypothesis anticipated that lower temperatures (below 5°C) would hinder molecular motion and result in weak binding, while higher temperatures (above 45°C) would destabilize the complex. Therefore, the results only partially support the hypothesis.

The results indicate that temperature plays a critical role in binding affinity, but the data shows some unexpected trends. As expected, the binding affinity generally increases with temperature, with the strongest binding affinity occurring at 45°C, which supports the hypothesis that higher temperatures improve thermodynamic stability and binding strength. The negative slope for the line of best fit ($m = -0.1084$) further confirms that elevated temperatures enhance the stability of the 4H11-scFv-MUC16^{ecto} complex, likely due to improved conformational flexibility and hydrogen bonding. Moreover, the observation of reduced hydrogen bonds and salt bridge interactions at 5°C, with the diminishment of key hydrogen bonds (GLN30th-ASN103 and LEU26th-SER53), further supports the

idea that the complex may not be as stable at lower temperatures as initially anticipated. The change in binding free energy for this simulation temperature illustrates a mild upward sloping trend, representing a less ideal and feasible binding activity, aligning with the hypothesis.

While the complex shows improved stability and binding at 45°C, the variability (SD = 8.0164 kcal/mol) at this temperature suggests that prolonged exposure to high temperatures might lead to structural disruptions or instability. This introduces the possibility that the complex may not remain consistent at higher temperatures for a long time, as the hypothesis anticipated. Additionally, unlike what the hypothesis expected to witness, the complex didn't have an evident optimal temperature point or peak in the data around 35°C to 45°C. Instead, the data points were distributed identically for the broad temperature range in the simulation. The data from 15°C, 25°C, 35°C, and 45°C all possess a slight downward sloping trend and change in binding free energy at approximately -82 to 92 kcal/mol, indicating a similarly energetically feasible and favorable binding activity across the temperature difference of 30°C. In fact, the average change in the free binding energy of the three trials for 5°C is lower than the results from 15°C and 35°C, contradicting the hypothesis's anticipation of an evident peak performance at 35°C to 40°C. The complex is overall more stable than expected.

These results and data have various strengths. Firstly, it illustrates a clear temperature dependency. The consistent trend of increasing binding affinity or decreasing ΔG as temperature rises supports the hypothesis that temperature influences the stability of the complex. The complex is thermodynamically stable across a wide range of temperatures, suggesting that the interaction can occur spontaneously without the input of external energy. Secondly, the visualization using Excel and PyMOL confirmed that the 4H11-scFv-MUC16^{ecto} complex maintained its overall integrity across the temperature range, with two amino acid sites torn down at 5°C, supporting the hypothesis. Finally, the control of pH, ionic strength, and pressure ensures that temperature is the primary variable influencing the binding affinity. This strengthens the reliability of the findings and their relevance to real-world applications in diverse climates.

Regardless, the results are flawed in some manners. Firstly, the insilico nature of the experiment eradicated many confounding variables that wouldn't be able to be in real life. Since simulations are heavily dependent on calculations and algorithms, the data can't be confidently applied in the real world directly without further wet lab's validation, which can account for the menstrual blood's nature as whole blood. Secondly, as the hypothesis addressed, where it expected the optimal temperature range to be 35-40°C, this wasn't fully determinable due to the wide intervals between increments. The selected simulation temperatures for this study are from 5°C to 45°C, with 10°C in between each increment. This is insufficient for generating an adequate understanding of the protein's interactions at a minor scale, as unique and pivotal transition points might be neglected subsequently. The identification of such stage may aid in the 4H11-scFv's advancement for diagnostics. Finally, the short period of simulation (50ns) seems insufficient for some temperature points, such as at 5°C, where the linear upward trend seems to be constant and may offer intriguing insights if the simulation was conducted for longer.

In summary, temperature is a substantial factor in antibody-antigen binding stability and affinity. However, it appears that the 4H11-scFv-MUC16^{ecto} complex is relatively stable across a wide range of temperatures and no prominent temperature point particularly stood out, disagreeing with the hypothesis.

Evaluation of Method

Overall, the computational approach using molecular dynamics simulations (AMBER, PyMOL) to assess the temperature effect on the structural conformation and binding free energy of the 4H11 scFv-MUC16 complex is valid for the research question. These tools are well-suited for detailed molecular-level analysis under controlled environmental conditions. However, the validity is

compromised to an extent because the simulations are conducted in silico and may not account for complex in vivo environmental factors like fluid dynamics in menstrual blood, which is whole blood with extraneous substances that may influence the binding and structural integrity of the protein complex.

Limitation / Problem explained	Effects of the issue and its significance	Specific solution
This method adopted an insilico simulation that rely heavily on idealized models and controlled variables, which may not accurately reflect the complexity of real biological systems. For example, the effects of proteases, cervical mucus, and endometrial tissues, or the interactions with other biomolecules are not considered in such method. This reduces the certainty and confidence in the direct applicability of findings in the real-world without further experimental validation.	Specifically to the LFIA screening system, this limitation significantly decreases its reliability. Since the insilico models do not account for the confounding variables and neglects the complexity of biological environments, the observed binding affinities and structural stabilities might differ in practice. This could lead to an increased risk of false negatives or positives, when the normal antibody is replaced by the 4H11-scFv. Also, the system may fail to predict unexpected interactions or degradation patterns that could compromise its usability and accuracy, especially in resource-limited or variable environmental conditions, which is what this simulation is attempting to address.	The most direct and beneficial method of addressing this limitation of the insilico simulation is conducting physical binding affinity tests using purified proteins and menstrual blood samples under controlled lab conditions for further validation and verification. For instance, surface plasmon resonance (SPR) is a testing method in the lab. It measures the binding of an analyte (in this case, the antigen MUC16) to a ligand (the antibody, 4H11-scFv) immobilized on a sensor chip in real-time by detecting changes in the refractive index. This provides direct kinetic data, such as association and dissociation rates and affinity constants under the manipulated abiotic factors. Another commonly used testing method is Enzyme-Linked Immunosorbent Assay (ELISA). It is a cost-effective method that measures binding indirectly by detecting antigen-antibody complexes using enzyme-linked secondary antibodies that generate a colorimetric signal, signifying the presence of binding and its strength. Finally, an Isothermal Titration Calorimetry (ITC) can be used, too, for directly determining binding affinity (K_D), enthalpy (ΔH), and entropy (ΔS). Thus, this offers complementary parameters to the Gibbs free energy

		calculations from the simulations. Altogether, these lab experiments may address the uncertainty in real-world scenarios, validating or complementing the results from the simulation, increasing the complex's reliability when utilized in a screening system.
The increment in this method can be improved. Currently, the method involves five different simulation temperatures, starting from 5°C, then every 10°C until 45°C (5°C, 15°C, 25°C, 35°C, 45°C). The interval between these temperature points is relatively large, and this is a problem because it may fail to capture subtle changes in the binding affinity and structural stability of the antibody-antigen complex that occur between the chosen increments.	The interactions between antibodies and antigens may exhibit non-linear responses to temperature changes. The wide gap can cause important transitions or tipping points in the binding reaction to be neglected or missed, leading to an incomplete understanding of the optimal temperature range for the LFIA system and how the antibody can be modified to increase its binding affinity with the target antigen. Maintaining accuracy and sensitivity across diverse temperature ranges is essential for regular and effective disease screening in these point-of-care applications. Therefore, neglecting such nuances may reduce its practicality the real-world.	This limitation can be addressed by employing temperature increments with smaller intervals. Instead of testing only at 5°C, 15°C, 25°C, 35°C, and 45°C, the temperature range could be broken up into smaller intervals, such as every 2°C or 5°C, each temperature at least 3 trials, to capture more wholistic data. This approach would allow the identification of subtle changes in binding affinity and structural stability that may occur between the current increments. Additionally, this limitation can also be tackled by continuous temperature gradient simulations, rather than points. These methods can potentially provide a more comprehensive understanding of the behavior of the 4H11-scFv-MUC16 interaction, ensuring that the LFIA system performs reliably under distinct and subtle changes in the environmental conditions. But most importantly, the results would be more insightful if they are conducted in labs with purified protein and menstrual blood for greater certainty.
Another limitation of this method is its temporal resolution. The current simulation captures the ΔG of	In real-world settings, the diagnostic tool will function over minutes or hours, during which dynamic changes may	To mitigate this limitation, extended simulation can be conducted to ensure that the protein complex remains stable

the protein complex binding for 50ns. In many cases, this is more insightful than a mere snapshot of the binding behavior over fixed intervals, yet slightly insufficient if the 4H11-scFv was to be implemented on the LFIA.	occur. A 50ns snapshot might miss potential destabilizing factors that occur over longer timescales. If over a longer period, minutes or hours, the 4H11-scFv-MUC16 complex might undergo structural changes or degradation due to the fluctuation in temperature. These changes could reduce binding affinity, causing the proteins to unbind. This could lead to false negatives, where the antigen is present but goes undetected, or inconsistent results across different environmental conditions. Such inconsistencies undermine the reliability of the LFIA system, particularly in point-of-care applications where environmental control is limited and prolonged operation is expected. It is also sensible to note that users will not observe the test strip's reaction within 50ns or any similarly brief timeframe. Hence, the diagnostic tool must demonstrate stability and reliability over a significantly longer duration, aligned with typical user interactions in practical settings.	and functional overtime. For instance, the simulation time can be extended to 100ns, 500ns, or even microseconds, providing insights into long-term binding dynamics and structural integrity. This can be achieved by modifying the input files used in the simulation software, such as adjusting the “prod.in” in AMBER to increase the simulation duration. The simulation step size (dt) and the number of steps (nsteps) can be increased to achieve the desired extended timeframe while ensuring computational efficiency. For example, if the original simulation runs for 50ns with nsteps=50000 and dt=2 fs, increasing nsteps to 500,000 would extend the simulation to 500ns while maintaining the same time-step resolution.
--	---	--

Together, this simulation offers a basic understanding of the 4H11-scFv-MUC16^{ecto} complex's binding under various temperatures, though with some limitations due to its insilico nature. Further testing should focus on wet lab experiments, such as SPR, ITC, and ELISA, to verify the insilico findings. Moreover, later testing can focus on the effects of environmental factors such as humidity and extended exposure to air on the protein complex to gain a more holistic understanding of its feasibility for diagnostics. To simulate real-world usage more accurately, experiments can implement dynamic abiotic profiles, like cycling between high and low temperatures. This approach mimics the operational environments where users may store the LFIA device in non-optimal conditions. Noticing any performance degradation under these conditions may help refine the system's design and stability. Ideally, if the 4H11-scFv can be immobilized on LFIA test strips, real-time degradation studies can also be conducted by exposing it to various environmental conditions and monitoring performance metrics like binding strength, specificity, and signal intensity. These advanced tests can reveal whether the LFIA system can maintain reliability across diverse climates and storage conditions.

As a next step, the LFIA applicability could be expanded by incorporating additional biomarkers like IL-6 or HE4, both critical in gynecological health. Testing for multiple biomarkers simultaneously would enhance accuracy and allow for broader disease screening. Finally, if the 4H11-scFv can be manufactured into LFIA, field testing in diverse climatic regions and resource-limited settings is essential to ensure its feasibility. Distributing these prototypes and collecting data on diagnostic accuracy, usability, and user feedback would provide valuable insights for final design adjustments. Subsequently, the studies should also include assessments of the tool's cost-effectiveness and scalability, ensuring it remains accessible and affordable in low-resource settings.

With these steps, the LUNA Pad's LFIA system would exhibit a greater chance of optimization for reliable, real-world applications in gynecological health early screening.

Works Cited

- Akiyama, Noriko, et al. "Ending Period Shame and Poverty in Asia." *Stanford Social Innovation Review*, 14 Oct. 2021, ssir.org/articles/entry/ending_period_shame_and_poverty_in_asia. Accessed 12 Dec. 2024.
- "Breaking Period Stigma with a New Report from Always." *Always*, www.always.co.uk/en-gb/breaking-period-stigma/. Accessed 12 Dec. 2024.
- Brunner-Ziegler, Sophie, et al. "Predictors of Participation in Preventive Health Examinations in Austria." *BMC Public Health*, vol. 13, no. 1, Dec. 2013, <https://doi.org/10.1186/1471-2458-13-1138>. Accessed 12 Dec. 2024.
- Cao, Yukun, et al. "The Global Burden of Gynecological Diseases from 1990 to 2019." *American Journal of Preventive Medicine*, vol. 67, no. 5, Nov. 2024, pp. 698-704, <https://doi.org/10.1016/j.amepre.2024.06.022>. Accessed 12 Dec. 2024.
- Crotti, Robert, et al. *Global Gender Gap Report 2021 : Insight Report*. 2021. *We Forum*, www3.weforum.org/docs/WEF_GGGR_2021.pdf. Accessed 12 Dec. 2024.
- Dosnon, Lucas, et al. "A Wearable In-pad Diagnostic for the Detection of Disease Biomarkers in Menstruation Blood." *medRxiv*, 24 Mar. 2024, <https://doi.org/10.1101/2024.03.22.24304704>. Accessed 12 Dec. 2024.
- Encarnação, João Crispim, et al. "Impact of Assay Temperature on Antibody Binding Characteristics in Living Cells: A Case Study." *Biomedical Reports*, vol. 7, no. 5, 14 Sept. 2017, pp. 400-06, <https://doi.org/10.3892/br.2017.982>. Accessed 12 Dec. 2024.
- Hong, Sae Yong, et al. "The Effect of Dilution, PH and Ionic Strength of Plasma on T-PA Precipitation in Euglobulin Fraction." *The Korean Journal of Internal Medicine*, vol. 7, no. 2, 31 July 1992, pp. 73-81, <https://doi.org/10.3904/kjim.1992.7.2.73>. Accessed 12 Dec. 2024.
- "An Introduction to Antibodies: Antibody-Antigen Interaction." *Merck*, www.sigmaaldrich.com/SG/en/technical-documents/technical-article/protein-

[biology/elisa/antibody-antigen-interaction?srsId=AfmBOopfNY1-Zf9_mT2qDt5umid2Vqv7-wOOvAJWjhnJx4c13yShc2TJ](https://biology.elisa/antibody-antigen-interaction?srsId=AfmBOopfNY1-Zf9_mT2qDt5umid2Vqv7-wOOvAJWjhnJx4c13yShc2TJ). Accessed 12 Dec. 2024.

Kottasová, Ivana. "It's 2021 and Women around the World Are Still Struggling to Safely Manage Their Periods | CNN." *CNN*, 1 July 2021, edition.cnn.com/2021/07/01/world/menstrual-health-period-poverty-intl/index.html. Accessed 12 Dec. 2024.

Laitner, Melissa. "Ovarian Cancer: Outdated Diagnostics for a Deadly Disease." *Society for Women's Health Research*, 4 Feb. 2020, swhr.org/ovarian-cancer-outdated-diagnostics-for-a-deadly-disease/. Accessed 12 Dec. 2024.

Larson, Sharon, and Rosaly Correa-de-Araujo. "Preventive Health Examinations: A Comparison along the Rural–urban Continuum." *Women's Health Issues*, vol. 16, no. 2, Mar. 2006, pp. 80-88, <https://doi.org/10.1016/j.whi.2006.03.001>. Accessed 12 Dec. 2024.

Lebozec, Kristell, et al. "Quality and Cost Assessment of a Recombinant Antibody Fragment Produced from Mammalian, Yeast and Prokaryotic Host Cells: A Case Study Prior to Pharmaceutical Development." *New Biotechnology*, vol. 44, Sept. 2018, pp. 31-40, <https://doi.org/10.1016/j.nbt.2018.04.006>. Accessed 12 Dec. 2024.

Lee, Kwangkook, et al. "Structural Basis for Antibody Recognition of the Proximal MUC16 Ectodomain." *Journal of Ovarian Research*, vol. 17, no. 1, 19 Feb. 2024, <https://doi.org/10.1186/s13048-024-01373-9>. Accessed 12 Dec. 2024.

Mehrotra, Ateev. "Preventive Health Examinations and Preventive Gynecological Examinations in the United States." *Archives of Internal Medicine*, vol. 167, no. 17, 24 Sept. 2007, p. 1876, <https://doi.org/10.1001/archinte.167.17.1876>. Accessed 12 Dec. 2024.

"PAP Smear." *Turquoise Health*, turquoise.health/services/pap-smear-2/. Accessed 12 Dec. 2024.

"Period Stigma Facts and Figures." *Citron Hygiene*, 6 Oct. 2023, www.citronhygiene.co.uk/resources/period-stigma-facts-figures#What_is_period_stigma. Accessed 12 Dec. 2024.

"Project Description." *IGEM Wiki*, 2024.igem.wiki/bwya/description. Accessed 12 Dec. 2024.

Smith, Lori. "Everything You Need to Know about the Pap Smear." Edited by Debra Rose Wilson.

Medical News Today, 20 Sept. 2023, www.medicalnewstoday.com/articles/311995.

Song, Yaan, et al. "Update Value and Clinical Application of MUC16 (cancer Antigen 125)." *Expert Opinion on Therapeutic Targets*, vol. 27, no. 8, 3 Aug. 2023, pp. 745-56,

<https://doi.org/10.1080/14728222.2023.2248376>. Accessed 12 Dec. 2024.

Tindal, Kirstin, et al. "The Composition of Menstrual Fluid, Its Applications, and Recent Advances to Understand the Endometrial Environment: A Narrative Review." *F&S Reviews*, vol. 5, no. 3, July 2024, p. 100075, <https://doi.org/10.1016/j.xfmr.2024.100075>. Accessed 12 Dec. 2024.

Torre, Lindsey A., et al. "Ovarian Cancer Statistics, 2018." *CA: A Cancer Journal for Clinicians*, vol. 68, no. 4, 29 May 2018, pp. 284-96, <https://doi.org/10.3322/caac.21456>. Accessed 12 Dec. 2024.

"Vaginal Health: What Causes pH Imbalance?" *Dr. Daniel S. Kushner*,

www.drdanielkushner.com/blog/vaginal-health-what-causes-ph-imbalance#:~:text=Menstruation,change%20pads%20and%20tampons%20frequently.

Accessed 12 Dec. 2024.

Wang, Hao, et al. "Overexpression of MUC16 Predicts Favourable Prognosis in MUC16-mutant Cervical Cancer Related to Immune Response." *Experimental and Therapeutic Medicine*, vol. 20, no. 2, 4 June 2020, pp. 1725-33, <https://doi.org/10.3892/etm.2020.8836>. Accessed 12 Dec. 2024.

"What Is Air Pressure?" *National Data Buoy Center*, www.ndbc.noaa.gov/education/pressure.shtml.

Accessed 12 Dec. 2024.

Yi, Du. *Analysis of Chinese Contemporary Young Females' Attitudes and Experiences toward Menstruation and Feminine Products*. U of Richmond, MA thesis.

scholarship.richmond.edu/cgi/viewcontent.cgi?article=2638&context=honors-theses. Accessed 12 Dec. 2024.

Zhang, Phoebe. "Why China's Women Are Talking Back like Never before." *South China Morning Post*, 26 Sept. 2024, www.scmp.com/opinion/china-opinion/article/3279833/why-chinas-

[women-are-talking-back-never?module=perpetual_scroll_0&pgtype=article](#). Accessed 12 Dec. 2024.

Criterion B: Inquiring & Designing

Level	strand	Achievement descriptor. <i>"I have shown that I can..."</i>
1-2	i.	state a problem or question to be tested by a scientific investigation
	ii.	outline a testable hypothesis
	iii.	outline the variables
	iv.	design a method, with limited success.
3-4	i.	outline a problem or question to be tested by a scientific investigation
	ii.	formulate a testable hypothesis using scientific reasoning
	iii.	outline how to manipulate the variables, and outline how relevant data will be collected
	iv.	design a safe method in which he or she selects materials and equipment.
5-6	i.	describe a problem or question to be tested by a scientific investigation <i>Descriptive title</i> <i>RQ/statement includes IV and DV and maybe key CVs</i> <i>Background knowledge includes theory of the science</i> <i>Background knowledge is supported by cited sources</i>
	ii.	formulate and explain a testable hypothesis using scientific reasoning <i>Logical prediction</i> <i>Scientific reason for prediction</i> <i>Hypothesis includes trends and patterns expected</i> <i>Sketch graph supports hypothesis</i>
	iii.	describe how to manipulate the variables, and describe how sufficient, relevant data will be collected <i>IV correctly identified, with units</i> <i>IV levels presented, with method</i> <i>DV correctly identified, with units</i> <i>DV measurement method described</i> <i>DV calculations outlined (if needed)</i> <i>Controlled variables correctly identified</i> <i>Method for controlling variables described</i>
	iv.	design a complete and safe method in which he or she selects appropriate materials and equipment <i>Safety issues identified and addressed with methods for keeping safe.</i> <i>Materials and equipment (with quantities) correctly presented</i> <i>Methods for carrying out the experiment presented</i> <i>Labeled diagram or photo of experiment set-up included, if needed.</i>
7-8	i.	explain a problem or question to be tested by a scientific investigation <i>Background knowledge is explained correctly, with support from cited, reliable sources..</i>
	ii.	formulate and explain a testable hypothesis using correct scientific reasoning <i>Hypothesis is mathematical, with justification. For example, might include possible ratios or relationships.</i> <i>A sketch of an IV vs DV graph is included and described to show the expected relationship.</i>
	iii.	explain how to manipulate the variables, and explain how sufficient, relevant data will be collected <i>IV increments presented, with clear reason for the range and divisions</i> <i>Calculations (if needed) for manipulating IV are included</i> <i>DV is explained, including any calculations needed to determine the DV from the raw data</i> <i>Controlled variables are explained how they could affect the DV</i>
	iv.	design a logical, complete and safe method in which he or she selects appropriate materials and equipment. <i>Logical: very clear to the reader, can be easily replicated.</i> <i>Materials and equipment: specific, clear and complete.</i> <i>Relevant safety considerations are described and how to best follow them are included</i>

Criterion C: Processing & Evaluating

Level	strand	Achievement descriptor. <i>"I have shown that I can..."</i>
L1-2	i.	collect and present data in numerical and/or visual forms
	ii.	interpret data
	iii.	state the validity of a hypothesis based on the outcome of a scientific investigation
	iv.	state the validity of the method based on the outcome of a scientific investigation
	v.	state improvements or extensions to the method.
L3-4	i.	correctly collect and present data in numerical and/or visual forms
	ii.	accurately interpret data and explain results
	iii.	outline the validity of a hypothesis based on the outcome of a scientific investigation
	iv.	outline the validity of the method based on the outcome of a scientific investigation
	v.	outline improvements or extensions to the method that would benefit the scientific investigation.
L5-6	i.	correctly collect, organize and present data in numerical and/or visual forms <i>Raw quantitative data correctly recorded</i> <i>Qualitative data accurately recorded</i> <i>Units and uncertainties correct</i> <i>Tables include title, units, uncertainties, consistent decimal points and are neatly presented.</i> <i>Graphs include title, axis labels, units, uncertainties, neat accurate plotting, appropriate trendlines</i>
	ii.	accurately interpret data and explain results using scientific reasoning <i>Trends patterns and relationships are appropriately described</i> <i>Results are explained using reasoning</i>
	iii.	discuss the validity of a hypothesis based on the outcome of a scientific investigation <i>Validity: to what extent do the results support your hypothesis?</i>
	iv.	discuss the validity of the method based on the outcome of a scientific investigation <i>Validity: to what extent did the method help you answer the research question?</i>
	v.	describe improvements or extensions to the method that would benefit the scientific investigation. <i>Describes specific improvements to significant limitations</i> <i>Extensions: where could you go next with this investigation? What further questions do you have?</i>
L7-8	i.	correctly collect, organize, transform and present data in numerical and/or visual forms <i>Tables and graphs meet all standards</i> <i>Example calculations are clear and appropriate for presentation and accuracy</i>
	ii.	accurately interpret data and explain results using correct scientific reasoning <i>Trends patterns and relationships are appropriately explained</i> <i>Results are explained using correct reasoning</i> <i>Uncertainties/error considered, including outliers and/or specific measurements</i> <i>Qualitative data are used where appropriate</i>
	iii.	evaluate the validity of a hypothesis based on the outcome of a scientific investigation <i>Validity: to what extent do the results support your hypothesis?</i> <i>Give specific examples in your interpretation</i>
	iv.	evaluate the validity of the method based on the outcome of a scientific investigation <i>Validity: to what extent did the method help you answer the research question?</i> <i>Include evidence from the qualitative and quantitative data</i>
	v.	explain improvements or extensions to the method that would benefit the scientific investigation. <i>Explanation includes potential impact on the results, evaluated as high/medium/low impact.</i> <i>Improvements are detailed and realistic</i> <i>Discuss further investigations inspired by this investigation, with reasoning</i>

Criterion D (iv): Documenting Sources

Level	strand	Achievement descriptor. <i>"I have shown that I can..."</i>
1-2	iv.	Document sources, with limited success
3-4	iv.	Sometimes document sources correctly
5-6	iv.	Usually document sources correctly ○ <i>Includes a properly formatted Works Cited list</i> ○ <i>Includes some in-text citations</i>
7-8	iv.	Document sources completely ○ <i>Includes a properly formatted and complete Works Cited list</i> ○ <i>Includes all necessary in-text citations including images</i>