A Genome-wide Search for Essential SUMOylation E3 ligase for Influenza A Virus through qFRET Technology

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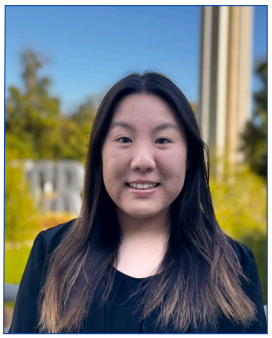
ABSTRACT

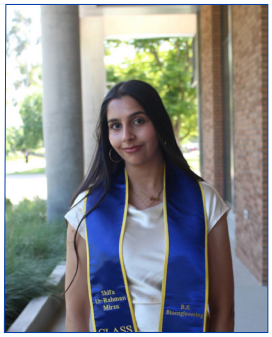
Influenza is one of the most prevalent viruses that has plagued millions worldwide. Every year health organizations encourage the public to get their flu vaccines to combat the flu season. Although the flu vaccines and medicines are widely available, it is estimated that flu viruses still cause 300,000-500,000 deaths every year. The two most common influenza virus strains are influenza A and B viruses, or IAV and IBV, respectively. It has been discovered that drug resistance develops very soon after a new drug is launched. It is highly demanded that anti-flu virus drugs with novel mechanisms be developed. Our lab has discovered that SUMOylation, a post-translational modification, is essential to the viral IAV and IBV life cycle. In this study, we have screened all the E3 ligases in the human genome to discover the SUMO E3 ligase responsible for the essential SUMOylation of IAV M1 protein using our Quantitative Fluorescence Energy Transfer(qFRET). We first determined the FRET spectrum of all E3 ligases with M1 protein and then quantified the FRET signals to provide a first-line examination of interactions. We then determined the E3-M1 interaction affinities, K_D, to ensure the real interactions. We found the E3 ligase PIAS1 has the highest affinity to M1 among other E3s. By understanding the interaction affinity between IAV M1 protein with SUMOylation E3 ligase, we hope to block the interaction between the PIAS1-M1 for novel anti-flu medicine development.

KEYWORDS: SUMOylation, influenza, quantitative Förster resonance energy transfer, viral proteins, protein interaction



NIKKI WONG

Nikki Wong is a fourth year Bioengineering major. Under the guidance of Dr. Liao, she has been researching the interaction between E3 ligases with IAV M1 protein for the past two years. Nikki is a member of the University Honors program and is a Undergraduate Education Minigrant recipient. After graduation, Nikki will be pursuing her Master's in Cellular and Molecular Engineering.



SHIFA MIRZA

Shifa Mirza is a fourth year Bioengineering major. For the past year, she has worked in Dr. Liao's lab, studying the interaction of E3 ligases with IAV M1 Protein. Shifa is also the head of public relations for the Biotechnology Organization of Riverside and a mentor for the Bioengineering Graduate Student Associate. After graduation, Shifa will be pursuing a Masters in Bioengineering, with a concentration in Cellular and Molecular Biology.

FACULTY MENTOR - Dr. Jiayu Liao, Department of Bioengineering

Dr. Liao's research focuses on the development of a novel quantitative FRET(qFRET) technology platform for both basic research, such as dissecting host–virus and Ubl E3-substrates interactions, and translational research, such as SUMOylation inhibitor for anti-virus and anti-cancer therapeutics.

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INTRODUCTION

Influenza, a highly contagious respiratory virus, has and continues to pose significant challenges to public health. Categorized into four distinct subtypes - A, B, C, and D - it is responsible for causing both seasonal epidemics and sporadic pandemics worldwide. Specifically, the Influenza A virus (IAV) is known to trigger widespread outbreaks, exemplified by notable historical events such as the Spanish Flu of 1918 and the pandemic of H1N1 in 2009. During these global pandemics, the virus spreads swiftly from its origin, infecting millions across different regions in successive waves throughout the year. The impact of influenza is substantial, with the World Health Organization (WHO) estimating approximately 1 billion infections, 3–5 million severe cases, and 300,000–500,000 deaths annually.[1] The severity of the illness is contingent on multiple factors, including the specific viral strain and the cytokine storms in human immune responses.

Despite the development of seasonal flu vaccines, their efficacy remains suboptimal, particularly against IAV variants, which can mutate quickly with limited pre-existing human immunity. Additionally, the cyclical occurrence of pandemic strains, which antigenically differ from existing types—emerging approximately every 10–50 years—underscores the persistent threat posed by the disease. While significant strides have been made in public health awareness and virus technology, influenza continues to evolve and develop drug resistance to existing therapeutics, highlighting the pressing need for further research.[2]

Understanding the molecular mechanisms underlying influenza infection requires elucidating human-virus interactions represented as protein-protein interactions, which are fundamental to numerous biochemical and physiological processes. However, studying these interactions faces significant challenges, as over 80% of proteins exist in complex formations rather than in isolation, and it is very difficult to express viral proteins in other systems, such as bacterial cells or mammalian cells, necessitating sophisticated technologies for analysis. Förster Resonance Energy Transfer (FRET) is a phenomenon in which two fluorophores with overlapped excitation and emission spectrums can transfer energy through dipole-dipole interaction when they are close enough. As depicted in Figure 1, when proteins are in close proximity of 1-10 nm, FRET occurs between the two fluorophores, and the FRET spectrum can be elucidated.[4,13]

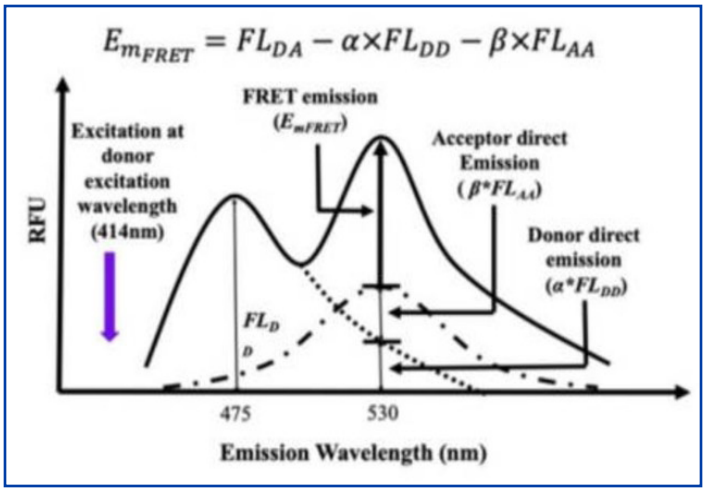

Figure 1: Fluorescent emission spectra explaining the energy transfer phenomenon between FRET donor and acceptor when the two fluorophores with overlapped excitation and emission spectrums are close to each other.[12]

Quantitative FRET (qFRET) technology emerges as a promising tool, as it is quicker, cheaper, and often less destructive to proteins than alternative assays, and it can be implemented as a high-throughput assay format. Specifically, qFRET can be used to assess protein interaction affinity (K_D), allowing further study into mitigating these interactions. This technology is developed explicitly towards post-translational modifications, such as SUMOylation, a pathway critical for the IAV M1 life cycle, as it utilizes the pathway for replication.[4] Using qFRET, we characterized the

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interaction affinities of E3 ligase molecules within the SUMOylation pathway with the influenza IAV virus M1 protein. This research holds the potential to identify novel targets for antiviral drug development and deepen our understanding of the host-virus interactions, ultimately aiding in developing more effective strategies for combating influenza infections.

MATERIALS AND METHODS

Molecular Cloning of Constructs

pET28b plasmids encoding the fluorescent fusion protein, CyPet-TRAF6, PIAS3, PIAS4, RHES, hSTUB1, hRNF4, hMApl, parkin, PIAS1, hCBX4, hHDAC4, hHDAC4, hTRIM28, Dcst, Fbxw7, hCRBN, SOCS1, YPet-hSCNA, and YPet-M1, were cloned into *E.Coli* using varying strains of Electrocomp *E.Coli* cells via electroporation. Followed by a 1-hour recovery, Luria-Bertani (LB) agar plates with 50 µg/mL kanamycin were used to plate the transformed E.Coli cells. The following strains were used to amplify all the transformed plasmid DNA constructs followed by an extensive screening protocol to determine the highest strain value used for protein expression: BL21-CodonPlus (DE3)-RIL, ArticExpress(DE3)RP, BL21-CodonPlus, OverExpress™ C43(DE3), BL21(DE3), Shuffle®T7, BL21(DE3) pLysS, Rosetta (DE3)pLysS and OverExpress™ C41(DE3).

Protein Expression and Characterization of IAV M1 and E3 Ligases

The previously identified highest expressing strain was inoculated into a starting culture at 1:8 v/v of LB broth with 50 µg/mL Kanamycin, for resistance selectivity. Grown at a smaller culture overnight at 37°C and placed in a shaker at 250 RPM overnight, the culture was then transferred to 1 L of 2XYT media supplemented with 50 µg/mL Kanamycin and placed into a shaker at 250 RPM at 37°C until a proper optical density (O.D.) of 0.4-0.6 was reached at 600 nm absorbance. At the desired OD, protein expression was induced with 1M IPTG, a final concentration of 0.375 mM, and left to shake overnight at 16°C and 200 RPM.

After the induced culture was left to shake for 12-15 hours, the bacterial cells were collected by centrifugation at 4°C, 8000 xg for 5 minutes. The collected bacterial pellet was resuspended in centrifuge bottles physically, with the addition of 30 mL of Binding Buffer (20 mM Tris HCl, pH 7.4, 0.5 mM NaCl, 5 mM Imidazole). The resuspended bacterial pellet underwent sonication at ultrasonic frequencies to lyse the cells at alternating on and off phases of pulses for 7 minutes. Subsequently, the sonicated cells underwent 2 cycles of centrifugation at 4°C, 35,000 xg for 30 minutes, after which the supernatant was transferred into columns containing Ni^{2+}-NTA agarose beads while ensuring that pellet fibers were not included to prevent clogging. The attached protein and beads underwent two-column volumes of Wash Buffer 1 (20mM Tris HCl, pH 7.4, 300mM NaCl), two-column volumes of Wash Buffer 2 (20mM Tris HCl, pH 7.4, 1.5 M NaCl, 0.5% Triton-100), one-column volume of Wash Buffer 3 (20 mM Tris HCl, pH 7.4, 0.5M NaCl, 10mM Imidazole), and one-column volume of Interaction Buffer (150mM NaCl, 25mM Tris HCl pH 8, 5% glycerol) to strip unwanted bounded components to reduce non-specific binding.

After washing, proteins were eluted with 300 µL to 1mL 450 mM Elution Buffer (1 M Imidazole, Milliq Water) depending on the expected yield; 300 µL of Elution Buffer was allowed to flow through before collection. Dialysis Buffer (150 mM NaCl, 25 mM Tris HCl pH 8, 5% glycerol, DTT to a final concentration of 1 mM) was prepared in a glass beaker with a dialysis membrane bag prepared for each protein. Eluted proteins were pipetted into the dialysis bags and left to dialyze overnight at 4°C to remove excess salts.

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Protein concentration was determined using the FlexStationI1384 to measure fluorescence intensities at Excitation 414 nm / Emission 475 nm (CyPet range) and Excitation 475 nm / Emission 530 nm (YPet Range). Purified protein underwent a 1:6 dilution before being pipetted into Greiner 384-well plates. The acquired fluorescence readings were calculated based on CyPet and YPet fluorescence standards to determine the concentration of the purified fluorescent-tagged proteins. Protein size was determined and confirmed via SDS gel electrophoresis; gel samples were prepared by taking 5 µg of the protein sample, 15 µL of SDS, and 15 µL of MilliQ H_2O. Samples were heated at 100°C for 5 minutes before loading into the polyacrylamide gel (Acrylamide, 10% APS, Temed, 1.5M Tris HCl pH 8.8, 1.5M Tris HCl pH 7.4, 10% SDS) with 3 µL of the DNA ladder. Electrophoresis was conducted at 100V for 3 hours; the gels were stained overnight with a Staining Buffer (Coomassie Blue R350, Methanol, Acetic Acid), then with a Destaining Buffer (Methanol, Acetic Acid) to better visualize the gel.

Em_{FRET} Assay

A 1µM:1µM CyPet and YPet fused with E3 Ligase and M1, respectively, assay was performed to generate preliminary interaction data. Varying excitation and emission peak wavelengths at 414 nm /475 nm and 475 nm/ 530 nm, were used for CyPet and YPet, respectively. When the fluorescent pair (CyPet and YPet) are placed in close contact at 2-10 nm with favorable orientations, then the excitation of the donor will excite the energy transfer from the acceptor. The coupling between the two fluorophores occurs due to the excitation of the donor, CyPet, which induces an energy transfer to the emission of the acceptor, YPet. As a result, it quenches the donor while exciting the acceptor. Preparing a 1 µM sample of CyPet and YPet with Interaction Buffer (150 mM NaCl, 25 mM Tris HCl pH 8, 5% glycerol) and 1 M DTT. Control samples were prepared with CyPet alone for the alpha value (α) and with YPet alone for the beta values (β); the ratio coefficient, α, is calculated to account for the emission peak of CyPet at 475 nm, as the ratio coefficient, β, is calculated to account for the emission peak of YPet at 530 nm. The prepared samples were pipetted in triplicate into 384 well plates.

Em_{FRET} was determined by utilizing Equation 1 to calculate true FRET emission.

Equation 1:

$$EmFRET = EmTotal - ((a * FLdonor) + (\beta * FLacceptor))$$

Spectrum reading is generated for qualitative interaction data collection. The following parameters are set for CyPet and YPet with 414 nm excitation, 455 nm cutoff and 475 nm excitation, 515 nm cutoff, respectively. For both samples, it was measured from 400 to 600 nm. Spectrum readings are to be performed separately for CyPet and YPet intensities.

K_D Determination

The dissociation constant (K_D) was determined by keeping the donor protein concentration at a constant of 0.1 µM and titrating the acceptor protein concentration from 0 µM to 25 µM. The fluorescent fusion protein pairs were combined into a total volume of 60 µL with Interaction Buffer (150mM NaCl, 25mM Tris HCl pH 8, 5% glycerol) and 1M DTT. Each titration was repeated in triplicates, again, to account for errors introduced due to pipetting variation. The prepared samples were incubated for 15 minutes in a 55°C water bath before being transferred to a Greiner 384-well plate. FlexStationII384 was used to measure fluorescence intensities at 414 nm /475 nm, 475 nm /530 nm, and 414 nm/ 530 nm. The settings necessary for the FlexStationII384 are selecting the "Endpoint" settings, setting the correct fluorescence intensities, and selecting the wells to be analyzed, and PMT constant gain at "Low" to allow for mixing.

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Three wavelengths were recorded and the relationship between K_D and Em_{FRET} was determined by Equation 2.

Equation 2:

$$EmFRET * \left(\frac{[Acceptor]total - [Donor]total - KD + \sqrt{([Donor]total + KD - [Acceptor]total)^2 + 4 * KD * [Acceptor]total}}{[Donor]total + KD - [Acceptor]total + \sqrt{([Donor]total - [Acceptor]total + Kd)^2 + 4 * KD * [Acceptor]total}} \right)$$

Prism5 (GraphPad Software) was used to fit the Em_{FRET} values into Equation 2 to determine the K_D value. A non-linear regression fit was set with the donor concentration set at 0.1 uM and the initial K_D and $Em_{FRETmax}$ conditions set to zero.

RESULTS

The potential all human SUMOylation E3 ligases and as control, Ubiquitin E3 ligase, in human genome, TRAF6, PIAS3, PIAS4, RHES, hSTUB1, hRNF4, hMApl, parkin, PIAS1, hCBX4, hHDAC4, hHDAC4, hTRIM28, Dcst, Fbxw7, hCRBN, SOCS1, hSCNA, and IAV M1 genes were synthesized and cloned into pET28b vector with CyPet and YPet tag, respectively. Following an extensive screening process, strains exhibiting an expression ratio above 3 (induced vs. uninduced) were chosen to express proteins. Among these, hTRIM28 and PIAS1 were chosen for K_D determination because their FRET spectrum and EmFRET signals were positive for potential interaction. The proteins underwent purification through Ni-His affinity columns, and the eluted samples were collected for qFRET determination. An SDS gel electrophoresis was performed to confirm the fluorescent full-length proteins were maintained.

The FRET spectral analyses were conducted prior to determining the dissociation constant (K_D) for the interaction between the E3 ligase and the IAV M1 protein. The interaction spectra were examined at concentrations of 0.1 μM, 0.5 μM, and 1.0 μM/each protein, aiming to discern the specificity of the binding between the two proteins. In the initial investigation, CyPet-tagged E3 ligase at a concentration of 0.1 μM was engaged with YPet-tagged IAV M1 protein (Figure 2a-f). Subsequently, a parallel study was conducted wherein both CyPet-tagged E3 ligase and YPet-tagged IAV M1 protein were present at concentrations of 0.5 μM (Figure 3a-f). Finally, a third examination involved both entities at concentrations of 1.0 μM (Figure 4a-f). The graphical representations derived from these experiments offer qualitative insights into the interaction dynamics before the quantitative determination of K_D. An increase of each of the substrate loadings shows a clearer emission peak at the YPet fluorescent emission at 475 nm indicating an energy transfer between the two protein pairs.

A select set of E3 ligases were chosen for assessment at lower concentrations of 0.1 μM and 0.5 μM, in addition to the standard 1.0 μM, to discern potential concentration-dependent effects on their interactions. However, all E3 ligases were evaluated at the 1.0 μM concentration alongside the determination of Förster resonance energy transfer (FRET) efficiency (Em_{FRET}) to investigate their binding characteristics comprehensively.

A quantitative absolute FRET signal value, Em_{FRET}, can provide additional information about the binding nature between the E3 ligase and IAV M1 protein. The Em_{FRET} was obtained to determine the sensitized FRET signal resulting from the binding of two proteins. The fluorescent pairs were excited at excitation wavelengths of 414 nm and 475 nm for CyPet and YPet, respectively. The α coefficient, necessary for determining Em_{FRET}, was derived from the donor fluorescent protein excited at 414 nm. Consequently, the β coefficient was derived from the acceptor fluorescent protein excited at 475 nm. The α and β coefficients were multiplied by the fluorescent emission of the donor and acceptor and subtracted by the total emission, as described in Equation 1.

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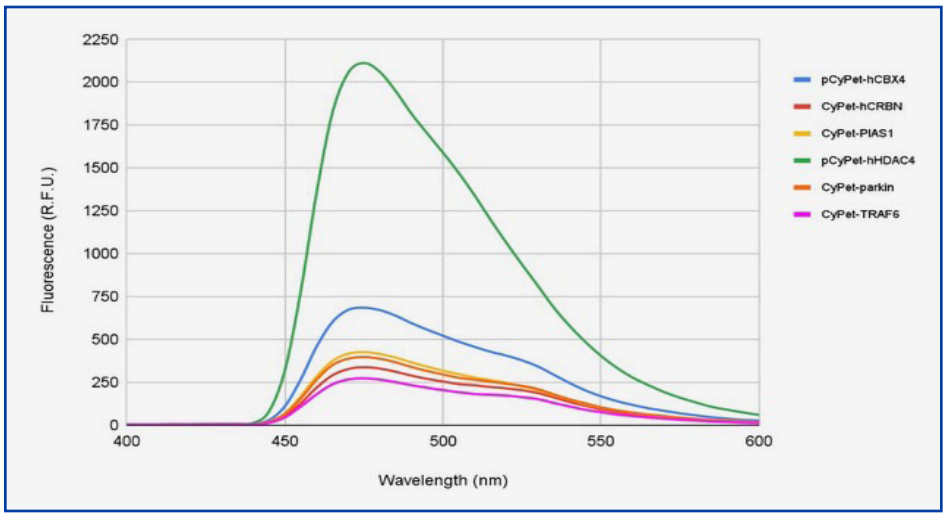

Figure 2: 0.1 uM CyPet : 0.1 uM YPet IAV M1 interaction spectrum graph determined from EmFRET assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink).

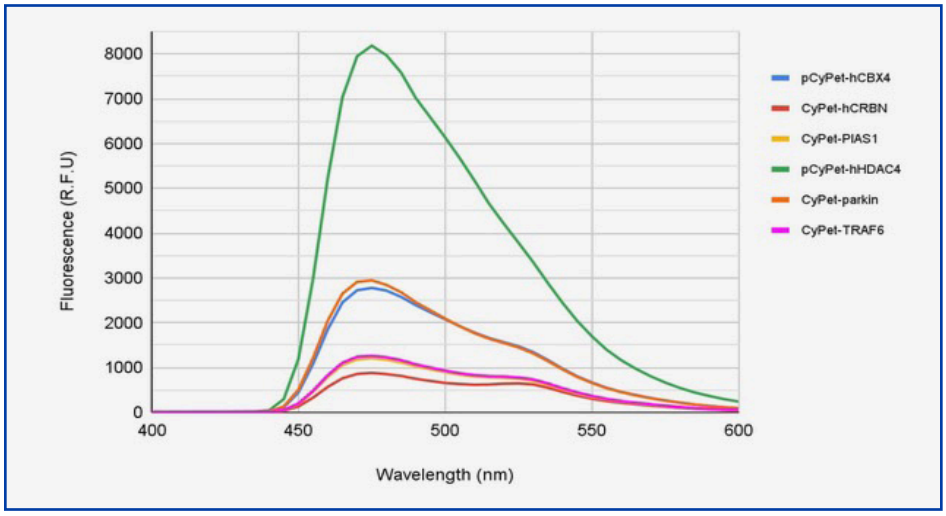

Figure 3: 0.5 uM CyPet : 0.5 uM YPet IAV M1 interaction spectrum graph determined from Em_{FRET} assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink).

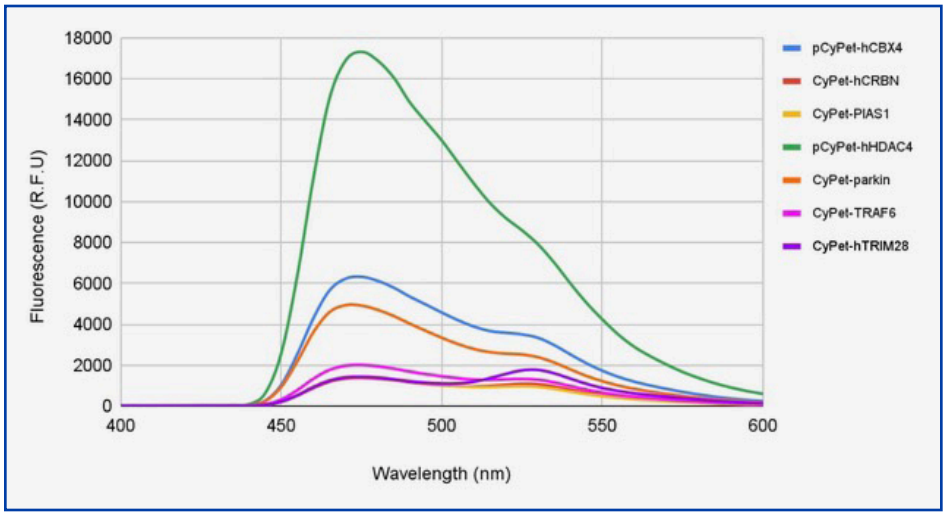

Figure 4: 1.0 uM CyPet : 1.0 uM YPet IAV M1 interaction spectrum graph determined from Em_{FRET} assay. CyPet-hCBX4 (blue), CyPet-hCRBN (red), CyPet-PIAS1 (yellow), CyPet-hHDAC4 (green), CyPet-parkin (orange), CyPet-TRAF6 (pink), CyPet-hTRIM28 (purple).

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The acquired Em_{FRET} values were then subjected to comparative analysis, facilitating the assessment of the binding affinity between the E3 ligase and the IAV M1 protein. Consistent throughout (Figure 2b, 3b & 4c) there was a clear energy transfer between the pair with an evident peak at 414 nm and 475 nm, however the Em_{FRET} value is considerably low at 89.74 (Figure 5). This comparative evaluation was depicted graphically in Figure 5, offering a visual representation of the binding characteristics between the protein entities under investigation. The Em_{FRET} value for pCyPET-PIAS1 and pCyPet-M1 was 565.92, exhibiting a high binding affinity. In addition, the Em_{FRET} values for PIAS3, PIAS4 hSCNA, and TRM28 were very high too, indicating potential interactions. The generated Em_{FRET} values also provide a clue for further investigations.

We determined the K_D values between PIAS1 and TRIM28, and IAV M1 in order to determine its binding affinity for further characterizations. The binding affinity between the fused CyPet hTRIM28 and PIAS1 with YPet IAV M1 was determined by holding the FRET donor at a set concentration of 0.1 μM. The FRET acceptor was titrated in varying concentrations from 0 to 25 μM. The K_D value was determined by quantifying the absolute FRET signal between the interactions of the two fluorescent pairs.[12] The curves generated in Figure 6 exhibit the binding affinity difference between hTRIM28 with IAV M1 and PIAS1 with IAV M1. The calculated K_D values for (Figure 6a & b) were 24.2 μM and 2.9 μM, respectively. The determined values indicate that the E3 ligase PIAS1 exhibits a notably higher affinity for IAV M1 protein, indicating a real SUMOylation E3 ligase for IAV M1, whereas the E3 ligase hTRIM28 is not. We will examine the E3 ligase activity of PIAS1 in the future study.

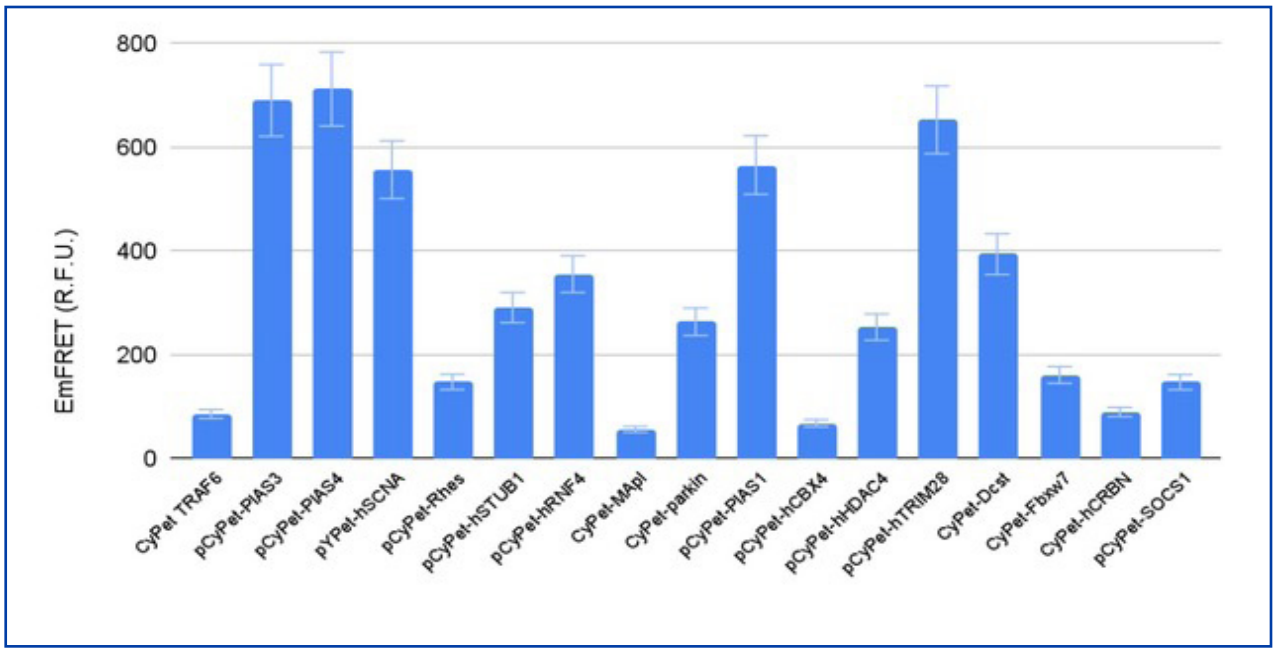

Figure 5: Results of varying 1uM E3 Ligase with 1uM YPet IAV M1 to check for interaction prior to K_D determination.

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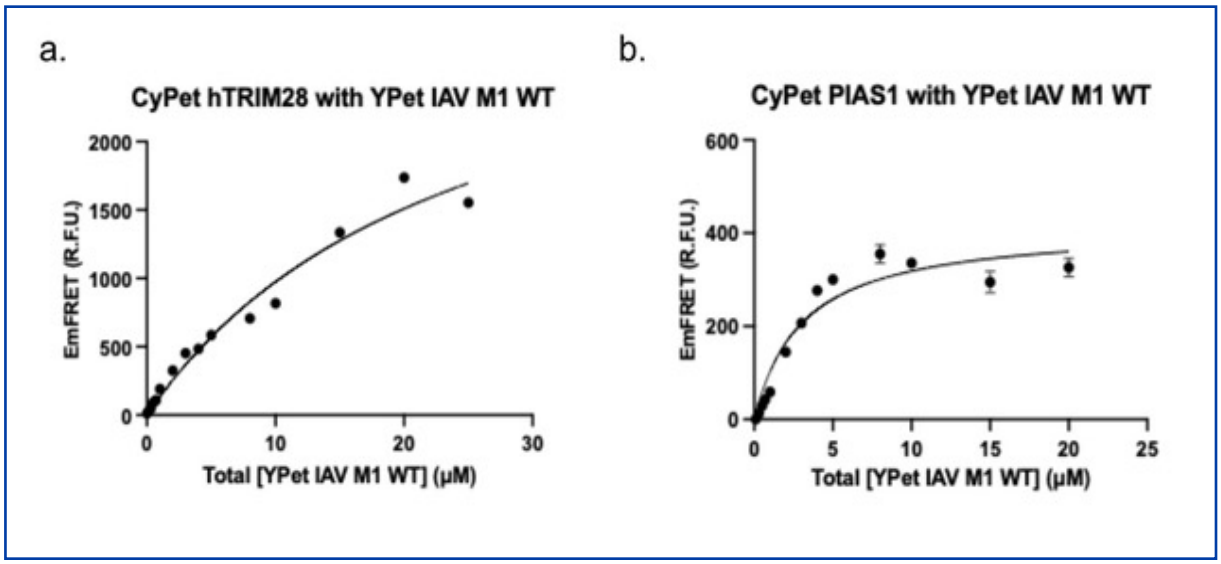

Figure 6: K_D determination results. (A) The interaction between IAV M1 and hTRIM28 was determined, with a K_D value of 24.2 μM. (B) The interaction between IAV M1 and PIAS1 was determined, with a K_D value of 2.9 μM.

DISCUSSION

SUMOylation is a post-translational modification process capable of regulating protein function *in vivo*. Viruses, such as the influenza virus, have been shown to utilize the SUMOylation process to replicate once infecting human cells. Viral proteins exploit the SUMOylation process to enhance their assembly and block the immune response of the host protein.[14] However, similar to the viral protein's ability to utilize the SUMOylation pathway to regulate its replication, the host protein can combat the viral infection by modulating its immune response through SUMOylation. We were able to isolate the fluorescence-labeled proteins of interest for K_D determination to understand their interaction better by taking molecular engineering approaches. After preliminary spectrum and Em_{FRET} analysis, CyPet PIAS1 and hTRIM28 were chosen to determine its K_D because they showed high FRET signals. However, hTRIM28 with the initial high Em_{FRET} value showed a very high K_D value of 24.2 μM, indicating a low binding affinity for IAV M1. Whereas PIAS1 was consistent with its preliminary results and had a low K_D value of 2.9 μM. The results suggest that PIAS1 exhibits a high affinity for IAV M1 protein and could be a real E3 ligase for M1 protein. Once this is validated, chemical inhibitors can be developed to block the host-viral interaction.

We were surprised to find that the same protein appeared to exhibit varying degrees of affinity when comparing protein-protein interactions using three different methods of FRET. Due to the presence of non-specific interactions between proteins, high concentration can force two proteins to interact spatially rather than based on their true interaction affinity. Many types of interfaces exist in physiological fluid media, such as important protein fibers, membranes, and interfaces between condensed phases of solutions, in which interfacial interactions with solute molecules (in this case proteins) in aqueous solution can occur.[5] Depending on the distance between the solute and the surface, the solution layer can be considered close enough to the interface to include a

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microenvironment separate from the whole solution. As such, qualitatively assessing FRET interaction alone is not sufficient to accurately determine the affinity between proteins, especially large-sized proteins that are more susceptible to non-specific binding at high concentrations. Unavoidable protein degradation in our expressed protein purification makes the final product contain some non-fluorescent tagged proteins. These proteins also have interferent effects when squeezed in tight spaces, all of which can lead to inaccurate results in the final calculation of FRET values. In measuring the affinity between 1 μM CyPet hTRIM28 and 1 μMYPet IAV M1 WT, an EmFRET of 653 R.F.U. was obtained, especially in measurements using a 384-well plate non-specific binding was observed due to the large size of the molecule and high concentration of CyPet hTRIM28 (145,040 Da). To solve this problem, we used qFRET to measure the affinity of the proteins. The FRET values for true target protein interactions were first calculated by subtracting the interference of nonspecific interactions and non-target protein interactions. Secondly, by gradually increasing the amount of YPet IAV M1 WT and fixing the amount of 0.1 μM CyPet hTRIM28, when the binding between the target proteins reaches saturation, depicted as a plateau, where no more binding can be achieved despite the continuous increase in the amount of YPet IAV M1 WT. Furthermore, at higher concentrations of this protein, it is harder to avoid any non-specific protein-protein interactions that may lead to inaccurate results, as well as saturated fluorescent signals. Therefore, even though strong interaction is observed in measuring FRET alone, more accurate affinity calculations using qFRET ultimately yield substantially weaker interactions among these proteins.

Our study is highly clinically relevant. We aim to elucidate the emphasis on screening for host factors that viruses, such as influenza, exploit for the replication of their genome rather than on the viral protein itself if we are to engineer an avenue of effective therapeutics that bypass viral evasion strategies.[6] While the IAV M1 protein is essential for viral assembly, replication, and spread within the host, its SUMOylation, dependent on host factors such as E3 ligases, may be as crucial for IAV M1 protein function.[7,8] As such, SUMOylation is a multi-step cascade of reactions in which the SUMO post-translational modifier (PTM) is activated and conjugated by a known E1 heterodimer and an E2 enzyme, respectively.[9] Further probing into the importance and mechanism of E3 ligases, which catalyze the transfer of conjugated SUMO to the viral protein (M1), is still lacking, as specific and physiologically relevant candidates have not yet been identified until this screening approach using qFRET. In this study, we clarified the importance of quantitative analysis of these interactions using qFRET, as seen with the aforementioned discrepancy between CyPet hTRIM28 and YPet IAV M1 interaction compared with their biochemical activity. Thus, we have gained insights into yet another potential host factor as a tunable target for developing therapeutics that can modulate and hopefully attenuate influenza replication. However, the degree to which these *in vitro* results agree with *in vivo* interactions remains to be seen; luckily, we present qFRET again as a solution for investigating and comparing the effect of other E3 ligases in both experimental settings.[10,11]

The qFRET technology used in this study is carried out in a solution without a high purity requirement, and this condition can mimic physiological and pathological conditions. Therefore, the qFRET-based measurements are closer to physiological events in living cells. In addition, the qFRET assay is very sensitive, and the concentrations of fluorescence-tagged proteins required in the qFRET assay can be as low as nM; therefore, a minimal amount of proteins is needed for the interaction affinity determinations. Furthermore, the qFRET assay is environmentally friendly and does not contain any radioisotopes or chemicals. Applications of the qFRET technology should provide high-quality

protein interaction and catalytic affinities of systems, networks, and proteomes and provide comprehensive quantitative biological and biomedical maps without the need for laborious protein purification, especially for those difficult-to-be-expressed proteins, such as SUMOylation E3 ligase in this study. The genome-wide search for SUMOylation E3 ligase for influenza virus M1 protein is not only important for research but also for novel anti-virus therapeutics development in the future.

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