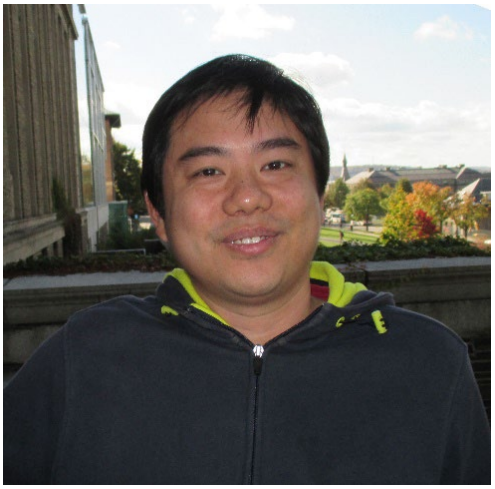
COVID Information Commons (CIC) Research Lightning Talk

Transcript of a Presentation by Liqi Alex Lai (Cornell University), July 16, 2021



Title: SARS-CoV-2 Fusion Peptide has a Greater Membrane Perturbating Effect than SARS-CoV with Highly Specific Dependence on Ca.

Liqi Alex Lai CIC Database Profile

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YouTube Recording with Slides

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Transcript:

Liqi Alex Lai:

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Hello everybody and thank you for the organizers. So, my topic is about the SARS-CoV-2 fusion peptide which is but- more like a bio physical study.

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So, this is the- how the SARS virus enter the host cells. So basically, it is a common process shared by a lot of envelope virus. So, the virus first attaches to the cell membrane receptors and then they will possibly go through two pathways. The first one is to go through the plasma membrane. So, there is a fusion between the virus envelope and the plasma membrane. And then the second one is the virus first engulfed by the host cell to go into the endosome and then the fusion between the endosomal membrane and the viral envelope will release the genetic material into the host cell.

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So, during this process, the spike protein or other called- similar proteins or another virus are very important. So basically, the spike protein is a trimer of the S1-S2 heterodimers. So, the S1 is basically responsible for the binding of the receptors and then the S2 subunit is responsible for the membrane fusion. So, as we can see here, there is a very small part which we call the fusion peptide that's actually

inserted into the membrane, and this insertion is very important because it will initiate the membrane fusion and this is a required step in the viral infection. So that's why we need to find out the mechanism of how distribution peptide interacts with the membrane and how they initiate membrane fusion.

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The method we mainly use is the- we call ESR [erythrocyte sedimentation rate] which is magnetic spectrometer method. So basically, that we want to put some spin- a free spin labeled lipids onto the membrane as we're showing here and then this beam will locate our different position in the membrane. And then we mix the fusion peptide with the membrane and detect the change of the structure of the membrane, which is reflected by the spectrum. So, we collect this spectrum and then we repeat this experiment in the condition of different peptide concentration. And then, from this spectrum, we do a simulation and then we extract the parameters. So, one of the most important parameters in this study is called the order parameters. As you can show in this figure, if the concentration of the peptide increased, and then the- this x not very increased as well so there is the x type of jump. We have repeated this experiment for a very- a wide range of virus fusion peptides and what we find is that the active fusion peptide will induce this as junk- as shape junk- while the mutants and then the non-active mutants cannot induce this kind of junk. So, what we think is that this membrane ordering effect is a prerequisite for the membrane fusion in the viral entry process.

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So, when it goes to the SARS-CoV glycoprotein, the S protein, then the- it's a little bit difficult to determine which part is the fusion peptide because the for the SARS COVID, the S protein has several distinctive cleavage sites. So, the most important are tool that- what we call is the S1H2 side and then the S2 prime side which looking here. So, the first thing we need to do is to determine which one is the real fusion peptide. So, and actually for- we have several candidates and then for these candidates if we put it in the artificial system, they can induce a kind of artificial membrane fusion. So, it's very- it's not very effective. They use the traditional method. That's why we think the membrane ordering can be a criterion to distinguish which one to identify the real fusion peptide.

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So, what we find here is that the FP1, it can show a very significant jump while the other two candidates cannot have this kind of certain degree of increase. So, and then we finally can identify- determine that the FP1 that which we immediately after the S2 prime position cleavage site is the real fusion peptide. And during this process, we also found a very interesting thing that is quite not uncommon in the virus- that is dependent on the calcium. So, as you can show here, without the calcium there is no big jump. And then in here if we fix the concentration of the fusion peptide and increase the concentration of the calcium, we can see the calcium significantly increase the ability of- for the fusion peptide to induce the membrane ordering.

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And then when it goes to the SARS-2 fusion peptide, we first compare with the SARS the sequence with SARS-1, SARS-2 and then the MERS and we identify the homologous sequence of the SARS-2. And then we think that is the SARS-2 fusion peptide. And then we do this experiment and we really found that it can also induce the membrane ordering and in the condition of the calcium. And then we compare the ability to induce the membrane ordering between the SARS-1, SARS-2, and the MERS, and we found that the SARS-2 has a higher activity. And we also have detected the calcium dependency of the SARS-2 fusion peptide is very specific as we showed here. The other ions do not have- cannot induce the membrane ordering as much as the calcium. So, and with some other method, we can also know that one SARS-2 fusion peptide binds two calcium ions and then the interaction between the SARS-2 fusion peptide and calcium is stronger than those- this last one is MERS fusion peptide.

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And we also go one step forward that form a separate fusion peptide to the host by protein trimer. So, remember that the fusion peptide is only part of the whole protein and we need to know whether the whole protein- the fusion peptide domain of the whole protein functions as the separate fusion peptide. So, what we use is a pseudotype virus particle which we call pp which express the spike protein trimers on the membrane. And we use this to interact with the SUV which have the spin label on the membrane and then we detect this- the increase of the membrane ordering in real time. So, as we can shown here, after we trigger with the calcium we can find out the jump, and then if we use some other ions then we cannot observe that. So that means that the whole S protein trimer anchor of the membrane also induced the membrane ordering as the fusion peptide and also is a very specific calcium-dependent factor.

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So, this is a conclusion then. The SARS-2 fusion peptide locates in the downstream of the S2 prime cleavage site and it can induce the membrane ordering in a calcium-dependent fashion and it binds to the calcium in specifically in one peptide to two calcium ratio and has a higher fighting affinity and stronger membrane ordering effect than the SARS-1 fusion peptide. And then we also notice that the SARS S2- S protein trimer also induced the membrane ordering as the separate fusion peptide. So, this study will help us to understand the mechanism of the viral entry in the whole cell and also indicate how- give some hints to how to develop a drug and vaccines. So, yeah, that's my talk. Thank you so much.