Hi Katia,

Before we answer each question, I would like to point out our results are not denying binding avidity adaptation. Our model results support the average binding avidity at population level will change according to population immunity. However, the variance between individual hosts is limited. Overall, it can be thought as there are two different components, similar to the Brownian motion random process, one component is called drift or trend, the other is the variance. The trend is the average change. The variance is the individual changes. The average changes demonstrate importance of binding adaptation. The limited individual changes, lead to the short sighted evolution.

Hi Sean, and James,

Thanks again for sending this manuscript, and sorry about my delayed response. I’ve carefully read through it, and have a bunch of comments. See below. My main concern is my confusion of the model structure. I think the first thing we need to do together is work through this model and the associated text to finalize the overall model structure. I know parts of it are related to our PhilTrans paper, and that the other part of it is related to your work with Steve – do you have a published paper on this that I can look at?

Ans:

James and I decide to rephrase some paragraphs and one figure to describe the model schema, virus properties, antibody responses, and immune history. We will use a top down approach to describe the scenario of natural infection in a population first. Then we describe epidemiological parameters, and then how we define and capture human immunity. Both our work (under reviewed) and Adam J Kucharski et al., The impact of stratified immunity on the transmission dynamics of influenza, use a Poisson distribution to capture antibody responses. It is a more realistic way to incorporate to capture and to produce different levels of immunities.

My one other concern is that, with your simulations, you seem to be looking only at a seasonal epidemic. Since immunity profiles are changing over the time course of the epidemic, there is no single ‘optimal’ binding avidity value for the virus. This makes some of the presentation confusing, I think, because, while it is still stabilizing selection, the optimum is moving. Showing simulations for an endemic scenario (additionally) would significantly strengthen the presented work. Also, since it is well known that less seasonal E/SE Asian places play a critical role in H3N2, with the trunk of the HA phylogeny located in these regions, I think looking at only seasonal epidemics doesn’t seem to make much sense to me.

**Ans:**

One of the main point from our results is that we can calculate optimum binding avidity through the changes of the population immunity. Therefore the optimum is moving higher to adapt to higher population immunity. In our model, the optimum binding avidity will change or increase, as long as more individuals acquire more antibody immunity and produce stronger partial protection. This mechanism really reflect the binding avidity events from serial passaging.

Moreover, SE Asia showed the chaotic patterns (<http://jmsc.hku.hk/reportinghealth2016/2016/02/25/hk-children-are-more-vulnerable-to-flu-this-year>), rather than due to an ideal endemic transmission (R0=1). The endemic pattern is the outcome from a simplified SIR compartment model, and here after we adapt individual changes on both virus and human host, the pattern would reflect more in real scenarios but not endemic transmission.

If we use a simpler SIR model (e.g., the model I created 3 years ago) to fix the population immunity then it will lose the reality. I remember at that time, other researchers/reviewers argued we ignore certain mechanisms and suggested us to track individual changes. In this more realistic model we use now, the only scenario more ‘similar to’ endemic we can generate is that what we we run later for multiple seasons without binding avidity adaptation. However, it is an extreme case which is not able to explain the short-sighted evolution.

I like the within- vs between-host selection argument at the core of the paper, and think that we could incorporate some very simple population genetic models to illustrate the main argument. I can think about this if you would be OK with this.

**Ans: Please let us know your ideas. We are interested in knowing this.**

Here are my more specific comments:

1. Net charge is a good marker of receptor binding avidity

My main concern here is that there is no clear indication for why receptor-destroying enzyme (RDE) activities should reflect binding avidity. Is this obvious? More background text on this is needed.

**Ans:** We will find the references to explain the usages of RDE.

Supp Table 1 referenced from the results section here is awkwardly placed since no phylogenetic analysis has yet been presented

Including another subplot (A) with figure 1 that has RDE change on x-axis and net charge change on y-axis would be visualizing convincing.

**Ans:** We use the chart to show RDE distribution by net charge. However, statistically, we use TableS1 and Fisher’s exact test with the categorical data to test the correlation. This categorial F test should be sufficient to indicate whether these two variables are correlated.

Figure S1 – I am unclear why you would have change (log ratio) on the y-axis and then absolute net charge value on the x-axis. It seems that both x and y-axes need to be in absolute values or in relative values (change effect), but not one of one and one of the other. This doesn’t make sense to me.

**Ans:** Good point. I agree with you. I will regenerate a new figure to use a same strain as a standard binding avidity reference.

1. Net charge is a phenotype under immune-mediated selection

I think this figure 2 can be significantly improved upon by showing the individual point data by age (x-axis = age, y-axis = netcharge), and then fitting a spline through these data points, as was done in Fig 2 in Kucharski et al. PloS Biology (2015). Aggregating by large age groupings doesn’t make much sense to me.

Ans: I will add one figure and use both figures. Previously you have suggested us to change to age group because Kucharski et al, used age groups and we can make comparison with their data. Let me try 10 or 15. Age group 20 is commonly used when contact mixing, or age specific serological attack rate is considered. Also it doesn’t show a huge variance, so it shows some information. The other main reason is that because Kucharski et al. data is based on seroprevalence but not simply on different level of titres. It would make more sense to compare prevalence of higher net charge to seroprevalence by age groups than simply using average net charge.

I think making a comment here that these age-related patterns could come about from differences in susceptibility to certain viruses vs. evolution within these individuals is important

**Ans:** We can recall Hensley’s words. I think they have described the view about ‘immunity’ to certain viruses vs. evolution. However, if you mean the impact of the aged structure population, it would be better to put in the discussion.

1. Model: receptor binding adaptation lengthens epidemic season; trait under stabilizing selection in heterogeneous host population (binding avidity changes – short-sighted evolution)

A much more detailed description of the epi model is definitely needed – from the text, I don’t follow what you’ve done. I also am unclear about why Fig 3B vs. 3C reflect different levels of differential selection and what this has to do with bottleneck size.

Ans:

1. As our previous response. We will rephrase our model structure.
2. Will describe Rin more and cite this from the previous work.
3. Will add few lines to describe how I generate fig3b and c and what does the parameter value indicate.
4. About the differential selection vs bottlenecks. In figure 3B, the number of transmitted virions is less than in 3C. Transmission bottlenecks is present when only a few individual pathogens are transmitted, which is like figure 3B. Given a fewer number of transmitted virions, a much narrower or triangle like peak is present, representing a stronger differential selection. On the other hand, in figure3C, the curve is more square like, which means no difference in selection among a wide range of binding avidity.

Lengthening the epidemic – are you thinking about this in the context of a seasonal epidemic or a pandemic? I believe the former, but the text should be clear on this.

**Ans:**

I am not quite sure whether we need to make clear the distinction between epidemic and pandemic here. If we need, I can make it clear to be a single seasonal epidemic with partial immunity. Worth to keep in mind that here in the model, the difference of seasonal epidemic or a pandemic will only be pre-existing immunity. We still can imagine it is a pandemic if not so many people has partial immunity. Since I produced the pre-existing, I will assume it is a seasonal epidemic while an antigenic strain was introduced in this population.

I think the simulations in Fig 4 are interesting (what assumptions are you making, though, of bottleneck size, parameterization, etc.) and I think showing the dynamics over a single season is a good idea. Why does the adaptive binding simulations have that strange bump in the confidence intervals?

Why doesn’t fig 4 show the adaptive binding avidity dynamics with simulations that are started with a late-adapted binding avidity value?

**Ans:**

1. The bump is because it’s a stochastic simulation, and I think we’re only running 100 trajectories. If we run more, this should smooth out. Agree that we need to add one simulation with different initial condition, such as a higher initial binding avidity but still able to produce the proper peaks. Keep in mind that this is a more complex model. Certain parameter settings might lead to stochastic die out.
2. The most important result is the prolonged tail with binding avidity. Also, the peak incidence is not optimized.

The one problem I see with only showing a seasonal epidemic scenario is that, because immunity levels are changing throughout the period, the late-adapted optimal value and the early-adapted optimal values differ from one another, and there is no overall optimal value as there would be in an endemic population. I think running a similar analysis for a non-seasonal, endemic case would be a good idea, to show similar patterns.

**Ans:**

This is key property we demonstrated in our model that the optimum binding avidity will adapt through the changes of the population immunity. We didn’t deny, on the contrary, our results support average level of binding avidity will adapt to population immunity. However,

Fig 5 never cited. Also, it is not clear to me at all what the y-axis (J) represents – again, a much more thorough description of the model is necessary.

**Ans:** J is the immunity when antigenic drift is included. Will add the description. This is showing how the distribution and the mean population immunity changes over a single epidemic, which will put increased selection pressure for immune escape on the viruses.

Paragraph starting with ‘Within-host binding avidity evolution…’ at bottom of p, 9: seems like it would be short-sighted in any heterogeneous host population, no? The paragraph that follows (p9-10) is incredibly confusing to me. I like the distinction between within-host selection and between-host selection, and have seen it previously, for example, in Christophe’s recent HIV paper in eLife. I don’t understand Fig 7 though… this is not to say that it is not worthwhile to include – I just don’t understand what it is showing, given the text.

**Ans:** A Heterogeneious population will lead to short-sighted evolution. That is right, generally. Hard to answer whether in ‘any’, situation. However, I will modify figure 7 to demonstrate the situations in both more homogeneous (when population is more naive) and more heterogenous (i.e., with more partial immunity). Figure shows that the fitness within host and the fitness of population will force the binding avidity toward different directions when there are partial immunity in the population.

Fig 8 – is this for the seasonal epidemic simulation? I think it would be much more worthwhile to do this same analysis for an endemic situation.

Ans: As mentioned before. We are not able to capture an endemic situation here.

Fig 9 – this is interesting, and makes sense given that fitness variation introduced through population-level differences in binding avidity lowers the effective population size and therefore diversity. Again, is this for the epidemic simulation? Would be better, in my opinion, for an endemic situation.

1. Phylogenetic analysis – supports stabilizing selection on binding avidity phenotype

It is unclear to me why you should see overall lower net charge on external branches (I can see why the variance should be higher). This overall lower binding avidity on these branches seems to be consistent with your simulations (Fig 8), but it is unclear to me whether you are finding this in your simulations just because you are starting at significantly-below-the-optimal binding avidity. It seems that this is the case. If you started your simulation at optimal early-adapted binding avidity (dotted value in Fig 8 at an early time point), I don’t think you would see overall lower binding avidity on external branches. So, the explanation for seeing this in the empirical data (Fig 10) is still missing, I feel.

**Ans:** The nodes with lower binding represent most of them transmitted to naive individuals. To answer your question, we have to compare figure 8 to figure S3, with ‘no differential selection’, then we know that viruses in naive individuals largely decrease binding and undergo short-sighted evolution. On the other hand, if there is no differential selection (as figure S3), viruses with low binding can still infect to individuals with partial immunity with similar chance, so it won’t evolve to dead end and short-sighted evolution not exist. I would put figure 8 with figure S3, in order to give better explanation. The initial condition only affect short period transiently patterns. We would do another simulation with different initial binding avidity.

1. Conclusion – compensatory effects of binding avidity are important in eliciting antigenic jumps

In Abstract – this conclusion seems unwarranted, given that you hadn’t mentioned any analysis on compensatory effects of binding avidity. There is also no analysis on this in the results section.

**Ans:** Yes. This could be one of the future work. I will rephrase them.

Other comments: Prior to sending this manuscript to Christophe and Simon, I would definitely like to go through the text and modify certain sentences for clarity and at times grammar, as well as modify the flow of some paragraphs and between-paragraph transitions

**Ans:** Would discuss this with you through email.

p. 5- To understand how virus binding avidity evolves… by comparing the marker of binding avidity to antibody prevalence. – do you do this anywhere? Seems like the first part of the results section does not do exactly this

**Ans:** We listed the prevalence of higher net charge vs age and it showed the similar pattern with the serosurveillance vs age. I will rephrase it to “by the similarity between the proportion of higher net charge to seroprevalence.”

p. 5- viral lineages with less variability would persist longer during the epidemic – this is confusing to me in relation to (3) above. Maybe just rephrase? Next sentence, emphasize that the viruses also appear to be subject to stabilizing selection, with binding avidity changes towards lower or higher binding avidities via short-sighted evolution exacting a fitness cost

**Ans:** To make it less confused, we would like to define two kinds of adaptations? One is average adaptation at population level. One is between hosts variance. It is more like diffusion theorem, the trend (average binding by time) is adapting to the increased of the population immunity. At the same time, the variance (between hosts adaptation at a given time), is limited.

p. 6- Results – the flow of this paragraph is confusing – you start off with a conclusion sentence

Ans: We can discuss again. Here at IC I learned to put the statement first, and then describe what we do. Eventually the conclusion will match the statement. PI at IC said it would let readers easy to read.

p. 7- Results – same comment for starting off with a conclusion sentence, and also in the following results sections

What is your timing/schedule going forward? I would also really like to see this work published.

Best, Katia