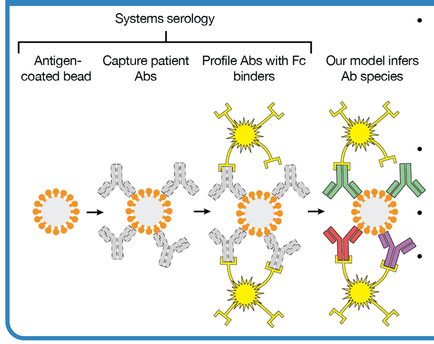
Multivalent binding model quantifies antibody species from systems serology

Armaan A. Abraham[1], Cyrillus Tan[2], Priyanka Shrestha[3], Emily Bozich[2], Aaron S. Meyer[1 2]

[1]Department of Bioengineering, [2]Bioinformatics Interdepartmental Program, University of California, Los Angeles; [3]Stanford University

Introduction



- Systems serology aims to broadly profile the antigen binding, Fc biophysical features, immune receptor engagement, and effector functions of antibodies.
  - Excels at identifying disease-relevant antibody effector functions.
- A crucial limitation is its incomplete description of the antibody structural features responsible for the effector functions we observe.
- We address this limitation with a binding model to quantify antibody species in systems serology.
- With the model, we discover novel patterns of IgG fucosylation.
  - E.g., COVID-19 vaccination is more protective when it induces afucosylated antibodies.

Method

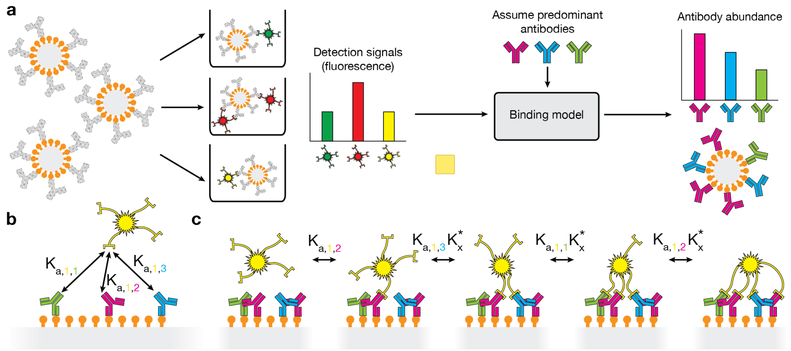


Fig. 1. (a) In systems serology, fluorescently tagged detection reagents are used to profile antibodies that are captured with bead-coupled antigen. The binding model uses the detection signals to infer the abundance of each antibody Fc species. **(b)** Each detection has a known binding affinity to each antibody species, which we use to quantify the equilibrium constant for the initial monovalent binding event. **(c)** We consider all binding events leading to a binding configuration. Subsequent multivalent binding events are quantified using the monovalent binding affinity multiplied by a crosslinking constant, K_x^*, which encapsulates steric and local concentration effects.

Validation

The binding model effectively imputes unseen measurements.

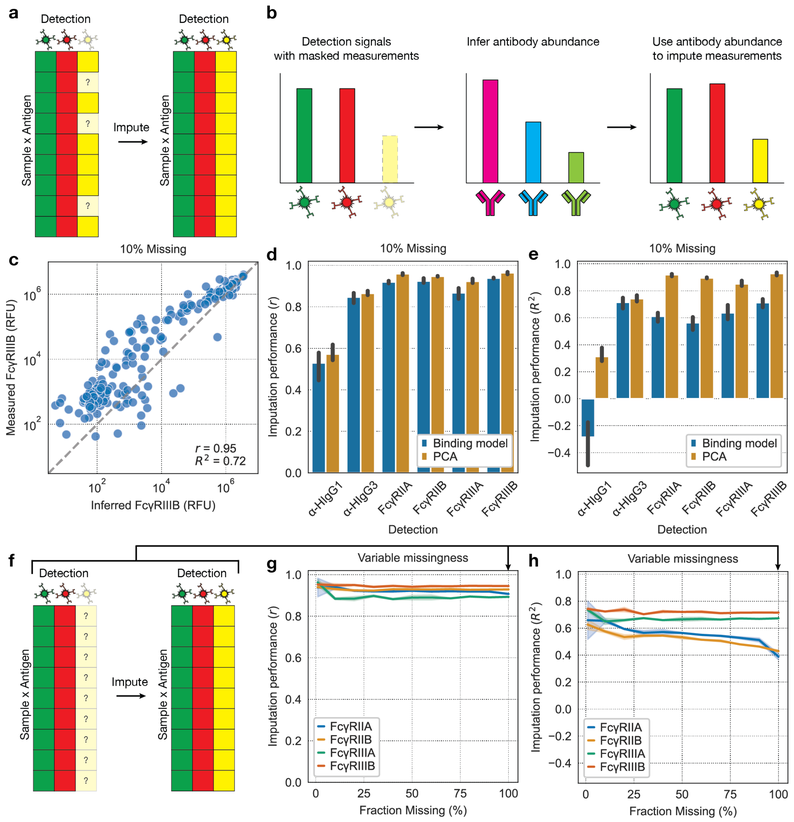


Fig. 2. (a) We mask measurements corresponding to a particular detection in real systems serology data and use the model to impute them. **(b)** The model imputes detection signals by using the incomplete data to infer the antibody abundances, which are then used to infer the left-out signals. **(c)** Imputed versus actual measurements. **(d)** Pearson correlation and **(e)** coefficient of determination between actual and imputed values for binding model and PCA. **(f)** Imputation at 100% missingness. **(g)** Pearson correlation and **(h)** coefficient of determination between actual and imputed values for the binding model at various missingness.

Validation

The binding model infers IgG fucosylation.

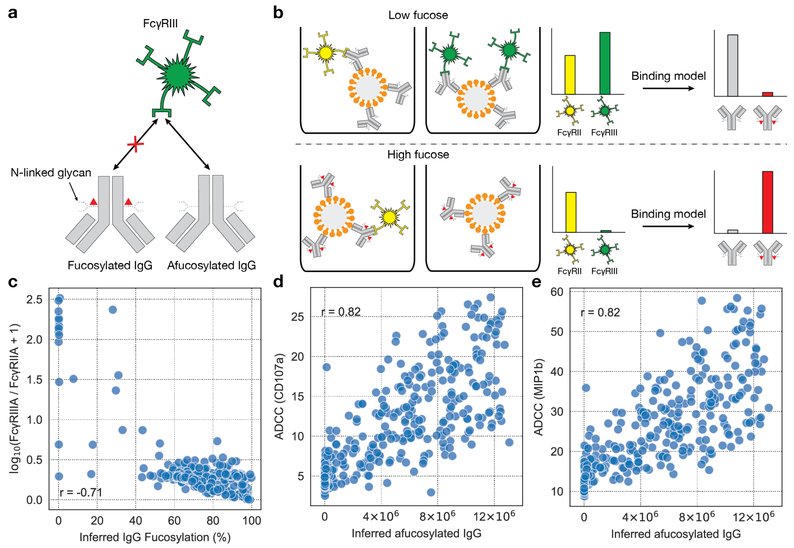


Fig. 3. (a) IgG fucosylation blocks binding to FcγRIIIA with little effect on binding to the other FcRs, such as FcγRIIA. **(b)** Higher fucosylation of bead-bound antibodies leads to a lower FcγRIIIA signal relative to FcγRIIA signal, which the binding model uses to infer how many antibodies are fucosylated. **(c)** Inferred IgG fucosylation versus measured FcγRIIIA signal / FcγRIIA signal. **(d)**, **(e)** Inferred abundance of afucosylated IgG versus ADCC measured by two markers of natural killer (NK) cell activation: **(d)** CD107a and **(e)** MIP1b.

Results

Inferred IgG fucosylation correlates with HIV severity and is lower for membrane-associated antigens.

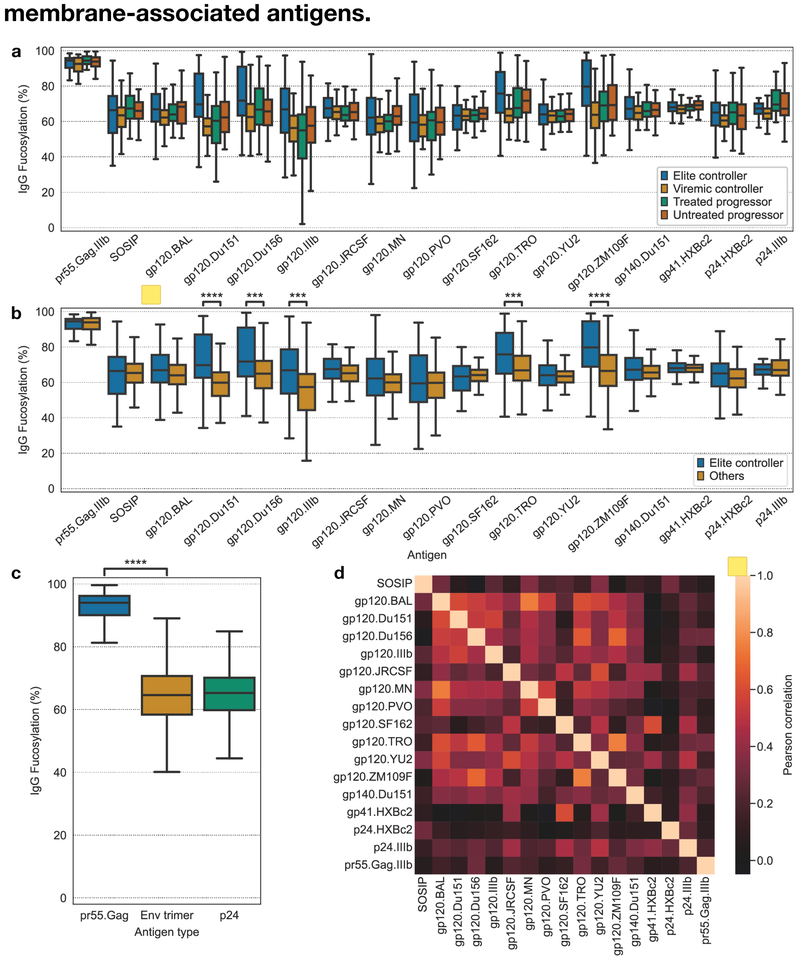


Fig. 4. (a), (b) Inferred IgG fucosylation by target antigen and patient status for HIV-infected subjects. **(c)** Inferred IgG fucosylation by antigen type. **(d)** IgG fucosylation was inferred for each sample and antigen. The fucosylation inferences for each antigen were compared across samples and used to compute a Pearson correlation coefficient. The pairwise correlation in IgG fucosylation between each antigen is shown.

- The afucosylation of p24-targeting antibodies indicates that p24 is susceptible to antibody targeting on the viral envelope or host cell membrane [2].
- The high degree of variation in IgG fucosylation by antigen across subjects indicates that antigen-specific IgG fucosylation cannot be inferred using the IgG fucosylation for another antigen from the same pathogen.

Results

In COVID-19, inferred IgG fucosylation varies by target antigen, symptom severity, and vaccine efficacy.

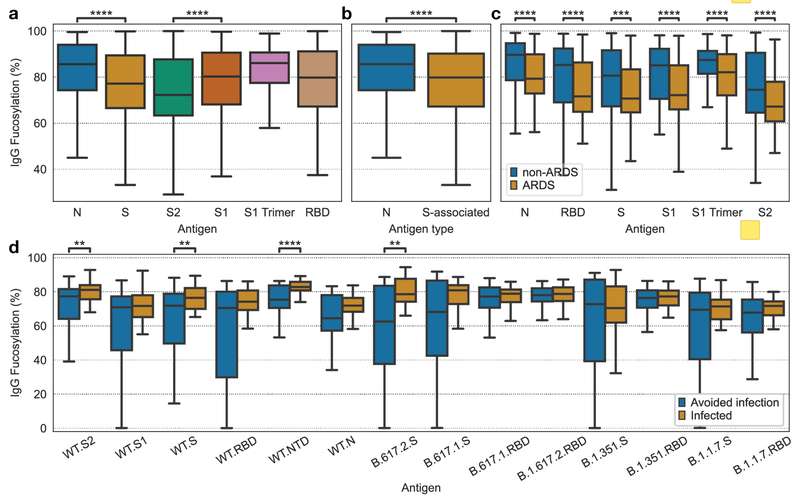


Fig. 5. Inferred fucosylation of IgG by **(a)** target antigen, **(b)** target antigen type, **(c)** target antigen and presence of ARDS. **(d)** Inferred fucosylation of IgG by SARS-CoV-2 vaccine protection and target antigen.

- More effective COVID-19 vaccines could be designed by inducing afucosylated spike-targeting antibodies.

The binding model enables optimization of systems serology assays.

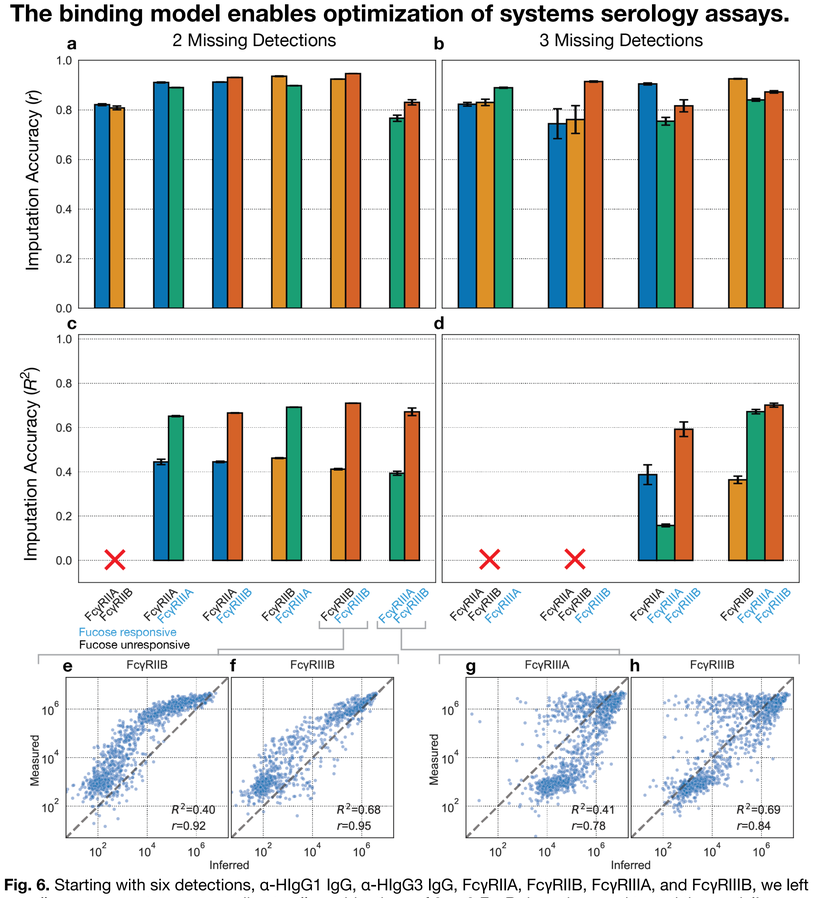


Fig. 6. Starting with six detections, α-HIgG1 IgG, α-HIgG3 IgG, FcγRIIA, FcγRIIB, FcγRIIIA, and FcγRIIIB, we left out all measurements corresponding to all combinations of 2 or 3 FcγR detections and tested the model's ability to impute them. **(a)** Pearson correlation and **(c)** coefficient of determination between imputed and actual measurements when combinations of two FcγRs are left out. **(b), (d)** The same metrics shown in **(a)** and **(c)** when three FcγRs are left out. An 'x' indicates that one of the specified values exceeds the lower y axis limit.

Conclusion

- Multivalent binding model accurately quantifies antibody species in systems serology.
- The quantification of antibody species better informs the engineering of antibody-based therapies.
- The model identifies redundancy in these assay measurements and suggests strategies to make these assays more efficient.

Future Directions:

- Experimental validation of model inferences.
- Apply the binding model to other assays within systems serology.
- Research into how to tune antibody glycosylation in vaccination [3].

References

[2] Larsen et al. 2021. "Afucosylated IgG Characterizes Enveloped Viral Responses and Correlates with COVID-19 Severity." Science 371 (6532): eabc8378.

[3] Mahan, et al. 2016. "Antigen-Specific Antibody Glycosylation Is Regulated via Vaccination." Edited by Alexandra Trkola. PLOS Pathogens 12 (3): e1005456.