pH Modulated Binding Characteristics of HIV Broadly Neutralizing Antibodies

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Abstract

Human Immunodeficiency Virus (HIV) research has focused on prevention via vaccination or clearing infection through treatments involving broadly neutralizing antibodies (bnAbs), but results have been unable to alleviate chronic infection or completely prevent new infection. One potential reason for the virus' ability to escape immune system response is environmental factors such as pH during HIV transmission and understanding how pH modulates the underlying protein-protein interactions requires in-depth analysis across a broad combination of HIV proteins and bnAbs. Traditional lab experiments may be too expensive or time consuming to screen all sequence variations, highlighting the need to analyze such interactions theoretically. To fill this need, we generate models of HIV-1 envelope glycoprotein, gp120, in complex with bnAbs in computational simulations to the binding energy between the two proteins as environmental pH varies. These results are validated against lab data performed on the same structures at pH 5.5 and 7.4. Comparing experimental and theoretical data presents an 80\% agreement between the two approaches and supports the hypothesis that binding is stronger at low pH for bnABs that specifically target the CD binding site. We then make observations of binding energy predictions across broad spectrum pH which may provide insight into factors limiting the effectiveness of bnAbs in vivo.

Introduction

The challenge that scientists face with human immunodeficiency virus (HIV) is the overwhelming rate of evolutionary change. The following facts lead researchers to conclude an effective vaccine requires a greater understanding of the mechanics involved with the infection process [5]–[8]:

- > **The largest population of quasi-species is not responsible for transmission from host to host**
- > **Broadly neutralizing antibodies (bnAbs) attack multiple regions of envelope protein gp120 [9]–[13]**
- > **The most promising target region is the CD binding site of gp120**
- > bnAbs 3BNC117 and VRC01, show forward direction towards a vaccine [14]–[18].

Background

Engineering bnAbs to improve breadth and potency of neutralizing capacity have provided promising results:

- > **Modifications of protein structures has a direct effect on surface charges which can be determined in computational simulations [22]-[26]**
- > Engineered variants of bnAb 10E8 increased potency without diminishing functional breadth [14]
- > Engineered variants of CH235 that display increased potency [9]

Comparison of Experimental and Theoretical Data

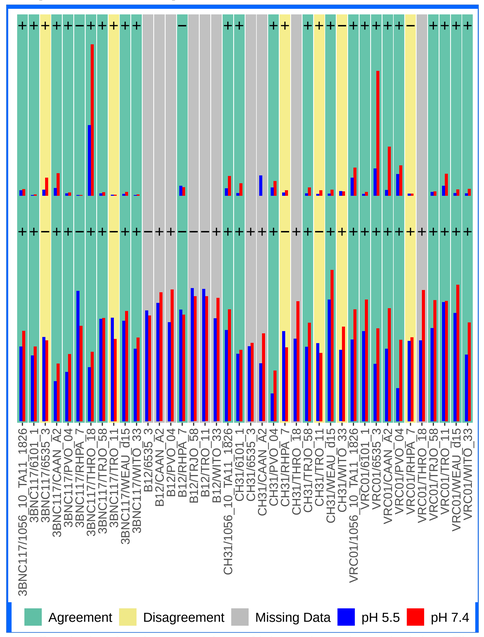

Figure 1 Graph showing the comparison of lab results to theoretical data. The two sub panels represent lab experiments (top) and theoretical results (bottom) with blue representing pH 5.5 and red indicating pH 7.4. The + / - markers represent the direction of change from lower to higher pH. The background color for each method set of results indicates agreement between theory and experiment using a green shade, disagreement using yellow shading while gray indicates indeterminate lab results. Complexes are represented as bnAb/gp120 along the horizontal axis.

Purpose

We apply a computational method of analyzing binding energies and the effect pH has on the interactions of bnAbs and gp120. Observations of theoretical data across broad spectrum pH of 3.0 to 9.0 in 0.1 increments provide researchers the ability to observe how the addition, removal, replacement or transposition of any residue in bnAb and gp120 structures effects binding efficacy across a wide range of pH.

Results

Figure 1 is a comparison of lab results to theoretical data. The two sub panels represent lab experiments (top) and theoretical predictions (bottom) with blue representing pH 5.5 and red indicating pH 7.4. The + / - markers represent the direction change in binding energy from lower to higher pH. The background color for each method set of results indicates agreement between theory and experiment using a green shade, disagreement using yellow shading and gray to indicate indeterminate lab results.

Figure 2 shows four panels with binding patterns of bnAbs: (A) 3BNC117, (B) B12, (C) CH31, and (D) VRC01 across all gp120 assemblies. The red vertical bar is conservatively placed at the approximate point where unpredictable binding energies end and predictable outcomes begin with a more positive result occurring as pH increases; red shading indicates the functional range of each bnAb.

Our observations mostly agree with Mascola et al [8] in terms of breadth and potency vs functional range and starting pH as observed in Figure 2:

- > 3BNC117 has good breadth and good potency (+++ and +++) Mascola et al. and good range and good starting pH (5.9–8.5) panel (A)
- > B12 has low breadth and moderate potency (+ and ++) Mascola et al. and good range and moderate starting pH (6.2–8.9) panel (B)
- > VRC-CH30-34 lineages have good breadth and good potency (+++ and +++) Mascola et al. and low range and moderate starting pH (6.3–8.5) panel (C)
- > VRC01-03 lineages have wide breadth and good potency (++++ and +++) Mascola et al. and wide range good starting pH (5.6–8.5) panel (D)

Binding Energy Motifs of Broadly Neutralizing Antibodies

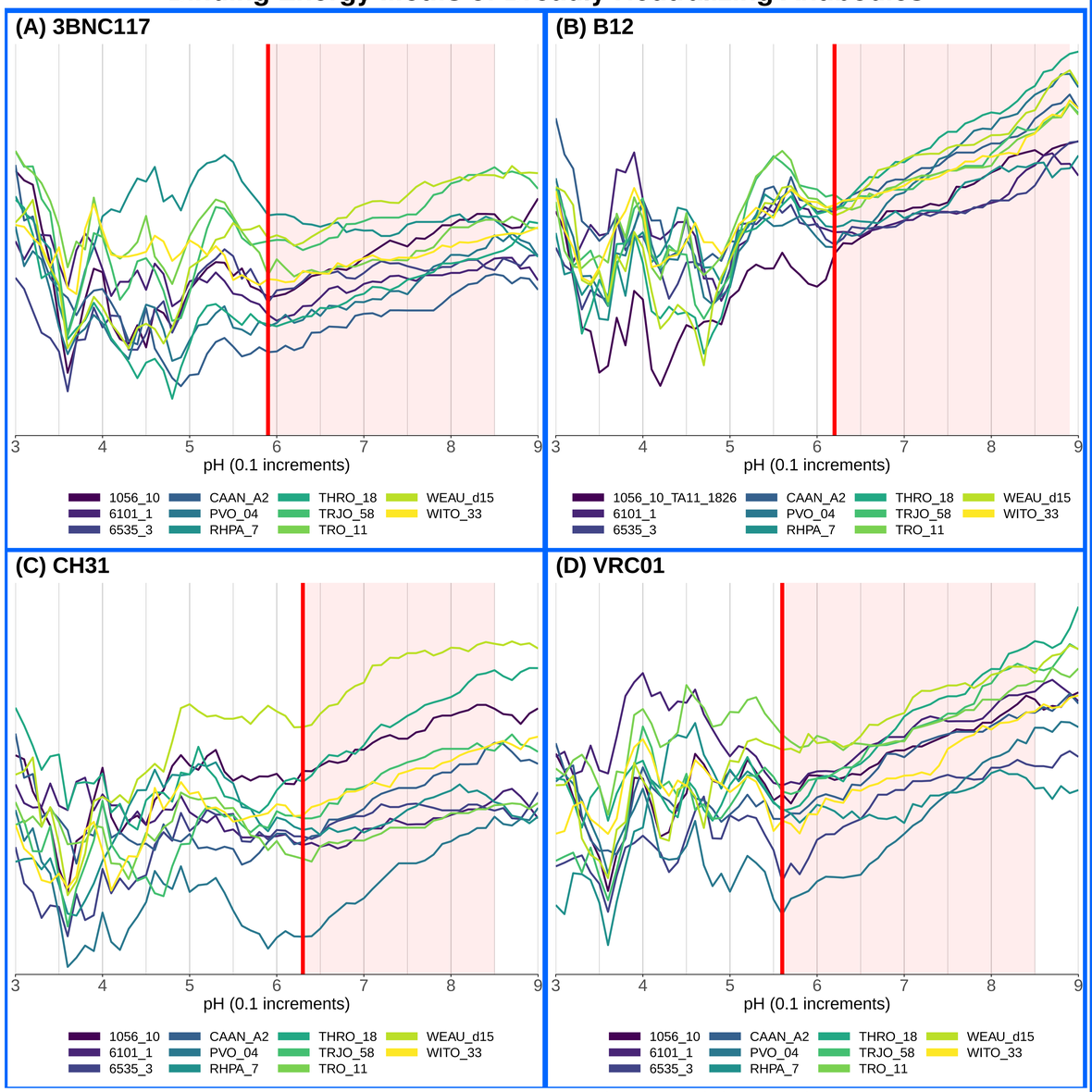

Figure 2 Broad spectrum binding energy motifs of broadly neutralizing anti-bodies (*bnAbs*) (A) 3BNC117, (B) B12, (C) CH31, (D) VRC01 displaying the affinity each has binding to the eleven Env gp120 proteins analyzed. The red vertical bar is conservatively placed at the approximate pH value where, to the right, outcomes become predictable in their positive movement as pH rises. The shaded background indicates the functional range of predictable activity. Data is the normalized mean of ten models per Complex.

Methods

- > Models of bnAbs and gp120 are computationally generated from x-ray crystallography representations or similar methods
- > The protein models are perturbed into conformations based on other crystal structures of similar proteins in their naturally occurring states
- > Structures of each Complex are placed in individual simulation boxes filled with solvent at specific pH values across the range of pH 3.0 and 9.0 in 0.1 increments.
- > Binding energies were calculated through the advanced Poisson-Boltzmann solver (APBS) [19][20] to determine the binding free energy of the bound Complex and the individual components separately.

The following formula determines the binding energy of the Complex at each pH value:

$$G_{be} = G_{complex} - G_{bnAb} - G_{gp120}$$

Where G_{be} is the resulting binding energy of the Complex, $G_{complex}$ is the binding free energy of the proteins in the bound state, G_{bnAb} is the binding free energy of the bnAb and G_{gp120} is the binding free energy of the gp120. All methods are detailed by Stieh et al., Morton et al., Howton et al. in [21] – [26].

Conclusion

We established a foundation to study binding energies of HIV gp120 in computational simulations. We focused this research on bnAbs which bind to gp120 CD4bs

- > **The results are complimented by laboratory experimentation and indicate an 80% agreement between the two approaches**
- > **Observations of theoretical predictions point to pH as a component in binding efficacy of bnAbs and gp120**
- > **Results and observations agree with past research in this field where an observable range of predictable outcomes indicates a functional range of bnAbs that coincides with potency and breadth observations of wild type bnAbs**

The information presented here suggests our methods can be used to reduced turn around time and provide broader representation of binding function that will assist the reverse engineering of bnAbs to mature lineages towards a vaccine.

Acknowledgments

I would like to personally thank Dr. Joshua Phillips for his guidance and open-minded approach to problems and solutions. I would also like to acknowledge and thank the entire Computer Sciences Department, the Computational Sciences program and all the professors I have had the privilege of studying under at MTSU for providing a great opportunity to learn and grow as a human being.

References

1. D. I. Boeras, P. T. Hraber, M. Hurlston, T. Evans-Strickfaden, T. Bhattacharya, E. E. Giorgi, J. Mulenga, E. Karita, B. T. Korber, S. Allen, C. E. Hart, C. a. Derdeyn, and E. Hunter, "Role of donor genital tract HIV-1 diversity in the transmission bottleneck," Proceedings of the National Academy of Sciences, vol. 108, no. 46, pp. E1156–E1163, nov 2011.
2. C. Derdeyn, J. Decker, F. Bibollet-Ruche, J. Mokili, M. Muldoon, S. Denham, M. Heil, F. Kasolo, R. Musonda, B. Hahn, G. Shaw, B. Korber, S. Allen, and E. Hunter, "Envelope Constrained Neutralization Sensitive HIV-1 after Heterosexual Transmission," Science, vol. 303, no. 5666, pp. 2019–2022, 2004.
3. R. E. Haaland, P. A. Hawkins, J. Salazar-Gonzalez, A. Johnson, A. Tichacek, E. Karita, O. Manigart, J. Mulenga, B. F. Keele, G. M. Shaw, B. H. Hahn, S. A. Allen, C. A. Derdeyn, and E. Hunter, "Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1," PLoS pathogens, vol. 5, no. 1, p. e1000274, jan 2009.
4. B. F. Keele, E. E. Giorgi, J. F. Salazar-Gonzalez, J. M. Decker, K. T. Pham, M. G. Salazar, C. Sun, T. Grayson, S. Wang, H. Li, X. Wei, C. Jiang, J. L. Kirchherr, F. Gao, J. A. Anderson, L.-H. Ping, R. Swanstrom, G. D. Tomaras, W. A. Blattner, P. A. Goepfert, J. M. Kilby, M. S. Saag, E. L. Delwart, M. P. Busch, M. S. Cohen, D. C. Montefiori, B. F. Haynes, B. Gaschen, G. S. Athreya, H. Y. Lee, N. Wood, C. Seoighe, A. S. Perelson, T. Bhattacharya, B. T. Korber, B. H. Hahn, and G. M. Shaw, "Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection," Proceedings of the National Academy of Sciences, vol. 105, no. 21, pp. 7552–7557, may 2008.
5. B. F. Haynes and J. R. Mascola, "The quest for an antibody-based HIV vaccine," Immunological Reviews, vol. 275, no. 1, pp. 5–10, jan 2017.
6. A. S. Fauci, "An HIV Vaccine," JAMA, vol. 316, no. 2, p. 143, jul 2016.
7. B. Haynes, G. Shaw, B. Korber, G. Kelsoe, J. Sodroski, B. Hahn, P. Borrow, and A. McMichael, "HIV-Host Interactions: Implications for Vaccine Design," Cell Host & Microbe, vol. 19, no. 3, pp. 292–303, mar 2016.
8. J. R. Mascola and B. F. Haynes, "HIV-1 neutralizing antibodies: understanding nature's pathways," Immunological Reviews, vol. 254, no. 1, pp. 225–244, jul 2013.
9. C. C. LaBranche, R. Henderson, A. Hsu, S. Behrens, X. Chen, T. Zhou, K. Wiehe, K. O. Saunders, S. M. Alam, M. Bonsignori, M. J. Borgnia, Q. J. Sattentau, A. Eaton, K. Greene, H. Gao, H.-X. Liao, W. B. Williams, J. Peacock, H. Tang, L. G. Perez, R. J. Edwards, T. B. Kepler, B. T. Korber, P. D. Kwong, J. R. Mascola, P. Acharya, B. F. Haynes, and D. C. Montefiori, "Neutralization-guided design of HIV-1 envelope trimers with high affinity for the unmutated common ancestor of CH235 lineage CD4bs broadly neutralizing antibodies," PLOS Pathogens, vol. 15, no. 9, p. e1008026, sep 2019.
10. P. D. Kwong, J. R. Mascola, and G. J. Nabel, "Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning," Nature Reviews Immunology, vol. 13, no. 9, pp. 693–701, sep 2013.
11. R. Wyatt, P. D. Kwong, E. Desjardins, R. W. Sweet, J. Robinson, W. A. Hendrickson, and J. G. Sodroski, "The antigenic structure of the HIV gp120 envelope glycoprotein," Nature, vol. 393, no. 6686, pp. 705–711, jun 1998.
12. R. Pantophlet and D. R. Burton, "GP120: Target for neutralizing HIV-1 antibodies," Annual Review of Immunology, vol. 24, pp. 739–769, 2006.
13. J. Overbaugh and L. Morris, "The Antibody Response against HIV-1," Cold Spring Harbor Perspectives in Medicine, vol. 2, no. 1, pp. a007 039–a007 039, jan 2012.
14. Y. D. Kwon, G.-Y. Chuang, B. Zhang, R. T. Bailer, N. A. Doria-Rose, T. S. Gindin, B. Lin, M. K. Louder, K. McKee, S. O'Dell, A. Pegu, S. D. Schmidt, M. Asokan, X. Chen, M. Choe, I. S. Georgiev, V. Jin, M. Pancera, R. Rawi, K. Wang, R. Chaudhuri, L. A. Kueltzo, S. D. Manceva, J.-P. Todd, D. G. Scorpio, M. Kim, E. L. Reinherz, K. Wagh, B. M. Korber, M. Connors, L. Shapiro, J. R. Mascola, and P. D. Kwong, "Surface-Matrix Screening Identifies Semi-specific Interactions that Improve Potency of a Near Pan-reactive HIV-1-Neutralizing Antibody," Cell Reports, vol. 22, no. 7, pp. 1798–1809, feb 2018.
15. K. J. Bar, M. C. Sneller, L. J. Harrison, J. S. Justement, E. T. Overton, M. E. Petrone, D. B. Salantes, C. A. Seamon, B. Scheinfeld, R. W. Kwan, G. H. Learn, M. A. Proschan, E. F. Kreider, J. Blazkova, M. Bardsley, E. W. Refsland, M. Messer, K. E. Clarridge, N. B. Tustin, P. J. Madden, K. Oden, S. J. O'Dell, B. Jarocki, A. R. Shiakolas, R. L. Tressler, N. A. Doria-Rose, R. T. Bailer, J. E. Ledgerwood, E. V. Capparelli, R. M. Lynch, B. S. Graham, S. Moir, R. A. Koup, J. R. Mascola, J. A. Hoxie, A. S. Fauci, P. Tebas, and T.-W. Chun, "Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption," New England Journal of Medicine, vol. 375, no. 21, pp. 2037–2050, nov 2016.
16. M. Caskey, F. Klein, J. C. C. Lorenzi, M. S. Seaman, A. P. West, N. Buckley, G. Kremer, L. Nogueira, M. Braunschweig, J. F. Scheid, J. A. Horwitz, I. Shimeliovich, S. Ben-Avraham, M. Witmer-Pack, M. Platten, C. Lehmann, L. A. Burke, T. Hawthorne, R. J. Gorelick, B. D. Walker, T. Keler, R. M. Gulick, C. Fätkenheuer, S. J. Schlesinger, and M. C. Nussenzweig, "Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117," Nature, vol. 522, no. 7557, pp. 487–491, jun 2015.
17. J. F. Scheid, J. A. Horwitz, Y. Bar-On, E. F. Kreider, C.-L. Lu, J. C. C. Lorenzi, A. Feldmann, M. Braunschweig, L. Nogueira, T. Oliveira, I. Shimeliovich, R. Patel, L. Burke, Y. Z. Cohen, S. Hadrigan, A. Settler, M. Witmer-Pack, A. P. West, B. Juelg, T. Keler, T. Hawthorne, B. Zingman, R. M. Gulick, N. Pfeifer, G. H. Learn, M. S. Seaman, P. J. Bjorkman, F. Klein, S. J. Schlesinger, B. D. Walker, B. H. Hahn, M. C. Nussenzweig, and M. Caskey, "HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption," Nature, vol. 535, no. 7613, pp. 556–560, jul 2016.
18. T. Schoofs, F. Klein, M. Braunschweig, E. F. Kreider, A. Feldmann, L. Nogueira, T. Oliveira, J. C. C. Lorenzi, E. H. Parrish, G. H. Learn, A. P. West, P. J. Bjorkman, S. J. Schlesinger, M. S. Seaman, J. Czartoski, M. J. McElrath, N. Pfeifer, B. H. Hahn, M. Caskey, and M. C. Nussenzweig, "HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1," Science, vol. 352, no. 6288, pp. 997–1001, may 2016.
19. Dolinsky, Todd J., et al. "PDB2PQR: Expanding and Upgrading Automated Preparation of Biomolecular Structures for Molecular Simulations." Nucleic Acids Research, vol. 35, no. SUPPL.2, May 2007, pp. 522–25, doi:10.1093/nar/gkm276.
20. Dolinsky, Todd J., et al. "PDB2PQR: An Automated Pipeline for the Setup of Poisson-Boltzmann Electrostatics Calculations." Nucleic Acids Research, vol. 32, no. WEB SERVER ISS., July 2004, pp. 665–67, doi:10.1093/nar/gkh381.
21. D. J. Stieh, J. L. Phillips, P. M. Rogers, D. F. King, G. C. Cianci, S. A. Jeffs, S. Gnanakaran, and R. J. Shattock, "Dynamic electrophoretic fingerprinting of the HIV-1 envelope glycoprotein," Retrovirology, vol. 10, no. 1, p. 33, 2013.
22. S. Morton, J. Phillips, and J. Phillips, "High-throughput structural modeling of the HIV transmission bottleneck," in Proceedings – 2017 IEEE International Conference on Bioinformatics and Biomedicine, BIBM 2017, vol. 2017-Janua, 2017.
23. J. Howton, "A Computational Electrostatic Modeling Pipeline for Comparing pH-dependent gp120-CD4 Interactions in Founder and Chronic HIV Strains," Ph.D. dissertation, Middle Tennessee State University, Murfreesboro, TN, 2017.
24. J. Howton and J. L. Phillips, "Computational Modeling of pH dependent gp120-CD4 Interactions in Founder and Chronic HIV Strains," in Proceedings of the 8th ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics – ACM-BCB '17. Boston, MA, USA: ACM Press, 2017, pp. 644–649.
25. S. P. Morton, J. Howton, and J. L. Phillips, "Sub-Class Differences of PH-Dependent HIV GP120-CD4 Interactions," in Proceedings of the 2018 ACM International Conference on Bioinformatics, Computational Biology, and Health Informatics - BCB '18. New York, New York, USA: ACM Press, 2018, pp. 663–668.
26. S. P. Morton, J. L. J. B. Phillips, and J. L. J. B. Phillips, "The Molecular Basis of pH-Modulated HIV gp120 Binding Revealed," Evolutionary Bioinformatics, vol. 15, p. 117693431983130, jan 2019. [Online]. Available: http://journals.sagepub.com/doi/10.1177/1176934319831308