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Vaccines in Modern Era: New Paradigms to Address Unmet Needs

ITMAT Symposium
University of Pennsylvania School of Medicine
Philadelphia, PA

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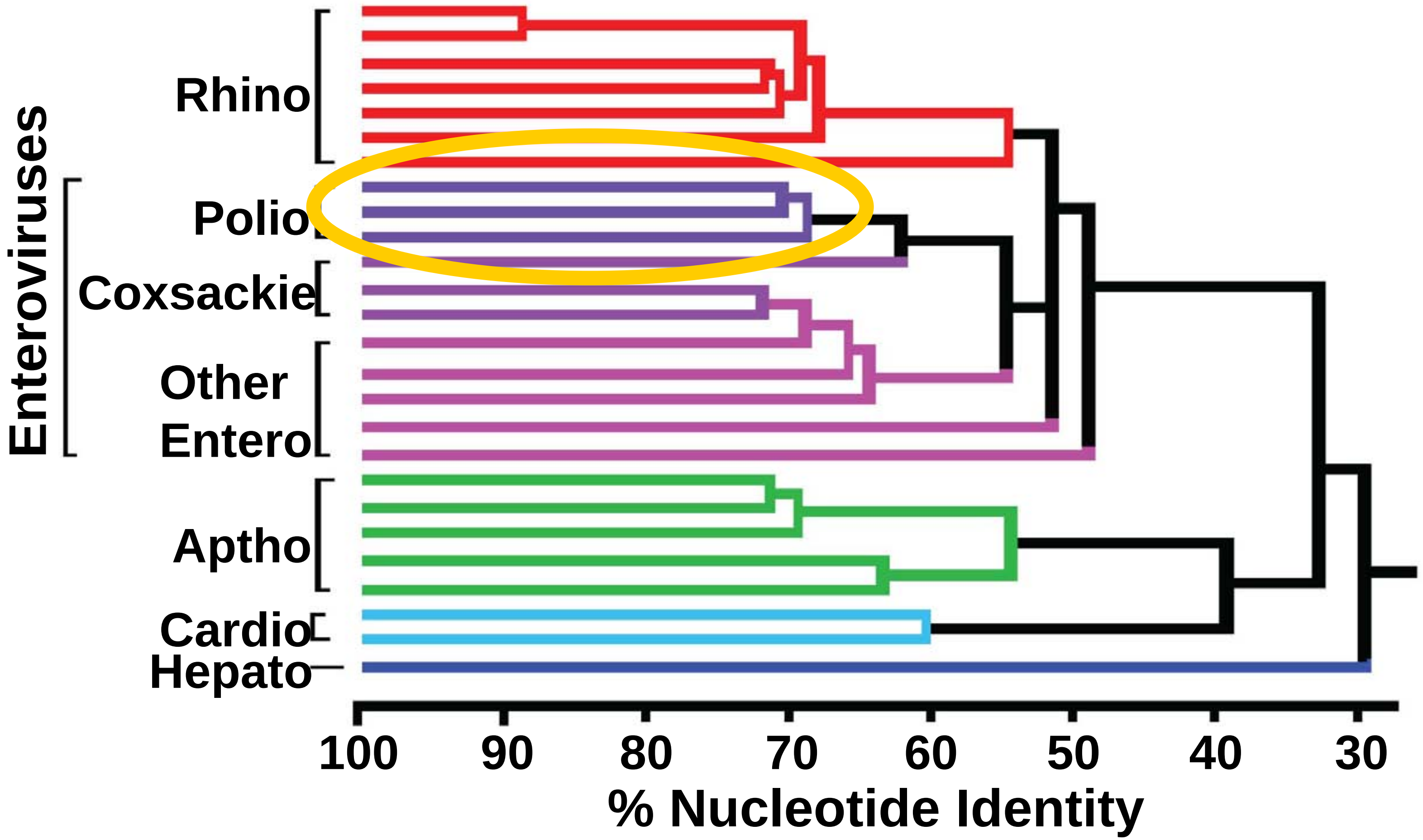
Oct. 27, 2010

A Biomarker for Successfully Licensed Vaccines: Serotypes

Three poliovirus strains found in Nature: three serotypes are required for a protective vaccine



28 Licensed Vaccines to 24 Infectious Diseases

- Anthrax
- Diphtheria
- *Haemophilus influenzae* type b
- Hepatitis A
- Hepatitis B
- Herpes Zoster (shingles)
- Human papillomavirus
- Influenza A, B
- Pertussis
- Pneumococcal disease
- Polio
- Rabies
- Rotavirus
- Rubella
- Smallpox
- Tetanus

[redacted]

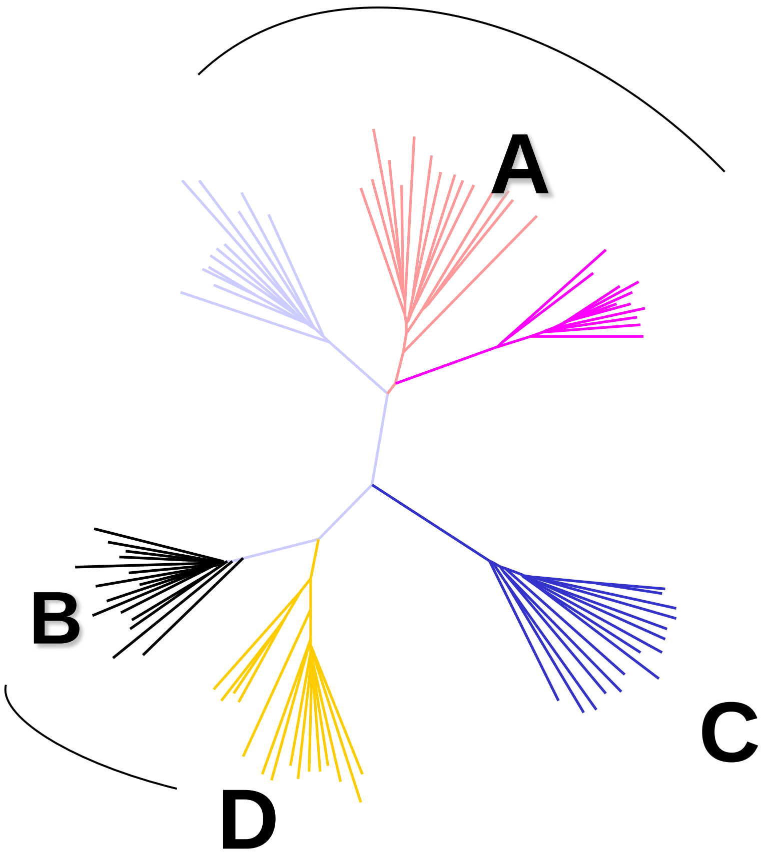
(BCG)
- Japanese Encephalitis
- Tuberculosis

The Burden of Infectious Diseases without Vaccines

	Prevalence	Deaths
HIV/AIDS	33.4 million infected	2.0 million
Tuberculosis	~ 2 billion infected 9.4 million active cases	1.8 million
Malaria	243 million cases	863,000

Sources: UNAIDS, WHO

Can HIV-1 Be Serotyped? Contrast with Polio

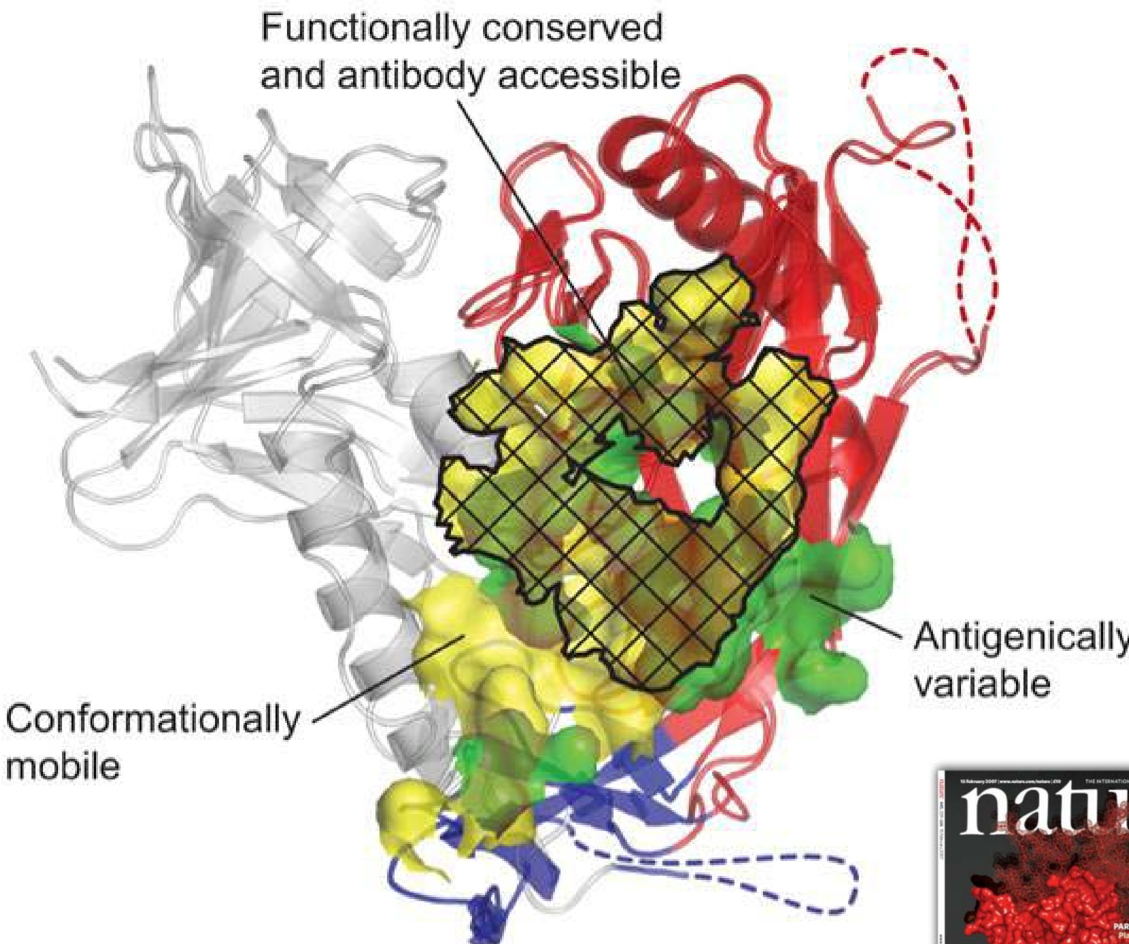


Infinite number of viruses

? Role of Abs in immunity

Evolving neutralization profiles

A Site of Vulnerability to Antibody

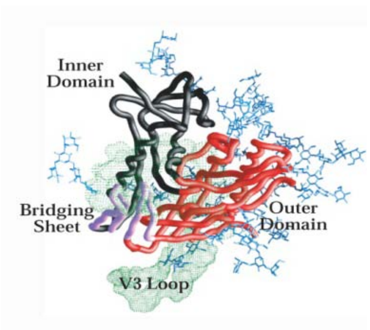


Structural Definition of a Conserved Neutralization Epitope on HIV-1 gp120

T Zhou, L Xu, B Dey, AJ Hessell, D Van Ryk, S-H Xiang, X Yang, M-Y Zhang, MB Zwick, J Arthos, DR Burton, DS Dimitrov, J Sodroski, R Wyatt, GJ Nabel & PD Kwong

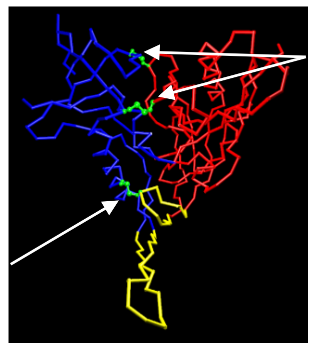
Strategy for Isolation of New Monoclonal Antibodies Based On HIV Protein Structure

Designer Envelopes



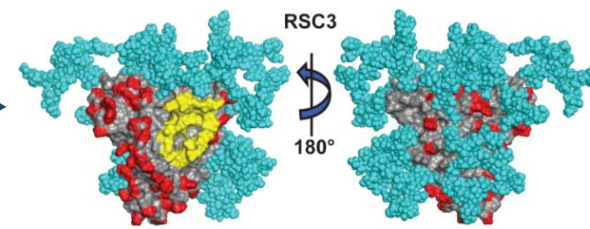
Core

Stabilizing inner domain and bridging sheet



Stabilizing the inner/outer domains

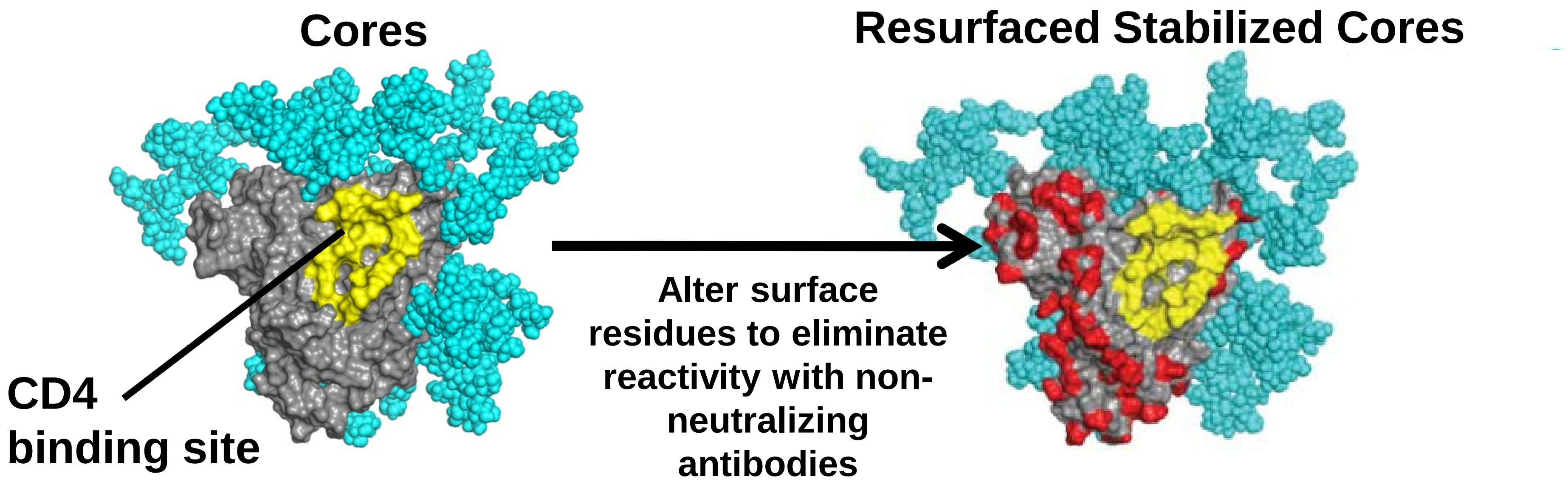
Stabilized Core



Resurfaced Stabilized Cores (RSC)

Nabel, Schief, Kwong, Mascola

Resurfaced Stabilized Cores: Probes for Human Abs and Templates for Immunogens

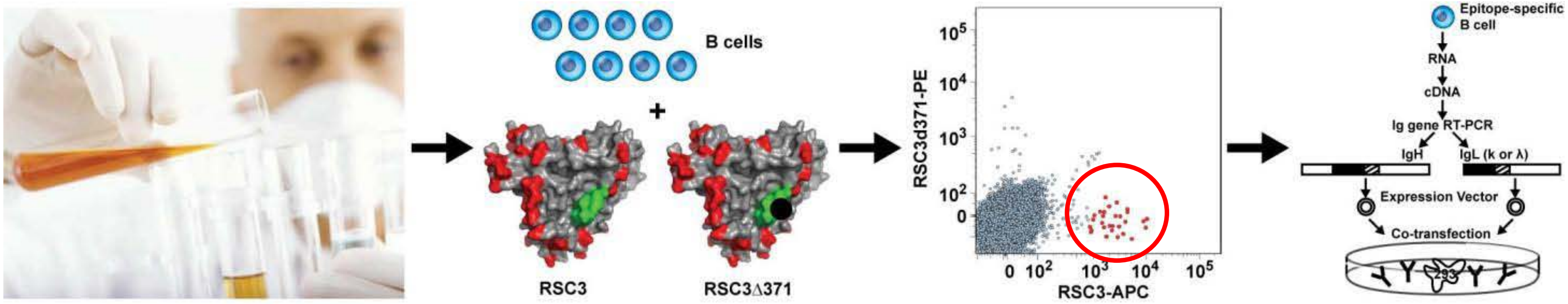


1. Probe to isolate B cells and clone broadly neutralizing abs

2. Prototype immunogens to elicit antibodies to the highly conserved CD4 binding site

Nabel, Schief, Kwong, Mascola

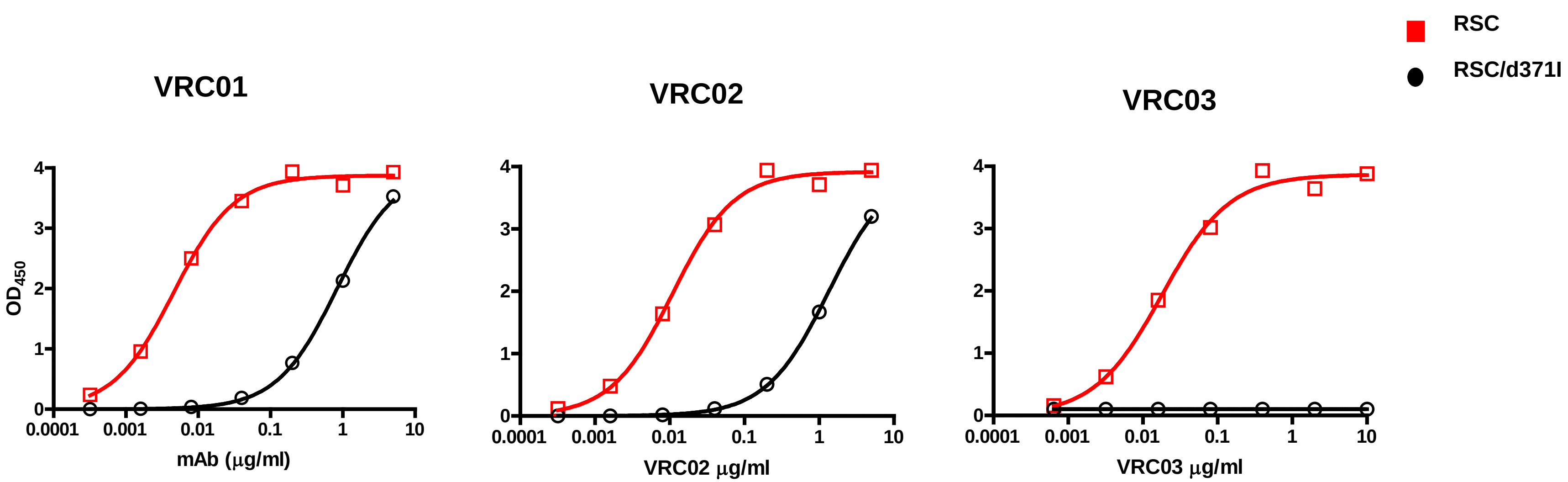
Strategy for Isolation of New Monoclonal Antibodies Based On HIV Protein Structure: Rescue of Antigen-Specific B Cells



1. Select special subjects with broadly neutralizing, potent antisera
2. Incubate B cells with wild type and CD4 binding site mutant resurfaced cores
3. Select for CD4 binding site-specific B cells by flow cytometry with positive selection on wild type and negative selection on mutant resurfaced cores
4. PCR amplify and express IgG of HIV-1 specific neutralizing antibodies

Wu et al. (2010) *Science* 329, 856.

Three mAbs bind to the RSC protein

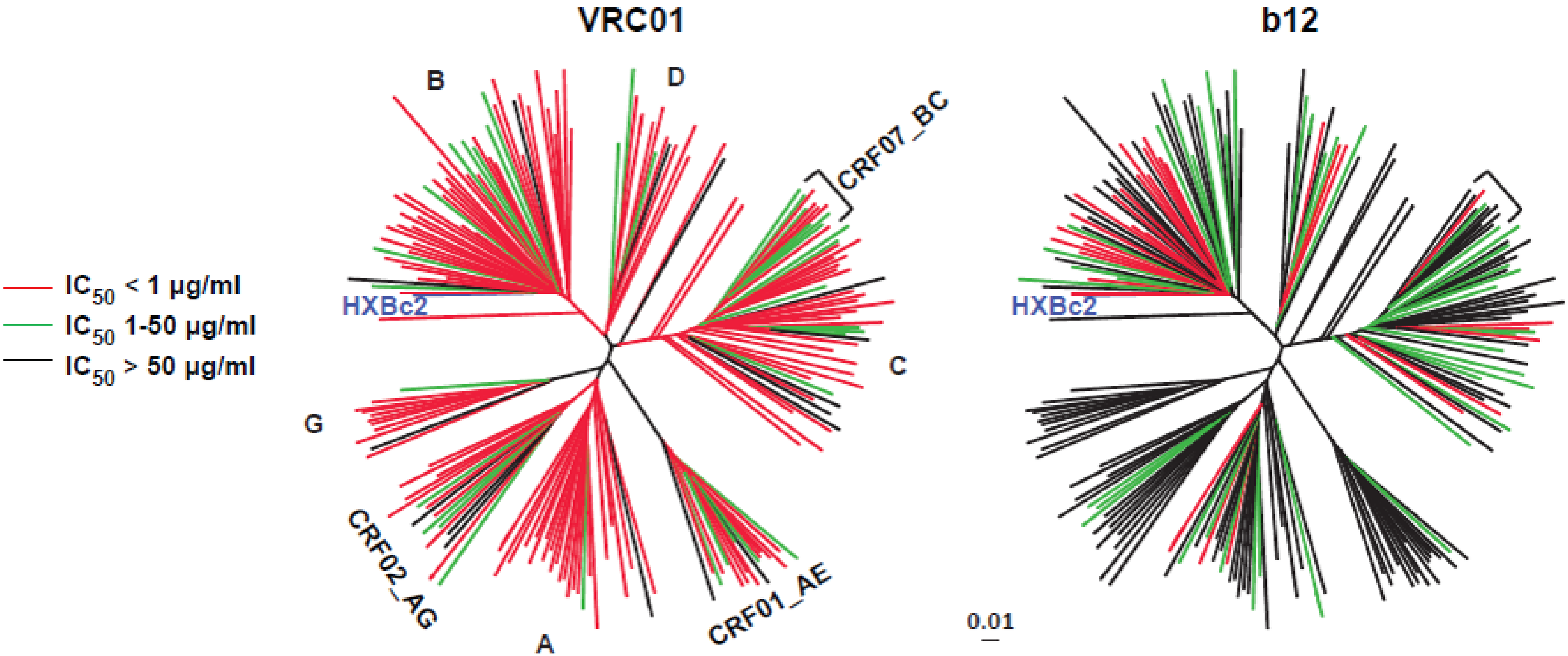


- Two closely related somatic variants (VRC01, VRC02)
  - bind to CD4bs region of gp120
  - Neutralize ~90% viruses, often < 1ug/ml

- 1 additional mAb (VRC03)
  - CD4bs directed
  - Neutralizes ~ 60% viruses

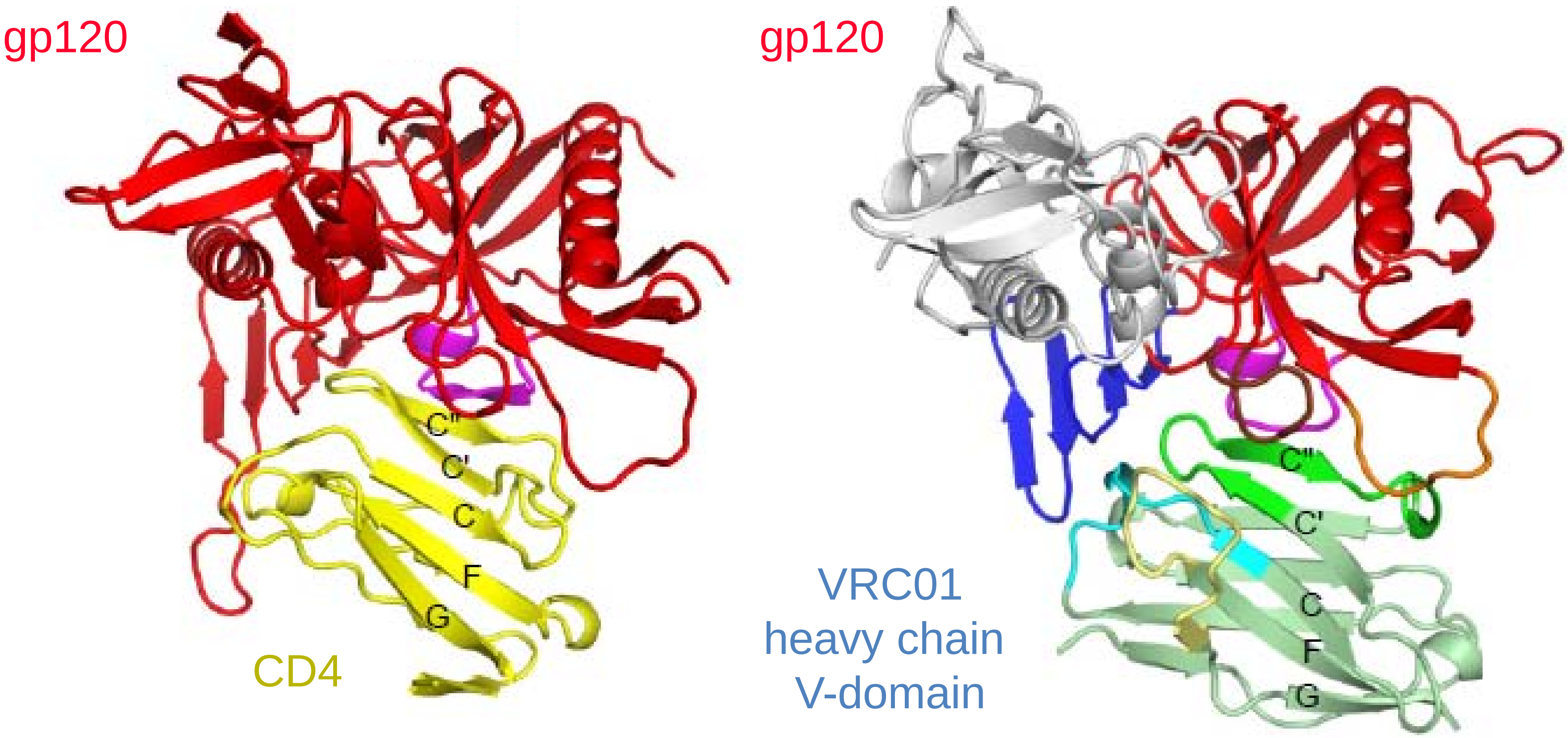
Wu et al. Science (2010) 329:856

Pan-Reactive Antibody VRC01



Wu et al. (2010) *Science* 329, 856.

Mimicry of CD4 Receptor by Antibody VRC01

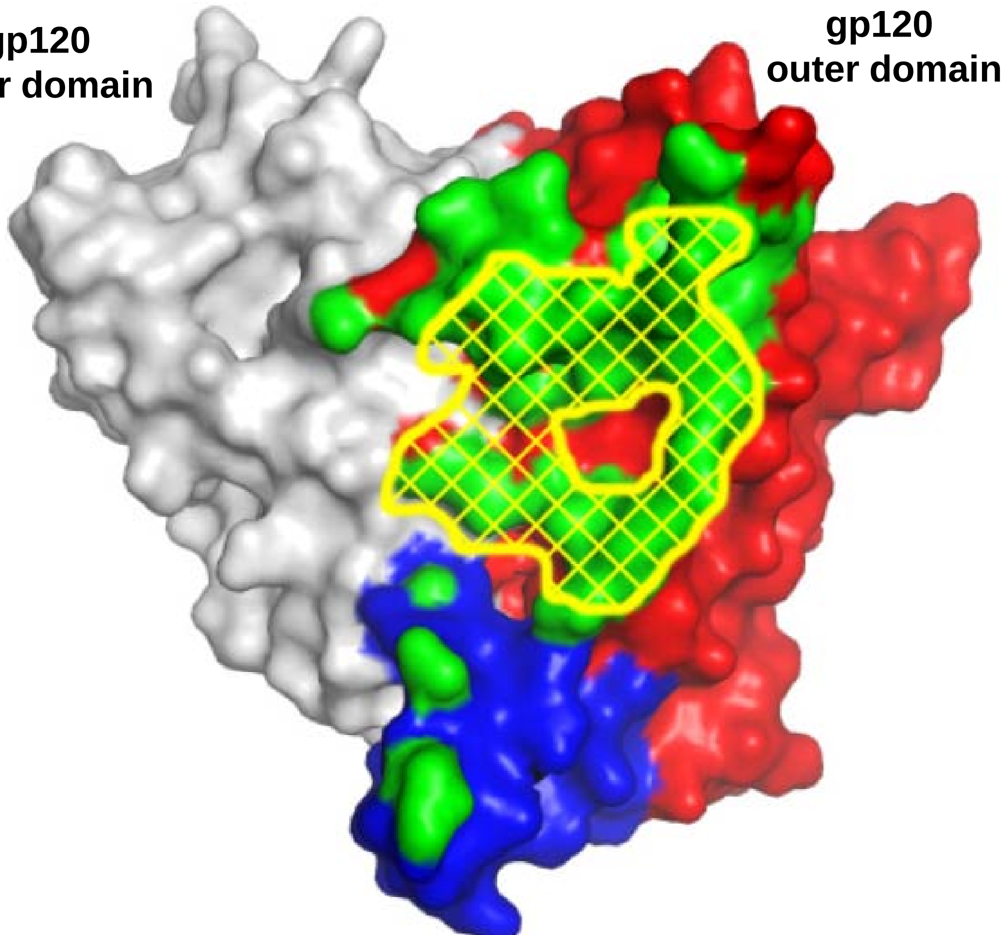


CD4 and VRC01 in highly similar positions

Why does VRC01 Work So Well?

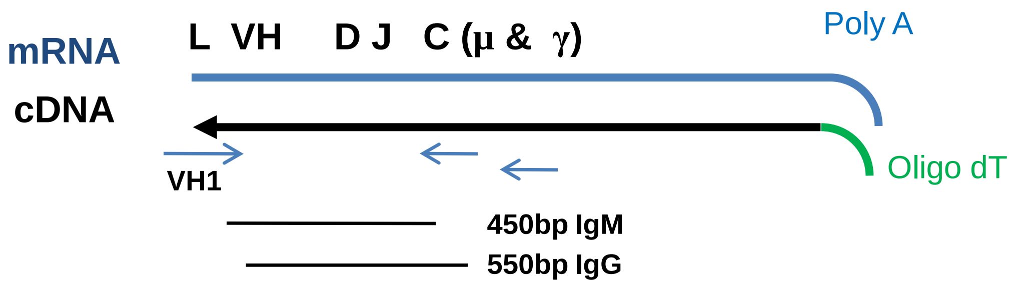
1. Partial mimicry of CD4 binding to gp120

2. Binding focused on the conformationally invariant site of initial CD4 attachment.



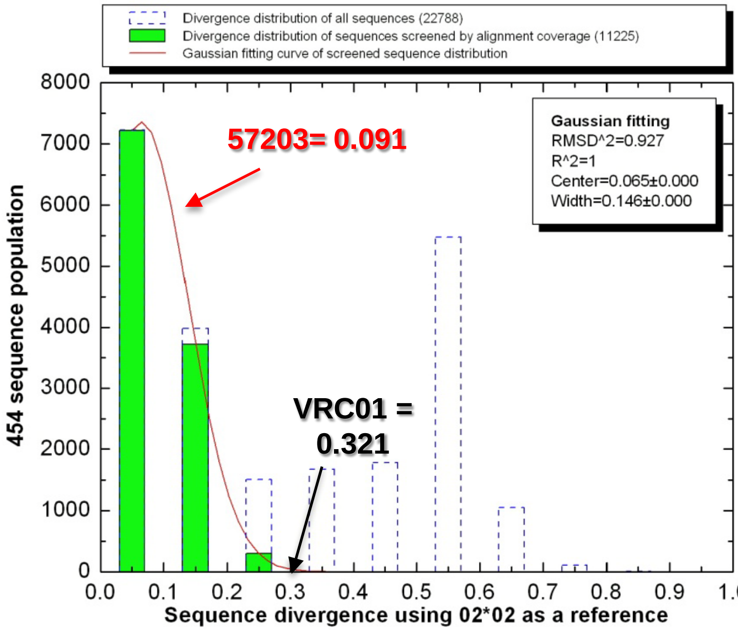
454 pyrosequencing to Identify Additional VRC01-like Antibodies

- ❑ **Known mAbs (VRC01 – 03): Use knowledge of specific gene usage and structural motifs to identify and study the family of related antibodies in a specific donor**
- ❑ **cDNA library from donor B cells; isolate antibody heavy chain sequences; analyze sequence and predicted structural motifs – to find VRC01-like antibodies**
- ❑ **Understand lineage and evolution of affinity maturation of antibody responses**

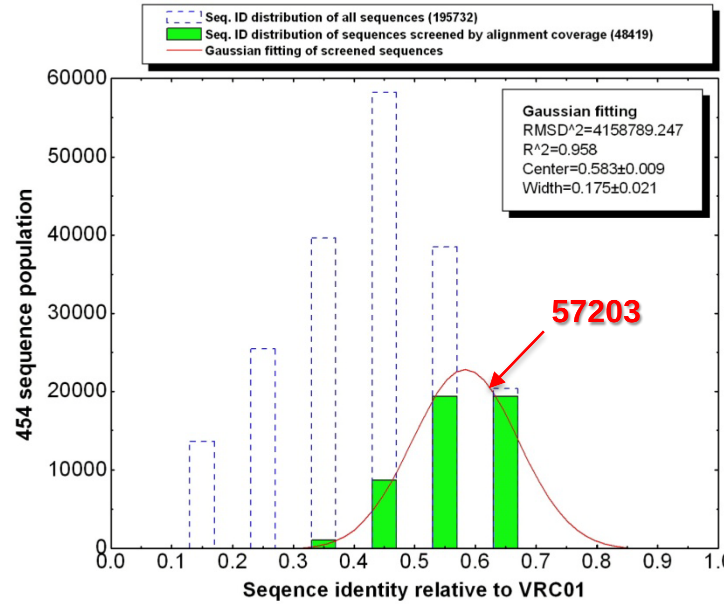


Evaluation of 454 sequences

Distribution of IGHV1-2*02 divergence

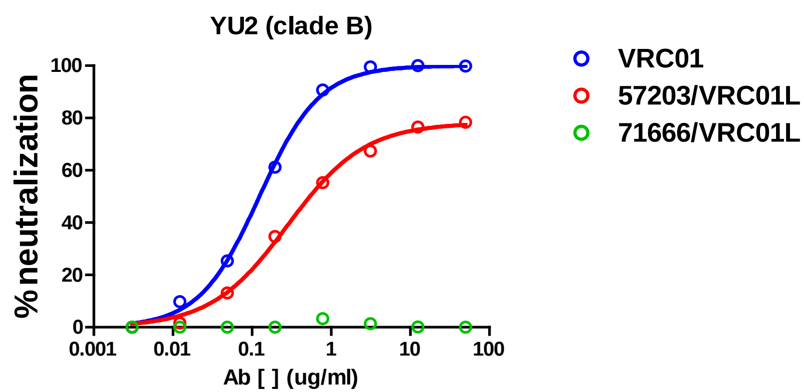


Sequence similarity to VRC01



57203 heavy chain

- Only 59% aa sequence homology to VRC01
- Only 9% divergence from germline

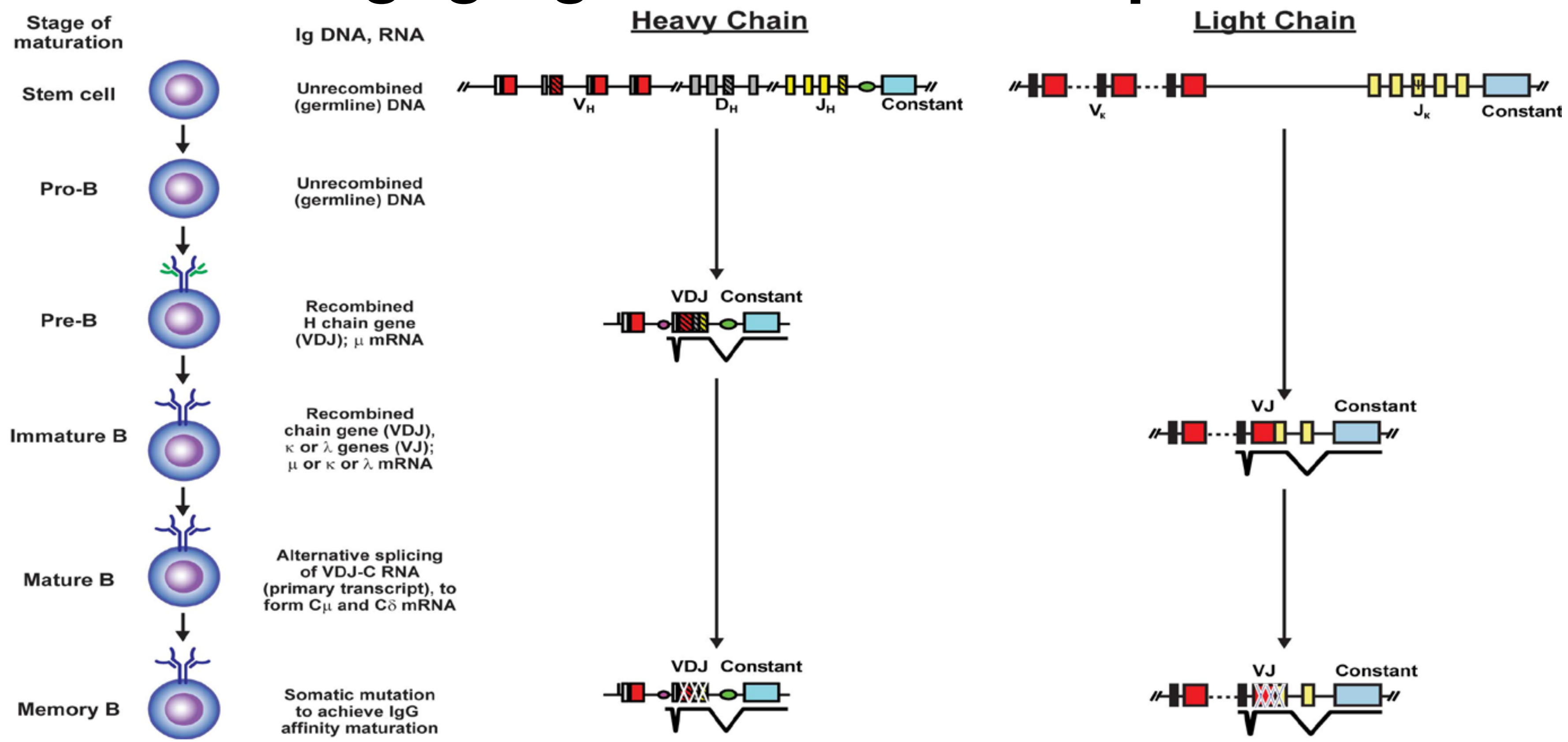


J Zhu, L Shapiro, T Zhou (Kwong lab)

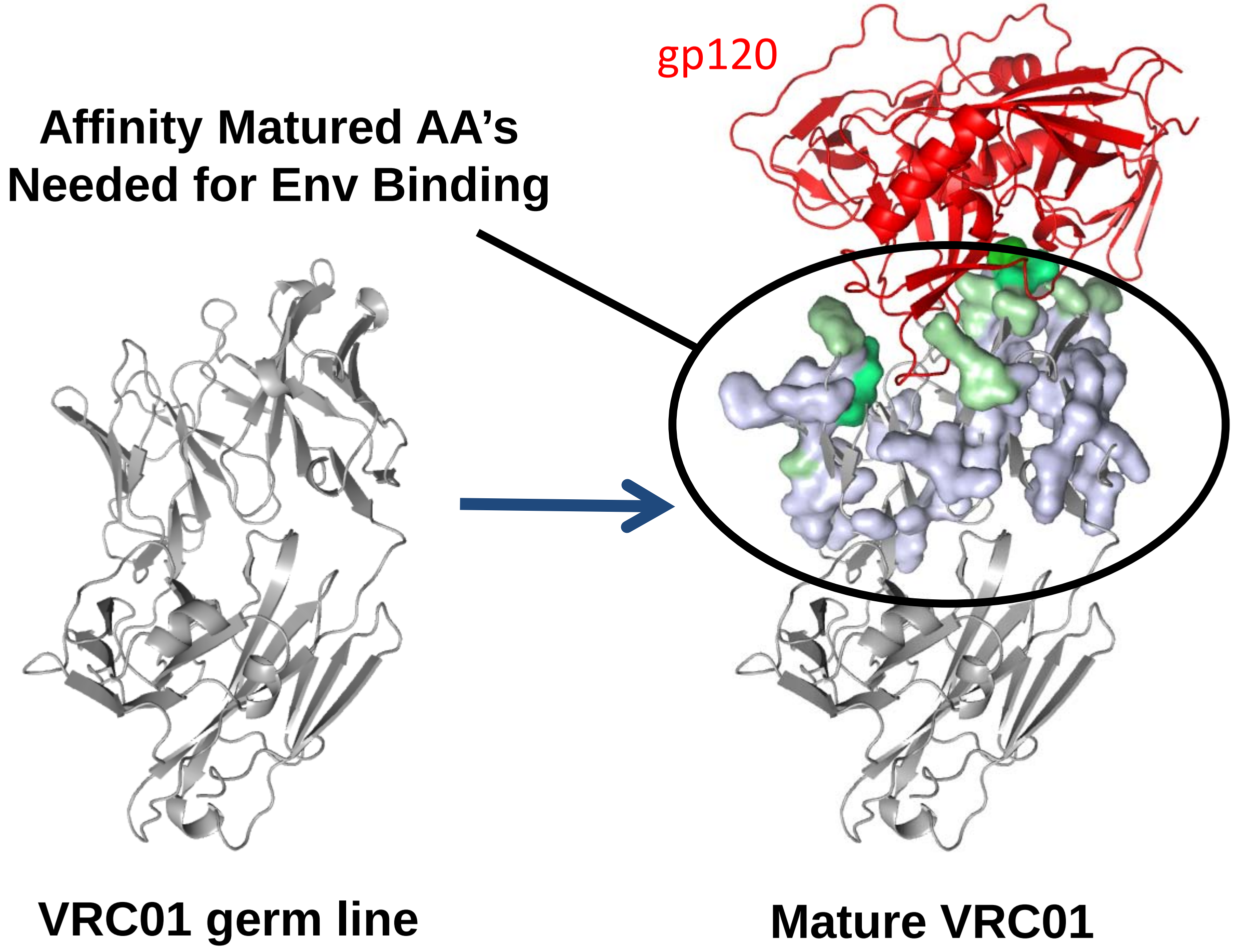
Eliciting VRC01-like Antibodies…

Elicitation depends on three stages of antibody development: recombination, deletion of autoreactive antibodies, and affinity maturation.

Engaging the B Cell Receptors



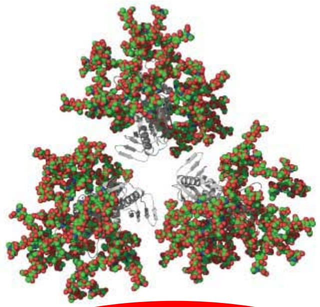
Affinity Maturation and VRC01 Affinity



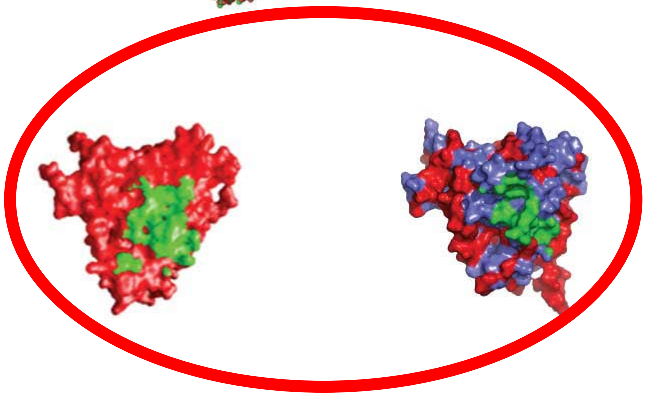
Design of Immunogens to Elicit Broadly Neutralizing Abs to the CD4 Binding Site

Structure-based design:

1. Trimers



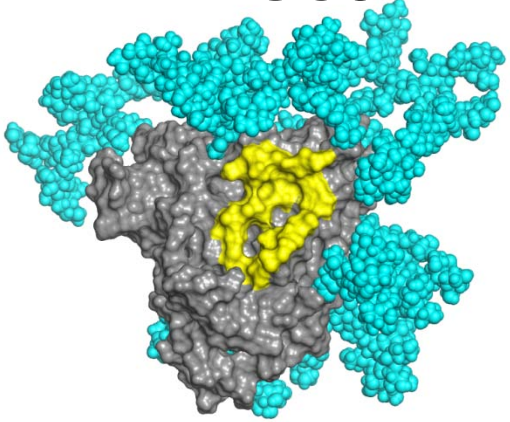
2. **Monomers**

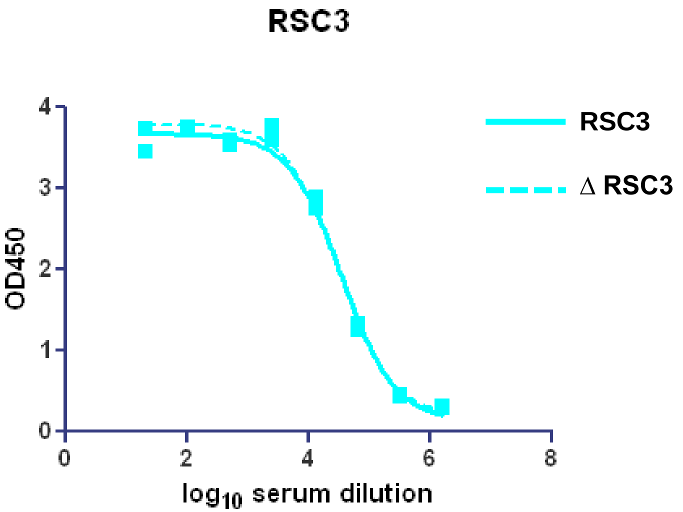


3. Outer Domains

Induction of CD4 BS Antibodies by Glycan Modified RSC3: Y5

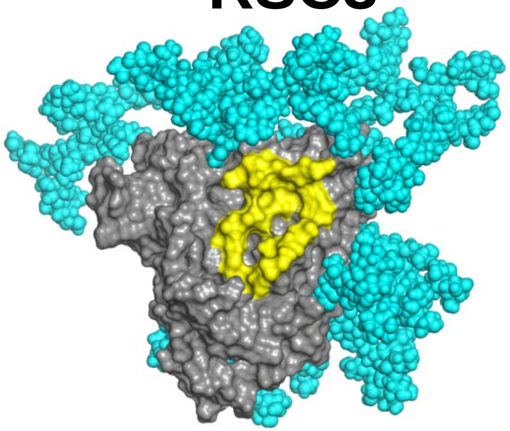
RSC3

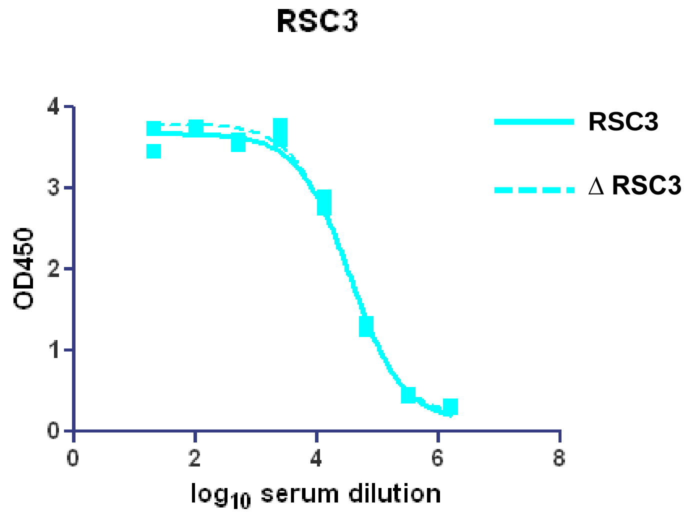




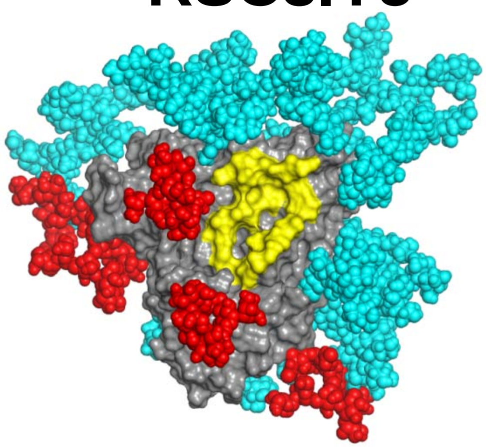
Induction of CD4 BS Antibodies by Glycan Modified RSC3: Y5

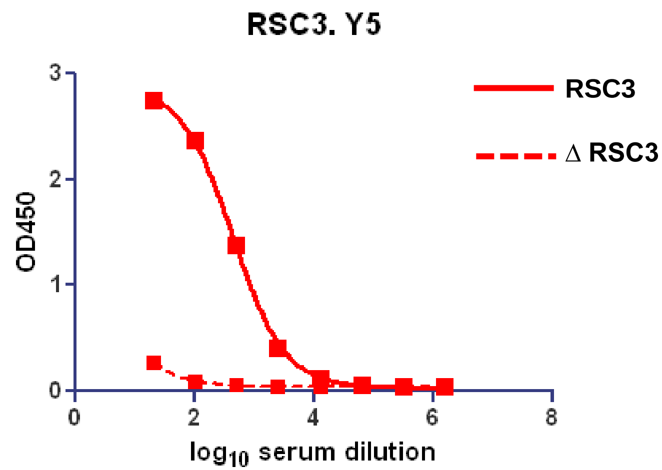
RSC3





RSC3.Y5





Summary

1. An understanding of HIV-1 “serotypes” has presented a major conceptual challenge to the AIDS vaccine scientific community. A solution to this problem is developing through increased success of the field in identifying broadly neutralizing human monoclonal antibodies.
2. Definition of the specificities and targets of broadly neutralizing antisera and monoclonal antibodies have facilitate the identification of “structural” serotypes.
3. It is now possible to elicit CD4 BS neutralizing abs through structure-based vaccine design with trimeric Env proteins, modified core protein (RSCs), and possibly with arrayed ODs. Further modifications of these prototypes are in progress that may improve their breadth of neutralization.

Scope of Clinical Applications of Anti-HIV Neutralizing Antibodies

Scope

- **Prevention**
- **Therapy**
- **Eradication of reservoir**

Influenza Vaccines-The Yearly Cost

New vaccine every year

120-150 million doses per year

2.8-4.0 billion dollars total expenditure

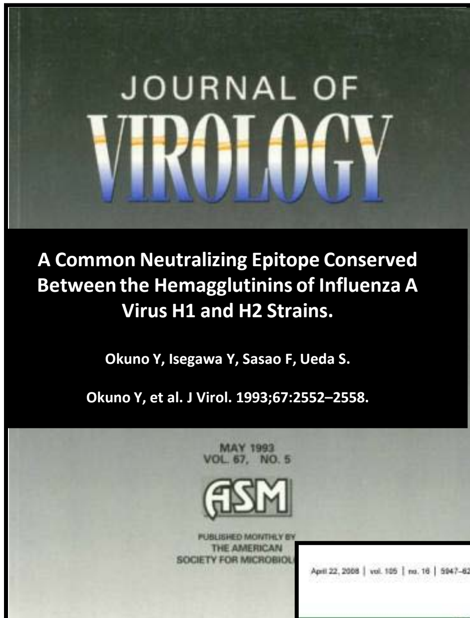
Can We Make a Better Vaccine?

Improve potency

Increase breadth

Can we make a universal influenza vaccine that is administered during childhood and lasts a lifetime?

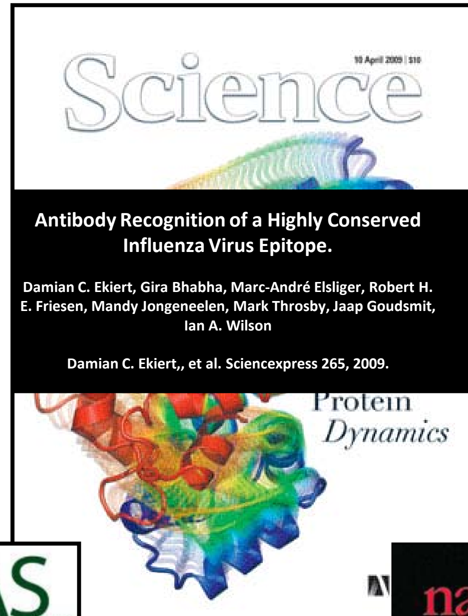
Influenza: Broadly Neutralizing Antibodies



A Common Neutralizing Epitope Conserved Between the Hemagglutinins of Influenza A Virus H1 and H2 Strains.

Okuno Y, Isegawa Y, Sasao F, Ueda S.

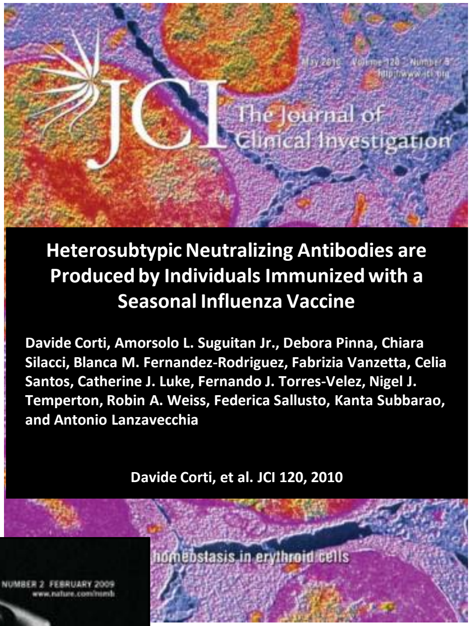
Okuno Y, et al. J Virol. 1993;67:2552–2558.



Antibody Recognition of a Highly Conserved Influenza Virus Epitope.

Damian C. Ekiert, Gira Bhabha, Marc-André Elsliger, Robert H. E. Friesen, Mandy Jongeneelen, Mark Throsby, Jaap Goudsmit, Ian A. Wilson

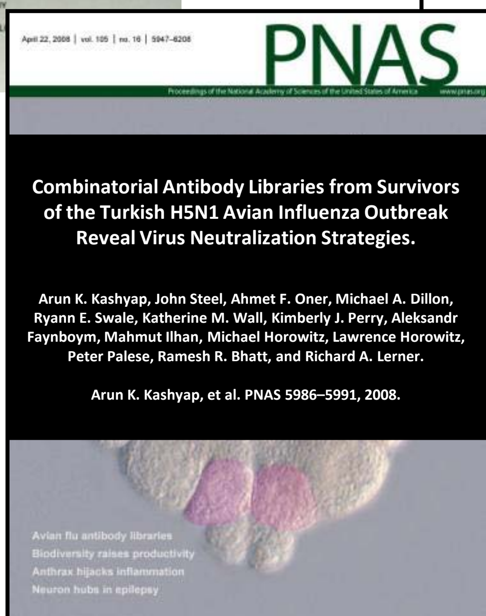
Damian C. Ekiert,, et al. Sciencexpress 265, 2009.



Heterosubtypic Neutralizing Antibodies are Produced by Individuals Immunized with a Seasonal Influenza Vaccine

Davide Corti, Amorsolo L. Suguitan Jr., Debora Pinna, Chiara Silacci, Blanca M. Fernandez-Rodriguez, Fabrizia Vanzetta, Celia Santos, Catherine J. Luke, Fernando J. Torres-Velez, Nigel J. Temperton, Robin A. Weiss, Federica Sallusto, Kanta Subbarao, and Antonio Lanzavecchia

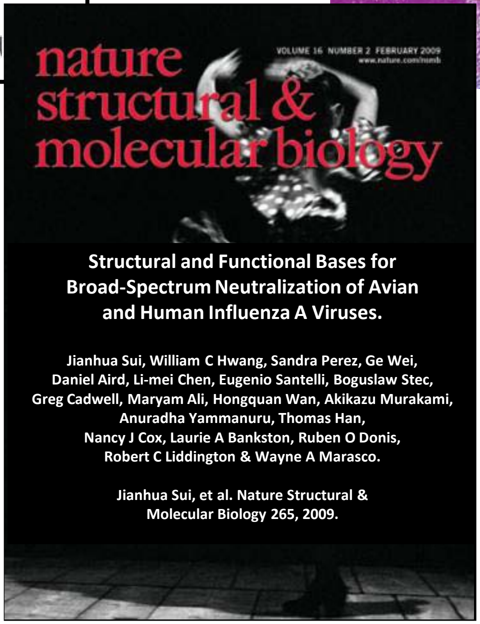
Davide Corti, et al. JCI 120, 2010



Combinatorial Antibody Libraries from Survivors of the Turkish H5N1 Avian Influenza Outbreak Reveal Virus Neutralization Strategies.

Arun K. Kashyap, John Steel, Ahmet F. Oner, Michael A. Dillon, Ryann E. Swale, Katherine M. Wall, Kimberly J. Perry, Aleksandr Faynboym, Mahmut Ilhan, Michael Horowitz, Lawrence Horowitz, Peter Palese, Ramesh R. Bhatt, and Richard A. Lerner.

Arun K. Kashyap, et al. PNAS 5986–5991, 2008.

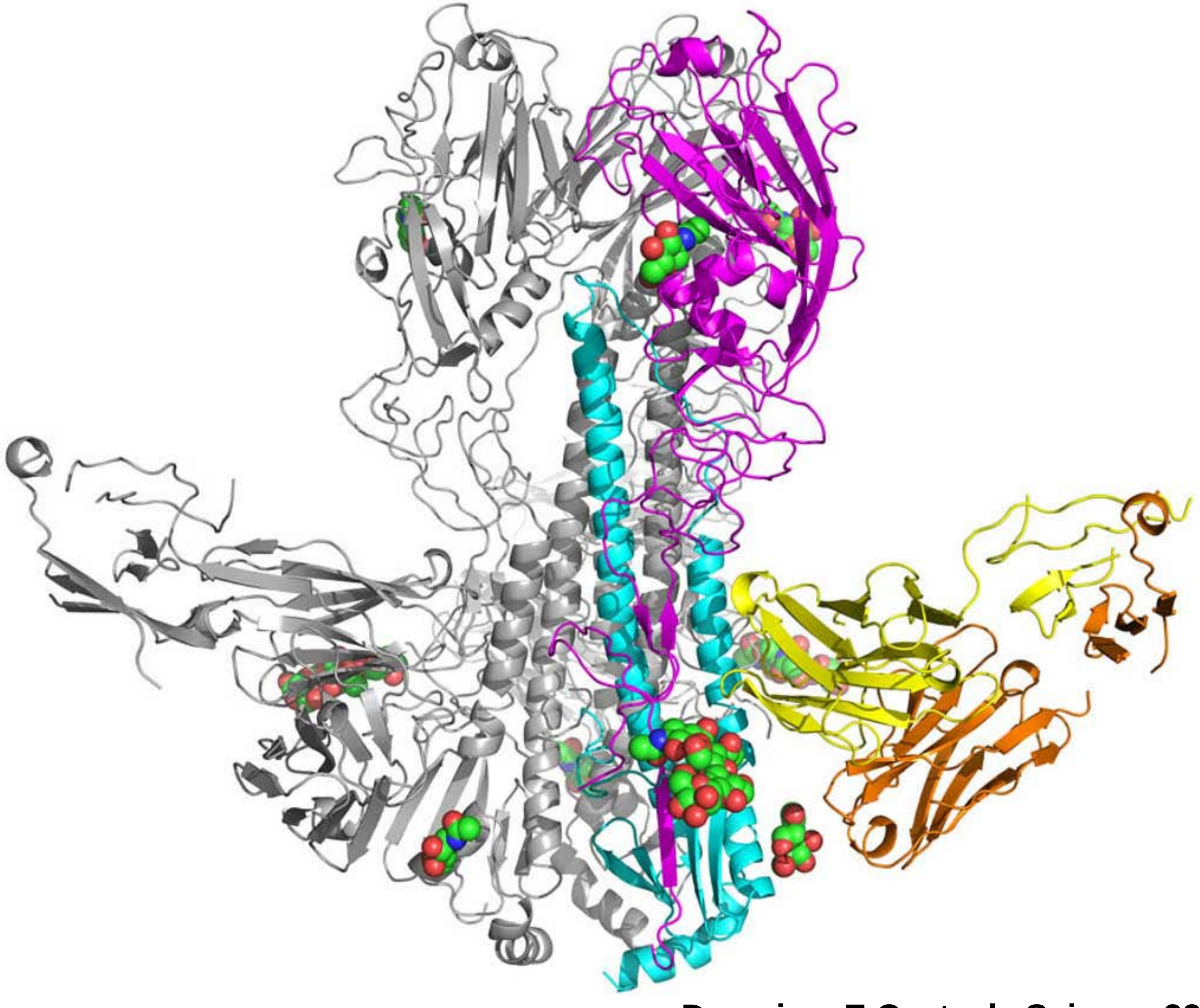


Structural and Functional Bases for Broad-Spectrum Neutralization of Avian and Human Influenza A Viruses.

Jianhua Sui, William C Hwang, Sandra Perez, Ge Wei, Daniel Aird, Li-mei Chen, Eugenio Santelli, Boguslaw Stec, Greg Cadwell, Maryam Ali, Hongquan Wan, Akikazu Murakami, Anuradha Yammanuru, Thomas Han, Nancy J Cox, Laurie A Bankston, Ruben O Donis, Robert C Liddington & Wayne A Marasco.

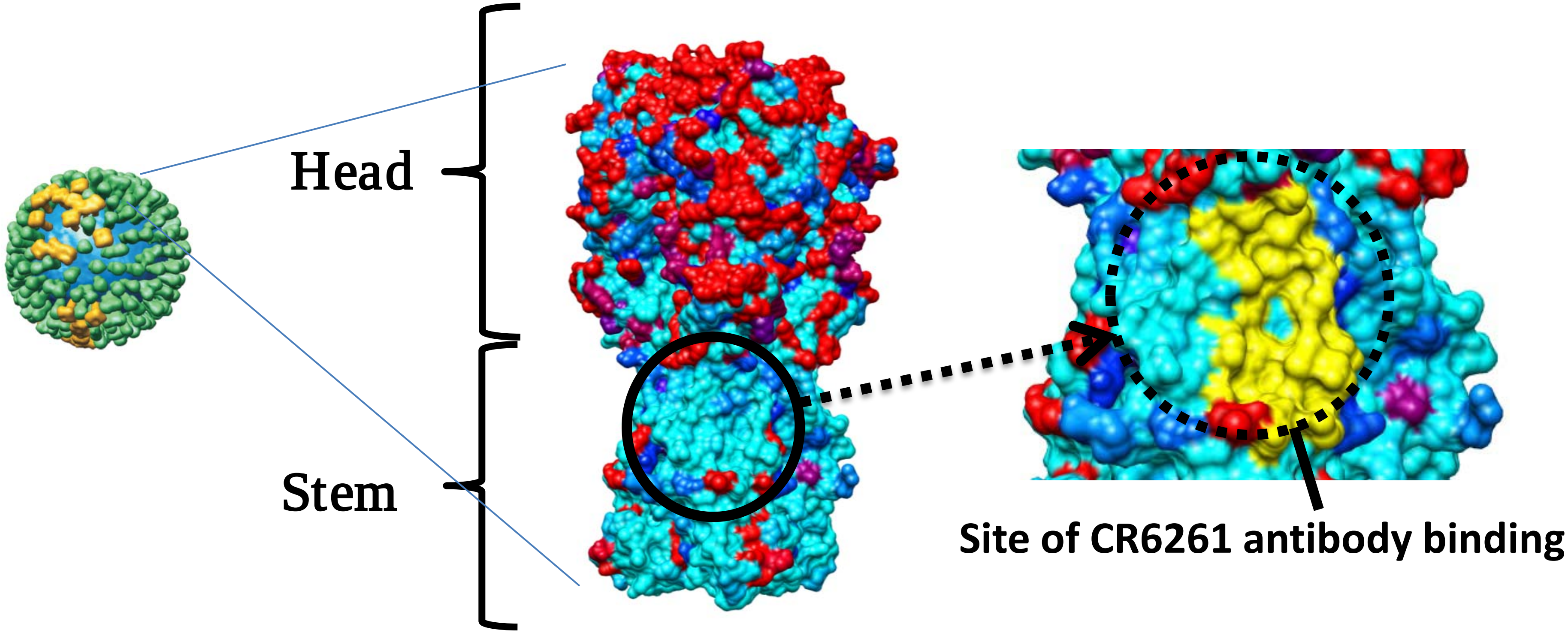
Jianhua Sui, et al. Nature Structural & Molecular Biology 265, 2009.

Interaction of a Broadly Neutralizing Influenza Antibody with Hemagglutinin



Damnian, E.C. et. al., Science 324, 246 (2009)

Structural Basis for Broad Recognition of HA



>700 human H1N1 strains; Cyan, 100% conservation; Red, 98% conservation

Jeffrey Boyington and Gary Nabel

Questions

- **Can we elicit broadly neutralizing HA antibodies through immunization?**

  -DNA/Seasonal vaccine or DNA/rAd

- **Can this prime-boost regimen increase the breadth of neutralizing antibodies against other H1 HAs?**

Increased Breadth of Neutralization by Prime-Boost Immunization

Immune Mouse

Immunization \ Virus	1934 PR8	1986 Sing	1995 Bei	1999 NC	2006 SI	2007 Bris
DNA	0	0	631	879	<100	<100
Vaccine	0	693	677	330	574	0
Vaccine/Vaccine	<100	366	625	2778	851	728
DNA/Vaccine	574	735	3083	>12800	1808	1251

Immune Ferret

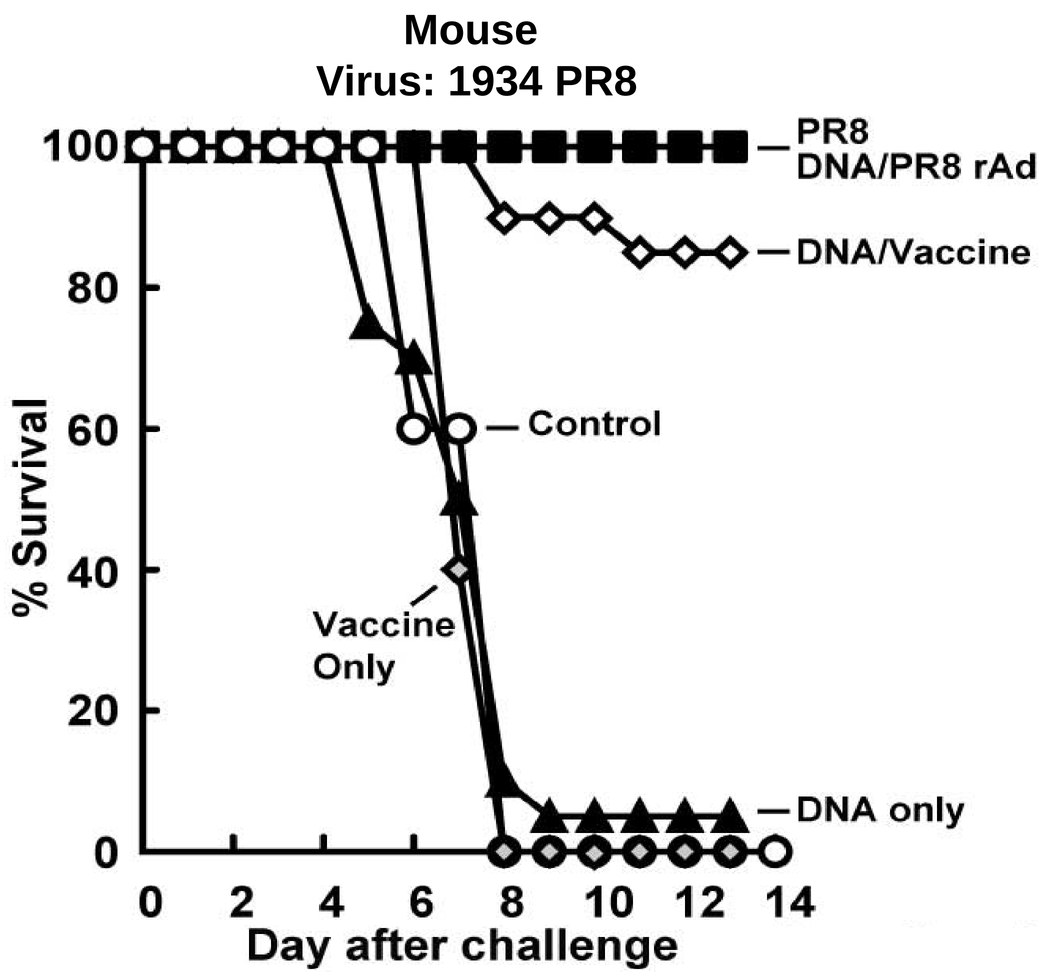
Immunization \ Virus	1934 PR8	1986 Sing	1995 Bei	1999 NC	2007 Bris
DNA/Vaccine	<100	576	2683	1287	105
DNA/rAd	246	552	16497	48951	1584

Immune NHP

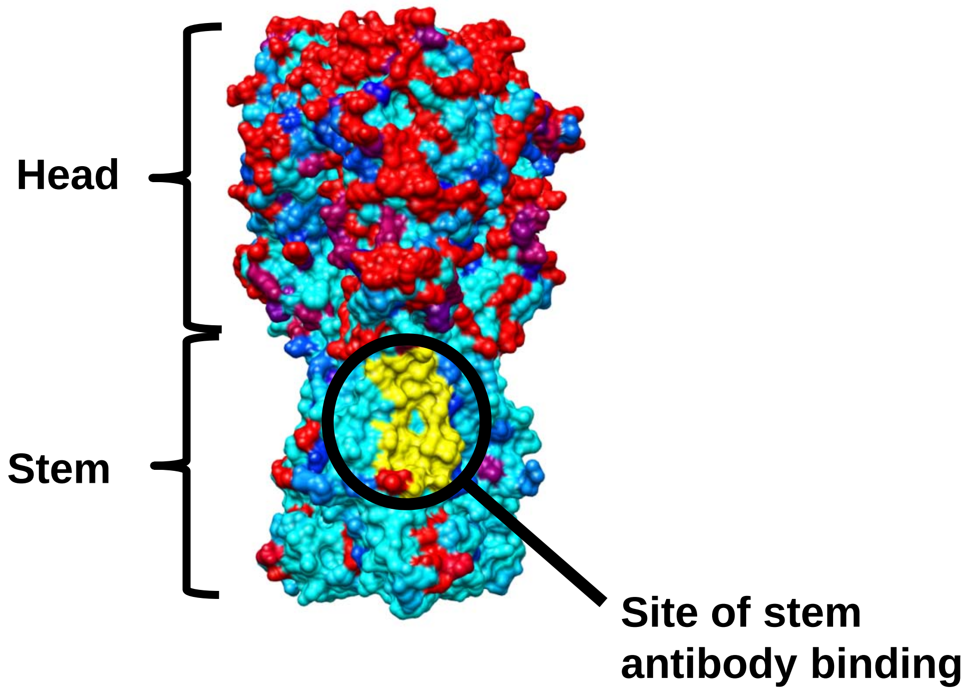
Immunization \ Virus	1986 Sing	1995 Bei	1999 NC	2007 Bris
DNA	<50	223	100	<50
Vaccine	<50	<50	<50	<50
DNA/Vaccine	485	4182	1176	334

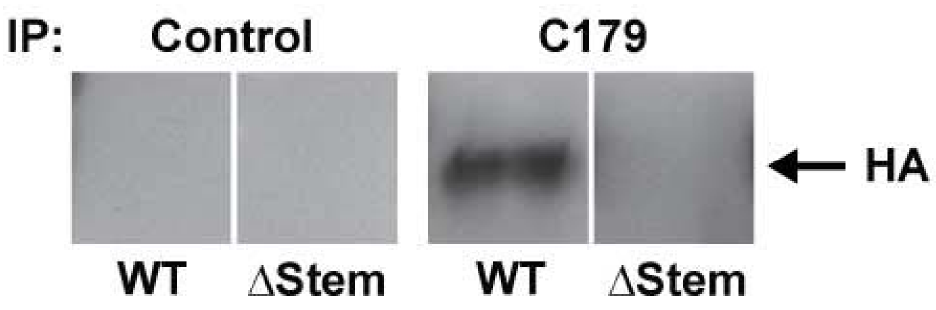
Pseudotyped IC_{50} titers

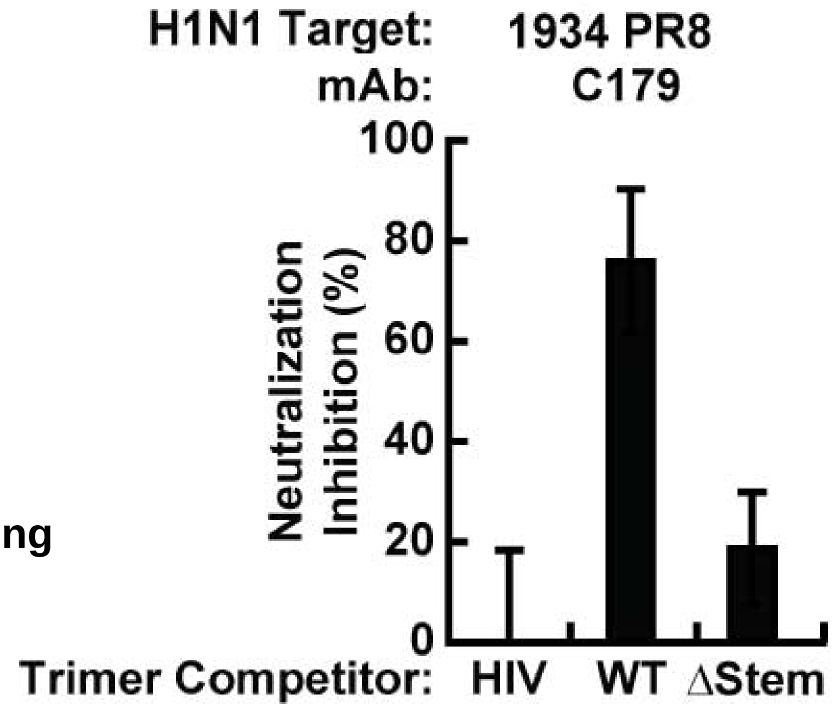
1999 NC HA DNA/Vaccine Prime-Boost Immunization Protected Mice against 1934 PR8 Challenge



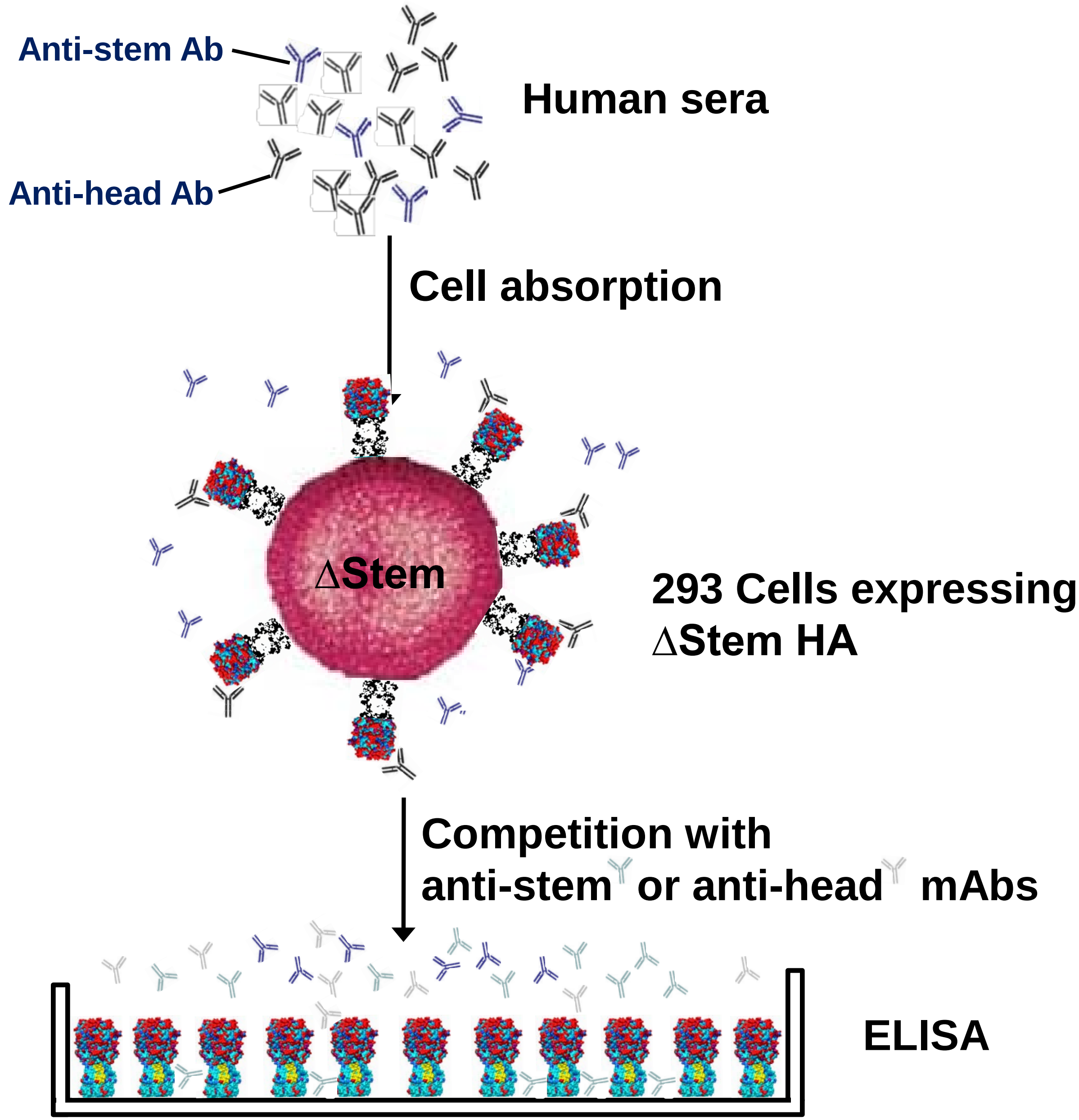
Anti-Stem mAb C179 Binds to Wild-type 1999 NC Trimer but Does Not React with Stem Mutant (ΔStem)



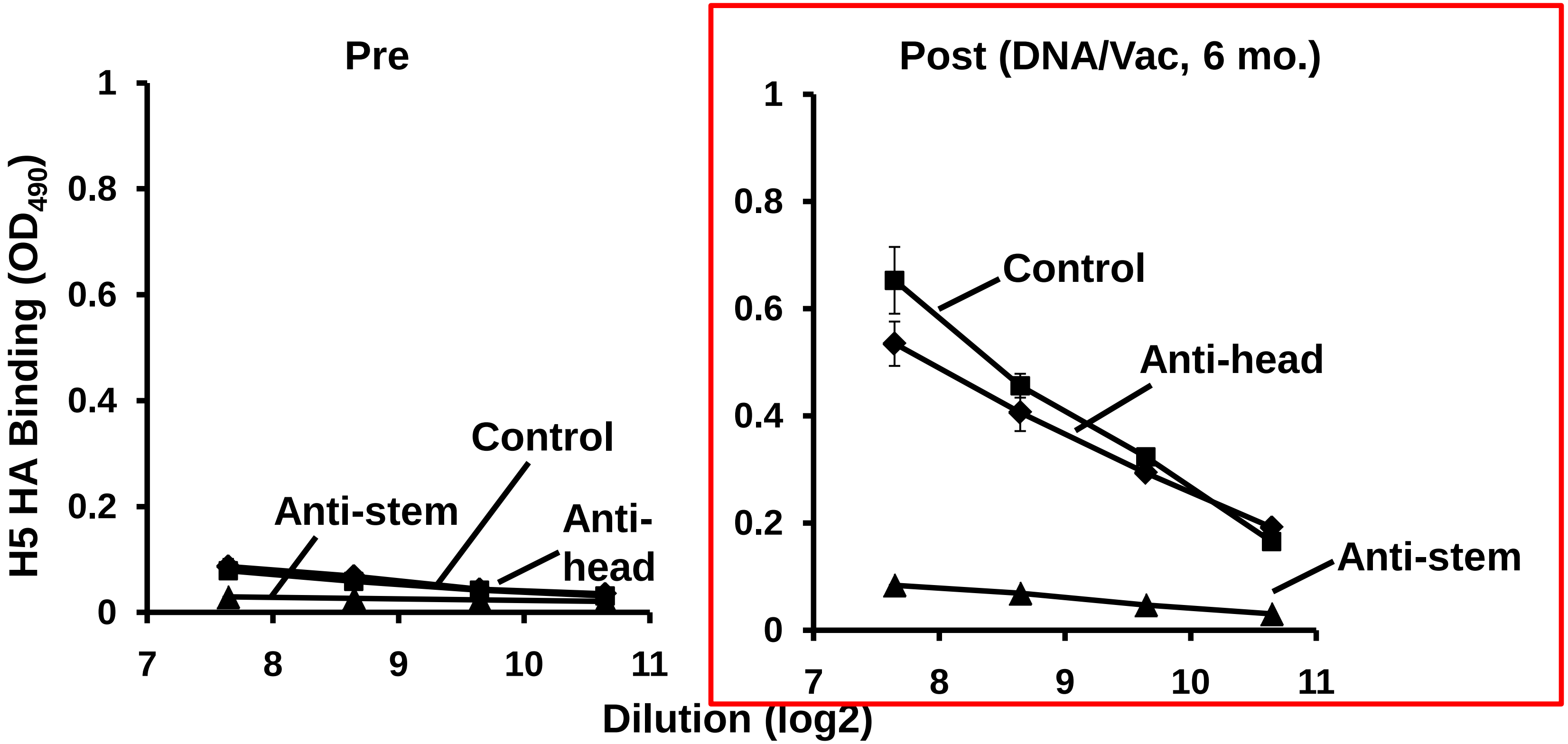




Cell Absorption and mAb Competition Assay



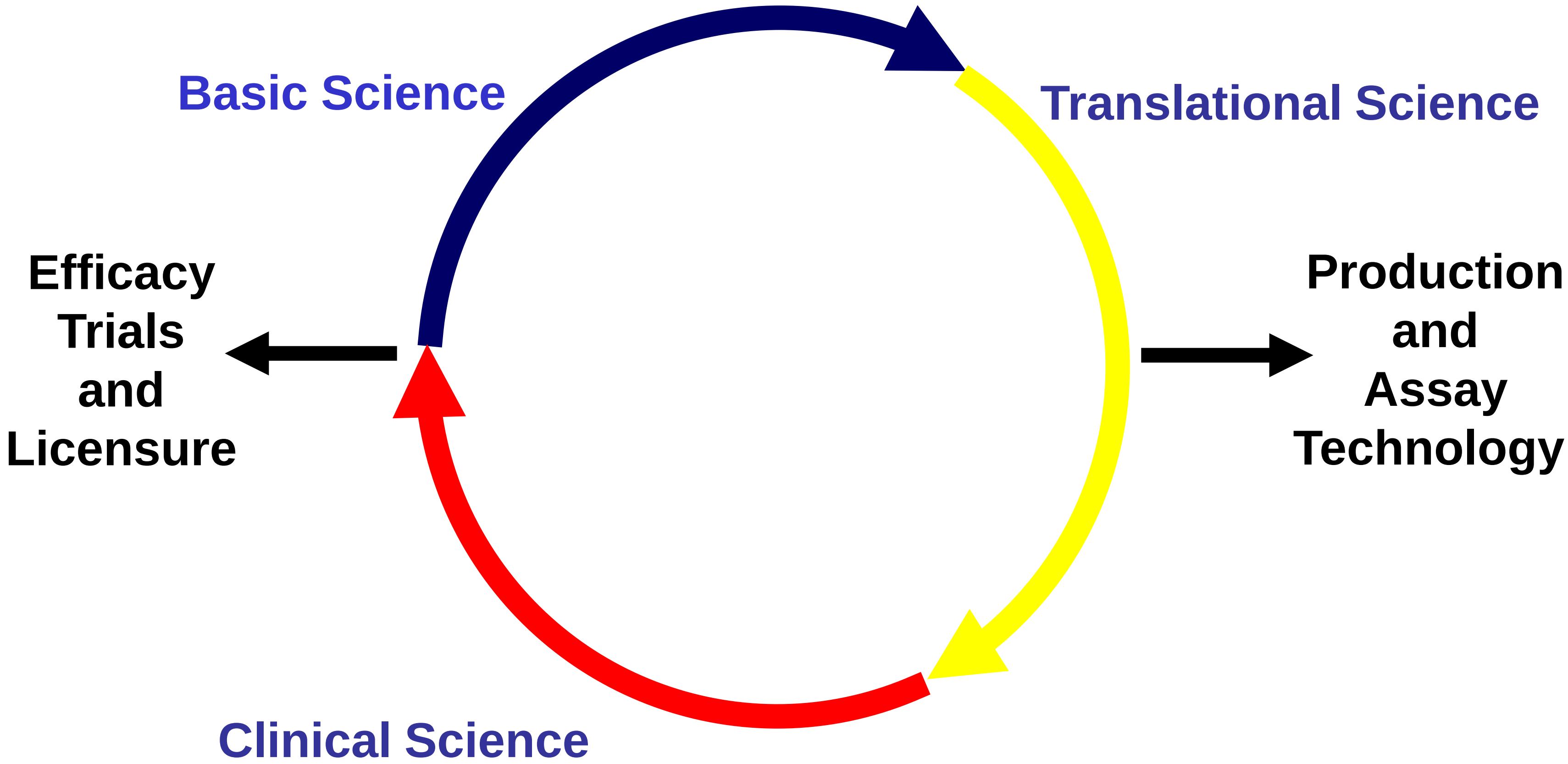
Evidence of Stem-Directed Antibodies Elicited by DNA/Vaccine Immunization in Humans



Summary

1. Vaccination with plasmid DNA encoding H1N1 influenza HA and boosting with seasonal vaccine or rAd stimulated the production of broadly neutralizing influenza antibodies in mice, ferrets, and NHPs.
2. This vaccine protected mice against lethal challenge by a seasonal strain dating back to 1934, and also conferred protection against divergent H1N1 viruses from 1934 and 2007 in ferrets.
3. These broadly neutralizing antibodies were directed to the conserved stem region of the HA and were also elicited in monkeys and humans and provide the basis for a first-generation universal flu vaccine.

The Product Development Cycle for Challenging Vaccines



Vaccine Development at the VRC