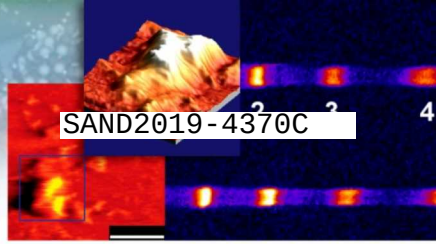
SANDIA BIOSCIENCE



SAND2019-4370C

Rapid development of neutralizing antibody cocktails

Mike Kent, Brooke Harmon

Sandia National Labs

DHS S&T Technology Surprise Summit,
Mitre Corp, McLean Va, Oct 2018

Sandia National Laboratories is a multi-mission laboratory managed and operated by National Technology and Engineering Solutions of Sandia, LLC., a wholly owned subsidiary of Honeywell International, Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA-0003525.

Tech surprise

Technology is becoming available soon that will allow

a. adversaries to introduce mutations into viruses that render ineffective existing neutralizing antibodies (Abs)

b. DHS to rapidly develop cocktails derived from a neutralizing antibody that are effective against a broad range of related viral types and subtypes.

(pertains to toxins and bacterial pathogens as well as viruses)

SANDIA BIOSCIENCE

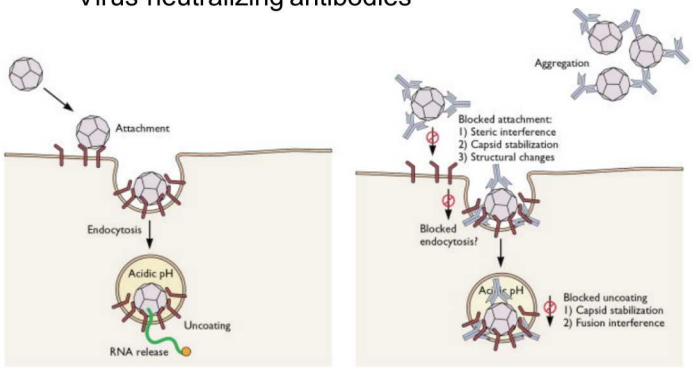
Background

Journal of Bioterrorism & Biodefense
ISSN: 2157-2526
Hu and Nagata, J Bioterror Biodef 2016, 7:3
DOI: 10.4172/2157-2526.1000149
Review Article Open Access

Opportunities and Challenges of Therapeutic Monoclonal Antibodies as Medical Countermeasures for Biodefense

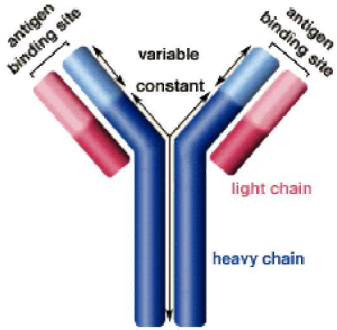
Wei-Gang Hu* and Les P Nagata

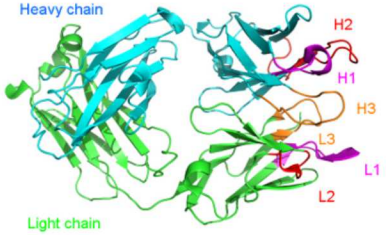
Virus-neutralizing antibodies



http://www.virology.ws/2009/07/24/virus-neutralization-by-antibodies/

antibody structure





Complementarity-determining region (CDR) loops

SANDIA BIOSCIENCE

Sandia National Laboratories

Idea

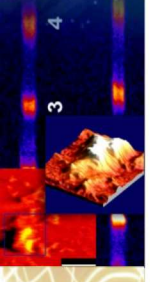
Rapid and efficient method to broaden the spectrum of a neutralizing antibody by developing variants effective against other types and subtypes

-For neutralizing Ab: identify Ag epitope, sequence of Ab

-Establish Ab-Ag structure from cryoEM or in-silico modeling

-Use in-silico analysis of structure to focus experimental screening library

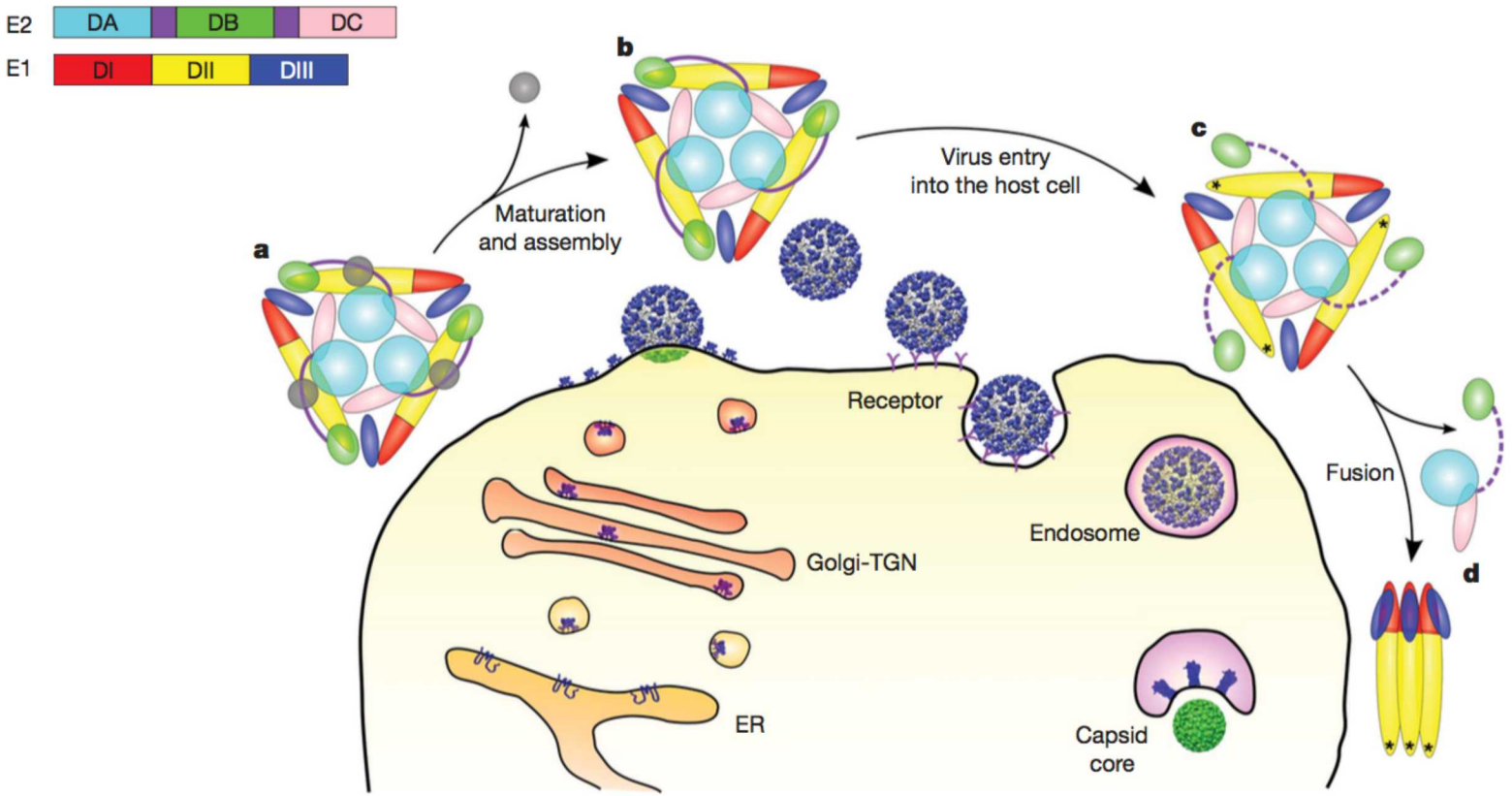
-Screen phage-displayed synthetic antibody libraries for variants effective against related virus types and subtypes (including mutants)



SANDIA BIOSCIENCE

Example: Neutralizing antibodies for equine encephalitis viruses (VEEV, WEEV, and EEEV)

maturation and fusion of alphavirus



Example: Neutralizing antibodies for equine encephalitis viruses (VEEV, WEEV, and EEEV)

JVI Journals.ASM.org

Locking and Blocking the Viral Landscape of an Alphavirus with Neutralizing Antibodies

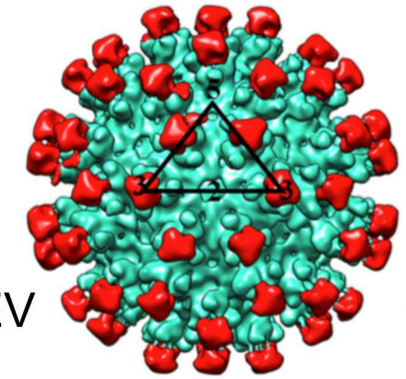
Jason Porta,[a] Joyce Jose,[a] John T. Roehrig,[b] Carol D. Blair,[c] Richard J. Kuhn,[a] Michael G. Rossmann[a]

Department of Biological Sciences, Purdue University, West Lafayette, Indiana, USA[a]; Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, Colorado, USA[b]; Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, USA[c]

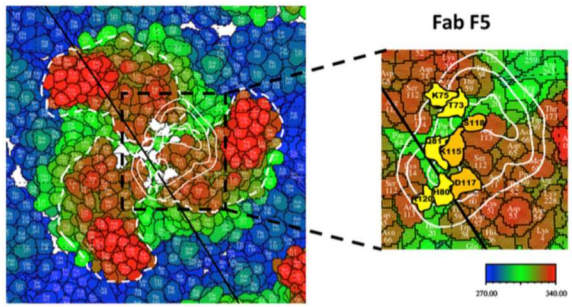
F5 isolated from human bone marrow donors

F5 binds to E1E2 heterotrimer spikes and blocks fusion

F5 (red)



VEEV



View of the VEEV surface spike - F5 contact region. Footprint of a single F5 Fab projected radially onto the viral surface spike. Solid white contours represent the projection of the Fab density. From Porta et al 2014.

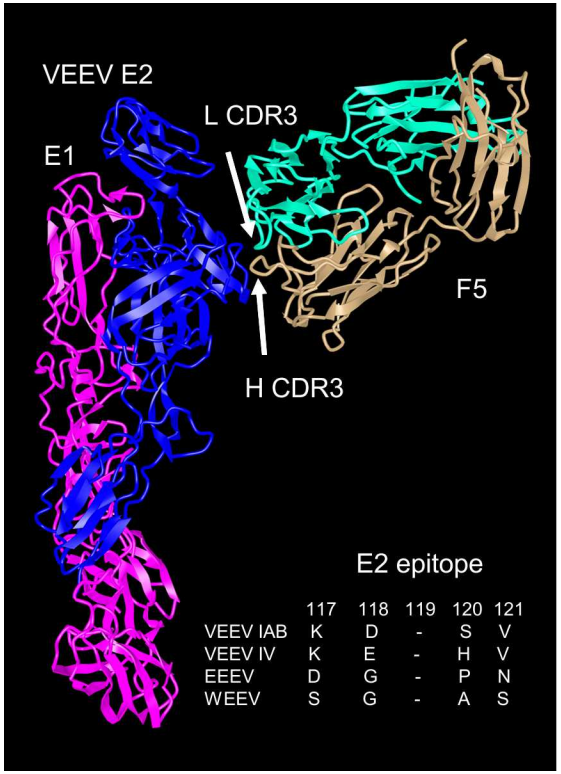
F5 neutralizes VEEV subtypes IAB, IC, ID, IE, II IIIA, IIIB, IIIC and VI, but not IV and V, or EEEV and WEEV

Can variants be found to neutralize VEEV subtypes IV and V, and also EEEV and WEEV (all subtypes)?

SANDIA BIOSCIENCE

Find analogues of F5 for all subtypes of VEEV, EEEV, and WEEV: Experimental and computational methods

Light chain contacts second E2 within the same span of residues (117-121)



Find F5 variants that bind EEEV and WEEV

	VEEV E2	EEEV E2	WEEV E2	CHIKV E2	SINV E2
VEEV E2	-	48%	41%	36%	40%
EEEV E2	48%	-	45%	40%	44%
WEEV E2	41%	45%	-	38%	67%
CHIKV E2	36%	40%	38%	-	37%
SINV E2	40%	44%	67%	37%	-

Exptl htp screens: 10^8 diversity

Use structural information to focus the experimental library on sequences with high probability of binding

SANDIA BIOSCIENCE

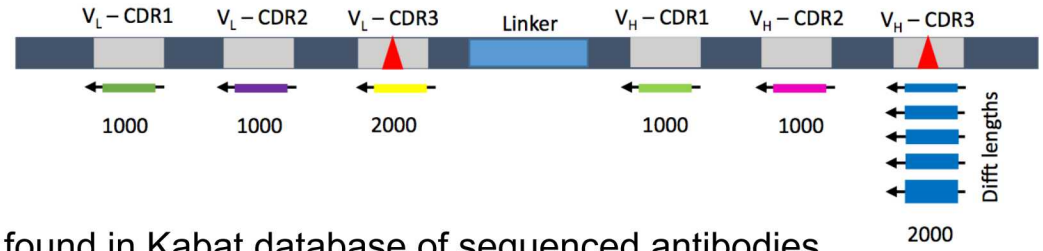
Sandia National Laboratories

Phage displayed synthetic antibody libraries

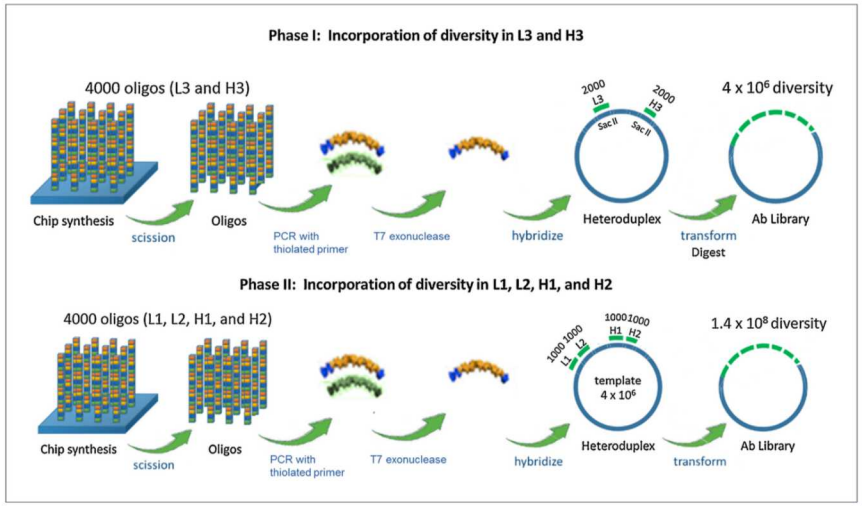
New Biotechnol 33, 565, 2015

scFv based on human frameworks Vh5-3 and VL3-10

pre-defined CDR regions



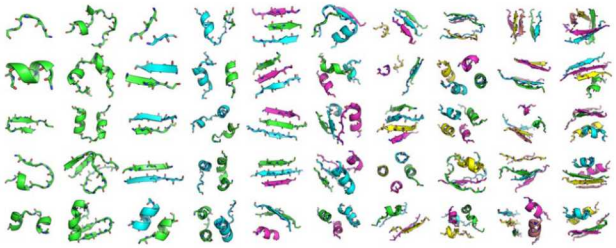
Follow statistics found in Kabat database of sequenced antibodies
-frequency of germline residues at each position
-probabilities of each residue type vs position along loop



Does not use any information from the Ab-Ag structure!

Two ways to use information from the Ab-Ag structure

1. Informatics: tertiary structural motifs



600 terms sufficient to describe 50% of the PDB at sub-Å resolution

460,000 terms describe 99% of the PDB

Resolve Ab-Ag structural interface into TERMS

Use informatics analysis to determine sequences of CDR loops expected to be compatible with binding (defining compatible sequence space)

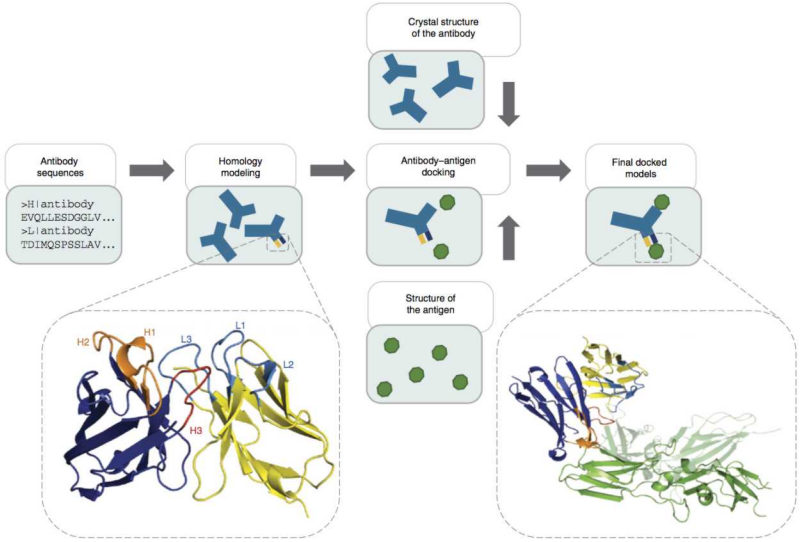
Diverse as possible to sample compatible sequence space more broadly

Can also specify aa-propensities as each position along the loops

Advantage – less dependent on structural accuracy

Two ways to use information from the Ab-Ag structure

2. Structural modeling, screen mutations using scoring functions (Rosetta)



Nature Protocols 12, 401, 2017

Trying to find specific high binding sequences

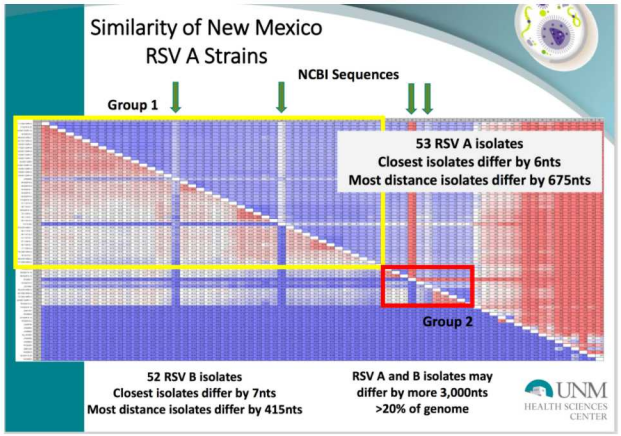
disadvantage – highly dependent on structural accuracy

SANDIA BIOSCIENCE

Account for mutants

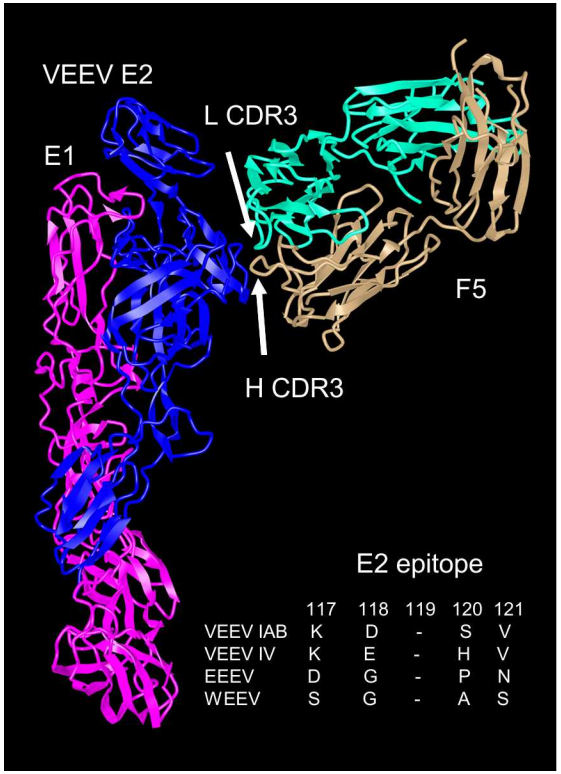
Predict (structural modeling) or measure (Next-Gen sequencing) mutations that block binding in parent Ab, develop variants effective against those

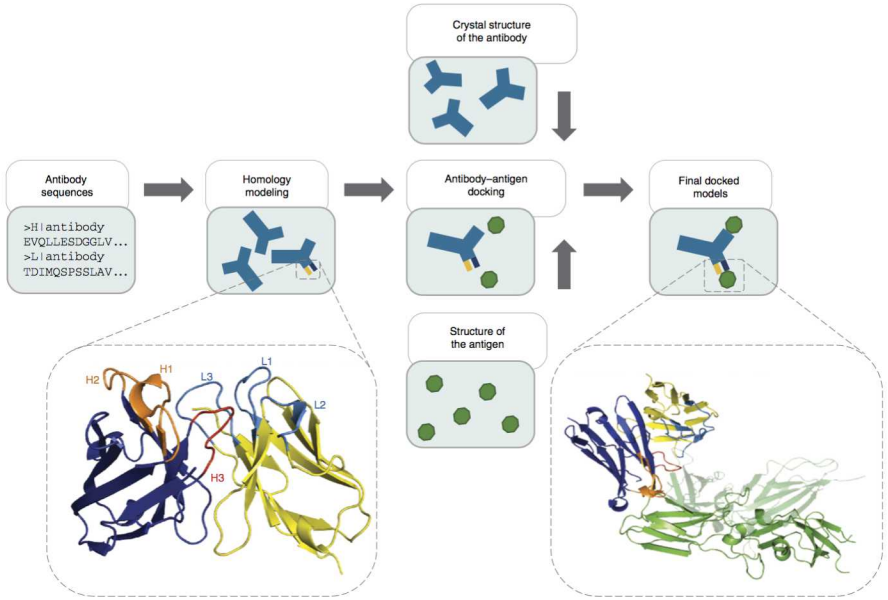
Next Gen sequencing of RSV strains during recent outbreak shows high degree of sequence variability within each subtype



https://academic.oup.com/ofid/article/3/suppl_1/81/2637639

The same methods can be used by adversaries to design mutants to evade neutralizing Ab





Can determine mutations to evade existing neutralizing Ab

SANDIA BIOSCIENCE

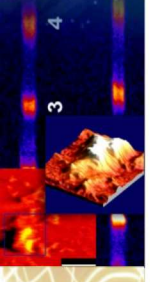
Sandia National Laboratories

Summary

Therapeutic monoclonal antibodies have great promise as medical countermeasures for biodefense

Multiple technologies are advancing that will soon allow rapid development of cocktails derived from a neutralizing antibody that are effective against a broad range of related viral types and subtypes

The same methods can be used by adversaries to design viral mutants to evade a single therapeutic neutralizing Ab



SANDIA BIOSCIENCE