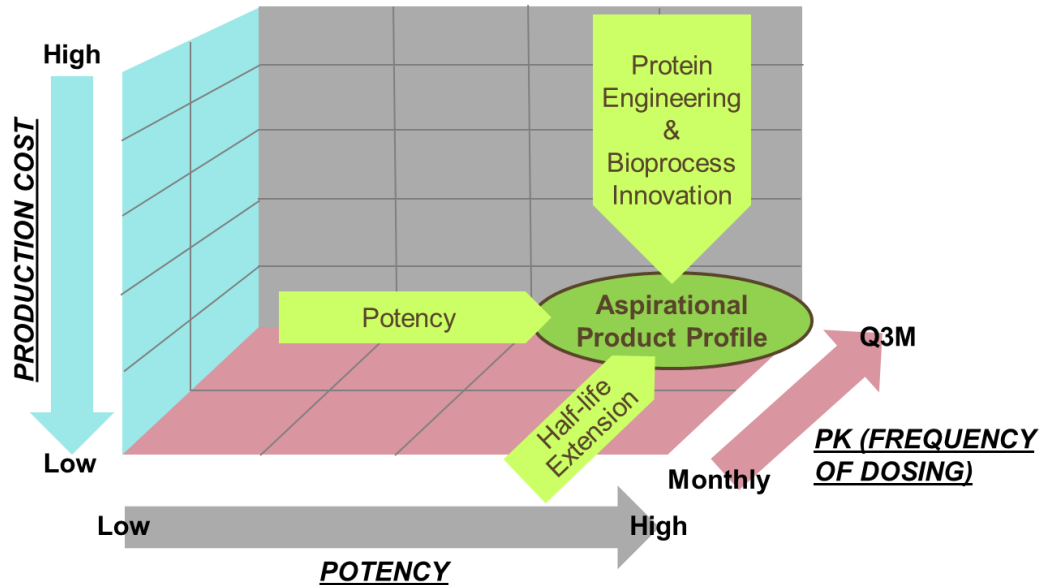


# Antibody Engineering: Optimization of Antibodies for Commercialization

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Stephen Hadley, PhD  
2018 GVRIF  
20-22 March 2018  
Bangkok, Thailand

# ENGINEERING mAbs FOR USE IN HIV PREVENTION IS NECESSARY TO MEET A CHALLENGING TPP



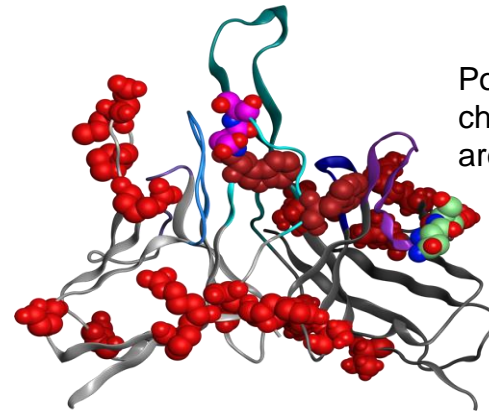
## Additional TPP Challenges:

1. Mixture of 2 mAbs targeting different epitopes
2. SC administration – 150mg/mL.
3. Robust stability profile.

# SOMATIC HYPERMUTATION LEADS TO SPECIFICITY AND ACTIVITY, BUT CAN ALSO DEGRADE STABILITY AND MANUFACTURABILITY

mAbs as products benefit from design and engineering of the variable region to eliminate or reduce potentially problematic sequences:

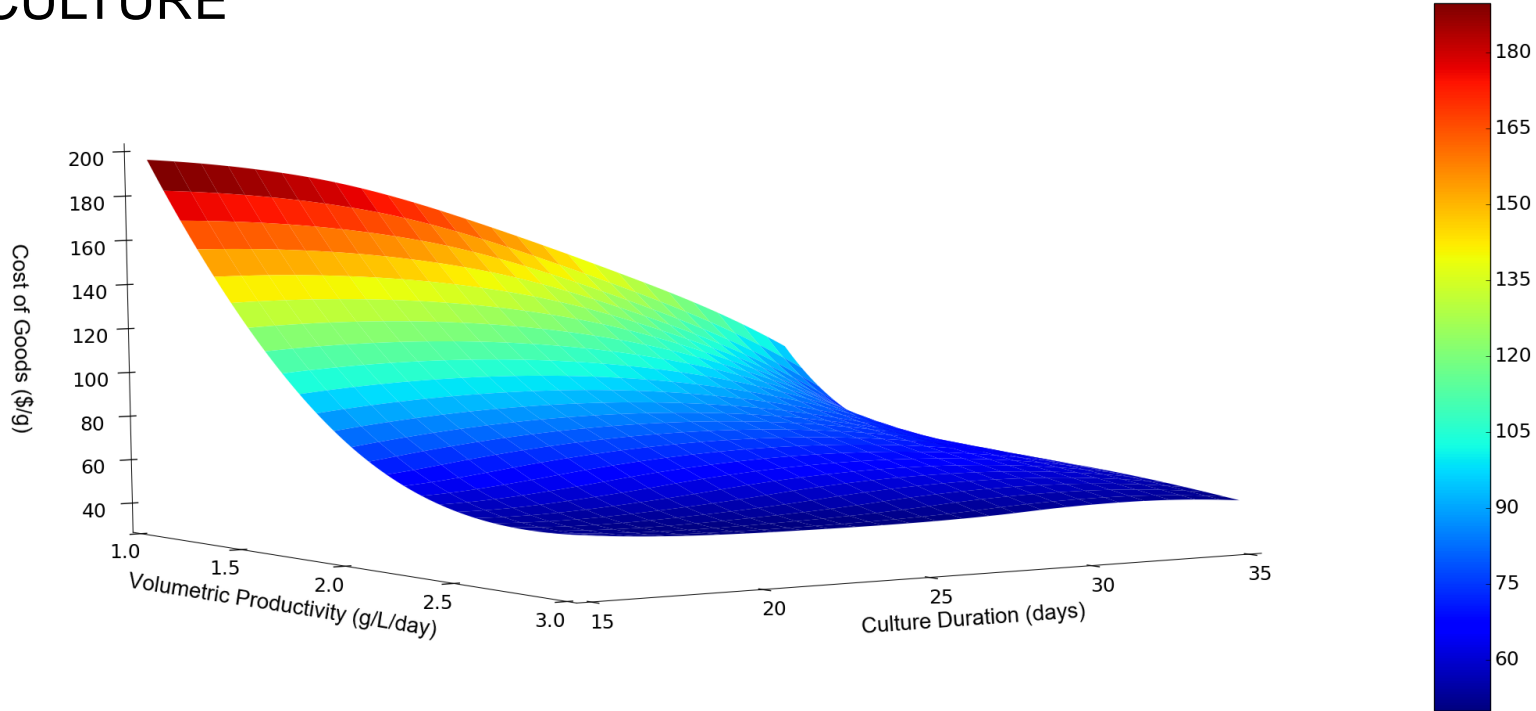
- Deamidation/Isomerization sites
- Alternate glycosylation sites
- Non-standard cysteines
- Folding/thermal stability



Potentially destabilizing and chemically unstable residues are highlighted in red

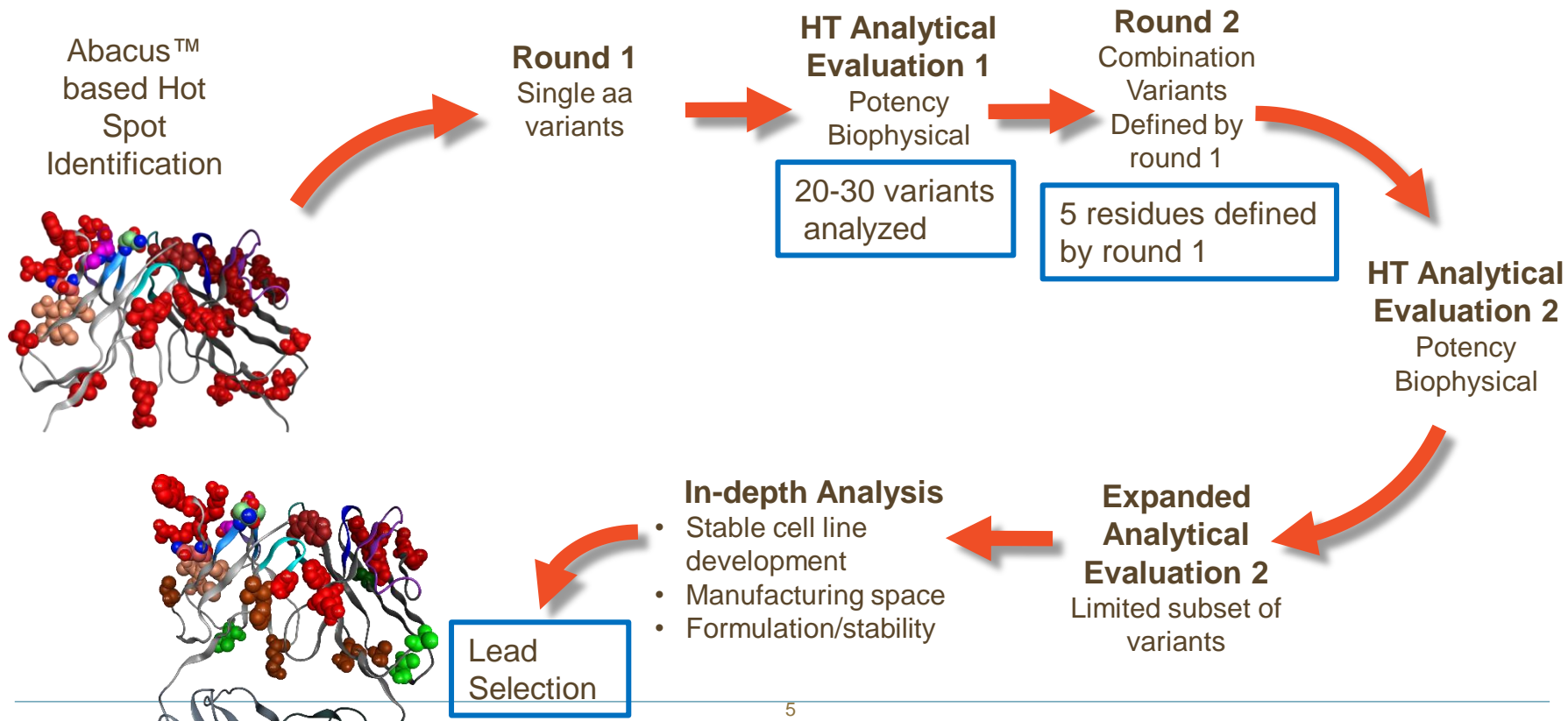
Repair of these sites utilizing structure based models and computational analysis is possible while retaining activity

# THE PRIMARY DRIVER TO DECREASE COST OF PRODUCTION FOR mAbs IS INCREASING PRODUCTIVITY DURING CELL CULTURE

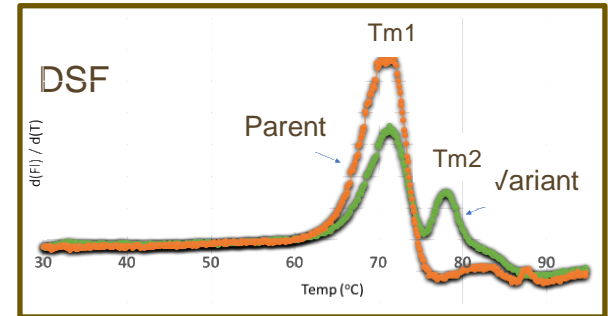
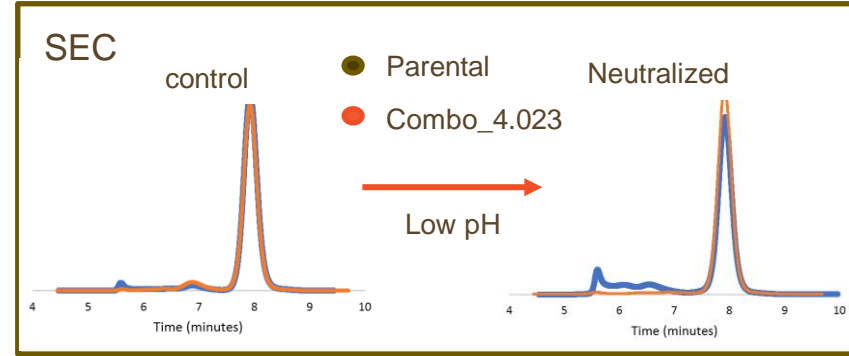
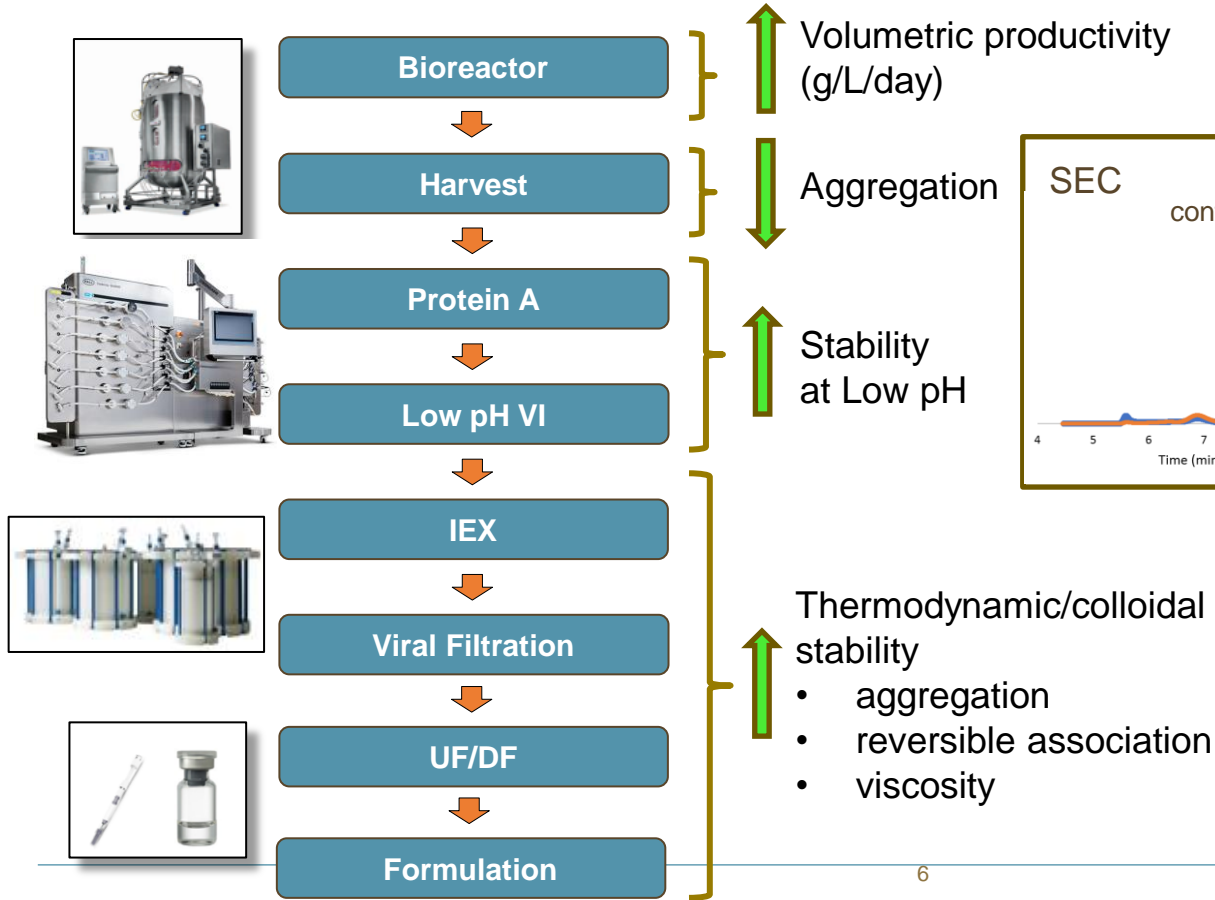


Process model developed for Just Biotherapeutics J.Pod (4 x 500L Brx) Facility

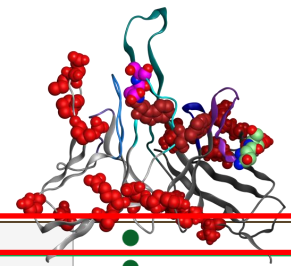
# SEQUENCE OPTIMIZATION STRATEGY TO IDENTIFY IMPROVED VARIANTS: JUST BIOTHERAPEUTICS J. MOD PLATFORM



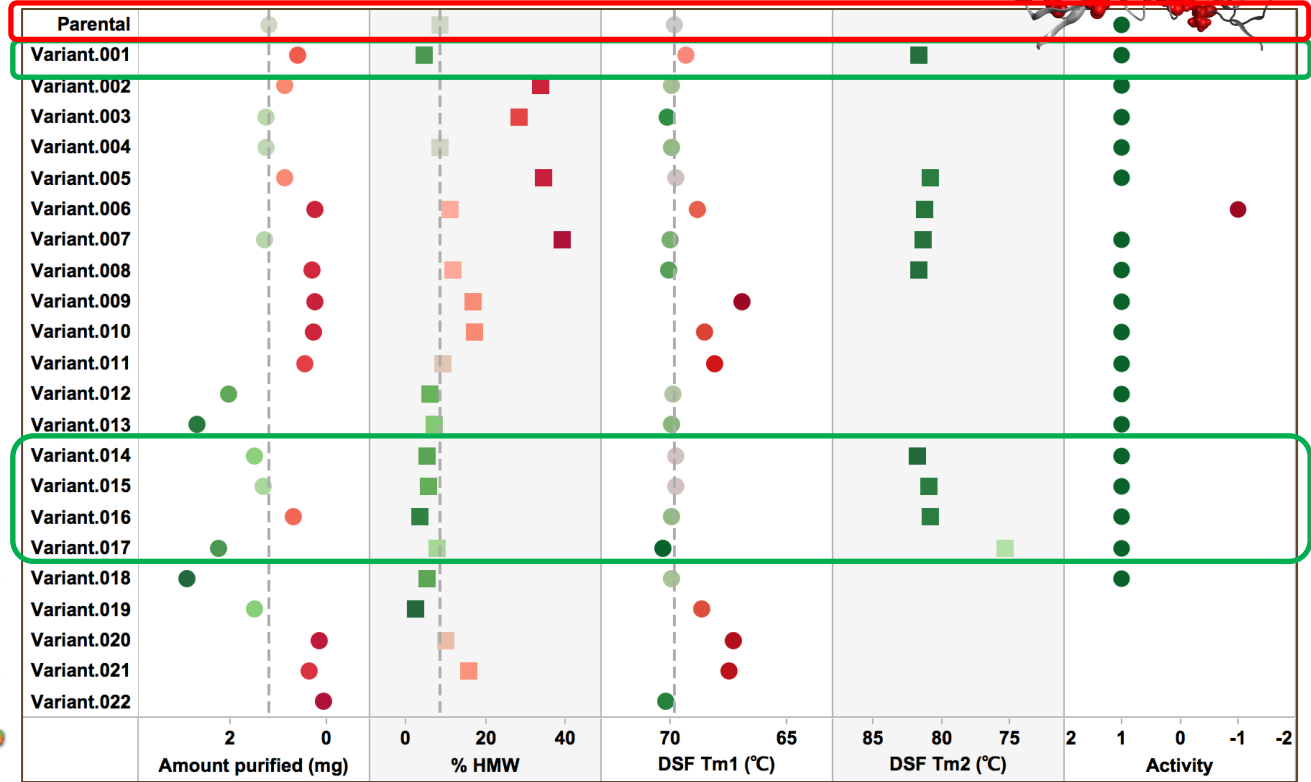
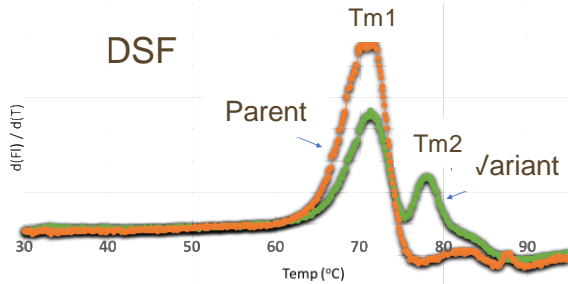
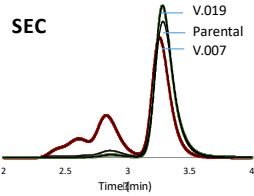
# BIOPHYSICAL ANALYSIS OF VARIANTS IS USED FOR EVALUATING POTENTIAL BEHAVIOR DURING PRODUCTION AND STORAGE



# ROUND 1: SINGLE VARIANTS ALLOW HOT SPOT SITE FILTERING FOR ACTIVITY AND DEVELOPABILITY

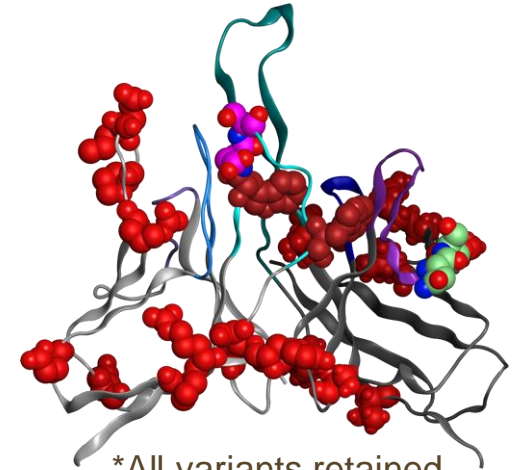


Select variants with consideration of maintained activity, positive expression, and biophysical characteristics



# ROUND 2: COMBINATORIAL VARIANTS DEMONSTRATE IMPROVEMENTS ACROSS MULTIPLE CHARACTERISTICS

	% Main	% Dimer	% Oligomer		DSF Score	GuHCl (M) Inflection Pt	pH 3.3 %HMW
Parental	92	3.7	3.9	8	2.4	40	
Combo_2.001	89	3.1	7.8	7	2.4	40	
Combo_2.002	93	3.5	3.0	8	2.5	14	
Combo_2.003	90	3.1	6.7	8	2.5	28	
Combo_2.004	95	3.6	1.0	16	2.6	9	
Combo_2.005	93	4.0	2.6	9	2.6	10	
Combo_2.006	94	3.4	2.6	10	2.6	12	
Combo_2.007	95	3.9	0.8	21	2.6	3	
Combo_2.008	95	3.5	1.9	10	2.6	9	
Combo_2.009	95	3.9	0.6	20	3.0	2	
Combo_2.010	97	2.9	0.4	28	3.0	3	
Combo_3.011	93	3.5	3.8	8	2.6	14	
Combo_3.012	93	3.8	3.2	8	2.5	22	
Combo_3.013	95	4.0	1.4	16	2.6	5	
Combo_3.014	92	3.9	4.3	8	2.6	31	
Combo_3.015	95	4.6	0.8	16	2.9	3	
Combo_3.016	96	3.4	0.7	22	3.0	3	
Combo_3.017	95	3.6	1.5	10	2.7	10	
Combo_3.018	94	4.9	0.7	21	3.0	3	
Combo_3.019	95	4.7	0.7	29	3.1	2	
Combo_3.020	95	4.5	0.7	24	2.9	2	
Combo_4.021	92	4.5	3.7	8	2.6	10	
Combo_4.022	94	5.0	1.0	16	2.9	3	
Combo_4.023	94	5.0	1.1	22	3.1	4	
Combo_4.024	93	5.6	1.0	19	3.1	3	
Combo_4.025	95	4.6	0.5	22	3.3	2	
Combo_5.026	94	4.9	0.8	18	2.9	3	



\*All variants retained neutralization activity

3 optimized variants were interrogated by more in-depth analysis

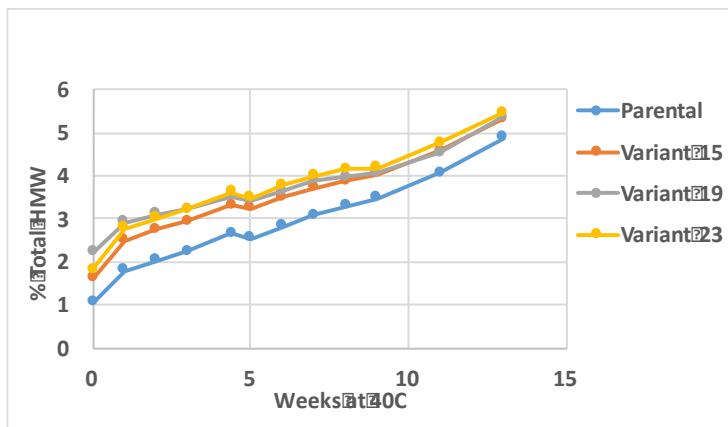


# SEQUENCE OPTIMIZATION IMPROVED THERMAL STABILITY IN A PRODUCT FORMULATION

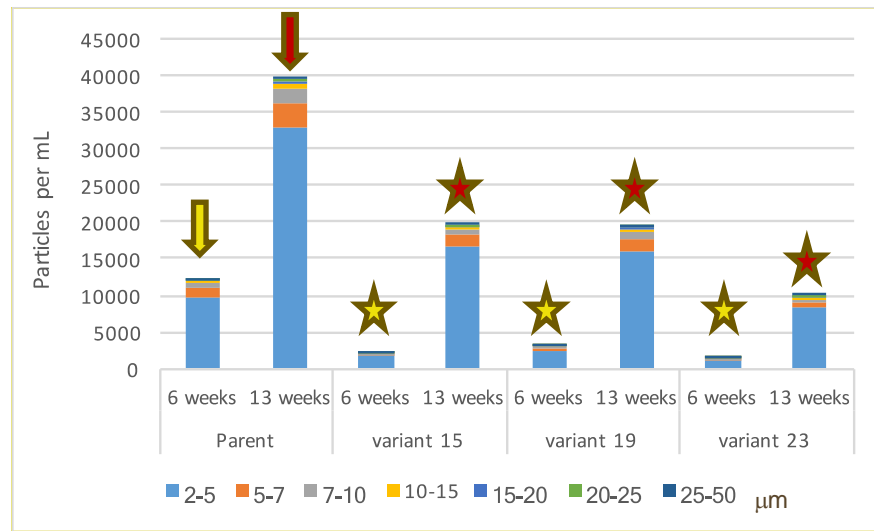


Incubation at 40°C

- 20mM Acetate pH 5.2, 9% Sucrose and 0.01% polysorbate 80, 100 mg/mL bNAb

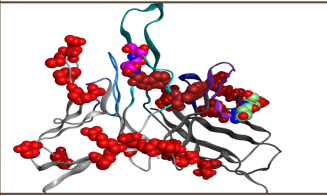
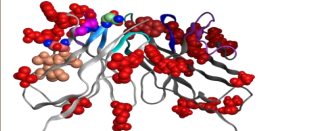

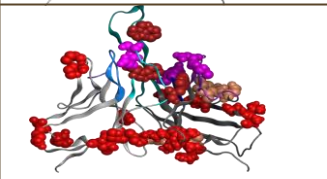


Sub-visible particle analysis after storage



Improved thermal stability allows for time outside the cold chain

# NOT ALL mAbs ARE AMENABLE TO SEQUENCE OPTIMIZATION

		# of potentially destabilizing residues	# of residues affecting potency
	bNAb 1	18	1
	bNAb 2	22	10
	bNAb 3	19	2
	bNAb 4	19	4

# LOW-COST mAb INVESTMENT STRATEGY

Innovation, Risk Level and Time to Impact

Traditional mAb  
Manufacturing

*Where  
commercial  
technology  
is today*

## CHO Platform Innovation

Innovation in molecule optimization, process intensification (including continuous processing) and facility design to drive **production cost to \$10/g**

**Just**<sup>®</sup>

## Alternative Hosts

**Non-mammalian/non-bacterial hosts** offer the potential to transform mAb production leading to low cost

 Biogen/Mit

 DU PONT DANISCO

## Novel Expression Systems

- Synthetic Biology
- Nucleic Acid Delivery

# ACKNOWLEDGEMENTS



## **Just Biotherapeutics**

Bruce Kerwin

Randy Ketchem

Rutilio Clark

Christine Siska

Alison Gillespie

Alaina Floyd

Ken Timmons

Jeremy Shaver

Yan Brodsky

Megan McClure

Lisa Connell-Crowley

Dean Pettit

## **Rockefeller University**

Michel Nussenzweig



**Beth Israel Deaconess  
Medical Center**

## **Beth Israel Deaconess Medical Center**

Dan Barouch

Mike Seaman