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Creating Tomorrow's Vaccines Today

Global Comparability/Characterization Strategy for NVX-CoV2373

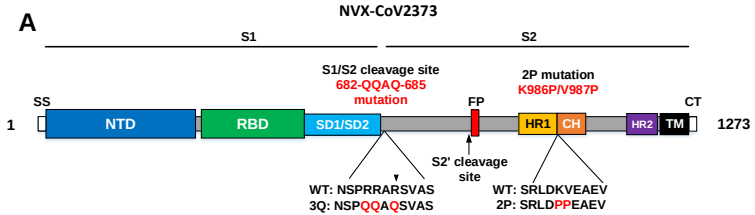
NVX-CoV2373 Vaccine Design

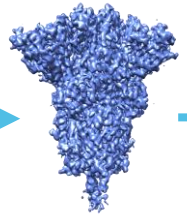
Vaccine Platform Technology: Nanoparticle vaccine formulated with Matrix-M1 adjuvant

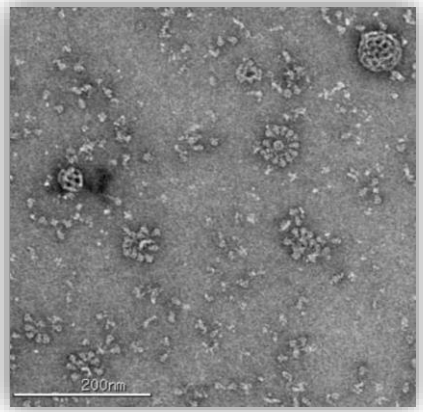
Antigen expressed in baculovirus-*S. frugiperda* system
- Codon-optimized
- Full-length protein, including transmembrane domain
- Furin cleavage site mutated and protein stabilized

Drug Substance
- Native conformation trimers
- Stable PS80 nanoparticle

Drug Product
- Co-formulated with adjuvant
- Dispensed in vial
- Stored 2-8^{O} C

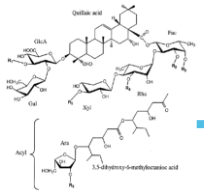


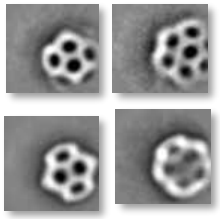




Matrix-M adjuvant
- Purified from *Quillaja saponaria molina*

Matrix-M Adjuvant

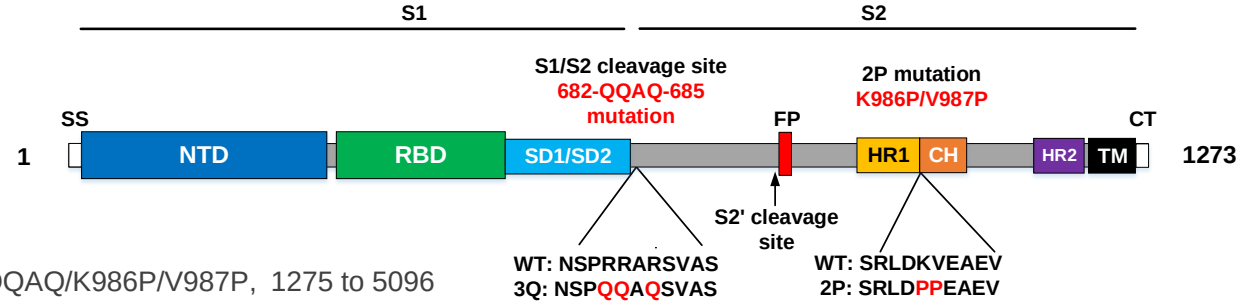




Bangaru et al. bioRxiv 06 August 2020 and Tian et al. bioRxiv 30 June 2020

NVX-CoV2373 genetic clone is full length S with furin site QQAQ and S2 PP mutations

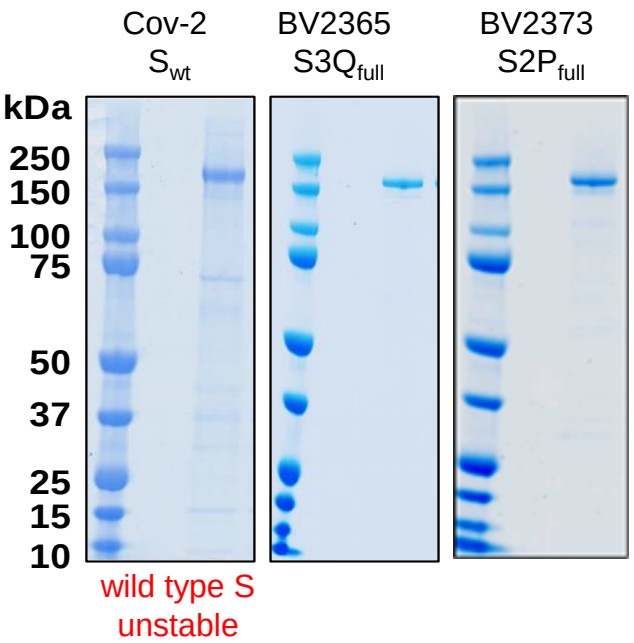
2019-nCoV/USA-WA1

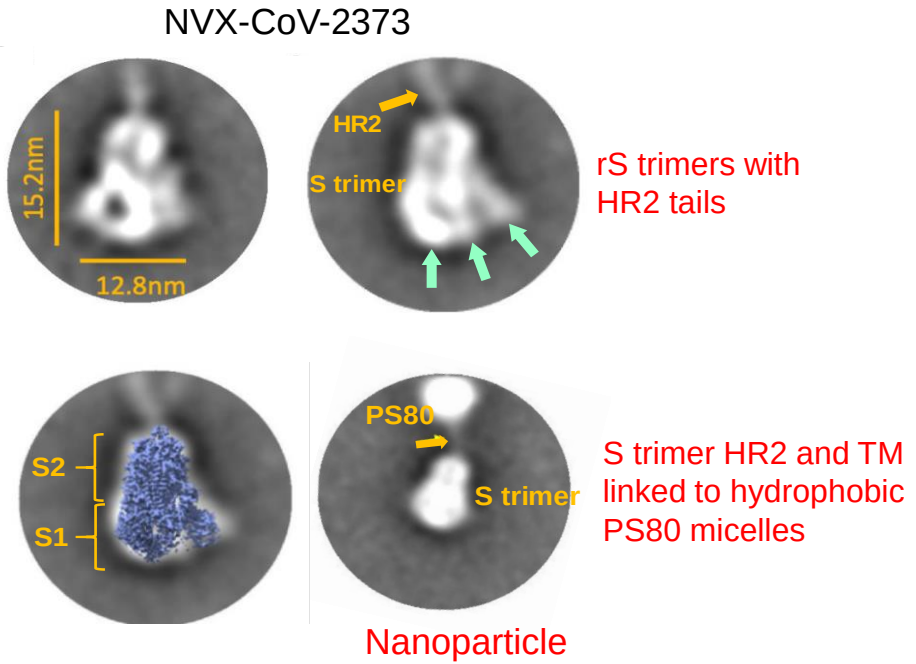


SARS-Cov-2/QQAQ/K986P/V987P, 1275 to 5096
Translation product 1273 aa, Mol Wt 141058.8, pI 5.89
Extinction coefficient: 1.067

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDS
KTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSAL
EPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPF
GEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGN
YNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFL
PFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGIC
ASYQTQTNSP**QQAQ**SVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVF
AQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQI
PFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLD**PP**EAEVQIDRLITGRLQSLQTY
VTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIIT
TDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVM
VTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

Recombinant NVX-CoV2373 rS SDS-PAGE and 2D Class Averaging





Tian and Patel et al., ***Nat Comm, December 2020*** SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 elicits immunogenicity in baboons and protection in mice.

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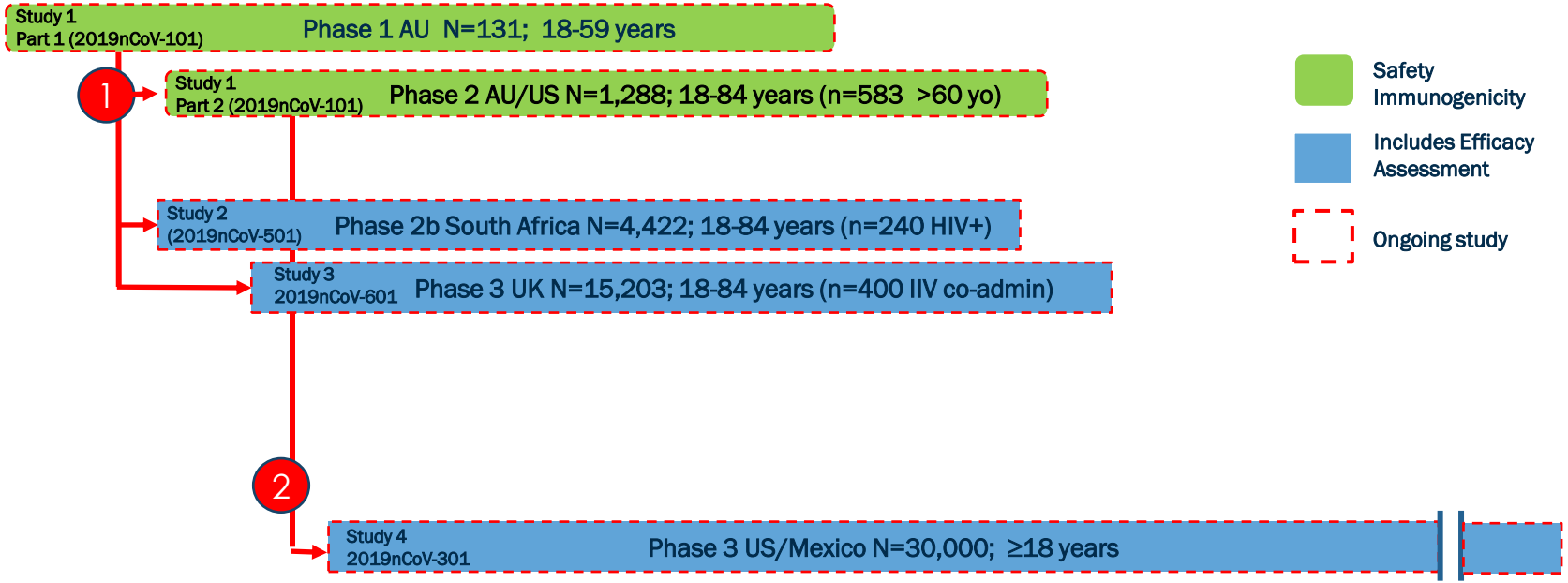
Administration of NVX-CoV2373

- Intramuscular injection, standard needle and syringe
- Antigen (rSpike) dose 5 µg
- Adjuvant (Matrix-M1) 50 µg dose
- Drug product contains antigen and adjuvant in aqueous suspension in 10-dose vials
- No preservatives
- Stored and transported at 2-8^{0}C
- Two dose regimens administered at day 0 and 21

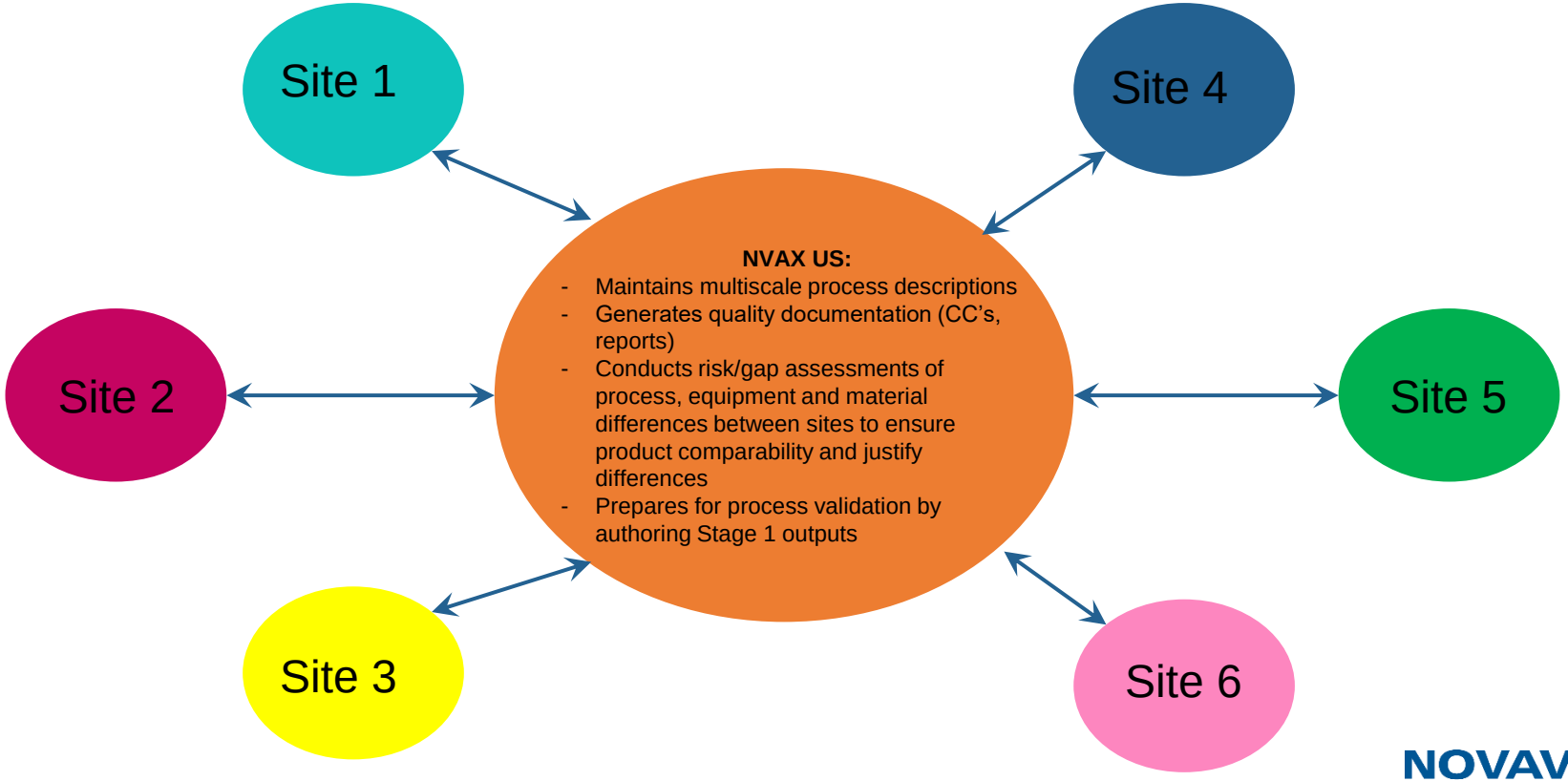
Clinical Development Plan



1 Dose confirmation based on Phase 1 data

2 Dose confirmation in adults >60 y based on Phase 2

Hub and spoke model for manufacturing



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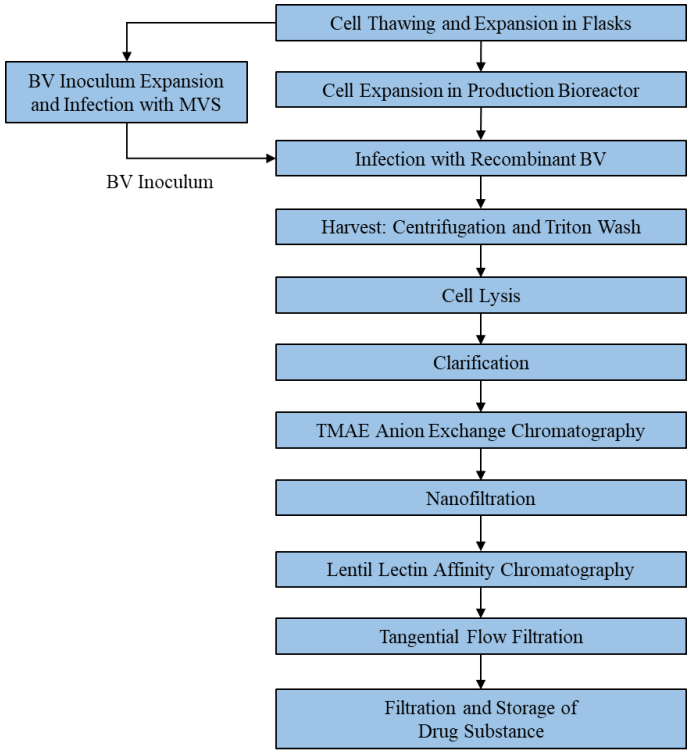
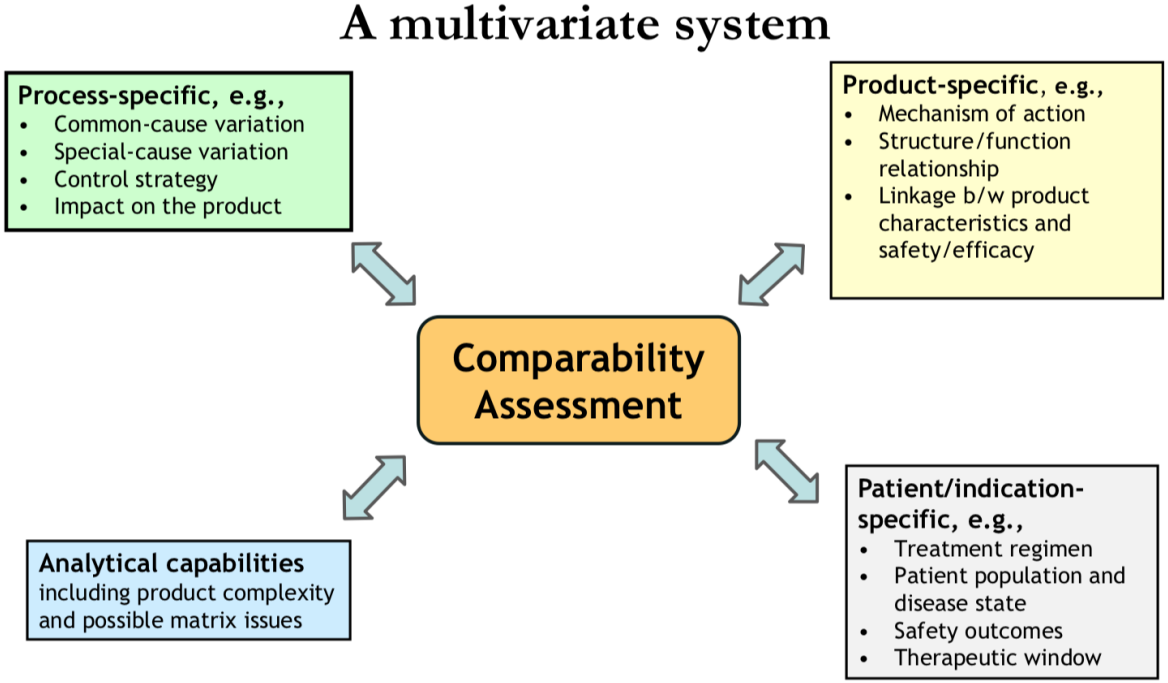
Process Overview



Comparability Assessment



From presentation by Ingrid Markovic (FDA-CBER) at the CMC Strategy Forum July, 2016
(https://cdn.ymaws.com/www.casss.org/resource/resmgr/2016_CMCS_MarkovicIngrid.pdf)

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Designing meaningful comparability studies

- Perform risk assessment to evaluate if a change in manufacturing could impact product quality- what is most likely to be affected and to what degree?
  - Leverage what you already know from product development
  - Include the most critical parameters for comparison purposes, and rank them for your study design and analysis
  - Where does known variability exist and how will you try to control for that?
- Justify in your submission the number and types of samples, tests, acceptance criteria, and the analysis/statistics you will perform
- What limits/assumptions does the study design place on interpretation?

From presentation by Ingrid Markovic (FDA-CBER) at the CMC Strategy Forum July, 2016
(https://cdn.ymaws.com/www.casss.org/resource/resmgr/2016_CMCS_MarkovicIngrid.pdf)

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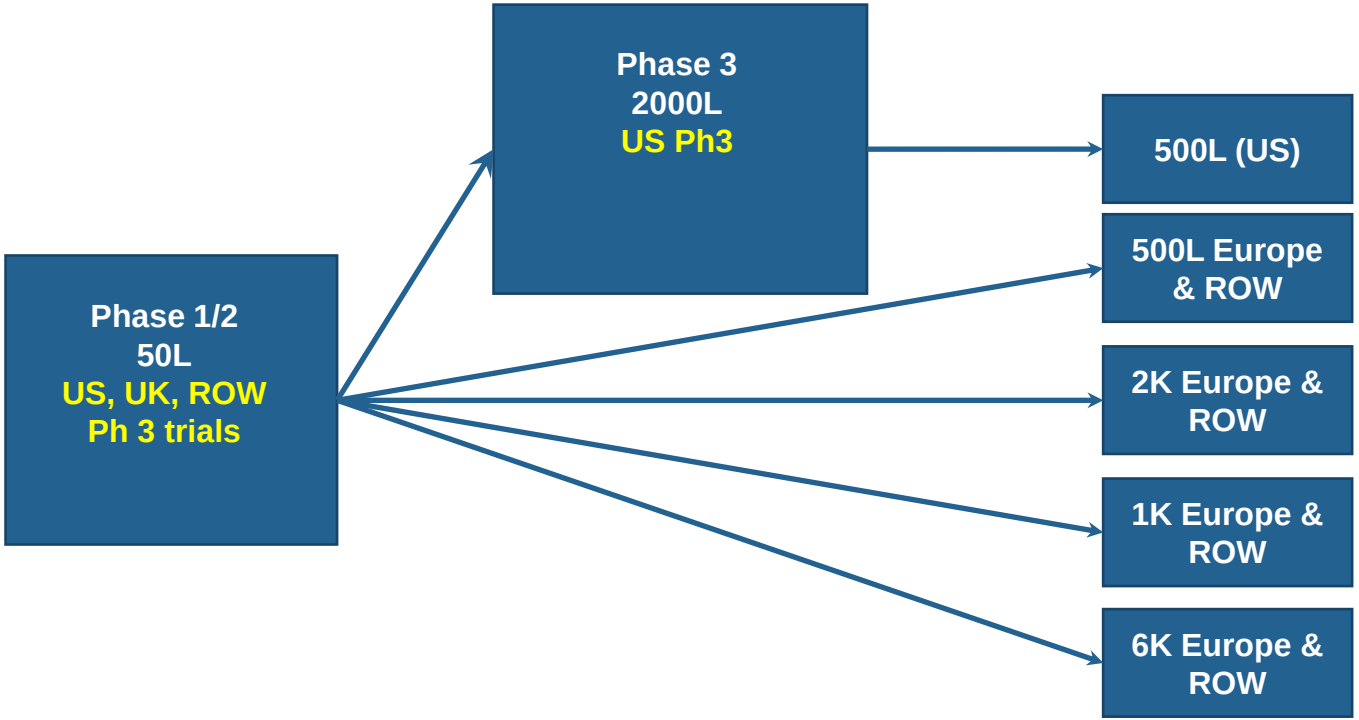
Content of Comparability Protocol

DS Comparability Strategy



Rationale: clinical trials in US, Europe & ROW were initiated using 50L materials. It is, therefore, the primary source of comparison for other manufacturing sites producing for the US, Europe & ROW.

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Comprehensive Data Analysis of NVX-CoV2373 Spike Protein

Characterization of NVX-CoV2373 DS using multiple orthogonal assays

- Higher Order Structure – TEM, DSC, CD, DLS, nanoparticle tracking
- Size – SDS-PAGE, CE-SDS, DLS, nanoparticle tracking, HPSEC
- Potency – ACE2 ELISA, ACE2 Octet
- Purity – Quantitative mass spectrometry (MS) for rS and gp64
- Identity and Integrity – Peptide mapping with MS, SDS-PAGE, CE-SDS, Western Blot
- Characterization – Peptide mapping with MS, mouse immunogenicity, oligosaccharide profiling

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Comparability Criteria for DS

Test Method	Classification	Quality Attribute	Acceptance Criteria
Appearance	Release	Color, Clarity, Visible Particles	
pH	Release	Physiochemical	
Total Protein (A280)	Release	Quantity	
PS-80 Content (HPLC)	Release	Excipient Content	
SARS-CoV-2 rS Binding ELISA	Characterization/ Release	Potency	
Kinetics of SARS-CoV-2 rS Binding to ACE2 by BLI	Characterization	Potency	
α-rS Western Blot	Release/ Characterization	Identity / Product Variants	
α-gp64 Western Blot	Characterization	Purity / Process Related	
SDS-PAGE (reduced) w/ Densitometry	Release/ Characterization	Purity / Product & Process Related	
Purity by Peptide Mapping Mass Spectrometry	Characterization/ Release	Purity / Process Related	
Host Cell Protein Mass Spectrometry	Characterization	Purity / Process Related	
Total DNA by PicoGreen	Release	Purity / Expression System	
BV/Sf9 DNA by qPCR	Characterization	Purity / Expression System	
Particle Size (DLS)	Characterization	Higher Order Structure	
Peptide Mapping	Characterization	Primary Structure / Product Variants	
Oligosaccharide Profile	Characterization	Primary Structure	
Thermal Stability by DSC	Characterization	Higher Order Structure	
Circular Dichroism Spectroscopy	Characterization	Higher Order Structure	
Bioburden	Release	Contaminants	
Endotoxin	Release	Contaminants	
Residual Baculovirus by HS Plaque Assay	Release	Purity / Process Related	

Supplementary DS Characterization Testing

- Nanoparticle Tracking
- TEM with 2D image classification
- AF4-MALS
- Side-by-side comparisons for PPQ
  - SDS-PAGE (3 x 3)
  - Western blot with anti-gp64 (3 x 3)
  - Western blot with anti-rS (3 x 3)
  - Silver stain (3 x 3)

<u>Proposal for Initial Expedited Approvals</u> : Perform 3 x 1 comparisons and provide data to Health Authorities as soon as available.

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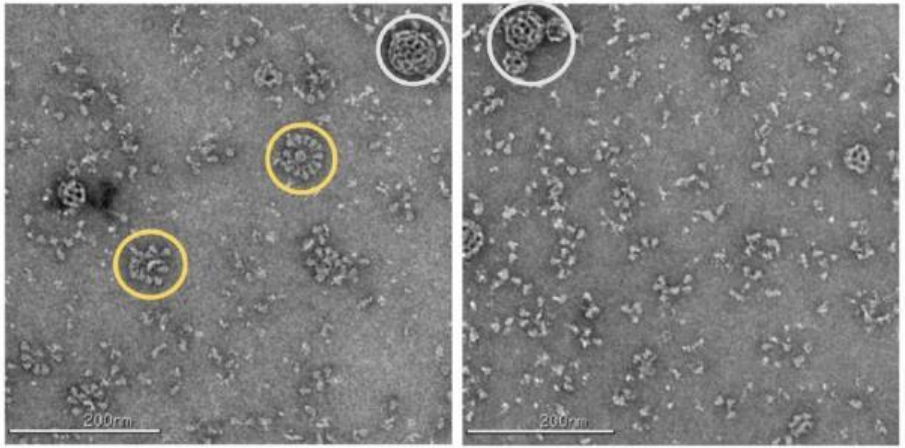
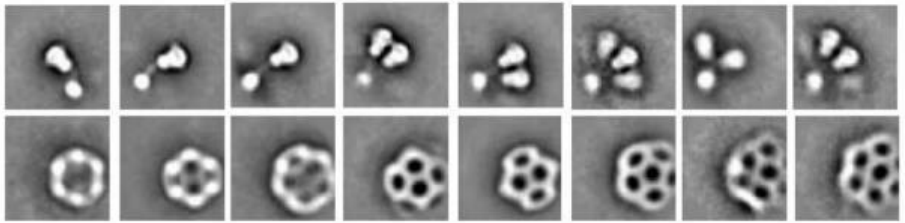
NVX-CoV2373 DP – TEM Co-formulated Vaccine

S:PS80 nanoparticles mixed with Matrix-M cage-like particles

- No visible interactions between S and Matrix-M
- Essentially all S particles consistent with prefusion timers with 3-axis of symmetry
- No elongated postfusion S structures
- No monomers can be identified



TEM NVX-CoV2373 with Matrix-M



2D class averages

Matrix-M

Bangaru, Andrew Ward et al., *Science*, **2020**, Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate.

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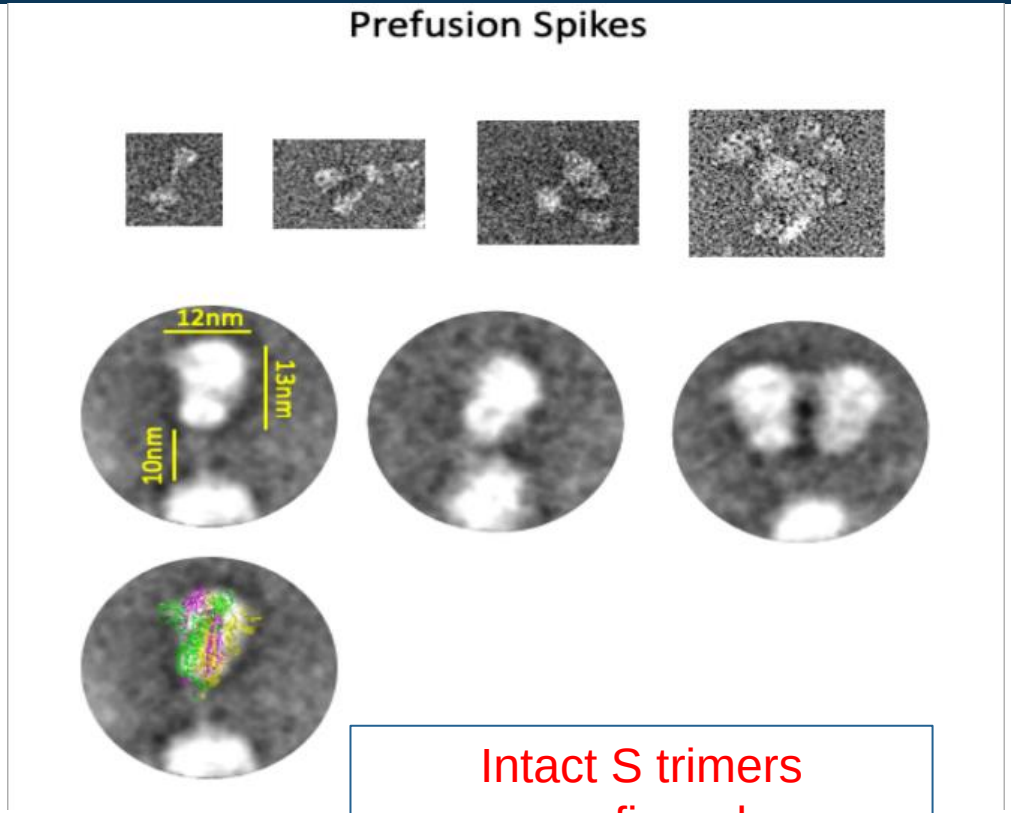
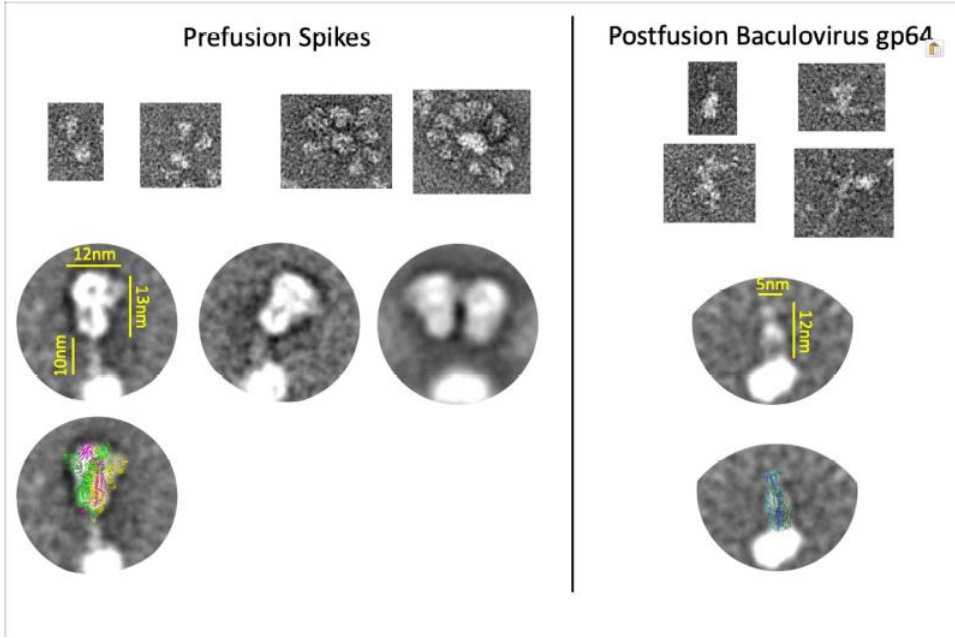
TEM Images of 50L lot: Observation consistent with literature



Intact S trimers confirmed

TEM Images of 2000L lot: Observation consistent with literature



Intact S trimers confirmed

gp64 spikes

Typical TEM Field for 2000L lot

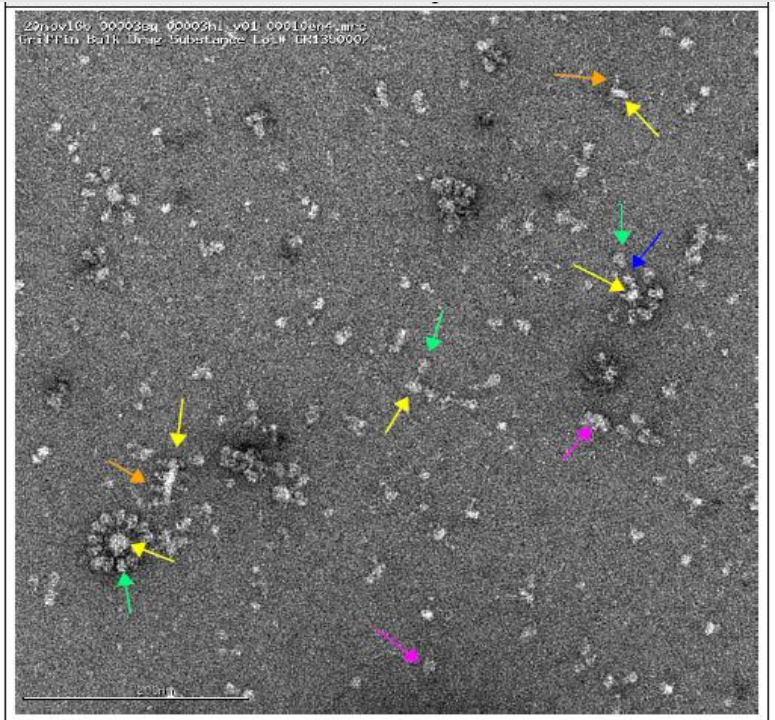


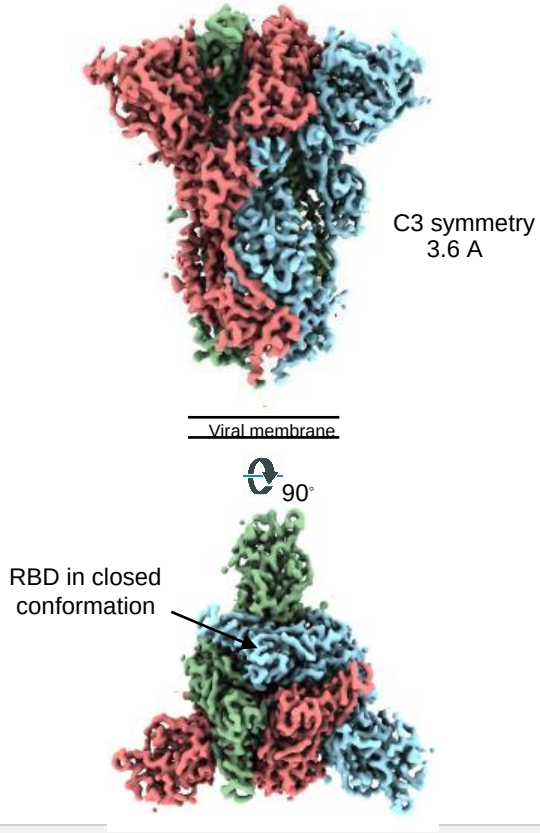
Figure 3c: Observed in the sample at a magnification of 67,000x are: lightbulb shaped particles consistent with the expected size and shape of the SARS CoV-2 prefusion spike protein (green arrows) that were associated with core particles of variable shape and size (yellow arrows). These spike proteins appeared to be attached via a narrow protrusion (blue arrow). Single spike trimers or multiple trimers could be attached to these core particles. Also observed were short, narrow assemblies (orange arrows) associated with the core particles (yellow arrows). Again, one or multiple of these narrow assemblies could be associated with the variable particles. Additional, unidentifiable material was present in the sample (magenta arrows).

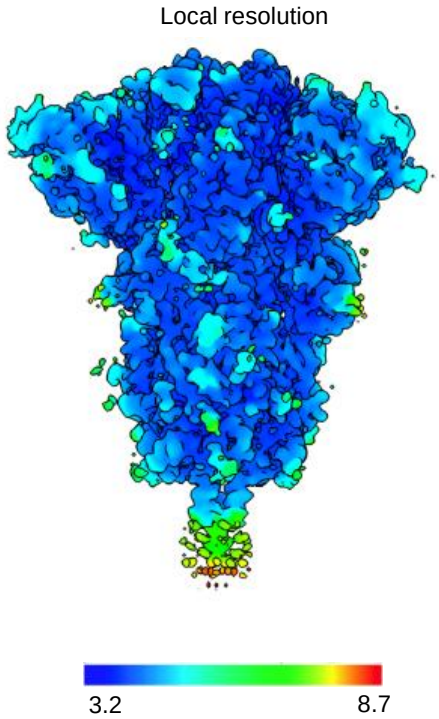
As expected, observe:

- Intact trimers
- Dimers of trimers
- Trimers of trimers
- Rosettes of trimers

NVX- CoV2373 S High Resolution Prefusion Trimer Structure

Cryo-EM S 3.4 Å atomic model S are trimers in prefusion RBD-down conformation





Bangaru, Ward et al. *Science*, Oct 2020. “NVX-CoV2372 is stable, homogeneous, and locked in the antigenically preferred prefusion conformation”

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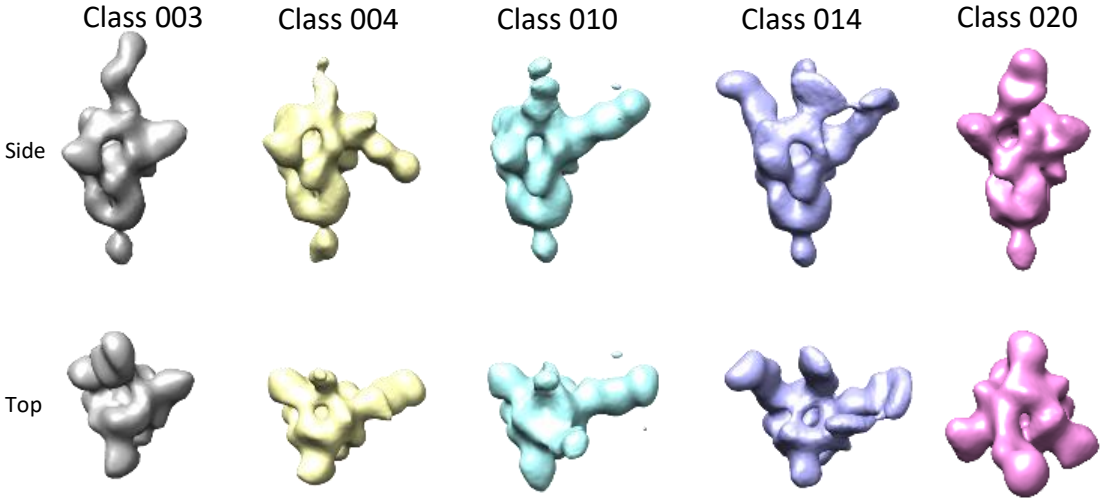
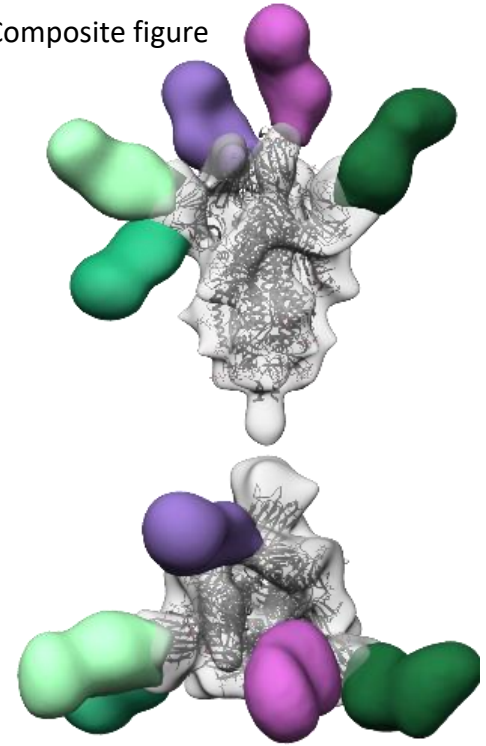
Scripps, Andrew Ward, et al: Polyclonal Fabs reveal 3 different NTD specificities and 2 different RBD specificities NVX-CoV2373 NHP immune sera

Refined Classes



Composite figure



Fitted with 6VYB

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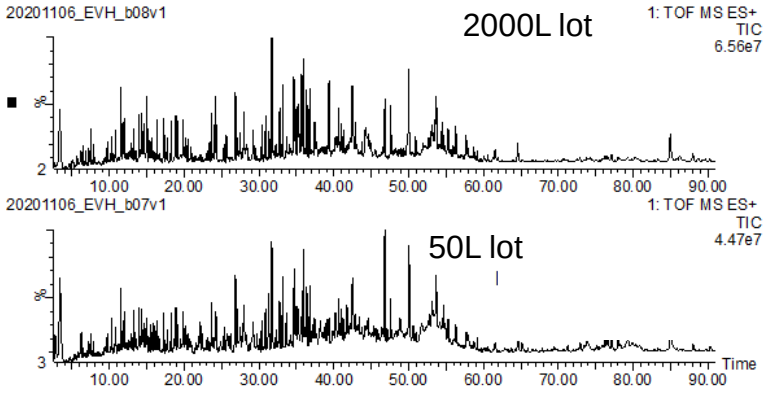
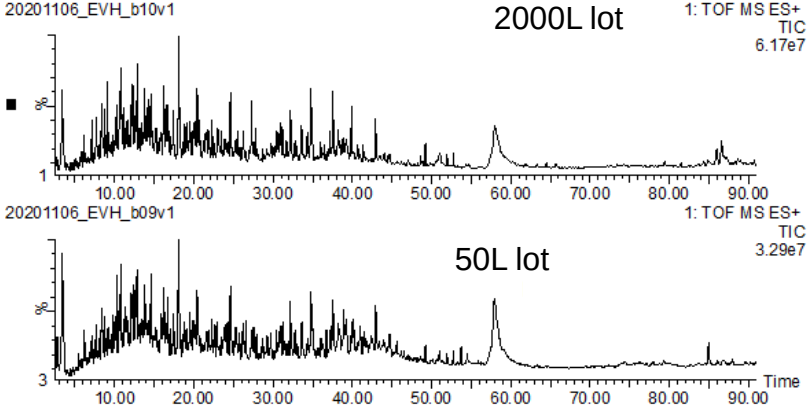
Single-particle CryoEM polyclonal epitope mapping: 2 RBD and 3 NTD epitopes identified in NVX-CoV2373 vaccine immune NHP sera

Peptide Mapping: Sequence Coverage

Lys-C/Trypsin Digestion TICs



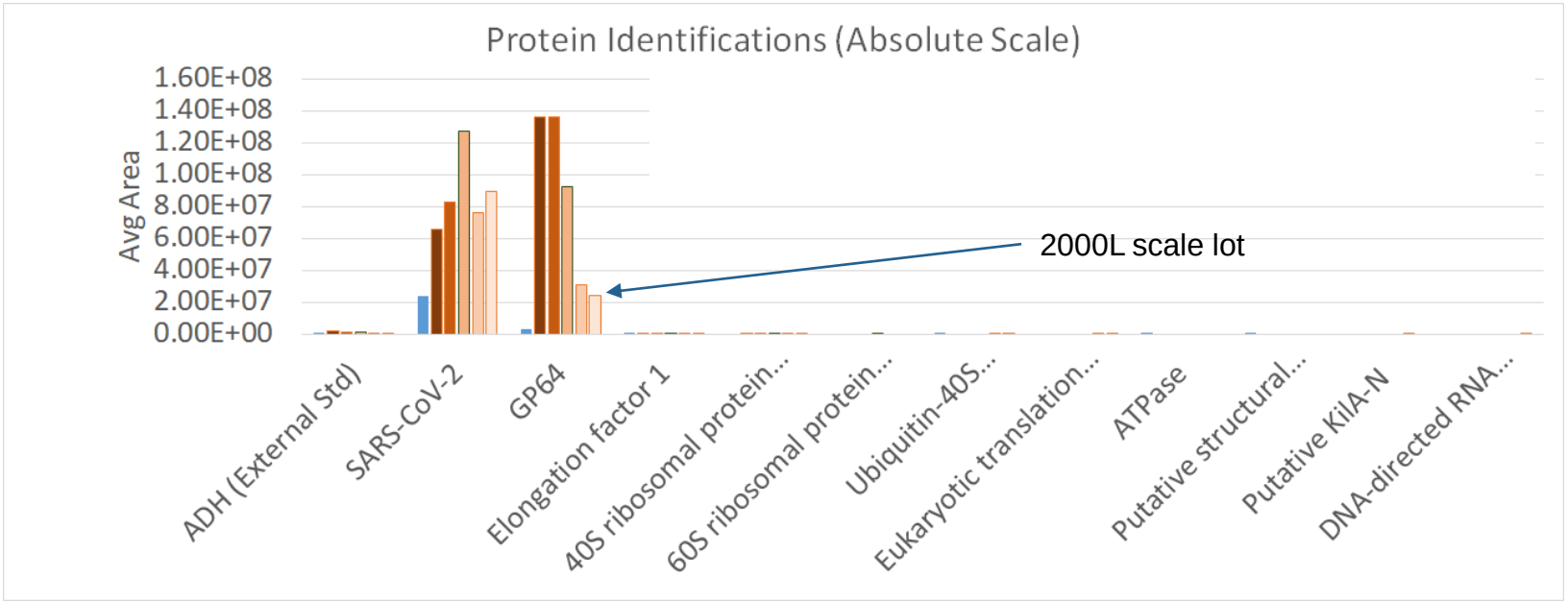
Chymotrypsin Digestion TICs



Overall Sequence Coverage

Lot	Expected Residues	Covered Residues	Sequence Coverage (%)
50L lot	1273	1198	94.1
2000L lot	1273	1208	94.9

Host Cell Protein-Mass Spectrometry



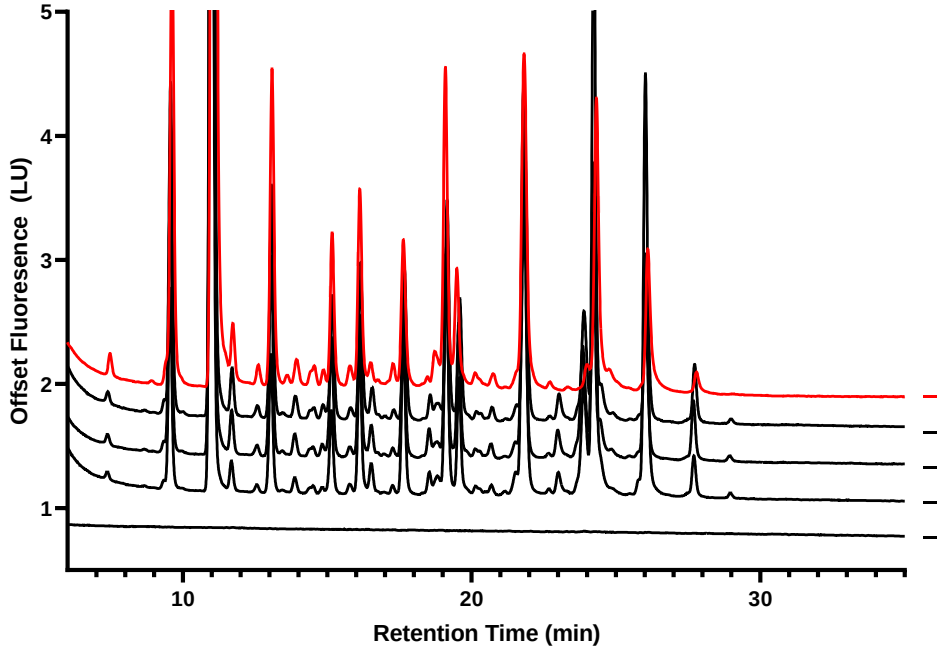
- gp64 is greater than 99.95% of all impurities
- Remainder are trace amounts of Sf9 proteins

Host Cell/Viral Protein Impurities in Site 1 and Site 2 batches

Sample	Lots	Relative gp64 (HCP) Impurity (%)*	Relative Other HCPs Impurities (%)*
Site 1	50L lot 1	98.35	1.65
	50L lot 2	96.10	3.90
	50L lot 3	96.26	3.74
Site 2	2000L lot 1	99.56	0.44

*% of total impurities by weight

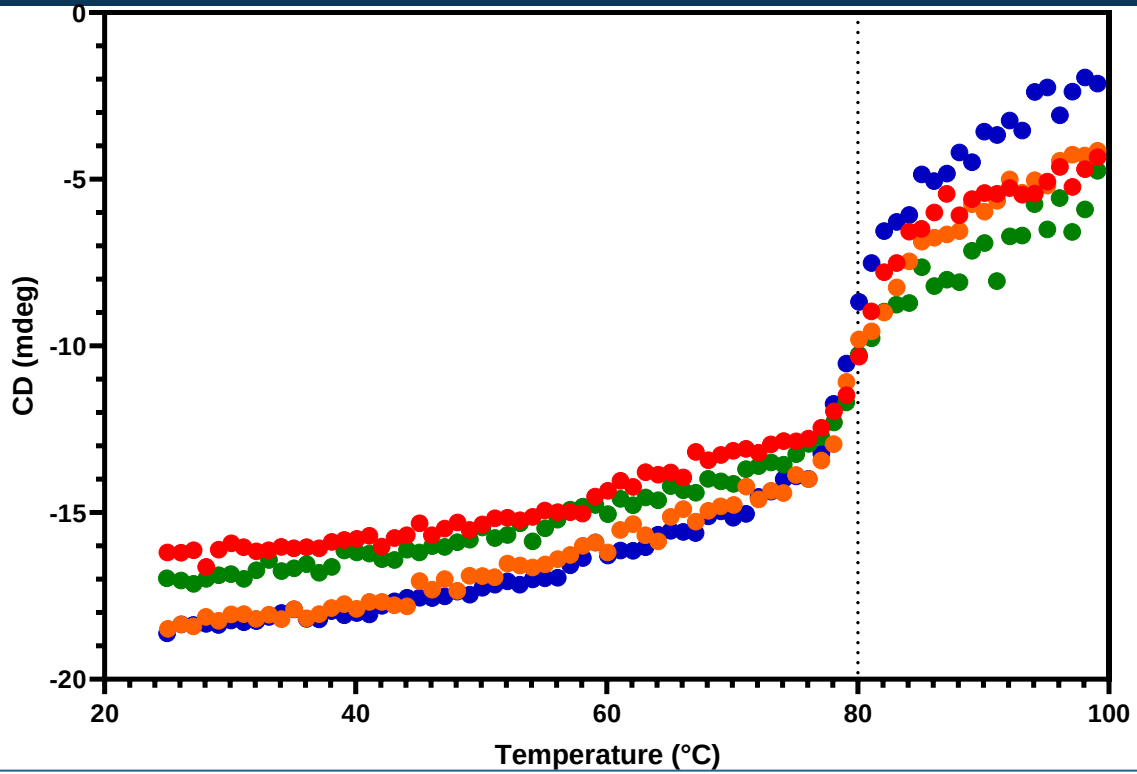
Oligosaccharide Profile Overlays



- Predominantly high mannose and pauci-mannose structures, as expected for insect cell lines

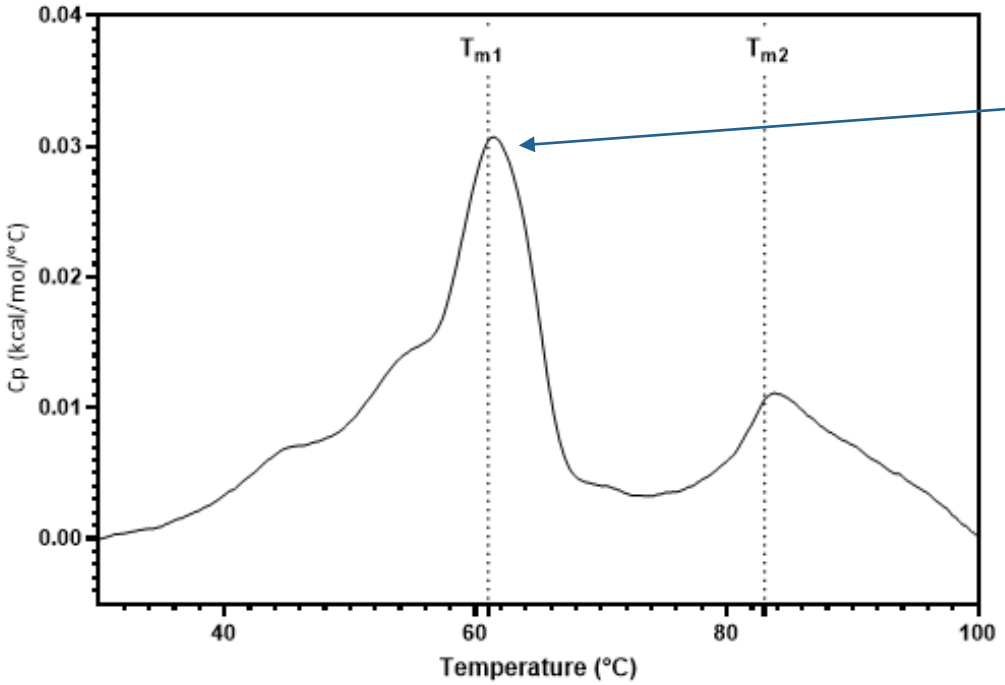
50L and 2000L lots show consistent oligosaccharide profiles; important for proper folding

Far UV CD with Thermal Denaturation



50L and 2000L lots are highly stable (thermal transition ~80ºC)

Representative DSC Thermogram of 2000L Lot



Tm1 consistent with published values for rS trimers

Consistent with properly folded thermostable trimeric spike protein

Purity of rS by Mass Spectrometry

Lot	Summed MS Intensities of Matched Peptides (counts)		Relative Mass Spectral Intensity (%) (molar ratio)		Converted Relative Mass Abundance (%) (mass ratio)	
	gp64	rS	gp64	rS	gp64	rS
50L lot 1	233292	1828579	11.3	88.7	4.6	95.4
50L lot 2	156938	1148295	12.0	88.0	4.9	95.1
50L lot 3	194285	785503	19.8	80.2	8.6	91.4
2000L lot 1	12609048	20085207	38.6	61.4	19.2	80.8

Estimated MW of gp64: 62011 g/mol

Estimated MW of rS: 163997 g/mol

- Purity of 50L batches is similar to 2000L batch (all within specifications)
- Only impurity detected is gp64 (baculovirus spike protein) for which there is substantial prior knowledge

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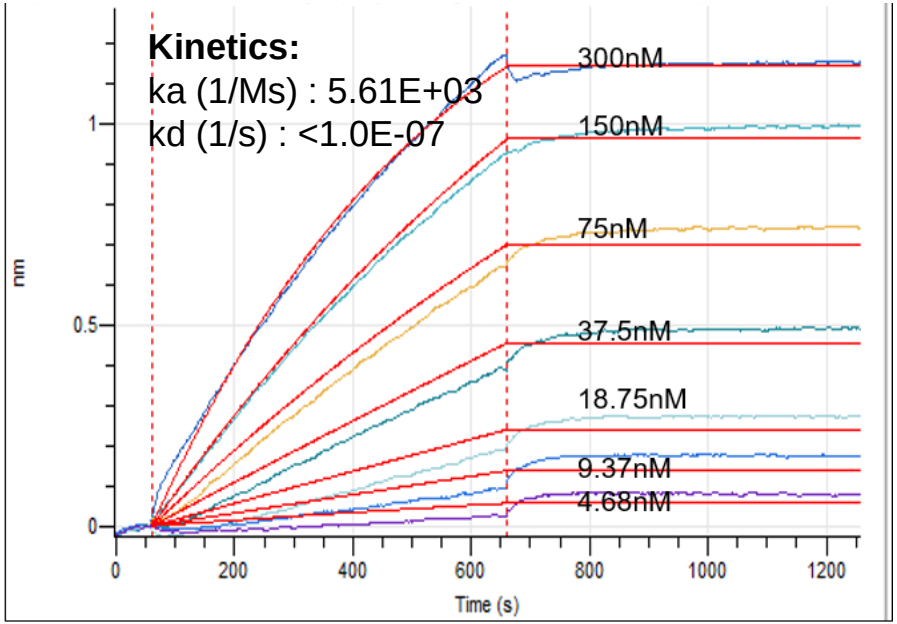
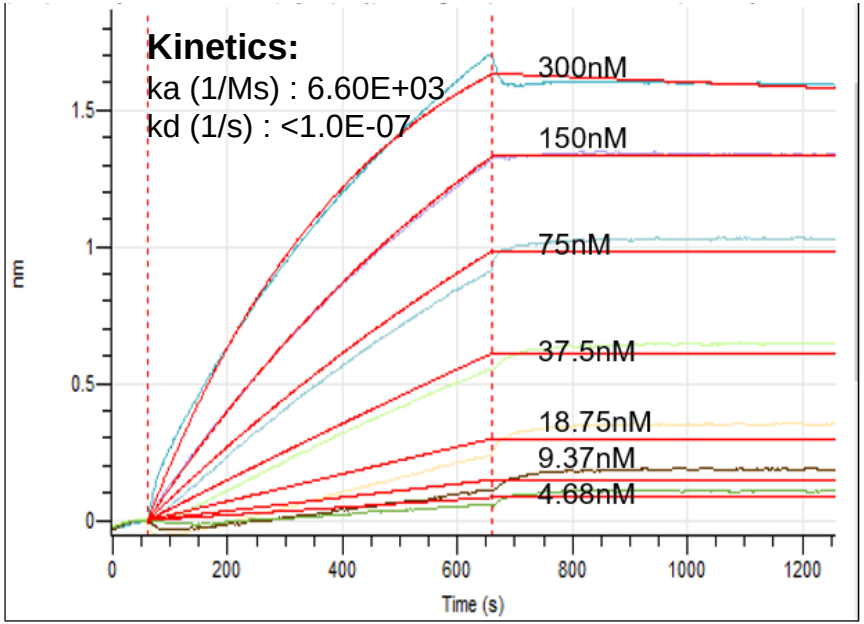
Octet hACE2 Receptor Binding Kinetics

50L lot



2000L lot



Comparable high affinity between 50L and 2000L batches with no measurable disassociation

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Stressed DS Stability Study

- 40°C: 0, 1, 2, 3, 4, 6, 8 **weeks**
- Stability assays
  - Appearance
  - pH
  - Total protein
  - SDS-PAGE
  - Potency ELISA
- Acceptance criteria: Degradation profile consistent with RS

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Comparability Criteria for DP

Test Method	Classification	Quality Attribute	Acceptance Criteria
Appearance	Release	Color, Clarity, Visible Particles	
pH	Release	Physicochemical	
Osmolality	Release	Physicochemical	
Total Protein (CBQCA)	Release	Quantity	
SARS-CoV-2 rS Binding ELISA	Release	Potency / Identity	
α-rS Western Blot	Release	Identity / Product Variants	
Matrix-A Content (rpHPLC)	Release	Excipient Quantity	
Matrix-C Content (rpHPLC)	Release	Excipient Quantity	

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Supplementary DP Characterization

- TEM with 2D classification
- PC
- Cholesterol
- Saponin integrity
- Additional methods under evaluation
  - DLS
  - AUC
  - MFI
  - AF4-MALS

<u>Proposal for Initial Expedited Approvals</u>: Perform 3 x 1 comparisons and provide data to EMA as soon as available.

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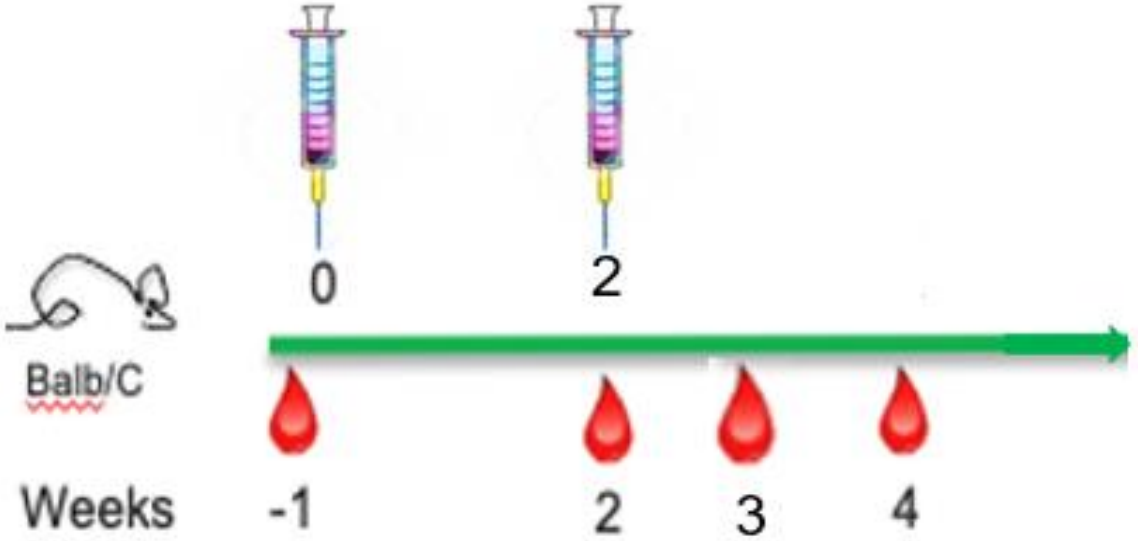
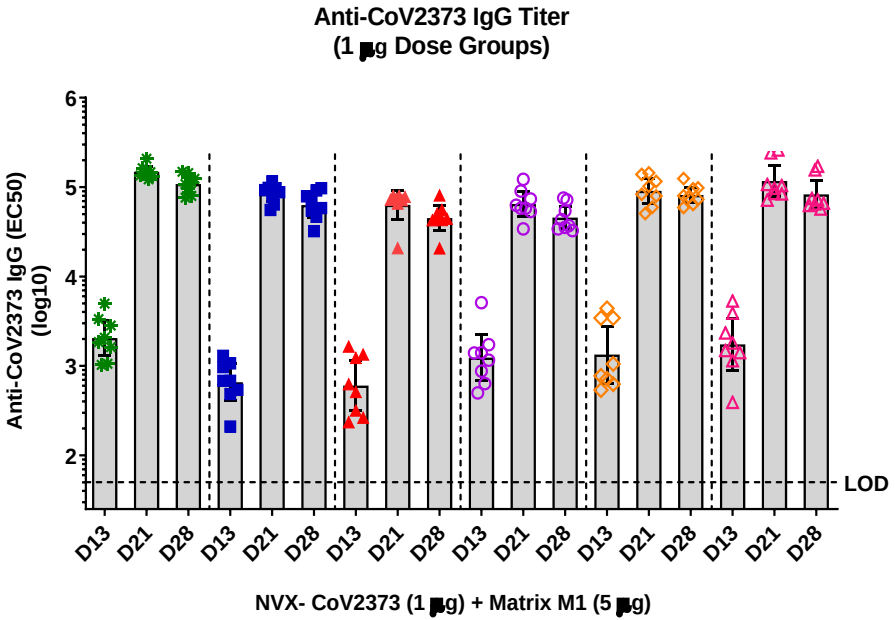
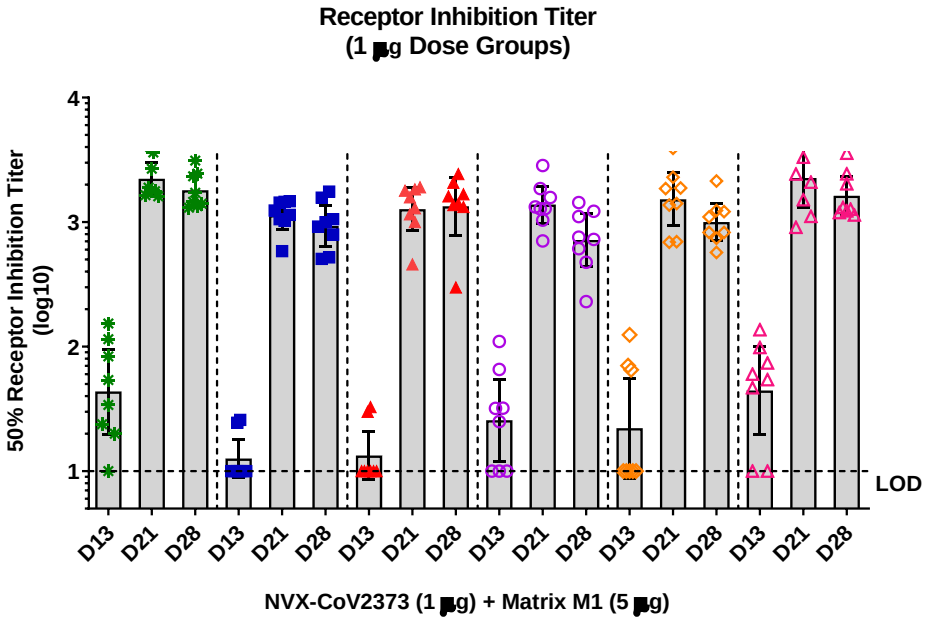
GMP Material Mouse Immunogenicity Study

Dose : 1 µg + 5 µg Matrix M



Mouse Immunogenicity: Anti-S IgG and Receptor Inhibition Titers





Stressed DP Stability Study

- 40°C: 0, 1, 2, 3, 4, 6, 8 **weeks**
- Stability assays
  - Appearance
  - pH
  - Total protein
  - Western blot
  - Potency ELISA
- Acceptance criteria: Degradation profile consistent with RS

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Step 1: 50 L to 2 KL

- Lot release + initial characterization tests for all batches prior to start of PPQ
- All batches meet lot release; characterization tests are “consistent with Reference Standard”
- Accelerated stability comparison; degradation profiles are consistent
- Provides the model for other CMOs
- Continue to collect other characterization data to build knowledge
- Approach for the initial batches submitted to Health Authorities; formal comparability protocol implemented prior to start of PPQ

Step 2: DP site 1 to DP site 2

- Lot release + characterization tests
- Accelerated stability comparisons of rate of degradation
- Formal QA-approved protocol to be discussed with Health Authorities

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Step 3: DP Site 2 to Other DP sites

- Lot release + characterization tests
- Acceptance criteria based on experience with Step 2
- Accelerated stability comparisons of rate of degradation
- Formal QA-approved protocol to be discussed with Health Authorities

Summary

- The data demonstrate that NVX Cov-2373 is a full length, **conformationally correct trimeric rS protein**
- 50L and 2000L products are comparable, based on the data from physicochemical and biological assays
- Full battery of tests will continue to be performed on each lot manufactured
  - Physicochemical assays
    - TEM, CD with thermal denaturation, DSC, Peptide mapping with MS, Oligosaccharide profile
  - Biological assays
    - ACE2 binding ELISA, ACE2 Octet binding kinetics, Mouse immunogenicity

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