

6-15-2016

Improving global human health through norovirus virus-like particle manufacturing

Scot Shepard
Takeda Vaccines, USA

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Recommended Citation

Scot Shepard, "Improving global human health through norovirus virus-like particle manufacturing" in "Vaccine Technology VI", Laura Palomares, UNAM, Mexico Manon Cox, Protein Sciences Corporation, USA Tarit Mukhopadhyay, University College London, UK Nathalie Garçon, BIOASTER Technology Research Institute, FR Eds, ECI Symposium Series, (2016). http://dc.engconfintl.org/vaccine_vi/25

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A black and white photograph of dandelion seed heads. One large seed head is in the foreground on the left, with many seeds blowing away. Other smaller seed heads are scattered across the background against a light, hazy sky.

Improving Global Human Health through Norovirus Virus-like-particle Manufacturing

Vaccine Operations, Vaccines Business Unit

Presentation for Vaccine Technology VI, Albufeira Portugal (June 2016)

Scot Shepard, Director, Biologics Process Design



Improving Global Human Health through Norovirus Virus-like Particle Manufacturing

- 1 Background information and Norovirus Illness
- 2 Design and Manufacturing of Norovirus Virus-like Particles
- 3 Manufacturing Scalability and Reproducibility
- 4 Summary

Takeda is a Leading Global Pharmaceutical Company



Takeda Pharmaceutical Company Limited

- Takeda is a research-based global pharmaceutical company founded in 1781 with global headquarters in Osaka, Japan.
- Takeda is the largest pharmaceutical company in Japan with operations in more than 70 markets worldwide and approximately 31,000 employees.
- For more than two centuries, Takeda has continued its mission to strive towards better health for patients worldwide through leading innovation in therapeutic and preventative medicine.



Chobei Takeda I

Why is Takeda Pursuing a Global Vaccine Business?



...because it is high-impact, focused on prevention, and driven by innovation

“The most successful and cost-effective health care intervention, second only to clean drinking water”

“Vaccines prevent 3-4M deaths / year”

“New vaccines / better global use of existing vaccines could prevent another 4-7M deaths”

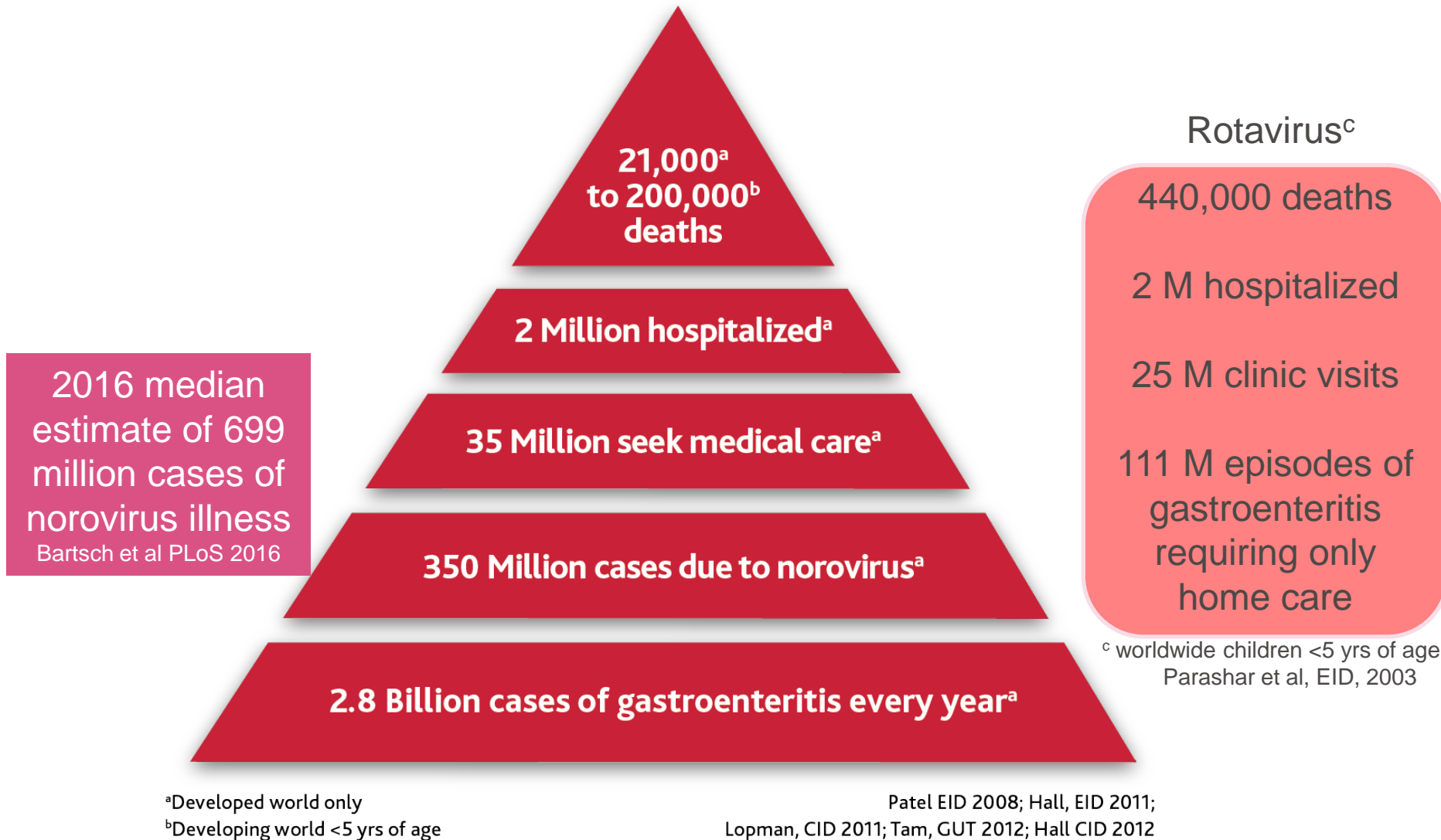
“The single most important tool to reach the 2000 UN Millennium Development Goals”

“We must make this the Decade of Vaccines”



Quotes from Gro Brundland, Jeffrey Sachs, Kofi Annan, Julio Frenck, Bill Gates and others

Noroviruses are currently recognized as the leading cause of acute gastroenteritis (AGE) worldwide



According to the CDC, *“The perfect human pathogen”*

Norovirus – “The Perfect Human Pathogen”



- Genetically diverse and environmentally stable
 - Family Caliciviridae; RNA genome of ~7,500 nucleotides
 - High mutation and recombination rates → high genetic variability
 - Non-enveloped virus with one major capsid protein
 - Survives heating and freezing, remains viable on dry surfaces for weeks

- Highly contagious
 - As few as 18 particles can cause illness
 - Transmission by fecal-oral and aerosolized routes
 - Ingestion of contaminated foods or water
 - Handling of contaminated objects

- Prolifically shed
 - Up to 5 billion doses of infectious virus can be shed by an ill person over the course of a few weeks, most of which is an asymptomatic period

Norovirus –The Burden of Disease¹



- One-fifth of all diarrhea cases globally
- Greater than 200,000 deaths annually in kids < 5 years of age living in developing countries
- Principle cause of foodborne disease outbreaks in the United States
- A key health care-acquired infection
- A common cause of travel-associated diarrhea

¹ Lopman, et al. (2016) PLoS Med 13(4)



Disruption due to Norovirus



- The norovirus disease burden is substantial, often causing extensive and wide-ranging disruption ¹⁻³
- Settings where norovirus infection is **particularly disruptive** ^{2,3}



Care institutions

- Long-term health care facilities, nursing homes
- Hospitals: ER, in- & outpatients
- Immuno-compromised



Other institutions

- Nursery, school
- Prisons
- Refugees (e.g. hurricanes)



Community

- Restaurant, food outlet
- Party, events



Military

- Military camps
- Deployed soldiers



Travelers

- Cruise ships
- Air, train and bus travel

- The disruptive impact of norovirus-related disease is underestimated in outbreak data and national surveillance statistics ^{4,5}

1. Glass RI NEJM 2009; 2. CDC MMWR 2011; 3. Koo HL J Clin Microbiol 2010; 4. Tam CC Gut 2012; 5. Beersma MF J Clin Microbiol 2012

Global Economic Impact of Norovirus Illness¹



Model Simulation ¹	Annual Economic Burden (2013 USD) ¹
Direct Health System Costs ²	\$4.2 billion
Societal costs ³	
•Total	\$ 60.3 billion
•Children < 5 years	\$ 39.8 billion
Cost per illness varied by region and age and was highest among adults ≥ 55 years	

¹ Bartsch, SM et al (2016) PLoS ONE 11(4). Model simulation of 233 countries/ areas

² Outpatient visits and hospitalization – see reference (1)

³ Productivity losses due to absenteeism and mortality costs – see reference (1)



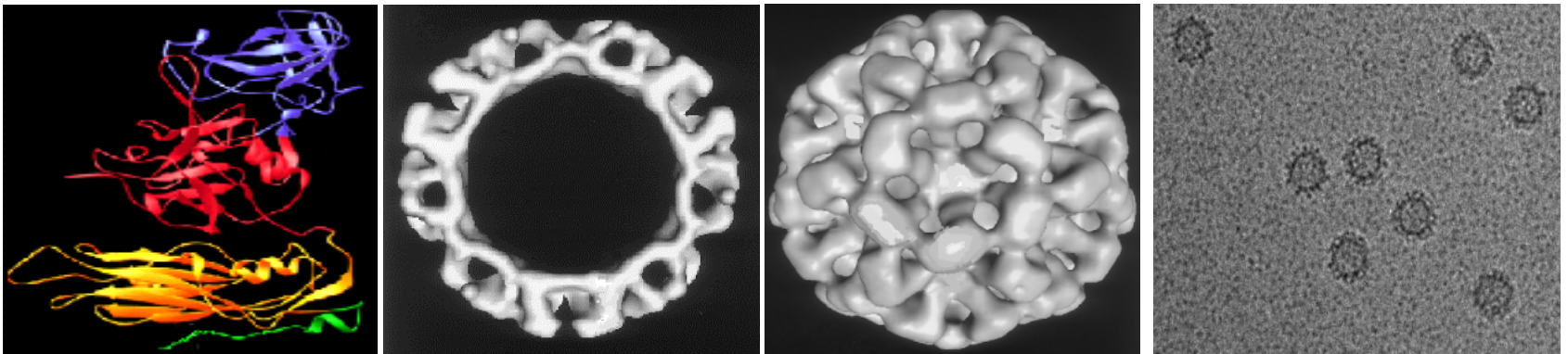
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Takeda's Norovirus Antigens are Virus-Like-Particles



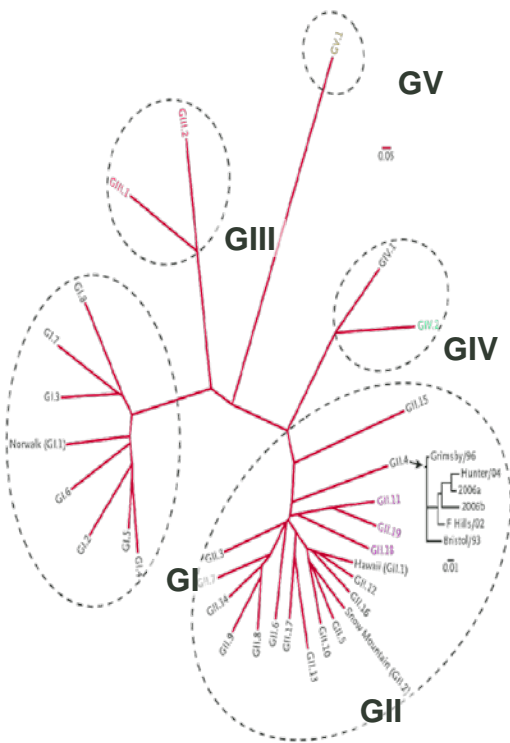
- VLPs are engineered and expressed using state-of-the-art recombinant protein technologies
- Stable, icosahedral T3 capsid assemblies (native conformation)
- Diameter of about 40 nanometers; Mass of about 10 mDa
- No viral genetic material, can not replicate and are thus non-infectious
- Expression level is high using a scalable manufacturing platform
- Well-behaved, stable during purification and long-term storage



Example of VLP Design – The GII.4 Consensus VLP

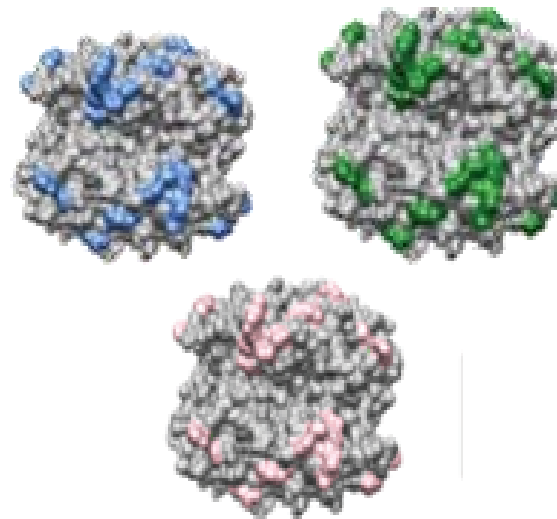


Noroviruses are classified into 7 genogroups and >25 human genotypes¹



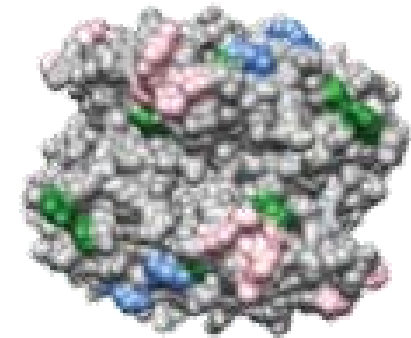
Norovirus Classification²

Different Norovirus genotypes contain largely conserved VP1 capsid protein structures



Molecular models of a conserved Norovirus structural protein that highlight amino acid differences. Courtesy of GI Para, NIH.

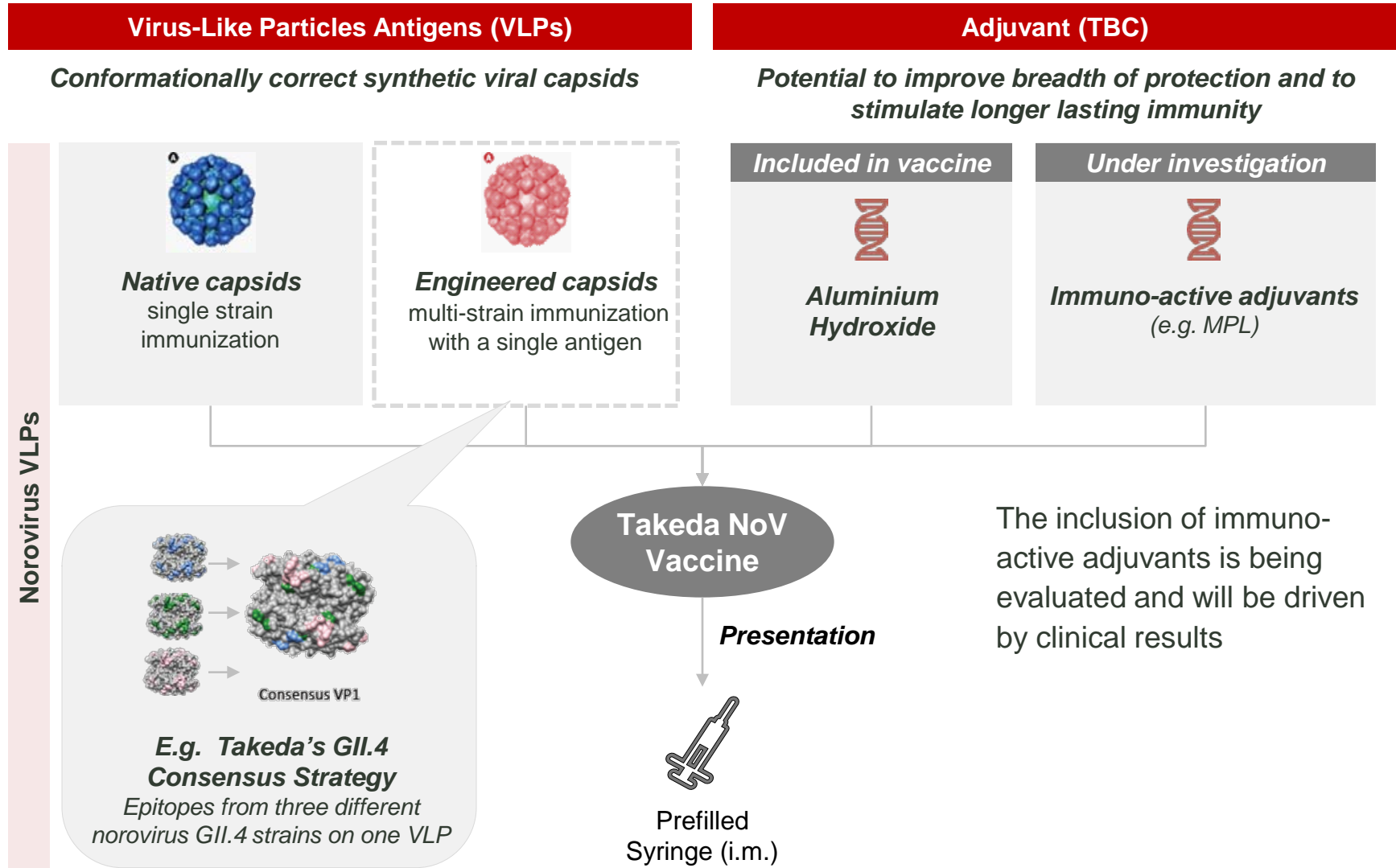
Multiple genotype-specific sequences are combined into a single synthetic VP1 capsid protein



Molecular model of a synthetic protein containing antigenic determinants from three Norovirus genotypes. Courtesy of GI Para, NIH.

¹Vinje J et al. J Clin Microbiol 2015; ² Glass et al., NEJM 2009;361:1776-85

Takeda's Norovirus Vaccine Approach





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Commercial-scale Manufacturing



- **Protein expression**
 - Common platform for all Norovirus VLPs
 - Small footprint, single-use stirred-tank reactor
 - Serum-free media, suspension culture
 - Standard BSL-1 / Grade D recombinant protein facility
- **Protein purification**
 - Chromatography and UF/DF steps performed with automated, disposable flow path skids
 - Bulk purified antigen stable long-term prior to Alum adsorption
 - VLPs characterized using contemporary protein analytical methods
- **Manufacturing operations**
 - Standard, multi-product, multi-host Mfg facility
 - VLP productivity supports global supply from one small-scale Mfg train
 - Drug product manufacturing successfully scaled to meet global demand



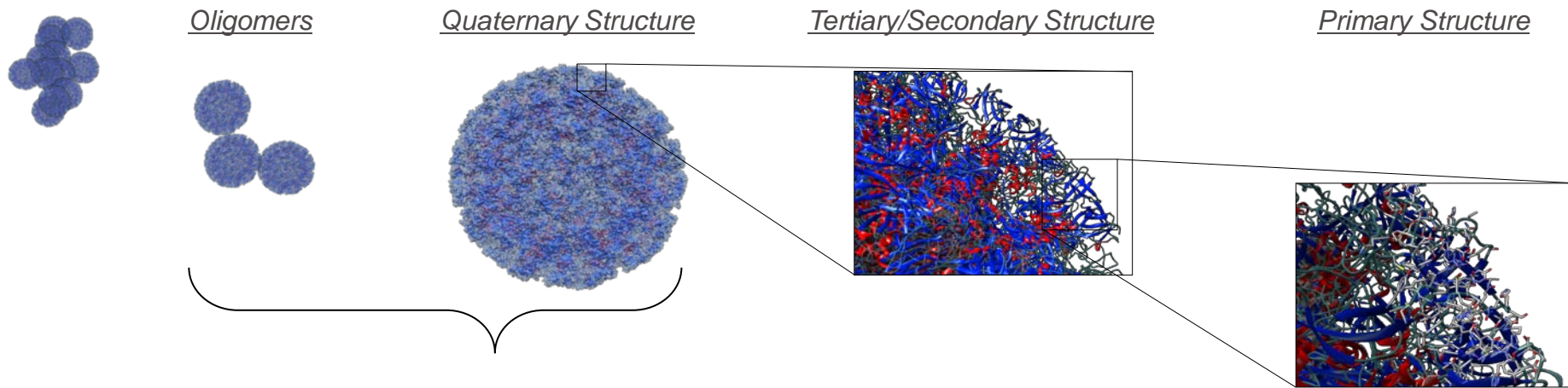
Representative VLP Purity Data



Attribute	Specification	Scale-Down Model (N=3)	Commercial Scale (N=4)
Product Purity Test 1	≥ 90%	100	100
Product Purity Test 2	≥ 90%	98	99
Host Impurity Test 1	≤ 10 µg/mg	≤ 0.52	< 0.001
Host Impurity Test 2	≤ 10 ng/mg	≤ 0.96	≤ 0.001
Host Impurity Test 3	≤ 10 ng/mg	≤ 0.063	≤ 0.001
Process Impurity Test 1	≤ 100 µg/mg	Not tested	< 0.14
Process Impurity Test 2	≤ 100 µg/mg	Not tested	< 0.02
Process Impurity Test 3	≤ 10 µg/mg	Not tested	< 0.24

- Commercial scale VLPs are highly purified; similar results for other VLPs
- Results from a 1/20th scale development model are comparable to full scale

VLP aggregates Amino acid sequence



Laser Diffraction
Electrophoretic mobility
Micro Flow Imaging
FFF-MALS

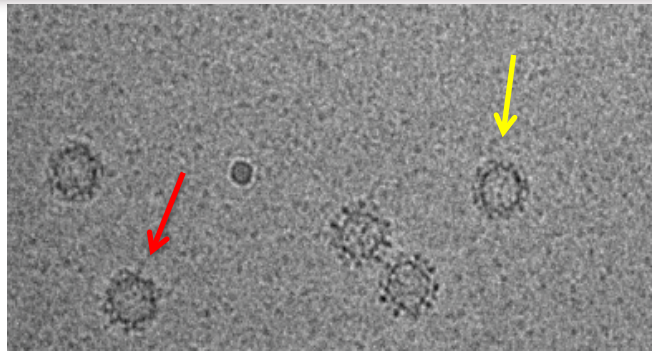
SEC-MALS
Batch MALS
DLS
Cryo-TEM

FTIR Spectroscopy
Fluorescence Spectroscopy
UV Spectroscopy
DSC

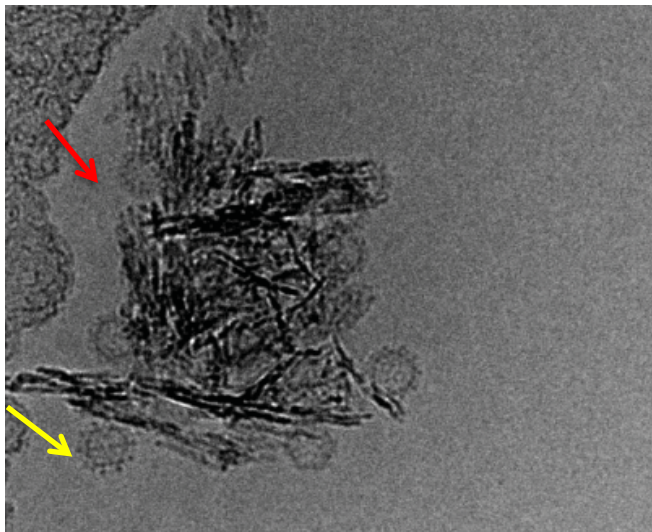
Mass Spectrometry



Cryo-TEM Micrograph Reconstructions

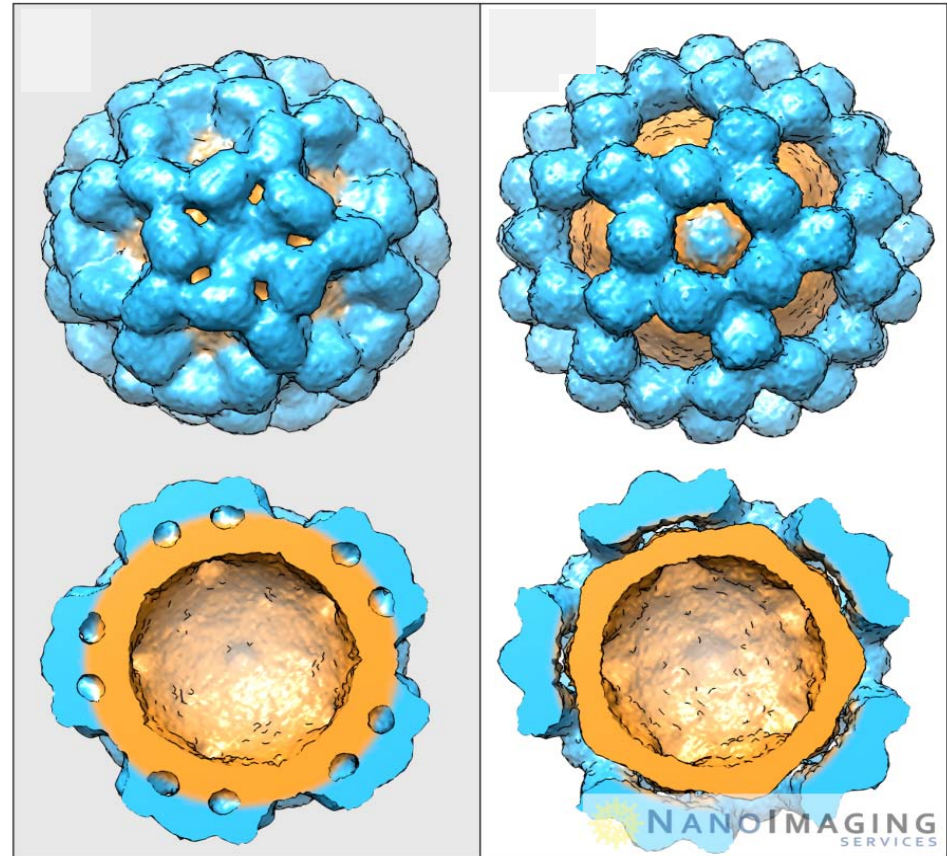


VLP 1 (red)
VLP 2 (yellow)



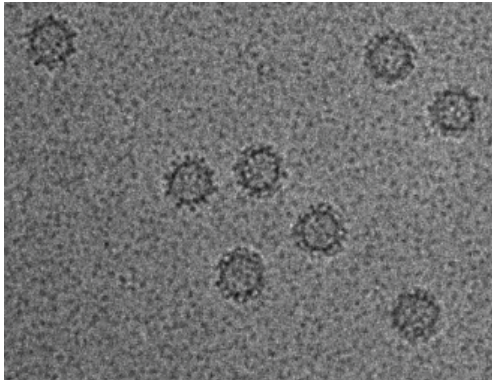
VLP 1 and VLP 2 adsorbed to adjuvant

Transmission Electron Microscopy can distinguish between VLPs with different primary structures

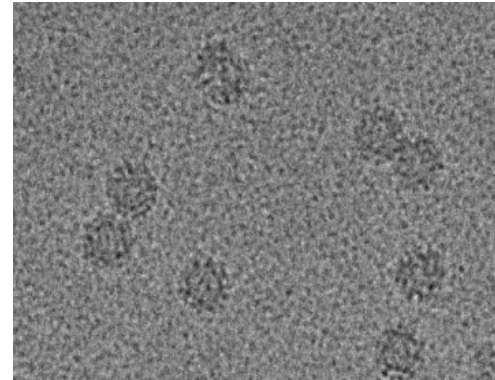


Reconstruction from TEM images showing differences between VLP 1 and VLP 2

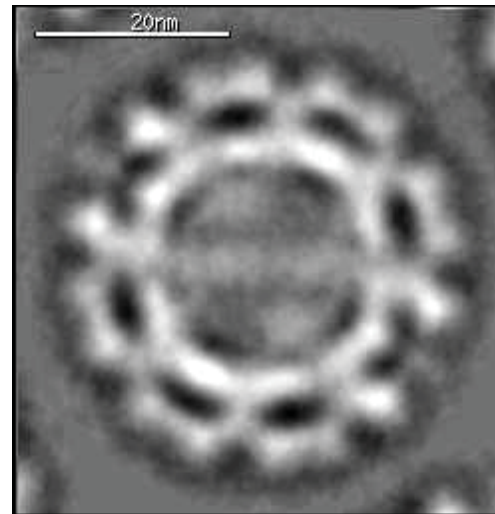
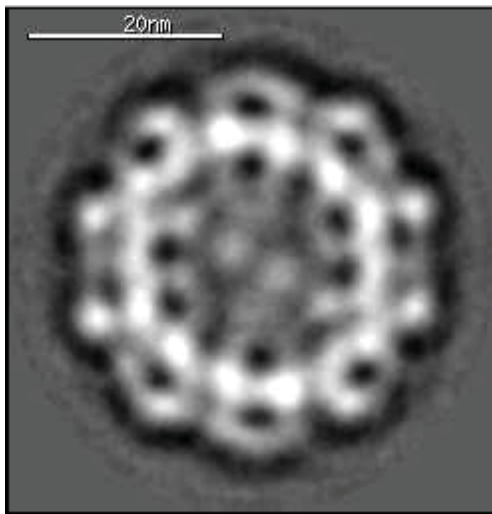
Cryo-TEM GI.1 and GII.4 VLPs



GI.1 VLP

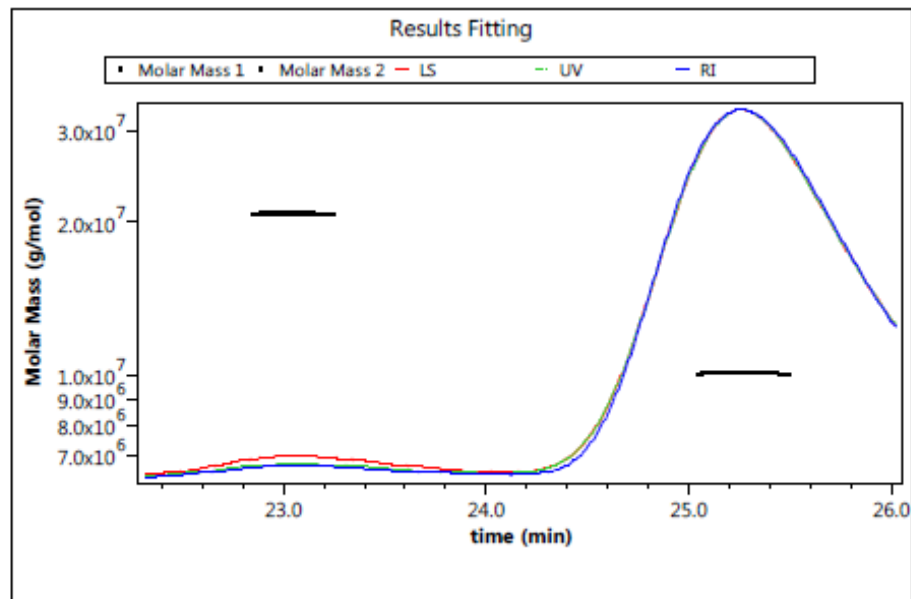
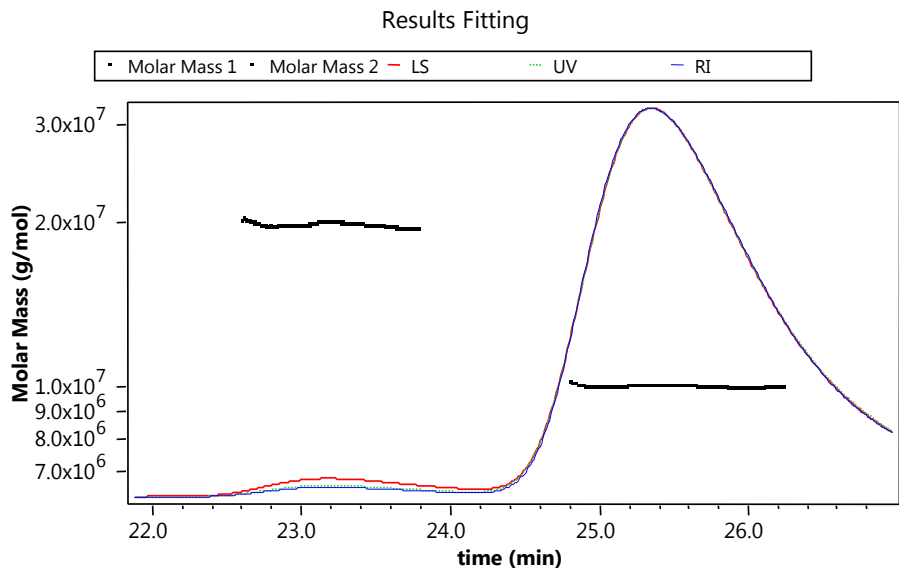


GII.4 VLP



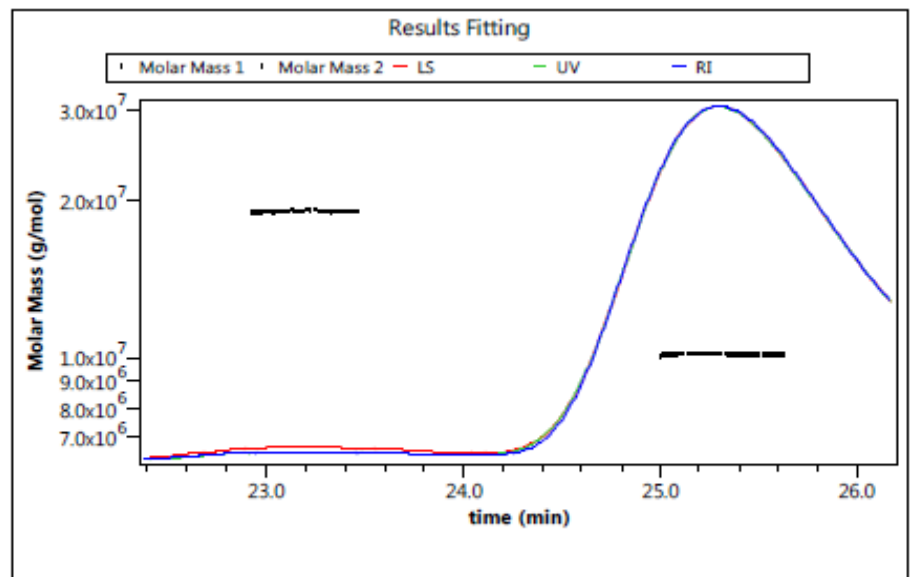
Dependent on vaccine-specific amino acid sequences, particle shapes may vary

Manufacturing Reproducibility: SEC-MALS¹



The majority of the nanoparticle population (97-98%) is monodisperse (10mDa) with 2-3% of the population existing in a dimeric state (20 mDa).

¹ SEC-MALS = size exclusion chromatography – multi angle light scattering



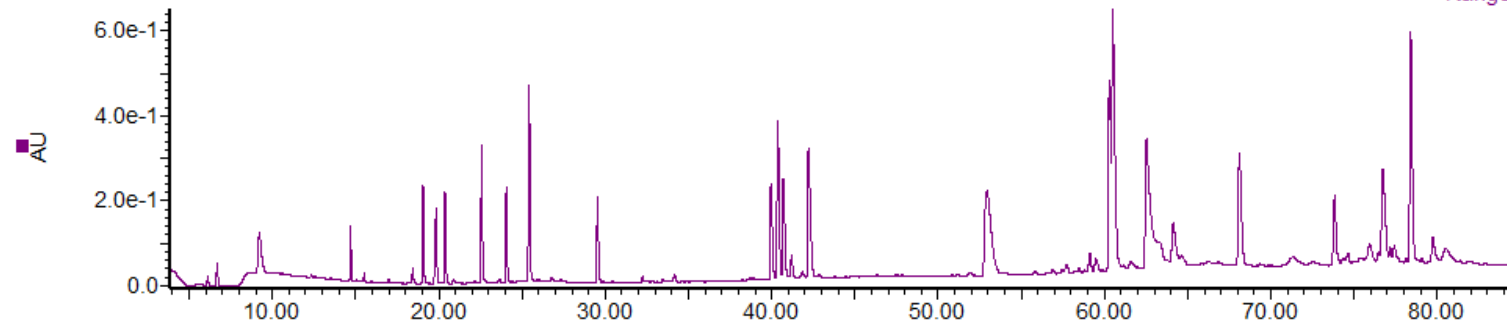
Manufacturing Reproducibility: Peptide mapping



02_Reference

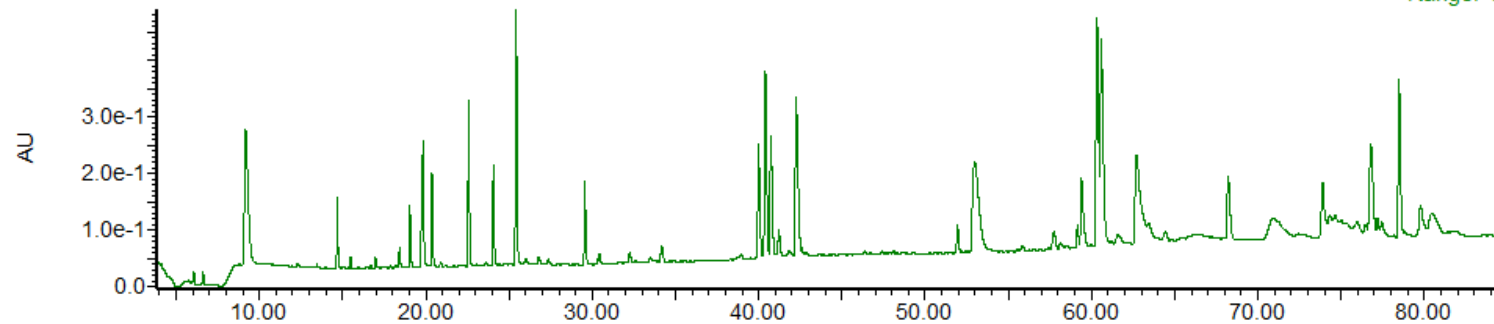
02_Reference

4: Diode Array
Range: 6.6e-1



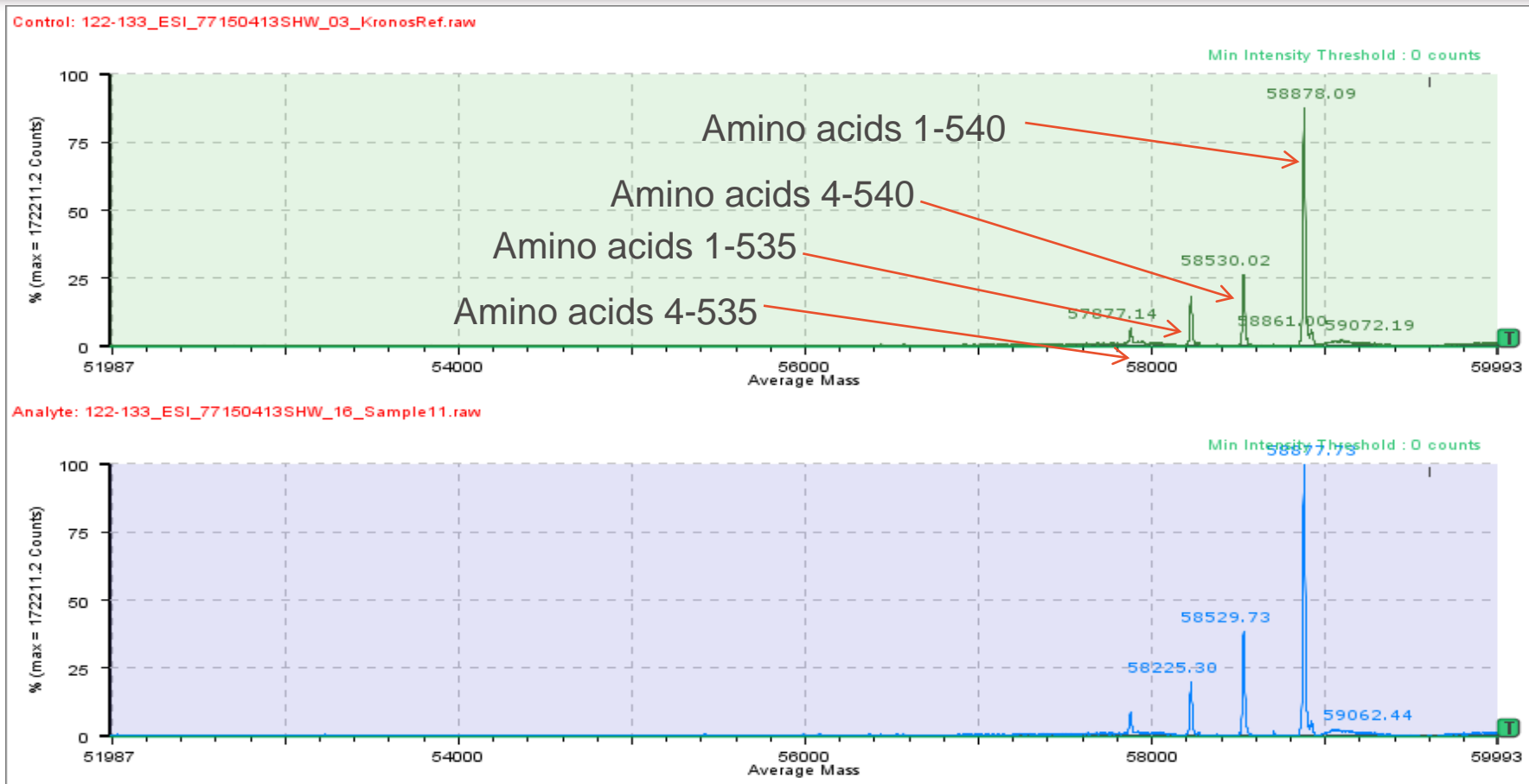
05_Gaia_Map_15GA5777001_5PPQ1Gaia

4: Diode Array
Range: 4.947e-1



When digested with a mixture of proteases and then analyzed by mass spectrometry, the resulting VLP “fingerprint” was reproducible demonstrating consistent protein sequence

Manufacturing Reproducibility: intact mass analysis



- The VLPs are composed of a reproducible distribution of four capsid protein species
- The species vary based on minor differences in the N- or C- terminal regions



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Summary: Takeda's Norovirus VLP Technology



- Norovirus is highly contagious and is the leading cause of acute gastroenteritis worldwide. There are hundreds of millions of cases of illness each year with up to 200,000 deaths in children < 5 years of age.
- Takeda's norovirus vaccine candidate is based on synthetic ~40 nm VLPs manufactured by a proprietary platform process that is highly portable and is designed to fit in standard recombinant protein facilities.
- Tens of millions of highly purified VLP dose equivalents have already been produced at commercial scale.
- VLPs have been characterized by a suite of advanced particle and protein analytical methods.
- Drug product manufacturing for multiple formulations is operating at commercial scale at a sustainable cost-of-goods.
- Takeda's VLP manufacturing platform can be applied to multiple norovirus strains and other VLP-based vaccine candidates.

Thank you



Vaccine Business Unit