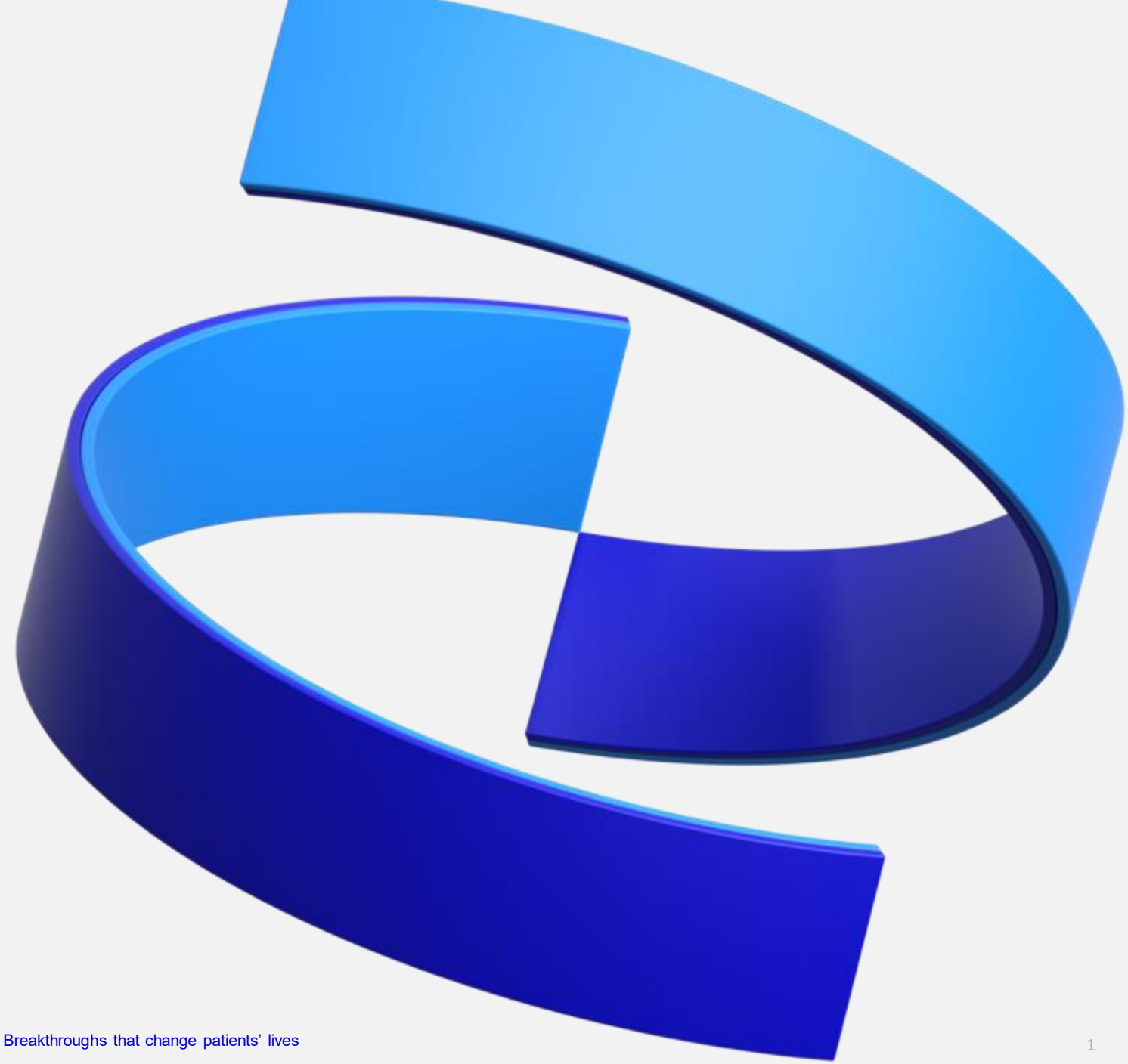
Enhanced Quality Attribute Understanding to Enable Accelerated Development of an RSV Vaccine



John Davis

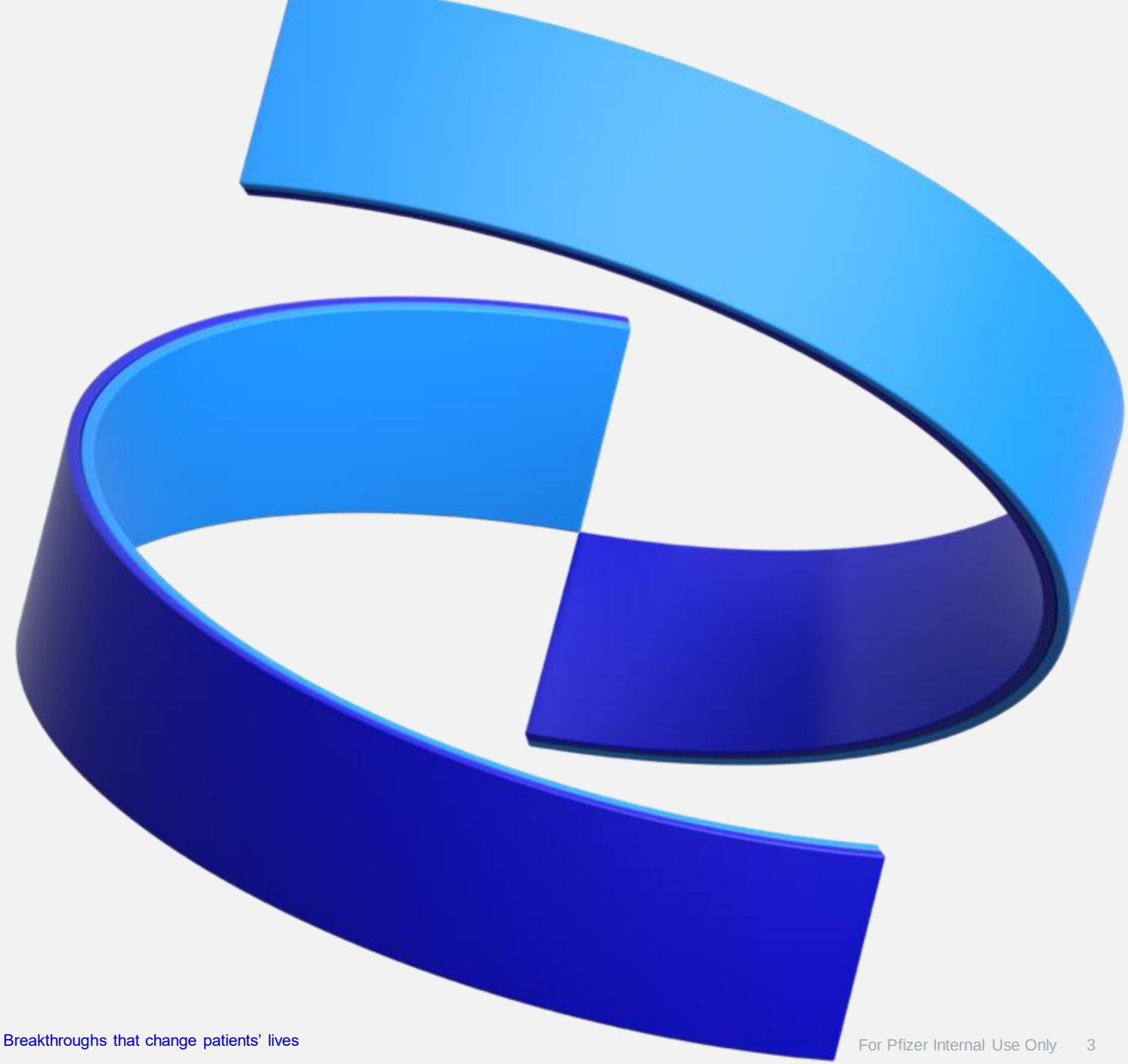
23Jan2024

Pfizer Research & Development

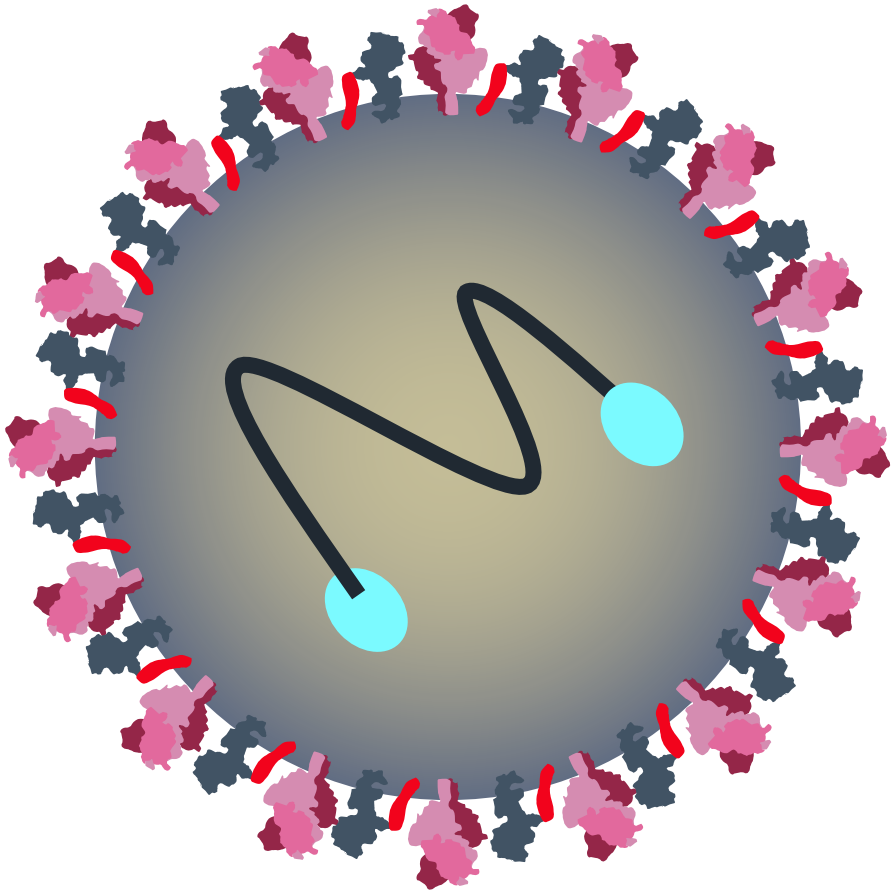
Agenda

- Respiratory Syncytial Virus
- Pfizer’s RSVpreF Subunit Vaccine
- A Case Study in Attribute, Process, and Analytical Understanding
- Summary and Conclusions

Respiratory Syncytial Virus



Respiratory Syncytial Virus



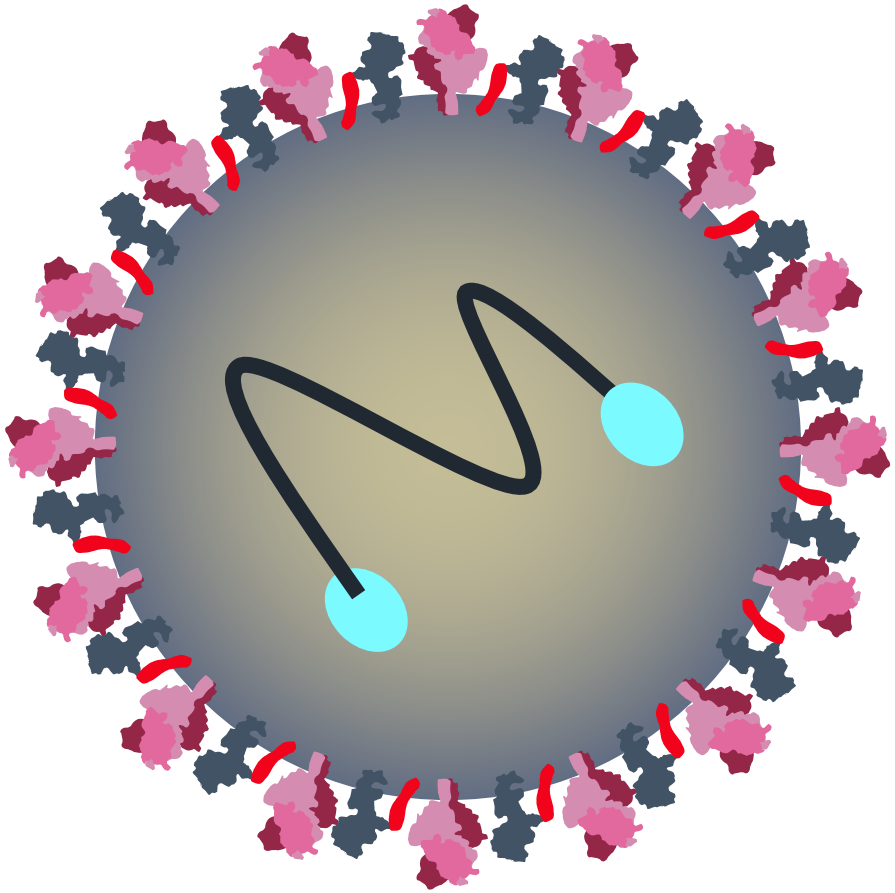
Respiratory syncytial virus (RSV) infection is a common cause of lower respiratory tract illness worldwide, with a risk of severe illness among infants, young children, and older adults.[1]

Second only to malaria as cause of death by a single pathogen in children < 1 year of age[2]

Two major antigenic subtypes of the virus (A & B) [2]

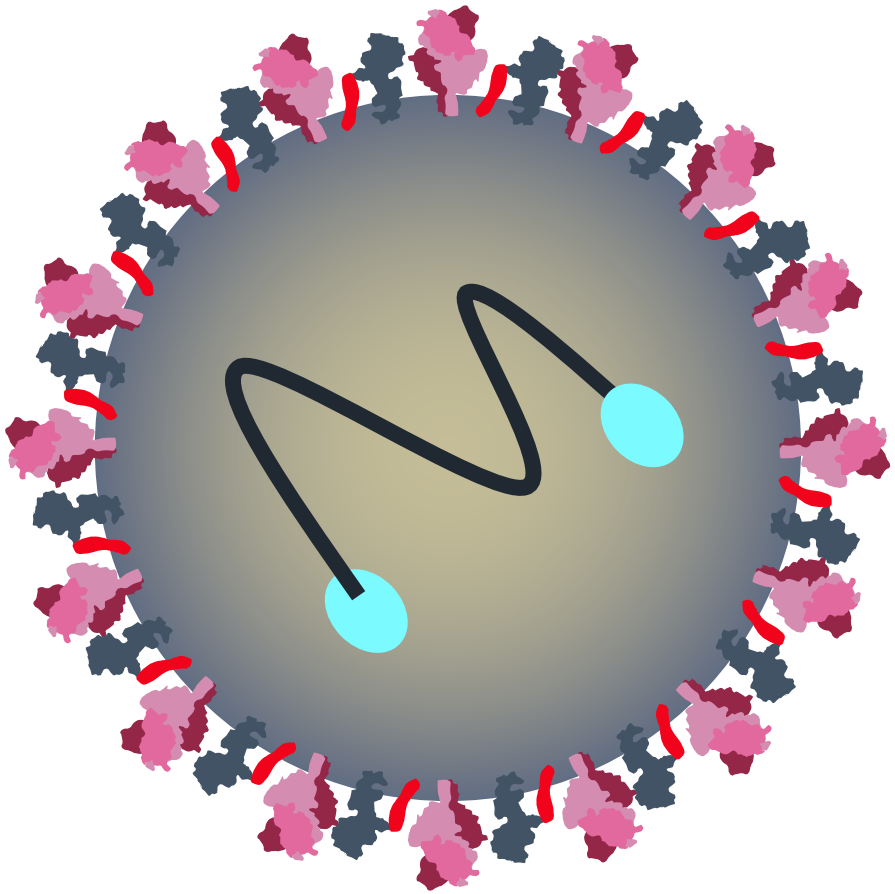
1: World Health Organization. Respiratory syncytial virus (RSV) disease. 2022 (https://www .who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/respiratory-syncytial-virus-disease).
2: Tian D, Battles MB, Moin SM, et al. Nature Communications **volume 8**, Article number: 1877 (2017)

Respiratory Syncytial Virus



Despite more than 50 years of development, no RSV vaccine had been licensed prior to 2023.

Respiratory Syncytial Virus

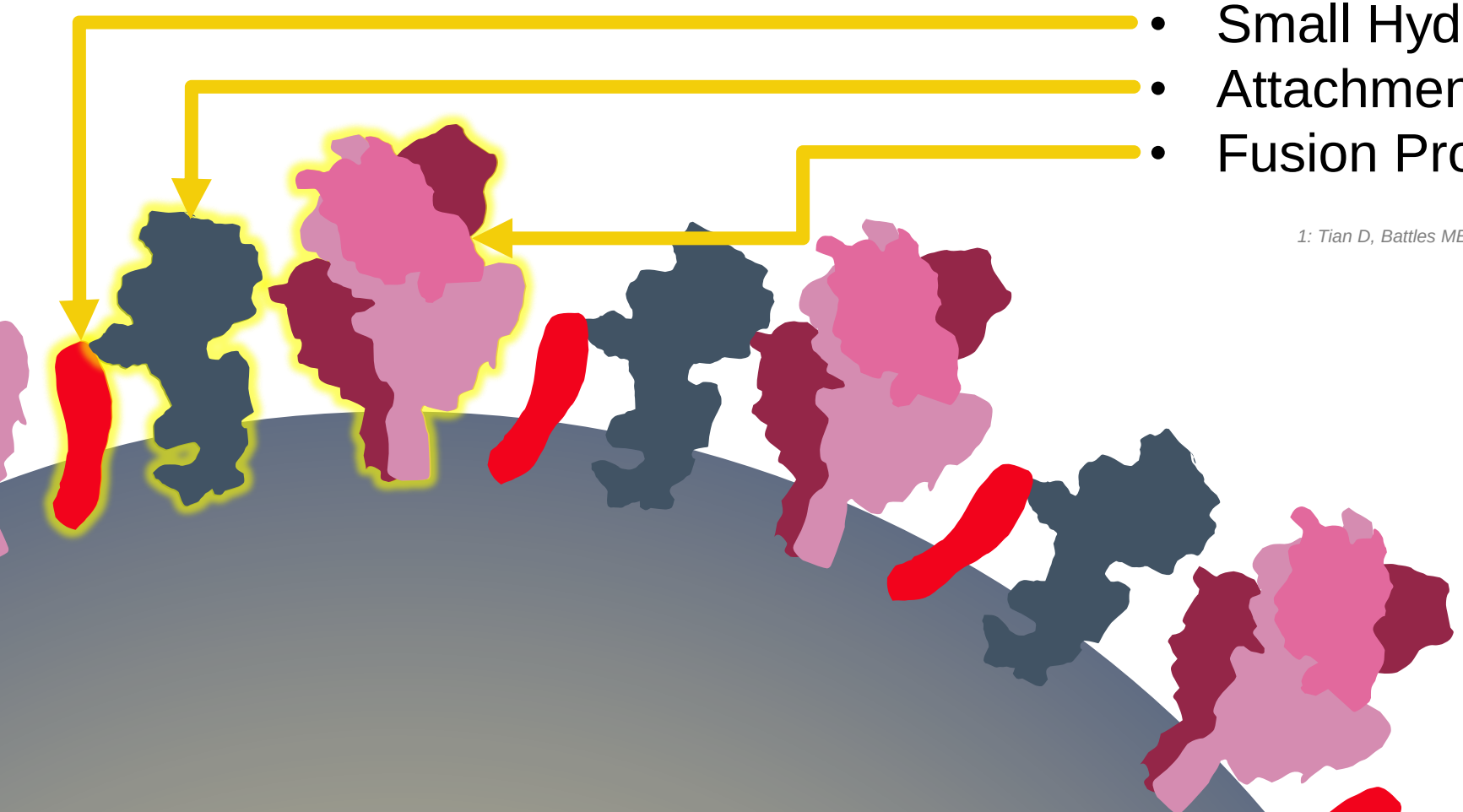


- Enveloped, negative-sense RNA virus[1]

1: Tian D, Battles MB, Moin SM, et al. Nature Communications **volume 8**, Article number: 1877 (2017)

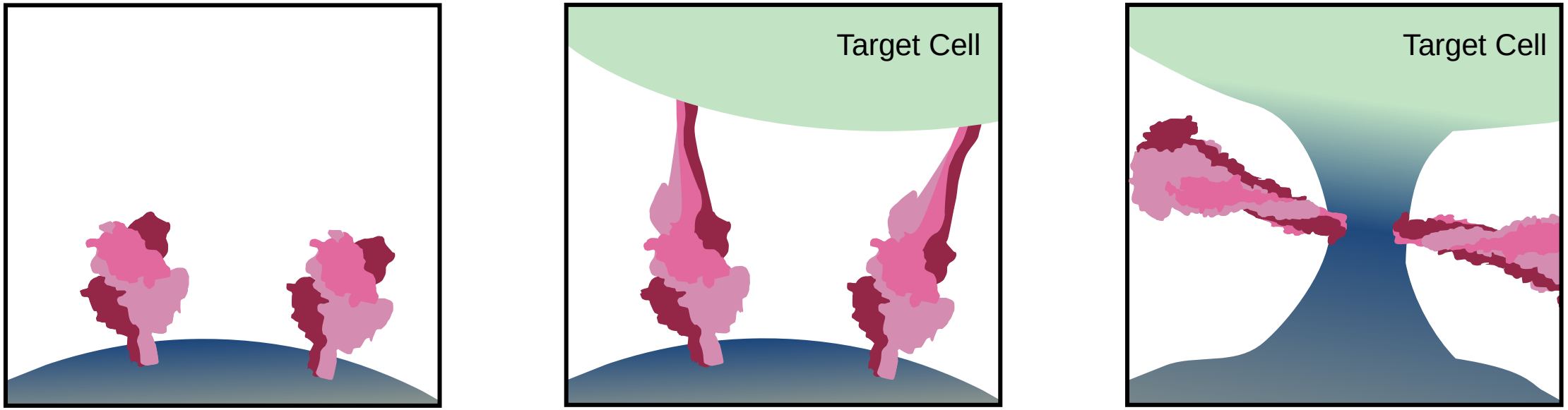
Respiratory Syncytial Virus

- Enveloped, negative-sense RNA virus[1]
- There are three proteins on the virion surface:
  - Small Hydrophobic Protein (SH)
  - Attachment Protein (G)
  - Fusion Protein (F)



1: Tian D, Battles MB, Moin SM, et al. Nature Communications **volume 8**, Article number: 1877 (2017)

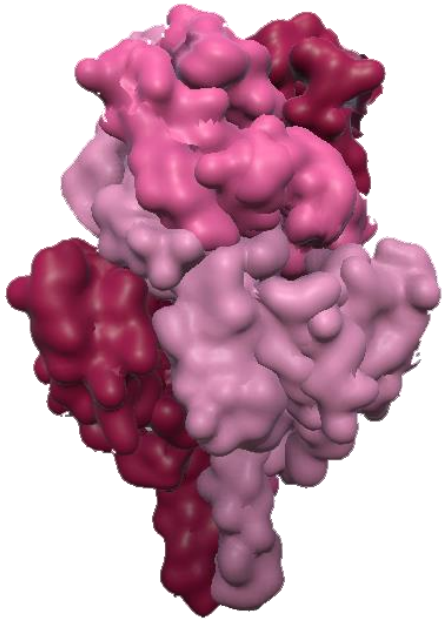
Respiratory Syncytial Virus



Adapted from Chang A, Dutch RE, Viruses 2012, 4(4), 613-636, *Zhao X, Singh M, Malashkevich VN, et al. PNAS 2000; 97(36):14172, and Russell CJ, Luque LE, TRENDS in Microbiology, Vol.14 No.6 June 2006*

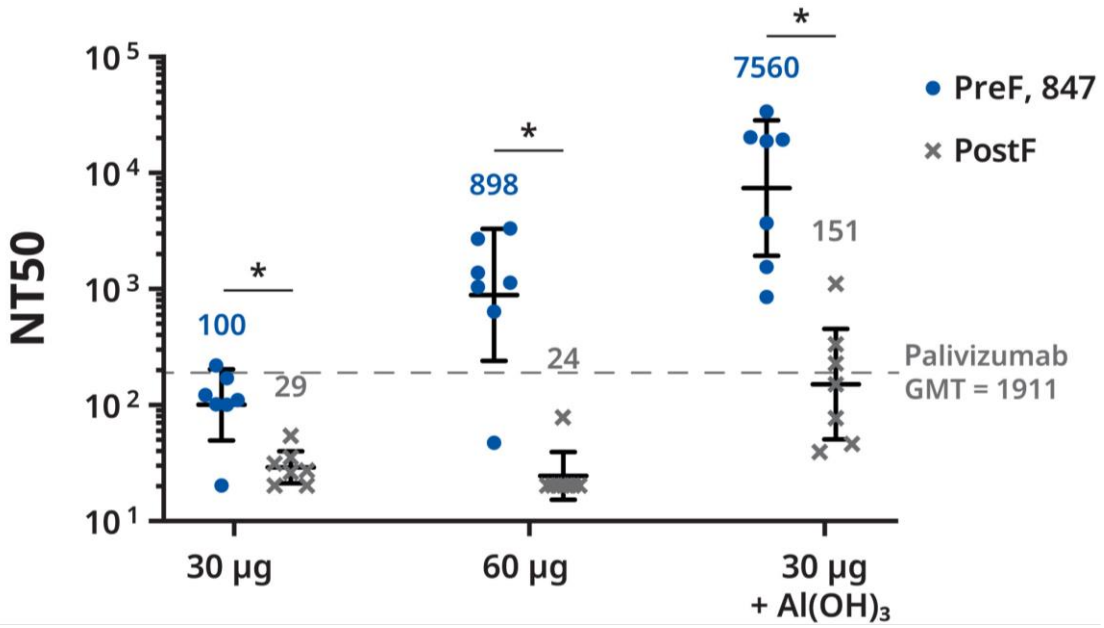
Respiratory Syncytial Virus

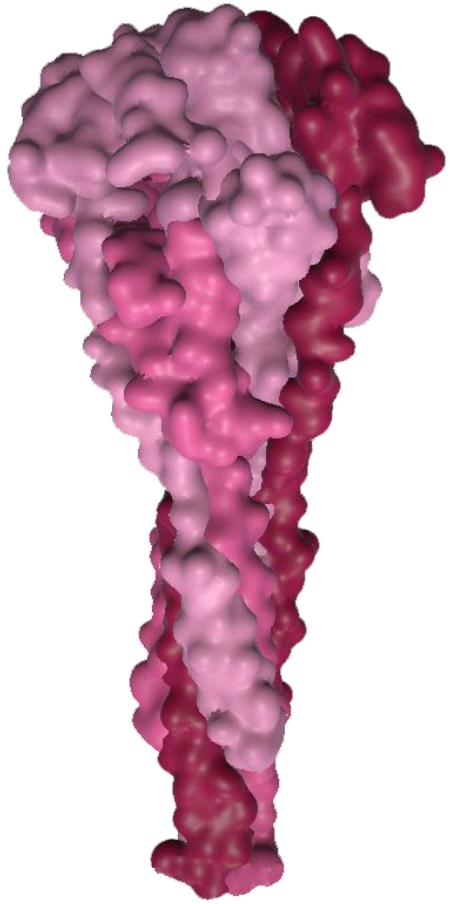
- RSV protein-subunit vaccines development has focused on the fusion protein.
- The metastable prefusion form (preF) is a major target of the most potent virus neutralizing antibodies.[1, 2, 3, 4]
- Thus, stabilized preF is a key vaccine antigen.



Prefusion

Prefusion F elicits 50-fold higher neutralizing titers than postfusion F in non-human primates[5]

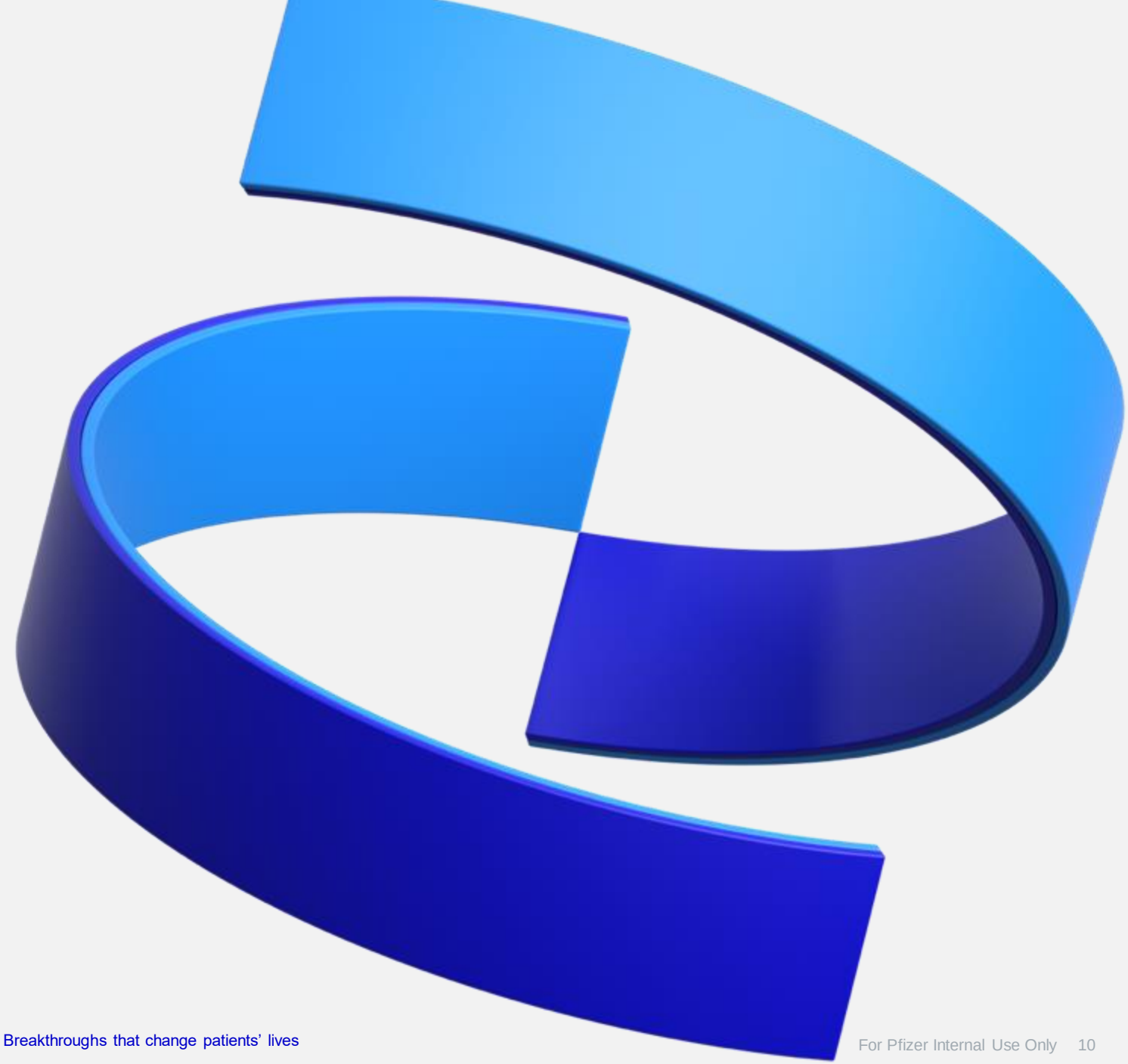




Postfusion

1: *Zhao X, Singh M, Malashkevich VN, et al. PNAS 2000; 97(36):14172 , 2: Russell CJ, Luque LE, TRENDS in Microbiology, Vol.14 No.6 June 2006 , 3: McLellan JS. Curr Opin Virol 2015; 11:70-5, 4: Ngwuta JO, Chen M, Modjarrad K, et al. Sci Transl Med 2015;7(309): 309ra162, 5*: Che Y, Gribenko AV, Song X, et al. Sci Transl Med . 2023 Apr 26;15(693):eade6422

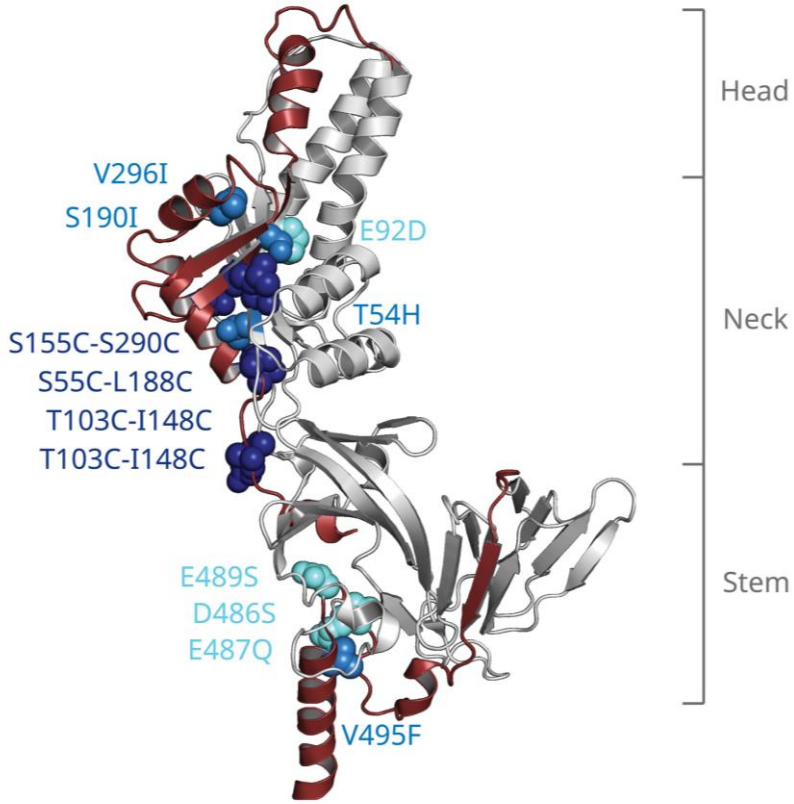
Pfizer's RSVpreF Subunit Vaccine

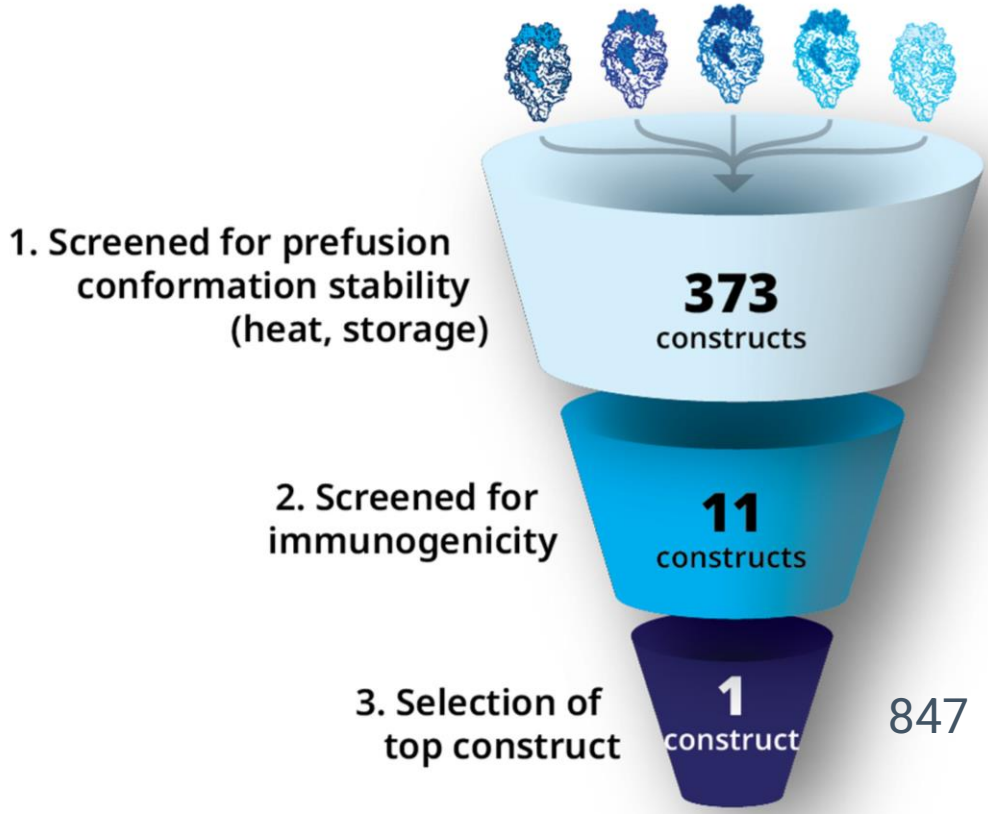


Rational design of a highly immunogenic prefusion-stabilized F glycoprotein antigen for a respiratory syncytial virus vaccine

- Pfizer vaccine research evaluated multiple mutation options to stabilize the F protein in the prefusion conformation
- Through screening of 373 constructs, a final construct (847) was selected as the basis for the Abrysvo bivalent RSVpreF vaccine

Mutations:
- disulfide (dark blue)
- cavity-filling (cyan)
- electrostatic charge (light blue)

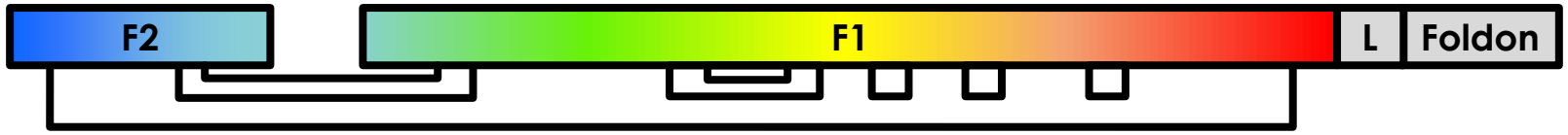


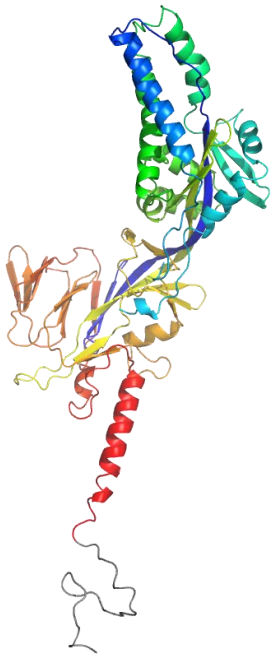


847

Che Y, Gribenko AV, Song X, et al. Sci Transl Med . 2023 Apr 26;15(693):eade6422

ABRYSVO – RSVpreF Vaccine

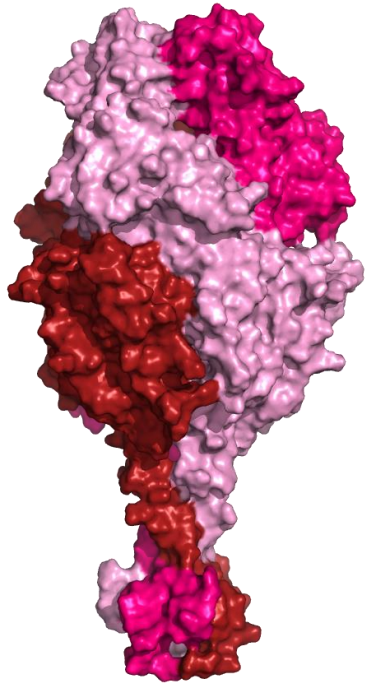




RSVpreF Bivalent Vaccine

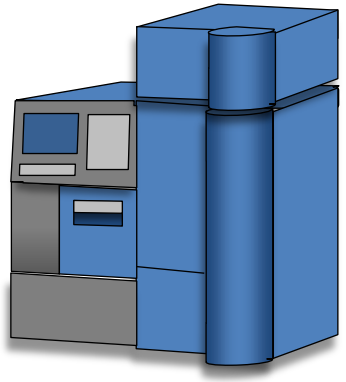
"847" mutations transferred to backbone of RSVA and B strains to create 847A and 847B antigens[1]

- 847A and 847B each are comprised of two covalently bound subunits (F1 and F2)
- Engineered to trimerize through inclusion of bacteriophage T4 foldon domain

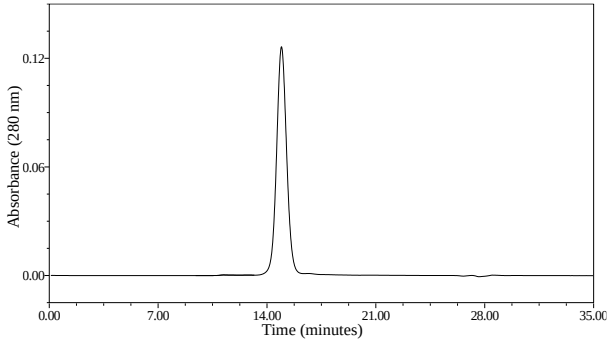


1: Che Y, Gribenko AV, Song X, et al. Sci Transl Med . 2023 Apr 26;15(693):eade6422

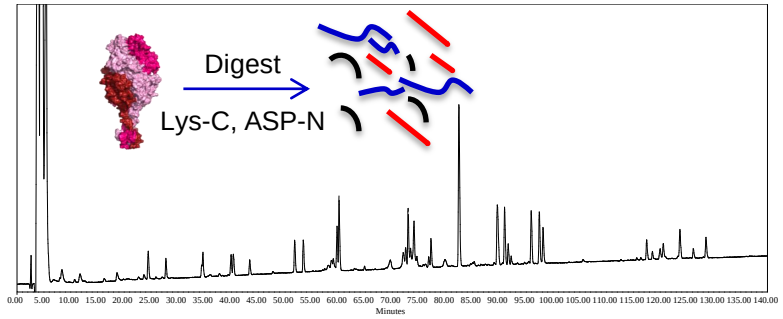
Characterization of RSVpreF 847A and 847B

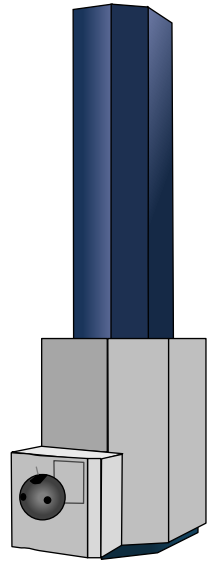


Native Size Heterogeneity

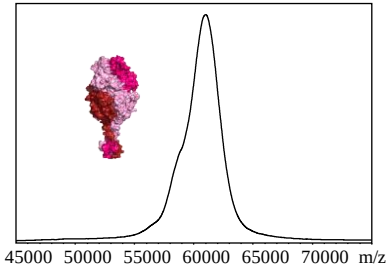


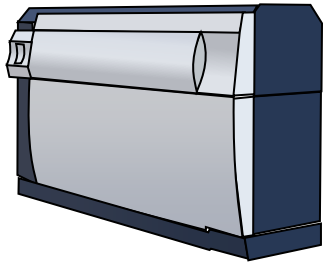
LC-MS Peptide Mapping

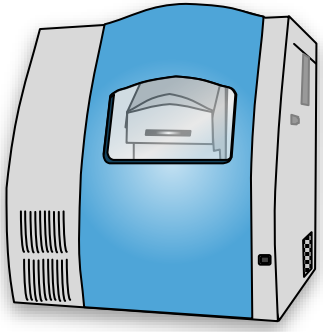




Intact MS

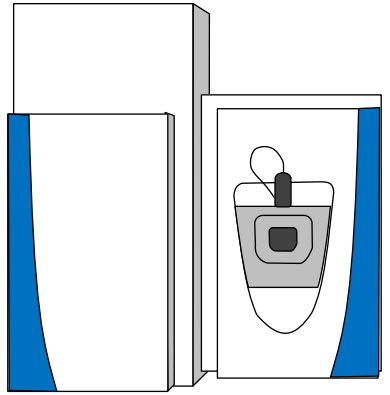






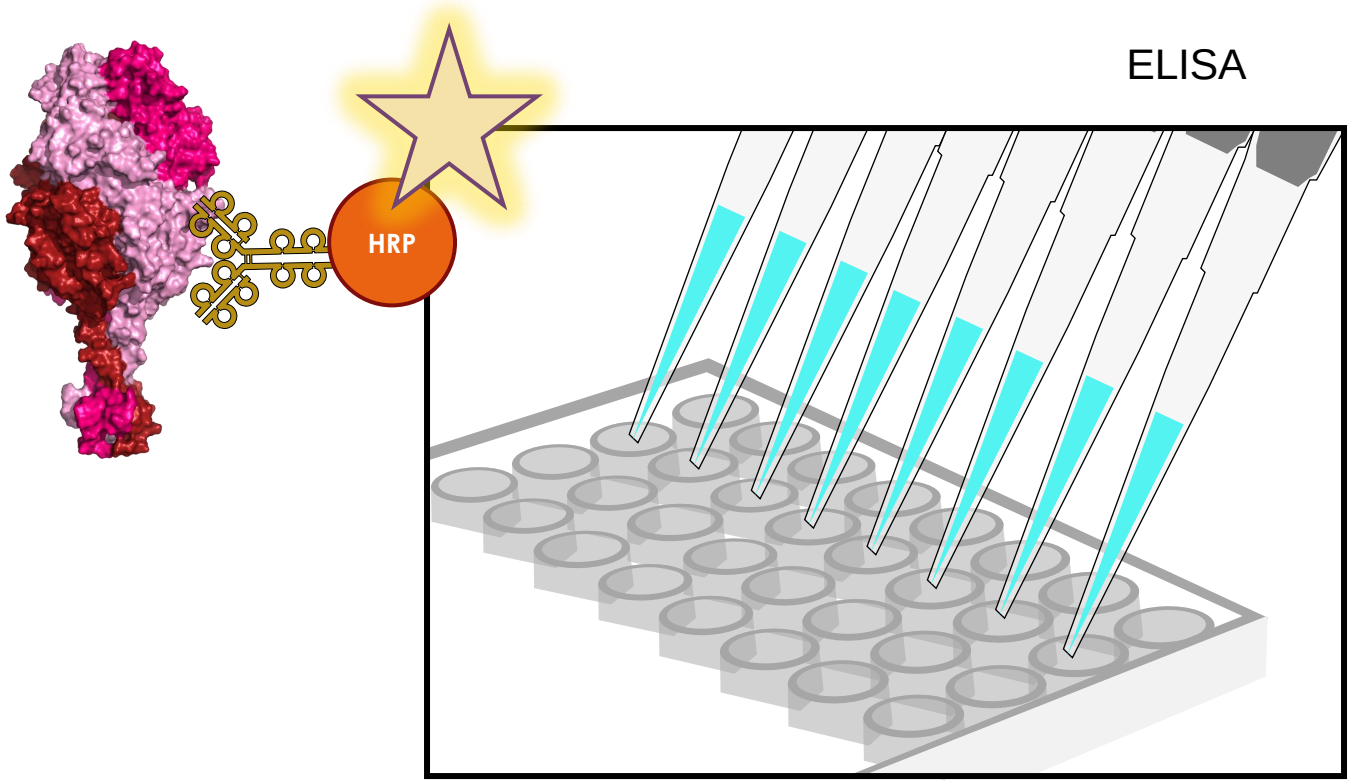
Reduced/Denatured Size Heterogeneity

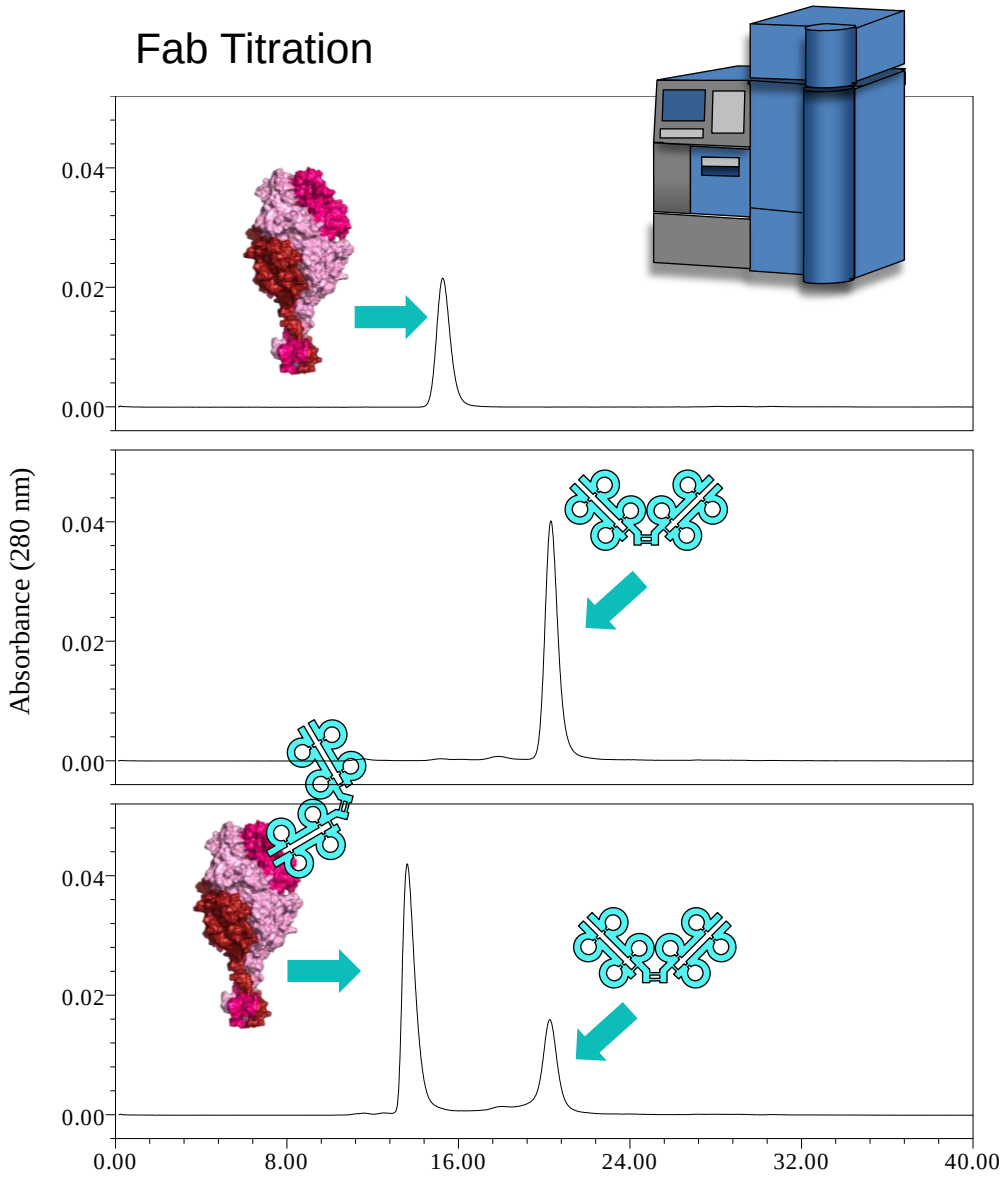
LC/MS-N-linked Glycan Profiling



Prefusion Content

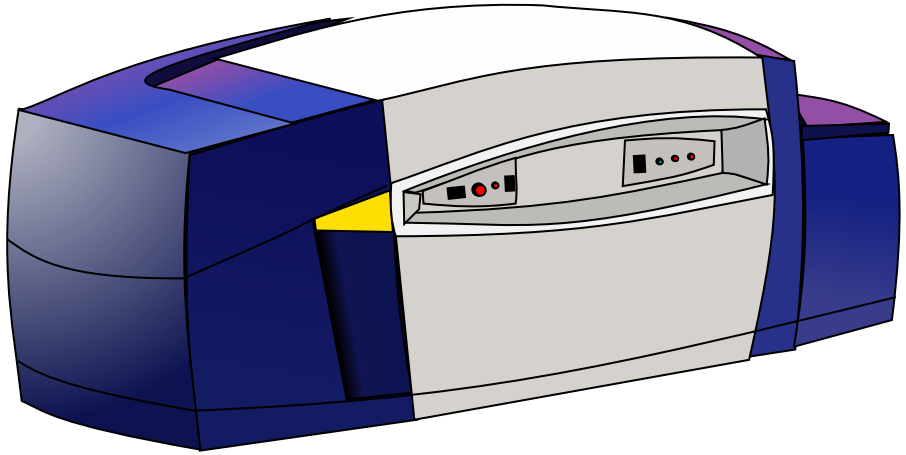
Prefusion Specific Antibody/Fab Binding

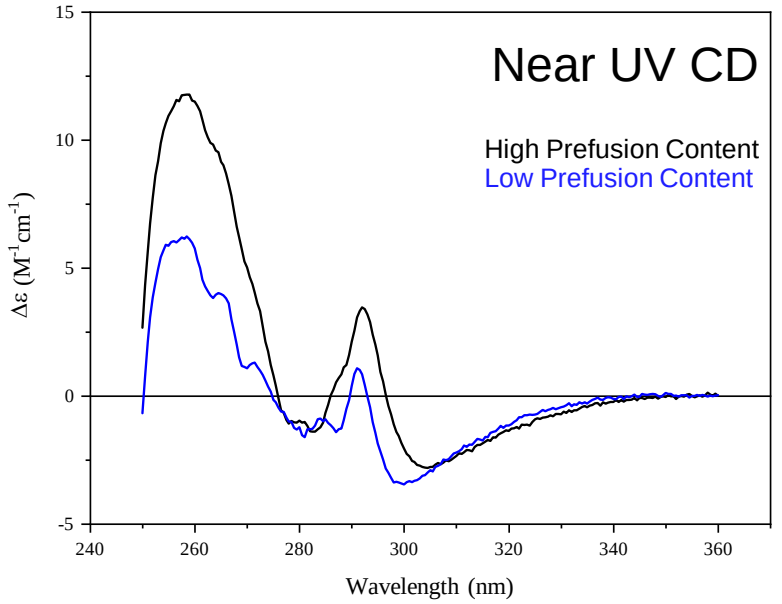




Prefusion Content

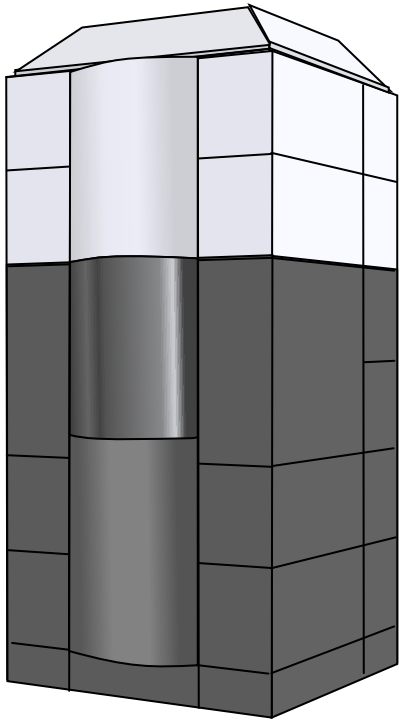
Tertiary Structure by Near UV CD

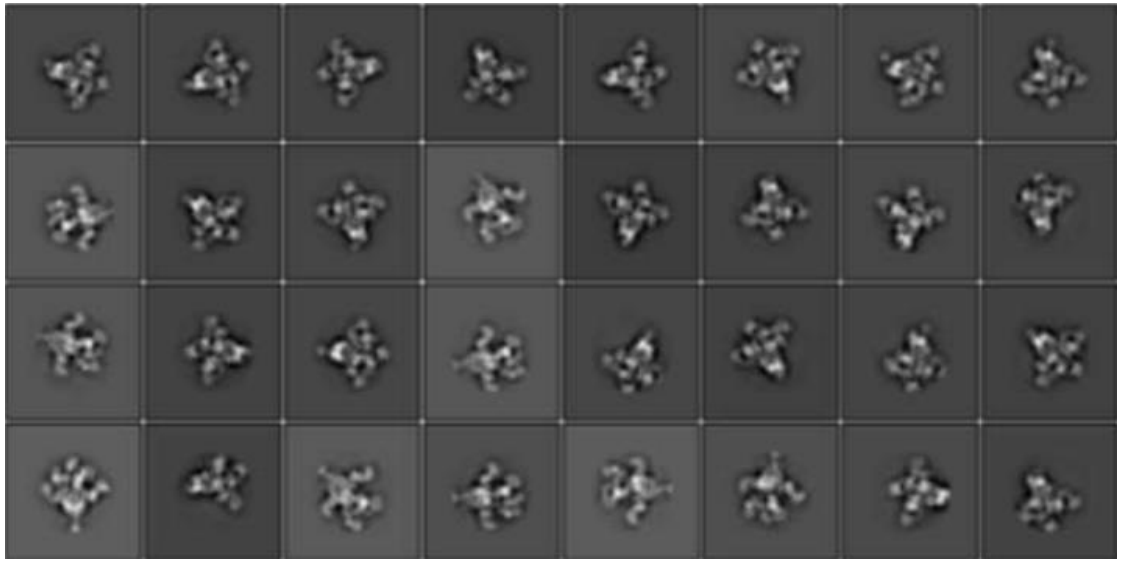




Prefusion Content

Cryo-EM Characterization of RSVpreF





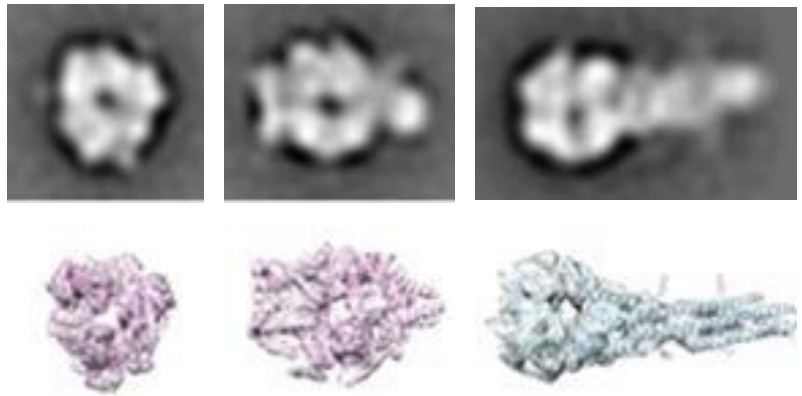
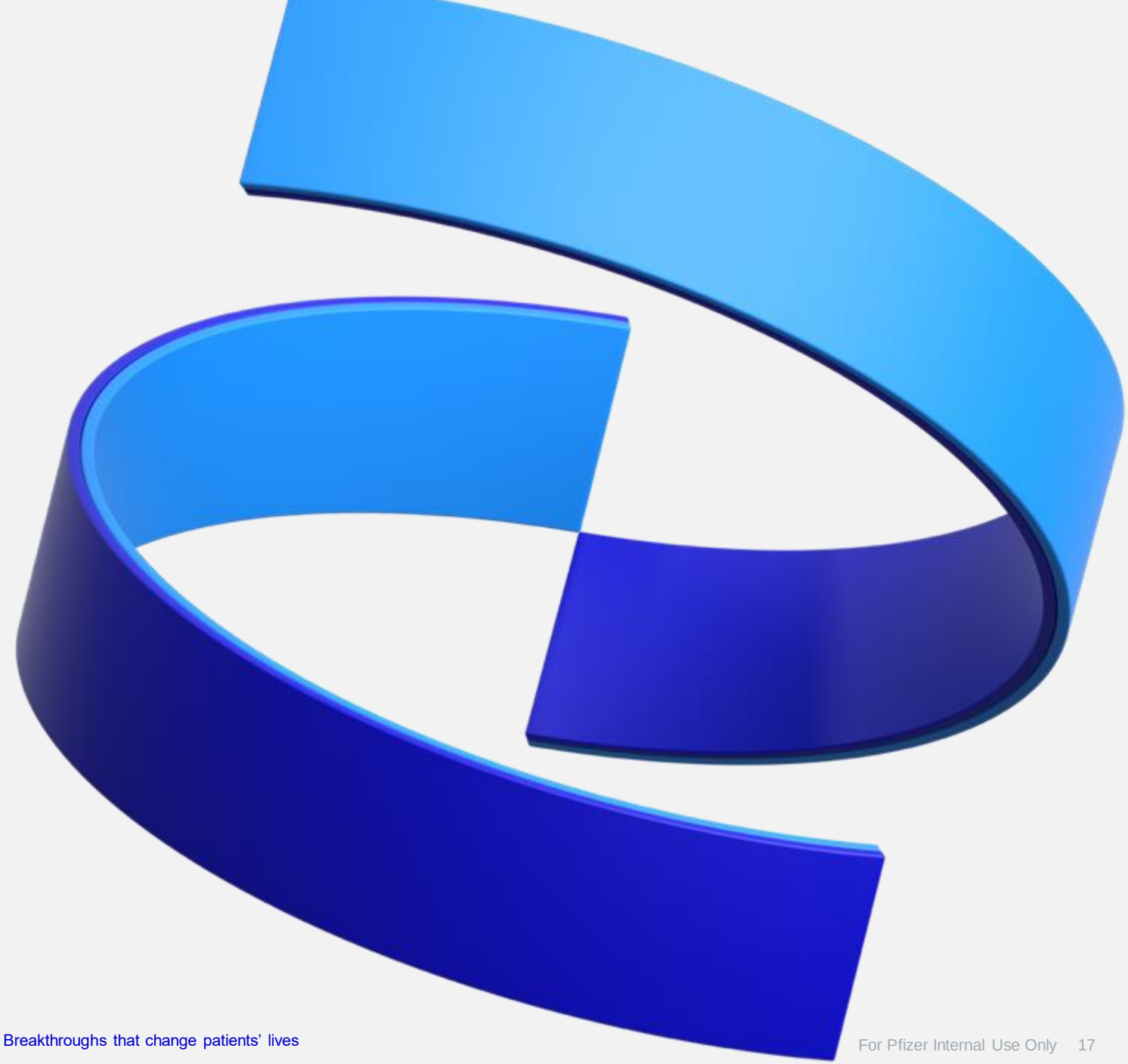


Figure by Mark Ammirati

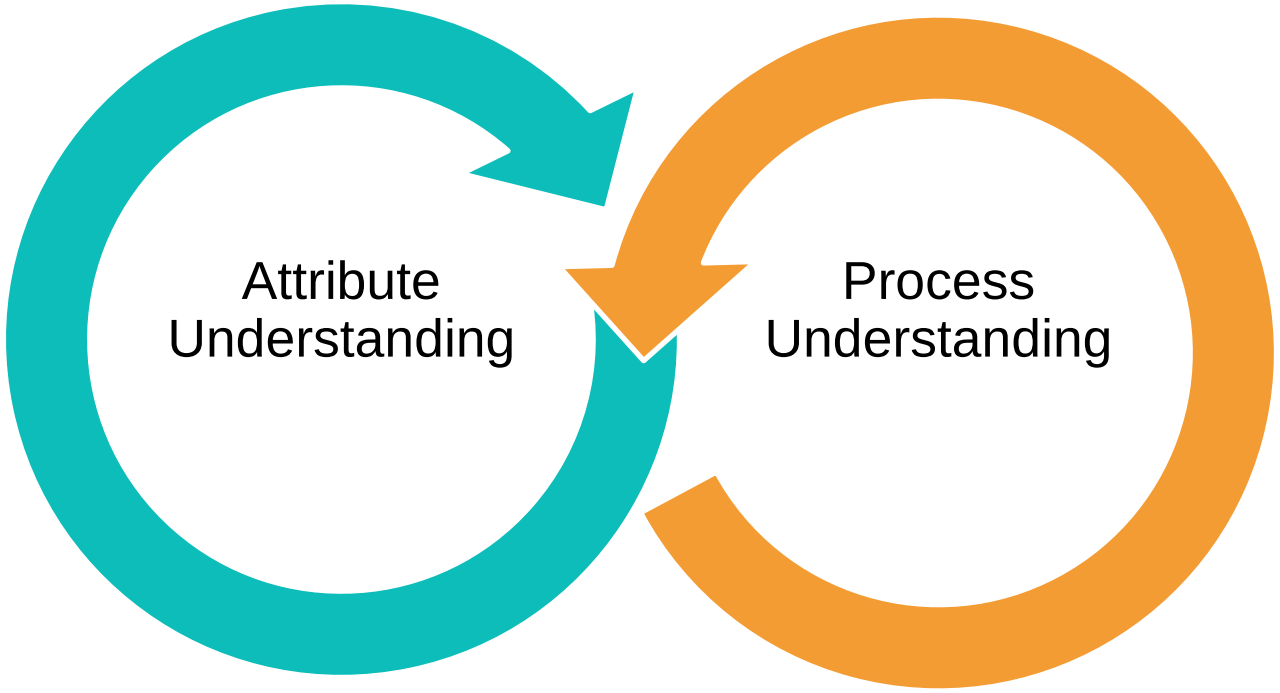
Che Y, Gribenko AV, Song X, et al. Sci Transl Med . 2023 Apr 26;15(693):eade6422

A Case Study in Attribute, Process, and Analytical Understanding

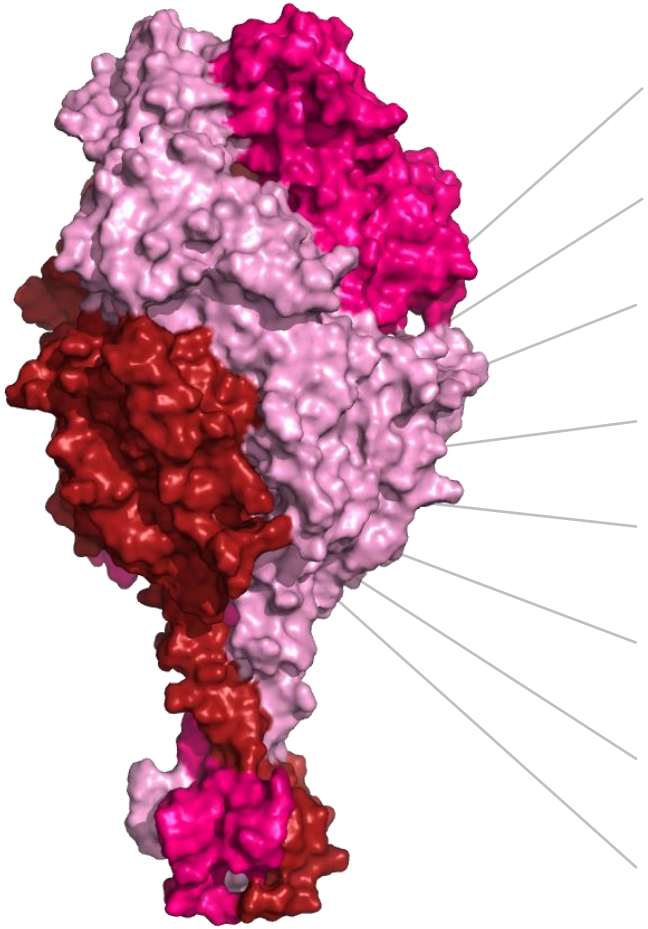


Pathway to Control Strategy

The key to an effective control strategy is understanding how the process impacts product critical quality attributes.



Pathway to Control Strategy

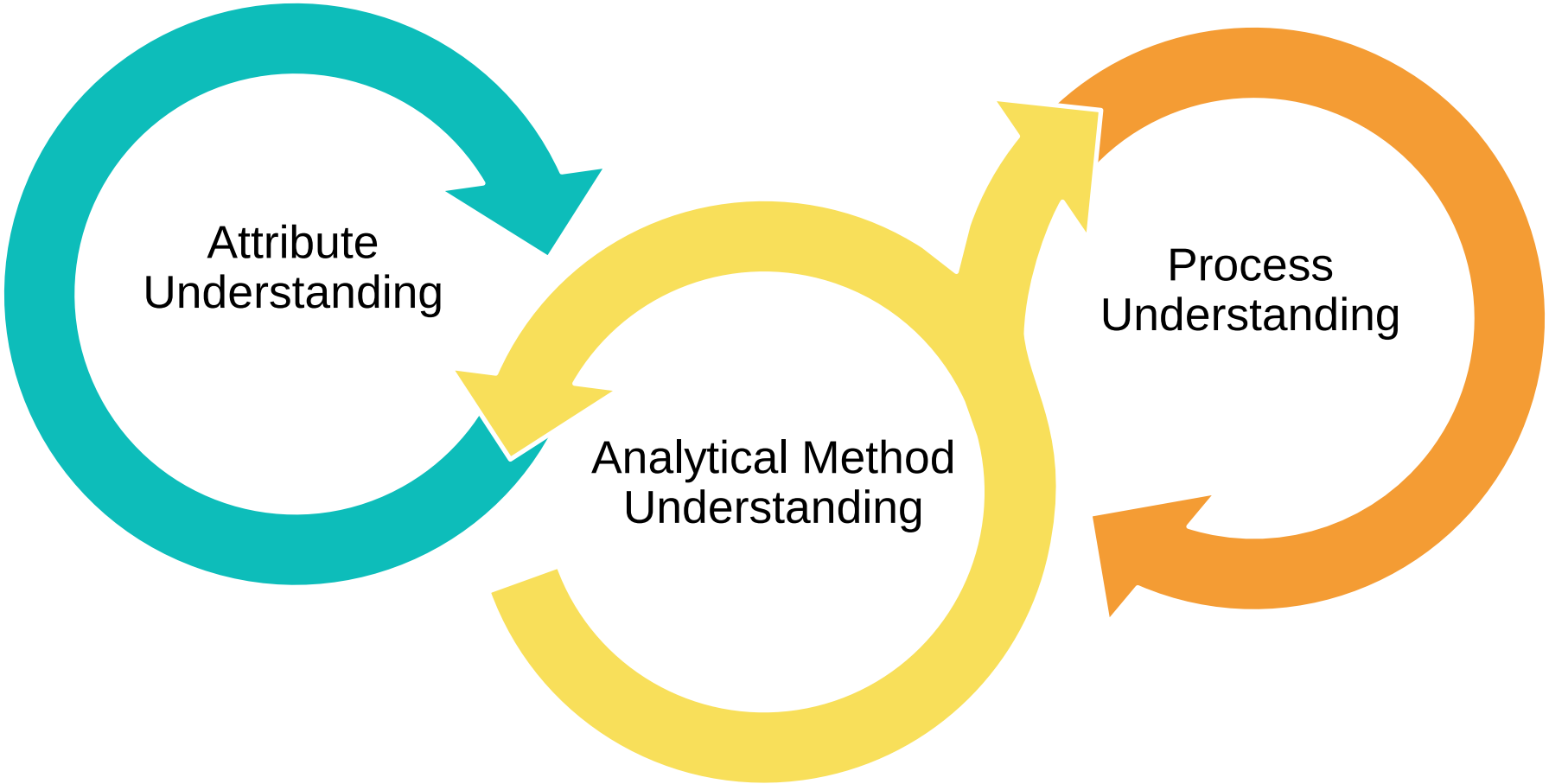


Critical Quality Attributes (CQAs)

Critical Process Parameter (CPP)

Pathway to Control Strategy

Prior to fully understanding the relationship between process parameters and critical product quality attributes, it is necessary to understand the analytical toolbox used to monitor those attributes across the process.



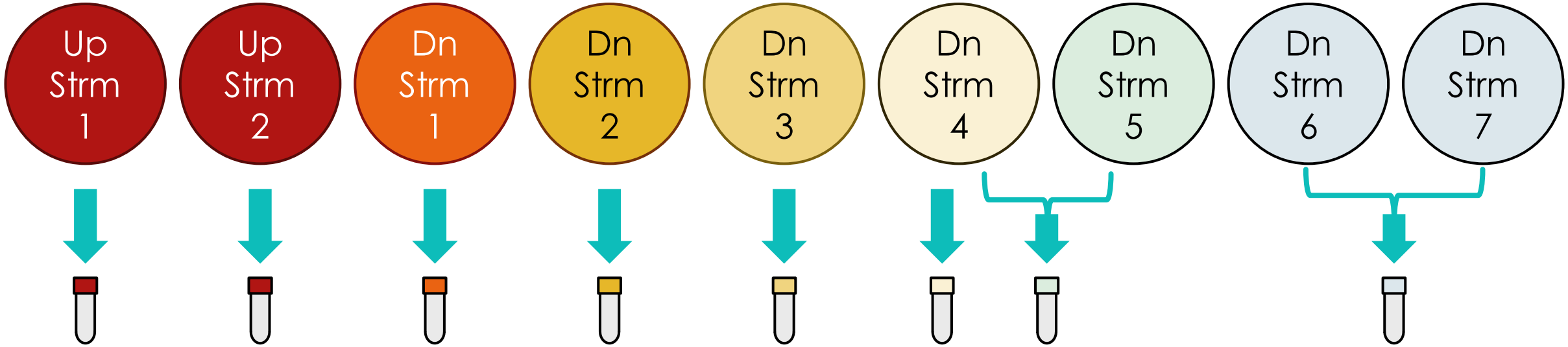
Analytical Method Understanding

Questions to Answer:

1. Does each required method provide accurate results for the desired attribute in each sample type?
2. If not, is there a sample handling / storage condition that will enable analysis?
3. If not, is there a purification process to enable analysis?
4. Does that purification change the attribute result?

RSVpreF Drug Substance Process

- The upstream bioprocessing steps include the thawing of a working cell bank, followed by multiple step expansion and harvest.
- The downstream purification process comprises of seven steps with ultrafiltration/diafiltration (UF/DF) steps, chromatography steps, a viral retaining filtration (VRF) step, a formulation and filtration step.



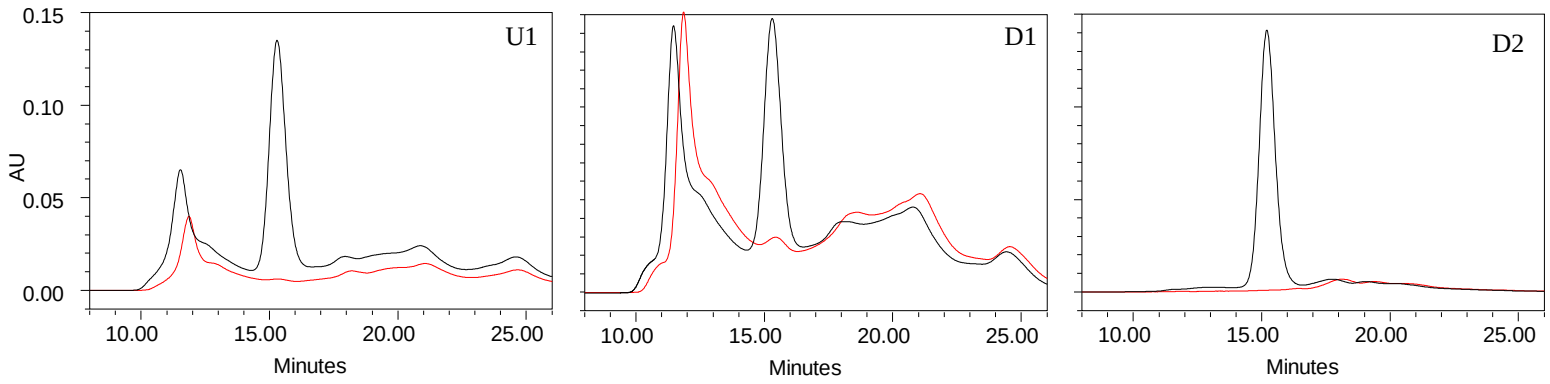
- Together, these steps produce 8 different sample matrices

Analytical Method Understanding

Question 1: Does each required method provide accurate results for the desired attribute in each sample type?

Created assessment samples of known product quality for each sample type

- Null media (cell culture from cells that do not express prefusion F protein) was harvested and taken through the purification process to create “null” process pools.
- 847A and 847B materials enriched with species of interest were used to created stocks of varying attribute levels (two of 847A and two of 847B).
- Each pool was spiked with the 847A and 847B stocks and assessed for accuracy of attribute determination for each sample type.



Null Media Process Pool

Prefusion F Spiked Null Media Process Pools

Analytical Method Understanding

Question 1: Does each required method provide accurate results for the desired attribute in each sample type?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Analytical Method Understanding

Question 1: Does each required method provide accurate results for the desired attribute in each sample type?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

CQA4	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+++	+++	+++	+++
U2	+++	+++	+++	+++
D1	+++	+++	+++	+++
D2	+++	+	+	+
D3	+	+	+	+
D4	+++	+++	+++	+
D5	+	+	+	+

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Analytical Method Understanding

Question 1: Does each required method provide accurate results for the desired attribute in each sample type?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

CQA4	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+++	+++	+++	+++
U2	+++	+++	+++	+++
D1	+++	+++	+++	+++
D2	+++	+	+	+
D3	+	+	+	+
D4	+++	+++	+++	+
D5	+	+	+	+

QA3	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+	+	+	+++
U2	+	+	+	+++
D1	+	+	+	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	NT	+	+++
D5	+	+	+	+

NT = Not tested

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Analytical Method Understanding

Question 1: Does each required method provide accurate results for the desired attribute in each sample type?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

CQA4	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+++	+++	+++	+++
U2	+++	+++	+++	+++
D1	+++	+++	+++	+++
D2	+++	+	+	+
D3	+	+	+	+
D4	+++	+++	+++	+
D5	+	+	+	+

QA3	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+	+	+	+++
U2	+	+	+	+++
D1	+	+	+	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	NT	+	+++
D5	+	+	+	+

NT = Not tested

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Purification step sufficient to enable analysis

Analytical Method Understanding

Question 2: Is there a sample handling or storage condition to enable analysis?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

CQA4	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+++	+++	+++	+++
U2	+++	+++	+++	+++
D1	+++	+++	+++	+++
D2	+++	+	+	+
D3	+	+	+	+
D4	+++	+++	+++	+
D5	+	+	+	+

QA3	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+	+	+	+++
U2	+	+	+	+++
D1	+	+	+	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	NT	+	+++
D5	+	+	+	+

NT = Not tested

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Determined to require real-time analysis

Analytical Method Understanding

Question 3: Is there a purification process to enable analysis?

Example Read Outs: Accuracy of result compared to starting material.

CQA1	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	++	++	+	+
U2	+	+	+	+
D1	+++	+++	++	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	+	+	+
D5	+	+	+	+

CQA4	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+++	+++	+++	+++
U2	+++	+++	+++	+++
D1	+++	+++	+++	+++
D2	+++	+	+	+
D3	+	+	+	+
D4	+++	+++	+++	+
D5	+	+	+	+

QA3	847A Sample 1	847A Sample 2	847B Sample 1	847B Sample 2
U1	+	+	+	+++
U2	+	+	+	+++
D1	+	+	+	+++
D2	+	+	+	+
D3	+	+	+	+
D4	+	NT	+	+++
D5	+	+	+	+

NT = Not tested

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

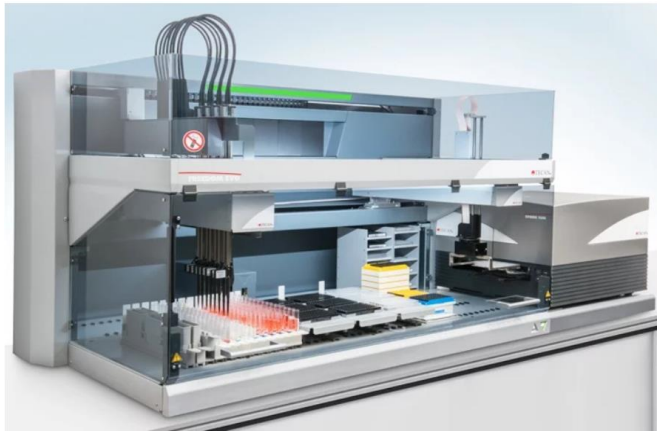
Sample required buffer exchange to remove matrix interference prior to analysis

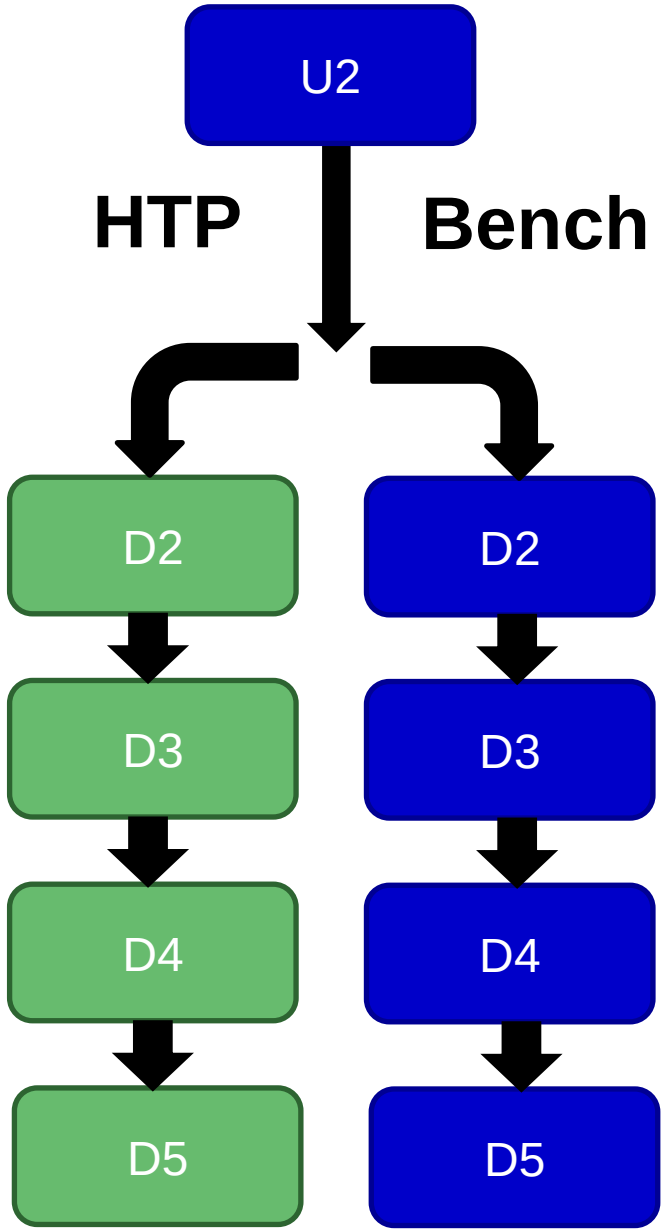
Analytical Method Understanding

Question 3: Is there a purification process to enable analysis?

Bioprocess Purification group developed an automated high-throughput (HTP) sample purification scheme to aid in analysis of sample types that needed additional purification.

The HTP process was compared to the bench scale process using 1L bioreactor harvests.





Analytical Method Understanding

Question 4: Does the purification change the attribute result?

Materials of known product quality from different processes were taken over the HTP process to evaluate impact on quality attributes

Effect of Purification Process and Impurities on CQAs

CQA1	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+
D2	+	+	+
D3	++	++	+++
D4	+	+	+

CQA4	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+++
D2	+	+	+
D3	+++	+	+++
D4	+	+	+

QA3	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+
D2	+	+	+
D3	+	+	+
D4	+	+	+

Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Analytical Method Understanding

Question 4: Does the purification change the attribute result?

Materials of known product quality from different processes were taken over the HTP process to evaluate impact on quality attributes

Effect of Purification Process and Impurities on CQAs

CQA1	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+
D2	+	+	+
D3	++	++	+++
D4	+	+	+

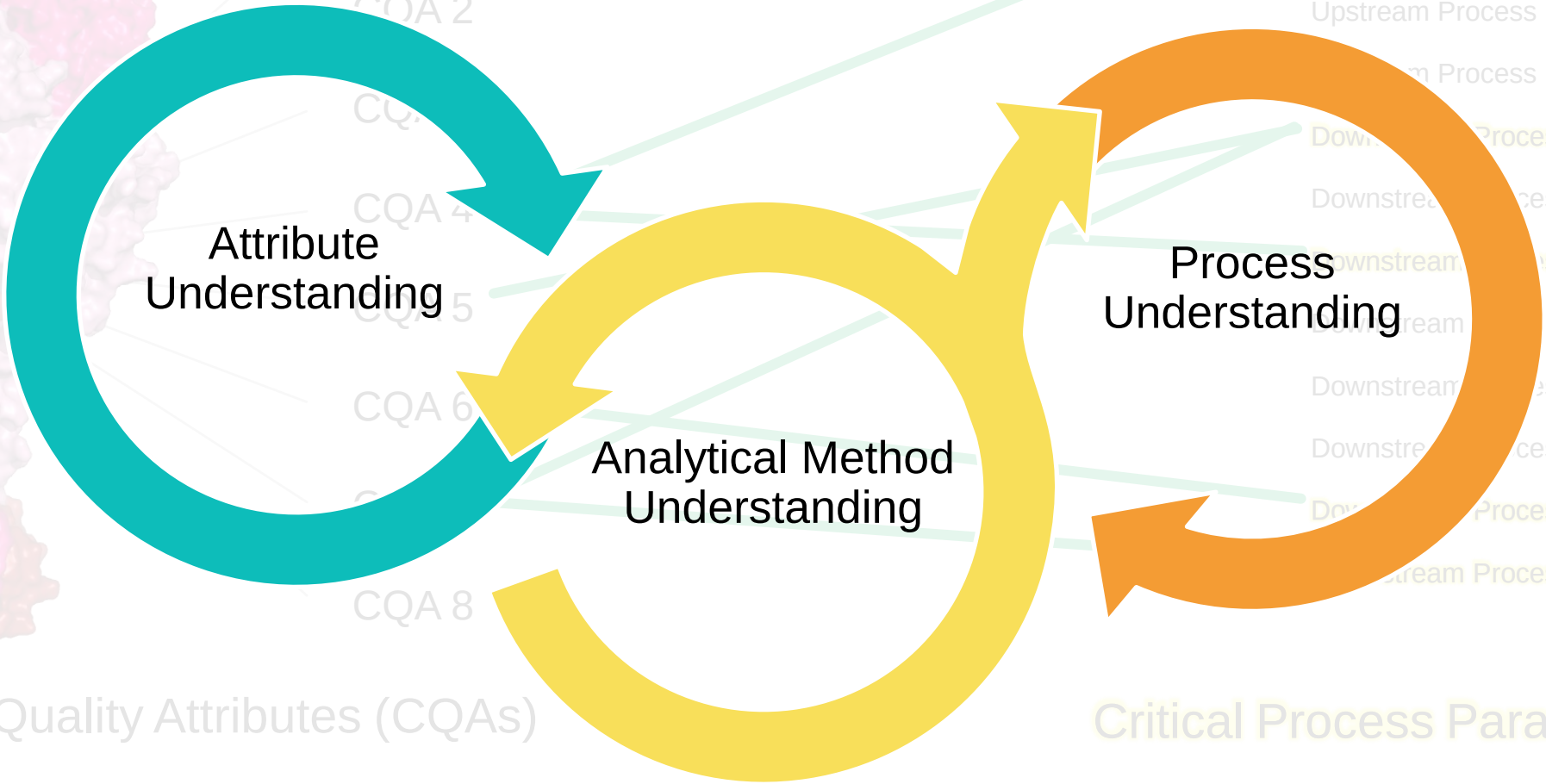
CQA4	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+++
D2	+	+	+
D3	+++	+	+++
D4	+	+	+

QA3	Process 1 DS	Process 2 DS	Process 1 DS in Null Media
D1	+	+	+
D2	+	+	+
D3	+	+	+
D4	+	+	+

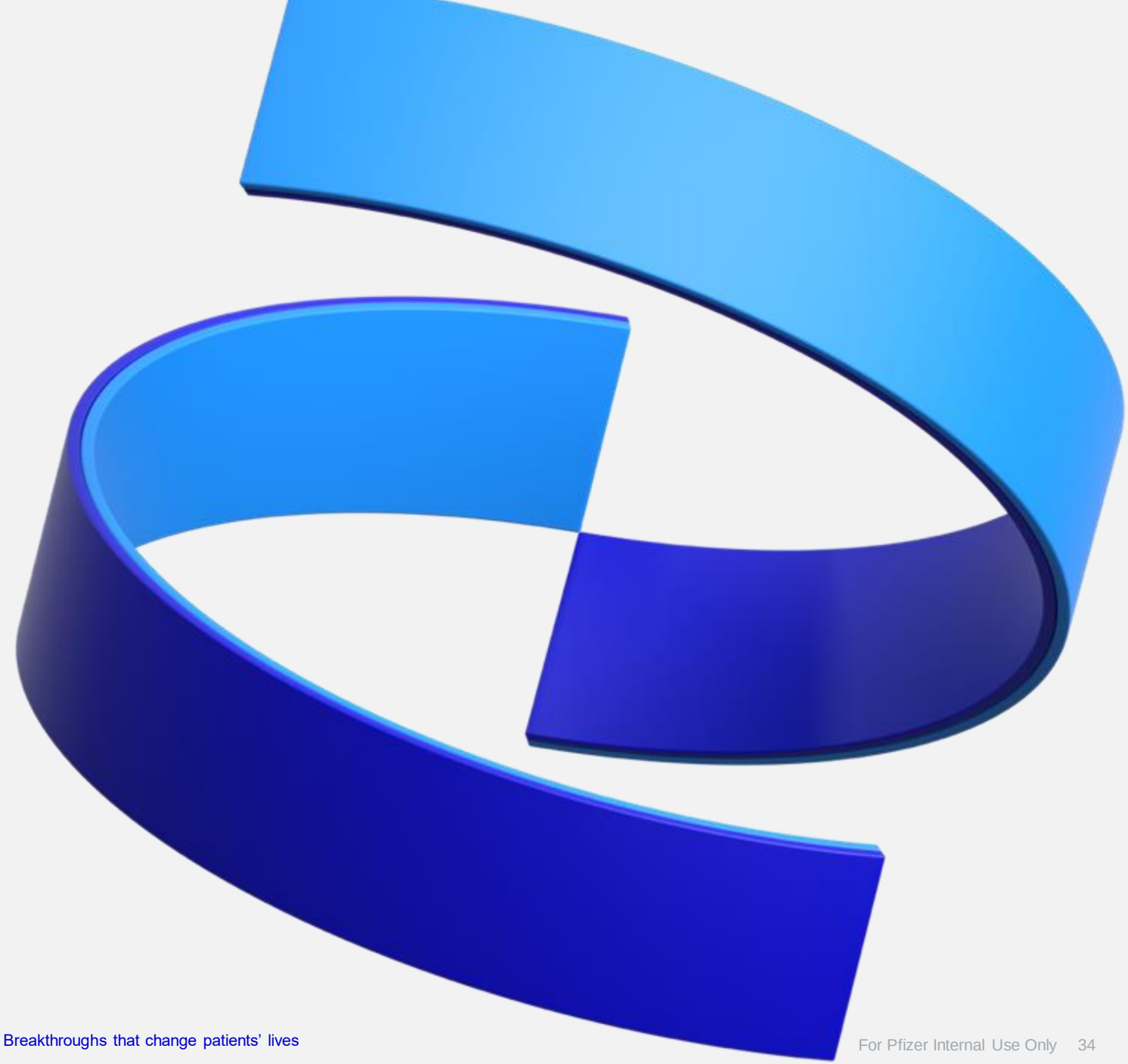
Key	
Not Impacted	+
Mild Impact	++
Heavily Impacted	+++

Pathway to Control Strategy

With a deep understanding of analytical method capability with respect to attributes of interest across the process, the RSVpreF team was able to execute an expansive process evaluation to identify the process parameters critical to ensure the target product profile is maintained.



Summary and Conclusions



Summary and Conclusions

- Early and extensive characterization of RSVpreF subunits, 847A and 847B, enabled a greater understanding of the molecule attributes throughout clinical development.
- Advanced characterization tools including high resolution mass spectrometry, biophysical characterization, and electron cryo-microscopy enabled a deeper understanding of the primary, secondary, and tertiary structures of the molecules.
- Understanding the performance of the analytical methods through all stages of the drug substance process allowed for greater understanding of the process itself, making it possible to subsequently assess the process and identify CPPs with confidence.
- The attribute and analytical understanding facilitated the development of a process that underwent relatively minor changes between phase II and commercial registration.
- This early yet deep understanding of the product and process provided the means to bring a greatly needed medicine to patients in an accelerated manner.

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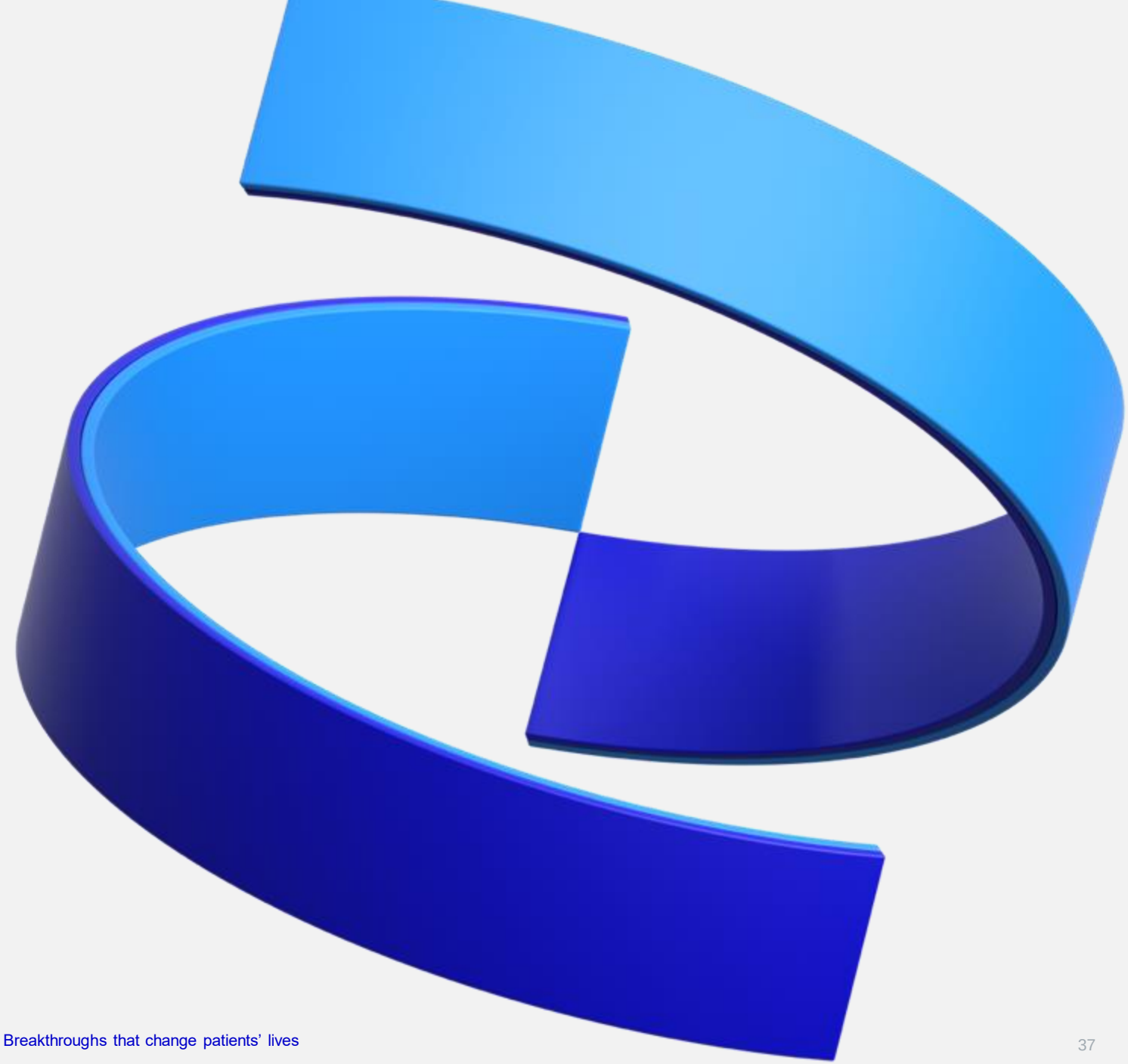
Thank You



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Questions?