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Delivering on the Promise of the Proteome

OCTOBER 29, 2024

Safe harbor

This presentation and the accompanying oral presentation contain forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, including, among others, statements regarding the size and growth of the protein analysis market; Nautilus Biotechnology's anticipated total addressable market; the performance, value and enabling nature of Nautilus Biotechnology's proteomics and proteoform analysis technology platform; statements regarding Nautilus Biotechnology's future development milestones and timing; statements regarding the timing and nature of Nautilus Biotechnology's potential engagements with partners in proteoform analysis; Nautilus Biotechnology's business and operational strategy and financial targets; Nautilus Biotechnology's prospective products; Nautilus Biotechnology's business development plans and opportunities; Nautilus Biotechnology's anticipated customer mix and collaborations plans; and objectives of management for future operations are forward looking statements. Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current expectations and projections about future events and financial trends that we believe may affect our financial condition, results of operations, business strategy, and financial needs. All statements other than statements of historical facts contained in this presentation, including, without limitation, statements regarding our future performance and our market opportunity, could be deemed forward-looking statements. The words "may," "will," "expect," "anticipate," "aim," "estimate," "intend," "plan," "believe," "is/are likely to," "potential," "continue" and other similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words.

Forward-looking statements are subject to numerous risks and uncertainties that could cause actual results to differ materially from currently anticipated results, including but not limited to risks relating to the development and commercialization of our Nautilus platform; macroeconomic conditions; regional conflicts; Nautilus Biotechnology's dependence on certain sole and single source suppliers; competition; market acceptance of Nautilus Biotechnology's current and potential products; Nautilus Biotechnology's ability to manage the growth and complexity of its organization; Nautilus Biotechnology's ability to maintain, protect and enhance its intellectual property; and Nautilus Biotechnology's ability to continue to stay in compliance with its material contractual obligations, applicable laws and regulations. Information on these and additional risks and uncertainties and other information affecting Nautilus Biotechnology's business and operating results is contained in Nautilus Biotechnology's Annual Report for the year ending December 31, 2023, filed February 28, 2024, and in its other filings with the Securities and Exchange Commission. These forward-looking statements speak only as of the date hereof. Except as required by applicable law, Nautilus Biotechnology does not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise. No representations or warranties (expressedor implied) are made about the accuracy of any such forward-looking statements.

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Nautilus seeks to revolutionize biomedicine



Defining a new gold standard for single-molecule protein analysis



Proteins are key drivers of biology





Interrogating the proteome is challenging

Proteins span a wide dynamic range

From single-digit numbers of molecules to millions of copies per cell (or drop of blood)



Anderson, N. L. *Molecular & Cellular Proteomics* 1, 845–867 (2002).

There is no PCR for proteins

Amplification isn't possible





Proteins exist in a range of modified states (proteoforms)





Current analysis methods can't see most proteins and proteoforms





There is no solution today to measure and quantify intact proteoforms



Nautilus is reinventing proteomics from the ground up

What is necessary to identify and quantify the proteome and proteoforms?



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Core platform components





Simple and robust sample preparation workflow

Arraying single protein molecules onto a hyper-dense chip



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Multiple applications from the same core platform





Integration of breakthrough innovations across the platform

Designed to allow access to full resolution digital proteomic data for Broadscale Proteome Analysis



Designed to comprehensively quantify the proteome

Human Proteome Coverage Across 300 Unique Multi-affinity Probes

Capable of Achieving >95% Proteome Coverage

¹Estimates based on Nautilus computational analysis projecting the number of Nautilus designed short epitope probe binding events necessary to identify the SwissProt reference proteome.

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Multi-affinity probes bind to buried/structured regions in denatured proteins

- Binding of a multi-affinity probe to its intended peptide target is required
- Multi-affinity probes must also be able to bind to short epitopes in denatured, full-length proteins
- We have demonstrated binding to short epitopes in internal portions of denatured, but full-length proteins (red) using both aptamer- and antibody-based multi-affinity probes

Transferrin

G6PI

Binding patterns to transferrin and G6PI at single-molecule resolution

Samples consisting of transferrin (TRFE), glucose 6 phosphate (G6PI), pyruvate kinase M2 (PKM2), a model protein, no protein (negative control) or mixtures thereof were deposited into flowcell lanes for PrISM analysis.

Shown top are the 5 most prevalent binding patterns from these experiments for transferrin and G6PI.

From these binding patterns, machine learning tools identify each molecule. Each additional cycle builds additional information about protein identity as transferrin and G6PI have different binding patterns, indicated by the triangles.

The resulting difference in probability between the best-matching protein, and the next best protein in the database leads to confident protein identifications.

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- G6PI)

Ultra-sensitive quantification of transferrin

(LEFT) Sensitive single-molecule measurements: Lower limit of detection in the high-yocto to low-zeptomole range

Reproducible single-molecule measurements:

The highest abundance Transferrin measurement (2 attomoles) was repeated 7 times across 4 days and 7 flow cells with a CV of **7.7%**

(RIGHT) High fidelity quantification: Multi-cycle decoding data is significantly more sensitive and error tolerant than achievable with any one multi-affinity probe alone

This improvement arises from the ability of the machine learning software to better identify proteins whose identifications were derived from either false positive or false negative bindings

Transferrin dilution series in a background of alternate protein, or null scaffolds

Nautilus: Revealing unseen proteoforms

¹International Journal of Analytical Chemistry. 2016; 2016: 7436849. The Size of the Human Proteome: The Width and Depth, Elena A. Ponomarenko et al. Nautilus Biotechnology – All Rights Reserved 17

Peptide-centric proteomics methods are unable to differentiate mixtures of proteoforms

High-resolution proteoform quantitation: a core application of Nautilus' platform

Which drugs work and to what extent is defined not by just the total amount of PTMs and splice forms, but instead by how combinations of specific alterations operate together. Creating a technology to see these PTM patterns, and measure their relationship to one another, has the potential to hugely advance precision medicine.

Dr. Ruedi Aebersold, Head of IMSB, Swiss Federal Institute of Technology (ETH) and Nautilus Scientific Advisory Board Member

Integration of breakthrough innovations across the platform

Designed to allow access to full resolution digital proteomic data for Proteoform Mapping

Research collaborations

Genentech

A Member of the Roche Group

- Signed a pilot study Research Collaboration Agreement in December 2020.
- Collaborating with Genentech using the Nautilus platform to analyze and map the proteoform landscape of a Genentech protein target of interest.

AMGEN

- Signed a pilot study Research Collaboration Agreement in October 2021.
- Collaborating with Amgen using the Nautilus platform across a number of projects to investigate proteins and proteoforms of interest to the company.

THE UNIVERSITY OF TEXAS MDAnderson Cancer Center

- Signed a Research Agreement in October 2021.
- Collaborating with MD Anderson using the Nautilus platform to measure the quantity and patterns of posttranslational modifications on specific oncology protein targets of interest across different settings.

- Signed a Research Agreement in January 2023.
- Collaborating with TGen using the Nautilus platform to analyze specific protein targets in diffuse intrinsic pontine glioma (DIPG), a rare and often fatal childhood cancer.

Sharing insights about our platform...

bioRχiv

A theoretical framework for proteome-scale single-molecule protein identification using multi-affinity protein binding reagents

[b] Jarrett D. Egertson, [b] Dan DiPasquo, Alana Killeen, [b] Vadim Lobanov, [b] Sujal Patel, [b] Parag Mallick doi: https://doi.org/10.1101/2021.10.11.463967

This article is a preprint and has not been certified by peer review [what does this mean?].

Full Text

Abstract

Info/History Metrics

🗅 Preview PDF

Abstract

The proteome is perhaps the most dynamic and valuable source of functional biological insight. Current proteomic techniques are limited in their sensitivity and throughput. A typical single experiment measures no more than 8% of the human proteome from blood or 35% from cells

Key Takeaways:

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Demonstrates the potential to efficiently decode greater than 95% of the proteome.

Demonstrates potential dynamic range of eleven and a half orders of magnitude in plasma, far exceeding the capabilities of other approaches

Details the ability of our platform to work across multiple organisms, critical for translational research

Experimental Findings Presented at October 2024 HUPO World Congress

What are the factors that influence the sensitivity and dynamic range in a single molecule assay?

Number of molecules measured	>	In general, the more molecules measured the wider the dynamic range	
Peptides vs Proteins	>	Each protein can give rise to 10s-100s of peptides thus increasing the number of molecules that must be measured to achieve comparable dynamic range	
False Positive Rate	>	The higher the false positive rate the worse the sensitivity and dynamic range	
Measurement Time	>	The longer the measurement time, the longer it takes to measure N molecules, which leads to a trade-off between throughput and dynamic range	
Number of measurements required to make a confident identification	>	The more measurements required to identify a peptide/protein the longer it takes to measure N molecules, which leads to a trade-off between throughput and dynamic range	
Number of molecules interrogated per measurement	>	Sparsely patterned molecules require more imaging and longer acquisition times to measure N molecules, which leads to a trade-off between throughput and dynamic range	

Scale is critical: dynamic range is directly related to number of molecules measured

- As one increases the number of molecules measured with larger flowcells, one increases the dynamic range of the platform
- (LEFT) In medium blue is the distribution of abundances of proteins in the proteome and in dark blue – the estimate of which of those would get detected if the platform measured either 100,000 or 10,000,000 protein molecules.
- (RIGHT) Modeling the relationship between number of protein molecules measured and dynamic range shows a target of 1-10 billion to be optimal to cover substantively all of the proteome

In orange is shown the dynamic range as the difference between the lowest abundance and highest abundance protein.

In grey is shown the dynamic range where greater than 90% of the proteins at a given concentration are measured.

Multi-affinity probes undergo extensive characterization

Candidates are first evaluated for binding to the selection target

Probe raised against epitope WNK. Kd ~ 1nM

Leads are then tested using millions of peptides to define binding profiles

DCPRYDPRFVGFGWNKVAHIVELDAQARQARQPLLLCPTLQEYELLVLP RFVGFGWNKVAHIVELDAQEYELLVLPEAFTIHLPHAPSLDISRFRSSP KQFWNKSAHRPHVVGAKWLLECFSKGYMLSEEPYIHANYQPVEIPVSHK ERIKNTISYSLQDYIFQSYWGEWNSYYSKILGRPTTLCETMGKAEIWLI WLDNPERWNKVKMVVSREEVELAYQEAMFNMATLNRTAAGLMHTFNAHA WSKVKDKVESDPRYKAVDSSSMREDLFKQYIEKIAKVRSSDVSWSDTRR YNHAAANQNSNATSNIRKEFVPKWNKPSDVSATERTAKYTMEGKGRAAH YRVQWAANYEPYVVVPRDCPRYDPRFVGFGWNKVAHIVELDAQEYELLV WGTPAQNTGTNLPSVEWNKLPSNQHSNDSANGNGKTFTNGWKSTEEEDQ

Candidates are next evaluated for binding to epitope-containing proteins

Tau protein exists in many forms

- Tau has multiple functions in a diverse range of molecular pathways including cell signaling, synaptic plasticity, and regulation of genomic stability
- Tau forms a group of six highly soluble protein isoforms and is highly post-translationally modified, which is pivotal in defining and modulating tau localization and its roles in health and disease
- Tau proteoforms are directly linked with Alzheimer's disease (AD) phenotype in addition to contributing to numerous other tauopathies
- The stoichiometries of tau modifications over the course of the development of AD pathology remain uncharacterized
- Technical limitations prohibit the characterization of the co-occurrence of these PTMs on molecules of tau to date

Quantification of mixtures of proteoforms

Tau proteoforms	Molar ratio		
0N	25		
0N ERK (181 & 396)	50		
2N	12.5		
2N PKA (214)	12.5		

Exploiting the massively parallel nature of our platform, the relative abundances of seven Tau proteoforms were accurately quantified. This measurement is intractable on both traditional and emerging peptide-based platforms.

We additionally showed how the platform can be applied to measure Tau proteoforms.

Expanded panel for tau proteoform quantification

11 reagents enables measurement of 2,048 proteoform groups of tau

New additional detail into the proteoform landscape of model systems enabled by Nautilus Platform

NAUTILUS™ BIOTECHNOLOGY

Large market opportunity ready for disruption

Addressable markets & applications

Longitudinal Monitoring of Proteome Dynamics

> Precision Medicine Development

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Clinical Research

& Development

Drug Rescue & Repurposing

Planned sales model and key customer segments

Planned strategic elements of the platform designed to create competitive advantage in the field

Phases leading to commercial launch planned for 2025

Every step represents a fundamentally new and unprecedented use of our technology

Today	2025			
Note: timeline not to scale	3. Launch of Proteome Analysis Platform (Expected in late 2025) Shipment of First Instruments & Consumables Early Access Beta Testing, and Full Commercial Launch			
	2.b. First Broadscale Proteome Decoding Data			
	Early Access Program for High-Output Discovery Proteomics Launch in-house data production facility, support customer proof of concept studies			
2.a. Tau Protect Collaborations and Accelerate engage	oform Data nd Partnerships Jements with pharma partners and key academic collaborators using Tau as our first biomarker			
1. Leveraging Single-Molecule	Multi-Cycle Data Read-out			

Continue to Establish Collaborations & Partnerships Focused on Targeted Proteomics Engage early through research collaborations, build a foundation of publications

Patent Portfolio Summary

(as of October 29, 2024)

Overall Process (3 Families)3 US Pending1 PCT8 US GrantedEP, CN, JP, IN									
APPLICATIONS & SAMPLE PREP 12 Families	ARRAYS 15 Families	INSTRUMENT HARDWARE 6 Families	PROBES & REAGENTS 17 Families	INSTRUMENT SOFTWARE 2 Families	DECODING & BIOINFORMATICS 6 Families				
12 US Pending 1 US Granted	21 US Pending 8 US Granted	7 US Pending 1 US Granted	24 US Pending 3 US Granted	2 US Pending	6 US Pending 4 US Granted				
4 PCT EP, CA, AU	5 PCT EP,CN, JP, CA, AU, IN, IL	2 PCT EP, CA, AU, HK	5 PCT EP, CN, JP, IN, HK, CA, AU, KR	EP, CA, AU	3 PCT EP, CN, JP, CA, AU, IN, BR, MX, HK, IL, KR,HK				

Why Nautilus?

We believe that humanity needs a dramatic acceleration of drug development and that a bold scientific leap is required to make possible a new world of precision and personalized medicine.

To deliver, we need to radically reinvent proteomics, a large untapped opportunity in biological science today.

Potential for revolutionizing biomedicine

Proven team, driven to win

Significant new potential market opportunity

Designed to address what the market wants – the proteome at single molecule resolution, enabling unprecedented sensitivity and scale

NAUTILUS^M BIOTECHNOLOGY

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