NAUTILUS™

BIOTECHNOLOGY

Delivering on the Promise of the Proteome

MARCH 12, 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 of Nautilus Biotechnology's proteomics technology platform; statements regarding Nautilus Biotechnology's future development milestones and timing; 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 (expressed or implied) are made about the accuracy of any such forward-looking statements.

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Nautilus seeks to revolutionize biomedicine

Single-molecule proteome analysis platform

with integrated machine learning designed to enable unprecedented **sensitivity and scale**

Potential to unlock a massive market

$55+ Billion opportunity across proteomics and adjacent markets by 2027
(Source: BCC Research)

Research collaborations

Genentech
A Member of the Roche Group
AMGEN
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
tgen part of City of Hope

Defining a new gold standard for single-molecule protein analysis

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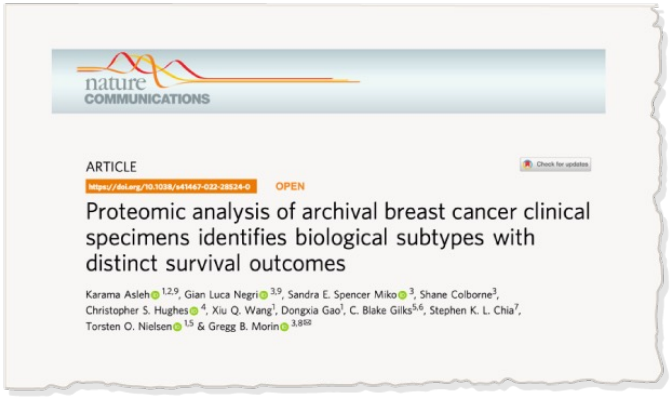
Proteins are key drivers of biology

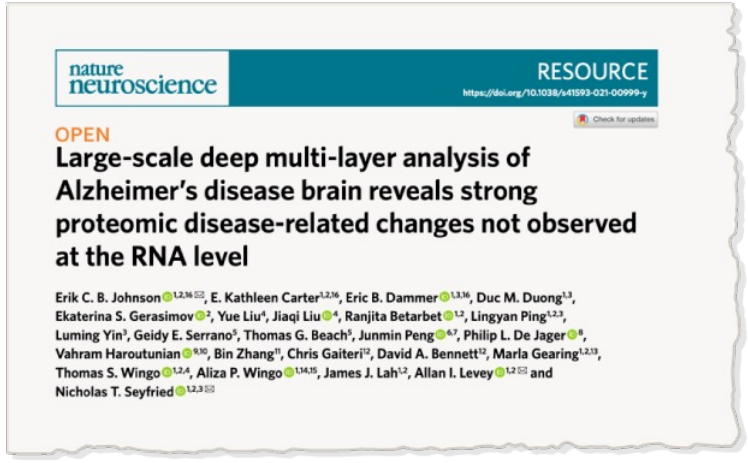

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-28524-0 OPEN

Proteomic analysis of archival breast cancer clinical specimens identifies biological subtypes with distinct survival outcomes

Karama Asleh[1,2,9], Gian Luca Negri[3,9], Sandra E. Spencer Miko[3], Shane Colborne[3], Christopher S. Hughes[4], Xiu Q. Wang[1], Dongxia Gao[1], C. Blake Gilks[5,6], Stephen K. L. Chia[7], Torsten O. Nielsen[1,5] & Gregg B. Morin[3,8✉]


nature neuroscience

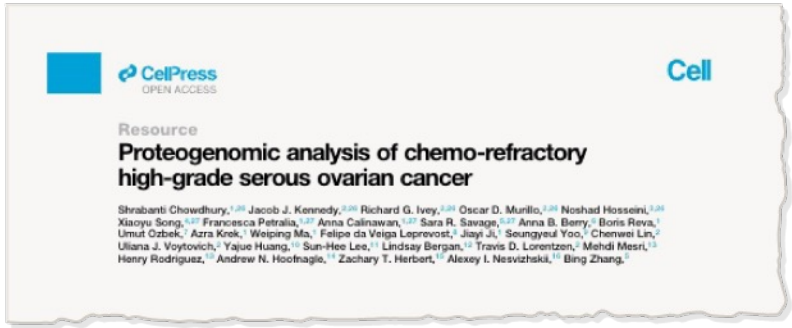
RESOURCE

https://doi.org/10.1038/s41593-021-00999-y

OPEN

Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level

Erik C. B. Johnson[1,2,16✉], E. Kathleen Carter[1,2,16], Eric B. Dammer[1,3,16], Duc M. Duong[1,3], Ekaterina S. Gerasimov[2], Yue Liu[4], Jiaqi Liu[4], Ranjita Betarbet[1,2], Lingyan Ping[1,2,3], Luming Yin[3], Geidy E. Serrano[5], Thomas G. Beach[5], Junmin Peng[4,7], Philip L. De Jager[6], Vahram Haroutunian[9,10], Bin Zhang[11], Chris Gaiteri[12], David A. Bennett[12], Marla Gearing[1,2,13], Thomas S. Wingo[1,2,4], Aliza P. Wingo[1,14,15], James J. Lah[1,2], Allan I. Levey[1,2✉] and Nicholas T. Seyfried[1,2,3✉]

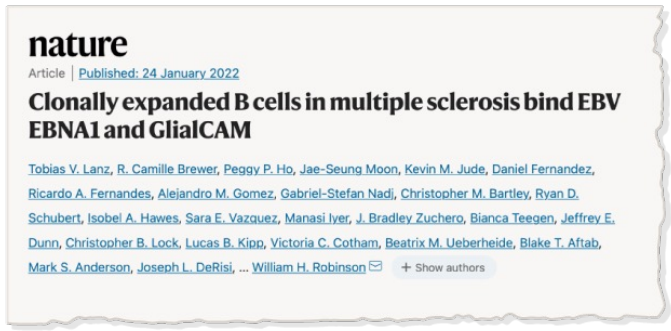

CellPress OPEN ACCESS

Cell

Resource

Proteogenomic analysis of chemo-refractory high-grade serous ovarian cancer

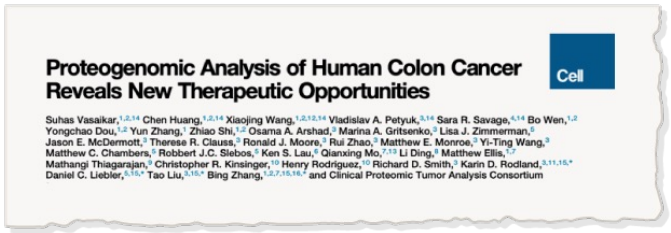
Shrabanti Chowdhury,[1,20] Jacob J. Kennedy,[2,20] Richard G. Ivey,[2,20] Oscar D. Murillo,[2,20] Noshad Hosseini,[2,20] Xiaoyu Song,[4,21] Francesca Petralia,[1,21] Anna Calinawan,[1,21] Sara R. Savage,[2,21] Anna B. Berry,[2] Boris Reva,[1] Umut Ozbek,[1] Azra Krek,[1] Weiping Ma,[1] Felipe da Veiga Leprevost,[2] Jiayi Ji,[1] Seungyeul Yoo,[1] Chenwei Lin,[2] Uliana J. Voytovich,[2] Yajue Huang,[12] Sun-Hee Lee,[11] Lindsay Bergan,[12] Travis D. Lorentzen,[2] Mehdi Mesri,[13] Henry Rodriguez,[10] Andrew N. Hoofnagle,[14] Zachary T. Herbert,[15] Alexey I. Nesvizhskii,[16] Bing Zhang,[5]


nature

Article | Published: 24 January 2022

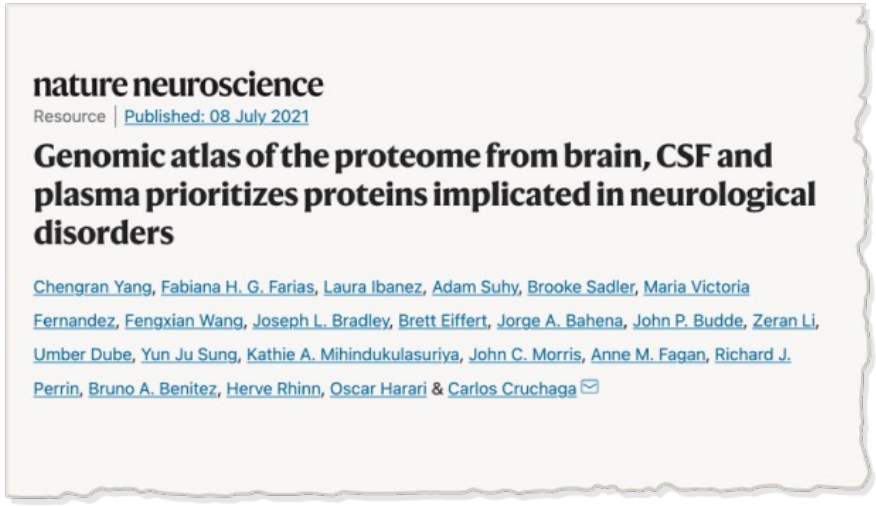
Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM

Tobias V. Lanz, R. Camille Brewer, Peggy P. Ho, Jae-Seung Moon, Kevin M. Jude, Daniel Fernandez, Ricardo A. Fernandes, Alejandro M. Gomez, Gabriel-Stefan Nadi, Christopher M. Bartley, Ryan D. Schubert, Isobel A. Hawes, Sara E. Vazquez, Manasi Iyer, J. Bradley Zuchero, Bianca Teegen, Jeffrey E. Dunn, Christopher B. Lock, Lucas B. Kipp, Victoria C. Cotham, Beatrix M. Ueberheide, Blake T. Aftab, Mark S. Anderson, Joseph L. DeRisi, ... William H. Robinson ✉ + Show authors


Proteogenomic Analysis of Human Colon Cancer Reveals New Therapeutic Opportunities

Cell

Suhas Vasaikar,[1,2,14] Chen Huang,[1,2,14] Xiaojing Wang,[1,2,12,14] Vladislav A. Petyuk,[3,14] Sara R. Savage,[4,14] Bo Wen,[1,2] Yongchao Dou,[1,2] Yun Zhang,[1] Zhiao Shi,[1,2] Osama A. Arshad,[2] Marina A. Gritsenko,[3] Lisa J. Zimmerman,[3] Jason E. McDermott,[3] Therese R. Clauss,[3] Ronald J. Moore,[3] Rui Zhao,[3] Matthew E. Monroe,[3] Yi-Ting Wang,[3] Matthew C. Chambers,[5] Robbert J.C. Slebos,[5] Ken S. Lau,[6] Qianxing Mo,[7,13] Li Ding,[8] Matthew Ellis,[1,7] Mathangi Thiagarajan,[9] Christopher R. Kinsinger,[10] Henry Rodriguez,[10] Richard D. Smith,[3] Karin D. Rodland,[3,11,15,*] Daniel C. Liebler,[5,15,*] Tao Liu,[3,15,*] Bing Zhang,[1,2,7,15,16,*] and Clinical Proteomic Tumor Analysis Consortium


nature neuroscience

Resource | Published: 08 July 2021

Genomic atlas of the proteome from brain, CSF and plasma prioritizes proteins implicated in neurological disorders

Chengran Yang, Fabiana H. G. Farias, Laura Ibanez, Adam Suhy, Brooke Sadler, Maria Victoria Fernandez, Fengxian Wang, Joseph L. Bradley, Brett Eiffert, Jorge A. Bahena, John P. Budde, Zeran Li, Umber Dube, Yun Ju Sung, Kathie A. Mihindukulasuriya, John C. Morris, Anne M. Fagan, Richard J. Perrin, Bruno A. Benitez, Herve Rhinn, Oscar Harari & Carlos Cruchaga ✉

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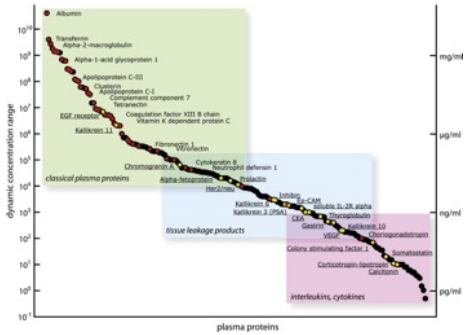
Interrogating the proteome is challenging

Proteins span a wide dynamic range

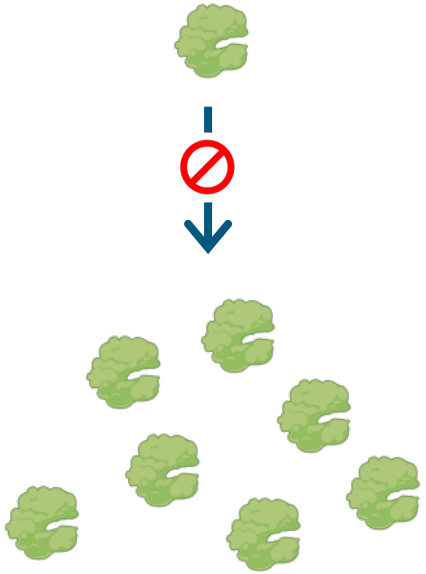
From single-digit numbers of molecules to millions of copies per cell (or drop of blood)



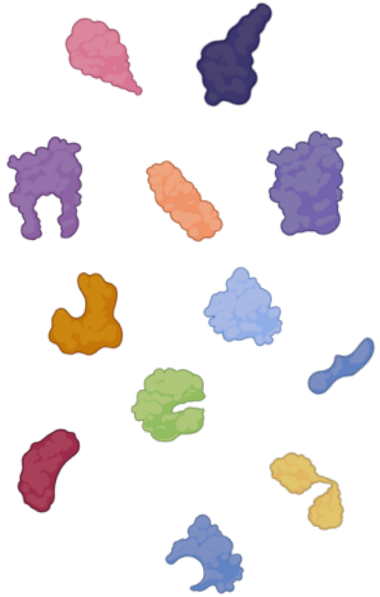
Anderson, N. L. *Molecular & Cellular Proteomics* 1, 845–867 (2002).

There is no PCR for proteins

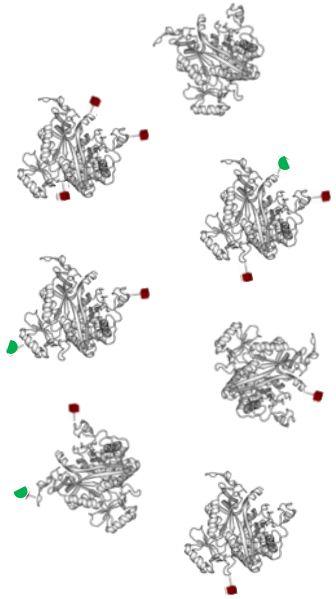
Amplification isn't possible



Proteins are biophysically extremely diverse



Proteins exist in a range of modified states (proteoforms)



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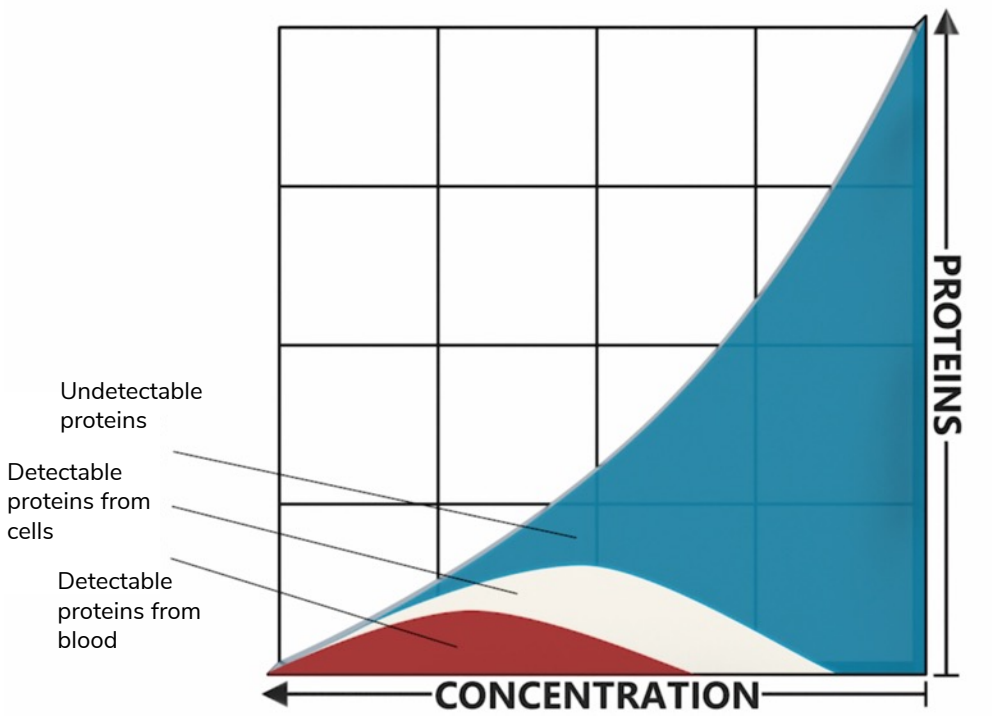
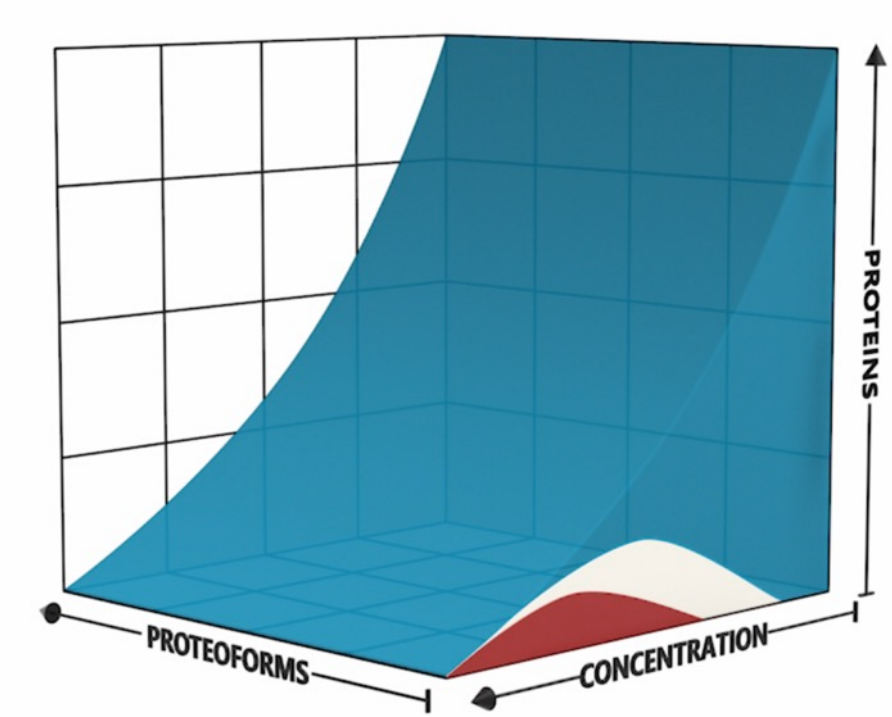
Current analysis methods can't see most proteins and proteoforms



Mass spec solutions today only capture a fraction of the proteome from blood or cells



There is no solution today to measure and quantify intact proteoforms

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Nautilus is reinventing proteomics from the ground up

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

Comprehensive	→	Measure substantively all the proteins and proteoforms in a sample
Sensitive	→	Single-molecule detection
Wide dynamic range	→	Match the scale of the proteome
Reproducible and robust	→	Path to clinical translation of discoveries
Rapid run time	→	Process a large number of samples
Easy to use	→	Any lab can run it

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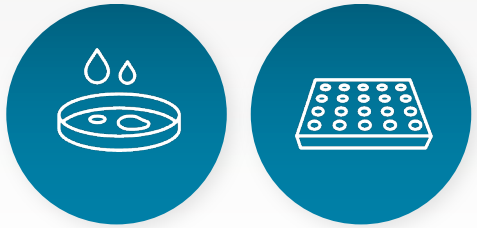
Core platform components

Sample Preparation & Single-Molecule Protein Deposition



HYPER-DENSE SINGLE MOLECULE ARRAY

Instrumentation and Reagents for Iterative Affinity Reagent Hybridization and Imaging

DIGITAL PROTEOMIC DATA

Machine Learning-Based Analysis

PROTEIN DECODING ANALYTICS

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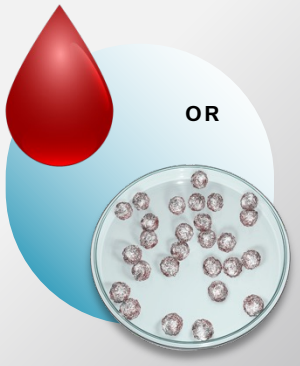
Simple and robust sample preparation workflow

Arraying single protein molecules onto a hyper-dense chip

STEP 1

Blood or cells



STEP 2

Extracted protein sample

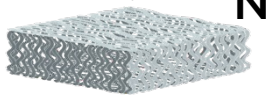
STEP 3

Functionalization of protein on cysteines and lysines

STEP 4

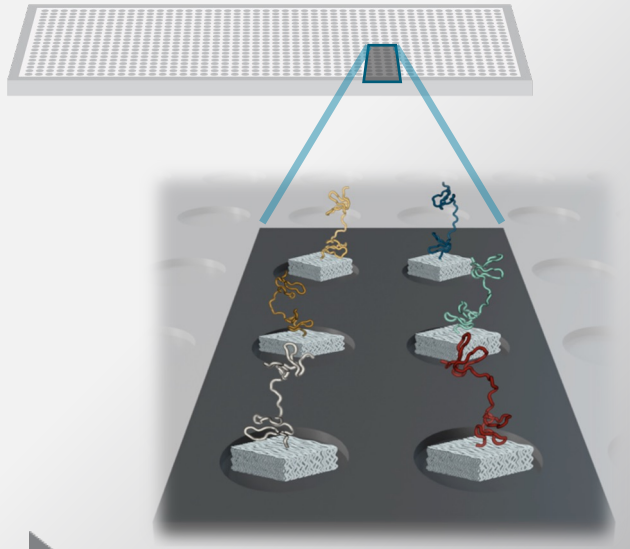
Click-based conjugation of protein to scaffold

Nanoparticle scaffold



STEP 5

Deposition on Nanofabricated Chip



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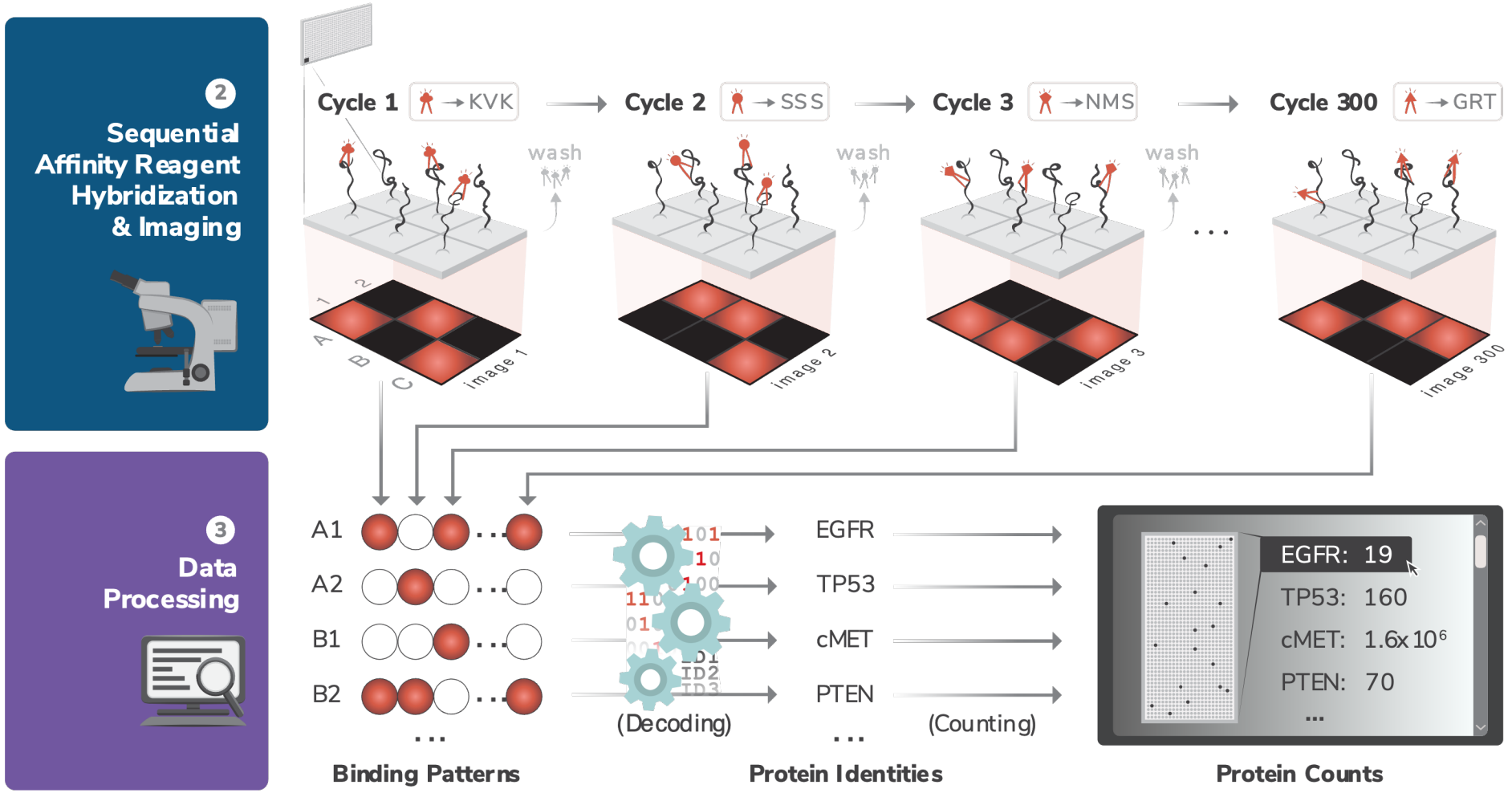
Integration of breakthrough innovations across the platform

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



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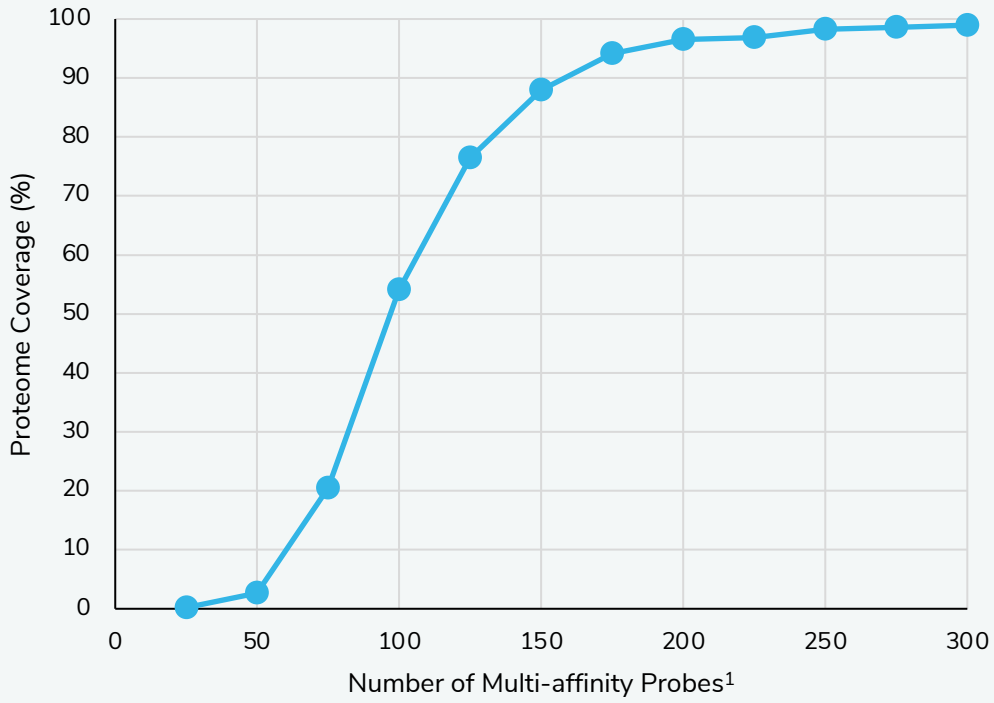
Designed to comprehensively quantify the proteome

Human Proteome Coverage Across 300 Unique Multi-affinity Probes



Capable of Achieving

>95% Proteome Coverage

[1]Estimates 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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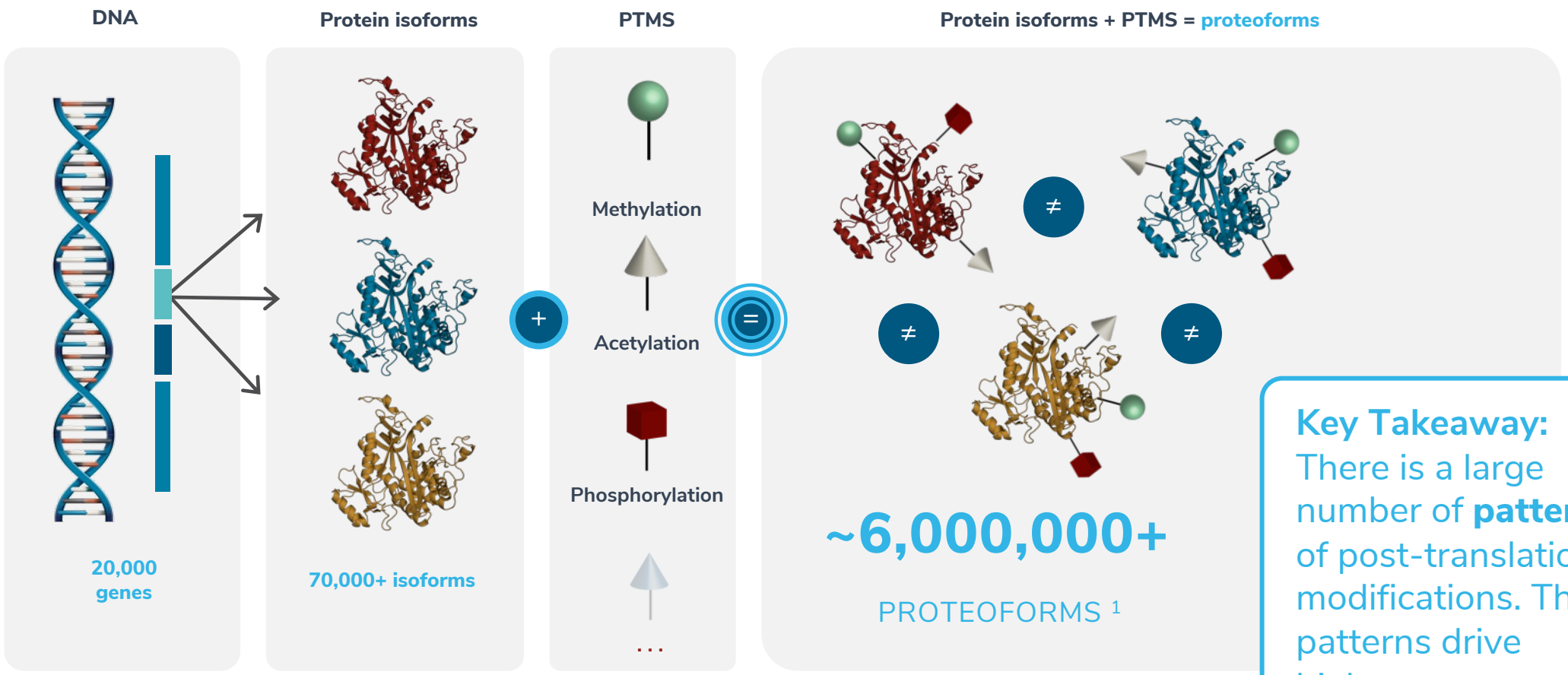
Nautilus: Revealing unseen proteoforms



Key Takeaway:
There is a large number of **patterns** of post-translational modifications. These patterns drive biology.

[1]International Journal of Analytical Chemistry. 2016; 2016: 7436849. The Size of the Human Proteome: The Width and Depth, Elena A. Ponomarenko et al.

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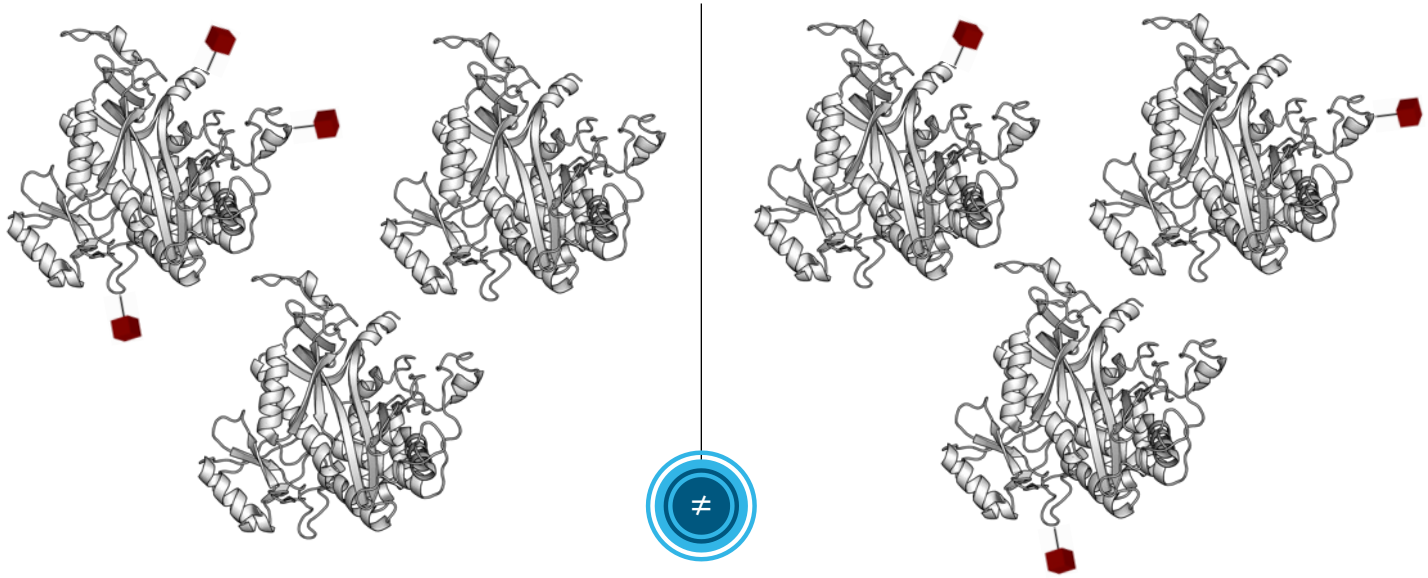
High-resolution proteoform (PTM) quantitation: a core application of Nautilus' platform

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



> "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**

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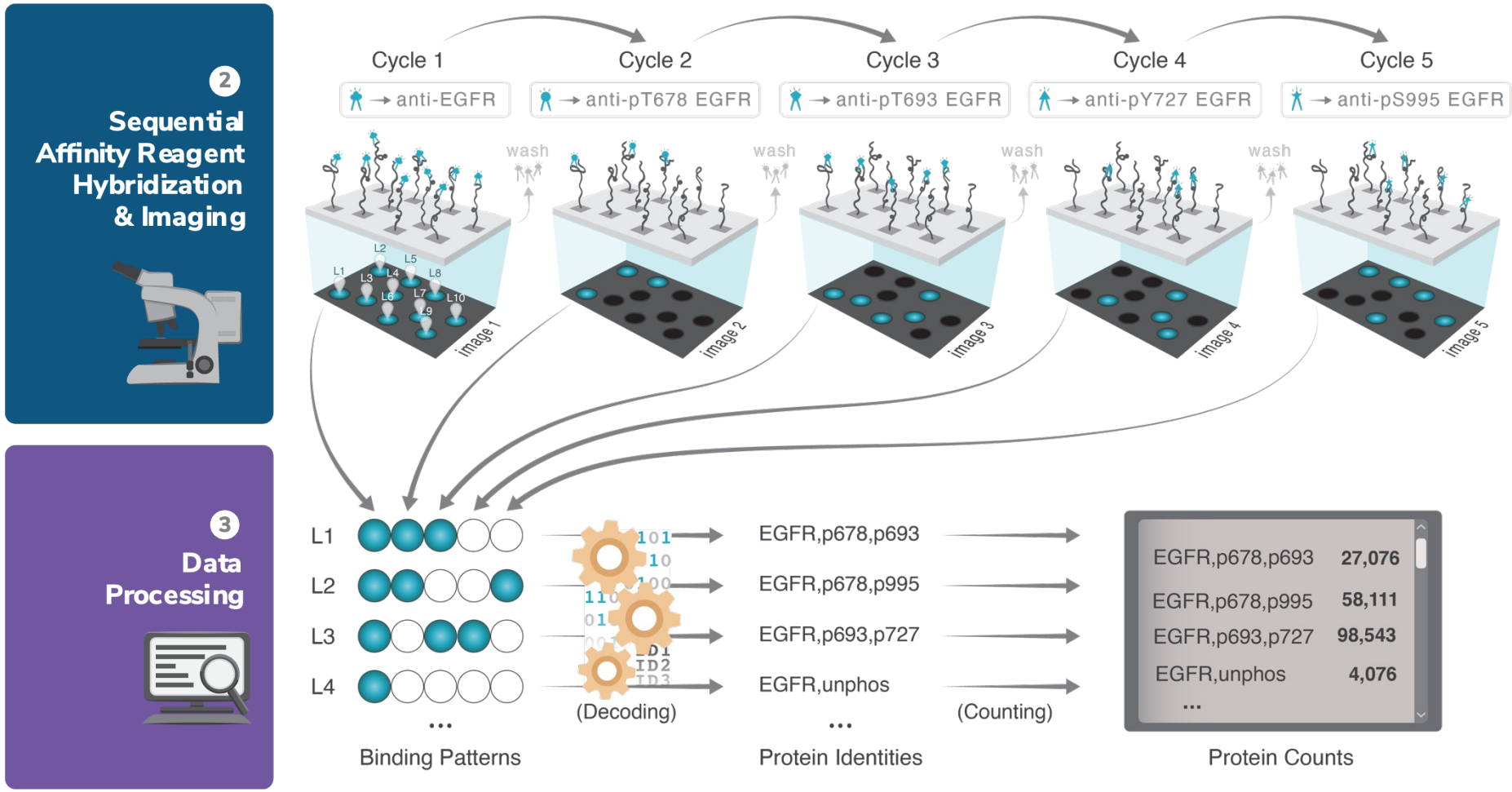
Integration of breakthrough innovations across the platform

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



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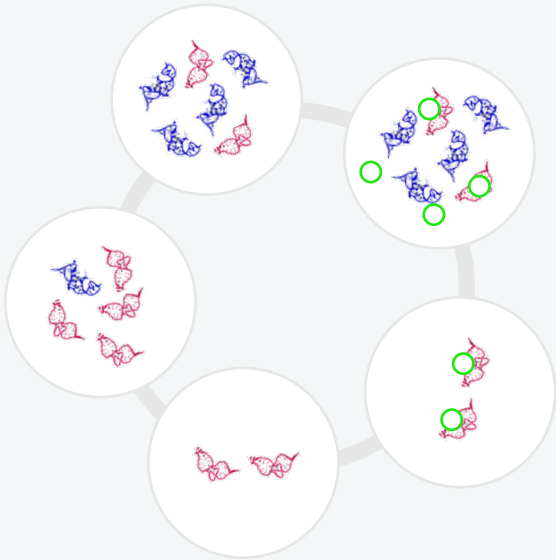
Open platform technology
designed to be compatible with many affinity reagent options

Off-the-shelf reagents for targeted proteoform analysis

Designed to be compatible with off-the-shelf antibodies

Nautilus affinity reagents designed for comprehensive proteome analysis



Multi-affinity aptamer

Engineered antibodies

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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 MD Anderson Cancer Center

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

tgen part of City of Hope

- 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…

Key Takeaways:

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

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

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

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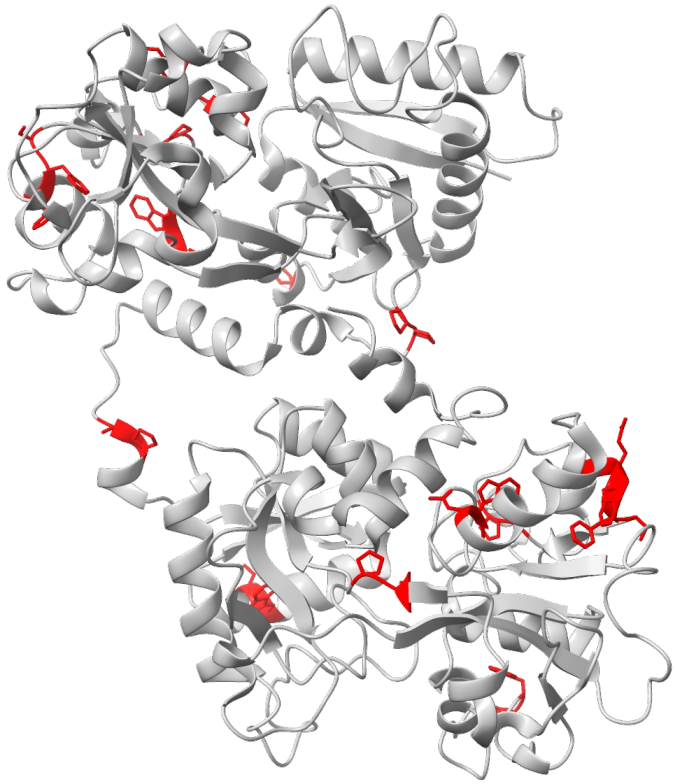
Experimental Findings Presented at March 2024 US HUPO Annual Conference

(5 slides)

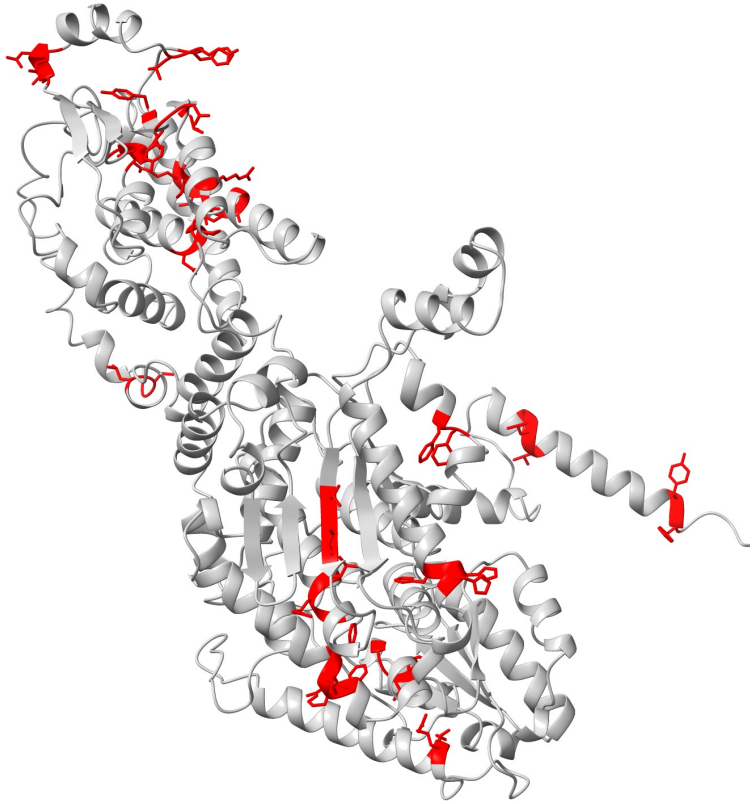
Multi-affinity probes bind to buried/structured regions in proteins

March 2024

- 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 full-length proteins
- We have demonstrated binding to short epitopes in internal portions of full-length proteins (red) using both aptamer- and antibody-based multi-affinity probes



Transferrin



G6PI

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False discovery rate in PrISM

March 2024

Identification	P-val
KRAS	.99998
P53	.9995
⋮	⋮
SMP1	.998
DECOY	DECOY
EGFR	.998

We apply a target-decoy based method to estimate what % of identifications are false

- Generate a database with decoy proteins that *look* like real proteins
- Analyze how often decoy IDs occur to estimate the false ID rate

In our system, the most likely failure mode occurs when proteins with high sequence similarity are mistaken as one another

Therefore, we want to generate decoys that:

Reflect the sequence structure of real proteins

Capture the relative likelihood of one protein being mis-identified as another

We demonstrated that decoys either generated with shuffled protein sequences or alternative proteomes effectively estimated the false discovery rates

We also demonstrate that the FDR estimation is not impacted by possible failure modes of the system, including mis-prediction of the binding rates between probes and proteins from the proteome

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Binding patterns to transferrin and G6PI at single-molecule resolution

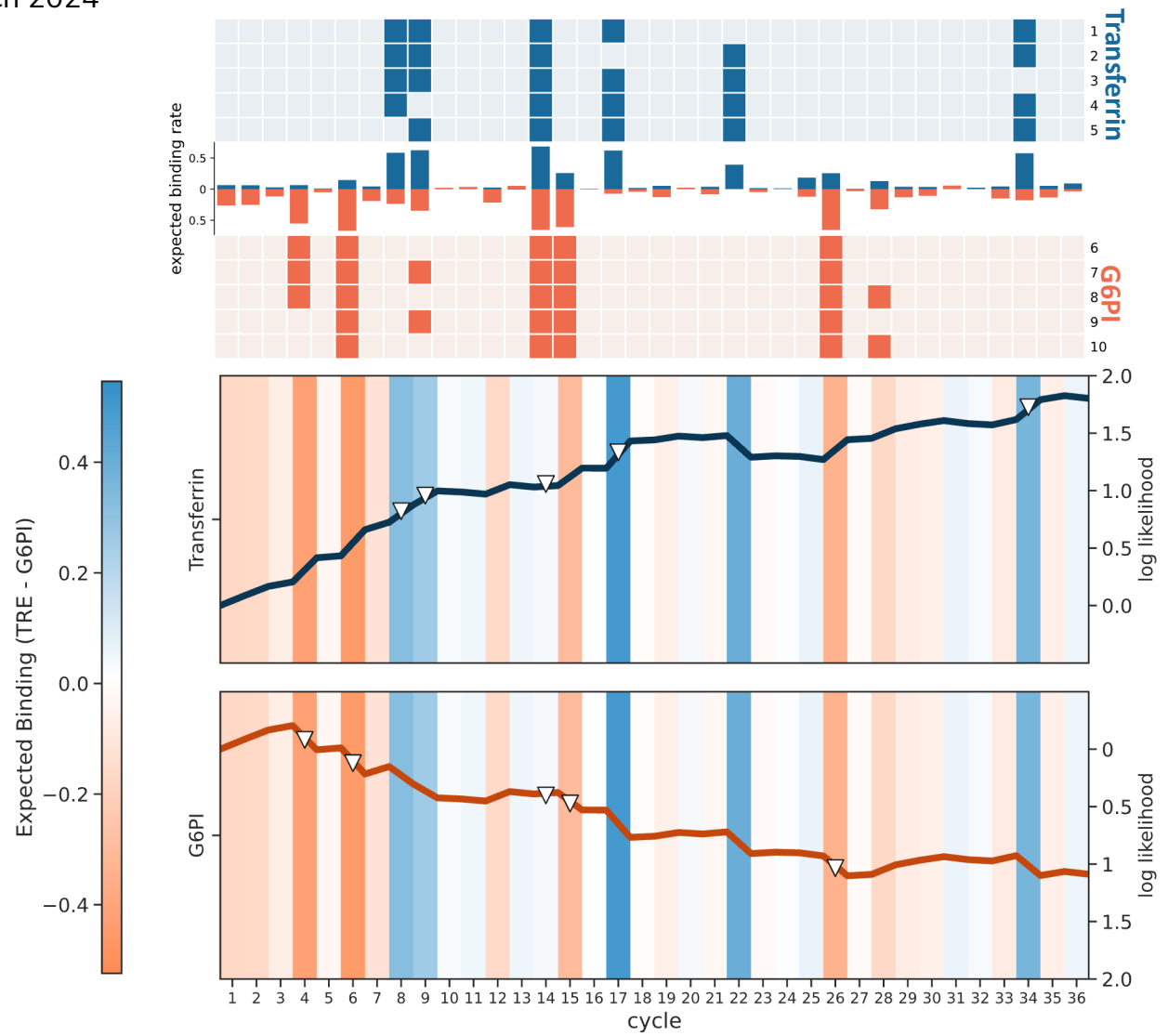
March 2024

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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Ultra-sensitive quantification of transferrin

March 2024

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

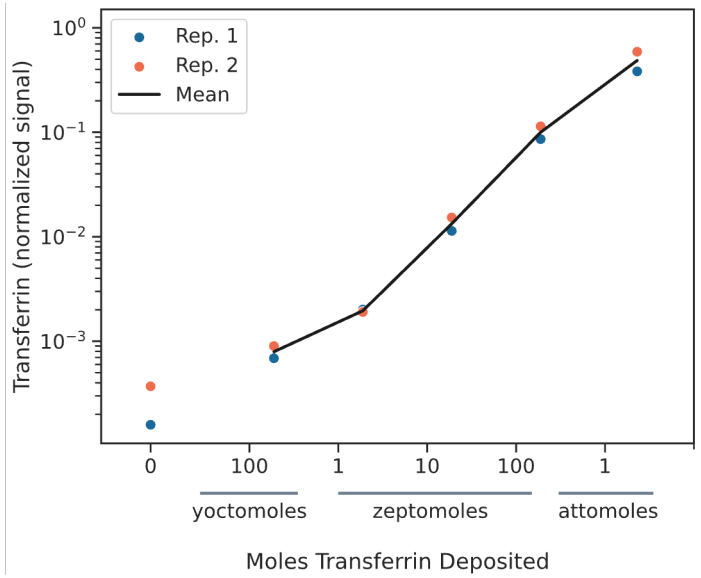
Reproducible single-molecule measurements: The highest abundance Transferrin measurement (2 attomoles) was repeated 7 times across 4 days and 7 flow cells with a CV of **7.7%**

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

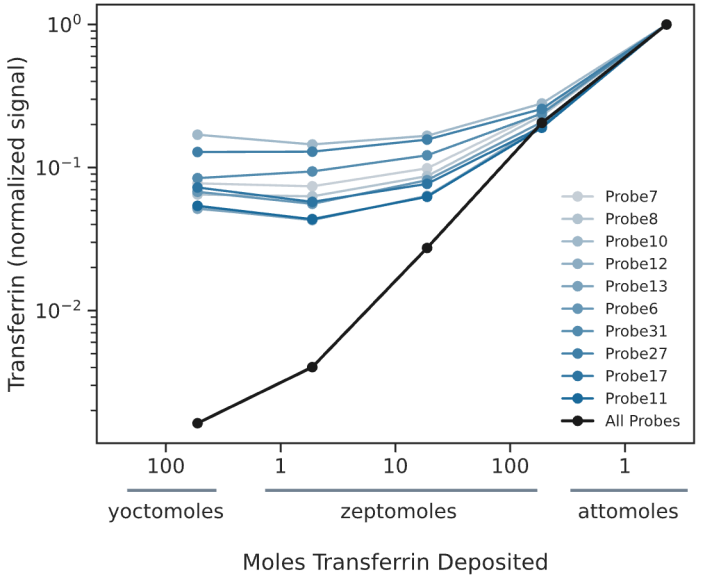
> This improvement arises from the ability of the machine learning software to better identify proteins whose identifications were derived from either false positive or false negative bindings

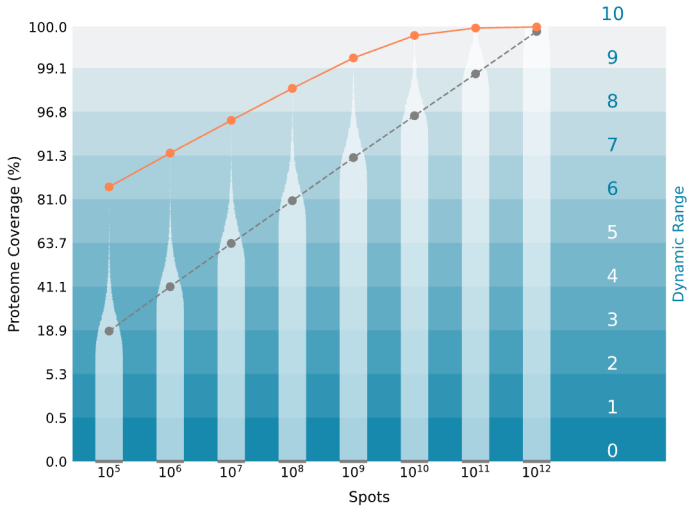
(BOT-LEFT) : As one increases the number of molecules measured with larger flowcells, one increases the dynamic range of the platform

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



Multi-probe decode vs single-probe





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.

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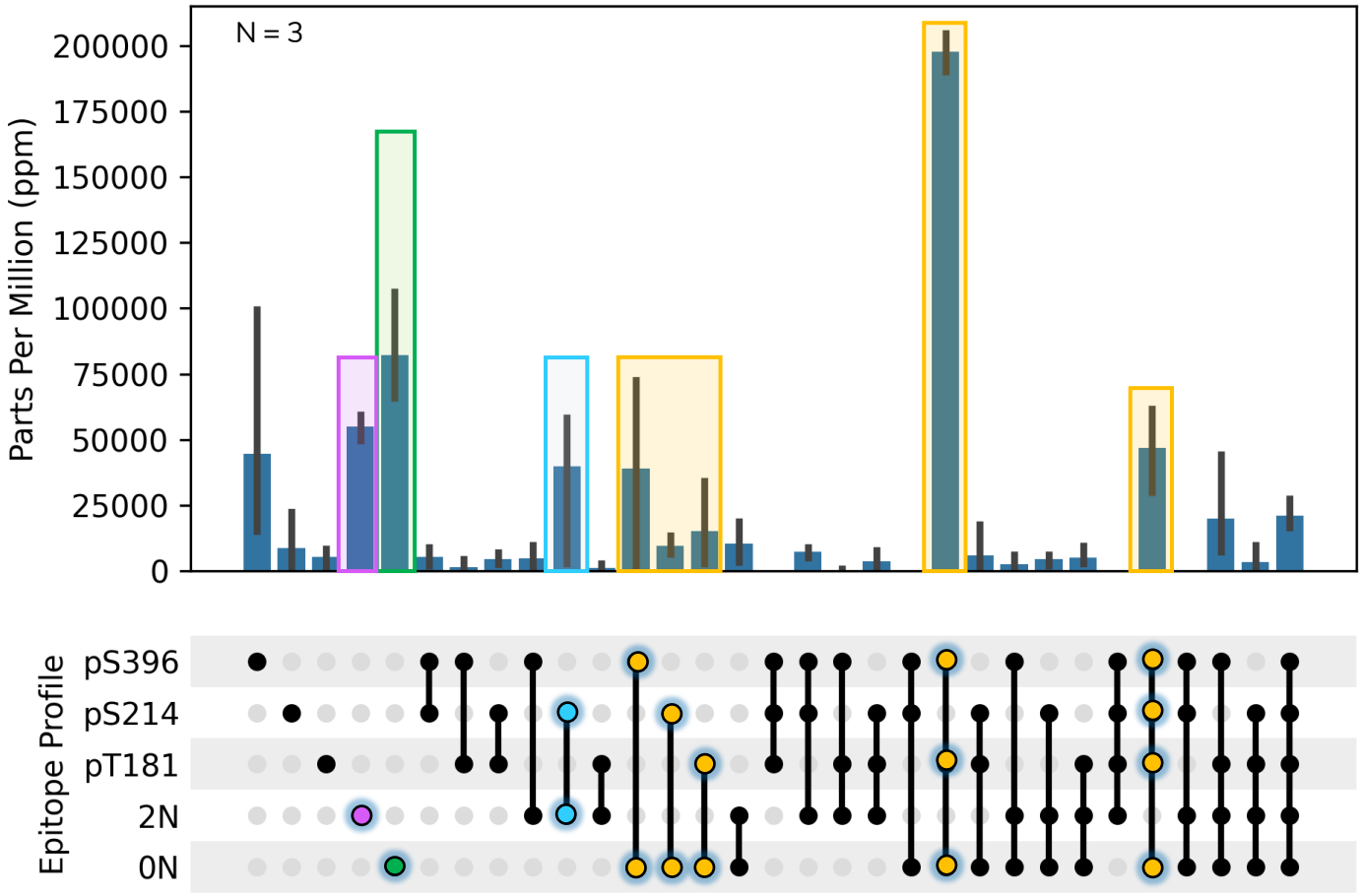
Quantification of mixtures of proteoforms

March 2024



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 EGFR proteoforms.

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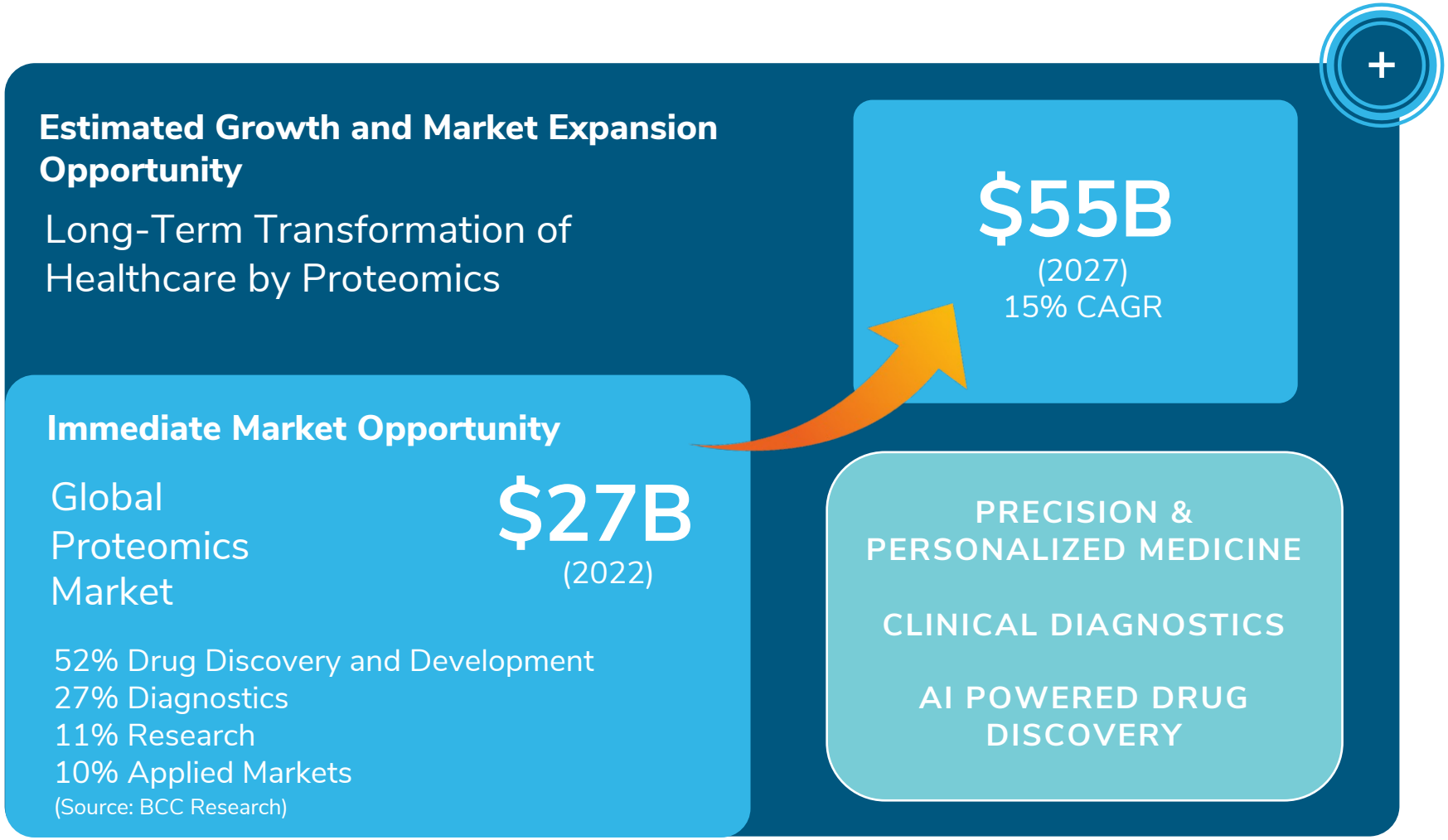
Large market opportunity ready for disruption



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Addressable markets & applications

Basic Sciences

Multi-Omics & Systems Biology

Proteoform Composition & Landscape

Proteome Profiling (species agnostic)

Translational Research

Biomarker & Drug Target Discovery

Mechanism of Action Studies

Toxicity Profiling and Prediction

Clinical Research & Development

Longitudinal Monitoring of Proteome Dynamics

Precision Medicine Development

Drug Rescue & Repurposing

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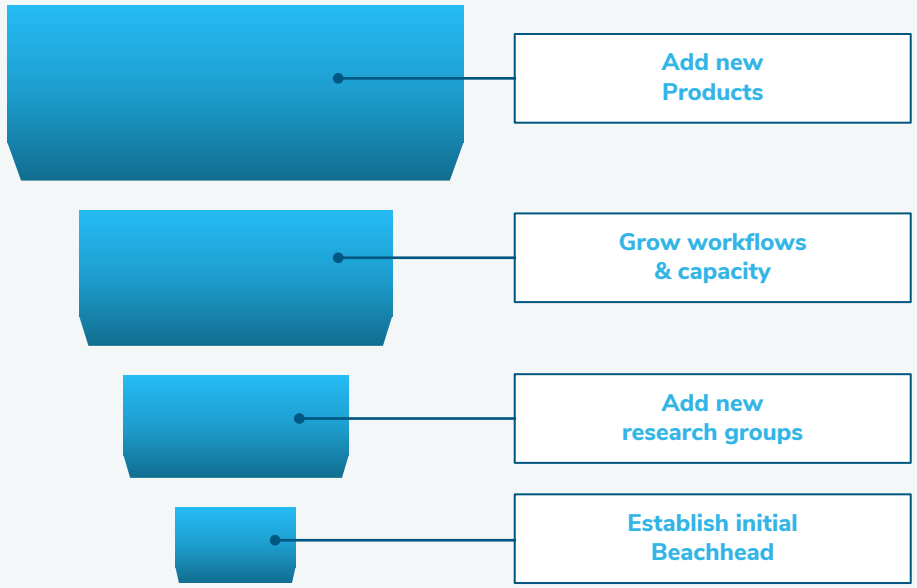
Planned sales model and key customer segments

Land and expand sales model



Target customers

- Pharmaceutical & Biotech
- Academic & Research Community
- Large-Scale Omics Core Laboratories
- Contract Research Organizations
- B2B Diagnostic Service Partnerships

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

First to Market with Novel Detection Platform

First mover advantage in a large and expanding market

Highly Disruptive Technology

Unlocks new sources of primary biological information

Immense Data Production Capacity

Drives discovery potential and technology ubiquity

Data is an asset

Proven Commercial Model

Average selling price enables efficient direct sales model (>$1M ASP)

Start in North America and then start building international footprint with distribution partners

Diversified and Recurring Revenue Sources

Partnerships

Instrumentation

Consumables

Service and support

Software as a service

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Phases leading to commercial launch planned for 2025

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

Note: timeline not to scale

III: Launch of Proteome Analysis Platform (Expected in 2025)

Shipment of First Instruments & Consumables

Early Access Beta Testing, and Full Commercial Launch

II: 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

I: 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

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Patent Portfolio Summary
(as of February 28, 2024)

Overall Process (3 Families)
3 US Pending
8 US Granted
EP, CN, JP, IN

APPLICATIONS & SAMPLE PREP	ARRAYS	INSTRUMENT HARDWARE	PROBES & REAGENTS	INSTRUMENT SOFTWARE	DECODING & BIOINFORMATICS
7 Families	12 Families	4 Families	15 Families	2 Families	6 Families
7 US Pending	17 US Pending 5 US Granted	5 US Pending	22 US Pending 1 US Granted	3 US Pending	6 US Pending 4 US Granted
5 PCT EP, CA, AU	5 PCT EP, CN, JP, CA, AU, IN, IL, KR, HK	2 PCT EP, CA, AU, HK	6 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

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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

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