MERCK
INVENTING FOR LIFE

Leveraging computation and HTP experimentation to engineer biologics

Grant Murphy

Executive Director,

Discovery Biologics

27 June 2023

Merck Research Labs Mission

To translate breakthroughs in fundamental biomedical research into meaningful new therapeutics and vaccines that improve and extend the lives of people, worldwide.

We're conducting R&D to address some of the world's most urgent global health challenges.

Merck Research Laboratories

Rahway, NJ

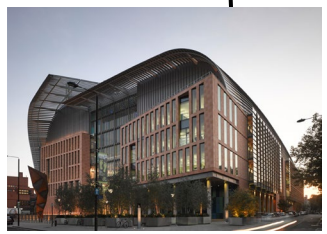
Boston, MA

San Francisco, CA

Cambridge, MA

Kenilworth, NJ

West Point, PA



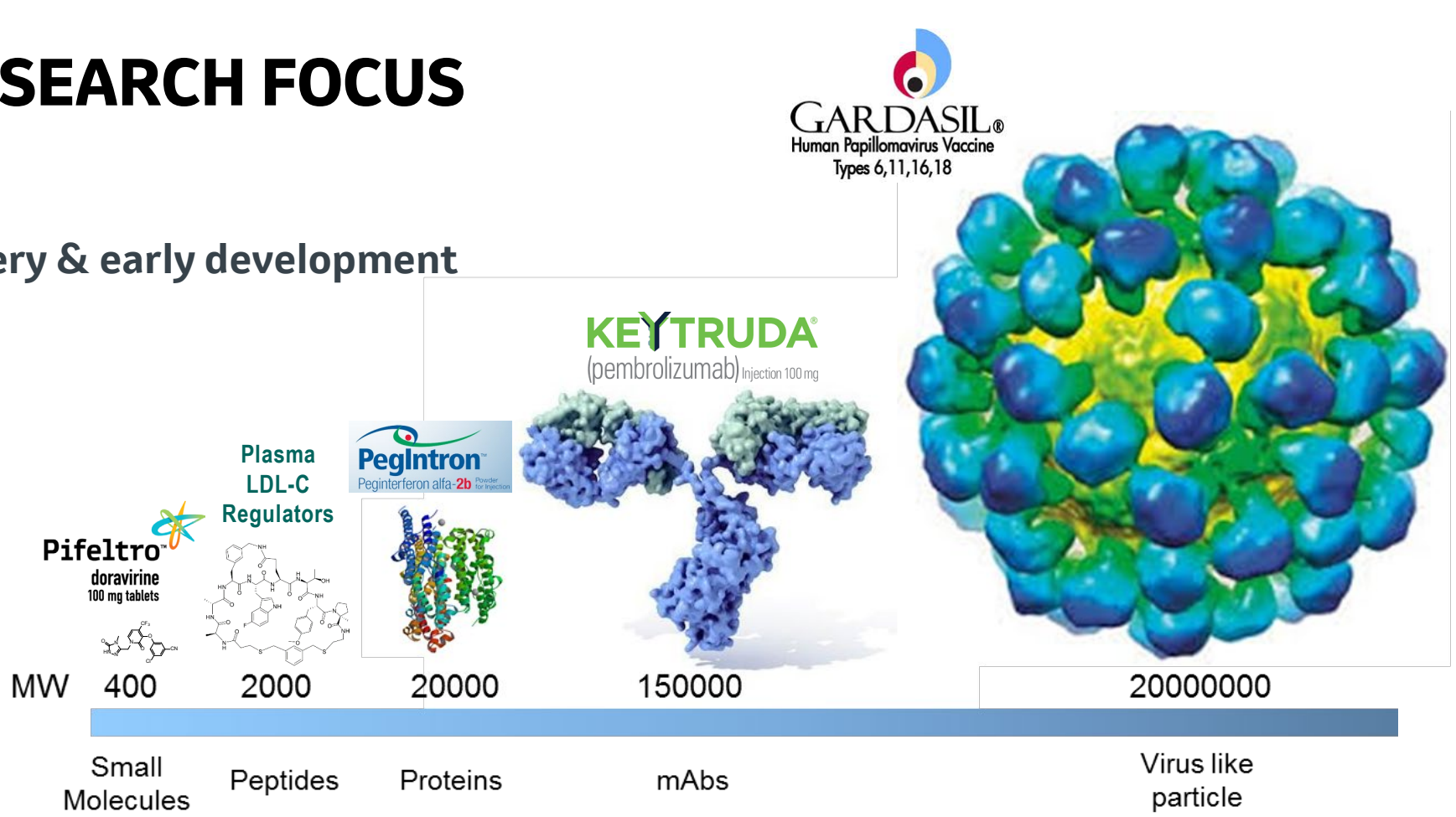
London, UK

OUR AREAS OF RESEARCH FOCUS

Diverse and Robust Pipeline

- **Over 150 programs in discovery & early development**
  - Oncology
  - Infectious Diseases
  - COVID-19
  - Vaccines
  - Cardio-metabolic Disorders

- **We take a modality-agnostic approach to solving human health challenges:**



- **Clinical Development**:

OUR GLOBAL LEADERSHIP IN ONCOLOGY

KEYTRUDA® (pembrolizumab) Injection 100 mg

Foundational cancer treatment

Lynparza® olaparib tablets 150 mg

First-in-class poly ADP ribose polymerase (PARP) inhibitor (collaboration with AstraZeneca)

LENVIMA® (lenvatinib) capsules 10 mg and 4 mg

Broad-based tyrosine kinase inhibitor (collaboration with Eisai to develop novel combination therapies with Keytruda)

- Committed to establishing strategic partnerships to develop the most effective therapies for our patients
- Alliance to Advance Patient-Centered Cancer Care
  - $15M, five-year (2017-2021) commitment from Merck Foundation
  - Aim to increase timely access to patient-centered care and reduce disparities in cancer care for underserved communities
- American Cancer Society (ACS) Global Navigation
  - Nearly $2M, five-year (2019-2023) commitment from Merck Foundation
  - Helps ACS bring its patient navigation expertise to countries in resource-limited settings and with a growing burden of cancer

30+	**2K+**	**1M+**
Cancers that our products are being studied in	Clinical trials involving our cancer medicines	Patients treated with commercially-available Merck oncology medicines (past 5 yrs)

Public

Our View of Protein Engineering

Multiple HPC Centers

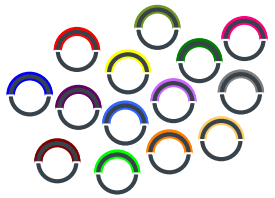
Internal PDB/Blast/etc

https://github.com/Merck/

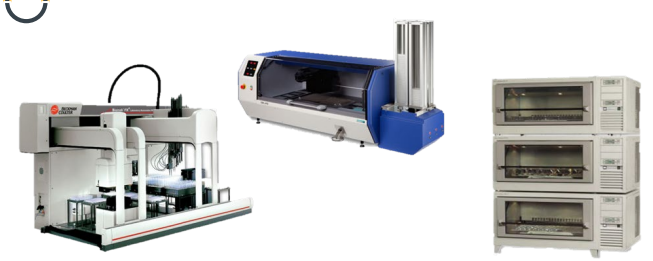
Broad collaborations

Computational Based Methods

Evolution Based Methods



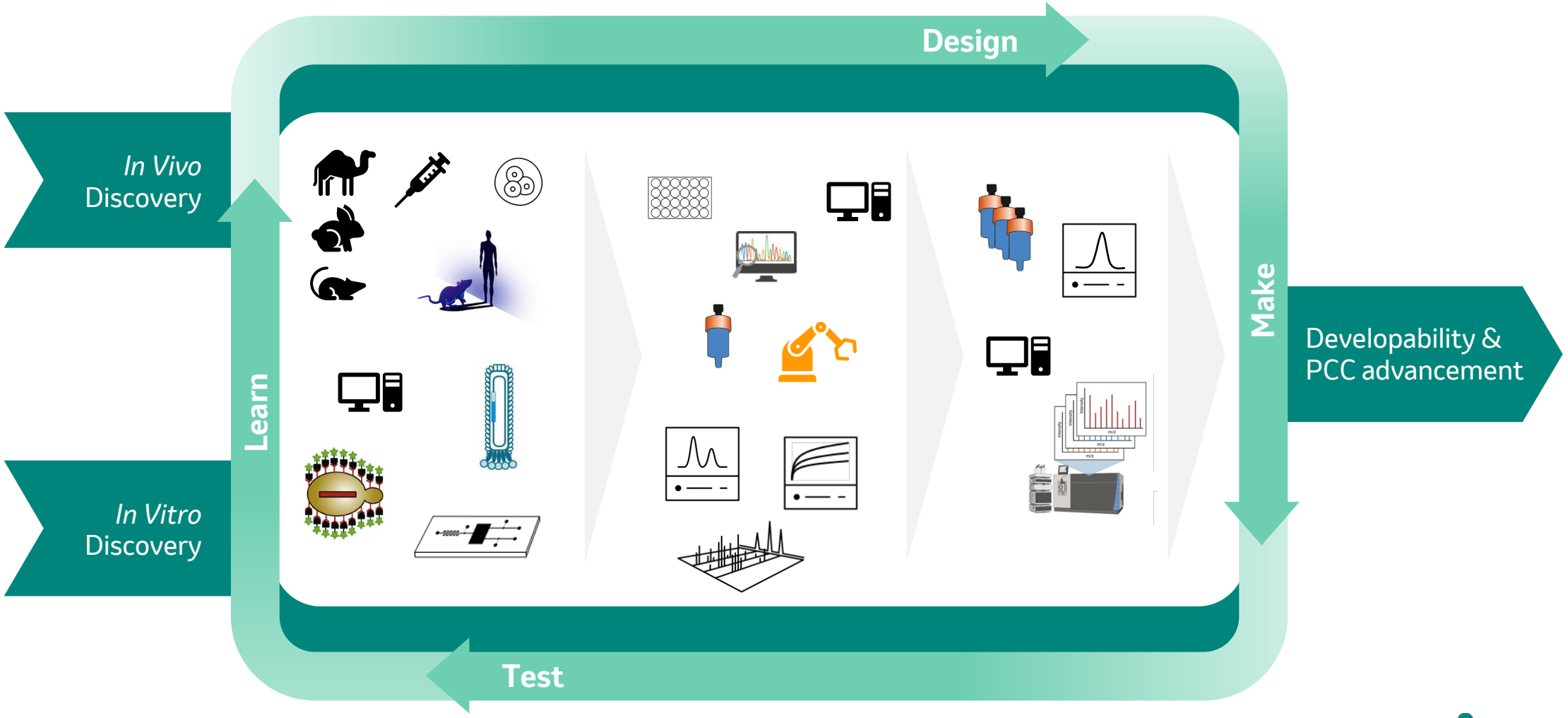
Machine Learning
Based Methods



Functional Protein

Public

Merck Discovery Biologics



Public

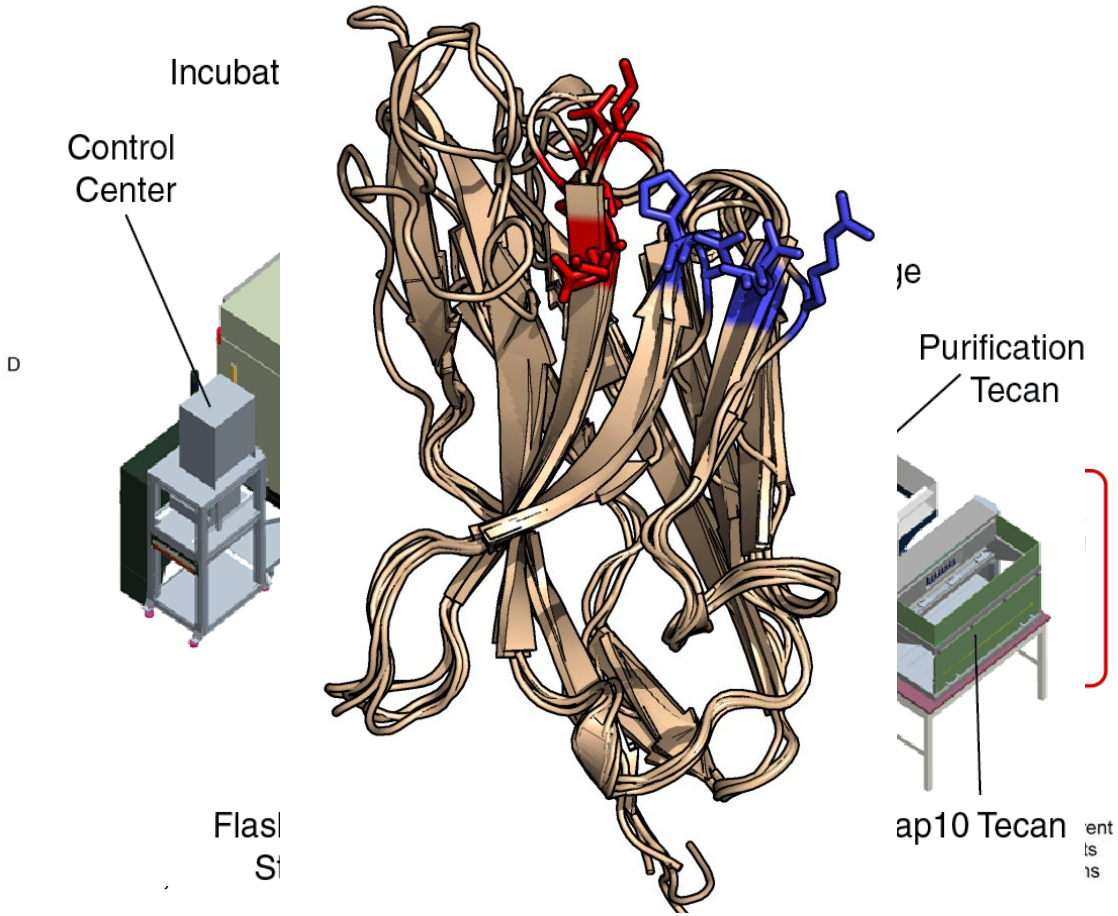
Discovery Biologics Technologies

Yeast Display & Phage Display

GlycoFi yeast strains w/ humanized glycosylation

Advanced Automation

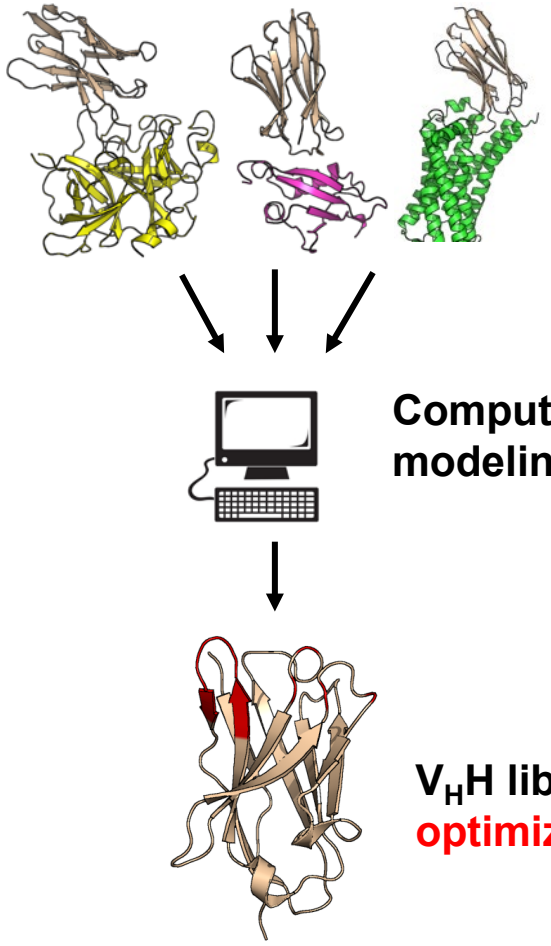
Computational Design & Machine Learning



Public

Structure- and sequence-based analysis of VHH

Structural analysis of known VHH



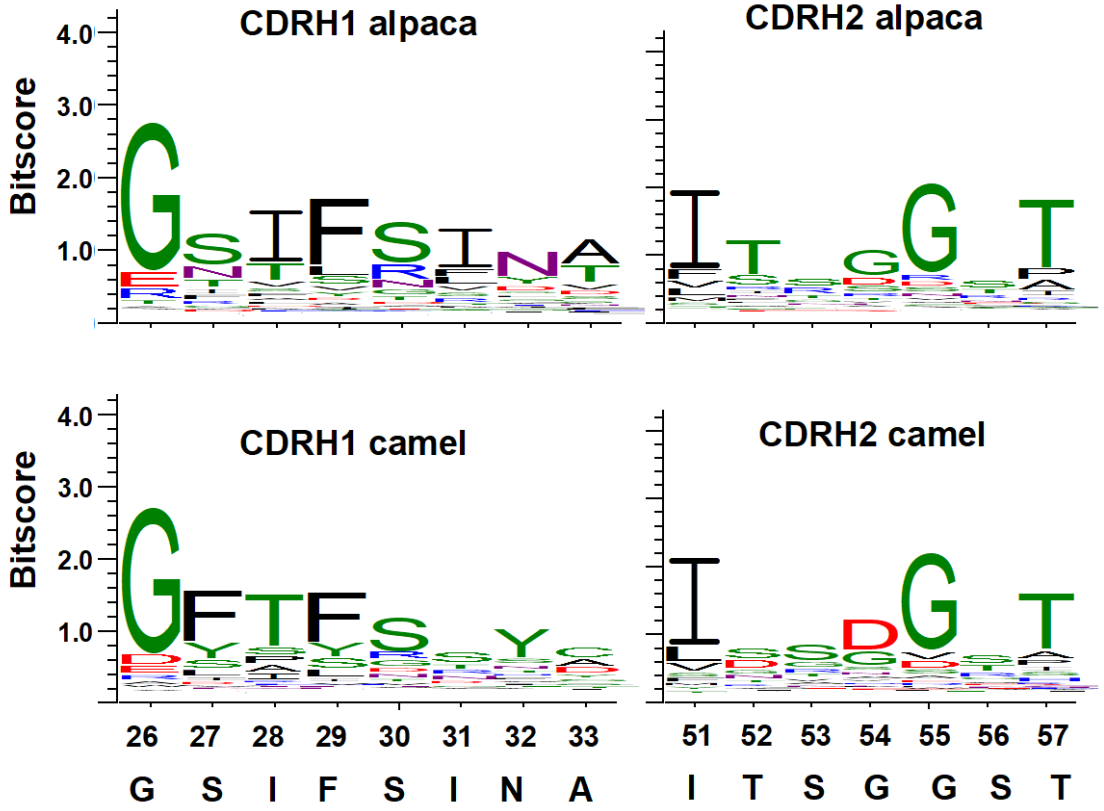
V_HH-antigen structures

Computational modeling

V_HH library with optimized diversity

Dataset: 208 VHH-antigen complex structures

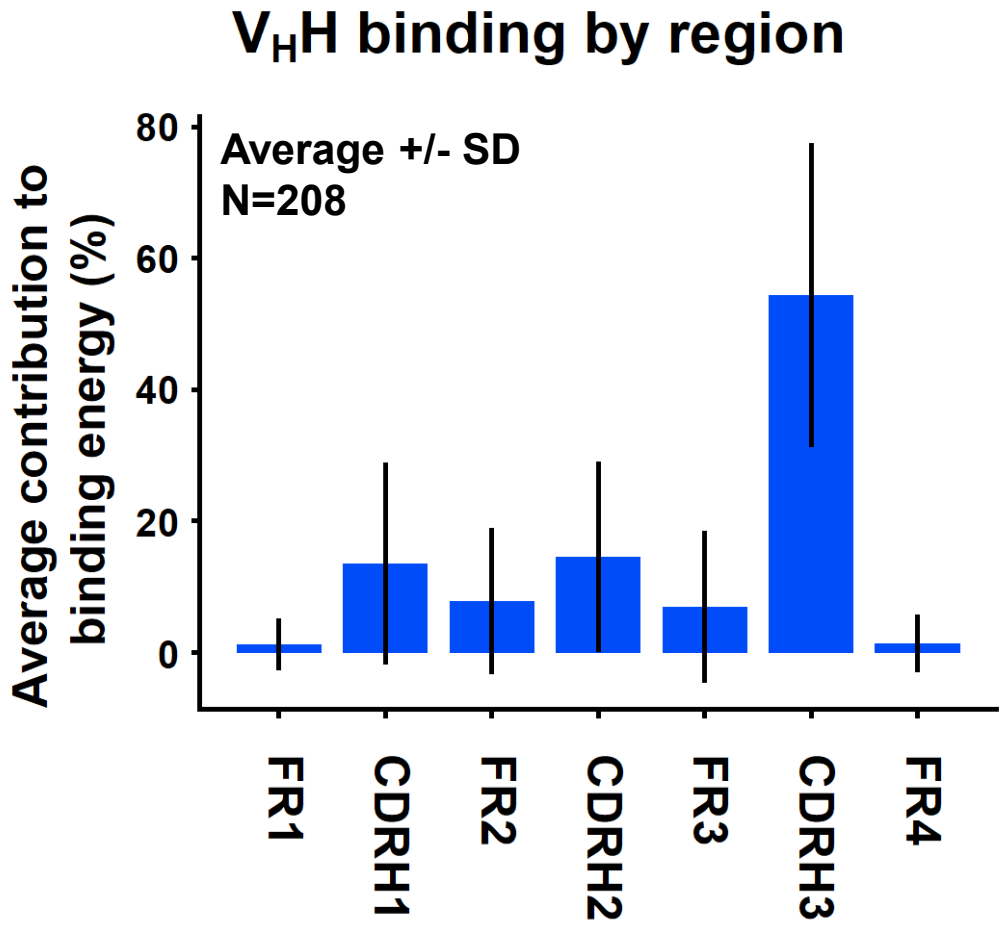
Sequence analysis of camelid repertoires



Sevy, PEDS, 2020

Public

Which region of the V_HH is responsible for binding?

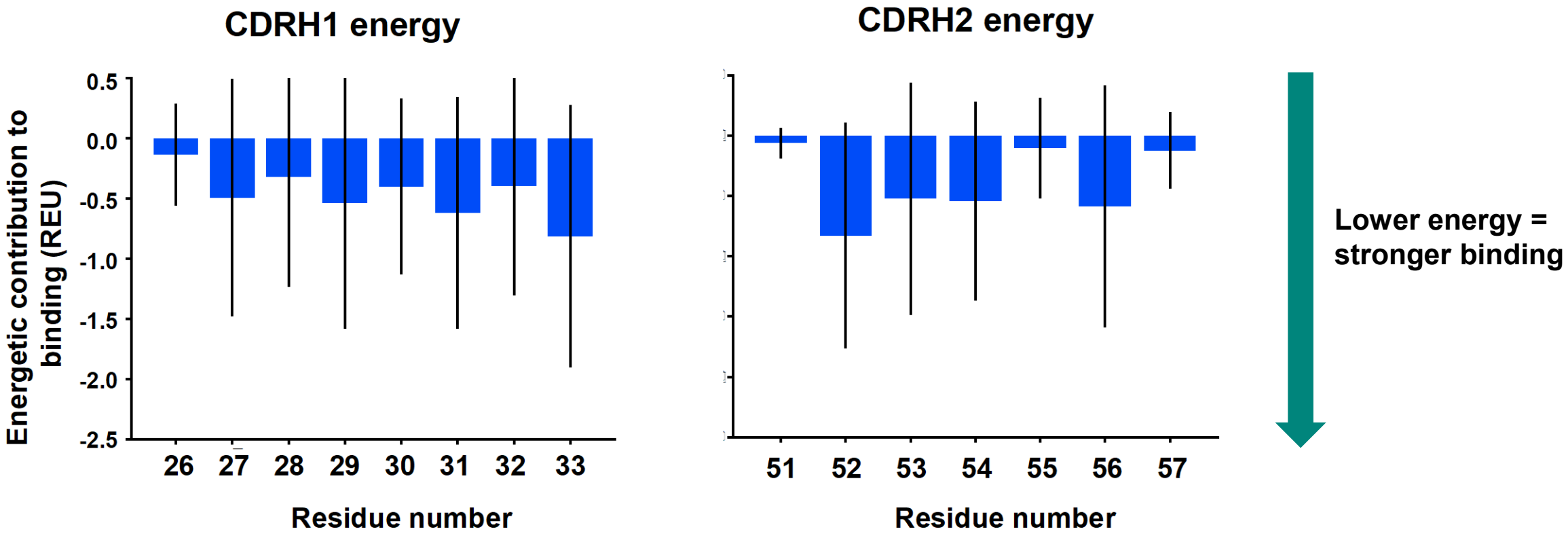


- Calculated the computational binding energy of each residue along the V_HH and grouped by region
- CDRH3 dominates interaction with antigen
- CDRH1 and 2 contribute roughly the same amount of interaction on average

Dataset: 208 VHH-antigen complex structures

Public

Which residues along the V_HH tend to contribute to binding?



Dataset: 208 VHH-antigen complex structures

Error bars = mean +/- SD

Public

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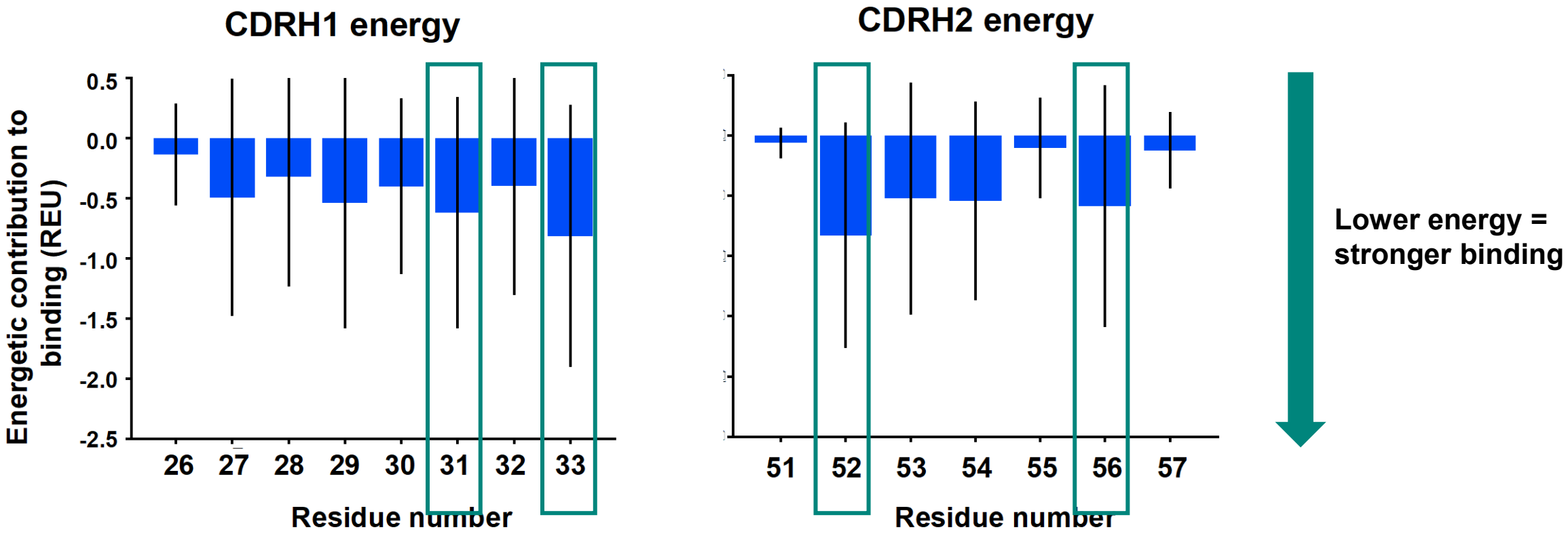


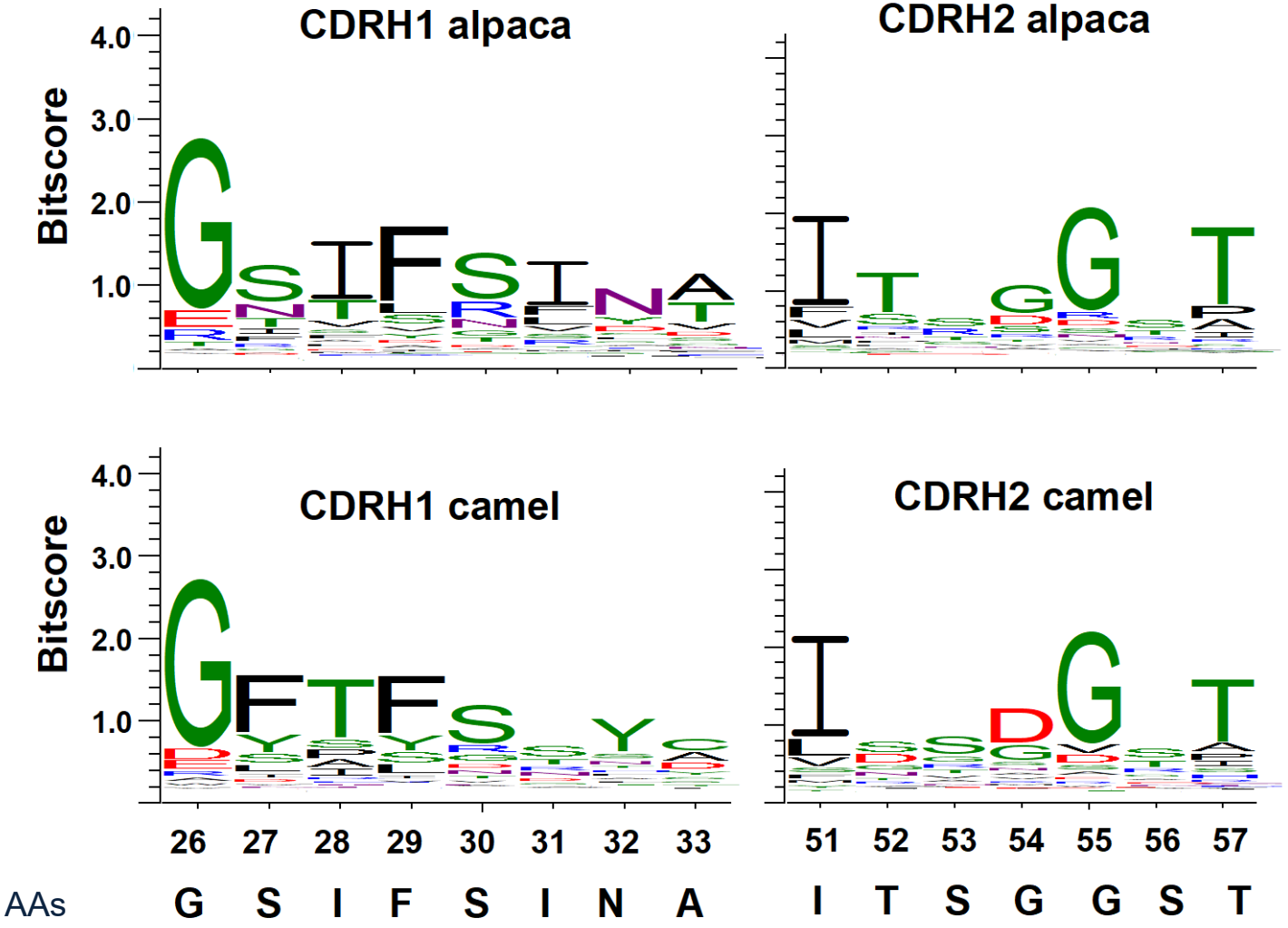
Candidates for diversity

Dataset: 208 VHH-antigen complex structures

Error bars = mean +/- SD

Public

Which positions in the CDR1 and 2 can tolerate randomization in a library?



Public

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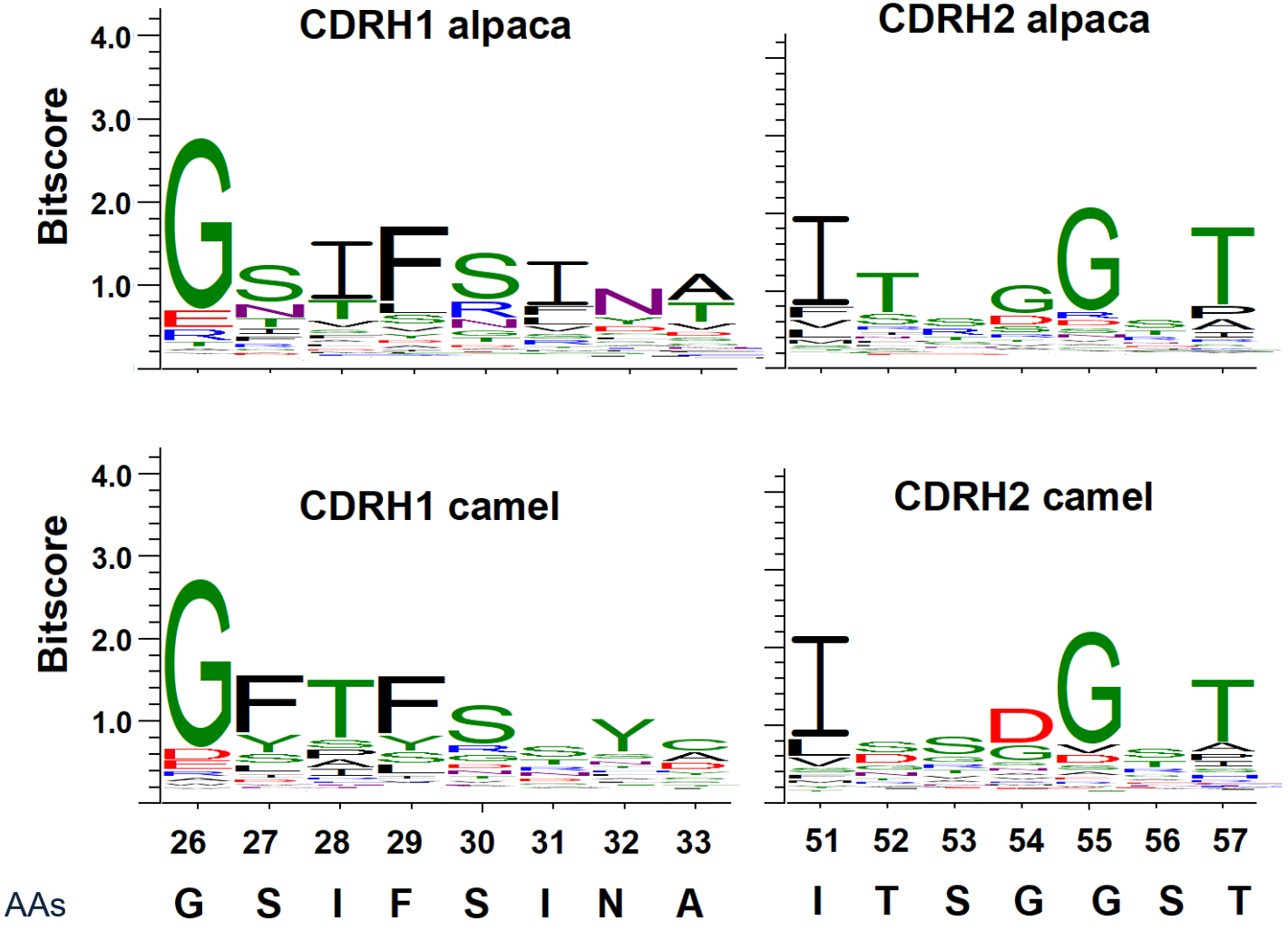
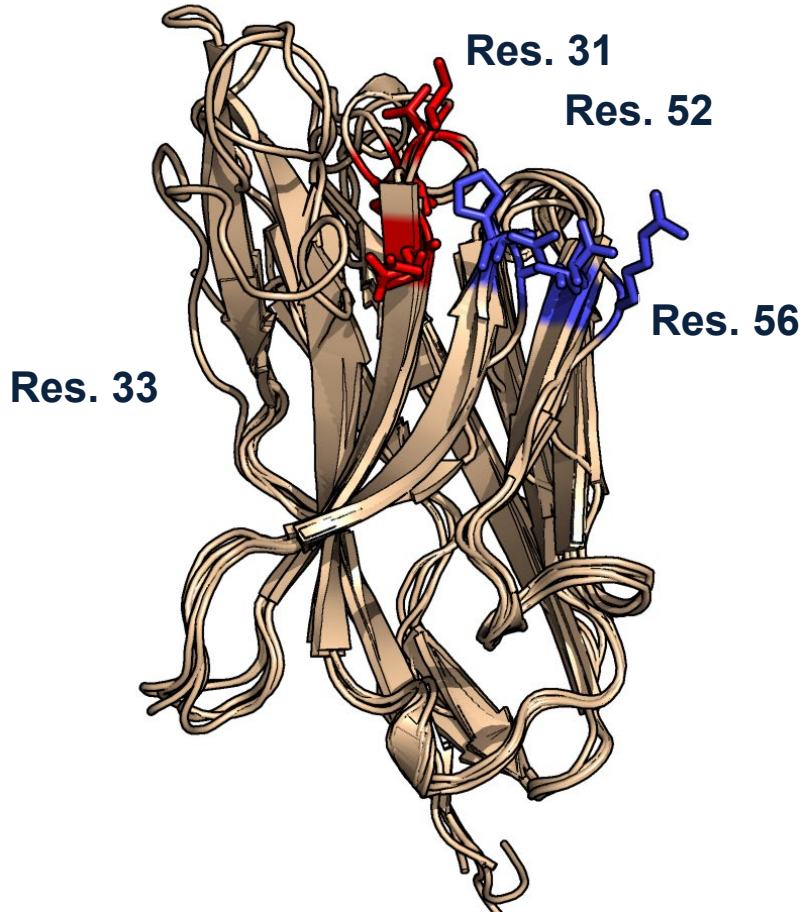




Public

Final **yeast** library designs

Library	Framework	CDR1+2 diversity	CDR1+2 Theoretical diversity	Transformed library size
Alp_LowDiv	Alpaca	Low	6.5×10^5	1.2×10^9
Hum_LowDiv	Humanized-4AA	Low	6.5×10^5	1.5×10^9
Alp_HighDiv	Alpaca	High	1.5×10^{12}	0.9×10^9
Hum_HighDiv	Humanized-2AA	Medium	1.6×10^7	1.1×10^9
Kruse[1]	Llama consensus	High	2.3×10^{10}	1×10^9

Synthetic CDR3 fragment used for internal libraries

[1]McMahon, C.,et al. (2018). *Nature Structural and Molecular Biology*, *25*(3), 289–296. https://doi.org/10.1038/s41594-018-0028-6

Public

Final phage library designs

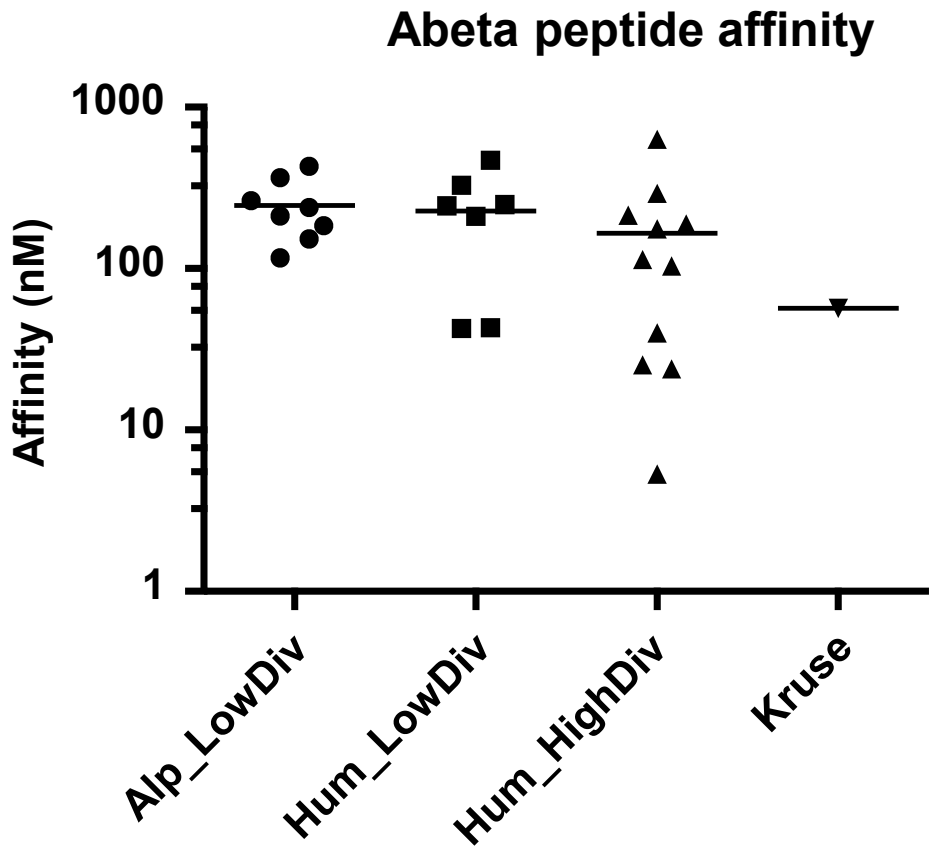
Library	Framework	CDRH3 Definition	Library Size
LD-01	Alpaca	Synthetic (6-10aa)	1.20E+11
LD-02	Alpaca	Synthetic (11-14aa)	2.10E+11
LD-08	Alpaca	Synthetic (15-18aa)	9.50E+10
LD-04	Alpaca	Natural/human	4.70E+09
LD-06	Humanized-2AA	Synthetic (6-10aa)	2.00E+11
LD-05	Humanized-2AA	Synthetic (11-14aa)	9.50E+10
LD-07	Humanized-2AA	Synthetic (15-18aa)	1.40E+11
LD-03	Humanized-2AA	Natural/human	6.20E+09

No CDR1+2 diversity incorporated in phage libraries

Public

Abeta affinity from V_HH campaign

- 42 recombinant VHH produced from four libraries
  - Library Alp_HighDiv gave only reagent binders
- Affinity measured by ForteBio
- Protein produced for 33 clones, binding detected for 27
- Best VHH has affinity of 5 nM (Hum_HighDiv)



New Technologies

MERCK

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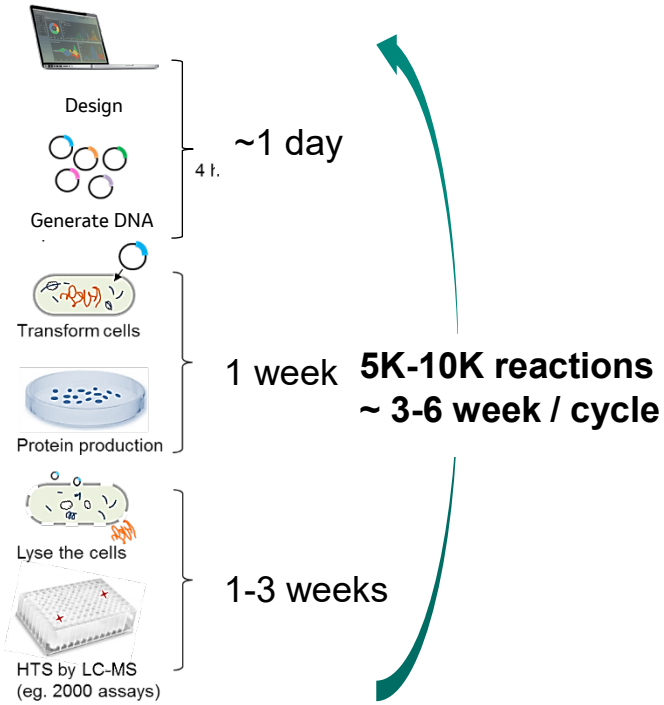
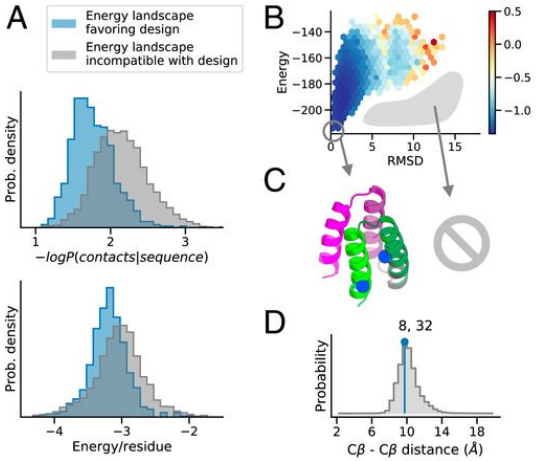
The Future of Protein Engineering

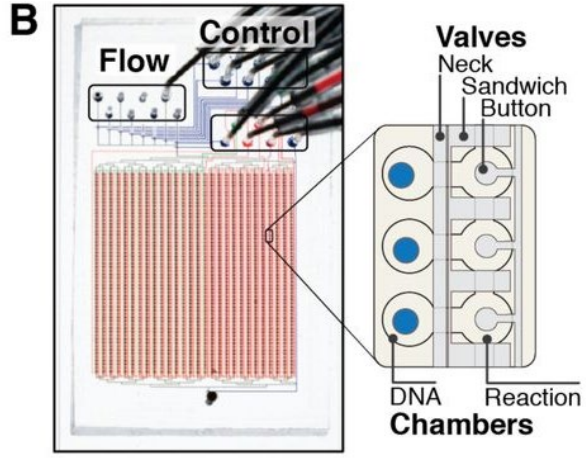
Our protein engineering workflow is approaching its limits, even with automation



Computational protein design and ultra HTP experimentation are transforming the experiments considered possible



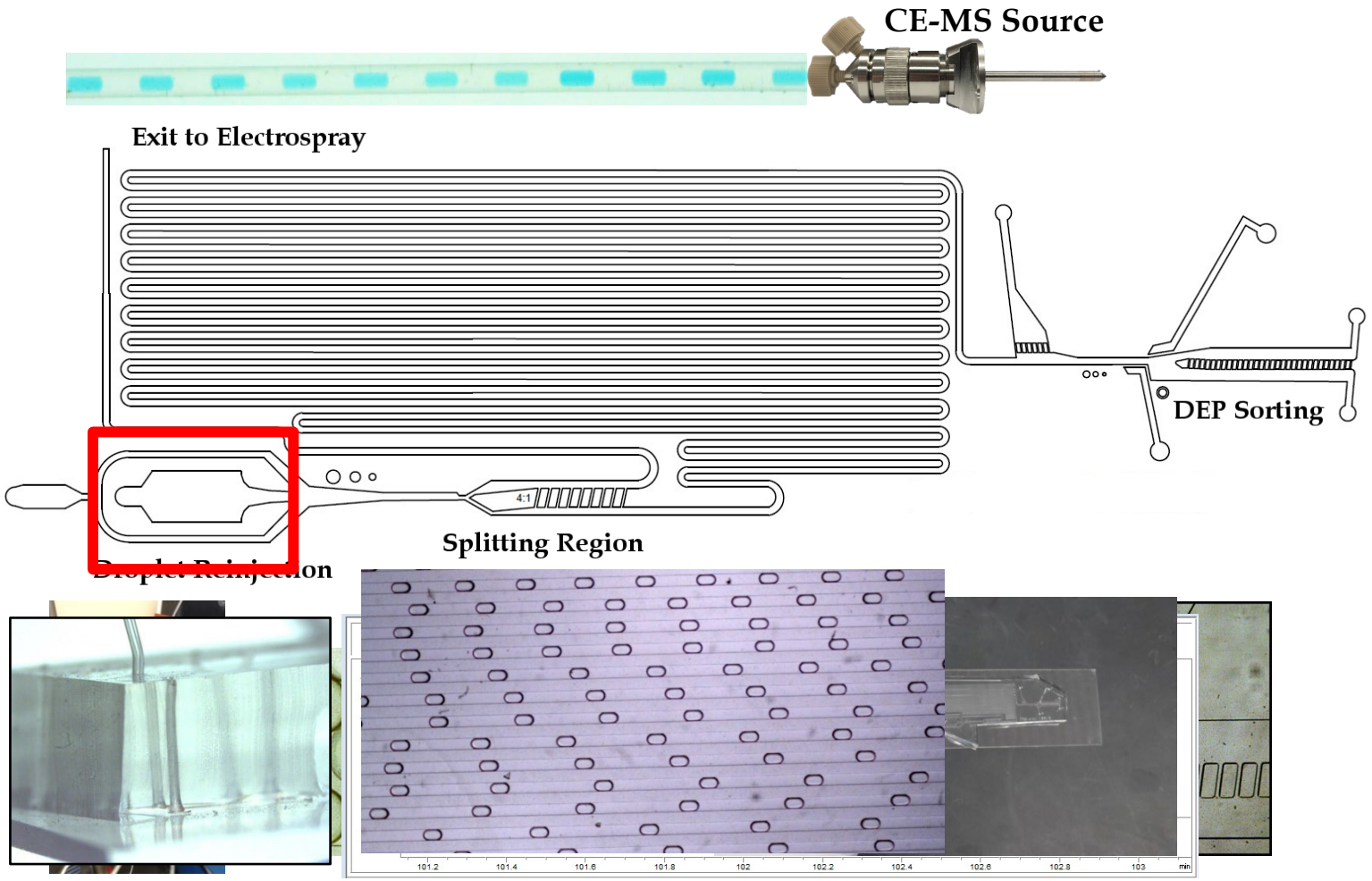
Protein sequence design by conformational landscape optimization
Norn, et al. PNAS 2021



Revealing enzyme functional architecture via high-throughput microfluidic enzyme kinetics
Fordyce Lab, Science 2021

Public

Mass Activated Droplet Sorting



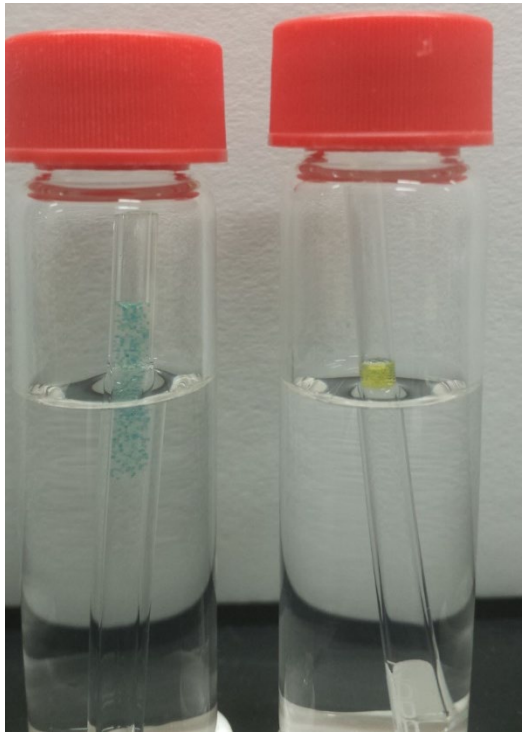
Public

Mass Activated Droplet Sorting

Starting Pool
10% Hits
20% Marker

Collected Negatives
2.5% Hits
22% Marker

Collected Positives
99% Hits
0% Marker



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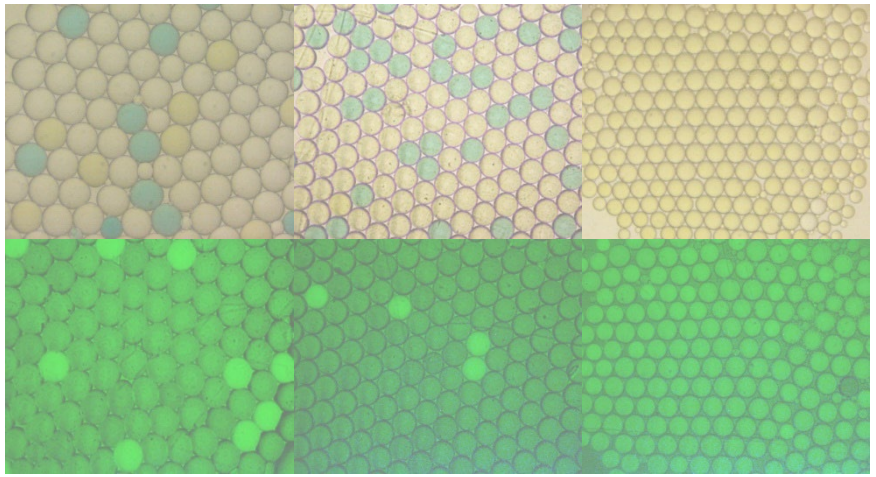

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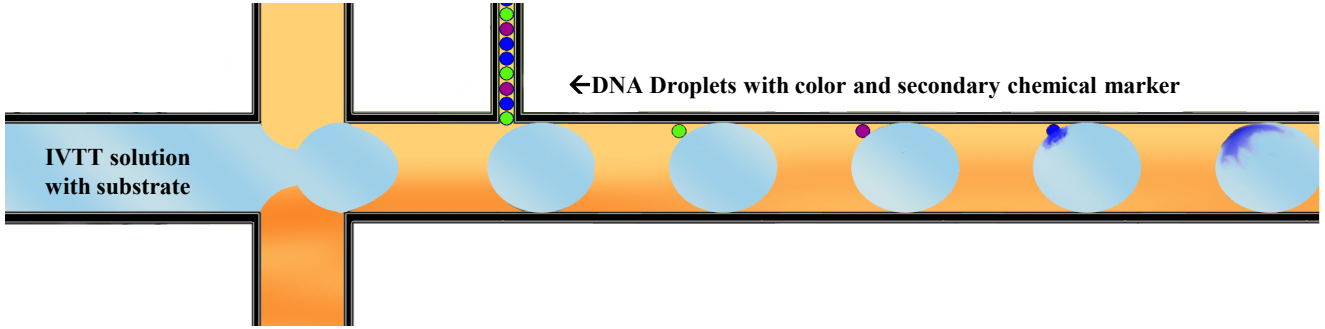
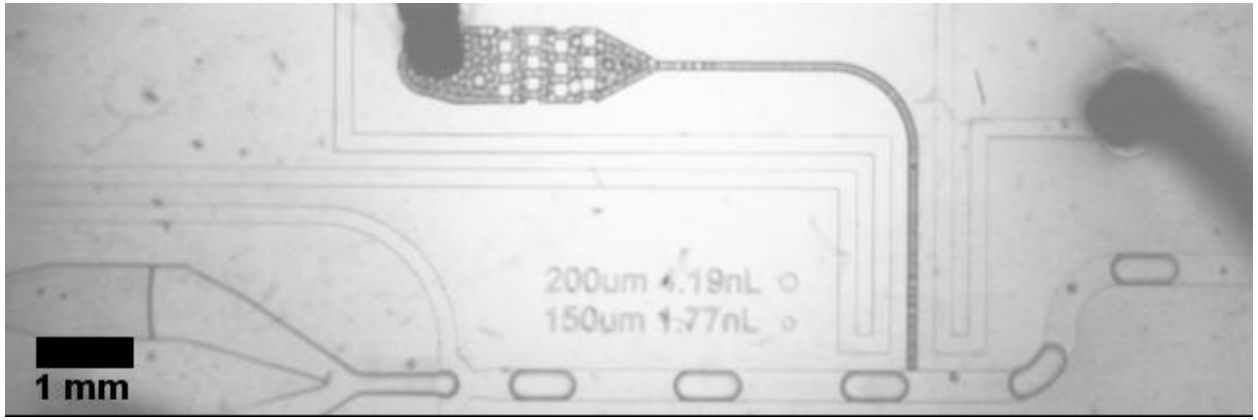
ProMADS Setup





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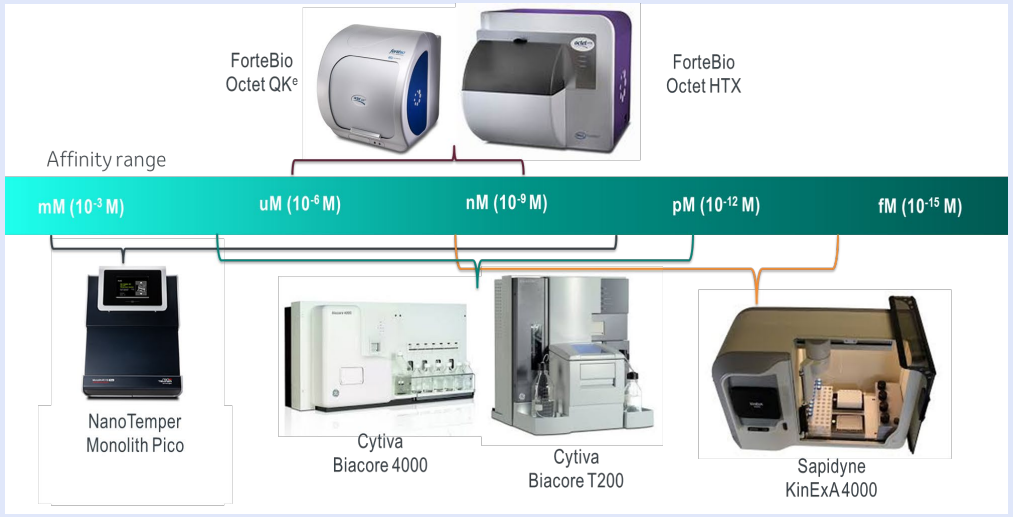
The Carterra LSA enables true High-Throughput Surface Plasmon Resonance (HT-SPR) analysis

- Screen more clones simultaneously in a single experiment
- Results in substantially less time
- Use only a small amount of sample

carterra®

MERCK

Evolution of our interaction analysis capabilities

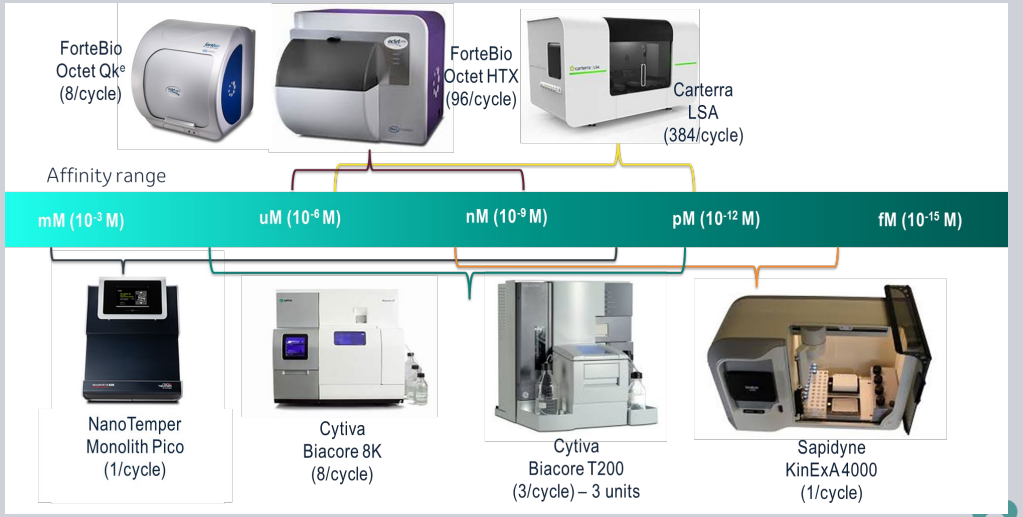


Pre-2023 state:

- Array of technologies spanning a wide affinity ranges
- Generally higher quality, multicycle, kinetics experiments utilized lower throughput instruments (Biacore T200)
- Octet HTX offered maximal throughput with 96 single point interactions however tighter K_D sensitivity of the instrument is limited
- Octet is main instrument for binning analysis however large matrix binning panels are challenging to run, consume reagent and requires highly manual characterization with outdated software

Post-2023 state:

- Array of technologies spanning a wide affinity ranges
- Higher quality, multicycle, kinetics experiments can be tailored to appropriate instrument based on throughput demands
- Carterra LSA offers maximal throughput capacity of 384 interactions with improved sensitivity range
- Carterra LSA is main instrument for classical and premix epitope binning analysis, large matrix binning panels are straightforward to set-up and data analysis is enabled with leading edge epitope binning analysis software



Public

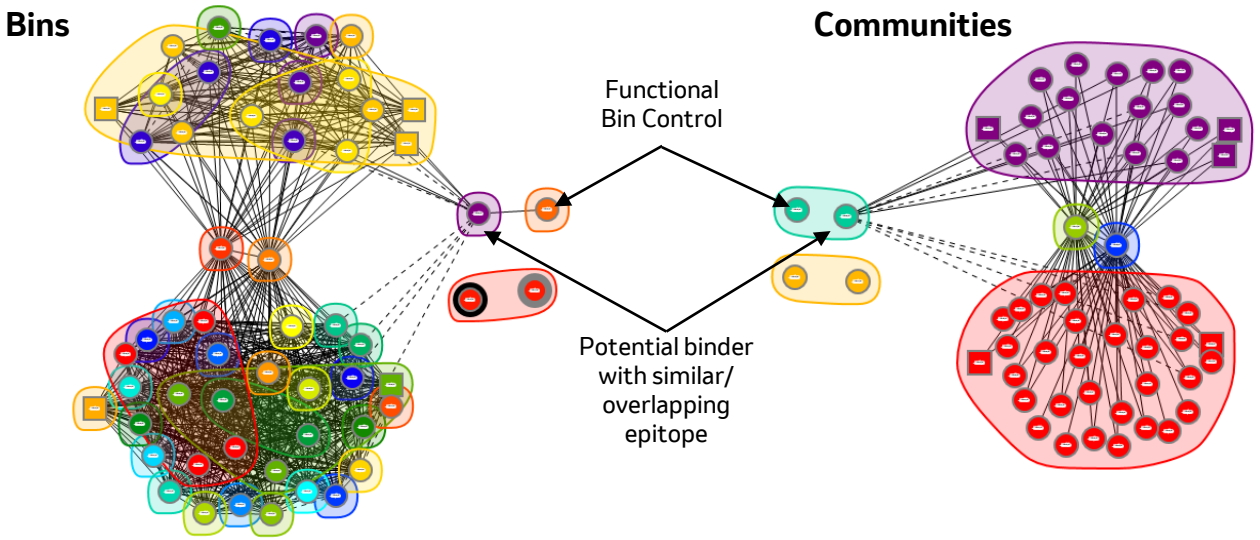
203 x 203 matrix binning experiment

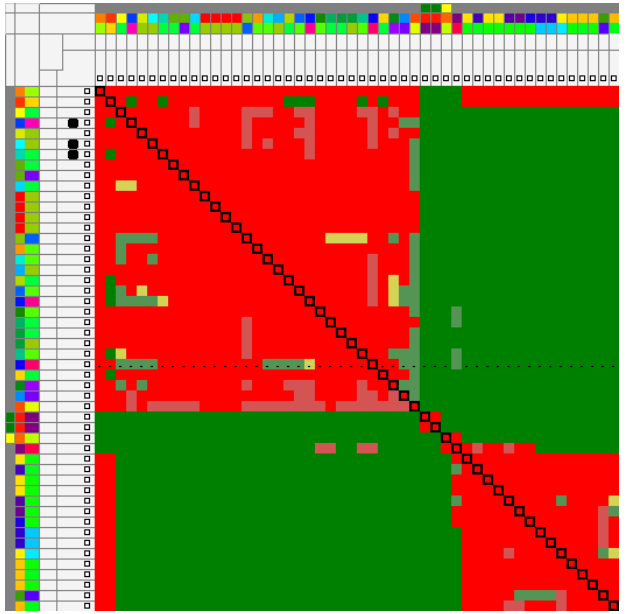
- Mammalian expressed HT VHH-Fc discovered via phage utilized for direct coupled classical epitope binning expt. using pre-known binning controls
- Leveraged initial experiment to assess panel binding to 200nM Ag and regeneration conditions
- Executed full binning experiment, 203 x 203 (>5-day expt. run time)
- Entire run used ~15ug per VHH-Fc and ~350ug Ag to run experiment

Experimental Conditions

- Sensor chip: HC200M
- [Ligand] – 10ug/mL – direct amine coupled to sensor surface
- [Ag] is 200nM, monomeric protein
- [Analyte] – 30ug/mL
- Regeneration - 10mM Glycine 2.0, 30s pulse injection, x2
- Standard classical epitope binning settings (5 min Ag, 5 min mAb)

Data triaging resulted in final 54 x 54 bi-directional binning matrix





Excluded data

- Non-expressors
- Replicates
- Multiple Batches
- Non-binders
- Low Ag binding

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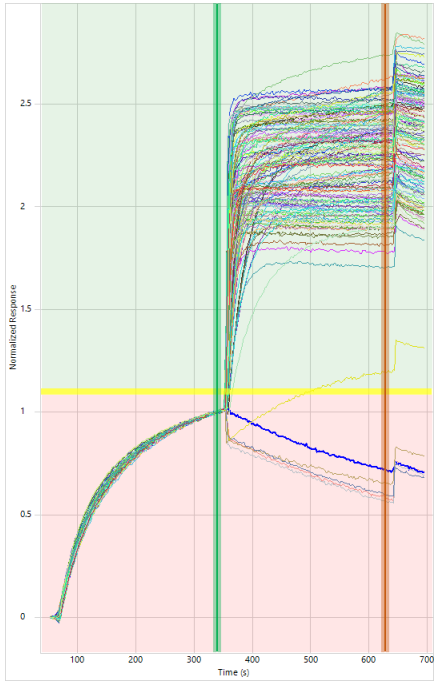
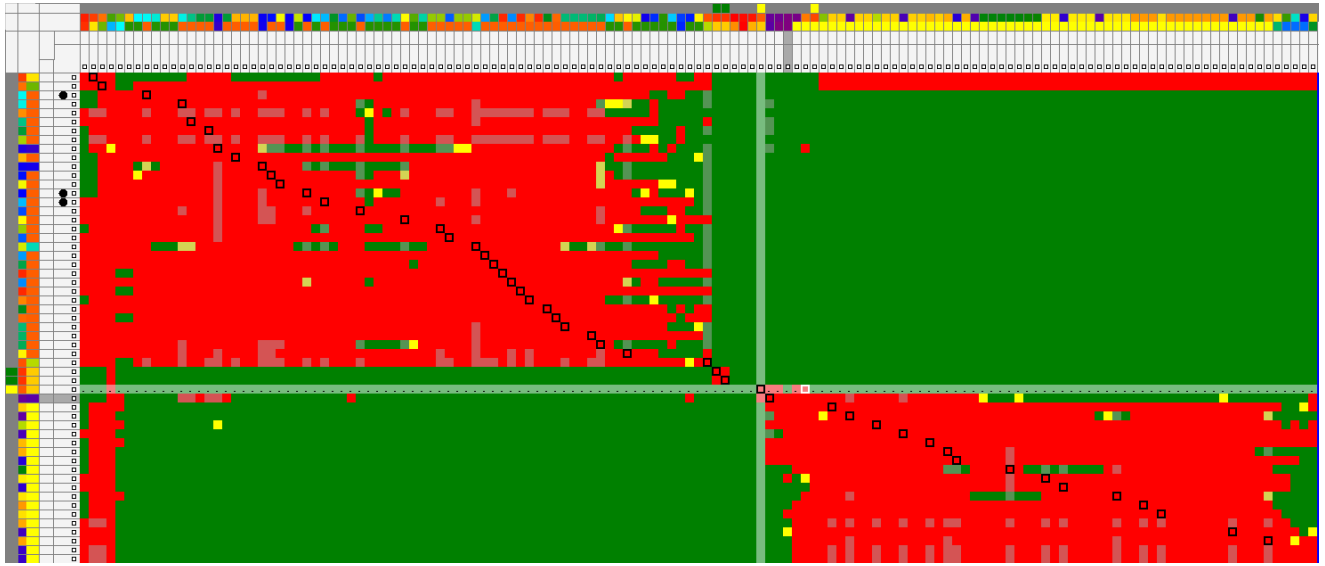
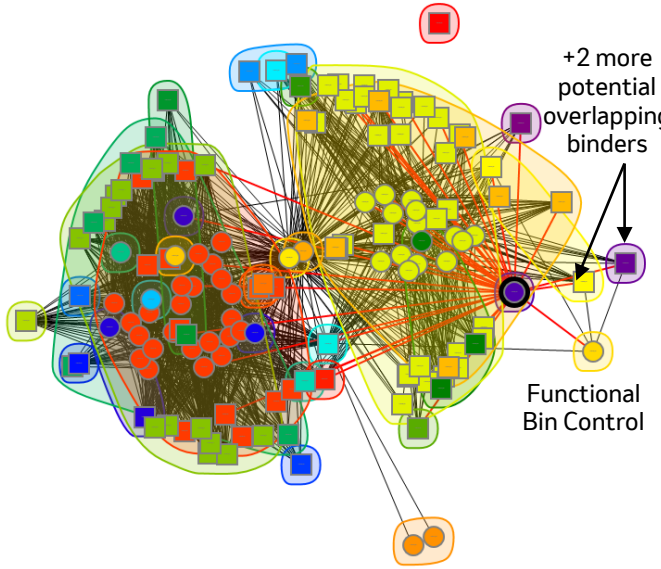
Addition of uni-directional data to matrix reveals other *potential* binders



Singlicate 54 x 140 binning matrix



Communities



- Binders that have demonstrated binding to Ag were analyzed uni-directionally as analytes as they performed poorly as immobilized ligands
- Further analysis of the single functional bin control identified two new *potential* binders
- **Carterra enabled the identification of 3 *potential* new functional binders out of 166 total binders for "challenging to find" epitope bin in <1 week with minimal setup time and minimal sample**

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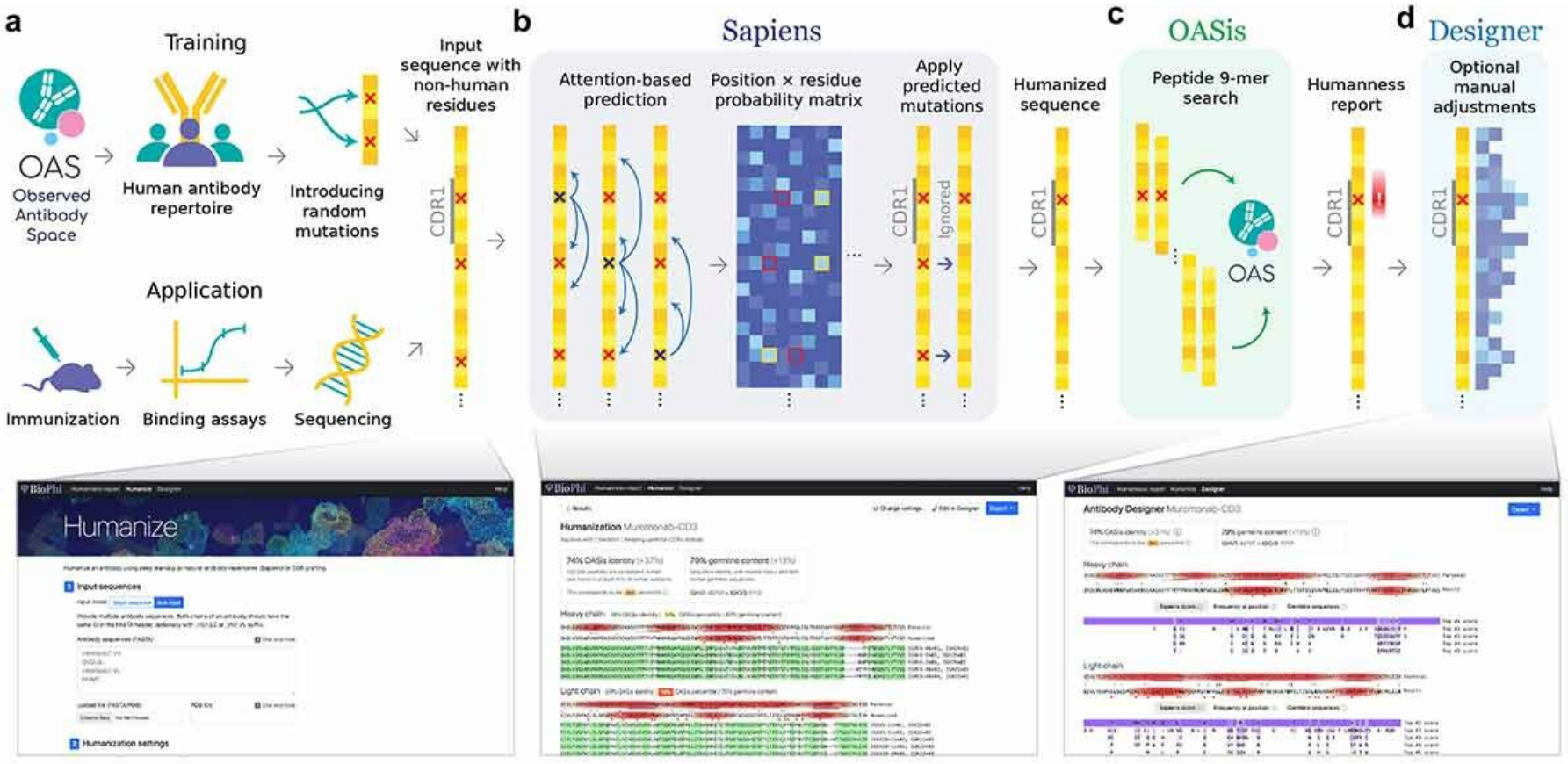
BioPhi

1. Open-source platform for Antibody design and humanization



Visit BioPhi at http://biophi.dichlab.org/
GitHub repository: https://github.com/Merck/BioPhi

Prihoda, et al 2022
BioPhi: A platform for antibody design, humanization, and humanness evaluation based on natural antibody repertoires and deep learning

Public

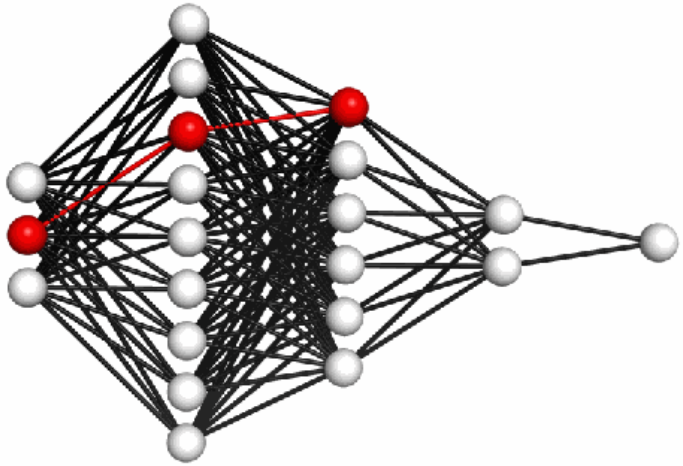
Where are we going next?

Even greater investment in automation, microfluidics, & machine learning

Our department is integrating all of these capabilities under ‘one roof’

Recently hired several machine learning, data science, & automation positions in Discovery Biologics

plus additional positions in Computational & Structural Chemistry, Data Science, IT, and Software Engineering



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Acknowledgements

Merck Discovery Biologics

Merck Protein Engineering

Merck Modeling & Informatics

Merck Structural Chemistry

Professor Bob Kennedy

UNIVERSITY OF MICHIGAN

Discovery Biologics: SSF, Boston, and Cambridge

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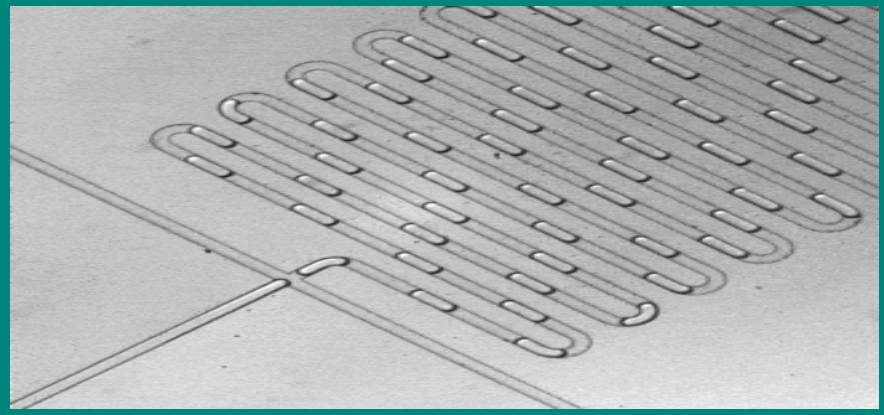
Thank you!



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Email: grant.murphy@merck.com