Immunogenicity of engineered enzymes:
strategies for risk management with alpha-galactosidase A as a case example

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EIP Symposium

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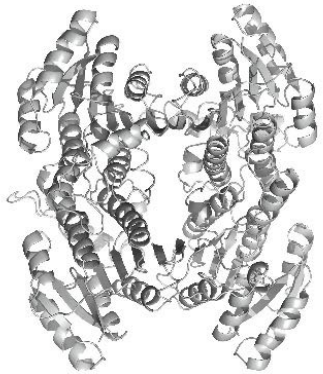
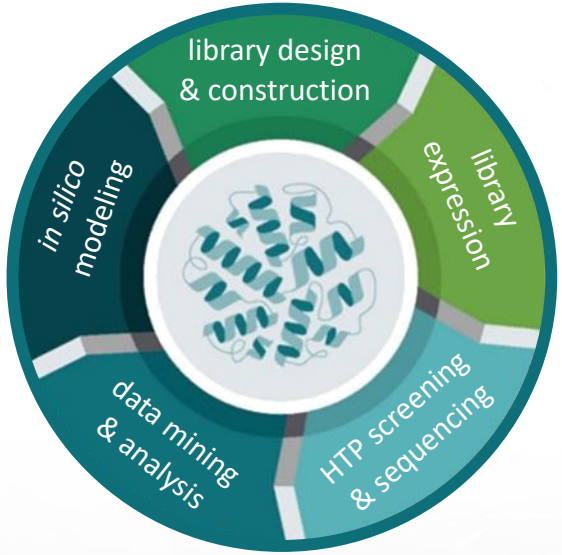
Codexis optimizes proteins with the end in mind

- stability in human (e.g., blood, GI tract)
- delivery (into cell, across BBB)
- reduced immunogenic risk

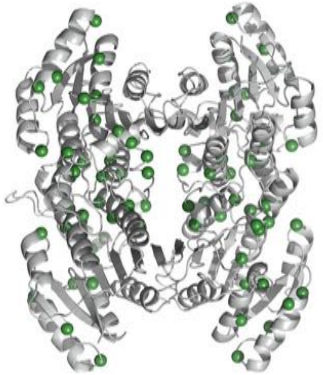
Natural protein

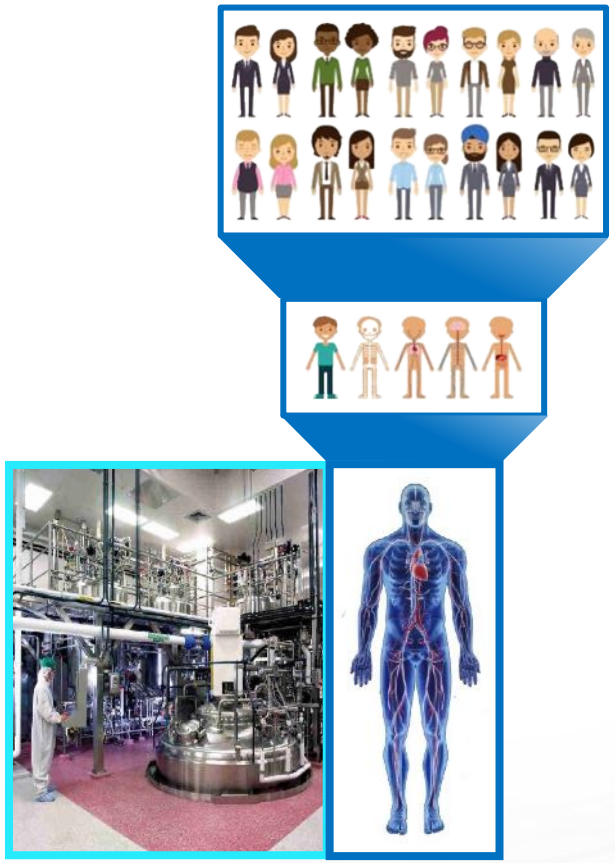


Directed Evolution
CodeEvolver® Technology



Optimized Protein





Recent example – engineered GLA for treatment of Fabry Disease

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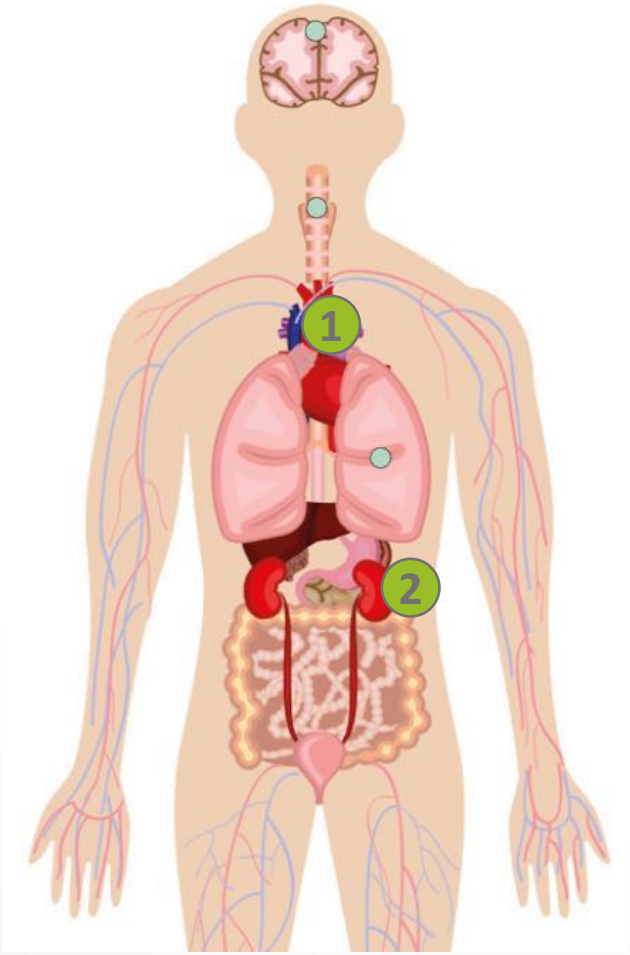
Fabry Disease Overview



- **Disease overview:** rare lysosomal storage disorder caused by a buildup of globotriaosylceramide (Gb3)
- **Disease cause**: mutation in the gene (X-linked) that codes for alpha-galactosidase A (GLA).
- **Pathophysiology**: Gb3 accumulation causes multifaceted dysfunction in many organ systems, including
  1. Heart: complications including angina, CKD, cardiomyopathy, and heart failure
  2. Kidney: progressive kidney failure
- **Current Standard of Care**: Agalsidase alfa (EU) and Agalsidase beta (US) administered by an IV infusion every 2 weeks.

Despite available treatment, key unmet needs still exist

- Patients receiving ERT still undergo significant cardiac pathophysiologic changes *(J. Int. Med. 2013, J. Med. Gen. 2015)*
- Progressive kidney disease and failure *(J. Med. Gen. 2015)*
- 70-80% of patients develop IgG antibodies that can reduce efficacy within first three months of exposure *(PLOS One 2012)*

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Optimization of GLA at Codexis using the CodeEvolver® platform

Overall goal: to develop an engineered GLA biotherapeutic for the treatment of Fabry disease with a reduced frequency of administration and *reduced immunogenic risk*

Optimization goals (screening)

- Improved serum stability
- Improved lysosomal stability
- Improved cellular uptake in key cell types
- Reduced number of predicted T cell epitopes

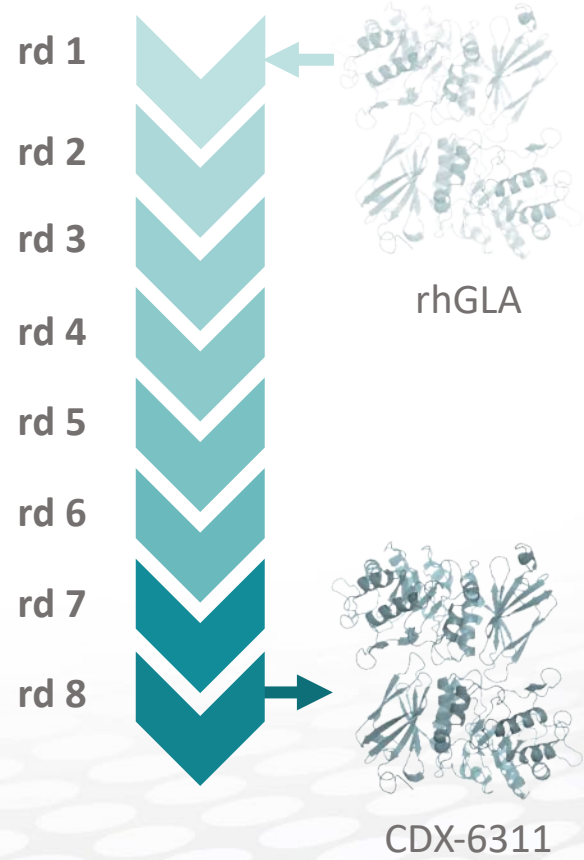
Optimization statistics

8 rounds of evolution

>50,000 wells assayed

>12,000 GLA variants screened

>3,000 GLA variants sequenced



Mutational coverage across GLA primary sequence (based on sequencing data)

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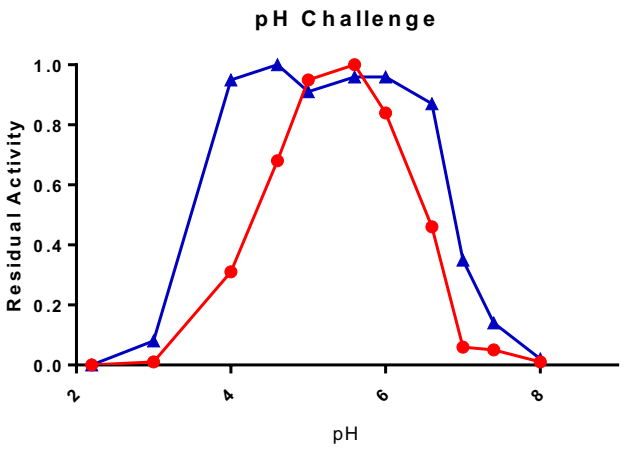
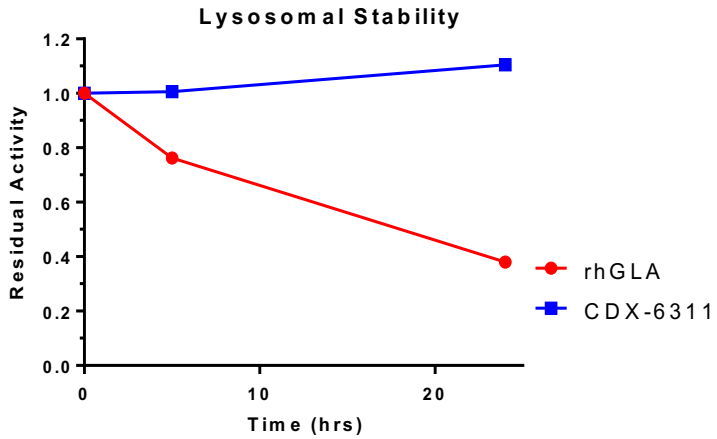
Improved *in vitro* stability of CDX-6311 compared to rhGLA

Improved low pH stability



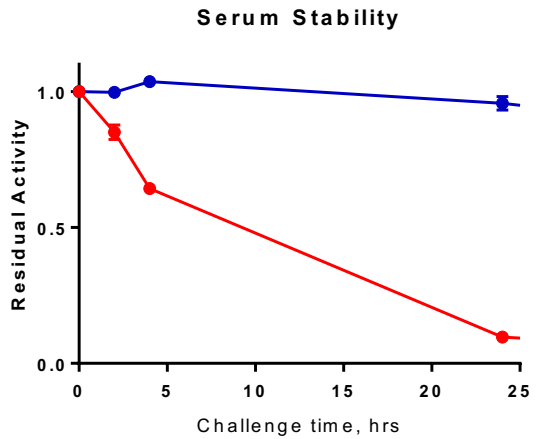
CDX-6311 exhibits higher stability at acidic and neutral pH after 1 hr exposure.

Improved lysosomal lysate stability



CDX-6311 is more stable in lysosomal extracts (liver) than rhGLA

Improved *in vitro* serum stability



CDX-6311 is more tolerant to serum than rhGLA

CDX-6311 exhibits superior stability to acidic and neutral pH, temperature (data not shown), human serum, lysosomal extracts, and expression (data not shown)

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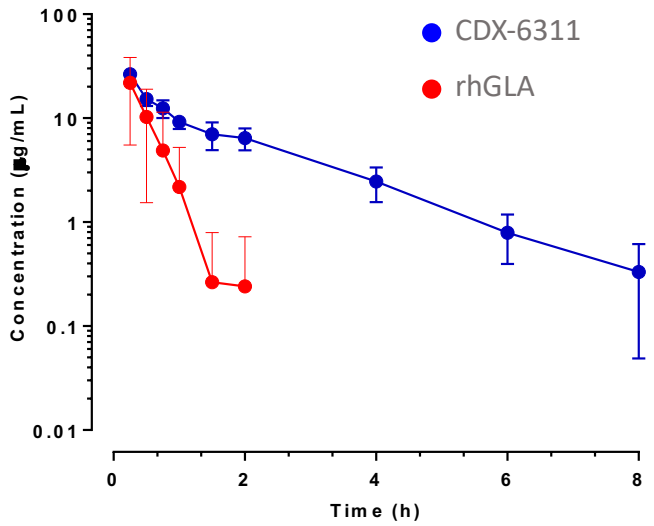
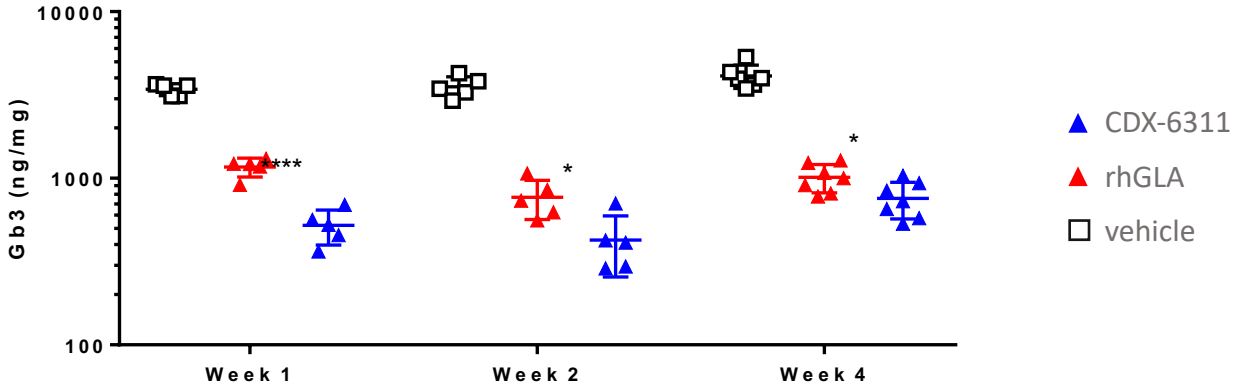
CDX-6311 shows an improved PK/PD profile in non-human primates and the Fabry mouse model

circulating half-life in non-human primates



rhGLA and CDX-6311 were administered at a dose of 1 mg/kg IV

reduction of Gb3 in the heart



*: p <0.05, ***: p <0.001 , ****: p <0.0001

Results from a single dose administration

CDX-6311 compared to rhGLA:

- Improved circulating half-life in rodents and non-human primates
- Reduced Gb3 and higher GLA activity in the heart (Fabry mouse)
- Similar Gb3 reduction and GLA activity in the kidney (Fabry mouse)

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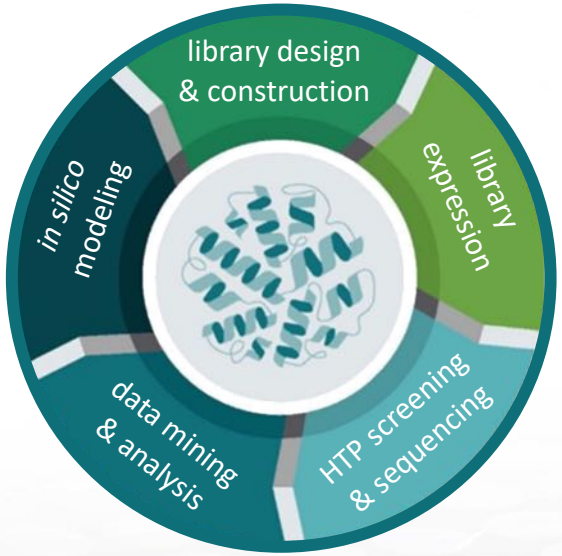
Our risk mitigation strategy for GLA immunogenicity

1. Collect info on starting protein:
- Identification of TCEs[1]
- Selection of positions to mutate

2. Apply CodeEvolver Technology:
- Guided by predictions

3. Assess engineered variants:
- MAPPs assay

in silico predictions



MAPPs assay

1. TCE = T Cell Epitope

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MAPPs assay overview

MAPPs assay

- Monocyte-derived dendritic cells from 10 healthy donor PBMCs
- MHC II profile of donors closely matched the panel of MHC II alleles used in silico
- Samples tested: rhGLA, CDX-6311
- Blank

Which step(s) along this pathway are influenced by CDX-6311 mutations?

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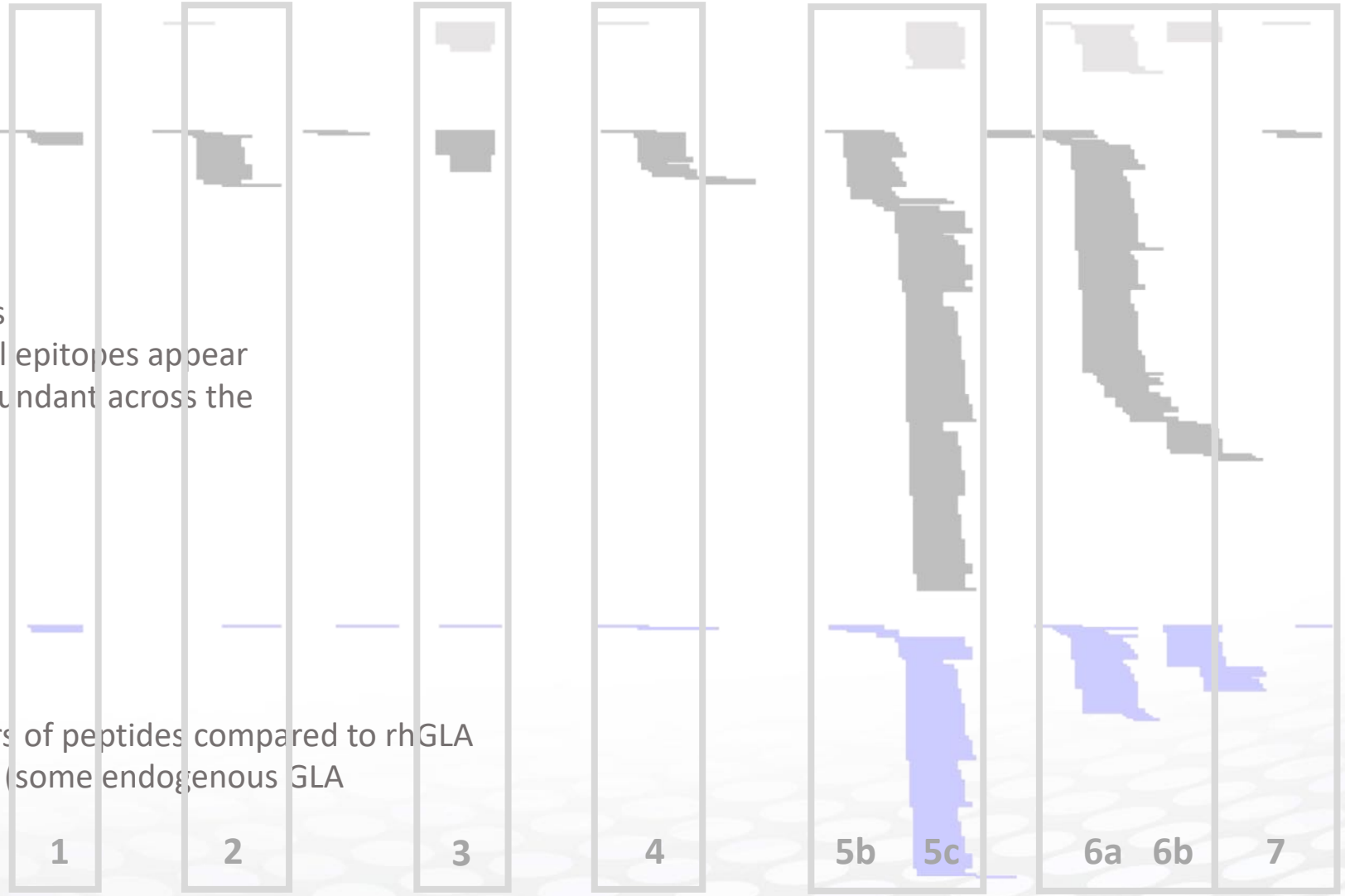
CDX-6311 has fewer presented peptides in MAPPs compared to rhGLA

- Blank:
  - 42 GLA peptides detected across donors
  - 4 main epitopes are present in healthy donors (endogenous GLA)
- rhGLA:
  - 233 GLA peptides detected across donors
  - compared to the blank, several additional epitopes appear
  - Two epitopes near the C-terminus are abundant across the majority of donors
- CDX-6311:
  - 94 GLA peptides detected across donors
  - Has similar profile as rhGLA
  - Nearly all epitopes have reduced numbers of peptides compared to rhGLA
  - Mutations are not present in all peptides (some endogenous GLA background exists)



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Each donor has fewer presented peptides for CDX-6311 compared to rhGLA

rhGLA-treated samples

	epitope #						
Donor ID	1	2	3	4	5	6	7
AIV00741	0	4	1	0	0	1	1
AIV01121	3	3	1	3	19	16	4
AIV01295	0	0	2	3	2	4	0
AIV01317	0	0	2	0	1	1	2
AIV01343	0	4	2	0	0	6	0
AIV01345	0	1	2	1	22	8	1
AIV01359	0	0	2	0	11	4	1
AIV01372	1	1	0	1	13	5	0
AIV01373	0	1	2	2	10	4	0
AIV01390	0	0	2	2	21	18	4

6311-treated samples

	epitope #						
Donor ID	1	2	3	4	5	6	7
AIV00741	0	0	0	0	0	0	0
AIV01121	2	1	0	0	9	5	9
AIV01295	0	0	0	0	1	2	0
AIV01317	0	0	0	0	2	0	0
AIV01343	0	0	0	0	0	0	0
AIV01345	0	0	0	0	18	2	0
AIV01359	0	0	1	0	4	2	1
AIV01372	0	0	0	0	2	0	0
AIV01373	0	0	0	1	3	3	0
AIV01390	0	0	0	1	17	16	4

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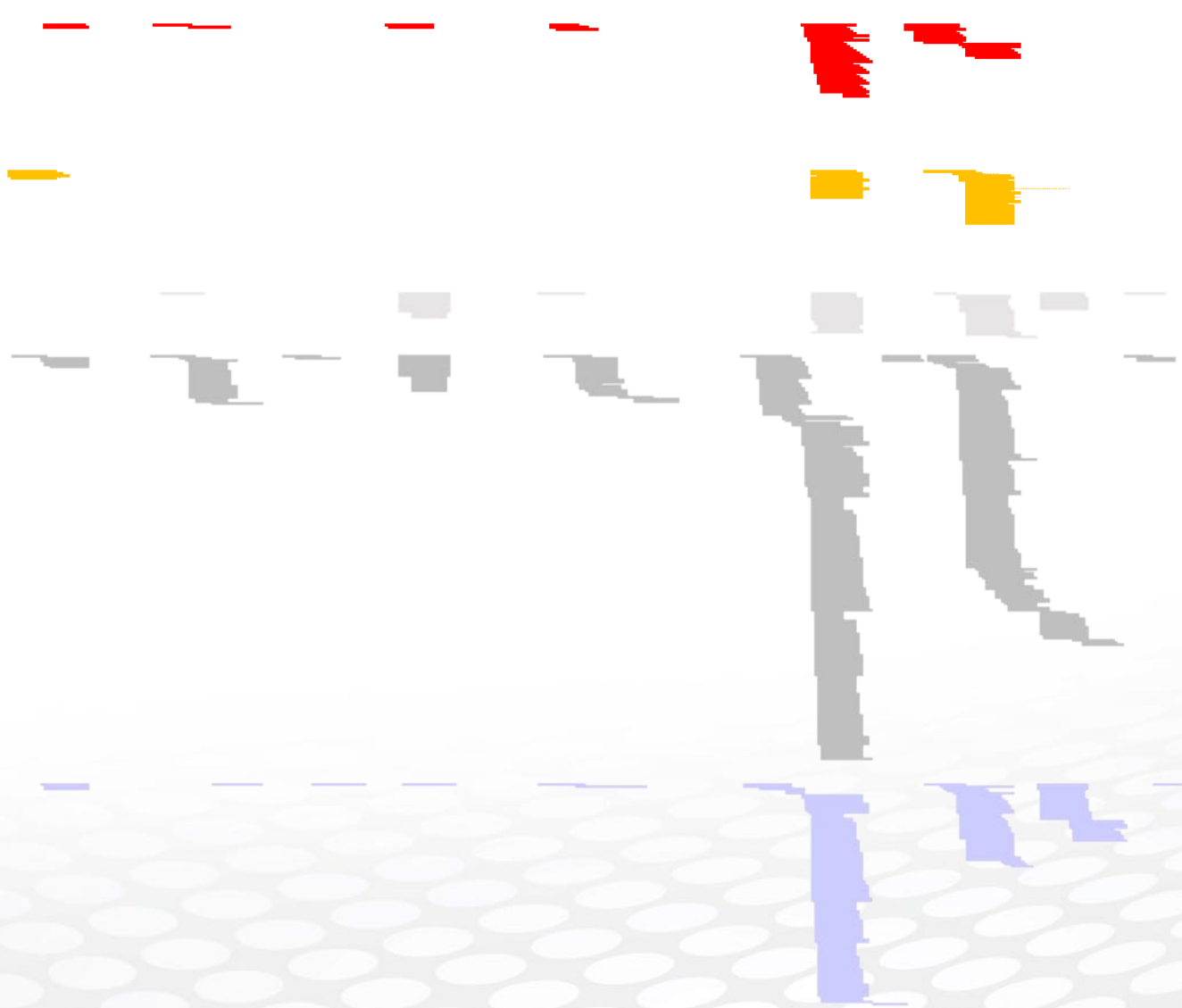
Supplemental GLA epitope information aligns well with MAPPs assay

- MAPPs-like assay with GLA$^{-/-}$ Raji B cell (Stanford)
  - Correlates well with MAPPs in location of peptides and abundance
- Database information (IEDB and literature)
  - Most abundant peptides are again in same regions
- Blank
- rhGLA
- CDX-6311



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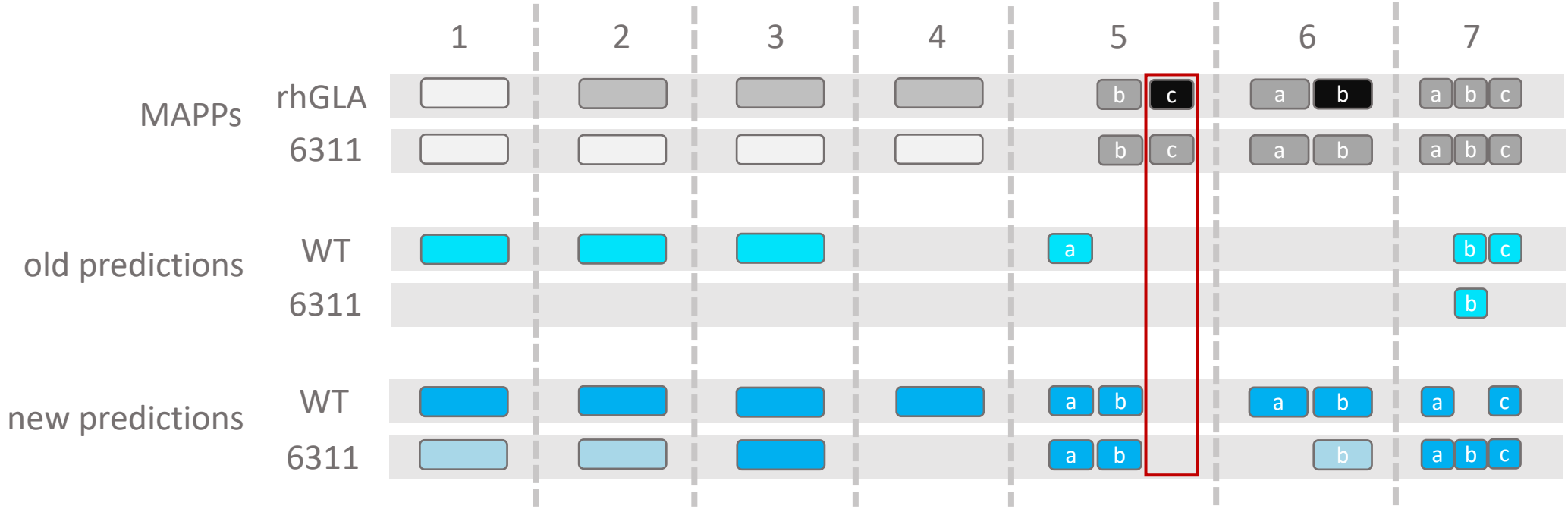
How do *in silico* predictions compare to experimental data?



- The version of the predictive tools used during evolution (“old predictions”) aligns with 4 of the 7 experimental epitopes
- Newer version of predictive tools aligns better with experimental data (predicted epitopes remain although reduced # MHC IIs)
- One of the two abundant regions (epitope 5c) is NOT predicted by either version of the tools

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Observations and conclusions

Observations

- The regions of peptide presentation are similar for rhGLA MAPPs and CDX-6311 MAPPs
- There is less presentation for CDX-6311 compared to rhGLA. *Why*?
  - Introduced mutations ➔ reduced MHC II binding?
  - Increased stability ➔ reduced processing?

- The newer version of predictive tools aligns better with experimental data, but still misses highly abundant epitopes (ex. epitope 5c)

Conclusions:

- Improved stability may translate to reduced processing and presentation
- Experimental data (MAPPs, T cell assays) are ideally used to inform the de-epitoping strategy

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Proposed de-epitoping strategy for future evolution programs

1. Collect info on starting protein:
- Identification of TCEs[1] with MAPPs
- Test WT epitopes for T cell activation
- Select positions to mutate

2. Apply CodeEvolver Technology:
- Guided by predictions, MAPPs, and T cell assays
- Test mutations in T cell assays

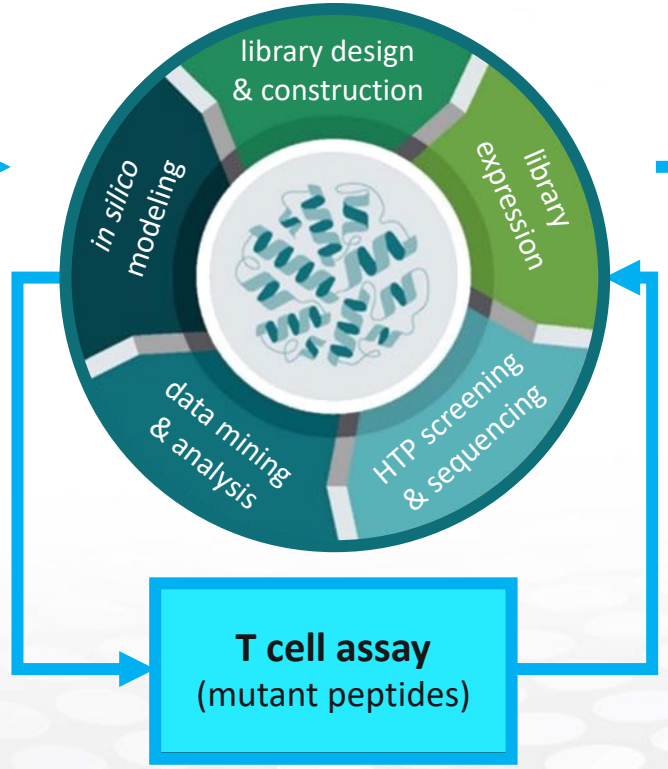
3. Assess engineered variants:
- T cell assay with full length protein

MAPPs assay

T cell assay
(WT peptides)

in silico
predictions

1. TCE = T Cell Epitope



T cell assay (protein)

Peptide T cell assay challenges
- Sensitivity
- Fluctuating responses
- Throughput

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