

# HLA antibody epitopes in organ transplantation

Eric Spierings (e.spierings@umcutrecht.nl)

# disclosure:

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- the UMCU has filed patent applications on the prediction of an alloimmune response against mismatched HLA
- I am listed as inventor on these patent applications

# Learning objectives of this presentation

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## HLA antibody algorithms

- To understand the basic principles of antibody recognition
- To understand the basic concepts of HLA antibody epitope matching algorithms
- To understand the most important differences between the various HLA antibody epitope matching algorithms

## HLA T-cell epitope algorithms

- To understand the basics of T-cell epitope recognition
- To understand the difference between PIRCHE-I and PIRCHE-II and the potential immunological consequences
- To understand how PIRCHE-II affects transplant outcome

# Learning objectives of these presentations

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# HLA antibody epitopes: The evolution of algorithms

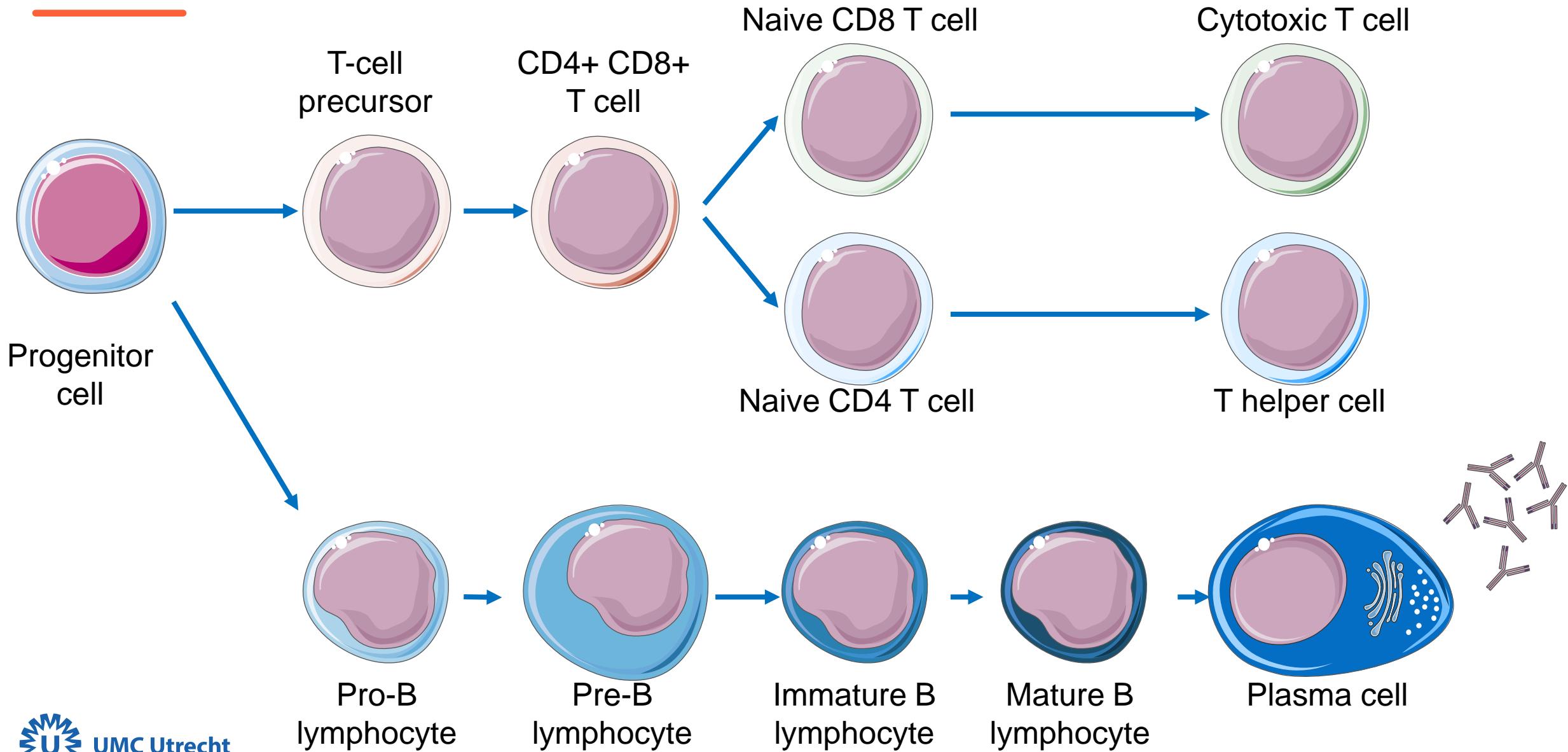
Epitope workshop ABHI  
Sao Pauloa – Wednesday, December 13, 2023

# Characteristics of the adaptive immune system

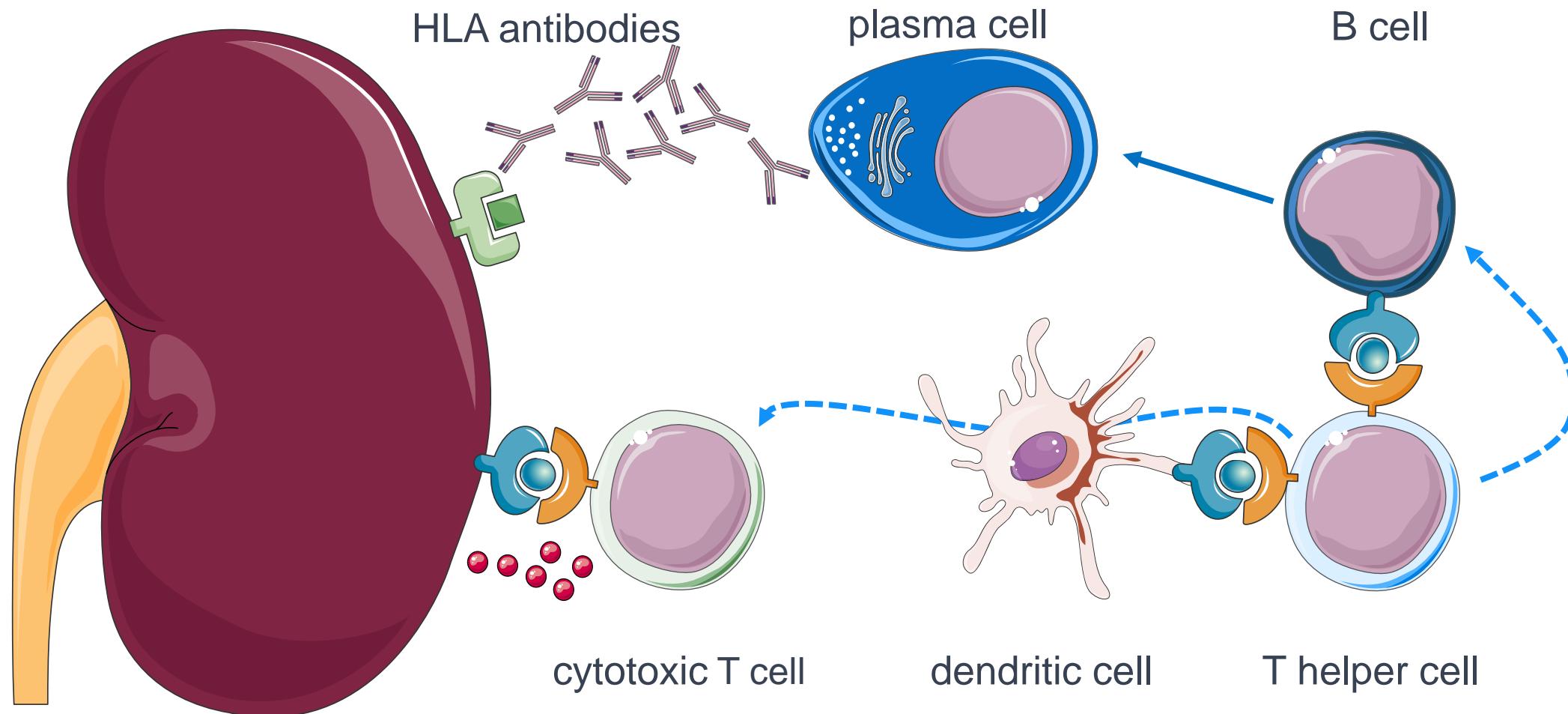
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- The adaptive immune system **anticipates on** specific pathogenic threads
- This system produces **antibodies, B cells and T cells**
- These components **recognize** foreign substances, like bacteria and viruses, and **remember** those for an effective future defense
- The adaptive immune system supports a **long-lasting** immunity

# Adaptive immune responses – the players

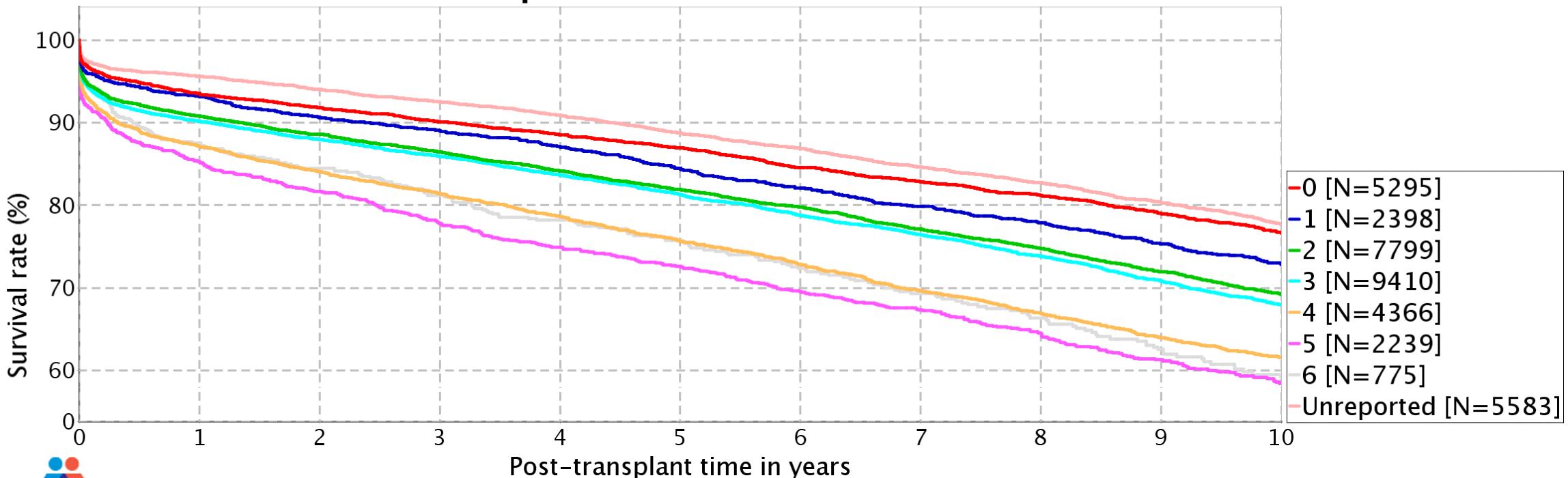


# Adaptive immune responses after transplantation



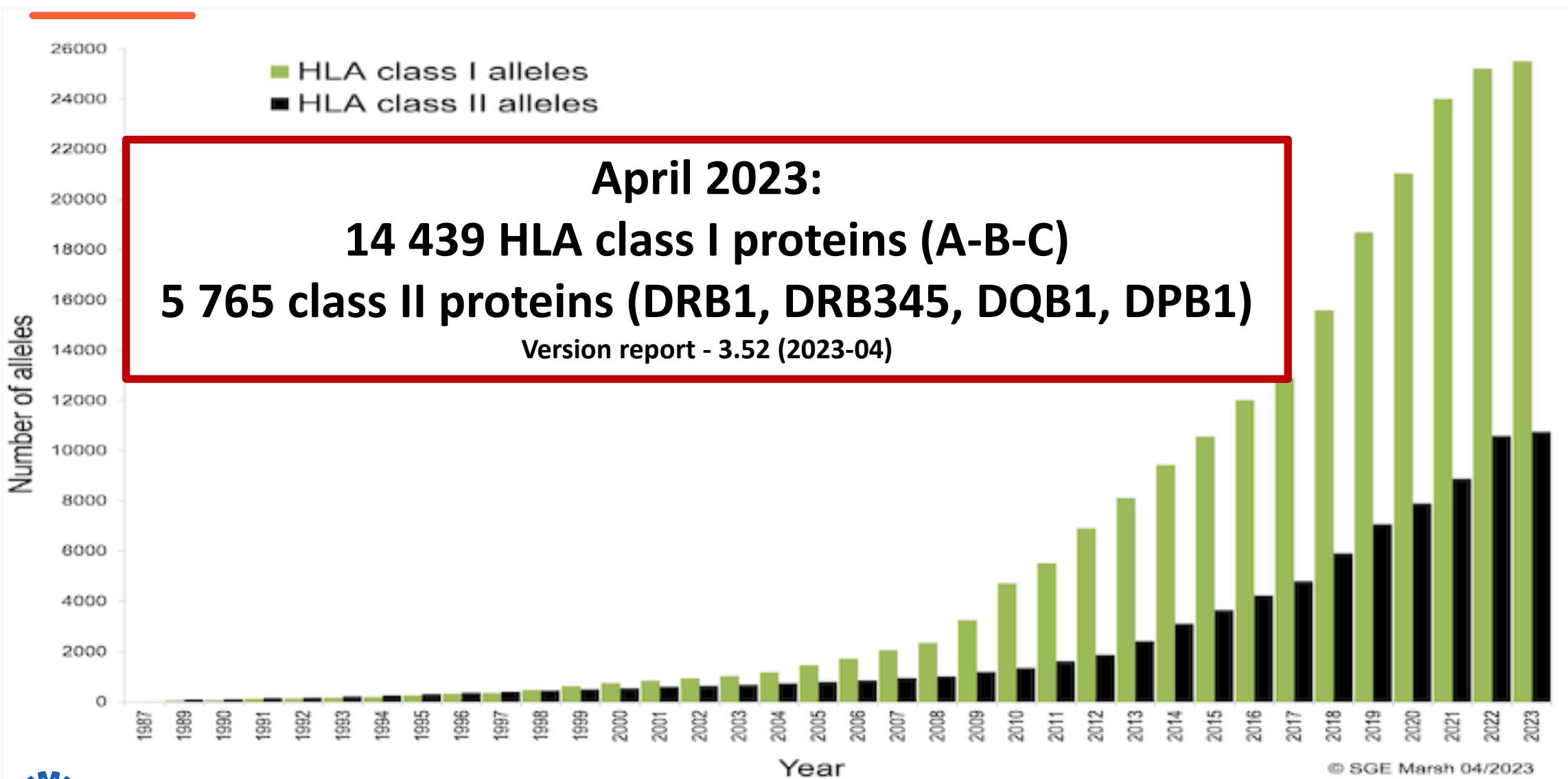
# HLA mismatches reduce graft survival

Kidney only transplant death censored graft survival rates stratified by HLA Mismatches. Transplants: 01.01.1999 to 01.01.2009



Eurotransplant  
Database date : 2019-09-11

# HLA and big data



# HLA and big data – an example

HLA Class I			
Gene	A	B	C
Alleles	7793	9274	7761
Proteins	4548	5580	4311

IPD-IMGT/HLA database  
Version report - 3.52 (2023-04)

- HLA-A has **4548 protein variants**
- Without any matching, this could theoretically lead to approximately  **$20 \times 10^6$  mismatched HLA-A combinations**.
- A **reduction of the complexity** is required to identify permissive and non-permissive mismatches.
- This reduction can be achieved by dissecting each protein variant into **units** that can be **recognized by the immune system**

# definition of an epitope

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## epitope

[ ēp'ī-tōp' ]

*noun.*

the **surface portion**  
(τοπος) on (επι) an  
antigen

## B-cell epitope

[bi sĕl ēp'ī-tōp' ]

*noun.*

epitope that is capable  
of eliciting a **B-cell**  
immune response

## T-cell epitope

[ti sĕl ēp'ī-tōp' ]

*noun.*

epitope that is capable of  
eliciting a **T-cell** immune  
response

# definition of an epitope

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**epitope**

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[bi sĕl ēp'i-tōp' ]

*noun.*

epitope that is capable  
of eliciting a B-cell  
immune response



recognition by the B-cell  
receptor and by  
antibodies

**T-cell epitope**

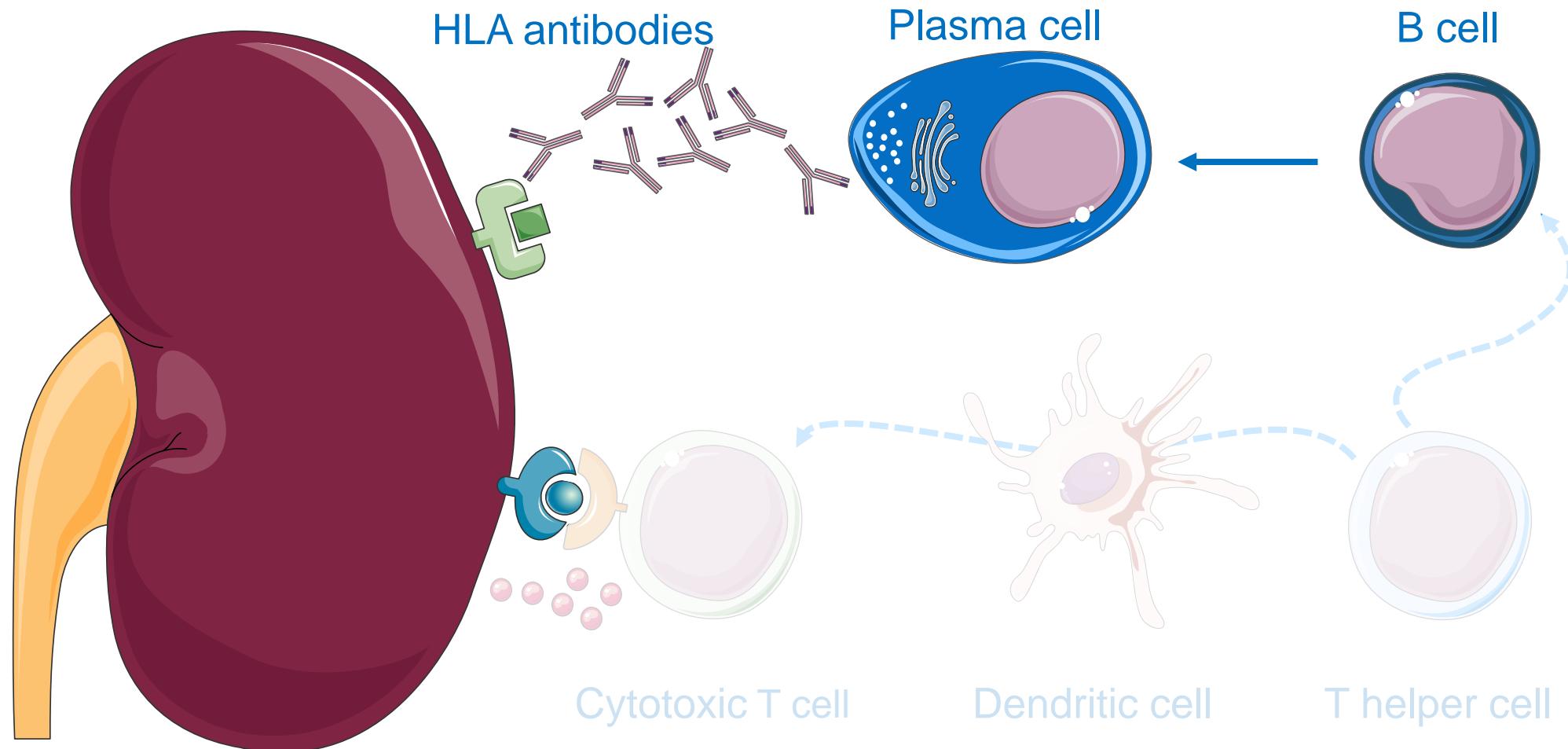
[ti sĕl ēp'i-tōp' ]

*noun.*

epitope that is capable of  
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response

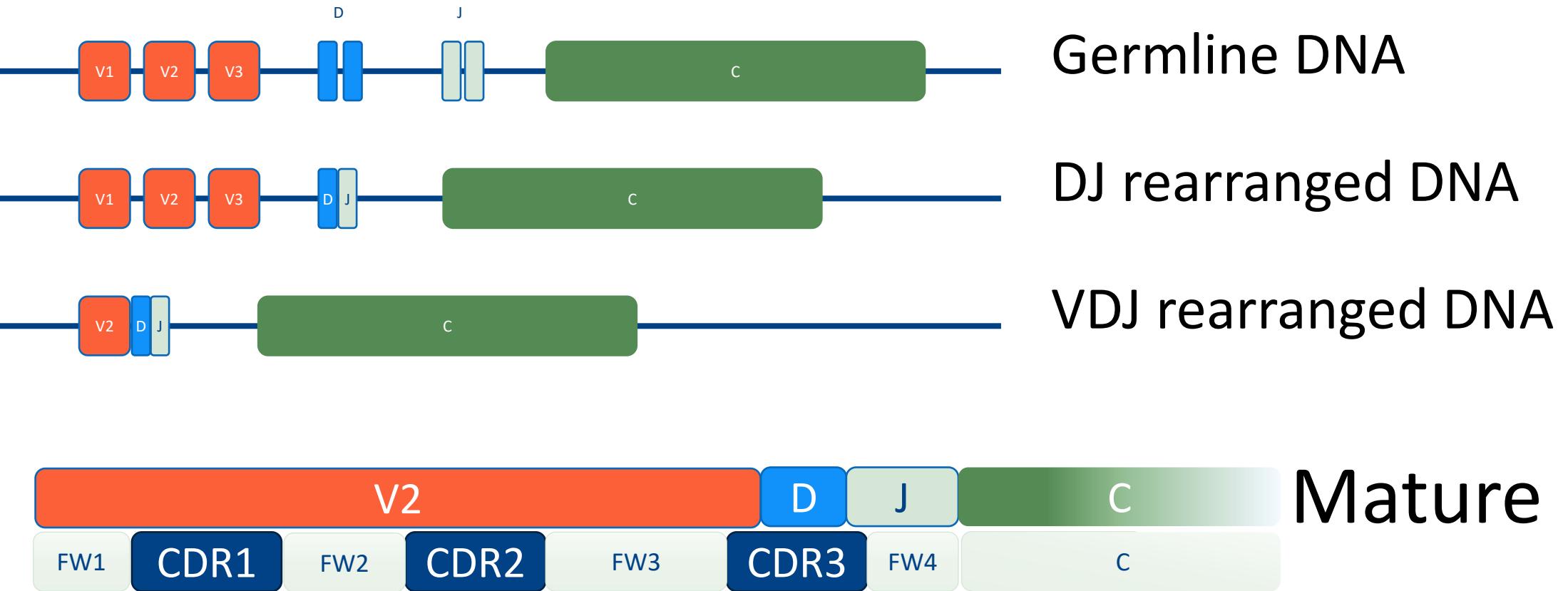
# adaptive immunity and graft rejection

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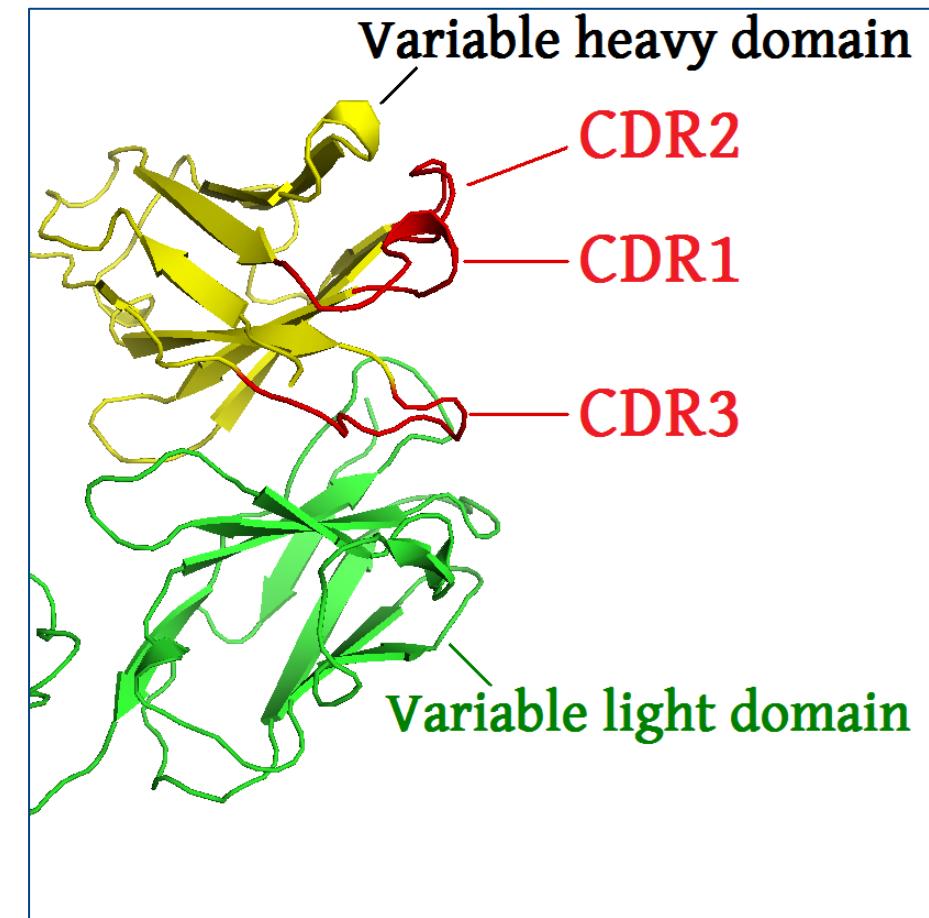
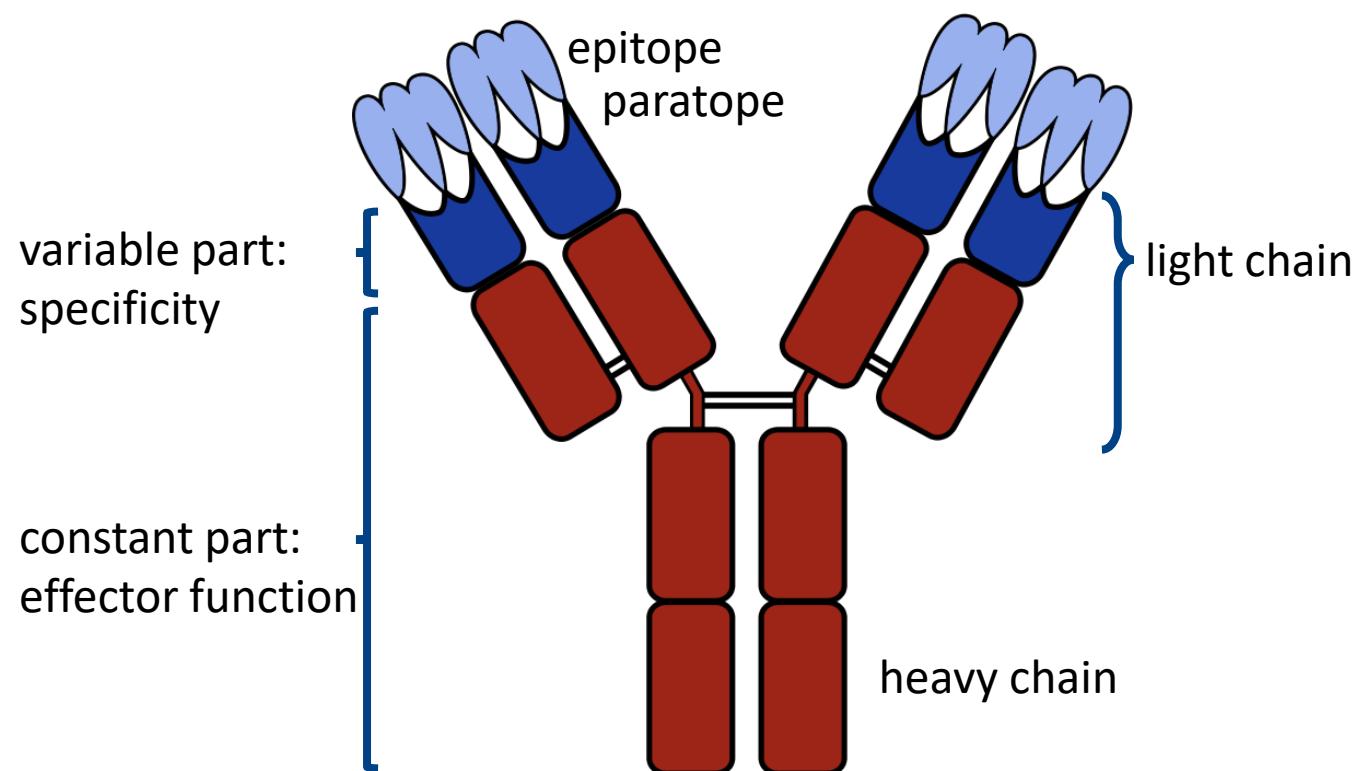
# Antibodies and BCR VDJ rearrangement

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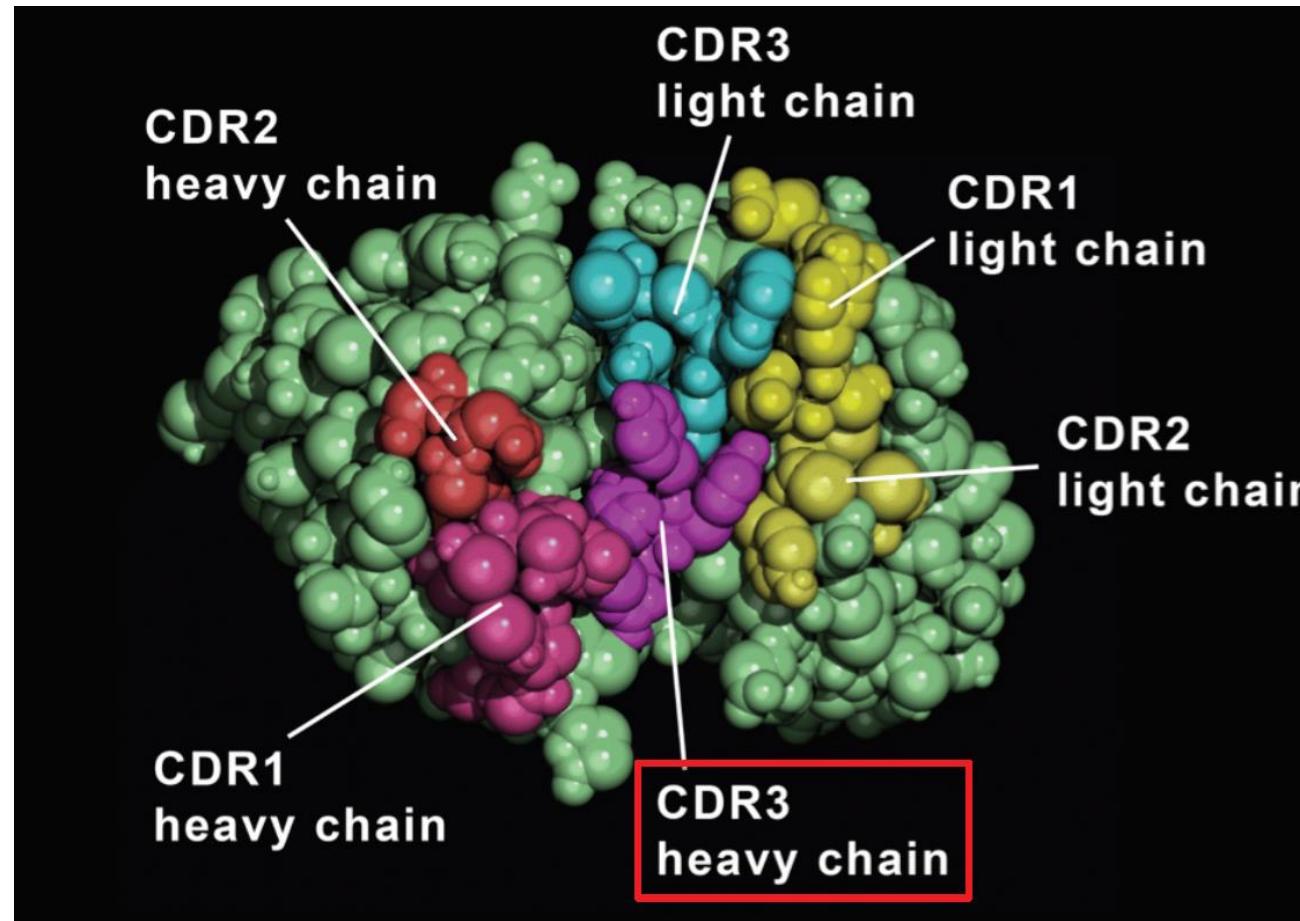


# anatomy of antigen recognition by antibodies

antibodies/B-cell receptors recognize epitopes via their CDR regions on the distal parts of the Ig-heavy and -light chains

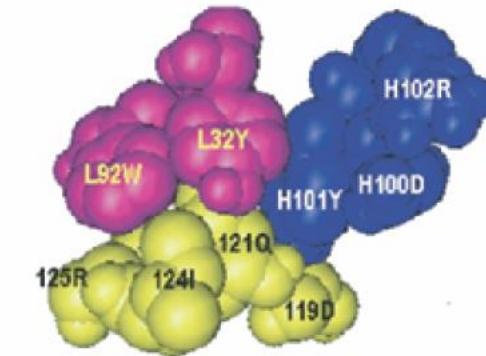


# Antigen-antibody interaction in a 3D structure



Epitope-Paratope Interface

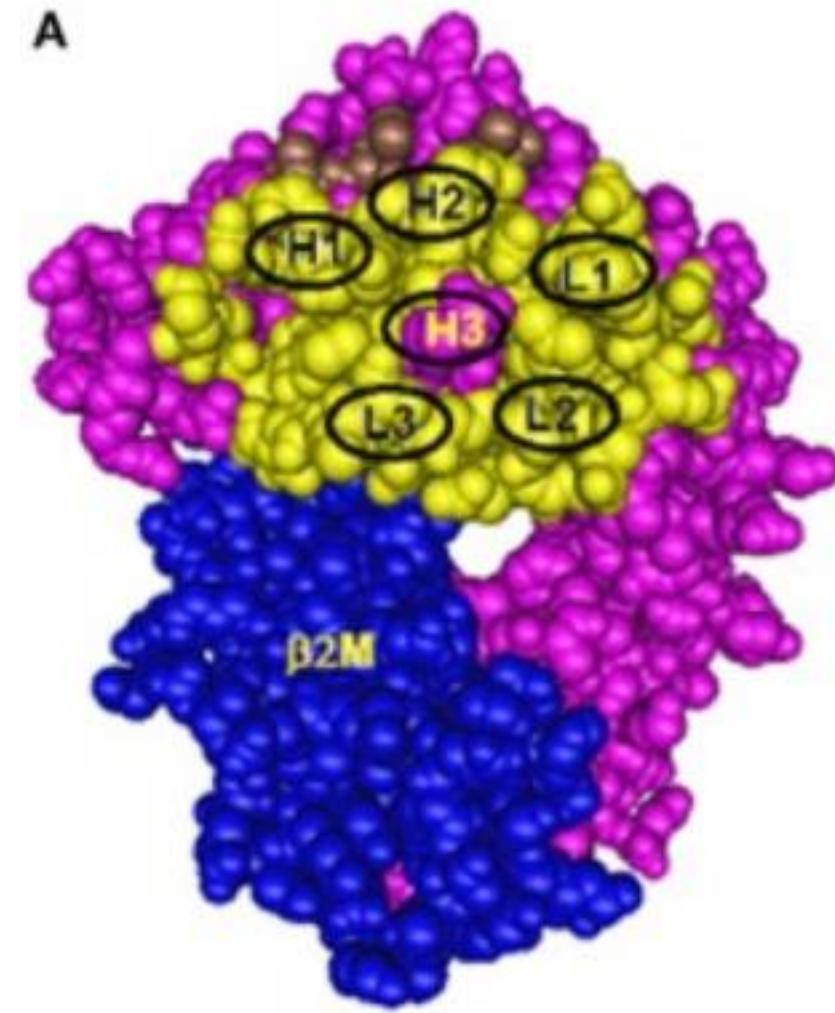
Single Patch



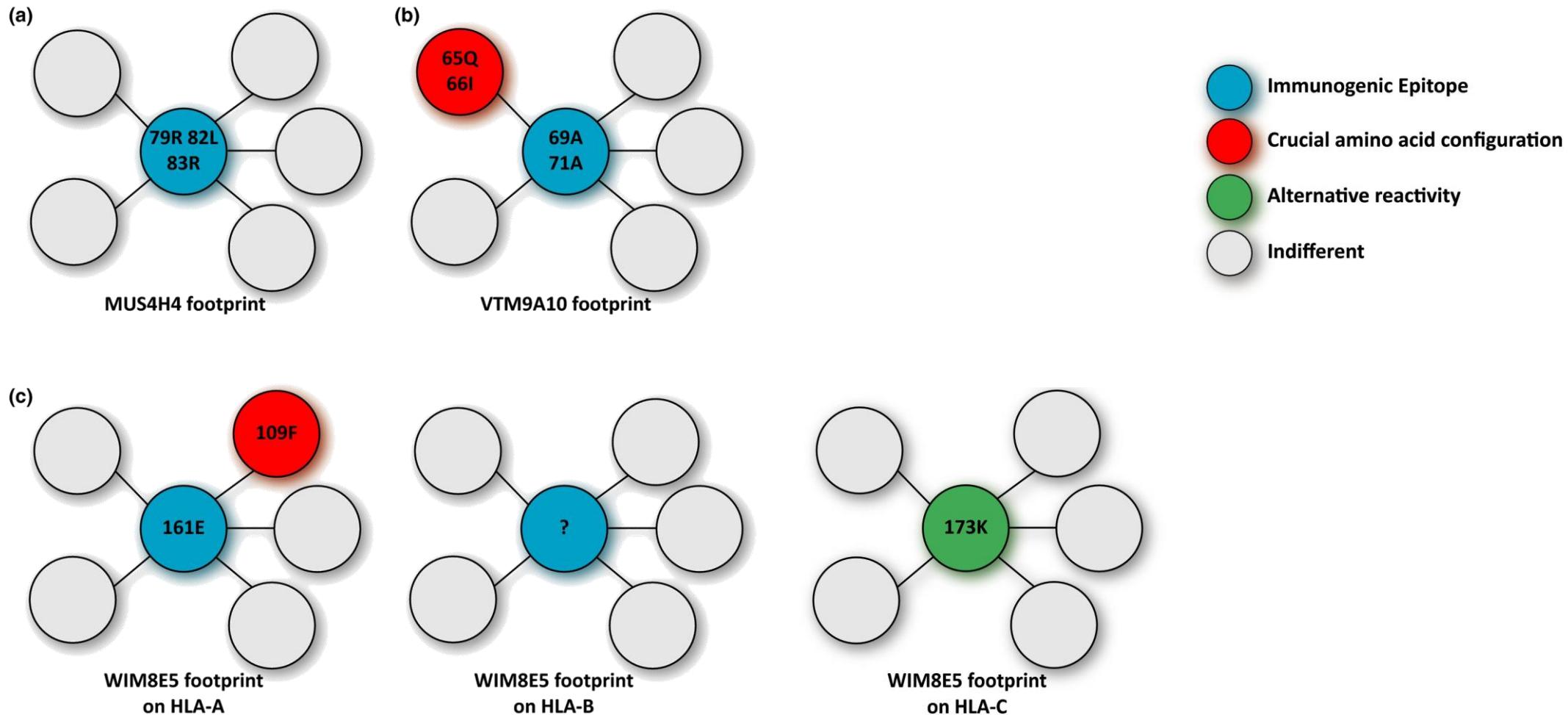
# models for HLA antibody epitopes – eplets

**Eplet** – 3 Å polymorphic area on the antigen surface that determines antibody specificity (interacts with IgH CDR3 region)

**Epitope** – the entire area on the antigen surface which makes contact with an antibody paratope

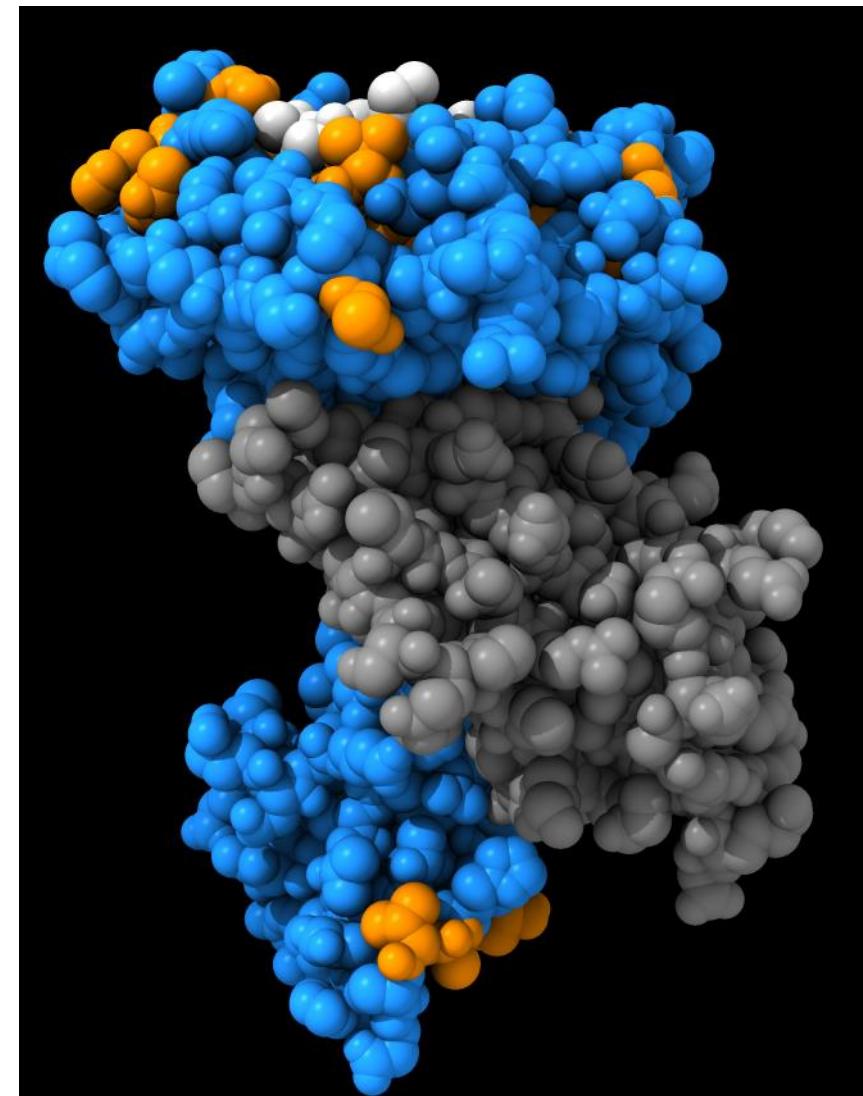


# anatomy of antigen recognition by antibodies



# HLA variants differ only for a few amino acids

AA Pos.	10	20	30	40	50
A*01:01	GSHSMRYFFT	SVSRPGRGEP	RFIAVGYVDD	TQFVRFDSDA	ASQKMEPRAP
A*02:01	-----	-----	-----	R	-----
AA Pos.	60	70	80	90	100
A*01:01	WIEQEGPEYW	DQE <del>T</del> TRNMKAH	SQTDRANLGT	LRGGYNQSED	GSHTI <del>Q</del> IMYG
A*02:01	G	KV	HVD	A	V-R
AA Pos.	110	120	130	140	150
A*01:01	CDVGP <del>D</del> GRFL	RGYRQDAYDG	KDYIALNEDL	RSTWAADMAA	QITKRKWEAV
A*02:01	S W	H Y	K	T H	A
AA Pos.	160	170	180	190	200
A*01:01	HAAE <del>Q</del> RRVYL	EGR <del>C</del> V <del>D</del> GLRR	YLENGKETLQ	RTDPPKTHMT	HHPISDHEAT
A*02:01	V L A	T EW	A	A V	-----
AA Pos.	210	220	230	240	250
A*01:01	LRCWALGFYP	AEITLTWQRD	GEDQTQDTEL	VETRPAGDGT	FQKWA <del>A</del> VVVP
A*02:01	S	-----	-----	-----	-----
AA Pos.	260	270	280	290	300
A*01:01	SGEE <del>Q</del> RYTCH	VQHEGLPKPL	TLRWE <del>L</del> SSQP	TIPIVGIIAG	LVLLGAVITG
A*02:01	Q	-----	P	-----	F
AA Pos.	310	320	330	340	
A*01:01	AVVAAVMWRR	KSSDRKGGSY	TQAASSDSAQ	GSDVSLTACK	V
A*02:01	-----	S	-----	-----	-----



# Epitope rule 1: adaptive immune system tolerates “self”

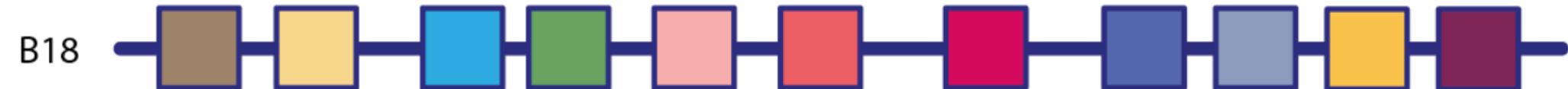
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- B cells and T cells that recognize self proteins, will be eliminated during their training
- For B cells: bone marrow
- For T cells: thymus
- This elimination is essential in preventing auto-immun reactivity

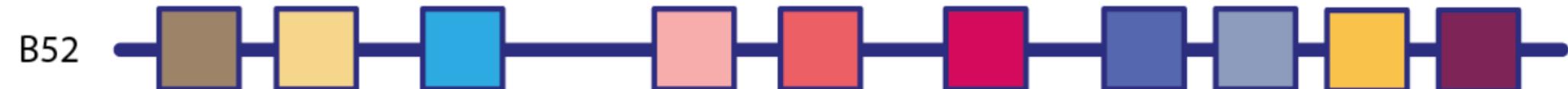
➔ **Identical epitopes** shared between mismatched will be **ignored**

# HLA as epitope/eplet blocks

Donor mm

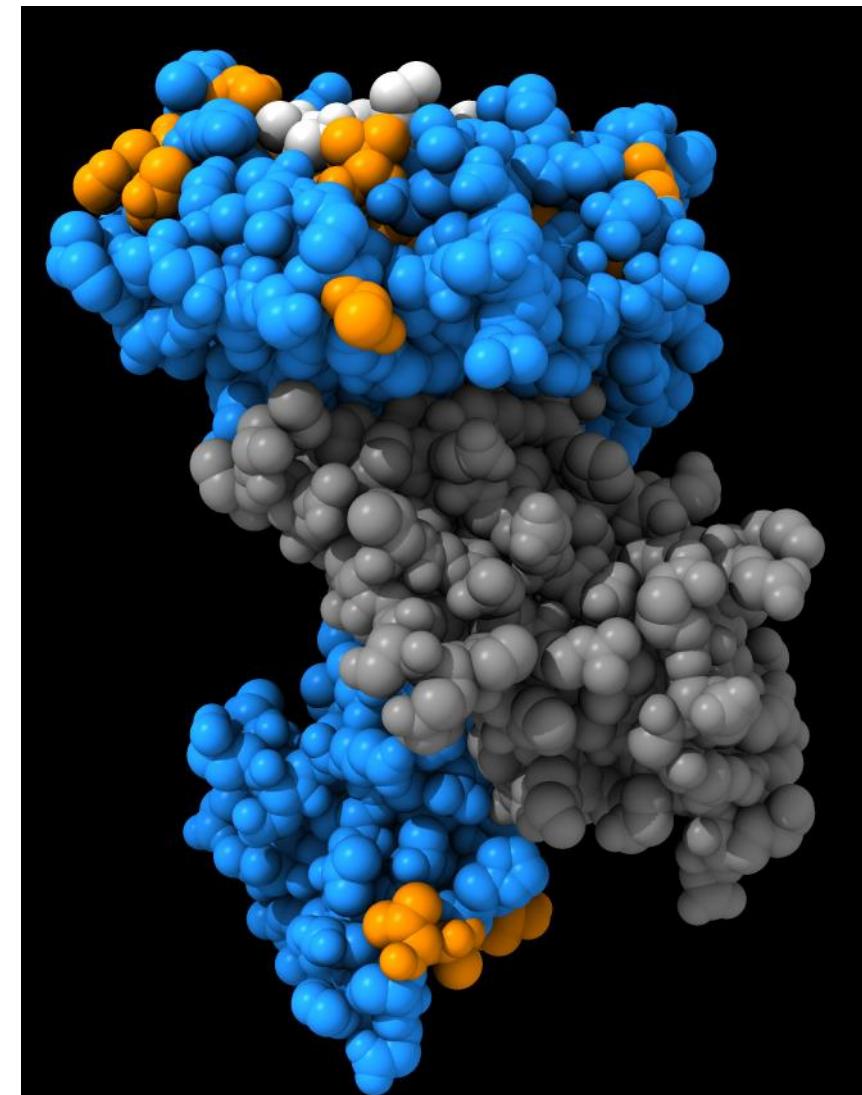


Patient: B7



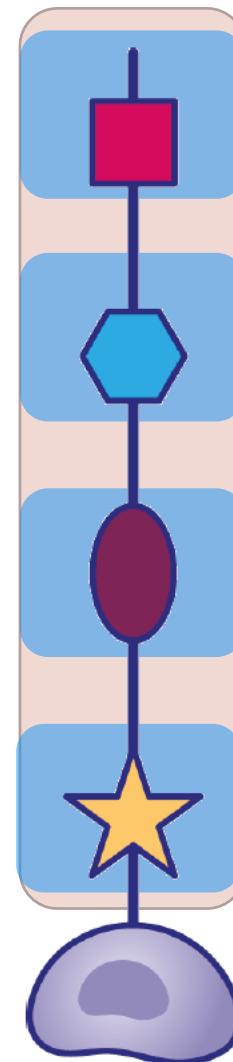
# HLA variants differ only for a few amino acids

AA Pos.	10	20	30	40	50
A*01:01	GSHSMRYFFT	SVSRPGRGEP	RFIAVGYVDD	TQFVRFDSDA	ASQKMEPRAP
A*02:01	-----	-----	-----	-----	R
AA Pos.	60	70	80	90	100
A*01:01	WIEQEGPEYW	DOETRNMKAH	SQTDRANLGT	LRGGYNQSED	GSHTIQIMYG
A*02:01	G KV	H VD	-----	A	V R
AA Pos.	110	120	130	140	150
A*01:01	CDVGPDPDGRFL	RGYRQDAYDG	KDYIALNEDL	RSTWAADMAA	QITKRKWEAV
A*02:01	S W	H Y	K	T H	A
AA Pos.	160	170	180	190	200
A*01:01	HAAEQRKVYL	EGRCVVDGLRR	YLENGKETLQ	RTDPPPKTHMT	HHPISDHEAT
A*02:01	V L A	T E W	-----	A	AV
AA Pos.	210	220	230	240	250
A*01:01	LRCWALGFYP	AEITLTWQRD	GEDQTQDTEL	VETRPAGDGT	FQKWAADVVP
A*02:01	S	-----	-----	-----	-----
AA Pos.	260	270	280	290	300
A*01:01	SGEEQRTCH	VQHEGLPKPL	TLRWEELSSQP	TIPIVGIIAG	LVLLGAVITG
A*02:01	Q	-----	P	-----	F
AA Pos.	310	320	330	340	
A*01:01	AVVAAVMWRR	KSSDRKGGSY	TQAASSDSAQ	GSDVSLTACK	V
A*02:01	-----	-----	S	-----	-----

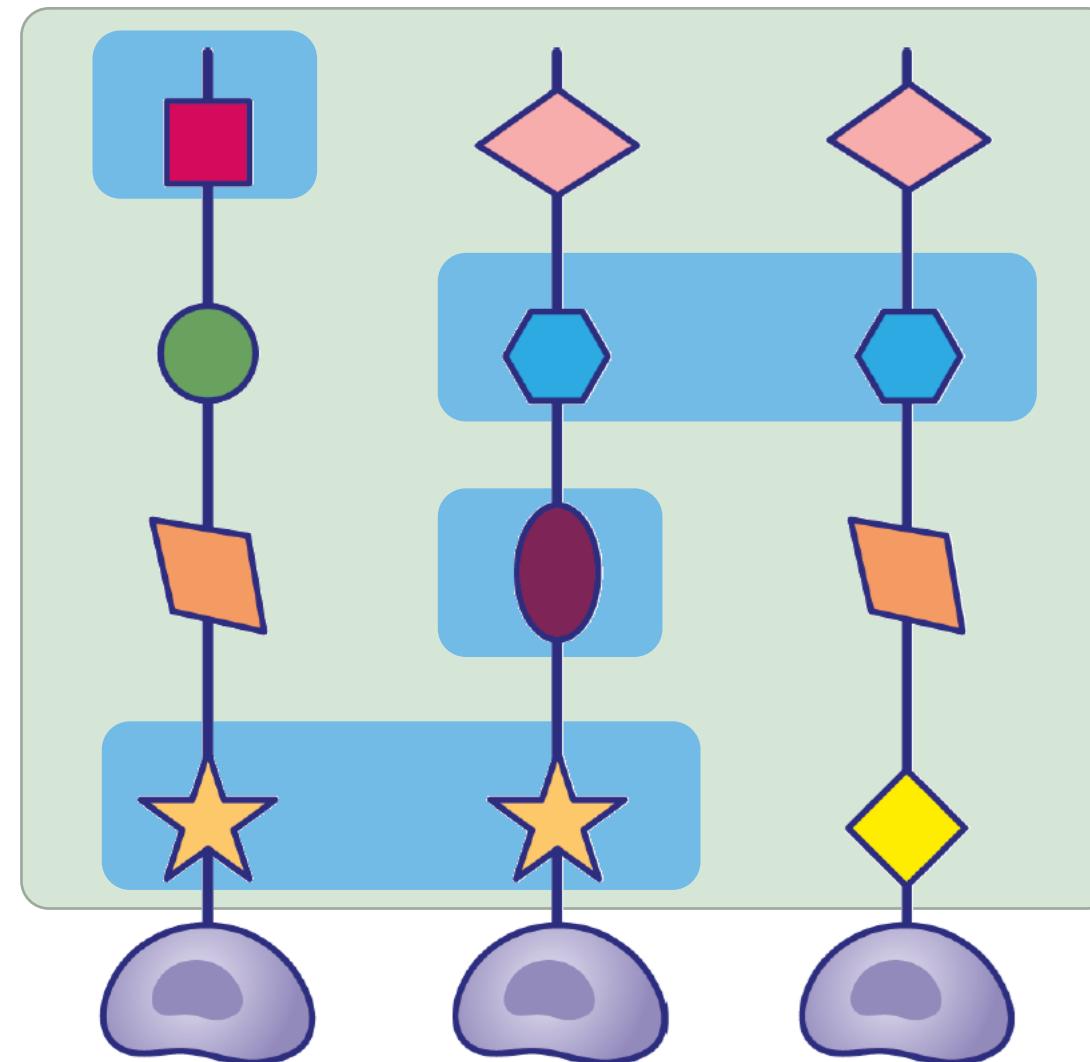


# All self epitopes/eplets will be ignored

Mismatch

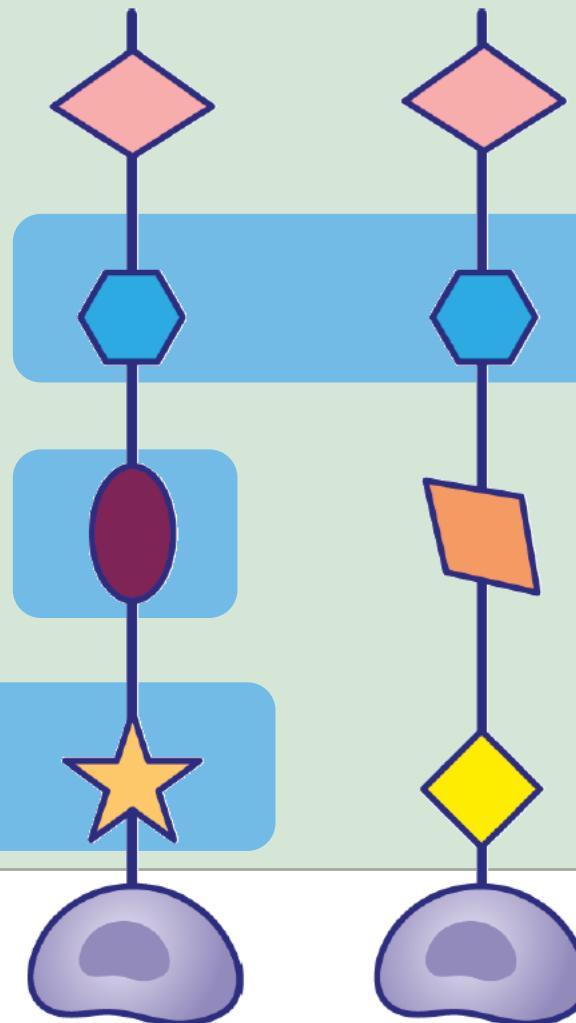


HLA antigen 1

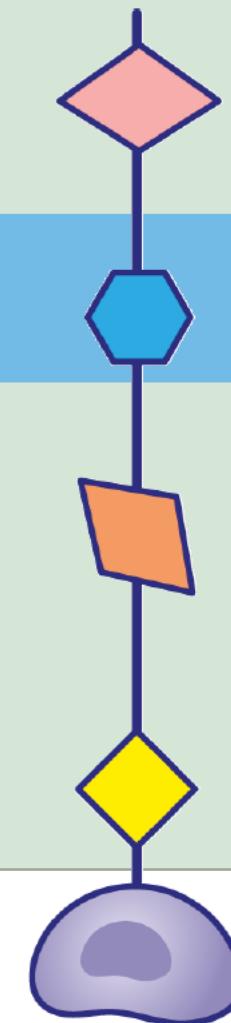


HLA antigen 2

Self HLA



HLA antigen 3



HLA antigen 4

# All self epitopes/eplets will be ignored

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	10	20	30	40	50
B*51:01	GSHSMRYFYT	AMSRPGRGEP	RFIAVGYVDD	TQFVRFDSDA	ASPRTEPRAP
B*35:01	-----	-----	-----	-----	-----
B*41:01	-----H-	-----	-T-----	-L-----	T---K-----

# Epitope rule 2: antibodies interact with accessible parts

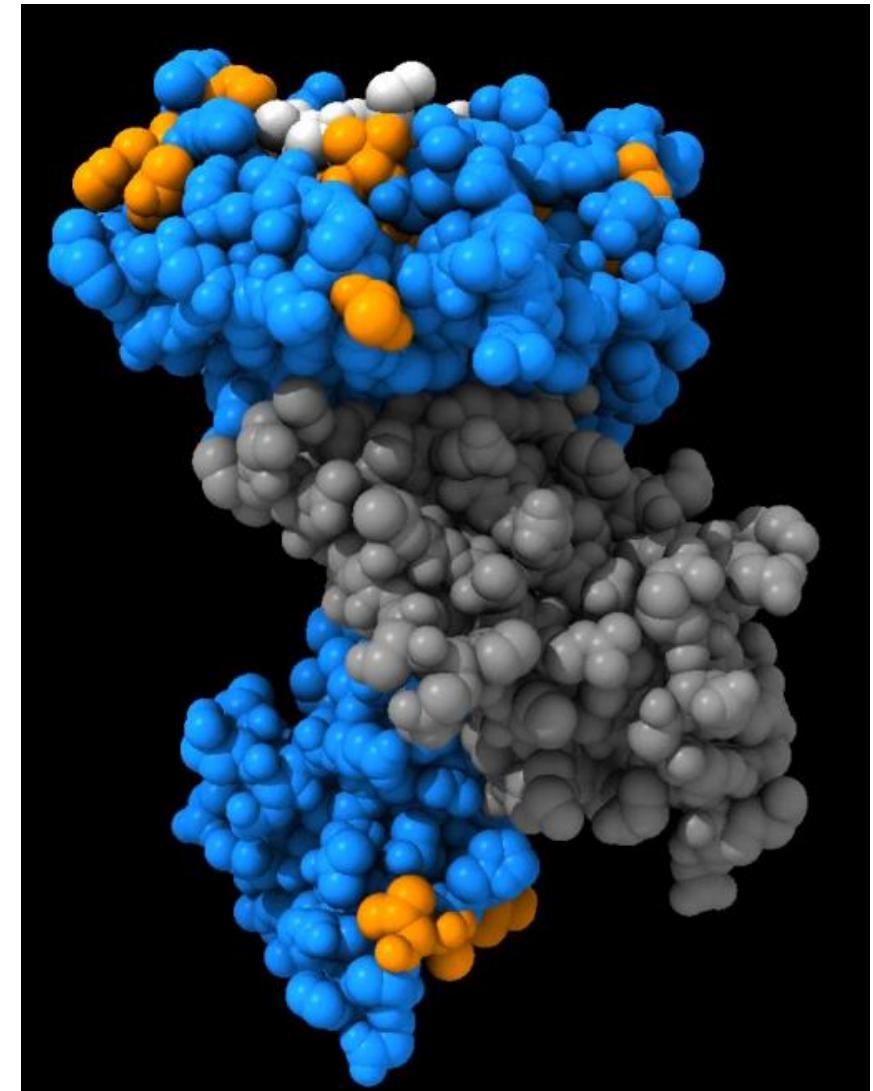
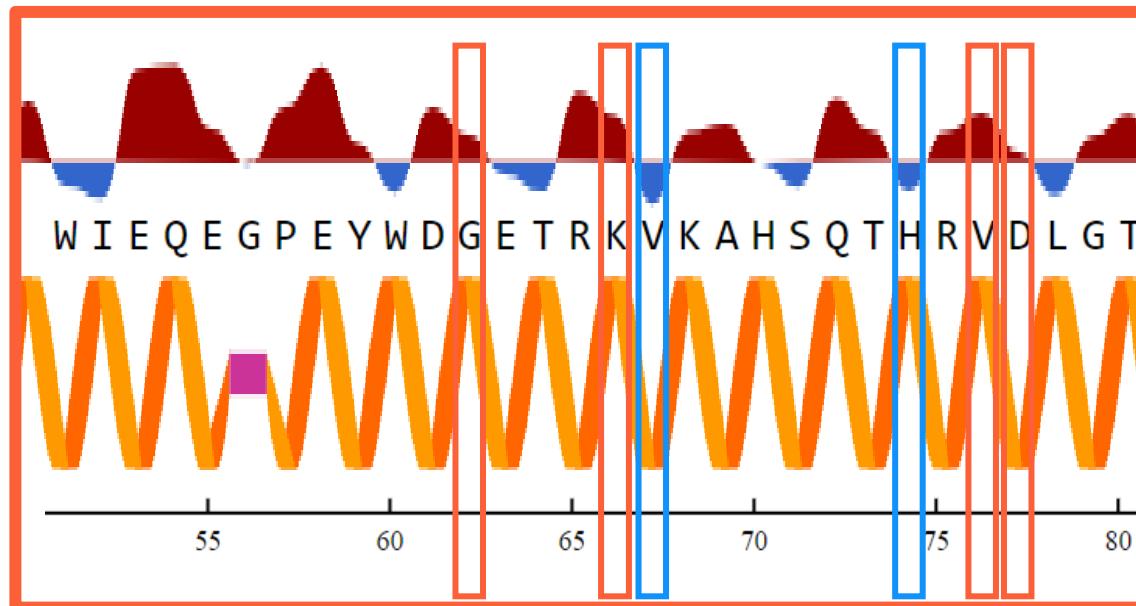
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- Amino acids inside the “**interior**” of a protein are **not accessible**
- Amino acids outside the “**exterior**” of a protein are **accessible**
- To be recognized by an **antibody**, an amino acid needs to be located on the surface of a protein

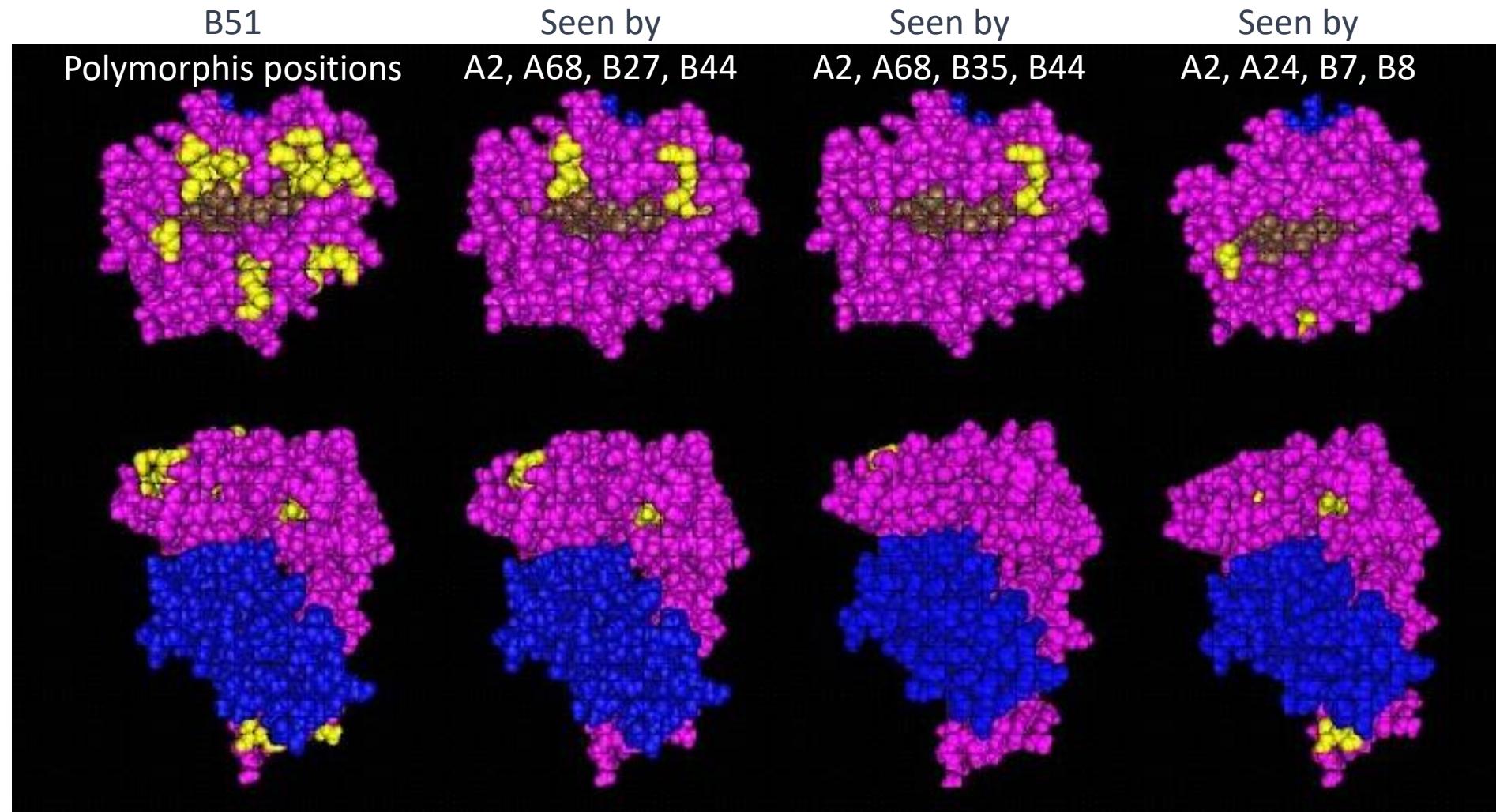
# Which foreign amino acids are accessible?

AA Pos.	10	20	30	40	50
A*01:01	GSHSMRYFFT	SVSRPGRGEP	RFIAVGYVDD	TQFVRFDSDA	ASQKMEPRAP
A*02:01	-----	-----	-----	-----R-----	

AA Pos.	60	70	80	90	100
A*01:01	WIEQEGPEYW	DQETRNMKAH	SQTDRANLGT	LRGGYNQSED	GSHTIQIMYG
A*02:01	-----G-----KV-----H-VD-----			-----A-----	-----V-R-----



# The same HLA antigen form a different point of view



# Basic principles of HLA antibody epitope algorithms

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1. Position/region needs to be polymorphic (foreign)
2. Polymorphism needs to be antibody accessible / solvent accessible

# the birth of the HLA matchmaker algorithm



## 15<sup>th</sup> International Workshop Project on Epitope Immunogenicity

- Analyze post-allograft nephrectomy sera for antibodies against donor class I and class II epitopes
- Serum screening with single alleles (Luminex) and by CDC
- So far, 40 laboratories worldwide will contribute informative cases

**Determination of Structurally Defined Immunogenic HLA Epitopes**  
Rene Duquesnoy, Frans Claas

# HLA Matchmaker and eplets

 HLA Eplet Registry

Home Databases Mismatch Calculator Publications Submit pattern new Contact us

Home > Mismatch Calculator

 **HLA Eplet Mismatch Calculator**

VERSION 2023-11-24. UPDATED WITH IPD-IMGT/HLA 3.53

**Multiple Patients** **Multiple Donors** **Multiple Immunizers**

 **HLA TYPINGS TO MISMATCH**

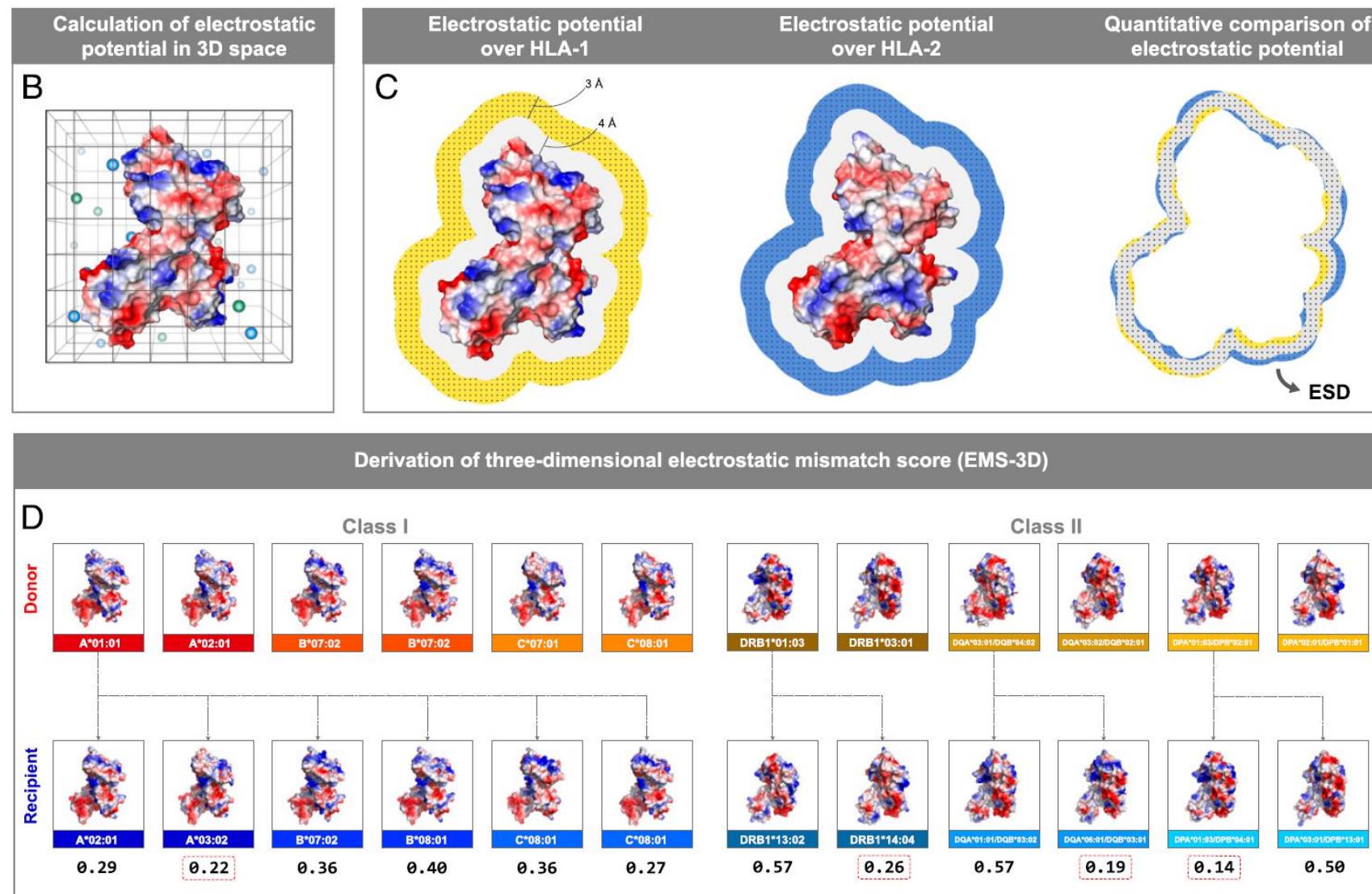
Donor/Immunizer	A*02:01	<ul style="list-style-type: none"> <li>Click at any HLA locus or eplet to see its details.</li> <li>Eplets between [brackets] are the antibody-verified ones.</li> <li>If you have any tips on improving this calculator, please <a href="#">get in contact</a>.</li> <li><b>Service not working? Please, get in contact.</b></li> </ul>
Patients	A*01:01, A*68:01, B*07:01, B*08:01, DR1, DR3	

Donor/Immunizer Vs. Patient 1	A/B/C	DRB1/3/4/5	DQA1/DQB1	DPA1/DPB1	MICA	TOTAL
	11					11

**HLA LOCI GROUP** **MISMATCHES QTY.** **MISMATCHES DETAILS**

**A/B/C** 11 All mismatches: [62GE], [62GK], 65RK, 66K, 66KA, 66KH, 95V, 97R, [107W], 114H, 156L  
Single-allele mismatch load: A\*02:01 11

# structural and physicochemical dissimilarity



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Revised: 23 March 2020

Accepted: 25 March 2020

DOI: 10.1111/tan.13883

**ORIGINAL ARTICLE**

**HLA**  
Immune Response Genetics

**WILEY**

# **HLA-EMMA: A user-friendly tool to analyse HLA class I and class II compatibility on the amino acid level**

**Cynthia S. M. Kramer<sup>✉</sup>** | **Johan Koster** | **Geert W. Haasnoot** |  
**Dave L. Roelen** | **Frans H. J. Claas** | **Sebastiaan Heidt**

# HLA-EMMA: immunological concept

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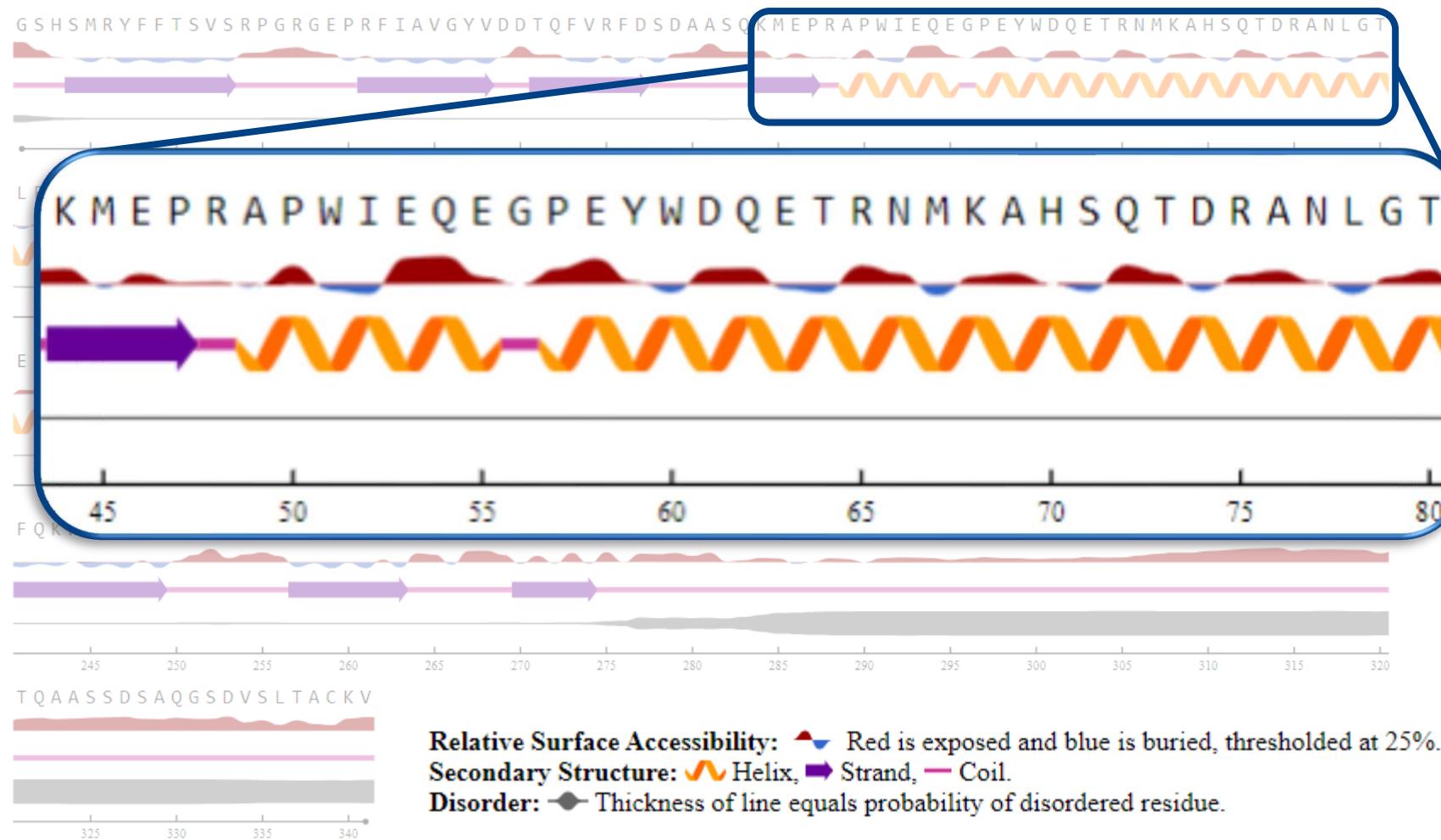
The software program is based on the hypothesis that any polymorphic amino acid exposed on the surface of an HLA molecule can trigger an antibody response.

# HLA-EMMA: HLA amino acid sequences

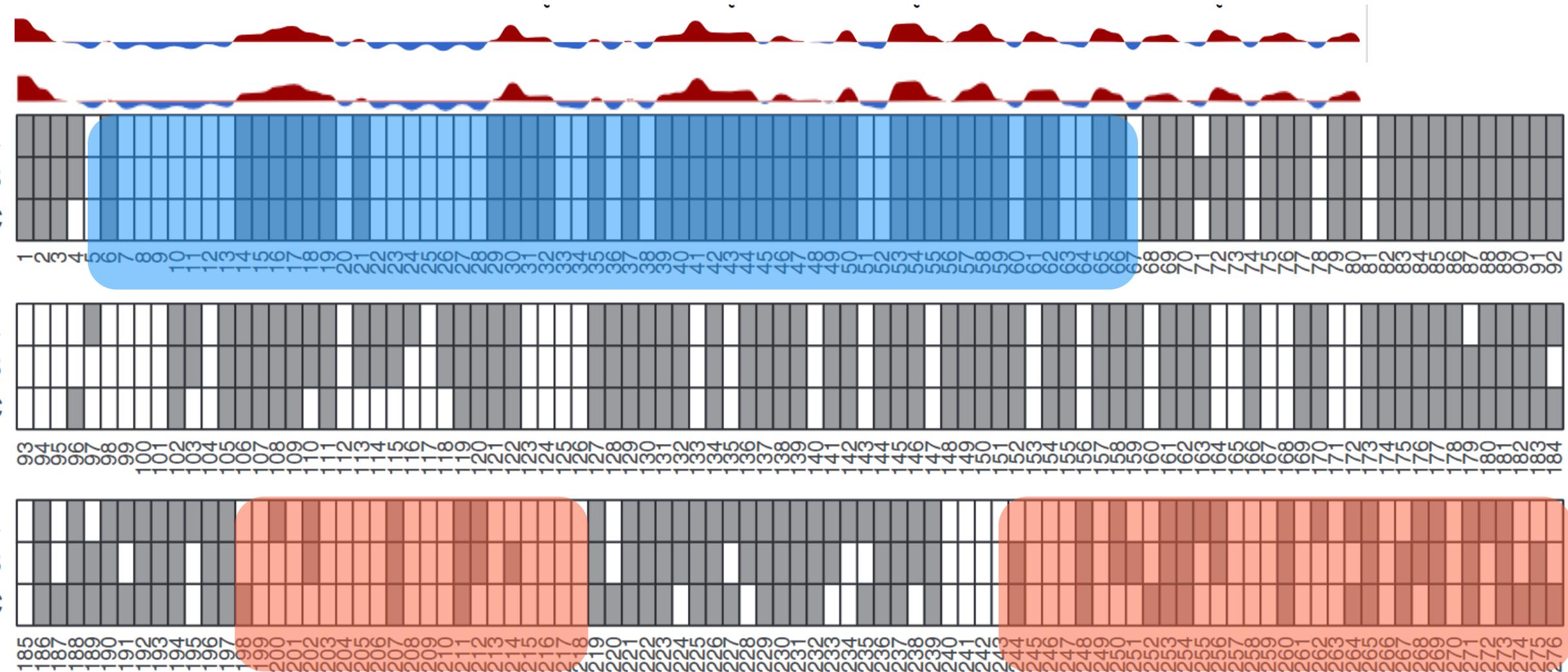
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- Alleles grouped by identical second field identity
- Inclusion of all amino acid sequences covering position 1-275 for class I and 1-226 for class II
- Inclusion of all null alleles
- Incomplete sequences are included, but considered rare and marked in the analyses

# An example of Netsurf2.0 output



# HLA-EMMA: a *per locus* surface accessibility map



# Allele-specific structures

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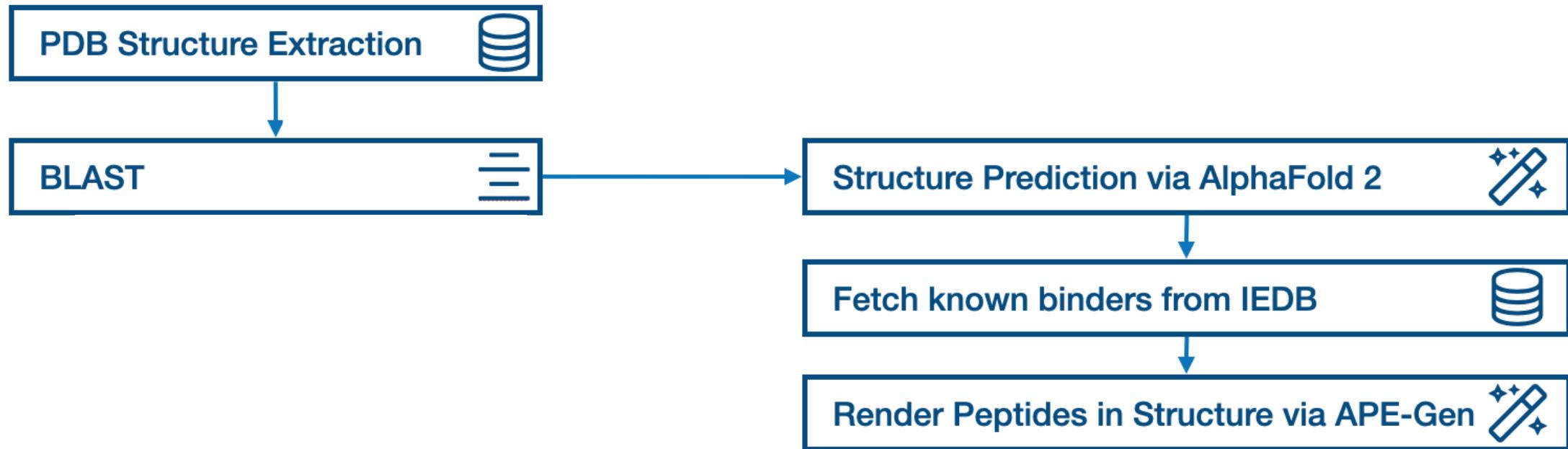
Frontiers in Immunology

TYPE Original Research  
PUBLISHED 29 July 2022  
DOI 10.3389/fimmu.2022.937587

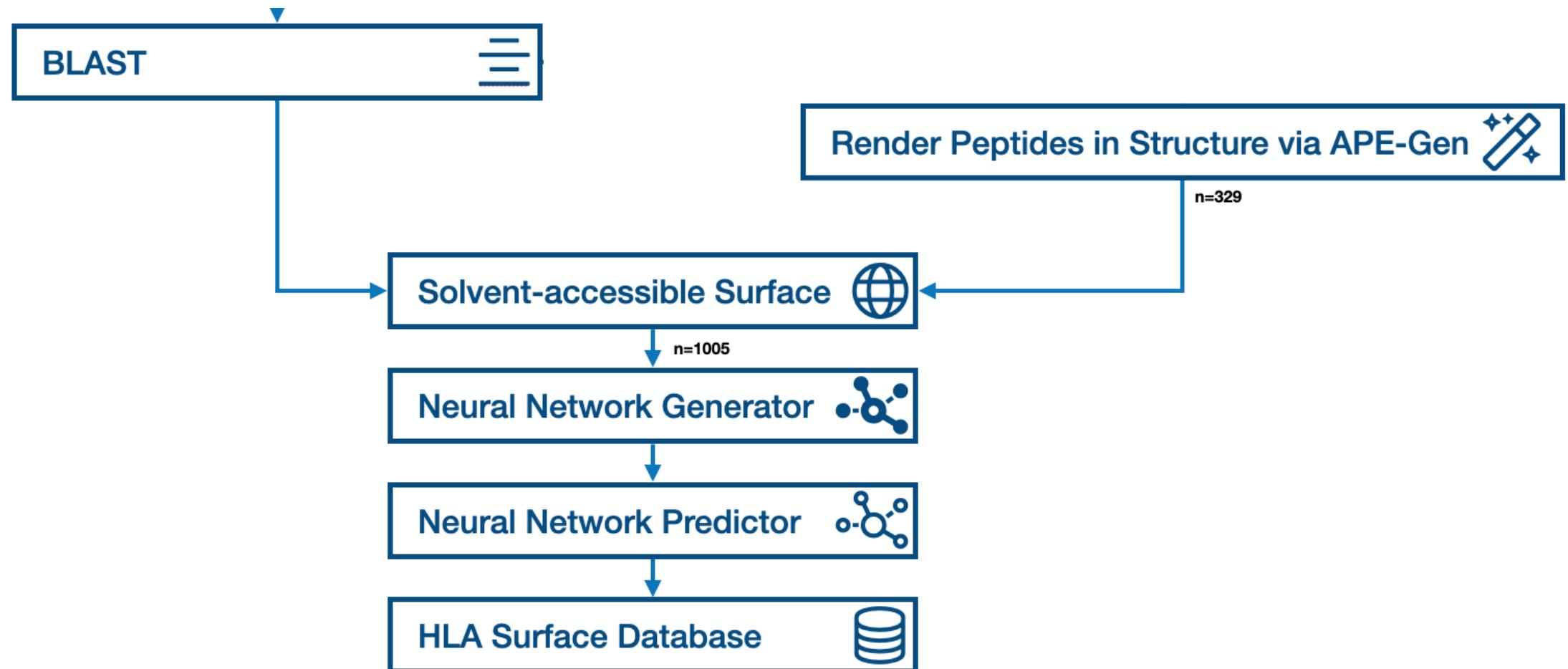
## Snowflake: A deep learning-based human leukocyte antigen matching algorithm considering allele-specific surface accessibility

Matthias Niemann<sup>1\*</sup>, Benedict M. Matern<sup>2</sup> and Eric Spierings<sup>2,3</sup>

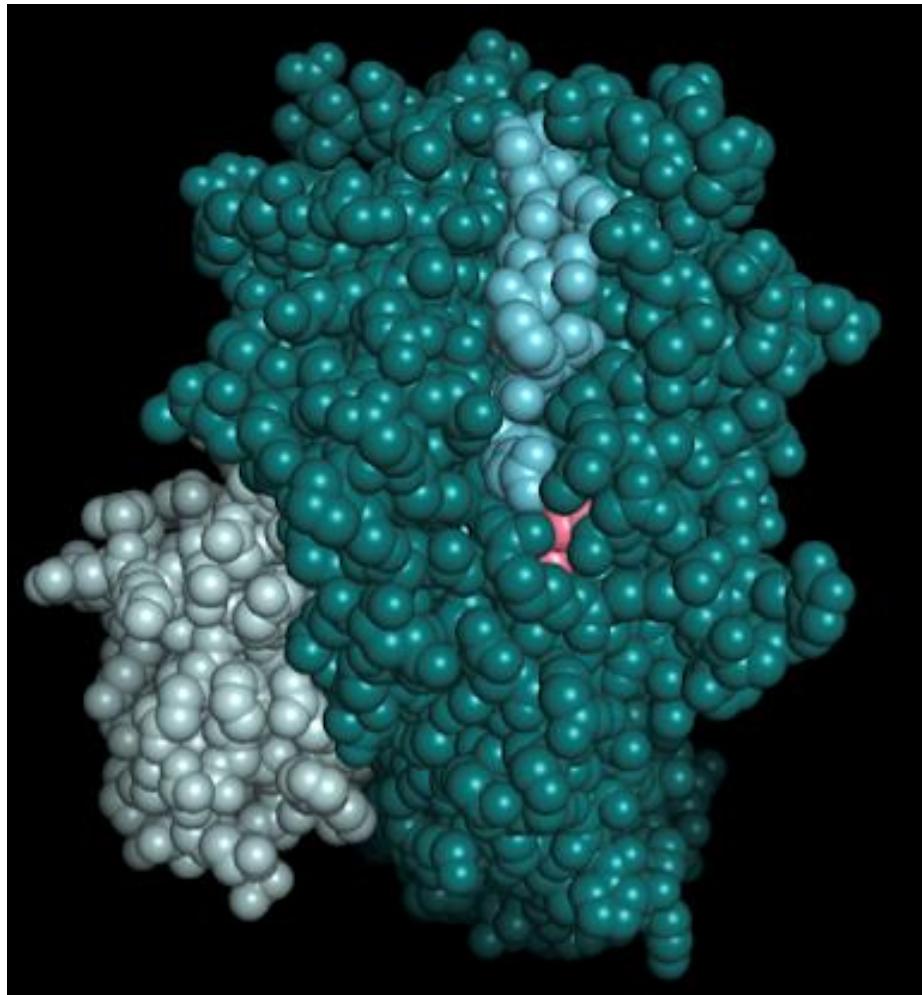
# Snowflake: a deep-learning prediction pipeline



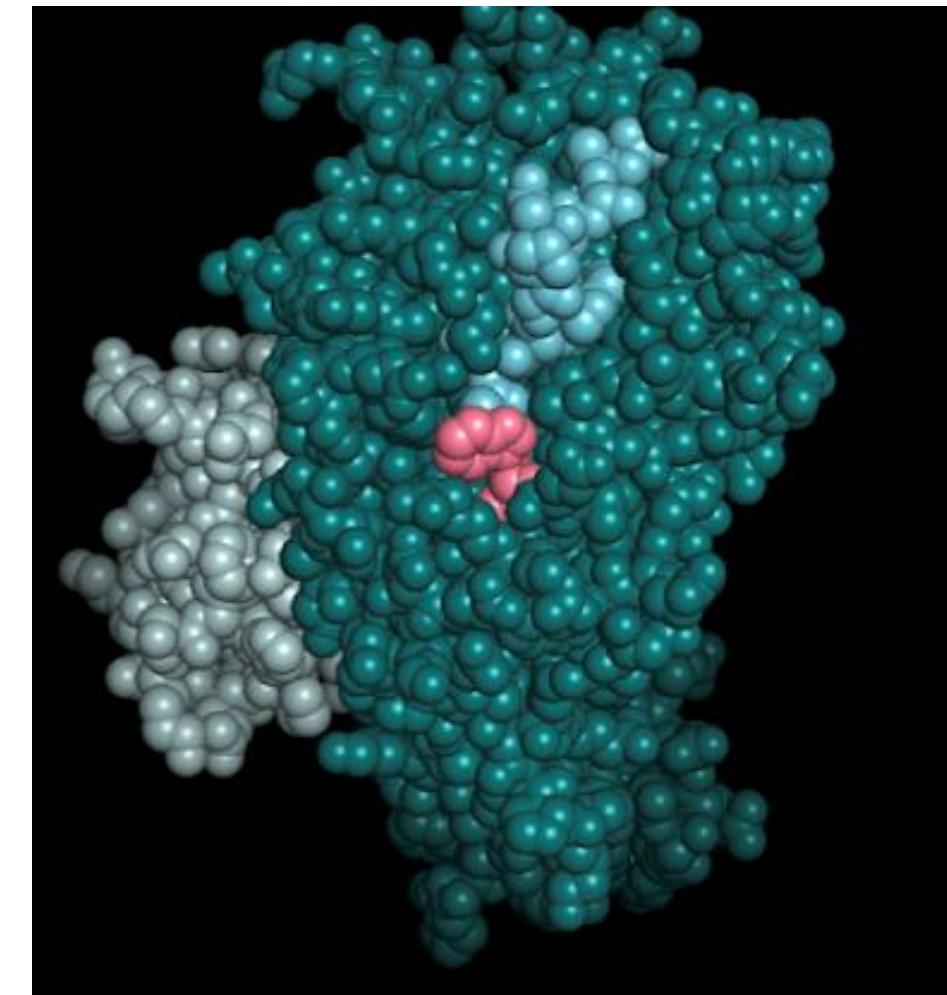
# Solvent accessibility



# Accessibility may differ per allele, even within one locus

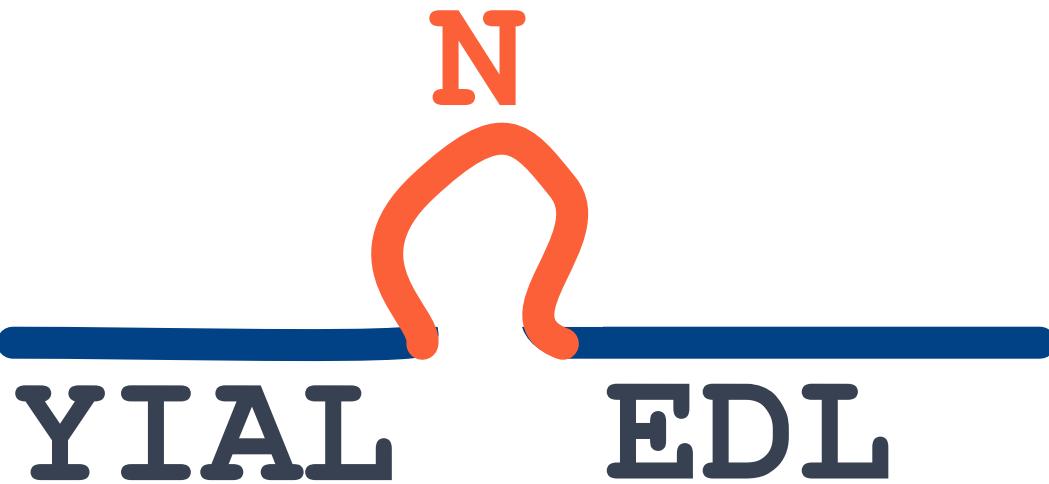


HLA-A\*01:01



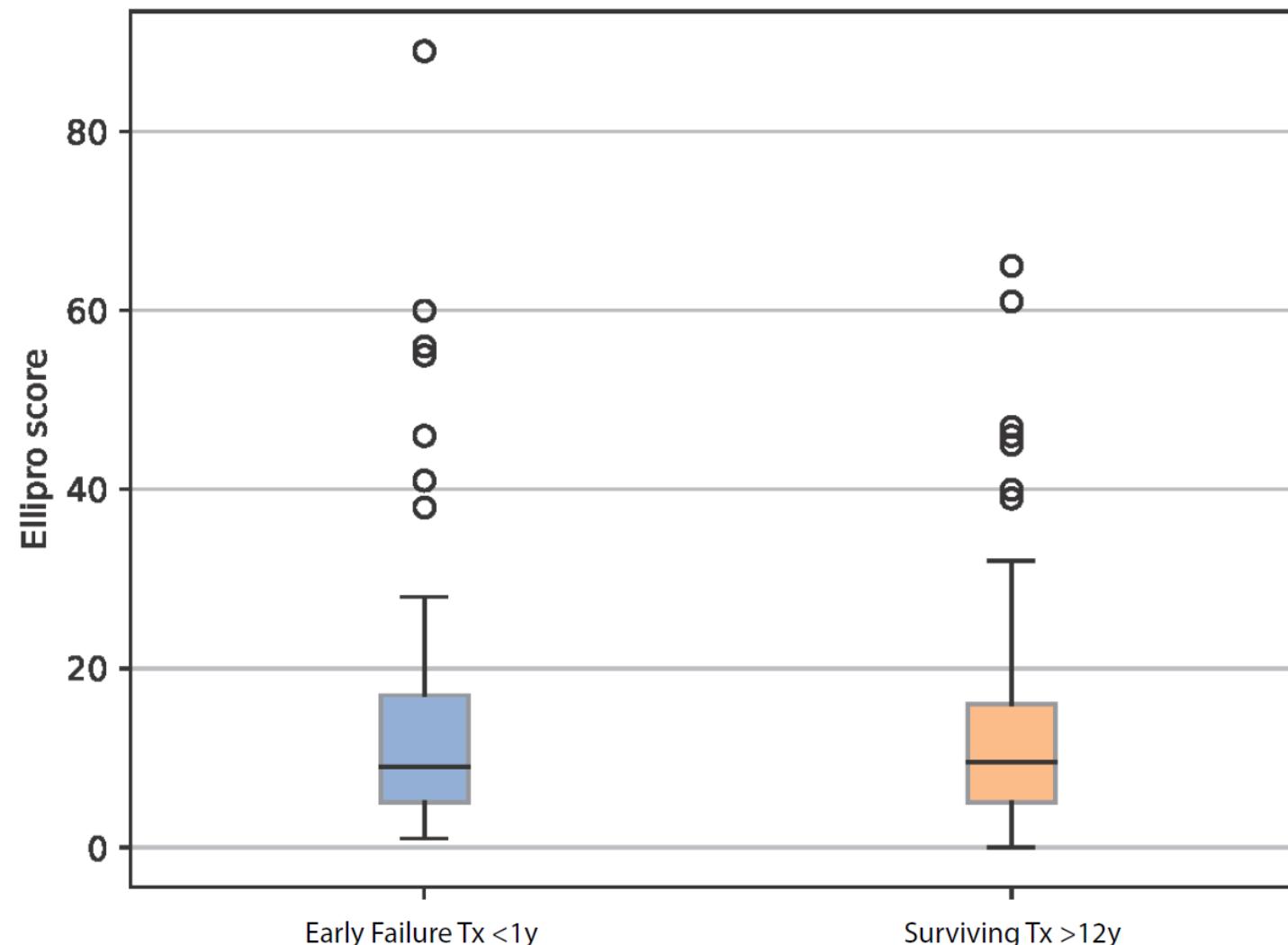
HLA-A\*02:01

# Protrusion enhances antibody interaction – ellipro scores



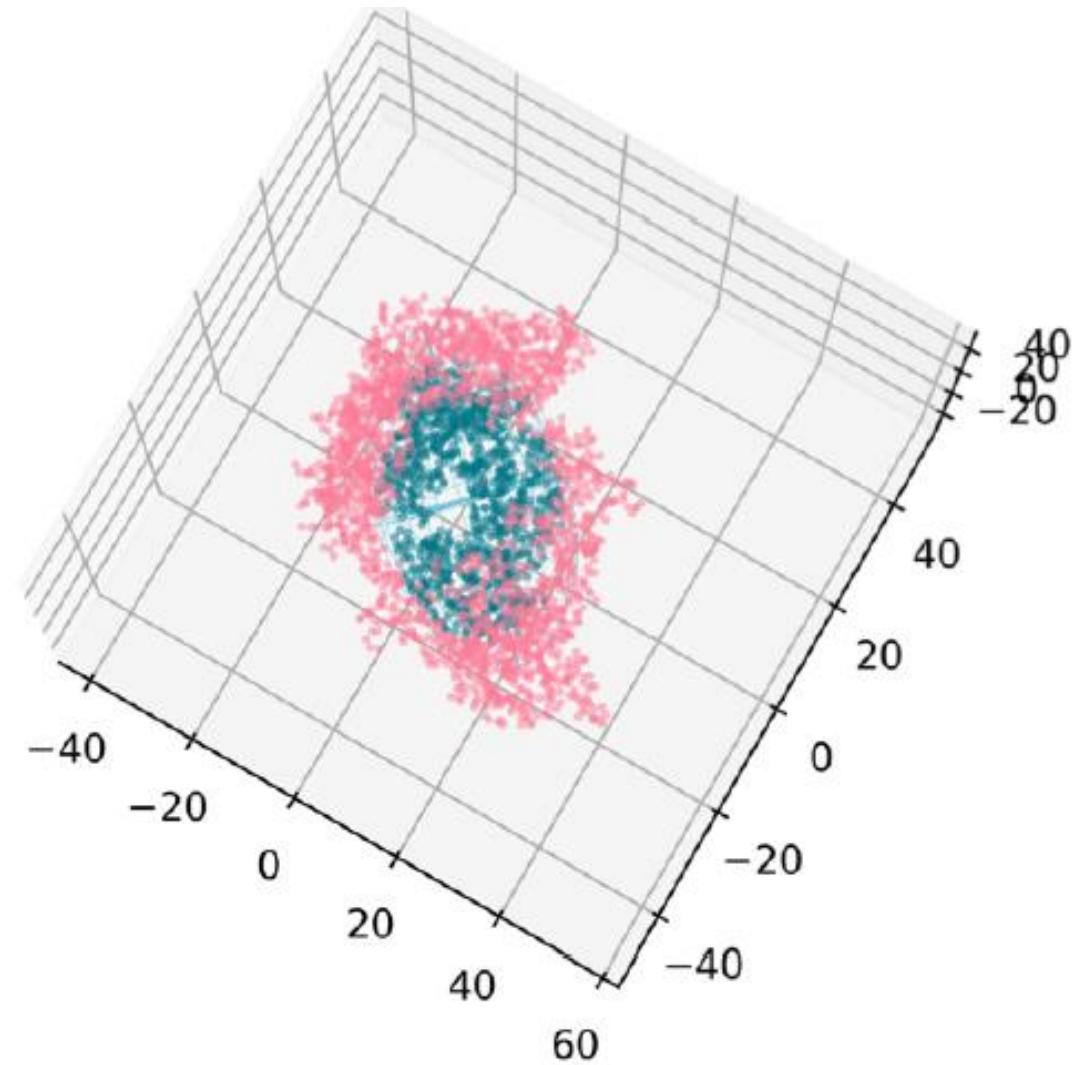
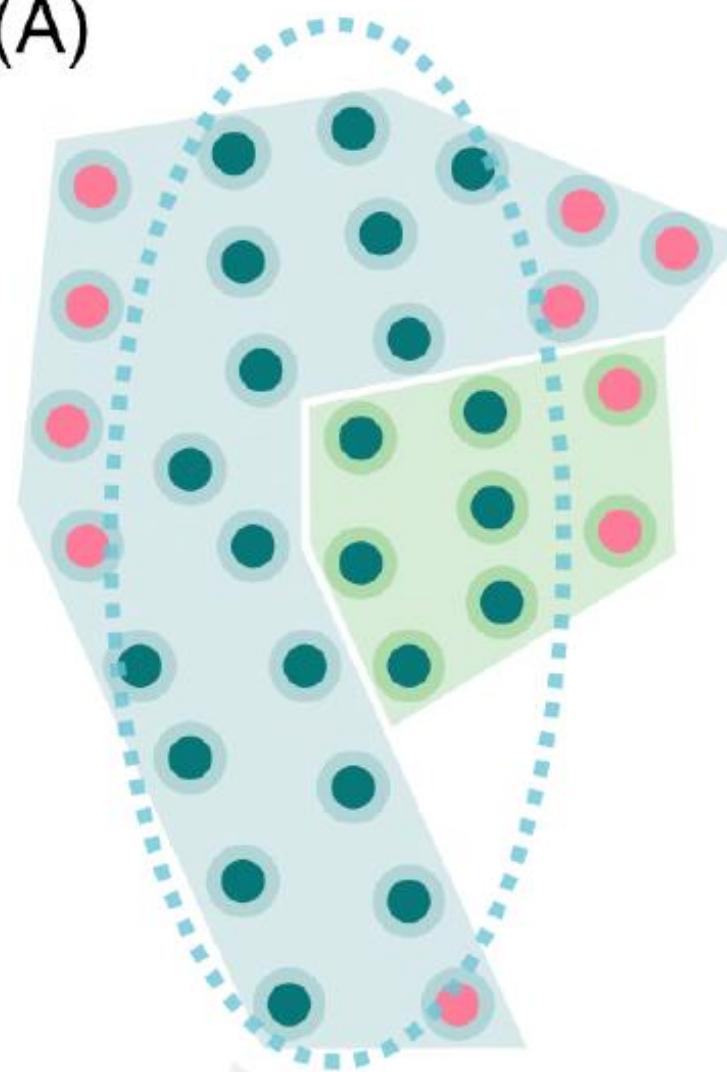
# No association between Ellipro scores and graft loss

Figure 2



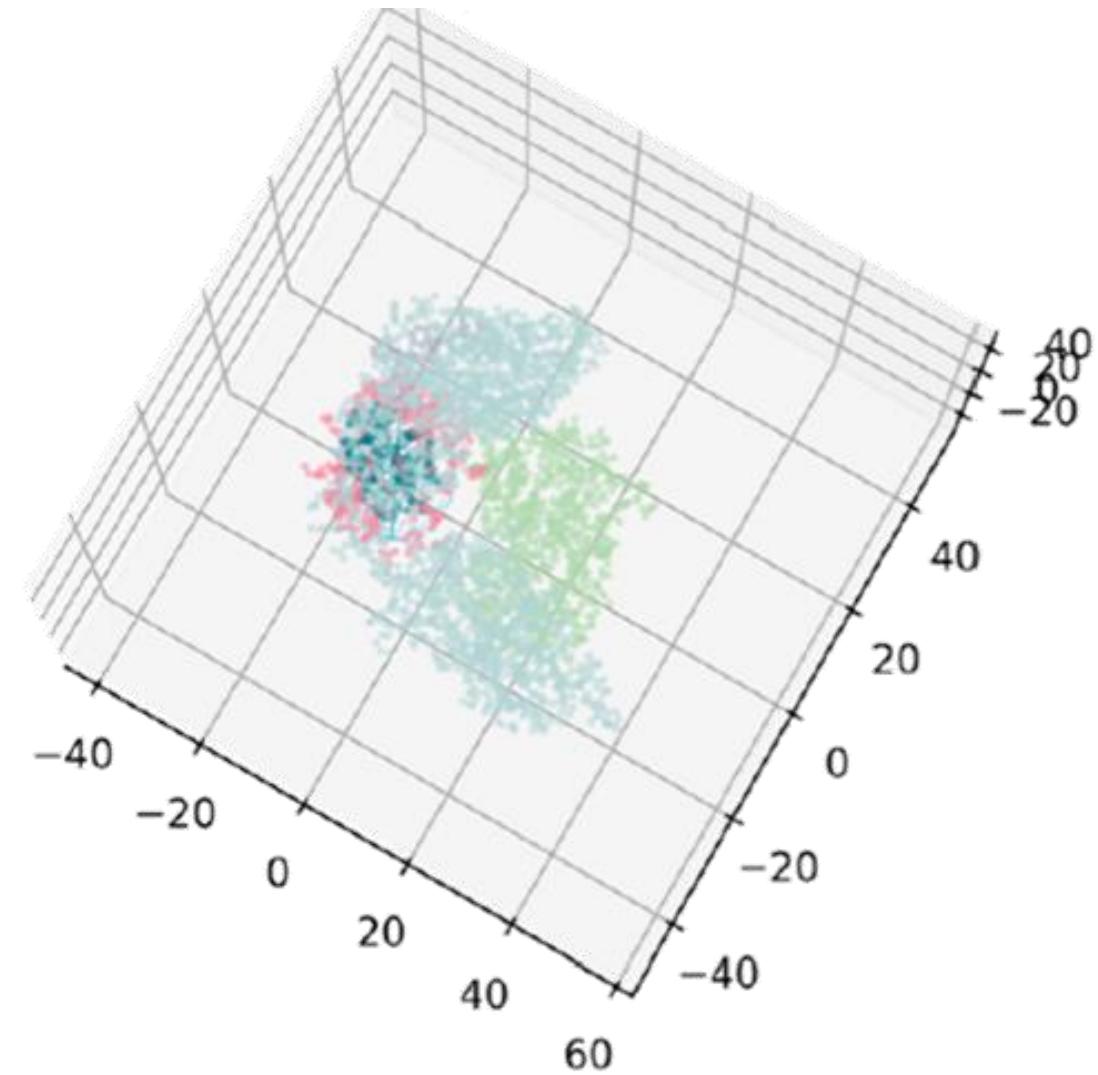
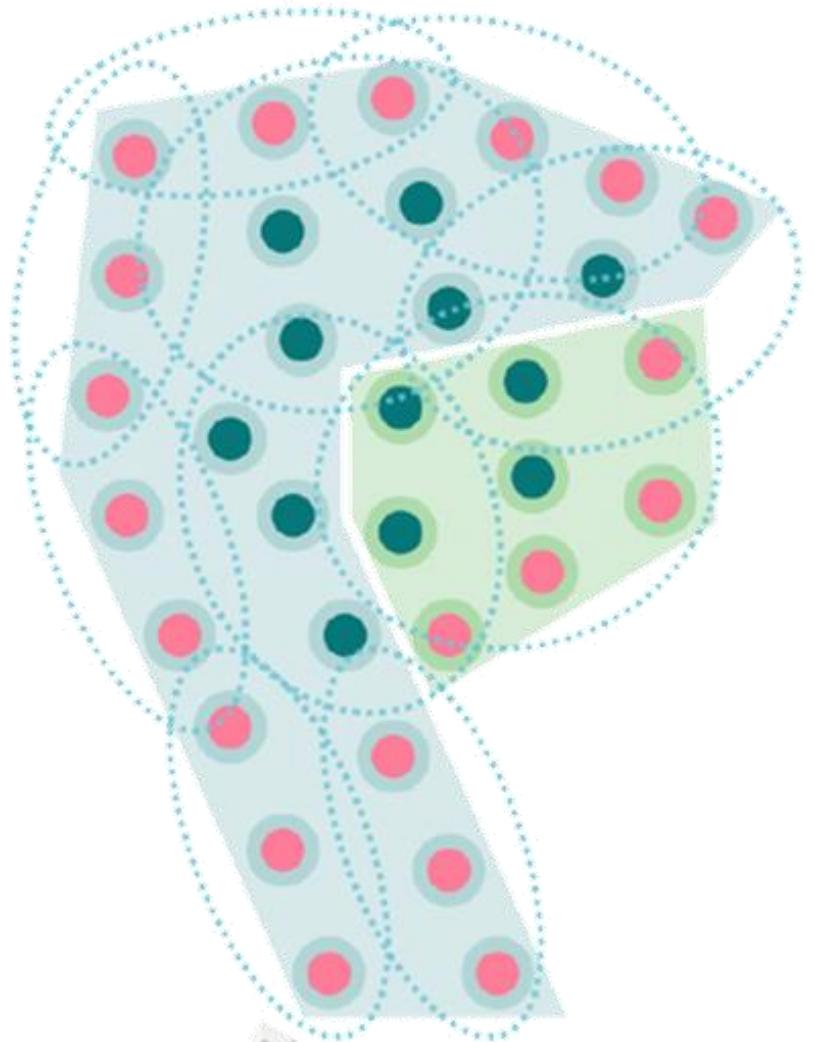
# HLA molecules are not shaped like American footballs

(A)

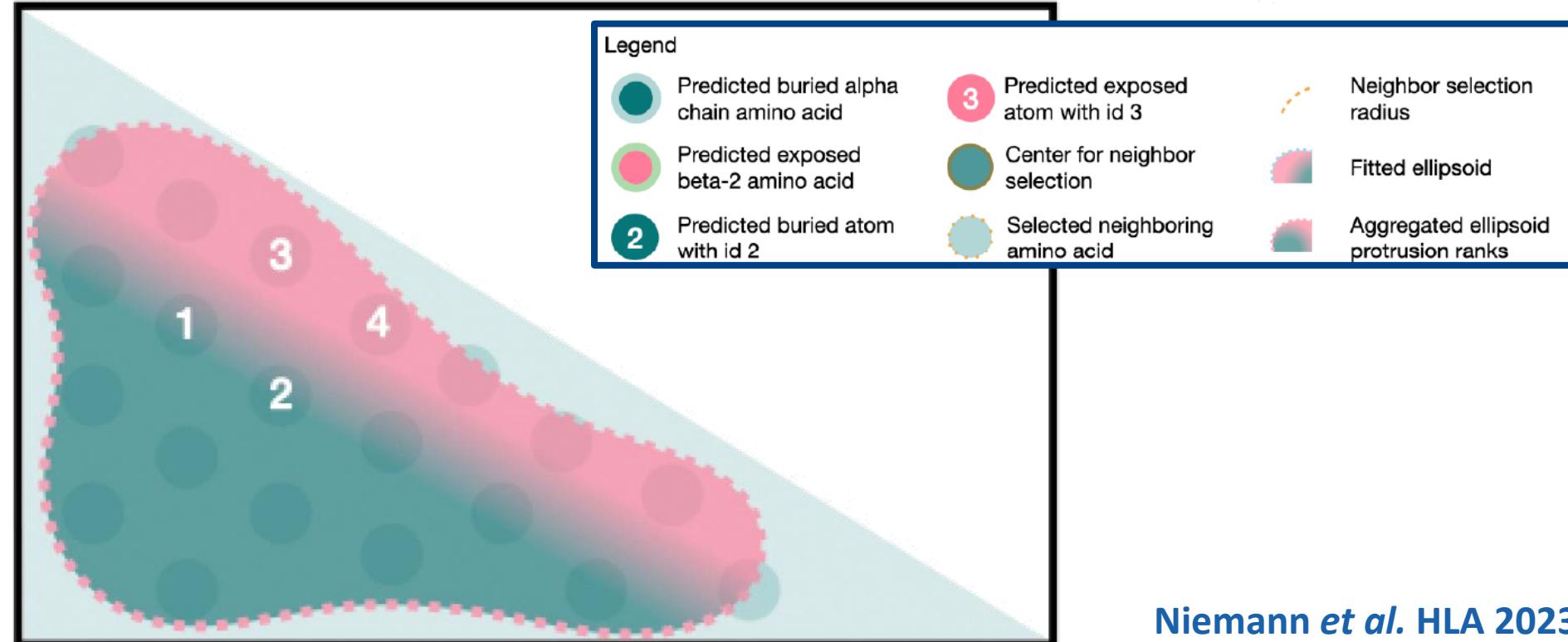
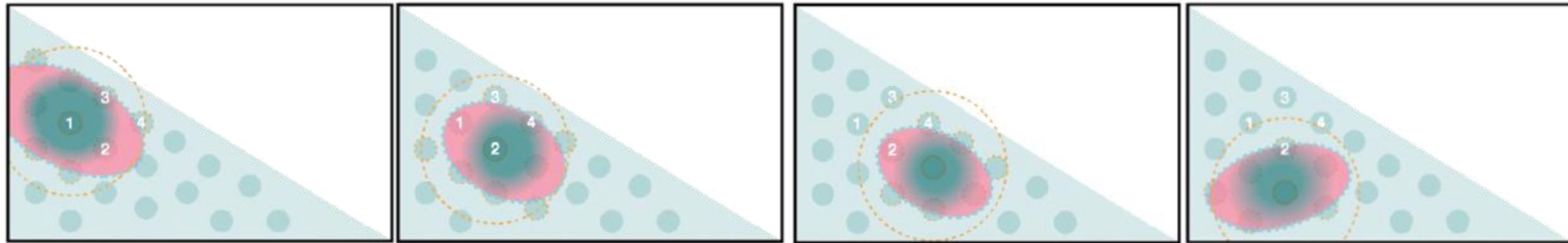


# Fitting smaller ellipsoids on the HLA molecule

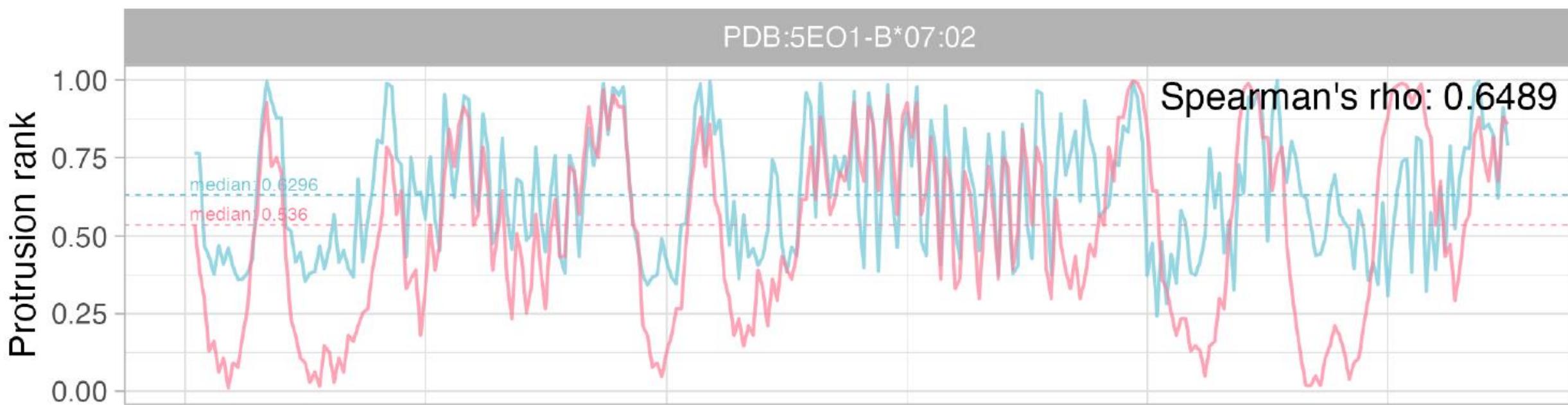
B)



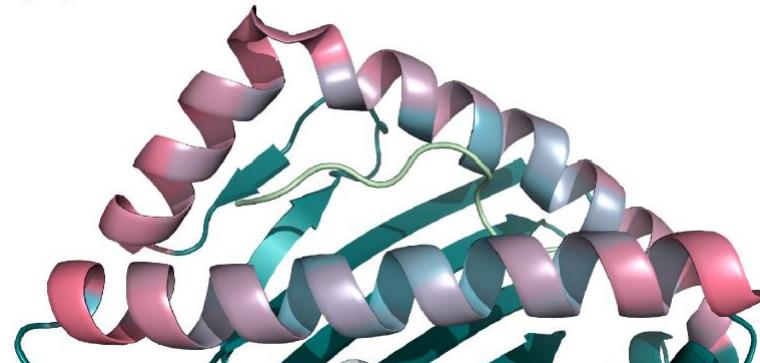
# Accumulating sub-ellipsoid data – Snowball algorithm



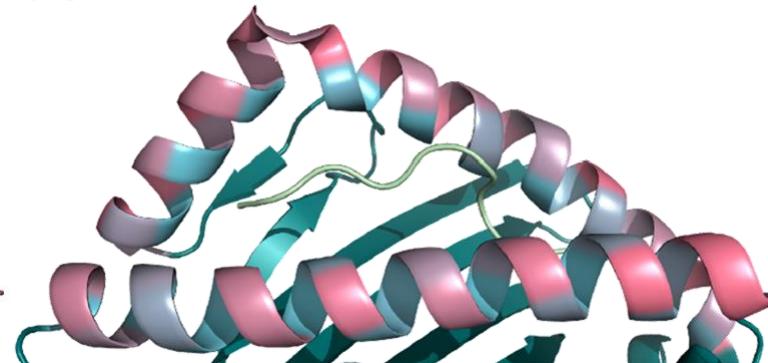
# Accumulating sub-ellipsoid data – Snowball algorithm



(C) ElliPro-based colors in helices



(D) Snowball-based colors in helices



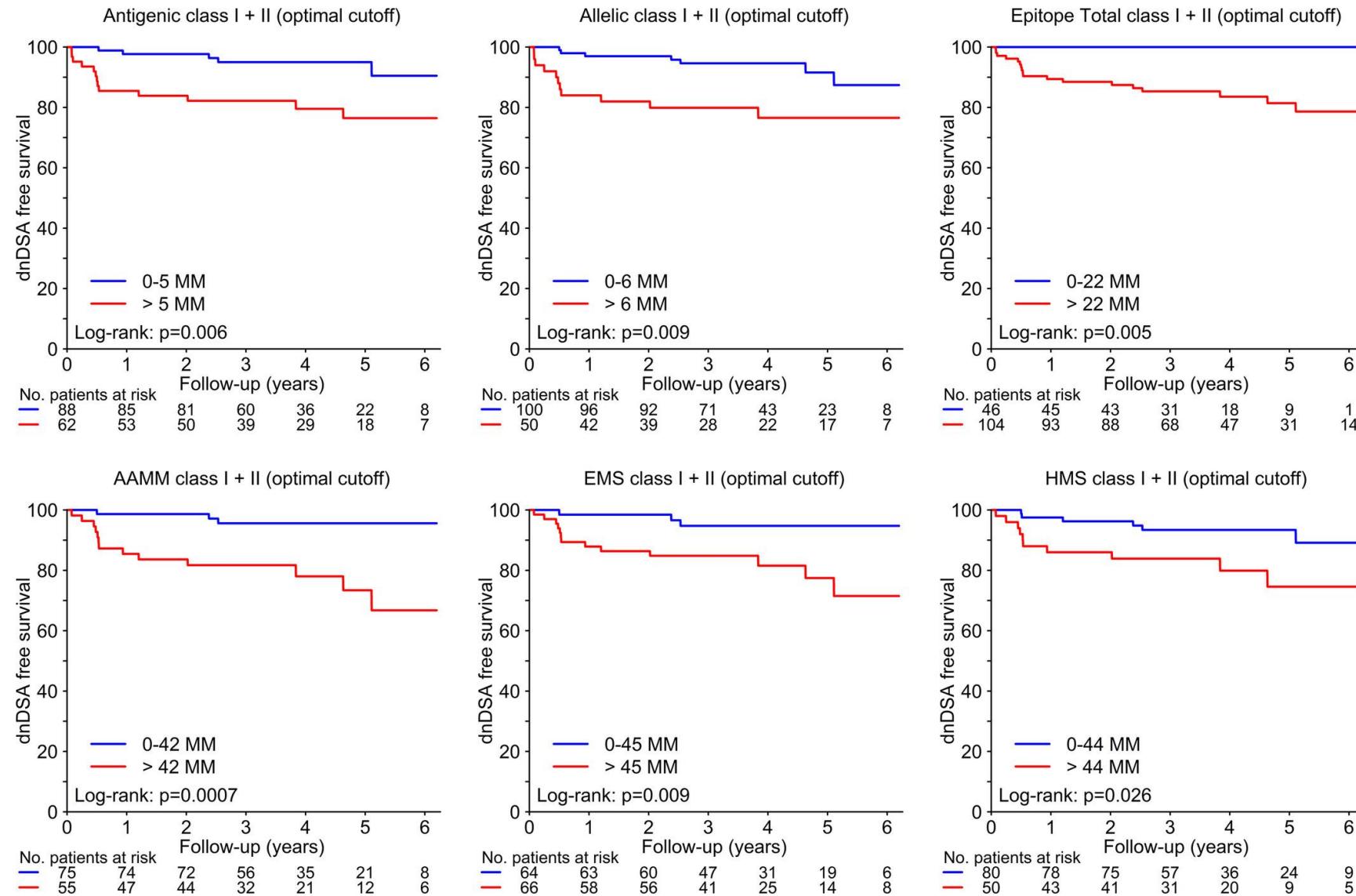
variable

- Snowball
- ElliPro

# HLA Antibody epitope models

	Amino Acid Mismatch	HLA Matchmaker	Electrostatic mismatch 3D	HLA-EMMA	ElliPro	Snowflake
<b>3D structure</b>	✗	✓	✓	✓	✓	✓
- Experimental	✗	✓	✓	✓	✓	✓
- Predicted	✗	✗	✗	✓	✗	✓
- Allele specific	✗	✗	✗	✗	✗	✓
<b>Solvent accessibility</b>	✗	✗	✗	✓	✓	✓
<b>Physiochemical characteristics</b>	✗	✗	✓	✗	✗	✗
<b>Protrusion</b>	✗	✗	✗	✗	✓	✗
<b>Standalone</b>	✓	✓	✓	✓	✗	✗
<b>Online</b>	✗	✓	✗	✗	✓	✓

# an example of a study – DSA formation



# Learning objectives of this presentation

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## HLA antibody algorithms

- To understand the basic principles of antibody recognition
- To understand the basic concepts of HLA antibody epitope matching algorithms
- To understand the most important differences between the various HLA antibody epitope matching algorithms

## HLA T-cell epitope algorithms

- To understand the basics of T-cell epitope recognition
- To understand the difference between PIRCHE-I and PIRCHE-II and the potential immunological consequences
- To understand how PIRCHE-II affects transplant outcome



# HLA antibody epitopes in organ transplantation

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Eric Spierings ([e.spierings@umcutrecht.nl](mailto:e.spierings@umcutrecht.nl))