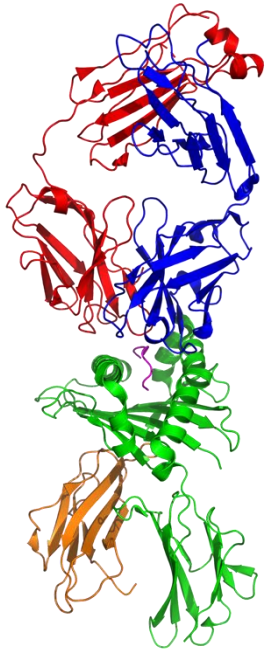
TCR-pMHC interaction: from molecular principle to therapeutic development

Xinbo Yang, Ph.D.

Memorial Sloan Kettering Cancer Center



- Concept and method overview of protein engineering
- Overview of how T cell mediated immune system works
  - Antigen receptor diversification
  - Antigen receptor signaling
    - Signal 1
    - Signal 2
    - Signal 3
- Cytokine based immunotherapy (signal 3)
  - principal of cytokine signaling
  - Strategies for designing effective cytokine therapies
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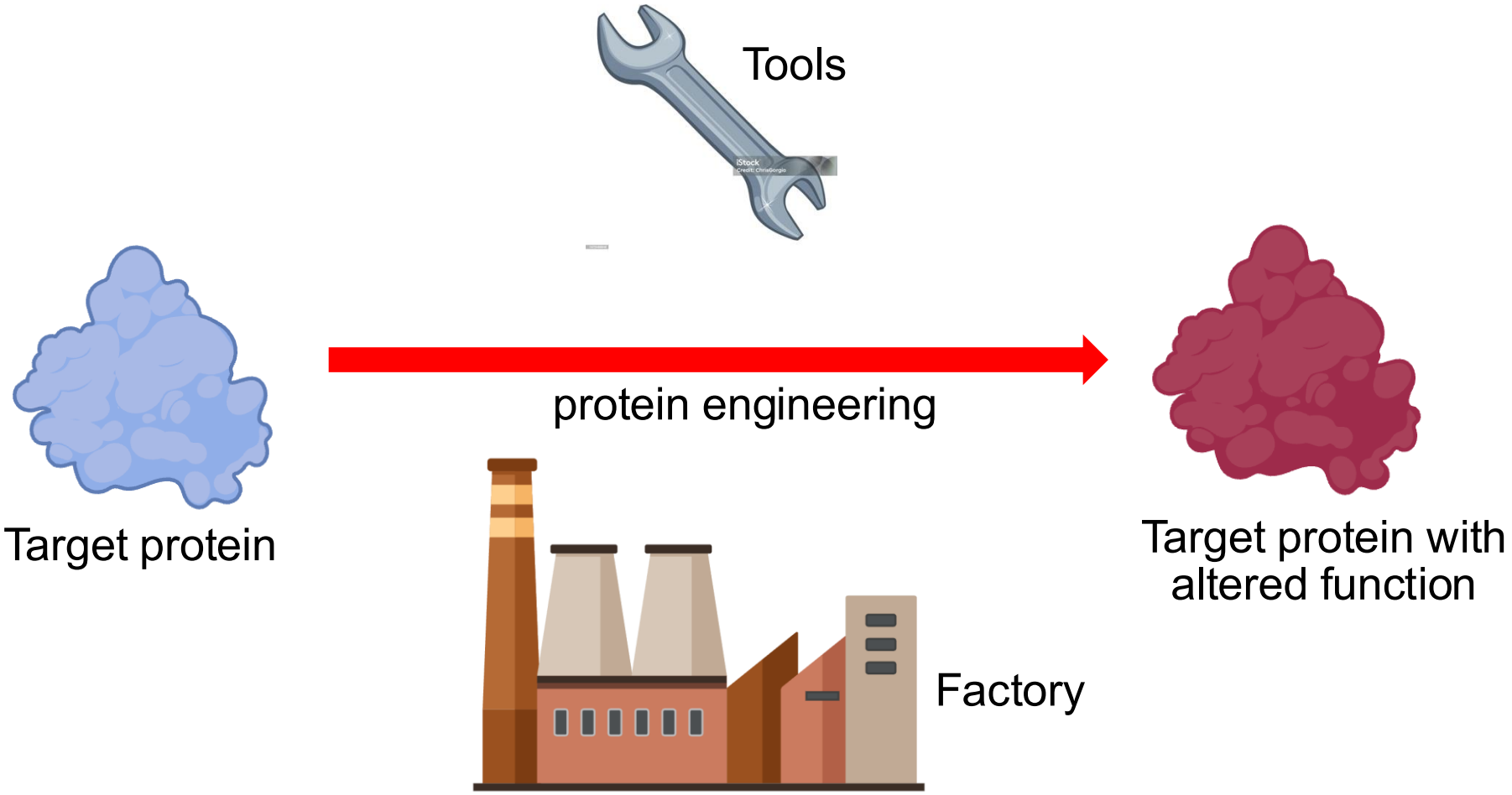
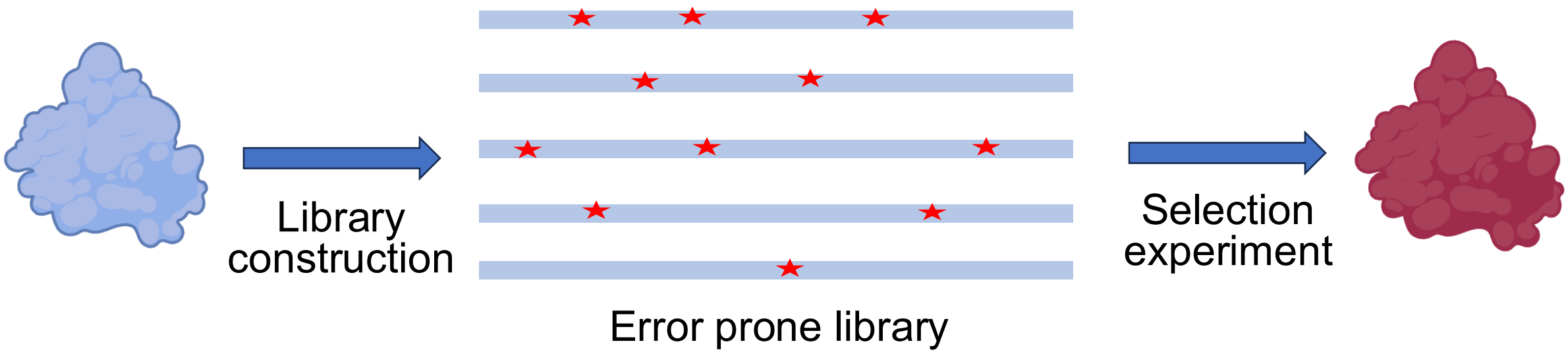
Concept of protein engineering

- A process that uses genetic technology to create or modify proteins for specific purposes



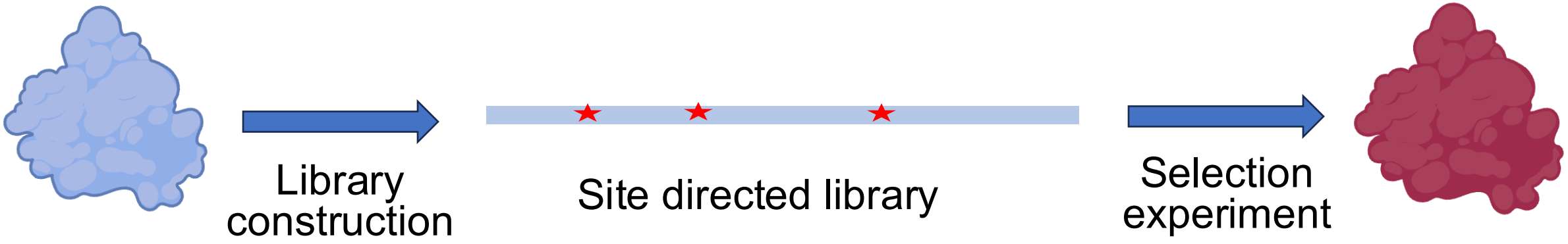
Protein engineering tools



Advantages: no prior information requirement

Disadvantages: low success rate; large library size required

Protein engineering tools



Advantages: high success rate; small library size

Disadvantages: Often requires reliable structural information

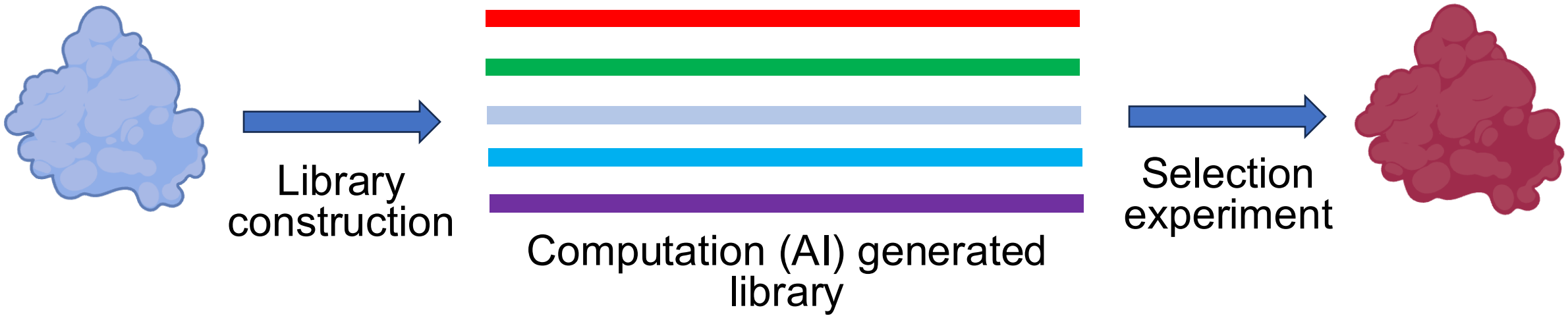
Functional diversity vs theoretical diversity

Saturated libraries for 6 positions:

Functional diversity: 20^6

Theoretical diversity: 32^6 NNK (4X4X2)

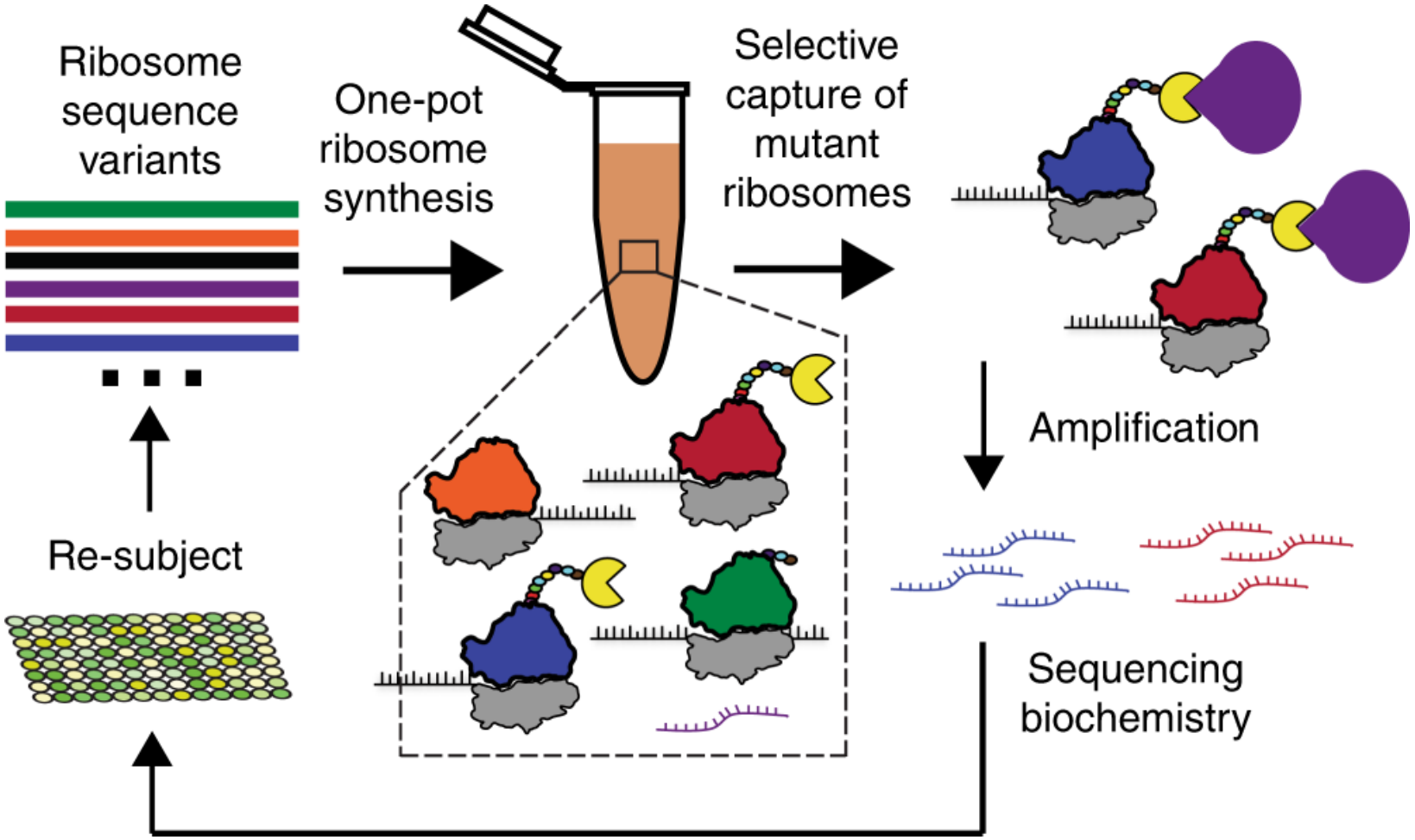
Protein engineering tools



Advantages: multiple template generated

Disadvantages: small construct only (<100 AA); single purpose only (binding)

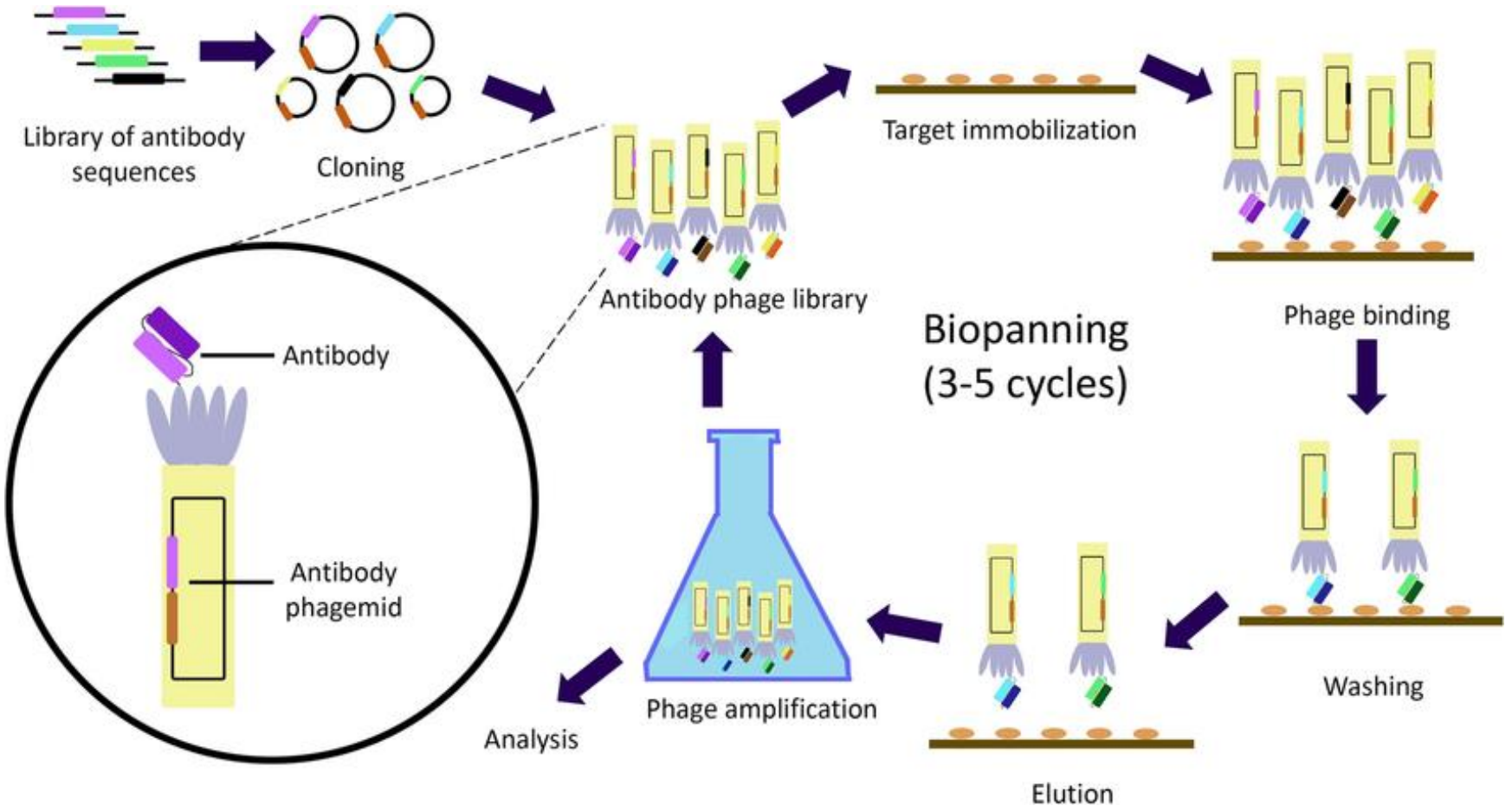
Protein engineering factories (ribosome)



- Protein folding: No
- Protein translational modification: No
- Protein valency: low
- Transduction efficiency: No Limit
- Library size: >10^13
- Selection method: binding

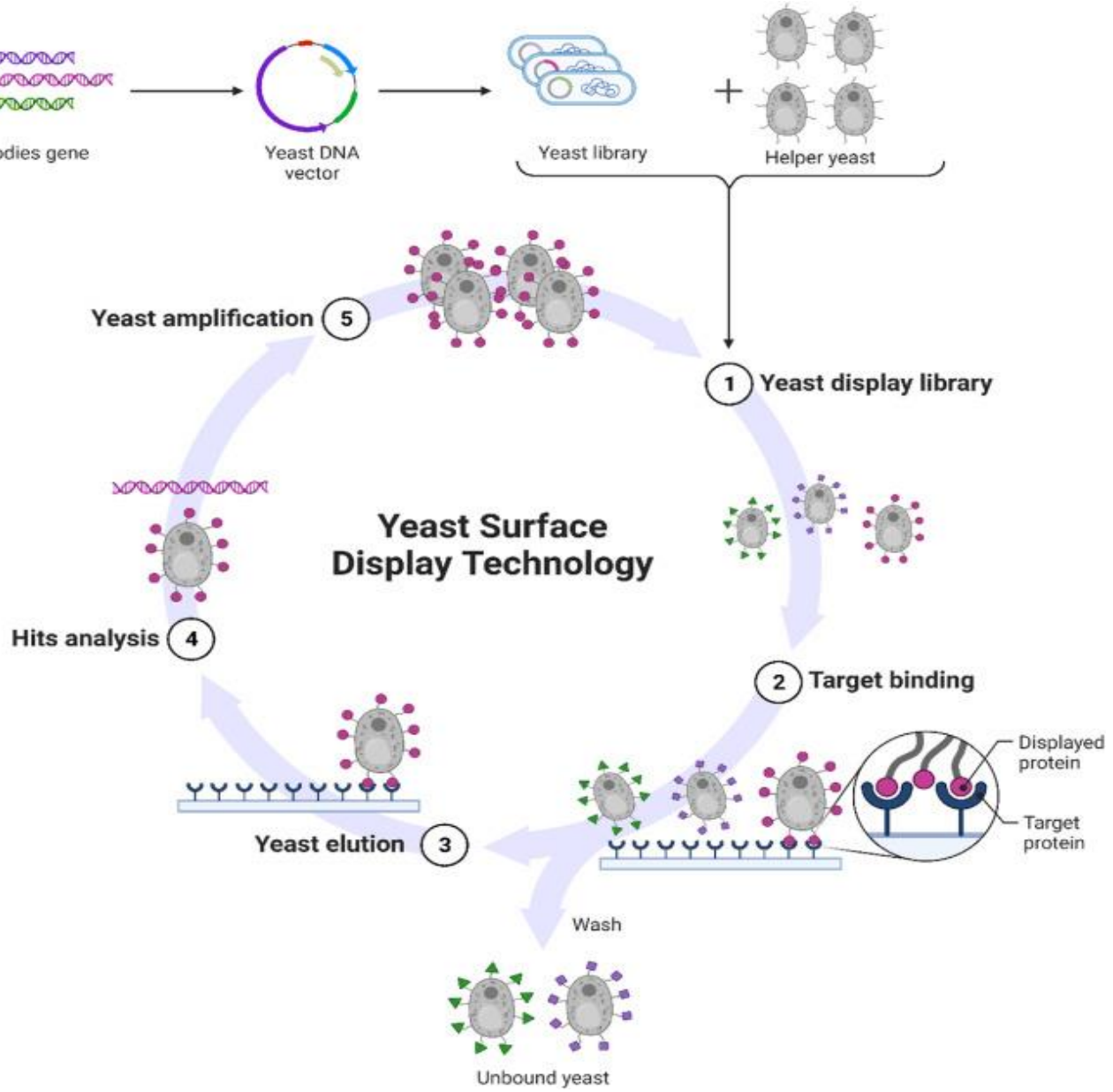
Hammerling MJ, et al. Nature communications 2020

Protein engineering factories (phage)



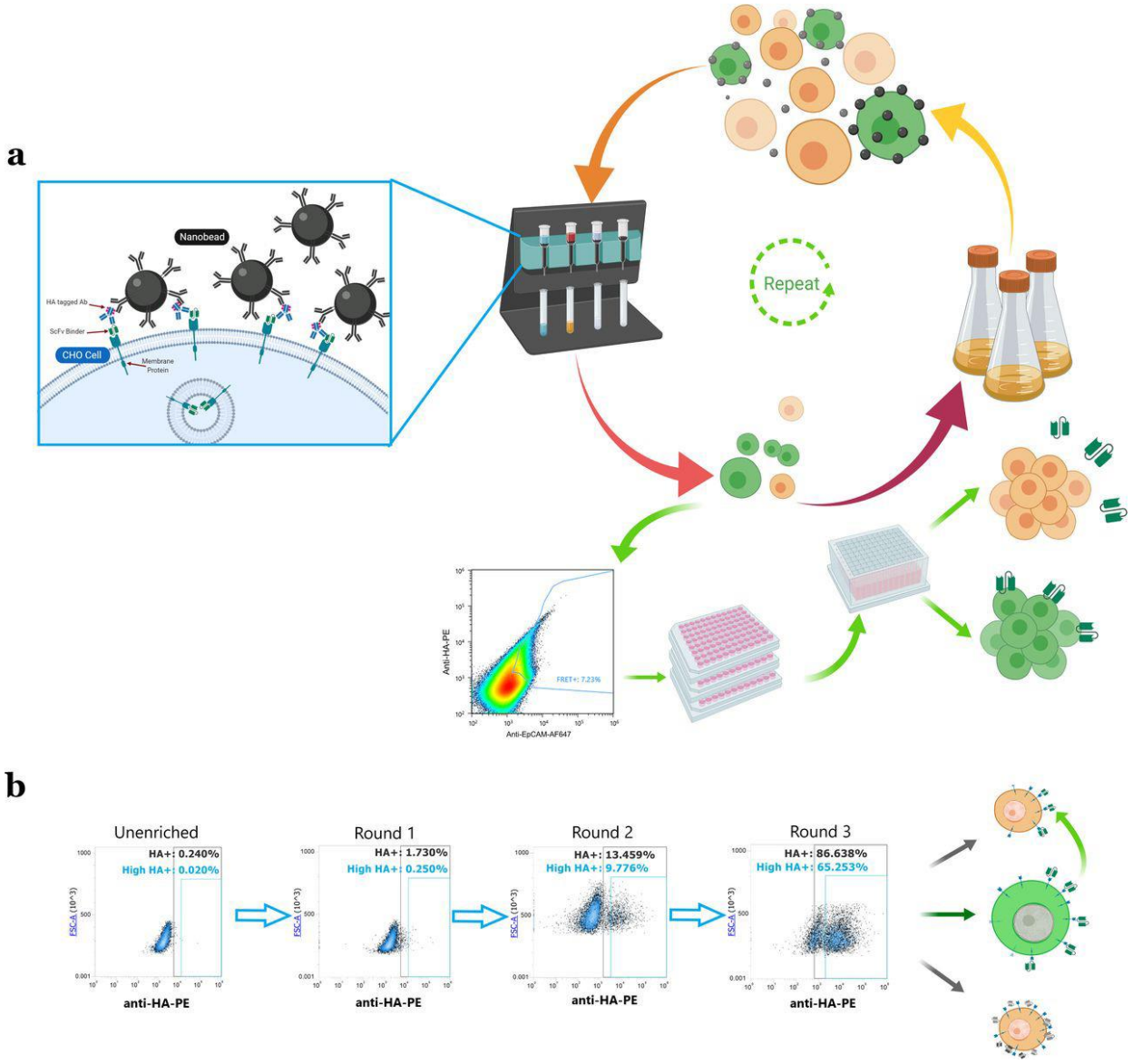
- Protein folding: simple
- Protein translational modification: No
- Protein valency: low
- Transduction efficiency: High
- Library size: >10^10
- Selection method: binding

Protein engineering factories (yeast)



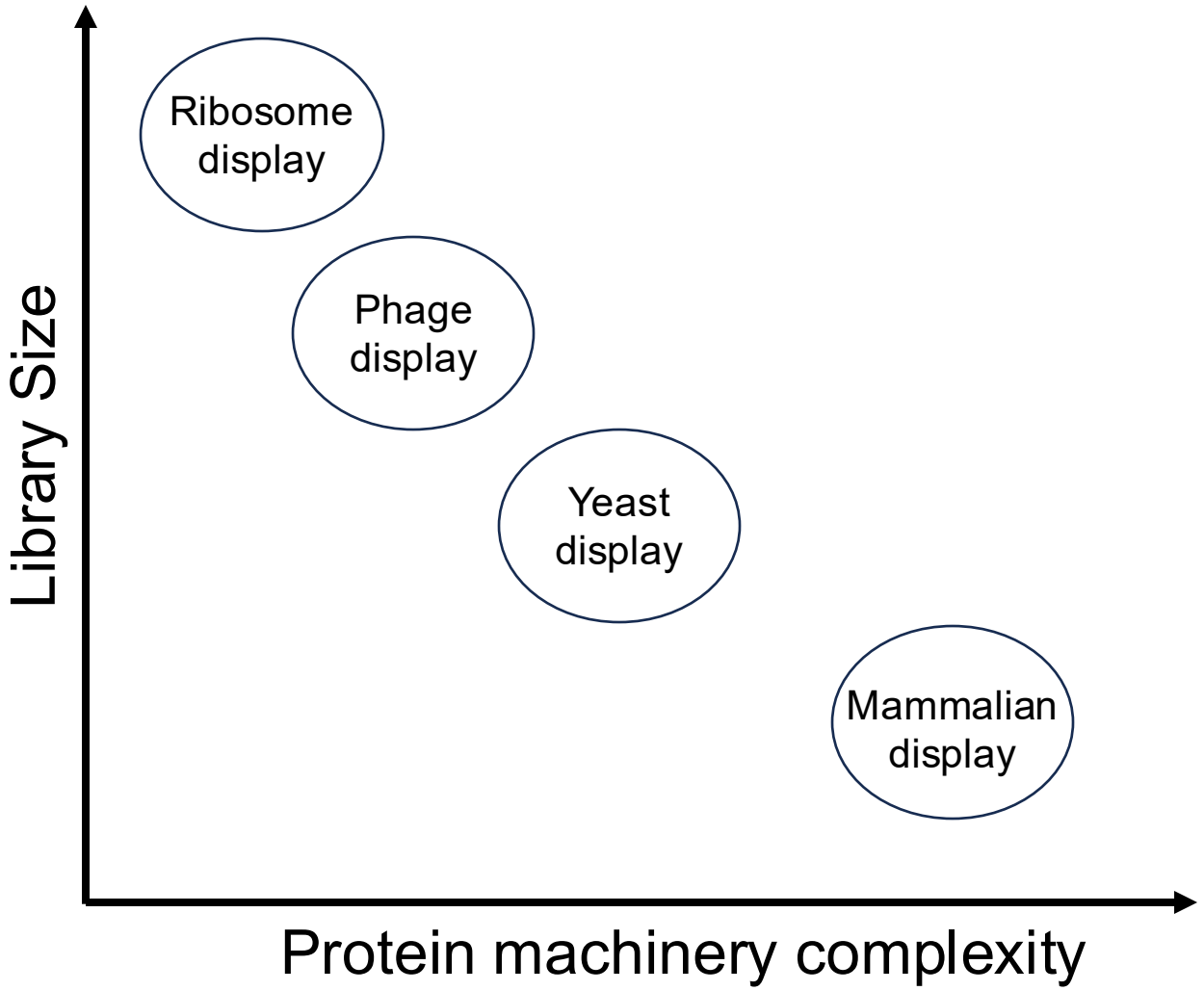
- Protein folding: simple
- Protein translational modification: No
- Protein valency: intermediated
- Limit by transduction efficiency: Yes
- Library size: >10^9
- Selection method: binding; limited functional selection

Protein engineering factories (mammalian)



- Protein folding: simple
- Protein translational modification: No
- Protein valency: high
- Limit by transduction efficiency: Yes
- Library size: >10^6
- Selection method: binding; functional selection

Protein engineering factories



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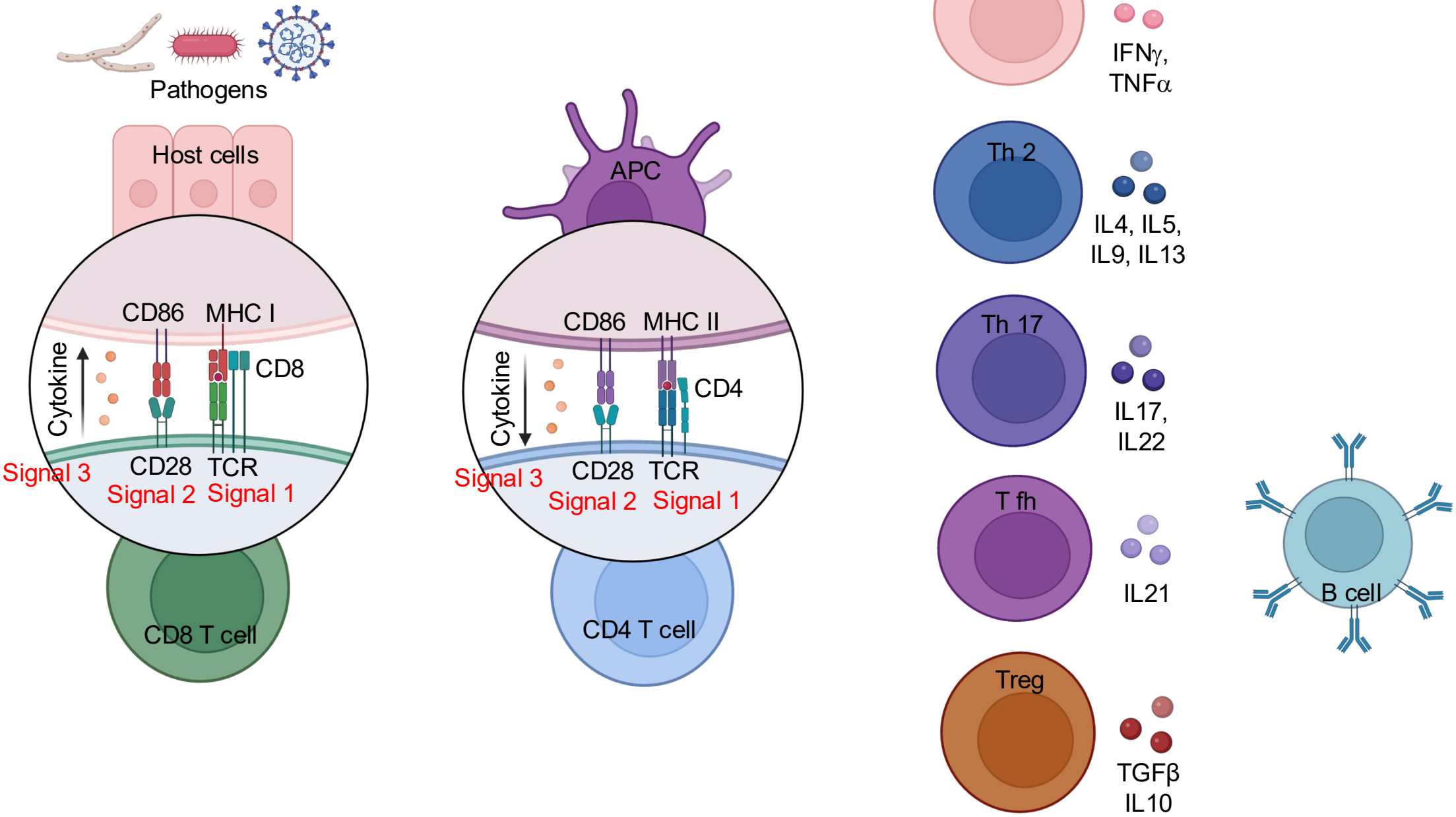
A simplified overview of T cell mediated immune response



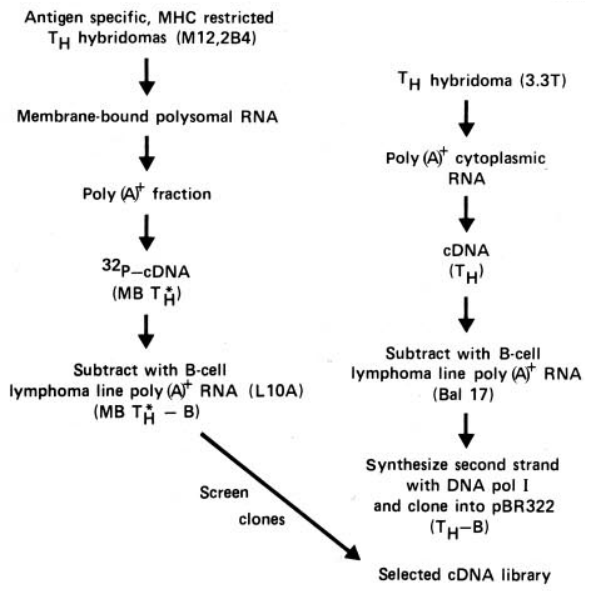
surface of T lymphocytes.

T LYMPHOCYTES, like B lymphocytes, are capable of recognizing a wide range of different antigens[1–3]. As with B cells, the ability to recognize a given antigen is fixed in any particular clonal line of T cells. However, unlike B cells, T cells appear to recognize antigens in combination with self major histocompatibility (MHC) determinants[4–6]. In view of the similarities to B cells, early ideas as to how T cells recognize antigens centred on the use of either entire antibody molecules[7] or at least some of the separately encoded (in the germ-line genome) segments that make up the antigen-binding sites of immunoglobulin heavy and light chains[8–12]. But despite early reports that antibodies against immunoglobulin antigen-binding sites can react with T cells [13–15] and recognize a target closely linked to the immunoglobulin heavy-chain locus[16,17], attempts to demonstrate an involvement of immunoglobulins in T-cell antigen recognition have proved consistently negative[8–12].

More recent investigations have taken a route largely independent of the antibody models and have succeeded in raising at

polypeptides to attach to the endoplasmic reticulum by a leader peptide, or signal sequence[27]. (3) That like immunoglobulin genes those that encode the T-cell receptor, proteins should be rearranged in T cells as a mechanism of generating diversity and consequently increasing the antigen-recognition repertoire. (4) That like immunoglobulin genes they should have constant regions (as they presumably share at least some functions) and variable regions, would confer the antigen-binding specificity.

An experimental strategy could be developed on the basis of these assumptions, as B and T cells differ in only a small fraction of their gene expression (~2%, or 200–300 different sequences[28]; M.M.D. and W. E. Paul, manuscript in preparation) and only a small proportion of lymphocyte mRNAs appear to be in the membrane-bound polysomal fraction (~3%)[29]. Thus, by synthesizing ^{32}P-labelled DNA copies (cDNAs) of the membrane-bound polysomal RNA of antigen-specific T cells and removing by RNA hybridization those sequences also expressed in B cells, one should be left with a



- Expressed in T but not B cells
- Membrane bound
- Encoded by genes that rearrange like genes encoding Ig
- Composed of constant and variable domains

Massachusetts 02138, USA
† Department of Medical Microbiology, Stanford University, Stanford, California 94305, USA
‡ Present addresses: Department of Medical Microbiology, Stanford University, Stanford, California 94305, USA (P.J.B.) and Max-Planck-Institute für Molekulare Genetik, Abteilung Wittmann, D-1000 Berlin 33, FDR (W.S.B.)
§ To whom correspondence should be addressed

The class I histocompatibility antigen from human cell membranes has two structural motifs: the membrane-proximal end of the glycoprotein contains two domains with immunoglobulin-folds that are paired in a novel manner, and the region distal from the membrane is a platform of eight antiparallel β-strands topped by α-helices. A large groove between the α-helices provides a binding site for processed foreign antigens. An unknown 'antigen' is found in this site in crystals of purified HLA-A2.

HLA (human leukocyte antigen) molecules are polymorphic membrane glycoproteins found on the surface of nearly all cells. Multiple genetic loci within the major histocompatibility complex (MHC) encode these proteins, and one individual simultaneously expresses several polymorphic forms from a large pool of alleles in the population. HLA molecules (also known as class I histocompatibility antigens) are the targets of antibodies and cytotoxic T lymphocytes (CTL) during rejection of foreign transplants[1,2]. They are also recognized by T cells together with viral antigens on infected cell surfaces, a phenomenon known as MHC restricted recognition[3]. In contrast

ated with a particular HLA molecule. Because one individual expresses a limited set of different HLA molecules, a central question has been how these few HLA molecules can interact with so many foreign antigens. Limitations in the ability of a particular HLA molecule to associate with all antigens may explain the linkage of histocompatibility antigens to variations in susceptibility to human diseases[4], and the immune system's responsiveness to particular antigens[5].

Recent work has shown that virus-specific CTL will lyse an uninfected target cell of appropriate class I specificity to which peptide fragments of a viral protein have been added[6]. T-helper

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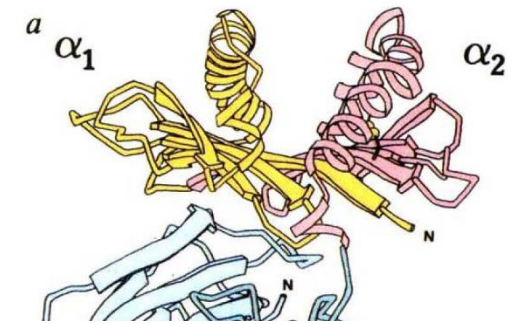


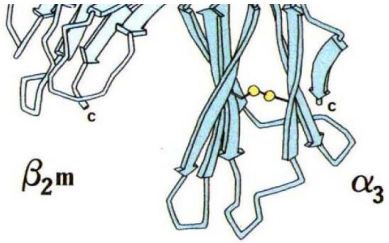
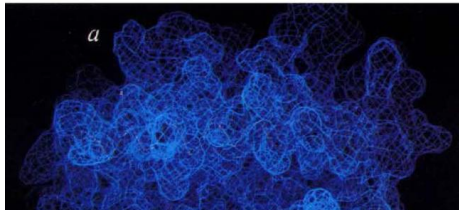
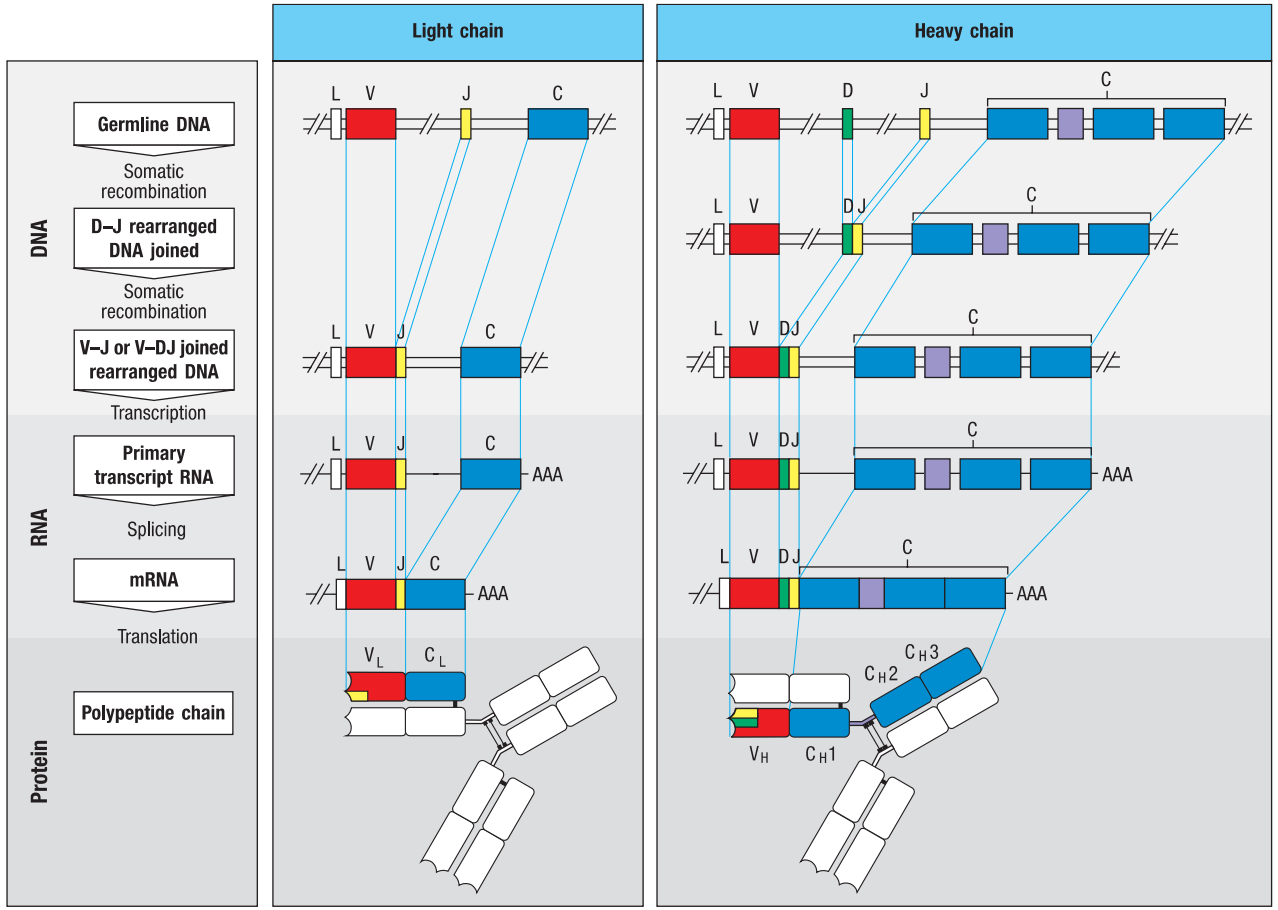
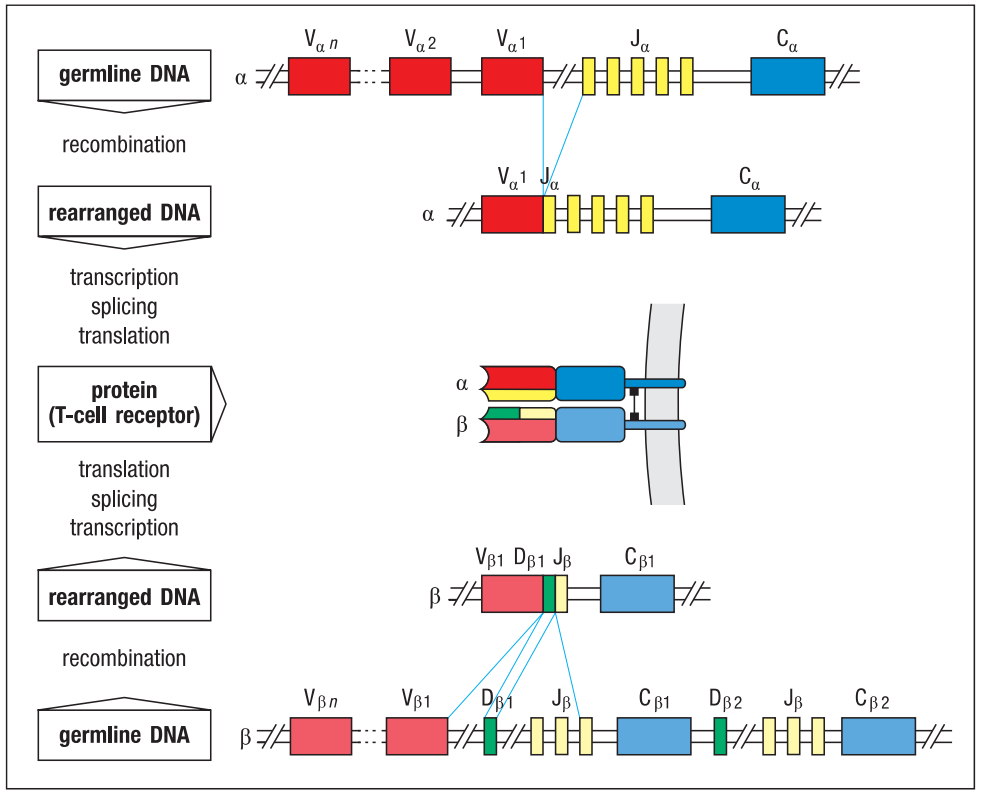


Fig. 2*a*



Molecular basis of Antigen receptor diversity

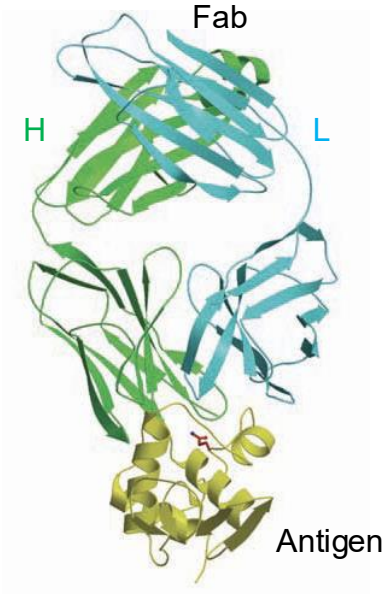




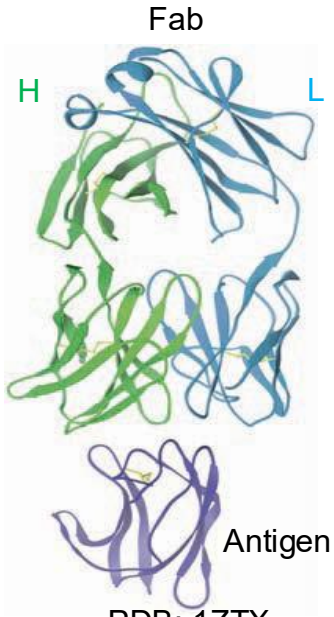
Differences between B cell and T cell antigen diversity

Element	Immunoglobulin		α:β T-cell receptors	
	H	κ+λ	β	α
Number of variable segments (V)	~40	~70	52	~70
Number of diversity segments (D)	23	0	2	0
Number of D segments read in three frames	rarely	–	often	–
Number of joining segments (J)	6	5(κ) 4(λ)	13	61
Number of joints with N- and P-nucleotides	2 (VD and DJ)	50% of joints	2 (VD and DJ)	1 (VJ)
Number of V gene pairs	1.9×10^6		5.8×10^6	
Number of junctional diversity	$\sim 3 \times 10^7$		$\sim 2 \times 10^{11}$	
Number of total diversity	$\sim 5 \times 10^{13}$		$\sim 10^{18}$	

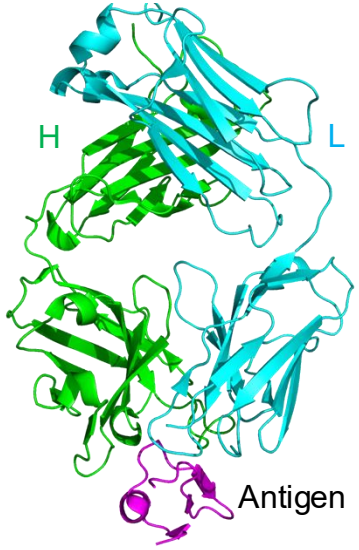
Structural basis of antigen receptor specificity



PDB: 1MLC

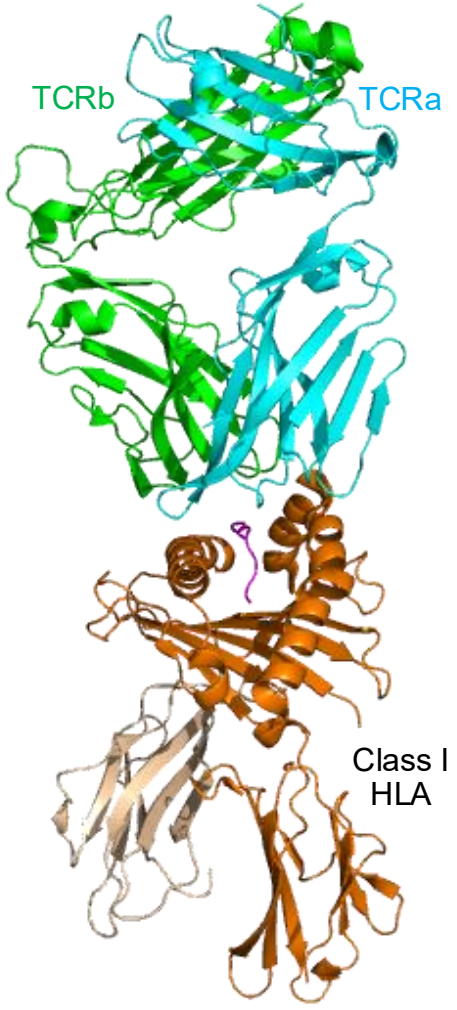


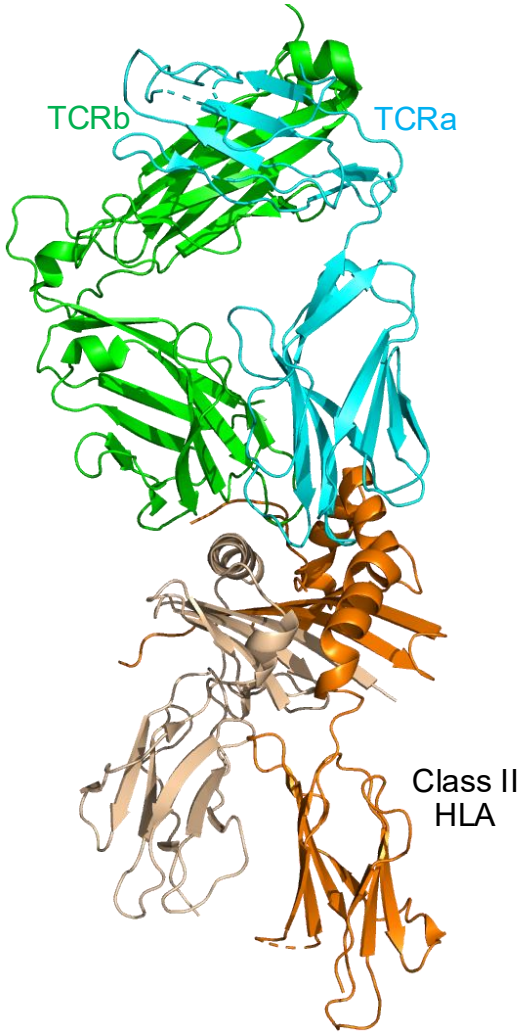
PDB: 1ZTX



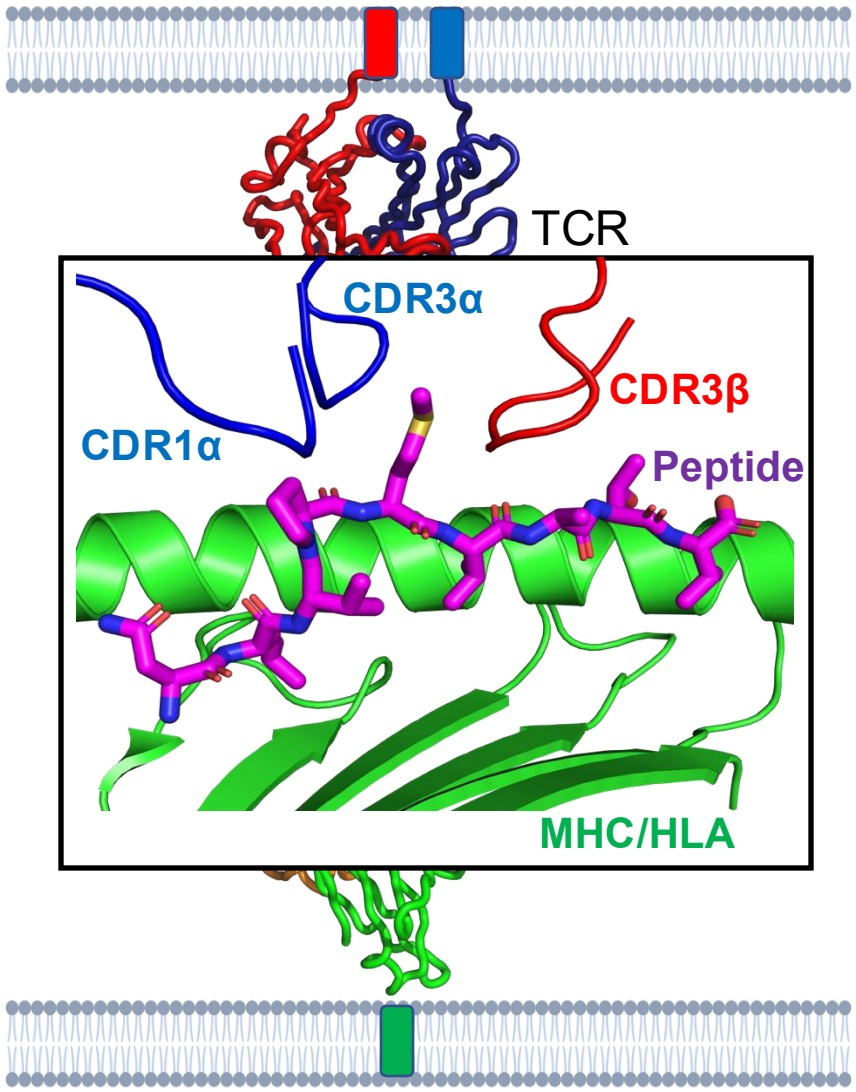
PDB: 2J4W

PDB: 8F5N





Size of T cell repertoire

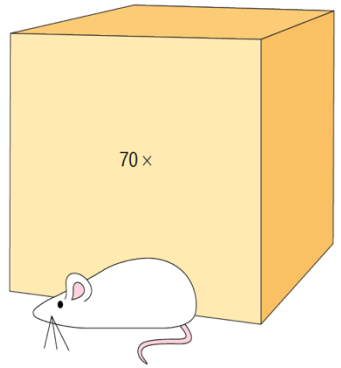


- T cell receptors 10^{15}
- Possible pMHC-I>10^{11}; pMHC-II> 10^{12}
- MHC alleles: 7000

- T cells in a human 10^{10}
- T cells in a mouse 10^{8}

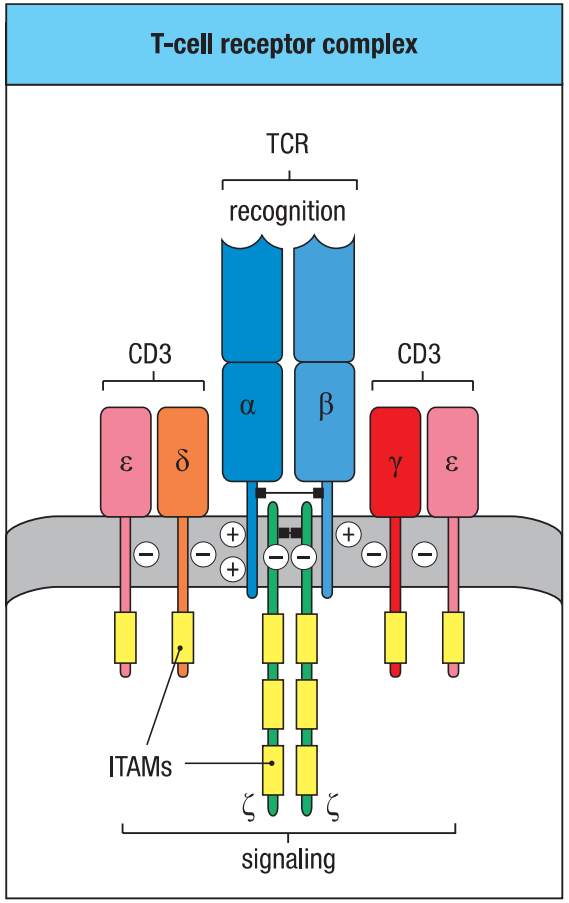
A very high level of crossreactivity is an essential feature of the T-cell receptor

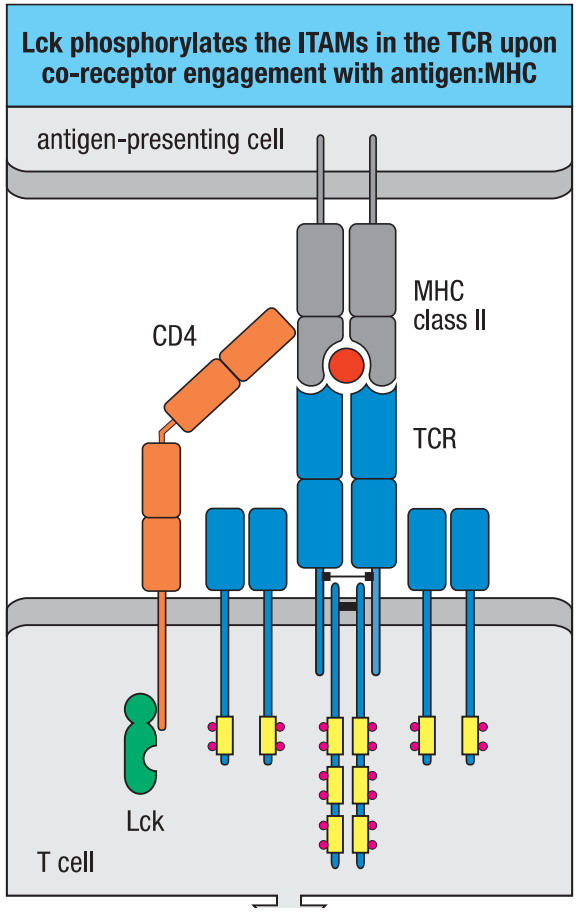
Don Mason

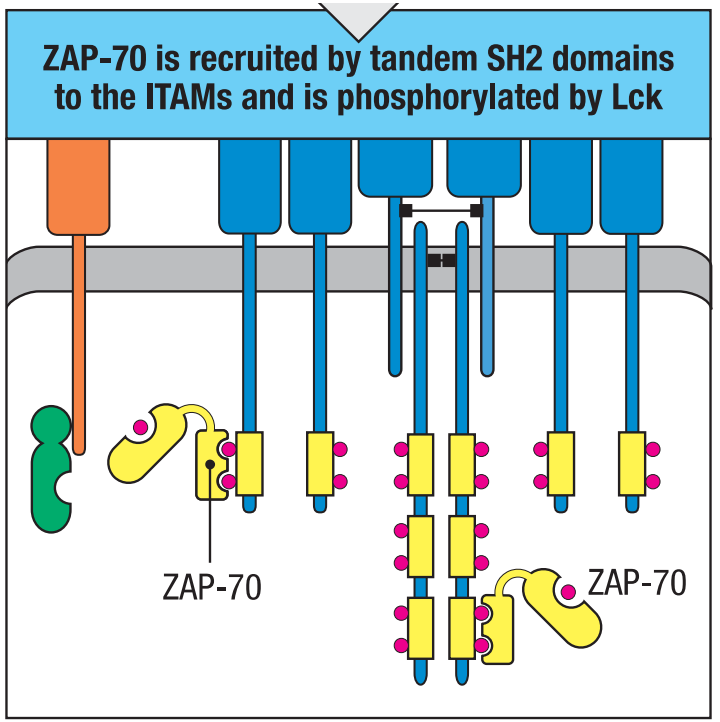


Mason 1998. Immunol. Today

Signal 1. antigen receptor signaling

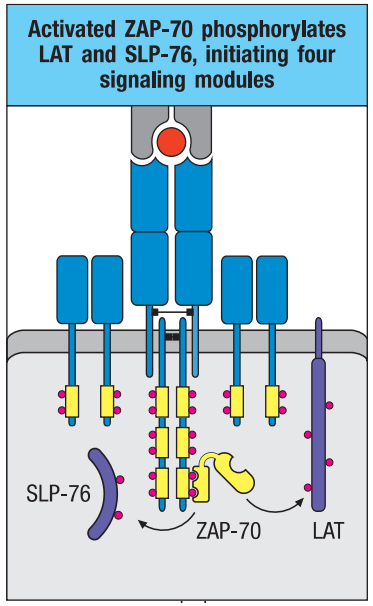


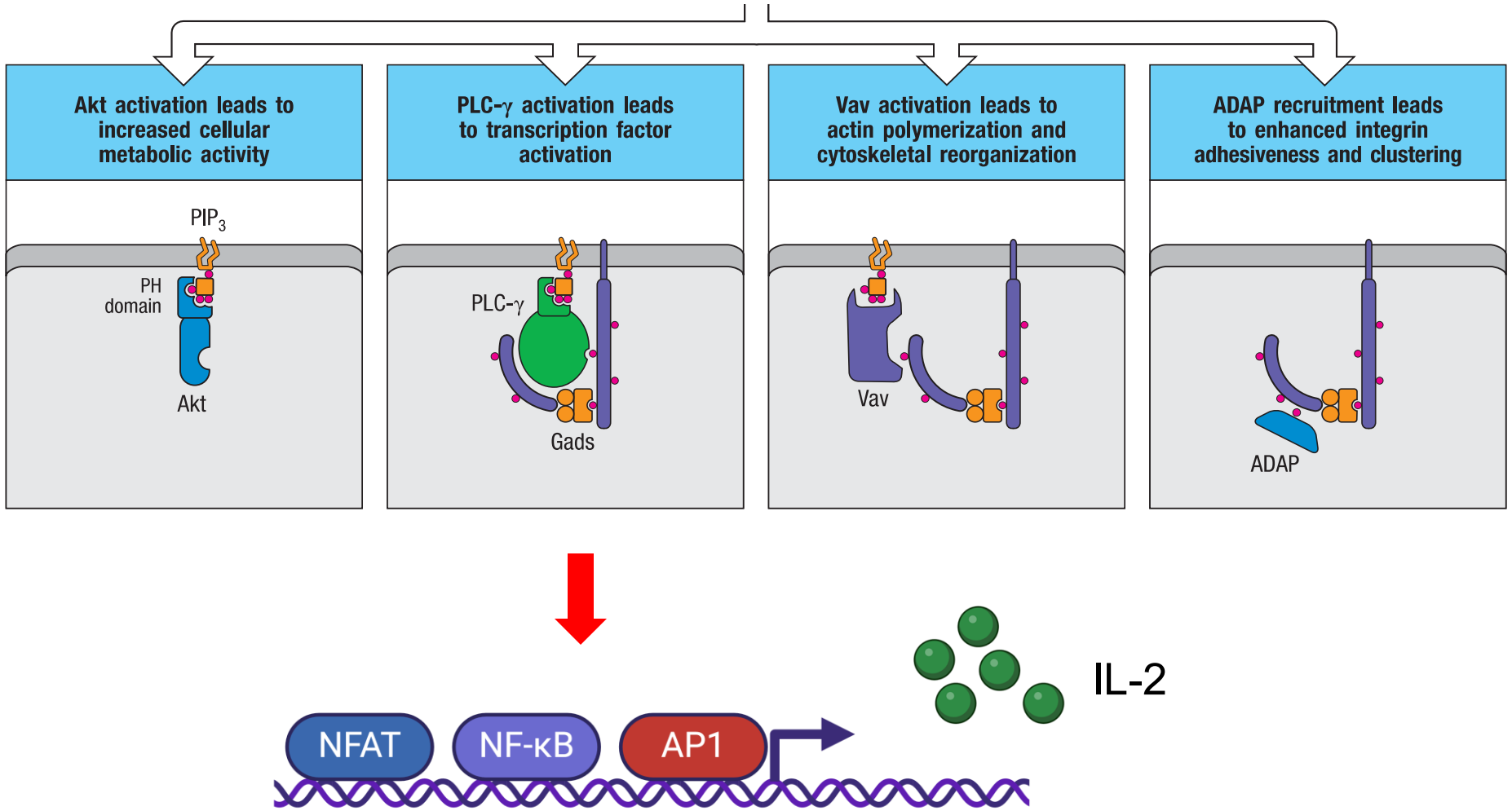




Janeway's Immunobiology 9th edition

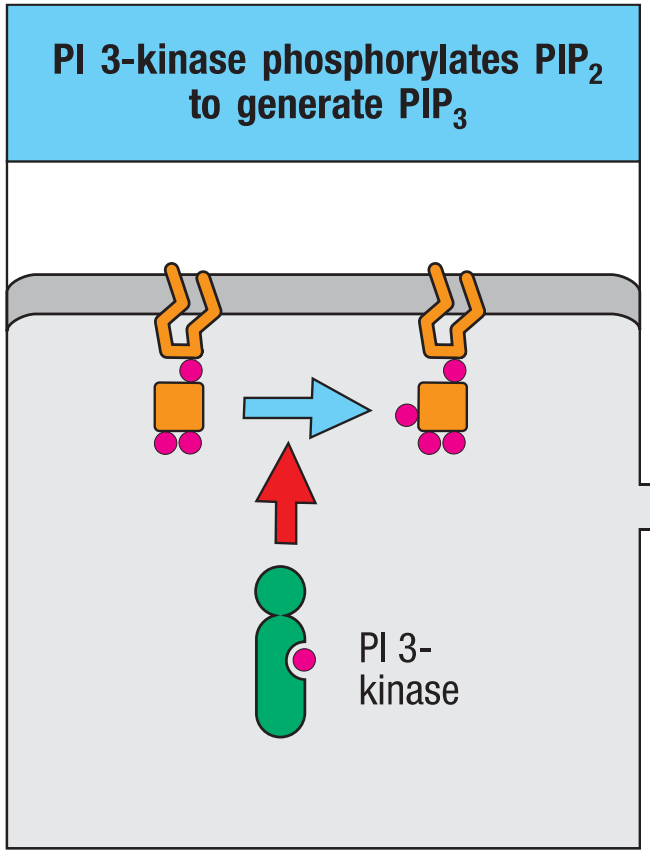
TCR signaling leads to downstream effector function production



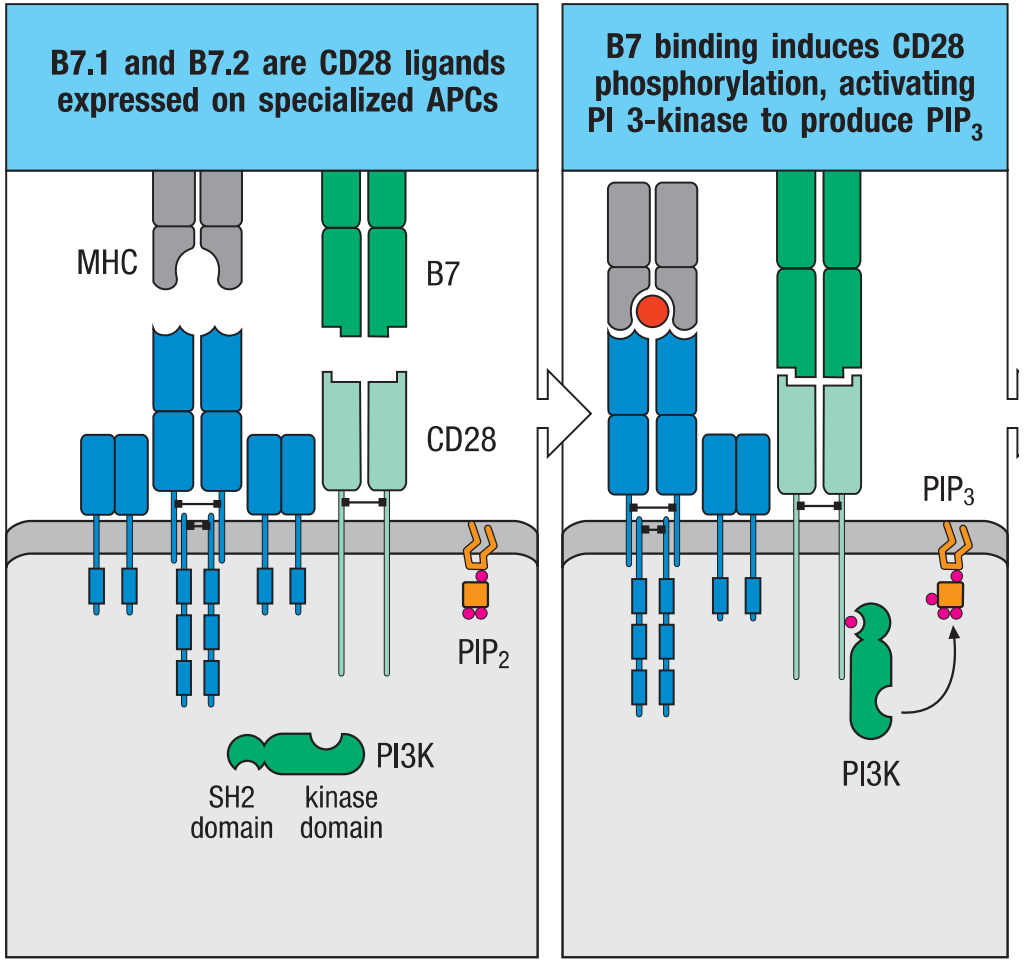


Janeway's Immunobiology 9th edition

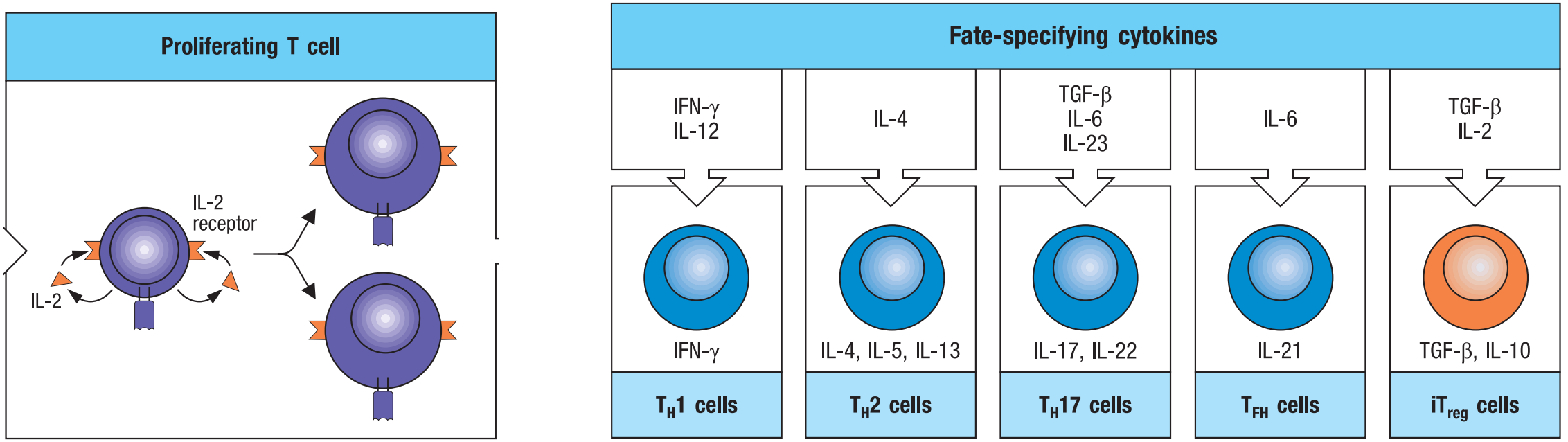
Signal 2. CD28 cascades co-stimulatory signal



Converting ubiquitous membrane molecule PIP_2 (Phosphotidylinositol 4,5 bisphosphate) to PIP_3 (Phosphotidylinositol 3,4,5 trisphosphate) is absolutely essential for TCR mediated signaling



Signal 3. cytokine signaling leads to T cell proliferation and differentiation



IL-2 is the key T cell ‘growth hormone’.

- The most proliferative cytokine for T cells and NK cells
- The first cytokine used for cancer treatment
- Open the door of immunotherapy

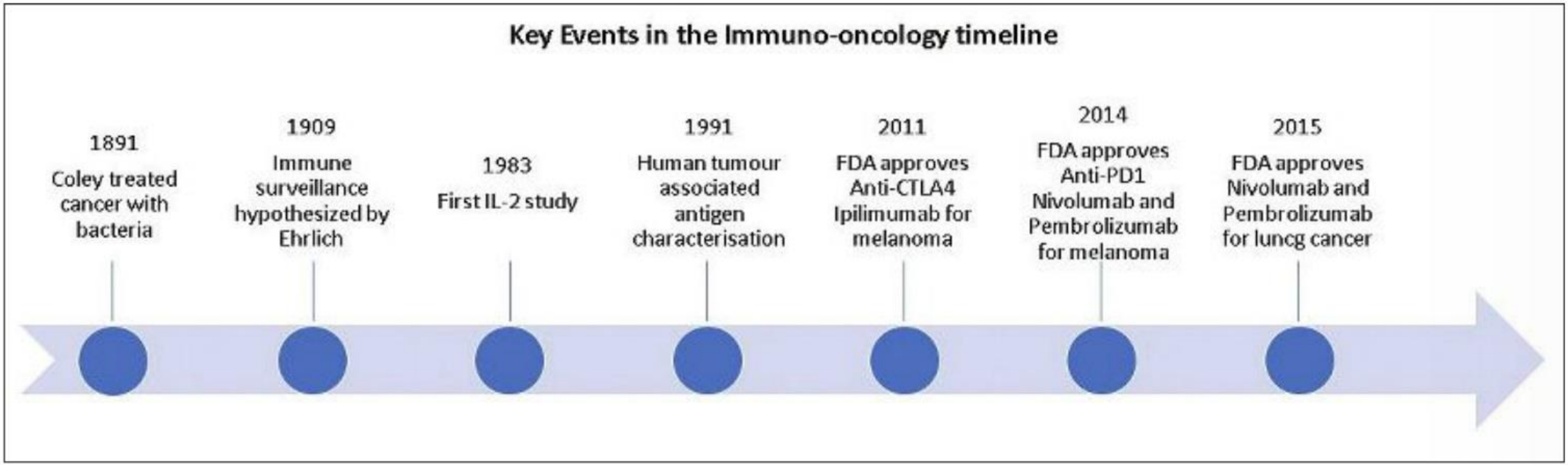


Figure 1 Key events in the immuno-oncology timeline. From Coley's early discoveries through to some of the recent approvals for melanoma and lung cancer. *Timeline adapted from Morrissey et al*

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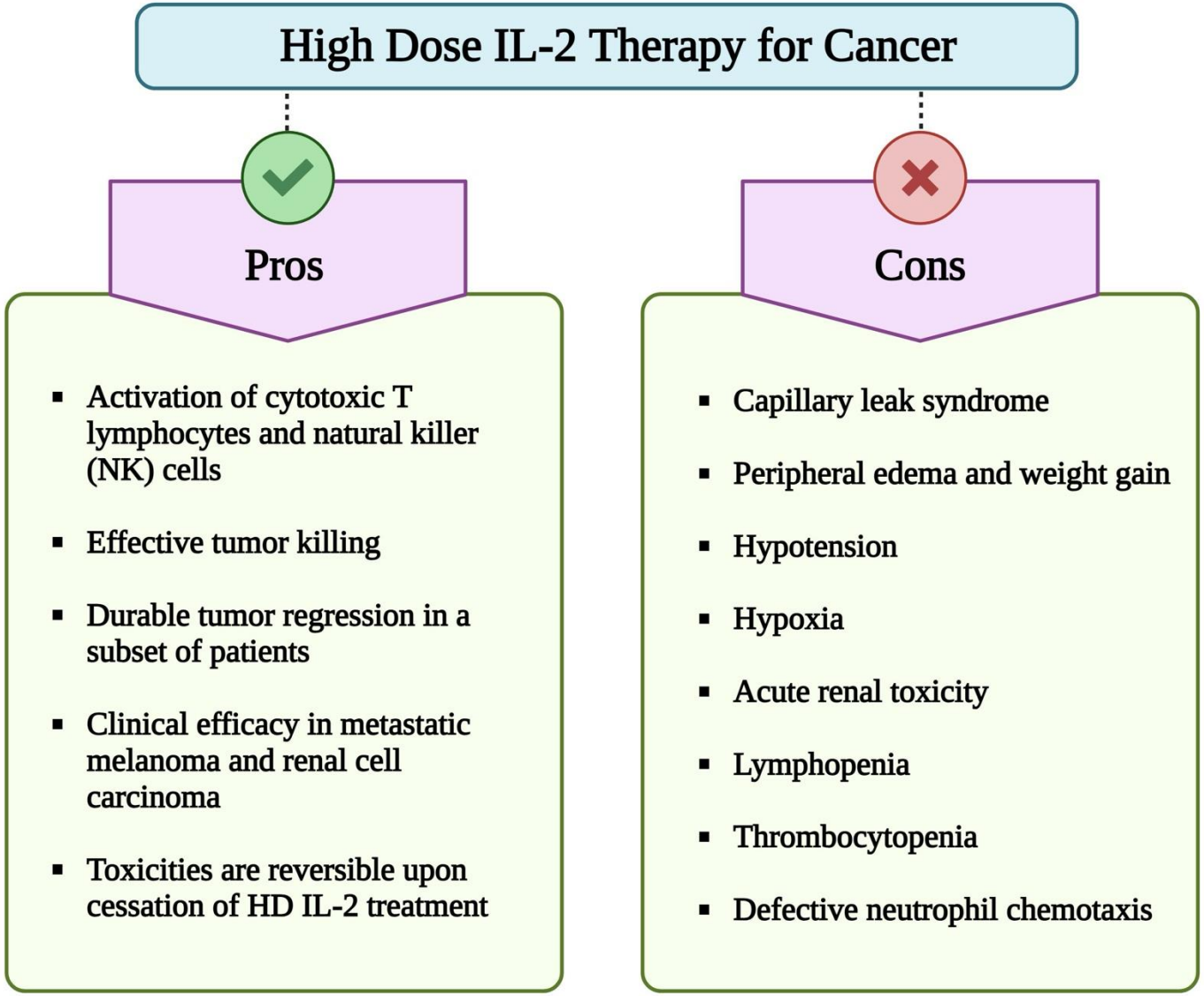
- Cytokine based immunotherapy (signal 3)
  - principal of cytokine signaling
  - Strategies for designing effective cytokine therapies
- Check point blockade-based immunotherapy (signal 2)
  - landscape of co-stimulatory and co-inhibitory signaling
  - CTLA4 vs PD1
- Antigen receptor-based immunotherapy (signal 1)
  - Antigen identification
  - Engineering therapeutics

IL-2 treatment showed moderate effect in treating metastatic melanoma

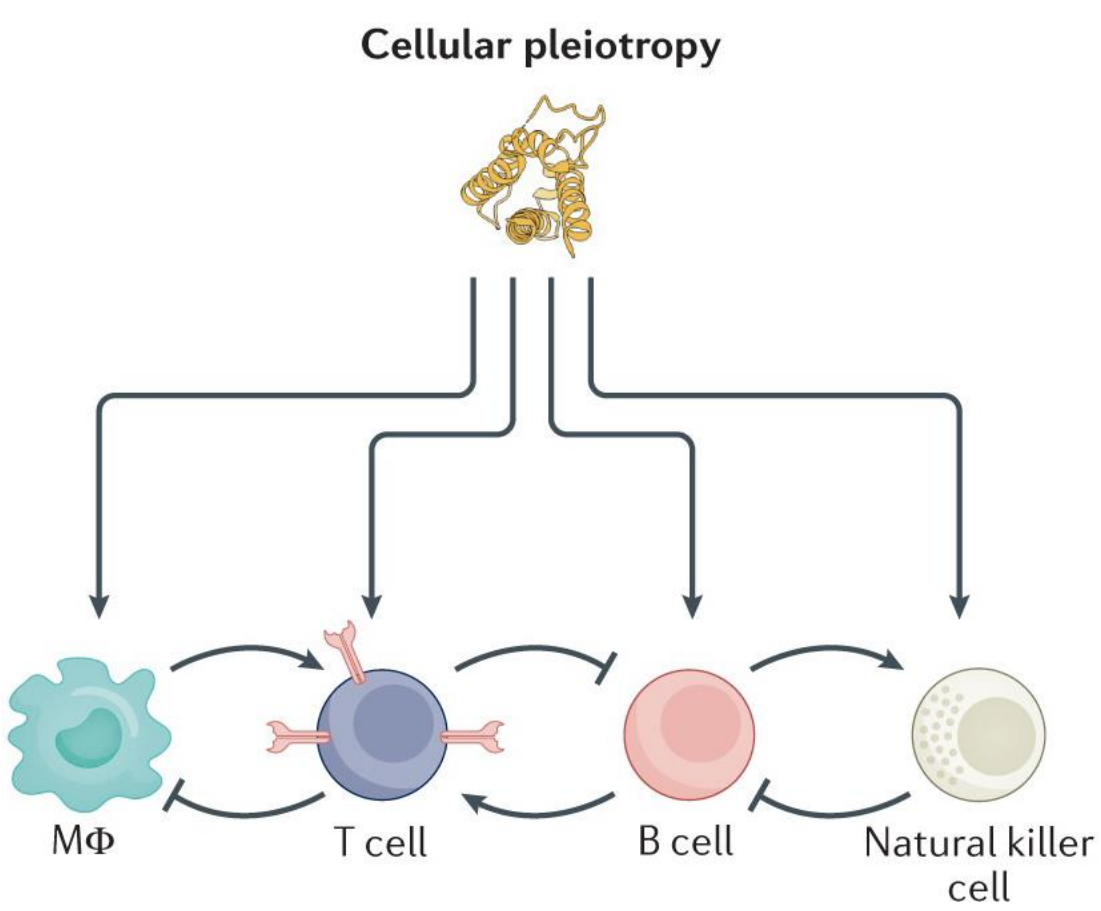
Treatment of Metastatic Melanoma Using Interleukin-2 Alone or in Conjunction with Vaccines

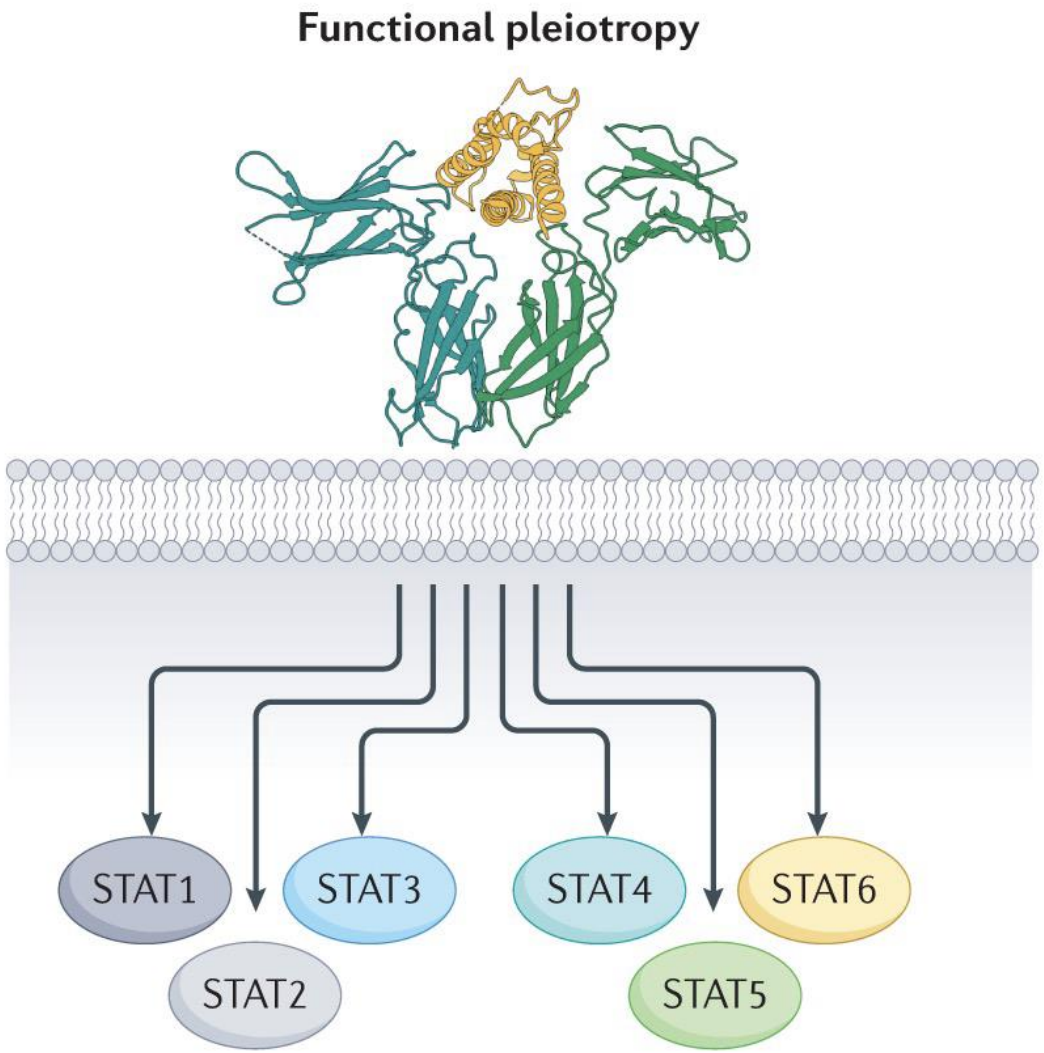
Franz O. Smith,[1] Stephanie G. Downey,[1] Jacob A. Klapper,[1] James C. Yang,[1] Richard M. Sherry,[1] Richard E. Royal,[1] Udai S. Kammula,[1] Marybeth S. Hughes,[1] Nicholas P. Restifo,[1] Catherine L. Levy,[1] Donald E. White,[1] Seth M. Steinberg,[2] and Steven A. Rosenberg[1]

- Overall response rate 15% with cases more than 30 months free of disease progression
- 4% of patients died from adverse events (lethal capillary leaking syndrome)



Pleiotropy





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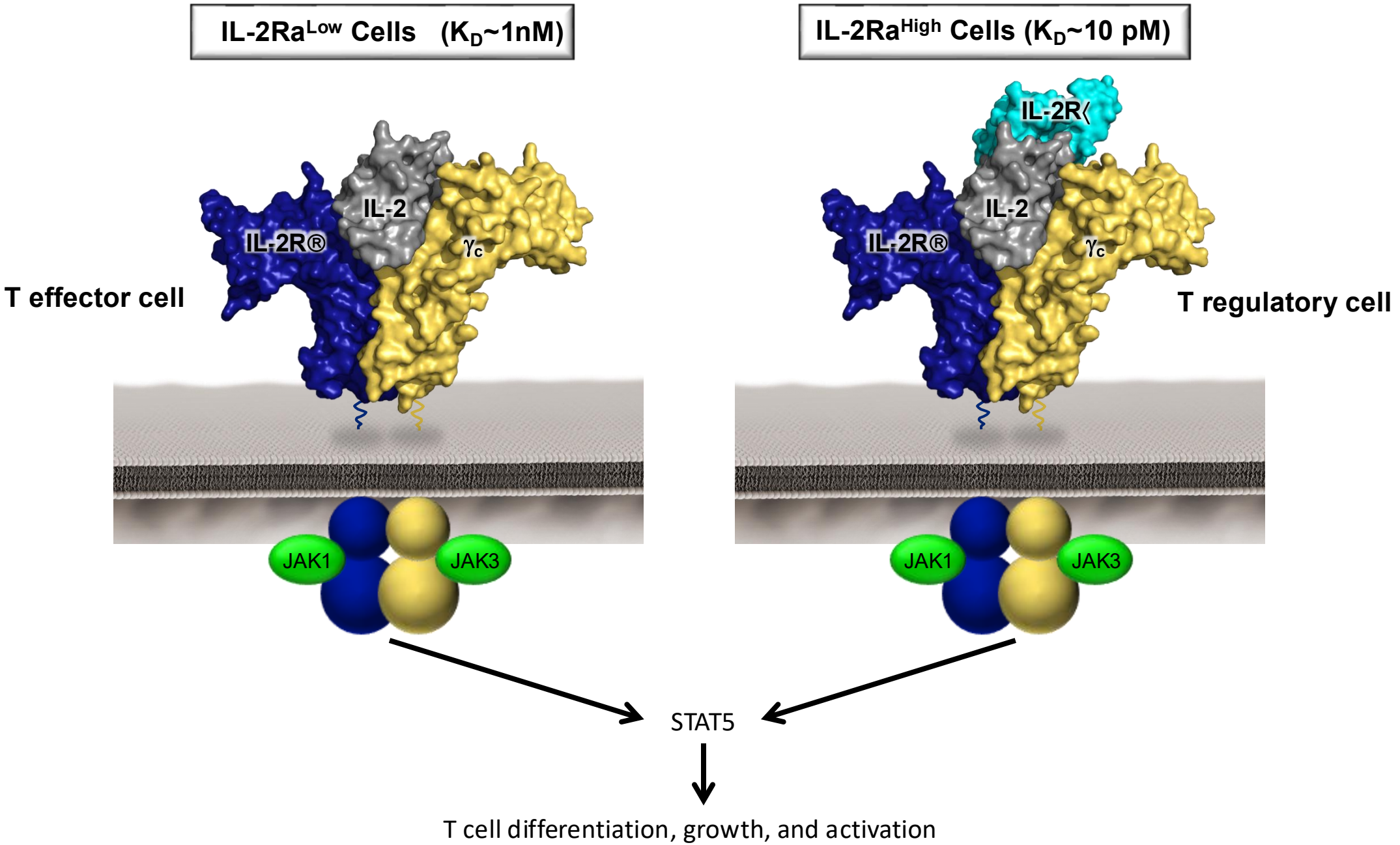
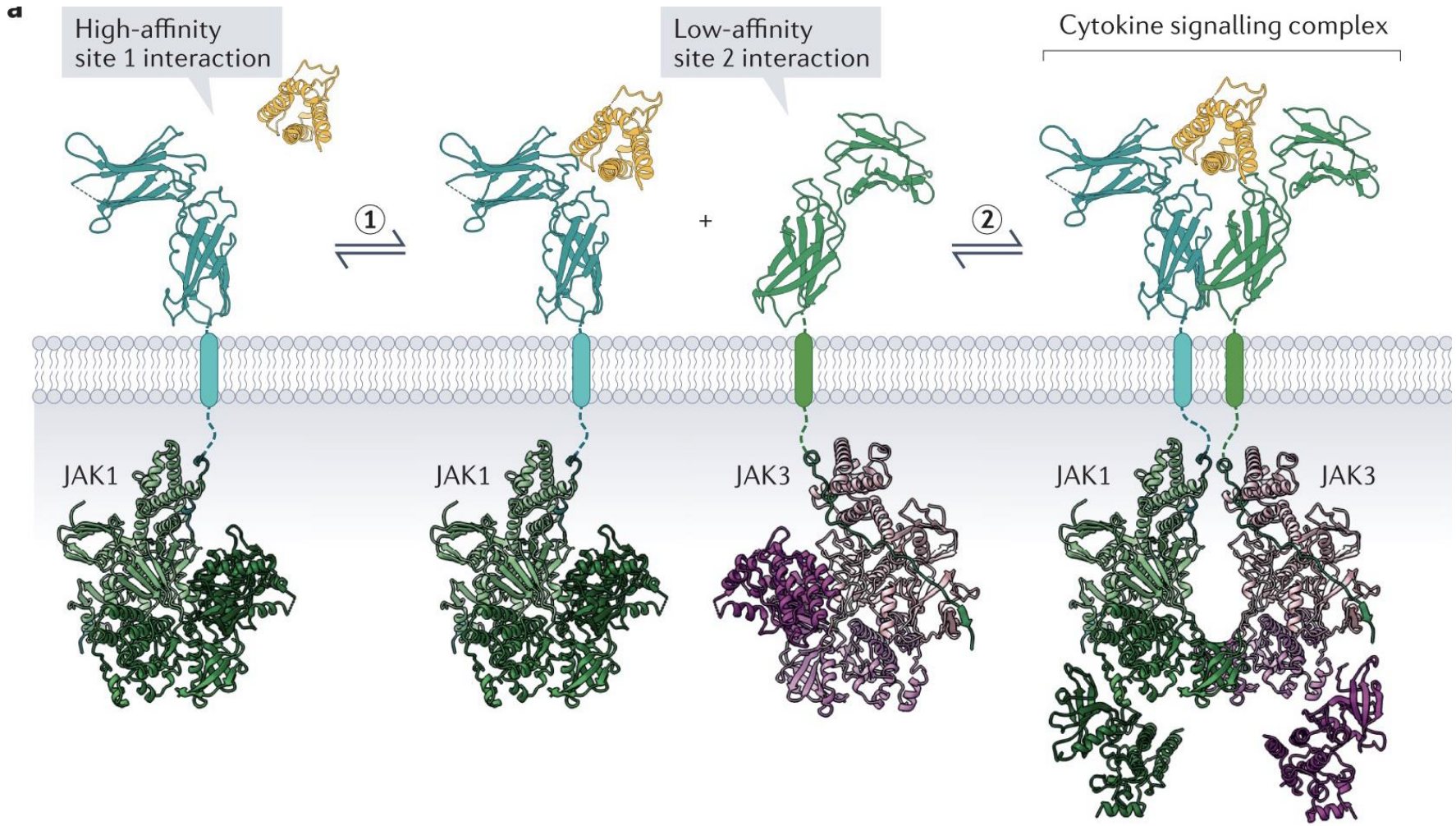
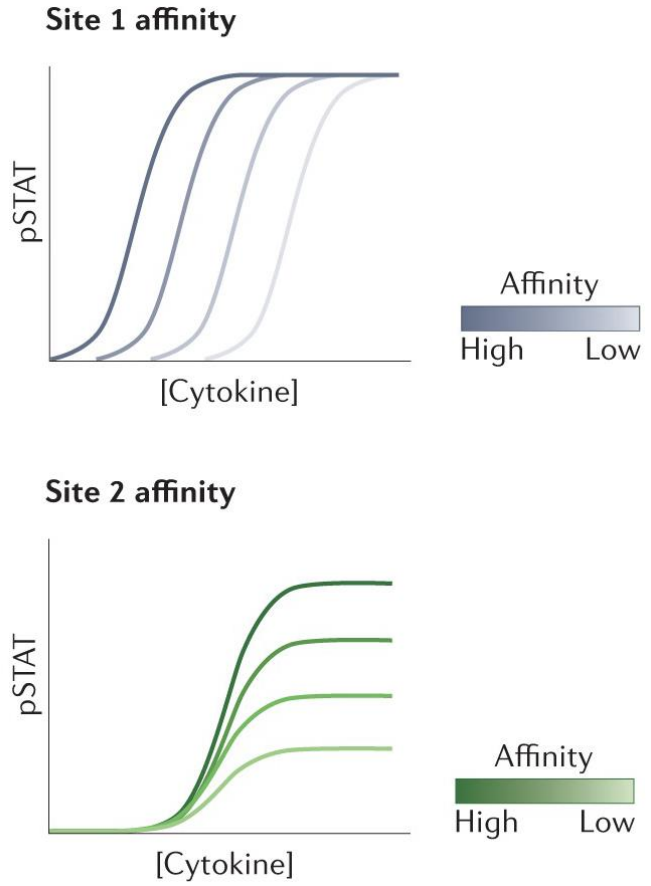
Interleukin-2 signals through a dimeric or trimeric receptor complex



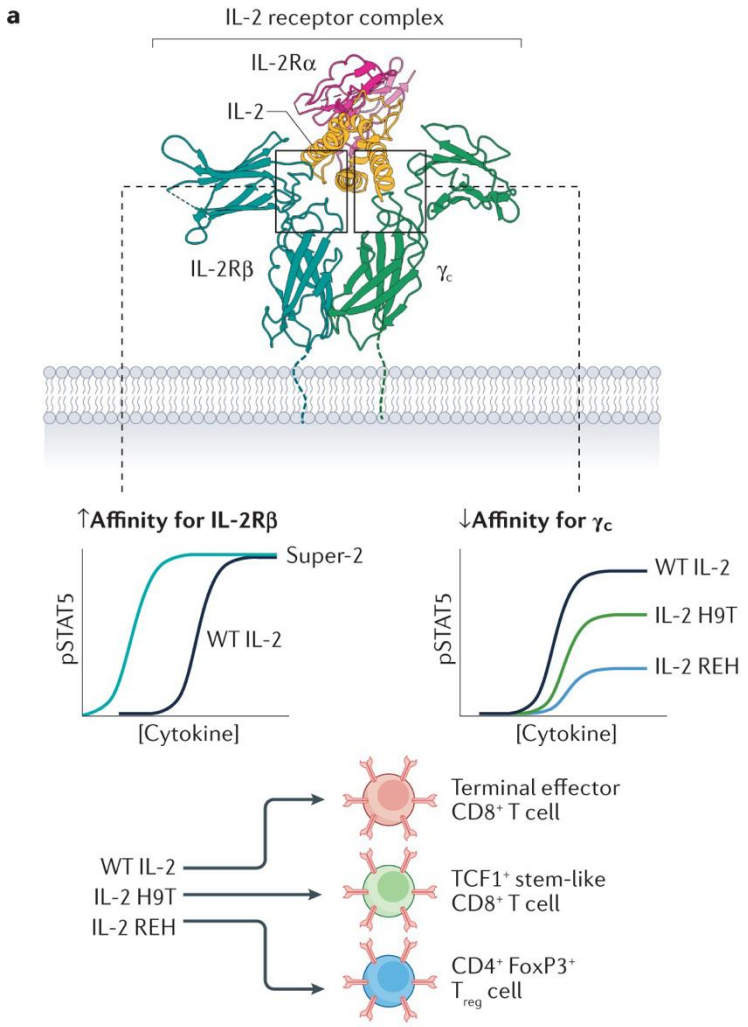
Molecular principal of cytokine signaling reveals strategy to engineer next-gen cytokine therapeutic

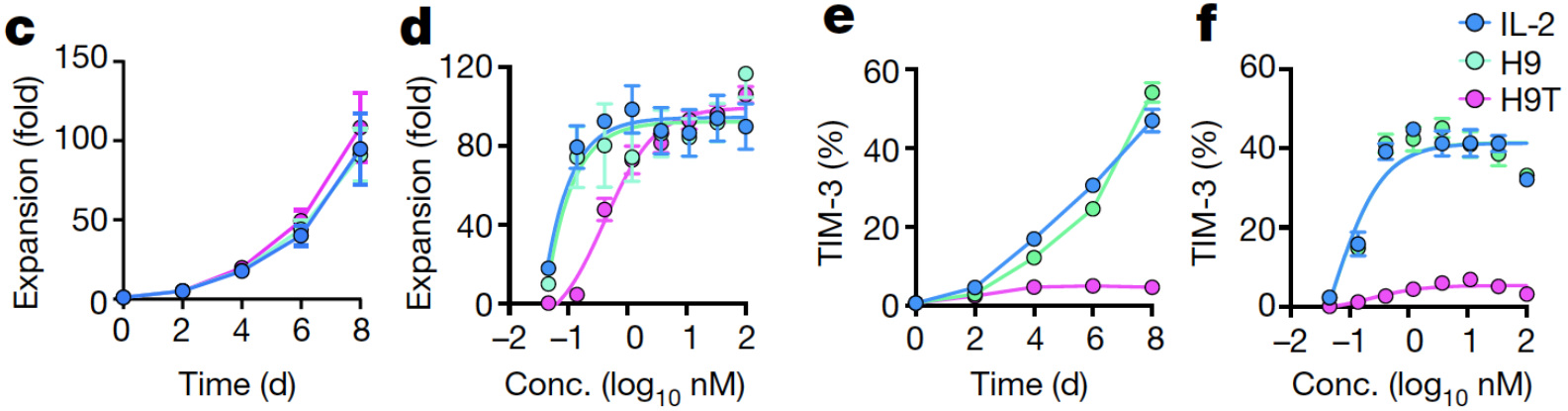


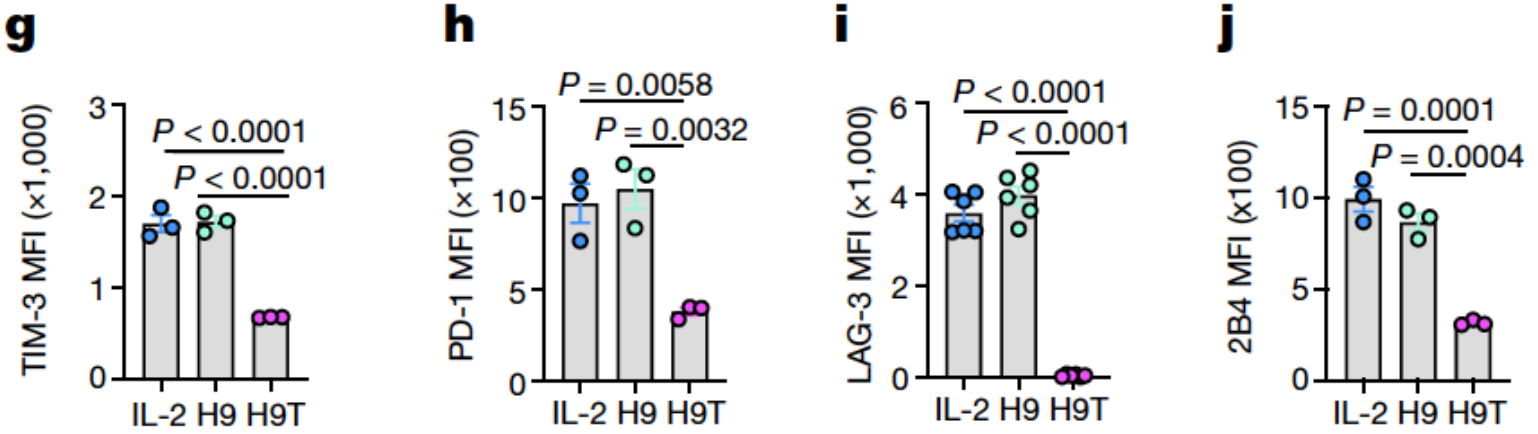


Saxton et al. Nat. Reviews Drug Discovery 2023

Strategies to create more precise cytokine drugs: 1. modulate cytokine affinity

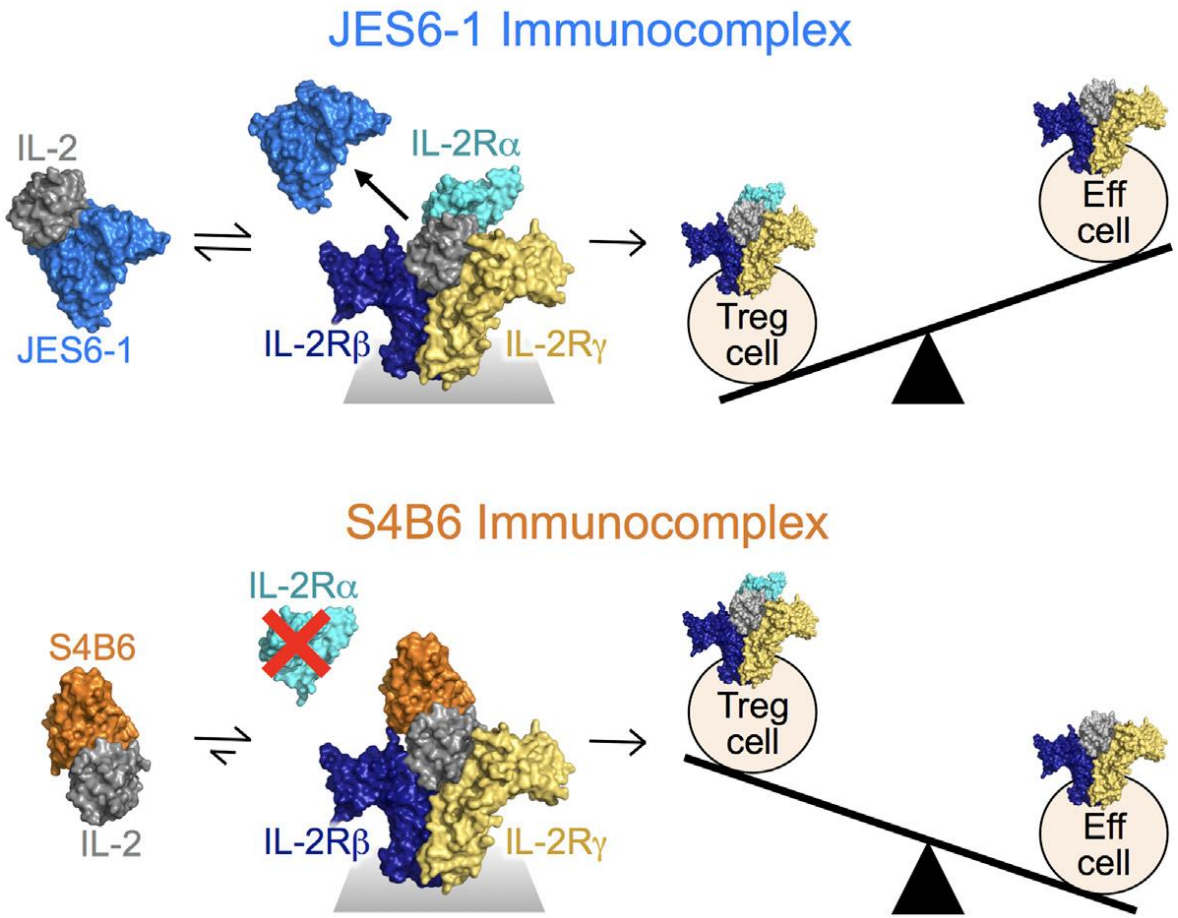


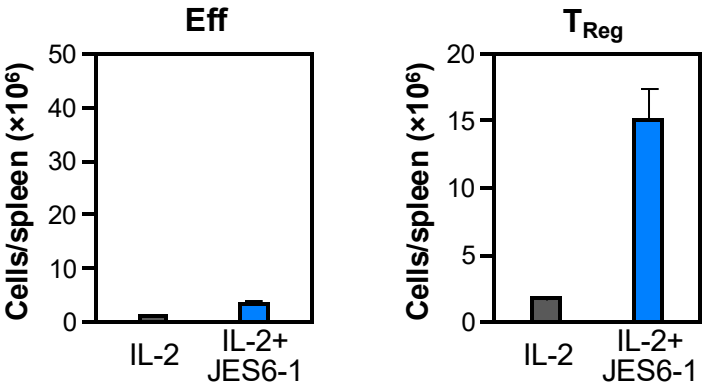


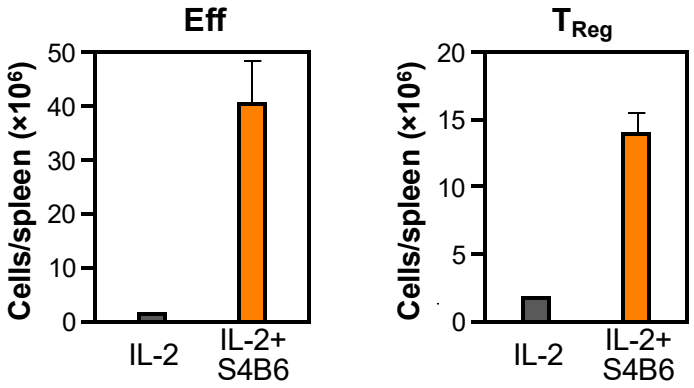


Mo et al. Nature 2021
Spangler et al. Immunity 2015
Sockolosky et al. Science 2018
Allen et al. Science 2022

Strategies to create more precise cytokine drugs: 2. Cytokine antibody complex



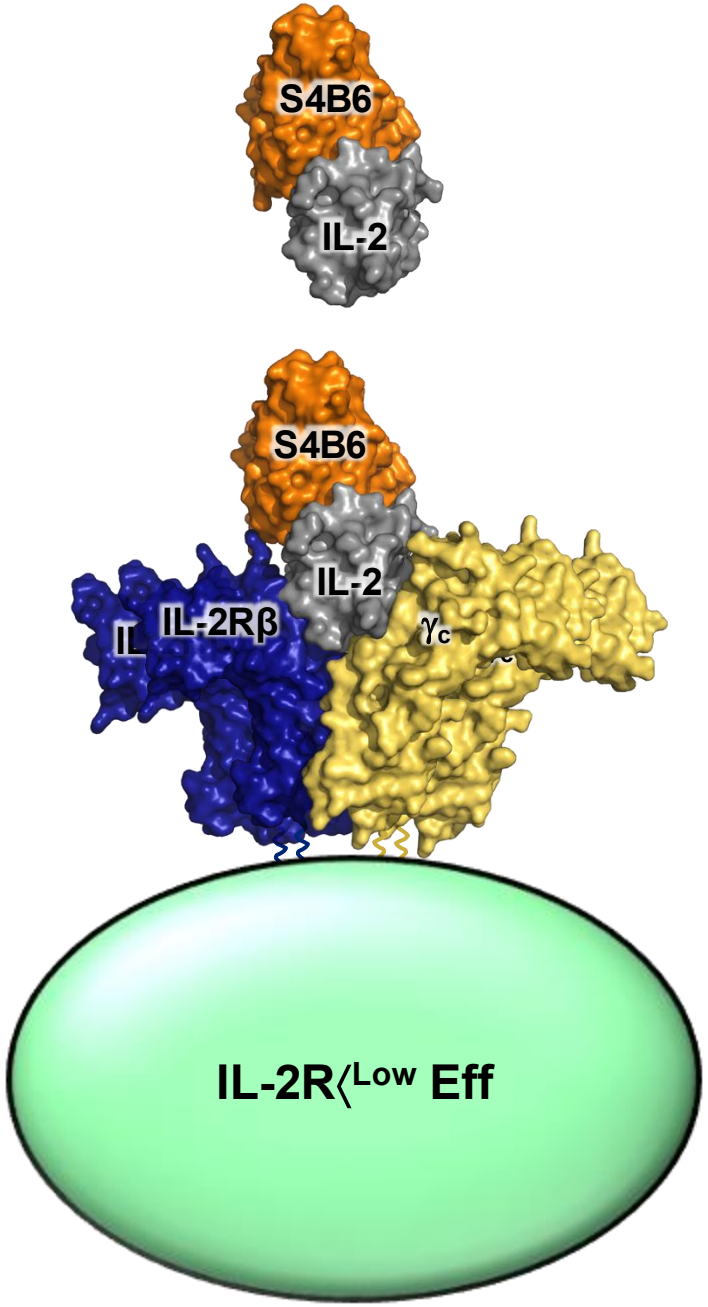




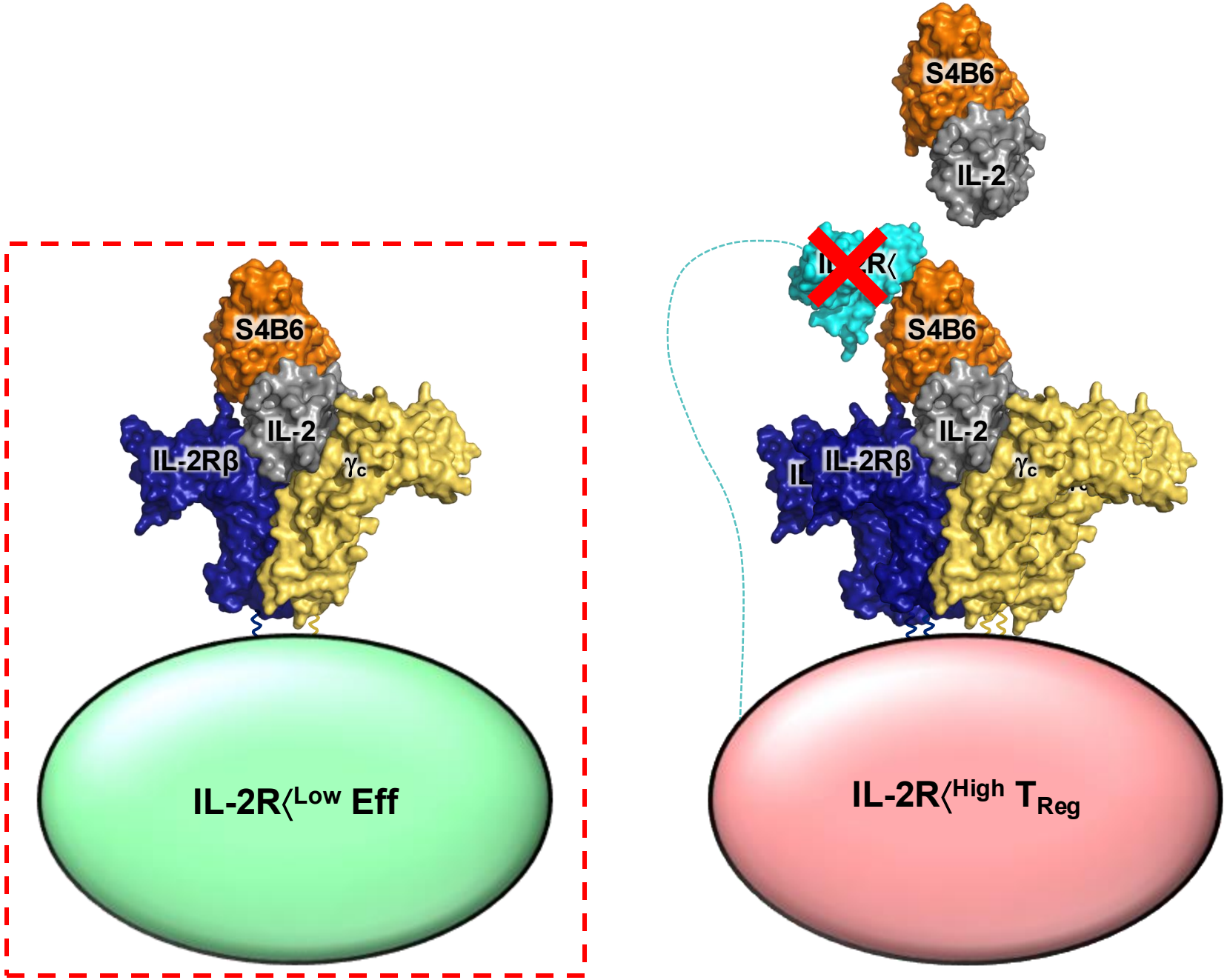
2. Cytokine antibody complex

Spangler et al. Immunity 2015

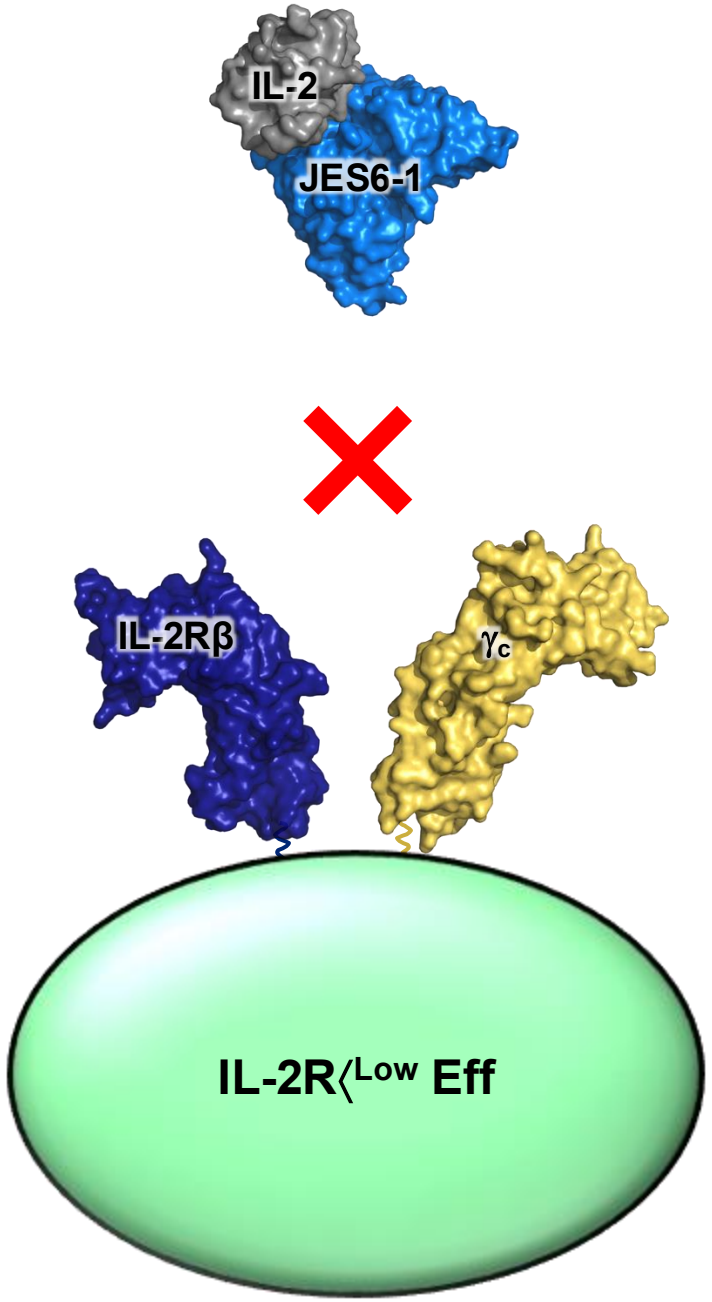
S4B6 stimulates both Effs and T_{Reg}s, favoring IL-2Rβ^{High} Effs



S4B6 stimulates both Effs and T_{Reg}s, favoring IL-2Rβ^{High} Effs



JES6-1 selectively stimulates IL-2Rα^{High} cells



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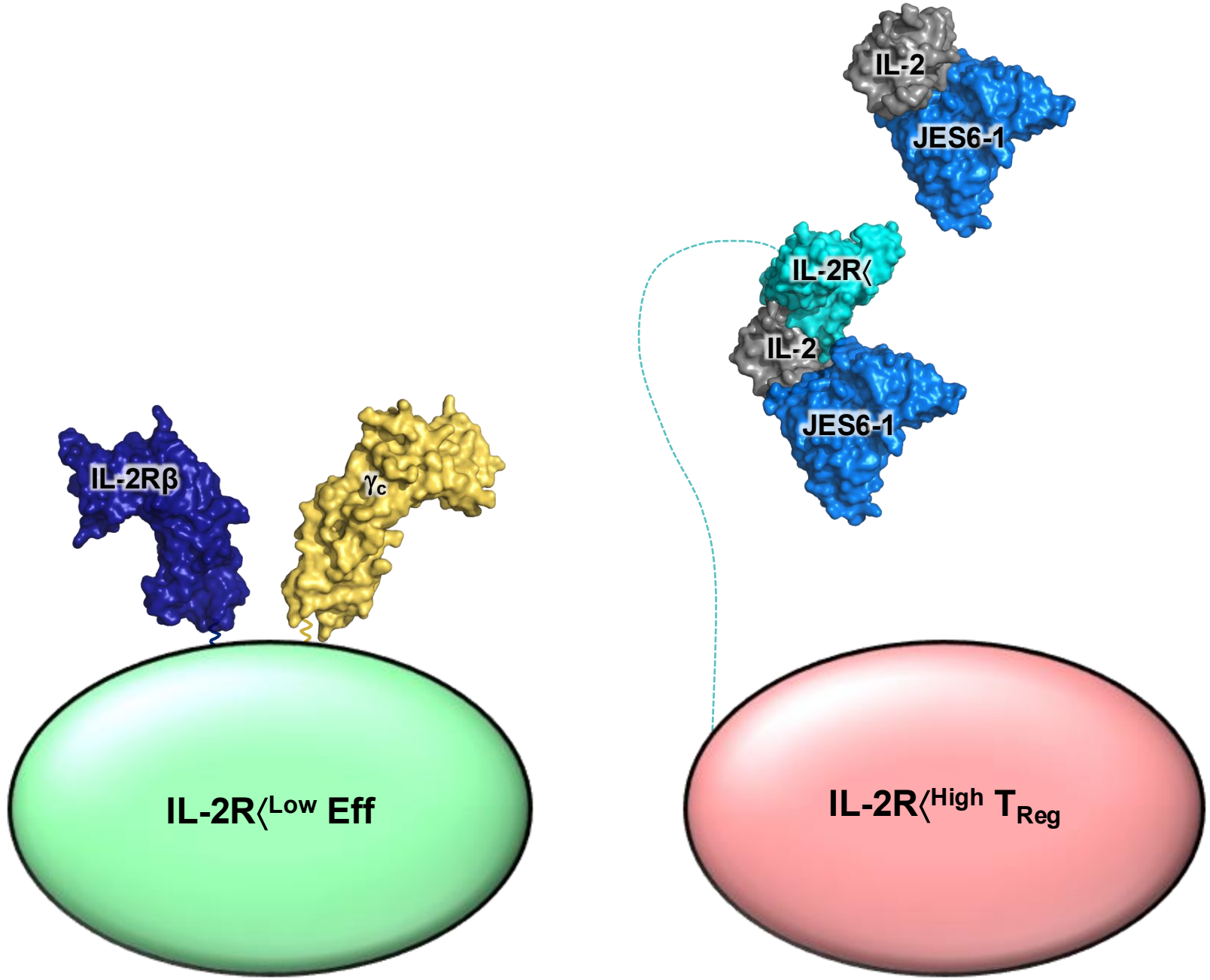

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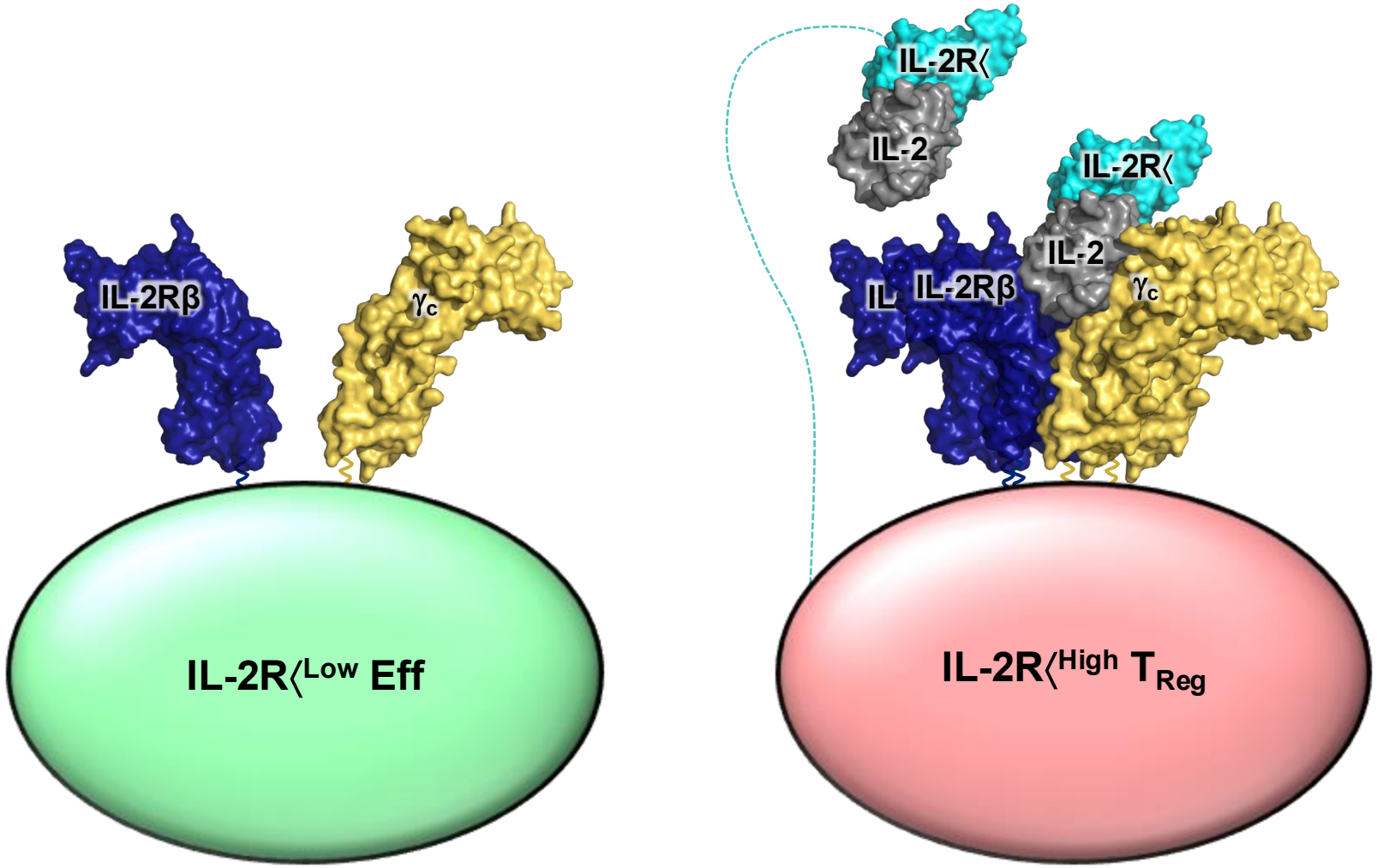
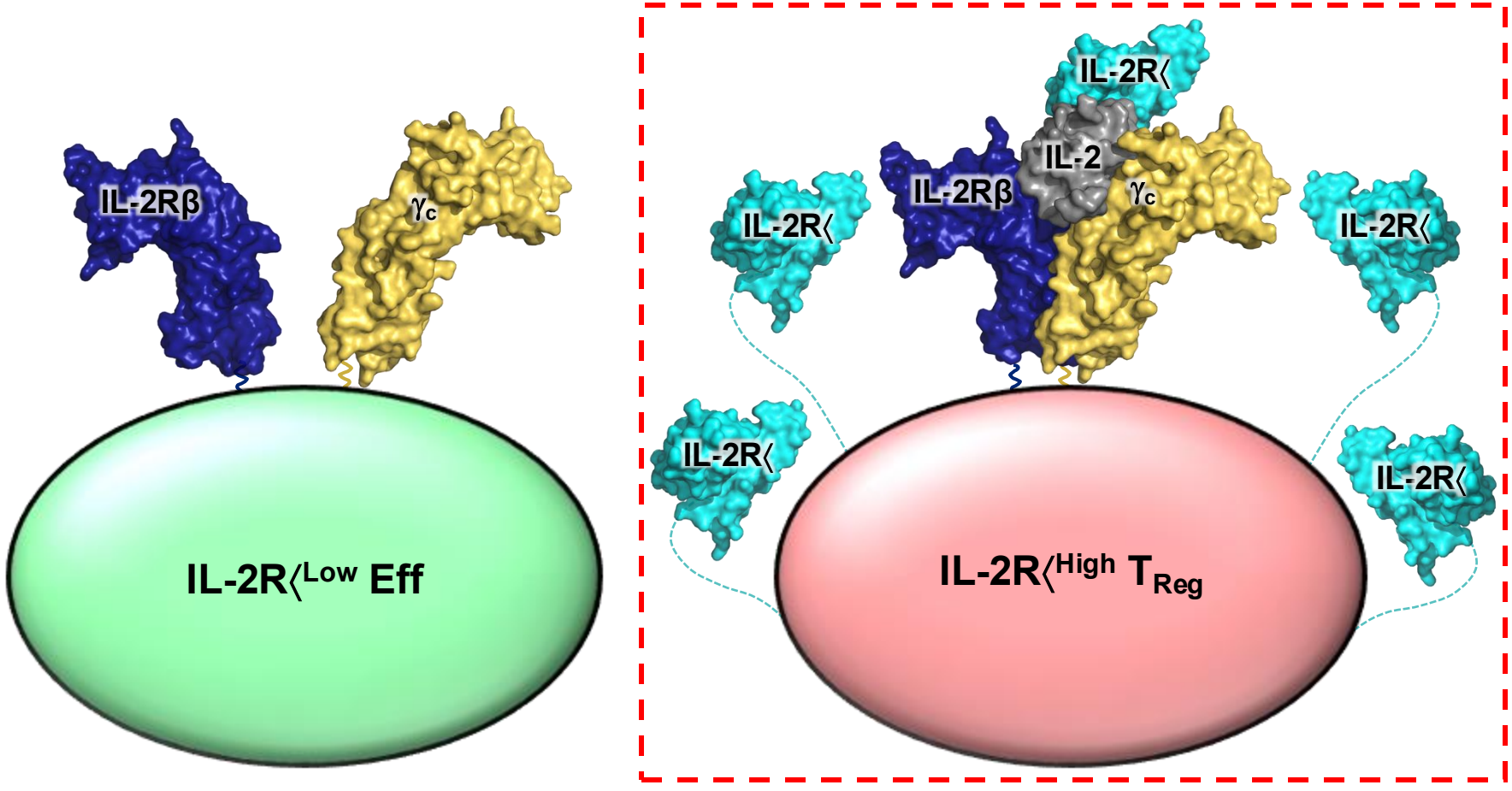
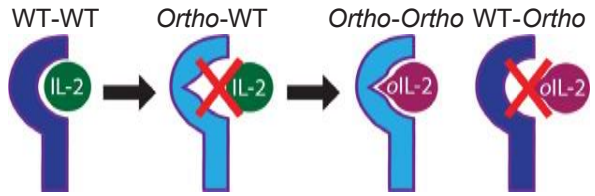
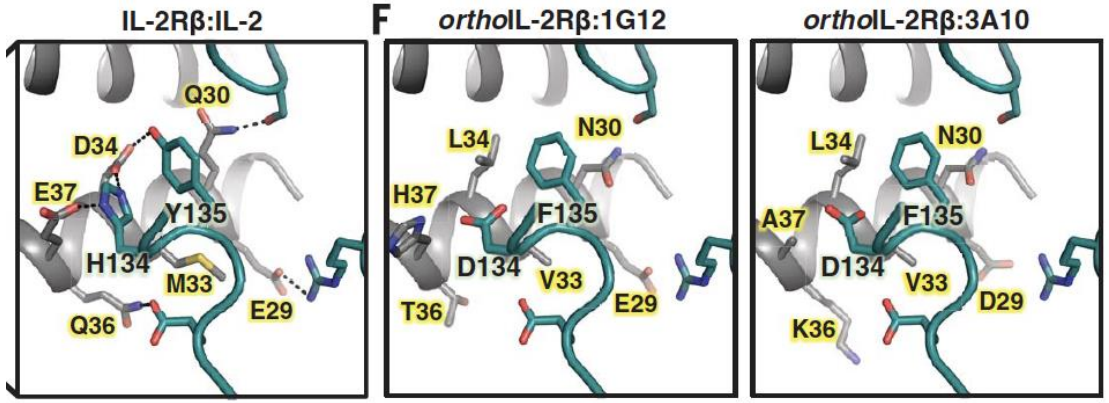


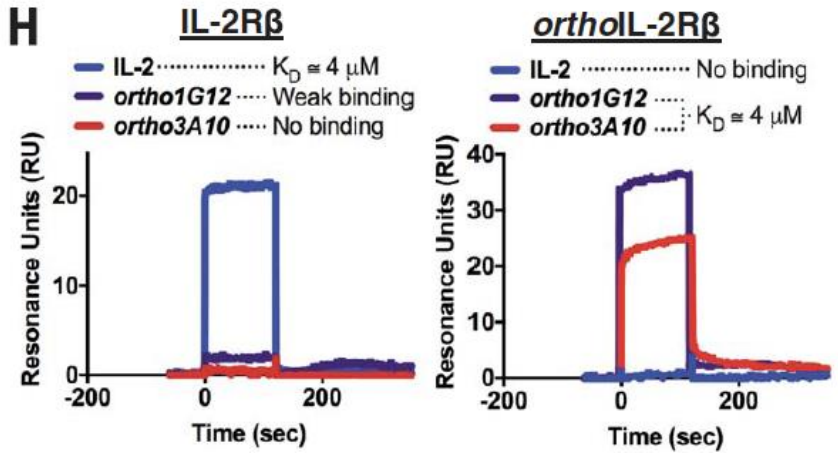
JES6-1 selectively stimulates IL-2Rα^{High} cells

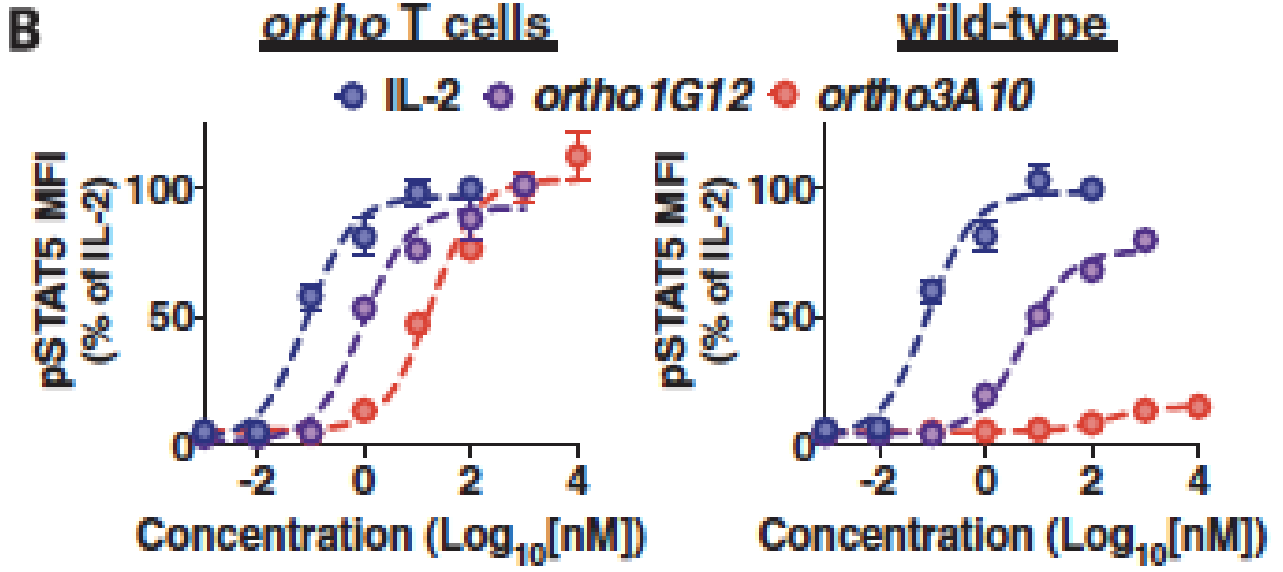


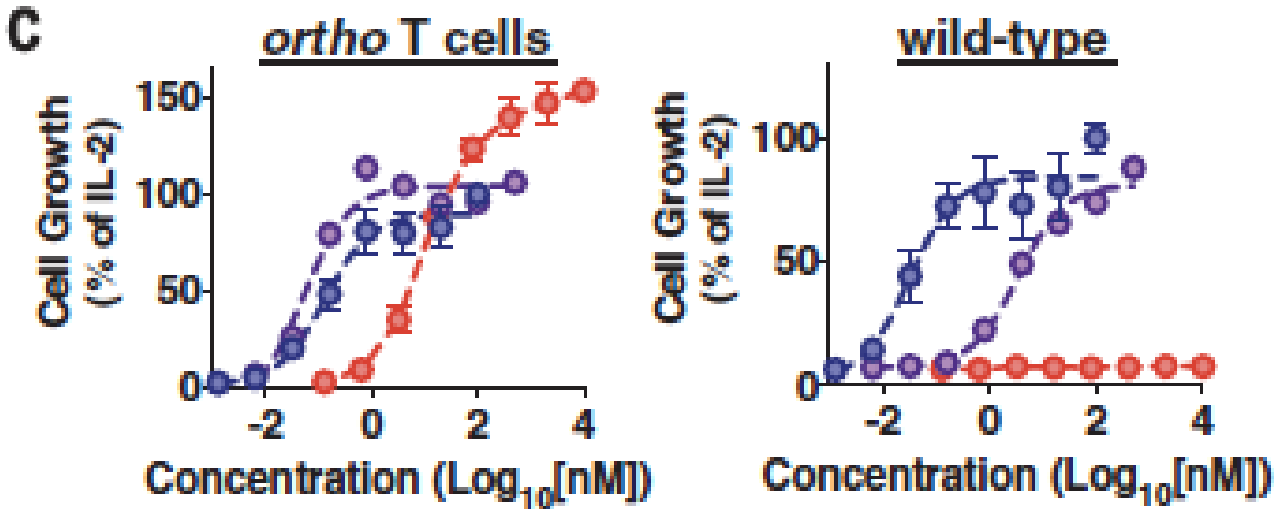
Strategies to create more precise cytokine drugs: 3. Engineering orthogonal IL-2-IL-2R complexes





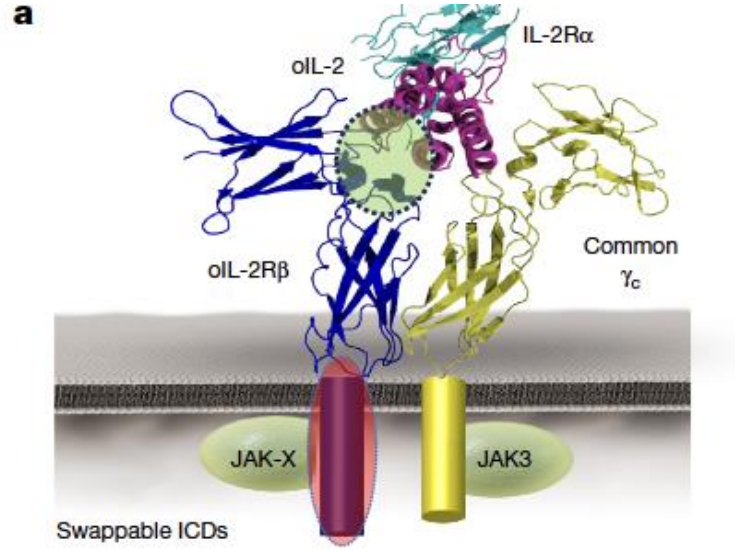


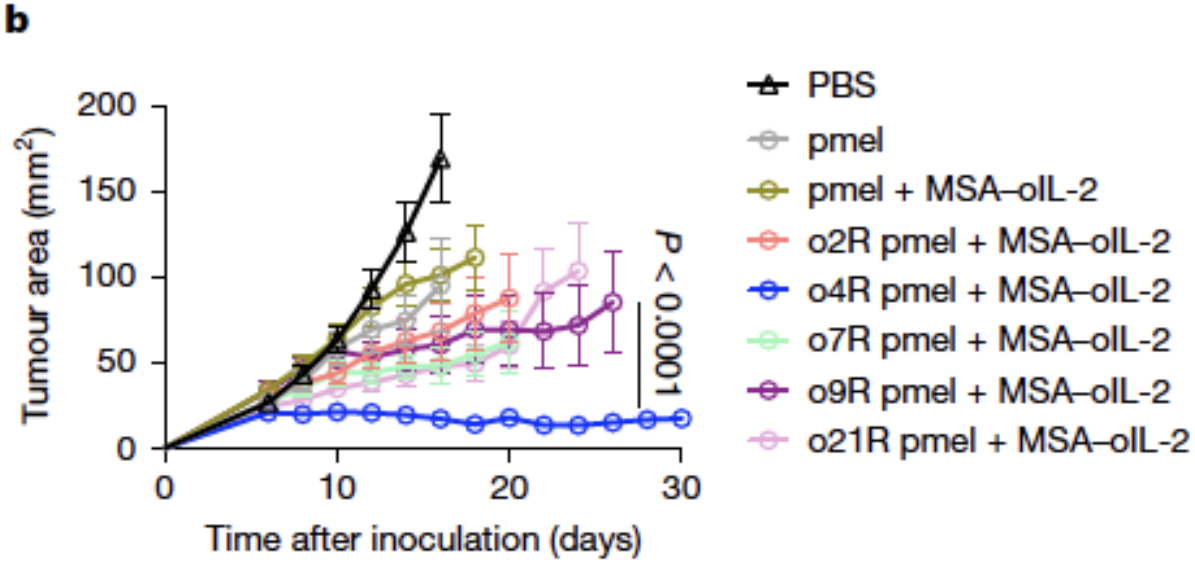


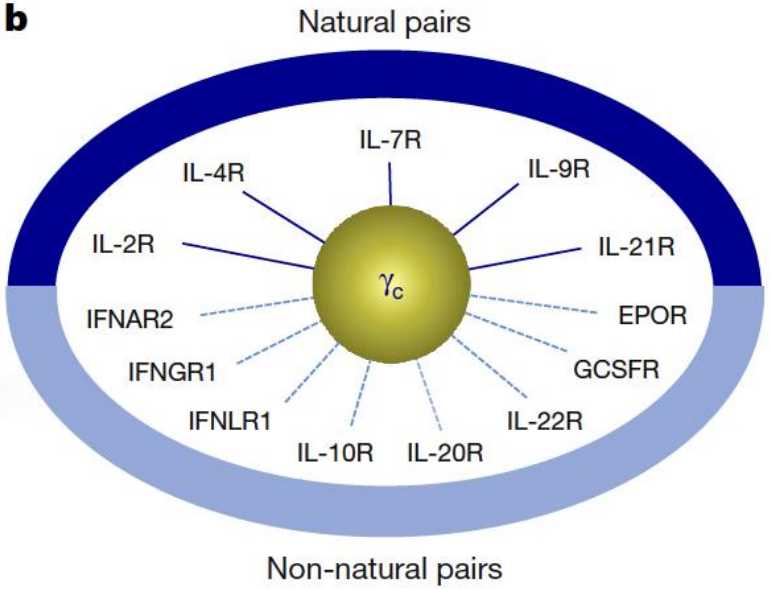


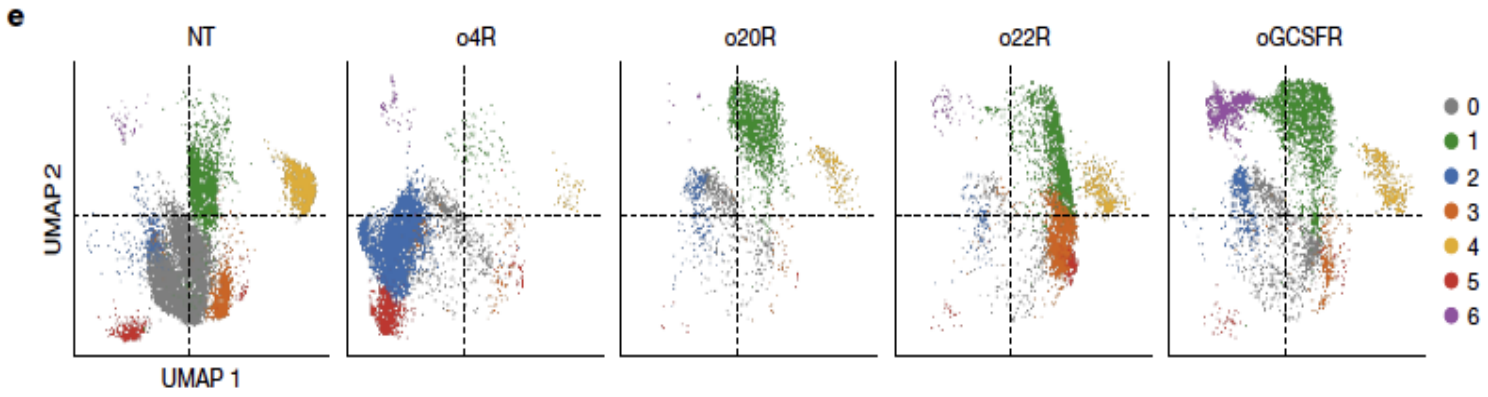
Sockolosky et al. Science 2018

Strategies to create more precise cytokine drugs: 3. Engineering orthogonal IL-2-IL-2R complexes







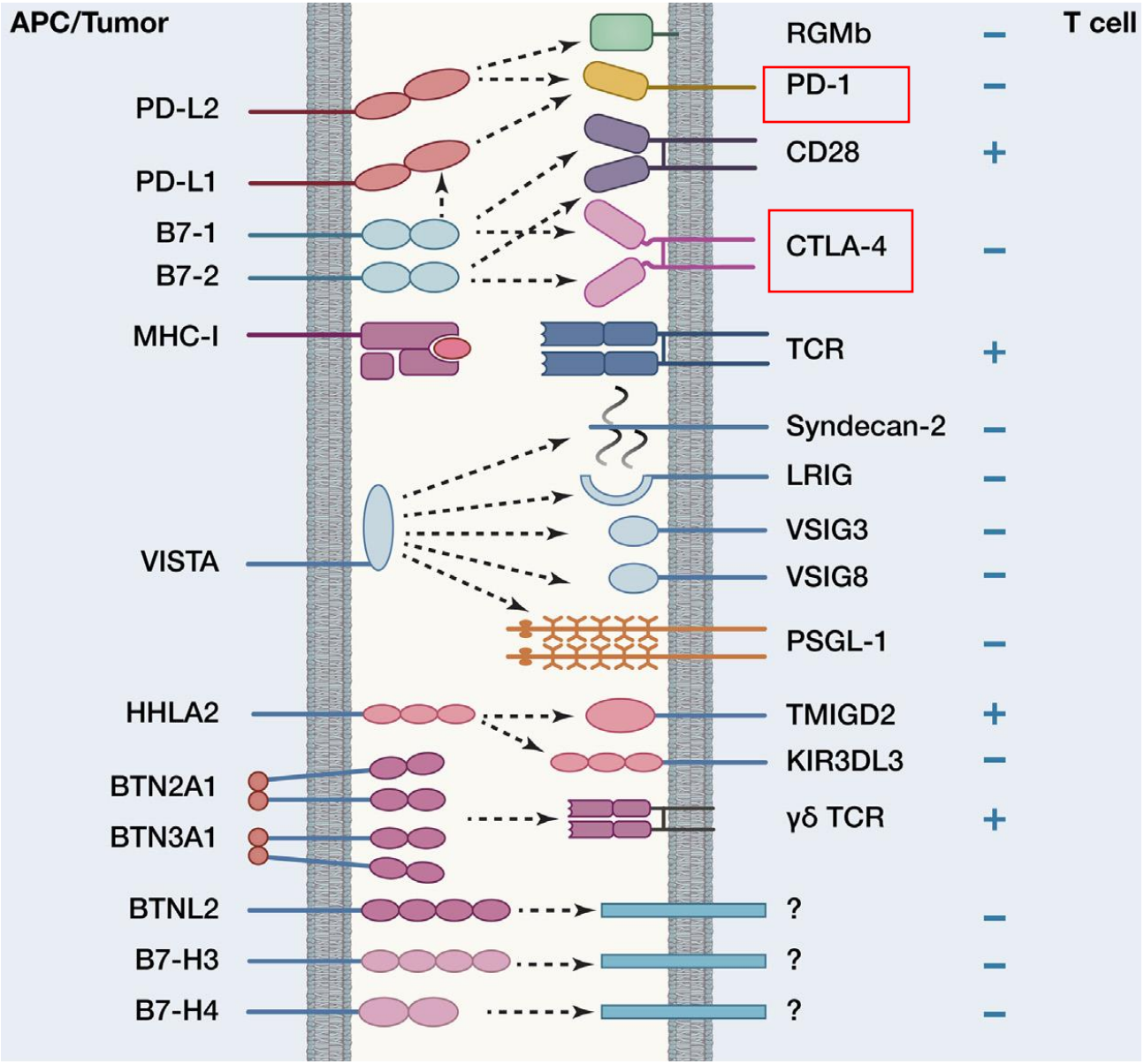


Engineered ortho T cells not only acquire better anti-tumor activity but also display distinct cell fate

Zhao et al. Science 2018

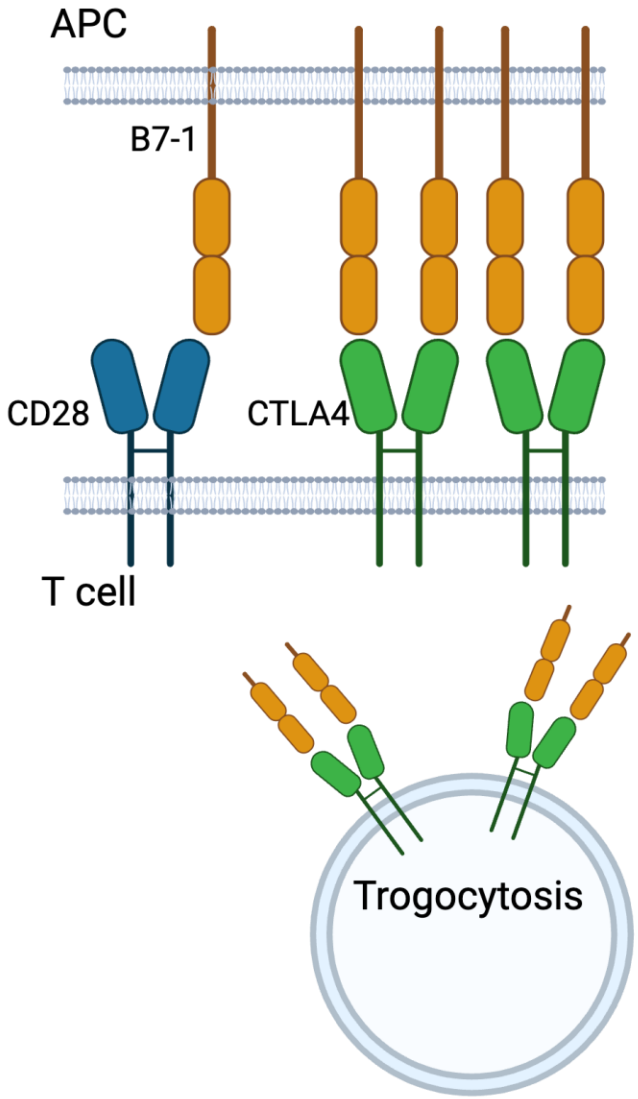
- Overview of how T cell mediated immune system works
  - Antigen receptor diversification
  - Antigen receptor signaling
    - Signal 1
    - Signal 2
    - Signal 3
- Cytokine based immunotherapy (signal 3)
  - principal of cytokine signaling
  - Strategies for designing effective cytokine therapies
- Check point blockade-based immunotherapy (signal 2)
  - landscape of co-stimulatory and co-inhibitory signaling
  - CTLA4 vs PD1
- Antigen receptor-based immunotherapy (signal 1)
  - Antigen identification
  - Engineering therapeutics

Landscape of Co-stimulatory and Co-inhibitory Signals for T cell function



Burke et al. Immunity 2024

CTLA4 and PD1 inhibits T cell function through different mechanisms (extracellular vs intracellular)



CTLA4:CD80(B7-1) affinity: K_D=0.4uM

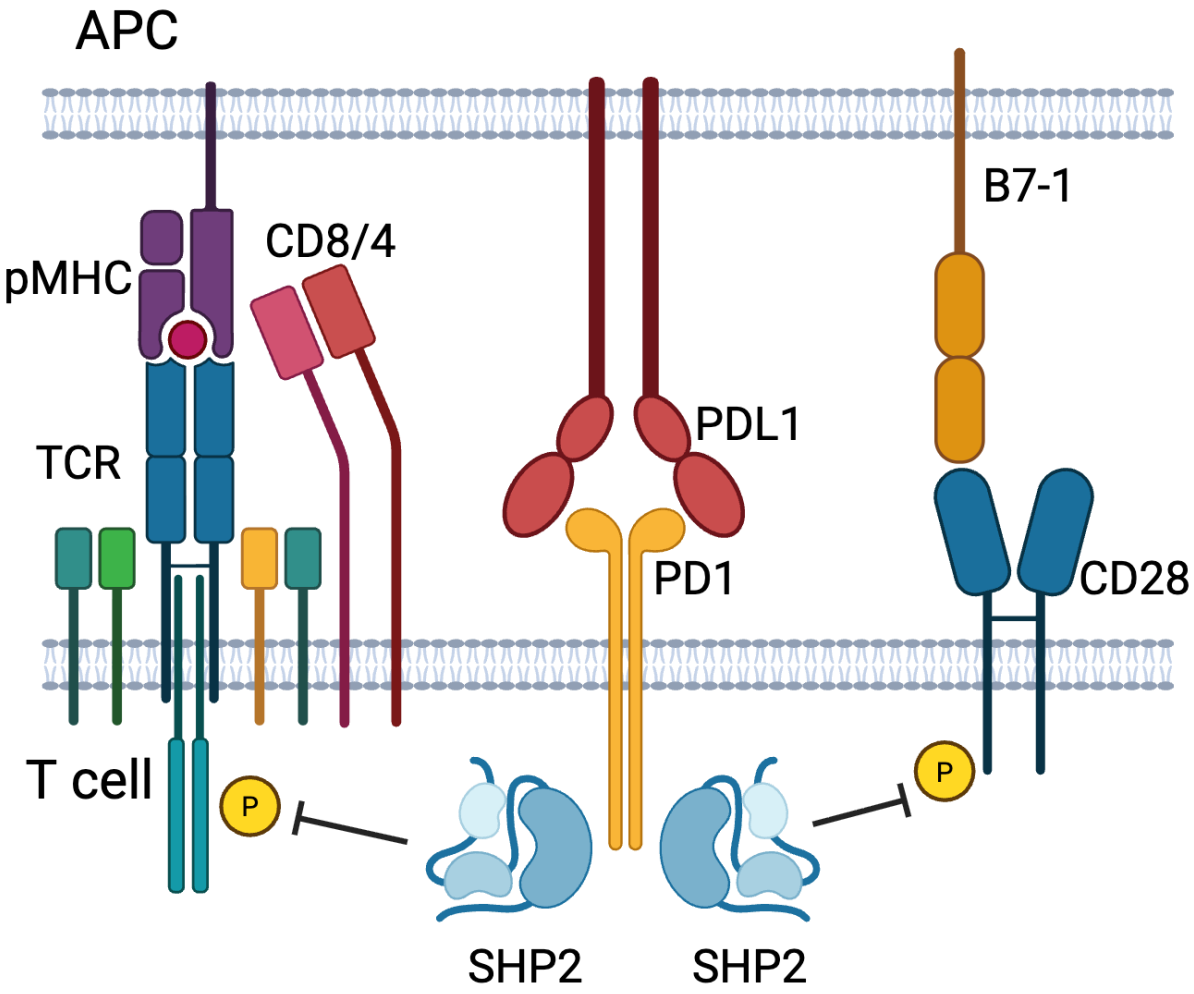
CD28:CD80(B7-1) affinity: K_D=4uM

CTLA4 competes CD80/CD86 on APC surface

CTLA4 down-regulate CD80/CD86 through trogocytosis

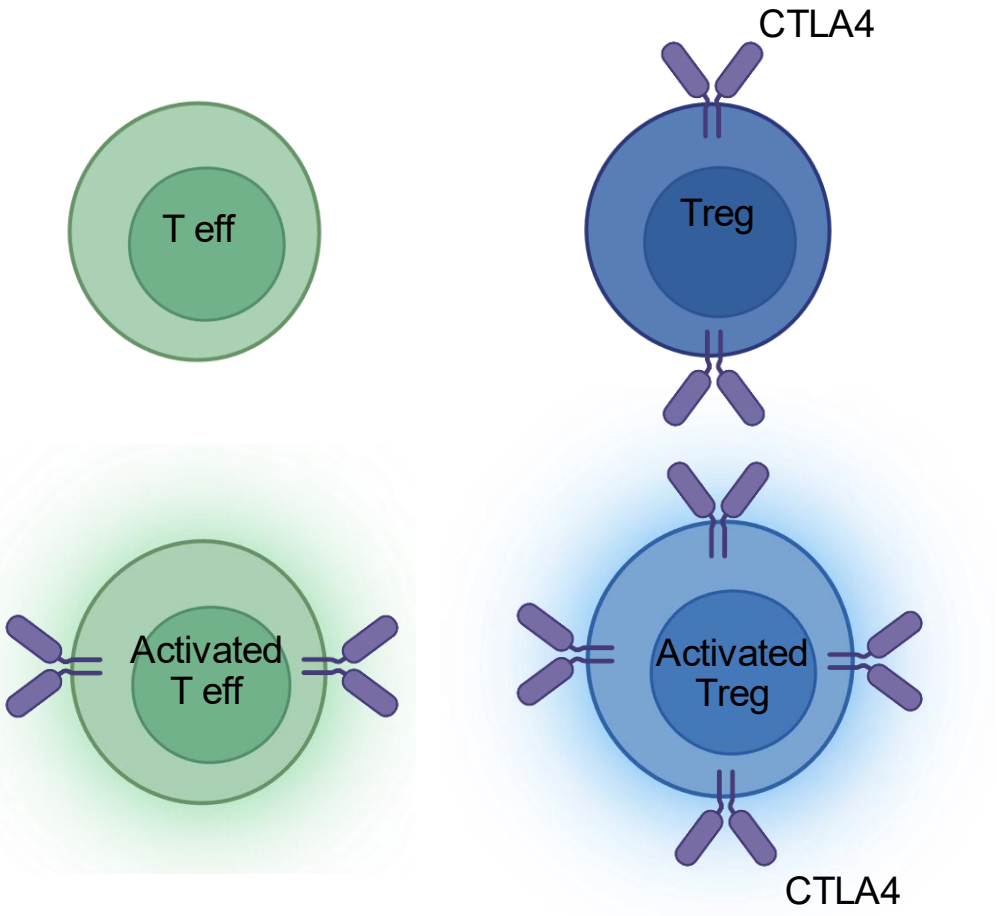
Burke et al. Immunity 2024

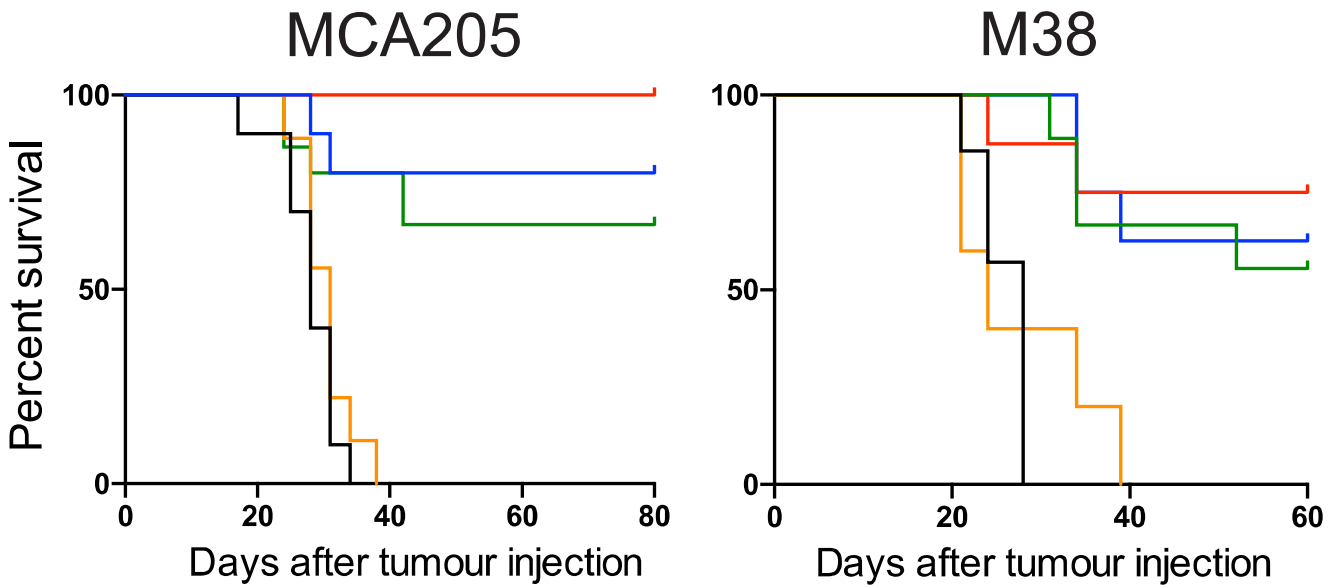
CTLA4 and PD1 inhibits T cell function through different mechanisms (extracellular vs intracellular)



PD1 recruits SHP2 to immune synapse to dephosphorylate TCR CD3 and CD28 intracellular domains

Immune check point ‘blockade’?
Anti-CTLA4 mechanism of action; Fc-FcR interaction is extremely important!

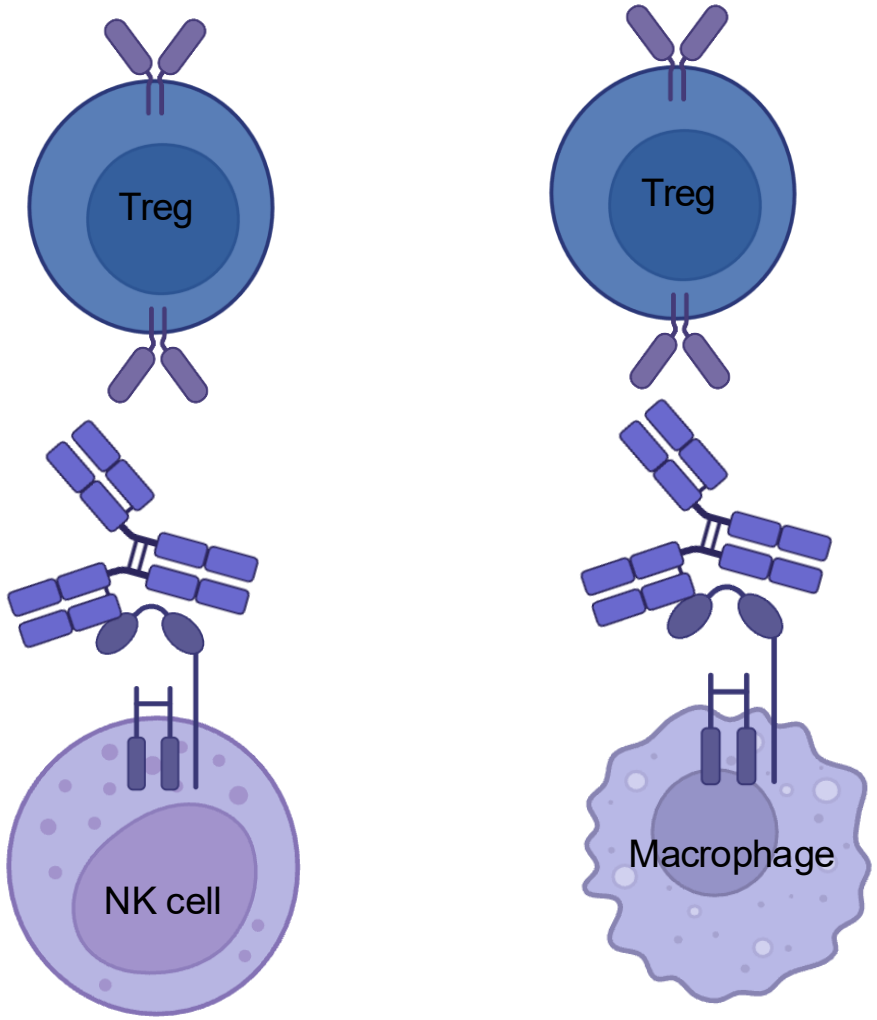


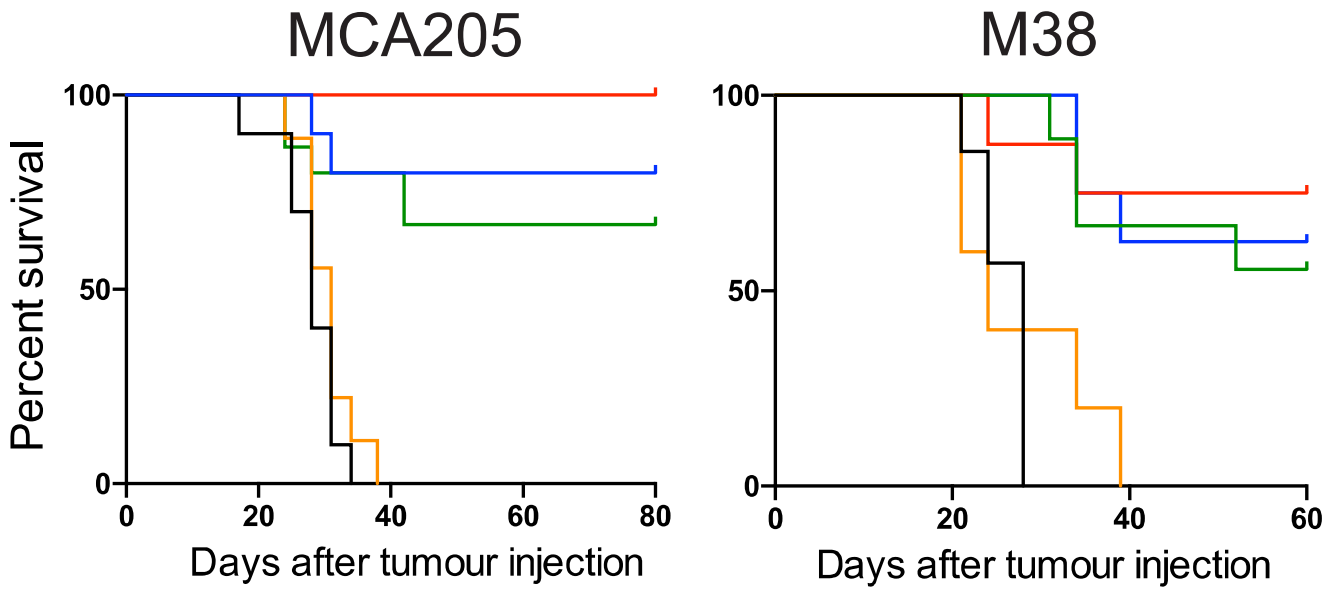


n =	MCA205	M38
No tx	10	7
$IgG1_{N297A}$	9	5
IgG1	15	9
IgG2	10	8
$IgG1_{SDALIE}$	15	8

Takahashi et al. Journal of Experimental Medicine 2000
Arce Vargas et al. Cancer Cell 2018

Anti-CTLA4 mechanism of action (antibody dependent cytotoxicity of Treg cells)





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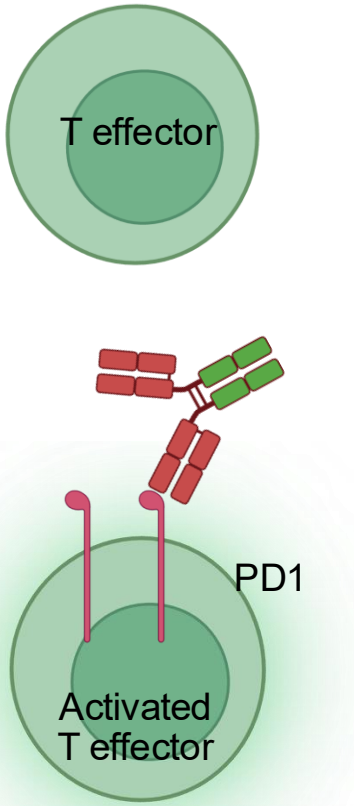
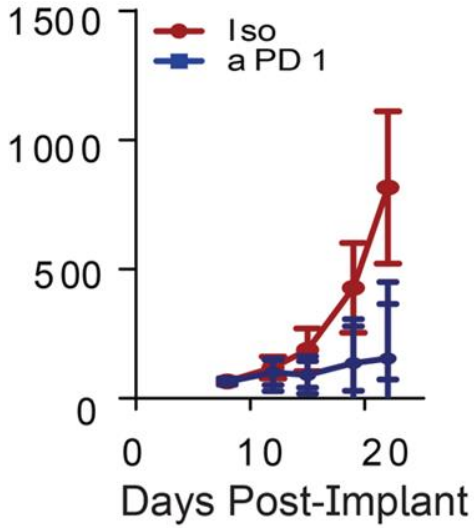
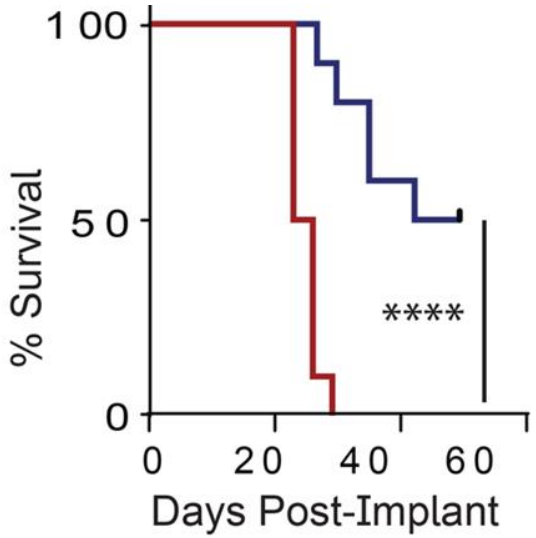
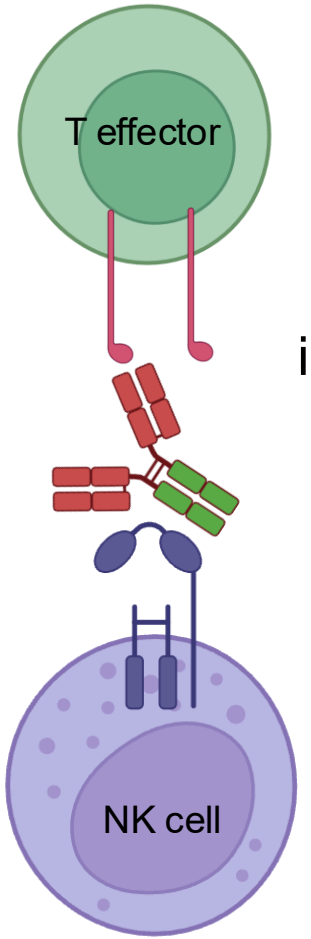
Immune check point ‘blockade’?
Anti-PD1 mechanism of action (blocking PD1-PDL1 interaction)









No Fc-FcR interaction is good for anti-PD1 antibody

Some Anti-PD1 antibody is made with IgG4 Fc which has no or little FcR binding ability

Kim et al. Cancer Immunology, Immunotherapy 2023

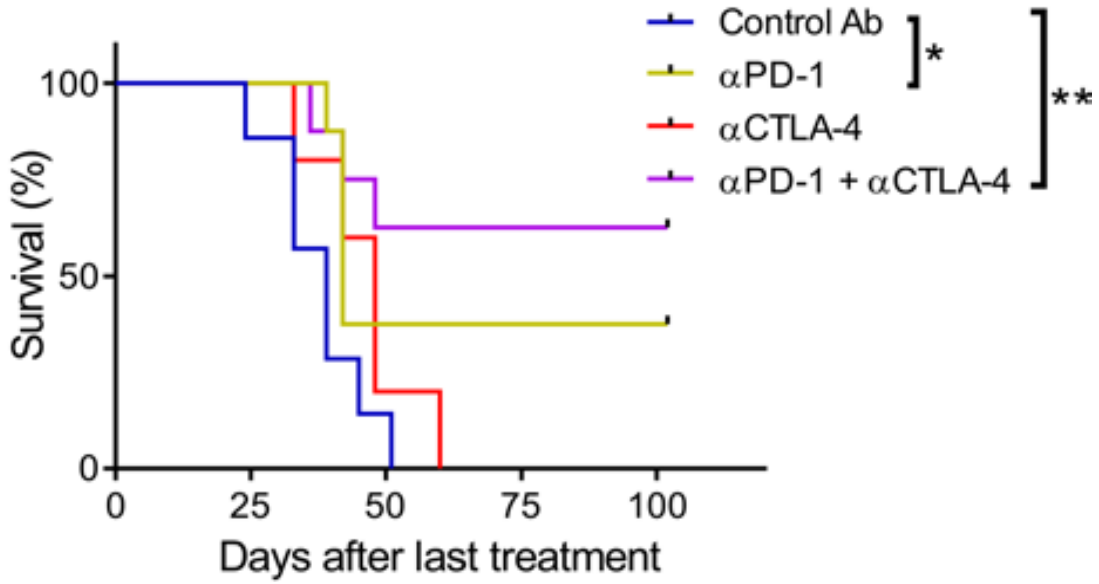
Anti-CTLA4 vs Anti-PD1

Anti-CTLA4

- Targets CD28 extracellular complex
- Expands clonal diversity
- Responses often slow
- Primarily affects CD4 Treg cell
- ADCC or ADCP
- Adverse events are frequent and severe

Anti-PD1

- Targets TCR and CD28 intracellular signaling
- Expands clonal diversity to less extent
- Responses often rapid
- Primarily affects CD8 T cells
- Blockade PD1-PDL1 interaction
- Adverse events are less frequent and manageable



Liu et al. JCI Insight. 2023

More than blockade…

Article

Immune receptor inhibition through enforced phosphatase recruitment

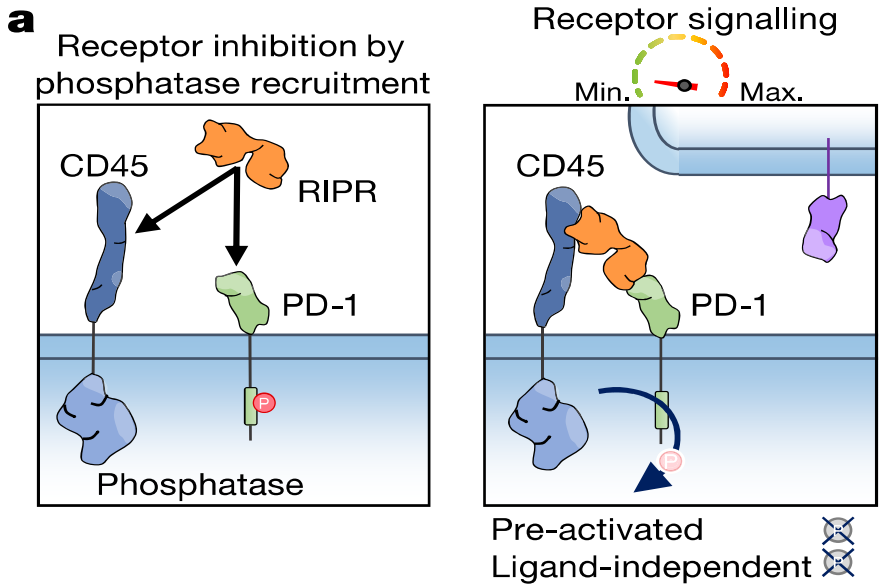
https://doi.org/10.1038/s41586-020-2851-2

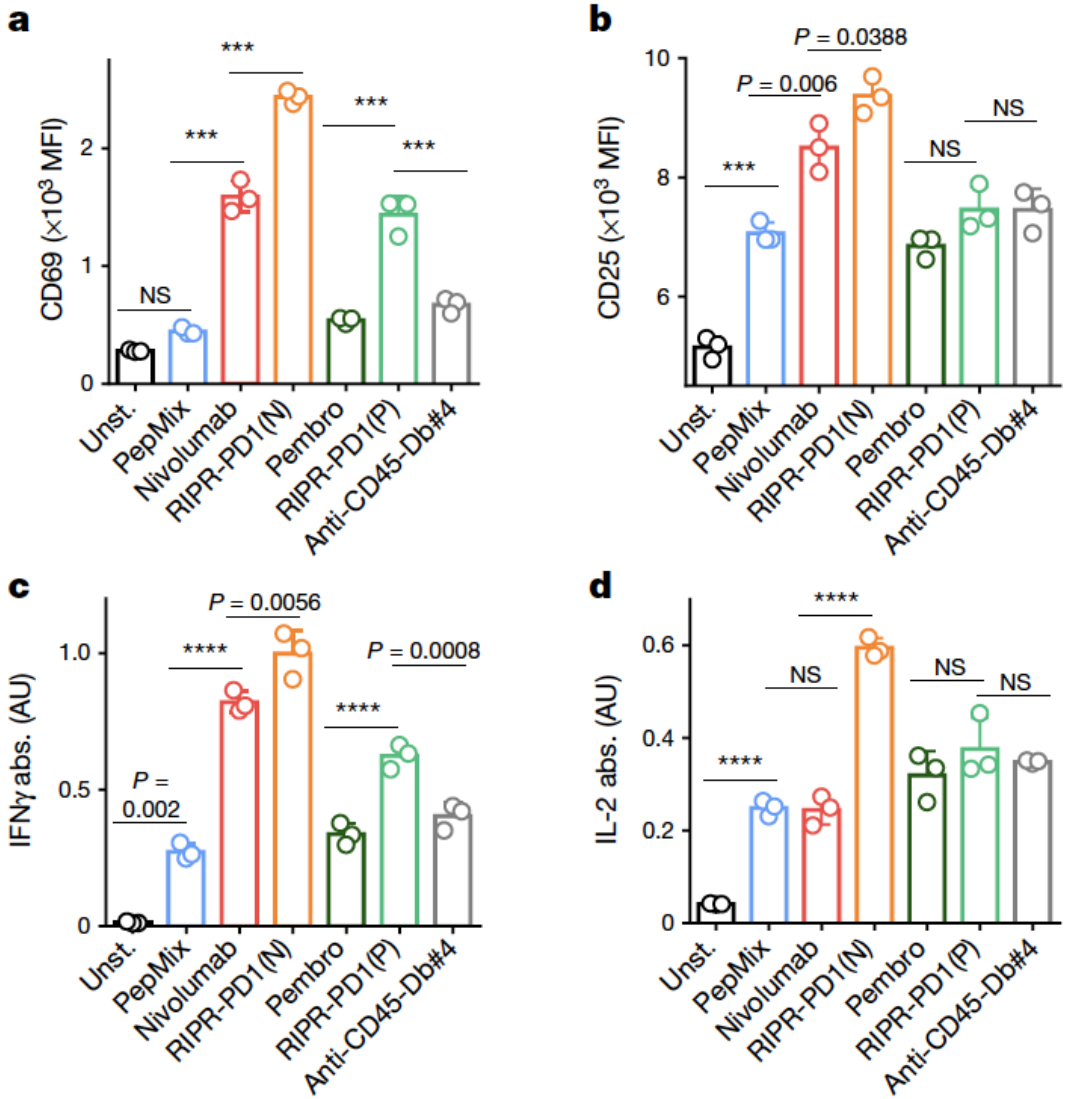
Received: 21 June 2019

Accepted: 24 July 2020

Published online: 21 October 2020

Ricardo A. Fernandes[1,2], Leon Su[1,2], Yoko Nishiga[3,4], Junming Ren[1,2], Aladdin M. Bhuiyan[5], Ning Cheng[6], Calvin J. Kuo[6], Lora K. Picton[1,2], Shozo Ohtsuki[1,2], Robbie G. Majzner[3,7], Skyler P. Rietberg[7], Crystal L. Mackall[3,7,8], Qian Yin[9], Lestat R. Ali[10], Xinbo Yang[1,2], Christina S. Savvides[1,2], Julien Sage[3,11], Michael Dougan[5,10] & K. Christopher Garcia[1,2,12]✉





Fernandes et al. Nature. 2020

More than blockade…

ACS Synthetic Biology

pubs.acs.org/synthbio

Research Article

Receptor Elimination by E3 Ubiquitin Ligase Recruitment (REULR): A Targeted Protein Degradation Toolbox

Dirk H. Siepe, Lora K. Picton, and K. Christopher Garcia*

Cite This: *ACS Synth. Biol.* 2023, 12, 1081−1093

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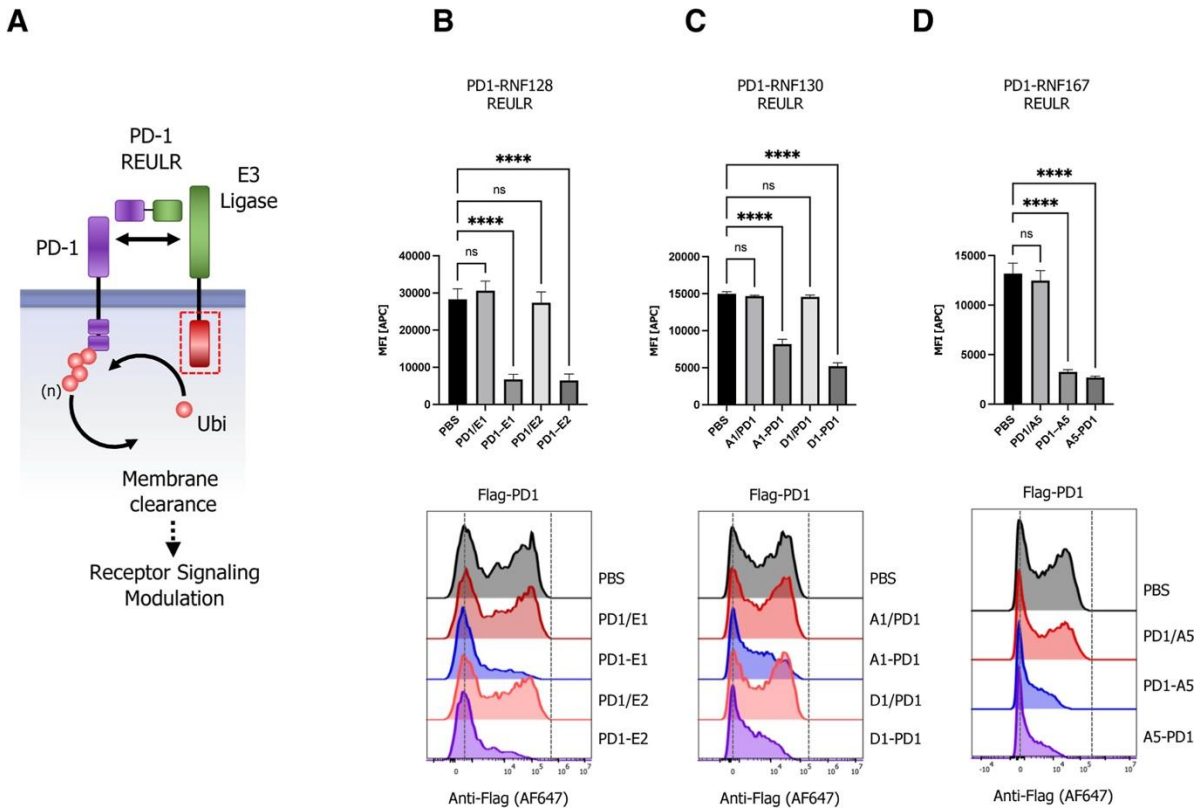


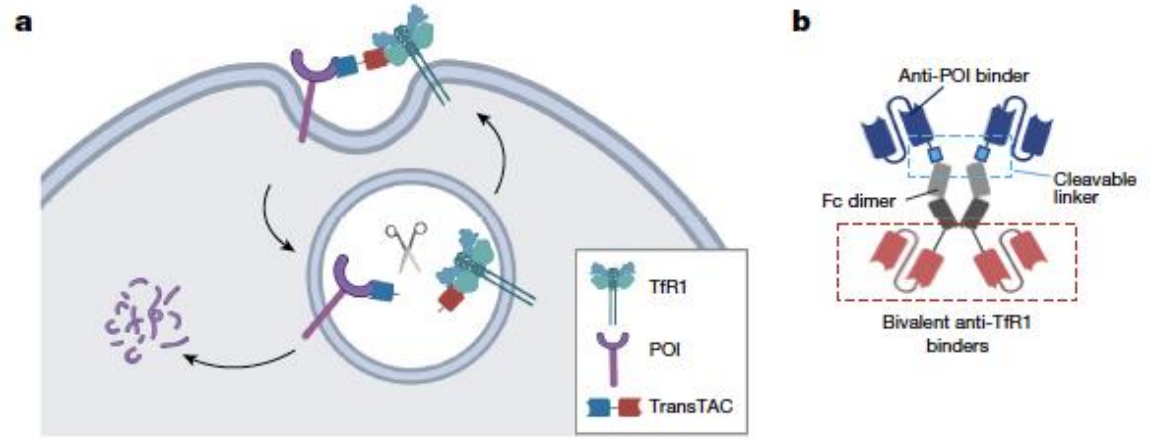
Transferrin receptor targeting chimeras for membrane protein degradation

https://doi.org/10.1038/s41586-024-07947-3

Received: 10 May 2023

Accepted: 14 August 2024

Dingpeng Zhang[1,2], Jhoely Duque-Jimenez[1], Francesco Facchinetti[3,4,5], Garyk Brixi[6], Kaitlin Rhee[1,2], William W. Feng[3,4,5], Pasi A. Jänne[3,4,5,7] & Xin Zhou[1,2]✉



Siepe et al. ACS synthetic bio. 2023
Zhang et al. Nature. 2025

- Overview of how T cell mediated immune system works
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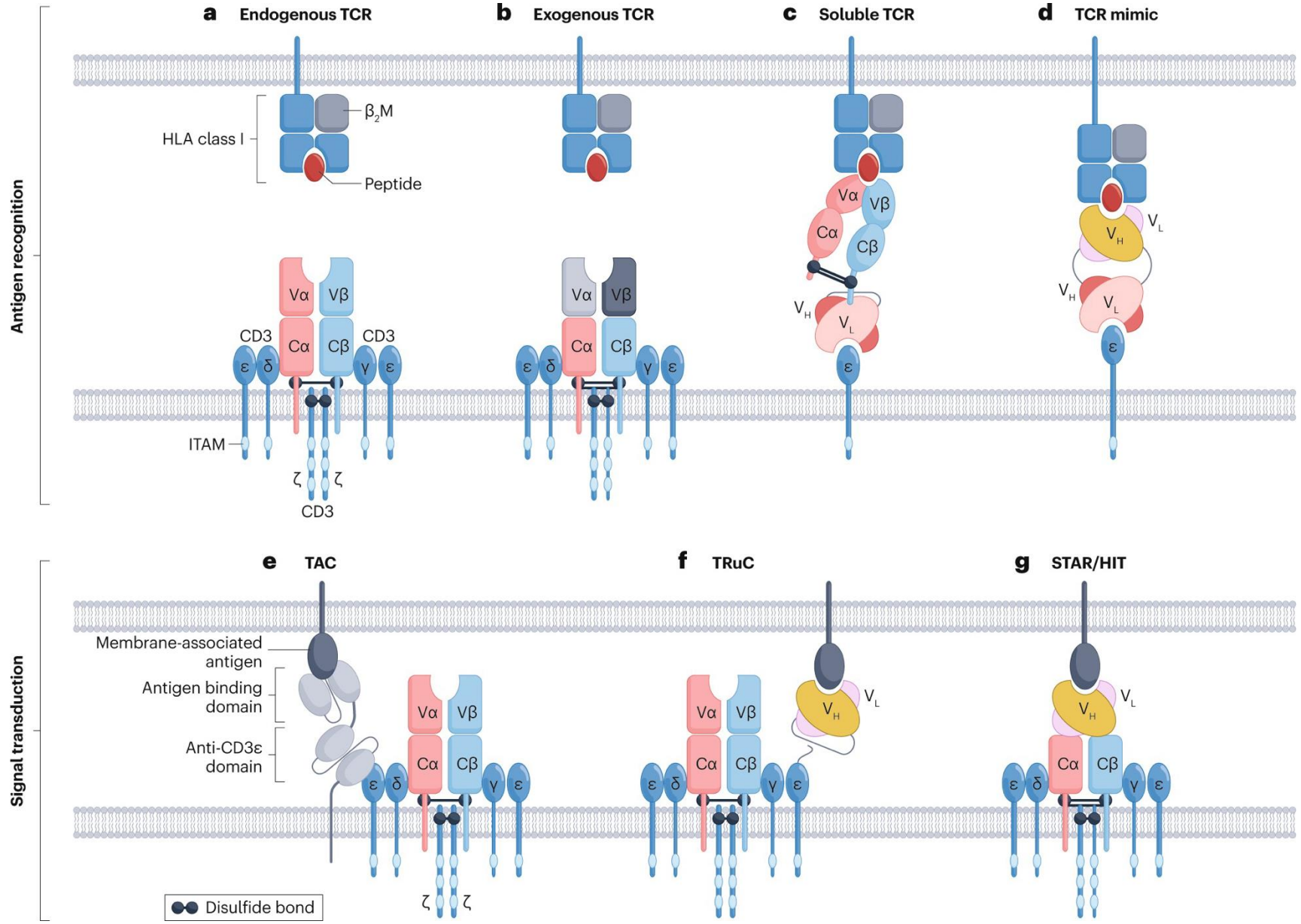
  - Antigen identification
  - Engineering therapeutics

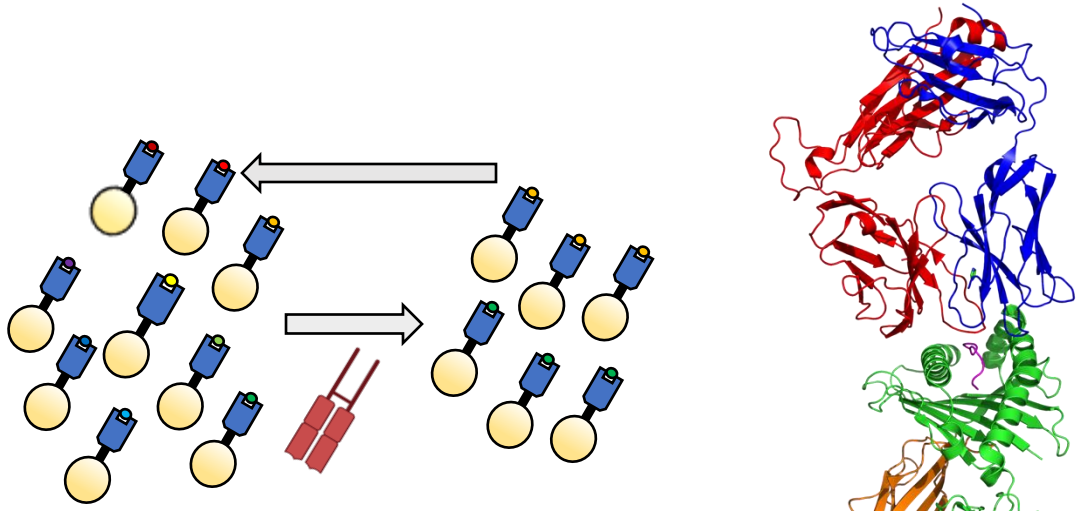
Antigen receptor based immunotherapeutics



Klebanoff et al. Nat. Rev. Drug Discovery. 2023

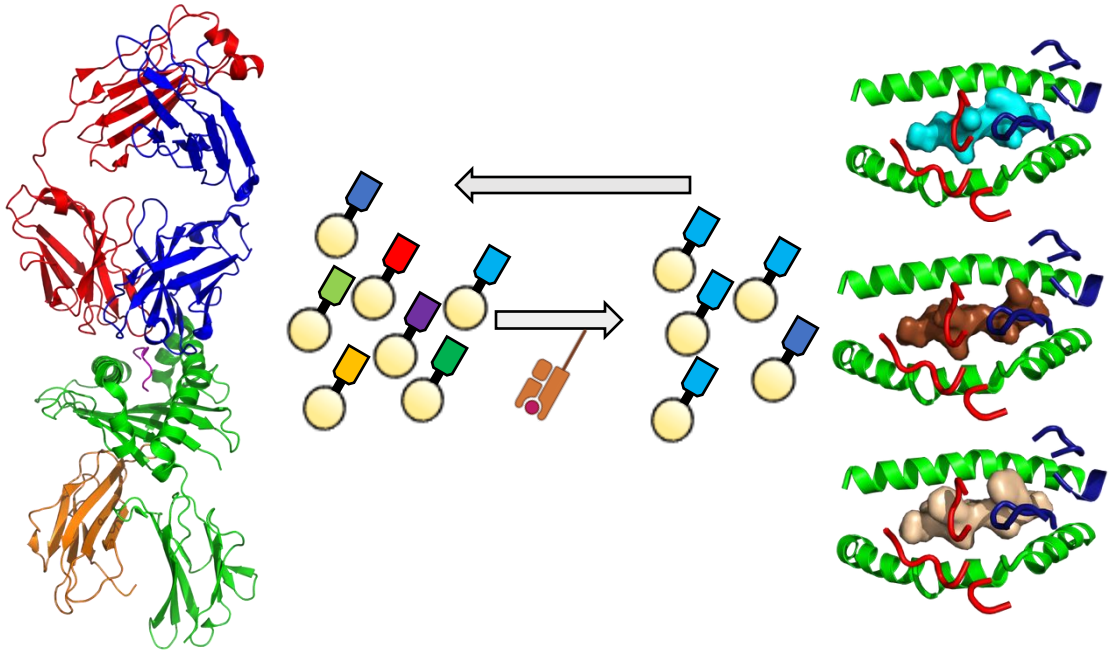
Understanding and manipulating T cell receptor (TCR) specificity and function

Identifying TCR ligand



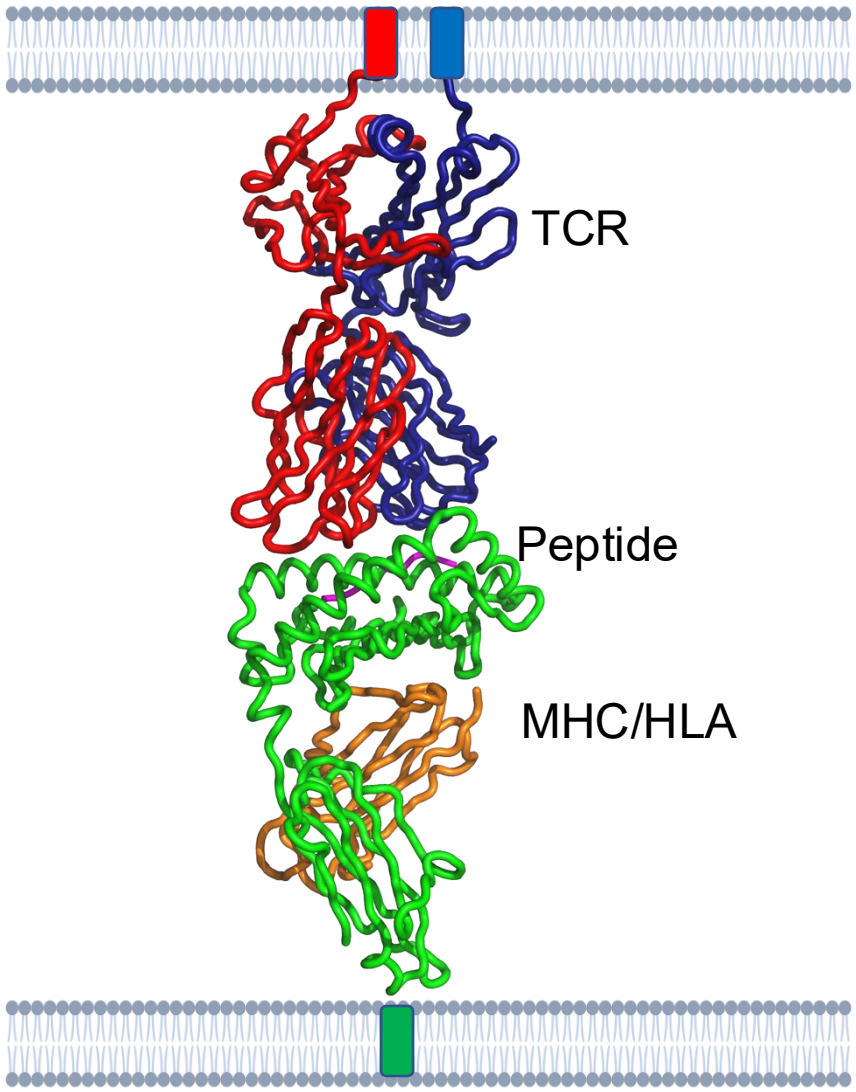
- What do T cells recognize in certain disease?

Engineering TCR or TCRmimic



- Can we rapidly isolate therapeutic TCRm Abs?
- Can the TCRm format for ADCC, BiTE and CAR-T

Size of T cell repertoire

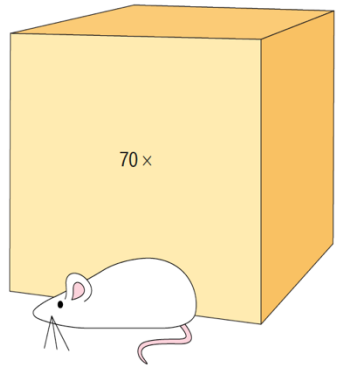


- T cell receptors 10^{15}
- Possible pMHC-I>10^{11}; pMHC-II> 10^{12}
- MHC alleles: 7000

- T cells in a human 10^{10}
- T cells in a mouse 10^{8}

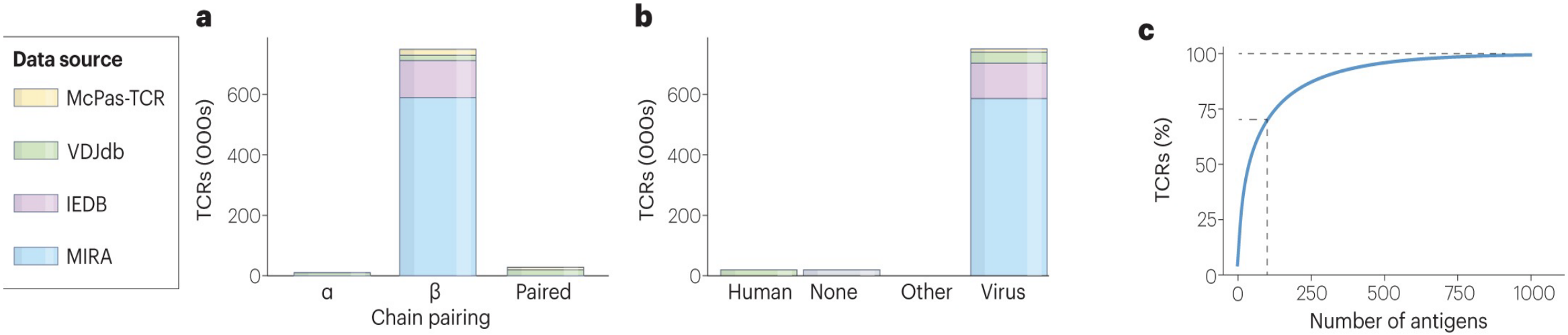
A very high level of crossreactivity is an essential feature of the T-cell receptor

Don Mason



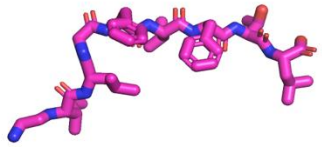
Mason 1998. Immunol. Today

Lack of high-quality TCR and antigen-MHC pairs

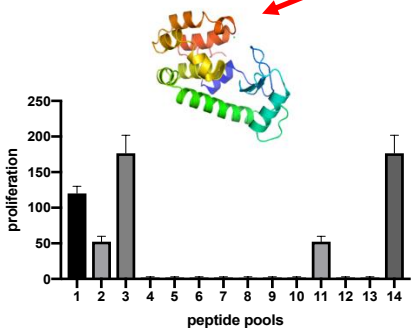


Hudson et al. Nat. Rev. Immunol. 2023

Methodology for peptide identification

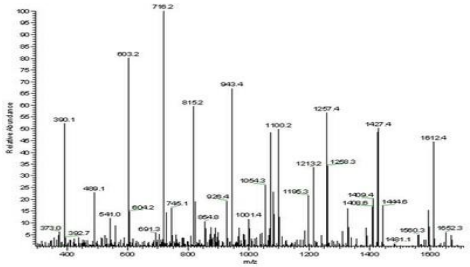


Peptide pool-based approach



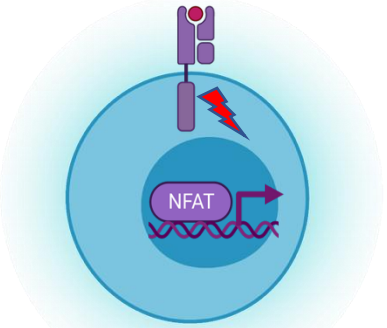
Pros: TCR-pMHC linkage; Easy implementation
Cons: Low throughput; Low diversity

Mass spec-based approach



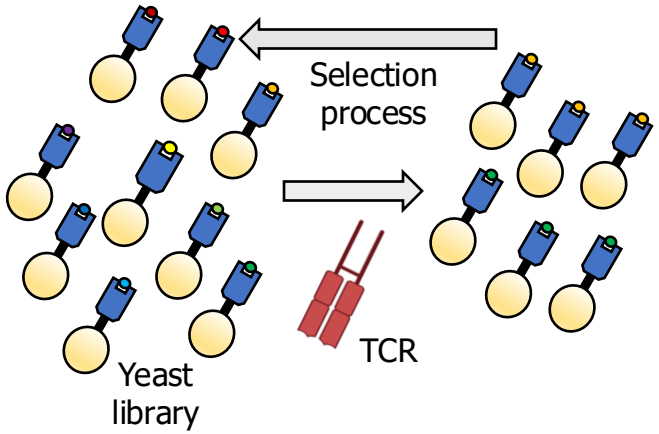
Pros: High throughput
Cons: Loss of TCR-pMHC linkage; Hard implementation

Cell-based approach



Pros: Semi-high throughput; TCR-pMHC linkage; Semi-easy implementation
Cons: Semi-high diversity; relatively high false positive rate

Combinatorial library-based approach

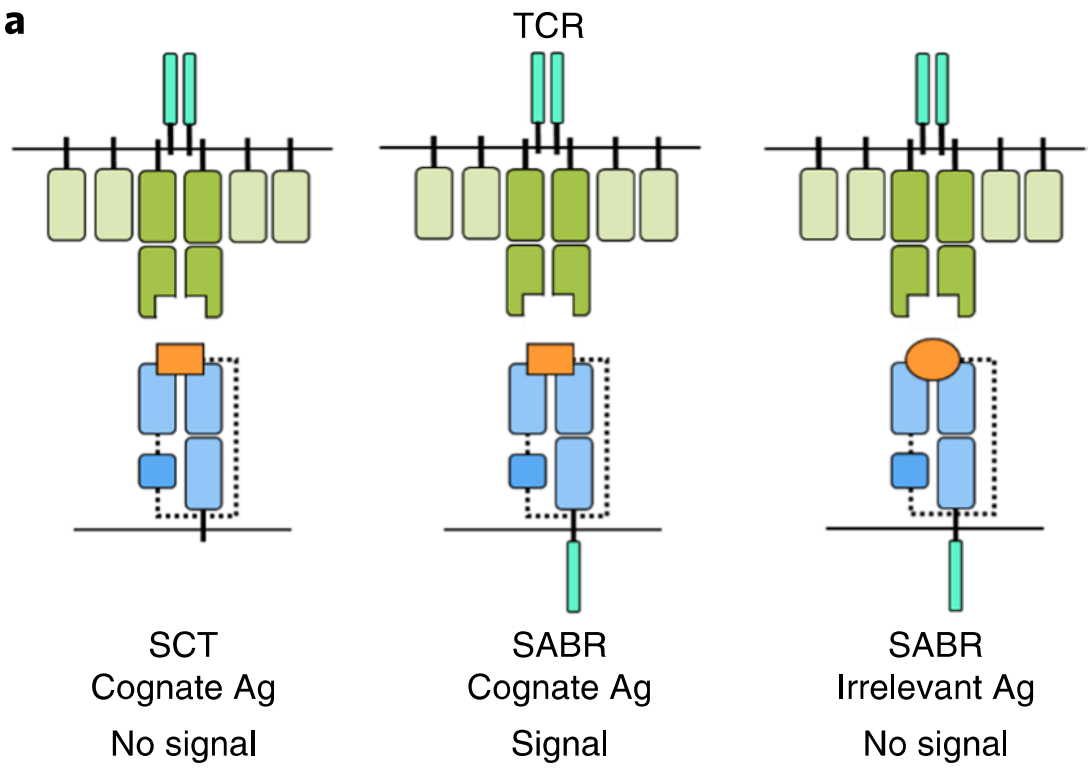


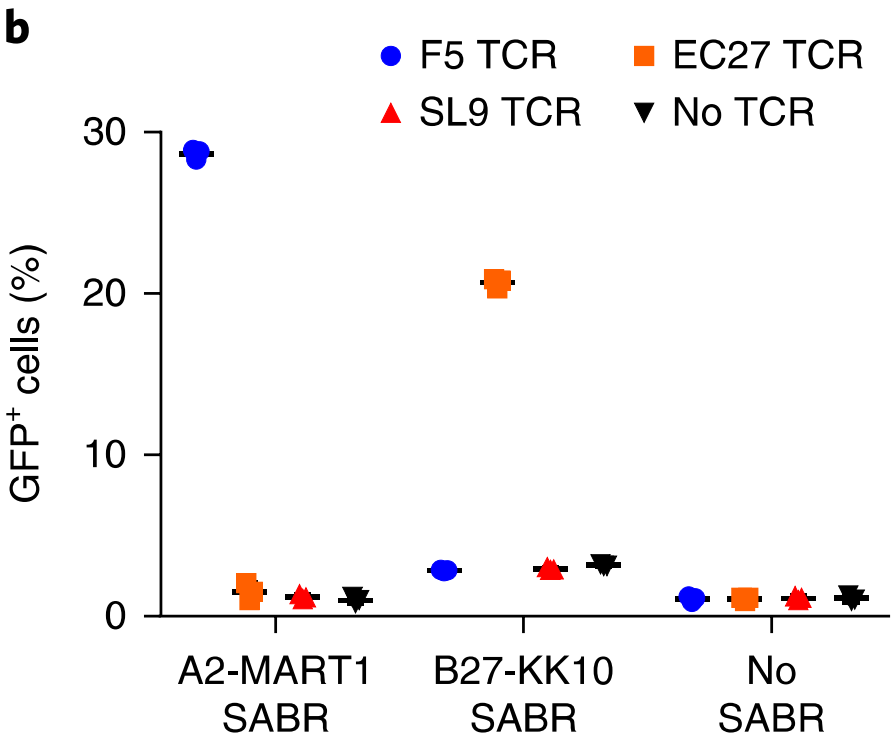
Pros: Semi-high throughput; TCR-pMHC linkage; Very high diversity; Low false positive rate
Cons: Hard implementation

Joglekar & Li. Nat. Methods 2021

T cell antigen discovery via signaling and antigen-presenting bifunctional receptors

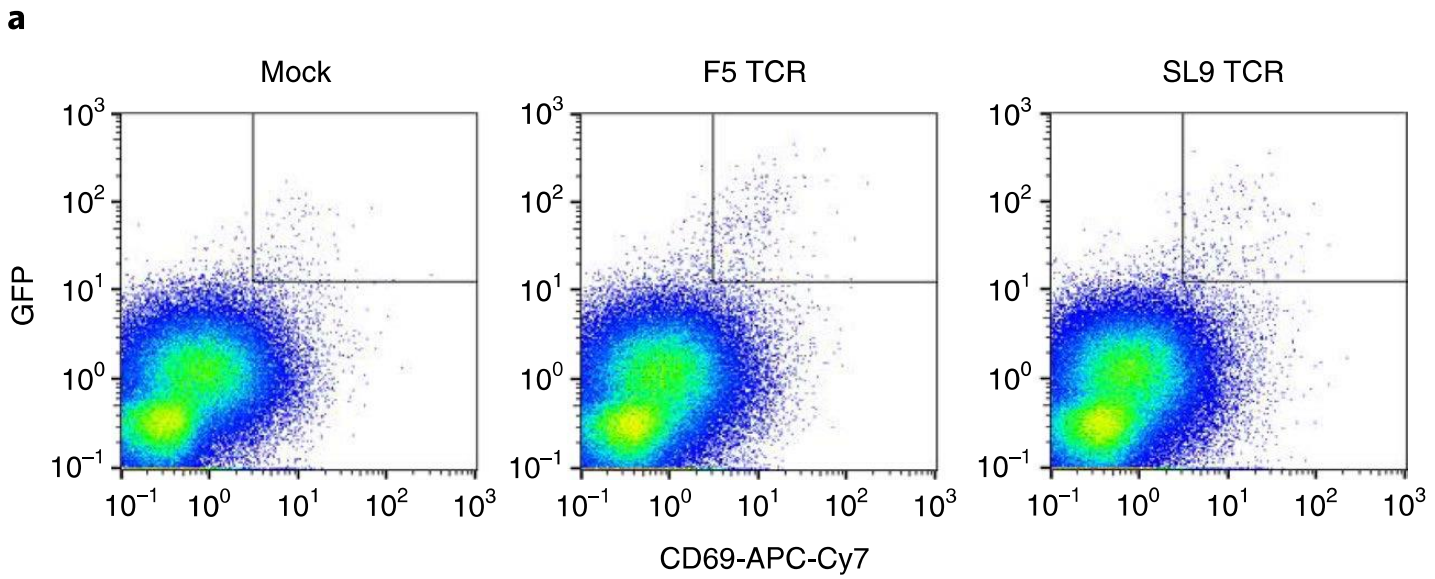
Alok V. Joglekar[1*], Michael T. Leonard[1], John D. Jeppson[1], Margaret Swift[1], Guideng Li[1,2,3], Stephanie Wong[1], Songming Peng[4], Jesse M. Zaretsky[5], James R. Heath[4,6], Antoni Ribas[5,6,7,8], Michael T. Bethune[1] and David Baltimore[1,6*]

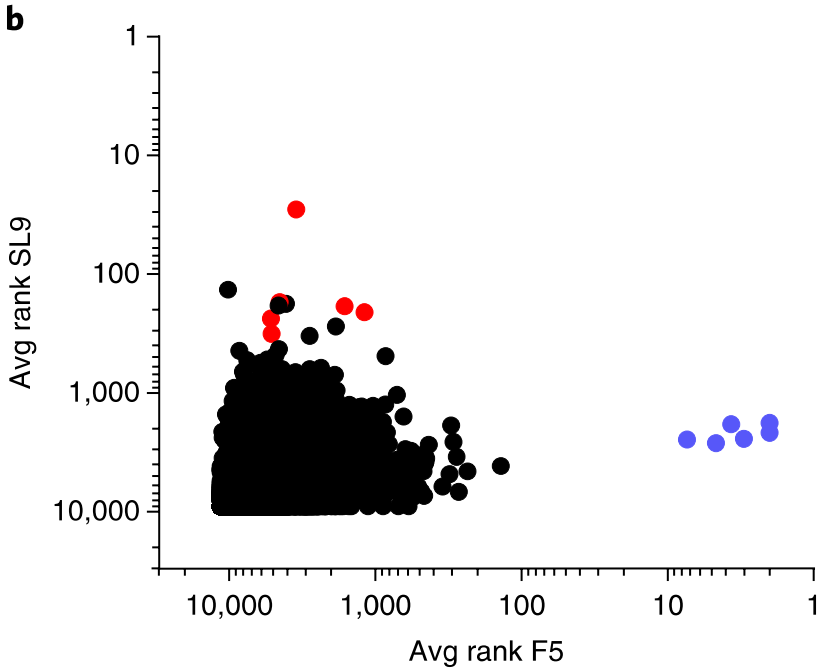




Signaling and antigen-presenting bifunctional receptors (SABRs)

Library size: 1X10^4

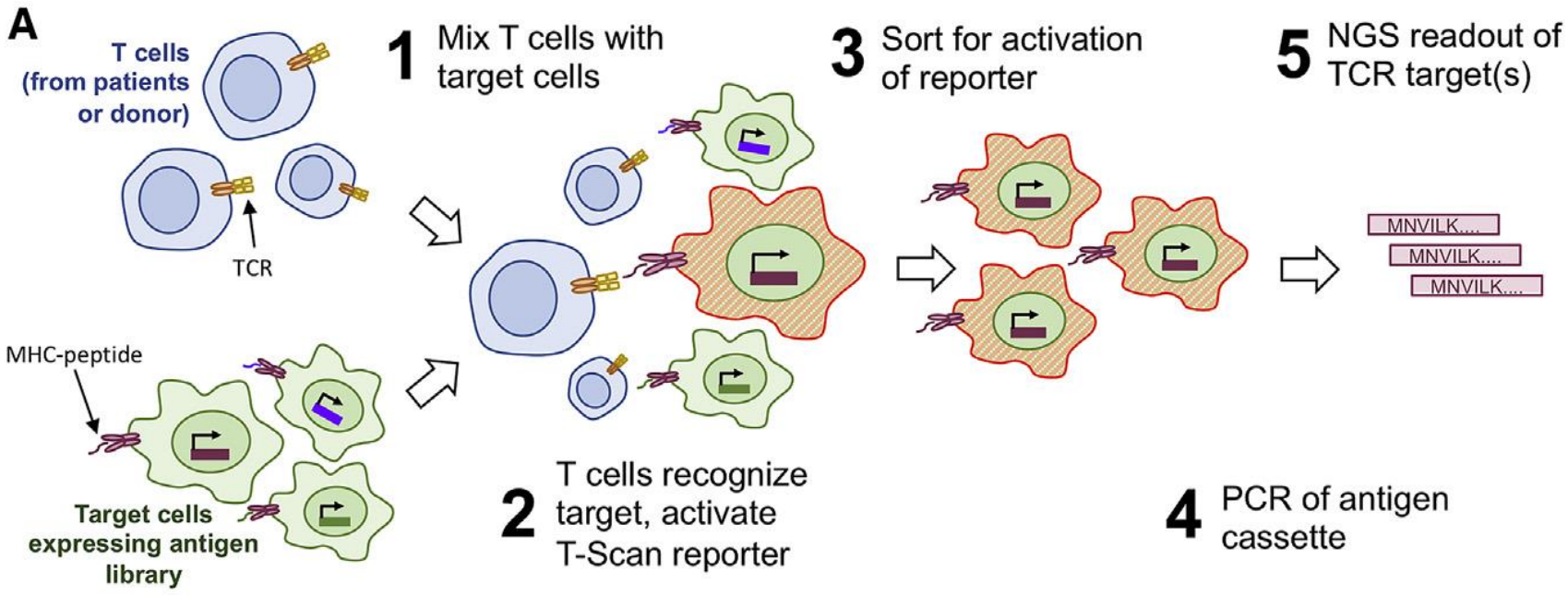


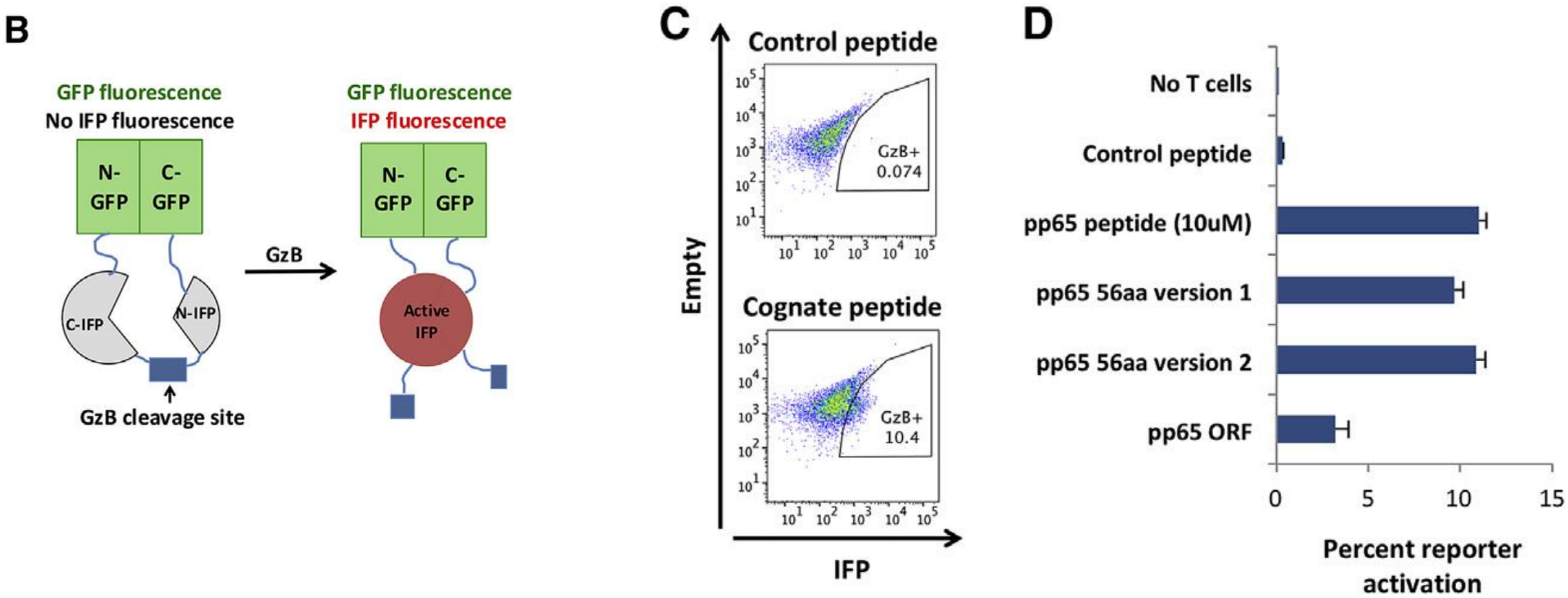


F5: ELAGIGILTV (MART-1)
SL9: SLYNTVATL (HIV gag)

T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes

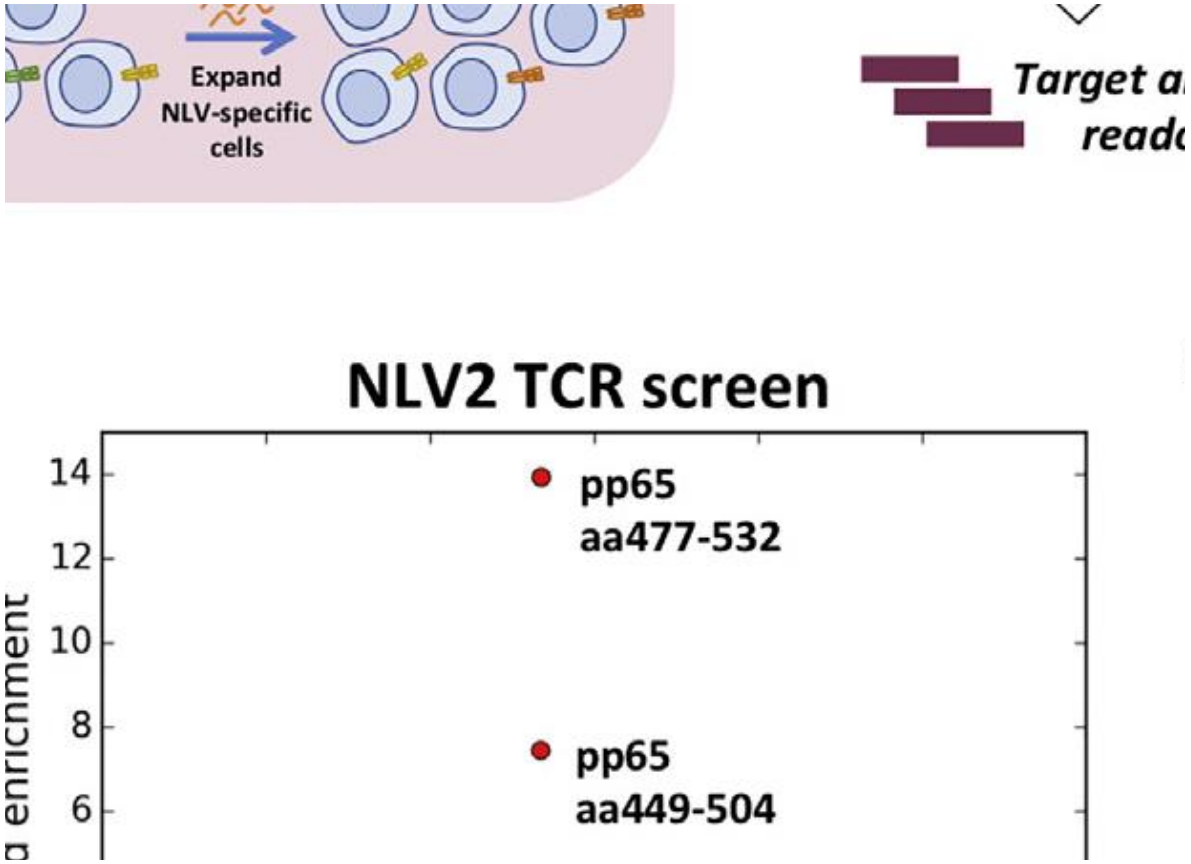
Tomasz Kula,[1,2] Mohammad H. Dezfulian,[1,2] Charlotte I. Wang,[1,2,3] Nouran S. Abdelfattah,[1,2] Zachary C. Hartman,[4] Kai W. Wucherpfennig,[5] Herbert Kim Lyerly,[6] and Stephen J. Elledge[1,2,7,*]



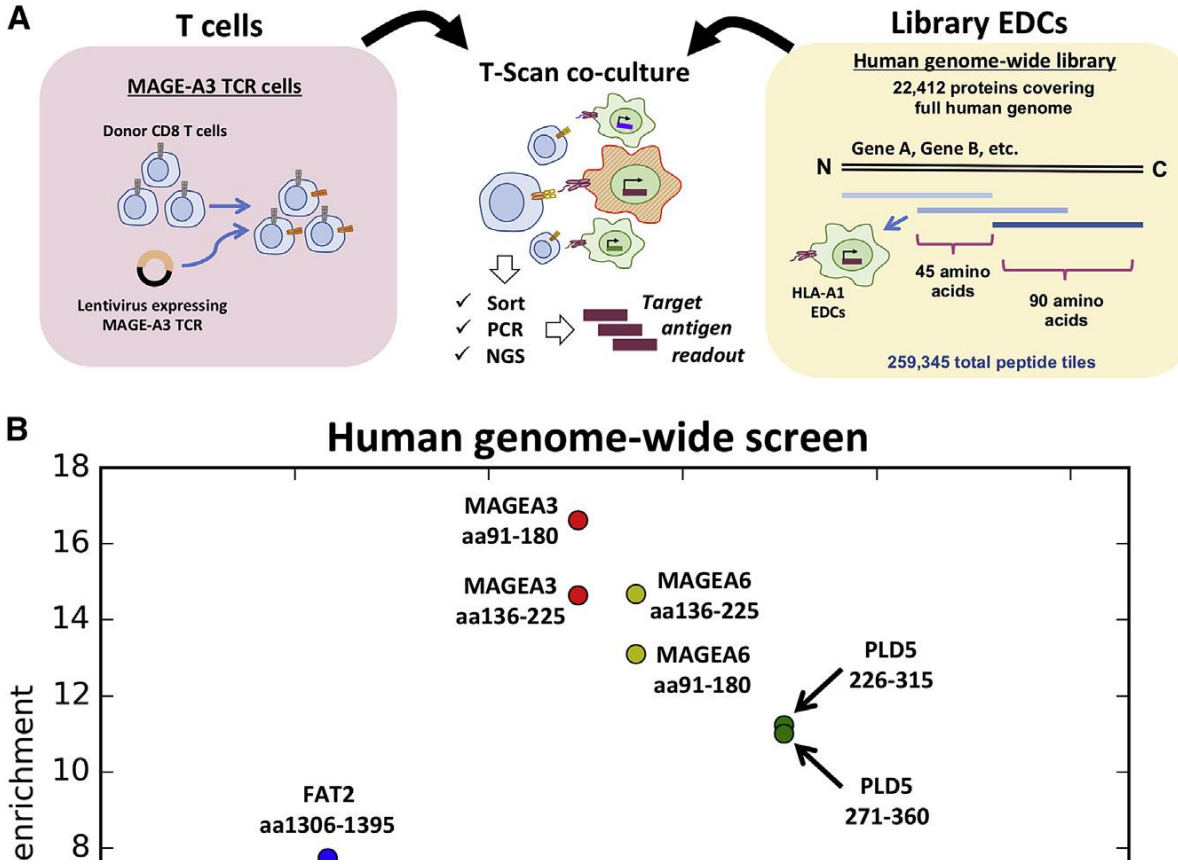

B
GFP fluorescence
No IFP fluorescence
N-GFP
C-GFP
C-IFP
N-IFP
GzB cleavage site
GzB
GFP fluorescence
IFP fluorescence
N-GFP
C-GFP
Active IFP
C
Control peptide
GzB+ 0.074
Empty
Cognate peptide
GzB+ 10.4
IFP
D
No T cells
Control peptide
pp65 peptide (10uM)
pp65 56aa version 1
pp65 56aa version 2
pp65 ORF
0
5
10
15
Percent reporter activation

T-Scan can identify antigens for viral and cancer specific TCRs

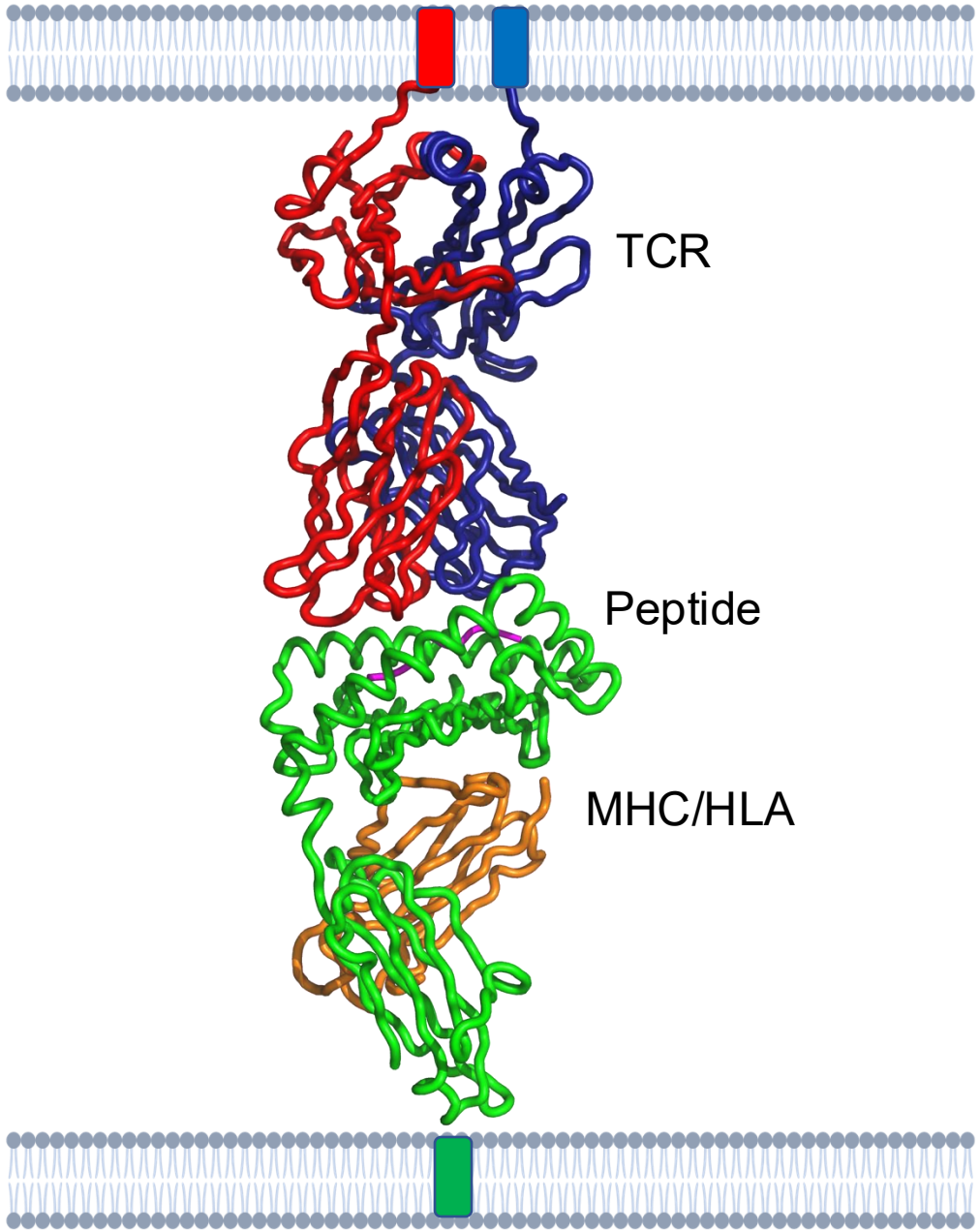
Library size: $5X10^3$

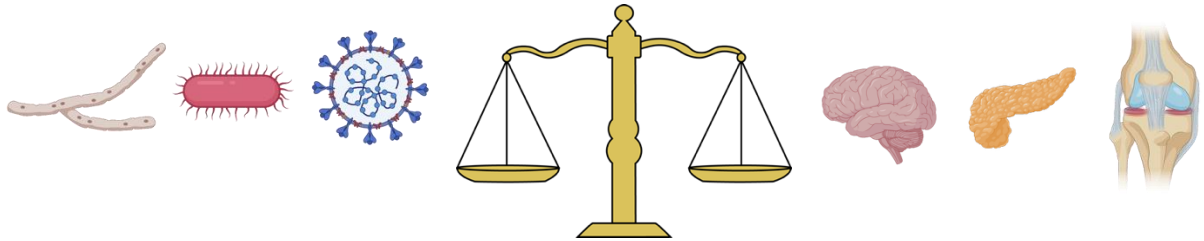


Library size: $2.5X10^5$



TCR Cross-reactivity could lead to autoimmune disease

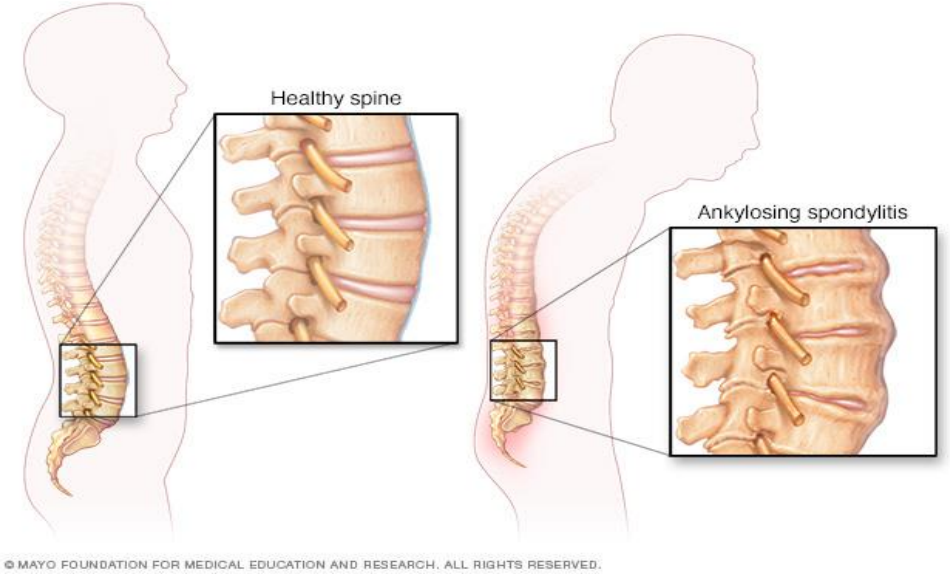




HLA alleles	Disease	Relative risk	Peptide antigen
HLA-B27	Ankylosing spondylitis	>90	Un-identified
HLA-DQ6	Narcolepsy	~50	HCRT
HLA-DQ2.5 HLA-DQ8	Celiac disease	~30	Gluten
HLA-DQ8	T1D	~14	Pro-Insulin; Hybrid peptide
HLA-DR4	Rheumatoid arthritis	~12	Citrullinated vimentin; Citrullinated fibrinogen
HLA-DR15	Multiple Sclerosis	~12	Myelin Basic Protein (MBP)
HLA-DR3	Systemic lupus erythematosus	~10	Histone

Dendrou et al. 2018. Nat. Rev. Immunol.

HLA-B27 is associated to ankylosing spondylitis (AS), a leading form of Spondylarthritis (SpA)



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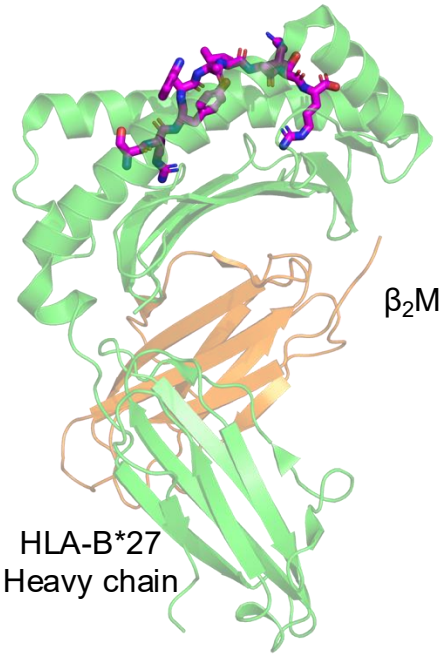
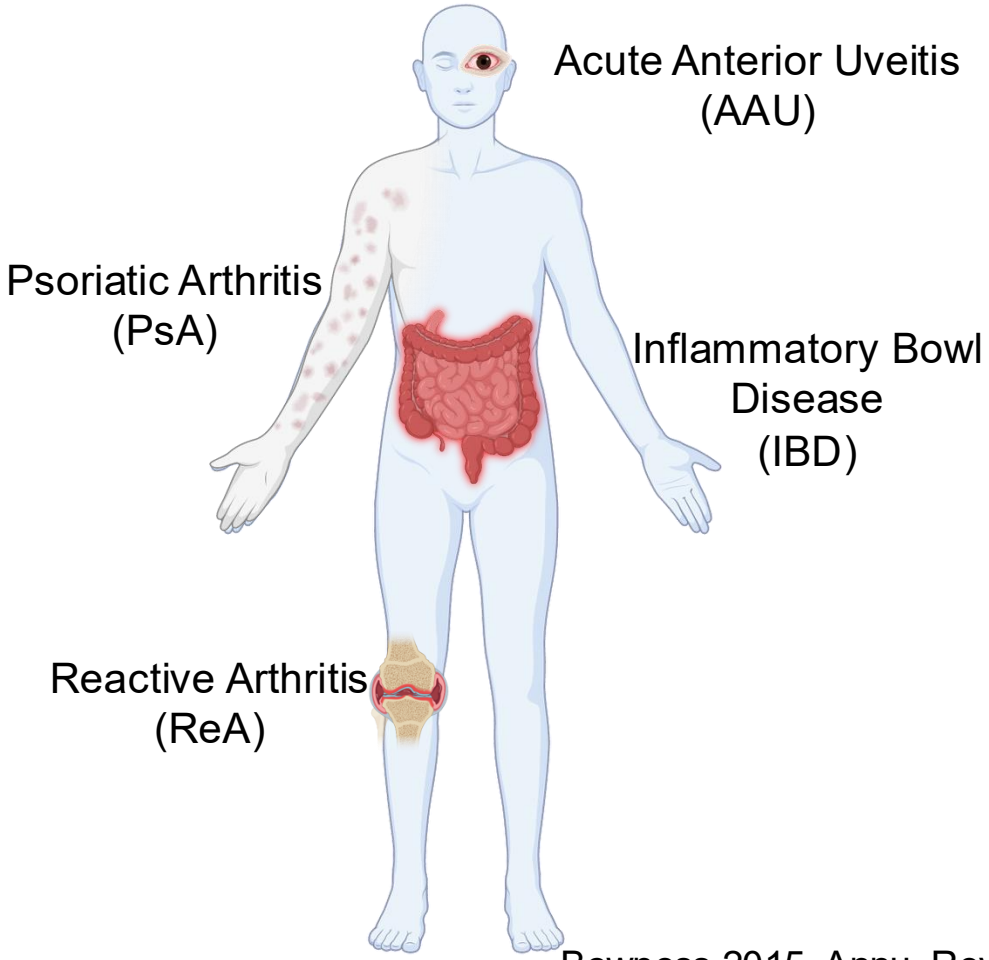
- Inflammatory back pain and other joint pain
- Symptoms usually strike early age of adulthood
- More common in men; underdiagnosed in women
- Treated by a rheumatologist not orthopedics doctor
- Treated with NSAIDs or biologcis



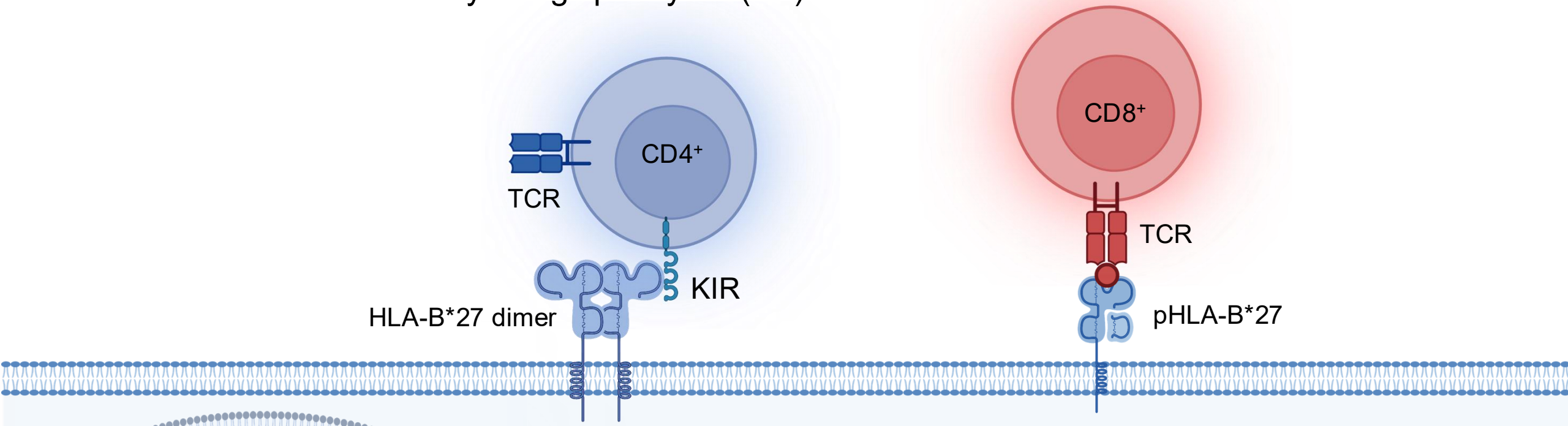
including the HL-A system, and the identification of the HL-A antigens is an essential part of tissue typing. In Caucasians it is now usually possible to identify four HL-A antigens, and these are believed to be genetically determined, probably transmitted by two related loci on each of a pair of chromosomes. Relationships between diseases and particular HL-A antigens are now being studied extensively. Associations have been reported in lymphoma (HL-A 5 and W 18),[4–8] multiple myeloma (W 18),[8] adult cœliac disease (HL-A 1 and 8),[9] systemic lupus erythematosus (HL-A 13 and W 17),[10–12] lymphoblastic leukæmia (HL-A 27),[13] and psoriasis (HL-A 13 and W 17).[14] No definite association has been established with rheumatoid disease.[15–17]

There are several reports of ankylosing spondylitis in 2 or more patients in the same family,[18–22] with a few instances of the disease in pairs of identical twins.[23] Hersh, Stecher, and their colleagues [13,19]



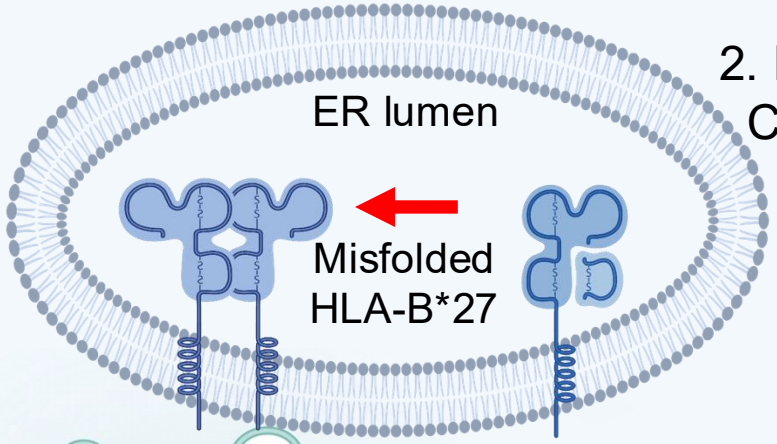
Bowness 2015. Annu. Rev. Immunol.

HLA-B*27 is associated to ankylosing spondylitis (AS)



2. HLA-B*27 FHC dimer hypothesis: CD4+ T cells recognize HLA-B*27 dimer via KIR

3. Arthritogenic peptide hypothesis: CD8+ T cells recognize self-antigens presented by HLA-B*27

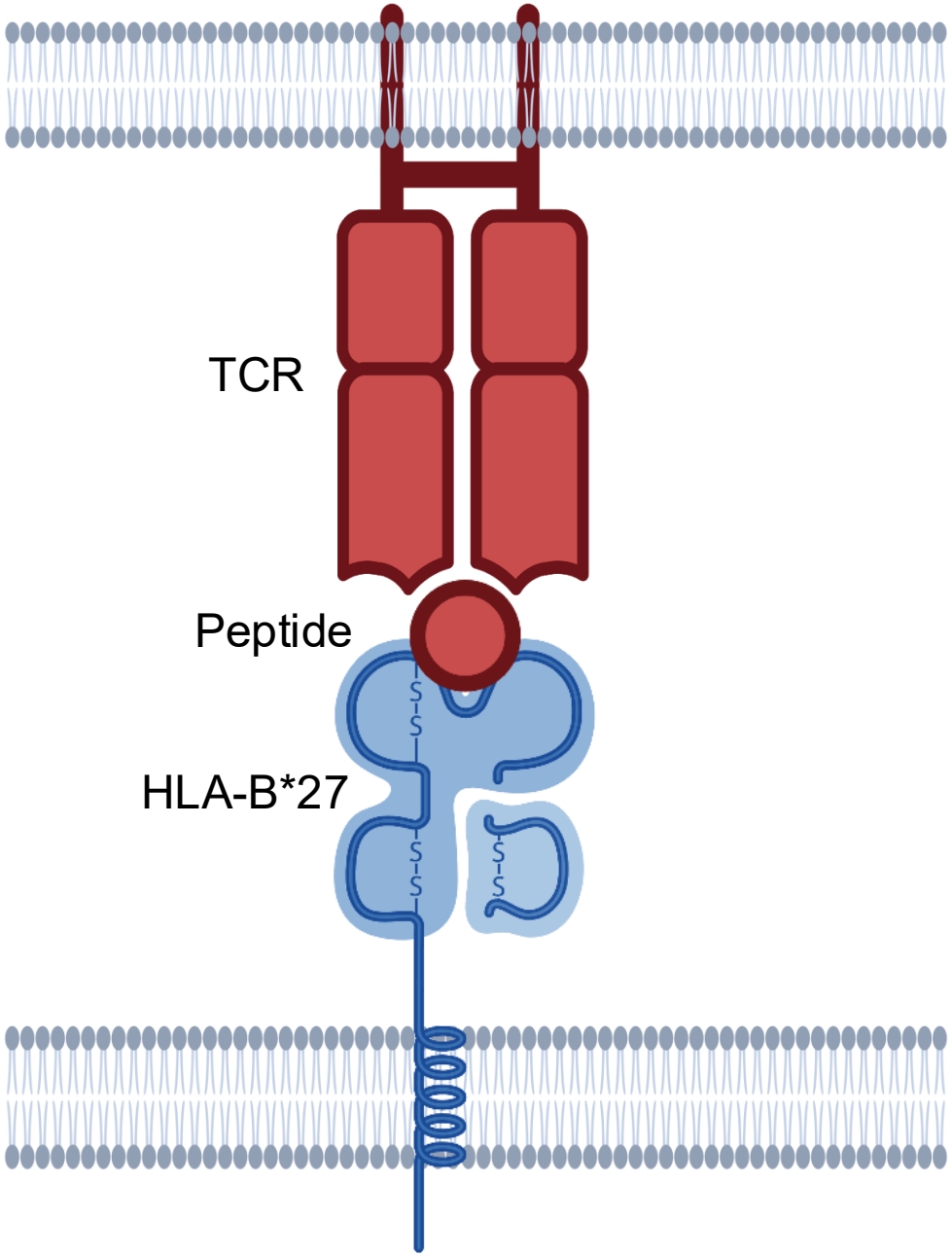


Not all HLA-B*27 alleles are predisposing to AS (B*2706; B*2709)

1. Misfolding hypothesis: Misfolded HLA-B*27 triggers unfolded protein response

Bowness 2015. Annu. Rev. Immunol.
Mauro et al. 2021. Nat. Rev. Rheumatol.

Identification of AS related ‘public’ TCRs



Arthritogenic peptide hypothesis

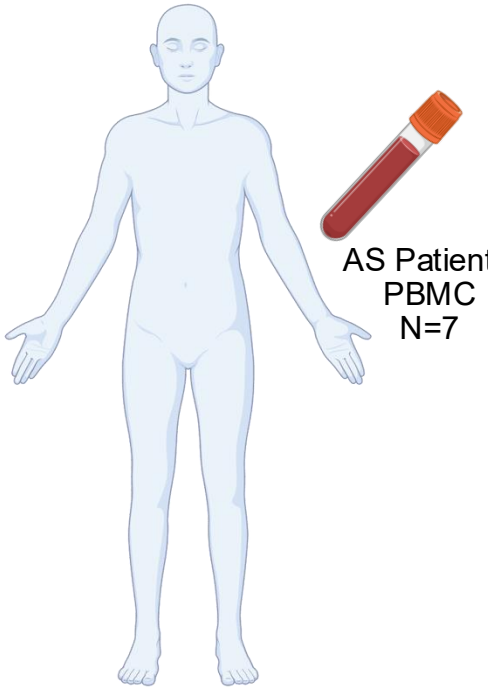
- **What are the disease relevant TCRs?**
- **What are the peptide ligands for disease relevant TCRs?**

	Vβ	CDR3β	Jβ
Clone1	TRBV9	CASSVGLYSTDTQ	TRBJ2-3
Clone2	TRBV9	CASSVGLFSTDTQ	TRBJ2-3

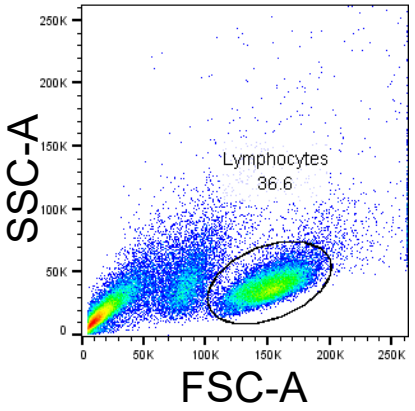
Faham et al. 2017. Arthritis & Rheumatology
Komech et al. 2018. Rheumatology
Zheng et al. 2019. eBioMedicine
Hanson et al. 2020. Arthritis & Rheumatology

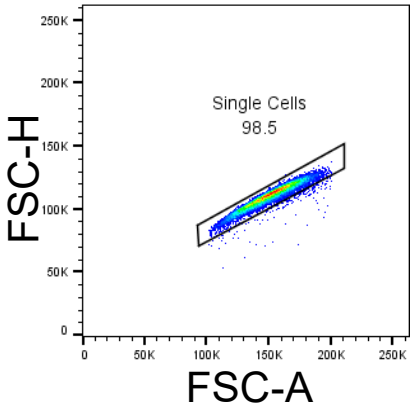
Dulphy et al. 1999. Journal of Immunology
May et al. 2002. Tissue Antigens

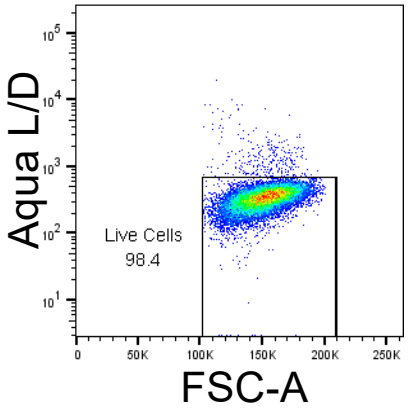
Isolation and sequencing AS related ‘public’ TCRs

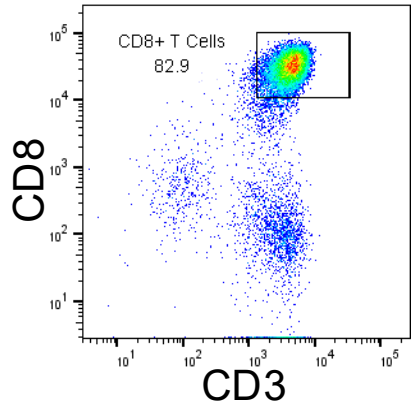


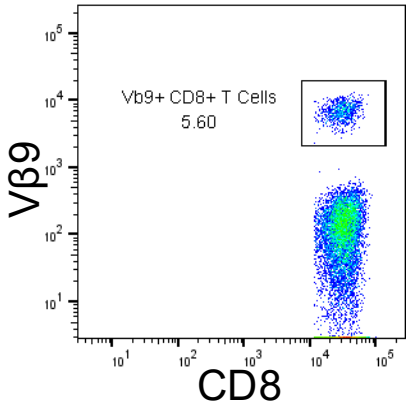
AS Patients’
PBMC
N=7

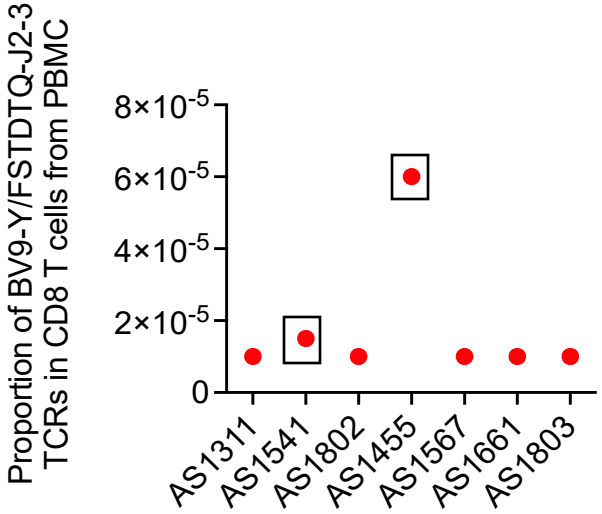








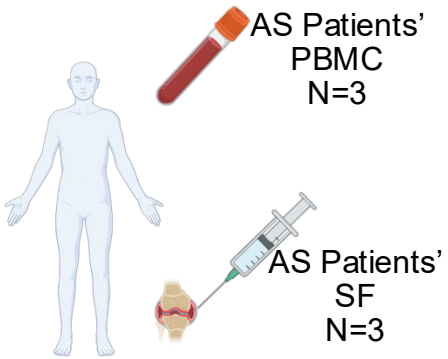


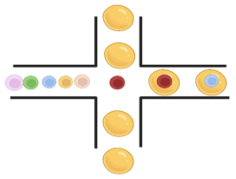


	Vβ	CDR3β	Jβ	Vα	CDR3α	Jα	Expression
AS3.1	TRBV9	CASSVGLYSTDTQ	TRBJ2-3	TRAV21	AVSLGTGAGSYQLT	TRAJ28	Yes
AS4.1	TRBV9	CASSVGLYSTDTQ	TRBJ2-3	TRAV21	AVSSPQGGSEKLV	TRAJ57	Yes
AS4.2	TRBV9	CASSVGLFSTDTQ	TRBJ2-3	TRAV21	AVLSPVQETSGSRLT	TRAJ18	Yes
AS4.3	TRBV9	CASSVATYSTDTQ	TRBJ2-3	TRAV21	AVSNFNKFY	TRAJ21	Yes
AS4.4	TRBV9	CASSVGLYSTGELF	TRBJ2-2	TRAV21	AVSFFDKLI	TRAJ34	Yes

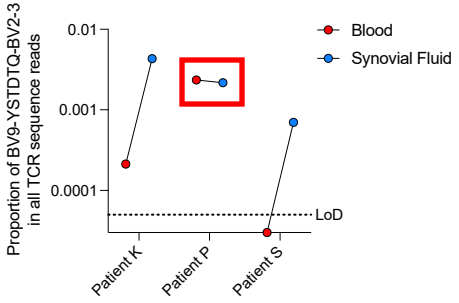
Geraldine Gillespie Group at Oxford

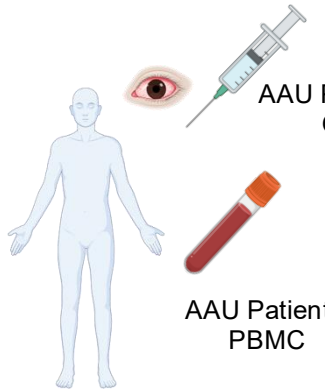
AS TCRs isolated from patients' synovial fluid and AAU TCRs isolated from patients' ocular fluid

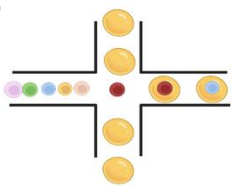




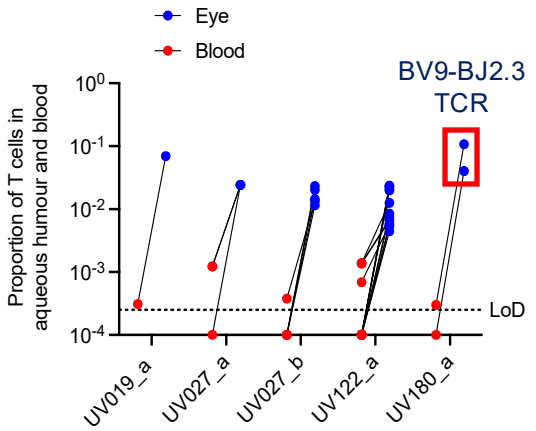
10X Genomics







10X Genomics



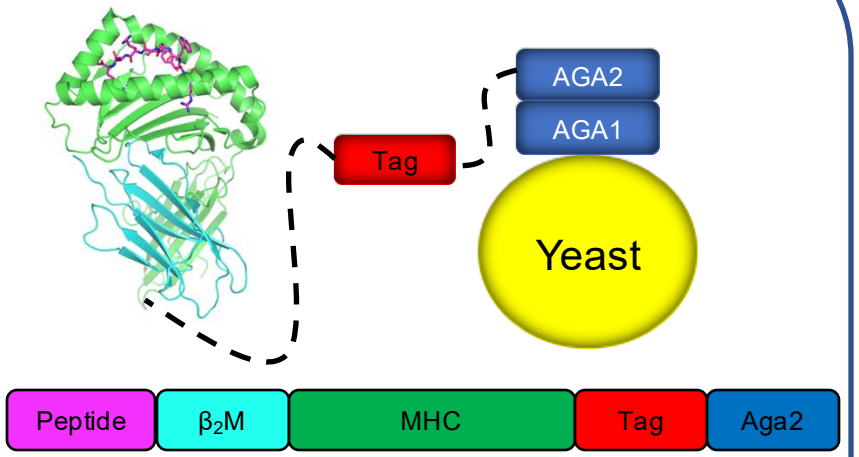
	Source	Vβ	CDR3β	Jβ	Vα	CDR3α	Jα
AS8.2	Synovial fluid	TRBV9	CASSVGLYSTDTQYF	TRBJ2-3	TRAV21	CAVPNQAGTALIF	TRAJ15
AS8.3	Synovial fluid	TRBV9	CASSVGLYSTDTQYF	TRBJ2-3	TRAV21	CAATSPRRQGGSEKLVF	TRAJ57
AS8.4	Synovial fluid	TRBV9	CASSVGTYSTDTQYF	TRBJ2-3	TRAV21	CAVNSPGSGAGSYQLTF	TRAJ28
AS8.5	Synovial fluid	TRBV9	CASSVATYSTDTQYF	TRBJ2-3	TRAV21	CAVMDQDGANSKLTF	TRAJ56
AS9.1	Synovial fluid	TRBV9	CASSVGLYSTDTQYF	TRBJ2-3	TRAV21	CAVLSQTGANSKLTF	TRAJ56
AS9.2	Synovial fluid	TRBV9	CASSVATYSTDTQYF	TRBJ2-3	TRAV21	CAADSGSARQLTF	TRAJ22

	Source	Vβ	CDR3β	Jβ	Vα	CDR3α	Jα
AU1.1	Ocular fluid	TRBV9	CASSVATYSTDTQYF	TRBJ2-3	TRAV21	CAVMGTTDSWGKLQF	TRAJ24
AU1.2	Ocular fluid	TRBV9	CASSVATYSTDTQYF	TRBJ2-3	TRAV21	CATYNFNKFYF	TRAJ21
AU1.3	Ocular fluid	TRBV9	CASSPGLYSTDTQYF	TRBJ2-3	TRAV21	CAVRPSDSWGKLQF	TRAJ56
AU2.1	Ocular fluid	TRBV9	CASSVGLYSTDTQYF	TRBJ2-3	TRAV21	CAVGEGEGGGFKTIF	TRAJ9
AU2.2	Ocular fluid	TRBV9	CASSVGLYSTDTQYF	TRBJ2-3	TRAV21	CAASSTQGGSEKLVF	TRAJ57

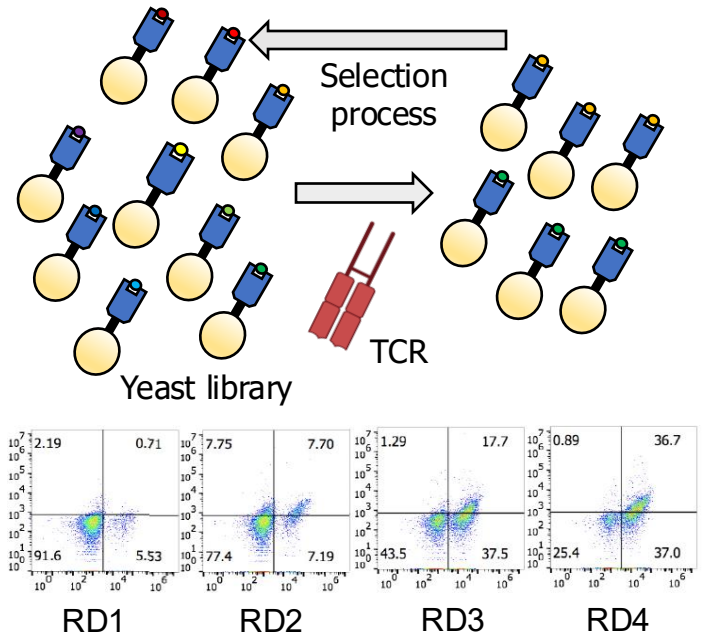
Ivan Zvyagin group RAS & Yokoyama group WUSTL

Overview of yeast display pMHC library

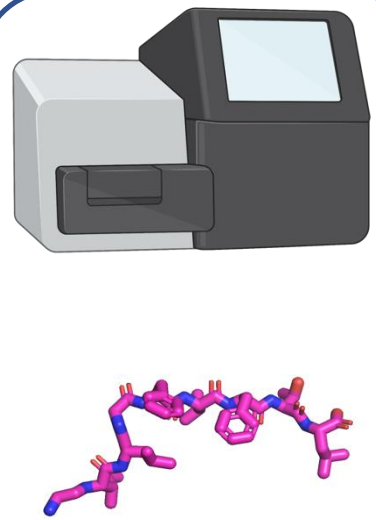
- No prior information was available on antigens
- Predicted low potency as auto-reactive TCR
- No tissue samples are available
- The novel cell-based antigen discovery tools are not available



Yeast library construction



Yeast library selection

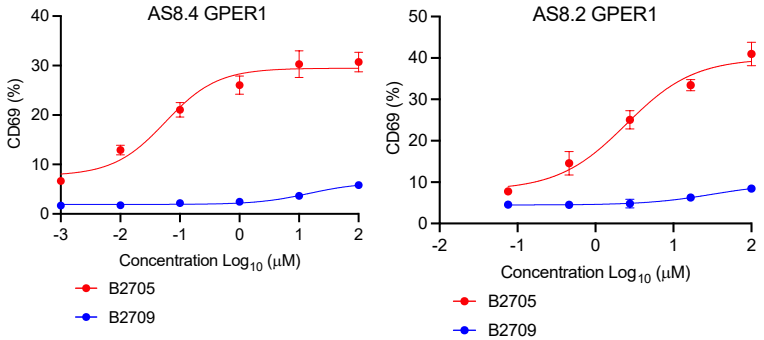


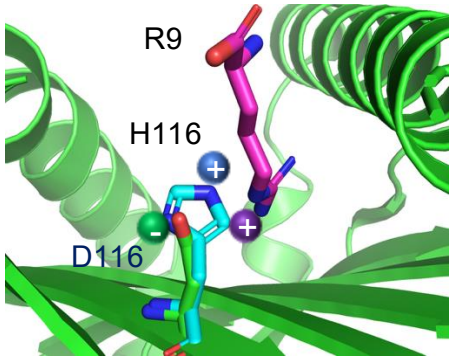
Peptide ID

Birnbaum et al., Cell 2014
Gee et al., Cell 2018

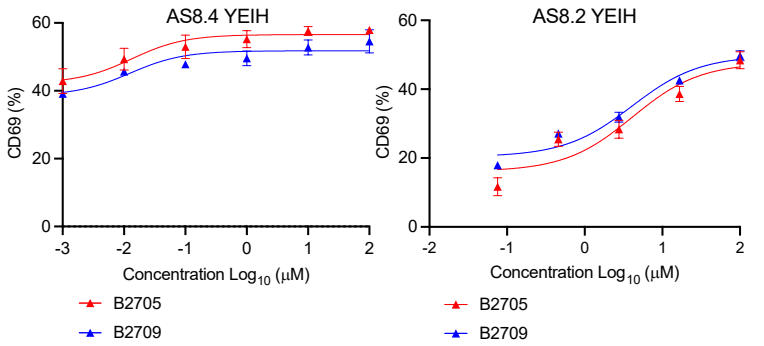
Self and microbial peptides are identified to activate AS and AAU TCRs

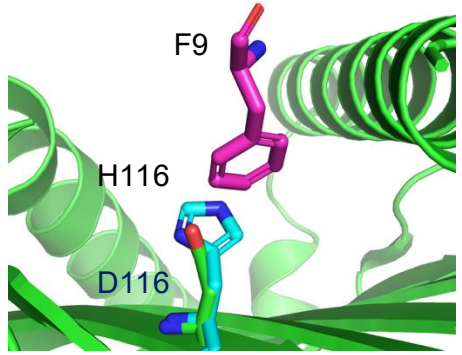
	AS 3.1	AS 4.1	AS 4.2	AS 4.3	AS 4.4	AS 8.2	AS 8.3	AS 8.4	AS 8.5	AS 9.1	AS 9.2	AU 2.1	AU 1.2
PRPF3	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓
JAK3				✓									
GPER1	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓
HELQ			✓	✓									
IPO9				✓									
GLRB				✓								✓	
RNASEH 2B			✓	✓		✓	✓		✓	✓	✓	✓	✓
MPP4				✓								✓	
SEC14L2				✓	✓								
UvrABC			✓										
YEIH	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓
gspD				✓		✓	✓		✓	✓	✓	✓	✓





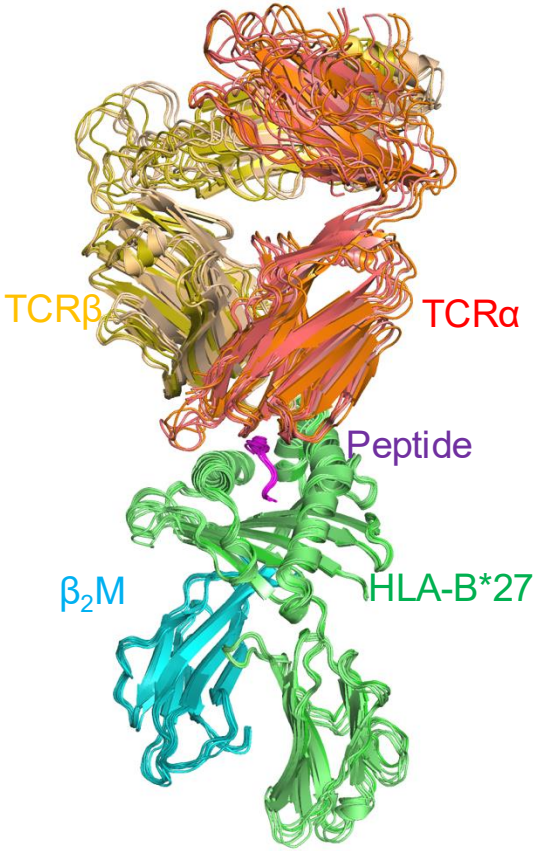
GPER:GQMWLLAPR

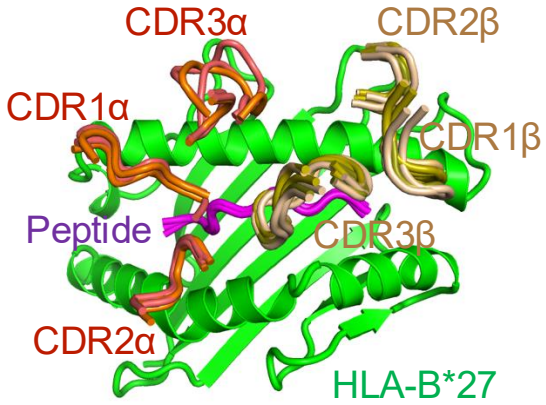


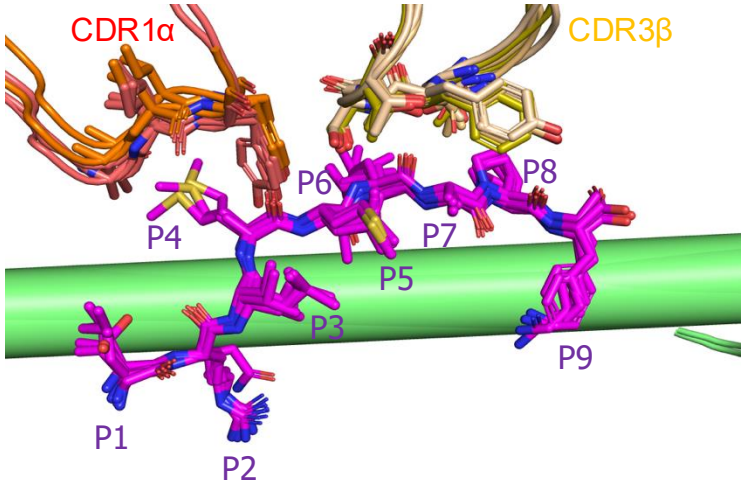


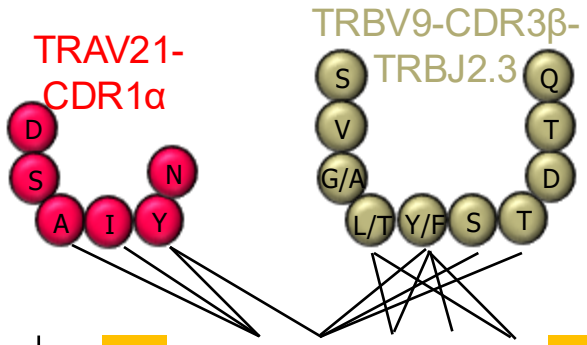
YEIH: LRVMMLAPF

Identical AS TCR structural solution for p-HLA-B27 recognition

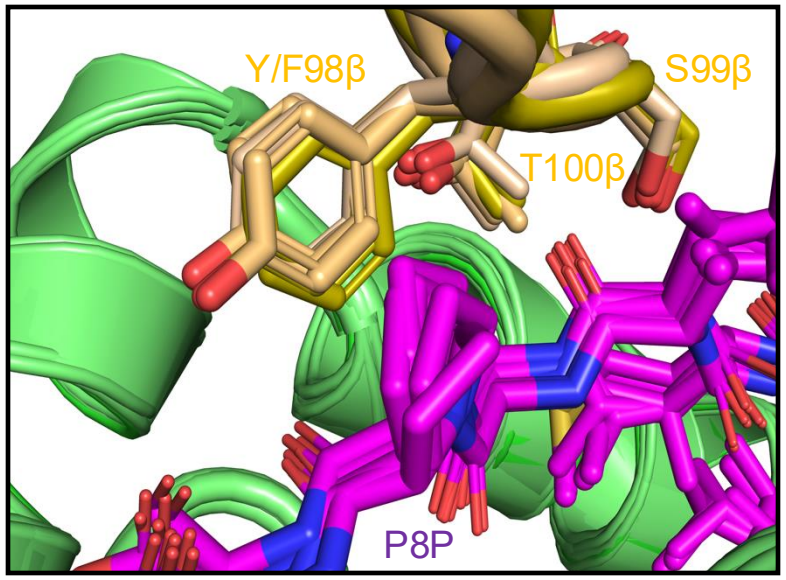






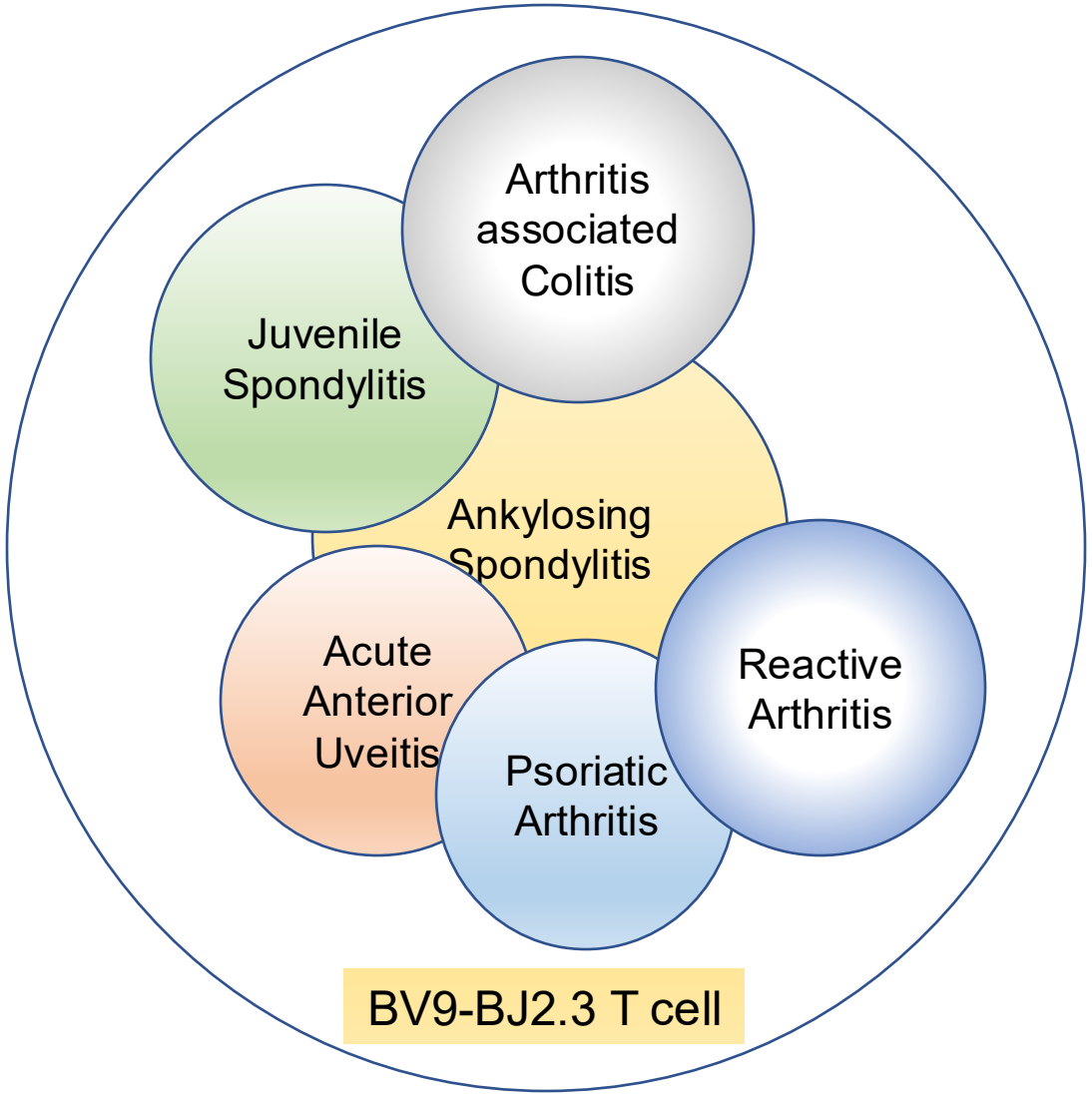


	P1	P2	P3	P4	P5	P6	P7	P8	P9
PRPF3	T	R	L	A	L	I	A	P	K
GPER1	G	Q	M	W	L	L	A	P	R
RNASEH2B	G	Q	V	M	V	V	A	P	R
HELQ	R	R	V	I	L	R	A	P	Y
IPO9	T	Q	M	P	L	V	A	P	V
GLRB	V	Q	V	M	L	N	N	P	K
JAK3	D	R	Q	Q	L	P	A	P	K
SLEC14L2	G	R	V	G	D	L	S	P	R
YEIH	L	R	V	M	M	L	A	P	F
gspD	G	K	T	E	L	L	A	P	F



- CDR3β (SVGLY/FSTDTQ) Y/F docked into the hydrophobic pocket between P8 and HLA-B*27 α2 helix.
- CDR3α is not used for peptide recognition.
- CDR1α is used for peptide recognition.
- **'Molecular mimicry' is the underlying mechanism for TCR cross-reactivity**

Could BV9-BJ2.3 T cells play a central role to initiate Spondyloarthrities (SpA)?



- Can we generate B*27 Tg Mice with TCR tg mice to mimic SpA?
- Can we specifically target BV9-BJ2.3 T cells as potential therapeutics?

Targeted therapy for treating SpA patients

Brief Communication

https://doi.org/10.1038/s41591-023-02613-z

Targeted depletion of TRBV9$^+$ T cells as immunotherapy in a patient with ankylosing spondylitis

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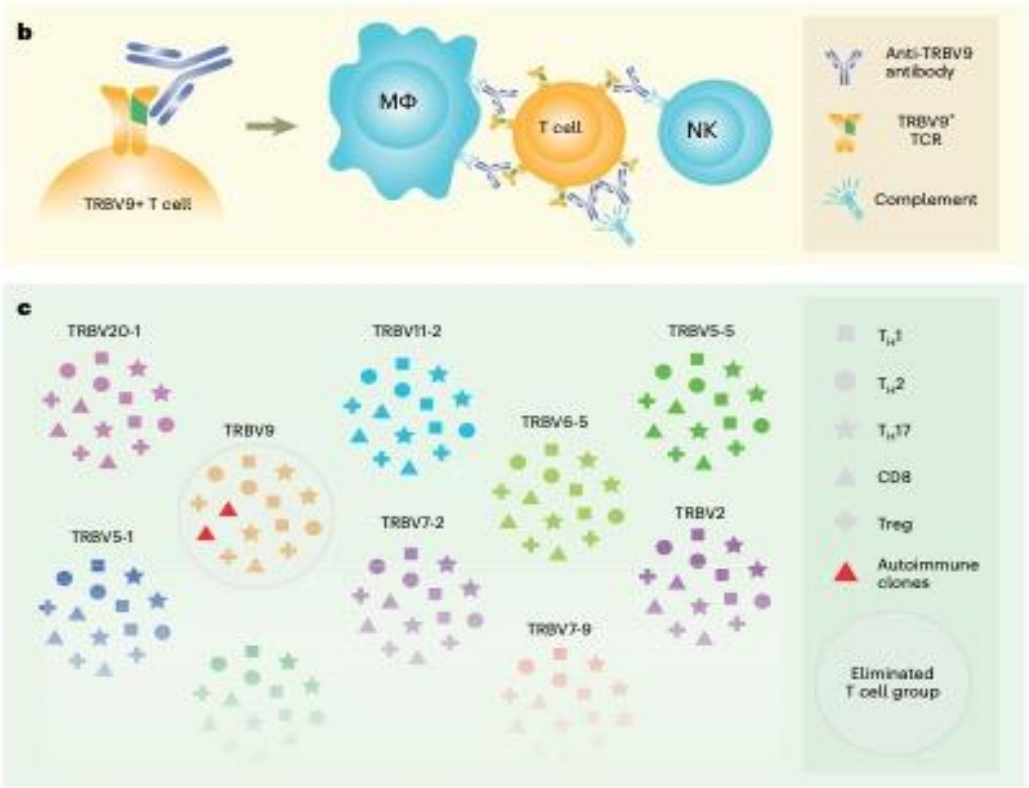
Olga V. Britanova[1,2,11], Kseniia R. Lupyr[1,3,11], Dmitry B. Staroverov[1,2,11], Irina A. Shagina[1,2,11], Alexey A. Aleksandrov[4], Yakov Y. Ustyugov[4], Dmitry V. Somov[1], Alesia Klimenko[1], Nadejda A. Shostak[1], Ivan V. Zvyagin[1,2], Alexey V. Stepanov[2], Ekaterina M. Merzlyak[1,2], Alexey N. Davydov[5,6], Mark Izraelson[6], Evgeniy S. Egorov[2,9], Ekaterina A. Bogdanova[1], Anna K. Vladimirova[4], Pavel A. Iakovlev[4], Denis A. Fedorenko[7], Roman A. Ivanov[4], Veronika I. Skvortsova[1,10], Sergey Lukyanov[1,2] & Dmitry M. Chudakov[1,2,5,8]

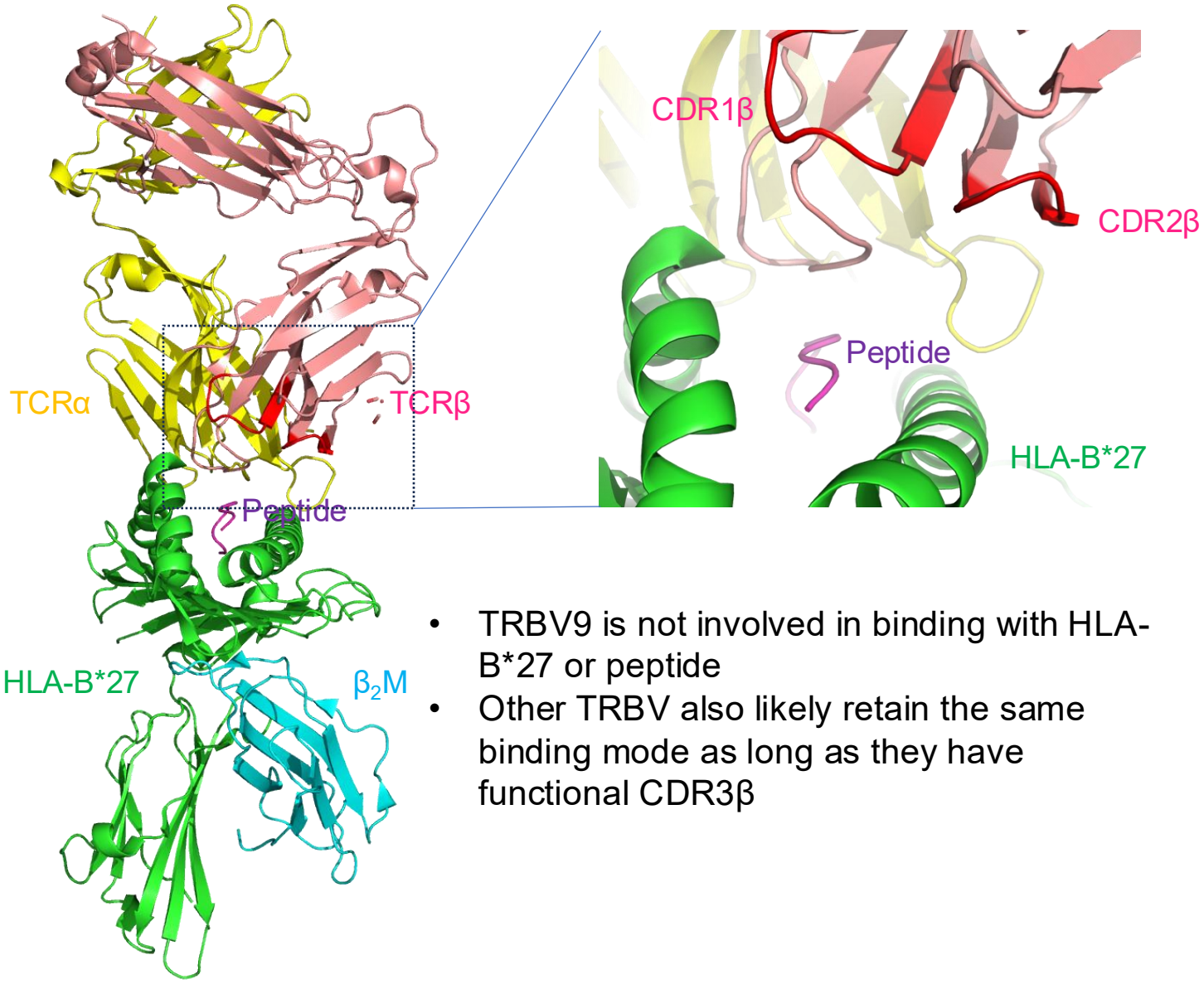


Mucosal signatures of pathogenic T cells in HLA-B*27$^+$ anterior uveitis and axial spondyloarthritis

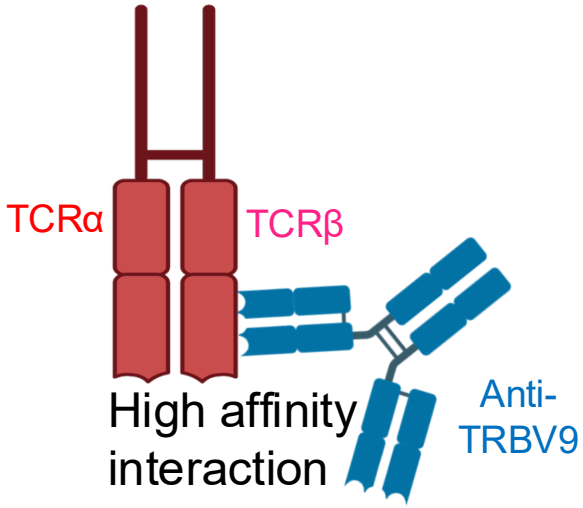
Michael A. Paley,[1] Xinbo Yang,[2] Lynn M. Hassman,[3] Frank Penkava,[4] Lee I. Garner,[5,6] Grace L. Paley,[3] Nicole Linskey,[1] Ryan Agnew,[1] Paulo Henrique Arantes de Faria,[1] Annie Feng,[1] Sophia Y. Li,[1] Davide Simone,[4] Elisha D.O. Roberson,[1,7] Philip A. Ruzycki,[3,7] Ekaterina Esaulova,[8] Jennifer Laurent,[1] Lacey Feigl-Lenzen,[1] Luke E. Springer,[1] Chang Liu,[8] Geraldine M. Gillespie,[5,6] Paul Bowness,[4] K. Christopher Garcia,[2,9] and Wayne M. Yokoyama[1,10]

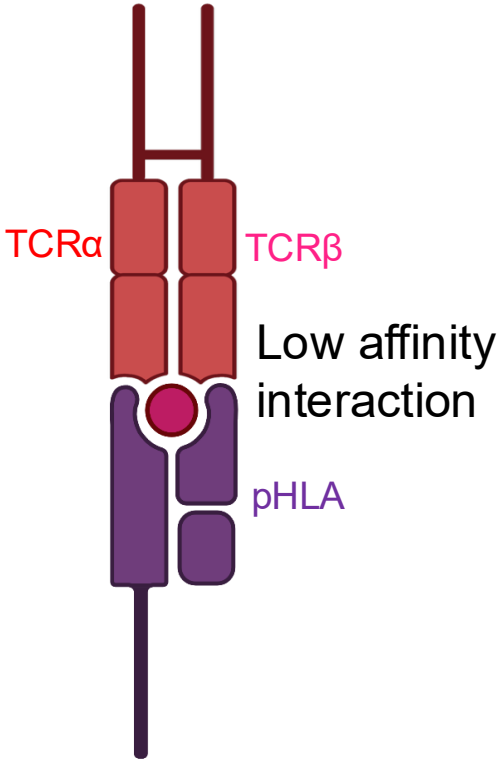
TRBV	CDR3β	TRAV	CDR3α
TRBV9	CASSVATYSTDTQYF	TRAV21	CAVYNFNKFYF
TRBV5-5	CASSLGLYSTMEQYF	TRAV21	CAVSGGSNYKLTF
TRBV9	CASSVALFSTDTQYF	TRAV21	CAVSSATGANSKLTF
TRBV9	CASSVGTYSTDTQYF	TRAV21	CAVTSFSAGAGSYQLTF
TRBV9	CASSVGLYSTDTQYF	TRAV21	CAASLPQGGSEKLVF
TRBV9	CASSVATYSTDTQYF	TRAV21	CAVTLSSGGSNYKLTF
TRBV9	CASSVGLYSTDTQYF	TRAV21	CAATSTQGGSEKLVF
TRBV5-5	CASSFGLYSTYEQYF	TRAV21	CAVGYSGAGSYQLTF
TRBV9	CASSVGLFSTDTQYF	TRAV21	CAVSLGVEGGSEKLVF
TRBV5-4	CASSTGLYSTDTQYF	TRAV21	CAVGVSGGSNYKLTF
TRBV9	CASSVGLFSTDTQYF	TRAV21	CAVTLGLLKSETSGSRLTF
TRBV9	CASSVGLFSTDTQYF	TRAV21	CAVGAAFSDGQKLLF
TRBV5-4	CASSTGLYSTDTQYF	TRAV21	CAALRPITGTASKLTF
TRBV9	CASSSGLYSTDTQYF	TRAV21	CAVESQSGANSKLTF
TRBV9	CASSVGLYSTDTQYF	TRAV21	CAVDNQGGKLIF
TRBV9	CASSVGLYSTDTQYF	TRAV21	CAVGEGEGGGFKTIF
TRBV9	CASSPGLYSTDTQYF	TRAV21	CAVRPSDSWGKLQF
TRBV9	CASSVGLYSTDTQYF	TRAV21	CAASSTQGGSEKLVF
TRBV7-3	CASSLGLYSTDTQYF	TRAV21	CAVKGFGNVLHC
TRBV9	CASSVATYSTDTQYF	TRAV21	CAVMGTTDSWGKLQF

Targeted therapy for treating SpA patients



- TRBV9 is not involved in binding with HLA-B*27 or peptide
- Other TRBV also likely retain the same binding mode as long as they have functional CDR3β





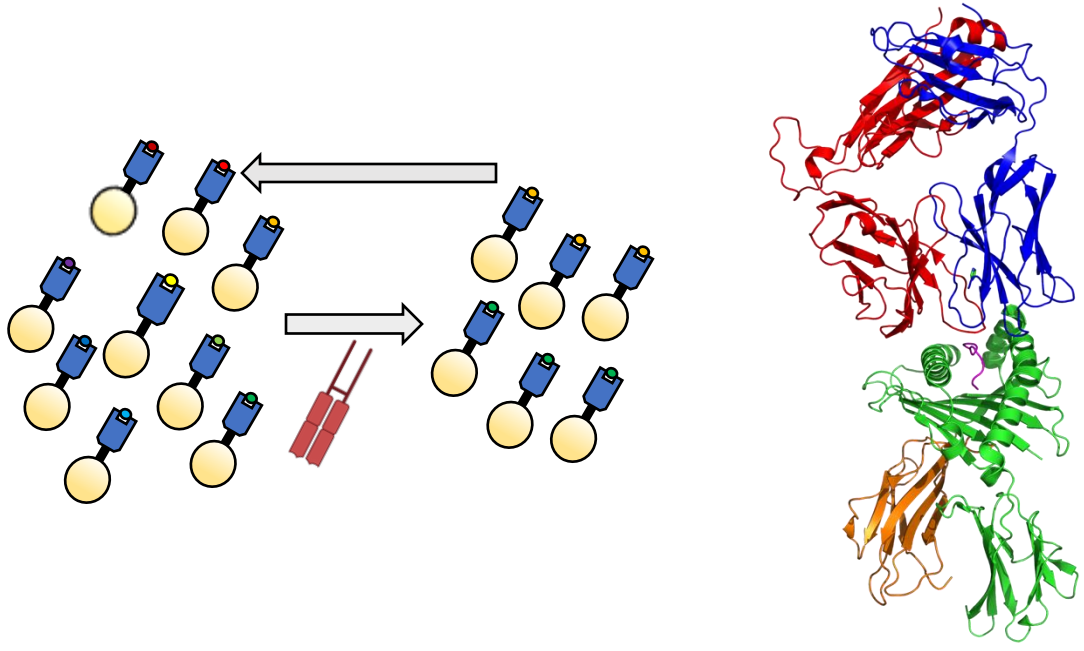
Antigen identification for ‘MHC-I-opathy’ and future therapeutic development

Autoimmune disease antigen ID

HLA alleles	Disease	Relative risk	Peptide antigen
HLA-B27	Ankylosing spondylitis/ Anterior Uveitis	>90	GPER1; PRPF3; RNASEH2B
HLA-B51	Behcet’s disease	>90	Un-identified
HLA-B29	Birdshot Uveitis	>90	Un-identified
HLA-C06/ HLA-B27	Psoriatic arthritis	>70	Un-identified
HLA-C06	Psoriasis	>70	LL37; ADAMTSL5
HLA-B15/ HLA-A31	Carbamazepine SJS/TEN	>70	Un-identified
HLA-B58	Allopurinol SJS/TEN	>70	Un-identified

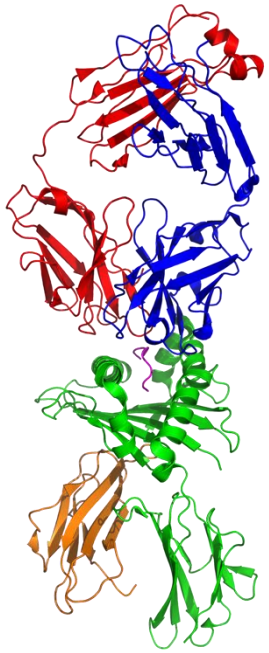
Identifying antigen: what's next?

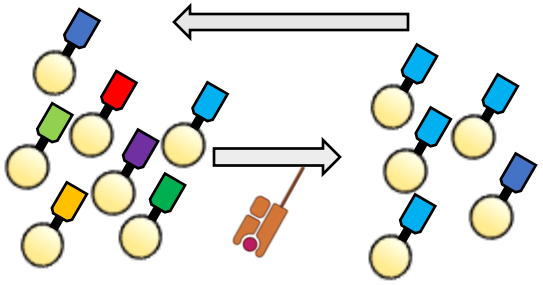
Identifying TCR ligand

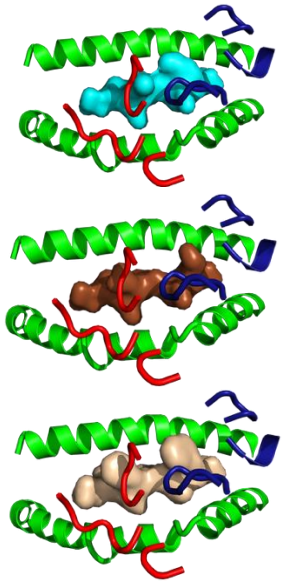


- What do T cells recognize in certain disease?

Engineering TCRm Ab







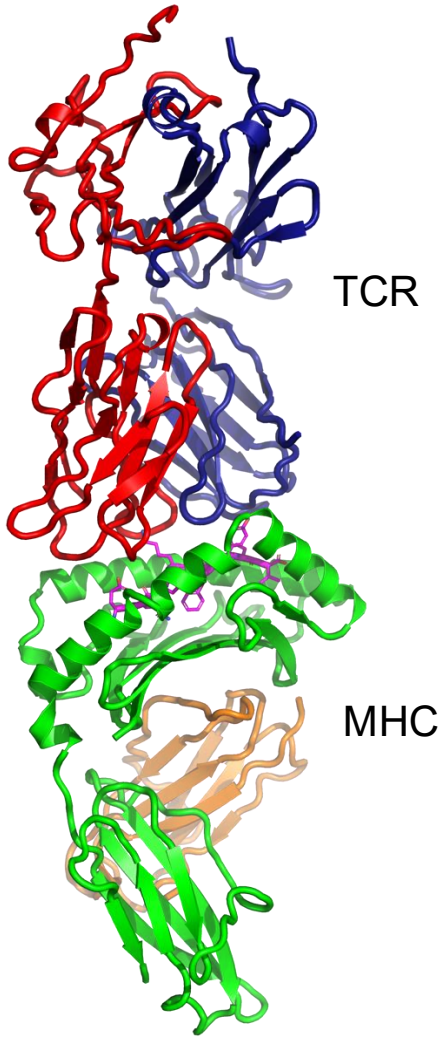
- Can we rapidly isolate therapeutic TCRm Abs?
- Can the TCRm format for ADCC, BiTE and CAR-T

From TCR-to-TCR mimic Abs

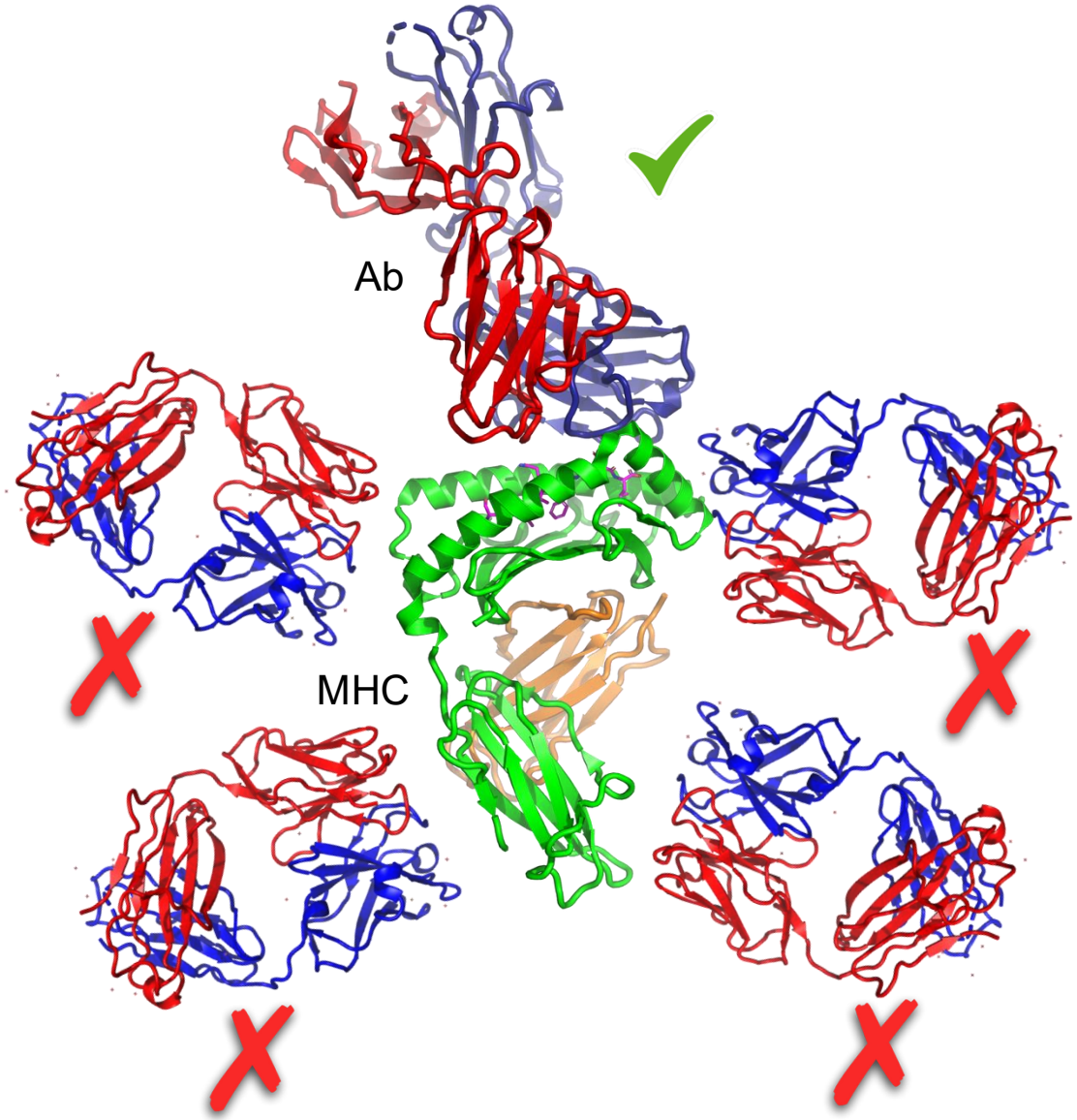
Cell surface antigen

Intracellular antigen

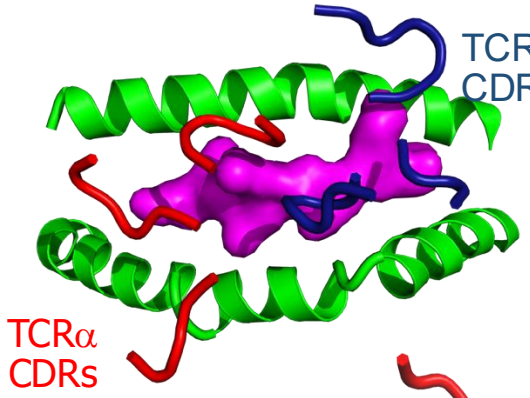
TCRs are ‘MHC-restricted’



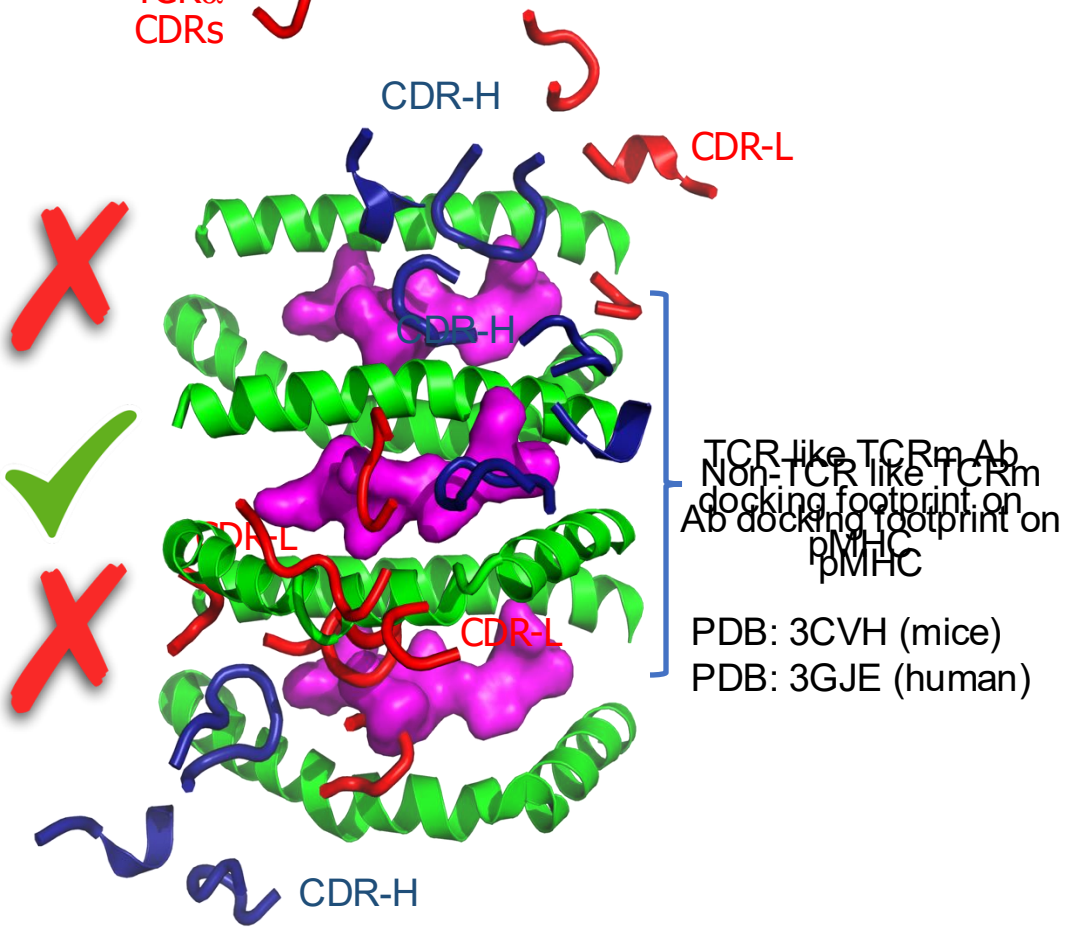
Abs are not ‘MHC-restricted’



Strategy to repurpose TCR mimic Abs



Classical TCR docking footprint on pMHC

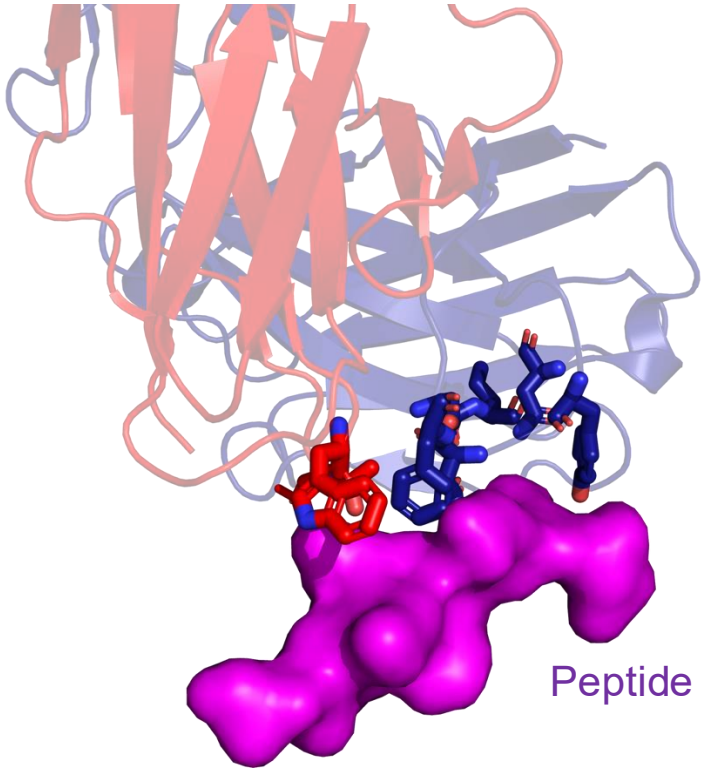


TCR-like TCRm Ab docking footprint on pMHC

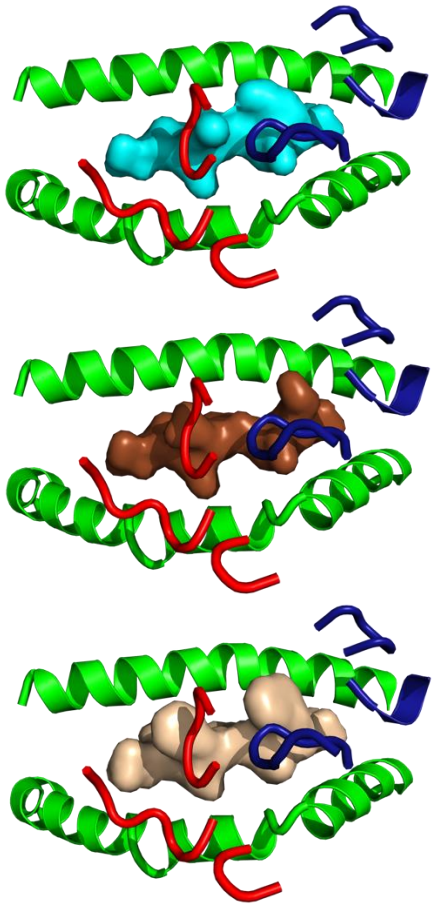
Non-TCR like TCRm Ab docking footprint on pMHC

PDB: 3CVH (mice)
PDB: 3GJE (human)

'MHC-restricted' TCRm Ab: scFv library focused only on peptide-contacting CDR residues

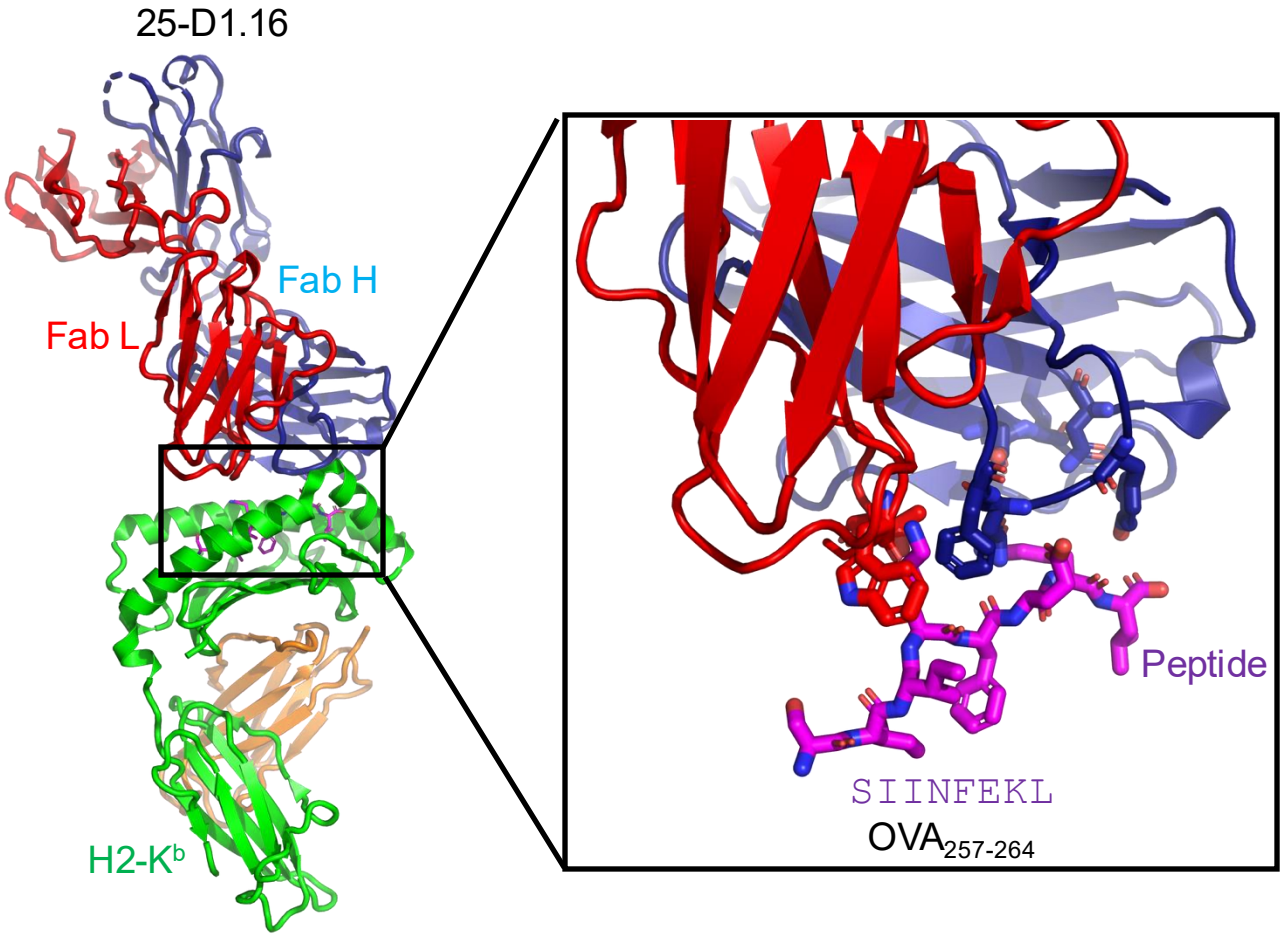


re-engineered MHC-restricted Abs preserve TCR-like docking



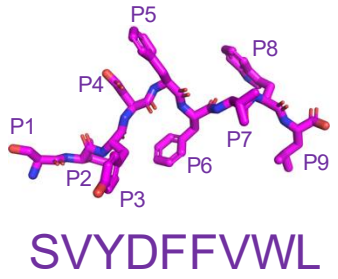
Yang et al., Nat. Biotechnol. 2023

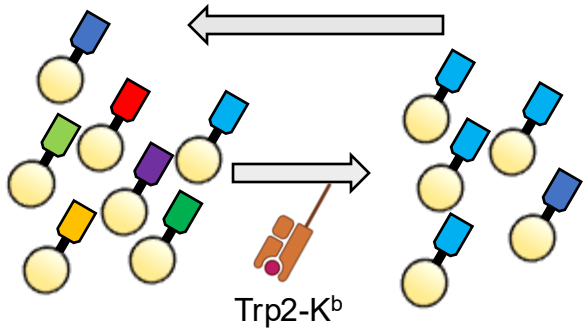
Repurposing OVA specific TCR mimic to melanoma associated tumor antigen Trp2

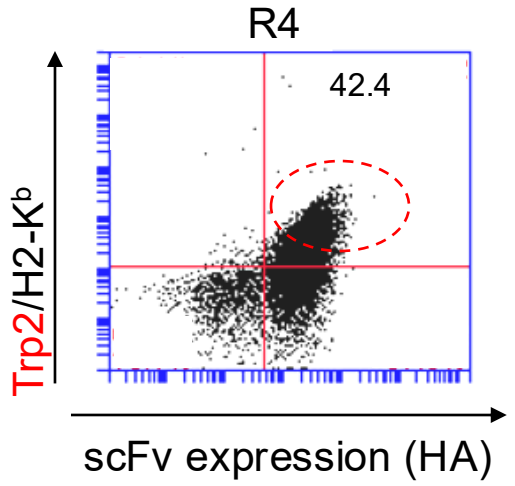


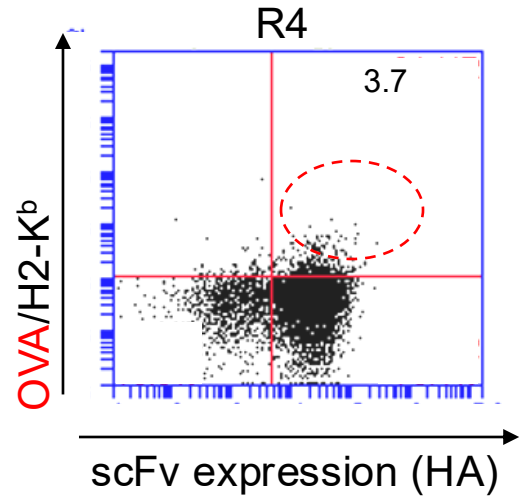
CDR	H1	H2		H3			L3		
#	33	50	58	102	104	105	92	93	94
aa	N	D	I	Y	N	F	W	S	T
Interaction	P	P	P	P/M	P/M	P	M	P	P

Generation TCR mimic scFv yeast library



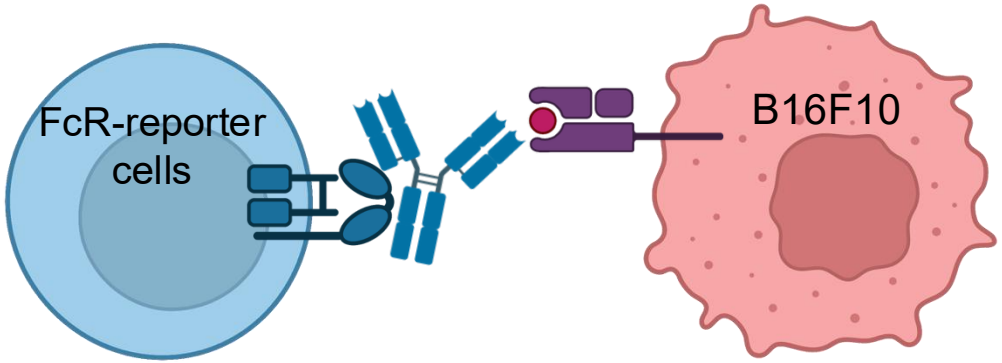


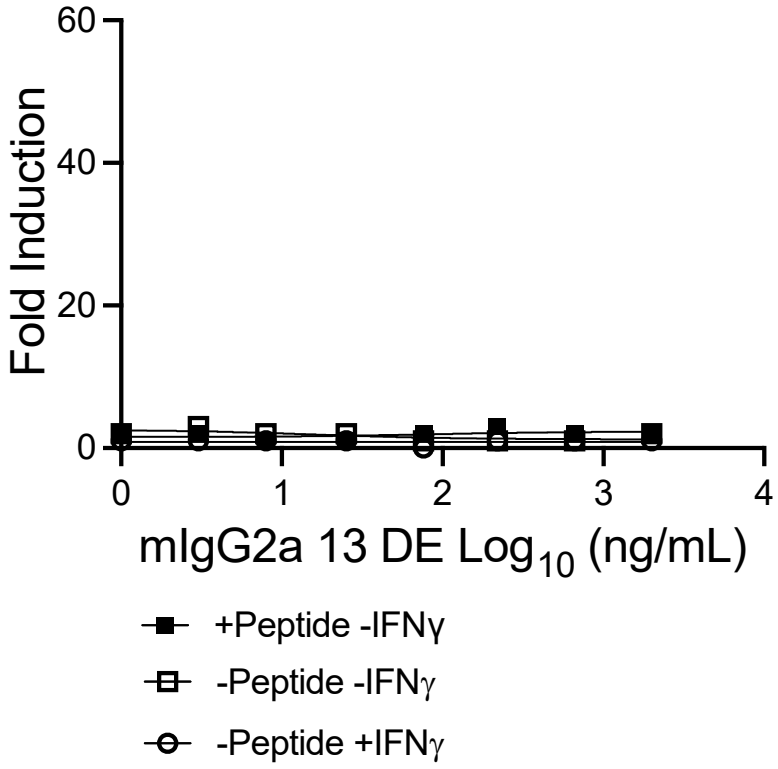


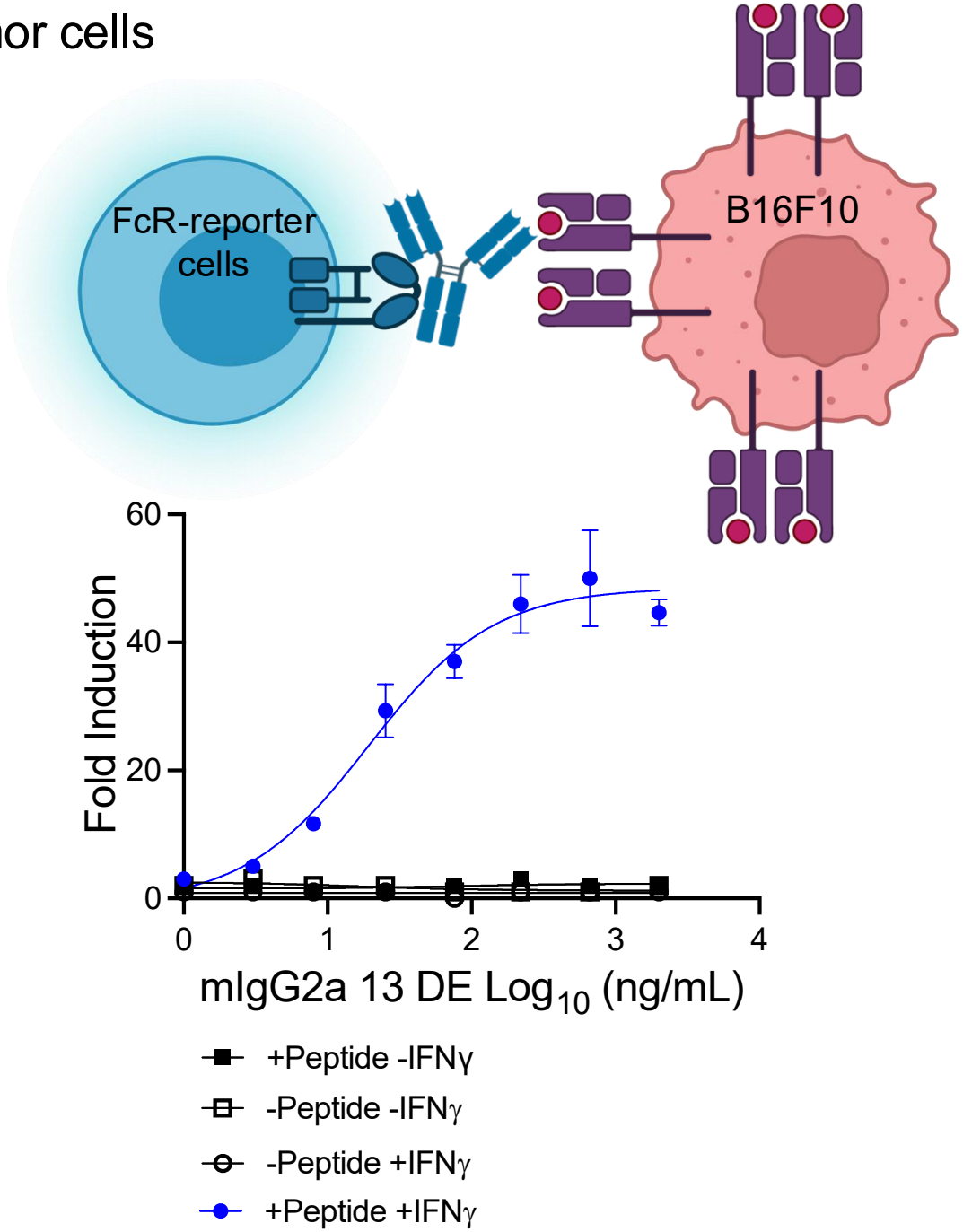


Repurposing scFv from OVA-Kᵇ to Trp2-Kᵇ

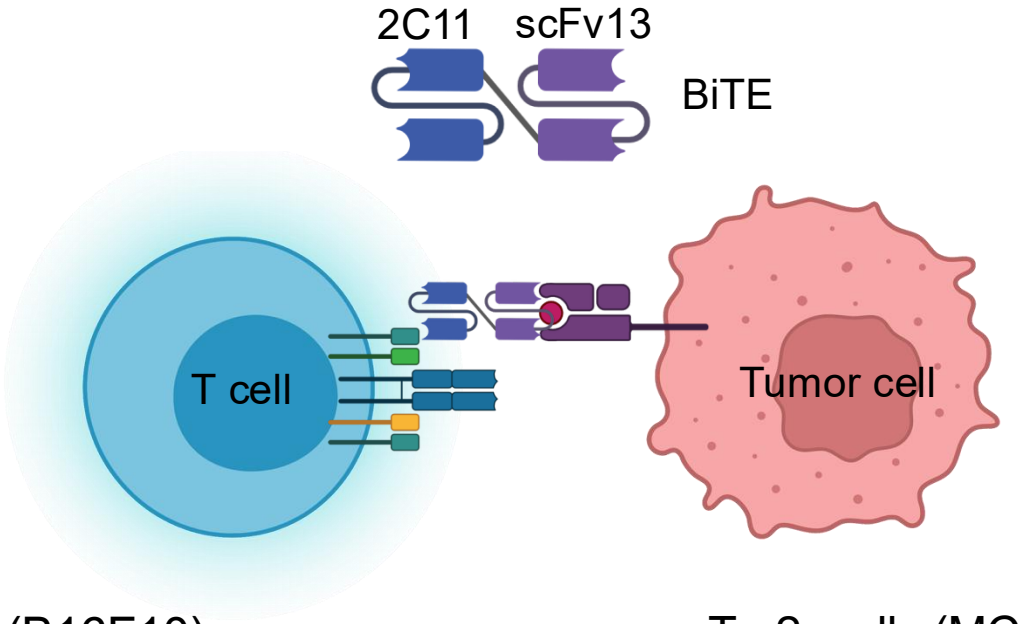
Effective ADCC requires high density of antigen on tumor cells

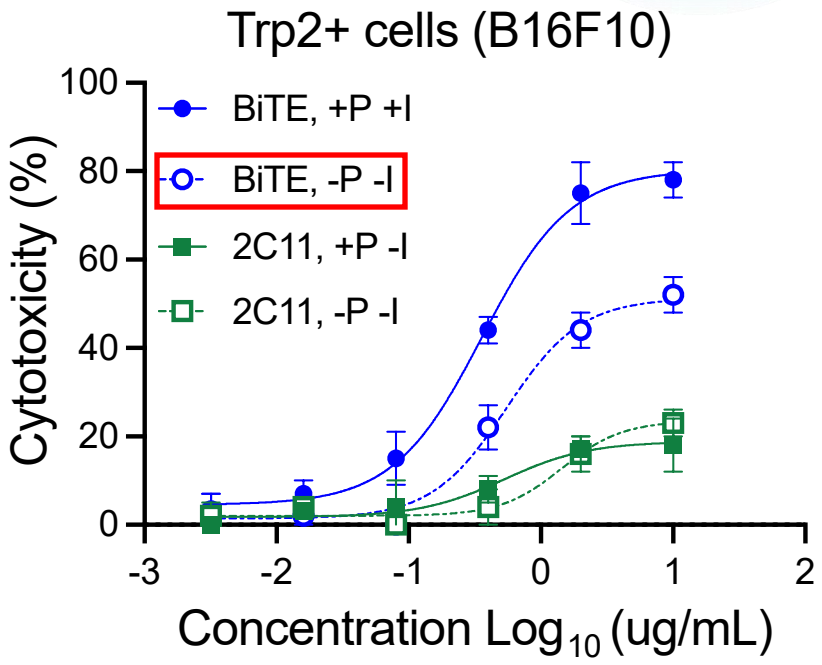


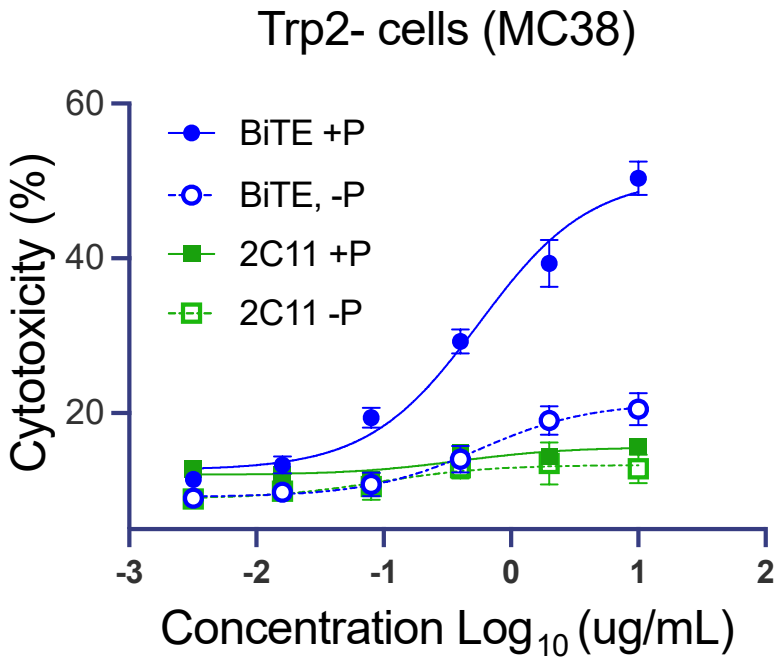




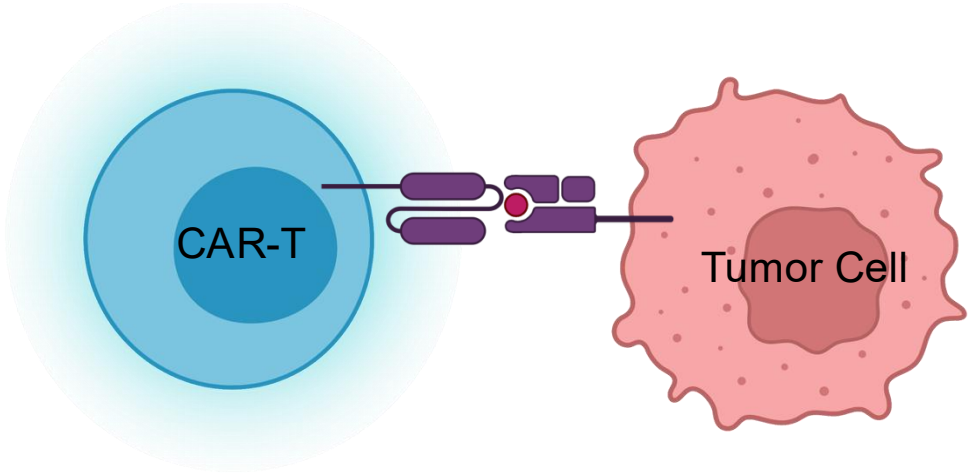
T cell-based therapeutics (BiTE)

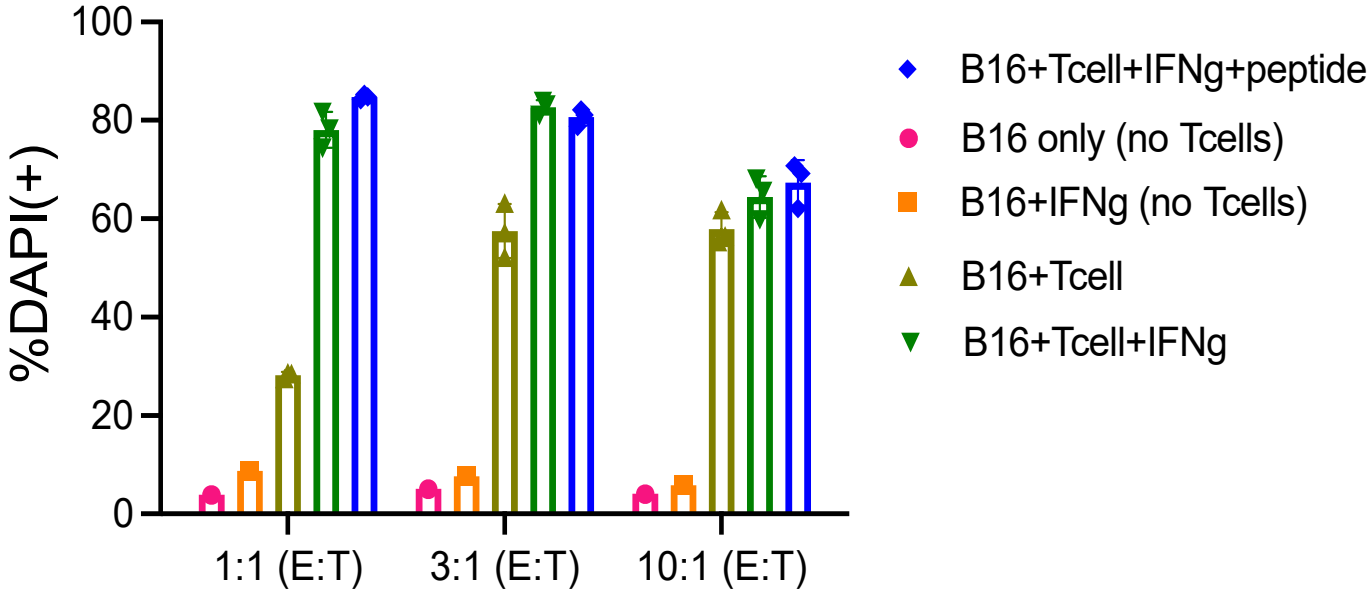


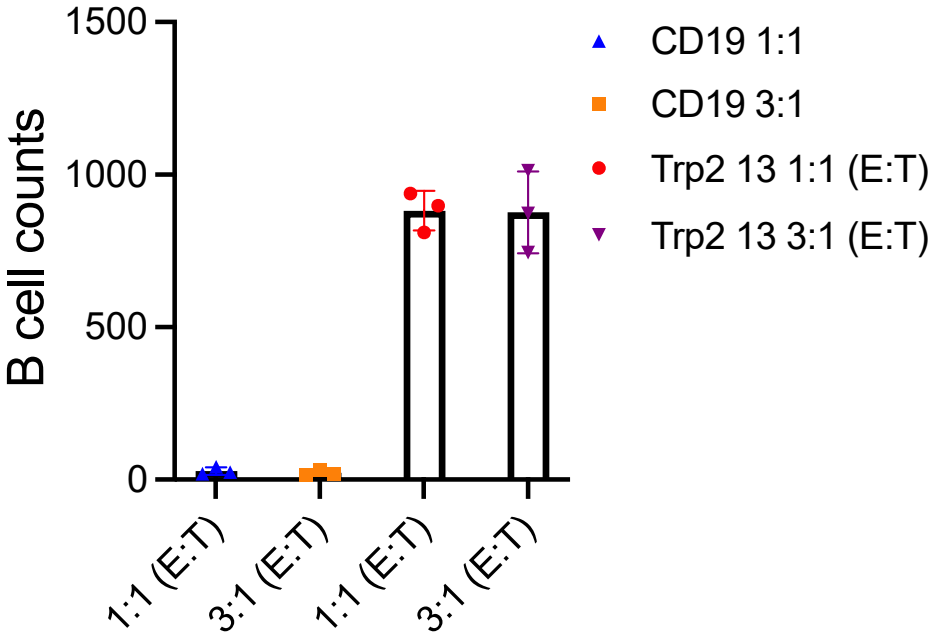




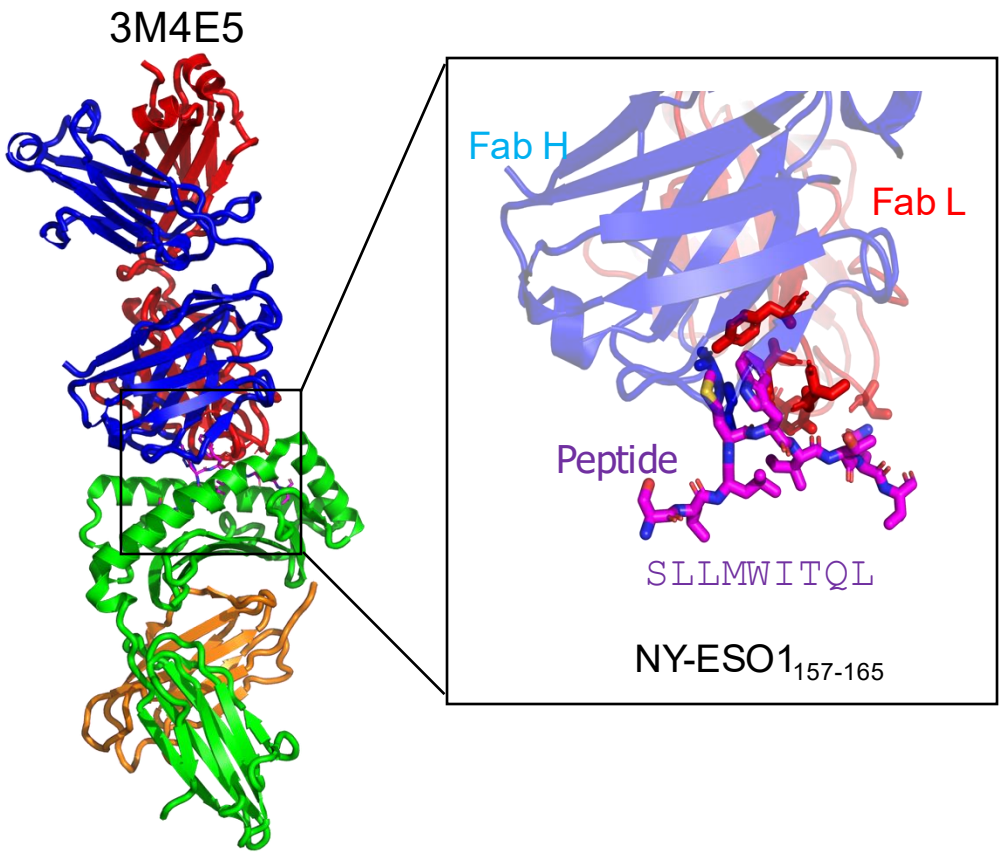
T cell-based therapeutics (CAR-T)



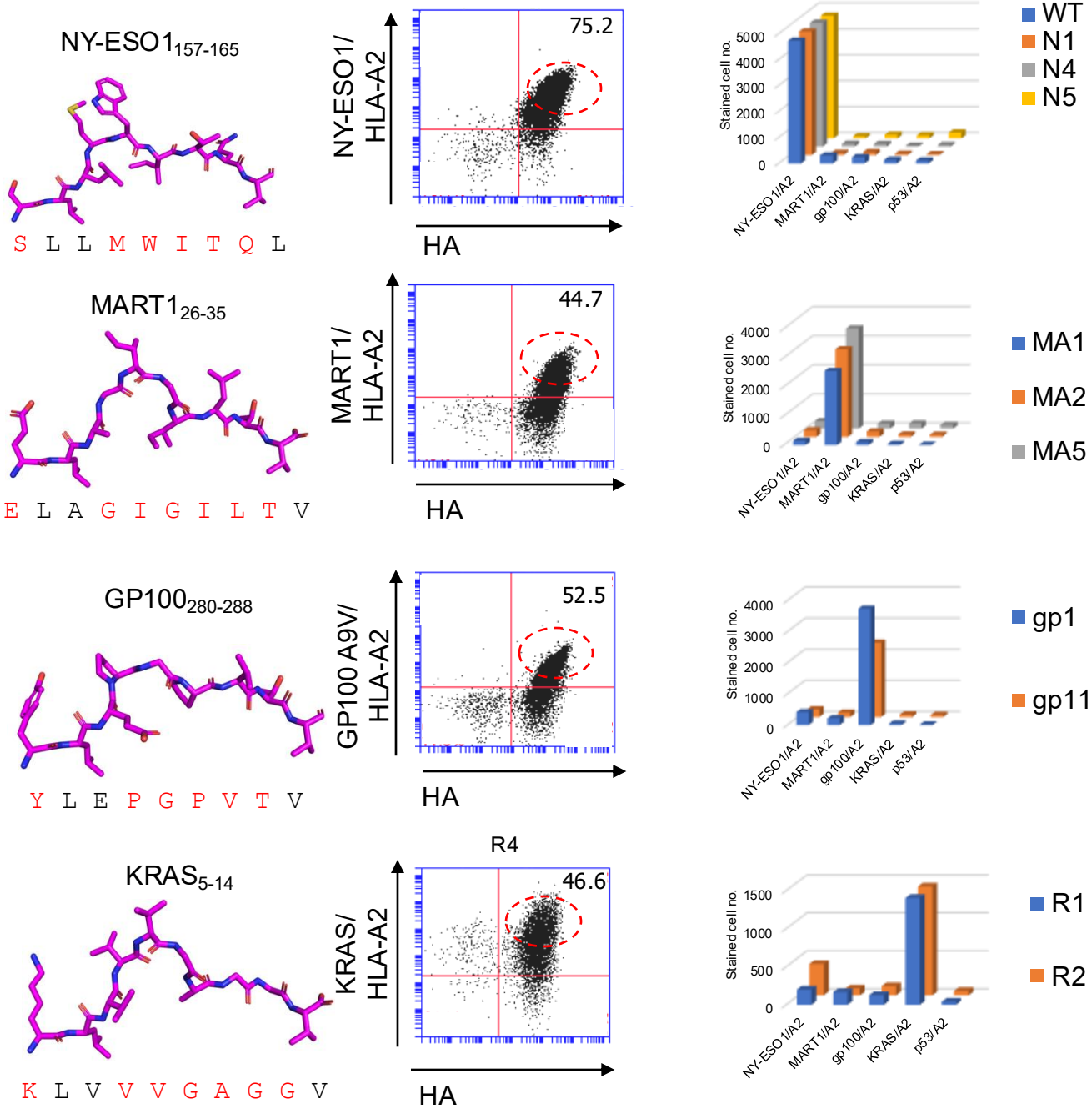




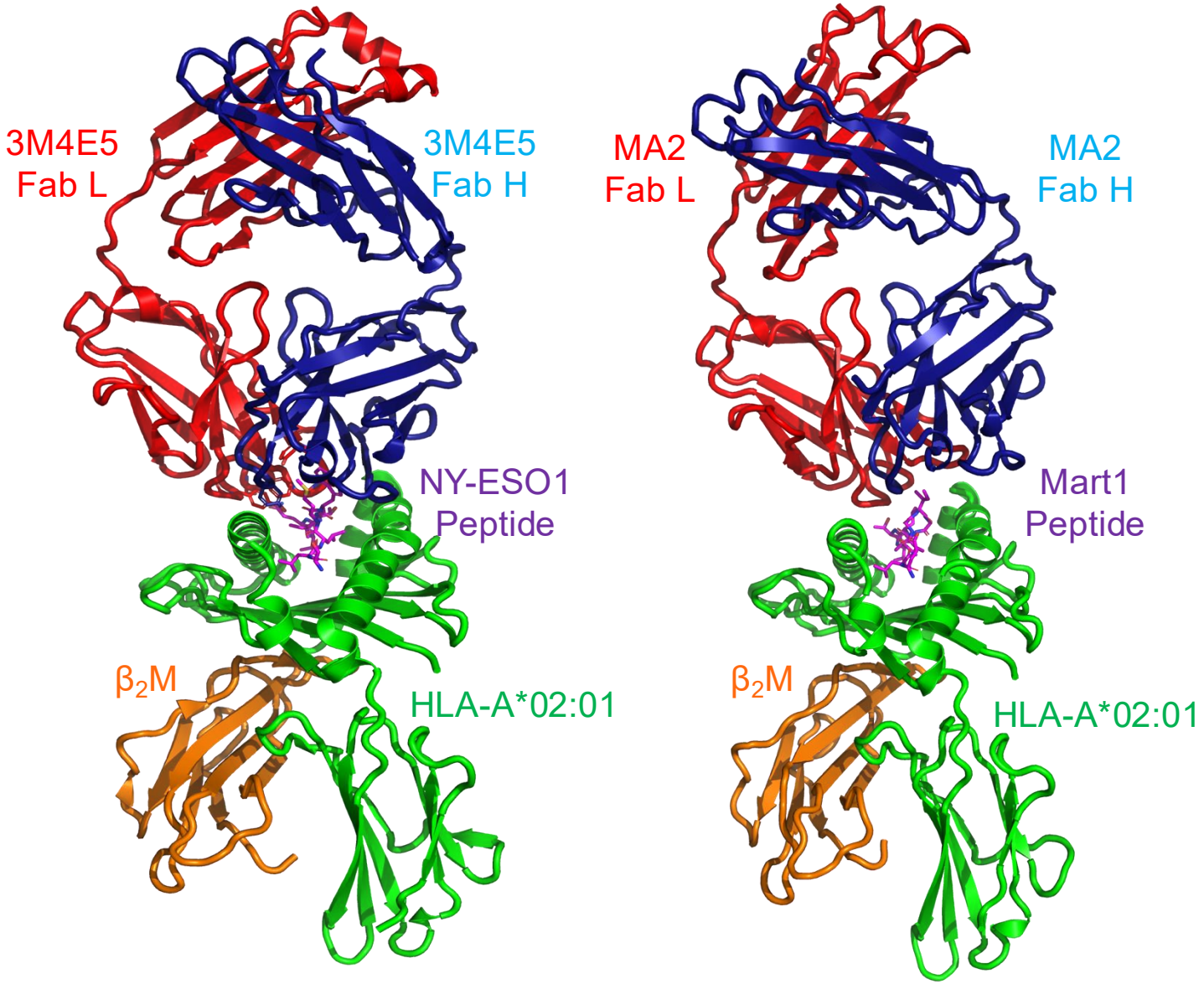
Repurposing the human TCR mimic antibody

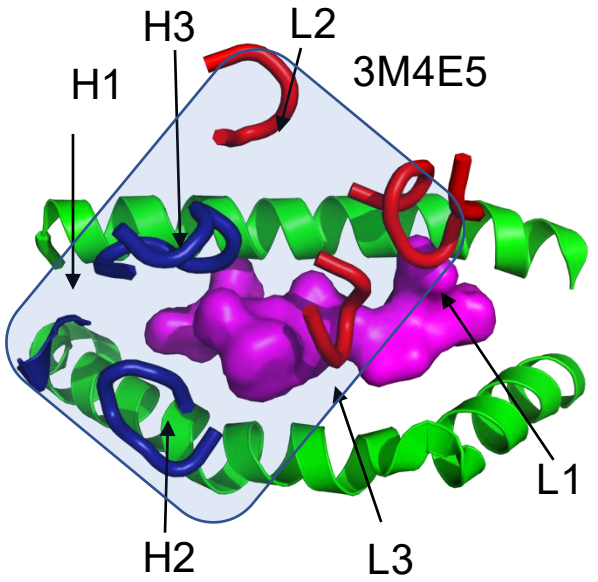


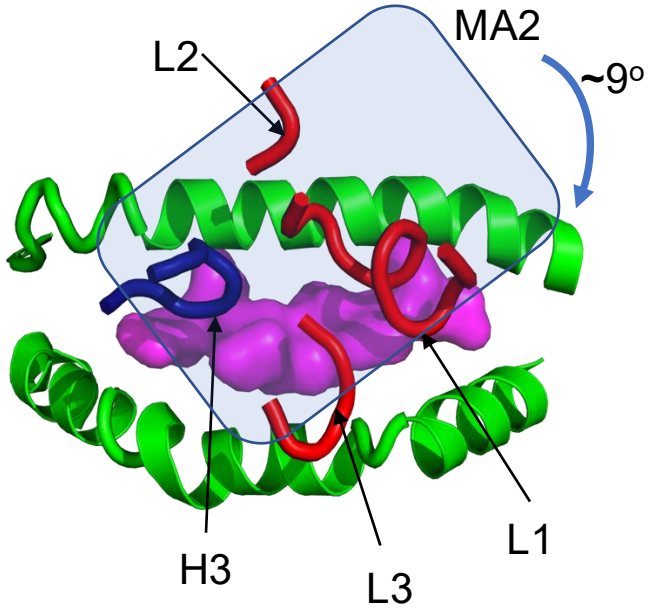
CDR	H3	L1		L3			
#	103	26	32	93	95	96	98
aa	Y	S	Y	F	G	S	Y
Interaction	P/M	P	P	P	P	P/M	P



Structural basis repurposed TCR mimic antibody

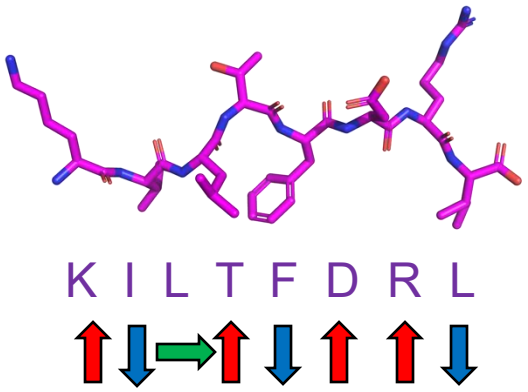




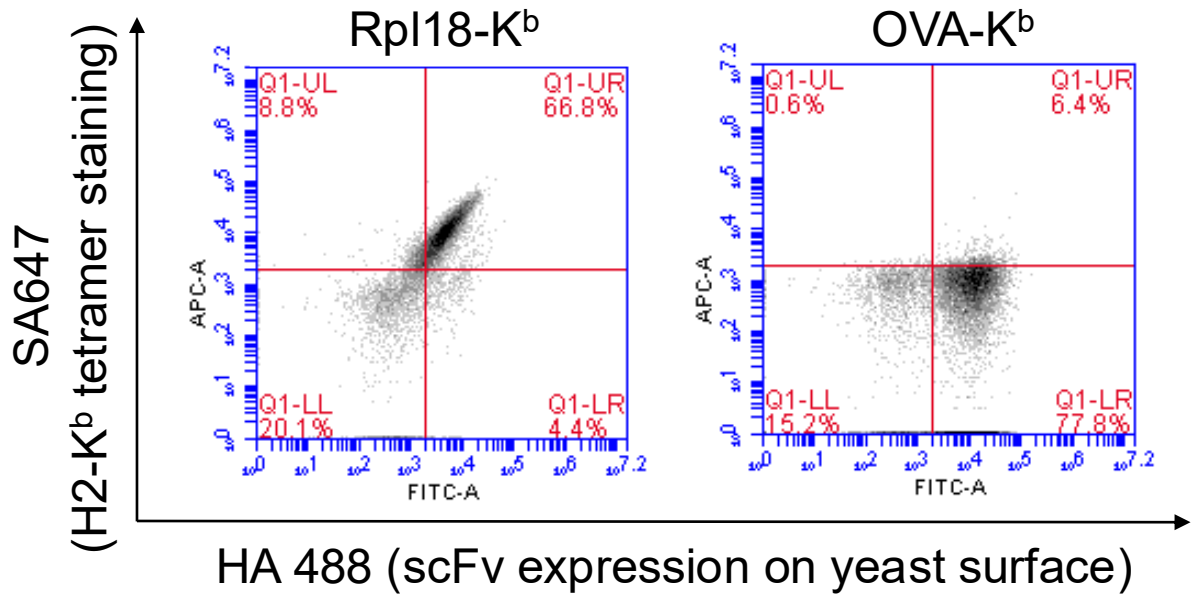


Screening of $Rpl18_{mut}$ peptide-specific TCRm Ab yeast library

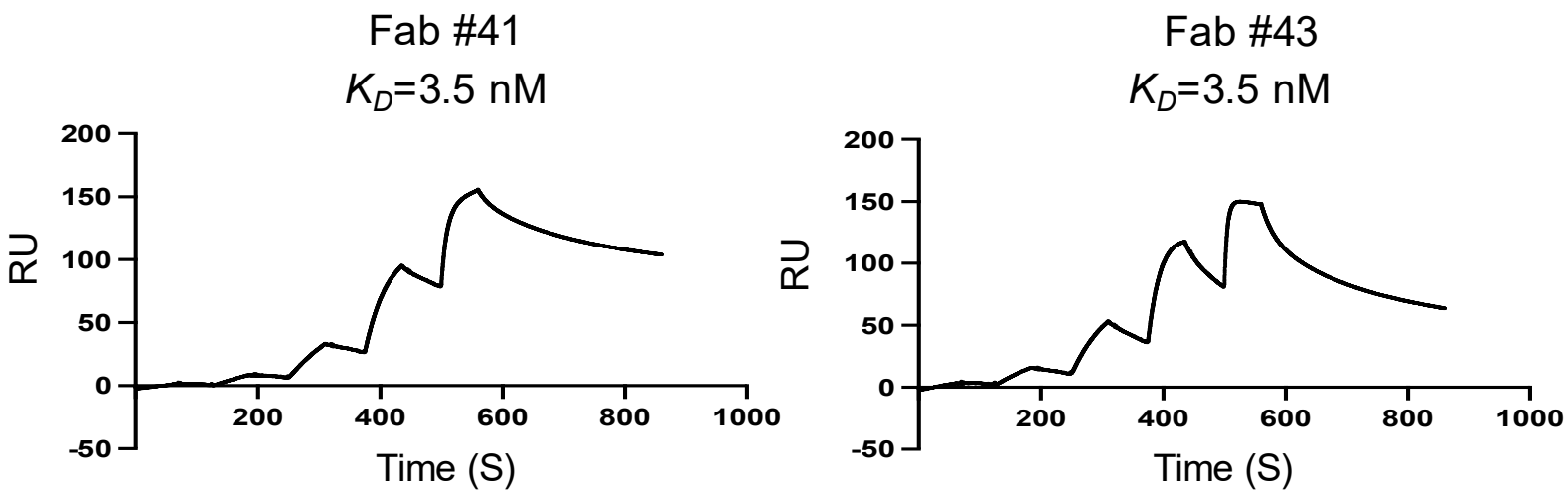
$Rpl18_{wt}$: KILTFDQL
$Rpl18_{mut}$: KILTFDRL

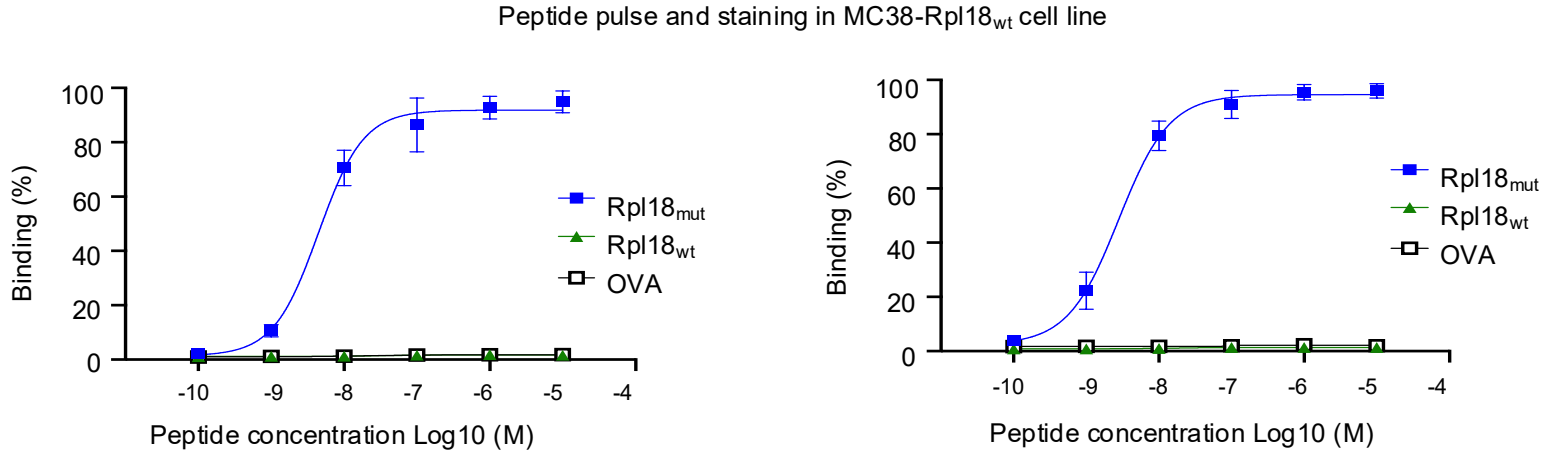


TCRm yeast library after 4 round selection

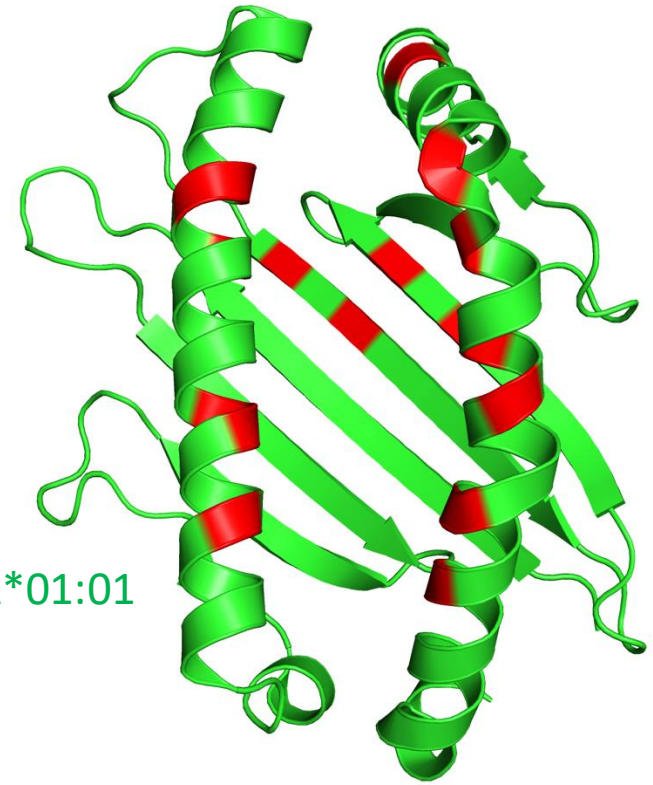


Binding of RPL18-K^{b}-specific TCRm antibodies





Cross-allele TCR mimic antibody generation



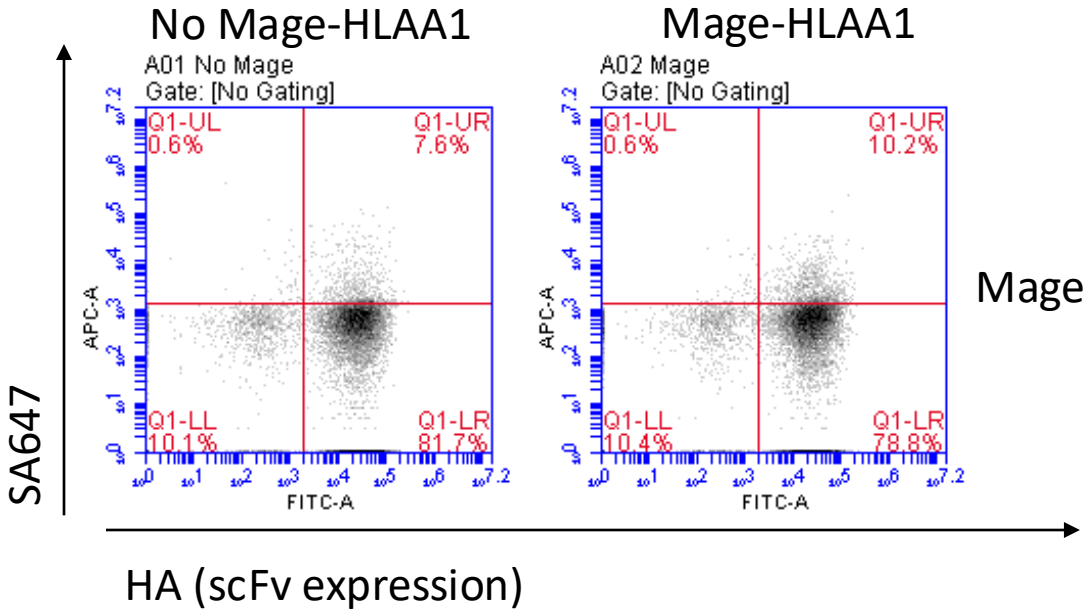
HLA-A*01:01

- The red regions are polymorphism between HLA-A*02:01 and HLA-A*01:01

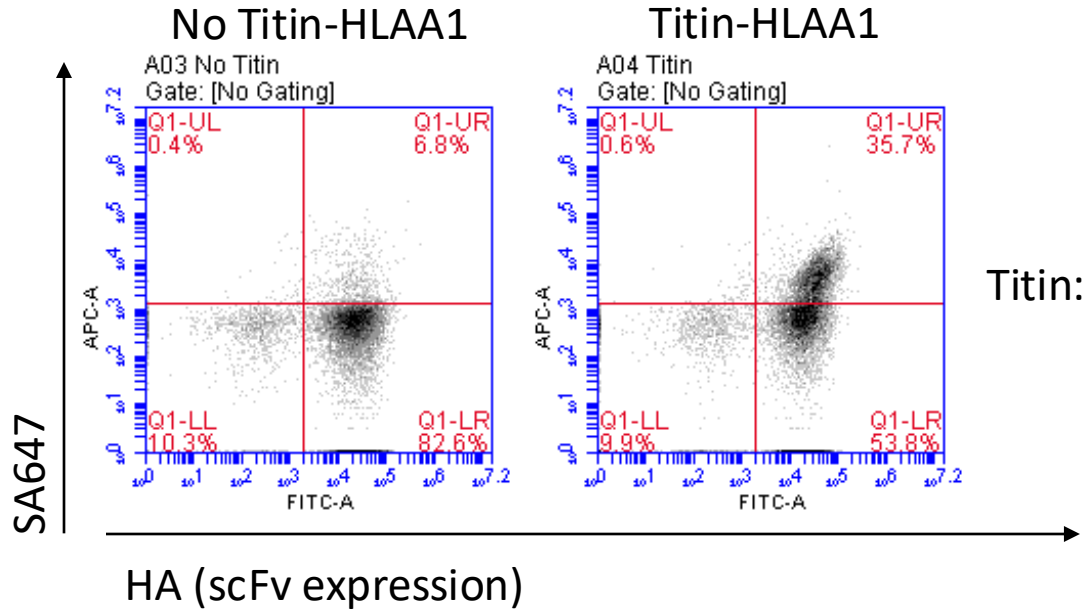
CDR	H3	L1		L3			
#	103	26	32	93	95	96	98
aa	Y	S	Y	F	G	S	Y
Interaction	P/M	P	P	P	P	P/M	P

- The library design has incorporated several residues that allow bona-fide residue to elicit cross-allele TCRm antibody.
- Positive selection reagent: MAGE-HLA-A*01:01
- Negative selection reagent: Titin-HLA-A*01:01
- Outcome: we may be able to generate the first TCRm that target MAGE-HLA-A*01:01 without cross-reactive to Titin-HLA-A*01:01.

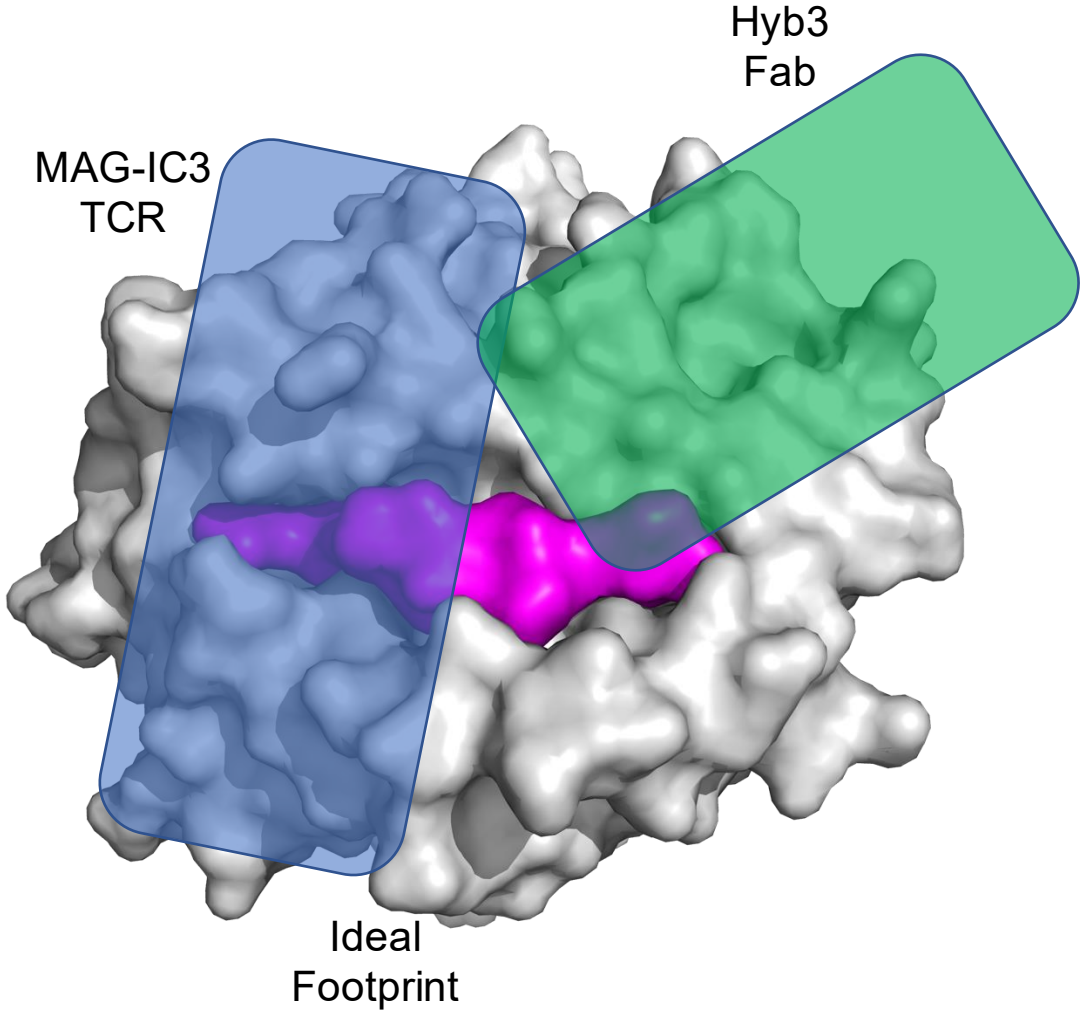
TCR mimic antibody selection on HLA-A1 allele



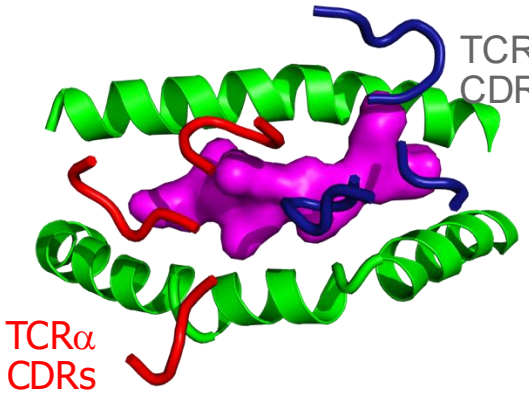
Mage: EVDPIGHLY



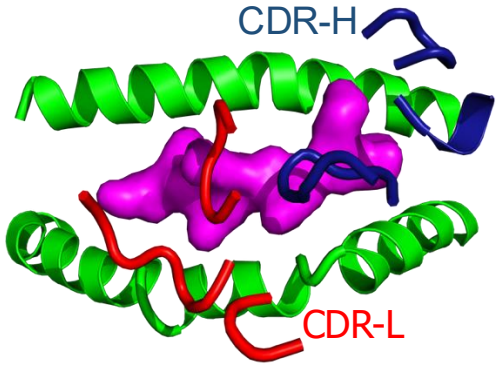
Titin: ESDPIVAQY



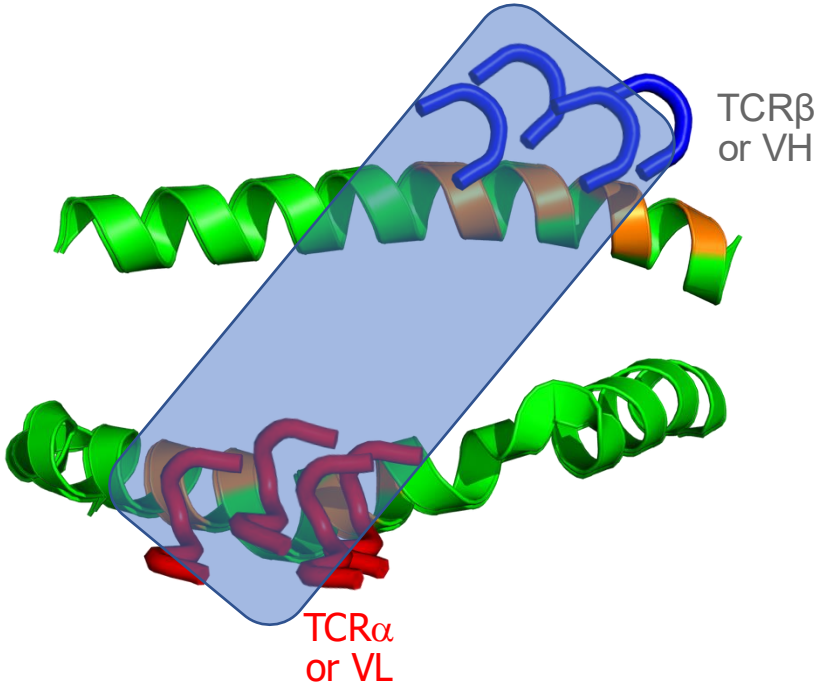
Strategy to isolate panHLA TCR mimic Abs

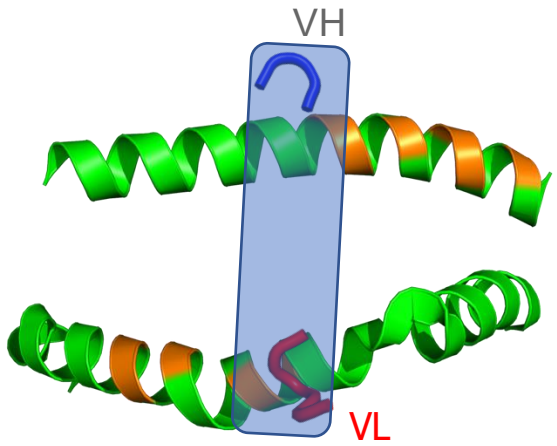


Classical TCR docking footprint on pMHC

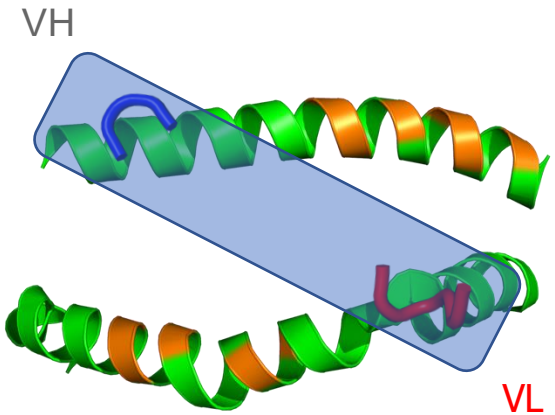


Current TCRm Ab docking footprint on pMHC





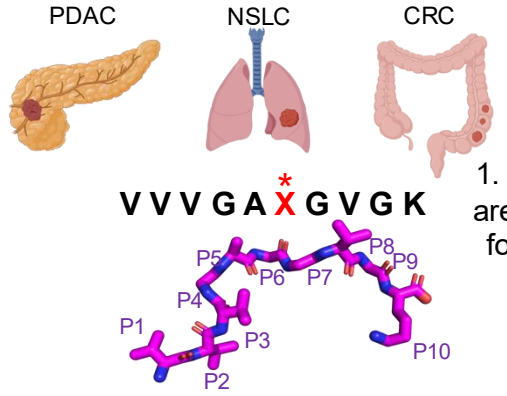
TCRm-AFP-HLA-A*02:01
PDB: 7RE7
scFv1



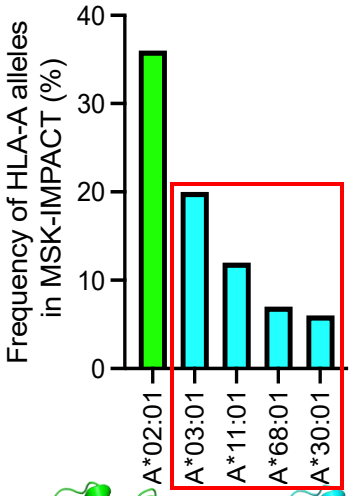
H2-TP53-HLA-A*02:01
PDB: 6W51
scFv2

Yang et al., Nat. Biotechnol. 2023

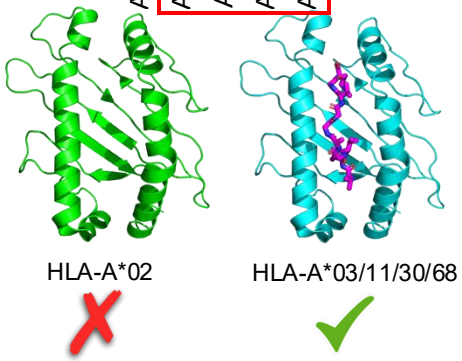
Isolation TCRm modalities with broad reactivity to KRAS-HLA complex

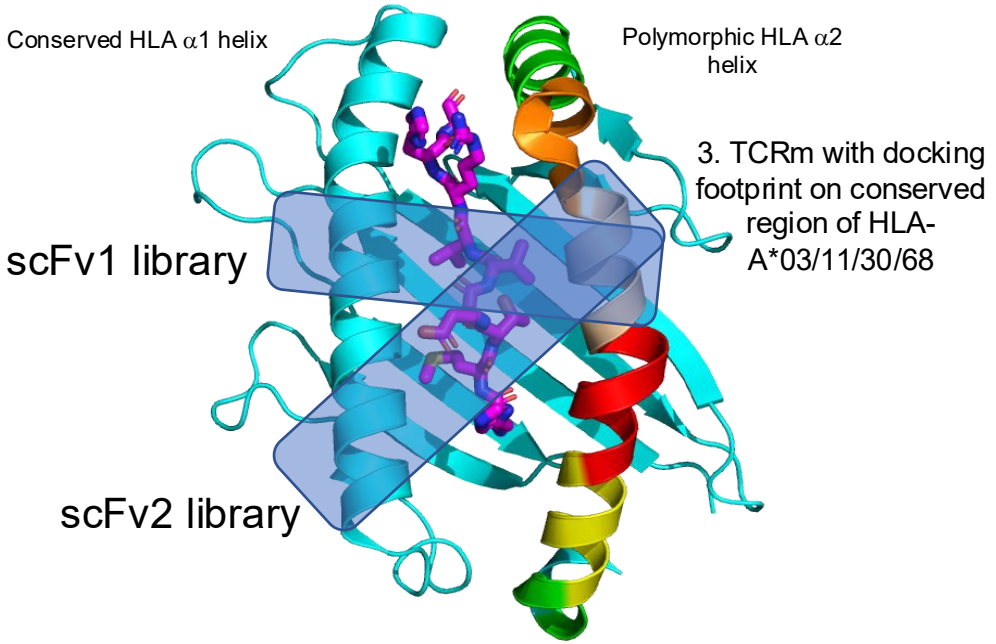


1. KRAS neo-antigen are oncogenic drivers for multiple cancers

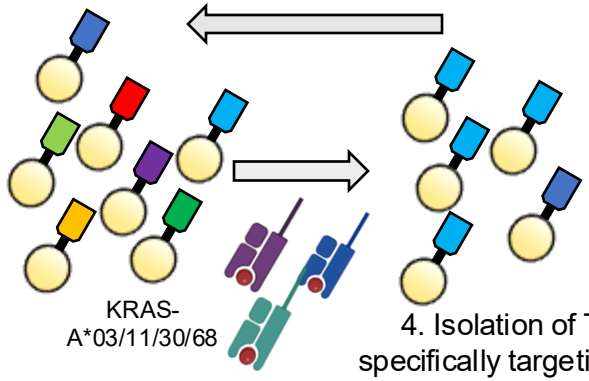


2. KRAS neo-antigens are efficiently presented on HLA-A*03/11/30/68 (40% of patients) but not HLA-A*02 (35% of patients)



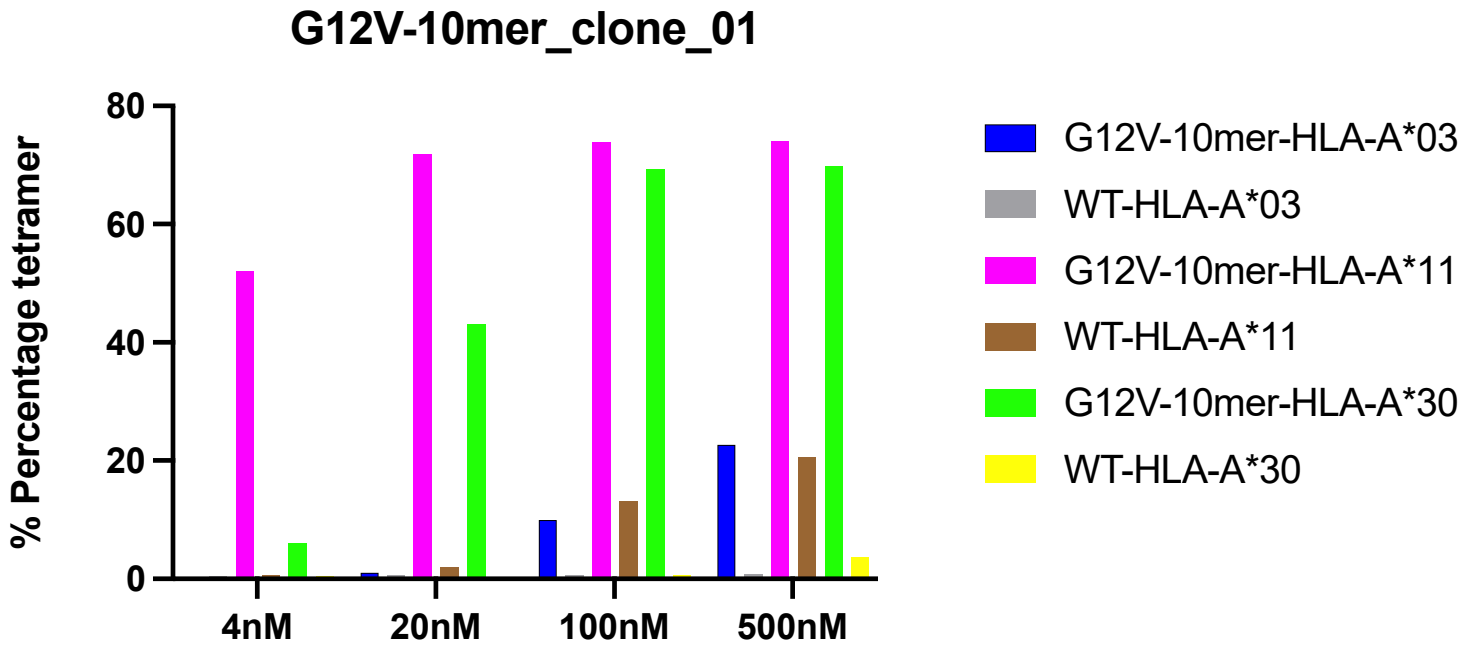


3. TCRm with docking footprint on conserved region of HLA-A*03/11/30/68



4. Isolation of TCRm specifically targeting KRAS neo-antigen presented on HLA-A*03/11/30/68

Neo-antigen specific TCR mimic clones



New library allows us to isolate multi-HLA allele neo-antigen specific TCR mimics

- Overview of how T cell mediated immune system works
  - Antigen receptor diversification
  - Antigen receptor signaling
    - Signal 1
    - Signal 2
    - Signal 3
- Cytokine based immunotherapy (signal 3)
  - principal of cytokine signaling
  - Strategies for designing effective cytokine therapies
- Check point blockade-based immunotherapy (signal 2)
  - landscape of co-stimulatory and co-inhibitory signaling
  - CTLA4 vs PD1
- Antigen receptor-based immunotherapy (signal 1)
  - Antigen identification
  - Engineering therapeutics

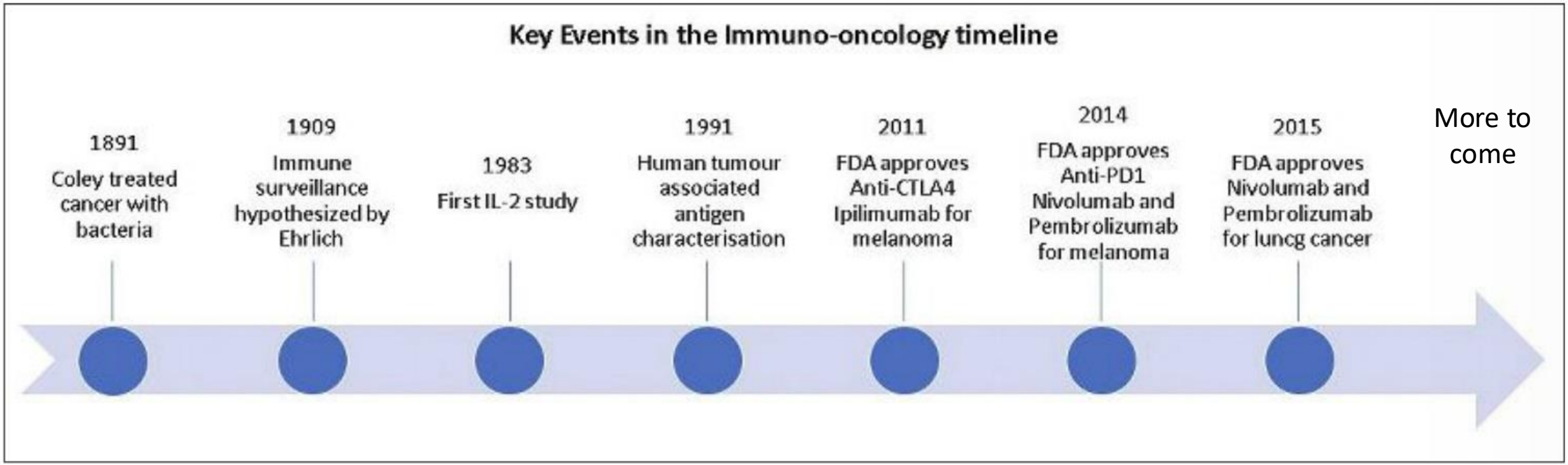


Figure 1 Key events in the immuno-oncology timeline. From Coley's early discoveries through to some of the recent approvals for melanoma and lung cancer. *Timeline adapted from Morrissey et al*