Tumor Neoantigens and Presentation and Recognition

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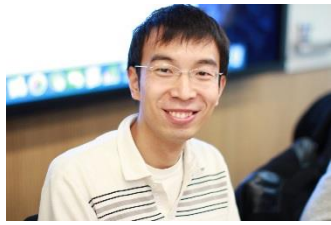

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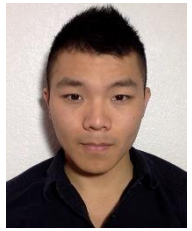
NATIONAL CANCER INSTITUTE
Jean Perkins Foundation
PARKER INSTITUTE for CANCER IMMUNOTHERAPY
S↑2C

Won Jun Noh



William Chour

Plus Prof. Chris Garcia (Stanford) and Leah Sibener (Garcia lab)

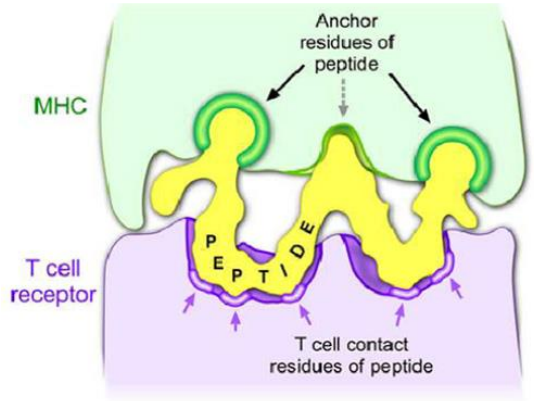
Presenter Disclosure Information

James R. Heath

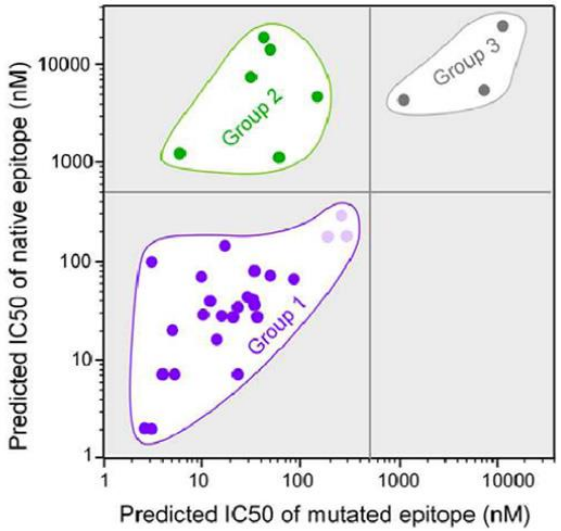
The following relationships exist related to this presentation:

Isoplexis, Inc. Founder, Board member
PACT Therapeutics Founder, Board Member
Indi Molecular Founder, Board Member
Sofie Biosciences Founder, Board Member

Potential Neo-antigens: MHC Binding Prediction



Fritsch EF et al. Canc. Imm. Res. 2014



Strong Binder < 50 nM
Weak Binder > 100 nM

#	Neo-antigen	Kd (nM)
1	F(S)LGSLILV	5
2	RLS(P)SCFDYV	9
3	S(P)LMNEDFIL	11
4	F(S)LGSLLILVV	11
5	S(P)LMNEDFILA	11
6	SLHD(G)LTDGV	12
7	KAWENF(S)PNV	14
8	LLSEFF(S)SCL	18
9	KLLSEFF(S)SCL	20
10	LQ(R)DSGLWFPV	21
11	S(P)LMNEDFILAV	25
12	Y(D)LYHRVDVI	28
13	FVANLFNTYL(P)	29
14	GLFH(R)SLYRSV	29
15	GLSE(G)KCSLV	36
16	HLQ(R)DSGLWFPV	39
17	TLANRFS(P)AV	45
18	FLVIV(A)PLSTI	48
19	GLSE(G)KCSLVV	57
20	F(P)LHGNSLYQKV	61
21	F(S)LRESQETL	65
22	LLSEFF(S)SCLA	66
23	QLDS(P)GTLIV	67
24	WMGLL(P)DLEV	67
25	FVLE(D)HEDGL	71
26	TL(P)VSLATETV	75
27	KMGKTIYKY(H)V	76

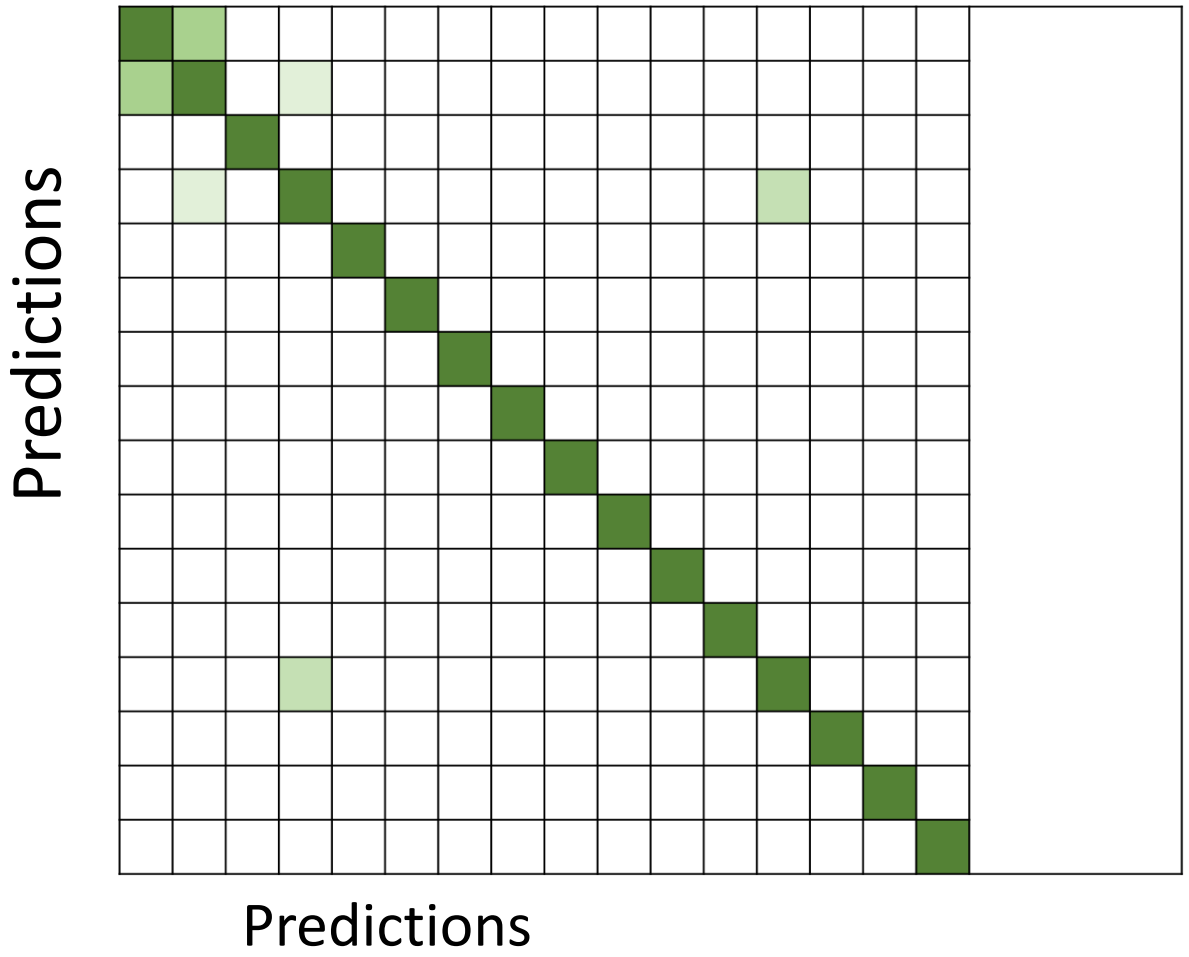
#	Neo-antigen	Kd (nM)
28	NLFNTYL(P)CL	77
29	RLSEV(A)MARM	82
30	VLTEIF(S)LGSL	105
31	ALYKEE(G)EQEPV	133
32	VLIDLIQRTKV(D)	134
33	MVC(R)TFCPPPL	138
34	LLFHS(P)PRAHL	139
35	V(M)LLHAFEGYNV	147
36	VTSSIVTL(P)V	158
37	SL(P)APPRTPEL	212
38	F(S)FVEASMSV	223
39	S(C)MLTARSWDSV	248
40	FVLE(D)HEDGLNL	261
41	SLQT(A)NVQRL	273
42	KVKCIPF(Y)AV	313
43	FVFSKYC(R)HRA	366
44	S(N)LVPEDEANI	368
45	ILPFF(L)YLGSA	380
46	RI(N)AGEEVTLTV	416
47	VLT(A)RLALLQL	418
48	LLEYRI(S)SENPV	440
49	MQQPSPQ(P)IPPV	449
50	GLFH(R)SLYRS	463

USP7 D789Y

		Predicted Affinity (nM)
Wild Type:	…PTAKEYFRDLYHRVDVI…	
Mutant:	…PTAKEYFRYLYHRVDVI…	
	PTAKEYFRY	28230
	TAKEYFRYL	14373
	AKEYFRYLY	26447
	KEYFRYLYH	26160
	EYFRYLYHR	26499
	YFRYLYHRV	8833
	FRYLYHRVD	28595
	RYLYHRVDV	15575
	YLYHRVDVI	**28 (WT peptide = 10865 nM; Group 2)**

Beyond NET MHC: Many Neoantigen Prediction Algorithms

Somatic mutations, Splice Variants, …



My approximation of the correlation matrix showing the overlap of Neoantigen Predictions for 4 melanoma cancer patients

22 different sets of predictions

TESLA Program, Parker Institute

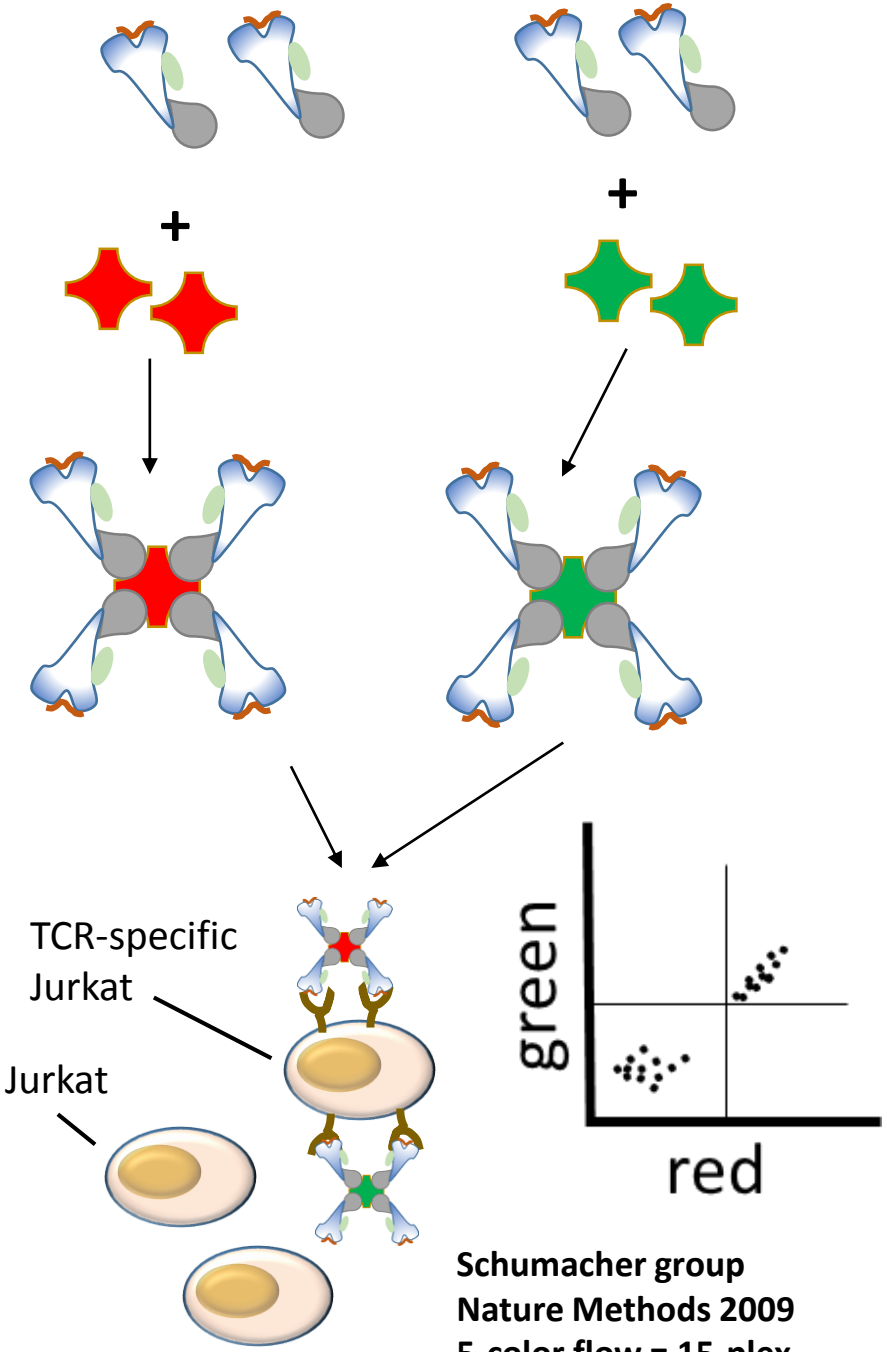
Any individual *non-expanded* population of neoantigen-specific CD8+ T cells, especially in a challenging patient will likely be extremely rare

Example: From tumor infiltrates, one might separate 10,000 viable CD8+ T cells
Assume patient has 6 HLA alleles (typical)
Likely 100 candidate neoantigens per allele that exhibit reasonable binding to MHC

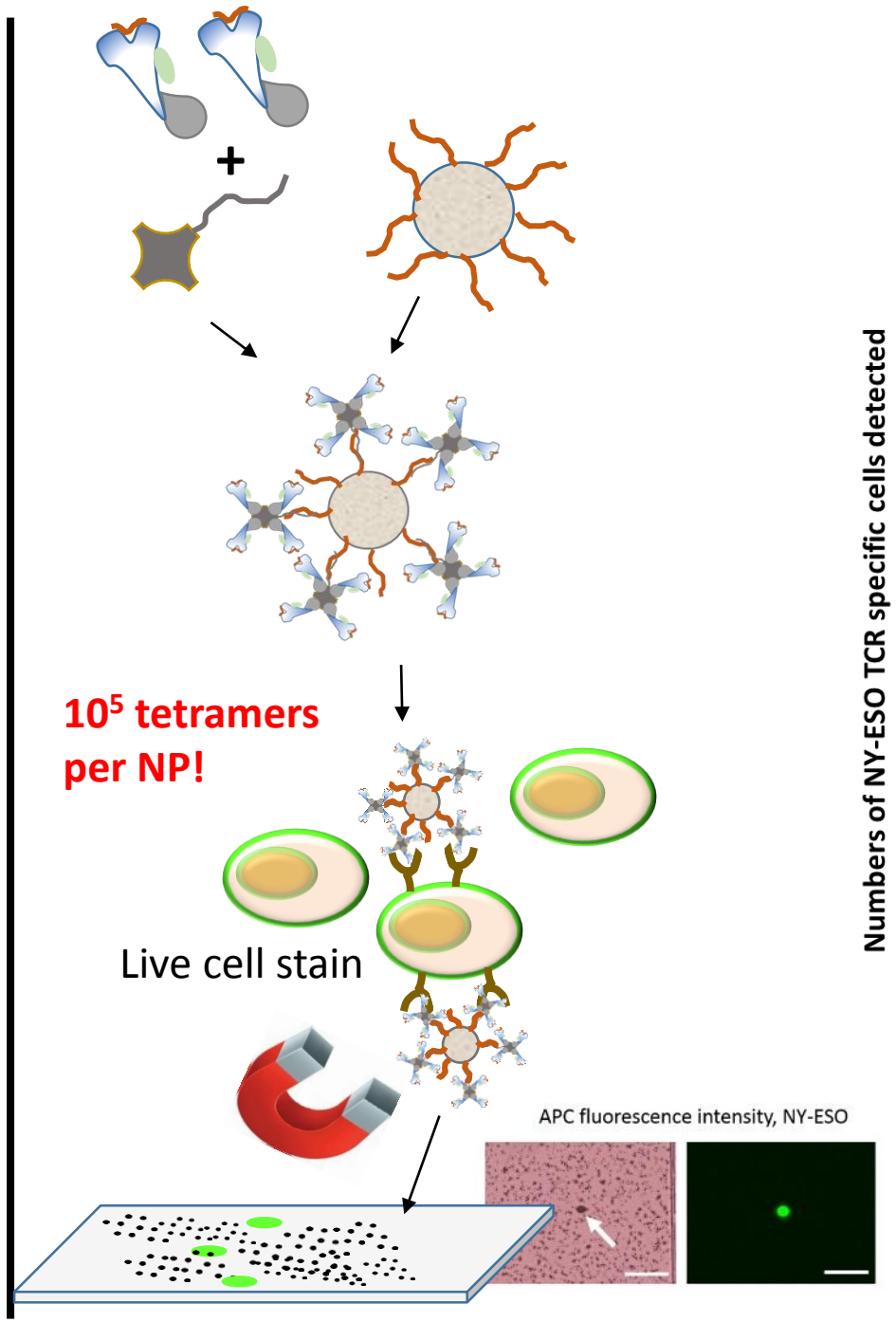
Neoantigen-specific populations likely exist in single digit numbers per 10^4 CD8+ tumor infiltrates
Abundance in the blood will be 10-fold lower

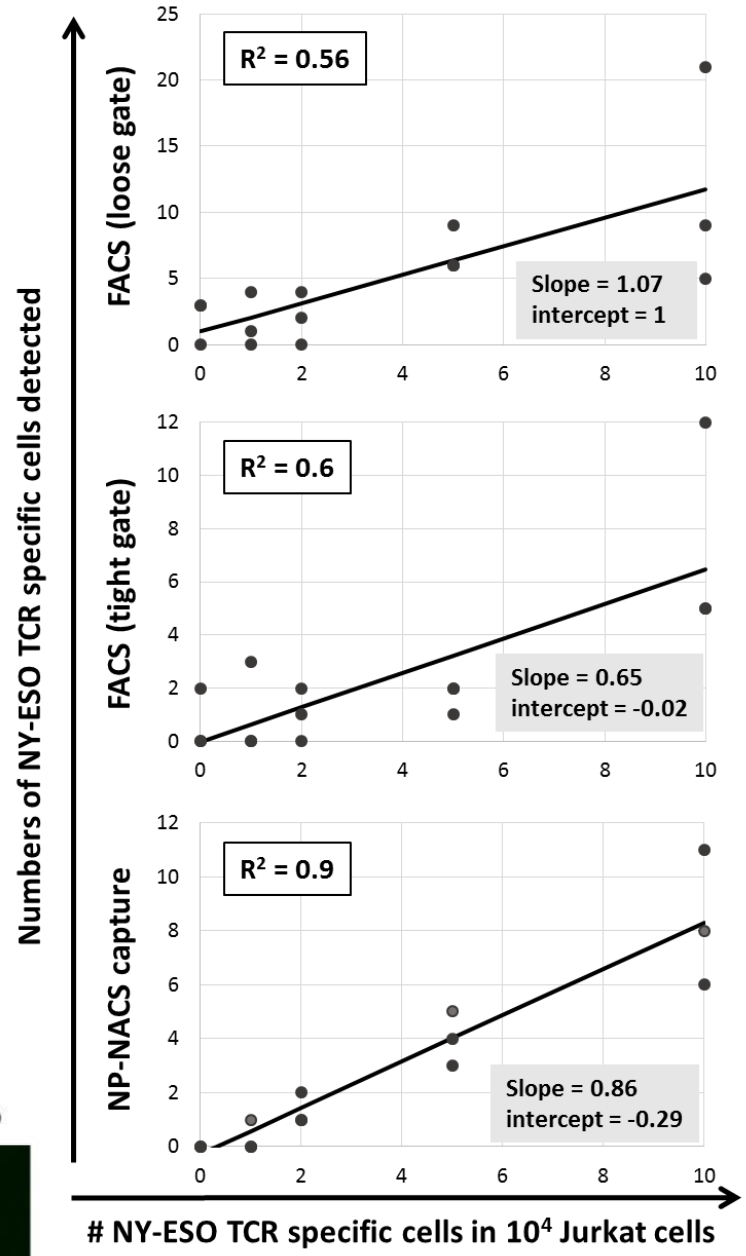
This presents a highly challenging sample for analysis

For R_X applications (vaccines, TCR-engineering), the analysis must be rapid and accurate

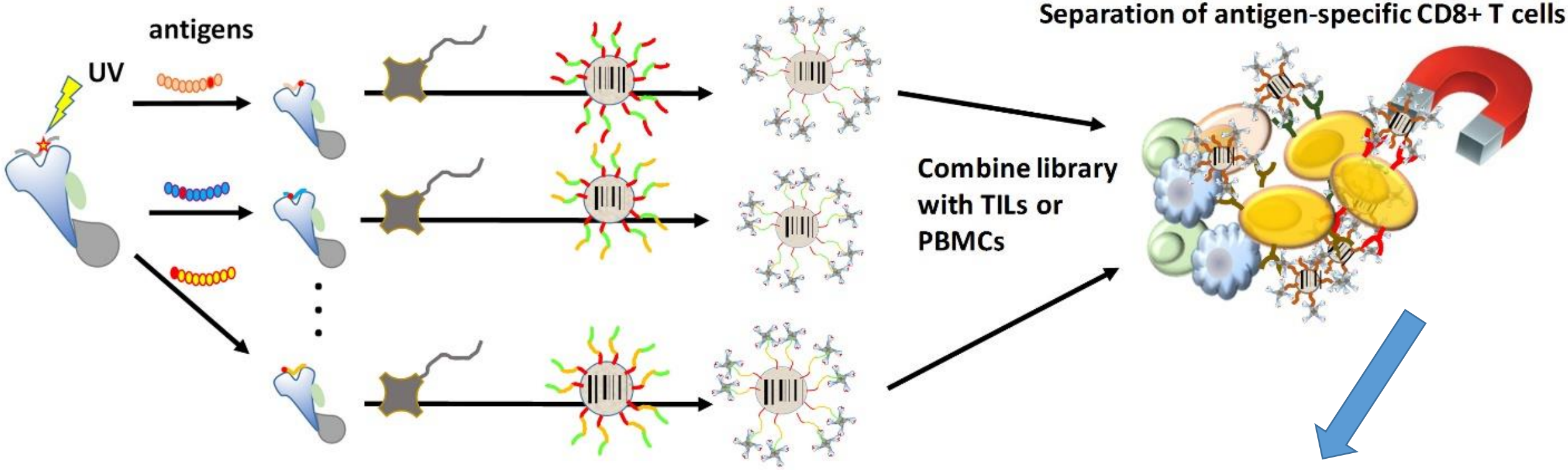


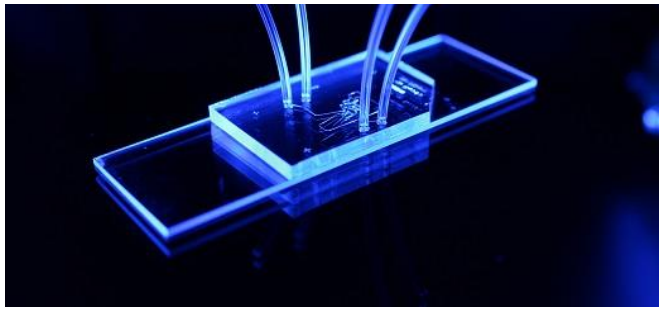
Schumacher group
Nature Methods 2009
5-color flow = 15-plex





Barcoded Nanoparticle Nucleic Acid Cell Sorting *(barcoded NP-NACS)*





- Conditional Antigens

(Schumacher, T.N. *Nat. Med.* **2006**)

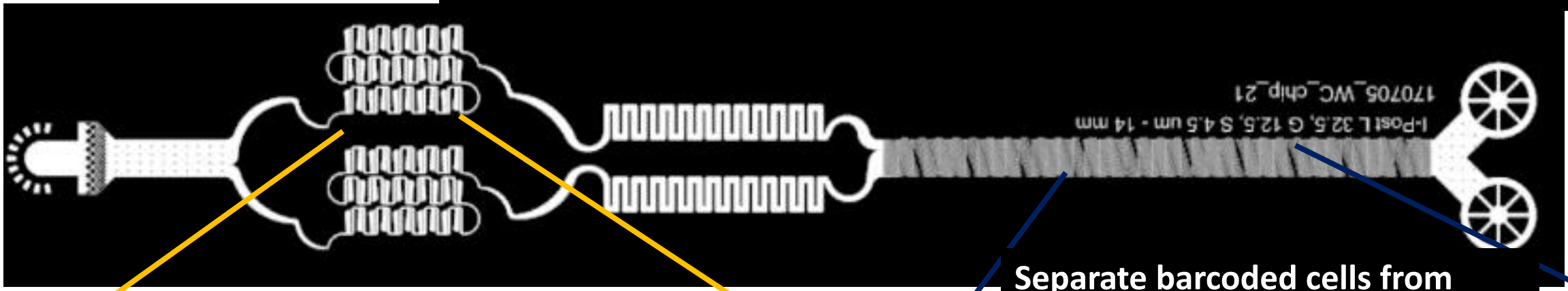
- cysteine-labeled streptavidin scaffold

*(*Altman, *J Immun Meth* **2007***)*

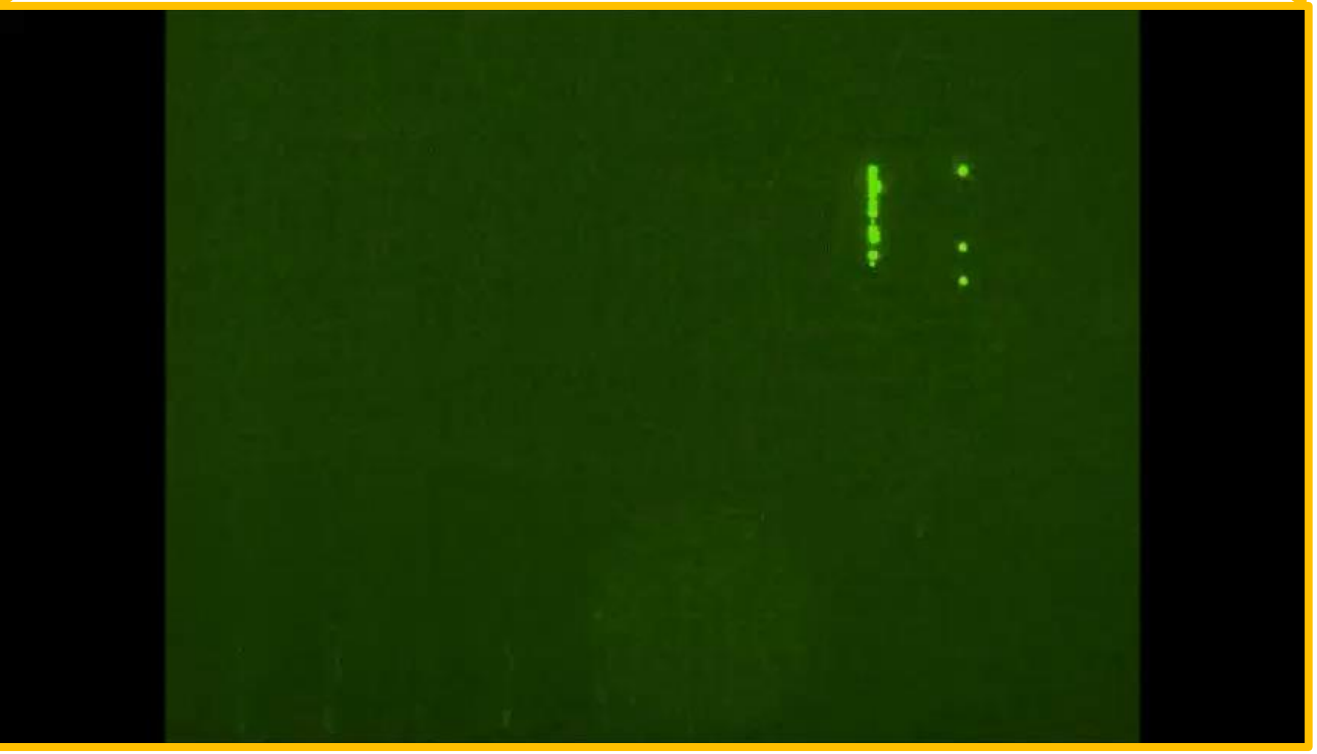
- DNA-labeled cys-Strep tetramers

(Kwong et al., *JACS* 2009)

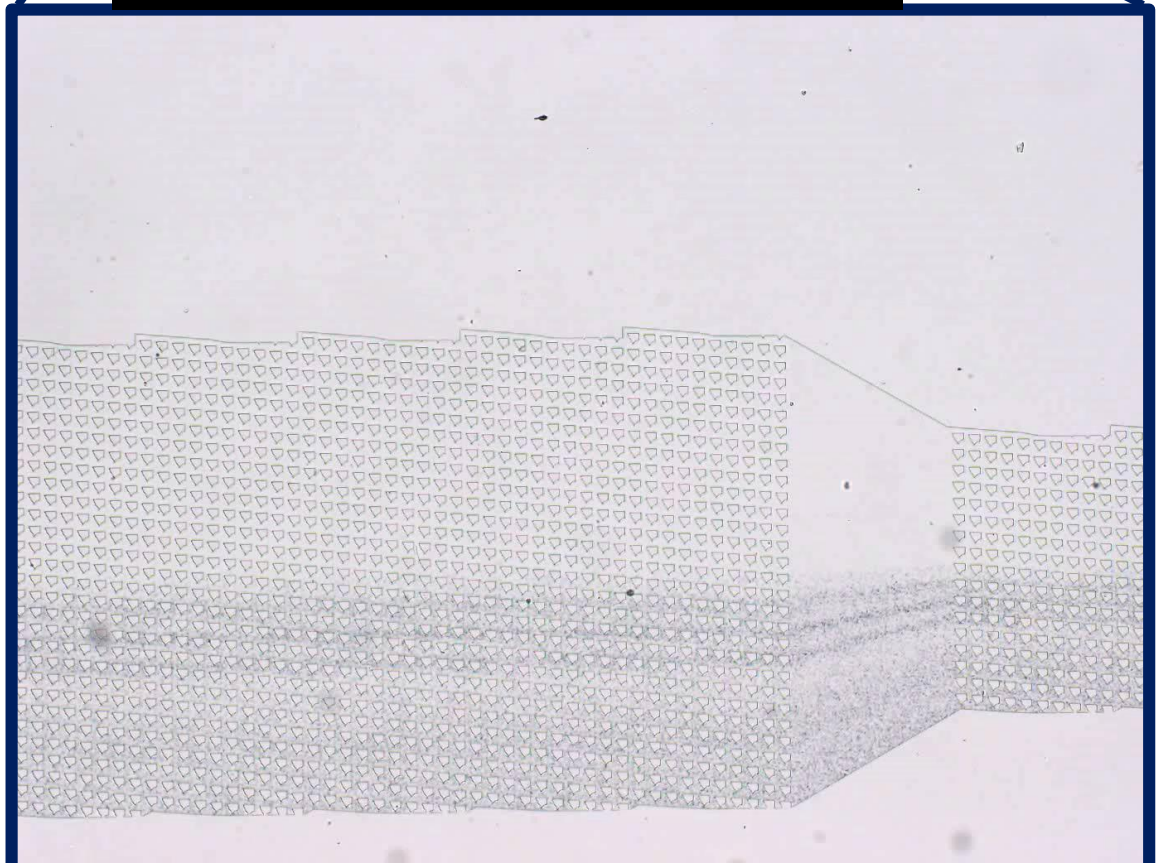
Microchip Design for Analyzing Neoantigen-specific T cell populations

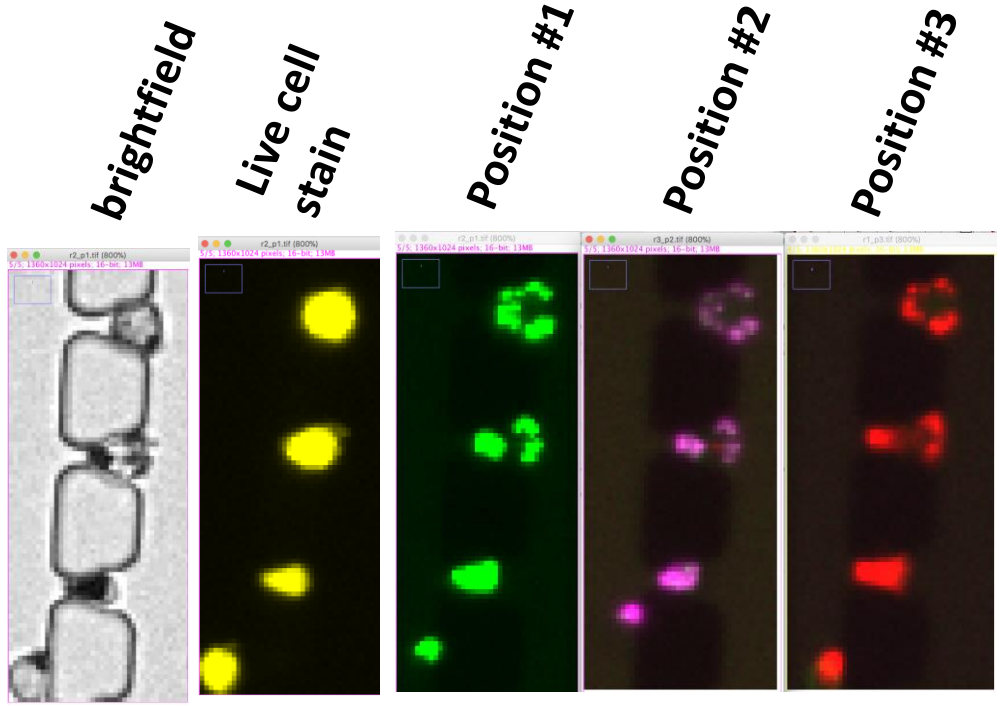


Trap Barcoded Cells



Separate barcoded cells from unbound nanoparticles

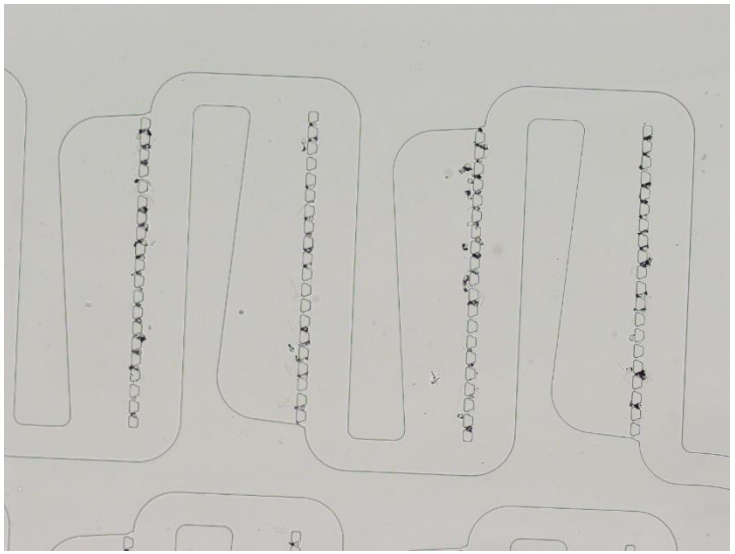


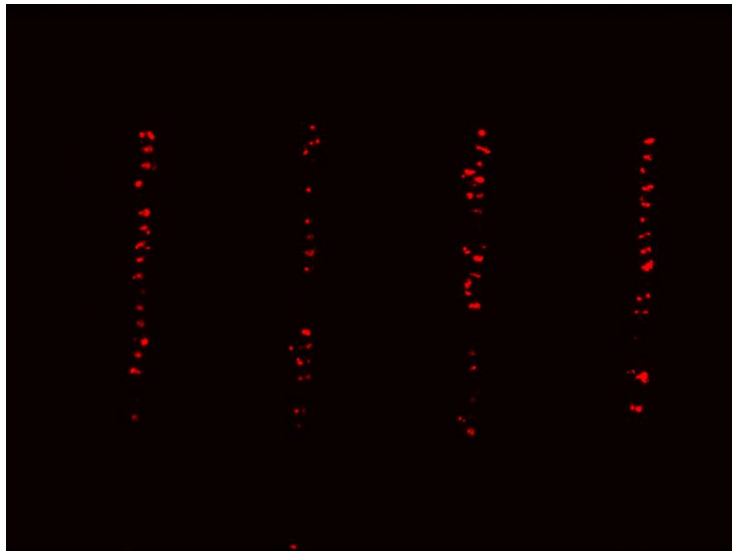


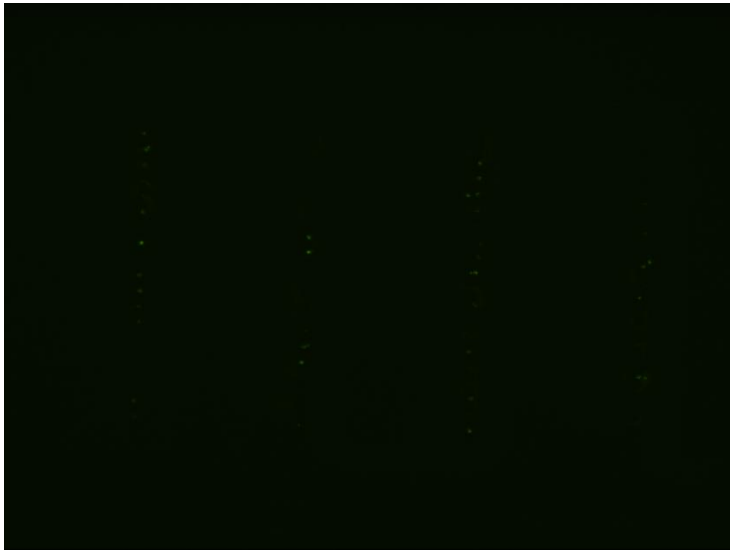
peptide seq	Barcode	p1	p2	p3
ALDHMFMYFL	1			
FLDPDLTNI	2			
FLGSLLILV	3			
FLNCDIMLGV	4			
FVANLFNTYL	5			
FVLEHEDGL	6			
KAWENFPNV	7			
KLLSEFFSCL	8			
LLAPLIATL	9			
LLSEFFSCL	10			
LMMHSATSA	11			
RLSEVMARM	12			
RVYDALNLL	13			
VLASLCLYV	14			
YLYHRVDVI	15			
FLGSLILV	16			
RLSCFDYV	17			
NLFNTYLCL	18			
VLTEIFLGSL	19			
VTSSIVTLV	20			
ILPFFYLGSA	21			
VLTRLALLQL	22			
RIAGEEVTLTV	23			
LLEYRISENPV	24			
SKQTNVQRL	25			
SLMNEDFILAV	26			
control MART-1	27			

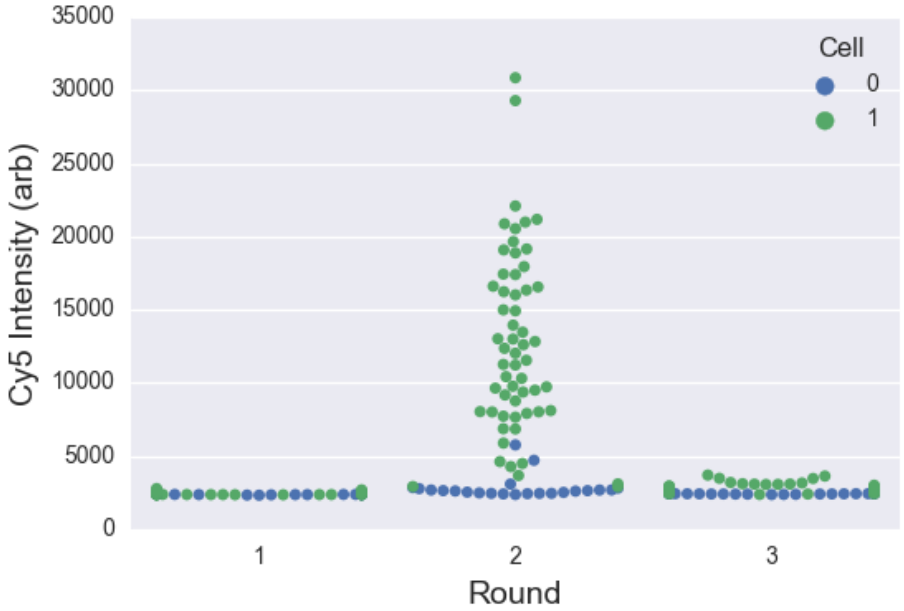
Round 2: Orange (TRITC) – Red (Cy5) – Green (GFP)

73 % Cell occupancy

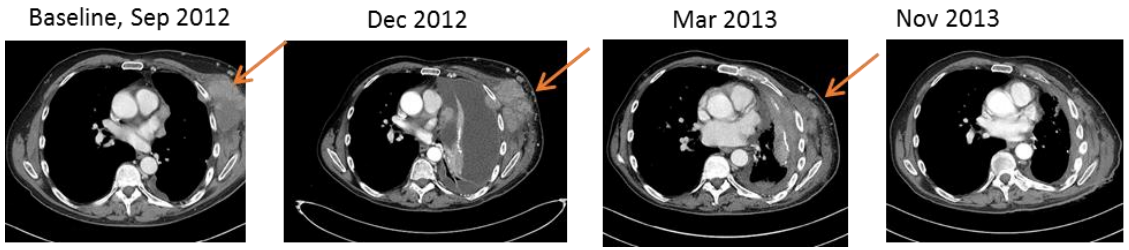


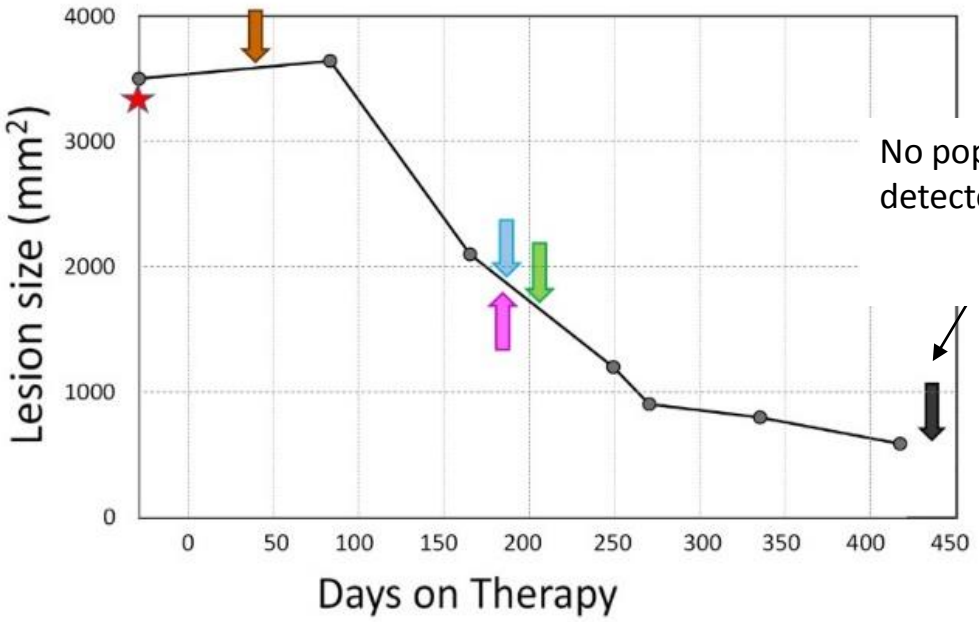






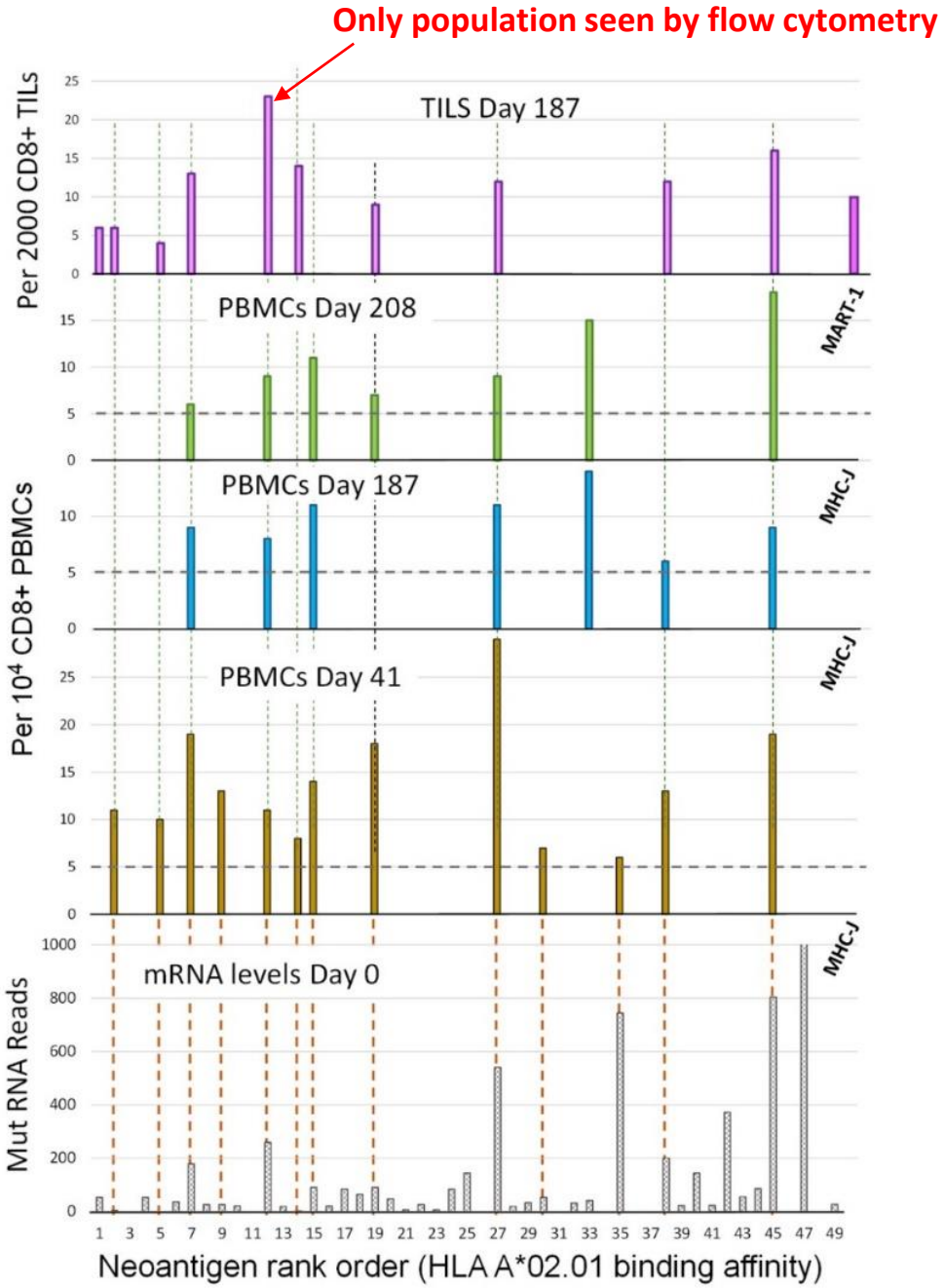
Kinetic study of neoantigen-specific PBMCs



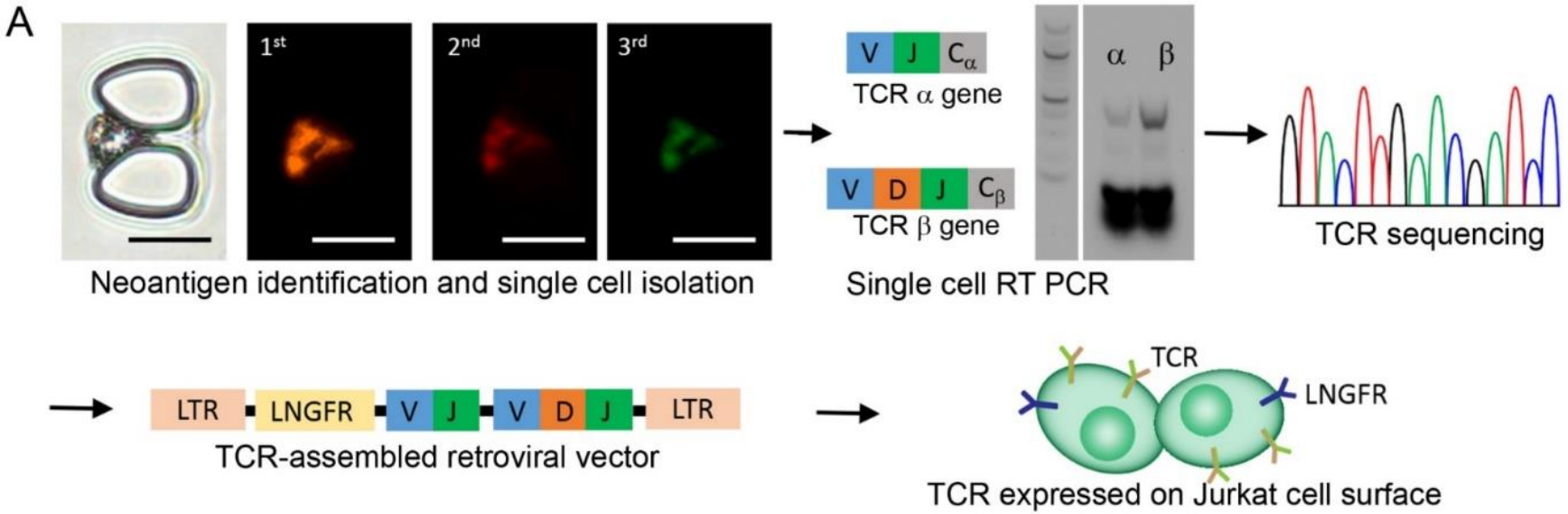


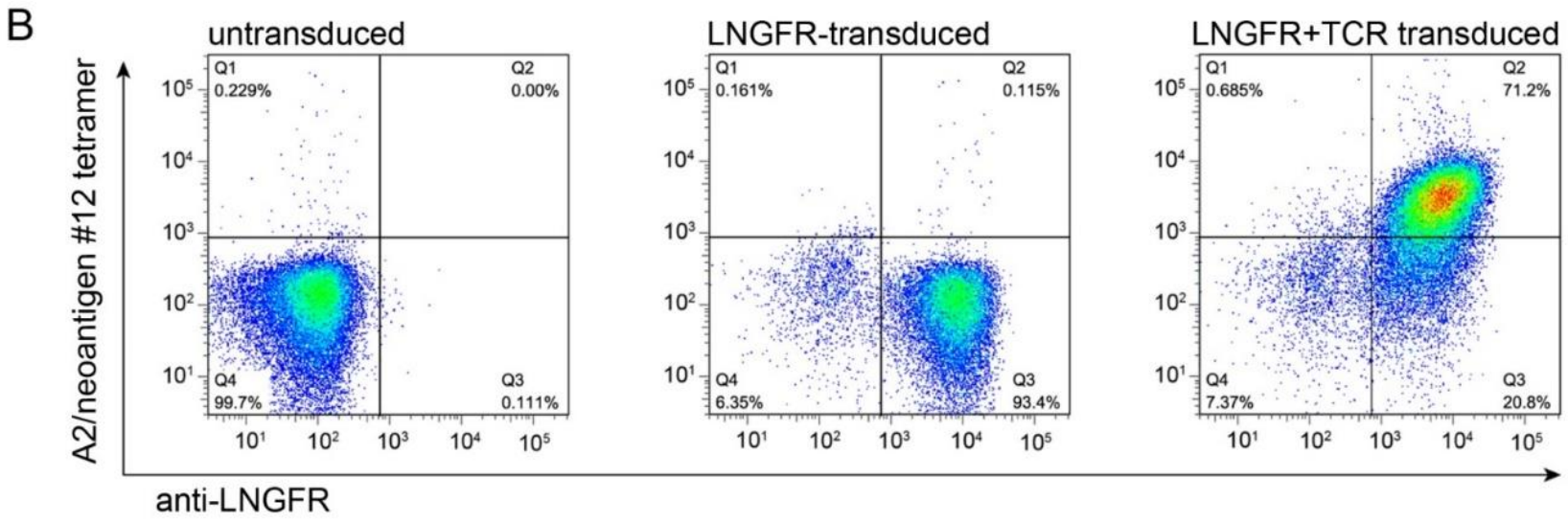
No populations detected

- **8 of 12 most highly expressed transcripts yield associated neoantigen-specific populations**
- **For top 15 MHC binders, 7 (~45%) yield populations**
- **For bottom 35 binders, 6 (~15%) yield populations**



Capturing the T cell receptor α/β genes





Melanoma Research Alliance

S↑2C

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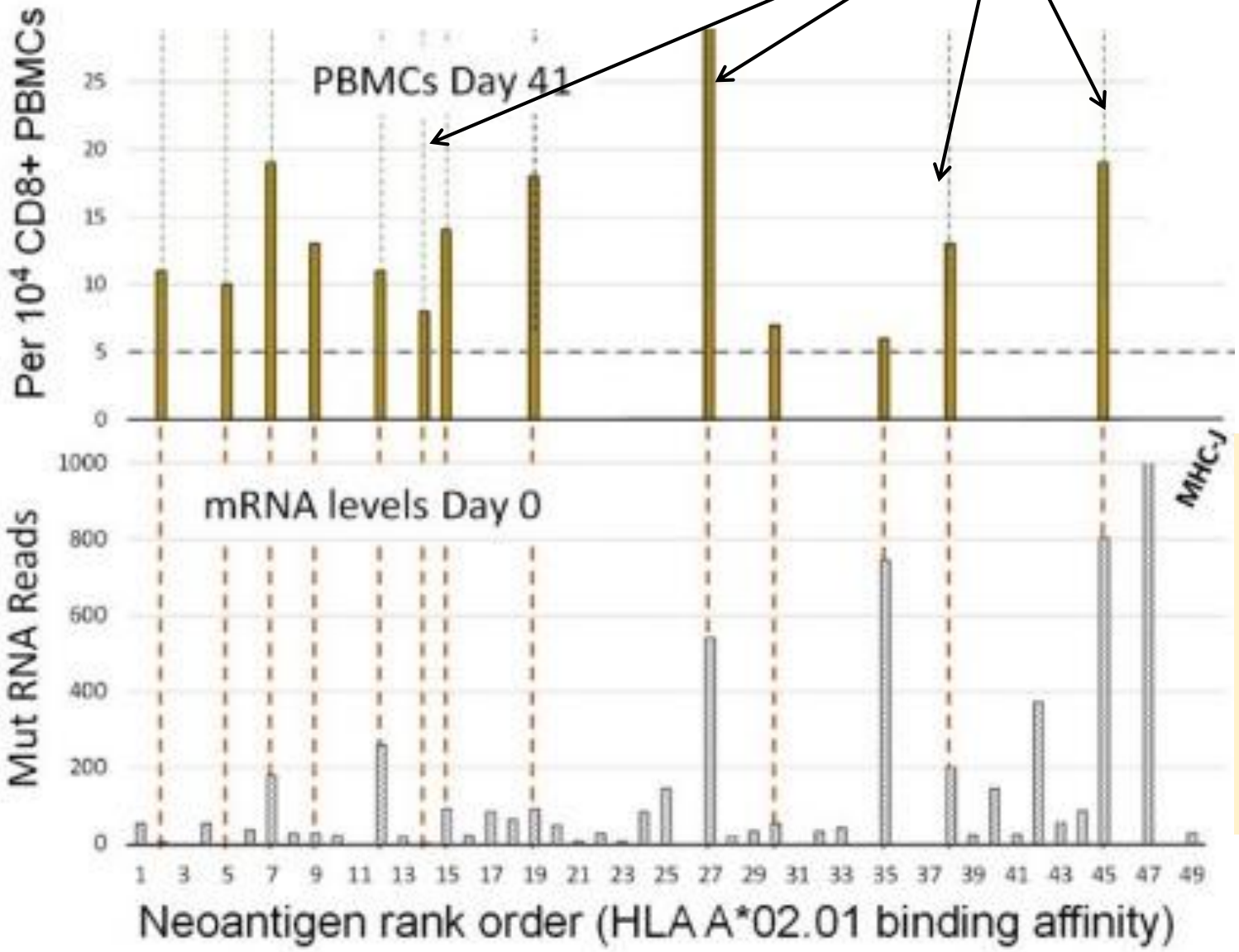
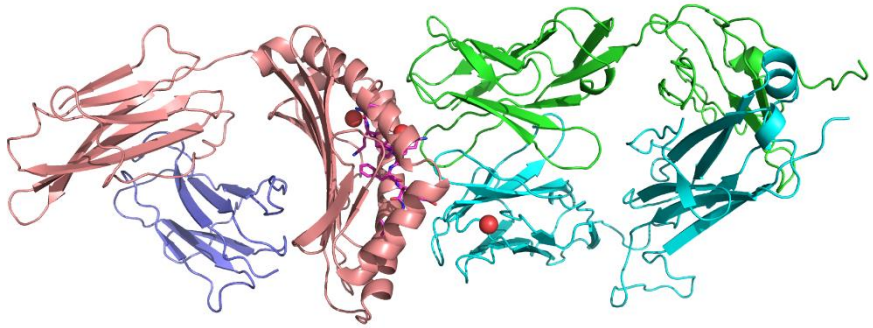
Matching these (likely) polyclonal antigen-specific T cell populations with the TCRa/b genes

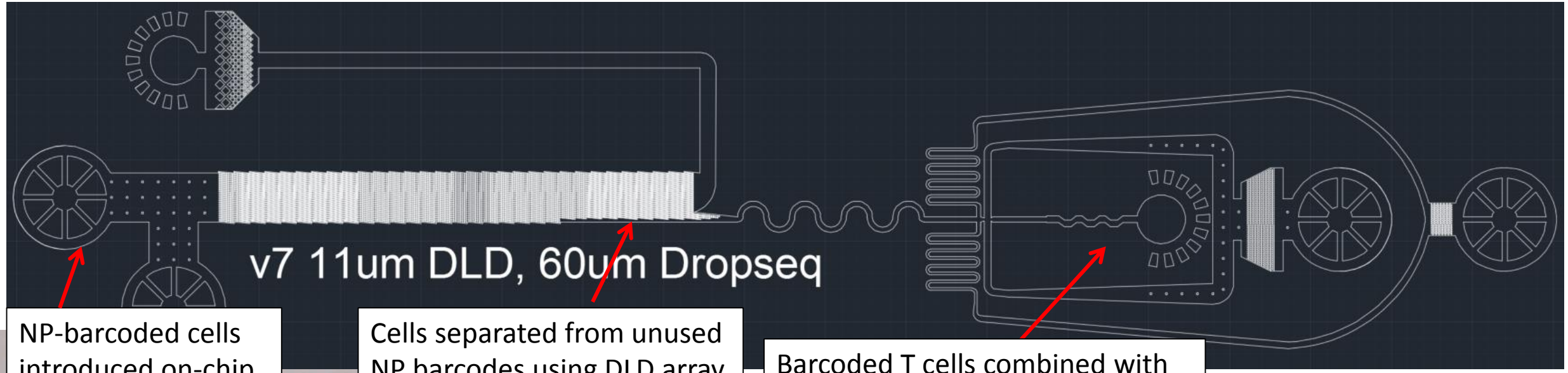




Mark Davis (Stanford) has developed a method to do this for relatively high abundance antigen-specific T cells

Ton Schumacher has shown that most CD8+ T cells within a tumor are NOT tumor reactive

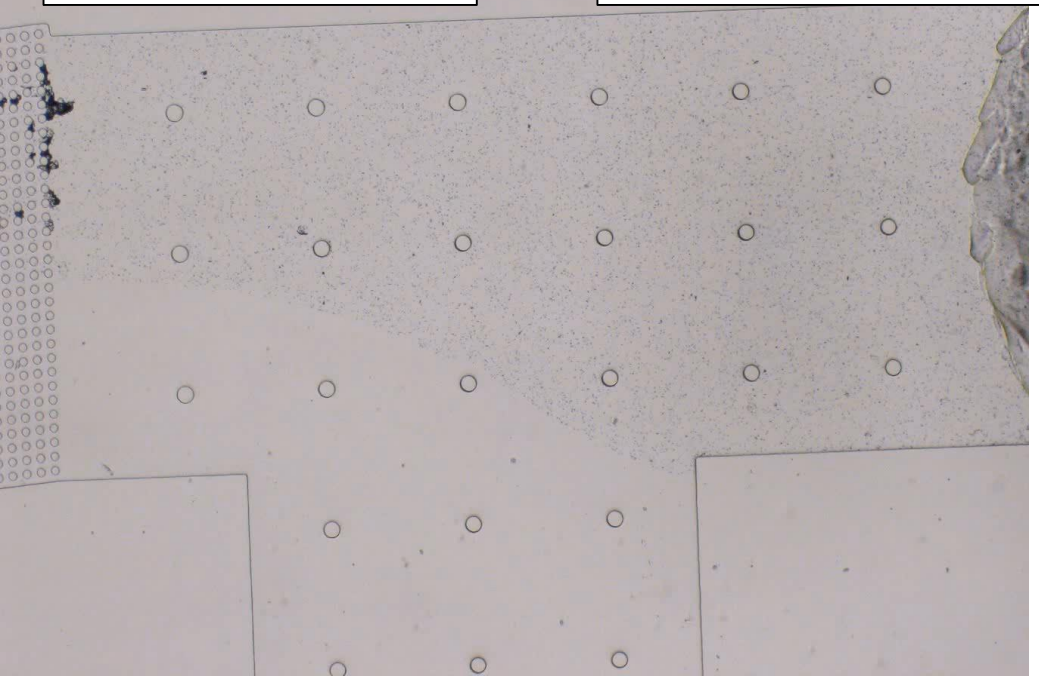
Microchip to Facilitate MHC/Antigen/TCRα/β gene Pairing



NP-barcoded cells introduced on-chip

Cells separated from unused NP barcodes using DLD array

Barcoded T cells combined with RT-PCR reagents, entrained in microdroplets, and collected here



Won Jun Noh

Songming Peng

60 micrometer droplet

RT + PCR (30 cycles) done in intact droplet

Lysed cell

NP

Biotin-photocleavable group

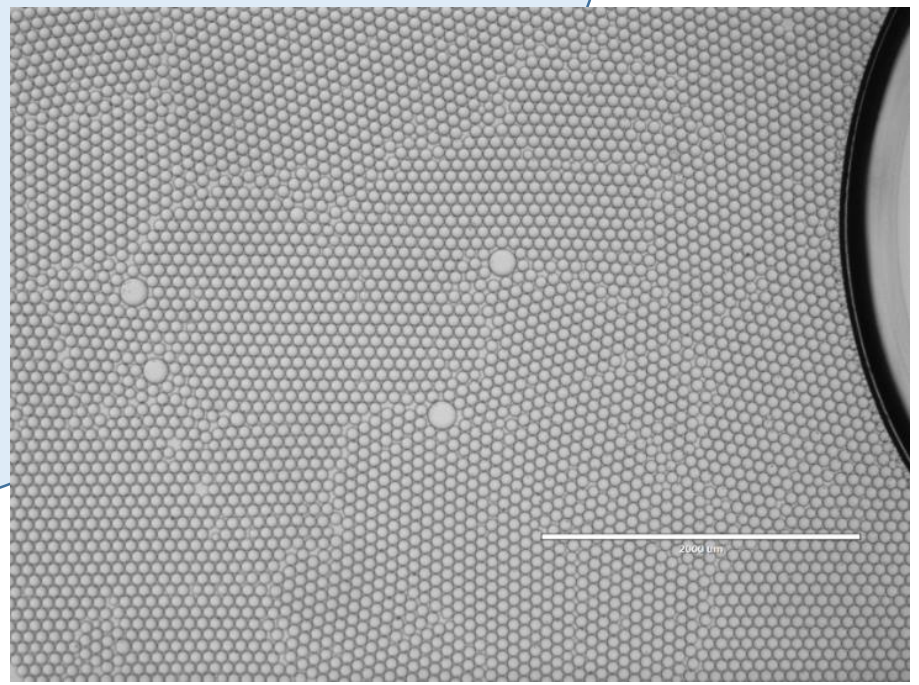
Universal Sequence (for illumina sequencer)

UMI (8-mer)

Neoant i.d. 6-mer

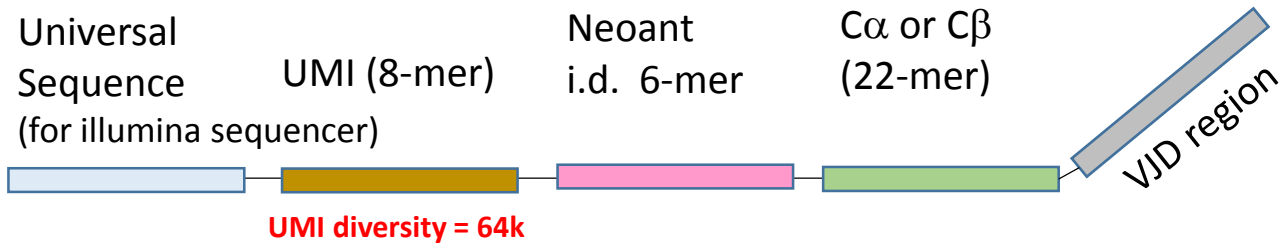
Cα or Cβ (22-mer)

VJD region



Uniform droplet size and intact droplets after 35 cycles PCR (RT- PCR done within droplets – big difference from drop-RNA-seq methods)

Analysis pairs multiple antigens with cognate TCR a/b chains in a single sequencing run



TGTGCCAGCAGTACCGTCTCCGGGGCCCCCAGCGAGCAGTTCTTCCASSTTGTGCCAGCAGTACCGTCTCCGGGGCCCCCAGCGAGCAGTTCTTCCASST

TGTGCCACGAATACCGTCTCCGGGGCCCCCAGCGAGCAGTTAGCAGTTCTTGTGCCAGCAGTACCGGTGCCAGCAGTACCGTCGAGCAG

We get millions of reads that look similar to this; all 64k UMIs are represented

1. Get rid of reads of < 100 base pairs
2. Given UMI at least 80% single neoantigen. Lowers UMI count to 40k.
3. Define S/N > 10 for a given UMI (lowers count to 30k).

TCR Sequencing Data after cleaning algorithm applied

CMV	UMI	CDR3 AA	V.gene
1	1113	CASSYQTGTIYGYTF	TRBV6-5
2	177	CLE*IME~SQGNLIF	TRAV4
3	86	CSARDRIGNTIYF	TRBV20-1
4	8	CAEDKDSTLTF	TRAV5
5	3	CAISAPTGPNTEAFF	TRBV10-3
6	2	CASSRALASGIDEQYF	TRBV4-3
7	1	CASSQSGP~DRAQIRYF	TRBV23-1
8	1	CA[illegible]DSAEADTQYF	TRBV5-4
9	1	CS[illegible]ALASGIDEQYF	TRBV4-3
10	1	CAS[illegible]ANRQQNEQFF	TRBV7-9
11	1	CASSYPTGPIYGYTF	TRBV6-5
12	1	CASSY[illegible]	TRBV6-5
13	1	CASSPKTGTTYEQYF	TRBV6-5
14	1	CASREGVAVNTEAFF	TRBV7-6
15	1	GASSDQTGTSYGDPG	TRBV6-5
16	1	CASSAQSINQPQHF	TRBV7-9
17	1	CASSLRHW*PQHF	TRBV7-3
18	1	CASSLRHWQPQHF	TRBV7-3

noise

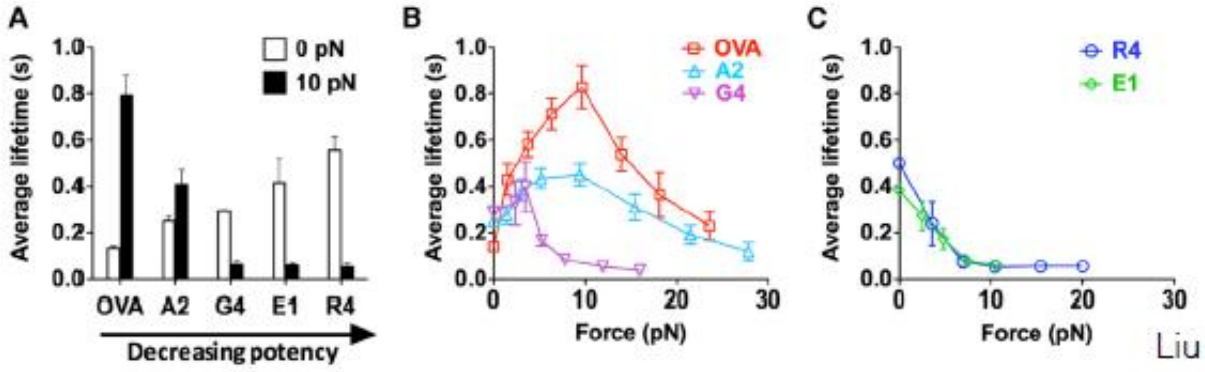
EBV	UMI	CDR3 AA	V.gene
1	16832	CSARDRIGNTIYF	TRBV20-1
2	11799	CAEDKDSTLTF	TRAV5
3	48	CASSYQTGTIYGYTF	TRBV6-5
4	1	CASSLRGIGA~LAGVNEQFF	TRBV7-8
5	1	CAIS[illegible]GPNTEAFF	TRBV10-3
6	1	CTP[illegible]GNTIYF	TRBV20-1
7	1	CRA[illegible]GKTIYF	TRBV20-1
8	1	WQCKDKVGNTKIF	TRBV20-1
9	1	CAEDKDSTLAF	TRAV5
10	1	CAEATA[illegible]	TRAV5
11	1	CAEDQDSTRTG	TRAV5

noise

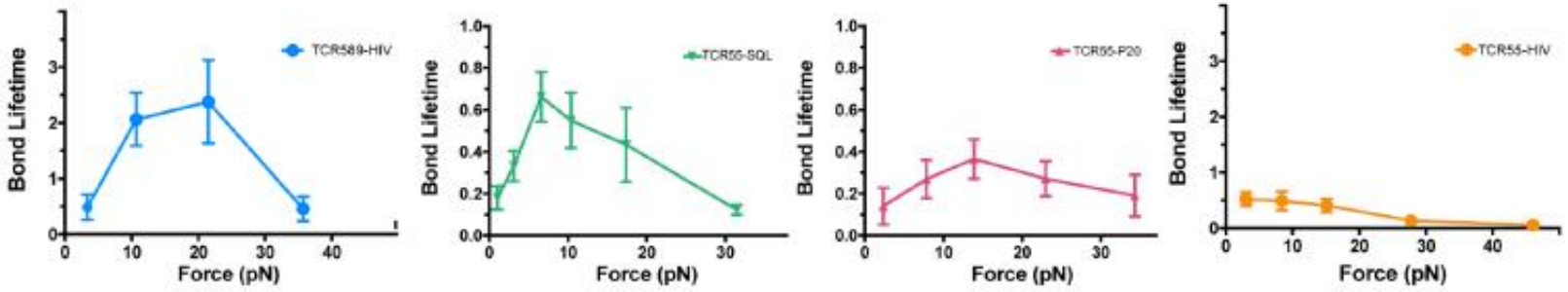
MHC-J	UMI	CDR3 AA	V.gene
1	6	CSARDRIGNTIYF	TRBV20-1

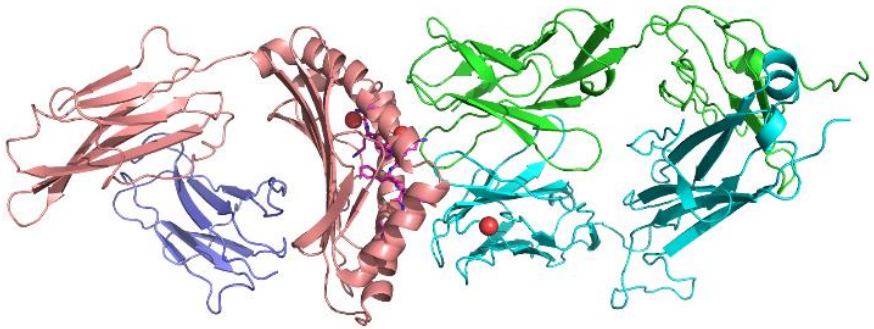
What makes a good TCR for TCR-engineered Adoptive Cell Therapy?

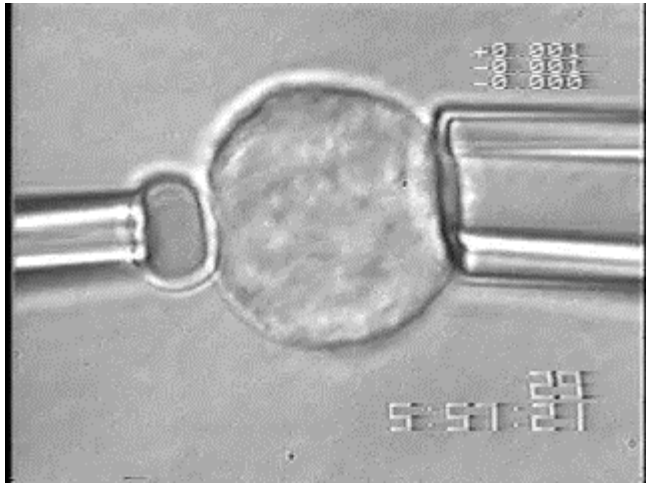
Do TCRs require a "catch bond" to activate?



Liu et al., Cell 2014

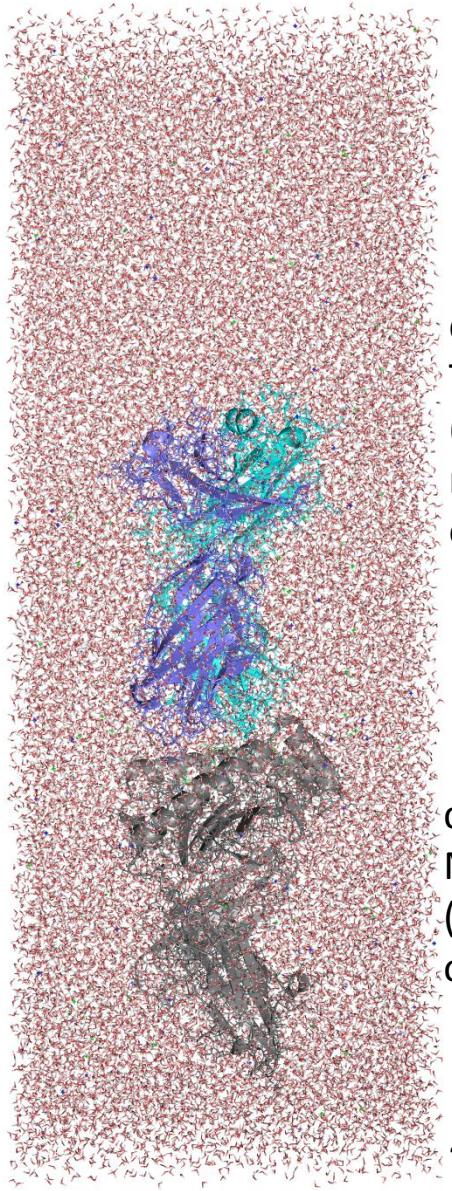






- Dembo, M., D. C. Torney, ., D. Hammer. 1988. The reaction-limited kinetics of membrane-to-surface adhesion and detachment. Proc. R. Soc. Lond. B Biol. Sci. 234:55–83.
- Thomas, W. E., V. Vogel, and E. Sokurenko. 2008. Biophysics of catch bonds. Annu. Rev. Biophys. 37:399–416.
- Marshall, B. T., M. Long, ., C. Zhu. 2003. Direct observation of catch bonds involving cell-adhesion molecules. Nature. 423:190–193.
- Liu, B., W. Chen, ., C. Zhu. 2014. Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. Cell. 157:357–368.
- V. Luca, … T.J. Ha, K.C. Garcia 2017. Notch-Jagged complex structure implicates a catch bond in tuning ligand sensitivity Science.

Molecular dynamics simulations: Inputs and outputs

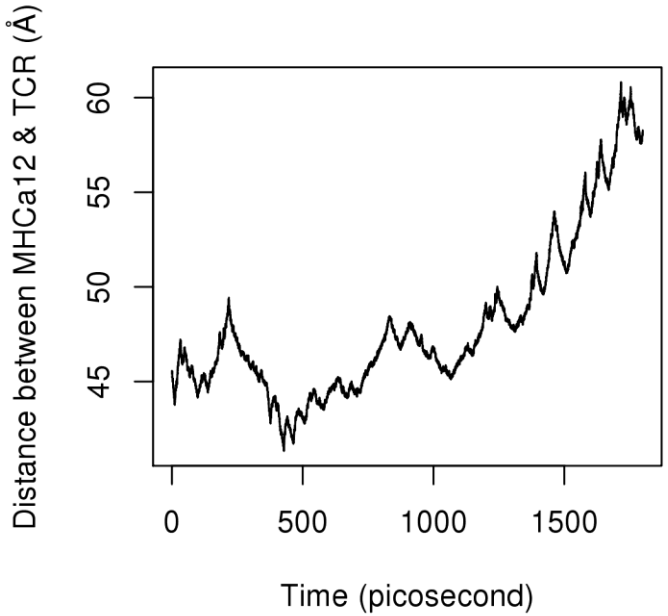


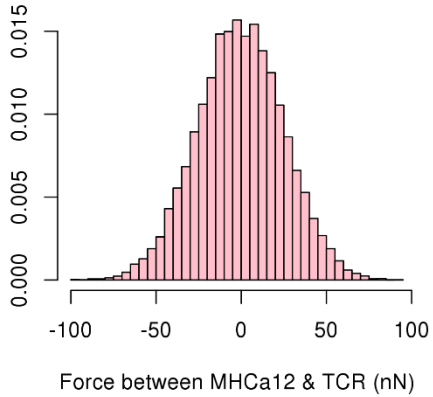
center of mass of TCRαβ (constant force or no force is applied on the COM)

"Bond length"

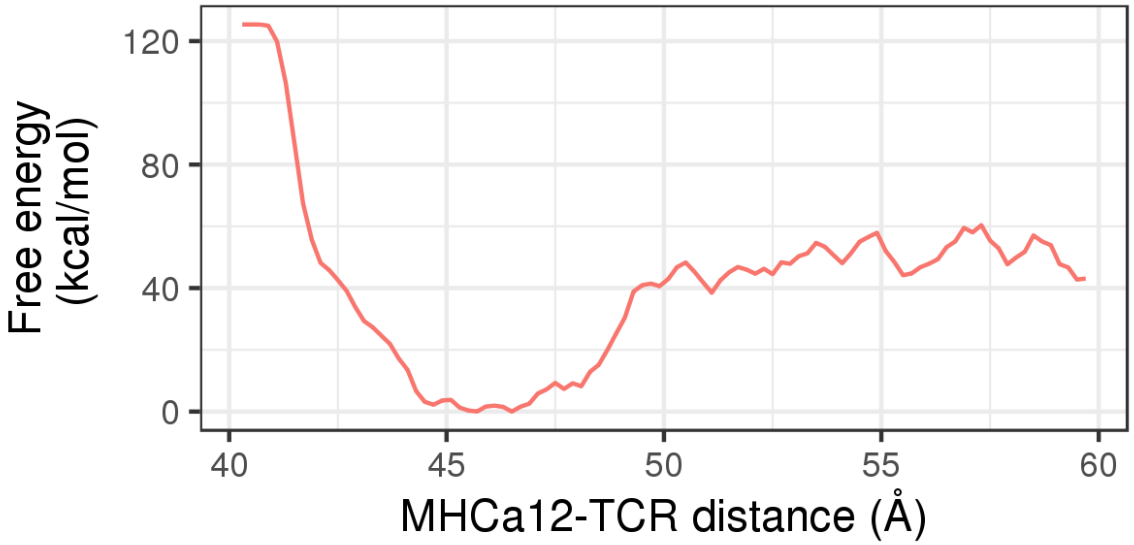
center of mass of MHC α12 domains (position and orientation fixed)

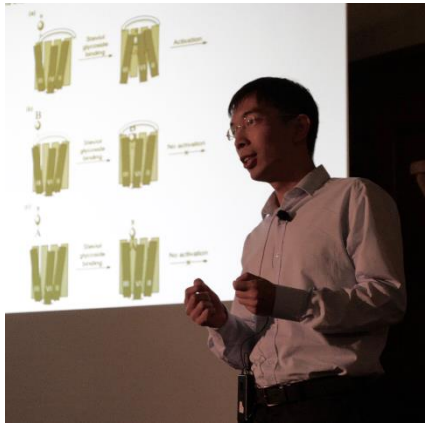
124K atoms
~5 hours on 12cpus & 4gpus



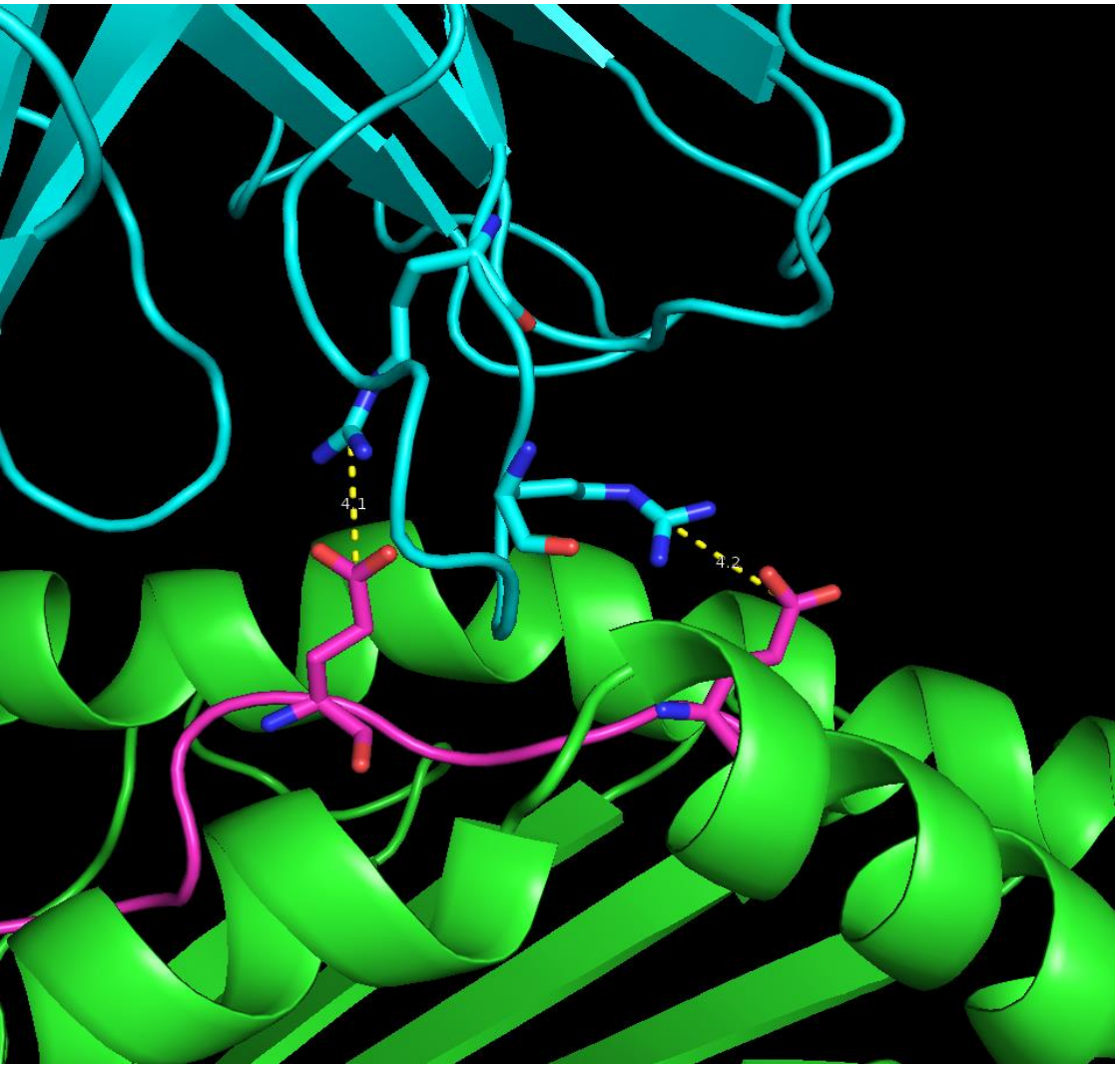


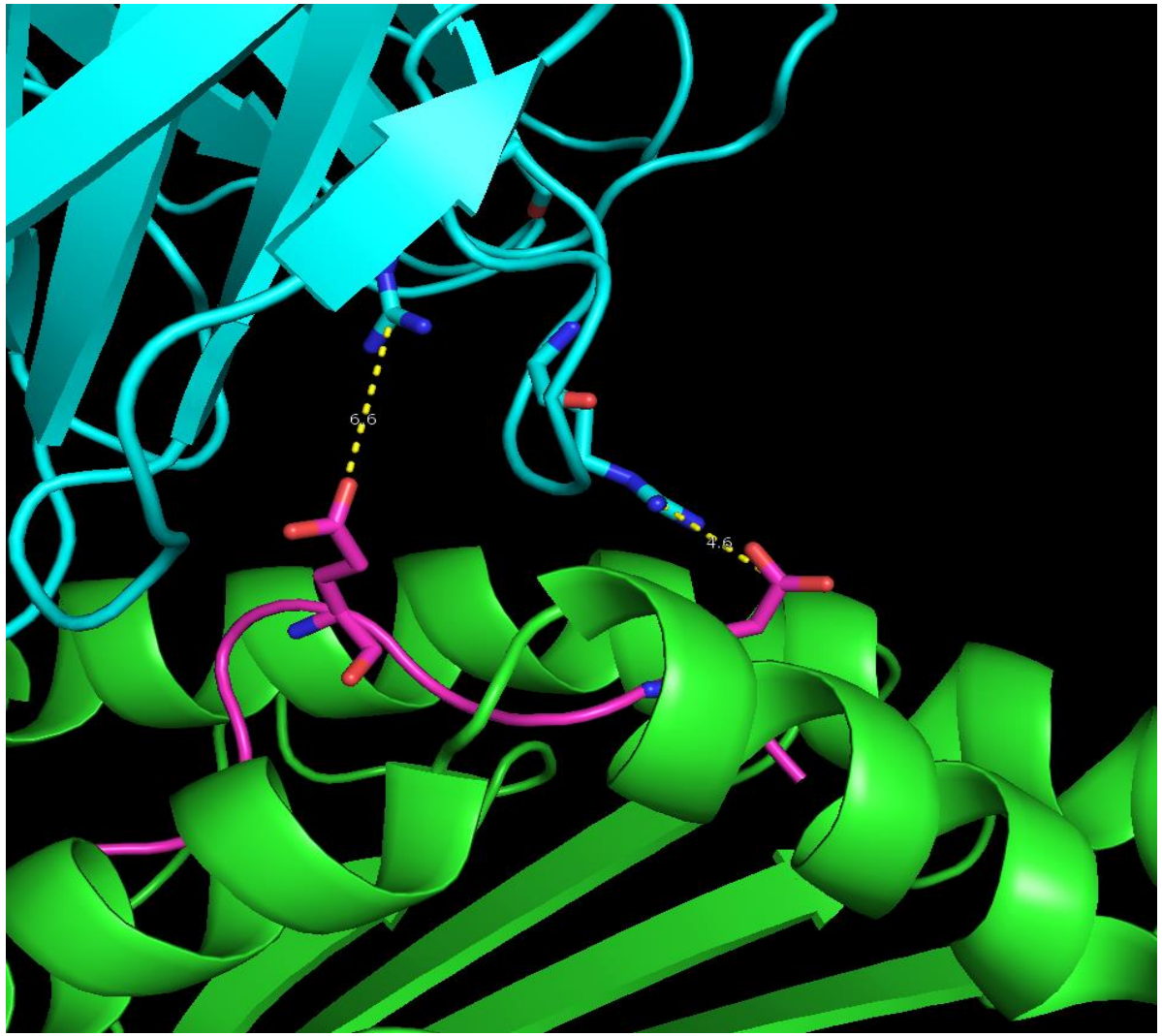
Fluctuating force is orders of magnitude larger than the applied force (~15pN)

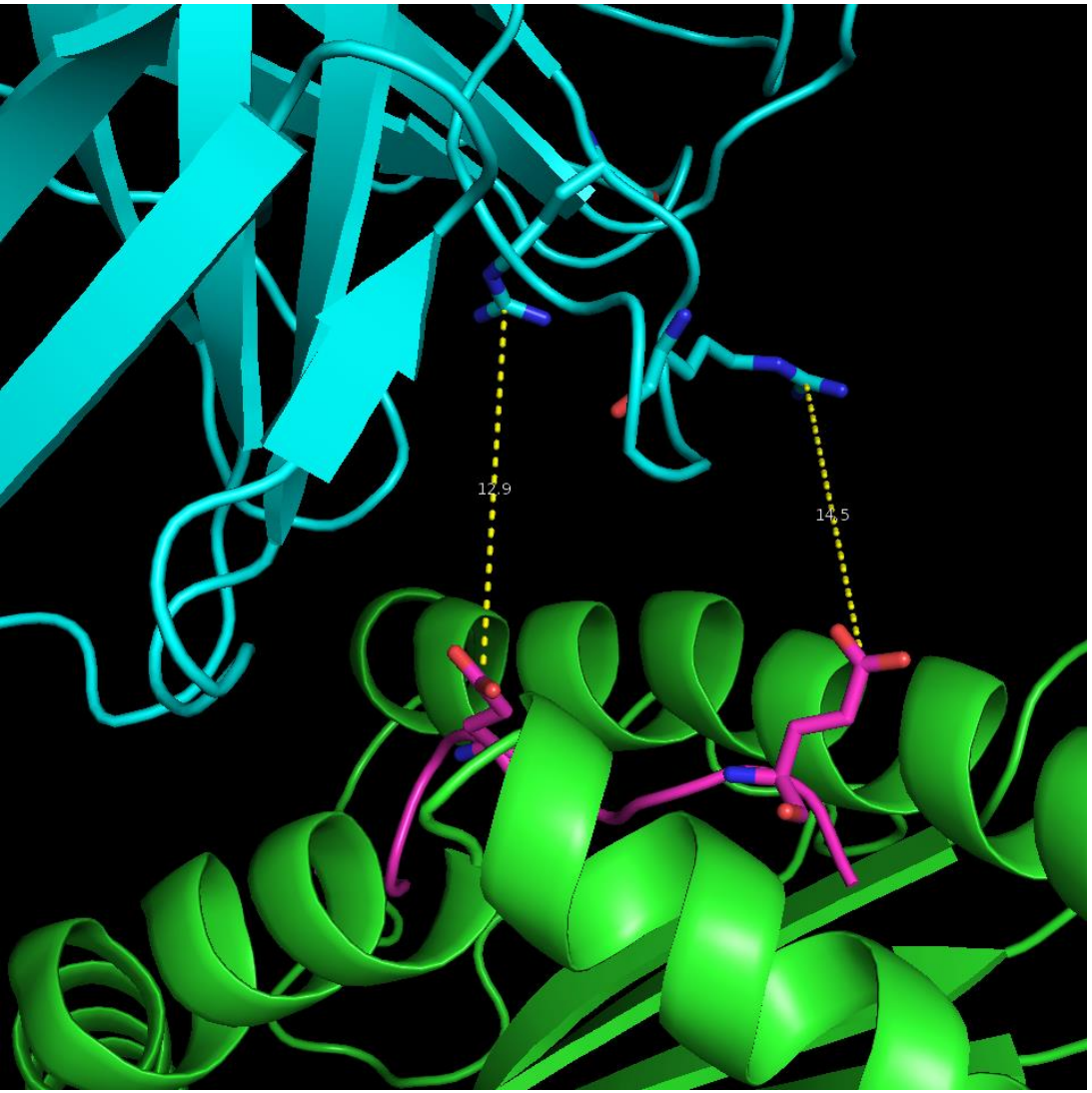


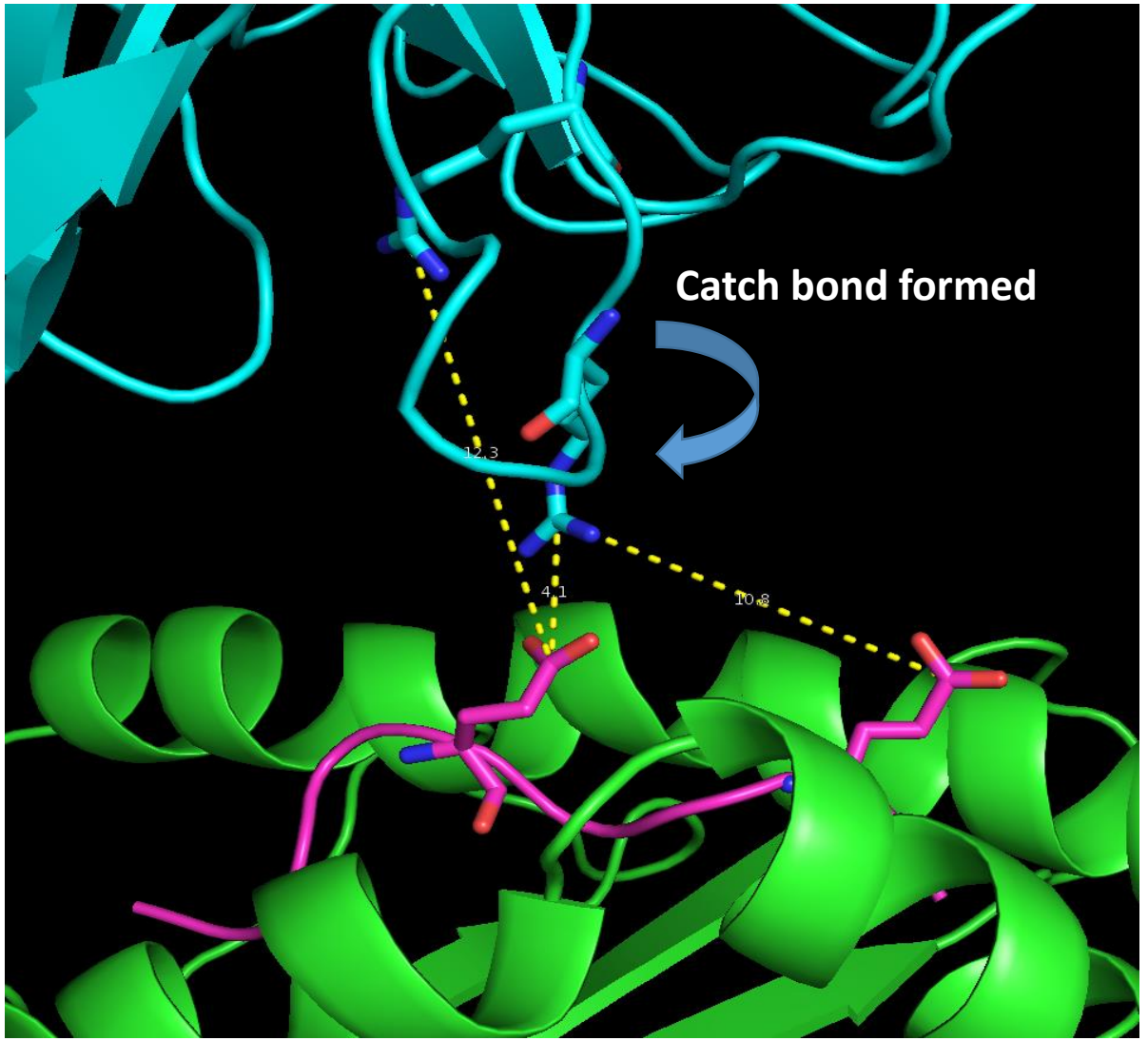


Dr. Fan Liu

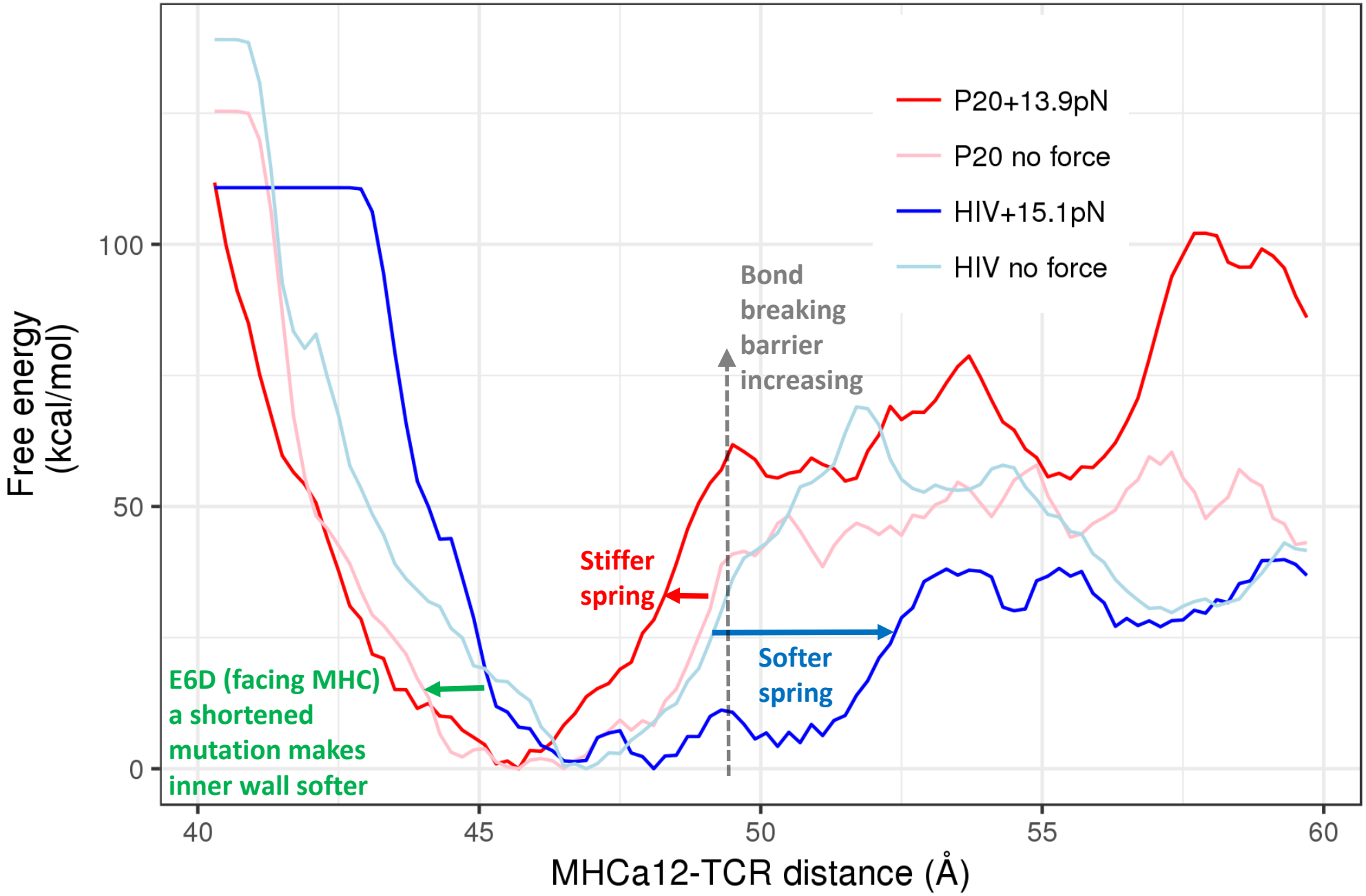

4.1
4.2


6.6
4.6


12.9
14.5

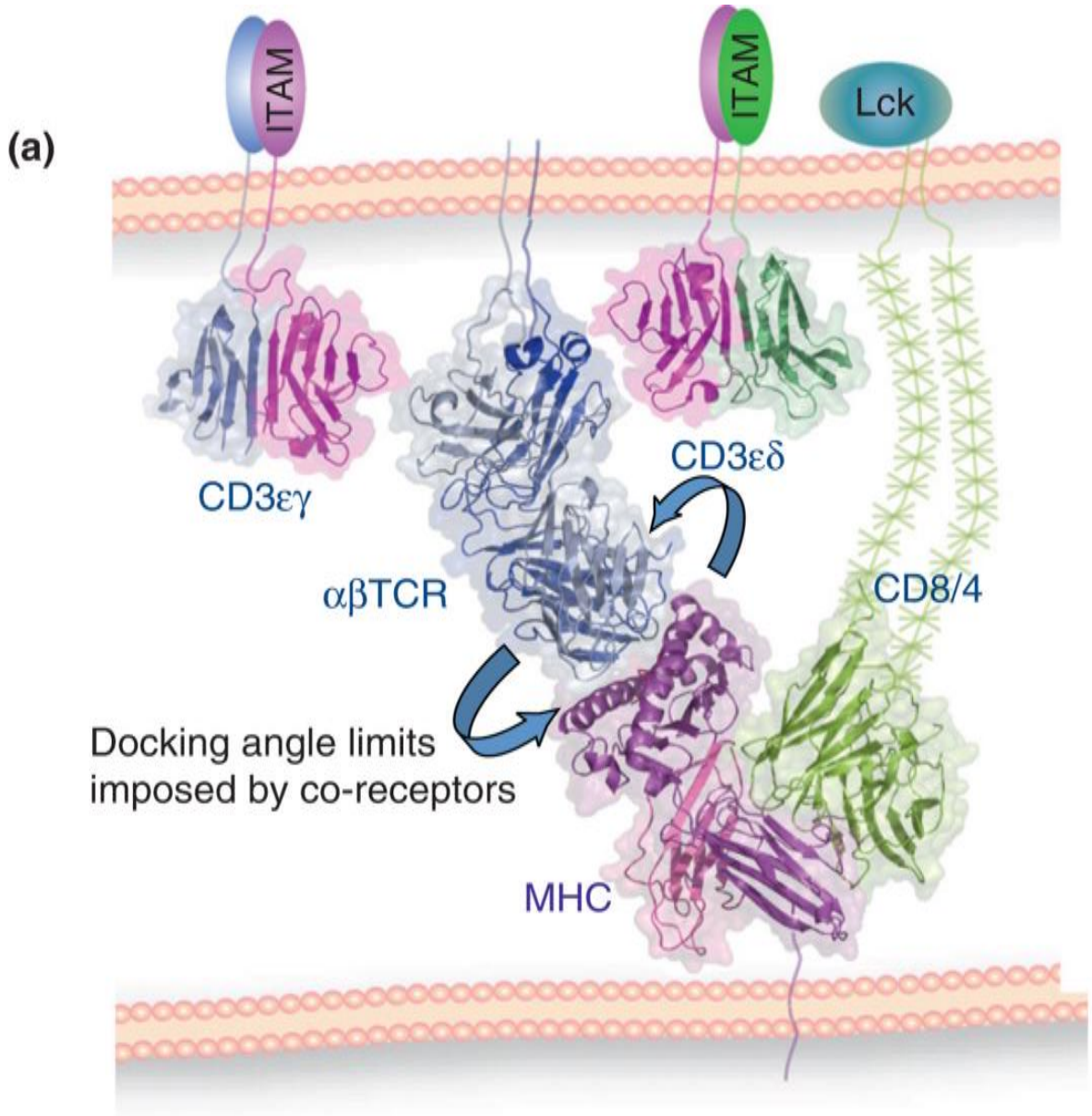

Catch bond formed
12.3
4.1
10.8

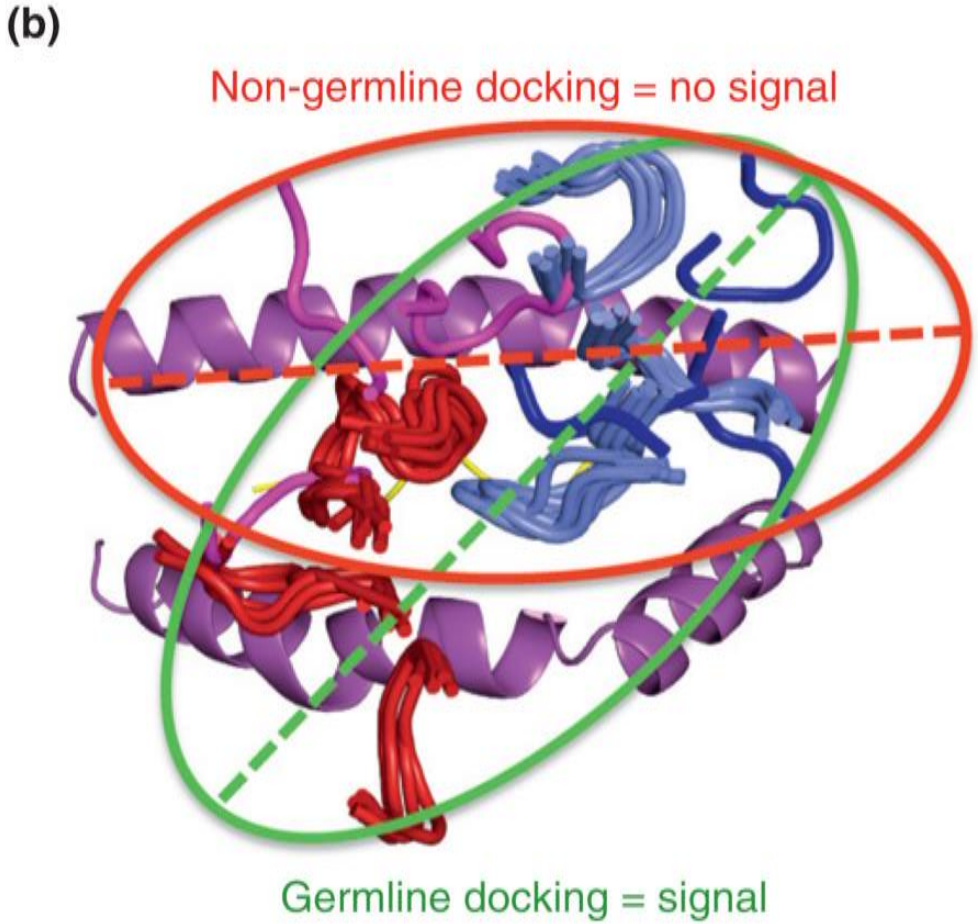
Applying constant forces changes the “spring stiffness” of the “pMHC-TCR bond”



CD8 imposes shear force on the TCR-pMHC interface

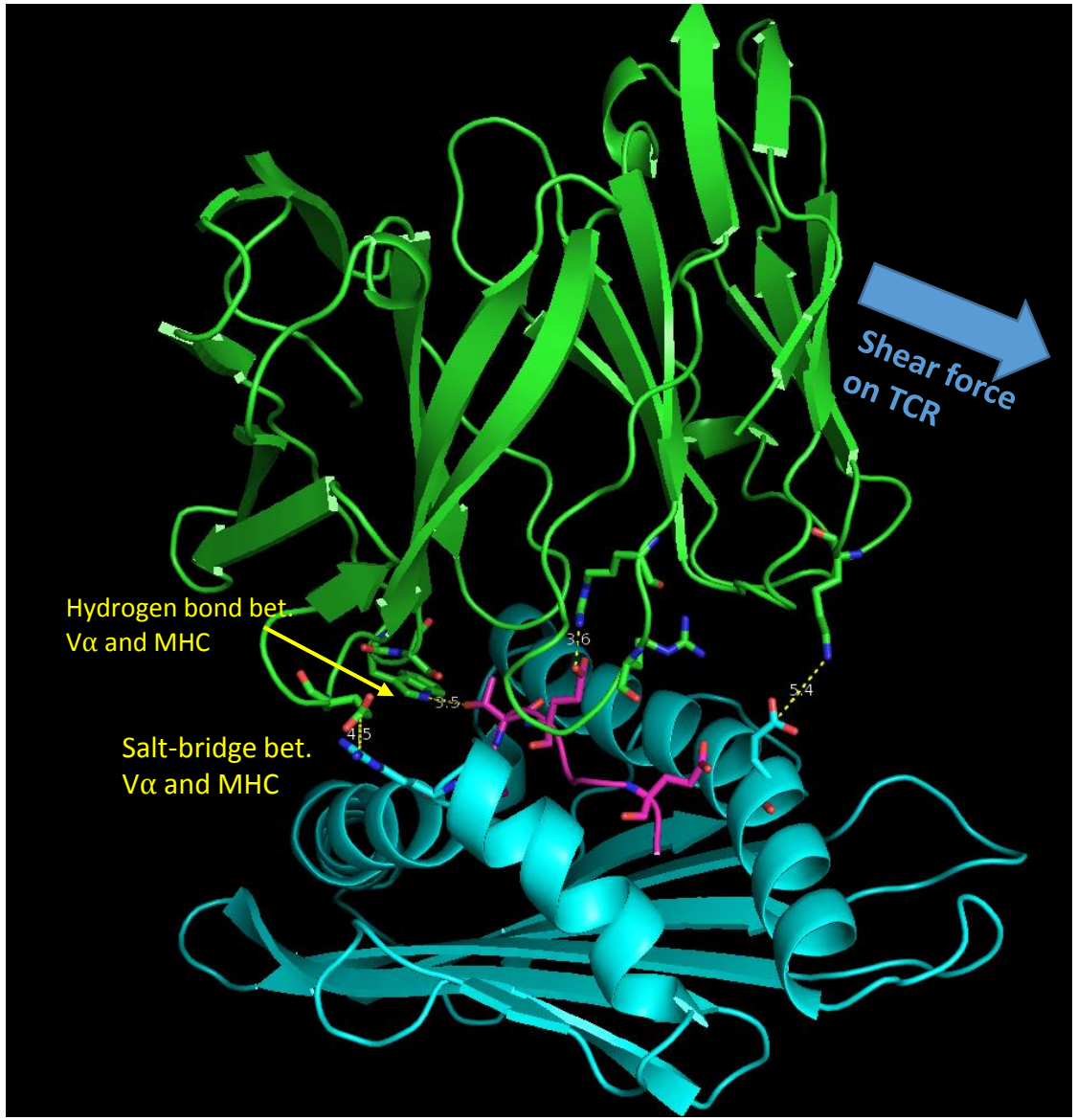
TCR docking angles from crystal structures vary between agnoists vs non-agonists antigens





Chris Garcia, Trends Immunol. 2012

+23 deg. Signal angle minimum



-20 deg. Signal angle minimum