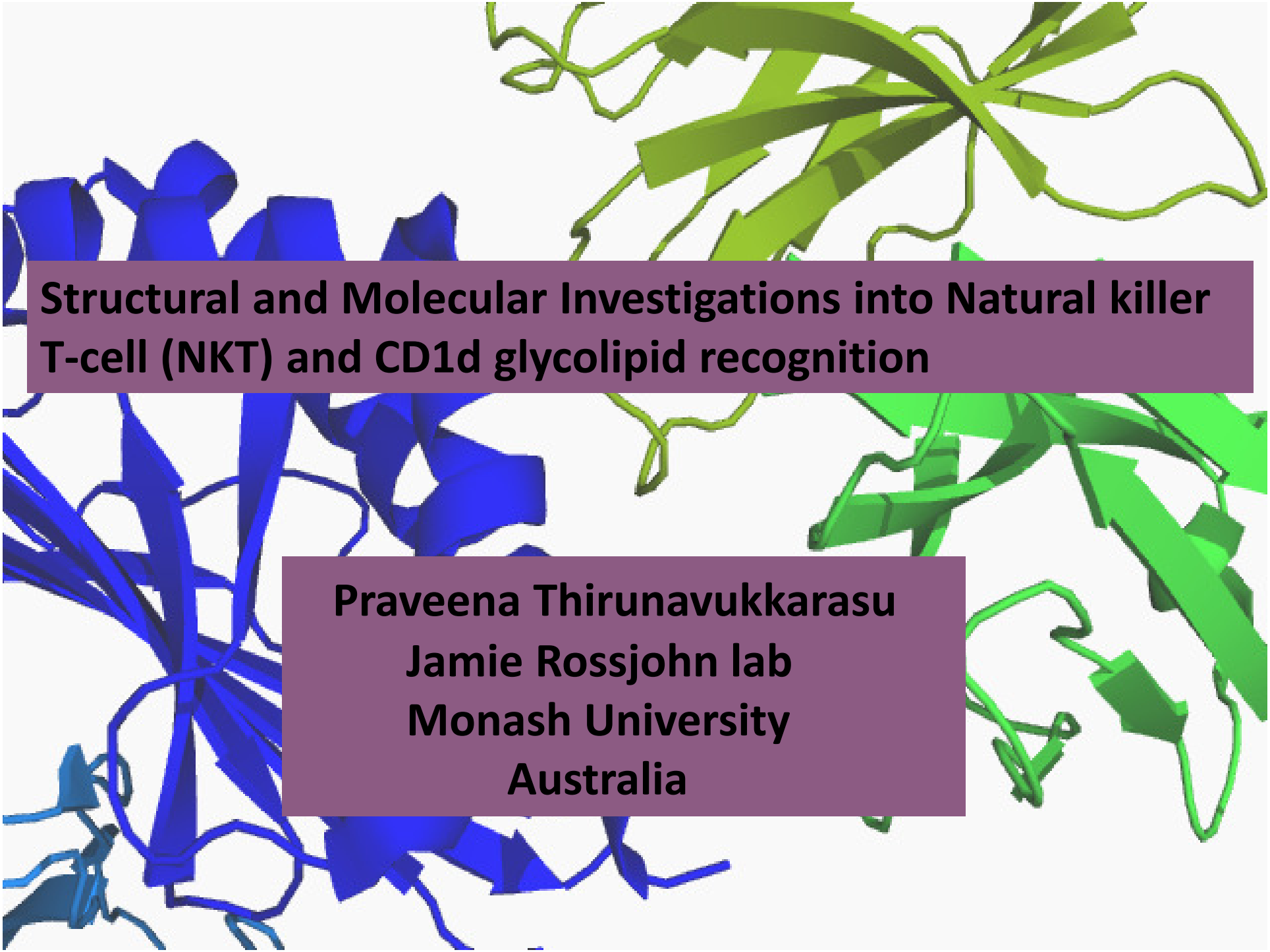


Structural and Molecular Investigations into Natural killer T-cell (NKT) and CD1d glycolipid recognition

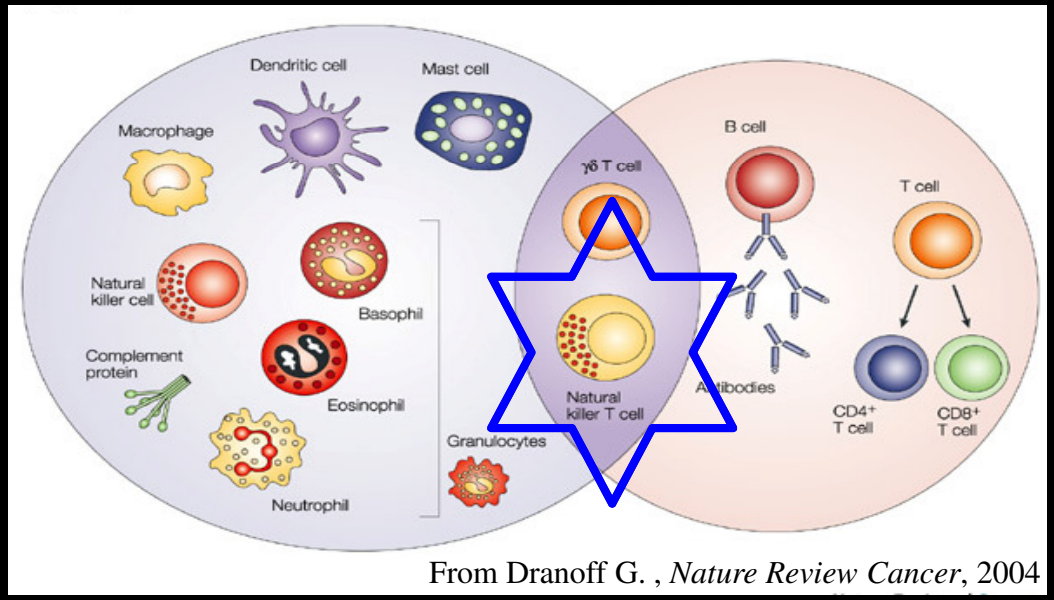
Praveena Thirunavukkarasu
Jamie Rossjohn lab
Monash University
Australia

Human Immune System

Pathogens

Innate Immunity

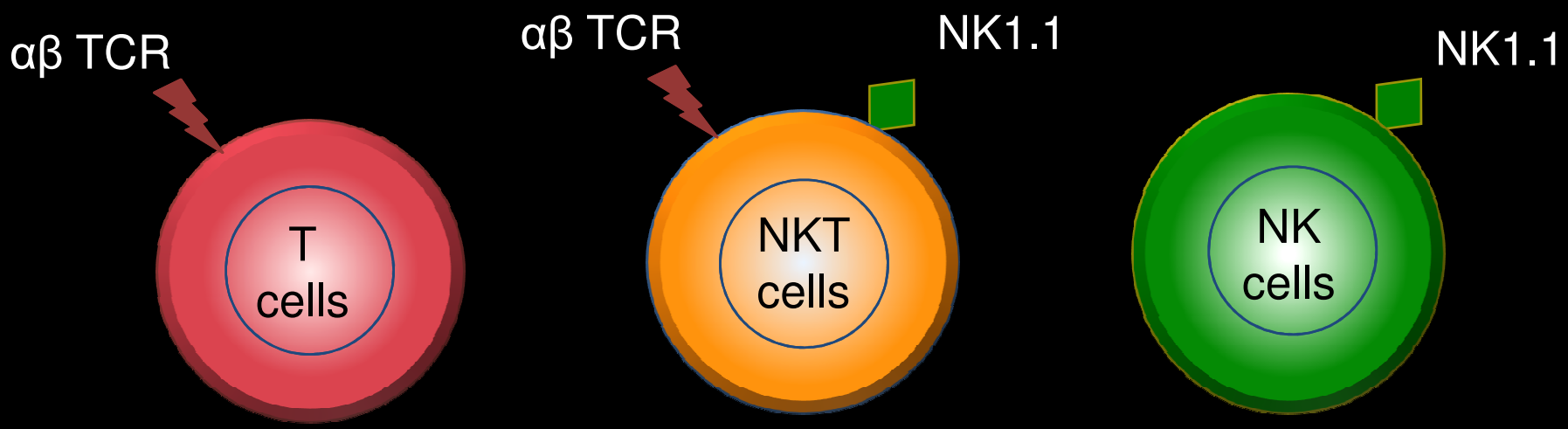
Adaptive Immunity



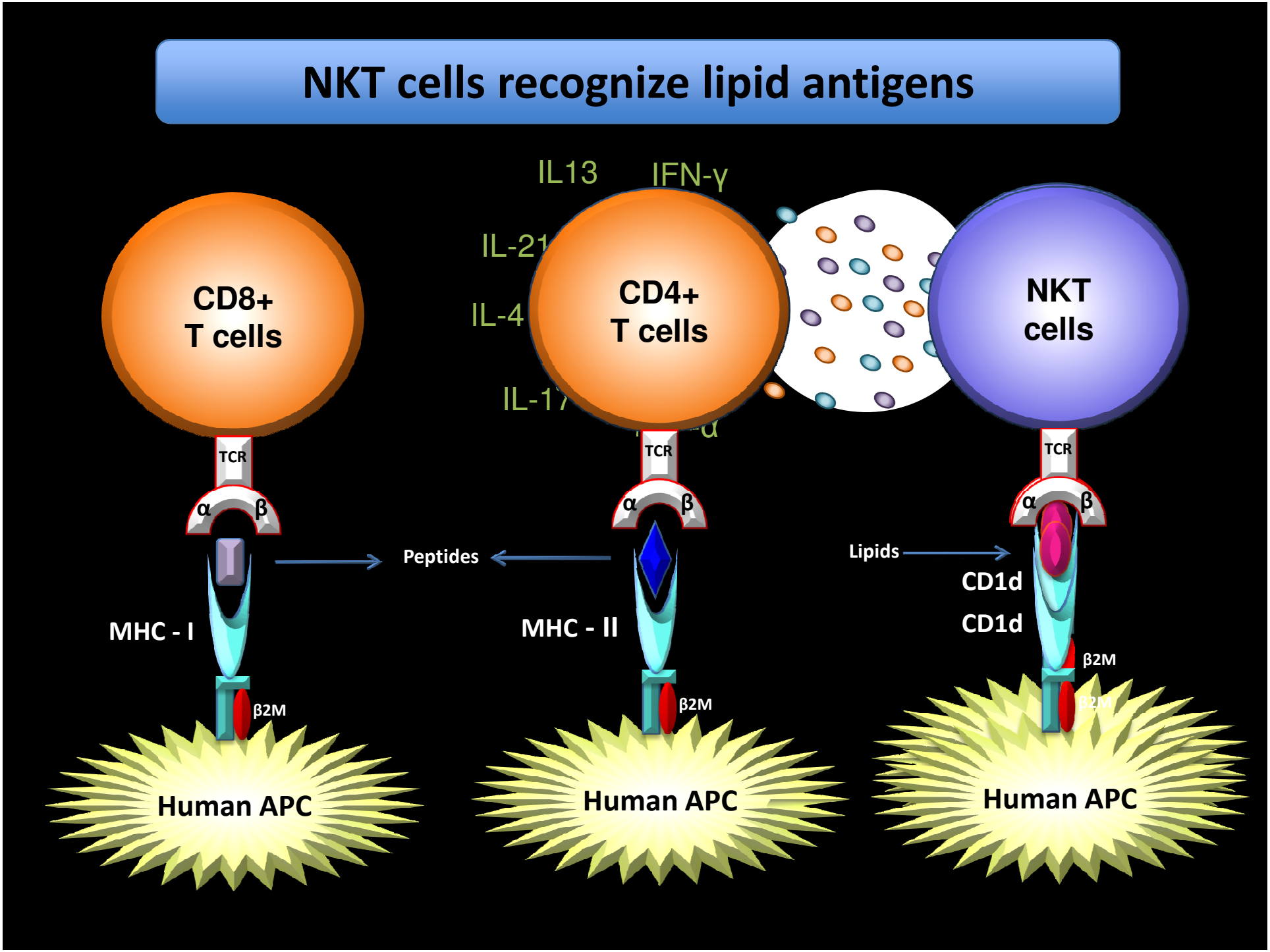
From Dranoff G. , *Nature Review Cancer*, 2004

- Bridge the gap between innate and adaptive immunity
- Function as `Innate-adaptive hybrids'
- Possess immunomodulatory potential

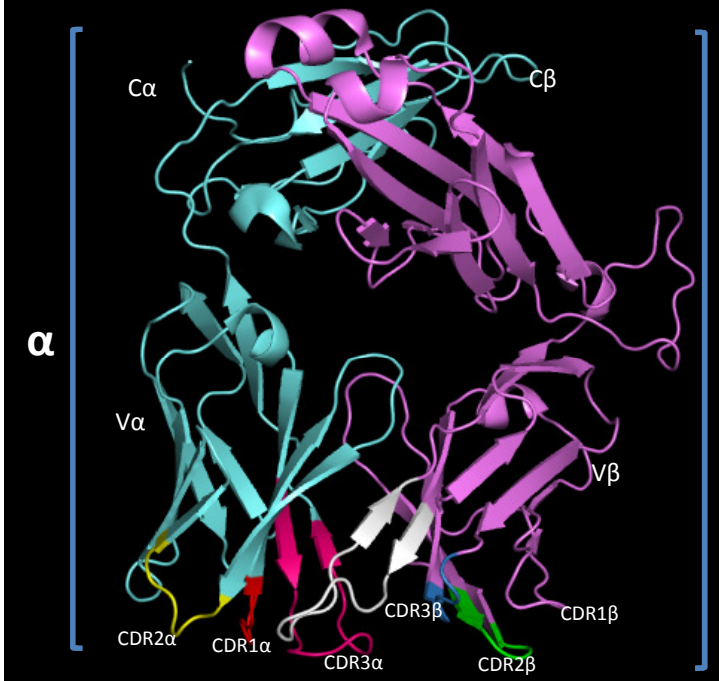
Natural Killer T (NKT) cells

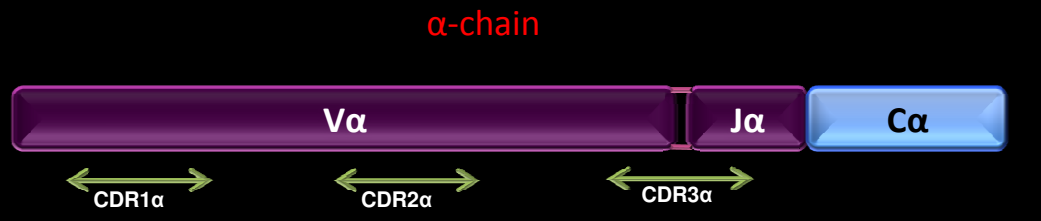


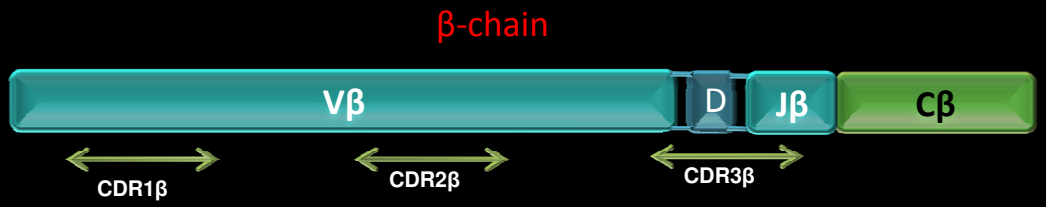
- Share properties of both conventional T cells and Natural Killer (NK) cells
- Express a T cell receptor (TCR) that allows them to recognize antigens
- Constitute ~ 0.1% of all peripheral blood T cells

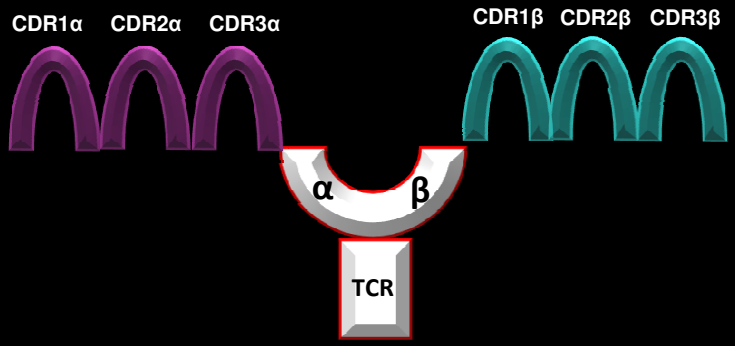

NKT cells recognize lipid antigens
IL13
IFN-γ
IL-21
IL-4
IL-17
CD8+
T cells
CD4+
T cells
NKT
cells
TCR
α
β
Peptides
Lipids
MHC - I
MHC - II
CD1d
CD1d
β2M
β2M
β2M
Human APC
Human APC
Human APC

T cell receptor (TCR)

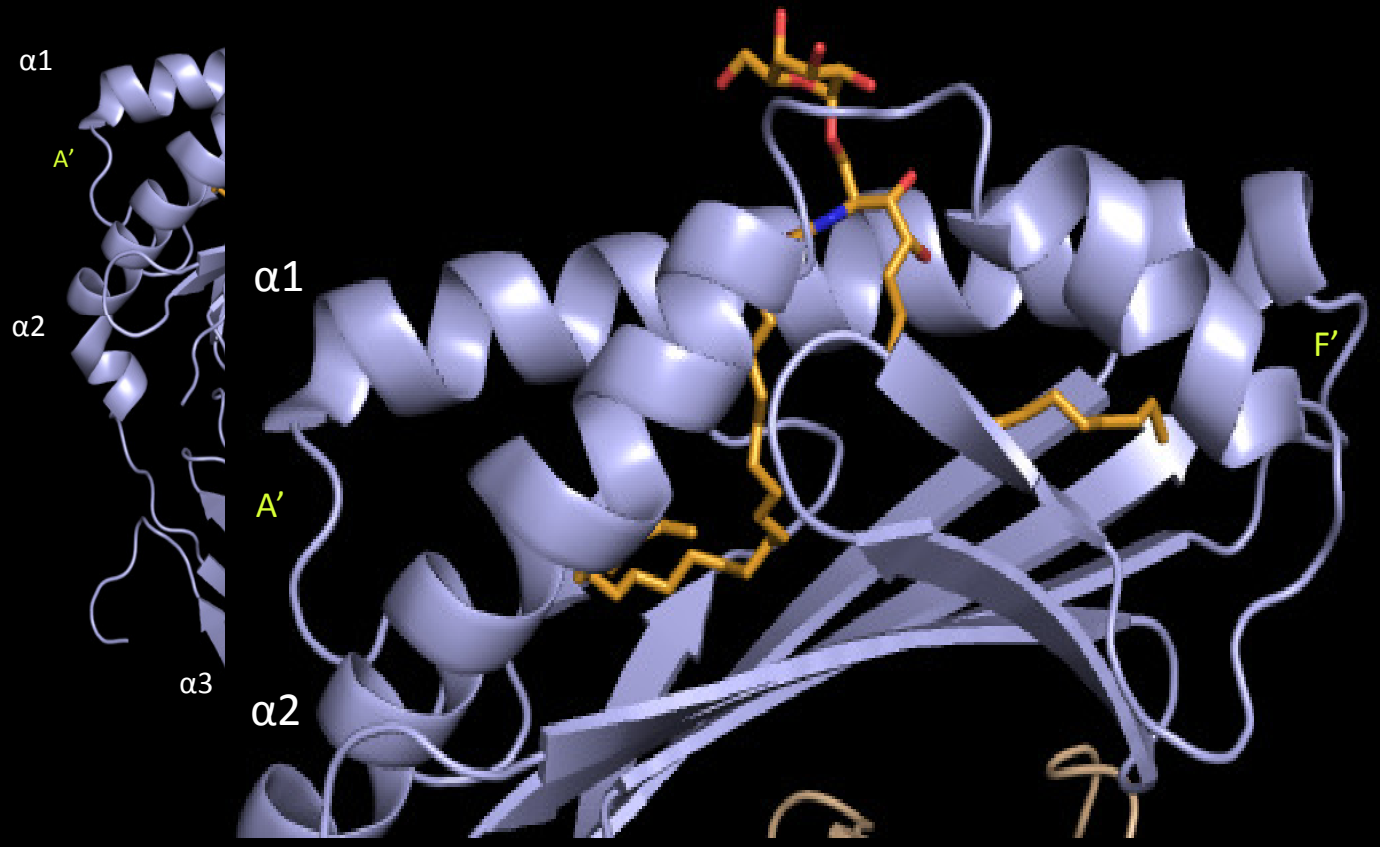








Structure of CD1d

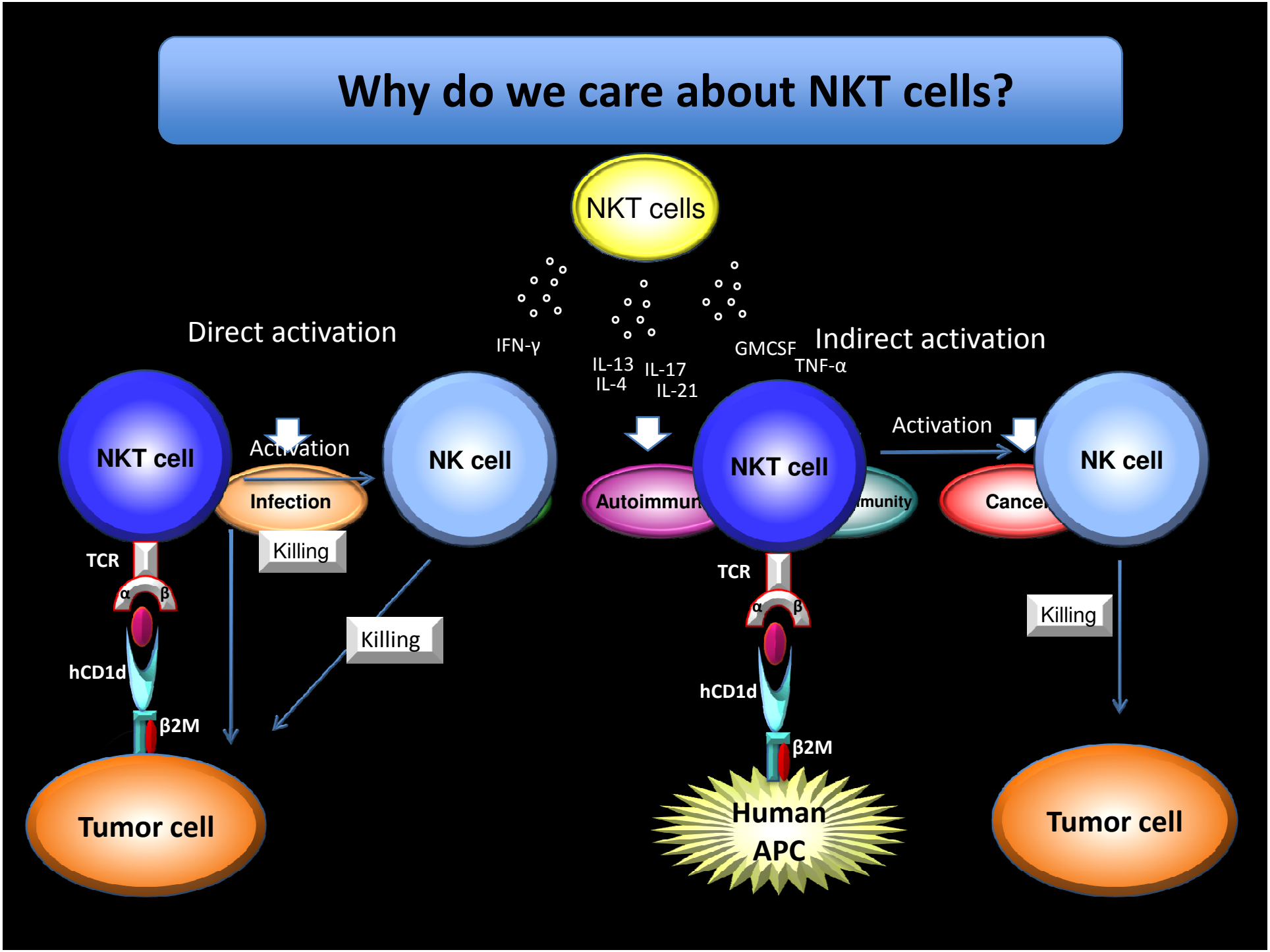


n

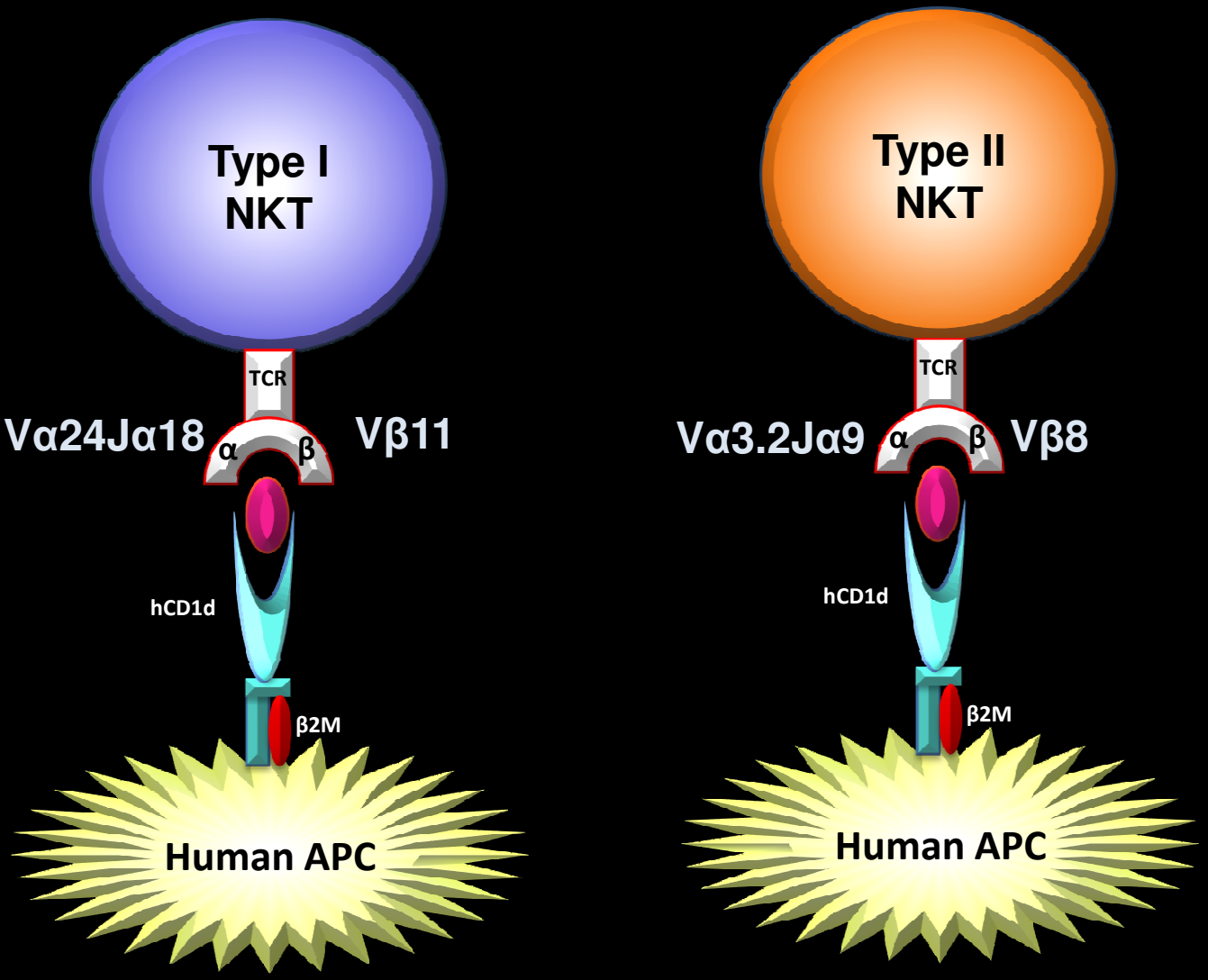
α2 and α3

ι2 domains

ımely A’ and F’


Why do we care about NKT cells?
NKT cells
IFN-γ
IL-13
IL-4
IL-17
IL-21
GMCSF
TNF-α
Direct activation
Indirect activation
NKT cell
Activation
Infection
NK cell
Killing
Killing
TCR
α
β
hCD1d
β2M
Tumor cell
NKT cell
Autoimmuni
mmunity
Activation
Cancer
NK cell
TCR
α
β
hCD1d
β2M
Killing
Human APC
Tumor cell

Types of NKT cells



Lipid antigen - α-Galactosylceramide

α-Gala

Immunity
Article

CellPress

The Identification of the Endogenous Ligands of Natural Killer T Cells Reveals the Presence of Mammalian α-Linked Glycosylceramides

Lisa Kain,[1] Bill Webb,[2] Brian L. Anderson,[3] Shenglou Deng,[3] Marie Holt,[1] Anne Costanzo,[1] Meng Zhao,[4] Kevin Self,[1] Anais Teyton,[1] Chris Everett,[1] Mitchell Kronenberg,[4] Dirk M. Zajonc,[4] Albert Bendelac,[5] Paul B. Savage,[3] and Luc Teyton[1,*]
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[2]Department of Molecular Biology, the Scripps Research Institute, La Jolla, CA 92037, USA
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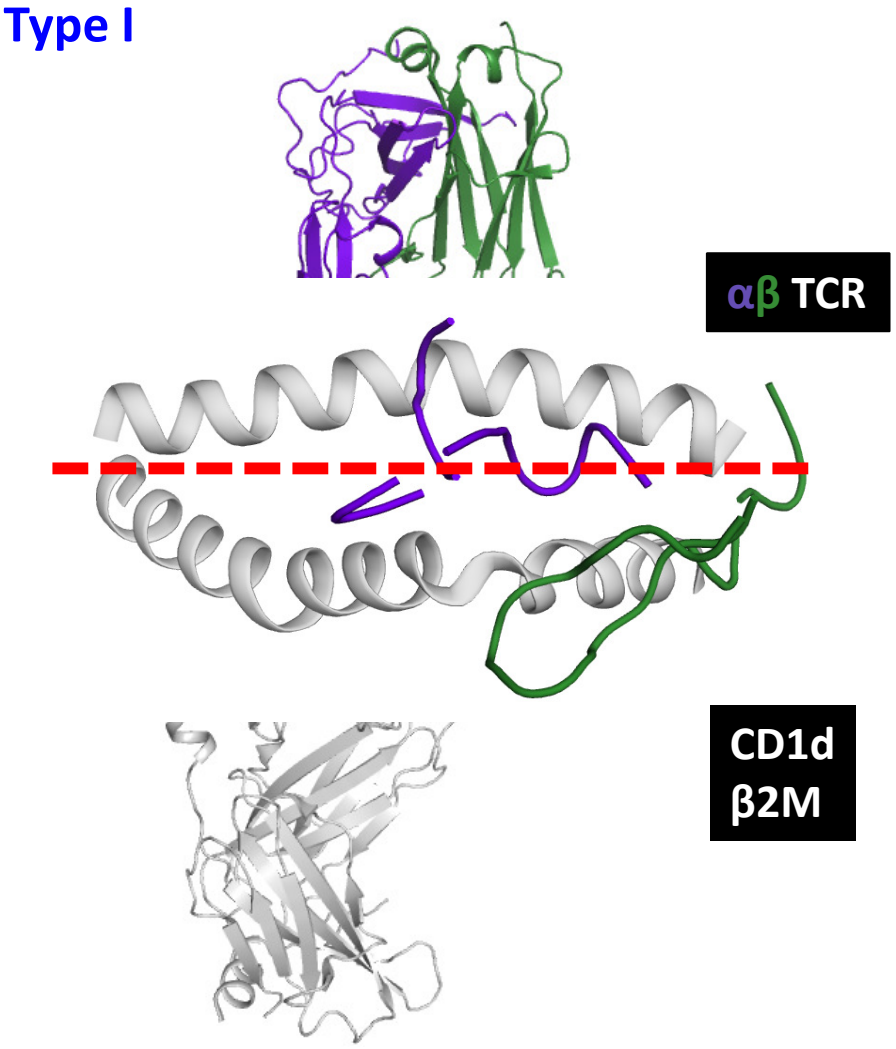
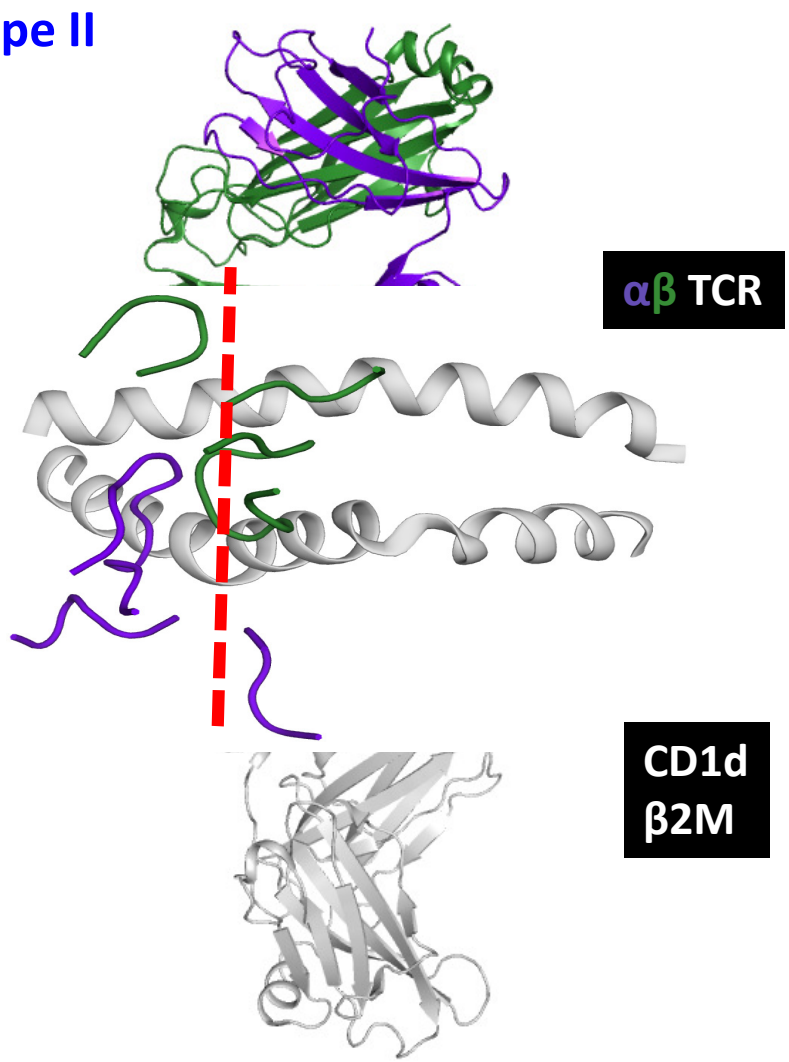
http://dx.doi.org/10.1016/j.immuni.2014.08.017

- Prototy
- Marine
- Lipid pc
- Carbohydrate head group interacts with TCR
- Currently in human phase I/II trials as anti-cancer agent

Type I and Type II NKT TCRs docking modes



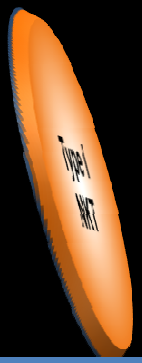
Borg *et al.*, *Nature*, 2007



Patel *et al.*, *Nature Immun.*, 2012

What does docking orientation signify?

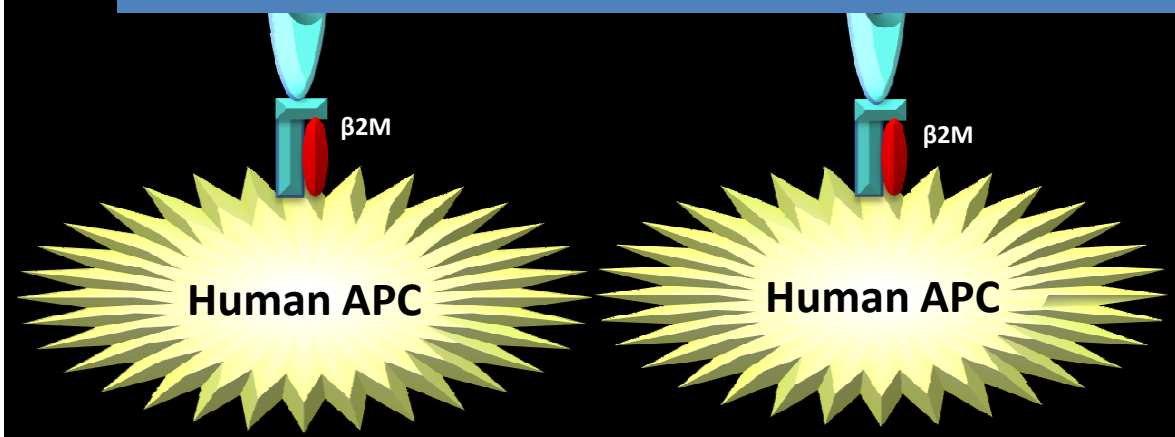
Type I



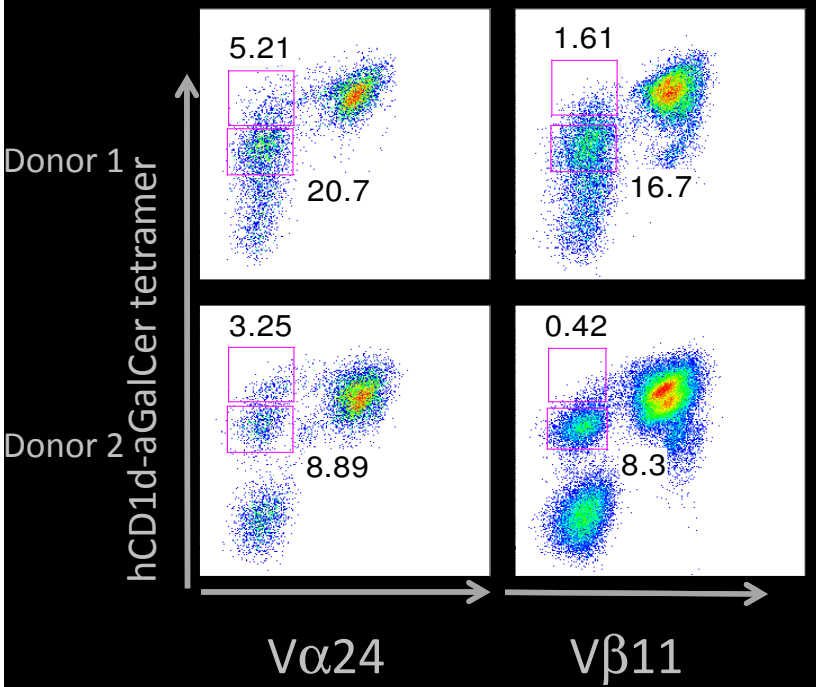
Type II

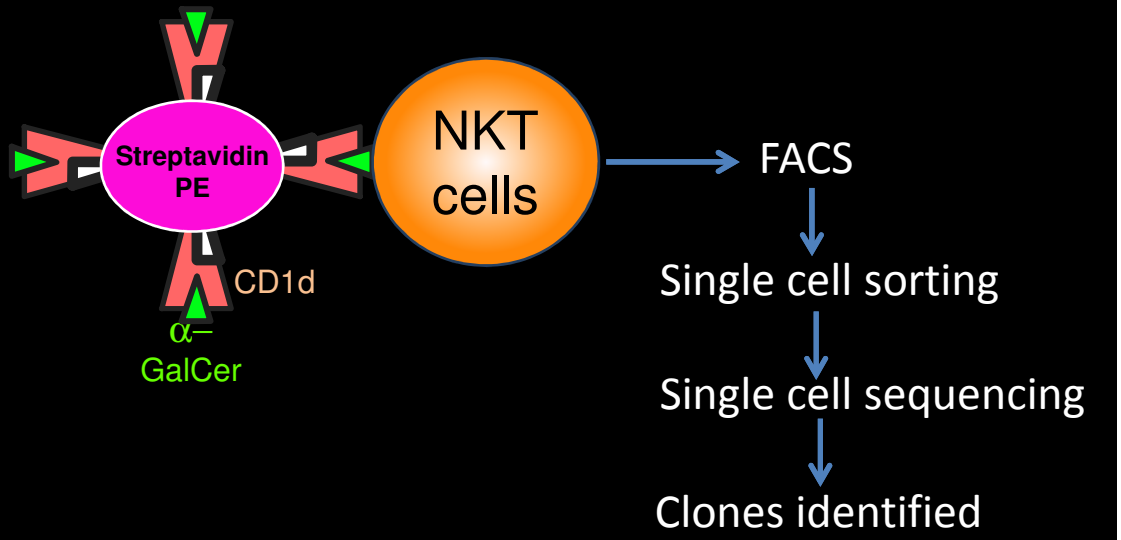
- TCRs dock on to peptide/MHC or lipid/CD1d in a conserved orientation

Are there any other CD1d-restricted α-Galcer reactive NKT subsets in humans and do they dock in a conserved manner?



New subset of NKT cells exist in humans





		TCRα		TCRβ		
Sequence	Donor	Vδ-Jα	CDR3α	Vβ-Jβ	CDR3β	
1	1	Vα14.2-Jα48	CAFILFGNEKLTF	Vβ1-Jβ2.3	CASSVDRGRPDTQYF	9B1
2	1	Vα2.2-Jα27	CAMSGDLNTNAGKSTF	Vβ13.1-Jβ1.5	CASSQGPFQPQEF	9B2
3	1	Vα8.2-Jα24	CAEKGMTTDSWGKLQF	Vβ2-Jβ1.6	CSAQTRGDSYNSPLHF	9B3
4	1	Vα8.1-Jα52	CAASSGGTSYGKLTF	Vβ7.1-Jβ2.5	CASSSQLLPGAPETQYF	
5	1	(a) Vα4.2-Jα42	CIVRGSMMNYGGSQGNLIF	Vβ7.1-Jβ2.2	CASSQDPSGVTGELFF	
		(b) Vα15-Jα29	CAESSNLSGNTPLVF			
6	1	Vα17-Jα30	CAAELVRDDKIIF	Vβ7.1-Jβ2.1	CASSQERERRILAGGPNEQFF	9B6
7	1	Vα4.1-Jα44	CILRDPLRGTASKLTF	Vβ22-Jβ1.4	CASTSNTGTGGFANEKLFF	9B7
8	2	Vα4.2-Jα48	CILRDGFGNEKLTF	Vβ7.1-Jβ2.1	CASSQVSSPGVPKEQXF	
9	3	Vα7.1-Jα21	CAVSRSLNFNKFYF	Vβ3-Jβ1.5	CASSLPGQGRQPQHF	
10	4	Vα24-Jα18	CVVSDRGSTLGRLYF	Vβ21.3-Jβ2.7	CASSPRDSYEQYF	9B10
11	4	Vα2.2-Jα20	CAMIPFNDYKLSF	Vβ8.3-Jβ1.1	CASGLVGTGEAFF	
12	5	Vα6-Jα43	CAMREGPGDMRF	Vβ2-Jβ1.6	CSARDPTEDRKKGLNSPLHF	9B12
13	6	Vα23-Jα8	CAGVNTGFQKLVF	Vβ6.2-Jβ2.7	CASSSRDLEQYF	

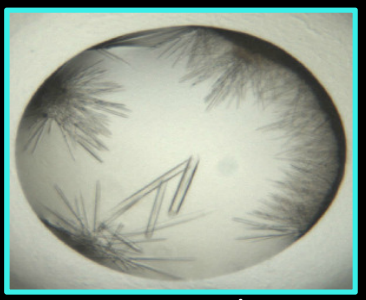
Collaboration with University of Melbourne

Experimental Flow

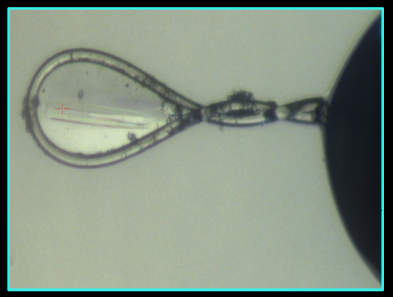
Clone and express protein (Bacterial system)

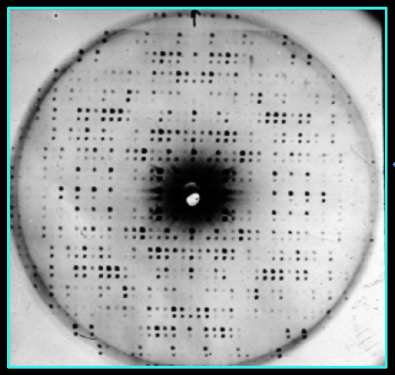
Protein Purification

Protein Crystallization

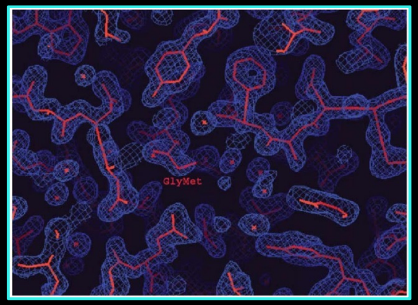


Crystals



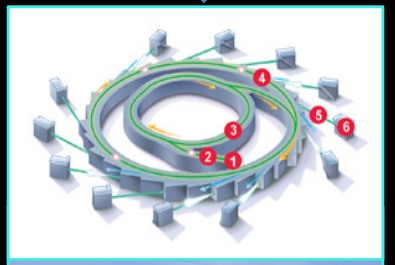


Diffraction pattern



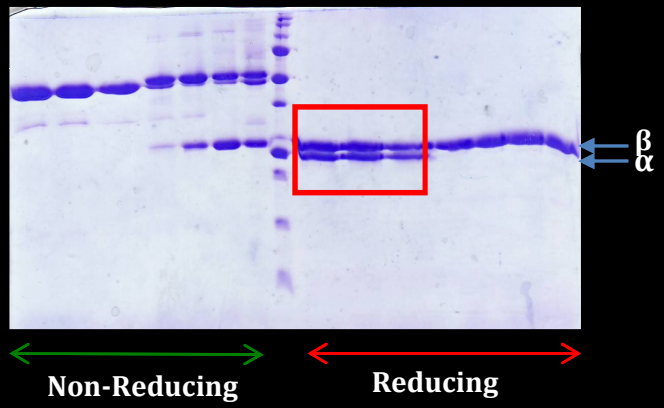
Electron density map

Protein Structure



Expression and Purification of TCR

- Cloned into pET-30 vector
- Expressed in BL21 *E.coli* cells
- Inclusion body preparations were performed



Refolding

Two injections of α and β chain

Dialysed 3 days

Purification

Ion exchange chromatography (DEAE)

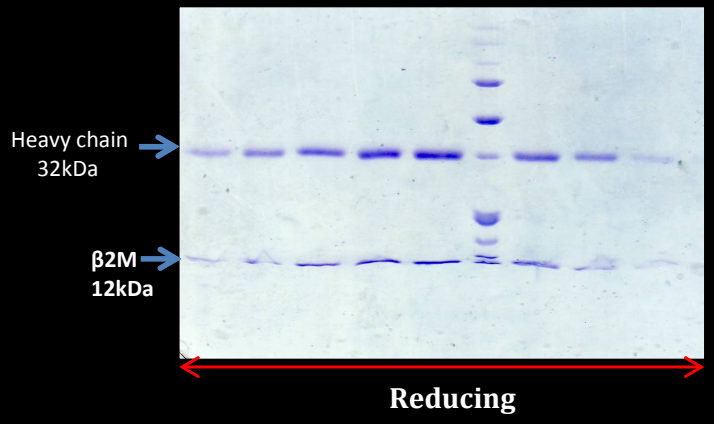
Size exclusion chromatography

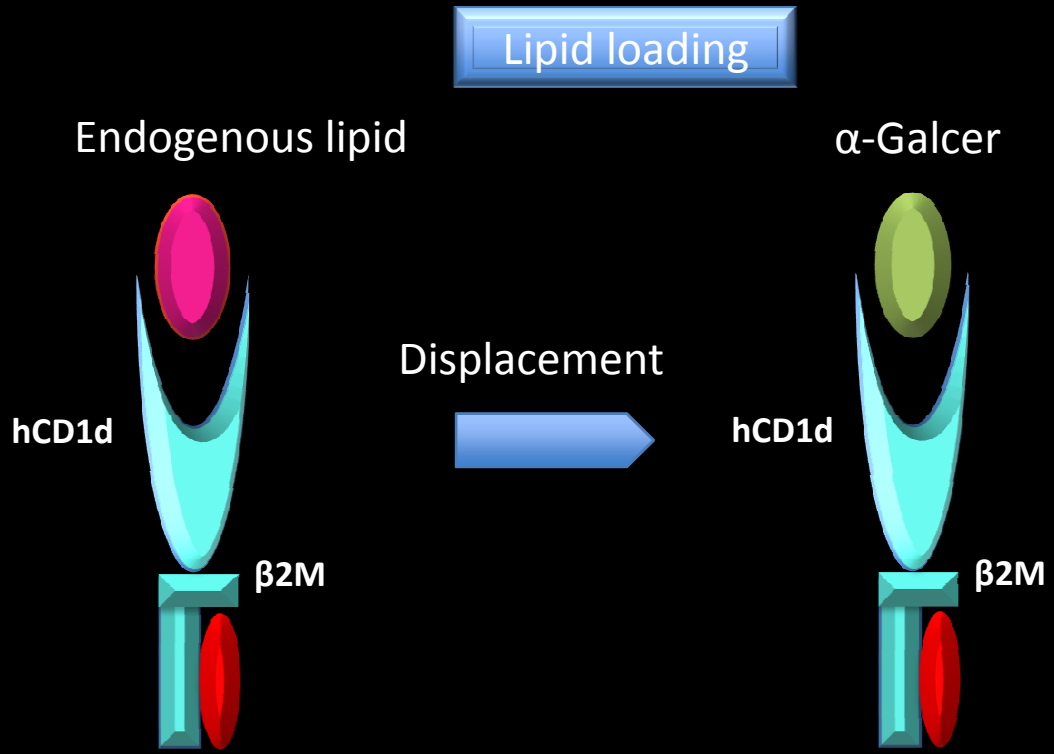
Anion exchange chromatography (Hitrap Q)

Hydrophobic interaction chromatography (HIC)

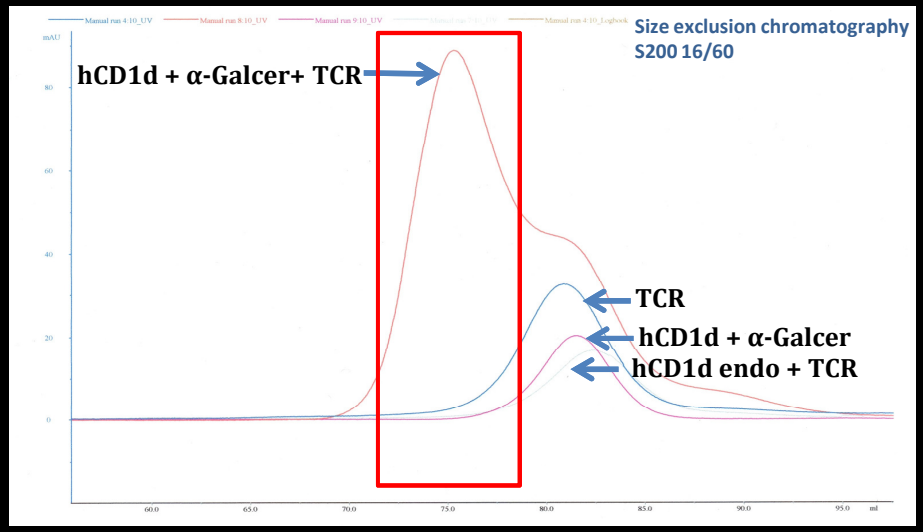
Expression and purification of Human CD1d

- Cloned into a dual promoter baculovirus transfer vector pBacp10pH.
- Expressed in Hi5 insect cells.

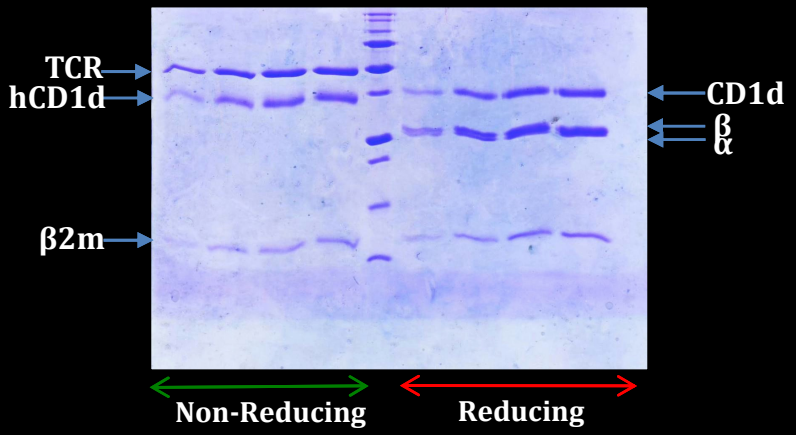




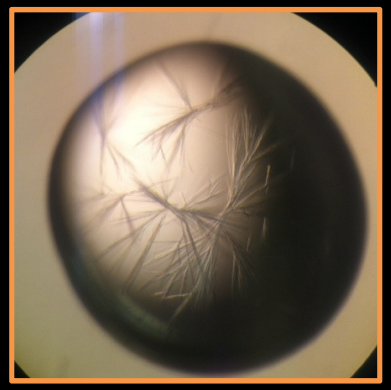
9B2 TCR-hCD1d/α-Galcer co-complexation



- A Shift in peak of 5ml indicated complex formation



Structure of 9B2 ternary complex

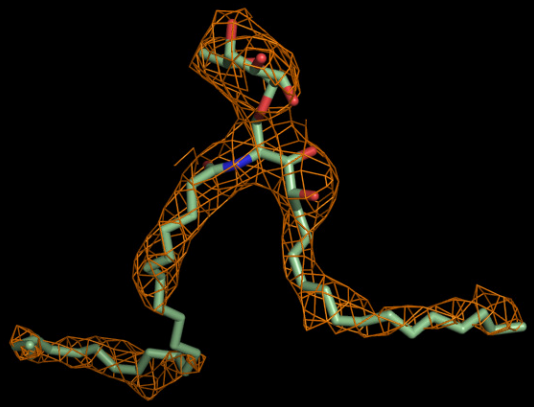


20% PEG 8000
0.1M CHES pH 9.5

α - chain
β - chain
α - Galcer
CD1d
β2M

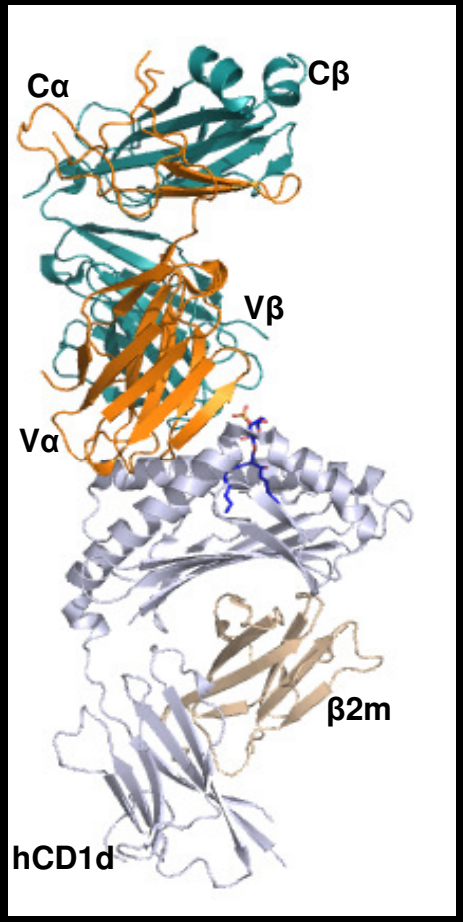
Novel docking mode
Docking angle ~110°

α-GalCer (Fo-Fc electron density map)



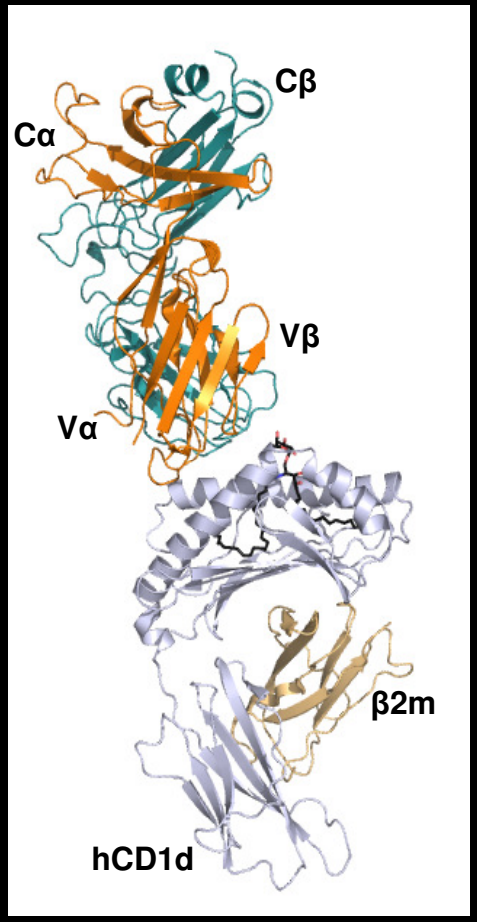
α-GalCer (2Fo-Fc electron density map @ 0.8σ level)

Comparison of docking modes of different types of NKT TCRs

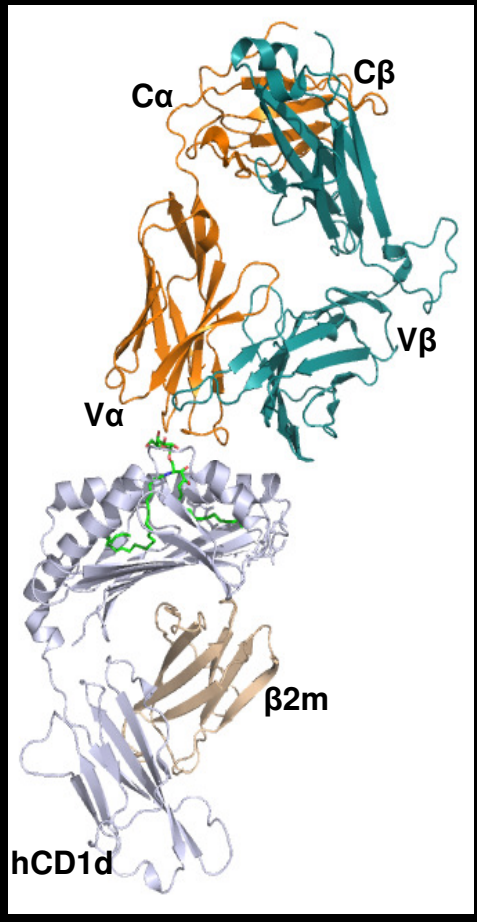


Type II

Patel *et al.*, 2012 Nat.Immunology



9B2



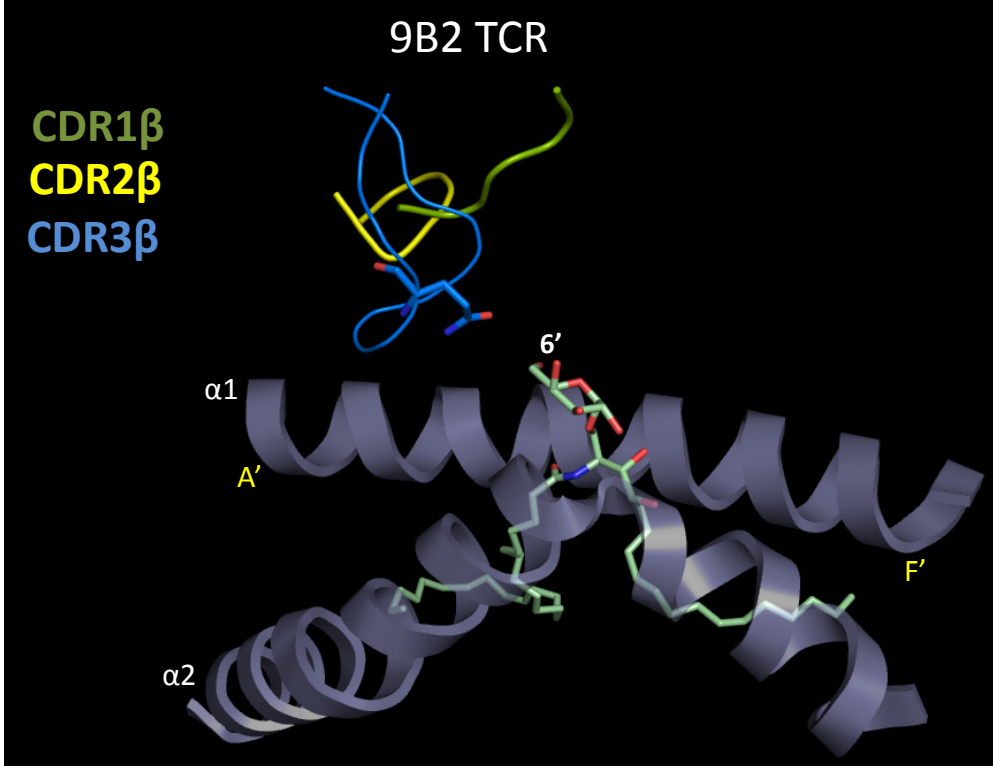
Type I

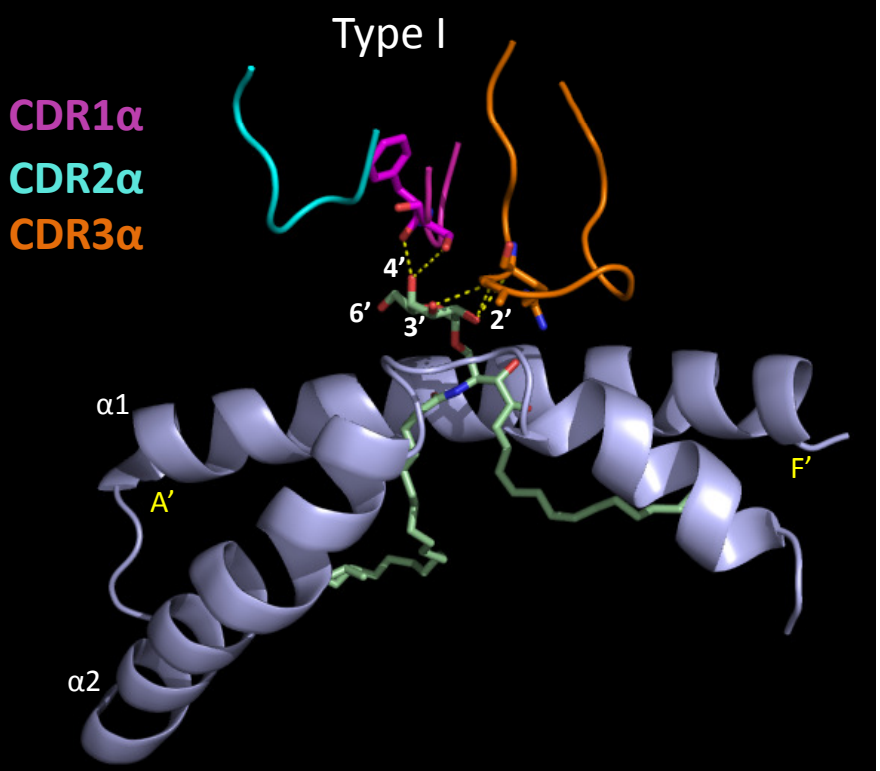
Borg *et al.*, 2007 Nature

Interactions of 9B2 TCR with α-Galcer

- Dominated by CDR3β loop
- Q99 interacts with O6 of galactose moiety by Van der waals interaction.

- Dominated by CDR1α and CDR3α loops.
- G96, F29 and S30 are H-bonded to O2, O4 and O3 respectively.

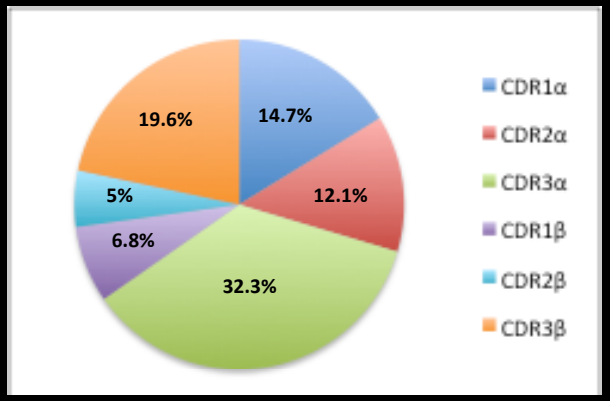




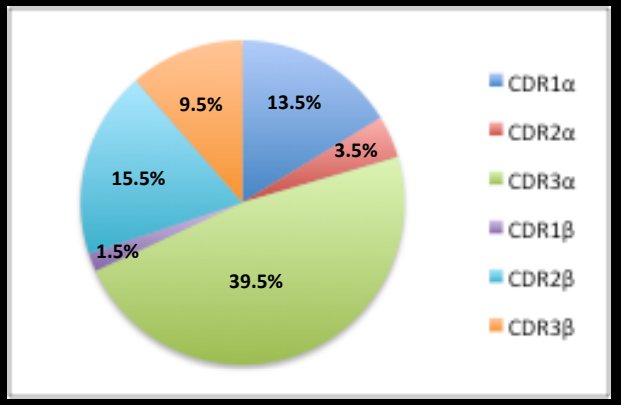
9B2 TCR-CD1d interactions

Buried surface area

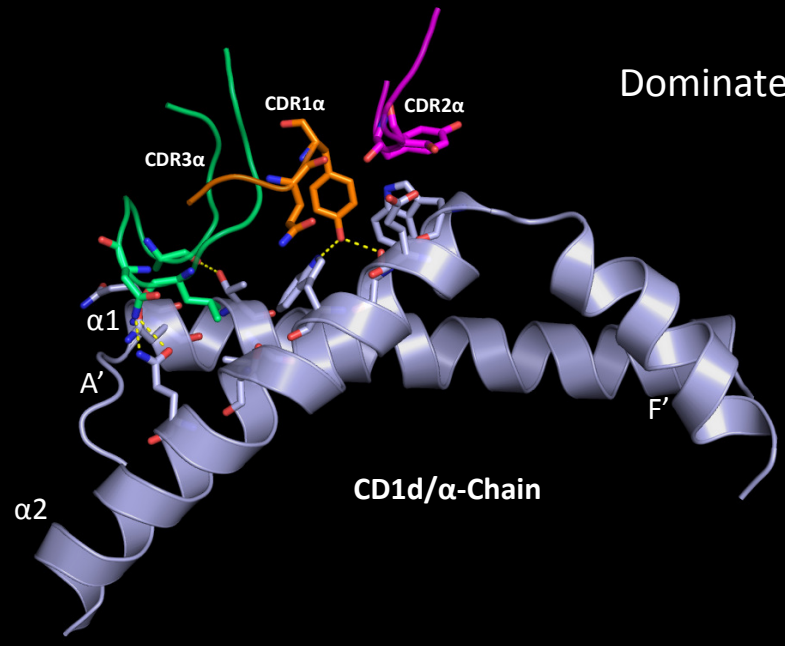
9B2 TCR

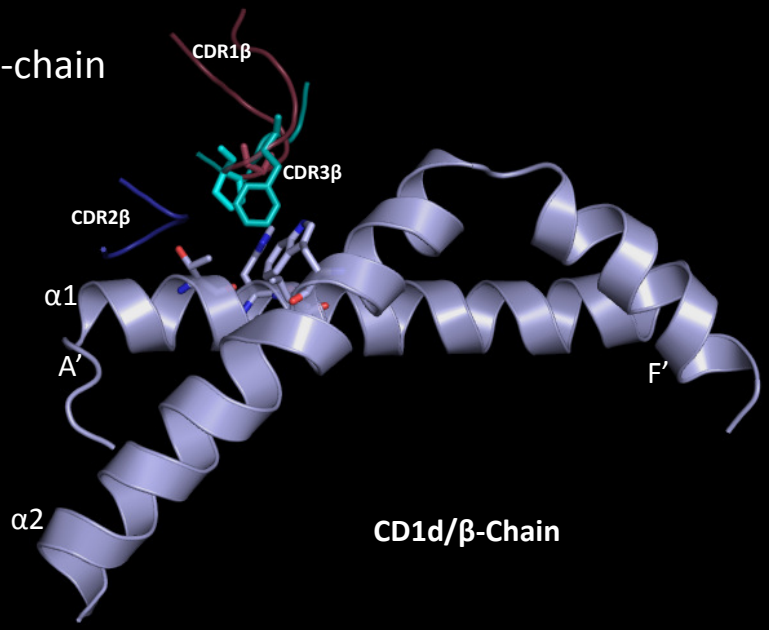


Type I TCR



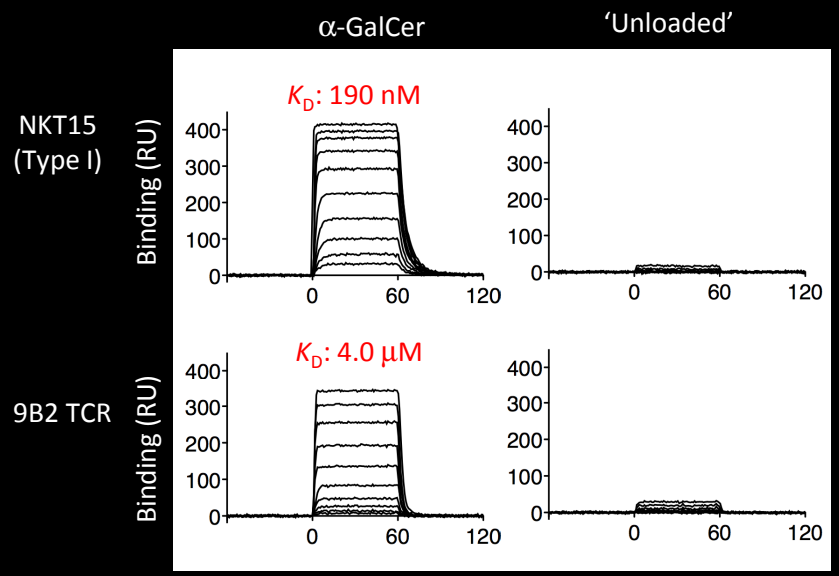
Dominated by α-chain

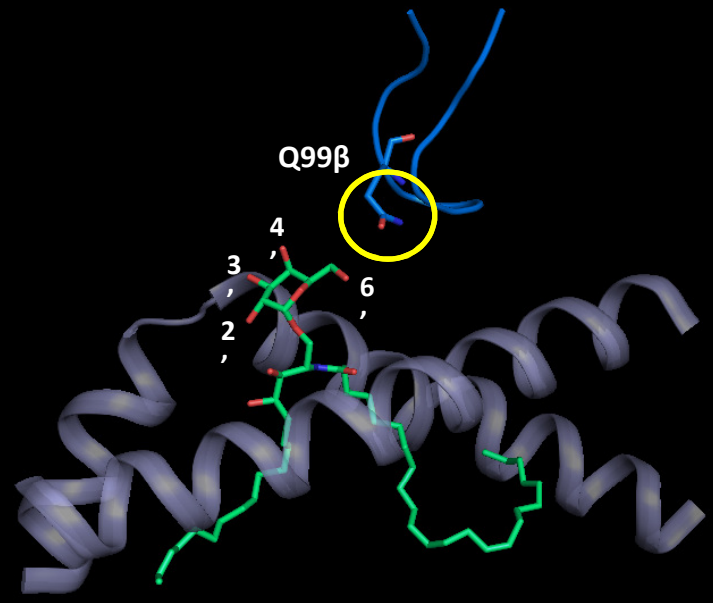




Affinity measurements of 9B2 TCR with CD1d-α-Galcer

Surface plasmon resonance (SPR)





- Q99A TCR mutant showed 2-fold reduction in affinity compared with wild type

Collaboration with University of Melbourne

Summary

- A new subset of CD1d-restricted NKT TCRs were identified in humans and termed as 'Atypical NKT cells'
- The ternary structure of 9B2 TCR revealed a novel docking mode (orthogonal) in clear contrast to Type I but comparable with Type II TCR
- 6'-OH of galactose moiety interacted merely with Q99 residue of TCRβ chain
- SPR studies showed the affinity of interaction of 9B2 TCR (wild type) with hCD1d- α-Galcer is 4.0μM and 2-fold reduction in affinity for Q99 mutant
- Diverse TCR repertoire broadens the spectrum of glycolipids recognised and thus leading to stimulation of NKT cells

Acknowledgements

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University of Melbourne

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Adam Uldrich

Daniel Pellicci

MONASH University

Australian Government
National Health and Medical Research Council
NHMRC

Australian Government
Australian Research Council

Australian Synchrotron
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