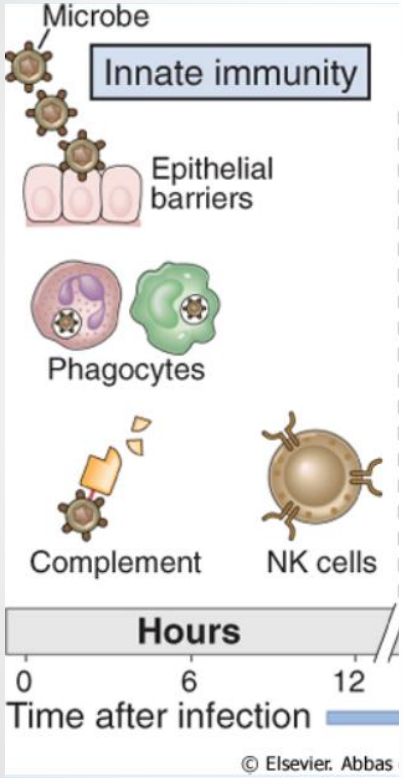
Monomeric TCRs Drive T-Cell Antigen Recognition

Mario Brameshuber, TU Wien
Institute of Applied Physics - Biophysics

Fighting (currently) a common cold…



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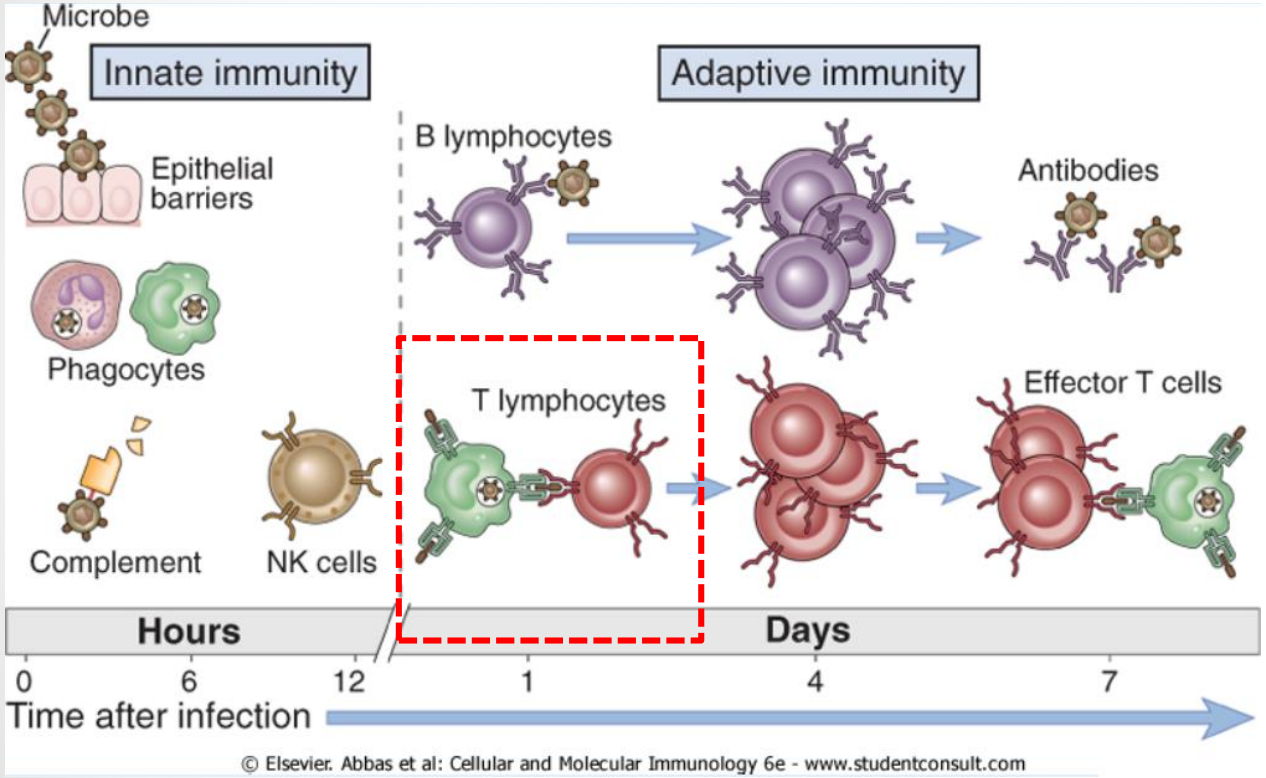
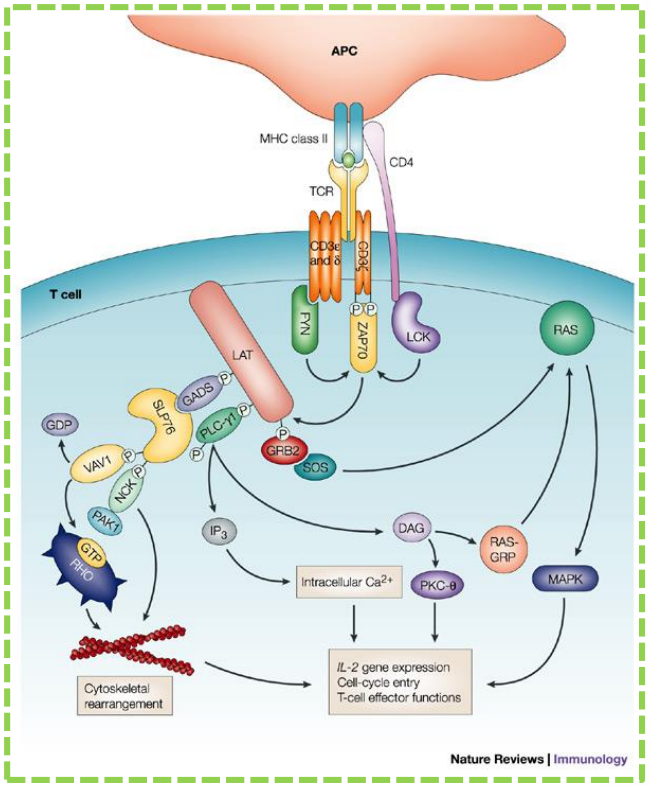
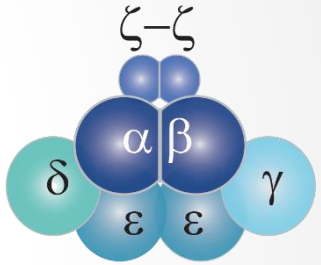


Antigen presenting cells (APCs) present pathogen fragements via their mayor histocompatibility complex (MHC)

→ Dicrimination between self and foreign peptides

The T-cell receptor signalling complex





T cells detect presented antigen with the T-Cell Receptor (TCR) protein complex

→ Dicrimination between self and foreign peptides

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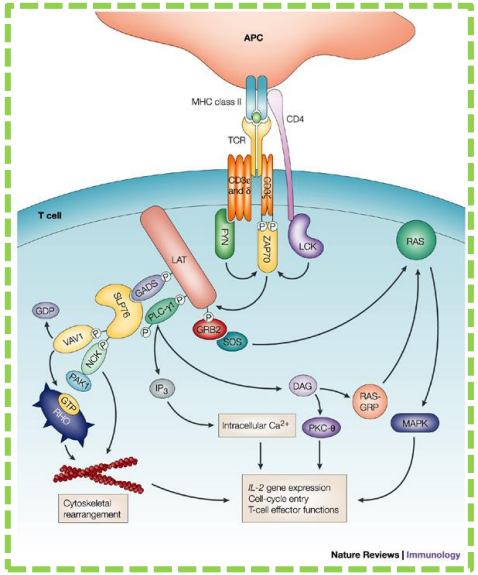

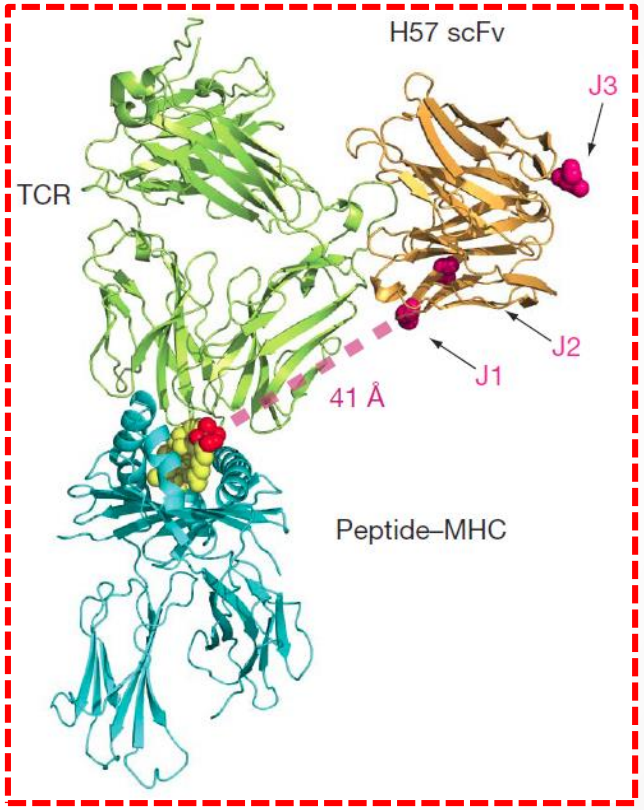

Huppa et al., *Nature* (2010)

Single molecule FRET experiments yielded interaction life times of ~ 100 ms.

→ Dicrimination between self and foreign peptides

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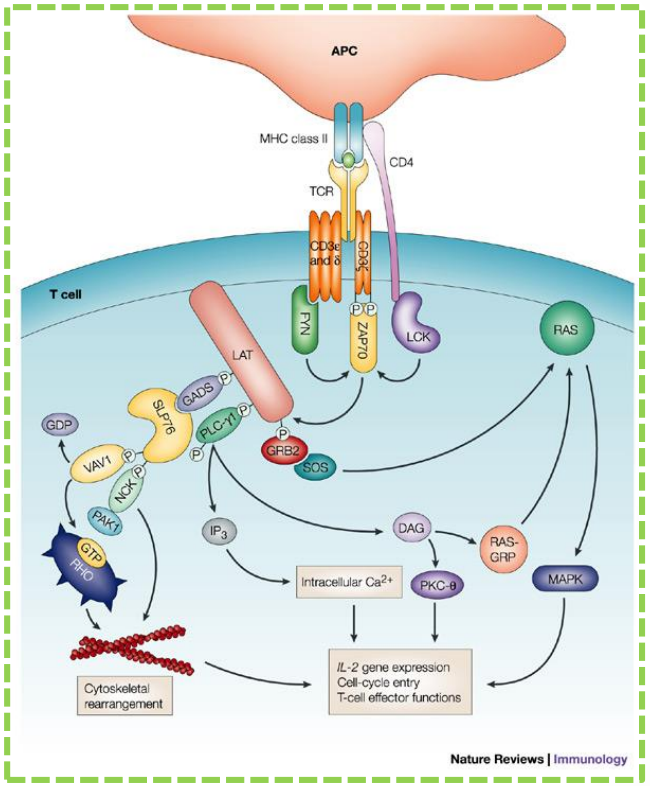
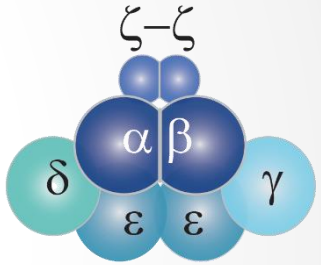




A single (antigenic) pMHC molecule is able to activate a T cell

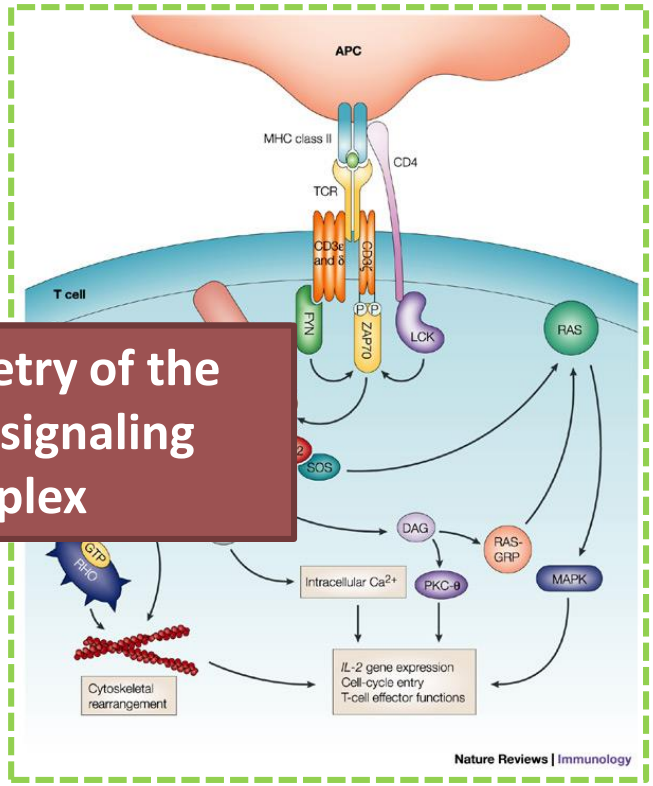
→ Incredible T-cell specificity and sensitivity

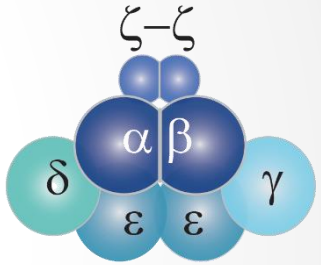
Huang et al., Immunity (2013)

Huppa et al., Nature (2010)

The T-cell receptor signalling complex

Stoichiometry of the TCR/CD3 signaling complex





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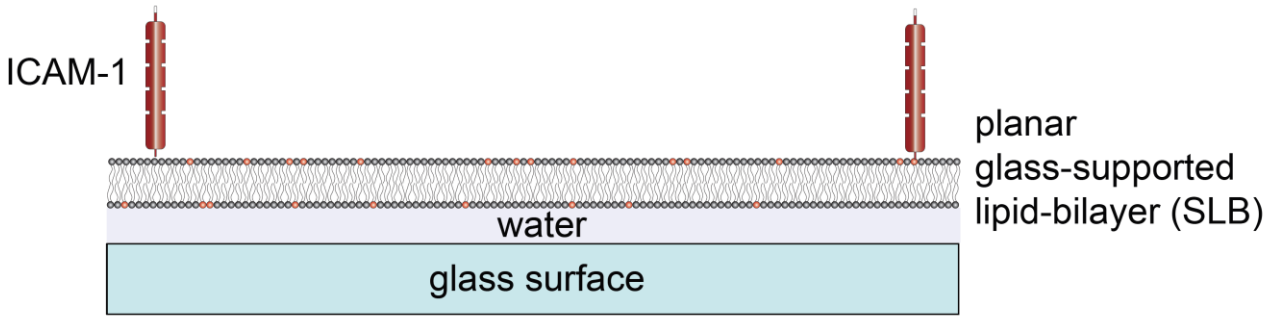
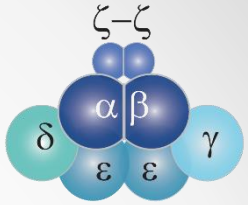
Model system for studying T-cells

- ICAM-1 on SLBs (90% POPC + 10% DGS-NTA(Ni))



Replace antigen presenting cell (APC) by a 2D-supported lipid bilayer (SLB) system



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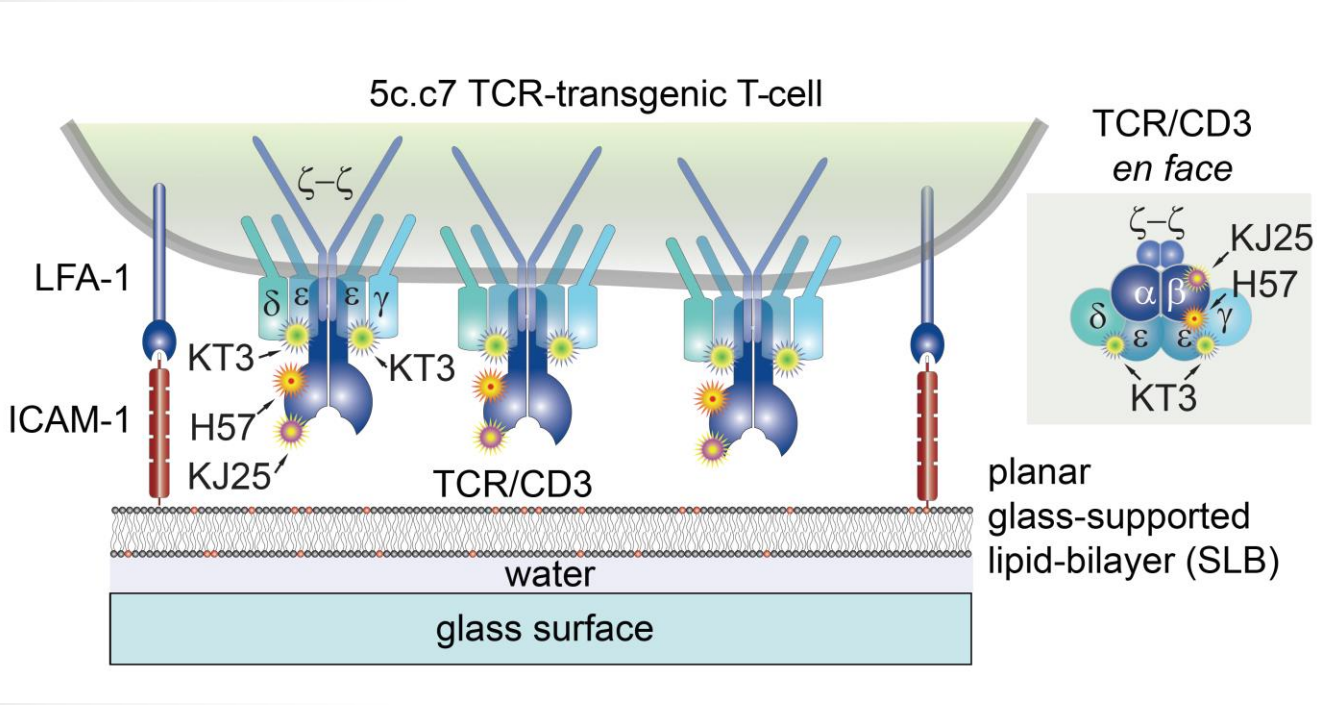

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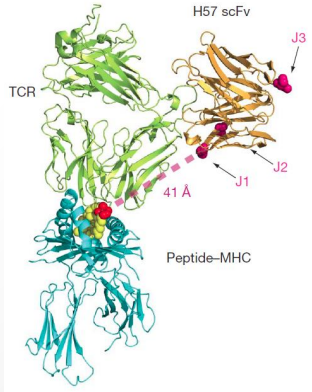
- 1:1 stoichiometric TCR labeling

Huppa et al., *Nature* (2010)



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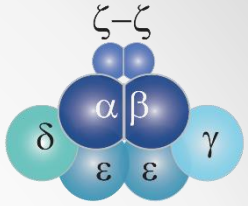

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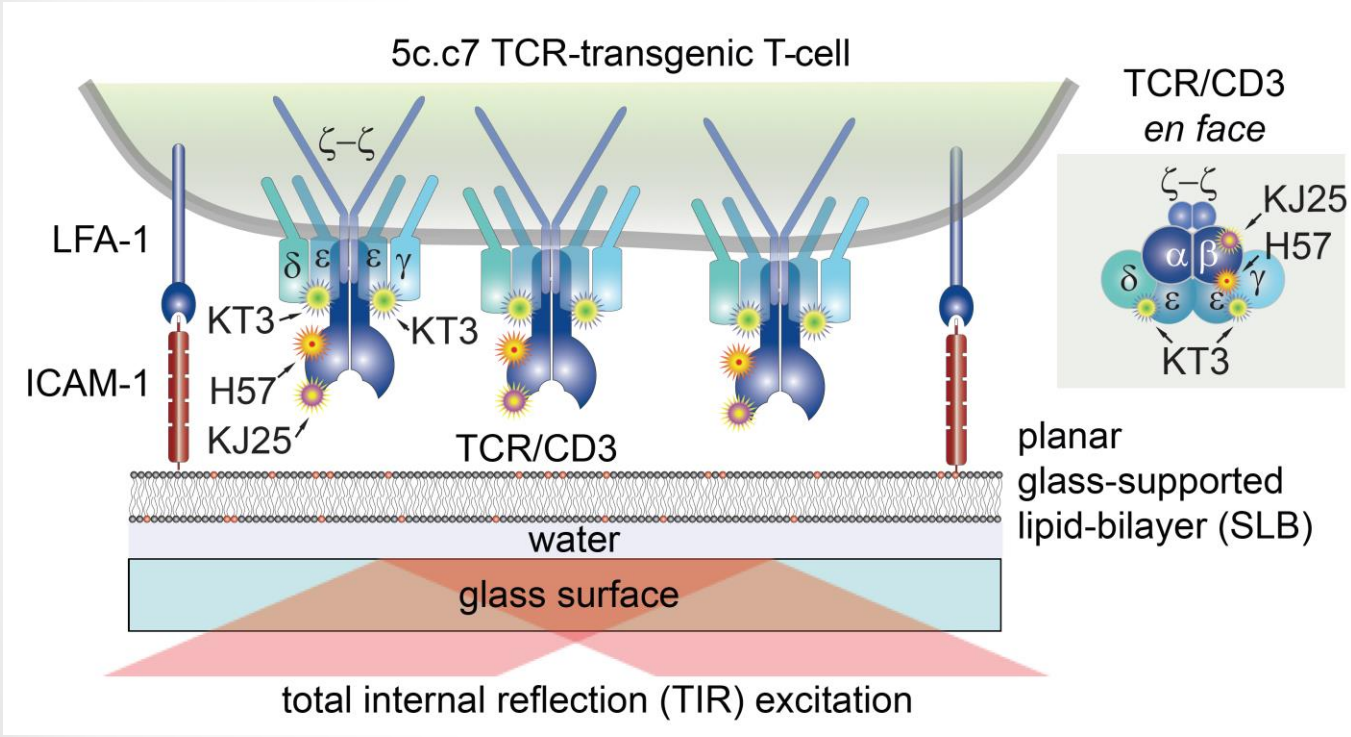

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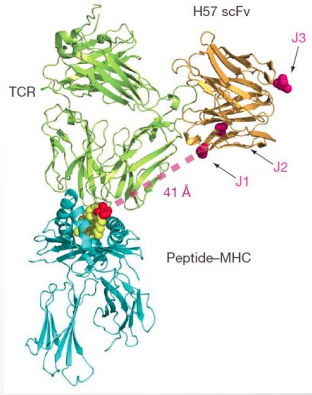

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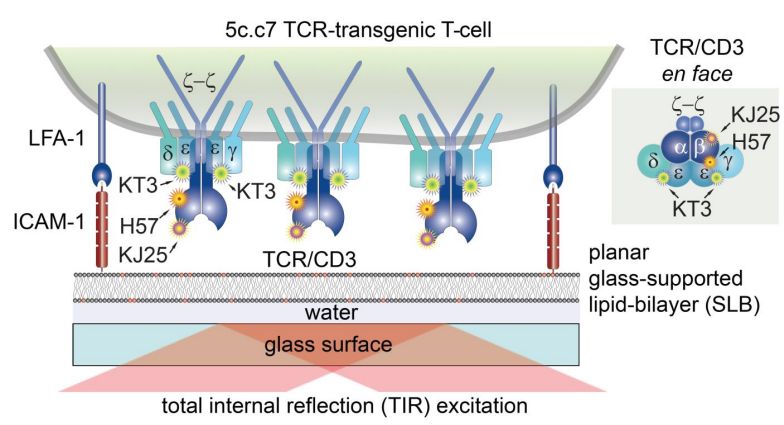
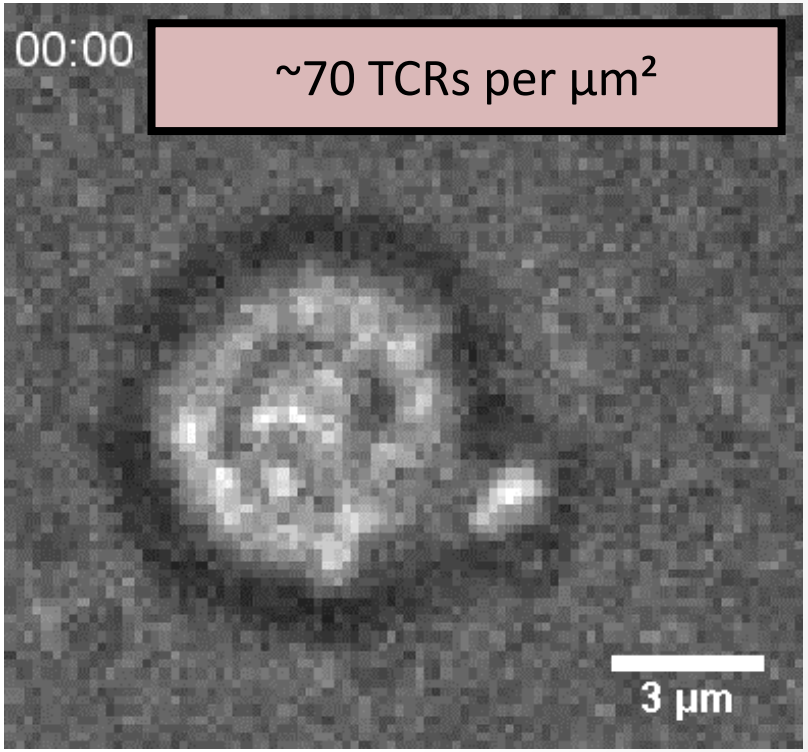


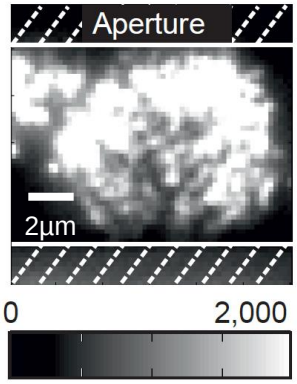


TIRF imaging of AlexaFluor568-H57-scF$_V$ bound to 5c.c7 T cells (blasted)

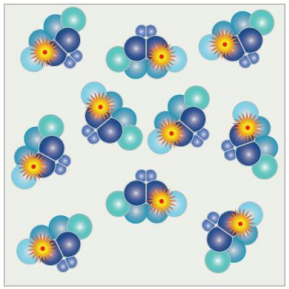
Laterally Mobile TCR/CD3 Complexes Feature One TCRβ and Two CD3ε Subunits

TOCCSL experiment – **T**hining **O**ut **C**luters while **C**onserving **S**toichiometry of **L**abeling

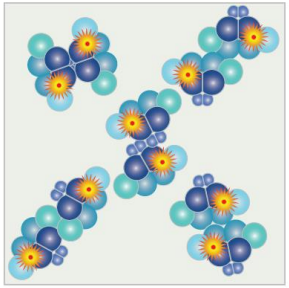
TOCCSL



Monomeric TCR-CD3



Dimeric TCR-CD3



Single camera pixel

Brameshuber et al., JBC (2010)

Brameshuber et al., Meth. Enzym. (2012)

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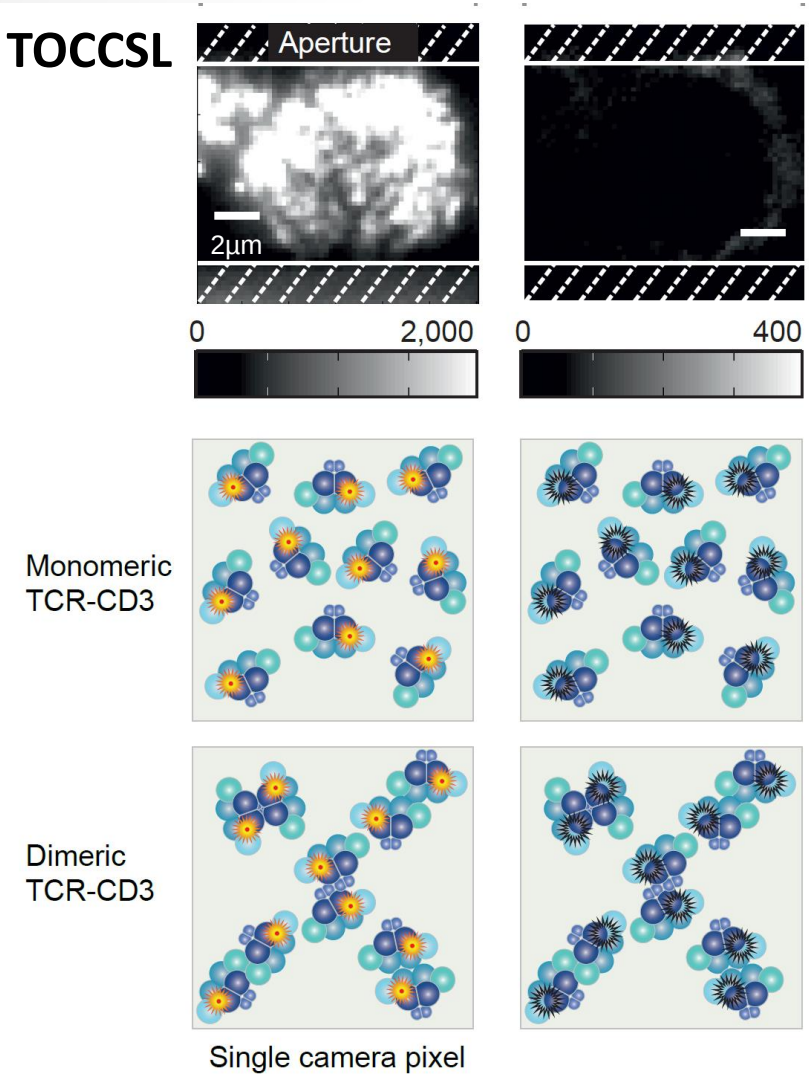

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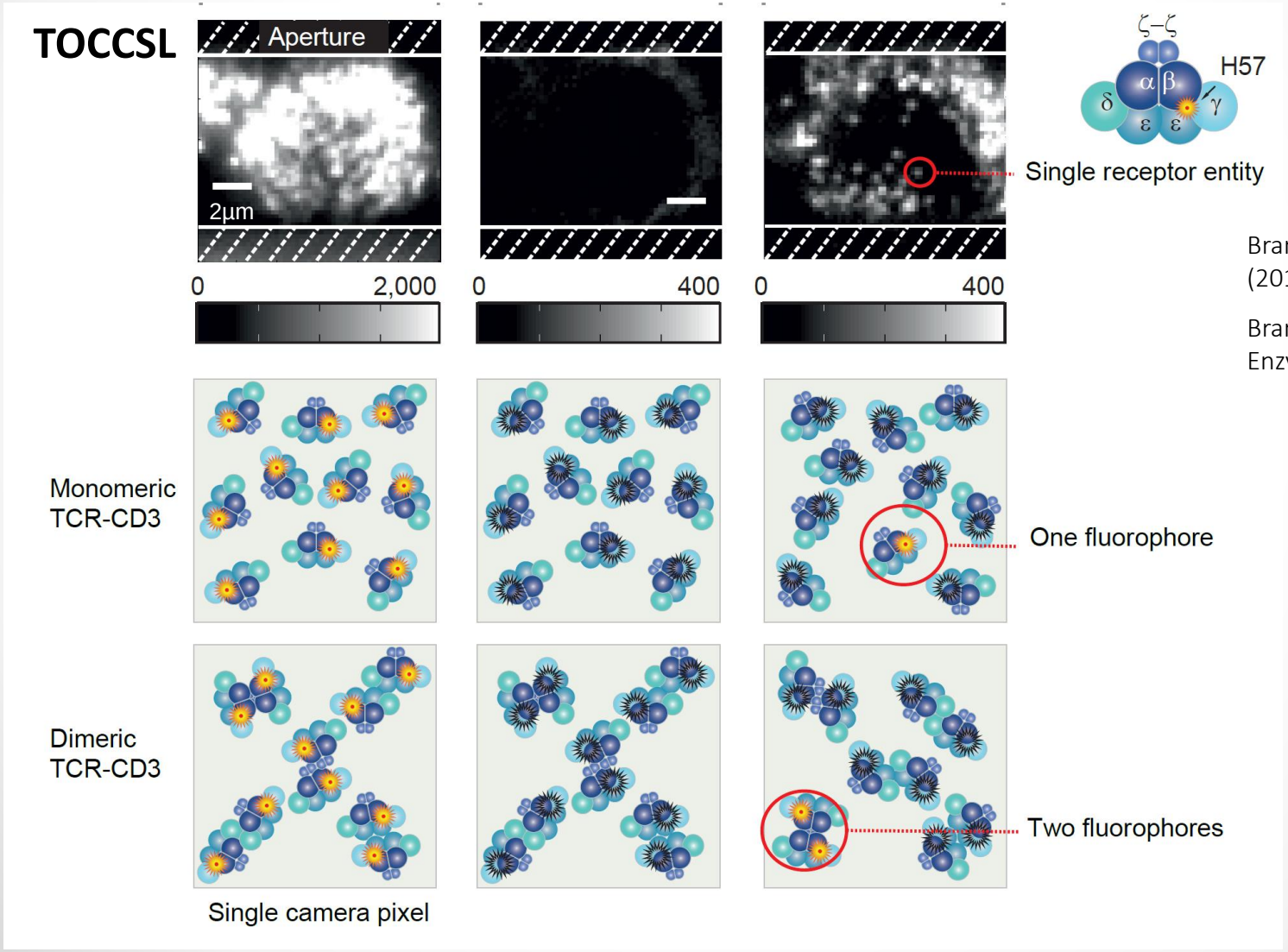

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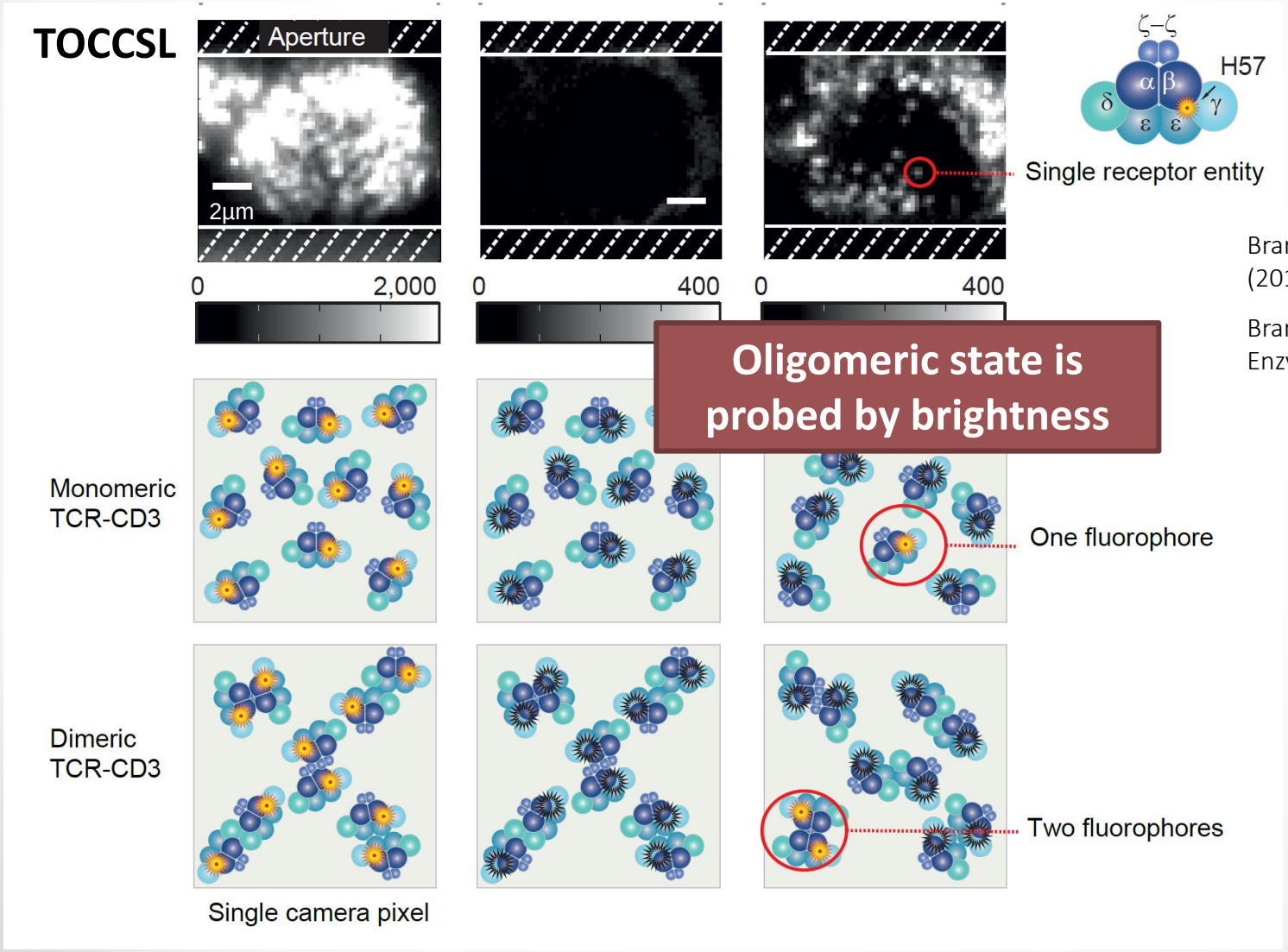

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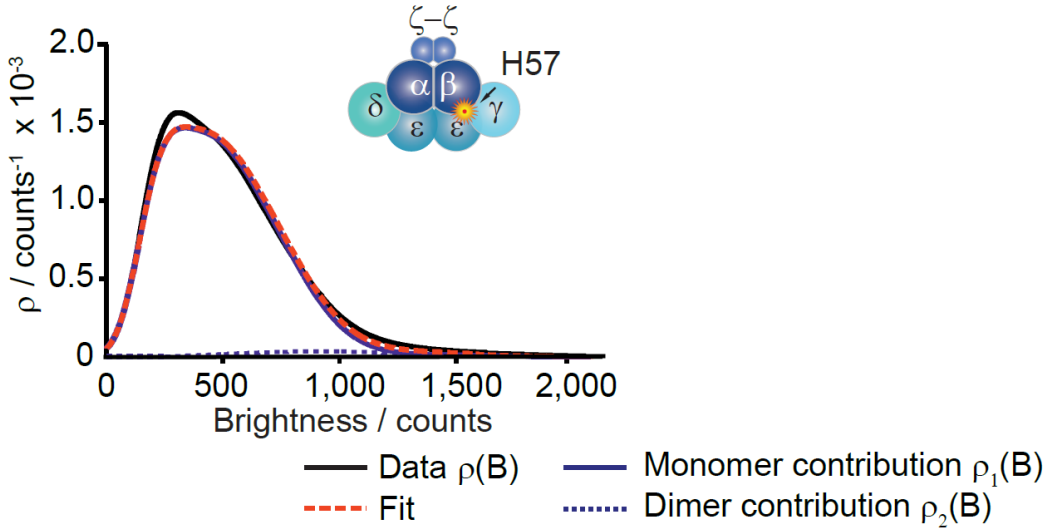

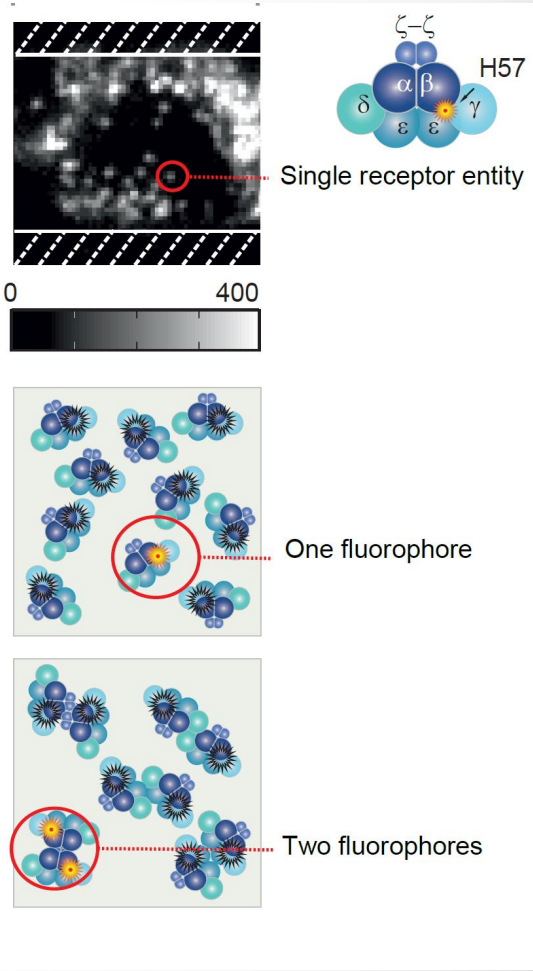

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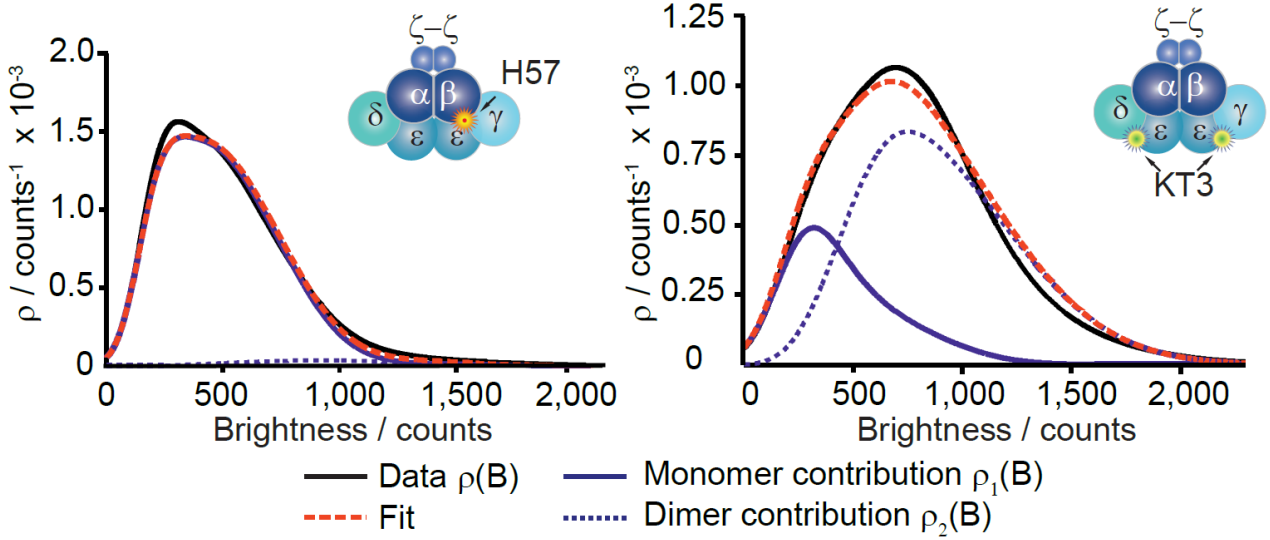

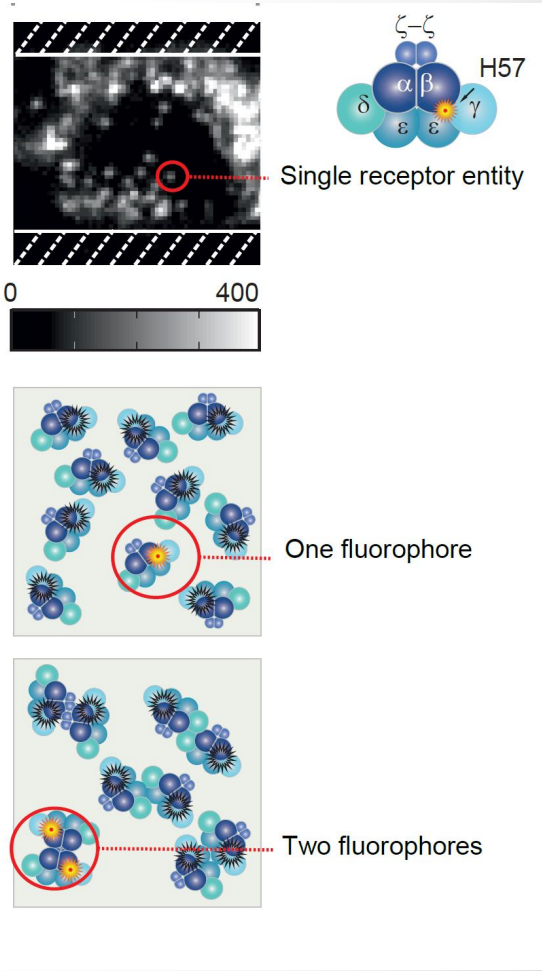

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How to increase the sensitivity: 2-color TOCCSL

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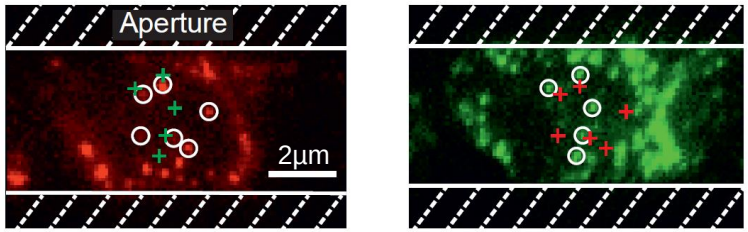
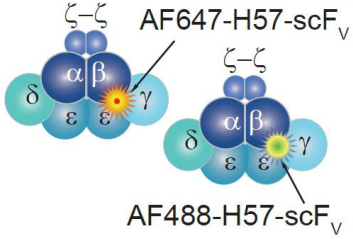
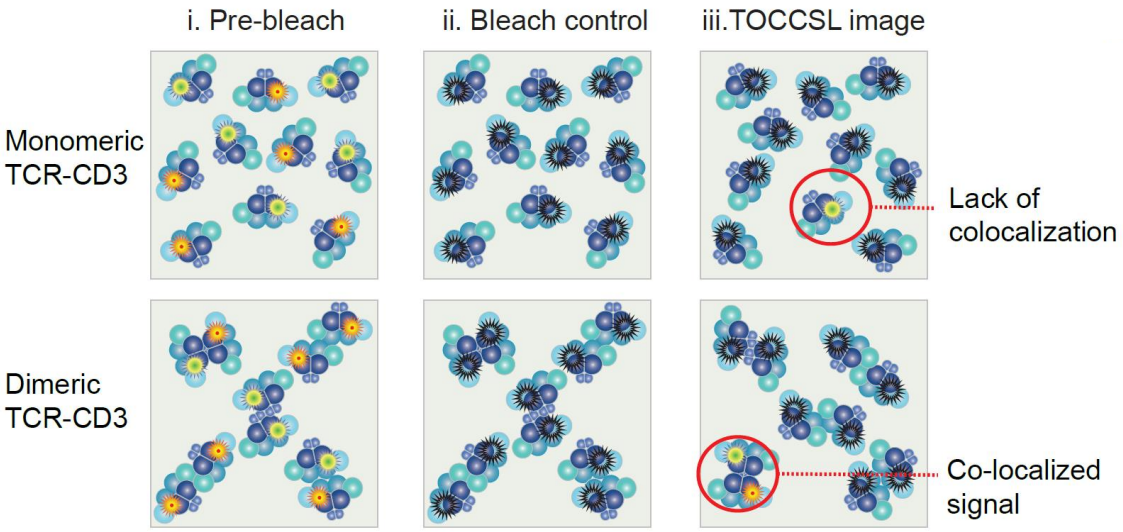




Oligomeric state is probed by colocalization



Ruprecht et al., Soft Matter (2010)

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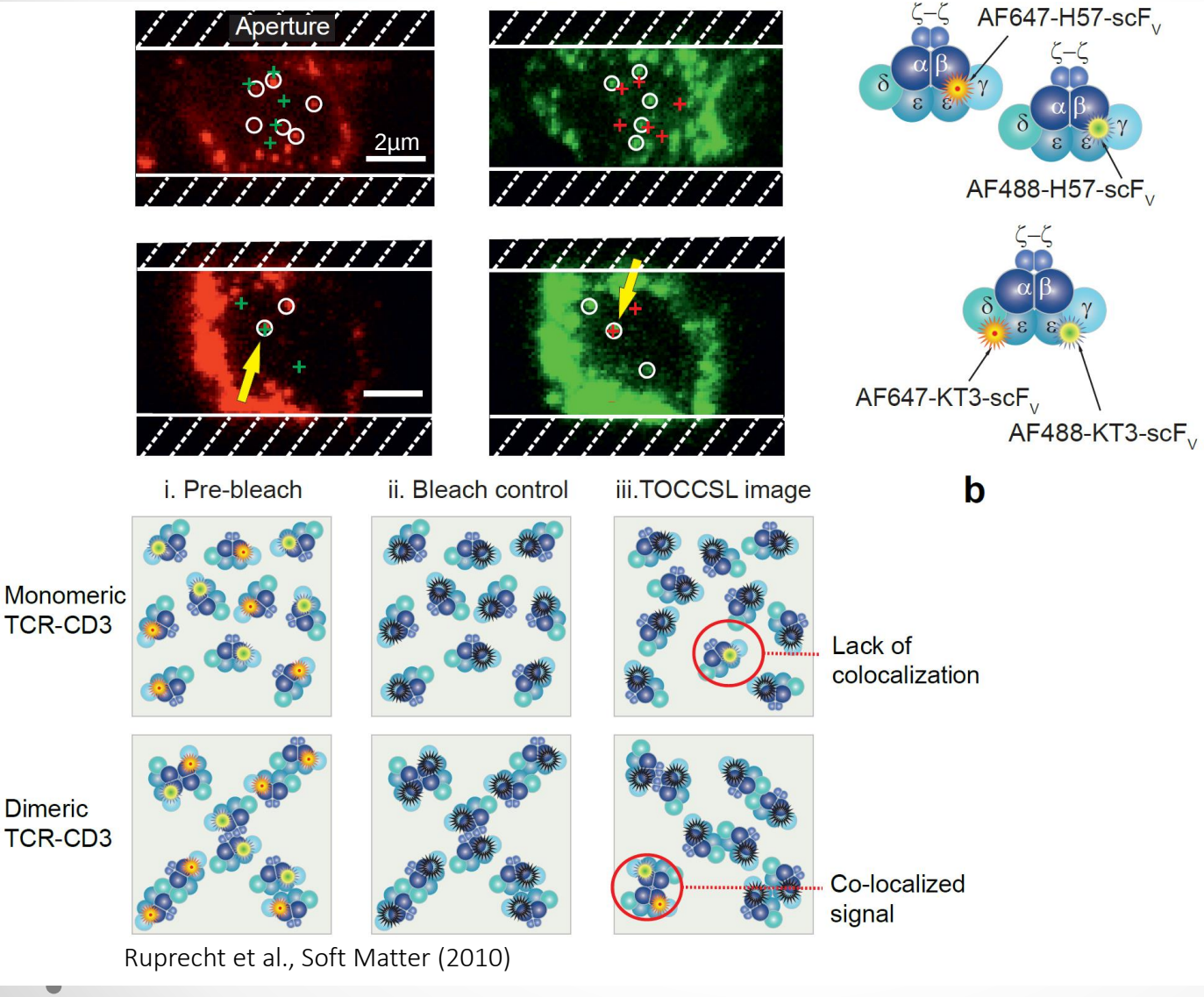

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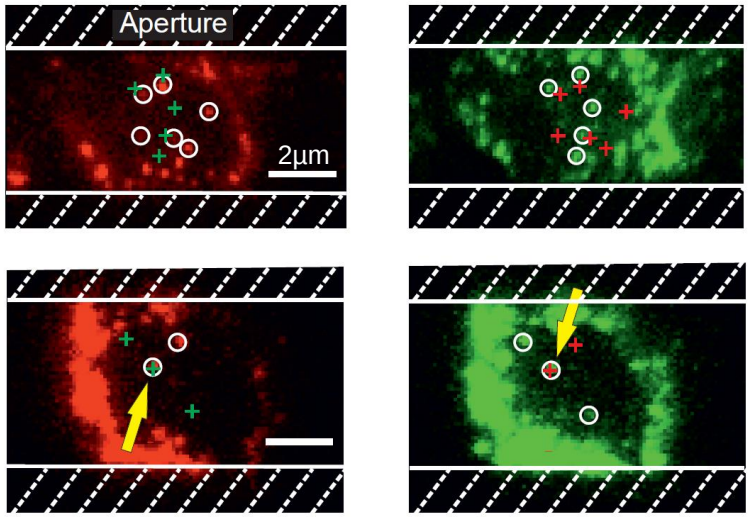

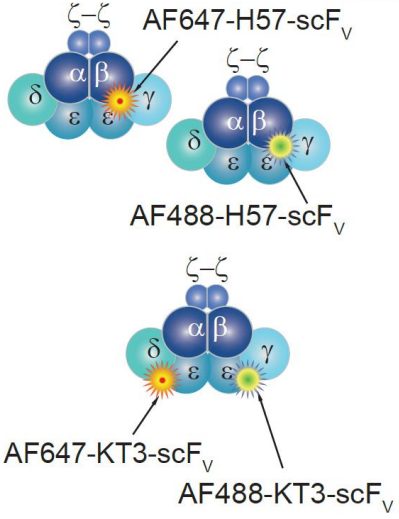

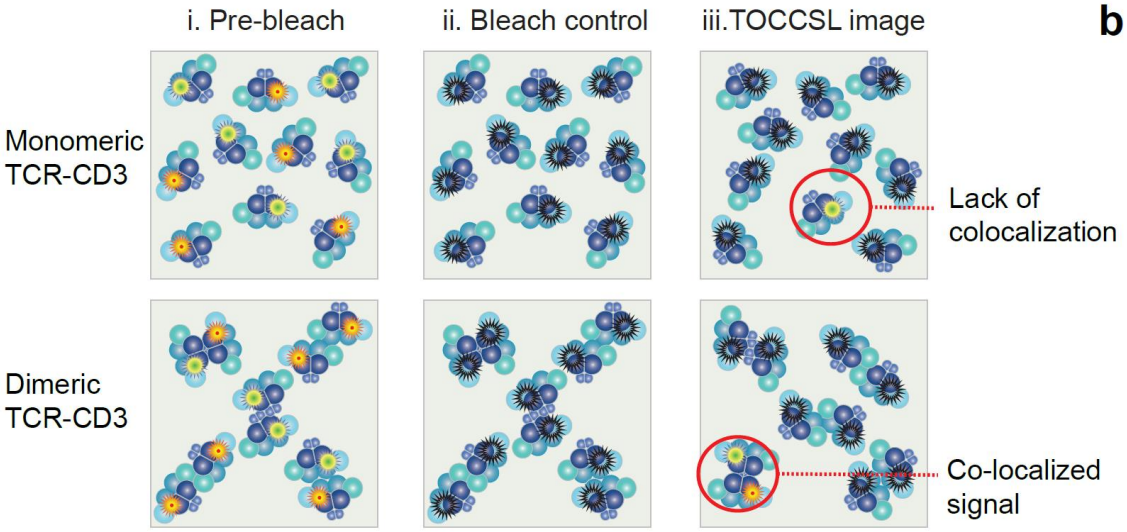

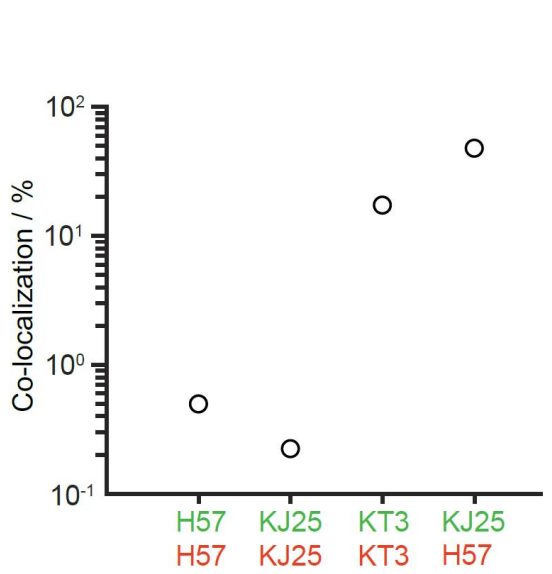

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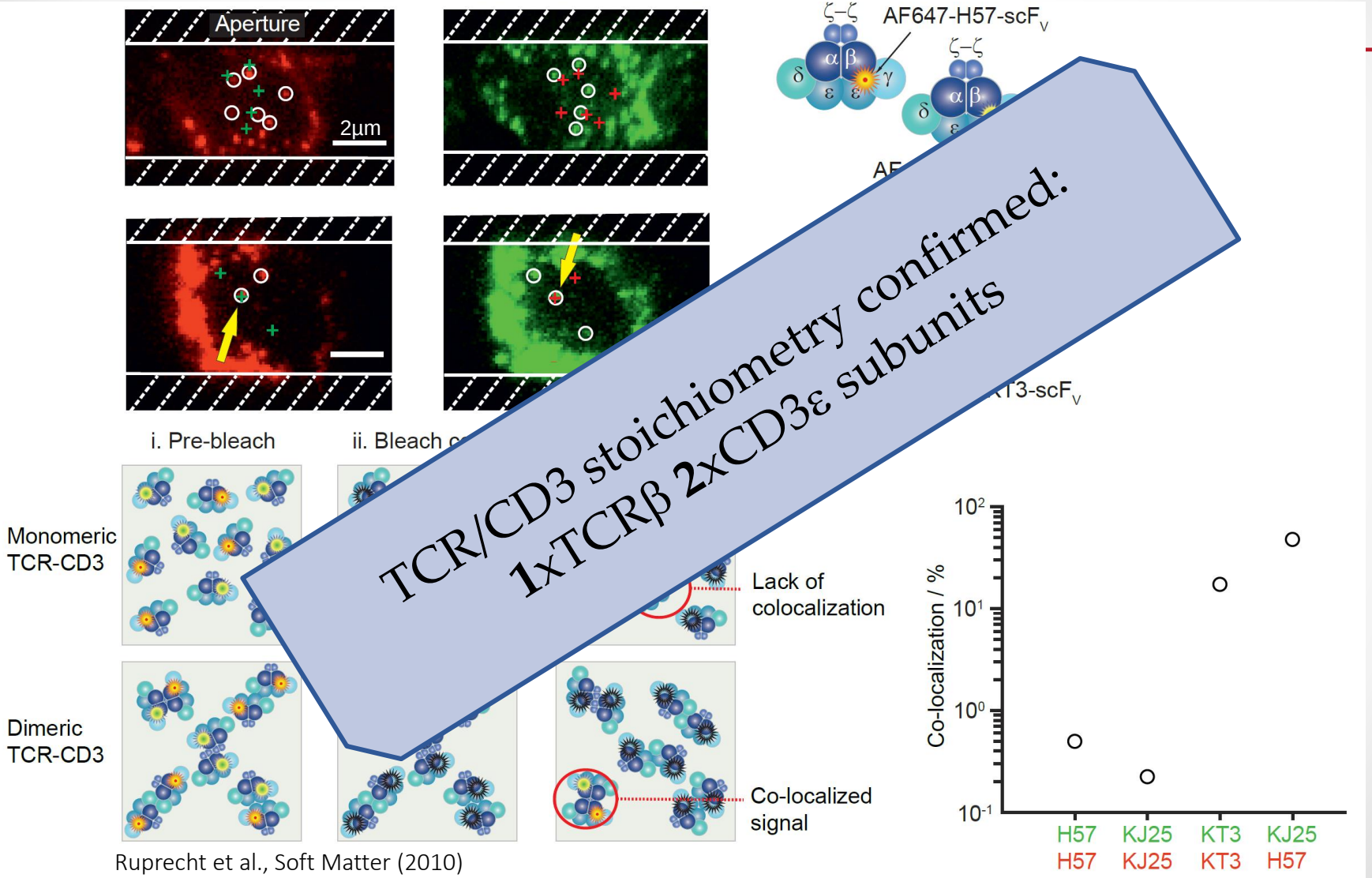

TCR/CD3 stoichiometry confirmed:
1xTCRβ 2xCD3ε subunits

Ruprecht et al., Soft Matter (2010)

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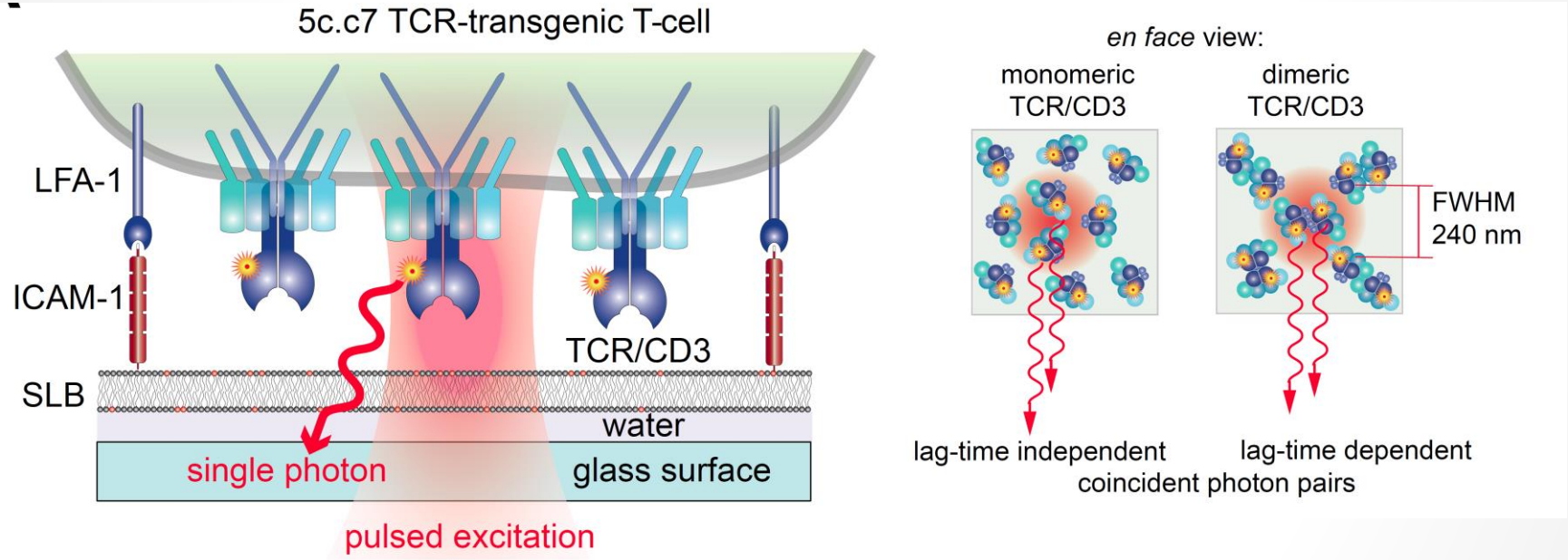
Photon-antibunching FCS experiments

Detection of potentially less stable complexes



Oligomeric state is probed by coincident photon pairs

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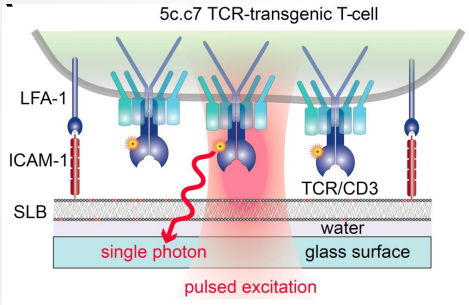

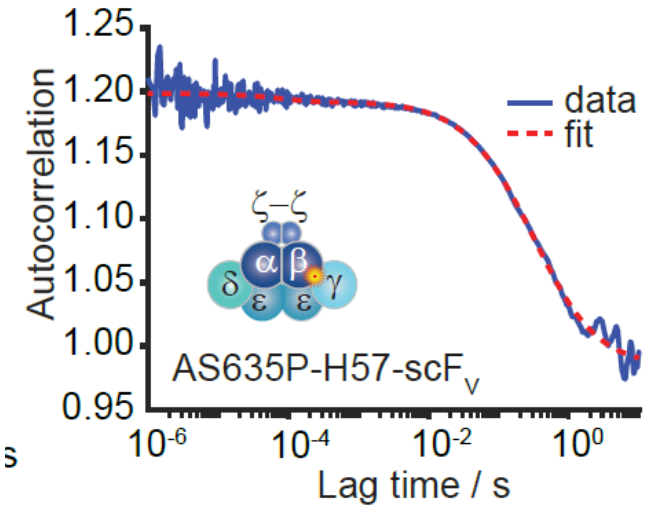

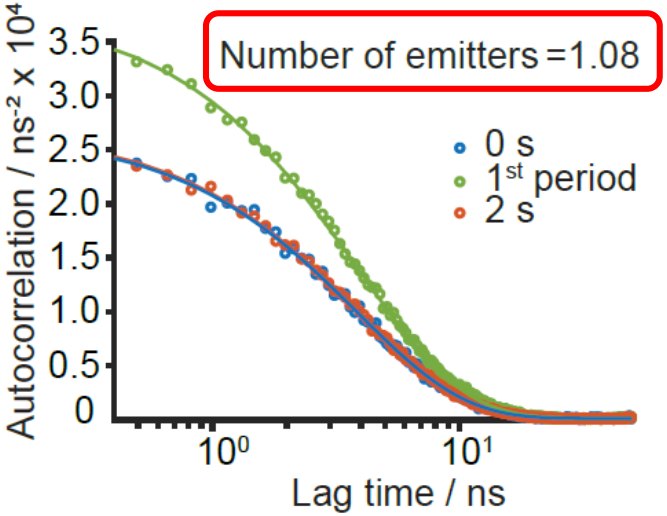

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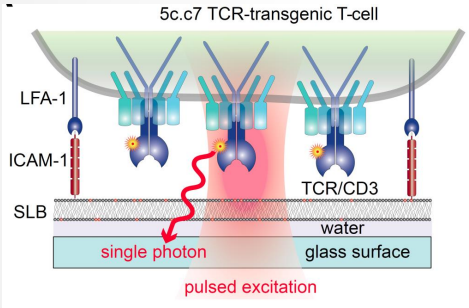
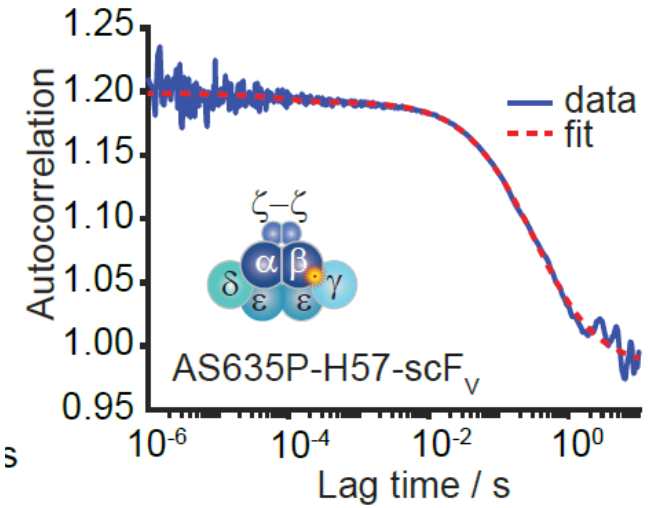
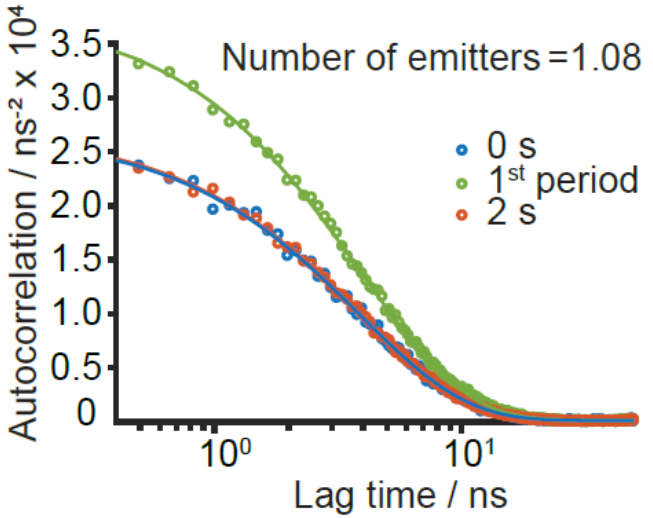
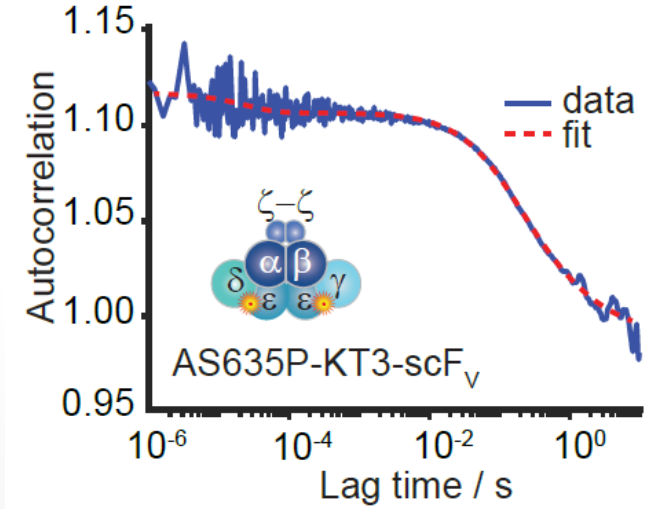
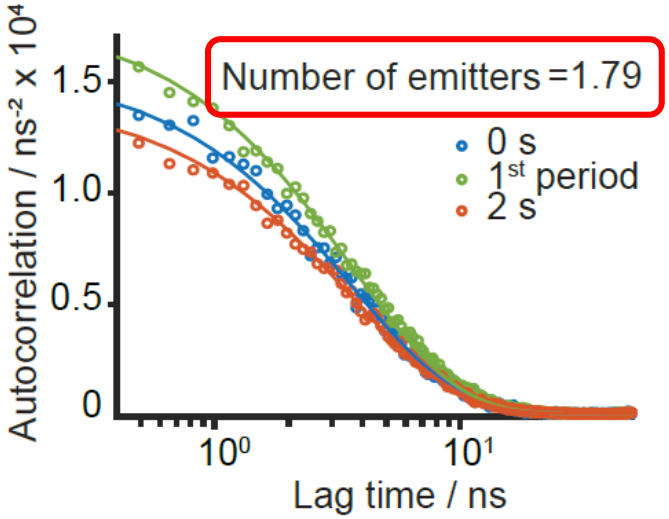






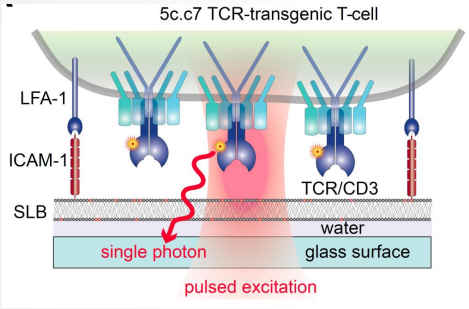


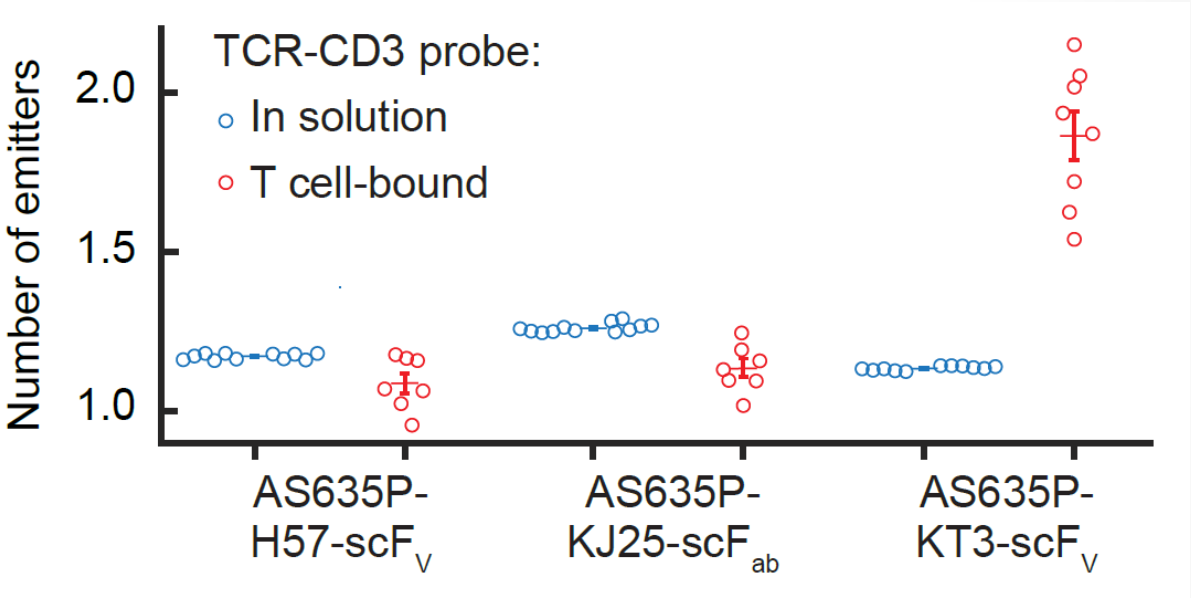


Laterally Mobile TCR/CD3 Complexes Feature One TCRβ and Two CD3ε Subunits

Photon-antibunching FCS experiments

Detection of potentially less stable complexes

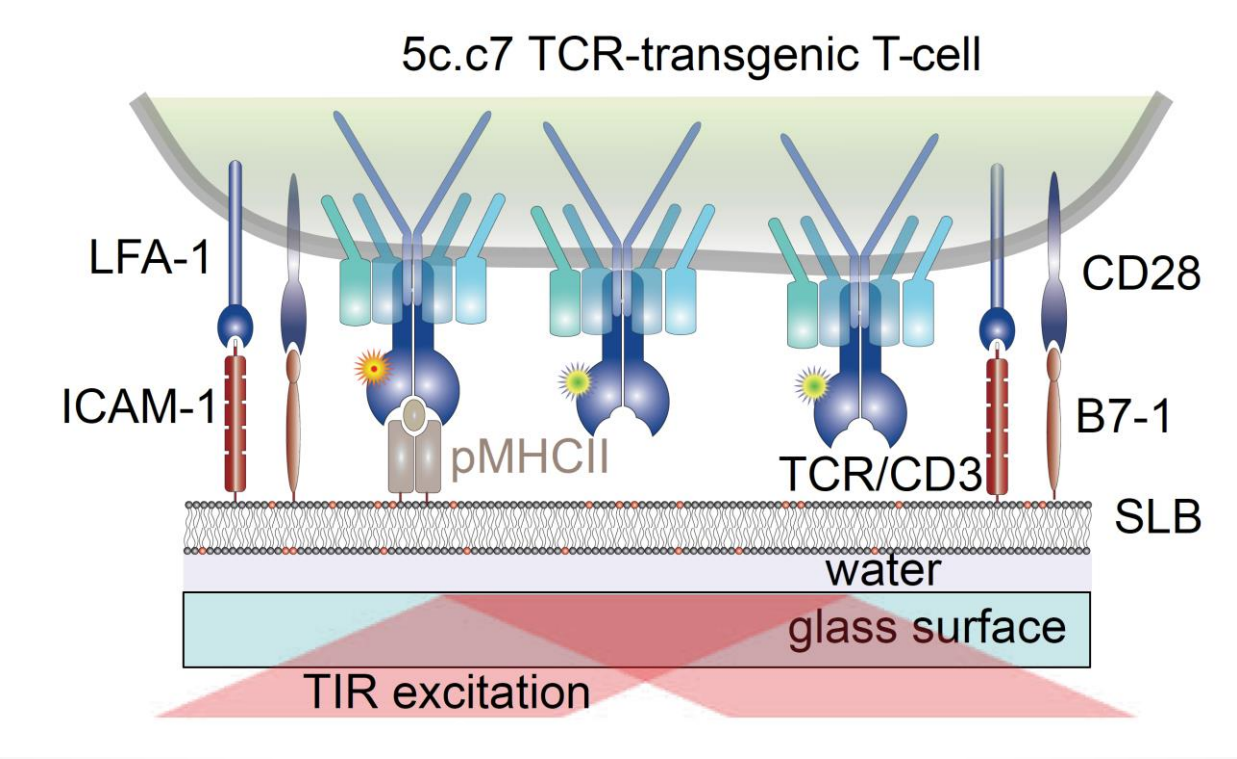




How to address immobile TCR/CD3 complexes?

FRET – **F**örster **R**esonance **E**nergy **T**ransfer Experiments

Detection of immobile/mobile complexes



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FRET – Förster Resonance Energy Transfer Experiments

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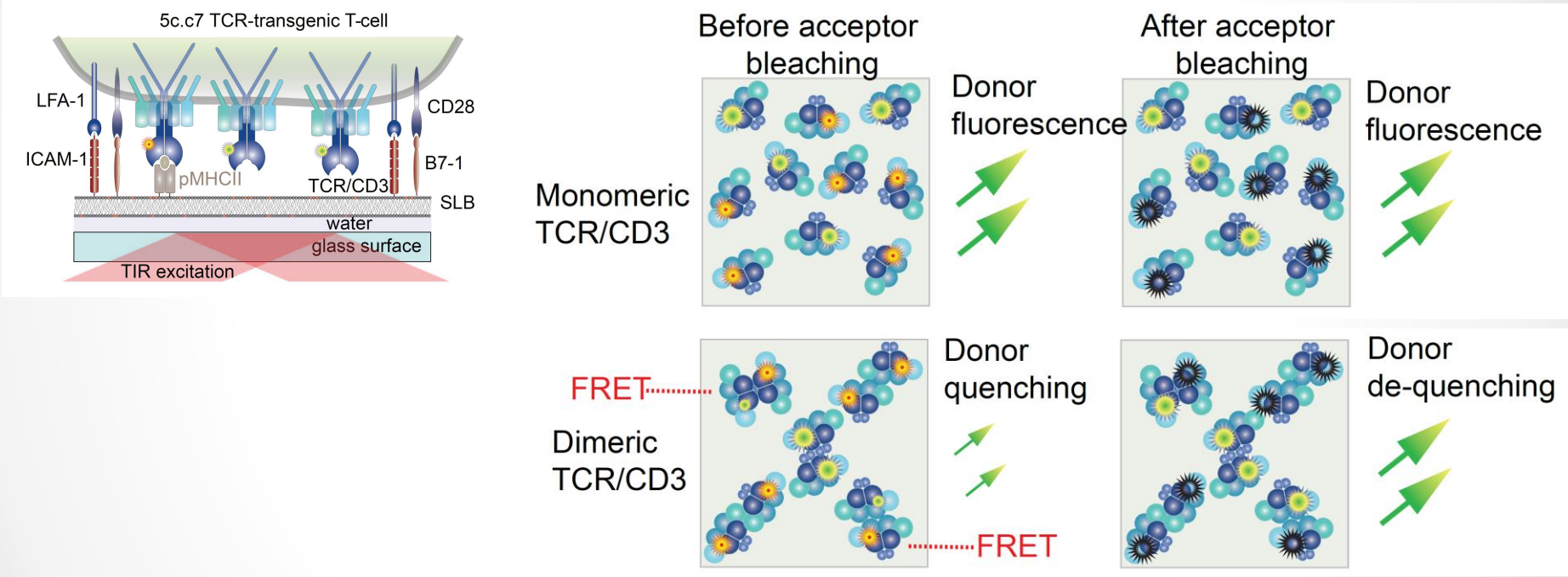

FRET yields determined with Donor Recovery After Acceptor Photobleaching

How to address immobile TCR/CD3 complexes?

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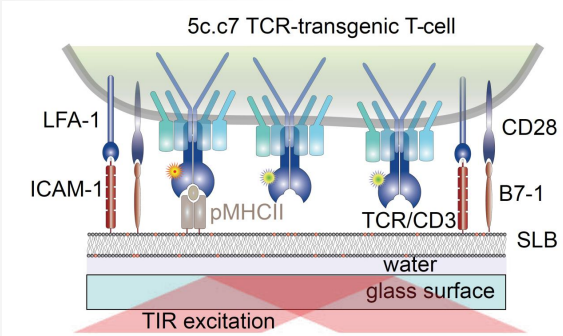

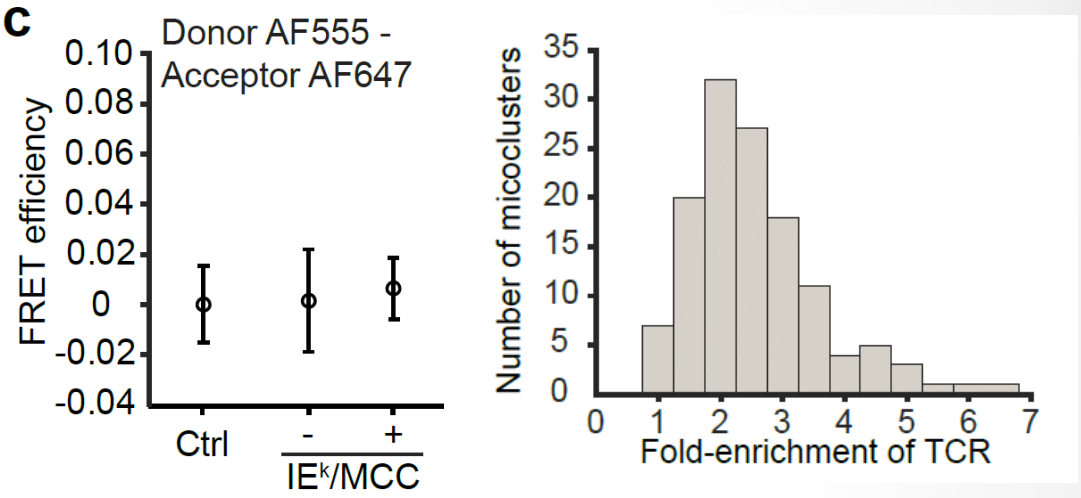

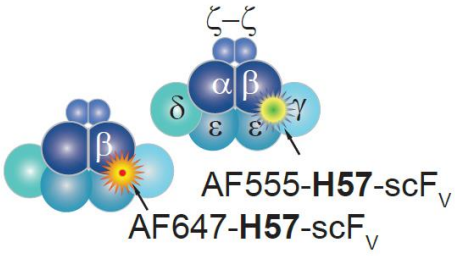

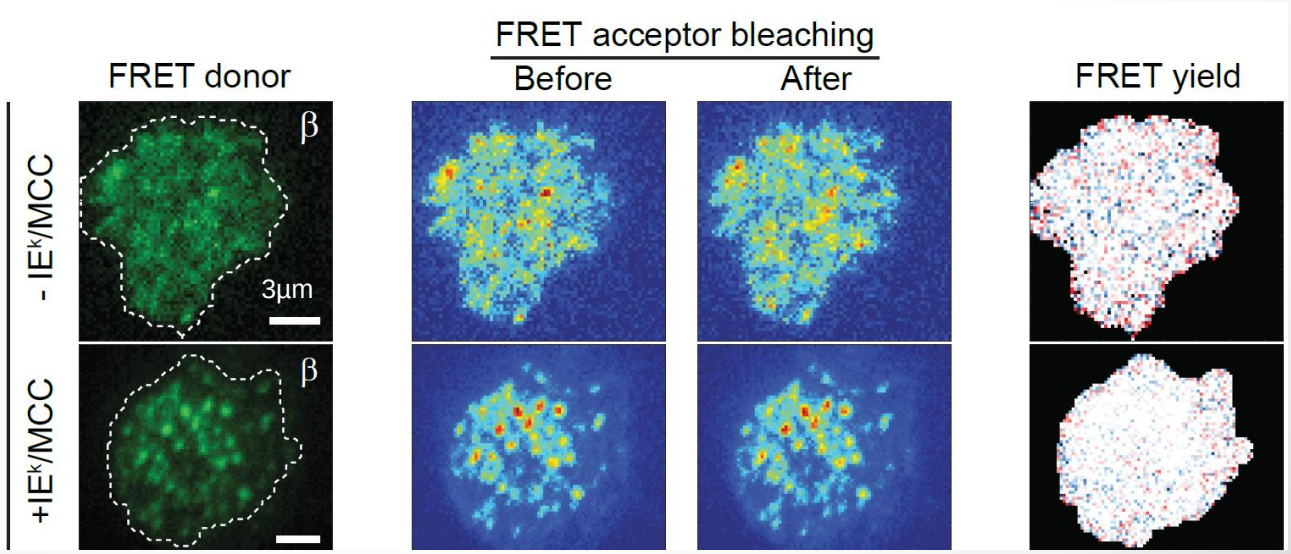

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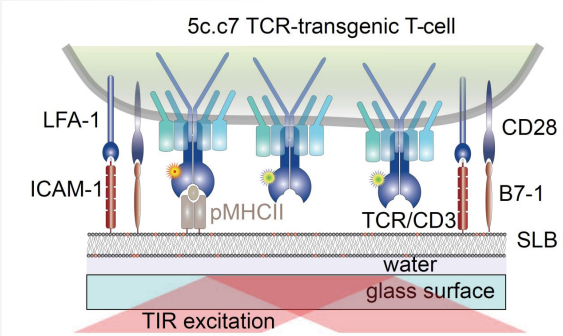
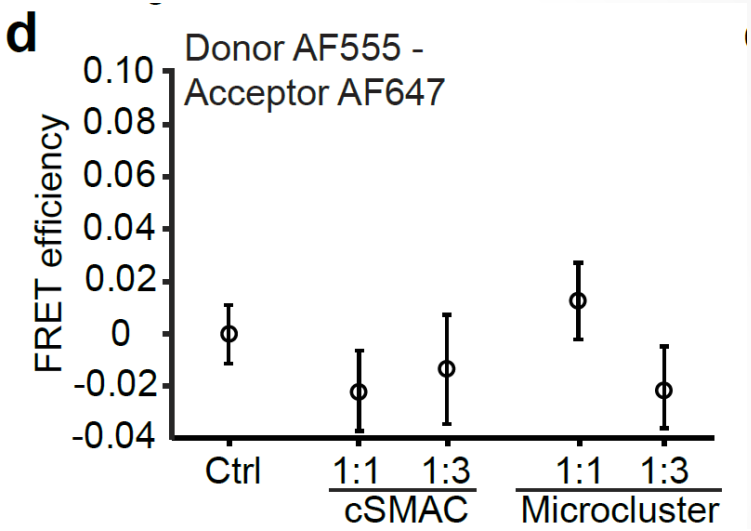
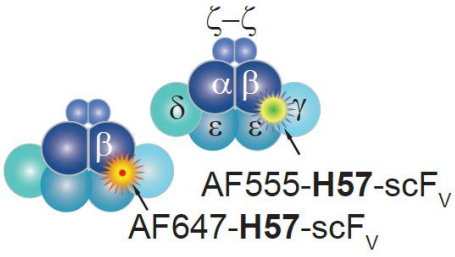
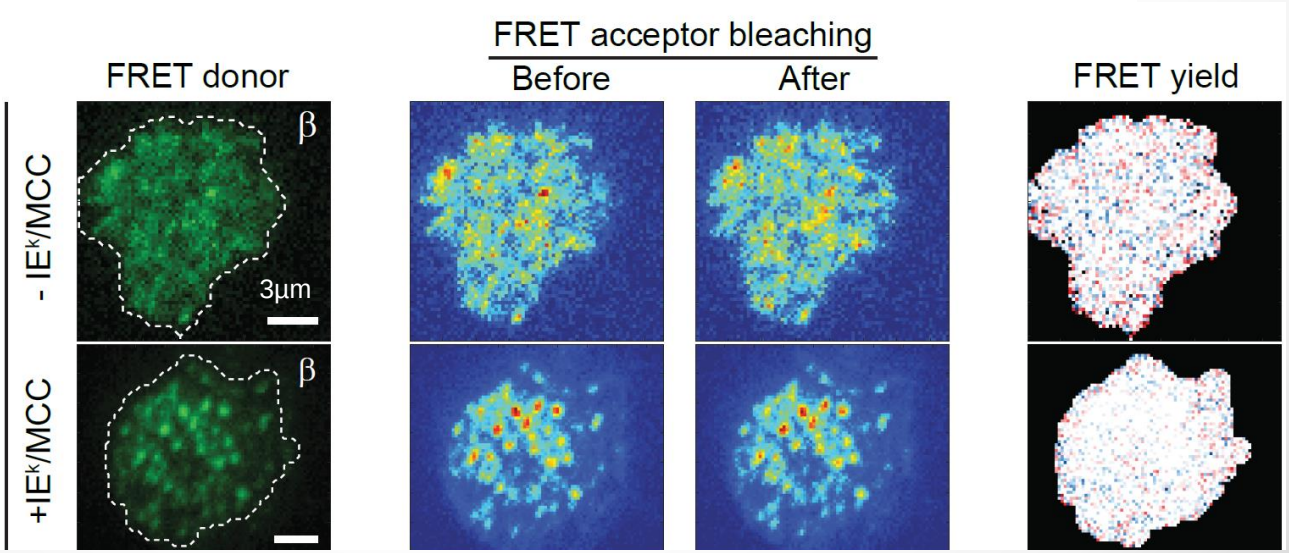




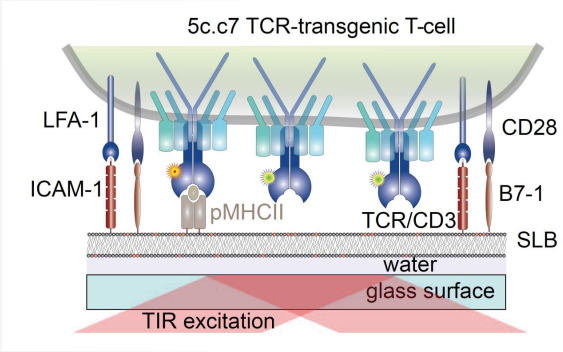




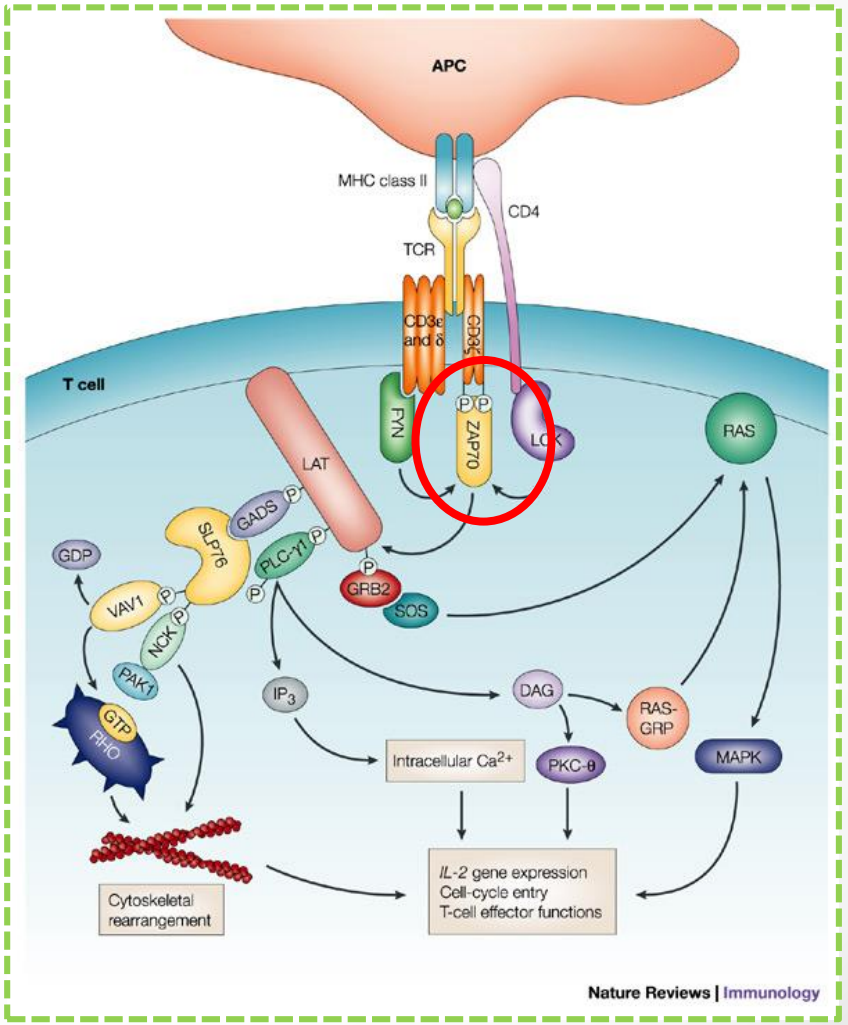
TCR oligomers at the inital contact of T-cells with bilayer ?

FRET – **F**örster **R**esonance **E**nergy **T**ransfer Experiments

Initial signaling events



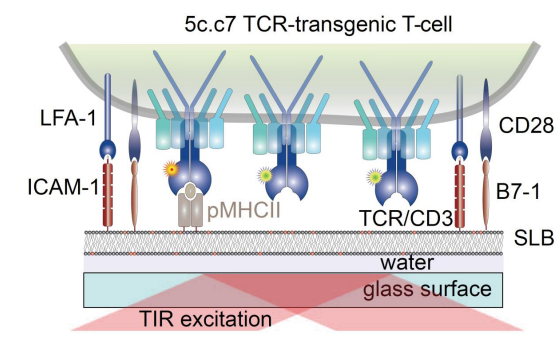
Recruitment of ZAP70 shows initial signaling events

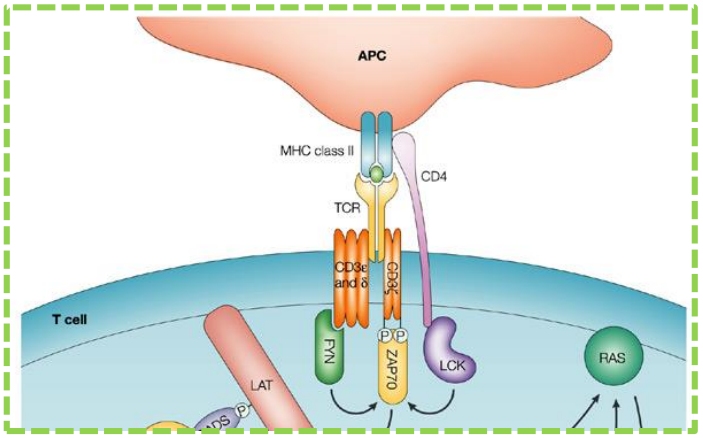


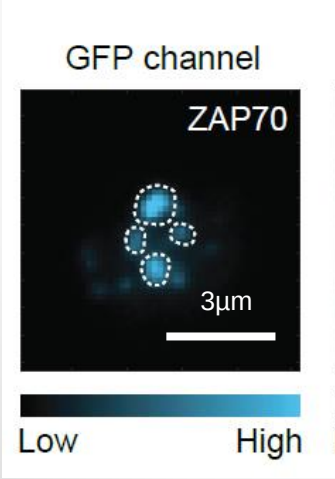
TCR oligomers at the inital contact of T-cells with bilayer ?

FRET – Förster Resonance Energy Transfer Experiments

Initial signaling events







TCR oligomers at the inital contact of T-cells with bilayer ?

FRET – Förster Resonance Energy Transfer Experiments

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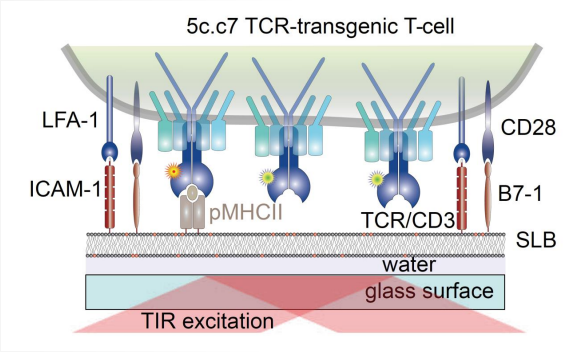

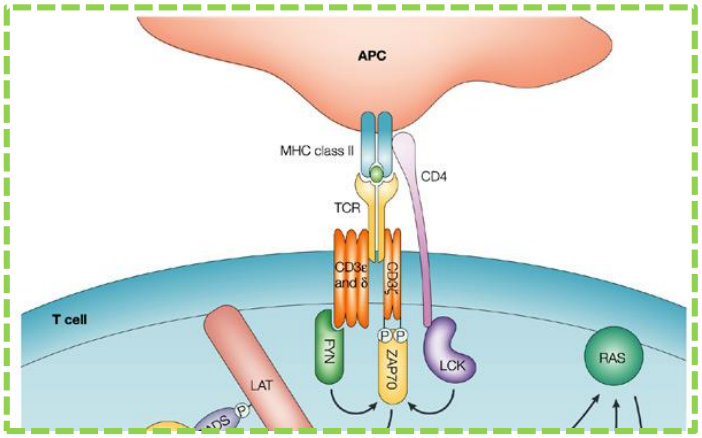

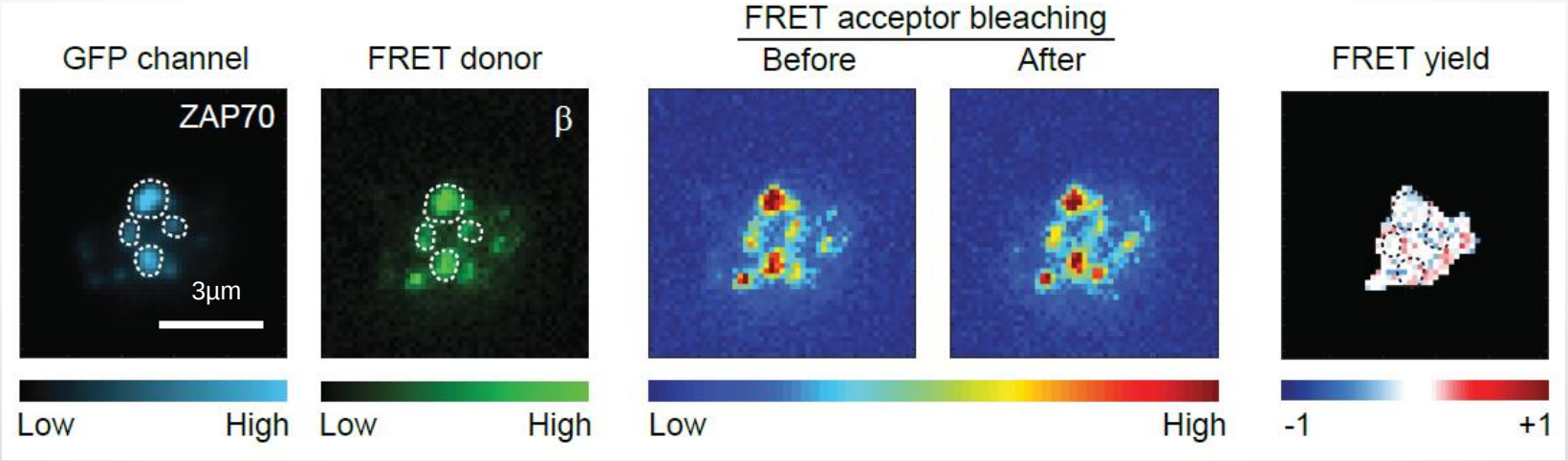

TCR oligomers at the inital contact of T-cells with bilayer ?

FRET – **F**örster **R**esonance **E**nergy **T**ransfer Experiments

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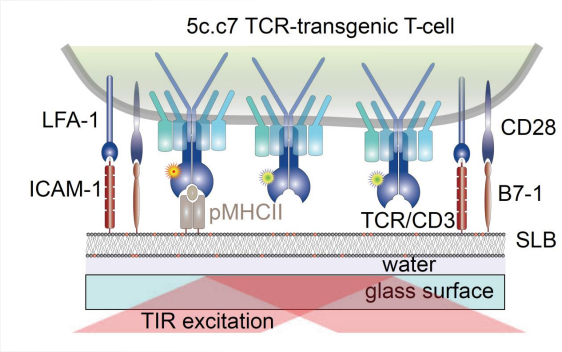
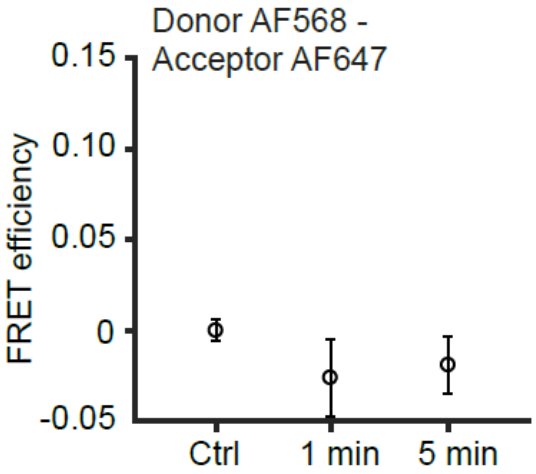
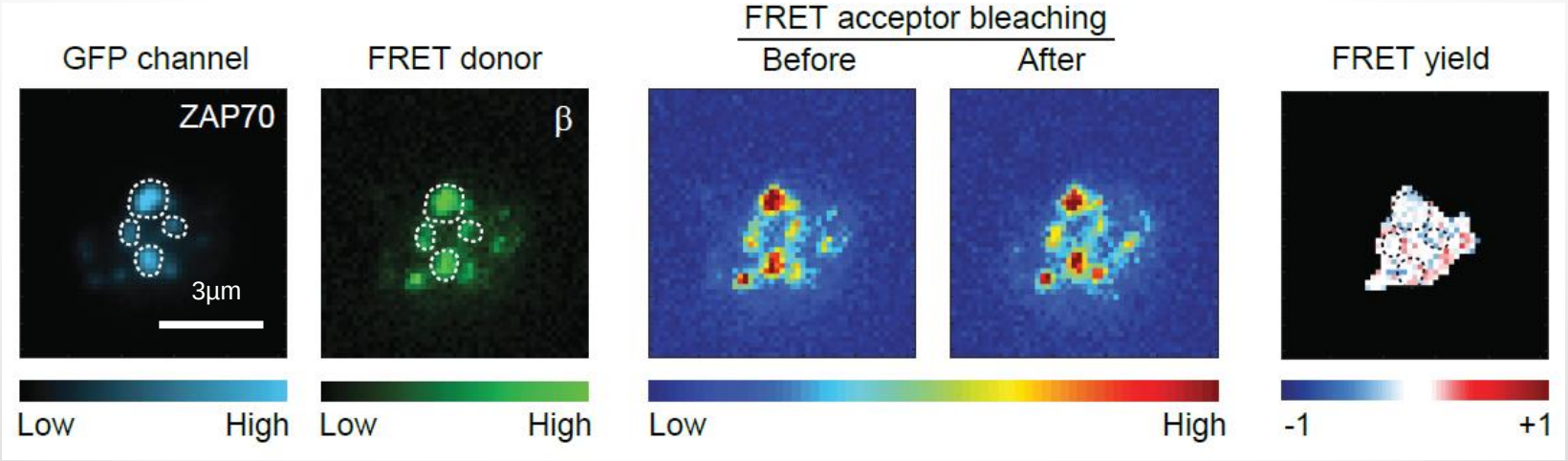
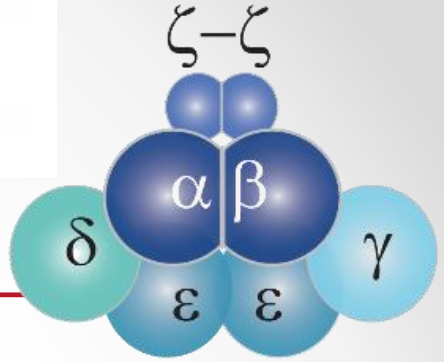






Conclusions

TOCCSL, PA/FCS & FRET experiments



No indication for other stoichiometry than one αβTCR per complex

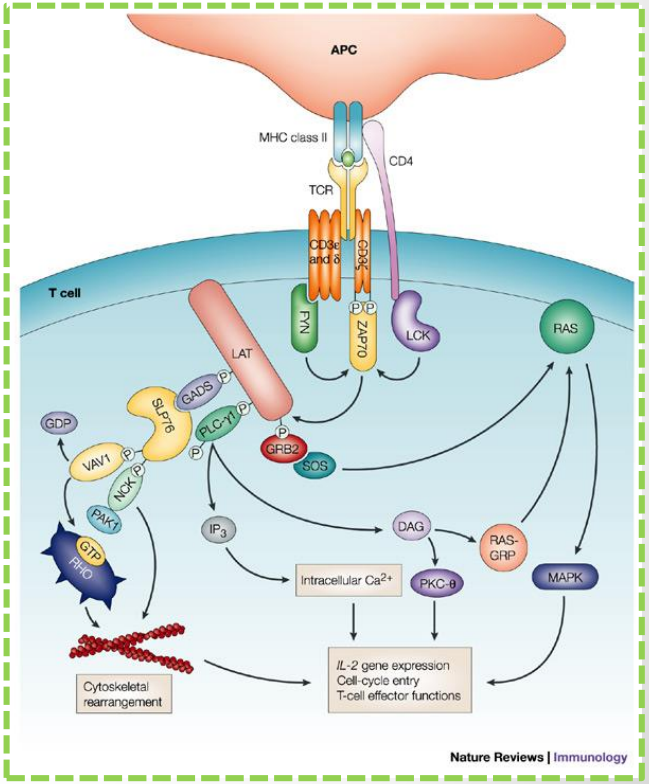
i. **Mobile fraction**: TOCCSL, two color-TOCCSL, single-molecule FRET and Photon-Antibunching FCS experiments

ii. **Immobile fraction**: FRET between TCRs, FRET between pMHCs

iii. **Activation via pMHC**: FRET between TCRs, FRET between pMHCs

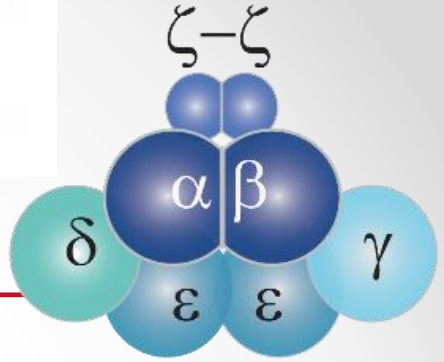
iv. **Early signaling events**: ZAP70 recruitment and FRET btw. TCRs.

v. **Late signaling events**: FRET between TCRs, FRET between pMHCs



Conclusions

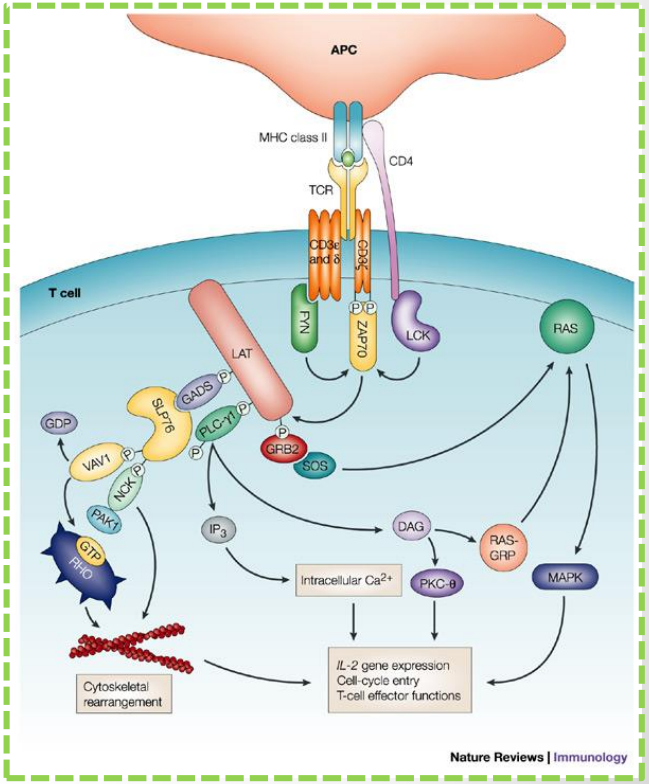
TOCCSL, PA/FCS & FRET experiments



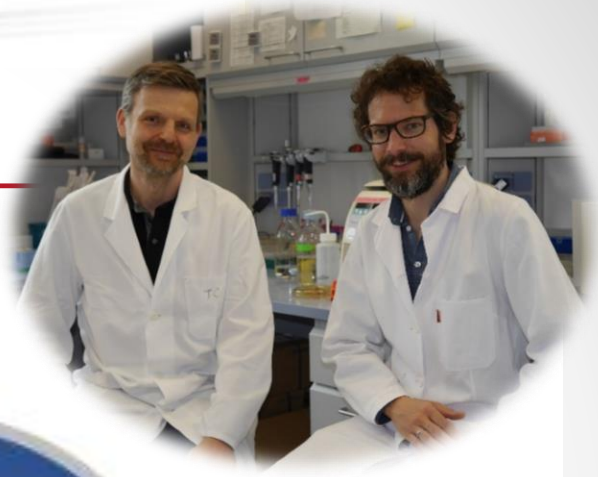
No increased FRET signal in TCR-enriched microclusters

Active separation of TCRs in microclusters

- → **Monomeric TCRs are responsible for the activation of T-cells, not TCR oligomers**
- → **Models for explaining high sensitivity/specificity still needed**



Brameshuber et al., Nature Immunology (2018), **19** (5), 487-96



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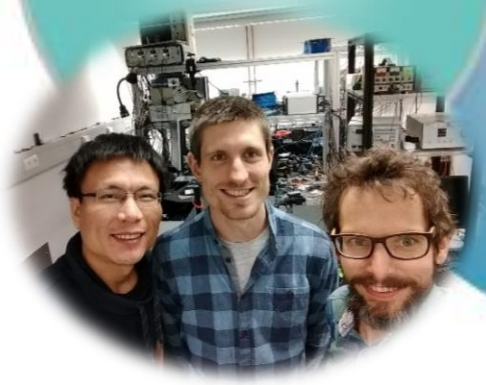

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