Saarland University

SFB 1027

Biophysics of Killing

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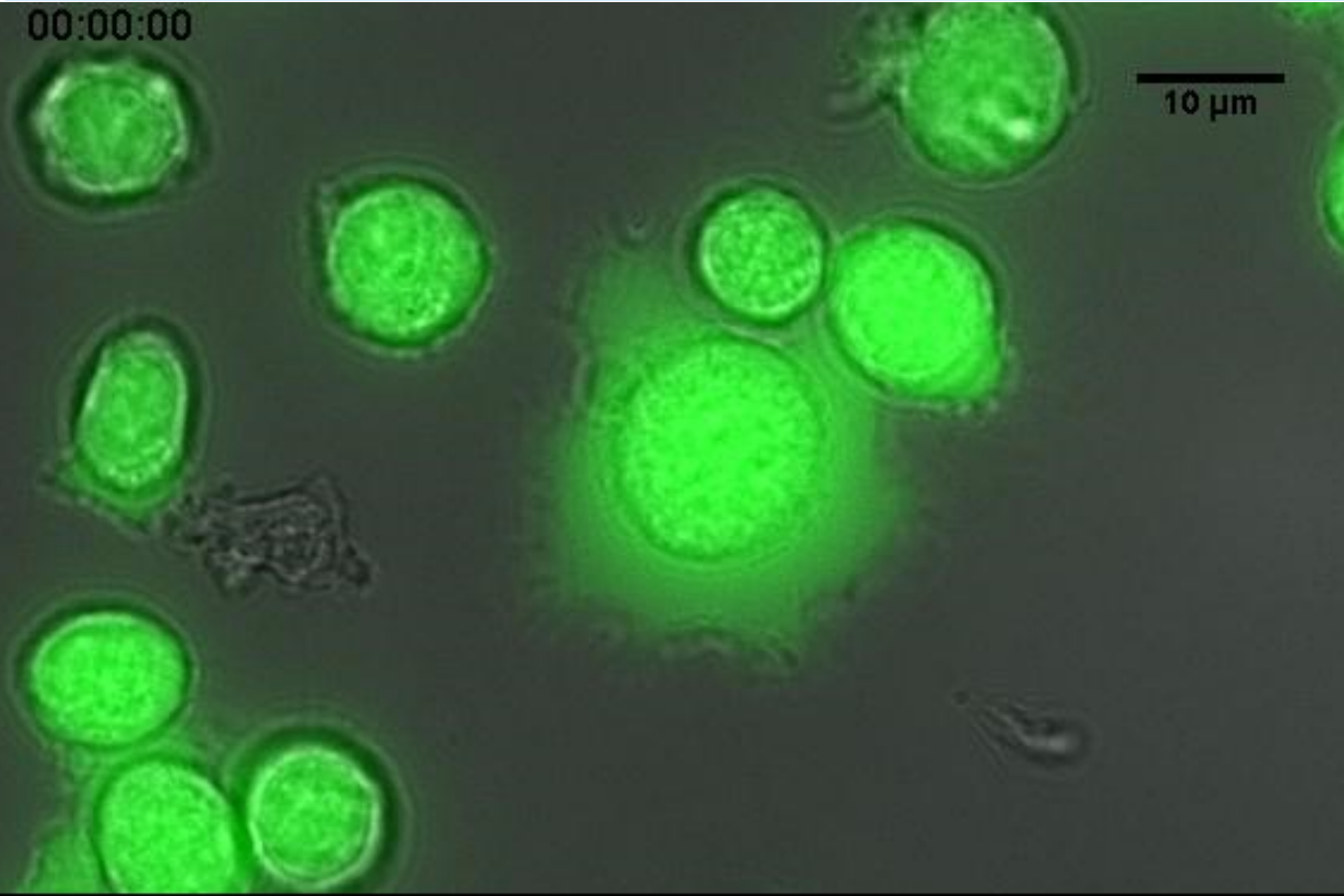
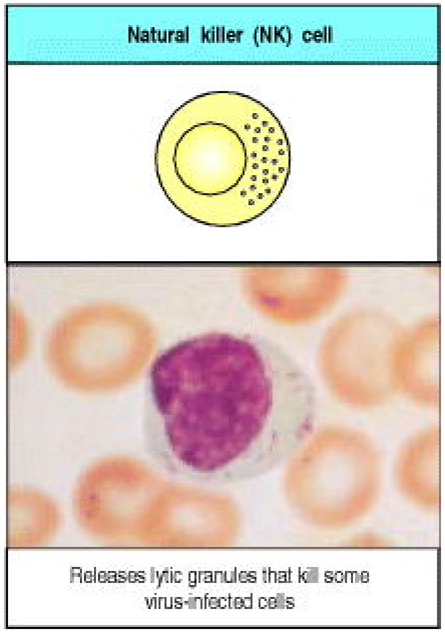
ICTS program „Statistical Biological Physics: From Single Molecule to Cell",
Bangalore, 11.-22.10.2022

Killer cells in action



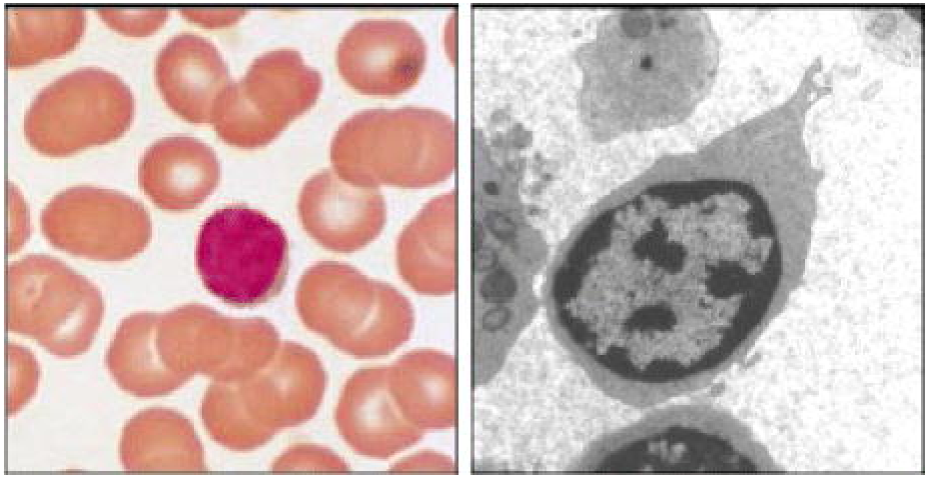
Natural killers & T cells

Natural killer cells (NK)



Part of the
innate immune system
lack antigen specific receptors
recognize & kill some abnormal cells

Cytotocix T Lymphocytes (T cells)



Part of the
adaptive immune system
Specific immune response against any foreign antigen

Both **kill** pathogenic cells via release of cytotoxins (lytic granules)

NK / T cell killing

- Friend or foe ?
- T cell activation
- Target search
- Making contact:

  - immunological synapse

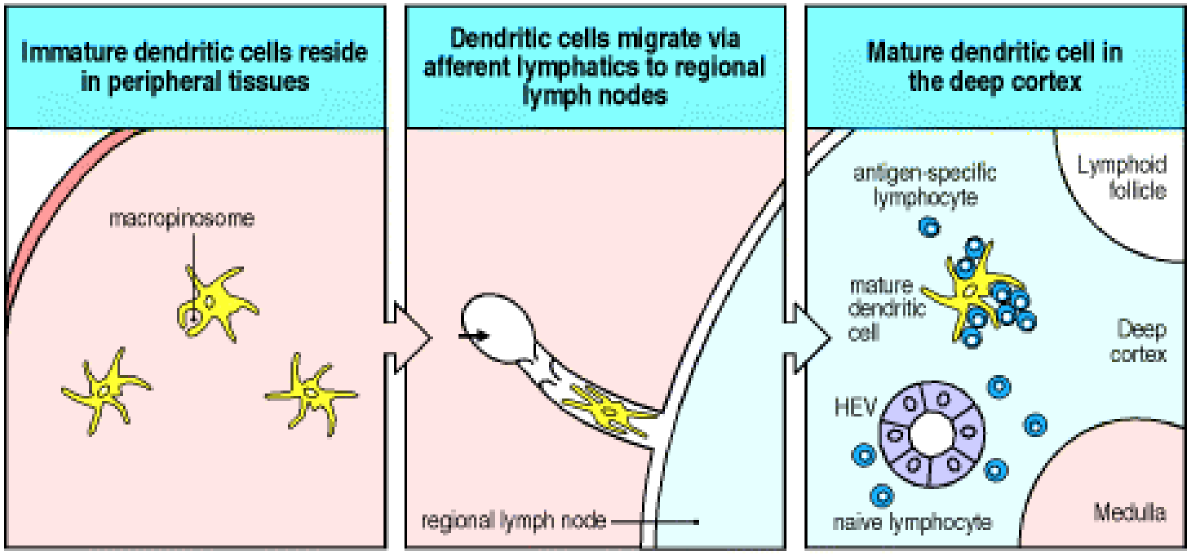
  - cell polarization
- Shooting: secretion of toxins
- Immune evasion counter measures …

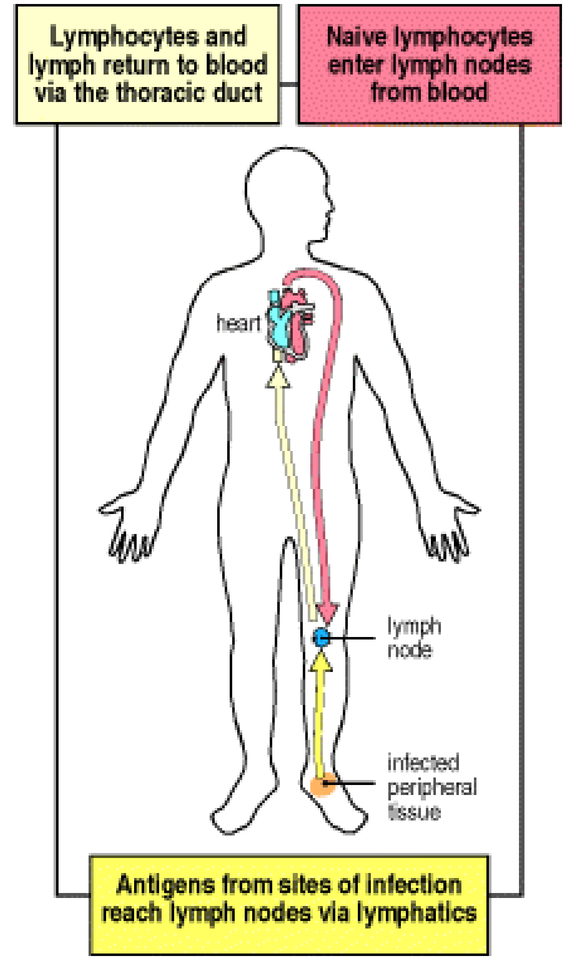
Immune system - basics

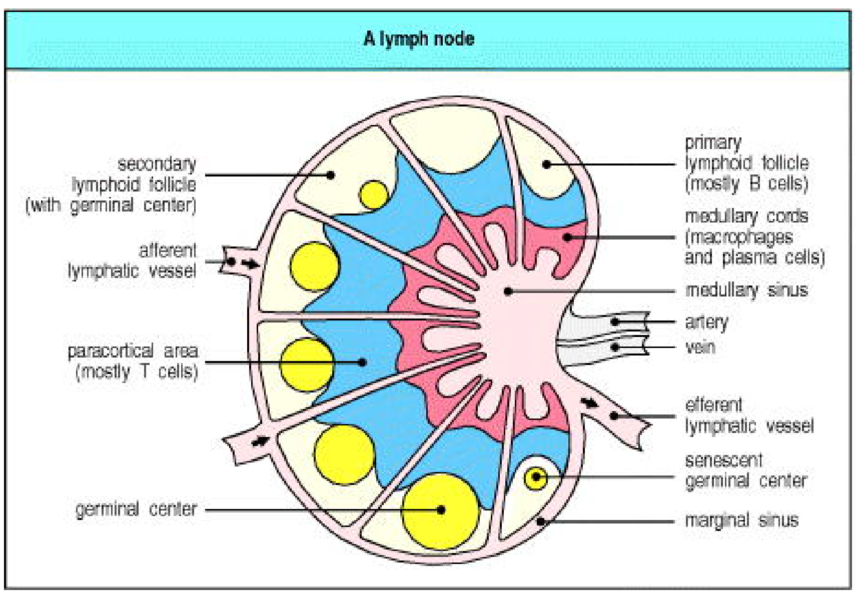
from “Janeway Immunbiology”

pdf of 8th edition available at
www.mta.ca/pshl/docs/janewayimmunobiology8.pdf

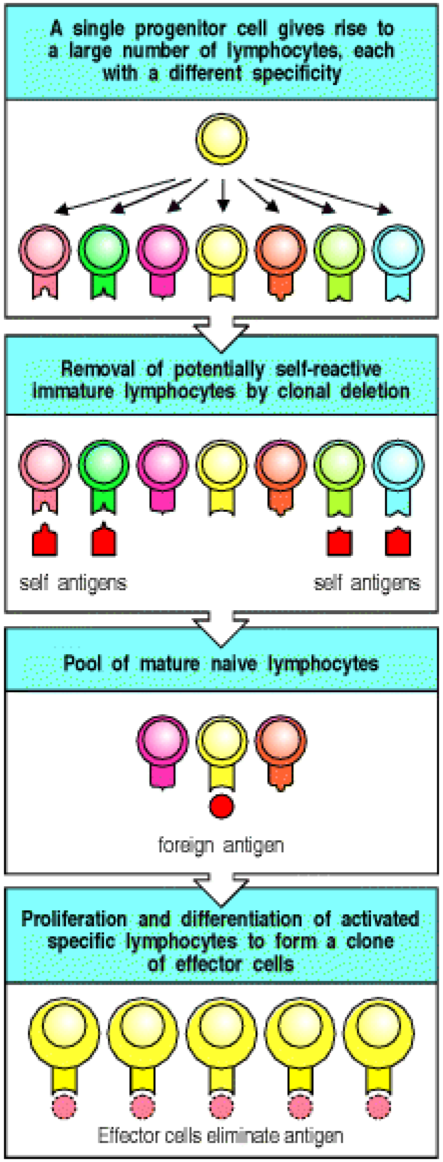
Activation of T cells by dendritic cells

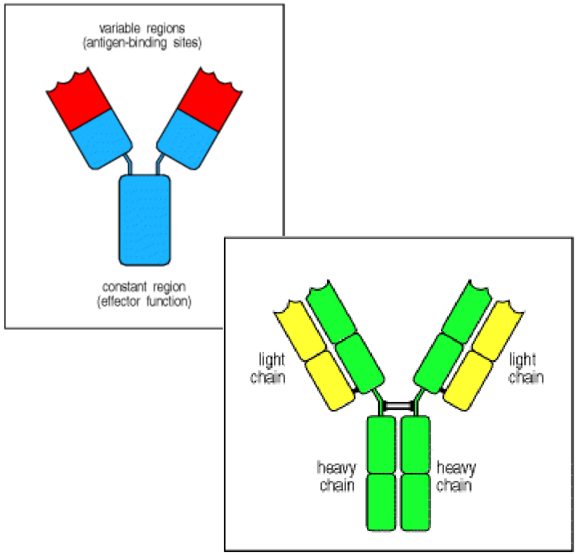


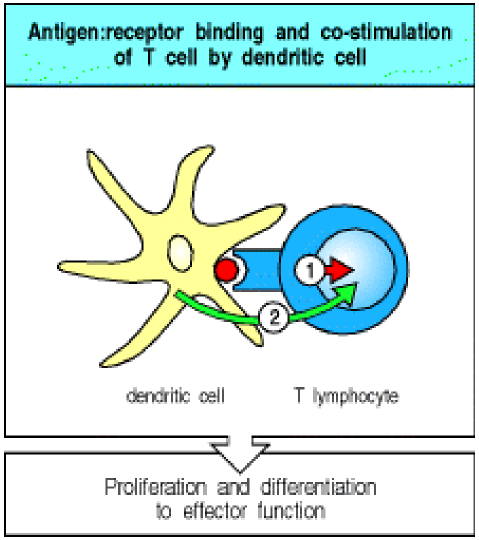


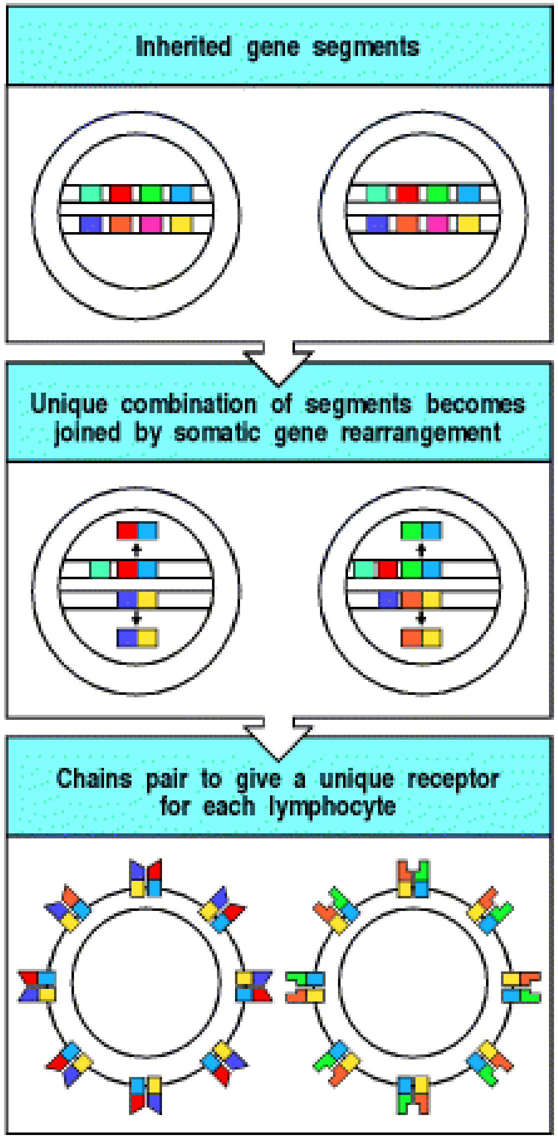


Clonal selection of T cells









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Friend or Foe ?

molecular mechanism

Stochastic generation of a T cell receptor

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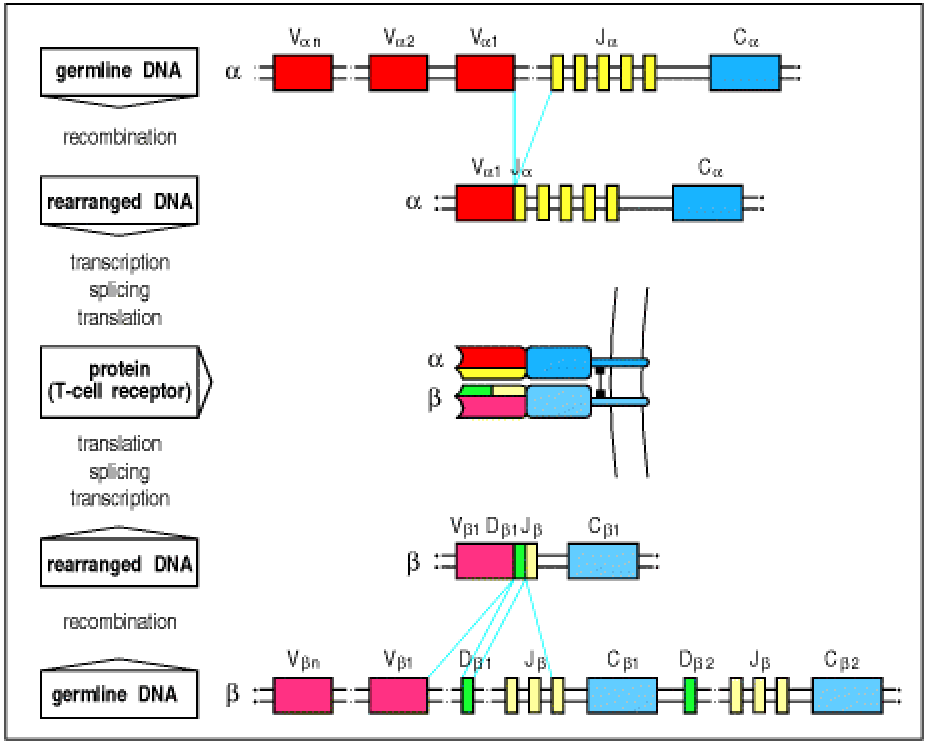
T cell receptor α- and β- chain gene rearrangement and expression



Element	α:β receptors β	α:β receptors α
Variable segments (V)	52	~70
Diversity segments (D)	2	0
D segments read in 3 frames	often	–
Joining segments (J)	13	61
Joints with N- and P-nucleotides	2	1
Number of V gene pairs	5.8×10^6	
Junctional diversity	$\sim 2 \times 10^{11}$	
Total diversity	$\sim 10^{18}$	

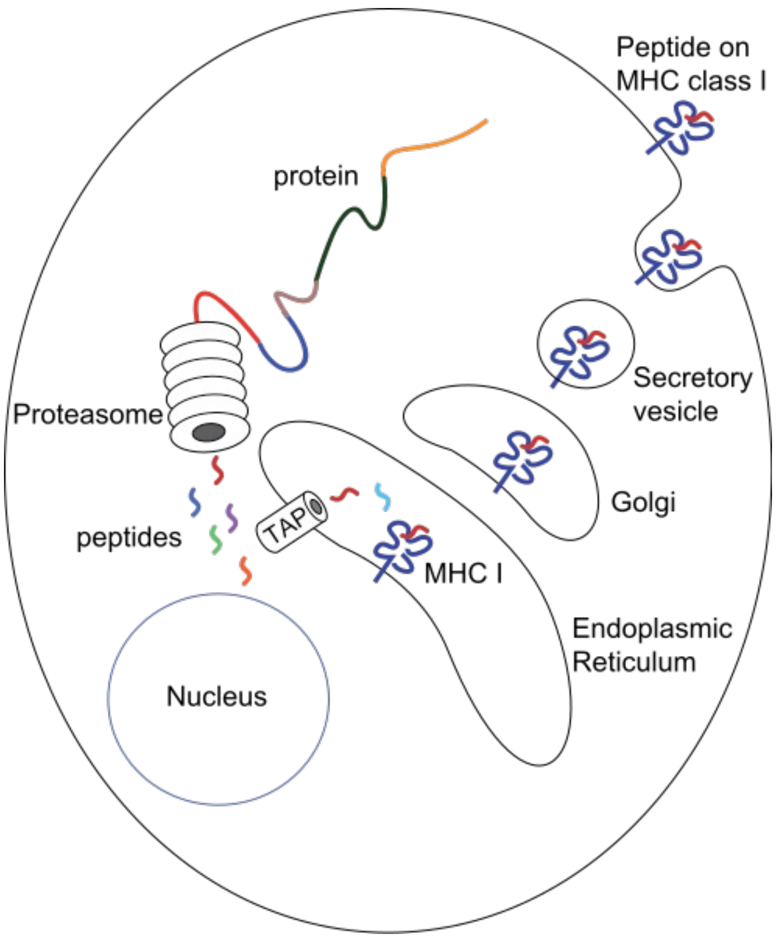
Statistical inference of the generation probability
Murugan et al. PNAS 109, 16161 (2012)

See also A. Walczak's talks on YouTube: https://www.youtube.com/watch?v=5Xw5BvlDI_o
https://www.youtube.com/watch?v=tWdx0uI6GCY

Major Histocompatibility Complex (MHC)

MHC displays antigens derived from pathogens on the cell surface **to the appropriate T cells**.

The ligand recognized by the **T cell receptor** is a **peptide** derived from the foreign antigen bound to a **MHC molecule** on the cell surface.



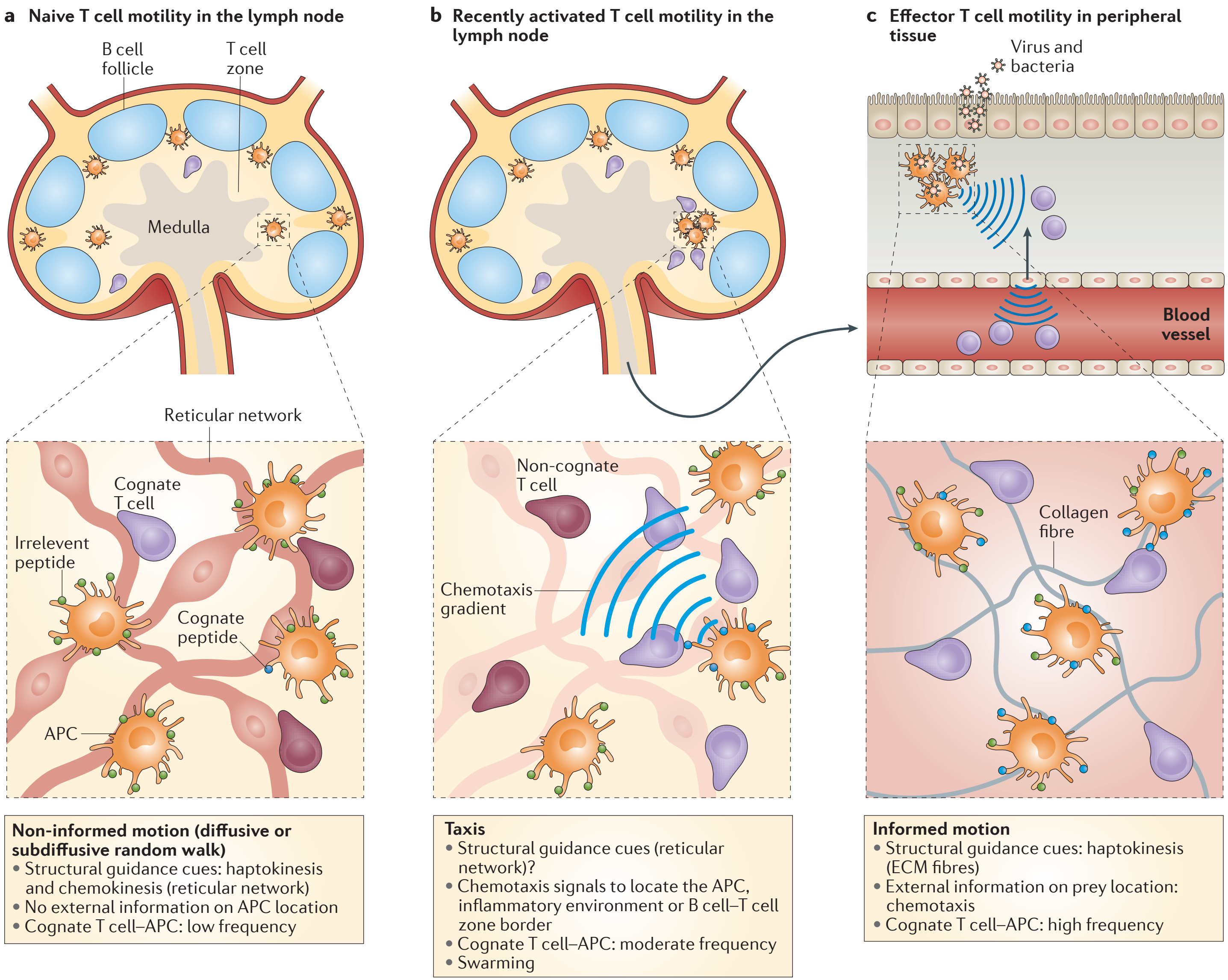
Two classes:

MHC I: occurs on all nucleated cells
activates T cell receptor
inhibits NK cell receptor

MHC II: occurs on APCs
(dendritic cells, macrophages, B cells)

Target search

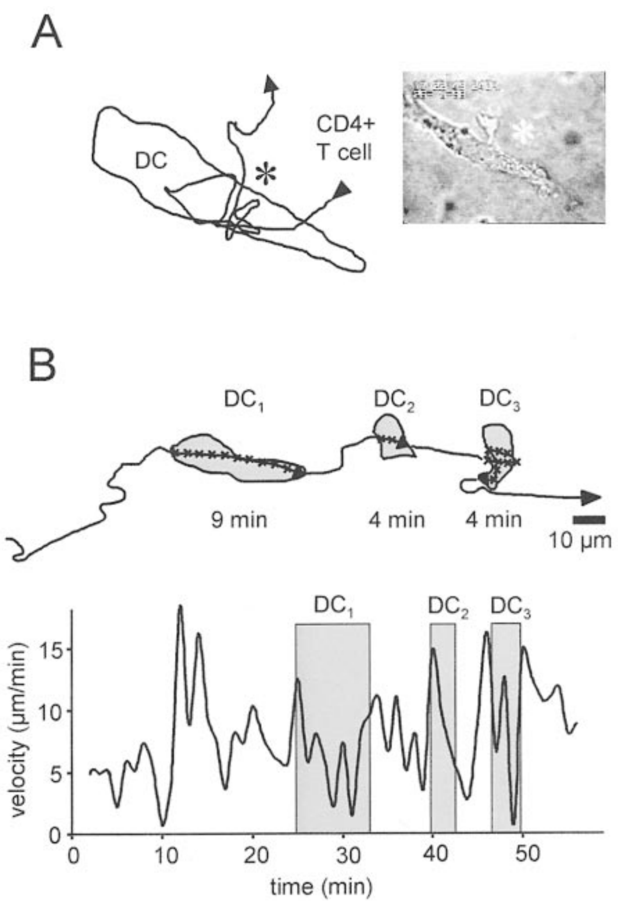
T cell motility according activation state

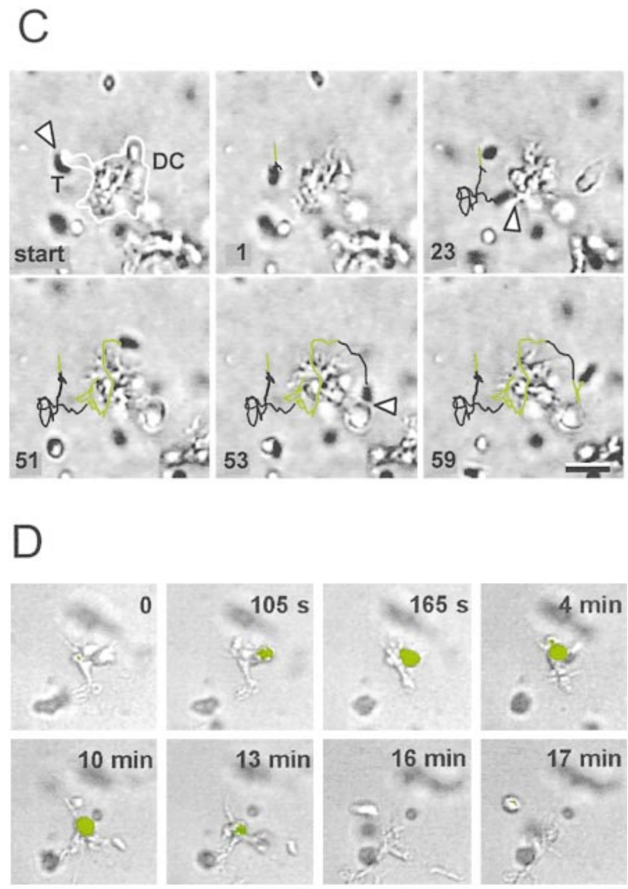


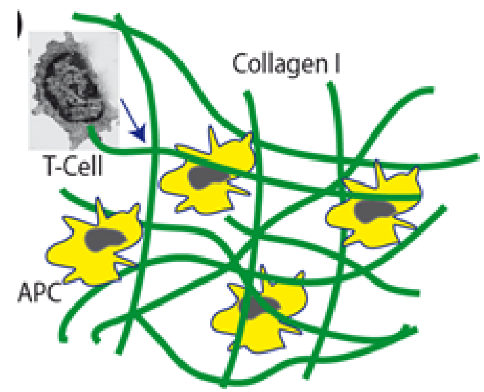
[Krummel et al., Nat. Rev. Imm. (2016)]

Interaction of T cells with dendritic cells

(in 3d collagen matrix)







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Multiple contacts

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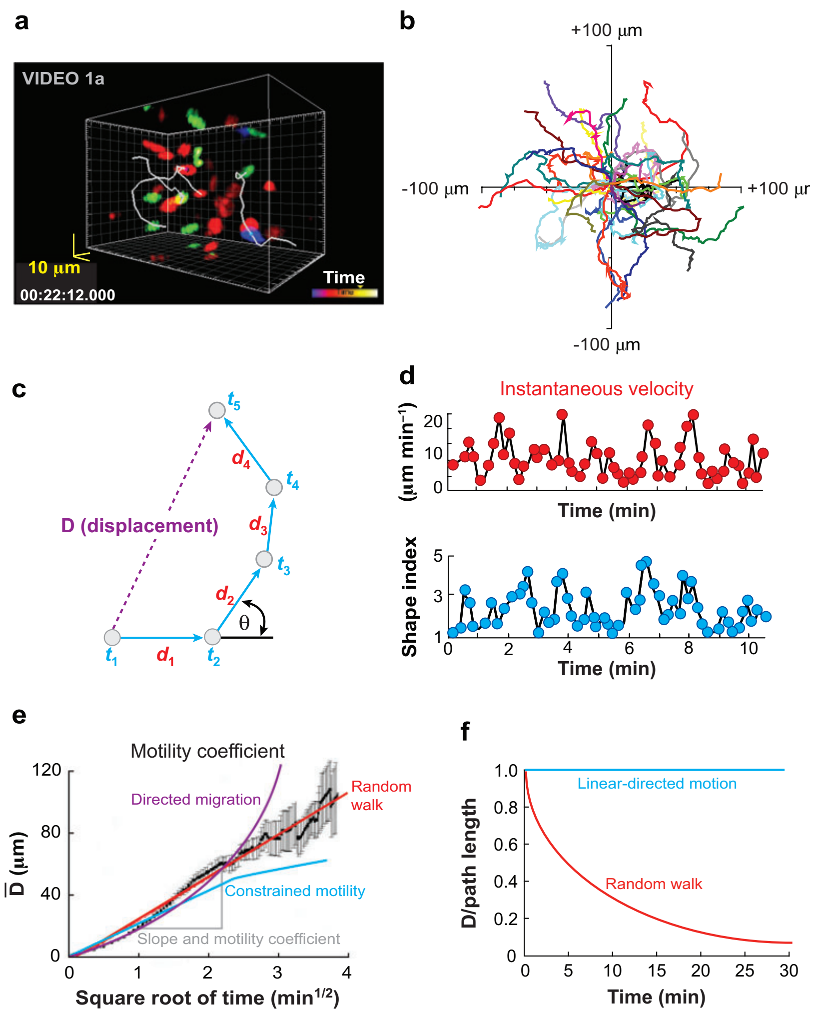
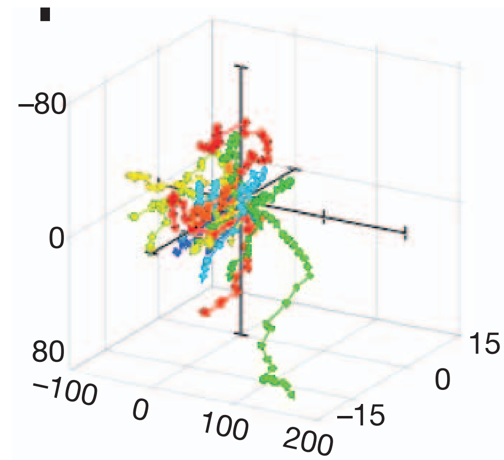
Calcium influx

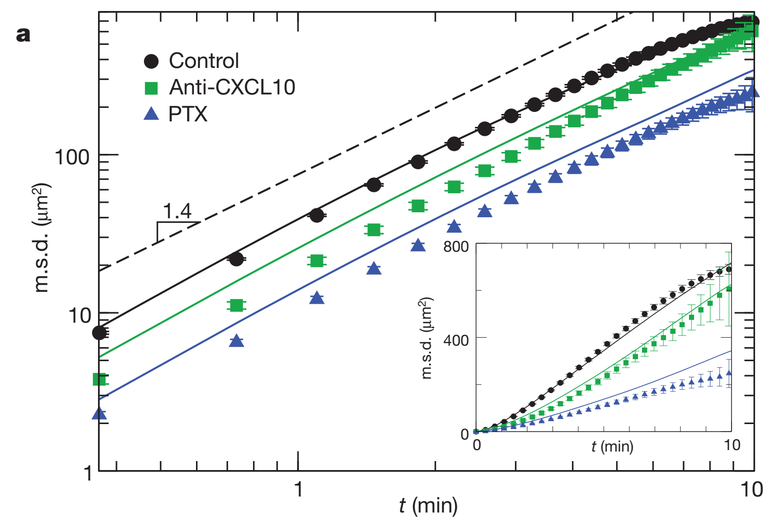
[Gunzer et al, Immunity 13, 323 (2000)]

T cells in tissue: Random walk vs. Lévy flight

(experimental technique: two-photon microscopy)



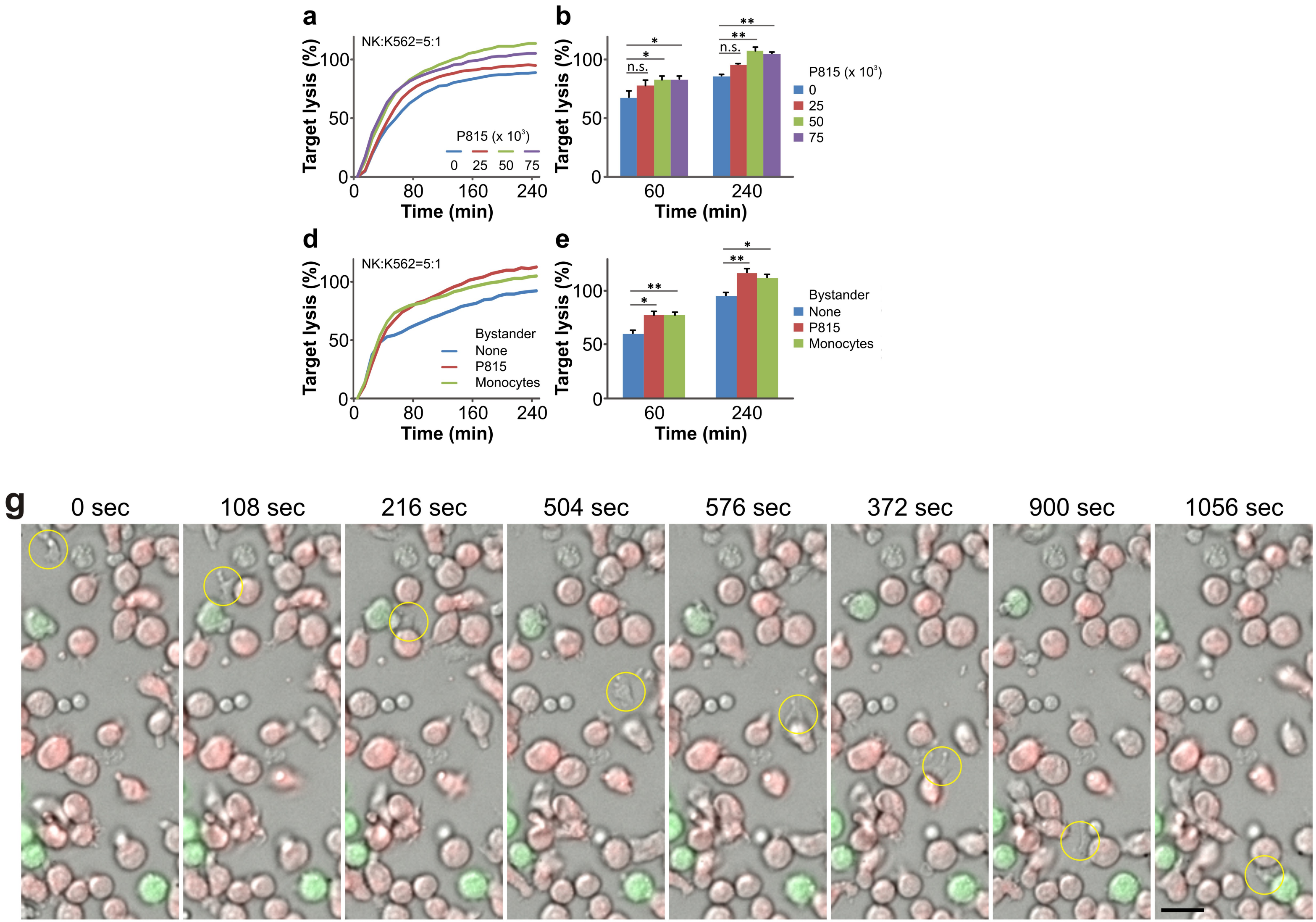




[Miller et al, Science 296, 1869 (2002)]

[Harris et al., Nature 486, 545 (2012)]

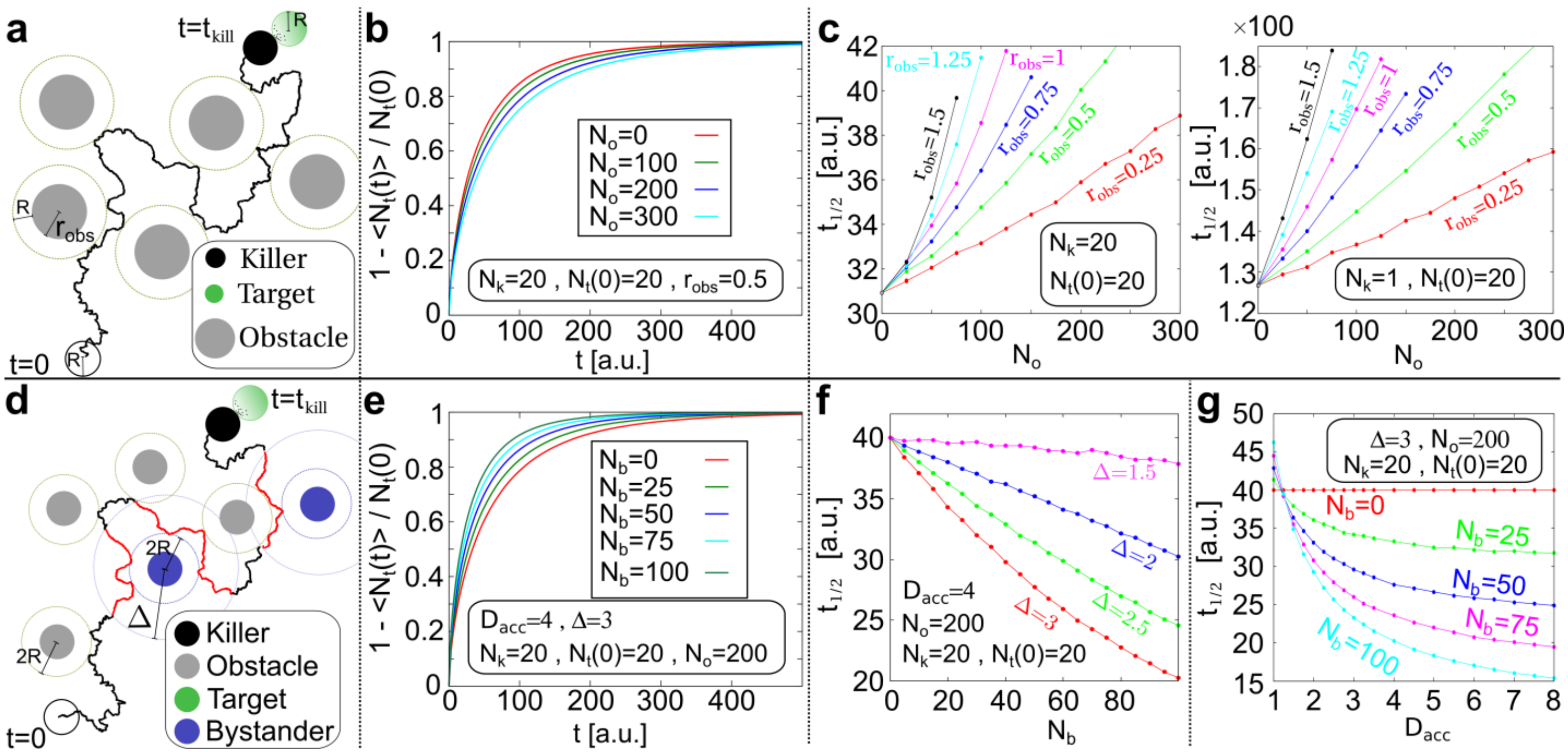
Bystanders increase search / killing efficiency



Zhou et al., Sci. Rep. 7, 44357 (2017)

Theoretical model

Passive bystanders are obstacles – impede target search

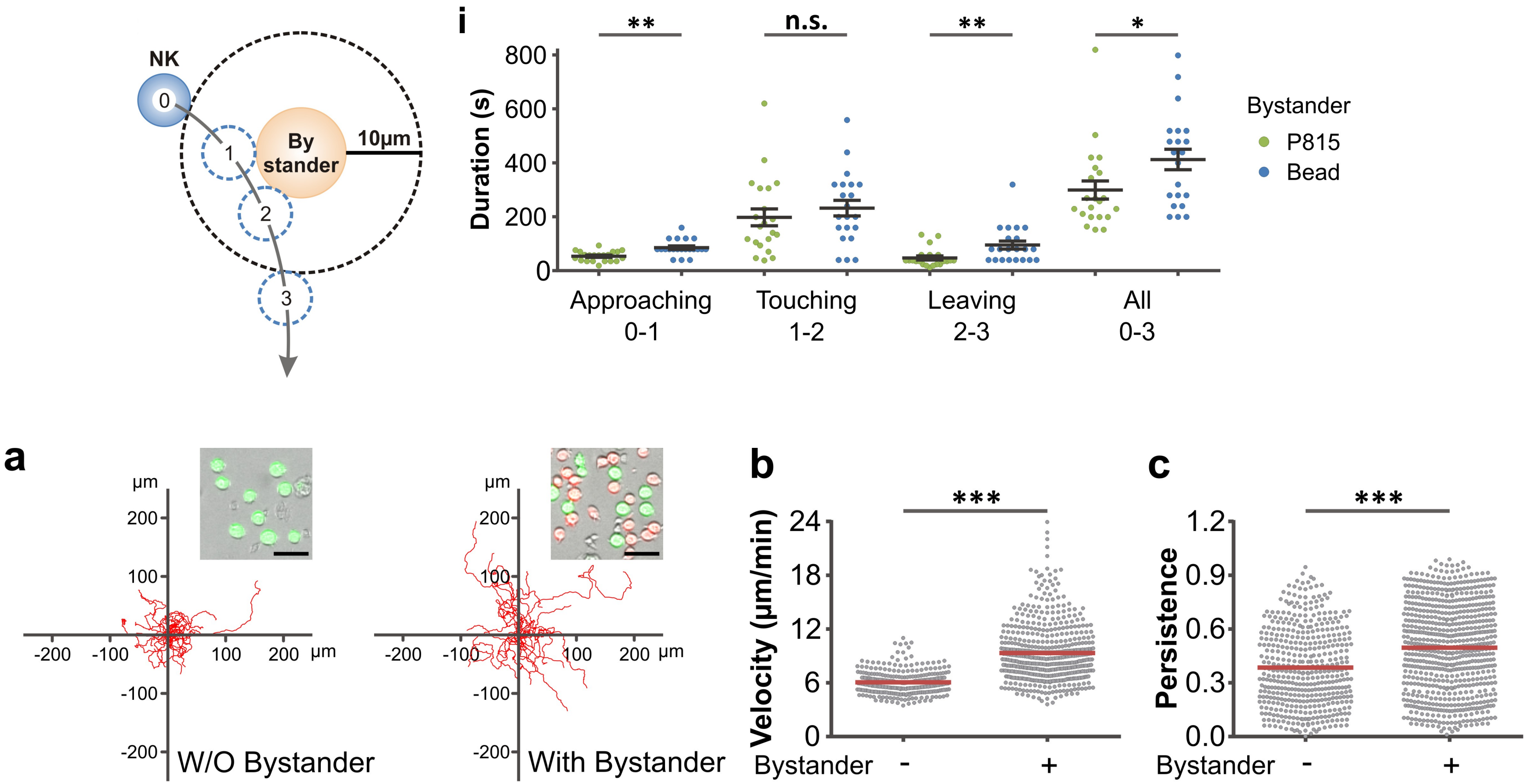


Bystanders increase locally diffusion constant of searchers –

- target search accelerated

Zhou et al., Sci. Rep. 7, 44357 (2017)

Experiment: Bystander accelerate killers



Zhou et al., Sci. Rep. 7, 44357 (2017)

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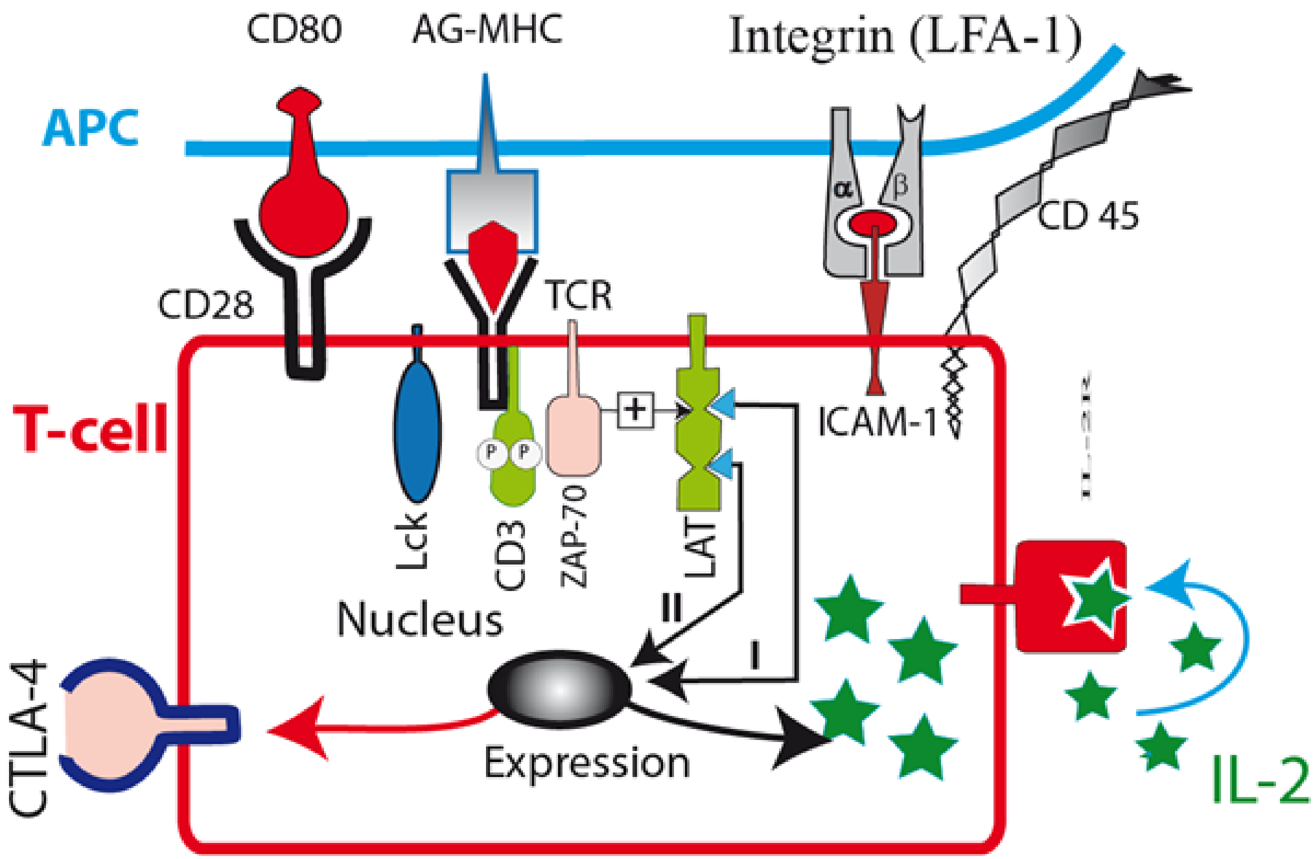
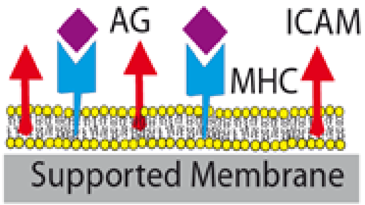
Formation of the immunological synapse (IS)

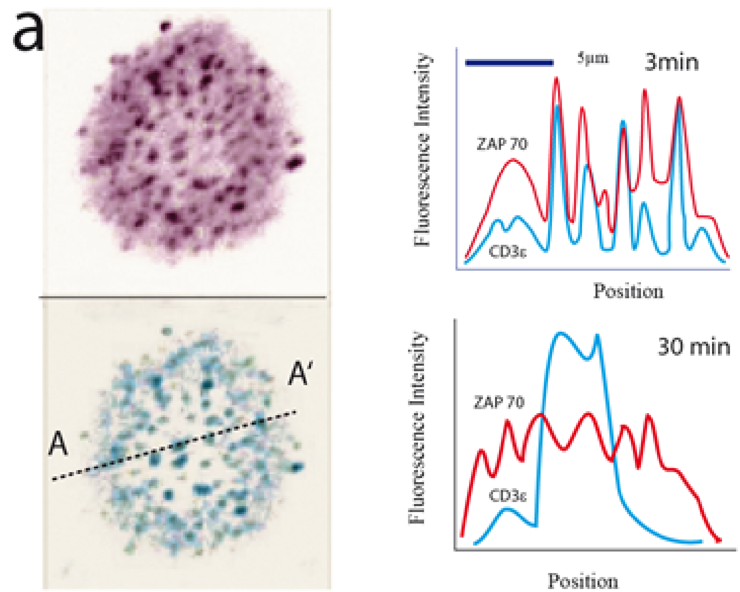
Molecular players during T cell activation by APC

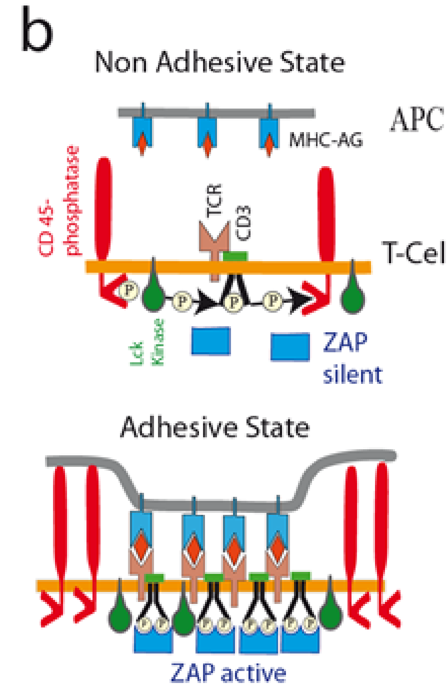


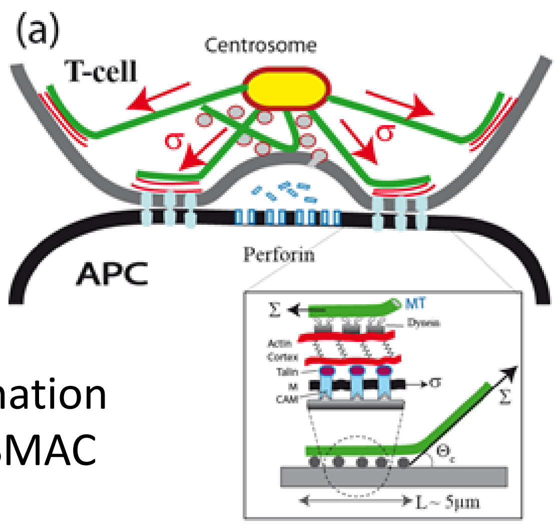
Formation of the adhesion domain and SMAC

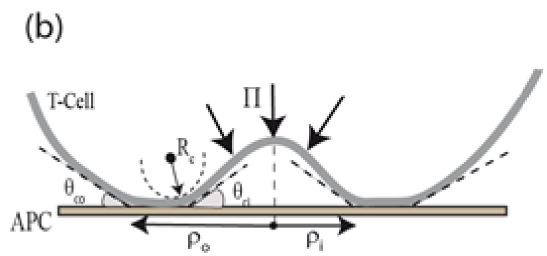


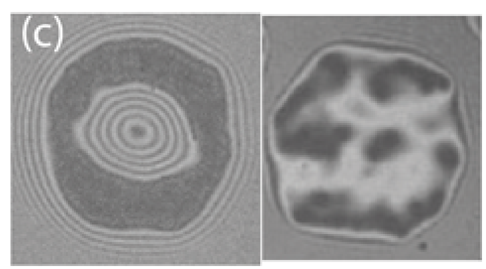
Experimental setup to study IS formation with TIRF







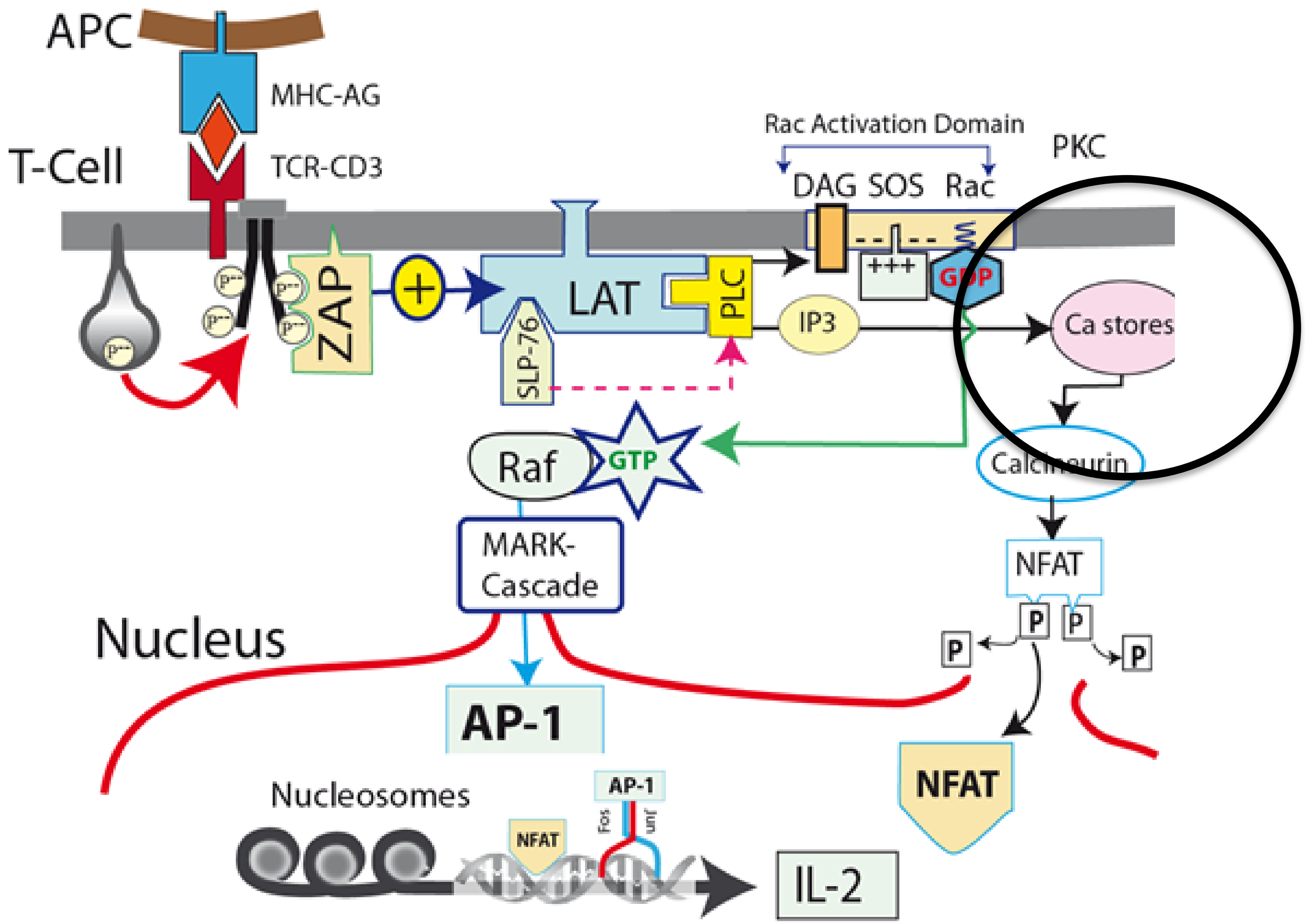




Pattern formation at the IS -> SMAC

[Sackmann/Merkel, Lipowsky]

T cell activation is Calcium dependent



Calcium / MAPK pathway to genetic expression of IL2

Dynamics of T cell polarization

T-cell killing – polarization and IS

IS = Immunological Synapse

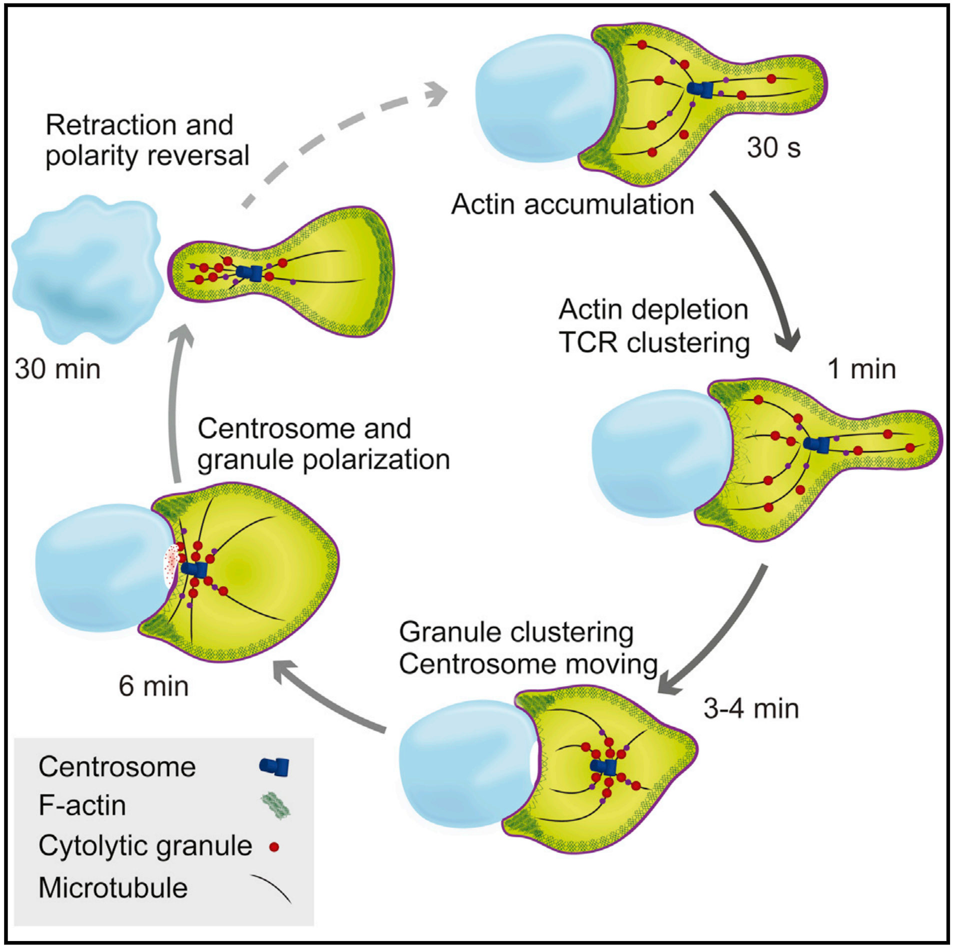
1. Bright orange:
   living target cells
   (Jurkat cells)

2. Red:
   primary human NK cells
   (labeled w. LysoTracker)

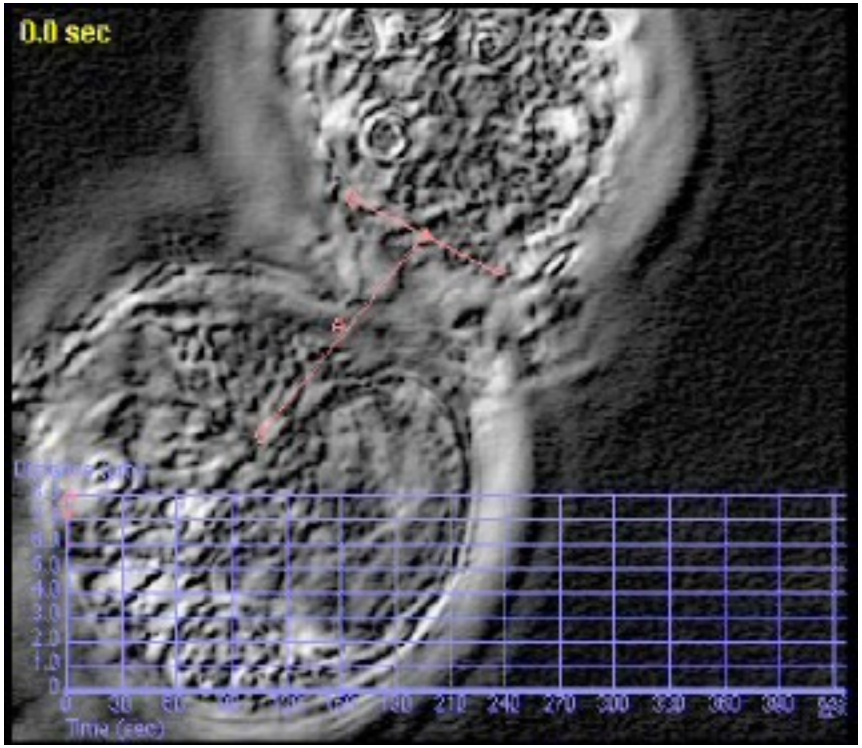
3. Orange-> green:
   apoptosis
   (often paralleled
   with blebbing)

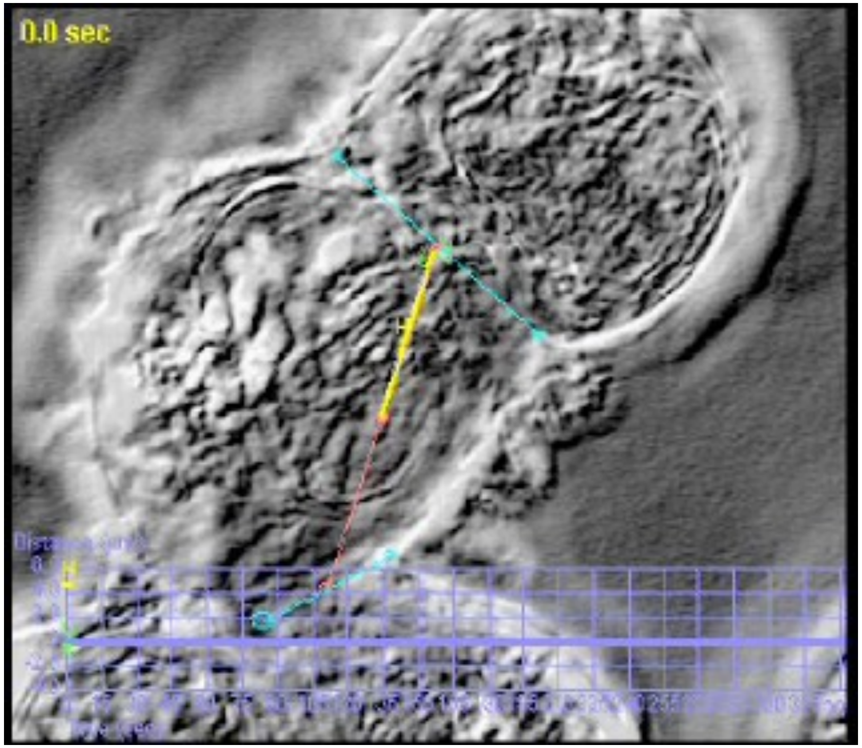
4. Sudden loss of orange
   –> Necrosis
   (Bursting open)

Polarization of T cells during killing



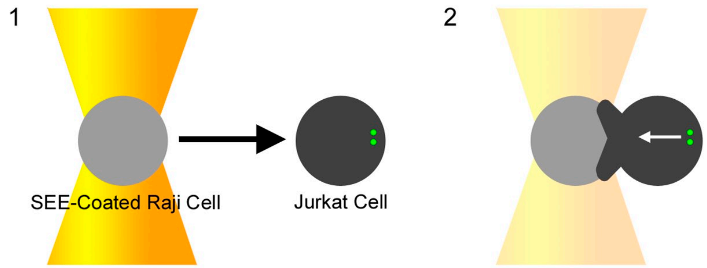
Polarization of MT cytoskeleton during killing

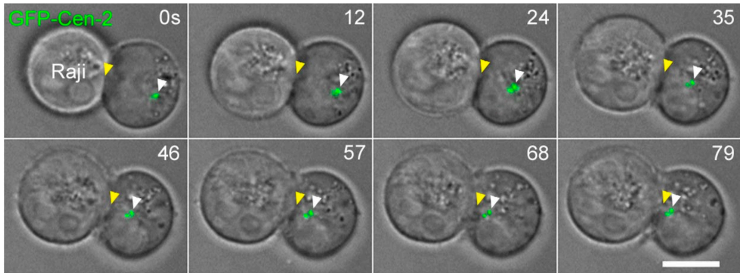


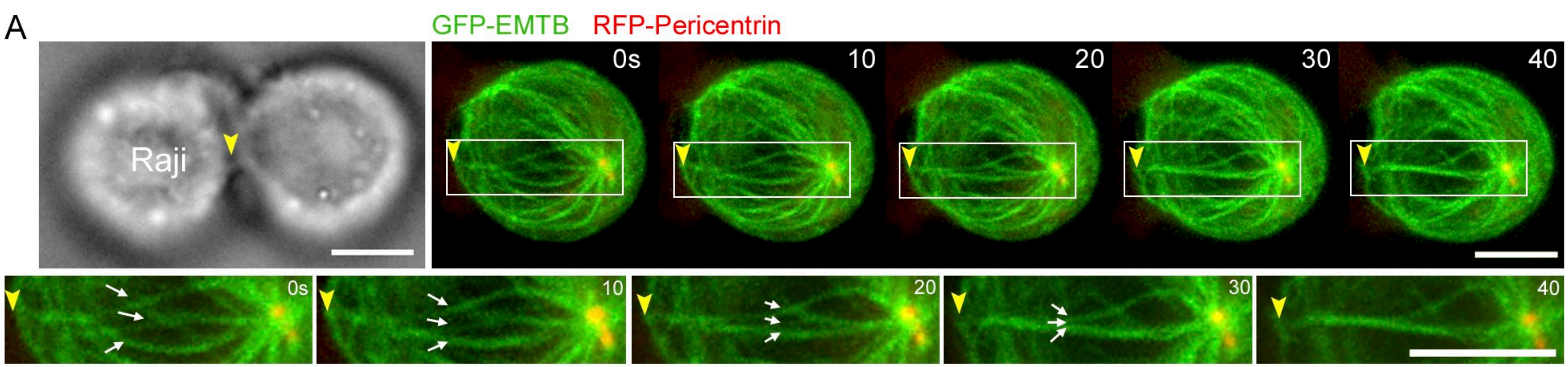


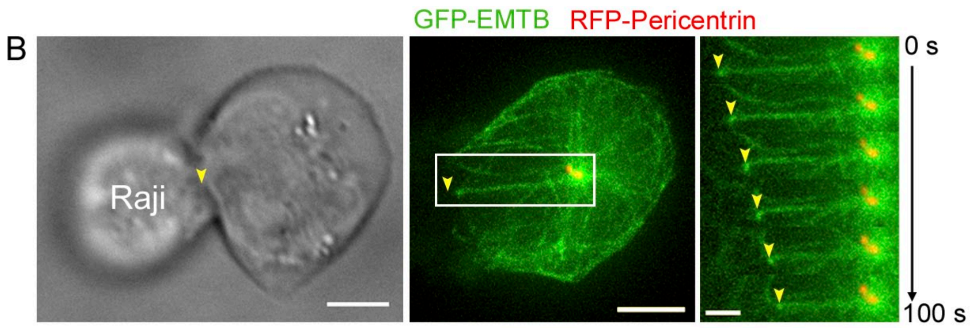
[Kuhn & Poenie, Immunity 2002]

MTOC relocation during T-cell polarization





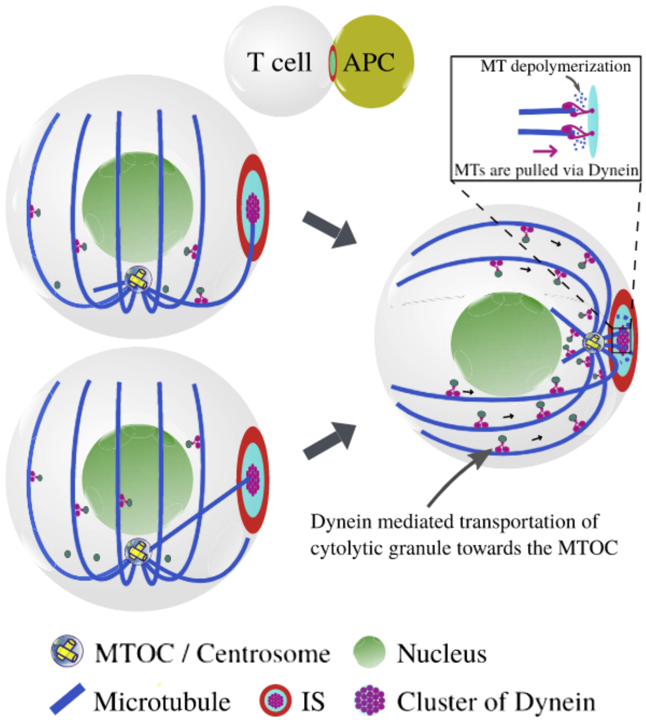


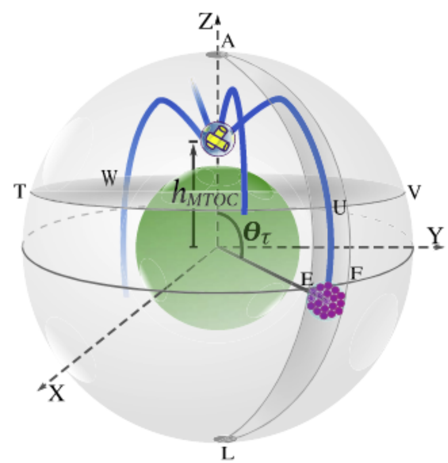


MT end-on capture-
shrinking mechanism

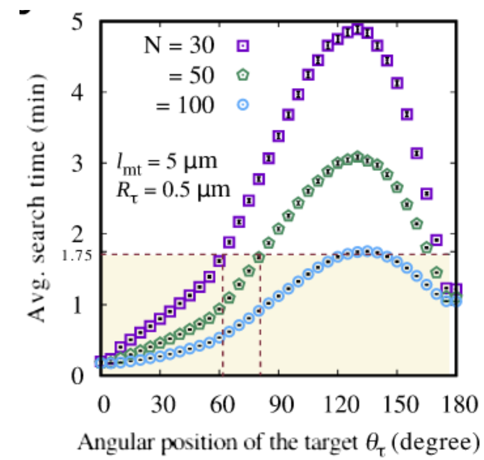
[Yi et al, JCB 2013]

Anchoring MTs at the IS: Search & Capture



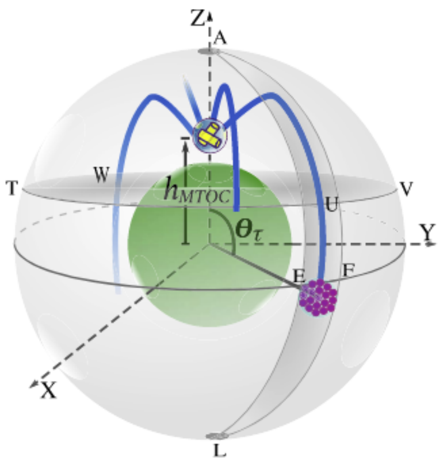


Average search time:



[Sarkar, HR, Paul, Biophys. J. (2019)]

Time scale estimate for search & capture



R_{cell} = 5µm, R_{target} = 0.5µm
$\langle L_{MT} \rangle = \pi R_{cell}$ ~ 15µm

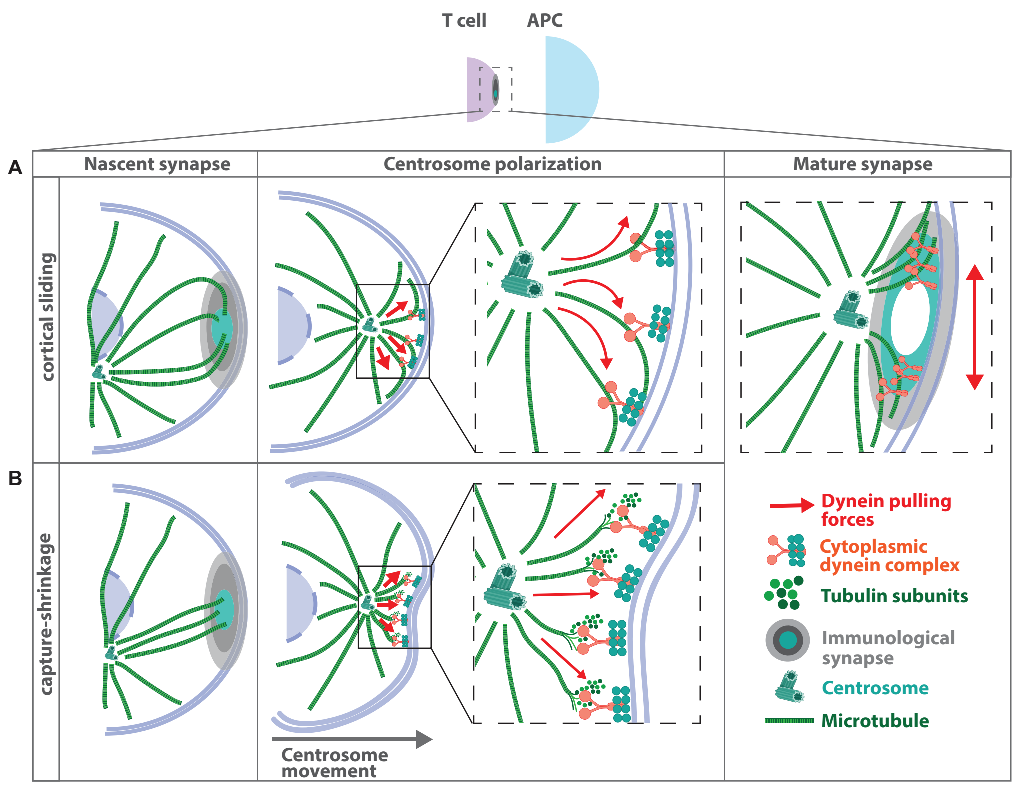
v_{growth} = 15µm/min, $v_{shrink} \sim v_{growth}$
T_{trial} ~ 2min

$P_{capture} = 2R_{target}/2\pi R_{cell}\sin\theta_{target} > R_{target}/\pi R_{cell} = 0.032$
-> $N_{trials} = 1/P_{capture} < 31$
-> $T_{capture}(1MT) = T_{trial}N_{trial} < 62min$

N MTs: $T_{capture} = T_{capure}(1MT) / N$,
e.g. 30 MTs: $T_{capture}$ ~ 2min

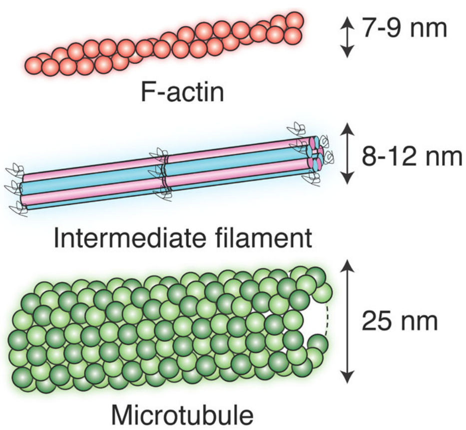
Two pulling mechanisms:

Capture shrinkage & cortical sliding



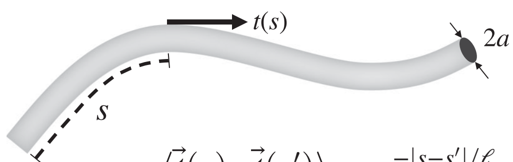
Quantitative description of MTOC relocation

Semiflexible filaments:



$$H_{\text{bend}} = \frac{\kappa}{2} \int ds \left| \frac{\partial \vec{t}}{\partial s} \right|^2$$

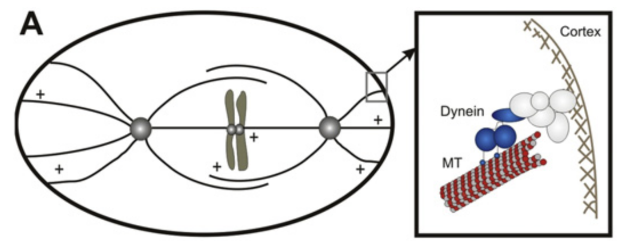
$$\vec{t} = \frac{\partial \vec{r}}{\partial s}$$



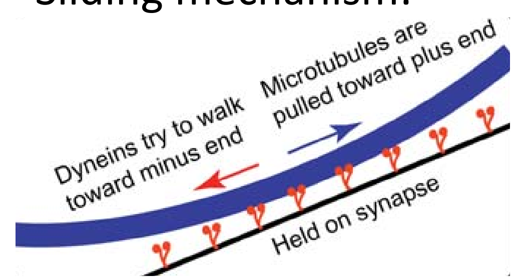
$$\langle \vec{t}(s) \cdot \vec{t}(s') \rangle = e^{-|s-s'|/\ell_p}$$

Force generators at cell cortex:

capture shrinkage mechanism



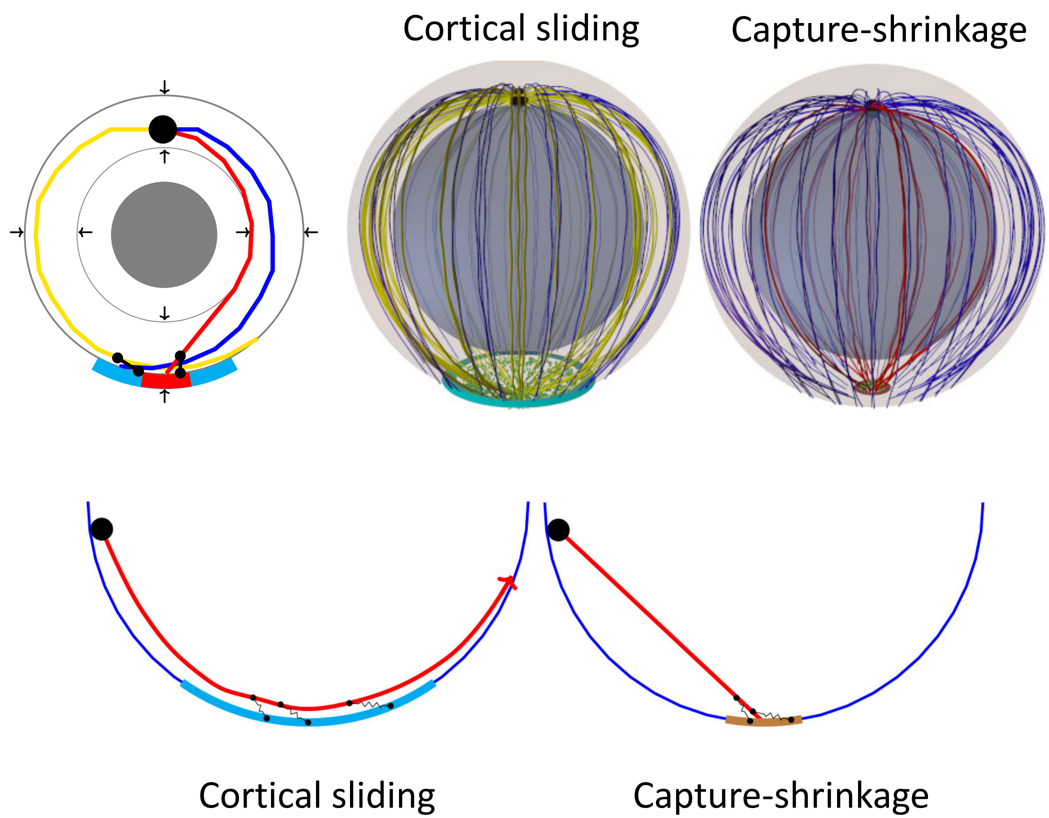
Sliding mechanism:



Numerical implementation:

- discrete set of filament segments
- constrained Langevin dynamics
- overdamped limit
- cell membrane and nucleus:
  repulsive forces

Relocation of the MTOC: Theoretical model



[Hornak, HR, Biophys. J. (2020)]

Estimate of time scales for MTOC repositioning

drag force acting on 1 MT: $F_{drag}(1MT) = \gamma_{MT}\, v$, v=MT velocity

drag coefficient of 1 MT: $\gamma_{MT} = 4\pi\mu L/(\ln(L/d)+0.84)$, $L \sim 10\mu m$, $d \sim 25nm$

cytosol viscosity: $\mu \sim 30\mu_{water} \sim 0.03\ Nsec/m^2$

$\gamma_{MT} = \mu * 18.4\mu m \sim 0.5\ pN\ sec/\mu m$

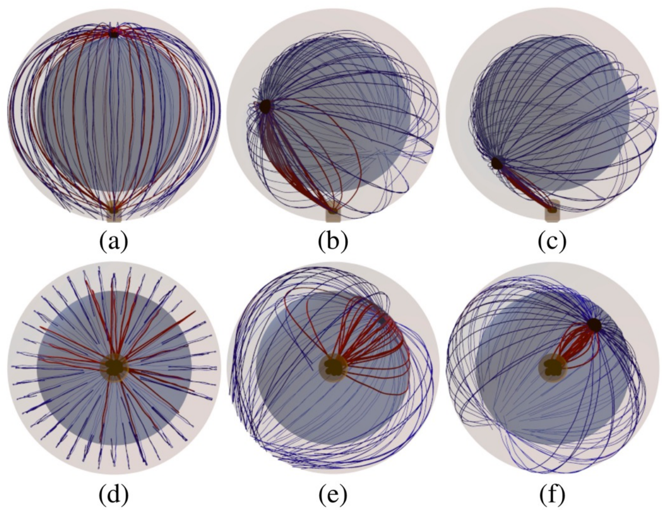
Total drag force $F_{drag}(cyto) = \gamma_{eff}\, v$, $\gamma_{eff} \sim 3\,\gamma_{cyto}$ (attached organelles), $\gamma_{cyto} = N_{MT}\,\gamma_{MT}$

pulling force of attached dyneins: $F = N_{dynein}\, F_{dynein}$, $F_{dyn} \sim 1pN$

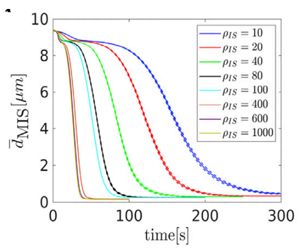
$v = F/\gamma_{eff} \sim 0.5\ N_{dynein}/N_{MT}\ \mu m/min$

e.g.: $N_{MT}=100$, $N_{dynein}=10\text{-}50$ -> $v=3.6\text{-}18\mu m/min$ -> $\boxed{T_{reposition} = \pi R_{cell}/v = 1\text{-}4min}$

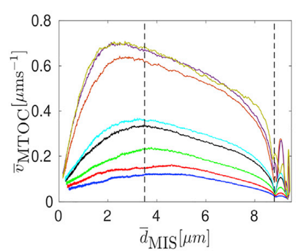
Simulation of stochastic model: Capture-Shrinkage



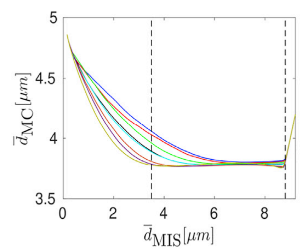
MTOC-IS distance



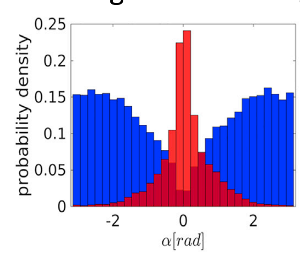
MTOC-IS velocity



d_{MC} vs d_{MIS}

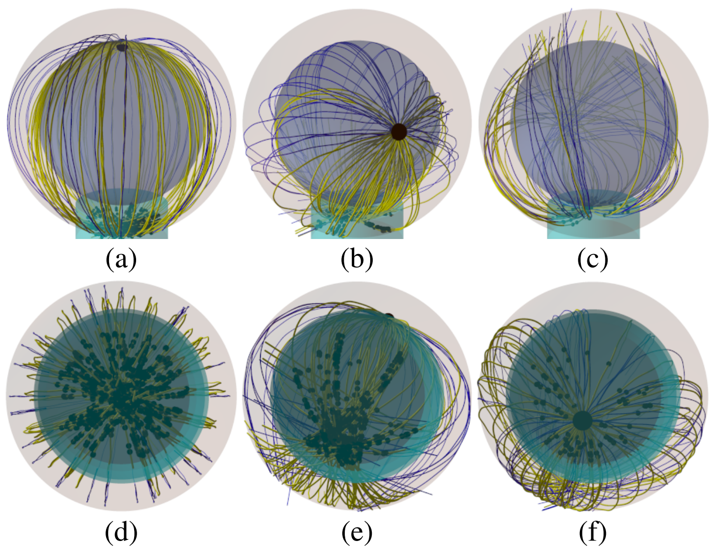


Histogram of MT angles

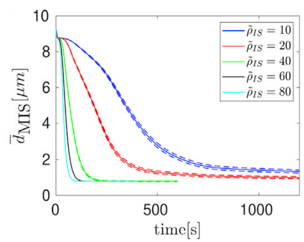


[Hornak, HR, Biophys. J. (2020)]

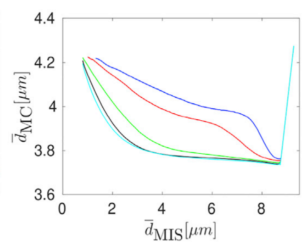
Simulation of stochastic model: cortical sliding



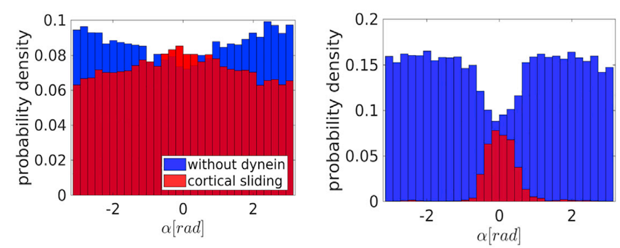
MTOC-IS distance



d_{MC} vs d_{MIS}



initial - MT angles - final



[Hornak, HR, Biophys. J. (2020)]

Both combined: Synergy mechanism

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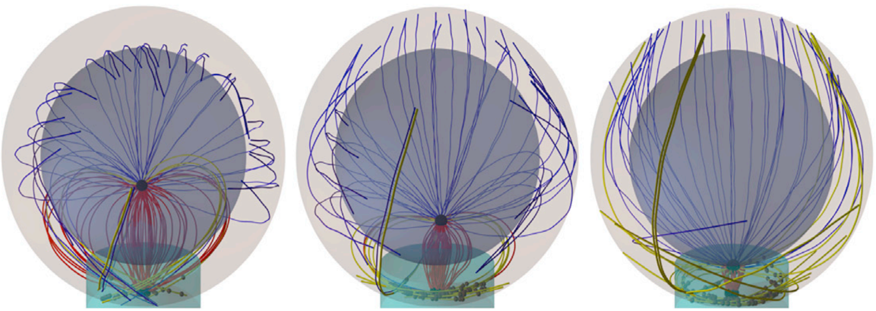
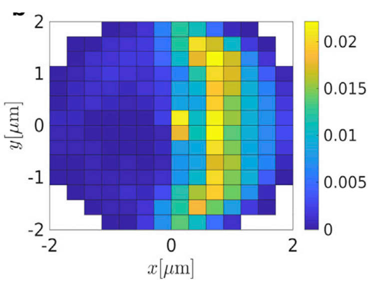
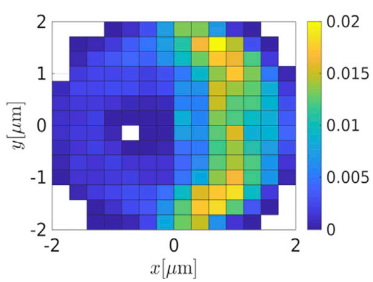
Cortical sliding & capture shrinkage combined



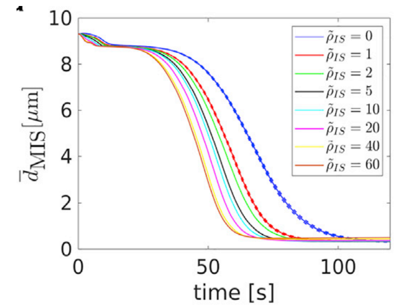
Density plot of attached cortical sliding dynein

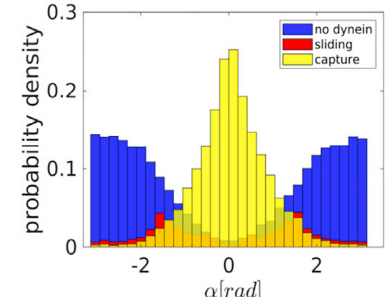


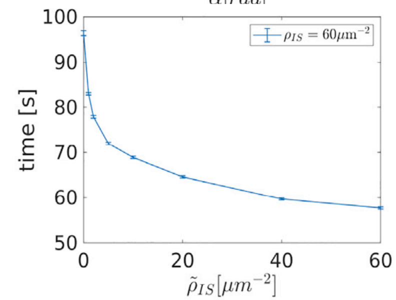
t=50 sec



t=65 sec

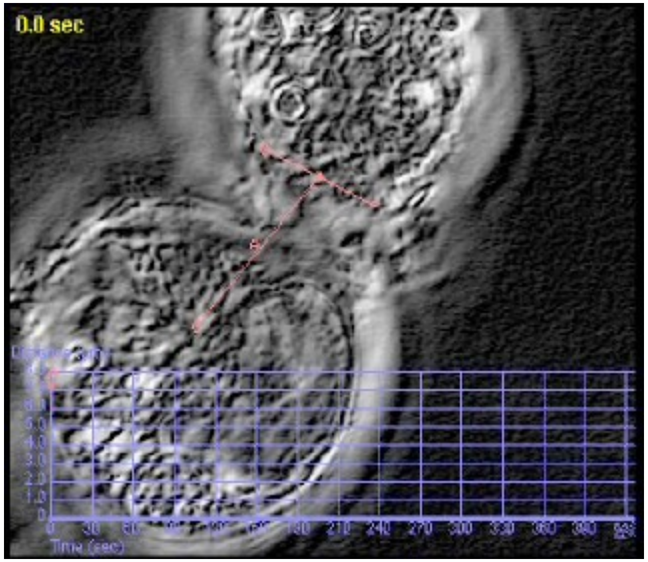


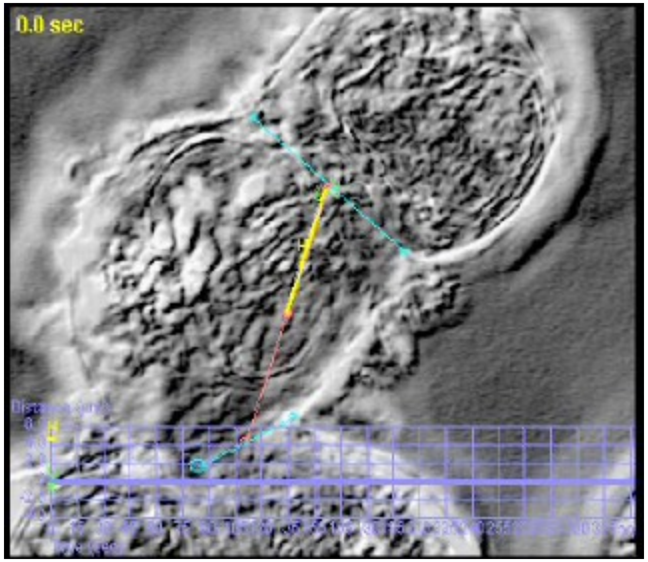




[Hornak, HR, Biophys. J. (2020)]

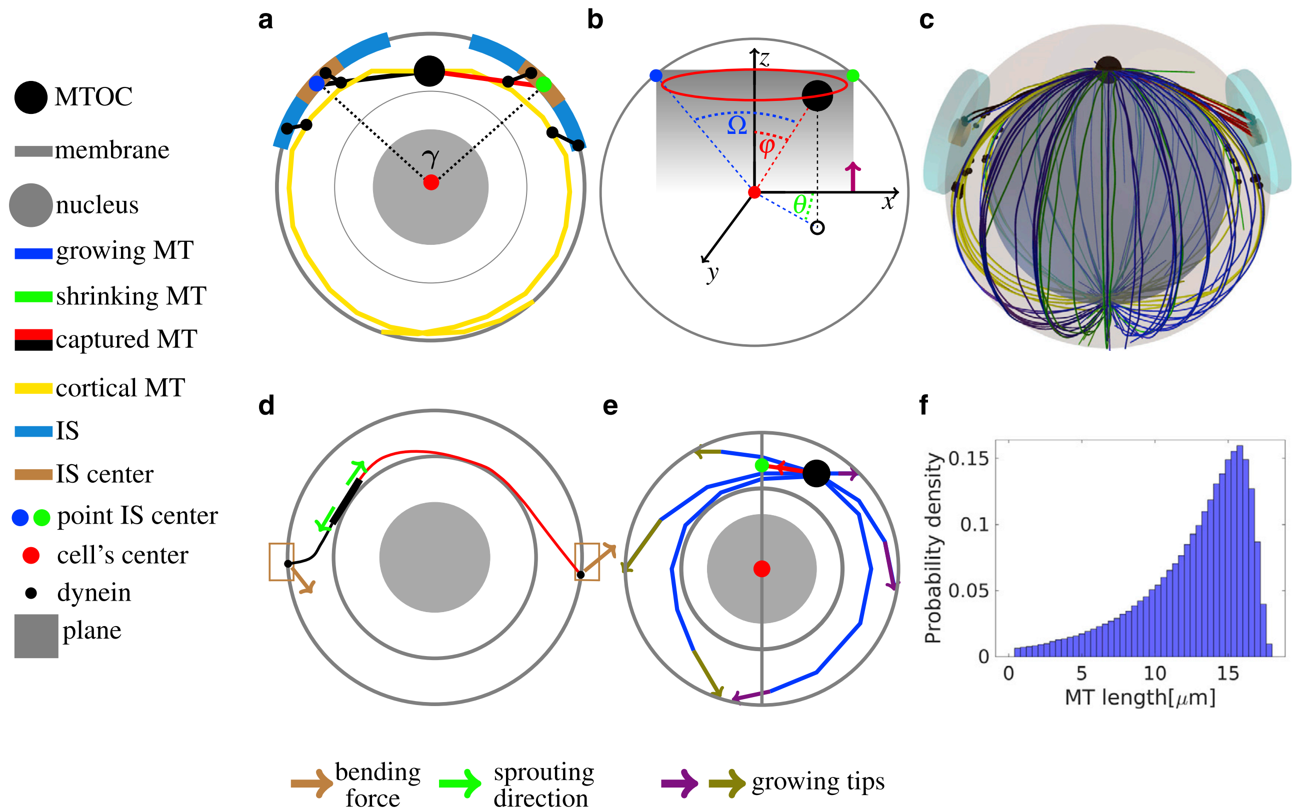
Polarization of MT cytoskeleton during killing with two IS





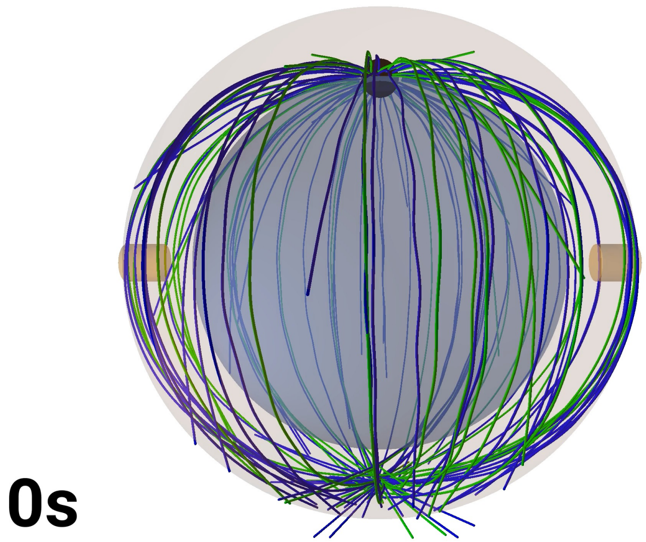
[Kuhn & Poenie, Immunity 2002]

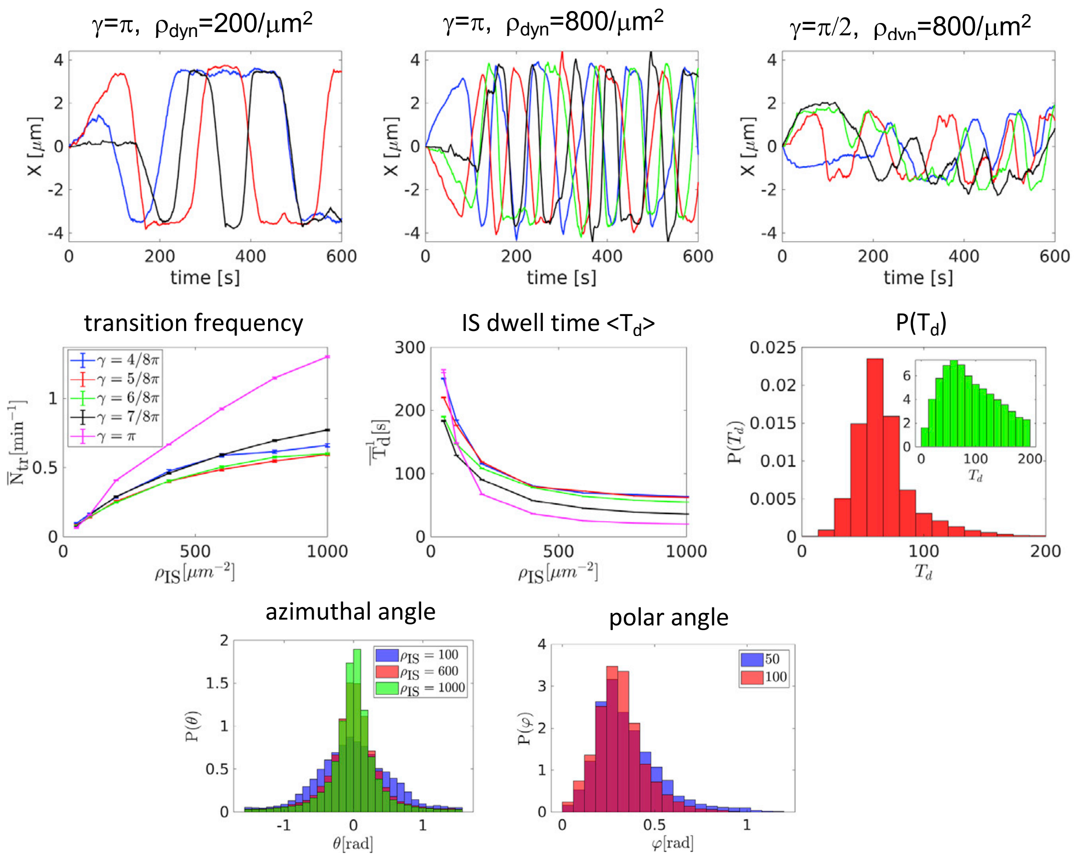
MTOC dynamics for two IS



[Hornak, HR, Biophys. J. (2022)]

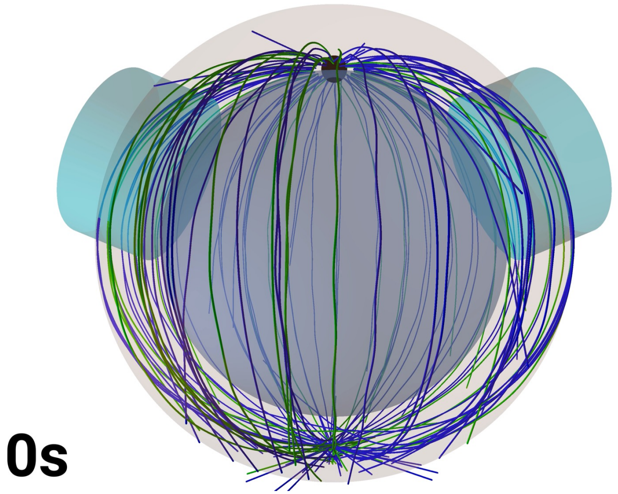
MTOC dynamics for two IS: capture-shrinkage

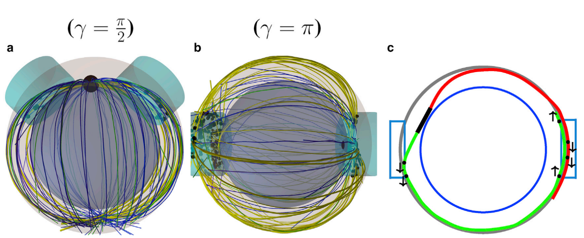


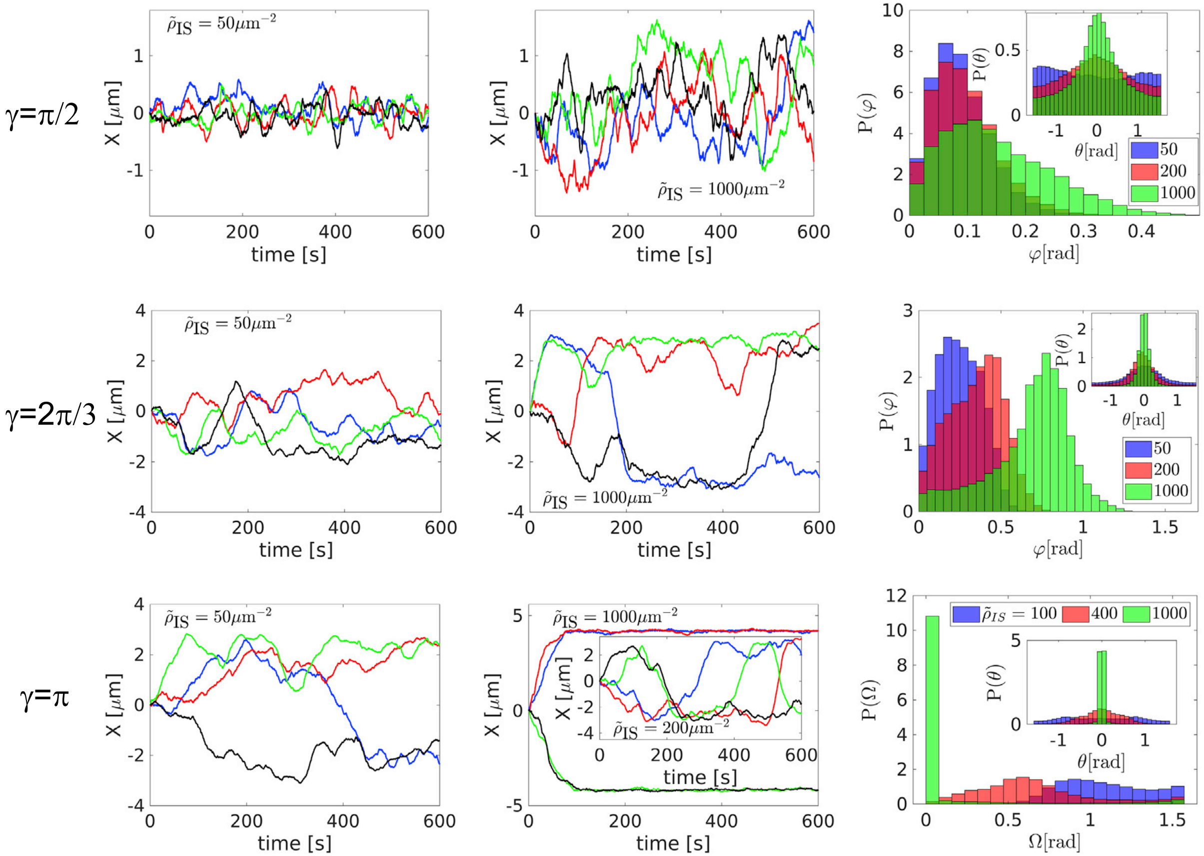


[Hornak, HR, Biophys. J. (2022)]

MTOC dynamics for two IS: cortical sliding



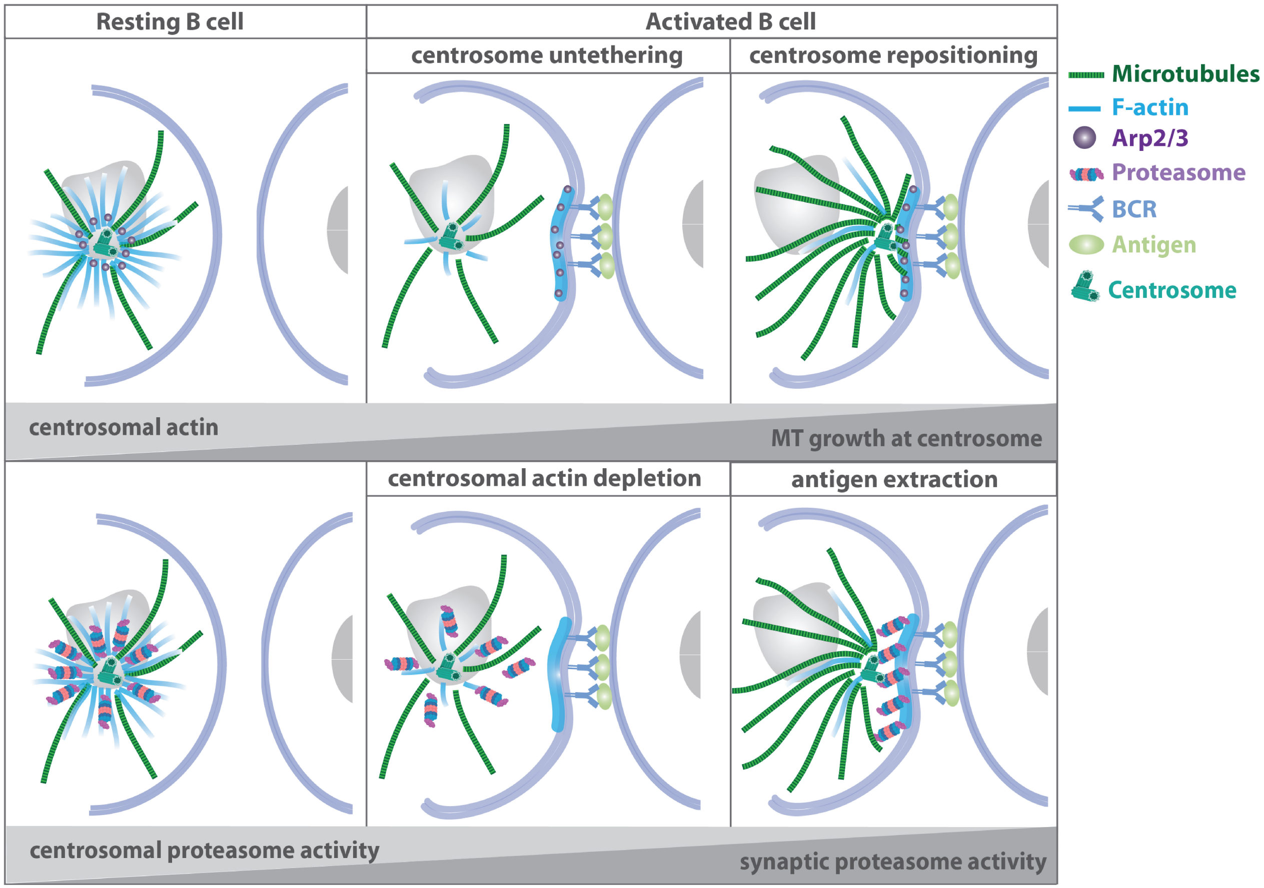




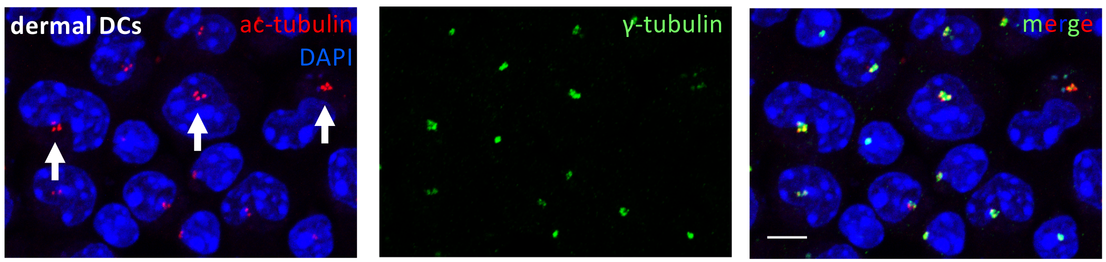
Synopsis: two IS

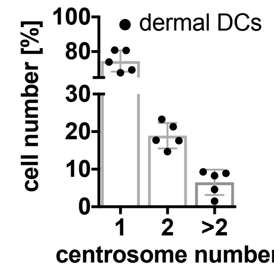
	Capture shrinkage	Cortical sliding	Different mechanisms	Combined mechanisms
Transition frequencies N_{tr}	N_{tr} increases with ρ_{IS} and maximal for $\gamma = \pi$	N_{tr} decreases with γ, and $\tilde{\rho}_{IS}$ for $\gamma \geq \frac{2\pi}{3}$	N_{tr} increases with ρ; $\gamma < \pi$. N_{tr} depends non-monotonously on ρ for $\gamma \approx \pi$	N_{tr} increases with ρ, maximal for $\gamma = \pi$
Dwell times T_D	T_D decreases with ρ_{IS}	MTOC does not come close to one of the two IS for $\tilde{\rho}_{IS} < 600\ \mu m^2$ and $\gamma < \frac{2\pi}{3}$, only fluctuates between the two hemispheres	T_D decreases and increases with ρ at the sliding, shrinkage IS, respectively	T_D decreases and then increases with ρ
Angles: azimuthal θ,Polar φ; MTOC-IS Ω	fluctuations of θ decrease with ρ_{IS} for $\gamma < \frac{2\pi}{3}$, but increase for $\gamma \approx \pi$	fluctuations of φ and Ω decrease for increasing $\tilde{\rho}_{IS}$ when $\gamma \approx \pi$	Ω decreases and MTOC is closer to shrinkage IS as ρ increases	fluctuations of φ increase with ρ, except when $\gamma \approx \pi$, when they decrease
MT cytoskeleton morphology	MTs form a stalk connecting the MTOC and the IS. Dyneins in IS can remain unattached for a time	MTs always intersect the IS. MTOC stays at one of the two IS for $\tilde{\rho}_{IS} > 600\ \mu m^{-2}$	MT stalk connects MTOC and s hrinkage IS. Capture shrinkage becomes dominant as ρ increases	captured MTs shrink and detach. Sliding dynein acts on reduced number of MTs at close IS

Centrosome repositioning in B cells

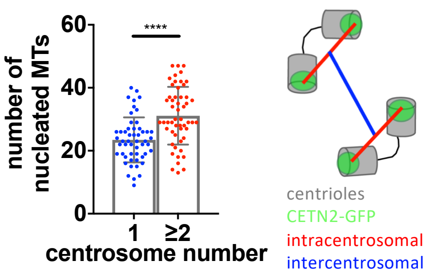


Dendritic cells: multiple centrosomes

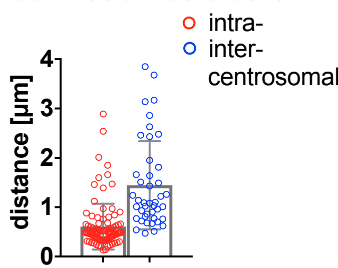




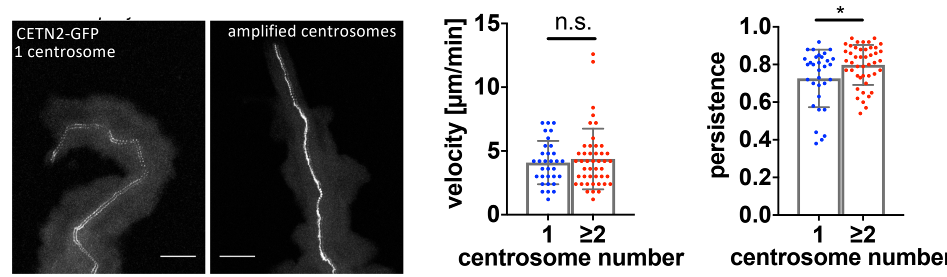
MTs is enhanced



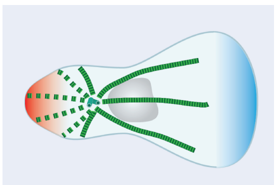
centrosomes cluster



Migration of DCs with amplified centrosomes is more persistent



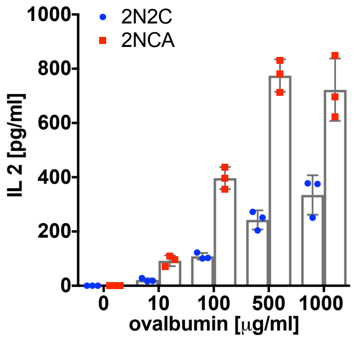
MTs & centrosome clustering <-> polarization <-> persistence ?

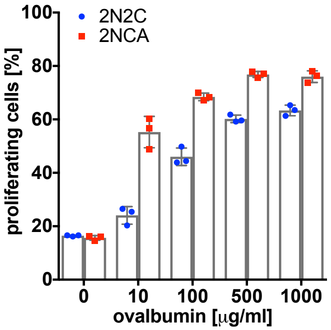


[Kiermaier, JCB 2022]

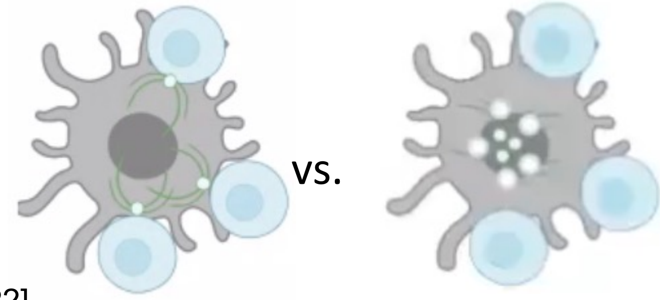
DCs with amplified centrosomes: T cell activation

DCs with amplified centrosomes activate T cells more efficiently



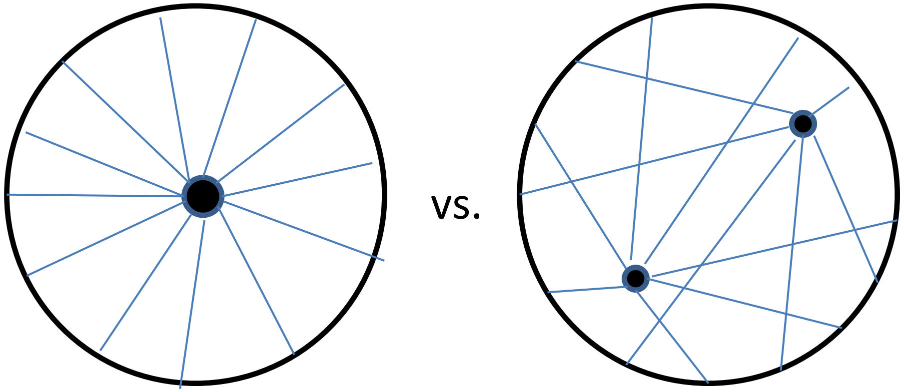


[Kiermaier, JCB 2022]



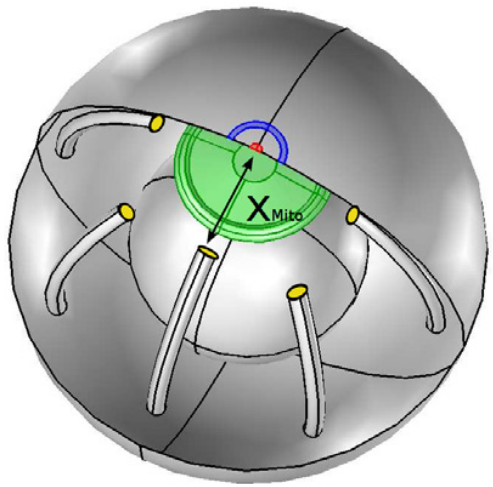
Hypothesis:
activation efficiency related
to MT mediated transport to IS:

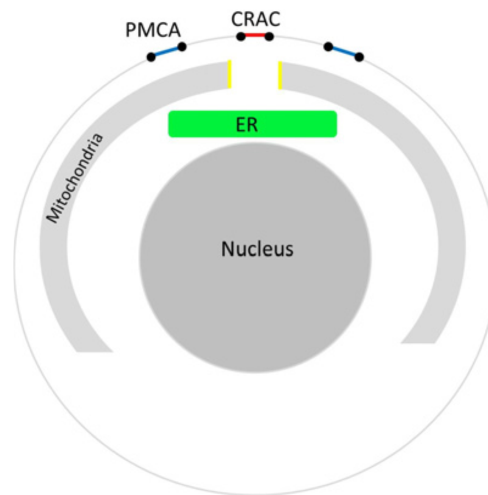
-> centrosome clustering in cell center
has minimal average distance
to cell periphery

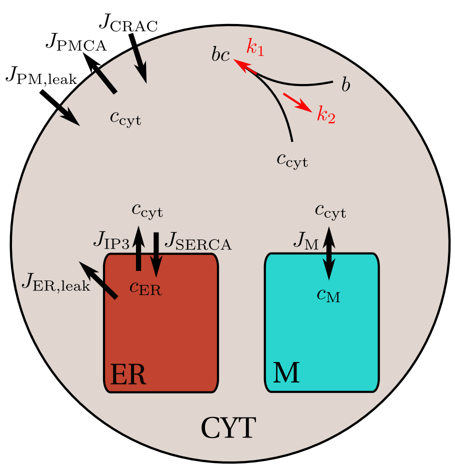


Calcium dynamics during T cell polarization

Theoretical model for Calcium dynamics







Differential Equation System

$$\frac{\partial c_{\mathrm{cyt}}}{\partial t} = D_{\mathrm{cyt}}\,\Delta c_{\mathrm{cyt}} - k_1\, b\, c_{\mathrm{cyt}} + k_2\, bc$$

$$\frac{\partial b}{\partial t} = D_{\mathrm{b}}\,\Delta b - k_1\, b\, c_{\mathrm{cyt}} + k_2\, bc$$

$$\frac{\partial bc}{\partial t} = D_{\mathrm{bc}}\,\Delta bc + k_1\, b\, c_{\mathrm{cyt}} - k_2\, bc$$

$$\frac{\partial c_{\mathrm{ER}}}{\partial t} = D_{\mathrm{ER}}\,\Delta c_{\mathrm{ER}}$$

$$\frac{\partial c_{\mathrm{M}}}{\partial t} = D_{\mathrm{M}}\,\Delta c_{\mathrm{M}}$$

Boundary Conditions

$\mathbf{n_r}$

$$D_{\mathrm{cyt}}\frac{\partial c_{\mathrm{cyt}}}{\partial \mathbf{n_r}} = J_{\mathrm{PMCA}} - J_{\mathrm{CRAC}} - J_{\mathrm{PM,leak}}$$

$\mathbf{n_r}$

$$D_{\mathrm{ER}}\frac{\partial c_{\mathrm{ER}}}{\partial \mathbf{n_r}} = J_{\mathrm{IP3}} - J_{\mathrm{SERCA}} + J_{\mathrm{ER,leak}}$$

$$D_{\mathrm{cyt}}\frac{\partial c_{\mathrm{cyt}}}{\partial \mathbf{n_r}} = J_{\mathrm{SERCA}} - J_{\mathrm{IP3}} - J_{\mathrm{ER,leak}}$$

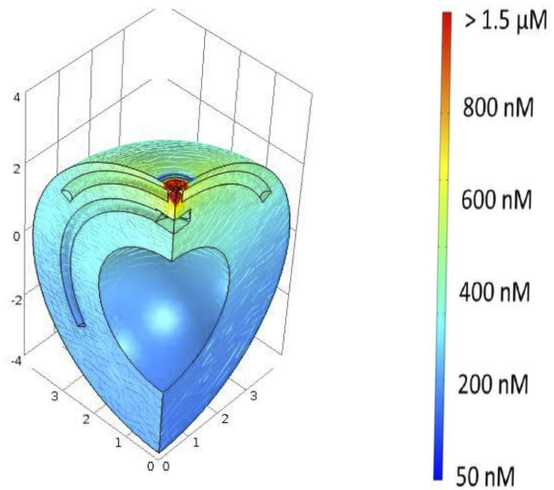
$\mathbf{n_r}$

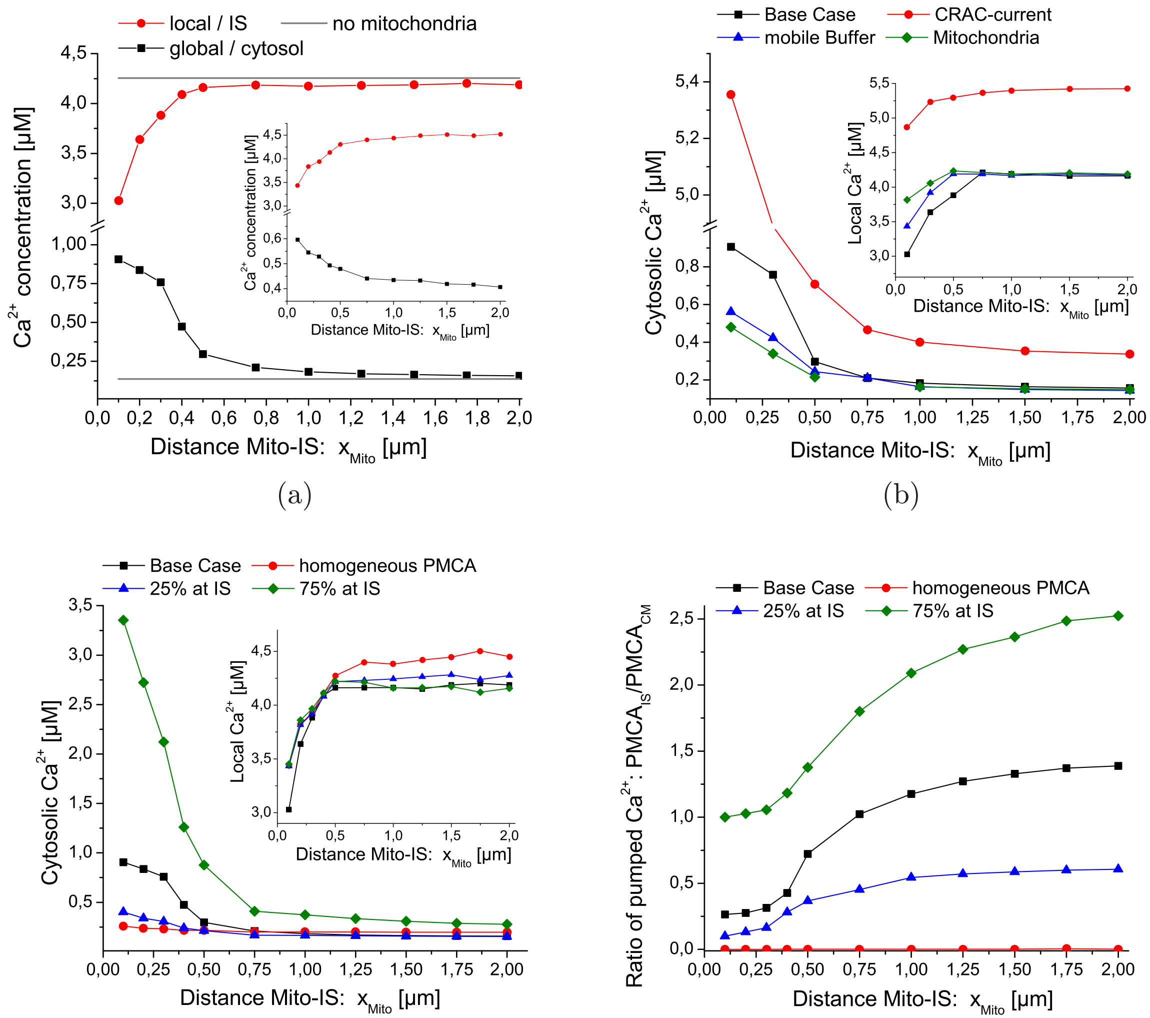
$$D_{\mathrm{cyt}}\frac{\partial c_{\mathrm{cyt}}}{\partial \mathbf{n_r}} = -J_{\mathrm{M}}$$

$$D_{\mathrm{M}}\frac{\partial c_{\mathrm{M}}}{\partial \mathbf{n_r}} = J_{\mathrm{M}}$$

for all boundaries: $\frac{\partial b}{\partial \mathbf{n_r}} = 0$ and $\frac{\partial bc}{\partial \mathbf{n_r}} = 0$

Calcium cc depends on distance mito-IS





(a) (b) (c) (d)

Lytic granule delivery to the synapse

Lytic granules

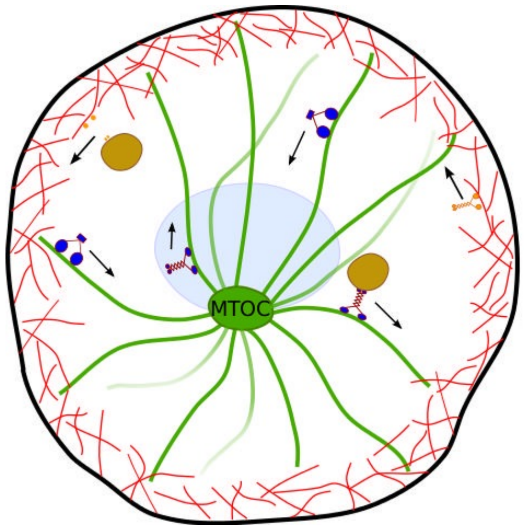
- Lytic granules are vesicles contain cytotoxic material,
  granzyme (inducing apoptosis),
  perforin (perforating the PM), and others

- Target cell killing (by NKs and T cells) is completed by
  directed secretion of lytic granules at the IS

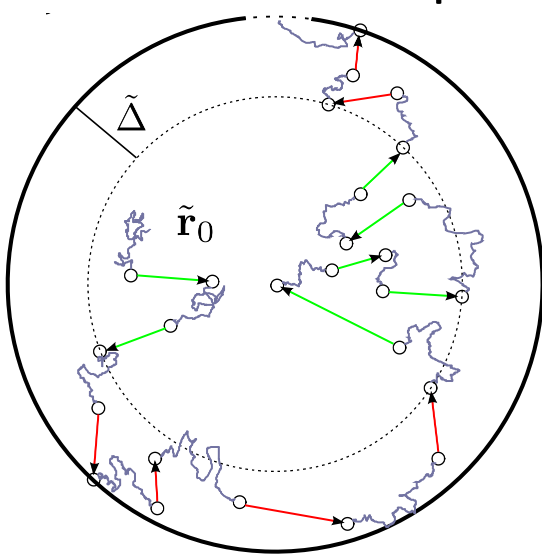
- Secretion proceeds via exocytosis analogous to
  neurotransmitter release in neuronal signal transmission

- Vesicles (lytic granules) have to be delivered to IS
  via molecular motor assisted transport along microtubules / actin filament

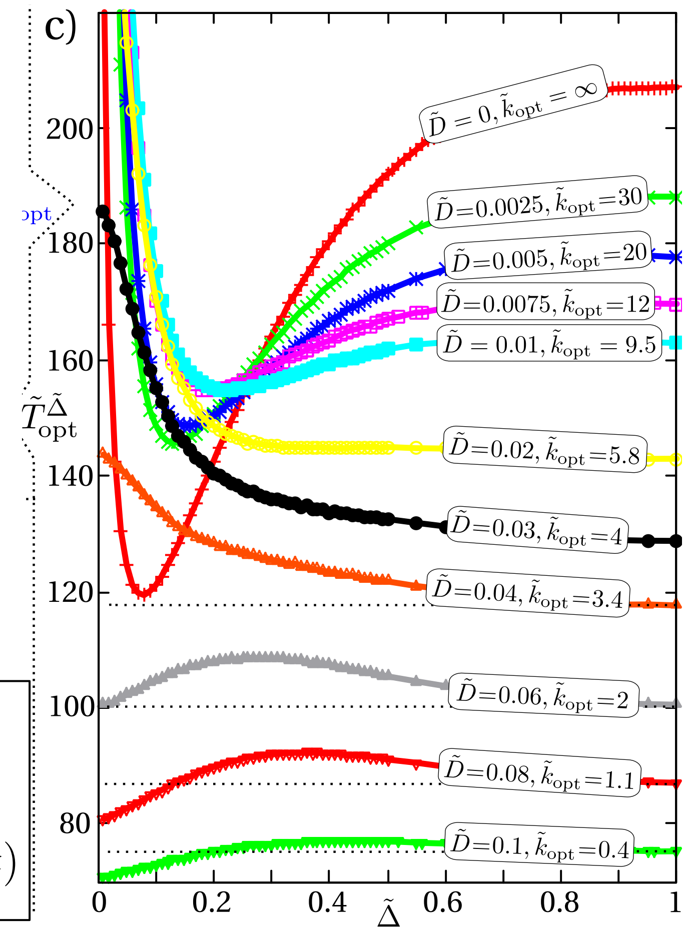
Cytoskeleton organization suuports delivery



↓ Escape region



MFPT as function of Δ



Random search for narrow escape region, ballistic along cytoskelton filaments, diffusive otherwise:

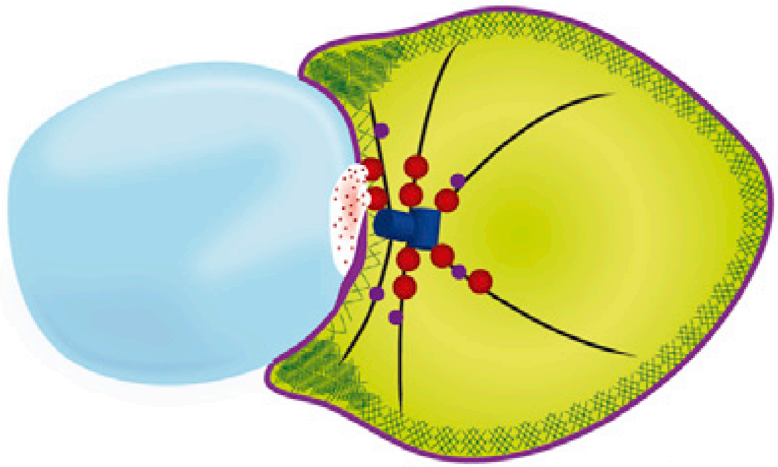
$$\frac{\partial}{\partial t}P_0(\mathbf{r},t) = D\Delta P_0(\mathbf{r},t) - kP_0(\mathbf{r},t) + k'\int d\Omega\, P_{\mathbf{v}_\Omega}(\mathbf{r},t)$$

$$\frac{\partial}{\partial t}P_{\mathbf{v}_\Omega}(\mathbf{r},t) = -\nabla\cdot(\mathbf{v}_\Omega P_{\mathbf{v}_\Omega}(\mathbf{r},t)) + k\,\rho_\Omega(\mathbf{r})P_0(\mathbf{r},t) - k'P_\Omega(\mathbf{r},t)$$

Thin (small Δ) actin cortex advantageous for vesicle delivery to IS

Schwarz et al.: PRL 117, 068101 (2016)

Vesicle delivery in polarized cells



- Vesicles to be released at the IS move towards the MTOC
- Equipment with dynein depends on tethering with LROs

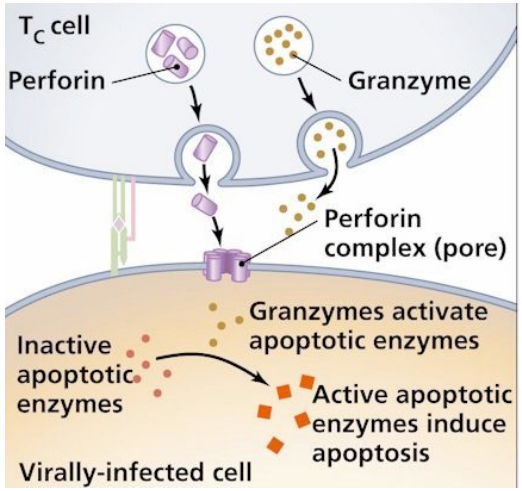
- In NKs some vesicles (IL-2) are released multidirectional -> equipment with kinesin after tethering with LROs
- Vesicle delivery dependent upon: MTOC relocation, motor equipment, tethering with LROs

[Qu et al., t.b.p.]

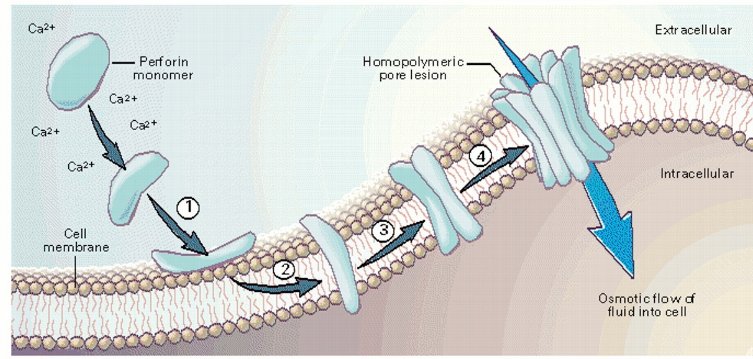
Killing Strategies

The actual kill: perforin induces necrosis

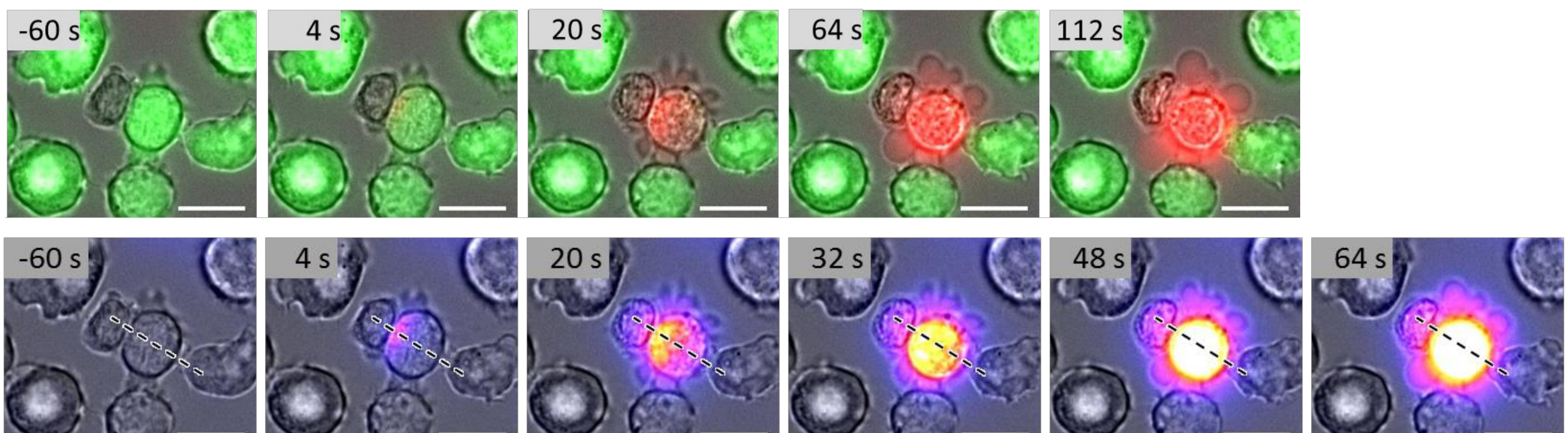
Lytic granules contain perforin and granzyme



Perforin is a pore forming protein



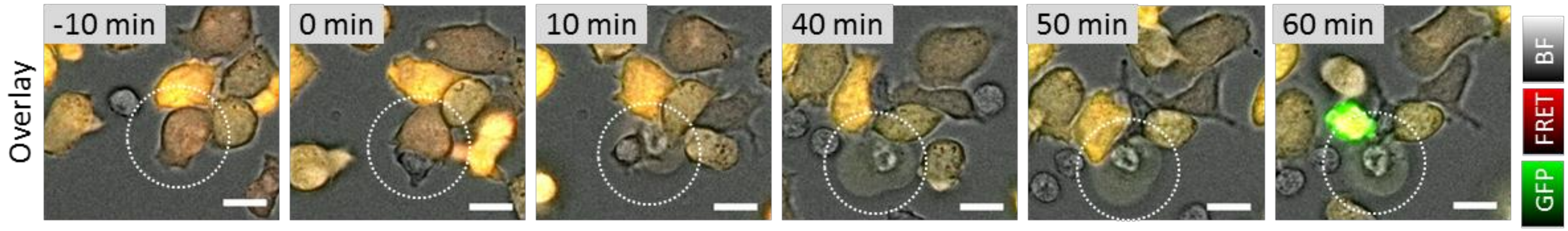
Killing via necrosis (fast)



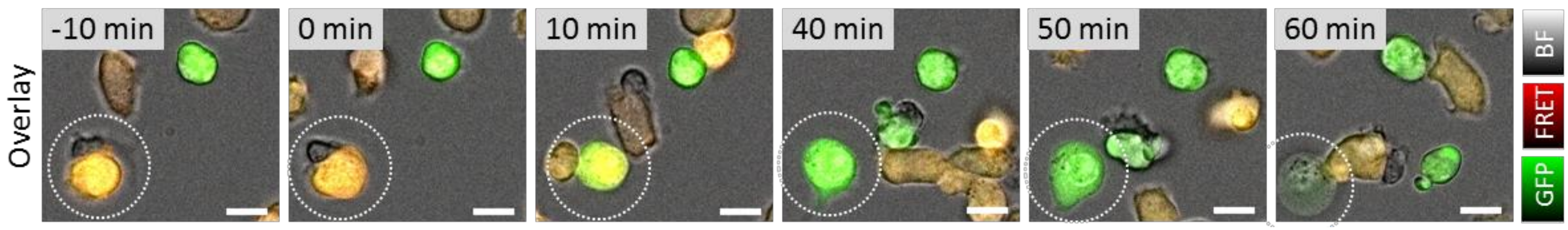
Various forms of target cell killing

SFB 1027

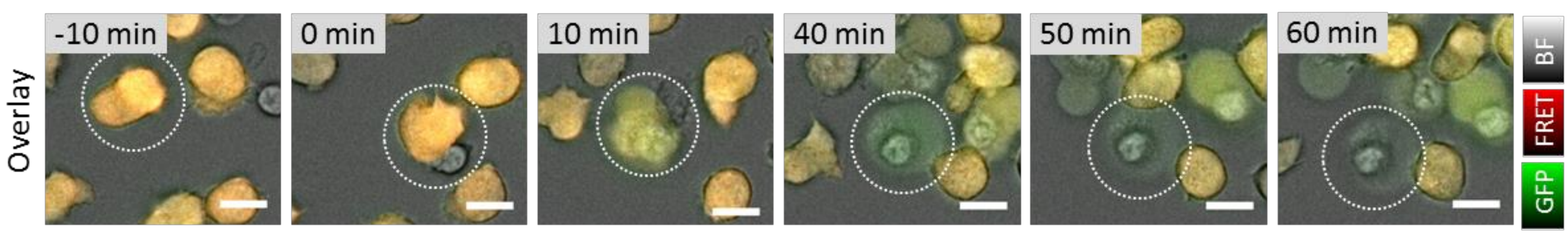
Nekrosis - kill via perforin



Apoptosis (with secondary necrosis indep. of NK) - kill via FAS ligand



Nekrose with caspase activity - kill via perforin and granzyme



Killing sequences

Experimental record of killing sequences of individual killer cells:

N = Nekrosis

A = Apoptosis

G = Mixed

N
NN
NN
N
NN
NN
NN
NNNNN
NN
NN
NNN
NNN
NNNN
NN
NN
NN
NN
NN
NN
NN
NN

[C. Backes, Diss. and t.b.p.]

Probabilistic generation of killing sequences

Are there several **NK phenotypes**

– or only one with varying killing capacity ?

Probabilistic model:

- Distribution of killing sequence length
- Necrosis kills faster than Apoptosis -> N first
- Distribution of number of necrosis kills
- Probability for mixed kills in the N->A transition zone
- Probabilities for N->A, A->N, N->G

Parameter optimization via maximum likelihood method

-> fits data well !

Mechanisms of immune evasion

Tumor growth and immune responses

Interaction between tumor formation and immune responses

- Infection, stress, inflammation → Tumor growth ↑, Survival ↑, Angiogenesis ↑ [3, 6-9]
- Autoimmune diseases → Tumor growth ↑ [6,10-12]

Tumor progression and immunity

- Immunoediting → Tumor ↑ [13-15]

Immune evasion by cancer

- Tregs [17-22]
- MDSCs [23,24, 26]
- Cytokines [22,28,30,39-44]
- M2 macrophages [28-30]
- Defective Ag presentation [32-38]
- Immune suppressive mediators (VEGF, RCAS1, tumor gangliosides, IDO, arginase, IKK2 [31, 45,47-58])
- Tolerance and immune deviation [59,60]
- Apoptosis [66,67]

From Vinay et al., Sem. Canc. Biol. 35, S185 (2015)

Summary

(Bio)-physical aspects of killing (T cells & NKs):

- stochastic generation of the immune repertoire
- lymphocyte migration in 3d environment (e.g. fiber network)
- search strategies – environmental cues / interactions in swarm search
- formation of immunological synapse and cell polarization:

  interplay of cell membrane, actin polymerization, microtubules & motors
- calcium dynamics during activation and killing:

  reaction-diffusion dynamics with dynamically changing sources and sinks
- delivery of vesicles (lytic granules) to the IS or to periphery
- exocytosis of cytotoxic material
- etc.

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- Ariel Quintana
- Ilaria Maccari
- Raja Paul
- Jae-Dong Noh
- Eva Kiermaier

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