ON/OFF Controls within the Dynein-Dynactin Machine
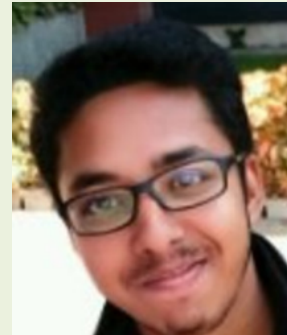
Roop Mallik

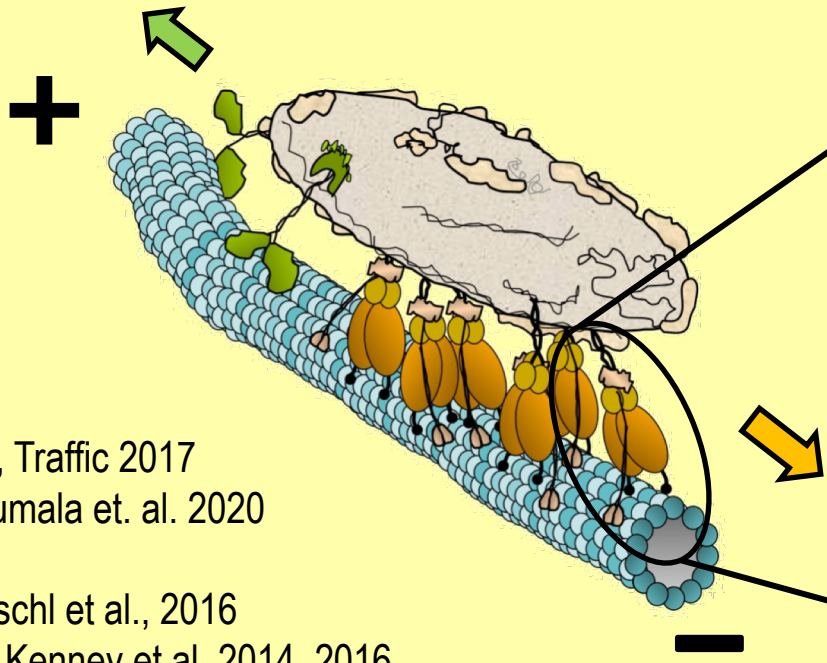Paulomi Sanghavi   Pankaj Kumar   Ankit Roy   M. S. Madhusudhan

Department of Biological Sciences, Tata Institute of Fundamental Research,
Homi Bhabha Road, Mumbai 400005, India

Department of Biology, Indian Institute of Science
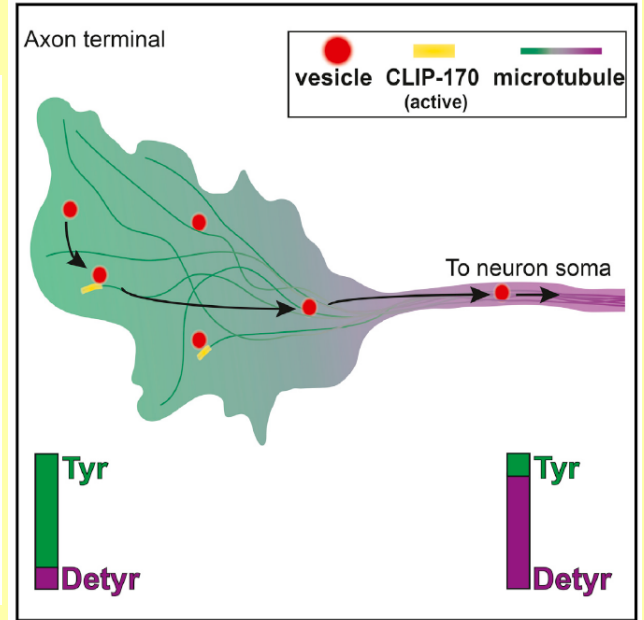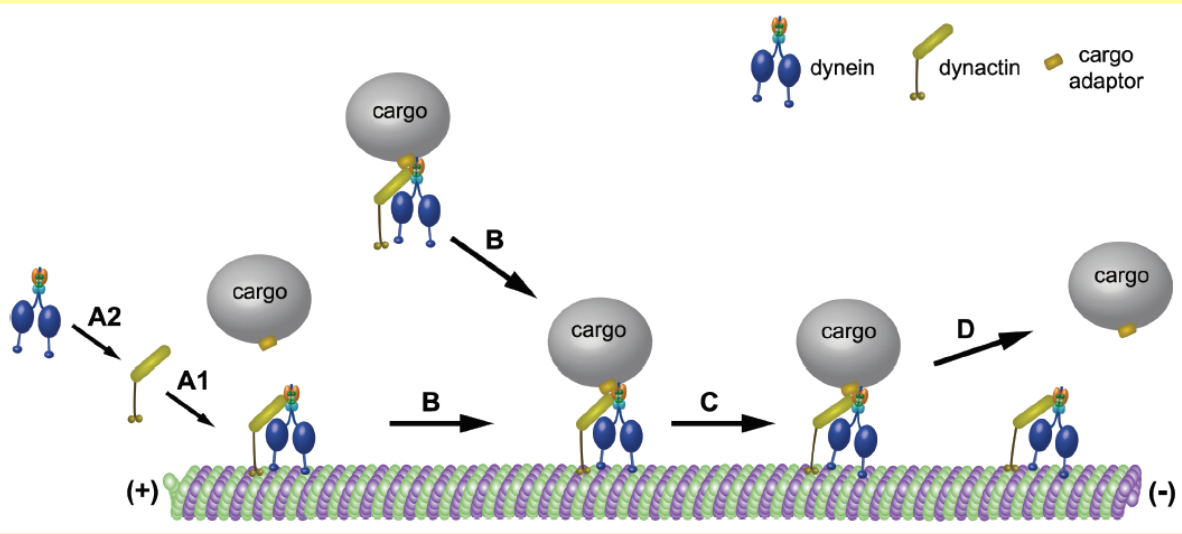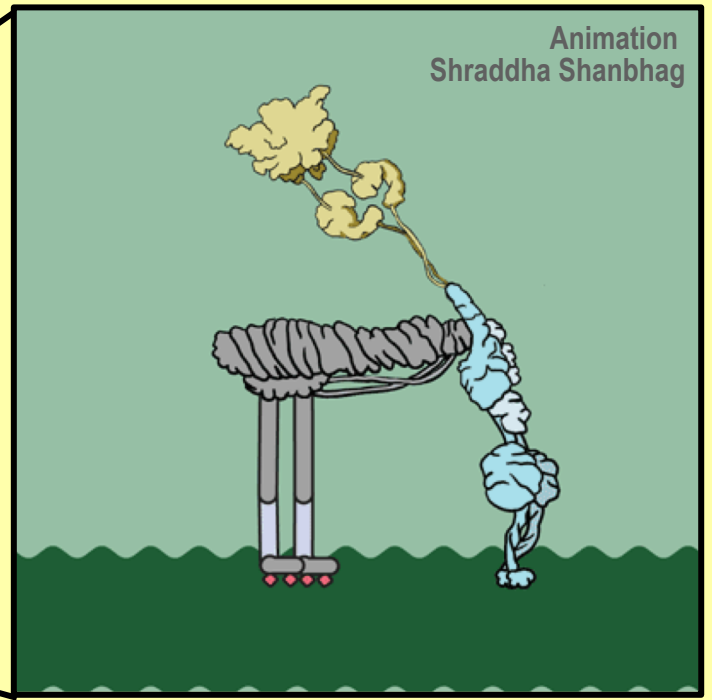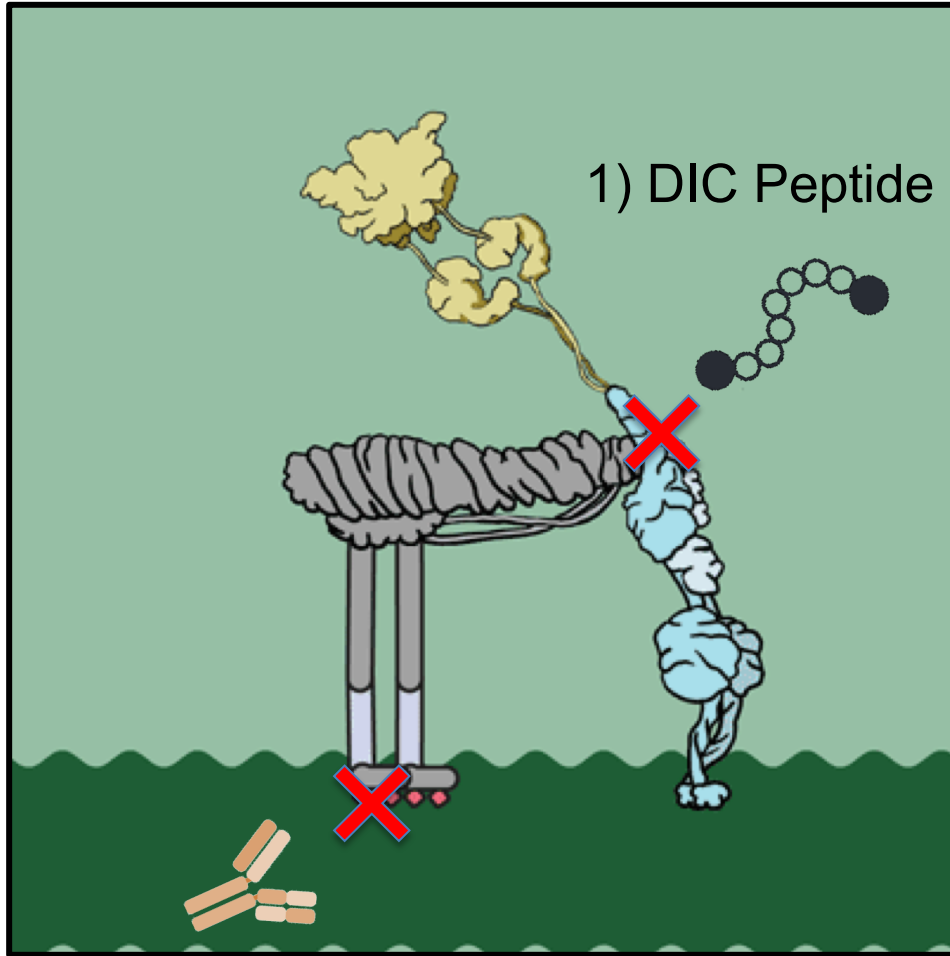*Education and Research, Pune 411008, India.*

Motor - Teams

Dynein – Dynactin

+

Liu, Traffic 2017
Tirumala et. al. 2020

Nirschl et al., 2016
Mc Kenney et al, 2014, 2016
Kardon et al, 2009, Kim et al, 2007

Animation
Shraddha Shanbhag
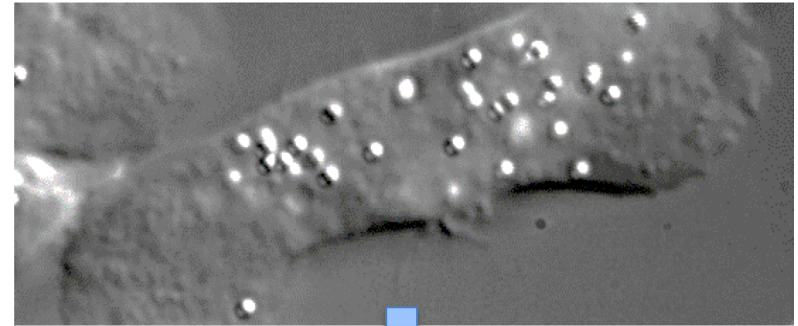
dynein   dynactin   cargo adaptor

cargo

cargo

A2

A1

B

B

C

D

cargo

cargo

cargo

(+)

(-)

Axon terminal

vesicle   CLIP-170 (active)   microtubule

To neuron soma

Tyr
Detyr

Tyr
Detyr

Disable Specific Interactions within "DD"
on Native Cargoes ...

1) DIC Peptide

2) Anti-CAPGly

Late Phagosomes (LPs)

Extract From Cells

Analyse

Force = Distance * K_{TRAP}
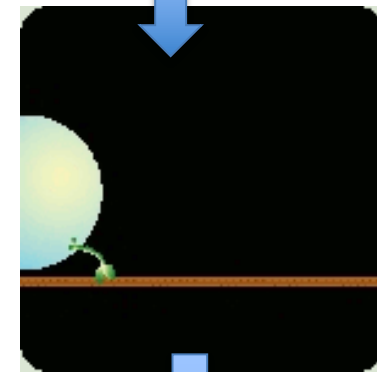
1) DIC Peptide
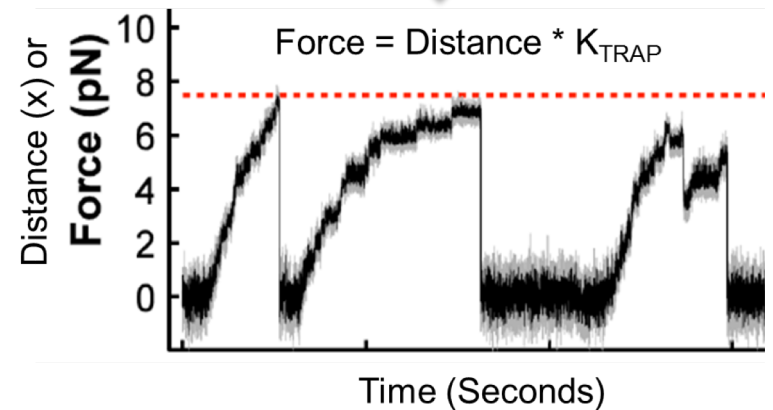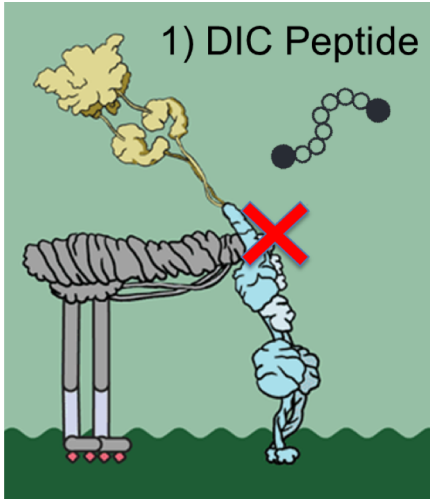
✔ DIC-WT does NOT Remove Dynein or Dynactin from LPs

✔ **DIC-WT localizes to Clusters of Dynein on LPs**

EPs 1 2 3

LPs 1 2 3

2 μm

Towns et al 2009
Vaughan et al 2001

✔

No ATP
→ K_{OFF} low

MT Pelleting
Brain Lysate + MTs

Retained in MT pellet → | +mDIC Mut | +mDIC WT |

Dynein

Dynactin

Tubulin

Starting Trouble
(K_{ON} reduced)

1) DIC Peptide

$P = 0.164$

$P < 0.001$

N=5 n=60	N=5 n=98	N=5 n=92

Untreated +DICMut +DICWT
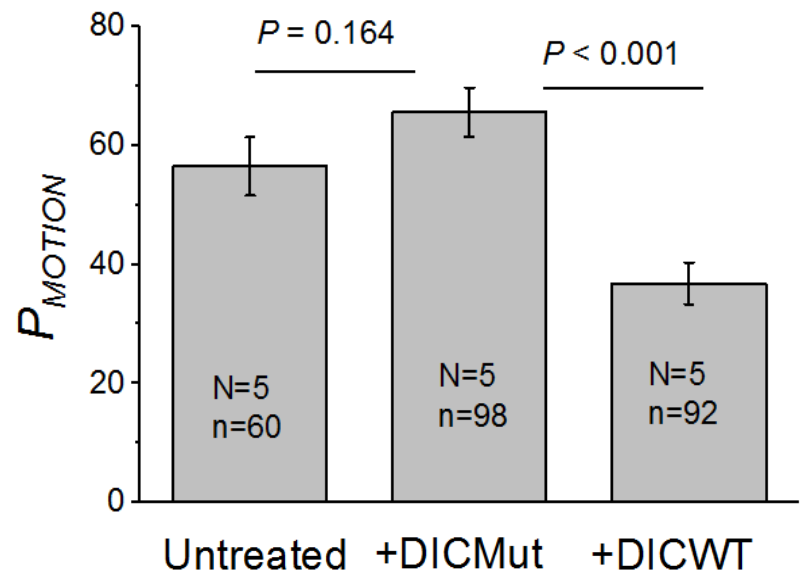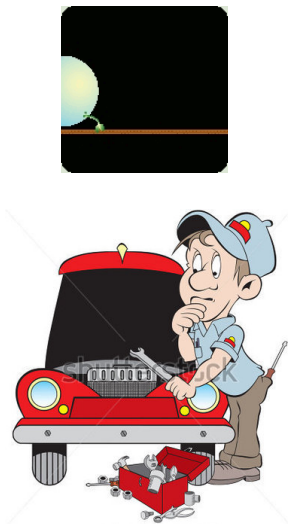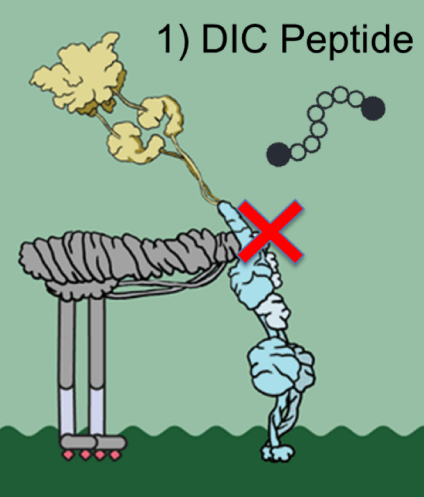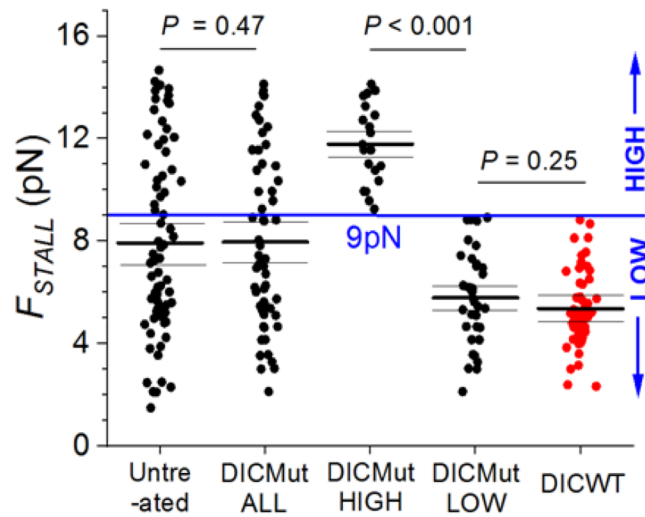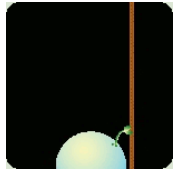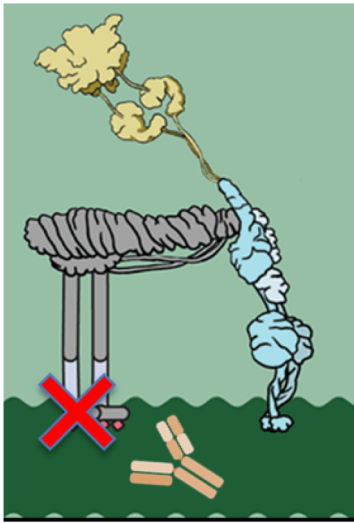
Agrees with McKenney et al. 2014, Zhang et al. 2017

But, works fine once it gets started
(No Change in K_{OFF})

F_{STALL}

T_{STALL}

F_{STALL} (pN)

$P = 0.47$ $P < 0.001$ $P = 0.25$

9pN HIGH LOW

Untre-ated DICMut ALL DICMut HIGH DICMut LOW DICWT

T_{STALL} (sec)

$P = 0.63$ $P < 0.001$ $P = 0.76$

Untre-ated DICMut ALL DICMut HIGH DICMut LOW DICWT

2) Anti-CAPGly

✔

No ATP
→ K_{OFF} low
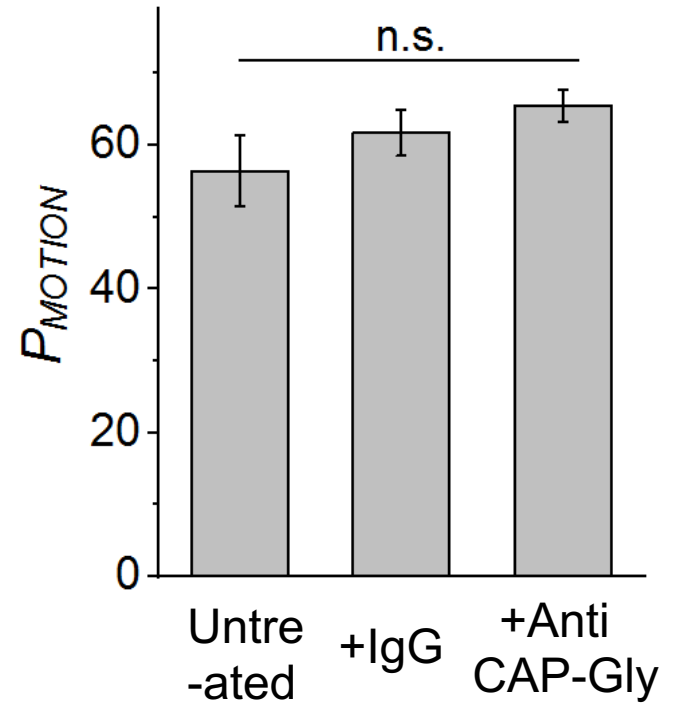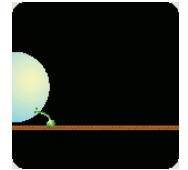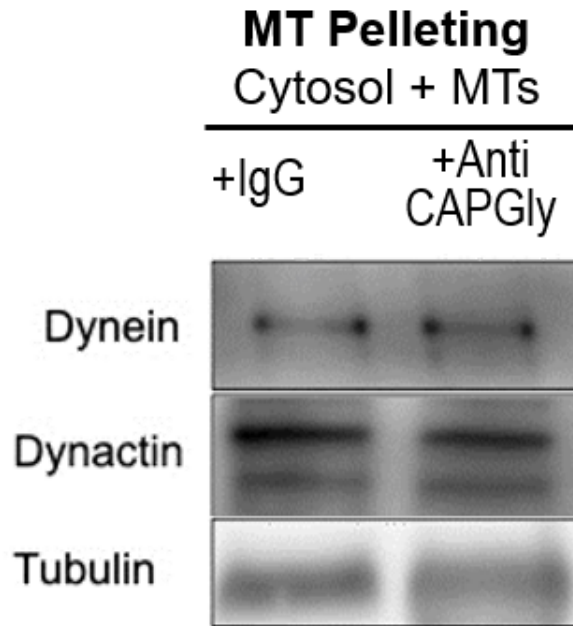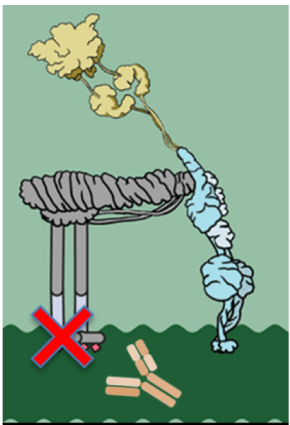
✔

Anti-CAPGly does NOT Remove Dynein or Dynactin from LPs

MT Pelleting
Cytosol + MTs

+IgG	+Anti CAPGly

Dynein

Dynactin

Tubulin

n.s.

P_{MOTION}

60

40

20

0
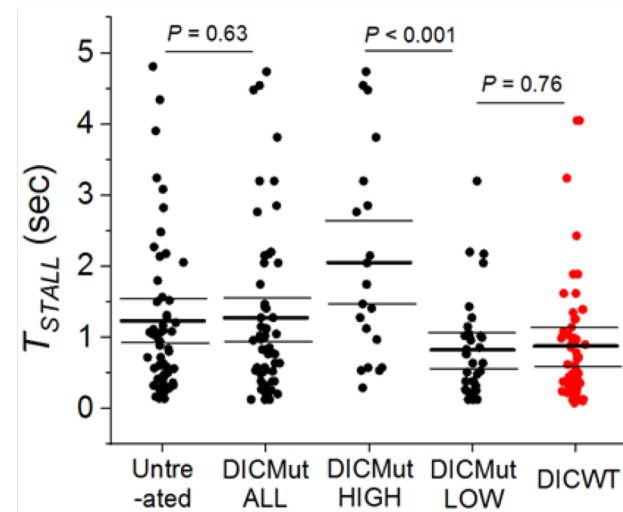
Untre-ated | +IgG | +Anti CAP-Gly

Anti-CAPGly has no effect on K_{ON} of DD

2) Anti-CAPGly

Force (pN) vs +IgG (All), +IgG(Low), +Anti CAP-Gly

$P = 0.59$
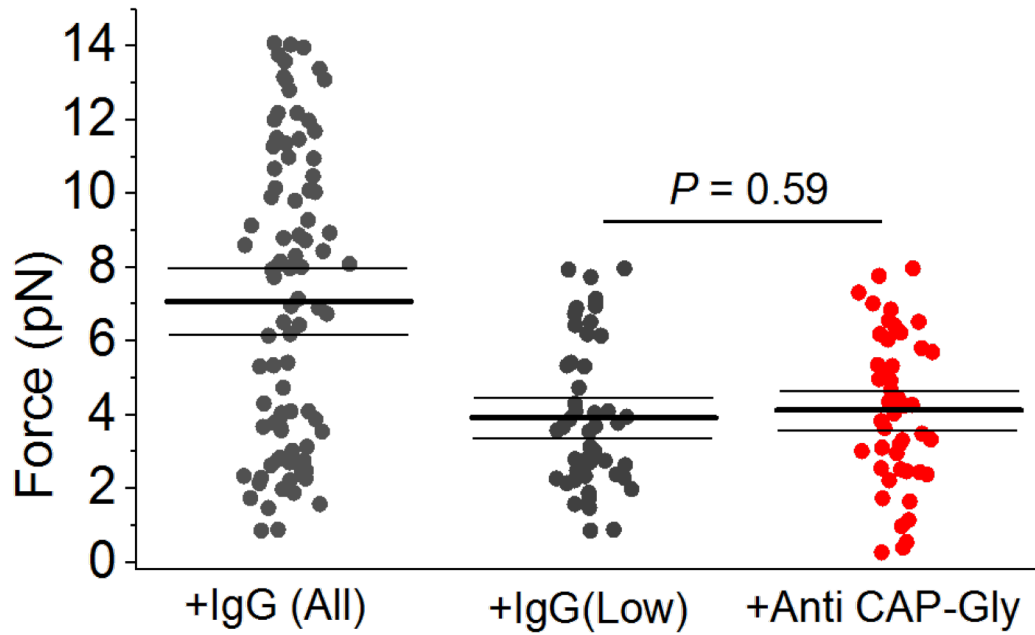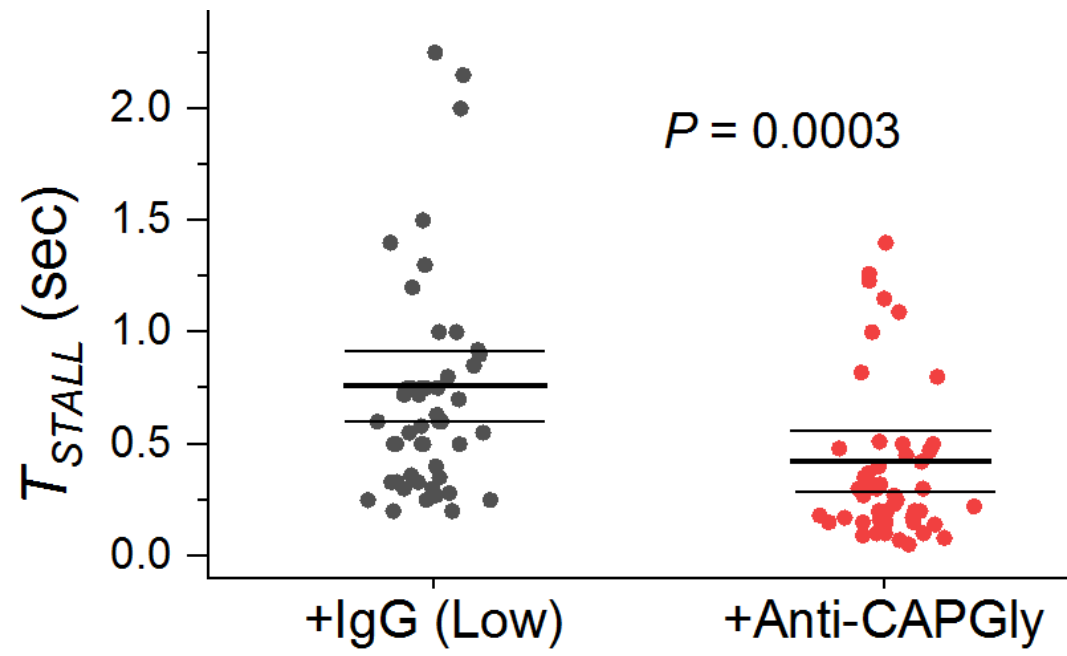
No Change in K_{ON}

But still Low force

$\rightarrow K_{OFF}$ Increased?

Yes, indeed

T_{STALL} (sec) vs +IgG (Low), +Anti-CAPGly

$P = 0.0003$

Reconstitution of Endogenous Dynein Dynactin Driven motion on Supported Lipid Bilayers

Dynactin – DIC Link
"ON" Control

Initiates DD
(High K_{ON} state)

Little role in
persistence
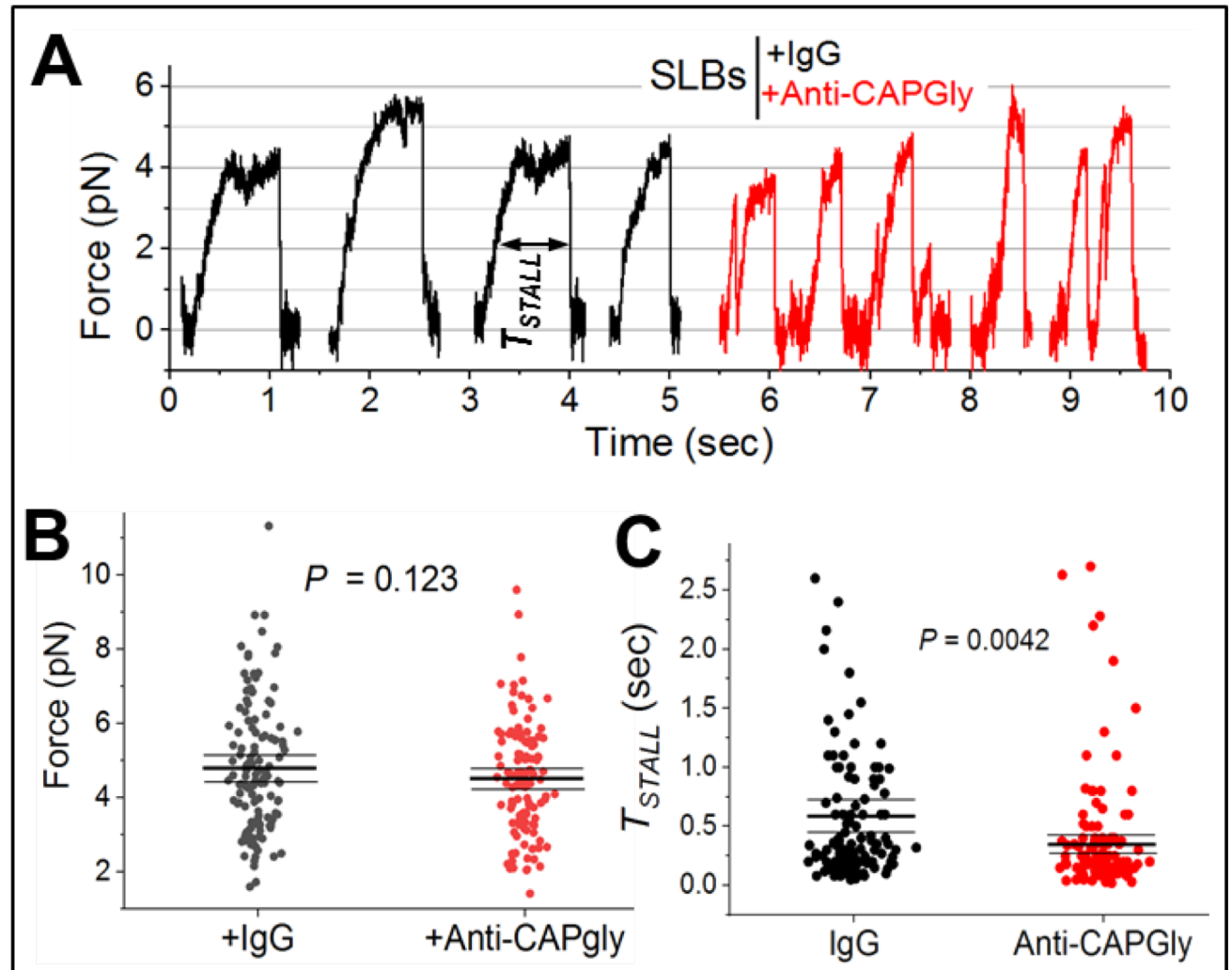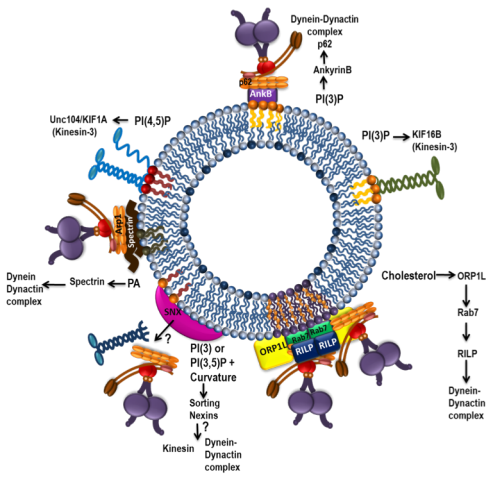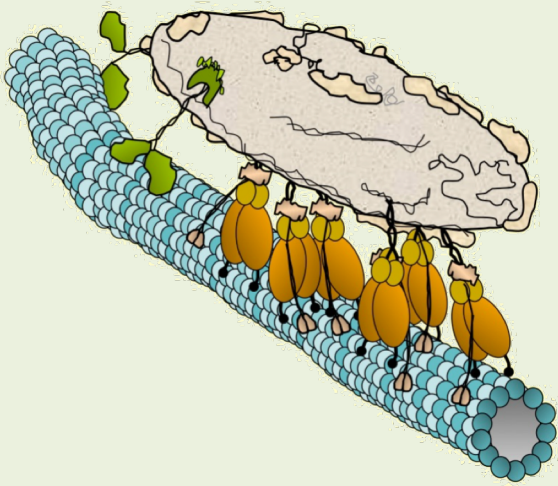
CAPGly - MT Link
"OFF" Control

Little role in initiation

Maintains DD in
persistent force
generation state
(Low K_{OFF})
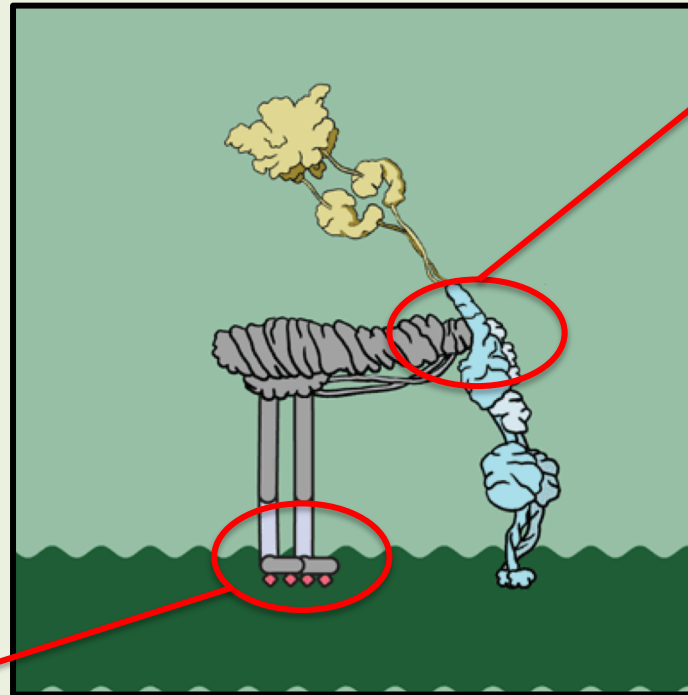
Animation : Shraddha Shanbhag

Not discussed …
DD motion on SLBs
Obstacles Experiment
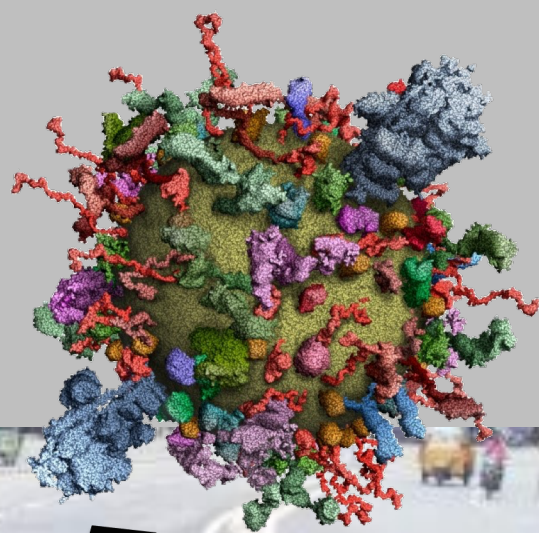
TIFR-DAE
DBT-Wellcome Trust IA
Wellcome Trust UK

Rupam Jha
Ashwin Dsouza

Meg Titus
K. Vaughan
E. Holzbaur