# Modulating Epithelial Drug Delivery through Controlled NanoInterfaces

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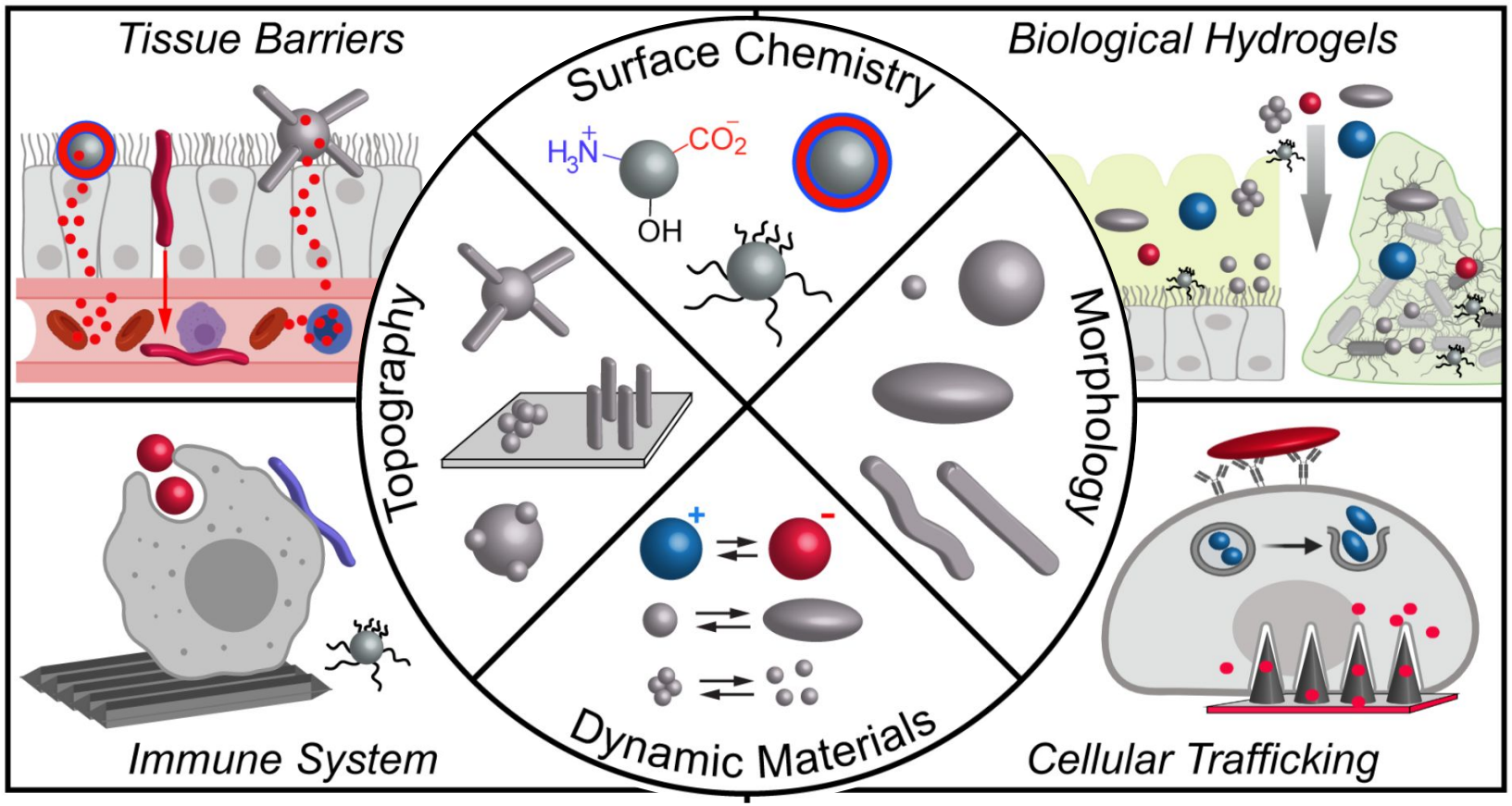
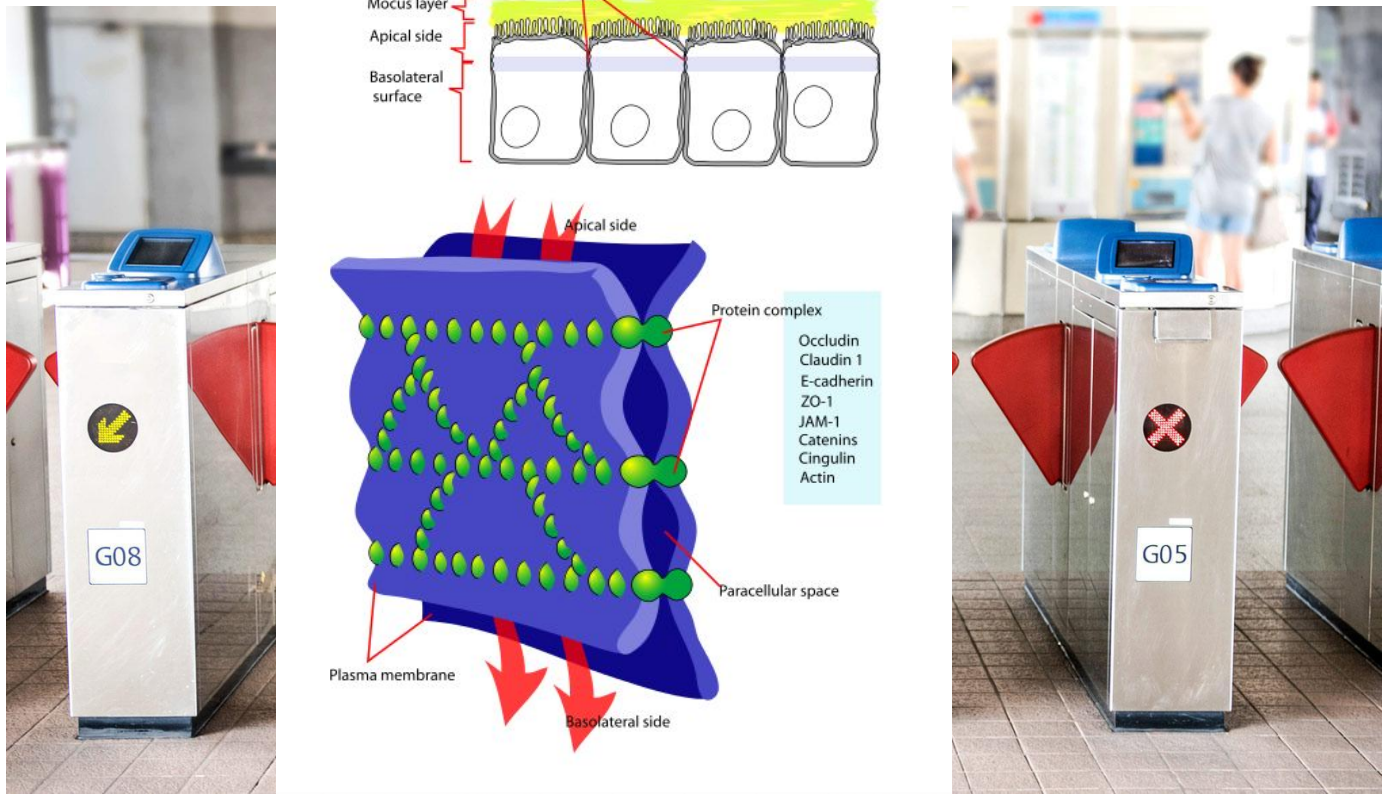
Advanced Delivery Science



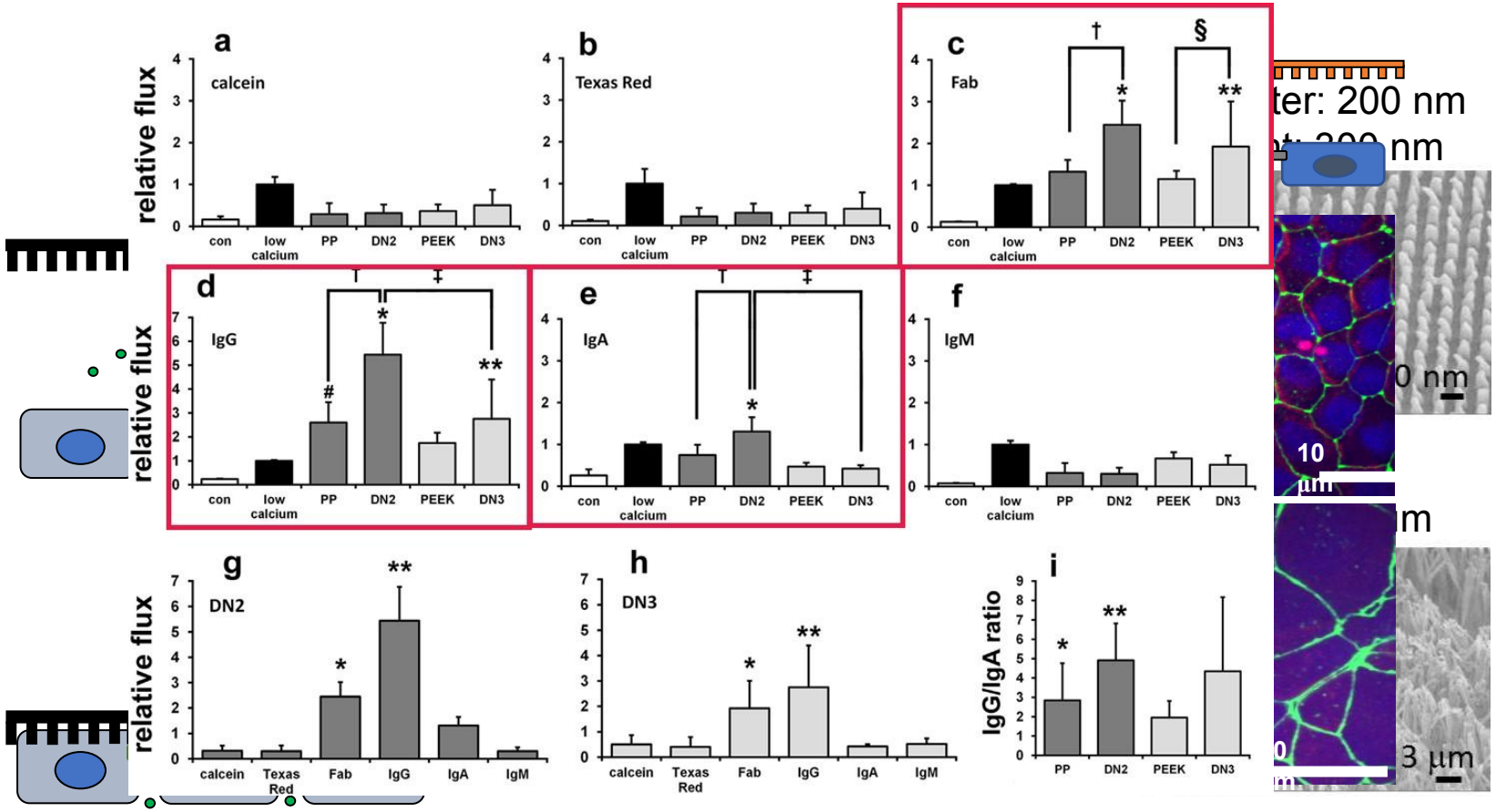

*J.A. Finbloom et al. Adv. Drug Deliv. Rev. 2020*

# Modulating Epithelial Barriers




Sun et al. Physiological Reviews, 2017.

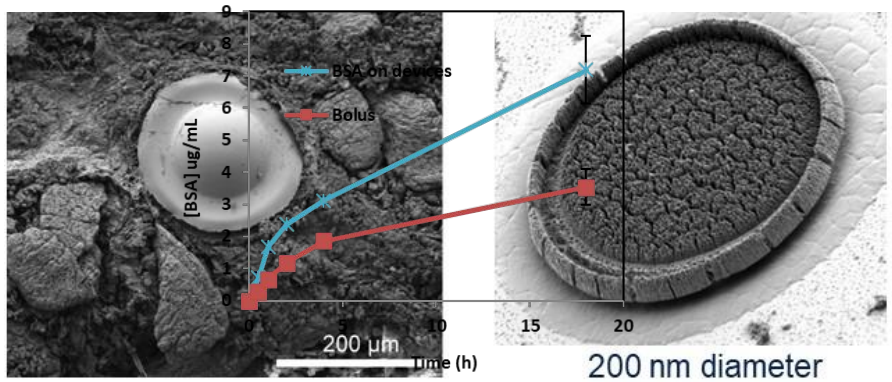
# Nanotopographical cues can enhance the permeation of protein therapeutics



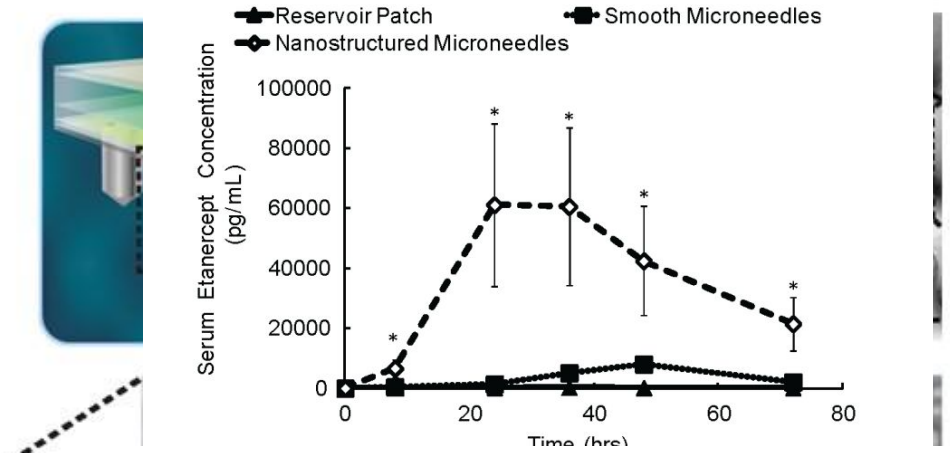

*Kam et al., Nanoletters, 2014; Stewart et al., Exp. Cell Res, 2017*

# Nanostructured Delivery Devices for increased permeability



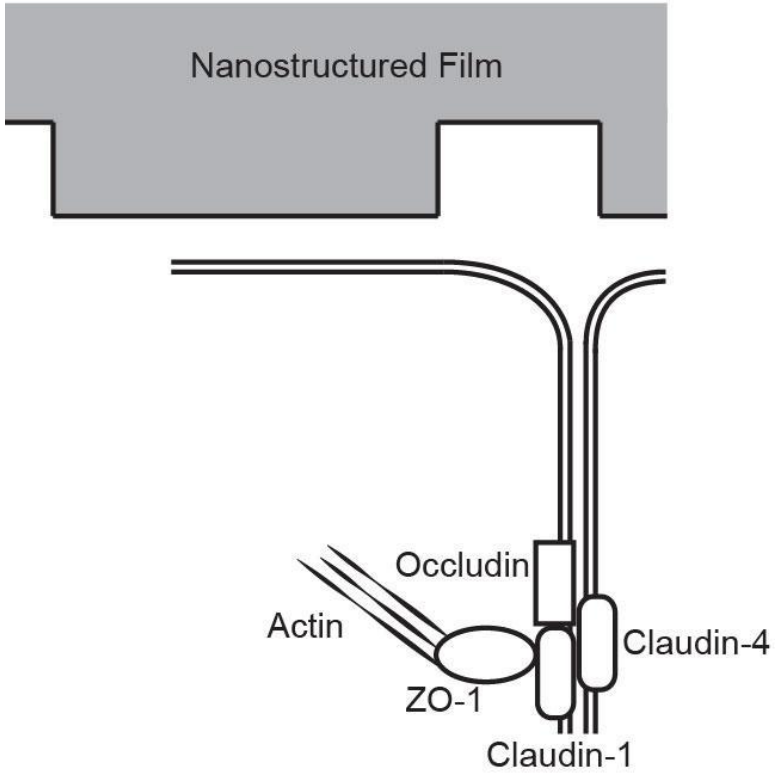

*Fox et al. JCR 2015, Fox et al. ACS Nano 2016; Stewart et al., 2017*




| | $T_{max}$ (hrs) | $C_{max}$ (pg/mL) | AUC |
|---|---|---|---|
| Reservoir Patch | 32 | 258 | 12,000 |
| Smooth Microneedles | 42 | 5,800 | 253,623 |
| Nanostructured Microneedles | 30 | 80,712 | 2,680,535 |

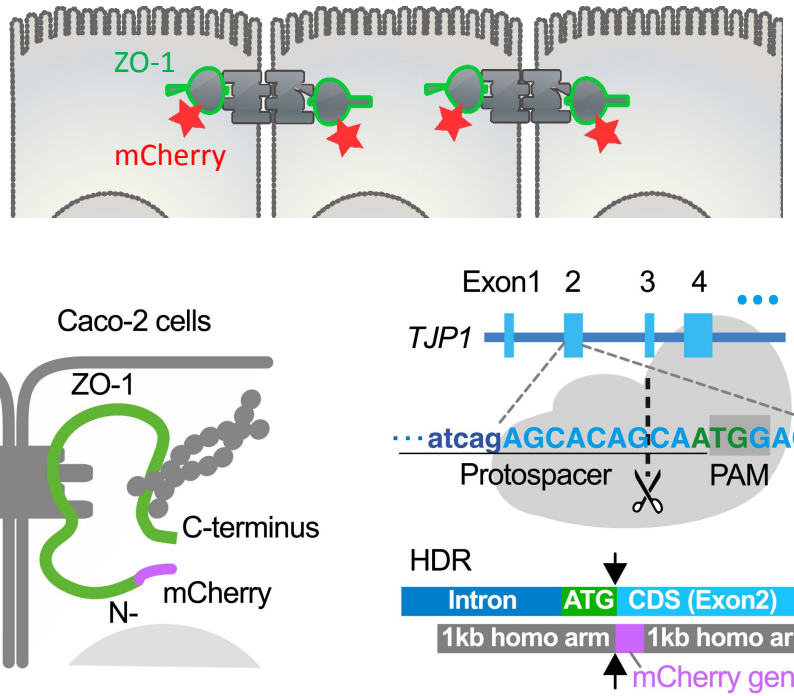
*Walsh and Ryu, Nanoletters, 2015*

# How does nanotopography induce cytoskeleton and tight junction remodeling?



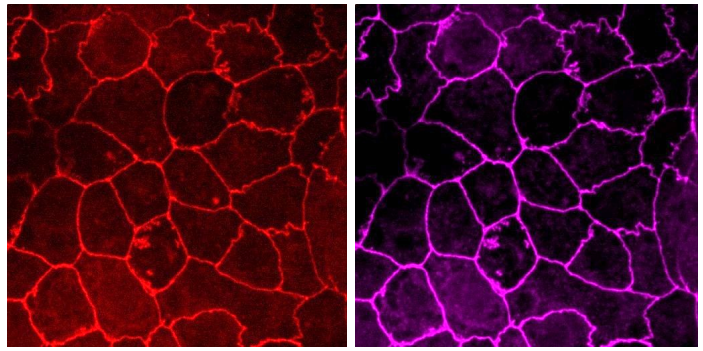
# CRISPR-based gene editing to visualize tight junctions

**Fluorescent protein fusion of TJP-ZO-1**




ZO-1 proteins fused with mCherry-reporter at N-terminus through CRISPR

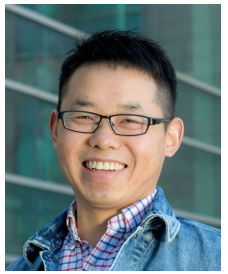
**After *In Vitro* barrier model screening**

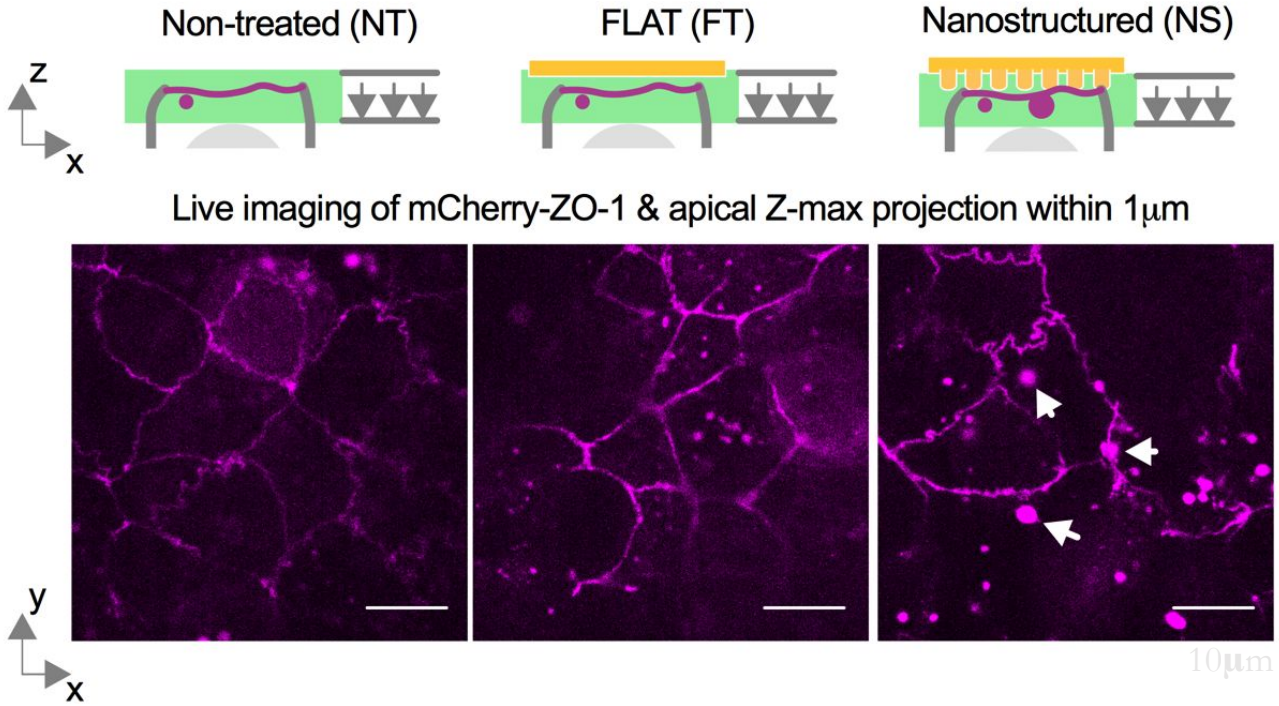


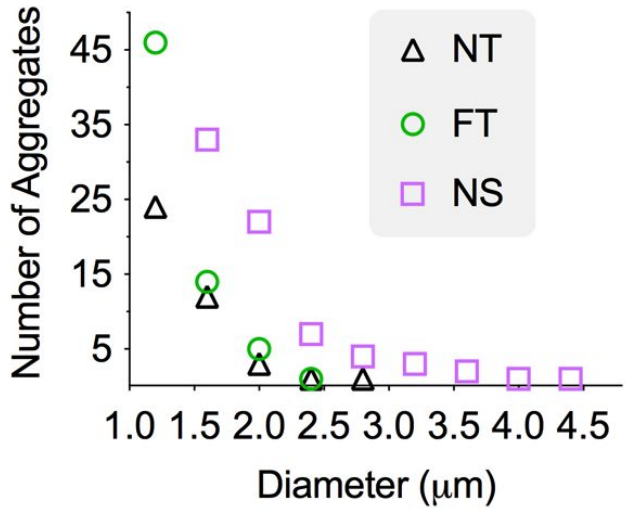
**Endogenous mCherry-ZO1**

**ZO-1 ICC**

# Nanostructure induces cytosolic ZO-1 liquid complexes at apical contact



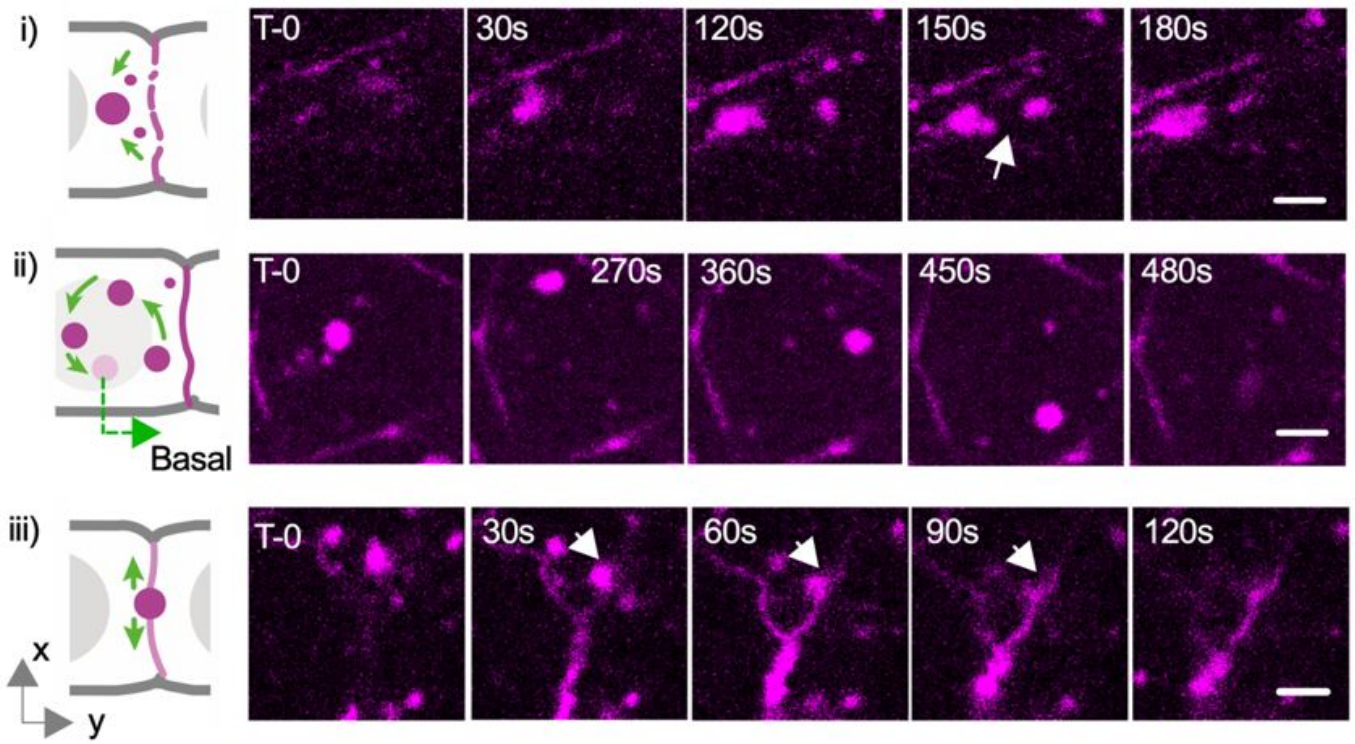


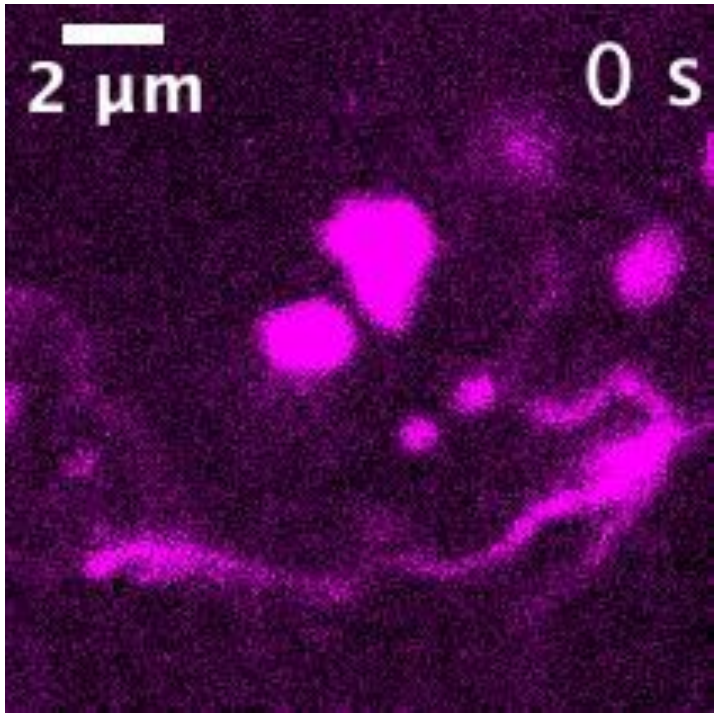




n = 25, from 5 independent experiments

*Huang, et al. ACS Nano, 2020*

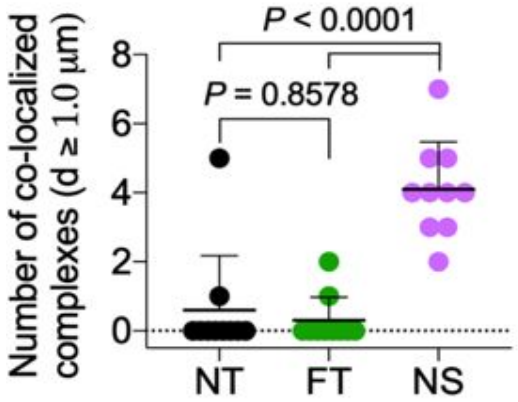
# These cytosolic complexes actively remodel and interact with tight junctions

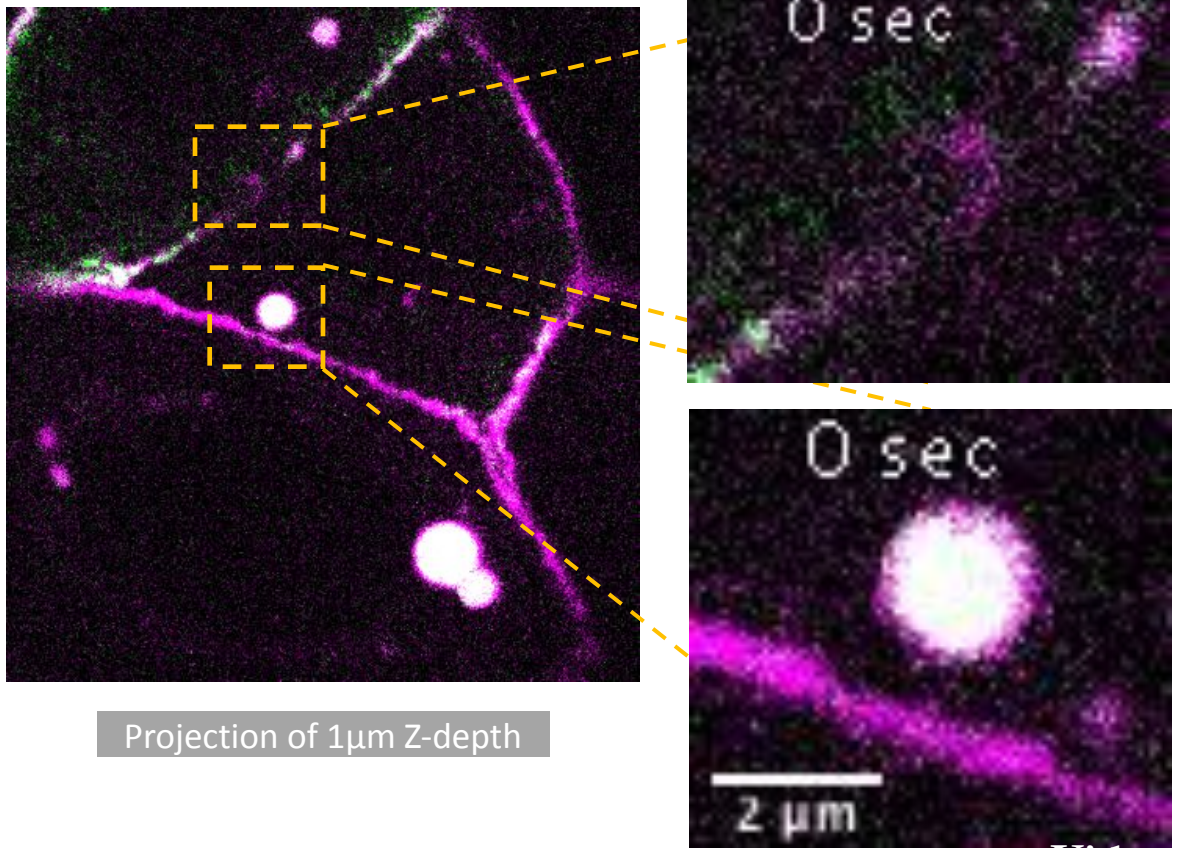



*Huang, et al. ACS Nano, 2020*

# Junctional protein Claudin-4 colocalizes with ZO-1 liquid structures

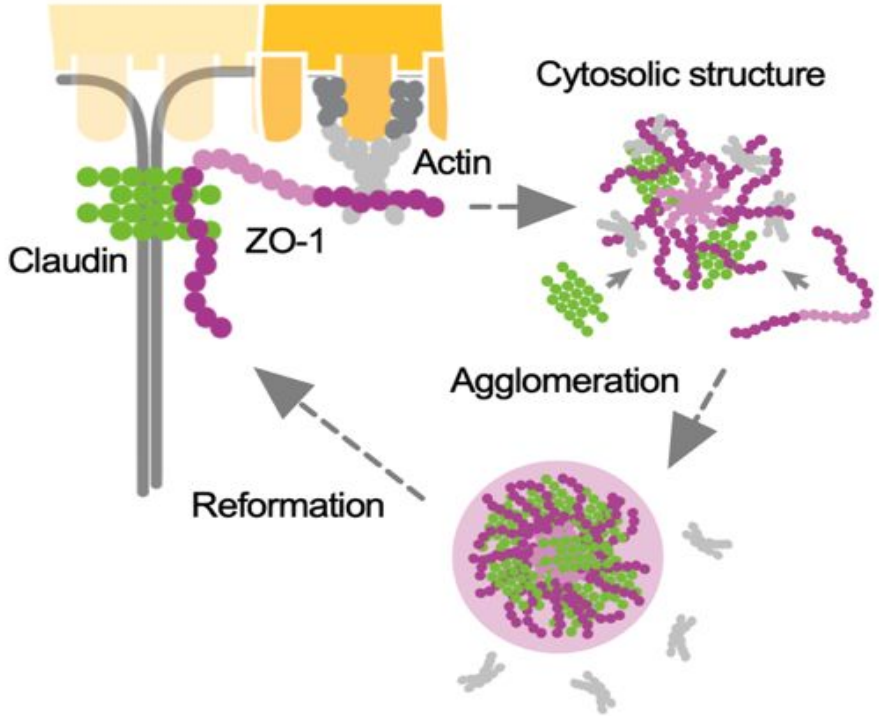



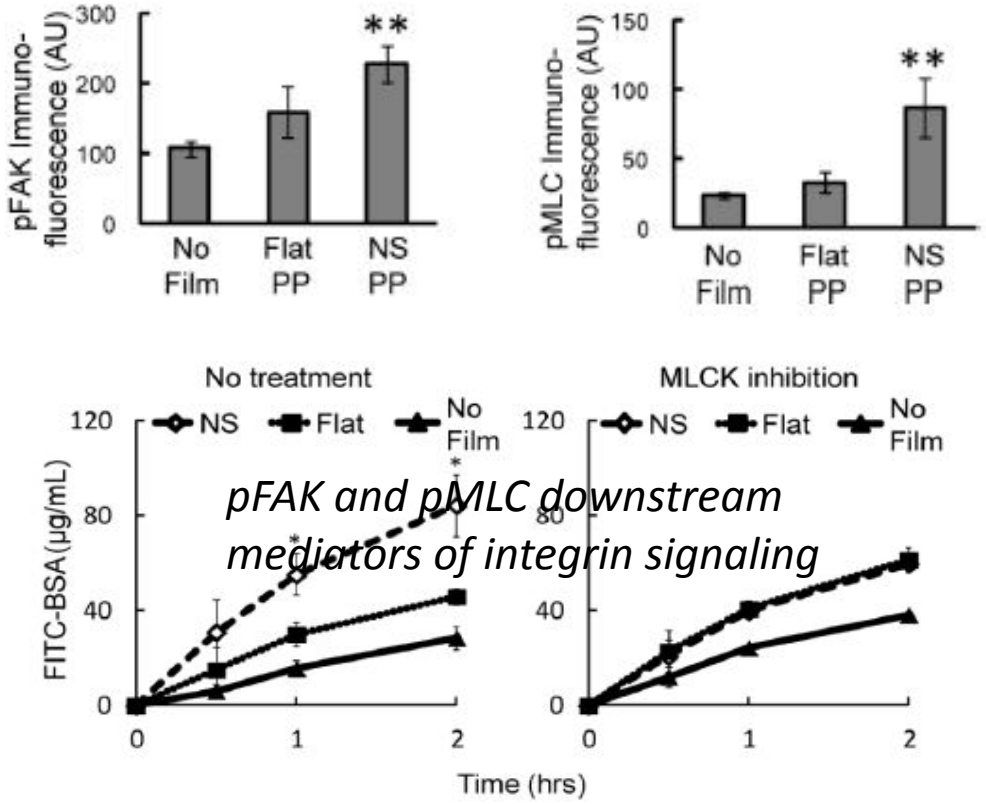

Projection of 1µm Z-depth

*Huang, et al. ACS Nano, 2020*

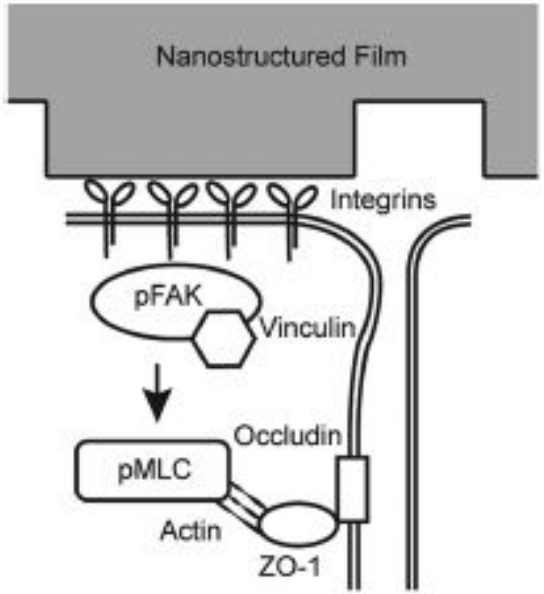
# Nanotopography induces dynamic remodeling of tight junctions



# Nanostructure-mediated transepithelial permeability alters mechanotransduction

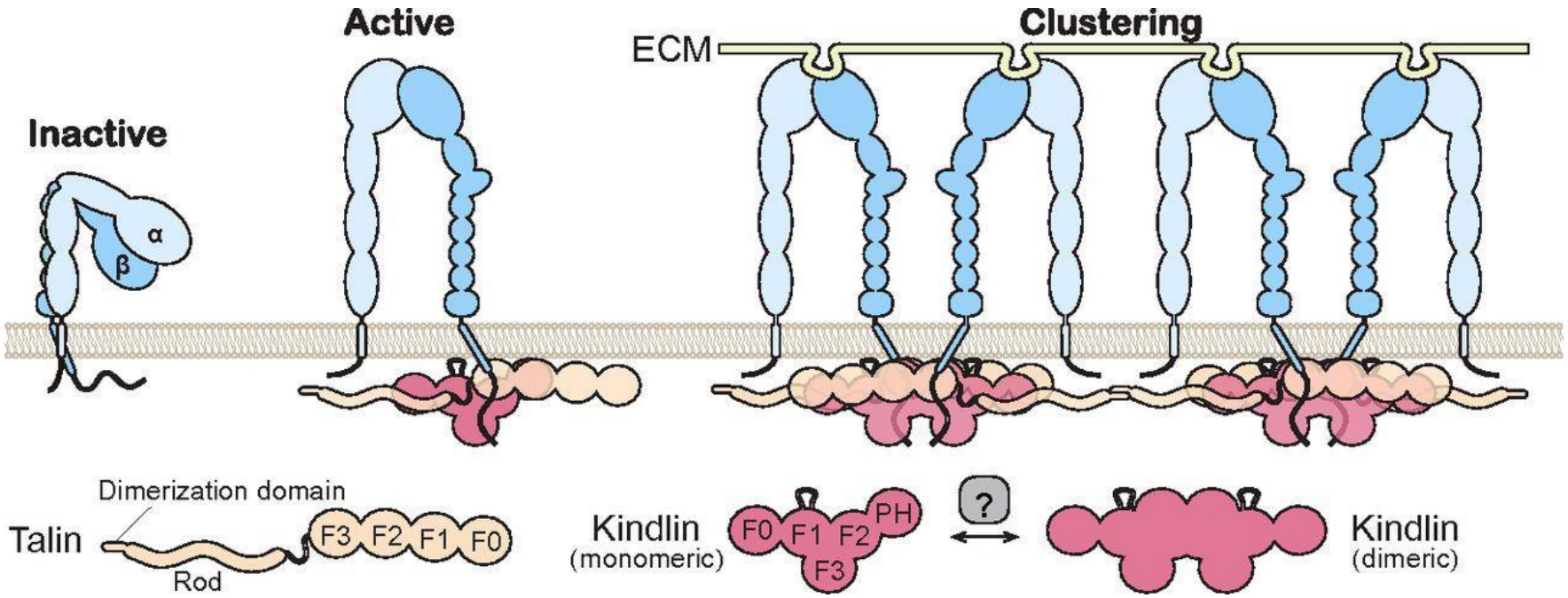



*pFAK and pMLC downstream mediators of integrin signaling*



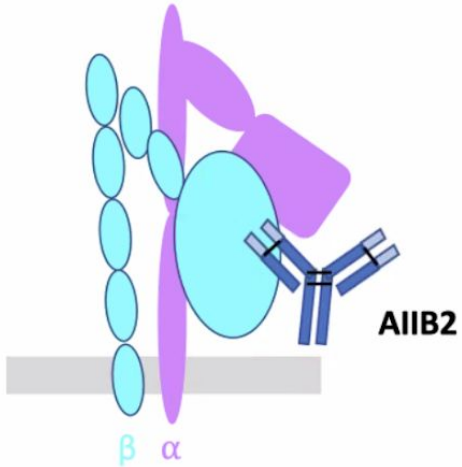

*Walsh, L.. et al, Nano Letters*

# Can we recapitulate nanotopography stimulation of tight junctions through multivalent integrin clustering?

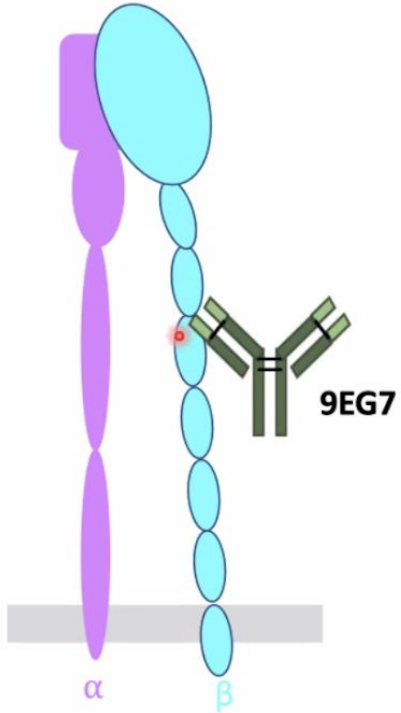



*Li. et al, PNAS 2017*

# Functional Anti-integrin Antibodies

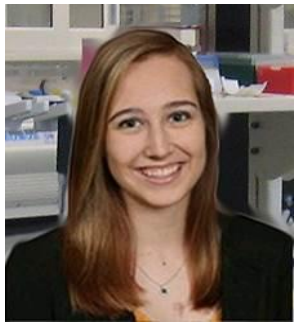


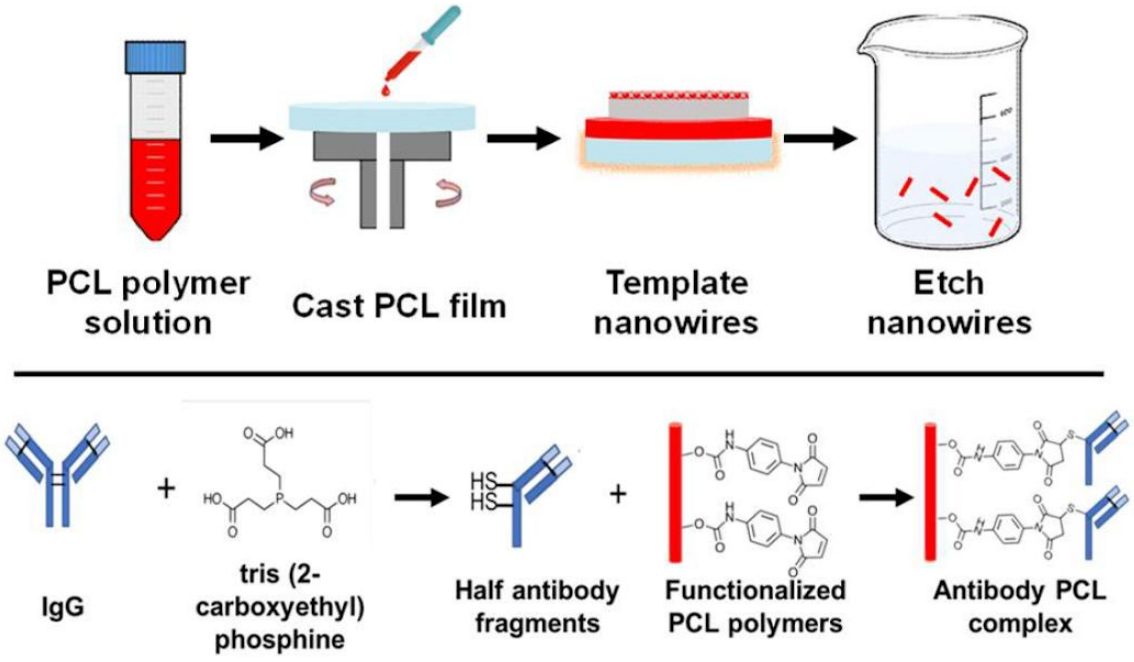

***Blocking*** **anti-integrin antibodies**
**Maintain closed conformation**

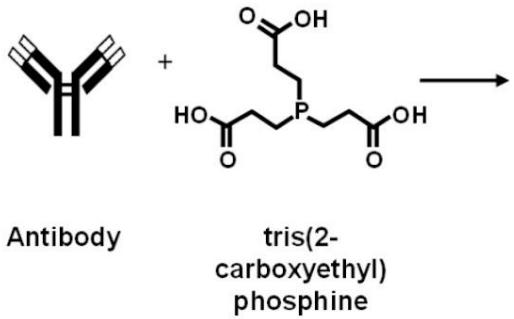


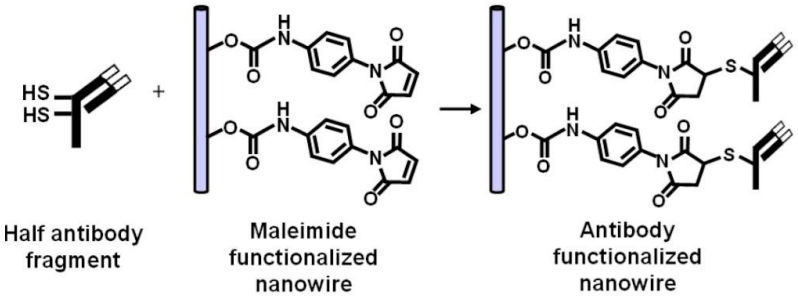

***Activating*** **anti-integrin antibodies**
**Maintain open conformation**

# Evaluation of β1 integrin antibodies on nanowires



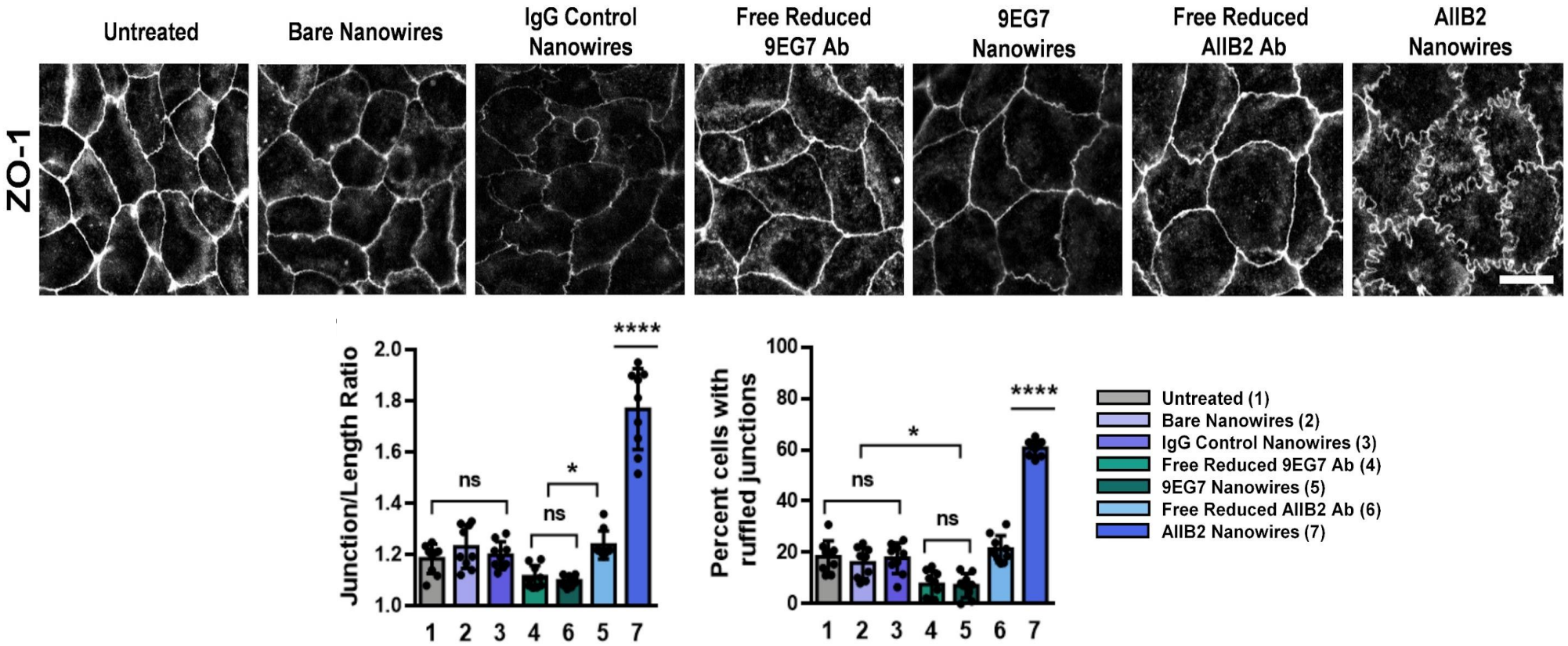



*Collaboration with Mike Koval at Emory*

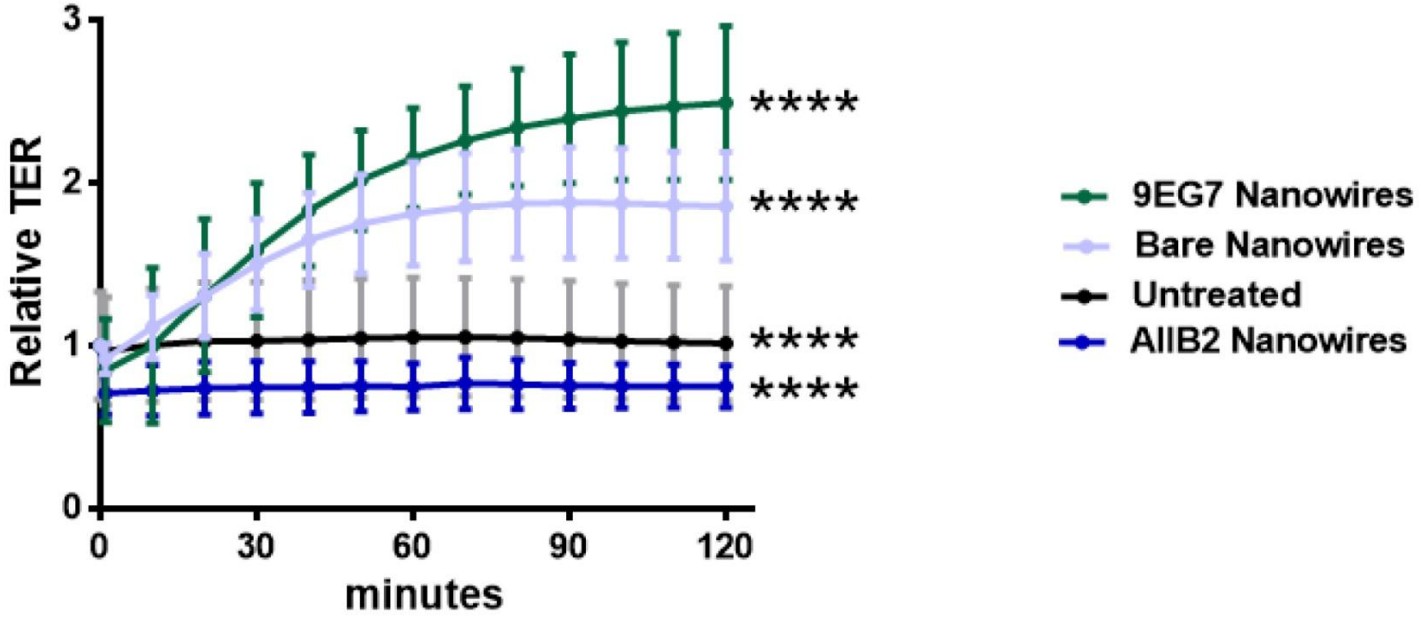
# Blocking antibodies cause TJ Ruffling




*Raven Peterson et al., in prep*

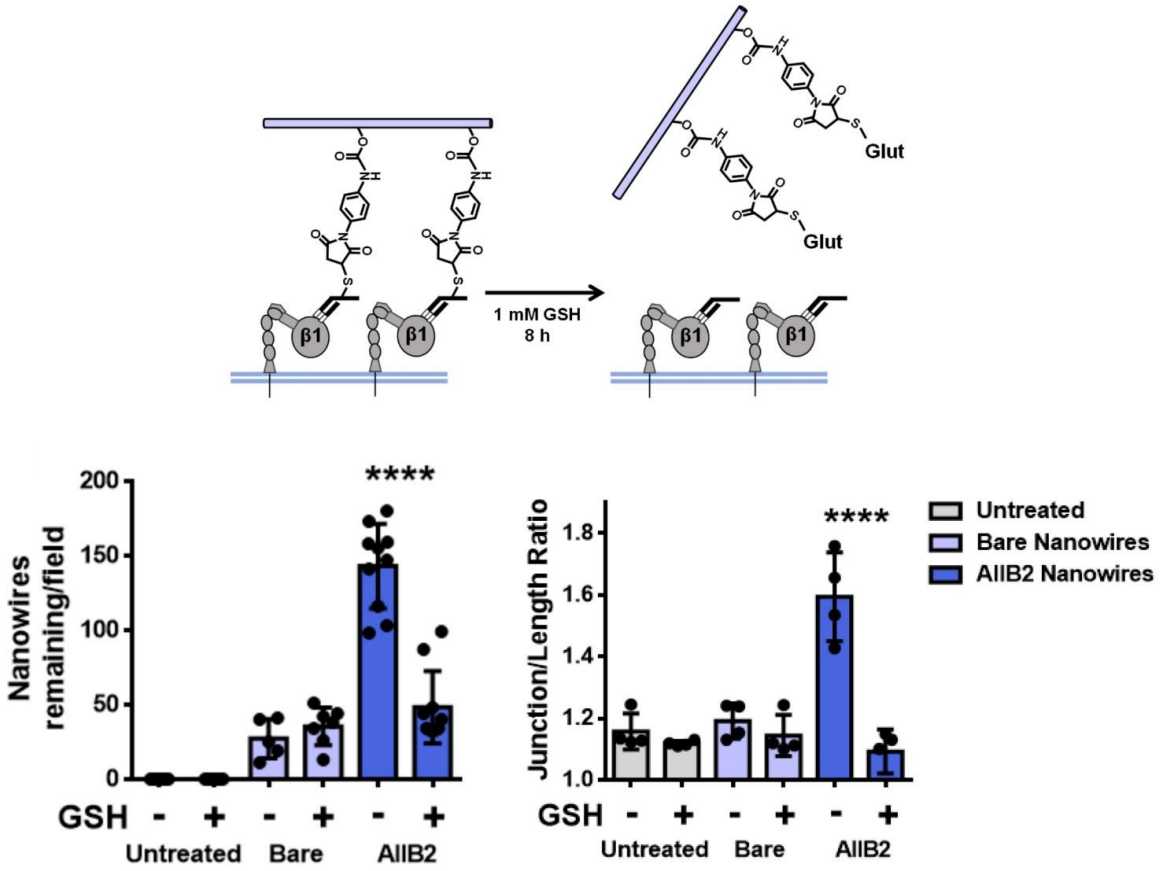
# Anti-integrin nanowires modulate TER:

## *Blocking Integrin nanowires Increase TER while Activating Integrin Nanowires Decrease TER*



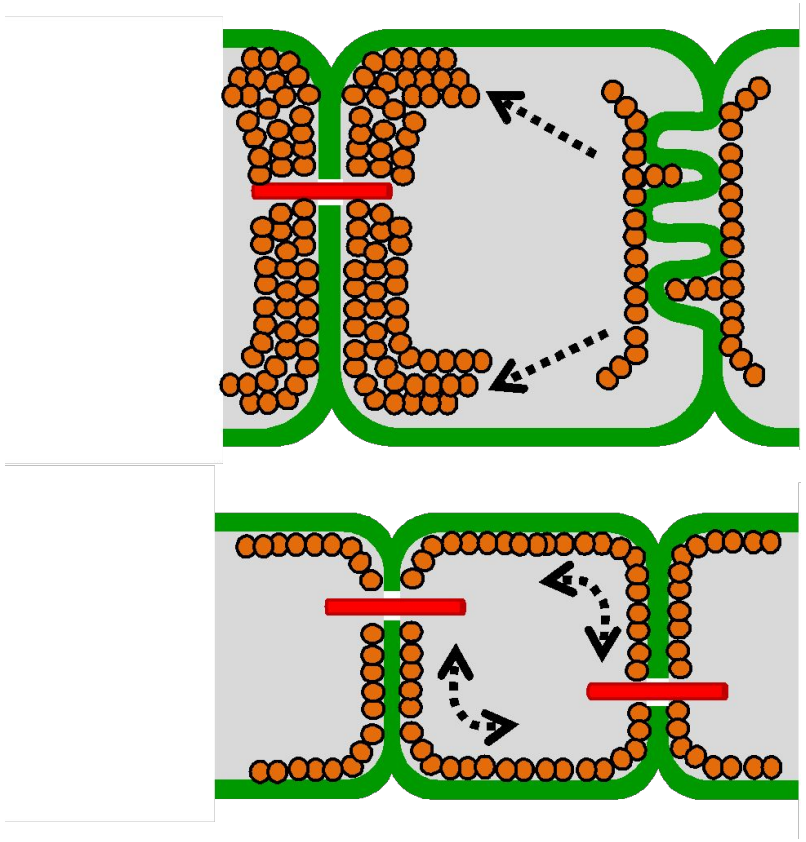

*Raven Peterson et al., unpublished*

# Apical integrins as a switchable target to regulate the epithelium

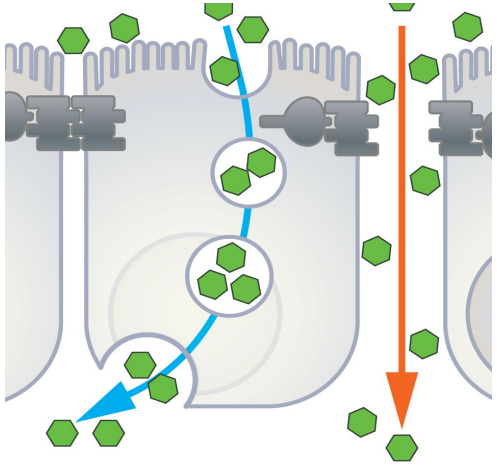


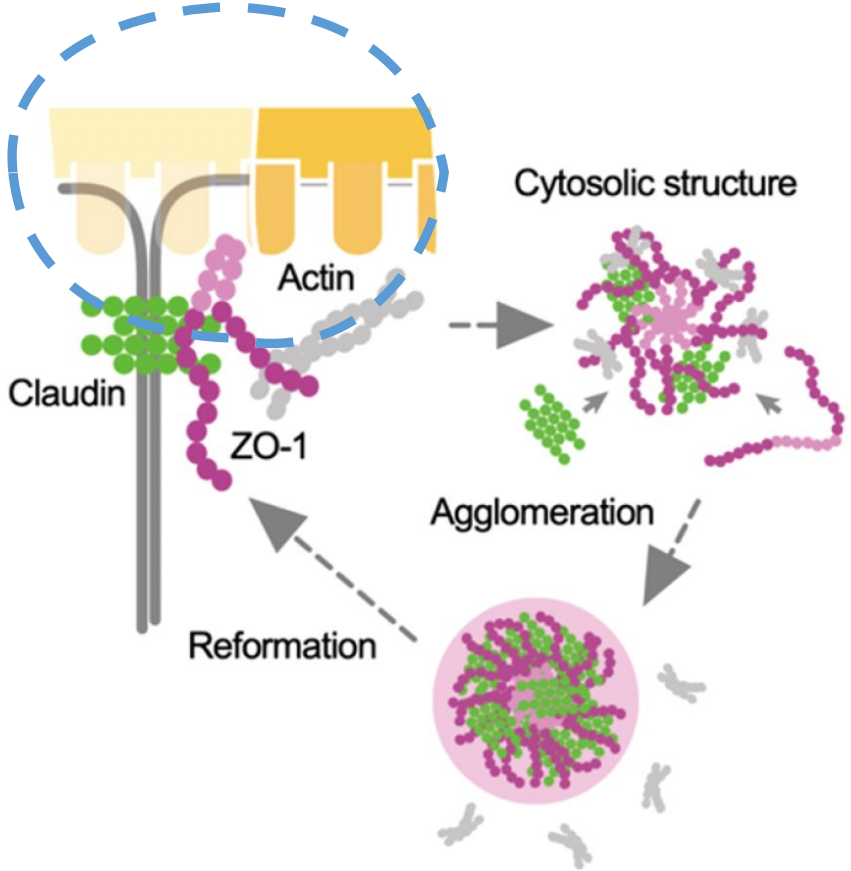

*Raven Peterson et al., unpublished*

# Differential effects of anti-β1 integrin complexes on tight junction morphology (?)

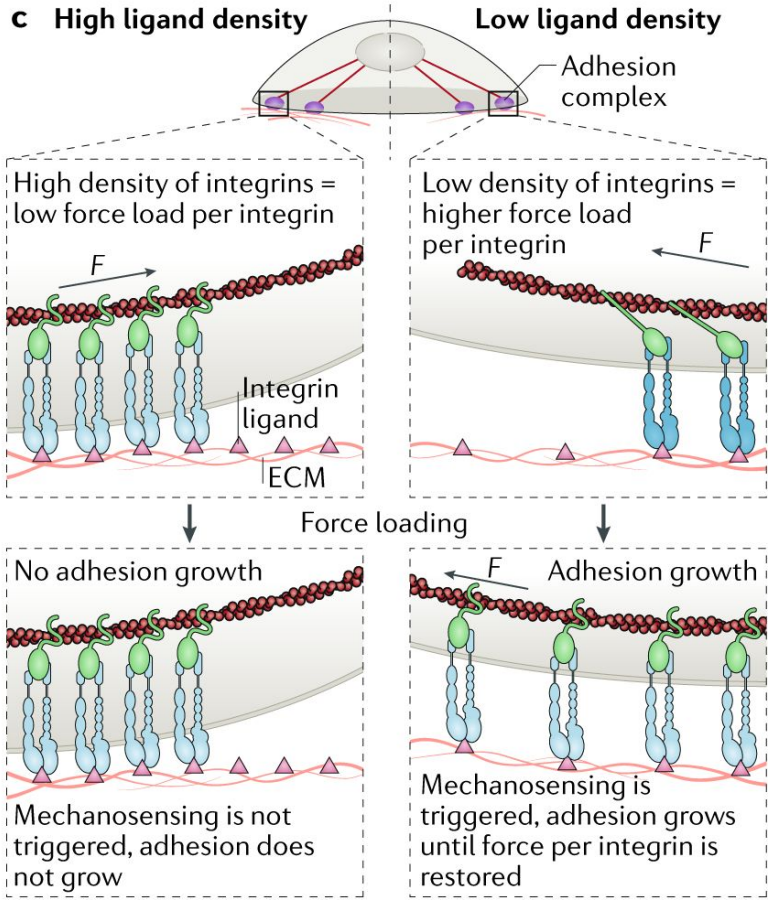


# Nanotopography can create multivalent integrin clustering that modulates TJ remodeling

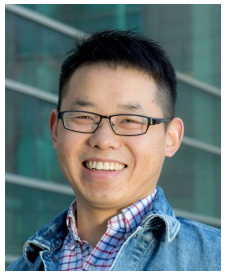


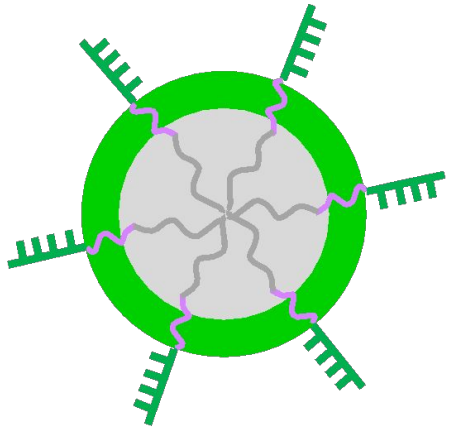
# Integrin clustering & signaling require precise ligand density

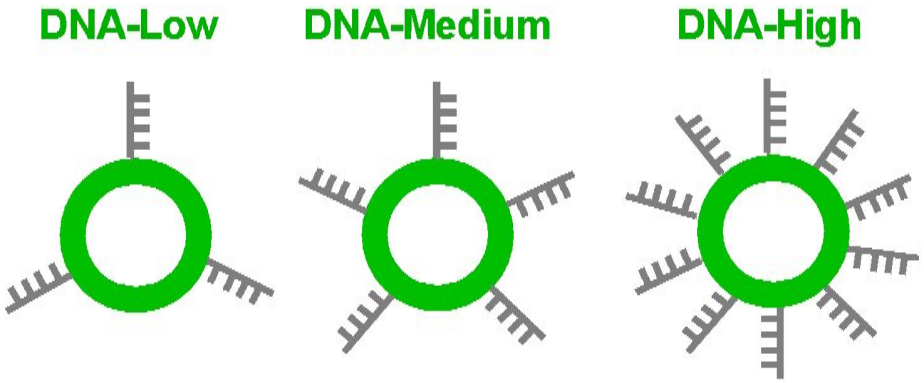


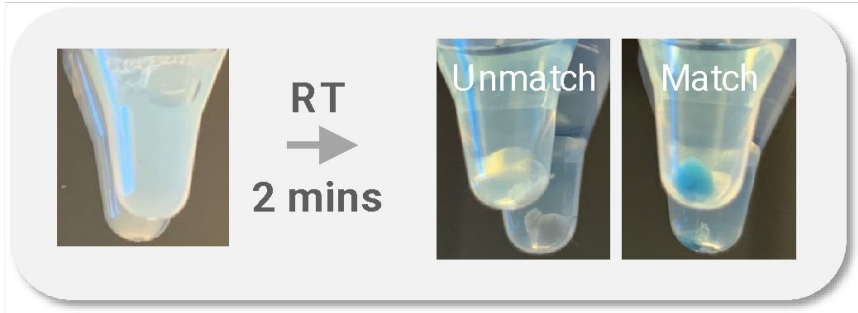

*Kechagia, et al. Nature Reviews Molecular Cell Biology, 2019*

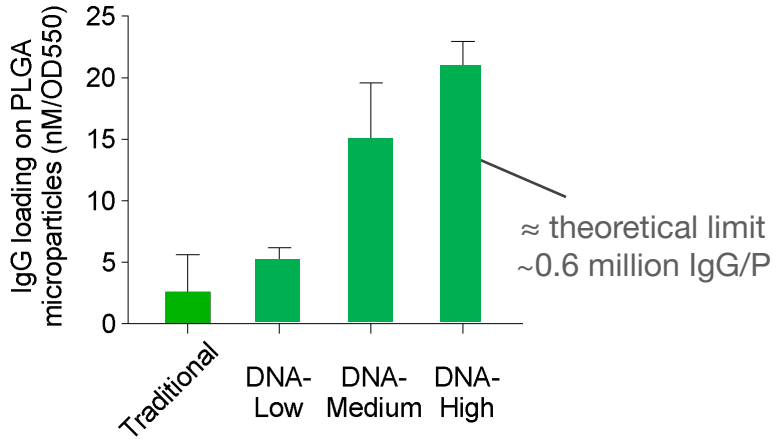
# Synthetic DNA scaffolds on particles enable ratiometric control over surface loading





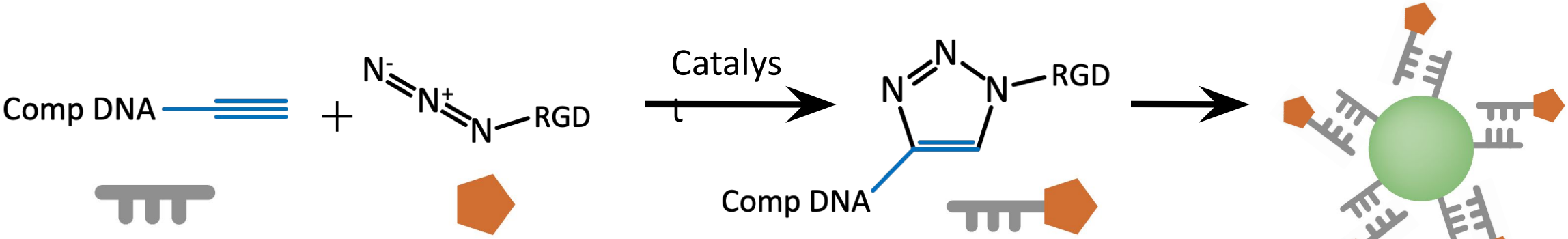




*Huang, et al. Nature Nanotech, 2021*

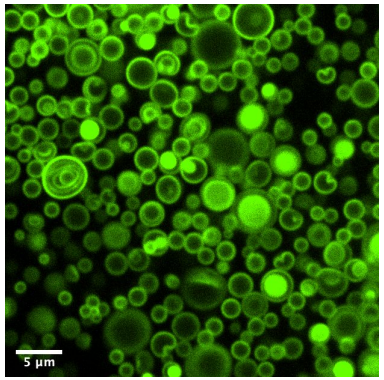
# Decoration of particles with RGD peptide through DNA scaffold hybridization




*Copper Catalyzed Click Chemistry used to conjugate comp DNA to RGD*

RGD - Comp DNA

Complimentary DNA

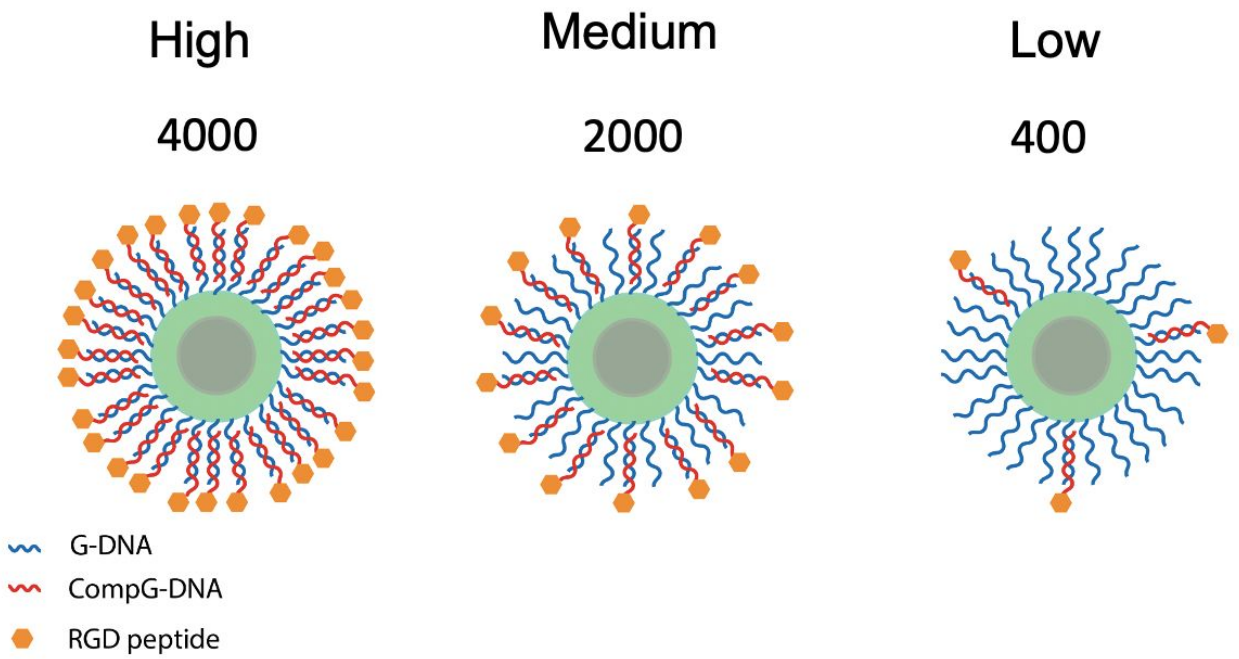


# DNA-scaffolded particles facilitate tuning of particle RGD-density

Diameter: 212 nm

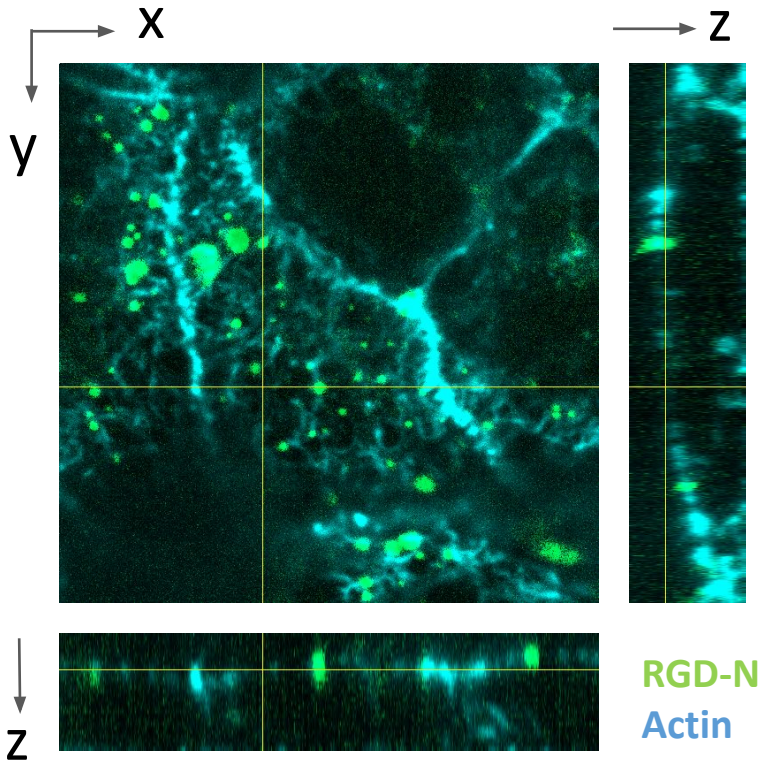
Surface DNA density:
4000 DNA/particle

Estimated RGD/particle:

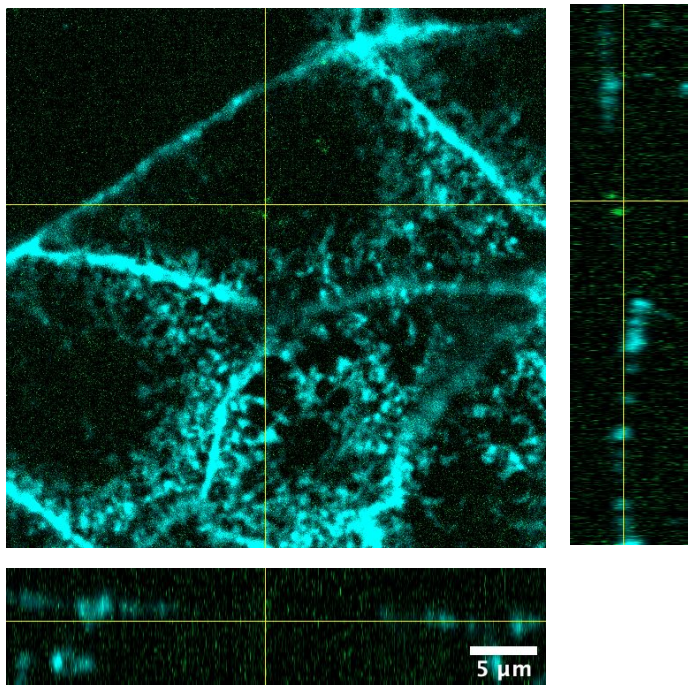


# Particle RGD-density alters extent of nanoparticle internalization

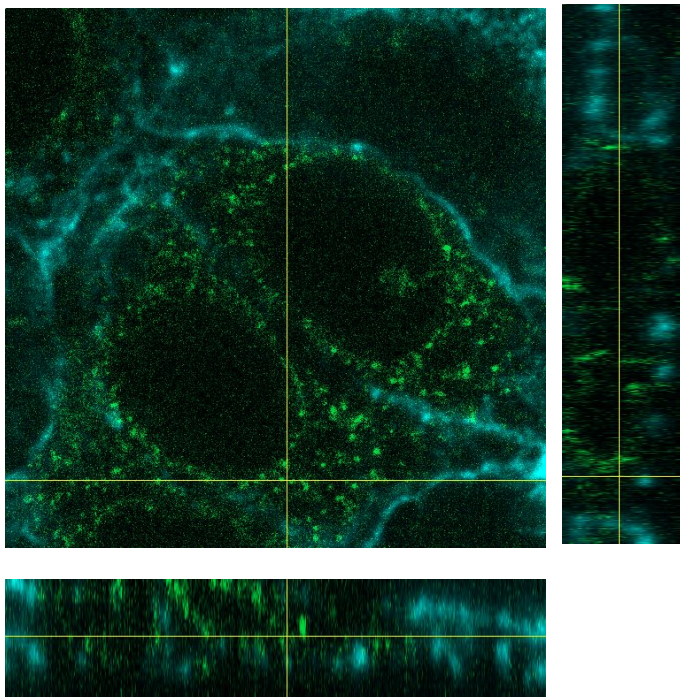
*Nanoparticle Control*



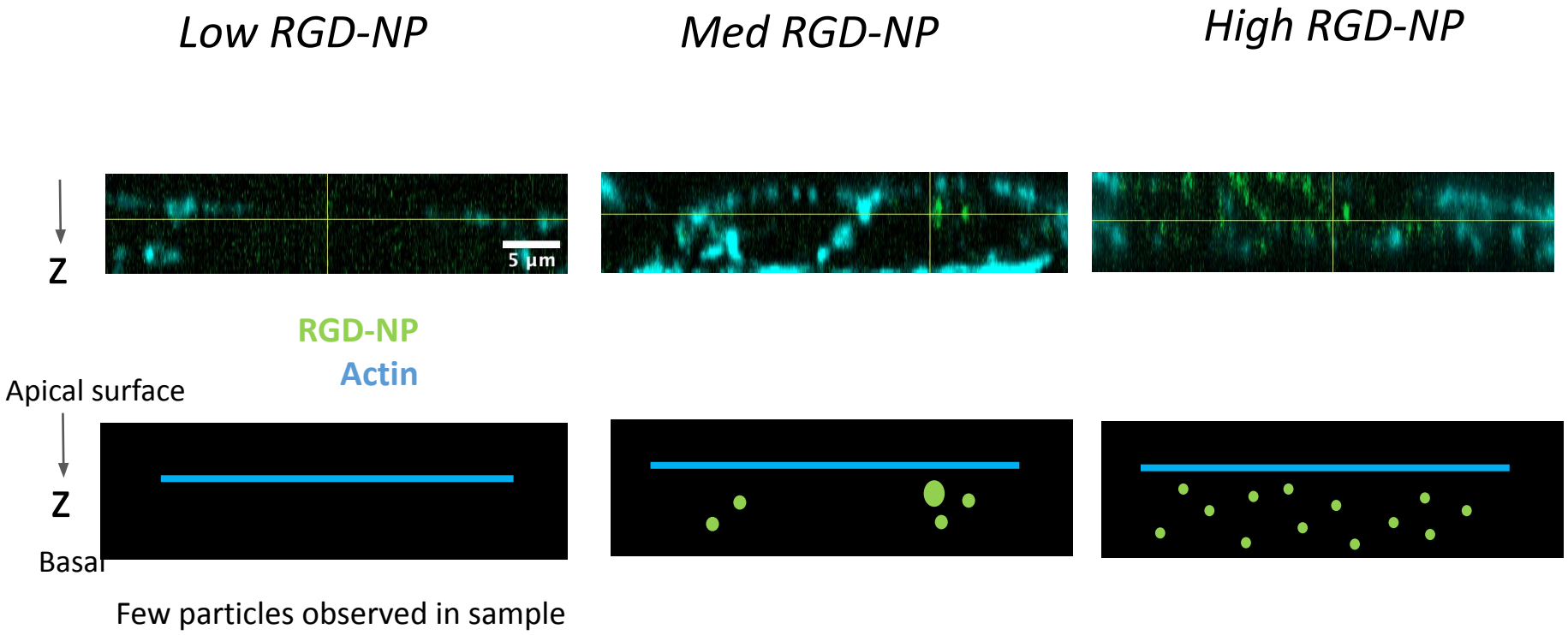

*Low RGD-Nanoparticle*



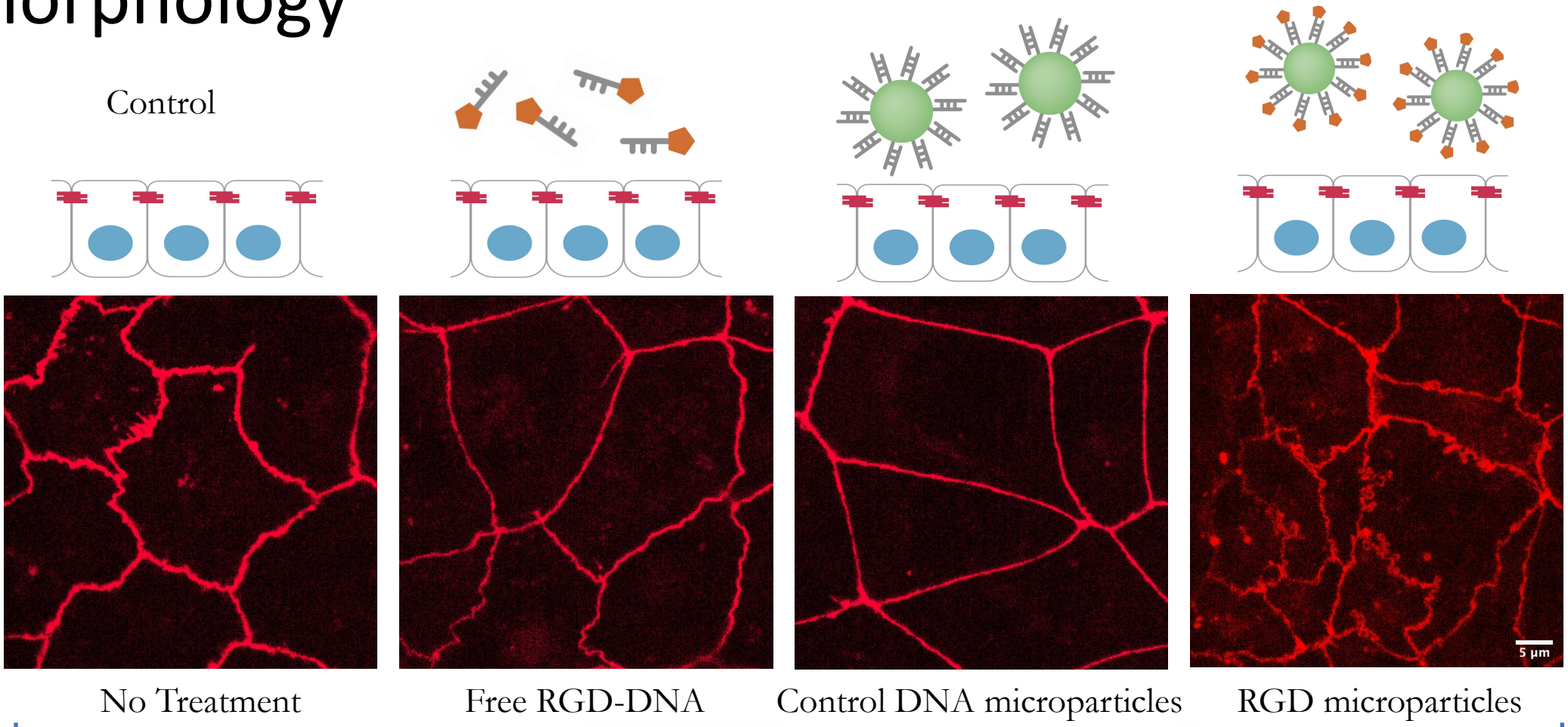

*High RGD-Nanoparticle*



# Particle surface RGD-density alters extent of particle internalization



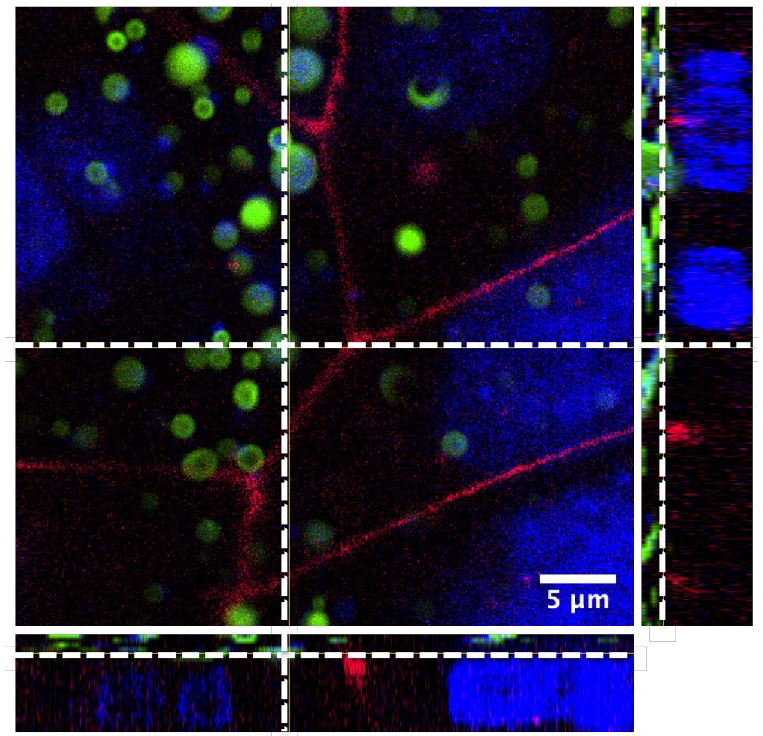
# RGD-microparticle treatment alters junctional morphology




mCherry ZO-1 Caco-2 Cells. *Images shown are z-projection of apical 0.9 um*

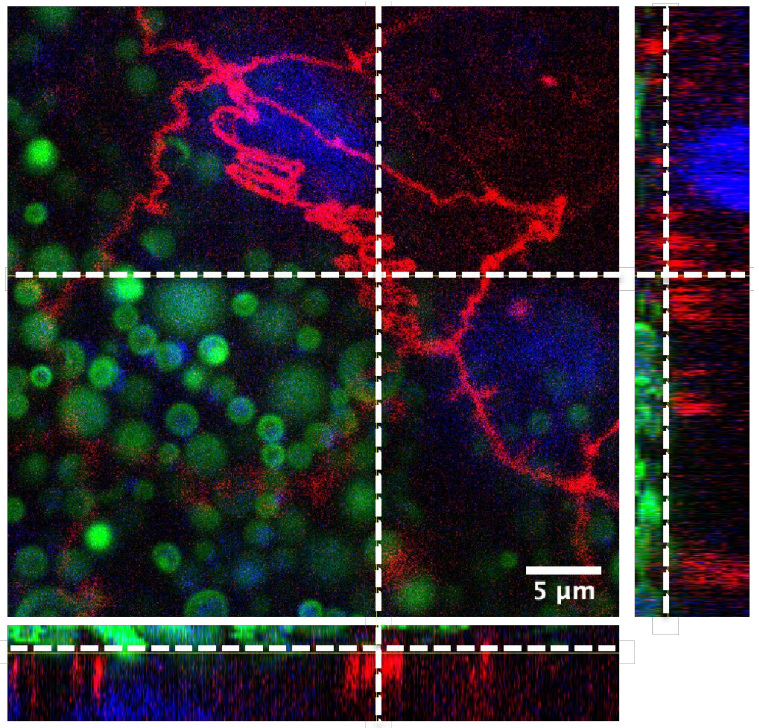
# Orthogonal view of RGD-microparticle interaction with mCherry-ZO-1 engineered Caco-2 cells

*DNA only -Microparticle*




Particle
ZO-1
Nucleus

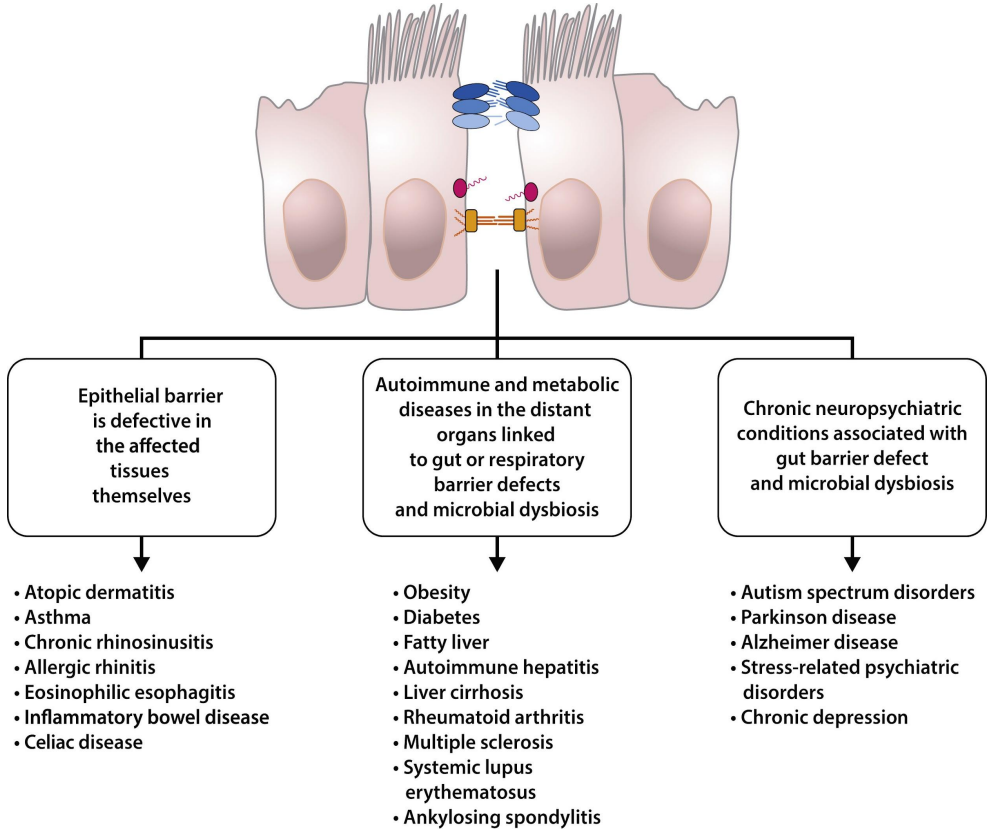
*RGD-Microparticle*



# Why does this matter in the context of health and disease?

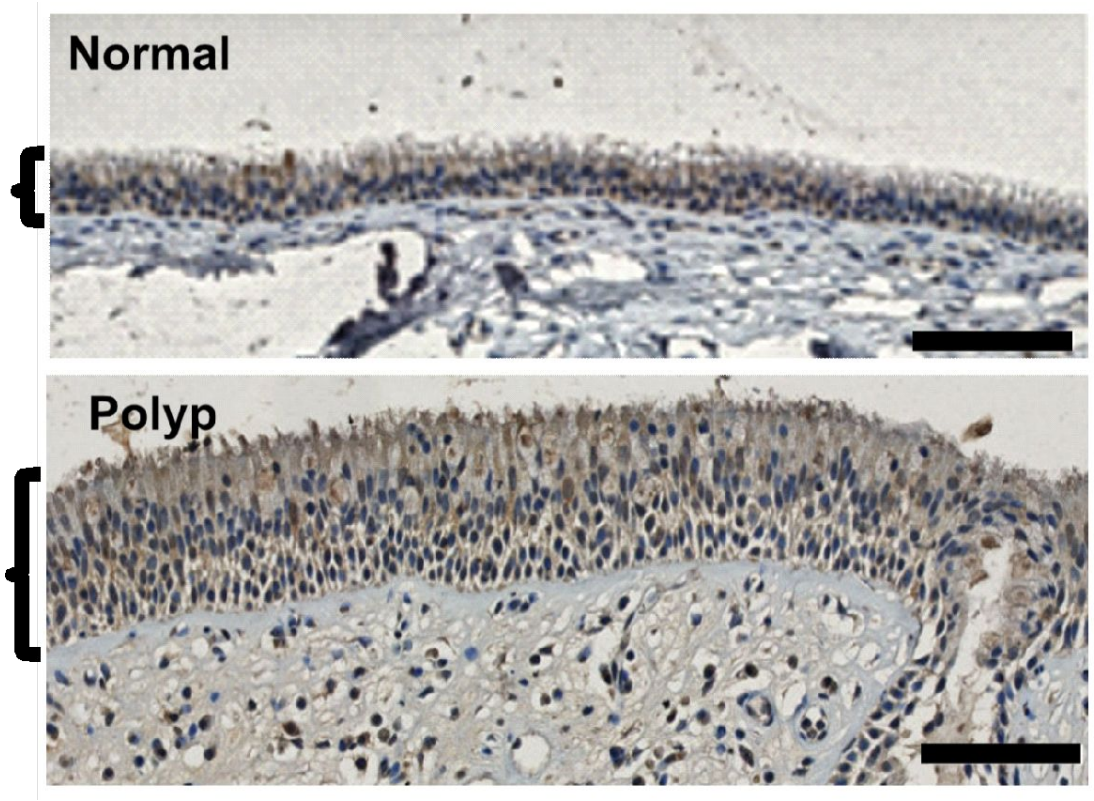
*Misregulation of epithelial permeability is the hallmark of many diseases*

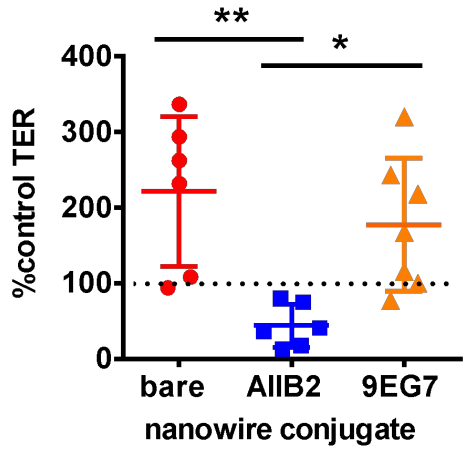
*IBD (UC and Crohn's), Esophageal Disease, Cystic Fibrosis, etc.*

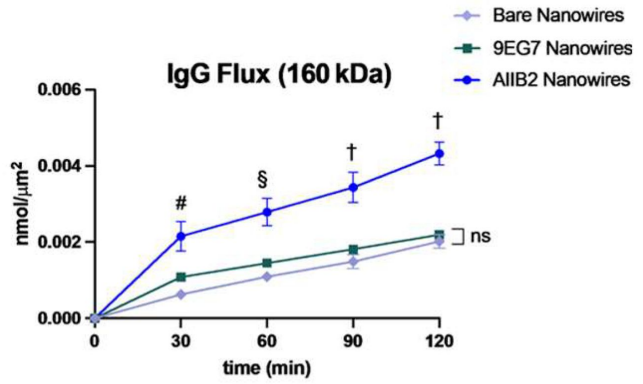



*Journal of Allergy and Clinical Immunology 2022*

# Application to Nasal Epithelium -- Polyps



# The Therapeutic Micro and Nanotechnology Laboratory at UCSF

Mike Koval, Emory
Raven Peterson, Emory
Thea Mauro, UCSF
Bo Huang, UCSF

Funding from NIH and Roche