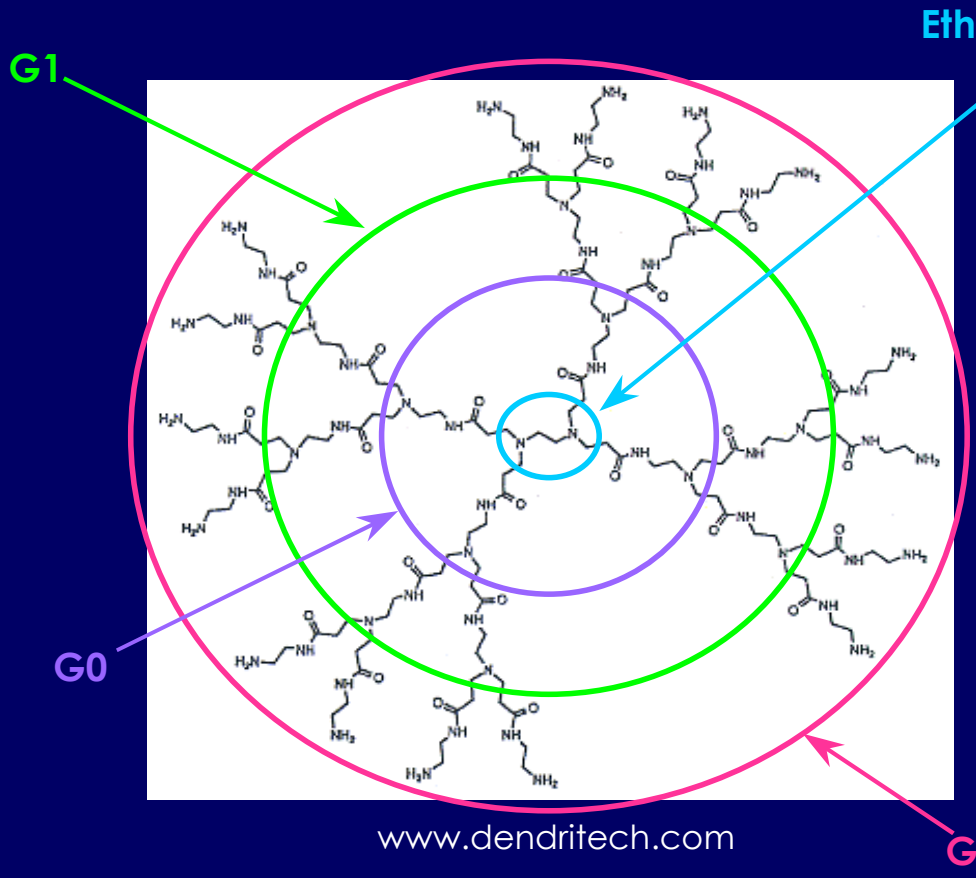
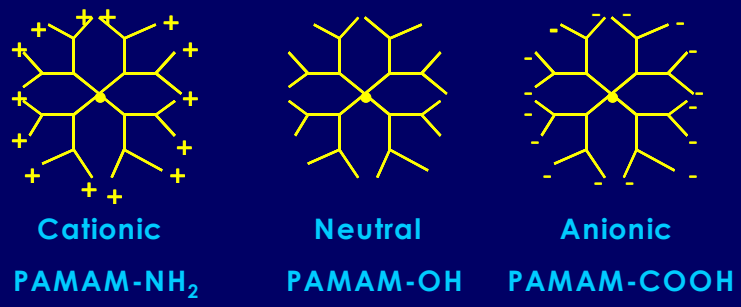
Evaluation of Poly (Amidoamine) Dendrimer Permeability and Transport Mechanisms across Caco-2 Cell Monolayers

Kelly Marie Kitchens
Center for Nanomedicine and Cellular Delivery
Department of Pharmaceutical Sciences
University of Maryland, Baltimore

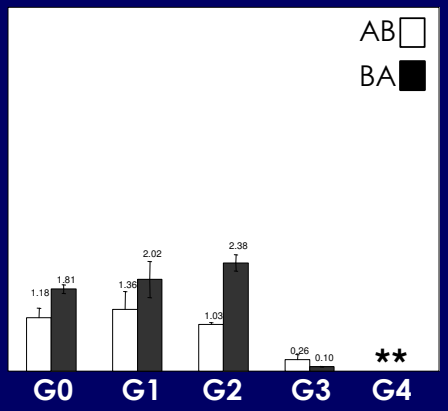
Poly (Amidoamine) Dendrimers



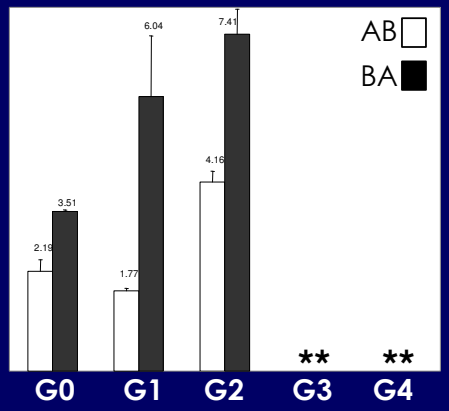


- Defined mass, size, shape and surface chemistry
- Potential use as oral drug delivery systems

Florence (ed.) *Adv Drug Del Rev*, (2005)



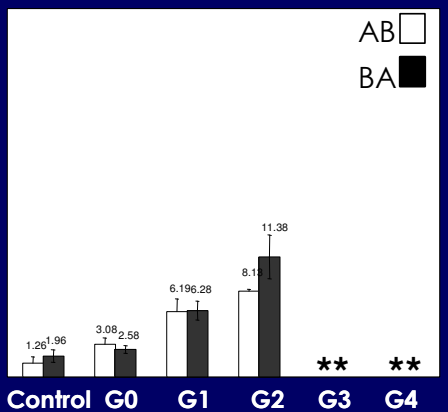
1.0 mM @ 90 minutes



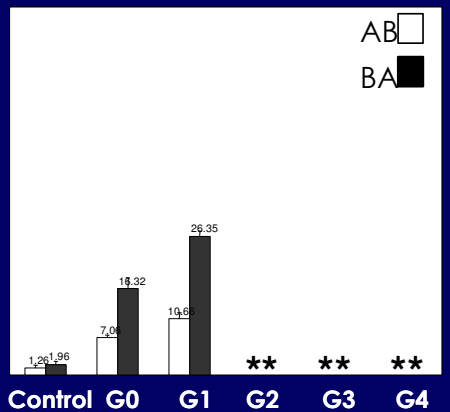
1.0 mM @ 210 minutes

PAMAM-NH_2 Permeability

El-Sayed et al., *J Control Rel*, (2002)



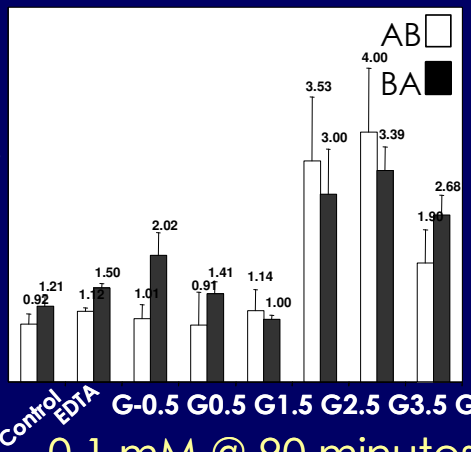
0.1 mM @ 210 minutes



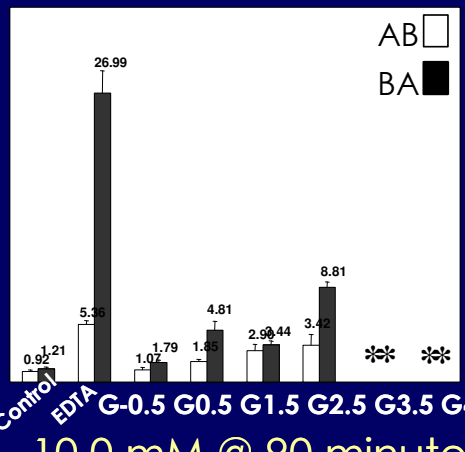
10.0 mM @ 210 minutes

^{14}C-Mannitol Permeability

El-Sayed et al., *J Bioactive Compat*, (2003)



0.1 mM @ 90 minutes



10.0 mM @ 90 minutes

^{14}C-Mannitol Permeability

El-Sayed et al., *J Bioactive Compat*, (2003)

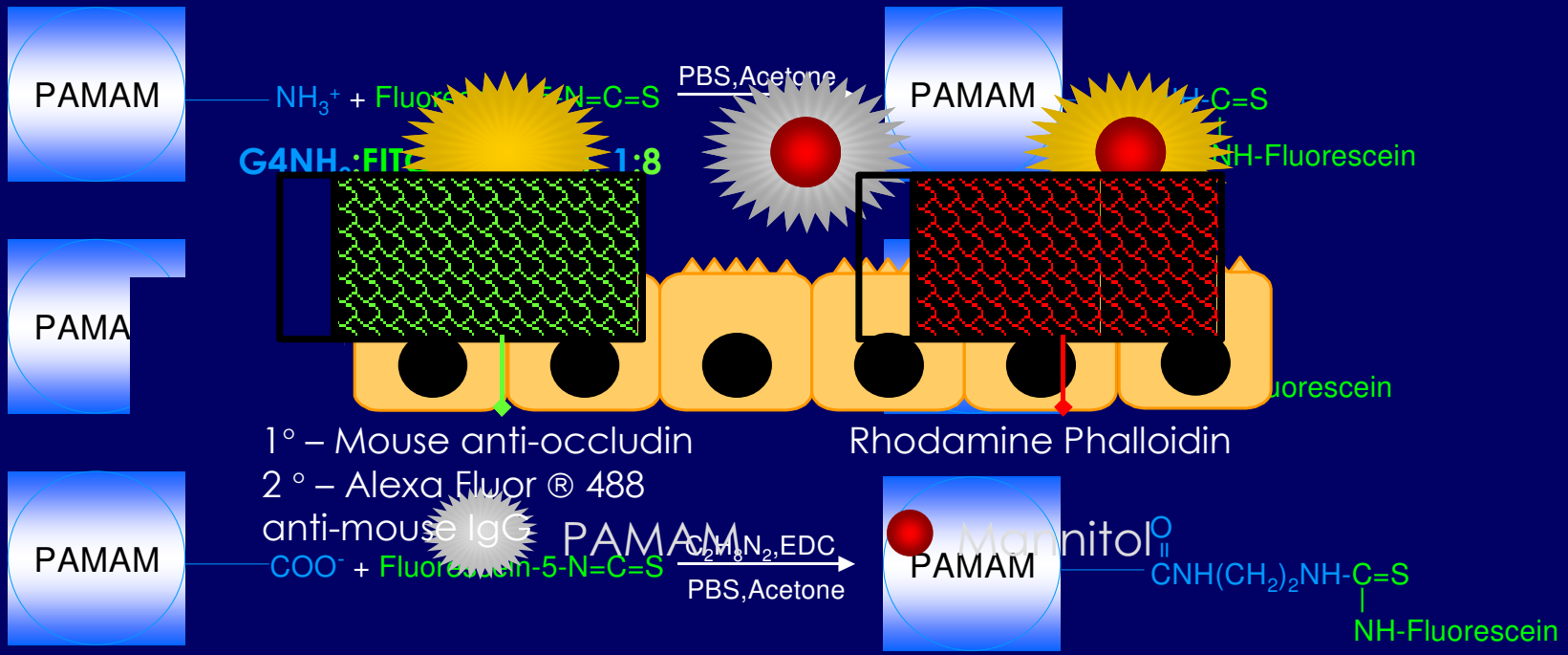
Research Objectives

I. Influence of surface charge and size

II. Effect of hydrophobic drug loading

III. Transport mechanisms of PAMAM

Experimental Methodology: PAMAM Permeability



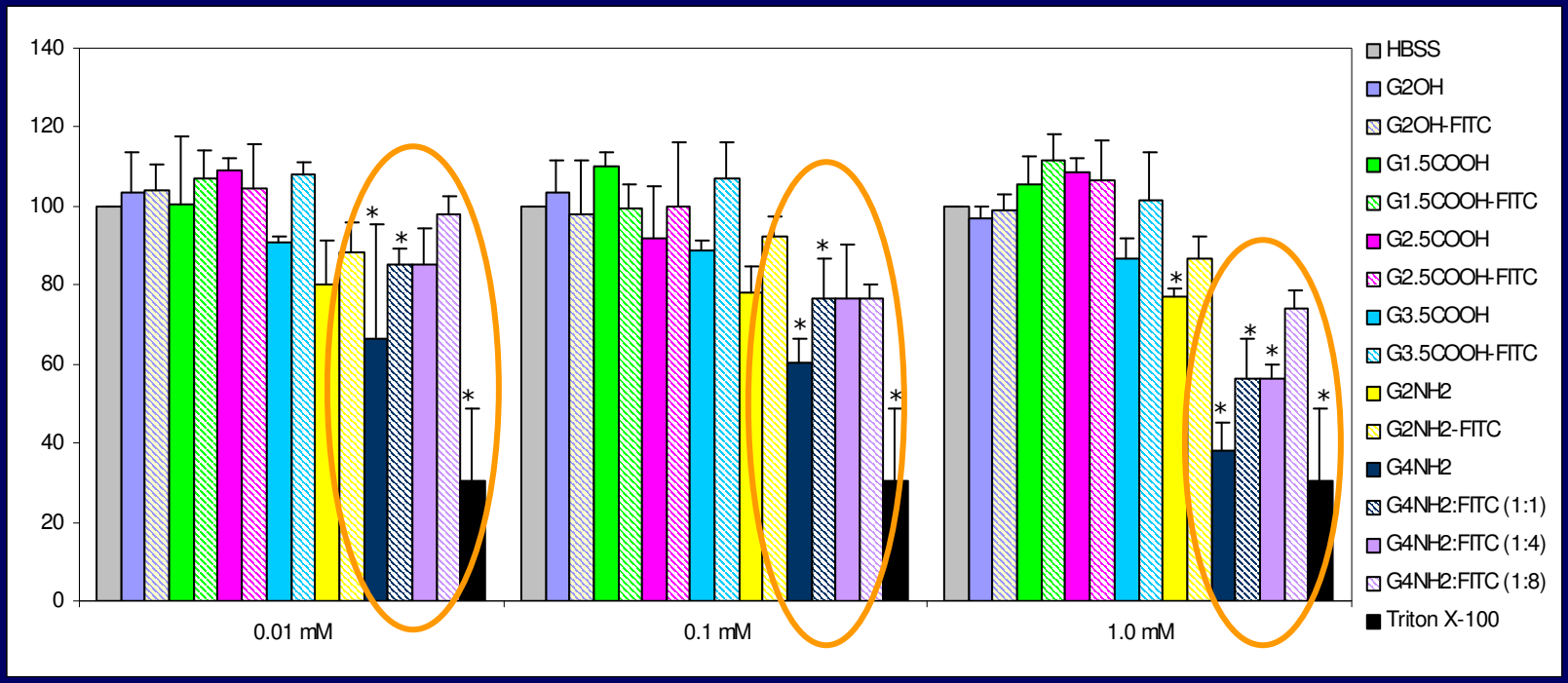
Structural Features of PAMAM Dendrimers

Generation	Surface Group	# Surface Groups	M_W (Da)[a]	Label Content (mmol FITC / g dendrimer)[b]
G2	$-NH_2$	16	3,256	0.3896 ± 0.0288
G2	-OH	16	3,272	0.0604 ± 0.0001
G1.5	-COOH	16	2,935	0.0447 ± 0.0008
G2.5	-COOH	32	6,266	0.0016 ± 0.0001
G3.5	-COOH	64	12,928	0.0044 ± 0.0003
G4:FITC (1:1)	$-NH_2$	64	14,215	0.0520 ± 0.0004
G4:FITC (1:4)	$-NH_2$	64	14,215	0.2179 ± 0.0020
G4:FITC (1:8)	$-NH_2$	64	14,215	0.4148 ± 0.0090

[a]Reported by the manufacturer, Dendritech, Inc., Midland, MI
[b]Label content is reported as mean ± SD (*n* = 3)

Kitchens et al., *Pharm Res*, (2006)

Cell Viability via WST-1 Assay

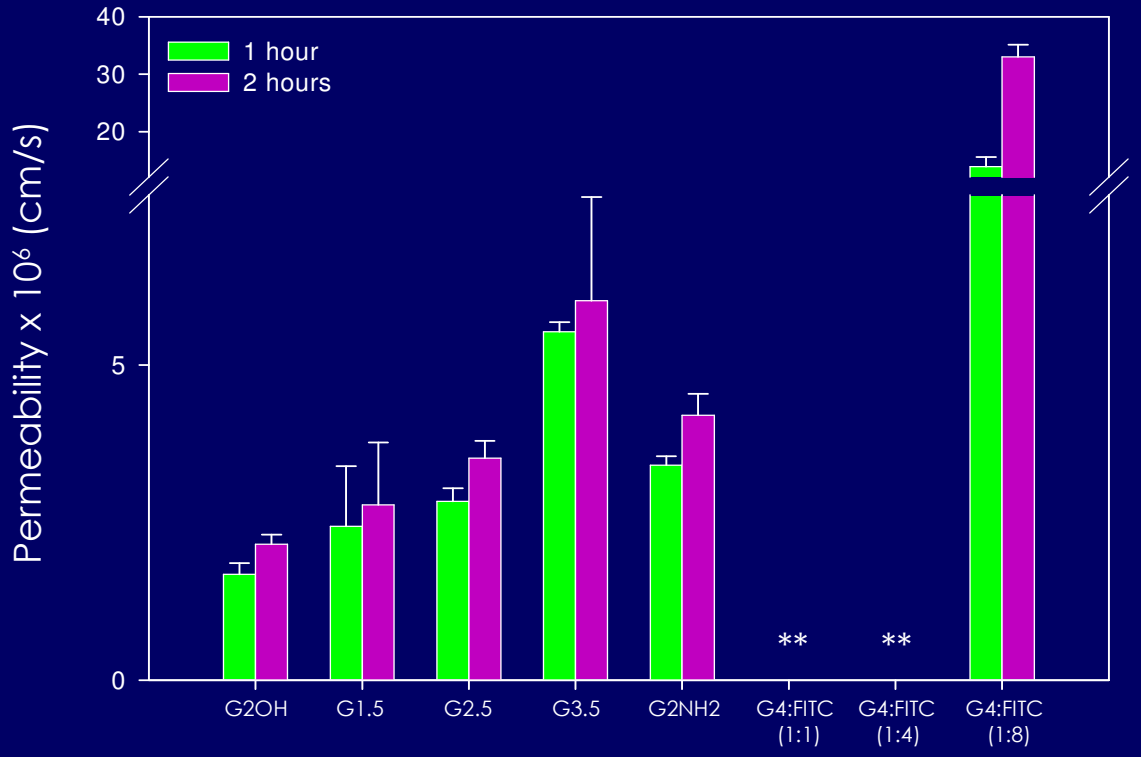


PAMAM Concentration

Mean ± SD (n = 3)
* $P < 0.05$, reduction in viability

Kitchens et al., *Pharm Res*, (2006)

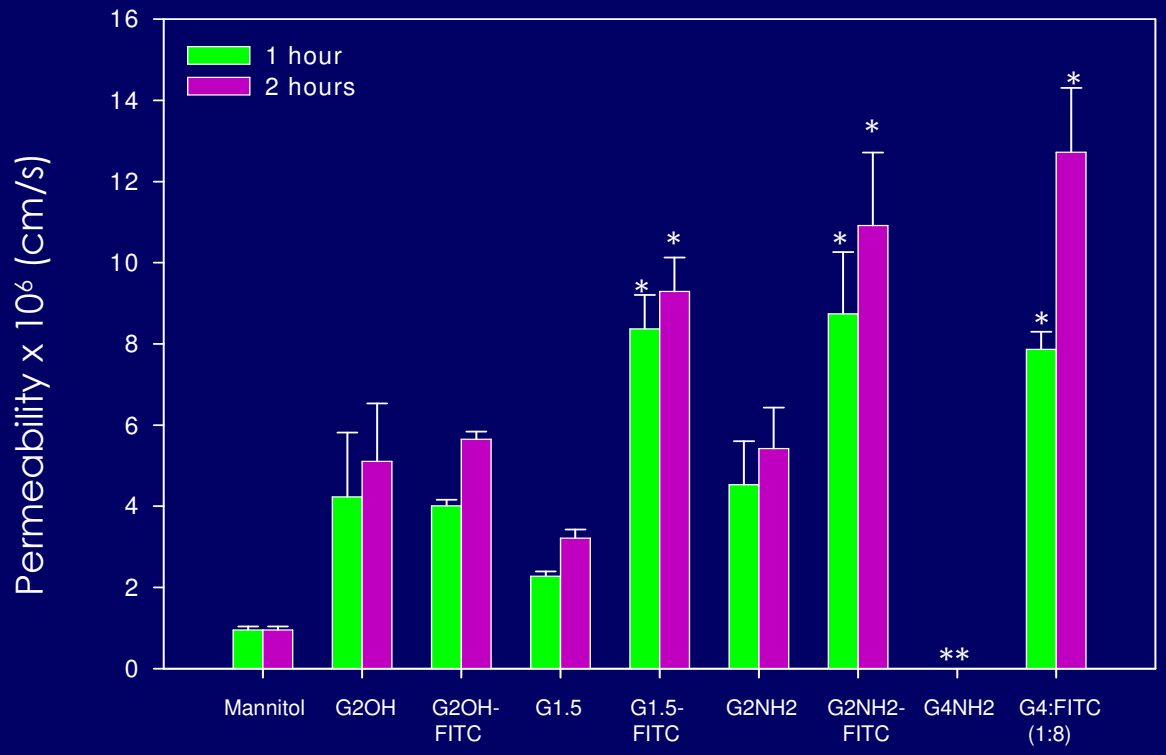
Permeability across Caco-2 Cell Monolayers



Mean ± SEM (*n* = 9)
** Toxic (WST-1 assay)

Kitchens et al., *Pharm Res*, (2006)

Mannitol Permeability in the Presence of PAMAM



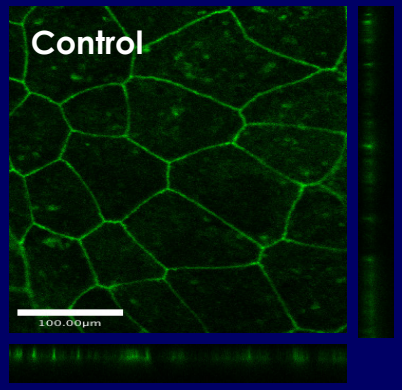
Mean ± SEM (*n* = 9)
** Toxic (WST-1 assay)
* $P < 0.05$, increase in P_{app}

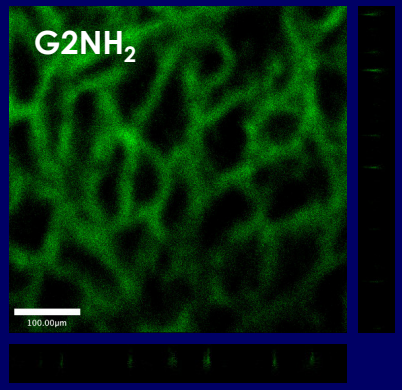
Kitchens et al., *Pharm Res*, (2006)

Influence of PAMAM on Tight Junction Protein Occludin

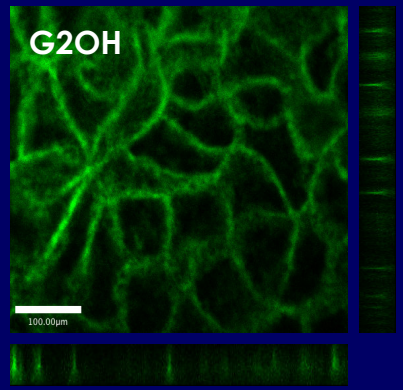
Mean ± SD
(n = 3)
* P < 0.05, increase in signal

Bars = 100 μm



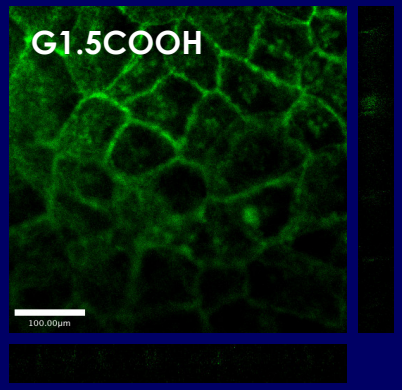


*34.2 ± 13.4%

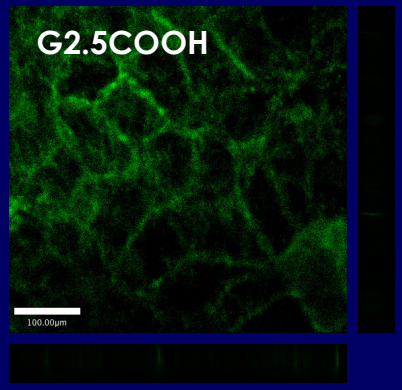


*14.4 ± 4.0%

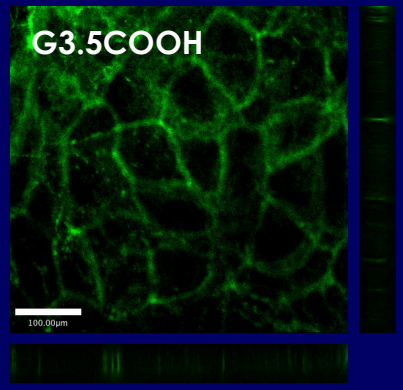
60x oil objective
100 μm pinhole
0.40 μm z-step size



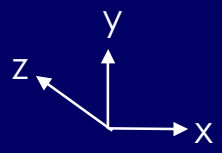
*50.8 ± 8.6%



*56.6 ± 5.9%



*51.9 ± 7.5%

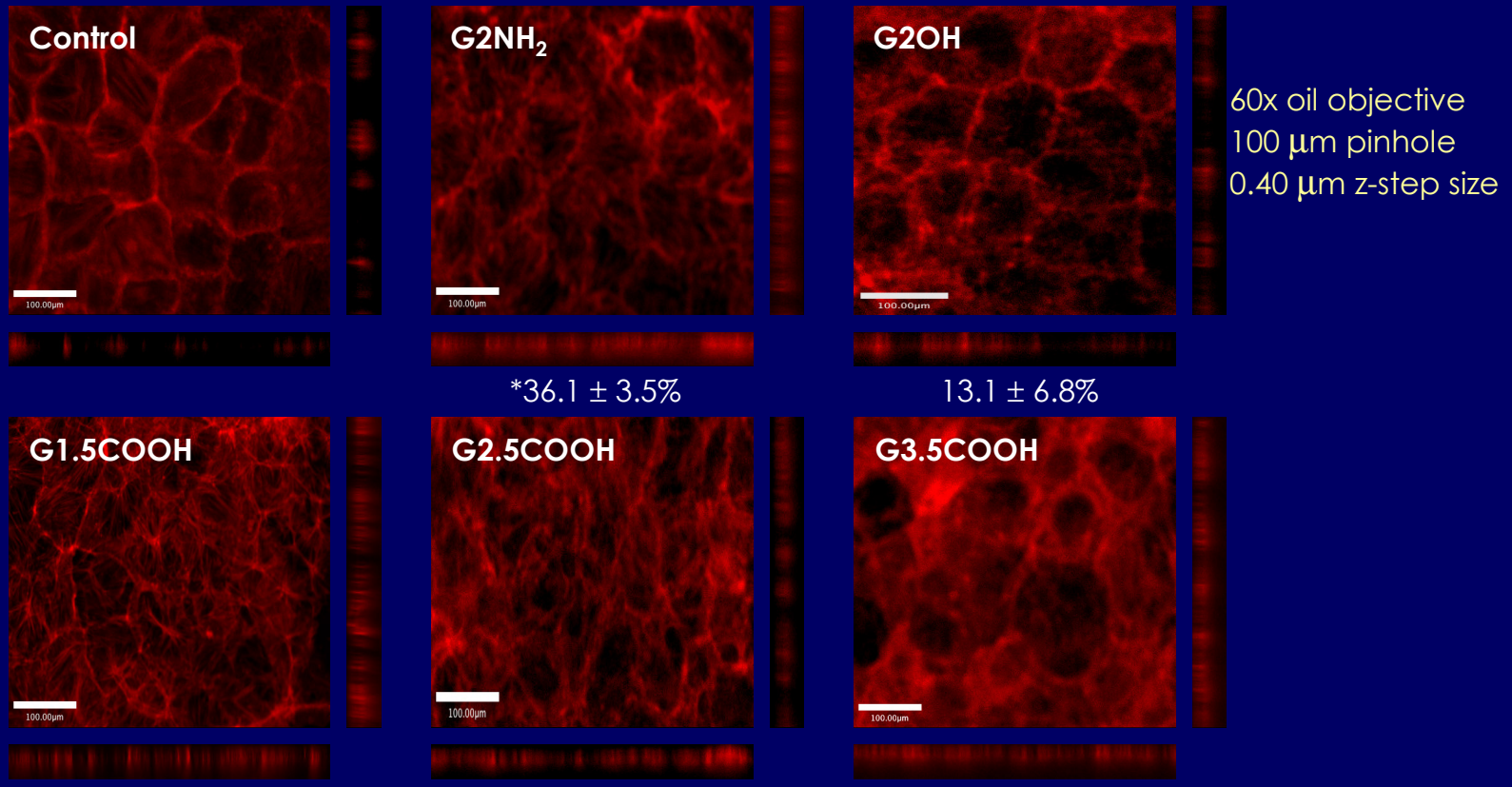


Kitchens et al., *Pharm Res*, (2006)

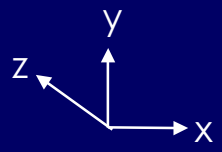
Influence of PAMAM on Tight Junction Protein Actin

Mean ± SD
(n = 3)
* P < 0.05, increase in signal

Bars = 100 μm

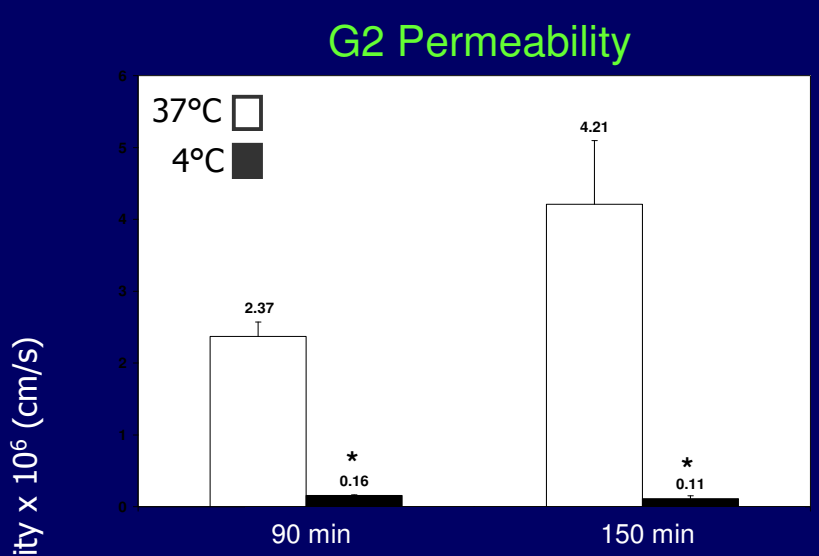


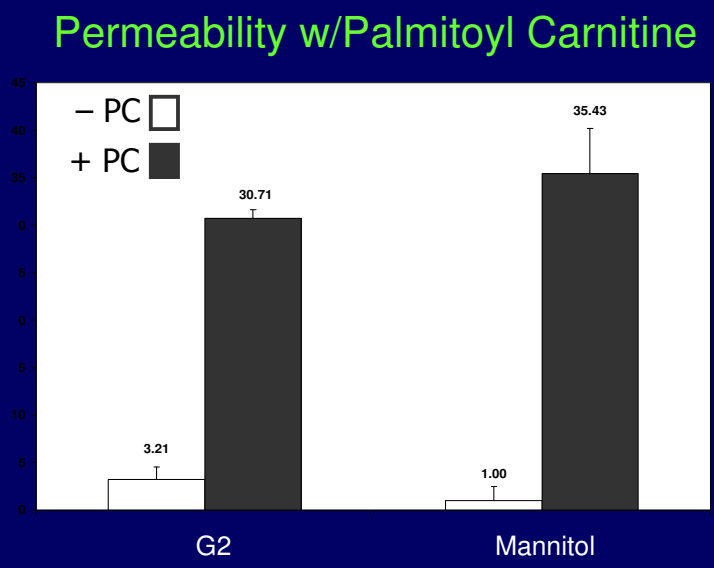
60x oil objective
100 μm pinhole
0.40 μm z-step size

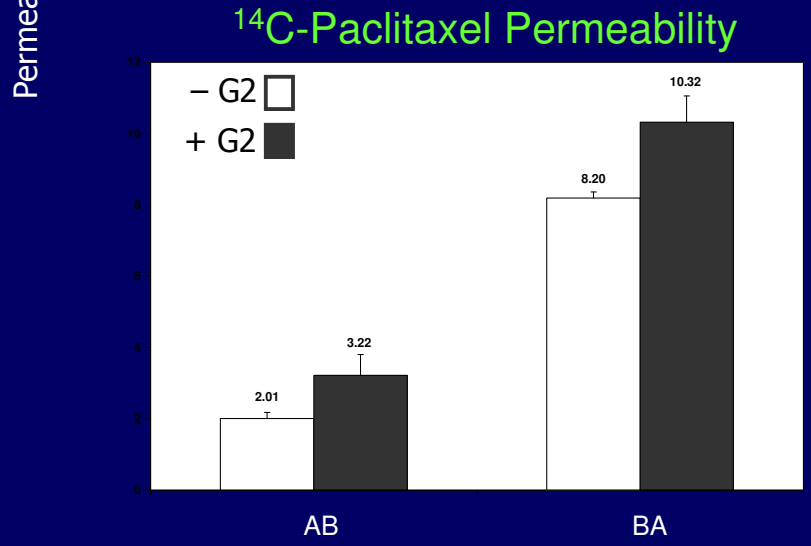


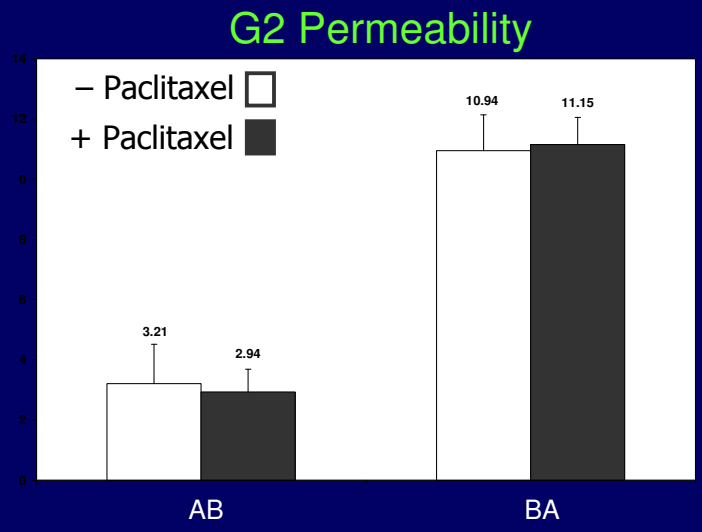
Kitchens et al., *Pharm Res*, (2006)

Suggested Transport Mechanisms





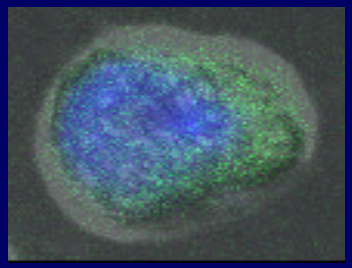




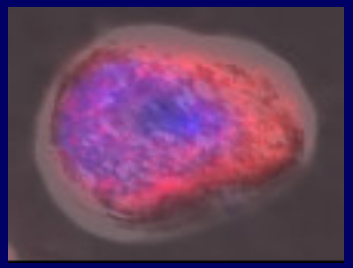
El-Sayed et al., *Int J Pharm*, (2003)

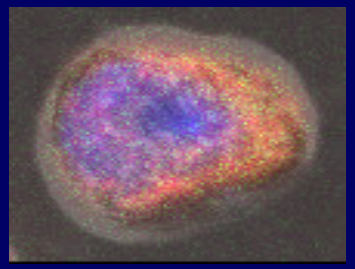
Experimental Methodology: Transport Mechanisms

G2-FITC &
G1.5-FITC



1° - Antibodies against endocytosis markers
2 ° - Alexa Fluor® 568 goat anti-mouse IgG
LysoTracker Red





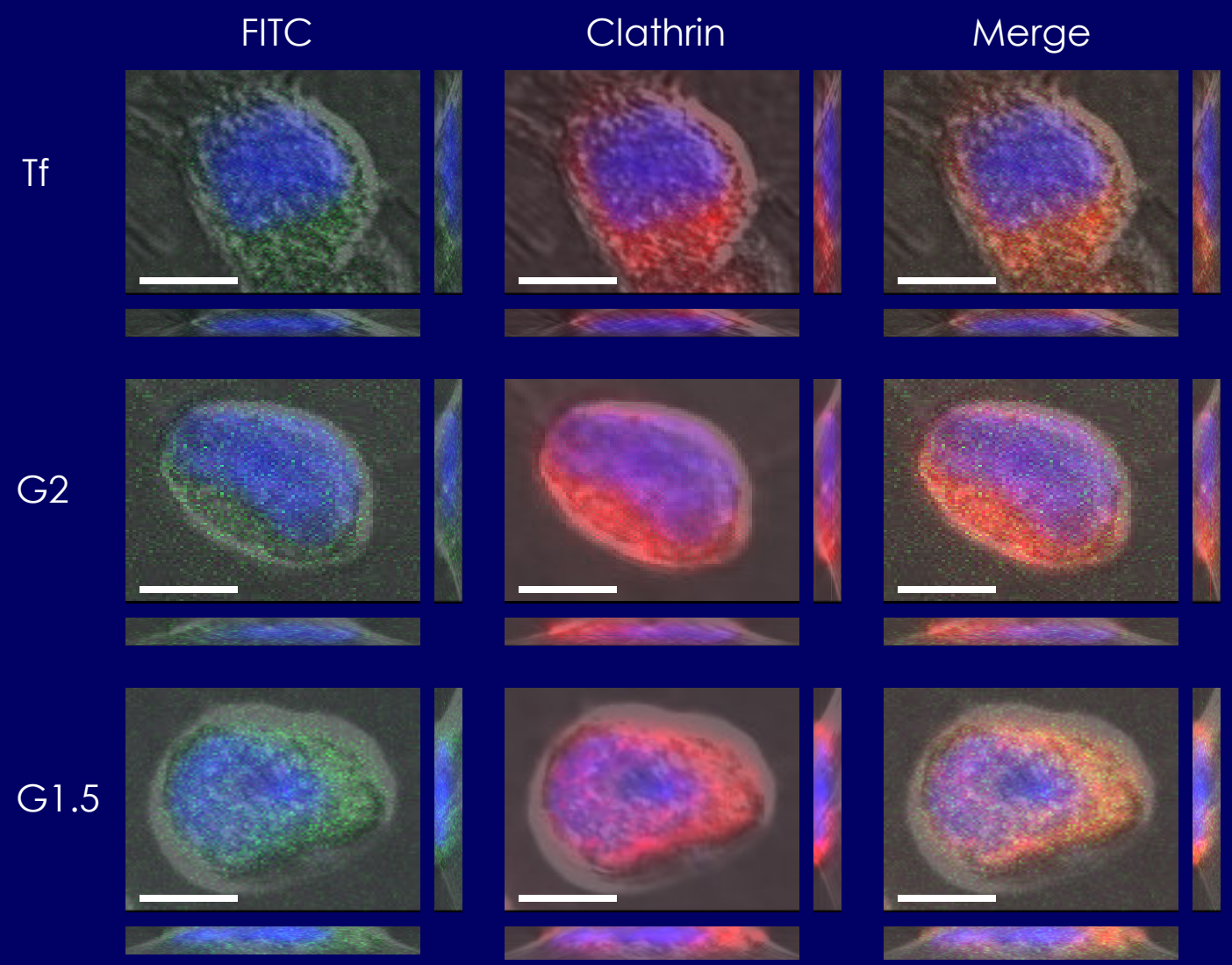
Merge/Colocalization

Colocalization Coefficient

$$*M_x = \frac{\sum_i x_{i,\,coloc}}{\sum_i x_i}$$

*Manders et al., *J Microsc,* (1993)

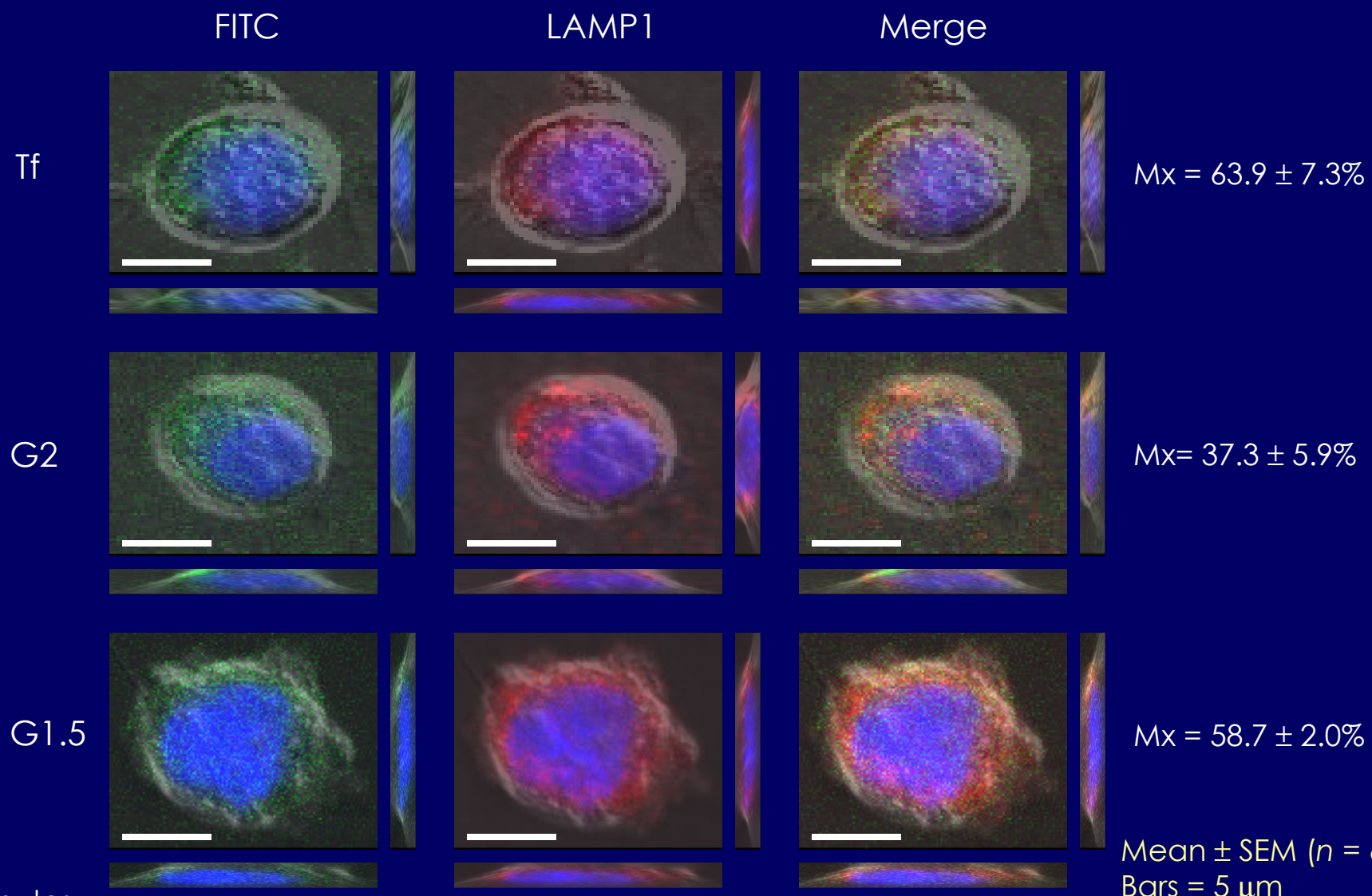
Colocalization with Clathrin



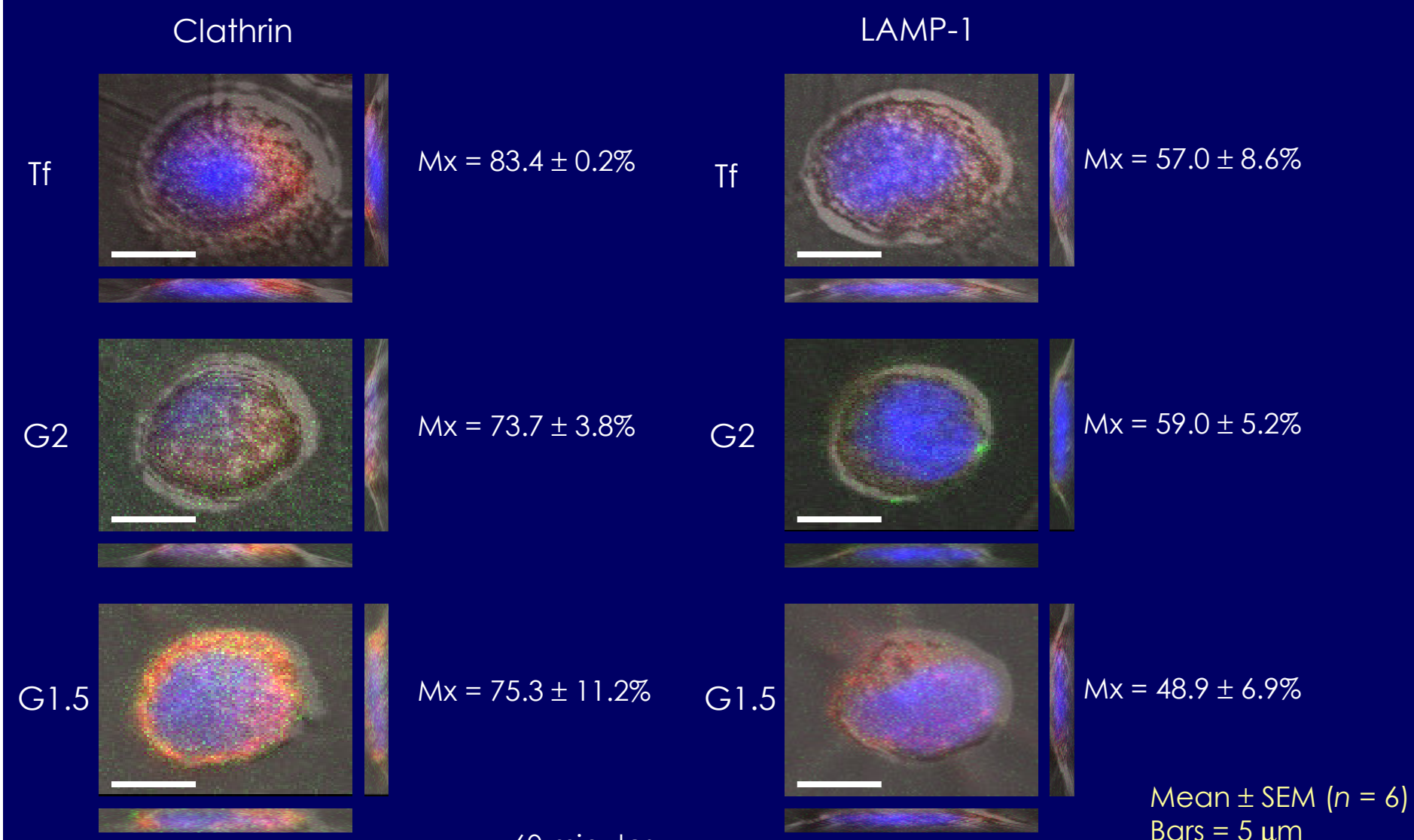
Mean ± SEM (*n* = 6)
Bars = 5 µm

20 minutes

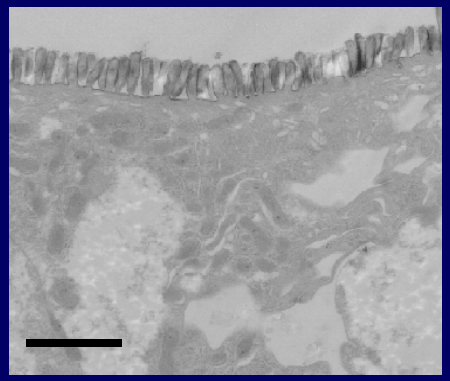
Colocalization with LAMP-1



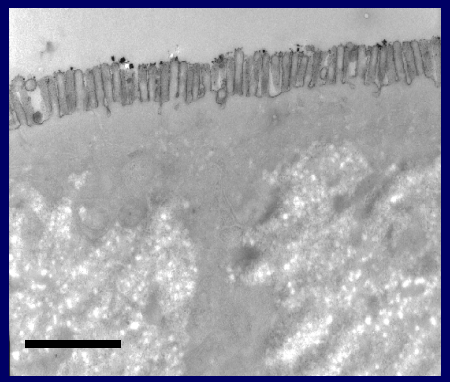
Colocalization: Clathrin & LAMP-1



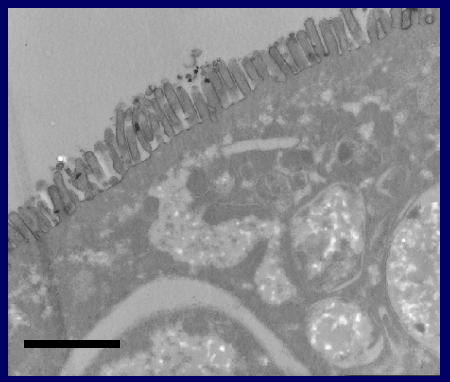
PAMAM Effect on Microvilli



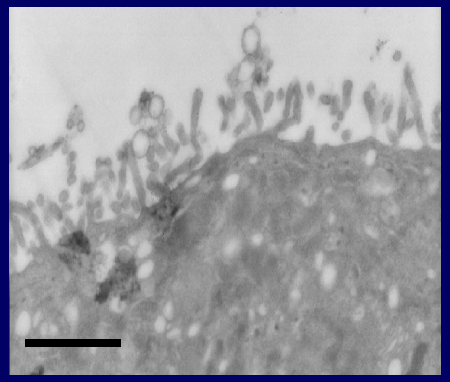
Control



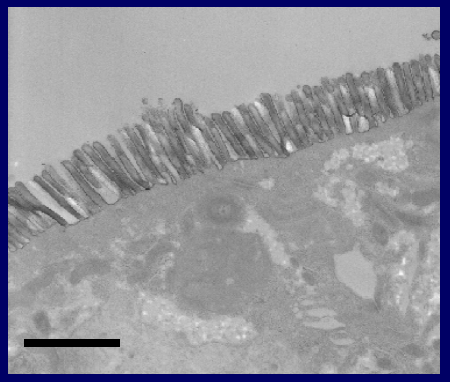
$G2NH_2$ (1 mM)



G1.5COOH (1 mM)



$G4NH_2$ (1 mM)

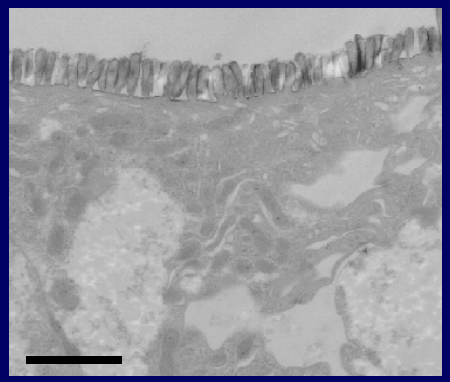


G3.5COOH (1mM)

12,500x Magnification
Bars = 1 μm

Generation-dependent effect

$G4NH_2$ Effect on Microvilli



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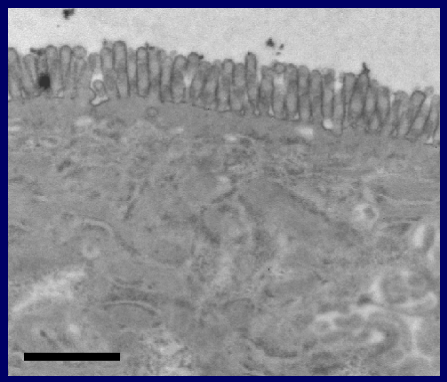
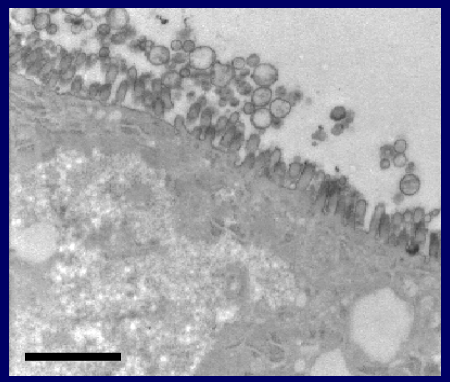
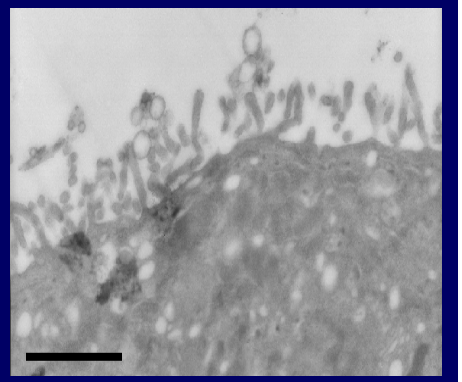


$G4NH_2$ (0.01mM)



$G4NH_2$ (0.1 mM)



$G4NH_2$ (1 mM)

12,500x Magnification
Bars = 1 μm

Concentration-dependent effect

Conclusions

- Cationic and large generation dendrimers had greater permeability than anionic and neutral
- Hydrophobic "drug" loading reduced toxicity and enhanced permeability
- PAMAM dendrimers modulate tight junctions
- PAMAM dendrimers colocalize with endocytosis markers
- Cationic dendrimers cause cytotoxicity by compromising microvilli integrity

Design dendrimers for optimized oral drug delivery applications

Acknowledgements

- Hamid Ghandehari, Ph.D.
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- UMB Pharmaceutical Sciences

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