Advancing Nephrology Through 2-Photon Microscopy

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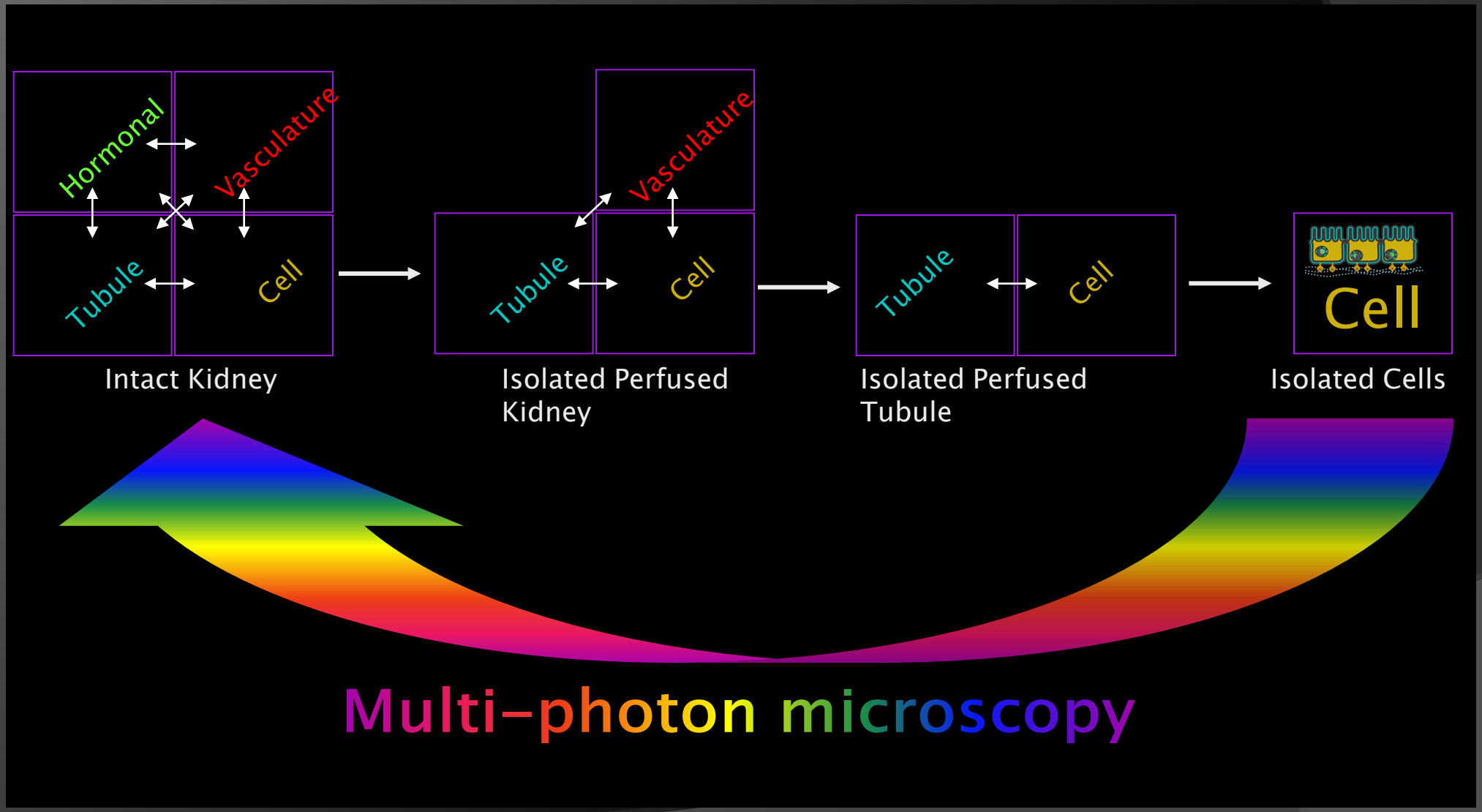
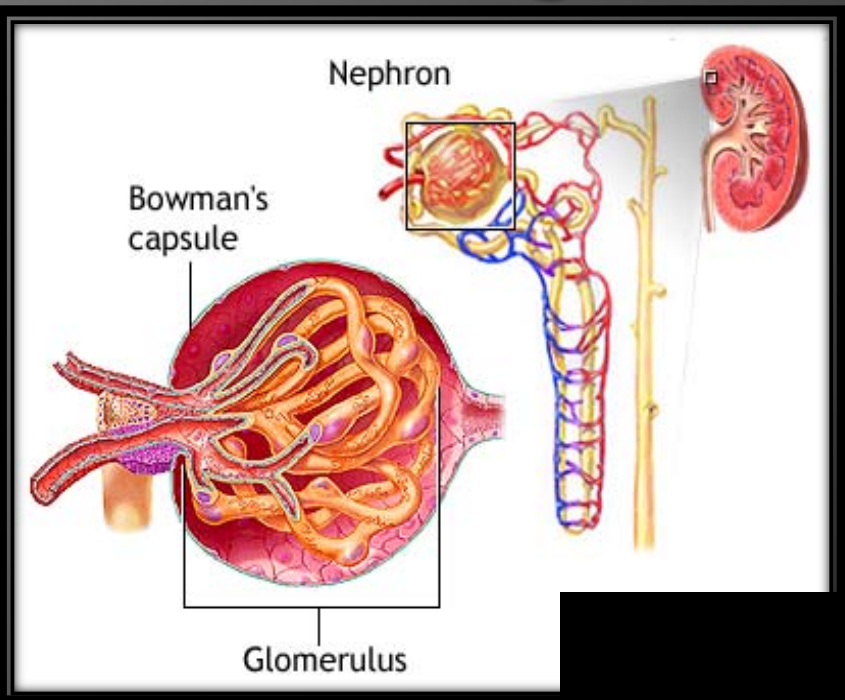
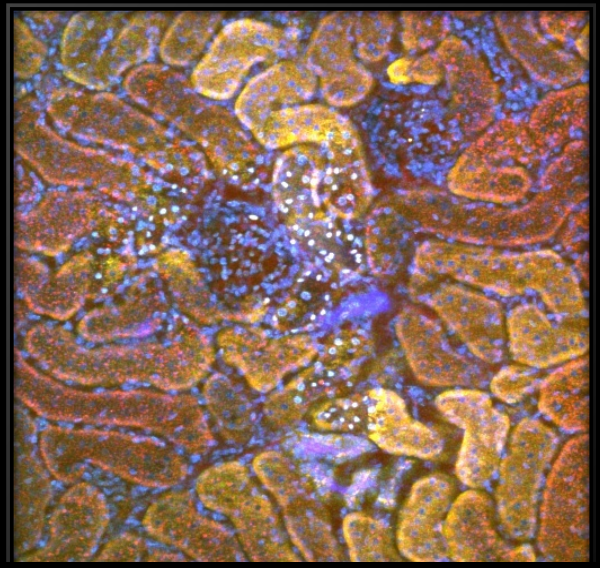
Reversing Reductionism

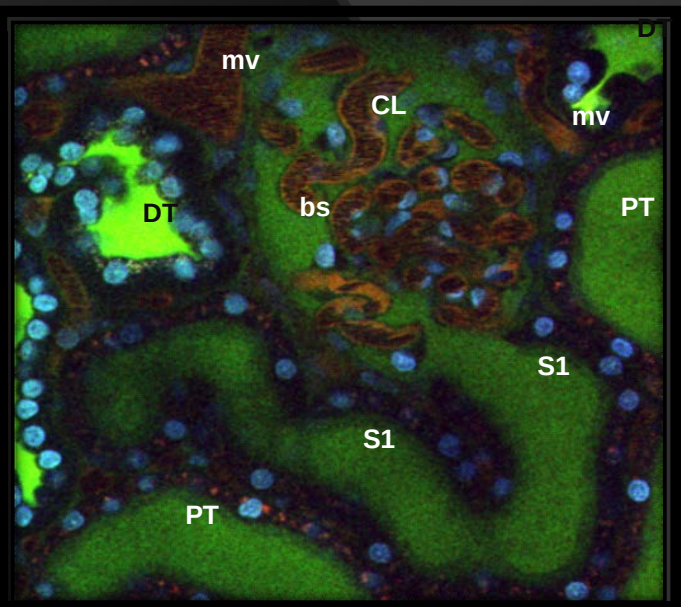


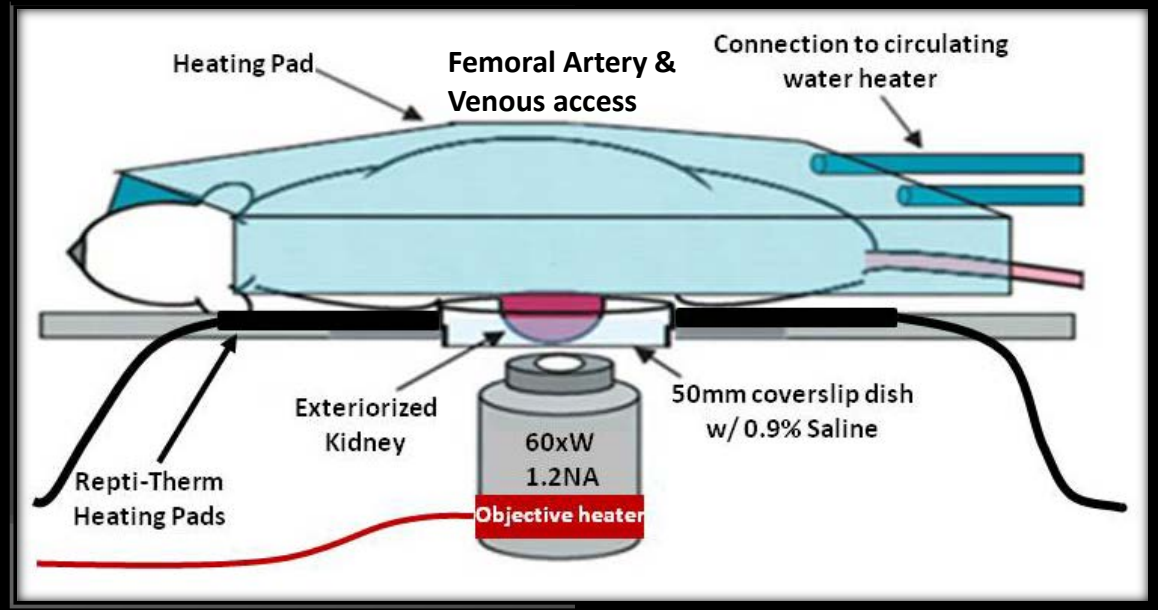
Sandoval et.al. AJP 2005

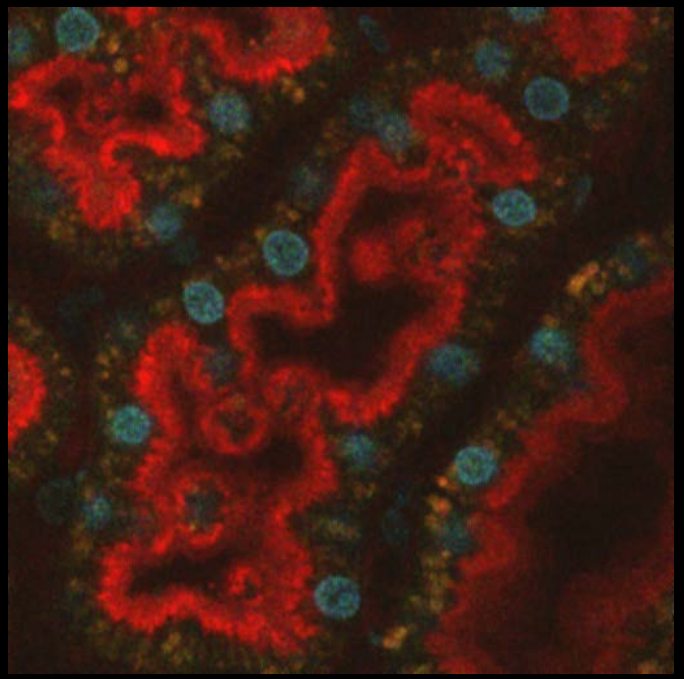
Visualizing Glomerular & Nephron Function



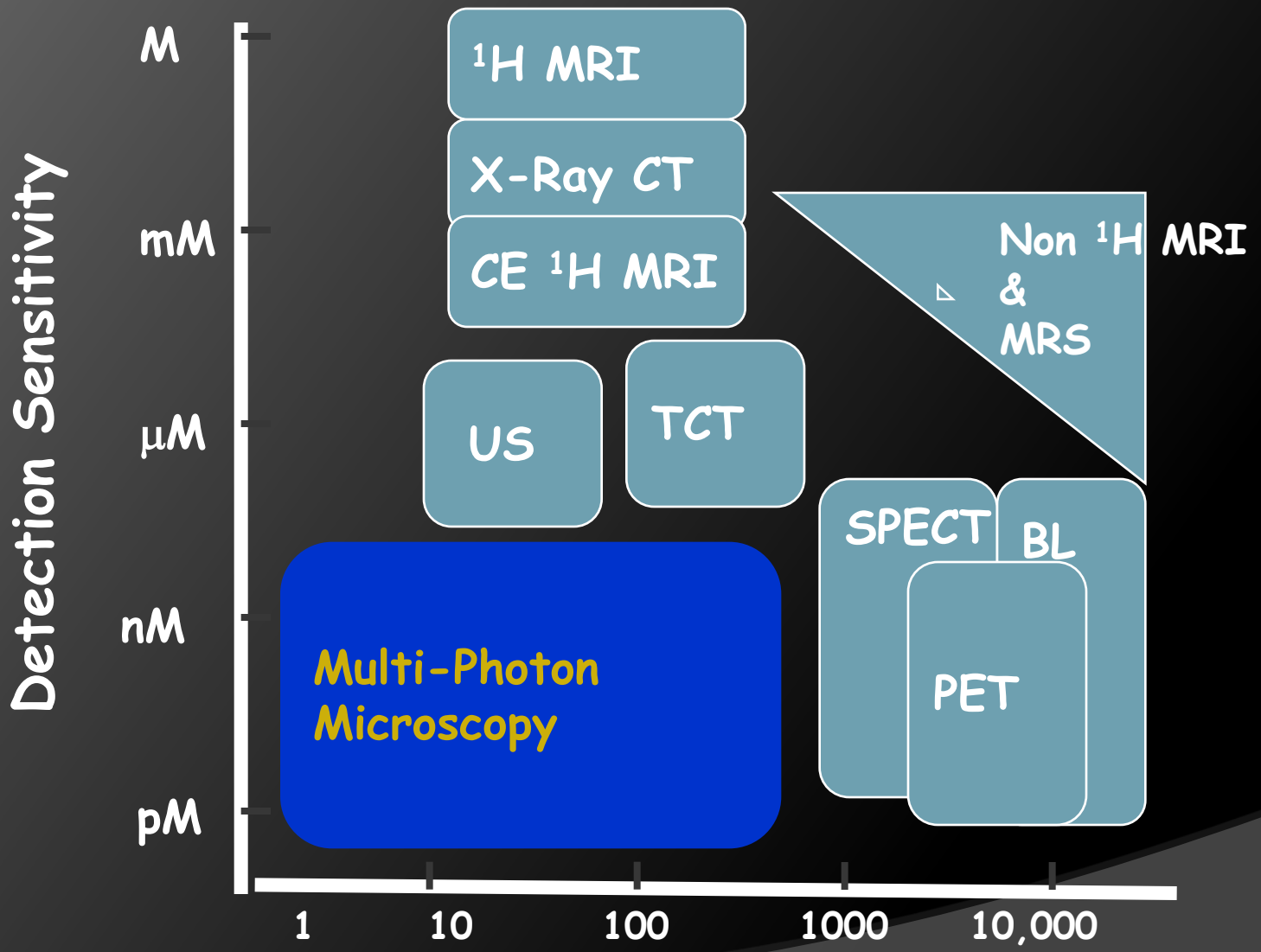




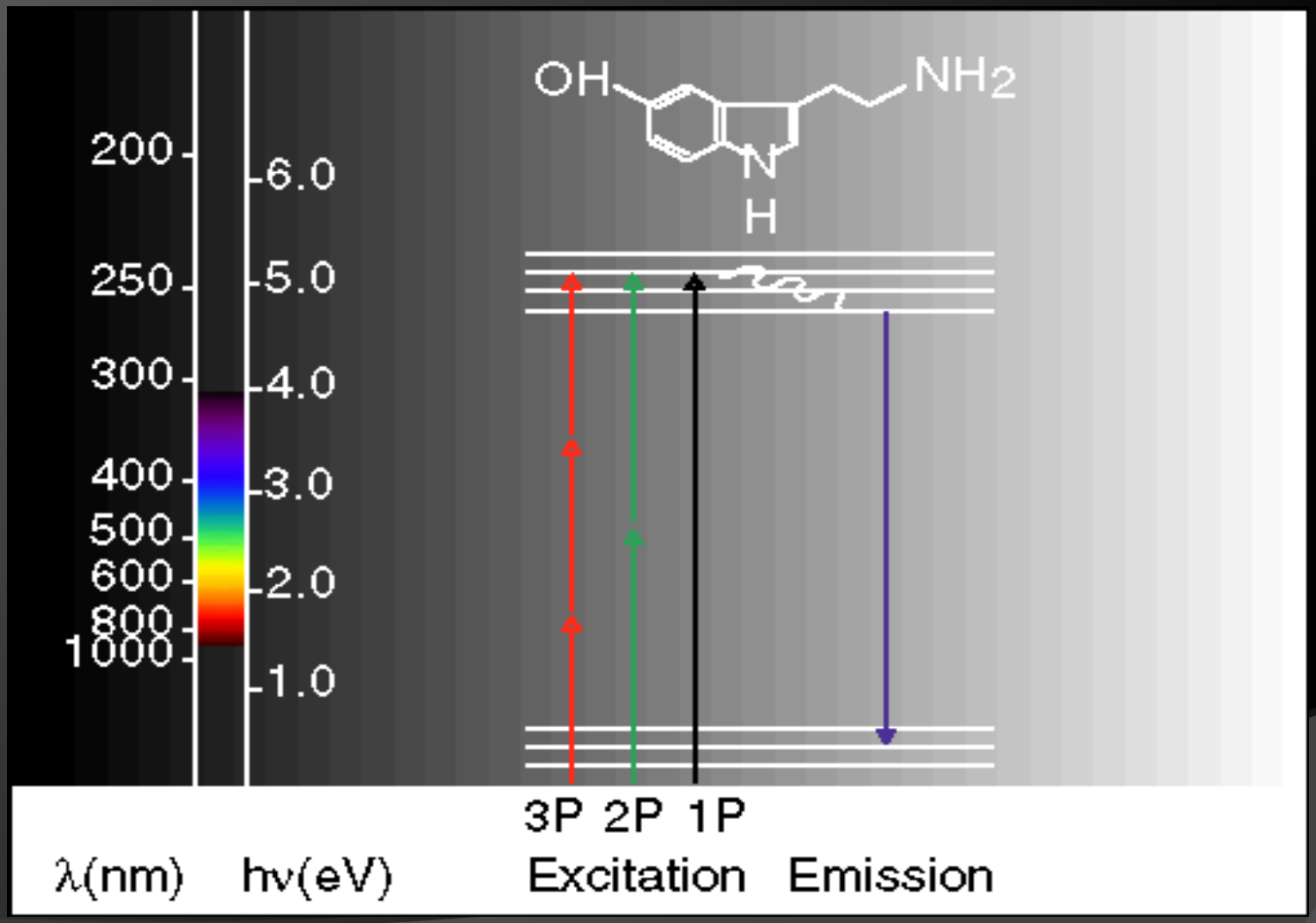




Intra-Vital Imaging Sensitivity vs Resolution



TWO-PHOTON MICROSCOPY PRINCIPLE:



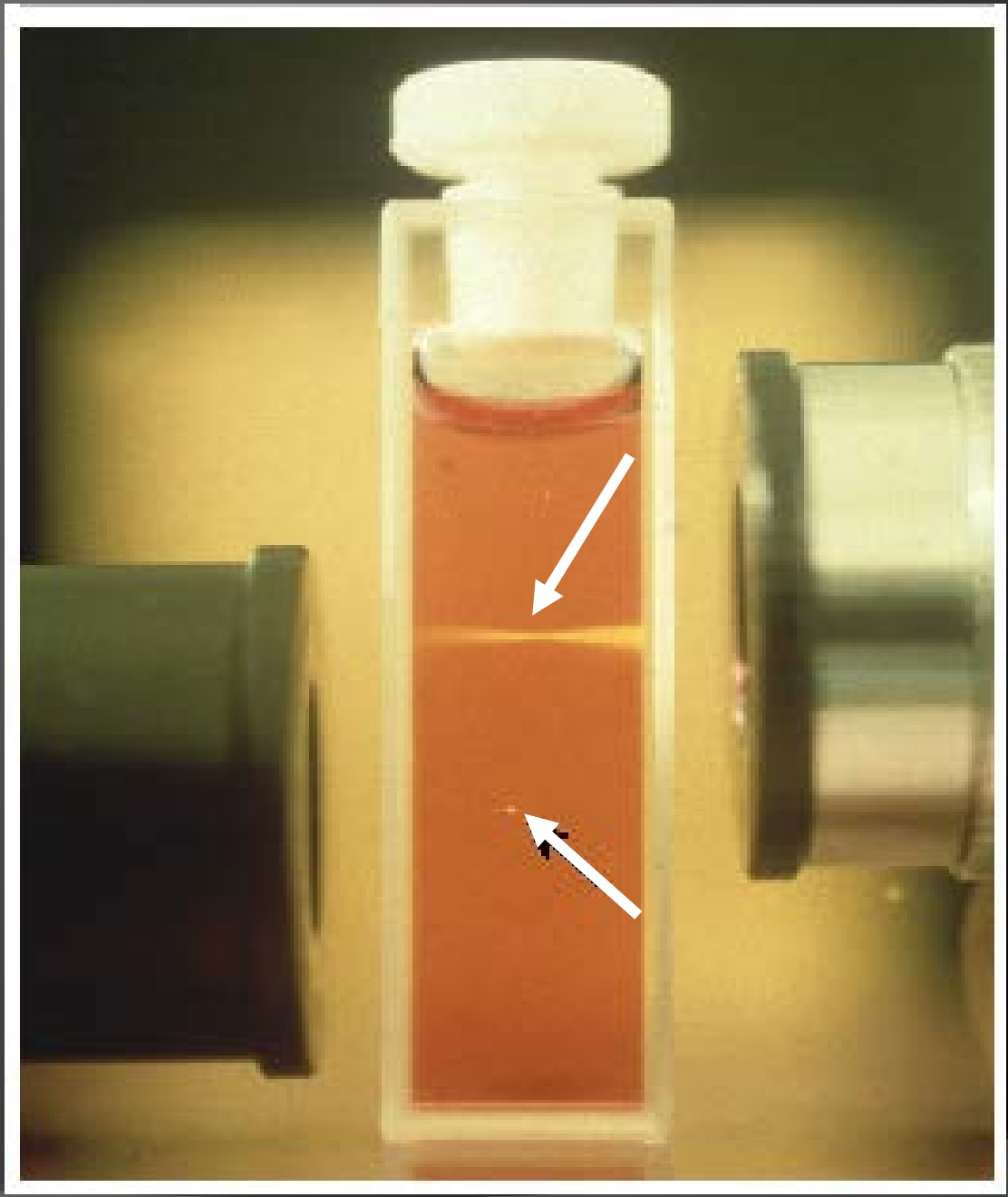
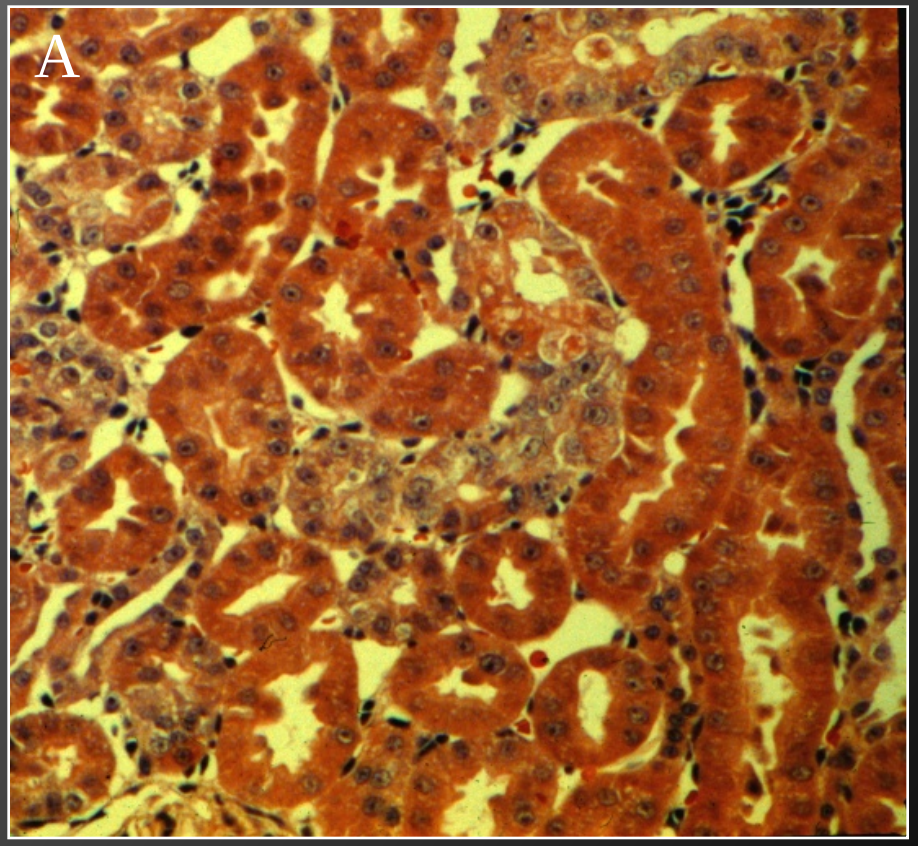


Figure courtesy of Brad Amos, MRC Laboratory

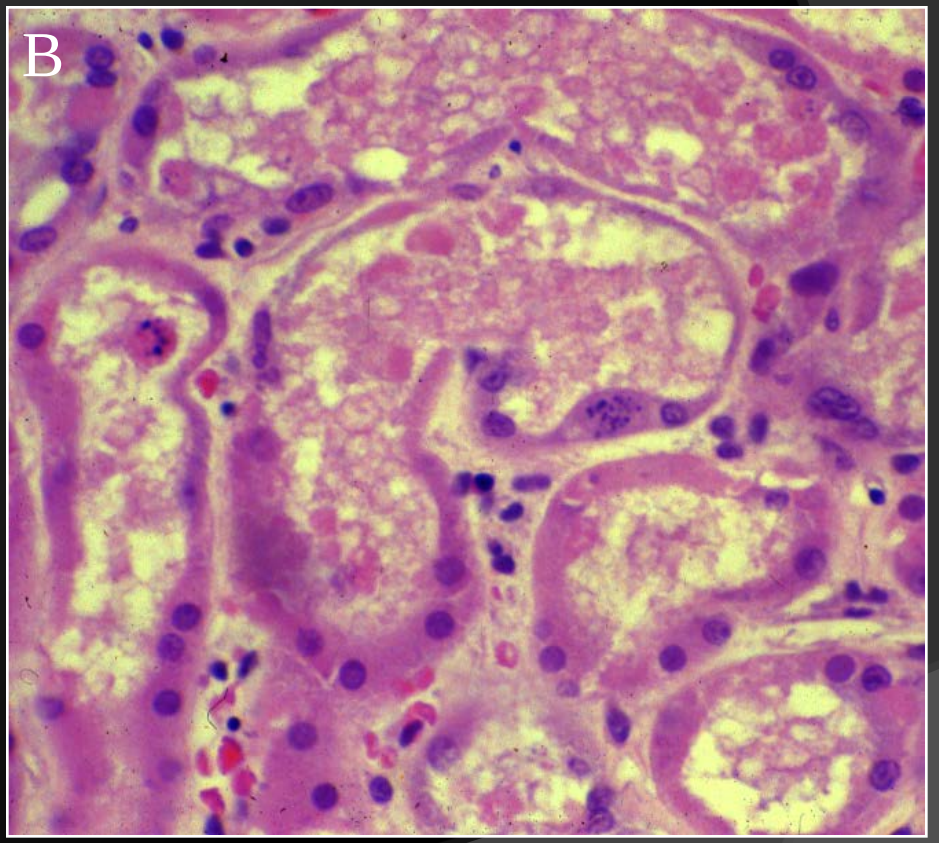
Volume of fluorescence excitation –

Confocal versus 2-photon microscopy

Human Renal Ischemia



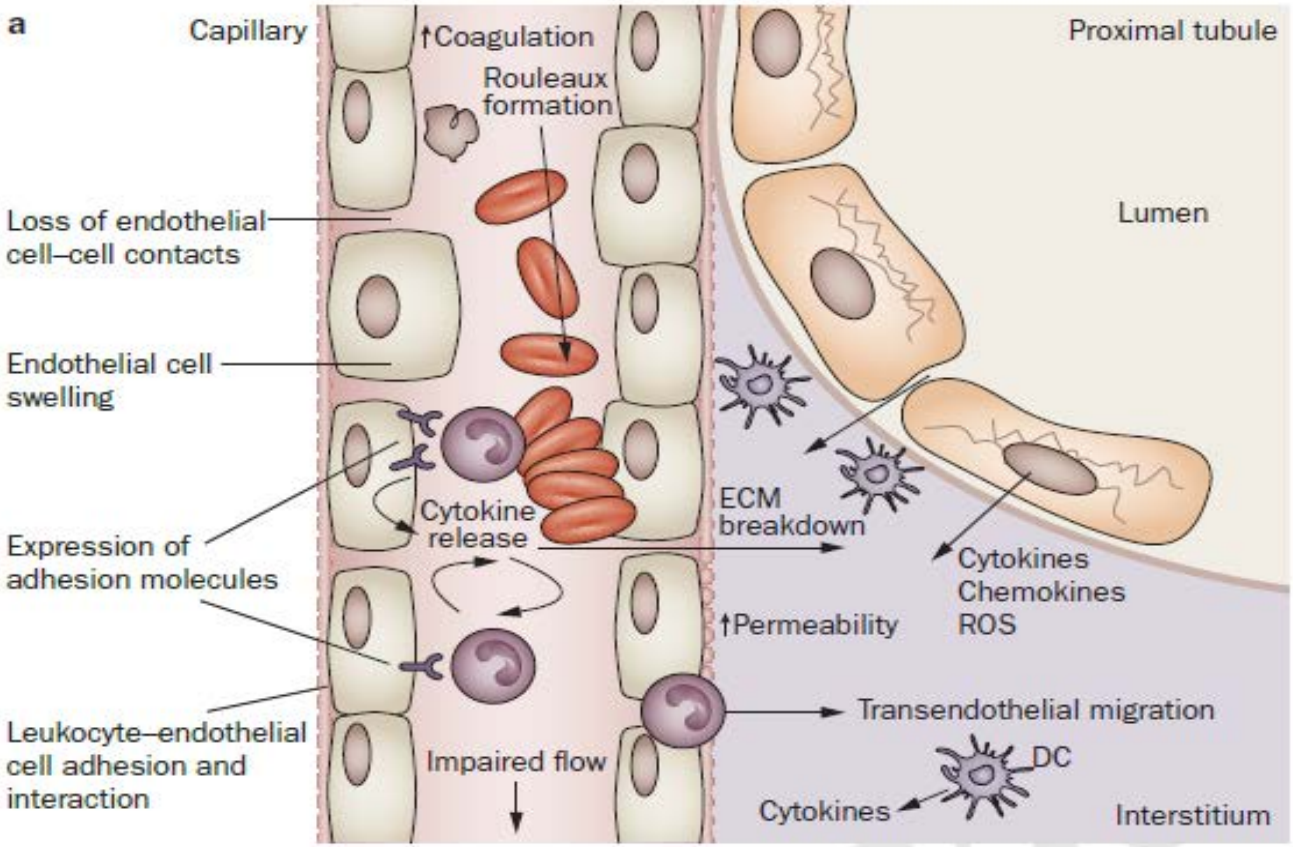
Control

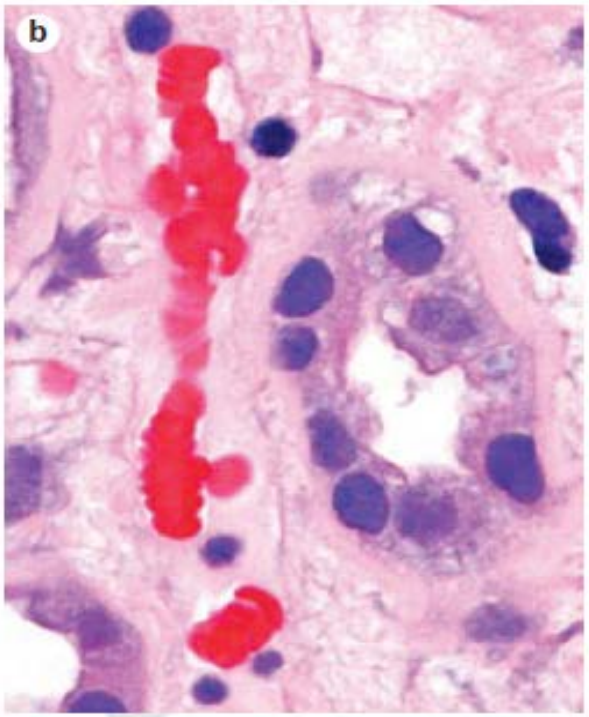


Ischemic

Available Clinical Data Insufficient to Understand the Disease !

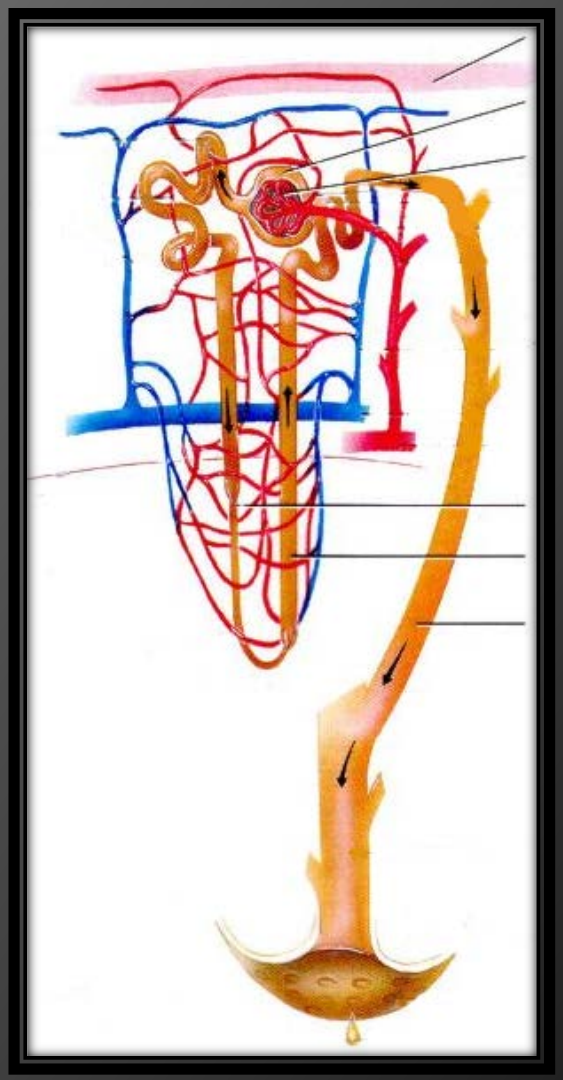
Endothelial Pathophysiologyic Events in AKI

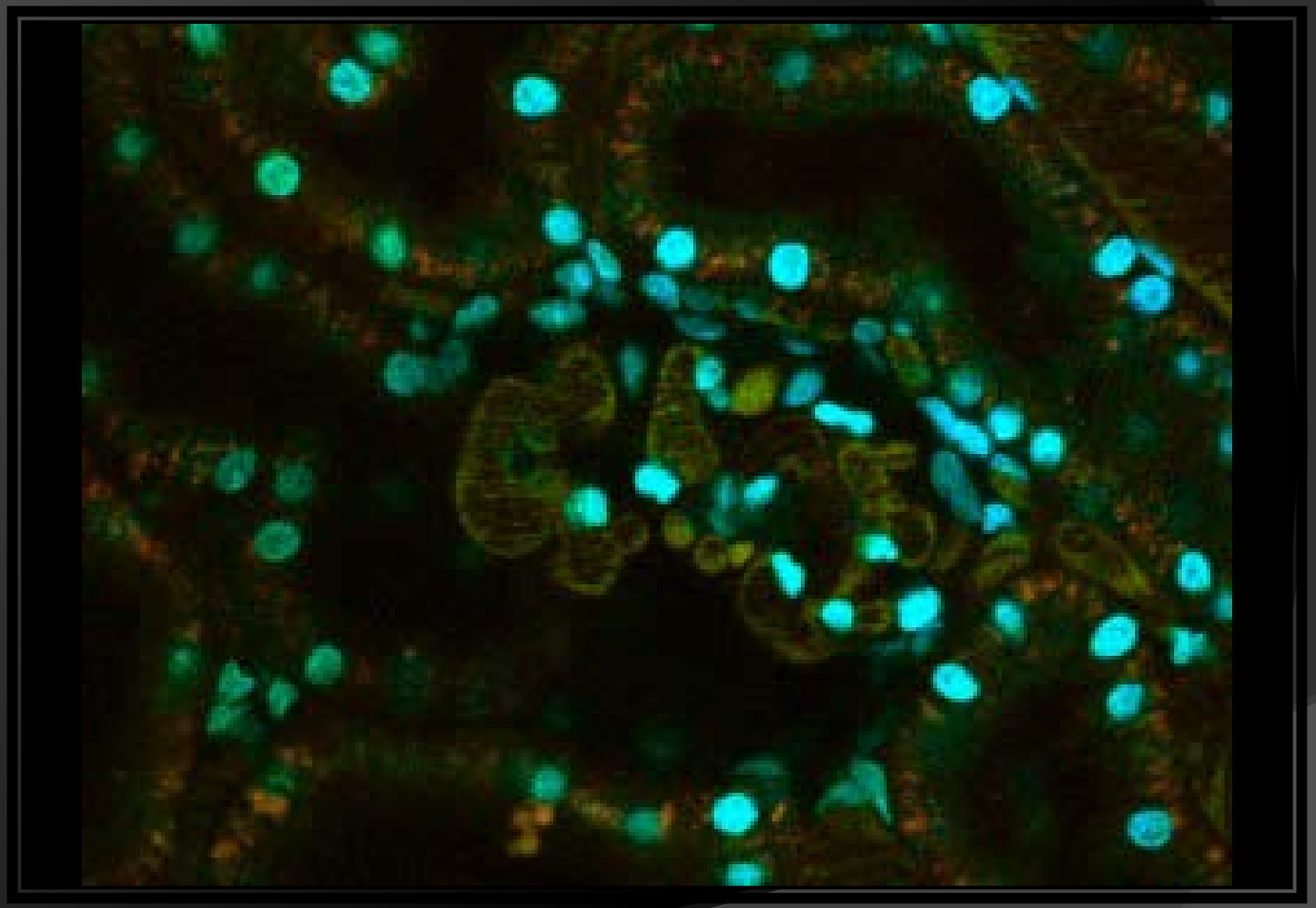




Sharfuddin and Molitoris Nature Neph Reviews 2011

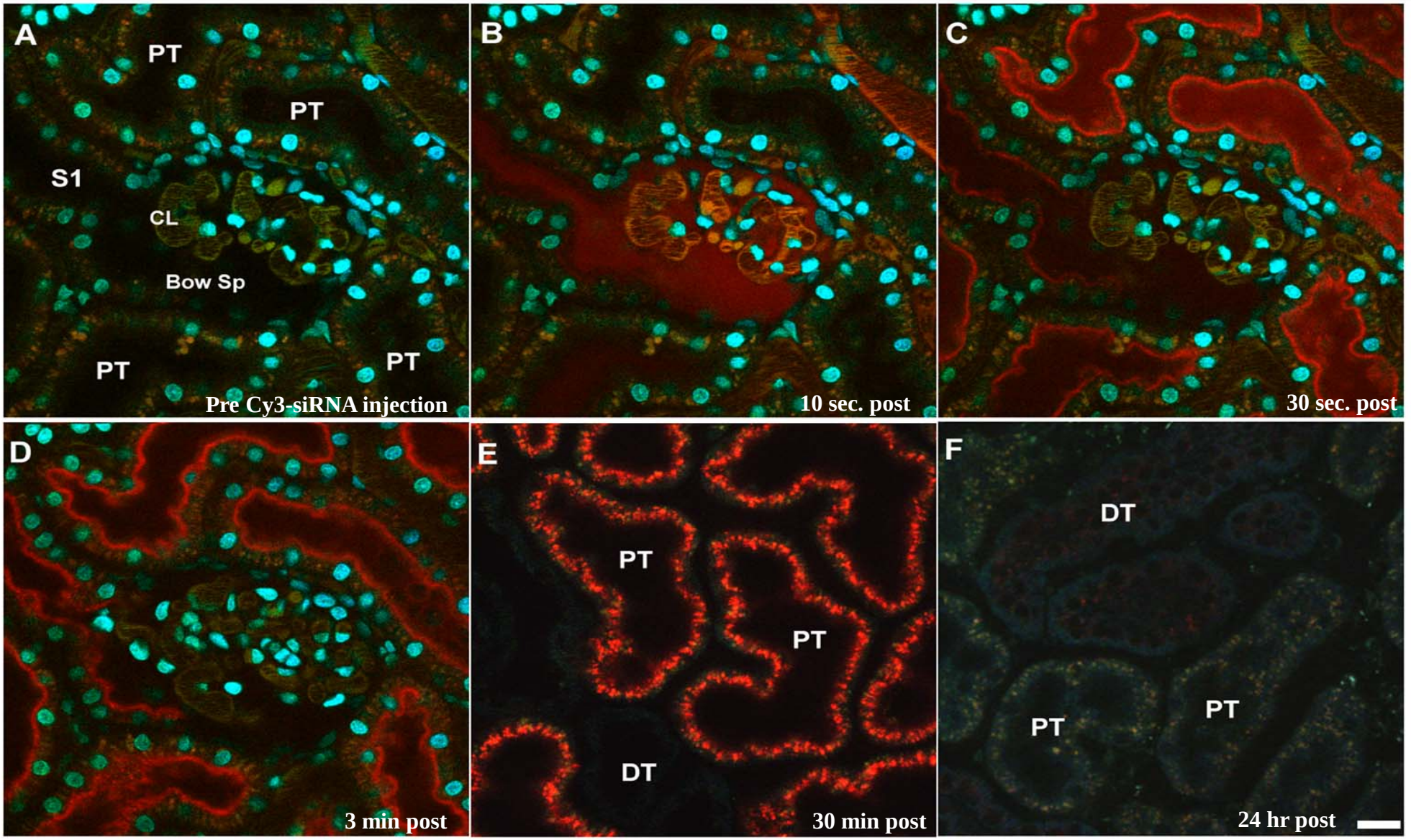
Cy3-siRNA Filtration and Reabsorption by PTCs

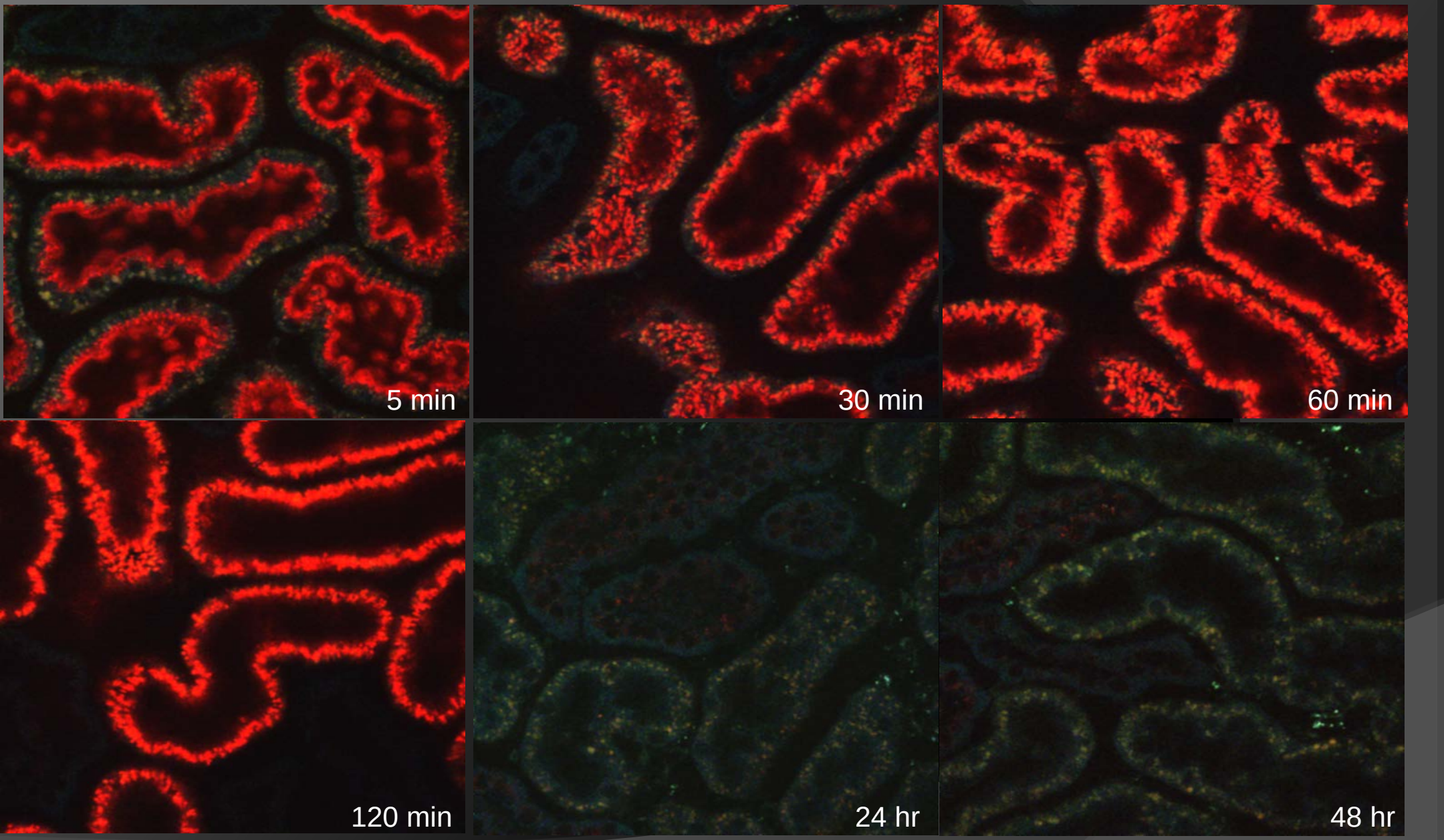




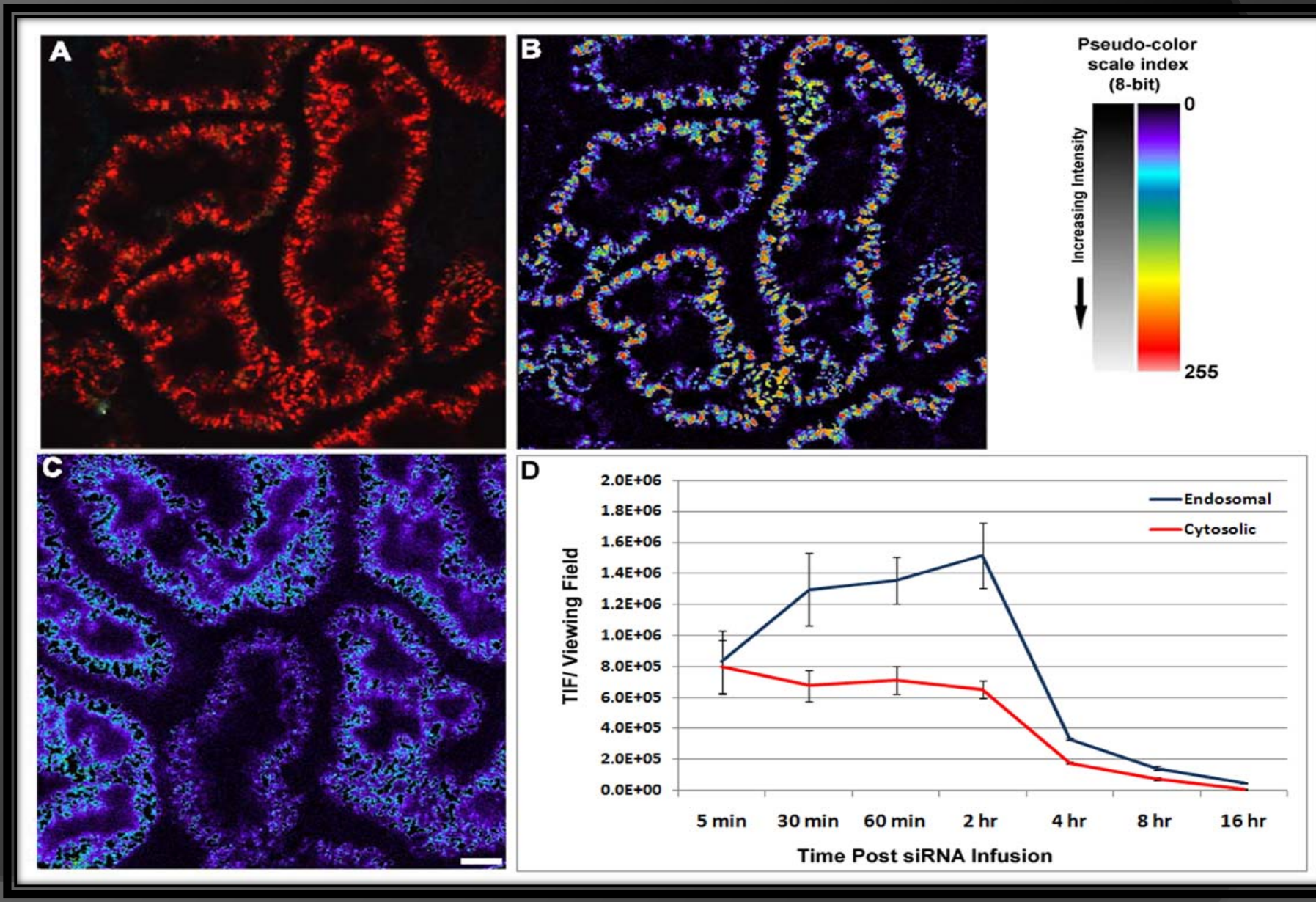
Molitoris et.al. JASN 2009

PTC Uptake and Metabolism of Cy3-siRNA



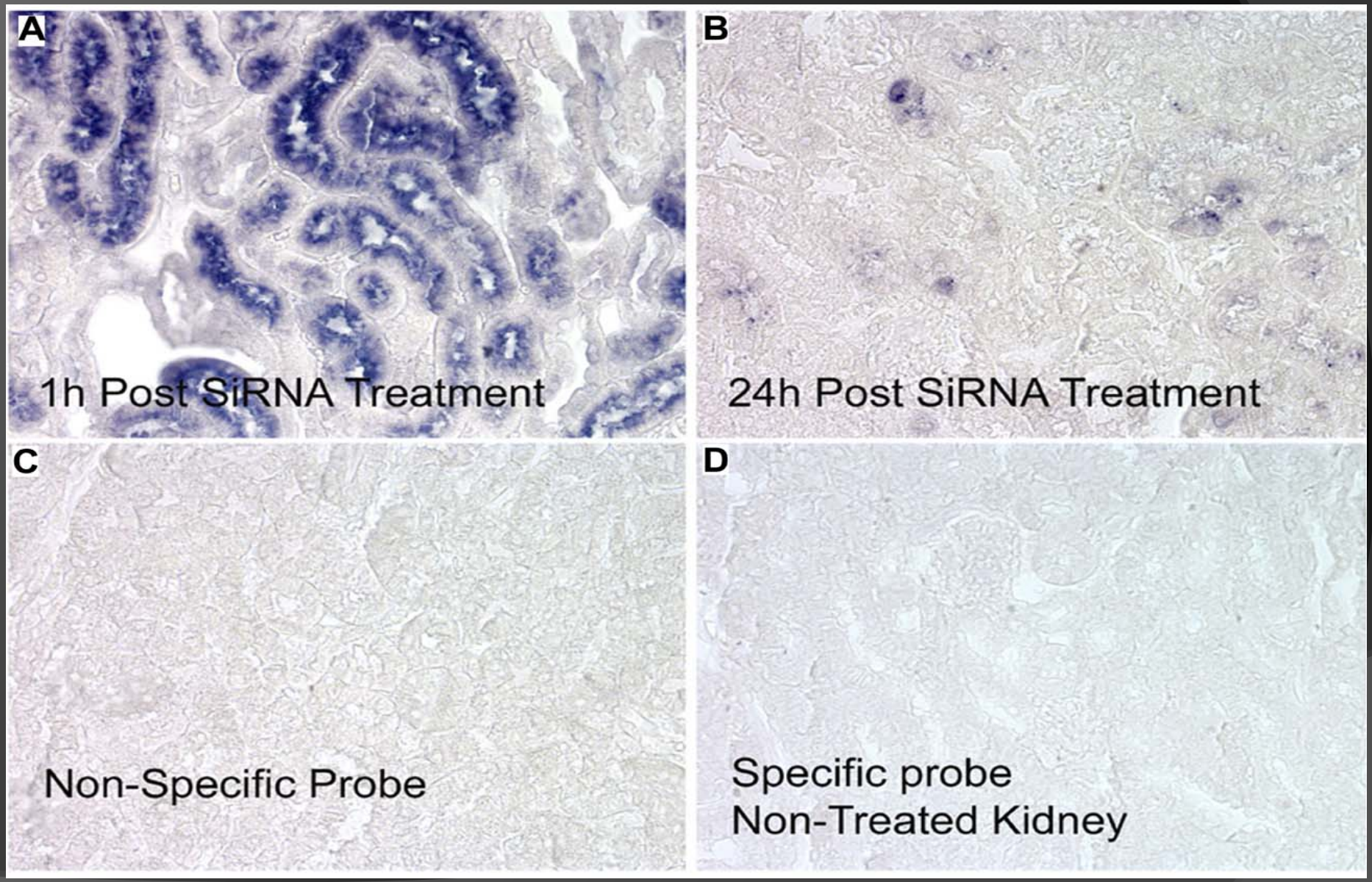

5 min
30 min
60 min
120 min
24 hr
48 hr

Quantifying Vesicular vs Cytosolic Cy3-siRNA in PTCs



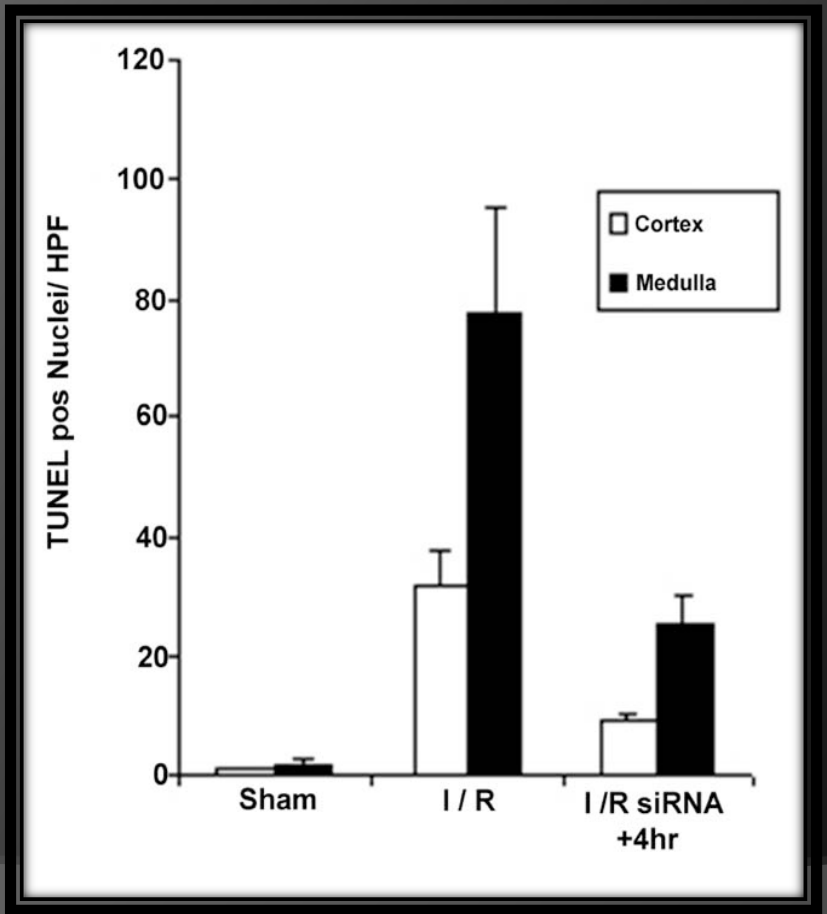
Molitoris et. al. JASN 2009

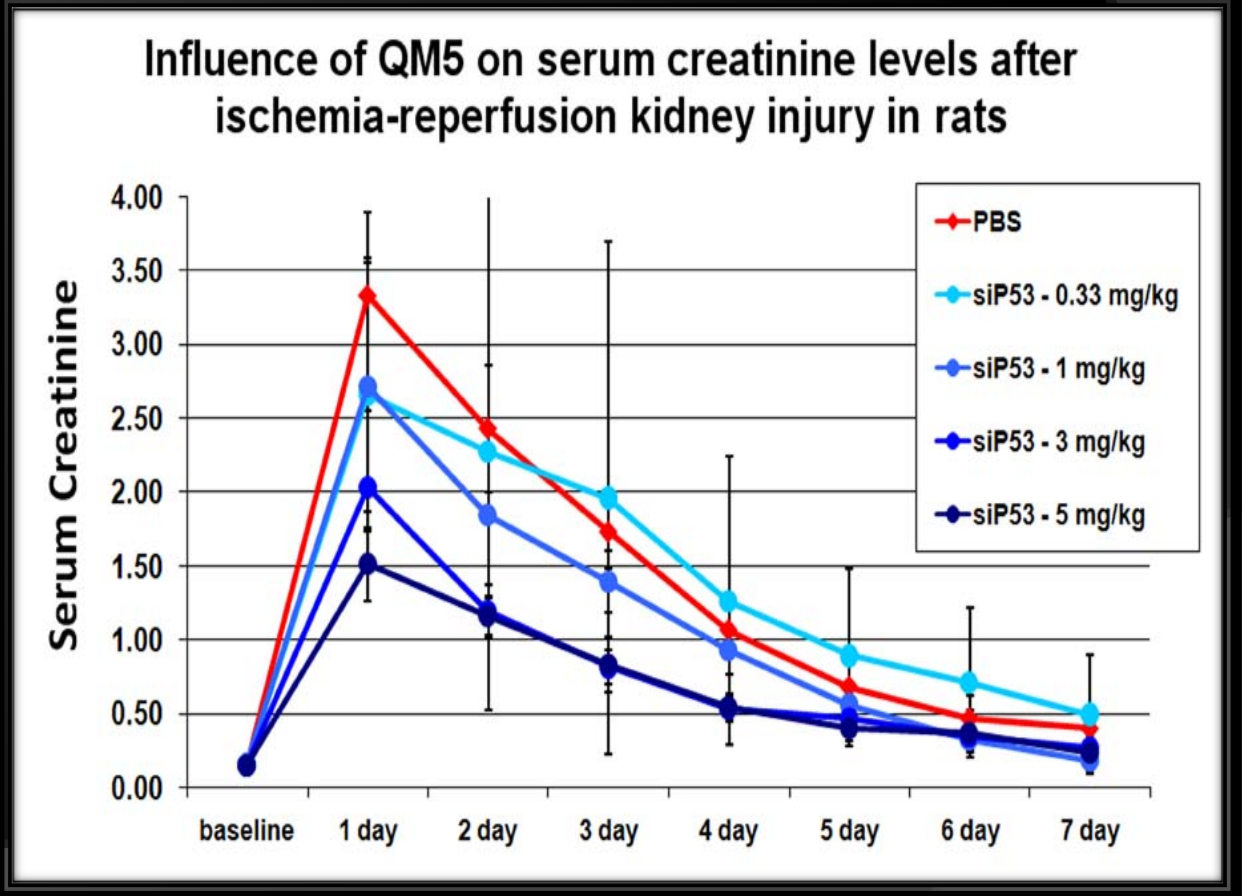
Rapid Metabolism of siRNA in PTC by In situ Hybridization



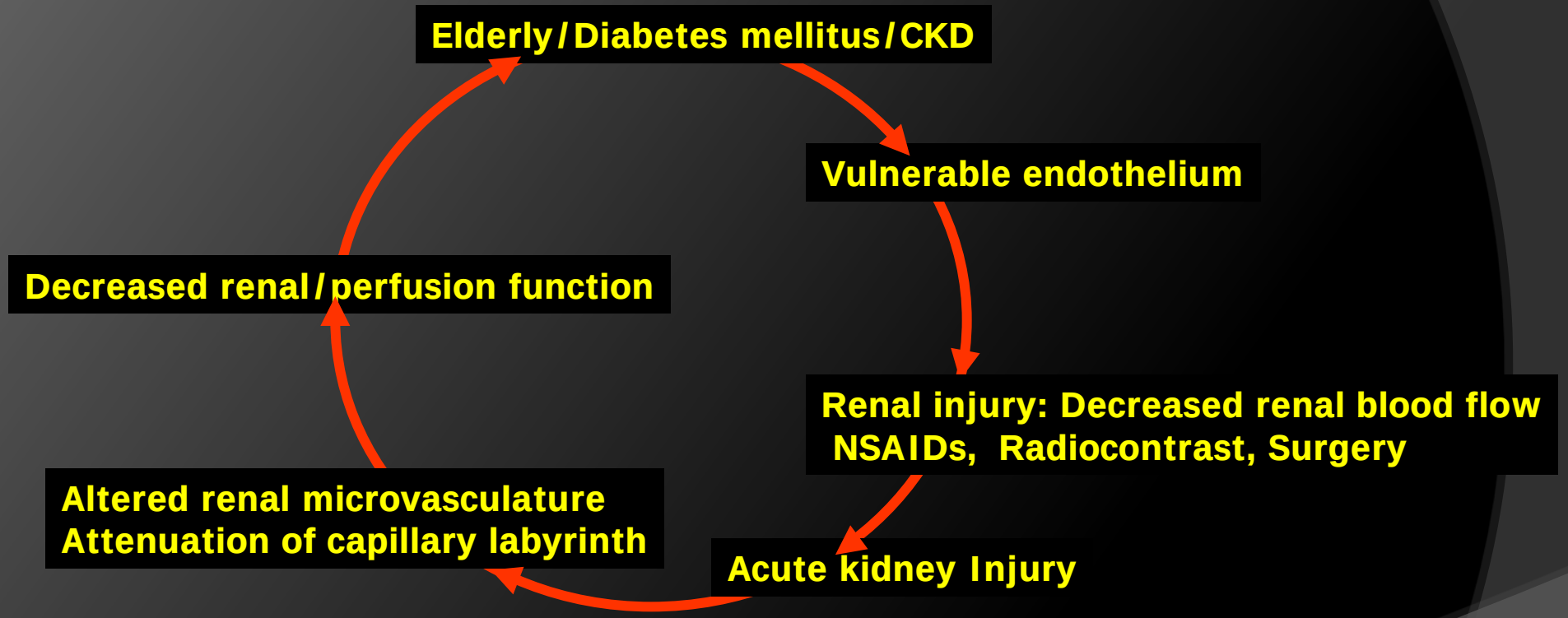
Molitoris et.al JASN 2009

Effect of siRNA to P53 on Expression, Apoptosis and Kidney Function

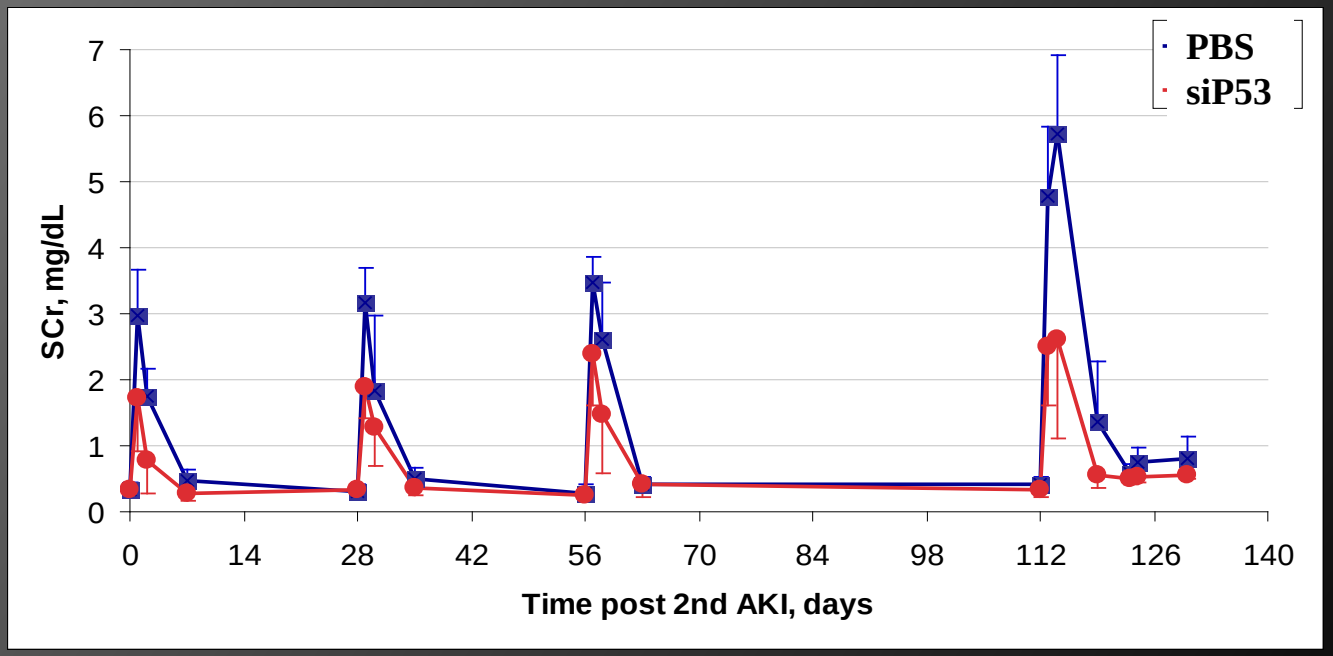


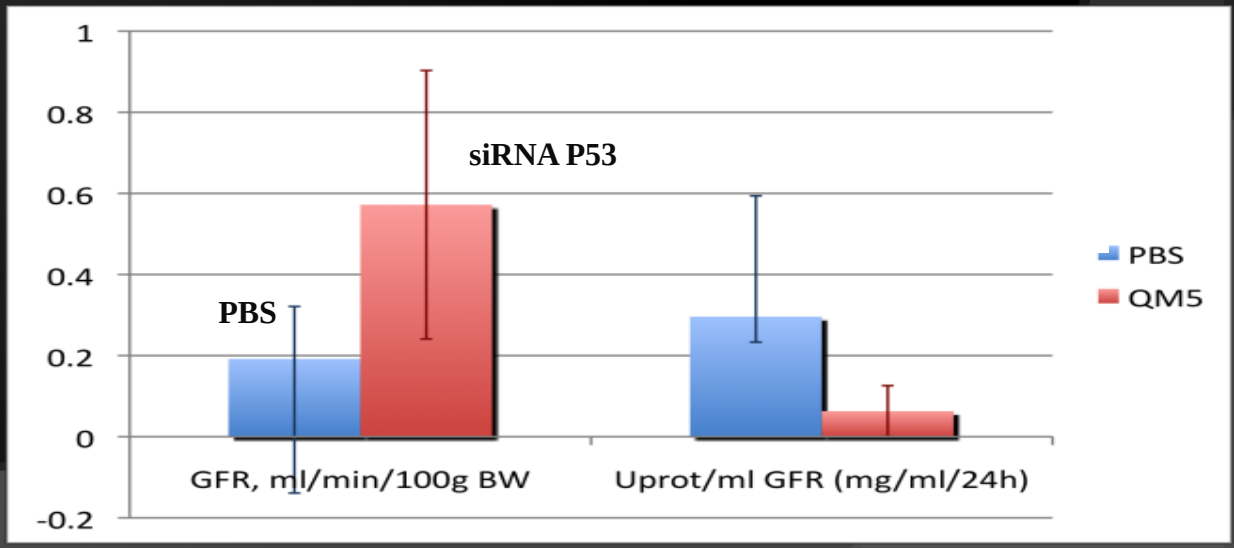


A vicious cycle

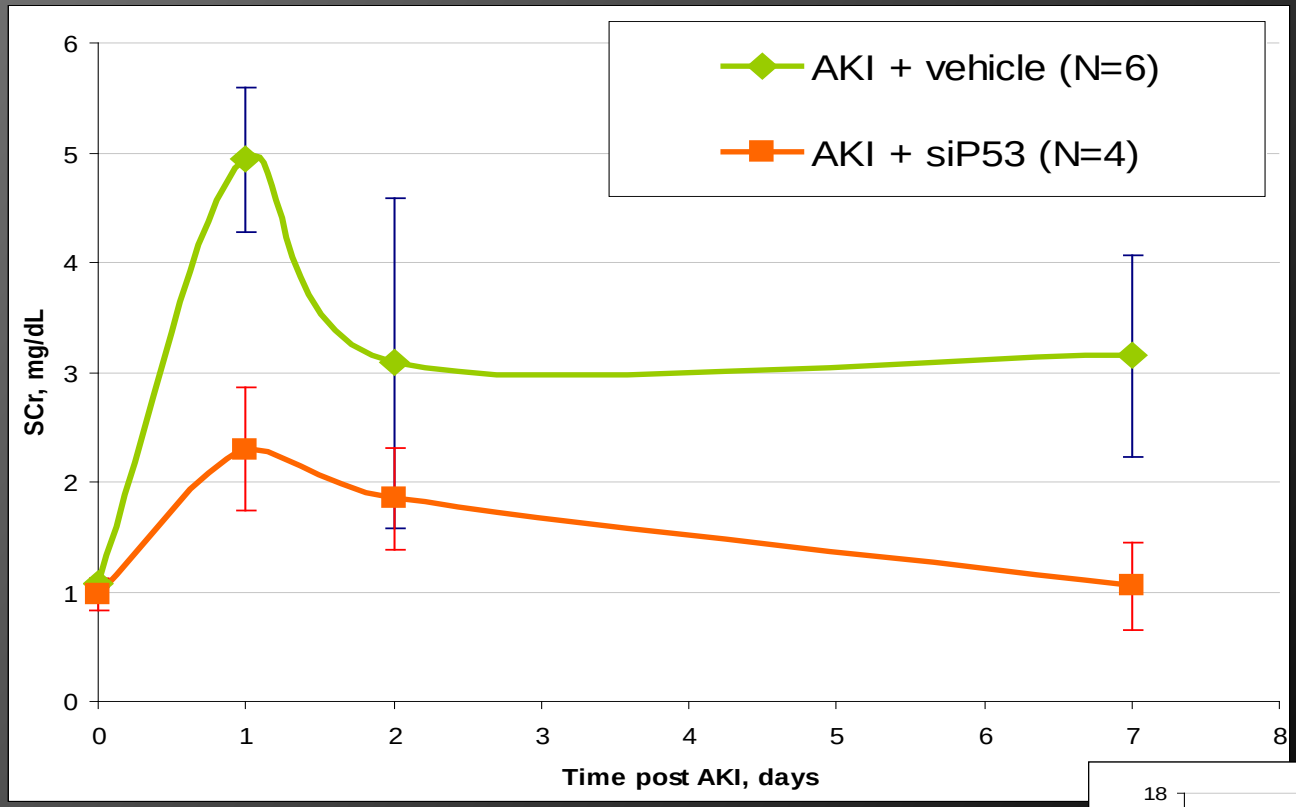


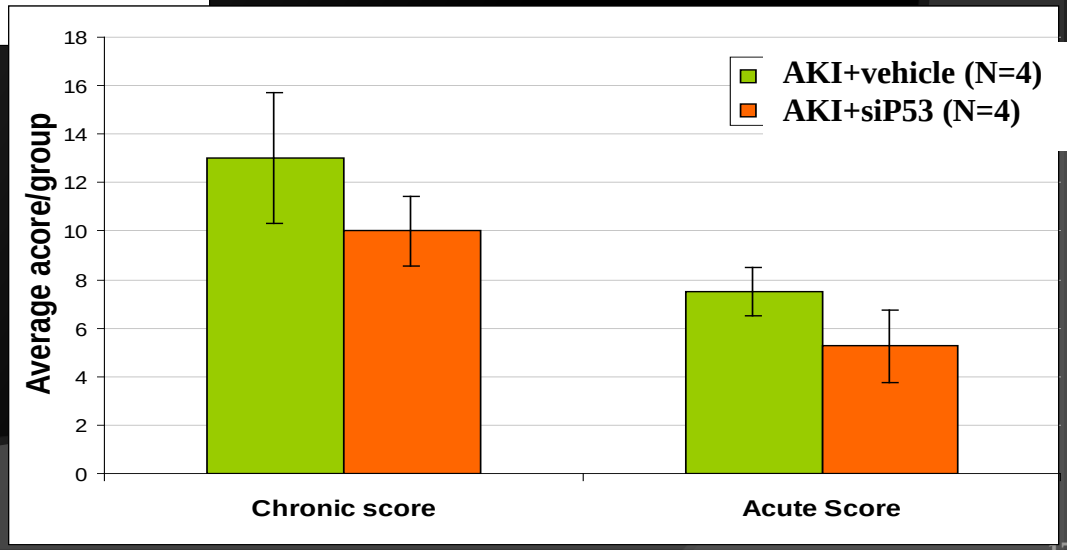
siP53 Protects GFR and Minimizes Proteinuria



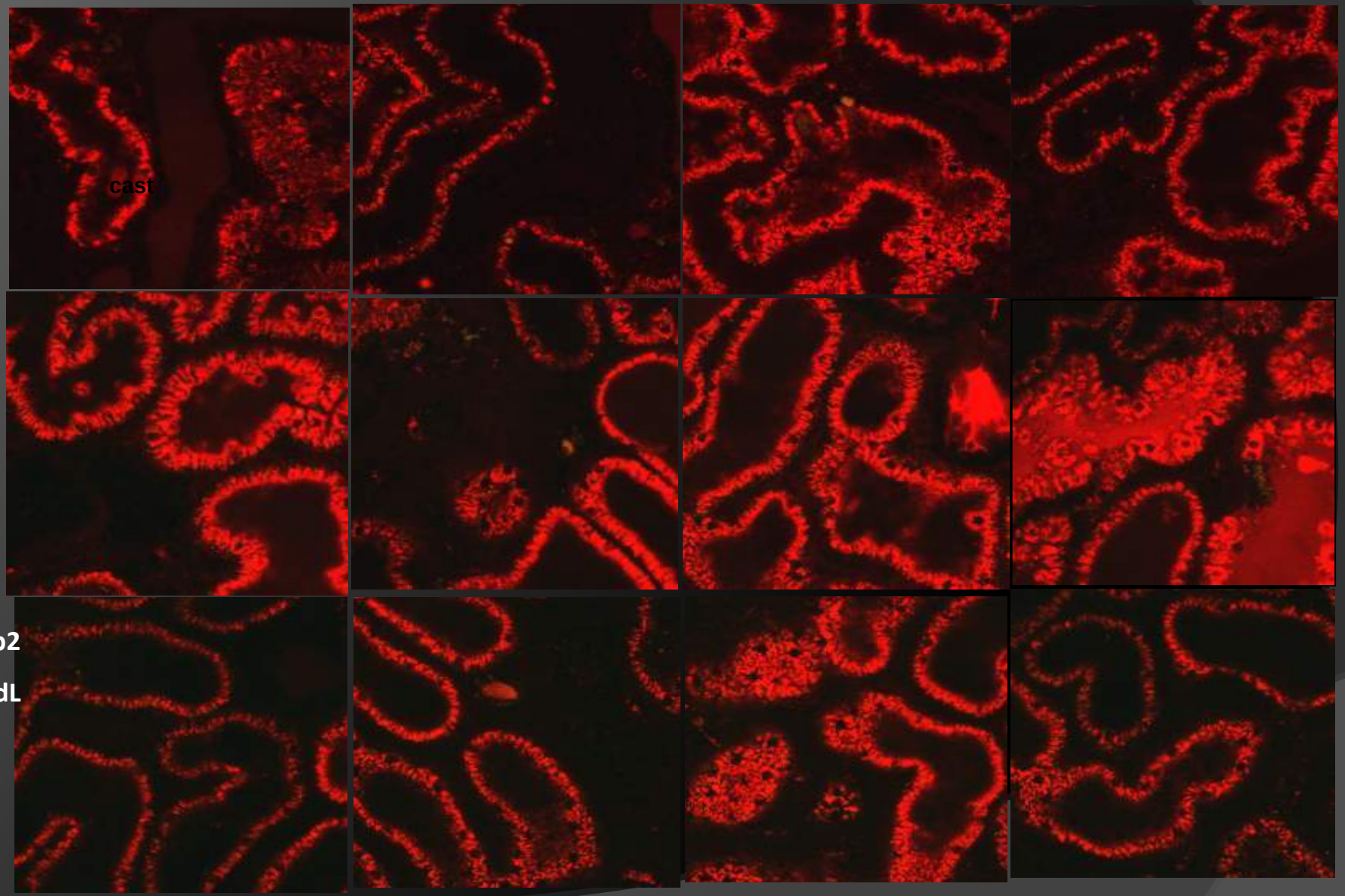


siP53 Attenuates AKI in pre-existing CKD



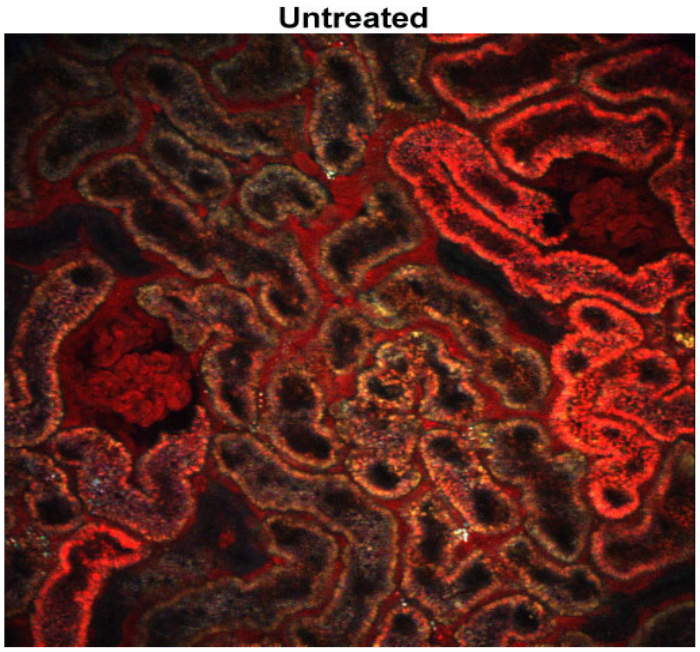


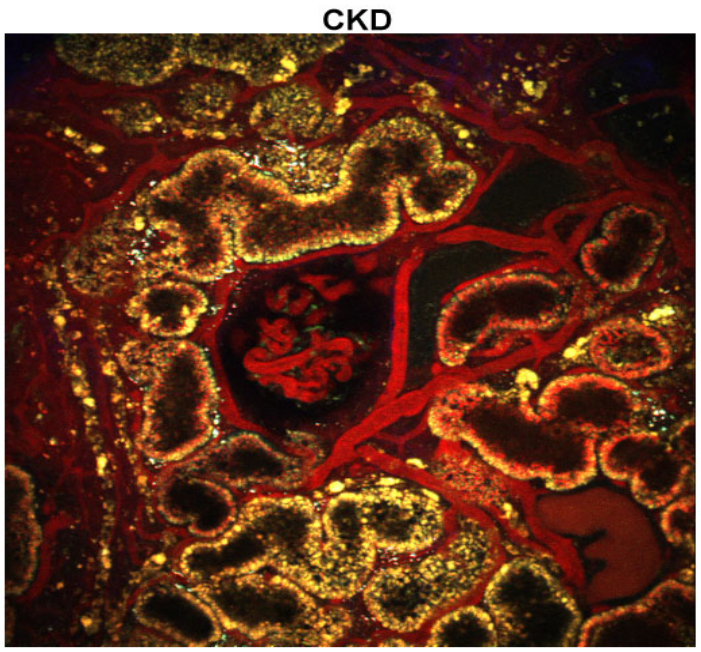
siRNA is Reabsorbed by PTC in CKD Proteinuric Rats



0.9mg Cy3-siRNA 1 hr post Injection Cy3-siRNA

Proteinuric Model Post AKI and Atrophy



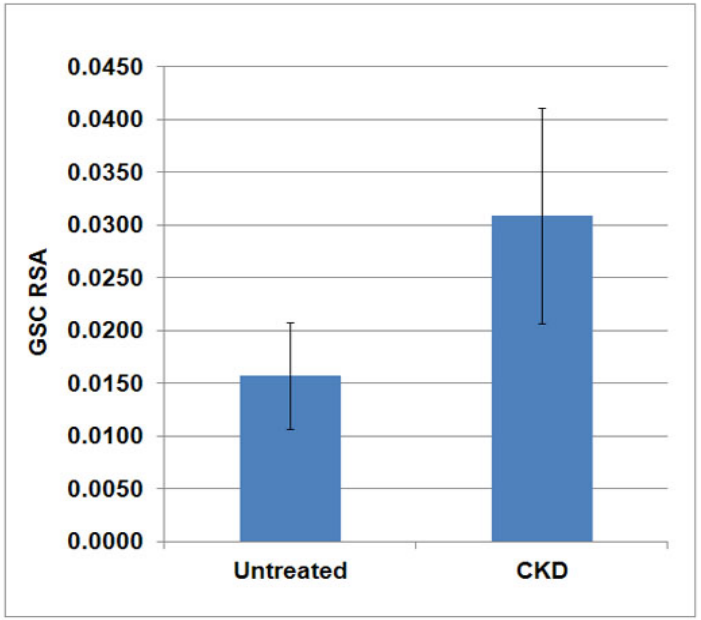


Male MW-F CKD rats,

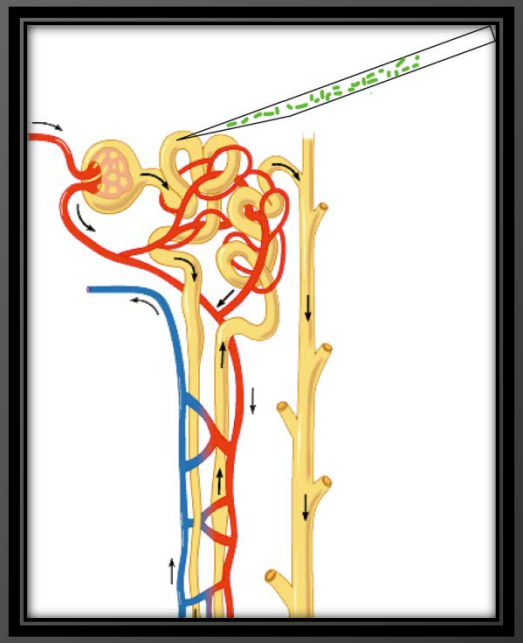
Mean SCr 1.4 (n=3)

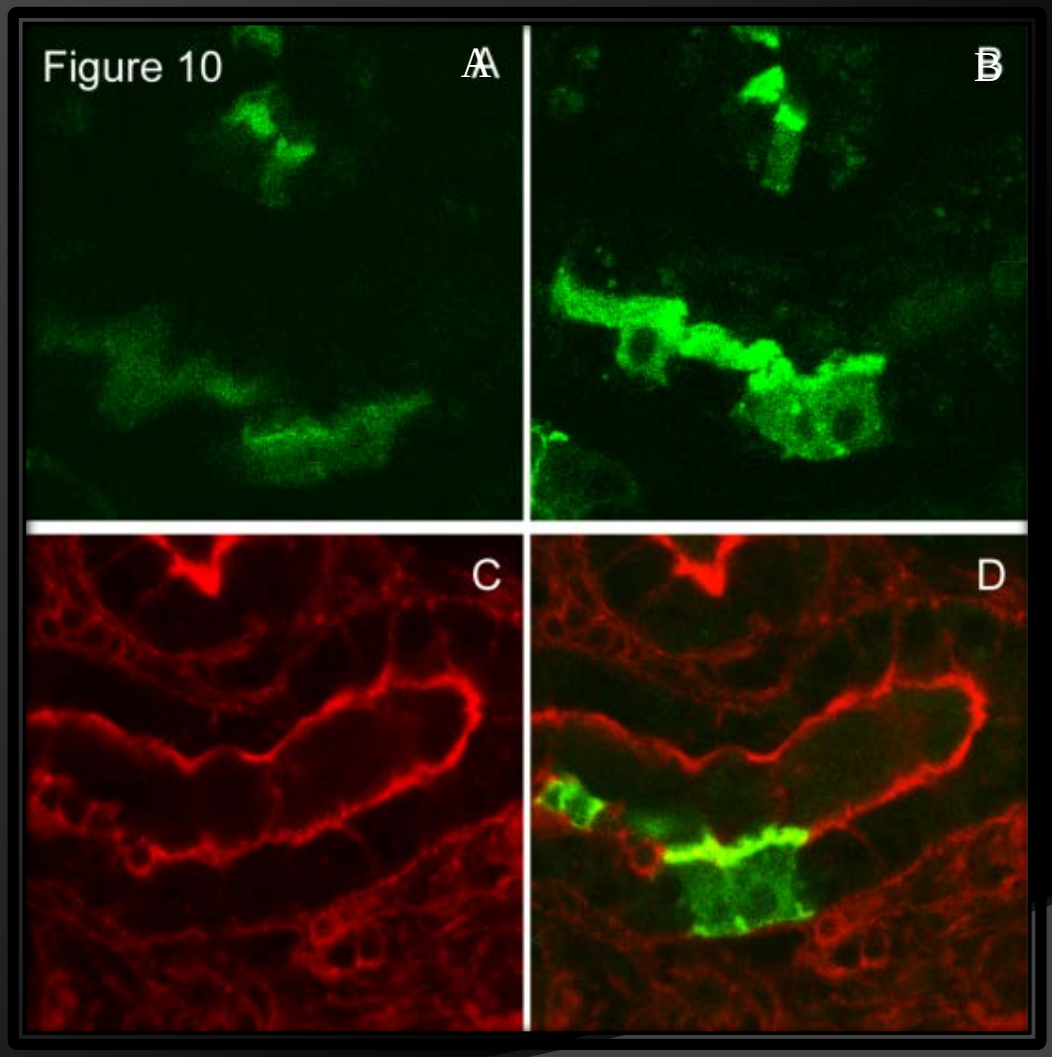
GFR 0.18 ml/min/100g

Uprot 230 mg/24 hr



Micropuncture Delivery of Adeno-eGFP Actin



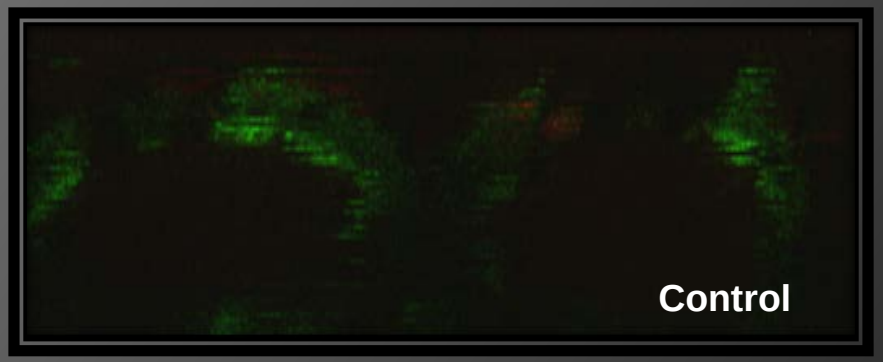


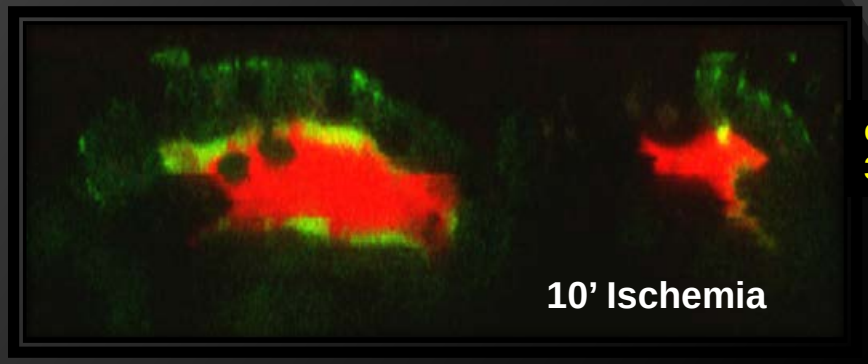
Proximal Tubules 48 hr post Viral Injection

Proximal Tubules Post Fixation and rhodamine Phalloidin Staining;.

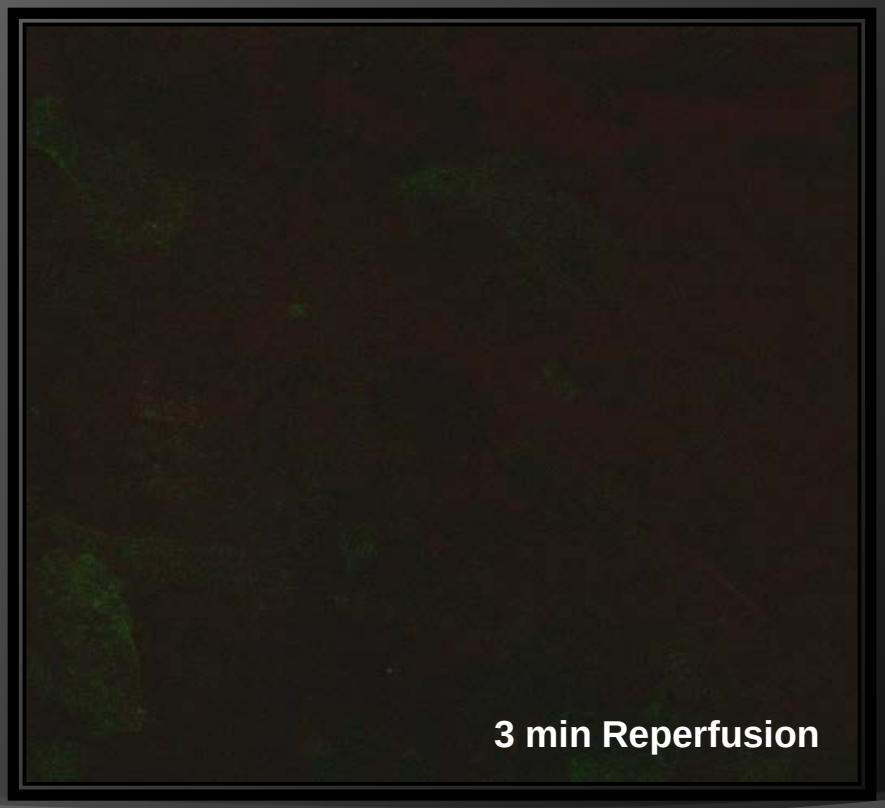
Tanner et.al. *AJP-Renal* 2005

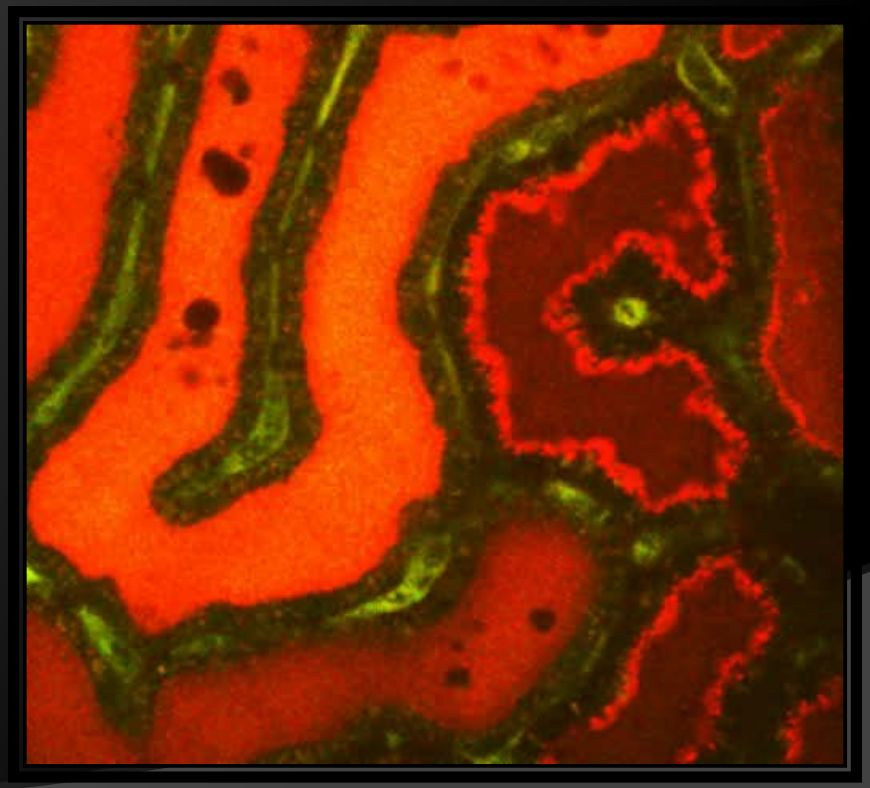
Apical Membrane Bleb and Tubular Cast Formation in Ischemia





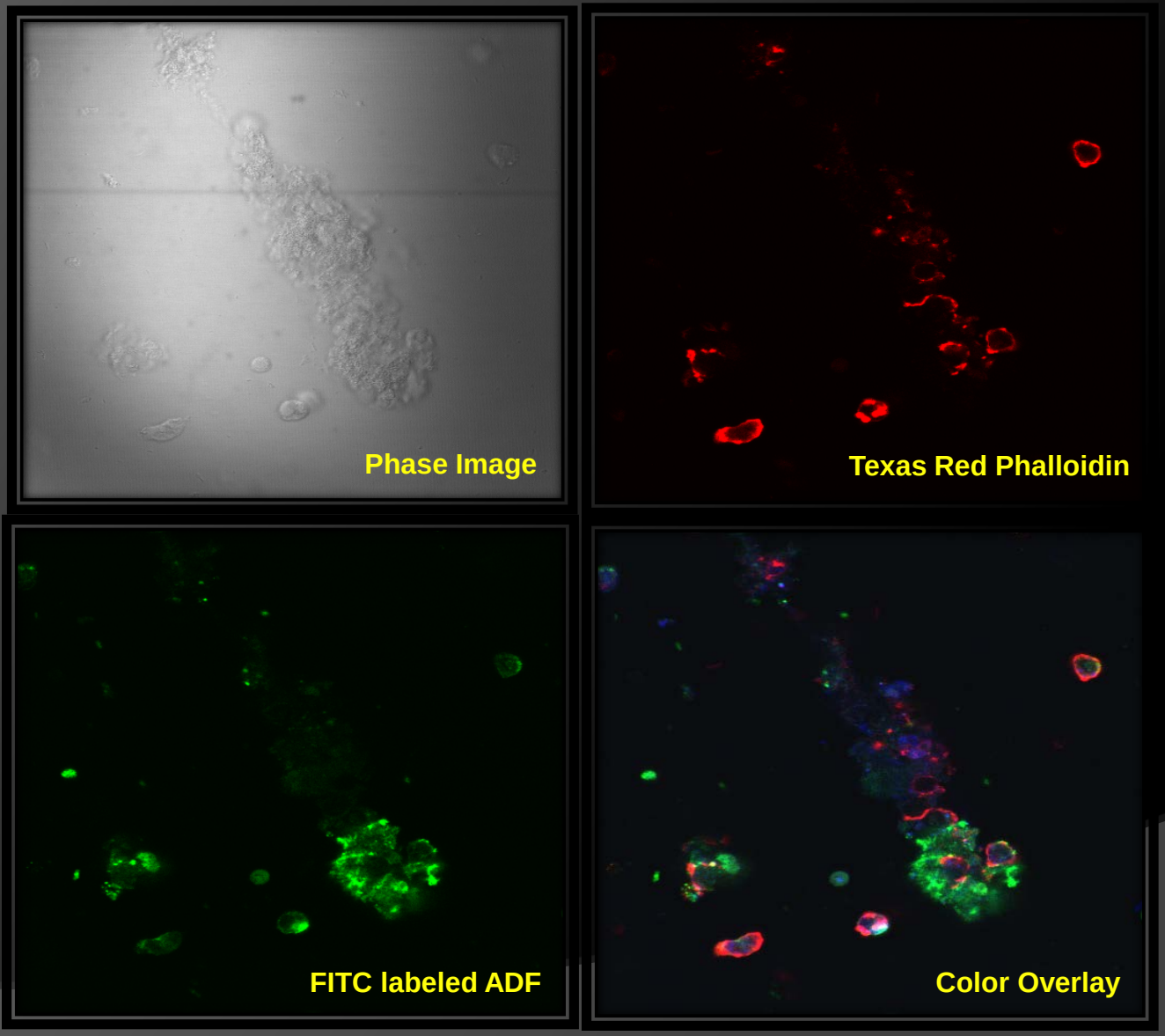
eGFP-Actin and
3kDa TR Dextran





Ashworth et.al. *Kidney Int*. 2007

Actin Components of a Urinary Cast in Acute Renal Failure



Molitoris , *Kidney Int*. 2004

Summary

The Proximal Tubule cell is a long lived cell with avid endocytosis

Endocytosis is necessary for recycling filtered materials

Unfortunately, this includes toxins that accumulate and cause cell injury

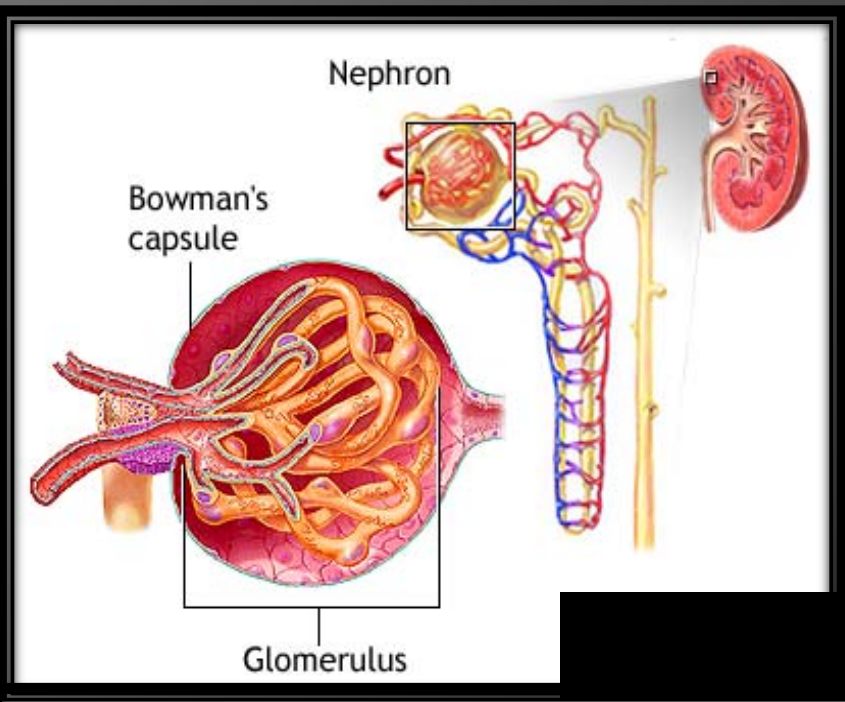
RNAi therapy is perhaps best applied to the Proximal Tubule

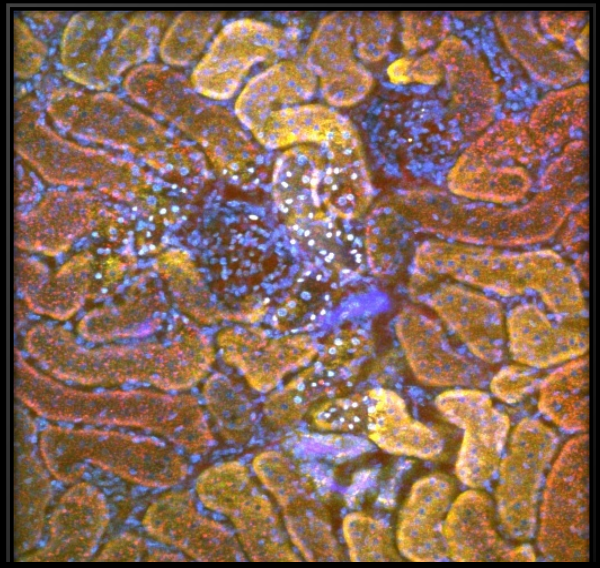
Presently it is possible to inhibit upregulation of specific proteins

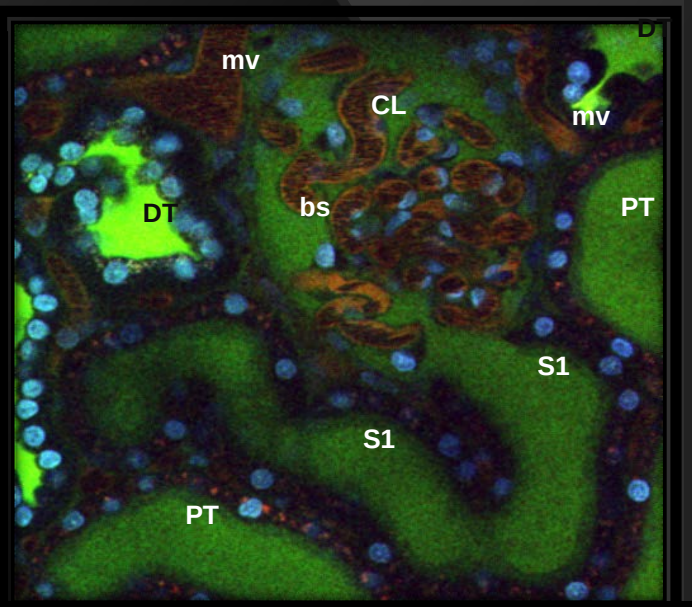
It is also possible to down regulate specific proteins

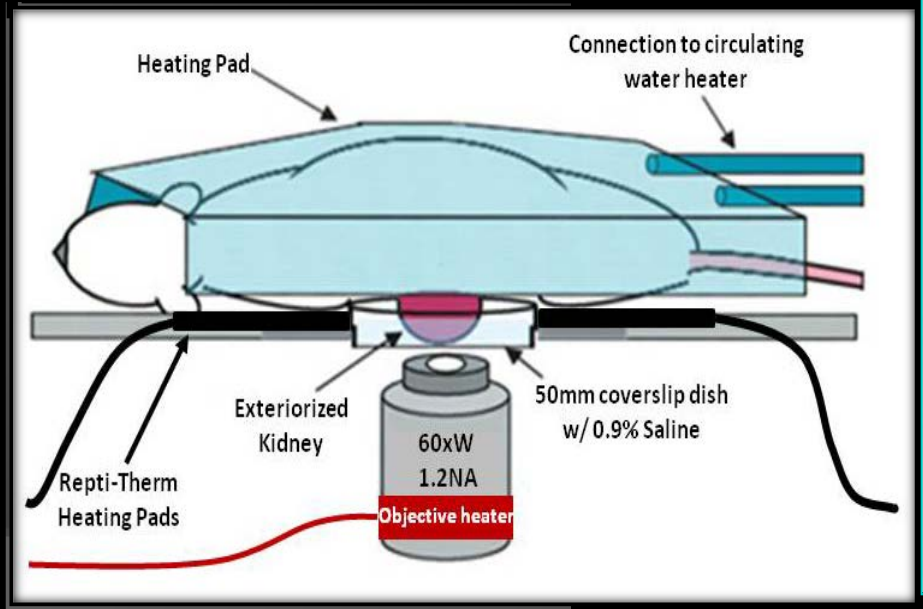
There are many untested potential targets for endocytic processes in PTCs

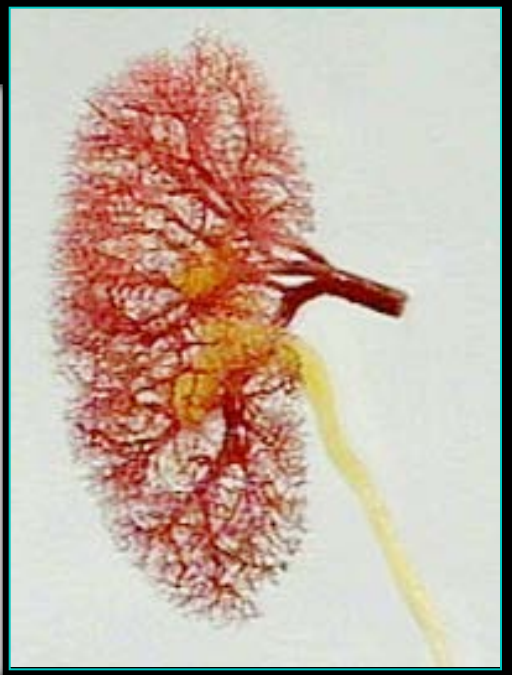
Visualizing Vascular, Glomerular & Nephron Function

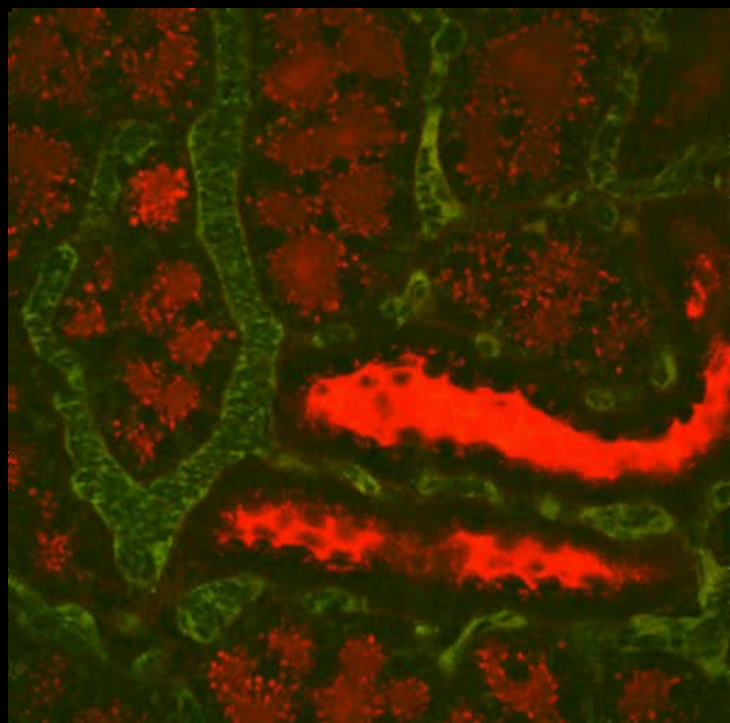






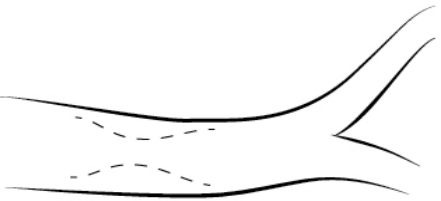






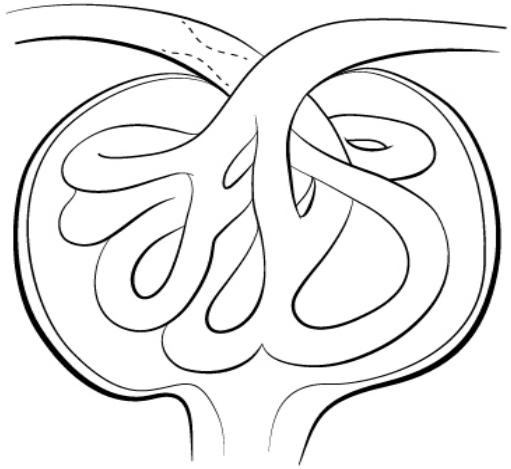
Series and Parallel Resistors to Vascular Blood in the Kidney

A. Macro-vasculature



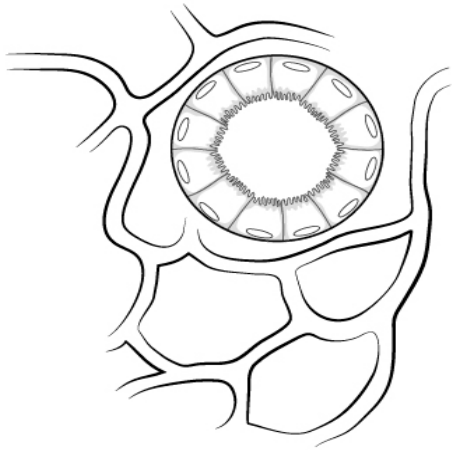
Volume Depletion, CHF
Liver Disease, NSAIA
Renal Artery Stenosis
Thrombosis, Sepsis with
Reduced PVR

B. Glomerular



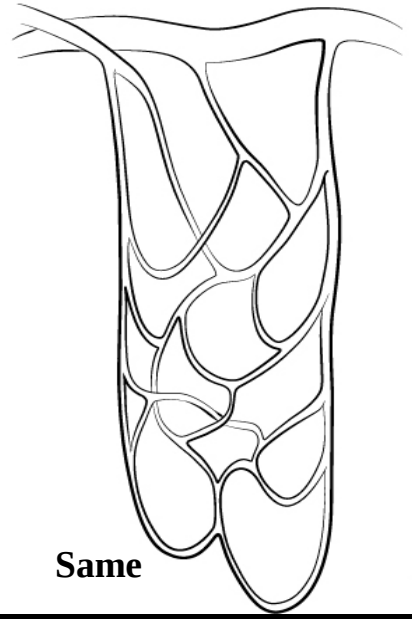
Vasculitis, Inflammation
Sclerosis, Fibrosis

C. Peritubular



Endothelial Dysfunction,
Coagulopathy, Sepsis,
Ischemia, Hyperviscosity

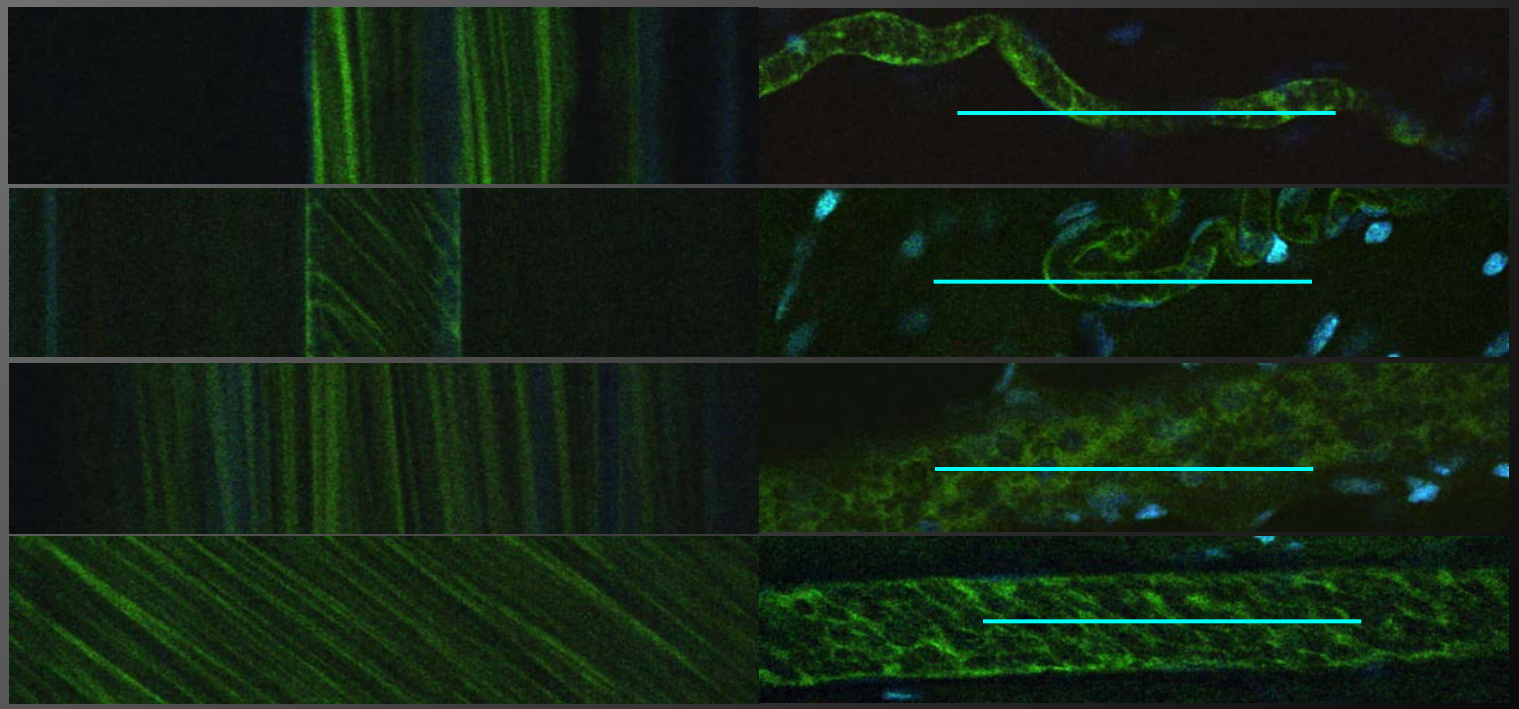
D. Vaso Recti



Same

D. Venous

High Venous
Pressure
CHF, ACS
Thrombosis

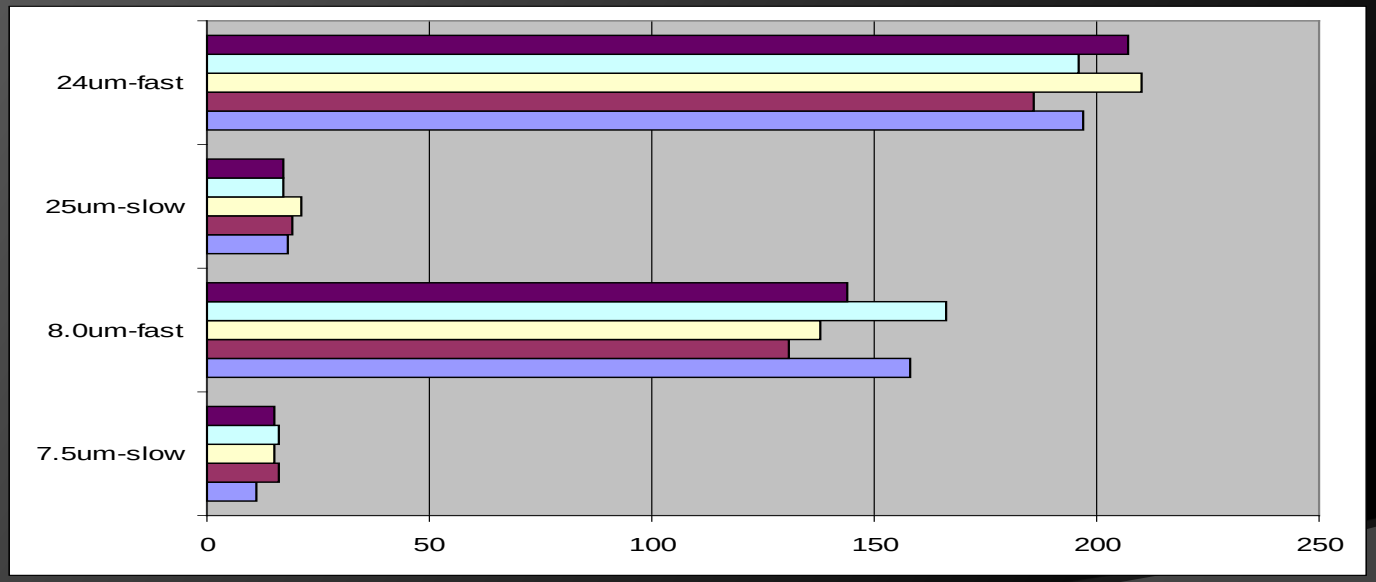


Vessel Diam.=7.5 um
Ave.Speed=14um/sec

Vessel Diam.=8 um
Ave Speed=147um/sec

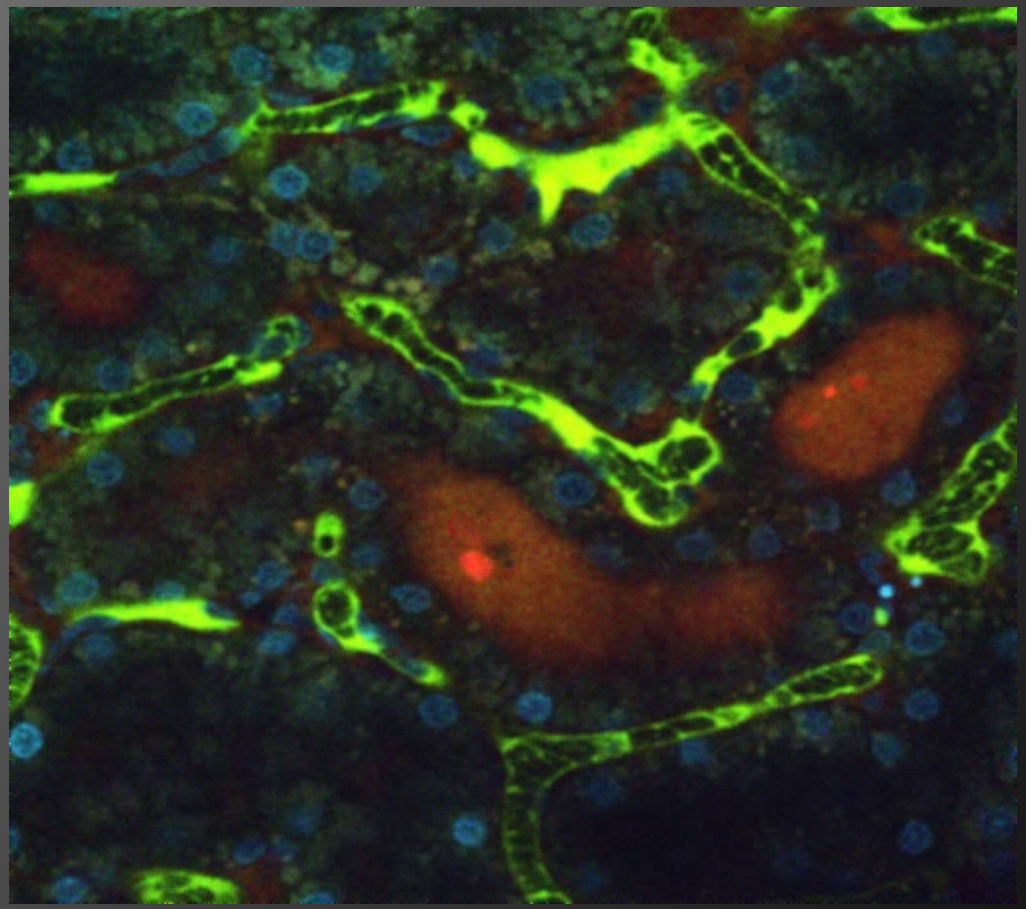
Vessel Diam.=23 um
Ave Speed=18um/sec

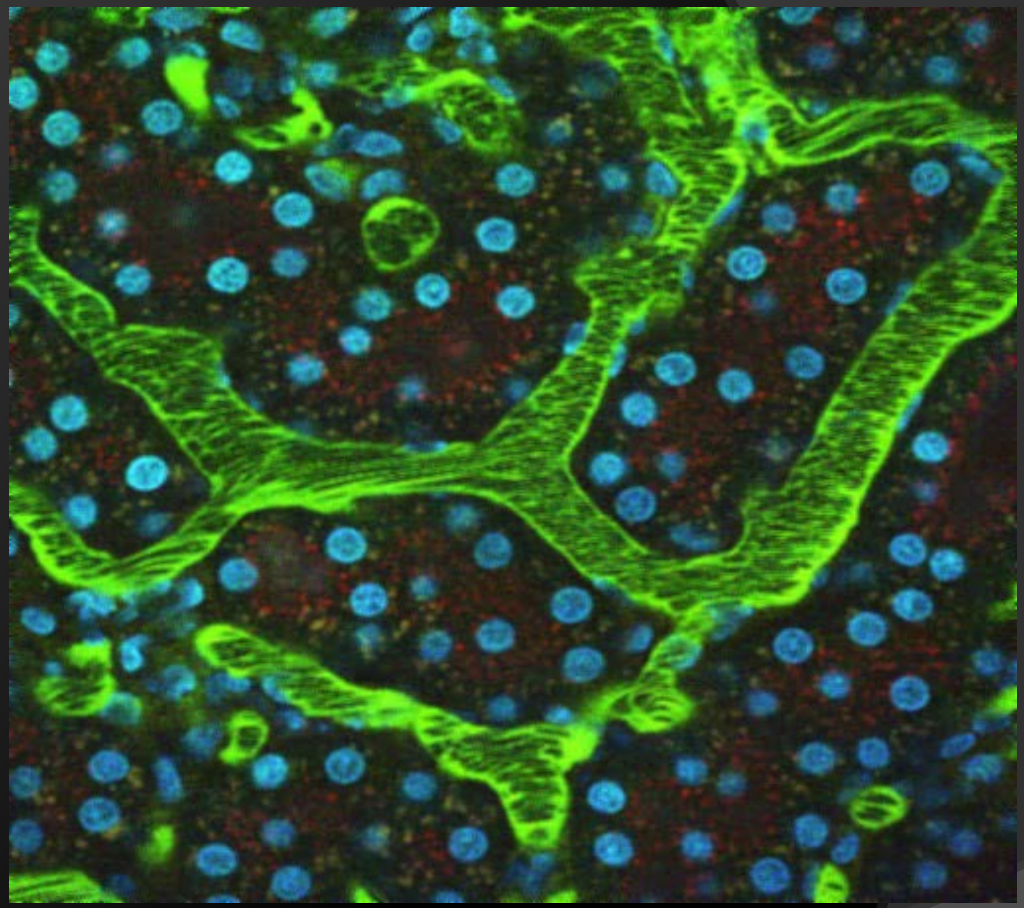
Vessel Diam.=24 um
Ave Speed=199um/sec



Vessel Diam. relative speed	Ave Speed in um/sec	St. Dev
7.5um-slow	14.6	2.07364414
8.0um-fast	147.4	14.3805424
25um-slow	18.4	1.67332005
24um-fast	199.2	9.5760117

Microvascular Blood Flow at 24h Post Ischemia Effect of sTM





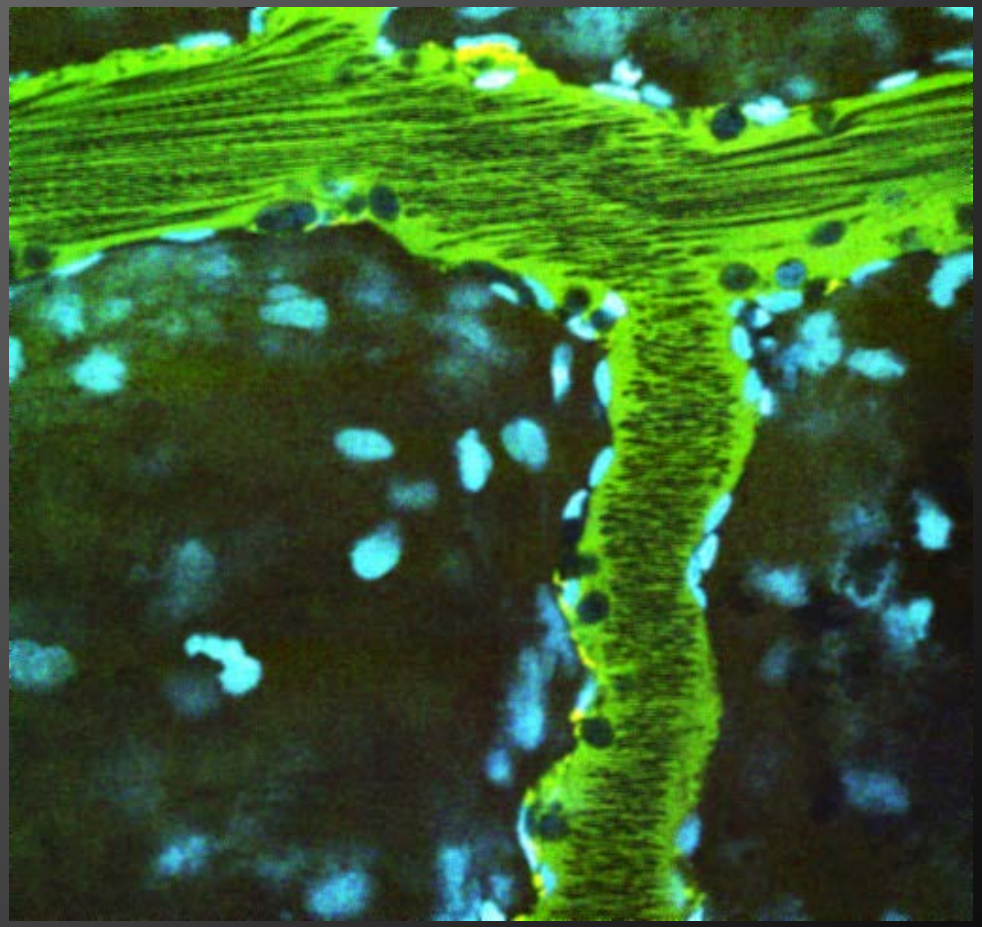
Saline treated

sTM treated

Blood Flow velocity (µm/sec)	253.36+/-95.01	786.75 +/- 280.75 * *P < 0.05

Sharfuddin et.al JASN 2009

Leukocyte-Endothelial Interactions – Intra-Vital 2-Photon



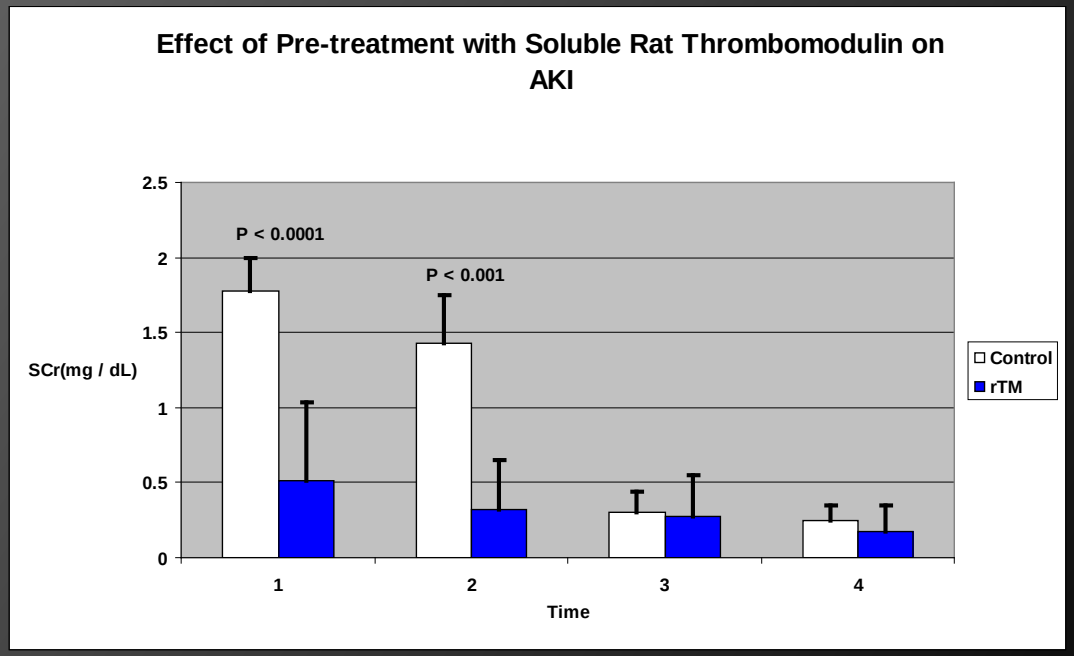
Ischemic – Saline treated rat at 24h

	Saline	sTM treated
Flowing (%)	69.5	88.3 *
Rolling (%)	18.2	8.3 *
Static (%)	12.9	3.3 *

* $p<0.05$

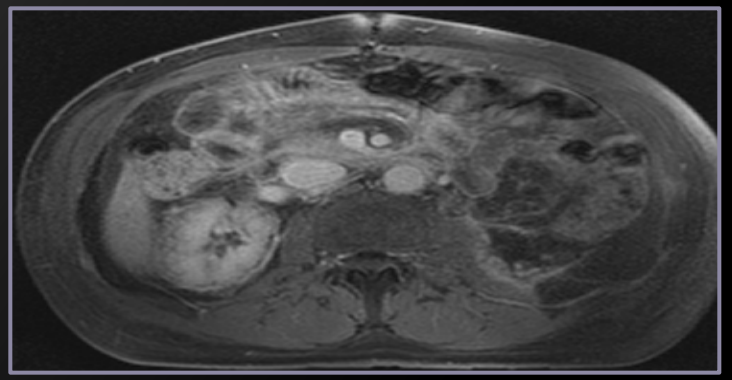
Sharfuddin et.al. JASN 2009

Effect of sTM Therapy on Kidney Function in Acute Kidney Injury

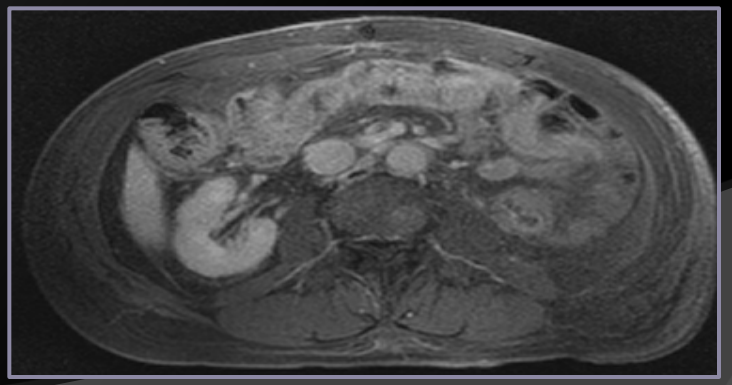


Sharfuddin et.al. JASN 2009

NMR Prior to Kidney Donation



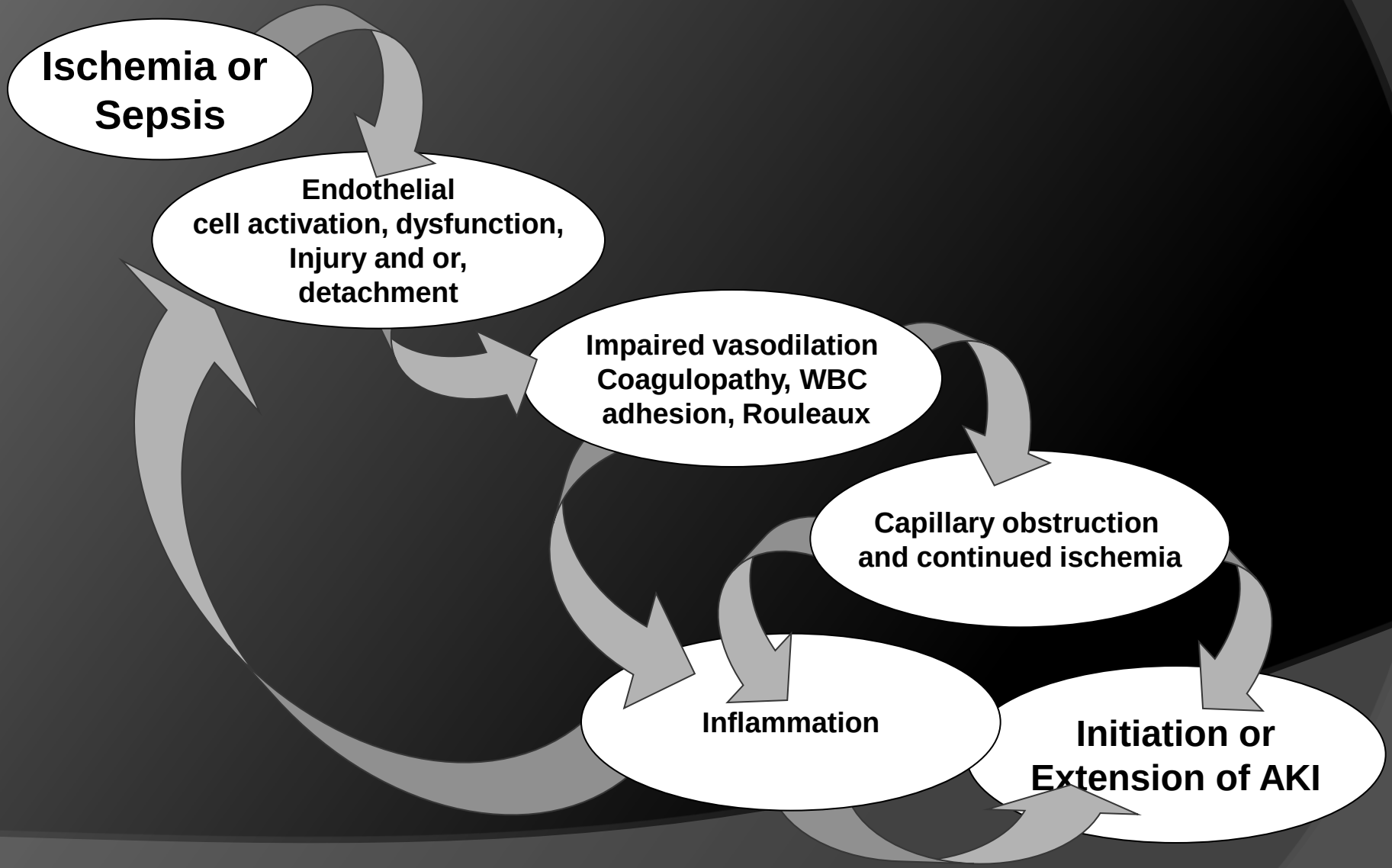
Acute Kidney Injury



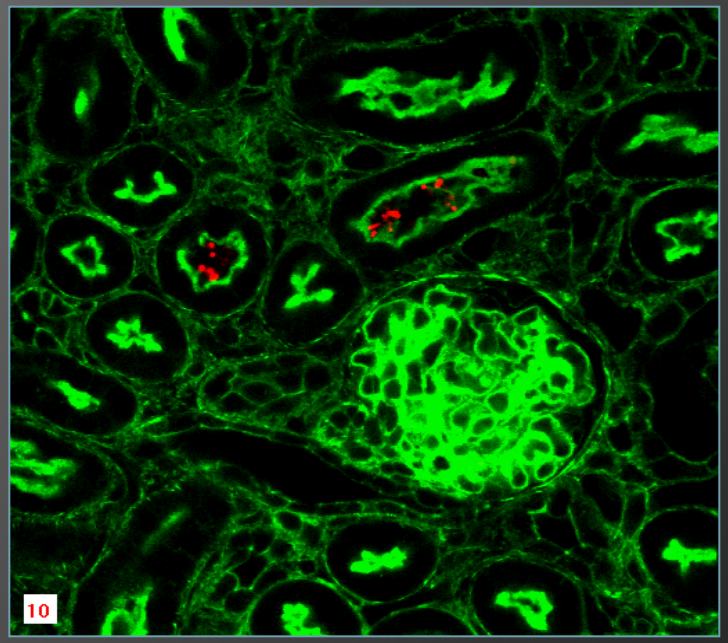
Resolution of AKI

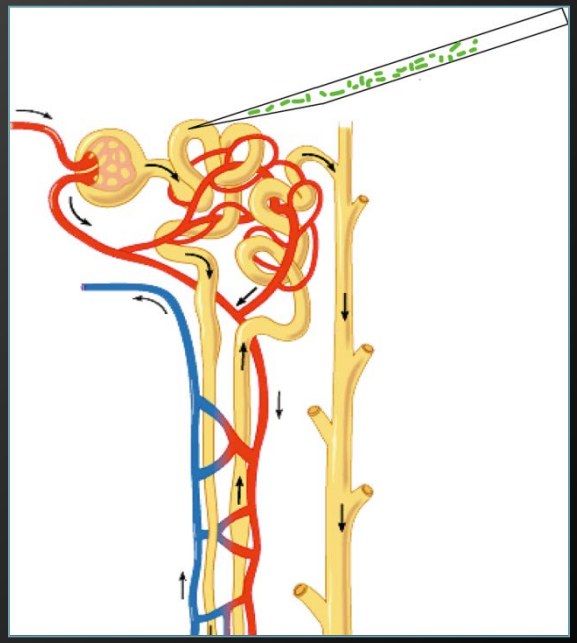
Rosenthal et.al JASN, 2003

Small Vessel Injury in Acute Kidney Injury



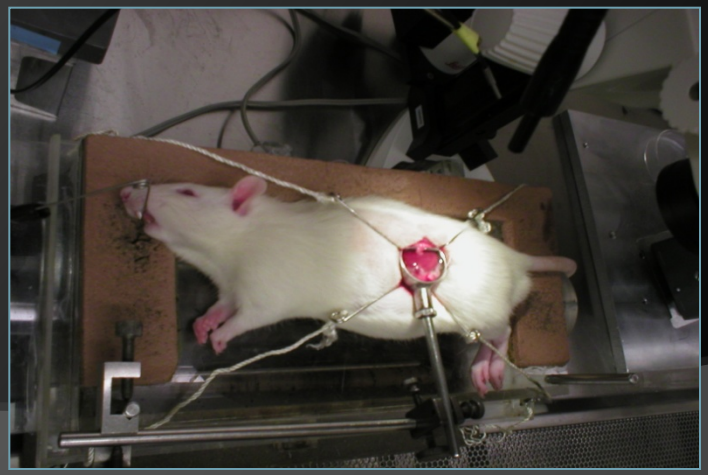
Spatial Specificity Achieved by Micro-Infusion of Bacteria into Proximal Tubules

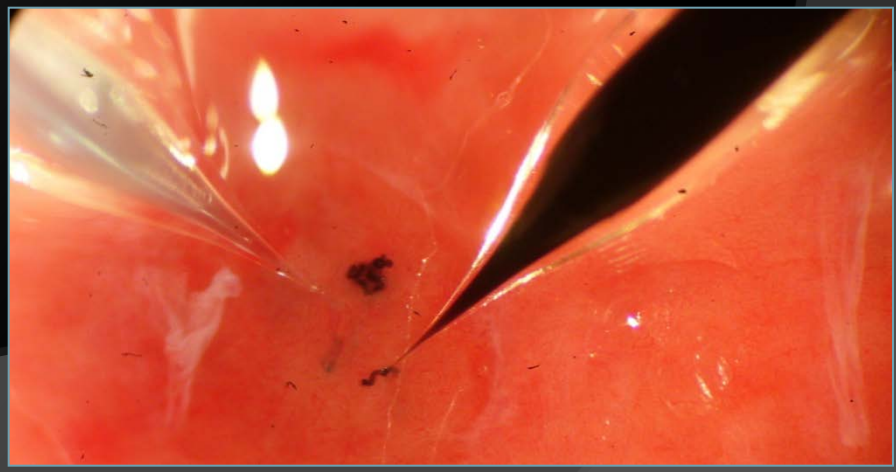




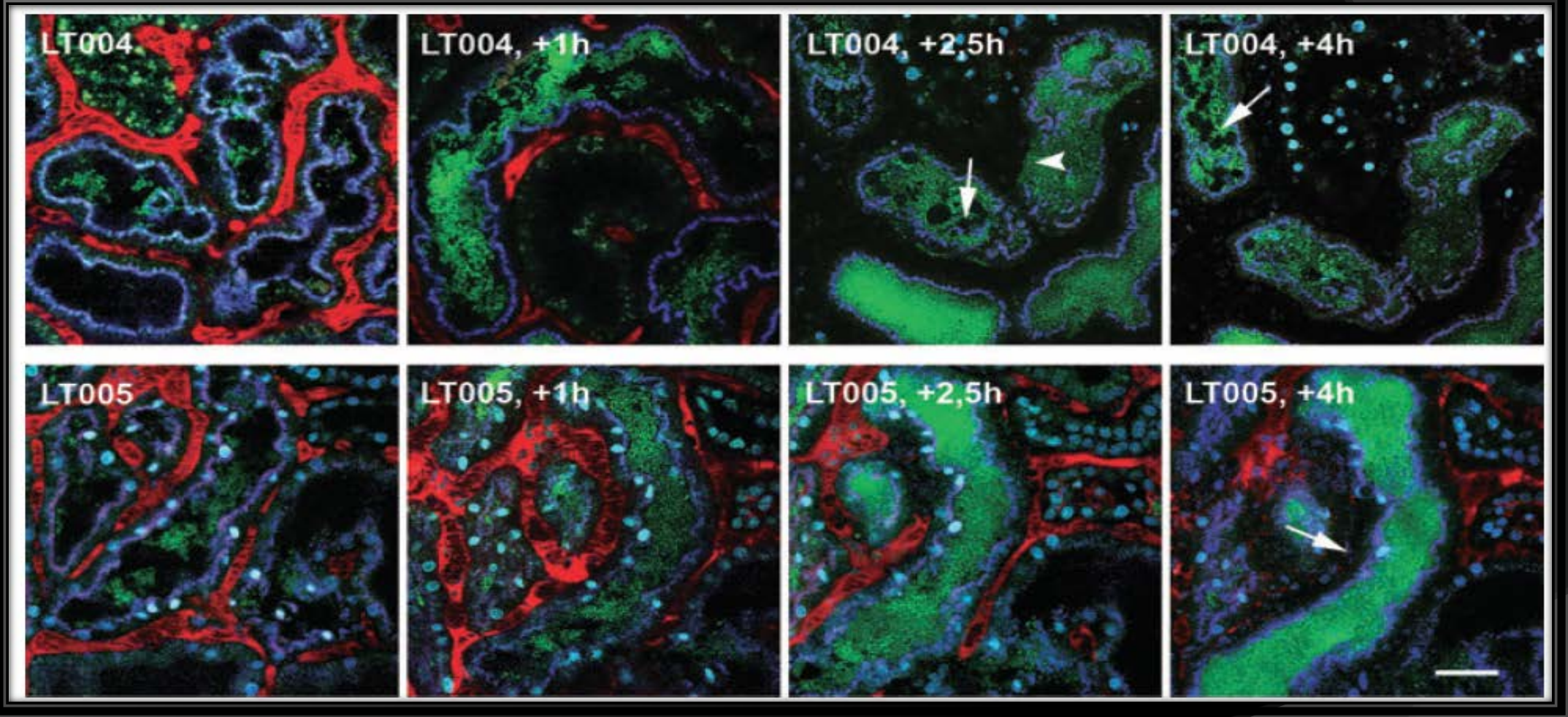
- 10^5 cfu UPEC GFP^+
- 0.1 to 0.7 µl injected

Agneta Richter-Dahlfors,
Lisa E. Mansson and Keira Melican
Karolinska Institutet





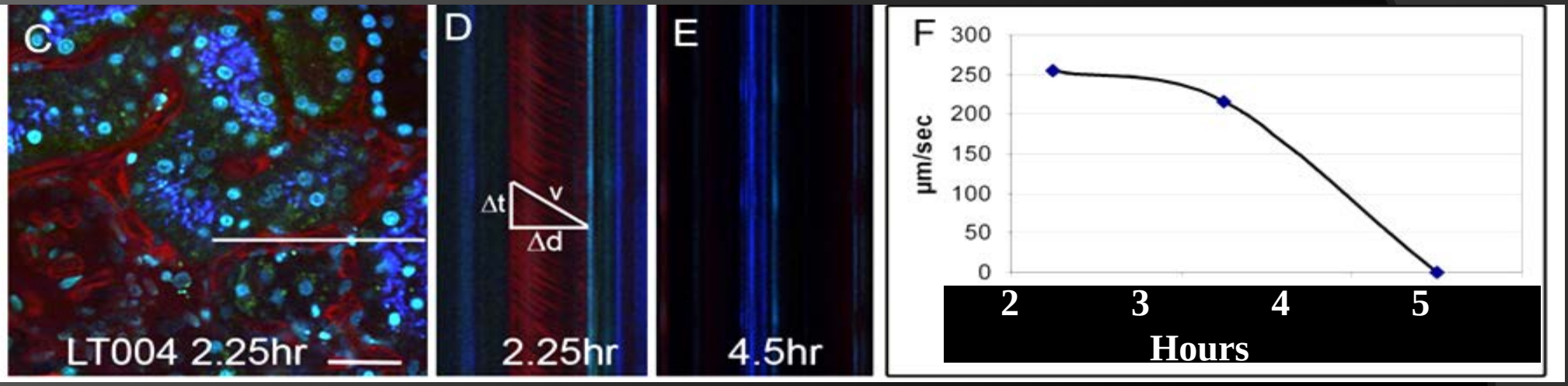
Proximal Tubule *E. coli* Infection: Effect of Virulence Factor



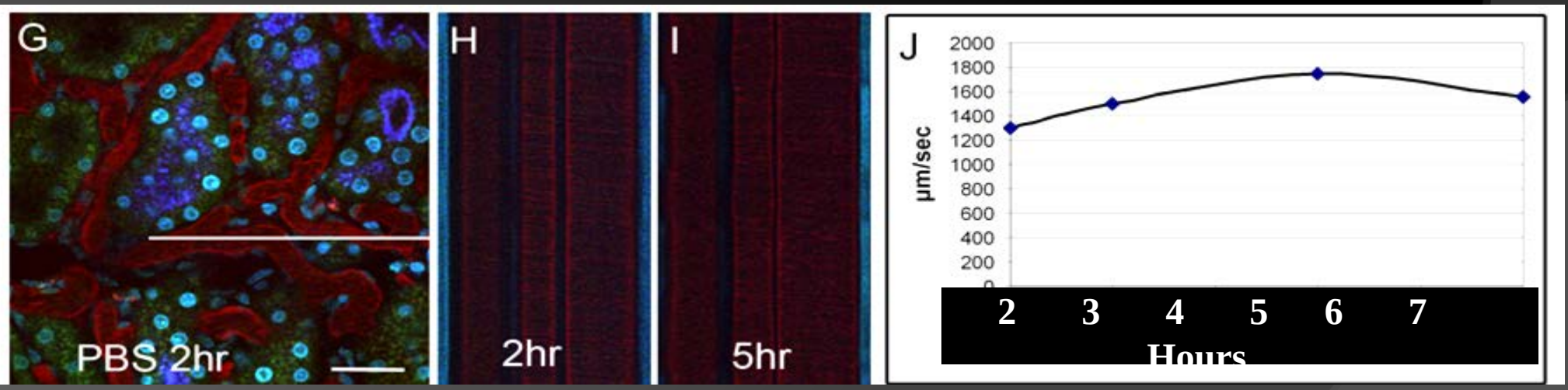
LE Månsson et al, Cell Microbiol 2007 Feb; 9(2) 413-24

Determining blood flow rates *in vivo*

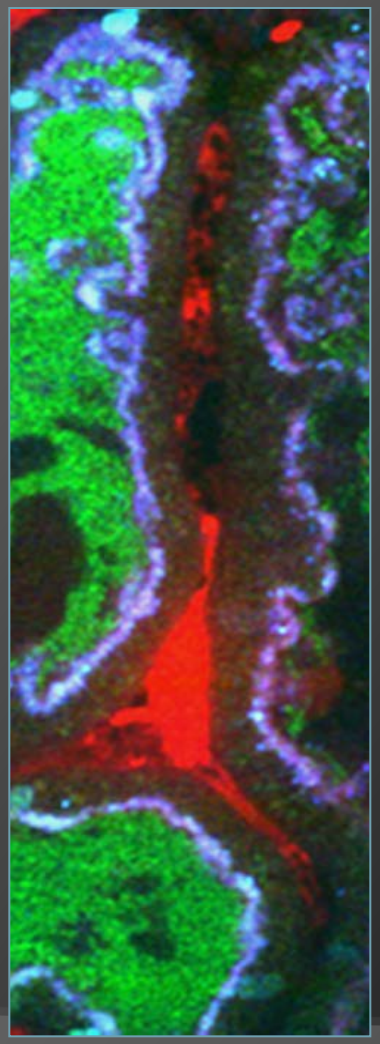
UPEC wt

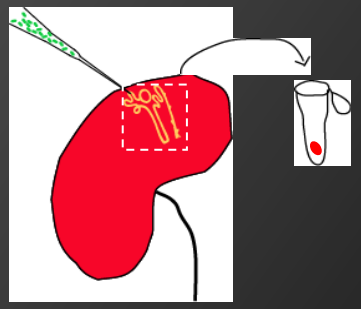


PBS

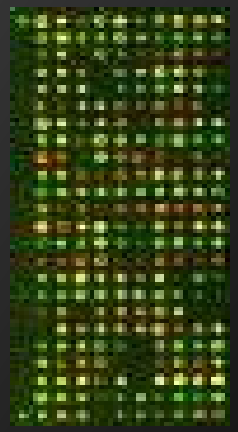


Clotting Cascade Genes are Up-Regulated in Infected Kidneys

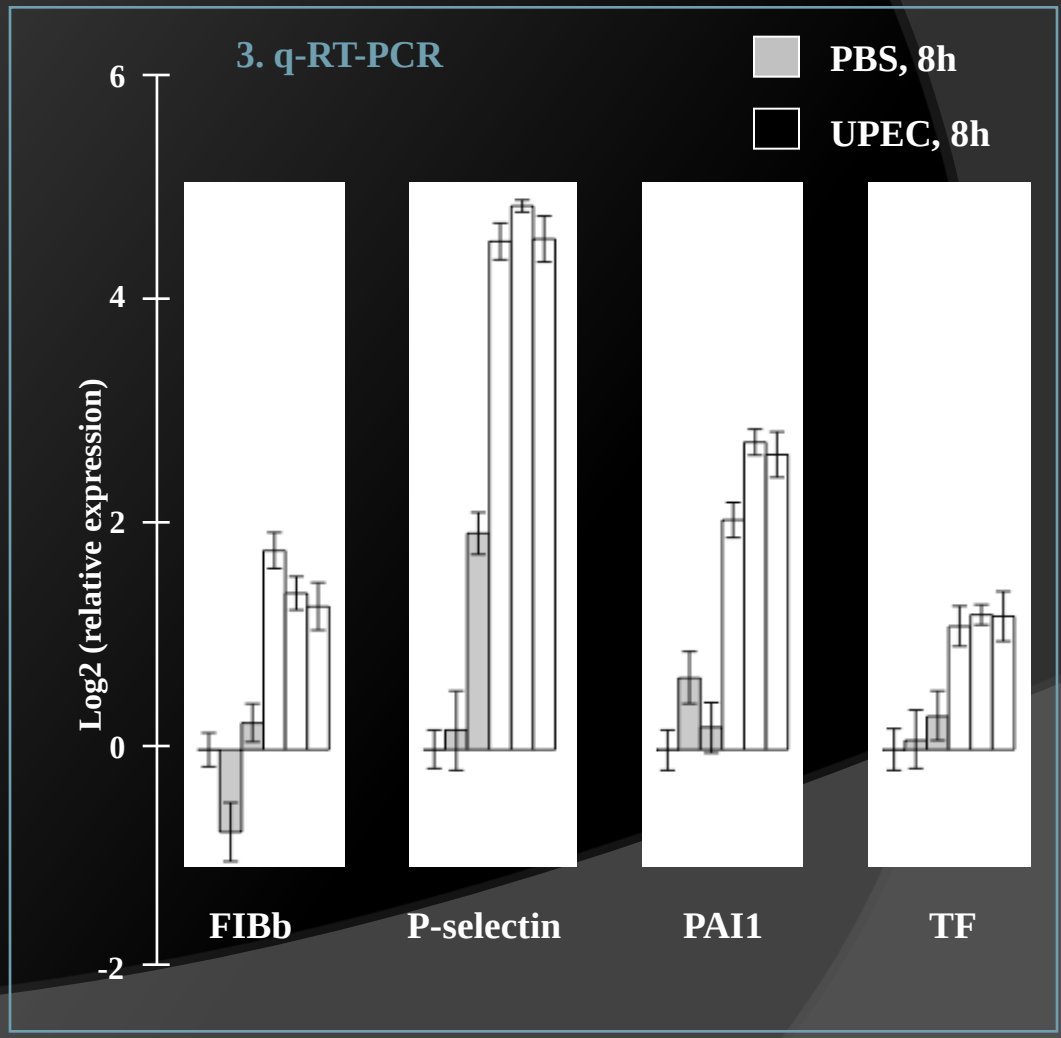




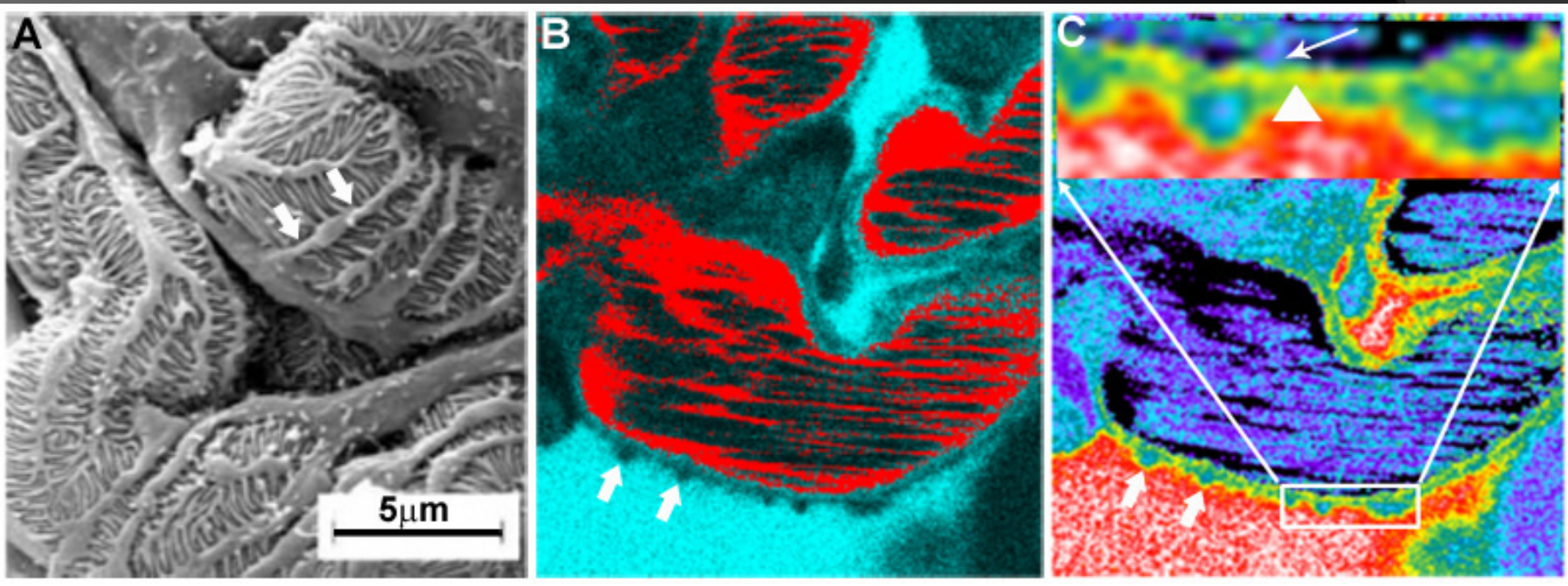
1. Precise dissection to enrich for local mRNA



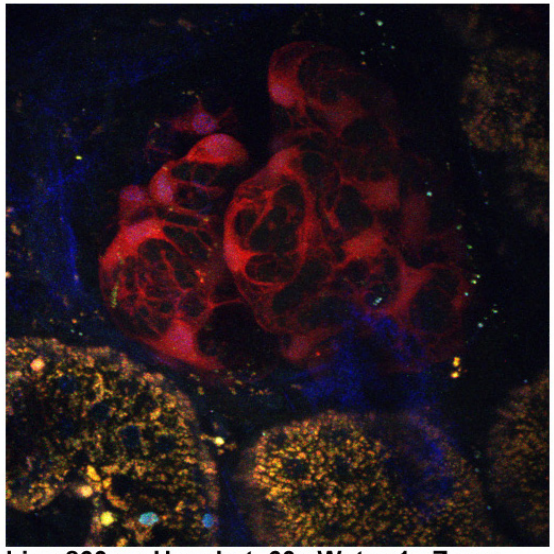
2. Gene expression array



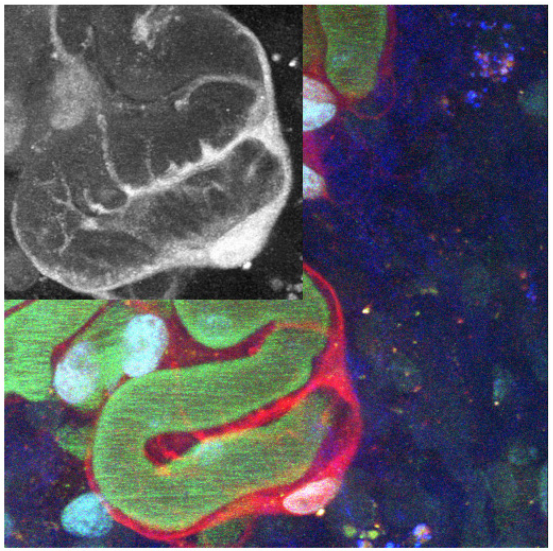
2-Photon Resolution of Glomerular Filtration



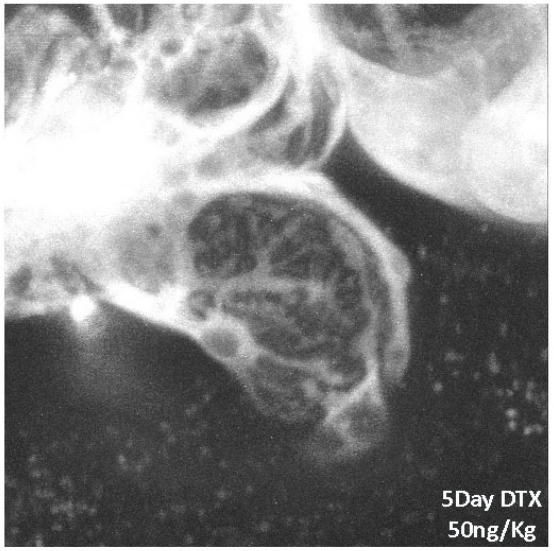
DS Red Labeled Podocytes *in vivo*



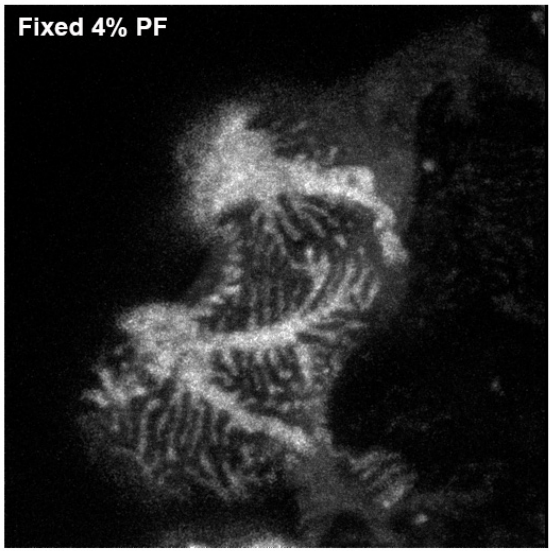
Live-860nm Hoechst, 60x Water-1x Zoom



Live-800nm, 60x water-2x
150K FITC dextran, inset B/W podocyte

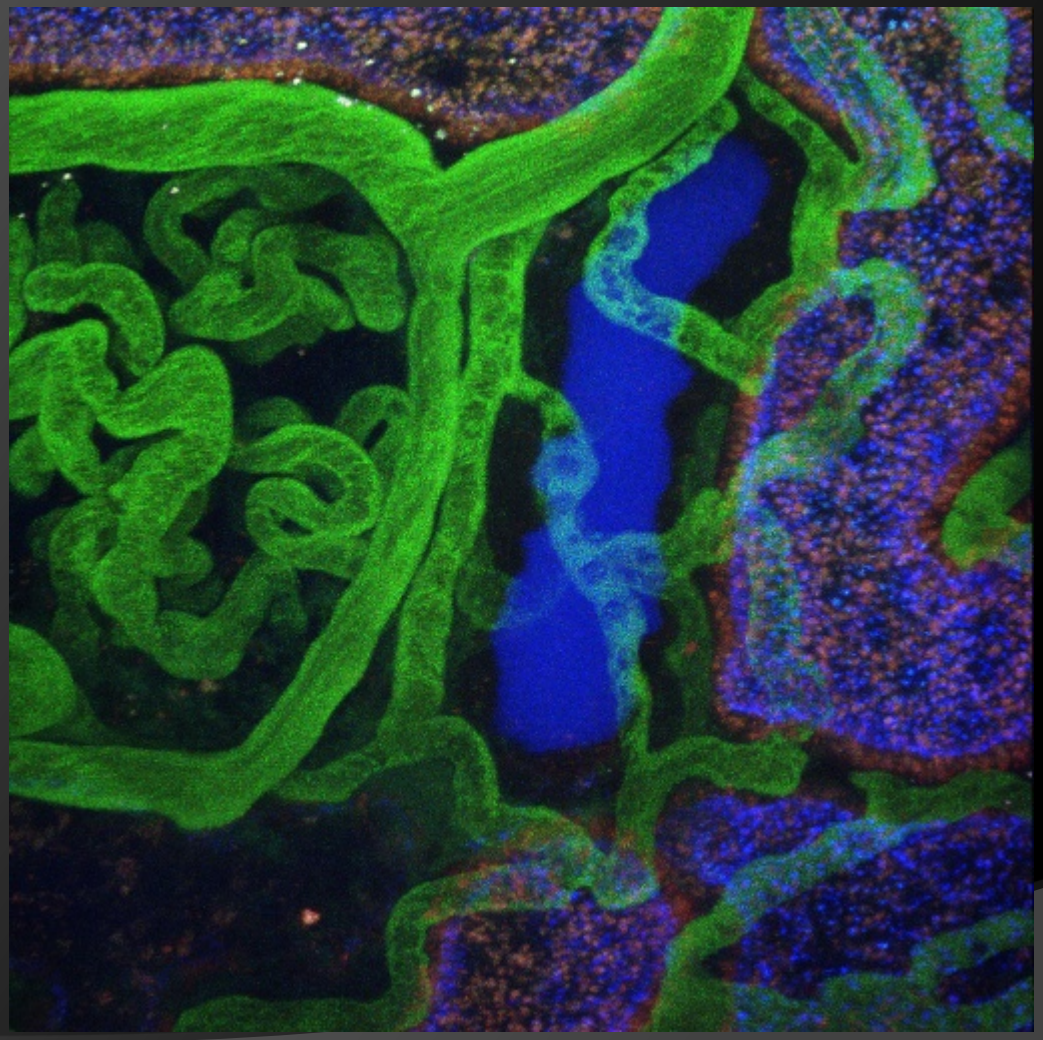


Live-860nm-100x Oil, 4.0x Zoom



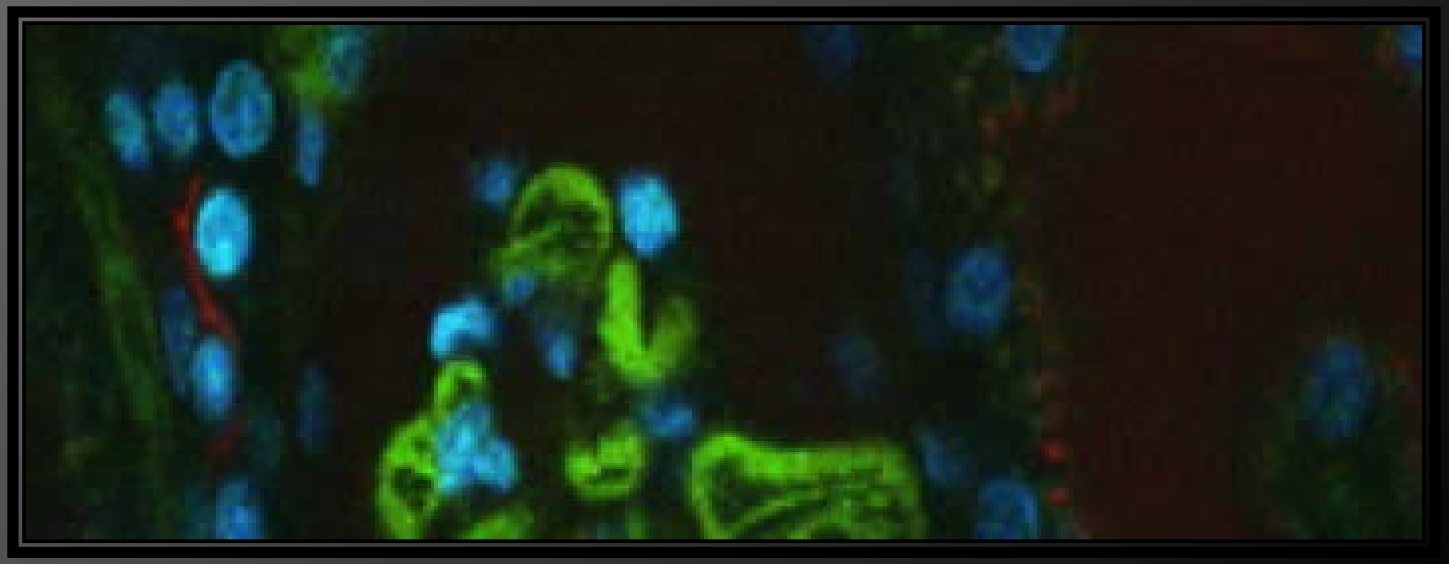
100x Oil, 4.0x Zoom

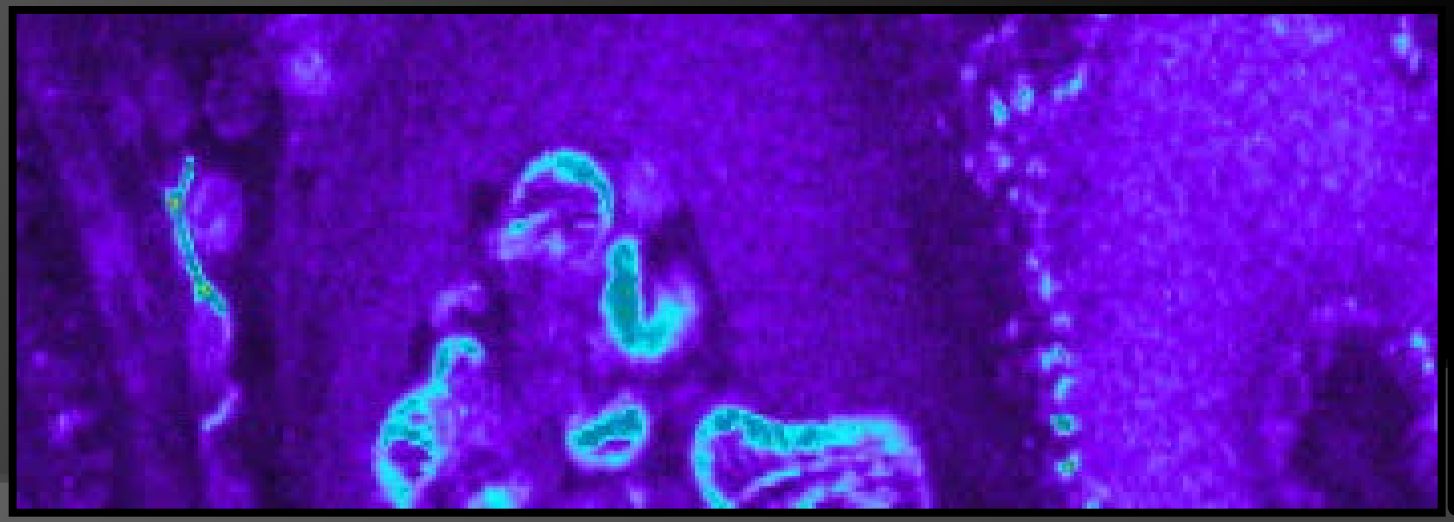
4-D Rat Glomerulus

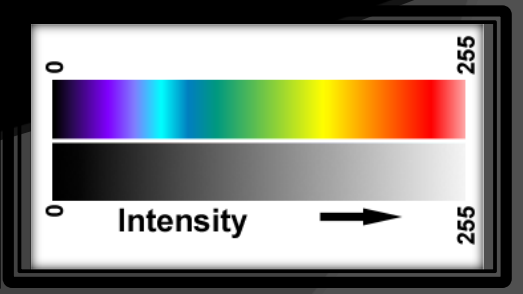


Reducing Scan Size

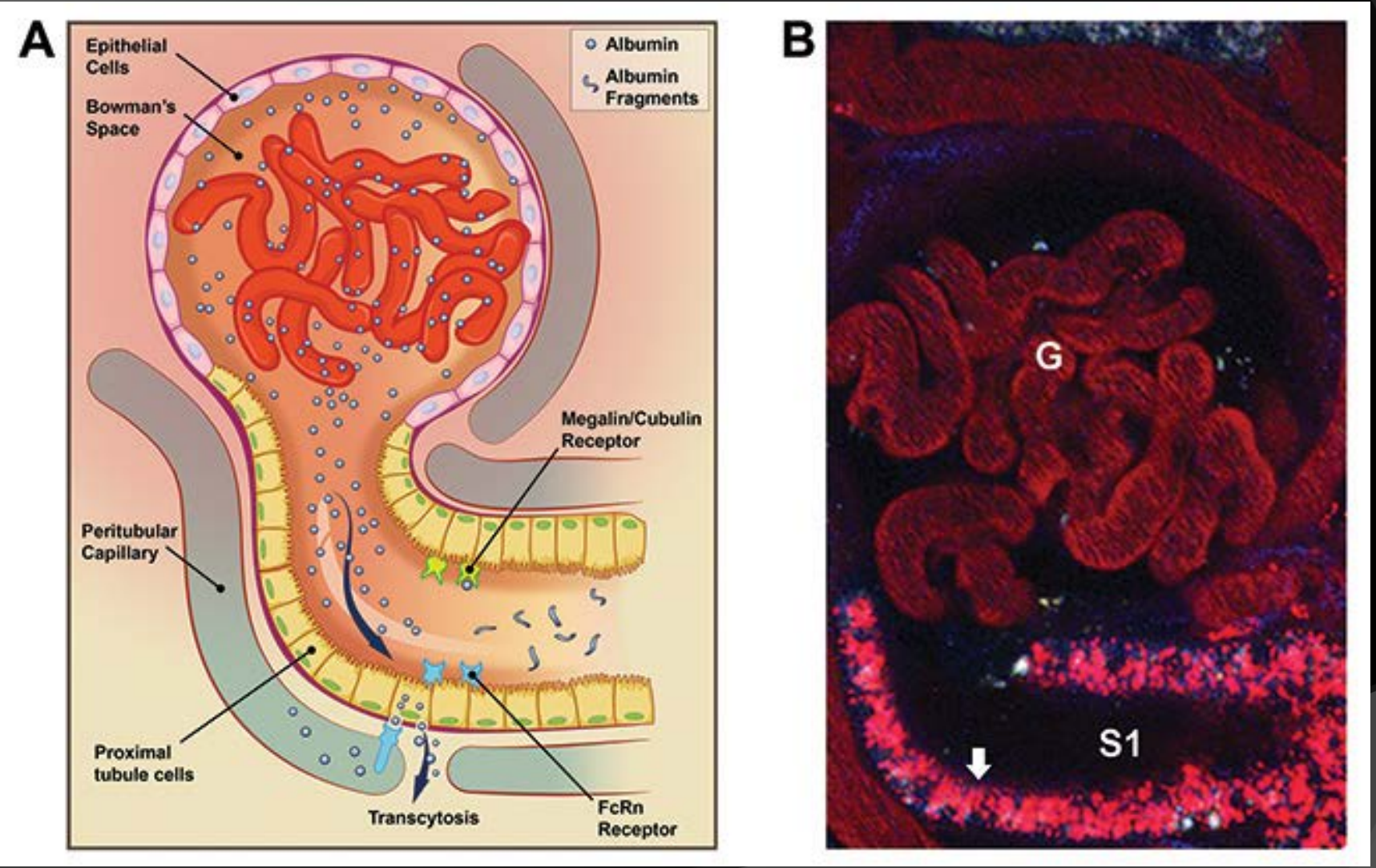
5 Frames/sec
500kDa FITC Dextran with
3kDa TR Dextran Injection







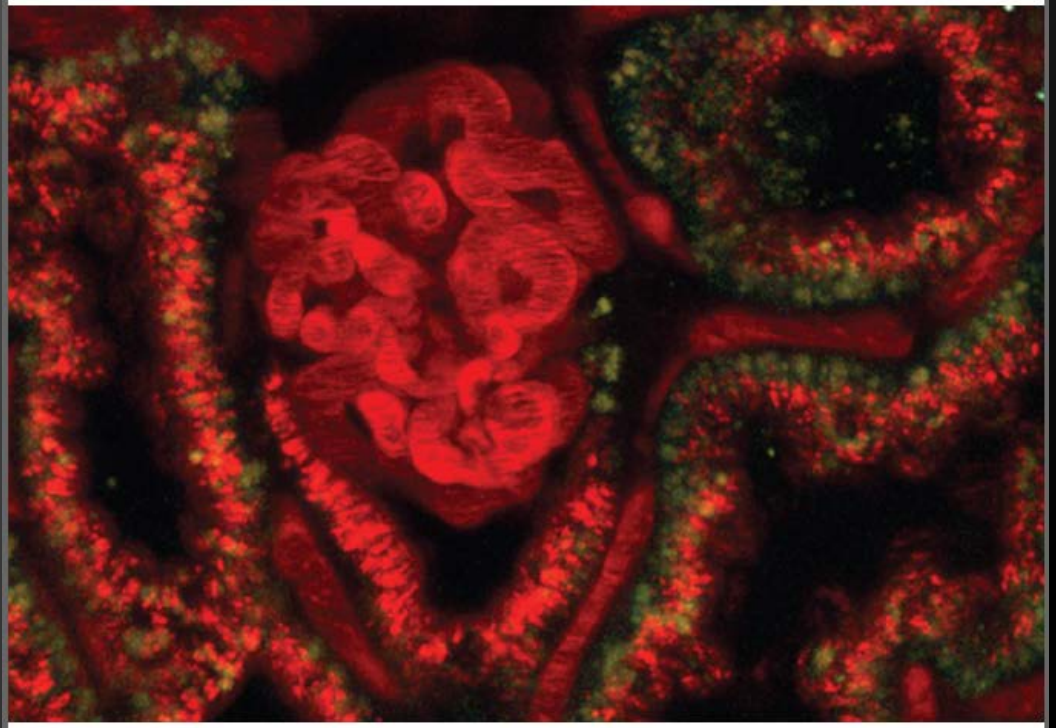
Renal Handling of Albumin by the PCT



Dickson et.al. JASN 2014

OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

kidney INTERNATIONAL



VOLUME 71 | ISSUE 6 | MARCH (2) 2007
http://www.kidney-international.org

Albumin filtration

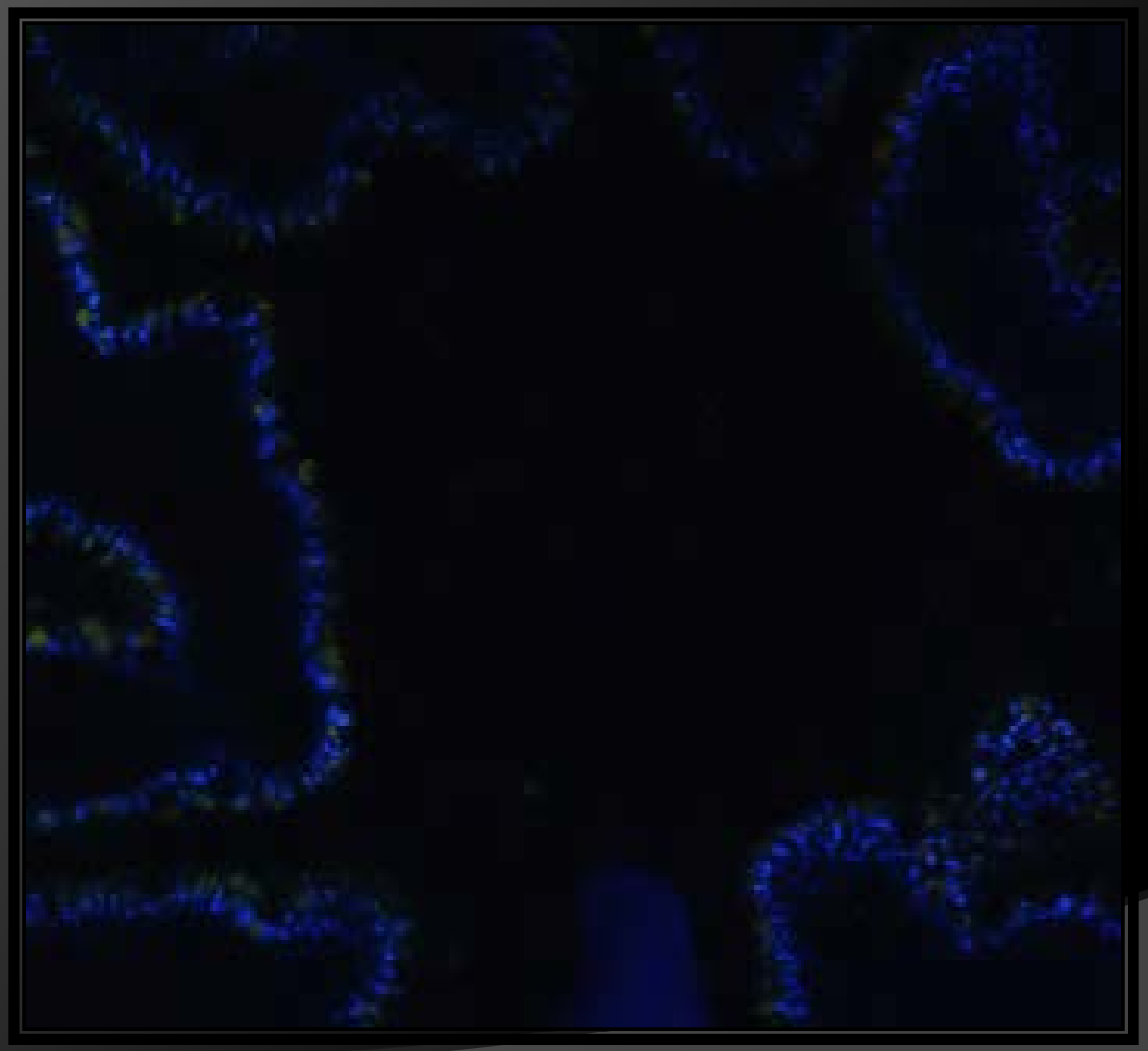
Classification of lupus nephritis

Peritoneal dialysis solutions

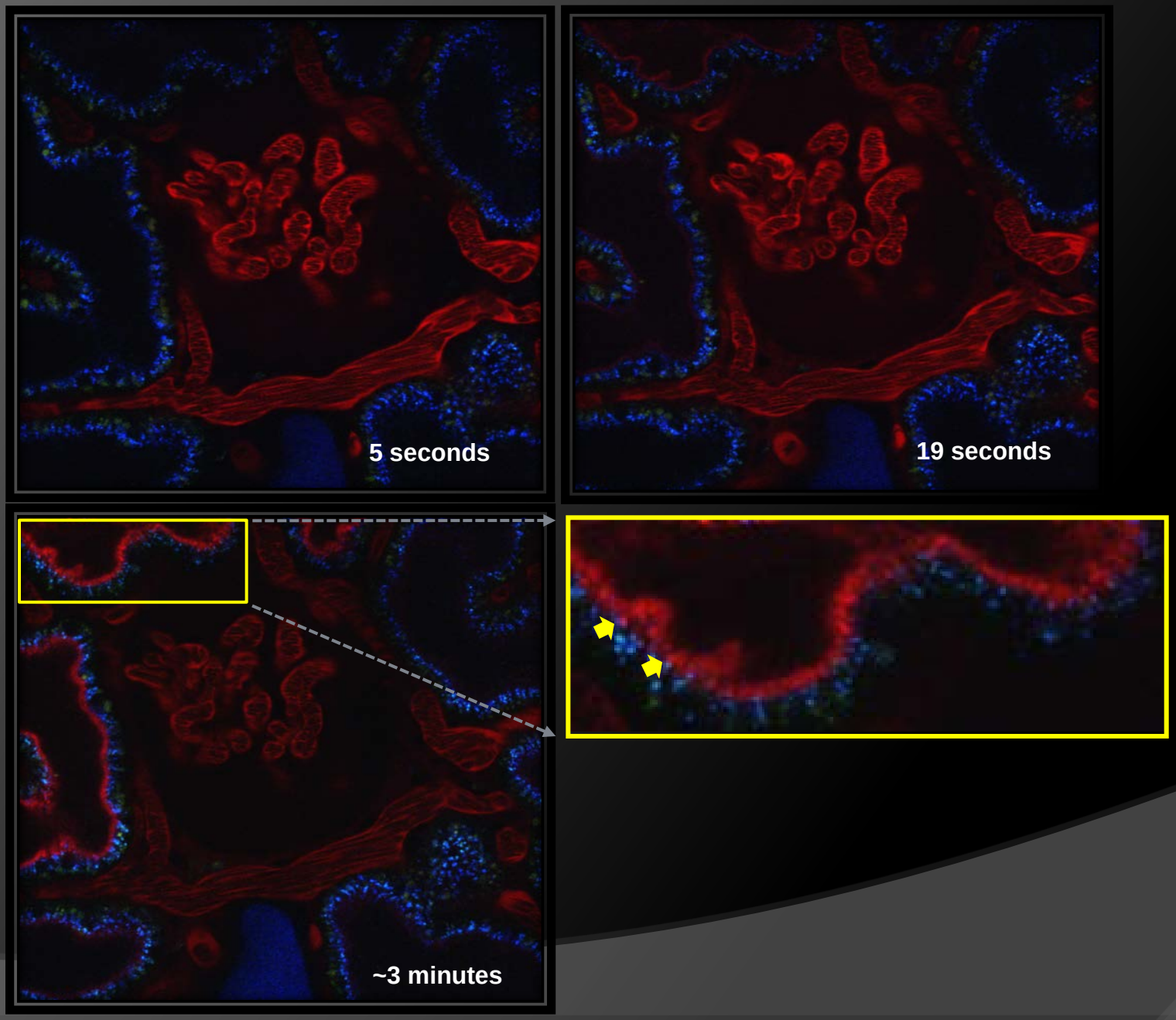
Challenges

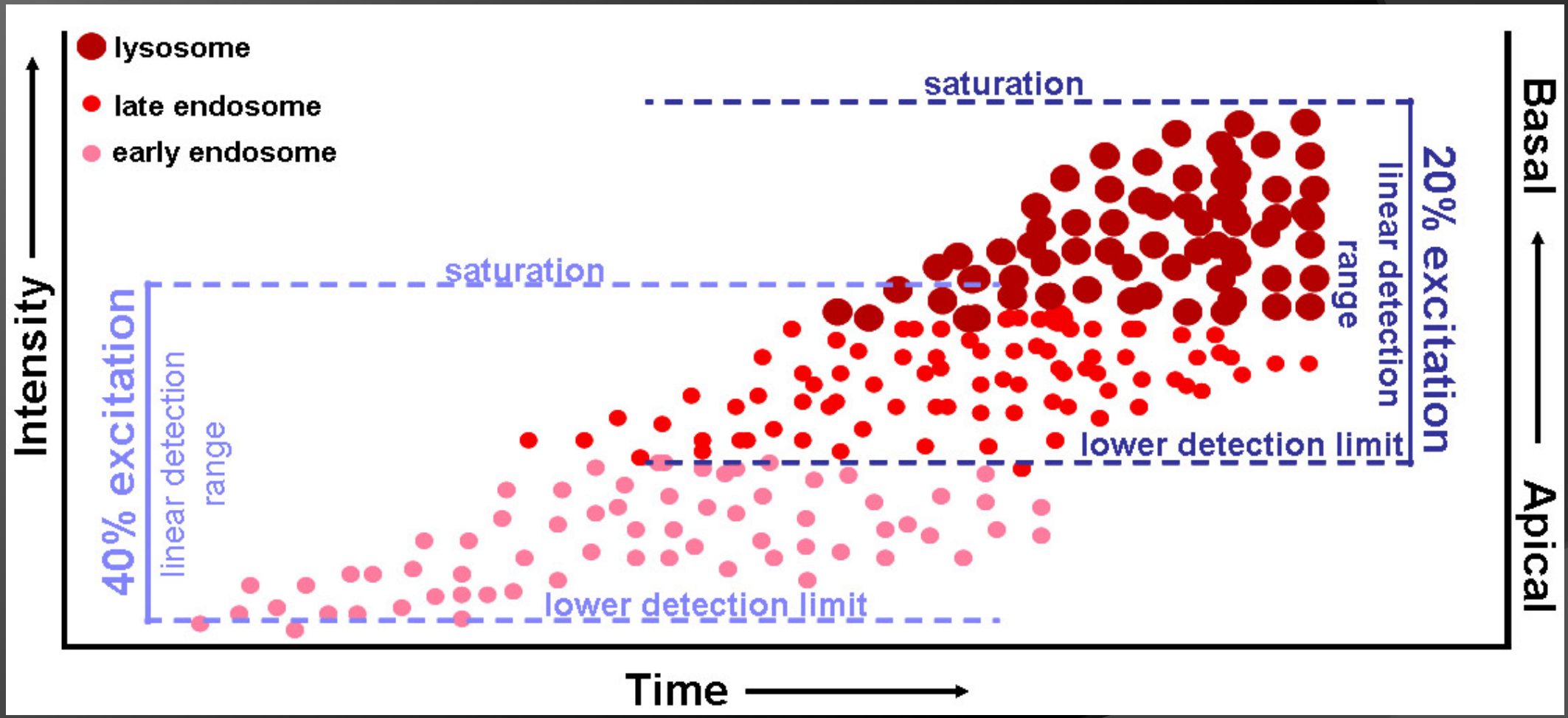
1. Dogma, Assumptions, Biology, Reagents, Sensitivity
2. Quantitative Analysis without Gold Standards
3. You See What you are Looking For
4. Correcting for Depth of Field
5. Out of Focus Fluorescence
6. Physiologic state of the rat

Albumin Filtration and Reabsorption in the Rat

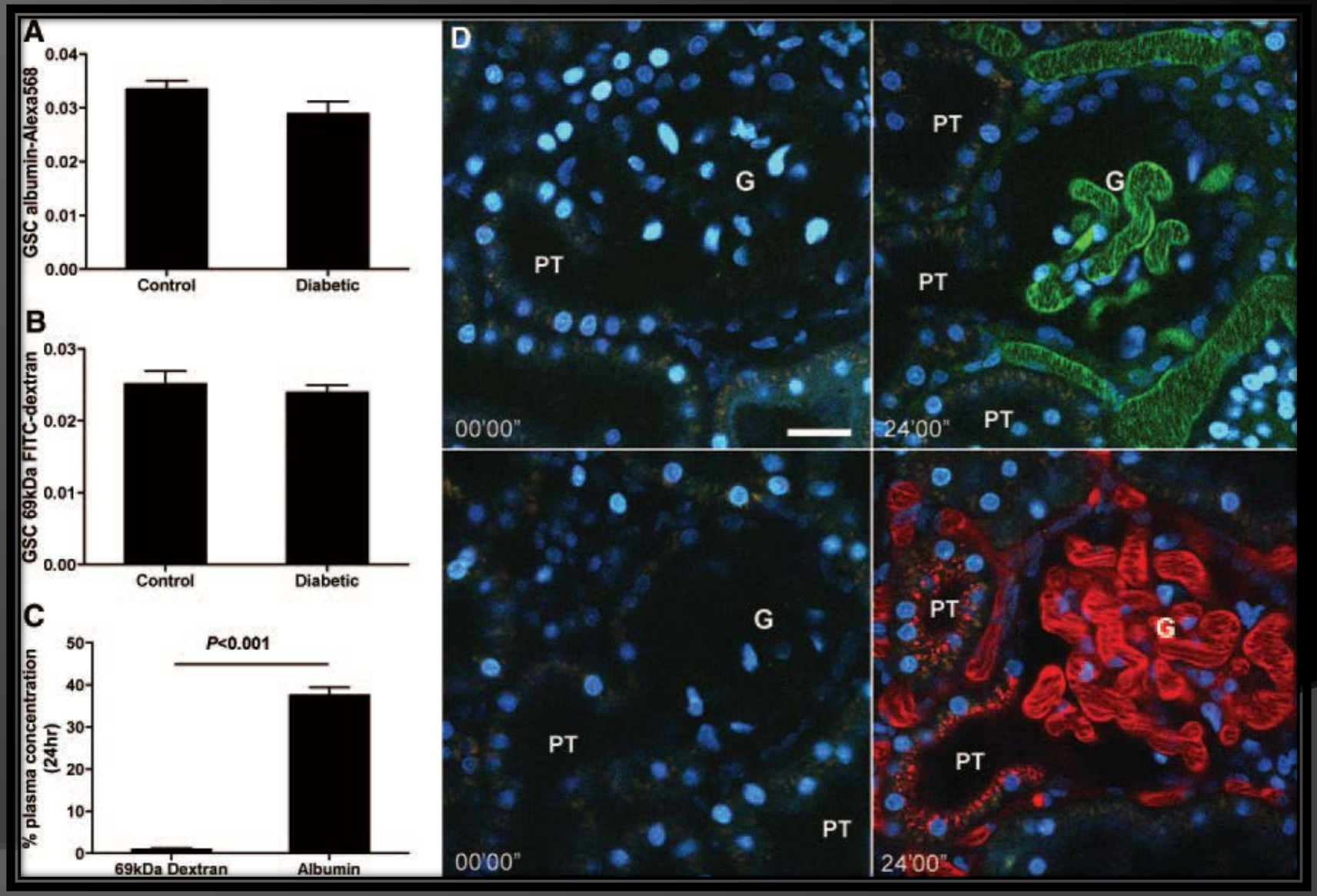


Albumin Filtration and Reabsorption in the Rat



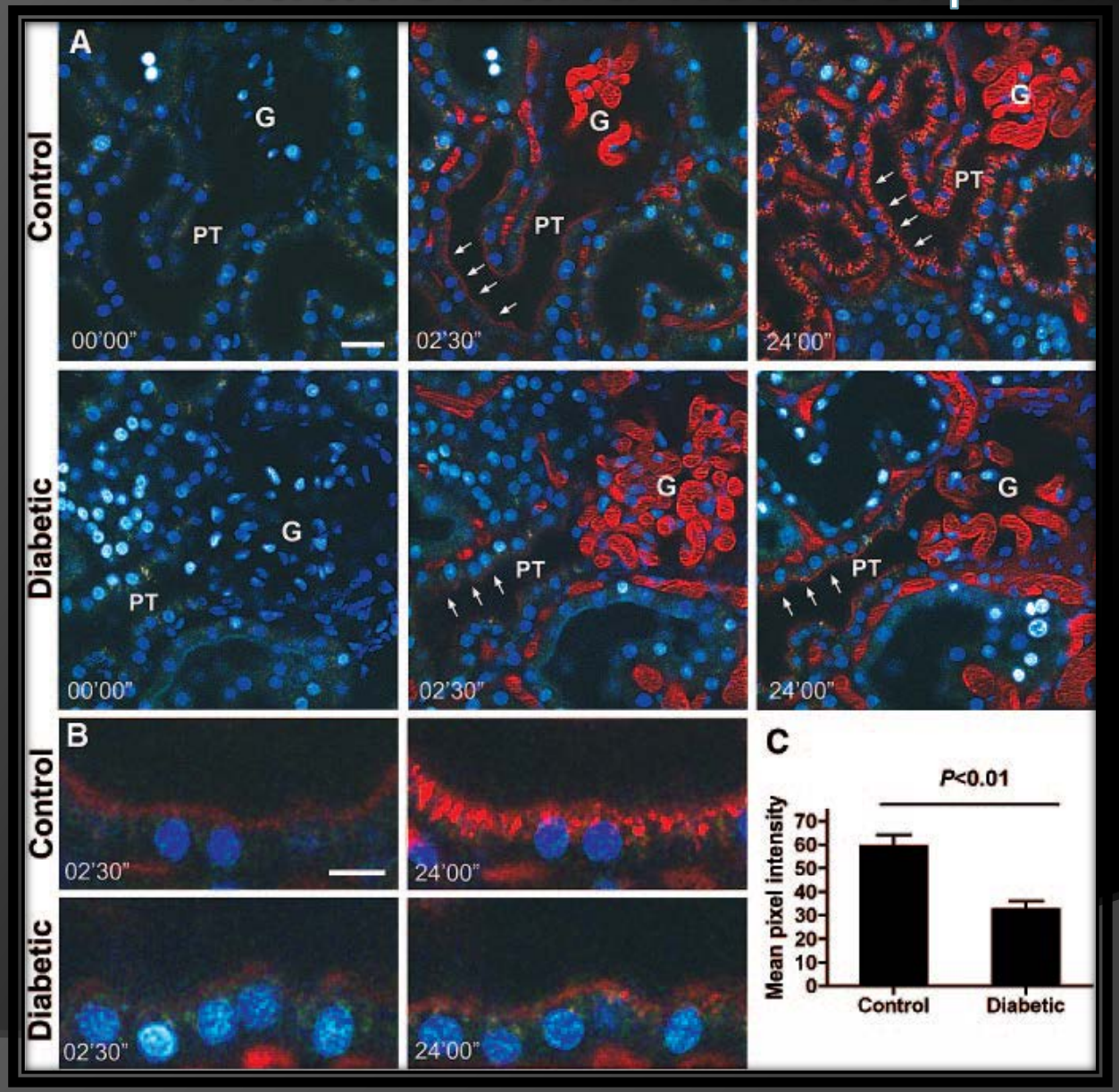

lysosome
late endosome
early endosome
saturation
20% excitation
linear detection range
lower detection limit
saturation
40% excitation
linear detection range
lower detection limit
Intensity
Time
Basal
Apical

Effect of Early Diabetes in the Rat on Albumin Filtration and Reabsorption



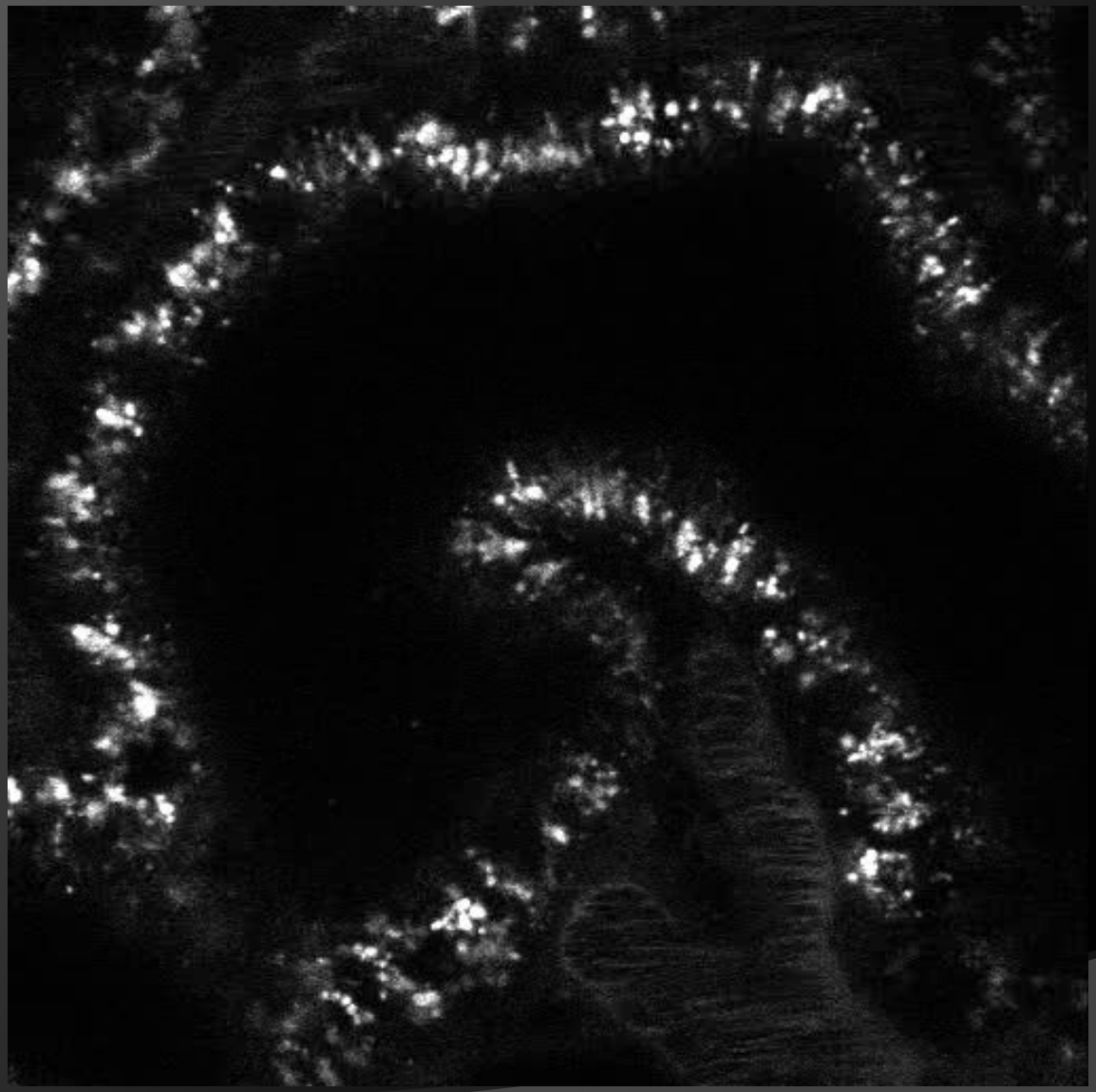
Russo et.al. JASN 2009

Effect of Early Diabetes in the Rat on Albumin Filtration and Reabsorption



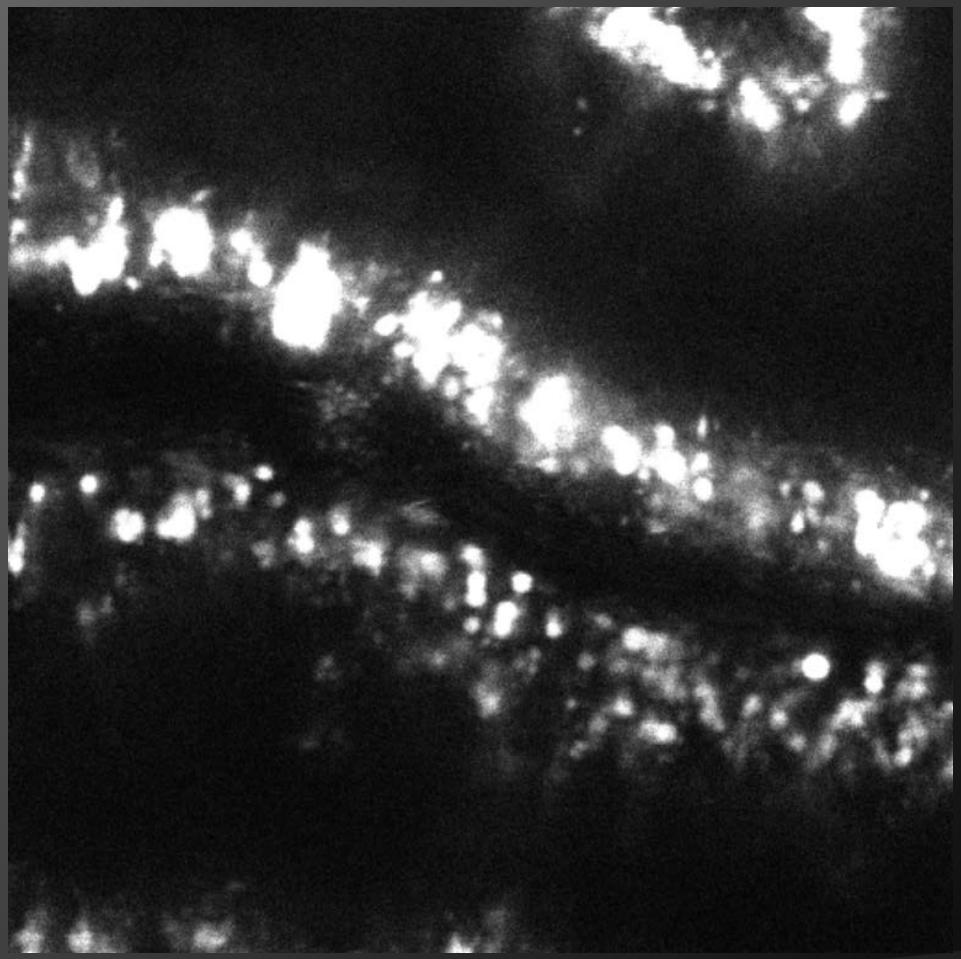
Russo et.al. JASN 2009

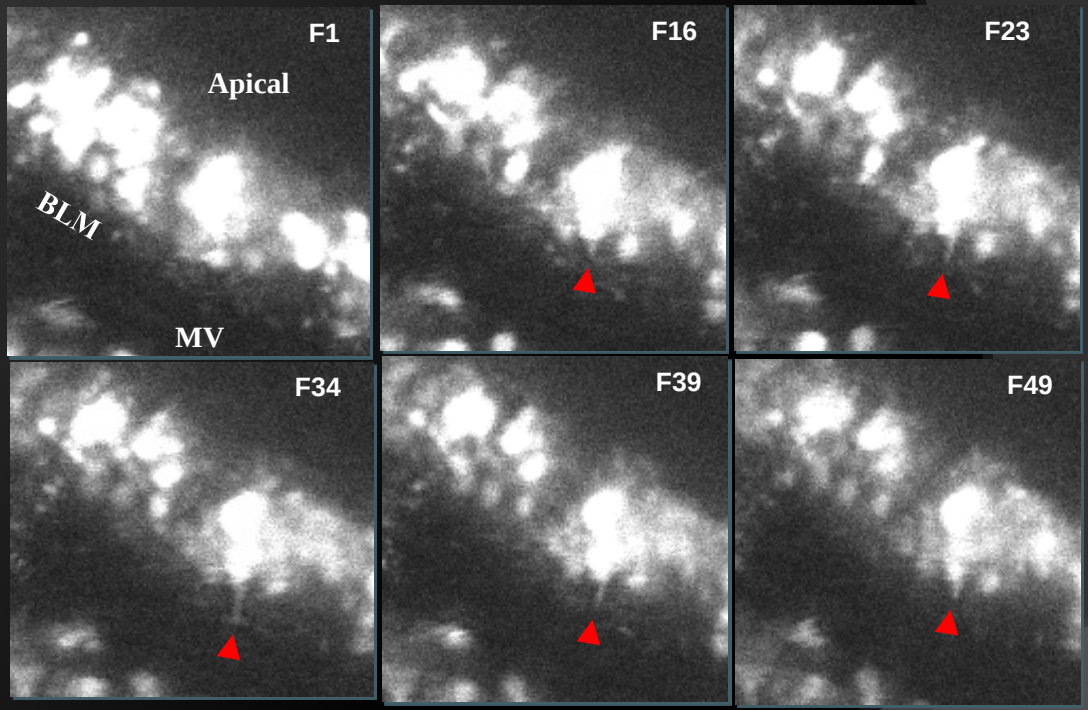
Albumin Transcytosis



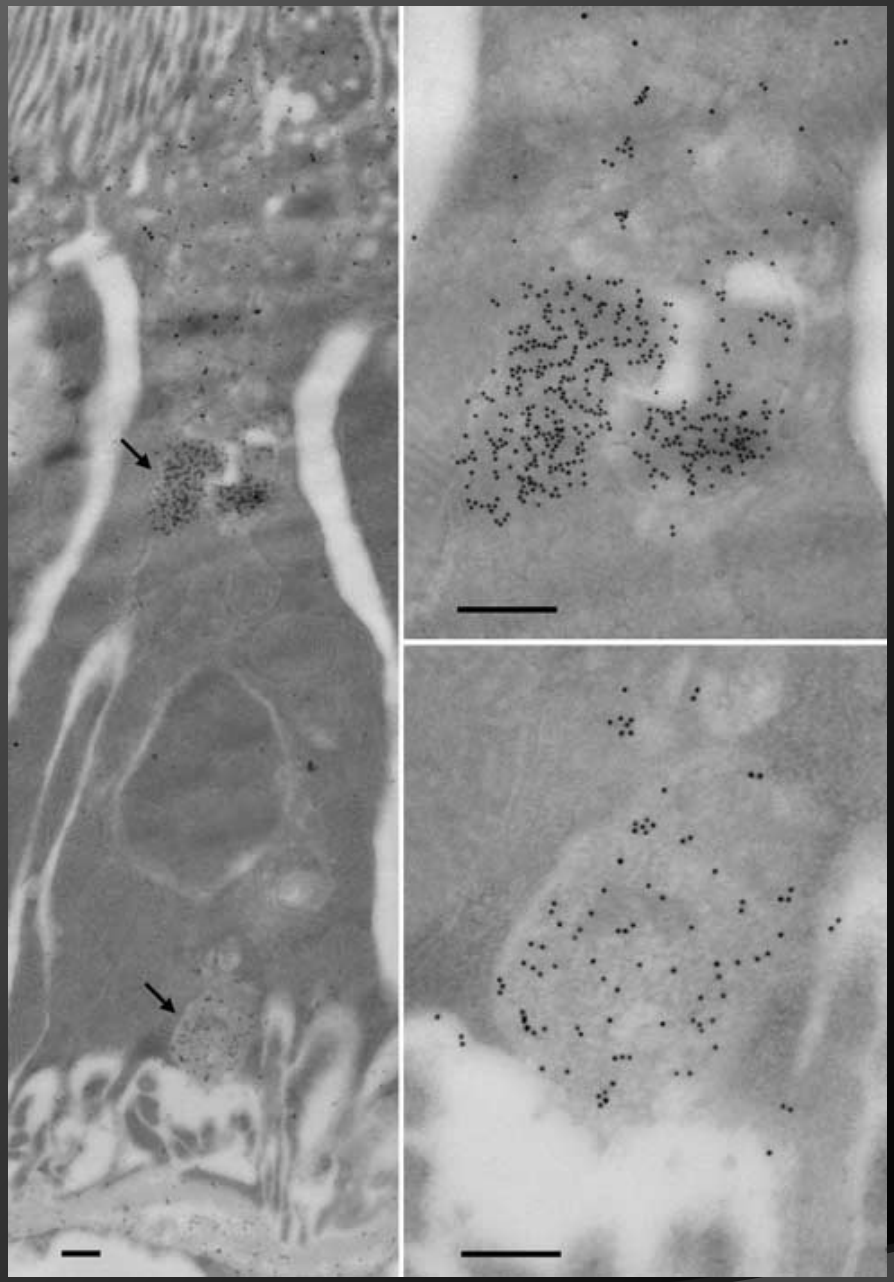
Sandoval et.al. JASN 2012

PTC Albumin Transcytosis





Sandoval et.al. JASN 2012



EM Gold Visualization of RSA Endocytosis and Transcytosis in a Rat PTC

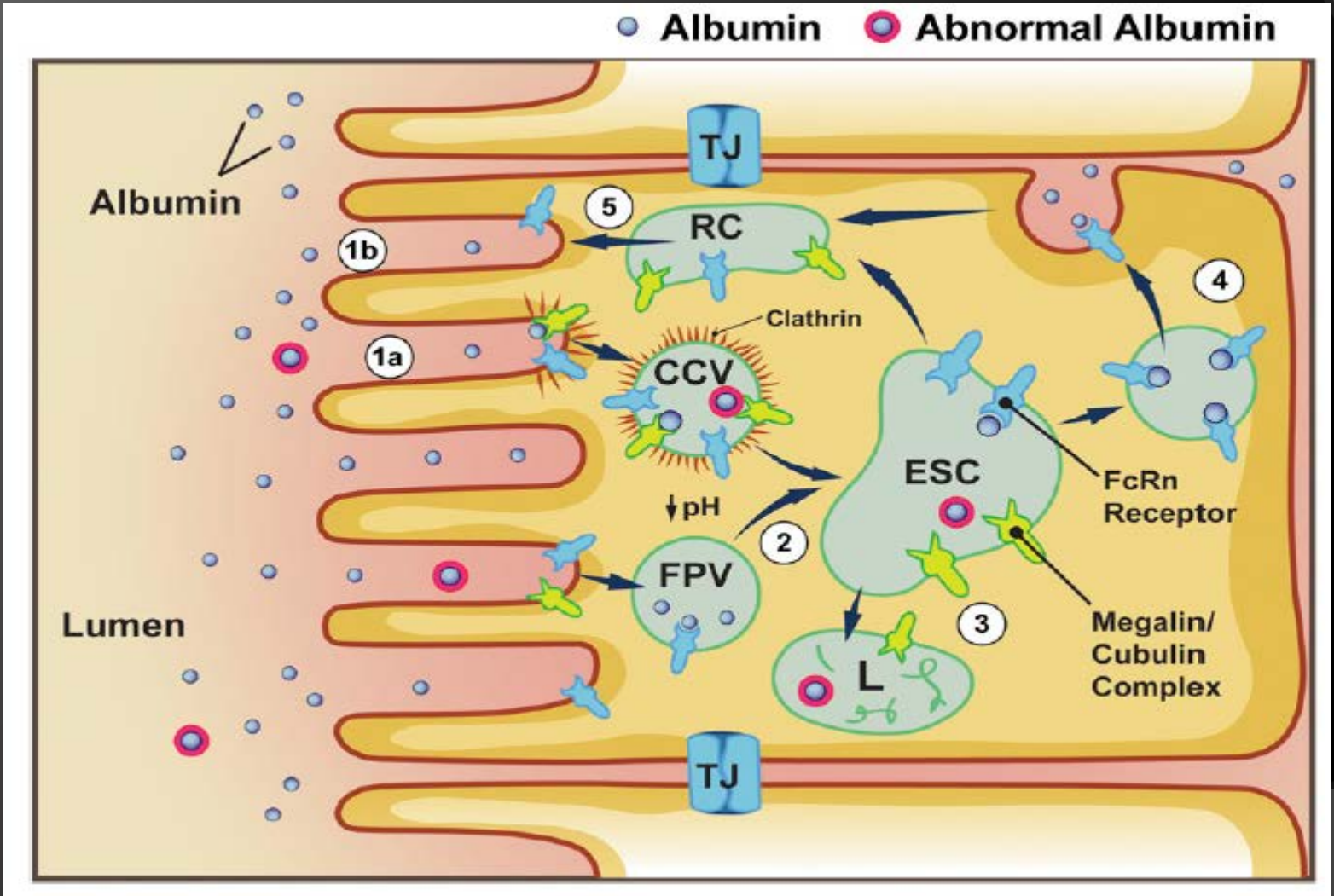
Russo, LM et.al. Kidney Inter 2007

JASN ASN.2013010018; published ahead of print August 22, 2013,

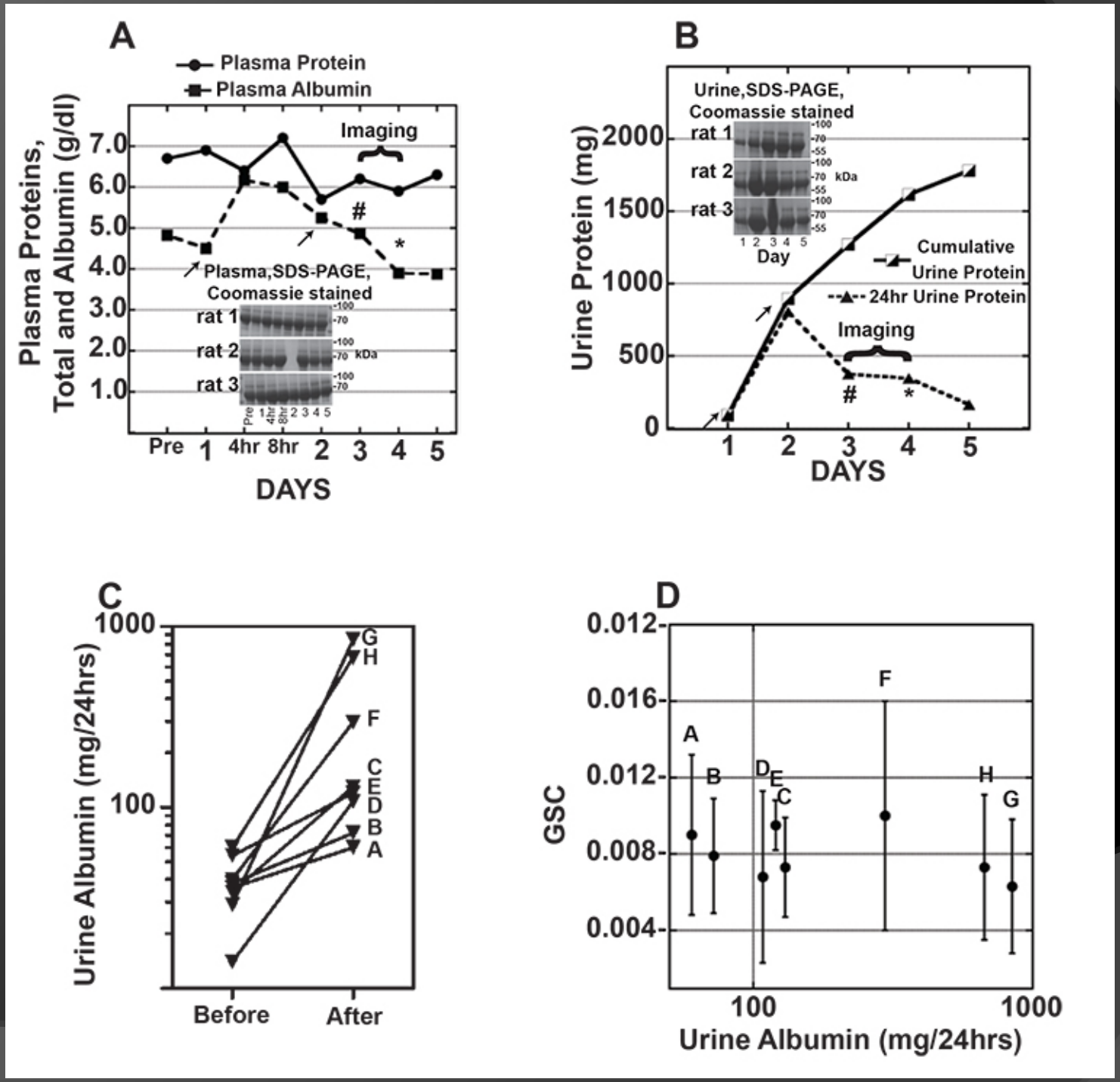
Albumin Is Recycled from the Primary Urine by Tubular Transcytosis.

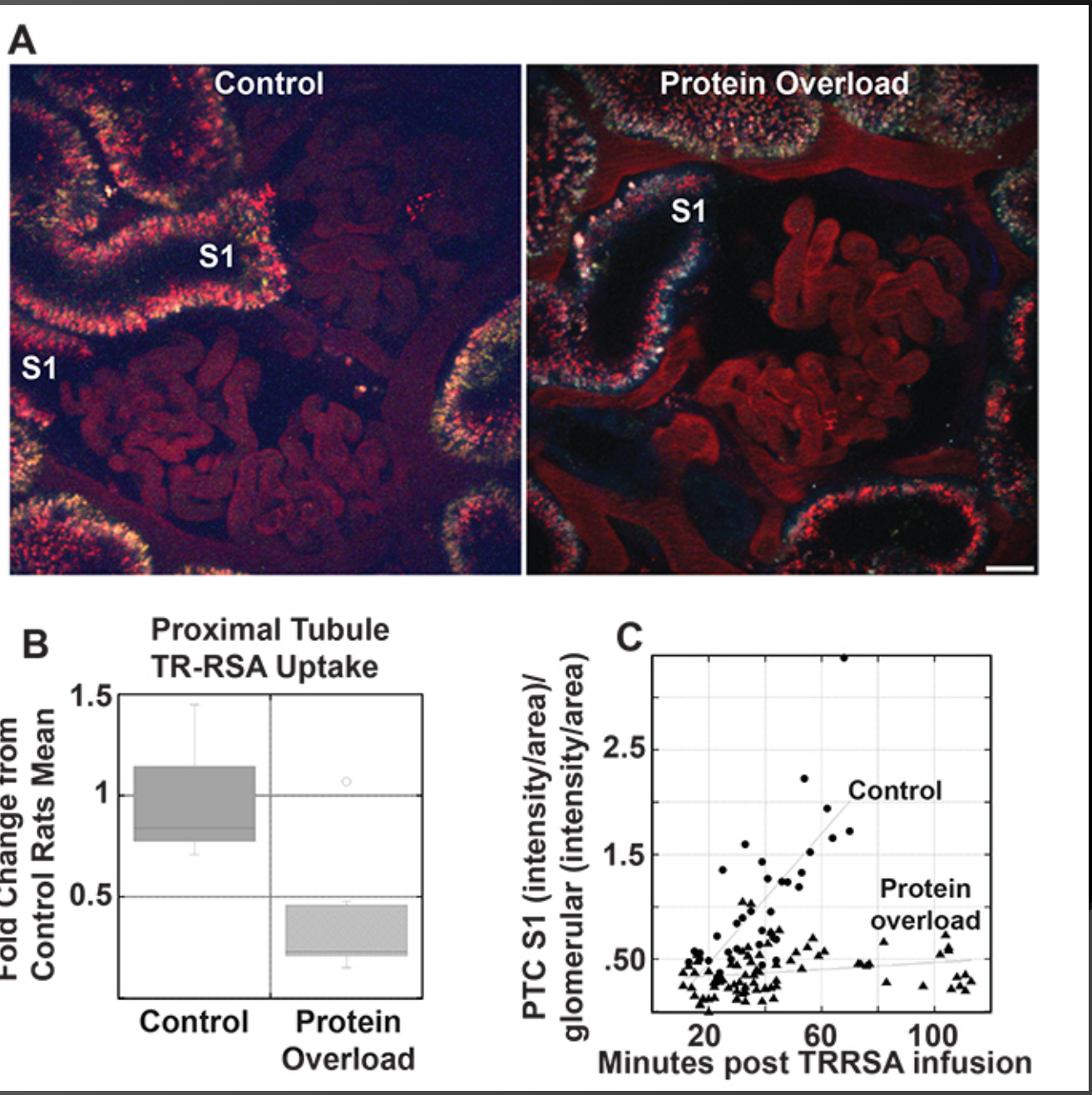
Tenten V, Menzel S, Kunter U, Sicking EM, van Roeyen CR, Sanden SK, Kaldenbach M, Boor P, Fuss A, Uhlig S, Lanzmich R, Willemsen B, Dijkman H, Grepl M, Wild K, Kriz W, Smeets B, Floege J, Moeller MJ.

J. Am. Soc. Nephrol., Aug 2013



Dickson LE et al, JASN 2014


A
Plasma Protein
Plasma Albumin
Plasma Proteins, Total and Albumin (g/dl)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
Imaging
#
*
Plasma,SDS-PAGE, Coomassie stained
rat 1
rat 2
rat 3
-100
-70
kDa
Pre 1 4hr 8hr 2 3 4 5
Pre 1 4hr 8hr 2 3 4 5
DAYS
B
Urine,SDS-PAGE, Coomassie stained
rat 1
rat 2
rat 3
-100
-70
-55
kDa
1 2 3 4 5
Day
Urine Protein (mg)
2000
1500
1000
500
0
Cumulative Urine Protein
24hr Urine Protein
Imaging
#
*
1 2 3 4 5
DAYS
C
Urine Albumin (mg/24hrs)
1000
100
G
H
F
C
E
D
B
A
Before
After
D
GSC
0.012
0.016
0.012
0.008
0.004
A
B
D
E
C
F
H
G
100
1000
Urine Albumin (mg/24hrs)


A
Control
S1
S1
Protein Overload
S1
B
Proximal Tubule
TR-RSA Uptake
Fold Change from
Control Rats Mean
1.5
1
0.5
Control
Protein
Overload
C
PTC S1 (intensity/area)/
glomerular (intensity/area)
2.5
1.5
.50
Control
Protein
overload
20
60
100
Minutes post TRRSA infusion

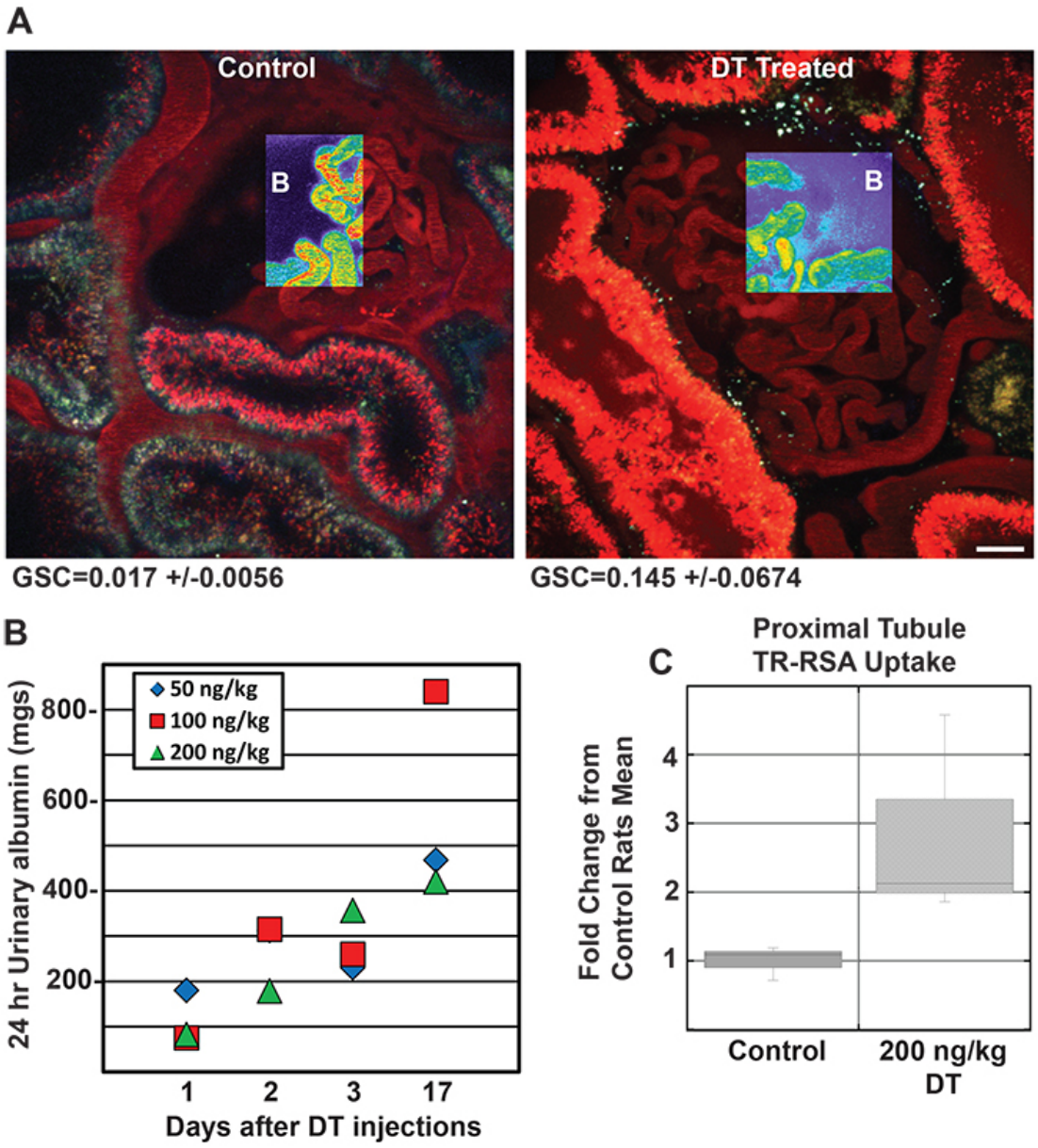
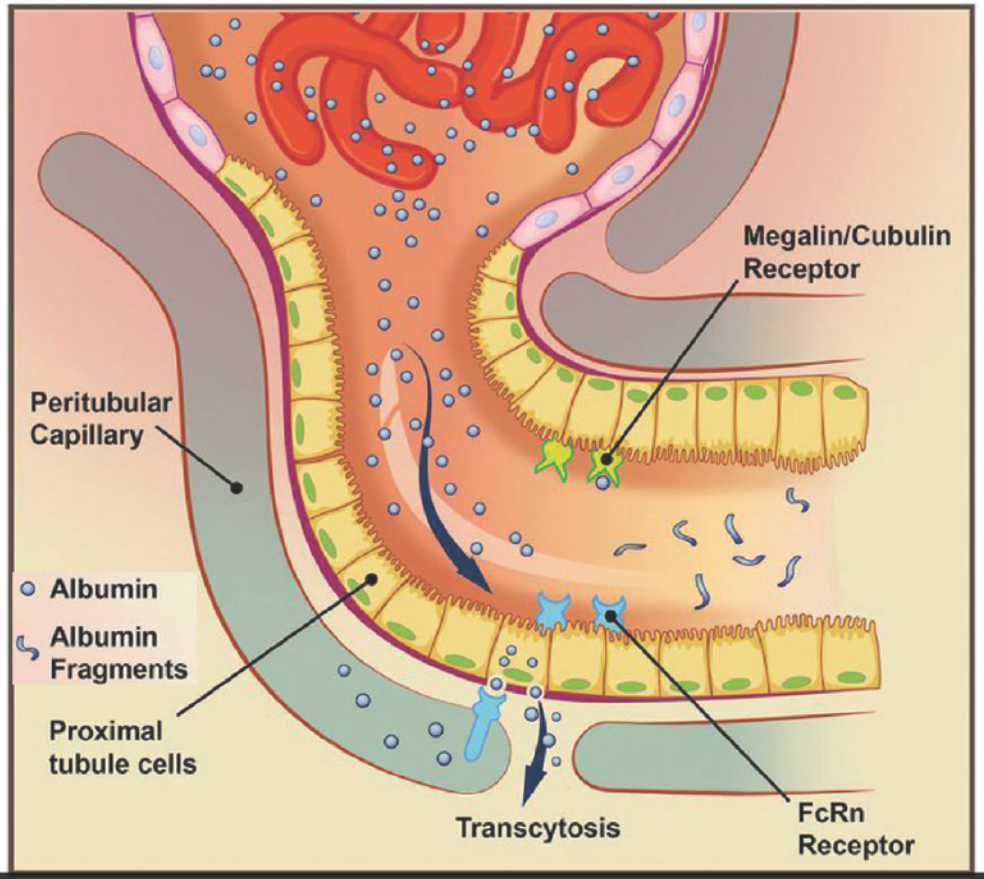

A
Control
DT Treated
B
B
GSC=0.017 +/-0.0056
GSC=0.145 +/-0.0674
B
50 ng/kg
100 ng/kg
200 ng/kg
24 hr Urinary albumin (mgs)
800
600
400
200
1
2
3
17
Days after DT injections
C
Proximal Tubule
TR-RSA Uptake
Fold Change from
Control Rats Mean
4
3
2
1
Control
200 ng/kg
DT

Figure 3

Figure 5



	GFR (ml/min)	Serum Alb (mg/ml)	GSC_A	Albumin Filtered (mg/24hr)	PT Uptake (%)	Urine Alb Calculated (mg/24hr)	Urine Alb Observed (mg/24hr)
Control	1.4	45	0.008	725	95	X	38
Protein Overload	1.4	45	0.008	725	37	457	329


MD

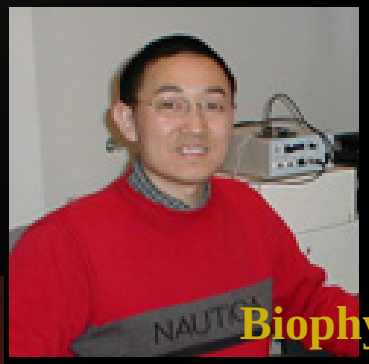

Cell Biologist

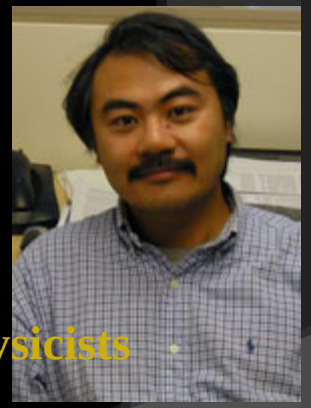

MD

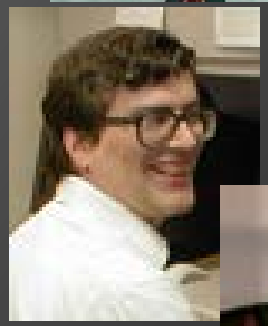

Research Associate

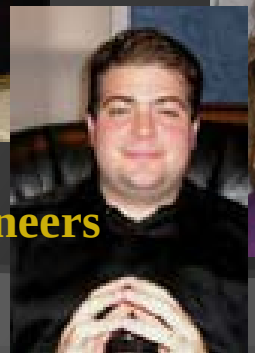

Physiologist


MD PhD




Biophysicists

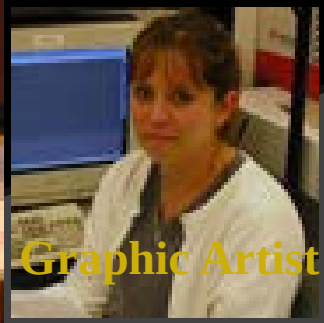

Engineers

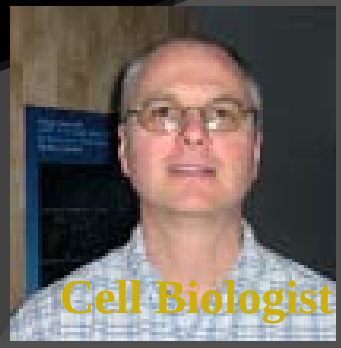



MD


Molecular Biologist


Cell Biologist


Graphic Artist


Cell Biologist