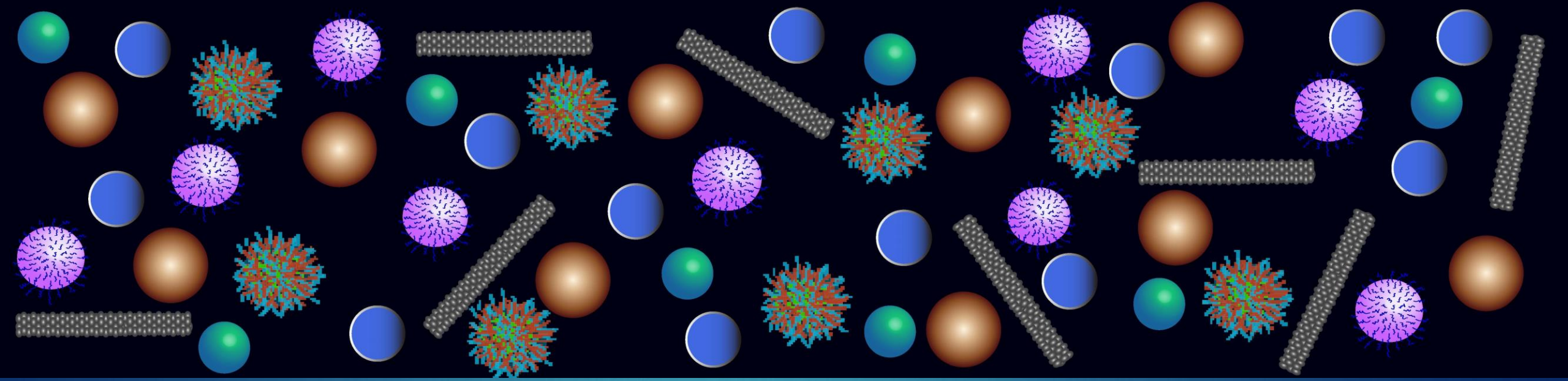
Polymeric Mesoscale Nanoparticles Selectively Target Gene Therapies to the Kidneys

Ryan M. Williams, PhD

CRS 2025 ANNUAL MEETING & EXPOSITION
PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS



Polymeric Mesoscale Nanoparticles Selectively Target Gene Therapies to the Kidneys

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Controlled Release Society Annual Meeting

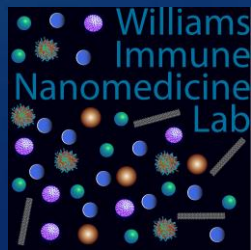
Tech Session IV: Nanomedicine and Nanoscale Delivery (Focus: Gene)

July 16, 2025

The City College of New York
The Grove School of Engineering

Disclosures

- Founder and Director of Zipcode Therapeutics



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Julia Morris, NSF RaMP Postbac

Shakuntala Sookraj, BME Undergrad

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Williams Immune Nanomedicine Lab

Williams
Immune
Nanomedicine
Lab

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Shengyu Mu, MD, PhD, University of Arkansas College of Medicine
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Lilach Lerman, MD, PhD, Mayo Clinic
And others just getting started…

Williams Immune Nanomedicine Lab

Acknowledgements

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Stand up to Cancer DISRUPT Project
The Grove School of Engineering, CCNY
The City University of New York

Williams Inflammatory Nanomedicine Lab

My lab focuses on the design of nanomedicines to treat and detect chronic inflammation associated with cancer and renal disease

Biomaterials, nanotechnology, materials science, chemistry, collaboration with engineers and chemists

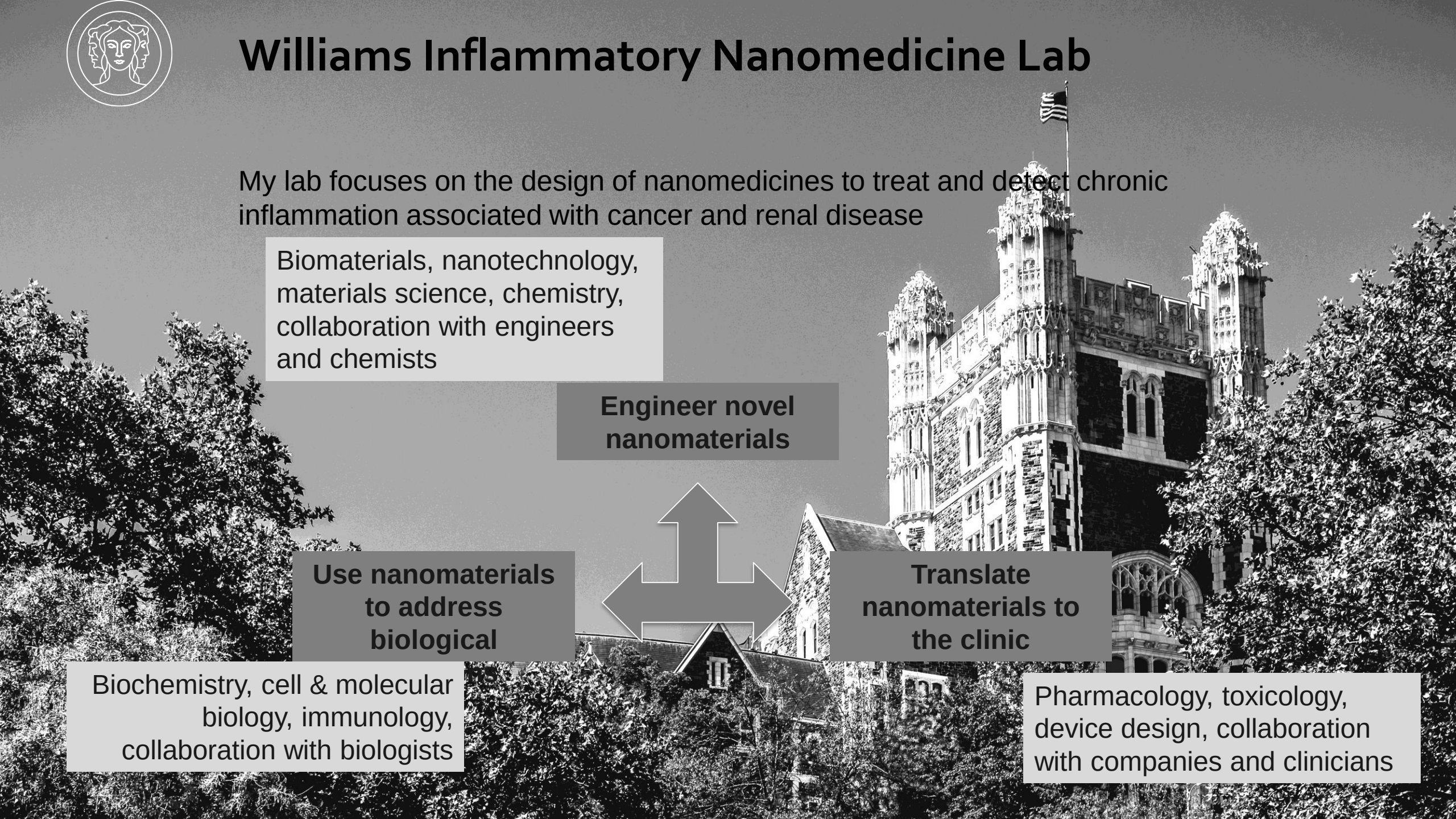
Engineer novel nanomaterials

Use nanomaterials to address biological

Translate nanomaterials to the clinic

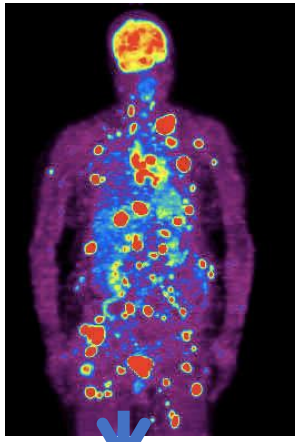
Biochemistry, cell & molecular biology, immunology, collaboration with biologists

Pharmacology, toxicology, device design, collaboration with companies and clinicians

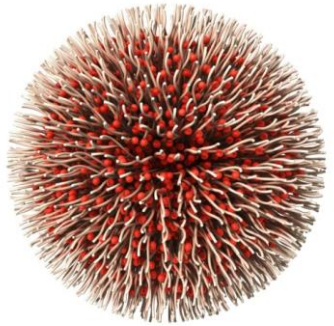


Challenges We are Addressing in our Lab with Nanomedicine

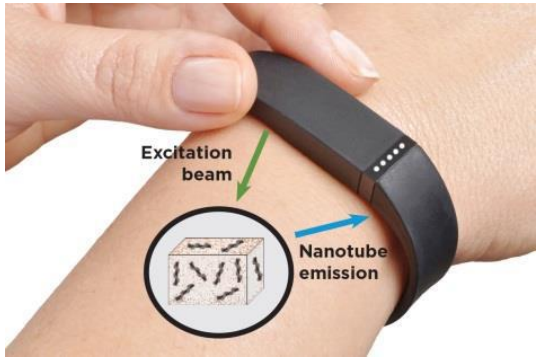
Targeting Therapies to Disease Sites



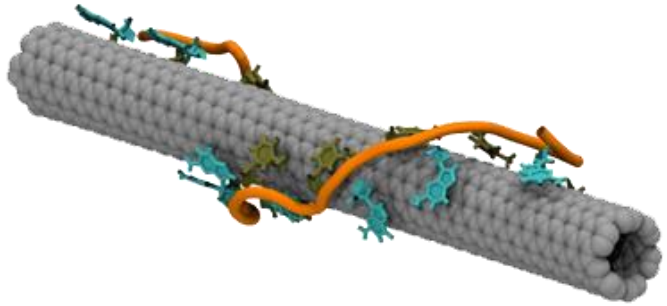
Targeted Nanoparticles



Early Disease Detection/ Therapeutic Monitoring



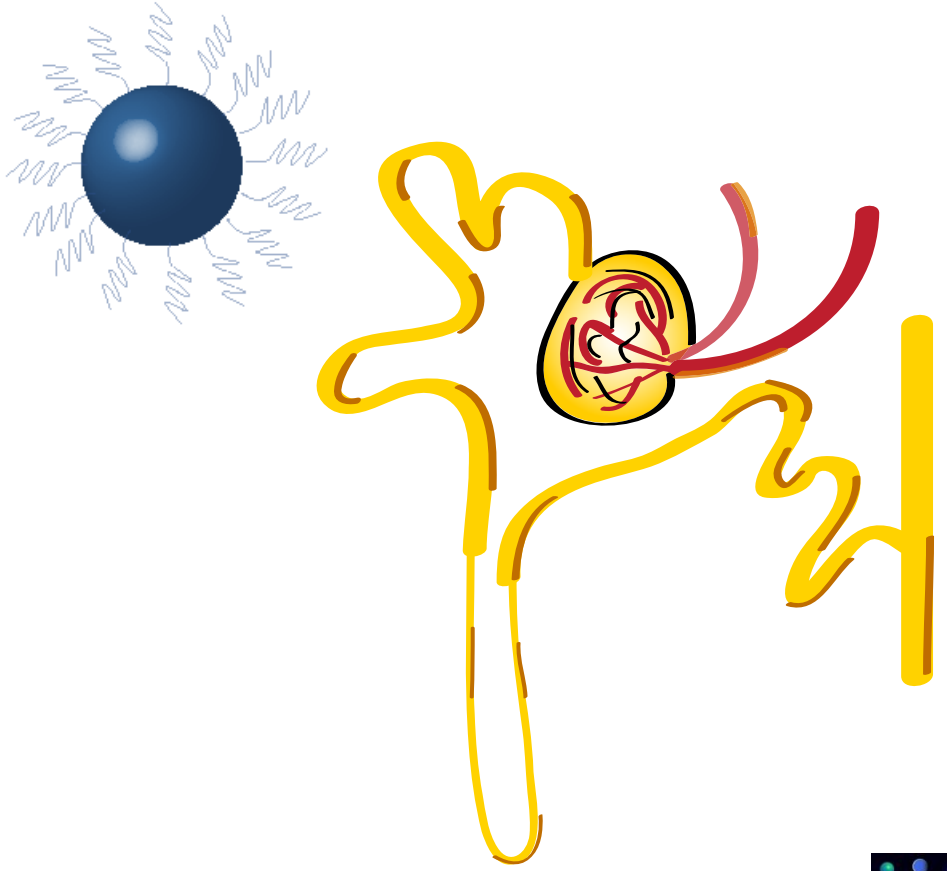
Optical Nanosensors



Williams Immune Nanomedicine Lab

MNPs Demonstrate Selective Kidney Delivery

- Polymeric mesoscale nanoparticles (MNPs) selectively target the kidneys in preclinical models due to their size and charge, resulting in liver avoidance
- Our data demonstrates kidney-selectivity and slowing CKD progression
  - MNPs have demonstrated efficacy in 6+ animal disease models with 6+ classes of cargo in 14+ publications
- MNPs are well-tolerated, biodegradable, and localize into tubular epithelial cells, where they release their cargo over time
- Expanding the platform…



Williams Immune Nanomedicine Lab

Kidney-Targeted Nanomedicine

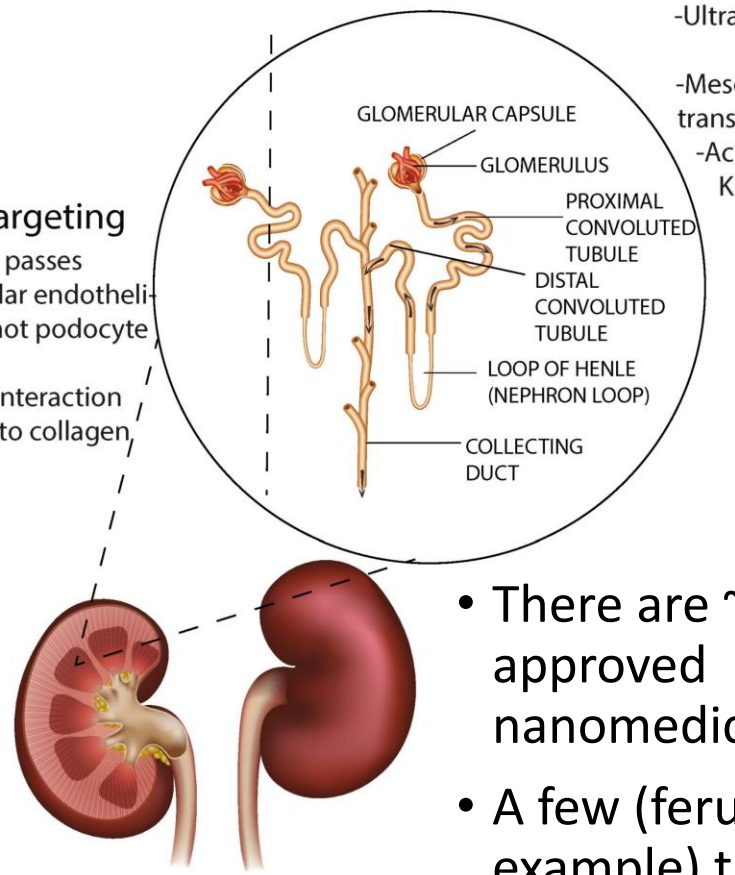
Lipid NPs/Liposomes
Liposomes
Phospholipids
Lipid Micelles

Polymer NPs
PLGA-PEG Mesoscale Nanoparticles
PLL-PEG; PEG-PCL-PEI; PVP
Chitosan; Sorbitol-PEI;
Phosphoester; Pluronic
PAMAM; PLGA

Protein, Peptide, and Nucleic Acid NPs
Albumin
DNA origami
Cell penetrating peptide
Protein nanocage

Inorganic NPs
Perfluorocarbon
Quantum dots
Selenium; Iron oxide
Gold; Germanene
SWCNT; Zeolite
Mesoporous Silica

Glomerular targeting
-Size (10-100 nm) passes through glomerular endothelium fenestra but not podocyte foot processes
-Cationic charge interaction
-Active targeting to collagen



Tubular targeting
-Ultra-small NPs (<10 nm) pass through glomerulus
-Mesoscale NPs (300 - 500 nm) transcytosed from vasculature
-Active targeting to megalin, Kim-1, CD44, CXCR4, folate receptor
-Increased permability in disease models

- There are ~100 FDA approved nanomedicines
- A few (ferumoxytol, for example) treat kidney diseases, but do not act directly on the kidney

- As of last year, there were ~36 particle systems in the literature that purported to target the kidney or directly treat the kidney disease
- ~5 were published prior to 2015, and 66% were published over the past 2 years (my lab keeps a running list but haven't updated our tally!)
- This list does not guarantee kidney "specificity" or safety/efficacy

Vasylaki *et al.* 2024 *ASN Kidney360* (Review)

Patients with Kidney Diseases Have High Unmet Needs

700 million Chronic Kidney Disease (CKD) patients worldwide

$66 Bn market in 2022

CAGR of 5.4% to $100 Bn through 2030

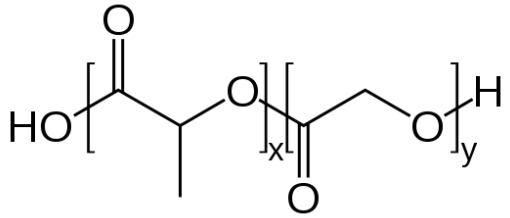
Slowing down kidney damage is a major unmet need

Market Research Future, 2023

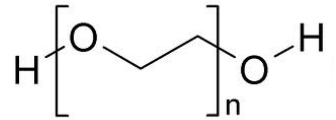
The Polymer MNP Platform is Unique in Size and Surface Chemistry

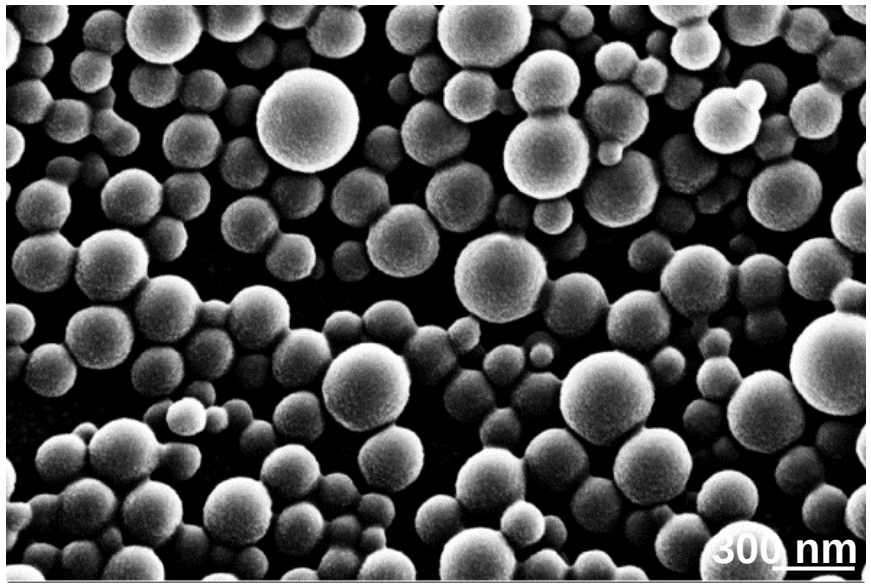
- **300-500 nm diameter biodegradable polymer mesoscale nanoparticles**; advanced and validated over ten years in our lab and with collaborators
- Mesoscale nanoparticles are larger than typical LNPs (100 nm) and smaller than microparticles (1000 nm)
- Made from biodegradable materials used in other FDA-approved products
- The MNP formulation is lyophilized for long-term storage and ambient-temp shipping

Poly(lactic-*co*-glycolic) acid (PLGA)
Degradable though ester bond cleavage

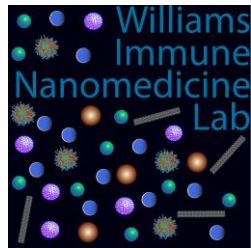


Polyethylene Glycol (PEG)
Prevents opsonization





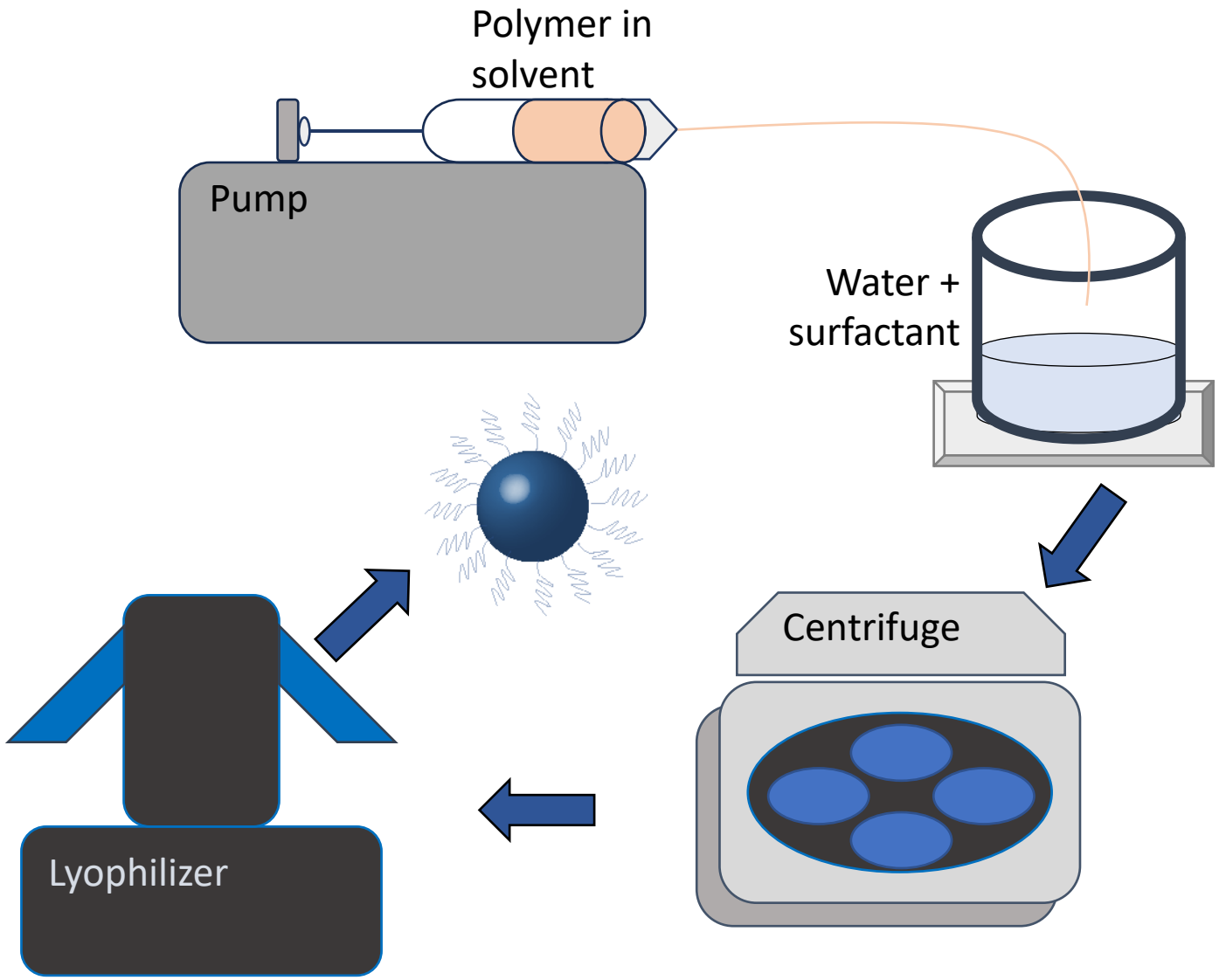
Scanning Electron Micrograph



Williams *et al.*, *Nano Letters*, 2015

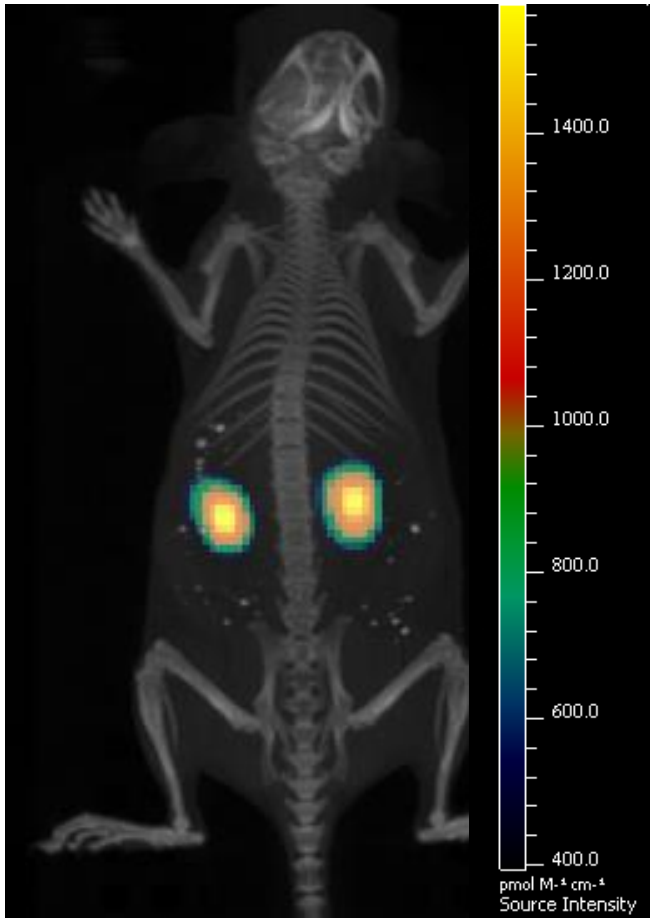
The MNP Formulation Process is Straightforward and Scalable

- MNPs are formulated by a simple nanoprecipitation method
  - The polymer and cargo are dissolved in a solvent
  - This solution is added dropwise to a water + surfactant solution
  - MNPs form through miscibility of solvent/water causing surface tension
- MNPs are stirred to solidify, centrifuged to purify, then lyophilized for long-term storage
- Ratios of polymer, solvent, and water are controlled for size precision and yield
- This formulation process is scalable

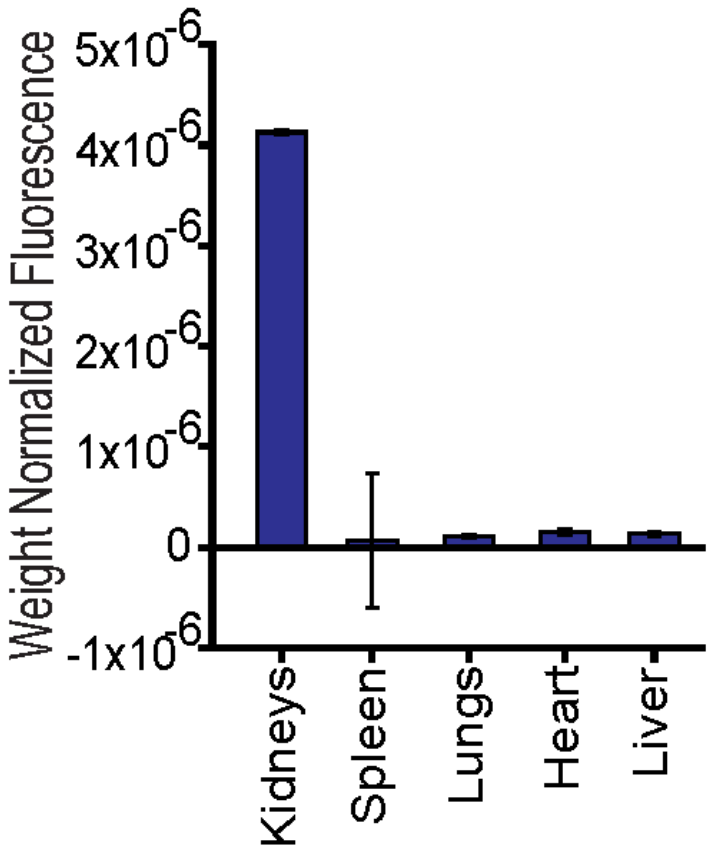


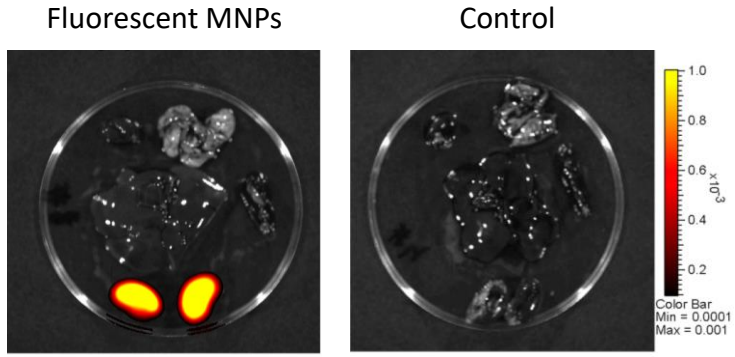
Williams *et al.*, *Nano Letters*, 2015

MNPs Preferentially Localize to the Kidneys



Fluorescent Cy5 Dye Loaded in MNPs IV injected into a mouse





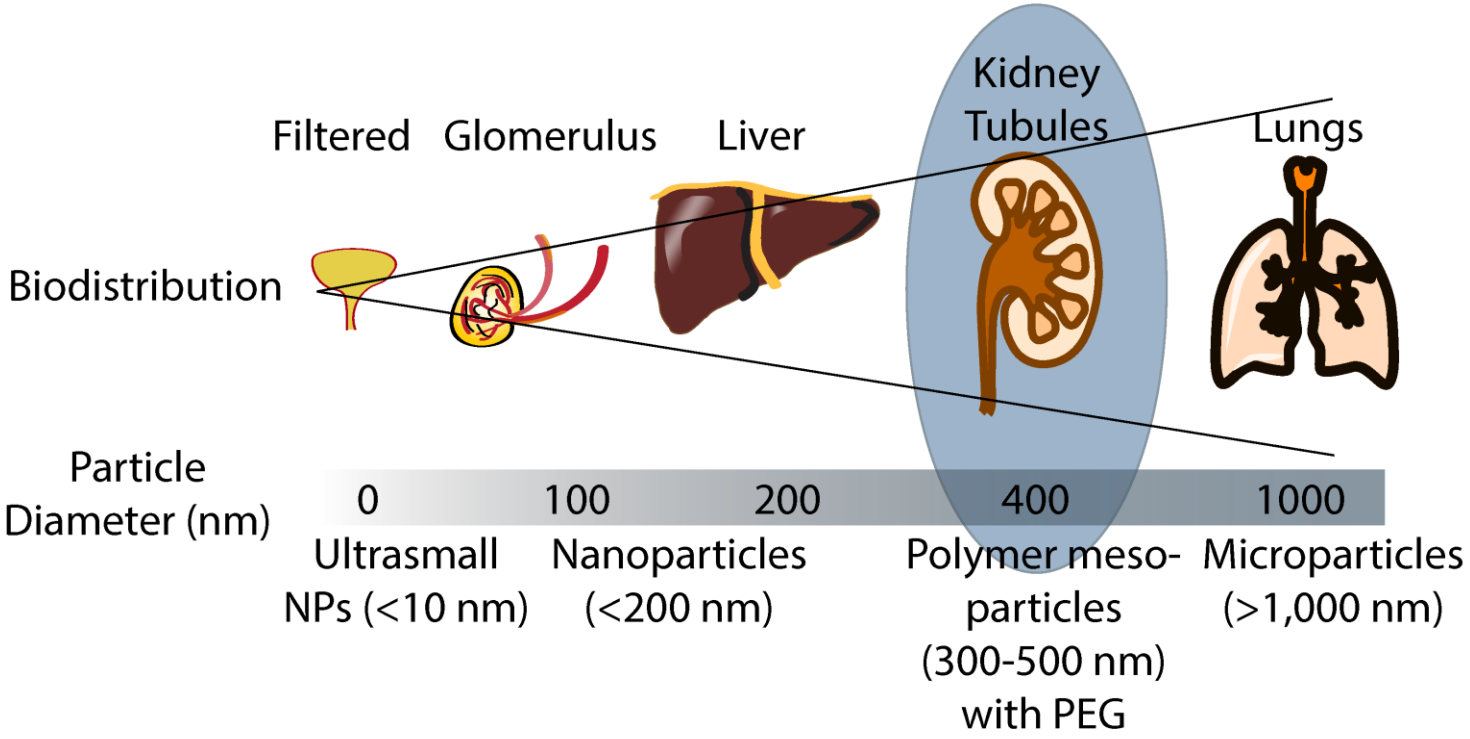
MNPs target the kidneys up to **<u>26x more than any other organ</u>** following IV injection

This uniquely-designed delivery system **<u>avoids uptake in the liver</u>** and other organs due to its size and charge

Williams *et al.*, *Nano Letters*, 2015

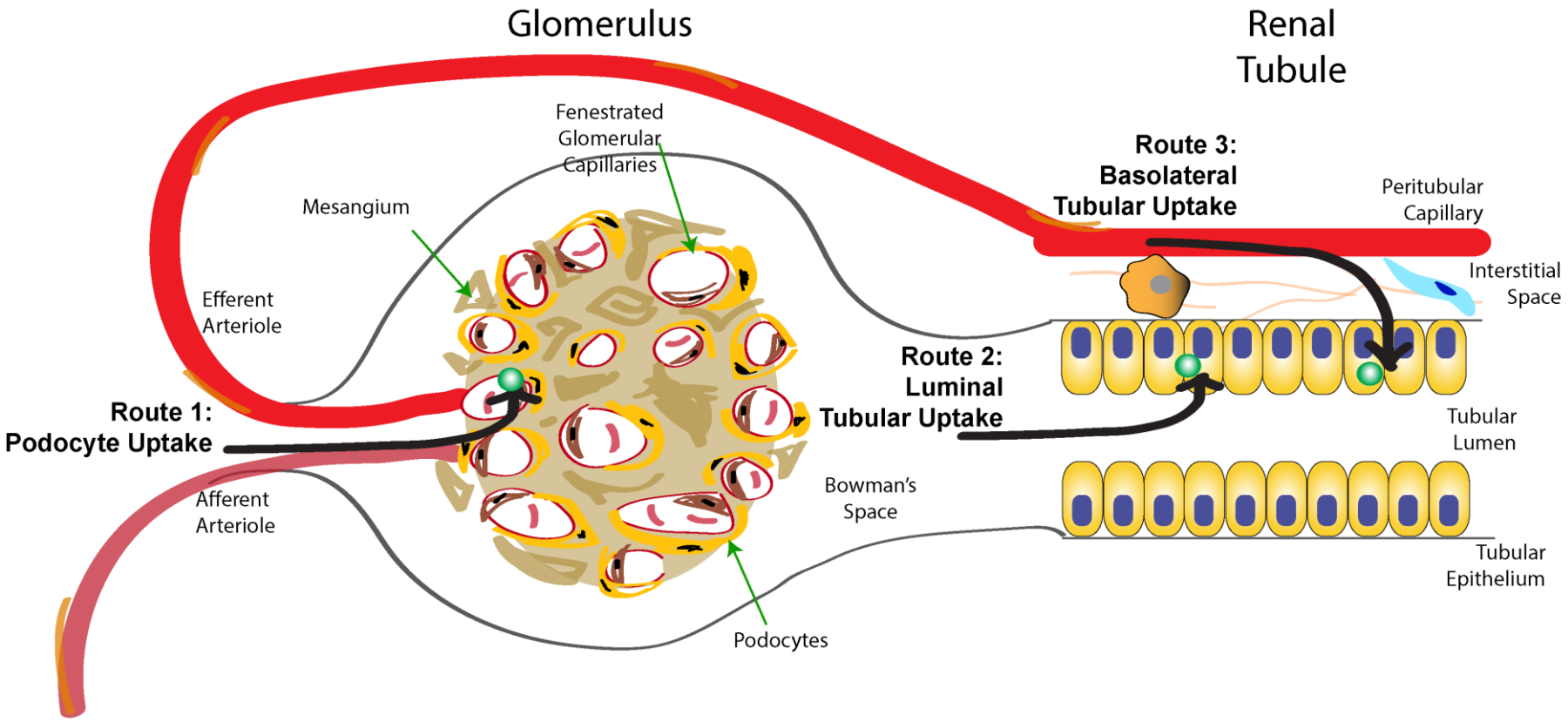
Williams *et al.*, *Hypertension*, 2017

MNPs Target the Kidneys Due to Their Size and Surface PEGylation



- Ultrasmall NPs (<10 nm) are filtered into the urine
- 10-80 nm NPs localize to the liver and glomerulus
- Most nanoparticles (80-200 nm LNPs and polymer NPs) localize to the liver because they pass through liver sinusoidal vasculature fenestrations and/or are taken up by Kupffer Cells
- **Polymer mesoscale nanoparticles (300-500 nm) were engineered to avoid the liver** due to their size and PEGylation, ultimately accumulating in the kidney tubules
- Microparticles (>1,000 nm) accumulate in the lungs

MNPs Target the Tubular Epithelium Through Peritubular Transcytosis

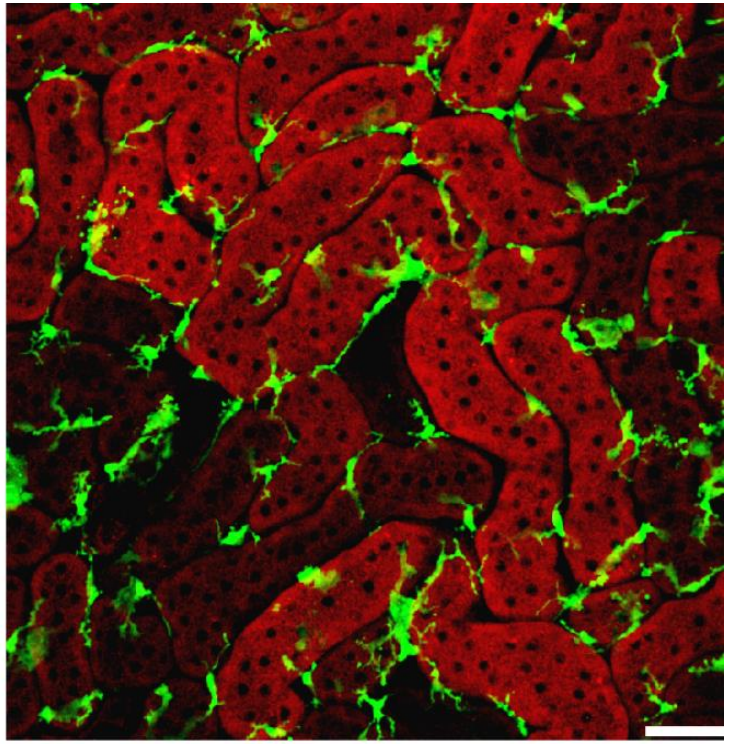


Routes of kidney targeting:

1) Podocyte Uptake: NPs pass through glomerular fenestrations (typically 10-80 nm and positively-charged)
2) Luminal Tubular Uptake: Glomerular filtration of particles and uptake at the tubular lumen (<10 nm)
3) **<u>Basolateral Tubular Uptake: Glomerular exclusion leads to tubular uptake via peritubular transcytosis (the novel mechanism of 300-500 nm MNPs)</u>**

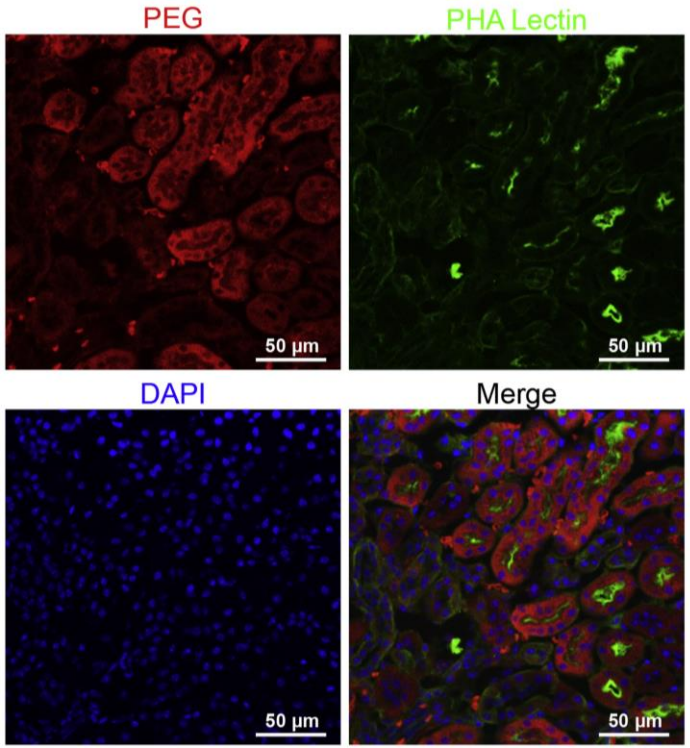
Williams *et al.* 2021 Kidney-Targeted Nanomedicines in "Regenerative Nephrology" (Book Chapter)

MNPs Primarily Target Tubular Epithelial Cells



Intravital Microscopy
72 hours post-injection
Green: Renal Macrophages
Red: Cy5 Dye Loaded in MNPs

The distribution is approximately 2/3 proximal and 1/3 distal tubules



Immunofluorescence Microscopy
24 hours post-injection
Red: PEG antibody staining MNPs
Green: Proximal Tubule Lumen
Blue: Cell Nuclei

In Collaboration with Columbia University Medical Center

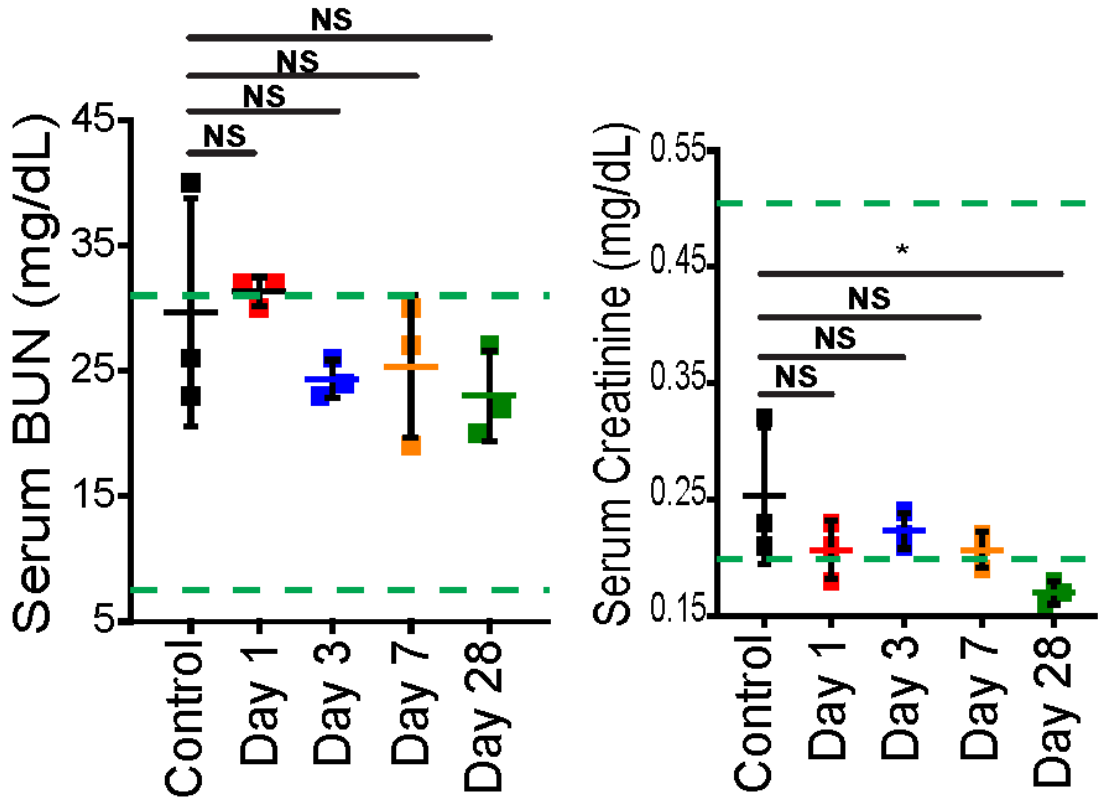
Williams *et al.*, *Nano Letters*, 2015

Williams *et al.*, *Hypertension*, 2017

Williams Immune Nanomedicine Lab

Biodegradable MNPs are Well-Tolerated

- MNPs are composed of polymers approved by the FDA in other formulations
- Short- and long-term renal function in mice is preserved after MNP injection
- No detectable changes in liver function or multi-organ histology
- No detectable changes in local or systemic inflammation due to MNPs
- We performed multi-dose regimens in several mouse therapeutic efficacy studies



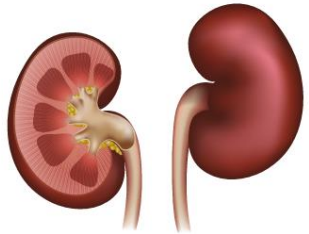
Williams *et al.*, *Hypertension*, 2017

Our Versatile Kidney-Targeting MNP Platform Can Carry Multiple Drug Classes

RNAi therapy
Benson et al. 2022 Circulation Research
Veiras et al. 2022 Circulation Research

Therapeutic mRNA
Skelton *et al.* 2022 *Pharmaceutical Research*

Therapeutic peptides
Han et al. 2020 JCI Insight
Guo et al. 2022 JASN



Small Molecules
Williams et al. 2015 Nano Letters
Williams et al. 2018 Hypertension
Mrug et al. 2020 ASN Abstract
Williams et al. 2022 Frontiers in Pharmacology

Gene editing
Manuscript In Revision

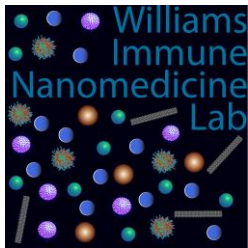
Immune-modulating nucleic acids
Han et al. 2020 Kidney International

Academic collaborations represented here include MSKCC, Cornell, Yale, Columbia, Cedars-Sinai LA, and U Arkansas for Med Sciences. Five other validation pubs from external labs

Vasylaki *et al.* 2024 *ASN Kidney360* (Review)

We Published Several Proof-of-Concept Rodent Studies Using MNP Delivery

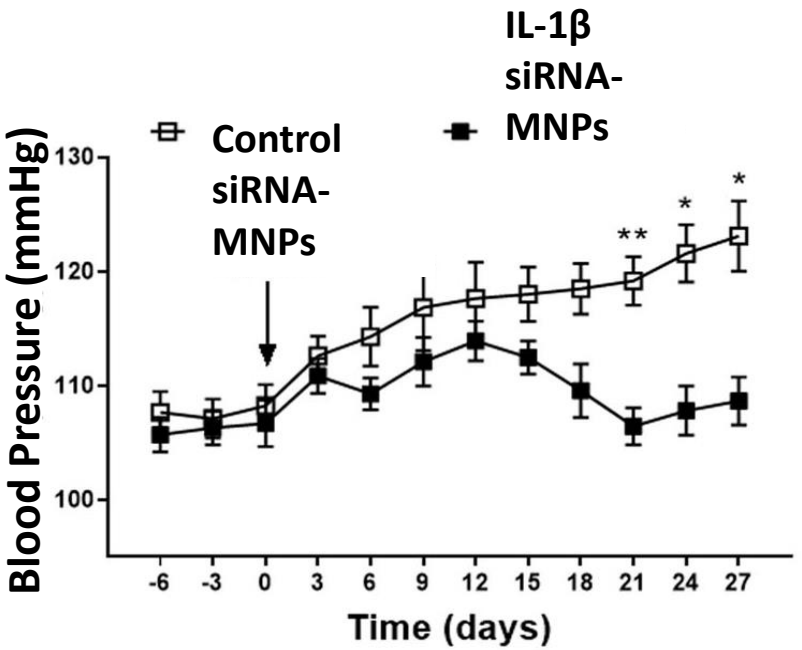
- Hypertensive chronic kidney disease
- Salt-sensitive diabetes
- Ischemic acute kidney injury
- Cisplatin-induced chronic kidney disease
- Cisplatin-induced acute kidney injury
- Polycystic kidney disease

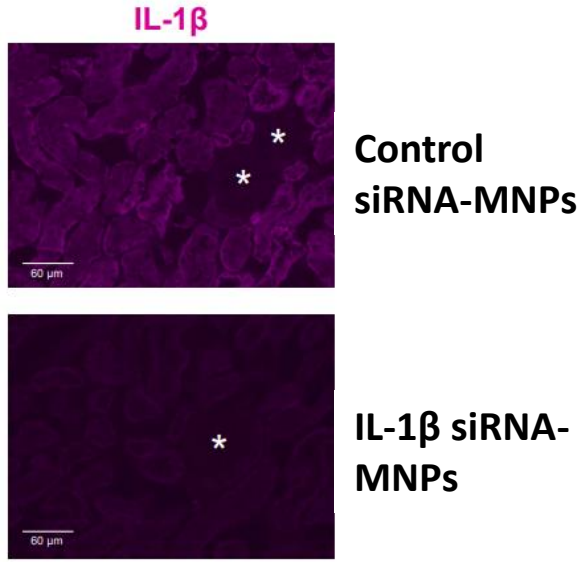


IL-1β siRNA-MNPs are Effective in a Mouse Model of Diabetic Kidney Disease

In Collaboration with Cedars-Sinai Medical Center Los Angeles

- Diabetic mice (db/db) were fed a high-salt diet
- Mice were treated every other week with an IV injection
- IL-1β siRNA-MNPs **significantly reduced hypertension**





Control siRNA-MNPs

IL-1β siRNA-MNPs

Top image shows **IL-1β expression** in control mice

Bottom image shows **lack of IL-1β expression** after siRNA-MNP therapy

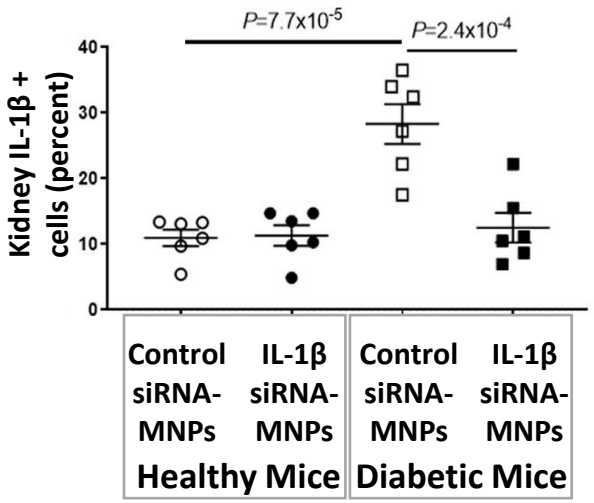
Williams Immune Nanomedicine Lab

Veiras *et al. 2022 Circulation Research*

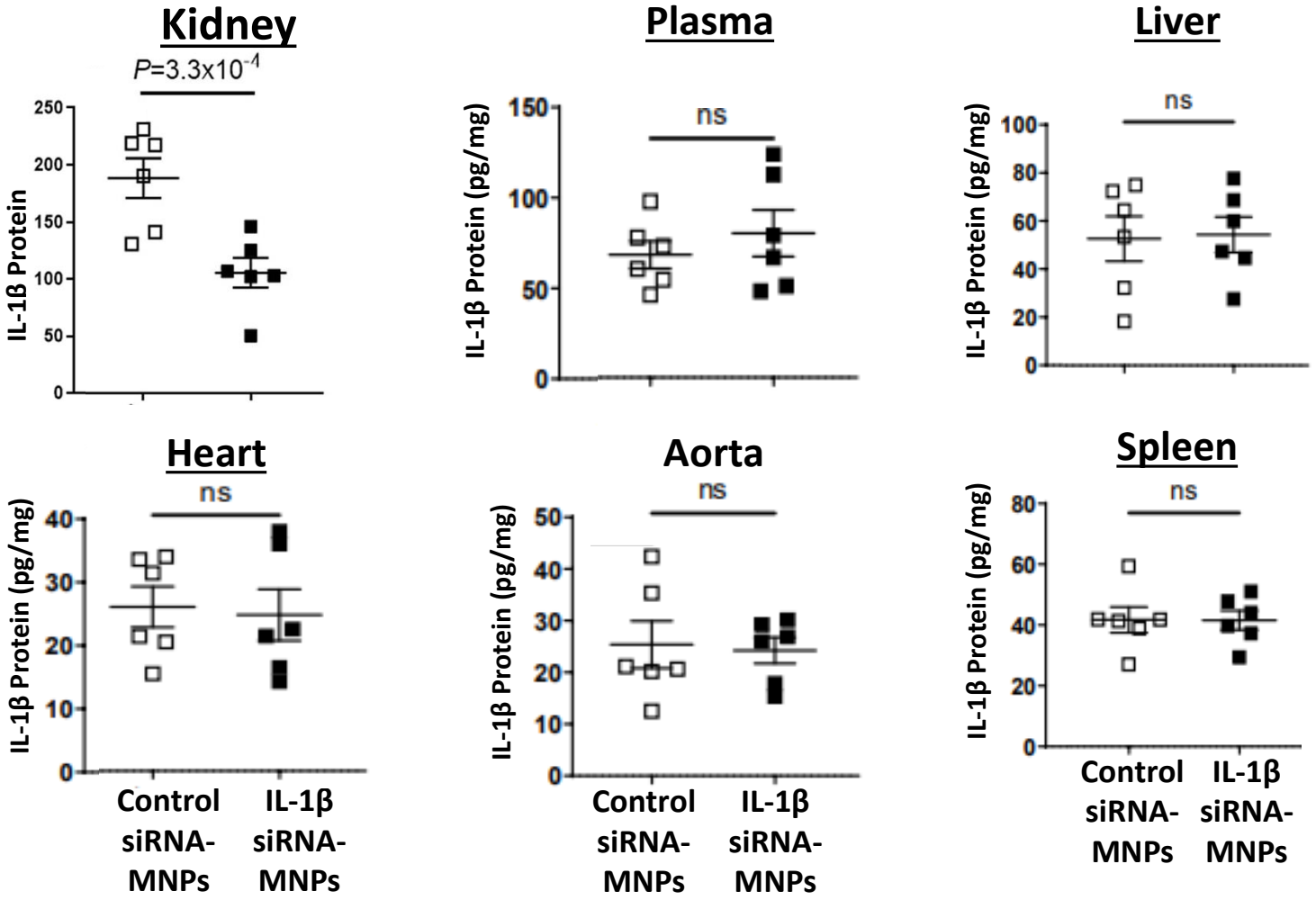
IL-1β siRNA-MNPs Demonstrate Kidney-Specific Target Knockdown for Several Weeks

In Collaboration with Cedars-Sinai Medical Center Los Angeles

IL-1β Positive Kidney Cells are Reduced to Baseline



IL-1β Protein Knockdown Only in the Kidneys

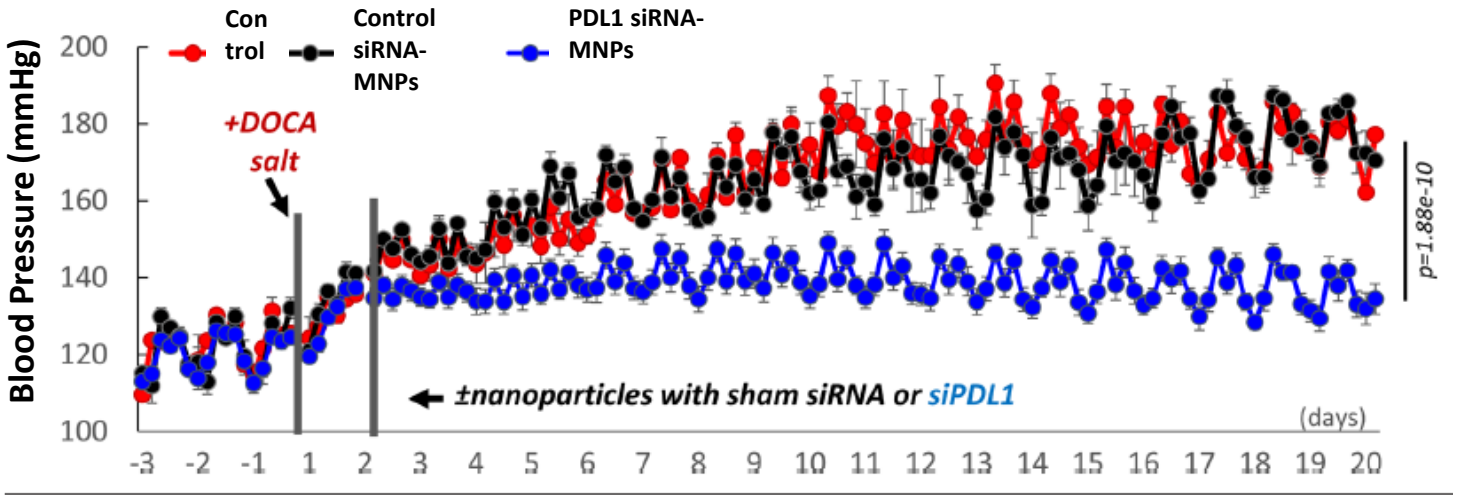


Veiras *et al.* 2022 *Circulation Research*

PDL1 siRNA-MNPs Treat Hypertensive Kidney Disease in a High-Salt Uninephrectomy Model

In Collaboration with U Arkansas for Medical Sciences

- Salt sensitive hypertension was induced in mice
- Mice were treated one time and sacrificed 18 days later
- PDL1 siRNA-MNPs **significantly reduced hypertension**



Benson *et al.* 2022 *Circulation Research*

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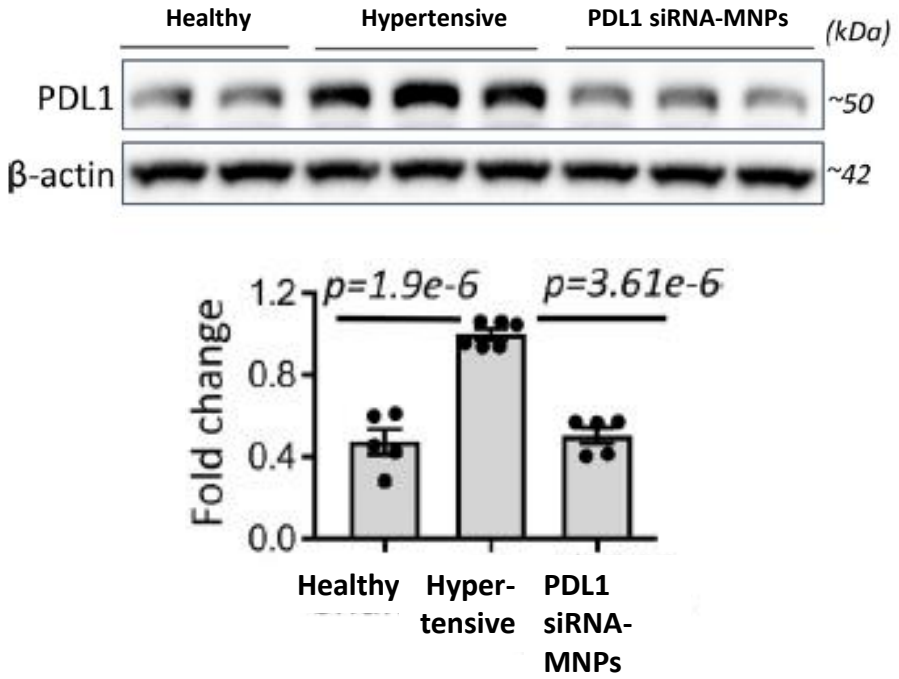
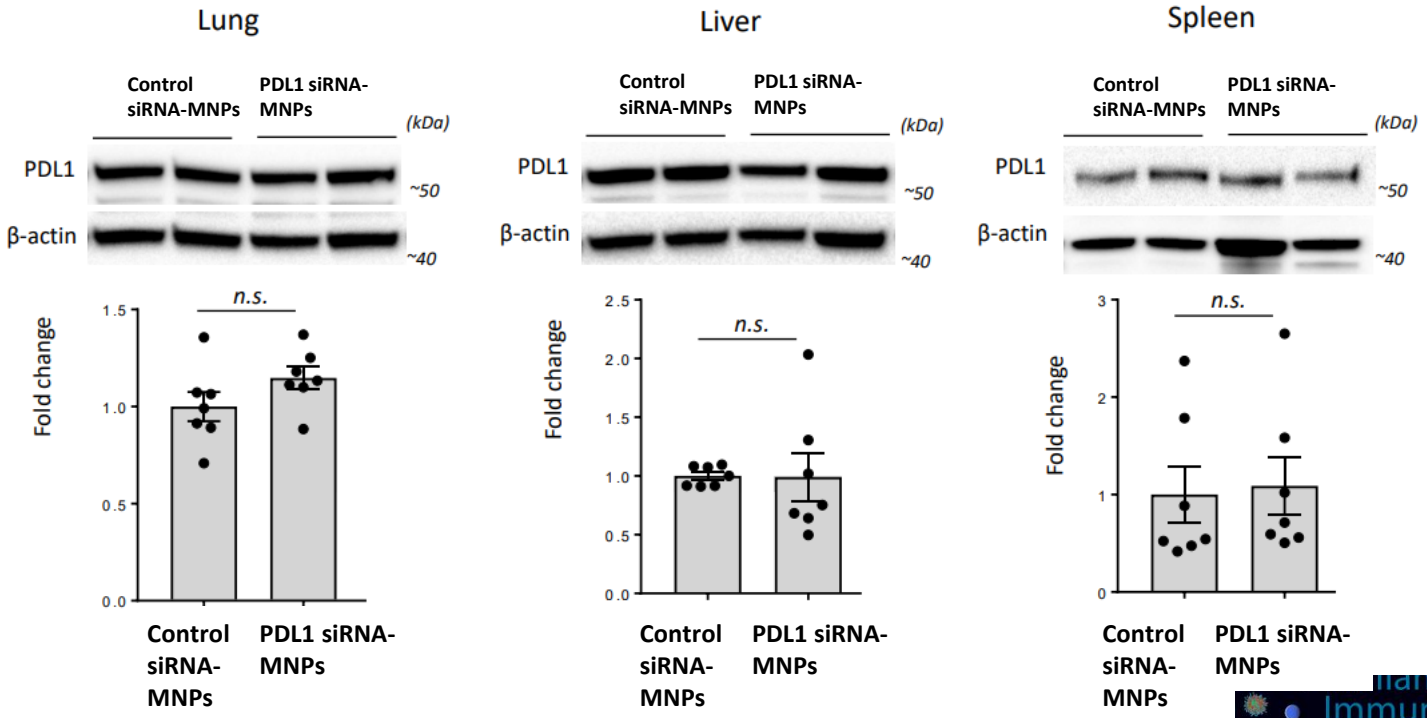
PDL1 siRNA-MNPs Induced Kidney-Specific Knockdown for 18 Days After One Dose

In Collaboration with U Arkansas for Medical Sciences

PDL1 expression in kidneys is knocked down to baseline



PDL1 expression is unchanged in other organs

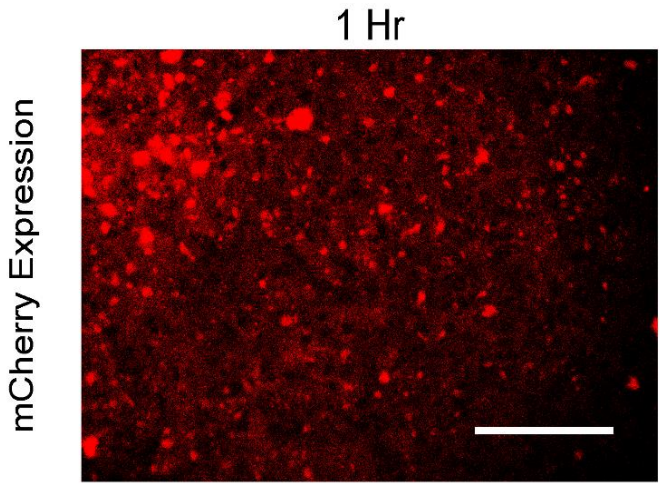


Benson *et al.* 2022 *Circulation Research*

Immune Nanomedicine Lab

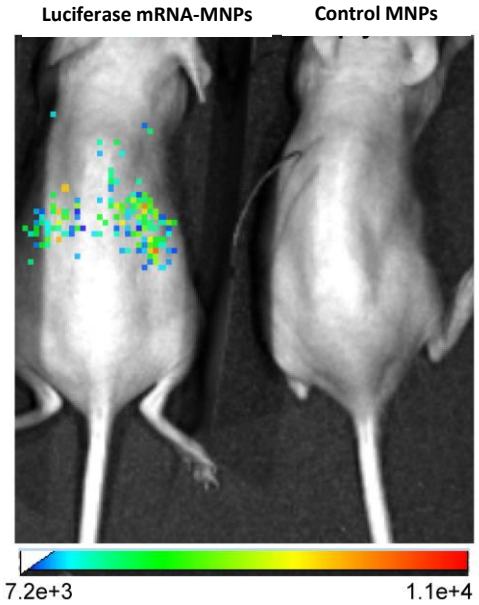
Ongoing work…Reporter mRNA Loaded into MNPs is Expressed In Vitro and In Vivo

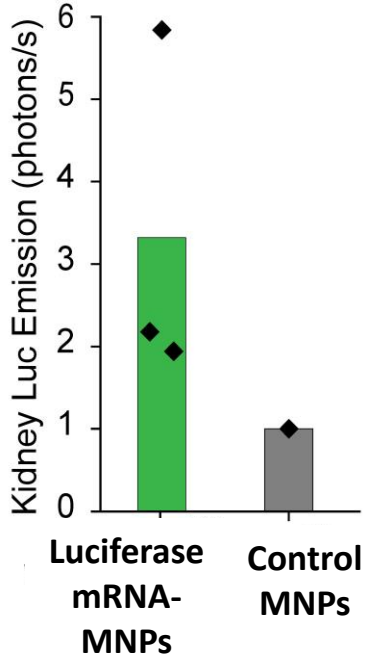
mCherry mRNA-MNP formulation was optimized and validated in vitro



[Skelton and Roach *et al.* 2022 *Pharmaceutical Research*]

Luciferase mRNA-MNP formulation was optimized and validated in vivo





unpublished

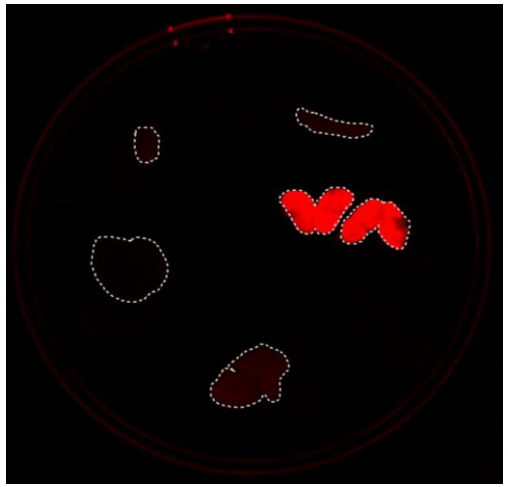
Additional data with a therapeutic enzyme mRNA has been generated with collaborators and is near publication…

Williams Immune Nanomedicine Lab

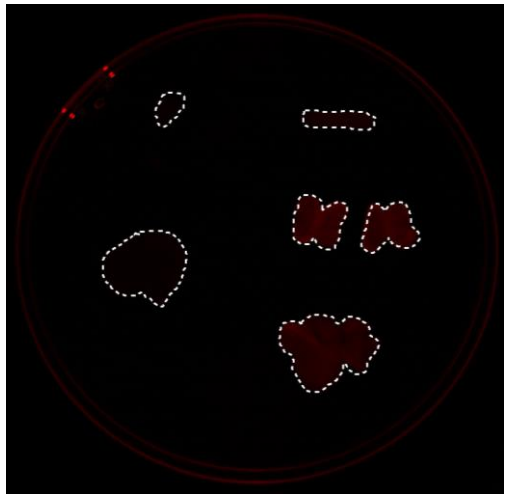
Ongoing work…developing Lipid Mesoscale Nanoparticles

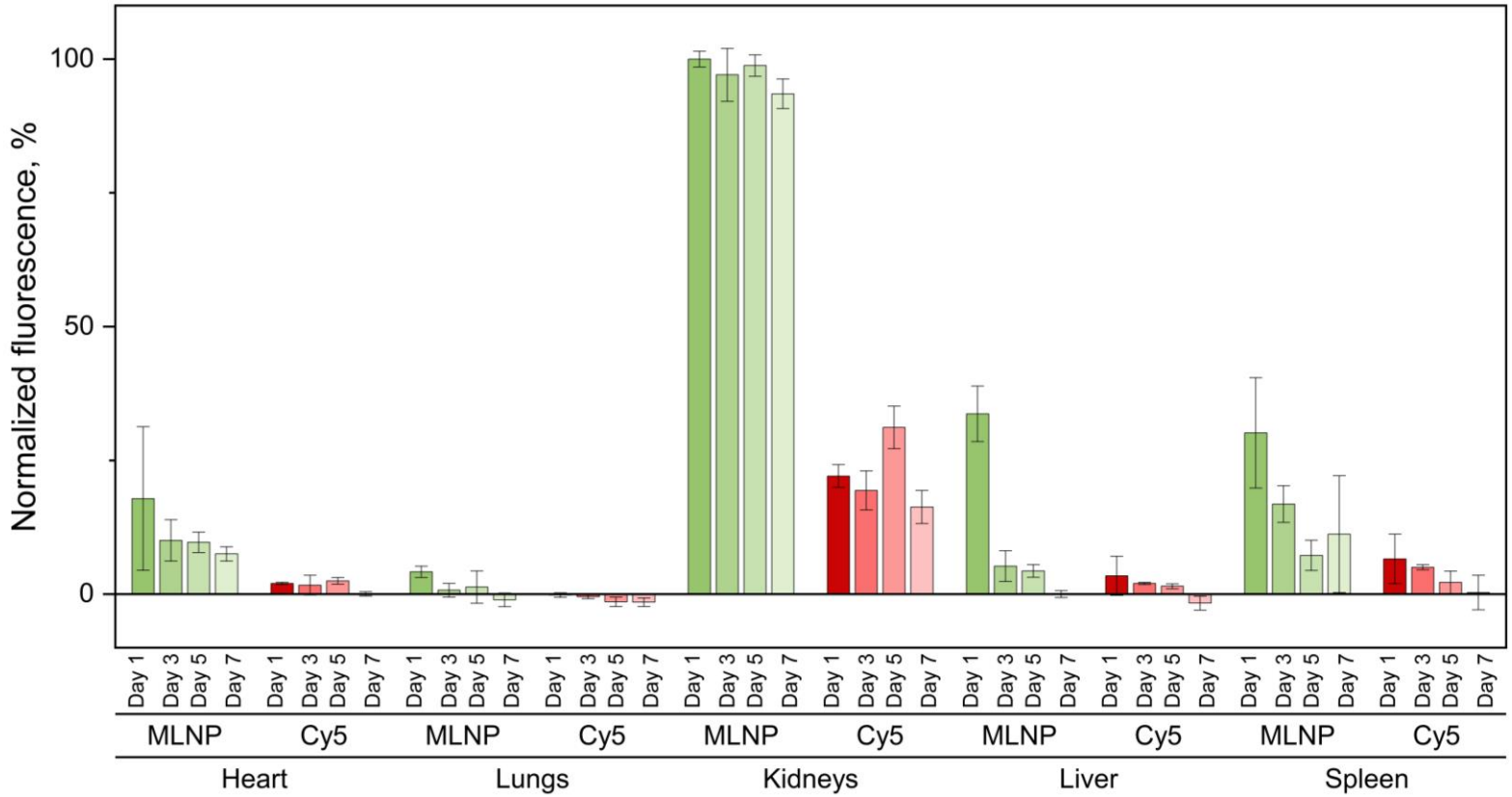
Day 7

MLNP



Free DEDC

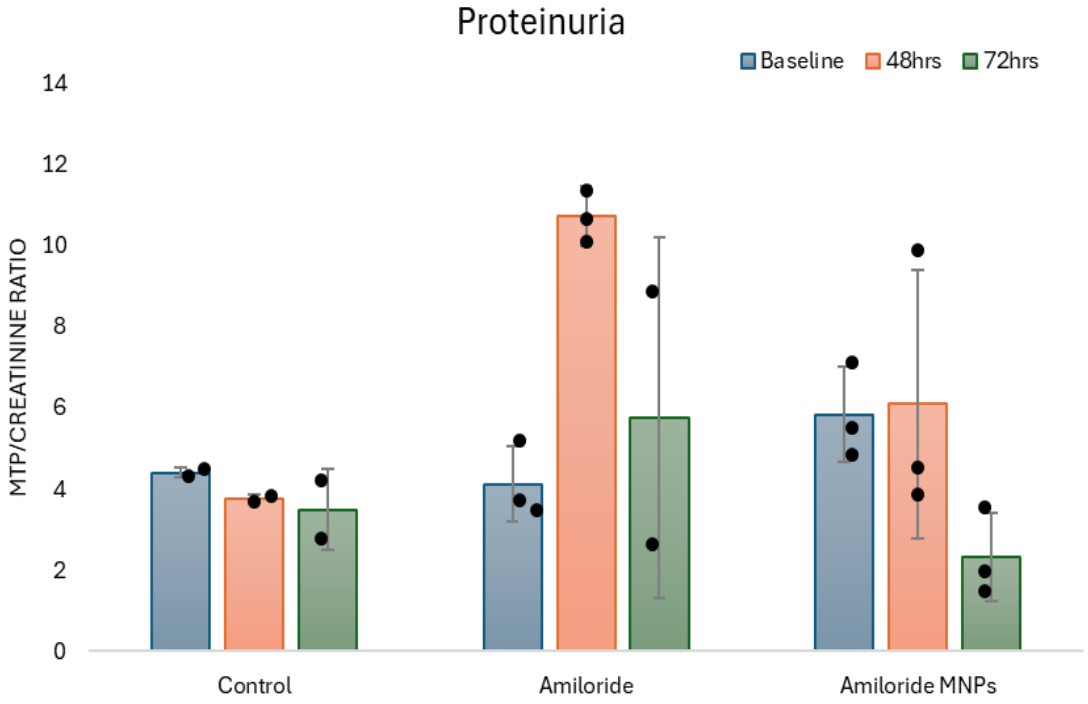




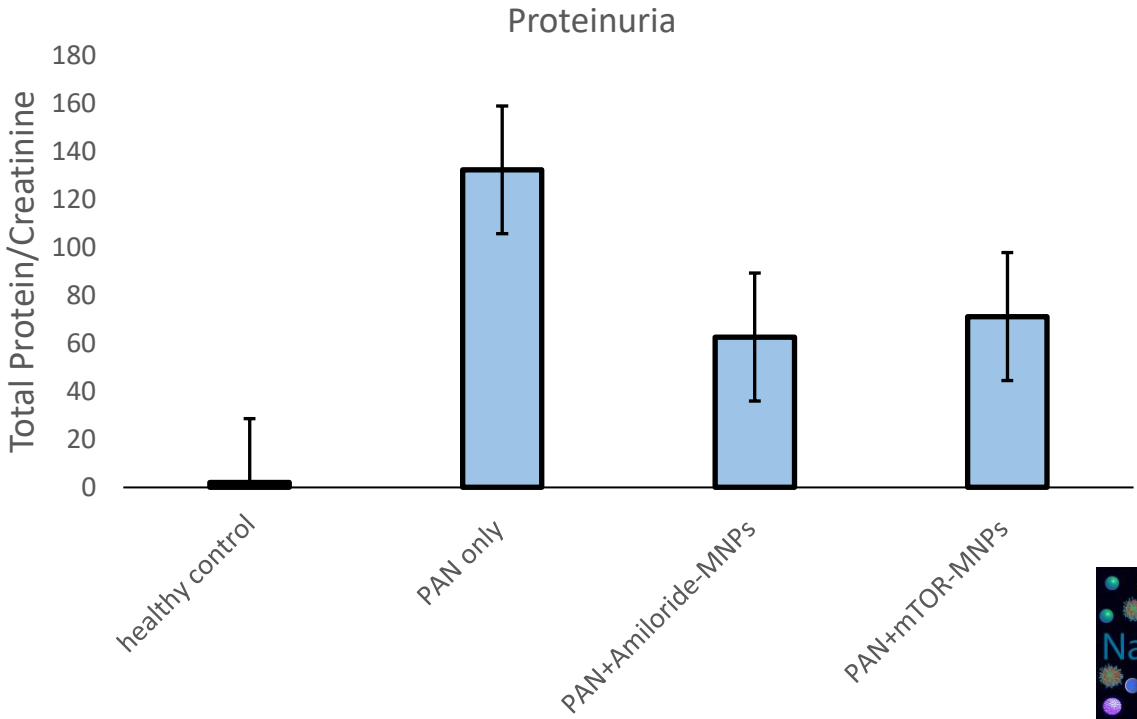
Williams Immune Nanomedicine Lab

Ongoing work…Glomerular localizing MNPs

Lipopolysaccharide (LPS)-induced glomerular injury in mice



Puromycin aminonucleoside (PAN)-induced glomerular injury in rats



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MNPs are Generating Excitement in the Kidney Disease Community

Editorial Commentary

Mesoscale Nanoparticles
An Unexpected Means for Selective Therapeutic Targeting of Kidney Diseases!

May Lin Yap, Xiaowei Wang, Geoffrey A. Pietersz, Karlheinz Peter

commentary

Targeted protection of proximal tubular cells by nanoparticle-enhanced delivery of a TLR9-antagonist

Zoltán H. Endre[1,2] and Jonathan H. Erlich[1,2]

The kidney target site for injury that leads to acute kidney injury (AKI) is the proximal tubule. Nanoparticle-encapsulation enhanced delivery of a selective Toll-like receptor 9 antagonist to mouse proximal tubules and attenuated experimental ischemia-reperfusion injury in a mouse model of AKI.

Kidney International (2020) **98,** 48–50; https://doi.org/10.1016/j.kint.2020.04.024

EDITORIAL www.jasn.org

Cisplatin-Induced Kidney Injury: Delivering the Goods

Joshua N. Curry and James A. McCormick
Division of Nephrology and Hypertension, Oregon Health and Science University, Portland, Oregon

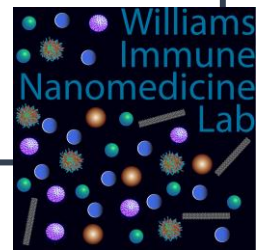
JASN 33: 255–256, 2022.
doi: https://doi.org/10.1681/ASN.2021121591

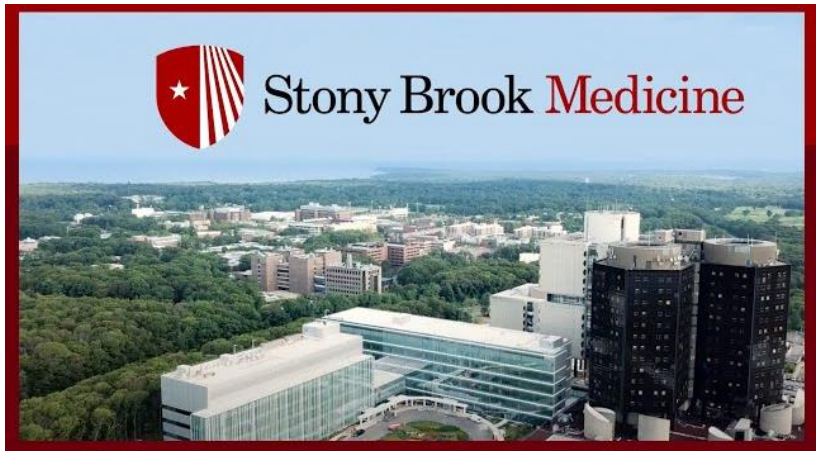
Circulation Research

EDITORIAL

Kidney Tubular IL-1β ENaCtivation in Diabetes and Salt-Sensitive Hypertension

Ashley Pitzer, Thomas R. Kleyman, Annet Kirabo





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Questions?

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