CBC | CHICAGO BIOMEDICAL CONSORTIUM

# CBC Catalyst Award Proposal Title Page

**Title of the proposal** *(no more than 100 characters including white space)*

Cell-penetrating quantum dots for lung transplant assay

**Name, degree, title, institution, and contact information including the email address of each PI**

Dr. Preston T. Snee, Associate Professor, University of Illinois at Chicago. Email: sneep@uic.edu Phone: (312) 413-2566

Dr. Ankit Bahrat, Chief of Thoracic Surgery in the Department of Surgery, Feinberg School of Medicine. Email: ankit.bharat@northwestern.edu Phone: (312) 695-3800

**Do you have any current or pending grant applications that potentially overlap with this application?**
**Check ONE:**

**YES** ☐ **NO** ☒

If YES, please identify them.

(ENTER THE OVERLAP HERE)

Explain the overlap in the Biosketch section.

| | | | |
|---|---|---|---|
| **Does the proposed research involve animal subjects?** | **Check ONE:** | **YES** ☒ | **NO** ☐ |
| **Does the proposed research involve human subjects?** | **Check ONE:** | **YES** ☐ | **NO** ☒ |
| **Does the proposed research involve embryonic stem cells?** | **Check ONE:** | **YES** ☐ | **NO** ☒ |

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

## LAY SUMMARY (150 words)

Lung transplants are necessary for patients with end stage diseases. Transplanted tissues may suffer from ischemia reperfusion injury (IRI) resulting from re-oxygenation after surgery, which causes primary graft dysfunction (PGD) and short-term mortality [1, 2]. IRI is identified by the presence of the patient's "first responder" neutrophils (white blood cells) in the tissue. The neutrophils and other white blood cells from the spleen are recruited into the lung by dead graft cells and residual donor white blood cells in the transplanted organ [3-6]. We seek to study the mechanisms that drive this recruitment using semiconductor quantum dots ("QDs"), which are the brightest fluorophores created to date. QDs add value to biomedical research by enabling multiplexing of biological assays due to their narrow emission profiles [7]. We will create QDs that stain live monocytes to track cells from the bone marrow, spleen, and finally to the transplanted lung in murine models.

Traditional (federal) funding sources are not available to support this research. The NSF does not support the development of imaging for translational purposes; likewise, the NIH does not fund projects using II-VI semiconductor nanoparticles. Both agencies require substantial preliminary data to minimize risk. Funding for this proposal will also address the stagnation of the application of quantum dots for biomedical research. This is due to the fact that there are no reproducible and robust methods to impart competency to QDs as cytosolic delivery and imaging agents for live cells [8, 9]. Success will reinvigorate the application of QDs for biomedical research and result in future NIH-fundable imaging applications.

## TRANSLATIONAL POTENTIAL

Primary graft dysfunction affects over 50% of lung recipients within the first 72 hours [1, 2], and current therapies are largely ineffective and increase the risk of immunosuppression [10]. We will elucidate the mechanisms of patient monocyte recruitment into donor lung tissue that has been implicated in PGD. These findings will, in turn, help guide the development of clinically relevant interventions to reduce neutrophil extravasation and attenuate ischemia-reperfusion injury, lung allograft dysfunction, and reduce long-term allograft rejection and fibrosis by inhibiting development of monocyte-derived profibrotic macrophages.

## i. DESCRIPTION OF PROPOSED RESEARCH

**Rationale and Specific Aims.** In the last decade, the indications for lung transplantation have expanded significantly, resulting in over 50% increase in the number of procedures. However, the survival rate is the worst amongst solid organs with only 80% and 50% of patients alive at 1 and 5 years, respectively [11]. The success of lung transplantation is currently limited by high rates of PGD resulting from IRI. PGD affects over 50% of patients within 24 hours of transplantation, is associated with pneumonitis and hypoxemic respiratory failure, and has emerged as the strongest risk factor for both early post-transplant mortality as well as chronic allograft rejection.

Understanding the impact of the cellular mechanisms and pathways involved in PGD and IRI in mouse models for humans is a major focus to this translational work that is currently limited by the number and reach of available genetic and molecular techniques. Using two-photon imaging and murine lung transplants, in combination with multifunctionalized QDs, we will elucidate the mechanisms by which immune cells drive the pathogenesis of these syndromes with a focus on pathways that can be targeted in the donor lung prior and after transplantation,

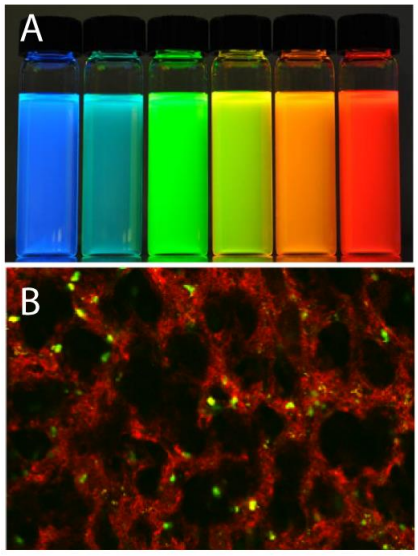


**Fig. 1 A.** Semiconductor quantum dots emit over the visible to NIR with very narrow fluorescent profiles that enable multiplexing of biological assays. **B.** Two-photon image of blood vessels labeled with CdSe/ZnS QDs (red) in native lung of CCR2-GFP mice (green).

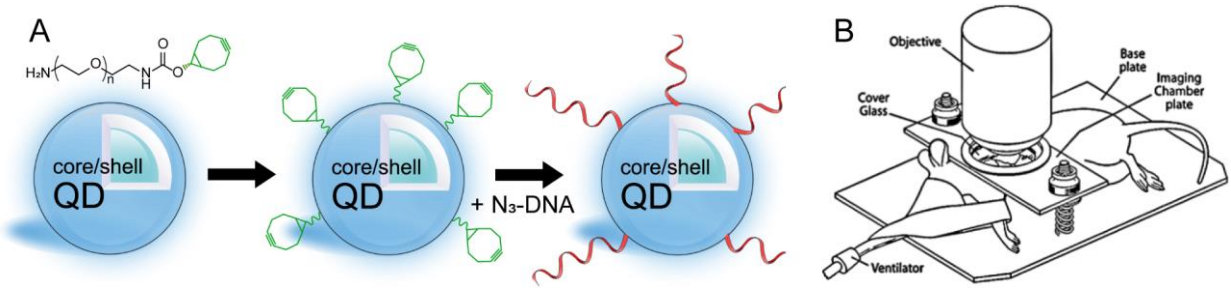


**Fig. 2 A.** Conjugation approach to multifunctionalize QDs with DNA. **B.** Diagram of mouse intravital two-photon imaging.

thereby avoiding recipient toxicity. The Bharat and Snee groups are poised to realize the potential solution(s) described herein by using their extensive experience and expertise in intravital imaging, lung, transplant biology, and development and characterization of nanomaterials for biomedical research. The Aims of this application will be to **1) Develop**, and **2) Test these agents in combination with each other and alone with and without.**

**Aim 1: DNA-coated QDs for cytosolic delivery and tracking.** The Snee group has developed visible to near-IR emissive CdS/ZnS and CdSe/ZnS (**Figure 1A**) that can be delivered to live cells using a novel anionic cell penetrating peptide [12]. The preparation of such imaging agents is challenging because QDs precipitate from solution upon biological functionalization using traditional approaches. As a result, the Snee group developed new technologies that enabled high yield when functionalizing with several chem- and biological vectors. This allowed the group to be one of the first to demonstrate live cell staining with QDs coated with a novel cell penetrating peptide. The group has recently begun to develop cadmium-free QDs such as InP/ZnS and $AgInS_2$/ZnS that are of interest to the NIH.

For this proposal we will develop a new method for live cell staining to address two shortcomings of the present method, specifically the high cost of our current technology (~$700 per sample) and lack of flexibility in the design. We will investigate the use of DNA coatings that delivery of gold nanoparticles into live cells [12, 13] via scavenger receptors that are found in human and mouse monocytes. It is interesting to note that DNA-mediated cellular delivery of gold nanoparticles was reported in 2006, yet the same could not be applied to semiconductor quantum dots which is due to the fact that Au and CdSe/ZnS QDs have orthogonal conjugation chemistries. The Snee group has recently developed a protocol that addresses this shortcoming. Specifically, they first coat a water soluble CdSe/ZnS QD with a click chemistry vector and then conjugate azide functional DNA to the resulting product as shown in **Figure 2A**. They have thus far demonstrated a record-breaking loading of 5 DNAs per QD at ~80% yield. Support from the CBC will enable the group to create a variety of quantum dot / DNA conjugates for monocyte labeling; and if any difficulties are encountered the group can simply return to the method of (DSS) conjugation to nanoparticles.

**Aim 2: Application of cytoplasmic staining QDs to elucidate mechanisms involved in murine/human primary graph disfunction and ischemia reperfusion injury for therapeutic development.** A paramount goal of our research is to rapidly translate our findings from murine models to the treatment of patients with lung transplants. To that end, the Bharat laboratory has been at the forefront of innovation and has consistently and repeatedly published work using novel techniques to advance our understanding for potential treatments for lung transplantation and disease. [3, 4, 10]

Our understanding of cellular and molecular cues that regulate the recruitment of neutrophils and monocytes to inflamed pulmonary tissue has been advanced by our development of intravital imaging techniques that allow for real-time visualization of leukocyte trafficking in lungs and spleen [14, 15]; the experimental set up is shown in **Figure 2B**. Using flow cytometry, two-photon imaging, confocal microscopy, and vascularized heterotopic spleen transplantation, in combination with

murine lung transplants, we have also made great advances in our understanding of the role of the spleen and bone marrow-derived classical monocytes to injury after transplantation. [4, 10, 16]. In this application, our experimental design will take advantage of our models of lung and spleen transplantation, in conjunction with QDs technology, to facilitate the study of the mechanisms involved in IRI and PGD in mouse models. Using two-photon imaging and vascularized heterotopic spleen transplantation, in combination with murine lung transplants, we will determine the role of the spleen in bone marrow-derived monocyte differentiation and recruitment after lung transplant. We will make use of the new functionalized QDs, developed in Aim 1, to label and track cells from the bone marrow, spleen, and finally transplanted organs to elucidate the mechanisms by which monocytes are educated and recruited to promote lung injury. Although not the main focus of this application, we have already begun preliminary testing of these semiconductor quantum dots for enhanced biocompatibility and labeling of blood vessels in live imaging, see **Figure 1A**.

In creating a molecular atlas of cellular pathways and interactomes of human IRI and PGD after lung transplantation, the single-cell multifunctionalized QD cytoplasmic staining of neutrophils, classical and non-classical monocytes, and QD-antibody conjugation approaches for associated proteins to be used in these studies will provide: 1) unique insights into the expression and recruitment of individual lung cell populations that emerge with injury after transplantation and 2) allow for the examination of multiple labeled cell populations such as neutrophils and specific classical monocytes and non-classical monocytes simultaneously. Which is therefore useful for reconstructing and studying multiple cellular pathways and interactions during disease.

The overall results from these experiments will not only introduce and provide novel tools to study mechanisms of disease and cellular interactions but will also have the potential to introduce biomedically and clinically applicable strategies to improve lung and solid organ transplantation outcomes. All of our murine experiments are tied to clinically applicable strategies that could be applied to humans. Therefore, these studies will facilitate the rapid translation of our findings to clinical practice.

**ii. NATURE OF INTER-INSTITUTIONAL COLLABORATION** Our groups are in close proximity and have been holding meetings at NU Lurie Children's Hospital, which has enabled the foundation for this proposal. Future interactions under CBC support will emphasize graduate student education. Specifically, trainees who specialize in nanoparticle synthesis or biological imaging will interact to understand the nature of each other's research. This will benefit all laboratories and their trainees.

**iii. CRITERIA FOR SUCCESS.** We consider the following as Milestones: **A)** Synthesis of DNA coated CdSe/ZnS at high (~20:1) valences. If difficulties are encountered we will use a cell-penetrating peptide-QD conjugate as an alternative vehicle; **B)** Simultaneous delivery of nanomaterials into the cytosol of live cells confirmed by 2-photon imaging; **C)** Direct *in-vivo* QD labeling of bone marrow monocytes (source) with high affinity and high specificity (nonspecific binding less than 5% of total binding) confirmed with two-channel in vivo flow cytometry and fluorescence microscopy. If difficulties are encountered, we will instead apply *ex vivo* isolation and purification of monocyte populations from donor animals, QD labeling of cells, followed by adoptive transfer into recipient animals.; **D)** Tracking, identification, and live 2-photon imaging of bone marrow-derived QD-labeled monocytes in spleen and lungs; **E**) Publication and/or IP protection; and **F)** Obtaining long-term funding support.

**iv. LONG-TERM FUNDING PLAN.** With proof-of-principle data we will apply the technology to Cd-free quantum dots and seek National Institute of Biomedical Imaging and Bioengineering (NIBIB) and National Heart, Lung, and Blood Institute (NHLBI) funding; alternatively, focusing on the imaging and materials science would lead to NSF proposals targeting CHE/CMI or CBET.

**References:**


1. Bharat, A., et al., *Immunological link between primary graft dysfunction and chronic lung allograft rejection.* Ann Thorac Surg, 2008. **86**(1): p. 189-95; discussion 196-7.
2. Bharat, A. and D. Kreisel, *Immunopathogenesis of Primary Graft Dysfunction After Lung Transplantation.* Ann Thorac Surg, 2018. **105**(3): p. 671-674.
3. Li, W., et al., *Necroptosis triggers spatially restricted neutrophil-mediated vascular damage during lung ischemia reperfusion injury.* Proc Natl Acad Sci U S A, 2022. **119**(10): p. e2111537119.
4. Kurihara, C., et al., *Crosstalk between nonclassical monocytes and alveolar macrophages mediates transplant ischemia-reperfusion injury through classical monocyte recruitment.* JCI Insight, 2021. **6**(6).
5. Kreisel, D., et al., *Bcl3 prevents acute inflammatory lung injury in mice by restraining emergency granulopoiesis.* J Clin Invest, 2011. **121**(1): p. 265-76.
6. Sayah, D.M., et al., *Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation.* Am J Respir Crit Care Med, 2015. **191**(4): p. 455-63.
7. Tyrakowski, C.M. and P.T. Snee, *A primer on the synthesis, water-solubilization, and functionalization of quantum dots, their use as biological sensing agents, and present status.* Phys Chem Chem Phys, 2014. **16**(3): p. 837-55.
8. Boeneman, K., et al., *Selecting improved peptidyl motifs for cytosolic delivery of disparate protein and nanoparticle materials.* ACS Nano, 2013. **7**(5): p. 3778-96.
9. Jing, H., et al., *Cytosolic delivery of membrane-penetrating QDs into T cell lymphocytes: implications in immunotherapy and drug delivery.* Nanoscale, 2021. **13**(10): p. 5519-5529.
10. Yang, W., et al., *IL-1beta-dependent extravasation of preexisting lung-restricted autoantibodies during lung transplantation activates complement and mediates primary graft dysfunction.* J Clin Invest, 2022. **132**(20).
11. Zheng, Z., et al., *Donor pulmonary intravascular nonclassical monocytes recruit recipient neutrophils and mediate primary lung allograft dysfunction.* Sci Transl Med, 2017. **9**(394).
12. Giljohann, D.A., et al., *Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles.* Nano Lett, 2007. **7**(12): p. 3818-21.
13. Rosi, N.L., et al., *Oligonucleotide-modified gold nanoparticles for intracellular gene regulation.* Science, 2006. **312**(5776): p. 1027-30.
14. Kreisel, D., et al., *In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation.* Proc Natl Acad Sci U S A, 2010. **107**(42): p. 18073-8.
15. Looney, M.R., et al., *Stabilized imaging of immune surveillance in the mouse lung.* Nat Methods, 2011. **8**(1): p. 91-6.
16. Hsiao, H.M., et al., *Spleen-derived classical monocytes mediate lung ischemia-reperfusion injury through IL-1beta.* J Clin Invest, 2018. **128**(7): p. 2833-2847.

Program Director/Principal Investigator (Last, First, Middle): Snee, Preston Todd

| DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY | FROM | THROUGH |
|---|---|---|
| | 6/01/2023 | 05/31/2025 |

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

| NAME | ROLE ON PROJECT | Cal. Mnths | Acad. Mnths | Summer Mnths | INST.BASE SALARY | SALARY REQUESTED | FRINGE BENEFITS | TOTAL |
|---|---|---|---|---|---|---|---|---|
| Preston Snee | PD/PI | 0 | 0 | 0.13 | 107,155 | 1,584 | 550 | 2,134 |
| TBA Graduate Student | GS | | 4.5 | 1.5 | 45,180 | 30,120 | 1,689 | 31,809 |
| | | | | | | | | |
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| | | | | | | | | |
| **SUBTOTALS** → | | | | | | 31,704 | 2,239 | 33,943 |

| | |
|---|---|
| CONSULTANT COSTS | |
| EQUIPMENT *(Itemize)*<br>N/A | 0 |
| SUPPLIES *(Itemize by category)*<br>$10K is requested per year based on our general supply needs. An additional $10K is requested to replace small equipment (stir / hot plates, glove box catalyst etc); funds to replace these items has been unavailable to us for over a decade. | 20,000 |
| TRAVEL<br>Conference fees (will support GS) | 1,000 |
| INPATIENT CARE COSTS | |
| OUTPATIENT CARE COSTS | |
| ALTERATIONS AND RENOVATIONS *(Itemize by category)* | |
| OTHER EXPENSES *(Itemize by category)*<br>We need use of the UIC RRC for materials characterization purposes (typically $500 / yr). The TBA GS will require an additional $813 fee for training purposes in 1st year. | 1,313 |
| CONSORTIUM/CONTRACTUAL COSTS — DIRECT COSTS | |
| **SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD** *(Item 7a, Face Page)* | $ 56,256 |
| CONSORTIUM/CONTRACTUAL COSTS — FACILITIES AND ADMINISTRATIVE COSTS | |
| **TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD** | $ 56,256 |

Program Director/Principal Investigator (Last, First, Middle): Snee, Preston Todd

## BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
## DIRECT COSTS ONLY

| BUDGET CATEGORY TOTALS | INITIAL BUDGET PERIOD *(from Form Page 4)* | 2nd ADDITIONAL YEAR OF SUPPORT REQUESTED | 3rd ADDITIONAL YEAR OF SUPPORT REQUESTED | 4th ADDITIONAL YEAR OF SUPPORT REQUESTED | 5th ADDITIONAL YEAR OF SUPPORT REQUESTED |
|---|---|---|---|---|---|
| PERSONNEL: *Salary and fringe benefits. Applicant organization only.* | 33,943 | 34,996 | | | |
| CONSULTANT COSTS | | | | | |
| EQUIPMENT | 0 | 0 | | | |
| SUPPLIES | 20,000 | 10,000 | | | |
| TRAVEL | 1,000 | 1,000 | | | |
| INPATIENT CARE COSTS | | | | | |
| OUTPATIENT CARE COSTS | | | | | |
| ALTERATIONS AND RENOVATIONS | | | | | |
| OTHER EXPENSES | 1,313 | 500 | | | |
| DIRECT CONSORTIUM/ CONTRACTUAL COSTS | | | | | |
| **SUBTOTAL DIRECT COSTS** *(Sum = Item 8a, Face Page)* | 56,256 | 46,496 | | | |
| F&A CONSORTIUM/ CONTRACTUAL COSTS | | | | | |
| **TOTAL DIRECT COSTS** | 56,256 | 46,496 | | | |

| **TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD** | **$** 102,752 |
|---|---|

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

The Snee group will synthesize blue CdS/ZnS as well as green and red emitting CdSe/ZnS quantum dots that match the filter set being used by the NU group for 2-photon imaging. The materials will be water solubilized and functionalized with single stranded DNA for live cell staining. Supply funding includes the costs of biologicals ($450 for ssDNA). If problems are encountered, we will return to our established technology (functionalization with (DSS)10 peptide, see Jing et al., Nanoscale, 2021, 13, 5519), $2310 for peptide synthesis). In the latter period of support we will synthesize Cd-free nanomaterials such as InP/ZnS (visible) and AgInS2/ZnS (red to NIR).

The supply funding is based on our current consumption, although additional 1st year of support costs will be incurred. These include the training fee for a graduate student on the electron microscope (to characterize materials synthesized at the UIC RRC) and an increase to help supply small equipment that hasn't been replaced in >10 years. These items include stir / hot plates, centrifuges, glove box equipment such as the blower, catalyst bed and filters, and a large number of glassware items.

Program Director/Principal Investigator (Last, First, Middle): Bahrat Ankit

| DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY | FROM | THROUGH |
|---|---|---|
| | 6/01/2023 | 05/31/2025 |

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

| NAME | ROLE ON PROJECT | Cal. Mnths | Acad. Mnths | Summer Mnths | INST.BASE SALARY | SALARY REQUESTED | FRINGE BENEFITS | TOTAL |
|---|---|---|---|---|---|---|---|---|
| Ankit Bahrat | PD/PI | 0 | 0 | | | | | |
| Feliz Nunez | Postdoc | | | | | 10,000 | | 10,000 |
| | | | | | | | | |
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| | | | | | | | | |
| **SUBTOTALS** → | | | | | | 10,000 | | 10,000 |

| | |
|---|---|
| CONSULTANT COSTS | |
| EQUIPMENT *(Itemize)*<br>$15,000 Bio-imaging<br>$10,000 Flowcytomery | 25,000 |
| SUPPLIES *(Itemize by category)*<br>$5,000 Software Purchase<br>$10,000 Mice<br>$15,000 Lab Supplies | 30,000 |
| TRAVEL | |
| INPATIENT CARE COSTS | |
| OUTPATIENT CARE COSTS | |
| ALTERATIONS AND RENOVATIONS *(Itemize by category)* | |
| OTHER EXPENSES *(Itemize by category)* | |

| | | |
|---|---|---|
| CONSORTIUM/CONTRACTUAL COSTS | DIRECT COSTS | |
| **SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD** *(Item 7a, Face Page)* | | $ 65,000 |
| CONSORTIUM/CONTRACTUAL COSTS | FACILITIES AND ADMINISTRATIVE COSTS | |
| **TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD** | | $ 65,000 |

Program Director/Principal Investigator (Last, First, Middle): Bahrat Ankit

## BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
## DIRECT COSTS ONLY

| BUDGET CATEGORY TOTALS | INITIAL BUDGET PERIOD *(from Form Page 4)* | 2nd ADDITIONAL YEAR OF SUPPORT REQUESTED | 3rd ADDITIONAL YEAR OF SUPPORT REQUESTED | 4th ADDITIONAL YEAR OF SUPPORT REQUESTED | 5th ADDITIONAL YEAR OF SUPPORT REQUESTED |
|---|---|---|---|---|---|
| PERSONNEL: *Salary and fringe benefits. Applicant organization only.* | 10,000 | 10,000 | | | |
| CONSULTANT COSTS | | | | | |
| EQUIPMENT | 25,000 | 25,000 | | | |
| SUPPLIES | 30,000 | 30,000 | | | |
| TRAVEL | | | | | |
| INPATIENT CARE COSTS | | | | | |
| OUTPATIENT CARE COSTS | | | | | |
| ALTERATIONS AND RENOVATIONS | | | | | |
| OTHER EXPENSES | | | | | |
| DIRECT CONSORTIUM/ CONTRACTUAL COSTS | | | | | |
| **SUBTOTAL DIRECT COSTS** *(Sum = Item 8a, Face Page)* | 65,000 | 65,000 | | | |
| F&A CONSORTIUM/ CONTRACTUAL COSTS | | | | | |
| **TOTAL DIRECT COSTS** | 65,000 | 65,000 | | | |

| **TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD** | **$** 130,000 |
|---|---|

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

The Bahrat lab will perform all imaging in vivo and in vitro studies. Costs are estimated based on annual lab expenditures and estimated effort.

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ankit Bharat

eRA COMMONS USER NAME (credential, e.g., agency login): abharat

POSITION TITLE: Professor of Surgery

EDUCATION/TRAINING

| INSTITUTION AND LOCATION | DEGREE | COMPLETION DATE | FIELD OF STUDY |
|---|---|---|---|
| **Medical School**, Christian Medical College, Vellore, India | Graduated MBBS | 02/2003 | Medicine |
| **Post-doctoral Research Fellowship,** Washington University, St Louis, MO | | 08/2003-06/2006 | Immunology and Molecular Biology |
| **Internship and Residency**, Washington University, St Louis, MO | | 7/2006-06/2011 | General Surgery |
| **Fellowship**, Washington University, St Louis, MO | | 7/2011-06/2013 | Cardiothoracic Surgery |

**A. Personal Statement**

I am a tenured Professor, Chief of Thoracic Surgery, and the surgical director of lung transplantation at Northwestern University. I investigate the pathogenesis of lung allograft dysfunction in human subjects and murine models with an overarching goal of improving survival following lung transplantation. *Scope of Research*: We use state-of the art murine and tissue culture models to detect immunopathogenesis of lung disease with a focus on transplant immunobiology. Using contemporary research tools such as single cell transcriptomics, top-down proteomics, intravital imaging, and multichannel flow cytometry, in combination with bioinformatics and computational biology, we identify clinically actionable mechanisms underlying lung injury. We follow a team science approach and I co-lead, with Dr. Scott Budinger – Chief of Pulmonary Medicine, a research group that includes senior investigators from surgery, medicine, rheumatology, and immunology. We have developed high-throughput processes within our group that allows us to perform next generation sequencing on large number of patients. Our highly collaborative multidisciplinary team allows us to successfully complete investigations on complex clinical problems as evident by our academic productivity (Grant A et al, Nature, 2021; Querrey M et al, JCI, *In Press*). I am an associate editor for the *Journal of Clinical Investigation* and *American Journal of Transplantation*. Additionally, I serve on the editorial board of *American Journal of Respiratory Cell & Molecular Biology* and *Journal of Surgical Research*. Relevance to current proposal: At Northwestern University, we have one of the largest clinical lung transplant experiences in the United States for acute respiratory distress syndrome (ARDS). Our underlying expertise in ARDS enabled us to perform the *first successful double lung transplant in the United States for COVID-19 ARDS* and we have done the most of such transplants to date (Bharat A et al, Science Transl Medicine, Dec 2020, **Cover;** Kurihara C, JAMA, 2022). We subsequently led global efforts to develop consensus guidelines for lung transplantation for ARDS (Bharat A et al, Lancet Resp Med, 2021; Bharat & Hoetzenecker, Thor Surg Clin, 2022; and Vanberkel A et al, JTCVS, *In press*). Lung transplantation is a highly successful life-saving procedure for critically ill patients with ARDS but the post-transplant course is protracted and complicated due to the unusually high (>70%) incidence of post-transplant primary graft dysfunction, the strongest risk for chronic lung rejection and poor long-term outcomes. Recognizing the growing importance of transplants for ARDS for both non-COVID-19 and COVID-19 etiologies and based on the discoveries made by our previously funded NIH projects we performed rigorous preliminary experiments that revealed targetable mechanisms for post-transplant graft dysfunction in patients with ARDS resulting in the hypotheses outlined in this proposal.

Current projects I would like to draw attention to:

R01HL145478-01A1
Bharat (PI)
01/01/19- 12/31/23

*Donor nonclassical monocytes initiate lung injury following reperfusion*

R01HL153312
Misharin (PI), Role co-investigator
06/01/20-08/31/24
*Lung transplant injury drives chronic lung allograft dysfunction via recruitment of monocyte-derived alveolar macrophages*

Relevant Citations:

1. A. Bharat et al, Lung transplantation for patients with severe COVID-19 (**Cover**). *Science TM*. 2020 Dec 16; 12(574) Epub ahead of print. PMID:33257409.
2. A. Bharat et al, Early outcomes after lung transplantation for severe COVID-19 from four countries. *Lancet Respir Med* 2021; 9: 487-97. PMID: 33811829.
3. R. Grant et al, Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. *Nature* 2021 Feb;590(7847):635-641. PMID: 33429418
4. C. Kurihara et al, Clinical Characteristics and Outcomes of Patients With COVID-19-Associated Acute Respiratory Distress Syndrome Who Underwent Lung Transplant. *JAMA* 2022 Feb 15;327(7): 652-661. PMID:35085383.

**B. Positions, Scientific Appointments, and Honors**

**Positions and Employment**:

10/2022- Present Reviewer Bioengineering, Technology, and Surgical Sciences (BTSS) Study Section - NIH
10/2021-Present Executive Director, Canning Thoracic Institute, Northwestern Medicine, Chicago, IL
06/2014–12/2024 Board-Certified by The American Board of Thoracic Surgery (Certificate No: 8003)
06/2006–07/2022 Board-Certified by The American Board of Surgery (Certificate No: 57104)
03/2019- Present Chief, Division of Thoracic Surgery, Feinberg School of Medicine, Northwestern University.
1/2016 – Present Program Director, Advanced Lung Failure Fellowship Program, Feinberg School of Medicine, Northwestern University, Chicago, IL
07/2013- Present Surgical Director, Lung Transplantation, Feinberg School of Medicine, Northwestern Medicine, Chicago, IL
09/2017- Present Associate Professor (tenure-track), Department of Surgery/ Thoracic, Feinberg School of Medicine, Northwestern University, Chicago, IL
07/2013-08/2017 Assistant Professor (tenure-track), Department of Surgery/ Thoracic, Feinberg School of Medicine, Northwestern University, Chicago, IL
01/2010-06/2013 Early Specialization Program Cardiothoracic Surgery Clinical Fellow, Washington University, St Louis, MO
07/2006-12/2009 General Surgery Resident, Washington University, St Louis, MO
08/2003-06/2006 Post-doctoral Research Fellowship, Washington University, St Louis, MO

**Other Experience and Professional Memberships**

2021- Present Elected Member, American Surgical Association (ASA)
2019- Present Elected Member, American Society of Clinical Investigation (ASCI)
2016- Present Elected Member, Society of University Surgeons
2015- Present Member, Society of Thoracic Surgeons, American Society of Transplantation, International Society of Heart and Lung Transplantation
2015- Present Fellow of American College of Surgeons
2013- Present Member, Association of Academic Surgery, International Heart and Lung Transplant Society, American Thoracic Society.

**Honors**

2020, 2021 **Top Doctor**, Castle Connelly and Chicago Magazine
2020, 2021 **Outstanding teacher award**– Northwestern University
2017 **ASCI Young Physician-Scientist Award**, the American Society for Clinical Investigation
2017 **Harold & Margaret Method Research Endowed Chair**, Northwestern University
2016 **Jacobson II Promising Investigator Award,** American College of Surgeons

2015 **Visiting Professorship** to Royal Australasian Surgical Society
2015 **Outstanding teacher award**– Northwestern University
2015 **Best Junior Faculty Research Award**- International Society of Heart and Lung Transplant
2013-2016 **Gibbon Research Scholar,** American Association of Thoracic Surgery
2013 **Richard E. Clark Award**: Society of Thoracic Surgeons annual meeting
2011 **Distinguished Fellow,** American & Canadian Society Transplant
2011 **Philip K Caves Award,** International Society of Heart & Lung Transplantation
2009 **American Transplant Society Distinguished Fellow** 2009
2008 **J. Maxwell Chamberlain Memorial Award,** Society of Thoracic Surgeons
2007 **Excellence in Research Award**. American College of Surgeons
2005 **Research excellence award**. Transplantation Society
2005&2007 **Young Investigator Award,** American Transplant Congress

**C. Contributions to Science**

1. Mechanisms of ischemia-reperfusion injury (IRI) and its impact on lung transplantation: During my post-doctoral research training, I was among the first to show that IRI was associated with the initiation of inflammatory cascade within the lung allograft, which induced donor-specific alloimmunity and predisposed to the development of chronic lung allograft rejection, thereby linking early post-transplant events with long-term allograft outcomes. Recipient neutrophils are the key effector cells which mediate IRI. However, the mechanisms that drive the neutrophils into the transplanted lungs previously were unknown. We discovered the immune mechanisms that initiate and drive the pathogenesis of IRI. Specifically, we have discovered that nonclassical monocytes, retained in the donor pulmonary vasculature, are activated upon allograft reperfusion to release CXCL2 which recruits host neutrophils. Additionally, these donor non-classical monocytes activate the donor alveolar macrophages to secrete MCP-1 to enable the influx of classical monocytes from the host spleen into the lung graft. The classical monocytes permeabilize the vascular endothelium to allow neutrophil extravasation that initiates NETosis and IRI. Importantly, tissue necroptosis after reperfusion can enhance the activation of donor nonclassical monocytes to worsen IRI. Based on these data, clinical trials targeting the pathways described in our reports, for example depleting donor nonclassical monocytes or anti-IL1β therapy, are underway to mitigate IRI.

a) Z. Zhikun et al, Donor pulmonary intravascular nonclassical monocytes recruit recipient neutrophils and mediate primary lung allograft dysfunction. (**Cover**). *Science TM.* 14 June 2017: 9; 394, eaal4508. PMCID: PMC55688853.
b) H Hsiao et al, Spleen-derived classical monocytes mediate lung ischemia-reperfusion injury through IL1β. *J Clin Invest*. 2018 Jul 2;128(7):2833-2847. PMID: 29781811
c) C. Kurihara et al, Crosstalk between nonclassical monocytes and alveolar macrophages mediates transplant ischemia-reperfusion injury through classical monocyte recruitment. *JCI Insight*. 2021 Mar 22;6(6):e147282. PMID33617207.
d) M. Querrey et al, CD11b suppresses TLR activation of nonclassical monocytes to reduce primary graft dysfunction after lung transplantation. *J Clin Invest*. In Press.

2. Etiology of primary graft dysfunction: Primary graft dysfunction (PGD) which affects over 50% of lung transplant recipients has emerged as the predominant cause of both short-term mortality as well as chronic rejection following lung transplantation. While we and others have shown that ischemia-reperfusion injury (IRI) is a common cause of PGD, we have identified several clinically treatable etiologies that can lead to PGD, independently of IRI. We discovered that over a third of patients with end-stage lung diseases develop *de novo* lung-restricted autoantibodies which can bind to the cognate antigens after extravasation from pulmonary vasculature and mediate PGD through the activation of classical and alternate complement pathway, independent of IRI. Based on these findings, autoantibodies are being prospectively screened for and complement inhibition has been introduced into the treatment paradigm of PGD. We have also found that pathogen-associated molecular patterns persist in the donor lungs despite antimicrobial therapy and activate ischemia-reperfusion primed donor alveolar macrophages to mediate PGD. Current trials are underway at our center to determine whether scavengers of oxygen free radicals and abrogate the priming of alveolar macrophages during IRI. We have also demonstrated that necroptosis associated with prolonged ischemia of the allograft can contribute to PGD introducing the possibility of necroptosis-inhibition for lungs being procured at distant donor hospitals.

a) M. Akbarpour et al, Residual endotoxin induces primary graft dysfunction through ischemia-reperfusion primed alveolar macrophages. *J Clin Invest*. 2020 Aug 3;130(8):4456-4469. PMID: 32692317.

b) S. Tanaka et al, Bacterial products in donor airways prevent the induction of lung transplant tolerance. *Am J Transplant*. 2021 Jan; 21(1): 353-361. PMID: 32786174.
c) W. Li et al, Necroptosis triggers spatially restricted neutrophil-mediated vascular damage during lung ischemia-reperfusion injury, *Proc Natl Acad Sci (PNAS)*. 2022 Mar 8; 119 (10); e2111537119.
d) W. Wang et al, IL1β dependent extravasation of pre-existing lung-restricted autoantibodies activates classical and alternate complement pathways to promote primary graft dysfunction. *J Clin Invest*. In Press.

3. Identification of *Ureaplasma* as a fatal infection following lung transplantation: About 5% of lung transplant recipients develop fatal hyperammonemia within the first month of transplantation. This was previously attributed to unmasking of an inborn error of urea cycle metabolism by calcineurin inhibitors such as tacrolimus, typically administered as part of lung transplant immunosuppressive regimen. However, despite medical therapy, mortality associated with this syndrome approached 100%. We discovered that donor-derived *Ureaplasma* infection is causally linked to hyperammonemia. This was validated in our multi-institutional clinical study as well as animal models to fulfil Koch's postulates for disease causation. Further, *Ureaplasma* infection in lung transplant recipients was found to be associated with other morbidities including acute renal failure, sepsis, and dehiscence of bronchial anastomosis. These findings have led to changes in practice with regards to *Ureaplasma* screening in donors and successful treatment of hyperammonemia across the country. Ongoing investigations in our laboratories have demonstrated that *Ureaplasma* affects a variety of immunosuppressed hosts such as those undergoing cardiac and renal transplantation suggesting that this may be an unrecognized pathogen with a larger disease burden. Based on our discovery, this fatal complication is treatable.

a) A Bharat et al. Disseminated Ureaplasma infection as a cause of fatal hyperammonemia in humans. *Science TM*. 2015 Apr 22; 7(284). PMCID: PMC4677674
b) A Bharat et al. Donor-derived Ureaplasma is a potentially lethal infection in lung allograft recipients. *Journal of Heart and Lung Transplantation*. August 2017, Volume 36, Issue 8, 917 – 918. PMID: 28483300.
c) R Fernandez et al. Sequelae of Donor-derived Mollicutes Transmission in Lung Recipients. *American Journal of Respiratory and Critical Care Medicine*. 2017 Mar 01. 195(5), pp. 687–689. PMID: 27925760;
d) S. Roberts et al, Impact of screening and treatment of Ureaplasma species on hyperammonemia syndrome in lung transplant recipients. *Clin Infect Dis*. 2021 Nov 2;73(9):2531-2537. PMID: 33068392.

4. Role of CD4+CD25+foxp3+ regulatory T cells in transplantation tolerance and chronic rejection: Despite the improvements in surgical techniques and immunosuppression, the incidence of chronic lung allograft has not improved largely due to the medically refractory chronic rejection. We have demonstrated a crucial role of $CD4^+CD25+$ regulatory T cells in mediating transplant tolerance as well as the development of lung-restricted autoimmunity. We elucidated the mechanisms underlying the homing of the donor-specific regulatory T cells into the allograft. We further demonstrated that, once tolerized, the regulatory T cells are self-replicating and can drive tolerance to donor tissues implanted at distant sites. Additionally, we have described the role of autoantigens containing exosomes in the development of *de novo* autoantibodies when regulatory T cells are impaired by respiratory viruses following transplantation. This lays important groundwork for the development of strategies to mitigate chronic rejection.

a) M. Rahman et al, Novel role for tumor suppressor gene, liver kinase Bi, in epithelial-mesenchymal transition leading to chronic lung allograft dysfunction. *Am J Transplant*. 2022 Mar; 2022(3): 843-852. PMID: 34859569.
b) S. Bansal et al, Circulating exosomes induced by respiratory viral infections in lung transplant recipients activate cellular stress, innate immune pathways and epithelial to mesenchymal transition. *Transpl Immunol*. 2021 Dec; 69:101489. PMID: 34619318.
c) W. Li et al, Lymphatic drainage from bronchus-associated lymphoid tissue in tolerant lung allografts promotes peripheral tolerance. *J Clin Invest*. 2020 Dec 1; 130(12):6718-6727. PMID: 33196461.
d) W. Li et al, Bronchus-associated lymphoid tissue-resident Foxp3+ T lymphocytes prevent antibody-mediated lung rejection. *J Clin Invest*. 2019 Feb 1;129(2): 556-568. PMID: 30561386.

4. Mechanisms for development of lung-restricted autoimmunity in lung transplant recipients. We have investigated the pathogenesis of *de novo* lung-restricted autoimmunity following lung transplantation as well as in patients with end-stage lung disease, awaiting transplantation. Our initial studies demonstrated that clonal expansion of self-reactive $CD4^+$ T lymphocytes against lung-restricted self-antigens preceded the development of chronic lung allograft rejection. $CD4^+CD25^+Foxp3^+$ regulatory T cells were found to dynamically suppress these self-reactive

lymphocytes. Concomitant loss of regulatory T cells was necessary for the clonal expansion of self-reactive lymphocytes and development of lung-restricted autoimmunity. Respiratory viruses were clinically associated with apoptosis of regulatory T cells. Using murine models, we found that respiratory viruses indeed induced apoptosis of regulatory T cells by upregulating Fas-L on the infected airway epithelium which predisposed the host to lung-restricted, but not systemic, autoimmunity. However, loss of regulatory T cells alone was not sufficient to induce lung-restricted autoimmunity in normal hosts. We found that lung injury, for example by acid aspiration or HLA antibodies, was necessary along with simultaneous loss of regulatory T cells, for example due to respiratory viral infection, to trigger lung tissue-restricted autoimmunity. Based on these observations, we proposed a "two-hit hypothesis" for the development of *de novo* tissue-restricted autoimmunity in transplant recipients.

a) A Bharat et al. Lung-restricted antibodies mediate primary graft dysfunction and prevent allo-tolerance following murine lung transplantation. *American Journal Respiratory Cell & Molecular Biology*. 2016 Oct;55(4):532-541. PMCID: PMC5070112.
b) S Chiu et al. Lung injury combined with loss of regulatory T cells leads to de novo lung-restricted autoimmunity. *Journal Immunology*. 2016 Jul 1;197(1):51-7. PMCID: PMC4912911.
c) S. Bansal et al, The role of miRNA-155 in the immunopathogenesis of obliterative airway disease in mice induced by circulating exosomes from human lung transplant recipients with chronic lung allograft dysfunction. *Cell Immunol*. 2020 Sep;355:104172.PMID: 32707293.
d) Y.Itabashi et al, Decline in Club Cell Secretory Proteins, Exosomes Induction and Immune Responses to Lung Self-antigens, Kα1 Tubulin and Collagen V, Leading to Chronic Rejection After Human Lung Transplantation. *Transplantation*. 2021 Jun 1;105(6):1337-1346.PMID: 32890135.

5. Role of pleural hypercapnia in the development of delayed lung healing and alveolopleural fistula: My K08 proposal outlined experiments we have completed to evaluate the role of carbon dioxide in lung healing. We developed a technology to measure pleural gas milieu and used it to show that pleural hypercapnia following lung resection is associated with delayed lung healing and the development of alveolopleural fistula. A clinical trial to reduce carbon dioxide levels in the pleural space was associated with significant improvement in lung healing. Each 1% increase in pleural carbon dioxide level was associated with a delay of 8 hours in lung healing. Laboratory investigation revealed that injury-triggered production of CXCL12 was necessary for epithelial cell migration and repair of lung wound healing. Carbon dioxide inhibited NFkB mediated transcription through proteosomal activation, which reduced CXCL12 production and impaired wound healing following injury. We went on to show that carbon dioxide impairs the migration of epithelial cells from the airway and alveolus, independent of cell proliferation, and reduces the clearance of apoptotic epithelial cells by alveolar macrophages (efferocytosis), both of which slow lung injury repair. These data have implications in the clinical management of patients with acute lung injury, both surgical trauma and acute respiratory distress syndrome and have resulted in a new form of chest drainage system being developed to incorporate analysis of intrapleural gas analysis.

a) J Kanter et al, Decreased CXCL12 is associated with impaired alveolar epithelial cell migration and poor lung healing following lung resection. *Surgery*, 2015. 158(4):1073-80. PMCID: PMC4567440
b) A Bharat et al, Pleural hypercarbia after lung surgery is associated with persistent alveolopleural fistula. *CHEST*. 2016 Jan;149(1):220-7. PMCID: PMC5831571.
c) A Bharat et al, High CO2 impairs lung wound healing. *Am J Respir Cell Mol Biol.* 2020 Aug; 63(2):244-254.
d) E Ceco et al, Elevated CO2 levels delay skeletal muscle repair by increasing fatty acid oxidation. *Front Physiol*. 2021 Jan 21;11:630910. PMID: 33551852.

**Complete List of Published Work in MyBibliography:** (total 170, h factor 36, Google Scholar)
https://www.ncbi.nlm.nih.gov/myncbi/1bYq6QgoLsGk5/bibliography/public/

**D. Additional Information: Research Support and/or Scholastic Performance**
**Pending**

Title: Necroptotic cell death as a driver of primary graft dysfunction after lung transplantation

Major Goals: To determine the role of mitochondrial ROS and RIPK3 signaling in the development of PGD after lung transplantation for ARDS in mice and humans.

Source of Support: National Heart, Lung, and Blood Institute

Primary Place of Performance: Northwestern University

Project/Proposal Start and End Date: 02/01/2023-01/31/2028

Total Award Amount (including Indirect Costs): $3,976,242

**Active and approved**

Title: Donor nonclassical monocytes initiate lung injury following reperfusion

Major Goals: The goal of this project is to identify pathways that are activated by donor non- classical monocytes which could be clinically targeted to ameliorate PGD without causing recipient toxicity.

Project Number: 1R01HL145478-01 Name of PD/Pl: Bharat

Source of Support: National Heart, Lung, and Blood Institute

Primary Place of Performance: Northwestern University

Project/Proposal Start and End Date: (MMIYYYY) (if available): 1/1/2019 -12/31/2023

Total Award Amount (including Indirect Costs): $2,756,133

*Title: Pathogenesis of lung injury mediated by lung-restricted antibodies

Major Goals: The goal of this project is to examine the mechanisms of neutrophil recruitment and complement activation and their role in autoantibody-mediated lung injury.
Status of Support: Active
Project Number: 1R01HK147290-01 Name of PD/Pl: Bharat
Source of Support: National Heart, Lung, and Blood Institute
Primary Place of Performance: Northwestern University
Project/Proposal Start and End Date: (MMIYYYY) (if available): 4/01/2019-3/31/2024
Total Award Amount (including Indirect Costs):$2,454,786

Title: Role of Spleen educated monocytes in mediating ischemia-reperfusion injury following lung transplant
Major Goals: The goals of the project is to examine the mechanisms through which classical monocytes are educated in the host spleen and subsequently recruited to the transplanted lungs where they enable neutrophils to leave the blood vessels and mediate lung injury.
Status of Support: Active
Project Number: 1R01HL147575-01
Name of PD/Pl: Bharat (Contact), Budinger
Source of Support: National Heart, Lung, and Blood Institute
Primary Place of Performance: Northwestern University
Project/Proposal Start and End Date: (MM/YYYY) (if available:) 4/01/2019-3/31/2024
Total Award Amount (including Indirect Costs): $2,366,872

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Snee, Preston T.

eRA COMMONS USER NAME (credential, e.g., agency login): SNEEPT

POSITION TITLE: Associate Professor of Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | Completion Date MM/YYYY | FIELD OF STUDY |
|---|---|---|---|
| University of North Carolina at Chapel Hill | B.S. | 05/1995 | Chemistry |
| University of California at Berkeley | Ph.D. | 12/2002 | Chemistry |
| Massachusetts Institute of Technology | Postdoctoral | 05/2006 | Chemistry |
| University of Illinois at Chicago | MBA | 05/2021 | Finance |

### A. Personal Statement

The goals of the Snee group are to develop robust semiconductor nanocrystal quantum dot (QD) technology and to make the materials accessible for biological imaging applications. PI Snee is an expert in the synthesis of a variety of highly emissive quantum dots and subsequent water-solubilization and functionalization. The QDs are made from a variety of core/shell materials such as visible emitting ZnSe/ZnS, CdS/ZnS, CdSe/ZnS, as well as near-IR InAs. A recent focus has been on the synthesis of cadmium-free nanocrystals such as InP/ZnS and $AgInS_2$/ZnS. They are also experts at functionalizing QDs for biological imaging applications and have demonstrated the highest reaction yields reported to date. Due to use of highly refined chemical precursors and solvents, QD syntheses are very robust which aids technology transfer.

### B. Positions, Scientific Appointments, and Honors

The Research Triangle Institute, The Research Triangle Park, NC
1995-1997 Analytical Chemist I
Massachusetts Institute of Technology, Cambridge, MA
2003-2006 Postdoctoral Research Associate (Advisors: Profs. Moungi G. Bawendi, Daniel G. Nocera)
University of Illinois at Chicago, Chicago, IL
2006-2013 Assistant Professor, Department of Chemistry
2013-pres. Associate Professor with Tenure, Department of Chemistry

### C. Contributions to Science

Snee has published 80 peer-reviewed papers, reviews, and book chapters and has produced four issued patents. His publications have registered over 4500 Google Scholar citations to date, with an H index of 35. Some important work includes:

***1) Non-toxic semiconductor quantum dot synthesis (2007 to present)***

The use of quantum dots for biological studies has increased rapidly since their discovery in 1983. However, most materials are synthesized from cadmium-containing semiconductors. As such, there are concerns for future application of these dots in clinical trials due to toxicity issues. An obvious solution is to develop less toxic QDs for biological applications. In this regard, Snee has examined the synthesis of non-toxic visible and NIR-emitting QDs via doping of benign host materials and by synthesizing cadmium-free systems.

Work in this area continues with addressing the problems with the synthesis of core/shell cadmium-free dots, which is necessary to develop biological imaging applications.

Thakar, R.; Chen, Y.; **Snee, P. T.** "Efficient Emission from Core / (Doped) Shell Nanoparticles: Applications for Chemical Sensing" *Nano Lett.,* 2007, *7*, 3429–3432.

Shamirian, A. Appelbe, O.; Zhang, Q.; Ganesh, B.; Kron, S. J; **Snee, P. T.** "A toolkit for bioimaging using near-infrared $AgInS_2$/ZnS quantum dots" *J. Mater. Chem. B*, 2015, 3, 8188-8196.

Das, A.; Hsu, B.; Shamirian, A.; Yang, Z.; **Snee, P. T.**, "Synthesis of High-Quality $AgSbSe_2$ and $AgBiSe_2$ Nanocrystals with Antimony and Bismuth Silylamide Reagents" *Chem. Mater* 2017, 29, 4597–4602.

Chandrasiri, H. B.; Kim, E.; Snee, P. T. "Sterically Encumbered Tris(trialkylsilyl) Phosphine Precursors for Quantum Dot Synthesis" *Inorg. Chem.* 2020, *21*, 15928–15935.

***2) Fluorescent quantum dot ratiometric sensors (2006 to present)***

Snee was the first chemist to prepare a quantum dot ratiometric (or color-changing) sensor. This study demonstrated that the continuous and strong absorption manifold of the QDs resulted in an excitation-wavelength independent response. Furthermore, QD-based FRET sensors are generally reversibly sensitive to specific analytes as well as resistant to photobleaching. They can be further functionalized to target specific biological entities or coated with poly-ethylene glycol to reduce toxicity and non-specific biological interactions. Recently, the group has examined the development of several systems for the analytical examination of targets including toxic mercury, thiols, and proteins.

**Snee, P. T.;** Somers, R. C.; Bawendi, M. G.; Nocera, D. G. "Fluorescent Sensor and Methods," US Patent 20090221088. Licensed to Life Technologies.

**Snee, P. T.**; Somers, R. C.; Nair, G.; Zimmer, J. P.; Bawendi, M. G.; Nocera, D. G. "A Ratiometric CdSe/ZnS Nanocrystal pH Sensor." *J. Am. Chem. Soc.*, 2006; *128*, 13320–13321.

Krooswyk, J. D.; Tyrakowski, C. M.; **Snee, P. T.** "Multivariable Response of Semiconductor Nanocrystal-Dye Sensors: the Case of pH," *J. Phys. Chem. C.* 2010, 114, 21348–21352.

Tyrakowski, C. M.; **Snee, P. T.** "Ratiometric CdSe/ZnS Nanocrystal Protein Sensor" *Anal. Chem.* 2014, 86, 2380−2386.

Shamirian, A.; Samareh Afsari, H.; Wu, D.; Miller, L. W.; **Snee, P. T.** "Ratiometric QD-FRET Sensing of Aqueous $H_2S$ *in vitro*.", *Anal. Chem.* 2016, 88, 6050–6056.

Shamirian, A.; Samareh Afsari, H.; Hassan, A.; Miller, L. W.; **Snee, P. T.** "*In vitro* Detection of Hypoxia using a Ratiometic Quantum Dot-Based Oxygen Sensor" *ACS Sensors*, 2016**,** 1*,* 1244-1250.

Maloney, V. P.; Wu, D.; Pan, L.; Shamirian, A.; **Snee, P. T.** "Ratiometric Sensing Compound and Device Made from the Compound," US Patent Application US15464643.

***3) Efficient quantum dot functionalization (2007 to present)***

While the synthesis of very bright water-soluble quantum dots has been addressed by our group and others, the materials are useless unless they can be functionalized for biological imaging or sensing purposes. Unfortunately, there are a limited number of functionalization protocols / reagents that are effective in water, and many of them fail when applied to quantum dots due to their colloidal instability. Snee was the first to show that this was due to the electrostatic nature of quantum dot colloids. This knowledge allowed the group to develop new reagents and to demonstrate some of the first high-yielding (>95%) protocols for quantum dot surface functionalization. These protocols were applied to create QD-based sensing and imaging systems.

Chen, Y.; Thakar, R.; **Snee, P. T.** "Imparting Nanoparticle Function with Size-Controlled Amphiphilic Polymers*." J. Am. Chem. Soc*., 2008, 130, 3744-3745.

Shen, H.; Jawaid, A. M.; **Snee, P. T.** "Poly(ethylene glycol) Carbodiimide Coupling Reagents for the Biological and Chemical Functionalization of Water Soluble Nanoparticles" *ACS Nano*, 2009, 3, 915–923.

Zhang, X. Mohandessi, S.; Miller, L. W.; **Snee, P. T.** "Water soluble CdSe/ZnS Nanocrystal Functionalization Using Small Chemical Activators," *Chem. Commun.* 2011, 47, 3532–3534*.*

Ravindran; S.; **Snee, P. T.**; Eapen, A. S.; Sundivakkam, P.; Tiruppathi, C.; George, A. "Acidic Domains in DPP Facilitates Endocytosis: Implications for Targeted Protein Delivery" *J. Bio. Chem.,* 2013, 288, 16098-16109*.*

Padovano, J. D.; Ravindran, S.; **Snee, P. T.**; Ramachandran, A.; Bedran-Russo, A. K., George, A. "DMP1-derived Peptides Promote Remineralization of Human Dentin.", *J. Dent. Res.,* 2015, 94, 608–614.

**Snee, P. T.** "The Role of Colloidal Stability and Charge in Functionalization of Aqueous Water-Soluble Quantum Dots.", *Acc. Chem. Res.,* 2018, 51, 2949–2956.

Jing, H.; Pálmai, M.; Saed, B.; George, A.; **Snee, P. T.**; Hu, Y. S† "Cytosolic Delivery of Membrane-penetrating QDs into T Cell Lymphocytes: Implications in Immunotherapy and Drug Delivery" *Nanoscale*, 2021, *13*, 5519-5529.

Jing, H.; Saed, B.; Pálmai, M.; Gunasekara, H.; **Snee, P. T**.; Hu, Y. S. "Fluorescent Artificial Antigens Revealed Extended Membrane Networks Utilized by Live Dendritic Cells for Antigen Uptake" *Nano Lett.* 2022, *22*, 4020.

***4) Quantum dot water solubilization with cap exchange (2011 to present)***

The brightest, most stable quantum dot materials are synthesized in hydrophobic solvents that render them soluble in non-aqueous solutions only. To impart water-solubility, the dots can be encapsulated in polymers or cap exchanged with new ligands. The cap exchange process produces the smallest QD colloids; however, they are also notoriously unstable and lost a significant level of emission efficiency. Snee was able to manipulate the mechanism of cap exchange by chemically reacting new ligands on the surface of dots to render them water soluble with enhanced brightness and stability. Recently, the group developed a method to cross link the surface-bound ligands to create cap-exchanged colloids that are stable on an unprecedented several month timescale. Furthermore, the materials have built-in chemical "handles" that facilitates chemical functionalization.

Liu, D.; **Snee, P. T.** "Water Soluble Semiconductor Nanocrystals Cap Exchanged with Metallated Ligands," *ACS Nano* 2011, *5*, 546–550.

Tyrakowski, C. M.; Isovic, A.; **Snee, P. T.** "Water-solubilization and Functionalization of Semiconductor Quantum Dots" chapter of Methods in Molecular Biology. Humana Press, Springer, Netherlands, 2013, ISSN: 1064-3745.

Tyrakowski, C. M.; **Snee, P. T.** "A primer on the synthesis, water-solubilization, and functionalization of quantum dots, their use as biological sensing agents, and present status" *Phys. Chem. Chem. Phys.*, 2014, 16, 837-855.

Zhang, X.; Jawaid, A. M.; Tyrakowski, C. M.; Page, L. E.; Chen, O.; Isovic, A.; Hassan, A.; Shamirian, A.; **Snee, P. T.** "Near Single-Layer $SiO_2$ Coated Aqueous Semiconductor Quantum Dots" *Small*, 2015, 11, 6091–6096.

**SciENcv profile:**

https://www.ncbi.nlm.nih.gov/myncbi/preston.snee.1/cv/89506/

**Google Scholar profile:**

https://scholar.google.com/citations?hl=en&user=Hhv5F4kAAAAJ

**D. Additional Information: Research Support and/or Scholastic Performance**

**Pending**

"Exploring Photo-Induced Triplet Energy Transfer in Organic-Semiconductor Hybrid Materials for Catalysis Applications" NSF/CHE/MSN, $125,527, 08/23-7/26.
The goal of this project is to develop semiconductor nanocrystal photocatalysts for production of $H_2$ gas.
Role:co-PI

**Active and approved**

"Metal Phosphide Catalysts Prepared by Direct Conversion of P4", American Chemical Society Petroleum Research Fund, $110,000, 01/20-08/23.
The goal of this project is to create nickel phosphide nanocrystals that may have utility as catalysts for denitrification of shale oil. No overlap with the current proposal.
Role: PI