CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

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

Prognostic Imaging of T Cell Health (PITCH) for CAR T Therapy

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

Ying S. Hu, PhD
Assistant Professor of Chemistry, University of Illinois at Chicago
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Do you have any current or pending grant applications that potentially overlap with this application? Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

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.)

Summary of project. **Chimeric antigen receptor T-cell (CAR T) therapy** has revolutionized the treatment of several types of blood cancers, but the treatment has reported mixed response rates. Since CAR T therapy harvests a patient's immune T cells and engineers them to target cancer, evaluating the health status of a patient's T cells before the treatment is an attractive strategy for improving the therapeutic outcome. Existing T-cell functional assays are labor-intensive, costly, and inaccessible in diagnostic clinics. Therefore, there is an unmet need for rapid and cost-effective assays. This CBC Catalyst proposal develops a pilot imaging technique, **Prognostic Imaging of T Cell Health (PITCH)**, to help predict the outcomes of CAR T therapy.

Innovation. This high-risk, high-reward project represents unique challenges for conventional funding sources. Primarily, the technology development is still in its conceptual stage. While the connections between imaging data and T-cell fitness and those between cell lines and primary T cells isolated from patients are highly complex and multifactorial, our work sets the pathway to demonstrate the feasibility of a reductionist approach. If successful, the technology will cast a paradigm change in immunotherapy by providing patient-accessible and cost-effective screening assays with a drop of blood.

Description of proposed research. Cellular therapies have revolutionized the treatments of hematologic malignancies. The FDA has approved six different autologous chimeric antigen receptor T-cell (CAR T) therapy products (Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, and Carvykti) for commercial use to treat acute lymphoblastic leukemia, non-Hodgkin lymphomas, and multiple myeloma.[1] Despite the amazing successes of these CAR T therapies, a significant portion of patients either fail to respond or have a short duration of response.[2–4] Furthermore, the total cost of care can exceed a whopping $1.5M per patient.[5] Clinical factors, such as age, bulky disease, prior lines of therapy, high lactate dehydrogenase, and specific genetic alterations, have been studied to predict CAR T therapy outcomes. However, these factors are indirectly related to the CAR T therapy and lack specificity. Evaluating T cell fitness is an attractive strategy because it has the potential to predict CAR T therapy outcomes from the upstream source. However, existing methodologies to measure T cell fitness, such as flow cytometry and cytokine analysis, are labor and time intensive and costly.[6] Other single-cell techniques generate static snapshots of information. Cell sorting, immunolabeling, and the use of other machines also add to the cost. Currently, outside of the cell viability as a quality metric of transduced T cells, no other T cell activity parameters are evaluated before cell infusion into the patients. Therefore, there is an unmet need for a rapid and cost-effective system to guide patient selection for CAR T therapy.

To this end, intracellular calcium (Ca^{2+}) imaging has been extensively used to study T cell activation.[7] Engagement of the T cell receptor (TCR) with the agnostic peptide triggers the emptying of calcium stores from the endoplasmic reticulum (ER), which then causes an influx of Ca^{2+} across the plasma membrane (Fig. 1A).[8] Sustained Ca^{2+}signaling is paramount to the effector function of T cells, including proliferation, cytokine production, and cytotoxicity (Fig. 1B), all of which correlate with T cell health. Therefore, the overall objective of this CBC Catalyst Award is to develop a cost-effective Ca^{2+}-imaging technology that paves the way for a rapid quantitative evaluation of T-cell health. We termed the technique Prognostic Imaging of T Cell Health or PITCH. Our central hypothesis is that the endoplasmic reticulum (ER) calcium storage correlates with T-cell functional status and, thus, the clinical response of CAR T therapy.

To test this hypothesis, we will measure ER Ca^{2+} storage using a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), thapsigargin. We have previously demonstrated the feasibility of this platform in studying the ER Ca^{2+} storage in mouse embryonic fibroblasts.[9] Briefly, T cells will be incubated with membrane-permeable Fura-2-acetoxymethyl ester (Fura-2AM), immobilized on glass surfaces coated with CD45 antibodies, and imaged using a specialized 40x/1.3 oil-immersion objective with UV transmission on a Nikon Ti-2E inverted microscope. The microscope system is housed in Dr. Hu's research group at UIC. The ER Ca^{2+} concentration will be obtained using the Fura-2AM Ca^{2+} imaging calibration kit.

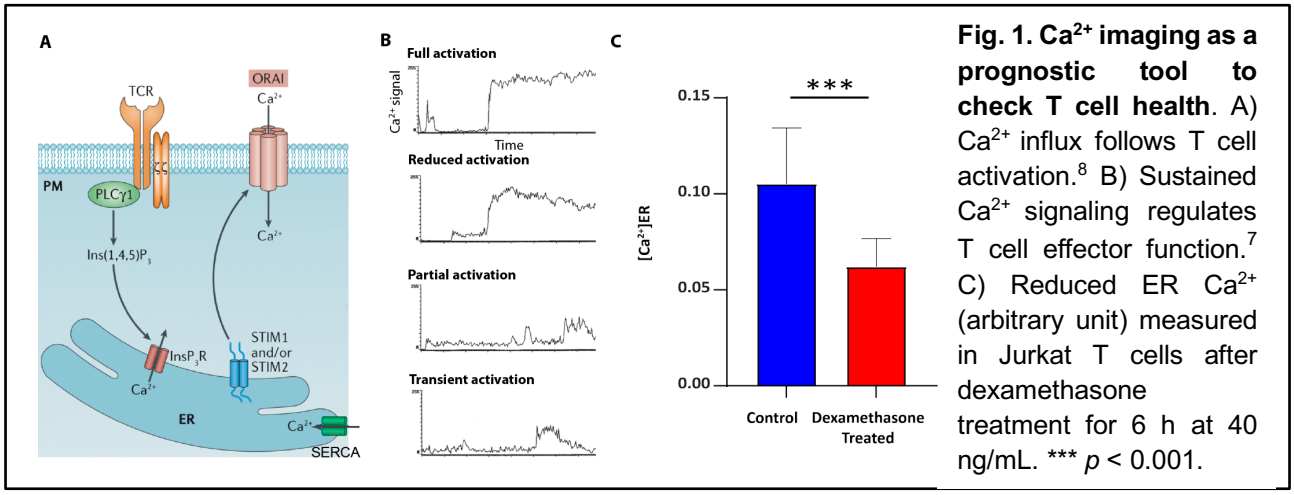

Fig. 1. Ca^{2+} imaging as a prognostic tool to check T cell health. A) Ca^{2+} influx follows T cell activation.[8] B) Sustained Ca^{2+} signaling regulates T cell effector function.[7] C) Reduced ER Ca^{2+} (arbitrary unit) measured in Jurkat T cells after dexamethasone treatment for 6 h at 40 ng/mL. *** $p < 0.001$.

Aim 1: Establishing correlations between ER Ca^{2+} storage and CAR T cell fitness.
The goal of Aim 1 is to benchmark PITCH against standard T-cell fitness assays *in vitro*.

Objective 1.1. Determining the quantitative relationship between ER Ca^{2+} storage and immunosuppressive culture conditions of Jurkat T cells. Wild-type Jurkat T cells and a Jurkat-based CAR T cell line (BPSBiosceinces, Cat # 79853) will be cultured with media containing the steroid (dexamethasone), chemotherapy agent (cyclophosphamide), inhibitory cytokine (interleukin 10, IL-10), or activation beads (exhaustion) to mimic immune-suppressing conditions in clinical scenarios. Corticosteroids, including dexamethasone, have been used to treat cytokine-release syndrome or immune effector cell-mediated neurotoxicity syndrome during CAR T-cell treatment.[10] Our supporting data suggest a 40% reduction in ER Ca^{2+} with dexamethasone treatment (Fig. 1C). Similarly, cyclophosphamide is used in various standard-of-care lymphoma therapy regimens and conditioning prior to CAR T therapy. IL-10 represents a family of potent anti-inflammatory cytokines for treating autoimmune diseases.[11] The ER Ca^{2+} store data will be compared with standard methods to measure T cell fitness, such as flow cytometry for immunophenotyping[12] and cytokine assays (interferon-gamma, IL-1, and tumor necrosis factor). Furthermore, the imaging data will be correlated with CAR-T functional assays on manipulated and unmanipulated CAR T cells. The anti-CD19 CAR T cells will undergo multiple functionality testing, which includes proliferation (CellTrace CSFE and MTS kits) and cytokine release (cytokine array kits).

Objective 1.2. Determining the quantitative relationship between ER Ca^{2+} storage of primary T cells from healthy and cancer donors. T cells will be isolated from the peripheral blood of healthy donors and patients with hematologic malignancies (leukemia, lymphoma, and myeloma), excluding T cell lymphoma/leukemias. The initial phase will include five healthy donors and 15 patient samples, which is well under previous accruals for peripheral blood collection within the hematologic malignancy group at the Robert H Lurie Comprehensive Cancer Center (RHLCCC) at Northwestern University. The final sample size determination will depend on the differences in T cell Ca^{2+} data from the above experiments.

Expected outcome, potential pitfalls, and alternative strategies. This activity will establish the connection between ER Ca^{2+} storage and the health status of T cells for making CAR-T products. Importantly, cancer patients' T cells may be less healthy than those from healthy donors; the latter has been utilized to make generic allogeneic CAR-T products.[13] Using patient-derived T cells, however, has the distinct advantage of minimizing the immune rejection of CAR-T products. To this end, our PITCH technology optimizes the resource allocation to harvest the T cells that display ER Ca^{2+} signatures that correlate with healthier status. Moreover, the CAR-T response may deviate from the health status of T cells. To address this potential limitation, we will perform the CAR T-cell

functional assay and establish correlations between the health status based on the Ca^{2+} readout and the CAR T-cell functionality.

Aim 2: Evaluating PITCH as a predictive marker for CAR T therapy.
The goal of Aim 2 is to evaluate PITCH as a predictive technology for patients receiving CAR T therapy for treating lymphoma and myeloma.

Objective. Establishing a correlation between ER Ca^{2+} stores as a function of days after cell infusion and patient survival. Patients who qualify for CAR T therapy will be consented to collect a vial of peripheral blood during apheresis, the day of cell infusion (day 0), day 30, and day 90, as well as leftover CAR T cells in the bag after infusion. T cells will be isolated from these samples. ER Ca^{2+} stores will be recorded using PITCH. The primary objective is to correlate ER Ca^{2+} signatures during apheresis with progressive free survival (PFS) after matching other patient characteristics. Secondary objectives include correlating ER Ca^{2+} storage at day 0, 30, or 90 with PFS and ER Ca^{2+} signatures at apheresis day 0, 30, or 90 with overall survival. The patient samples will also undergo standard flow cytometry to measure T cell fitness.

The RHLCCC at Northwestern University, where Dr. Lin is a PI, performed around 100 cellular therapies in 2021. In addition, 30-60 patients were enrolled in previous studies evaluating the correlation between T cell phenotype *via* flow cytometry with CAR T therapy outcomes[14]. To ensure adequate patient accrual to evaluate the PITCH platform, this pilot study will initially enroll 30 eligible patients, and the sample size will be adjusted depending on correlation analysis, which will be performed by the Quantitative Data Sciences Core at the RHLCCC.

Potential pitfalls and alternative strategies. While Aim 2 represents an ambitious goal, our objective is to leverage the unique strength of our team and the CBC funding mechanism to collect clinical data in this pilot study. If the pilot study is inconclusive, our strategy is to use the preliminary data and seek collaborations with pharmaceutical companies with access to large tissue banks with known patient records. While PITCH may not be the *sole* technique to predict CAR T therapy outcome, our strategy is to develop *a* patient-accessible and cost-effective strategy to complement the existing technologies.

Nature of inter-institutional collaboration and how the success of the Catalyst project requires the expertise of each PI. We have assembled a team of junior PIs with complementary expertise; both areas of expertise are critical to ensure the success of this CBC Catalyst project. Dr. Hu specializes in immune imaging, and the lab creates impactful imaging-driven solutions to improve the standard of care in immuno-oncology. Dr. Lin specializes clinically in lymphoma and cellular therapies and has access to the target patient population for this grant. Drs. Hu and Lin have previously collaborated on developing goal-based nanoparticles for cancer theranostics.[15,16]

Criteria for measuring success. We anticipate quantitatively establishing the connection between ER Ca^{2+} storage and CAR T-cell product functionality and activities. We anticipate that the healthier T cells determined from Aim1 will enable more function and improved lymphoma-killing activity in corresponding CAR T cells. Moreover, an 80% correlation between PITCH results and patient outcomes defines a benchmark milestone for Aim 2.

Long-term funding plan. Supporting results from this study will enable the team to apply for multi-PI R33: Advanced Development (RFA-CA-22-002) and R01 Integration & Validation (RFA-CA-22-005) in the Innovative Molecular Analysis Technologies (IMAT) Program at the National Cancer Institute in late 2023 or early 2024. We will also seek funding from RHLCCC and CAR T fabrication companies such as Bristol Myers Squibb (Breyanzi and Abecma), with which the PIs have established collaborations.

Bibliography

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2 Ahle S. *Are CAR T-Cell Therapies Worth the Costs?*. ASH Clinical News. 2020. URL: https://www.ashclinicalnews.org/spotlight/drawing-first-blood/car-t-cell-therapies-worth-costs/ (Accessed 11 November 2022).

3 Abreu TR, Fonseca NA, Gonçalves N, Moreira JN. Current Challenges and Emerging Opportunities of CAR-T Cell Therapies. *J Control Release* 2020;**319**:246–61.

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6 Maryamchik E, Gallagher KME, Preffer FI, Kadauke S, Maus MV. New Directions in Chimeric Antigen Receptor T Cell [CAR-T] Therapy and Related Flow Cytometry. *Cytometry B Clin Cytom* 2020;**98**:299–327.

7 Wülfing C, Rabinowitz JD, Beeson C, Sjaastad MD, McConnell HM, Davis MM. Kinetics and Extent of T Cell Activation as Measured with the Calcium Signal. *J Exp Med* 1997:1815–25. https://doi.org/10.1084/jem.185.10.1815.

8 Trebak M, Kinet J-P. Calcium Signalling in T Cells. *Nat Rev Immunol* 2019:154–69. https://doi.org/10.1038/s41577-018-0110-7.

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10 Liu S, Deng B, Jing PAN, Yin Z, Lin Y, Ling Z, *et al.* Corticosteroids Do Not Influence the Efficacy and Kinetics of CAR-T Cells for B-Cell Acute Lymphoblastic Leukemia. *Blood* 2019; **134**(Suplement_1):228–228. https://doi.org/10.1182/blood-2019-123051.

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13 Graham C, Jozwik A, Pepper A, Benjamin R. Allogeneic CAR-T Cells: More than Ease of Access? *Cells* 2018;**7.**: https://doi.org/10.3390/cells7100155.

14 Monfrini C, Stella F, Aragona V, Magni M, Ljevar S, Vella C, *et al.* Phenotypic Composition of Commercial Anti-CD19 CAR T Cells Affects In Vivo Expansion and Disease Response in Patients with Large B-cell Lymphoma. *Clin Cancer Res* 2022;**28.**: https://doi.org/10.1158/1078-0432.CCR-22-0164.

15 Chen AL, Jackson MA, Lin AY, Figueroa ER, Hu YS, Evans ER, *et al.* Changes in Optical Properties of Plasmonic Nanoparticles in Cellular Environments are Modulated by Nanoparticle PEGylation and Serum Conditions. *Nanoscale Res Lett* 2016;**11**:303.

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

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH

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
	PD/PI							
SUBTOTALS →								

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
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)*		**$**
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**$**

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

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.*					
CONSULTANT COSTS					
EQUIPMENT					
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TRAVEL					
INPATIENT CARE COSTS					
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ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS					

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$**

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

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

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY	FROM	THROUGH

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
	PD/PI							
SUBTOTALS →								

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
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CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$

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

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
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ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES					
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS					

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$**

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

BIOGRAPHICAL SKETCH

NAME: Hu, Ying Samuel

eRA COMMONS USER NAME: yingshu1

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Houston, Houston, TX	B.S.	05/2006	Electrical Engineering
Rice University, Houston, TX	Ph.D.	12/2011	Bioengineering
California Institute of Technology, Pasadena, CA	Postdoctoral	03/2012	Electrical Engineering
Salk Institute for Biological Studies, La Jolla, CA	Postdoctoral	08/2018	Bioimaging and T Cell Biology

A. Personal Statement

The central research theme of the Hu laboratory is to probe nanoscale immunobiophysics using single-molecule and superresolution fluorescence imaging. Current research encompasses four major areas: 1) developing antibody-based imaging probes for quantitative superresolution molecular census, 2) investigating spatial and temporal membrane regulation of cytokine release at the nanoscale; 3) probing antigen uptake and cross-presentation in dendritic cells, and 4) leveraging computational approaches to extract immune cell functions from fluorescence images. The multidisciplinary research is at the forefront of bioimaging, chemical biology, bioanalytical chemistry, and nanotechnology.

This CBC Catalyst proposal leverages Dr. Hu's research expertise in applying cutting-edge imaging (1) and analysis techniques (2) to study T cells. Calcium imaging has been previously established and the Hu group has developed a workflow for imaging ER calcium release (3). In collaboration with a physician scientist, Dr. Lin, Dr. Hu's research group will translate the T-cell imaging technology for predicting patient outcomes of CAR T therapy. Of note is that Drs. Hu and Lin have previously collaborated on developing gold-based nanoparticles as imaging contrast agents for cancer theranostics (4,5).

Selected citations:

1. **Hu YS**, Cang H, Lillemeier BF. Superresolution imaging reveals nanometer- and micrometer-scale spatial distributions of T-cell receptors in lymph nodes. *Proc Natl Acad Sci U S A*. 2016;113(26):7201-6. PMID: 27303041; PMCID: PMC4932922.
2. Saed B, Munaweera R, Anderson J, O'Neill WD, **Hu YS**. Rapid statistical discrimination of fluorescence images of T cell receptors on immobilizing surfaces with different coating conditions. *Sci Rep.* 2021;11(1):15488. PMID: 34326382; PMCID: PMC8322097.
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4. Chen AL, Jackson MA, **Lin AY**, Figueroa ER, **Hu YS**, Evans ER, Asthana V, Young JK, Drezek RA. Changes in Optical Properties of Plasmonic Nanoparticles in Cellular Environments are Modulated by Nanoparticle PEGylation and Serum Conditions. *Nanoscale Res Lett*. 2016;11(1):303. PMID: 27316744; PMCID: PMC4912538.
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experienced by plasmonic nanoparticles in a cellular environment to inform biomedical nanoparticle design. *Nanoscale Res Lett*. 2014;9(1):454. PMID: 25258596; PMCID: PMC4164329.

Ongoing and recently completed projects that I would like to highlight include:

Current:

NIGMS/NIH — Hu (PI) — 8/1/22 – 5/31/27
R35 GM146786

The R35 proposal will develop ultrasensitive quantification tools to characterize cytokine release from the plasma membrane. Using T cells and interleukin-2 as a model system, the research will probe the temporal and directional cytokine release profiles, proximity-based membrane regulation, and the distribution between soluble and extracellular-vesicle-associated cytokines during T cell activation. The research program will enhance the fundamental understanding of the cytokine release at the molecular level and enable the search for membrane mechanisms to modulate the process.

Research Corporation for Science Advancement — Hu (PI) — 8/1/22 – 7/31/23
Scialog Advancing BioImaging Award

The proposal develops a live tissue clearing technique for lymph nodes using dendritic cells with programmable optical properties.

Completed:

Chicago Biomedical Consortium — Swartz, Scott, Hu, Hubbell (Multi-PI) — 9/1/20 – 8/31/22
COVID-19 Response Award

The purpose of this grant is to develop novel functional nanomaterials to target the vaccine to specific cells that regulate immune response, specifically dendritic cells to induce immunity and lymphatic endothelial cells to induce enhanced memory. The collaborative proposal will employ advanced microscopy to gain better understanding of how specific cell types affect the generation and breadth of the response.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2018 - Assistant Professor, Department of Chemistry, University of Illinois at Chicago, Chicago, IL
2022 - Affiliate Faculty, Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL
2022 - Full Member (Associate Member, 2019-22), Cancer Biology, University of Illinois Cancer Center, Chicago, IL

Other Experience and Professional Memberships

2019 - Member, American Chemical Society (ACS)
2013 - Member, Screening and Selection Committees, US Department of Energy (DOE) Computational Science Graduate Fellowship (CSGF)
2007 - Member, The International Society for Optical Engineering (SPIE)

Honors

2022 Scialog Fellow, Advancing BioImaging, Research Corporation for Science Advancement
2016 Young Investigator Award, The American Association of Immunologists (AAI)
2013 Young Investigator Award, Single Molecule Spectroscopy and Superresolution Imaging, PicoQuant (Germany) and SPIE Photonics West
2012 -17 DOE Allocation Awards, National Energy Research Scientific Computing Center (NERSC)
2012 -15 California Institute for Regenerative Medicine (CIRM) postdoctoral fellowship
2009 -11 DOE Computational Science Graduate Fellowship (CSGF)
2008, 09 Graduate Scholarship in Optical Science and Engineering, SPIE
2007 NSF East Asia and Pacific Summer Institute Fellowship in Australia

C. Contributions to Science

I. Ultrasensitive imaging techniques to study nanoscale immunity. I have established a niche for my research group by developing and applying highly sensitive imaging techniques to dissect spatial and temporal mechanisms of immunity. Current research develops single-molecule and superresolution microscopy techniques to understand how membrane proteins (1-2) regulate T cell signaling. In parallel, my group employs single molecule and superresolution techniques to investigate nano-membrane interactions for vaccine design and drug delivery (3-4).

1. **Hu YS**, Cang H, Lillemeier BF. Superresolution imaging reveals nanometer- and micrometer-scale spatial distributions of T-cell receptors in lymph nodes. *Proc Natl Acad Sci U S A*. 2016;113(26):7201-6. PMID: 27303041; PMCID: PMC4932922.
2. Saed B, Munaweera R, Anderson J, O'Neill WD, **Hu YS**. Rapid statistical discrimination of fluorescence images of T cell receptors on immobilizing surfaces with different coating conditions. *Sci Rep*. 2021;11(1):15488. PMID: 34326382; PMCID: PMC8322097.
3. Jing H, Pálmai M, Saed B, George A, Snee PT, **Hu YS**. Cytosolic delivery of membrane-penetrating QDs into T cell lymphocytes: implications in immunotherapy and drug delivery. *Nanoscale*. 2021;13(10):5519-5529. PMID: 33688882; PMCID: PMC8029070.
4. Jing H, Saed B, Pálmai M, Gunasekara H, Snee PT, **Hu YS**. Fluorescent artificial antigens revealed extended membrane networks utilized by live dendritic cells for antigen uptake. *Nano Lett*. 2022; 22(10): 4020–4027, PMID: 35499493.

II. Technology development for quantitative superresolution imaging. My group has invented antibody-based probes to enable quantitative molecular census (5). In parallel, my research develops light-sheet instrumentation (6-7) and algorithms (8) for superresolution microscopy.

5. Gunasekara H, Munaweera R, Novotná L, Lillemeier BF, **Hu YS,** Chaotropic perturbation of noncovalent interactions of the hemagglutinin tag monoclonal antibody fragment enables superresolution molecule census, *ACS Nano.* 2022;16(1):129-139. PMID: 34797055.
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III. Contrast enhancement and label-free imaging using tunable plasmonic nanoparticles. Plasmonic nanoparticles are about one hundredth the width of a human hair but possess uniquely tunable scattering and absorption properties much stronger than what their physical dimensions afford. Using Mie and finite-element methods, my research has demonstrated enhanced scattering from gold-based nanoparticles of multilayered geometry for contrast enhancement in diagnostic imaging (9-10). I also experimentally demonstrated the use of nanoengineered substrates for enhanced label-free imaging (11).

9. **Hu Y**, Noelck SJ, Drezek RA, Symmetry breaking in gold-silica-gold multilayer nanoshells, *ACS Nano*. 2010;4(3):1521-8. PMID: 20146507.
10. **Hu Y**, Fleming RC, Drezek RA, Optical properties of gold-silica-gold multilayer nanoshells, *Opt Express.* 2008;16(24):19579-91. PMID: 19030045.
11. **Hu YS**, Jeon J, Seok TJ, Lee S, Hafner JH, Drezek RA, Choo H, Enhanced Raman scattering from nanoparticle-decorated nanocone substrates: a practical approach to harness in-plane excitation, *ACS Nano.* 2010;4(10):5721-30. PMID: 20836500.

IV. Imaging research in low-resource settings. My research group cultivates a supportive environment to train future engineers and scientists. Undergraduate researchers contributed to several publications cited above. Of note is an independent research project led by undergraduate researchers that developed an innovative imaging platform using evaporation-resistant droplets of cooking oils (12). Our undergraduate

researchers have been funded by the UIC Honors College Research Grant and Summer Research Opportunities Program.

12. Szydlowski NA, Jing H, Alqashmi M, **Hu YS**, Cell phone digital microscopy using an oil droplet, *Biomed Opt Express.* 2020;11(5):2328-2338. PMID: 32499926; PMCID: PMC7249838.

Complete list of publications:
https://www.ncbi.nlm.nih.gov/myncbi/1zoxdZlzT_GIf8/bibliography/public/

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: Adam Lin

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

POSITION TITLE: Assistant Professor

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 California, Irvine, Irvine, CA	B.S.	06/2007	Biomedical Engineering
Baylor College of Medicine, Houston, TX	M.D.	12/2014	MSTP
Rice University, Houston, TX	Ph.D.	06/2013	Bioengineering
Northwestern University, Chicago, IL		06/2017	Internal Medicine
Northwestern University, Chicago, IL		06/2021	Hematology/Oncology

A. Personal Statement

I am an assistant professor at Feinberg School of Medicine, Northwestern University, and the Robert H Lurie Comprehensive Cancer Center. My clinical focus is on **lymphoma, hematopoietic stem cell transplant, and cellular therapy**. My research program, the Nanotechnology & Biomaterials Lab for Blood Cancers, is focused on translating nanotechnologies into the diagnosis and treatment of hematologic malignancies. The project that is proposed for the CBC Catalyst Award combines my unique background in bioengineering technology and cellular therapy experts and Dr. Hu's microscopy expertise in calcium signaling imaging. Success of this project will allow a new rapid, low-cost, predictive tool to help guide physicians regarding results of cellular therapies.

Nanotechnology has made significant advancements in medicine in the last 15-20 years though mostly in preclinical work. My training allows me to bridge the gap between technological advancements and their use in clinical or personalized medicine. I have extensive experience with modifications of nanoparticles and its utilization in medicine. Currently, I have 15 first/co-first authorships publications (*1/3 in journals with IF >10 and 1/3 in journals with IF between 5-10)* and another 10 publications as a co-author.

For various nanoparticle platforms, I was awarded the **American Society of Hematology** (ASH) Research Training Award for Fellows and the **Lymphoma Research Foundation** Postdoctoral Fellowship during my training. I was also selected for the **AACR** Molecular Biology in Clinical Oncology (Snowmass) as well as the ASH Clinical Research Training Institute for clinical trial training. During my first year of faculty, I was also awarded the **Cancer Research Foundation** Young Investigator Award.

Aside from my interest in clinical medicine, I am very passionate about education. I am currently a mentor for the MSTP at Northwestern and a speaker for the "A Day in Life of a STEM-itist," which is a virtual STEM career series for middle and high school students in Chicago. Previously, I enjoyed mentoring undergraduate students and medical students both in the clinical and research settings. I want to continue mentoring and educating graduate or medical students while encouraging participation in research, in emphasis to provide opportunities for underrepresented students.

Selected Citations (*denotes co-first author; Impact factors (IF) from 2021 Journal Citation Reports)

- Lin AY*, Choi BS*, Sim TH, Yang E, Choi HJ, Behdad A, Kim DH, Gordon LI. In Situ Vaccination by Photothermal Therapy with CpG Deoxynucleotide Coated Gold Nanoparticles in a Lymphoma Model. Blood Advances. 2022; PMID: 35687489 (*IF 7.367*)
- Lin AY, Rink JS, Karmali R, Xu JH, Kocherginsky M, Thaxton CS, Gordon LI. Tri-ethylene glycol modified class B and class C CpG conjugated gold nanoparticles for the treatment of lymphoma. Nanomedicine: Nanotechnology, Biology, and Medicine. 2020. 30:102290. PMID: 32798731 (*IF 6.458*)
- Lin AY*, Young JK*, Nixon AV, Drezek RA: Encapsulated Fe3O4/Ag Complexed Cores in Hollow Gold Nanoshells for Enhanced Theranostic Magnetic Resonance Imaging and Photothermal Therapy. *Small*. 2014; 10(16): 3246-3251. PMID: 24729414 (*IF 15.15*)
- Lin, AY*, Young JK*, Nixon AV, Drezek RA: Synthesis of a quantum nanocrystal–gold nanoshell complex for near-infrared generated fluorescence and photothermal decay of luminescence. *Nanoscale*. 2014; 6: 10701-10709. PMID: 25096858 (*IF 8.307*)

Ongoing and recently completed projects that I would like to highlight include:

Cancer Research Foundation Young Investigator Award (Lin) 07/01/22-6/30/24
Combination of CpG Nanoparticles for the Treatment of Aggressive Non-Hodgkin Lymphoma

Our preliminary data suggest that combination of class B-CpG nanoparticles and class C-CpG nanoparticles generated the strongest anti-lymphoma effect. Here, we aim to uncover the immune mechanism for these improved effects delivered by injecting the nanoparticles intratumorally or intravenously.

Lymphoma Research Foundation Postdoctoral Fellowship Grant (Lin) 07/01/20-06/30/22
Engineering CpG deoxynucleotide-conjugated gold nanoparticles for enhanced anti-lymphoma effects

The goal of this project was to synthesize and characterize the anti-lymphoma effects of class B and class C CpG deoxynucleotide when conjugated on gold nanoparticles.

American Society of Hematology RTAF (Lin) 07/01/19-06/30/20
Enhancing apoptosis and immunotherapy with CpG coated hollow gold nanoshells for aggressive B cell lymphoma

The goal of this project was to engineer hollow gold nanoshell-CpG deoxynucleotide complexes and characterize the physical properties as well as the cytotoxic effects this novel agent.

NRSA F30 NCI CA165686 (Lin) 07/31/12-12/31/14
Gold Nanoparticle Assisted Delivery of Cancer Vaccines and Immune Stimulants

The goal of this project was to design and test a novel gold based nanovaccines that can be prepared using this simple, cost effective, bottom-up conjugation method to generate effective anti-tumor immune responses with limited toxicity and improved lymphatic drainage.

B. Positions, Scientific Appointments, and Honors

Positions:

2021-present Assistant Professor, Division of Hematology-Oncology, Northwestern University

License: Physician and Surgeon in the State of Illinois (2017)
Board: Certified in Internal Medicine (2018), Medical Oncology (2021), Hematology (2021)

Honors and Awards:

2020 CAHON Young Investigator Award
2020 ASH Annual Meeting Abstract Achievement Award
2019 Translational Bridge Award, Robert H Lurie Comprehensive Cancer Center, NU
2019 ASH Annual Meeting Abstract Achievement Award
2019 Jameson Research Award, Department of Medicine, Northwestern University
2018-19 Chief Hematology/Oncology Fellow
2018-19 ASH Research Training Award for Fellows Finalist
2017 Hershel M. Rich Invention Award, Rice University
2015-16 Medical Student Teaching Award
2014 Medical Scientist Training Program Thesis Publication Award
2009-10 SPIE Scholarship in Optical Science and Engineering

2007-15 Edward and Josephine Hudson Scholarship
2007-08 Jack Welhausen Scholarship
2007-08 Dr. & Mrs. Alfred S. Wolfe '38 Scholarship
2007-08 Robert C. Carman Scholarship
2007-08 MEDELLAS Scholarship
2005-07 UC Leadership Excellence through Advanced Degrees (UCLEADS)
2005-06 Life Sciences Industry Council (LINC) Scholar
2005-06 Outstanding Academic Achievement in Biomedical Engineering Award

Accepted Programs and Workshops:
2021 ECOG-ACRIN Young Investigator Symposium
2020-21 ASH Clinical Research Training Institute (CRTI)
2019-20 Feinberg School of Medicine Leadership Program with the Center for Leadership at NU
2018 AACR Molecular Biology in Clinical Oncology Workshop (Snowmass)

C. Contributions to Science

1. Cancer Vaccines gold nanoparticles: Our gold nanovaccine design used a simple bottom-up conjugation scheme to form chained peptides on the particle surface, thus enabling the particles to carry large amounts of peptides while maintaining sub-100 nm in diameter. The design can be assembled with multiple epitopes or even antigens to accommodate natural variations between patients. The design is not restricted for cancer vaccines and can be used for any type of vaccine antigen such as viral or bacterial. In addition, we designed a novel triethylene glycol modified CpG coated AuNPs for enhanced adjuvant function. This design outperformed traditional DNA AuNP constructs at stimulating macrophages per CpG delivered and decreased tumor growth and increased survival in comparison to free CpG. My role in these projects include designing and synthesizing, testing, and validating the efficacy of the particles in murine models.

- Lin AY, Rink JS, Karmali R, Xu JH, Kocherginsky M, Thaxton CS, Gordon LI. Tri-ethylene glycol modified class B and class C CpG conjugated gold nanoparticles for the treatment of lymphoma Nanomedicine: Nanotechnology, Biology, and Medicine. 2020. 30:102290. PMID: 32798731
- Lin AY*, Mattos Almeida JP*, Langsner RJ, Eckels P, Foster AE, Drezek RA. In Vivo Immune Cell Distribution of Gold Nanoparticles in Naïve and Tumor Bearing Mice. Small. 2013; 10(4): 812-819.
- Lin AY*, Mattos Almeida JP*, Bear A, Liu N, Luo L, Foster AE, Drezek RA: Gold Nanoparticle Delivery of Modified CpG Stimulates Macrophages and Inhibits Tumor Growth for Enhanced Immunotherapy. PLOS One. 2013; 8(5): e63550.
- Lin AY, Lunsford J, Bear AS, Young JK, Eckels P, Foster AE, Drezek RA: High Density Sub-100nm Peptide-Gold Nanoparticle Complexes Improve Vaccine Presentation by Dendritic Cells In Vitro. Nanoscale Research Letters. 2013; 8:72.

2. Theranostic nanoparticles combined with photothermal therapy: We have designed two types of theranostic gold nanoshells, which allows for photothermal ablation. First, we have developed a triple function theranostic nanoparticle complex that could be potentially used as a diagnostic, treatment, and therapeutic confirming agent by combining the near infrared and photothermal properties of hollow gold nanoshells with the luminescent properties of NIR-based quantum nanocrystals. The plasmonic response of the nanoshells and proximity of the nanocrystals to the gold surface could potentially serve to enhance the luminescent properties. This complex design and functionality could greatly aid in the identification of tumor margins and satellite tumors while allowing for ablative confirmation. For the other theranostic particle, we designed, fabricated, and tested a novel magnetic hollow gold nanoshell that incorporates small iron oxide nanoparticles (IONP) in the center of the nanoshell. The collaborative effect of the smaller IONPs improved the overall magnetic properties of the design and thus improved the particle's capability as a MRI contrast agent. The magHGNs were able to perform well as MRI T2 contrast agents and were able debulk tumors and improve survival with PTT.

- Lin AY*, Choi BS*, Sim TH, Yang E, Choi HJ, Behdad A, Kim DH, Gordon LI. In Situ Vaccination by Photothermal Therapy with CpG Deoxynucleotide Coated Gold Nanoparticles in a Lymphoma Model. Blood Advances. 2022; PMID: 35687489
- Lin, AY*, Young JK*, Nixon AV, Drezek RA: Synthesis of a quantum nanocrystal–gold nanoshell complex for near-infrared generated fluorescence and photothermal decay of luminescence. Nanoscale. 2014; 6: 10701-10709.

- Lin AY*, Young JK*, Nixon AV, Drezek RA: Encapsulated Fe3O4/Ag Complexed Cores in Hollow Gold Nanoshells for Enhanced Theranostic Magnetic Resonance Imaging and Photothermal Therapy. Small. 2014; 10(16): 3246-3251.

3. Microfluidic cell separation: My undergraduate career was focused on microfluidic devices for liposomal particle synthesis and cell separation. One project, in particular, involved designing a microfluidic device that improved magnetic cell separation efficiency. My role was to design, fabricate, and test the device to separate CD20+ B cells. We were able to generate a strong magnetic field density gradient over a small length by introducing a channel of nickel particles in close proximity to the separation channel where the sample flows through. The channel is made with PDMS, peeled from a SU-8/Si mold, and plasma bonded with glass. By using a channel with nickel particles, we did not have to deposit or electroplate nickel on the glass surface and thus we avoid possible problems with alignment of the nickel and the separation channel. We were able to set up a proof of principle device and file for a patent.

- Lin AY, Wong TS: Particle-Based Microfluidic Device for Providing High Magnetic Field Gradients. US Patent. US20100044232A1, US8551333B2, WO2009008925A2, WO2009008925A3. April 7 2008.
- Lin AY, Wong TS, Bhardwaj U, Chen JM, McCabe E, Ho CM: Formation of high electromagnetic gradients through a particle-based microfluidic approach. Journal of Micromechanics and Microengineering 2007; 17:1299-1306.

*co-first authorship

Link for full publication list:
https://www.ncbi.nlm.nih.gov/myncbi/adam.lin.1/bibliography/public/