Proteases:

Powers, Problems, and Opportunities in Conditions that Impact Women

Manu O. Platt, Ph.D.

Senior Investigator
Distinguished Scholar
Head, MATRICES Lab, NIBIB
Director, BETA Center, NIH/NIBIB
Associate Director for DEIA, NIBIB

NATIONAL ACADEMIES Sciences Engineering Medicine

October 1, 2024

Board on Health Sciences Policy
Forum on Regenerative Medicine
Forum on Temporomandibular Disorders

MATRICES LAB
Mechanics And Tissue Remodeling Integrating Computational + Experimental Systems

BETA
CENTER for BIOMEDICAL ENGINEERING TECHNOLOGY ACCELERATION

NIH National Institute of Biomedical Imaging and Bioengineering

Engineering the Future of Health℠

BETA

CENTER for BIOMEDICAL ENGINEERING TECHNOLOGY ACCELERATION

Manu Platt, Ph.D.
Director

Nicole Morgan, Ph.D.
Deputy Director

Goals:

The BETA Center is a NIH-wide resource housed by the NIBIB intramural research program and will serve as a new NIH campus model for **accelerating technology-driven interdisciplinary research and clinical translation.**

The BETA Center community will **bring researchers together** with engineering and imaging expertise including biomedical imaging, biosensing, biomechanics, engineered/synthetic biology, nano/biomaterials, artificial intelligence, modeling, computation, and informatics.

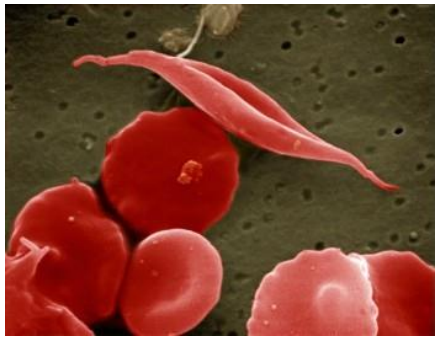
Central to BETA Center's mission is employing evidence-driven approaches to **expand diversity, equity, and inclusion** within NIBIB's Intramural Research Program and serving as a model for recruiting diverse biomedical engineering talent to NIH.

Manu O. Platt, Ph.D.
Head, MATRICES, NIBIB
manu.platt@nih.gov

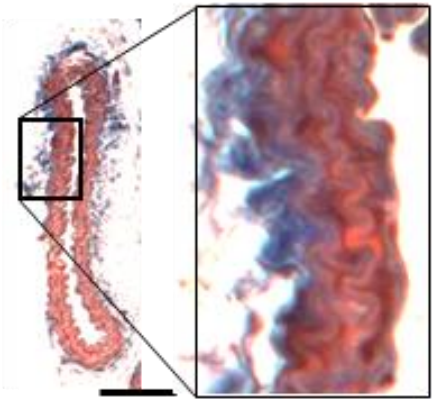
MATRICES LAB

Mechanics And Tissue Remodeling Integrating Computational + Experimental Systems

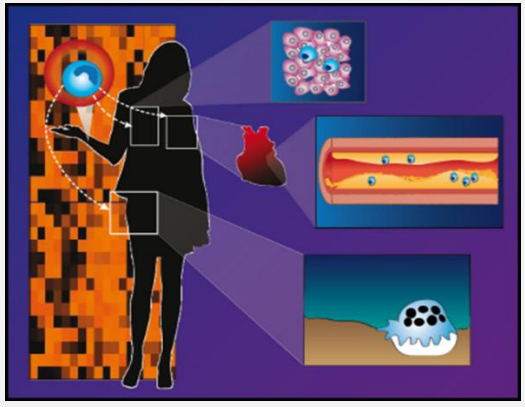
PEDIATRIC STROKES IN SICKLE CELL DISEASE



EXTRACELLULAR MATRIX REMODELING AND ARTERIAL MECHANICS

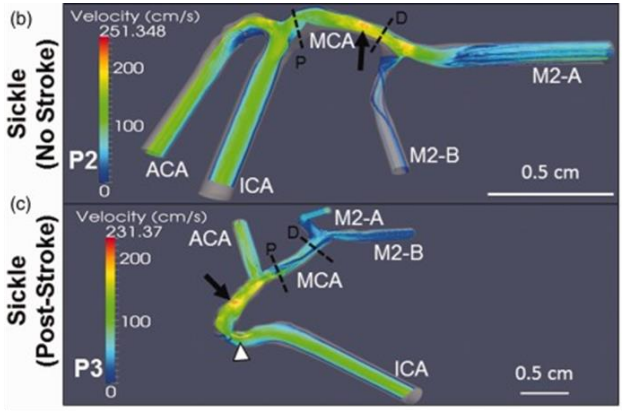


SYSTEMS BIOLOGY AND PREDICTIVE MEDICINE

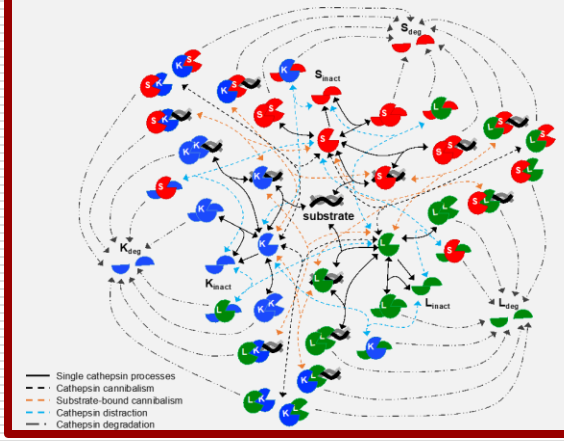


LABEL FREE MR IMAGING

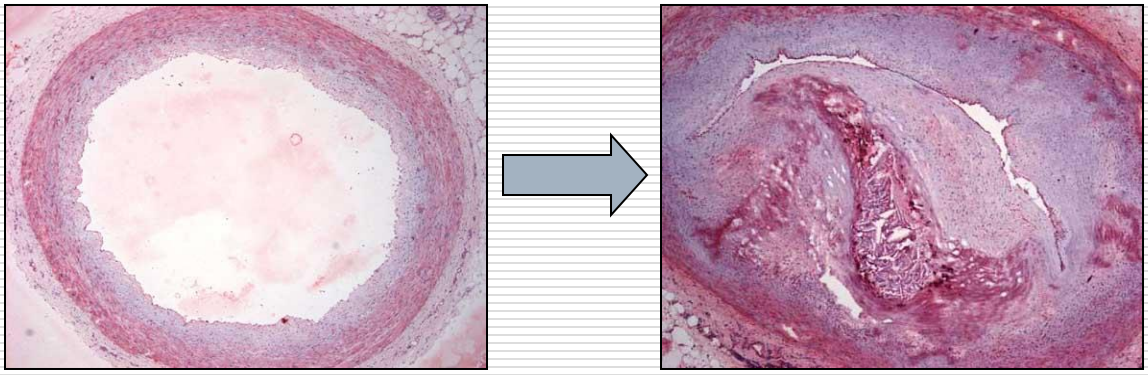
COMPUTATIONAL HEMODYNAMICS



INTERROGATION OF MATRIX PROTEOLYTIC NETWORKS



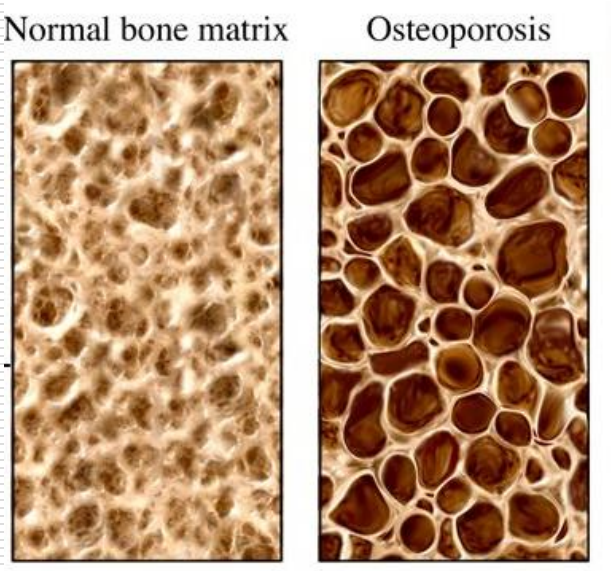
What is Tissue Remodeling?

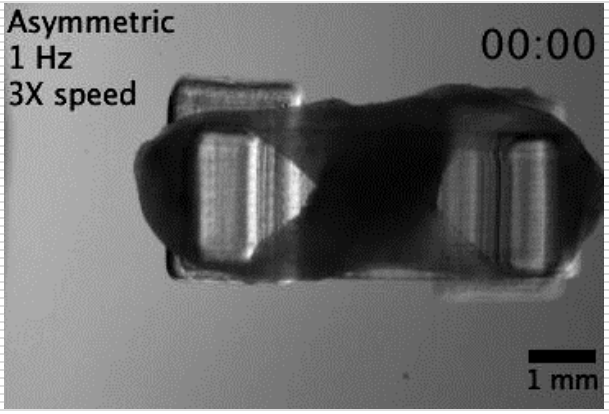


protein degradation

protein synthesis

cell infiltration

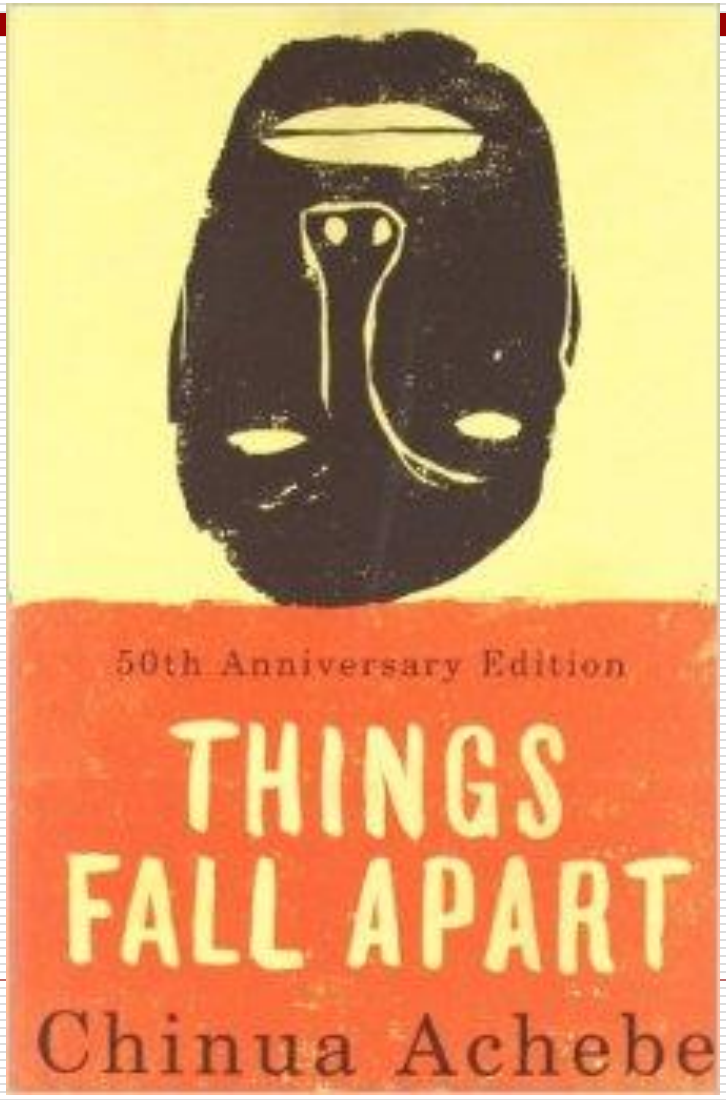




www.ebics.net

Collab with Bashir, UIUC

Proteases as a Non-Cellular Innovation in Women's Health



PROTEASES

- Functional biomarkers
- Low cost diagnostics from biopsies / fluids
- Predictive and personalized medicine strategies

Conditions of Interest:

HIV/AIDS
Breast Cancer
Endometriosis

Sickle Cell Disease: Single point mutation, Multi-factorial disease

REGULAR ARTICLE

blood advances

Sickle cell disease promotes sex-dependent pathological bone loss through enhanced cathepsin proteolytic activity in mice

Jada Selma,[1,*] Hannah Song,[1,*] Christian Rivera,[1] Simone Douglas,[1] Abhiramgopal Akella,[1] Keval Bollavaram,[1] Nishone Thompson,[1] Manu O. Platt,[1,2] and Edward A. Botchwey[1,2]

[1]Wallace H. Coulter Department of Biomedical Engineering at Georgia Institute of Technology and Emory University, Atlanta, GA; and [2]Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA

42 for females

1 in 400 African-Americans (SS)
1 in 12 have sickle trait (AS)

100,000 Americans with sickle cell disease

Proteases in manufacturing contexts

Roche

CUSTOMBIOTECH

Proteases for Industrial Applications

Safe, Consistent Processing of Polypeptides

Nonfood Protease Applications

GENENCOR® A Danisco Division

Medicine

Pharmacology & drug manufacture

Laundry & dishwashing detergents (#1)

Hard surface cleaning formulations

Contact lens cleaning formulations

Waste treatment

Industrial applications

Fermentation (fuel EtOH, etc.)

Chondroitin & heparin production

Animal feed additives

Digestive supplements

Control of *in vivo* proteolysis in the production of recombinant proteins

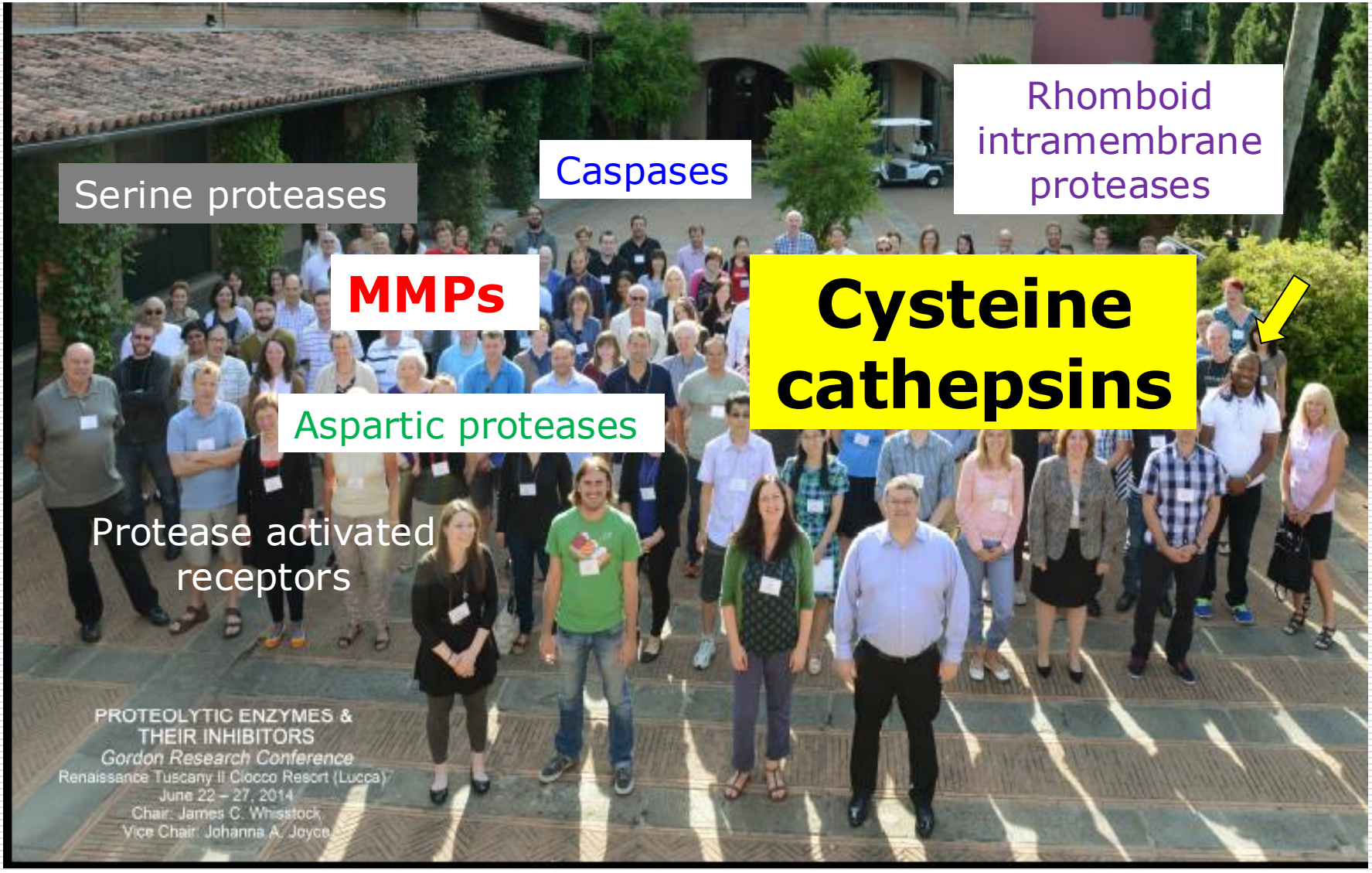
Sven-Olof Enfors

Table 1. Strategies adopted for the control of proteolysis

Stage employed	Strategy used
Downstream processing	Low temperature Protease inhibitors Early protease elimination Fast product isolation
Fermentation	Limiting substrate control Temperature optimization Inclusion body control
Cell	Protease-negative mutants Secretion Expression of intracellular protease inhibitors Heat shock modulation
Protein	Sequence modification Fusion for inclusion-body formation Protective fusion

Proteolytic degradation of protein products causes many problems in the bioprocess industry. The increasing production of proteins in heterologous hosts through the use of recombinant DNA technology, has recently brought the problem into focus, since it seems that heterologous proteins are more prone to proteolysis. The result of proteolytic attack may vary from complete hydrolysis of the product to minor truncation of the protein without impairment of its biological function. The degree to which such partial proteolysis is a problem depends on the requirements of the ultimate use of the protein product.

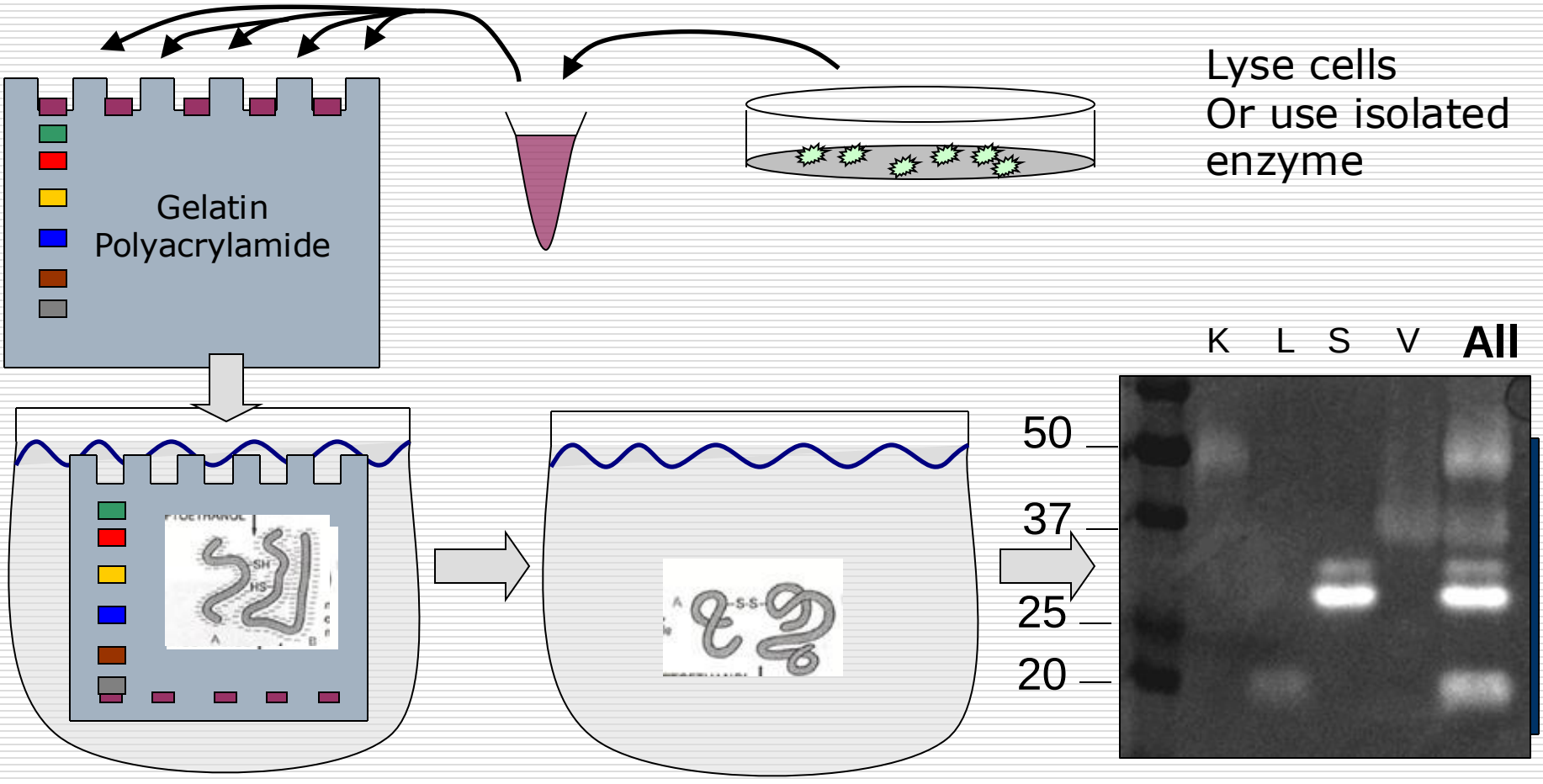
Proteases: not just MMPS anymore
460 human proteases known



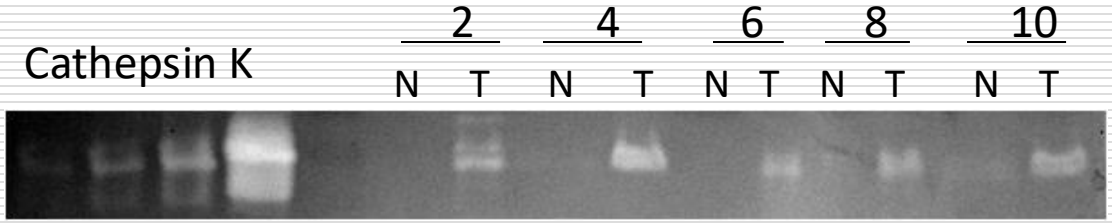
Cysteine cathepsins: pathologies and challenges

- Pathologies include osteoporosis, atherosclerosis, tendinopathy, and tumor metastasis
- **Most potent mammalian collagenases and elastases**

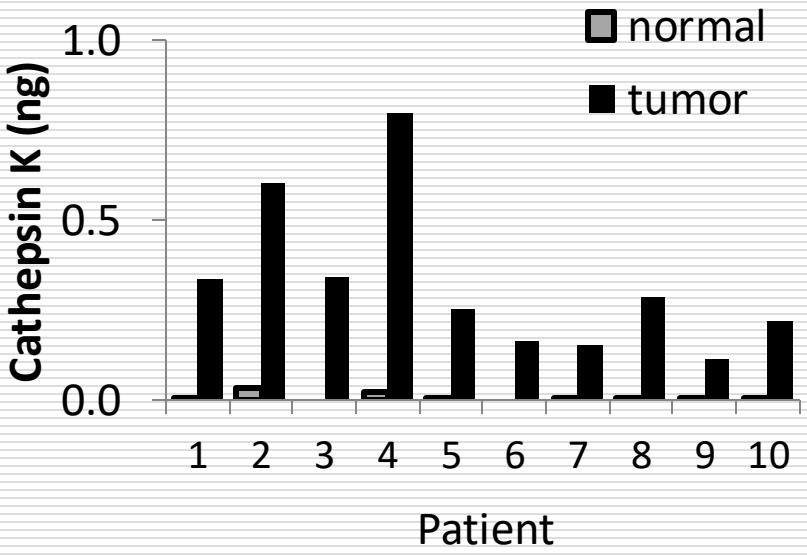
Low cost assays with increased sensitivity: multiplex cathepsin zymography

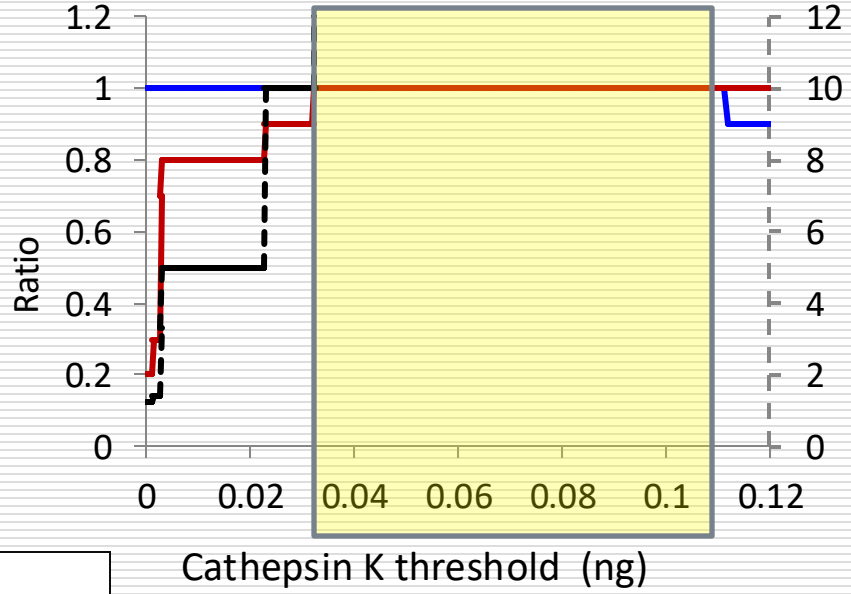


Cathepsin K is 50-fold increased in human breast cancer



N-normal
T- tumor





Chen and Platt *Journal of Translational Medicine* 2011, **9**:109
http://www.translational-medicine.com/content/9/1/109

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH **Open Access**

Multiplex Zymography Captures Stage-specific Activity Profiles of Cathepsins K, L, and S in Human Breast, Lung, and Cervical Cancer

Binbin Chen and Manu O Platt*

Keon-young Park, MD, PhD

SCIENTIFIC REPORTS

OPEN

Monocyte-derived macrophage assisted breast cancer cell invasion as a personalized, predictive metric to score metastatic risk

Received: 21 April 2015
Accepted: 06 August 2015
Published: 09 September 2015

Keon-Young Park, Gande Li & Manu O. Platt

Sickle Cell Disease Activates Peripheral Blood Mononuclear Cells to Induce Cathepsins K and V Activity in Endothelial Cells

Philip M. Keegan, Sindhuja Surapaneni, and Manu O. Platt

Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA

Annals of Biomedical Engineering, Vol. 42, No. 6, June 2014 *(© 2014)* pp. 1185–1194
DOI: 10.1007/s10439-014-1005-9

BMES BIOMEDICAL ENGINEERING SOCIETY™ www.bmes.org

Pro-Atherogenic Shear Stress and HIV Proteins Synergistically Upregulate Cathepsin K in Endothelial Cells

Ivana Kennedy Parker,[1,4] Ladeidra Monet Roberts,[2] Laura Hansen,[2,4] Rudolph L. Gleason Jr.,[1,2,4] Roy L. Sutliff,[3] and Manu O. Platt[2,4]

REGULAR ARTICLE

blood advances

Sickle cell disease promotes sex-dependent pathological bone loss through enhanced cathepsin proteolytic activity in mice

Jada Selma,[1,*] Hannah Song,[1,*] Christian Rivera,[1] Simone Douglas,[1] Abhiramgopal Akella,[1] Keval Bollavaram,[1] Nishone Thompson,[1] Manu O. Platt,[1,2] and Edward A. Botchwey[1,2]

Annals of Biomedical Engineering, Vol. 43, No. 9, September 2015 *(© 2015)* pp. 2036–2046
DOI: 10.1007/s10439-014-1245-8

BMES BIOMEDICAL ENGINEERING SOCIETY™ www.bmes.org

CrossMark

Cathepsins in Rotator Cuff Tendinopathy: Identification in Human Chronic Tears and Temporal Induction in a Rat Model

Song P. Seto,[1] Akia N. Parks,[1] Yongzhi Qiu,[1] Louis J. Soslowsky,[2] Spero Karas,[3] Manu O. Platt,[1,4] and Johnna S. Temenoff[1,4,5]

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

ELSEVIER

CrossMark

Differential cathepsin responses to inhibitor-induced feedback: E-64 and cystatin C elevate active cathepsin S and suppress active cathepsin L in breast cancer cells

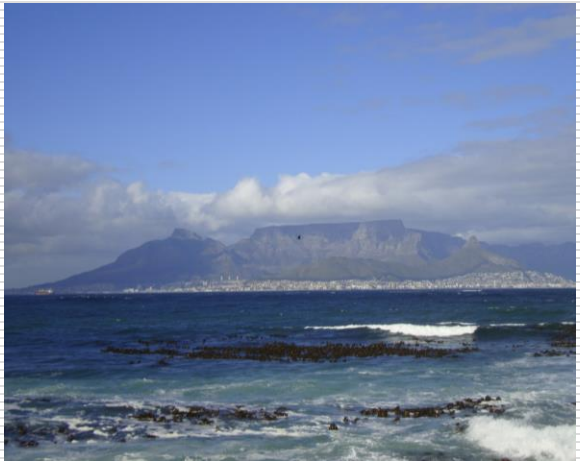
Catera L. Wilder[a], Charlene Walton[a], Valencia Watson[a], Fermin A.A. Stewart[a], Jade Johnson[a], Shelly R. Peyton[b], Christine K. Payne[c], Valerie Odero-Marah[d], Manu O. Platt[a,*]

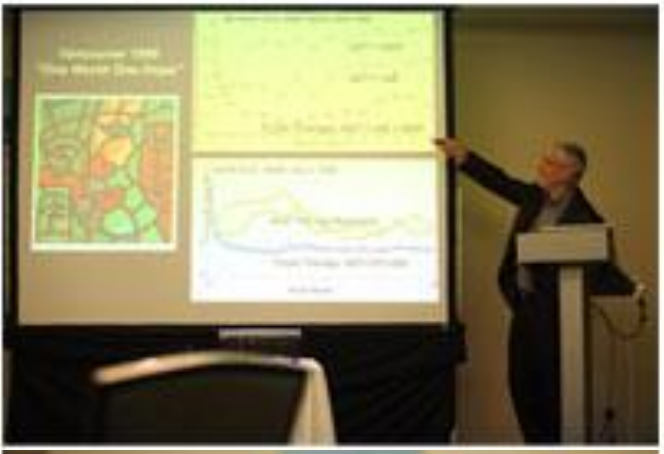
Cathepsin Protease Inhibition Reduces Endometriosis Lesion Establishment

Reproductive Sciences
1-7

DOI: 10.1177/1933719115611752
rs.sagepub.com
SAGE

Kristi M. Porter, PhD[1], Friedrich A. Wieser, MD[2], Catera L. Wilder[1], Neil Sidell, PhD[2], and Manu O. Platt, PhD[1]





NATIONAL INSTITUTES OF HEALTH

International AIDS Society
Stronger Together

July 2009


RIGHTS HERE. RIGHT NOW.

VIENNA, AUSTRIA, AIDS 2010

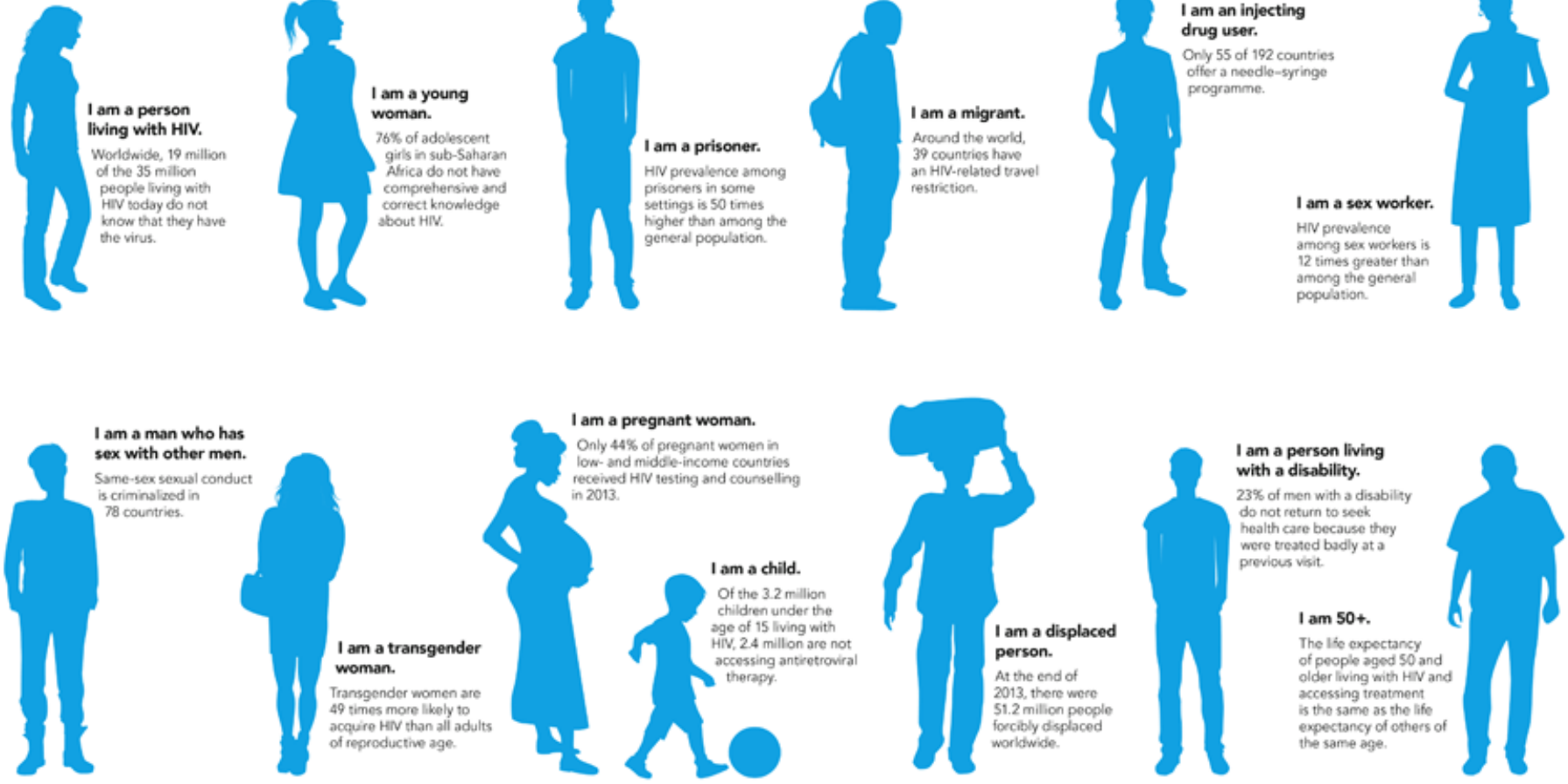
CNHR

International AIDS Society
Stronger Together

Be a scientist-activist!

1. Think globally, act locally. Then act globally.
2. Global health concerns are health disparities for underrepresented minorities in the U.S.
3. Understand the importance of becoming a scientist-activist when studying health disparities.
4. *Gender health inequities are intersectional.*

12 populations being left behind



UNAIDS

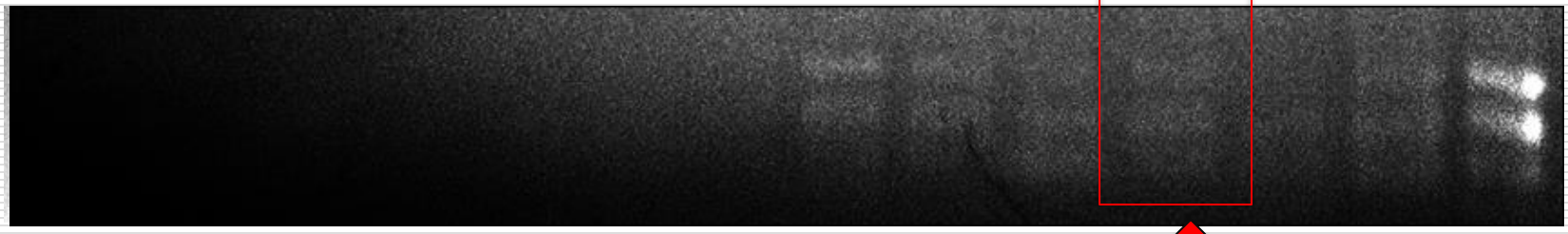
Source: UNAIDS Gap report

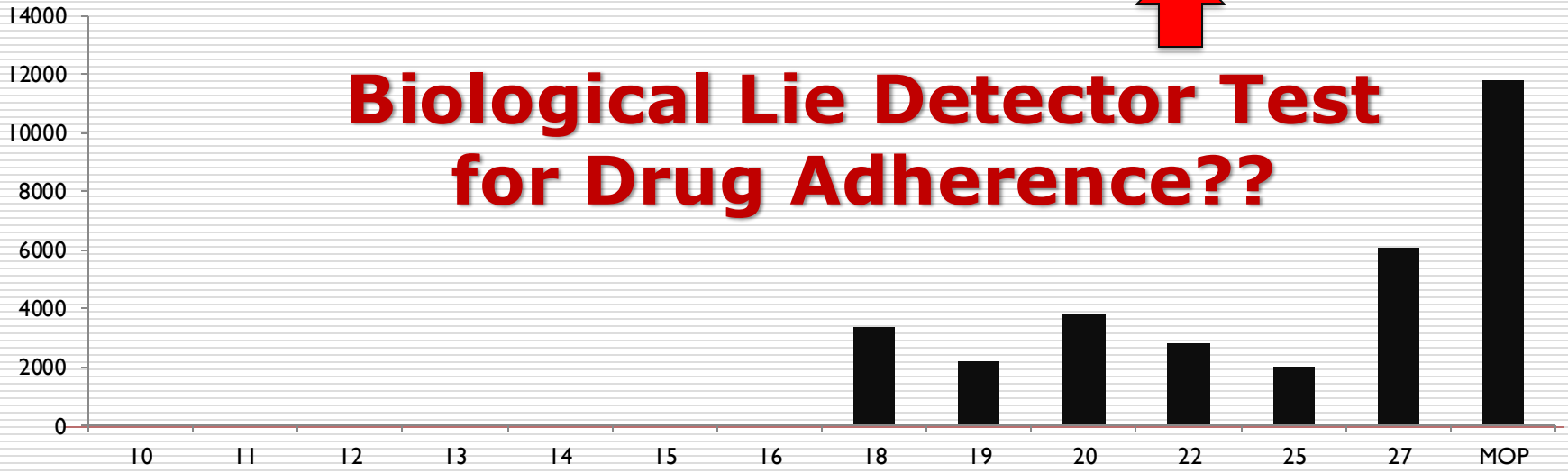
Searching for Solutions in South Africa

HIV+ patients on antiretrovirals have reduced proteolytic activity

On ARVs

10 11 12 13 14 15 16 18 19 20 22 25 27 MOP





Biological Lie Detector Test for Drug Adherence??

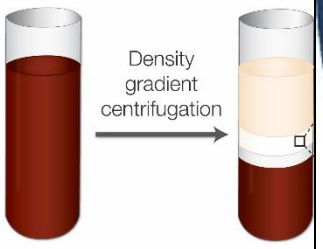
Prospectiv

Baseline

(ARV naïve)

months

Blood and cel

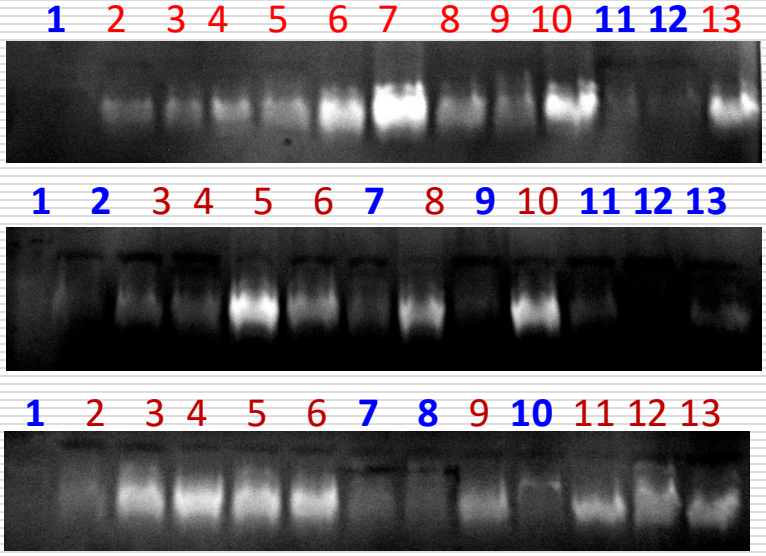


Whole blood

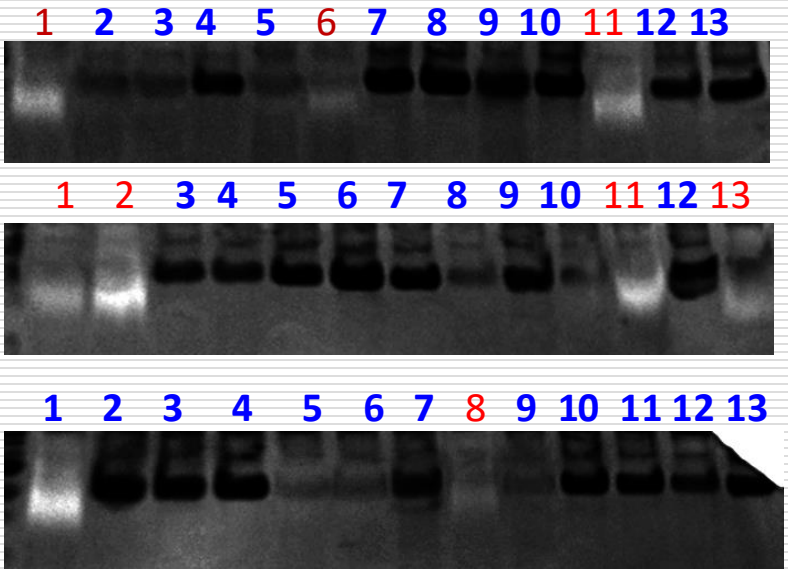
Isola
PBM

More zymo negative specimens after 6 month of ART

Baseline (ART naïve)



6 months



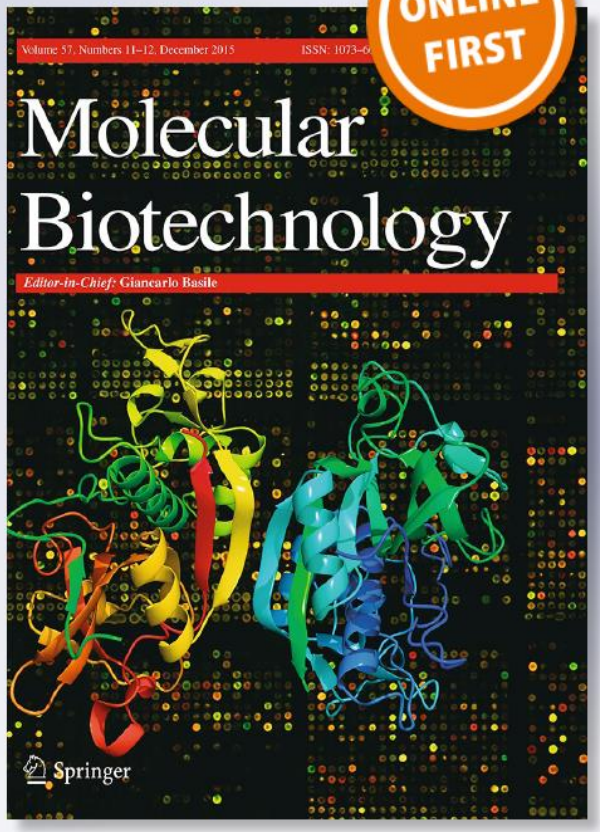
Patients with cathepsin **zymo positive** signals were **twice** as likely to have detectable viral loads

Mol Biotechnol
DOI 10.1007/s12033-015-9903-0

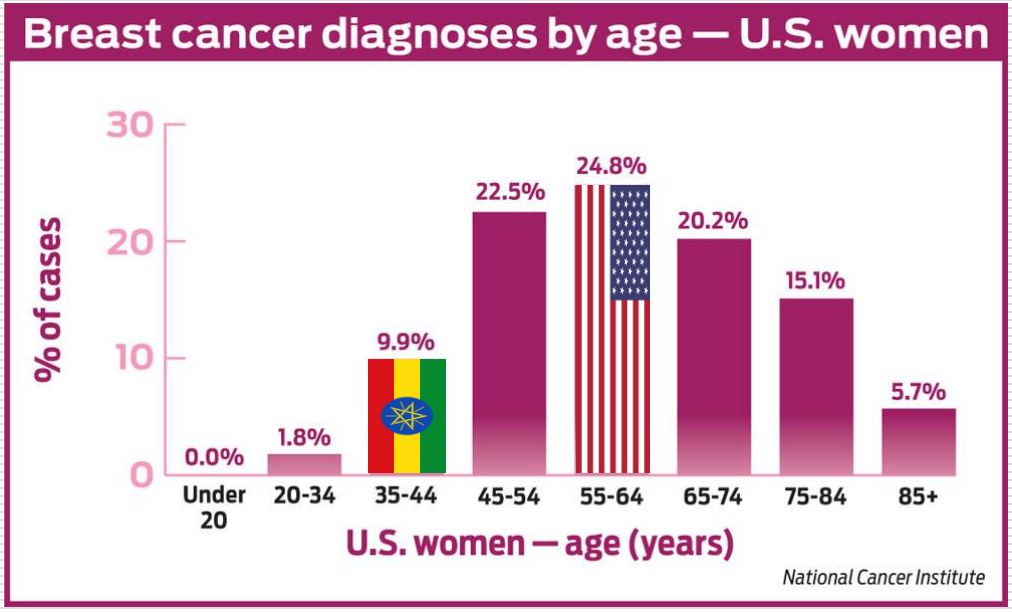
ORIGINAL PAPER

Low-Cost Method to Monitor Patient Adherence to HIV Antiretroviral Therapy Using Multiplex Cathepsin Zymography

Manu O. Platt[1,2] · Denise Evans[3] · Philip M. Keegan[1,2] · Lynne McNamara[4] · Ivana K. Parker[2,5] · LaDeidra M. Roberts[1,2] · Alexander W. Caulk[2,5] · Rudolph L. Gleason Jr.[1,2,5] · Daniel Seifu[6] · Wondwossen Amogne[7] · Clement Penny[8]



Ethiopian women face aggressive and early onset breast cancer



Median Age of Breast Cancer Diagnosis:

United States

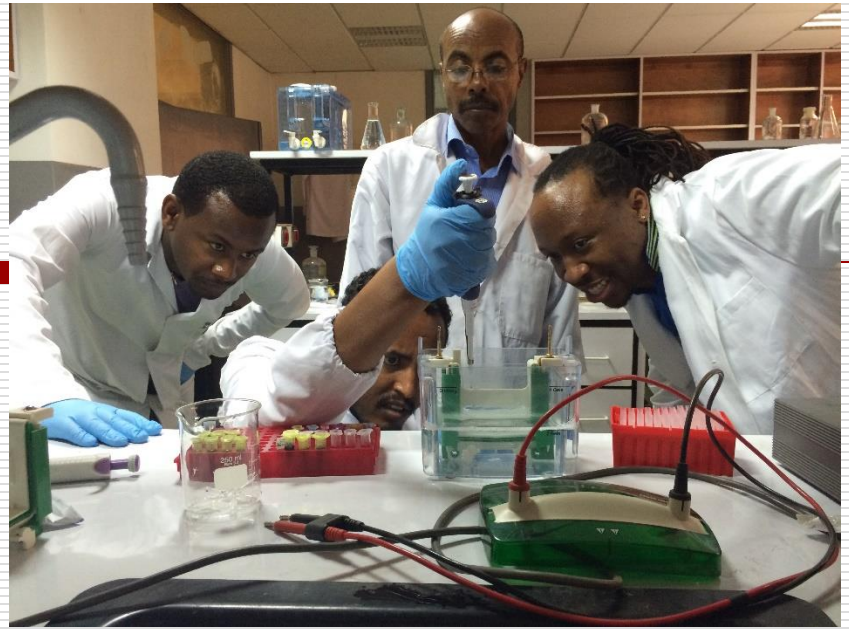
White women = 63 years

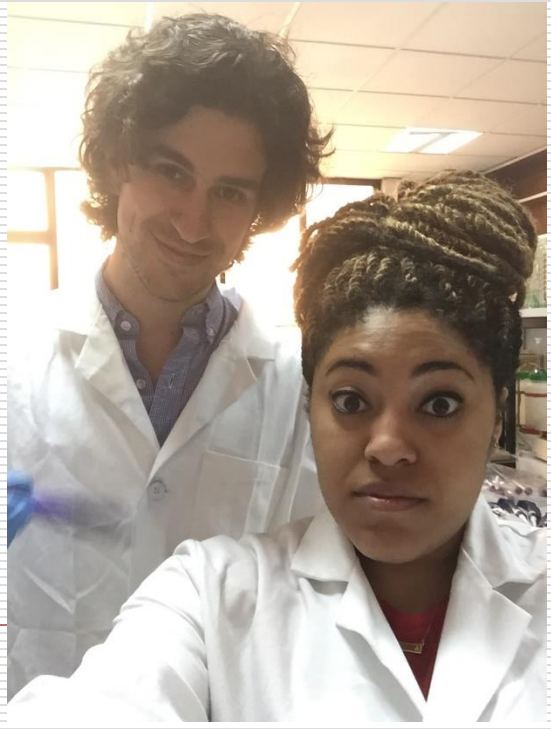
Black women = 59 years

Ethiopia

Women = 40 years

Howlader et al. (2017). *SEER Cancer Statistics Review, 1975-2014*. Bethesda, MD.





The team in Addis Ababa, Ethiopia!

Collecting samples and training graduate students in Addis Ababa, Ethiopia

Breast Cancer: Targets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Cysteine Cathepsins and Matrix Metalloproteases Among Breast Cancer Patients

Solomon Tsegaye[1]
Mohammed Mehdi[2]
Wajana L Labisso[3]
Daniel Seifu Melka[2]

[1]Department of Biochemistry, College of Health Sciences, Assela University, Addis Ababa, Ethiopia; [2]Department of Biochemistry, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; [3]Department of Pathology, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

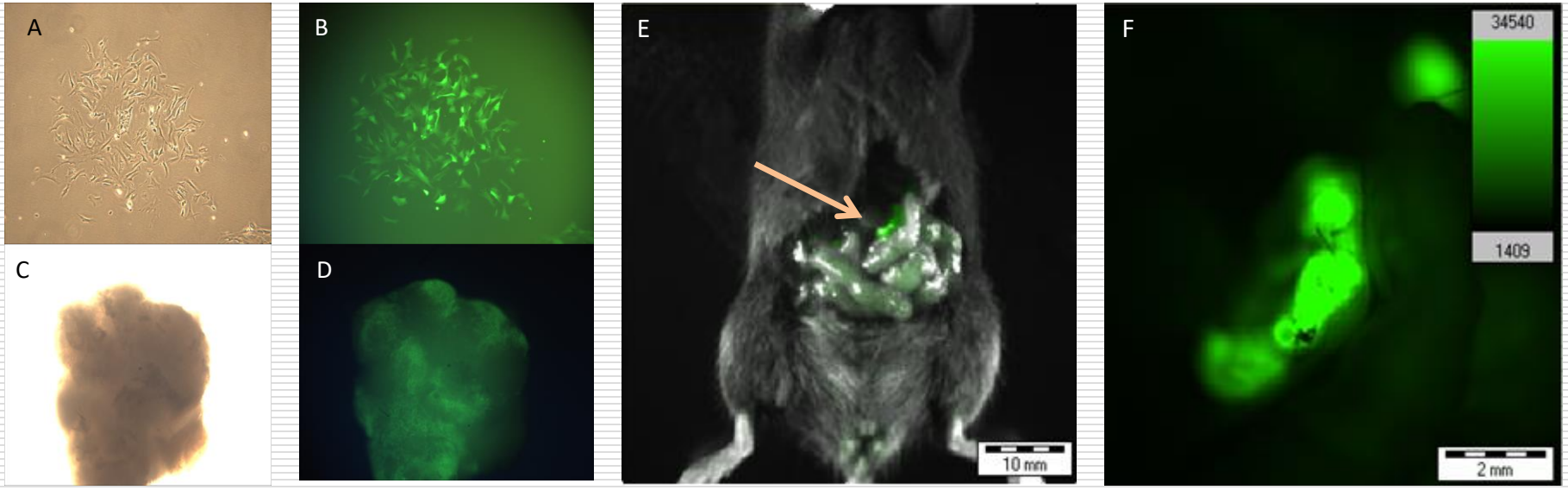
What is the best use of your professional time?

Annual Atlanta Sickle Cell Road Race, 2018

With Dr. Linda Griffith

Director of MIT Center for Gynepathology Research

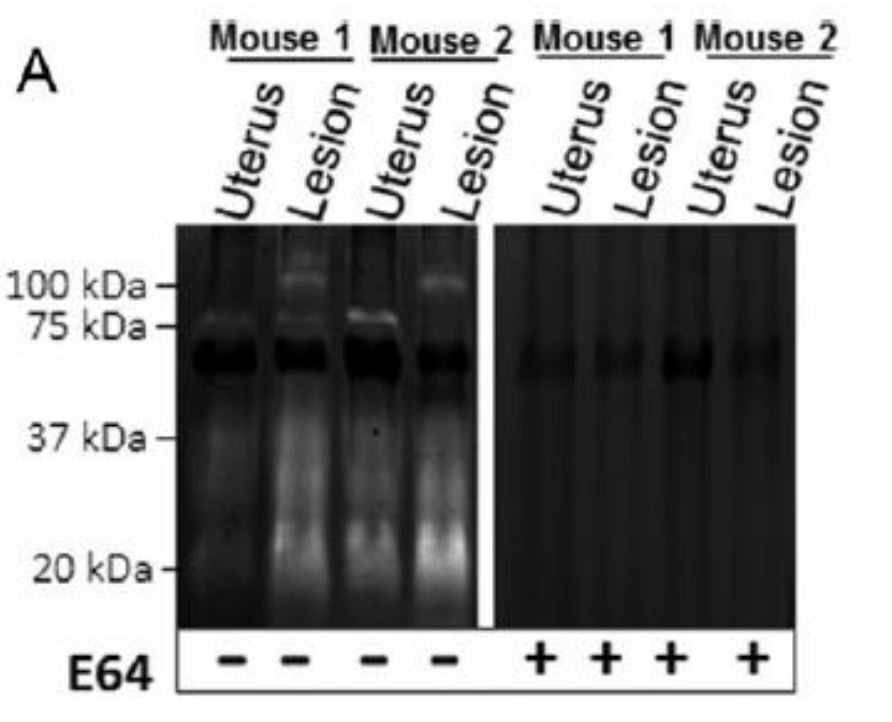
Endometriotic implant mouse model



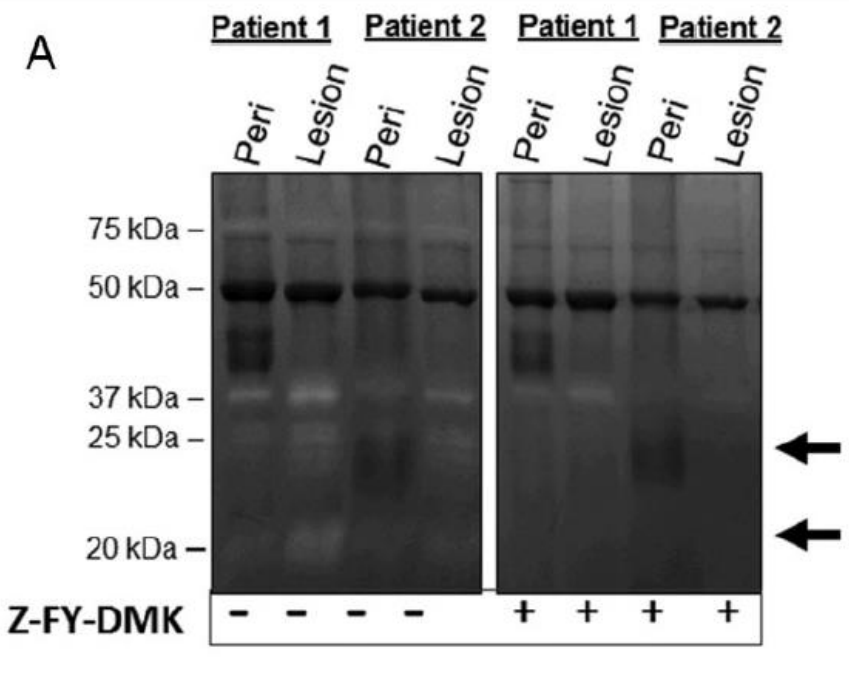
Endometrial tissue taken from a GFP transgenic mouse and cultured for 3 days. We also harvested endometriotic implants from the peritoneal cavity of mice after injection of endometrial fragments. Macroscopic picture of an excised endometriotic implant (2x3 mm) after 10 days of injection with endometrial cells under normal light (C) under fluorescent light (D)

Increased amounts of active cathepsins in the lesions than in eutopic uterine tissue

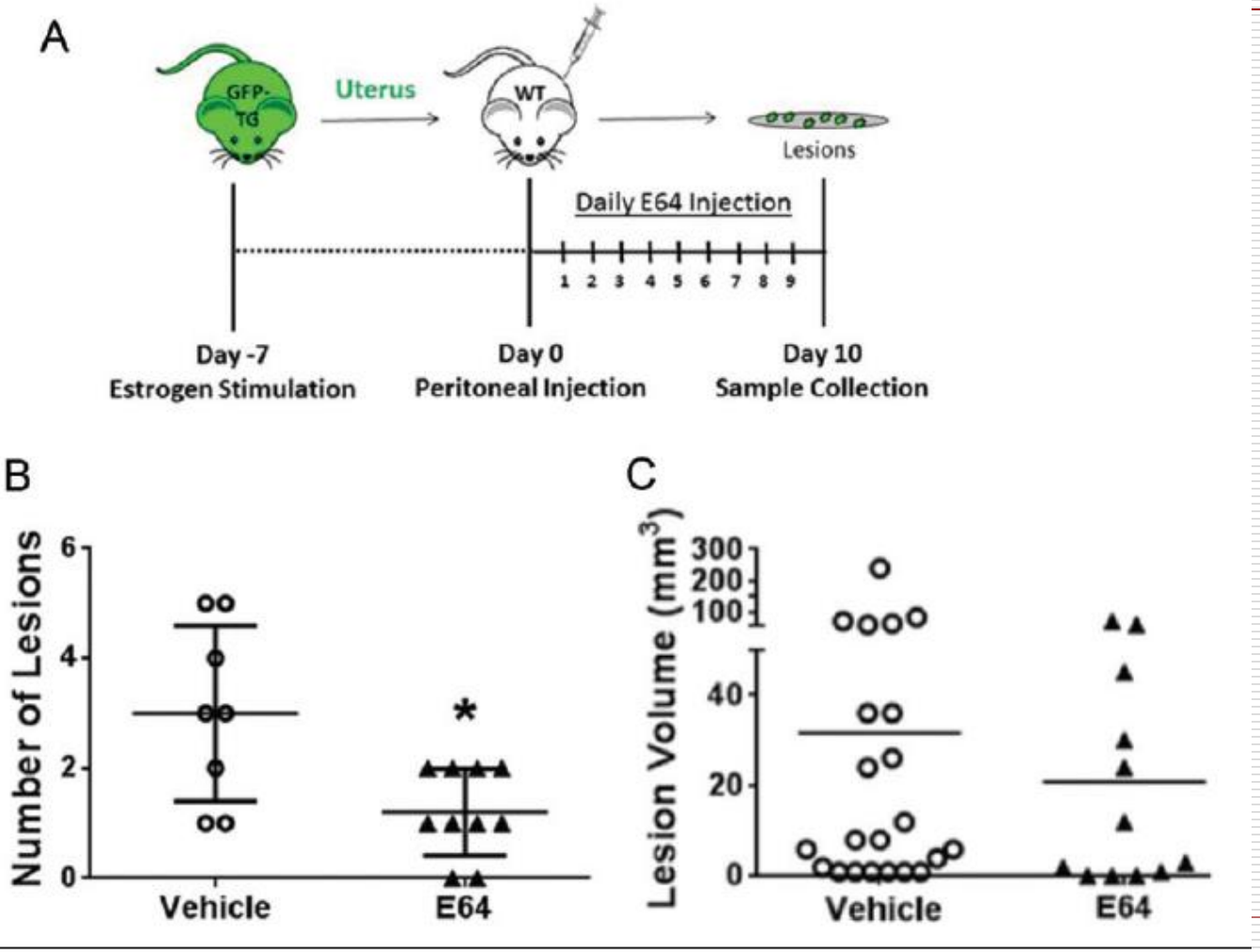
Mouse model



Human specimens



Inhibiting cathepsins reduces endometriotic lesion establishment in murine model



2 of the mice had no lesions at all!

Summary

Our data also suggest that cathepsin activity may be important in this initial establishment of lesions, and one of these patient specific differences distinguishing women at risk for endometriosis could be inherent cathepsin activity.

Systemic administration of these inhibitors may be detrimental, but repurposing them for targeted gynecologic or peritoneal delivery to mitigate endometriosis may revive their utility.

Conclusions

- Think proteases as therapeutic targets for tissue destructive and tissue remodeling diseases that impact women's health.
- Develop protease detection assays for low cost diagnostics useful in low resource settings where diseases have disparate impacts on women.
- Consider sex-specific differences across multiple diseases which might indicate new mechanisms for therapeutic targeting.
- Use enzymatic activity and mathematical biology for personalized medicine or predictive medicine strategies to help distinguish patient-to-patient variability in women's health issues.

Acknowledgements

NIH DIRECTOR'S NEW INNOVATOR AWARD

American Heart Association. Learn and Live

National Heart Lung and Blood Institute

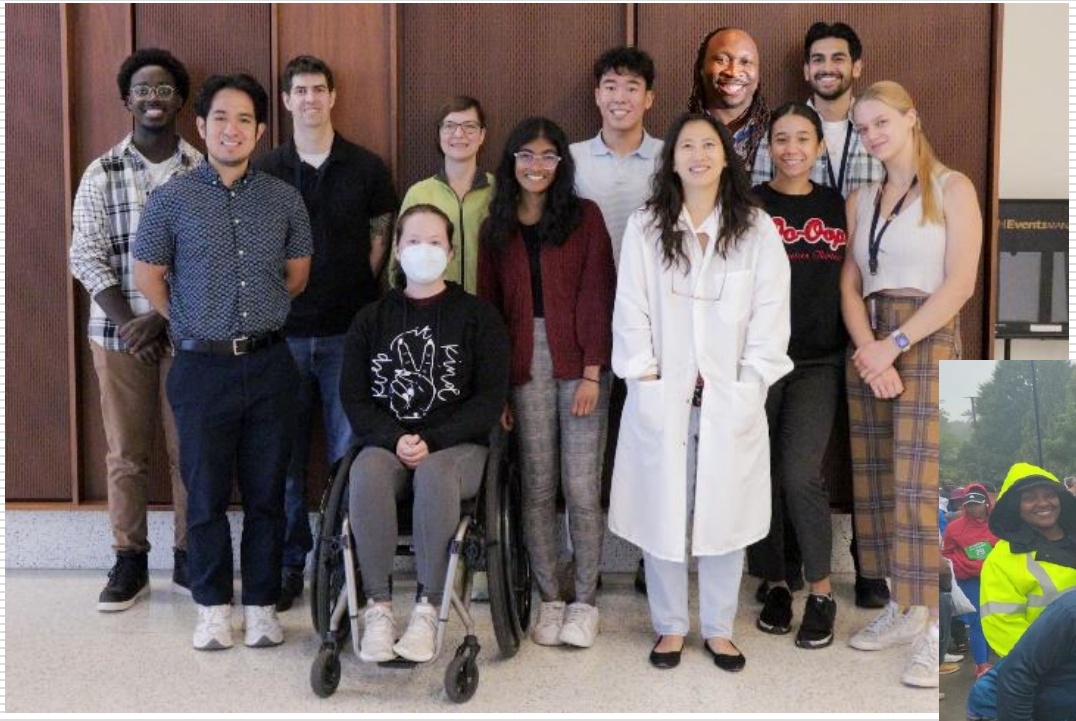
NSF

Integrated Cellular Systems

DARPA

NIH National Institute of Biomedical Imaging and Bioengineering

Engineering the Future of Health℠



manu.platt@nih.gov

@DrPlattLab

MATRICES LAB
Mechanics And Tissue Remodeling Integrating Computational + Experimental Systems

BETA
CENTER for BIOMEDICAL ENGINEERING TECHNOLOGY ACCELERATION