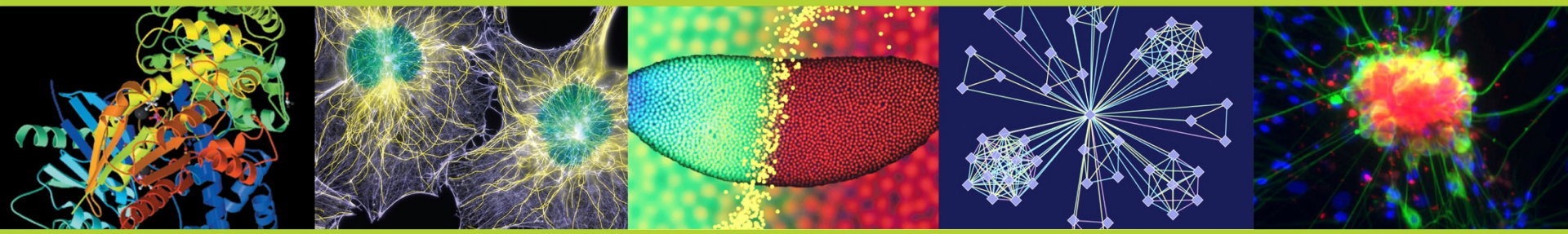
Transformative High Resolution Cryo-Electron Microscopy

Jon Lorsch, PhD
Director
National Institute of General Medical Sciences

NIH Working Group: NEI, NIGMS, NCI, NHLBI, NIAID, NIDA, NIDDK, NINDS, ORIP, NIAAA, NIBIB

Inter-agency Working Group: NSF, DOE, Beckman



Working Group Members

Co-Chairs

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Working Group Coordinators:

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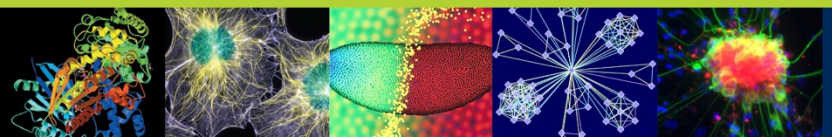
Manjit Hanspal (NHLBI)

Jue Chen (NHLBI)

James Luo (NHLBI)

Margaret Sutherland (NINDS)

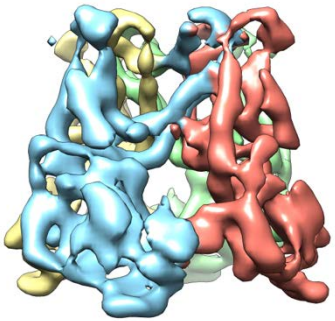
Malgorzata Klosek (ORIP)

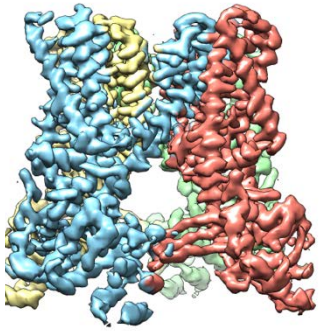


Why Now? New Technological Breakthroughs in Cryo-EM

1) **New electron microscopy technology dramatically improves our ability to see biological molecules**

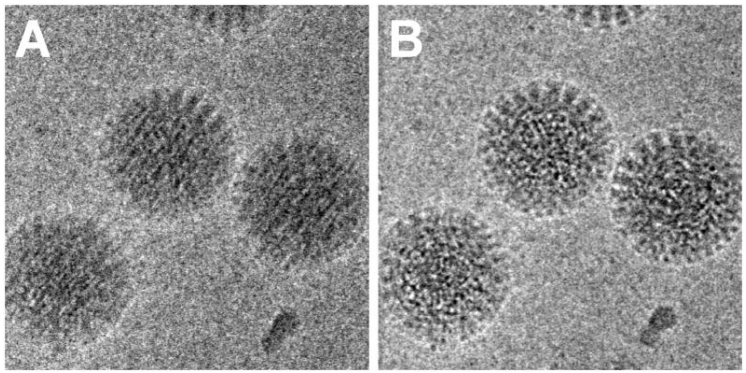
New Methods





TRPV1 Ion Channel:
Mediates burn sensation,
Yifan Cheng UCSF

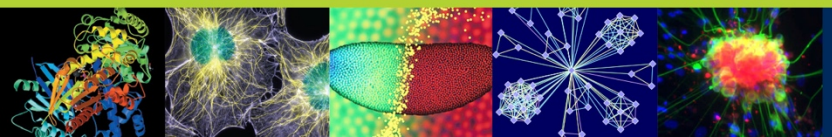
2) **New motion correction methods resolve blurring of images due to movement of particles in electron beam**



Rotavirus Particles
Niko Grigorieff, Janelia Farms

The U.S. is Rapidly Falling Behind Europe and Asia in Cryo-EM Infrastructure

- Initial Investment, 1-2 CryoEM microscopes, local facility
- Significant Investment, 3-4 CryoEM microscopes, regional facility
- Major Investment, 5+ CryoEM microscopes, HTP comprehensive facility
- Planned investment



National Institute of General Medical Sciences

Request for Information (RFI): Transformative High-Resolution Cryo-Electron Microscopy

Notice Number: NOT-RM-16-022

Release Date: June 24, 2016
Response Date: August 8, 2016, 46 responses

Need and Capacity

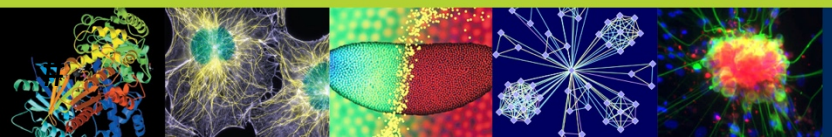
- Overwhelming support for National Centers for automated high-resolution cryoEM data collection for SPA
- US is falling behind
- CryoET expertise, resources also needed

Training and Workforce

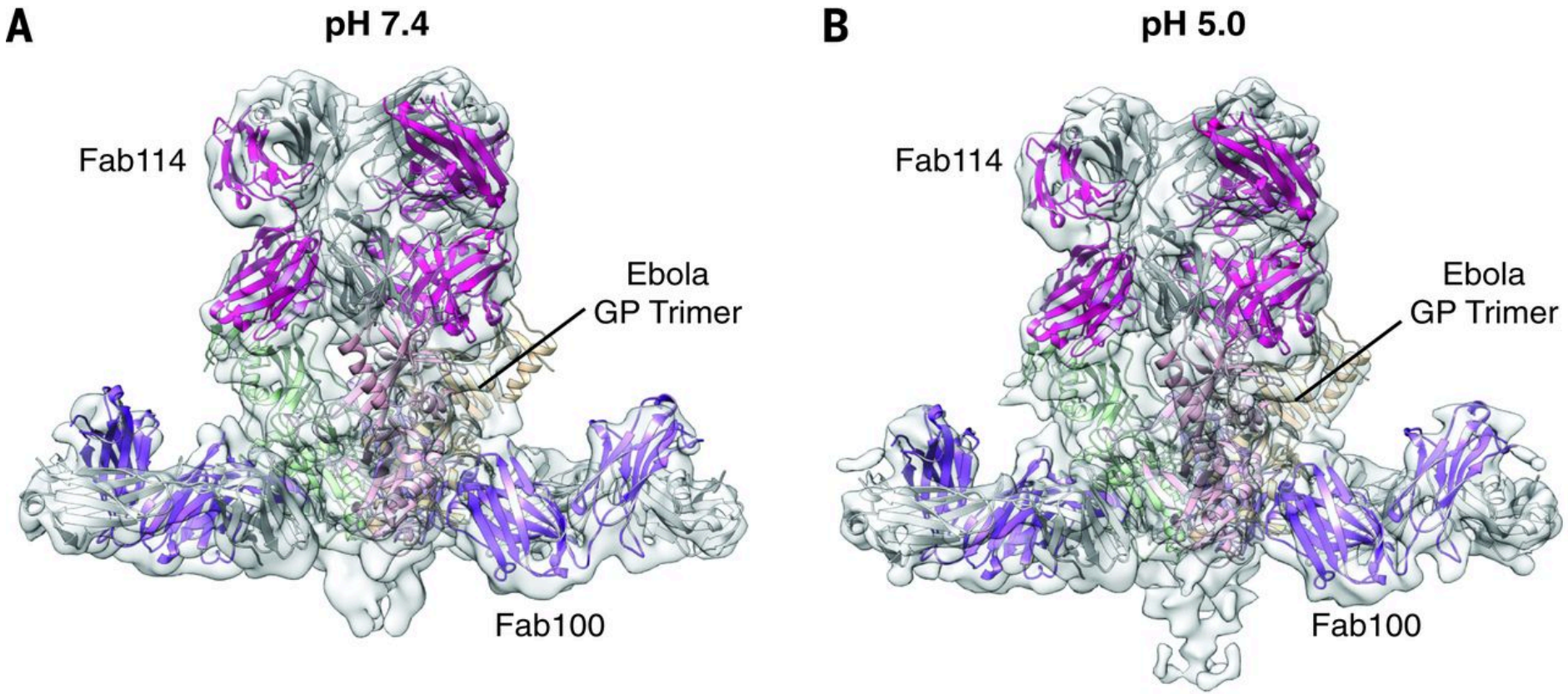
- Expertise in all stages required for success
- 2 levels of trainees:
  Structural biologists
  Neophyte biologists
- Hands-on training at Centers essential
- Tutorials, online materials at home

Technology Development

- HTP pipeline for thin sections of cells
- Optics and instrumentation
- Segmentation and subtomogram averaging
- Data management and storage needs



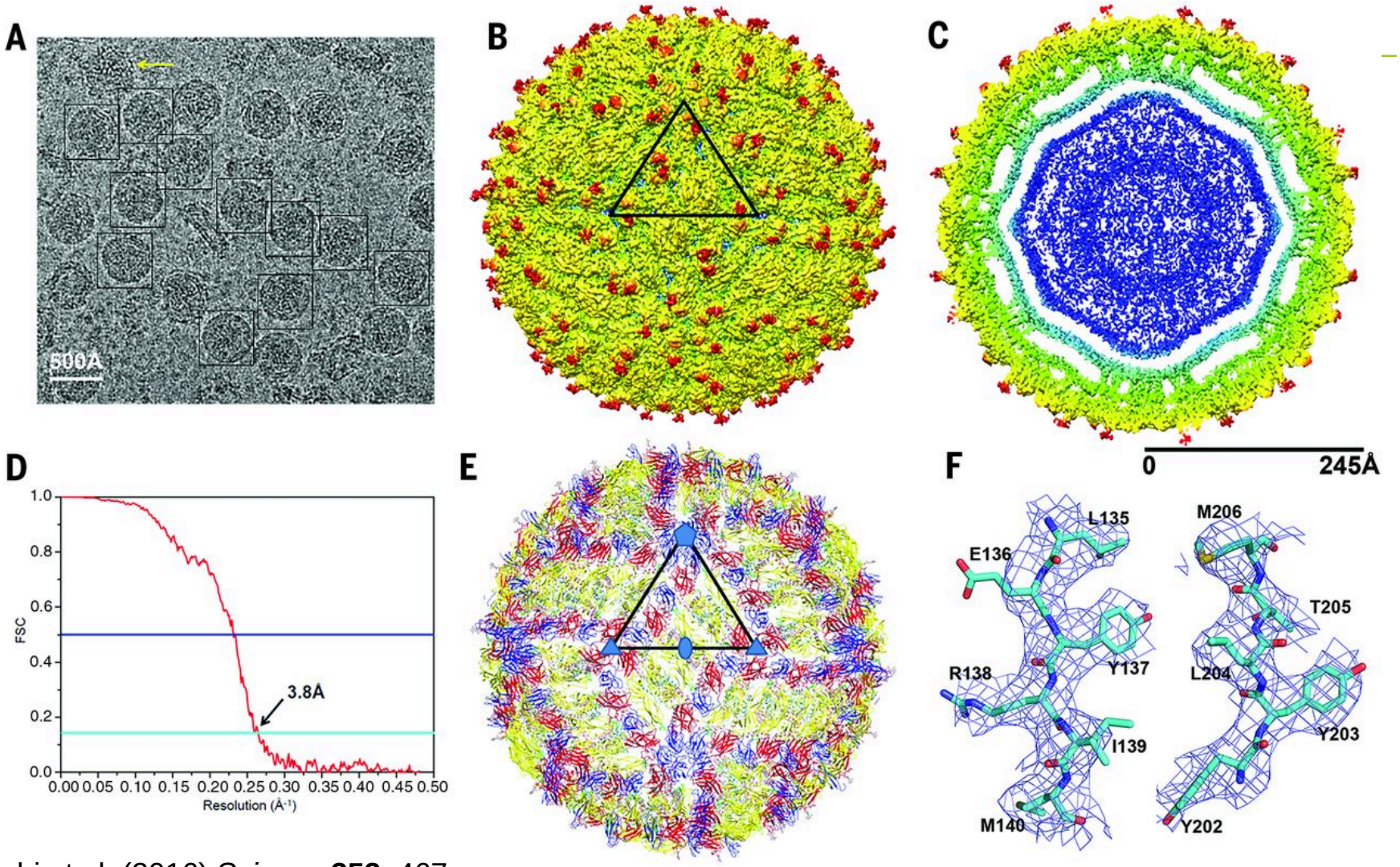
Ebola virus neutralization by protective human antibodies (9Å)



Two human monoclonal antibodies, mAb100 and mAb114 in combination, protect nonhuman primates against all signs of Ebola virus disease.

Misasi et al. (2016) *Science* **351**: 1343

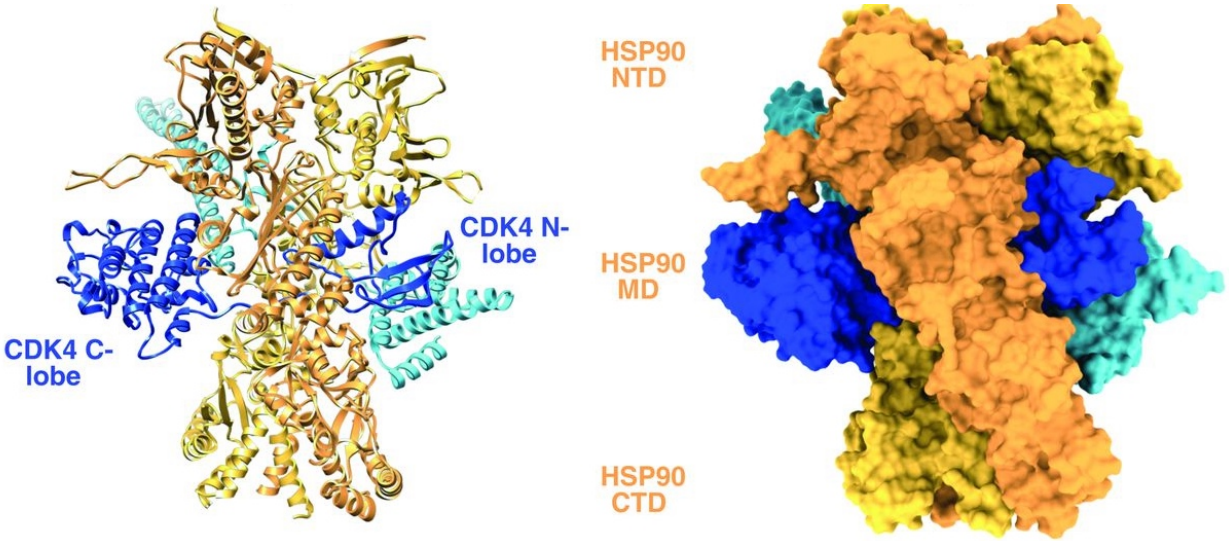
Zika Virus 3.8Å



Sirohi et al. (2016) *Science* **352**: 467,

HSP90-Cdc37-Cdk4 Complex at 3.9Å

- HSP90 Interacts with 60% of human kinome.
- Cdk4 kinase is trapped and stabilized in the complex.
- HSP90 inhibition leads to degradation of kinases, including oncoproteins vSrc, bRafV600E, Her2.
- HSP90 inhibitors are undergoing clinical trials as cancer therapeutics.



Verba, K.A. et al. (2016) *Science*, **352**:1542

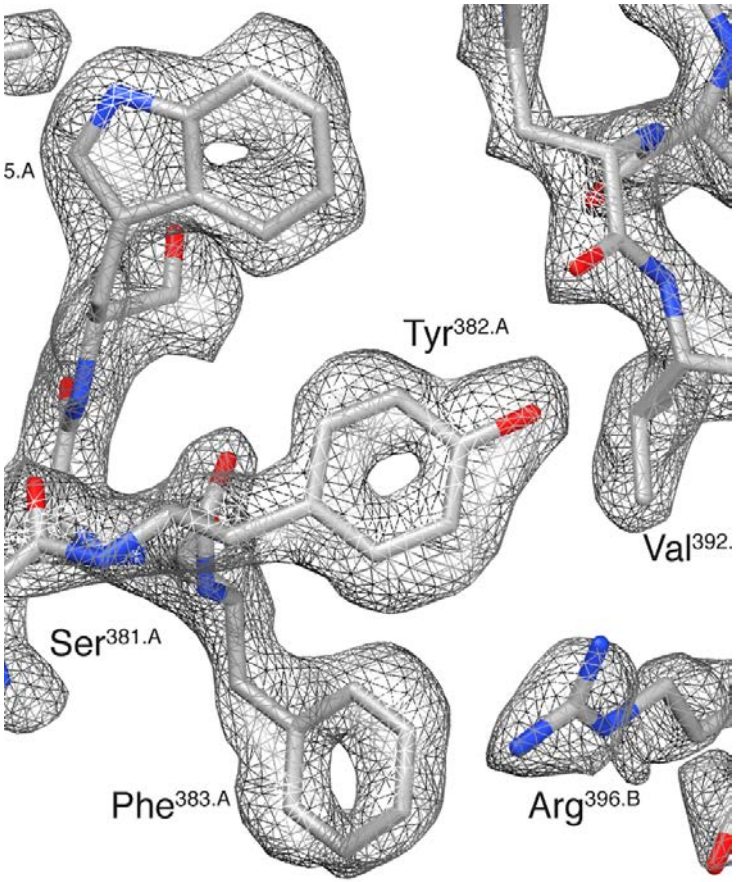
Challenges and Strategies

Challenges ● Cost of instrumentation and upkeep ● Access to high performance data collection ● Limited base of expert investigators

Strategy ● Open access to state of the art instrumentation ● Build an expert workforce ● Improve and extend technology ● Create economies of scale

Comprehensive Centers ● Three centers ● Research assistance and training 20-30 labs/year ● High-throughput data collection services ● Each center 4 microscopes, 7-8 FTEs, $4M TC/year ● R01/R21s to develop new technology & methods

Long Term Plan ● High throughput data collection services at three centers, each $2M TC/year ● Interagency WG (NIH, NSF, DOE, Beckman)



Glutamate dehydrogenase (1.8A)
Merk et al. Cell 165, 1698, 2016

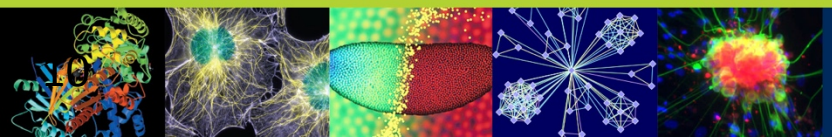
Proposed Budget

Comprehensive Centers (3)	Year 1	Year 2	Year 3	Year 4	Year 5	**Total**
Equipment (4 microscopes/center)	$22M	$22M	$22M	0	0	**$66M**
Operations	$4M	$6.4M	$10.5M	$10.5M	$10.5M	**$41.9M**
User Training & Service (4 FTEs/center)	$0.6M	$1.2M	$2.5M	$2.5M	$2.5M	**$9.3M**
	$26.6M	**$29.6M**	**$35M**	**$13M**	**$13M**	**$117.2M**

Research Grants (R21,R01)	TC Yearly	**Total**
Cryoelectron Tomography R&D	$3M	**$15M**
Single Particle R&D	$2.4M	**$12M**
	$5.4M	**$27M**

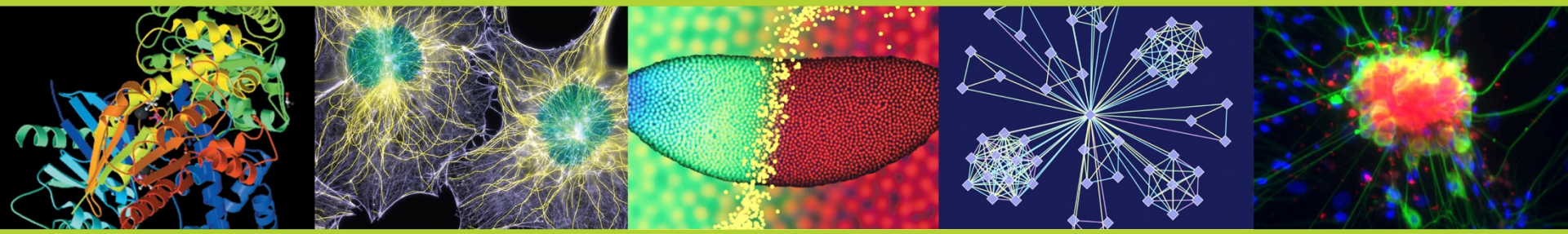
All Activities	Year 1	Year 2	Year 3	Year 4	Year 5	**Total**
	$32M	**$35M**	**$40.4M**	**$18.4M**	**$18.4M**	**$144.2M**

Possible jumpstart program with FY2017 funding of Comprehensive Centers?

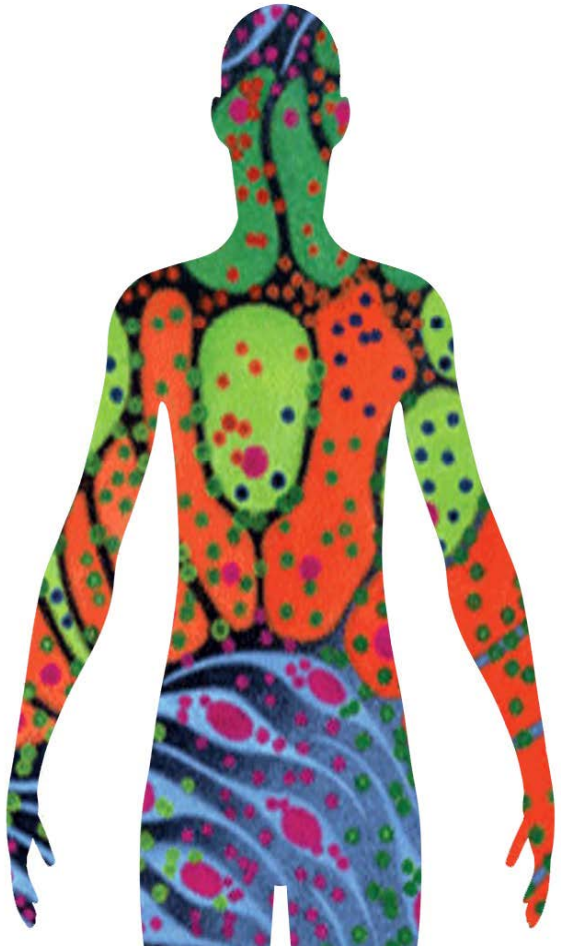


Thank You!

Questions?



NIH National Institute of General Medical Sciences



The Human BioMolecular Atlas Project "HuBMAP"

Robert Carter, MD, NIAMS

on behalf of the trans-NIH HuBMAP WG

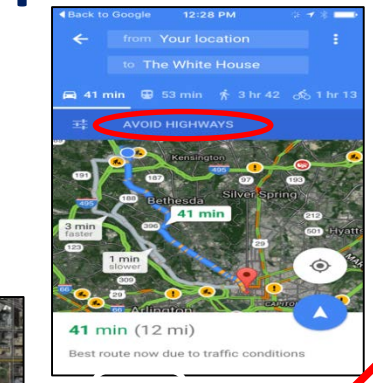
HuBMAP NIH Working Group

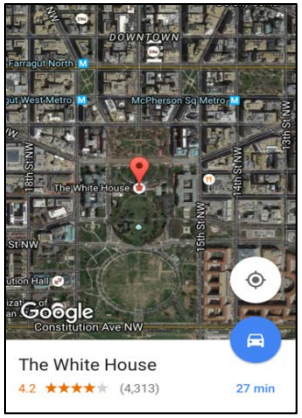
- **Common Fund Program Lead:** Ananda Roy, Ph.D. Office of Strategic Coordination (OSC)

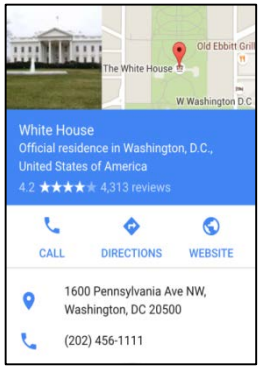
Members representing:

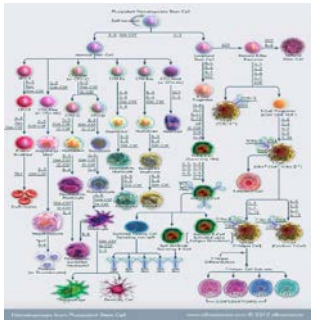
- **Center for Scientific Review (CSR)-** David Balasundaram, Ph.D
- **Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)** - Reiko Toyama, Ph.D.
- **National Cancer Institute (NCI) -** Jennifer Couch, Ph.D., J. Randy Knowlton, Ph.D., Jerry Li, Ph.D.
- **National Heart, Lung, and Blood Institute (NHLBI)** - Zorina Galis, Ph.D. , Pothur Srinivas, Ph.D., Sara Lin, Ph.D.
- **National Institute of Aging (NIA) -** Jose Velazquez, Ph.D.
- **National Institute of Allergy and Infectious Diseases (NIAID) -** Elizabeth Church, Ph.D., Katarzyna Bourcier, Ph.D**.**
- **National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)** - Robert Carter, M.D.
- **National Institute of Biomedical Imaging and Bioengineering (NIBIB) -** Richard Conroy, Ph.D.
- **National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)** - Deborah K. Hoshizaki, Ph.D., Krystyna Rys-Sikora, Ph.D
- **National Institute of General Medical Sciences (NIGMS) -** Sarah Dunsmore, Ph.D., Joseph G. Gindhart, Ph.D
- **National Human Genome Research Institute (NHGRI) -** Ajay Pillai, Ph.D**.,** Jeff Schloss, Ph.D.
- **National Institute of Mental Health (NIMH) -** Andrea Beckel-Mitchener, Ph.D., Yong Yao, Ph.D.
- **National Institute of Neurological Disorders and Stroke (NINDS) -** Francesca Bosetti, Ph.D**.**
- **Office of Strategic Coordination (OSC) -** Jessica Smith, Ph.D., Tony Casco

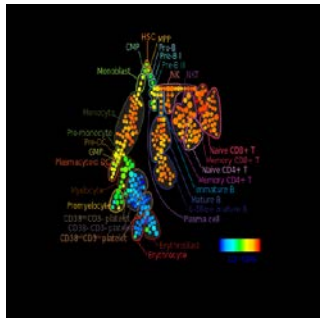
Aspiration: Building from Single Cells to Context to Organizational Principles Across the Human Body

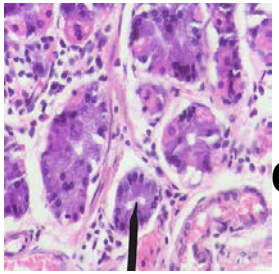












Distance

BODY

Understand Principles

Tissue

Understand Patterns

Neighborhood

Understand Relations

Cell in-situ

Amount of data

Reality check..

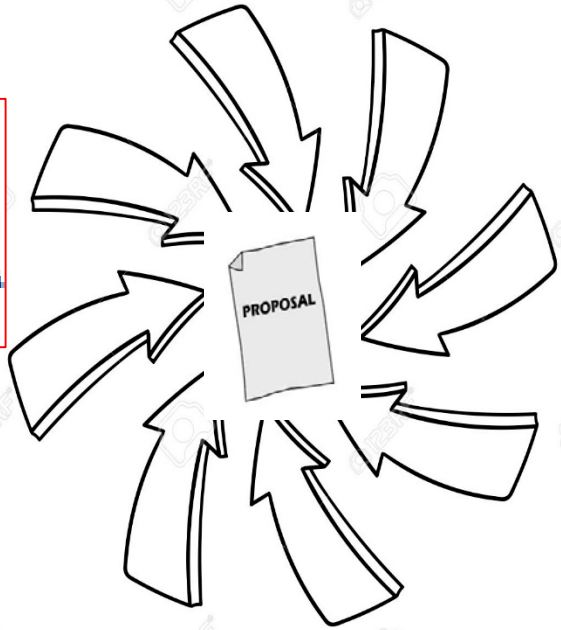
Identifying Key Areas in a Human BioMolecular Atlas (HuBMAP) WS, June 15, 2016

the WHITE HOUSE
BRAIN INITIATIVE
BRAIN RESEARCH THROUGH ADVANCING INNOVATIVE NEUROTECHNOLOGIES

GTEx

LungMAP
Molecular Atlas of Lung Development Program

NIH RePORTER



GUDMAP

PubMed.gov

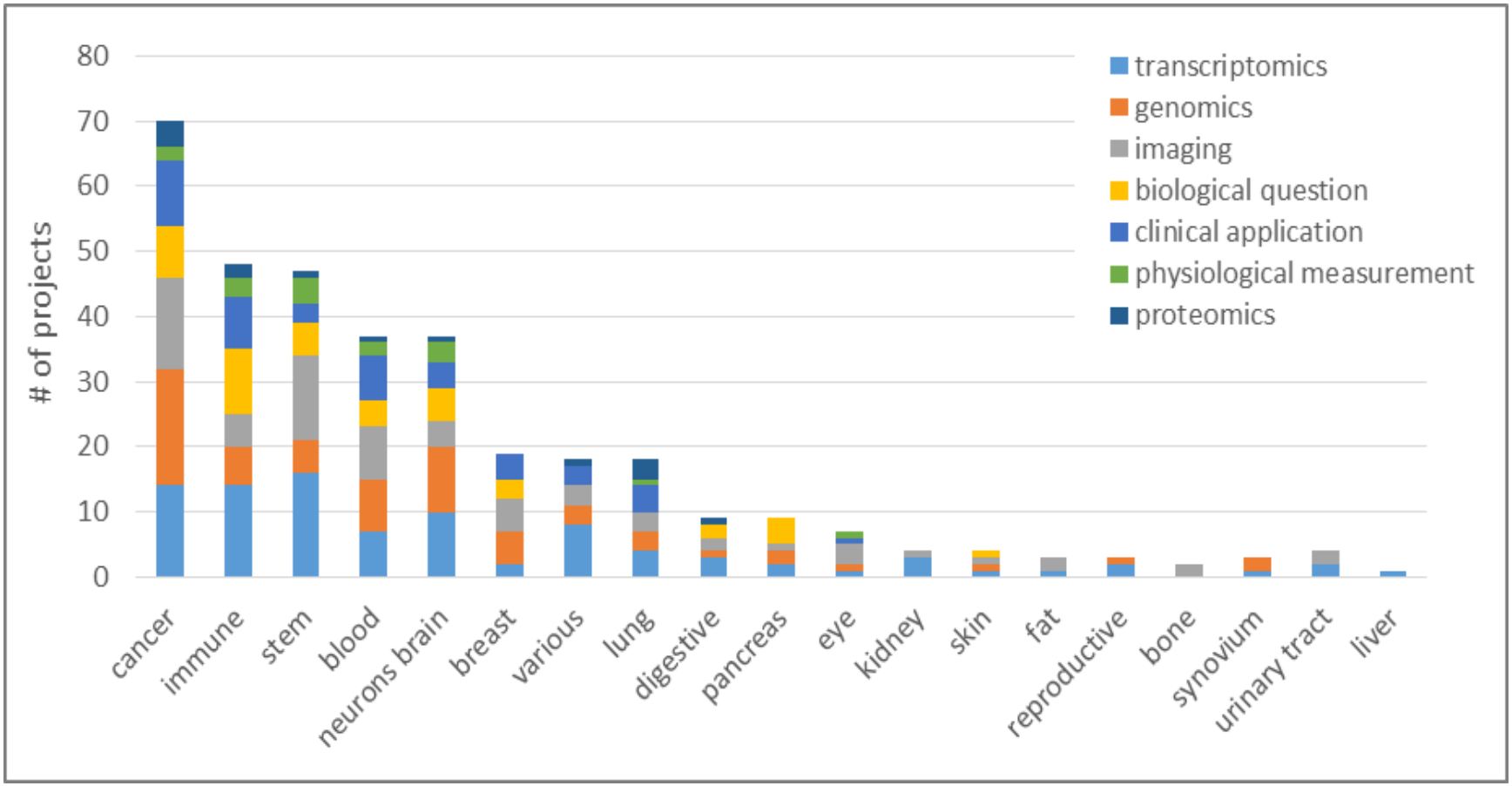
THE HUMAN PROTEIN ATLAS

CellFinder

Request for Information (RFI): Characterizing and Understanding the Organization of Individual Cells within Human Tissues

Notice Number: NOT-RM-16-025

Current Landscape of NIH-Funded Research



Cell or Tissue Type and Project Focus or Technology

NIH Query, View, and Report (QVR), June 28th, 2016
169 projects, 17 IC's. Total investment of $97M.

Why the HuBMAP?

	HuBMAP	GTEx	GUDMAP	LungMAP	BRAIN	SGMAP	HPA
Primary Species	**Human**	Human	Mouse moving to Human	Human / Mouse	Mouse	Mouse	Human
Tissues	**Phase 1: ~10 Phase 2: ~40**	~53	Kidney / Prostate	Lung	Brain	Salivary glands	~44
Focus	**Inter-individual variability**	eQTLs	Early development	Early development	Cell census	Early development	Proteome
Tech	**FISH, RNA-Seq, IMS**	RNA-Seq	FISH, RNA-Seq	FISH, RNA-Seq, MS, CT	RNA-Seq	Microarray / RNA-Seq	60,000+ Antibody
Single cell focus?	**Yes**	No	Yes	Yes	Yes	No	Moving towards
Spatial?	**Yes**	No	Yes	Yes	No	No	Yes
Across Body?	**Yes**	**Yes**	**No**	**No**	**No**	**No**	**Yes**

Opportunities

Synergistic Collaborations

GUDMAP

SHOGoiN
Human Omics database for the Generation of iPS and Normal Cells

CellFinder

THE HUMAN PROTEIN ATLAS

LungMAP

GTEx

THE BRAIN INITIATIVE

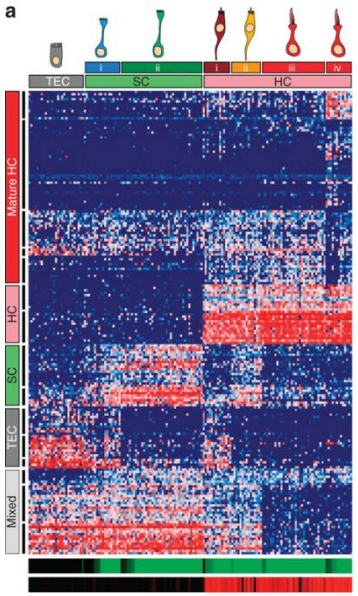
Accelerating Medicines Partnership
Rheumatoid Arthritis
Type 2 Diabetes

ALLEN INSTITUTE for CELL SCIENCE

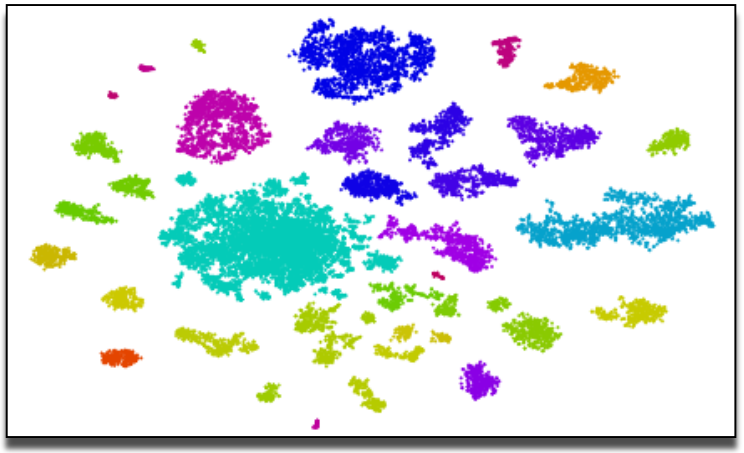
NIH LINCS PROGRAM

The Society for Single-Cell Surveyor
Understanding Life at the Molecular Level

Single Cell Technologies

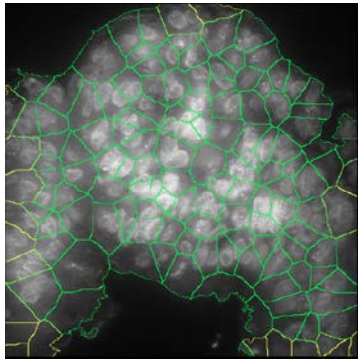


RNA-Seq identifies unique cell types in mouse utricle (Kelley Lab)

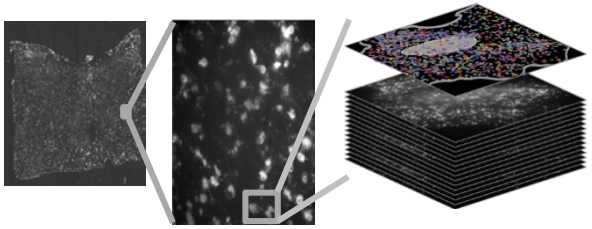


Retina Drop-Seq (48,808 cells) – 3 new cell types identified (Regev Lab)

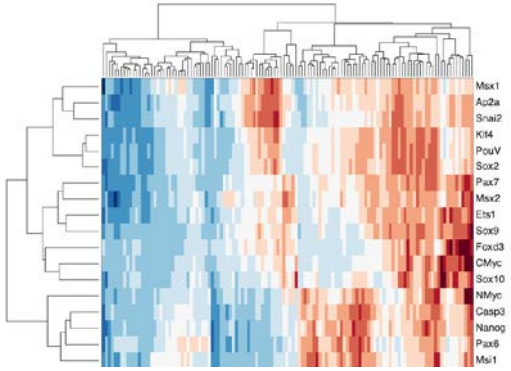
Emerging In-situ Technologies



FISH Imaging

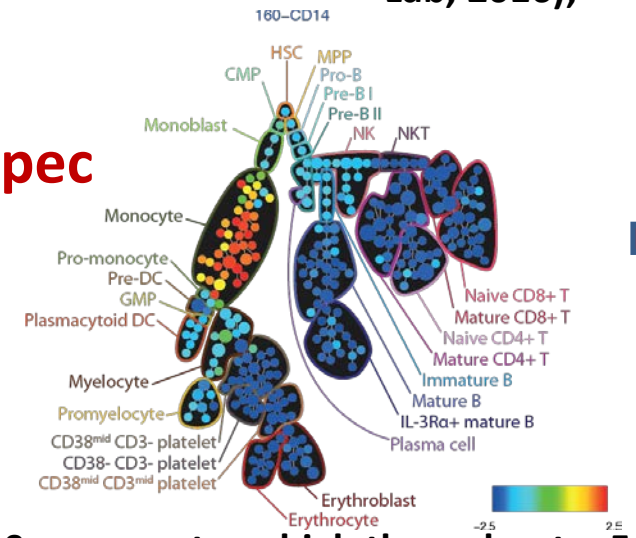


MERFISH – Imaging 1000+ genes in tissue (Zhuang Lab, 2016);

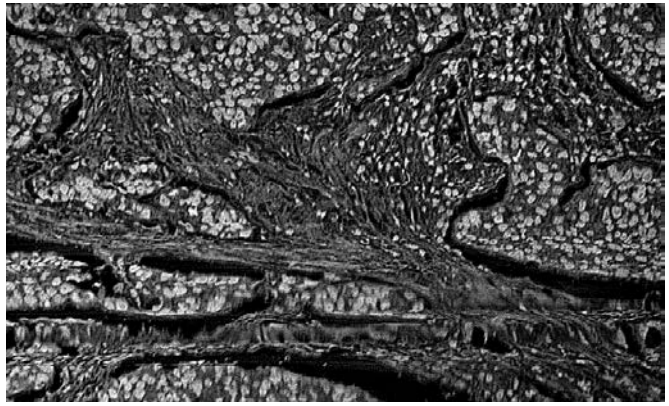


SeqFISH– Sequential barcoding, 100+ parameters, single molecule sensitivity (Cai Lab)

Mass Spec & CyTOF



CyTOF – 30+ parameters, high throughput, <5 Ab sensitivity (Nolan Lab)



MIBI-TOF – up to 50 parameter imaging, down to 20nm (Angelo Lab, 2016)

Proposed Goals for the HuBMAP

To understand:

1) The principles behind the organization of cells in human tissues across the body
2) The role of this organization in orchestrating short and long-range communication between individual cells

Will lead to better understanding:

1) The role played by specific individual variations and changes across the lifespan and health/disease continuum

Outputs of the HuBMAP

Phase 1:

1. A standardized pipeline to create multiscale multidimensional molecular maps
2. Next generation tools (high-resolution, high-content and high-throughput) to map tissue organization
3. Census of major cell types in multiple tissues to understand inter-individual variability
4. Characterization and mapping of the 3D biomolecular architecture of all cells in ~10 human tissues / systems
5. Understanding of “normal” inter-individual variation

Phase 2:

1. Extension of cell census and mapping projects to lifespan and health / disease continuum
2. Validated models of organizational / functional relationships in tissue
3. Next generation tools to explore tissue dynamics (4D)

Initiatives

Phase 1 & 2

1. **Tissue Core:** Human tissue from multiple donors (>20) and multiple sites (>20) to 1) study inter-individual variability, 2) changes in development & disease
2. **Cell Census and Deep Profiling:** High-throughput single cell RNA-seq and FISH imaging, chemistry, validation and benchmarking. Accelerate the development, validation and dissemination of in situ analysis. Mapping the organizational and functional relationship between tissue-specific cells of each organ and immune cells, progenitor cells, endothelial/vascular cells, and the stroma.
3. **Data Coordination and Organizational Hub:** Track, store, and display all data generated by the HuBMAP and assist with development of ontologies, metrics, standards and analytical tools. Integrate with complementary programs to make data interoperable. Promote cross-site interactions, managing working groups and committees of the consortium (e.g. the Steering Committee), the website, meetings and outreach

Phase 2 Only

1. **Visualizing and Modeling:** Build statistical and analytic techniques and models of cellular organization and communication in tissues. Compare signatures of tissues from healthy individuals to those with different diseases
2. **Tissue Dynamic Mapping:** Accelerate the development of technologies and systematic approaches for mapping spatio-temporal changes within human tissues

Next steps

- Refine boundaries based on continued community input
- Decide which components will be prioritized by peer review
- Build synergies with ongoing similar NIH and international programs
- Continue gathering best practices for management and evaluation of the HuBMAP consortium in phase I and phase II
- Develop detailed implementation plans for the HuBMAP program

Backup Slide

Proposed HuBMAP Budget

Initiatives		Phase 1			Phase 2				
	Lead IC	FY18	FY19	FY20	FY21	FY22	FY23	FY24	FY25
Initiative 1: Tissue Core	TBD	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5
Initiative 2: Census of Human Cell Types	TBD	6.0	6.0	6.0	10.0	10.0	10.0	10.0	10.0
Initiative 3: Deep Profiling of Human Tissues	TBD	6.0	6.0	6.0	10.0	10.0	10.0	10.0	10.0
Initiative 4: Technology Development for in situ Analysis	TBD	5.0	5.0	5.0	3.0	3.0	3.0	3.0	3.0
Initiative 5: Data Coordination Center	TBD	1.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0
Initiative 6: Organizational Hub	TBD	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5
Initiative 7: Visualizing and Modelling Large-Scale Cell Networks	TBD				3.0	3.0	3.0	3.0	3.0
Initiative 8: Tissue Perturbation Mapping	TBD				5.0	5.0	5.0	5.0	5.0
RMS:	TBD	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
TOTAL		**20.5**	**21.5**	**22.5**	**37.5**	**37.5**	**37.5**	**37.5**	**37.5**

THANK YOU.

QUESTIONS?

Mechanisms of Fatigue

A PROPOSED COMMON FUND PROGRAM

Carlos Blanco, MD, PhD
NIDA/NIH
Presented on behalf of the Mechanisms of Fatigue Common Fund Working Group

Common Fund "Mechanisms of Fatigue" Working Group

NINDS – Vicky Whittemore, PhD & Walter Koroshetz, MD
NIAID – Joseph Breen, PhD & Katarzyna Bourcier, PhD
NINR – Leorey Saligan, PhD, RN, CRNP & Martha Matocha, PhD
NIA – Basil A. Eldadah, MD, PhD
NIDA – Carlos Blanco, MD, PhD & Nora Volkow, MD
NHLBI – Cheryl McDonald, PhD & Michael Twery, PhD
NIAMS – James Witter, MD, PhD FACR
NICHD – Karen Lee, MD, Danuta Krotoski, PhD & Mary Ellen Michel, PhD
NIMH - Mi Hillefors, PhD
NCCIH – Wen Chen, PhD
NCI – Sandra Mitchell, PhD, RN
OBSSR – William Ellwood, PhD
OER – Cheryl Kitt, PhD
OSC – Patricia Labosky, PhD, Rebecca Lenzi, PhD

Why a Common Fund Program?

- Fatigue is a normal mechanism in healthy individuals, and also a symptom of many diseases that cut across NIH Institutes and Centers
- Fatigue research would benefit from strategic planning and coordination
- Feasible to include milestones and goals for the research
- Research on fatigue would encourage collaboration across areas of interest to many ICs
- Defining fatigue and developing common, validated measures would transform the field
- Understanding the mechanisms of fatigue across the lifespan and in healthy and disease populations will lead to improved therapies and/or prevention of fatigue

Input and Analyses

- NIH Portfolio analysis indicates that $163 million was awarded in general fatigue-related research (not excluding chronic fatigue) since FY14
  - Less than one-third of the funds directed at fatigue explored any type of biological mechanism. Most of the research focuses on fatigue as a symptom of aging or disease
- Three conference calls (with 8-12 investigators per call) were held to determine the current challenges/roadblocks in fatigue research and to obtain input from extramural and intramural researchers about research opportunities and barriers. Investigator's background and expertise included:
  - Neuroscience
  - Oncology
  - Sleep Medicine
  - Immunology (HIV/AIDS)
  - Cognition/Psychiatry
  - Chronobiology

Focus Group Conference Calls

- **Call 1:**
  - Stephen Anton, PhD; University of Florida
  - Robert Dantzer, DVM, PhD; University of Illinois
  - John DeLuca, PhD; Kessler Foundation
  - Helen Genova, PhD; Kessler Foundation
  - Monika Haack, PhD; Harvard/BI
  - Jason Leonard, PhD; DePaul University
  - Rachel Manber, PhD; Stanford
  - Sigrid Veasey, MD; University of Pennsylvania
  - Glen Wylie, DPhil; Kessler Foundation
- **Call 2:**
  - Adriana Andrade, MD, MPH; Johns Hopkins University
  - Debra Barton, RN, PhD, AOCN, FAAN; University of Michigan
  - Allison Harvey, PhD; University of California, Berkeley
  - Paul Jacobsen, PhD; University of South Florida
  - Keith Kelley, PhD; University of Illinois
- **Call 2** (con't.):
  - Leorey Saligan, PhD, RN, CRNP; National Institute of Nursing Research, NIH
  - Christine Miaskowski, RN, PhD, AOCN, FAAN; University of California, San Francisco
  - Janet Mullington, PhD; Beth Israel/Harvard
- Call 3:
  - Torbjorn Åkerstedt, PhD; Karolinska Institute
  - Andrea Barsevick, PhD; Thomas Jefferson University
  - Ann Berger, PhD; University of Nebraska
  - Mary Harrington, PhD; Smith College
  - Sarah (Holly) Lisanby, MD; National Institute of Mental Health, NIH
  - Mark Rapaport, MD; Emory University
  - Amita Sehgal, PhD; University of Pennsylvania
  - Fred Turek, PhD; Northwestern
  - Brian Walitt, MD; National Institute of Nursing Research, NIH

Opportunities and Barriers

- The investigator identified the following issues:
  - Investigators studying fatigue are working in silos (cancer, HIV/AIDS, ME/CFS, etc.)
  - Many investigators commented on the lack of correlation between subjective and objective measures of fatigue
  - Need for taxonomy of fatigue considering the different domains (cognitive, emotional, physical)
  - Significant need for validated measures of fatigue to be used across study populations
  - No consensus in field about role/importance of restorative sleep and the link to fatigue
  - Very little ongoing research on underlying mechanisms of fatigue

Conclusions from Calls

- Investigators examining fatigue are working in silos (cancer, HIV/AIDS, ME/CFS, etc.) and are using different measures of fatigue
- No agreed definition of fatigue
- Need for taxonomy for fatigue
- Need for validated measures of fatigue where there is a correlation between subjective and objective measures
- No consensus in field about role/importance of restorative sleep and the link to fatigue
- Very little ongoing research on underlying mechanisms of fatigue

Fatigue Workshops in FY17
Workshop 1

- Facilitate workshops to bring investigators together from all areas of fatigue research
- Goals of the first workshop would be to:
  - Define the metrics by which investigators measure and study fatigue (both subjective and objective measures in both humans and animal models)
  - Identify measures/instruments used to study the domains of fatigue
  - Determine which measures/instruments need to be validated
  - Identify wearables and innovative ways to study fatigue

Fatigue Workshops in FY17
Workshop 2

- Second workshop to focus on the relationship between sleep and fatigue
  - Discuss mechanisms by which sleep alleviates fatigue
  - Identify knowledge of how energetics and metabolomics impact sleep and fatigue
  - Determine the role of glymphatics in the mechanisms of fatigue and impact of sleep
  - Review animal models utilized to study sleep and circadian rhythms that can be used to study fatigue

Questions and Comments