Navigating the NIH:

Getting the Most from Your NIH Summer Internship

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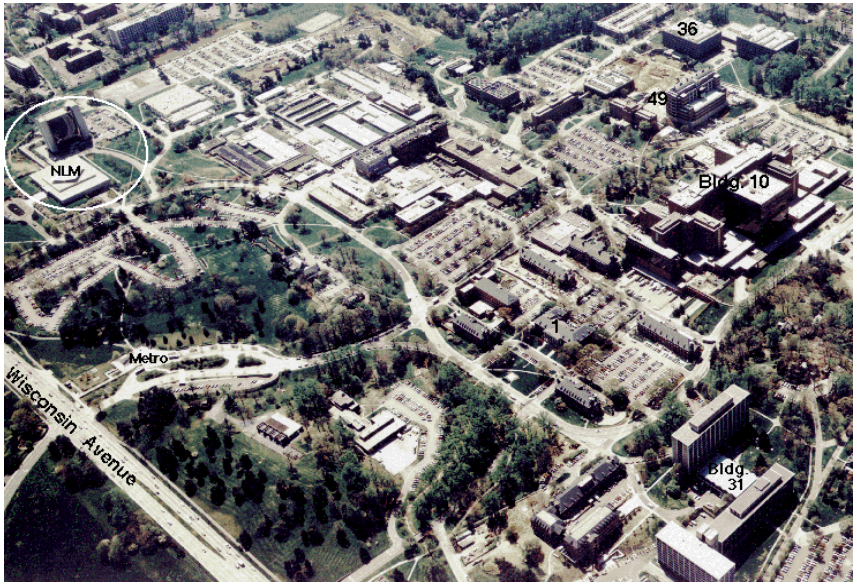
Office of Intramural Training and Education

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

Four Sections

- Understanding the NIH
- Settling into your research group and meeting your science goals
- Using NIH resources to meet your career and professional goals
- Understanding the research culture

What is the NIH?



The Nation's Biomedical Research Institution

- 27 Institutes &Centers (ICs)
- Biomedical, behavioral, and social science research at all levels - basic, translational and clinical
- Campuses in MD, NC, MT, AZ, MI and MA
- Two main divisions: **intramural** and **extramural**
- Learn more at www.nih.gov

Bench-To-Bedside Research At NIH

- 240 beds
- 7,000 inpatient admissions/yr
- 72,600 outpatient visits/yr
- 900 active clinical protocols

For information on clinical trials, please visit: http://clinicaltrials.gov/
We seek diversity in our clinical trials!

Researchers at NIH

- 1100 Faculty (PIs)
- 1500 Staff Scientists & Clinicians
- 3800 Postdoctoral Fellows
- 480 Clinical Fellows
- 485 Graduate Students
- 100 Medical Students
- 600 Postbacs
- YOU! ~1000 Summer interns

People You May Meet in Your IC

- Your Summer Coordinator, Training Director and Training Office staff
  - Be sure to attend your IC orientation
- The SD and Deputy SDs
- Your Branch or Lab Chief
- Other PIs in your Lab/Branch
- Your AO

First -- Define your goals

Focus on Development In Three Areas:

- Science
- Career
- Personal

To get the most out of your summer:

Make a plan

Research goals

Professional goals

Personal goals

Share it with your mentor!

Important Research Relationships

- Supervisor
  - Someone who directs the work of another
  - Is responsible for ensuring that someone does their job

- Mentor
  - Someone who passes on skills, knowledge, and wisdom to another person
  - Works to help develop someone's career by providing honest feedback, encouragement and guidance

There is No Typical Research Experience

- Differences in lab and research groups
  - Group dynamics and atmosphere
  - Level of day-to-day engagement of the PI
  - Level of independence, at experimental level and beyond
  - Types of rules and how they are imposed
  - Type and frequency of feedback
- Work hours, work environments and expectations vary; be sure you understand expectations in your group

Expectations Your Supervisors and Mentors Have of You

- Engagement in planning your research
- Honest communication regarding research progress
- Careful thought and regard for all elements of scientific ethics
- Energy and focus on your lab notebook
- Active participation in group activities and attention to fostering collegial relationships in your research group
- Good work ethic; balance internship and outside responsibilities effectively

Expectations You Should Have of Your Supervisors and Mentors

- Intellectual support and guidance
- Assistance in developing a project within the framework of on-going work in the group
- Availability when needed; guidance in planning the next step
- Honest but supportive feedback
- Support for professional development activities outside of lab
- Honest feedback regarding letters of recommendation

Mentoring Relationships Are Key To Your Success

- Successful scientists have many mentors -- in all areas
- Mentoring is a two-way relationship
- There is help to resolve issues:
  - Your IC Summer Coordinator and/or Training Director
  - Branch Chiefs and others in your research group
  - OITE staff and career counselors
  - Office of the Ombudsman, Center for Cooperative Resolution
  - NIH Employee Assistance Program (EAP)

To Achieve Your Science Goals

- Appreciate that first impressions are key
- Meet with your supervisors - EARLY & OFTEN
- Read papers in your field; work to be sure you understand how to read a paper
- Focus on the “big picture” AND the details of your work
- Use “down-time” in lab wisely
- Actively participate in research group meetings
- Attend seminars, in and outside of your field
- Present your work at Summer Poster Day

To Achieve Your Career Goals

- Use the online Summer Handbook and IC materials to find information on useful career development activities
- Work with your mentor to create a summer plan
- Attend the Graduate & Professional School Fair if appropriate
- Use informational interviews to explore careers

Take Care of Your Whole Self

- Make certain you always feel your best
  - Exercise
  - Eat well
  - Stay connected to family/friends
- Get to know interns in your research group and IC
- Explore your local area

Since you are here…

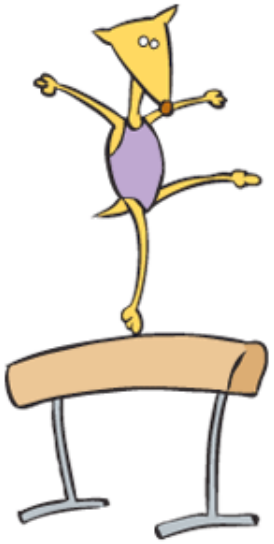
- Check out the NIH Recreation & Welfare Association (R&W)
  - Fitness centers
  - Discount tickets
- Join ClubPCRmini social listserv
- Explore Washington, DC
  - Summer Handbook has pages of things to do
  - Even if you are from area…explore it

A tale of 2 PIs

- Hands-on
- Found in the lab/office or close-by
- Talks with students and fellows all the time
- Called by his/her first name

- Hands-off
- Locked in his/her office or away at meetings
- Meets with trainees for formal meetings scheduled in advance
- Called Dr._____

Interacting with your mentor



- Be respectful and courteous
- Adapt to his/her mentoring style
- Accept criticism with grace
- Ask when you have questions
- Find balance between being independent and asking for help
- Address problems before they escalate

Different research groups have their own culture

- How loud is the room?
- How clean/organized is their research space?
- What hours do people work?
- How do people dress?
- Do group members use Ipods or cell phones?
- Do group members eat lunch together?
- Do group members go to seminars together?

Mandatory activities

- Group meeting?
- Journal clubs?
- Seminars?

Be a good lab citizen

- Keep common space clean
- Don’t disturb other people’s work
- Be extremely careful with common reagents and equipment
  - Don’t contaminate!
  - Replace common reagents if they are running low
  - **Ask for help** if you don’t know how to use equipment
  - Tell somebody if equipment is broken

“Research is formalized curiosity. It is poking and prying with a purpose.”

Zora Neale Hurston

Scientific research . . .

- Is a team effort

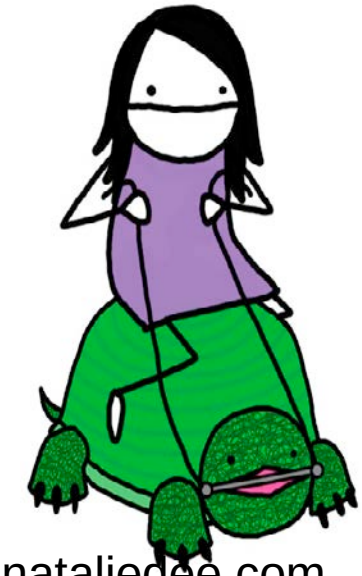
www.barcelonafootballblog.com

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions

Scientific research . . .

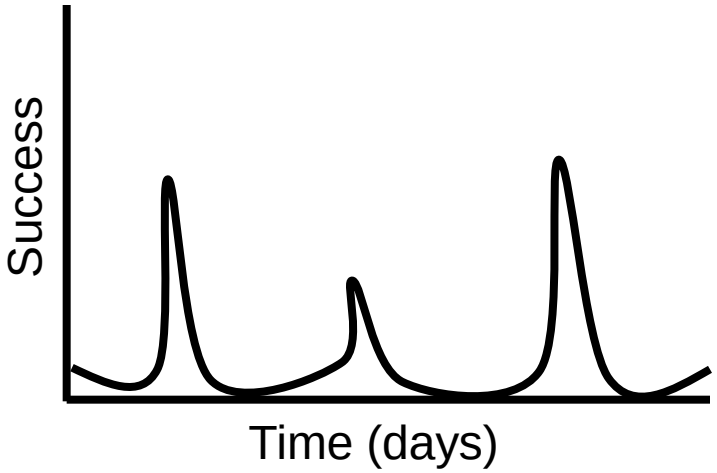
- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly



nataliedee.com

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly
- Contains periods of frustration between successes



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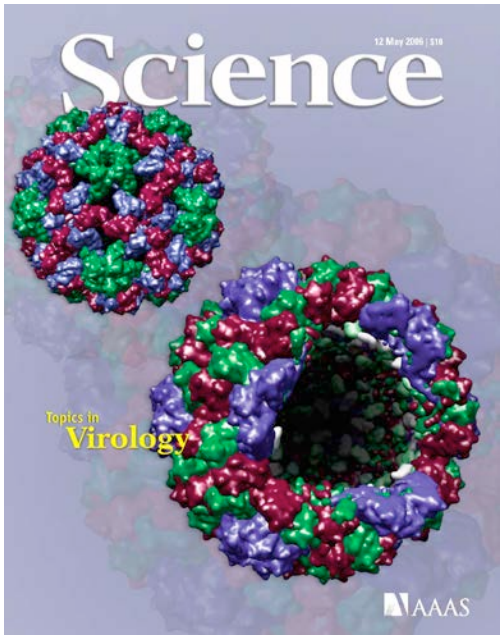
- Is not a 9-5 job

Scientific research . . .

- Is a team effort
- Involves asking small, manageable questions
- Often moves slowly
- Contains periods of frustration between successes
- Is not a 9-5 job
- Must always be documented

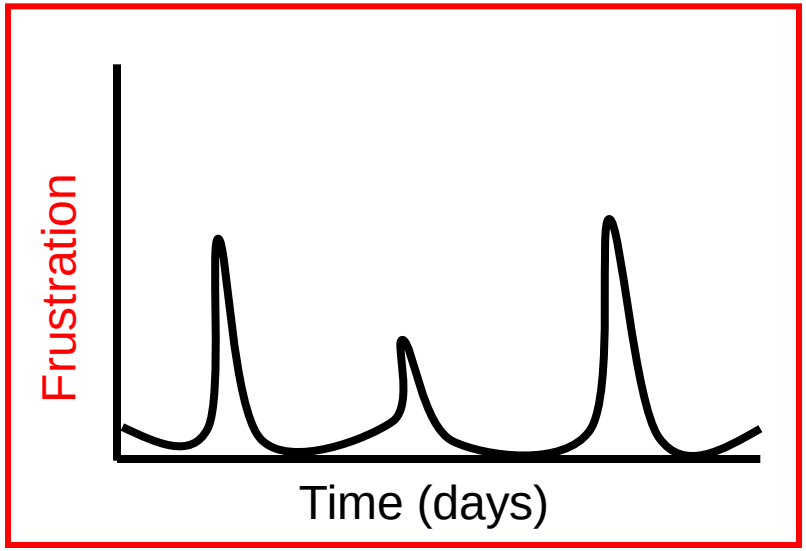
For data to be publishable, your work must be:

- Meticulous
- Reproducible
- Well-documented



Science can be unpredictable

- Don’t get frustrated!
- Ask for help
- Be creative
- Be flexible with your research plan



A typical day of research

- At the bench
  - Doing experiments
  - Making reagents
- Away from the bench:
  - Planning experiment
  - Analyzing data
  - Thinking about your project
  - Reading papers

What if you don’t have any work to do?

- Read papers
- Think about your project
- Plan experiments
- Learn about other people’s projects
- Volunteer to help others
- Start your poster

To achieve your research goals

- Meet with your mentor(s) early and often!
- Be clear about expectations- yours and your supervisor's
- Read papers related to your work
- Attend the Summer Lecture Series
- Make sure you understand both the big picture and the details of your research
- Pay attention and participate during group meeting
- Volunteer to give a talk in your group meeting
- Present a poster at Summer Poster Day
- Attend a Summer Journal Club

This is your summer job . . . But it's also a training experience!

- Attend seminars and workshops
- Take advantage of NIH resources
- Talk to other researchers
- Network
- Have fun

Summary: What your research group expects of you:

- Attention to safety
- Respect for diversity
- Respect for animal models
- Professionalism, especially when dealing with patients
- Participation in all lab/group activities
- Engagement in your research project
- Honest communication about your research

Looking out for yourself

Reasonable expectations:

- To be treated fairly and with respect
- To have some guidance from a mentor
- To have appropriate training when working with dangerous equipment or hazardous chemicals
- To have (at least a little) time to work on professional development

If problems arise

- Be mature and rational
- Try to address the problem early, by talking calmly with the person you are conflicting with
- If necessary, talk to your PI or other mentors
- If necessary, seek help from your IC training director, OITE, or the NIH Office of the Ombudsman

What if something really bad happens?

You are not alone!

- Your principle investigator
- Your IC training director
- Dr. Sharon Milgram, OITE Director
  milgrams@mail.nih.gov
- Dr. Pat Sokolove, Director Postbaccalaureate and Summer Intern Programming
  sokolovp@mail.nih.gov

Final Thoughts: ALL Summer Interns Should

- Use the online Summer Handbook
- Check that your name is on the OITE-SIP listserv
- Attend appropriate summer intern activities
- Make use of online resources from OITE
  - Follow us on twitter @NIH_OITE
- Participate in Summer Poster Day
- E-mail me if you have questions: ryanp@od.nih.gov

Event

NIH SACNAS CHAPTER: SUMMER KICK-OFF EVENT

SPECIAL EVENT: NETWORKING OPPORTUNITIES

Building 40, Room 1201/1203

Jun 22, 2012 4:00 pm - 5:00 pm

Science Skills Boot Camp

Workshop 4: Resources Available to Frederick National Lab Summer Students

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

www.training.nih.gov

Resource categories

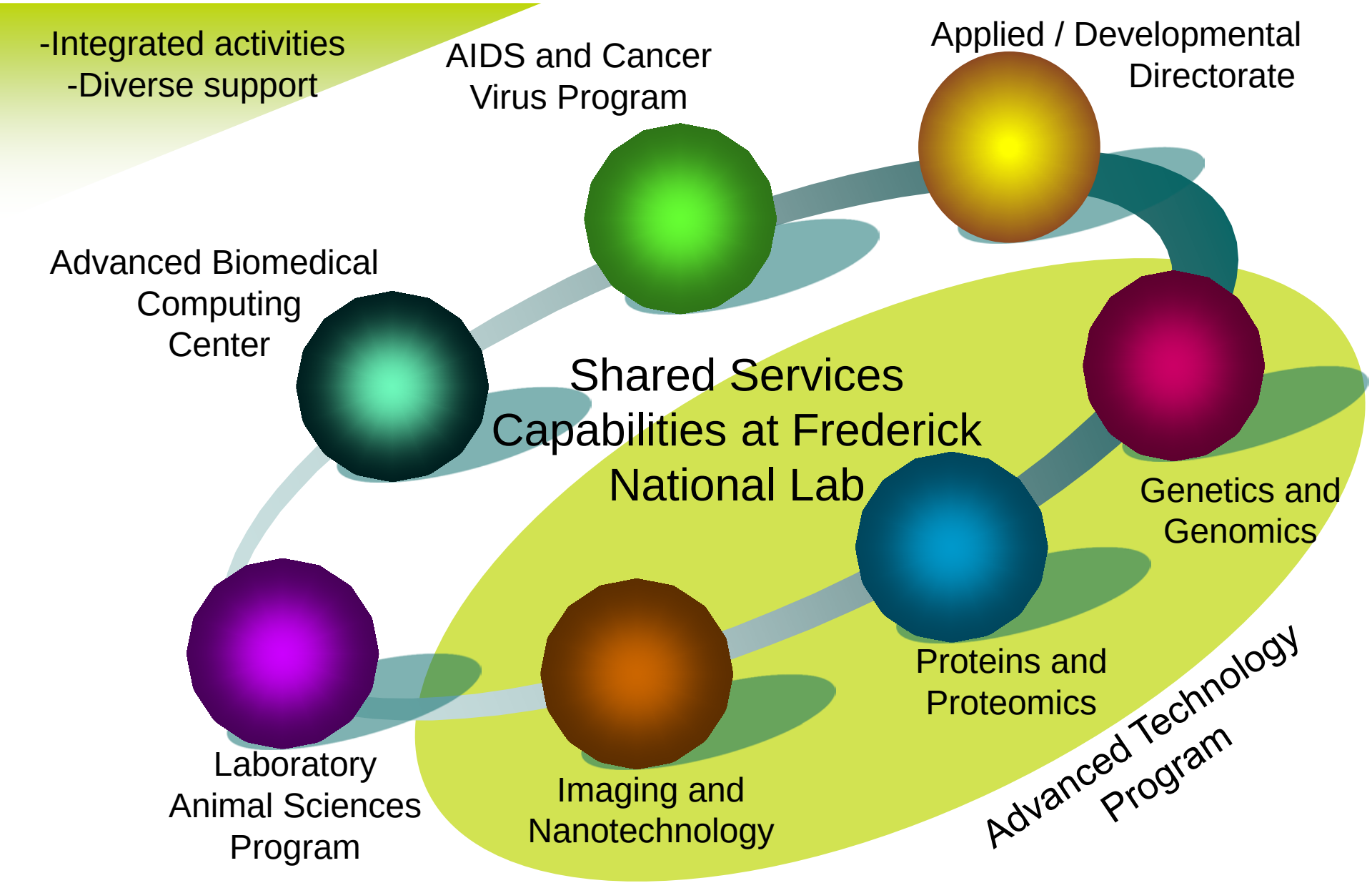
- Research resources
- Science skills
- Career development
- Administrative problems
- Technical problems
- Personal health and happiness
- Interpersonal support
- Social resources

Research resources (1): NCI-Frederick Library (Building 549)

- Access to >7,000 journals, >12,000 books, Internet resources, and > 220 online databases
- Document delivery
- Reference and research assistance
- Training programs
- Translation services
- Writing assistance services
- Library tours

Research resources (2): World Class Laboratories

-Integrated activities
-Diverse support



Research resources (3): The people

Your best resource for everything!!

- Your mentor(s)
- Members of your research group
- Other students and fellows
- People you meet at seminars, workshops, and journal clubs

For a list, refer to the NCI-SIP App or visit http://ncifrederick.cancer.gov/News/Feature/StudentActivities.aspx

Science skills resources

- Your mentor and research group members
- IC training events
- OITE workshops
- OITE Webinars (www.training.nih.gov)
  - Keeping a lab notebook
  - Guidelines for writing professional email
  - Guidelines for attending your first scientific meeting
  - Lab math

Career development resources

- Your IC training office – Dr. Jonathan Wiest
- OITE
  - Workshops and seminars
  - Pre-professional advisor Dr. William Higgins
  - Career counseling
  - Career library
- Everybody around you!

Administrative problems

- Your lab secretary/manager
- Summer coordinator – Julie Hartman
  hartmanjb@mail.nih.gov
- Your administrative officer (AO) –Administrative Resource Center (ARC)
- Debbie Cohen, OITE summer coordinator
  cohend@mail.nih.gov

Technical problems

- Computer help:
  - Frederick National Lab Computer Helpdesk
  - Submit Online requests:

http://css.ncifcrf.gov/helpdesk/

Personal health and happiness (1)

- Frederick Memorial Hospital – 400 West 7th Street, 240-566-3300
- Occupational Health Service (OHS), 301-846-1096
  - Preplacement evaluations
  - Occupation-related medical care
- CPT Jennifer J. Shafer Odom Fitness Center
  - Building 1507 (Fort Detrick)
  - No Charge

Personal health and happiness (2)

- NIH Employee Assistance Program (EAP)
  - Short-term counseling
  - Coaching
  - Disability management
  - Health and wellness consultation
  - Crisis intervention
  - Referrals

Free, confidential services!

http://ncifrederick.cancer.gov/Staff/Eap.aspx

Interpersonal support resources

- Your mentor and alternate mentors
- IC training directors
- OITE staff
  - Sharon Milgram, Director
    milgrams@mail.nih.gov
  - Pat Sokolove, Deputy Director
    sokolovp@mail.nih.gov
  - Debbie Cohen, Summer Program Coordinator
    cohend@mail.nih.gov
- Office of Ombudsman, Center for Cooperative Resolution

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- Dr. Pat Sokolove, OITE Deputy Director

Diversity resources (1)

- Frederick National Lab Employee Diversity Team: http://diversity.ncifcrf.gov/
- Office of Equal Opportunity and Diversity Management (OEODM)

  http://oeodm.od.nih.gov/
- NIH Black Scientists and Friends Network

  Contact Dr. Roland Owens

  owensrol@mail.nih.gov (301) 594-7471
- NIH Hispanic Employee Organization

  http://heo.nih.gov/

Diversity resources (2)

- SACNAS- Advancing Hispanics/Chicanos and Native Americans in Science

  Contact Dr. Erika Barr

  Erika.Barr@nih.gov (301) 451-2164
- Asian and Pacific Islander American Organization (APAO) http://www.recgov.org/r&w/apao/
- Association for Women in Science (AWIS)

  http://www.awisbethesda.org/
- NIH salutaris

  NIH gay, lesbian, bisexual, transgender employees' forum: http://www.recgov.com/salutaris/index.html

How do I know what's going on this summer?

- NCI SIP App (search for NCI SIP on device)
- Summer handbook - https://www.training.nih.gov/2011_summer_intern_news
- Frederick National Lab Activities Page: http://ncifrederick.cancer.gov/News/Feature/StudentActivities.aspx
- OITE Web site

  www.training.nih.gov

Ways to meet other summer students

- Summer Student Seminar Series – Tuesday's at Noon in Building 549 Auditorium (with pizza!)
- Summer Student Journal Clubs
- Research seminars
- The many activities listed on the NCI SIP App and Summer Student Activity web page.

Reading a Scientific Paper

2012 Summer Student Boot Camp

06.26.2012

Overview

- Rationale for reading journal papers
- Types of papers
- The elements of the paper
- How to approach reading the paper
- Example Paper
- Critical Analysis of the paper

Why Read Scientific Papers?

- Journal articles are one of the primary methods of communication between scientists
- Cutting Edge, Up to date information
- This is where you can examine the original data/findings
- Allows for replication of the study
- Develop your critical thinking skills

Different Types of Scientific Papers

- Primary - Original research
  1. Experimental
  2. Observational, e.g. case control, cohort
- Secondary
  - Reviews
  - Editorials/Opinion/Commentary/Perspectives
  - Meta analysis

Finding your Paper

NCBI Resources How To My NCBI Sign In

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed | KIR3DL1 | Search

RSS Save search Advanced Help

Choose additional filters

Text availability
Abstract available
Free full text available
Full text available

Publication dates
5 years
10 years
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Species
Humans
Other Animals

Article types
Clinical Trial
Randomized Controlled Trial
Review
more ...

Languages
English
more ...

Clear all

Choose additional filters

Display Settings: Summary, 20 per page, Sorted by Recently Added

Send to:

See 154 articles about KIR3DL1 gene function
See also: KIR3DL1 killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1 in the Gene database
kir3dl1 in Homo sapiens | Mus musculus | Rattus norvegicus | All 8 Gene records

Results: 1 to 20 of 232

<< First < Prev Page 1 of 12 Next > Last >>

1. Influence of Cytokines on HIV-Specific Antibody-Dependent Cellular Cytotoxicity Activation Profile of Natural Killer Cells.
Wren L, Parsons MS, Isitman G, Center RJ, Kelleher AD, Stratov I, Bernard NF, Kent SJ.
PLoS One. 2012;7(6):e38580. Epub 2012 Jun 11.
PMID: 22701674 [PubMed - in process] Free PMC Article
Related citations

2. CD158k/KIR3DL2 and NKp46 are frequently expressed in transformed mycosis fungoides.
Ortonne N, Le Gouvello S, Tabak R, Marie-Cardine A, Setiao J, Berrehar F, Nghe-Tang A, Martin N, Bagot M, Bensussan A.
Exp Dermatol. 2012 Jun;21(6):461-463. doi: 10.1111/j.1600-0625.2012.01489.x.
PMID: 22621189 [PubMed - as supplied by publisher]
Related citations

3. Possible KIR-driven genetic pressure on the genesis and maintenance of specific HLA-A,B haplotypes as functional genetic blocks.
Capittini C, Tinelli C, Guarene M, Pasi A, Badulli C, Sbarsi I, Garlaschelli F, Cremaschi AL, Pizzochero C, Monti C, Salvaneschi L, Martinetti M.

Filters: Manage Filters

Related searches
kir3dl1 allele

Titles with your search terms
Variable NK cell receptors exemplified by human KIR3DL1/S1. [J Immunol. 2011]
Common HIV-1 peptide variants mediate differential binding of KIR3DL1 to H [J Virol. 2011]
Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. [Nat Genet. 2007]
See more...

46 free full-text articles in PubMed Central
Influence of Cytokines on HIV-Specific Antibody-Dependent Cellular Cytotoxicity [PLoS One. 2012]
Enrichment of variations in KIR3DL1/S1 and KIR2DL2/L3 among H1N1/09 I [PLoS One. 2011]
Copy number variation of KIR genes influences HIV-1 control. [PLoS Biol. 2011]
See all (46)...

http://www.ncbi.nlm.nih.gov/pubmed/

The Structure of a Scientific Paper

Title

Abstract

Introduction

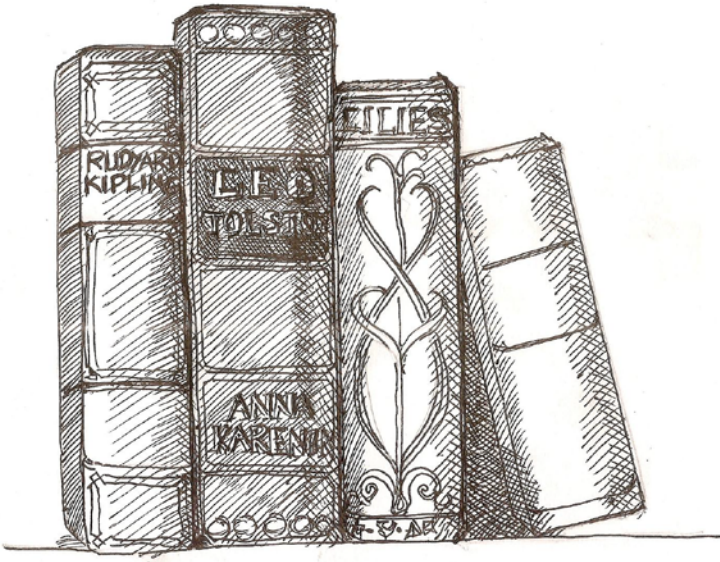
Methods

Results

Discussion

References

IMRaD



- Author Affiliations, acknowledgements, author contributions, competing interests

- Variations
  - Combined Results and Discussion
  - Methods included at the end
  - On-line supplements – supplemental data, methods, references

Abstract:

An abstract summarizes, in one paragraph, the major aspects of the entire paper including the question explored, the methodology used, the results and interpretation/conclusion of the findings.

Introduction:

This sections gives the context of the work, including what is currently known, states the purpose of the work (what is the question/hypothesis under investigation), relevance to the field and the rationale and approach of the current study

Methods:

Described the steps and methodology used to gather and analyze data, including statistical methods. Should be of sufficient detail for replication of the study.

Results:

- This is where the data is. Reported as tables, graphs, images etc. Statistical results are included here. Minimal interpretation of the data.
- First experiment might have several possible interpretations, and the later ones are designed to distinguish among these
- Often several different experimental approaches combine to support a particular conclusion.

Discussion:

Interpretation of the data – Answer to research question? Confirmation/rejection of hypothesis?

Relation to other literature, possible pitfalls/caveats, broader implications, future directions.

References:

List of publications cited throughout the paper. Style differs by journal.

Vivian, J. P., R. C. Duncan, R. Berry, G. M. O'Connor, H. H. Reid, T. Beddoe, S. Gras, P. M. Saunders, M. A. Olshina, J. M. Widjaja, C. M. Harpur, J. Lin, S. M. Maloveste, D. A. Price, B. A. Lafont, D. W. McVicar, C. S. Clements, A. G. Brooks, and J. Rossjohn. 2011. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature* 479:401-405.

How to Approach a Scientific Paper

- Have a clear idea of what you want to achieve by reading the paper.
- Reading a scientific paper from start to finish in a linear manner is *not* recommended
- Start with the **abstract** – based on this decide if this paper is of interest to you.
- **Discussion**
- **Introduction**
- **Results –** Often broken into *headed sections* based on findings. Next look at the figures and figure legends. Read text of results.

Adapted from http://www.lib.purdue.edu/phys/inst/scipaper.html

Guide Questions

Increase your comprehension of scientific articles by taking notes

1. What questions do I hope this article will answer?
2. What do the authors conclude?
3. Why did the authors do the study?
4. What data/results emerged from the study?
5. How did the authors do this study?
6. What is the significance of these findings
7. How does this article relate to other article I've read?
8. Did this article answer my questions? If so, what the answers are…
9. List other article cited here that I should read:

Taken from http://www.istl.org/09-fall/article4.html

Group Activity

- Read the abstract of the provided paper.
- In groups of 3-4 find the answers to the following questions about the provided paper [Hint: decide which section is likely to contain the required information]
  - Describe in one sentence the aim of this study
  - What was the model system used?
  - Why do the authors believe this protein has relevance for *human* disease
  - The authors examine Muc1 – describe this protein in 1-2 sentences
  - Describe in your own words what is measured in Figure 1A
  - How did the authors isolate CD11c+ dendritic cells?

GASTROENTEROLOGY 2012;142:865–874

The Membrane-Bound Mucin Muc1 Regulates T Helper 17-Cell Responses and Colitis in Mice

ATSUSHI NISHIDA,[*,‡] CINDY W. LAU,[*] MEI ZHANG,[§,‖] AKIRA ANDOH,[¶] HAI NING SHI,[§,‖] EMIKO MIZOGUCHI,[#,**,‡‡] and ATSUSHI MIZOGUCHI[*,‡,‡‡]

[*]Molecular Pathology Unit, Massachusetts General Hospital, Charlestown; [‡]Department of Pathology, Harvard Medical School, Boston; [§]Mucosal Immunology Laboratory, Massachusetts General Hospital, Charlestown; [‖]Department of Pediatrics, Harvard Medical School, Boston, Massachusetts; [¶]Division of Mucosal Immunology, Graduate School of Medicine, Shiga University of Medical Science, Shiga, Japan; [#]Gastrointestinal Unit, Massachusetts General Hospital, Boston; [**]Department of Medicine, Harvard Medical School, Boston; and [‡‡]Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital, Boston, Massachusetts

BACKGROUND & AIMS: T helper (Th) 17 cells produce the effector cytokine interleukin (IL)-17, along with IL-22, which stimulates colonic epithelial cells to produce a membrane-bound mucin, Muc1. Muc1 is a component of the colonic mucus, which functions as a lubricant and a physiologic barrier between luminal contents and mucosal surface. The gene *MUC1* has been associated with susceptibility to inflammatory bowel disease; we investigated the role of Muc1 in development of colitis in mice. **METHODS:** *Muc1* and *RAG1* were disrupted in mice (*Muc/RAG* double knockout mice); Th1-mediated colitis was induced by intravenous injection of $CD4^+CD45RB^{high}$ T cells. We also studied Th2-mediated colitis using mice with disruptions in *Muc1* and *T-cell receptor α chain* (*Muc/TCR* double knockout mice). **RESULTS:** Muc1 deficiency led to the development of more severe forms of Th1- and Th2-induced colitis than controls. Loss of Muc1 increased colonic permeability and the Th17-cell, but not Th2 or Th1 cell, response in the inflamed colon. Loss of Muc1 also promoted expansion of an innate lymphoid cell population (Lin^- $ckit^-$ $Thy1^+$ $Sca1^+$) that produces IL-17. The expansion of Th17 adaptive immune cells and innate lymphoid cells required the commensal microbiota. **CONCLUSIONS: Muc1, which is up-regulated by Th17 signaling, functions in a negative feedback pathway that prevents an excessive Th17 cell response in inflamed colons of mice. Disruption of this negative feedback pathway, perhaps by variants in Muc1, might contribute to inflammatory bowel disease in patients.**

Keywords: Susceptibility; Risk Factor; Mouse Model; Immune Regulation.

commensal microbiota, interleukin (IL)-23 receptor (IL-23R)/T helper cell (Th) 17 axis, and immune regulation.[2,6,7]

An attractive cell population that links the major IBD pathways together may be Th17 T-cell subset. Th17 subset develops through a reciprocal differentiation. Individual T-cell precursor can differentiate into effector Th17 cells vs forkhead box P3 ($Foxp3^+$) regulatory T cells,[8–10] suggesting a relation of Th17 subset to the initial event of immune regulation pathway in IBD. Polymorphisms of IL-23 receptor are negatively associated with the development of IBD.[11] Indeed, pathogenic role of IL-23 has been well documented in several IBD models,[12] and beneficial effect of anti-IL-12p40 monoclonal antibodies (mAbs), which can neutralize both IL-12 (p35/p40) and IL-23 (p19/p40) activities, has been shown in active CD patients.[13] The IL-23 is required for the maintenance of Th17 cells,[8–10] highlighting the involvement of Th17 cells in the IL-23 pathway in IBD.[1–3,7,12] In addition, Th17 cells are considered to closely participate in the pathway of epithelial defense through production of IL-22.[8] Polymorphisms of IL-10R2, which is shared by IL-22R1 to make a heterodimer receptor for IL-22, are positively associated with IBD,[14] and IL-22 gene is located within an UC susceptibility locus.[5] Indeed, we and others previously demonstrated the protective role of IL-22 in some colitis models, including Th2-mediated colitis of T-cell receptor α knockout (TCRα KO) mice[15] and Th1-mediated colitis of CD45RB model.[16] IL-22 serves as a major activator of signal transducer and activator of transcription 3 (STAT3) in epithelial cells under intestinal inflammatory conditions[15,17] and possesses the ability to stimulate the epi-

BASIC AND TRANSLATIONAL AT

Th1, Th2, Th17: types of T cell immune response

Colitis: Inflammation of the colon

Colonic epithelium: cells that line the colon

Gut Microbiota: collection of microorganisms found in the gut

Critical Review of a Paper

What **questions** does the paper address?

What are the main **conclusions** of the paper?

What **evidence** supports those conclusions?
Do the data actually **support** the conclusions?

What is the **quality** of the evidence?

Why are the conclusions **important**?

What **evidence** supports those conclusions?

- Is the data sufficient to merit the conclusion?
  You need to understand the methods – what are the *limitations* of the method

  If multiple approaches, different model systems, different lines of evidence, supporting the conclusions, then more credible.

- Are there other interpretations of the data?
  Identify any implicit or hidden assumptions ,Separate fact from interpretation

What is the **quality** of the evidence?

- Are all the controls present?
  - This varies widely for different techniques, need to be familiar with methodology.
- What is the gold standard in the field?
- Look at details, assess them for plausibility
  - Is there an obvious experiment missing?
  - Any expected result not found?
  - Is there excessive manipulation of the data?

Why are the conclusions **important**?

- So what?
- What does this add to what we already knew?
- Does it suggest a new mechanism, a new potential drug target….?

Summary

- The ability to read and assess scientific literature is an important skill
- Decide what you are looking for (e.g. overview of a topic, description of a new model, methodological detail)
- Read the abstract carefully to gauge if the paper is appropriate
- Abstract. Introduction. Discussion. Results.
- Do not blindly accept findings. Are *you* convinced?

Extra Slides

A badly written paper

- Cluttered with jargon, acronyms
- Lack of clear road-map through the paper
  - Central hypothesis/question not stated clearly, and revisited
- No logical consistency
  - Rationale for progression of experiments not described
- Difficulties determining what was done
  - Ambiguous, sketchy or missing description
  - Endless citation trail back to first paper
  - The devil is in the details!
- Data mixed up with interpretation and speculation

http://www.nature.com/scitable/ebooks/english-communication-for-scientists-14053993/writing-scientific-papers-14239285

The Measure of a Journal?

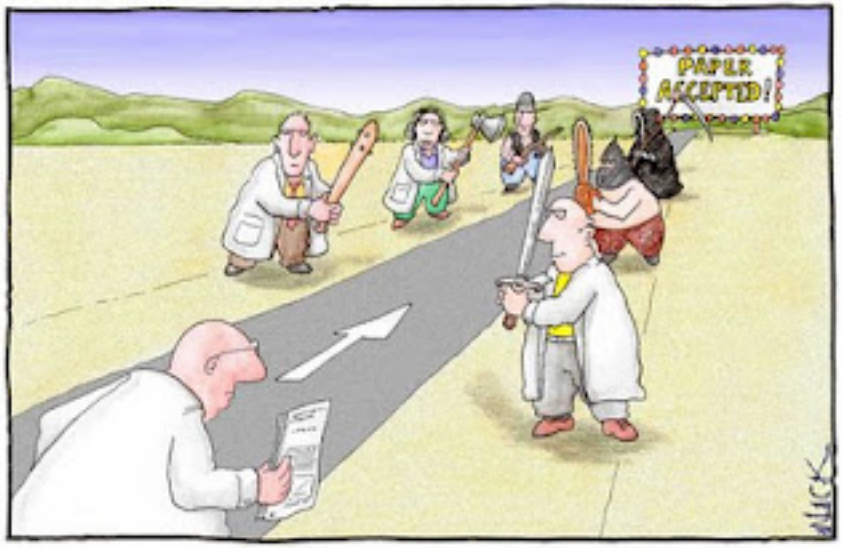
- Impact Factor
  - it is a measure of the frequency with which the "average article" in a journal has been cited in a particular year or period.
  - A = the number of times articles published in 2010-2011 were cited in indexed journals during 2012

    B = the number of articles, reviews, proceedings or notes published in 2010-2011

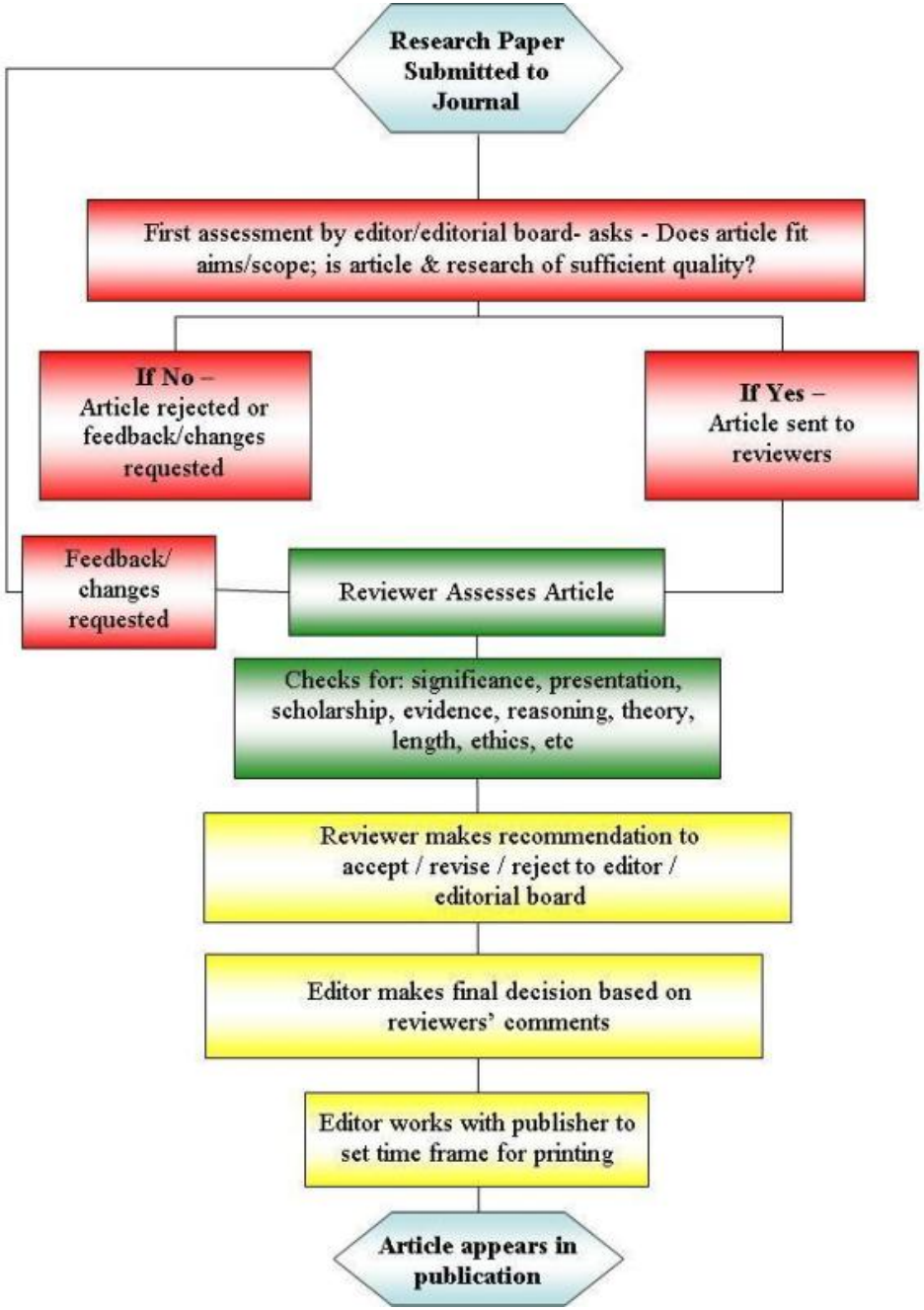
    impact factor 2012 = A/B

Peer Review

- Peer review is the process commonly used to determine whether scientific papers are worthy of publication.



Most scientists regarded the new streamlined peer-review process as 'quite an improvement.'



Taken from http://www.elsevier.com/wps/find/reviewershome.reviewers/peer_diagram

Google

KIR3DL1

Scholar

About 2,320 results (0.16 sec)

My Citations

Articles

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Any time
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Since 2008
Custom range...

Recent additions

include patents
include citations

Innate partnership of HLA-B and **KIR3DL1** subtypes against HIV-1 — Full-Text Plus!
MP Martin, Y Qi, X Gao, E Yamada, JN Martin… - Nature genetics, 2007 - nature.com
Abstract Allotypes of the natural killer (NK) cell receptor **KIR3DL1** vary in both NK cell expression patterns and inhibitory capacity upon binding to their ligands, HLA-B Bw4 molecules, present on target cells. Using a sample size of over 1,500 human ...
Cited by 272 - Related articles - BL Direct - All 8 versions

Different NK cell surface phenotypes defined by the DX9 antibody are due to **KIR3DL1** gene polymorphism — [HTML] from jimmunol.org Full-Text Plus!
CM Gardiner, LA Guethlein… - The Journal of …, 2001 - Am Assoc Immnol
KIR3DL1 and KIR3DL2 are NK cell receptors for polymorphic HLA-B and-A determinants. The proportion of NK cells that bind anti-**KIR3DL1**-specific Ab DX9 and their level of binding vary between individuals. To determine whether these differences are due to KIR ...
Cited by 170 - Related articles - BL Direct - All 6 versions

Cutting edge: allele-specific and peptide-dependent interactions between **KIR3DL1** and HLA-A and HLA-B — [HTML] from 9med.net Full-Text Plus!
H Thananchai, G Gillespie, MP Martin… - The Journal of …, 2007 - Am Assoc Immnol
Although it is clear that **KIR3DL1** recognizes Bw4+ HLA-B, the role of Bw4+ HLA-A allotypes as **KIR3DL1** ligands is controversial. We therefore examined the binding of tetrameric HLA-A and–B complexes, including HLA* 2402, a common Bw4+ HLA-A allotype, to **KIR3DL1*** ...
Cited by 114 - Related articles - BL Direct - All 5 versions

The protein made from a common allele of **KIR3DL1** (3DL1* 004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig … — [HTML] from jimmunol.org Full-Text Plus!
MJ Pando, CM Gardiner, M Gleimer… - The Journal of …, 2003 - Am Assoc Immnol
KIR3DL1 is an inhibitory HLA-B receptor of human NK and T cells that exhibits genetic and phenotypic polymorphism. **KIR3DL1*** 004, a common allotype, cannot be detected on the surface of PBLs using the **KIR3DL1**-specific Ab DX9. The nature of this phenotype was ...
Cited by 118 - Related articles - BL Direct - All 5 versions

How to Keep a Lab Notebook

ALYSON K. FREEMAN, PH.D.

Why is this important?

- Collect all of your protocols and data in one place
- Analyze results
- Think through your experiments
- Keep a permanent record

- You will not remember every little detail in the future

Who is your audience?

- Many people may need to read your notebook
  - You – repeating experiments is a critical part of research
  - Your lab members – After you leave at the end of the summer, someone may pick up your project
  - Outside reviewers – Funding agencies and journal editors may need to see original work
  - Patent offices and pharmaceutical companies

Who owns your lab notebook?

- Your lab
- The government
- Not you

When to write in your notebook

- As soon as possible!
- Try to take notes throughout the day as you work
- At the very least, take notes at the end of the day
- Be sure to add in data, results, and conclusions

Format

- Can be a bound book or binder
  - A book where pages cannot be taken out is better
  - Use what your lab uses
- Things to include
  - On the cover:
    - Your name
    - Lab/PI name
    - Telephone number
    - Address
    - Dates used
  - In the notebook:
    - Page numbers
    - A table of contents
    - Dates

Table of contents

- Dates
- Page numbers
- Experiment numbers
- Experiment description

What to include for each experiment

- Page number/experiment number
- Title
- Dates
- General protocol
- Protocol modifications/mistakes
- Important calculations
- Reagents used – include company names, catalog numbers, lot numbers
- Data
- Results/conclusions

What to include for each experiment

- Loose material
  - Printouts from lab equipment – plate reader, spectrophotometer, flow cytometer, gel dock, etc.
  - Printouts from your computer – Excel spreadsheets, Prism graphs, microscopy images
- Attach to notebook itself or keep in a separate notebook and reference location
- Mark file location in computer

Common protocols

- The first time you use a protocol, write out in detail
- Each subsequent time, write the important details and mark any changes (intentional or unintentional)
- Reference the original protocol so it can be easily accessed

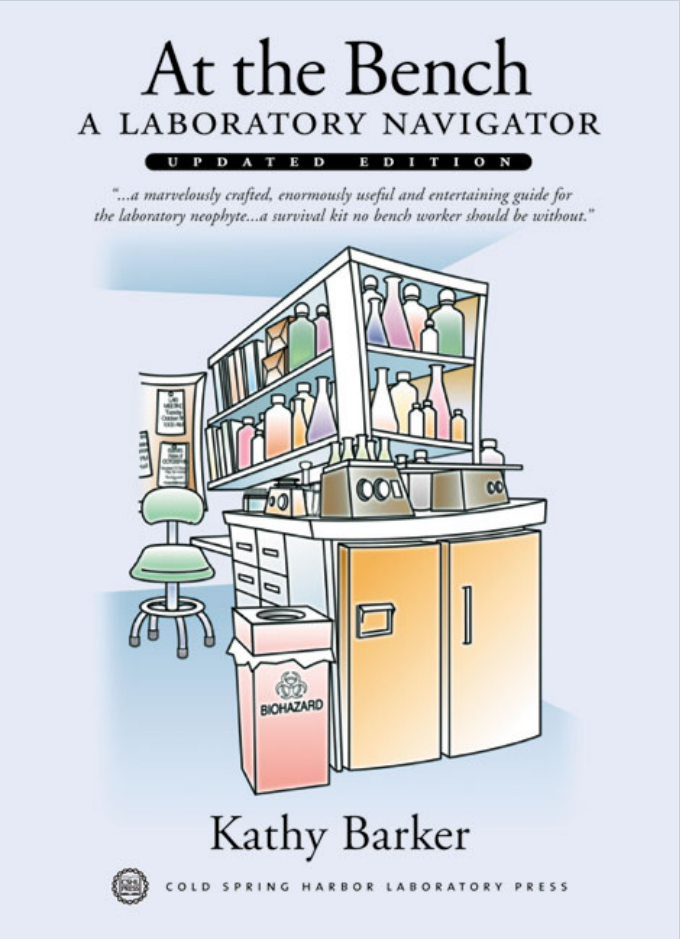
What *not* to do

- Anything that looks suspicious
- Use white-out
- Erase errors
- Rip out pages
- Write on scrap paper to transfer later
- Wait until the end of the week or month to take notes
- Take your notebook out of the lab

What *to* do

- Write as soon as possible, throughout the day as you work
- Hand write as much as possible
- Use a ball point pen
- Write legibly
- If you make a mistake, draw a line through it
- Use a different notebook for each project
- Leave notebooks in the lab

Questions?


At the Bench
A LABORATORY NAVIGATOR
UPDATED EDITION
"...a marvelously crafted, enormously useful and entertaining guide for the laboratory neophyte...a survival kit no bench worker should be without."
BIOHAZARD
Kathy Barker
COLD SPRING HARBOR LABORATORY PRESS

“Giving Group Meeting”

Science Skills Bootcamp
Building 426, Conference Room
26 June 2012

Lillian Kuo, Ph.D.
kuols@mail.nih.gov
HIV Drug Resistance Program
National Cancer Institute

What are the objectives of a group meeting?

1. Opportunity for you to share your work
2. Highlight the great work you have been doing
3. Show and tell
4. Ask questions to the whole group
5. Discuss your problems and achievements
6. Status update for everybody
7. Receive invaluable feedback from your group

Different Types and Styles of Meetings

Sub-group Roundtable	Single Lab/Group	Multiple/Larger Group
Informal	Semi-informal, semi-formal	Formal
Not entire lab necessarily present	Just your lab group	Your lab and others
PI likely present	Just one PI	Multiple PIs
Just your notebook and chalk talk	PowerPoint or chalk talk	PowerPoint
Everybody shares their data	You are the star	You are the star
Minimal introduction	Basic introduction	Thorough introduction
Data focused	Data and project focused	Project focused
Extensive group discussion	You talk with group discussion	You talk with a few questions

Before your group meeting…

1. Discuss with your mentor/PI expectations
2. Organize your lab notebook
3. What do you want to get out of the meeting?
4. Think about your journey:
   a. How did I get here?
   b. Why did I do these experiments?
   c. What happened?
   d. What did I learn?
   e. What am I going to do next?

5. Draft your talk/slides to show your mentor/PI in advance
6. Your group meeting presentation should fit the specific sub-culture of your group

Basic Components

1. Introduction
2. Objectives and Hypotheses
3. Methods
4. Data and Results
5. Conclusions
6. Future Directions
7. Summary

Introduction

- Review of the most salient literature
- Description of your mentor’s research interests, and how your projects fits in
- What is the question or problem you are addressing?
- Show a couple illustrations or diagrams

Objectives and Hypothesis

- What are the goals and objectives ?
- Why is this significant?
- What are the questions you are asking?
- Based on the previous work described in your introduction, what is the hypothesis?

Methods

- Description of experimental materials, methods and techniques
- Be sure to understand the methods you are using
- Extent of methods discussion is relative to audience
- What is unique or novel about the methods employed?
- Why did you chose these methods? Are there alternatives?

Data and Results

- More figures and pictures, less text
- Demonstrate logical progression of data, not necessarily chronological
- Show graphs and statistical analyses as necessary
- Be sure to adequately label graphs, gels, images, etc.
- Explain why you did the experiments
- It’s okay to show negative data
- What do these data and results tell you?

Conclusions

- What does the data tell you?
- What did you learn from these experiments?
- What are the answers to the experimental questions you asked?
- Is the data inconclusive? If so, how can you change your experimental methods to obtain more useful data?

Future Directions

- Science NEVER STOPS, there are always more questions and problems to address
- Based on your data, what future experiments will you conduct?
- What new questions arose from your data and results?
- How would you use your data and results to write a fellowship or grant proposal?

Summary

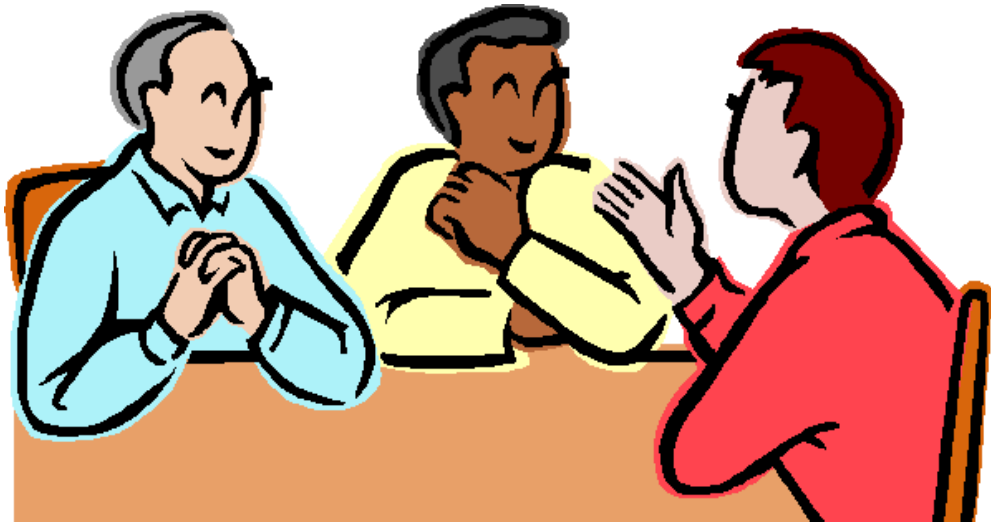
- Take-home message
- Re-emphasize the key findings of your project
- Highlight the significance of your work
- End with acknowledgements

Tips and Concepts to Keep in Mind

1. You will be amongst friends
2. This is a mutually beneficial exercise
3. It’s not all about showing lots and lots of data
4. Communicate the who, what, when, where, and why?
5. Put things in the context of your audience
6. Practice will help you be more comfortable
7. Your group meetings presentation will be relative to each unique lab group

After your group meeting…

1. Congratulate yourself on a job well-done!
2. Follow-up with your mentor/PI
   a. What experiments do you need to do next?
   b. What directions should we follow next?
3. Address questions asked
4. Utilize the feedback you received
5. Plan for the future



Questions? Let’s discuss!

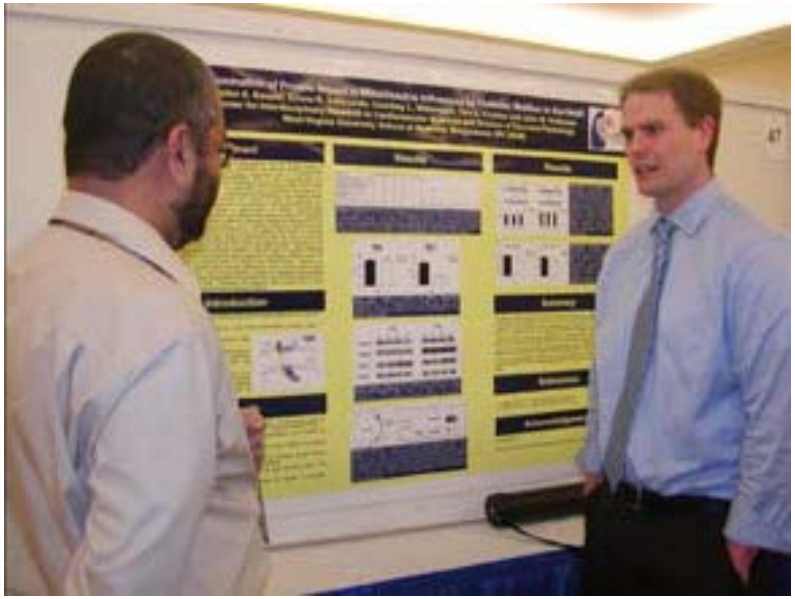
How to Prepare and Present a Scientific Poster

Walter Baseler

Undergraduate Bootcamp

June 26th, 2012

Purpose



1. Disseminating scientific data quickly and efficiently at conferences of other venues
2. Great way to interact with peers/experts in the field
3. Sharpen public speaking skill set

Poster’s Appearance

1. Are figures/tables clearly labeled?
2. How is the poster organized?
3. Appropriate font size?
4. Amount of text appropriate?
5. Any large amount of open space?

Structure and Layout

- Orientation: Landscape
- Width: 56 inches
- Height: 28 inches

- **Size of Font- Can be read from 3-5 feet**
  - Title: 90-120
  - Authors: 48-60
  - Headings: 70-80
  - Main Text: 36-40

Generic Poster Outline

1

Overexpression of phospholipid hydroperoxide glutathione peroxidase (MPHGPx) attenuates cardiac mitochondrial proteomic loss and reverses protein import detriments observed with type 1 diabetes mellitus.

Walter A. Baseler, Erinne R. Dabkowski, Dharendra Thapa, Rajaganapathi Jagananathan, Dharendra Thapa, Cody E. Nichols, Danielle L. Shephard, Tara L. Croston, David M. Schnell, John M. Hollander
Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences.
West Virginia University, School of Medicine, Morgantown, WV 26506.

2

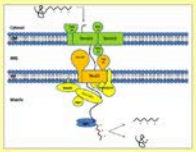
Abstract

Mitochondrial dysfunction is a contributor to diabetic cardiomyopathy. Previously, we observed proteomic decrements within the mitochondrial inner membrane (IMM) and matrix of diabetic cardiac interfibrillar mitochondria (IFM) correlating with dysfunctional mitochondrial import. The goal of this study was to determine whether overexpression of MPHGPx, an antioxidant capable of scavenging membrane-associated lipid peroxides, could preserve the mitochondrial proteome and import process. MPHGPx transgenic mice and controls were made diabetic by multiple low-dose streptozotocin. Proteomic analyses revealed MPHGPx overexpression preserved proteins decreased within the diabetic IFM. Ingenuity Pathway Analyses indicated that OXPHOS, TCA, and FAO processes most influenced in diabetic IFM were preserved by MPHGPx overexpression. Further, IMM proteins were the most highly preserved (63%) followed by matrix proteins (34%). Mitochondrial protein import decrements were also preserved in the MPHGPx diabetic IFM ($P<0.05$) which correlated with increases in protein import constituents. The results indicate overexpression of MPHGPx can protect essential mitochondrial complexes in both IMM and matrix and may provide cardioprotective benefits to the diabetic heart.

3

Introduction

- Cardiac Interfibrillar mitochondrial (IFM) dysfunction resulting from type 1 diabetic insult has been linked to decrements in contractile function.
- Mitochondria-destined nuclear-encoded proteins constitute >99% of the organelles proteomic makeup and must be imported into the mitochondrion. The mitochondrial import process has been shown to be dysfunction in diabetic IFM (1)
- Mitochondrial phospholipid hydroperoxide glutathione peroxidase (mPHGPx) can directly reduce hydroperoxide groups of phospholipids, lipoproteins and cholesterol esters, potentially protecting mitochondrial phospholipid membranes from oxidative damage.
- The goal of this study was to determine whether overexpression of mPHGPx with lead to the preservation of mitochondrial import constituents, correct mitochondrial import dysfunction and preserve the mitochondrial proteome, specifically in the diabetic IFM

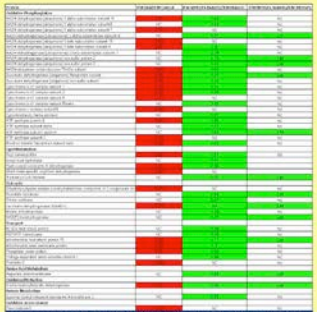


4

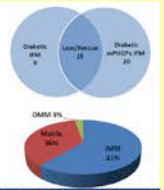
Methods

- FVB mice were made diabetic through multiple low-dose intraperitoneal injections (50mg/kg) of streptozotocin. Experiments were performed 5 week post diabetes onset.
- Cardiac tissue was excised and mitochondrial subpopulations were isolated by differential centrifugation and enzymatic digestion (2).
- Proteomic profiles were examined by LC-MALDI MS/MS using i-TRAQ™ technology (Protea Biosciences).
- Mitochondrial protein import assay was performed to compare protein import efficiencies of control and diabetic mitochondrial subpopulations.
- Canonical mitochondrial pathways and protein networks were analyzed using Ingenuity Pathway Analyses (IPA).
- Western blot analyses were performed to evaluate the content of essential mitochondrial import proteins and translocases.

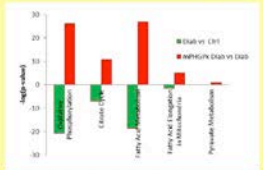
Results



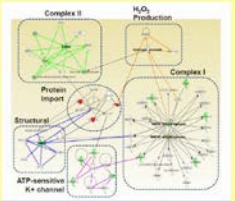
Proteomic Assesment. iTRAQ analysis revealed many proteins significantly decreased in the diabetic IFM as compared to control. Further, overexpression of mPHGPx in the diabetic hearts attenuated majority of changes. *P<.05 for control vs. diabetic IFM and mPHGPx diabetic vs. diabetic IFM. Red = downregulated, Green = upregulated



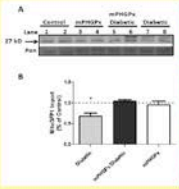
Proteomic alterations in diabetic and mPHPGx diabetic IFM. (A) Total amount of proteins decreased solely in the diabetic IFM (left), common proteins decreased in diabetic IFM and preserved within mPHGPx diabetic IFM (middle), and proteins increased solely within the mPHGPx diabetic IFM (B). Breakdown in approximate percentages of protein contents preserved in the mPHGPx diabetic heart IFM. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane



Mitochondrial canonical pathways. Identification of essential mitochondrial canonical pathways negatively influenced in diabetic IFM and preserved within mPHGPx diabetic IFM. Oxidative phosphorylation, Citrate cycle, fatty acid metabolism, and fatty acid elongation were negatively affected (green) by diabetes mellitus. Conversely, these pathways as well as pyruvate metabolism were preserved (red) within the mPHGPx diabetic IFM.



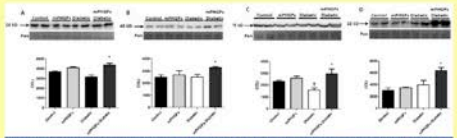
Mitochondrial protein networks. Identification of mitochondrial proteomic networks negatively influenced in diabetic IFM and preserved within mPHGPx diabetic IFM. Red indicates proteins positively enhanced in mPHGPx diabetic IFM. White indicates proteins unchanged by diabetes mellitus but are part of their respective networks.



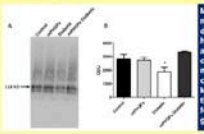
Mitochondrial protein import. Effect of type 1 diabetes mellitus and mPHGPx overexpression on MitoGFP1 import in IFM. Cardiac mitochondrial subpopulations from control, diabetic, mPHGPx, and diabetic mPHGPx mice were isolated and incubated with 2µl of MitoGFP1 protein lysate at 1 and 2 minute time points. Representative Western blots from IFM (A) protein import assay. Graphical representation of mitochondrial protein import performed in control (C), diabetic (STZ), mPHGPx (M), and diabetic mPHGPx IFM (M + STZ) (B). Both time point percentages were averaged to show total import efficiency. Control for protein loading was confirmed by Ponceau staining. Values are presented as means ± SE; *P < 0.05 for IFM STZ vs. all IFM groups. N=5 per group.

5

Results



Mitochondrial protein import constituents. Western blot analysis of protein import constituents. Key proteins involved in mitochondrial protein import were assessed using Western blot analysis. Representative Western blot and densitometry analysis for Tom20 (A), Tim50 (B), MtHsp70 (C), and Tim23 (D) from control, mPHGPx, diabetic and diabetic MPHGPx IFM. Control for protein loading was confirmed by Ponceau staining. Values are presented as means ± SE; *P < 0.05 for mPHGPx diabetic vs. all groups; †P < 0.05 for IFM mPHGPX diabetic vs. diabetic. N=4 per group.



MtHsp70 protein complexing. Isolated mitochondrial subpopulations from control, diabetic, mPHGPx, and diabetic mPHGPx hearts were partially lysed in digitonin buffer and subjected to BN-PAGE. Protein complexes were transferred to nitrocellulose membrane and probed for MtHSP70. A complex of MtHsp70 and Tim44 exists at 119 kD (A). MtHsp70 content was analyzed through densitometric analysis (B). *P < 0.05 for IFM STZ vs. all IFM groups. N=4 per group.

6

Summary

- The mitochondrial proteome was significantly dysregulated in the diabetic IFM subpopulation and preserved in diabetic mPHGPx IFM subpopulation.
- The majority of the proteins/proteins networks influenced by diabetes mellitus and preserved within mPHGPx IFM were of IMM origin.
- Nuclear-encoded mitochondrial import was significantly decreased in the diabetic IFM and preserved within the mPHGPx diabetic IFM.
- Key outer and inner membrane mitochondrial translocases Tom20, Tim50, MtHsp70, and Tim23 protein content were significantly increased in mPHGPx IFM during type 1 diabetic insult.
- These results suggest that mPHGPx is capable of preserving the IFM proteome through a mechanism of nuclear-encoded mitochondrial protein import preservation during a type 1 diabetic insult.

References

1. Baseler WA., et al., AJP Reg Integr Comp Physiol. 300:R186-R200, 2011
2. Palmer JW., et al., *J. Biol. Chem.* 252:8731-8739, 1977.

Acknowledgements

Supported by:

NIH DP2DK083095, AHA 0855484D, NIH T32HL090610, AHA 10PRE3420006, AHA 815406D

Title

1. Title of Poster
2. Authors in order of importance
3. Institution/address

Abstract

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- Brief description of entire study (Usually a word limit)
- Includes
  - Introduction
  - Goal of study
  - Methods
  - Results
  - Conclusion

Introduction

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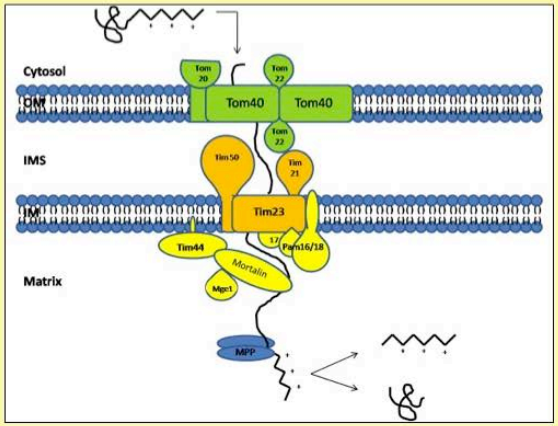
- The goal of this study was to determine whether overexpression of mPHGPx with lead to the preservation of mitochondrial import constituents, correct mitochondrial import dysfunction and preserve the mitochondrial proteome, specifically in the diabetic IFM



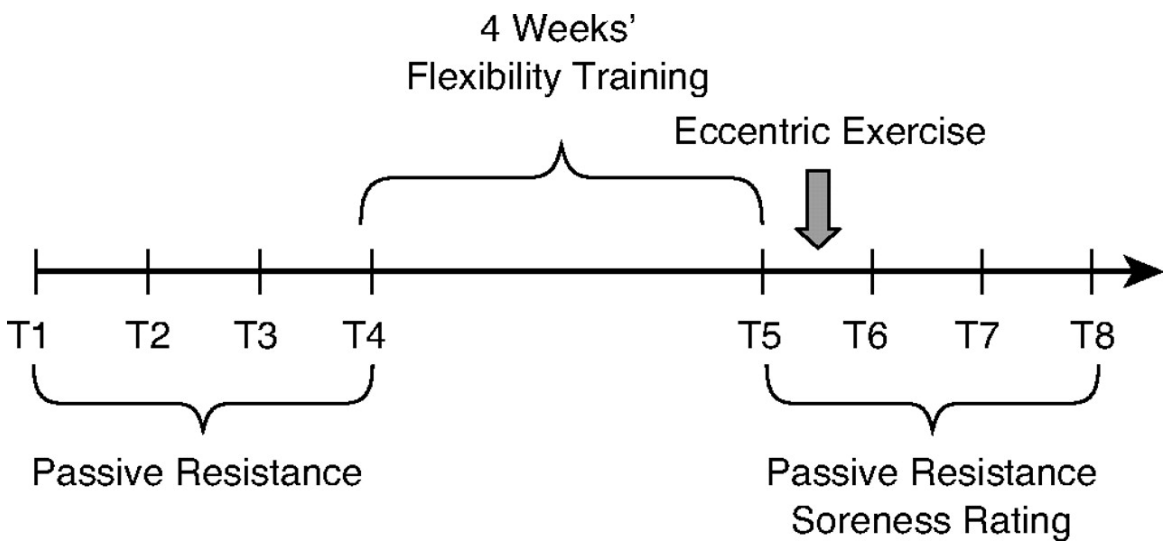
- As broad of a description as possible to give background and relevance to your study
- 3-5 sentences
- Can be advantageous to restate goal of the study and hypothesis
- Simple diagram of pertinent information/processes can be helpful

Methods

- FVB mice were made diabetic through multiple low-dose intraperitoneal injections (50mg/kg) of streptozotocin. Experiments were performed 5 week post diabetes onset.
- Cardiac tissue was excised and mitochondrial subpopulations were isolated by differential centrifugation and enzymatic digestion (2).
- Proteomic profiles were examined by LC-MALDI MS/MS using i-TRAQ™ technology (Protea Biosciences).
- Mitochondrial protein import assay was performed to compare protein import efficiencies of control and diabetic mitochondrial subpopulations.
- Canonical mitochondrial pathways and protein networks were analyzed using Ingenuity Pathway Analyses (IPA).
- Western blot analyses were performed to evaluate the content of essential mitochondrial import proteins and translocases.

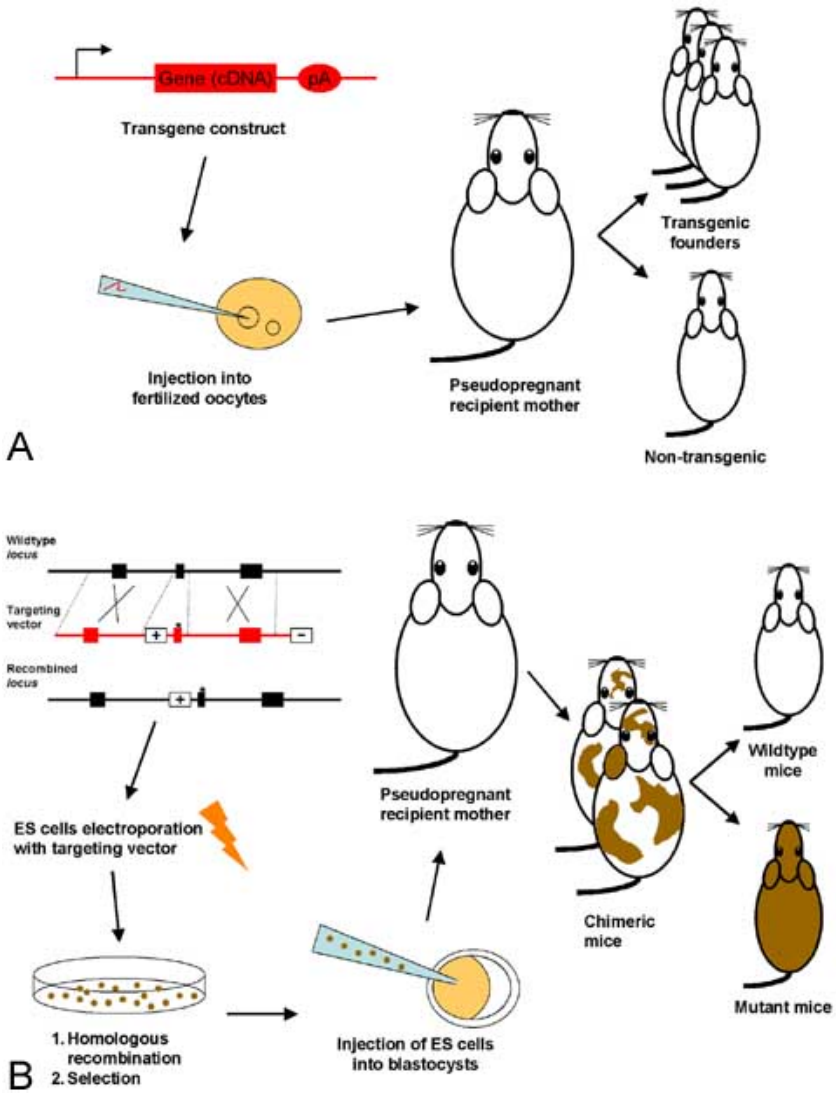
- Summarized version of pertinent experimental methods
- Do not get into specifics (examples)
  - Western blots – No need to say what secondary antibody you used
  - RTQ-PCR – No need to say how much cDNA used

Methods – What's Acceptable?

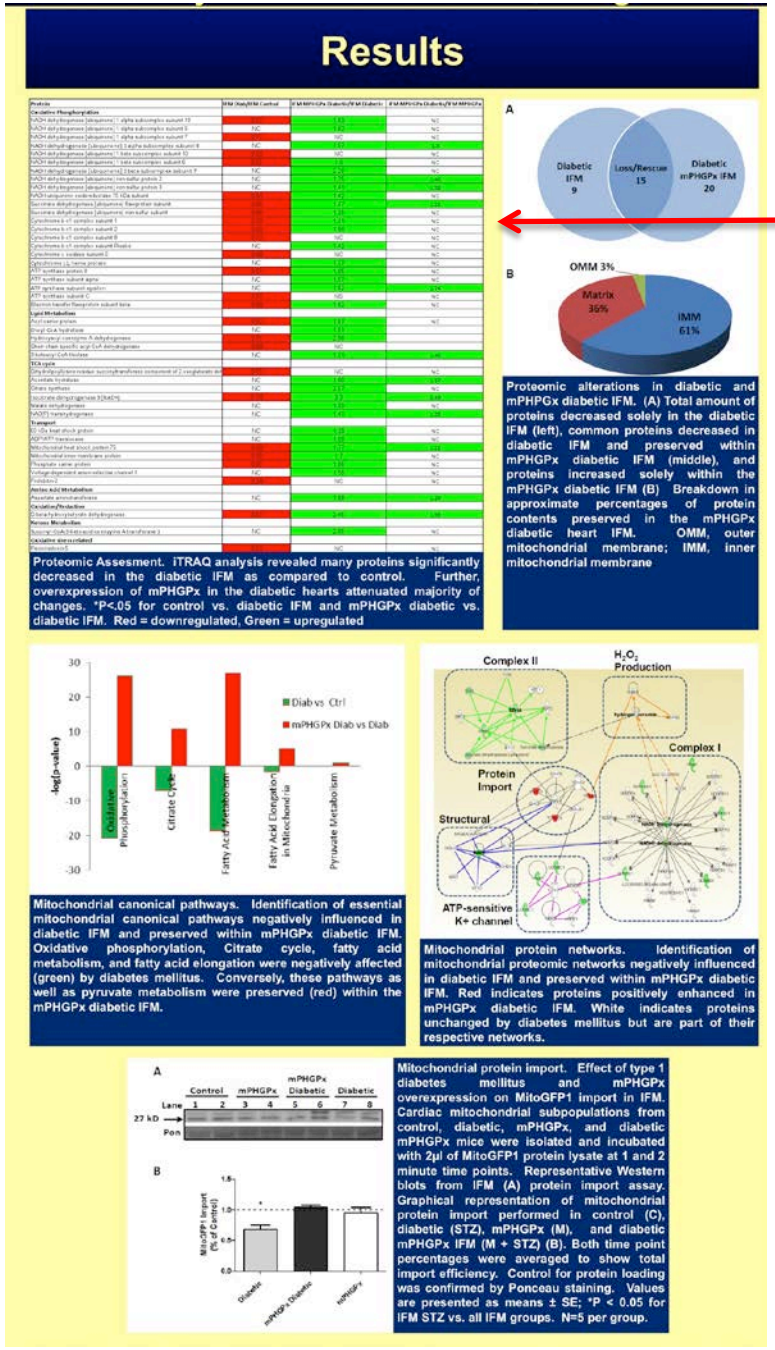


- Experimental time line

Methods – What's Acceptable?



- Novel or complicated experimental methodology may benefit from a figure/diagram for clear understanding



- Should come in the form of figures or tables
- Tables should be avoided (if possible)
- Make navigating through the results section logical
- Be sure to clearly label all tables and figures

- All figures will have legends
  - Title
  - Brief summation of each result/results
  - Identify abbreviations/color schemes
  - Include mean and statistical analyses performed

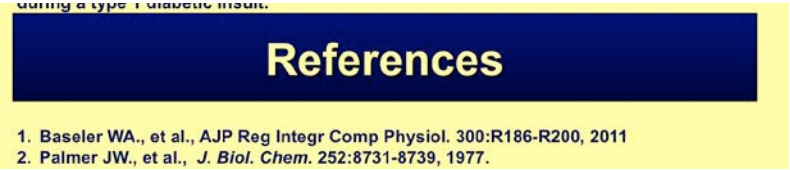
Summary

- The mitochondrial proteome was significantly dysregulated in the diabetic IFM subpopulation and preserved in diabetic mPHGPx IFM subpopulation.
- The majority of the proteins/proteins networks influenced by diabetes mellitus and preserved within mPHGPx IFM were of IMM origin.
- Nuclear-encoded mitochondrial import was significantly decreased in the diabetic IFM and preserved within the mPHGPx diabetic IFM.
- Key outer and inner membrane mitochondrial translocases Tom20, Tim50, MtHsp70, and Tim23 protein content were significantly increased in mPHGPx IFM during type 1 diabetic insult.
- These results suggest that mPHGPx is capable of preserving the IFM proteome through a mechanism of nuclear-encoded mitochondrial protein import preservation during a type 1 diabetic insult.

- Can also be called “conclusions”
- Brief rundown of pertinent findings from the poster
- Should end with
  - These results suggest…….
  - Our findings revealed…..
  - Combined these results may influence……

Optional Sections

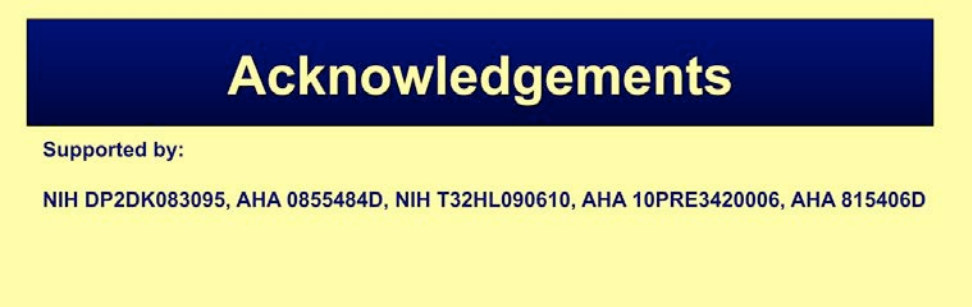
- Hypothesis
- References



References

1. Baseler WA., et al., AJP Reg Integr Comp Physiol. 300:R186-R200, 2011
2. Palmer JW., et al., *J. Biol. Chem.* 252:8731-8739, 1977.

- Acknowledgements
- Funding



Acknowledgements

Supported by:

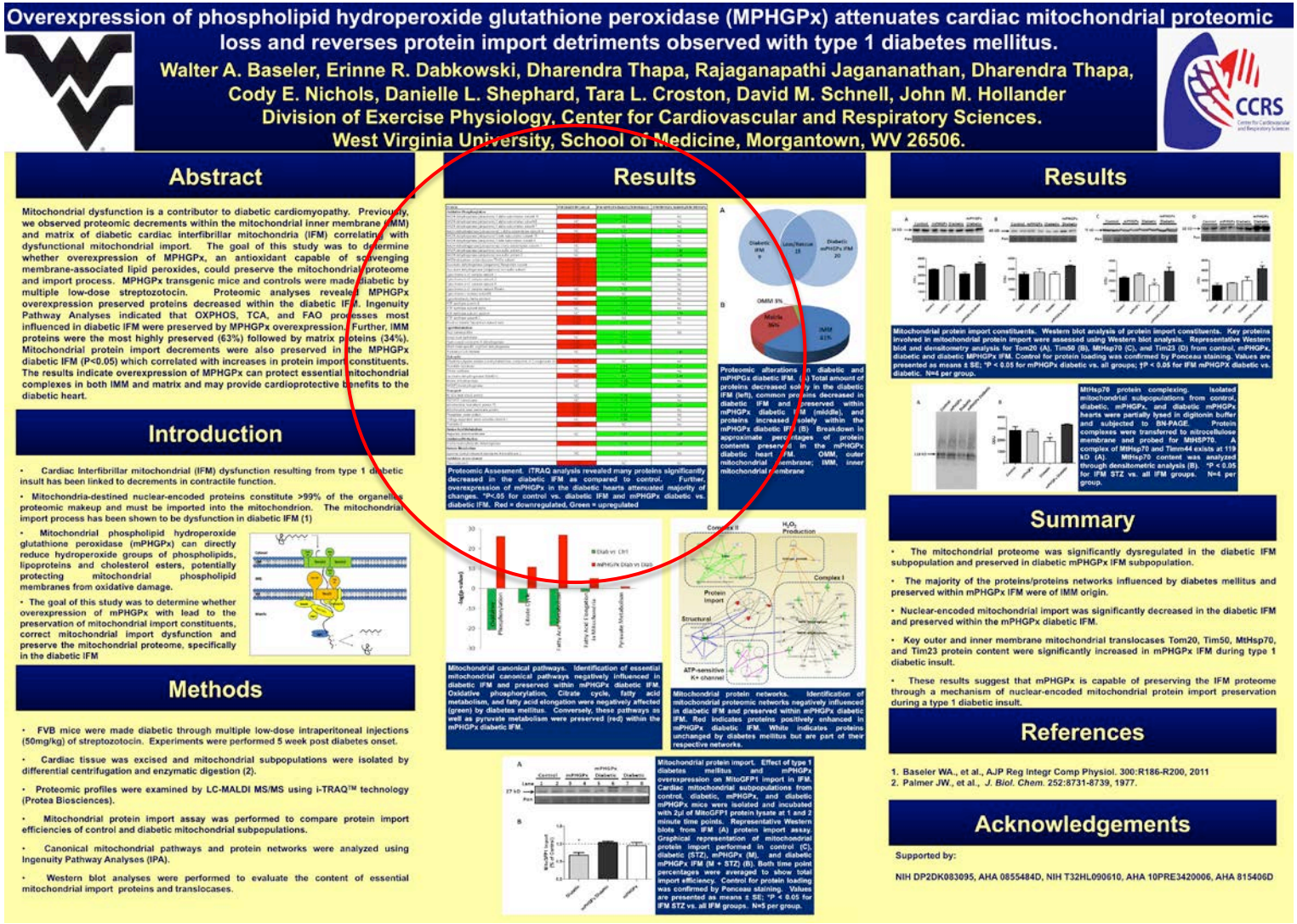
NIH DP2DK083095, AHA 0855484D, NIH T32HL090610, AHA 10PRE3420006, AHA 815406D

Remember!

- There is no “right way” to make a poster

- Some PI’s/Mentors are particular about how to make a poster. Please consult with them.
  - General Layout
  - Color Scheme
  - Background Scheme

- However, there are plenty of “wrong ways”
- Let’s Identify what a poorly made poster looks like



Overexpression of phospholipid hydroperoxide glutathione peroxidase (MPHGPx) attenuates cardiac mitochondrial proteomic loss and reverses protein import detriments observed with type 1 diabetes mellitus.

Walter A. Baseler, Erinne R. Dabkowski, Dharendra Thapa, Rajaganapathi Jagananathan, Dharendra Thapa, Cody E. Nichols, Danielle L. Shephard, Tara L. Croston, David M. Schnell, John M. Hollander
Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences.
West Virginia University, School of Medicine, Morgantown, WV 26506.

Abstract

Mitochondrial dysfunction is a contributor to diabetic cardiomyopathy. Previously, we observed proteomic decrements within the mitochondrial inner membrane (IMM) and matrix of diabetic cardiac interfibrillar mitochondria (IFM) correlating with dysfunctional mitochondrial import. The goal of this study was to determine whether overexpression of MPHGPx, an antioxidant capable of scavenging membrane-associated lipid peroxides, could preserve the mitochondrial proteome and import process. MPHGPx transgenic mice and controls were made diabetic by multiple low-dose streptozotocin. Proteomic analyses revealed MPHGPx overexpression preserved proteins decreased within the diabetic IFM. Ingenuity Pathway Analyses indicated that OXPHOS, TCA, and FAO processes most influenced in diabetic IFM were preserved by MPHGPx overexpression. Further, IMM proteins were the most highly preserved (63%) followed by matrix proteins (34%). Mitochondrial protein import decrements were also preserved in the MPHGPx diabetic IFM (P<0.05) which correlated with increases in protein import constituents. The results indicate overexpression of MPHGPx can protect essential mitochondrial complexes in both IMM and matrix and may provide cardioprotective benefits to the diabetic heart.

Introduction

- Cardiac Interfibrillar mitochondrial (IFM) dysfunction resulting from type 1 diabetic insult has been linked to decrements in contractile function.
- Mitochondria-destined nuclear-encoded proteins constitute >99% of the organelles proteomic makeup and must be imported into the mitochondrion. The mitochondrial import process has been shown to be dysfunction in diabetic IFM (1)
- Mitochondrial phospholipid hydroperoxide glutathione peroxidase (mPHGPx) can directly reduce hydroperoxide groups of phospholipids, lipoproteins and cholesterol esters, potentially protecting mitochondrial phospholipid membranes from oxidative damage.
- The goal of this study was to determine whether overexpression of mPHGPx will lead to the preservation of mitochondrial import constituents, correct mitochondrial import dysfunction and preserve the mitochondrial proteome, specifically in the diabetic IFM

Methods

- FVB mice were made diabetic through multiple low-dose intraperitoneal injections (50mg/kg) of streptozotocin. Experiments were performed 5 week post diabetes onset.
- Cardiac tissue was excised and mitochondrial subpopulations were isolated by differential centrifugation and enzymatic digestion (2).
- Proteomic profiles were examined by LC-MALDI MS/MS using i-TRAQ™ technology (Protea Biosciences).
- Mitochondrial protein import assay was performed to compare protein import efficiencies of control and diabetic mitochondrial subpopulations.
- Canonical mitochondrial pathways and protein networks were analyzed using Ingenuity Pathway Analyses (IPA).
- Western blot analyses were performed to evaluate the content of essential mitochondrial import proteins and translocases.

Results

Proteomic Assessment. iTRAQ analysis revealed many proteins significantly decreased in the diabetic IFM as compared to control. Further, overexpression of mPHGPx in the diabetic hearts attenuated majority of changes. *P<0.05 for control vs. diabetic IFM and mPHGPx diabetic vs. diabetic IFM. Red = downregulated, Green = upregulated

Proteomic alterations in diabetic and mPHGPx diabetic IFM. (A) Total amount of proteins decreased solely in the diabetic IFM (left), common proteins decreased in diabetic IFM and preserved within mPHGPx diabetic IFM (middle), and proteins increased solely within the mPHGPx diabetic IFM. (B) Breakdown in approximate percentages of protein contents preserved in the mPHGPx diabetic heart IFM. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane

Mitochondrial canonical pathways. Identification of essential mitochondrial canonical pathways negatively influenced in diabetic IFM and preserved within mPHGPx diabetic IFM. Oxidative phosphorylation, Citrate cycle, fatty acid metabolism, and fatty acid elongation were negatively affected (green) by diabetes mellitus. Conversely, these pathways as well as pyruvate metabolism were preserved (red) within the mPHGPx diabetic IFM.

Mitochondrial protein networks. Identification of mitochondrial proteomic networks negatively influenced in diabetic IFM and preserved within mPHGPx diabetic IFM. Red indicates proteins positively enhanced in mPHGPx diabetic IFM. White indicates proteins unchanged by diabetes mellitus but are part of their respective networks.

Mitochondrial protein import. Effect of type 1 diabetes mellitus and mPHGPx overexpression on MitoGFP1 import in IFM. Cardiac mitochondrial subpopulations from control, diabetic, mPHGPx, and diabetic mPHGPx mice were isolated and incubated with 2µl of MitoGFP1 protein lysate at 1 and 2 minute time points. Representative Western blots from IFM (A) protein import assay. Graphical representation of mitochondrial protein import performed in control (C), diabetic (STZ), mPHGPx (M), and diabetic mPHGPx IFM (M + STZ) (B). Both time point percentages were averaged to show total import efficiency. Control for protein loading was confirmed by Ponceau staining. Values are presented as means ± SE; *P < 0.05 for IFM STZ vs. all IFM groups. N=5 per group.

Results

Mitochondrial protein import constituents. Western blot analysis of protein import constituents. Key proteins involved in mitochondrial protein import were assessed using Western blot analysis. Representative Western blot and densitometry analysis for Tom20 (A), Tim50 (B), MtHsp70 (C), and Tim23 (D) from control, mPHGPx, diabetic and diabetic MPHGPx IFM. Control for protein loading was confirmed by Ponceau staining. Values are presented as means ± SE; *P < 0.05 for mPHGPx diabetic vs. all groups; †P < 0.05 for IFM mPHGPX diabetic vs. diabetic. N=4 per group.

MtHsp70 protein complexing. Isolated mitochondrial subpopulations from control, diabetic, mPHGPx, and diabetic mPHGPx hearts were partially lysed in digitonin buffer and subjected to BN-PAGE. Protein complexes were transferred to nitrocellulose membrane and probed for MtHSP70. A complex of MtHsp70 and Timm44 exists at 119 kD (A). MtHsp70 content was analyzed through densitometric analysis (B). *P < 0.05 for IFM STZ vs. all IFM groups. N=4 per group.

Summary

- The mitochondrial proteome was significantly dysregulated in the diabetic IFM subpopulation and preserved in diabetic mPHGPx IFM subpopulation.
- The majority of the proteins/proteins networks influenced by diabetes mellitus and preserved within mPHGPx IFM were of IMM origin.
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References

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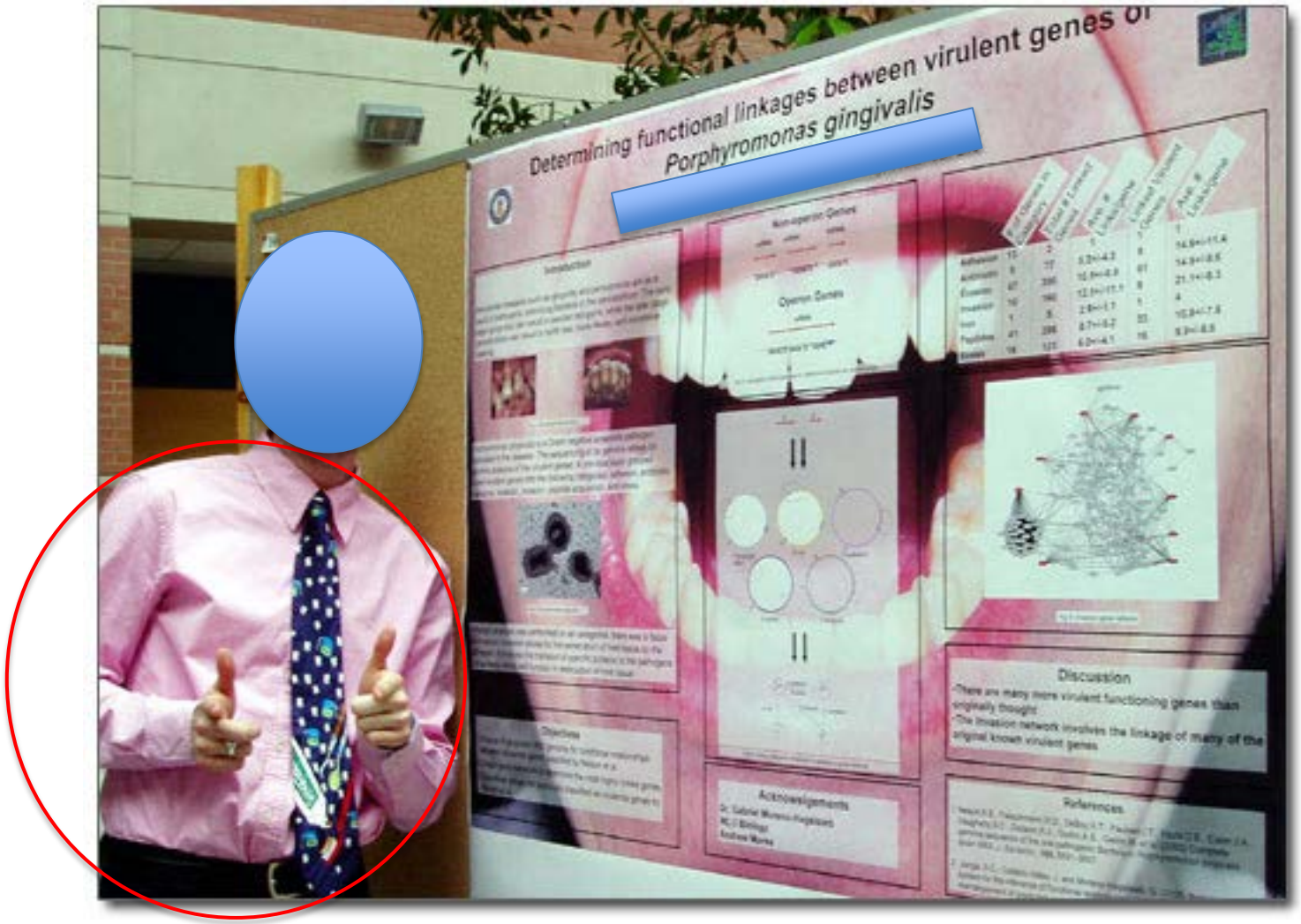
  - Too much condensed information/hard to explain
- Figures not clearly labeled



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O^6-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

MD ANDERSON CANCER CENTER ORLANDO

SUPPORTED BY THE CHARLES LEWIS INSTITUTE

Abstract

Endocrine therapies using anti-estrogens are least toxic and very effective for breast cancers, however, tumor resistance to tamoxifen remains a stumbling block for successful therapy. Based on our recent study on the involvement of the DNA repair protein MGMT in pancreatic cancer (Clin Cancer Res. 15, 6087, 2009), here, we investigated whether MGMT overexpression mediates tamoxifen resistance. Specifically, we determined whether administration of MGMT inhibitor [O^6-benzylguanine (BG)] at a non-toxic dose alone or in combination with the anti-estrogens (tamoxifen/fulvestrant) curtails human tamoxifen resistant breast cancer cell growth. Further, we also determined whether BG sensitizes breast cancers to tamoxifen using tamoxifen resistant cells.

MGMT expression was found to be increased in breast cancer cells relative to normal breast epithelial cells. Also, MGMT levels were significantly higher in tamoxifen resistant MCF-7 compared to the parent cells. Silencing of the ER-α expression using a specific siRNA resulted in augmentation of MGMT mRNA and protein levels by 2 fold. We also observed an inverse correlation between MGMT and p53 levels in breast cancer cell lines; moreover, p53 downregulation was accompanied by increased MGMT expression. Other experiments showed that BG alone or BG in combination with tamoxifen or fulvestrant decreased ER-α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the p21^{WAF} mRNA and protein expression significantly. BG inhibited tamoxifen resistant breast cancer growth in a dose-dependent manner and it also resensitized resistant breast cancer cells to anti-estrogen therapy (TAM/ICI). These combinations also enhanced the cytochrome C release and the PARP cleavage, indicative of apoptosis. In breast cancer xenografts, BG alone or a combination of BG with tamoxifen or fulvestrant caused significant tumor growth delay and immunohistochemistry revealed that BG inhibited the expression of MGMT, ER- α, ki-67 and increased p21^{WAF} staining. These findings suggest that MGMT inhibition may provide a novel and effective approach for overcoming tamoxifen resistance.

Introduction

Recent advances in breast cancer research have identified key pathways involved in the repair of DNA damage induced by chemotherapeutic agents. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism for therapeutic resistance and has a negative impact on therapeutic efficacy. A number of DNA-damaging alkylating agents attack the nucleophilic O^6 position on guanine, forming mutagenic and highly cytotoxic interstrand DNA crosslinks. The DNA repair enzyme O^6-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. MGMT is expressed constitutively in normal cells and tissues. In breast tumors, MGMT gene expression is elevated and levels are up to 4-fold higher than in the normal breast. Interestingly, it has been shown that tamoxifen accelerates proteasomal degradation of MGMT in human cancer cells. In 1991, Pegg, Moschel, and Dolan observed that O^6 benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both chloroethylating agents and methylating agents. In a series of important observations, they fully characterized the interaction between BG and AGT and its therapeutic impact. They showed that BG binds AGT, transferring the benzyl moiety to the active-site cysteine [29]. The reaction is very rapid and more potent than any other previously known AGT inhibitor. BG is not incorporated into DNA in living cells and reacts directly with both cytoplasmic and nuclear AGT. Because BG is a pseudosubstrate for MGMT which results in the covalent transfer of benzyl group to the active site cysteine, the MGMT protein is degraded after each reaction. This stoichiometric reaction mechanism effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing clinical trials in various cancers to increase the efficacy of alkylating agents.

Interestingly, several observations suggest an inverse correlation between the levels of MGMT and p53 tumor suppressor proteins where wild-type p53 suppresses transcription of human MGMT expression. Unfortunately, p53 function is often inactivated or suppressed in human cancers; therefore, restoration of wt-p53 activity is essential for the success of some treatments. However, whether or not this is mediated by suppression of MGMT expression has yet to be determined. To date, the cross-talk between MGMT and ER-alpha (and the link to p53 expression) has not been explored in drug (i.e., tamoxifen) resistant breast tumors. The anti-estrogen tamoxifen is the most commonly used treatment for patients with estrogen receptor positive breast cancer. Although many patients benefit from tamoxifen in the adjuvant and metastatic settings, resistance to this endocrine therapeutic agent is an important clinical problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is increased in TAM-resistant breast cancers and inhibition of MGMT by BG significantly improves TAM-sensitivity.

Results

Prolonged Treatment of Tamoxifen Increases MGMT Expression: We developed a tamoxifen resistant MCF-7 cell line by using prolonged treatment of tamoxifen on the parental ER-positive breast cancer cell line, MCF-7. Tamoxifen-resistant MCF-7 cells proliferate at rates similar to the parental MCF-7. Prolonged treatment of tamoxifen onto MCF-7 cells increased MGMT expression compared to parental MCF-7 cells by 2 fold (Fig.1).

Knocking Down ERα Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ERα and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ERα has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ERα using specific siRNA significantly reduced ERα protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows that silencing of ERα increases MGMT expression in these cells, and interestingly, the results in the right panel (Fig.2B) show increased MGMT mRNA levels were increased as assessed by qRT-PCR. These data suggest that ERα–mediated signaling functions to repress MGMT gene expression in breast cancer cells.

Transcriptional Regulation Between MGMT and p53: Previously, it was reported that p53 negatively regulates MGMT in breast cancer cells. Therefore, we addressed whether or not silencing the p53 enhances endogenous MGMT transcription. Tamoxifen resistant MCF-7 cells were transfected with either p53 siRNA (p53-KD) (Fig.2C) or MGMT siRNA (MGMT-KD) (Fig.2D) along with Non-specific siRNA (NS). MGMT expression was consistently increased in p53 knock down cells, with different experiments showing a ~ fold augmentation (Fig. 2A) and as expected, knocking down MGMT decreased MGMT transcription where as p53 mRNA levels were unaffected in MGMT knockdown cells (Fig.2D). These results confirm that p53 can regulate MGMT at the transcriptional level.

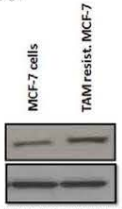


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were prepared, proteins were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

O^6-Benzylguanine Plays a Dual Role in Tamoxifen Resistant MCF-7 Cells: Contrasting with the experiments above, next, we studied whether or not knocking down MGMT has any effect on ERα transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ERα gene transcription was also reduced after MGMT silencing. (Fig.2E). These data demonstrate that BG has the ability to attenuate the not only the MGMT, but also the ERα transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

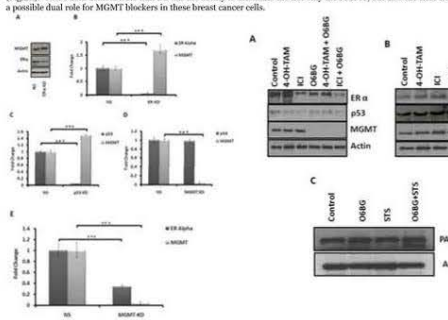


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total proteins were isolated and ERα and MGMT expression was determined by western blot analysis. MGMT protein was significantly increased in ERα knock down cells (B) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and MGMT and ERα transcription was determined by qRT-PCR. MGMT transcription was significantly increased in ERα knock down cells. (C)Total RNA was isolated from non-specific siRNA (NS) (100nM) and p53 siRNA (20 nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. (D) Total RNA was isolated from non-specific siRNA (NS) (100nM) and MGMT siRNA (100nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. There is an inverse correlation between MGMT and p53 in tamoxifen resistant breast cancer cells (C & D).

O6-Benzylguanine Modulates p53 Down-Stream Targeted Protein Expressions: Encouraged by the results reported, we investigated the effect of combination therapy on endogenous MGMT, p53, and ERα protein expressions. As expected, BG decreased MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ERα expressions. BG alone or in combination with tamoxifen or ICI decreased ER-α expression, whereas tamoxifen alone and ICI alone increased and decreased the same respectively (Fig.3A). p53 expression was slightly altered after ICI treatment. The reduction in p53 expression by ICI alone was reversed when BG was combined (Fig.3A). We investigated the effect of BG on proteins which are involved in cell cycle regulation, apoptosis in tamoxifen resistant breast cancer cells. Although treatments significantly increased the p21^{WAF} protein expression (Fig.3B). PUMA expression was also increased with these treatments. Hence, PUMA may have translocated to the mitochondria, cytochrome C is released (Fig.3B), and apoptosis was triggered in these cells in presence of combination therapy. PARP cleavage is seen in BG treated cells in presence of staurosporin as an indicative of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) and 48h post treatment 4-OH-TAM (1μM), ICI (1μM) either alone or in combination with BG. 24h post treatment cells were harvested and proteins were isolated and western blot analysis was performed. (A) ERα, p53 and MGMT expressions (B) Cytochrome C, PUMA and p21 was determined by western blot analysis (C) tamoxifen resistant MCF-7 cells were treated with or without BG for 48h and later treated with staurosporin (5 μM/L) for 6 hrs PARP cleavage was determined by western blot analysis.

O6-Benzylguanine Modulated Transcriptional Targets in Tamoxifen Resistant Breast Cancer Cells: The effect of combination therapy on endogenous MGMT mRNA levels wa aso studd. Quantitative real-time PCR (qRT-PCR) resulted that anti-estrogens (TAM/ICI) increased the MGMT expression while the combination therapy decreased it compared to control levels. ERα transcription was decreased compared to controls with all these treatments (Fig.4A). Surprisingly, p21 and PUMA mRNA was significantly increased in the presence of combination treatments (Fig.4B &C). These results suggests that p53 mediated target gene transcription was affected by the drug combinations in breast cancer cells (Fig. 3 & 4).

O6-Benzylguanine Enhances p21 Transcriptional Activity in Tamoxifen Resistant Breast Cancer Cells: In order to investigate the effect of BG on p53 function, we performed luciferase reporter assays. Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21 luc promoter construct in presence or absence of BG (target gene of p53). These results clearly demonstrate that BG significantly enhanced p21 transcriptional activity by 4-5 fold in these cells (Fig.4D).

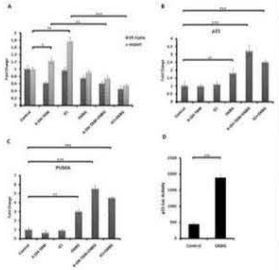


Figure 4. Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) for 48h and later 4-OH tamoxifen and ICI (1μM) was either alone or in combination with BG and 24h later cells were harvested and total RNA was isolated. (A) MGMT and ERα (B) p21 transcription (C) PUMA transcription was determined by qRT-PCR. 4-OH tamoxifen and ICI induces MGMT transcription. BG induced PUMA and p21 transcription. (D) Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21-luc constract and 6h later treated with BG and 24h later cells were harvested. p21 transcriptional activity was significantly increased by BG in these cells.

O6-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Increase Resistant Breast Cancer Cell Sensitivity to Anti-Estrogen Therapy (TAM/ICI): Detailed necropsy revealed that all the mice had tumors in the breast. The data summarized in Table 1 show the daily BG alone or in combination with twice weekly tamoxifen/ICI significantly decreased median tumor volume and weight as compared with that seen in tamoxifen/ICI treated and control mice. The combination of BG with tamoxifen or ICI produced the greatest decrease in median tumor volume as compared with control mice (83.99 mm^3, 9.33 mm3 (TAM+BG), respectively; p< 0.0001); (83.99 mm^3, 31.60 mm^3 (ICI+BG), respectively; p<0.0001). Tumor weight was also significantly reduced in mice treated with combination therapy as compared with control mice (81.23 mg, 22.30 mg (TAM+BG), respectively, p<0.0005); (81.23 mg, 51.57 mg (ICI+BG), respectively, p<0.0005). (Table.1). Body weight was not changed among all treatment groups as compared with control mice. No visible liver metastases were present (enumerated with the aid of a dissecting microscope) in all treatment groups.

Histology and IHC Analysis: We next determined the *in vivo* effects of BG (alone or in combination) with tamoxifen/ICI. Tumors harvested from different treatment groups were processed for routine histological and IHC analysis. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI exhibited a significant decrease in MGMT, ERα, ki-67 as compared with tumors treated with tamoxifen/ICI alone or control group. p53 expression was not much altered in these treatment groups. In sharp contrast, the expression of p21 was significantly increased in tumors from mice treated with BG either alone or in combination with tamoxifen/ICI. The images were analyzed by ImageJ (NIH) and MGMT, ERα, p53, p21 and ki-67 expressions were quantified by the ImmunoRatio plugin. (Fig.5).

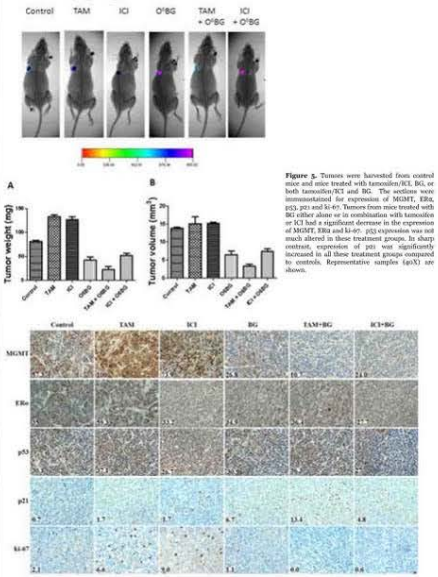


Figure 5. Tumors were harvested from control mice and mice treated with tamoxifen/ICI, BG, or both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ERα, p53, p21 and ki-67. Tumors from mice treated with BG either alone or in combination with tamoxifen or ICI had a significant decrease in the expression of MGMT, ERα and ki-67. p53 expression was not much altered in these treatment groups. In sharp contrast, expression of p21 was significantly increased in all these treatment groups compared to controls. Representative samples (40X) are shown.

Conclusions

1. In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O^6-methylguanine DNA methyltransferase (MGMT).
2. Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (tamoxifen and ICI 182,780).
3. We also observed that combination therapy of anti-estrogens and MGMT blockers not only overcame the MGMT derived drug (tamoxifen and ICI) resistance but also increased the efficacy of anti-estrogen therapy by decreasing estrogen receptor expression and restoration of the functional activity of p53 in tamoxifen-resistant breast cancer cells.
4. Combination therapy inhibited tamoxifen resistant breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program 09B80-10 for their funding of this project.

- Wall of text. Who will read this?

VITAMIN C: THE MULTIFUNCTIONAL ANTIOXIDANT

Rice University

BACKGROUND

Vitamin C (Ascorbic Acid) is an essential nutrient discovered in 1932 by Albert Szent-Györgyi, who isolated the antiscorbutic factor as pure crystalline material from lemon juice. In the past 25 years, much of the vitamin's biochemical functions have been elucidated, inducting vitamin C to the treatment of viral infections, diabetes, and even cancer prevention. Today, scientists' growing knowledge of ascorbic acid uncovers the significance of its antioxidant property, making its organic synthesis one of high demand for research and public consumption.

ANTIOXIDANT PROTECTION

- Stability of antioxidant free radicals
- Resonance delocalization
- Further oxidation of antioxidant radicals
- Reduction of radical species

REACTION MECHANISMS

Antioxidant Radical Formation

$R\cdot + AH \rightarrow RH + A\cdot$

$RO\cdot + AH \rightarrow ROH + A\cdot$

$ROO\cdot + AH \rightarrow ROOH + A\cdot$

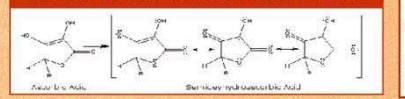
Radical Chain Termination

$R\cdot + A\cdot \rightarrow RA$

$RO\cdot + A\cdot \rightarrow RA$

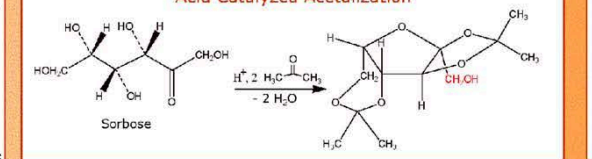
$ROO\cdot + A\cdot \rightarrow RA$

ANTIOXIDANT RADICAL STABILITY

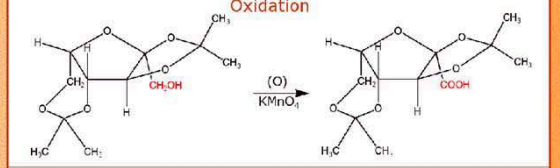


ORGANIC SYNTHESIS OF VITAMIN C

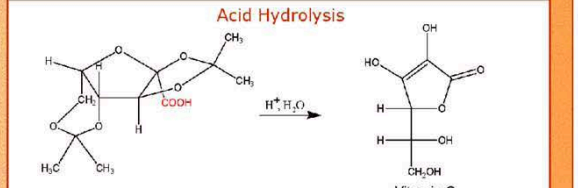
Acid Catalyzed Acetalization



Oxidation



Acid Hydrolysis



CHEMICAL FUNCTIONS

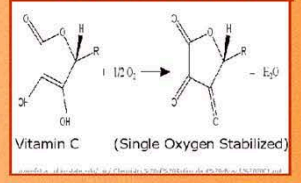
Antioxidant

- Hydrogen donation to lipid radicals
- Removal of molecular O
- Quenching of singlet O
- Regeneration of tocopherol radicals

Prooxidant

- Reduction of Fe^{+3} to Fe^{+2}

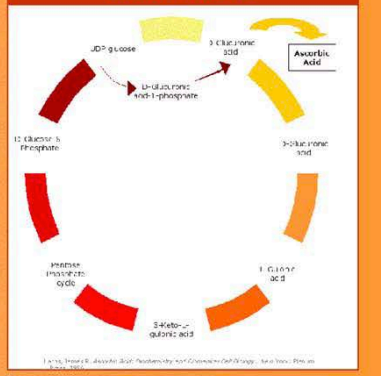
OXYGEN SCAVENGER



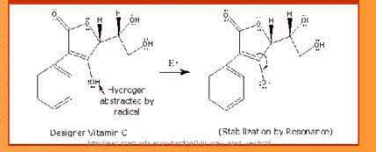
BIOLOGICAL BENEFITS

- Defense against common cold
- Collagen formation
- Absorption of inorganic iron
- Metabolism of folic acid, amino acids, and hormones
- Protection of DNA, cell membranes, and critical molecules from radicals

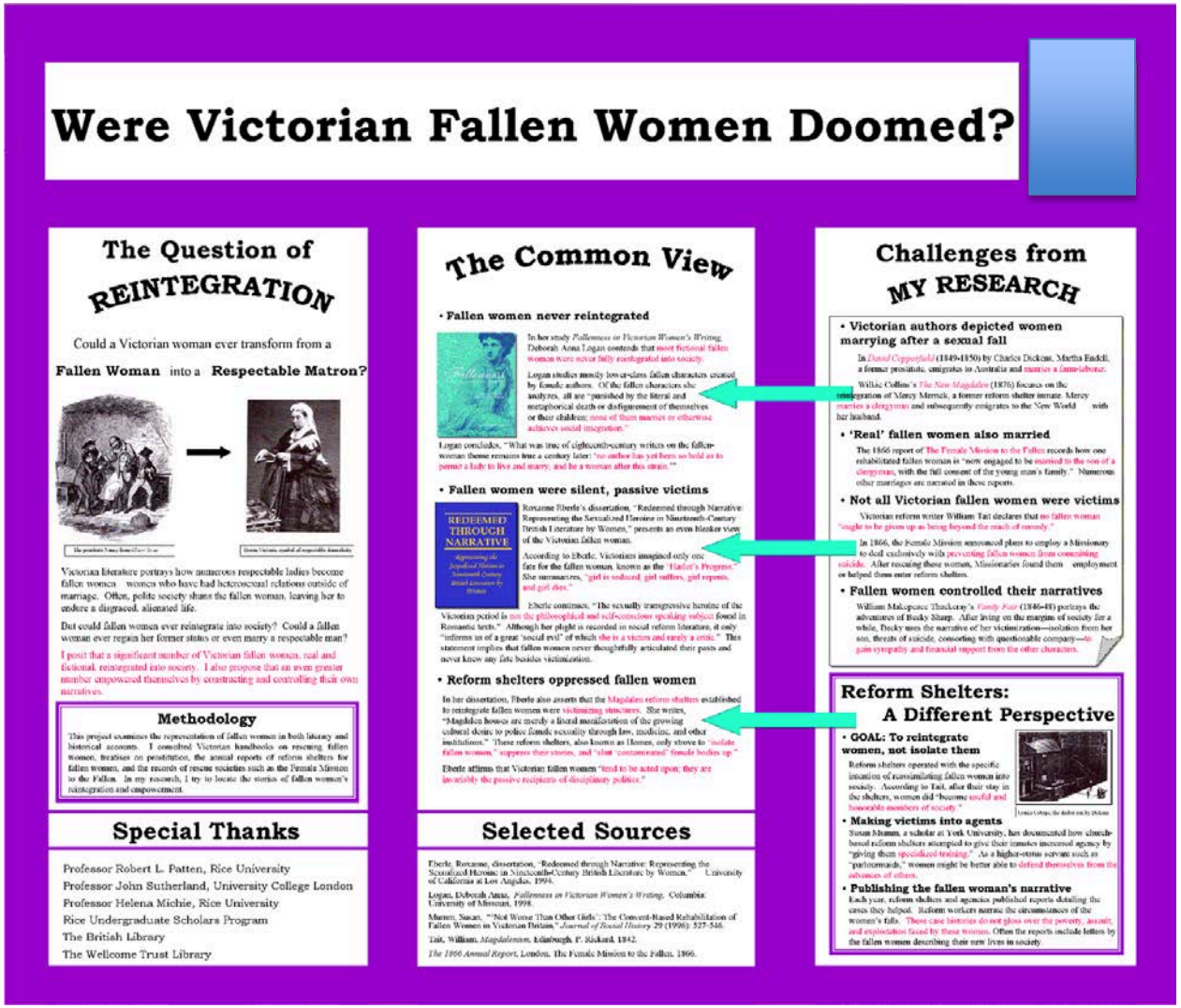
BIOSYNTHESIS



DESIGNER VITAMIN C



- Logical Progression?
- Not enough writing (legends?)
- Conclusions?



- Distracting color
- Confusing layout with the arrows. Where do I start or finish?

Giving the Presentation

- Has anyone given a presentation?
- Difficulties encountered?
- Things you learned that you didn’t know prior to presentation?

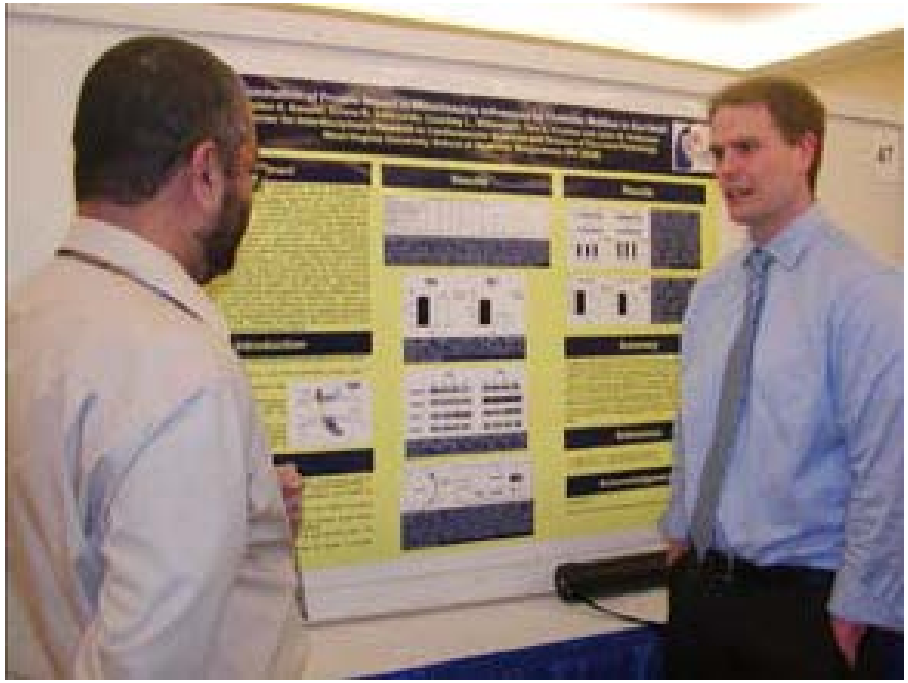
Giving the Presentation

- Time frame: Subjective (5-10 minutes)

- Positioning/Posture:
  - Eye Contact

Correct

Incorrect



What is Important?

- **The presentation is a "conversation"**
  - Try to read as little as possible from your poster
- **Start with something easy to get the ball rolling** (Examples)
  - Introduce yourself
  - Read the title of your poster
  - Start with a basic sentence like……
    - Our lab is interested in……..
- **Skip the abstract**
  - For someone to get a quick overview of your presentation if you aren't present
- **Go directly into the Introduction**
  - This is where these key sentences act as a guide

Navigating the Introduction

- The introduction can make or break your presentation

- **Key components** –

  1. Statement of problem/issue you are working on
  2. Clear and concise summary of relevant literature
  3. Goal of Study
  4. Hypothesis

Navigating Methods

- Don’t spend a large amount of time explaining the minutia of each methods.
  - Want to be able to give an solid explanation as to why you chose certain methods

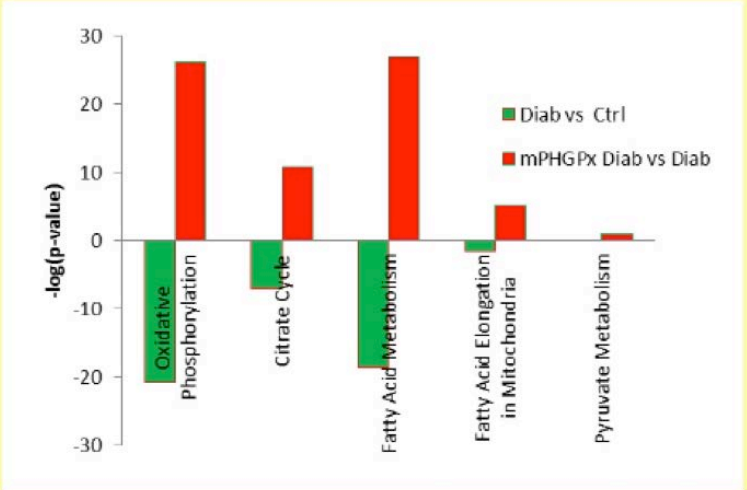
> • **Western blot analyses were performed to evaluate the content of essential mitochondrial import proteins and translocases.**

- Exceptions:
  - It is a novel/complex methodology critical to the poster
  - You have a timeline diagram
  - These can act as a nice transition into your results section

Navigating Results

- Make sure your figure progression (fig1-6) are logical and build upon on another
- Give summarized version of the results. Explain:
  - HOW you performed the experiment
  - WHY you performed the experiment
  - WHAT the experimental conclusion was

- Judges are looking for
  - Strong material
  - Material supporting purpose of study
  - Strong conclusions



Mitochondrial canonical pathways. Identification of essential mitochondrial canonical pathways negatively influenced in diabetic IFM and preserved within mPHGPx diabetic IFM. Oxidative phosphorylation, Citrate cycle, fatty acid metabolism, and fatty acid elongation were negatively affected (green) by diabetes mellitus. Conversely, these pathways as well as pyruvate metabolism were preserved (red) within the mPHGPx diabetic IFM.

Navigating Summary/Conclusion

- Home stretch
- Reiterate what was discovered in the results
  - Don't be afraid to use phrases like
    - This is a unique finding……
    - We uncovered a novel finding……..
    - Our group was the first to…..
- Always end with a broad statement that gives strength to your findings/research

- **These results suggest that mPHGPx is capable of preserving the IFM proteome through a mechanism of nuclear-encoded mitochondrial protein import preservation during a type 1 diabetic insult.**

Final Thoughts

- Questions? You'll get them
  - Answer enthusiastically and to the point
  - Some people will not know your field
    - Speaking in "layman's terms" is beneficial. If they probe further, then hit them with the scientific jargon.
  - It's OK to not know an answer. It's going to happen.
  - Redirect them to what you do know
    - "That's a great question. I'm not sure there's one simple answer although……"
    - "Currently, the literature is not clearly define, however in this model….."

Thanks and Good Luck!

- Questions?

What Makes a Good Scientific Question?

Philip Ryan, PhD
Director of Student Services
NIH Graduate Partnerships Program
Office of Intramural Training and Education

Office of Intramural TRAINING & EDUCATION
NATIONAL INSTITUTES OF HEALTH

Biology

- What is the question?
  - What is Biology?
  - What's the answer?
    - The Study of Life
- What are the next questions?
  - What is life?
  - How do we study it?
- Life is defined by a set of criteria
  - Once we know that criteria, we can ask the question…
  - "Is X alive?

What Makes a Good Question?

- Has not already been answered!
  - Background information is paramount
  - Literature searches, database searches
  - Reading scientific papers

- Can be answered
  - With the resources you have
    - Time, technology, reagents, expertise, etc.
  - Conclusively
    - While some evidence may suggest that prehistoric toads liked softer lily pads to more coarse ones….one may never know for sure.

Relevance

- So what?
  - How does it relate to the big picture?
  - Who will be impacted?

- Builds on what is already known
  - That is why we publish…so others can add to it
    - Background information is important
  - If there is previous work, it is likely important to someone

Hypothesis Driven

- There is reason to believe that the answer is X?
  - Builds on what is already known
    - Background information: notice a pattern yet?
  - Logically fits with what is understood
- Proteins Y and Z interact. Protein Z has been found in the nucleus.
    - Hypothesis: Protein Y can be found in the nucleus.
    - Question: Is Protein Y in the nucleus under the same conditions as when Protein Z is in the nucleus?

Different Approaches

- More than one way to approach answering the question.
  - Sometimes your first approach does not give you an answer either way
  - Answering a question only one way, often is misleading
- Different approaches let you adjust the question as you go along
  - A “no” to the original question can be redirected to a related question to answer

Further Questions

- Good scientific questions lead to more questions when they are answered
- If “no” means the end of the project…then its not a good question
  - Note: That does not mean it should not be asked and answered! Just not something to base a project on

Answers lead to Questions

- Proteins Y and Z interact. Protein Z has been found in the nucleus and affects transcription of gene B.
  - Hypothesis: Protein Y can be found in the nucleus.
  - Question: Is Protein Y in the nucleus under the same conditions as when Protein Z is in the nucleus?
    - If yes, hypothesis two: Protein Y can affect transcription of gene B
    - Question: Does Protein Y affect transcription of gene B?
      - If yes…how?
      - If no, hypothesis three: Protein Y can affect the transcription of other genes
      - Question: Does Protein Y affect the transcription profile in cell X?

Animal Models

Kimberly Boelte

June 26, 2012

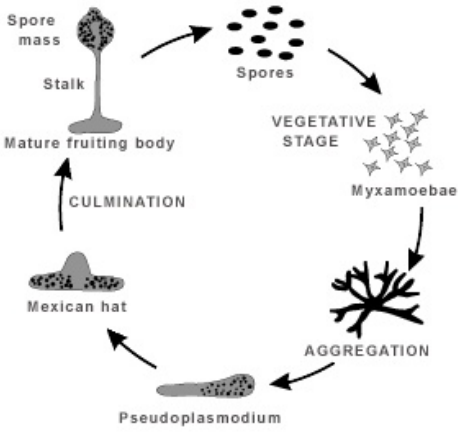
What is a model organism?

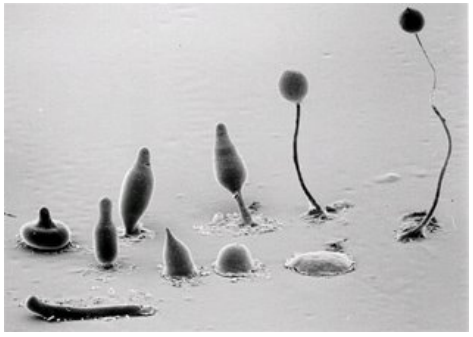
- Definition – non-human species that is used to study biological processes with the thought that any discoveries made can be applied to other species, particularly humans

- Why are they important?

Non-animal models

- *Dictyostelium discoideum*
  - Slime mold (Protist)
  - Used to study cell-cell communication, differentiation, cell death, and migration
  - Pros
    - Easy to grow in lab
    - Reproduces quickly
    - Genome has been sequenced
  - Cons
    - Not animal cells
    - How analogous to human cells?





http://www.sciencemusings.com/2006/08/beauty-of-slime.html

Non-animal models

- Yeast
  - *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*
  - Used to study signaling, cell cycle, DNA damage, DNA replication, etc
  - Pros
    - Easy to cultivate
    - Quickly multiplies
    - Easy to manipulate genetically
    - Genomes are sequenced
  - Cons
    - Many differences from mammalian cells, so how analogous to humans?

Animal Models

- Necessary to study biological processes involving interactions of different types of cells, or different organ systems, etc.
- Often used to model diseases
- Can be classified in various ways
  - Vertebrate vs invertebrate
  - Mammalian vs non-mammalian

Types of animal models

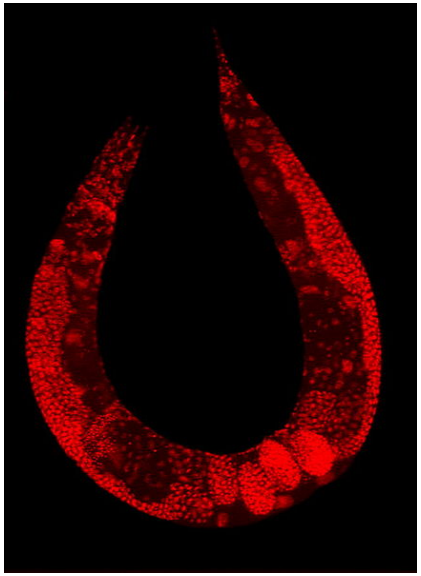
- Non-mammalian
  - Worms
  - Fruit flies
  - Zebrafish
  - Lampreys
  - Frogs
- Non-human primates
  - Rhesus macaques
  - Chimpanzees
  - Baboons
- Mammalian, non-primate
  - Rats
  - Mice
  - Guinea pigs
  - Hamsters
  - Rabbits
  - Cats
  - Dogs
  - Pigs

Animal Models

- Which model should you use?
- What should you consider when making this decision?
  - Ideally, you should use the lowest organism possible.
  - Budget
  - Available facilities
  - Does the organism have the genes you are interested in?
  - Is the resulting disease similar to that seen in humans?

Worms

- *Caenorhabditis elegans*
  - Nematode
  - Used to study genetics, development, physiology, cell death
  - Pros
    - Easy to culture
      - Can be frozen
    - Are transparent, so can watch cells inside
    - Easy to manipulate genes with RNAi
    - Genome is sequenced
  - Cons
    - Invertebrate
    - Not as complex as mammal



http://en.wikipedia.org/wiki/Caenorhabditis_elegans

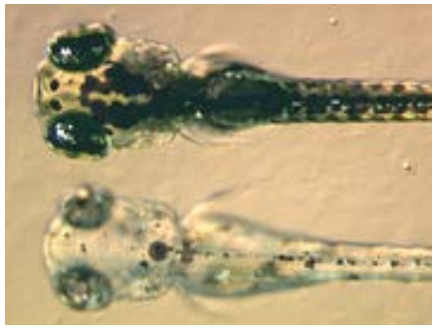
Fruit Flies

- *Drosophila melanogaster*
  - Used to study genetics, development, behavior
  - Pros
    - Easily grown in lab
    - Multiplies quickly
    - Easy to mutate
    - Genome has been sequenced
    - Many homologues with human genes
  - Cons
    - Invertebrate
    - Not mammalian

http://en.wikipedia.org/wiki/Drosophila_melanogaster

Zebrafish

- *Danio rerio*
  - Used to study development, toxicology, genetics, regeneration, etc
  - Pros
    - Easy to culture in lab (aquariums, many eggs at once)
    - Do not take as much space as mice
    - From egg to juvenile development is rapid
    - Fertilized eggs are transparent, as are juvenile fish
    - Sequenced genome
  - Cons
    - Not mammalian



http://en.wikipedia.org/wiki/Zebrafish

Frogs

- *Xenopus laevis*
  - Used to study development, DNA replication, cell cycle
  - Pros
    - Easy to house and breed
    - Large oocytes
    - Large embryos
    - Oocyte extract can support DNA replication outside of a cell
  - Cons
    - 1-2 years to mature
    - Not mammalian

http://en.wikipedia.org/wiki/African_clawed_frog

Rodents

- Most commonly used type of model animal
- Breed pretty quickly, with lots of babies
- Mature quickly
- Require a relatively small amount of space to maintain

http://taylorlifescience.pbworks.com/w/page/19600874/Hamsters

http://animal.discovery.com/guides/baby-animals/mammals/guinea_pig.html

http://www.isgtw.org/feature/mice-men

http://jbwhisler.wordpress.com/zoology/

Mice

- *Mus musculus*
  - The archetypal animal model
  - Used for pretty much any type of study
  - Pros
    - Inbred and outbred strains
    - Many different strains, some bred for specific disease studies
    - Breed quickly (every 19-21 days), large litters (~6-8 pups)
    - Many knockouts and transgenics already made
    - Smaller than non-human primates (easier and cheaper to work with)
    - Genome is sequenced
    - Quite homologous to humans
  - Cons
    - More expensive than previous models
    - Do not model human disease as well as we would like

http://nsatng.wordpress.com/200
02/19/obesity-americas-growing-
epidemic/

http://en.wikipedia.org/wiki/House_Mouse

Rats

- *Rattus norvegicus*
  - Used for many types of studies
  - Pros
    - Different strains, inbred and outbred
    - Reproduce quickly, large litter sizes
    - Smaller than non-human primates (easier and cheaper to work with)
    - Bigger than mice
    - Genome sequenced
  - Cons
    - Not as easy to genetically manipulate as mice
    - Bigger than mice

http://en.wikipedia.org/wiki/Laboratory_rat

Rabbits

- *Oryctolagus cuniculus*
  - Used to study respiratory diseases, toxicology, antibody production, etc.
  - Pros
    - Small mammal
    - Breed quickly
    - Less expensive to work with than non-human primates
    - Sequenced genome
  - Cons
    - More expensive to work with than mice or rats

http://www.nwabr.org/sites/default/files/learn/animalresearch/Lesson_2_Science_and_Ethics_of_Animal_Research-NWABR.pdf

Dogs

- *Canis lupus familiaris*
  - Used for studying respiratory and cardiovascular systems, diabetes, conditioning/behavioral responses, surgical techniques
  - Pros
    - Organ systems are larger
    - Interact with researchers
    - Sequenced genome
  - Cons
    - More expensive to work with
    - Do not breed as quickly as rodents

http://www.nwabr.org/sites/default/files/learn/animalresearch/Lesson_2_Science_and_Ethics_of_Animal_Research-NWABR.pdf

Non-human primates

- Used for behavioral, developmental, vaccine and infectious disease, and drug safety studies (to name a few)
- Pros
  - Most like humans
  - Some have sequenced genomes
- Cons
  - Expensive!
  - Require a lot of care
  - Breed slowly
- Several species in use
  - Rhesus macaques (*Macaca mulatta*)
  - Chimpanzees (*Pan troglodytes*)
  - Baboons (*Papio hamadryas*)

http://www.nwabr.org/sites/default/files/learn/animalresearch/Lesson_2_Science_and_Ethics_of_Animal_Research-NWABR.pdf

Rules and Regulations

- Animal Welfare Act and Regulations
  - Applies to all U.S. research with dogs, cats, rodents (except mice and rats), rabbits, and non-human primates
  - Involves regulations for procurement, handling, treatment, and transportation
  - Enforced by USDA
  - http://awic.nal.usda.gov/government-and-professional-resources/federal-laws/animal-welfare-act

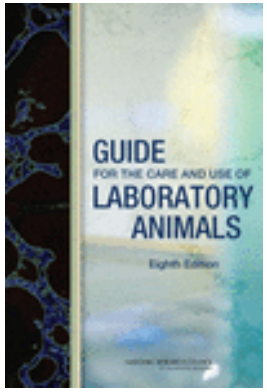
Rules and Regulations

- PHS Policy on Humane Care and Use of Laboratory Animals
  - Applies to the use of live, vertebrate animals (including mice and rats) in any activity supported or conducted by agencies of the Public Health Services
  - Oversight of policies by Office of Laboratory Animal Welfare
  - Requires PHS-funded institutes to establish and maintain an Institutional Animal Care and Use Committee
  - http://grants.nih.gov/grants/olaw/references/phspol.htm

Rules and Regulations

- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training
  - 9 principles forming the ethical foundation of U.S. animal research
  - Emphasizes the minimization and alleviation of pain or distress and the use of alternatives when possible

- Guide for the Care and Use of Laboratory Animals
  - Written by experienced scientists and veterinarians
  - Widely accepted as the authoritative reference
  - PHS Policy requires use of the Guide



Rules and Regulations

- Animal Care and Use Committee Policies and Guidelines
  - Required by AWA and PHS Policies
  - Specific to the institution

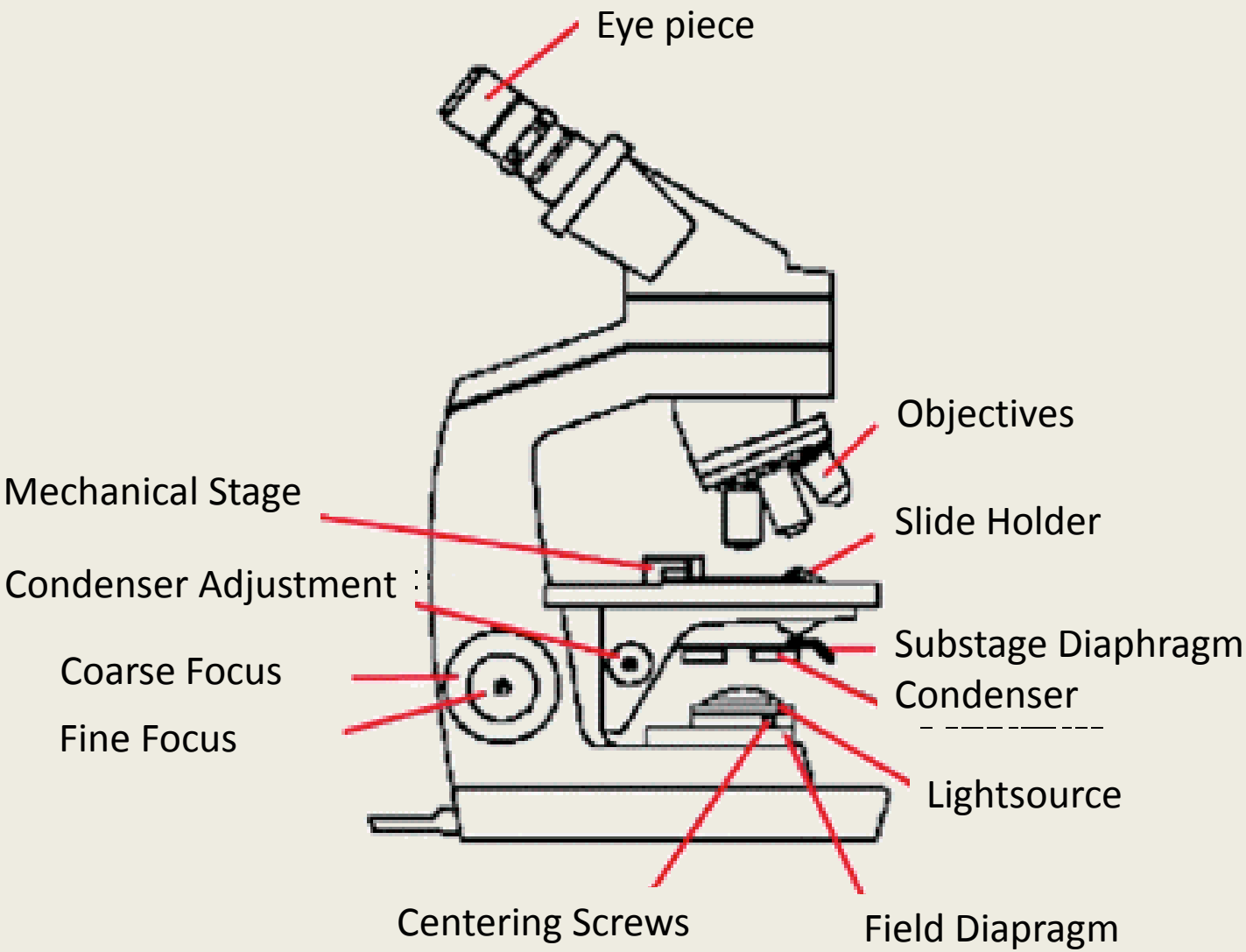
- AAALAC
  - Association for the Assessment and Accreditation of Laboratory Animal Care International
  - Voluntary
  - http://www.aaalac.org/

Summary

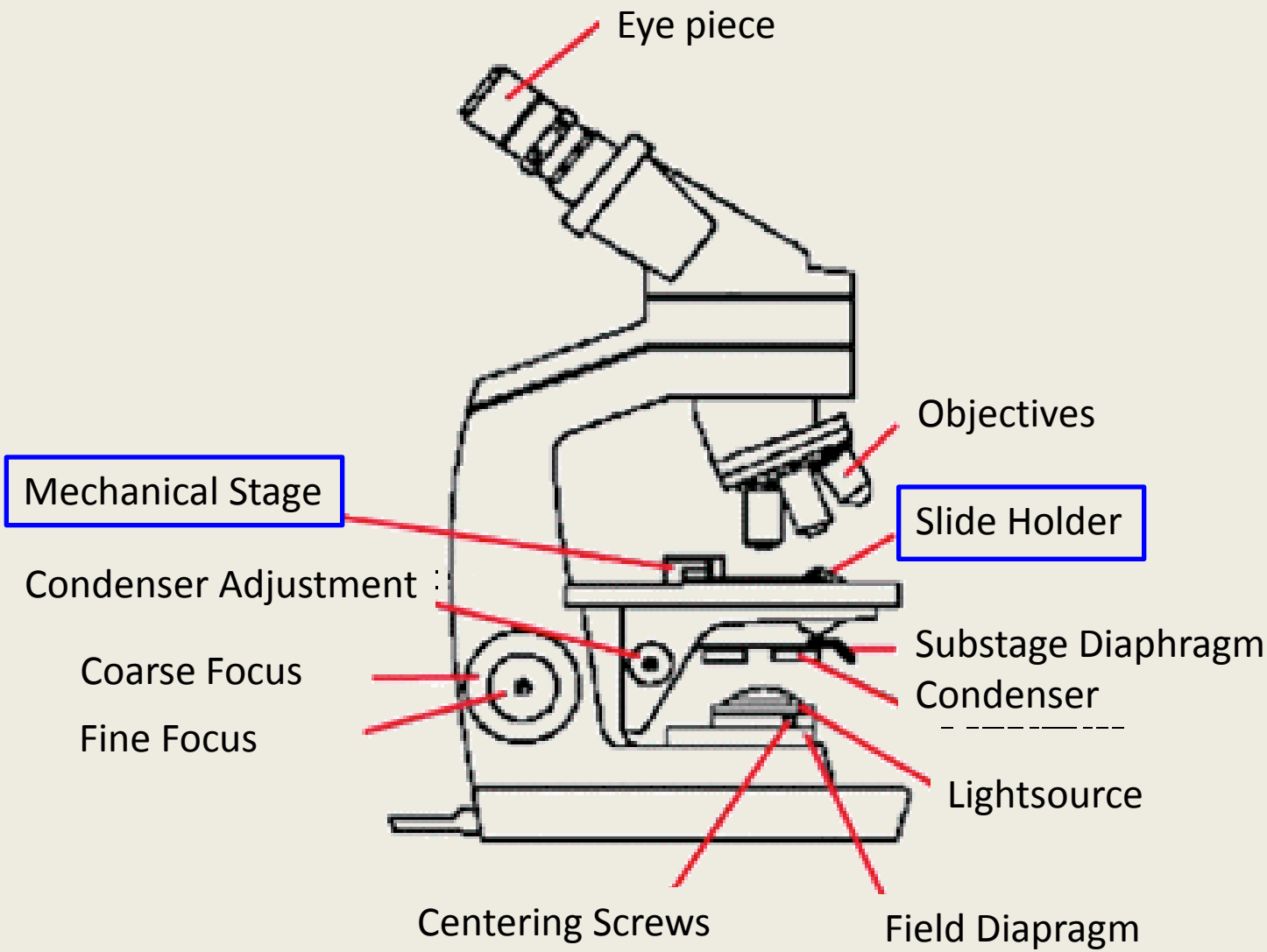
- Animal models are an important aspect of biomedical research.
- Several models are in use, and special care goes into choosing the appropriate model.
- There are rules and regulations about the proper care and use of animals in research that must be followed.

Introduction to Microscopy

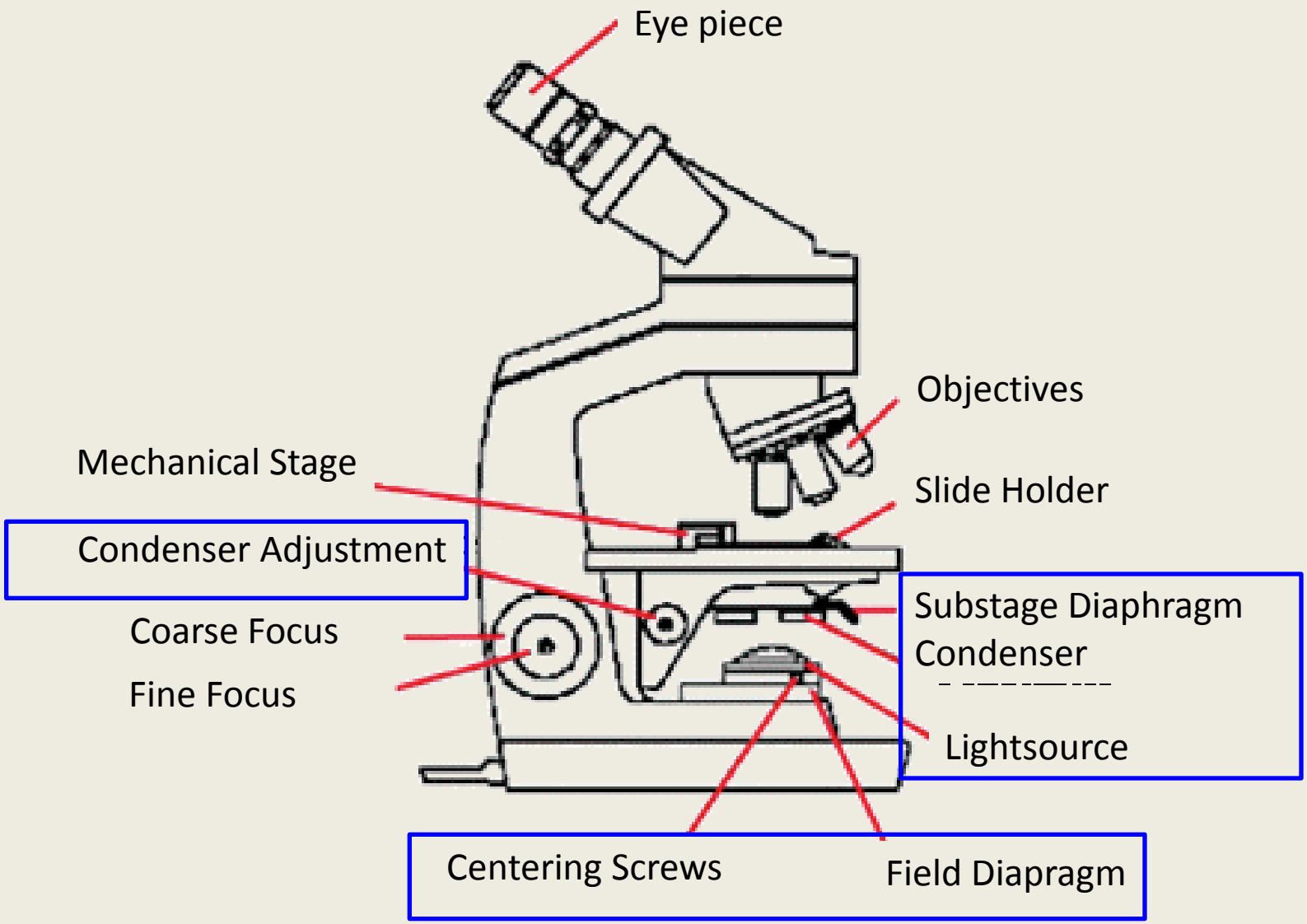
The Microscope



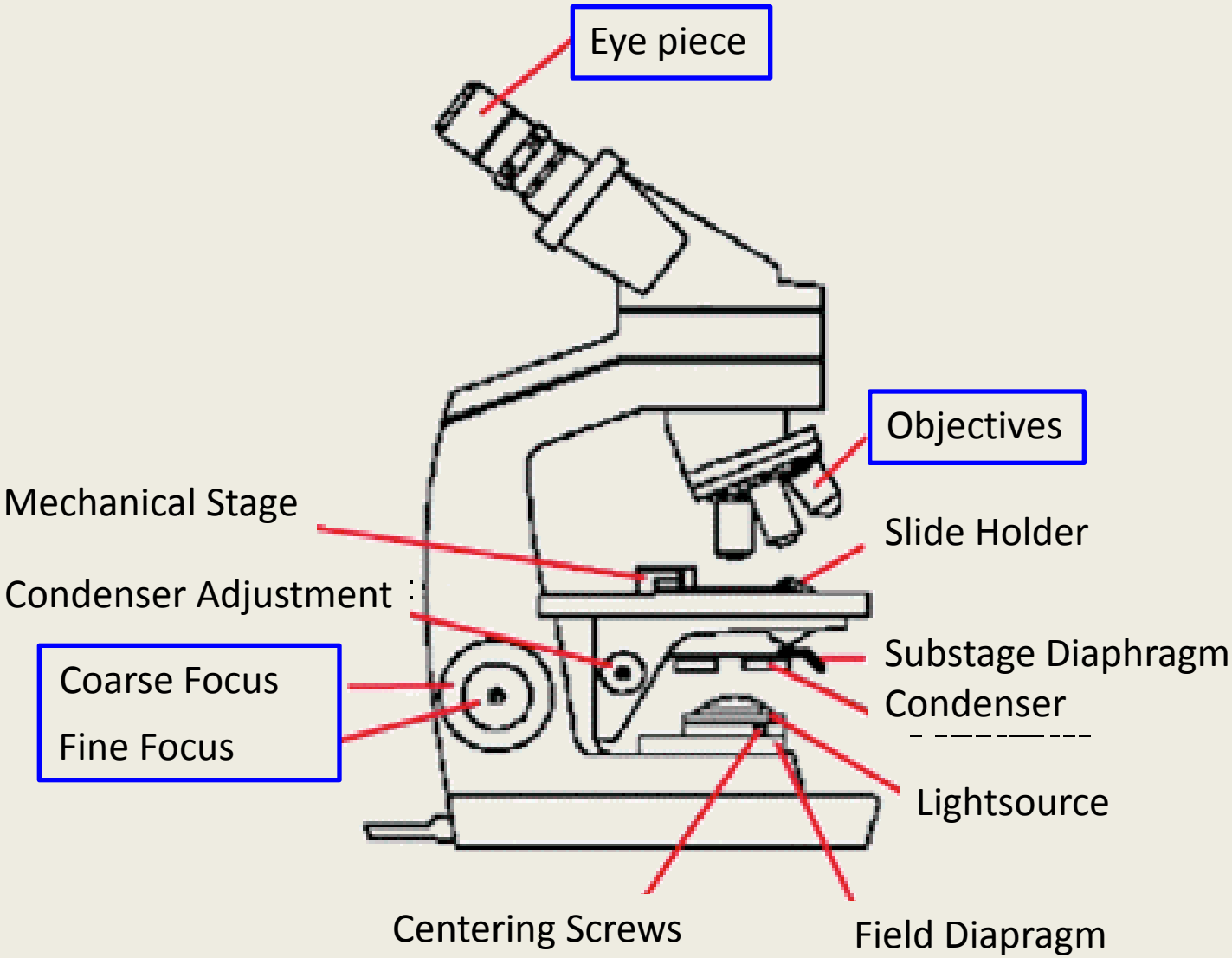
The Microscope



The Microscope



The Microscope



The First Microscopes

1664

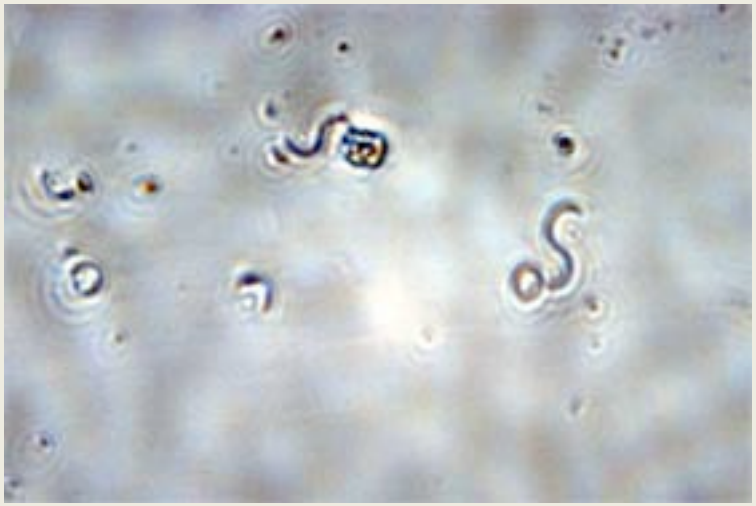
Robert Hooke

1684

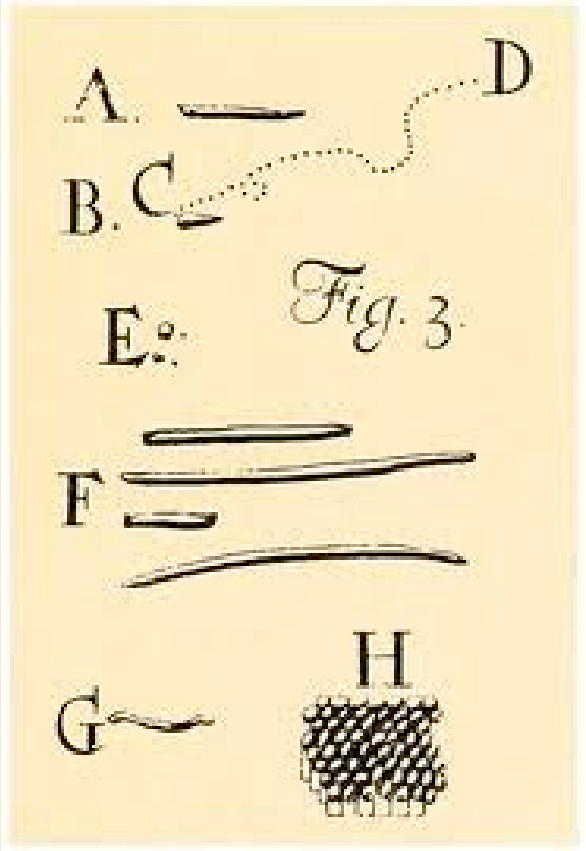
Antonie van Leeuwenhoek

The Conclusion of Early Microscopy?

Wow, small stuff!

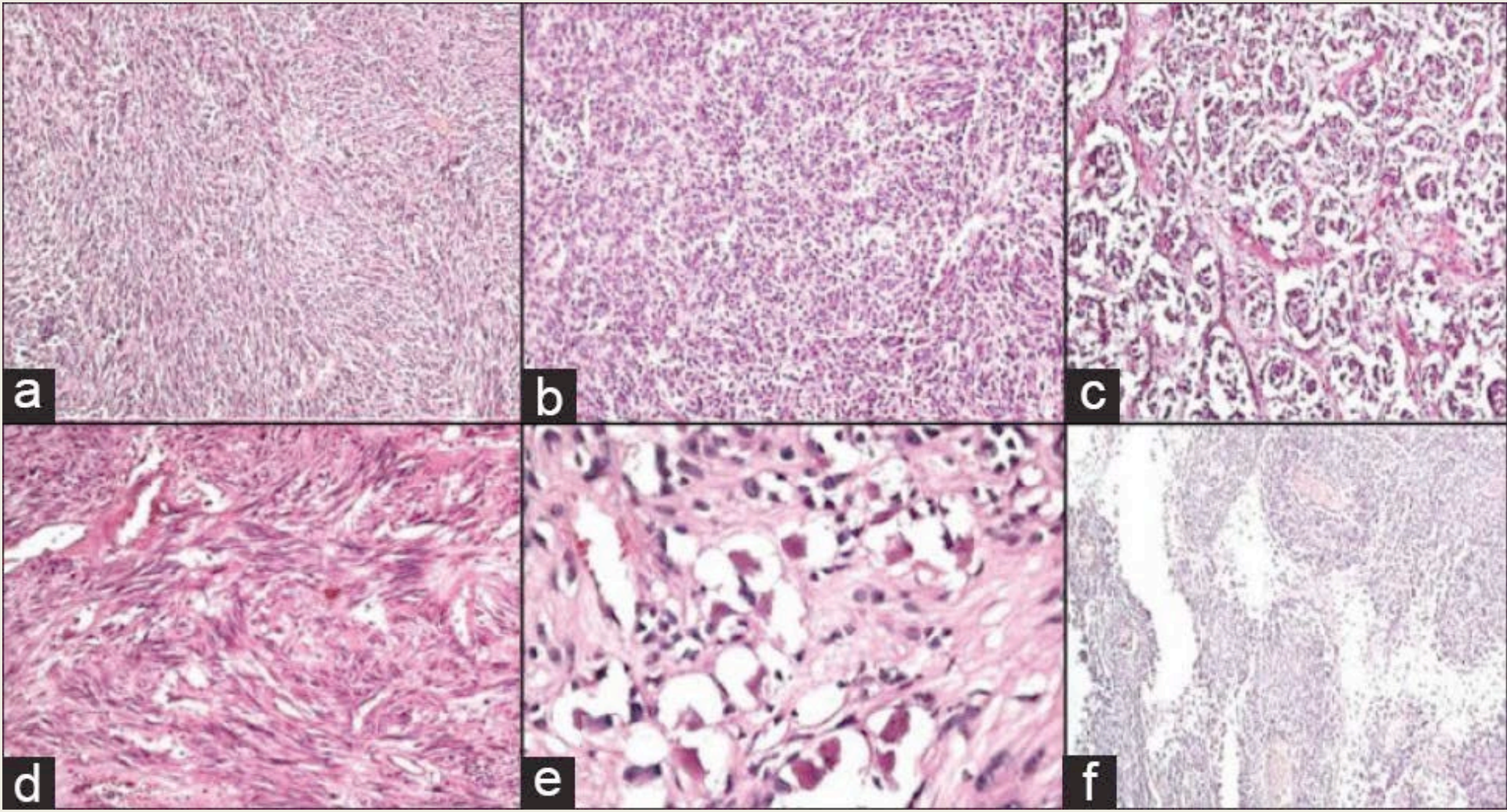


Spirillia



Modern Light Microscopes

Histological Staining



Hematoxylin and Eosin stain

Ravikumar *et al.* Indian J Cancer 2011

Fluorescence Microscopy

Typically used for imaging regions and structures within a cell

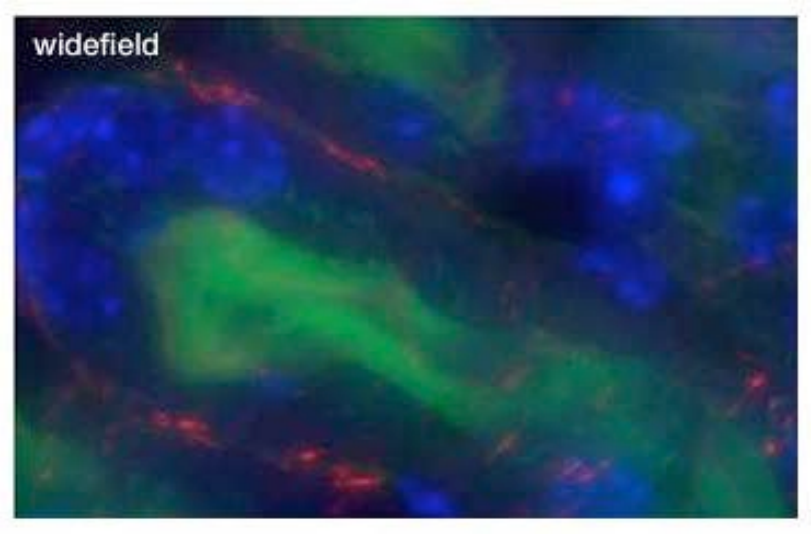
Fluorescence comes from:

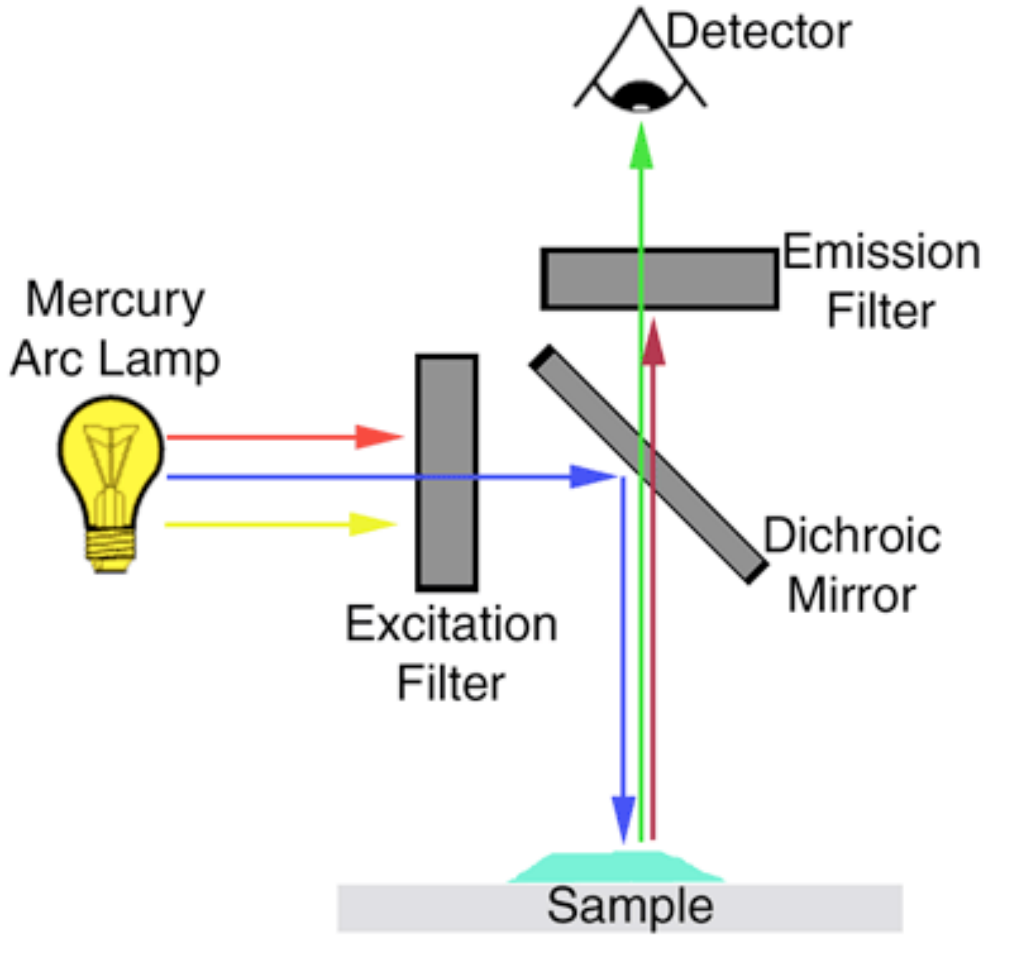
- fluorescent dye
- fluorophore conjugated to an antibody (fix and permeabilise)
- fluorescent fusion protein (live cells can be imaged)

RESOLUTION!

Fluorescence Microscope

Fluorescence Microscopy

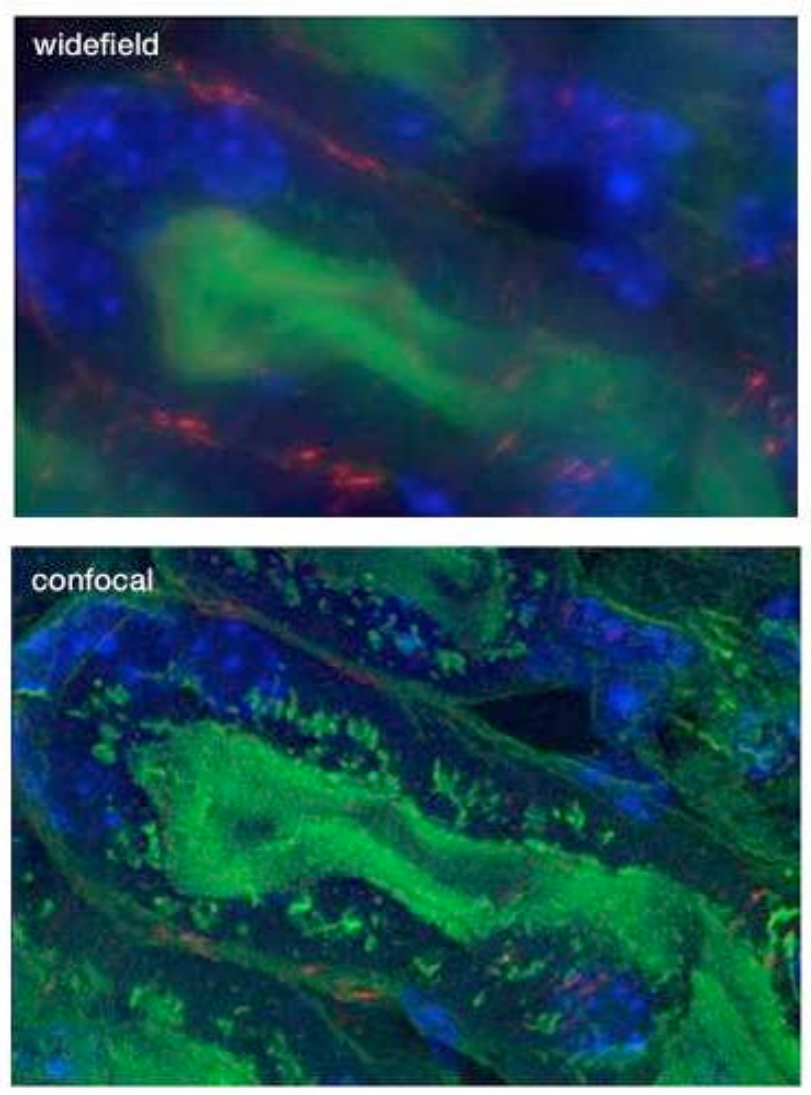


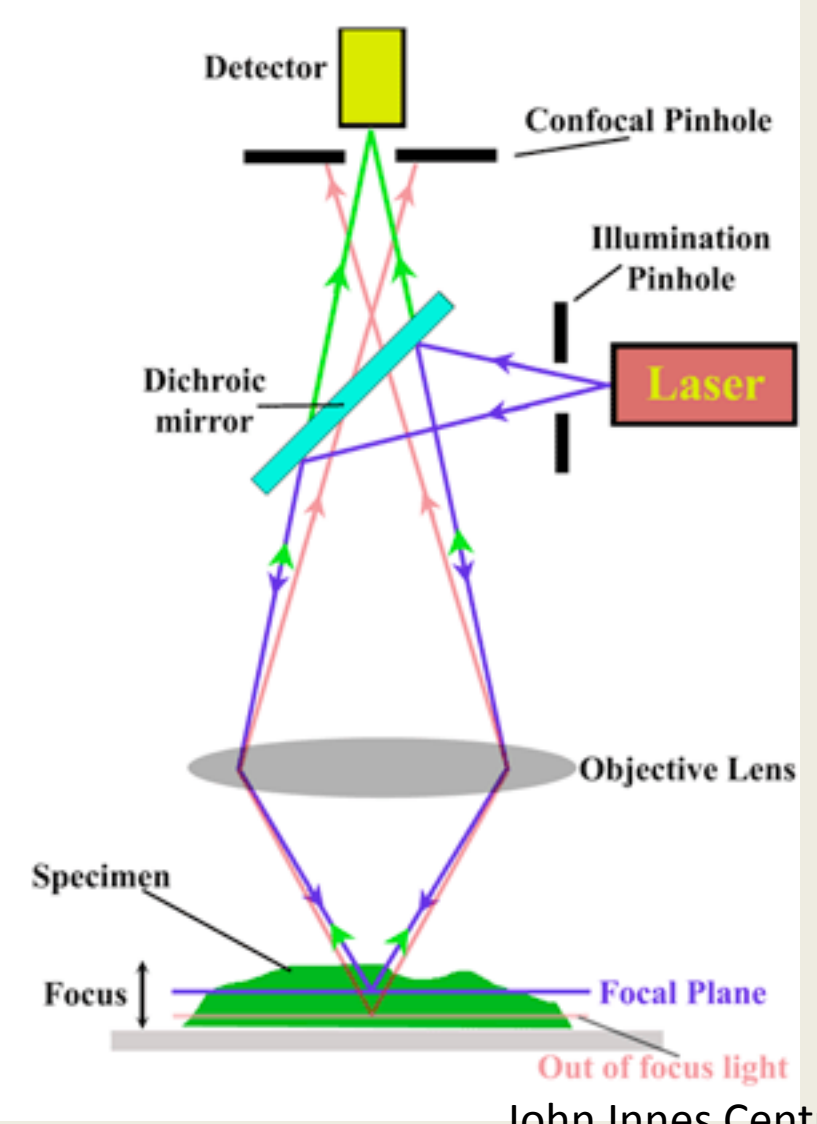


John Innes Centre

Confocal Microscope

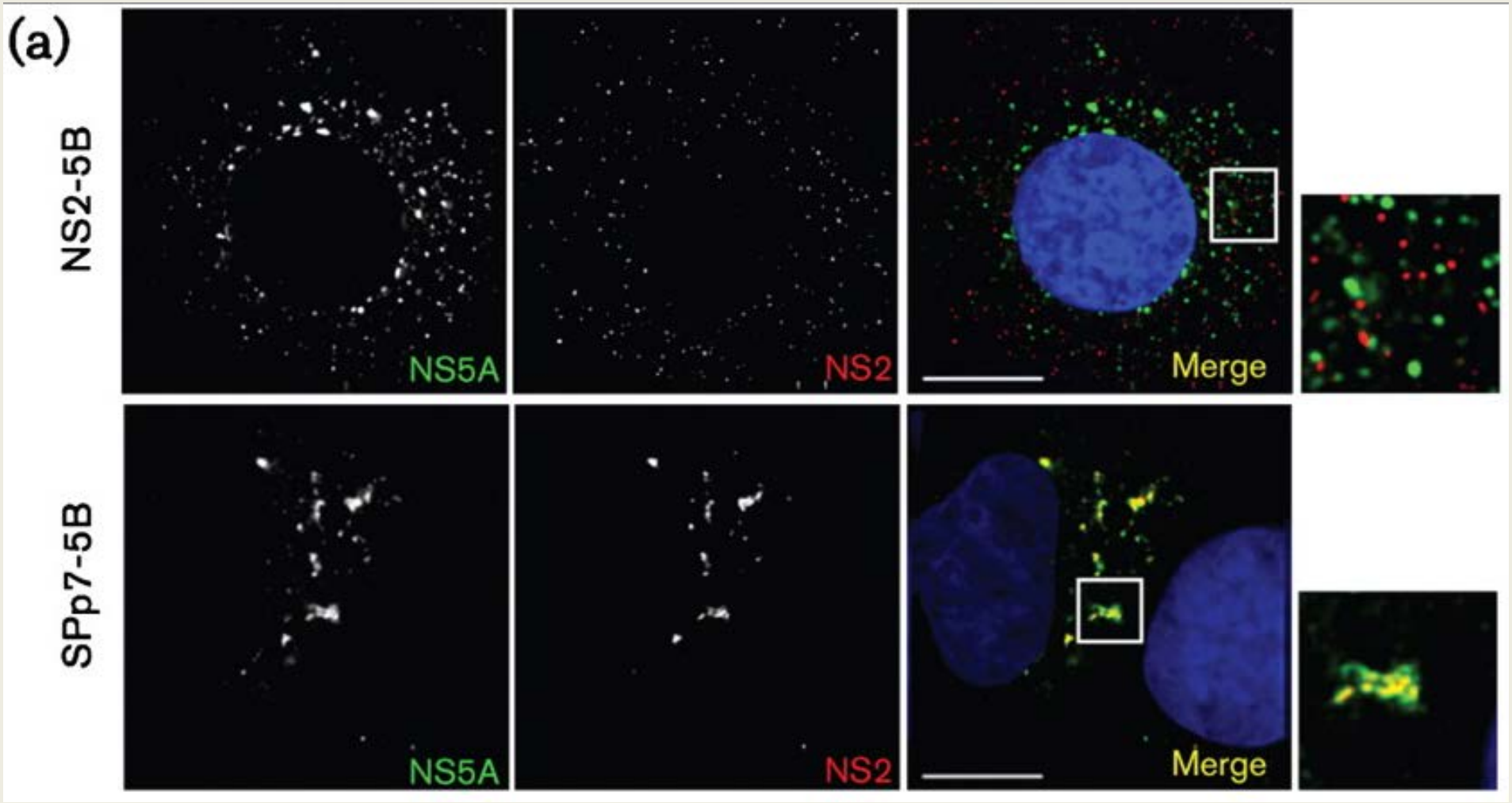
Confocal Microscopy





John Innes Centre

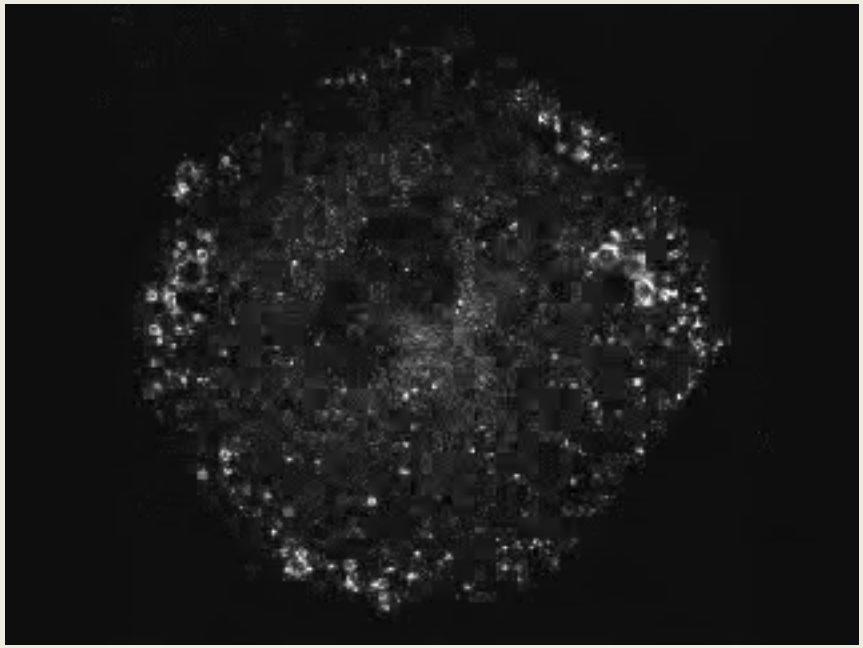
Colocalization Studies



Using fixed cells stained with fluorescent antibodies

Live Cell Studies

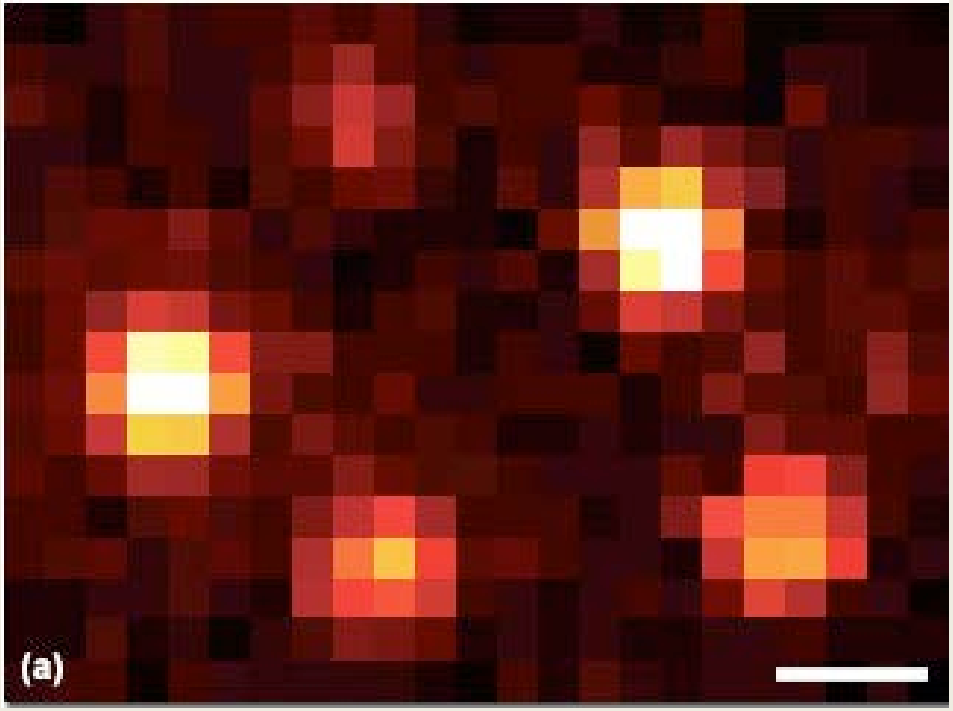
Live Cell Studies



Using live cells and a Rab5-Green Fluorescent Protein (GFP) fusion.

Super-Resolution Microscopy

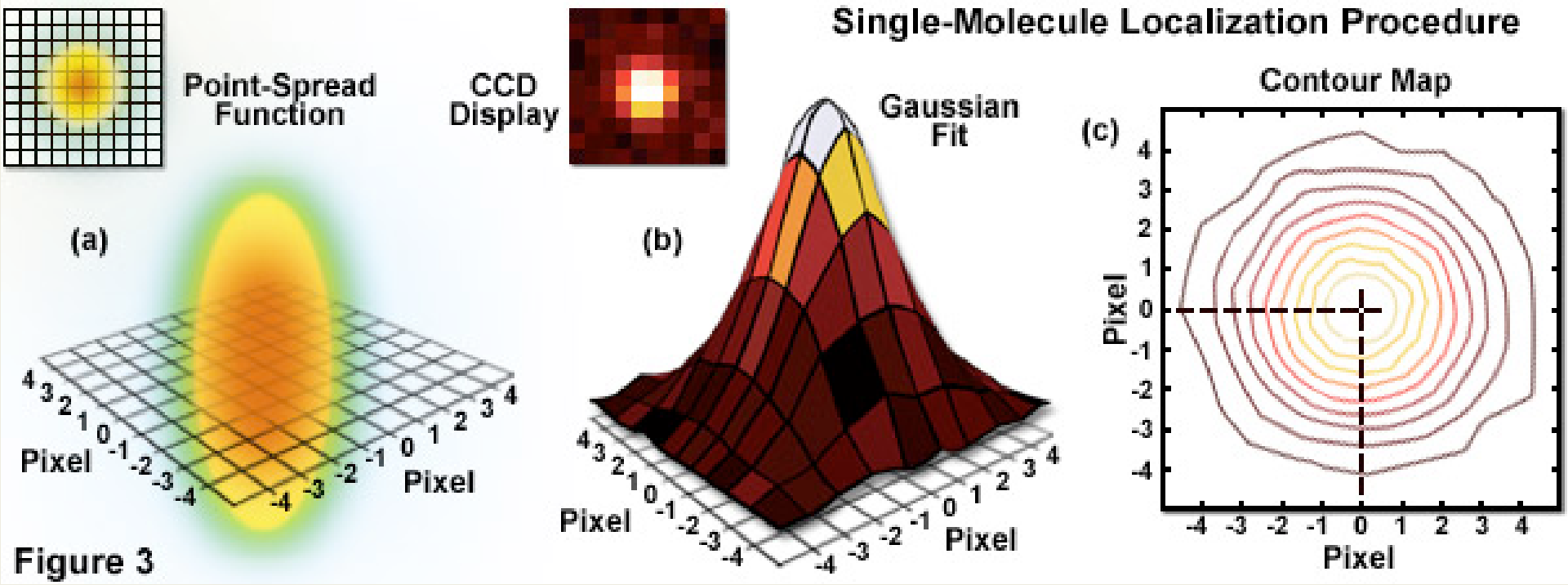
Confocal



200 nm

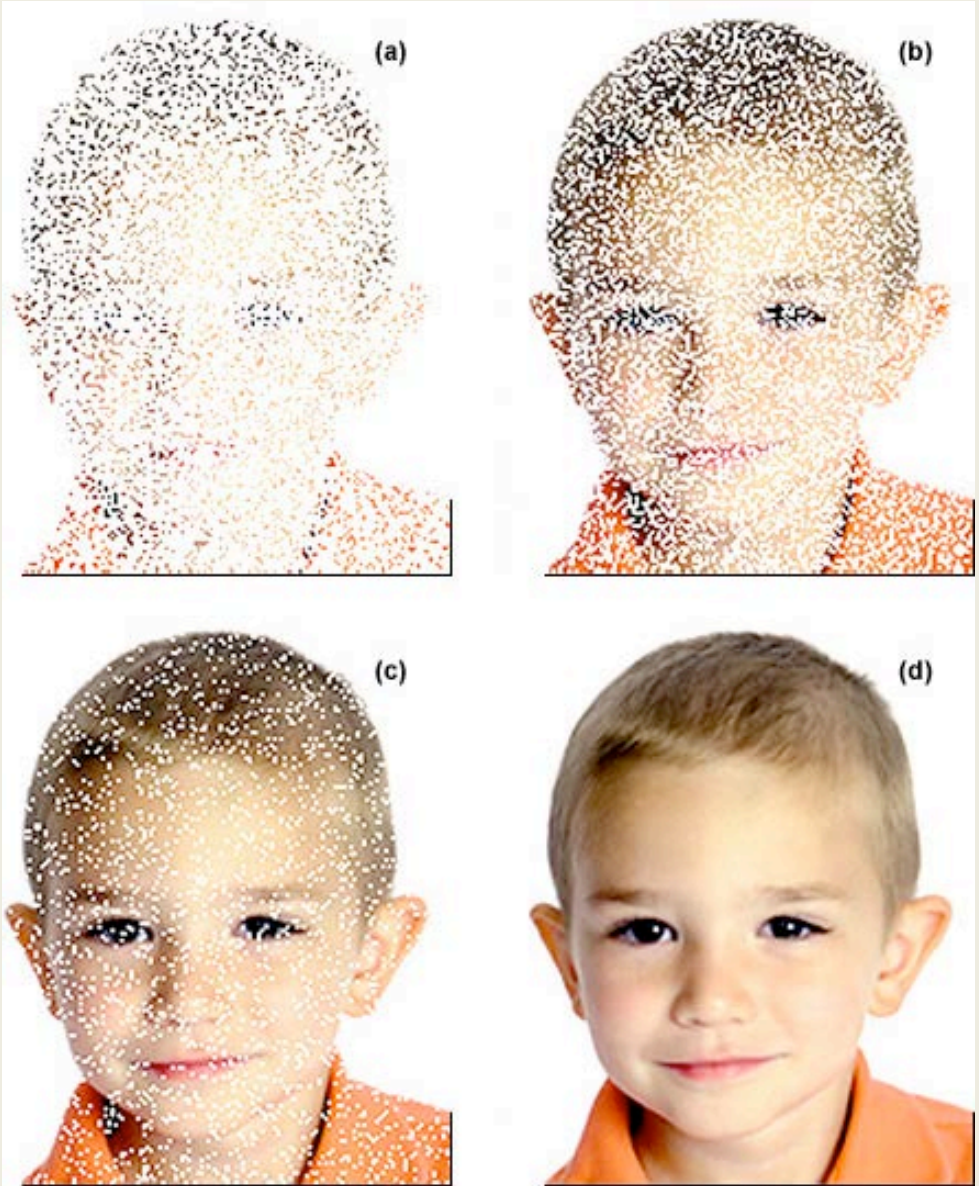
Zeiss

Super-Resolution Microscopy



Zeiss

Super-Resolution Microscopy

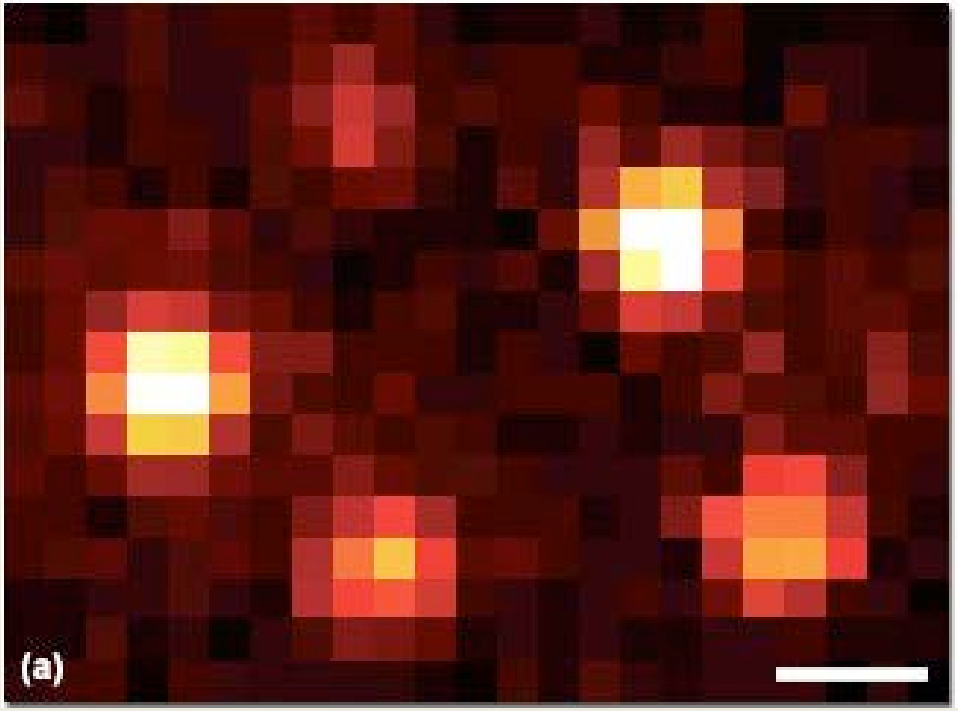


Zeiss

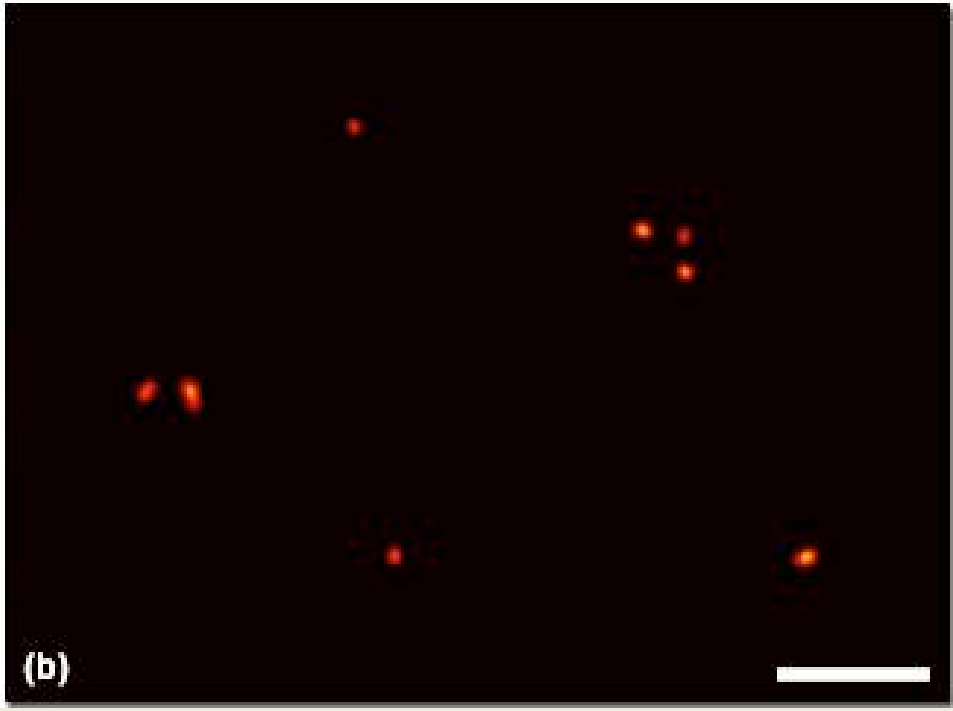
Super-Resolution Microscopy

Confocal

Photo-Activated Localization Microscopy (PALM)



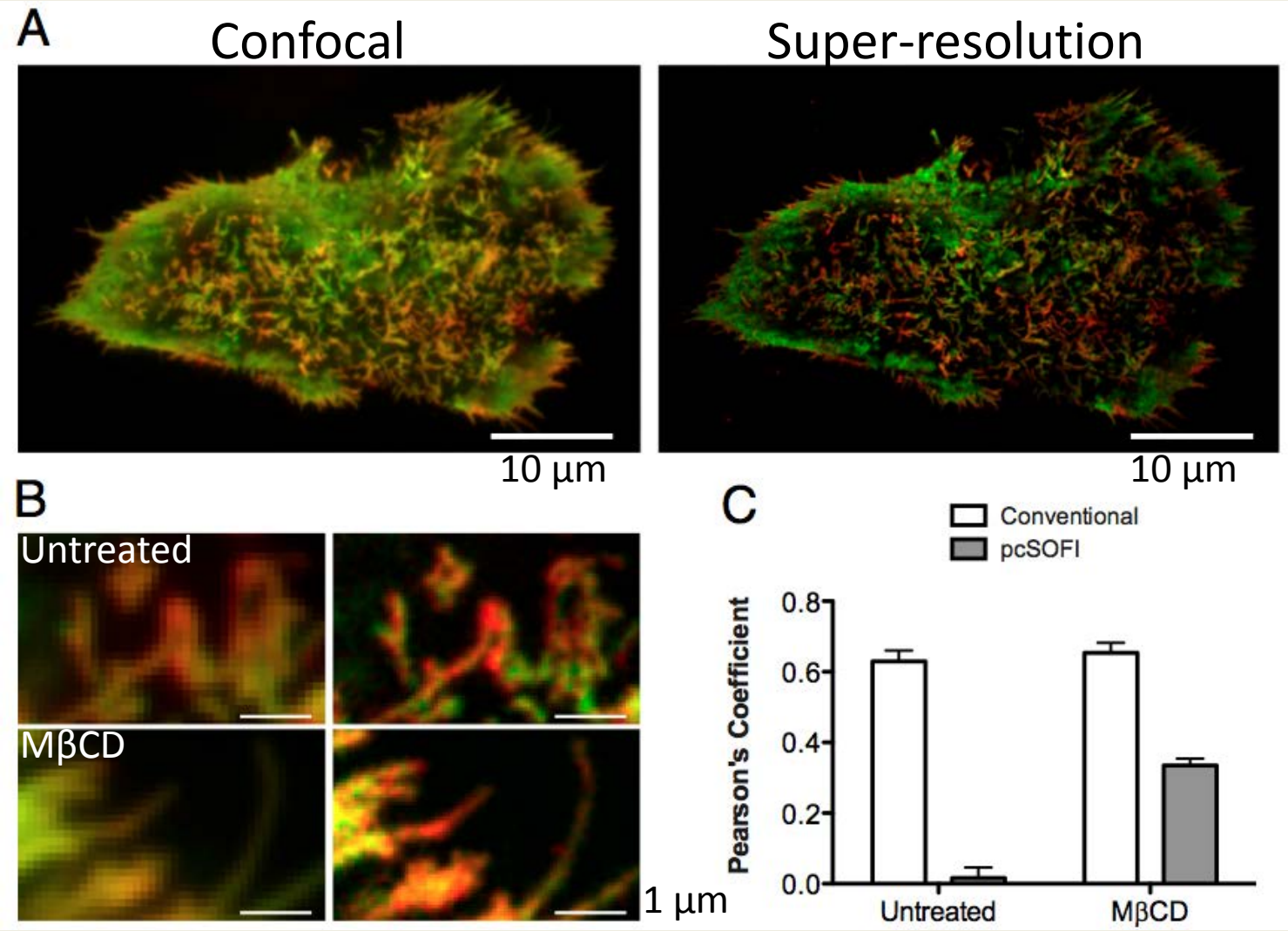
200 nm



200 nm

Zeiss

(Super)Colocalization Studies



Lyn – Green
Kras – Red

Dedecker *et al.* PNAS 2012

Electron Microscope

Electron Microscopy

How does it work?

$E = h\nu$ (Planck) $\lambda = h/p = h/(mv)$ (de Broglie)

Electron Microscopy

How does it work?

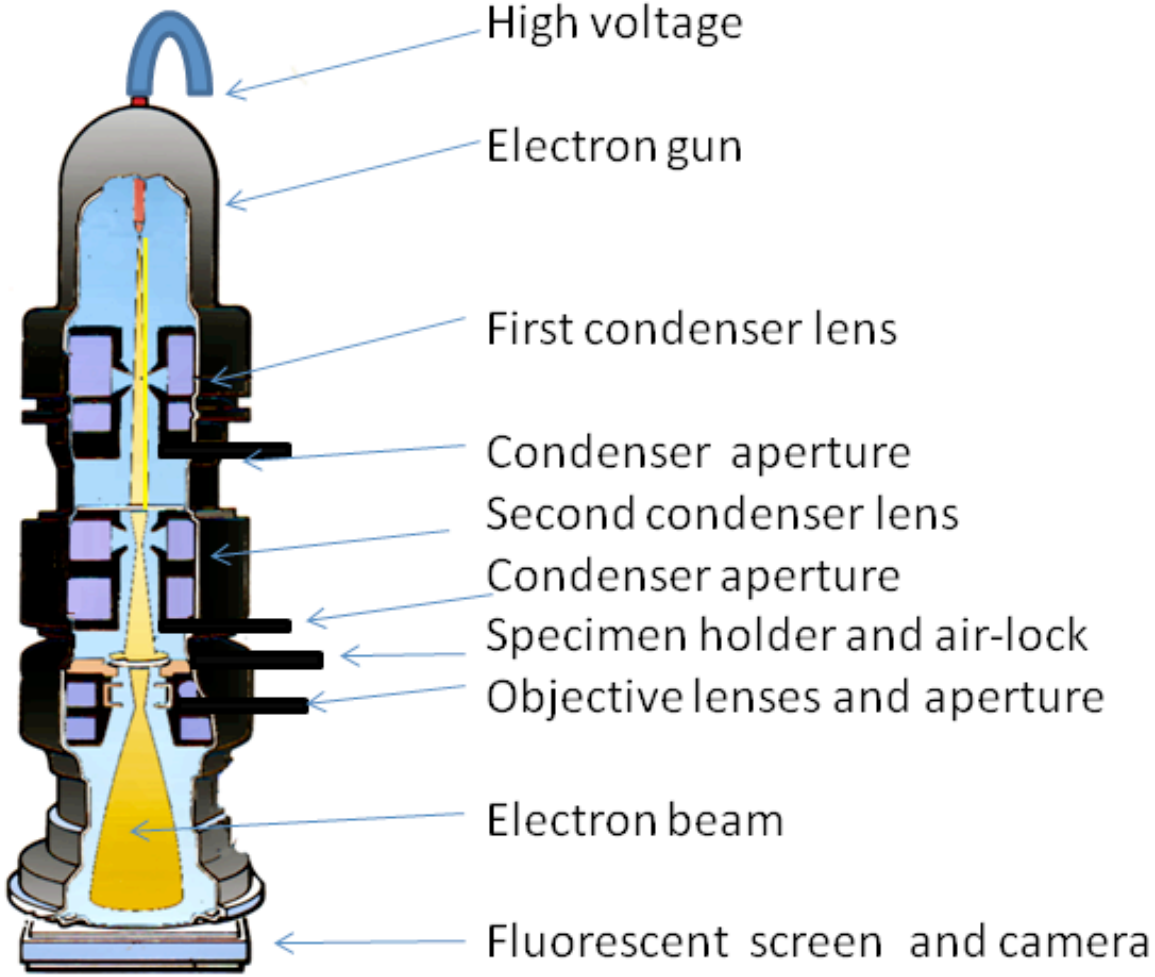
$E = h\nu$ (Planck) $\lambda = h/p = h/(mv)$ (de Broglie)

Mass and energy are interchangeable.

The faster a particle travels the higher it's energy and the lower its wavelength.

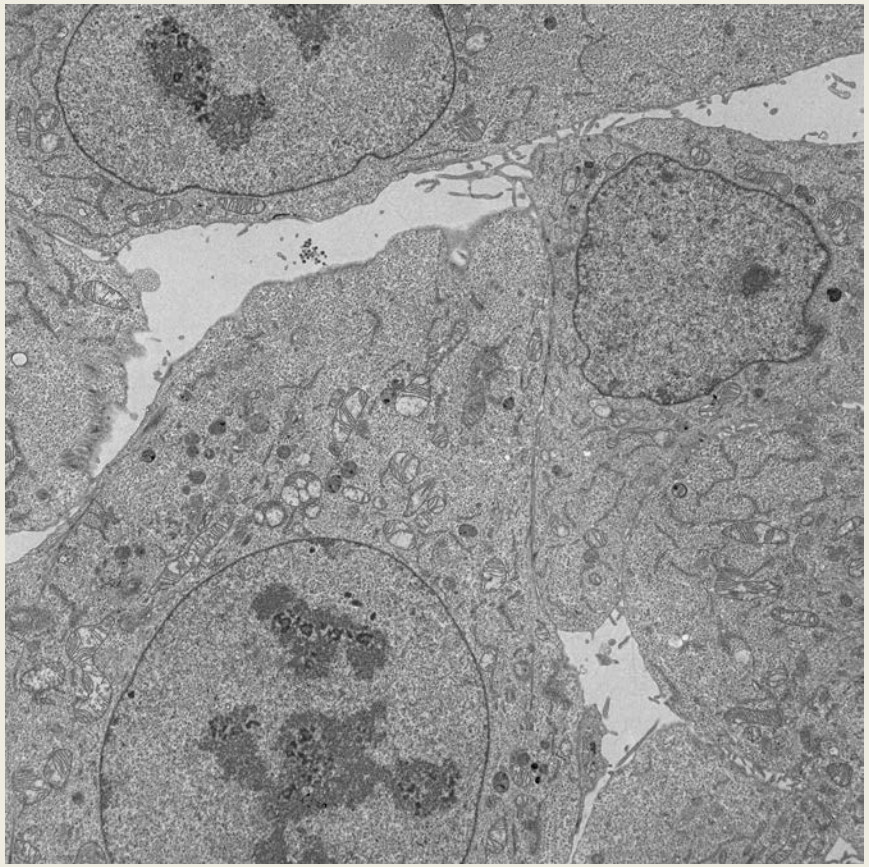
For an electron accelerated by 100 kV, the wavelength is 4 nm.

Electron Microscopy

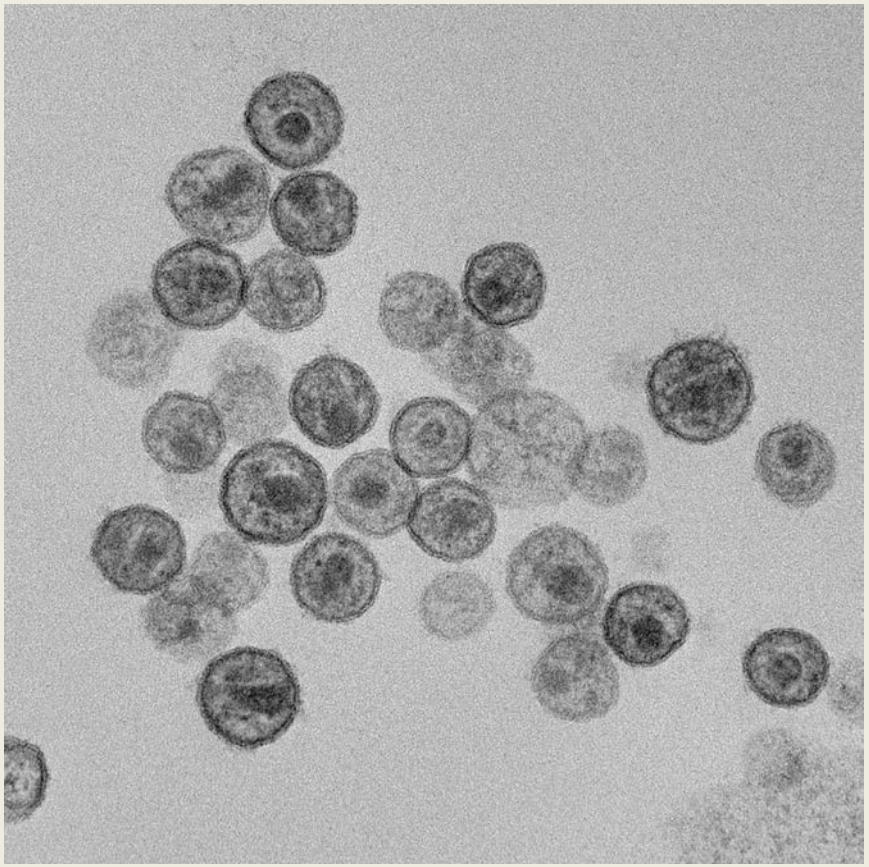


Transmission Electron Microscope

Electron Microscopy



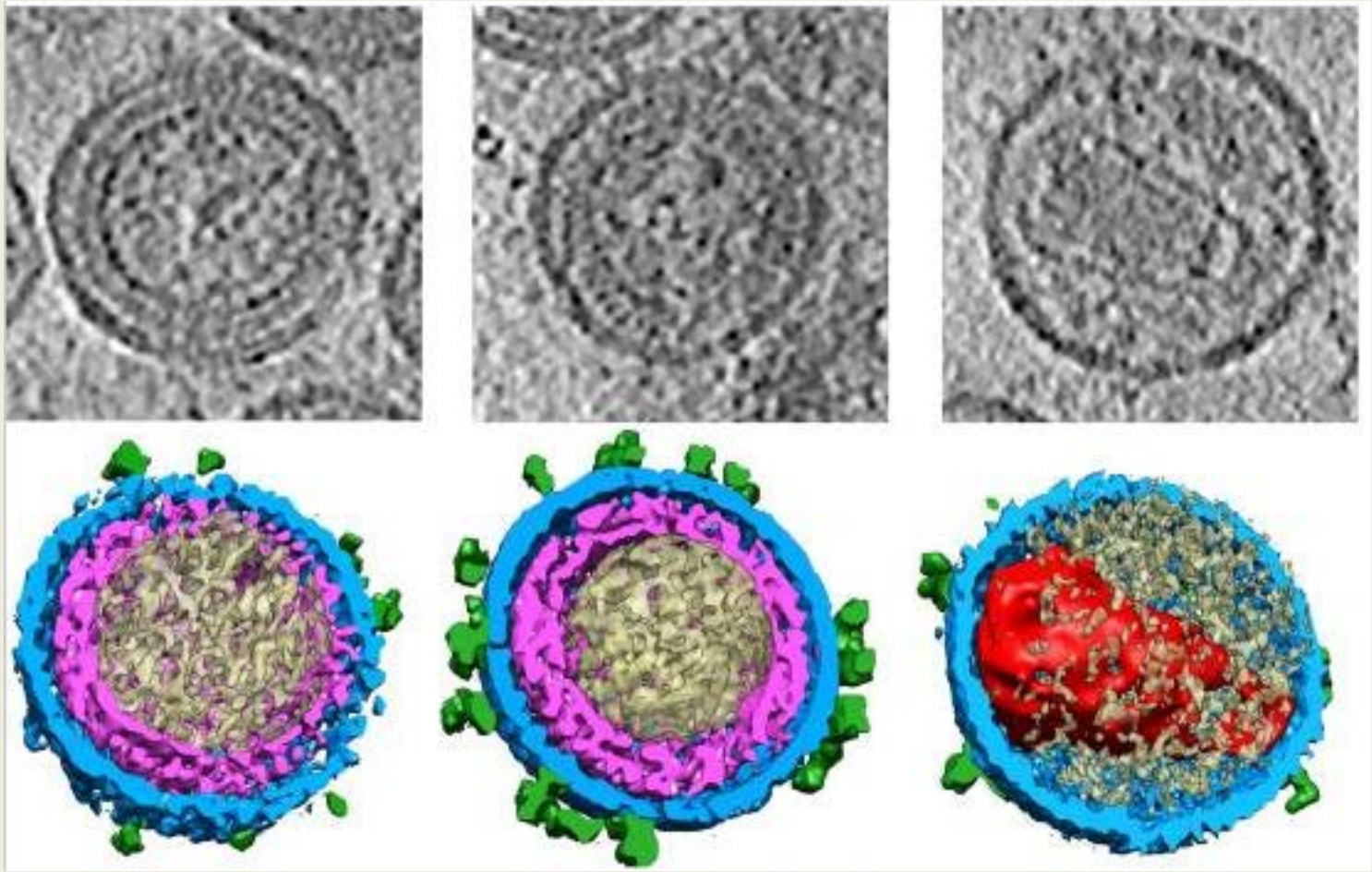
10 µm



200 nm

Using fixed cells stained with heavy metals (Os, U, Pb).

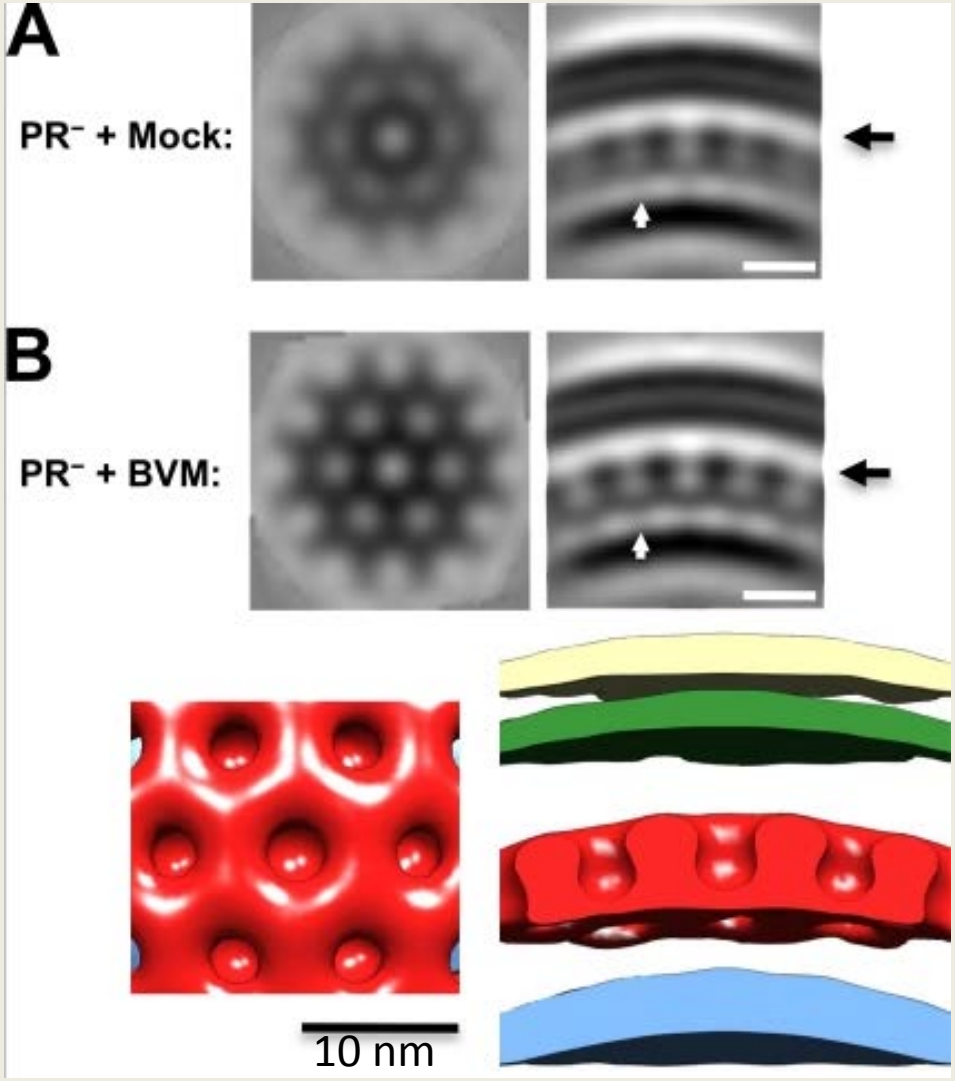
Cryo-EM Tomography



Immature Drug treated Mature

Keller *et al.* JVI 2011

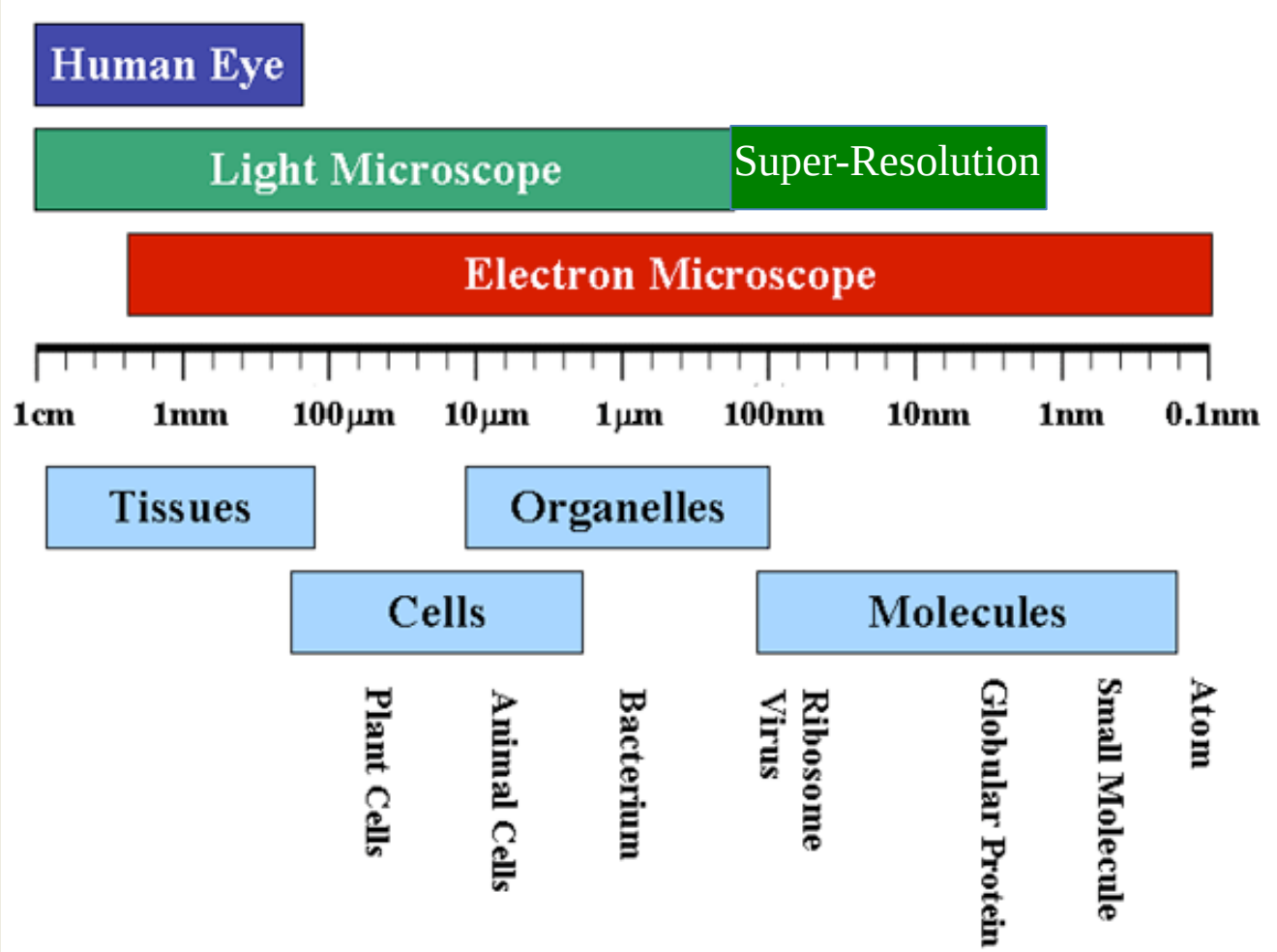
Sub-Tomographic Averaging



Keller *et al.* JVI 2011

Which is best?

What level of resolution do you require?



Which is best?

What level of resolution do you require?

What type of structure are you trying to visualize?

Is it a dynamic process?

Artefacts…
Sometimes what you see is a product of the way you did the experiment.

- Fixation
- Fusions/Tags