Academic skills

Elena Ambrosino, PhD

Maastricht University

Introduction round

Elena Ambrosino

MSc in Medical Biotechnology
PhD in Immunology and Cellular Biology
Advanced MSc in International Health

Italy, USA, France, The Netherlands + field research Burkina Faso, Tanzania

Research experience	Tumor immunology Infectious diseases Implementation & translational research
Editorial experience	Associate Editor Member of Editorial Boards Reviewer

Introduction round

- You background
- Your experience
- …

Core academic skills

- Academic writing
- Academic reading
- Presentation skills
- Communication
- Academic integrity
- Critical thinking
- Critical reading
- Searching for information
- …

Why sharing scientific knowledge?

- Share lessons (evidence, strategies, practices) learned in different contexts
- Contribute to knowledge equity
- Knowledge is a global public good and should travel in all directions
- Promote your career, establish yourself as expert
- Promote your organization

Dissemination of scientific knowledge

- Written – Articles (abstracts…)
- Oral – Presentations (interviews, …)
- Visual – Posters (infographics, …)

Scientific content

- Relevant
- Evidence-based
- Scientifically sound
- Clear and synthetic

Possible items for this week

- Intro on scientific writing
- Discuss published articles
- Discuss your examples/practice
- Discuss oral & poster presentations
- Examples/practice

Your expectations…

Academic writing

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Maastricht University

KEY POINTS

- What to publish
- How to prepare a manuscript
- Where to publish
- Publication process

You experience

.....

KEY POINTS

- What to publish
- How to prepare a manuscript
- Where to publish
- Publication process

Peer-reviewed scientific articles

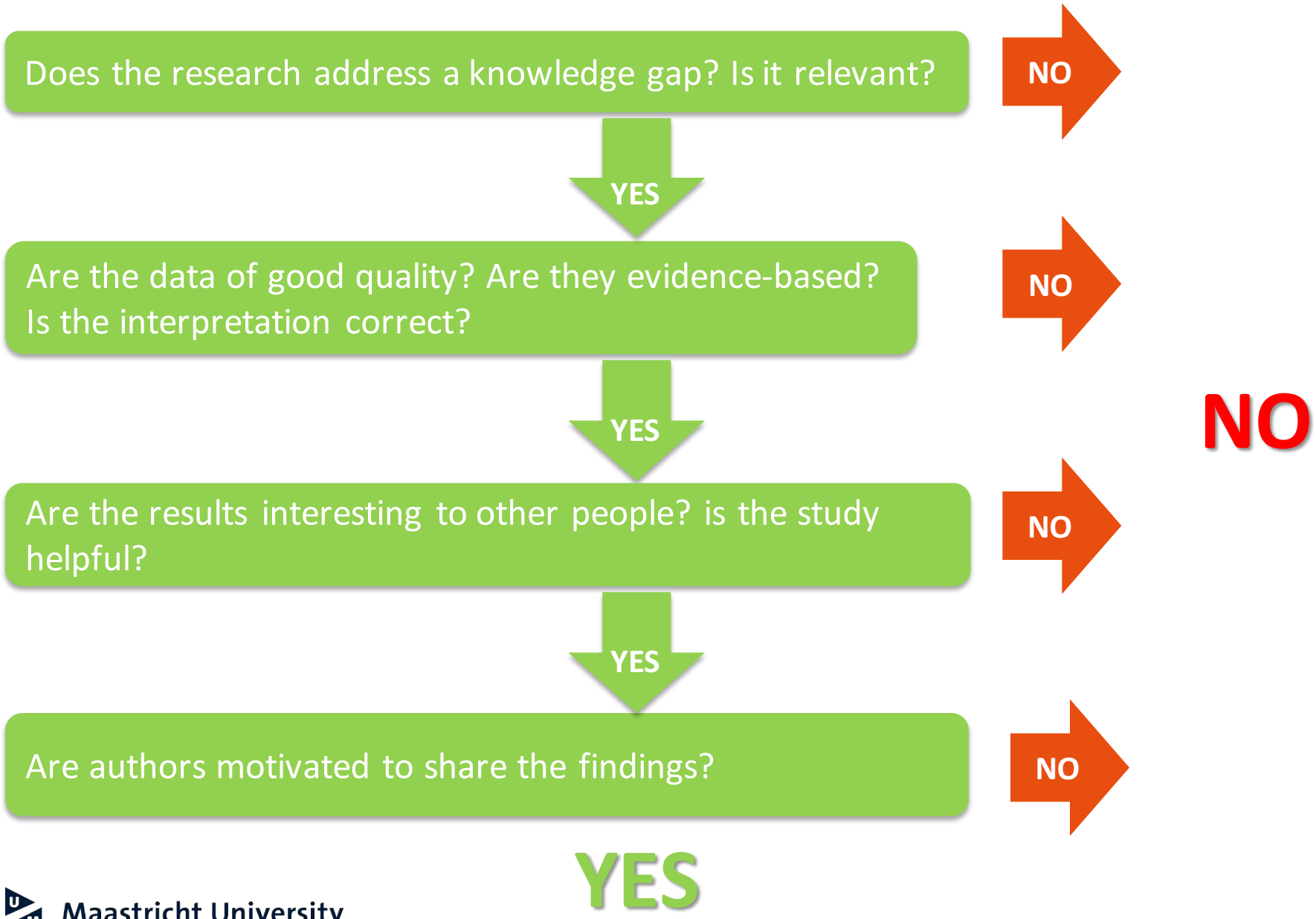
https://www.change.org/

https://skeptoid.com

Why peer-reviewed scientific articles?

- Ensure quality, validity, integrity
- Wide/global circulation
- Easy to retrieve and access

Should the idea be published?



Should the idea be published?

Evaluate a proposed research question/aim

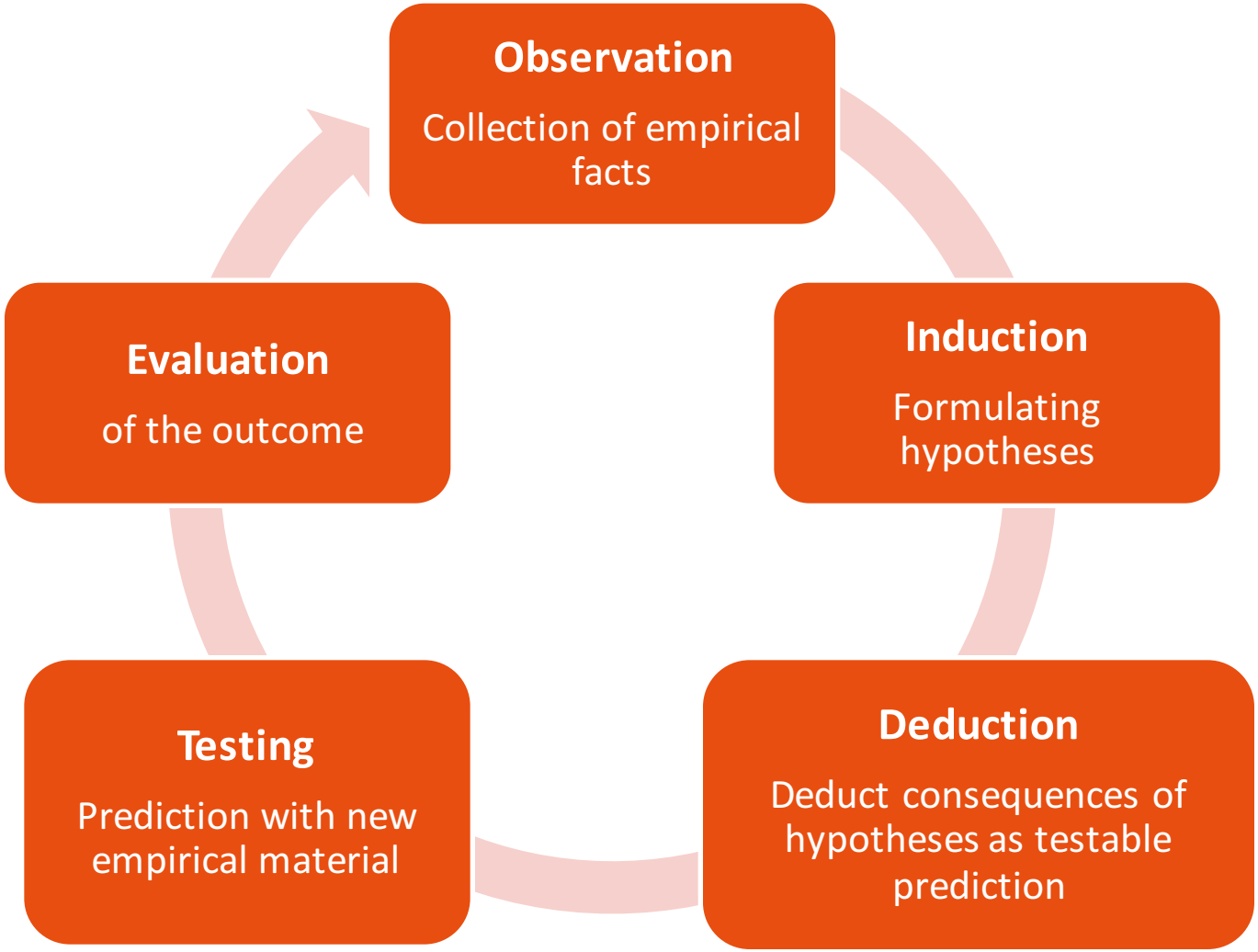
Is it clear, concise, and complete? Can it be answered with the data you have?

Does it identify the **P**opulation/**P**atients of interest?
Intervention or **I**ndicators/exposure being studied?
Comparison group/**C**ontrol?
Outcome of interest?
Time frame of the study?

***PICOT** criteria*

Should the idea be published?

It is based on the empirical cycle?



Modified from A.D. De Groot

In what form should be idea be published?

- Research articles: full articles (qualitative/quantitative research)
  communications (brief/short)
  implementation research
- Reviews: narrative
  systematic
  meta-analysis
- Others: perspectives
  debates
  commentaries
  case study
  field report
  letter to the editor

Different aim, content, length, structure

KEY POINTS

- What to publish
- How to prepare a manuscript
- Where to publish
- Publication process

Content of a scientific article

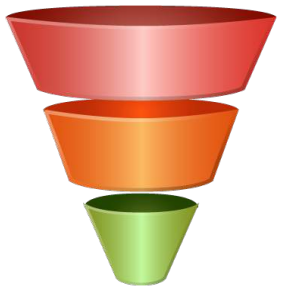
Introduction -1

Start with formulating your RELEVANT (eg PICOT) research question/aim

Which themes can be found in the research question/aim?

→ Search for background information about these themes

→ Write your introduction by using this information



Start with general information and become more specific

End your introduction with your research question/aim (should be logical to the reader!)

Introduction -2

Try to include all important items by answering the following questions:

What is the (health/scientific) problem?

What is know about the problem (theories)

What is the knowledge gap?

What is your hypothesis?

What is your research question/aim and what are your objectives?

What is the relevance?

What are the expected outcomes?

BREAST CANCER IS THE MOST COMMON cancer in women in the United States and worldwide.[1] Hormone-receptor–positive, axillary node–negative disease accounts for approximately half of all cases of breast cancer in the United States.[2] Adjuvant chemotherapy reduces the risk of recurrence,[3-5] with effects that are proportionally greater in younger women but that are little affected by nodal status, grade, or the use of adjuvant endocrine therapy.[6,7] These findings led a National Institutes of Health consensus panel to recommend adjuvant chemotherapy for most patients,[8] a practice that has contributed to declining breast cancer mortality.[9] However, the majority of patients may receive chemotherapy unnecessarily.

The 21-gene recurrence-score assay (Oncotype DX, Genomic Health) is one of several commercially available gene-expression assays that provide prognostic information in hormone-receptor–positive breast cancer.[10,11] The recurrence score based on the 21-gene assay ranges from 0 to 100 and is predictive of chemotherapy benefit when it is high, whether a high score is defined as 31 or higher[12,13] or 26 or higher[12,14]; when the recurrence score is low (0 to 10), it is prognostic for a very low rate of distant recurrence (2%) at 10 years that is not likely to be affected by adjuvant chemotherapy.[12,14] Although expert panels recommend the use of the 21-gene assay,[15,16] uncertainty remains as to whether chemotherapy is beneficial for the majority of patients, who have a mid-range recurrence score.

The Trial Assigning Individualized Options for Treatment (TAILORx) was designed to address these gaps in our knowledge by determining whether chemotherapy is beneficial for women with a mid-range recurrence score of 11 to 25. It was a prospective clinical trial, a type of trial that provides the highest level of evidence supporting the clinical usefulness of a biomarker.[17] Another objective of the trial was to prospectively confirm that a low recurrence score of 0 to 10 is associated with a low rate of distant recurrence when patients are treated with endocrine therapy alone.[18]

Health problem

What is known

Knowledge gap

Objectives

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Practice

→Make your own example

-AIM/Objectives

-possible list of items to include in the intro

Introduction -3

Avoidable mistakes

Research question/ aim is not specific enough

No use of references… use references to support your claims!

Insufficient depth / not state-of-the-art

Start is too general

Methods -1

Synthetic and clear

Enough detail to evaluate their appropriateness and repeat the process

Skip methods used to collect data not included

Present the information in logical (possibly chronological) order

Use clear subheads, you can delete/change them, based on journal guidelines

Use the past tense

Methods -2

Avoidable mistakes

Copying the whole protocol

Mentioning thinks that are obvious or not relevant

- Results were recorded by writing them in lab journal
- pH-meter was calibrated before use
- Solutions were made in a beaker
- Beakers were labelled as A, B, C…
- Mention what is in the tube/ glassware, not what is written on it

Explaining calculations of known statistical outputs (average, standard deviation)

Practice

→ List possible methods for your study example

Results -1 quantitative

Present all data (and only that) needed to answer the research questions

Organize the data (e.g. following the study objectives)

Highlight key findings including statistical significance

Use tables and figures to summarize large amounts of data

Use the text to highlight the patterns

Do not repeat the entire contents of tables and figures in the text

Results -1 qualitative

Present all data (and only that) needed to answer the research questions

Organize the data (e.g. following the study objectives, categories, theory)

Each quote should serve a purpose

Use a clear and consistent system to label quotes

Use italics or indents to visually separate quotes

Try to describe patterns/themes without quotes to limit the word count

Discussion -1

1 Start with your research question **summary**

- What was the main research question?
- What was the answer, based on your results?
- What additional research questions and findings were there?

1-4 sentences

2 Compare your results with other studies **discussion**

- Do the findings agree with past studies? If not, how can one explain the differences (methods, setting, participants)?
- Were any results surprising? If so, why?
- If the results are generally what would be expected, how does your study contribute to the literature?
- What were the strengths of the study?
- What were the limitations of the study?

do not review/describe other studies

3 Outline the study's implications and new understanding of the research area **conclusion**

- What are the benefits or implications of the findings for patients, practitioners, health care organizations, researchers, or policymakers?
- How do the findings impact current policy and/or practice?
- What is our new understanding of the problem statement outlined in the Introduction?
- What will be the next step in the research?

1-4 sentences

Discussion -2

Avoidable mistakes

The discussion presents new results

It does know acknowledge limitations

It does not have a clear take-home message at the end

Abstract

Take sentences from each section of your manuscript. Then revise for smooth flow

Follow journal guidelines (word limit, sub-sections…)

Avoid abbreviations or acronyms

Avoid citations, tables, figures, or illustrations

Do not include information not present in the paper

Title

Create a title that summarizes your article and draws attention

Include keywords

Be as specific as possible (e.g. intervention, outcome, study population, setting, and study design,…)

Check the journal's guidelines (word limit, including study type,…)

References

Include references each time you mention evidence from other studies

Try to refer always to the original article (not reviews)

Only mention references that are also mentioned in the text

Only use scientific literature

In what order do you write your articles?

....

Writing style -1

Avoid typos, check grammar, punctuation and spelling

Be clear and concise

Be precise, avoid ambiguity

Do not make unsupported claims

Use formal and subject-specific language (do not use slang or contractions ‘isn’t’, ‘won’t’…)

Do not use the following construction: ‘This is because…’ or ‘it can be said…’

Try to avoid series of short sentences , merge sentences if possible, being synthetic

NaOH was added to the solution. The solution was mixed. The pH was measured immediately.

The solution was mixed after adding NaOH and the pH was measured immediately.

Writing style -1

No number in digits to start sentences

Define acronyms the first time (do not have them in abstract)

Usually do not use the first person

Use past tense for most of the text -> *when past, present or future?*

Do not use direct questions

Avoid bullet points

Avoid phrasal verbs (e.g. ‘look at’, ‘get on with’…)

→ Avoid plagiarism (paraphrase, quote, cite)

READ YOUR ARTICLE AGAIN AND AGAIN

KEY POINTS

- What to publish
- How to prepare a manuscript
- Where to publish
- Publication process

Enhance the credibility of your article

Publish in peer-reviewed journals

Check where key experts in your field are publishing

Check what your focus audience reads (local journals?)

Consider the journal's IF (new journals do not have an IF)

Consider journals indexed in PubMed and other databases

Publish open access (fee required) → Beware of predatory journals

Open access publication

Directory of Open Access Journals (DOAJ) https://doaj.org/
community-curated online directory that indexes and provides access to high quality, open access, peer-reviewed journals

Bioline International http://www.bioline.org.br/
not-for-profit scholarly publishing cooperative committed to providing open access to quality research journals published to low-resources countries

AAS Open Research https://aasopenresearch.org/
platform for publication and open peer review for researchers supported by AAS (African Academy of Sciences) and its funding platform AESA (Alliance for Accelerating Excellence in Science in Africa)

Research4Life https://www.research4life.org/
collective name for the five programmes – Hinari, AGORA, OARE, ARDI and GOALI– that provide low-resources countries with free or low cost access to peer-reviewed content online

→ Institutions can apply to join

Open access publication

African journals online (AJOL) https://www.ajol.info/
world's largest online collection of African-published, peer-reviewed scholarly journals.

Scientific African https://www.journals.elsevier.com/scientific-african
Peer-reviewed, open access, inter- and multidisciplinary scientific journal that is dedicated to expanding access to African research, increasing intra-African scientific collaboration, and building academic research capacity in Africa

Where to find a journal's Impact Factor?

http://www.bioxbio.com/if/

https://www.scopus.com/sources

(free journal metrics, CiteScore, not IF)

www.webofknowledge.com/

(IF – from Journal Citation Reports (JCR) of items in the Web of Science (WoS) database by Thomson Reuters. NOT FREE)

KEY POINTS

- What to publish
- How to prepare a manuscript
- Where to publish
- Publication process

Work in a team

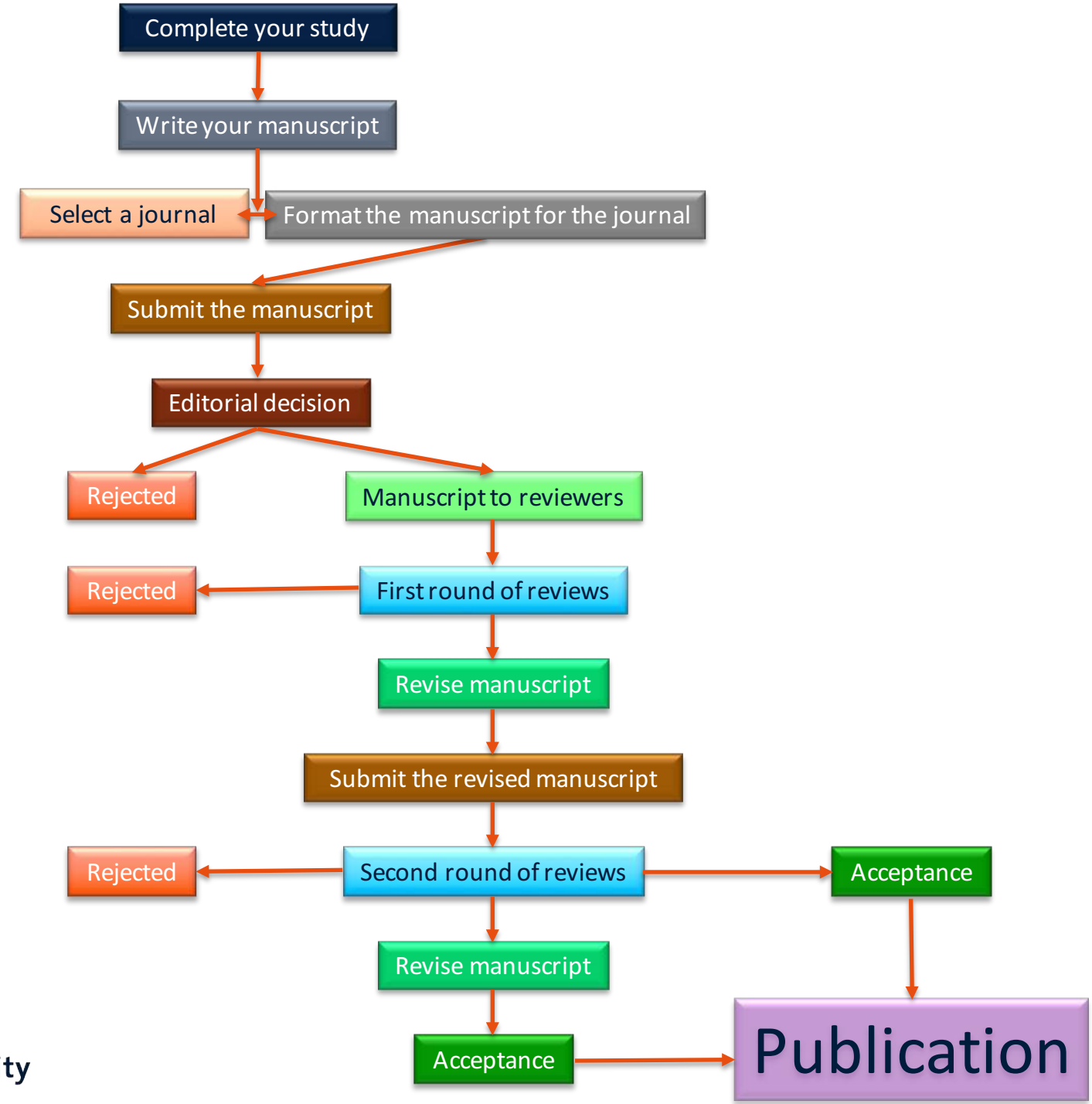
Communicate with all authors

Aknowledge all authors in the authors' list

→ mind the order

→ some journals want description of each one's contribution

Publication process



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Revision process

Take a systematic approach to peer review comments

- In your ***manuscript***, address each comment using track/changes

- In you ***response letter***, start by thanking the reviewers for their comments

  Write down your answer to each individual comment


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Practice

Look at a piece of published work and discuss

Is the aim clear?

Is the study relevant?

Is the methodology sound?

Do the results make sense? Are they in line with the aim?

Are the results clearly discussed?

What are the study limitations?

Are the conclusion in line with the aim?

Which aspects of the author's writing style help or hinder the reader?

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Scientific presentations

Elena Ambrosino, PhD

Maastricht University

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https://www.chemistryworld.com/careers/how-to-give-a-scientific-presentation/3008021.article
https://biysc.org/awards/be-passionate-attitude-award
https://newsroom.aua.am/2012/12/11/public-health-seminar-explores-latest-scientific-breakthroughs-in-blood-malignancies/
https://sites.google.com/a/umn.edu/geog8260-the-art-of-scientific-presentations/home

Scientific presentation

Structure

Important items

1) Scientific content

2) Form and style of slides

3) Presentation style

-tips

-engaging your audience

Pitfalls

Scientific presentation

Structure

- Title (include event location, date, your name, affiliation, logo)
- Background
- Methods
- Results
- Conclusion
- Acknowledgments
- *Q&A*

Scientific presentation

Important items

1) **Scientific content**

2) **Form and style of slides**

3) **Presentation style**

→ ***All important the same way?***

Scientific presentation

‘Thought Leader’ gives talk that will inspire your thoughts

1) Scientific content

- Adapt the content to your audience
- Tell up front why the audience should care
- Tell a story (with a beginning, middle and end)
  - Start with the ***context***
  - Frame the ***problem***
  - Provide ***highlights*** of what you did in a logical order
  - Conclude with ***key points***
  - Give a peek into your ***next steps***
  - ***Acknowledge*** collaborators and include your ***contact*** details
- Optimize the content (visuals for methods/timelines, simplify results!)

2) Form and style of slides -1

- Keep it simple
- Less is more (1 slide every 2/3 min)
- Start with an outline of what you'll cover and stay focused
- Create sections
- Only include images with a purpose

2) Form and style of slides -2

- Avoid clutter
  - -bullet points with keywords
  - -readable font
- Use visuals (simple & understandable)
- Check the spelling
- Use repeated slide templates + consistent color schemes and font

3) Presentation style

Tips -1

Upload the presentation and run through it

Practice in advance (in front of peers)

Emphasize your (1 or 2) main points

Take your time to gather your thoughts or move to a new topic

Do not read your slides

3) Presentation style

Tips -2

Talk to the audience, not the screen. Look (at people) at the back of the room

Use your laser pointer effectively

Stick to your time frame

Don't drift off at the end

--> "That concludes my presentation. Thank you for your attention."

--> ask for questions (if appropriate)

1 person on stage!

3) Presentation style

Engaging your audience

- Dress for success (casual local meeting or an international conference?)
- Walk energetically to the podium
- Start by saying good morning/afternoon

3) Presentation style

Engaging your audience

- Never express uncertainty, project confidence
- Smile (to appear more relaxed and reduce stress)
- Sound excited to make your audience excited
- Make it personal. Tell a brief anecdote or describe the “aha” moment

Scientific presentation

Pitfalls -1

Rushing through all your slides

Not leaving time for discussion

Not practicing in advance

Copy/paste journal article content (graphics, discussion)

Scientific presentation

Pitfalls -2

Showing raw data (always convey a message)

Trying to surprise the audience. The conclusion shouldn't be unexpected

Failing to put yourself in the audience's shoes

Thinking a collection of slides is enough (you need a narrative!)


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Poster presentation

Elena Ambrosino, PhD

Maastricht University

Poster presentation

What is it?

Structure

Key items to include

Where to start in making a poster

Poster format

Tips for a good poster

Poster presentation

Examples

What is a scientific poster?

Advertisement of your work

Illustrated abstract

Scientific poster structure

- Title + authors + logo
- Background
- Aim
- Methods
- Results
- Conclusion / take-home messages
- Future direction
- Acknowledgement (funding) + contact

Key points to include

Why should anyone care

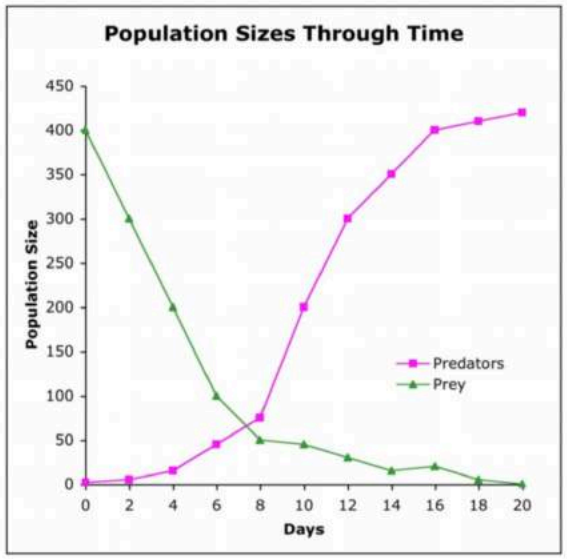
What are you adding to current knowledge

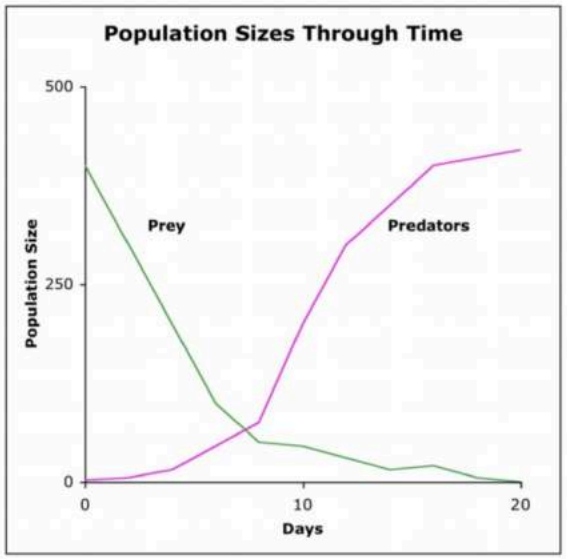
What you found

How you found it

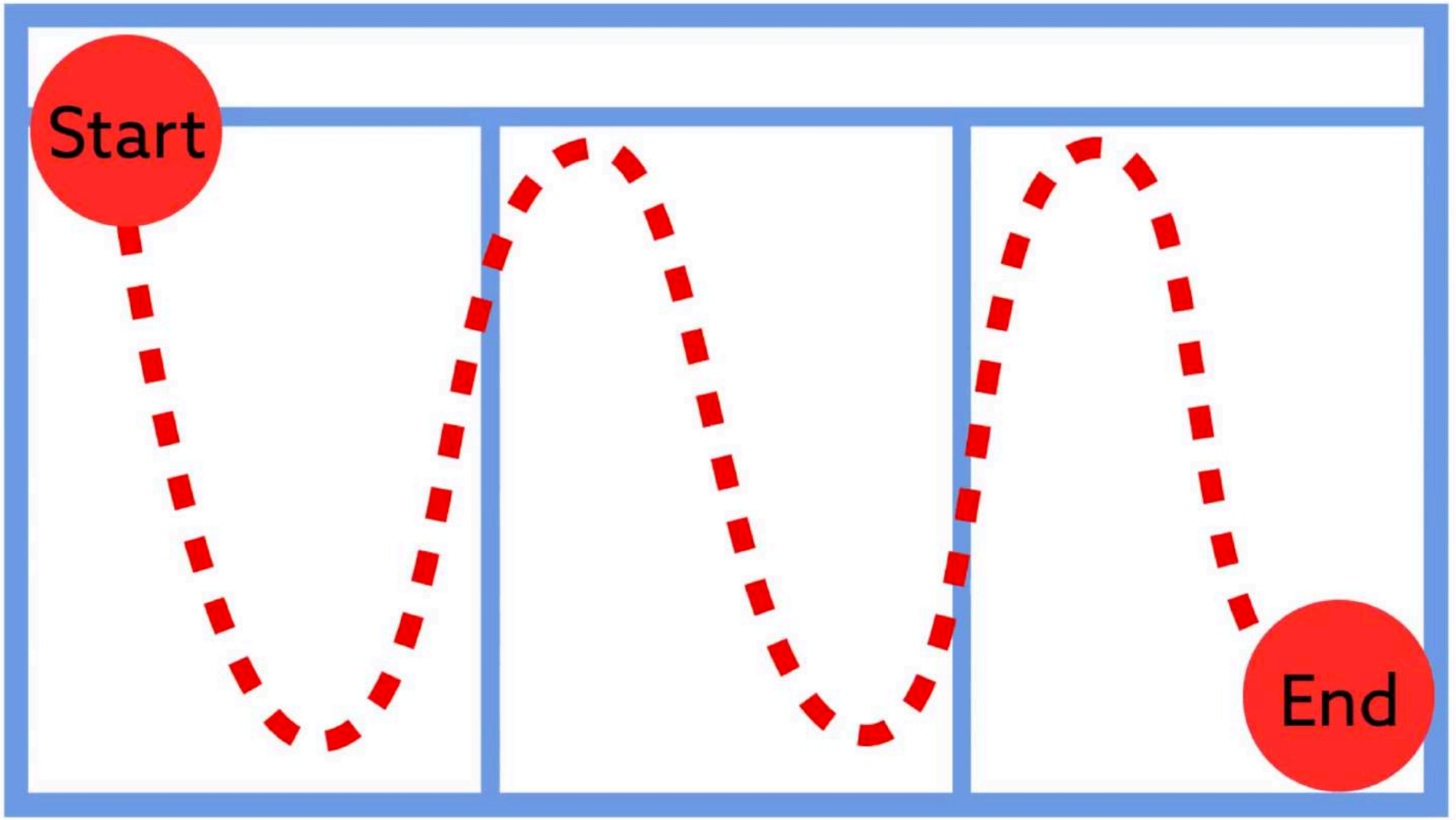
What you recommend

Where to start?





- Main results --> simplify graphs
- Fill in small blocks of supporting text around that
- Make the order logical (eg. use numbers)



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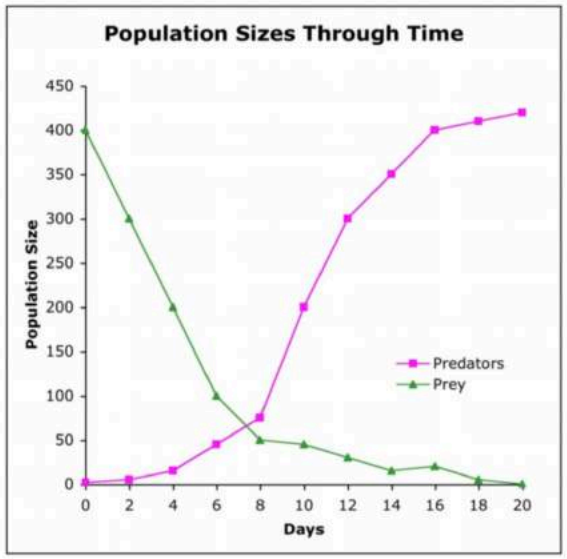
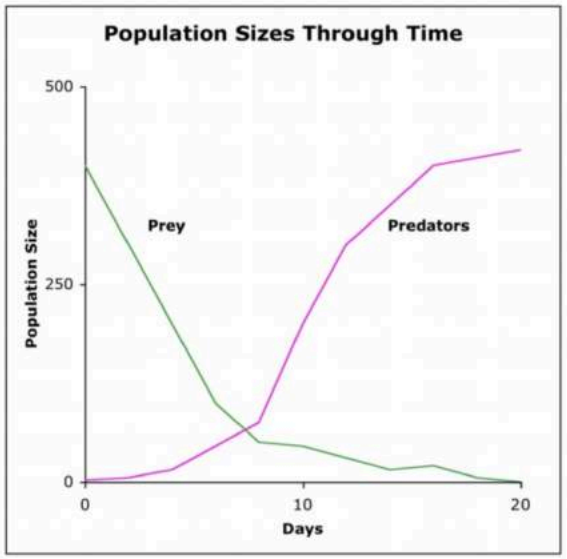
Logical order

Where to start?

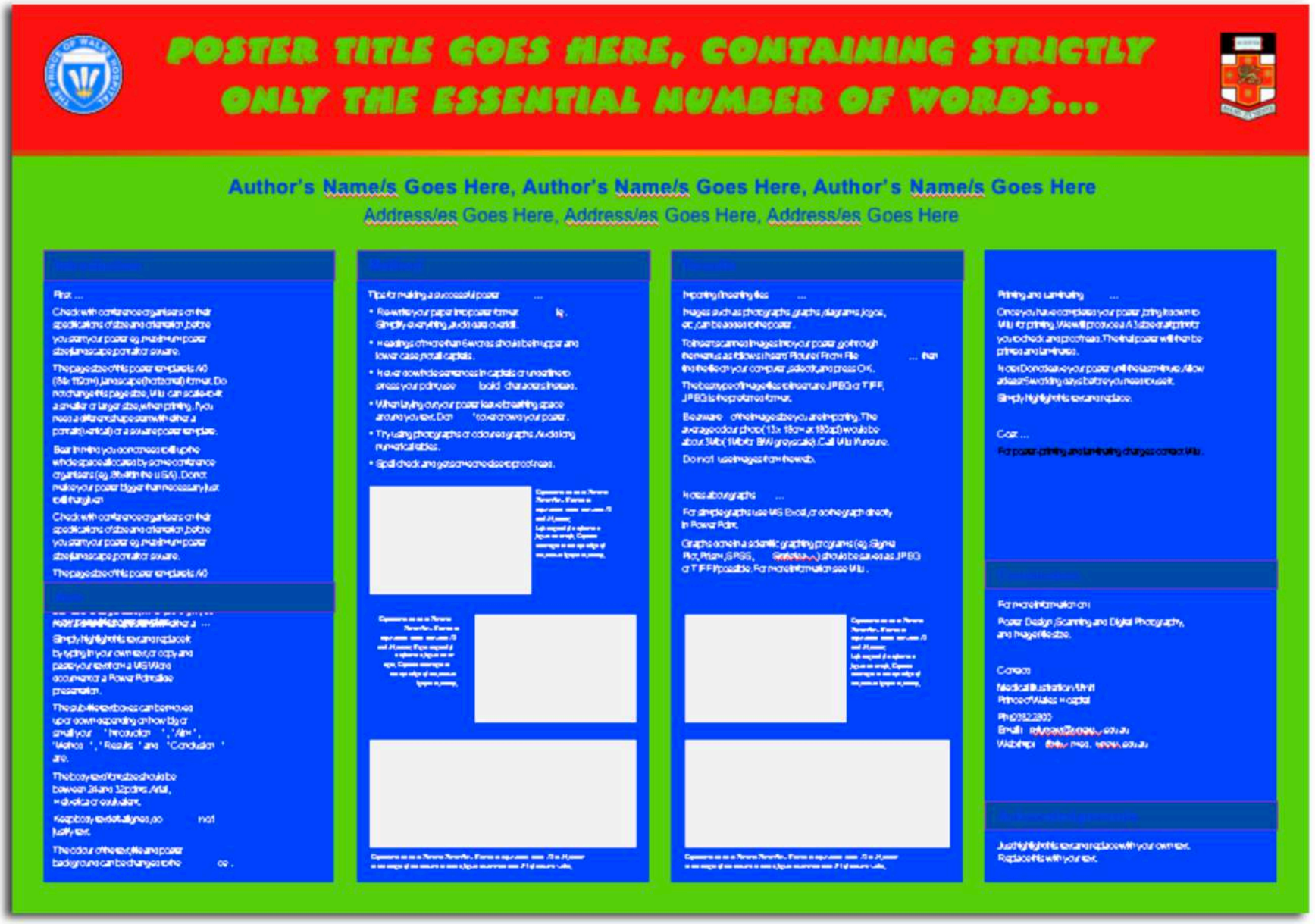




- Main results --> simplify graphs
- Fill in small blocks of supporting text around that
- Make the order logical (eg. use numbers)
- Attractive title

Poster format -1

- Split the layout into columns
- Plain font (serif for text, title and headings non serif)
- Same size and style (readable from 1-2m away)
- Left aligned
- Use color (best not primary colors)



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Primary colors

Poster format -2

- Decent resolution for pictures
- Print an A4 draft (size & color ok? Too busy?)
- Not busy backgrounds

NC STATE UNIVERSITY

Snook Growth in Habitats with Differing Abiotic Variability

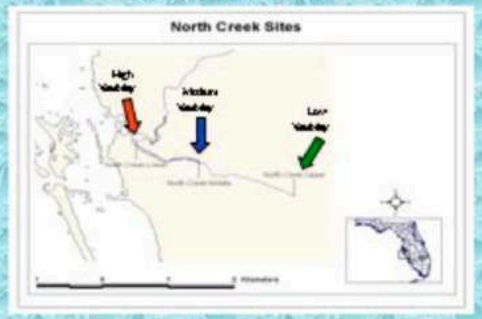
Alesia Read, North Carolina State University, anread@unity.ncsu.edu

PROPOSED OBJECTIVE

To create a useful tool for assessing potential stocking habitats based on degree of variability in water quality.

- Snook are a popular game fish found in the estuarine creeks of Florida
- Snook population has been on the decline due to overfishing and habitat degradation
- Numerous stock enhancement endeavors are currently underway without sufficient preliminary research
- Abiotic variability is a prominent feature of these estuaries
- Temperature, dissolved oxygen and salinity might play influential roles in the survivorship of the juvenile snook

STUDY SITES

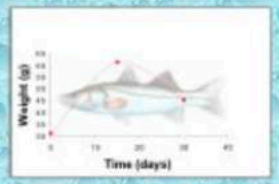


METHODS

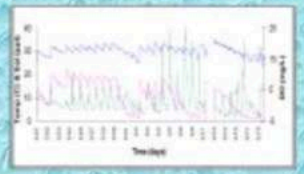
1. Juvenile snook are raised to fingerlings (100-200 mm) in the aquaculture facility
2. All snook are tagged with identifying markers for individual growth measurements
3. Fish are placed in cages within variable habitats at the research sites for 40 days
4. Fish are weighed and measured for growth

RESULTS

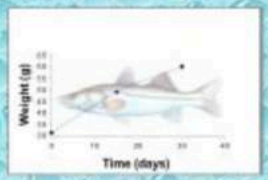
North Creek Lower (High Variability)



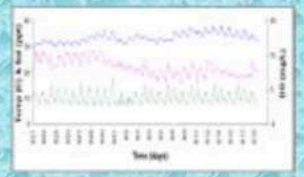
Negative Growth:
Dissolved Oxygen (mg/L)
0-22
Salinity (ppt)
2-21
Temp (°C)
25-34



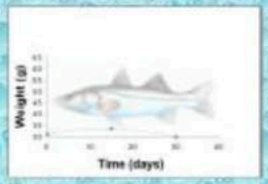
North Creek Middle (Medium Variability)



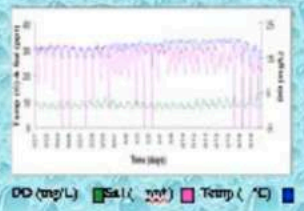
Positive Growth:
Dissolved Oxygen (mg/L)
0-8
Salinity (ppt)
16-28
Temp (°C)
30-38



North Creek Upper (Low Variability)



Slow Growth:
Dissolved Oxygen (mg/L)
0-4
Salinity (ppt)
16-30
Temp (°C)
26-33



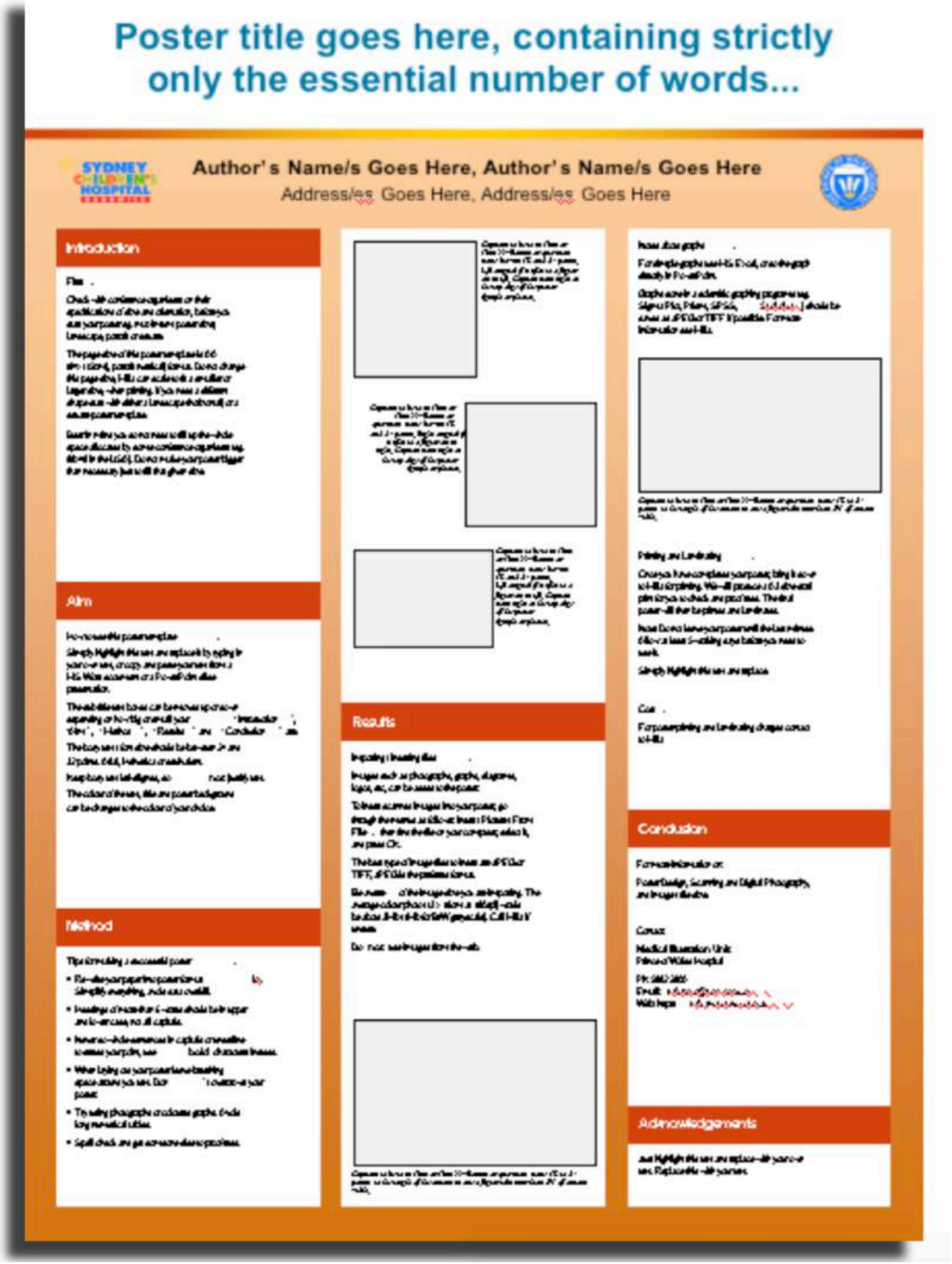
CONCLUSION

- Snook exhibit increased growth in habitats with a medium degree of abiotic variability
- Stock enhancement projects will be more efficient by releasing juvenile snook primarily in nursery habitats with a medium degree of abiotic variability

Busy background

Poster format -2

- Decent resolution for pictures
- Print an A4 draft (size & color ok? Too busy?)
- Not busy backgrounds
- Use white space to lead from one element to the other and make it easier to read



Use of white space

Short Title

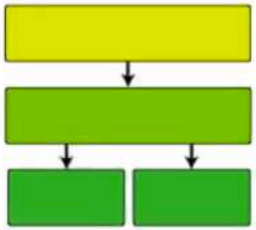
Introduction

Body of the introduction section

Pie chart title

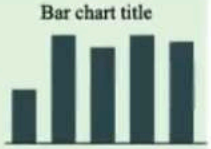
Objectives

- Bullet 1
- Bullet 2
- Bullet 3

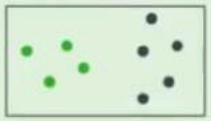


Results

Bar chart title



Scatterplot title



Line graph title

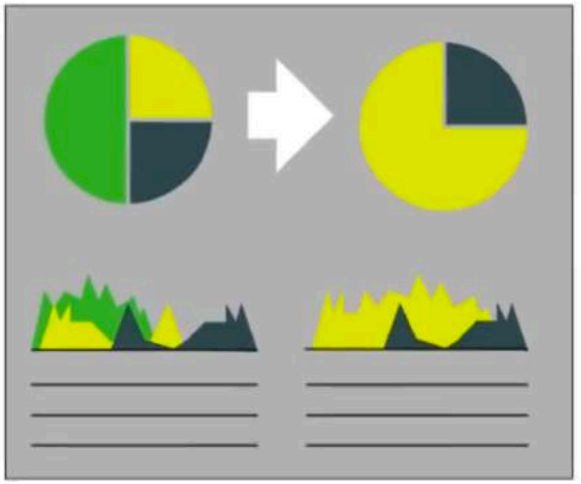
Discussion

1.First point

2. Second point

Conclusions

- Bullet 1
- Bullet 2
- Bullet 3
- Bullet 4
- Bullet 5
- Bullet 6



Maastricht University

Use of white space

Tips for a good poster

Short story → less is best! (max 800/1000 words – 20% text)

Describe a few major points (not at the bottom of the page)

Know your audience

Find out the format required

Get feedback

For the poster presentation

Prepare:

- 3-5 minutes verbal presentation
- Mini-sized poster handouts

Examples

Good or bad….or in between?

NC STATE UNIVERSITY

Southern Flounder Exhibit Temperature-Dependent Sex Determination

J. Adam Luckenbach*, John Godwin and Russell Borski
Department of Zoology, Box 7617, North Carolina State University, Raleigh, NC 27695

Introduction

Southern flounder (*Paralichthys lethostigma*) support valuable fisheries and show great promise for aquaculture. Female flounder are known to grow faster and reach larger adult sizes than males. Therefore, information on sex determination that might increase the ratio of female flounder is important for aquaculture.

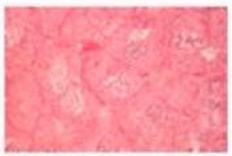
Objective

This study was conducted to determine whether southern flounder exhibit temperature-dependent sex determination (TSD), and if growth is affected by rearing temperature.

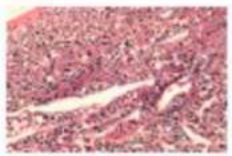
Methods

- Southern flounder broodstock were strip spawned to collect eggs and sperm for *in vitro* fertilization.
- Hatched larvae were weaned from a natural diet (rotifers/*Artemia*) to high protein pelleted feed and fed until satiation at least twice daily.
- Upon reaching a mean total length of 40 mm, the juvenile flounder were stocked at equal densities into one of three temperatures 18, 23, or 28°C for 245 days.
- Gonads were preserved and later sectioned at 2-6 microns.
- Sex-distinguishing markers were used to distinguish males (spermatogenesis) from females (oogenesis).

Histological Analysis

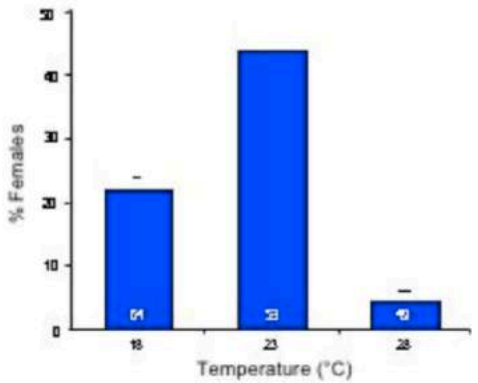


Male Differentiation



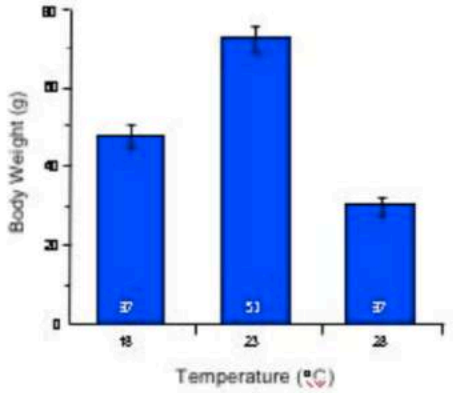
Female Differentiation

Temperature Affects Sex Determination

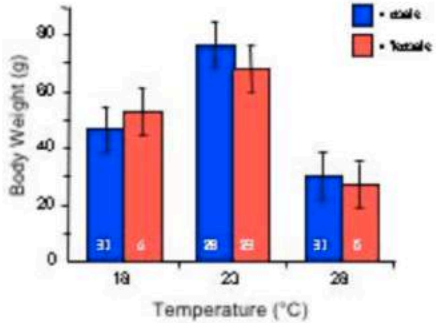


(* = P < 0.05 and *** = P < 0.001 represent significant deviations from a 1:1 male:female sex ratio)

Rearing Temperature Affects Growth



Growth Does Not Differ by Sex



Results

- Sex was discernible in most fish greater than 120 mm long.
- High (28°C) temperature produced 4% females.
- Low (18°C) temperature produced 22% females.
- Mid-range (23°C) temperature produced 44% females.
- Fish raised at high or low temperatures showed reduced growth compared to those at the mid-range temperature.
- Up to 245 days, no differences in growth existed between sexes.

Conclusions

- These findings indicate that sex determination in southern flounder is temperature-sensitive and temperature has a profound effect on growth.
- A mid-range rearing temperature (23°C) appears to maximize the number of females and promote better growth in young southern flounder.
- Although adult females are known to grow larger than males, no difference in growth between sexes occurred in age-0 (< 1 year) southern flounder.

Acknowledgements

The authors acknowledge the [illegible] for providing support for this research. Special thanks to [illegible] for help with the work.

PREVALENCE OF OBESITY AMONG INNER CITY LATINO CHILDREN AND ADOLESCENTS

Nazrat M. Mirza MD. ScD. Jill Merchant MS. Leila Beker, PhD

Children's National Medical Center and George Washington University School of Medicine and Health Sciences, Washington, DC

Background

Obesity is a major clinical and public health problem facing children and adolescents in the U.S. US particular significance is the increasing prevalence of obesity and its complications among the Latino population. Among this ethnic group there is a strong sense of family and children are a priority. However all the pressures placed on children, does may be a weakened acceptance that children should not be denied food or other luxuries such as TV. Obesity in children and adolescents is concerning not only because of the associated health and psychosocial consequences, but also because obese children tend to become obese adults. Since obesity is associated with many chronic diseases, it will have an enormous impact on the healthcare system.

Purpose of Study: To estimate the extent of obesity among inner city Latino children and adolescents (6-19) the overall goal of assessing the need for an obesity intervention program.

Study Design

One hundred and twenty five charts of children and adolescents aged 6 to 19 years were randomly selected from well child visits to Children's Hospital's Adams Morgan Clinic for the calendar year 2006. This clinic sees an average of 8-10 (?) patients a week, approximately 90-100% are Latinos, predominantly from El Salvador. Information extracted from the charts included weight, height, blood pressure, Tanner classification, history and physical findings consistent with obesity complications (body mass index (BMI) was calculated from recorded weight and height. Data analysis was done using SAS version 8.1.

Results

The distribution of the study sample is shown in Table 1. About 54% were females. The mean age was 11.6 years with a SD of 3.3 and a range of 4.8 to 18.7 years. The mean BMI was 20.8 with a SD of 5.8 and a range of 13.3-47.6. Overall 40% of the children and youth were overweight (BMI $\geq$ 95th percentile) or at risk for overweight (BMI $\geq$ 85-94th percentile), with an almost equal distribution between the two categories (Table 2). Males were more overweight and at risk for overweight than females, but the gender difference was not statistically significant. The prevalence of overweight was highest for youth ages 10 to 14 years.

Table 1 – Patient statistics

Variable	Frequency %
Gender	
Male	46.4
Female	53.6
Age Category (years)	n=125
4-5	8.0
6-9	22.4
10-13	27.2
14-15	19.2
16-17	16.8
18-19	6.4

Table 2 – BMI distribution

BMI Category	Frequency %
At Risk for overweight (BMI= 85-94th)	
1 Both Sexes (n=125)	20.8
2 Males (n=58)	22.4
3 Females (n=67)	19.4
Overweight (BMI >95th Percentile)	
1 Both Sexes (n=125)	22.4
2 Males (n=58)	24.1
3 Females (n=67)	20.9

Table 3 – At Risk for Overweight and Overweight by Age Category

Age Category (Yrs)	At Risk for Overweight (%) (BMI 85-94th %ile)	Overweight (%) (BMI >95th %ile)
4<=5 y (n=10)	20.0	10.0
6<=9 y (n=28)	28.6	21.4
10<=13 y (n=34)	8.8	38.2
14<=15y (n=24)	16.7	37.5
16<=17y (n=21)	23.8	19.0
18<=19 y (n=8)	25.0	50.0

Results continued:

Table 3 shows the distribution of overweight and at risk for overweight by age category. These data show that prevalence of overweight and at risk for overweight is high in children as young as 4 to 5 years. Although the prevalence of overweight and at risk for overweight was lowest in the age group 10-13 years, the differences were not statistically significant (Fisher Exact test p= 0.063 and p=0.05 respectively).

Asthma frequency was higher among the overweight than the non-overweight children and youth (p=0.048 Fishers Exact Test). There was no difference in the frequency of occurrence of other symptoms such as obstructive sleep apnea, learning difficulties, behavioral problems, seizures, and ADHD between the overweight and non-overweight group. Only 2% of all the overweight children had their cholesterol levels checked. The cholesterol levels ranged from 112-210 mg/dl. Ten percent of the children had their serum triglycerides assayed, and the range was 67-147mg/dl. There was no significant association between overweight and systolic or diastolic blood pressure in this small sample. Only 20% of the overweight children and youth were diagnosed and verification made in their charts regarding their overweight status by their health care providers. There were no referrals for overweight interventions noted in their charts.

Conclusions & Recommendations

The prevalence rate for overweight and at risk for overweight among children and youth in this inner city Latino community is more than twice the national average. Primary health care providers need to acknowledge and correct the presence of obesity and overweight in children and adolescents early and provide appropriate management of the problem. Targeted interventions and preventive strategies for overweight and obesity in children and adolescents are urgently needed to halt this prevalence.

Multidimensional NMR Spectroscopy of Proteins in Living Cells

Leonard D. Spicer,[1] Patrick Reardon,[2] Anne Marie Augustus[2]

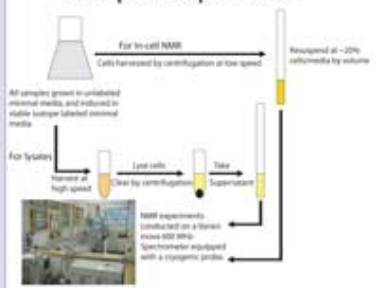
[1] Department of Biochemistry and Department of Radiology

[2] Department of Biochemistry
Duke University Medical Center
Department of Biochemistry
Box 3711 DUMC
Durham, NC 27710

Abstract

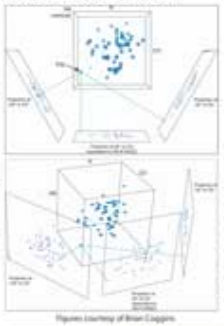
We have successfully implemented a suite of 3D NMR spectroscopy experiments which provide sufficient data to assign the full backbone resonances of the protein GB1 in live E. coli cells. This represents a unique demonstration that proteins can be studied with detailed NMR characterization in a living cell where the crowded and complex environment likely influences their behavior. The purified and homogeneous samples of proteins and other macromolecules normally studied in structural biology may not reveal this functionally important information. Experiments of this type are essential for de novo assignment of proteins undergoing structural changes in response to perturbing influences such as intermolecular interactions. In-cell multidimensional NMR experiments depend critically on both recent advances in NMR probe performance at high magnetic fields and NMR techniques which considerably reduce the time needed for data acquisition. For the protein GB1, the data were collected using fast projection reconstruction (PR) NMR methods and cryogenically cooled probes on the 600 MHz and 800 MHz NMR spectrometers in the Duke NMR Spectroscopy Center. We have also studied the behavior of the methionine repressor protein, MetJ, using this strategy. Our data suggest that MetJ is primarily associated nonspecifically with DNA in intact cells. This indicates that the repressor likely undergoes linear diffusion along DNA in finding its specific mettbox recognition sequences rather than the slower random 3D diffusion associated with free cytoplasmic proteins. Support for the NMR Center and the instrumentation used in this research was provided by grants from the NIH and NSF.

Sample Preparation



Projection Reconstruction

PR-NMR is a reduced dimensionality technique in which data are normally collected in the appropriate orthogonal planes as well as in a series of radially projected planes through the multi-dimensional space.
A projection reconstruction method is then used to combine the 2D data sets into a higher dimensional construct which produces a true multi-dimensional spectrum as illustrated. Since the data are collected in only two dimensions, considerable time is saved in the experiment.



Figures courtesy of Brian Coggins

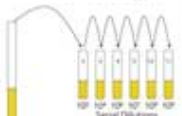
Model Systems

GB1
GB1 is the 56 residue IgG binding domain from Streptococcus protein G.

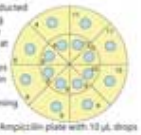
MetJ (shown on DNA)
MetJ is a 24 kDa dimeric repressor protein that regulates the methionine biosynthesis pathway in E. coli.

HCA II
Human Carbonic Anhydrase II is a 29 kDa monomeric enzyme that catalyzes the formation of bicarbonate.

Cell Viability



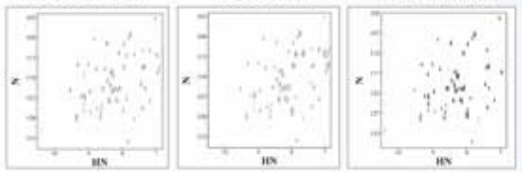
Viability studies were conducted using a micro-drop plating assay. 10 µL aliquots were taken from the NMR tube at various time points, and serially-diluted. 10 µL drops are placed on an ampicillin LB agar plate, incubated overnight, and colony forming units counted.



Ampicillin plate with 10 µL drops

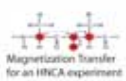
GB1

Assignment Spectra

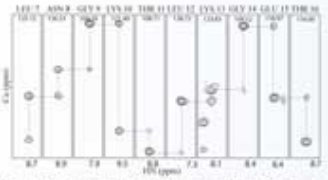


2D plots from (A.) purified GB1, (B.) GB1 in cell, and(C.) a 3D HNCA spectrum of GB-1in cell projected along Ca axis onto HN,N plane. HSQC experiments were collected in ~7 minutes. HNCA was collected in ~2 hrs. In cell spectra were acquired on a ~20% cell slurry. Axis units are ppm.

3D HNCO of GB-1 collected with 5 tilt angle projections and two orthogonal planes on a ~20% cell slurry. The experiment was completed in ~1hr.

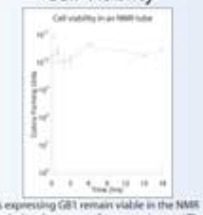


Magnetization Transfer for an HNCA experiment



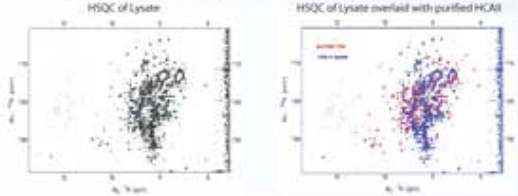
HNCA strip plot showing scalar couplings between HN and Ca carbons in the i and i-1 positions for residues 7-16. The y-axis is ^{13}C chemical shift. The ^{15}N chemical shift for each plane is noted at the top of the strip. In addition an HA(CA)NH data set was also required to complete the assignment. The HA(CA)NH experiment took ~ 3hrs to acquire.

Cell Viablity



Cells expressing GB1 remain viable in the NMR tube during the course of our experiments. The first three hours of the data are the average of 2 experiments, while the later time points result from one experiment. Standard deviations are shown for the first three hours.

Human Carbonic Anhydrase II

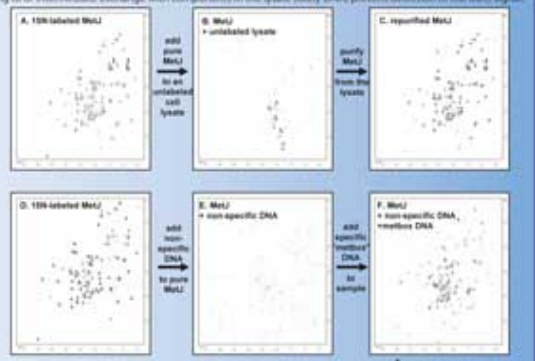


Some HCA II resonances can be observed in a whole cell lysate. The above spectra were acquired on a whole cell lysate of a 500 ml culture. The aquisition time was ~ 2 hours.

Human Carbonic Anhydrase II is generally considered a cytoplasmic enzyme. However, earlier biochemical data have indicated that HCA II may be membrane associated. Association with the membrane could render the protein undetectable by in-cell NMR, due to the increase in rotational correlation time or intermediate exchange. The weak signals detected in this experiment suggest that this carbonic anhydrase isoform maintains some level of membrane association.

MetJ

The characteristic HSQC spectrum for MetJ was not visible in whole or lysed cells over-expressing MetJ.
A. Therefore, pure 15N-labeled MetJ was used in order to ensure there was enough well-folded, well-behaved material for NMR analysis, as well as to eliminate the non-specific background labeling in the cells.
B. When pure 15N labeled MetJ was added to an unlabeled cell lysate, the spectrum completely changed, and the MetJ fingerprint was lost.
C. The fact that MetJ could be re-purified from this lysate, however, shows that MetJ was not being degraded. Instead, binding to or intermediate exchange with components in the lysate (likely DNA) prevent detection of the MetJ signal.



D. To test the hypothesis that MetJ is interacting non-specifically with genomic DNA in the lysate, sonicated salmon sperm DNA (200-3000 bp fragments) was added to pure 15N-MetJ.
E. The spectrum of MetJ disappears, just as in the case of the lysate.
F. MetJ can be rescued from these non-specific interactions with small pieces (20 bp) of "mettbox" DNA corresponding to MetJ's preferred binding motif.
G. The recovered spectrum looks like the standard fingerprint of MetJ bound to mettbox DNA.

Summary

In summary, we have demonstrated the de novo assignment of the small protein GB1 from 3D data collected on whole, living E.coli cells. This was enabled by new NMR experiments incorporating fast projection reconstruction data acquisition and the enhanced sensitivity of cryogenic probes. The implementation of heteronuclear 3D data aquisition in living cells opens a route to previously inaccessible studies of proteins in the biological milieu inside the cell. Furthermore, we have demonstrated how in-cell NMR can yield new insights into the behavior of proteins in their natural environment. In the case of MetJ, the protein may be associated with DNA non-specifically, allowing it to undergo linear diffusion to locate its specific cognate binding site. For HCA II, our data support biochemical results that suggest association with membranes and/or membrane-bound proteins.

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WHICH IS MORE IMPORTANT: NUMBER OF PATCHES OR CONNECTIVITY?

Darin Kalisak, PBS Student

Contact: [illegible]

INTRODUCTION AND OBJECTIVES

[illegible]

[illegible]

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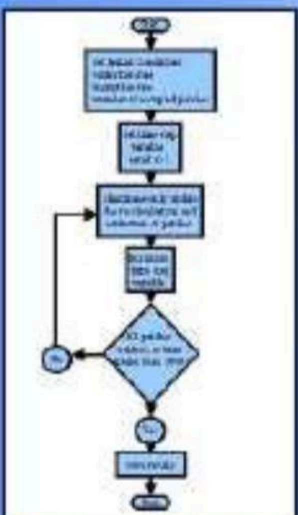
THE PROGRAM



ASSUMPTIONS AND LIMITATIONS

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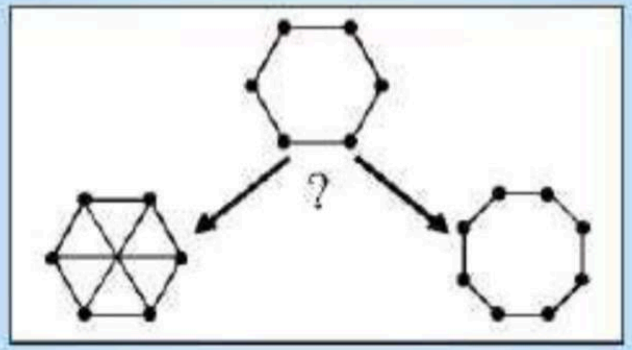
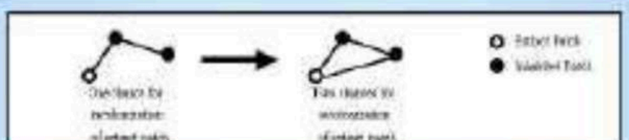
THE ISSUE



A metapopulation is a collection of discrete population patches, in which individual patches may typically go extinct and be recolonized. Is the long-term viability of the metapopulation helped more by adding new patches or by increasing the number of migration pathways between existing patches?

Adding patches increases the overall population of the organism, and makes a total extinction less likely by increasing the sheer number of patches which would have to go extinct.

Adding migration pathways increases the likelihood of recolonization of extinct pathways, by giving extinct patches more sources for immigration.



RESULTS

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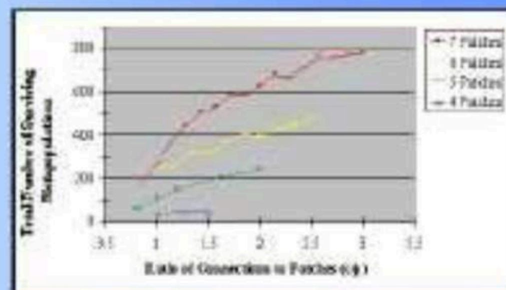

CONCLUSIONS

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A SHARED PROPENSITY TOWARDS
FOOD AND ALCOHOL

Patricia N. Darmoko, Jenna R. Cummings, A. Janet Tomiyama

University of California, Los Angeles

BACKGROUND

Overeating and **binge drinking** are two of the most common health problems among college students.
- 48% students reported binge-eating symptoms
- 63% females and 83% males had binge drinking episodes[1]

Most research examines the two problems in isolation, but food and alcohol might be more related than we realize.

Is alcohol similar to food?
- Alcohol is derived from sugar → similar chemical bases with food
- Both eating and drinking alcohol activate **dopaminergic** pathways[2]
- **Addiction** models have been applied to both food and alcohol use[3]
- Correlations between food and alcohol **intake** in animal studies[4]

Eating and drinking behaviors are highly driven by one's expectancies of food and alcohol. The **more positive expectancies** one has about the psychological effects of food and alcohol, the **higher their consumption** of food and alcohol respectively[5,6].

Since eating and drinking share similar **biological** pathways, could they also share similar **psychological** pathways?

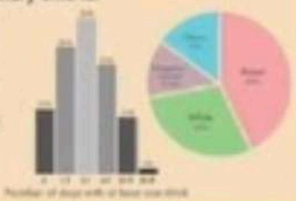
HYPOTHESIS

Individuals who have **high expectancies** of the psychological effects of **food** would also have **high expectancies** of those of **alcohol**.

In other words, **the propensity towards food would be positively correlated with the propensity towards alcohol**.

METHOD

200 UCLA undergraduates (76% females; Mean age = 22.1) filled out an **online survey** in one-sitting as part of a larger experimental study with the following **exclusionary criteria**:
- Less than 21 years old
- Self-reported history of eating disorders or substance abuse
- Abstinence from drinking beer
- A strict diet
- Food allergies to experimental stimuli
- Lack of proficiency in English



Number of days with at least one drink

MEASURES

ALCOHOL EXPECTANCY QUESTIONNAIRE (AEQ)

68-item questionnaire measuring one's anticipatory effects of drinking alcohol

Relaxation and Tension Reduction ("Alcohol makes me worry less")
Arousal and Aggression ("After a few drinks, it is easier to pick a fight")
Increased Social Assertiveness ("A few drinks makes it easier to talk to people")
Physical and Social Pleasure ("Drinking adds a certain warmth to social occasions")
Global Positive Changes ("Alcohol seems like magic")
Sexual Enhancement ("I often feel sexier after I have had a couple of drinks")

DUTCH EATING BEHAVIOR QUESTIONNAIRE (DEBQ)

36-item questionnaire assessing one's eating behaviors (since expectancies predict consumption[5,6], food expectancies are implicitly implied)

External Eating: sensitivity to anticipated conformity benefits of eating ("If you see others eating, do you also have the desire to eat?")

Emotional Eating: sensitivity to anticipated emotional benefits of eating ("Do you have a desire to eat when you are depressed or discouraged?")

RESULTS

AEQ \ DEBQ	External Eating	Emotional Eating
Relaxation and Tension Reduction	.316***	.239***
Arousal and Aggression	.244***	.223**
Increased Social Assertiveness	.233***	.069
Physical and Social Pleasure	.229**	.027
Global Positive Changes	.224**	.168*
Sexual Enhancement	.192**	.160*

*p<.05, **p<.01, ***p<.001

CONCLUSIONS

DEBQ External Eating scale was correlated to **all** AEQ scales, while DEBQ Emotional Eating scale was only correlated to **some**, but not all, AEQ scales. This showed that:

- **External eating had a more consistent relationship with alcohol expectancies**. We speculated that **impulsivity**, which is defined as tendency to act without adequate thought, might be implicated in both external eating and drinking behavior[7].
- **Emotional eating had a less consistent relationship with alcohol expectancies**. We speculated that **depressive symptoms** are more tightly associated with sensitivities to food[8] than to alcohol.

AEQ Relaxation and Tension Reduction and AEQ Arousal and Aggression had the highest correlations with DEBQ scales. We inferred that the **anticipatory pharmacological effects of alcohol are strongly associated to those of food**.

Non-significant correlations between DEBQ Emotional Eating scale and AEQ Increased Social Assertiveness and AEQ Social and Physical Pleasure might imply that **social factors driving eating and those driving alcohol use might be less associated**.

In general, the results support our hypothesis that **food expectancy is positively correlated to alcohol expectancy**

Intervention efforts for overeating and binge drinking among college students should thus consider the **possibility that addressing one of the two problems might directly or indirectly address the other**.

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LESSONS LEARNED FROM AIRWAY PRESSURE RELEASE VENTILATION (APRV)

Lewis J. Kaplan, MD[*†], Heatherlee Bailey, MD, FAAEM[†‡]
Medical College of Pennsylvania-Hahnemann University
Departments of Surgery[*] and Emergency Medicine[†‡], Philadelphia, PA USA

INTRODUCTION

Airway Pressure Release Ventilation (APRV, a.k.a. BIPAP) has been previously demonstrated to be a useful modality to manage patients with acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS). As this is a fundamentally different mode than conventional cyclic ventilation, we reviewed a single institution's experience with APRV to determine safety, complication detection, and efficacy at resolving hypoxemia and hypercarbia.

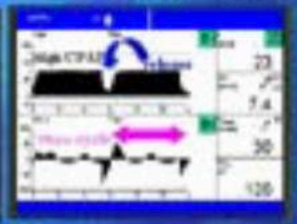
METHODS

Consecutive patients transitioned from either volume or pressure targeted ventilation to APRV (Dräger Evita 4 Pulmonary Workstation) at a University hospital surgical ICU were retrospectively reviewed. Patients initially ventilated with APRV were excluded. Initial ARPV settings to correct for hypoxia ($pO_2 \leq 60$ torr on $FIO_2 \geq 0.9$) were a P_{High} at the prior plateau pressure, a T_{High} of 6.0 sec and a T_{low} of 0.8 sec. Hypercarbic ($pCO_2 \geq 55$ torr and $pH \leq 7.3$) patients were set at a T_{High} of 5.0 sec and a T_{low} of 1.0 sec. Settings were adjusted to resolve hypoxemia and hypercarbia. IRB approved abstracted data included principal diagnoses, ventilation parameters, laboratory values and ventilator associated complications. Data before and after APRV were compared using a two-tailed paired t-test or Chi-square as appropriate; significance was assumed for $p < 0.05$ ($^{\alpha}$).

RESULTS

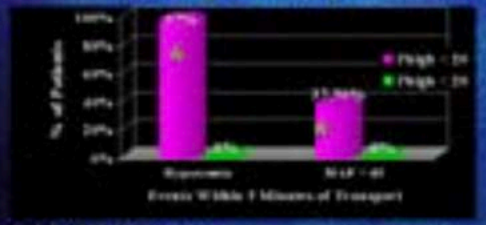
Demographics

APRV

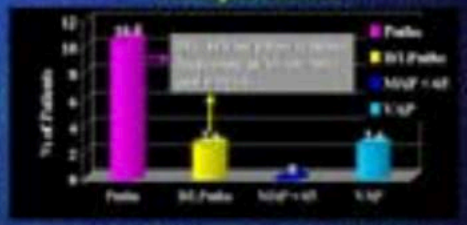


Element	Value
% Hypoxemia	89%
% Hypercarbia	12%
Time to $S_pO_2 \geq 92\%$	7 ± 4 min
Time to $FIO_2 \leq 0.6$	5.2 ± 0.9 hr
Time to $pCO_2 \leq 40$ torr	42 ± 7 min
Time to max Δ pCO_2	76 ± 12 min
Mean change in V_t	-3.3 ± 0.9 L/min[α]

Transport Safety



Complications



CONCLUSIONS

1. APRV is a safe rescue mode for hypoxemic or hypercarbic respiratory failure and requires a significantly lower V_t than conventional ventilation.
2. Decreasing release phase volumes and a rising pCO_2 are strong indicators of pneumothorax in a patient on APRV. Routine end-tidal CO_2 monitoring is recommended.
3. Preparation for safe intra-hospital transport may be keyed to the P_{high} required for oxygenation and ventilation. Patients requiring a $P_{high} > 20$ cm H_2O should be transported on the ventilator.

Diverging aspects of HDAC inhibitors: transcription and metabolism

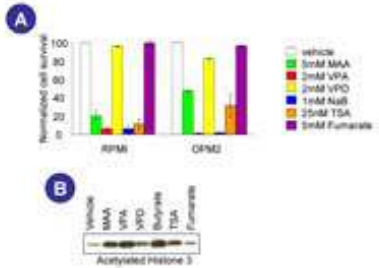
Suzanne E. Wardell, Olga R. Ilkayeva, Christopher B. Newgard, Huey-Jing Huang and Donald P. McDonnell
Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710

Abstract

Multiple myeloma is a hematological neoplasm caused by an expansion of malignant plasma B cells. Standard treatment includes corticosteroids, which induce apoptosis of the myeloma cells, but frequently results in resistance. Experimental alternative therapies for myeloma include histone deacetylase inhibitors (HDACi). We find that valproic acid (VPA), an HDACi widely used to treat seizures, efficiently induces apoptosis in myeloma cells. While HDACi can potentiate transcriptional activity of steroid hormone receptors (1), VPA affects myeloma cells independent of glucocorticoid receptor activity and efficiently induces apoptosis regardless of glucocorticoid resistance. HDACi are known to induce apoptosis in hematopoietic tumor cells concurrent with induction of p21 and TRAIL death ligand (2,4). In addition, HDACi's rapidly reduce mRNA and protein expression of growth factor receptors associated with growth and suppression of apoptosis in myeloma, including interleukin-6 receptor (IL-6R) α, fibroblast growth factor receptor (FGFR) 3, and B cell maturation antigen (BCMA) (2,3,5). However, HDACi have additional activities independent of their role in transcription. HDACi treatment reduces the available cellular pool of acetyl CoA. In response, the cells turn to protein degradation and metabolism of amino acids for energy, decreasing cellular levels of individual amino acids by up to ten fold. mRNAs encoding arginase II and carbamoyl phosphate synthase (CPS1), enzymes involved in amino acid metabolism and nitrogen clearance, are correspondingly induced after 24 hrs of HDACi treatment. Supplementation with additional amino acids increases the induction of apoptosis, suggesting that buildup of nitrogen metabolites of amino acid degradation contributes to HDACi mediated apoptosis. Organic acid analysis of cells following HDACi treatment indicates a significant drop in α-ketoglutarate, a key component of the TCA cycle that is also a required intermediate in the metabolism of amino acids and β-oxidation of fatty acids. These data together indicate that while HDACi can modulate transcription of select genes, an additional facet to their action is the profound effect on cellular metabolism initiated by a significant reduction in the cellular pool of acetyl CoA.

Results

Figure 1. HDACi induce apoptosis in myeloma cells regardless of dexamethasone sensitivity. (A) Multiple myeloma cells lines RPMI (dex sensitive) and OPM2 (dex resistant) were treated for 96hrs in complete media with the indicated compounds – methoxyacetic acid (MAA), valproic acid (VPA), valpromide (VPD), sodium butyrate (NaB), trichostatin A (TSA), or fumarate. Apoptosis was analyzed by annexin-PE and 7-AAD staining followed by flow cytometry. (B) Lysates of OPM2 cells treated for 24 hrs with the indicated compounds were analyzed for HDAC activity by Western blot analysis of acetylated histone 3.



Results

Figure 2. HDACi treatment rapidly down-regulates mRNA and protein expression of growth factor receptors previously demonstrated to participate in myeloma cell growth and resistance to apoptosis. Three indicated myeloma cell lines were treated 0-24 hrs with 2mM VPA followed by (A) Western blot or (B) real time qPCR analysis of lysates or RNA, respectively, analyzing expression of growth factor receptors demonstrated to be essential for each respective cell line.

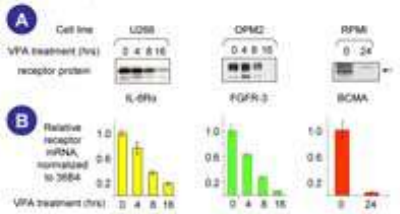


Figure 3. HDACi treatment reduces the cellular pool of available acetyl CoA. (A) Cellular processes that tightly regulate cellular levels of acetyl CoA through its contribution and utilization. (B) OPM2 cells were treated for 44hrs with VPA (2mM), MAA (5mM), butyrate (NaB – 1mM), suberoylanilide hydroxamic acid (SAHA – 5uM), or Dexamethasone (Dex – 100nM). Cells were lysed by sonication and MS/MS analysis was performed on clarified lysates to determine levels of acetyl carnitine (in equilibrium with acetyl CoA) The reduction of acetyl carnitine indicates a significant drop in the normally tightly regulated levels of acetyl CoA.

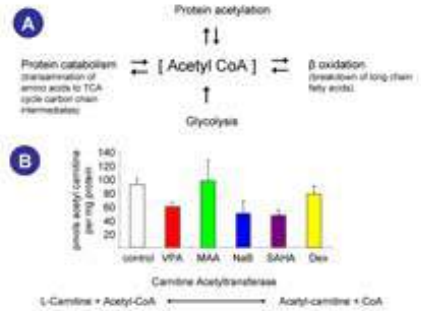
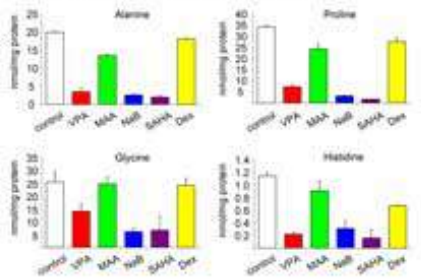


Figure 4. HDACi treatment increases metabolism of amino acids. Lysates from Figure 3B were examined using MS/MS analysis for amino acid content. No significant change in glycolytic intermediates or long chain fatty acids was observed, while a significant reduction in the levels of all 17 amino acids analyzed was evident, four of which are shown below. These finding indicate that OPM2 cells preferentially utilize transamination to replace acetyl CoA.



Results

Figure 5. Toxicity of amino acid metabolism in the presence of HDACi suggests a buildup of nitrogen intermediates. (A) Ammonia created through amino acid degradation is cleared physiologically through the indicated pathways. No significant production of urea was measured from HDACi-treated cells in culture (data not shown). (B and C) OPM2 cells were treated for 96hrs with VPA (2mM) in the presence of absence of the indicated compounds. Apoptosis was analyzed as in Figure 1. Increased apoptosis in the presence of VPA and supplemental amino acids suggests a buildup of a toxic nitrogen product. (D) OPM2 cells were treated 24 hrs with the indicated compounds as in Figure 3, and expression of arginase II and carbamoyl synthetase 1 (enzymes involved in nitrogen disposal) were analyzed by real time qPCR.

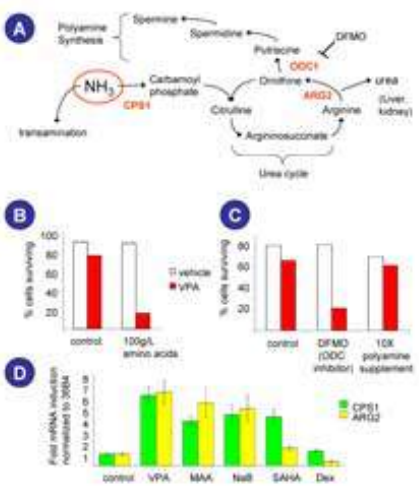
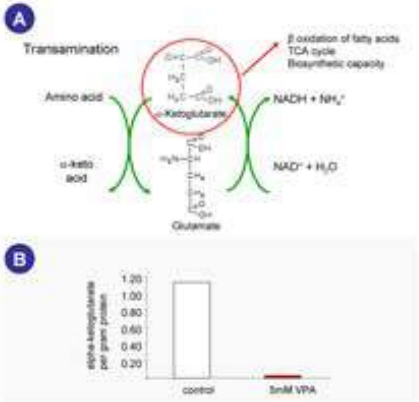
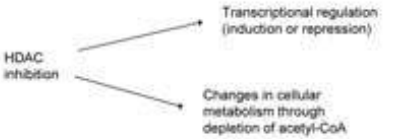


Figure 6. Toxicity of amino acid metabolism in the presence of HDACi suggests a buildup of nitrogen intermediates. (A) Model of transamination, the process by which amino groups are removed from amino acids to allow metabolism of the carbon skeleton. (B) NB4 cells were treated 24 hours with or without 1mM VPA prior to acidic extraction of the cells followed analysis of organic acids.



Conclusion



- HDAC inhibitors effectively induce apoptosis in multiple myeloma cell lines as well as myeloma patient isolates (not shown), and their ability to induce apoptosis appears to be proportional to their activity as HDAC inhibitors.
- In addition, HDAC inhibitors rapidly down-regulate growth factor receptors important for myeloma cell growth and survival, at both the mRNA and protein levels.
- HDAC inhibitor treatment reduces levels of acetyl carnitine, suggesting a corresponding reduction in the available cellular pool of acetyl CoA that may result in stalling of the TCA cycle and utilization of amino acids by the cell as an energy source.
- Breakdown of amino acids to salvage the carbon chains for energy forces the cell to dispose of ammonia released by deamination of the amino acids. Myeloma cells utilize both transamination and production of polyamines to sequester the released nitrogen.
- The contribution of the polyamine pathway may be small, because addition of excess polyamines does not significantly affect cell survival itself or the apoptotic activity of VPA. However, inhibition of the pathway increases the apoptotic potential of VPA, likely because of additional use of transamination.
- While transamination potentially sequesters the ammonia produced, it depletes the cell of α-ketoglutarate, further crippling the TCA cycle and ultimately preventing transamination.
- Physiologically, amino groups would be incorporated into arginine, glutamine, or alanine, and ultimately converted to urea in the liver. However, the rate of ammonia production that may be occuring in myeloma cells may contribute to the clinical effectiveness observed for HDAC inhibitors.
- Because cancer cells, as opposed to normal cells, rely primarily on glycolysis for energy and do not significantly utilize β-oxidation of fatty acids even in the presence of oxygen, the effect of HDACi on metabolism may ultimately be specific to cancer cells, accounting for the low toxicity observed clinically.

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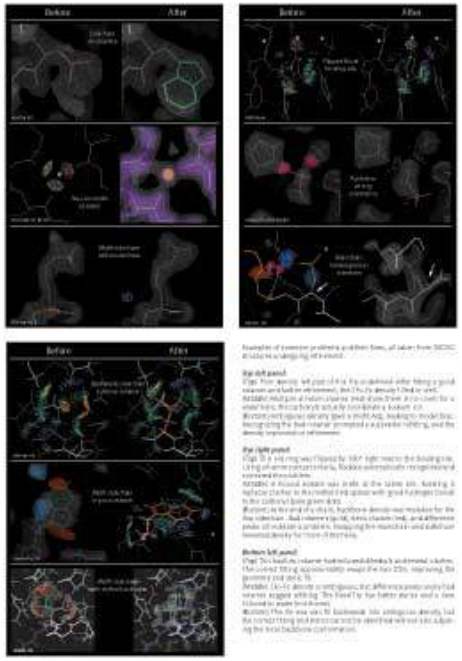
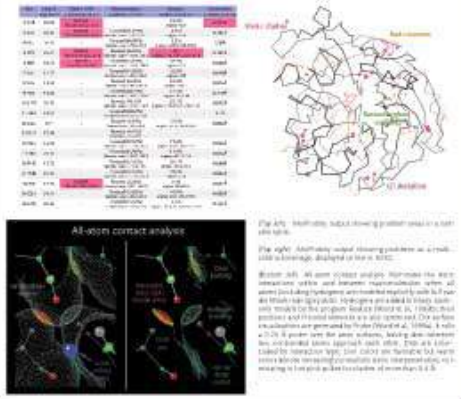
MolProbity

Ian W. Davis, W. Bryan Arendall III, Laura W. Murray, Jeremy N. Block,
Jane S. Richardson, David C. Richardson
Department of Biochemistry, Duke University, Durham, NC

Things that go "bump" in proteins:

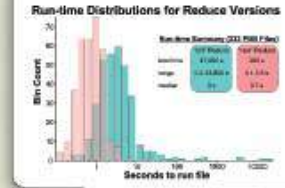


...and how to find them:

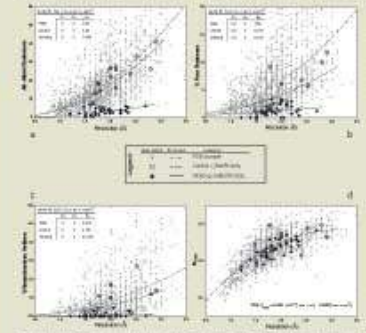


Overview

New! Faster Hydrogens!



Results @ SECSG



Results with RNA

http://kinemage.biochem.duke.edu

Conclusions & References

Maastricht

A Compartment Model for the Transport and Storage of Folate

Mentor: Dr. H. Frederik Nijhout Biology Department, Duke University
Tiffany J. Chen

Objectives:

- To estimate the average pool sizes of folate distributed within the plasma, the cell, and the mitochondria.
- To develop mathematical models that represent these pool sizes and mimic real bodily responses to day-to-day changes in diet and metabolism.
- To test these models against experimental data, as well as make predictions.

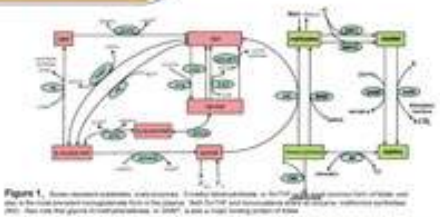


Figure 1.

Background:

Folate, or vitamin B9, is important for the synthesis of thymidine, a pyrimidine, and purines. Deficiency in folate is associated with megaloblastic anemia, cancer, cardiovascular disease, neurological disorders, and neural tube defects in infants. Folate metabolism provides the rate-limiting step for DNA synthesis and DNA and histone methylation (Fig. 1). Reduced folate status affects these critical cellular activities and also increases the level of homocysteine, a highly reactive amino acid that is associated with cell damage. It has been shown that increased folate intake by pregnant women can help reduce the risk of infant neural tube defects, presumably due to a reduction in plasma homocysteine levels. Folate metabolism occurs within cells, but their levels are typically measured in the plasma. It is therefore critical to understand the relationship between the concentrations of folate in the plasma and the cell.

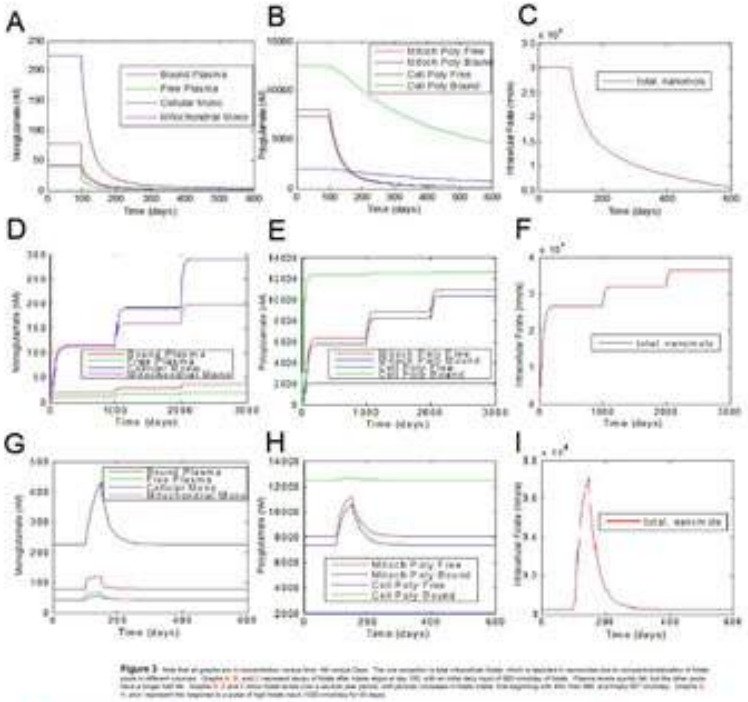


Figure 3

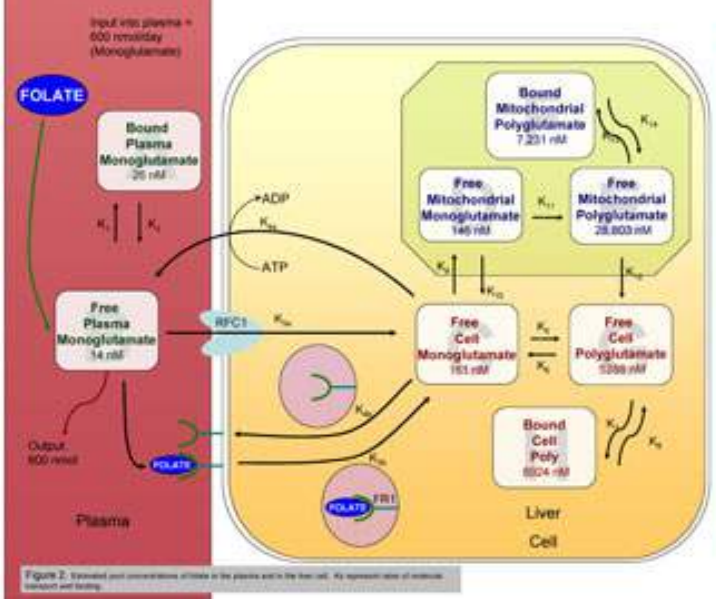


Figure 2.

Methods:

Various pool values for plasma and intracellular folate were collected from experimental data (Figure 2). We made predictions for pool values that are not readily available. These predictions were based on known distribution of the various folate pools within the body. For example, 50% of body folate is stored in the liver – the liver contains 2 compartments. These are the cytosol and the mitochondria, each containing three general pools, monoglutamate, free polyglutamate, and bound polyglutamate. These individual pools have different proportions in the cytosol and the mitochondria.

After pool values were established, we assumed that transport of molecules between pools were based on first-order mass-action kinetics. We used Michaelis-Menten equations for the bound polyglutamate pools, because there is a limited amount of protein that will bind to folate – mainly glycine N-methyltransferase (GNMT), one of the enzymes in the methionine cycle (Fig. 1). In addition, we used Michaelis-Menten kinetics for the transport of folates in and out of the cell via Reduced Folate Carrier 1 (RFC1), Folate Receptor 1 (FR1), and an ATP-dependent exporter (Fig. 2).

Rate constants, or k-values, were calculated by assuming certain fluxes between pools. These fluxes were determined by known rates of gain and loss of folate in different compartments where these rates were known, and by adjusting the relative rates of input and output to obtain the right pool sizes between compartments in cases where the absolute rates were not known.

Experiments were performed by varying folate input. These were performed to determine half-lives of the pools, as well as to determine how the pools reacted to example experimental conditions from the literature.

Results:

1. The Model

The model correctly simulates the sizes of the folate pools in the various compartments, including the cytosol, the mitochondria and the fractions bound to proteins in those compartments.

2. Predicted half-life of folate.

After we removed the constant input of folate into the system, all pools diminished over time, some more quickly than others (Figures 3A, 3B). We can also see in figure 3C that the approximate half-life for total intracellular folate is 80 days, which is close to predicted values of around 80-100. Bound polyglutamate seems to decrease at a much slower rate than the other pools.

3. Reaching steady-state values.

The time for the total intracellular pools to reach steady-state typically ranged from 300 to 500 days, which corresponds well with data from the literature. Consistent with the idea that there is a correlation between intracellular folate pool size, polyglutamation, and protein binding, all types of polyglutamate pools do in fact take longer to reach a steady-state value (Figures 3D, 3E).

4. Response to pulsed folate input.

The input of folate was increased to 1000 nmol/day for 50 days. Model plasma levels were quick to rise and fall with the sudden changes, which predicts that free as well as loosely bound monoglutamates will react quickly to changes in folate intake (Fig. 3G). Out of the polyglutamate pools, the model predicts that both bound pools will take longer to return to steady-state, although the mitochondrial bound polyglutamate will take the longest of all of the pools (Fig. 3H).

Conclusions:

We have constructed a mathematical compartment model for folate that takes into account the different methods of transport, as well as retention in the plasma, cell, and mitochondria. We have compared the output of this model with results from current experiments, and have found that the model accurately simulates data from the literature. This model will for the foundation for future studies on the metabolism, transport and sequestration of folates under various genetic and environmental conditions.

Many thanks to Dr. H.F. Nijhout for his guidance and his patience, as well to both Dr. Nijhout and Dr. M.C. Reed for the use of their folate and methionine cycle programs and diagrams. Initial research was supported (in part) by a Howard Hughes Summer Research Fellowship.

Key References

27-hydroxycholesterol is a Novel Endogenous Regulator of Estrogen Receptor Activity

Carolyn D. DuSell and Donald P. McDonnell.
Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710

Introduction

There are two isoforms of the estrogen receptor (ER), ERα and ERβ, which upon ligand binding regulate the transcription of target genes. In general, it is thought that the proliferative effects of estrogens are mediated primarily through ERα, whereas the anti-proliferative ones are through ERβ. There are three endogenous estrogen receptor agonists that activate both ERα and ERβ, the most potent being 17β-estradiol (E2).

The majority of breast cancers express ERα and rely on E2 as a growth stimulus, prompting the development of therapies aimed at reducing E2 levels, such as aromatase inhibitors. Unfortunately, many breast cancers become resistant to aromatase inhibitors, but continue to rely on ERα for growth. Tumor-infiltrating macrophages (TAMs), which are associated with increased tumor growth and decreased patient survival, possess high cytochrome P450 27A1 (CYP27A1) enzymatic activity and are therefore capable of producing 27-hydroxycholesterol (27HC), which we have shown to be a novel endogenous partial agonist for ER. It is therefore possible that macrophage infiltration of a breast tumor provides an alternate estrogenic ligand to promote tumorigenic behavior. Furthermore, the production of 27HC by either TAMs and tumor epithelial cells, and the estrogenic activities of 27HC, presents a potential mechanism for the development of resistance to aromatase inhibitors. Therefore, understanding the intricacies of the regulation of ER by 27HC and how this impinges on estrogen signaling will have a profound impact on estrogen-regulated biology, especially in breast cancer.

Hypothesis & Objectives

Hypothesis:

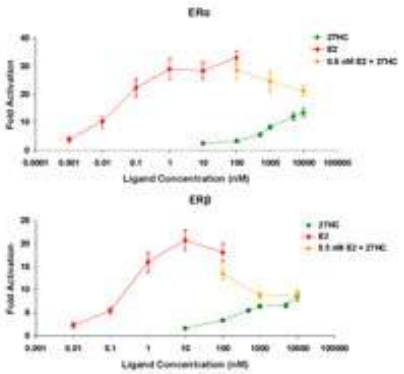
We hypothesize that 27HC is a partial agonist for ERα and ERβ that may contribute to therapeutic resistance in breast cancer.

Objectives:

- Determine whether 27HC is an ER agonist, antagonist, or Selective Estrogen Receptor Modulator (SERM).
- Define the role of 27HC in regulating ERα activity in ERα-positive breast cancer cell lines.

Results

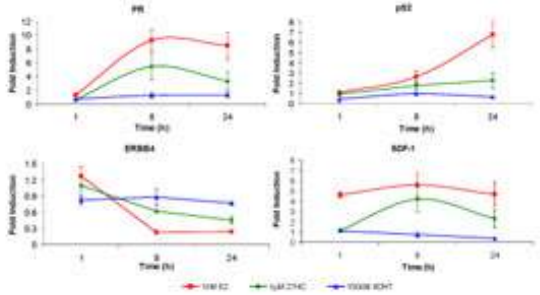
Figure 1. 27HC activates exogenous ERα and ERβ



ER-negative HeLa cells were transfected with pCDNA3.1nv5-ERα or -ERβ, a 3xERE-TATA-luc reporter, and a CMV-β-gal transfection control for 24 hours. Cells were treated with vehicle or increasing concentrations of E2 or 27HC for 22-26 hours, then harvested and assayed for luciferase and β-gal expression. Data is presented as mean ± SEM for three independent triplicate experiments.

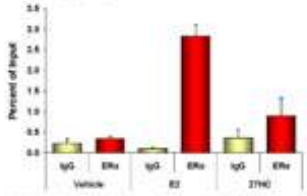
Results

Figure 2. 27HC regulates endogenous ERα target genes



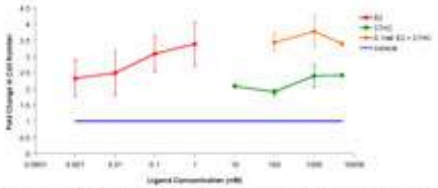
ERα-positive MCF-7 cells were plated in phenol-red free media containing 8% charcoal-stripped FBS at 2x10^5 cells/ml. After 48 hours, the cells were treated with vehicle or ligand. At the indicated time, cells were harvested for RNA isolation and ER target gene expression was analyzed by RT-PCR. Threshold cycle values were normalized to the housekeeping gene 36B4. Data was then normalized to vehicle and is presented as mean ± SEM for three independent experiments.

Figure 3. 27HC treatment leads to recruitment of ERα at the estrogen response element in the pS2 promoter



MCF-7 cells were plated in phenol-red free media containing 8% charcoal-stripped FBS at 3.5x10^5 cells/ml. After 72 hours, the cells were treated with vehicle, 100nM E2, or 10μM 27HC for 45 minutes. After cross-linking DNA and protein, protein and associated chromatin was harvested and subjected to immunoprecipitation with either IgG or ERα antibody. Following immunoprecipitation, cross-linking was reversed and DNA harvested for analysis by RT-PCR. Data is presented as the percent of DNA immunoprecipitated with ERα compared to the total amount of DNA present in the input.

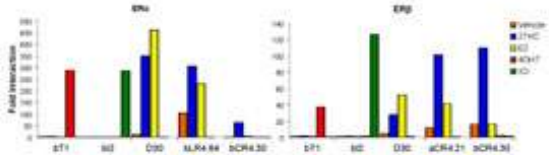
Figure 4. 27HC stimulates proliferation in ERα-positive breast cancer cells



MCF-7 cells were plated in phenol-red free media containing 8% charcoal-stripped FBS at 5x10^4 cells/ml. After 48 hours, the cells were washed and the media was replaced with serum-free media for 24 hours. Cells were then treated with vehicle, E2, or 27HC for 48 hours. Treatment was replenished after 48 hours. On day 8, the increase in cell number was assayed using a DNA dye. Data is presented as the mean ± SEM for three independent triplicate experiments.

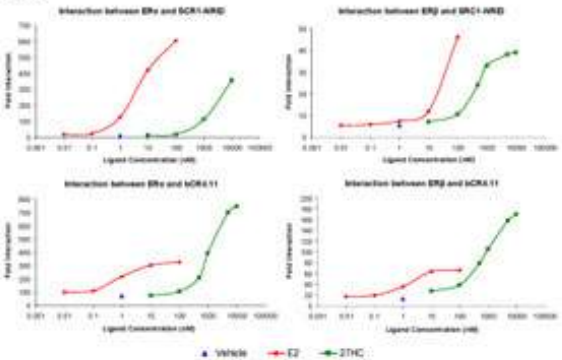
Results

Figure 5. 27HC induces a unique conformation of ERα and ERβ



ER-negative HepG2 cells were transfected with ER-interacting peptides fused to Gal4 to use as conformational probes of the receptor structure. VP16-ERα or ERβ, a 5xGAL4-TATA-luc reporter, and a CMV-β-gal transfection control were co-transfected with the peptides for 24 hours. Cells were subsequently treated with vehicle, 10μM 27HC, 100nM E2, 100nM 4OHT, or 100nM ICI for 22-26 hours. Cells were then harvested and assayed for luciferase and β-gal expression. Data is presented as the mean of one representative triplicate experiment.

Figure 6. 27HC allows for differential peptide recruitment to ERα and ERβ



HepG2 cells were transfected with VP16-ERα or ERβ, Gal4-peptide, 5xGAL4-TATA-luc reporter, and CMV-β-gal transfection control for 24 hours. Cells were subsequently treated with vehicle or increasing concentrations of 27HC or E2 for 22-26 hours. Cells were then harvested and assayed for luciferase and β-gal expression. Data is represented as the mean of one representative triplicate experiment.

Conclusions

- 27HC is a novel partial agonist that regulates both ERα and ERβ.
- 27HC stimulates proliferation in an ERα-positive breast cancer cell line.
- 27HC induces a unique conformation of both ERα and ERβ that allows for differential recruitment of ER-interacting peptides.

Future Directions

- Determine how 27HC influences breast tumor growth.
- Establish which cells produce 27HC within the tumor microenvironment and determine whether this ligand acts in an autocrine or paracrine manner.

Acknowledgements

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O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

Joshua Smith[1], George C Bobustuc[1], Rafael Madero-Visbal[1], Jimmie Colon[1], Beth Isley[1], Jonathan Ticku[1], Kalkunte S. Srivenugopal and Santhi Konduri[1]

[1]Cancer Research Institute of M.D Anderson Cancer Center Orlando [2]Texas Tech University Health Sciences Center, Amarillo, TX

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 [O⁶-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 $p21^{cip1}$ 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 $p21^{cip1}$ 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 psuedosubstrate 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.

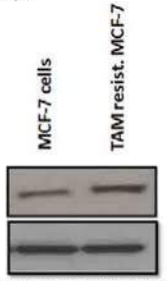


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were prepared, proteins were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

O⁶-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.

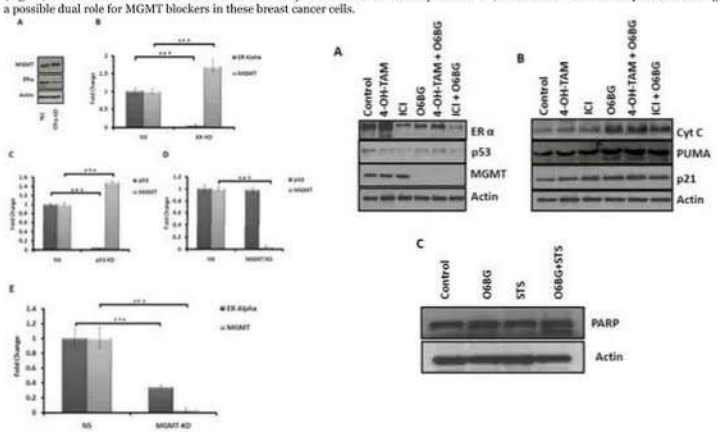


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total proteins were isolated and ERα and MGMT expression was determined by western blot analysis. MGMT protein was significantly increased 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).

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 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. All these treatments significantly increased the $p21^{cip1}$ 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.

O6-Benzylguanine Modulated Transcriptional Targets in Tamoxifen Resistant Breast Cancer Cells: The effect of combination therapy on endogenous MGMT mRNA levels ws aso stuid. 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).

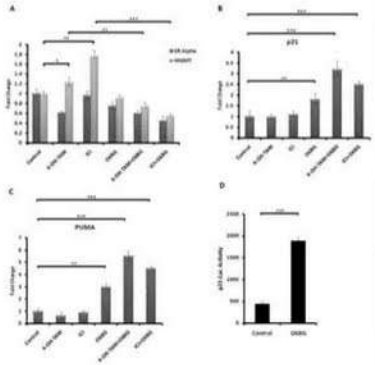
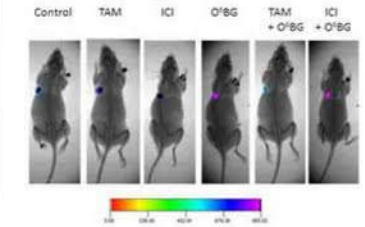


Figure 4. Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) for 48h and later 4-OH tamoxifen and ICI (1μM) was either alone or in combination with BG and 24h later cells were harvested and total RNA was isolated. (A) MGMT and ERα (B) p21 transcription (C) 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 construct 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).



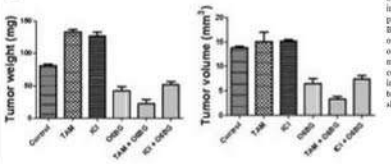
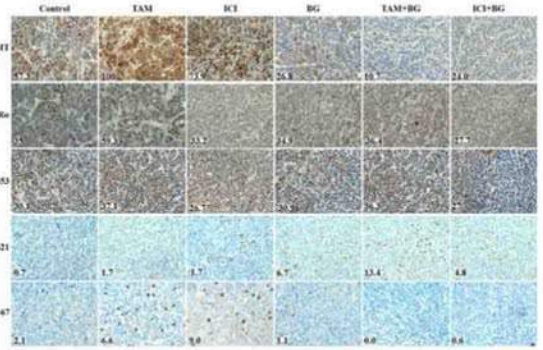


Figure 5. Tumors were harvested from control mice and mice treated with tamoxifen/ICI, BG, or both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ERα, p53, p21 and ki-67. Tumors from mice treated with BG either alone or in combination with tamoxifen or ICI had a significant 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 09BN-10 for their funding of this project.

Serological evaluation of the exposure to *Anopheles gambiae* bites and malaria transmission by a multiple antigen assay

Elena Ambrosino[1], Chloé Dumoulin[1], Pierre Druilhe[2], Thierry Fusai[1], Franck Remoué[3], Aissatou Toure-Baldé[4], Adama Tall[4], Jean-François Trape[5] and Christophe Rogier[1]

[1]URBEP and UMR6236-IRBA,BP60109,13262 Marseille Cedex 07, France;[2]Insitut Pasteur, Paris, France; [3]IRD, Montpellier, France;[4]Institut Pasteur de Dakar, Dakar, Senegal; [5]IRD, Dakar, Senegal and UMR6236, Marseille, France

Objectives:

Assessment of malaria exposure and immunity is an important step in the fight against the disease. Increased frequency in malaria infection in non immune travelers under anti-malaria chemoprophylaxis, as well as spreading of malaria elimination programmes in endemic countries raised new issues in such process. Notably, monitoring malaria immunity has become more difficult in people with weak antibody titres or taking medications against *Plasmodium* blood stages. Indeed, commonly available techniques in malaria seroepidemiology have limited sensitivity, as anti-circumsporozoite protein (CSP) antibody (Ab) detection by ELISA, or evaluate the immune response against blood stages of the parasite. Thus, the aim of this study was to develop a sensitive tool to examine the exposure to the vector *Anopheles gambiae*'s bites or to *Plasmodium falciparum* malaria transmission, despite anti-malaria treatment intake. Ab responses to multiple *P. falciparum* pre-erythrocytic antigens (Ags) and one *A. gambiae* saliva protein were investigated in people living in African villages and in travelers.

Study population:

NON EXPOSED	TRANSIENTLY EXPOSED	EXPOSED 253 people living in 3 Senegalese villages		
		Diama low malaria transmission ~2 infective bites/ person/yr 85 people (38 adults)	**Ndiop** moderate malaria transmission ~ 20 infective bites/ person/yr 86 people (40 adults)	**Dielmo** high malaria transmission ~ 200 infective bites/ person/yr 82 people (45 adults)
21 never exposed European adults	85 French soldiers traveling shortly to malaria endemic countries (travelers)			

Methods:

A Luminex-based multiplex assay was optimized to simultaneously test Abs to 16 Ags:

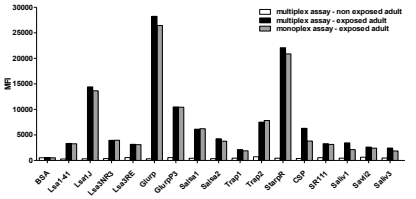
13 pre-erythrocytic *P. falciparum*-specific Ags belonging to the following proteins: Lsa1, Lsa3, Glurp, Salsa, Trap, Starp, CSP and Pf11.1

3 Ags specific for the *A. gambiae*'s saliva protein gSG6: Saliv1, Saliv2 and Saliv3

Synthetic Ags were covalently coupled to BSA-coated beads. The beads were then incubated with diluted sera.

Results:

1 - Multiplex assay technology allows comparison of exposed and non exposed serum samples and provides results equivalent to monoplex assay



Multiplex assay optimal experimental conditions:

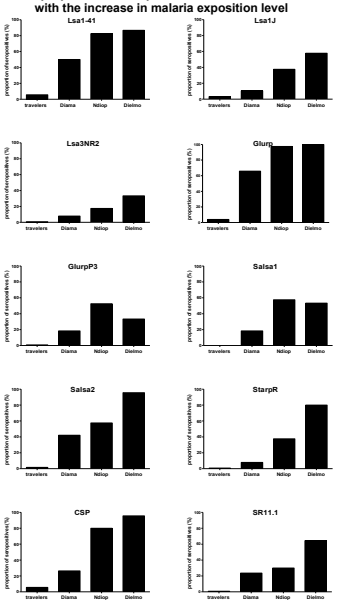
-0.3 nmol Ag concentration for bead coating;

-1:100 sera dilution.

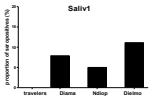
A serum from an exposed adult from Dielmo (black bars) is compared to one from a non exposed adult (white bars). **The assay allows a clear separation of Ag-specific mean fluorescence intensities between exposed and non exposed.**

The serum from the exposed individual from Dielmo was also tested for the same antibody responses by monoplex assay with single Ag-coated beads independently (gray bars). **The results with monoplex and multiplex assays are nearly identical with a correlation coefficient R= 0.9896.**

2 - The proportion of seropositives individuals increases with the increase in malaria exposition level

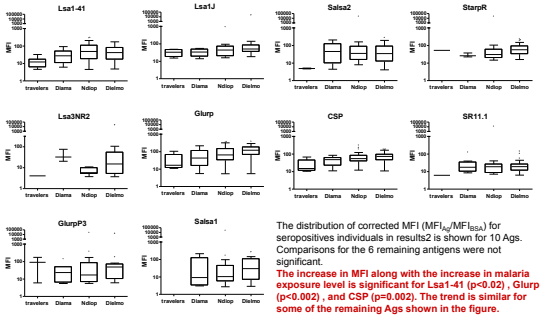


Adults from the transiently exposed and the exposed groups were tested for all 16 Ags. For the Ags shown above, the proportions differed significantly between groups (p≤0.01). For the remaining Ags the proportion of seropositives was too low for the differences to be significant. Nevertheless for the *A. gambiae*'s Saliv1 Ag (right) the proportion of seropositives was higher in exposed (the three villages together) compared to transiently exposed (travelers) adults (p=0.0035).



The proportion of seropositives increased significantly with the increase in the level of malaria endemicity to which people were exposed.

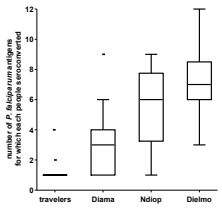
3 - Mean fluorescence intensity (MFI) response of seropositives increases in individuals more exposed to malaria



The distribution of corrected MFI (MFI_{Ag}/MFI_{BSA}) for seropositives individuals in results2 is shown for 10 Ags. Comparisons for the 6 remaining antigens were not significant.

The increase in MFI along with the increase in malaria exposure level is significant for Lsa1-41 (p<0.02) , Glurp (p<0.002) , and CSP (p=0.002). The trend is similar for some of the remaining Ags shown in the figure.

4 - The mean number of *P. falciparum* antigens for which individuals were seropositives is higher in people more exposed to malaria



The mean number of recognized Ags increased significantly with the increase in exposure to malaria transmission (p<0.0001).

The higher the malaria exposure to malaria transmission, the higher the mean number of Ags for which people were seropositive.

Conclusions:

The multiplex assay provides a useful tool to test immune responses to multiple Ags in large populations, even for small amounts of serum, or weak antibody titers, as in case of travelers.

The comparison of immune responses among different Senegalese villages and travelers enables the identification of exposition markers.

Lastly, the association of Ab responses to malaria endemicity levels provides a way to monitor exposure in autochthonous individuals, as well as in non-immune travelers, thus assessing the disease transmission and risk in the new eradication area.

email contacts: elena.ambrosino@gmail.com - christophe.rogier@wanadoo.fr

Maastricht University

Inorganic Biochemistry of Iron Proteins

Jared J. Heymann, Claire J. Parker Siburt, Katherine D. Weaver, and Alvin L. Crumbliss

Duke University – Department of Chemistry – Durham, NC

Purpose:

To study iron protein biochemistry from the perspective of the iron

Protein = Ligand

The Iron Paradox

Iron is needed for nearly every living cell

Iron is toxic and can produce reactive oxygen species & must be controlled

Iron Abundance In Humans

45-55 mg/kg in humans

70% in Red Blood Cells (Hemoglobin)

0.1% in Transferrin

However

Turnover of transferrin iron is ~30 mg / 24 hours with 80% of this Fe being transported to the bone marrow for hemoglobin synthesis

Bacteria can also target Tf as a source of iron

Proteins act as the 1st & 2nd coordination shell of iron and can modulate the kinetics and thermodynamics of reaction.

Techniques:

Spectroelectrochemistry
UV-Visible Spectroscopy
Fluorescence Spectroscopy
Difference Spectroscopy
Stopped-Flow Kinetics
SUPREX

TRANSFERRIN

A mechanistic study of the iron release by receptor-bound transferrin using spectroelectrochemistry

FERRIC BINDING PROTEIN

Role of a synergistic anion on modulating iron uptake in a bacterial transferrin by pathogenic bacteria: A study in kinetics and thermodynamics

HEMOGLOBIN

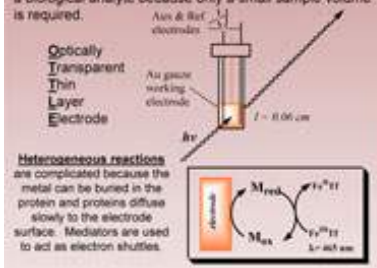
Effects of subunit cross-linking on hemoglobin oxidation states determined by spectroelectrochemistry

Transferrin

How is Fe^{3+} removed from Tf when $K_d = 10^{-22}$?

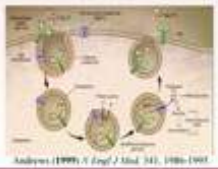
Hypothesis: When transferrin binds to a receptor, the reduction potential shifts into a biologically relevant range.

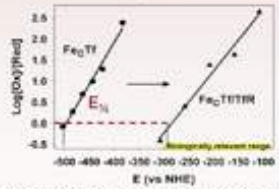
Spectroelectrochemistry utilizes a short pathlength created by an *OTTLE* cell, to measure the variations in visible spectra as the analyte is oxidized or reduced by an externally applied potential. This technique is ideal for a biological analyte because only a small sample volume is required.



Heterogeneous reactions are complicated because the metal can be buried in the protein and proteins diffuse slowly to the electrode surface. Mediators are used to act as electron shuttles.

Iron loaded Tf binds to the human **receptor** and is taken into the cell by **endocytosis**. Tf releases iron inside in the endosome where the conditions are acidic (Andrews, 1999). However, the chemical mechanism is unclear. The reduction potential of Fe-Tf in the plasma (pH 7.4) and in the endosome (pH 5.8) is too low for biological reducing agents.



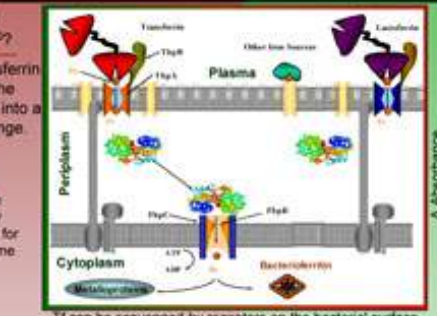


Using spectroelectrochemistry, we measured a positive shift in redox potential of Fe-Tf upon receptor binding.

The transferrin receptor is capable of shifting the reduction potential into the range accessible by biological reducing agents, allowing for a redox mechanism of Fe release.

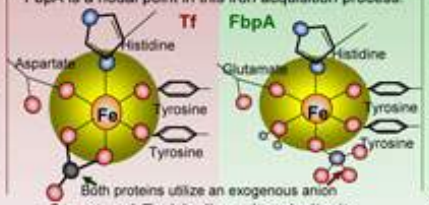
Transferrin not only supplies iron to mammalian cells, but has been identified as a target for pathogens to mechanistically steal iron from their host.

Kraiter, Zak, Aisen and Crumbliss. (**1998**) Inorg. Chem. 37, 964.
Dhungana, Taboy, Anderson, Vaughan, Aisen, Mietzner and Crumbliss. (**2003**) *PNAS* 100, 3659-64.
Dhungana, Taboy, Zak, Larvie, Crumbliss and Aisen. (**2004**) Biochem. 43, 205-9.



Tf can be scavenged by receptors on the bacterial surface

FbpA is a nodal point in this iron acquisition process.



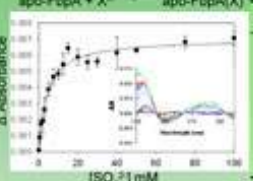
Both proteins utilize an exogenous anion

Structural Fe binding site similarity between Tf and FbpA

Like Tf, FbpA requires a synergistic anion to facilitate tight iron binding, which may play a role in ease and rate of Fe uptake by the bacteria.

1. FbpA acts as an anion binding protein

apo-FbpA + X^{n-} ⇌ apo-FbpA(X)

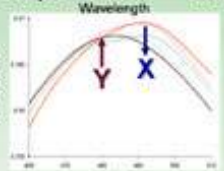


Anion	K_a (mM)
Sulfate	4.1
Phosphate	2.3
Arsenate	2.1
NTA	2.1
Oxalate	0.21
Citrate	0.089
Pyrophosphate	0.018

Anion preorganizes the Fe binding site

2. FeFbpA-X can exchange anions

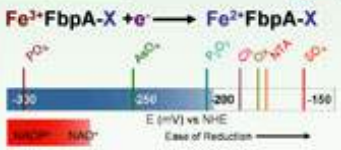
$FeFbpA\text{-}X + Y^{m-} \underset{K}{\rightleftharpoons} FeFbpA\text{-}Y + X^{n-}$



3. Anion identity modulates both thermodynamic stability and redox potential

$Fe^{3+} + FbpA\text{-}X \rightleftharpoons Fe^{3+}FbpA\text{-}X$

$Fe^{3+}FbpA\text{-}X + e^- \longrightarrow Fe^{2+}FbpA\text{-}X$



Redox potential varies by ~140 mV (14 kJ) based on identity of X

Anion	log K
Phosphate	18.6
Arsenate	18.1
Oxalate	17.6
NTA	17.3
Pyrophosphate	17.3
Citrate	17.1
Sulfate	16.2

Thermodynamic stability varies by two orders of magnitude (14 kJ) based on identity of X

Iron transport can occur by a redox or non-redox mechanism in the periplasm. The **thermodynamic stability** and **reduction potential** are both varied by the identity of the synergistic anion. **Kinetically labile** exchange is possible in the diverse anionic conditions of the periplasm.

Heymann, Weaver, Mietzner and Crumbliss. (**2006**) *unpublished*.
Dhungana, Anderson, Mietzner and Crumbliss. (**2005**) *Biochem.* 44, 9606-18.
Roulhac, Powell, Dhungana, Weaver, Mietzner, Crumbliss and Fitzgerald. (**2004**) *Biochem.* 43, 15767-74.
Dhungana, Taboy, Anderson, Vaughan, Aisen, Mietzner and Crumbliss. (**2003**) *PNAS* 100, 3659-64.

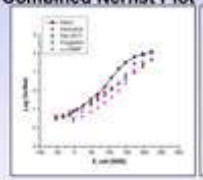
Chemically modified Hb

- Pyridoxalation
- Pegylation
- Conjugation to polysaccharides & proteins

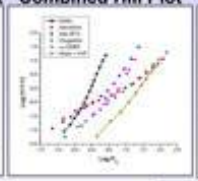
- Polymerization = Intermolecular cross-linking

- Intramolecular cross-linking

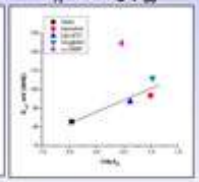
Combined Nernst Plot



Combined Hill Plot



$E_{½}$ vs Log p_{50}



Sample	$E_{½}$ mV (NHE)	Oxidation n_{50}	Log P_{50}	Oxygenation n_{50}	
HbA_0	83	1.3	-0.455	2.28	•
Hemolink	97	0.7	0.994	0.71	•
Dex-BTC	94	0.9	0.618	1.49	•
$OxygliO_2bin$	106	0.9	1.028	1.11	
aa-DBBF	125	1.0	0.461	1.56	

Implications

- Reengineering redox center not necessary
- Drive for autoxidation not thermodynamic
- Structural modifications perturb kinetics by altering exposure of heme cavity

Modified Hb Conclusions

Oxygen Transport
- Loss of cooperativity
- Lower oxygen affinity
- T-state stabilization

Anaerobic Reduction Potentials
- Loss of cooperativity
- $E_{½}$ potential increased vs HbA_0
- Normal physiological range
- Decreased tendency to form metHb

Bonaventura, Henkens, Weaver, Herrich, Pearce, Alayash and Crumbliss. (**2006**) *unpublished*.
Taboy, Bonaventura and Crumbliss. (**2002**) *Meth. In Enzymology* 353, 187-209.
Reiss. (**2001**) Chem. Rev. 101, 2797-2919.

Necrotising fasciitis due to *Mycobacterium kansasii* in a patient with rheumatoid arthritis on infliximab

Roger P. Clark, DO[1]; Eric T. Tolo, MD[2]; Gerald S. Harris, MD[2]; Sarah K. Zimmerman, MEd[2]; Daniel P. McQuillen, MD[2]

[1]Tufts University School of Medicine, Boston, MA; [2]Lahey Clinic, Burlington, MA

Abstract

A 49-year-old man with rheumatoid arthritis (RA) and gout treated for 4 years with infliximab, methotrexate and prednisone (5 mg daily) presented with a painful, swollen left arm. He had a staphylococcal right elbow ulcer after a gout flare and trauma one year earlier. The ulcer recurred and persisted despite antibiotics and intralesional steroids, biopsy was negative for bacteria. Three weeks prior to presentation nonpainful edema with two large distinct, deeply erythematous plaques developed on his left forearm with peripheral satellite papules. Right elbow biopsy taken 3 weeks earlier showed numerous elongated, beaded mycobacteria. Left arm tenderness ensued. On presentation there was fever to 102.5°F, substantial left extensor forearm fluctuance, and a swollen, tender right index finger PIP joint. No cardiac murmurs were present. No history of travel, swimming or aquarium exposure. WBC was 6000/mm³ and ESR 13. Left forearm aspirate yielded 57,500 WBC with 80 PMNs and 4+ AFB on smear. Extensive purulence and necrotising fasciitis were found at surgery, requiring debridement of left extensor and flexor forearm fascia and nonviable muscle. All intraoperative specimens grew *Mycobacterium kansasii* with rifampin MIC 0.12 µg/ml; bacterial and fungal cultures were negative. Blood cultures and echocardiogram were negative. Two weeks later debridement of the right index finger also grew *M. kansasii*. Treatment with rifampin, ethambutol, and isoniazid and VAC dressings followed by skin grafts resolved the infection. Methotrexate 7.5 mg weekly and prednisone 6 mg daily without infliximab were resumed 6 months after presentation. Approximately 40 cases of musculoskeletal infection with *M. kansasii*, mostly septic arthritis in compromised hosts or rheumatologic disease, have been described. This is the first reported case of necrotizing fasciitis due to *M. kansasii* and is notable for its subacute presentation and association with infliximab therapy.

Introduction

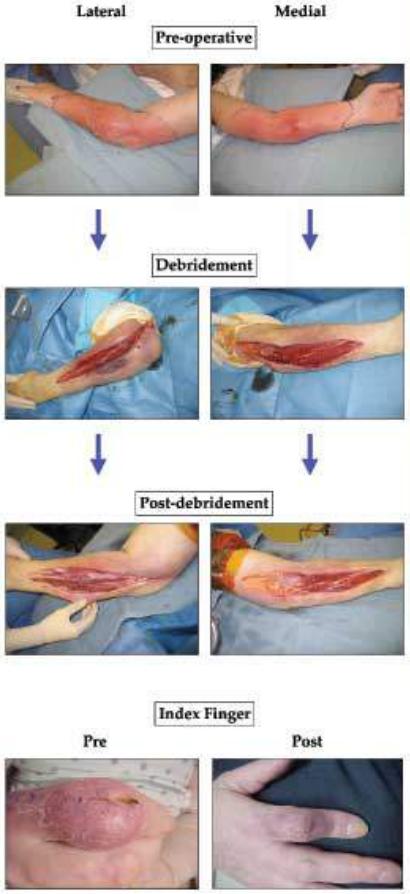
Mycobacterium kansasii is classified as a slow growing mycobacterium that most often causes pulmonary disease that clinically resembles tuberculosis. It is generally considered to be minimally contagious although it is abundant in the environment. In the United States *M. kansasii* disease occurs most commonly in the Midwestern and Gulf Coast states yielding rare case reports of extrapulmonary disease. The most common extrapulmonary disease is septic arthritis, generally affecting the upper extremities. *M. kansasii* musculoskeletal disease appears to be associated with intraarticular steroids as well as conditions and medications that may lead to an immunocompromised state.

Clinical Course

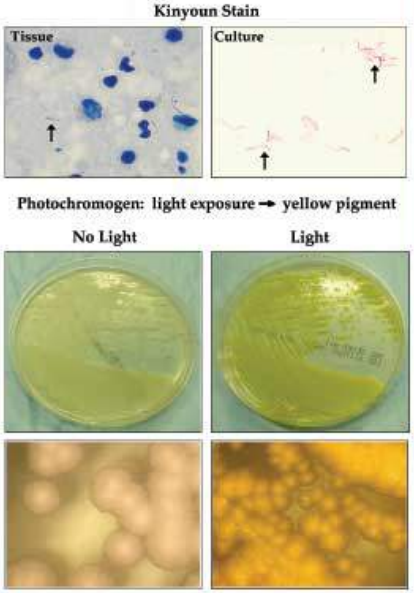
This case involves a 49-year-old man with rheumatoid arthritis, systemically treated for 4 years with infliximab (injections every 5 weeks), methotrexate (7.5 mg weekly) and prednisone (5 mg daily) who developed a relatively indolent left upper extremity soft tissue infection. This was preceded one year earlier by a traumatic staphylococcal right elbow ulcer approximately that healed but later became chronic despite antibiotics and intralesional steroids. Biopsy was negative for bacteria. Three weeks prior to presentation nonpainful edema with two large distinct, deeply erythematous plaques developed on his left forearm with peripheral satellite papules. Right elbow biopsy taken three weeks earlier showed numerous elongated, beaded mycobacteria. Left arm tenderness ensued.

On presentation there was fever to 102.5°F, substantial left extensor forearm fluctuance, and a swollen, tender right index finger PIP joint. No cardiac murmurs were present. No history of travel, swimming or aquarium exposure. WBC was 6000/mm³ and ESR 13. Left forearm aspirate yielded 57,500 WBC with 80 PMNs and 4+ AFB on smear. Due to the severity of the appearance and the progressive nature of the process, he was taken to the operating room. Extensive purulence and necrotising fasciitis were found at surgery, requiring debridement of left extensor and flexor forearm fascia and nonviable muscle. All operative specimens grew *M. kansasii* with rifampin MIC 0.12 µg/ml; bacterial and fungal cultures were negative. Blood cultures and echocardiogram were also negative. Two weeks later debridement of the right index finger yielded tissue that also grew *M. kansasii*.

Surgical Findings



Microbiology Methods and Results



Microbiology Timeline

Day 1: Tissue received. 4+ AFB were seen on direct Kinyoun Stain (Becton, Dickinson and Co., Sparks, MD). Plated directly to liquid and solid mycobacteriological media at room temperature and 37°C.

Day 4: Liquid Mycobacteria Growth Indicator Tube (MGIT) (Becton, Dickinson) at 37°C was positive for acid-fast bacilli. MGIT subbed to selective mycobacteriological media, Mitchinson 7H11 agar (Becton Dickinson).

Day 11: Original solid media positive for a rough, buff colony. Kinyoun stain positive. Culture yielded an atypical mycobacterium whose microscopic characteristics at 1000x were large-sized acid-fast rods with a cross-banding appearance (arrows). Identification as *M. kansasii* made by microscopic and macroscopic morphology, growth rate, pigment production and DNA sequencing. The isolate was rifampin sensitive (MIC 0.12 µg/ml; ARUP, Salt Lake City, UT).

Summary

Mycobacterium kansasii is an acid fast organism that generally causes pulmonary disease and is antigenically similar to *M. tuberculosis*. While an uncommon cause of extrapulmonary infections in humans, the most common manifestations of *M. kansasii* are arthritis and tenosynovitis. There are several risk factors associated with articular *M. kansasii* infections including immunosuppresive medications, rheumatologic conditions such as systemic lupus erythematosis (SLE) or rheumatoid arthritis (RA), psoriasis, AIDS, diabetes mellitus and intraarticular corticosteroids. (1,2)

M. kansasii is environmentally contracted, with the most likely source being tap water, where it has been found to survive for up to a year. (3) This organism is one of the so-called slow growing mycobacteria, and is characterized by being a photochromagen – that is it acquires a yellow pigmentation upon exposure to light. In fact, this bacterium was initially known as the "yellow bacillus" due to this phenomenon.

M. kansasii musculoskeletal infection generally follows a very indolent course, with a mean time to diagnosis of 14 - 17 months. Laboratory studies such as elevated leukocyte count and ESR are not helpful and PPD is often negative. (1,2) While rare, septic arthritis and tenosynovitis are the most common extrapulmonary infections caused by this organism. A literature search did not reveal any cases of necrotizing fasciitis caused by *M. kansasii*. Other atypical mycobacteria that have been reported to cause necrotizing soft tissue infections include *M. ulcerans*, *M. marinum*, and *M. haemophilum*. *M. ulcerans* (Buruli ulcer) is the most commonly reported, with rapid growth of cases in Africa, Australia, India, South America and other tropical regions. Buruli ulcer is characterized by large, well circumscribed necrotic areas of the deep dermis and panniculus.

This patient had multiple predisposing risk factors for mycobacterial infection including RA, gout, intralesional steroid injections, methotrexate, low dose prednisone and infliximab, a systemic tumor necrosis factor inhibitor. After surgical debridement and vacuum-assisted closure accompanied by 12 months of treatment with isoniazid, ethambutol, and rifampin his wounds are well healed with no signs of recurrent infection. Reasonable control of his arthritis was achieved with low dose prednisone and methotrexate resumed 6 months after surgery. Infliximab has not been restarted. This is the first reported case of necrotizing fasciitis due to *M. kansasii* and is notable for its subacute presentation and association with infliximab therapy.

Medial forearm after treatment

Literature Cited

1. Bernard L, Vincent V, Lortholary O *et al. Mycobacterium kansasii* septic arthritis: French retrospective study of 5 years and review. *Clin Infect Dis* 1999;29:1455-60.
2. Nakamura T, Yamamura Y, Tsuruta T *et al. Mycobacterium kansasii* arthritis of the foot in a patient with systemic lupus erythematosus." *Intern Med* 2001;40:1043-9.
3. Joynson, DH. Water: the natural habitat of *Mycobacterium kansasii*? *Tubercle* 1979;60:77-81.

Maastricht University

Determining the Wear Resistance of Occlusal Splints in a Prospective Clinical Study

P. Ottl, P. Schmelz, A. Piwowarczyk, H.-Ch. Lauer

Dept. of Prosthodontics, School of Dentistry (Director: Prof. Dr. H.-Ch. Lauer), ZZMK (Carolinum), J. W. Goethe University, Frankfurt, Germany

Objective

- To determine quantitatively the wear resistance of a newly developed light-curing splint resin over a period in situ of six months.

Materials and Methods

- **Patients**

  n = 20 consecutive patients

  (mean age: 34.7 years; 12 F, 8 M)

- **Inclusion criteria**

  - Natural dentition/fixed denture
  - Complete dentition to at least the 1st molar

  and

  for the stabilization splint sample:

  - Insufficient occlusal support
  - Increased occlusal loss of dental hard tissue

  for the distraction splint sample:

  - TMJ pain **and**
  - Complete anterior dislocation of the disk without reduction/with terminal reduction
  - TMJ osteoarthrosis

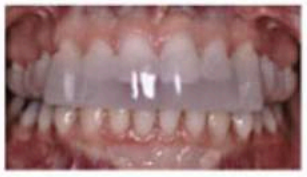


Fig. 1: Stabilization splint in situ

- **Resin splint material (Fig. 1)**
  - Light-curing (400–500 nm) resin made of high-molecular dimethacrylates with organic and inorganic fillers
  - Does not contain methyl methacrylate

- **Study design**
  - **Duration**: 6 months
  - **Types of splints** (maxilla, n = 10 each): stabilization splints, distraction splints
  - **Splint wear mode**: 24 hours
  - **Examinations**: before insertion (BI), at 4 weeks (4W), at 3 months (3M), at 6 months (6M)
  - **Occlusal adjustments** were restricted to the time before 4W.

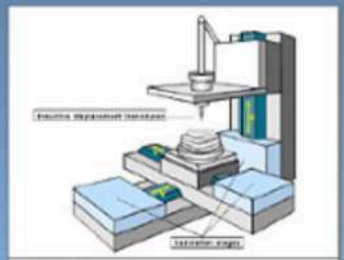


Fig. 2: Test setup

- **Measuring technology (Fig. 2)**
  - Vibration-isolated table framework
  - 3 translation stages (for directions x, y, and z) (DC-Motor) (Pl, Waldbronn)
  - DV 4 stereomicroscope (Zeiss, Oberkochen)
  - WA 20 inductive displacement transducer/ Spider8 digital 8-channel measurement unit/ Catman 32 software V2.1 (HBM, Darmstadt)
  - Local coordinate storage for occlusal contacts during baseline measurements
  - Ten measurements each in regions 13, 23, 16, 26 (BI, 4W, 3M, 6M)
  - Splint repositioned on remount cast

Results

- The medians of the occlusal vertical gains/losses (wear, resin torsion, water sorption, etc.) are shown in **Fig. 3** (stabilization splints) and **Fig. 4** (distraction splints).

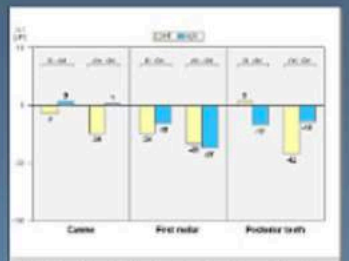


Fig. 3: Occlusal vertical gains/losses (medians) of the teeth in relation over a period in situ of six months (n = 10 stabilization splints)

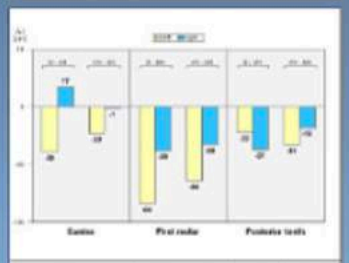
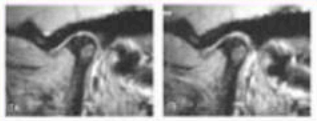


Fig. 4: Occlusal vertical gains/losses (medians) of the teeth in relation over a period in situ of six months (n = 10 distraction splints)

- **Statistical analysis** (Mann-Whitney U-test, $p \leq 0.05$) showed no significant differences when comparing the corresponding results of stabilization and distraction splints.



Figs. 5a and b: Sagittal oblique images (MRI) of the condyle/fossa relationship without splint (Fig. 5a) and with distraction splint inserted (Fig. 5b) following six months of wearing

Conclusions

- The present study *clinically* confirms the good wear resistance results of the new resin splint material obtained in a previous *in-vitro* study [OTTL et al., Dtsch Zahnärztl Z 52, 342 (1997)].
- Good wear resistance is of great importance for maintaining the therapeutic mandibular position during the treatment period (Figs. 5a and b).

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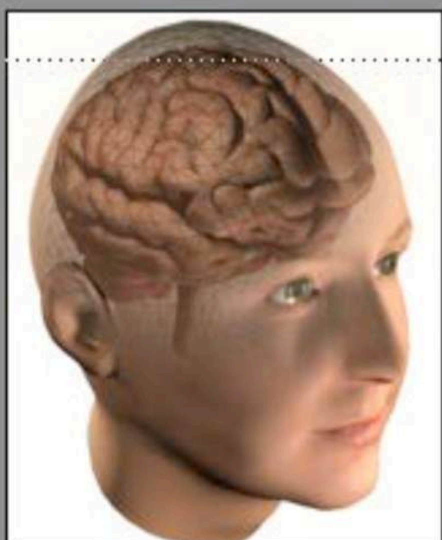
Your Ingenious Teaser Right Here to Woo Them Down to the Body

Thenameoftheauthors 28ptregular

Conclusions first: 44 pt bold

Always put the most important part - your conclusions - first! Place your conclusions in the upper left hand corner of your poster.

Prepare your material from the reader's perspective. What was done, by who and your conclusion has to be understood within a couple of second's reading! Use active voice when writing the text. textsize:: 34 pt regular



use pictures or illustrations! Image caption 28pt regular

Introduction

Posters are primarily visual presentations. Your poster should be dominated by self-explanatory illustrations such as graphs and pictures while the amount of text should be kept to the minimum. .

Your aim

Your poster is an advertisement for your research and as such it needs to be eye-catching and straight to the point. You only have seconds, or at best a few minutes to attract the attention of the visitor to a poster session. Keep your message short and clear

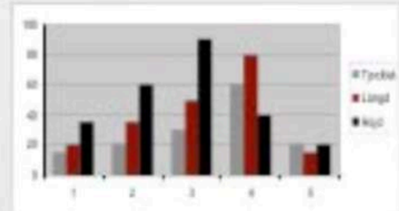
Your message

Keep your message clear and your text concise. Decide what is relevant for this poster and try to get your message across to your target group.

Layout, photos and print

Contact Mediahyrån at University Library for help with layout and Image enhancement. For printouts and professional photographers contact Bildmakarna. Fore more information: www.bildmakarna.kib.ki.se

Always write a descriptive caption 28pt regular



Always write a descriptive caption 28pt regular

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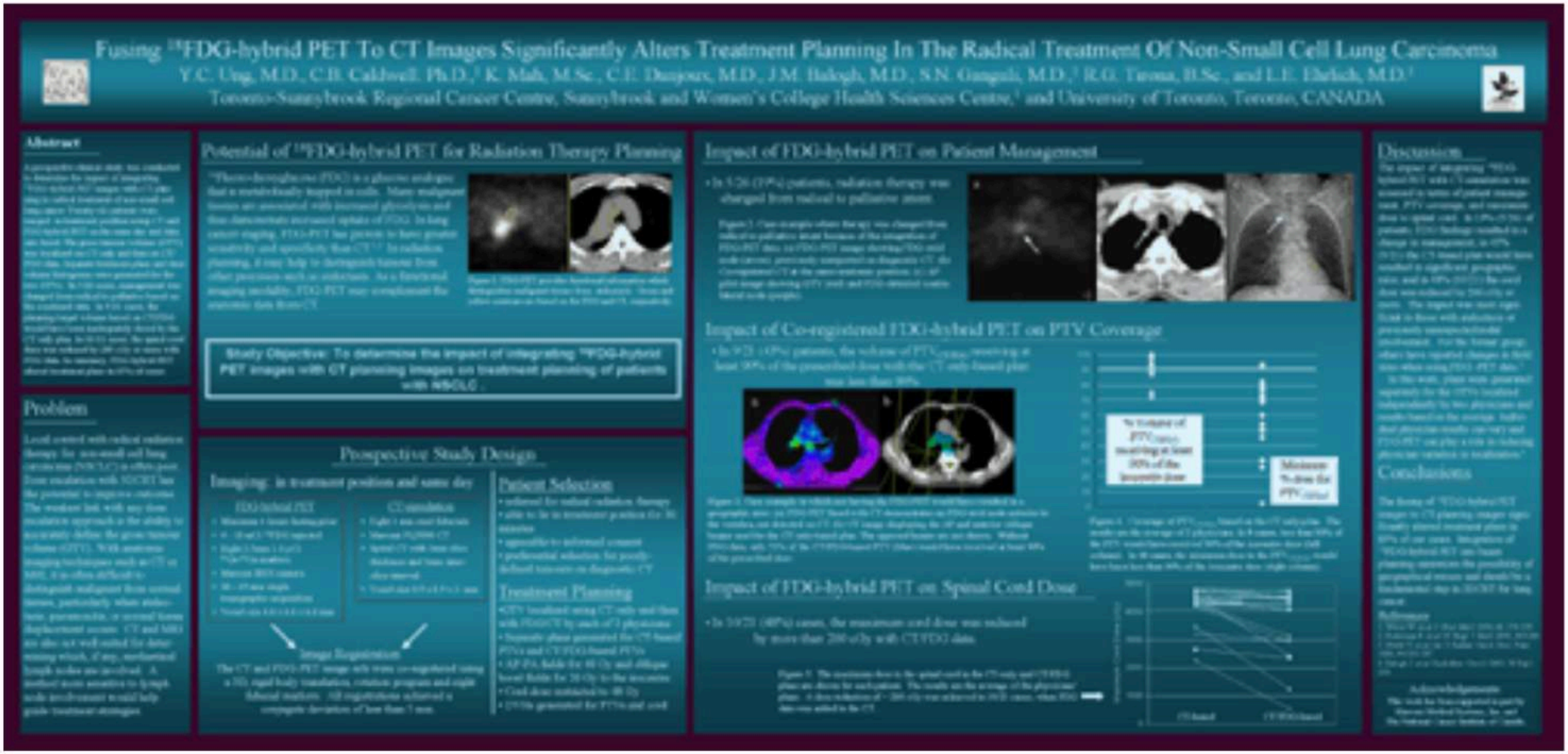
The best font for text blocks that are as short as they should be on a poster is a Sans Serif typeface family. Therefore, use sans serif fonts such as Arial or Mundo sans rather than serif fonts like Times or Courier.
AVOID CAPITAL LETTERS IN TEXTS THAT ARE LONGER THAN ONE LINE, SINCE THEY ARE MORE DIFFICULT TO READ.

Handouts

If you succeed in getting the reader's attention, provide her/him with more detailed information in the form of handouts or printed articles. Include references on your handout instead of your poster.

It is always nice to put in a picture and write some few short notes of what's going on in the future. Put handouts, business cards, nearby - on a table or in an envelope hung with the poster.



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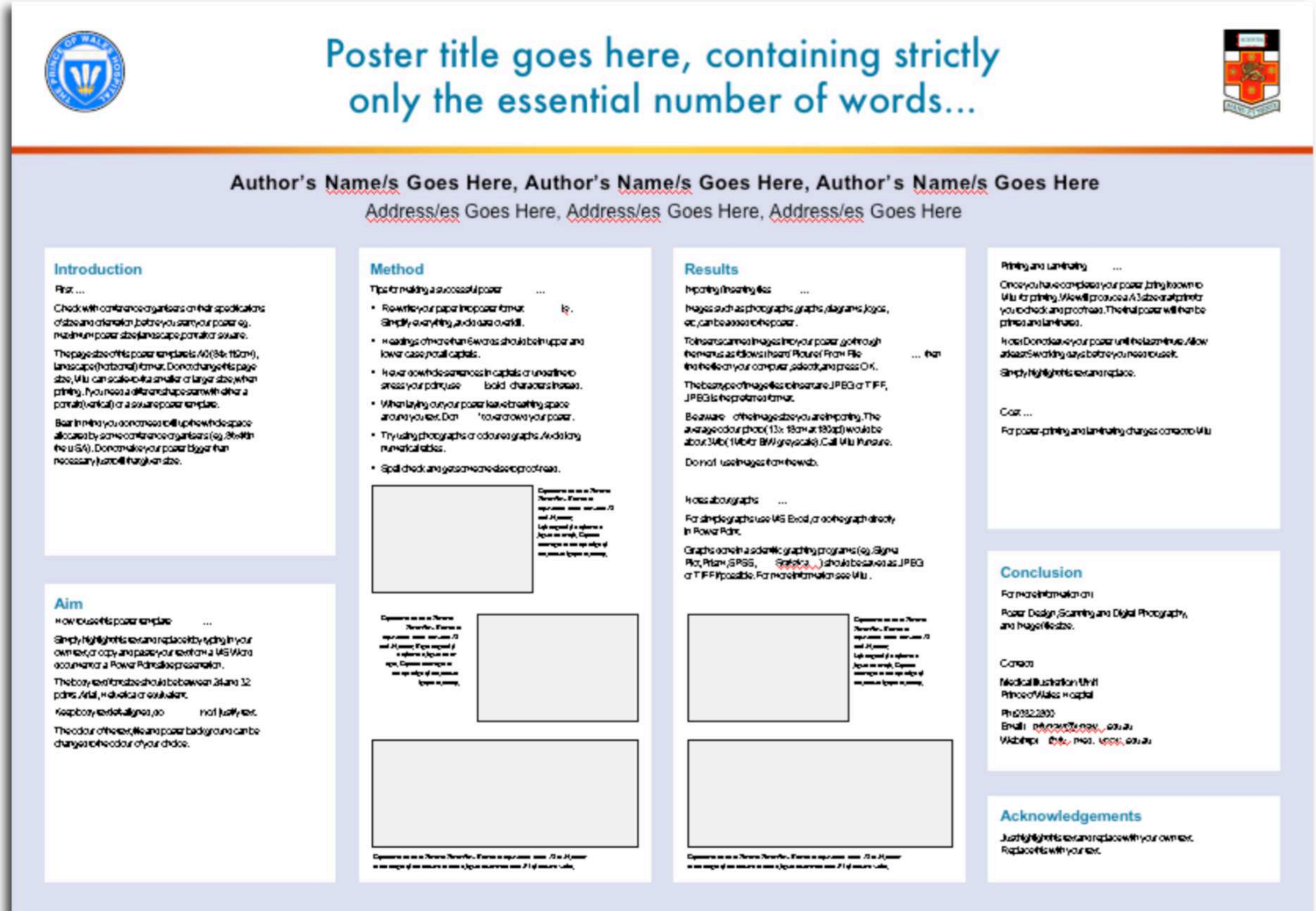
Poster title goes here, containing strictly only the essential number of words...
Author's Name/s Goes Here, Author's Name/s Goes Here, Author's Name/s Goes Here
Address/es Goes Here, Address/es Goes Here, Address/es Goes Here
Introduction
Aim
Method
Results
Conclusion
Acknowledgements


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Academic skills
Take-home messages

Elena Ambrosino, PhD

Maastricht University

No matter the communication form…

- Written – Articles (abstracts…)
- Oral – Presentations (interviews, …)
- Visual – Posters (infographics, …)

Take-home messages

- Know your audience
- Less is best (be synthetic and clear)
- Make a story and stick to it
- Follow the guidelines (written or time, size)
- *Practice in advance (for oral)*


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Resources:

Science and news
Free courses: https://www.scidev.net/global/content/script.html

Manuals: https://www.scidev.net/global/content/practical-guides.html

Smile for the camera Use interviews to promote your science, raise your profile and practise your media skills. Nature 562, 153-155 (2018)

Writing
Free course: Journal Manuscript Development for Global Health - Global Health eLearning Center

Angel Borja. 11 steps to structuring a science paper editors will take seriously. Elsevier

Bert Blocken. 10 tips for writing a truly terrible journal article. Elsevier

F. Ecarnot , M.-F. Seronde, R. Chopard, F. Schiele, N. Meneveau. Writing a scientific article: A step-by-step guide for beginners.. European Geriatric Medicine 6 (2015) 573–579

English for Writing Research Papers - Useful Phrases. Springer

Resources:

Examples of bad graphs
https://www.biostat.wisc.edu/~kbroman/topten_worstgraphs/

Scientific presentation
http://blogs.nature.com/naturejobs/2017/01/11/scientific-presentations-a-cheat-sheet/

http://blogs.nature.com/naturejobs/2016/02/10/a-david-letterman-like-countdown-to-the-10-biggest-pitfalls-in-scientific-presentations/

Marilynn Larkin. How to give a dynamic scientific presentation. Elsevier

Peer-reviewing
https://www.scisnack.com/wp-content/uploads/2018/10/A-Peer-Review-Process-Guide.pdf

Additional
Ignobel prize https://www.improbable.com