



qbio
quantitative
biology



QBIO MASTER PROGRAM
quantitative biology in practice

LAB1 - POSTER PRESENTATIONS

Emmanuel.Margeat (Margeat@cbs.cnrs.fr)

LAB 1 – POSTER PRESENTATIONS



- When and where will you do a poster presentation ?
- Preparing the poster
- Preparing your speech
- Practical examples

LAB 1 – POSTER PRESENTATIONS



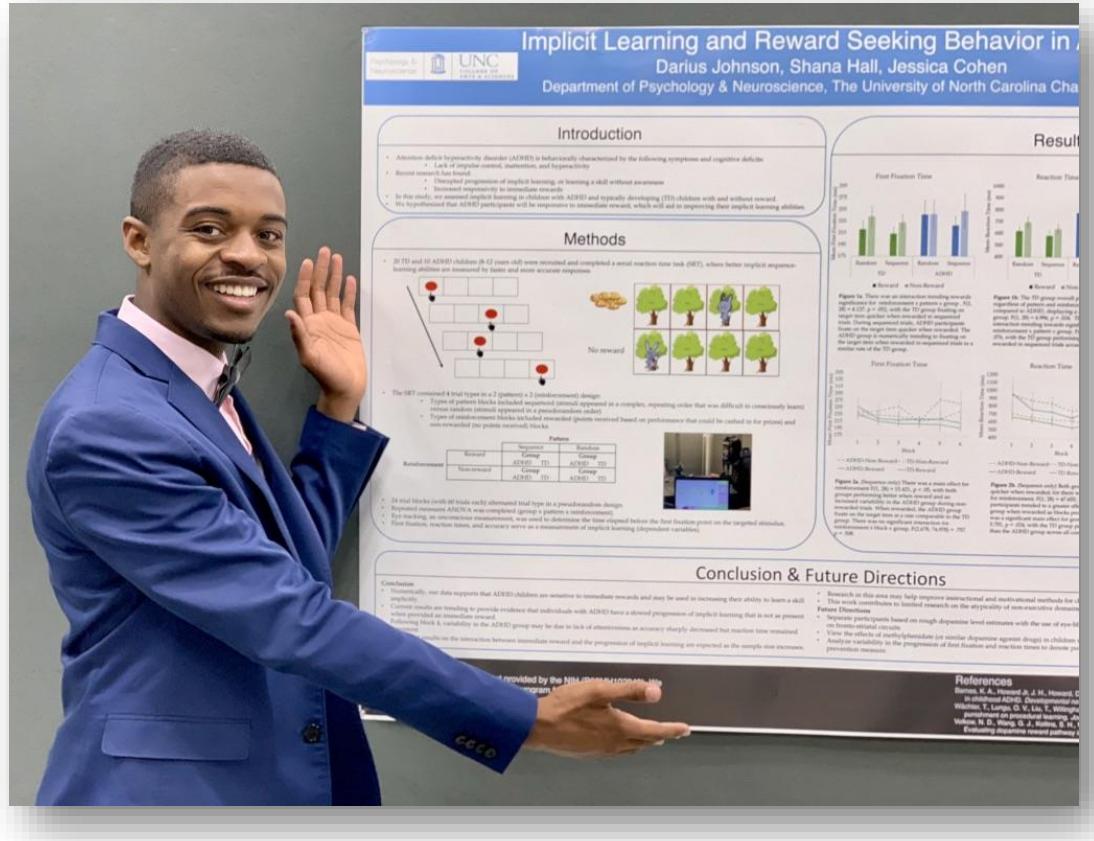
- When and where will you do a poster presentation ?
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When and where will you do a poster presentation ?



A typical poster presentation in a (very large) scientific meeting

When and where will you do a poster presentation ?



An effective poster operates on multiple levels ...

- Summary of your work
- Advertisement of your work
 - Reference of your work
 - Conversation starter

An effective poster is not just a standard research paper stuck to a board.

A poster uses a different, visual grammar.

It shows, not tells

Know Your Audience !!

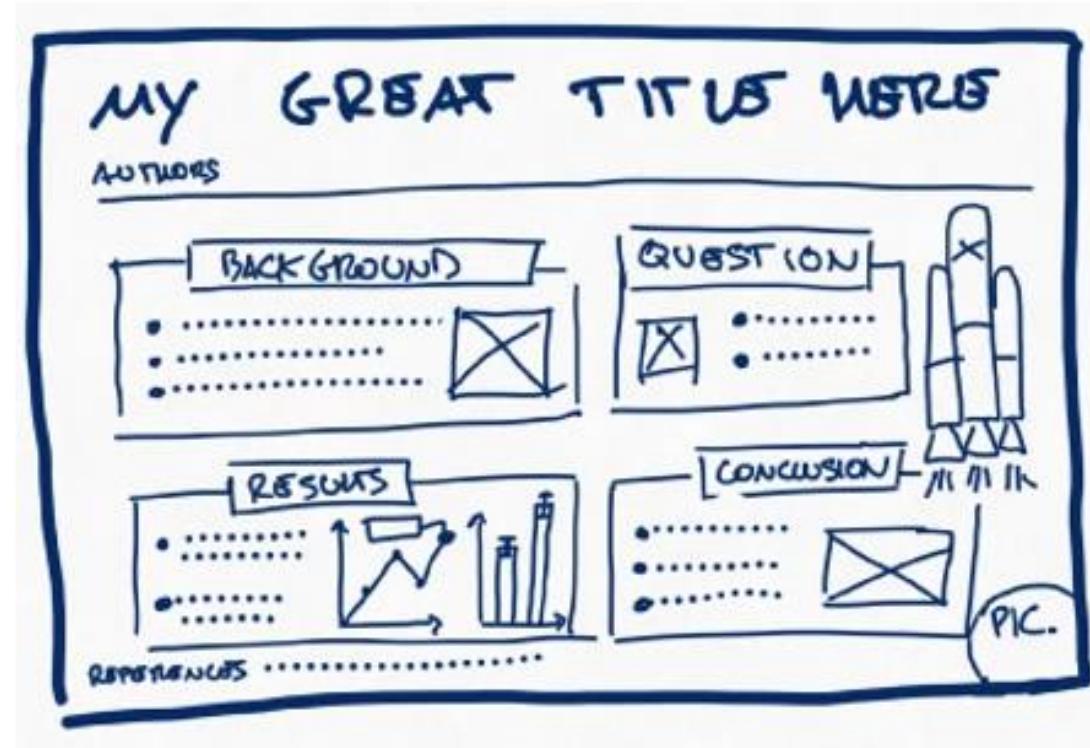
- When and where will you do a poster presentation ?
- **Preparing the poster**
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Content

MANDATORY :

- Title section
- Authors names and affiliations, contact
- Introduction / Aims and objectives
- Methods
- Results
- Discussion / Conclusion
- Acknowledgments / Sponsors
- References

YOU CAN ADD : abstract or summary



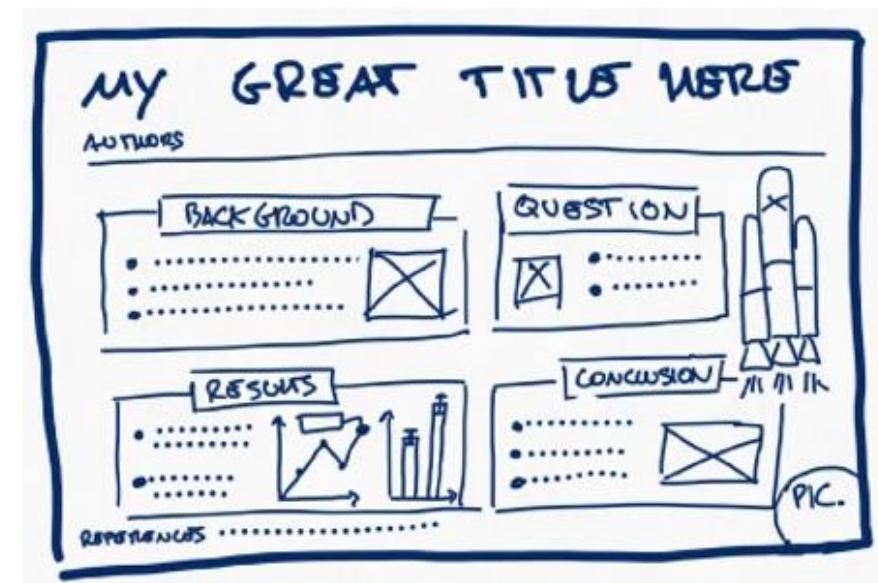
- Start with a sketch of the layout
- Dimensions, orientation
- Pick a software

Title

- Manuscript titles are often long and complex, as they describe the basic findings of the research paper
- ... but your poster title can be more creative. The goal is to catch a viewer's attention !

Paper title :
Solubilization and stabilization of several class-C G-protein receptors using circularized nanodiscs produced using total extracts from E. Coli

Poster title :
GPCR stabilization with circular nanodiscs



Authors names and affiliations

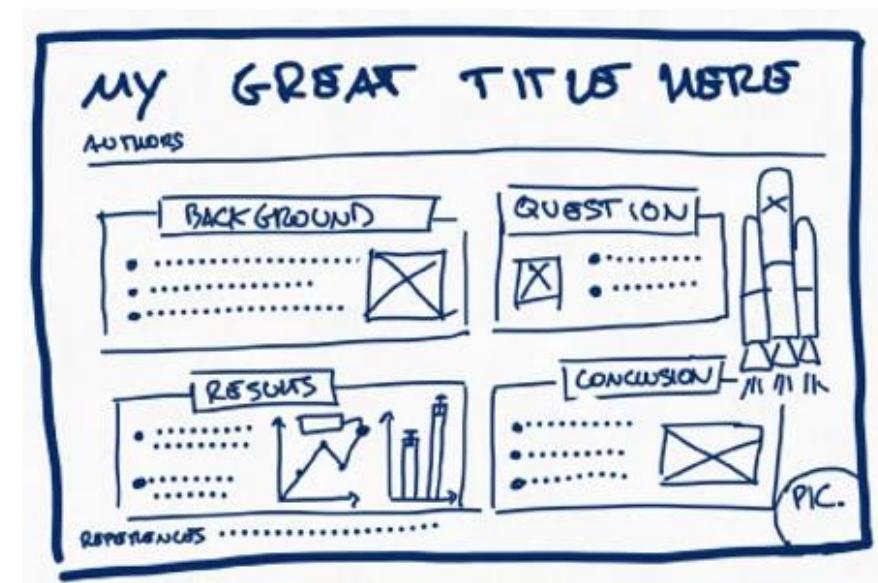
- Full names of all authors (incl. First name, not just the initial)
- Underline the presenter (you)
- Affiliations
- Contact
- Picture if you want

Emmanuel Margeat¹, The-Other Student¹, My Collaborator², My Boss¹

¹ Centre de Biologie Structurale, CNRS, Montpellier, France

² The other amazing lab, INSERM, Palavas, France

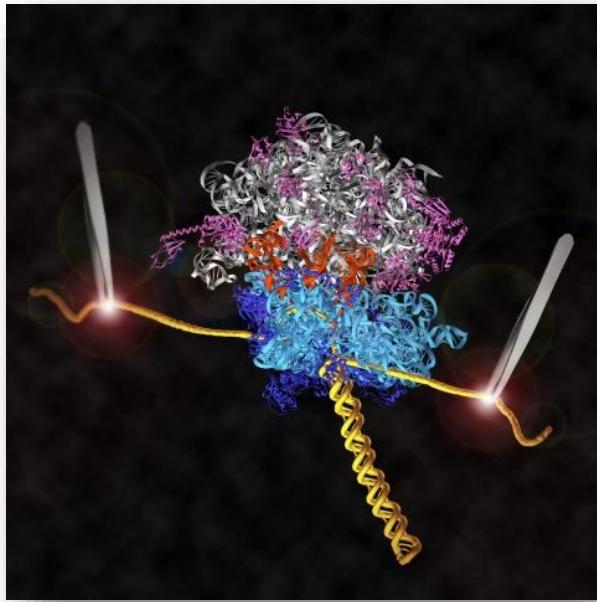
Contact : emmanuel.margeat@umontpellier.fr



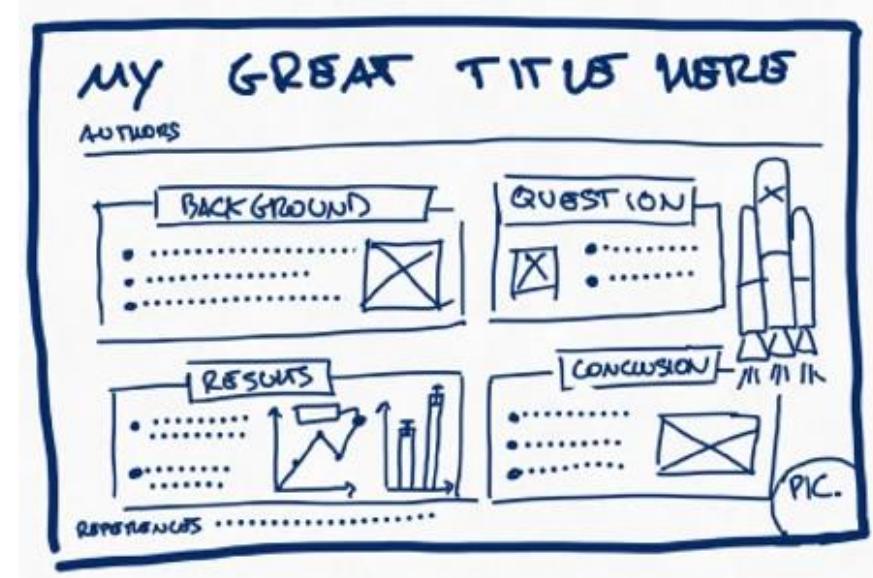
Introduction, Aims and Objectives

- Target here someone who is not necessarily in your field
- Get your viewer *interested* in the issue or question
- Use the absolute minimum of background information, definitions, and acronyms
- Pitch an interesting, *novel* hypothesis
- Describe (briefly) the experimental approach that can test your hypothesis.

A nice image can draw people in !



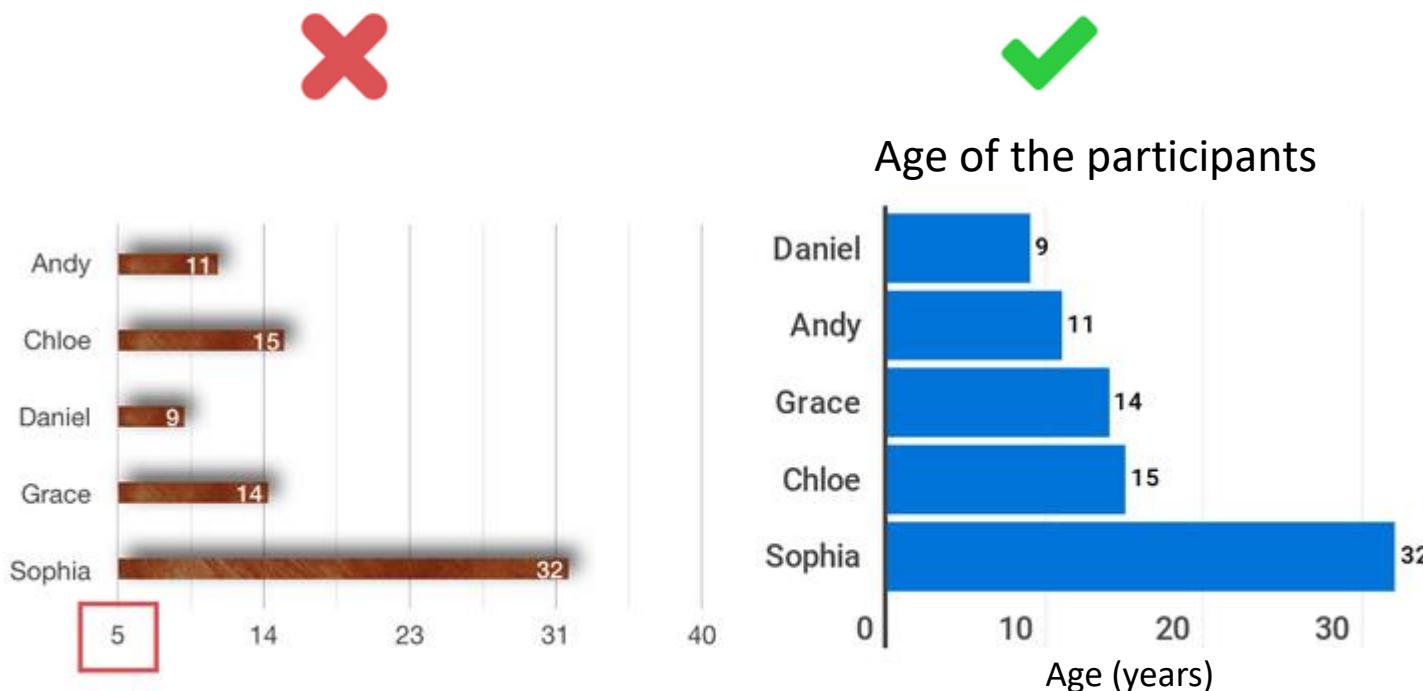
Courtney Hodges (UC Berkeley) and Laura Lancaster (UC Santa Cruz), Bustamante lab



Results

- This is where the action is !
- Remember – you don't need to include every experiment you've ever done.
- Just describe the results that help address the main question/hypothesis.

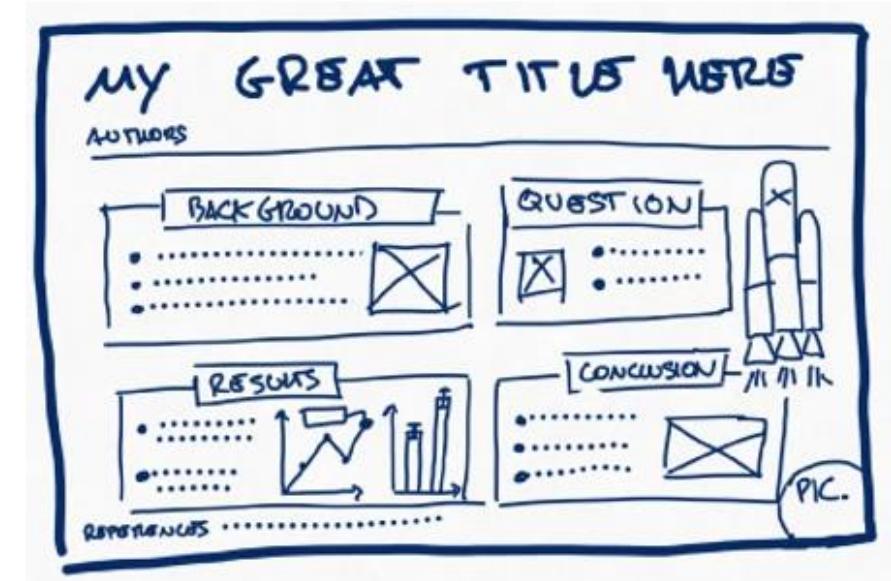
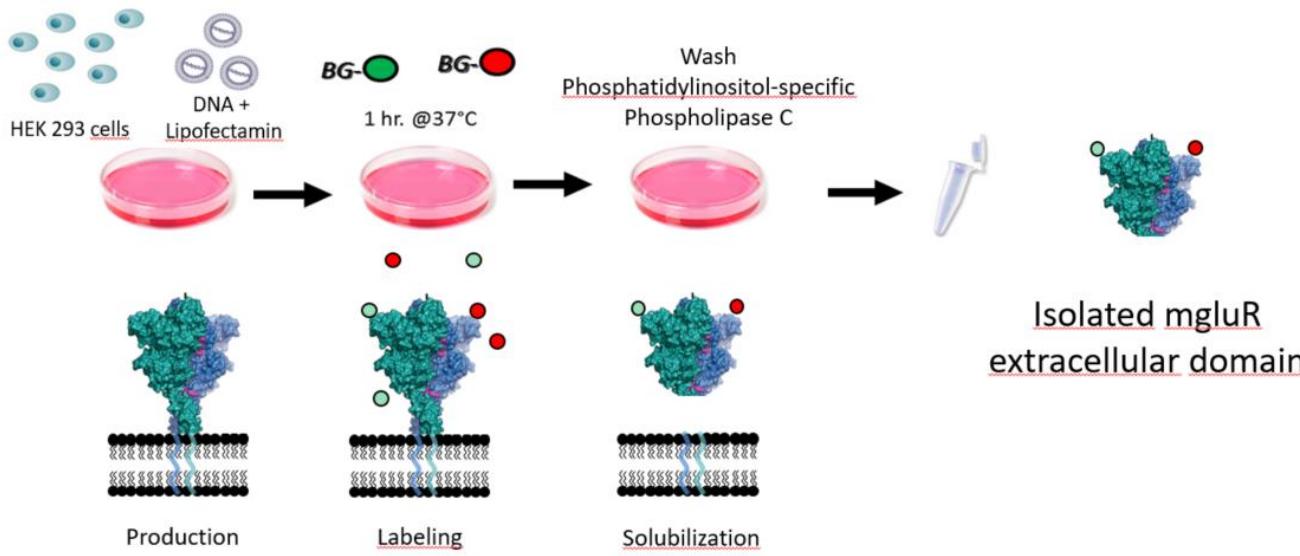
Figures, Images, Data representation are central here



Methods

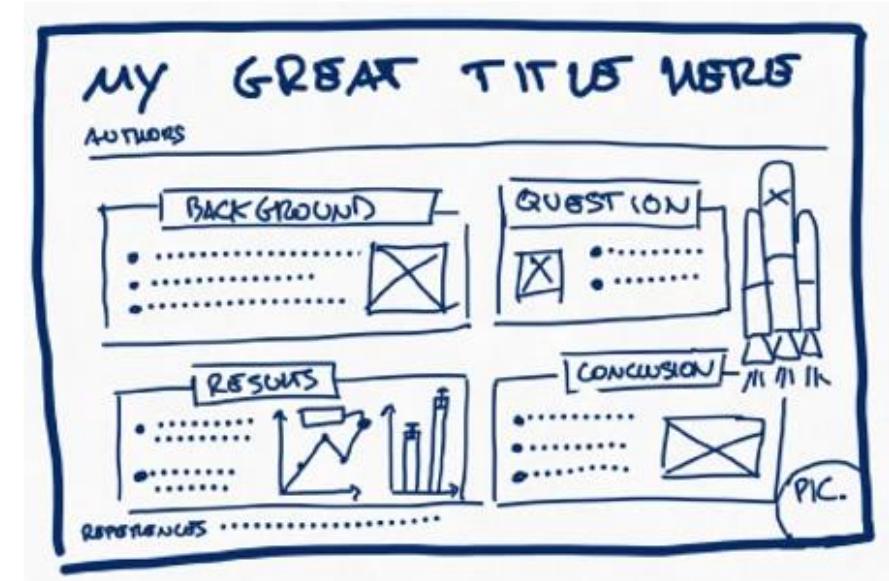
- Use this section to help the viewer understand your experimental approach to the question.
- You don't need to detail every last step – save that for the paper you publish!

Again, a diagram or figure works great here.



Conclusion / Discussion

- remind readers of the importance/relevance of your work
- Use 2-4 bullet points to summarize the meaning or implications of your results
- Did this address the objectives of the project ?
- Mention any alternative explanation for results or unanticipated results
- What are the potential outcomes ?



Layout and Format

- Top to bottom and from left to right.
- Common alternative layout : conclusions in the centre of the poster and supporting work radiating out from it
- No long and detailed sections of text. Bullet points are more effective and maintain the reader's interest.
- White-space is important, and will make the poster more readable.
- Printing on paper



Style

- Ensure that your font size is large enough to be legible from at least one-two meters away (e.g. 18-24 for the text and 24 - 36 for titles).
- Don't use CAPITAL LETTERS even in Title AS THEY ARE MORE DIFFICULT TO READ
- Try to use one or two font types at most. Too many font types can look messy and confusing,
- Choose fonts that are easy on the eye, such as Times Roman or Arial.

Text sizes:

Title: 85 point

Authors : 36pt

Sub-headings: 36pt

Body text: 24pt

Captions: 18pt

The poster features a purple header with the text "Your Ingenious Teaser Right Here to Woo Them Down to the Body" and "The name of your poster". Below the header is a logo for Karolinska Institutet. The main content area is divided into several sections: "Conclusions first: 44 pt bold" (with a note about placing conclusions in the top-left corner), "Introduction" (with a note about posters being visual presentations), "Your aim" (with a note about posters being advertisements), "Your message" (with a note about keeping messages clear), "Layout, photos and print" (with contact information for Mediebyrån and Blommakarta), "Tips" (with a note about using sans-serif fonts like Arial or Mundo sans), and "Handouts" (with a note about providing more detailed information). There are also two pie charts at the bottom left and a small portrait of a man at the bottom right. The footer contains copyright information for Karolinska Institutet.

LAB 1 – POSTER PRESENTATIONS



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Preparing your speech

- Introduce yourself
- You should have a short and a long version of your speech
 - The short one should be 1 minute / 3-4 sentences, covering
 1. What is your research topic?
 2. What have you found?
 3. Why is that important?
 - If you have caughted your audience attention, you can switch to the long version (+5 minutes) that covers :
 - Background information about your research, How did this lead you to your research question, what were you hoping to find out and why?
 - How did you get from your research question to your conclusion? What techniques did you use and why ? Were there any interesting twists ?
 - What are the conclusion of your work ? How does this open new avenues ?
- Be prepared for questions
- Practice !

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REFINEMENT OF ATOMIC MODELS OF HIV-1 CA OLIGOMERS

JUAN R. PERILLA[†], GONGPU ZHAO^{*}, DANIELLE CHANDLER[†],

ANGELA GRONENBORN^{*}, PEIJUN ZHANG^{*} AND KLAUS J. SCHULTE[†]

[†]BECKMAN INSTITUTE FOR ADVANCED SCIENCE AND TECHNOLOGY, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

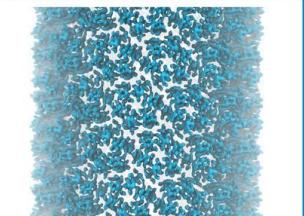
^{*}DEPARTMENT OF STRUCTURAL BIOLOGY, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE



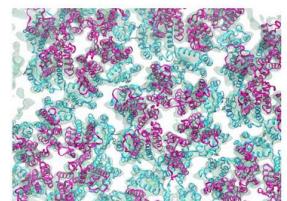
INTRODUCTION

Native capsids are made of higher-order structures of HIV-1 CA, involving thousands of CA proteins, but arranged in a lattice involving only two types of oligomers: hexamers and pentamers. High-resolution structures for the oligomers are available, but not in native conformations, thus they lack the intrinsic curvature. By using MDFF combined with cryo-EM data, we present a new structure of the hexameric form of HIV-1 CA. The MDFF-derived model accurately captures the inter- and intra-hexameric interactions. Using the MDFF-derived model of the hexameric form of HIV CA, we have also been able to model new interactions between pentamers and hexamers.

MDFF

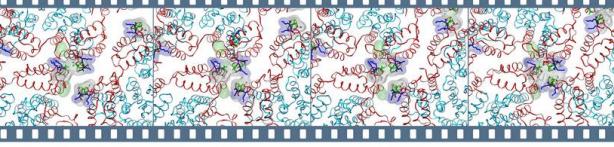


The hexameric structure 3H47 is used as the starting point, missing residues are built by homology modeling using Modeller, and are optimized via MD while constraining the solved residues. Dimers are modelled after the NMR structure 2KOD. Initial rigid body docking to the cryo-EM map is performed by using SITUS with a resulting cross correlation coefficient of 0.75.



Molecular dynamics flexible fitting (MDFF) incorporates the EM density map as a potential in a way that high density areas in the grid correspond to energy minima. MDFF yields a structure with a CCC of 0.96.

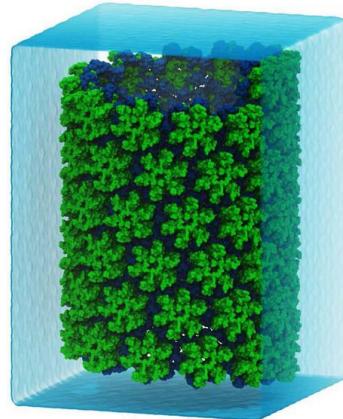
STRUCTURAL REFINEMENT



The MDFF-derived structure was allowed to equilibrate for 20ns. During equilibration the 7-hexamers were stabilized by the formation of hydrogen bonds, and by the presence of a hydrophobic core at the three-fold symmetry axis.

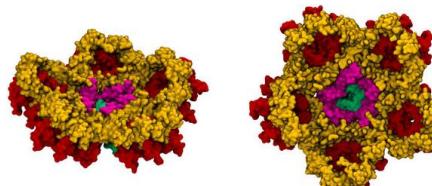
CYLINDRICAL MODEL

A total of 71 MDFF-derived hexamers were docked to the cryo-EM cylindrical map, solvated and ionized by NaCl (1M). The resulting system consisting of 15×10^6 atoms, was equilibrated for 100ns using the **NSF BlueWaters supercomputer**.



PENTAMER MODEL

Using the MDFF-derived model, and the pentameric structure 3P05. We were able to construct an all-atom pentamer-hexamer model. The system was equilibrated for 200ns.



The effects of sub-anesthetic doses of the non-competitive NMDA receptor antagonist ketamine on reconsolidation and expression of fear memory in Sprague Dawley rats



Delcellier, K.^{1,2}, Cayer, C.^{1,2}, Kent, P.¹ and Merali, Z.^{1,2}

¹Institute of Mental Health Research, ²University of Ottawa School of Psychology

Introduction

-Ketamine, a non-competitive NMDA receptor antagonist, has historically been used as a sedative in veterinary and human medicine.

-Recent reports suggest that it displays anti-depressant as well as anxiolytic effects at sub-anesthetic doses. Several non-competitive NMDA receptor antagonists that have been shown to disrupt fear memory processes, however surprisingly little work has been done on the effects of ketamine in this domain.

-The objective of this study was to investigate the effects of ketamine on reconsolidation and expression of fear memory in Sprague-Dawley rats.

Methods

Subjects: Male Sprague-Dawley rats (275-300 g) were maintained on a 12h light/dark cycle and given ad libitum access to food and water.

Drugs: Ketamine, dissolved in saline was administered intraperitoneally at doses 1, 3 or 10 mg/kg. The control (vehicle) animals received an equivalent volume of saline alone.

Procedure:
Acquisition: Rats were placed in conditioning chambers (Gulbeam Instruments) where they received either 1.0 footshock (1.0 mA; 1s duration) on a random schedule (contextual training) or 20 pairings of a 20-s tone with a 1.0 mA (1-s) continuous footshock delivered during the final second of the 20-s tone (cued training).

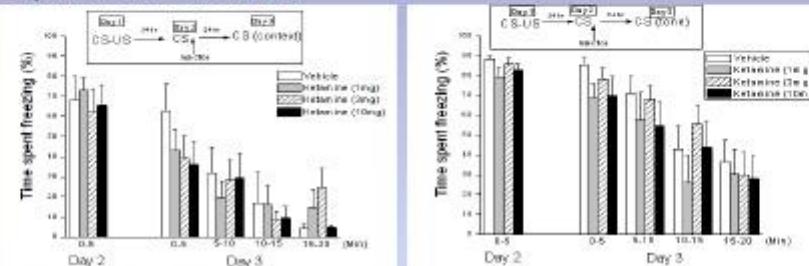
Experiment 1 (expression): 24 h after acquisition training, rats were randomly injected with one of the 3 doses of ketamine 20 min before testing. Contextual fear expression was assessed over 20 min by placing the rats back into the conditioning chamber where they had previously been shocked and freezing behavior monitored. To assess fear expression in the cued condition, rats were transferred to a novel environment and presented with the cue (tone previously paired with footshock). A total of 15 tones (each 20 s in duration) were presented at 1 min intervals.

Experiment 2 (reconsolidation): 24 hr after acquisition training rats were presented with the CS (either context or cue, as described previously) without the US for 5 min (reactivation). Immediately thereafter, rats were injected with one of three doses of ketamine or saline and returned to their home cage. The following day (Day 2), rats were tested for contextual or cued fear expression as described in Experiment 1.

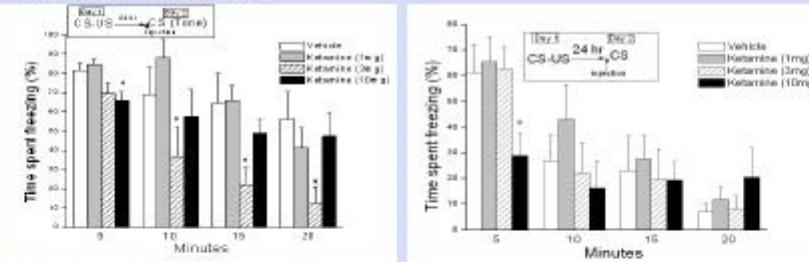
Experiment 3 (locomotor activity): rats were injected with one of two doses of ketamine (3 or 10mg/kg) or saline 20 minutes before testing. Locomotor activity was assessed over 30 minutes in the testing arena for the Open-Field test.

Results

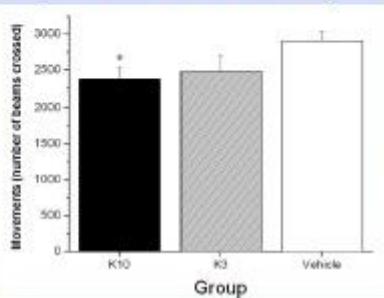
Experiment 1: Reconsolidation



Experiment 2: Expression



Experiment 3: Locomotor activity



Conclusions

-Ketamine did not disrupt reconsolidation, which is contrary to previous research on other non-competitive NMDA receptor antagonists using CER.

-Higher doses (3 and 10 mg/kg) of ketamine were shown to disrupt the expression of fear memory in both contextual and cued conditions.

-High dose (10mg/kg) of ketamine was shown to lower locomotor activity, leading to believe that its use would not be causing an increase in activity and therefore would not be interfering with the freezing behavior.

-The results of this study appear to indicate that ketamine is indeed implicated in the disruption fear memory processes, although there seems to be some differences with results previously reported on other non-competitive NMDA receptor antagonists using the same paradigm. Results such as these lead to the possibility of ketamine using a different mechanism.

Acknowledgements

I would like to thank everyone from Dr. Zul Merali's laboratory for all of the assistance and wonderful help they have given me throughout the year. I would like to especially thank Christian Cayer and Jonathan James for their most appreciated help in data collection and the invaluable guidance they have given me, as well as Pamela Kent, who has been an incredible source of support and knowledge throughout this experience.

Future research

-Future research into ketamine's effect on fear memory processes should focus on the possibility of its implication in a different mechanism in the amygdala, as well as its effects on fear memory acquisition.

-Implications of such studies could eventually lead to novel treatments for anxiety disorders, such as PTSD.

Structural determinants of binding of the human bile acid transporter SLC10A2 (ASBT)



Viktoria Gamsjäger¹, Claire Colas¹, Gerhard F. Ecker¹

¹Department of Pharmaceutical Chemistry, University of Vienna , Althanstrasse 14, 1090 Vienna, Austria

RESOLUTE
Research Empowerment on Solute Carriers

Introduction

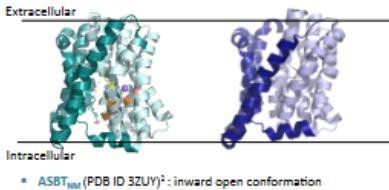
The human apical sodium-dependent bile acid transporter (hASBT, SLC10A2) is a membrane protein that is responsible for the uptake of bile acids across the enterocytes apical membrane. hASBT is a key drug target for the treatment of hypercholesterolemia. Additionally, hASBT is an interesting target for prodrugs.

Here we describe the interactions of this transporter with its ligands using computational methods. Our results improve our understanding on how substrate specificity is determined in hASBT, providing guiding rules for the development of new compounds targeting this pharmacologically important transporter.

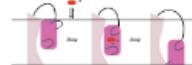
Goals

- To determine the specificity determinants of binding
- => Understanding the transporter's interaction with substrates and inhibitors at a molecular level
- Discovering new compounds
- => Used as chemical tools to understand function, or new scaffolds for the design of new drugs

Prokaryotic transporters ASBT_{NM} and ASBT_{yf}



- X-Ray structures of homologues in 2 conformations (ASBT_{yf} and ASBT_{NM})
- 22-26% sequence identity with human hASBT
- Elevator mechanism of transport³



Homology modeling



- Generation of a 3D model of a protein with an unknown structure ('target') based on an experimentally determined structure of a homolog protein ('template').
- The protocol generally includes several steps (c.f. flow chart) ranging from template selection to model validation.
- The process is iterative until a suitable model is obtained.

Substrate selectivity

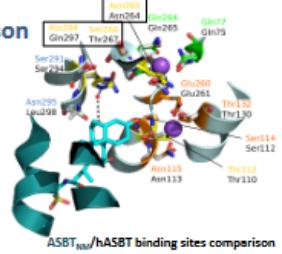


- Large discrepancy of affinities despite a similar scaffold
- Grouping of bile acids depending on their hydroxylation profile and substitutions

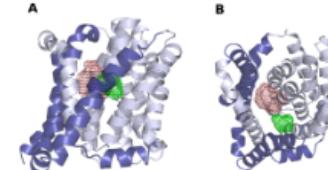
Binding sites comparison

- Mutagenesis studies on the template characterized essential residues for binding
- Hydrogen bond network with a water molecule
- Residues constituting the binding sites are conserved

=> Hypothesis : the substrate selectivity occurs in the outward open conformation



Outward open models reveal an horizontal orientation



Binding pockets in the outward open conformation

Future directions

Rationalize the substrate specificities of ASBT in the outward open conformation:

- => to reveal important residues involved in binding and transport
- => to identify conformation-specific compounds by virtual screening

Conclusions

- Our study reveals that the binding sites in the inward open conformation are conserved
- We suggest that selectivity occurs in the outward open conformation

1. Hu et al. Crystal structure of a bacterial homologue of the bile acid sodium symporter Asbt. *Nature* 478: 408 (2011)
2. Zhou et al. Structural basis of the alternating-access mechanism in a bile acid transporter. *Nature* 505: 509-513 (2013)

3. Colas et al. SLC Transporters: Structure, Function, and Drug Discovery *MedChemComm* 7(6):1069-1081 (2016)
4. Geyer et al. The solute carrier family SLC10: more than family of a bile acid transporters regarding function and phylogenetic relationships *Arch Pharmacol* 372: 413-431 (2006)



PIGS IN SPACE: EFFECT OF ZERO GRAVITY AND AD LIBITUM FEEDING ON WEIGHT GAIN IN CAVIA PORCELLUS



SPACE-EXES

ABSTRACT:

One ignored benefit of space travel is a potential elimination of obesity, a chronic problem for a growing majority in many parts of the world. In theory, when an individual is in a condition of zero gravity, weight is eliminated. Indeed, in space one could conceivably follow ad libitum feeding and never even gain an gram, and the only side effect would be the need to upgrade one's stretchy pants ("exercise pants"). But because many diet schemes start as very good theories only to be found to be rather harmful, we tested our predictions with a long-term experiment in a colony of Guinea pigs (*Cavia porcellus*) maintained on the International Space Station. Individuals were housed separately and given unlimited amounts of high-calorie food pellets. Fresh fruits and vegetables were not available in space so were not offered. Every 30 days, each Guinea pig was weighed. After 5 years, we found that individuals, on average, weighed nothing. In addition to weighing nothing, no weight appeared to be gained over the duration of the protocol. If space continues to be gravity-free, and we believe that assumption is sound, we believe that sending the overweight — and those at risk for overweight — to space would be a lasting cure.



INTRODUCTION:

The current obesity epidemic started in the early 1960s with the invention and proliferation of elastane and related stretchy fibers, which released wearers from the rigid constraints of clothes and permitted monthly weight gain without the need to buy new outfitts. Indeed, exercise today for hundreds of million people involve only the act of wearing stretchy pants in public, presumably because the constrictive pressure forces fat molecules to adopt a more compact tertiary structure (Xavier 1965).

Luckily, at the same time that fabrics became stretchy, the race to the moon between the United States and Russia yielded a useful fact: gravity in outer space is minimal to nonexistent. When gravity is zero, objects cease to have weight. Indeed, early astronauts and cosmonauts had to secure themselves to their ships with seat belts and sticky boots. The potential application to weight loss was noted immediately, but at the time travel to space was prohibitively expensive and thus the issue was not seriously pursued. Now, however, multiple companies are developing cheap extra-orbital travel options for normal consumers, and potential travelers are also creating new ways to pay for products and services that they cannot actually afford. Together, these factors open the possibility that moving to space could cure overweight syndrome quickly and permanently for a large number of humans.

We studied this potential by following weight gain in Guinea pigs, known on Earth as fond of ad libitum feeding. Guinea pigs were long envisioned to be the "Guinea pigs" of space research, too, so they seemed like the obvious choice. Studies on humans are of course desirable, but we feel this current study will be critical in acquiring the attention of granting agencies.

CONCLUSIONS:

Our view that weight and weight gain would be zero in space was confirmed. Although we have not replicated this experiment on larger animals or primates, we are confident that our result would be mirrored in other model organisms. We are currently in the process of obtaining necessary human trial permissions, and should have our planned experiment initiated within 80 years, pending expedited review by local and Federal IRBs.

ACKNOWLEDGEMENTS:

I am grateful for generous support from the National Research Foundation, Black Hole Diet Plans, and the High Fructose Sugar Association. Transport flights were funded by SPACE-EXES, the consortium of wives divorced from insanely wealthy space-flight startups. I am also grateful for comments on early drafts by Mariana Athletic Club, Corpus Christi, USA. Finally, sincere thanks to the Cuy Foundation for generously donating animal care after the conclusion of the study.

LITERATURE CITED:

- NASA. 1982. Project STS-XX: Guinea Pigs. Leaked internal memo.
Sekulić, S.R., D. D. Lukač, and N. M. Naumović. 2005. The Fetus Cannot Exercise Like An Astronaut: Gravity Loading Is Necessary For The Physiological Development During Second Half Of Pregnancy. Medical Hypotheses. 64:221-228.
Xavier, M. 1965. Elastane Purchases Accelerate Weight Gain In Case-control Study. Journal of Obesity. 2:23-40.

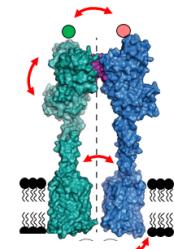
Structural dynamics of single metabotropic glutamate receptors dimers



Robert Quast¹, Anne-Marinette Cao¹, Fataneh Fatemi¹, Linnea Olofsson¹, Philippe Rondard², Jean Philippe Pin², Emmanuel Margeat¹.

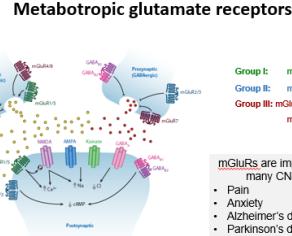
¹ Centre de Biochimie Structurale, CNRS UMR 5048, INSERM U1054, Université de Montpellier, Montpellier, France
² Institut de Génétique Fonctionnelle, CNRS, INSERM, Université de Montpellier, Montpellier, France
 Contact : margeat@cbs.cnrs.fr

Centre de Biochimie Structurale
 Montpellier, France



The activation mechanisms of GPCRs, where an external signal is propagated across the membrane through conformational rearrangements, have been extensively studied over the last decades by various biochemical, structural and biophysical methods. These have led to the conclusion that GPCR activation cannot be sufficiently explained by a simple on/off transition from an inactive to a distinct active state. Instead, it is rather a highly dynamic process where the equilibrium between multiple coexisting conformational states is altered by interacting molecules such as proteins, lipids, ions and others. Therefore, methods to monitor solubilized full-length receptor dimers.

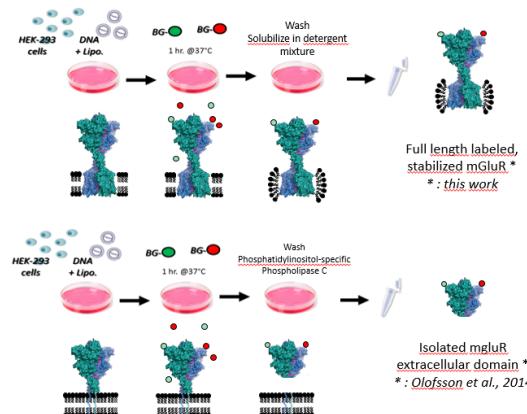
these conformational changes, preferentially at the single molecule level, are needed. Here, using single molecule Förster resonance energy transfer (smFRET) we are studying the structural dynamics that occur during activation of metabotropic glutamate receptors (mGluRs) in response to ligands. We have previously shown that isolated ligand binding domains oscillate between an active/open and an inactive/closed state in a time range of ~100 µs and that orthosteric ligands shift the equilibrium depending on their efficacy. We have now extended these observations to detergent-solubilized full-length receptor dimers.



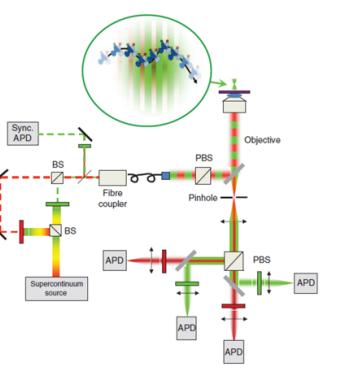
Conformational changes associated with activation



Sample preparation



Confocal nsALEX/PIE smFRET setup



In a proper detergent mixture (including MNG-3 and CHS), the solubilized, labeled receptor retains its pharmacological properties for several hours at RT.
 This preparation is suitable for single molecule FRET experiments

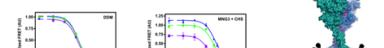
Stability of the full length receptor

Ensemble LRET on Living Cells



Agonist (Glu) binds to the extracellular domain and NAM are allosteric modulators, that bind to the 7TM domain and serve as reporters of receptor integrity

Ensemble LRET on solubilized receptor



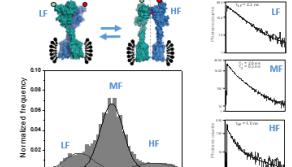
Effect of Cholesterol-HS concentration
 Allosteric modulation, that reports on the 7TM integrity, is only observed in MNG-3 + CHS



Stability vs. Time at RT

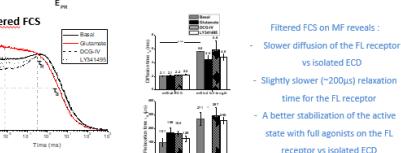
Structural dynamics of the apo receptor

Single molecules excited state lifetime analysis



3 populations are observed:
 - Static LF and HF single exponential decay
 - Dynamic MF state (double exponential decay)

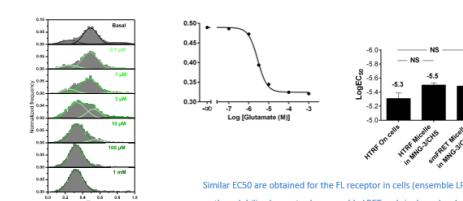
> Similar to the isolated extracellular domain (Olofsson et al., 2014)



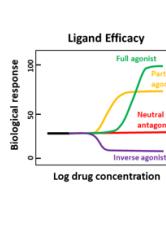
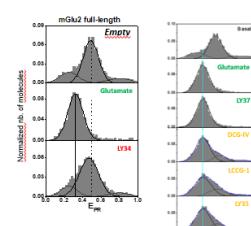
Filtered FCS on MF reveals:
 - Slower diffusion of the FL receptor vs isolated ECD
 - Slightly slower (~200µs) relaxation time for the FL receptor
 - A better stabilization of the active state with full agonists on the FL receptor vs isolated ECD

Effect of ligands - Pharmacology

Titration with glutamate



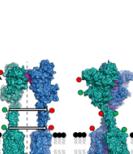
Effect of full agonists, partial agonists, and neutral antagonist



Full agonists strongly push the equilibrium toward the active state
Neutral antagonist reverses this effect
 The 7TM stabilizes the active conformation in the presence of **agonists**.
Partial agonists are less potent to shift this equilibrium

Conclusions and outlook

- Solubilization, labeling and stabilization of full length mGluR, suitable for smFRET
- Observation of fast conformational dynamics of the apo receptor
- Investigation of the effect of ligands. Explanation for the action of partial agonists
- Incorporation of unnatural amino acids at various positions to investigate the correlation between movements
- Decipher the mechanism of biased agonists
- Investigate the effect of lipid composition and/or curvature



Biophysical visual virtual reality in retinotopic visual areas

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bio-photonics

Previously, we have pointed out that biophoton production can be a controlled process that originates from regulated redox/radical reactions. Our biophoton experiments support the notion that various visual related phenomena such as discrete retinal noise, retinal phosphenes as well as negative afterimages are due to biophotons. We have also suggested a new model stating that the brain is able to create biophysical pictures in retinotopic visual areas via redox regulated biophotons of synchronized neurons according to our interpretation, visualisation (imager) is a special kind of representational (e.g., visual) imagery requires peculiar inherent biophysical processes. Our idea of biophysical visual virtual reality in retinotopic areas might be a possible biophysical basis of Kosykh's reality simulation principle in the case of visual imagery. Long-term visual memories are not stored as biophysical pictures but as epigenetic codes. During visual imagery top-down processes control the epigenetically encoded long-term visual information. Then, according to retrieved epigenetic information, synchronized retinotopic neurons generate dynamic patterns of biophotons via redox reactions that can produce biophysical pictures. We have also presented an iterative model involving a biophysical picture-representation without homunculus during visual imagery.

Abstract

1. Redox regulated biophotons

A great number of experiments have provided strong evidence that ROS (reactive oxygen species) and RNS (reactive nitrogen species) act as the second messengers at the level of regulated secondary messengers in diverse cells and neurons during intracellular signaling and intercellular communication processes¹. The delicate balance between beneficial and harmful effects of free radicals is essential for redox regulation and redox homeostasis of cells and organs².

Ultrastructural photon emission (biophoton) is continuously emitted by all living cells without any excitation. Since the production of ROS and RNS is not a random process, but rather a precise mechanism used in cellular signaling pathways, the biophoton emission can also be a redox regulated process in diverse cells and neurons³.

4-a. Biophysical pictures during visual perception and imagery

Based on the above mentioned functional roles of free radicals and regulated ultrastructural biophoton generation in cells and neurons, and also based on our previous findings⁴, we formulated molecular hypotheses regarding the natural biophysical substrate of visual perception and visual imagery⁵. It states that retinotopic electrical signals (spike-related electrical signals along classical axonal-dendritic pathways) can be converted into delayed biophoton signals by redox processes. Moreover it is possible to produce biophysical pictures in the visual system. In the highly organized mitochondria-rich cytochrome oxidase-rich visual areas during visual perception (see Fig.2) and visual imagery (see Fig.3). During visual imagery, top-down processes trigger and regulate the epigenetically encoded long-term visual information. These processes activate and regulate the mitochondrial networks in synchronized neurons generate dynamic patterns of biophotons via redox reactions. Thus, synchronized dynamic patterns of biophotons can produce biophysical pictures (depictive representation) in retinotopic visual neurons of V1 and V2 via iterative processes (see Fig.3 and Fig.4).

5. Visible light induced ocular delayed bioluminescence as a possible origin of negative afterimage

A generally accepted concept of negative afterimages is based on the photopigment bleaching hypothesis. However, there are several contradictions about photopigment bleaching idea. In addition, in a dark room, the photopigment bleaching idea cannot explain where the negative afterimage appears when we close our eyes without any external photon stimulation.

Based on our experiments about visible light induced delayed biophoton emission from isolated rat's whole eye, lens, vitreous humor and retina, we suggested that the phenomenon of negative afterimage may also occur within the eye by delayed bioluminescent photons. In other words, when we stare at a colored (or white) image for few seconds, external photons can induce excited electronic states within different parts of the eye that will emit photons. These photons can be emitted for several seconds. Finally, these emitted photons can be absorbed by non-bleached photoreceptors that produce a negative afterimage interpreted and modulated by cortical neurons.

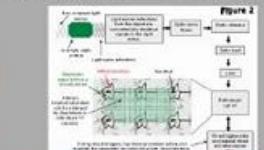
2. Retinal discrete dark noise via biophotons

Recently, we suggested that the discrete dark noise of rods (spontaneous phosphene activation in dark-adapted retinal cells) can be due to the bioluminescent biophotons generated continuously by retinal NPQ photoconversion and oxidative metabolism⁶.

Later, we presented the first experimental proof of the existence of spontaneous ultrawhite biophoton emission and visible light induced delayed biophoton emission from rat's whole eye, lens, vitreous humor and retina⁷. Our experimental results support that the retinal dark noise can result from bioluminescent photons (see Fig.1).

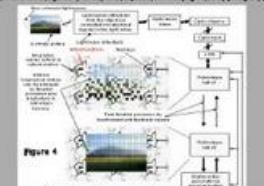
4-b. Biophysical pictures during visual perception

During visual perception, visual redox buffer can use bottom-up and top-down processes to display visual perceptions and makes it possible to visualize and identify an object. We believe that the visual biophoton signal can be much more dramatic than the visual photons compared to their surrounding environment and according to our rough calculations, the red biophoton intensity within retinotopic visual neurons may be sufficient to produce normal biophysical picture representation.



6. Biophysical picture-representation without homunculus during visual imagery

During visual imagery top-down processes activate and regulate the epigenetically encoded long-term visual memory. Next, according to retrieved long-term information, mitochondrial networks within synchronized neurons produce dynamic patterns of biophotons via redox reactions. These patterns can produce biophysical pictures (depictive representation) in retinotopic and mitochondrial rich visual neurons by iterative processes⁸ (see Fig.4). As a result, we could retrieve what we thought we would have seen or done in the analogous perceptual situation during visual imagery.

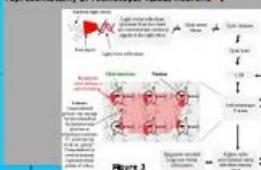


3. Retinal phosphenes by biophotons

Bókön proposed a new biophysico-visual concept of phosphene phenomenon⁹. He raised that various stimuli such as mechanical, electrical, magnetic, ionizing radiation etc., as well as random biophotons forming of cells in the visual pathway can elicit an unregulated overproduction of free radicals and exceed antioxidant capacity of the visual system. This can lead to phosphene. In different regions of the visual system, if this excess biophoton emission exceeds a certain threshold, it can appear as phosphene lights in our mind. However, our experiments about spontaneous and visible light induced delayed biophoton emission from rat's whole eye, lens, vitreous humor and retina support this new photo-biophysico-visual concept at least. In the case of retinal phosphenes, However, if it can be demonstrated that perception of cortical phosphene lights is also due to neuronal biophotons, intrinsic biophotonic retinotopic visual areas can serve as a natural biophysico-visual (redox molecular) substrate of visual perception and imagery.

4-c. Biophysical pictures during visual imagery

The long-term visual information can be stored as epigenetically encoded long-term visual information and not as biophysical pictures. During visual imagery, top-down processes trigger and regulate the epigenetically encoded long-term visual information. Then, according to retrieved epigenetic information, mitochondrial networks in synchronized neurons generate dynamic patterns of biophotons via redox reactions that can produce biophysical pictures (depictive representation) in retinotopic visual neurons¹⁰.



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Molecular Dynamics Simulation of Viral Glycan Binding Activity of Porcine/Human Lung Surfactant Protein D

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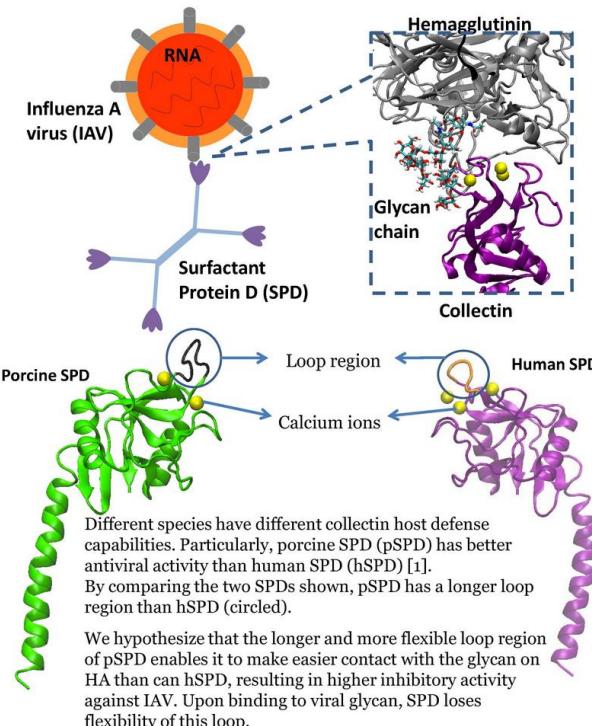
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Abstract

Lung collectin surfactant protein D (SPD) is a pulmonary host defense protein that contributes to innate, front-line defense against influenza A virus (IAV) and other inhaled pathogens. Collectins recognize viral glycans on the globular head of hemagglutinin (HA) on the IAV surface and initiate events leading to pathogen neutralization. Thus, effective pulmonary host defense requires fast recognition of IAV HA by collectins. In order to assist development of new approaches to collectin-based antiviral therapeutics, we investigated the mechanism underlying SPD recognition of IAV HA using molecular dynamics simulations. Comparing the binding likelihoods of SPDs of human and swine on different IAV HA proteins, we showed that swine's SPD has a higher binding likelihood towards the glycans of HA proteins.

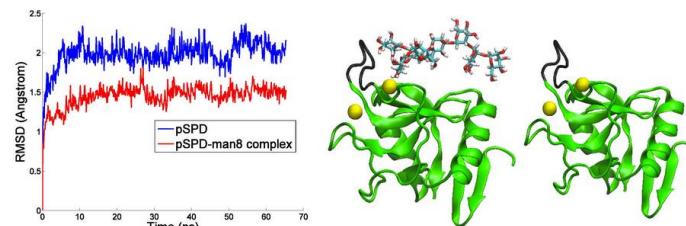
Introduction

Influenza is an infectious disease that continues to cause severe illness and deaths annually worldwide. To infect a host cell, HA on the surface of IAV's viral envelope, binds and subsequently fuses the IAV membrane to the host cell membrane. The collectin protein of SPD binds to the glycan chain of HA, which leads to functional inhibition of HA and reduction in virulence of IAV.



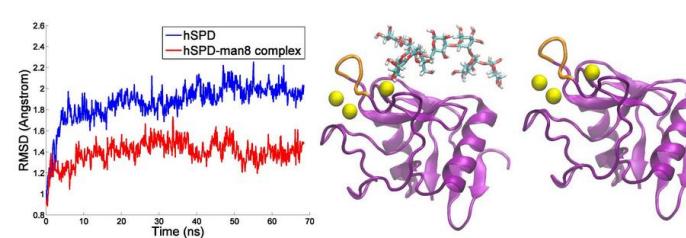
This computational work compares the flexibility of the loop region of porcine and human SPDs. It also shows reduction of flexibility upon glycan binding.

Porcine SPD (pSPD)-Glycan Interaction



Based on an rmsd calculation between the globular head of pSPD, simulations of pSPD-man8 complex and free pSPD revealed that the pSPD-man8 complex fluctuates less and therefore is conformationally more stable than pSPD alone.

Human SPD (hSPD)-Glycan Interaction



Calculations show that rmsd values for free hSPD are larger than for a hSPD-man8 complex.

The results of pSPD and hSPD are consistent with the hypothesis that glycan binding reduces the flexibility of SPD.

Simulation Methods

Protein	Glycan	Time (ns)
pSPD	Mannose-8	65
pSPD	None	65
hSPD	Mannose-8	68
hSPD	None	68

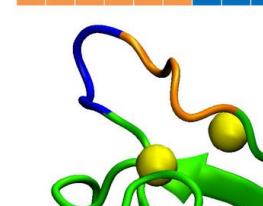
The pSPD-mannose-8 (man8) complex was determined by molecular docking with Glide using the pSPD crystal structure as the receptor for the man8 model. hSPD-man8 complex was obtained from overlay of the pSPD and hSPD crystal structures to align the docked man8 of the pSPD conformation in the lectin site of hSPD. The protein structures of pSPD and hSPD without man8 were generated by removing the mannose residues via VMD.

The simulations were performed using NAMD 2.8 [2] with CHARMM27 for proteins, CHARMM36 for carbohydrates and TIP3P water model. Na⁺ and Cl⁻ ions were added to neutralize the system. The complexes were simulated in NVT ensemble at temperature of 310K using a Langevin-Brownian thermostat and pressure of 1 atm via the Langevin Nosé-Hoover method.

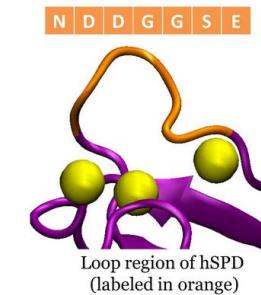
Flexibility Comparison of the Loop Region of the SPDs

Compared with the amino acid sequence of hSPD, pSPD has an insertion of three amino acids (329S,330G,331A) at the loop region close to collectin binding site. This insertion has been suggested to be functionally important [3]. We term this 3-residue-longer loop a 'lip' as we hypothesize this lip increases the likelihood of capturing the glycan chain of HA.

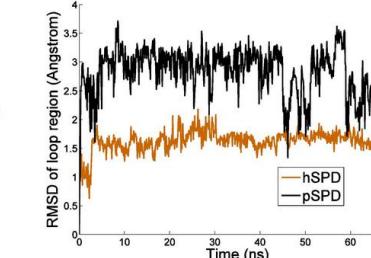
N N N G G S S G A E



N D D G G S E



Loop region of pSPD (labeled in orange) with the three-residue-insertion (labeled in blue)



Rmsd of the loop regions of free pSPD and hSPD are calculated. The lip of pSPD has higher rmsd values than hSPD almost throughout the simulation.

The three-residue-insertion introduces more flexibility to pSPD and increases the likelihood of binding to HA glycan of IAV.

Future Work

This comparative study between pSPD and hSPD allows one to understand the effect of amino acid insertion on the binding affinity of SPD.

Starting from docked structures between crystallized proteins of different strains of IAV and species of SPD, MD simulations will be performed to investigate the interaction between SPD and glycan of HA and identify the most stable docking configuration of the SPD-HA complex.

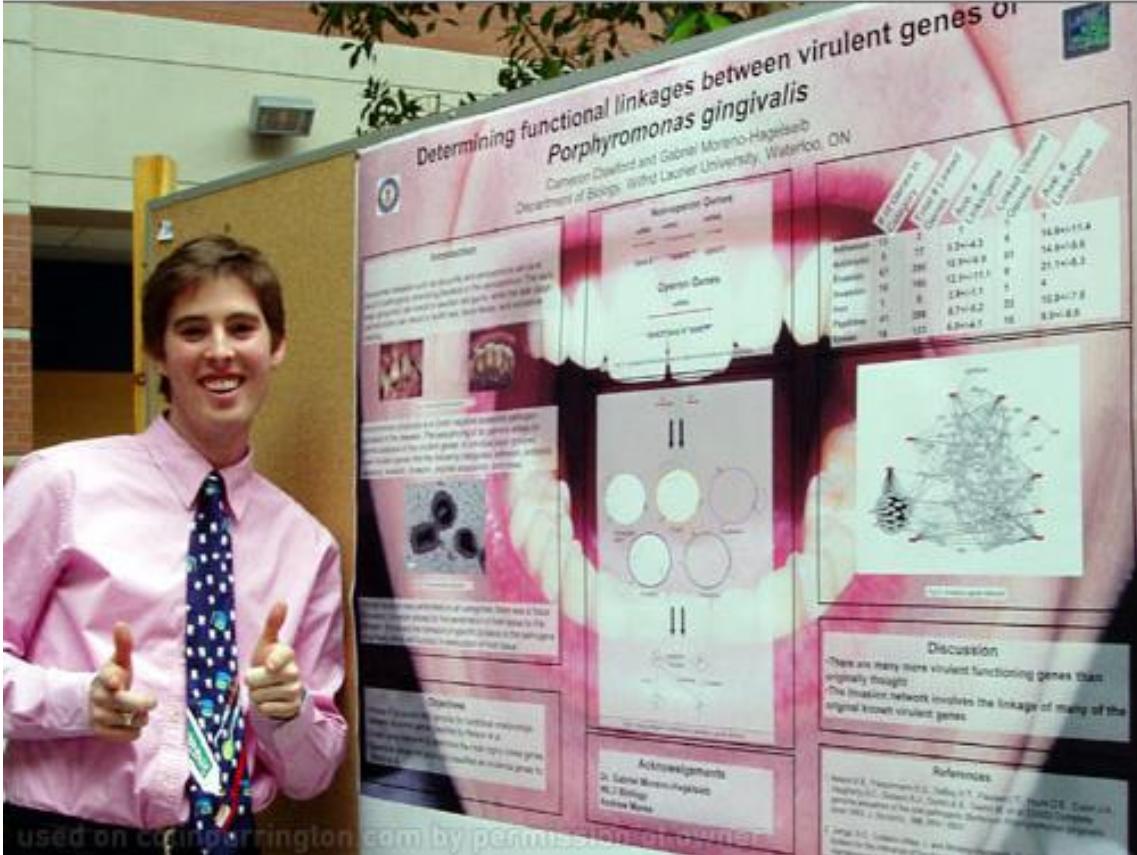
Acknowledgement

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For the next session (next Tuesday)



GOOD LUCK