Αναζήτηση βιβλιογραφίας - συγγραφή επιστημονικού κειμένου

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Τμήμα Μοριακής Βιολογίας και Γενετικής
«Διδακτική των Βιοεπιστημών»

Ενότητα Α

Αναζήτηση βιβλιογραφίας

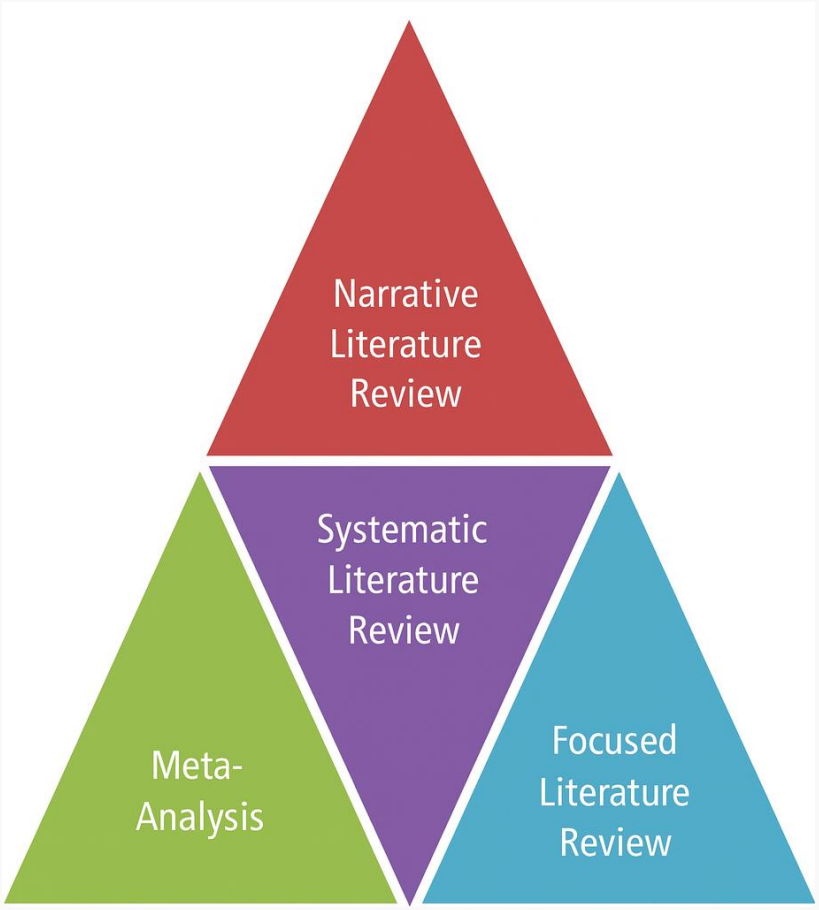
1 Γιατί είναι σημαντική;

- Κατανοούμε:

1. Ποια είναι η υπάρχουσα γνώση
2. Ποια είναι τα νεότερα και πιο αξιόπιστα δεδομένα για ένα αντικείμενο
3. Ποια είναι τα κενά → Τι δεν γνωρίζουμε

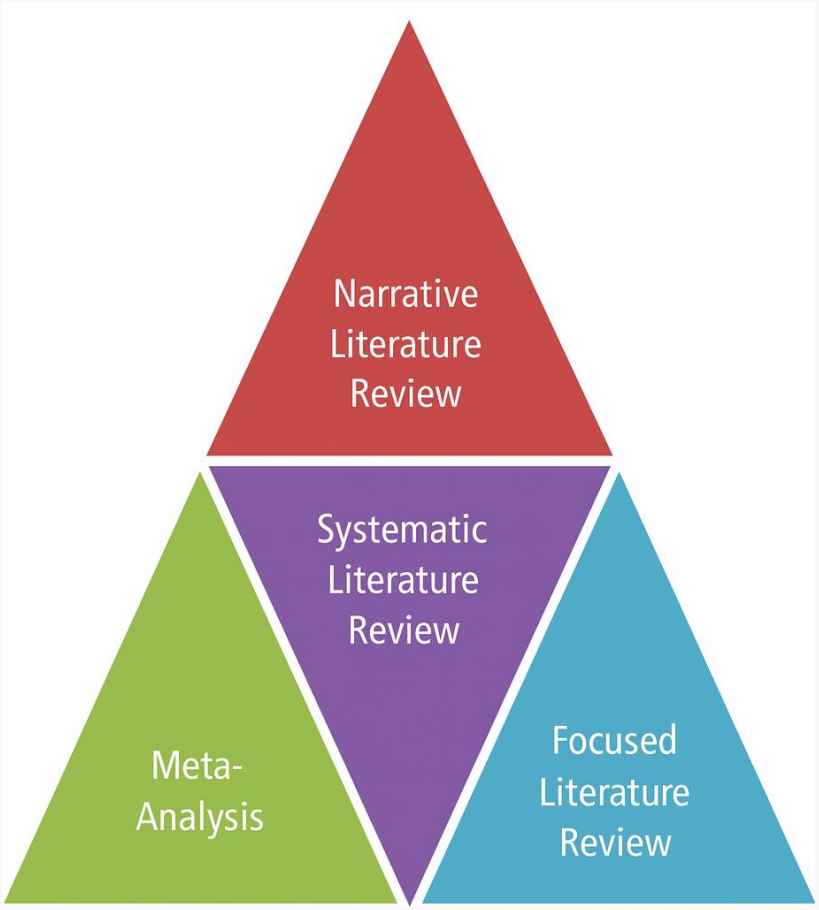
1 Γενική αναζήτηση vs. Συστηματική αναζήτηση βιβλιογραφίας

Γενική αναζήτηση: Ευρεία, διερευνητική, σημαντική για να αποκτήσουμε μια γενική και συνολική κατανόηση ενός θέματος



1 Γενική αναζήτηση vs. Συστηματική αναζήτηση βιβλιογραφίας

Συστηματικής αναζήτησης βιβλιογραφίας: Εστιασμένη, αναπαραγώγιμη διαδικασία για την εύρεση όλων των σχετικών μελετών που απαντούν σε ένα ερώτημα με καθορισμένα κριτήρια και βάσεις δεδομένων (ερευνητική διαδικασία)



1 Γιατί είναι σημαντική;

- Μια καλή αναζήτηση στην βιβλιογραφία:

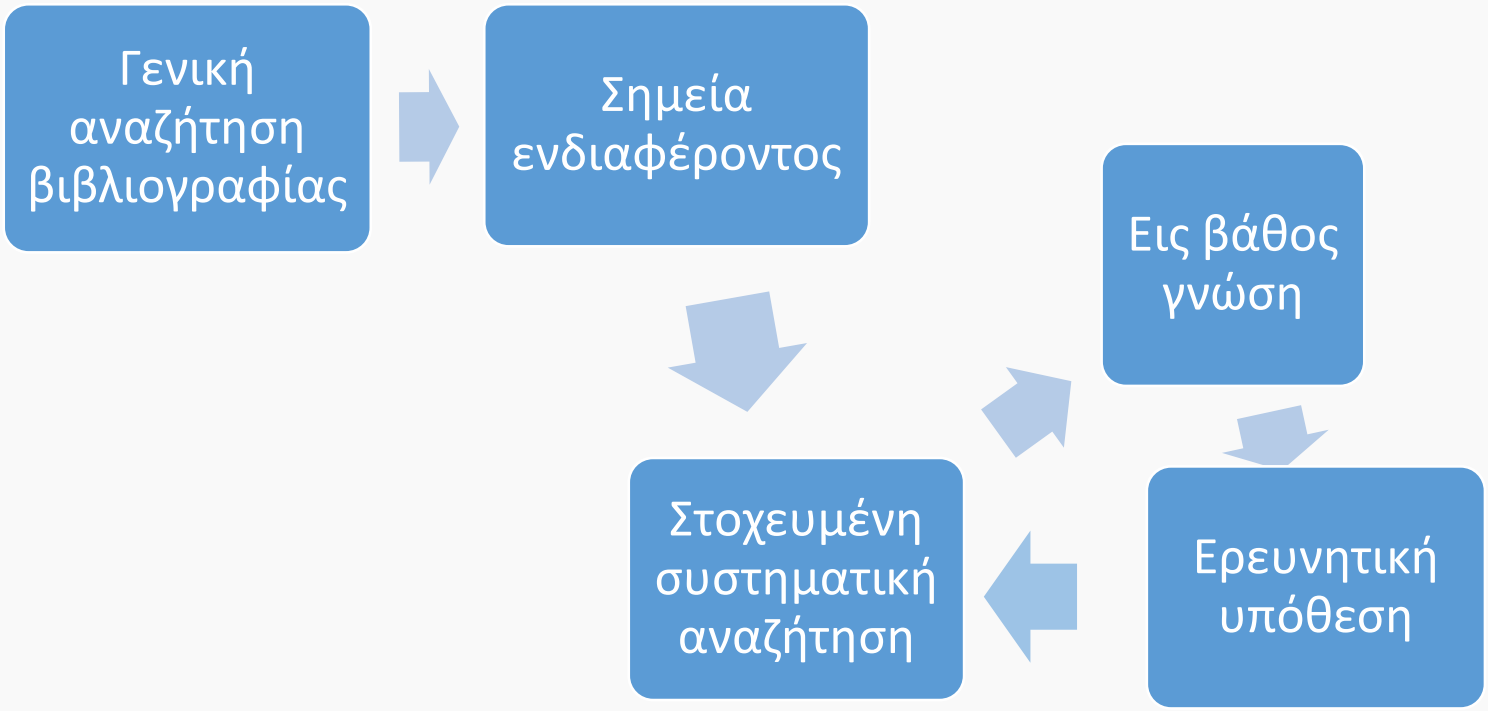
✓ Αποτρέπει την δημιουργία μη-πρωτότυπης δουλειάς/έρευνας

✓ Είναι καίρια για την διαμόρφωση μιας νέας και ξεκάθαρης υπόθεσης

✓ Καθορίζει την κατάλληλη μεθοδολογία για να διερευνηθεί μια υπόθεση

✓ Διασφαλίζει επιστημονική εγκυρότητα

1 Γιατί είναι σημαντική;



2 Τι θέλω να ψάξω;

- Έχοντας πλέον μια γενική εικόνα της βιβλιογραφίας:

✓ Λέξεις κλειδιά

✓ Φράσεις

Οι λέξεις κλειδιά και οι φράσεις που χρησιμοποιώ θα πρέπει να αναδιαμορφώνονται συνεχώς βάση των ευρημάτων μου ώστε να βελτιστοποιούνται

2 Τι θέλω να ψάξω;

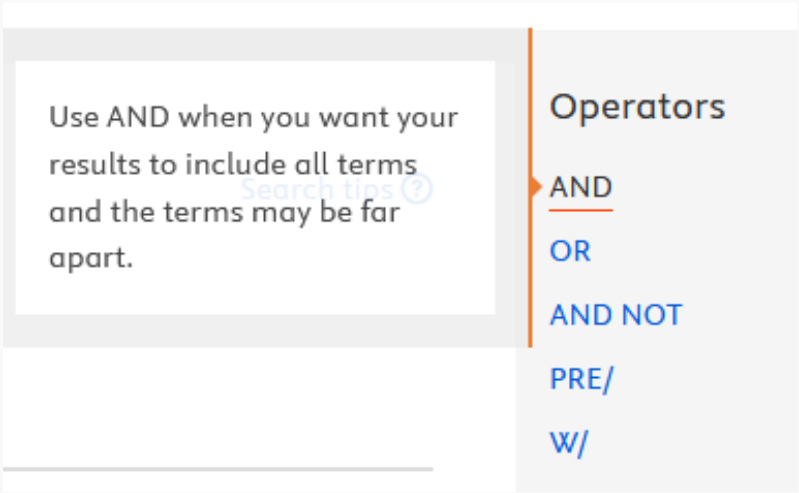
- Πως θα βρω τις λέξεις κλειδιά:

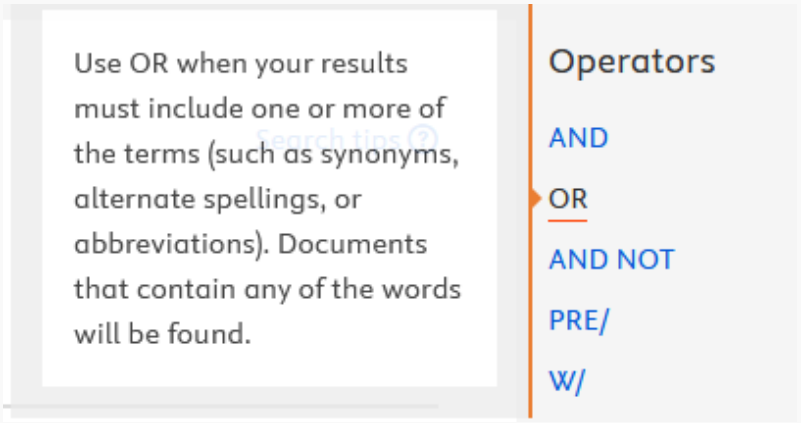
✓ Γράφω το θέμα μου

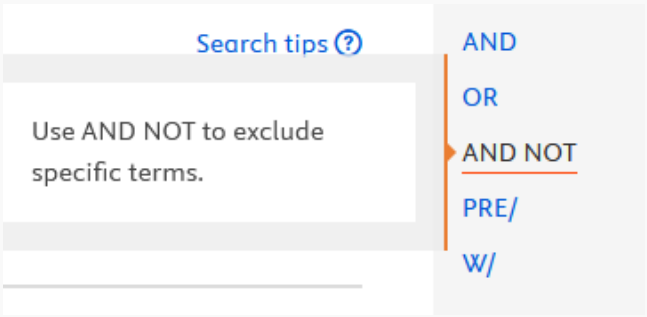
✓ Σημειώνω τις βασικές ιδέες

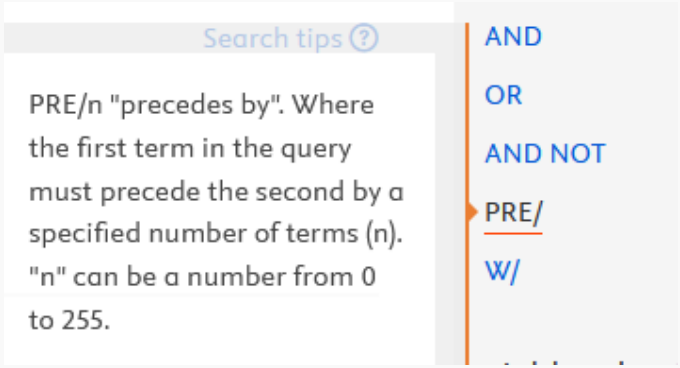
✓ Ψάχνω συνώνυμα ή έννοιες που μπορεί να σχετίζονται

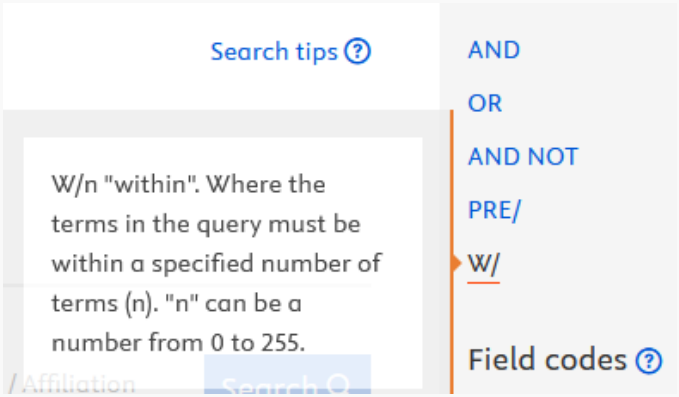
2 Τι θέλω να ψάξω;











Χρήση εισαγωγικών για αναζήτηση φράσεων “” (αναζήτηση της ακριβούς φράσης)

3 Μηχανές αναζήτησης

Google

Μπορεί να μας βρει πηγές αλλά θα πρέπει να προσέξουμε για την ποιότητα τους – γενικό εργαλείο

Google Μελετητής

Ειδικό εργαλείο για την εύρεση επιστημονικών δημοσιεύσεων

3 Μηχανές αναζήτησης

Εξειδικευμένες για επιστημονικές δημοσιεύσεις

PubMed®

Scopus

Επιστήμες υγείας

ScienceDirect®

4 Βελτίωση αποτελεσμάτων

Πολύ λίγα αποτελέσματα → Πιο ευρείς όρους

Πολλά αποτελέσματα → Πιο αυστηρά κριτήρια

4 Βελτίωση αποτελεσμάτων

- Φίλτρα:

✓ Εύρος ημερομηνιών (θέλουμε να δούμε όλες τις δημοσιεύσεις ή τις πιο πρόσφατες;)

✓ Τύπος δημοσίευσης (review, research article, meta-analysis)

Σημειώνουμε τον αριθμό των ευρημάτων σε κάθε αναζήτηση για να ελέγχουμε την ευαισθησία προς την ειδικότητα

5 Αξιοπιστία βιβλιογραφικών πηγών

- Ποιες πηγές είναι «κατάλληλες» για ένα επιστημονικό κείμενο;
- ✓ Peer-reviewed articles (golden-standard)
  - Το περιοδικό να έχει impact factor
  - Τα άρθρα να εμφανίζονται στις επίσημες επιστημονικές βάσεις αναζήτησης πχ pubmed
  - Ημερομηνία δημοσίευσης

5 Αξιοπιστία βιβλιογραφικών πηγών

- Ποιες πηγές είναι «κατάλληλες» για ένα επιστημονικό κείμενο;

✓ Επιστημονικά συγγράμματα

- Ο συγγραφές να έχει τα κατάλληλα προσόντα (επιστημονική ιδιότητα, εξειδίκευση)
- Έκδοση (είναι από επιστημονική έκδοση;)
- Παρέχει αξιόπιστες βιβλιογραφικές αναφορές;
- Η γνώση να είναι updated (πρόσφατη έκδοση)

5 Αξιοπιστία βιβλιογραφικών πηγών

- Ποιες πηγές είναι «κατάλληλες» για ένα επιστημονικό κείμενο;

✓ Διδακτορικές διατριβές

✓ Πτυχιακές εργασίες?

Προσοχή στις πληροφορίες που θα αντλήσουμε…

5 Αξιοπιστία βιβλιογραφικών πηγών

Προσοχή στο πως θα ενσωματώσουμε τις πηγές στην βιβλιογραφία

Βάζουμε την πρώτη αναφορά πάντα;

Figure 1. The process of malignant transformation. Cancer is a multi-staged procedure in which cells gradually acquire malignant characteristics. Initiation includes certain genetic/epigenetic changes resulting in the deregulated control of processes, such as cell-cycle progression, apoptosis, and proliferation. The clonal expansion of the initiated cell, which exhibits defective apoptosis, abnormal cell-cycle arrest, and excessive proliferation, leads to the formation of a preneoplastic lesion of closely attached cells. During progression, the genetically unstable preneoplastic cells progressively accumulate novel, malignant-related properties, such as the ability to escape from immune surveillance, migrate and invade new tissues, and form new tumors.

Initiation is an irreversible process that involves the deregulation of one or more genes associated with crucial regulatory pathways, either by chromosomal genetic or epigenetic alterations. These genes are considered to be either oncogenes, the activation of which results in increased cellular proliferation, or tumor-suppressing genes, the inhibition of which induces the inactivation of cell-cycle arrest and/or apoptosis [7,8]. A malfunction of these genes leads to the transformation of a healthy cell into a preneoplastic cell [9,10]. An important aspect of cancer research is identifyi g preneoplastic properties and the molecular context that support w, the origins of many cancers remain elusive. Recent experim n cells can initiate malignant transformation, the capacity to do so depends on the type of tissue and resulting cancer [11,12,13,14,15].

López-Lázaro, M. The stem cell division theory of cancer. *Crit. Rev. Oncol. Hematol.* **2018**, *123*, 95–113. [**Google Scholar**] [**CrossRef**]

Promotion refers to the selective clonal growth of the preneoplastic, initiated cell and its progeny as a result of their ability to evade apoptosis and/or their enhanced cell proliferation. At the promotion phase, the expanded clone of the preneoplastic cells forms a benign tumor in which the cells remain in close contact with each other; thus, they cannot detach from one another [9,16].

6 Χρήση βιβλιογραφικών πηγών

- Προσοχή στις οδηγίες για την μορφή που θα πρέπει να έχει η βιβλιογραφία (από το περιοδικό ή την επιτροπή του συνεδρίου ή την επιτροπή ενός μεταπτυχιακού)

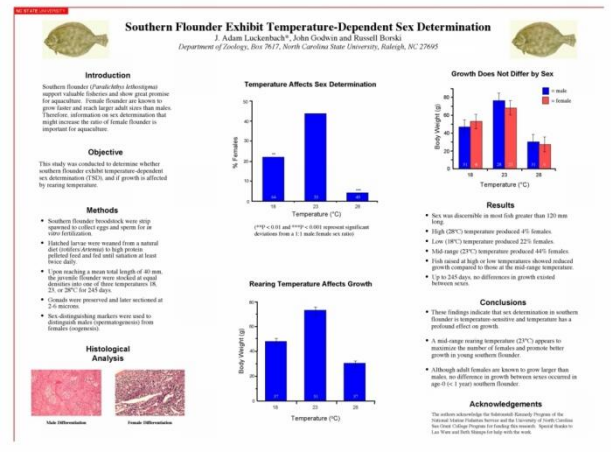
- Πολύ βοηθητική η χρήση προγραμμάτων/εργαλείων για την συλλογή και ενσωμάτωση της βιβλιογραφίας σε ένα κείμενο (δυνατότητα αυτόματης μορφοποίησης) (π.χ. Mendeley, EndNote κα)

Σχεδιασμός και παρουσίαση poster

Ενότητα 3

1 Σχεδιασμός και παρουσίαση poster

Γιατί κάνουμε poster;



Southern Flounder Exhibit Temperature-Dependent Sex Determination

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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) to high protein pelleted food 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

% Females

Temperature (°C)

(**P < 0.01 and ***P < 0.001 represent significant deviations from a 1:1 male:female sex ratio)

Rearing Temperature Affects Growth

Body Weight (g)

Temperature (°C)

Growth Does Not Differ by Sex

Body Weight (g)

Temperature (°C)

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 Saltonstall Kennedy Program of the National Marine Fisheries Service and the University of North Carolina Sea Grant College Program for funding this research. Special thanks to Lee Ware and Beth Matuga for help with the work.

Vs.

- Δεν είμαστε πάντα καλοί στις ομιλίες
- Αναφορά σε περισσότερες λεπτομέρειες
- Δυνατότητα άμεσης ανταλλαγή πληροφοριών
- Έκθεση της δουλείας μας για περισσότερη ώρα

1

Σχεδιασμός και παρουσίαση poster

Σημαντικά στοιχεία

- Ο πιο κοινός τρόπος παρουσίασης της έρευνας μας σε επιστημονικά συνέδρια
- Θα πρέπει να προσελκύει το κοινό
- Να έχει έναν εμφανή και χαρακτηριστικό τίτλο
- Να έχει αρκετές και καλά σχεδιασμένες φιγούρες

1

Σχεδιασμός και παρουσίαση poster

Σημαντικά στοιχεία

- Να έχει οργανωμένη και ξεκάθαρη διαμόρφωση

- Να έχει ακριβείς τίτλους στα επιμέρους κομμάτια

- Να κατευθύνει την προσοχή του αναγνώστη στα σημαντικά σημεία

1

Σχεδιασμός και παρουσίαση poster

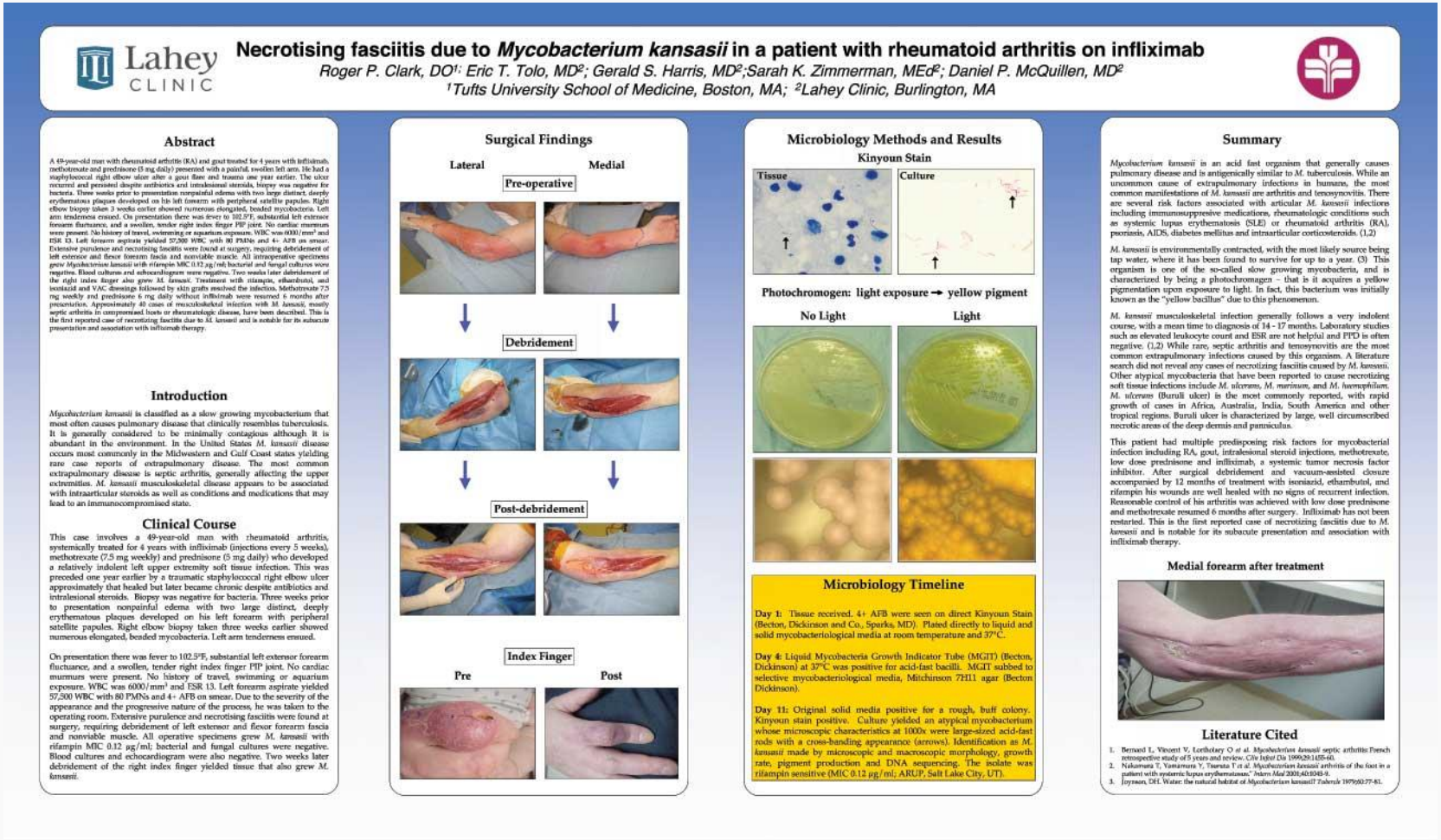
Σημαντικά στοιχεία

Τα poster πρέπει να έχουν περισσότερη πληροφορία και λεπτομέρειες από μια επιστημονική παρουσίαση και λιγότερη από μια επιστημονική δημοσίευση

Παράδειγμα 1

1 Σχεδιασμός και παρουσίαση poster

Λειτουργικά οργανωμένο poster



Lahey CLINIC

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

Lateral Medial

Pre-operative

Debridement

Post-debridement

Index Finger

Pre Post

Microbiology Methods and Results

Kinyoun Stain

Tissue Culture

Photochromogen: light exposure → yellow pigment

No Light Light

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

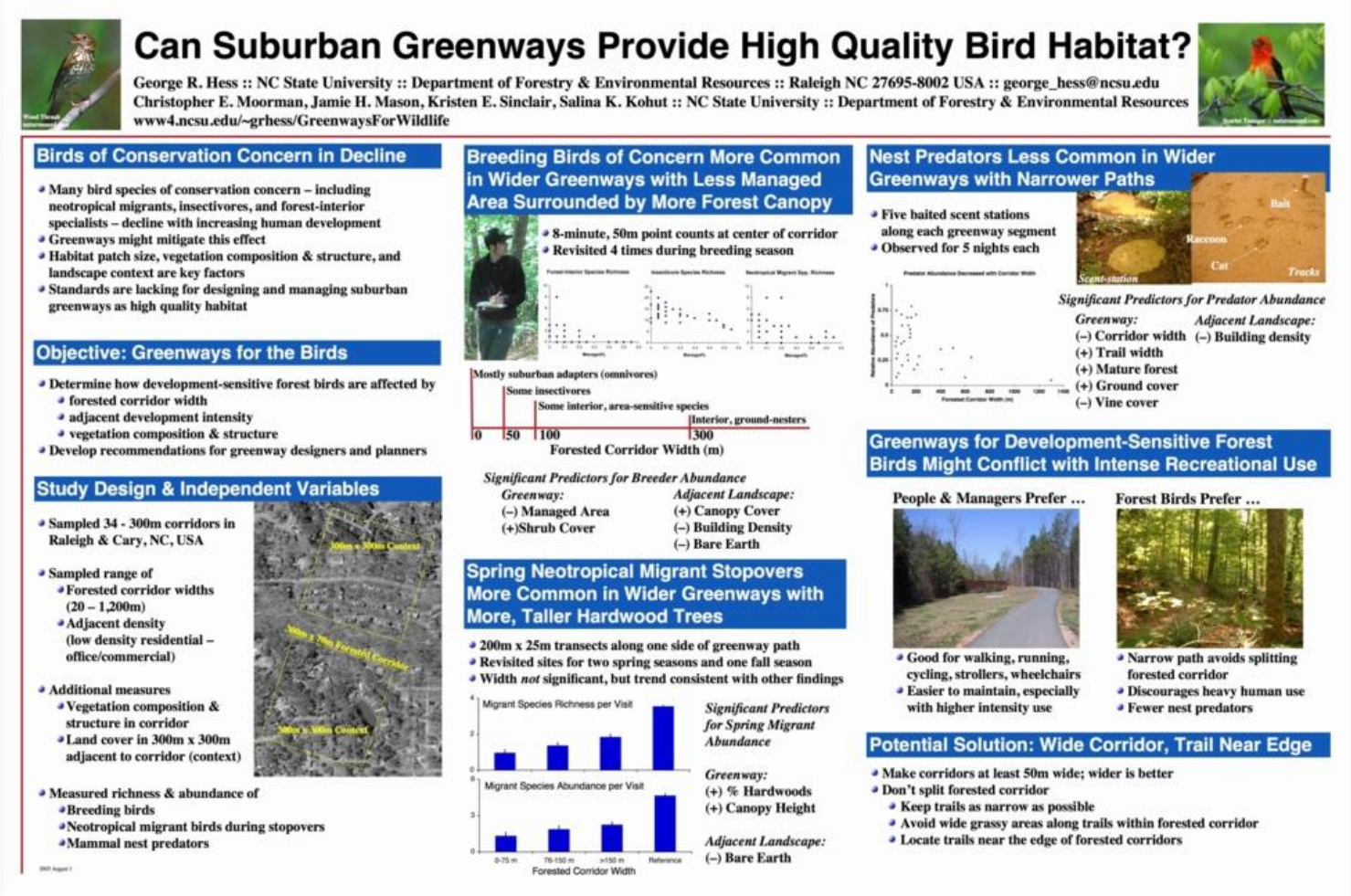
Literature Cited

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;40:1043-8.
3. Joynson, DH. Water: the natural habitat of *Mycobacterium kansasii? Tubercle* 1979;60:77-81.

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 2

Poster με οργάνωση που μπορεί να μπερδέψει



Can Suburban Greenways Provide High Quality Bird Habitat?

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Christopher E. Moorman, Jamie H. Mason, Kristen E. Sinclair, Salina K. Kohut :: NC State University :: Department of Forestry & Environmental Resources
www4.ncsu.edu/~grhess/GreenwaysForWildlife

Birds of Conservation Concern in Decline

- Many bird species of conservation concern – including neotropical migrants, insectivores, and forest-interior specialists – decline with increasing human development
- Greenways might mitigate this effect
- Habitat patch size, vegetation composition & structure, and landscape context are key factors
- Standards are lacking for designing and managing suburban greenways as high quality habitat

Objective: Greenways for the Birds

- Determine how development-sensitive forest birds are affected by
  - forested corridor width
  - adjacent development intensity
  - vegetation composition & structure
- Develop recommendations for greenway designers and planners

Study Design & Independent Variables

- Sampled 34 - 300m corridors in Raleigh & Cary, NC, USA
- Sampled range of
  - Forested corridor widths (20 – 1,200m)
  - Adjacent density (low density residential – office/commercial)
- Additional measures
  - Vegetation composition & structure in corridor
  - Land cover in 300m x 300m adjacent to corridor (context)
- Measured richness & abundance of
  - Breeding birds
  - Neotropical migrant birds during stopovers
  - Mammal nest predators

Breeding Birds of Concern More Common in Wider Greenways with Less Managed Area Surrounded by More Forest Canopy

- 8-minute, 50m point counts at center of corridor
- Revisited 4 times during breeding season

Mostly suburban adapters (omnivores)
Some insectivores
Some interior, area-sensitive species
Interior, ground-nesters
0 50 100 300
Forested Corridor Width (m)

Significant Predictors for Breeder Abundance

Greenway:
(–) Managed Area
(+)Shrub Cover

Adjacent Landscape:
(+) Canopy Cover
(–) Building Density
(–) Bare Earth

Spring Neotropical Migrant Stopovers More Common in Wider Greenways with More, Taller Hardwood Trees

- 200m x 25m transects along one side of greenway path
- Revisited sites for two spring seasons and one fall season
- Width *not* significant, but trend consistent with other findings

Migrant Species Richness per Visit
Migrant Species Abundance per Visit
0-75 m 76-150 m >150 m Reference
Forested Corridor Width

Significant Predictors for Spring Migrant Abundance

Greenway:
(+) % Hardwoods
(+) Canopy Height

Adjacent Landscape:
(–) Bare Earth

Nest Predators Less Common in Wider Greenways with Narrower Paths

- Five baited scent stations along each greenway segment
- Observed for 5 nights each

Bait
Raccoon
Cat
Tracks
Scent-station

Significant Predictors for Predator Abundance

Greenway:
(–) Corridor width
(+) Trail width
(+) Mature forest
(+) Ground cover
(–) Vine cover

Adjacent Landscape:
(–) Building density

Greenways for Development-Sensitive Forest Birds Might Conflict with Intense Recreational Use

People & Managers Prefer …

- Good for walking, running, cycling, strollers, wheelchairs
- Easier to maintain, especially with higher intensity use

Forest Birds Prefer …

- Narrow path avoids splitting forested corridor
- Discourages heavy human use
- Fewer nest predators

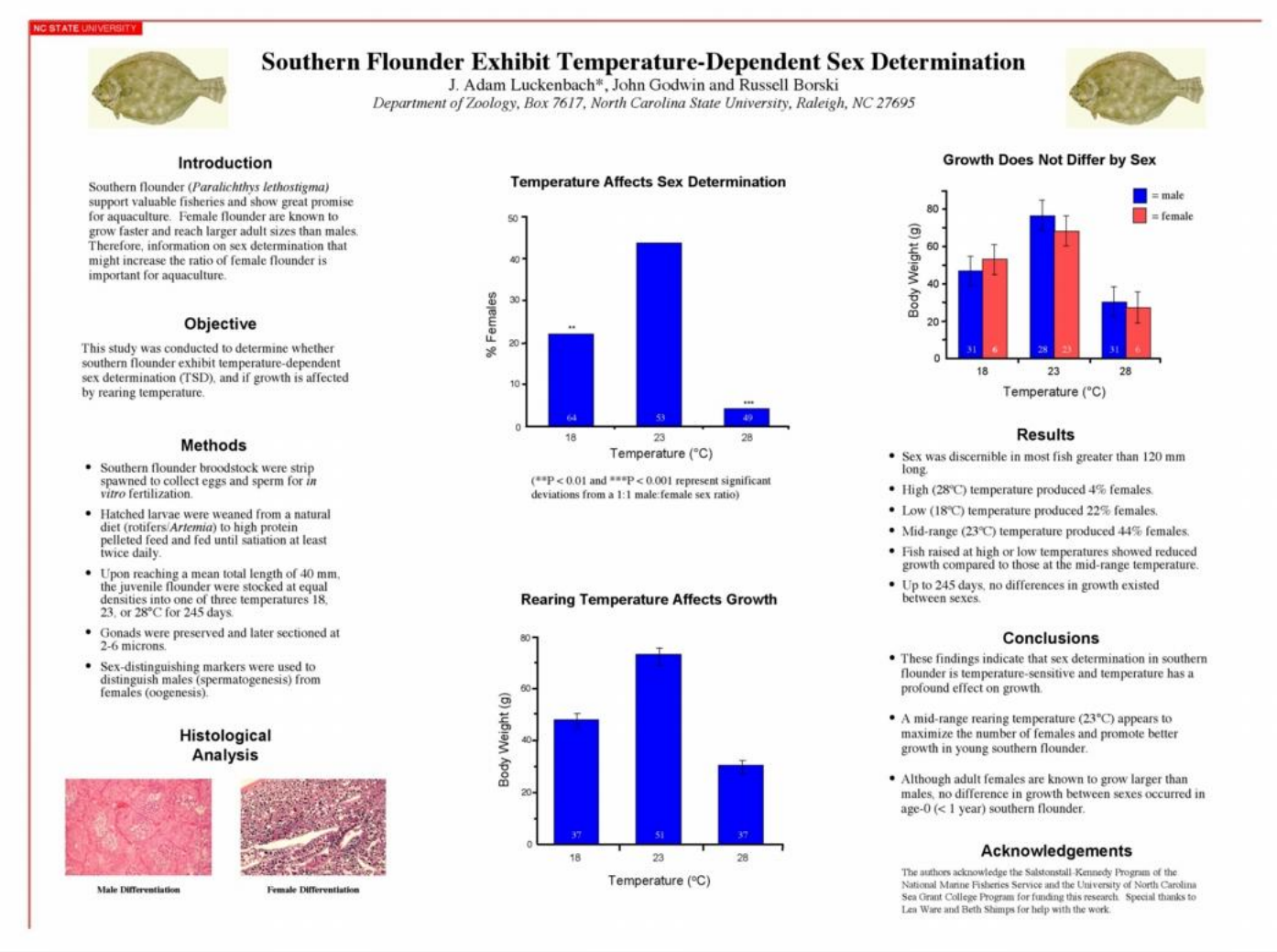
Potential Solution: Wide Corridor, Trail Near Edge

- Make corridors at least 50m wide; wider is better
- Don't split forested corridor
  - Keep trails as narrow as possible
  - Avoid wide grassy areas along trails within forested corridor
  - Locate trails near the edge of forested corridors

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 3

Poster με λίγη πληροφορία



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 < 0.01 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 Salstonstall-Kennedy Program of the National Marine Fisheries Service and the University of North Carolina Sea Grant College Program for funding this research. Special thanks to Lea Ware and Beth Shimps for help with the work.

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 4

Poster με πολύ πληροφορία

The Effects of Cumulative Life Stress on Cardiovascular Stress Reactivity

Alissa Der Sarkissian, Ji Min Jun, Holly Pham, Anthony Portolesi, Alexandra Dupont, M.A.

Department of Psychology, UCLA

Background

- The theory of psychophysiological toughness suggests that experiencing a moderate amount of stressors in the past will improve the person's ability to cope with stressors in the present (Dienstbier, 1989)
- The greater cumulative life stress an individual experienced, the greater dysregulation of cardiovascular reactivity to current acute stressors. (Evans, 2007)
- Chronic negative life stress has been found to be associated with greater diastolic blood pressure and increased heart rate reactivity (Low, Salomon, & Matthews, 2009)

Purpose

The purpose of this study is to examine how experiencing various amounts of cumulative life stress is associated with physiological stress responses to acute stress in young adulthood.

Hypothesis

We hypothesized that cardiovascular reactivity to a social stress task would reflect the amount of cumulative life stress experienced by an individual. Specifically, we hypothesized a U-shaped relationship, such that a moderate amount of stressful life events would be associated with responses of lower change in heart rate and blood pressure, while experiencing no stressors or many stressors would be associated with a greater increase in heart rate and blood pressure.

Methods

Male and female (m=27%, f=73%) undergraduate students (n=45) participated in the Noisy Neighbor task (Luecken, 2009), in which the participant attempted to resolve a social conflict with an uncompromising confederate. Before, during, and after this interaction, autonomic nervous system (ANS) data was collected via blood pressure cuff, ECG, and cardio-impedance. Participants also completed the Life Events Checklist, a measure that assesses cumulative life stress. Responses to the Life Events Checklist showed a mean response of 4.4 life events, a standard deviation of 3.6 and a range from 0 to 21.

Measures

Predictor Variables

•*Life Events Checklist*: A questionnaire measuring exposure to cumulative life stress. Participants indicate whether they actually experienced, witnessed, or learned about each event at any point throughout their lifetime.

○E.g., Natural disaster, fire or explosion, serious injury

○To form a total score, we added the total number of times each participant experienced or witnessed an event.

Outcomes

•*Blood pressure (BP)* = pressure exerted by circulating blood upon the walls of blood vessels

•*Heart rate (HR)* = the number of heartbeats per unit of time

As an individual feels stressed from a threatening situation, they will exhibit both a higher blood pressure and heart rate.

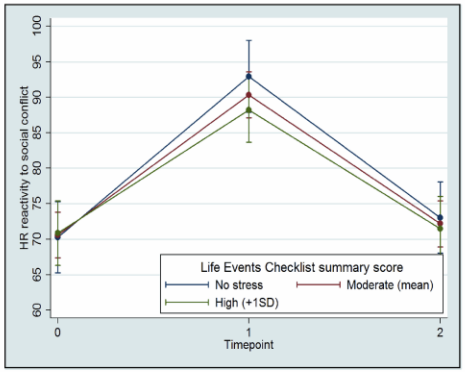


Figure 1: Patterns of heart rate reactivity during stress task based on level of cumulative life stress

Results

- Paired t-tests revealed a significant difference between the baseline and stress task in heart rate (M=19.70, SD=11.49, t = -13.32, p < .01), systolic blood pressure (M=25.19, SD=13.04, t= -15.01, p < 0.01), and diastolic blood pressure (M=16.03, SD=7.77, t = -15.89, p < 0.01).
- With the help of our graduate student advisor, we ran mixed models and found that the continuous variable of cumulative life stress significantly predicted change in heart rate (but not blood pressure) from baseline to the stress task.
- For visual interpretation, we modeled the heart rate reactivity patterns of individuals with no life stress (LEC = 0), moderate life stress (LEC = 4.4, mean), and high life stress (LEC = 8, mean + 1SD).
- Individuals who experienced no cumulative life stress had the greatest increase in heart rate from baseline to the stress task. Cumulative life stress was not associated with heart rate recovery or blood pressure reactivity/recovery.

Conclusions and Implications

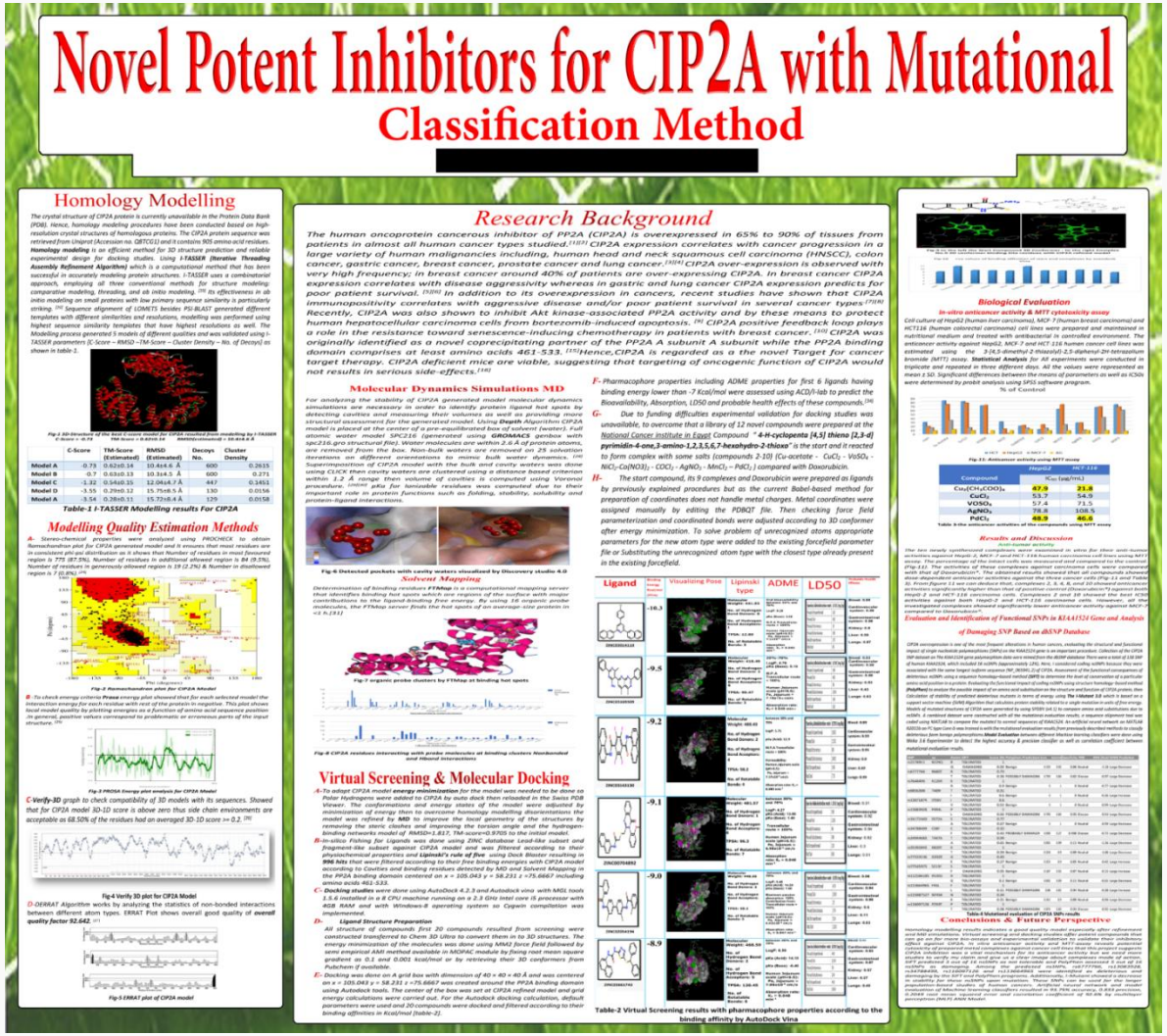
- Through our study we found that cumulative life stress is indeed associated with hemodynamic responses to stress in adults. However, this response is represented by a negative linear correlation with cumulative life stress, such that increasing amounts of cumulative life stress resulted in a decrease in heart rate reactivity, rather than a quadratic relationship.
- A possibility as to why we did not find a U-shape relationship between cumulative life stress and current cardiovascular responses could be due to the categorization of our high cumulative stress group as starting at only 4 life events. Future research with inclusion of participants who have experienced higher levels of cumulative life stress would provide the statistical power necessary to properly examine the current hypothesis.
- This linear relationship may suggest that stressful experiences may dampen future stress reactivity.
- The association that we found between cumulative life stress and cardiovascular reactivity could help to illuminate how past stress exposure may modulate an individual's ability to handle current stressors. The variability in stress reactivity between individuals could potentially lead to differences in future physical health outcomes.

Acknowledgement

We would like to thank our advisors, Alexandra Dupont, Julienne Bower, and Larissa Dooley, for all of their help and support.

Παράδειγμα 5

1 Σχεδιασμός και παρουσίαση poster



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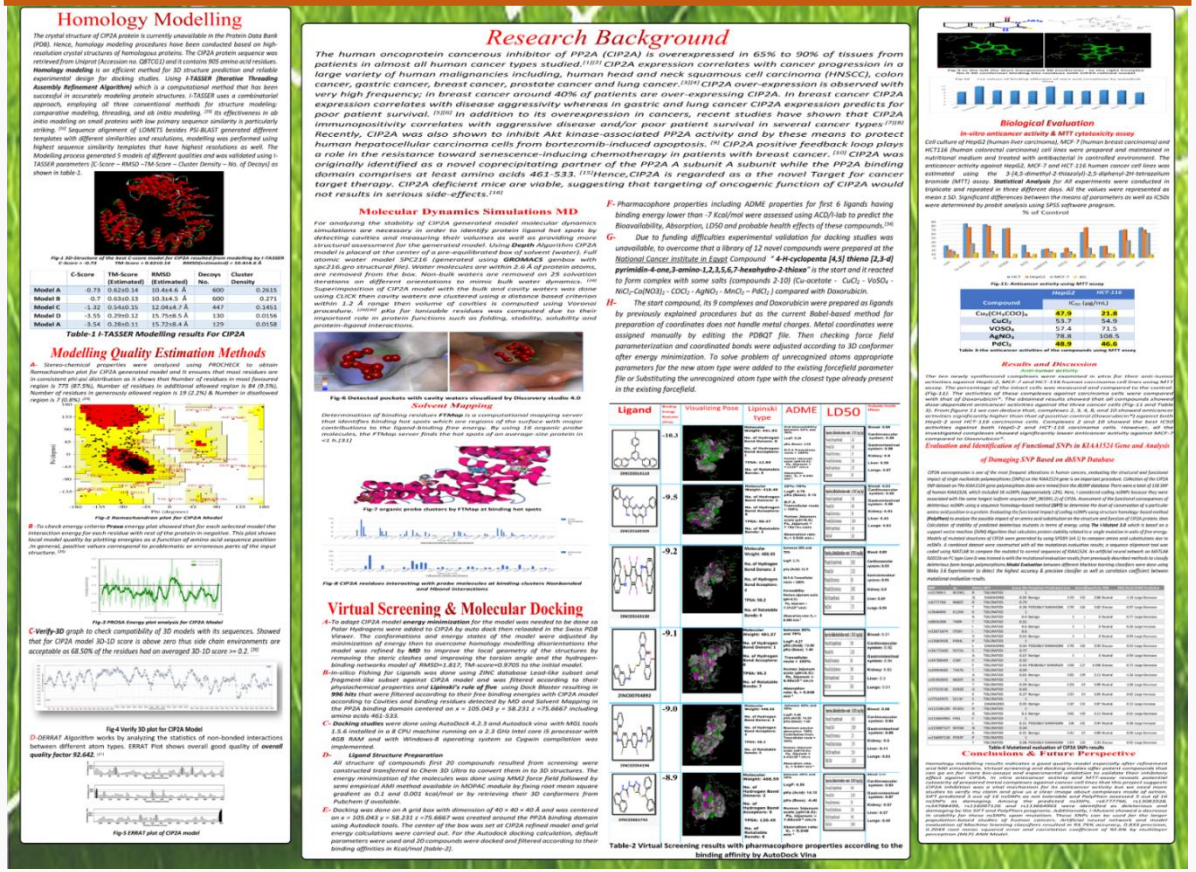
Σωστή γενική οργάνωση με διακριτές περιοχές

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 5

Περιοχή 1



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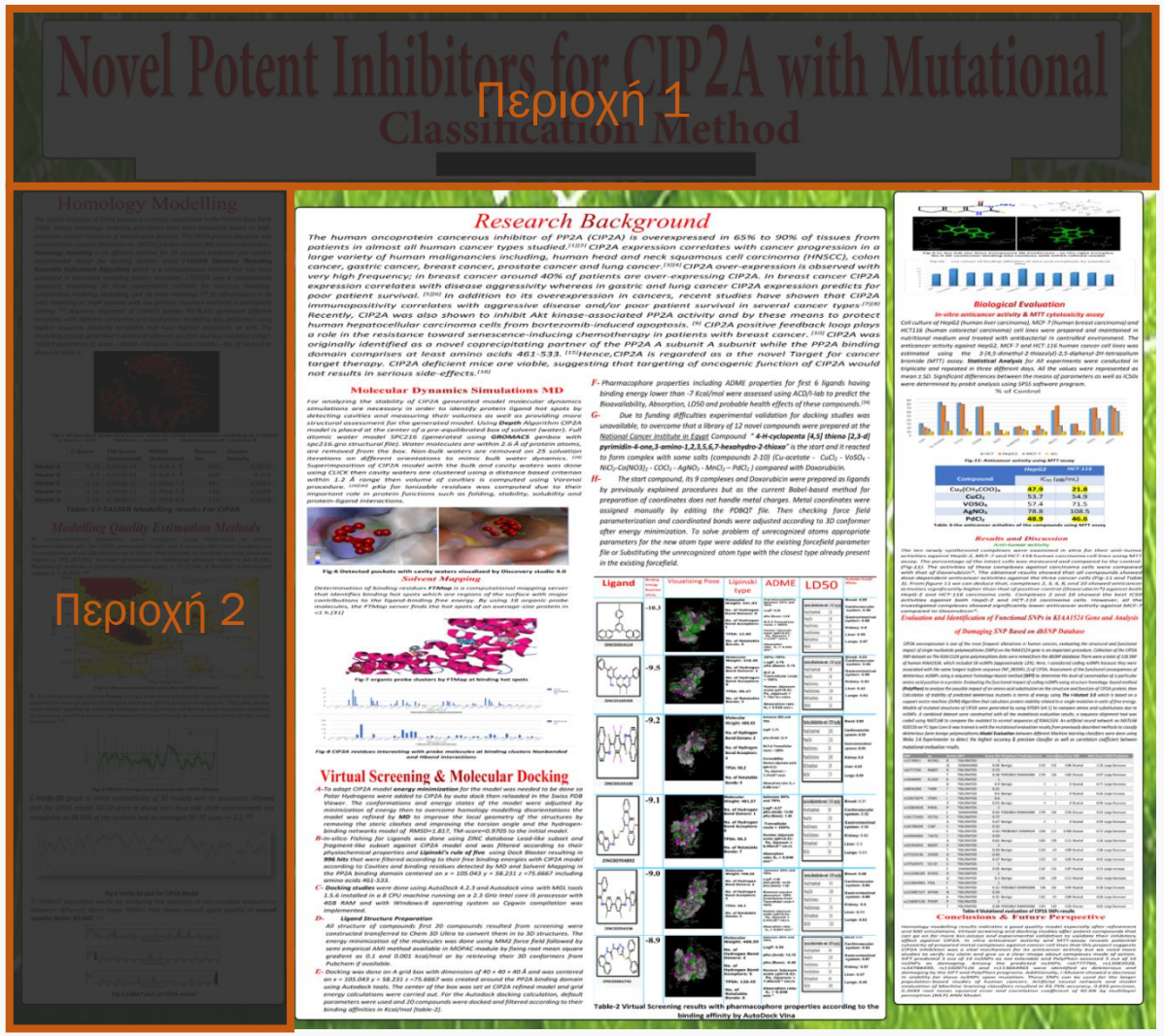
Σωστή γενική οργάνωση με διακριτές περιοχές

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 5



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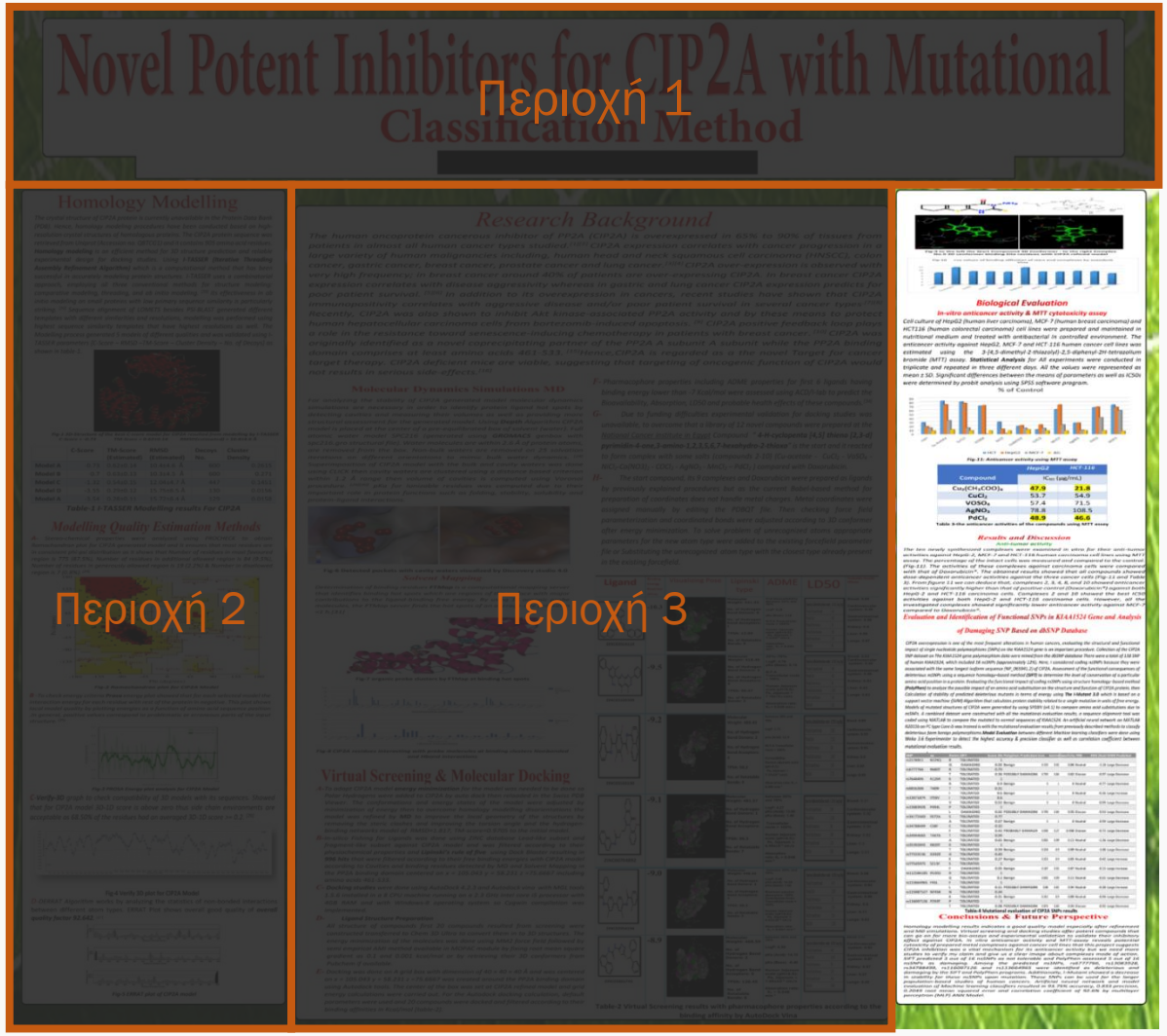
Σωστή γενική οργάνωση με διακριτές περιοχές

1

Σχεδιασμός και παρουσίαση poster

Παράδειγμα 5



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Σωστή γενική οργάνωση με διακριτές περιοχές

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 5

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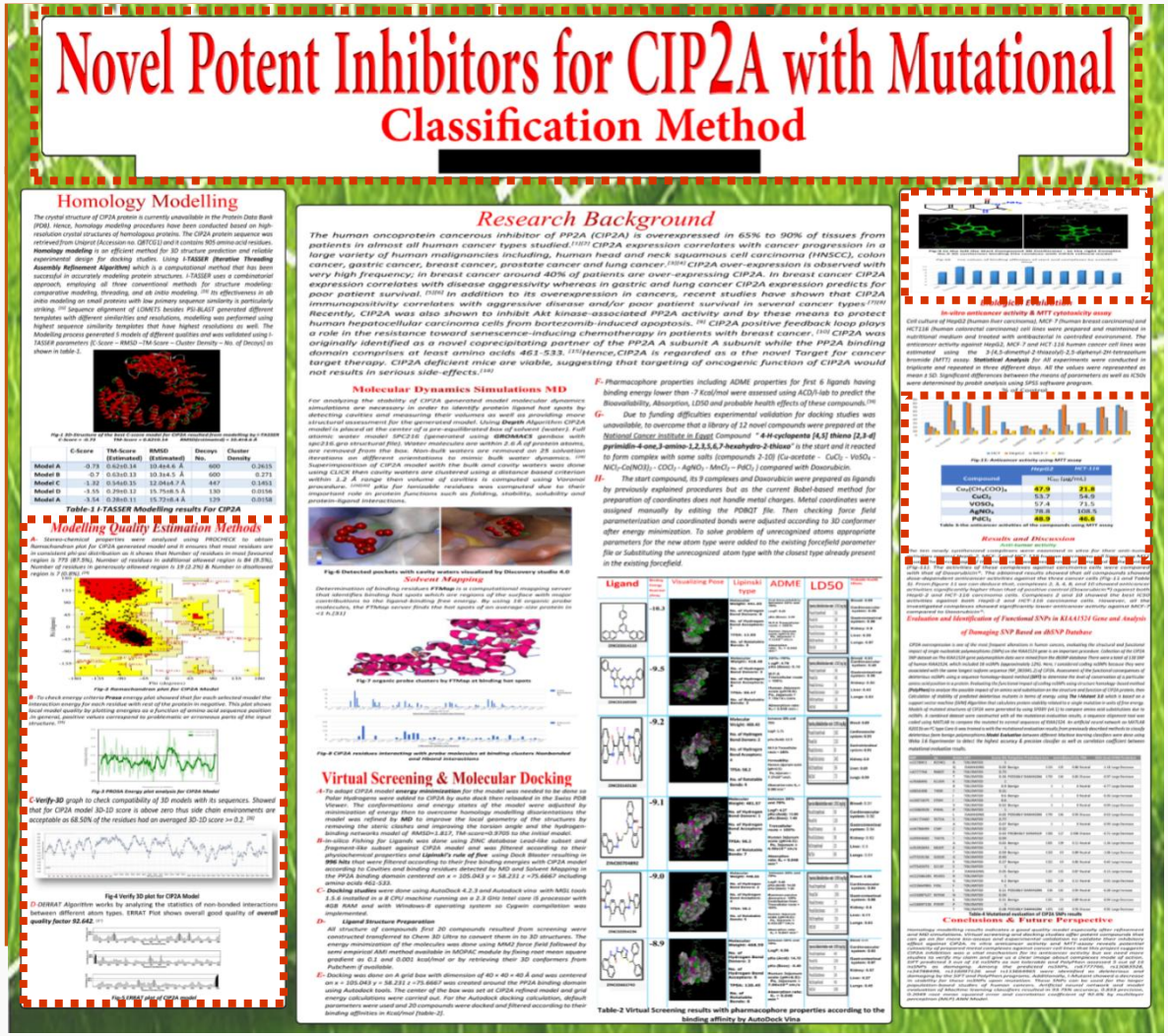
Σωστή γενική οργάνωση με διακριτές περιοχές

1 Σχεδιασμός και παρουσίαση poster

Παράδειγμα 5



- Πολύ και δυσανάγνωστο κείμενο
- Μικρά διαγράμματα
- Παραμορφωμένες εικόνες
- Κακή επιλογή φόντου

1 Σχεδιασμός και παρουσίαση poster

Προγράμματα σχεδίασης

Microsoft Powerpoint

+

Ευκολία στη χρήση

Πολύ διαδεδομένο

-

Έλλειψη ορισμένων εντολών

Διαφορά στο χρώμα κατα την εκτύπωση

Το πρόγραμμα είναι σχεδιασμένο για άλλο σκοπό (παρουσιάσεις σε projector)

Adobe Illustrator

+

Πλήθος εντολών

Διανυσματικά γραφικά (άπειρη λεπτομέρεια)

Δημιουργία προφιλ χρωμάτων για εκτύπωση

-

Μεγάλο κόστος απόκτησης

Σχετικά δύσκολη εκμάθηση

Inkscape

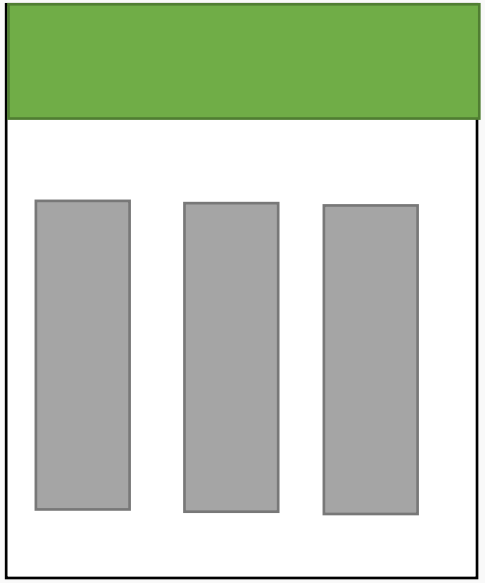
Freeware πρόγραμμα, παρόμοιες λειτουργείες με το Adobe Illustrator

2 Βασική διαμόρφωση του poster

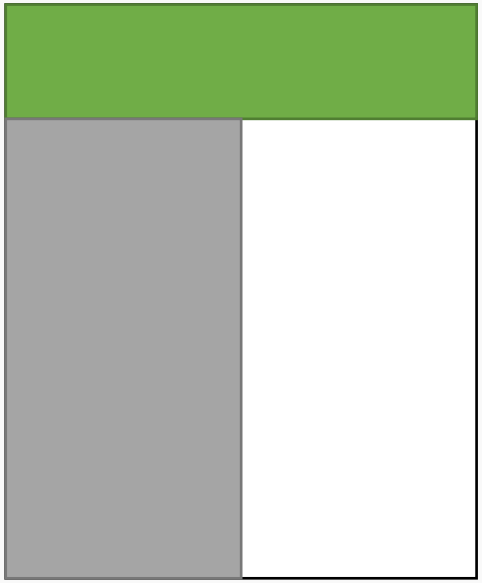
- Εισαγωγή
- Μέθοδοι
- Αποτελέσματα
- Συμπεράσματα
- Ευχαριστίες
- Βιβλιογραφία

2 Βασική διαμόρφωση του poster

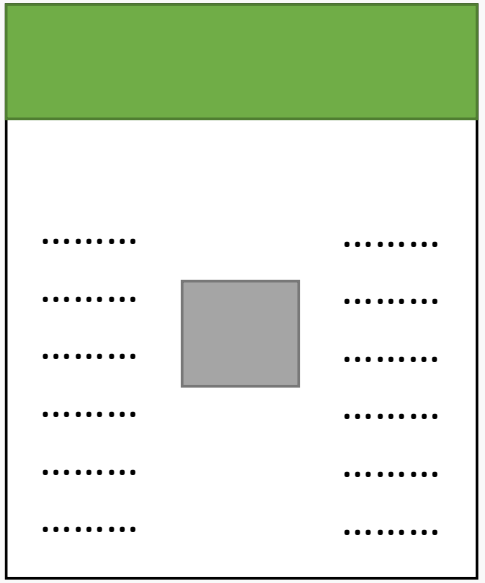
Γενικό πλάνο



Κάθετες στήλες



Αντίθετα πεδία

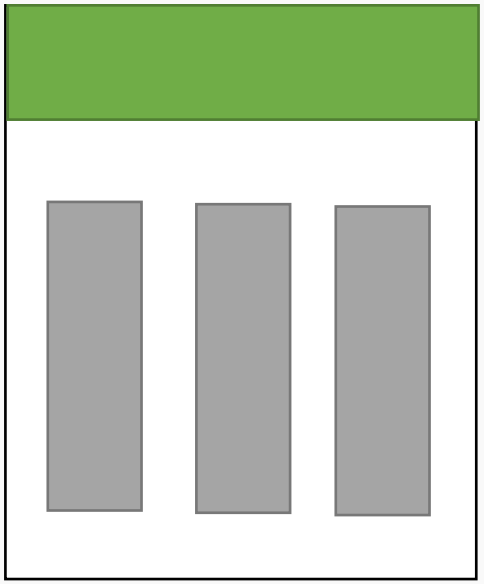


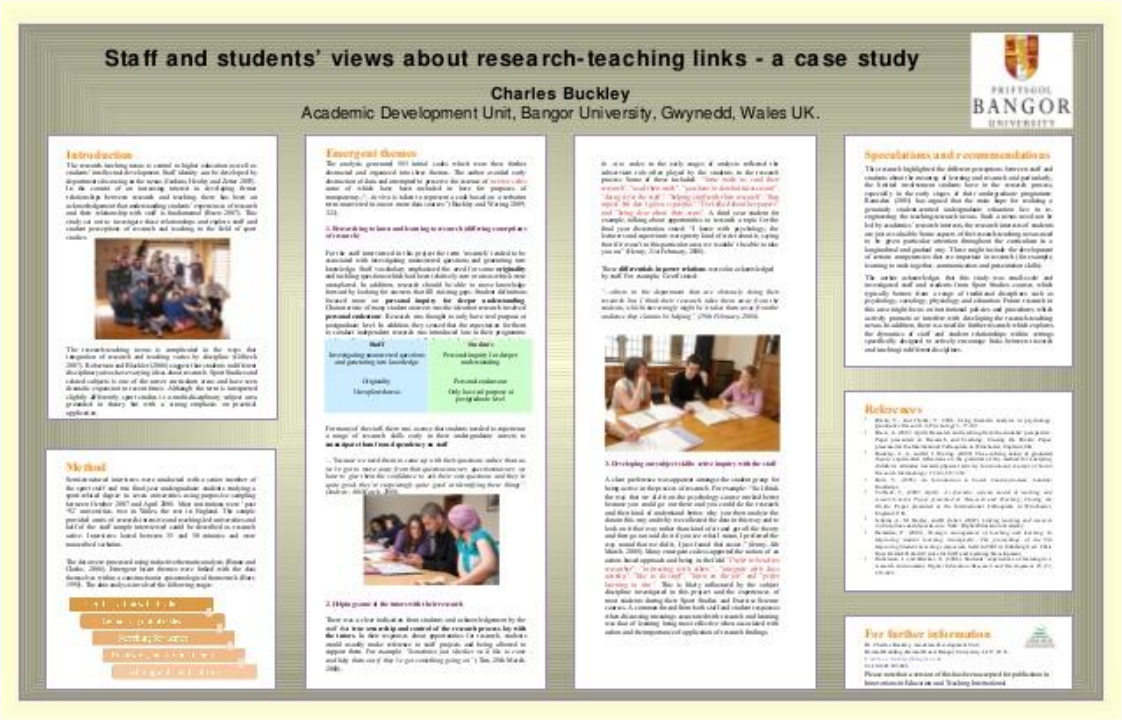
Κεντρική εικόνα

2 Βασική διαμόρφωση του poster

Γενικό πλάνο

Κάθετες στήλες



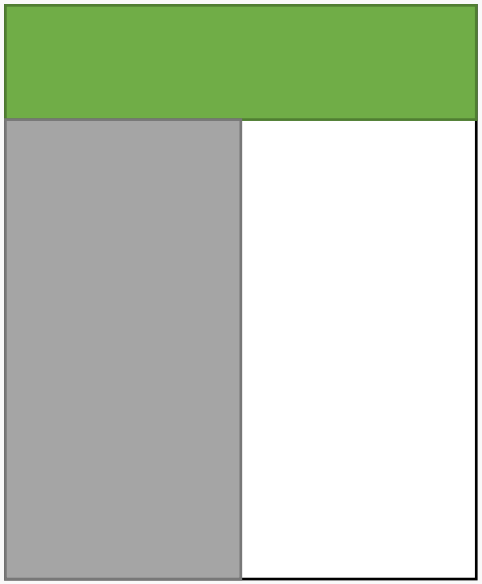


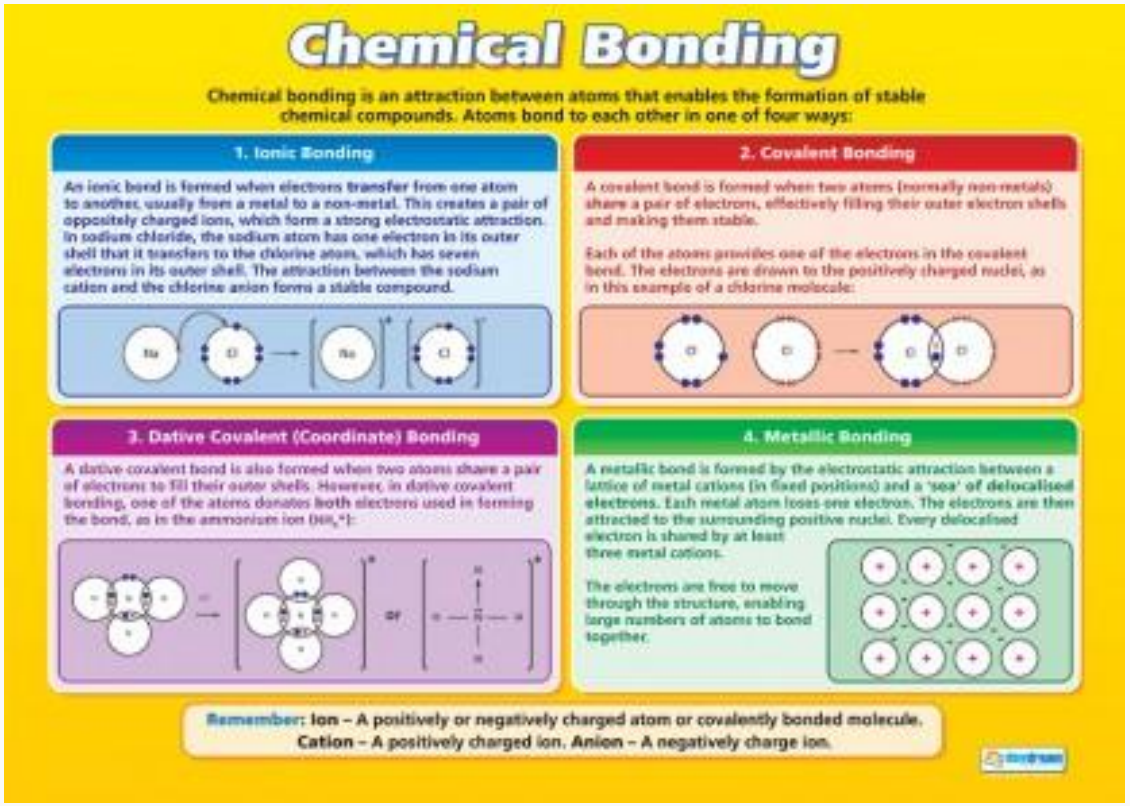
- Πιο διαδεδομένη δομή
- Πιο εύκολος διαχωρισμός
- Πιο εύκολη οργάνωση

2 Βασική διαμόρφωση του poster

Γενικό πλάνο

Αντίθετα πεδία



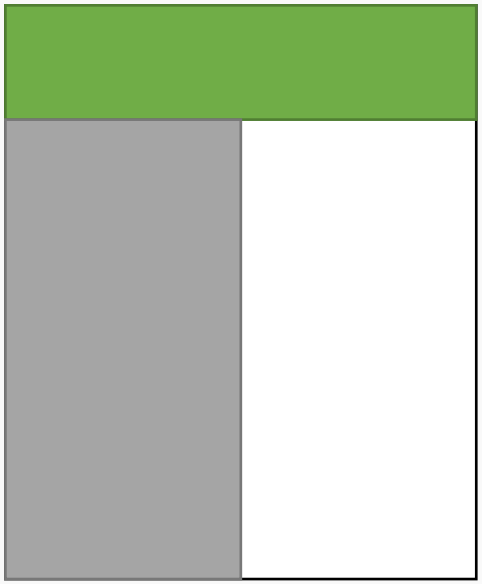


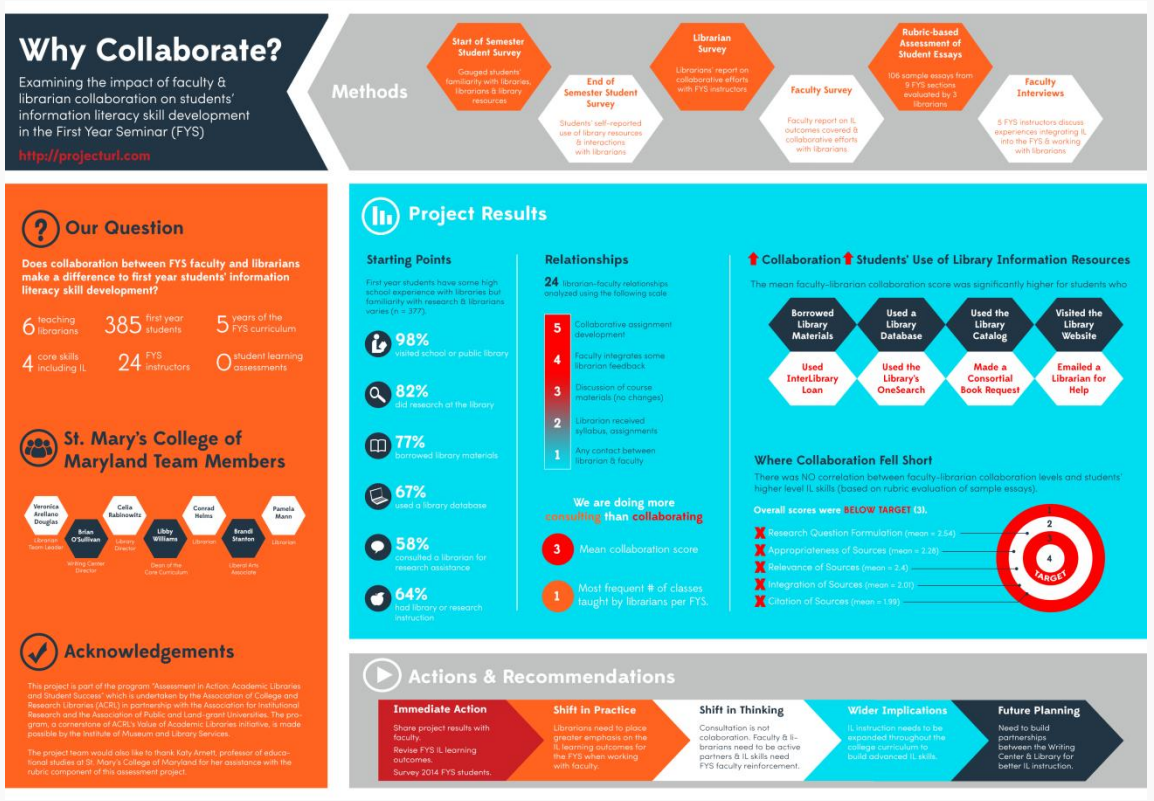
- Πιο δύσκολο στην οργάνωση από τις κάθετες στήλες
- Χρησιμοποιείται πολύ σπάνια

2 Βασική διαμόρφωση του poster

Γενικό πλάνο

Αντίθετα πεδία



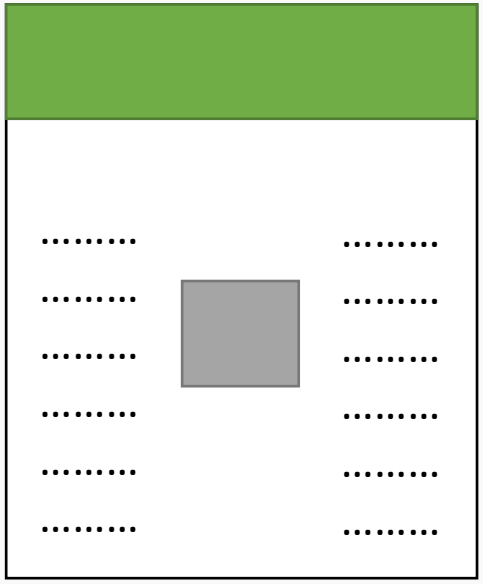


- Πιο δύσκολο στην οργάνωση από τις κάθετες στήλες
- Χρησιμοποιείται πολύ σπάνια

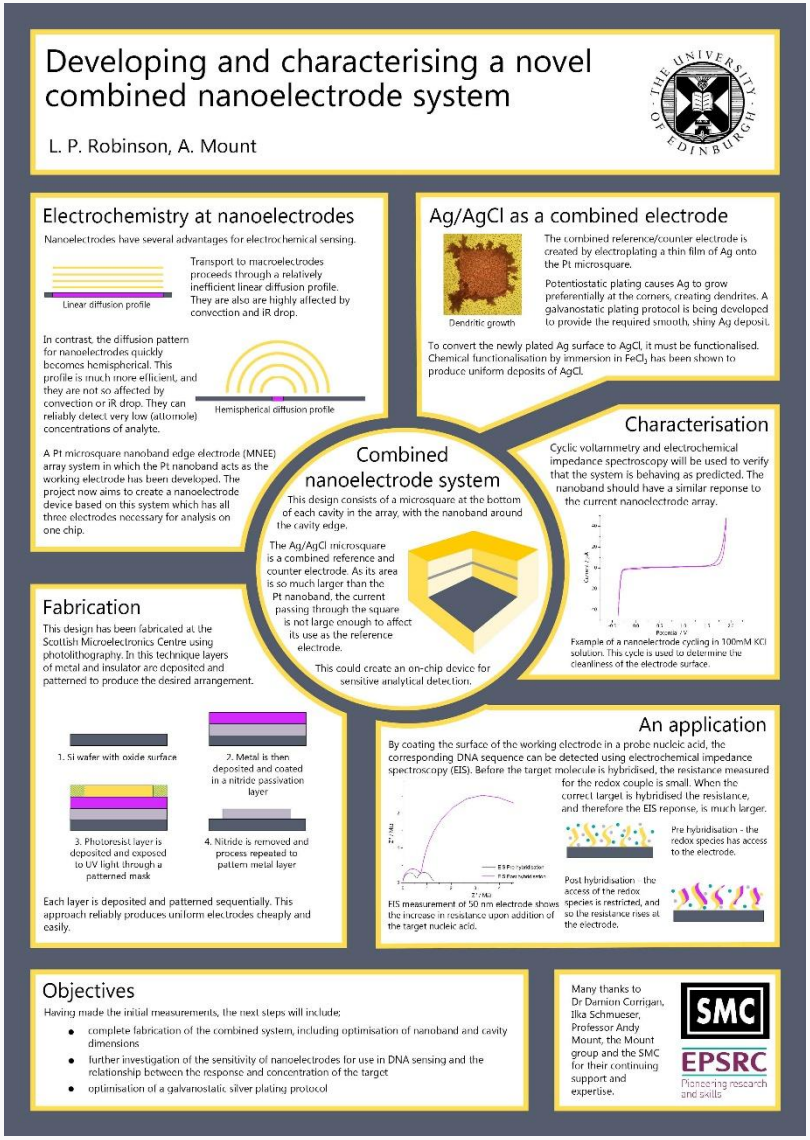
2 Βασική διαμόρφωση του poster

Γενικό πλάνο

Κεντρική εικόνα



- Δύσκολο στην οργάνωση
- Χρησιμοποιείται σπάνια



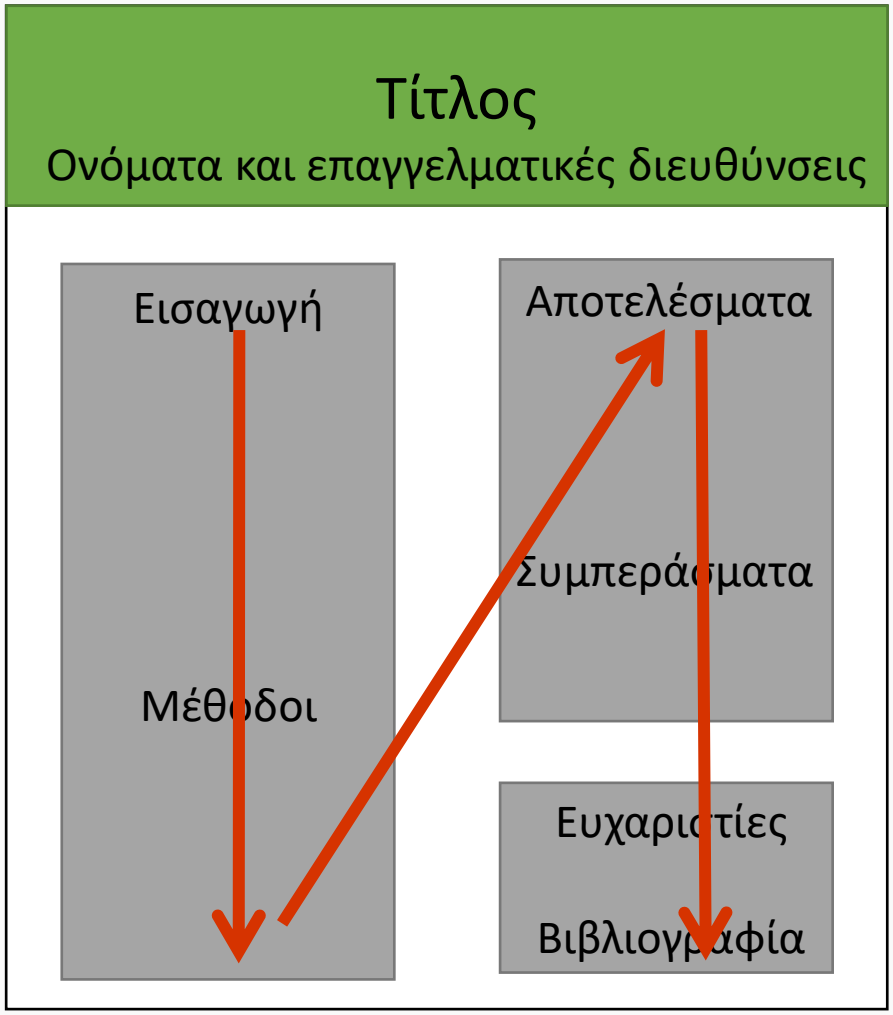
2 Βασική διαμόρφωση του poster

Πρόχειρη δομή

- Σχεδιάζουμε την βασική δομή
- Για κάθε πεδίο γράφουμε επιγραμματικά σε τι θα αναφερθούμε
- Επιλέγουμε τα αποτελέσματα που θα βάλουμε

2 Βασική διαμόρφωση του poster

Πρόχειρη δομή



- Θα πρέπει να είναι σαφής η κατεύθυνση με την οποία διαβάζεται το poster
- Το poster είναι μια ιστορία
- Θα πρέπει να έχει ξεκάθαρη αρχή μέση και τέλος

2

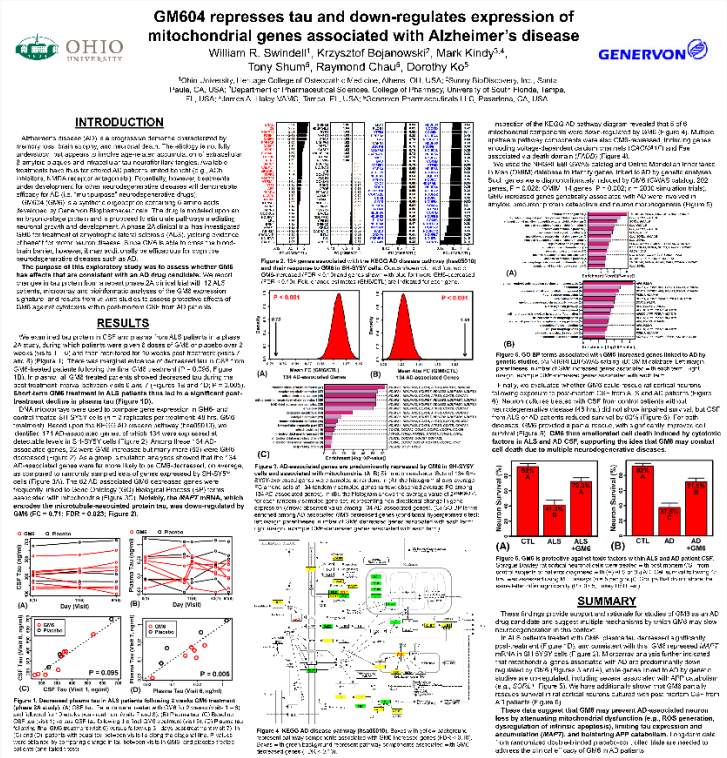
Βασική διαμόρφωση του poster

Το κείμενο

- Χρησιμοποιούμε «ξεκούραστες» γραμματοσειρές
- Τα πιο σημαντικά σημεία τονίζονται με μεγαλύτερη γραμματοσειρά
- Το λιγότερο 36 pt γραμματοσειρά

2 Βασική διαμόρφωση του poster

Το κείμενο



GM604 represses tau and down-regulates expression of mitochondrial genes associated with Alzheimer's disease

William R. Swindell[1], Krzysztof Bojanowski[2], Mark Kindy[3,4], Tony Shum[5], Raymond Chau[6], Dorothy Ko[5]

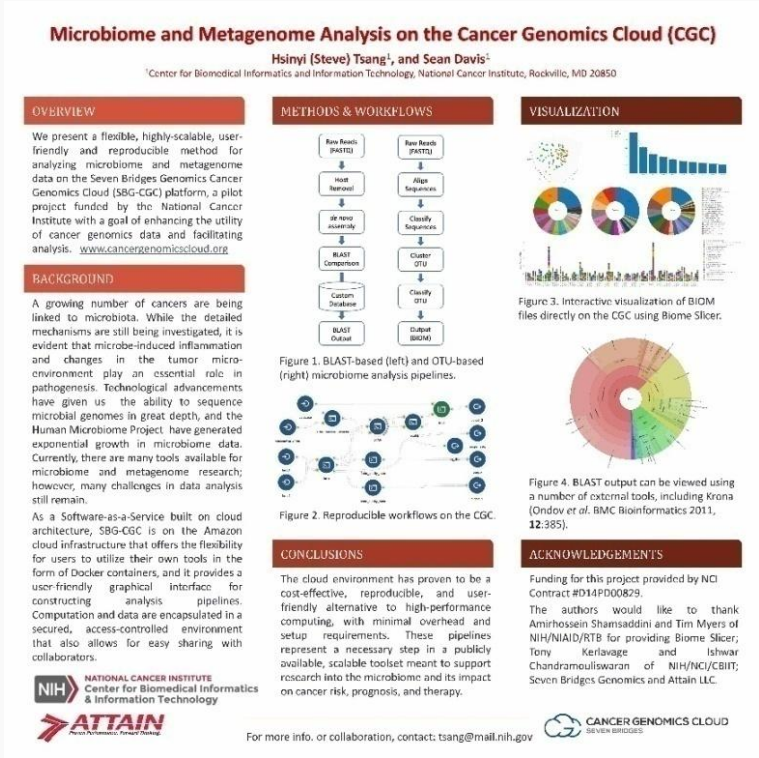
[1]Ohio University Heritage College of Osteopathic Medicine, Athens, OH, USA; [2]Sunny BioDiscovery, Inc., Santa Paula, CA, USA; [3]Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL, USA; [4]James A. Haley VAMC, Tampa, FL, USA; [5]Genervon Pharmaceuticals LLC, Pasadena, CA, USA

INTRODUCTION

RESULTS

SUMMARY

poster με πυκνό κείμενο



Microbiome and Metagenome Analysis on the Cancer Genomics Cloud (CGC)

Hsinyi (Steve) Tsang[1], and Sean Davis[1]

[1]Center for Biomedical Informatics and Information Technology, National Cancer Institute, Rockville, MD 20850

OVERVIEW

We present a flexible, highly-scalable, user-friendly and reproducible method for analyzing microbiome and metagenome data on the Seven Bridges Genomics Cancer Genomics Cloud (SBG-CGC) platform, a pilot project funded by the National Cancer Institute with a goal of enhancing the utility of cancer genomics data and facilitating analysis. www.cancergenomicscloud.org

BACKGROUND

A growing number of cancers are being linked to microbiota. While the detailed mechanisms are still being investigated, it is evident that microbe-induced inflammation and changes in the tumor microenvironment play an essential role in pathogenesis. Technological advancements have given us the ability to sequence microbial genomes in great depth, and the Human Microbiome Project have generated exponential growth in microbiome data. Currently, there are many tools available for microbiome and metagenome research; however, many challenges in data analysis still remain.

As a Software-as-a-Service built on cloud architecture, SBG-CGC is on the Amazon cloud infrastructure that offers the flexibility for users to utilize their own tools in the form of Docker containers, and it provides a user-friendly graphical interface for constructing analysis pipelines. Computation and data are encapsulated in a secured, access-controlled environment that also allows for easy sharing with collaborators.

METHODS & WORKFLOWS

Figure 1. BLAST-based (left) and OTU-based (right) microbiome analysis pipelines.

Figure 2. Reproducible workflows on the CGC.

VISUALIZATION

Figure 3. Interactive visualization of BIOM files directly on the CGC using Biome Slicer.

Figure 4. BLAST output can be viewed using a number of external tools, including Krona (Ondov *et al.* BMC Bioinformatics 2011, **12**:385).

CONCLUSIONS

The cloud environment has proven to be a cost-effective, reproducible, and user-friendly alternative to high-performance computing, with minimal overhead and setup requirements. These pipelines represent a necessary step in a publicly available, scalable toolset meant to support research into the microbiome and its impact on cancer risk, prognosis, and therapy.

ACKNOWLEDGEMENTS

Funding for this project provided by NCI Contract #D14PD00829.

The authors would like to thank Amirhossein Shamsaddini and Tim Myers of NIH/NIAID/RTB for providing Biome Slicer; Tony Kerlavage and Ishwar Chandramouliswaran of NIH/NCI/CBIIT; Seven Bridges Genomics and Attain LLC.

For more info. or collaboration, contact: tsang@mail.nih.gov

poster με ξεκάθαρο κείμενο

Ένα σωστά στημένο poster θα πρέπει να έχει διαβαστεί σε

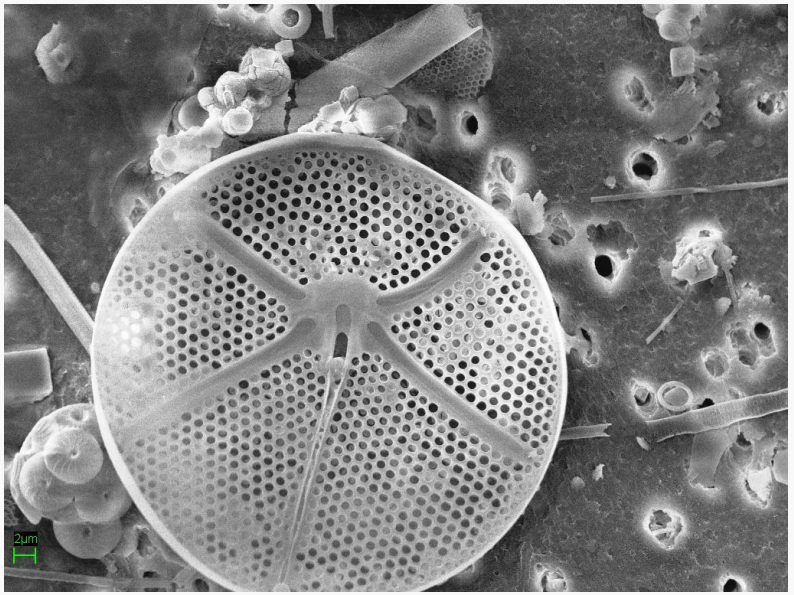
5 – 10 λεπτά

2 Βασική διαμόρφωση του poster

Ποιότητα εικόνων

- Χαμηλή ανάλυση εικόνων
- Επιρρεάζει αρνητικά το τελικό αποτέλεσμα

Low-res image



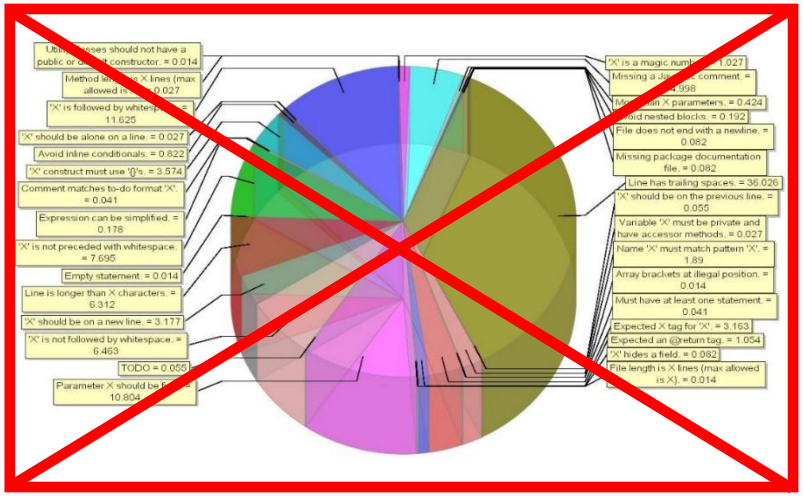
High-res image

- Ανάλυση 150 – 300 dpi (για αρχεία εικόνας)
- Αποθηκεύουμε σε τύπο αρχείου .tiff, .png
  (μικρότερη συμπίεση = μεγαλύτερη λεπτομέρεια)

2 Βασική διαμόρφωση του poster

Ποιότητα διαγραμμάτων

Τα διαγράμματα αποτελούν την απεικόνιση των αποτελεσμάτων μας. Αυτό σημαίνει ότι πρέπει να γίνοται κατανοητά και αντιληπτά εύκολα.



- Χρησιμοποιούμε όσο το δυνατόν απλούστερη μέθοδο απεικόνισης
- Δουλεύουμε σε υψηλή ανάλυση > 300 dpi
- Αποθηκεύουμε σε τύπο αρχείου .tiff, .png, .pdf (μικ. συμπίεση = μεγ. λεπτομέρεια)

2 Βασική διαμόρφωση του poster

Χρώματα

- Χρησιμοποιούμε χρώμα για να κατευθύνουμε τον αναγνώστη
- Δεν το παρακάνουμε με το χρώμα
- Δεν χρησιμοποιούμε έντονα χρώματα που κουράζουν
- Δεν χρησιμοποιούμε μοτίβα
- Ο συνδυασμός των χρωμάτων θα πρέπει να επιλεχθεί με προσοχή

2 Βασική διαμόρφωση του poster

- Δεν χρησιμοποιούμε μοτίβα

- Ο συνδυασμός των χρωμάτων θα πρέπει να επιλεχθεί με προσοχή

- Αποφεύγουμε να βάζουμε εικόνες ή σχέδια για φόντο
  i. Δημιουργούνται δυσανάγνωστα σημεία
  ii. Αποσπάται η προσοχή απο το κείμενο

Φόντο

2 Βασική διαμόρφωση του poster

Φόντο – Υψηλή αντίθεση

INSERT YOUR POSTER TITLE
ON THESE LINES HERE

Name of Author

Department Name and Institution Name can go here

BACKGROUND

- Insert your text here. You can change the font size to fit your text.
- You can also make this box shrink or grow with the amount of text. Simply double click this text box, go to the "Text Box" tab, and check the option "Resize AutoShape to fit text".
- The background of this template may appear blue on your screen, but it does print lavender.
- Insert your text here. You can change the font size to fit your text.
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MATERIALS AND METHODS

Title One

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Title Two

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Title Three

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RESULTS

Title Here

Insert your text here. You can change the font size to fit your text. You can also make this box shrink or grow with the amount of text. Simply double click this text box, go to the "Text Box" tab, and check the option "Resize AutoShape to fit text".

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PURPOSE

Insert your text here. You can change the font size to fit your text.

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CONCLUSIONS

Simply double click this text box, go to the "Text Box" tab, and check the option "Resize AutoShape to fit text". The background of this template may appear blue on your screen, but it does print lavender. Insert your text here. You can change the font size to fit your text.

Title Can Go Here

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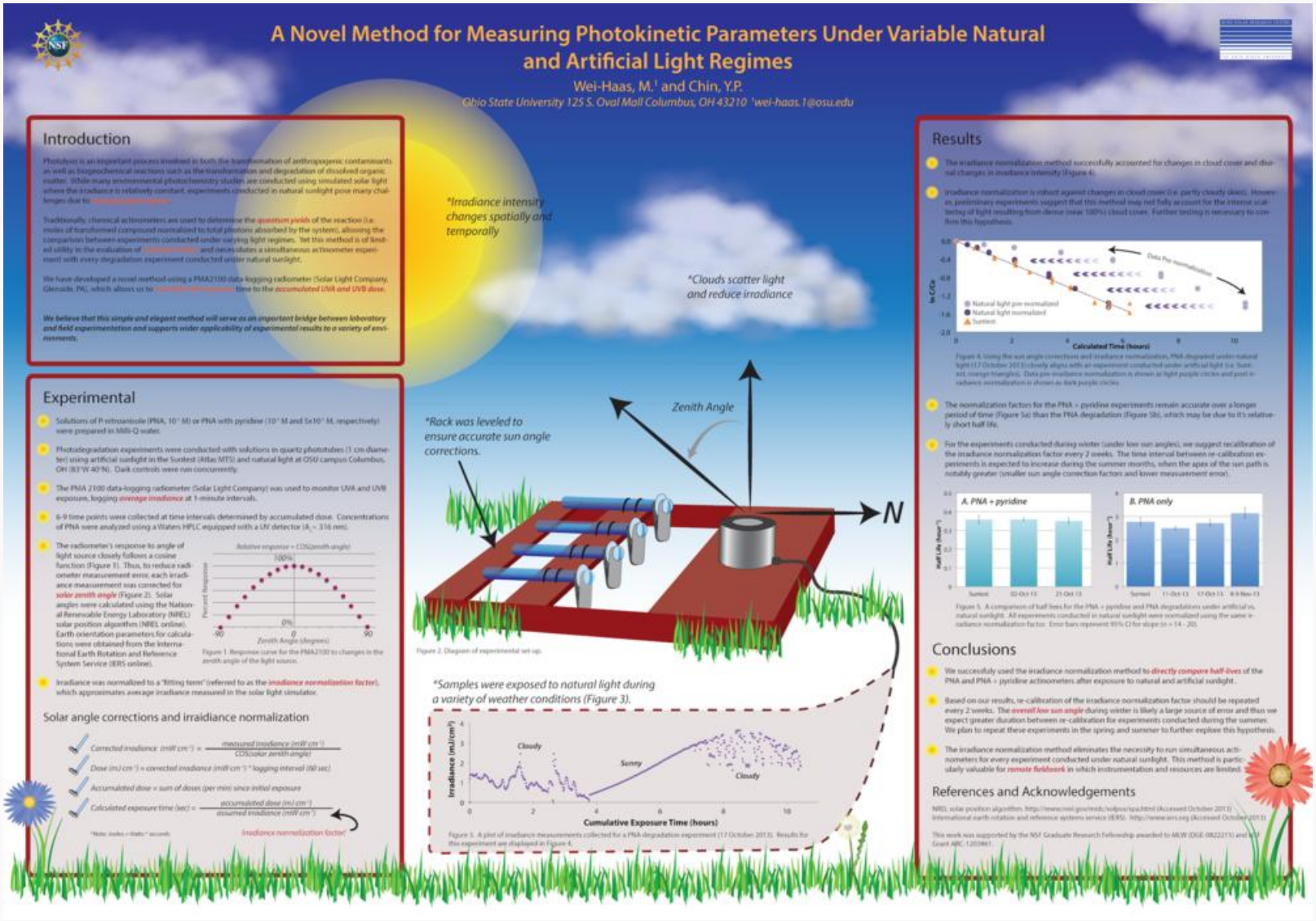
REFERENCES

1. Reference here
2. Second reference
3. Third reference

2 Βασική διαμόρφωση του poster

Φόντο – Φωτογραφία



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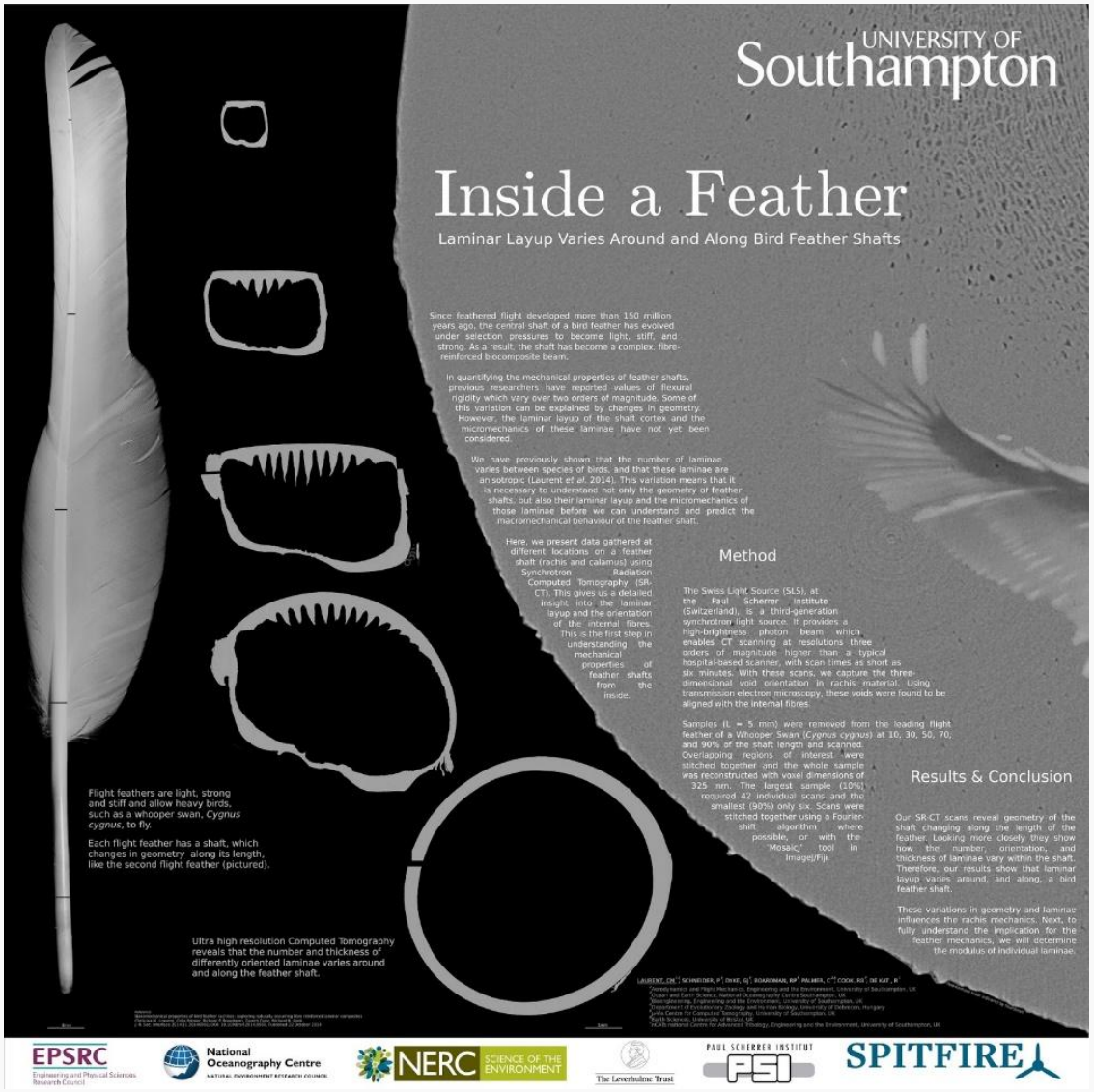

Μπορούμε όμως να είμαστε δημιουργικοί

Επιστημινκή δημοσίευση

Ενότητα

1 Επιστημονική δημοσίευση

Γιατί δημοσιεύουμε;

- Σημαντική η διάδοση των αποτελεσμάτων
- Η δουλειά μας κρίνεται ή ενισχύεται από άλλες επιστημονικές ομάδες
- Ελέγχουμε την απήχηση της έρευνας μας
- Προάγουμε την γνώση
- Ενισχύουμε την επιστημονική σκέψη
- Συμβάλλουμε στην εφαρμογή της γνώσης

1 Επιστημονική δημοσίευση

Γιατί δημοσιεύουμε;

- Σημαντικό για το βιογραφικό μας
- Βελτιώνονται οι πιθανότητες για εύρεση (καλύτερης) εργασίας
- Βελτιώνονται οι πιθανότητες χρηματοδότησης της έρευνας μας
- Προσδίδει κύρος στην ερευνητική ομάδα

1 Επιστημονική δημοσίευση

Ξεκινώντας...

- Επιλέγουμε το επιστημονικό περιοδικό
- Ελέγχουμε τις οδηγίες του επιστημονικού περιοδικού
- Σχεδιάζουμε το άρθρο
- Γράφουμε το άρθρο με την σωστή διαμόρφωση

Επιστημονική δημοσίευση

- Η εξειδίκευση του περιοδικού
- Impact factor και citation index
- Αξιοπιστία του περιοδικού
- Πιθανότητα αποδοχής της δουλειάς μας
- Χρόνος ανταπόκρισης του περιοδικού

1

Επιστημονική δημοσίευση

Επιλογή περιοδικού

- Κόστος δημοσίευσης
- Τύπος περιοδικού (free-access)
- Έχουμε ρεαλιστικές προσδοκίες
- Έχουμε επιμονή
- Είμαστε έτοιμοι για πειράματα εάν μας ζητηθούν

2 Σχεδιασμός και μορφοποίηση άρθρου

- Μικρός αλλά επεξηγηματικός
- Ελέγχουμε να είναι ακριβής
- Προσέχουμε να μην έχει ξαναχρησιμοποιηθεί

Τίτλος

2 Σχεδιασμός και μορφοποίηση άρθρου

Τίτλος

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PLOS ONE

Efficient *E. coli* Expression Strategies for Production of Soluble Human Crystallin ALDH3A1

Georgia-Persephoni Voulgaridou[1], Theodora Mantso[1], Katerina Chlichlia[1], Mihalis I. Panayiotidis[2], Aglaia Pappa[1]*

1 Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus, Dragana, Alexandroupolis, Greece, **2** Laboratory of Pathological Anatomy, Medical School, University of Ioannina, University Campus, Ioannina, Greece

Abstract

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

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The purpose of the present study was to employ *E. coli* recombinant methods in order to produce substantial amounts of human ALDH3A1 with the aim to direct future studies towards elucidating the biological functions of ALDH3A1. To this end, *E. coli* is the preferred organism for heterologous protein expression due to its many advantages including: (i) the ability to grow quickly into high cell densities, (ii) the requirement of non expensive carbon sources and (iii) its extensively studied physiology [13,14]. In addition, the wider range of commercial products available for all steps of expression and purification using *E. coli* makes this system even more operable. However, miss-folding and aggregation of recombinant proteins within inclusion bodies in bacteria hinders the successful production of many eukaryotic proteins [13,15–17]. Furthermore, on the occurrence of insolubility issues, both the type of fusion tag and the purification method to be used, constitute critical parameters. In addition, although known tags

Efficient *E. coli* Expression Strategies for Production of Soluble Human Crystallin ALDH3A1

2 Σχεδιασμός και μορφοποίηση άρθρου

Συμβολή συγγραφέων

- Τα ονόματα μπαίνουν με σειρά συμβολής
- Πρώτο το άτομο με την μεγαλύτερη συνεισφορά
- Τελευταίο το όνομα του correspondence author
- Στην σύλληψη και σχεδίαση της μελέτης
- Διεξαγωγή πειραμάτων

Σχεδιασμός και μορφοποίηση άρθρου

- Επινόηση της κεντρικής ιδέας
- Σχεδιασμός πειραμάτων
- Διεξαγωγή πειραμάτων
- Πρόσβαση σε εγκαταστάσεις
- Χειρισμός εξειδικευμένων μηχανημάτων

2

Σχεδιασμός και μορφοποίηση άρθρου

Συμβολή συγγραφέων

- Ανάλυση πειραμάτων
- Σχεδιασμός γραφημάτων και φιγούρων
- Συγγραφή
- Κριτική αναθεώρηση του άρθρου

2 Σχεδιασμός και μορφοποίηση άρθρου

Συμβολή συγγραφέων

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PLOS ONE

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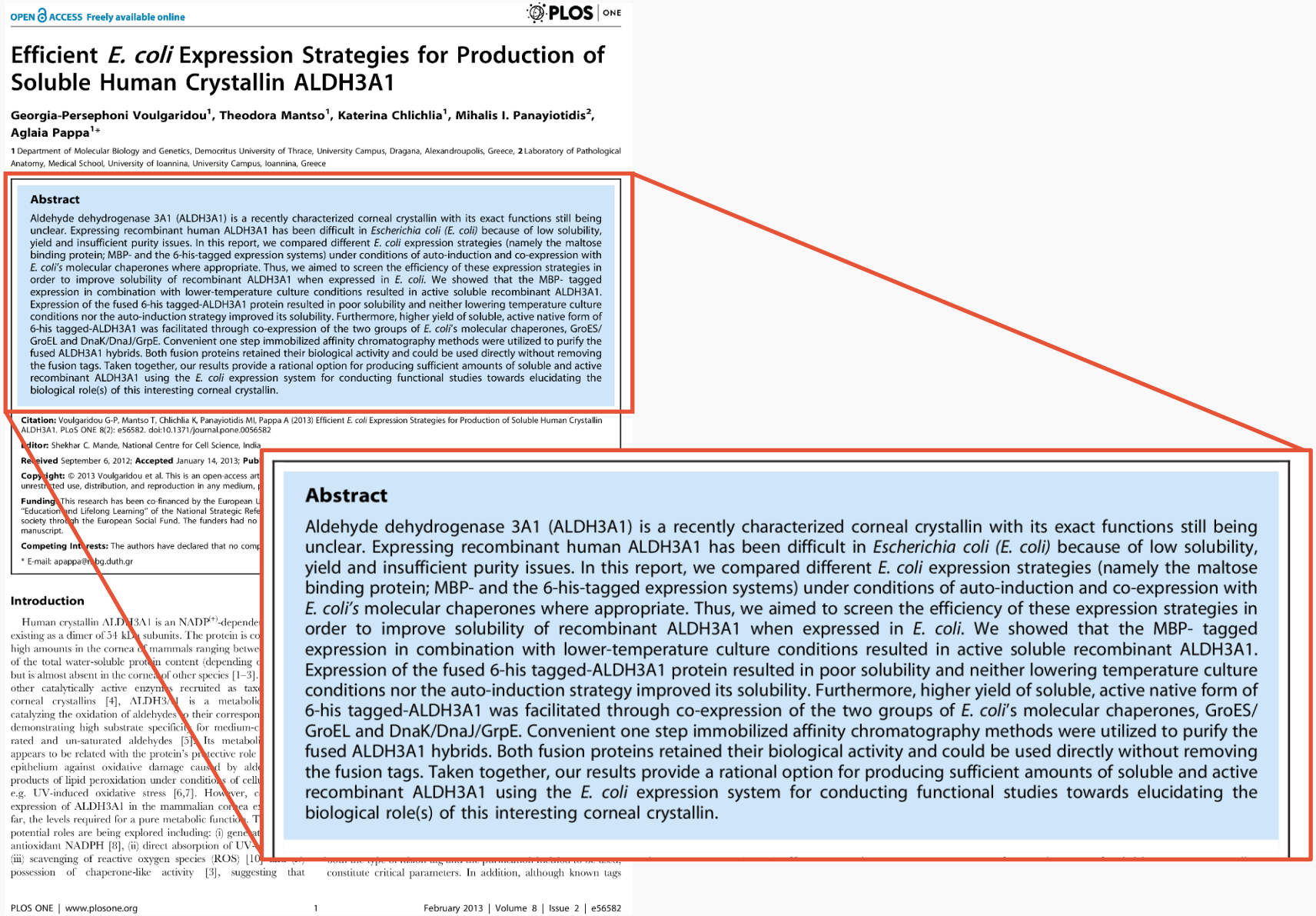
2 Σχεδιασμός και μορφοποίηση άρθρου

Περίληψη/Abstract

- Περιγράφω σύντομα (έως 250 λέξεις) την έρευνα
- Αναφέρω το πρόβλημα, τις μεθόδους, τα αποτελέσματα και τα συμπεράσματα
- Καλό είναι να γράφεται τελευταία
- Οι περισσότεροι αναγνώστες θα διαβάσουν μόνο την περίληψη

2 Σχεδιασμός και μορφοποίηση άρθρου

Περίληψη/Abstract



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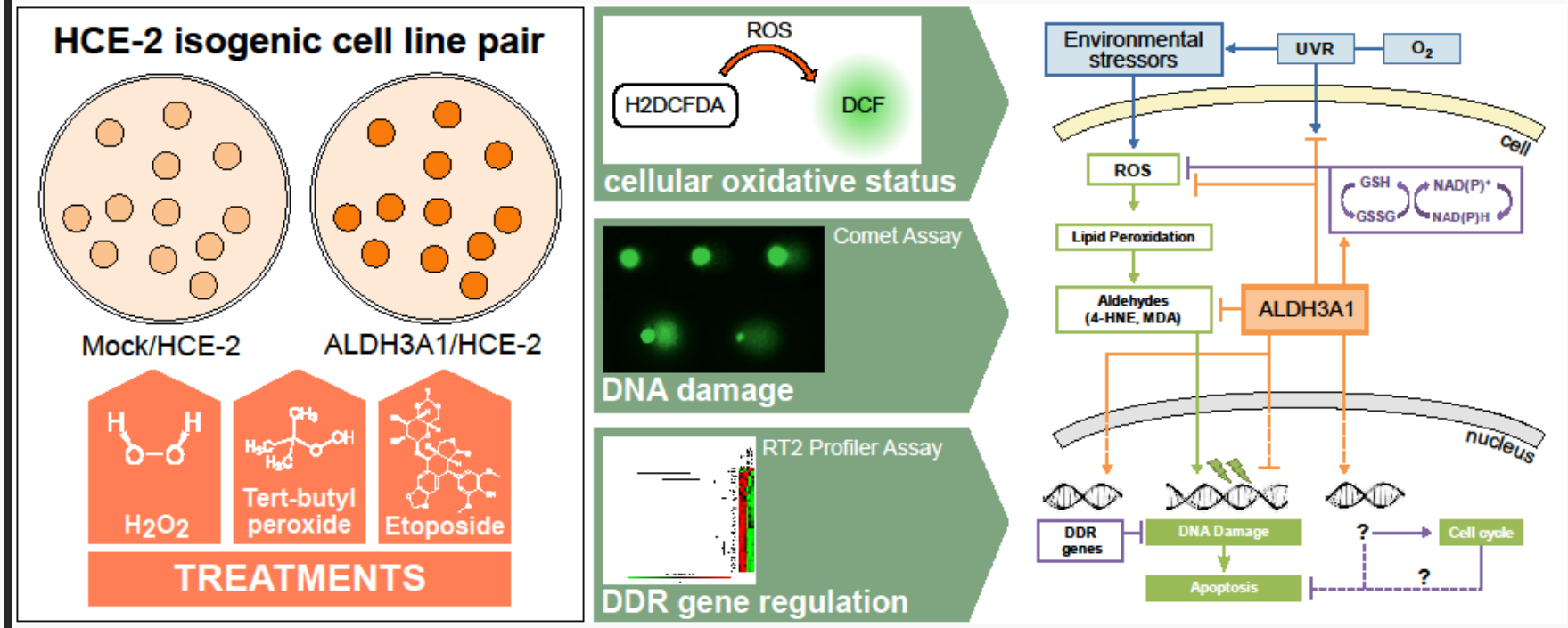

2 Σχεδιασμός και μορφοποίηση άρθρου

Εισαγωγή/Introduction

- Γιατί γίνεται η έρευνα
- Το θεωρητικό υπόβαθρο
- Τα κενά που θα καλύψει
- Οι στόχοι
- Περίπου 1 σελίδα

2 Σχεδιασμός και μορφοποίηση άρθρου

Εισαγωγή/Introduction

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Efficient *E. coli* Expression Strategies for Production of Soluble Human Crystallin ALDH3A1

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Abstract

Aldehyde dehydrogenase 3A1 (ALDH3A1) is a recently characterized corneal crystallin with its exact functions still being unclear. Expressing recombinant human ALDH3A1 has been difficult in *Escherichia coli (E. coli)* because of low solubility, yield and insufficient purity issues. In this report, we compared different *E. coli* expression strategies (namely the maltose binding protein; MBP- and the 6-his-tagged expression systems) under conditions of auto-induction and co-expression with *E. coli's* molecular chaperones where appropriate. Thus, we aimed to screen the efficiency of these expression strategies in order to improve solubility of recombinant ALDH3A1 when expressed in *E. coli*. We showed that the MBP- tagged expression in combination with lower-temperature culture conditions resulted in active soluble recombinant ALDH3A1. Expression of the fused 6-his tagged-ALDH3A1 protein resulted in poor solubility and neither lowering temperature culture conditions nor the auto-induction strategy improved its solubility. Furthermore, higher yield of soluble, active native form of 6-his tagged-ALDH3A1 was facilitated through co-expression of the two groups of *E. coli's* molecular chaperones, GroES/GroEL and DnaK/DnaJ/GrpE. Convenient one step immobilized affinity chromatography methods were utilized to purify the fused ALDH3A1 hybrids. Both fusion proteins retained their biological activity and could be used directly without removing the fusion tags. Taken together, our results provide a rational option for producing sufficient amounts of soluble and active recombinant ALDH3A1 using the *E. coli* expression system for conducting functional studies towards elucidating the biological role(s) of this interesting corneal crystallin.

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Introduction

Human crystallin ALDH3A1 is an $NADP^{(+)}$-dependent enzyme existing as a dimer of 54 kDa subunits. The protein is contained in high amounts in the cornea of mammals ranging between 5–50% of the total water-soluble protein content (depending on species) but is almost absent in the cornea of other species [1–3]. Similar to other catalytically active enzymes recruited as taxon-specific corneal crystallins [4], ALDH3A1 is a metabolic enzyme catalyzing the oxidation of aldehydes to their corresponding acids demonstrating high substrate specificity for medium-chain saturated and un-saturated aldehydes [5]. Its metabolic activity appears to be related with the protein's protective role in corneal epithelium against oxidative damage caused by aldehyde by-products of lipid peroxidation under conditions of cellular stress, e.g. UV-induced oxidative stress [6,7]. However, constitutive expression of ALDH3A1 in the mammalian cornea exceeds, by far, the levels required for a pure metabolic function. Thus, other potential roles are being explored including: (i) generation of the antioxidant NADPH [8], (ii) direct absorption of UV-energy [9], (iii) scavenging of reactive oxygen species (ROS) [10] and (iv) possession of chaperone-like activity [3], suggesting that ALDH3A1 may contribute to the optical properties of the cornea as well [1,3]. Furthermore, its absence has been linked with cataract phenotype and ocular oxidative damage in ALDH3A1-null mice [11], whereas recent studies implicate its involvement in cell homeostatic pathways, such as apoptosis, cell cycle regulation, proteasome degradation and DNA damage response [6,7,11,12].

The purpose of the present study was to employ *E. coli* recombinant methods in order to produce substantial amounts of human ALDH3A1 with the aim to direct future studies towards elucidating the biological functions of ALDH3A1. To this end, *E. coli* is the preferred organism for heterologous protein expression due to its many advantages including: (i) the ability to grow quickly into high cell densities, (ii) the requirement of non expensive carbon sources and (iii) its extensively studied physiology [13,14]. In addition, the wider range of commercial products available for all steps of expression and purification using *E. coli* makes this system even more operable. However, miss-folding and aggregation of recombinant proteins within inclusion bodies in bacteria hinders the successful production of many eukaryotic proteins [13,15–17]. Furthermore, on the occurrence of insolubility issues, both the type of fusion tag and the purification method to be used, constitute critical parameters. In addition, although known tags like maltose binding protein (MBP), can contribute to greater protein solubility [18,19], culture temperature conditions (during induction) also appear to be a detrimental factor in the production of native protein [20]. Furthermore, methods like auto-induction can be used for easier handling of cultures in combination with high protein yield [21] whereas co-expression of certain bacterial molecular chaperones can assist in the conformational process of the native protein [22].

In the present study, we report the expression of soluble MBP-fused and his-tagged recombinant human crystallin ALDH3A1 in substantial amounts, in *E. coli*, and their purification to homogeneity.

Materials and Methods

Materials

Vectors pMAL-c2X and pET-26b(+) were purchased by New England Biolabs (Beverly, MA, USA) and Novagen (EMD Millipore Corporation, Billerica, MA, USA) respectively. All primers were obtained by Invitrogen (Carlsband, CA, USA) while the restriction enzymes and the chaperone plasmid set were from Takara (Shiga, Japan). *Pfu* polymerase and the DNA ligase kit were purchased from Fermentas (Burlington, ON, Canada). Amylose resin was purchased by New England Biolabs (Beverly, MA, USA), while Ni-NTA resin by Qiagen (Venlo, Netherlands). Medium for bacterial cultures along with antibiotics and inducers were purchased either from Applichem (Darmstadt, Germany) or from Sigma-Aldrich Co. (Taufkirchen, Germany). Protease inhibitors as well as the chemicals for the ALDH3A1 enzymatic activity were obtained by Sigma-Aldrich Co. For western blotting, PVDF membranes were purchased from Millipore (Bedford, MA, USA), whereas the chemiluminescence reagents were from Thermo Scientific (Rockford, IL, USA) and the autoradiography films from Genesee Scientific (San Diego, CA, USA). Rabbit polyclonal antibody against human ALDH3A1 was obtained from Abgent (San Diego, CA, USA) and the goat anti-rabbit IgG horseradish peroxidise conjugated antibody was purchased by Millipore (Bedford, MA, USA).

pMAL/ALDH3A1 Vector Construction

To construct the pMAL/ALDH3A1 expression vector, the ΔpCEP4Δ/ALDH3A1 plasmid (containing a full-length of human ALDH3A1 cDNA) was used as the template for the polymerase chain reaction [6,23]. The following two primers were synthesized in order to: (i) amplify the entire coding sequence and (ii) introduce an *EcoRI* restriction site at the 5′ end and a *HindIII* site on the 3′ end: 1.forward primer: 5′-CT**GAATTC**AGCAAGATCAGC-GAG-3′ and, 2.reverse primer: 5′-CT**AAGCTT**T-CAGTGCTGGGTCAT-3′. The PCR conditions for the amplification were: 94°C for 60 sec and further 94°C for 30 sec, 60°C for 60 sec, 72°C for 90 sec (for 30 cycles) and a final step at 72°C for 10 min. The *EcoRI HindIII* fragment of the PCR product was inserted into the *EcoRI* and *HindIII* sites of the pMAL expression vector. The resulting vector, pMAL/ALDH3A1 (Figure 1A) was verified by restriction digestion and sequencing from both ends of the inserted fragment.

ALDH3A1-MBP Tagged Expression

For the expression of ALDH3A1 protein tagged with MBP, BL21(-) *E.coli* cells were transformed with the pMAL/ALDH3A1 construct and incubated in LB broth (with 0.2% glucose and 100 μg/ml ampicillin for selection). The medium was inoculated with an overnight culture (1:100 dilution) and the culture was incubated under shaking at 37°C until an $OD_{600} = \sim 0.5$ was reached. Subsequently, 0.5 mM IPTG was added and the culture was further incubated at 37°C or 25°C for 6 hours.

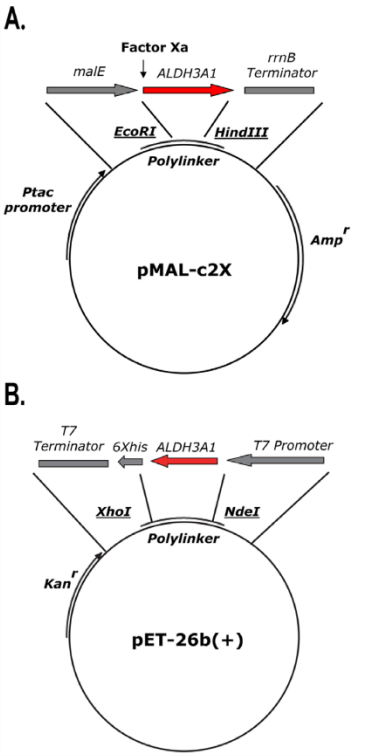

Figure 1. Cloning of the coding sequence of ALDH3A1 into the expression vectors. (A) Construction of the pMAL/hALDH3A1 vector. The HindIII/EcoRI fragment of the PCR product containing the coding region of human ALDH3A1 was inserted into the HindIII and EcoRI sites of the pMAL vector. (B) Construction of the pET-26b (+)/hALDH3A1 vector. The NdeI/XhoI fragment of the PCR product containing the coding region of human ALDH3A1 was inserted into the NdeI and XhoI sites of the pET-26b(+) vector.
doi:10.1371/journal.pone.0056582.g001

pET-26b(+)/hALDH3A1 Vector Construction

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Υλικά και μέθοδοι/Materials and methods

- **Πως διεξήχθη η έρευνα**
- Θα πρέπει οι αναγνώστες να καταλαβαίνουν πλήρως τι έχουμε κάνει
- Θα πρέπει, εάν θελήσουν, να είναι σε θέση να αναπαράγουν τα πειράματα
- Αναφορά στην μέθοδο στατιστικής ανάλυσης
- 2-3 σελίδες

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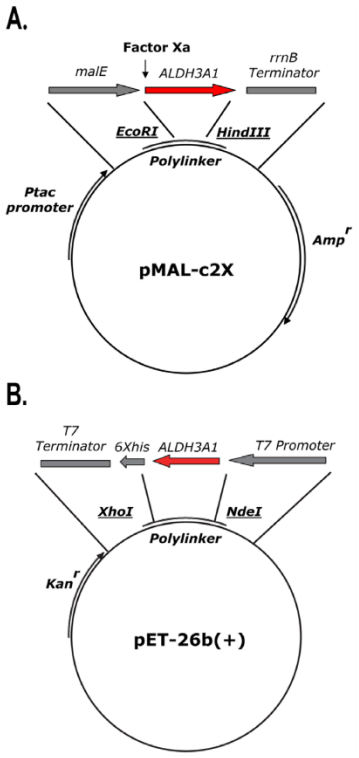

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doi:10.1371/journal.pone.0056582.g001

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ALDH3A1 his-tagged Expression

BL21(DE3) *E.coli* transformed with pET-26b(+)/ALDH3A1 were cultured at 37°C, in the presence of 30 μg/ml kanamycin for plasmid selection, and when OD_{600} reached ~ 0.5, 0.5 mM IPTG was added and incubation continued at 18–37°C for 6 hours.

Auto-induction

For auto-induction, ZYM-5052 medium was used containing 1% tryptone, 0.5% yeast extract, 25 mM Na_2HPO_4, 25 mM KH_2PO_4, 50 mM NH_4Cl, 5 mM Na_2SO_4, 2mM $MgSO_4$, 0.5% glycerol, 0.05% glucose and 0.2% α-lactose as previously described [21]. An overnight culture of BL21(DE3) transformed with pET-26b(+)/ALDH3A1 was used for inoculation at a dilution of 1:1000. Cultures were incubated (in ZYM-5052 medium with 100 μg/ml kanamycin) at 18°C, 25°C and 37°C, for 9 hours.

Molecular Chaperone Co-expression

The pET-26b(+)/ALDH3A1 transformed BL21(DE3) *E.coli* were re-transformed with one of the set's plasmids: pG-KJE8, pGro7, pKJE7, pG-Tf2 and pTf16 and cultured in LB broth with 20 μg/ml chloramphenicol, along with 30 μg/ml kanamycin for the selection of the transformed clones. For protein expression, cells were incubated in shaking cultures at 37°C and in the presence of the appropriate chaperone inducer (0.5 mg/ml L-arabinose and/or 5 ng/ml tetracycline) for allowing the chaperones to be already expressed at the time of ALDH3A1 induction (Table 1). When culture reached $OD_{600} \sim 0.6$, 0.5mM IPTG was added and the incubation proceeded for 6 hours at 25°C.

ALDH3A1 Enzymatic Activity

ALDH3A1 activity determinations were carried out using a spectrophotometer (Libra S22, Biochrom Ltd, Cambridge, UK) by monitoring NADPH production at 340 nm as described previously [6]. Briefly, a total of 1 ml reaction containing sodium pyrophosphate (100 mM, pH 8.0), 1 mM pyrazole and 2.5 mM $NADP^+$ (co-enzyme) and recombinant ALDH3A1 at various concentrations were prepared and incubated at 25°C. The reaction was initiated using benzaldehyde as a substrate (to a final concentration of 0.5 mM) and was monitored as an increase in NADPH at 340 nm for 5 min. Enzyme activity was calculated using a molar extinction coefficient of 6.22 mM^{-1}/cm^{-1} for NADPH. Enzyme specific activities are expressed as nmoles of NADPH/min/mg protein.

Purification of the ALDH3A1/MBP Recombinant Protein

Cells were harvested through centrifugation at 4.000xg, at 4°C for 20 min and lysed in 20 mM Tris-HCl pH 7.4, 200 mM NaCl and 1 mM EDTA (with the addition of the protease inhibitors: 100 μg/ml PMSF, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin and 1 μg/ml pepsatin) by sonication for 8s using the UP50H sonifier by Hielscher Ultrasonics GmbH (Teltow, Germany) at an intermediate setting (cycle 1, 70% amplitude). The lysates were cooled on ice for 30s and the procedure was repeated for a total of 6 cycles. Crude extract was isolated by centrifugation of samples at 9.000xg (4°C) for 30 min and further applied to an amylose resin column, already equilibrated with the lysis buffer. Recombinant protein was eluted after the addiion of lysis buffer with 10 mM maltose. The presence of ALDH3A1 throughout *E. coli* expression and purification steps was determined by SDS-PAGE and western blot analysis.

Purification of the ALDH3A1/6xHis Recombinant Protein

Cells were collected and placed in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 1% Tween-20, 20 mM imidazole, pH 8.0) in the presence of protease inhibitors as mentioned above. Purification was conducted *via* affinity chromatography, by Ni-NTA resin. For the two washes, the concentration of imidazole in the buffer was increased to 40 and 70mM respectively. Bounded protein was eluted through the addition of elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 300 mM imidazole, pH 8.0).

Western Blotting

Elution parts of ALDH3A1 purification were subjected to SDS-PAGE electrophoresis. The separated proteins were then transferred to a PVDF membrane (Polyvinylidene), which was subsequently blocked with 5% of non-fat dry milk in TBST solution (100 mM Tris, 150 mM NaCl, 0.1% Tween-20). Membrane was subsequently incubated overnight (at 4°C) with the primary, polyclonal anti-ALDH3A1 antibody at a dilution of 1:500 in 5% non-fat dry milk in TBST, while the secondary horseradish peroxidase conjugated goat anti-rabbit IgG was used in a dilution

Table 1. Description of the chaperone plasmids used in the study.

Plasmid	Resistance Marker	Inducer	Chaperones	Molecular Weights
pG-KJE8	Chloramphenicol (20 μg/ml)	L-arabinose (0.5 mg/ml) tetracycline (5 ng/ml)	dnaK-dnaJ-grpE/groES-groEL	dnaK-70 kDa dnaJ-40 kDa grpE-22 kDa groES-10 kDa groEL-60 kDa
pGro7	Chloramphenicol (20 μg/ml)	L-arabinose (0.5 mg/ml)	groES-groEL	groES-10 kDa groEL-60 kDa
pKJE7	Chloramphenicol (20 μg/ml)	L-arabinose (0.5 mg/ml)	dnaK-dnaJ-grpE	dnaK-70 kDa dnaJ-40 kDa grpE-22 kDa
pG-Tf2	Chloramphenicol (20 μg/ml)	tetracycline (5 ng/ml)	groES-groEL/tig	groES-10kDa groEL-60 kDa tig-56 kDa
pTf16	Chloramphenicol (20 μg/ml)	L-arabinose (0.5 mg/ml)	tig	tig-56 kDa

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of 1:5000 (1 hour incubation). Finally, the protein bands were visualized by the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) for alkaline phosphatase-conjugated secondary antibody as described by the manufacturer.

Results

Expression of MBP Fused ALDH3A1 Leads to High Yield and Sufficient Solubility

Initially, we tested the expression of the MBP tagged ALDH3A1, through the pMAL-c2X system, given that MBP is known to contribute to increased solubility of the heterologous produced proteins. As it is shown (Figure 2A), ALDH3A1 was expressed at high yields, but with extremely low solubility, when the induction occurred at 37°C (Table 2). After lowering cultivation temperature to 25°C, during induction time (a common strategy to overcome insolubility), significant improvement in ALDH3A1 solubility was observed by increasing from 2.5% at 37°C to 35.5% at 25°C (Figure 2B; Table 2). The recombinant MBP-fused ALDH3A1 was found to be functionally active when tested for the presence of ALDH3A1 enzymatic activity even though it was tagged with the 42kDa MBP (Table 3, specific activity of the crude extract).

Expression of his-tagged ALDH3A1 Leads to High Protein Yield but Insufficient Solubility

Although the MBP tag facilitated the expression of the target heterologous protein, there are further limitations considering the usage of the MBP-fused recombinant proteins including the fact that a number of assays do not allow for the use of a tag with the size of MBP. To this end, the usage of the specialized protease, factor Xa, could solve the problem but the required extra step of the purification process could be a limitation. Consequently, we sought to examine the expression of recombinant human ALDH3A1 fused with a rather smaller but very common 6-histidines tag. Although the resulted recombinant his-tagged ALDH3A1 was expressed at remarkably higher rates, almost the whole amount of the recombinant protein was insoluble and trapped into the inclusion bodies (Figure 3A, Table 2). Unlike the case of MBP-fused expression, lowering the induction temperature at 25°C (Figure 3B) and at 18°C (Figure 3C) did not improve significantly the solubility of the recombinant protein (Table 2).

Auto-induction Leads to Enhanced Expression Levels of his-tagged ALDH3A1 but Poor Solubility

In order to improve the solubility of the his-tagged recombinant ALDH3A1, we employed the auto-induction protocol described recently by Studier et al [21]. An important factor during heterologous protein expression is the need for a strict control of protein induction and the retention of cultures' viability for as long as possible. The auto-induction protocol requires specialized culture media which in combination with high rates of aeration allows for a firm hold of the induction, an equilibrated pH and a subsequent elongated viability even at extremely high cell densities. Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction is not applied in this case, as glucose, glycerol and lactose are all included in the media. Glycerol is used as an efficient carbon and energy source, which contributes to the growth of cells. On the other hand, as long as glucose is available in the media and usually until the late log phase, bacteria do not metabolize lactose. Near saturation, though, and when glucose is depleted, cells metabolize lactose to the inducer allo-lactose and the induction of the protein begins then manually with no extra addition. Due to the high viability of cultures, the yield of recombinant protein is extremely high.

In the case of ALDH3A1, while the expression of the protein was sufficiently rich under conditions of auto-induction, almost all of the produced ALDH3A1 was found in the inclusion bodies, as inactive aggregates (Figure 4). The soluble fraction of the expressed ALDH3A1 estimated to represent approximately 3% of the induced protein at 37°C (Table 2). Lowering the temperature from 37°C to 25°C and 18°C did not improve any further the solubility of the recombinant protein (Figure 4; Table 2).

Molecular Chaperones' Co-expression Facilitates the Production of Soluble Recombinant his-tagged ALDH3A1

Molecular chaperones play an important role in the conformation process of newly synthesized proteins. In the case of heterologous expressed proteins, which commonly exhibit solubility problems and misfolding, the presence of chaperones could become even more of a necessity. *E.coli* affords a variety of proteins that could be characterized as chaperones, and amongst them, the GroEL/GroES and the DnaK/DnaJ/GrpE are considered to be key groups. The above, along with the Trigger factor could be co-

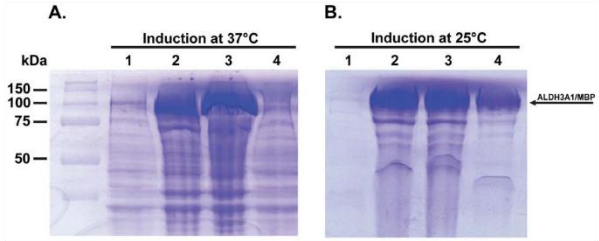

Figure 2. ALDH3A1 heterologous expression through the pMAL-c2X expression system. SDS-PAGE pattern showing induction of ALDH3A1 expression at (A) 37°C and (B) 25°C. Samples were subjected to SDS-PAGE and stained with Coomassie blue. Lane 1, whole cell lysate prior IPTG induction; Lane 2, whole cell lysate 6 hours after IPTG induction; Lane 3, insoluble fraction; Lane 4: soluble fraction, respectively, 6 hours after IPTG induction. The arrow indicates the position of ALDH3A1/MBP protein.
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2 Σχεδιασμός και μορφοποίηση άρθρου

Αποτελέσματα/Results

- **Τι βρέθηκε**
- **Τι δείχνουν τα αποτελέσματα**
- Φιγούρες και διαγράμματα
- Καλές επεξηγήσεις (Figure Legends) στις φιγούρες και τα διαγράμματα
- Κάνουμε περιγραφή των αποτελεσμάτων
- 2-3 σελίδες

Σχεδιασμός και μορφοποίηση άρθρου

- Τα διαγράμματα να έχουν μπάρες τυπικής απόκλισης (όπου είναι εφικτό)
- Τα διαγράμματα πάντα με σύμβολο για την στατιστική σημαντικότητα (όπου είναι εφικτό)
- Στην λεζάντα αναφέρουμε τον αριθμό των επαναλήψεων (τουλάχιστον 3)
- Προτιμούμε τα απαλά χρώματα ή κάνουμε τα γραφήματα ασπρόμαυρα (πιο οικονομικό)

Σχεδιασμός και μορφοποίηση άρθρου

- Οι πίνακες να είναι ξεκάθαροι
- Οι τίτλοι των γραμμών και των στηλών επεξηγηματικοί και απλοί
- Δεν ξεχνάμε να αναφέρουμε τις μονάδες

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of 1:5000 (1 hour incubation). Finally, the protein bands were visualized by the SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) for alkaline phosphatase-conjugated secondary antibody as described by the manufacturer.

Results

Expression of MBP Fused ALDH3A1 Leads to High Yield and Sufficient Solubility

Initially, we tested the expression of the MBP tagged ALDH3A1, through the pMAL-c2X system, given that MBP is known to contribute to increased solubility of the heterologous produced proteins. As it is shown (Figure 2A), ALDH3A1 was expressed at high yields, but with extremely low solubility, when the induction occurred at 37°C (Table 2). After lowering cultivation temperature to 25°C, during induction time (a common strategy to overcome insolubility), significant improvement in ALDH3A1 solubility was observed by increasing from 2.5% at 37°C to 35.5% at 25°C (Figure 2B; Table 2). The recombinant MBP-fused ALDH3A1 was found to be functionally active when tested for the presence of ALDH3A1 enzymatic activity even though it was tagged with the 42kDa MBP (Table 3, specific activity of the crude extract).

Expression of his-tagged ALDH3A1 Leads to High Protein Yield but Insufficient Solubility

Although the MBP tag facilitated the expression of the target heterologous protein, there are further limitations considering the usage of the MBP-fused recombinant proteins including the fact that a number of assays do not allow for the use of a tag with the size of MBP. To this end, the usage of the specialized protease, factor Xa, could solve the problem but the required extra step of the purification process could be a limitation. Consequently, we sought to examine the expression of recombinant human ALDH3A1 fused with a rather smaller but very common 6-histidines tag. Although the resulted recombinant his-tagged ALDH3A1 was expressed at remarkably higher rates, almost the whole amount of the recombinant protein was insoluble and trapped into the inclusion bodies (Figure 3A, Table 2). Unlike the case of MBP-fused expression, lowering the induction temperature at 25°C (Figure 3B) and at 18°C (Figure 3C) did not improve significantly the solubility of the recombinant protein (Table 2).

Auto-induction Leads to Enhanced Expression Levels of his-tagged ALDH3A1 but Poor Solubility

In order to improve the solubility of the his-tagged recombinant ALDH3A1, we employed the auto-induction protocol described recently by Studier et al [21]. An important factor during heterologous protein expression is the need for a strict control of protein induction and the retention of cultures' viability for as long as possible. The auto-induction protocol requires specialized culture media which in combination with high rates of aeration allows for a firm hold of the induction, an equilibrated pH and a subsequent elongated viability even at extremely high cell densities. Isopropyl β-D-1-thiogalactopyranoside (IPTG) induction is not applied in this case, as glucose, glycerol and lactose are all included in the media. Glycerol is used as an efficient carbon and energy source, which contributes to the growth of cells. On the other hand, as long as glucose is available in the media and usually until the late log phase, bacteria do not metabolize lactose. Near saturation, though, and when glucose is depleted, cells metabolize lactose to the inducer allo-lactose and the induction of the protein begins then manually with no extra addition. Due to the high viability of cultures, the yield of recombinant protein is extremely high.

In the case of ALDH3A1, while the expression of the protein was sufficiently rich under conditions of auto-induction, almost all of the produced ALDH3A1 was found in the inclusion bodies, as inactive aggregates (Figure 4). The soluble fraction of the expressed ALDH3A1 estimated to represent approximately 3% of the induced protein at 37°C (Table 2). Lowering the temperature from 37°C to 25°C and 18°C did not improve any further the solubility of the recombinant protein (Figure 4; Table 2).

Molecular Chaperones' Co-expression Facilitates the Production of Soluble Recombinant his-tagged ALDH3A1

Molecular chaperones play an important role in the conformation process of newly synthesized proteins. In the case of heterologous expressed proteins, which commonly exhibit solubility problems and misfolding, the presence of chaperones could become even more of a necessity. *E.coli* affords a variety of proteins that could be characterized as chaperones, and amongst them, the GroEL/GroES and the DnaK/DnaJ/GrpE are considered to be key groups. The above, along with the Trigger factor could be co-

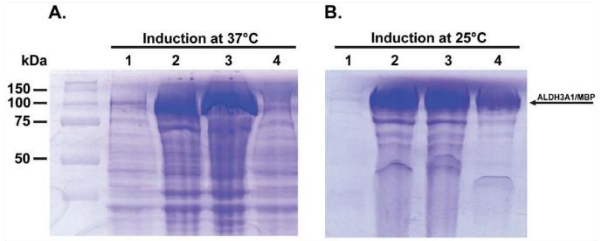

Figure 2. ALDH3A1 heterologous expression through the pMAL-c2X expression system. SDS-PAGE pattern showing induction of ALDH3A1 expression at (A) 37°C and (B) 25°C. Samples were subjected to SDS-PAGE and stained with Coomassie blue. Lane 1, whole cell lysate prior IPTG induction; Lane 2, whole cell lysate 6 hours after IPTG induction; Lane 3, insoluble fraction; Lane 4: soluble fraction, respectively, 6 hours after IPTG induction. The arrow indicates the position of ALDH3A1/MBP protein.
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Table 2. Comparison of different *E.coli* strategies for production of soluble recombinant ALDH3A1.

Method of expression	Induction temperature (°C)	Solubility (% of the induced protein)[a]
ALDH3A1/MBP tagged	37	2.5±0.59
	25	35.47±3.29
ALDH3A1/6xHis tagged	37	2.02±0.57
	25	3.45±1.26
	18	2.73±0.48
ALDH3A1/6xHis tagged (autoinduction)	37	3.11±0.34
	25	4.02±0.11
	18	2.96±0.59
ALDH3A1/6xHis tagged **pG-KJE8**	25	19.54±1.01
ALDH3A1/6xHis tagged **pGro7**	25	11.63±1.03
ALDH3A1/6xHis tagged **pKJE7**	25	4.36±0.36
ALDH3A1/6xHis tagged **pG-TF2**	25	3.75±0.48
ALDH3A1/6xHis tagged **pTF16**	25	6.29±1.01

[a]The % solubility values reported are mean of three different experiments.
doi:10.1371/journal.pone.0056582.t002

expressed with a heterologous protein and assist to its production in native and active forms. As these chaperone molecules are normally expressed at low levels in prokaryotic cells, heterologous over-expression of eukaryotic proteins with chaperones has been shown to improve the solubility of the overexpressed proteins in *E.coli* [24].

In this study, we used different combinations of chaperones to enhance the solubility of the recombinant his-tagged ALDH3A1 in BL21(DE3) *E. coli* (Table 1). The solubility was improved in the cases of co-expression with the pG-KJE8 (approximately 20%), and pGro7 (approximately 12%) plasmids (Figure 5; Table 2). However, the expression levels of soluble his-tagged ALDH3A1 were significantly lower (3.7%) when the recombinant protein was co-expressed in the presence of pG-Tf2 plasmid (Figure 5C; Table 2). On the other hand, negligible enhancement in solubility was observed when his-tagged ALDH3A1 was co-expressed with pTf16 (approximately 6%) and pKJE7 (approximately 4%) (Figure 5B/C; Table 2). Our results demonstrate that the presence of chaperone complexes especially those contain GroES and GroEL (e.g. plasmids pGKJE8 and pGro7) increased the solubility of the recombinant protein. In the presence of *Tig* chaperone, the solubility of his-tagged was significantly impeded. Combination of GroES/GroEL and dnaK/DnaJ/GrpE chaperone groups exhibited the most profound effect on his-tagged ALDH3A1 solubility. Lower induction temperatures or addition of ethanol did not facilitate any further the protein's solubility (data not shown).

Purification of the Recombinant Human Fused ALDH3A1 Hybrids

Recombinant human fused ALDH3A1 hybrids were produced and purified from *E.coli* using the MBP-fused expression strategy (under low temperature conditions during protein induction) and the his-tagged ALDH3A1 expression strategy (under conditions of co-expressing the pG-KJE8) both of which produced soluble recombinant ALDH3A1 at sufficient levels.

Purification of MBP-fused recombinant human ALDH3A1 was conducted with the use of affinity chromatography. Protein supernatant obtained from *E. coli* lysate was applied to amylose resin column in column buffer (see Methods). MBP-fused ALDH3A1 was then eluted from the column in the same buffer containing 10 mM maltose and appeared in the elution fractions (Figure 6). This convenient step of immobilized affinity chroma-

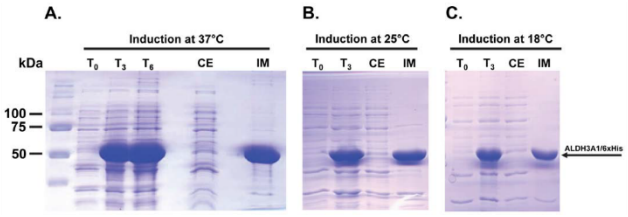

Figure 3. ALDH3A1 heterologous expression through the pET-26b(+) expression system. Induction at (A) 37°C, (B) 25°C and (C) 18°C. Samples were subjected to SDS-PAGE and stained with Coomassie blue. T_0: Total cell extract form bacterial culture prior of protein induction, T_3: Total cell extract 3 hours after induction, T_6: Total cell extract 6 hours after induction, CE: Crude extract of lysed cells 6 hours after induction, IM: Insoluble matter of lysed cells 6 hours after induction. The arrow indicates the position of ALDH3A1/6xHis protein.
doi:10.1371/journal.pone.0056582.g003

2 Σχεδιασμός και μορφοποίηση άρθρου

Συζήτηση/Discussion

- **Πως εξηγούνται τα αποτελέσματα**
- Κάνουμε επεξήγηση των αποτελεσμάτων
- Συσχετίζουμε τα αποτελέσματα με παλαιότερη βιβλιογραφία
- Με ποιον τρόπο συμβάλλουν τα αποτελέσματα στο βιολογικό ερώτημα

2 Σχεδιασμός και μορφοποίηση άρθρου

Συζήτηση/Discussion

- Μελλοντικοί στόχοι
- Δεν φοβόμαστε να αναφέρουμε έρευνες που είχαν αντίθετα αποτελέσματα
- Είμαστε ρεαλιστές με τα αποτελέσματα μας
- 2-3 σελίδες

2 Σχεδιασμός και μορφοποίηση άρθρου

Συζήτηση/Discussion

Table 3. Purification of MBP-tagged recombinant human ALDH3A1 from *E. coli*.

Purification Steps	Total Protein (mg)	Yield (%)	Specific Activity[b] (mU/mg protein)	Purification (fold)
Crude supernatant[a]	62.5	100	90	1
Amylose resin column	3.2	5.12	250	2.77

[a]The starting material was 250 ml of crude *E. coli* supernatant.
[b]One milliunit (mU) of activity was defined as the amount of activity that oxidized of 1 nmol of NADPH/min at 25°C. Representative results of three different isolation procedures.
doi:10.1371/journal.pone.0056582.t003

tagged recombinant human ALDH3A1 to homogeneity (Figure 7). The final yield was approximately 7% from the initial 36 mg of crude protein and purification of the recombinan protein was 5.8-fold (Table 4).

Discussion

Previous attempts to express active recombinant human ALDH3A1 in *E. coli* have failed primarily because of low solubility, yield and insufficient purity issues. In this study, we compared different *E. coli* fusion expression strategies (the MBP- and the 6-his-tagged expression) under various conditions intending to increase their efficiency for producing soluble recombinant ALDH3A1. We have shown that the MBP-tagged expression in combination with lower- temperature culture conditions resulted in active soluble recombinant ALDH3A1. Expression of the fused his-tagged ALDH3A1 protein resulted in poor solubility and neither lowering temperature culture conditions nor auto-induction conditions improved solubility. Furthermore, higher yield of soluble, fully active native form of his-tagged ALDH3A1 was facilitated through the co-expression of the two groups of *E.coli*'s molecular chaperones GroES/GroEL and DnaK/DnaJ/GrpE. Convenient one-step immobilized affinity chromatography methods were utilized to purify the fused ALDH3A1 hybrids to sufficient homogeneity. To our knowledge, this is only the second time that recombinant techniques have been used to produce human crystallin ALDH3A1. We have previously utilized the baculovirus expression system to produce recombinant human ALDH3A1 in order to overcome the insolubility problems occurred at our initial attempts to express the protein in *E. coli*. The baculovirus infection system in Sf9 cells in combination with 5′ AMP sepharose chromatography resulted to extremely high yield of recombinant ALDH3A1 with sufficient solubility [5]. However, while it is true that the baculovirus expression systems allow for high yields of structurally and functionally foreign proteins in insect cells, their high cost, complexity and requirement of specific equipment and trained personnel pose limitations thus providing reasoning for the development of alternative recombinant expression strategies.

Insolubility is the number one issue of recombinant proteins expressed in *E. coli*. The reason for this is believed to be their non-native, aggregation-prone conformation and their subsequent expression as inactive forms in the inclusion bodies. Important parameters include the interactions between the hydrophobic patches of newly synthesized unfolded polypeptides, which are influenced by the rate of protein synthesis along with the cellular microenvironment of expression. Therefore, factors considered important during protein synthesis are usually related with the expression vector used, the induction parameters and the cultivation conditions [17].

The selection of the appropriate tag for the fusion of the heterologous protein usually depends on the desired method of chromatography and the experimental needs of the protein's utilization. On the other hand, protein solubility depends on the type of the fusion tag to be used. Tags known for their contribution to solubility are the MBP, the NusA and the GST (glutathione S-transferase) [19]. MBP, in particular, is considered to be one the most effective and has been widely used. Although, MBP appears to facilitate the correct formation of disulfide bonds in the newly synthesized proteins, the exact mechanism through which it improves the solubility of the target heterologous proteins remains unknown [25]. Indeed, in our case, the expression of MBP-fused ALDH3A1 in combination with lower induction temperature in *E. coli* resulted in much better solubility compared to his-tagged ALDH3A1 expression under all different strategies tested.

While the establishment of a highly productive system with strict control elements is a well known and crucial issue, another prominent, but often underestimated factor is the need for high cell density cultures with enhanced viability [16]. Even though *E. coli* cultures are easy to be cultivated, the limited sources of oxygen and nutrition elements as well as the increased energy needs under conditions of protein induction, lead to stress and inadequately operated metabolism. This is the reason why the induced protein expression occurs during the exponential rather than the stationary phase, but even in this case, problems could also occur and the yield of production could be significantly restrained [13]. Auto-induction, a technique introduced by Studier et al [21], was designed so as to solve the above restrictions. Contrary to the classic method, protein expression is induced near saturation phase, when cultures have a high cell density, by lactose supplementation in the media instead of IPTG. Induction prior to saturation is prevented by glucose. The specially formulated media required along with proper aerization conditions are responsible for balancing the pH of the cell cultures and the growth of cells to extremely high densities without loss of viability. Bibliography is full of variable examples of correctly expressed proteins using the auto-induction method. The tumor necrosis factor family member APRIL [26], long repetitive resilin-based proteins [27], the psychrophilic TAB5 alkaline phosphatase [28], an Asc-1 homologue [29], the human epoxide hydrolase [30], the holotoxin Stx2 [31], and globin chains from Arenicola marina [32] are just a few of the recombinant produced proteins to name. In the case of recombinant ALDH3A1 (his-tagged ALDH3A1) however, the employment of auto-induction method resulted in higher protein expression levels but unfortunately it did not improve the protein's solubility.

Considering cultivation conditions, several different strategies have been demonstrated, in everyday laboratory practice, for the enhancement of protein solubility. The most commonly and easily tested one is with no doubt the low-temperature culture which was proved to be miraculous in a variety of cases [20,33] including ours as well by enhancing the solubility of the MBP-fused ALDH3A1. On the other hand, temperature is known to correlate with the production of active protein through a variety of mechanisms. Hydrophobic interactions, the basic driving force of inclusion bodies formation depend on temperature. Furthermore, (i) the temperature-dependent expression of molecular chaperones, (ii) the reduction of protein synthesis rate, (iii) the different folding kinetics and (iv) the lower activity of specific proteases [17,18] can also contribute to the enhanced yield of active recombinant proteins.

Co-expressing the recombinant protein with chaperones has been exploited as the most effective way and a quality control

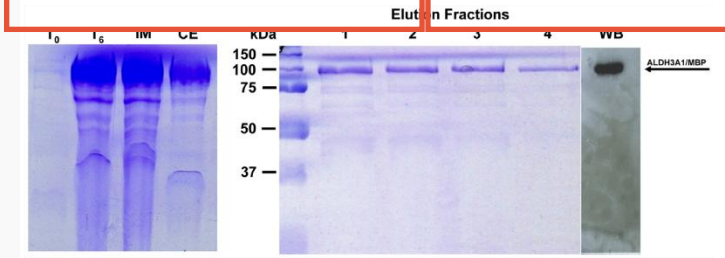

Figure 6. Protein expression and purification of recombinant MBP fused ALDH3A1. SDS-PAGE analysis at various stages of purification of recombinant MBP-fused ALDH3A1 using amylose resin chromatography (Coomassie blue staining). T_0: Total cell extract form bacterial culture prior of protein induction, T_6: Total cell extract 6 hours after IPTG induction, IM: Insoluble matter of lysed cells 6 hours after IPTG induction, CE: Crude extract of lysed cells 6 hours after IPTG induction, Elution fractions: purified recombinant ALDH3A1 eluted from amylose resin column. WB: western immunoblotting of purified recombinant ALDH3A1/MBP. The arrow indicates the position of the MBP-fused ALDH3A1 recombinant protein at approximately 92 kDa.
doi:10.1371/journal.pone.0056582.g006

Table 4. Purification of his-tagged recombinant human ALDH3A1 from *E. coli*.

Purification Steps	Total Protein (mg)	Yield (%)	Specific Activity[b] (mU/mg protein)	Purification (fold)
Crude supernatant[a]	36	100	200	1
Ni-NTA column	2.43	6.75	1150	5.75

[a]The starting material was 250 ml of crude *E. coli* supernatant.
[b]One milliunit (mU) of activity was defined as the amount of activity that oxidized of 1 nmol of NADPH/min at 25°C. Representative results of three different isolation procedures.
doi:10.1371/journal.pone.0056582.t004

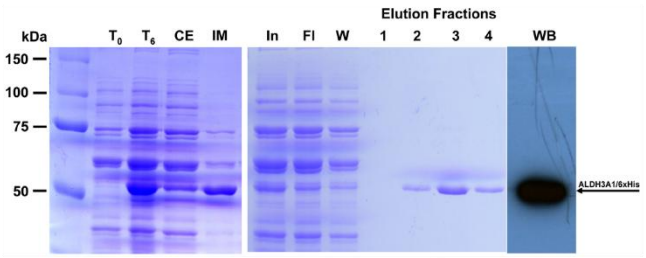

Figure 7. Protein expression and purification of recombinant his-tagged ALDH3A1. SDS-PAGE analysis at various stages of purification of recombinant his-fused ALDH3A1 using Ni-affinity chromatography (Coomassie blue staining). T_0: Total cell extract form bacterial culture co-expressing pG-KJE8 along with ALDH3A1 prior to IPTG induction, T_6: Total cell extract 6 hours after IPTG induction, CE: Crude extract of lysed cells 6 hours after IPTG induction, IM: Insoluble matter of lysed cells 6 hours after IPTG induction, In: Input of the column, Fl: flowthrough part, W: wash part, Elution fractions: purified recombinant protein eluted from Ni-NTA column. WB: western immunoblotting of purified recombinant ALDH3A1/6xHis. The arrow indicates the position of the recombinant his-tagged ALDH3A1 at approximately 51 kDa.
doi:10.1371/journal.pone.0056582.g007

2 Σχεδιασμός και μορφοποίηση άρθρου

Συζήτηση/Discussion

system to increase the solubility of recombinant proteins in *E. coli* [34–37]. Molecular chaperones facilitate the correct conformation of newly synthesized proteins and contribute in the retention of their native folding. Among them, DnaK/DnaJ/GrpE along with GroES/GroEL are the most commonly used systems for the expression of soluble proteins [22,38]. Basically, they are ATP-dependent folding chaperones which induce the partial unfolding and re-folding of non-native proteins [17]. Trigger factor, on the other hand, associates with the synthesized proteins as soon as they leave ribosome and through its interaction with their exposed hydrophobic patches averts their subsequent aggregation [14,38]. Co-expression of molecular chaperones resulted in enhanced solubility and production of recombinant rice plant catalase A [39] active ribonuclease inhibitor [40], human scramblase 1 [41] and zeta-crystallin [42]. Solubility of his-tagged ALDH3A1 was significantly improved under conditions of co-expressing the pG-KJE8) suggesting that dnaK/dnaJ/grpE and groES/groEL are the essential chaperones for the correct folding of recombinant human ALDH3A1 (his-tagged ALDH3A1) when over-expressed in *E. coli*.

In summary, soluble MBP-fused and his-tagged recombinant human ALDH3A1 proteins have been successfully expressed in *E. coli* and purified to homogeneity. Both fusion proteins retained their biological activity and so can be used directly without removing the fusion tags. The methods described in this study permit the production of substantial amounts of the recombinant human ALDH3A1 for conducting functional studies on the biological role of this interesting crystallin, which exists in high concentrations in the cornea of certain mammalian species.

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Author Contributions

Conceived and designed the experiments: AP MP GPV KC. Performed the experiments: GPV TM. Analyzed the data: GPV TM AP. Contributed reagents/materials/analysis tools: AP MP KC. Wrote the paper: GPV AP MP.

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2 Σχεδιασμός και μορφοποίηση άρθρου

Ευχαριστίες

- Δεν ξεχνάμε να αναφέρουμε όσους μας βοήθησαν

- Πάντα αναφέρουμε την χρηματοδότηση

2 Σχεδιασμός και μορφοποίηση άρθρου

Συζήτηση/Discussion

system to increase the solubility of recombinant proteins in *E. coli* [34–37]. Molecular chaperones facilitate the correct conformation of newly synthesized proteins and contribute in the retention of their native folding. Among them, DnaK/DnaJ/GrpE along with GroES/GroEL are the most commonly used systems for the expression of soluble proteins [22,38]. Basically, they are ATP-dependent folding chaperones which induce the partial unfolding and re-folding of non-native proteins [17]. Trigger factor, on the other hand, associates with the synthesized proteins as soon as they leave ribosome and through its interaction with their exposed hydrophobic patches averts their subsequent aggregation [14,38]. Co-expression of molecular chaperones resulted in enhanced solubility and production of recombinant rice plant catalase A [39] active ribonuclease inhibitor [40], human scramblase 1 [41] and zeta-crystallin [42]. Solubility of his-tagged ALDH3A1 was significantly improved under conditions of co-expressing the pG-KJE8) suggesting that dnaK/dnaJ/grpE and groES/groEL are the essential chaperones for the correct folding of recombinant human ALDH3A1 (his-tagged ALDH3A1) when over-expressed in *E. coli*.

In summary, soluble MBP-fused and his-tagged recombinant human ALDH3A1 proteins have been successfully expressed in *E. coli* and purified to homogeneity. Both fusion proteins retained their biological activity and so can be used directly without removing the fusion tags. The methods described in this study permit the production of substantial amounts of the recombinant human ALDH3A1 for conducting functional studies on the biological role of this interesting crystallin, which exists in high concentrations in the cornea of certain mammalian species.

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2 Σχεδιασμός και μορφοποίηση άρθρου

Βιβλιογραφία/References

- Δεν ξεχνάμε να αναφέρουμε τις πηγές μας
- Δεν παίρνουμε αυτούσια κομμάτια από άλλες δουλειές
- Όταν πρέπει αναγκαστικά να αναφέρουμε κάτι αυτούσια, πάντα βάζουμε εισαγωγικά

2 Σχεδιασμός και μορφοποίηση άρθρου

Βιβλιογραφία/References

- Οι πηγές αναφέρονται και για εικόνες που δανειζόμαστε και για τις μεθόδους
- Δεν βάζουμε αυτούσιες φιγούρες ή σχεδιαγράμματα από τις πηγές
- Μεγάλη προσοχή στην λογοκλοπή (plagiarism)

2 Σχεδιασμός και μορφοποίηση άρθρου

- Προσέχουμε οι πηγές μας να είναι ακριβείς
- Ελέγχουμε την εγκυρότητα των πηγών μας
- Προτιμούμε πηγές από αξιόπιστα περιοδικά
- Δεν χρησιμοποιούμε πολύ παλιά βιβλιογραφία
- Η μορφή της βιβλιογραφίας καθορίζεται από το περιοδικό
- Υπάρχουν ειδικά προγράμματα για την δημιουργία βιβλιογραφίας (π.χ. Endnote, Reference manager)

2 Σχεδιασμός και μορφοποίηση άρθρου

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system to increase the solubility of recombinant proteins in *E. coli* [34–37]. Molecular chaperones facilitate the correct conformation of newly synthesized proteins and contribute in the retention of their native folding. Among them, DnaK/DnaJ/GrpE along with GroES/GroEL are the most commonly used systems for the expression of soluble proteins [22,38]. Basically, they are ATP-dependent folding chaperones which induce the partial unfolding and re-folding of non-native proteins [17]. Trigger factor, on the other hand, associates with the synthesized proteins as soon as they leave ribosome and through its interaction with their exposed hydrophobic patches averts their subsequent aggregation [14,38]. Co-expression of molecular chaperones resulted in enhanced solubility and production of recombinant rice plant catalase A [39] active ribonuclease inhibitor [40], human scramblase 1 [41] and zeta-crystallin [42]. Solubility of his-tagged ALDH3A1 was significantly improved under conditions of co-expressing the pG-KJE8) suggesting that dnaK/dnaJ/grpE and groES/groEL are the essential chaperones for the correct folding of recombinant human ALDH3A1 (his-tagged ALDH3A1) when over-expressed in *E. coli*.

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E. coli Expression Strategies for Human ALDH3A1

Ενότητα Δ

Συγγραφή διατριβής - εργασίας

1 Dissertation

Τι είναι;

- Η τελική εργασία ενός προγράμματος σπουδών (π.χ. προπτυχιακό, μεταπτυχιακό)
- Επισφραγίζει τις επιστημονικές – ερευνητικές ικανότητες του φοιτητή
- Αποτελεί ερευνητική εργασία (παράγει νέα γνώση)
- Ερευνητική, βιβλιογραφική (συστηματική ανασκόπηση, μετα-ανάλυση)

1 Dissertation

Σε τι διαφέρει από την
δημοσίευση ή το poster;

- Πολύ πιο εκτενής σαν κείμενο
- Περισσότερες τεχνικές λεπτομέρειες
- Περισσότερη αναφορά στο θεωρητικό υπόβαθρο
- Αναφορά σε όλα τα αποτελέσματα της μελέτης (ακόμη και αυτά που ίσως δεν είναι δημοσιεύσιμα)
- Πιο άμεση και ολοκληρωμένη παρουσίαση της ερευνητικής δουλειάς ενός φοιτητή-ερευνητή

1 Dissertation

Σε τι διαφέρει από την
δημοσίευση ή το poster;

- Συχνά χρησιμοποιείται ως μέτρο για την «κρίση» ενός φοιτητή
- Δεν περνά διαδικασία peer-review αλλά συχνά κρίνεται από μια επιτροπή
- Πλέον δημοσιεύεται σε ιδρυματικά αποθετήρια διαθέσιμα για ανάγνωση
- Μπορεί να λειτουργήσει και ως βάση για μια επιστημονική δημοσίευση

1

Dissertation

Επιλογή θέματος

- Αντικείμενο σπουδών
- Προσωπική εξειδίκευση
- Πρακτικά δυνατό
- Επιβλέπων καθηγητής
- Μελέτη βιβλιογραφίας
- Πρωτοτυπία
- Ενδιαφέρον
- Πιθανές εφαρμογές

2 Σχεδιασμός και μορφοποίηση

- Συχνά υπάρχουν προδιαγραφές από το Τμήμα ή το ΜΠΣ
- Είναι προτιμότερη μια απλή και επίσημη γραμματοσειρά (Times New Roman, Calibri, Arial) εάν δεν υπάρχουν άλλες διευκρινήσεις
- Δίστιχο: 1,5 , πλήρης στοίχιση, μέγεθος 12
- Χρησιμοποιούμε την ίδια γραμματοσειρά σε όλο το κείμενο

- Τίτλος
- Ευχαριστίες
- Περίληψη
- Λέξεις κλειδιά
- Περιεχόμενα
- **Κύριο μέρος**
- Βιβλιογραφία
- Συντομεύσεις
- Παράρτημα/παραρτήματα

Σχεδιασμός και μορφοποίηση

- **Κύριο μέρος:**

✓Εισαγωγή

✓Υλικά και μέθοδοι

✓Αποτελέσματα

✓Συζήτηση

✓Συμπεράσματα

2 Σχεδιασμός και μορφοποίηση

Τίτλος

Συχνά τα ΜΠΣ έχουν συγκεκριμένες προδιαγραφές

- Αναφέρουμε το ίδρυμα στο οποίο πραγματοποιήθηκε η έρευνα
- Σχολή φοίτησης
- Το όνομα μας
- Την τοποθεσία
- Τον/τους επιβλέποντες
- Κάνουμε και ελληνική και αγγλική εκδοχή

2 Σχεδιασμός και μορφοποίηση

Ευχαριστίες

Συμπεριλαμβάνω όλους όσους μας βοήθησαν για την ολοκλήρωση της εργασίας

Π.χ. συναδέλφους, φορείς, άτομα που βοήθησαν συμβουλευτικά κτλ

2 Σχεδιασμός και μορφοποίηση

Περίληψη

- Μια σύντομη περιγραφή όλης της μελέτης:
- ✓ Ποια είναι η βασική υπόθεση
- ✓ Πως διαμορφώθηκε η βασική υπόθεση
- ✓ Ποια μεθοδολογία επιλέχθηκε
- ✓ Ποια ήταν τα αποτελέσματα
- ✓ Ποιο το βασικό συμπέρασμα
- Σύντομο και περιεκτικό (μέχρι 500 λέξεις περίπου—προσοχή στους εκάστοτε ξεχωριστούς κανόνες)
- Την γράφουμε στο **τέλος**
- Είναι από τα πιο σημαντικά κομμάτια της εργασίας
- Το πρώτο που διαβάσεις για να σχηματίσεις μια αρχική εικόνα για την εργασία είναι η περίληψη

2 Σχεδιασμός και μορφοποίηση

Λέξεις – κλειδιά

- Σημαντική η επιλογή τους
- Θα καθορίζουν τα αποτελέσματα στο αποθετήριο
- Ισορροπία: όχι πολύ ειδικά, όχι πολύ γενικά
- Σχηματίζουν μια περιγραφή του αντικειμένου της έρευνας

2 Σχεδιασμός και μορφοποίηση

Πίνακας περιεχομένων

- Κατάλογος όλων των υποκεφαλαίων και κεφαλαίων
- Τον ετοιμάζουμε στο **τέλος** αφού έχουν γίνει και όλες οι διορθώσεις
- Το Word έχει δυνατότητα αυτόματης ενσωμάτωσης
- Ένας σύνδεσμος είναι βοηθητικός στον αναγνώστη
- Μπορούμε να έχουμε και έναν κατάλογο με τις εικόνες, τα σχήματα και τους πίνακες του κειμένου

2 Σχεδιασμός και μορφοποίηση

Βιβλιογραφίες

- Ακολουθούμε τις οδηγίες
- Βοηθητικά τα ειδικά προγράμματα
- Συμπεριλαμβάνουμε όλες τις πηγές που χρησιμοποιήσαμε
- **ΠΡΟΣΟΧΗ στην λογοκλοπή**
- Δεν αντιγράφουμε αυτολεξεί προτάσεις
- Αναφέρουμε την πληροφορία με δικά μας λόγια

Σχεδιασμός και μορφοποίηση

- Ότι συντόμευση αναφέρεται στο κείμενο την προσθέτουμε σε ένα πίνακα στο τέλος
- Και στο κύριο μέρος του κειμένου την πρώτη φορά που θα αναφέρουμε την συντόμευση την επεξηγούμε

2 Σχεδιασμός και μορφοποίηση

Παράρτημα

- Οτιδήποτε δεν ενσωματώνεται στα βασικά αποτελέσματα της μελέτης
- Ότι σχετίζεται έμμεσα με το αντικείμενο της εργασίας και είναι απαραίτητο για να ενισχύσει τα αποτελέσματα

Σχεδιασμός και μορφοποίηση

- Αναλυτική παρουσίαση του θεωρητικού υπόβαθρου
- Περισσότερες πληροφορίες και από το poster και από την δημοσίευση
- Ξεκινάμε από το πιο γενικό και εξειδικεύουμε

Σχεδιασμός και μορφοποίηση

- Π.χ. Ο ρόλος της αλδεϋδικής αφυδρογονάσης $3^{A}1$ στην κυτταρική ομοιόσταση του κερατοειδούς

✓Τι είναι οι αλδεϋδικές αφυδρογονάσες → ALDH3A1

✓Κερατοειδής χιτώνας → οξειδωτικό στρες στον κερατοειδή → ALDH3A1 στον κερατοειδή χιτώνα

Σχεδιασμός και μορφοποίηση

- Πιο λεπτομερής αναφορά στα υλικά που χρησιμοποιήθηκαν και τις μεθόδους
- Αναλυτικά τα πρωτόκολλα
- Επεξήγηση του τρόπου λειτουργίας των τεχνικών και της μεθοδολογίας που ακολουθήθηκε

Σχεδιασμός και μορφοποίηση

- Πίνακες, εικόνες, γραφικά με στατιστική επεξεργασία
- Παρουσίαση των αποτελεσμάτων
- Τι παρατηρήθηκε
- Αναφορά των δεδομένων
- Μπορούμε να αναφέρουμε και δεδομένα τα οποία δεν θεωρούμε σημαντικά

2 Σχεδιασμός και μορφοποίηση

Συζήτηση

- Σύγκριση των αποτελεσμάτων με προηγούμενες έρευνες που μπορεί να σχετίζονται με την δική μας
- Δεν χρειάζεται να είναι πολύ εκτενής
- Μπορούμε να προσθέσουμε και θα έχει αξία τους περιορισμούς, τις αδυναμίες, τις μελλοντικές κατευθύνσεις που προκύπτουν από την εργασία μας

Σχεδιασμός και μορφοποίηση

- Μια μικρή παράγραφο με τα βασικά συμπεράσματα βάση και όσων αναφέρονται στην συζήτηση
- Απαντάμε στο βασικό ερώτημα;
- Πόσο σημαντικά είναι τα ευρήματα;
- Επιβεβαιώνονται από άλλες μελέτες;

Επιστημονικό κείμενο

- **ΠΡΟΣΟΧΗ στην χρήση AI**
- Δεν χρησιμοποιούμε AI για να μας ετοιμάσει την εργασία μας
- Υπάρχουν εργαλεία που αναγνωρίζουν εάν ένα κείμενο έγινε με AI