

## PREZENTAREA UNUI POSTER

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### **Ce este un poster?**

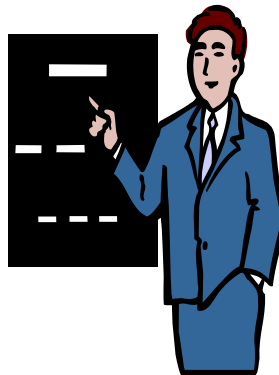
O modalitate de prezentare sintetica a unei comunicari sub forma de afis la o sesiune / conferinta / congres

### **De ce un poster ?**

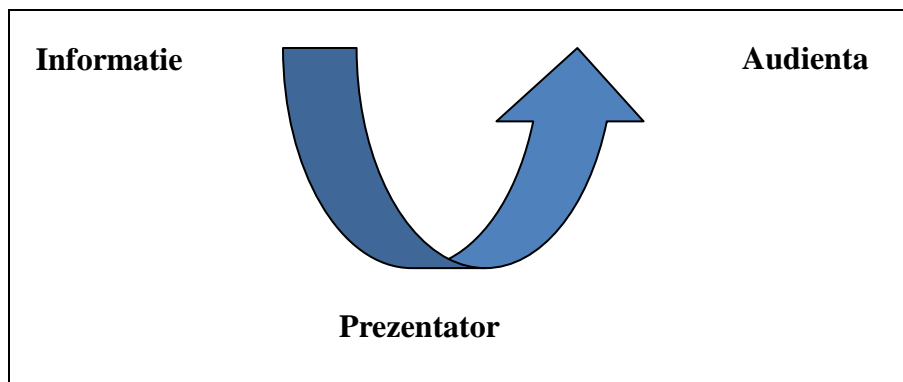
- ofera posibilitatea unui numar mare de autori sa-si prezinte lucrarile fara riscul de a fii respinse ca urmare a faptului ca sa depasit timpul alocat manifestarii sau capacitatea salii
- uneori un poster poate fi mai adecvat decat o prezentare orala

### **Scopurile unui poster bun**

- **Comunicare vizuala**
  - *Un rezumat ilustrat al lucrului dvs.*
- **Sa atraga si sa mentina atentia audientei**
  - *Sa fie usor de urmarit*
  - *Sa initieze discutii*
- **Concis si organizat**
  - *Sa aiba un mesaj clar*
  - *Sa prezinte cu succes date tehnice*
  - *NU este un articol intr-o revista*
- **Sa vorbeasca de la sine cand/daca autorul nu este langa el.**



### **Elemente esentiale ale unei prezentari poster**

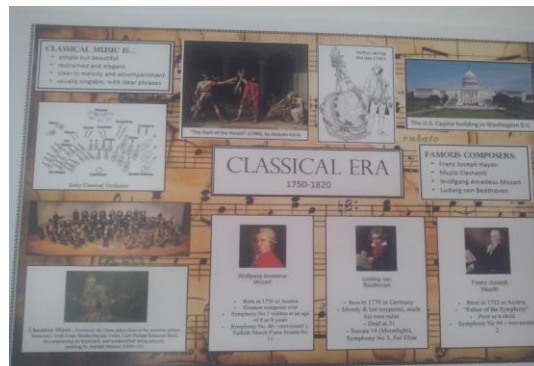
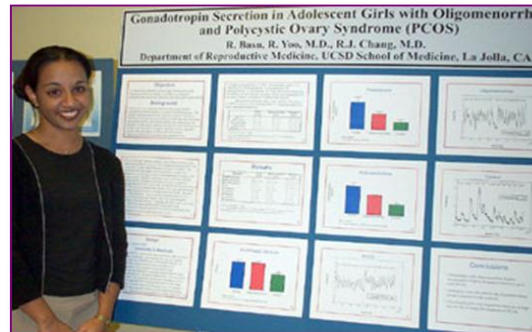


**Un poster este.....mai mult decat o prezentare de date**

- permite contribuția dumneavoastră la o manifestare stiintifica
- o parte semnificativă a educatiei profesionale
- furnizează informații
- va dezvoltă experiența
- construiește contacte și rețele de colaborare
- sursă buna de feedback

Varianta “veche”:

Mai multe pagini A4 montate pe un panou



Varianta “moderna”, lucrat in power point

**CARDIFF UNIVERSITY**  
PRIFYSGOL Y CERDD

**Expression, purification, and crystallization of recombinant mouse phospholipase c-zeta (PLC-ζ)**

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**ABSTRACT**

The aim of this study is to express and purify recombinant PLC-ζ protein for structural identification through X-ray crystallography. To date, there is no available structural data of the 3D structure of PLC-ζ. The identification of the structure is critical for understanding the protein mechanism and regulation, both of which are related to cancer. Bioinformatics analysis was also utilized to draw initial structural information, specifically on the domain differences of PLC-ζ and extensively identified isoforms PLC-δ1.

**INTRODUCTION**

Phospholipase C-ζ (PLC-ζ), a member of phospholipase C family, was identified as the specific factor responsible for initiating oocyte and thereby causing fertilization.

**RESULTS**

**Figure 1. Expression of PLC-ζ.** Horizontal blot of PIP<sub>2</sub> by PLC-ζ (released from sperm) produced DAG and IP<sub>3</sub>. IP<sub>3</sub> in turn activates Ca<sup>2+</sup> channels of ER to release calcium. This in turn binds to produce Ca<sup>2+</sup> oscillation and eventually fertilization.

**Figure 2. PLC Domain Organization.** PLC-ζ consists of 7 transmembrane domains (TM 1-7), seven intracellular loops (IL 1-7), and a cytosolic tail (CT). The cytosolic tail region of PLC-ζ is highly conserved with PLC-δ1. Bioinformatics analysis through sequence alignment and domain analysis revealed that the cytosolic tail region of PLC-ζ was expected to be significantly different from extensively determined PLC-δ1.

**Figure 3. Molecular cloning of PLCζ24 construct.** (A) Two new PLC-ζ constructs successfully produced in PLC-ζ construct with GFP and 3C protease cleavage site (3C-PLC-ζ) were used. Construct was ligated into pET20D(+)-3C vector. This is verified by restriction digest using ClaI. \*vector alone (V) showed a lower band compared to vector with the construct (2).

**Figure 4. Protein expression and purification.** (A) Molecular weight marker (kDa). (B) Protein band after IPTG induction (kDa). (C) Protein band after 3C protease cleavage (kDa). (D) Protein band after 3C protease cleavage (kDa). (E) Protein band after 3C protease cleavage (kDa). (F) Protein band after 3C protease cleavage (kDa).

**Figure 5. Crystallization of PLCζ24 Construct.** Six different conditions were tested for the ability to crystallize the protein. Crystals were confirmed to be present due to birefringence under polarized light. Protein crystals AC were needed to be optimized to obtain larger crystals. Protein crystals F were tested for X-ray diffraction. Preliminary analysis, however, revealed that only diffraction pattern was hindered by presence of high salt concentration.

**EXPERIMENTAL PROCEDURE**

PLCζ24 construct was generated using last best PCR to introduce 6- His and 3C protease recognition site. Construct was ligated into pET20D(+)-3C vector and transformed into E. coli BL21(DE3) using Prime Plus. Protein construct was isolated using Ni-NTA beads and cleavage of the protein from the His<sub>6</sub> tags was done by 3C protease. Further purification was carried out using PPLC (ion exchange) and gel filtration (chromatography). Crystallization of protein was carried out using sitting drop vapour diffusion method.

**REFERENCES**

1. Serrhini GM et al. (2002) PLC-ζ is a sperm-specific trigger of Ca<sup>2+</sup> oscillations in eggs and controls development. *Development* 130, 3503-4.
2. Partridge L, Parke D, and Brown H. (1996) Animal protein for Ca<sup>2+</sup> signaling at fertilization. *Curr Biol* 6, 101-10.
3. Durkin-Delgado L, Carré O, and Virelizier L. (2004) The topology of mammalian protein phosphatase 2A is a consequence of transmembrane (TM)1 (TM2) and TM2 (TM3) Protein Expression and Purification 37, 230-208.
4. Ewan LC, Pines G, Cheng R, Cohen M, and Williams RL. (1995) Crystal structure of a mammalian phosphoinositide-specific phospholipase C. *Nature* 376, 595-602.

**CONCLUSION**

- It was predicted from the bioinformatics analysis that PLC-ζ will be in the same general topology as PLC-δ1 (without Phosphatase).
- Specific differences were predicted to be in the location of transmembrane domain and CT domain.
- High salt concentration in preliminary X-ray studies could be protein crystallization. Various detergent additives may be added to address this problem.
- Further X-ray diffraction data from the crystals are to be expected in the future to verify the structural model generated.
- The recombinant mouse PLC-ζ was successfully expressed, purified and crystallized. However, the expression levels are low.
- It was estimated that the protein was relatively stable in buffer and not overexposed to avoid toxicity and degradation.
- To obtain higher protein expression, different water systems and buffer salts may be used. Future studies may use constructs with a shorter N-terminus to increase protein expression.
- The primary aim is to obtain the 3D structure of human PLC-ζ. However, the expression of the human PLC-ζ was much lower. It is possible though to construct a more accurate model if an improved 3D structure of mouse PLC-ζ is determined and used as a template.

**ACKNOWLEDGEMENTS**

I would like to thank Dr. A. Robinson for the antibody used in Western blotting, Dr. G.D. Cook for the PLCζ24 construct, Dr. Catherine Hill for supervision, Mr. Peter Thomas for technical support and Scott Higgins for help and assistance.

### **Pasul 1**

Pregătește, redactează și trimite rezultatul la conferința/congres, cei 4 C :

- *concis*
- *clar*
- *complet*
- *cursiv*

### **Pasul 2:**

Informează-te asupra manifestării științifice

- *consulta instrucțiunile, cerințele specifice ale organizatorilor manifestării științifice*
- *află informații despre publicul participant*

### **Pasul 3**

Redactează și construiește posterul

#### ***Condiții pentru un poster reușit***

Posterul va comunica optim conținutul științific al lucrării dacă:

- *Pondere textului 20% , elementele grafice 40% și restul spațiu liber*
- *Este menținut suficient spațiu alb în jurul blocurilor*
- *Se păstrează o aliniere logică a coloanelor*
- *Pastrarea numărului de cuvinte la un nivel de maxim 800 de cuvinte maximizează șansele ca posterul să fie parcurs în întregime*
- *Ideile principale organizate pe câteva direcții relevante, bine structurate pentru a da posibilitatea cititorilor informați să-și dea seama imediat de importanța conținutului posterului*
- *Utilizarea unui singur tip de font*
- *Simplitatea este cheia reușitei*
- *Textul cu caractere de maxim 4 mm*
- *Se utilizează 2-3 culori, culoarea de fond pentru tot posterul tonuri calde de gri sau bleu, culori intense pentru chenare sau sublinieri*

#### ***Componentele posterului:***

**Titlul** trebuie să fie scurt, să atragă atenția cât mai mult posibil și să fie lizibil de la o distanță de circa 3 m

**Marime:** cel puțin 30 mm

**Literele:** un caracter clasic, ușor lizibil de exemplu “ bold “

**Contrast** fond/ font

**Numele autorului** se scrie cu caractere mai mici decât cele ale titlului, maxim 20 mm

#### ***Partile posterului***

- Introducere
- Metode
- Rezultate
- Concluzii
- Referințe bibliografice
- Mulțumiri

### Introducere

- Utilizez un minim de informatii si definitii pentru a plasa rapid problema in contextual general si sa furnizez o indicatie succinta si o justificare asupra abordarii utilizate
- Maxim 200 cuvinte si 1-2 imagini
- Marime font pentru titlurile sectiunilor 1/2 din cea a titlului

### Metode

- Trebuie sa ofere o imagine sintetica asupra echipamentelor si metodelor utilizate
- Diagrame prezentand intercorelarea logica a diferitelor componente sau etape
- Fotografice pot oferi informatii subliminale asupra anvergurii si importantei cercetarii intreprinse
- Maxim 200 de cuvinte

### Rezultate

- Este sectiunea cea mai consistenta si trebuie sa poata furniza de sine statator informatia esentiala asupra cercetarii
- Imaginile si graficele transmit mai multa informatie decat textul
- Textul maxim 200 cuvinte

### Concluzii

- Sa treaca succint in revista rezultatele si sa precizeze daca acestea confirma ipotezele
- Maxim 300 cuvinte

### Referinte bibliografice

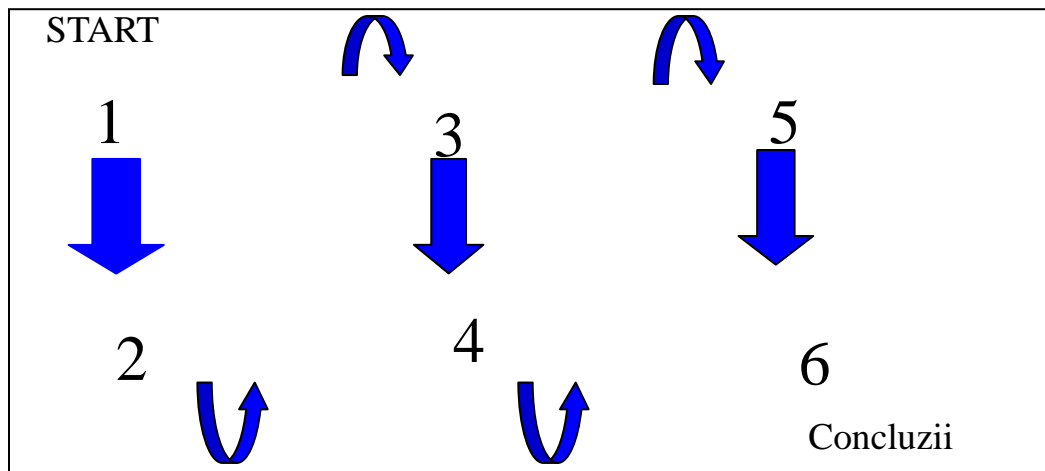
- Lista referintelor bibliografice care sustin rezultatele prezentate in lucrare maxim 5-10 titluri

### Mulumiri

- Este obligatoriu mentionarea institutiilor care au finantat cercetarea
- Fata de persoanele care au avut contributii directe sau indirecte
- Maxim 40 cuvinte

### Asamblarea elementelor:

De sus in jos si de la stanga la dreapta





Detalii importante:

- Planificare
- Modalitate de transport in tub de carton
- Modalitati de fixare a posterului
- Participa obligatoriu la sesiunea de prezentare si ... fii cat mai mult timp langa posterul tau
- Pregatiti cartea de vizita, pt contact.

**Pasul 4**

Bucura-te de prezentare!

Prezinta posterul cu entuziasm!

*Prezentarea...*

- ✓ Necesita pregatire
- ✓ Poate fi premiata...
- ✓ Prezinta datele obtinute, de etapa
- ✓ Aduce feed back din partea experților
- ✓ Poate fi premiata...

*Aminteste-ti!!!*

**Participarea cu un poster la o manifestare stiintifica este o investitie buna pentru dezvoltarea profesionala!**