UE De la conception à l'utilisation des kits en diagnostic Santé

Construire un projet, le mettre en forme et convaincre

04 12 2017, Nîmes

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« Pathogénèse et contrôle des infections chroniques »
Equipe 1- Innovations thérapeutiques et diagnostiques

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Construire un projet, le mettre en forme et convaincre

1/ Sélection et exploitation rationnelle de documents : méthodes

2/ Les questions à vous poser avant de vous lancer dans un projet collaboratif innovant

3/ Exemple de projet : appel d'offres ANR

4/ Mise en situation : projet d'études

- **Synthèse thématique (10-15p) + présentation orale**
- **Idée innovante :**
  - **Lettre d'intention (3-5p)**
  - **Présentation Poster devant jury**

Recherche et choix de documents scientifiques

Les documents à disposition : sélection des mots clés +++

- revues
- articles
- notes
- communications
- chapitres de livres
- internet
- brevets

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Recherche et choix de documents scientifiques

Les revues :

- **Intégrer, corréler, évaluer des résultats déjà publiés sur un sujet particulier:**

  - Rarement des nouveautés
  - Critique constructive
  - Rassembler des interprétations
  - Très souvent invité par un éditeur

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Recherche et choix de documents scientifiques

Les articles :

- **Description de données originales et d'importance significative**

Les notes:

- **Description de travaux originaux dont la portée reste limitée:**

  - **Amélioration d'une procédure expérimentale**
  - **Présentation d'une observation pertinente**
  - **Présentation de programme**

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Recherche et choix de documents scientifiques

Les communications:

➢ **Description de données originales et d'importance significative d'ordre urgent :**

- Seul un éditeur peut juger du caractère urgent

- Limitation stricte dans la taille du manuscrit

Les chapitres de livres:

- Articles originaux
- Revues de littérature
- En lien avec une communication orale de congres

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Recherche et choix de documents scientifiques

Internet

- Rapports nationaux (sénat, ministeres, …)
- Rapports européens et internationaux
- Rapports d'experts du domaine
- Penser à regarder les sites des organismes concernés par le sujet étudié (CEA, Inra, Inserm, CNRS, ANSM, OMS…) et des industriels du domaine.

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Recherche et choix de documents scientifiques

3- Analyse d'une publication

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Par où commencer ?

- Titre
- Auteurs et affiliations
- Résumé
- Introduction
- Matériels et Méthodes (parfois à la fin)
- Résultats (parfois combinés Résultats et discussion)
- Discussion
- Conclusions
- Remerciements
- Références
- Matériels supplémentaires

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Recherche et choix de documents scientifiques

Etapes recommandées pour l'analyse d'une publication ++++

1. Le titre
2. Les auteurs (les noms, leur origine)
3. Le résumé
4. L'introduction
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Recherche et choix de documents scientifiques

Exemples

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Electrical detection of pathogenic bacteria via immobilized antimicrobial peptides

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Edited* by Charles Lieber, Harvard University, Cambridge, MA, and approved September 3, 2010 (received for review June 21, 2010)

The development of a robust and portable biosensor for the detection of pathogenic bacteria could impact areas ranging from water-quality monitoring to testing of pharmaceutical products for bacterial contamination. Of particular interest are detectors that combine the natural specificity of biological recognition with sensitive, label-free sensors providing electronic readout. Evolution has tailored antimicrobial peptides to exhibit broad-spectrum activity against pathogenic bacteria, while retaining a high degree of robustness. Here, we report selective and sensitive detection of infectious agents via electronic detection based on antimicrobial peptide-functionalized microcapacitive electrode arrays. The semi-selective antimicrobial peptide magainin I—which occurs naturally on the skin of African clawed frogs—was immobilized on gold microelectrodes via a C-terminal cysteine residue. Significantly, exposing the sensor to various concentrations of pathogenic *Escherichia coli* revealed detection limits of approximately 1 bacterium/μL, a clinically useful detection range. The peptide-microcapacitive hybrid device was further able to demonstrate both Gram-selective detection as well as interbacterial strain differentiation, while maintaining recognition capabilities toward pathogenic strains of *E. coli* and *Salmonella*. Finally, we report a simulated "water-sampling" chip, consisting of a microfluidic flow cell integrated onto the hybrid sensor, which demonstrates real-time on-chip monitoring of the interaction of *E. coli* cells with the antimicrobial peptides. The combination of robust, evolutionarily tailored peptides with electronic read-out monitoring electrodes may open exciting avenues in both fundamental studies of the interactions of bacteria with antimicrobial peptides, as well as the practical use of these devices as portable pathogen detectors.

bacterial sensing | bioelectronic sensors | biorecognition | water monitoring | biomimetic devices

Bacterial infections remain the leading cause of death in developing countries, reducing the shelf life of antibody functionalized sensors. The high specificity of antibody–antigen interactions also requires a one-to-one pairing of antibody-based sensors for each target to be detected. Nucleic acid probe-based techniques such as PCR can reach single-cell detection limits, yet require the extraction of nucleic acids and are limited in portability.

By contrast, the ease of synthesis and intrinsic stability of antimicrobial peptides (AMPs) render them particularly interesting candidates for use as molecular recognition elements in electronic biosensing platforms (11, 12). AMPs appear in multiple niches in nature including the skin of higher organisms and the extracellular milieu of bacteria as the primary line of defense against bacteria and fungi (13). AMPs are much more stable than typical globular proteins—explaining how they can be continually exposed to the natural environment—and are exceptionally efficient at fending off bacterial infection (14). Indeed, some cationic antimicrobial peptides have shown activity toward pathogenic bacteria under harsh environmental conditions such as thermal (boiling/autoclaving) and chemical denaturants (15, 16). The replacement of current antibody-based affinity probes with more stable and durable AMPs in biological sensors may thus help to increase the shelf life of current diagnostic platforms. Finally, a major potential advantage of AMPs as recognition elements stems from their semiselective binding nature to target cells, affording each peptide the ability to bind a variety of pathogens.

The bioactivity of AMPs toward microbial cells is classified into groups according to their secondary structures (13). Many AMPs adopt amphipathic conformations that spatially cluster hydrophobic from cationic amino acids, thereby targeting the negatively charged head groups of lipids in the bacterial membrane. In contrast, the membranes of plants and animals seclude negative charges to the inner leaflet and contain cholesterols that reduce AMP activity (12). By aiming at the very foundation of the bacterial cell membrane, and remaining generically unrecog-

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Recherche et choix de documents scientifiques

Exemples

coagulation of horseshoe crab blood (4, 5). Microbial infections and drug-resistant supergerms are also a leading cause of military deaths, particularly in soldiers with burn injuries, and are considered potential biowarfare agents (6–8). Although containment strategies—such as vaccination and "broadband" antibiotic usage in hospitals—have helped reduce the severity of bacterial infections, these strategies have also inadvertently promoted the emergence of antibiotic resistance. Thus, the development of a sensor that can detect the presence of an infectious outbreak from a broad spectrum of pathogenic species would be highly desirable.

Current methods for detecting pathogenic bacteria include ELISA and PCR (9, 10). In the former case, the assays exploit antibodies as molecular recognition elements due to their highly specific targeting of antigenic sites. However, antibodies lack the stability needed to detect pathogenic species under harsh envir-

as a precursor to bactericidal activity (20). Magainin I also displays broad-spectrum activity toward other Gram-negative bacteria, which comprise the majority of pathogenic infection in humans.

A number of methods have been successful at detecting bacteria, including nanomechanical cantilever sensing (21, 22),

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The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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surface-enhanced Raman spectroscopy (23), and quartz crystal microbalance-based sensors (24). Similarly, recent attempts have utilized AMPs as biorecognition elements in fluorescent-based microbial detection with achievable detection limits of 5×10^4 cells/mL (25, 26). Yet, the development of an "all-in-one" solution that combines a high degree of portability, robustness, sensitivity, and selectivity toward pathogenic strains remains challenging. Among the various label-free signal transduction platforms that have been investigated, impedance spectroscopy is promising due to its simple instrumentation, ease of device assembly, and adaptability to multiplexed lab-on-a-chip applications (27, 28). A microcapacitive sensor detects impedance changes in the dielectric properties of an electrode surface upon analyte binding, where the variation in the impedance is directly proportional to the activity of analyte binding (29). Here, we report a label-free electronic biosensor based on the hybridization of the antimicrobial peptide magainin I with interdigitated microelectrode arrays for the sensitive and selective detection of pathogenic bacteria via impedance spectroscopy. We anticipate that the combination of compact, naturally bioselective AMPs with microcapacitive sensors may represent a highly robust and portable platform for fundamental studies of AMP-bacteria interactions, and for portable infectious disease threat agent signaling.

Results and Discussion

concentrations ranging from 10^3 to 10^7 cfu/mL. A "blank" device with no immobilized AMPs was also tested for comparison; the impedance of the blank device without immobilized AMPs is found to change negligibly upon exposure to various bacterial concentrations (see Fig. S2). Fig. 2*A* shows that at low frequencies, the different concentrations of bacterial cells have the effect of increasing the impedance in proportion to the number of cells present in the sample for concentrations greater than 10^5 cfu/mL. As the frequency increases, the contribution to the impedance from the bacterial cells decreases, leaving only the dielectric relaxation of small dipoles including water molecules in the buffer solution to affect the measured impedance. Fig. 2*B* thus depicts the impedance change at a fixed frequency of 10 Hz. The variation in the impedance is directly proportional to the number of bacterial cells bound to the immobilized AMPs and manifested in a logarithmic increase with respect to serially diluted bacterial concentrations. Significantly, the detection limit of response of the hybrid AMP-microelectrode device to *E. coli* was found to be 10^3 cfu/mL (1 bacterium/μL). This lowest limit of detection appears to be limited by the presence of impedance due to the electrical double layer resulting from the electrode polarization effect at low frequencies. Importantly, this sensitivity limit is clinically relevant (30) and compares favorably to AMP-based fluorescent assays (26), antibody-based impedance sensors (27), and to the LAL test (5).

To gain further insight into the activity of magainin I toward

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trodes in everyday applications, such as direct water sampling, the biosensor response was investigated in real time, as shown in Fig. 5. First, a microfluidic cell was bonded to the interdigitated biosensor chip (Fig. 5A) such that the electrodes were perpendicular to the direction of the sample flow (Fig. 5B) (44). Next, fluid was injected using a syringe pump connected to the inlet port and allowed to flow through to the outlet port at a flow rate of 100 μL/min. The flow cell was first flushed with buffer to establish a baseline. Various dilutions (10^2–10^7 cfu/mL) of

assays (23).

Conclusion

In summary, coupling of AMPs with microcapacitive biosensors has resulted in the implementation of a portable, label-free sensing platform for the detection of infectious agents. The achievable sensitivity approached 1 bacterium/μL—a clinically relevant limit—and the AMPs allowed for sufficient selectivity to distinguish pathogenic and Gram-negative bacteria, while retaining broadband detection capabilities. Finally, real-time

sensing results demonstrated the capability of the relatively simple impedance-based transduction architecture to directly detect bacteria, suggesting a promising alternative to traditional antibody-based immunoassays. We anticipate these results could provide a significant positive impact on the use of pathogenic sensors to test and monitor bacteria in reservoir water, or for use as biological threat agent detection systems. Yet, a number of key challenges remain. First, the detection of bacteria in real water samples has not yet been studied. Second, as confirmed with detection of *E. coli* in the presence of *Listeria*, the broadband selectivity of magainin-functionalized sensors complicates scenarios in which there are multiple infectious agents present, or when the concentrations of the target species are unknown. Finally, based on our previous work in coupling peptides to silicon nanowire sensors (45, 46), significantly enhanced sensitivity may be achievable by reducing the sensors down to the nanometer scale.

the immobilization procedure, the gold IMA electrodes were cleaned using acetone, isopropanol, and deionized (DI) water. Stock solutions of the AMPs were prepared in PBS, pH 7.4, consisting of 137 mM NaCl, 2.7 mM KCl, 4.4 mM Na_2HPO_4, and 1.4 mM KH_2PO_4 (35, 36). For the immobilization of the AMPs, 800 μg/mL (unless otherwise mentioned) of magainin 1 in PBS buffer was injected into the sensing chamber and incubated for 60 min under static conditions. The functionalized electrodes were then rigorously washed with 1 × PBS to remove any unbound AMPs, rinsed with deionized water and dried in liquid nitrogen. Gold surfaces covalently functionalized with magainins have shown antimicrobial binding activity persisting for at least 6 mo (54).

Fluorescent Microscopy. Stock solutions of PI, nucleic acid stain (Molecular Probes) was made from solid form by dissolving PI (molecular weight = 668.4) in deionized water at 1 mg/mL (1.5 mM) and stored at 4°C, protected from light. Heat-killed bacterial cells rehydrated in PBS were then incubated with 3 μM solution of PI (made by diluting the 1 mg/mL stock solution 1:500 in buffer) for 10–15 min (55). After incubation, the cells were pelleted by centrifugation and removal of the supernatant and were resuspended in

En savoir plus ?

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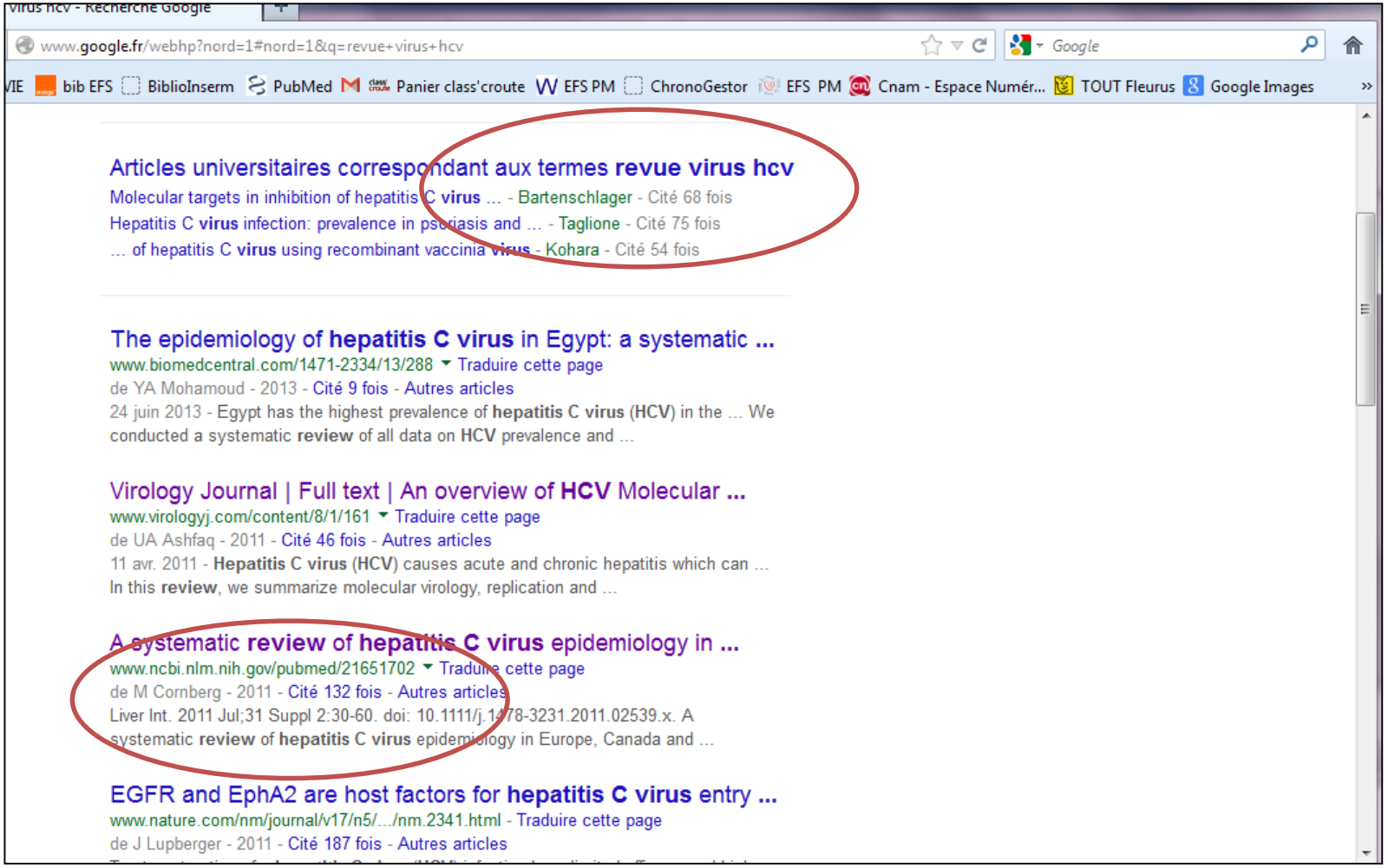

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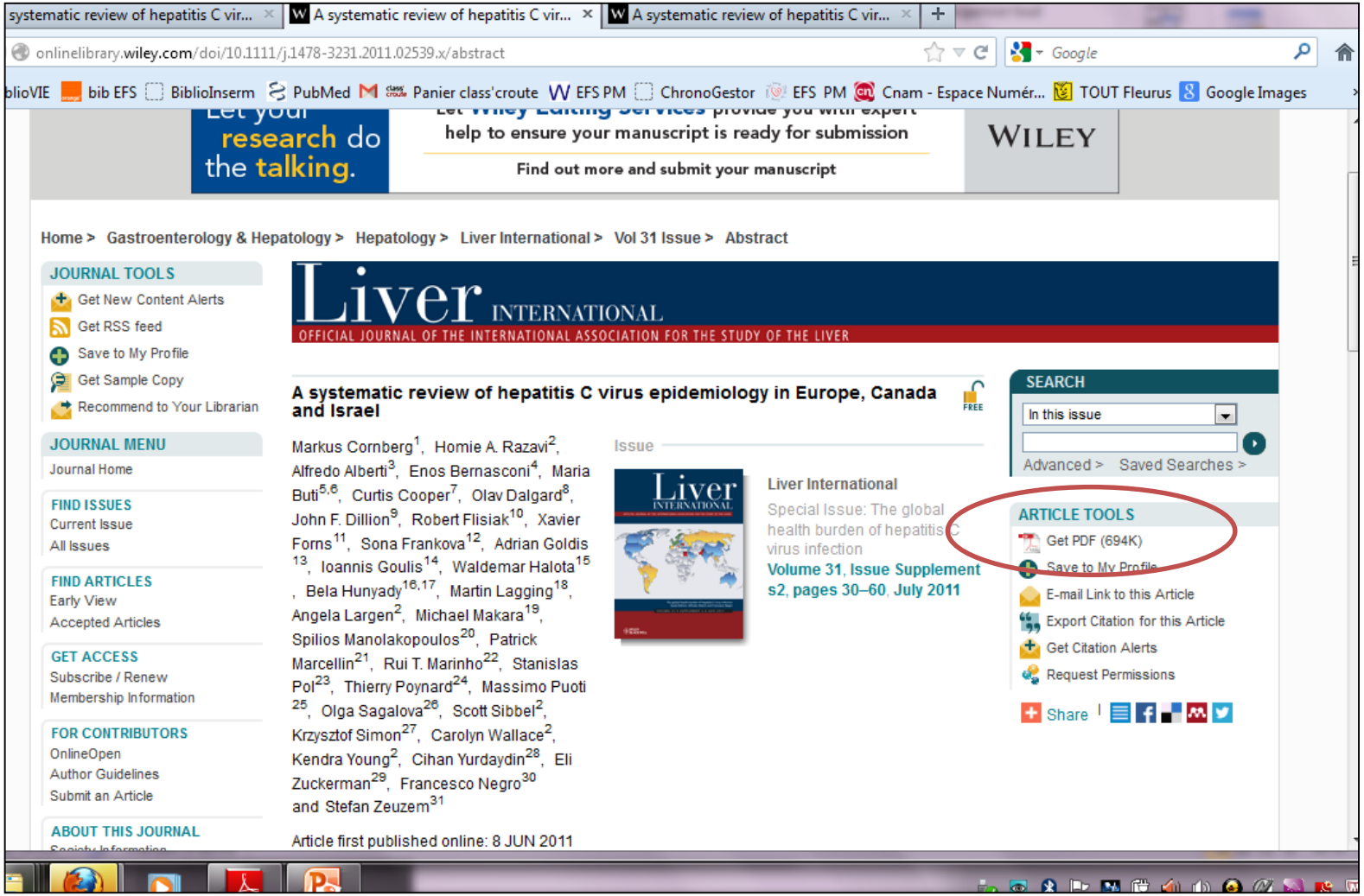

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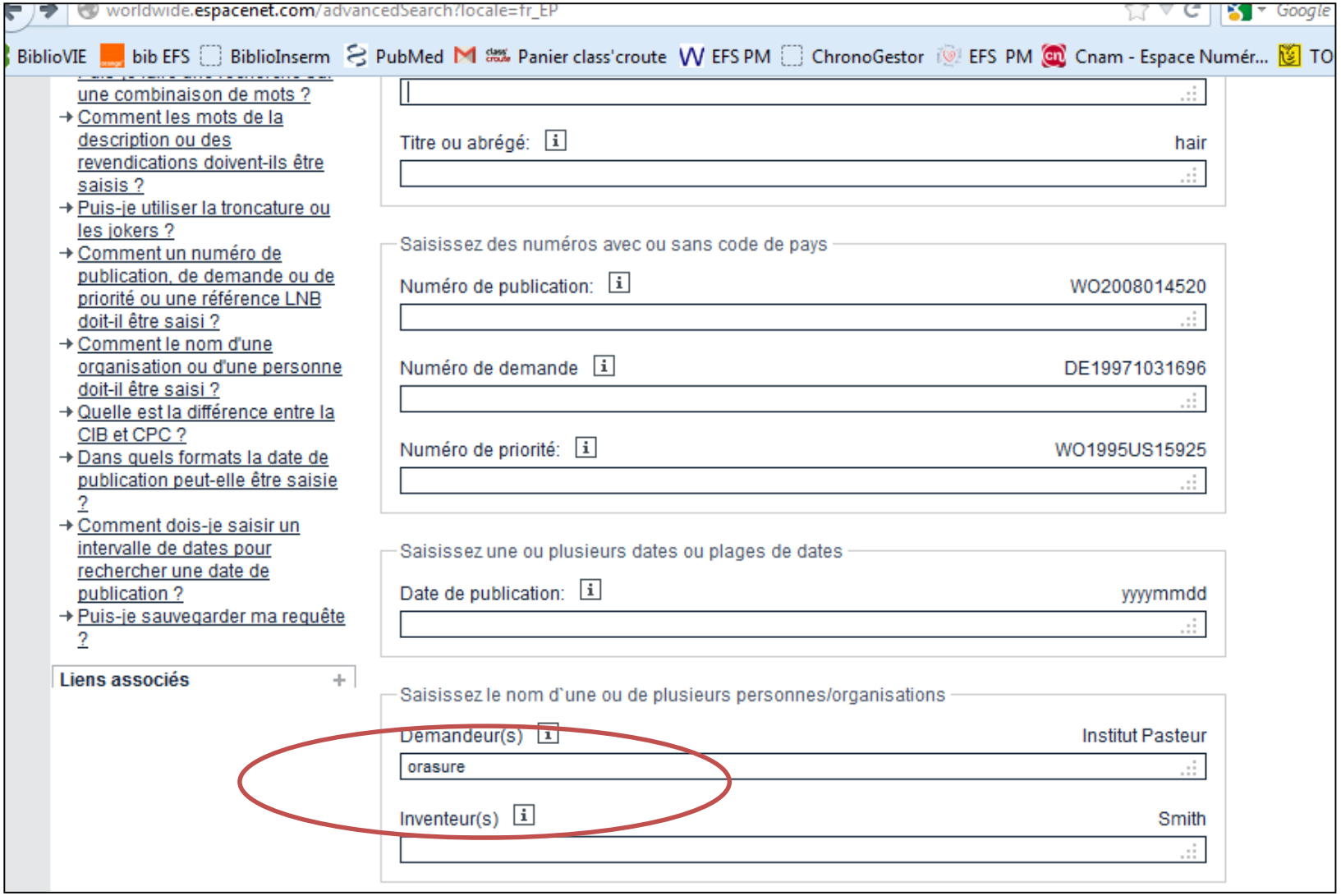

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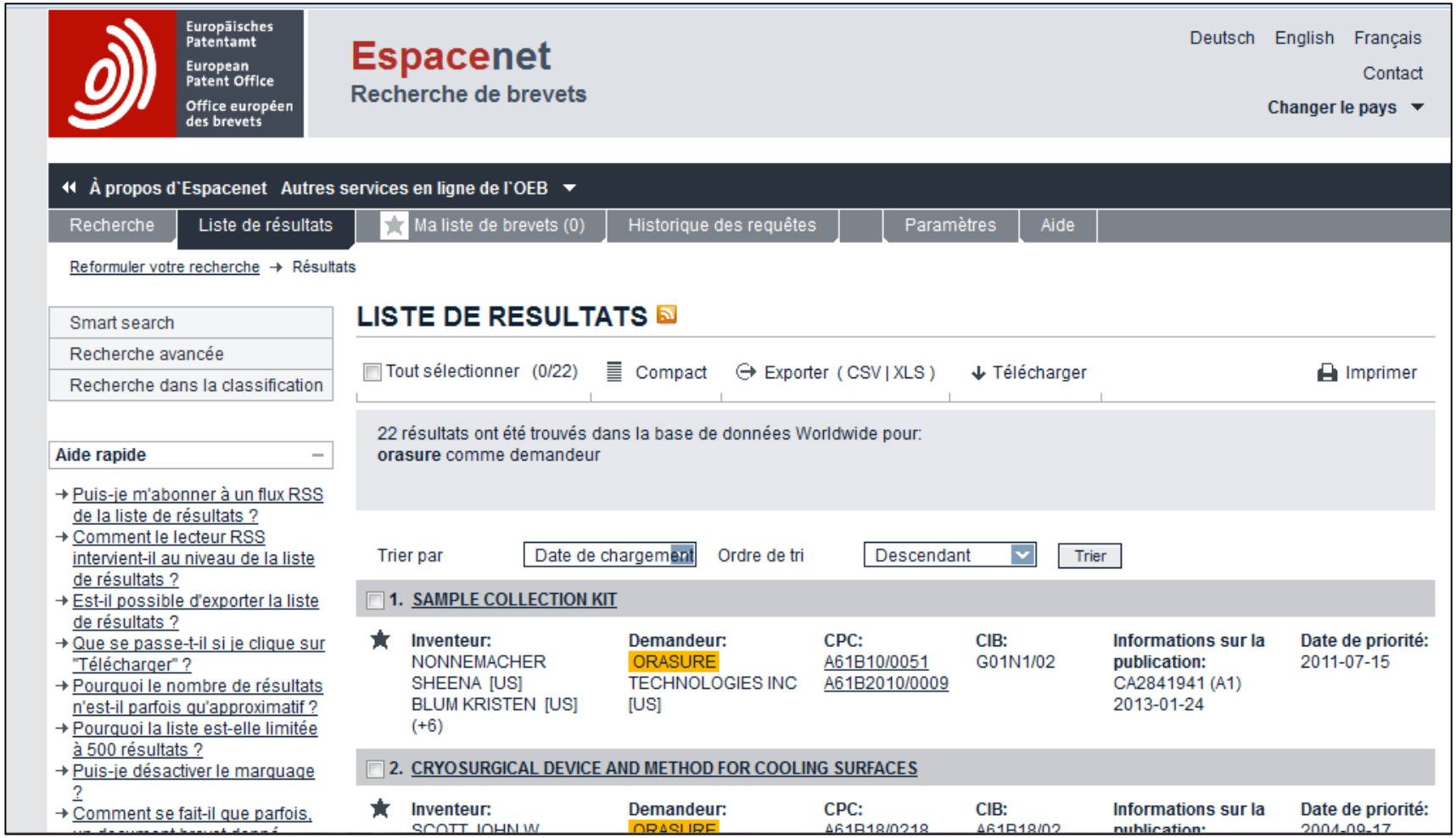

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Recherche et choix de documents scientifiques

Quelques conseils pour être efficace

- **lors de vos recherche sur internet ouvrir en parallèle un fichier powerpoint de travail** ou vous allez « copier-coller » l'écran de votre ordinateur pour retrouver facilement vos informations

- **Comment ?**

1/ Pour sauvegarder l'écran devant vous :

- Sur le clavier - touche « Fn » **+** touche « **Imp Ecran** »
  - touche Ctrl **+** touche C
- Sur votre fichier powerpoint coller avec : touche Ctrl **+** touche V

2/ Dans le menu windows, utiliser « **Outil capture** » pour sélectionner la zone à copier, puis coller sur le fichier powerpoint

- **Avantages ?**

Vous pourrez utiliser cette base de documents de travail pour monter les diapositives de votre présentation orale

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Recherche et choix de documents scientifiques

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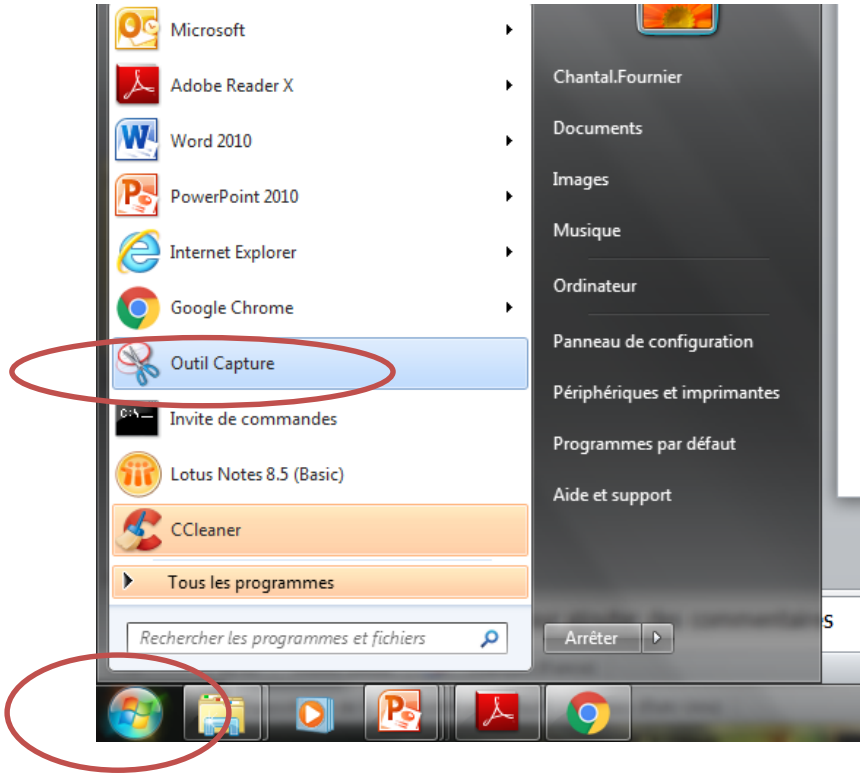

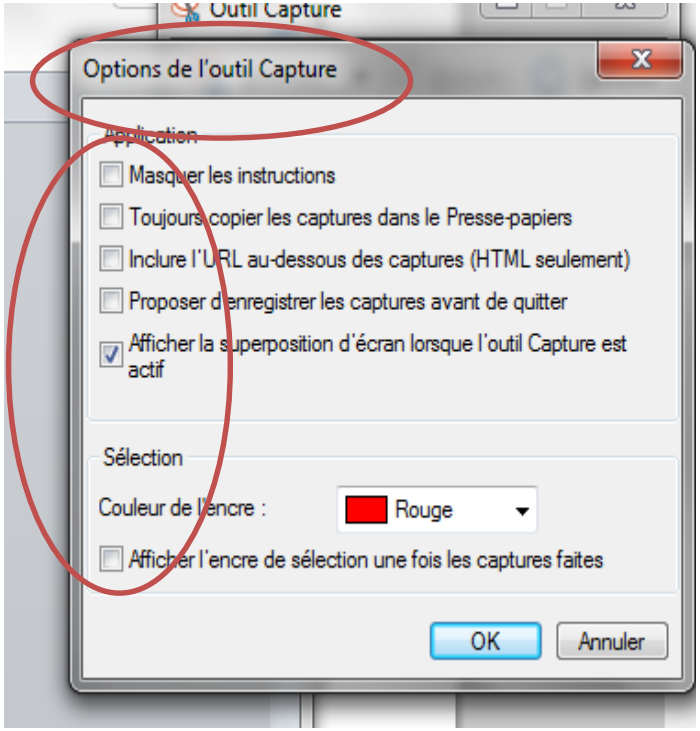

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Construire un projet, le mettre en forme et convaincre

1/ Sélection et exploitation rationnelle de documents : méthodes

2/ Les questions à vous poser avant de vous lancer dans un projet collaboratif innovant

3/ Exemple de projet : appel d'offres ANR

4/ Mise en situation : projet d'études

- Synthèse thématique (10-15p) + présentation orale
- Idée innovante :
  - Lettre d'intention (3p)
  - **Présentation Poster devant jury**

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Communiquer par affichage de poster

Permet de communiquer un message MAIS

- attention que le poster ne desserve pas les auteurs ou le projet

- ne pas divulguer des informations liées par le secret (accord de confidentialité…) ou susceptibles d'être brevetées
(risque d'auto-antériorité bloquant un dépôt de brevet)

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Communiquer par affichage de poster

- **Le poster doit être attractif, structuré et concis :**

le lecteur dispose

entre 30 secondes et 5 minutes par poster

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Communiquer par affichage de poster

Capturer l'attention

- ❑ **Capter le regard d'assez loin**

  ...pour donner **envie de venir voir** de plus près

- ❑ **Susciter l'intérêt scientifique**
- ❑ Être **bien composé**
- ❑ Être **agréable** à regarder
- ❑ Être **facile à lire**
- ❑ **Encourager la discussion**

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Communiquer par affichage de poster

Axer la communication sur le message

- **proscrire les détails inutiles**
  (il y a toujours trop de texte !!!) ;
- **privilégier les figures**
  aux tableaux de chiffres fastidieux;
- **utiliser des listes à puces** ;
- **se restreindre à des phrases courtes** (pas de gros « blocs » de texte) ;
- **utiliser la forme active**.

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Communiquer par affichage de poster

Valoriser le message principal

- Texte réduit **au minimum**
- **Introduction - Objectif** :
  Être clair et bref.
  Pas de revue de la littérature
- **Matériel et/ou méthodes** en bref et, si possible, illustré
- **Message : résultats ou lignes de force** de préférence sous forme d'**illustrations**)
- **Conclusion** : bien visible

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Communiquer par affichage de poster

Être lisible pour être lu

- Préférer les types de caractères **sans sérif** :

  Arial ou Helvetica

  plutôt que

  Times Roman ou Garamond ou Bookman

- Utiliser le **gras** mais éviter l'*italique* pour les mises en évidence.

- **Taille de caractère :**
  - lisible à 5m pour le titre;
  - lisible à 1-2 m pour le contenu.

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Communiquer par affichage de poster

Titre: Minuscules ou majuscules?

Faites l'expérience, lisez:

Communiquer les résultats de ses travaux de recherche	☺
COMMUNIQUER LES RESULTATS DE SES TRAVAUX DE RECHERCHE	☹

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Communiquer par affichage de poster

Dernières recommandations

- Les « plages » **blanches** sont importantes. Idéal :
  - 30 % de texte
  - 40 % d'illustrations
  - **30 % de vide**
- **Deux extrêmes à éviter** :
  - la **belle affiche** où l'information manque
  - l'information abondante mais **illisible** !

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Communiquer par affichage de poster

S'identifier

- **Où?**

  En bas du poster ou sous le titre

- **Qu'indiquer?**

  - **Noms et Prénoms** des auteurs ainsi que les coordonnées du laboratoire du(des) auteur(s) (attention à l'utilisation des sigles ou abréviations).
  - Le **logo** de l'université, de l'institut, du laboratoire.
  - Petits « plus »:
    - la **photo** de l'auteur qui présente le poster (pour pouvoir l'identifier facilement);
    - les adresses **mail** des auteurs (faciles à noter et pratiques pour communiquer)

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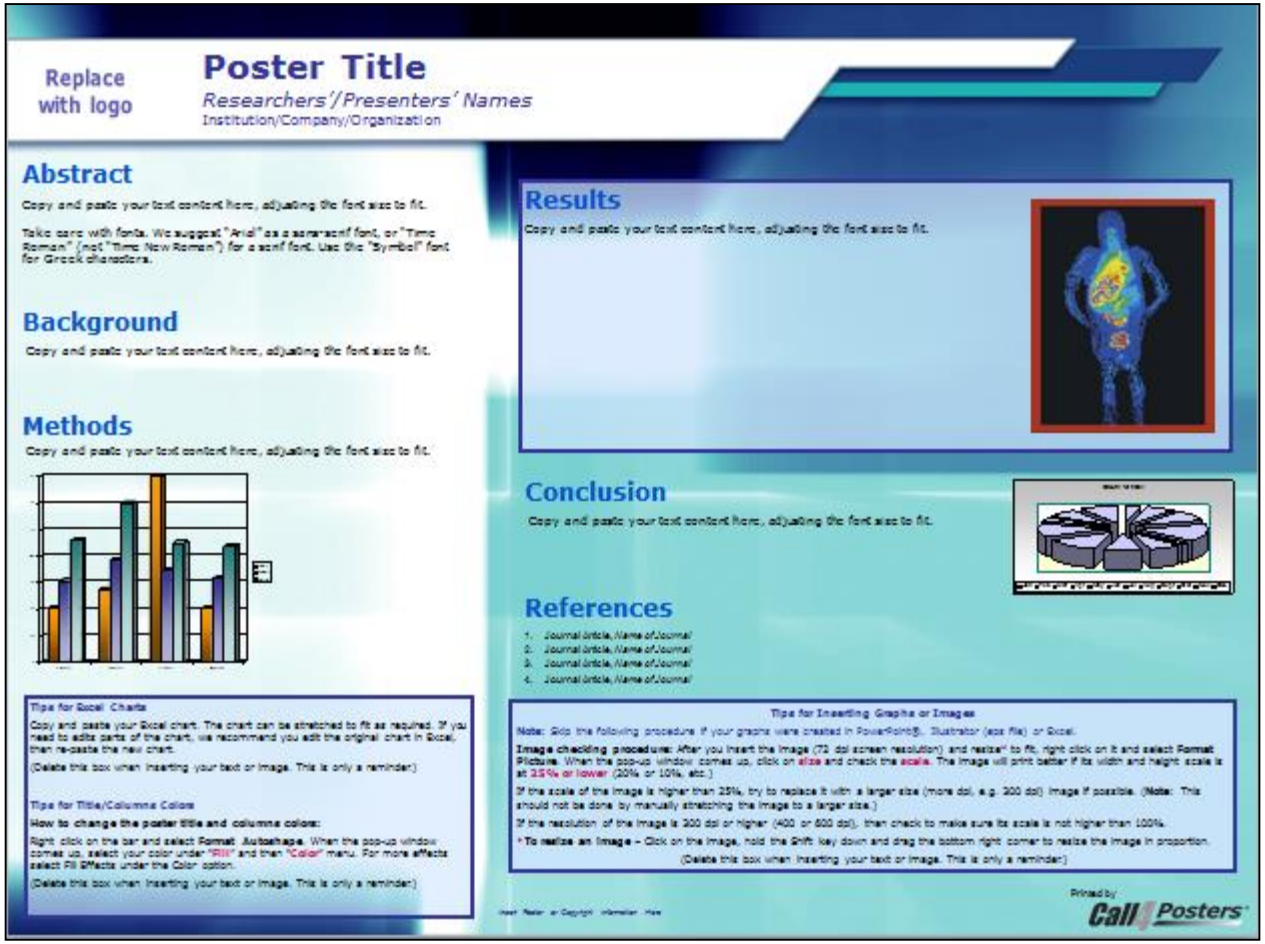
Monter un poster

Ex 2 colonnes

Ex« Modèles posters horizontaux »



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Monter un poster
Ex 4 colonnes

Ex « Modèles posters horizontaux »

Replace with logo

Poster Title

Researchers'/Presenters' Names
Institution/Company/Organization

Replace with logo

Abstract

Copy and paste your text content here, adjusting the font size to fit.

Take care with fonts.
We suggest "Arial" as a sans-serif font, or "Time Roman" (not "Time New Roman") for a serif font.
Use the "Symbol" font for Greek characters.

Background

Copy and paste your text content here, adjusting the font size to fit.

Methods

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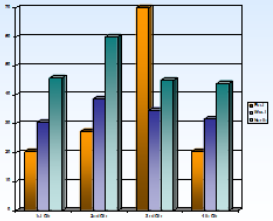

Results

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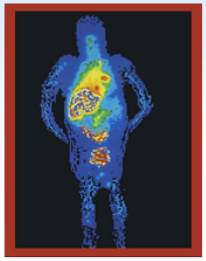

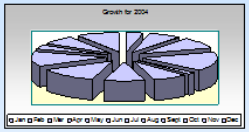

Conclusion

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References

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Note: Skip the following procedure if your graphs were created in PowerPoint®, Illustrator (eps file) or Excel.

Image checking procedure: After you insert the image (72 dpi screen resolution) and resize* to fit, right click on it and select **Format Picture**. When the pop-up window comes up, click on **size** and check the **scale**. The image will print better if its width and height scale is at **25% or lower** (20% or 10%, etc.)

If the scale of the image is higher than 25%, try to replace it with a larger size (more dpi, e.g. 300 dpi) image if possible. (**Note**: This should not be done by manually stretching the image to a larger size.)

If the resolution of the image is 300 dpi or higher (400 or 600 dpi), then check to make sure its scale is not higher than 100%.

***To resize an image** – Click on the image, hold the Shift key down and drag the bottom right corner to resize the image in proportion.

(Delete this box when inserting your text or image. This is only a reminder.)

Tips for Title/Columns Colors

How to change the poster title and columns colors:

Right click on the bar and select **Format Autoshape**. When the pop-up window comes up, select your color under "**Fill**" and then "**Color**" menu. For more effects select Fill Effects under the Color option.

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Copy and paste your Excel chart. The chart can be stretched to fit as required. If you need to edits parts of the chart, we recommend you edit the original chart in Excel, then re-paste the new chart.

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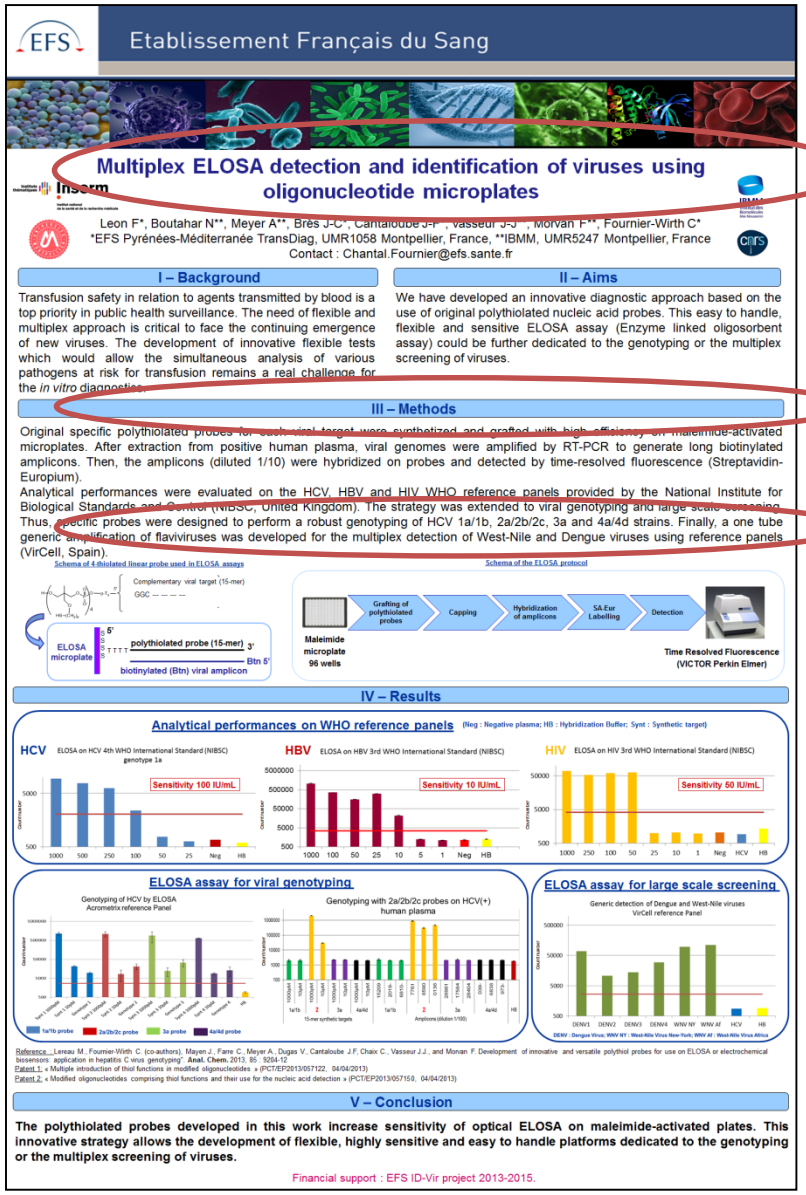
Monter un poster

Ex Powerpoint



Arial 60

Arial 40

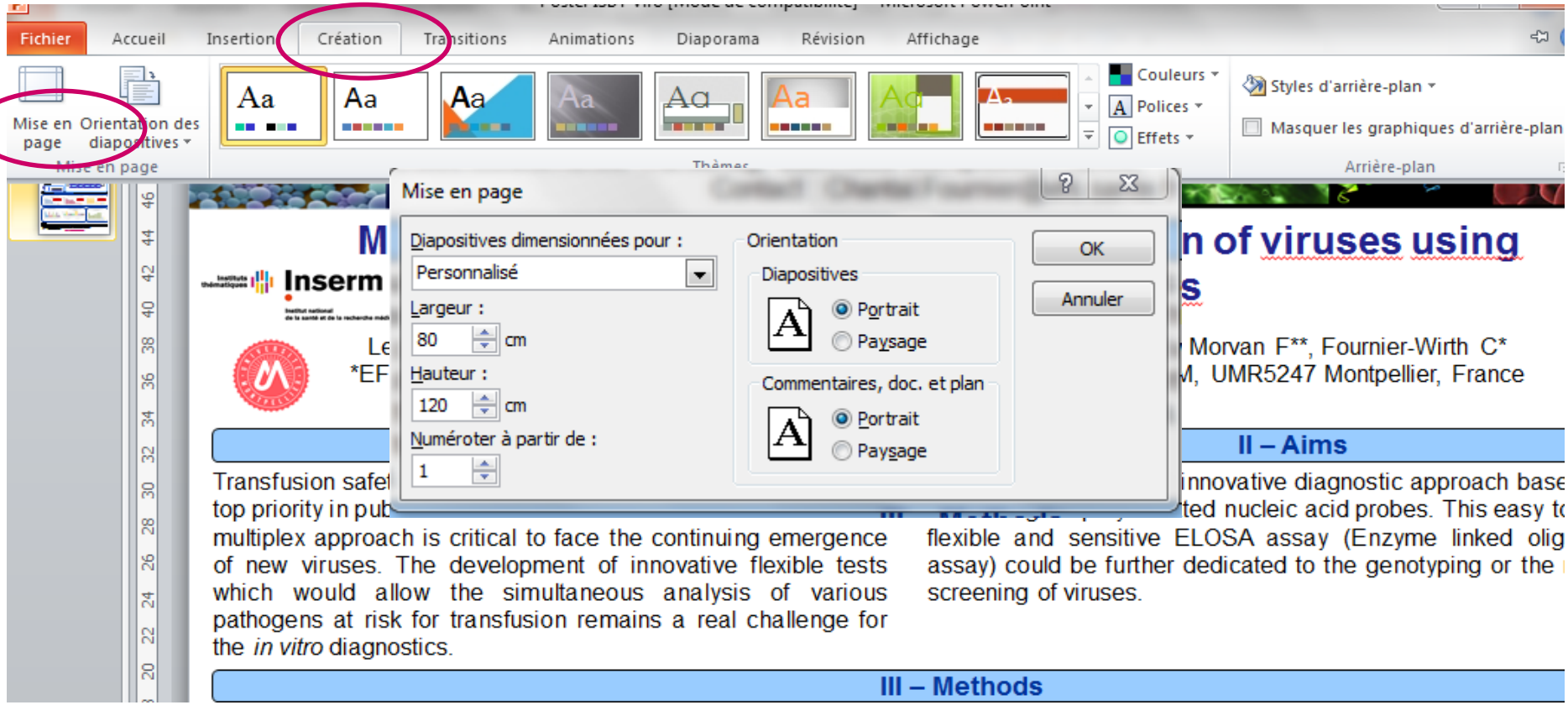
Arial 36

Background
Aims
Methods
Results
Conclusion

chantal.fournier@efs.sante.fr

Monter un poster

Powerpoint



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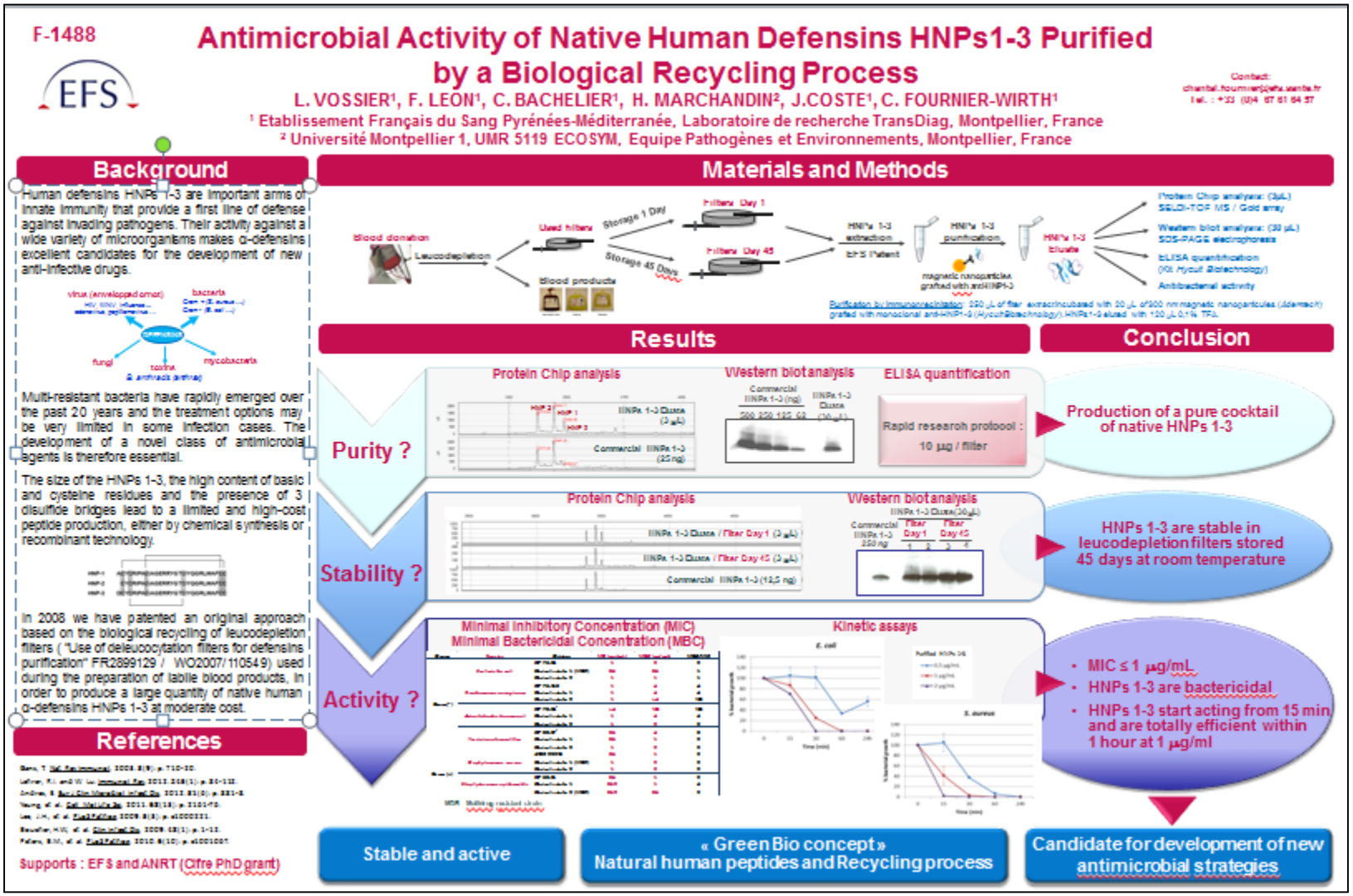
Monter un poster

Ex Powerpoint



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C’est parti ! 5 dates +++

IMPORTANT / PLANNING

Jeudi 14 décembre 2017 avant 17h : Rendu du titre du sujet choisi pour la soutenance de poster et de la lettre pour appel d’offres

Par mail à :
chantal.fournier@efs.sante.f
sandrine.bayle@mines-ales.fr
catherine.remy@chu-nimes.fr
christophe.hirtz@umontpellier.fr

Mercredi 17 janvier 2018 avant 17h : Rendu du Rapport de synthèse (10-15 pages)

ATTENTION : aucun envoi reçu après 17h ne sera accepté

Par mail à :
chantal.fournier@efs.sante.f
sandrine.bayle@mines-ales.fr
catherine.remy@chu-nimes.fr
christophe.hirtz@umontpellier.fr

Jeudi 18 janvier 2018 avant 17h : Rendu de la Lettre d’intention pour appels d’offres (3 pages)

ATTENTION : aucun envoi après 17h ne sera accepté

Par mail à :
chantal.fournier@efs.sante.f
sandrine.bayle@mines-ales.fr
catherine.remy@chu-nimes.fr
christophe.hirtz@umontpellier.fr

Lundi 22 janvier 2018 : Présentation orale Poster (9h-13h Site des Carmes)
Jury : C Fournier-Wirth, S. Bayle, C. Remy, C Hirtz

Mardi 23 janvier 2018 : Présentation orale Projet (9h-13h et 14h-17h Site des Carmes)
15 min / personne + 30 min questions/sujet
Jury : C Fournier-Wirth, S. Bayle, C. Remy, C Hirtz