



Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells

Aurélie Charazac, Célia Decondé Le Butor, Kévin Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, Jérôme Gilleron, Patrick Fenichel, Xavier Descombes, Frédéric Bost, Clavel Stéphan, et al.

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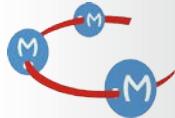
HAL Id: hal-01548783

<https://hal.science/hal-01548783>

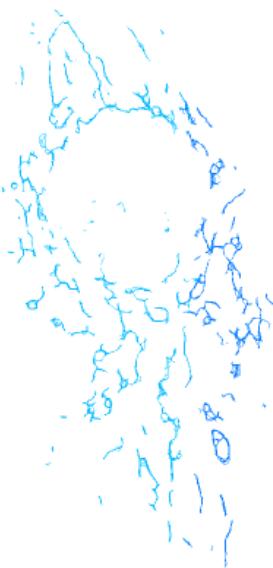
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Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.



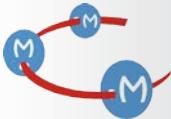
Charazac Aurélie, Decondé le Butor Célia, Giulietti Kévin, Lobaccaro Jean Marc, Baron Silvère, Gilleron Jérôme, Fénichel Patrick, Descombes Xavier, Bost Frédéric, Clavel Stéphan & Chevalier Nicolas.



AAP

Cancer et Environnement

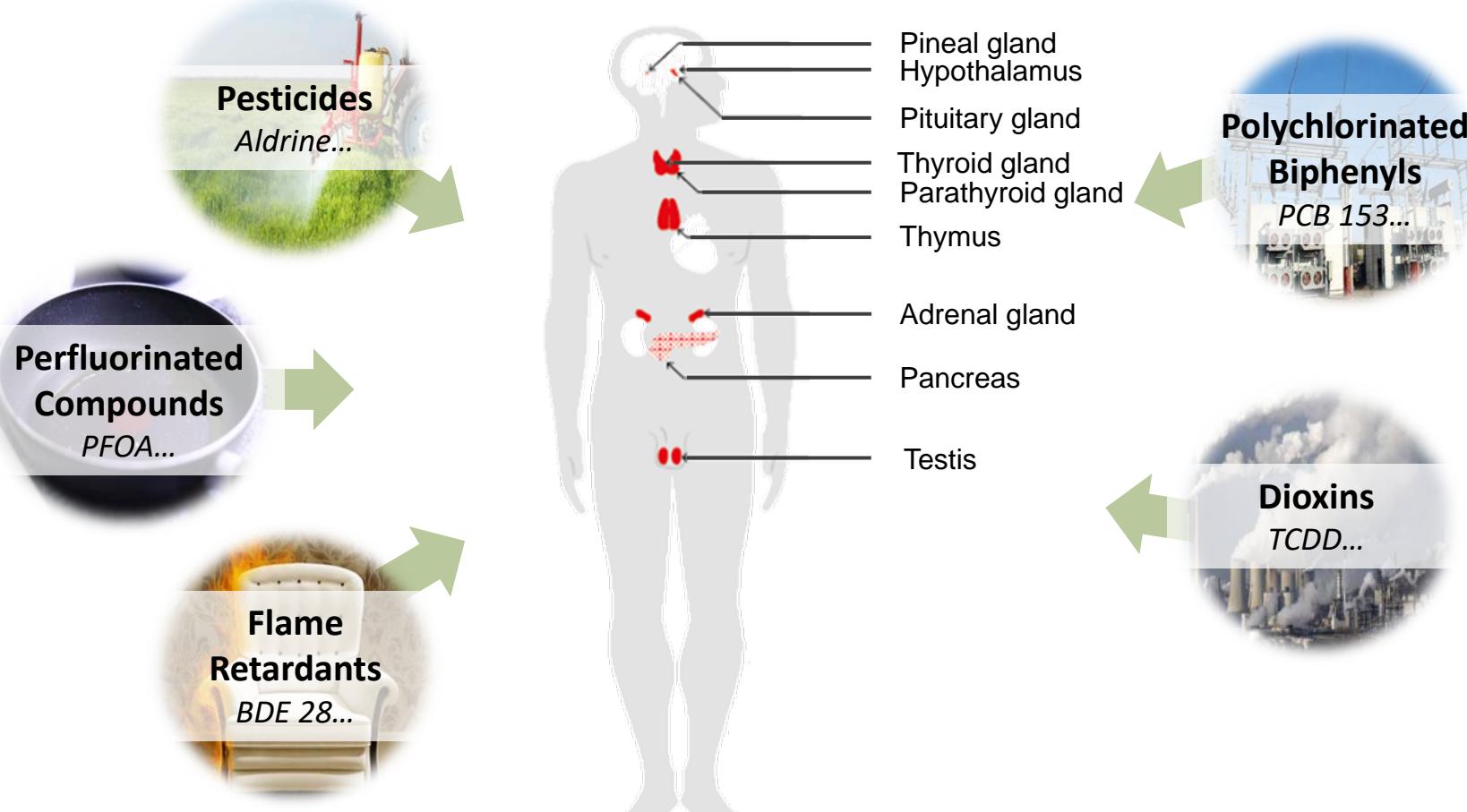


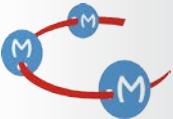


Endocrine Disruptor Compounds

« An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. »

World Health Organization





Major challenge

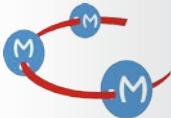
	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
Acceptation of WHO definition	✓	—	✗	✓
Based on risk assessment	✗	✗	✗	✗
List updating	✗	—	✓	✓

Adapted from EndocrineScience.org



Registration, Evaluation and Autorization of CHemicals

↳ Promotes alternative methods for the hazard assessment of substances.



Major challenge

	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
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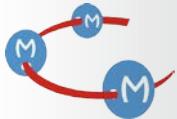


Registration, Evaluation and Authorization of CHemicals

↳ Promotes alternative methods for the hazard assessment of substances.

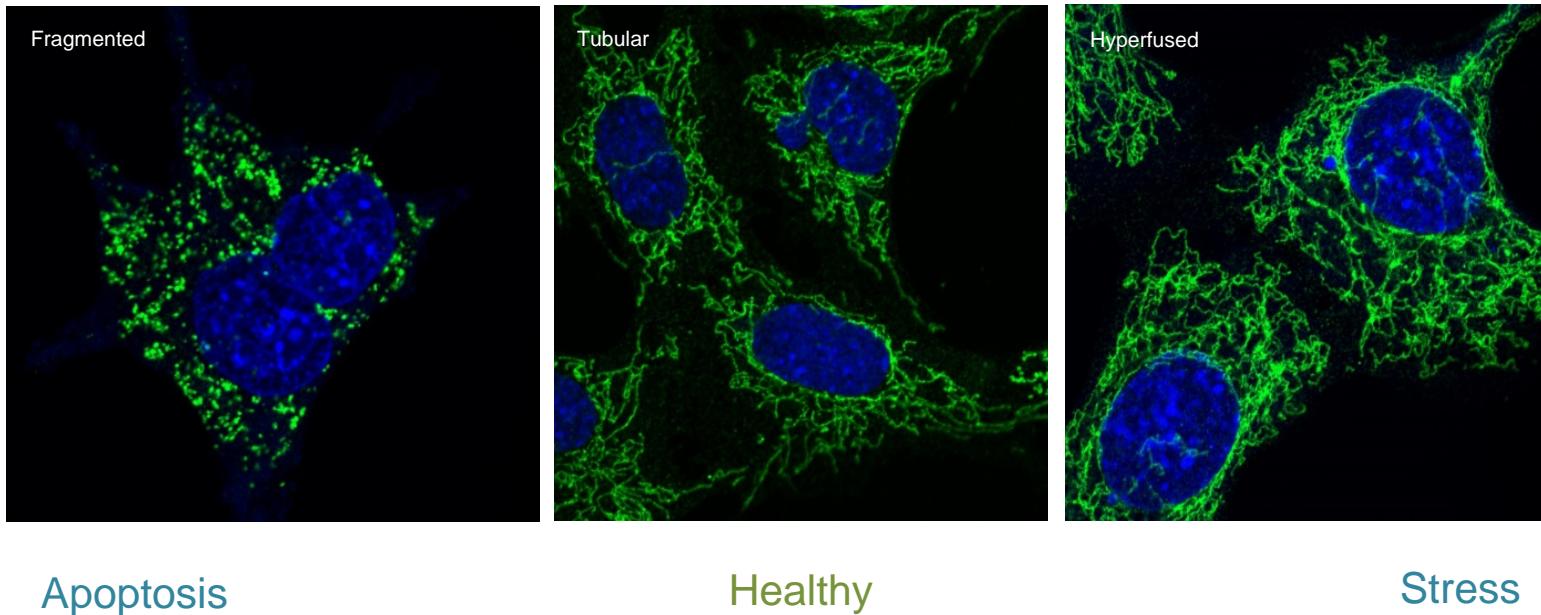
Urgent need for multiparametric, robust and high throughput cell-based assay :

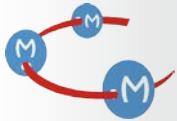
- To investigate the mechanisms underlying the adverse effects of known EDCs
- To identify new compounds with endocrine-disrupting potential



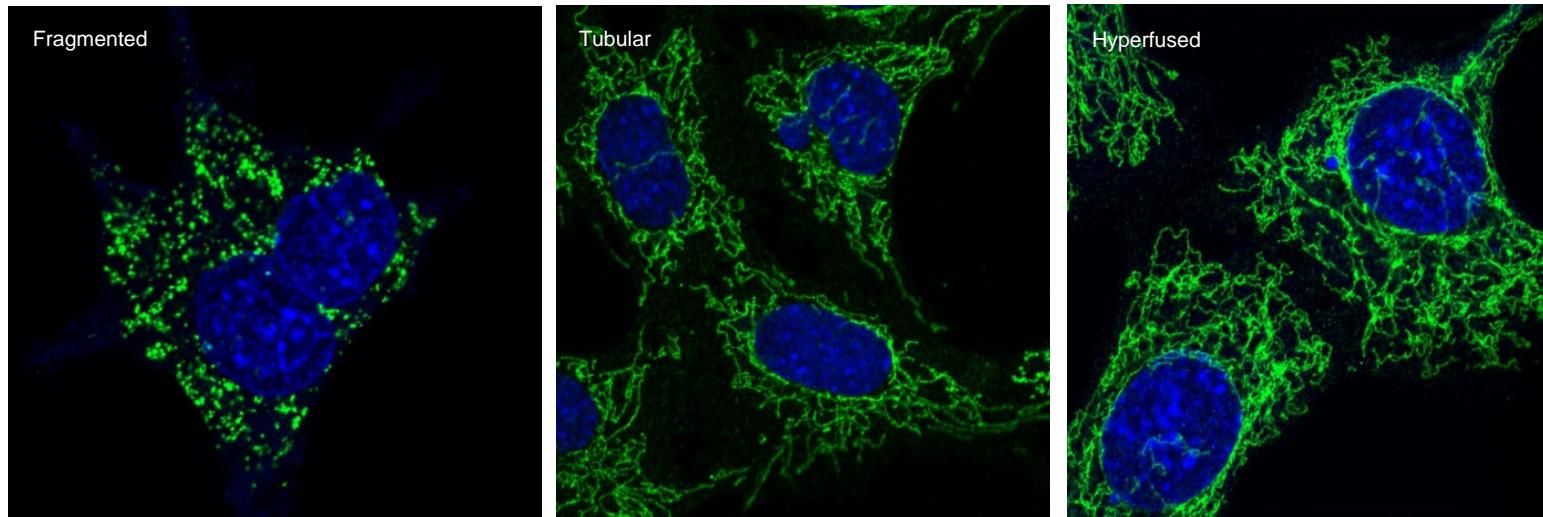
Introduction ~ Aim

Mitochondria, a cell sensor





Mitochondria, a cell sensor



Apoptosis

Healthy

Stress

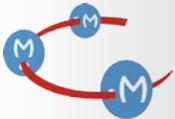
Calcium signalling

Cell growth and differentiation

Cell cycle control

Cell death

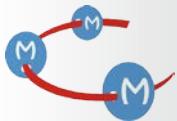




Mitochondria, a cell sensor

Alteration of mitochondrial forms and functions by EDCs ?

- 1) Quantitative image based analysis of mitochondrial functions
 - High throughput screening
- 2) Computational image based analysis of mitochondrial morphology
 - Image analysis and classification



Multiparametric cell-based assay

Automated image acquisition (Nikon A1R-20X)

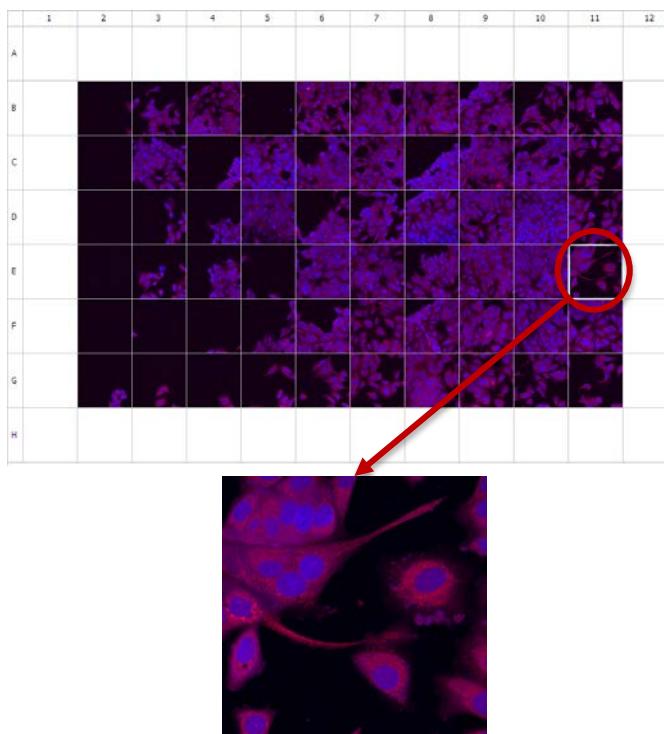
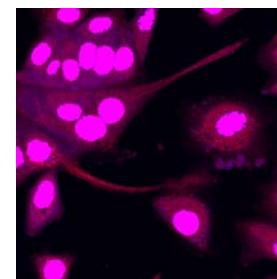
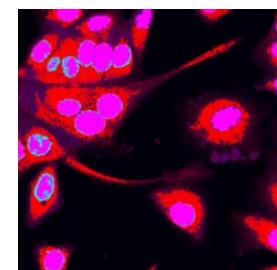


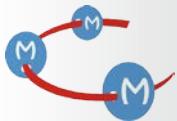
Image processing



→ 1) Dapi masking :
cell counting



→ 2) Dye masking :
fluorescence intensity



Multiparametric cell-based assay

Automated image acquisition

(Nikon A1R-20X)

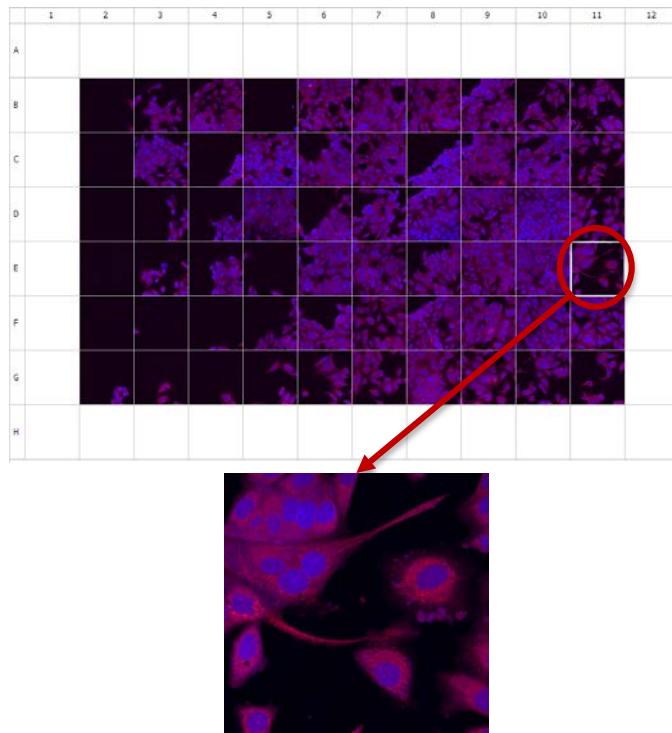
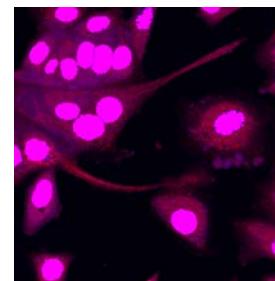
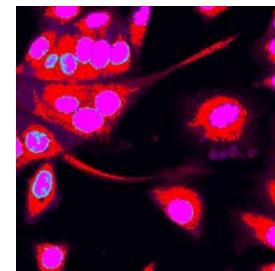


Image processing

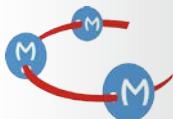


→ 1) Dapi masking :
cell counting



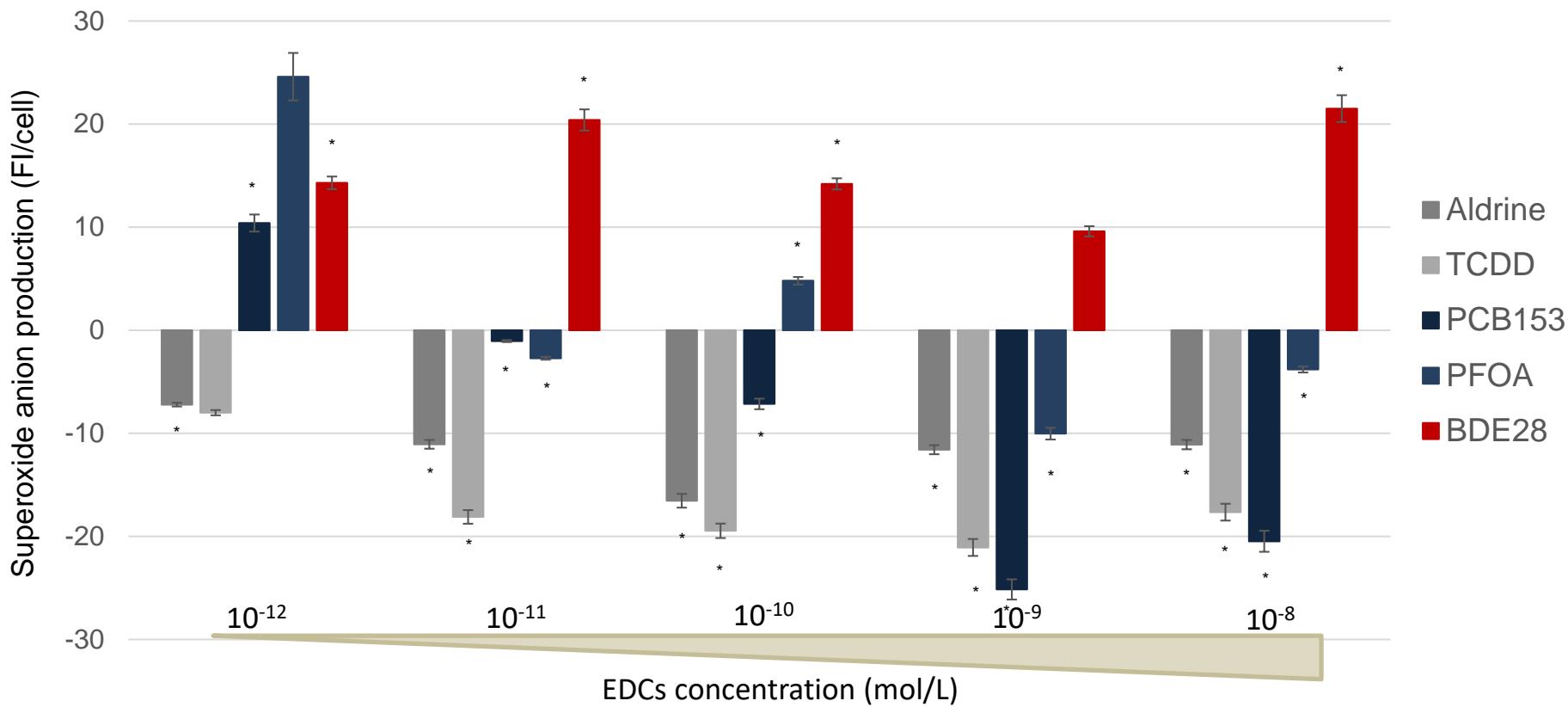
→ 2) Dye masking :
fluorescence intensity

- Multiparameter :
 - mitochondrial membrane potential (MitoTracker Red™)
 - superoxide anion production (MitoSox™)
 - mitophagy (Keima probe)

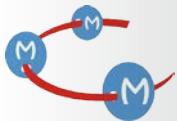


EDCs affect the mitochondrial function

- ROS production on DU145:

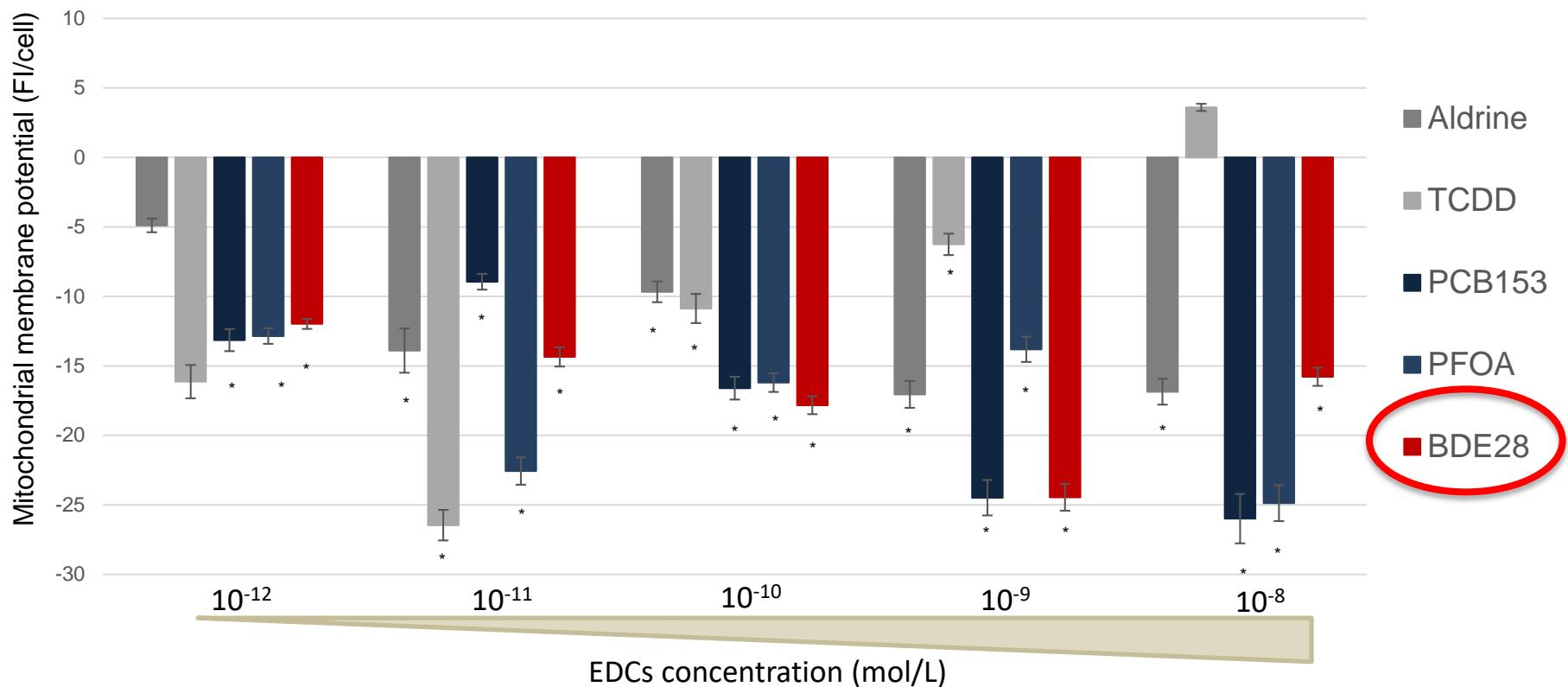


* significantly different from the control, $p \leq 0.005$.

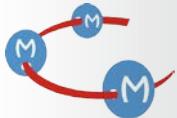


EDCs affect the mitochondrial function

- Mitochondrial membrane potential on DU145 :

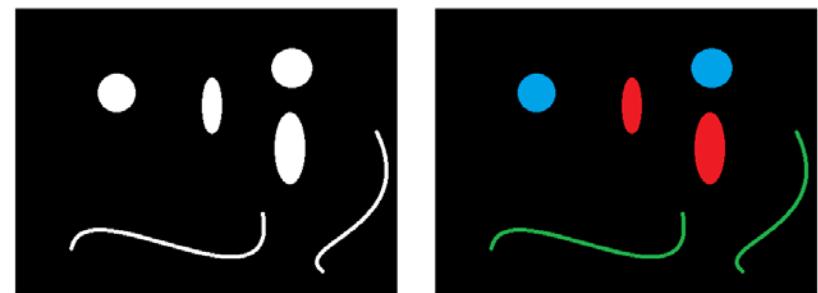
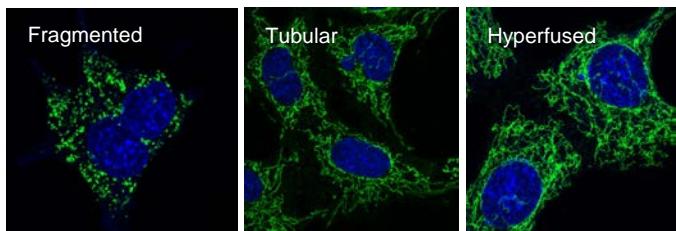


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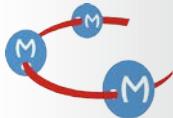


Forms classification of mitochondrial network

- Collaboration with

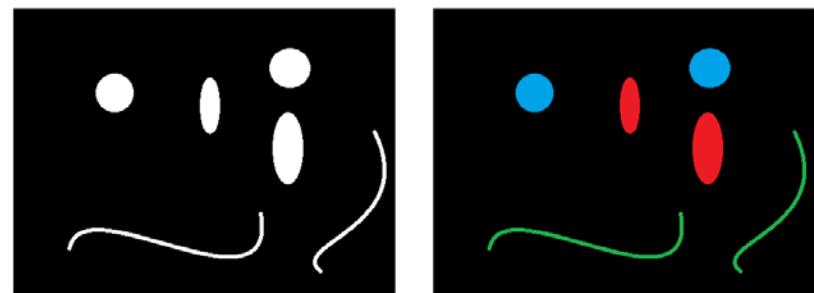
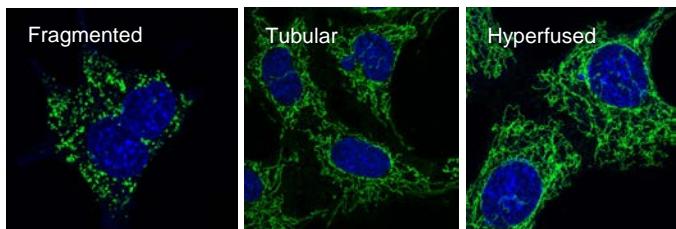


Fragmented, Tubular, Hyperfused



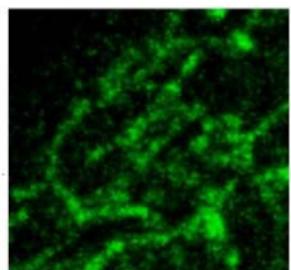
Forms classification of mitochondrial network

- Collaboration with

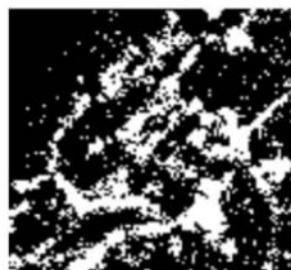


Fragmented, Tubular, Hyperfused

Image

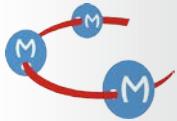


Basic
thresholding
method



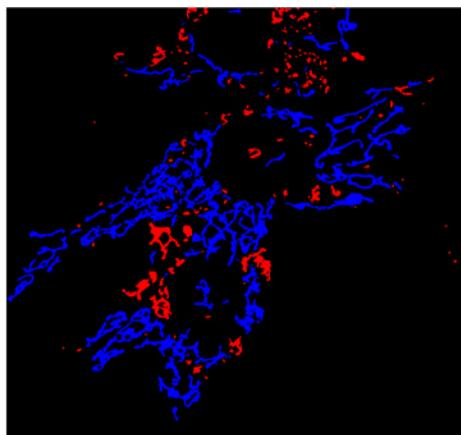
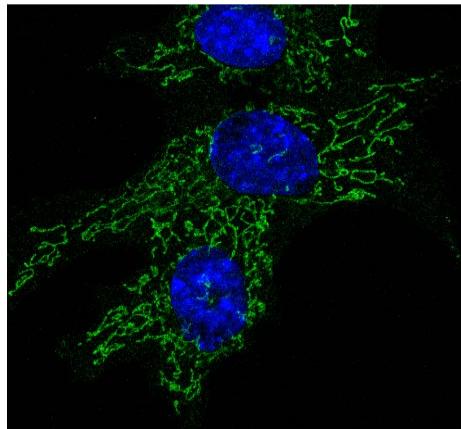
Developed
method





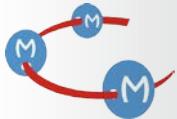
Exemple of developed method

Tubular



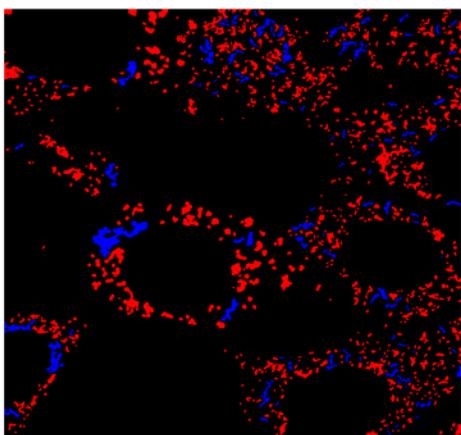
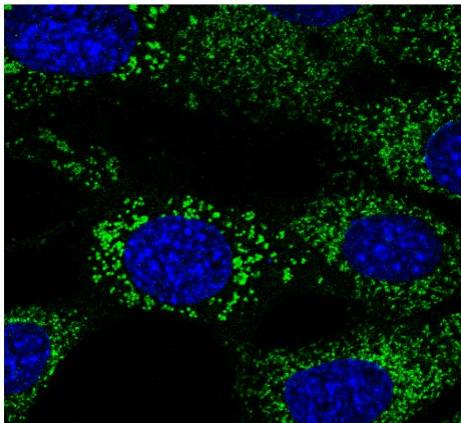
Aggregates Tubular Hyperfilamentous

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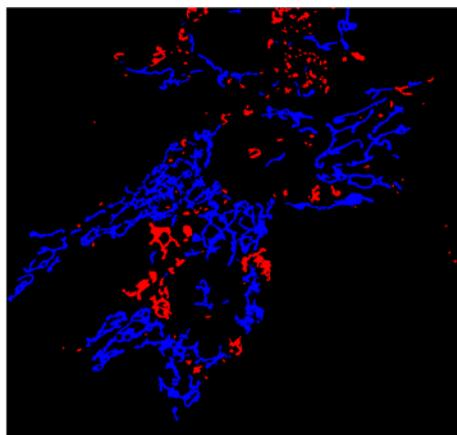
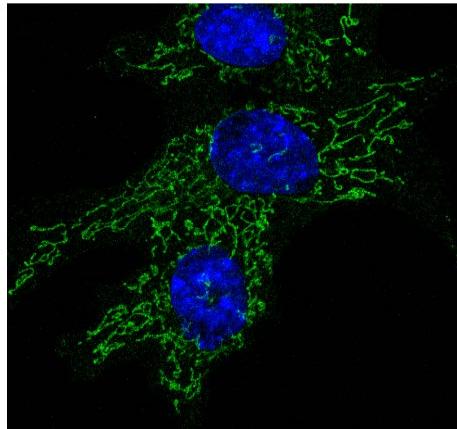


Exemple of developed method

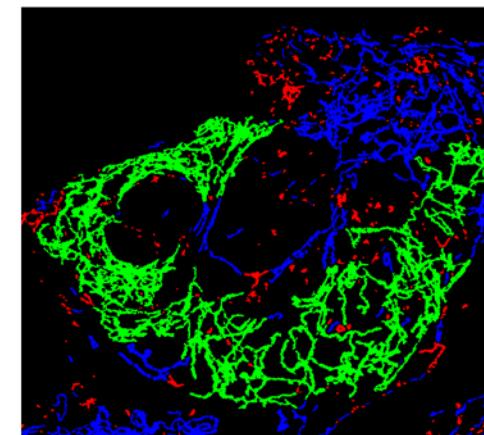
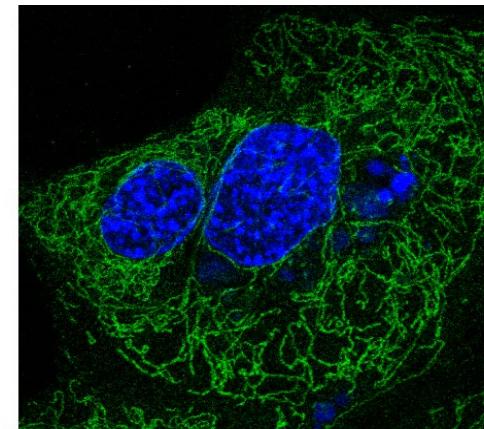
Fragmented
(Rotenone, 100nM)



Tubular

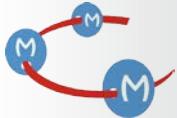


Fused
(Compound C, 20µM)



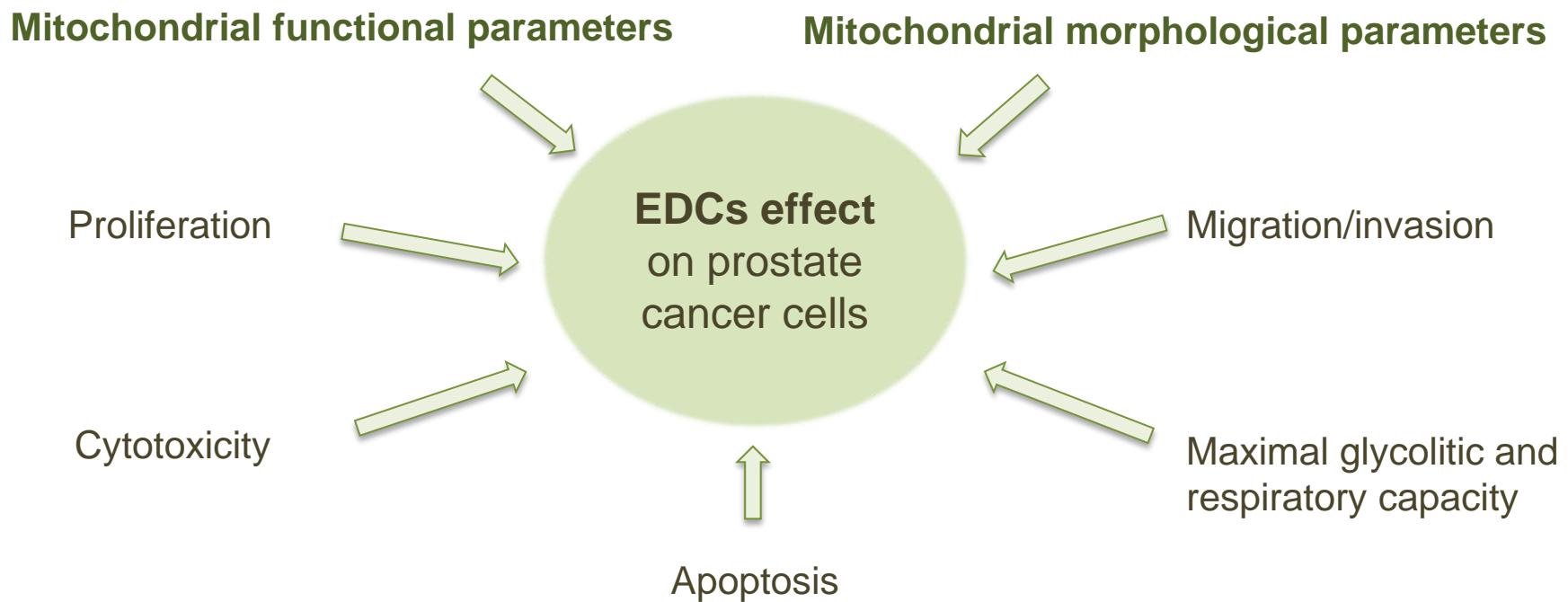
Aggregates Tubular Hyperfilamentous

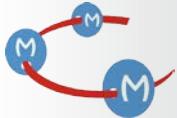
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Final Goal

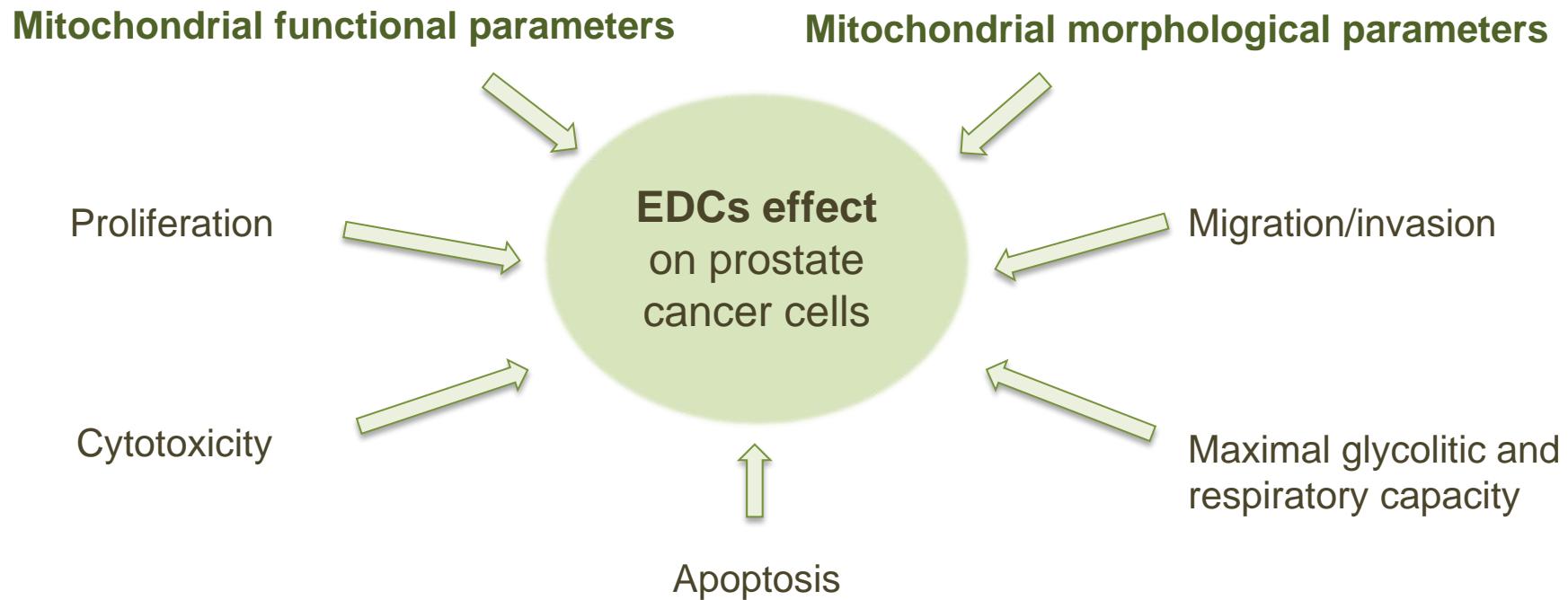
- Development of a multiparametric high throughput cell based screening :



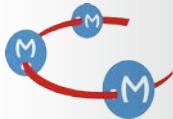


Final Goal

- Development of a multiparametric high throughput cell based screening :



- Signature of EDCs based on their effects on cell metabolism
- New perspective in identification and characterization of EDCs



Thanks...

Team 7

Frédéric Bost
Jean-François Tanti
Mireille Cormont
Stephan Clavel
Célia Deconde Le Butor
Lisa Kaminski
Kathiane Laurent
Nicolas Chevallier
Jérôme Gilleron
Jade Evrard
Bastien Vergoni
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Karine Dumas
Marine Bourcier
Mounia Tannour-Louet
Calypso Vacher-Chicane
Sophie Giorgetti-Péraldi
Sophia Fazio
Jean-François Louet
Stéphanie Torrino
Faustine Pastor



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alliance nationale
pour les sciences de la vie et de la santé

ITMO Cancer

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Écologique
Femelle
Anomalies
Spermie
Rameau
Travail
Testicule
Sécurité
Études
Oeuf
Pollution
Dose
Dioxines
Santé
Tests
Risque
Homme
Exposition
Substances
Endocrinien
Perturbateurs
Chimique
Faune
Produit
Poissons
Maladie
Maternal
Environnement
Problème
Périsant
Question

Caract.
Potentiel
Utero
Égoutage
Désordres
UV
Réputation
Progrès
Vénérables
Précipitation
Signification
Gestrophobie
Enfant
Organes
Régénération
Syndrome
Nigritude
Identification
Trifluorométhane
Hormonal
Cocktail
Cancer
Aliments
Évaluation
Inventaire
Possibilité
Surveillance
Lait
Vérité
Droits
Reproducteur
Reproduction
Droits
Retardateur
Reproduction
Problème
Environnement
Santé
Enfants
Mâle
Sécurité
Études
Oeuf
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Santé
Tests
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Question

Mer
Origine
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Jeunes
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