

# Mitochondrial dysfunction as intermediate endpoint to predict toxicity

Jochem Louisse, Peter Macko, Milena Mennecozzi, Jaroslav Novak, Francesca Pistollato, Susanne Bremer

## Introduction

- Mitochondria have pivotal roles in energy metabolism oxidative stress and apoptosis (Figure 1).
- Mitochondrial dysfunction is a 'key event' in several disorders, such as neuronal diseases and liver and cardiac dysfunction (Rosca and Hoppel, 2012).
- Mitochondria are important targets for toxicants (Nadanaciva and Will, 2011).
- Doxorubicin (dox) was chosen by the SEURAT-1 gold compounds working group as a model compound known to induce delayed cardiotoxicity in humans.
- Mitochondrial dysfunction has been identified in dox-exposed cardiomyocytes by partners in the DETECTIVE consortium, using -omics techniques.

## Aim

Select endpoints for mitochondrial dysfunction as predictive biomarkers for (delayed) cardiotoxicity.

## Technical objectives

- Set up protocols for measuring endpoints related to mitochondrial dysfunction using High Content Cell Imaging and Analysis technologies.
- Perform quality analyses of the used technologies and identify confounding parameters impacting data interpretation.

## Scientific objectives

- Compare sensitivity of cardiac cells to dox-induced mitochondrial and cellular dysfunction with sensitivity of other cell types, such as neuronal and liver cells.
- Relate chemical-induced mitochondrial dysfunction to effects on the cellular level in order to select predictive biomarkers for (delayed) cardiotoxicity.

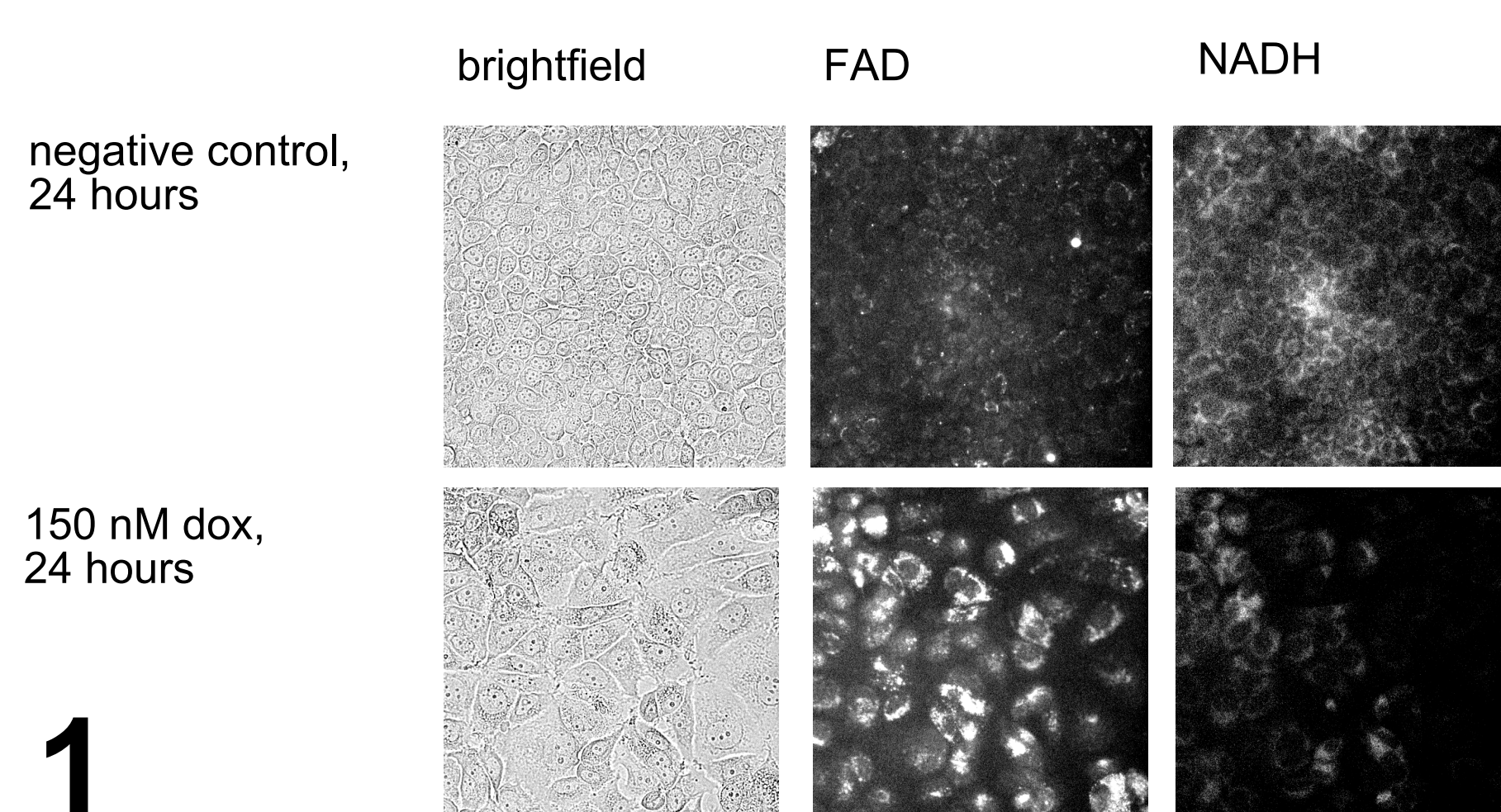
## Plans and perspectives

- Dox-induced mitochondrial and cellular dysfunction will be assessed in cardiac, liver and neuronal cells.
- The endpoints of mitochondrial dysfunction may be used to predict (delayed) cardiotoxicity induced by dox and other chemicals, and may serve as predictive in vitro biomarkers that can be applied in high throughput screening platforms.

## Measuring endpoints of mitochondrial dysfunction in doxorubicin-exposed MCF-7 cells

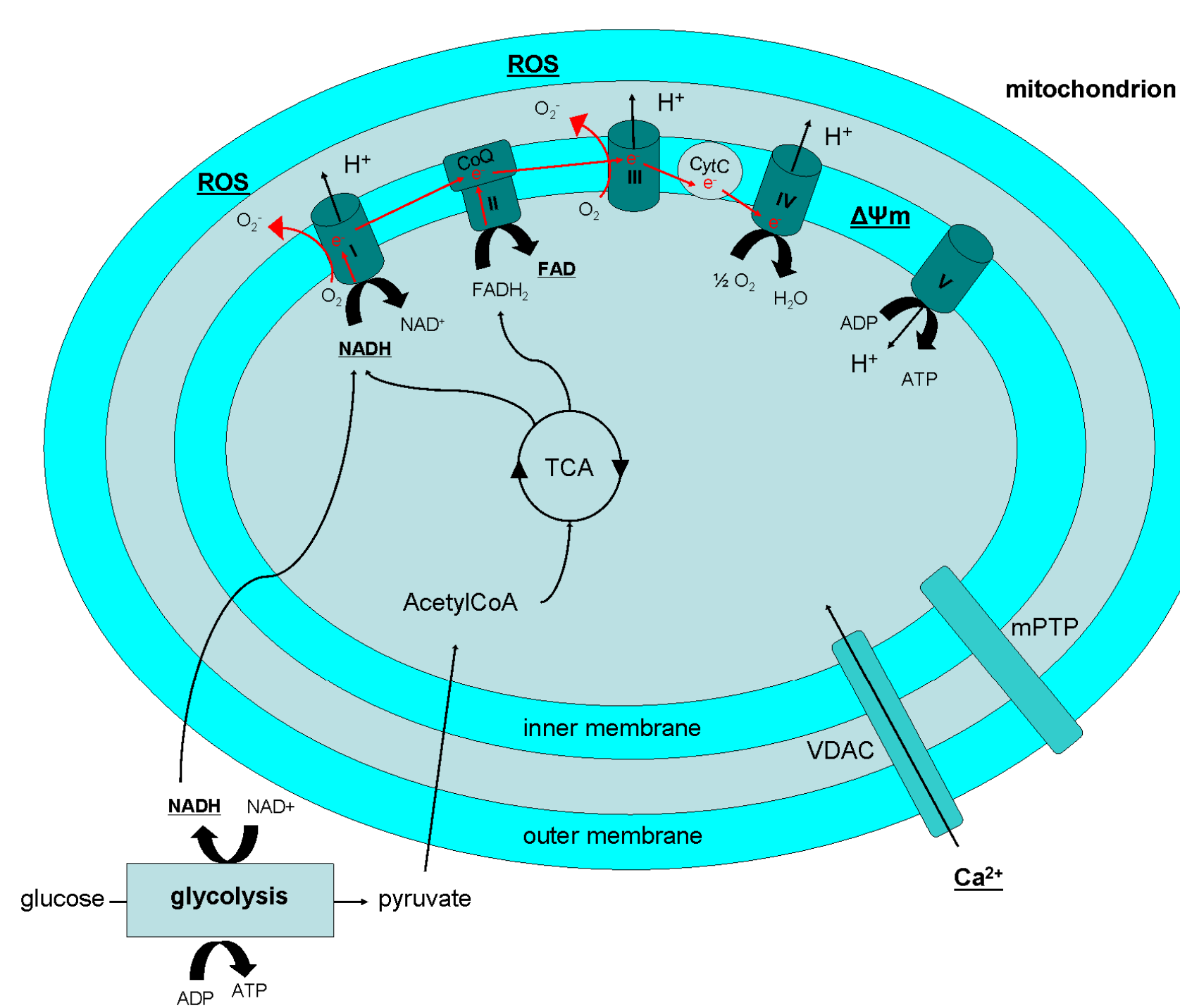
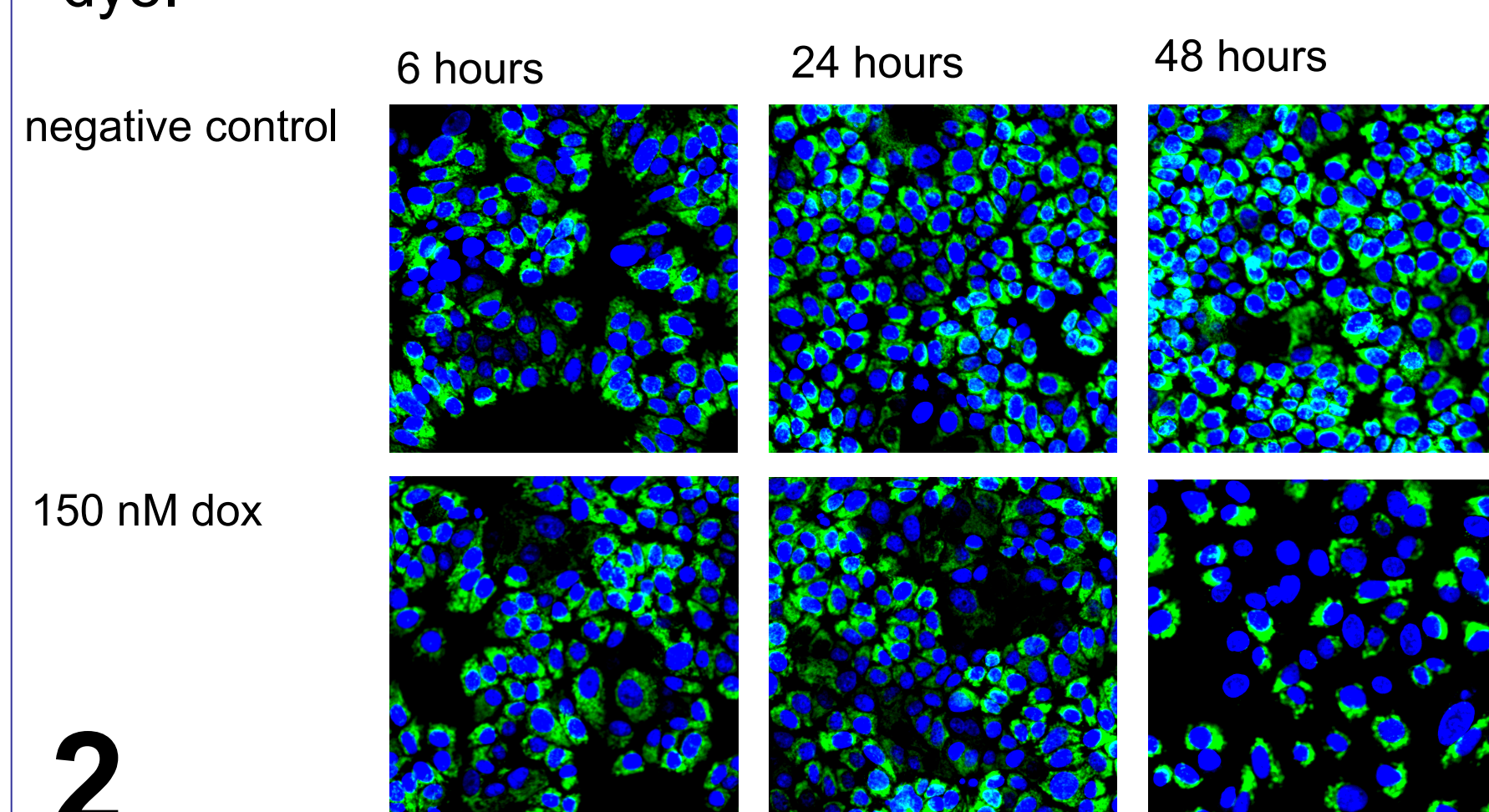
### Redox state

The cell's redox state can be defined as FAD/(NADH+FAD). In active mitochondria, NADH levels are relatively high and FAD levels relatively low, resulting in a low redox state, whereas this is the opposite in inactive mitochondria. FAD and NADH levels were determined using their autofluorescent characteristics.



### Mitochondrial membrane potential ( $\Delta\Psi_m$ )

The net accumulation of  $H^+$  outside the membrane generates the  $\Delta\Psi_m$ . During cellular stress, the  $\Delta\Psi_m$  may be altered and thereby change ATP production.  $\Delta\Psi_m$  was determined using the TMRE dye.

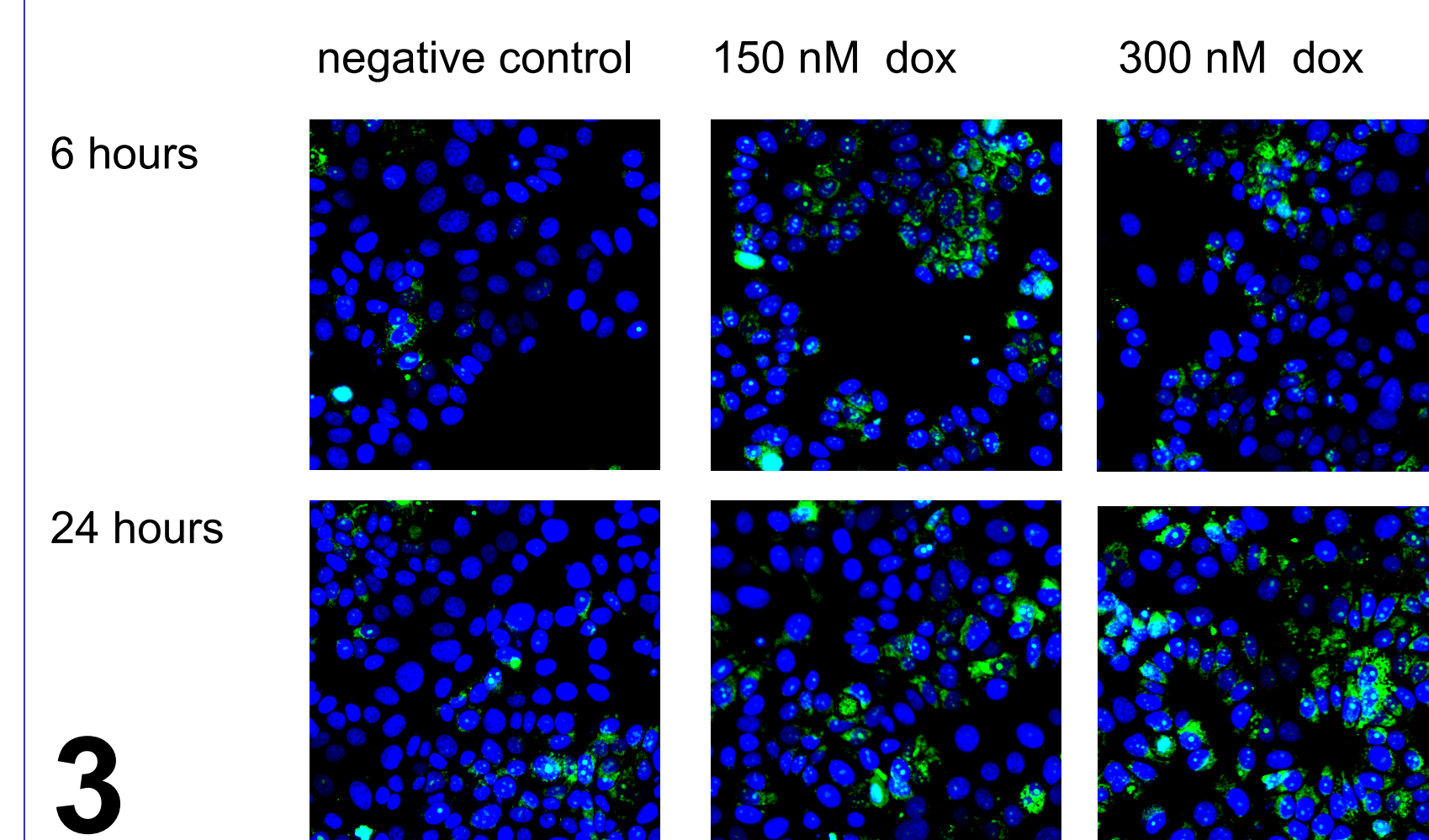


**Figure 1: Mitochondrial role in energy metabolism, oxidative stress and apoptosis.** In glycolysis, two ATP, two  $NAD^+$  and two pyruvate molecules are generated for each glucose molecule consumed. The pyruvate molecules are transported to the tricarboxylic acid cycle (TCA) in the mitochondrial matrix.  $FADH_2$  and  $NADH$  (box 1) are electron donors in the electron transport chain (ETC), in which they are oxidized to FAD (box 1) and  $NAD^+$ , respectively. A proton gradient across the inner mitochondrial membrane is generated (mitochondrial membrane potential ( $\Delta\Psi_m$ ; box 2)) that activates the ATP synthase (complex V), which provide the majority of cellular ATP. Free electrons in the ETC (especially from complex I and III) can generate ROS (box 3), which may cause oxidative stress and apoptosis. Mitochondrial  $Ca^{2+}$  (box 4) stimulates and controls the rate of oxidative phosphorylation, and plays a role in mitochondrial permeability transition, apoptotic cell death and the modification of cytosolic  $Ca^{2+}$  pulses or transients. The release of CytC from the inner mitochondrial membrane triggers apoptosis.

Modified from Heikal (2010). CoQ: Coenzyme Q; CytC: Cytochrome C; mPTP: Mitochondrial permeability transition pore; VDAC: Voltage-dependent anion channel.

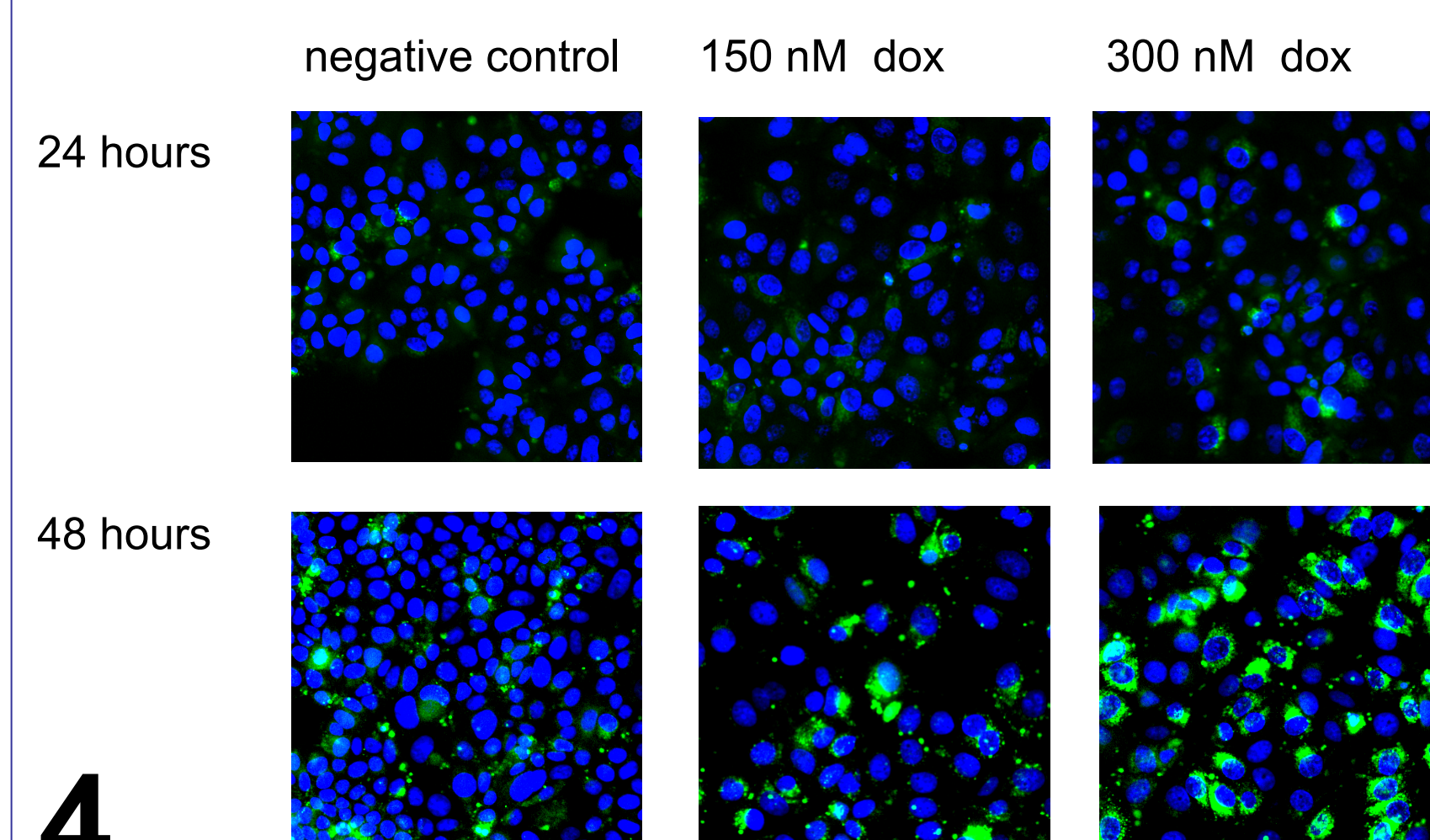
### ROS levels

ROS cause oxidative stress and the loss of  $\Delta\Psi_m$  in the absence of antioxidant defence mechanisms. ROS levels were determined using the DHE dye.



### $Ca^{2+}$ levels:

Mitochondrial dysfunction may change mitochondrial and cytosolic  $Ca^{2+}$  levels.  $Ca^{2+}$  levels were determined using the Fluo-4 dye.



## References

- Heikal (2010). Biomarkers in medicine **4**, 241-263.  
Nadanaciva and Will (2011). Current pharmaceutical design **17**, 2100- 2112.  
Rosca and Hoppel (2012). Heart failure reviews.

## Contact

Jochem Louisse  
European Commission • Joint Research Centre  
Institute for Health and Consumer Protection, Systems Toxicology  
Tel. +39 (0)332785993 • Email: jochem.louisse@ec.europa.eu