# Transfection of HeLa cells with a mitochondrial targeted probe

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**Introduction:**

Since mitochondria actively participate in cellular Ca2+ homeostasis both in physiological and in pathological conditions, our lab started a project aimed at studying calcium homeostasis in these organels.
In R.Tsien' laboratory a new family of Cameleons (GFP based calcium probes) was developed, and we thought these proteins could be a powerful tool allowing calcium measurement.
So we decided to use the mitochondrial targeted isoforms to study mitochondrial calcium homeostasis in HeLa cells.

**Materials:**

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). 2mt D2cpv in pcDNA3 was kindly provided by professor R.Tsien.

**Methods:**

The human cervical epithelial cancer cell line HeLa was cultured in Dulbecco's modified Eagle's MEM (DMEM) (Sigma) supplemented with 10% FCS (Sigma,), penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine (4 mM).

**Transfection protocol**

For transfection, HeLa were seeded in 2 ml of DMEM in 6-well culture plates (on glass coverslips, diameter 24 mm) one day before transfection, and used at approximately 70% confluence.
Cells were covered with 1 ml of the same medium.
Metafectene PRO was complexed with the 2mtD2cpv in pcDNA3 plasmid at reagent: DNA ratios of 3 µl:1 µg or 3 µl:1,5 µg DNA. Complexes were prepared by mixing Metafectene PRO with serum-free DMEM medium and by addition of DNA.

Since the transfected cDNA codes for a fluorescent protein, the efficiency of transfection was detected through a fluorescence microscope.
In fact cells expressing the fluorescent probes were analyzed using an inverted fluorescence microscope (Zeiss Axioplan) with an immersion oil objective (63 X, N.A. 1.40). Excitation light at the appropriate wavelength was produced by a monochromator (Polychrome II, TILL Photonics, Martinsried, Germany).
In parallel, 2mtD2cpv was transfected with the commonly used Ca2+-phosphate technique.

**Results and conclusion**

After transfection of our HeLa cells with the already described methods, we visualized our fluorescent probes with the previously described imaging system.
With the calcium phosphate technique, we could detect about 50% of positive cells, and mitochondria had a healthy rod-shaped morphology.
With the lipid transfection reagent, we had a much higher transfection efficiency (about 70 % positive cells), however mitochondria displayed a “dot” shape, typical of “stressed or unhealthy” mitochondria, and in some cells they were also clustered around the nucleus.
This last mitochondrial morphology let us deduce that mitochondria were probably suffering for some reason.
Since we transfected the same constructs with two different methods, we hypothesized that the unhealthy mitochondrial shape could be due to the transfection technique used, but this remains still a hypothesis.

**References:**

Palmer, A.E. *et al.* Ca2+ indicators based on computationally redesigned calmodulin-peptide pairs. *Chem. Biol.* 13, 521-530 (2006).