Chemical targeting of NEET/CISDs proteins reveals the central role of mitochondrial morphodynamics regulation in viral infection

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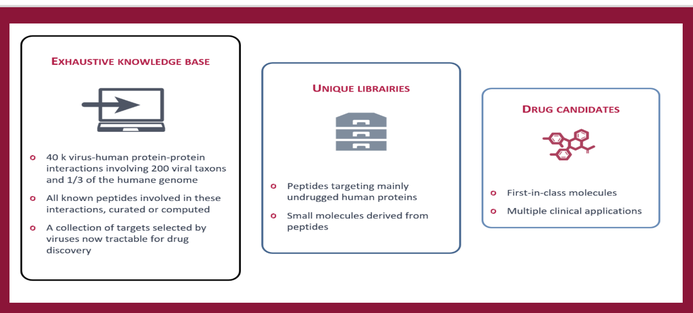
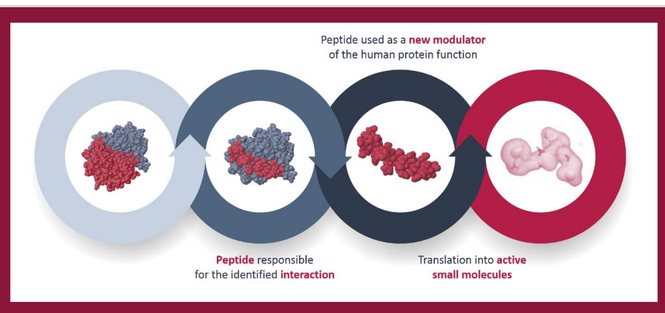
*/ # Equal contribution



ENYO Pharma has developed an innovative and disruptive drug discovery engine allowing to develop molecules directed against new therapeutic intracellular targets. The approach is inspired by viruses which are obligate intracellular pathogens. Over millions of years of evolution, they have perfected their ability to modulate and hijack the cell functions for their own benefit to complete their replication. Deciphering virus-host interactions is essential to built a unique concept based on virus bio-mimetism.

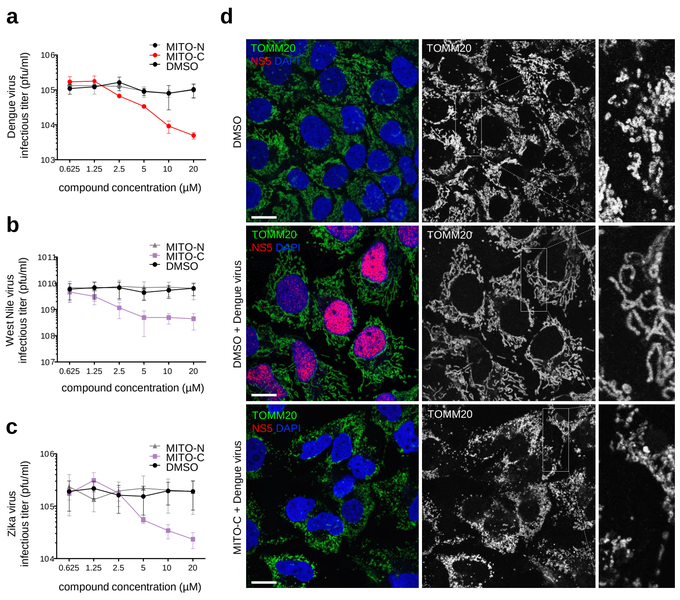


Identifying the right protein-protein interactions and the peptide sequence of a viral protein is sufficient to target and modulate the activity of a host protein. From the viral bioactive peptides, active small molecules were designed mimicking the structure and the activity of the peptide that can be easily developed. This allows to identify first-in-class therapeutics which are efficient against hitherto untapped targets and safe (the protein modulation is already used by viruses while keeping essential cellular functions intact to allow them time to replicate)

ABSTRACT

Several human pathologies, including neurological, heart and metabolic diseases as well as cancer and infections, have been associated with altered mitochondria morphodynamics. Here we designed and screened molecules able to target mitochondria and to rapidly modify their morphology. Among them we selected Mito-C, a potent compound which is able to counteract Dengue virus induced mitochondrial network hyperfusion, and blocks viral replication. Moreover, we show that Mito-C targets mitochondrial NEET/CISDs family, previously reported to regulate iron homeostasis in the mitochondria. Our study suggest that NEETs proteins could be a new potential therapeutic target for infectious diseases.

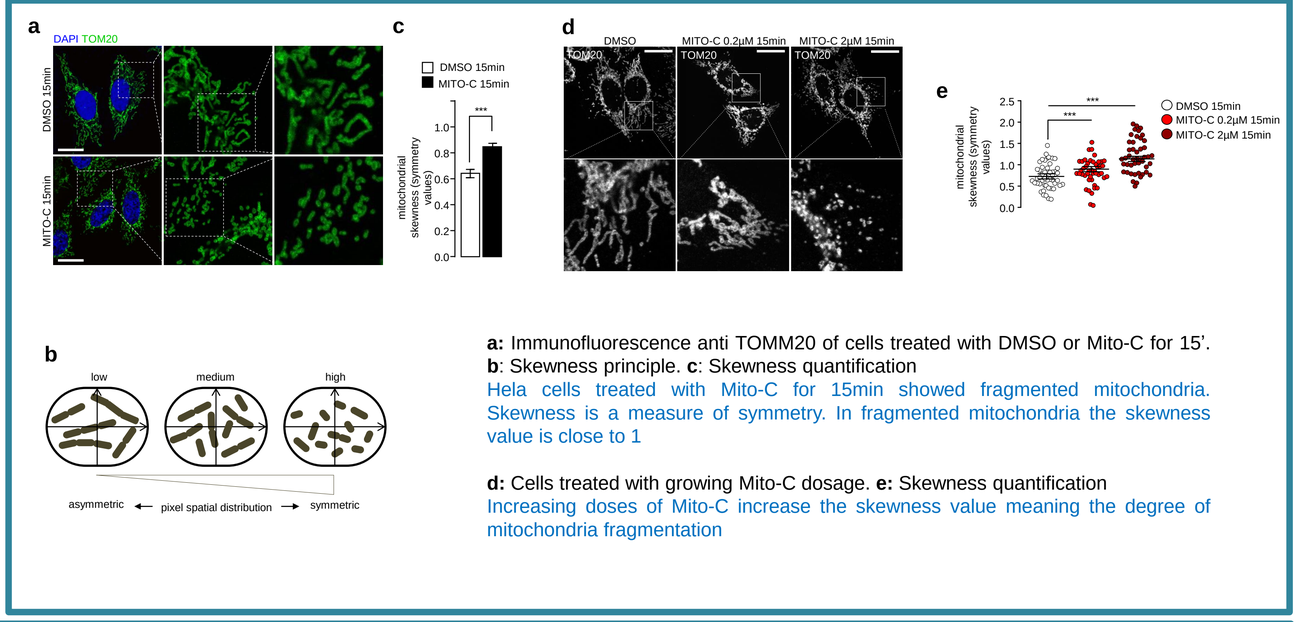
Mito-C counteracts flaviviruses replication



a, b, c: Dengue, West Nile and Zika infectious titers in growing Mito-C concentrations upon infection
Inhibition of viral titers confirms the antiviral effect of Mito-C in all three viruses

d: Immunofluorescence anti-TOMM20 (mitochondria) and NS5 in Dengue infected cells and treated with DMSO or Mito-C
Dengue virus has been described to cause hyperfused mitochondria. Mito-C treatment successfully counteracts this phenotype and the levels of NS5 viral protein are diminished as well

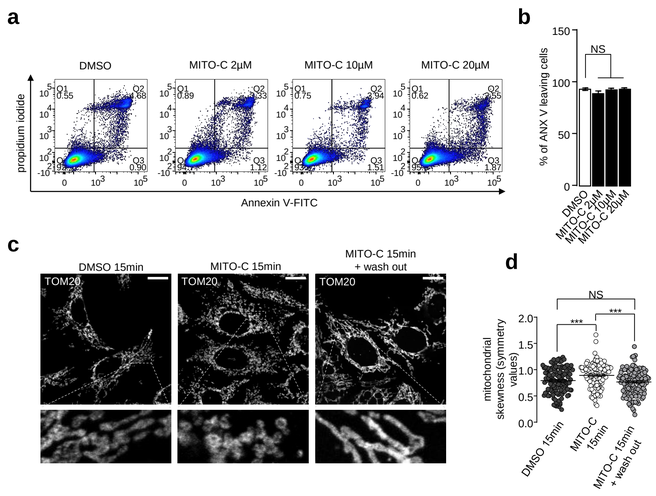
Mito- C induces mitochondria fragmentation



a: Immunofluorescence anti TOMM20 of cells treated with DMSO or Mito-C for 15'.
b: Skewness principle. **c**: Skewness quantification
Hela cells treated with Mito-C for 15min showed fragmented mitochondria. Skewness is a measure of symmetry. In fragmented mitochondria the skewness value is close to 1

d: Cells treated with growing Mito-C dosage. **e:** Skewness quantification
Increasing doses of Mito-C increase the skewness value meaning the degree of mitochondria fragmentation

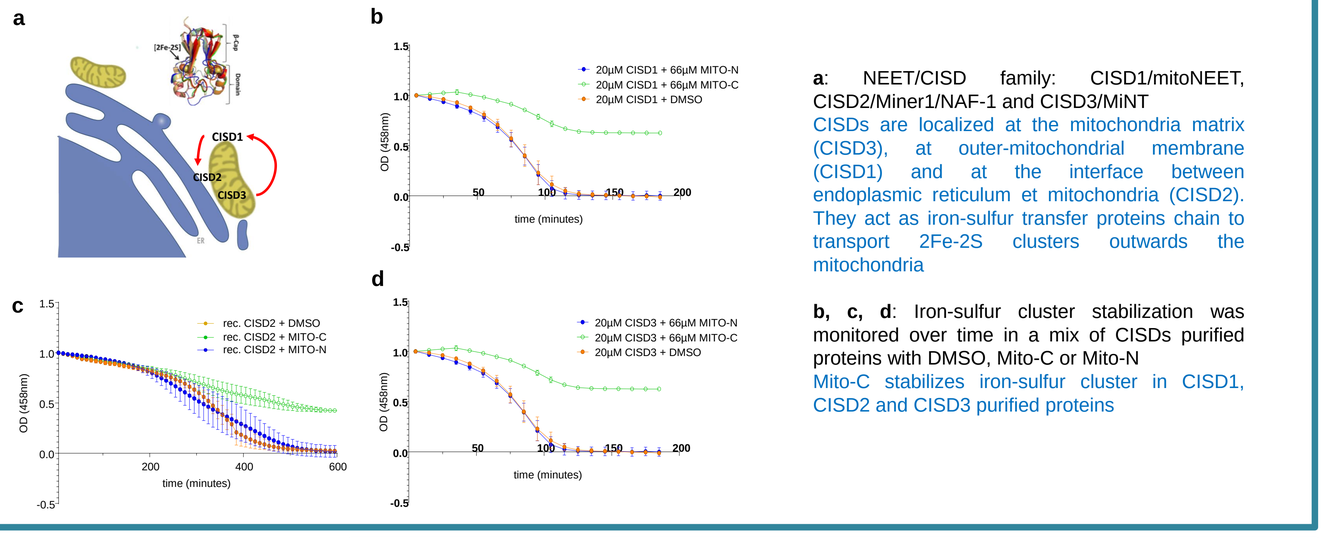
Mito-C treatment is reversible and does not induce cell death



a: Flow cytometry of cells treated with growing concentration of Mito-C stained with PI and Annexin V. **b:** Quantifications of triplicates of flow cytometry
Mito-C does not induce cell death on a range of 2µM to 20µM

c: Immunostaining anti-TOMM20 of cells treated with DMSO, Mito-C and treated and washed out overnight. **d:** Quantification of skewness
Fragmented mitochondria treated with Mito-C recover their normal shape after washing, suggesting reversibility

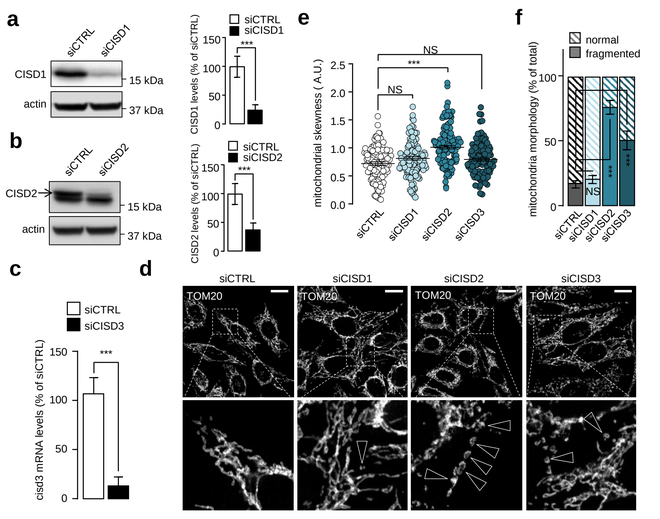
NEET/CISD family is targeted by Mito-C



a: NEET/CISD family: CISD1/mitoNEET, CISD2/Miner1/NAF-1 and CISD3/MiNT
CISDs are localized at the mitochondria matrix (CISD3), at outer-mitochondrial membrane (CISD1) and at the interface between endoplasmic reticulum et mitochondria (CISD2). They act as iron-sulfur transfer proteins chain to transport 2Fe-2S clusters outwards the mitochondria

b, c, d: Iron-sulfur cluster stabilization was monitored over time in a mix of CISDs purified proteins with DMSO, Mito-C or Mito-N
Mito-C stabilizes iron-sulfur cluster in CISD1, CISD2 and CISD3 purified proteins

NEET proteins knock down induces mitochondria fragmentation

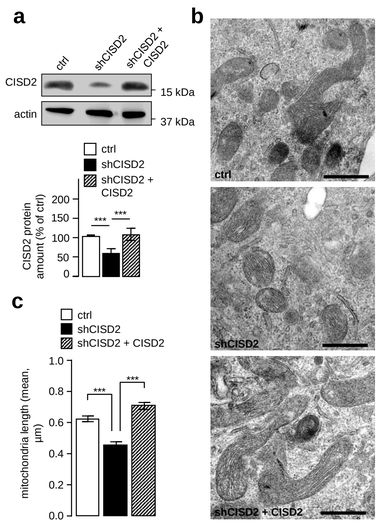


a, b: Western blots anti-CISD1 and anti-CISD2 and quantifications
Indicates the efficiency of the knock down for CISD1 and CISD2

c: Quantification of cisd3 mRNA levels by RT q-PCR
Indicates the efficiency of the knock down for CISD3

d: Anti-TOMM20 immunofluorescence of cells treated for silencing of each CISDs. **e:** Quantification of skewness. **f**: Quantification of normal versus fragmented mitochondria
CISD1 and CISD3 knock down does not cause significant changes in MT network symmetry, but CISD2 knock down does
Mitochondrial morphology changes were significant for cells with a knock down of CISD2 and CISD3

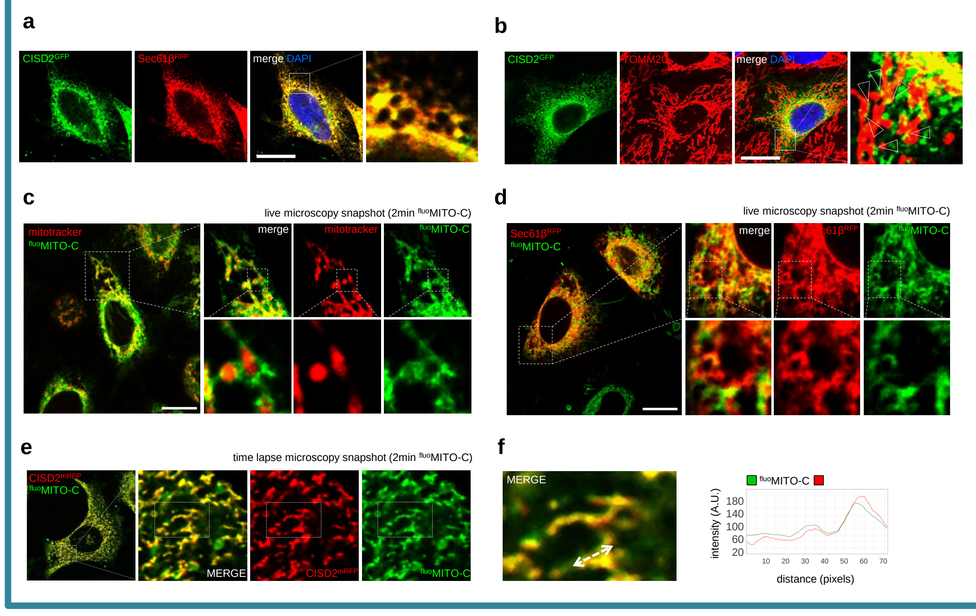
Rescue of CISD2 recovers mitochondria morphology



a: Western blot anti-CISD2 and quantification
Confirms the efficiency of the shRNA

b: Electron microscopy images from ctrl, shCISD2 and rescue vie re-expression of CISD2. **c**: quantification of mitochondria length of EM images
Cells overexpressing CISD2 in a knock down background show normal shape of mitochondria

FIGURE 7: Localization of Mito-C and CISD2



a,b: Anti-TOMM20 (mitochondria) immunofluorescence, CISD2-GFP and Sec61b (ER) staining
CISD2 is localized at the endoplasmic reticulum (ER) and at the mitochondria

c,d: Mitotracker (mitochondria) staining and Mito-C fluorescent compound
Mito-C is localized at the endoplasmic reticulum and at the mitochondria

e, f: CISD2-RFP staining and Mito-C fluorescent compound
Mito-C colocalize with CISD2 protein

SUMMARY / PERSPECTIVES

Mito-C treatment inhibits viral replication of Dengue, West Nile and Zika flaviviruses while it inhibits virus-induced mitochondria elongation

Targeting NEET/CISDs family with chemical compounds causes rapid and reversible mitochondrial fission

Like with Mito-C treatment, CISD2 knock down causes a mitochondrial fragmentation phenotype

Mito-C colocalizes with CISD2 at the interface between ER and mitochondria

The results suggest that NEET proteins are original targets to impact mitochondria morphodynamics

Targeting NEET proteins with our novel compound (Mito-C) opens potential applications that goes beyond infectious diseases