Cardiolipin-specific peptides, designed to possess cell penetrating ability, rescue mitochondrial dysfunction[1]

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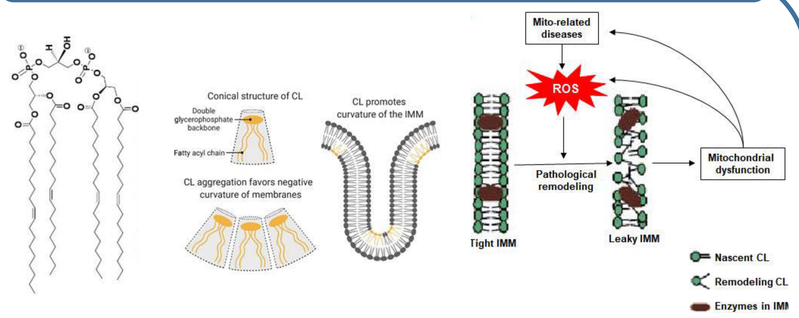
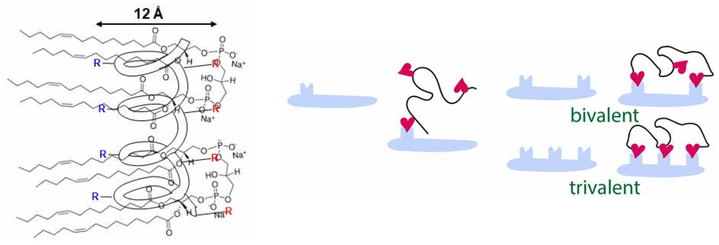
Pathogenic CL-remodeling makes leaky IMM, resulting in mitochondrial dysfunction[2]



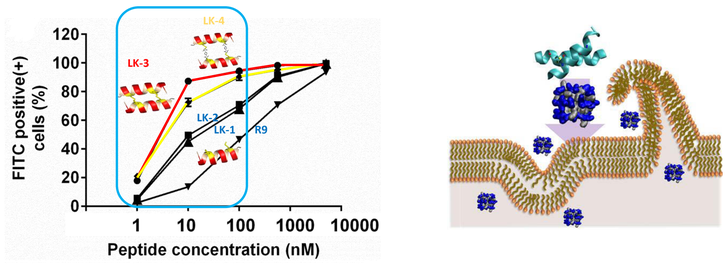
- Nascent CL (20% of total IMM) makes negative curvature, increasing surface area & fluidity of IMM, helps for proteins carrying out most chemical reactions in mitochondria
- CL is oxidized to be pathogenic remodeling, affording leaky IMM, where proteins begins to lose their functions
- Owing to the leaky IMM, dysfunctional mitochondria triggers a variety of degenerative diseases

Mitochondrial dysfunction can be restored by peptides, binding strongly to CLs, keeping tight IMM



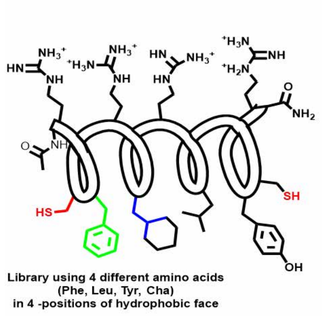
- More than 4 positive charges in amphipathic α-helix interacts with 4 negative charged phosphates in bivalent or trivalent CLs, increasing avidity
- α-Helical peptide has wider diameter to afford strong hydrophobic interactions with those parts in CLs
- Repairing all defective IMM is not necessary; A portion of active IMM is enough to restore normal tissue function; a small amount of CL-specific peptide may give enough efficacy

We have cell penetrable bundled α-helical peptides,[3] but lack of specificity to mitochondria



- α-Helical monomers are oxidized to form “dimer bundle” via 2 disulfide bonds[3]
- The “dimer bundle” has nano-molar cell penetrating ability, forming oligomers to facilitate endocytosis[4]
- It has >100-fold ability than the corresponding monomer peptides, which is penetrable only at micro-molar concentrations
- Introduction of mitochondria-specific residues in “dimeric bundle” would not ruin the cell penetrating ability

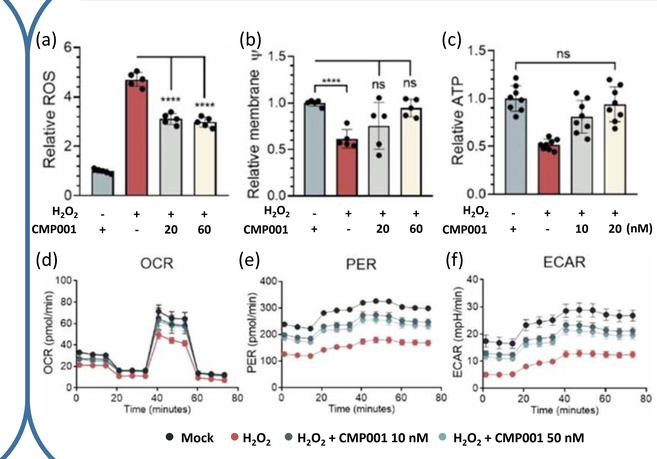
Synthesis of the deconvolution peptide library & selection for inhibition of ROS production



N-	hdf1	hdf2	hdf3	hdf4	-C
Ac-	Phe	Hdf	Hdf	Hdf	-amide
Ac-	Leu	Hdf	Hdf	Hdf	-amide
Ac-	Tyr	Hdf	Hdf	Hdf	-amide
Ac-	Cha	Hdf	Hdf	Hdf	-amide
Ac-	Hdf	Phe	Hdf	Hdf	-amide
Ac-	Hdf	Leu	Hdf	Hdf	-amide
Ac-	Hdf	Tyr	Hdf	Hdf	-amide
Ac-	Hdf	Cha	Hdf	Hdf	-amide
Ac-	Hdf	Hdf	Phe	Hdf	-amide
Ac-	Hdf	Hdf	Leu	Hdf	-amide
Ac-	Hdf	Hdf	Tyr	Hdf	-amide
Ac-	Hdf	Hdf	Cha	Hdf	-amide
Ac-	Hdf	Hdf	Hdf	Phe	-amide
Ac-	Hdf	Hdf	Hdf	Leu	-amide
Ac-	Hdf	Hdf	Hdf	Tyr	-amide
Ac-	Hdf	Hdf	Hdf	Cha	-amide

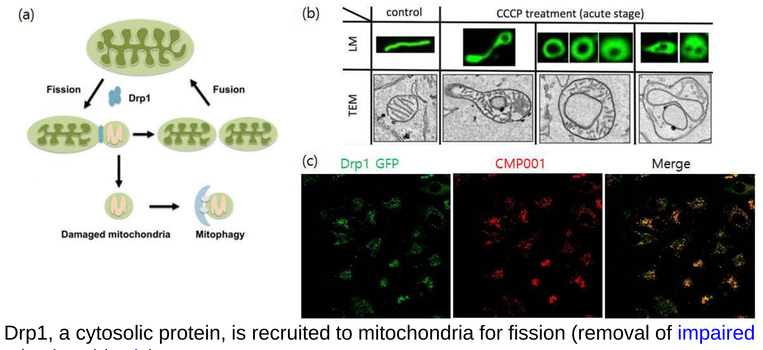
- Hdf; a mixture of known mitochondrial specific AA (Phe, Leu, Tyr & Cha) to increase diversity
- Each hydrophobic AA was position-scanned to make 16 sub-libraries (each sub-library contains 64 kinds, total 1,024 kinds of peptides)
- CMP001 was selected for the best inhibitor of ROS production

Maintaining mitochondrial function & protecting by CMP001 in oxidative damaged cells



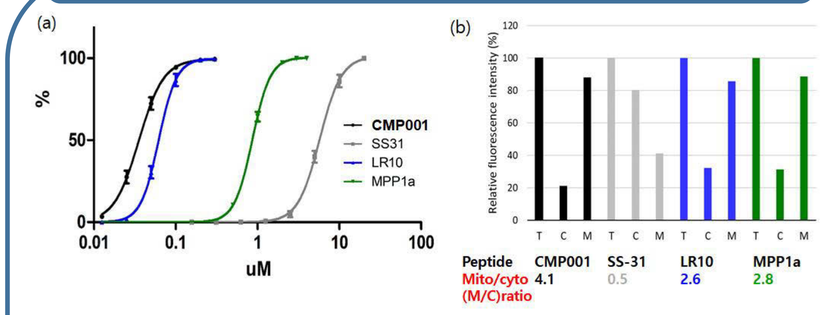
a and b, CMP001 inhibits ROS (a) production. Peptide pre-treated (3 h) cells were treated with H_2O_2 (0.5 mM) for 30 min. **b,** CMP001 preserves mitochondria membrane potential (ψ) from H_2O_2 stimulus. **c,** CMP001 causes resumption of ATP production. Peptide pre-treated (3 h) cells were treated with 1 mM of H_2O_2 for 1 h. **d-f**, CMP001 protects cellular respiration from ROS stimulus

CMP001 goes to the damaged (donut-shape) mitochondria



- Drp1, a cytosolic protein, is recruited to mitochondria for fission (removal of impaired mitochondria, (a)
- If CCCP is treated, mitochondria goes to donut- or blub-shape, less & shorter cristae (b)
- Drp1-GFP was transfected to cells; it went to the damaged mitochondria (c)
- CMP001 is colocalized with Drp1, suggesting that CMP001 goes to the damaged or dysfunctional mitochondria (c)

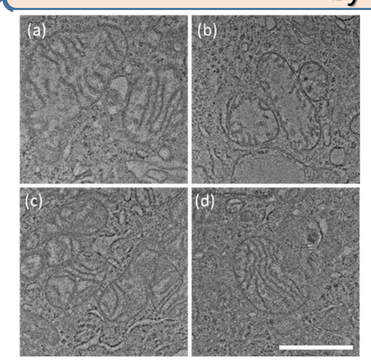
Mitochondrial fractionation of CMP001 & other peptides

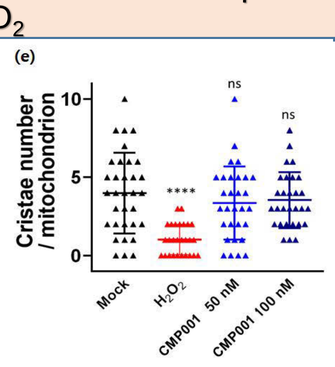


Peptide	CMP001	SS-31	LR10	MPP1a
Mito/cyto (M/C)ratio	4.1	0.5	2.6	2.8

- For the fair comparison, cell penetrating ability (EC_{50}, concentration of a peptide, at which it is inserted to 50% of the cells) of peptides (SS-31[5]; a typical CPP, LR10[6]; Kelley’s MPP[7]; CMP001) were determined
- CMP001 shows the lowest EC_{50} value; It has the best cell penetrating ability
- After peptides were inserted into cells, mitochondria were fractionated
- CMP001 shows the highest mito/cyto ratio (4.1) to compare with other peptides

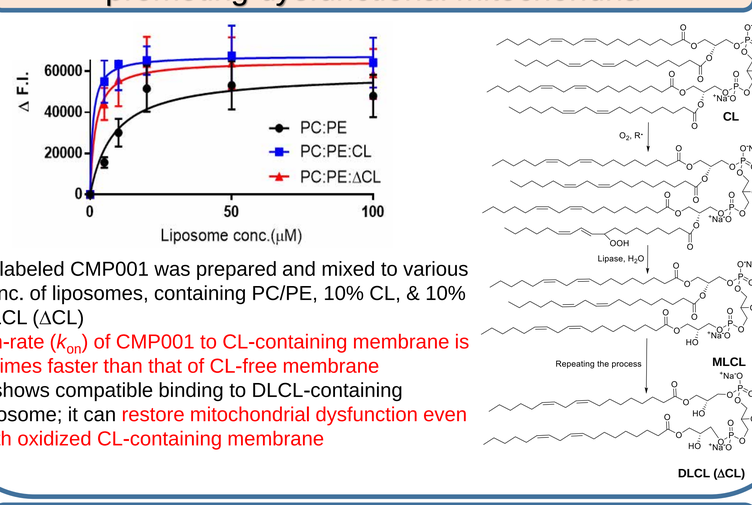
CMP001 protects and rejuvenates cristae depleted by H_2O_2





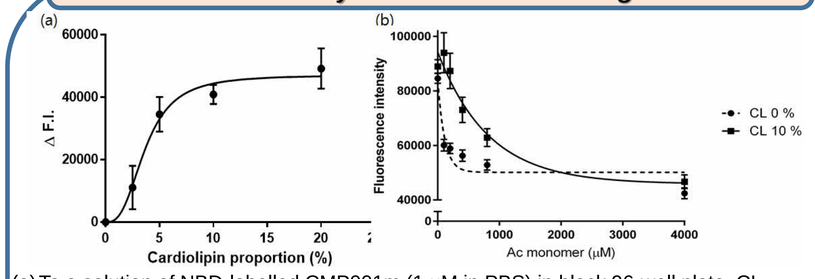
TEM images were observed after the following treatment conditions; (a) mock, (b) HeLa cells were treated with 0.5 mM of H_2O_2 for 2 h, (c-d) cells were pretreated with CMP001 (50 nM for c and 100 nM for d, respectively) for 2 h, followed by H_2O_2 for another 2 h, (e) more than 0.5 μm-long cristae was counted from at least 50 independent mitochondrial images. Scale bar = 1 μm.

CMP001m binds to the remodeled DLCL, possibly promoting dysfunctional mitochondria



- Fl-labeled CMP001 was prepared and mixed to various conc. of liposomes, containing PC/PE, 10% CL, & 10% DLCL (ΔCL)
- On-rate (k_{on}) of CMP001 to CL-containing membrane is 8-times faster than that of CL-free membrane
- It shows compatible binding to DLCL-containing liposome; it can restore mitochondrial dysfunction even with oxidized CL-containing membrane

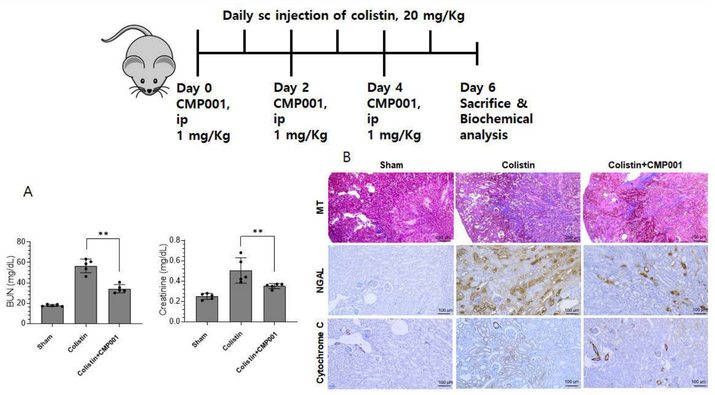
CMP001m strongly binds to membrane w/ > 5% CL & dissociates slowly from CL-containing membrane



(a) To a solution of NBD-labelled CMP001m (1 μM in PBS) in black 96-well plate, CL-containing liposomes (POPC:POPE = 2:1, 0 to 20 % various contents of CL) were added; After mixing for 10 min, fluorescent NBD was measured (Final liposome concentration = 5 μM); λ_{ex} = 467 nm, λ_{em} = 536 nm; n = 3; strong affinity between CMP001m & membrane is maintained if the membrane contains 5-20% CL

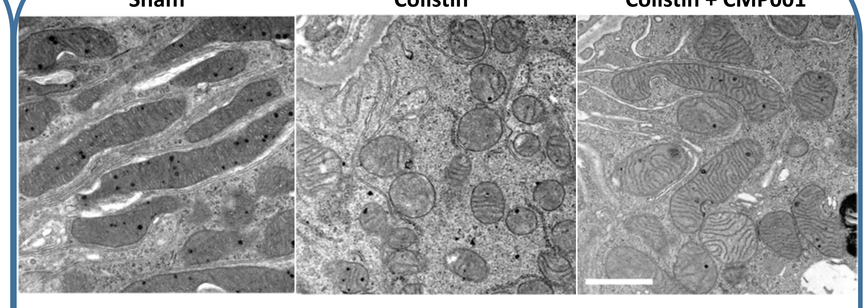
(b) A competition assay using non-labeled CMP001m was carried out for dissociation of the peptide from CL-containing or CL-free membrane; same as above conditions; Off-rate (k_{off}) of CMP001 monomer from CL-containing membrane is 8-times slower than that of CL-free membrane

CMP001 attenuates nephrotoxicity induced by colistin in mouse model



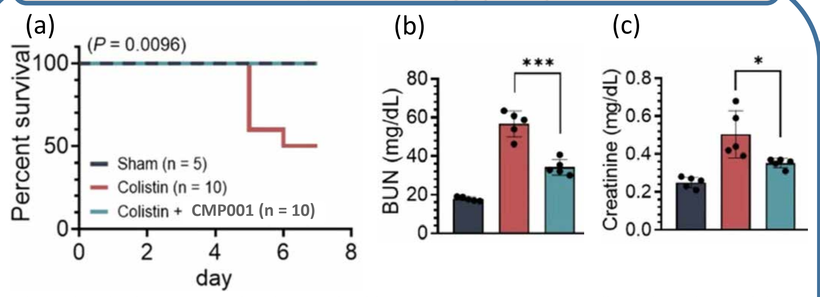
- AKI induced by colistin was rescued by small amount of CMP001 (1mg/Kg)
- BUN & creatinine levels are significantly reduced
- Biomarkers, such as NGAL & cytochrome C are greatly reduced

CMP001 protects mitochondria in the kidney section of colisitin induced kidney injury mice



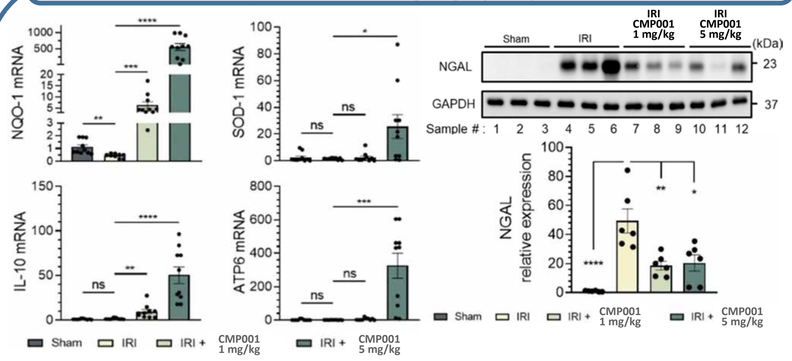
- TEM images were observed after sacrificing colistin-induced nephrotoxicity (CIN) mice. Scale bar = 1 mm. TEM images were observed after sacrificing colistin-induced nephrotoxicity (CIN) mice. Scale bar = 1 μm.
- In the CIN mouse model, CMP001 rejuvenates the kidney's morphology and cristae of mitochondria.

The survival curves of CMP001 treated on the colistin-induced nephrotoxicity (CIN) mouse model



- Five mice (5 days = 4 and 6 days =1) without CMP001 treatment died of nephrotoxicity-associated renal failure, with a percentage of survival of 50% (Long-rank, p-value = 0.0096), respectively
- CMP001-treated mice live beyond CIN mice, **showing a rate the same as the control**
- A dose of CMP001 promotes colistin-induced acute kidney injury reductions and is beneficial in mortality
- CMP001 protects the kidney function (BUN, Cr levels) on the CIN mouse model.

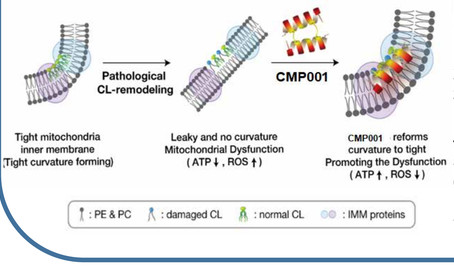
CMP001 increases anti-inflammatory effects on the Ischemia-reperfusion injury (IRI) mouse model



- In IRI mouse model, CMP001 increases mRNA of anti-inflammatory enzymes such as NAD(P)H dehydrogenase 1 (NQO-1), superoxide dismutase-1 (SOD-1).
- CMP001 decreases Neutrophil Gelatinase-Associated Lipocalin (NGAL), which is a early marker of kidney injury and inflammation.

Summary; CL-specific peptide holds IMM structure

- Chemically important events in mitochondria occur in the inner membrane, where cardiolipin (CL), a specific phospholipid, causes curvature needed for functional proteins to function properly
- Pathological CL remodeling, however, destroys the curvature, resulting in mitochondrial dysfunction
- The amphipathic dimeric α-helical peptide, CMP001, penetrates into cells, binds to multiple CLs, and regenerates mitochondrial curvature
- This results in gathering of mitochondrial proteins and correction of mitochondria dysfunction in many human diseases



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