Published in final edited form as:

Angew Chem Int Ed Engl. 2018 December 21; 57(52): 17183–17188. doi:10.1002/anie.201811940.

Non-Peptidic Cell-Penetrating Motifs for Mitochondrion-Specific Cargo Delivery

Appiah Kubi George,

Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 (USA)

Ziqing Qian,

Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 (USA)

Souad Amiar,

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 W Stadium Ave, West Lafayette, IN 47907 (USA)

Ashweta Sahni,

Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 (USA)

Robert V. Stahelin, and

Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, 575 W Stadium Ave, West Lafayette, IN 47907 (USA)

Dehua Pei*

Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210 (USA)

Abstract

Mitochondrial dysfunction is linked to a variety of human illnesses, but selective delivery of therapeutics into the mitochondrion has been challenging. We report herein a family of amphipathic cell-penetrating motifs (CPMs) consisting of four guanidinium groups and one or two aromatic hydrophobic groups (e.g., naphthalene) assembled through a central scaffold (e.g., a benzene ring). The CPMs and CPM-cargo conjugates efficiently enter the interior of cultured mammalian cells and are specifically localized into the mitochondrial matrix, as revealed by high-resolution confocal microscopy. With a membrane-impermeable peptide as cargo, the CPMs exhibited 170-fold higher delivery efficiency than previously reported mitochondrial delivery vehicles. Conjugation of a small-molecule inhibitor of heat shock protein 90 to a CPM resulted in accumulation of the inhibitor inside the mitochondrial matrix and greatly enhanced its anticancer activity. The CPMs showed minimal effect on the viability or the mitochondrial membrane potential of mammalian cells.

* pei.3@osu.edu.

Supporting information for this article is given via a link at the end of the document

Drug delivery:

A non-peptidic cell-penetrating motif is able to efficiently and specifically deliver small-molecule and peptidyl cargoes into the mitochondrial matrix of mammalian cells by crossing both plasma and mitochondrial membranes.

Keywords

Cell-penetrating motif; cytosolic entry; drug delivery; mitochondrial targeting

Mitochondria carry out a wide range of vital biochemical functions in eukaryotic cells including ATP production, calcium homeostasis, cell death, growth, differentiation, and catabolism and anabolism of secondary metabolites.^[1] Mitochondrial dysfunction is linked to many human diseases such as cardiovascular diseases (e.g., atherosclerosis, ischemia/reperfusion injury, heart failure, stroke), aging and neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Friedreich's ataxia), and metabolic diseases (e.g., diabetes and obesity).^[2] Therapeutic intervention of mitochondrial diseases is challenging, because a drug molecule must traverse the plasma as well as the two mitochondrial membranes before reaching the mitochondrial matrix. Current approaches to delivering bioactive cargo molecules into the mitochondria involve lipophilic cations [e.g., triphenylphosphonium (TPP) ion],^[3] cationic peptidomimetics and oligosaccharides,^[4] or mitochondrial-penetrating peptides (MPPs).^[5–8] To the best of our knowledge, these mitochondrial transporters have only been used to deliver membrane-permeable small-molecule cargoes (e.g., anticancer agents) into the mitochondrial matrix. To deliver larger, impermeable cargoes into the mitochondria, other investigators have fused the cargo (e.g., peptide nucleic acids) to naturally occurring

mitochondrial targeting sequences (MTSSs),^[9] which are recognized by the mitochondrial import machinery, or employed nanoparticle-based^[10] and cationic liposome-based^[11] transporter systems. In this work, we report a new class of non-peptidic cell-penetrating motifs (CPMs) capable of delivering small molecules and peptides into the mitochondrial matrix with unprecedented specificity and efficiency.

We previously discovered a family of small amphipathic cyclic peptides as highly efficient cell-penetrating peptides (CPPs), which are capable of delivering a wide variety of cargo molecules (e.g., small molecules, peptides and proteins) into the cytosol of mammalian cells.^[12, 13] The cyclic CPPs typically consisted of two aromatic hydrophobic amino acids and 3 or 4 arginine residues of both L- and D-configurations (e.g., CPP9 in Figure 1a). To gain further insight into the structure-activity relationship and potentially discover still more active cell-penetrating molecules, we designed a non-peptidic CPM consisting of 4 guanidinium groups anchored to a rigid scaffold (e.g., a benzene ring), to mimic the 4 arginine residues of cyclic CPPs (Figure 1a, CPM1). An aromatic hydrophobic amino acid [e.g., naphthylalanine (Nal)] was also appended to the benzene ring to render the CPM amphipathic, a feature critical for efficient cytosolic entry.^[13] To facilitate fluorescent labeling/cargo attachment and minimize any potential mutual interference between the CPM and the dye/cargo, a flexible linker, miniPEG-Lys, was added to the carboxyl group of Nal (Figure 1b). CPM1-miniPEG-Lys was readily synthesized on solid phase, starting with the miniPEG-Lys linker. After the sequential addition of Fmoc-Nal and 3,5-*bis*(bromomethyl)benzoic acid (Bmb) by standard peptide chemistry, the four guanidinium groups were installed by treating the resin-bound Bmb moiety with excess 1,1'-(azanediyldis(hexane-6,1-diyl))diguandine under mildly basic condition (pH 9). CPM1-miniPEG-Lys was cleaved from the resin and deprotected (at the lysine side chain) by treatment with trifluoroacetic acid (TFA).

To test whether CPM1 can enter the cytosol of mammalian cells, we labeled CPM1-miniPEG-Lys at its lysine side chain with a pH-sensitive dye, naphthofluorescein (NF; $pK_a = 7.8$), which is minimally fluorescent when entrapped inside the acidic endosomal/lysosomal compartments (pH 4.5–6.5) but becomes highly fluorescent upon escaping from the endosome/lysosome.^[14] Flow cytometry analysis of HeLa cells treated for 2 h with 5 μM CPM1^{NF} (see Figure S1 for detailed structure) gave a relative mean fluorescence intensity (MFI) of 404% [relative to CPP9^{NF} (100%), which is one of the most active CPPs known to date^[13]] (Figure 2a), suggesting that CPM1 efficiently enters the cytosol. To confirm cytosolic entry, we labeled CPM1-miniPEG-Lys with a pH-insensitive dye, tetramethylrhodamine (TMR), and examined HeLa cells treated with 2 μM CPM1^{TMR} by live-cell confocal microscopy. Surprisingly, CPM1^{TMR} exhibited exclusively punctate fluorescence in the cytoplasmic region (Figure 2b). These seemingly conflicting results led us to hypothesize that after cytosolic entry, CPM1 might become associated with subcellular structures such as the mitochondria, since certain lipophilic cations had previously been shown to accumulate inside the mitochondrial matrix.^[3,4,6–8,15] Indeed, incubation of HeLa cells with CPM1^{TMR} and a mitochondrion-specific dye (MitoTracker Green) showed co-localization of the two, with a Pearson's correlation coefficient of 0.73 (Figure 2b). In contrast, CPM1^{TMR} overlapped poorly with an endosomal marker, Alexa Fluor 488-labeled dextran, showing a correlation coefficient of 0.35 (Figure S2a). The fluorophore did not

affect the mitochondrial localization of CPM1, since fluorescein-labeled CPM1 also co-localized with MitoTracker Red (Pearson's correlation coefficient = 0.70; Figure S2b and vide infra).

In an attempt to improve the cytosolic entry efficiency and/or mitochondrial-targeting specificity of CPM1, we added a second hydrophobic amino acid, D-phenylalanine (D-Phe) or D-Nal, to the CPM1 structure to give CPM2 and CPM3, respectively (Figure 1a). Previous studies had shown that an alternating stereochemical configuration of the two hydrophobic residues improved the cytosolic entry efficiency of cyclic CPPs.^[13] Incorporation of a D-amino acid should also improve the proteolytic stability of the CPMs. As a control, we also generated CPM4, in which the Nal residue in CPM1 was replaced with an alanine (Figure S1). Flow cytometry analysis of HeLa cells treated for 2 h with 5 μ M NF-labeled CPM1–4 gave relative MFI values of 404%, 549%, 506%, and 21%, respectively (relative to CPP9^{NF}), confirming the critical role of hydrophobic groups for efficient cellular entry (Figure 2a). Live-cell confocal microscopic imaging showed that CPM2^{TMR} and CPM3^{TMR} co-localized with MitoTracker Green in HeLa cells, with Pearson's correlation coefficients of 0.73 and 0.89, respectively (Figure 2c,d). Interestingly, while each MitoTracker Green signal was matched by a corresponding CPM^{TMR} signal, the reverse was not true. A small fraction of CPM2^{TMR} (and CPM3^{TMR}) signals was not matched by MitoTracker Green signals. These signals were likely derived from CPMs still entrapped inside the endosomal and/or lysosomal compartments. CPM3 appears to be more efficient in endosomal escape than CPM1 and CPM2, resulting in better co-localization with the MitoTracker (Figure 2b–d). As expected, CPM4 showed much weaker intracellular fluorescence (Figure 2a) and poorer co-localization with MitoTracker Green than CPM1–3 ($R = 0.52$; Figure S2c). CPM3 was selected for further studies, because of its high cellular uptake efficiency and mitochondrial-targeting specificity.

To ascertain that mitochondrial localization is not caused by the TMR label, CPM3 was labeled with Alexa Fluor 488 (which carries two negative charges at physiological pH) and its cellular entry was again monitored by confocal microscopy. CPM3^{Alexa488} efficiently entered HeLa cells and co-localized with MitoTracker Red (Pearson's correlation coefficient = 0.90–0.97), demonstrating the ability of CPMs to deliver negatively charged cargoes into the mitochondria (Figure S3). Confocal imaging of HeLa cells treated with CPM3^{Alexa488} for shorter periods of time (e.g., 15 min) produced a punctate fluorescence pattern of minimal overlap with that of MitoTracker Red (Pearson's correlation coefficient = 0.12; Figure S4). This observation suggests that the CPMs enter cells by endocytic mechanisms and are largely entrapped inside endosomes/lysosomes after 15 min of treatment. On the other hand, once escaping from the endosome, translocation of CPMs from the cytosol into the mitochondria appears to be a rapid event.

To test whether the CPMs can deliver membrane-impermeable cargoes into the mitochondria, we designed an all D-peptide, ala-asn-ala-asn-ala-asn-miniPEG-Lys (ananan), as a mock cargo molecule. The choice of a D-peptide was to minimize any proteolytic degradation during experimentation and simplify data interpretation. The D-peptide was conjugated with CPM3 at its N-terminus and labeled with TMR at the C-terminal lysine (Figure S1). HeLa cells were treated with the peptides and MitoTracker Green, and imaged

by live-cell confocal microscopy. CPM3-ananan^{TMR} produced punctate fluorescence, which overlapped well with that of MitoTracker Green, having a Pearson's correlation coefficient of 0.80 (Figure 3a). A small amount of the peptide appeared to remain entrapped inside the endosomal/lysosomal compartments, causing the correlation coefficient to be <1. As expected, cells treated with the unconjugated D-peptide (Ac-ananan^{TMR}) showed no detectable intracellular fluorescence (Figure 3b). For comparison, we also conjugated the D-peptide with two of the most widely used (and most efficient) mitochondrion targeting agents, TPP and (F_xr)₃ (where F_x is cyclohexylalanine and r is D-arginine; Figure S1).^[6, 8] Under the same condition, (F_xr)₃-ananan^{TMR} showed partial co-localization with MitoTracker Green, as indicated by a Pearson's correlation coefficient of 0.47 (Figure 3c), which is similar to the previously reported values.^[6] A substantial fraction of (F_xr)₃-ananan^{TMR} was localized in subcellular structures other than the mitochondria, most likely the endosomes and/or lysosomes. TPP-ananan^{TMR} showed excellent co-localization with MitoTracker Green (Pearson's correlation coefficient = 0.74) but produced very weak intracellular fluorescence (Figure 3d). Next, the three peptide conjugates were labeled with NF and their cytosolic/mitochondrial entry efficiencies into HeLa cells were quantitated by flow cytometry. Consistent with the results obtained with CPM3^{NF} (Figure 2a), CPM3-ananan^{NF} entered the cytosol/mitochondria with remarkable efficiency, which was 170- and 1300-fold higher than that of (F_xr)₃-ananan^{NF} and TPP-ananan^{NF}, respectively (Figure 3e).

The sub-mitochondrial localization of the peptide was evaluated by high-resolution 3D-SIM imaging of HeLa cells treated with CPM3-ananan^{TMR} and transfected with the mitochondrial marker Grx2-mito-roGFP.^[16] The latter produces a green fluorescent protein (GFP) inside the mitochondrial matrix. CPM3-ananan^{TMR} appeared to be highly distributed throughout the mitochondrial network (Figure 4a). The zoomed insets (Figure 4b–d) showed highly similar fluorescence patterns for CPM3-ananan^{TMR} and mitochondrial GFP. Inner lamellar membrane accumulation is observed with dark regions and internal thin fluorescent gaps, corresponding to the structure of mitochondrial cristae (Figure 4b–d bottom panels). Treatment of HEK293 cells with CPM3-ananan^{TMR} and Grx2-mito-roGFP exhibited similar mitochondrial fluorescence patterns (data not shown). Similar mitochondrial structures were also previously observed where different mitochondrion probes and markers were analyzed by SD-SIM imaging.^[17] These data demonstrate that CPM3-ananan^{TMR} is efficiently delivered into the mitochondrial matrix.

We next tested whether CPMs can deliver a biologically active cargo into the mitochondrial matrix. Geldanamycin (GA) is a potent small-molecule inhibitor of heat shock protein 90 (Hsp90) and has anti-proliferative activity against cancer cells.^[18,19] Interestingly, treatment of cancer cells with GA causes only partial loss of cell viability, even at saturating concentrations. Delivery of GA into the mitochondrial matrix with a targeting moiety and subsequent inhibition of mitochondrial Hsp90, however, were previously shown to induce rapid apoptosis and cause complete killing of cancer cells.^[19] We covalently attached GA to CPM3 (Figure 5a) and assessed the anti-proliferative activity of CPM3-GA, GA alone, or CPM3 alone against HeLa cells. In agreement with previous reports,^[19] GA caused a maximum of ~40% inhibition in HeLa cell viability at saturating concentrations, although the maximum was reached at a low inhibitor concentration (0.3 μM; Figure 5b). In contrast, CPM3-GA reduced the viability of HeLa cells in a dose-dependent fashion (IC₅₀ ~4 μM)

and completely killed the cancer cells at 10 μM concentration. Annexin V and propidium iodine staining of the treated cells indicated apoptotic cell death (Figure S5). CPM3 alone showed no significant effect up to 10 μM concentration. To confirm mitochondrial localization of CPM3-GA, we labeled CPM3-GA with TMR (CPM3-GA^{TMR}) and treated HeLa cells with CPM3-GA^{TMR} and MitoTracker Green. Live-cell confocal microscopy of the treated cells showed co-localization of CPM3-GA^{TMR} and MitoTracker Green, with a Pearson's correlation coefficient of 0.74 (Figure 5c). These data suggest that CPM3 resulted in accumulation of GA inside the mitochondrial matrix, thereby enhancing its anticancer activity. The higher IC₅₀ value of CPM3-GA (relative to GA) is presumably due to reduced binding affinity of CPM3-GA to Hsp90; conjugation of GA to CPM3 through a releasable linker^[20] may produce a more potent anticancer agent. It is also possible that CPMs have lower endocytic uptake and endosomal escape efficiencies at lower concentrations.

The CPMs were tested for potential cytotoxicity. In MTT assays, up to 10 μM CPMs did not significantly affect the viability/proliferation of HeLa cells (Figures S6a). At 100 μM , CPM1–3 reduced the viability of HeLa cells by 40–60%. We next tested CPM1–3 for potential effect on mitochondrial membrane potential, a key indicator of cell health or injury. Briefly, HeLa cells untreated or treated with CPM were labeled with JC-10, a dye molecule which emits green fluorescence inside the cytosol but red fluorescence inside the mitochondria.^[21] The ratio of green/red fluorescence (F520/F590) provides an indicator of the mitochondrial membrane potential. Treatment of HeLa cells with a known mitochondrial membrane depolarizer, *p*-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP),^[22] caused retention of JC-10 in the cytosol and dose-dependent increase of the F520/F590 ratio, to up to 7-fold above the normal value at 20 μM FCCP concentration (Figure S6b,c). On the other hand, CPM1–3 did not perturb the membrane potential at 10 μM concentration. At 20 μM , only CPM3 caused a small (2.5-fold) increase in the F520/F590 ratio. These results indicate that the CPMs are relatively nontoxic to mammalian cells.

In conclusion, we have discovered a new family of non-peptidic CPMs which are capable of highly efficient and specific delivery of small molecules, peptides, and potentially other cargoes into the mitochondrial matrix of mammalian cells. They are metabolically stable (Figure S7) and appear to have minimal cytotoxicity to mammalian cells. These CPMs should provide a powerful research tool for specific delivery of chemical probes into the mitochondria. They may also be applied to deliver therapeutic agents into the mitochondrial matrix for potential treatment of mitochondrial diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Institutes of Health (GM122459). S.A. and R.V.S. acknowledge the use of the facilities of the Purdue University Bindley Bioscience Center, a core facility of the NIH-funded Indiana Clinical and Translational Sciences Institute.

References

- [1]. Ernster L, Schatz G, J. Cell. Biol. 1981, 91, 227. [PubMed: 7298718]
- [2]. For reviews on mitochondrial diseases, see: a) Malty RH, Jessulat M, Jin K, Musso G, Vlasblom J, Phanse S, Zhang Z, and Babu M, J. Proteome Res 2015, 14, 5; [PubMed: 25367773] b) Calvo SE, Mootha VK, Annu. Rev. Genomics Hum. Genet. 2010, 11, 25; [PubMed: 20690818] c) Toogood PL, Curr. Opin. Chem. Biol. 2008, 12, 457. [PubMed: 18602018]
- [3]. Murphy MP, Biochim. Biophys. Acta 2008, 1777, 1028. [PubMed: 18439417]
- [4]. a) Fernández-Carneado J, Van Gool M, Martos V, Castel S, Prados P, de Mendoza J, Giralte EJ, J. Am. Chem. Soc. 2005, 127, 869. [PubMed: 15656624] b) Zhao K, Zhao GM, Wu D, Soong Y, Birk AV, Schiller PW, Szeto HH, J. Biol. Chem. 2004, 279, 34682; [PubMed: 15178689] c) Wipf P, Xiao J, Jiang J, Belikova NA, Tyurin VA, Fink MP, Kagan VE, J. Am. Chem. Soc. 2005, 127, 12460. [PubMed: 16144372]
- [5]. Chuah J-A, Yoshizumi T, Kodama Y, Numata K, Sci. Reports 2015, 5, 7751.
- [6]. Jean SR, Ahmed M, Lei EK, Wisnovsky SP, Kelley SO, Acc. Chem. Res. 2016, 49, 1893. [PubMed: 27529125]
- [7]. a) Horton KL, Stewart KM, Fonseca SB, Guo Q, Kelley SO, Chem. Biol. 2008, 15, 375; [PubMed: 18420144] b) Cerrato CP, Pirisinu M, Vlachos EN, Langel U, FASEB J 2015, 29, 4589. [PubMed: 26195590]
- [8]. Hoye AT, Davoren JE, Wipf P, Acc. Chem. Res. 2008, 41, 87. [PubMed: 18193822]
- [9]. Chinnery PF, Taylor RW, Diekert K, Lill R, Turnbull DM, Lightowlers RN, Gene Ther. 1999, 6, 1919. [PubMed: 10637443]
- [10]. Pathak PK, Kolishetti N, Dhar S, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 2015, 7, 315. [PubMed: 25348382]
- [11]. a) Yamada Y, Harashima H, Biomaterials 2012, 33, 1589; [PubMed: 22105068] b) Weissig V, D'Sosza GG, Torchilin VP, J. Controlled Release 2001, 75, 401.
- [12]. a) Qian Z, Liu T, Liu Y-Y, Briesewitz R, Barrios AM, Jhiang SM, Pei D, ACS Chem. Biol. 2013, 8, 423; [PubMed: 23130658] b) Qian Z, LaRochelle JR, Jiang B, Lian W, Hard RL, Selner NG, Luechapanichkul R, Barrios AM, Pei D, Biochemistry 2014, 53, 4034. [PubMed: 24896852]
- [13]. Qian Z, Martyna A, Hard RL, Wang J, Appiah-Kubi G, Coss C, Phelps MA, Rossman JS, Pei D, Biochemistry 2016, 55, 2601. [PubMed: 27089101]
- [14]. Qian Z, Dougherty PG, Pei D, Chem. Commun. 2015, 51, 2162.
- [15]. Maiti KK, Lee WS, Takeuchi T, Watkins C, Fretz M, Kim DC, Futaki S, Jones A, Kim KT, Chung SK, Angew. Chem. Int. Ed. 2007, 46, 5880.
- [16]. Norcross S, Trull KJ, Snider J, Doan S, Tat K, Huang L, Tantama M, ACS Sens. 2017, 2, 1721. [PubMed: 29072071]
- [17]. Opstad S, Wolfson DL, Øie CI, Ahluwalia BS, Nanophotonics 2018, 7, 935.
- [18]. Drysdale MJ, Brough PA, Massey A, Jensen MR, Schoepfer J, Curr. Opin. Drug Discov. Devel. 2006, 9, 483.
- [19]. Kang BH, Plescia J, Song HY, Meli M, Colombo G, Keebe K, Scroggins B, Neckers L, Altieri DC, J. Clin. Invest. 2009, 119, 454. [PubMed: 19229106]
- [20]. Lei EK, Kelley SO, J. Am. Chem. Soc. 2017, 139, 9455. [PubMed: 28664723]
- [21]. Kirchhoff C, Cypionka H, Front. Microbiol. 2017, 8, 695. [PubMed: 28473821]
- [22]. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA, Biotechniques 2011, 50, 98. [PubMed: 21486251]

Figure 1. Design and synthesis of CPMs.

a) Comparison of the structures of a cyclic cell-penetrating peptide (CPP9) and non-peptidic CPMs. b) Solid-phase synthesis of CPM1-miniPEG-Lys. SPPS, solid-phase peptide synthesis.

Figure 2. Cellular entry and mitochondrial localization of CPMs.

(a) Cytosolic entry efficiency of CPM1–4 in to HeLa cells as analyzed by flow cytometry. MFI values of cells treated with 5 μ M NF-labeled CPM1–4 for 2 h are relative to that of CPP9 (100%). Data reported are the mean $\pm$ SD of three independent experiments. Control, untreated cells. (b) Live-cell confocal microscopic images of HeLa cells after 2 h treatment with 2 μ M CPM1^{TMR} (red) and 15 min incubation with MitoTracker Green. A merged image is shown on the right with the R value representing Pearson's correlation coefficient for co-localization. (c) Same as (b) but with CPM2. (d) Same as (b) but with CPM3.

Figure 3. Mitochondrial delivery of peptidyl cargo by CPM3, (F_{xr})₃, and TPP.

(a) Upper panel, live-cell confocal microscopic images of HeLa cells after treatment with 2 μ M CPM3-ananan^{TMR} for 2 h and MitoTracker Green for 15 min. Lower panel, zoomed-in images of the boxed areas in the upper panel. (b) Same as (a) but cells were treated with 2 μ M Ac-ananan^{TMR}. (c) Same as (a) but cells were treated with 2 μ M (F_{xr})₃-ananan^{TMR}. (d) Same as (a) but cells were treated with 2 μ M TPP-ananan^{TMR}. Different imaging conditions were used in a-d in order to detect the TMR signals. (e) Cytosolic entry efficiency of CPM3-, (F_{xr})₃-, and TPP-ananan^{NF} into HeLa cells as analyzed by flow cytometry. Data

shown are the mean $\pm$ SD of three independent experiments. Control, untreated cells. Scale bars, 20 μ m (10 μ m for the lower panel in a and b).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Mitochondrion CPM3-ananan^{TMR} DNA

Figure 4. High-resolution fluorescence images showing the mitochondrial distribution of CPM3-ananan^{TMR}.

(a) 3D-SIM reconstructed image of HeLa cells 48 h post-transfection with pGrx2-mito-roGFP (green) and treated with 2 μ M CPM3-ananan^{TMR} (red) for 2 h and 5 μ g/ml Hoechst 33342 dye (blue) for 15 min prior to imaging. (b-d) Zoom-in images of the boxed areas from (a) showing detailed mitochondrial structures. Top panel, zoom-in images of the boxed areas in (a); middle panel, localization of the mitochondrion marker (gray) within the stroma; and bottom panel, localization of CPM3-ananan^{TMR} (gray) at both the periphery (which corresponds to the mitochondrial membrane) and the interior of mitochondria (which correspond to the cristae). Scale bars: 2 μ m.

Figure 5. Delivery of Hsp90 inhibitor into the mitochondrial matrix by CPM3.

a) Structure of CPM3-GA. b) Effect of GA, CPM3, and CPM3-GA on HeLa cell viability as measured by the MTT assay. c) Live-cell confocal microscopic images of HeLa cells after 2 h treatment with 2 μM CPM3-GA^{TMR} (red) and 15 min incubation with MitoTracker Green. R, Pearson's correlation coefficient.