SUPPLEMENTARY INFORMATION FOR THE ARTICLE OF Y. N. ANTONENKO et al.

S1: Structures, Methods of Synthesis, and Certain Physicochemical Properties of Cationic Quinone Derivatives

Figures S1 and S2 show formulas of CoQH₂, plastoquinol, vitamin E, vitamin K₁, and compounds synthesized in our group, respectively. It is noteworthy that plastoquinol and vitamin K₁ inherent in chloroplasts, as well as the “professional” antioxidant vitamin E, in contrast to CoQ, contain no methoxy groups.

Plastoquinone differs from ubiquinone by (i) substitution of methyl for methoxy groups and (ii) absence of methyl group at fifth position of the quinone ring. SkQ3 contains no methoxy groups but retains, like ubiquinone, methyl at the fifth position. In SkQ2M, methylated carnitine substitutes for the phosphonium cation. In SkQ4 and SkQR1, decyltriphenylphosphonium cation was replaced by cations of decyltributylammonium and rhodamine 19, respectively. As shown in our group, the cation of ethyl rhodamine can penetrate through the lipid bilayer [1]. SkQR1 is unique among other SkQs since it is strongly fluorescing, so its fate in mitochondria, cells and the organism can be easily followed. SkQ5, containing a C₅ linker instead of C₁₀, was used as an example of a compound of lower hydrophobicity than the others. As to DMQ, it was tried as a compound, which, like SkQ3, has a structure intermediate between ubi- and plastoquinone (one methoxy group instead of two in ubiquinone). This compound was of certain interest since partial substitution of demethoxy-CoQ for CoQ was shown to prolong life of C. elegans and mice [2].

A number of antioxidative lipophilic compounds (SkQ1 (3a), SkQ3 (3b), MitoQ (3c), DMQ (3d), and SkQ5 (3e) in Fig. S3) were synthesized. In all of them, triphenylphosphonium cation was covalently attached to the substituted 1,4-benzoquinone via an aliphatic carbon chain of 10 (or 5) methylene groups. Benzoquinones (1a, 1b) were prepared from corresponding benzoquinols by their oxidation by aqueous solution of KBrO₃.

**General procedure of synthesis of alkyl-1,4-benzoquinones (1a-1d).**

Alkylhydroquinone (9 mmol), KBrO₃ (3 mmol), 9 ml H₂O, and 0.45 ml 5 N H₂SO₄ were stirred at 40-50°C for 30 min. The solution was extracted with diethyl ether. The organic extracts were dried over Na₂SO₄ and evaporated in vacuo. The yellow solid was purified by column chromatography on silica gel using chloroform as the eluent.

2,3-Dimethyl-1,4-benzoquinone (1a): the yield, 63%; TLC: Rᵢ (CHCl₃) 0.46; HPLC: tᵣ = 17.4 min (4.6 × 250 mm column Luna 5 µm C₁₈(2), 1 ml/min, 25°C, 0-90% B for 26.4 min; A: 10 mM H₃PO₄ in water; B: 10 mM H₃PO₄ in MeCN), m. p. 58°C (56.5-57.5°C) [3]; UV (CH₃OH): λₑₘₓ = 209, 256, and 344 nm; ESI MS: m/z calculated for C₈H₈O₂ 136.15; found 136.2.

2,3,5-Trimethyl-1,4-benzoquinone (1b), yield, 74%; TLC: Rᵢ (CH₂Cl₂) 0.67; HPLC: tᵣ = 7.8 min (4.6 × 150 mm column Luna 5 µm C₁₈(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN). UV (CH₃OH): λₑₘₓ = 198, 258, and 344 nm; ESI MS: m/z calculated for C₉H₁₀O₂ 150.18; found 150.4.

2,3-Dimethoxy-5-methyl-1,4-benzoquinone (1c) was from Aldrich (USA).

2-Methoxy-5-methyl-1,4-benzoquinone (1d) was obtained by refluxing methanol solution of p-toluquinone and ZnCl₂ [4]. Yield, 27.8%, m. p. 172°C.

Radical alkylation of the quinones (1a-1d) was done using the bromoalcanoic acid–ammonium persulfate–silver nitrate system [5].

**General procedure of the synthesis of bromoalkylenoquinones (2a-2e).**

11-Bromoundecanoic acid (2.1 mmol), 1a-1d (2 mmol), AgNO₃ (1 mmol) were dissolved in 7 ml of AcCN and H₂O (2:1) mixture at 80°C. Solution of (NH₄)₂S₂O₈ (2 mmol) in 3 ml of H₂O was added drop-wise with stirring at 70-89°C for 1.5 h. After dilution with water, the mixture was extracted with ether. The ether layer was washed with water, dried over Na₂SO₄ and evaporated in vacuo. The residue was applied to a silica gel column (225 × 35 mm) using chloroform as eluent. 10-(4,5-Dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decylbromide (2a): yield, 29.7%; TLC: Rᵢ (CHCl₃) 0.62; HPLC: tᵣ = 23 min (4.6 × 250 mm column Luna 5 µm C₁₈(2), 1 ml/min, 25°C, 0-90% B for 26.4 min; A:
10 mM H₃PO₄ in water; B: 10 mM H₃PO₄ in MeCN); UV (CH₃OH): λₓmax – 214, 258, and 344 nm. ESI MS: m/z calculated for C₁₈H₂₇O₂Br (M⁺ + H) 356.3; found 356.1. IR: 2928, 2336, 1600, 1496, and 1304 cm⁻¹.

10-(2,4,5-Trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decylbromide (2b): yield, 42%; TLC: Rf (CHCl₃) 0.79, Rf (CHCl₃–hexane, 3 : 1) 0.45. HPLC: tᵣ = 14.9 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 35-95% B for 18 min; A: 10 mM H₃PO₄ in water; B: 10 mM H₃PO₄ in MeCN); UV (CH₃OH): λₓmax – 214, 262, 268, and 352 nm; ESI MS: m/z calculated for C₁₉H₃₀O₂Br 369.34; found 369.6.

10-(4,5-Dimethoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decylbromide (2c): yield, 26%; TLC: Rᵣ (CH₂Cl₂–Et₂O, 20 : 1) 0.45; HPLC: tᵣ = 12.7 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C,
35-95% B for 26.4 min; A: 10 mM H$_2$PO$_4$ in water; B: 10 mM H$_3$PO$_4$ in MeCN; UV (CH$_3$OH): $\lambda_{max}$ 278 nm; ESI MS: m/z calculated for C$_{19}$H$_{29}$O$_5$Br 401.34; found 402.

10-(5-Methoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decdyli bromide (2d); yield, 37%; TLC: R$_f$ (CHCl$_3$) 0.59. Compound 2d was used for the next step without further purification.

5-(4,5-Dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)pentyl bromide (2e) was prepared as described for 2a with use of la (1 mmol), 6-bromohexamino acid (1 mmol), AgNO$_3$ (1 mmol), and (NH$_4$)$_2$SO$_4$ (1 mmol) in aqueous AcCN. Yield, 30%. Compound 2e was used for the next step without further purification.

Compounds 3a-3e were synthesized from triphenylphosphine and corresponding bromoalkylquinones (2a-2e).

**General procedure for the synthesis of the triphenylphosphonium bromides (3a-3e).** Triphenylphosphine (0.2 mmol), 2a-2e (0.2 mmol), and benzene or ethanol (0.3 ml) were sealed under argon in glass tube and kept at 85-95°C for 72 h. The residue after removing the solvent was precipitated by diethyl ether from CH$_2$Cl$_2$. The crude product was purified by column chromatography on silica gel with the mixture of CHCl$_3$–CH$_3$OH (at different ratio) as eluent. [10-(4,5-Dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decyl]triphenylphosphonium bromide, $SKQ1$ (3a); yield, 24%; TLC: R$_f$ (CHCl$_3$–CH$_3$OH, 4: 1) 0.66; HPLC: $t_R$ = 10.1 min (4.6 × 150 mm column Luma 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV (CH$_3$OH): $\lambda_{max}$ 278 nm; 1H-NMR: (CD$_3$OD, 300K, AV-600) 1.26-1.40 ppm (br. m, 15H, Ph); 13C-NMR: (CDCl$_3$, 300K, AV-600) 21.76 ppm (CH$_2$); 28.58, 29.04, 29.05, 29.13, 29.20, and 29.64 ppm (C 4', C6'); 188.36 and 188.55 ppm (C 3' and C6').

10-(5-Methoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decyl]triphenylphosphonium bromide, $MitoQ$ (3e) was described earlier [6]; the yield, 21%; HPLC: $t_R$ = 9.32 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV (CH$_3$OH): 275 nm ($\lambda_{max}$ 15,950 cm$^{-1}$·M$^{-1}$ proved to be slightly higher than that reported by Murphy [6] (10,400 cm$^{-1}$·M$^{-1}$); ESI MS: m/z calculated for C$_{37}$H$_{44}$O$_2$P, 583.3; found, 583.3; IR: 3357, 2927, 2857, 1650, 1609, 1438, 1266, 1113 cm$^{-1}$; 1H-NMR: (CD$_3$OH, 200K, AV-200) 2.64 ppm (H$_2$); 7.63 ppm (m, 15H, Ph); 13C-NMR: (CD$_3$OD, 30K, AV-300) 21.76 and 28.57 ppm (C 4', C6'); 188.36 and 188.55 ppm (C 3' and C6').

10-(5-Methoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decyl]triphenylphosphonium bromide, $DMQ$ (3d); yield, 25%; TLC: R$_f$ (CH$_3$OH–H$_2$O–H$_2$O, 65: 25: 4) 0.61; HPLC: $t_R$ = 9.11 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV (CH$_3$OH): $\lambda_{max}$ = 18.066 cm$^{-1}$·M$^{-1}$; ESI MS: m/z calculated for C$_{37}$H$_{44}$O$_2$P, 583.3; found, 583.3; 1H-NMR: (CD$_3$OD, 300K, AV-600) 2.64 ppm (H$_2$); 7.63 ppm (m, 15H, Ph); 13C-NMR: (CD$_3$OD, 300K, AV-600) 21.76 and 28.57 ppm (C 4', C6'); 188.36 and 188.55 ppm (C 3' and C6').
1.32 ppm (br. m, 12H, 1, 2, 3 - (CH2)3); 1.58-1.69 ppm (br. m, 12H, 4, 5, 6, 7, 8, 9 - (CH2)6); 2.47 ppm (t, J = 7.7 Hz, 2H, 10-CH2); 3.58-3.63 ppm (m, 2H, 1-CH2); 5.80 ppm (s, 3H, C4′); 7.63 ppm (m, 15H, Ph); 13C-NMR: (CD3OD, 303K): 12.17 ppm (2′-CH3); 22.68 ppm (d, J = 4.5 Hz, 2-CH2); 22.87 ppm (d, J = 49.2 Hz, 1-CH2); 26.26 (10-CH2); 28.46, 29.12, 29.20, 29.27, 29.30, and 29.67 ppm (4, 5, 6, 7, 8, and 9-(CH2)6); 30.40 ppm (d, J = 15.3 Hz, 3-CH2); 107.05 ppm (C2′); 118.47 ppm (d, J = 86.0 Hz, Cm of Ph); 130.49 ppm (d, J = 12.5 Hz, Cs of Ph); 133.74 ppm (d, J = 10.1 Hz, Co of Ph); 134.98 ppm (d, J = 2.7 Hz, Cp of Ph); 141.22 and 143.04 ppm (C1′ and Cp); 158.29 ppm (C5′); 182.09 ppm (C6′); 187.73 ppm (C3′).

The compound SkQ5 (3e) was identified as the corresponding quinol, [5-(3,6-dioxy-4,5-dimethyl-phen-1-yl)pentyl]triphenylphosphonium bromide (4), and prepared by reduction of 3e with NaBH4 in CH3OH. The yield (of 4) 18%; TLC: Rf (CHCl3–CH3OH–H2O, 65 : 25 : 4) 0.59. HPLC; tR = 7.0 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV: λmax – 268, 276, and 290 nm; ESI MS: m/z calculated for C31H34O2P, 469.57; found, 470.2; 1H-NMR: (DMSO-D6, 303K, AC-200) 1.45-1.62 ppm (br. m, 6H, 2, 3, and 4 - (CH2)3); 1.97 and 2.04 ppm (s and s, 3H and 3H, 4′- and 5′-(CH3)2); 2.41 ppm (br. t, Jt = 7 Hz, 2H, 5-CH2); 3.46-3.53 ppm (m, 2H, 1-CH2); 6.37 ppm (s, 1H, H2′); 7.06 (br. s, 1H, OH); 7.69-7.92 ppm (m, 15H, Ph); 8.13 ppm (br. s, 1H, OH); 13C-NMR: (DMSO-D6, 303K): 11.89 and 12.72 ppm (4′ and 5′-(CH3)2); 20.34 ppm (d, J = 49.5 Hz, 1-CH2); 21.74 ppm (d, J = 4.3 Hz, 2-CH2); 28.75 and 29.56 (4- and 5-CH2); 29.51 ppm (d, J = 16.4 Hz, 3-CH2); 113.01 ppm (C2′); 118.46 ppm (d, J = 85.6 Hz, Cs of Ph) 120.22 ppm (C1′); 124.84 and 126.61 ppm (Cx and Cx′); 130.09 ppm (d, J = 12.5 Hz, Cm of Ph); 133.41 ppm (d, J = 10.1 Hz, Cp of Ph); 134.71 ppm (d, J = 2.7 Hz, Cp of Ph); 144.65 and 147.83 ppm (Cy and Cy′).

Triphenyl(dodecyl)phosphonium iodide (5a) was synthesized from triphenylphosphine and 1-iodododecane by a similar procedure with 3a, the treatment of 5a by HCl/dioxane solution gave corresponding chloride (5b, C12TPP): yield, 62.5%; TLC: Rf (CHCl3–CH3OH, 4 : 1) 0.73, Rf (CHCl3–CH3OH, 9 : 1) 0.55; HPLC: tR = 10.5 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV (CH3OH): λmax – 200, 224, and 268 nm; ESI MS: m/z calculated for C30H40P, 431.6; found, 431.4; 1H-NMR: (CD3OD, 303K, AV-600) 0.883 ppm (t, Jt = 7.07 Hz); 1.23-1.36 ppm (br. m, 16H, 4, 5, 6, 7, 8, 9, 10, 11 - (CH2)8); 1.569 ppm (quintet, Jq = 7.50 Hz, 2H, 3-CH2); 1.671 ppm (sextet, Jsx = 8.0 Hz, 2H, 2-CH2); 3.48 ppm (m, 2H, 1-CH2); 7.74-7.78 ppm (m, 6H, Hm of Ph); 7.80-7.85 ppm (m., 6H, Hm of Ph); 13C-NMR: (CD3OD, 303K): 14.43 ppm (CH3); 22.84 ppm (d, J = 50.9 Hz, 1-CH2); 23.55 ppm (d, J = 4.3 Hz, 2-CH2); 29.89 (4-CH2); 30.34, 30.39, 30.51, 30.66, and 30.67 ppm (5, 6, 7, 8, and 9-(CH2)3); 31.55 ppm (d, J = 16.4 Hz, 3-CH2); 119.98 ppm (d, J = 86.2 Hz, Ci of Ph); 131.52 ppm (d, J = 12.5 Hz, Cm of Ph); 134.84 ppm (d, J = 9.9 Hz, Co of Ph); 136.21 ppm (d, J = 2.8 Hz, Cp of Ph).

The synthesis of [10-(4,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decyl]tributylammonium trifluoroacetate, SkQ4 (6), is outlined in Fig. S4.

11-Bromoundecanoic acid (7) was esterified with tert-butanol in the presence of N,N′-dicyclohexylcarbodiimide and N,N-dimethylaminopyridine to give tert-butylenyl ester (8), which was used for the synthesis of (9) followed by acid hydrolysis giving (10). Alkylation of 2,3-dimethyl-1,4-benzoquinone (1a) under the conditions described for 2a afforded crude SkQ4 (6), which was purified using a semi-preparative HPLC 10 × 250 mm column Ultrasphere 5 µm ODS, 5 ml/min, 25°C, 40-70% B for 20 min (A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); yield 5.4%; TLC: Rf (n-C4H9OH–H2O–CH3COOH, 4 : 1) 0.55; HPLC: tR = 10.06 min (4.6 × 150 mm column Luna 5 µm C18(2), 1.5 ml/min, 25°C, 5-95% B for 11 min; A: 0.05% aqueous TFA; B: 0.05% TFA in MeCN); UV (CH3OH): λmax – 202, 260, and 242 nm; ESI MS: m/z calculated for C30H54NO2, 460.75; found.
1-aminium chloride, chromatography with CHCl3–CH3OH–H2O (65 : 25 : 4) (11). The product 11 was purified by silica gel column described above for the synthesis of 2a to afford SkQ2M of hydroxyl group of carnitine methyl ester. The derivative oxalyldichloride to give (15), which was used for acylation -CH2 of Bu); 1.502 ppm (quintet Jqt = 7.0 Hz, 2H, 2-CH2); 1.416 ppm (sextet, Jsx = 6.510 ppm (t, Jt = 1.40 Hz, 1H, H (2')); 13C-NMR: (CD3OD, 300K): 11.93 and 12.33 ppm (4 6.510 ppm (t, Jt = 1.40 Hz, 1H, H (2')); 13C-NMR: (CD3OD, 300K, AV-600): 1.019 ppm (-CH2 of Bu); 22.76 ppm (2-CH2); 24.83 ppm (2-CH3); 2.771 and 2.806 ppm (m [AB-part of ABMXY-system: JMX = 8.51 Hz, JXY = 14.46 Hz], 1H, H(1''Y)); 5.639 ppm (q [M-part of ABMXY-system: Jq = 6.80 Hz], 1H, H(2'')).

Synthesis of lipophilic methyl carnitine-containing cation 2-[[11-(4,5-dimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)(decyl)oxy]carbonyl]oxy]-N,N,N-trimethyl-4-oxobutan-1-aminium chloride, SkQ2M (11) is shown in Fig. S5.

1,10-Decanedicarboxonic acid (14) reacted with the oxalylchloride to give (15), which was used for acylation of hydroxyl group of carnitine methyl ester. The derivative obtained (16) reacted with 1a under the conditions described above for the synthesis of 2a to afford SkQ2M (11). The product 11 was purified by silica gel column chromatography with CHCl3–CH3OH–H2O (65 : 25 : 4) as eluent, the final purification was done with a preparative HPLC; SkQ2M (11): the yield, 6.2%; TLC: Rf (CHCl3–CH3OH, 4 : 1) 0.41; tR = 24.4 min (4.6 × 250 mm column Luna 5 µm C18(2), 1 ml/min, 25°C, 0-90% B for 26.4 min; A: 100 mM H3PO4 in water; B: 100 mM H2PO4 in MeCN); M. p. 178-180°C (degr.); element anal.: calculated for C44H53ClN2O5: C, 72.53; H, 7.21; Cl, 4.22; N, 3.86; found: C, 72.53; H, 7.21; Cl, 4.22; N, 3.61; UV (C2H5OH): λmax ≈ 346 nm; ESI MS: m/z calculated for C44H53ClN2O5, 688.89; found, 689.4; IR(film): 3200, 2928, 2336, 1700, 1685, 1600, 1496, 1304 cm⁻¹; 1H-NMR (DMSO-D6, 303K): 6.57 ppm (s, 1H, H(5'''), and H(8''')); 7.44 ppm (dd, Jd = 7.8 Hz, Jd = 1.0 Hz, H(3''')); 13C-NMR (δ, ppm, DMSO-D6, 303K): 11.59 and 11.98 (4 6.513 ppm (t, Jt = 1.36 Hz, 1H, H (2')).

HOOC-(CH2)14-COOH

HOOC-(CH2)14-COCCI

14

15

HOOC-(CH2)14-COCCI

14

Fig. S5. Synthesis of SkQ2M.

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S5

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Stability of synthesized compounds in water solution at pH 6.5 and 37°C was found to decrease in the range MitoQ > SkQ3 > SkQ1 > SkQR1. A 50% decomposition of 0.1 mM SkQ1 took 54 h. For SkQR1, this value was 3.5 h. The stability decreased with increasing pH. All the compounds studied were stable in ethanol solution as well as in the solid state. In the latter case, $t_{1/2}$ for SkQ1 decomposition at 20°C was 4 months. Concentration of diluted water solution of all the above compounds was strongly affected by their sorption on the surface of some kinds of glass or plastic vessels where they were stored. Such an effect was absent if ethanol substituted for water by not less than 50%. Moreover, like other hydrophobic cations, decyltriphenylphosphonium derivatives of quinones are prone to occupy the air/water interface, so their solutions should be strongly shaken before use. The critical concentration for SkQ1 micelle formation measured with a Photocor Complex scattered laser goniometer (Photocor Instr., USA) was shown to be about 1.1 µM (Fig. S7).

Electronic spectra of oxidized and reduced SkQ1 are shown in Fig. S8a. The extinction coefficient for SkQ1 in ethanol at 267 nm was 21,730 cm$^{-1}$·M$^{-1}$.

In the next series of experiments, we compared the ESR spectra of semiquinone species of different ubiquinone and plastoquinone derivatives: MitoQ, CoQ1, SkQ1, decyl-PQ, PQ$_0$, SkQ3, and 2,3,5-trimethyl-1,4-benzoquinone. Some of these spectra are shown on Fig. S8b. The quinones were dissolved in ethanol and reduced by NaBH$_4$. The semiquinones were formed due to autooxidation of the corresponding quinols by O$_2$ at pH 8.

The EPR spectra of radical ions of MitoQ and short-chain analog of ubiquinol (CoQ$_1$) were found to be very similar. In both cases, the unpaired electron interacted with three protons of the methyl group at C5 and two protons of the methylene group at C6. However, hyperfine splitting due to methyl protons was about two times greater than hyperfine splitting due to methylene protons, leading to a more resolved spectrum for the methyl group.

Stability of synthesized compounds in water solution at pH 6.5 and 37°C was found to decrease in the range MitoQ > SkQ3 > SkQ1 > SkQR1. A 50% decomposition of 0.1 mM SkQ1 took 54 h. For SkQR1, this value was 3.5 h. The stability decreased with increasing pH. All the compounds studied were stable in ethanol solution as well as in the solid state. In the latter case, $t_{1/2}$ for SkQ1 decomposition at 20°C was 4 months. Concentration of diluted water solution of all the above compounds was strongly affected by their sorption on the surface of some kinds of glass or plastic vessels where they were stored. Such an effect was absent if ethanol substituted for water by not less than 50%. Moreover, like other hydrophobic cations, decyltriphenylphosphonium derivatives of quinones are prone to occupy the air/water interface, so their solutions should be strongly shaken before use. The critical concentration for SkQ1 micelle formation measured with a Photocor Complex scattered laser goniometer (Photocor Instr., USA) was shown to be about 1.1 µM (Fig. S7).

Electronic spectra of oxidized and reduced SkQ1 are shown in Fig. S8a. The extinction coefficient for SkQ1 in ethanol at 267 nm was 21,730 cm$^{-1}$·M$^{-1}$.
i.e. $a(3H) = 0.19$ mT, $a(2H) = 0.097$ mT. This means that unpaired electron density at C5 and C6 atoms of the quinone ring of both MitoQ and CoQ1 differed also about twofold. In the case of $Q_0$, unpaired electron density at C5-C6 atoms was shown to be equal to $a(3H) = 0.236$ mT and $a(1H) = 0.197$ mT.

The ESR spectra of SkQ1 and decyl-PQ can be characterized by almost identical interaction of unpaired electron with all nine protons of the quinone ring: six methyl protons at C2 and C3, one proton at C5 and two protons of methylene group at C6: $a(9H) = 0.18$ mT. The ESR spectrum of decyl-PQ was practically the same. Consequently, in both cases all carbon atoms of the quinone ring equally shared the unpaired electron.

The hyperfine structure of the ESR spectra mentioned above clearly demonstrates that the semiquinones of both MitoQ and SkQ1 exist predominantly in the form of anion radicals, which could be stabilized by hydrogen bonding.

The ESR spectrum of SkQ3 was more complicated than that of SkQ1. Replacement of the proton at C5 with a methyl group resulted in a situation when the unpaired electron became more unequally shared by the carbon atoms of the quinone ring: $a(9H) = 0.18$ mT, $a(2H) = 0.09$ mT. A similar result was observed in the case of $Q_0$ and 2,3,5-trimethyl-1,4-benzoquinone. The ESR spectra of these short-chain analogs of SkQ1 and SkQ3, respectively, demonstrated that unpaired electron density at C1-C6 atoms of the quinone ring were quite different (for $Q_0$, $a(6H) = 0.18$ mT, $a(2H) = 0.27$ mT; for 2,3,5-trimethyl-1,4-benzoquinone, $a(6H) = 0.181$ mT, $a(3H) = 0.225$ mT, $a(1H) = 0.191$ mT).

S2: SkQs in BLM and Isolated Mitochondria

When studied in BLM, the SkQ derivatives synthesized were found to destabilize the membrane at concentrations higher than about $5 \times 10^{-5}$ M. To study such high concentrations, thick planar phospholipid membrane (which is more stable than BLM) was used (Fig. S9). On
such membranes, the SkQ concentrations could be increased to \(10^{-3}\) M. Under these conditions, Nernstian diffusion potentials were obtained with SkQ1, SkQ3, MitoQ, C12TPP, and DMQ. As to SkQR1 (Fig. S9a), diffusion potential decreased below Nernstian at its concentrations above \(5 \times 10^{-5}\) M (most probably due to protonophorous activity of SkQR1). For SkQ2M and SkQ4, Nernstian potential was not observed even at high concentrations of these cations. However, it was increased in the presence of a penetrating anion, tetraphenyl borate (Fig. S9c).

In BLMs modified by gramicidin D, SkQ1 was shown to prevent inactivation of the gramicidin channels in the presence of a FeSO4, ascorbate, and tert-butyldihydroperoxide (Fig. S10).

Accumulation of SkQ1 by mitochondria in vitro is shown in Fig. S11. The SkQ1 concentration was measured by a TPP electrode [7]. One can see that the SkQ1 import by mitochondria takes about 15-20 min. Subsequent addition of uncoupler FCCP induces rather small but measurable release of SkQ1. Since the octanol/water partition coefficient for SkQ1 was about 13,000 : 1, a large portion of the SkQ1 uptake could be explained by a \(\Delta\psi\)-independent, uncoupler-insensitive, passive sorption of SkQ1 by mitochondrial membranes, whereas the rest (uncoupler-sensitive portion) was due to the electrophoretic SkQ1 accumulation inside mitochondria. In line with this reasoning, substitution of SkQ1 by less hydrophobic SkQ5 (pentane instead of decane as a linker between triphenylphosphonium and plastoquinone; octanol/water partition coefficient about 500) decreased energy-independent uptake whereas energy-dependent uptake was increased (cf. Fig. S11, a and b).

Experiments showed that SkQ1 can be reduced by the mitochondrial respiratory chain. In the cases of succinate as reductant (Fig. S12), malonate added 15 min after succinate induced oxidation of SkQ1H2, which was much slower than the preceding reduction of SkQ1 by succinate. This oxidation was inhibited by myxothiazol, indicating that Complex III is competent in the SkQ1H2 oxidation, the rate of oxidation being lower than the rate of SkQ1 reduction by succinate.

Similar relationships were revealed when glutamate and malate substituted for succinate as the SkQ1 reductant (Fig. 4a (main text; here and further figures designated without “S” refer to the main article)). Rates of reduction of SkQ1 and MitoQ by succinate were found to be similar (not shown). The data of Fig. S12 indicate that SkQ1 added to mitochondria should be maintained mainly in the quinol form, a feature favorable for antiox-
idant activity of this compound. In line with such a conclusion, we found that addition of myxothiazol, preventing quinol oxidation by the respiratory chain, increased only slightly the antioxidant activity of SkQ1 under the Fenton reaction conditions (Fig. S13a).

Results of a study of prooxidant activity of the cationic quinones are shown in Fig. S13, b-d. As follows from the data (Fig. S13, b and c), addition of μmolar MitoQ, SkQ1, or SkQ3 to mitochondria oxidizing glutamate and malate or succinate in State 4 strongly stimulates H$_2$O$_2$ production. C$_{12}$TPP fails to reproduce such an effect. The H$_2$O$_2$ generation is strongly inhibited by rotenone (Fig. S13, b and c) or uncoupler SF6847 (Fig. S13d). In the latter case, very low (C$_{1/2}$ = 6·10$^{-9}$ M) concentration of uncoupler is required. This value is close to that for inhibiting reverse electron transfer in Complex I and much smaller than that inducing half-maximal decrease in the Δψ level. This fact as well as the complete inhibition by rotenone of H$_2$O$_2$ formation with succinate indicate that ROS generation induced by cationic quinones is coupled to reverse electron transfer via Complex I. As to partial rotenone inhibition of H$_2$O$_2$ formation with NAD-substrates, it can be explained by a Δψ collapse (due to arrest of respiration), which prevents accumulation of cationic quinones in mitochondria.

**S3: SkQ1 in Human Cell Cultures**

As shown in the present paper, SkQ1 arrests apoptosis induced by low [H$_2$O$_2$] in cell cultures (Fig. 6, b and c). However, addition of large H$_2$O$_2$ amounts was shown to overcome the SkQ1-induced inhibition of apoptosis (Fig. S14a). As to a SkQ1-sensitive apoptosis induced by low [H$_2$O$_2$], it can be accounted for by the principle of ROS-induced ROS release when small amounts of ROS stimu-
Late formation of large ROS amounts (Fig. 6d). Quite recently, Pinton et al. described a molecular mechanism of such kind of event [8]. They reported that added H$_2$O$_2$ stimulates phosphorylation of p66shc, a “lifespan-shortening protein”, by protein kinase C$_\beta$. Phosphorylated protein (P-p66shc) becomes a target for proline isomerase Pin1, which converts P-p66shc to a conformer imported by mitochondria. In the mitochondrial intermembrane space, P-p66shc combines with cytochrome c, an event most probably resulting in that cytochrome c acquires ability to reduce O$_2$ to O$_2^-$ [9]. This, in turn, increases mitochondrial [ROS] and opens the permeability transition pore in the inner mitochondrial membrane. The latter event leads to release of cytochrome c and other pro-apoptotic proteins from mitochondria to cytosol, thereby initiating apoptosis.

One of steps of the above cascade, namely, H$_2$O$_2$-induced cytochrome c release, was already confirmed in our group (Fig. S14b). In the same experiment, the proapoptotic protein BAX was shown to migrate to mitochondria in the H$_2$O$_2$-treated cells. Both processes were
arrested by SkQ1. Identification of p66shc as a possible player in this episode is now under investigation.

Figure S14c shows that antiapoptotic effect of SkQ1 on the H₂O₂-treated cells cannot be reproduced by C₁₂TPP and decylplastoquinone (DPQ), compounds of similar hydrophobicity but lacking either quinone or cationic residue. Even more, C₁₂TPP proved to stimulate the H₂O₂-induced apoptosis.

Figure S15 shows that SkQ1 is more effective than MitoQ in protection against H₂O₂-induced fragmentation of mitochondria in HeLa cells. Prooxidant effect of SkQ1 was observed at higher concentrations and was prevented by Trolox.

In Fig. S16, concentration dependence of antinecrotic SkQ1 effect is seen, C₁/₂ being within \((2-5) \times 10^{-7} \text{ M}\) SkQ1.

REFERENCES

Fig. 5. (Y. N. Antonenko et al.) Co-localization of SkQR1 (a fluorescent SkQ derivative) and mitochondria-targeted jellyfish yellow fluorescent protein YFP fused with the leader sequence of cytochrome oxidase subunit VIII. HeLa cells were transfected with Mito-YFP (Clontech) and incubated for 15 min with 100 nM SkQR1. Bar, 15 µm.

Fig. 9. (Y. N. Antonenko et al.) SkQ1 initiates formation of a united mitochondrial electric network in HeLa cells. Arrow shows the place of illumination of a mitochondrion with a narrow argon laser beam of a Zeiss LSN 510 confocal microscope (488 nm; 50 sec; light spot, 6 × 60 pixels). Cells were stained with 100 nM TMRE for 15 min and analyzed 15 min after the laser illumination. Where indicated, cells were pre-treated with 20 nM SkQ1 for 7 days. Bar, 15 µm.