Electronic Supplementary Information

Synergic Nanoantioxidant Based on Covalently Modified Halloysite-Trolox Nanotubes with Intra-lumen Loaded Quercetin

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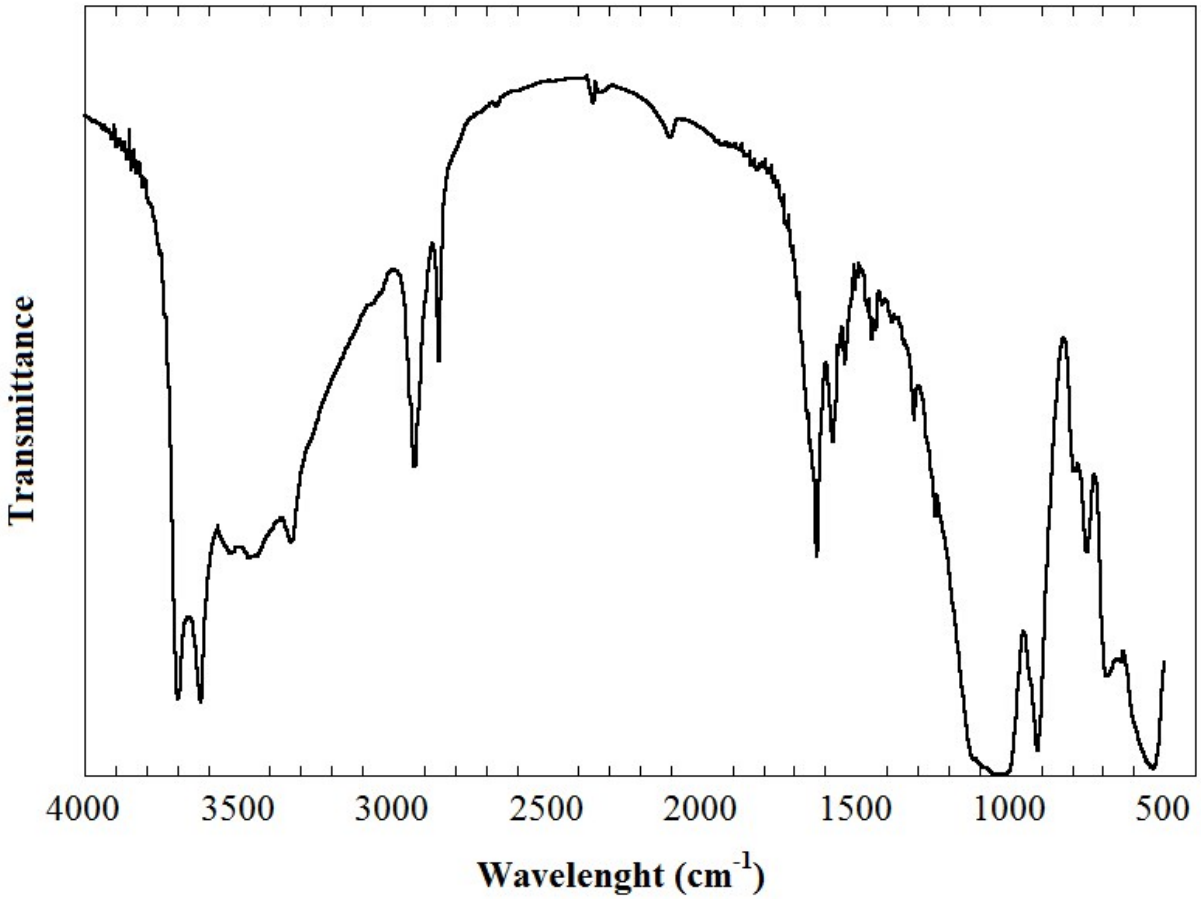

Figure S1. FT-IR spectrum of HNT-Trolox nanomaterial.

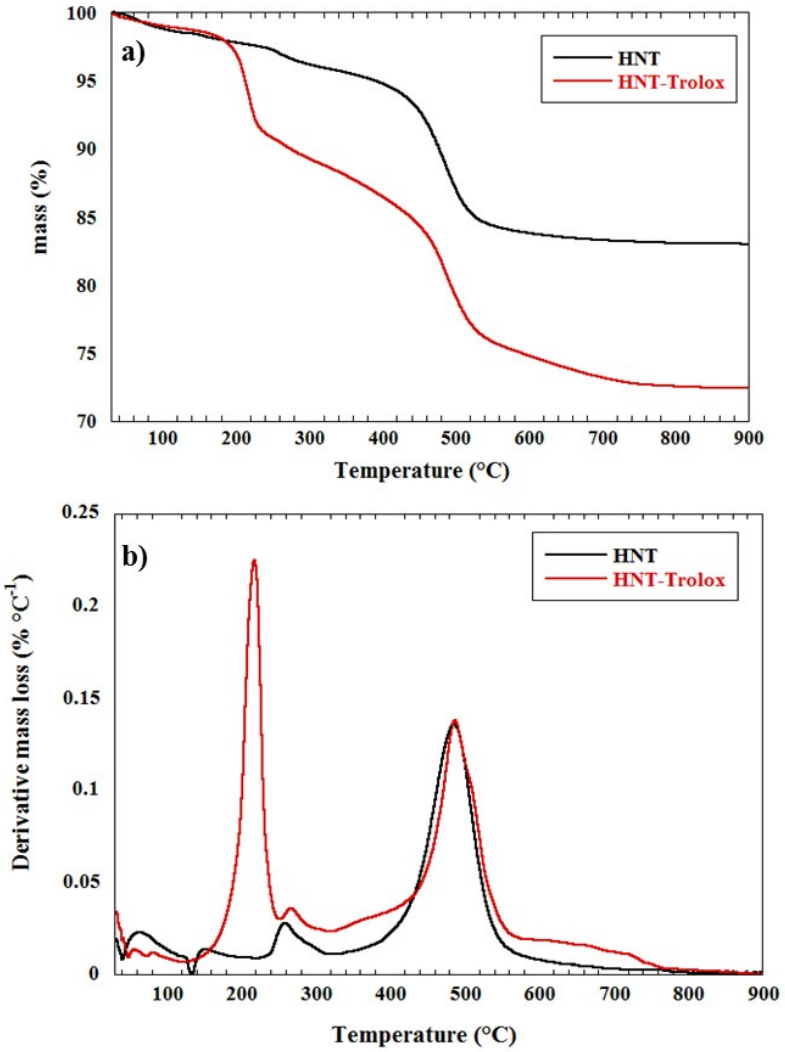

Figure S2 Thermogravimetric (a) and derivative thermogravimetric (b) curves of HNT and HNT-Trolox nanomaterials.

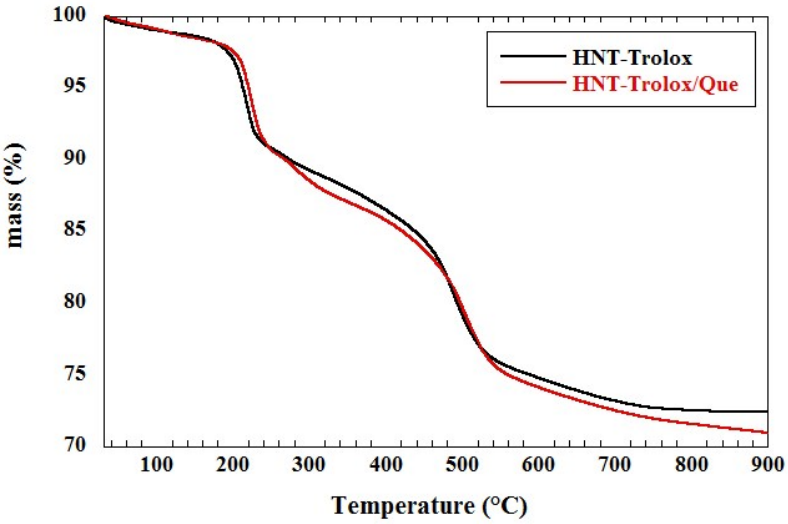

Figure S3 Thermogravimetric curves for HNT-Trolox before and after quercetin loading.

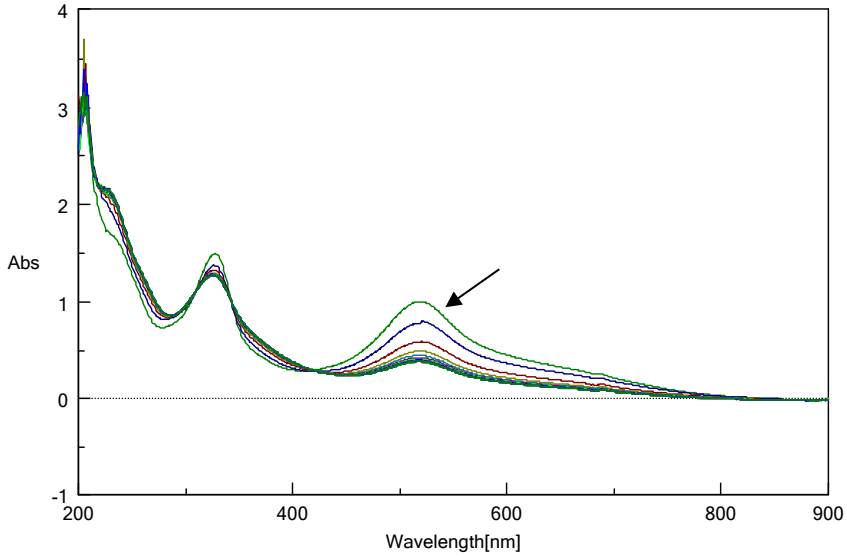

Figure S4. DPPH$^{\bullet}$ absorption spectra upon reaction with Trolox; DPPH$^{\bullet}$: 1.0 x 10^{-4} M, Trolox: 2.5 x 10^{-5} M. The arrow indicates the spectrum of DPPH$^{\bullet}$ before the addition of antioxidant.

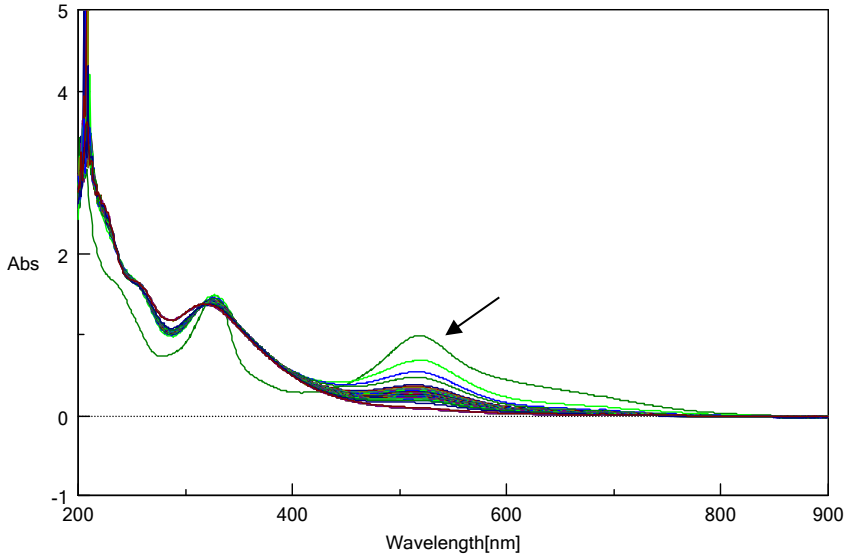

Figure S5. DPPH$^{\bullet}$ absorption spectra upon reaction with quercetin; DPPH$^{\bullet}$: 1.0 x 10^{-4} M, quercetin: 2.5 x 10^{-5} M. The arrow indicates the spectrum of DPPH$^{\bullet}$ before the addition of antioxidant.

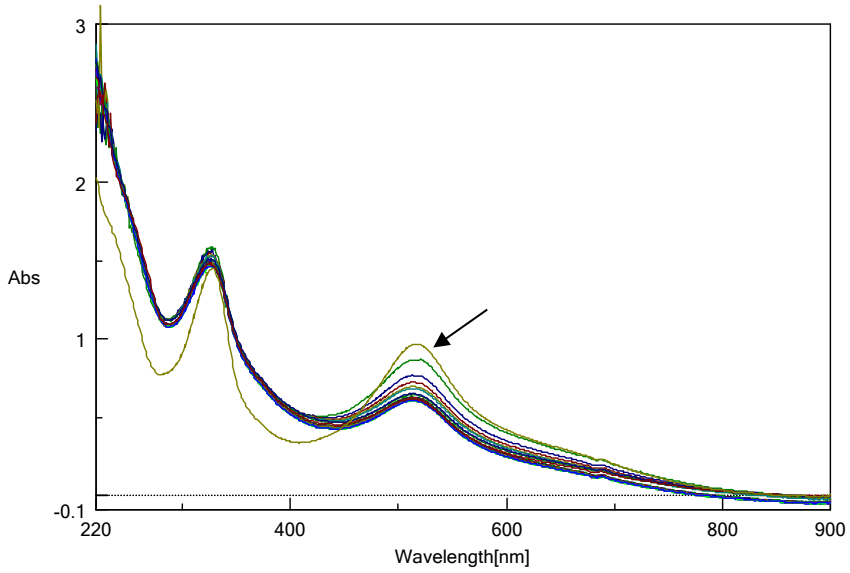

Figure S6. DPPH• absorption spectra upon reaction with HNT/Que; DPPH•: 1.0 x 10^{-4} M, HNT/Que: 2.5 x 10^{-5} M of loaded quercetin. The arrow indicates the spectrum of DPPH• before the addition of antioxidant.

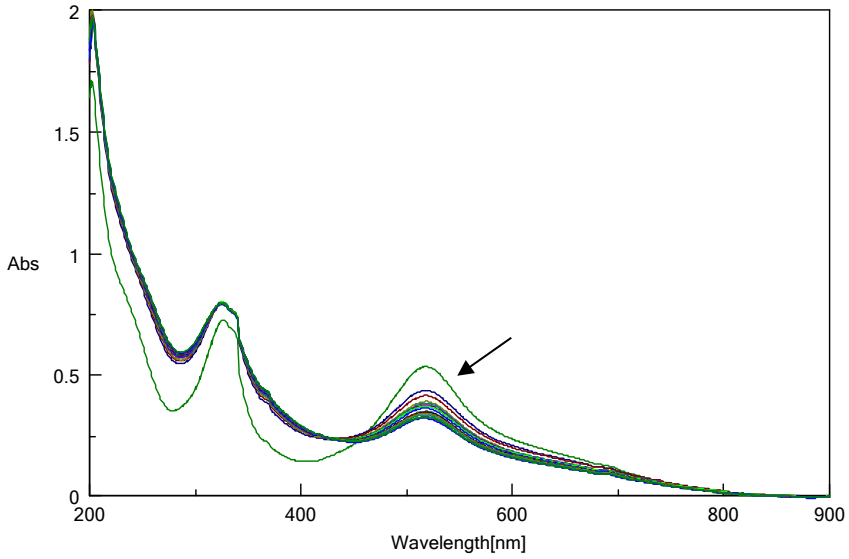

Figure S7. DPPH• absorption spectra upon reaction with HNT/Que; DPPH•: 5.0 x 10^{-5} M, HNT/Que: 6.2 x 10^{-6} M of loaded quercetin. The arrow indicates the spectrum of DPPH• before the addition of antioxidant.

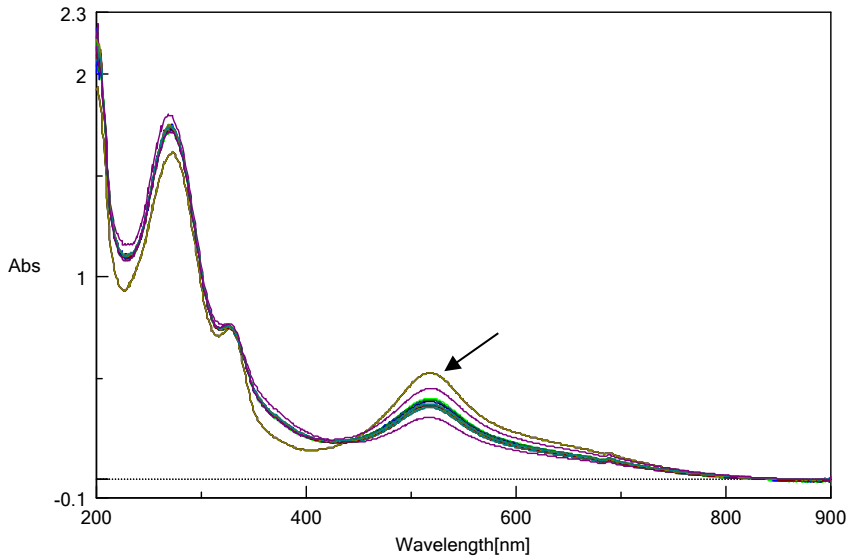

Figure S8. DPPH$^{\bullet}$ absorption spectra upon reaction with HNT-Trolox/Que; DPPH$^{\bullet}$: 5.0 x 10^{-5} M, HNT-Trolox/Que: 6.2 x 10^{-6} M of loaded Trolox + 1.7 x 10^{-6} M of loaded quercetin. The arrow indicates the spectrum of DPPH$^{\bullet}$ before the addition of antioxidant.

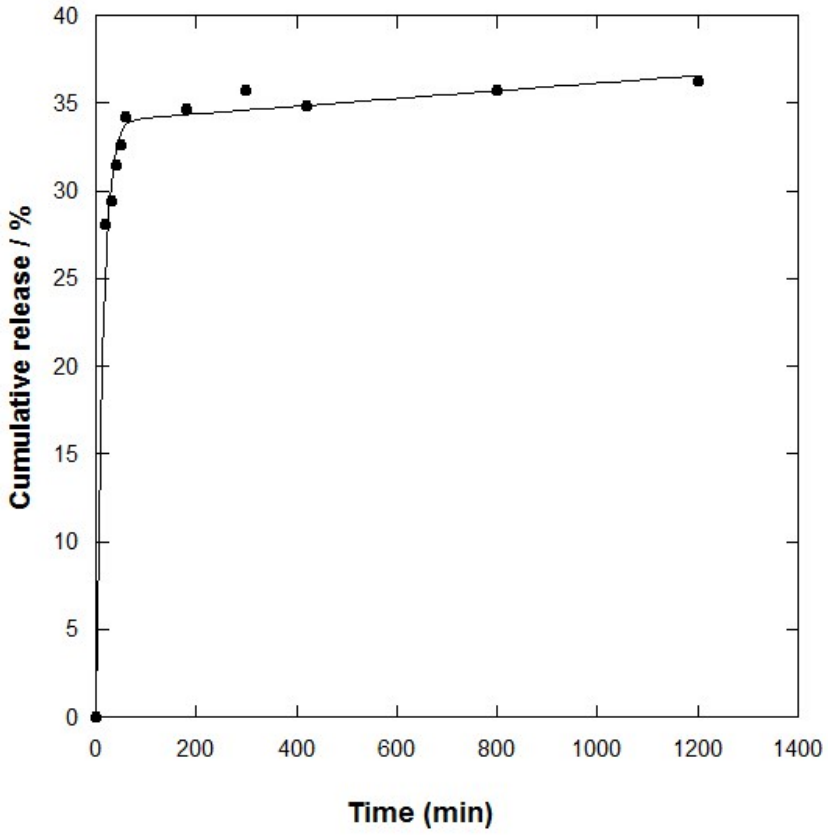

Figure S9. Release of quercetin from HNT/Que in acetonitrile, measured spectrophotometrically at 370 nm.

Numerical fitting of O_2 consumption traces

In order to gain mechanistic insight into the antioxidant effect of HNT/Que, the oxygen consumption plots were analyzed by means of Gepasi (http://www.gepasi.org), a chemical simulation software able to reproduce the transient concentrations of intermediates during chemical reactions. The reaction scheme used to simulate the autoxidation inhibited by quercetin included into halloysite nanotubes is reported below (Equations 1-11).

$$\text{Initiator} \xrightarrow{R_i} R^{\bullet} \quad (1)$$

$$R^{\bullet} + O_2 \xrightarrow{k_2} ROO^{\bullet} \quad (2)$$

$$ROO^{\bullet} + RH \xrightarrow{k_3} R^{\bullet} + ROOH \quad (3)$$

$$2\ ROO^{\bullet} \xrightarrow{k_4} \text{non radical products} \quad (4)$$

$$ROO^{\bullet} + AH_2BH_2 \xrightarrow{k_5} ROOH + AHBH_2^{\bullet} \quad (5)$$

$$AHBH_2^{\bullet} + ROO^{\bullet} \xrightarrow{k_6} ROOH + ABH_2 \quad (6)$$

$$ROO^{\bullet} + ABH_2 \xrightarrow{k_7} ROOH + ABH^{\bullet} \quad (7)$$

$$ABH^{\bullet} + ROO^{\bullet} \xrightarrow{k_8} ROOH + ABH_2 \quad (8)$$

$$L1AH_2BH_2 \underset{k_{-9}}{\overset{k_9}{\rightleftharpoons}} L1 + AH_2BH_2 \quad (9)$$

$$L2AH_2BH_2 \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} L2 + AH_2BH_2 \quad (10)$$

$$L3AH_2BH_2 \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} L3 + AH_2BH_2 \quad (11)$$

Equations 1-4 describe the autoxidation of a generic oxidizable substrate (RH), that in our case is represented by cumene or styrene, whose propagation and termination rate constants are k_3 and k_4, respectively, and are known from previous studies (see references in Table S1). The initiation rate

(R_i) is experimentally determined for each reaction condition ($3.0\pm0.2\times10^{-9}$ M s^{-1}), while k_2 is diffusion controlled (10^9 $M^{-1}s^{-1}$). Equations 5-8 represent the antioxidant effect of quercetin, which is described as a bifunctional compound (AH_2BH_2) able to trap 4 ROO• radical, but with different efficiency for the two units. The rate constants for the reaction of the two units of quercetin with ROO• radicals (k_5-k_7) are available from the studies performed in the absence of HNT, while k_6-k_8 are known to be fast reactions (10^8 $M^{-1}s^{-1}$) that occur at a nearly diffusion rate (E. T. Denisov, I. V. Khudyakov, *Chem. Rev.* **1987**, *87*, 1313–1357).

Table S1. Rate constants used for the fitting of the O_2-consumption plot.

	Rate constant ($M^{-1}s^{-1}$)	
	styrene[a]	**cumene[b]**
k_3	41	0.32
k_4	2.1×10^7	2.3×10^4

[a] J. A. Howard, K. U. Ingold, *Can. J. Chem.* **1965**, *43*, 2729-2736. [b] R. F. Enes, A. C. Tomè, J. A. S. Cavaleiro, R. Amorati, M. G. Fumo, G. F. Pedulli, L. Valgimigli, *Chem. Eur. J.* **2006**, *12*, 4646 – 4653

The release of quercetin from HNT is described by reactions 9-11. Several attempts were performed to properly simulate the O_2-consumption plots obtained from autoxidation experiments. In general, in both solvents in which it was studied, the quercetin release followed a two-phases process, comprising a fast release and a slower one. Although we succeeded in describing the autoxidation experiments by a two-sites model, the relative amount of quercetin released in the "fast" phase differed considerably depending on the solvent, being about 10% in chlorobenzene and about 40% in acetonitrile. In order to have a unique model for the antioxidant activity of HNT/Que, we combined information obtained experimentally in the two solvent systems and hypothesized that quercetin loaded on HNT is distributed into three sites, with low, medium and high affinity for

quercetin. The low affinity site (L1) contains 10% of the loaded quercetin and it is able to quickly release quercetin, either when the solvents are chlorobenzene or acetonitrile. The medium affinity site (L2) contains about 29% of quercetin, while the high affinity site (L3) contains the remaining 61%. From the numerical fitting of the experimental O_2-consumption plots according to this 3-stages model, it is evident that the medium affinity site (L2) releases quercetin only when the solvent is acetonitrile. On the other hand, the high affinity site L3 gives a negligible release of quercetin in both solvents.

In Figure S10 and S11 the simulated release of quercetin from HNT, is calculated on the basis of the kinetic data obtained from numerical fitting of the experimental O_2-uptake traces. In particular, we show the instantaneous quercetin concentration, and the overall percent released of quercetin during the time course of the autoxidation experiment.

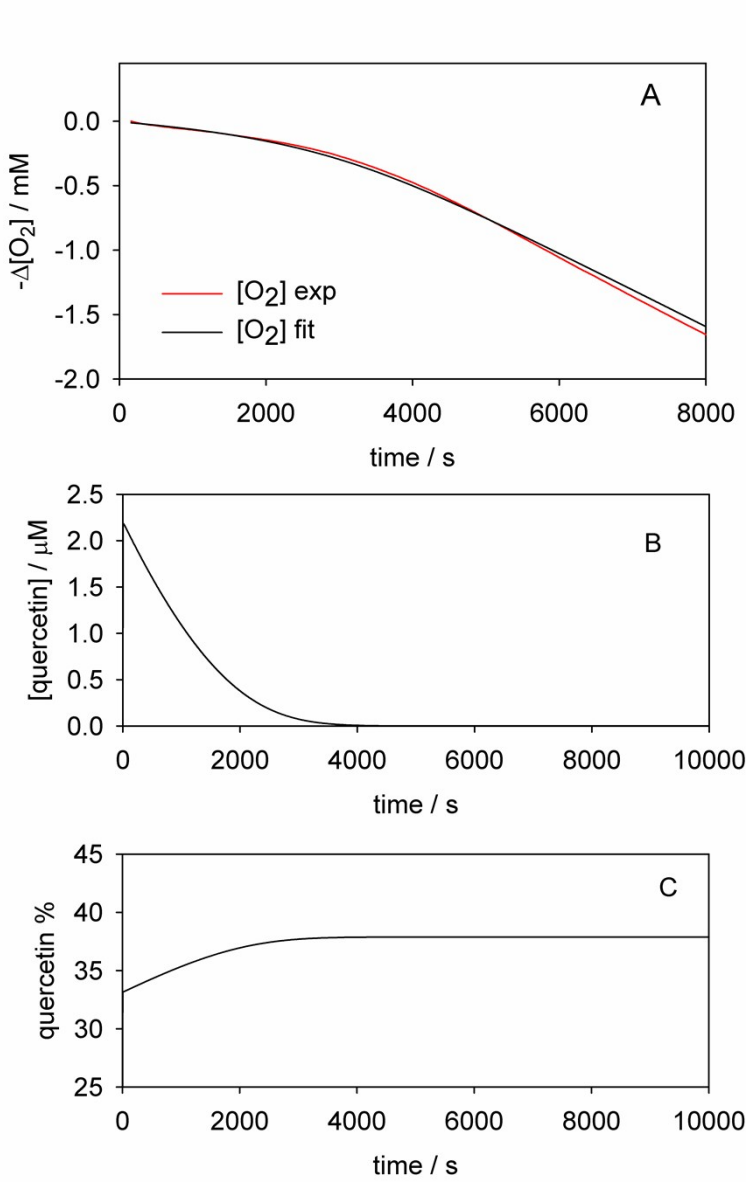

Figure S10. (A) Fitting of the O_2 consumption plot measured during the autoxidation of cumene (3.5 M) in acetonitrile initiated by AIBN (2.5×10^{-2} M) at 30°C, inhibited by HNT/Que corresponding to 6.6 μM of quercetin. (B) Calculated instantaneous concentration of free quercetin in solution during the autoxidation. (C) Calculated cumulative percent release of quercetin during the autoxidation.

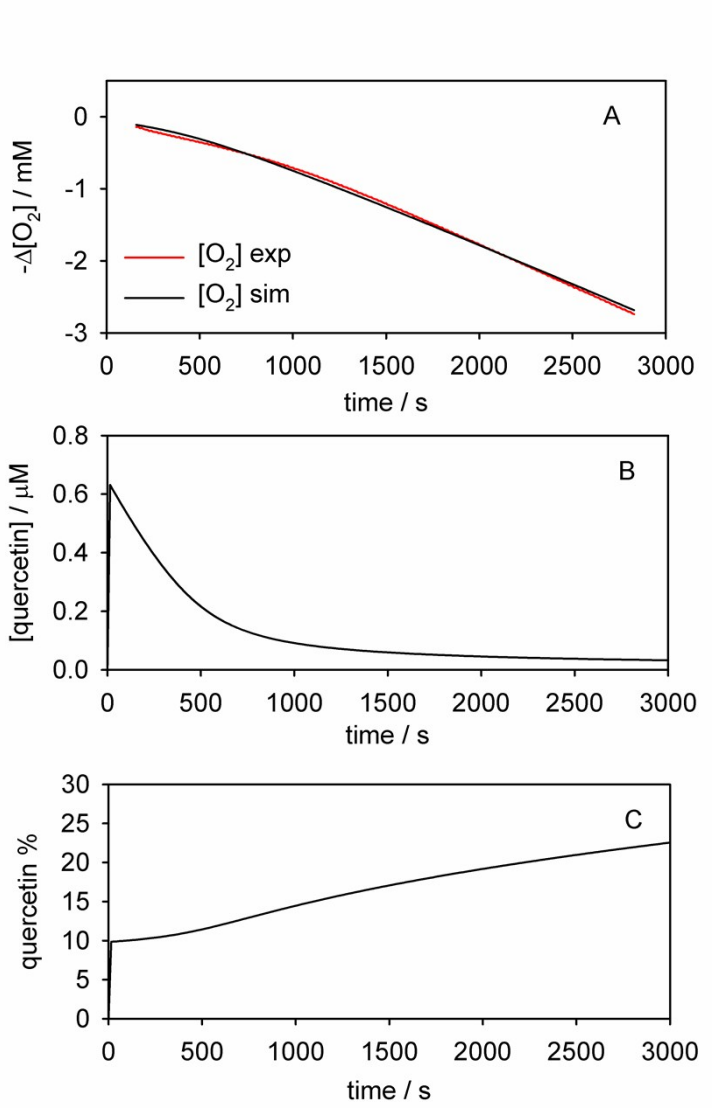

Figure S11. Results from the fitting of the O_2 consumption plot measured during the autoxidation of styrene (4.2 M) in acetonitrile initiated by AIBN (2.5×10^{-2} M) at 30°C, inhibited by HNT/Que corresponding to 6.6 μM of quercetin. (A) Fitting of the O_2 consumption. (B) Calculated instantanous concentration of free quercetin in solution. (C) Calculated cumulative percent release of quercetin during the autoxidation.

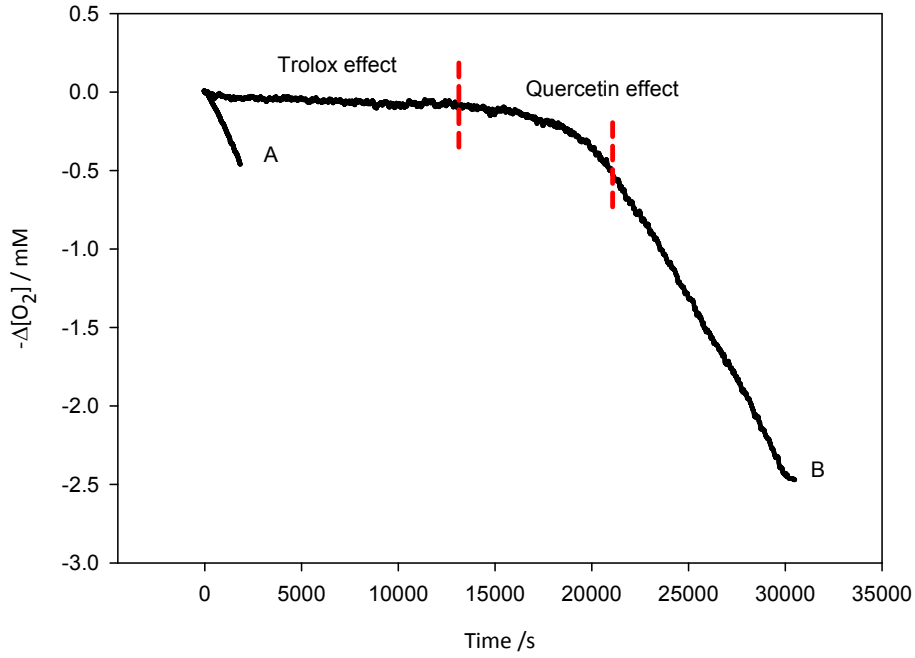

Figure S12. Representative oxygen consumption during the autoxidation of (A) cumene (3.6 M) initiated by AIBN (0.025 M) in ACN at 30° C in the absence of inhibitors; (B) or in the presence of Trolox 2.5 × 10^{-5} M and quercetin 6.6 X 10^{-6} M. The stoichiometric coefficient for the mixture is n = 1.8 ± 0.1.

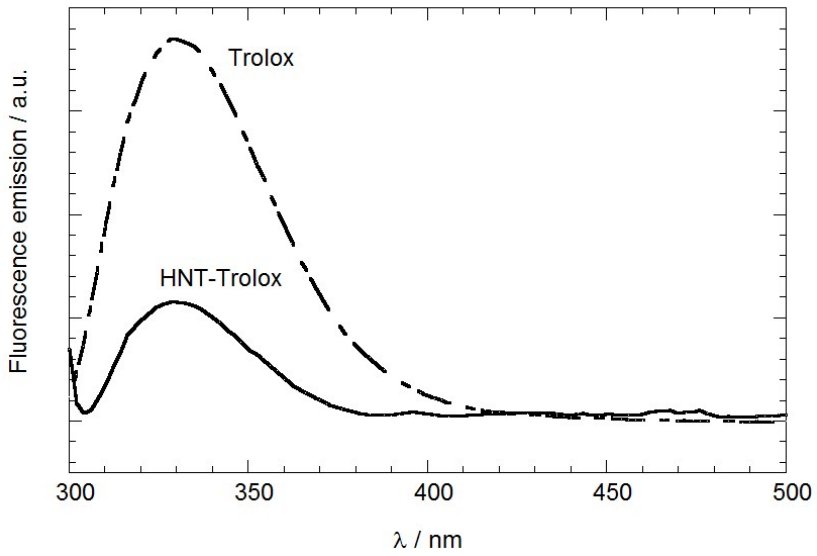

Figure S13. Fluorescence emission spectra for Trolox and HNT-Trolox hybrid in methanol.