Electronic Supplementary Material (ESI) for RSC Advances.

Formation and characterization of crosslinks, including Tyr-Trp species, on one electron oxidation of free Tyr and Trp residues by carbonate radical anion.

Supplementary Figures

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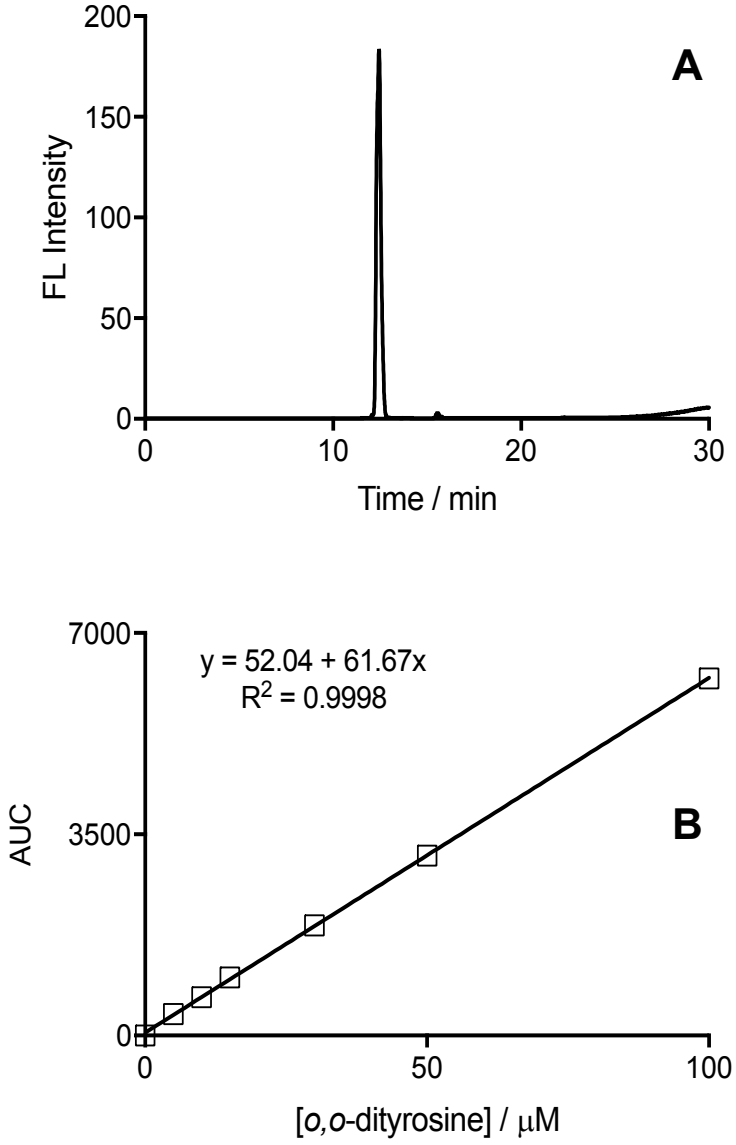
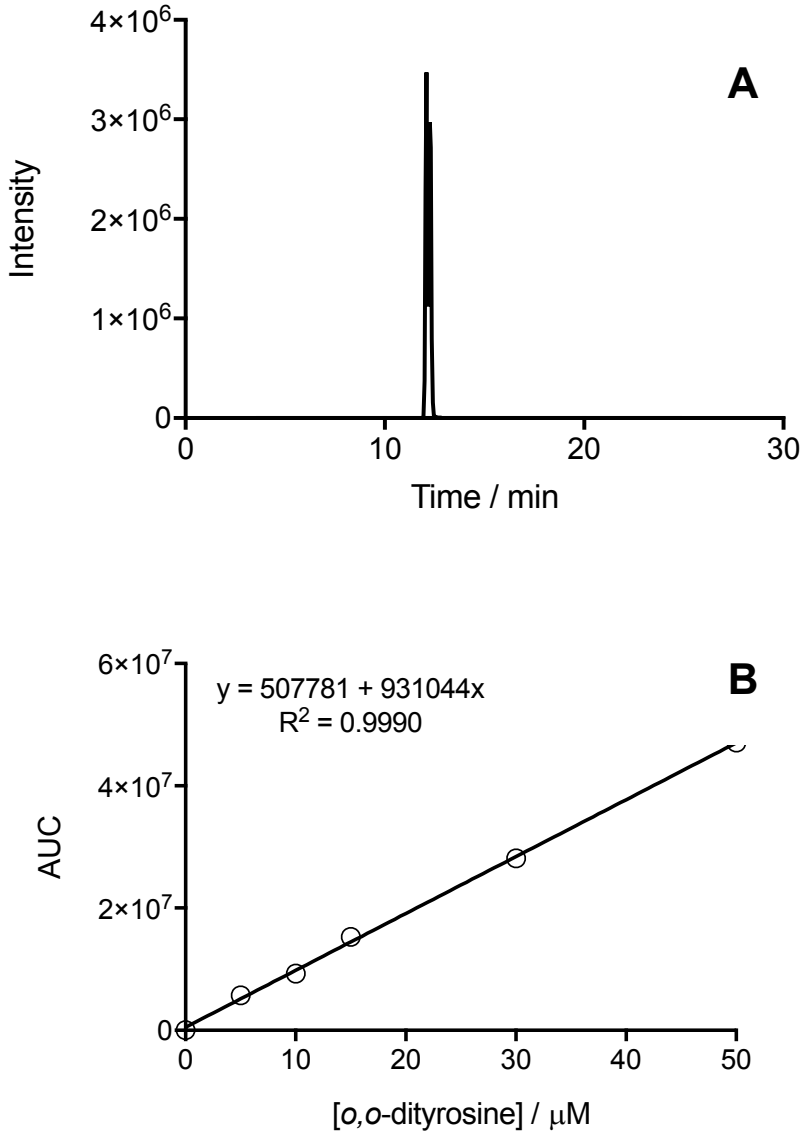
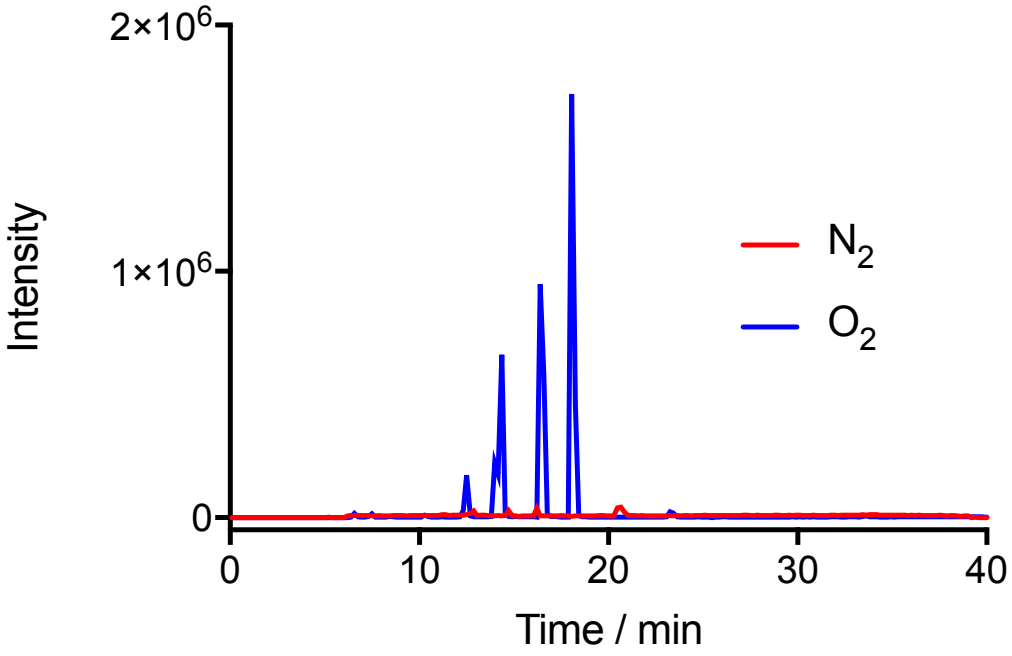


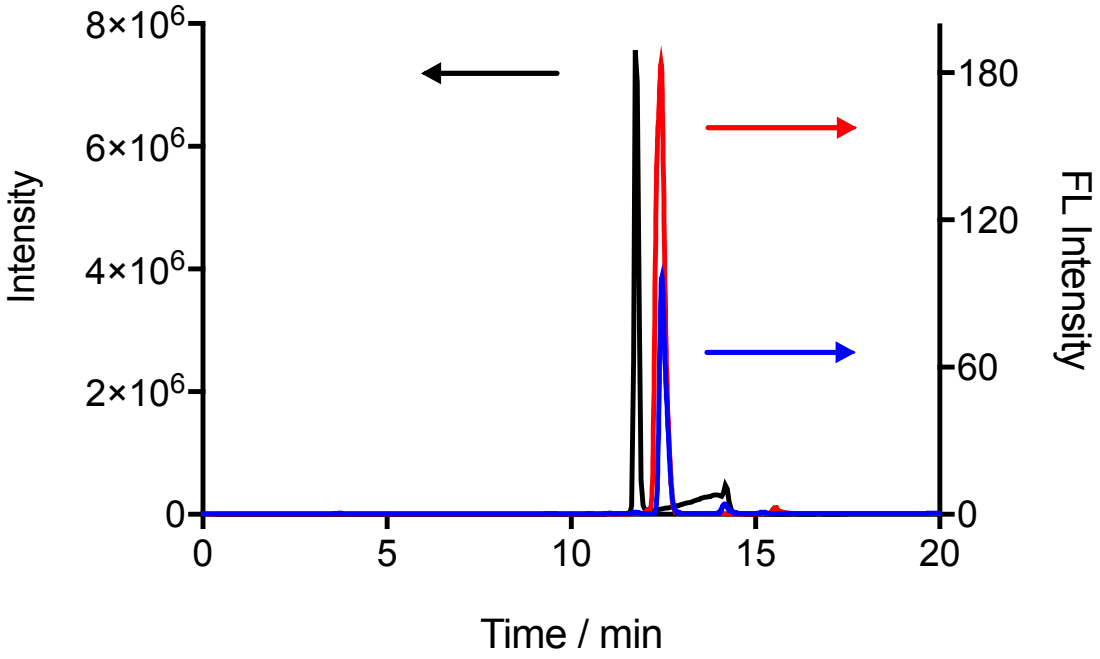
Supplementary Figure 1.
Quantification of *o,o*-dityrosine by liquid chromatography with fluorescence detection. Panel A shows a representative chromatogram, obtained using λ_{ex} 280 and λ_{em} 410 nm, obtained for a solution containing the commercial standard of di-Tyr at 50 μM (in phosphate buffer 75 mM, pH 7.4). Panel B depicts the calibration curve constructed using the area under the curve (AUC) of the HPLC peaks registered between 5 and 100 μM of *o,*o'-di-Tyr (commercial standard). Data represent the average of at least three independent experiments, each measured in triplicate.



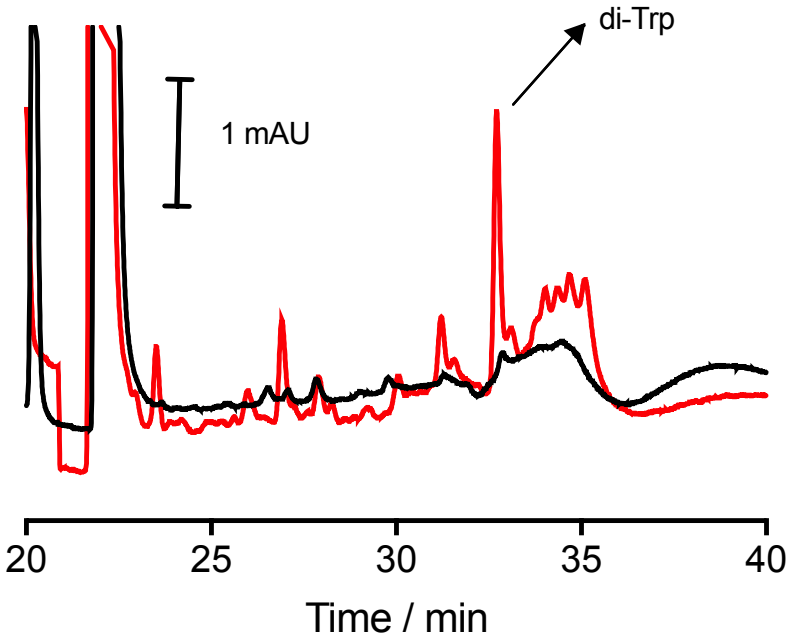
Supplementary Figure 2.
Quantification of *o,o'*-dityrosine by liquid chromatography (UPLC) with mass spectrometry (MS) detection. Panel A shows a representative chromatogram registered using the SRM mode (361 → 315) obtained for a solution containing the commercial standard of di-Tyr at 50 μM (phosphate buffer 75 mM, pH 7.4). Panel B depicts the calibration curve constructed from the area under the curve (AUC) of the UPLC peaks detected for 5 - 100 μM of *o,*o'-di-Tyr (commercial standard). Data represent the average of at least three independent experiments, each measured in triplicate.



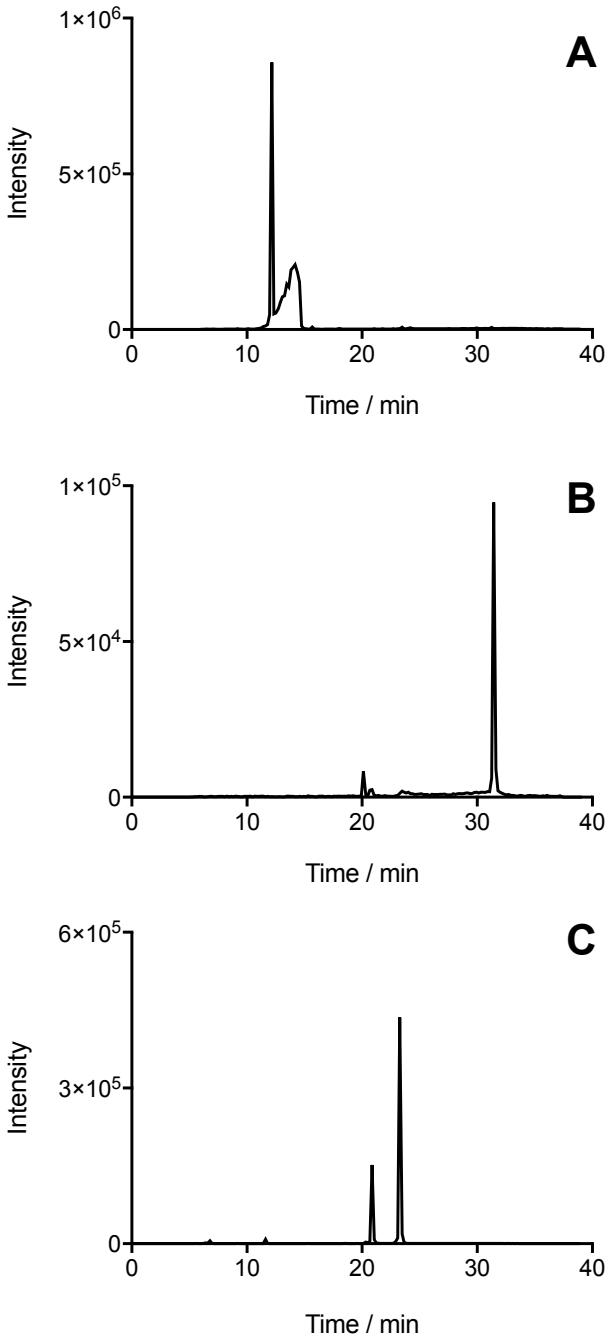
Supplementary Figure 3.
Extracted ions chromatograms at *m/z* 237, obtained from Trp (1 mM) samples illuminated (at 254 nm for 3 min) under aerobic (O_2 20%, blue trace), and anaerobic (N_2 bubbling; red trace) in the presence of the $[Co(NH_3)_5(CO_3)]NO_3$ complex (4 mM).



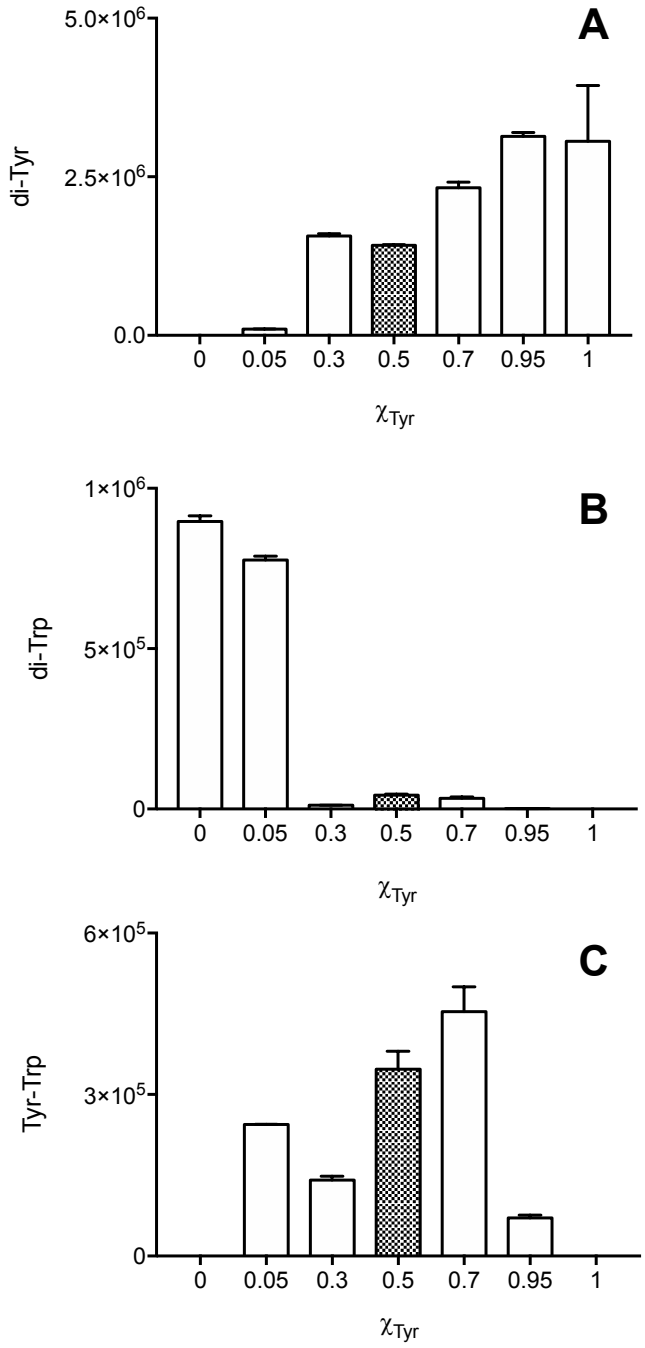
Supplementary Figure 4.
Comparative detection of di-Tyr in samples containing Tyr (1 mM) illuminated for 3 min in the presence of 4 mM $[Co(NH_3)_5(CO_3)]NO_3$ under an atmosphere of N_2. Black line shows a typical UPLC chromatogram obtained by MS fragmentation analysis (transition *m/z* 361 → 315) employing the SRM mode. Red and blue lines show HPLC chromatograms obtained with fluorescence detection (λ_{ex} 280 and λ_{em} 410 nm) for the *o,o*-dityrosine commercial standard (50 μM), and the illuminated Tyr-$[Co(NH_3)_5(CO_3)]NO_3$ solution, respectively. The observed differences between the retention times determined by UPLC-MS and HPLC-FL (12.4 versus 11.8 min) are attributed to the use of different instruments for these assays.



Supplementary Figure 5.
Comparative detection of di-Trp in samples containing Trp (1 mM) illuminated for 3 min in the presence of 4 mM $[Co(NH_3)_5(CO_3)]NO_3$ under an atmosphere of N_2. Red trace shows a typical HPLC chromatogram obtained with DAD detection (fixed at 280 nm). Black trace shows a HPLC-DAD chromatogram obtained for control solutions (not illuminated). Identification of the di-Trp peak was carried out based on the retention time.



Supplementary Figure 6.
Extracted ions chromatograms for detection of di-Tyr (panel A), di-Trp (panel B), and Tyr-Trp (panel C) obtained from solutions containing Tyr and Trp, each at 250 μM (500 μM total concentration) illuminated (at 254 nm for 3 min) in the presence of 4 mM $[Co(NH_3)_5(CO_3)]NO_3$, under an atmosphere of N_2. The SRM mode, using 361 → 315; 407 → 203, and 384 → 203, was employed for the detection of di-Tyr, di-Trp and Tyr-Trp, respectively. It should be noted that under these conditions (total concentration of 500 μM) only two peaks were registered for 384 → 203.



Supplementary Figure 7.
Formation of di-Tyr, di-Trp and Tyr-Trp depends on the concentration of Tyr and Trp. Solutions containing Tyr and Trp (1 mM total concentration) in phosphate buffer 75 mM, pH 7.4 (DTPA 0.1 mM) were illuminated for 3 min at 254 nm in the presence of 4 mM $[Co(NH_3)_5(CO_3)]NO_3$ under an atmosphere of N_2. The area under the curve of the chromatographic peaks for di-Tyr (MS transition *m/z* 361 $\rightarrow$ 315, panel A), di-Trp (*m/z* 407 $\rightarrow$ 203, panel B), and Tyr-Trp (*m/z* 384 $\rightarrow$ 367, panel C), were determined by MS as described in Material and methods. The concentrations of Tyr and Trp are expressed as molar fraction of Tyr (χ_{Tyr}), which means χ_{Tyr} = 0 a solution containing Trp 1 mM without Tyr, and χ_{Tyr} = 1, a solution containing Tyr 1 mM without Trp.