

Electronic Supporting Information

Gramicidin Conformational Changes During Riboflavin Photosensitized Oxidation in Solution and the Effect of *N*-methylation of Tryptophan Residues

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1. ¹H NMR spectra for 1-methyl-boc-*L*-tryptophan methyl ester

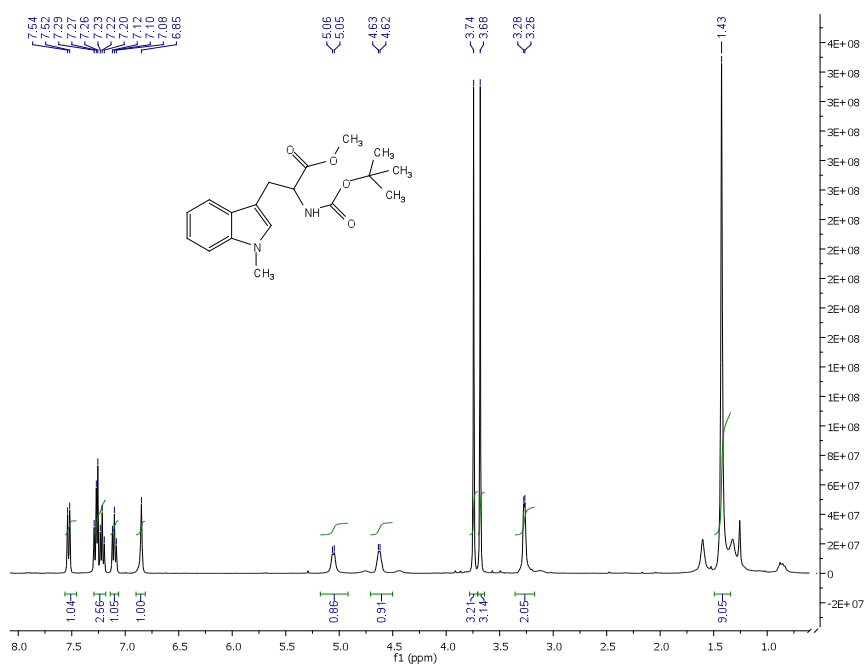


Figure S1. ¹H NMR for 1-methyl Trp derivative in CDCl₃.

2. ^{13}C NMR spectra for 1-methyl-boc-L-tryptophan methyl ester

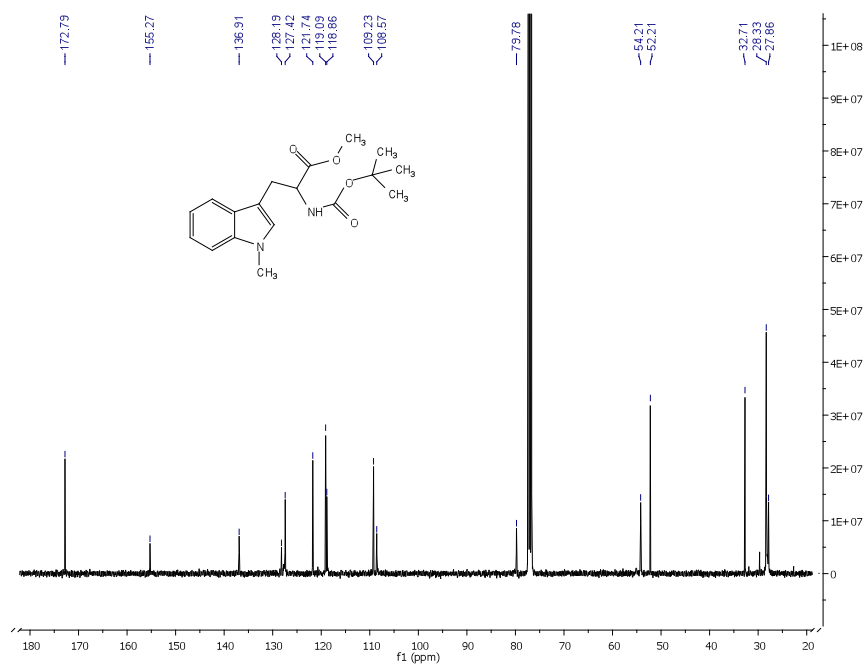


Figure S2. ^{13}C NMR for 1-methyl Trp derivative in CDCl_3 .

3. Gramicidin photo-oxidation in the presence of riboflavin in TFE

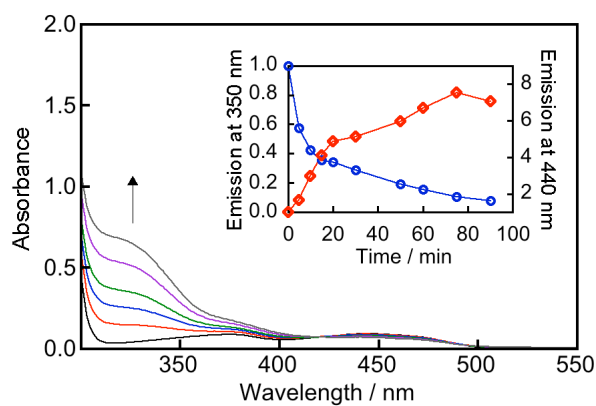


Figure S3. Absorption spectra for Gr (10 μM) irradiated in the presence of RF (10 μM) during 0, 5, 10, 15, 25 and 35 min in TFE. Inset: Fluorescence intensity decrease for the oxidation of Trp residues in Gr (Ex 280 / Em 350 nm) and fluorescence intensity increase for the formation of NFK (Ex 325 / Em 440 nm).

4. Mass spectra for Gr and MetGr

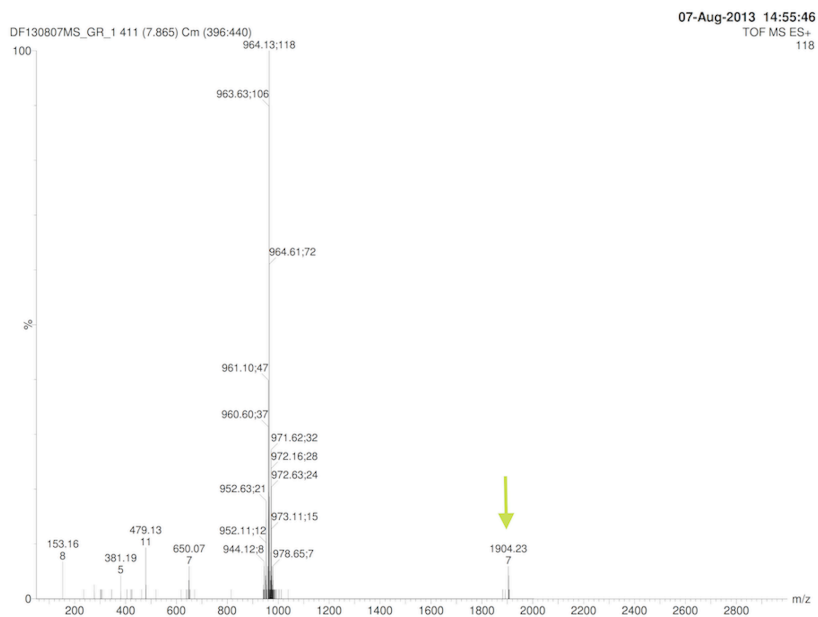


Figure S4. Mass spectrum for Gr (50 μ M in MeOH). The arrow indicates the zone that is expanded and shown on figure S5.

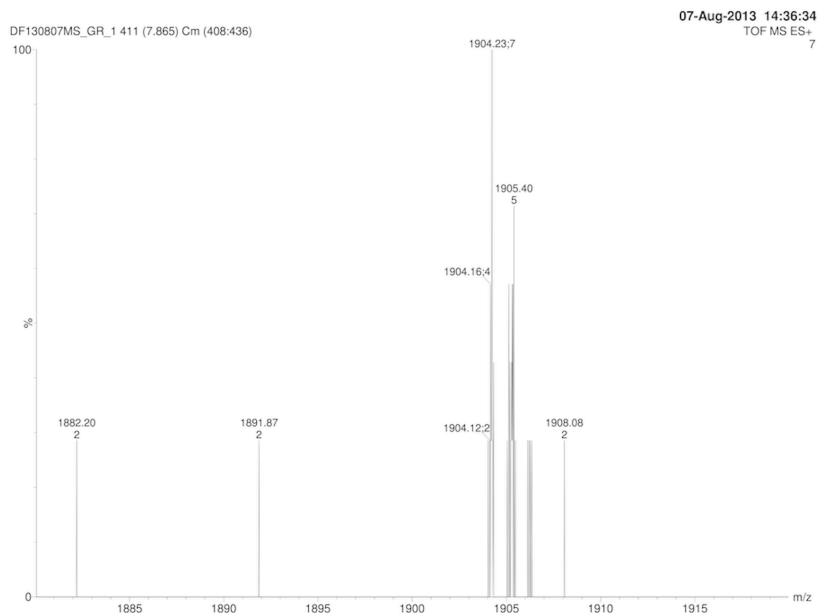


Figure S5. Mass spectrum for Gr (50 μ M in MeOH) expanded between 1880 and 1920 m/z.

Gramicidin obtained from the soil bacterium *Bacillus brevis* corresponds to a mixture of forms A, B and C. The major component in the mixture corresponds to gramicidin A, whose sequence is characterized by the presence of four tryptophan residues in the positions 9, 11, 13 and 15. When the Trp in position 11 is replaced by phenylalanine it is called gramicidin B and when it is replaced by tyrosine it is called gramicidin C. The variation in the sequence of gramicidin and the possibility of different degrees of methylation are reflected in the mass spectra for MetGr shown on figures S6 and S7.

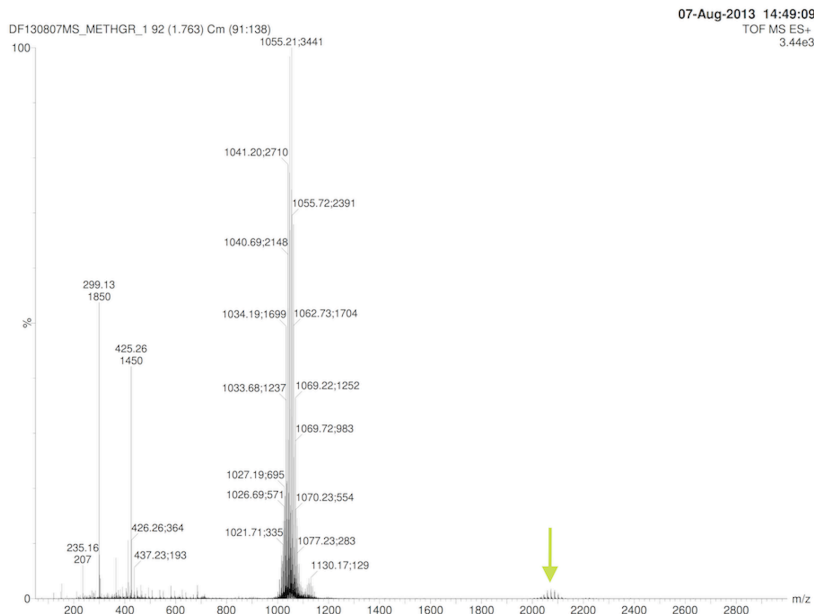


Figure S6. Mass spectrum for MetGr (50 μM in MeOH). The arrow indicates the zone that is expanded and shown on figure S7.

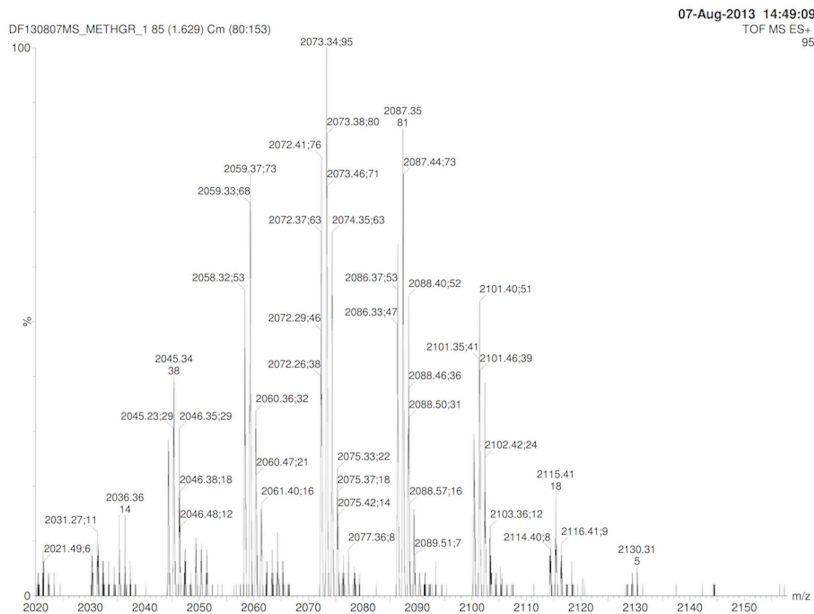


Figure S7. Mass spectrum for MetGr (50 μM in MeOH) expanded between 2020 and 2160 m/z.

5. Fluorescence excitation and emission spectra for 1-methyl Trp derivative

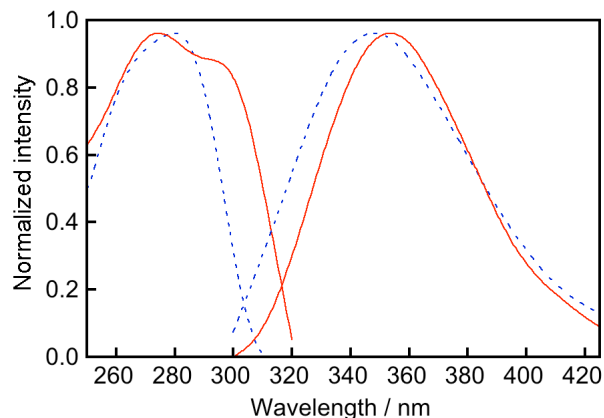


Figure S8. Normalized fluorescence excitation and emission spectra for 1-Met-Trp (red, solid line) and Trp (blue, dashed line) in MeOH (Ex 280 / Em 350 nm).

6. SDS-PAGE of MetGr irradiated in the presence of Riboflavin

Band 1 appeared at low molecular weights and it was very broad, which agrees with the wide range of molecular weights observed in the mass spectrum for this sample (see figures S6 and S7 above). As with Gr, a second band appeared around 9 kDa and it was attributed to aggregated MetGr. Both bands 1 and 2 increased in intensity over time, which was attributed to a change in the concentration of the sample during the irradiation.

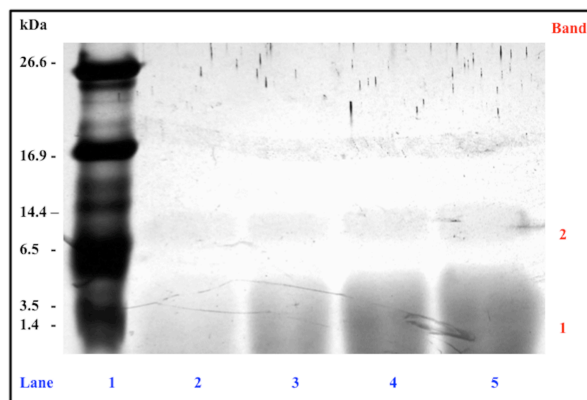


Figure S9. SDS-PAGE analysis of MetGr (3 mg mL^{-1}) irradiated in the presence of RF ($20 \text{ }\mu\text{M}$) in MeOH during 1 h. Lanes: 1, peptide molecular weight standards; 2, non-irradiated; 3, 20 min; 4, 40 min; 5, 80 min. Bands 1 and 2 correspond to monomeric Gr and aggregated Gr, respectively.