Correlated Imaging with C_{60}-SIMS and Confocal Raman Microscopy: Visualization of Cell-Scale Molecular Distributions in a Bacterial Biofilm

Eric J. Lanni[1], Rachel N.Masyuko[2], Callan M. Driscoll[3], Sage J. B. Dunham[1], Joshua D. Shrout[3], Paul W. Bohn[2], Jonathan V. Sweedler[1]

[1] Department of Chemistry and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[2] Department of Chemistry and Biochemistry and Department of Chemical and Biomolecular Engineering, University of Notre Dame, Notre Dame, IN 46556

[3] Department of Civil and Environmental Engineering and Earth Sciences and Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

Supporting Information

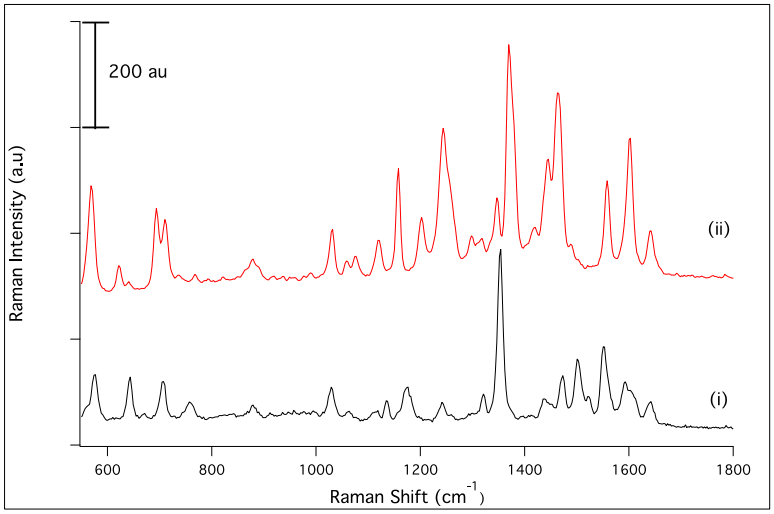

Figure S1. Raman spectra of quinolone standards. Raman spectra of quinolones (i) HHQ and (ii) PQS are uniquely characterized by contributions from ring deformation, ring breathing and ring stretching vibrations. Ring deformation vibrations are represented by the bands at 431 cm^{-1}, 490 cm^{-1} and 570 cm^{-1} in PQS and at 489 cm^{-1}, 573 cm^{-1}, 642 cm^{-1} and 753 cm^{-1} in HHQ.[1,2] The band at 1031 cm^{-1} in both PQS and HHQ is indicative of ring breathing vibrations. The bands found in the 1300 -1600 cm^{-1} region of the spectrum are characteristic of ring stretching vibrations which are associated with intense bands. In the PQS spectrum the major bands in this region are at 1371 cm^{-1}, assigned to the C-C stretching vibrations of the quinolone ring with contributions from CH bending vibrations,[1-3] 1557 cm^{-1} attributed to (C-C-C) and (C-N-C) ring stretching vibrations,[1,2] and at 1603 cm^{-1} assigned to symmetric C=C stretching vibrations of the quinolone ring.[1,2] In HHQ, the major bands are at 1353 cm^{-1} arising from C-C stretching vibrations of the quinolone ring with contributions from CH bending vibrations,[1,4] 1553 cm^{-1} attributed to (C-C-C) and (C-N-C) ring stretching vibrations,[1] 1501 cm^{-1} and 1593 cm^{-1} assigned to (C-C-C) and symmetric C=C ring stretching vibrations.[1] Other significant bands characterized by non-ring vibrations in PQS are 1158 cm^{-1} from CH bending vibrations[1,4] and 1245 cm^{-1} attributed to contributions from CH bending and CH twisting vibrations.[4] In HHQ, bands at 1173 cm^{-1} and 1239 cm^{-1} mark similar vibrations.[1,4] CH_2 deformation vibrations are observed at 1464 cm^{-1} and 1471 cm^{-1} in PQS and HHQ, respectively.[1,5,6]

Compound ID	Molecular formula	M_{ex}	$[M+H]^+$ theoretical	$[M+H]^+$ observed	ΔM
4-quinolone frag.	$C_{10}H_9NO$	159.07	-	159.07*	0.00
3-hydroxyquinolone frag.	$C_{10}H_9NO_2$	175.06	-	175.07*	0.01
4-quinolone frag.	$C_{11}H_{10}NO$	172.08	-	172.08*	0.00
3-hydroxyquinolone frag.	$C_{11}H_{10}NO_2$	188.07	-	188.07*	0.00
PC (phosphocholine)	$C_5H_{15}NO_4P$	184.07	-	184.08*	0.01
1-HP (1-hydroxyphenazine)	$C_{12}H_8N_2O$	196.06	197.07	197.09	0.02
1-MP (1-methoxyphenazine)	$C_{13}H_{10}N_2O$	210.08	211.09	211.10	0.01
HHQ (2-heptyl-4-quinolone)	$C_{16}H_{21}NO$	243.16	244.17	244.17	0.00
PQS (2-heptyl-3-hydroxyquinolone)	$C_{16}H_{21}NO_2$	259.16	260.17	260.17	0.00
HQNO (4-hydroxy-2-heptylquinolone-N-oxide)	$C_{16}H_{21}NO_2$	259.16	260.17	260.17	0.00
$C_{9:1}$-NHQ (2-nonenyl-4-quinolone)	$C_{18}H_{23}NO$	269.18	270.19	270.19	0.00
NHQ (2-nonyl-4-quinolone)	$C_{18}H_{25}NO$	271.19	272.20	272.19	-0.01
$C_{9:1}$-NQNO (4-hydroxy-2-nonenylquinolone-N-oxide)	$C_{18}H_{23}NO_2$	285.17	286.18	286.19	0.01
C_9-PQS (2-nonyl-3-hydroxyquinolone)	$C_{18}H_{25}NO_2$	287.19	288.20	288.20	0.00
C_9-NQNO (4-hydroxy-2-nonylquinolone-N-oxide)	$C_{18}H_{25}NO_2$	287.19	288.20	288.20	0.00
$C_{11:1}$-UHQ (2-undecenyl-4-quinolone)	$C_{20}H_{27}NO$	297.21	298.22	298.22	0.00

Table S1. Mass list for metabolites detected by C_{60}-SIMS analysis of *P. aeruginosa* biofilm. Compounds listed in bold typeface were confirmed with *in situ* tandem MS (spectra shown in Figure S3). * Indicates fragment ion detected as M^+ or $M^{\bullet+}$.

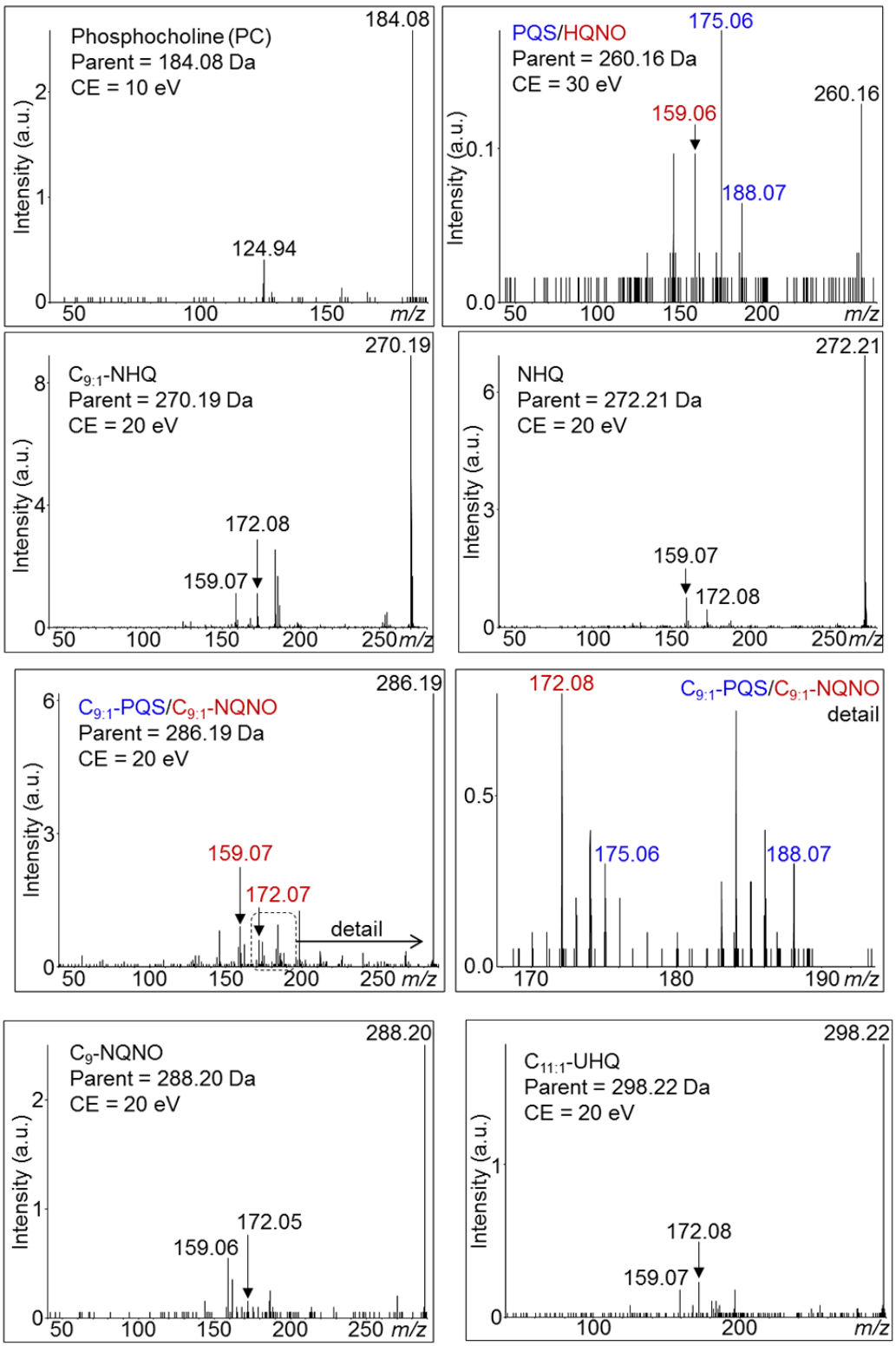

Figure S2. C_{60}-SIMS tandem MS spectra of biofilm metabolites. Spectra were collected directly from the untreated biofilm surface. Characteristic fragment ions matching those reported in previous work[7] are labeled in the spectra and color-coded in cases of isobaric species.

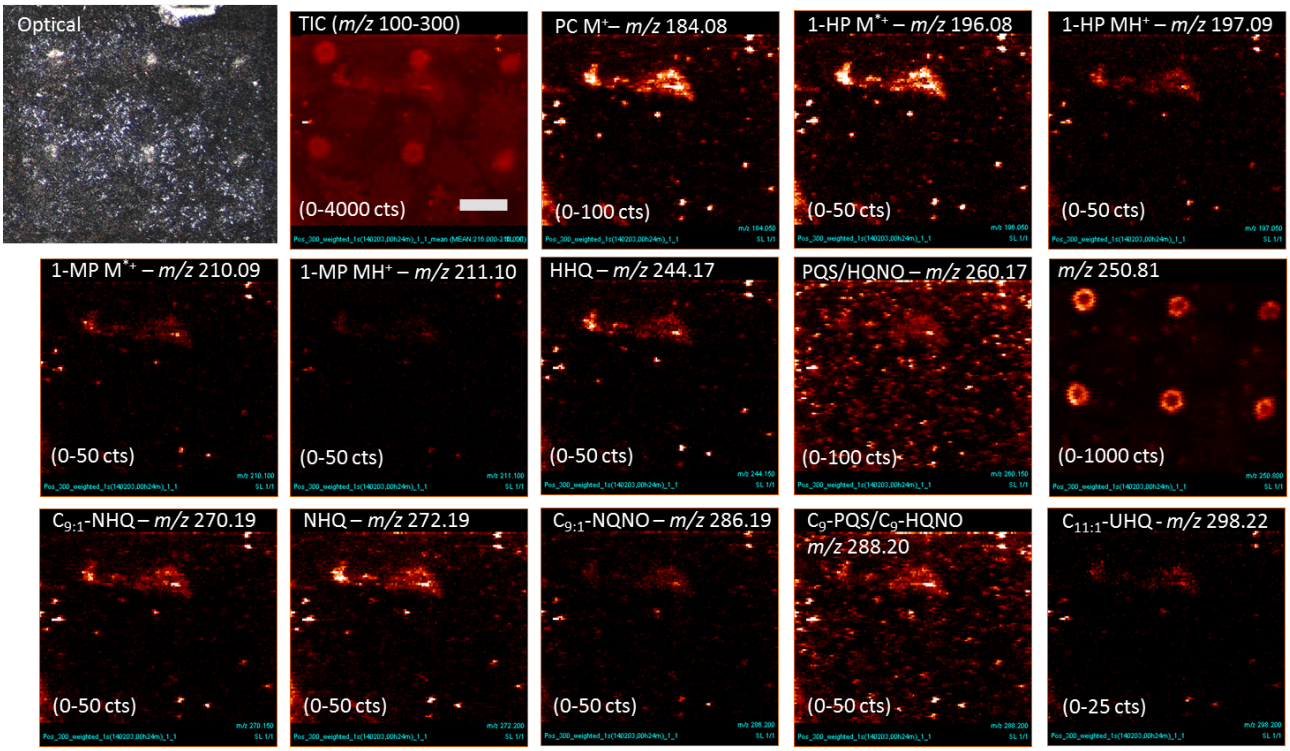

Figure S3. Full C_{60}-SIMS images of metabolites detected in Fig. 4 ROI. Scale bar = 250 μm.

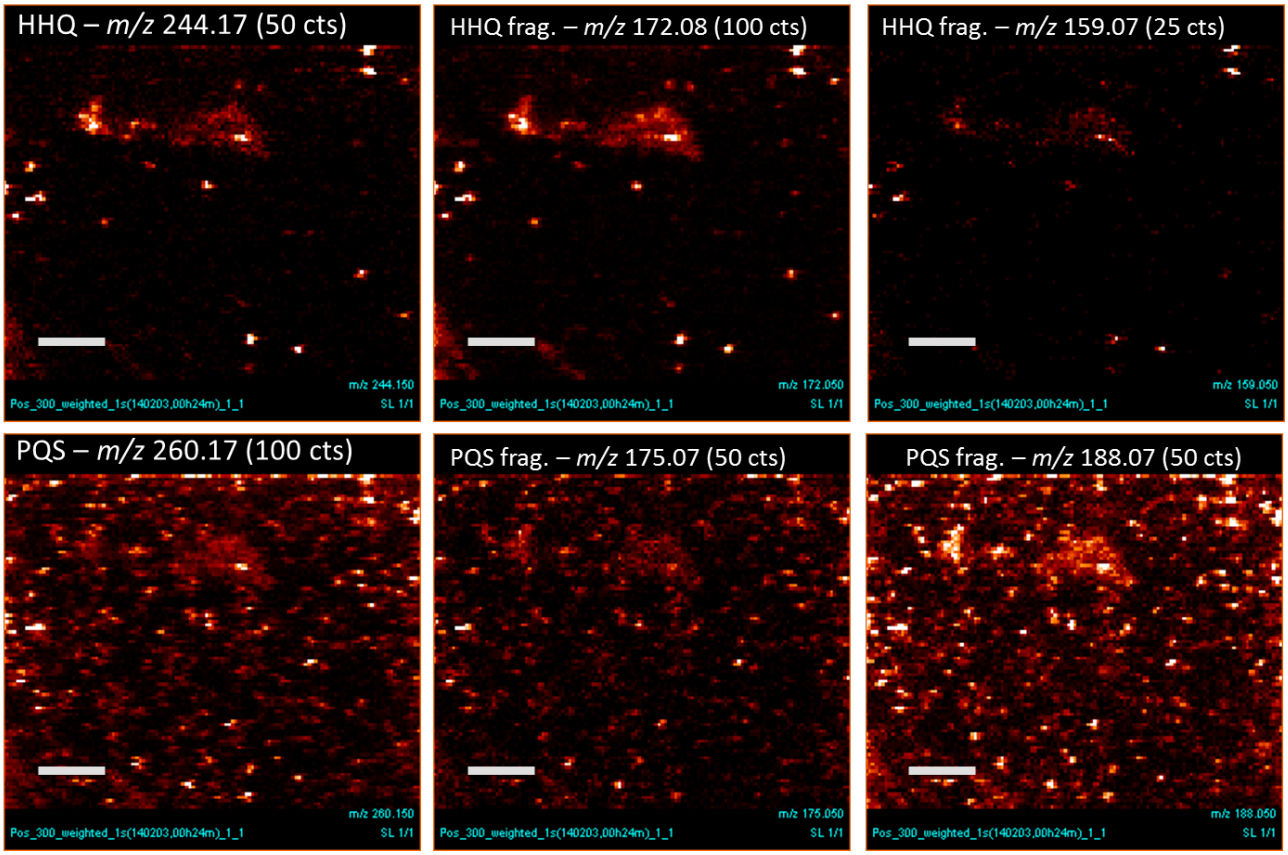

Figure S4. Spatial co-location of characteristic fragment ions with parent quinolone classes in Fig. 4 ROI. 4-quinolone HHQ and characteristic fragments *m/z* 172/159 show good agreement of spatial localization throughout the imaged region, likewise the 3-hydroxy-4-quinolone PQS and characteristic fragments *m/z* 175/188 are co-localized differently. Scale bars = 200 µm.

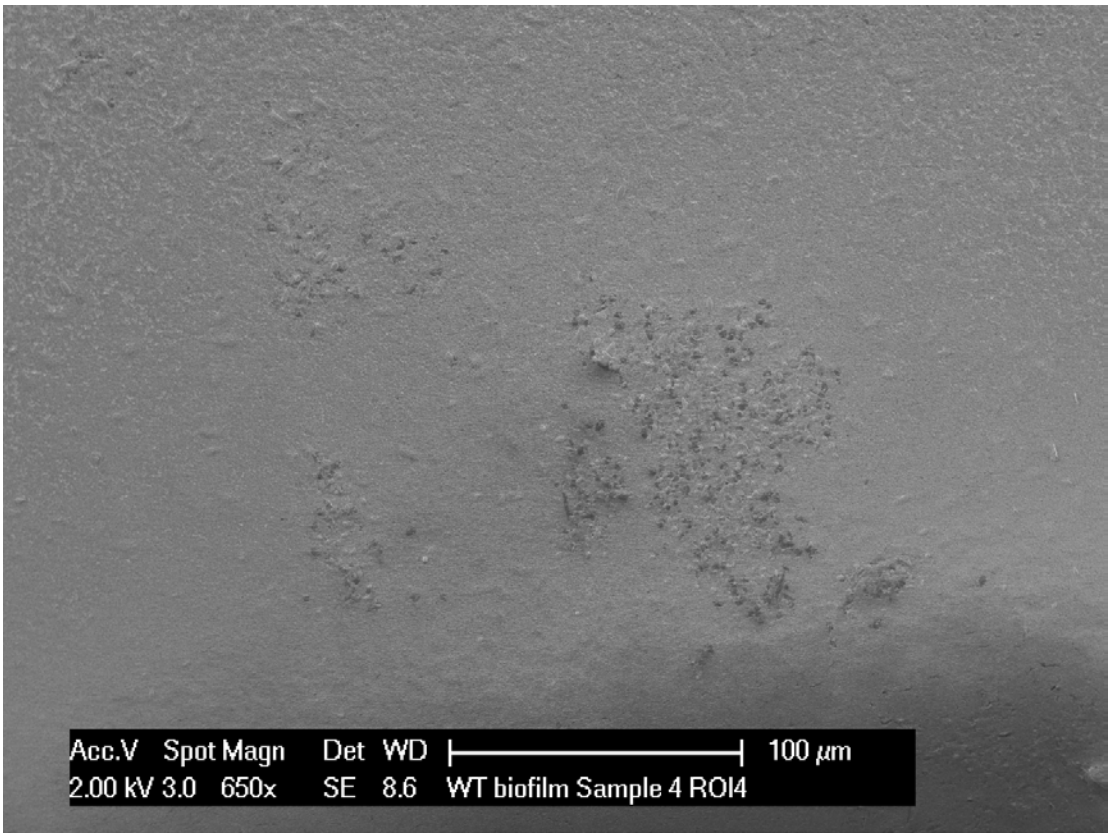

Figure S5: Low resolution SEM image of Figure 5 ROI. An electron micrograph of the region around the "quinolone hot spot" shown in Figure 5 shows smooth biofilm surface elsewhere, compared with a coarser texture and visible individual cells in the region of intense quinolone (and phosphocholine) signal.

References

(1) Bolboaca, M.; Kiefer, W.; Popp, J. *J. Raman Spectrosc.* **2002**, *33*, 207-212.

(2) Frosch, T.; Schmitt, M.; Popp, J. *Anal. Bioanal. Chem.* **2007**, *387*, 1749-1757.

(3) Neugebauer, U.; Szeghalmi, A.; Schmitt, M.; Kiefer, W.; Popp, J.; Holzgrabe, U. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **2005**, *61*, 1505-1517.

(4) Wang, Y.; Yu, K.; Wang, S. *Spectrochim. Acta. A. Mol. Biomol. Spectrosc.* **2006**, *65*, 159-163.

(5) Harz, M.; Rosch, P.; Peschke, K. D.; Ronneberger, O.; Burkhardt, H.; Popp, J. *Analyst* **2005**, *130*, 1543-1550.

(6) Neugebauer, U.; Schmid, U.; Baumann, K.; Ziebuhr, W.; Kozitskaya, S.; Deckert, V.; Schmitt, M.; Popp, J. *ChemPhysChem.* **2007**, *8*, 124-137.

(7) Lépine, F.; Milot, S.; Déziel, E.; He, J.; Rahme, L. G. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 862-869.