

SUPPLEMENTARY DATA

Polymer Masked-UnMasked Protein Therapy: Identification of the Active Species After Amylase-activation of Dextrin-Colistin Conjugates.

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Table S1. Characteristics of dextrin-colistin conjugates used for studying the effect of the succinylation rate on the released species.

Conjugate	M_w (g/mol) (M_w/M_n)	Degree of succinylation (mol %)	Protein content (%)	Molar ratio (dextrin:colistin)
Dextrin-colistin 1%	9,000 (1.7)	1.0	5.2	3.2:1
Dextrin-colistin 2.5%	9,500 (1.7)	2.5	9.7	1.6:1
Dextrin-colistin 7.5%	13,000 (2.2)	7.5	21.0	0.7:1

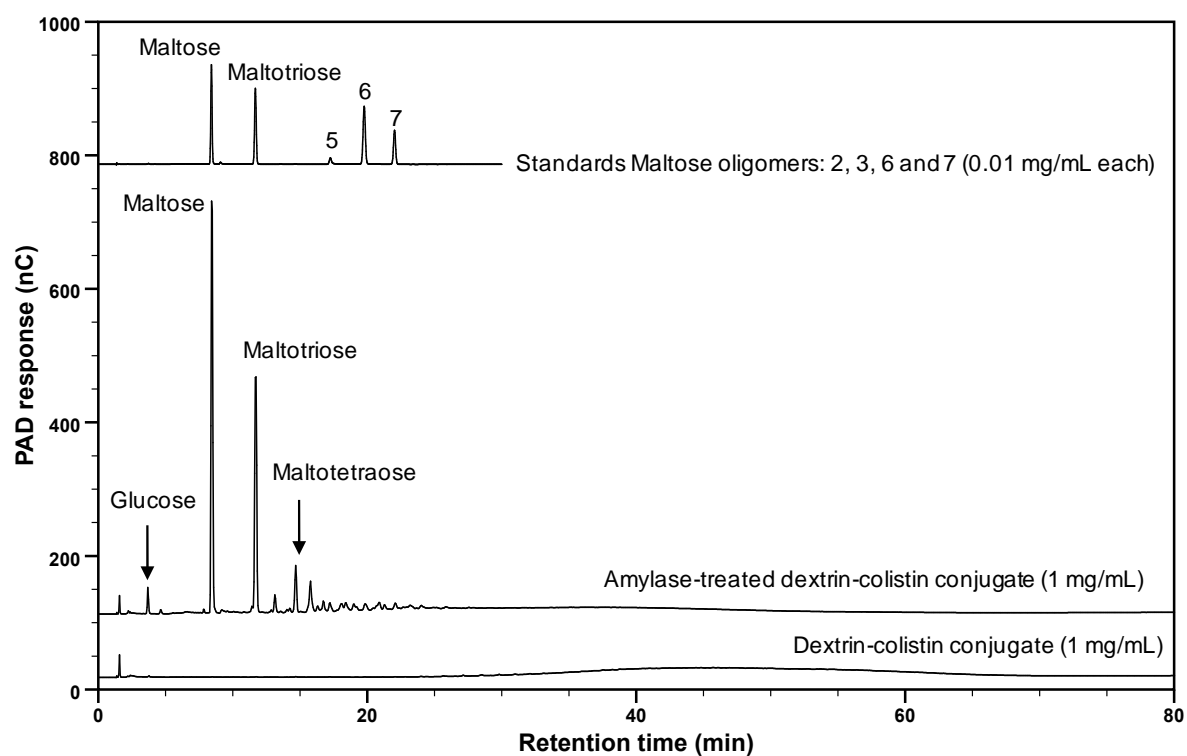


Fig. S1: HPAEC-PAD analysis of maltose oligomer standards (0.01 mg/mL) and oligosaccharides released from intact and amylase-degraded dextrin-colistin conjugates (1 mg/mL). Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.

Table S2: Identification of the detected species from UPLC-QTOF-MS analysis of colistin sulfate and fractions F6, F7 and F8.

Sample	RT (min)	Observed mass	Calculated mass	Species / Adducts	Molecular formula	Molecular Weight (g/mol)
Colistin	5.6	1155.76	1155.76	Colistin B [M+H] ¹⁺	C ₅₂ H ₉₈ N ₁₆ O ₁₃	1154.76
		578.38	578.38	Colistin B [M+2H] ²⁺		
	6.4	1169.77	1169.77	Colistin A [M+H] ¹⁺	C ₅₃ H ₁₀₀ N ₁₆ O ₁₃	1168.77
		585.39	585.39	Colistin A [M+2H] ²⁺		
F8	1.2	596.17	596.18	DP7 [M+K+H] ²⁺	C ₄₂ H ₇₂ O ₃₆	1151.38
		677.18	677.20	DP8 [M+K+H] ²⁺	C ₄₈ H ₈₂ O ₄₁	1314.43
		758.20	758.23	DP9 [M+K+H] ²⁺	C ₅₄ H ₉₂ O ₄₆	1476.49
		839.22	839.25	DP10 [M+K+H] ²⁺	C ₆₀ H ₁₀₂ O ₅₁	1638.54
		920.24	920.28	DP11 [M+K+H] ²⁺	C ₆₆ H ₁₁₂ O ₅₆	1800.59
		1001.25	1001.30	DP12 [M+K+H] ²⁺	C ₇₂ H ₁₂₂ O ₆₁	1962.64
		1082.27	1082.32	DP13 [M+K+H] ²⁺	C ₇₈ H ₁₃₂ O ₆₆	2121.70
	7.2	413.27	413.26	Colistin B + linker - H ₂ O [M+3H] ³⁺	C ₅₆ H ₁₀₀ N ₁₆ O ₁₅	1236.76
		619.38	619.39	Colistin B + linker - H ₂ O [M+2H] ²⁺		
	7.4	419.28	419.27	Colistin B + linker [M+3H] ³⁺	C ₅₆ H ₁₀₂ N ₁₆ O ₁₆	1254.80
		628.38	628.39	Colistin B + linker [M+2H] ²⁺		
	7.6	419.28	419.27	Colistin B + linker [M+3H] ³⁺	C ₅₆ H ₁₀₀ N ₁₆ O ₁₅	1236.76
		628.38	628.39	Colistin B + linker [M+2H] ²⁺		
	7.7	413.27	413.26	Colistin B + linker - H ₂ O [M+3H] ³⁺	C ₅₆ H ₁₀₀ N ₁₆ O ₁₅	1236.76
		619.38	619.39	Colistin B + linker - H ₂ O [M+2H] ²⁺		
	7.8	413.27	413.26	Colistin B + linker - H ₂ O [M+3H] ³⁺	C ₅₇ H ₁₀₂ N ₁₆ O ₁₅	1253.80
		619.38	619.39	Colistin B + linker - H ₂ O [M+2H] ²⁺		
	7.9	417.94	417.93	Colistin A + linker - H ₂ O [M+3H] ³⁺	C ₅₇ H ₁₀₂ N ₁₆ O ₁₅	1253.80
		626.39	626.39	Colistin A + linker - H ₂ O [M+2H] ²⁺		
	8.1	423.95	423.94	Colistin A + linker [M+3H] ³⁺	C ₅₇ H ₁₀₄ N ₁₆ O ₁₆	1268.78
		635.39	635.40	Colistin A + linker [M+2H] ²⁺		
	8.4	423.95	423.94	Colistin A + linker [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		635.39	635.40	Colistin A + linker [M+2H] ²⁺		
	8.6	423.95	423.94	Colistin A + linker [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		635.39	635.40	Colistin A + linker [M+2H] ²⁺		
	8.9	446.61	446.60	Colistin B + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		669.38	669.40	Colistin B + 2*linker - H ₂ O [M+2H] ²⁺		
	9.2	446.61	446.60	Colistin B + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		669.38	669.40	Colistin B + 2*linker - H ₂ O [M+2H] ²⁺		
	9.4	446.61	446.60	Colistin B + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		669.38	669.40	Colistin B + 2*linker - H ₂ O [M+2H] ²⁺		
	9.5	446.61	446.60	Colistin B + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		669.38	669.40	Colistin B + 2*linker - H ₂ O [M+2H] ²⁺		
	9.7	440.60	440.59	Colistin B + 2*linker - 2H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₂ N ₁₆ O ₁₇	1318.76
		660.38	660.39	Colistin B + 2*linker - 2H ₂ O [M+2H] ²⁺		
	9.9	446.61	446.60	Colistin B + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈	1336.77
		669.38	669.40	Colistin B + 2*linker - H ₂ O [M+2H] ²⁺		
	10.1	451.28	451.27	Colistin A + 2*linker - H ₂ O [M+3H] ³⁺	C ₆₁ H ₁₀₆ N ₁₆ O ₁₈	1350.79
		676.39	676.40	Colistin A + 2*linker - H ₂ O [M+2H] ²⁺		
	10.5	445.28	445.27	Colistin A + 2*linker - 2H ₂ O [M+3H] ³⁺	C ₆₁ H ₁₀₄ N ₁₆ O ₁₇	1332.78
		676.40	676.40	Colistin A + 2*linker - 2H ₂ O [M+2H] ²⁺		
F7	1.1	325.11	325.11	DP2 - H ₂ O [M+H] ¹⁺	C ₁₂ H ₂₀ O ₁₀	324.12
		487.17	487.17	DP3 - H ₂ O [M+H] ¹⁺	C ₁₈ H ₃₀ O ₁₅	486.16
		568.19	568.19	DP7 - H ₂ O [M+2H] ²⁺	C ₄₂ H ₇₀ O ₃₅	1136.38
		649.22	649.22	DP8 - H ₂ O [M+2H] ²⁺	C ₄₈ H ₈₀ O ₄₀	1296.42
		730.24	730.25	DP9 - H ₂ O [M+2H] ²⁺	C ₅₄ H ₉₀ O ₄₅	1458.48
		811.27	811.27	DP10 - H ₂ O [M+2H] ²⁺	C ₆₀ H ₁₀₀ O ₅₀	1620.53
		892.30	892.30	DP11 - H ₂ O [M+2H] ²⁺	C ₆₆ H ₁₁₀ O ₅₅	1782.58
		973.32	973.32	DP12 - H ₂ O [M+2H] ²⁺	C ₇₂ H ₁₂₀ O ₆₀	1944.63
		1054.35	1034.35	DP13 - H ₂ O [M+2H] ²⁺	C ₇₈ H ₁₃₀ O ₆₅	2106.69
		1135.38	1135.38	DP14 - H ₂ O [M+2H] ²⁺	C ₈₄ H ₁₄₀ O ₇₀	2268.74
		1216.40	1216.40	DP15 - H ₂ O [M+2H] ²⁺	C ₉₀ H ₁₅₀ O ₇₅	2430.79
		1297.43	1297.43	DP16 - H ₂ O [M+2H] ²⁺	C ₉₆ H ₁₆₀ O ₈₀	2592.85
		1378.45	1378.46	DP17 - H ₂ O [M+2H] ²⁺	C ₁₀₂ H ₁₇₀ O ₈₅	2754.90
		1459.48	1459.48	DP18 - H ₂ O [M+2H] ²⁺	C ₁₀₈ H ₁₈₀ O ₉₀	2916.95
	7.5	581.32	581.32	Colistin B + linker + 3Gluc [M+3H] ³⁺	C ₇₄ H ₁₃₂ N ₁₆ O ₃₁	1740.92
		635.33	635.33	Colistin B + linker + 4Gluc [M+3H] ³⁺	C ₈₀ H ₁₄₂ N ₁₆ O ₃₆	1902.98
		689.68	689.35	Colistin B + linker + 5Gluc [M+3H] ³⁺	C ₈₆ H ₁₅₂ N ₁₆ O ₄₁	2065.03
		743.70	743.37	Colistin B + linker + 6Gluc [M+3H] ³⁺	C ₉₂ H ₁₆₂ N ₁₆ O ₄₆	2227.08
		781.44	781.44	Colistin B + linker + 2Gluc - H ₂ O [M+2H] ²⁺	C ₆₈ H ₁₂₀ N ₁₆ O ₂₅	1560.86
		862.46	862.47	Colistin B + linker + 3Gluc - H ₂ O [M+2H] ²⁺	C ₇₄ H ₁₃₀ N ₁₆ O ₃₀	1722.91
		871.47	871.47	Colistin B + linker + 3Gluc [M+2H] ²⁺	C ₇₄ H ₁₃₂ N ₁₆ O ₃₁	1740.92
		952.49	952.50	Colistin B + linker + 4Gluc [M+2H] ²⁺	C ₈₀ H ₁₄₂ N ₁₆ O ₃₆	1902.98
		1033.52	1033.52	Colistin B + linker + 5Gluc [M+2H] ²⁺	C ₈₆ H ₁₅₂ N ₁₆ O ₄₁	2065.03
		1114.55	1114.55	Colistin B + linker + 6Gluc [M+2H] ²⁺	C ₉₂ H ₁₆₂ N ₁₆ O ₄₆	2227.08
	7.9	467.28	467.28	Colistin B + linker + 1Gluc - H ₂ O [M+3H] ³⁺	C ₆₂ H ₁₁₀ N ₁₆ O ₂₀	1398.81
		521.29	521.29	Colistin B + linker + 2Gluc - H ₂ O [M+3H] ³⁺	C ₆₈ H ₁₂₀ N ₁₆ O ₂₅	1560.86
		575.31	575.31	Colistin B + linker + 3Gluc - H ₂ O [M+3H] ³⁺	C ₇₄ H ₁₃₀ N ₁₆ O ₃₀	1722.91
		700.41	700.41	Colistin B + linker + 1Gluc - H ₂ O [M+2H] ²⁺	C ₆₂ H ₁₁₀ N ₁₆ O ₂₀	1398.81
		781.44	781.44	Colistin B + linker + 2Gluc - H ₂ O [M+2H] ²⁺	C ₆₈ H ₁₂₀ N ₁₆ O ₂₅	1560.86
		862.46	862.46	Colistin B + linker + 3Gluc - H ₂ O [M+2H] ²⁺	C ₇₄ H ₁₃₀ N ₁₆ O ₃₀	1722.91
F6	1.1	325.11	325.11	DP2 - H ₂ O [M+H] ⁺	C ₁₂ H ₂₀ O ₁₀	324.12
		487.17	487.17	DP3 - H ₂ O [M+H] ⁺	C ₁₈ H ₃₀ O ₁₅	486.16
		649.22	649.22	DP4 - H ₂ O [M+H] ⁺	C ₂₄ H ₄₀ O ₂₀	648.21
		811.27	811.27	DP5 - H ₂ O [M+H] ⁺	C ₃₀ H ₅₀ O ₂₅	810.26
		1054.35	1054.35	DP13 - H ₂ O [M+2H] ²⁺	C ₇₈ H ₁₃₀ O ₆₅	2106.69
		1135.38	1135.38	DP14 - H ₂ O [M+2H] ²⁺	C ₈₄ H ₁₄₀ O ₇₀	2268.74
		1216.40	1216.40	DP15 - H ₂ O [M+2H] ²⁺	C ₉₀ H ₁₅₀ O ₇₅	2430.79
		1306.42	1306.44	DP16 [M+2H] ²⁺	C ₉₆ H ₁₆₂ O ₈₁	2610.86
		1387.43	1387.46	DP17 [M+2H] ²⁺	C ₁₀₂ H ₁₇₂ O ₈₆	2772.91
		1468.44	1468.49	DP18 [M+2H] ²⁺	C ₁₀₈ H ₁₈₂ O ₉₁	2934.96
		1549.48	1549.51	DP19 [M+2H] ²⁺	C ₁₁₄ H ₁₉₂ O ₉₆	3097.01
		1630.54	1630.54	DP20 [M+2H] ²⁺	C ₁₂₀ H ₂₀₂ O ₁₀₁	3259.07
		1711.57	1711.57	DP21 [M+2H] ²⁺	C ₁₂₆ H ₂₁₂ O ₁₀₆	3421.12
		1792.55	1792.55	DP22 [M+2H] ²⁺	C ₁₃₂ H ₂₂₂ O ₁₁₁	3583.17
	7.9	743.37	743.37	Colistin B + linker + 6Gluc [M+3H] ³⁺	C ₉₂ H ₁₆₂ N ₁₆ O ₄₆	2227.08
		748.05	748.04	Colistin A + linker + 6Gluc [M+3H] ³⁺	C ₉₃ H ₁₆₄ N ₁₆ O ₄₆	2241.10
		797.39	797.39	Colistin B + linker + 7Gluc [M+3H] ³⁺	C ₉₈ H ₁₇₂ N ₁₆ O ₅₁	2389.14
		802.05	802.06	Colistin A + linker + 7Gluc [M+3H] ³⁺	C ₉₉ H ₁₇₄ N ₁₆ O ₅₁	2403.15
		851.40	851.40	Colistin B + linker + 8Gluc [M+3H] ³⁺	C ₁₀₄ H ₁₈₂ N ₁₆ O ₅₆	2551.19
		856.07	856.08	Colistin A + linker + 8Gluc [M+3H] ³⁺	C ₁₀₅ H ₁₈₄ N ₁₆ O ₅₆	2565.20
		910.09	910.09	Colistin A + linker + 9Gluc [M+3H] ³⁺	C ₁₁₁ H ₁₉₄ N ₁₆ O ₆₁	2727.26
		964.10	964.11	Colistin A + linker + 10Gluc [M+3H] ³⁺	C ₁₁₇ H ₂₀₄ N ₁₆ O ₆₆	2889.31
		1018.13	1018.13	Colistin A + linker + 11Gluc [M+3H] ³⁺	C ₁₂₃ H ₂₁₄ N ₁₆ O ₇₁	3051.36
		1072.14	1072.15	Colistin A + linker + 12Gluc [M+3H] ³⁺	C ₁₂₉ H ₂₂₄ N ₁₆ O ₇₆	3213.42
		1126.16	1126.16	Colistin A + linker + 13Gluc [M+3H] ³⁺	C ₁₃₅ H ₂₃₄ N ₁₆ O ₈₁	3375.47

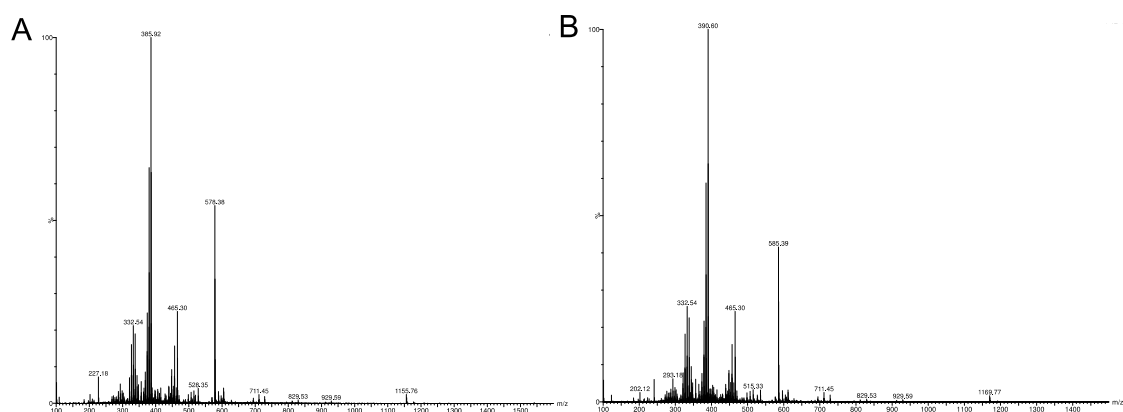
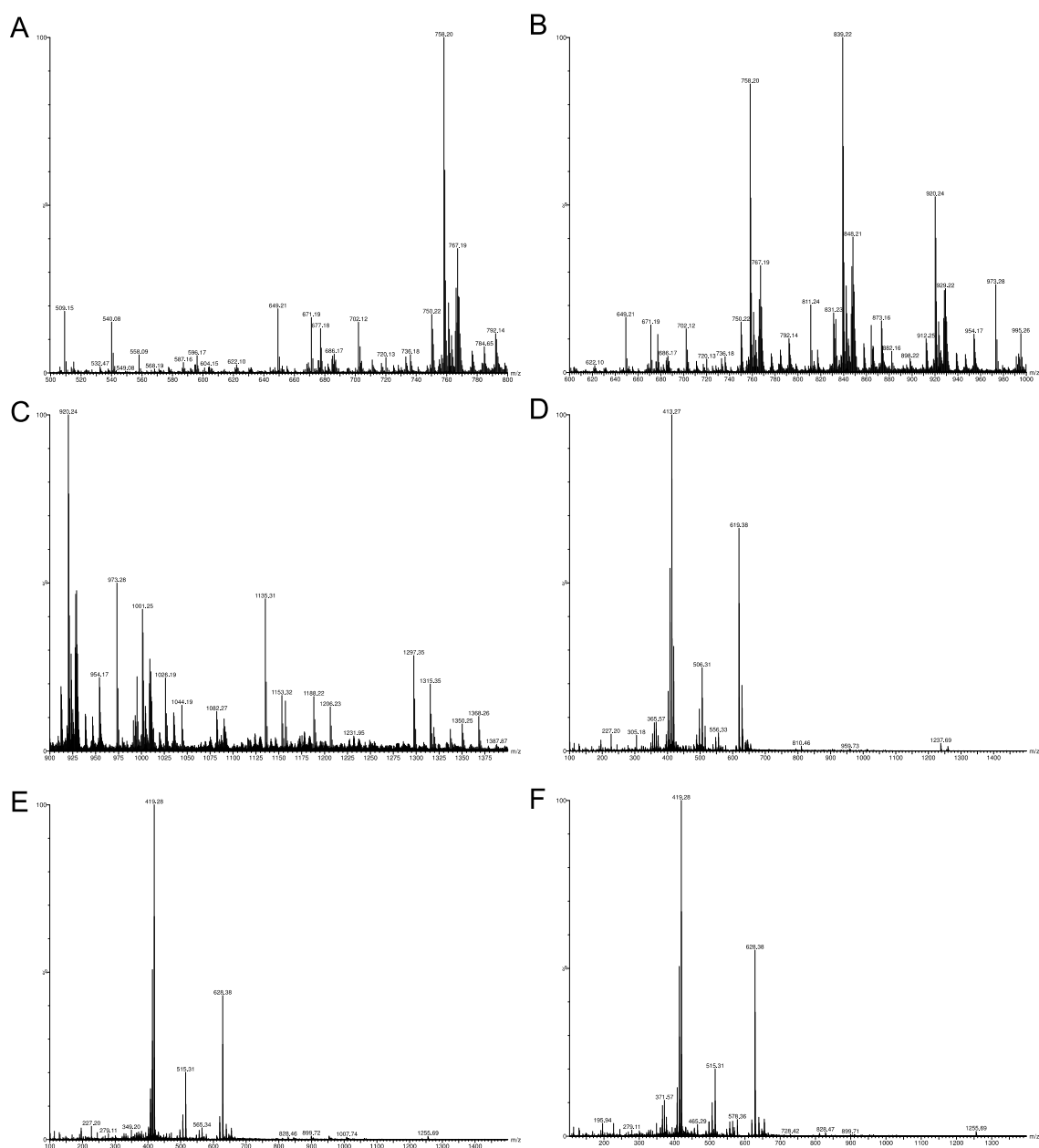


Fig. S2: QTOF-MS spectra of the isolated peaks from colistin with a retention of A. 5.6 min and B. 6.4 min.



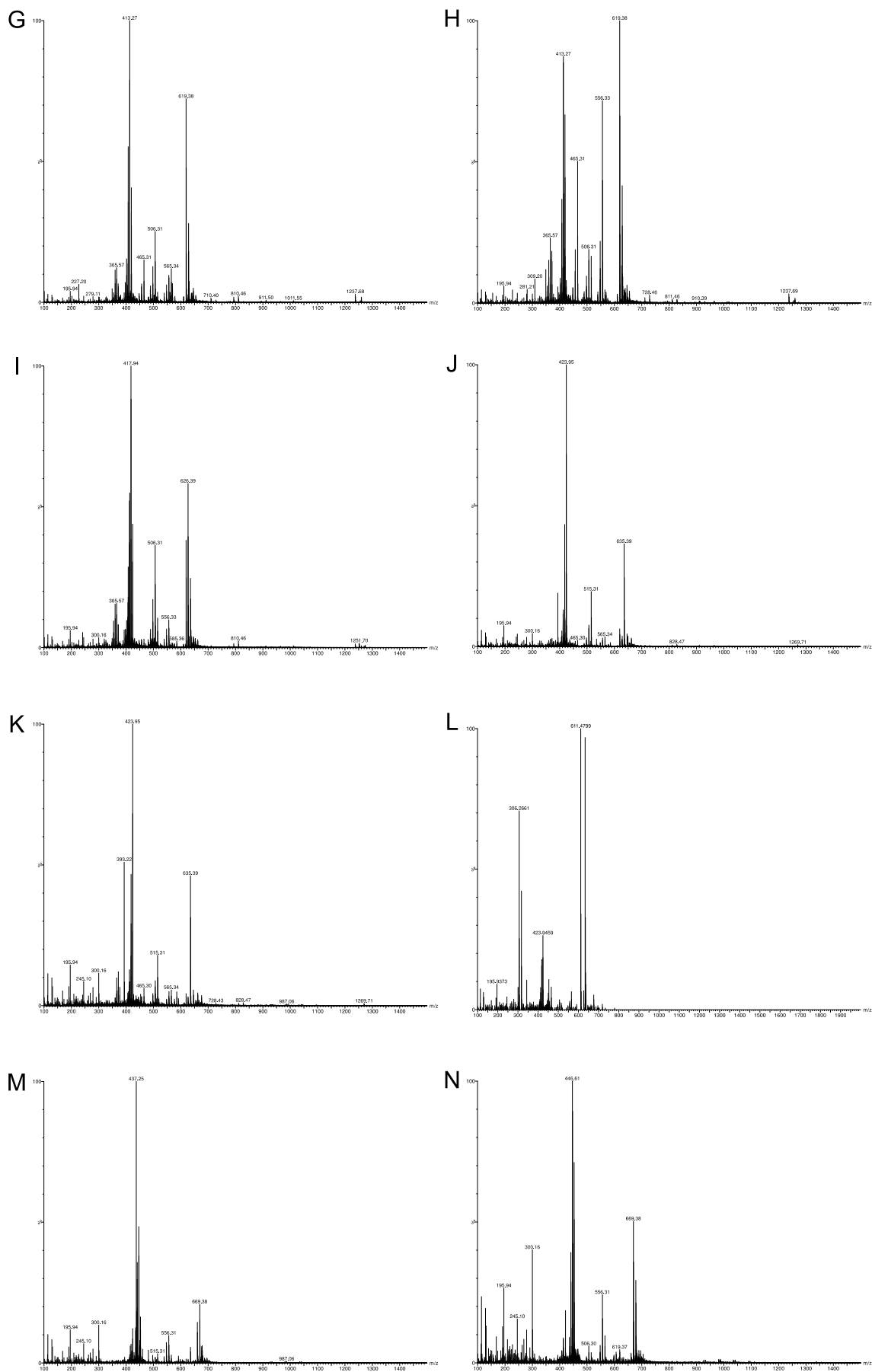


Fig. S3: continued.

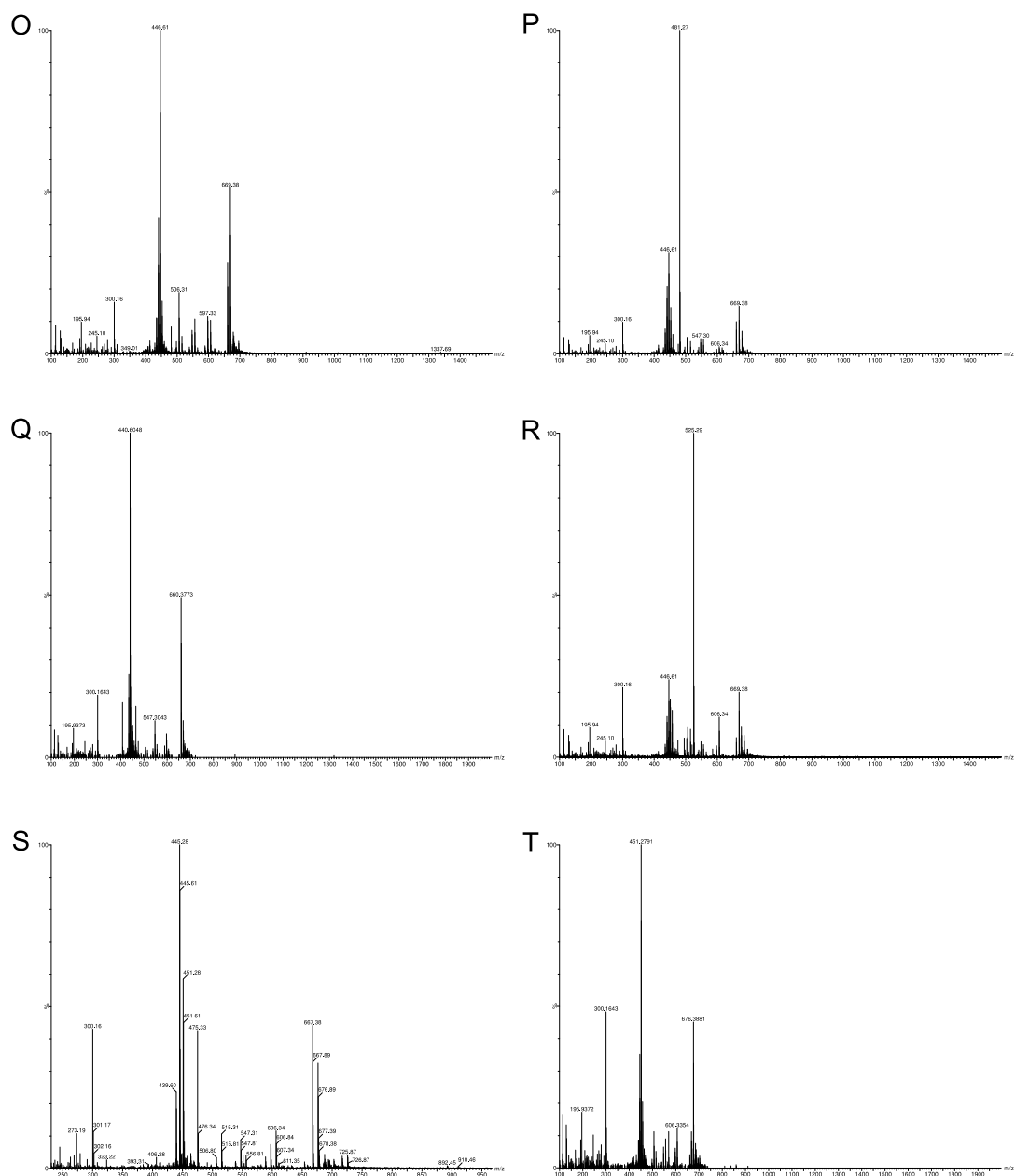


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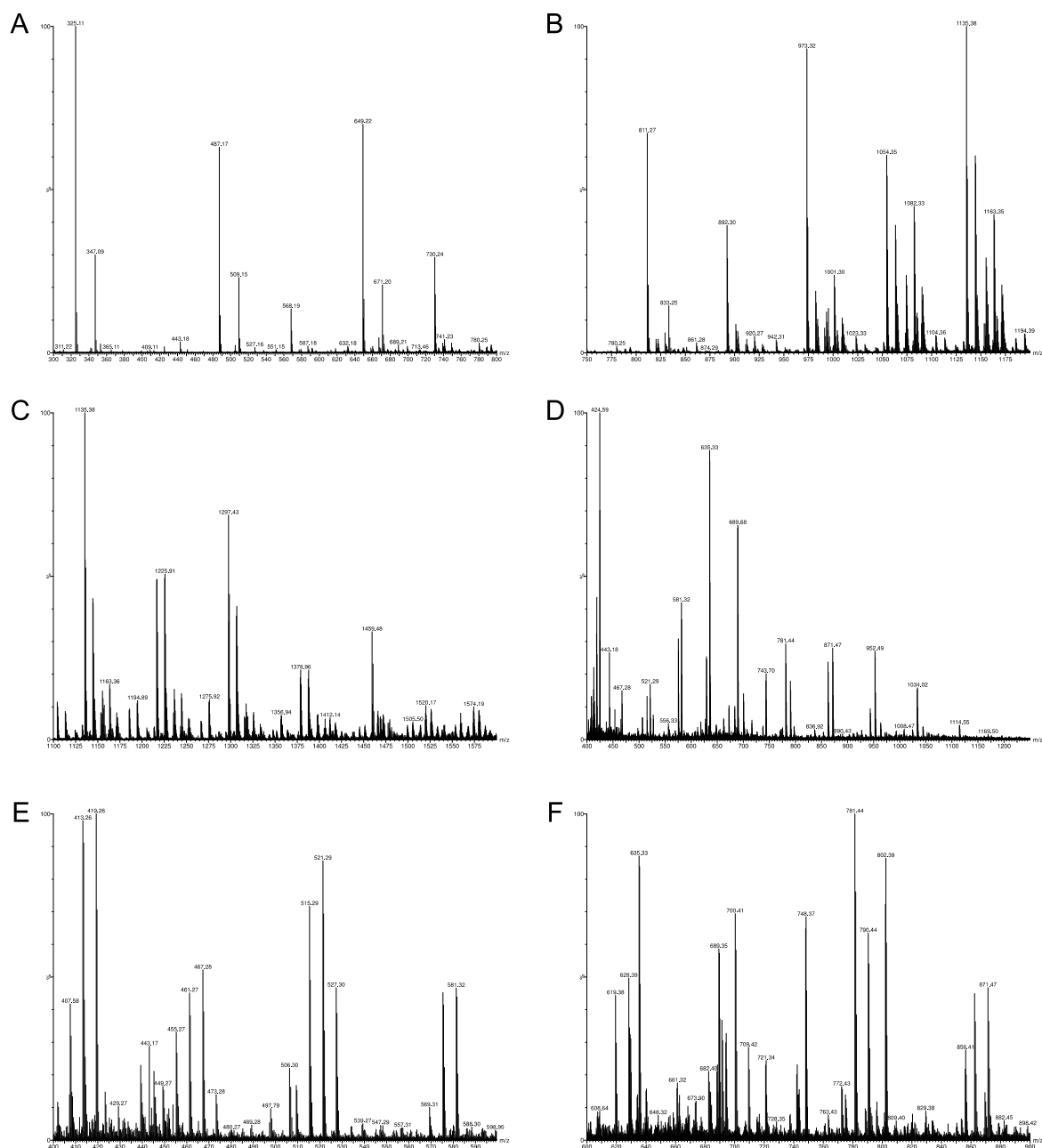


Fig. S4: QTOF-MS spectra of the isolated peaks from F7 with a retention time of A. 1.1 min (m/z = 300 to 800), B. 1.1 min (m/z = 750 to 1200), C. 1.1 min (m/z = 1100 to 1600), D. 7.5 min, E. 7.9 min (m/z = 400 to 600) and F. 7.9 min (m/z = 600 to 900).

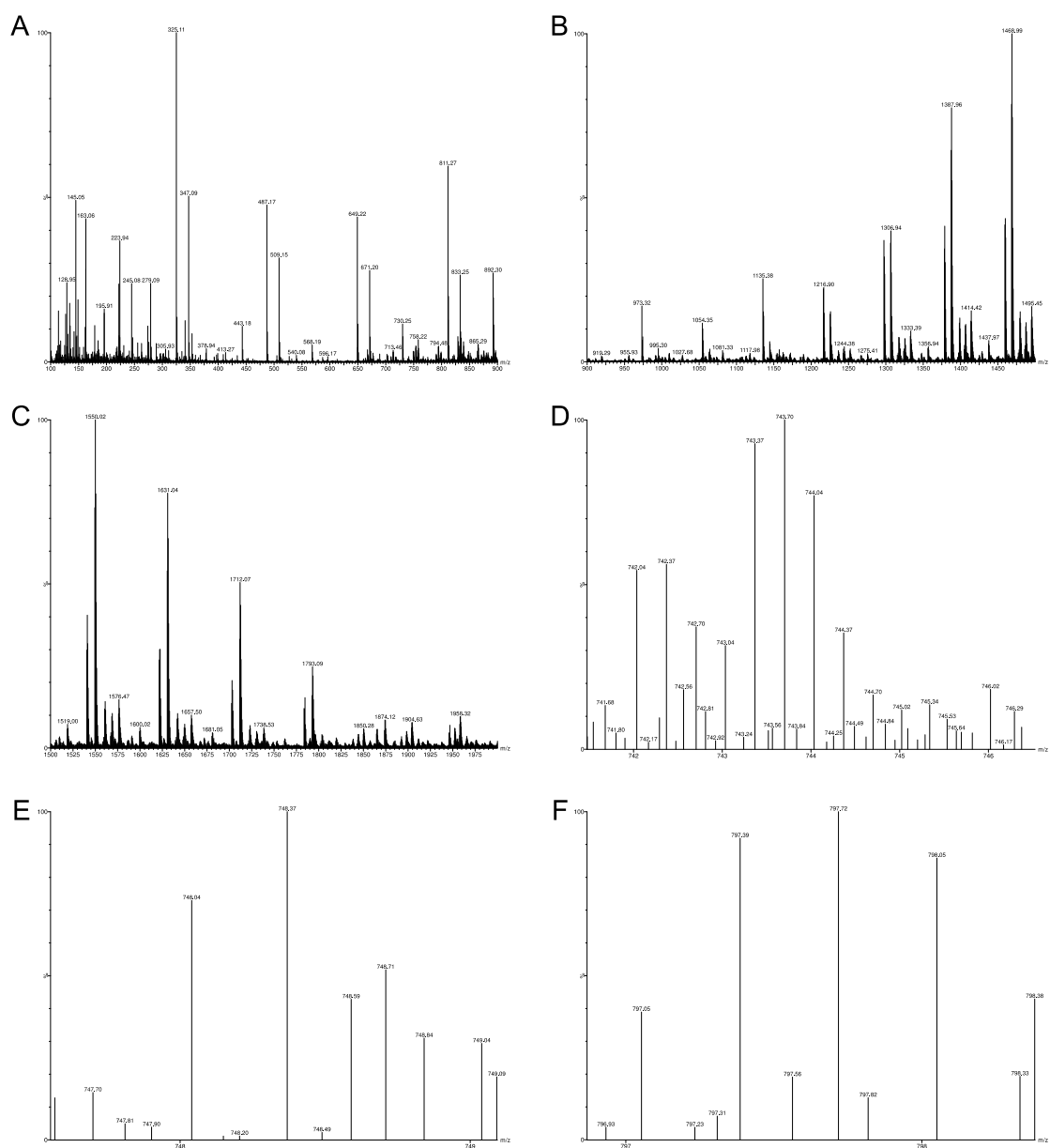


Fig. S5: QTOF-MS spectra of the isolated peaks from F6 with a retention time of A. 1.1 min ($m/z = 100$ to 900), B. 1.1 min ($m/z = 900$ to 1500), C. 1.1 min ($m/z = 1500$ to 2000), D. 7.9 min ($m/z = 743$), E. 7.9 min ($m/z = 743$), F. 7.9 min ($m/z = 797$), G. 7.9 min ($m/z = 802$), H. 7.9 min ($m/z = 851$), I. 7.9 min ($m/z = 856$), J. 7.9 min ($m/z = 910$), K. 7.9 min ($m/z = 964$), L. 7.9 min ($m/z = 1018$), M. 7.9 min ($m/z = 1072$) and N. 7.9 min ($m/z = 1126$).

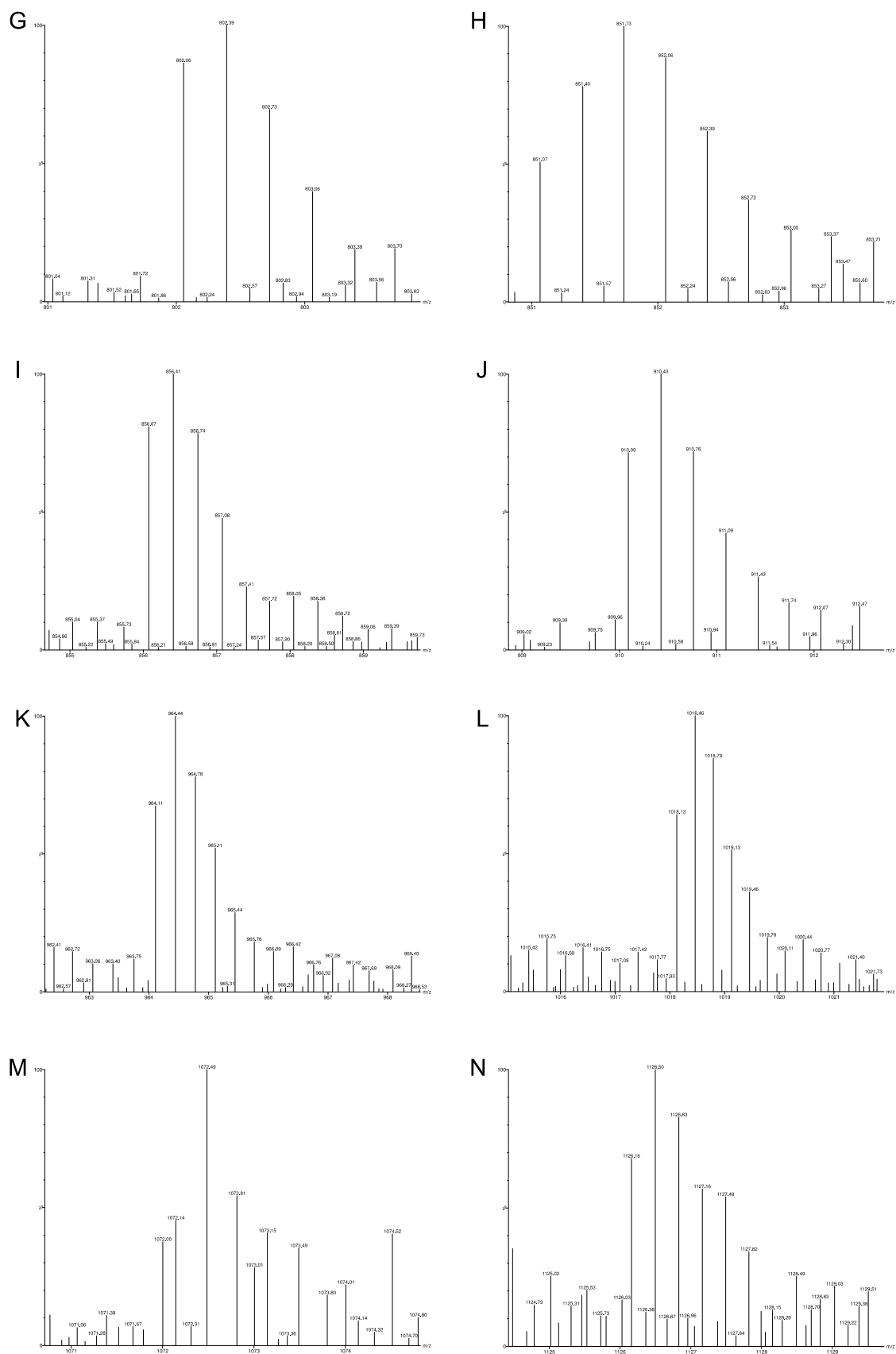


Fig. S5: continued.

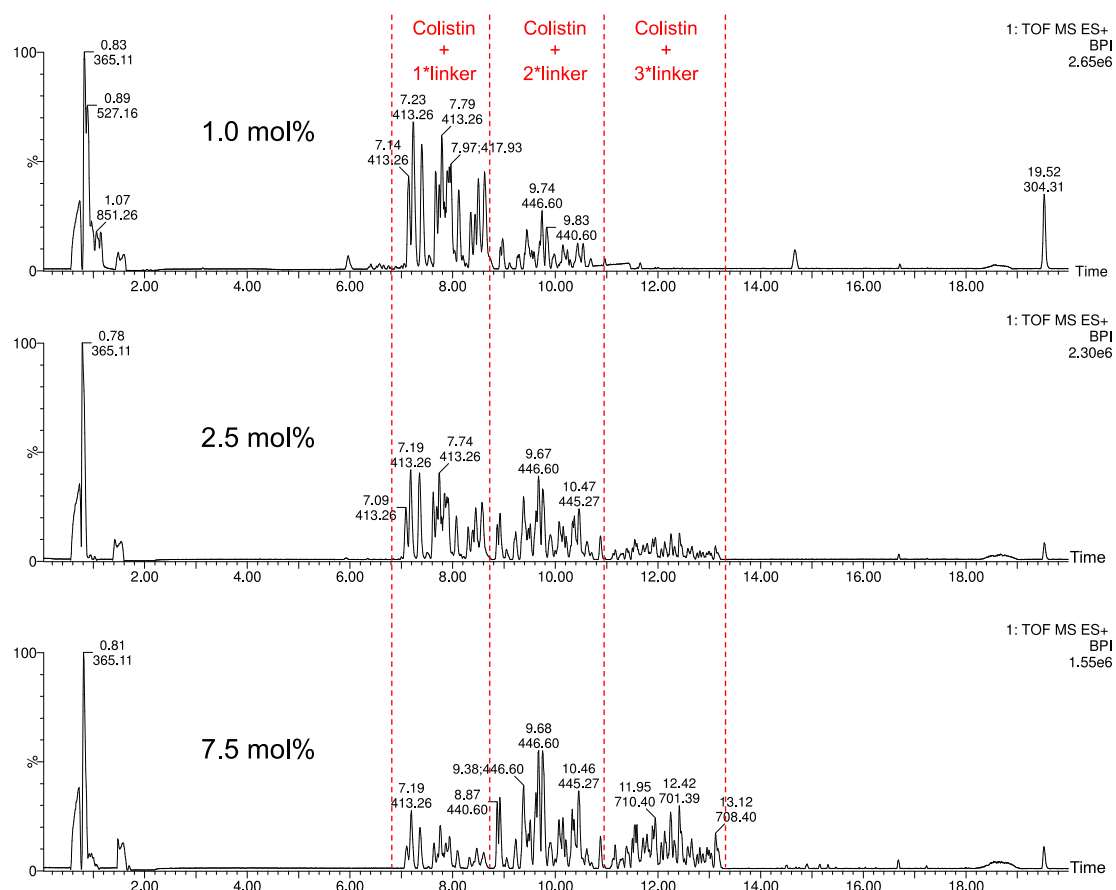


Fig. S6: UPLC-QTOF-MS chromatograms (Base Peak Intensity, BPI) of amylase-degraded dextrin-colistin conjugates (0.2 mg/mL colistin base) containing dextrin with 1.0, 2.5 and 7.5 mol% succinylation. Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.

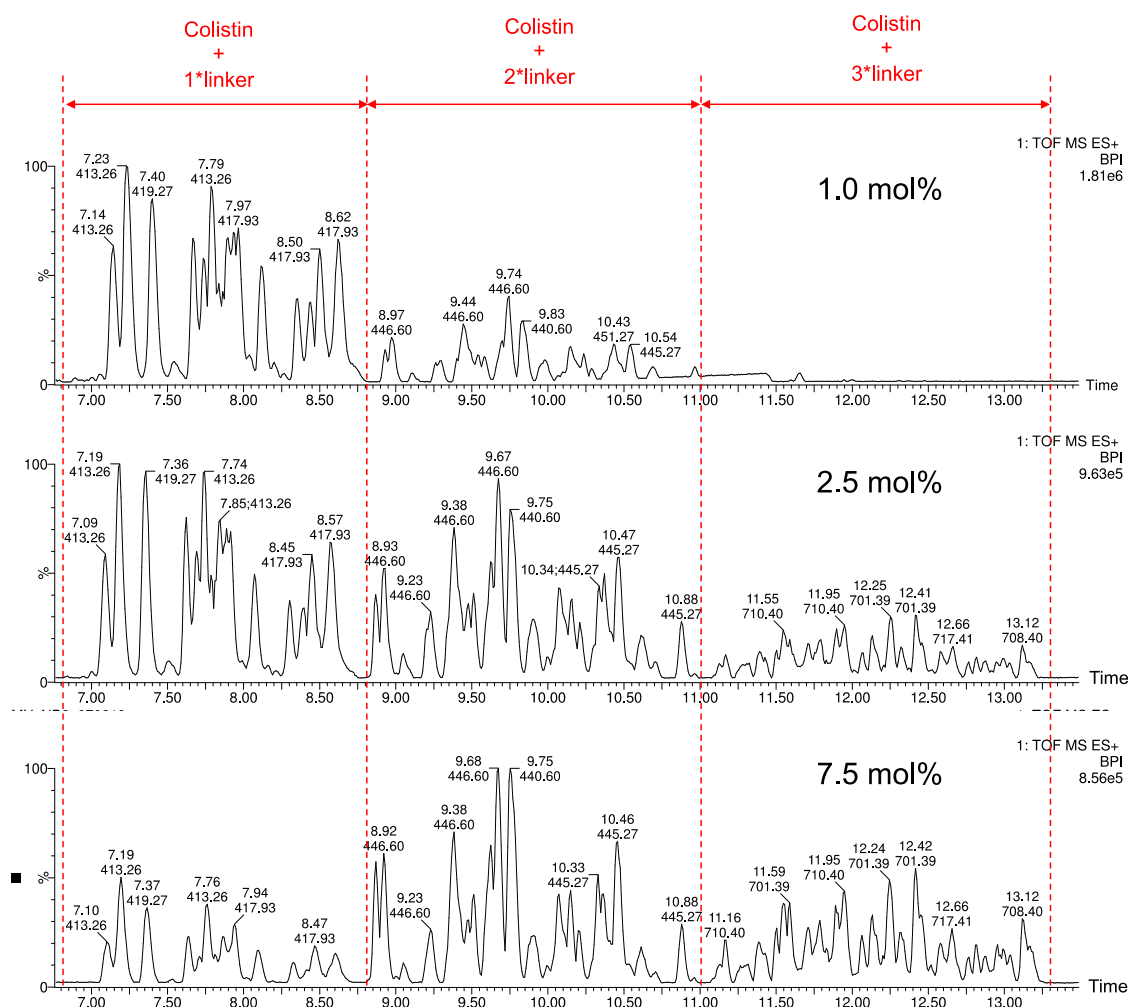


Fig. S7: Zoom view of UPLC-QTOF-MS chromatograms (Base Peak Intensity, BPI) between 7 and 13.5 min of amylase-degraded dextrin-colistin conjugates (0.2 mg/mL colistin base) containing dextrin with 1.0, 2.5 and 7.5 mol% succinylation. Degradation was performed by incubation of dextrin-colistin conjugate (3 mg/mL colistin base in PBS, pH 7.4) with amylase (100 IU/L) for 48 h at 37°C.

Table S3: Identification of the major detected species from UPLC-QTOF-MS analysis of amylase-degraded dextrin-colistin conjugates containing dextrin with 1.0, 2.5 and 7.5 mol% succinoylation.

Species	Adducts	RT (min)	Observed mass	Calculated mass	Molecular Formulae
Colistin B + linker	[M+3H] ³⁺	7.4 / 7.6	419.27	419.26	C ₅₆ H ₁₀₂ N ₁₆ O ₁₆
Colistin A + linker	[M+3H] ³⁺	8.1 / 8.4	423.93	423.94	C ₅₇ H ₁₀₄ N ₁₆ O ₁₆
Colistin B + linker - H ₂ O	[M+3H] ³⁺	7.1 / 7.2 / 7.8 / 7.9	413.26	413.26	C ₅₆ H ₁₀₀ N ₁₆ O ₁₅
Colistin A + linker - H ₂ O	[M+3H] ³⁺	7.9 / 8.5 / 8.6	417.93	417.93	C ₅₇ H ₁₀₂ N ₁₆ O ₁₅
Colistin B + 2*linker - H ₂ O	[M+3H] ³⁺	8.9 / 9.2 / 9.4 / 9.7 / 9.9	446.60	446.60	C ₆₀ H ₁₀₄ N ₁₆ O ₁₈
Colistin A + 2*linker - H ₂ O	[M+3H] ³⁺	10.1 / 10.6	451.27	451.27	C ₆₁ H ₁₀₆ N ₁₆ O ₁₈
Colistin B + 2*linker - 2H ₂ O	[M+3H] ³⁺	8.9 / 9.5 / 9.6 / 9.7 / 10.1	440.60	440.59	C ₆₀ H ₁₀₂ N ₁₆ O ₁₇
Colistin A + 2*linker - 2H ₂ O	[M+3H] ³⁺	10.2 / 10.3 / 10.5 / 10.9	445.27	445.27	C ₆₁ H ₁₀₄ N ₁₆ O ₁₇
Colistin B + 3*linker - H ₂ O	[M+2H] ²⁺	11.3	719.40	719.40	C ₆₄ H ₁₀₈ N ₁₆ O ₂₁
Colistin A + 3*linker - H ₂ O	[M+2H] ²⁺	12.0	726.41	726.41	C ₆₅ H ₁₁₀ N ₁₆ O ₂₁
Colistin B + 3*linker - 2H ₂ O	[M+2H] ²⁺	11.2 / 11.4 / 11.6 / 11.7 / 11.8 / 11.9 / 12.1	710.40	710.40	C ₆₄ H ₁₀₆ N ₁₆ O ₂₀
Colistin A + 3*linker - 2H ₂ O	[M+2H] ²⁺	12.6 / 12.7	717.41	717.40	C ₆₅ H ₁₀₈ N ₁₆ O ₂₀
Colistin B + 3*linker - 3H ₂ O	[M+2H] ²⁺	11.6 / 12.1 / 12.2 / 12.4	701.39	701.39	C ₆₄ H ₁₀₄ N ₁₆ O ₁₉
Colistin A + 3*linker - 3H ₂ O	[M+2H] ²⁺	12.8 / 12.9 / 13.1	708.40	708.40	C ₆₅ H ₁₀₆ N ₁₆ O ₁₉

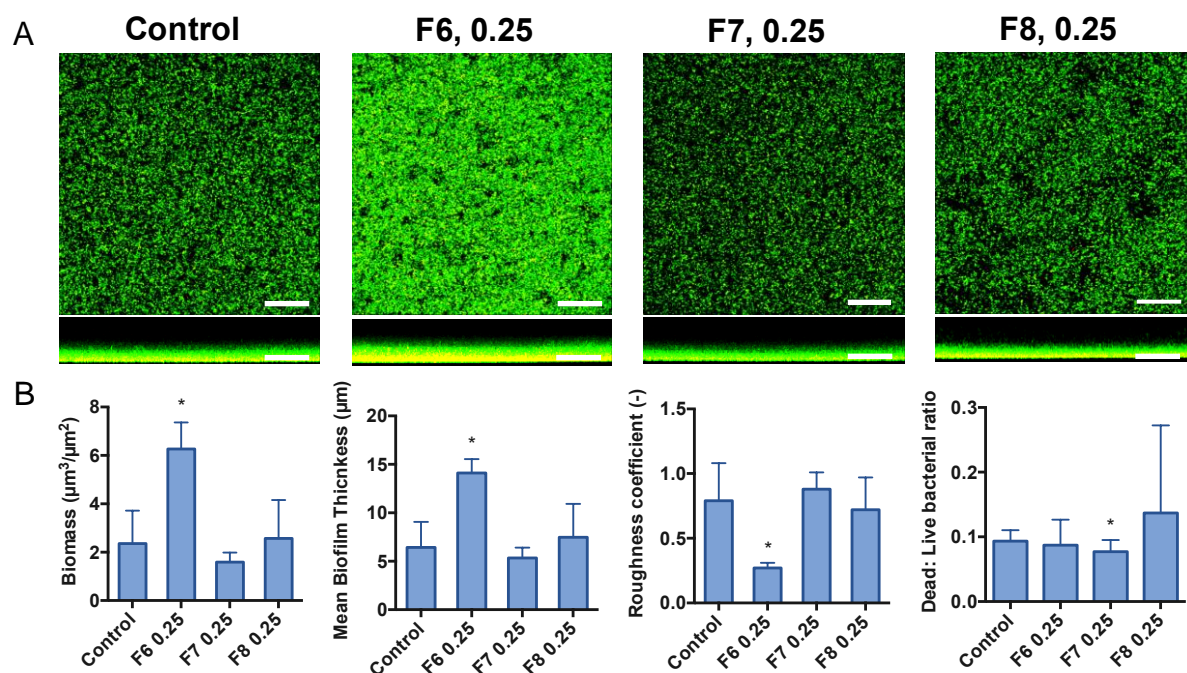


Fig. S8: A. Biofilm formation assay showing LIVE/DEAD® (green and red colors respectively) stained CLSM images of *E. coli* biofilms (aerial and side view, scale bar = 40 μm) grown for 24 h in the presence of fractions F6, F7 and F8 at 0.25 $\mu\text{g}/\text{mL}$ colistin base (equivalent to 2x fraction F8's MIC). B. COMSTAT image analysis of biofilm CLSM z-stack images. Data represents mean \pm SD; n = 3. Significant difference is indicated by *, where $*p < 0.05$, compared to untreated control.