

1 **Surface Enhanced Raman Spectroscopy (SERS) for *in-situ* Measurements of Signaling**
2 **Molecules (e.g., Autoinducers) Relevant to Bacteria Quorum Sensing**

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11 **NOTES:** Surface Enhanced Raman Spectroscopy (SERS), acyl-homoserine lactone (AHL),
12 silver colloid, Quorum Sensing, autoinducer molecules, chemical communication

13

14 **ABSTRACT**

15

16 Autoinducer (AI) molecules are used by Quorum Sensing (QS) bacteria to communicate
17 information about their environment, and are critical to their ability to coordinate certain
18 physiological activities. Studying how these organisms react to environmental stresses could
19 provide insight into methods to control these activities. To this end, we are investigating
20 spectroscopic methods of analysis that allow *in-situ* measurements of these AI molecules under
21 different environmental conditions. We found that for one class of AI's, *N*-acyl homoserine
22 lactones (AHLs), Surface Enhanced Raman Spectroscopy (SERS) is a method capable of
23 performing such measurements, *in-situ*. SERS spectra of seven different AHLs with acyl chain
24 lengths from 4 to 12 carbons were collected for the first time using Ag colloidal nanoparticles
25 synthesized via both citrate and borohydride reduction methods. Strong SERS spectra were

26 obtained in as little as 10 seconds for 80 μM solutions of AI that exhibited the strongest SERS
27 response, whereas 20 seconds was typical for most AI SERS spectra collected during this study.
28 Although all spectra were similar, significant differences were detected in the SERS spectra of
29 C4-AHL and 3-oxo-C6-AHL and more subtle differences were noted between all AHLs. Initial
30 results indicate a detection limit of $\sim 10^{-6}$ M for C6-AHL, which is within the limits of
31 biologically relevant concentrations of AI molecules (nM - μM). Based on these results, the
32 SERS method shows promise for monitoring AI molecule concentrations *in-situ*, within biofilms
33 containing QS bacteria. This new capability offers the possibility to “listen in” on chemical
34 communications between bacteria in their natural environment as that environment is stressed.

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36 **INDEX HEADINGS:** Surface Enhanced Raman Spectroscopy (SERS), acyl-homoserine
37 lactone (AHL), silver colloid, Quorum Sensing, autoinducer molecules, chemical communication

39 INTRODUCTION

40 Quorum Sensing (QS) is simply defined as a bacterial response dictated by a change in
41 the “sensed” cell population density of the bacteria.^{1,2} These responses may include but are not
42 limited to symbiosis, virulence, sporulation, and biofilm formation.¹⁻³ Quorum sensing bacteria
43 “sense” the changes in population density by the production and release of signaling compounds
44 called autoinducers.¹⁻⁴ QS has been a focus of many research groups over the past decade and is
45 prevalent in several reviews^{1,5} and the number of annual publications on QS has been growing
46 exponentially since the mid 1990’s.⁶

47 Acyl homoserine lactones (AHLs) are the most common and perhaps the most studied
48 autoinducer molecules⁵ and are central to the regulation of virulence in many Gram-negative

49 bacteria.^{1,7-10} As indicated in the basic structure of the AHLs, shown in Figure 1, individual
50 AHLs are identified by the length (n) of their *N*-acyl side chains where n = 4, 6, 7, 8, 10, 12 and
51 14,¹¹ and the *N*-acyl side chains may have either a carboxyl or hydroxyl substituent group (R) at
52 the C3 position.¹² To date several analytical techniques have been utilized to study AHLs.
53 These techniques include gas chromatography-mass spectrometry¹³ thin-layer
54 chromatography,^{14,15} proton nuclear magnetic resonance^{7,11,16} mass spectrometry¹¹, electrospray
55 ionization-ion trap mass spectrometry⁹, biological assays², two dimension correlation
56 spectroscopy,¹⁷ and the use of live bacterial sensors.¹⁴ So far, only the use of the live, green
57 fluorescent protein based bacterial sensors allows for detection of AHLs in the local
58 environment.¹⁴

59 Raman spectroscopy has continually demonstrated the ability to perform non-invasive
60 analysis, both remotely and *in situ*, and thus appears to be a prime candidate for the study of
61 AHLs in their natural environment. Unfortunately the Raman effect is very weak where only
62 one in $\sim 10^5$ photons are Raman, or inelastically, scattered.¹⁸ Therefore, many Raman based
63 techniques have been developed to increase the Raman signal. Surface Enhanced Raman
64 Spectroscopy (SERS),¹⁹⁻²¹ Resonance Raman Spectroscopy (RRS),²¹⁻²³ and Coherent Anti-Stokes
65 Raman Spectroscopy (CARS)²³ are a few such techniques that have been described in the
66 literature. Of the enhanced Raman techniques, SERS is relatively simple as it does not require a
67 tunable laser and it has previously demonstrated the ability to identify and analyze many
68 biological relevant molecules.^{20,22,24-28} The feasibility of using SERS for *in situ* analysis would
69 bring a unique capability to the study and understanding of *N*-acyl homoserine lactones in their
70 natural environment. The primary purpose for this work was to determine the viability of SERS
71 to detect, characterize, and quantify quorum sensing molecules, specifically *N*-acyl homoserine

72 lactones. We believe that this study is the first to specifically target and analyze these important
73 biological molecules with the use of SERS.

74 **EXPERIMENTAL**

75 *Materials*

76 All *N*-acyl homoserine lactones used in this study were purchased from Sigma-Aldrich
77 and are detailed in Table I. NaBH₄, Na₃C₆H₅O₇•H₂O, C₂H₆OS (DMSO), 30% H₂O₂ and AgNO₃
78 were also purchased from Sigma without further purification or modification. H₂SO₄ was
79 acquired from Fisher Scientific and KCl from Mallinckrodt. All H₂O was ~18MΩ from
80 Barnstead Nanopure water purification system.

81

82 *Instrumentation*

83 SERS spectra were recorded with the use of an Argon Ion laser (Coherent Innova-90)
84 using 514.5 nm excitation. The laser was delivered to the sample via a filtered fiber optic probe
85 (InPhotonics: Raman ProbeTM) with a 90 μm excitation fiber and a 200 μm collection fiber.
86 Probe head power for all measurements was ~150 mW. The collection fiber of the probe was
87 coupled to a Holospec *f*/1.8 imaging spectrometer (Kaiser Optical Systems Inc.) utilizing a
88 514nm SuperNotch Plus filter for further laser line rejection and a low frequency (LF)
89 holographic grating covering the spectral range of 50-2400 cm⁻¹. Raman spectra were recorded
90 using a liquid nitrogen cooled CCD (Princeton Instruments model: LN/CCD 1024EUV) using
91 WinSpec/32 software. Daily calibrations of the system were performed using cyclohexane as
92 described in the literature²⁹ and common gas chromatography target vials (National Scientific)
93 were used to hold all the samples. UV/Vis data was collected using a Cary 500 UV/Vis
94 Spectrometer and all SEM images were acquired using a Hitachi 8000 SEM.

95

96 *Borohydride Stabilized Colloidal Synthesis*

97 All glassware was cleaned using a piranha solution (4:1 H₂SO₄:30% H₂O₂) prior to use in
98 any colloidal synthesis. Borohydride stabilized particles were prepared following the procedure
99 described by Creighton et al.³⁰ Briefly, 30 mL of 2 mM NaBH₄(aq) was placed in a 50mL round
100 bottom flask which is submerged in an ice bath. After approximately one half hour, a stir bar
101 was added and the NaBH₄(aq) was vigorously stirred. 10mL of 1mM AgNO₃(aq) in an addition
102 funnel was added drop wise (~1 drop per second) to the borohydride solution. The hydrosol was
103 continuously stirred for approximately 30 minutes after the final drop of silver nitrate had been
104 added. The resultant Ag hydrosol was pale yellow and often darkened slightly to a deeper
105 yellow as it matured. The borohydride stabilized sols have a $\lambda_{MAX} = 391$ nm (Figure 2a) and
106 possessed a greater degree of mono-dispersity with regards to shape and had particle sizes of
107 approximately 20-50 nm (Figure 2a). These Ag hydrosols were stored in the reaction flask and
108 remained stable for several weeks.

109

110 *Citrate Stabilized Colloidal Synthesis*

111 Citrate stabilized silver hydrosols were synthesized using a modified Lee and Meisel
112 method.³¹ Briefly, 250 mL of 1.0 mM AgNO₃(aq) was placed in a 250 mL three neck round
113 bottom flask and brought to a rolling boil under vigorous stirring. The use of a condenser
114 reduced concentration effects due to loss of solvent during the synthesis. Once boiling, 5mL of
115 1% (w:v or 34 mM) sodium citrate was added via an addition funnel with the stopcock fully
116 open. The solution was brought back to a rolling boil and boiling was continued for 45 minutes.
117 It was then removed from heat and stirring was continued for an additional hour. The hydrosol

118 was then allowed to cool to room temperature and stored in Nalgene bottle. The resulting Ag
119 hydrosol was cloudy with a greenish yellow color and had a $\lambda_{MAX} = 426$ nm (Figure 2b) and had
120 a wide distribution of both particle size (around 100 nm) and shape and was stable for several
121 months. The SERS enhancement for both types of colloidal hydrosols tended to change with
122 time reaching a maximum after 10-14 days, probably due to aggregation of the colloidal sol over
123 time.

124

125 *Solvation of N-acyl homoserine lactones*

126 With the exception of the shorter ($C < 7$) acyl chain AHLs, all are slightly only soluble in
127 water mainly due to the hydrophobic nature of the acyl chains. However, AHLs are very soluble
128 in organic solvents like acetonitrile and ethyl acetate,³² but both of these solvents possess
129 significant Raman structure in the fingerprint region of the Raman spectrum ($\sim 500 - 1800$ cm^{-1})
130 and were therefore not considered. We decided to use either methylene chloride or dimethyl
131 sulfoxide (DMSO) to solvate the AHLs. Methylene chloride is relatively free of strong Raman
132 peaks can be easily sparged from an aqueous solution using nitrogen and DMSO has few sharp
133 Raman bands in the spectral region of interest.

134 For AHLs with an acyl chain length of 4-8 carbons, a stock solution of 10^{-2} M was
135 prepared by dissolving the AHL in 1.0 mL of methylene chloride. An aliquot of this stock
136 solution was added to a flask containing water and sparged for 10 minutes. The final AHL
137 concentrations ranged from 10^{-5} to 10^{-4} M. DMSO was used for AHLs with an acyl chain length
138 greater than 8 carbons due to the relatively poor solubility of these molecules in methylene
139 chloride. 10^{-2} M stock solutions of these AHLs were prepared in DMSO in the same manner
140 described above using methylene chloride. An aliquot of the stock AHL/DMSO solution was

141 then added very slowly to water under vigorous stirring with the use of a syringe. The final
142 concentration of these aqueous suspensions was between 10^{-5} and 10^{-4} M.

143

144 *Preparation of SERS samples*

145 All samples were prepared using 0.5 mL of Ag colloid, 1.0 mL of AHL, and a small
146 amount (μL) of 0.5M KCl solution. It is shown in the literature that the addition of chloride,
147 most often in the form of KCl, to a colloidal suspension can induce supplementary enhancements
148 when excited in the visible region.³³⁻³⁵ The effect of the chloride ions most likely induces a
149 reorientation of the adsorbate on the colloidal surface and results in the narrowing of some bands
150 and a change in the relative intensity of certain vibrational modes.³⁴ Furthermore, it is shown
151 that the addition of chloride leads to aggregation of the silver particles³³ as well as the formation
152 of colloidal clusters which significantly increases both the electromagnetic and chemical
153 enhancement factors.³⁵ Daily trial runs were conducted to determine the specific amount of KCl
154 required to obtain the greatest SERS response. In general, between 20 – 50 μL of KCl were
155 added to these samples although there appeared to be no common amount of KCl that produced
156 consistent results across all samples over the period of the study. The Ag colloid was added first
157 to the target vials followed by the KCl. Often the colloid/KCl was mixed using a fresh
158 disposable pipette prior to the addition of the AHL solution. The addition of 1.0 mL of AHL
159 appeared to provide adequate mixing of the solution when the colloid/KCl was not mixed as
160 described above. The final AHL concentrations were on the order of 10^{-5} M for all SERS
161 samples. No effort was made to control the pH of the sample solutions for these initial
162 experiments and therefore, all sample solutions were made fresh and measured within hours to
163 limit any possible hydrolysis of the parent AHL.

164 **RESULTS AND DISCUSSION**

165 *Collection of SERS Spectra*

166 All spectra shown were collected with a total of 2 minutes integration time (6
167 accumulations of 20 seconds) although spectra could be distinguished in as little as 10 seconds,
168 and a minimum of three spectra were collected for each one presented. As a general rule, no
169 baseline or background corrections were applied to the spectra shown, however, spectra were
170 often normalized to the amide I band at $\sim 1648\text{ cm}^{-1}$ to account for the varied SERS response of
171 each of the analyte runs. AHL spectra were collected using both citrate and borohydride
172 stabilized Ag sols to ensure there were no spectral artifacts from reaction by-products that might
173 have formed during the initial reduction reactions, and that might be misidentified as part
174 of the AHL structure. No significant differences were observed in AHL spectra using either type
175 of colloid.

176 Figure 3 shows two representative uncorrected SERS spectra of C6-AHL and C7-AHL
177 obtained using some of the most SERS active colloidal hydrosols. It is interesting to note that
178 the Ag colloidal hydrosol was different for these two samples; borohydride reduced colloid for
179 the C6-AHL and citrate reduced colloid for the C7-AHL. Other AHL spectra are shown in
180 Figure 4. It was further observed in this study that a single AHL produced identical SERS
181 spectra using either citrate or borohydride stabilized colloids. Two important observations are
182 drawn from the spectra shown and from others not presented here. First, the structure of the
183 SERS signal appears consistent for an individual AHL when using the two different types of
184 colloids albeit there are often overall intensity differences. Secondly, there appear to be no
185 major differences in the SERS spectrum of the individual AHLs though subtle differences such
186 as relative intensity ratios of bands do occur

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Comparison of SERS Response for the Different Ag Colloidal Sols

It is not surprising that the SERS response using both types of Ag colloids varied for the AHLs by as much as an order of magnitude. We feel that this is mainly due to the inherent irreproducibility of the SERS substrates, in this case the colloidal hydrosol, and not a function of the individual AHLs. On average, peak intensities for the AHLs were around 5-10 K counts/min above the baseline, but 100 – 250 K counts/min. peak intensities were also obtained for certain colloids during the study. However, spectra for each of the individual AHLs collected with both types of Ag colloids possessed no spectral differences when comparing the same analyte between the two different colloids. The interesting result of this observation is that we can reasonably expect similar SERS spectra using different colloidal hydrosols and excitation wavelengths tuned to those colloidal hydrosols. But it is not uncommon to observe bands that experience different degrees of enhancement because of different resonance effects which occur as a function of excitation wavelength or binding orientation to the colloidal surface. This does not seem to be an issue for the AHLs studied. The structure of the SERS spectra also remains unchanged as a function colloidal shelf-life time.

Tentative Assignments of Observed Peaks

The SERS spectra of all AHLs in this study are dominated by contributions of the amide portion of the molecule. We have tentatively assigned positions to the Amide I-IV bands as well as other bands attributed to the amide structure of the molecules. A detailed list of all band assignments is provided in Table II. The Amide I (1648 cm^{-1}), Amide II (1570 cm^{-1}), Amide III (1308 cm^{-1}), and Amide IV (771 cm^{-1}) are clearly identifiable in all of the SERS spectra obtained

210 during this study. We were initially surprised by the apparent lack of contribution to the SERS
211 spectrum of the AHLs from the γ -lactone portion of the molecule.^{37,40} However, it appears that
212 the Raman peak at 1360 cm^{-1} is most likely attributed to the C-O stretch of the lactone. Also, the
213 very strong Amide I band could contain contributions from both the amide C=O as well as the
214 C=O found in the lactone portion of the molecule. Comparing the Amide I band from the SERS
215 spectra of the AHLs to Raman spectrum of *N*-Ethylacetamide, a simple secondary amide, it is
216 interesting to note that the Amide I band in *N*-Ethylacetamide is less intense than either the
217 Amide II or III band and decreases further as the degree of hydrogen bonding increases.^{41,42} We
218 do observe some band shifts due to hydrogen bonding in other regions, yet the Amide I band is
219 consistently the strongest band observed in the SERS spectra of the AHLs. These observations
220 suggests the Amide I band experiences a greater SERS enhancement and thus would also suggest
221 an orientation of the molecule where the C=O from the amide is both closer to and nearly normal
222 to the metal surface. Additionally, based upon reported mesomeric effects of amides,⁴³ we
223 believe that the charge distribution as a result of a mesomeric isomer of the amide strongly
224 facilitates the bonding of the positively charged nitrogen to the metal surface. Furthermore, if
225 these two conditions are met, the C=O of the lactone portion of the molecule is virtually parallel
226 to the metal surface and thus would not experience a strong SERS effect consistent with the
227 weak contributions of the lactone ring to the SERS spectra.

228

229 *Test of Qualitative Capabilities of SERS*

230 SERS spectra of most AHLs were very similar but did show relative intensity differences.
231 Figure 4 shows the SERS spectra obtained for each AHL listed in Table I. The peaks marked
232 with an * are attributed to DMSO. Although the graph is cluttered, the intent for this graph is to

233 demonstrate the similarity of the SERS spectra for all the AHLs studied. But, there are a few
234 differences that can be readily seen even in this figure. Namely there are variations in, or in the
235 case of the 3-oxo-C6-AHL complete absence of, the structure in the 1000-1100 cm^{-1} region.
236 There is also a variation in the peak ratios in the region centered about 1200 cm^{-1} , specifically the
237 peaks at 1176 cm^{-1} and 1199 cm^{-1} . In this region, the 1176 cm^{-1} peak intensity is greater than the
238 1199 cm^{-1} peak for both C6 and C8 AHL samples. The opposite is the case for the C7 and C4
239 with the C4-AHL only possessing a very weak shoulder at the 1176 cm^{-1} position. Finally, the
240 intensities of the 1176 cm^{-1} and 1199 cm^{-1} are equal for the 3-oxo, C10, and C12 AHL samples.
241 Therefore, none of the spectral differences appear where there is obvious amide peaks, thus they
242 are indicative of changes in the acyl side chain of the molecule. Another spectral region of note
243 for observed differences in the SERS spectra of the AHLs is at 1533 cm^{-1} . Here either the
244 absence (3-oxo, C6, and C8) or presence of a peak (C4, C7, C10, and C12) may prove valuable
245 in identifying individual AHLs. To see these variations more clearly, three spectra (C8, C4, and
246 3-oxo AHL) are shown in Figure 5. In Figure 5, C8-AHL is shown it represents the majority of
247 the AHLs studied, while the C4-AHL and 3-oxo-C6-AHL showed the greatest variations among
248 the groups of AHLs studied. The 3-oxo-C6-AHL does not possess the peak at $\sim 1005 \text{ cm}^{-1}$ (# in
249 the graph), as previously mentioned. The C4-AHL possesses five additional peaks marked with
250 (*'s on Figure 5) at $\sim 1025 \text{ cm}^{-1}$, $\sim 1235 \text{ cm}^{-1}$, $\sim 1386 \text{ cm}^{-1}$, $\sim 1452 \text{ cm}^{-1}$, $\sim 1535 \text{ cm}^{-1}$. The two
251 bands at $\sim 1386 \text{ cm}^{-1}$ and $\sim 1452 \text{ cm}^{-1}$ are most likely attributed to C-H symmetric and asymmetric
252 deformation of the terminal methyl group respectively whereas the peak at $\sim 1535 \text{ cm}^{-1}$ is most
253 likely a result of CH_2 deformation. The final two peaks at $\sim 1025 \text{ cm}^{-1}$ and $\sim 1235 \text{ cm}^{-1}$ are left
254 unassigned but are most likely attributed to either a C-C skeletal stretch or CH_2 vibration.
255 Finally, Figure 5 clearly shows the variation of the peak ratios for the peaks around 1200 cm^{-1} as

256 detailed above. At present, the reason for these variations is unknown but could be a result of the
257 AHLs molecular vibrations, the binding orientation to the colloidal surface, or a combination of
258 both.

259

260 *Hydrolysis of N-acyl homoserine lactones*

261 As previously mentioned, no attempt was made to control the pH of the AHL solutions
262 and all solutions were analyzed immediately to minimize hydrolysis of the AHL samples. Once
263 we were confident in our ability to detect AHLs using SERS, a simple test was performed to
264 determine if it is possible to detect the hydrolysis of the AHLs using SERS. As shown in the
265 literature, hydrolysis of these *N*-acyl-homoserine lactones occurs more rapidly at basic pH's^{44,45}
266 and slower hydrolysis rates are observed at acidic pH.⁴³ Therefore, phosphate buffers (pH = 3.7
267 and pH = 9.1) were made and used in place of the B-Pure water for the make up of samples of
268 C7-AHL and Ag colloid was then added after 45 minutes. As can be seen in Figure 6, there is
269 the appearance of new peaks after approximately hour after the samples were prepared in the
270 basic buffer solution. The most obvious difference in the basic compared to the acidic sample is
271 observed at 1270 cm⁻¹ while subtle differences are also apparent as new shoulder peaks at 1143
272 cm⁻¹ and 1432 cm⁻¹. These new peaks are consistent with peaks commonly observed in
273 carboxylic acids which are the accepted product of hydrolysis of *N*-acyl-homoserine lactones.^{8,46}
274 It is possible that these new peaks are a result of only CH₂ deformation vibrations seen in long
275 aliphatic chain carboxylic acids, however, based upon relative intensities, we believe that the
276 1270 cm⁻¹ peak is attributed to carboxylic C-O stretch and the 1432 cm⁻¹ peak is a combination
277 band due to carboxylic C-O stretch and O-H deformation. It is clear that additional studies are

278 required in this particular area, yet it does appear that SERS is a viable alternative to the study of
279 hydrolysis reactions of *N*-acyl-homoserine lactones.

280

281 *Determination of Limit of Detection*

282 Using C6-AHL as a representative sample, two different experiments were run to
283 determine both a limit of detection and to get an idea of the nature of AHL binding to the Ag
284 nanoparticles. In the first experiment, an initial stock sample of Ag colloid and C6-AHL was
285 prepared and samples were made via several successive aqueous dilutions of this stock sample.
286 The final C6-AHL concentrations ranged from 4×10^{-4} M to 7×10^{-6} M with three replicates
287 taken at each sample concentration. Figure 7 shows the resultant calibration curve for this
288 experiment (error bars denoting $\pm 3\sigma$ of the measured peak intensity) with the baseline corrected
289 spectrum of 7×10^{-6} M C6-AHL shown as an insert. A second calibration curve (not shown) was
290 constructed with fresh samples of Ag colloid and AHL made for each sample concentration. The
291 sample concentrations for this experiment ranged from 3.3×10^{-3} M to 1.8×10^{-5} M and produced
292 spectra with larger S/N values. Using the Amide I peak @ 1648 cm^{-1} , the S/N ratio for this
293 lowest concentration C6-AHL sample was 6.87 with the noise defined as the standard deviation
294 of the spectral region beyond 1700 cm^{-1} which is devoid of any structure. Using a S/N of 3, the
295 detection limit is calculated to be 3.2×10^{-6} M. Again, using the Amide I peak, the S/N for $1.8 \times$
296 10^{-5} M was 23 with the noise defined as mentioned previously. A S/N of 3 equates to a slightly
297 lower detection limit of 2.4×10^{-6} M C6-AHL for this particular calibration run. The agreement
298 between these experiments suggests that AHL equilibrates between the Ag nanoparticle and
299 aqueous solution relatively quickly.

300

301 **CONCLUSIONS**

302 The viability of SERS as a possible method for analyzing *N*-acyl homoserine lactones in
303 their natural environment has been confirmed using Ag colloidal hydrosols. SERS clearly
304 provides a possible quantitative ability for the detection and monitoring of AHLs *in situ* as well
305 as a semi-qualitative ability to identify some individual AHLs. It is well understood that effect
306 of additional experimental conditions (changes in pH, decrease in the probe laser power, toxicity
307 of Ag, etc.) need to be addressed in future work to declare the SERS technique ready for use in
308 active biological environments. However, the intent in this study was only to determine the
309 viability of SERS as a possible technique to detect, characterize, and quantify quorum sensing
310 molecules, specifically *N*-acyl homoserine lactones. It is clear SERS has demonstrated the
311 ability to accomplish these goals.

312

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317

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389 **Figure Captions:**

390

391 **Figure 1.** Generic structure of *N*-acyl homoserine lactones indicating both the acyl chain length
392 (n) and the C3 substitution position (R).

393

394 **Figure 2.** UV/Vis spectra and SEM images of Ag colloids produced via a) borohydride and b)
395 citrate reduction methods. The scale bar for both images is 100nm.

396

397 **Figure 3.** Uncorrected SER spectra for a) C6-AHL obtained using a borohydride reduced Ag
398 colloidal sol (left hand scale) and b) C7-AHL obtained using a citrate reduced Ag colloidal sol
399 (right hand scale).

400

401 **Figure 4.** SER spectra for all AHLs listed in Table I. Peaks marked with an * are attributed to
402 DMSO.

403

404 **Figure 5.** Spectra highlighting the major differences observed in the SER of AHLs, spectra are
405 offset for clarity. 3-oxo-C6-AHL (a) shows the lack any bands at 1005 cm^{-1} as indicated by the
406 #. C8-AHL (b) is representative of SER spectrum observed for the majority of the AHLs
407 studied. Finally, C4-AHL (c) shows additional peaks compared to all other AHLs. The peak
408 ratio variations are also clearly visible in the structure around 1200 cm^{-1} .

409

410 **Figure 6.** C7-AHL SER spectra acquired in phosphate buffers at pH's of 3.7 (bottom trace) and
411 9.1 (top trace). The additional peaks (*) observed in the basic sample are believed to be
412 indicative of the hydrolysis of the lactone ring of the parent AHL.

413
414 **Figure 7.** Calibration curve constructed using successive aqueous dilutions. The Amide I peak
415 intensity at 1648 cm^{-1} was used to determine the signal at each concentration. Error bars denote
416 $\pm 3\sigma$ of the signal intensity and the insert shows a SER spectrum obtained at $7.22 \times 10^{-6}\text{ M}$.
417 Detection limit calculated from this curve is $\sim 1\ \mu\text{M}$.

418

419

<i>Name</i>	<i>Abbreviations</i>
N-Butyryl-DL-homoserine lactone	C4-AHL / C4
N-Hexanoyl-DL-homoserine lactone	C6-AHL / C6
N-Heptanoyl-DL-homoserine lactone	C7-AHL / C7
N-Octanoyl-DL-homoserine lactone	C8-AHL / C8
N-Decanoyl-DL-homoserine lactone	C10-AHL / C10
N-Dodecanoyl-DL-homoserine	C12-AHL / C12
N-(beta-Ketocaproyl)-DL-homoserine lactone (3-Oxo-hexanoyl-DL-homoserine lactone)	3-oxo-C6-AHL / 3-oxo

Table 1. *N*-acyl homoserine lactones used in this study along with abbreviations used throughout the text.

<i>Relative Wavenumber (cm⁻¹)</i>	<i>Proposed assignment</i>	<i>Notes / ref.</i>
565	C-C Skeletal vibration	
610	O=C-N Bending vibration	
658	2° amide NH deformation vibration	
701	NH deformation out of plane deformation	
771	C=O deformation	Amide IV, 35
928	C-O-C asymmetric stretch (cyclic)	
1006	C-C-C-C symmetric stretch	
1023	CH in plane bending	
1086	CH ₂ rock γ lactone	Ref. 36
1125	CH ₂ -N- stretch	
1179	C-N-C stretch 2° amide	
1308	C-N stretch (trans isomer)	Amide III, 35
1360	C-O Stretch	
1432	CH ₂ scissor γ lactone	Ref. 36
1468	CH ₂ deformation	
1507	NH bending vibration 2° amide dilute solution	Ref. 37
1571	NH deformation + C-N stretch (trans coplanar)	Amide II, 24,35
1596	NH deformation	
1646	C=O stretch with intermolecular H-bonding	Amide I, 24,35

Table 2. Proposed band assignments for SER spectra of AHLs studied. Unless otherwise noted, band assignments were made with the use of Ref. 38

Figure 1

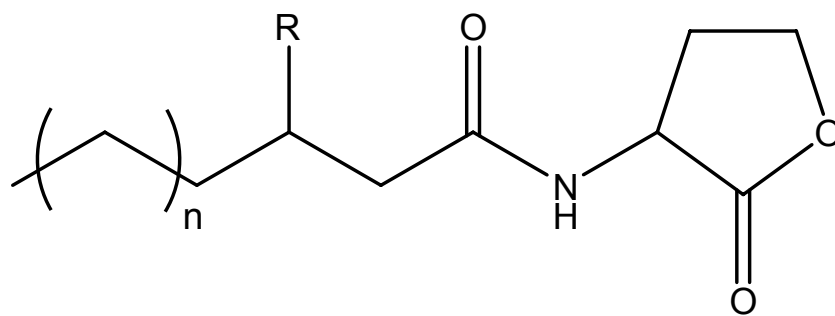


Figure 2

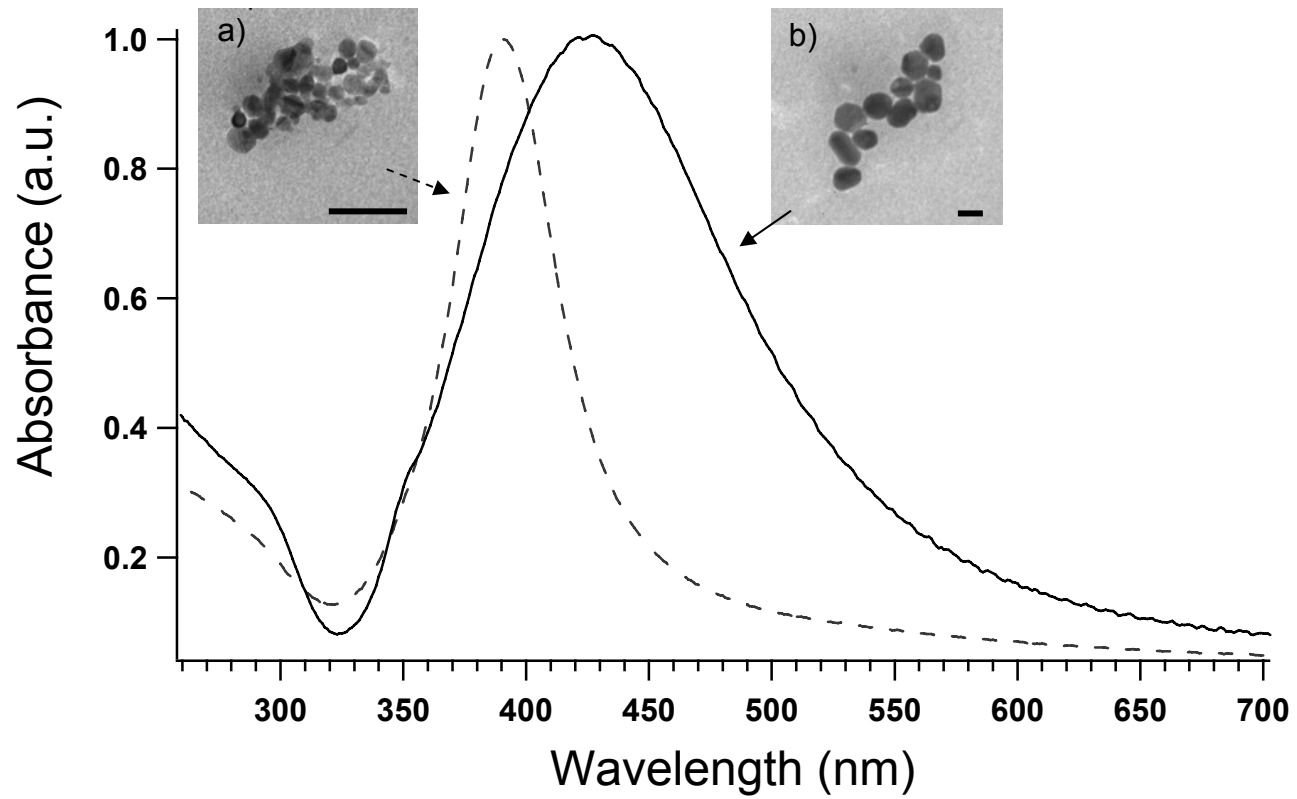


Figure 3

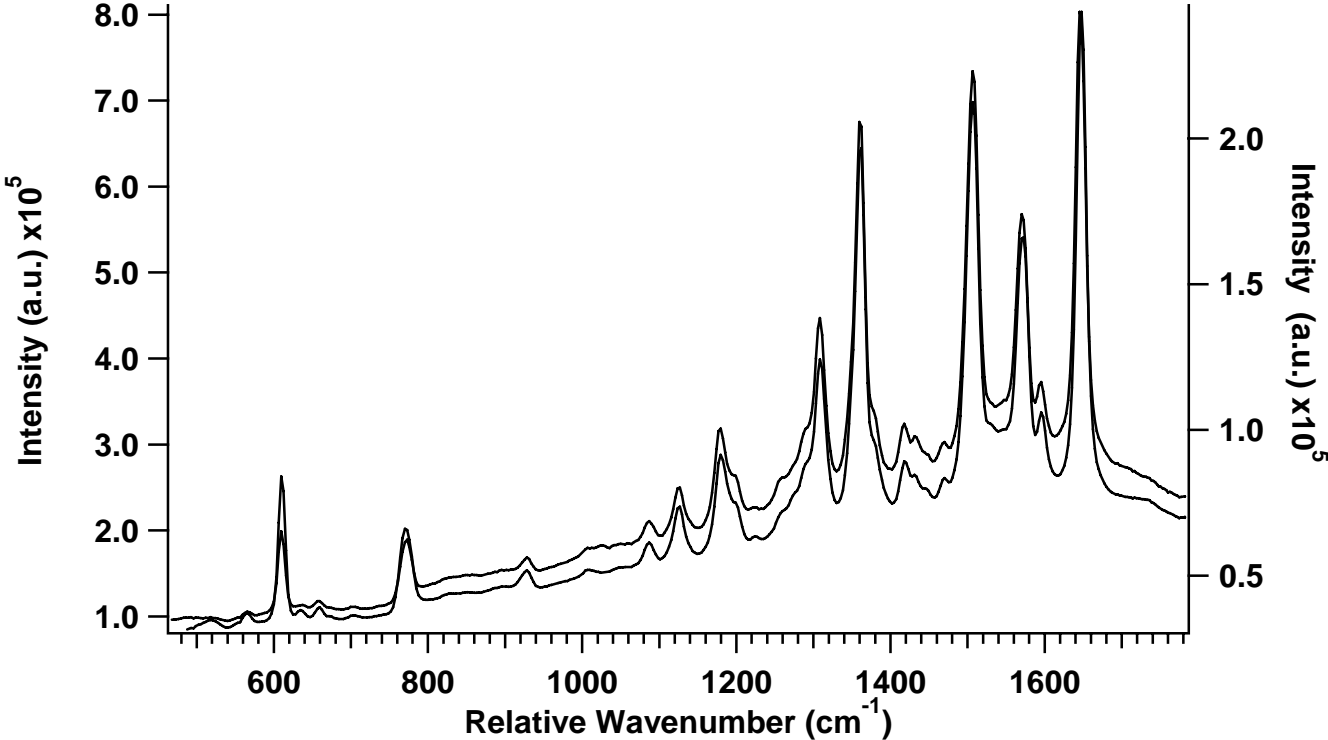


Figure 4

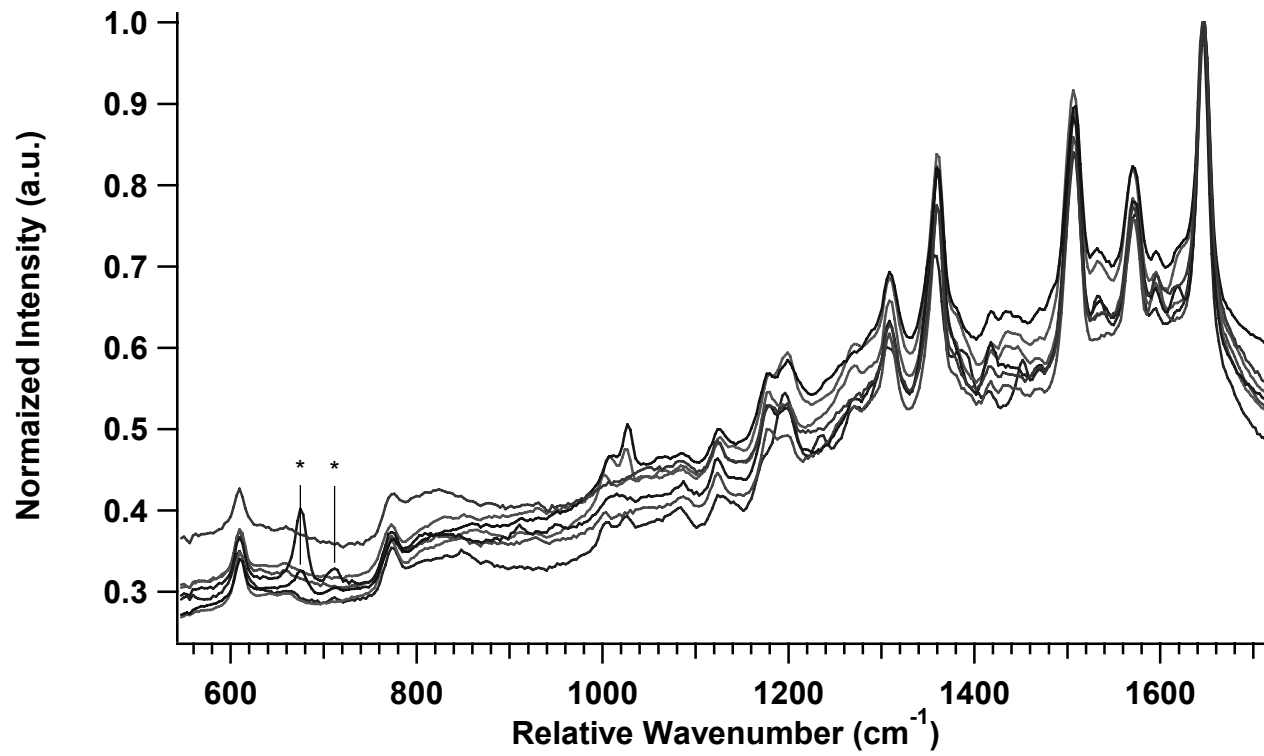


Figure 5

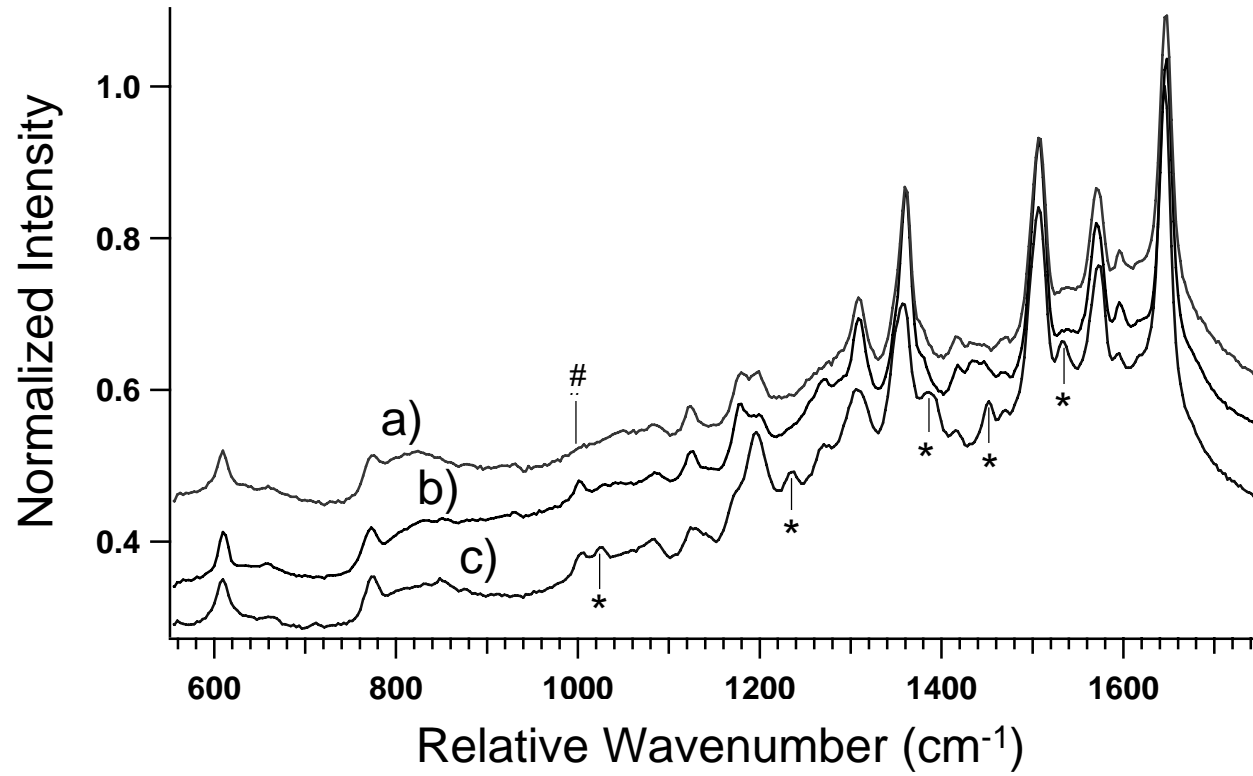


Figure 6

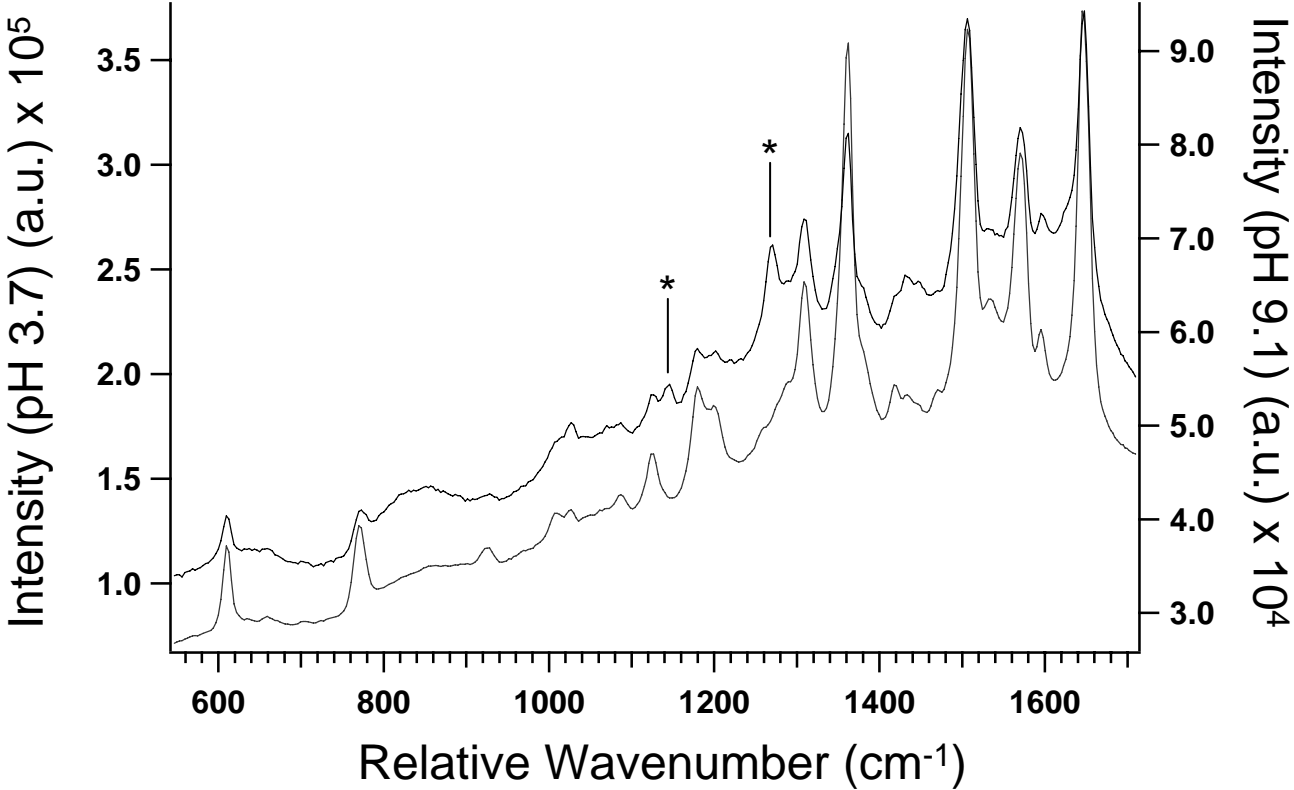


Figure 7

