SUPPORTING INFORMATION

Carboxylation and Decarboxylation of Active Site Lys 84 Controls the Activity of OXA-24 β-Lactamase of *Acinetobacter baumannii*: Raman Crystallographic and Solution Evidence

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Supplemental Figure Captions

Figure S1. Overall difference Raman spectra of OXA-24 β-lactamase complexed with SA-1-204 for selected time points.

Figure S2. Overall kinetic depiction of the main peaks intensities over time. A. Kinetic depiction of the substrate peaks intensities at 1695 $cm^{-1,}$ 1588 cm^{-1}, 1570 cm^{-1}, 1216 cm^{-1} over time. B. Kinetic depiction of the intermediate peaks intensities at 1659 cm^{-1} , 1550 cm^{-1}, 1447 cm^{-1}, 1322 cm^{-1} over time.

Figure S3. Difference absorbance spectrum of SA-1-204 in OXA-24 solution with 10 mM HEPES (pH 7.5)s. The spectrum of OXA-24 and SA-1-204 were first acquired separately. Once they were mixed, spectrum was acquired at the intervals of 30 seconds in a 30 minutes time range. A. The reaction undergoes one cycle in the absence of $NaHCO_{3.}$ 20 μM OXA-24 and 60 μM SA-1-204 were mixed. Without $NaHCO_{3,}$ the spectrum from 1 minutes to 15 minutes are from the mixture of product and unreacted substrates. B. The reaction undergoes more than one cycle in the presence of 100 mM $NaHCO_{3.}$ With NaHCO3 in solution, the reaction happens quickly and all the substrates (black line) are transformed into product (from 1 minute to 15 minutes).

Figure S4. Two views of the energy-minimized structure of intermediate X in Scheme 3. Color representations: white: hydrogen, gray: carbon, blue: nitrogen, red: oxygen, yellow: sulfur. A, from the front view; B, from the bottom view. The structure is very similar to that of the products shown in the active site of OXA-24 in Figure 2 of Bou *et al*.[1]

Figure S1

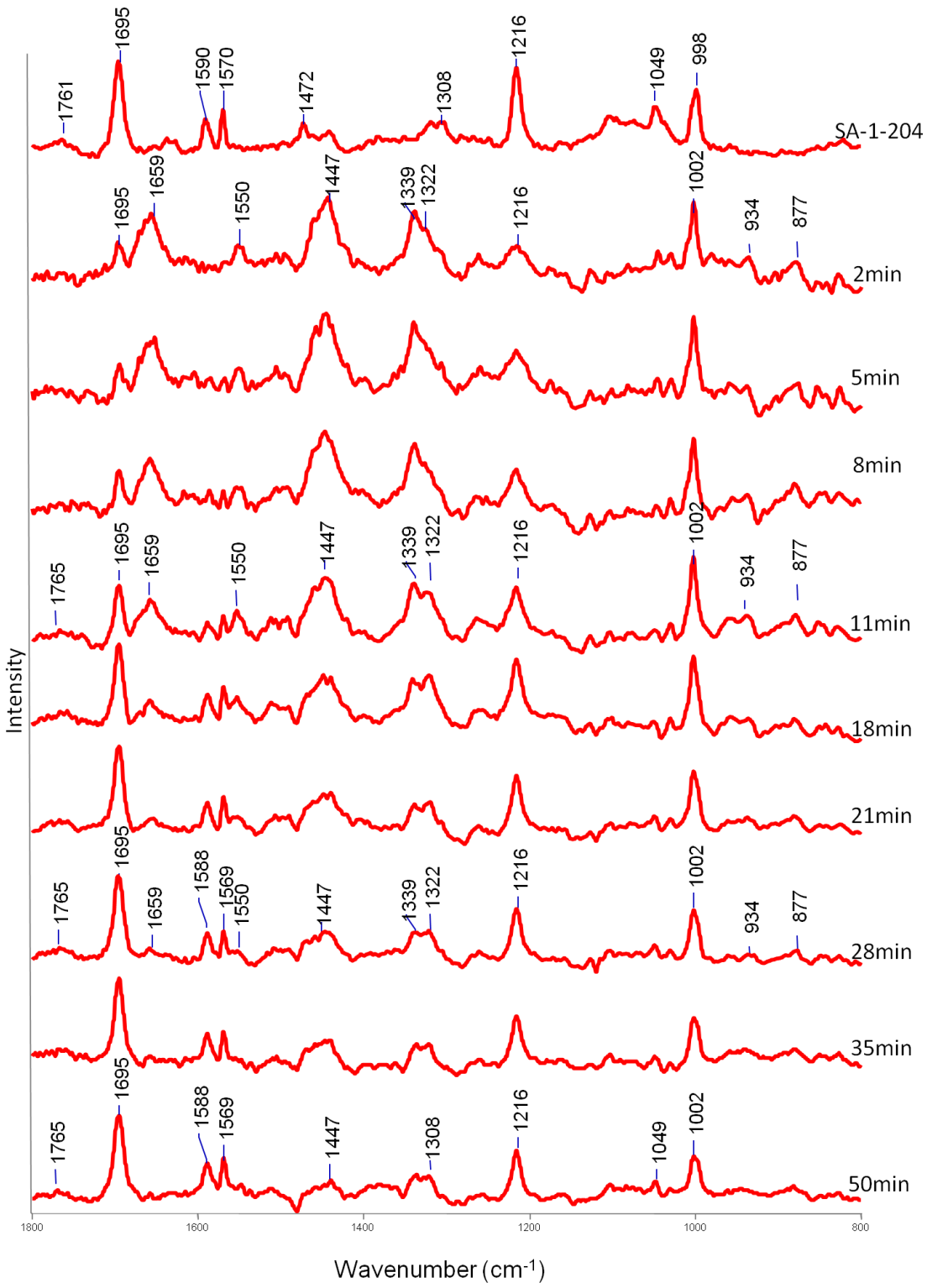

1761
1695
1590
1570
1472
1308
1216
1049
998
SA-1-204
1695
1659
1550
1447
1339
1322
1216
1002
934
877
2min
5min
8min
1765
1695
1659
1550
1447
1339
1322
1216
1002
934
877
11min
18min
21min
1765
1695
1659
1588
1569
1550
1447
1339
1322
1216
1002
934
877
28min
35min
1765
1695
1588
1569
1447
1308
1216
1049
1002
50min
Intensity
1800
1600
1400
1200
1000
800
Wavenumber (cm-1)

Figure S2

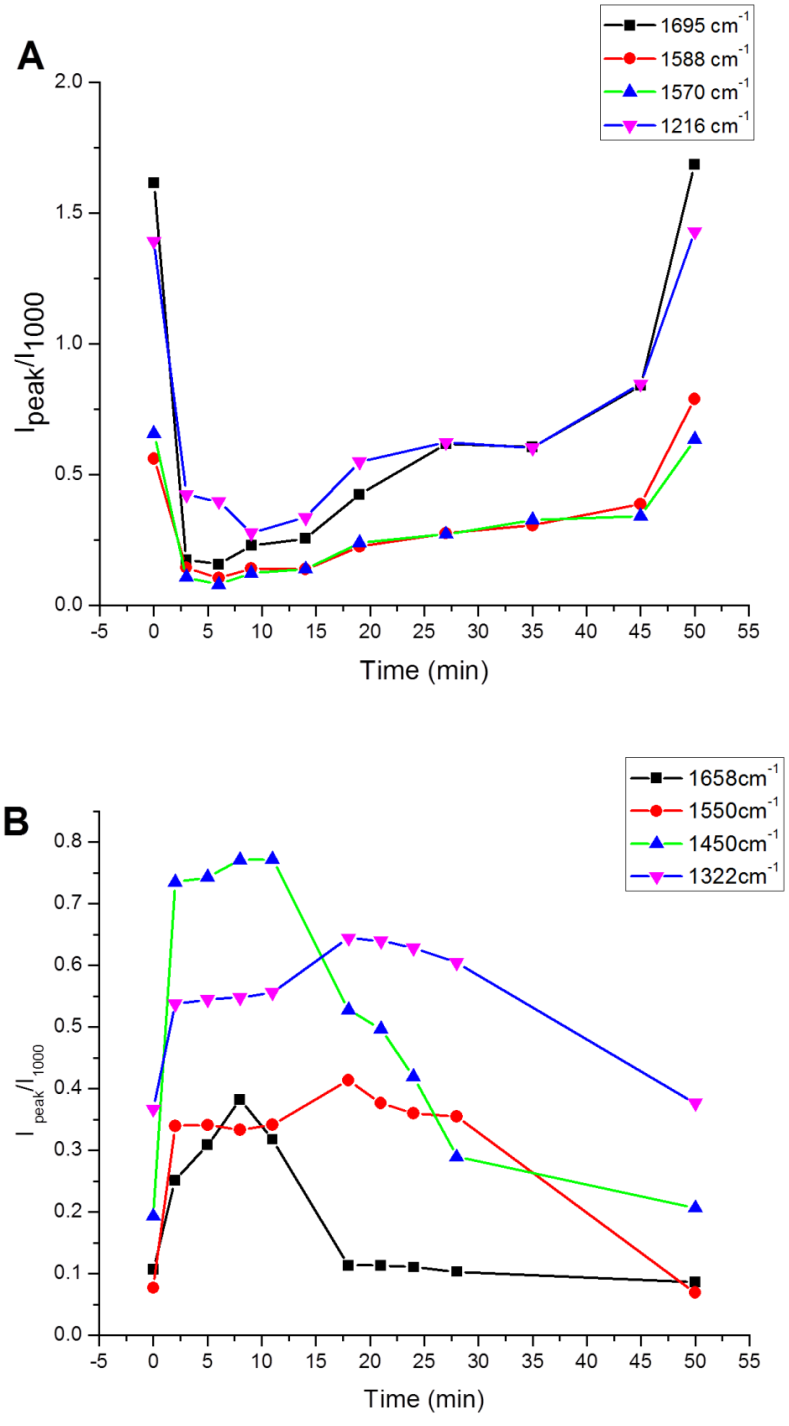

A
1695 cm-1
1588 cm-1
1570 cm-1
1216 cm-1
Ipeak/I1000
Time (min)
B
1658cm-1
1550cm-1
1450cm-1
1322cm-1
Ipeak/I1000
Time (min)

Figure S3

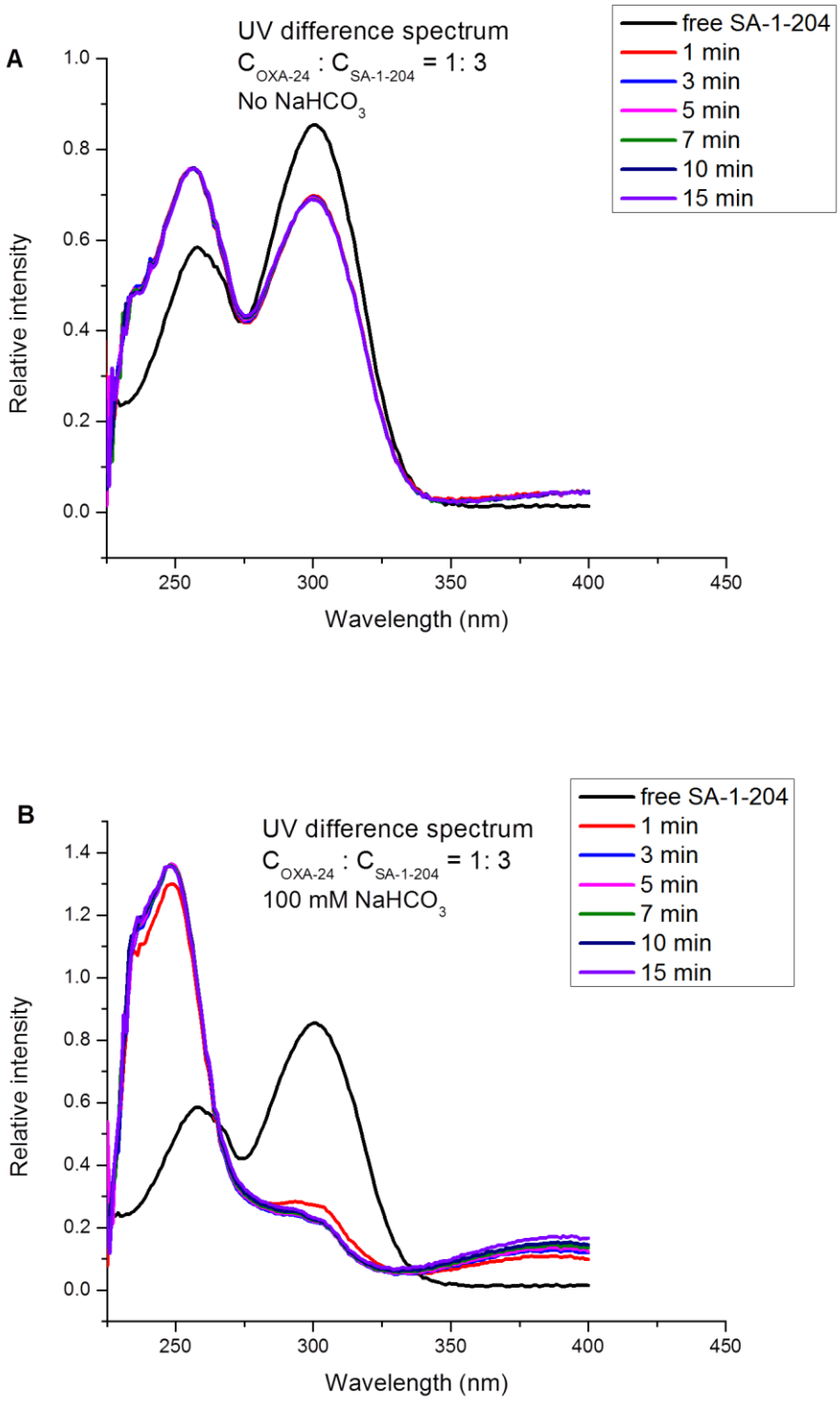
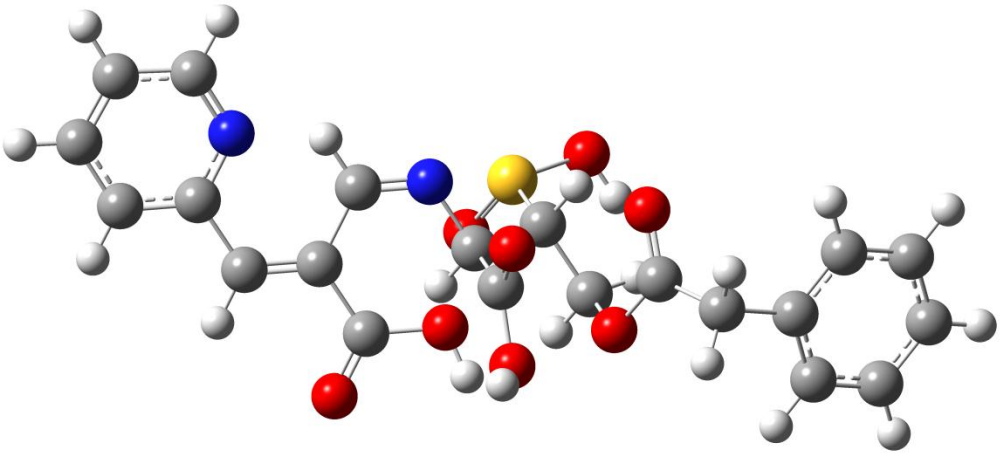
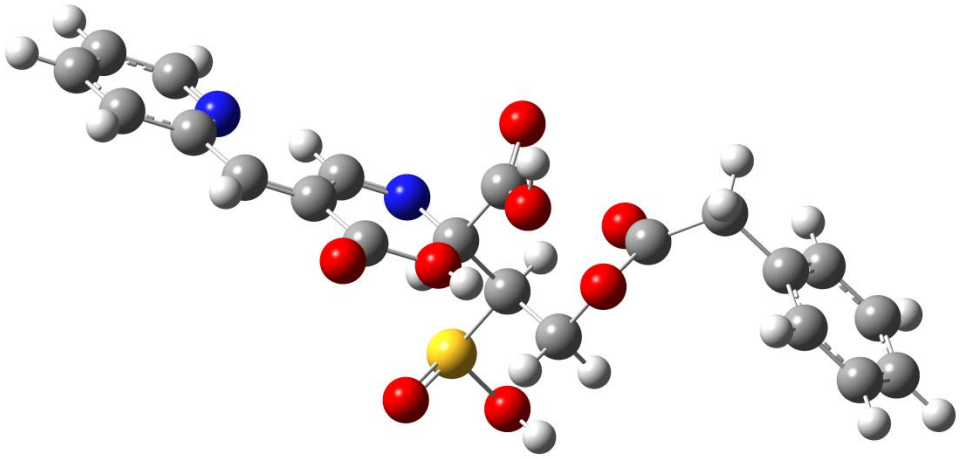

A
UV difference spectrum
C_{OXA-24} : $C_{SA-1-204}$ = 1: 3
No $NaHCO_3$
free SA-1-204
1 min
3 min
5 min
7 min
10 min
15 min
Relative intensity
Wavelength (nm)
B
UV difference spectrum
C_{OXA-24} : $C_{SA-1-204}$ = 1: 3
100 mM $NaHCO_3$
free SA-1-204
1 min
3 min
5 min
7 min
10 min
15 min
Relative intensity
Wavelength (nm)

Figure S4

A



B



Supplemental Text

1). Acquisition of Raman difference spectrum

For the spectrum of unreacted inhibitor SA-1-204, a buffer spectrum (W/O 28% PEG 2000) was obtained in a 5 μL hanging drop with a power of 80 mW (Spectrum 1). Next, spectrum of 5 mM inhibitor SA-1-204 was obtained in the same buffer as the above control, respectively (W/O 28% PEG 2000) (Spectrum 2). The Raman difference spectrum of inhibitor SA-1-204 was obtained by subtracting Spectrum 1 from Spectrum 2. Spectrum subtraction was performed using GRAMS/32 software (Galactic Industries, Inc.).

For the spectrum of bound inhibitor SA-1-204 in the active site of OXA-24, a spectrum of OXA-24 crystal in holding solutions containing 0.1 M HEPES (pH 7.5), 0.1M sodium acetate and 28% PEG 2000 was firstly recorded (Spectrum 3). Then, a spectrum of its mother liquor, obtained by shifting the hanging drop ~50 μm so that the laser focal point is away from the crystal (Spectrum 4). After Spectrum 4 was subtracted from Spectrum 3, the Raman difference spectrum of OXA-24 crystal was obtained (Spectrum 5). After inhibitor SA-1-204 was soaked in (final concentration is 5 mM), the time-dependent spectrum was recorded and the laser focal point on the crystal was at the same point as that before soak in. The spectrum of crystal after soak in (Spectrum 6) and new buffer (Spectrum 7) were obtained, respectively. A similar spectral subtraction was performed to give Raman difference spectrum of crystal after soak in (Spectrum 8). Finally, a Raman spectrum of bound SA-1-204 in the OXA-24 crystal plus any conformational changes of the enzyme was obtained by subtracting the following two spectra:

Raman difference spectrum of bound ligand = Spectrum 8 - Spectrum 5
= [(Spectrum 6 + Spectrum 7) - (Spectrum 7)] - [(Spectrum 3 + Spectrum 4) - Spectrum 4]

In this final trace, the intensities of the Raman peaks were ratiod against 1000 cm^{-1} phenyl ring mode to enable us to compare intensities among spectra. The intensities of the modes in Fig. 2 are about 3% compared to the intense peaks in the acyl-enzyme mother spectrum.

2). Concentration of active sites in a single crystal is calculated as follows:

$$\frac{\text{number of asymmetric subunits per unit cell}}{\text{volume per unit cell}} \Big/ (6.02 * 10^{23}\ \text{Mole}^{-1})$$

For OXA-24 crystal, it belongs to space group $P4_12_12$. There are 8 asymmetric subunits in a unit cell. The volume per unit cell is 102 × 102 × 86 Å^3. A value of ≈15 mM is obtained for the concentration of OXA-24 active sites in a single crystal.

(1) Bou, G.; Santillana, E.; Sheri, A.; Beceiro, A.; Sampson, J. M.; Kalp, M.; Bethel, C. R.; Distler, A. M.; Drawz, S. M.; Pagadala, S. R.; van den Akker, F.; Bonomo, R. A.; Romero, A.; Buynak, J. D. *J Am Chem Soc* **2010**, *132*, 13320.