

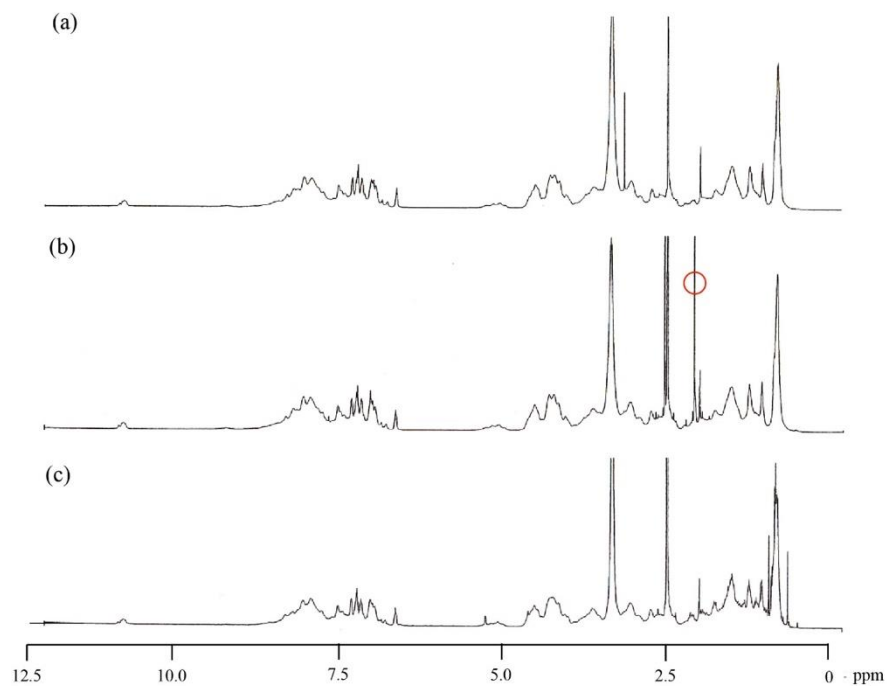
# Synthesis and Characterization of Cholesteryl Conjugated Lysozyme (CHLysozyme)

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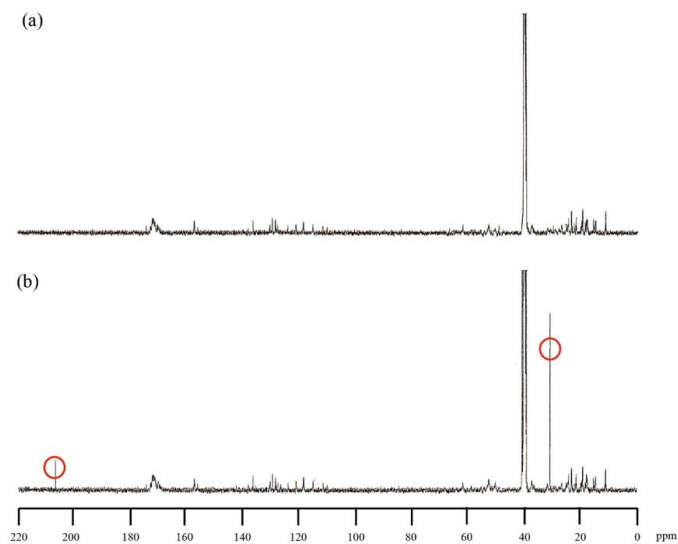
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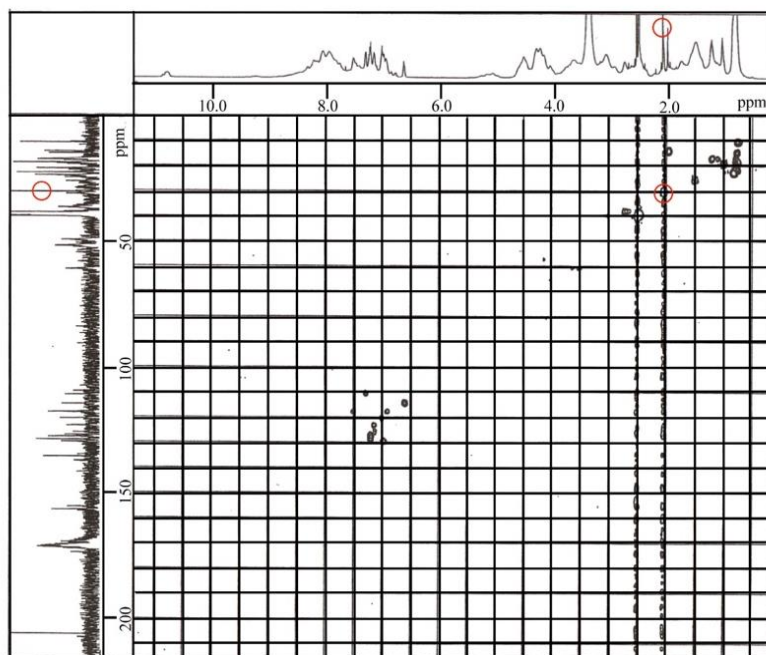
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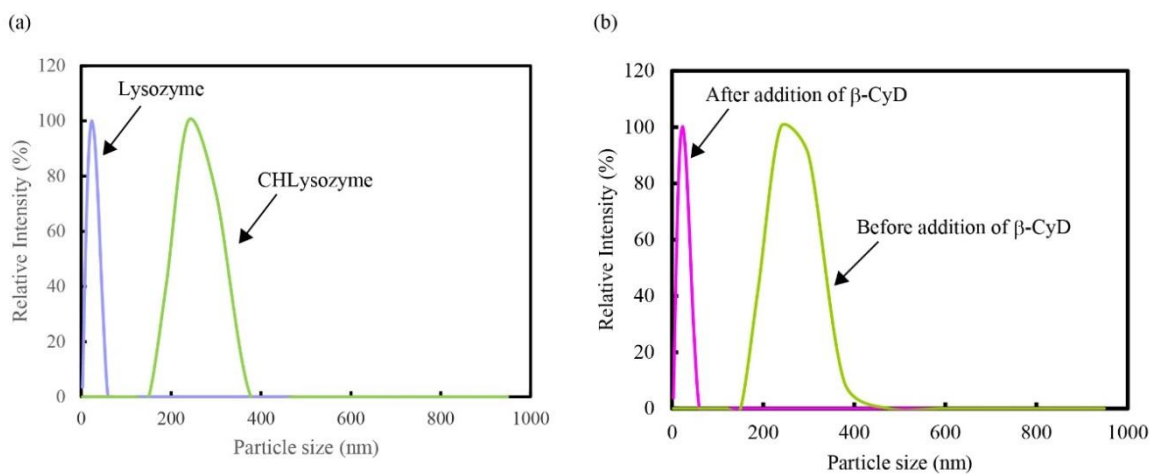
**Figure S1.** <sup>1</sup>H NMR spectra of each lysozyme in DMSO-d<sub>6</sub> at 25 °C. (a) Lysozyme, (b) CHLysozyme, and (c) physical mixture of lysozyme and cholesterol. Lysozyme and CHLysozyme concentration: 21.4 mg/mL; physical mixture of lysozyme and cholesterol (lysozyme [mol]/cholesterol [mol] = 1/12) concentration: 21.4 mg/mL.



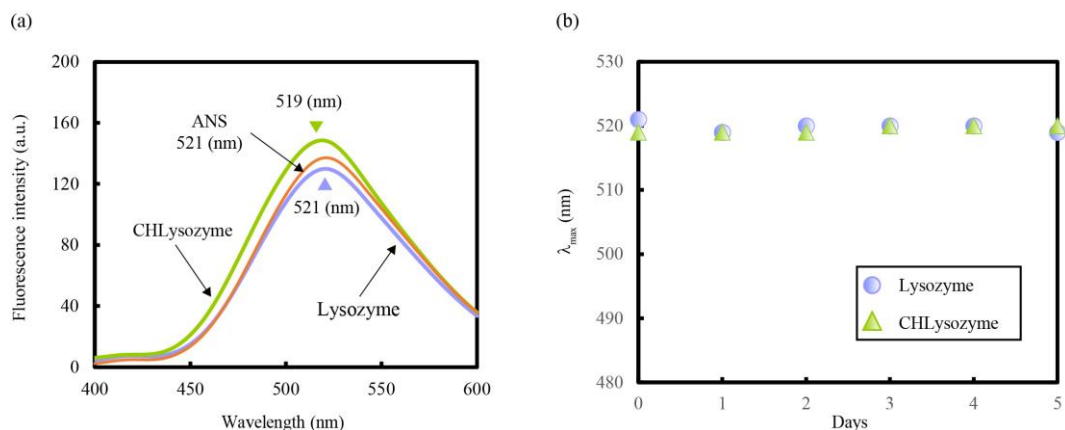
**Figure S2.** <sup>13</sup>C NMR spectra of lysozyme and CHLysozyme in DMSO-d<sub>6</sub> at 25 °C. (a) Lysozyme, and (b) CHLysozyme. Lysozyme and CHLysozyme concentration: 21.4 mg/mL.



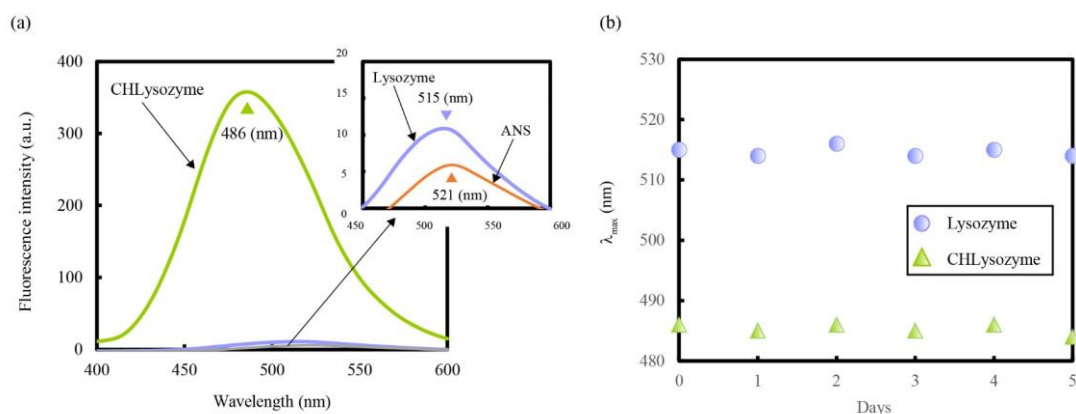
**Figure S3.**  $^1\text{H}$ - $^{13}\text{C}$  HMQC NMR spectrum of CHLysozyme in DMSO- $d_6$  at 25 °C. CHLysozyme concentration: 21.4 mg/mL.



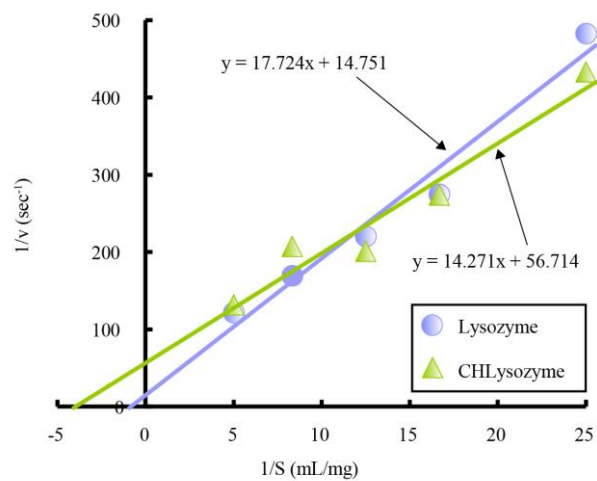
**Figure S4.** Particle size distribution of each lysozyme (a) and that of CHLysozyme before and after addition of  $\beta$ -cyclodextrin ( $\beta$ -CyD) (b). Lysozyme and CHLysozyme concentration: 1.0 mg/mL;  $\beta$ -CyD concentration:  $0.2 \times 10^{-3}$  mol/L. Preparation of the solution spiked with  $\beta$ -CyD: 0.1 mL of  $0.2 \times 10^{-3}$  mol/L  $\beta$ -CyD was added to 3.0 mL of CHLysozyme solution and the solution was analyzed 3 h post-preparation.



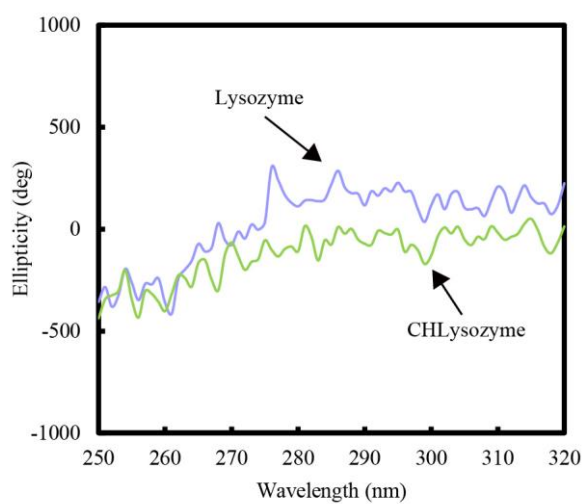
**Figure S5.** Fluorescence measurements of ANS alone and ANS with each lysozyme at 25 °C. **(a)** Fluorescence emission spectra of ANS alone and ANS with lysozyme and CHLysozyme at 25 °C. ANS concentration:  $1.0 \times 10^{-4}$  mol/L; lysozyme and CHLysozyme concentration: 0.02 mg/mL. Excitation wavelength: 365 nm. Gain value of fluorescence spectrometer: medium. **(b)** Change in  $\lambda_{max}$  of ANS with lysozyme and CHLysozyme against time at 25 °C. ANS concentration:  $1.0 \times 10^{-4}$  mol/L; lysozyme and CHLysozyme concentration: 0.02 mg/mL. Excitation wavelength: 365 nm. Gain value of fluorescence spectrometer: medium.



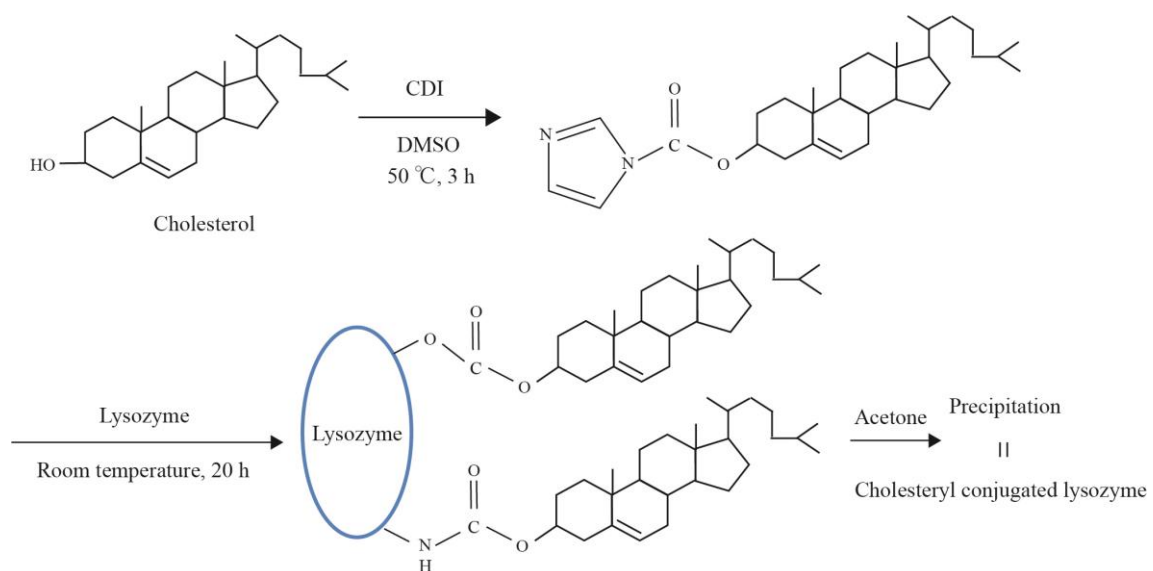
**Figure S6.** Fluorescence measurements of ANS alone and ANS with each lysozyme at 25 °C. **(a)** Fluorescence emission spectra of ANS alone and ANS with lysozyme and CHLysozyme at 25 °C. ANS concentration:  $1.0 \times 10^{-4}$  mol/L, lysozyme and CHLysozyme concentration: 2.0 mg/mL. Excitation wavelength: 365 nm. Gain value of fluorescence spectrometer: low. **(b)** Change in  $\lambda_{max}$  of ANS with lysozyme and CHLysozyme against time at 25 °C. ANS concentration:  $1.0 \times 10^{-4}$  mol/L; lysozyme and CHLysozyme concentration: 2.0 mg/mL. Excitation wavelength: 365 nm. Gain value of fluorescence spectrometer: low.



**Figure S7.** Lineweaver-Burk plots of lysozyme and CHLysozyme at 25 °C. Lysozyme and CHLysozyme concentration: 0.2 mg/mL; *Micrococcus lysodeikticus* concentration: 0.04–0.2 mg/mL.



**Figure S8.** CD spectra of each lysozyme in near UV (250 to 320 nm) region at 25 °C after two days. Lysozyme and CHLysozyme concentration: 0.2 mg/mL.



**Figure S9.** Synthetic procedure of CHLysozyme.