Architecture Effects on the Binding of Cholera Toxin by Helical Glycopolypeptides

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Supporting Information

Characterization of polypeptides and glycopolypeptides.

SDS-PAGE analysis. SDS-PAGE was employed to easily visualize the extent of reaction and homogeneity of the resulting glycopolypeptides. Images of polypeptides and related glycopolypeptides are shown in Figure S1. Before glycosylation, polypeptides electrophorese at an artificially high molecular weight owing to the high negative charge density of the glutamic acid-rich polypeptides. After glycosylation, almost all glutamic acid residues are no longer negatively charged, so the glycopolypeptides electrophorese close to their expected molecular weight. 35-H-6 electrophoreses at a molecular weight lower than that of 17-H-6, despite their similar

actual molecular masses, owing to the lower charge density of 35-H-6. There is therefore also not as significant a change in the apparent MW of the Cap 35-H-6 after glycosylation. In some gels, some species with a higher molecular weight appear (approximately the molecular weight of a dimer), most likely a result of low-order aggregation or due to some coupling of chains during chemical modification.

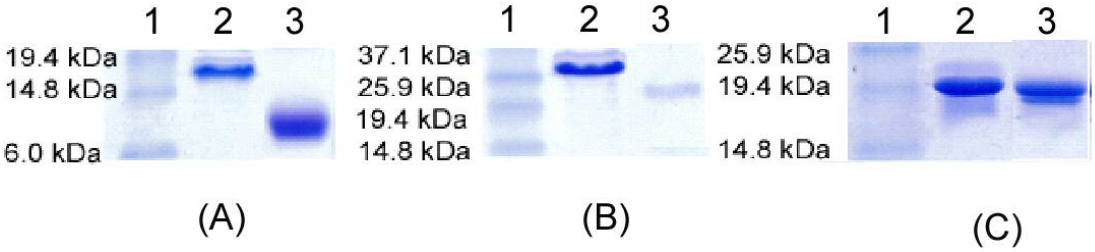

Figure S1. SDS-PAGE analysis of polypeptides and glycopolypeptides. (A) 1: Protein Ladder; 2:17-H-3; 3: Cap 17-H-3; (B) 1: Protein Ladder; 2:17-H-6; 3: Cap 17-H-6; (C) 1: Protein Ladder; 2:35-H-6; 3: Cap 35-H-6;

NMR spectra of polypeptides and glycopolypeptides. ^{1}H NMR spectra in D_2O were obtained for both polypeptides and glycopolypeptides characterization to confirm the composition and determine the number of saccharide ligands coupled to the polypeptide backbones. NMR spectra of 17-H-3 and Cap 17-H-3 are shown in Figure S2 and S3 as an example. In the spectra of 17-H-3 and Cap 17-H-3, protons of amino acid residues from polypeptide backbones have chemical shifts at 1.2-1.7 ppm (β protons of Ala), 1.8-2.1 ppm (β protons of Glu, Gln, Met and Ile), 2.1-2.6 ppm (γ protons of Glu, Gln and Met), 2.8-3.2 ppm (β protons of Tyr and His), 3.7-3.9 ppm (α protons of Gly, β protons of Ser), and 3.9-4.2 ppm (α protons of Ala, Glu, Gln and Ile). Other protons, such as α protons of His, Met, Tyr and Ser, are completely or partially

overlapped with the residual water peak. The 4 aromatic protons of Tyr show peaks at 6.75 and 7.0 ppm, which are used as an internal standard for integration.

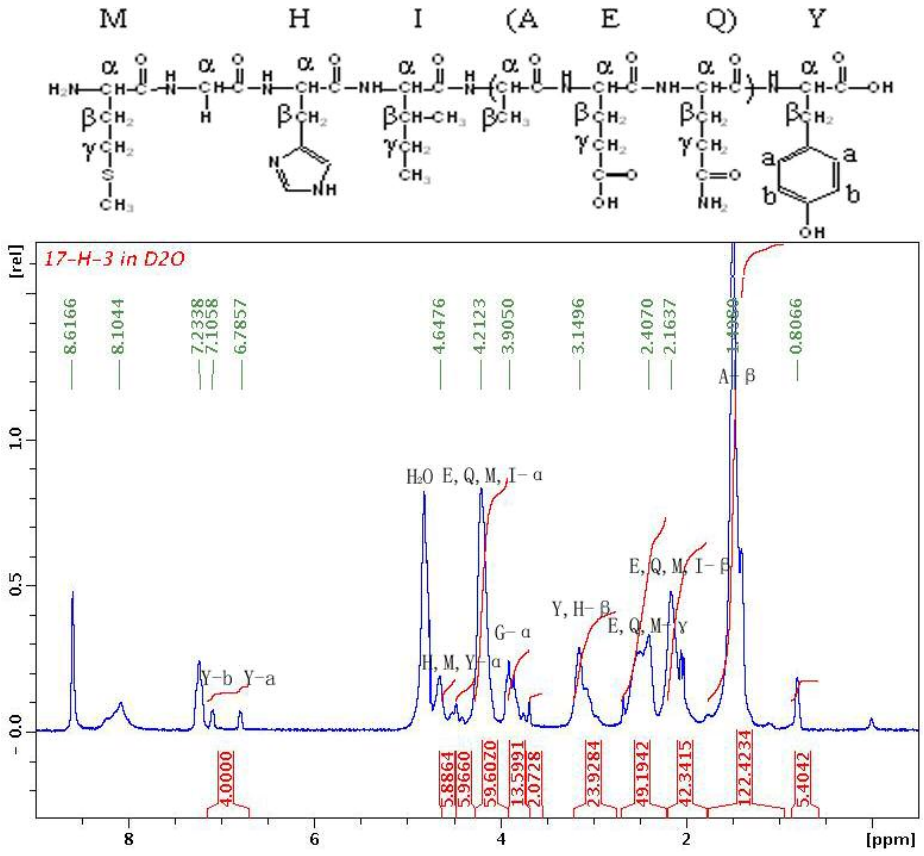

Figure S2. ^{1}H NMR spectrum of 17-H-3 in D_2O with the chemical shift assignments.

In NMR spectra of glycopolypeptides, such as Cap 17-H-3, 6 protons from the galactosyl group have chemical shifts in the region of 3.4-3.9 ppm, which overlap with the chemical shift of α protons of Gly and β protons of Ser at 3.7-3.9 ppm. With the integration of aromatic protons of Tyr as an internal standard, the number of saccharides ligands can be calculated. The β-anomeric proton chemical shift of galactose, which was reported at 4.81 ppm[1], can not be observed well because it overlaps with residue water peak in the spectra.

In the 6-carbon linker arm, there are 6 protons with chemical shifts of 1.1-1.6 ppm (from the central methylene groups of the linker arm), which overlap with chemical shift of Ala β protons, and the other 4 protons show chemical shifts at 2.3 and 2.7 ppm, which overlap with the chemical shifts of β and γ protons of Gln, Glu and Met. After correction for the contribution from the polypeptide based on the internal standard, the number of Cap saccharide ligands can be calculated according to the integration of these regions of the NMR spectrum.

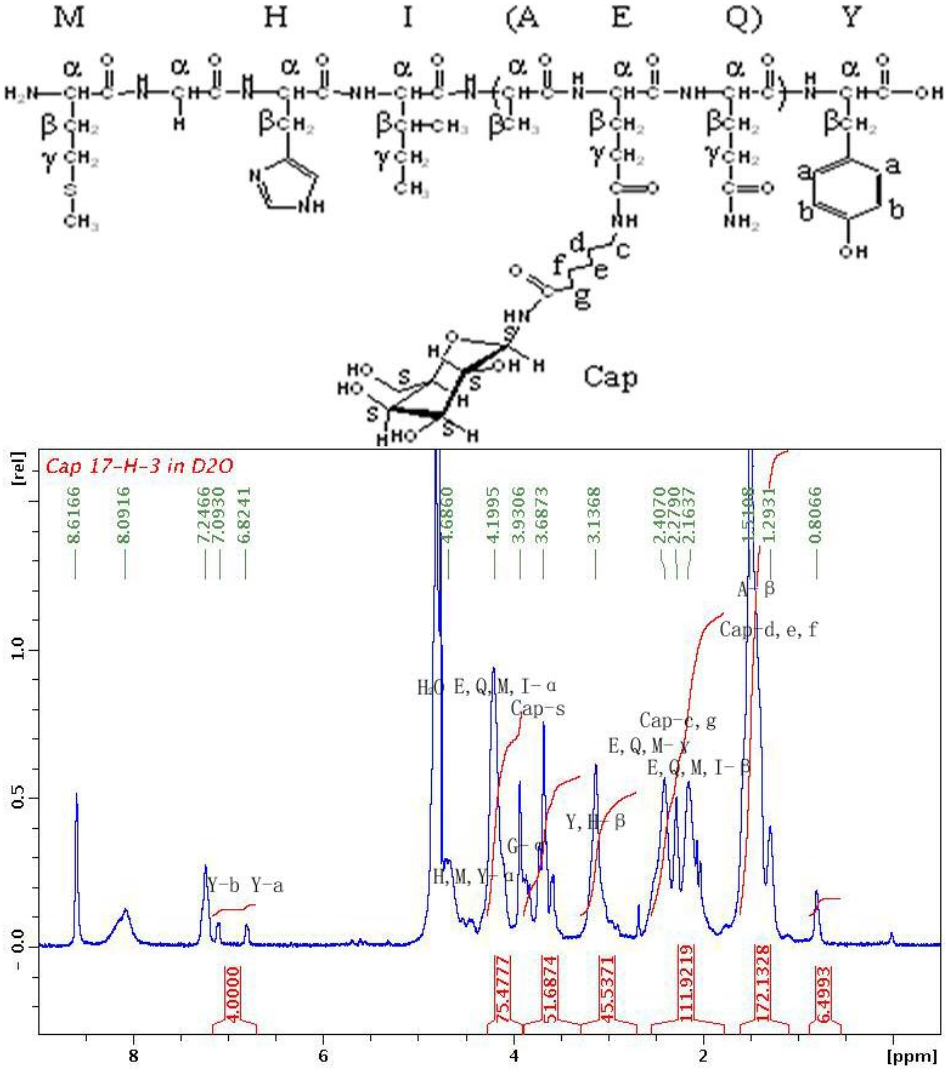

Figure S3. ^{1}H NMR spectrum of Cap 17-H-3 in D_2O with the chemical shift assignments.

MALDI-TOF for polypeptides and glycopolypeptides. Freshly prepared α-cyano-4-hydroxy cinnamic acid CHCA matrix was used for MALDI-TOF experiments to improve resolution in the MALDI-TOF experiments. As an example, MALDI spectra of 17-H-3 and Cap 17-H-3 are shown in Figure S4. The number of saccharide ligands coupled on the polypeptide backbones can be calculated based on the molecular weight difference between glycopolypeptides and corresponding polypeptides. The breadth of the peak is due to the high laser power needed to ionize the glycopolypeptides.

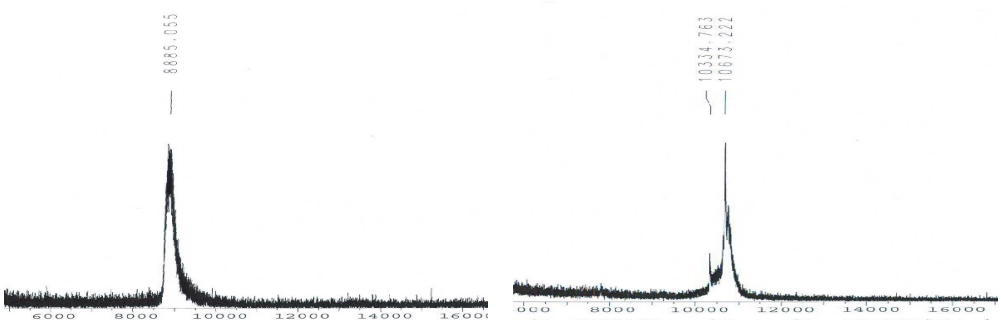

Figure S4. MALDI-TOF mass spectra of 17-H-3 and Cap 17-H-3.

Circular Dichroic spectroscopy (CD).

Helicity calculation. Helicity of alanine-rich polypeptides is length-dependent, and can be expressed according to the equation $[\theta_n]_{222} = [\theta_\infty]_{222}(1-\frac{x}{n})$.[2] $[\theta_n]_{222}$ represents the MRE value of an idealized 100% helical peptide with *n* amino acids. $[\theta_\square]_{222}$ is the limit of $[\theta_n]_{222}$ when *n* is infinite, and *x* is a length correction value that is set to 2.5.[3 4] The value of $[\theta_\infty]_{222}$, which is the helicity for infinitely long alanine-rich helices, is approximately -61,000 deg cm^2 $dmol^{-1}$ based on current theoretical models.[5] Once the $[\theta_n]_{222}$ value is obtained based on the length of the polypeptides, the fractional

helicities can be calculated as the ratio of the experimental value of $[\theta]_{222}$ over the $[\theta_n]_{222}$ value.

Thermal stability. The CD spectra of Cap 17-H-6 at various temperatures upon heating are shown in Figure S5, as an example for all the polypeptides and glycopolypeptides. The reversibility of thermal transition for polypeptides and glycopolypeptides is indicated by the observation of the same helicities before and after heating. CD spectra of Cap 17-H-6 shown in Figure S6 serve as an example.

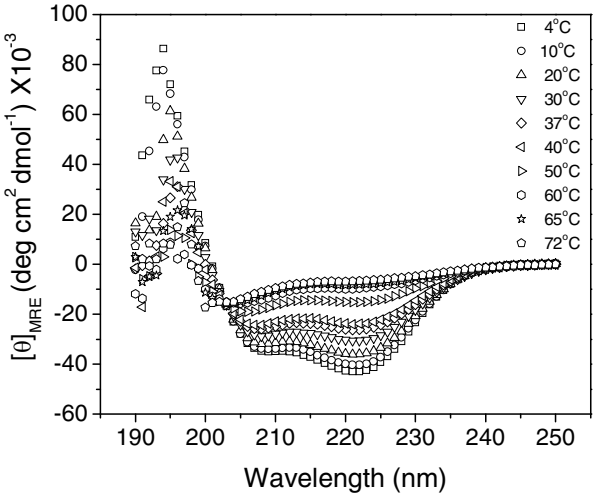

Figure S5. CD spectra of Cap 17-H-6 at a series of different temperatures.

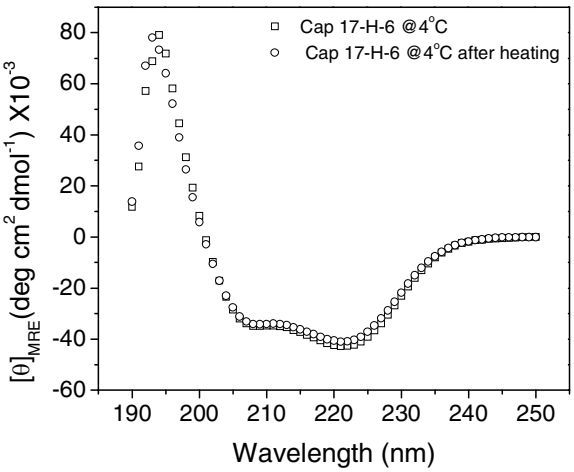

Figure S6. CD spectra of Cap 17-H-6 at 4^{o}C before and after heating

. **Two state function fitting Equation.** The protein folding-unfolding two state transition function can be used for calculating the transition temperature (T_m) and van't Hoff enthalpy values (ΔH_m) of the polypeptides and glycopolypeptides as reported previously.[6, 7] The equation shown below defines the native fraction of the protein as a function of temperature, where y_n and y_d are the fraction of native protein before and after the transition, respectively, m_n and m_d are the slopes of the pre-transition and post-transition curves, y_{obs} is the observed ellipticity at 222nm ($[\theta]_{222}$) for each sample. This equation was used to perform a nonlinear curve fitting of the data (using Sigma Plot 9.0 Software), and the results of the fit give the van't Hoff enthalpy (ΔH_m) and transition temperature (T_m) values.

$$y_{\text{obs}} = \frac{(y_{\text{n}} + m_{\text{n}}T) + (y_{\text{d}} + m_{\text{d}}T)\{\exp[\Delta H_{\text{m}}/R(1/T_{\text{m}} - 1/T)]\}}{1 + \exp[\Delta H_{\text{m}}/R(1/T_{\text{m}} - 1/T)]}.$$

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