Supplementary Information

Dramatically Increased Binding Constant of Water-Soluble Cyclodextrin Hyperbranched Polymers: Explored with Diffusion Ordered NMR Spectroscopy (DOSY)

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CDNP characterization of physical properties

*Determination of the weight average molecular weight (\(M_w\)) and hydrodynamic radius (\(R_h\))*

Size exclusion chromatography coupled with multiangle light scattering (SEC-MALS) and others SEC-MALS measurements were carried out for the CDNPs using a Shodex column (SB802.5 and SB806) at 40 °C with 10 mM aqueous NaCl as the eluent to measure \(M_w\). We prepared a sample solution of 1 mg/mL in 10 mM aqueous solution. The solution was optically purified with a PTFE (Polytetrafluoroethylene) membrane with 0.2 μm pores and injected into the column. The output from the column was passed sequentially through an MALS detector (Wyatt Technology, Dawn Heleos II, wavelength: \(\lambda = 658\) nm) and an RI detector (Wyatt Technology, 1.2 – 1.8 RIU, wavelength: \(\lambda = 488 – 690\) nm). The specific refractive index increments (\(\partial n/\partial c\)) of the CDNPs in 10mM aqueous NaCl was determined by a differential refractometer (Otsuka Electronics DRM-1020, wavelength: \(\lambda = 633\) nm). Data acquisition and molecular weight calculation were performed using the ASTRA software, version 7.3.2 (Wyatt Technology).

The DLS measurement was operated using the facility of DelsaMax Pro (Beckman Coulter, Inc.) to determine the hydrodynamic radius \(R_h\). We prepared a sample solution of 10 mg/mL in aqueous solution.

The typical example of GPC chromatogram and the angular dependence of static light scattering to determine \(M_w\) is shown in the Figure S1A. The distribution of \(R_h\) was unimodal as shown in the Figure S1B.

![Figure S1](image)

**Figure S1.** A: SEC-MALS fractogram of βCDNP\(_{2.7}\); B: Number size distribution of βCDNP\(_{2.7}\) in water

*Determinations of the carbohydrate concentration in CDNPs*
The CD weight percent in CDNPs was determined by the phenol sulfuric acid method. Dried CDNP (0.025 g) was refluxed at 100 °C for 8 h in 0.5M H$_2$SO$_4$ (15 mL) to break down the reacted CD to a pentose. Then, the solution was reacted with 5% (w/w) phenol and concentrated H$_2$SO$_4$. The final product was brown-orange in color, and the absorbance was detected by a UV–Vis spectrophotometer (Jasco, V-630) at 488 nm. The absorbance was converted into the CD weight percent (wt% CD) by use of a calibration curve established with glucose D(+). Here, we confirmed that polymerized ECH with poly vinyl alcohol samples did not show any absorbance after we treated them in the same manner. From the CD weight percent and $M_w$ of CDNP, we calculated the number of CDs in one CDNP particle ($N_{CD}$).

**NMR of CDNP in DMSO**
We carried out $^1$H-NMR spectra of $\beta$CDNP$_{0.9}$ in DMSO and its results showed disappearance of all -OH peaks in as shown in Figure S2.

![Figure S2](image)

**Figure S2.** $^1$H-NMR spectra of $\beta$CD and $\beta$CDNP$_{0.9}$ in DMSO

**Continuous variation method (Job plot) by NMR**
When the host-guest complexation equilibrium has a very fast exchange rate compared with the NMR time scale. The observed chemical shift of guest appears at the weighted average chemical shift of the free guest and complexed guest:

$$\delta_{G_{\text{obs}}} = \delta_G(1-x) + \delta_{HG}x$$

Here $\delta_{G_{\text{obs}}}$, $\delta_G$ and $\delta_{HG}$ are observed chemical shift of guest, chemical shift of free and complexed guest, respectively. $x$ is ratio of complexed guest at equilibrium [HG] over total guest [G].
\[ x = \frac{b[HG]}{[G]} \]  

Substitution of equation (2) into equation (1) results in the expression:

\[ [G] \Delta \delta_G = b[HG] \left( \delta_{HG} - \delta_G \right) \]  

Equation (3) means that \([G] \Delta \delta_G\) is proportional to \([HG]\), since \(b(\delta_{HG} - \delta_G)\) is constant. Consequently, the stoichiometry is obtained from the x-coordinate at the maximum in the modified Job’s plot, where \([G] \Delta \delta_G\) is plotted as y-coordinate and x-axis is \(r = [G]/([H]_i + [G]_i)\). The maximum value for \([G] \Delta \delta_G\) will occur at \(r = b/(a + b)\), where \(a\) and \(b\) are the proportions of CD and vanillin in the complex, respectively. For instance, if the stoichiometry of the complex is 1:1 \((a = b = 1)\), the maximum value for the examined parameter will be reached at \(r = 0.5\).

**Deviation from the 1:1 binding model in large ECH/CD polymers**

![Job plot](image)

**Figure S3.** Job plot for vanillin and \(\beta\)-CDNP\(_{2.7}\) in D\(_2\)O at 25 °C. The total concentration ([G]\(_i\)+[H]\(_i\)) was fixed at 4.9 mM.
Concentration dependence of D and K

**Figure S4.** A: Concentration dependence of diffusion coefficient of vanillin and βCDNP<sub>2.7</sub>; B: Concentration dependence of binding constant of CD and βCDNP<sub>2.7</sub> to vanillin

**Complex formation ability of CDNP and DXNP**

An amount of vanillin (3.35 mg) was added into the βCDNP<sub>0.9</sub> or DXNP solution (1ml, 50 mg/mL). The mixture was stirred in 1 day at room temperature. After centrifugation (12000 rpm for 5 min), the upper solution was collected and purified by spin dialysis (cut-off $M_w = 3500$ g/mol). The solution containing only complex finally was filtered before measuring UV-vis. The absorbance of vanillin was detected by UV-vis spectrophotometer.

**Figure S5.** The UV-vis spectra of the βCDNP<sub>0.9</sub>-Val and DXNP-Val complex
Figure S6. The NOESY spectra of βCDNP$_{0.9}$ in DMSO-d$_6$, 25°C

Figure S7. The $^1$H-NMR spectra of mixture βCDNP$_{0.9}$/βCD at 10mM, 20°C in D$_2$O