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Cationic and Anionic Reverse Micelles as the Molecular Crowding Container for G-quadruplex Structure

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In this study, we have tested the feasibility of using reverse micelles (RMs) as the molecular crowding containers for DNA G-quadruplex. The result suggested that the RMs formed by anionic surfactant should be avoid for the molecular crowding studies of G-quadruplex structures. In contrast to the molecular crowing condition created by polyethylene glycol, the human telomeric sequence d $[AG_3(T_2AG_3)_3]$ shows different conformations and drug binding stoichiometry in cationic RMs. In cationic RMs, the DNA can avoid the dehydration effect caused by polyethylene glycol, therefore are ideal containers for molecular crowding studies.

The d$[TTAGGG]_n$ repeating units exist in the human telomere region, and the sequences are able to form four-stranded G-quadruplex structures through the Hoogsteen hydrogen bond interactions.[1, 2] Recently, the conformation of human telomere DNA has attracted a lot of attention, because the formation of G-quadruplex can significantly reduce the activity of telomerase, which has been recognized as an important tumor promoter.[3] In the studies of G-quadruplex structure, one important question is the intracellular conformation of G-quadruplex. It has been known for many years that the intracellular environment is highly crowded and full of soluble (metabolites and metal ions) and insoluble (organelle) biomolecules.[4-6] Many studies suggested that the behaviour of the biomolecule in the crowded environment will significantly deviate from those in dilute solutions (,which is termed as molecular crowding effect).[7, 8] How to mimic the intracellular crowded conditions has become a critical issue for biochemistry studies. To investigate the molecular crowding effect, one strategy is adding inert co-solutes in solution.[6, 9, 10] However, recent findings suggested that the addition of co-solutes can also artificially change the conformations of the target biomolecules.[9, 11, 12] An alternative approach to mimic the molecular crowding condition is

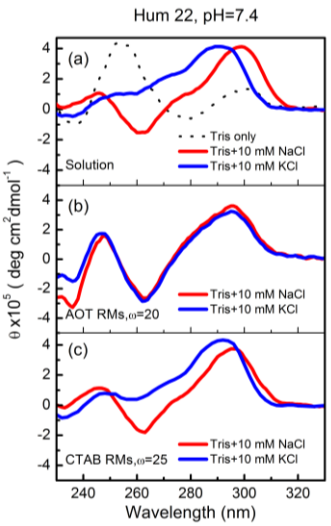

Figure 1: The CD spectra of Hum 22 in (a) Solution (b) AOT RMs and (c) CTAB RMs.

encapsulating the macromolecules in reverse micelles (RMs).[13-15] The RMs are composed of amphiphilic surfactants that assemble around a nanosized water pool in a nonpolar solvent. The radius of RMs ranges from few nm to tens of nm and can be easily controlled by changing the extent of water loading: ω=[H2O]/[surfactant]. Because the size of RMs is comparable with many biomolecules, it provides an ideal system for studying the intracellular crowding effect.[13] The highly structured and heterogeneous water pool inside the RMs closely resembles the biological interfacial water in membranes and protein interfaces. Therefore, the RMs are also recognized as the biomemberane mimic container.[16-18] Nowadays, the RM formed by anionic surfactants: sodium bis(2-ethylhexyl) sulfosuccinate (AOT) is

by far the most extensively investigated system, and many unique properties of the AOT RMs have been well documented in literatures.[18-20] Another attractive RMs system is the RMs formed by the cationic surfactant: cetyltrimethylammonium bromide (CTAB). The formation of CTAB RMs that usually requires the co-surfactant and the interfacial water in CTAB RMs is more flexible than that in AOT RMs.[21]

In this study, the molecular crowding effect of human telomere sequence d[$AG_3(T_2AG_3$] (the Hum 22) in AOT (isooctane) and CTAB (isooctane: 1-hexanol=7:1) RMs are investigated. The conformations of G-quadruplex are monitored by circular dichroism (CD) spectroscopy. In 10 mM Tris buffer (pH=7.4), the CD spectrum of the Hum 22 shows a positive band at ~256 nm and a negative band at ~238 nm, which indicates the formation of parallel tetramolecular G-quadruplex (Figure. 1a).[2, 22] In the Tris buffer containing 10 mM NaCl, the Hum 22 forms antiparallel G-quadruplex structure in solution,[2] and the CD spectrum is featured by a positive peak at 300 nm and a negative peak at 260 nm. In the Tris buffer that contains 10 mM KCl, the Hum 22 forms multiple G-quadruplex conformations in solution,[23, 24] and the spectrum is featured by a positive peak with a shoulder at 290 nm and 270 nm, respectively. In previous studies, the AOT RMs has been employed as the molecular crowding container by Li[14] and Sugimoto[15] groups. Their results suggested that the Hum 22 forms antiparallel G-quadruplex structures in the AOT RMs that contain bulk water or sodium phosphate buffer. It should be noted that the AOT is a sodium salt. Although the sodium ion in AOT RMs mainly stays at the Stern layer,[25-28] the water pools still contain certain amounts of sodium ions.[26] Therefore, the antiparallel conformation found in their experiment might simply due to the dissociated sodium ions in the nanosized water pool. To verify this argument, the Hum 22 was encapsulated in the AOT RMs (ω=20) that contained three different buffers: Tris only, Tris+10 mM NaCl, and Tris+10 mM KCl. The hydrodynamics radius (R_h) of the AOT RMs containing these

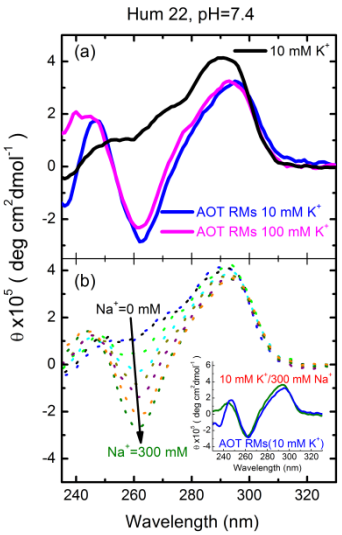

Figure 2: (a) The CD spectrum of Hum 22 in the AOT RMs containing 10 mM (blue) and 100 mM (magenta) KCl buffer. For comparison, the CD spectrum of Hum 22 in 10 mM KCl buffer is also indicated (black). (b) The Na+ titration experiment of Hum 22 in 10 mM KCl buffer. The inset shows the spectra of Hum 22 in AOT RMs and in the buffer containing 10 mM KCl and 300 mM NaCl.

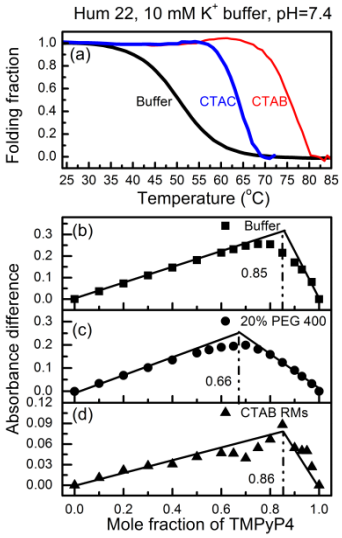

Figure 3: (a) The UV melting curves of Hum 22 in buffer(Tm=50.3°C), CTAC (Tm=63.8°C) and CTAB(Tm=75.5°C) RMs; The Job plots for Hum 22/TMPYP4 in (b) buffer (c) 20 % PEG 400 (d) CTAB RMs. All solution contains Tris+10 mM KCl.

buffers was estimated to be ~6 nm (measured by dynamic light scattering method, Fig. S_1, ESI†). The Hum 22 forms antiparallel conformation in the AOT RMs that contain Tris only and Tris+10 mM NaCl buffers, and the results are consistent with the previous reports; however, it is surprising that the Hum 22 forms similar antiparallel G-quadruplex in the AOT RMs containing Tris+10 mM KCl buffer (Figure 1b). Even in the AOT RMs that contain Tris+100 mM KCl buffer, the CD spectrum of the Hum 22 indicates that the majority parts of the Hum 22 still fold into the antiparallel conformation (Figure 2a). Because, the quadruplex is preferentially coordinated with K^+ over Na^+;[2] the antiparallel conformation found in the AOT RMs indicates that concentration of dissociated Na^+ must have been much higher than that of K^+ ions. Considering the size and the percentage of free dissociated Na^+ ions, it is reasonable for us to expected that the concentration of free Na^+ ions in AOT RMs (ω=20) is about 200-300 mM (supporting information available, ESI†). Our titration experiment revealed that the spectra of the Hum 22 in AOT RMs closely resemble those in the Tris buffer containing 10 mM KCl and 300 mM NaCl (Figure 2b). The result implies the elevated concentration of the dissociated Na^+ ion in AOT RMs. Because the conformation and the stability of G-quadruplex are extremely sensitive to the types and the concentration of cations in solution, our findings demonstrated that the AOT RMs are not suitable for studying the molecular crowding effect of the G-quadruplex. In order to avoid the interference of dissociated cations, the Hum 22 is encapsulated in the RMs formed by CTAB (ω=25). In contrast to the AOT RMs, the CD spectra of the Hum 22 in CTAB RMs are similar to those in the corresponding buffer solutions (Figure 1c). The result further confirmed that the antiparallel conformation found in AOT RMs is out of the dissociated Na^+ ions. Since the Hum 22 in K^+ containing solution will be adapted to the parallel conformation in dehydration condition,[11, 29] our findings also suggested that the dehydrating effect can be avoided in CTAB RMs. The UV-Vis melting curve shows that the melting

temperature (T_m) of the Hum 22 increases form 50.3°C in buffer solution to 75.5°C in CTAB RMs (Figure 3a). In different sizes of CTAB RMs, the T_m of Hum 22 decreases with the increasing of RMs sizes (Fig S2, ESI†). As the conformation of the Hum 22 in CTAB RMs is similar to that in buffer solution, the stabilization effect must have originated from the molecular crowding effect. Meanwhile, we also encapsulated the Hum 22 in the RMs formed by cetyltrimethylammonium chloride (CTAC, isooctane: 1-hexanol=7:1, ω=25). Like the case of CTAB RMs, encapsulating the Hum 22 in CTAC RMs would not change the conformation of G-quadruplex. (Fig S3, ESI†). However, the UV-Vis melting temperature analysis suggested that the Hum 22 in CTAC RMs is less stable than that in CTAB RMs. This finding can be directly associated with the faster hydrogen bond dynamics and the larger orientational mobility of the water in CTAC RMs.[21]

Another important issue is the molecular crowding effect on the binding of G-quadruplex stabilizing ligands. In this study, we employed the cationic porphyrin: TMPyP4 as the quadruplex binding ligand. The saturated binding numbers of TMPyP4 on the Hum 22 were estimated using the continuous variation method (Job's method).[30] Figure 3b through 3d present the Job plots for Hum22/TMPYP4 in buffer, 20 % PEG 400, and CTAC RMs (raw data in Fig S4, ESI†). As depicted, the saturated binding numbers in buffer (5.67) are similar to those in CTAB RMs (5.85). Our findings suggested that the molecular crowding effect would not change the binding stoichiometry of TMPyP4 on the Hum 22. In 20% PEG 400, the saturated binding number decreased to ~2, and similar findings have been reported in previous research.[31] We believe that the decrease in binding number is not due to the molecular crowding effect but is the result of different conformations of Hum 22 in PEG 400 (Fig S5, ESI†) .

Conclusions

In summary, we have investigated the molecular crowding effect of the Hum 22 encapsulated in RMs. Our result indicates that the dissociated Na^+ in the water pool of AOT RMs will affect the conformation of G-quadruplexs; therefore, the AOT RMs are not suitable for the molecular crowding study of G-quadruplex. In order to avoid the interference of dissociated cations, we have tested the feasibility of using cationic RMs as the molecular crowding container. By encapsulating the Hum 22 in CTAB and CTAC RMs, we have found that the thermal stability of the Hum 22 in RMs is directly associated with the flexibility and the strength of the hydrogen bond networking of the nano-sized water pool. In CTAB RMs, the binding stoichiometry between Hum 22 and TMPYP4 is similar to that in dilute solution, which implies that the molecular crowding condition would not change the binding stoichiometry of quadruplex-binding ligands. It overall leads to the conclusion that the CTAB RMs is ideal containers for studying the molecular crowding effect of the G-quadruplex.

This work is supported by the Ministry of Science and Technology of Taiwan (Project contracts: NSC 102-2113-M-018-005-MY2). We also thank Dr. Yi-Hong Lin (National Synchrotron Radiation Research Center) for his support in CD experiments.

Notes and references

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Electronic Supplementary Information (ESI) available: Materials and Methods; Fig S_1: the sized distribution of RMs; Fig S_2: The melting curve analysis of Hum 22 in different sizes of CTAB RMs;Fig S_3: CD spectrum of Hum 22 in CTAC RMs; Fig S_4: The raw data for Job plot experiments.; Fig S_5:The CD spectrum of Hum 22 in 20% PEG 400 solution. See DOI: 10.1039/c000000x/

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Supporting Information

Cationic and Anionic Reverse Micelles as the Molecular Crowding Container for G-quadruplex Structure

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Materials and Methods

Materials

The high performance liquid chromatography purified human teleomere sequence (5′-$AG_3(T_2AG_3)_3$, the Hum 22) was purchased from Sigma Aldrich and was used without further purification. The CTAB (BioXtra, >99%), AOT (BirXtra, >97%), TMPyP4 (>97%), NaCl (BioXtra, >99.5%) and KCl (For molecular biology, >99%) were also purchased from Sigma Aldrich and were used as received. The CTAC was purchased from Alfa Aser (96%). The isooctane (>99%) and 1-hexanol (>99%) were purchased from Macron™ and Alfa Aser, respectively. The deionized water (resistivity>18.2 MΩ-cm at 25°C) was obtained from Barnstead™ EasyPure™ II water purification system (Thermo Scientific™) and was used in all experiments.

Sample preparation

DNA preparation

The Hum 22 was dissolved in deionized water. The single strand concentration of oligonucleotide was estimated by measuring the absorbance at 260 nm (ε=228500 M^{-1} cm^{-1}). After determining the concentration of oligonucleotide, the proper volume of high concentration oligonucleotide was added to the Tris buffer solution (10 mM tris, pH=7.4) or the buffers that containing Na^+ or K^+ ions. The samples were heated to 95°C for 10 minutes and slowly cooled down to the room temperature. After cooling down to the room temperature, the samples were stored in the 4˚C refrigerator for at least 8 hours to ensure the formation of G-quadruplex structure.

Preparation of DNA encapsulated reverse micelles.

The cationic or anionic surfactants were dissolved in organic solvents (0.1M AOT in isooctane, 0.2 M CTAB and CTAC in isooctane: 1-hexanol=7:1 solution). The proper amounts of buffer or highly concentrate nucleic acid stock solution were directly injected into the solution (ω=20 in AOT, ω=25 in CTAB and ω=20 in CTAC RMs) and sonicated for 1 hr. In RMs, the finial concentration of DNA was control at 2 μM.

CD spectroscopy

The CD spectra were obtained using Jasco J-810 spectropolarimeter (JASCO, Japan). The spectra were obtained in a 1 cm path length quartz cuvette. The temperature was controlled at 25°C using temperature accessory (JASCO, PTC-423S). The spectra were recorded from 220 to 350 nm at a scanning rate of 100 nm/min. Each spectrum was scanned three times and the background CD spectrum was subtracted.

UV-Vis steady state spectra, melting curve and Job plot analysis

The UV-Vis absorption spectra were obtained using a Cary 100 spectrophotometer (Cary-100, Agilent) with 1 cm quartz cuvette. The temperature of the samples was

controled with a peltier thermostatted multicell holder accessory. The melting curves of the samples were monitored at 295 nm and increasing with the temperature gradient of 0.4˚C/nm. For the Job plot measurements, the temperature of the samples were controlled at 25˚C, and the total concentration of the Hum 22+TMPyP4 was 2μM in buffer or 20% PEG 400 and 1μM in CTAB RMs.

Estimation of the concentration of free Na^+ ions in AOT RMs

For the AOT RMs (ω=20), the hydrodynamics radius is 6 nm, considering the thickness of the surfactant monolayer is ~1 nm, the radius of the central water pool is ~5 nm, which corresponds to the volume of $5.24x10^{-22}$ liter. Because each droplet is composed of ~300 surfactants, the concentration of the total available Na^+

$$= \frac{300}{6.02\times10^{23}} \times \frac{1}{5.24\times10^{-22}} = 0.95\,\mathrm{M}$$

According to the MD simulation result, about 16 % of Na^+ ions (~150 mM) is free dissociated in ω=10 AOT RMs. Since the percentage of the dissociated Na^+ will also increase with the ω, we expected that the concentration of the dissociated Na^+ in ω=20 AOT RMs should be in the range between 200~300 mM, which is comparable to the 300 mM Na^+ ions we used.

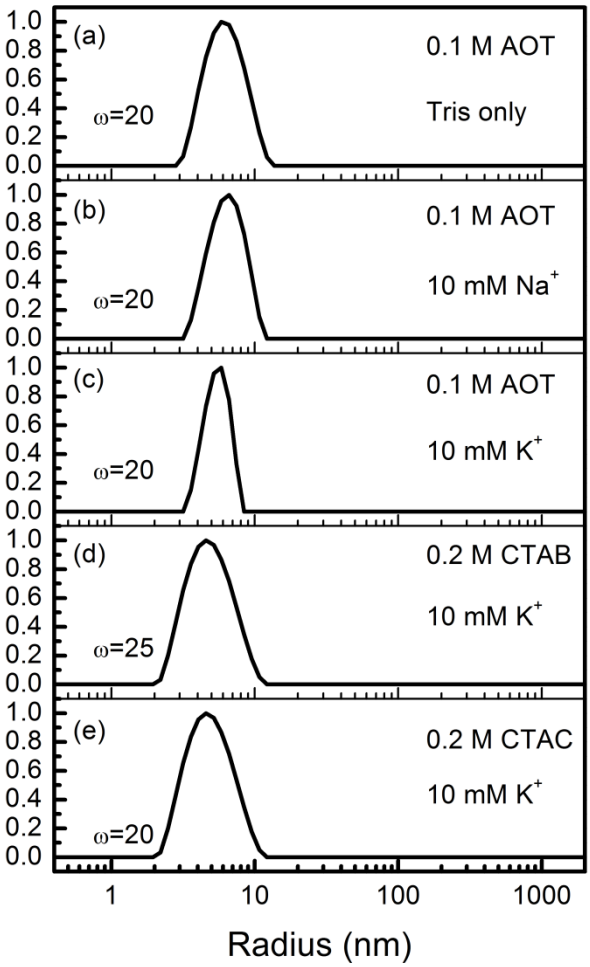

Figure S_1: The size distribution of (a-c) AOT, (d) CTAB and (e)CTAC reverse micelles. As depicted, the hydrodynamic radius of the reverse micelle was ~6 nm in all cases.

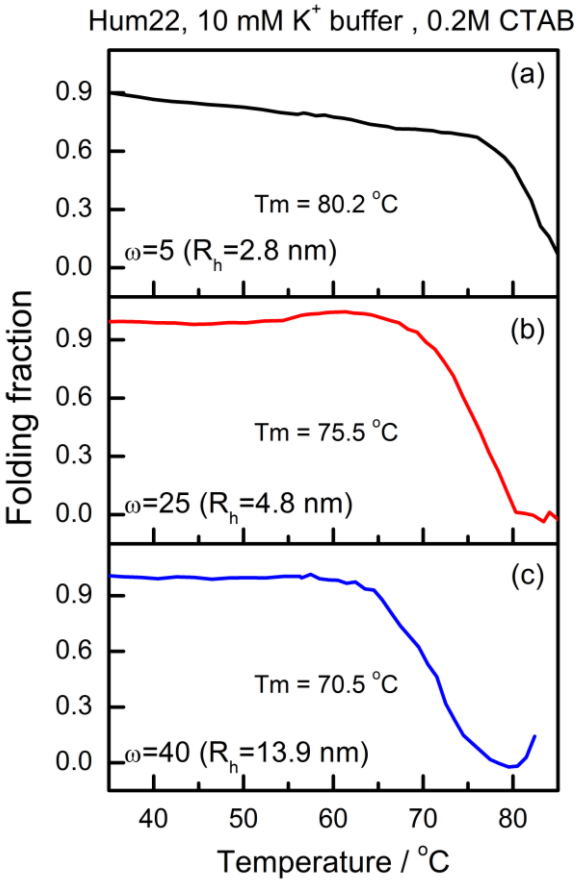

Figure S_2: The melting curve of Hum 22 in (a) ω=5 (b) ω=25 (c) ω=40 CTAB RMs. The hydrodynamics radius (R_h) was estimated by the DLS method.

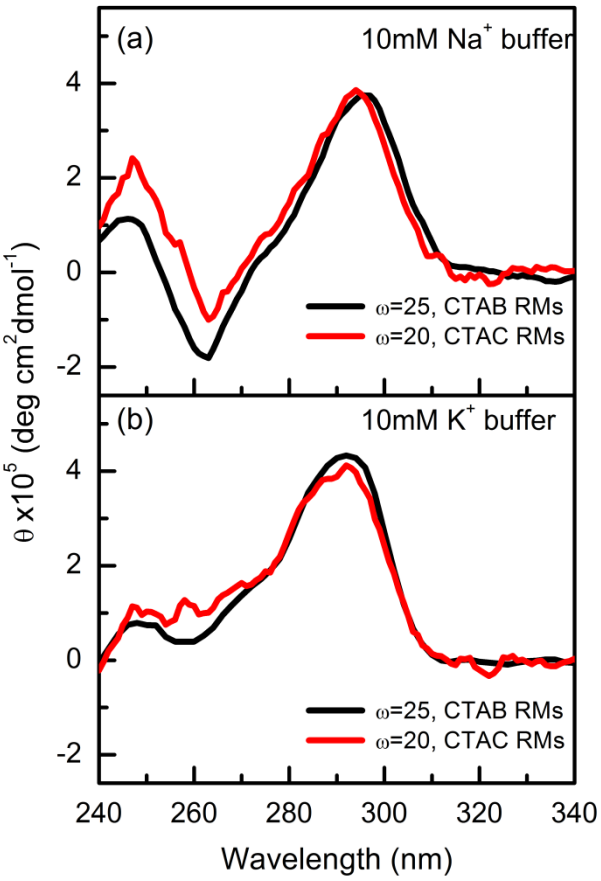

Figure S_3: The CD spectra of Hum 22 in CTAB (black) and CTAC (red) RMs containing 10 mM (a) NaCl and (b) KCl Tris buffer solutions.

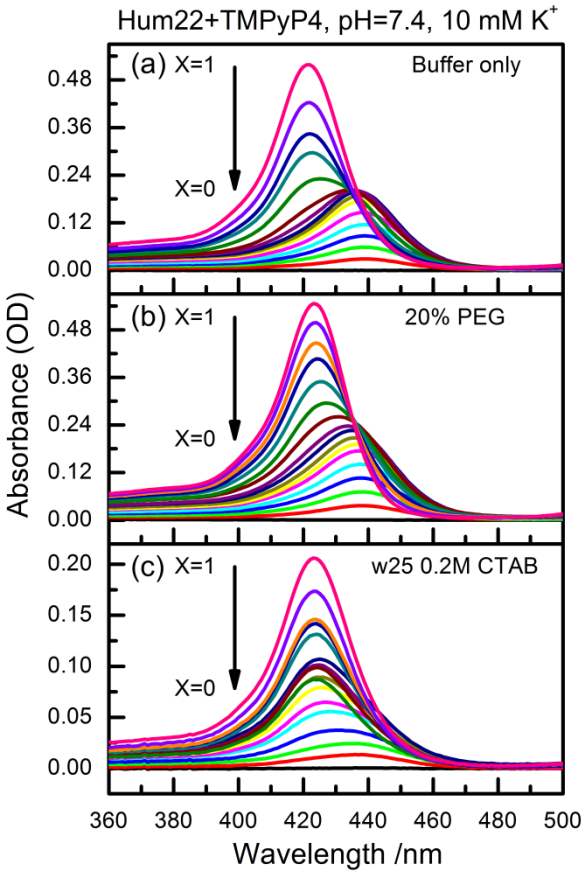

Figure S_4: The absorption spectra of TMPyP4 binding with different mole fraction of Hum 22 in (a) Buffer (b) 20% PEG 400 and (c) CTAB RMs.

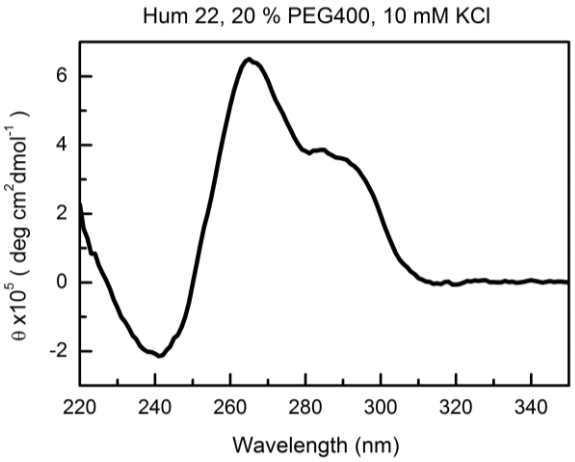

Figure S_5: The CD spectrum of Hum 22 in 20 % PEG400 solution.