

Supporting Information

Folding a Non-biological Polymer into a Compact Multi-helical Structure

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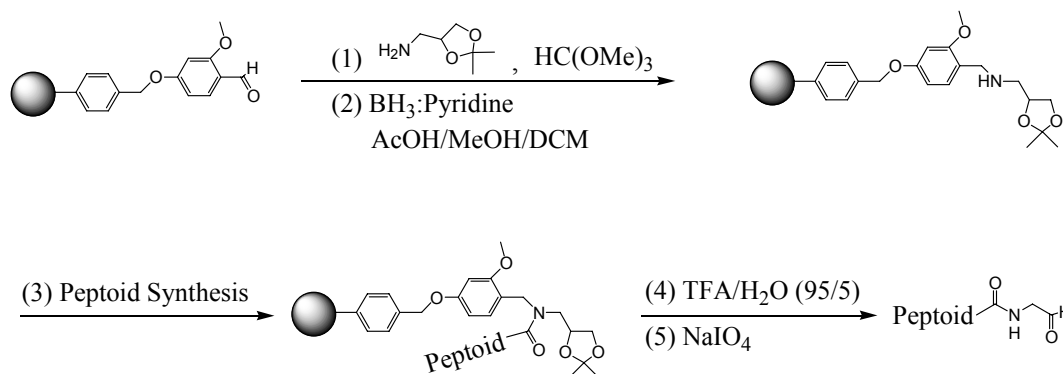
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(30) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M.A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. Discovery of nanomolar ligands for 7-transmembrane G-protein-coupled receptors from a diverse N-(substituted)glycine peptoid library. *J. Med. Chem.* **1994**, *37*, 2678-2685.

Coupling of 2,2-dimethyl-1,3-dioxolane-4-methaneamine into Sasrin resin. General scheme for generating C-terminus aldehyde group is as follows.



Scheme for making C-terminal aldehyde

Sasrin resin was coupled with 2,2-dimethyl-1,3-dioxolane-4-methaneamine in two steps via reductive amination. 5g of Sasrin resin was gently agitated for 24 hours with 5 mL of 2,2-dimethyl-1,3-dioxolane-4-methaneamine and 35 mL of trimethylorthoformate ($\text{HC}(\text{OMe})_3$). After the coupling reaction, the Sasrin resin was washed with dichloroethane (DCE). For the reduction of C=N double bond, 1 mL of BH_3 :pyridine was added to the Sasrin resin soaked in 40 ml of acetic acid (AcOH)/methanol (MeOH)/dichloromethane (DCM) (1:2:2). After incubation for 48 hours, the sasrin resin was washed with AcOH/MeOH/DCM (1:2:2), dimethylformamide (DMF), water, DCE and MeOH, and dried under vacuum.

Peptoids with C-terminal aldehydes were synthesized on this Sasrin resin. The crude peptoid product (50 μmol of resin) was cleaved from the Sasrin resin with 95:5 TFA/water (v/v) for 50 min. at room temperature. The peptoid product was then treated with 10 mL of 2 mM sodium periodate, 20% acetonitrile (v/v) in 50 mM sodium phosphate (pH 7.0) buffer for 30 min. to convert N-terminal diol to the aldehyde and directly subject to HPLC to stop the reaction and further purify the product.

Calculation of FRET efficiency. FRET efficiency (E) is obtained from the quenching of donor fluorescence with $E = (1 - I_{\text{da}}/I_{\text{d}})$, where I_{da} and I_{d} are the fluorescence intensities in the presence and absence of quencher, respectively. As a reference for measuring the FRET efficiency, the fluorescence of analogous peptoids with only a donor, with no quencher, were also measured as I_{d} . For example, fluorescence intensities of both **10** (30_Chi_CN_FQ) and **11** (30_Chi_CN_F) were measured at different concentration of acetonitrile as shown in Fig. S1. Then, FRET efficiency was obtained by using the equation of $E = (1 - I_{\text{da}}/I_{\text{d}})$.

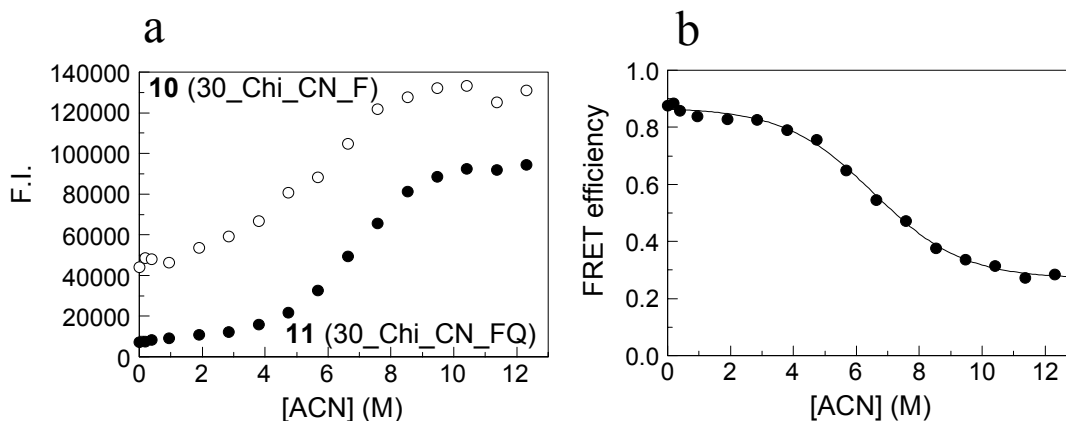


Figure S1. Calculation of FRET efficiency. (a) Fluorescence intensities of both **10** (30_Chi_CN_FQ) (I_{da}) and **11** (30_Chi_CN_F) (I_d) were measured at different concentration of acetonitrile. (b) FRET efficiency (E) was obtained by using the equation of $E=(1-I_{da}/I_d)$. FRET efficiencies of other peptoids were obtained by this procedure.

Distance between the donor and quencher in our peptoids. Ensemble average of distance between the donor and the quencher could be calculated using the equation $r = R_0(E^{-1}-1)^{1/6}$ where R_0 is a effective distance of donor-acceptor pair. R_0 in our peptoid corresponds 31 Å (see experimental section). Distance between the donor and the quencher in putative two-helix bundle peptoids (**10**; 30_Chi_CN_FQ and **13**; 30_Chi_NN_FQ) at pH 7 is around 22 Å or 23 Å, respectively (Fig. S2). Peptoids with achiral side chains (**15**; 30_Ach_CN_FQ and **17**; 30_Ach_NN_FQ) have also similar values of distance (22 Å) in the absence of denaturant even though their secondary structure is featureless (Fig. 8). Hydrophobic interaction is enough to bring the donor and quencher together in those peptoids. In the presence of 65% acetonitrile where the tertiary structures of peptoids are melted, the end-to-end distances of peptoids with achiral side chains (**15**; 30_Ach_CN_FQ and **17**; 30_Ach_NN_FQ) are shorter than those of peptoids with chiral side chains (**10**; 30_Chi_CN_FQ and **13**; 30_Chi_NN_FQ).

When the fluorescent donor and quencher are covalently constrained close to one another (**1**; 5_FQ_1) or separated by one and two residues (**2**; 5_FQ_2 and **3**; 5_FQ_3, respectively), the donor fluorescence is strongly quenched with a FRET efficiency more than 0.98 as expected (Fig. S2). Free fluorophore of amino-methylbenzamide in solution, which is an anthranilamide derivative, is not quenched by free quencher, amino-nitrophenol at the concentration of 2 μM same as that of peptoids used in here (Fig. S2).

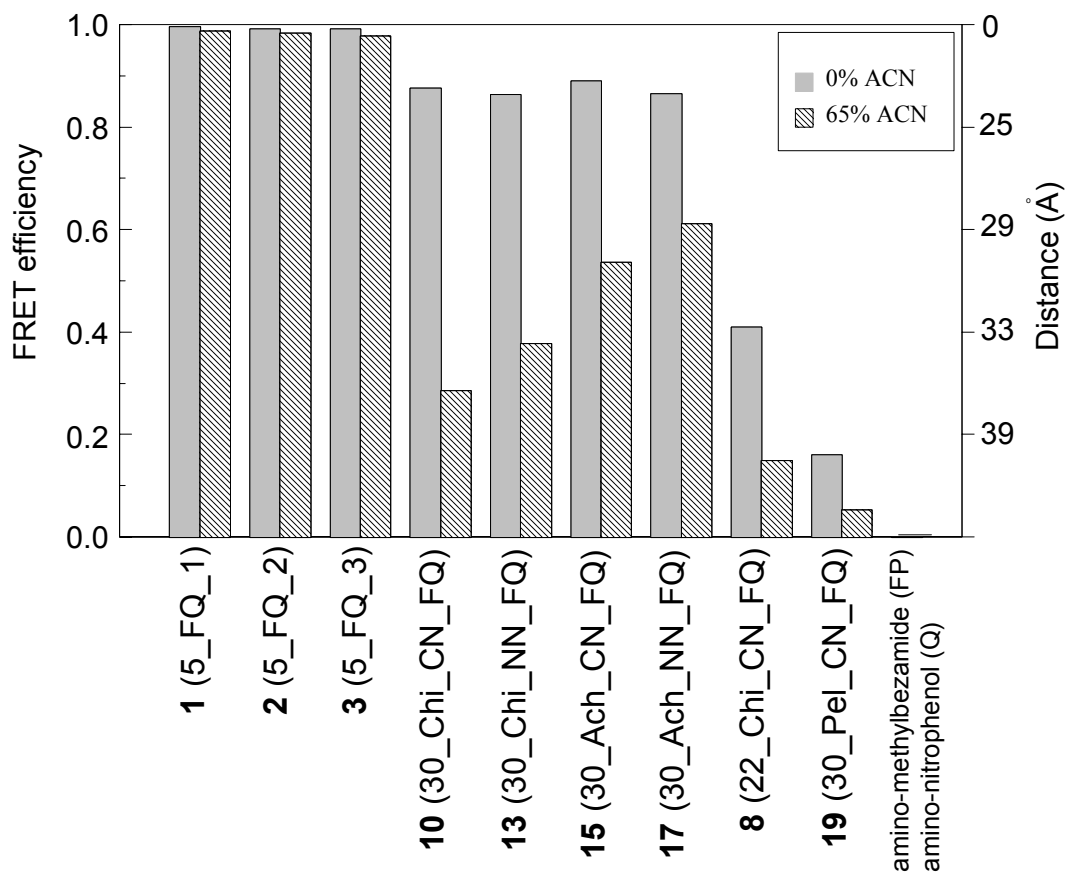


Figure S2. Comparison of two-peptoid conjugates in terms of FRET efficiency and distance between the fluorescent donor and quencher. Two different solvent conditions (0% and 65% of acetonitrile) are used to compare those peptoids in folded and unfolded states. The FRET efficiencies of control peptoids with the fluorescent donor and quencher next to each other or separated by one or two residues (**1**; 5_FQ_1, **2**; 5_FQ_2, and **3**; 5_FQ_3) are shown here for comparison. The FRET efficiency of free fluorophore and quencher in solution is also shown here. The concentration of all these peptoids is 2 μ M.

Reaction profiles of peptoid ligations. HPLC elution profiles of peptoid ligations and mass spectra of final products are shown in Fig. S3 – S9.

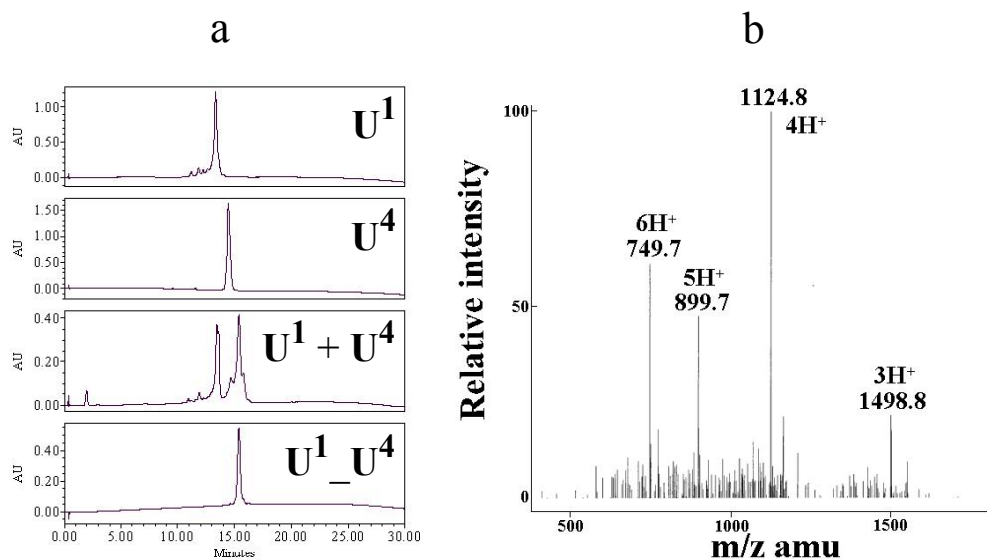


Figure S3. The generation of the peptoid **8** (22_Chi_CN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

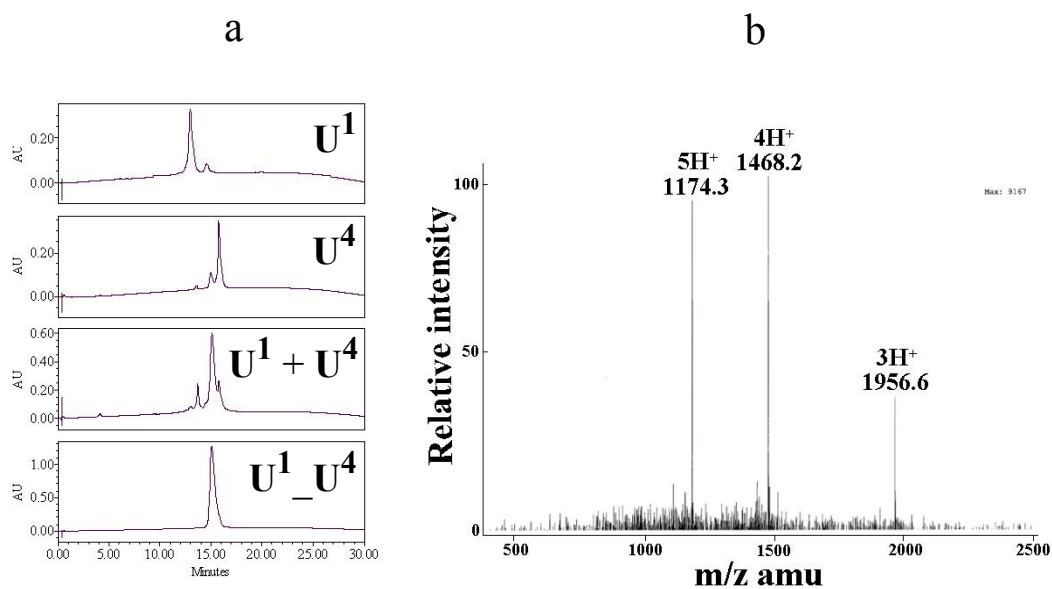


Figure S4. The generation of the peptoid **10** (30_Chi_CN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

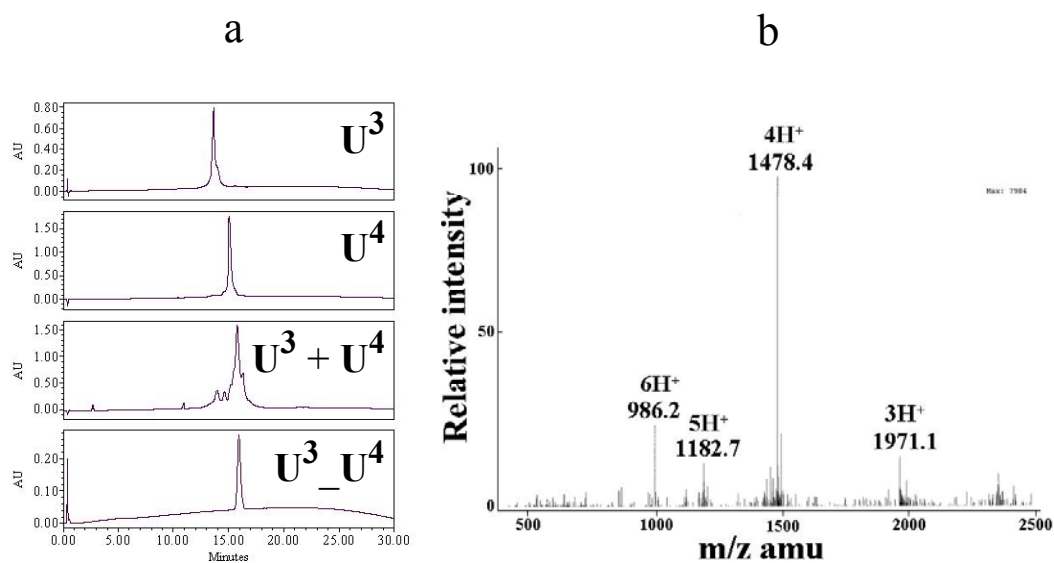


Figure S5. The generation of the peptoid **13** (30_Chi_NN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

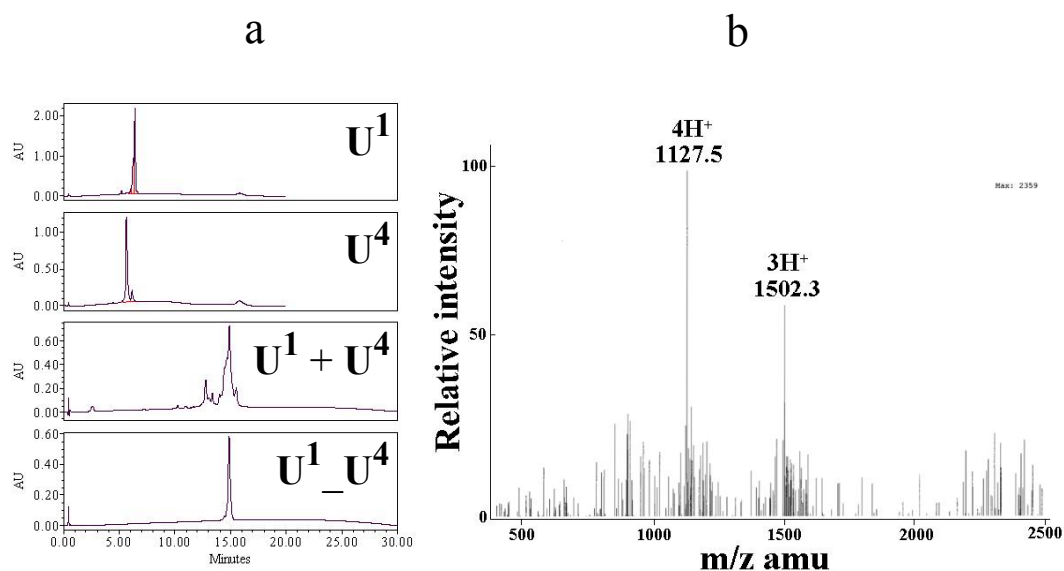


Figure S6. The generation of the peptoid **15** (30_Ach_CN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. First two samples were run by HPLC for 10 min. (b) Mass spectrum of the final product.

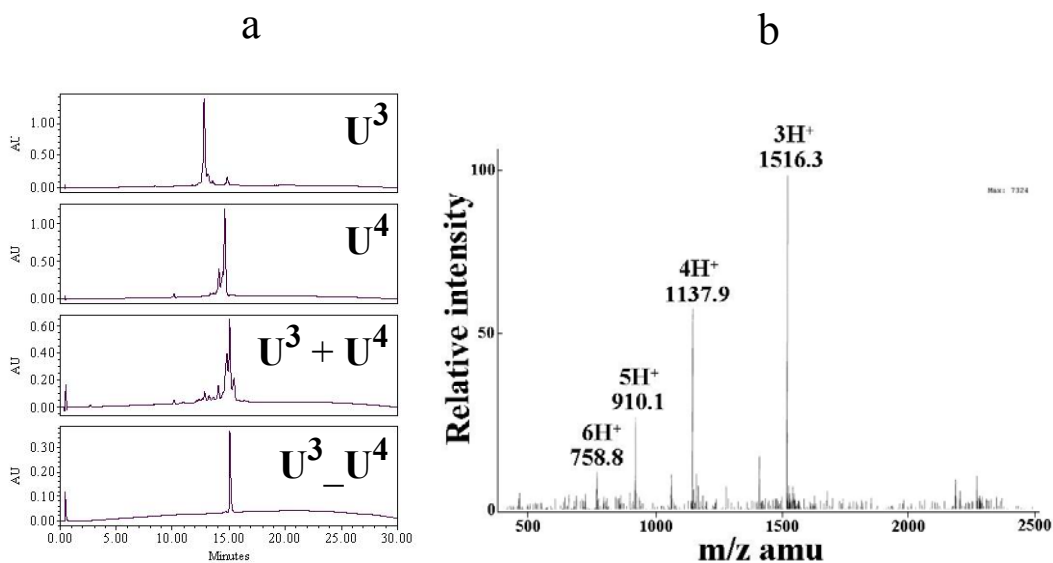


Figure S7. The generation of the peptoid **17** (30_Ach_NN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

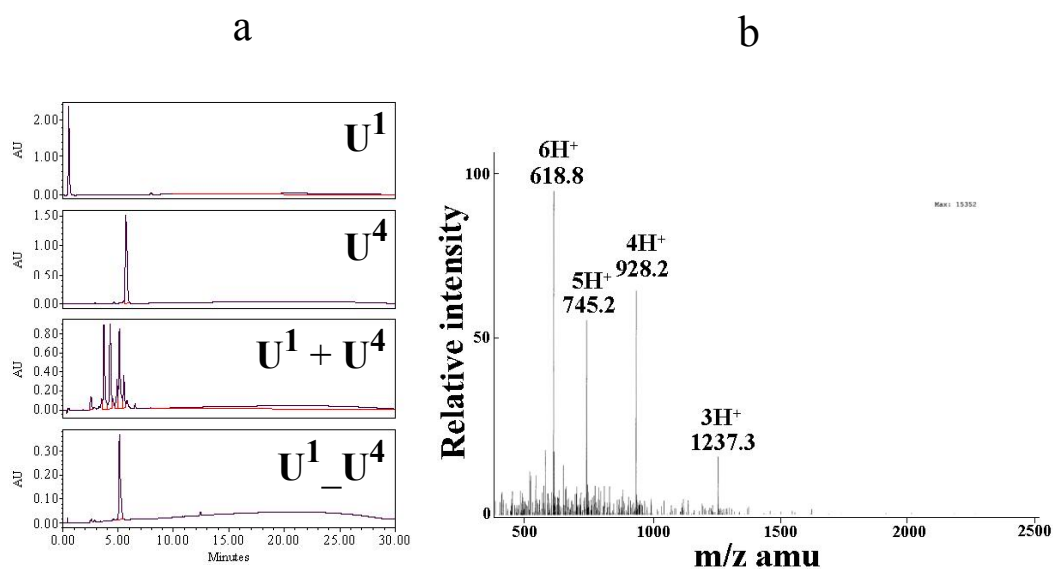
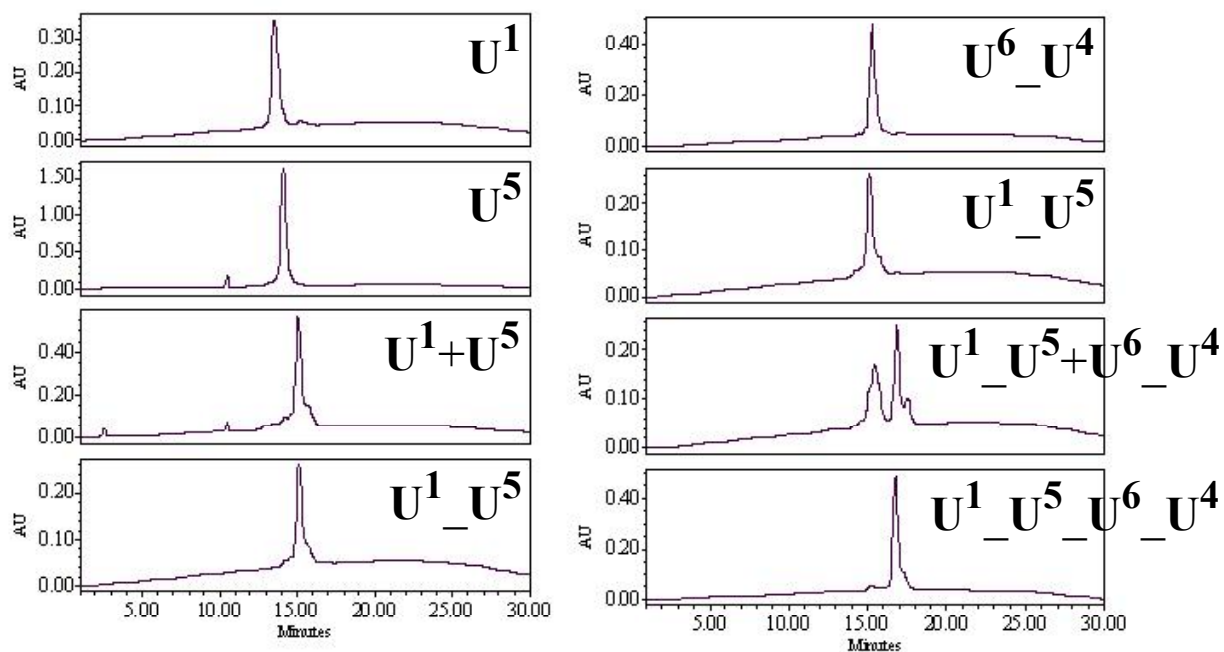


Figure S8. The generation of the peptoid **19** (30_Pel_CN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

a



b

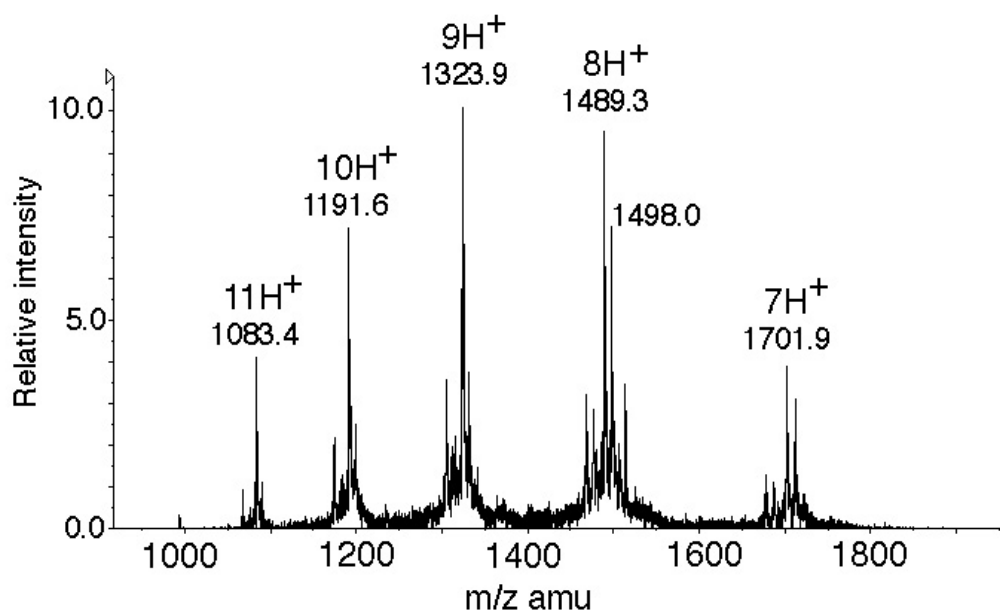


Figure S9. The generation of the peptoid **24** (60_Chi_CN_FQ). (a) HPLC profiles of peptoid ligation with starting materials and final product. (b) Mass spectrum of the final product.

Equilibrium GdnHCl titration. When some peptoids (**10**; 30_Chi_CN_FQ, **15**; 30_Ach_CN_FQ, **8**; 22_Chi_CN_FQ and **21**; 45_Chi_CN_FQ) were titrated with GdnHCl, they were not unfolded cooperatively although FRET efficiencies decrease at high concentration of GdnHCl (Fig. S10). It might indicate the expansion of the overall dimension of peptoids, but it remains to be resolved why GdnHCl gives non-cooperative transition, but acetonitrile does give cooperative transition (Fig. 8a and 10a).

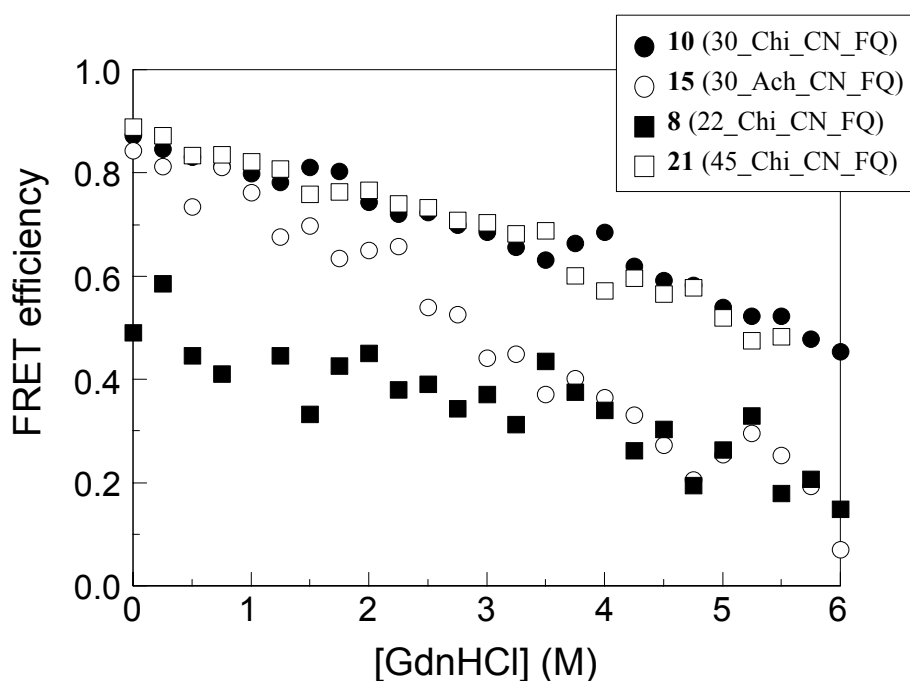


Figure S10. Equilibrium GdnHCl titration of **10** (30_Chi_CN_FQ), **15** (30_Ach_CN_FQ), **8** (22_Chi_CN_FQ) and **21** (45_Chi_CN_FQ).

Concentration-dependence of equilibrium acetonitrile titration. Different concentrations of the peptoid **10** (30_Chi_CN_FQ) were titrated with acetonitrile and probed by FRET (Fig. S11), essentially giving no difference in titration profiles.

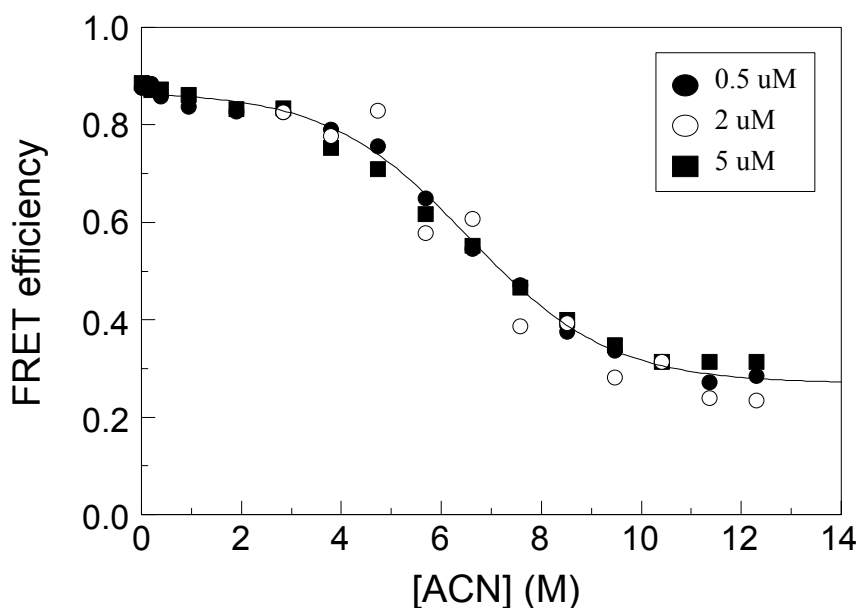


Figure S11. Equilibrium acetonitrile titration of **10** (30_Chi_CN_FQ) with different concentrations of the peptoid.

Analytical gel filtration of helix-bundle peptoids. TosoHaas G3000swxl (7.8mm x 30cm; 5 μ m) column was used for gel filtration with a flow rate of 0.5 mL/min. The elution profile was monitored by the absorbance at 214 nm. The buffer for gel filtration was 50 mM sodium phosphate (pH 7.0) with 0.2 M of sodium chloride. Molecular weight standards (BioRad GFC standards) were 670 kDa, 158 kDa, 44 kDa, 17 kDa and 1.35 kDa. 30 μ L of 100 μ M peptoid was loaded. All peptoids measured by the analytical gel filtration (**10**; 30_Chi_CN_FQ, **21**; 45_Chi_CN_FQ and **24**; 60_Chi_CN_FQ) run as a monomer (Fig. S12-S14). When retention times of these peptoids are fitted into the plot of the logarithm of molecular weights of standards vs. retention time, the peptoid **10** (30_Chi_CN_FQ) (molecular weight; 5868.8), **21** (45_Chi_CN_FQ) (molecular weight; 8842.2), and **24** (60_Chi_CN_FQ) (molecular weight; 11906.8) migrate in the gel filtration column as 6.1 Kda, 3.5 Kda, and 10.3 Kda, respectively. The retention time of the peptoid **21** (45_Chi_CN_FQ) deviates from the expected molecular weight. This suggests that the peptoid **21** (45_Chi_CN_FQ) is compactly packed.

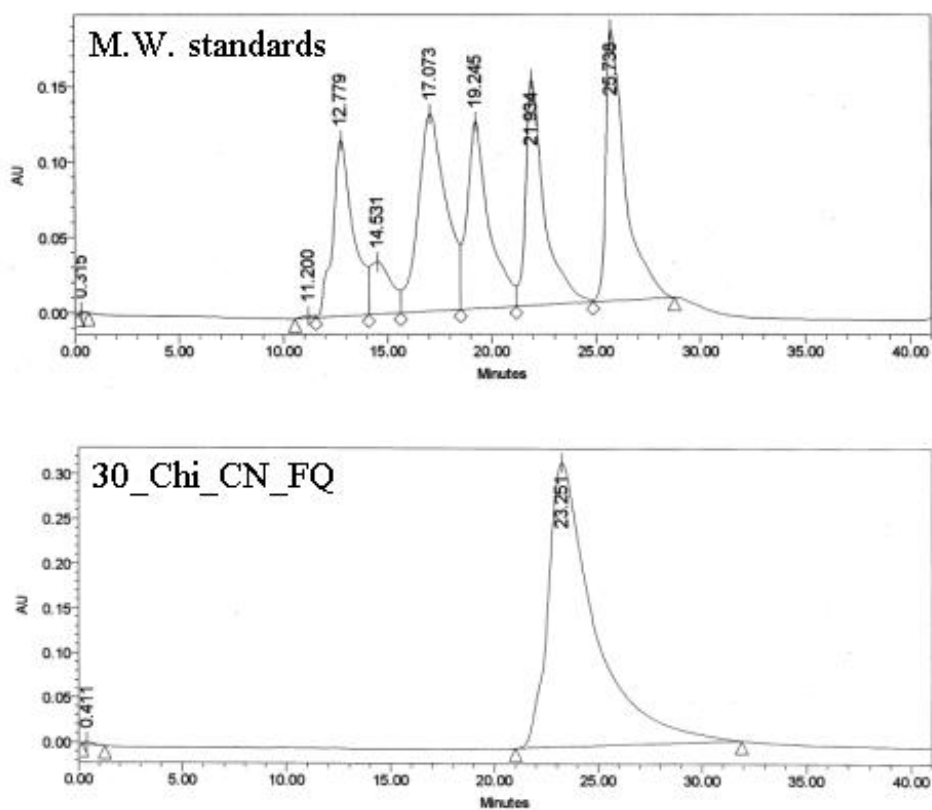


Figure S12. The profile of analytical gel filtration of the peptoid **10** (30_Chi_CN_FQ). Upper panel is retention times of molecular weight standards (670 kDa, 158 kDa, 44 kDa, 17 kDa and 1.35 kDa) and lower panel is the retention of the peptoid. The peptoid **10** (30_Chi_CN_FQ) migrates in the gel filtration column as 6.1 kDa.

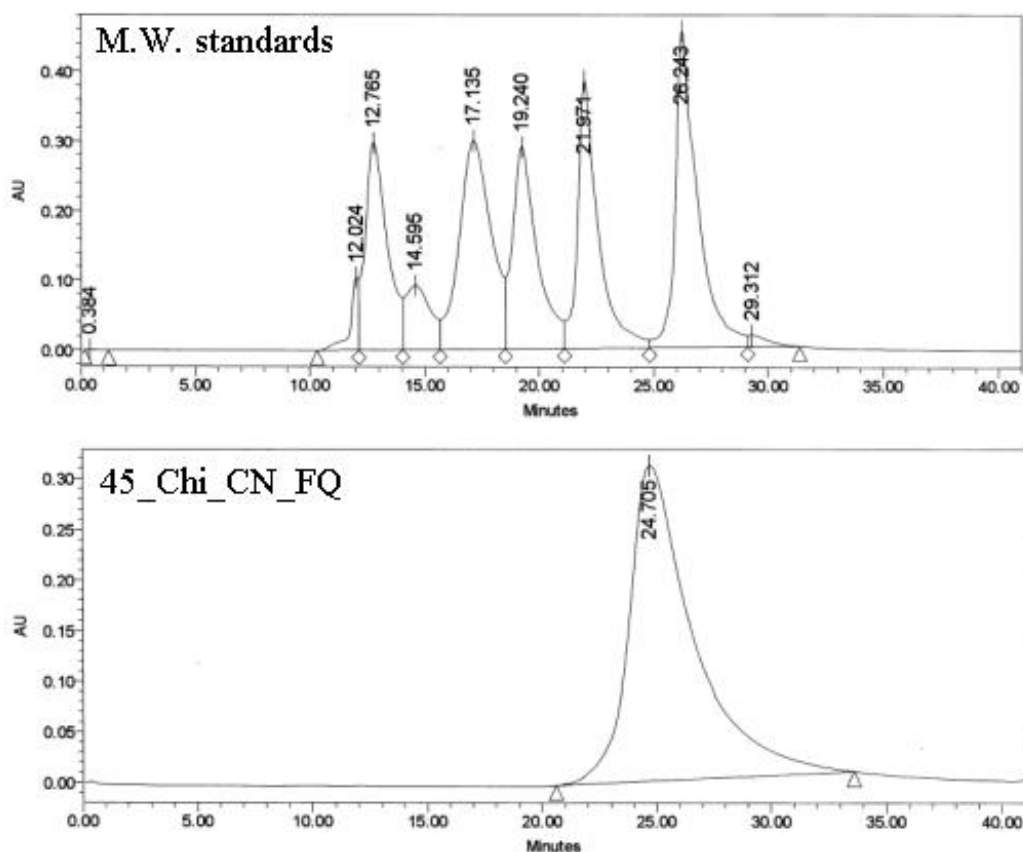


Figure S13. The profile of analytical gel filtration of the peptoid **21** (45_Chi_CN_FQ). Upper panel is retention times of molecular weight standards (670 kDa, 158 kDa, 44 kDa, 17 kDa and 1.35 kDa) and lower panel is the retention of the peptoid. The peptoid **21** (45_Chi_CN_FQ) migrates in the gel filtration column as 3.5 kDa.

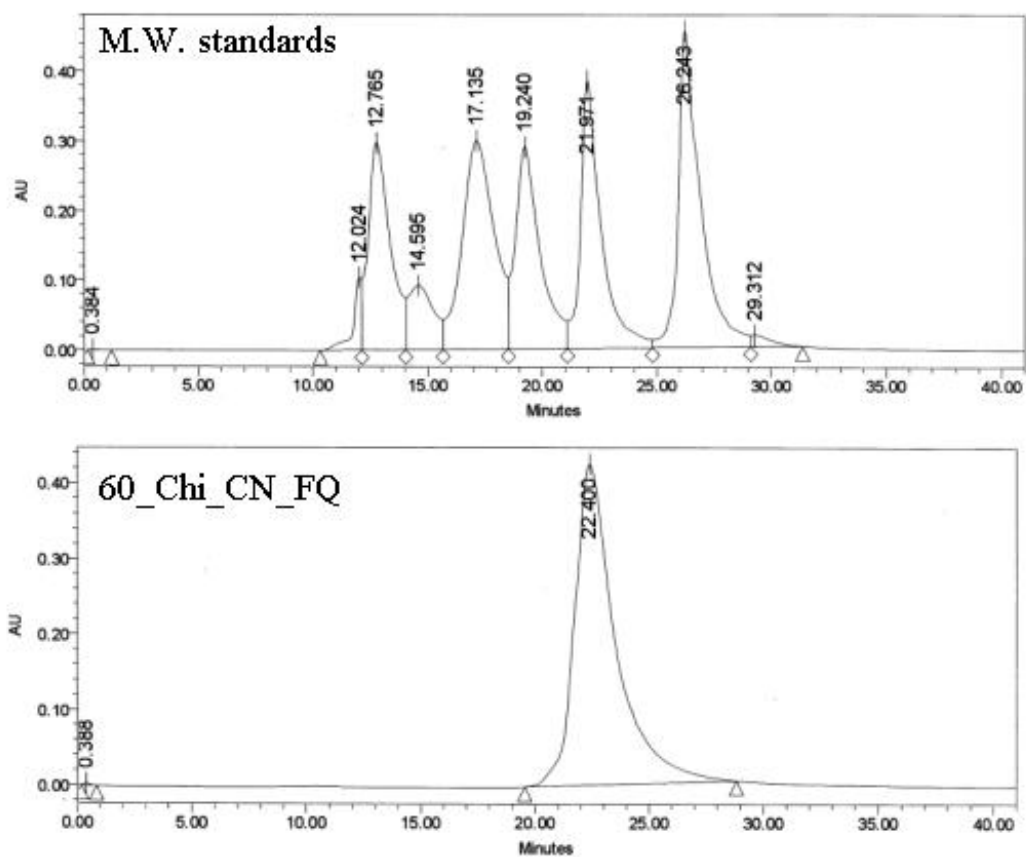


Figure S14. The profile of analytical gel filtration of the peptoid **24** (60_Chi_CN_FQ). Upper panel is retention times of molecular weight standards (670 kDa, 158 kDa, 44 kDa, 17 kDa and 1.35 kDa) and lower panel is the retention of the peptoid. The peptoid **24** (60_Chi_CN_FQ) migrates in the gel filtration column as 10.3 kDa.

The effect of acetonitrile on the secondary structure of peptoid. Far-UV CD spectra of some peptoids (**7**; 15_Chi and **10**; 30_Chi_CN_FQ) increase at high concentration of acetonitrile (Fig. S15). This result indicates that the secondary structures of these peptoids are not changed in the presence of acetonitrile.

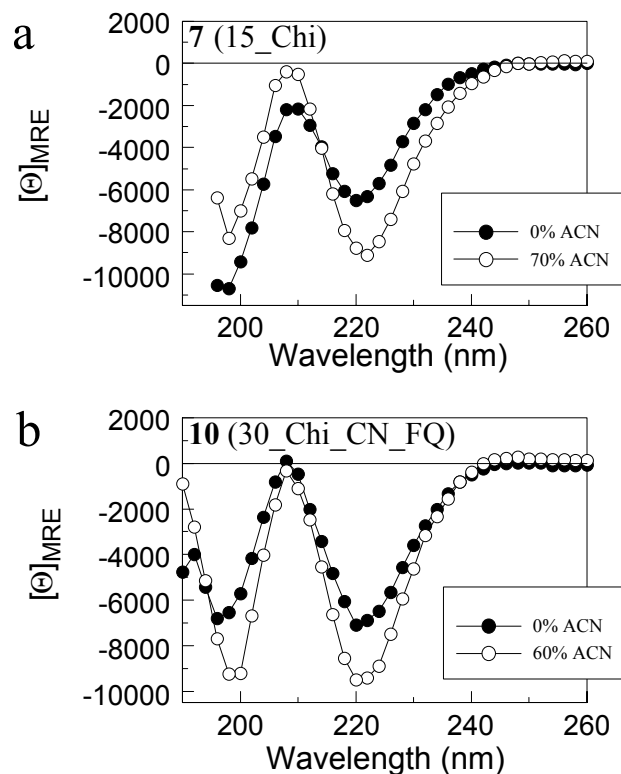


Figure S15. Effect of acetonitrile on the secondary structure of (a) the peptoid **7** (15_Chi) and (b) **10** (30_Chi_CN_FQ). Far-UV CD spectra of these peptoids were measured in the absence and presence of acetonitrile.

Equilibrium acetonitrile titration of putative three-helix bundle 45mers with a quencher at different positions. We synthesized a peptoid 45mer where the quencher is positioned at opposite end of other helical unit with respect to the donor (**22**; 45_Chi_CN_FQ_2). When compared with 45_Chi_CN_FQ where the quencher is located at same side of other helical unit with respect to the donor, the FRET efficiency of the peptoid **22** (45_Chi_CN_FQ_2) is less than that of the peptoid **21** (45_Chi_CN_FQ) in the absence of chemical denaturant as expected from the molecular conformation of three-helix bundle (Fig. 11 and Fig. S16). The profile of acetonitrile equilibrium titration of the peptoid **22** (45_Chi_CN_FQ_2) is same as that of the peptoid **21** (45_Chi_CN_FQ) (Fig. S16).

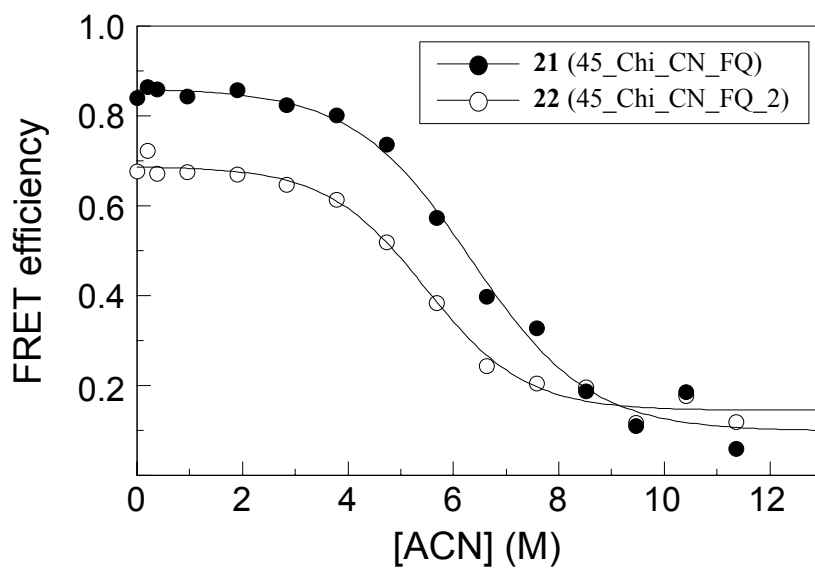


Figure S16. Equilibrium acetonitrile titration of two putative three-helix bundle peptoids. Two sequences are same except the position of the quencher.