Supporting Information

Biomimetic nanostructures: creating a high-affinity zinc-binding site in a folded non-biological polymer

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(13) 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.

Table S1. Molecular weight of each peptoid determined by mass spectrometry

peptoid	molecular weight (calc.:found)	peptoid	molecular weight (calc.:found)
1_FQ	4603.2:4601.3	**7_FQ**	4681.2:4683.1
1_F	4408.9:4407.9	**7_F**	4487.1:4488.7
2_FQ	4681.2:4680.4	**8_FQ**	4627.2:4627.6
2_F	4487.1:4486.3	**8_F**	4433.0:4434.0
2_Q	4462.0:4463.1	**8_Q**	4407.9:4408.1
2	4267.8:4269.0	**8**	4213.8:4214.2
3_FQ	4681.2:4679.5	**9_FQ**	5097.6:5098.4
3_F	4487.1:4488.1	**9_F**	4903.4:4905.0
3_Q	4462.0:4463.2	**9_Q**	4877.3:4879.5
3	4267.8:4268.4	**9**	4683.2:4684.8
4_FQ	4676.1:4676.9	**10_FQ**	5019.4:5020.9
4_F	4482.0:4482.8	**10_F**	4825.2:4826.9
5_FQ	4676.1:4677.0	**11_FQ**	4641.2:4641.9
5_F	4482.0:4482.2	**11_F**	4447.0:4446.9
6_FQ	4608.1:4609.1	**12 _FQ**	4588.1:4588.8
6_F	4414.0:4415.0	**12_F**	4393.9:4394.7
		13_FQ	4608.1:4606.2
		13_F	4414.0:4414.8

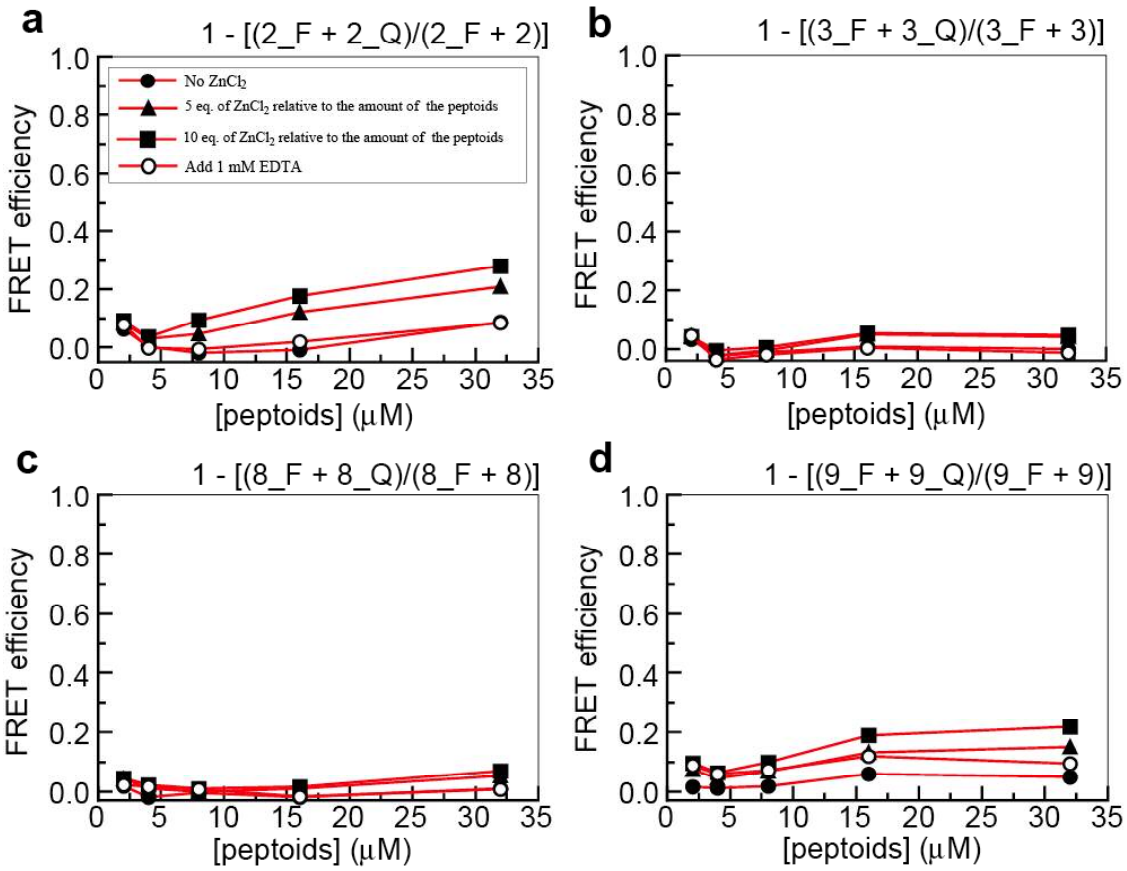

Figure S1. Concentration-dependent FRET efficiency for self-association of peptoids. The FRET efficiency was measured in the presence of 5 mM tris-HCl (pH 7.5) with 30 % acetonitrile. This same condition was used in the main text. Each sequence of four peptoids was tested for self-association at different peptoid concentrations from 2 μM to 32 μM. 5 eq. and 10 eq. of $ZnCl_2$ were added to see if there is a self-association between peptoids. Upon the addition of an excess amount of EDTA (1 mM) to remove all bound zinc ions, the FRET efficiency returned to the original value.

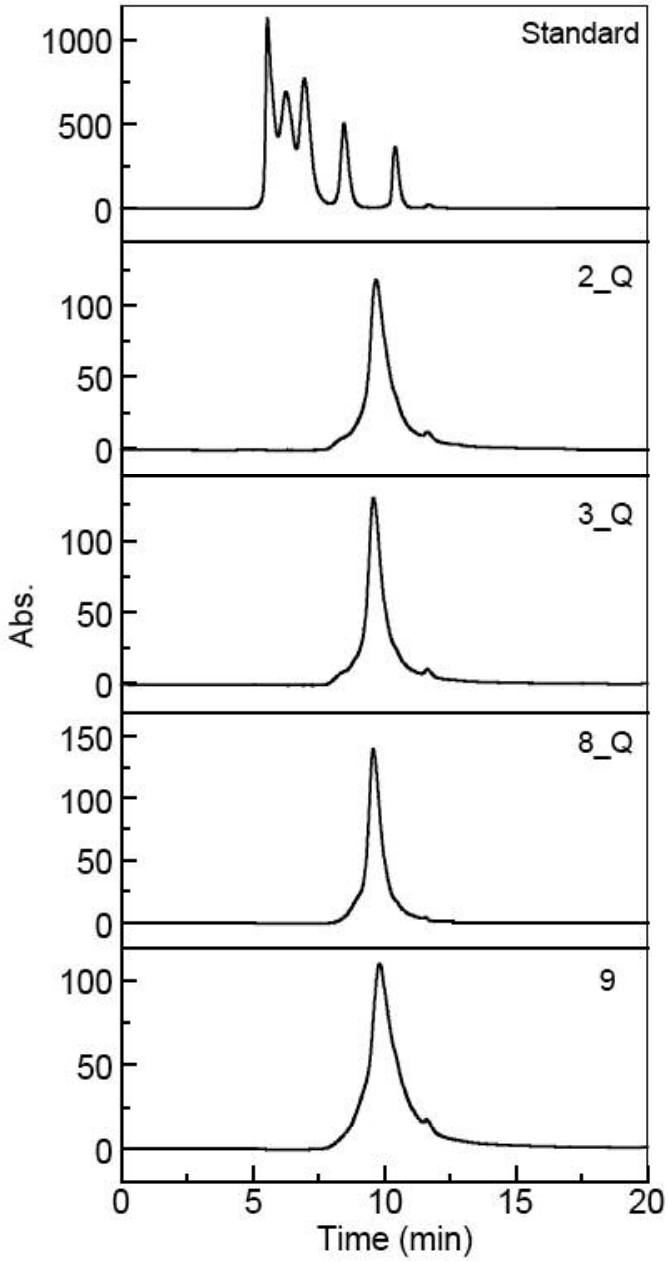

Figure S2. Analytical gel filtration on a Bio-Sil SEC-125 column for standard molecules and peptoids **2_Q**, **3_Q**, **8_Q** and **9**. The molecular weights of the standards (in order of elution) are 670 Kd (Thyroglobulin), 158 Kd (IgG), 44 Kd (Ovalbumin), 17 Kd (Myoglobin) and 1.35 Kd (Vitamin B12). Each of peptoids migrates as 3.5 Kd (**2_Q**), 3.9 Kd (**3_Q**), 3.9 Kd (**8_Q**) and 3.0 Kd (**9**), respectively. 20 μl of 50 μM peptoids were injected to the gel filtration column. The buffer used to run the column was 5 mM tris-HCl (pH 7.5) with 150 mM NaCl.

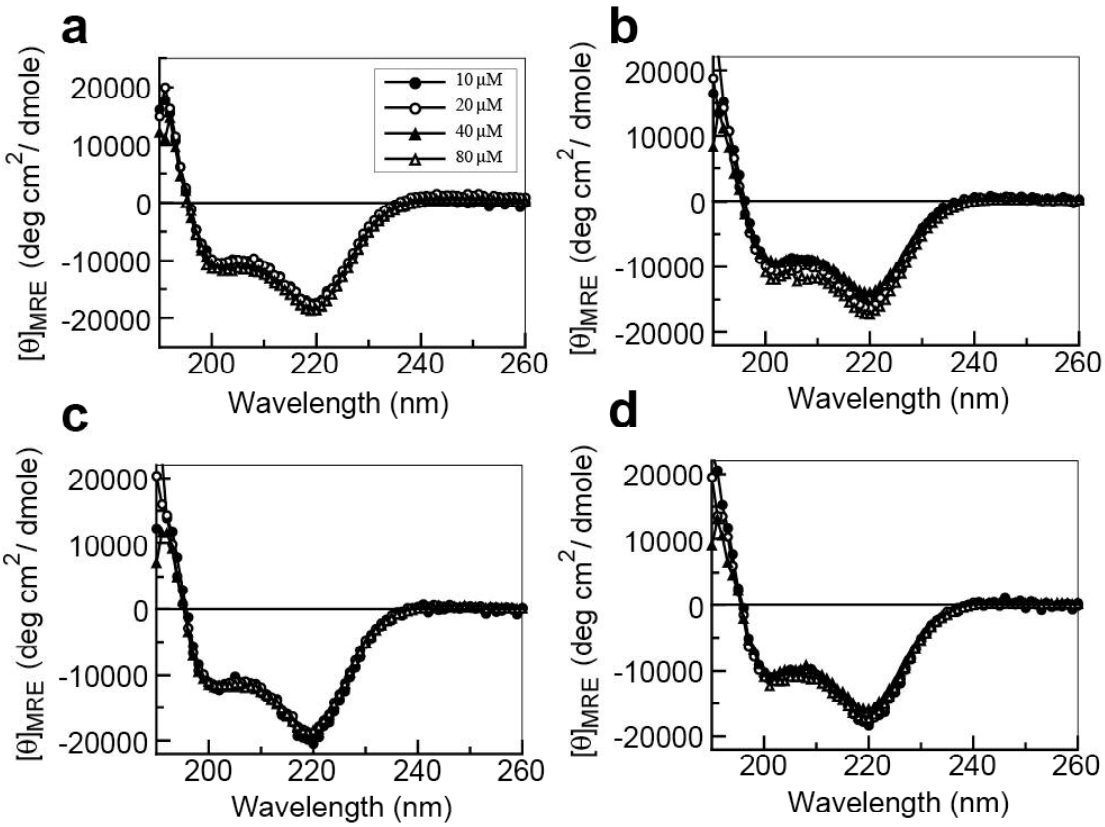

Figure S3. CD spectra of **2_FQ** and **3_FQ** at various concentrations of peptoid in the absence and presence of 5 molar equivalents of $ZnCl_2$ relative to the amount of peptoid. The buffer was 20 mM tris-HCl (pH 7.5) with 30 % acetonitrile. (**a**) **2_FQ** at four different peptoid concentrations (10, 20, 40 and 80 μM) in the absence of $ZnCl_2$. (**b**) **2_FQ** in the presence of 5 molar equivalents of $ZnCl_2$ relative to the amount of the peptoid. (**c**) **3_FQ** at various peptoid concentrations in the absence of $ZnCl_2$. (**d**) **3_FQ** in the presence of 5 molar equivalents of $ZnCl_2$ relative to the amount of the peptoid.

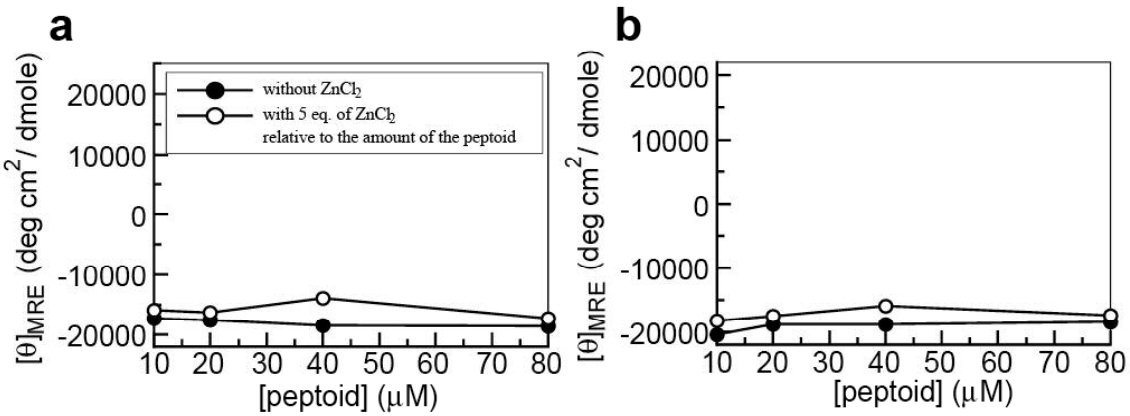

Figure S4. CD at 220 nm for **2_FQ (a)** and **3_FQ (b)**. Using the data presented at SI Fig. 10, the CD signal at 220 nm were plotted at different concentrations of the peptoids in the absence and presence of 5 eq. $ZnCl_2$ relative to the amount of the peptoid.

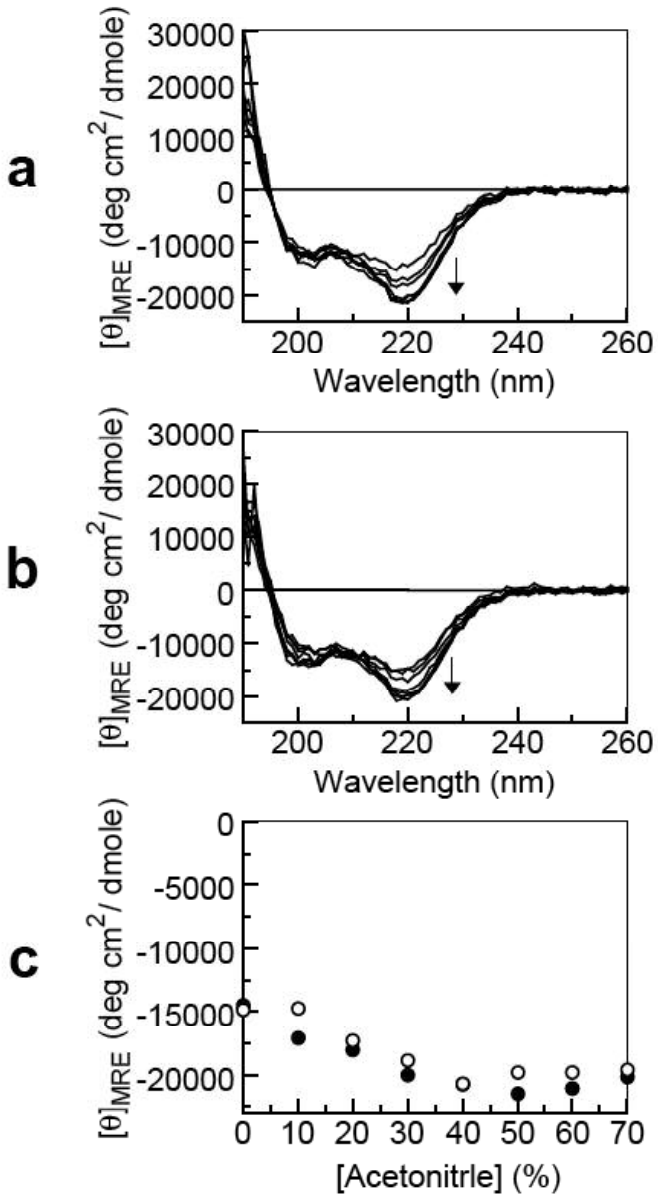

Figure S5. Effect of acetonitrile and $ZnCl_2$ on the secondary structure of peptoid **3_FQ**. 10 μM of **3_FQ** was used to measure the CD signal. The buffer was 20 mM tris-HCl (pH 7.5) with different concentrations of acetonitrile. 5 eq. (50 μM) of $ZnCl_2$ was added to see if there is a significant change of secondary structure. **(a)** Acetonitrile titration from 0 to 70 %. The arrow indicates the direction of change when the concentration of acetonitrile increases. **(b)** Acetonitrile titration in the presence of 5 eq. of $ZnCl_2$. **(c)** The CD signal at 220 nm was plotted at different acetonitrile concentrations in the absence (●) and presence (○) of 5 eq. of $ZnCl_2$.

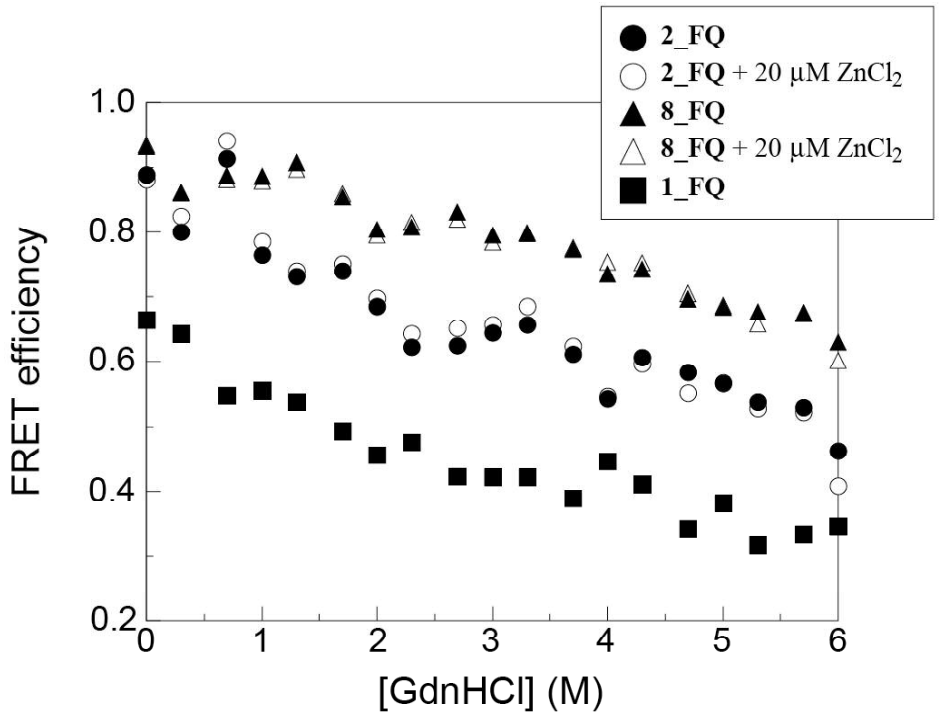

Figure S6. Equilibrium GdnHCl titration of **2_FQ**, **8_FQ** and **1_FQ**. 2 μM of each peptoid was titrated in the presence of 5 mM tris-HCl, pH (7.5).

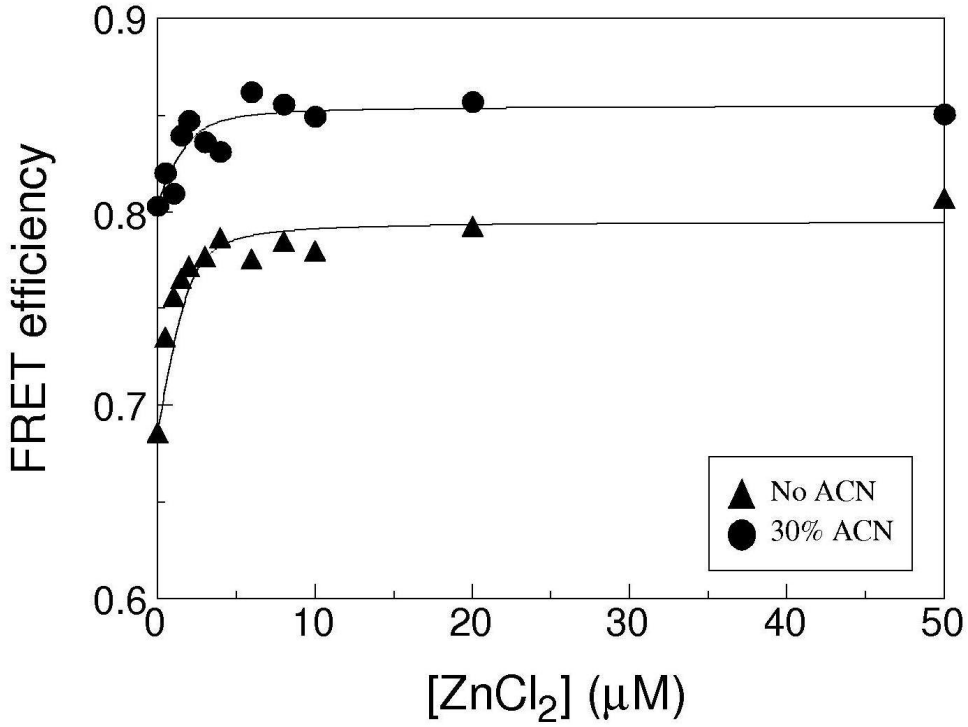

Figure S7. $ZnCl_2$ titration of the peptoid **9_FQ** in the absence (filled triangles) and presence (filled circles) of acetonitrile.

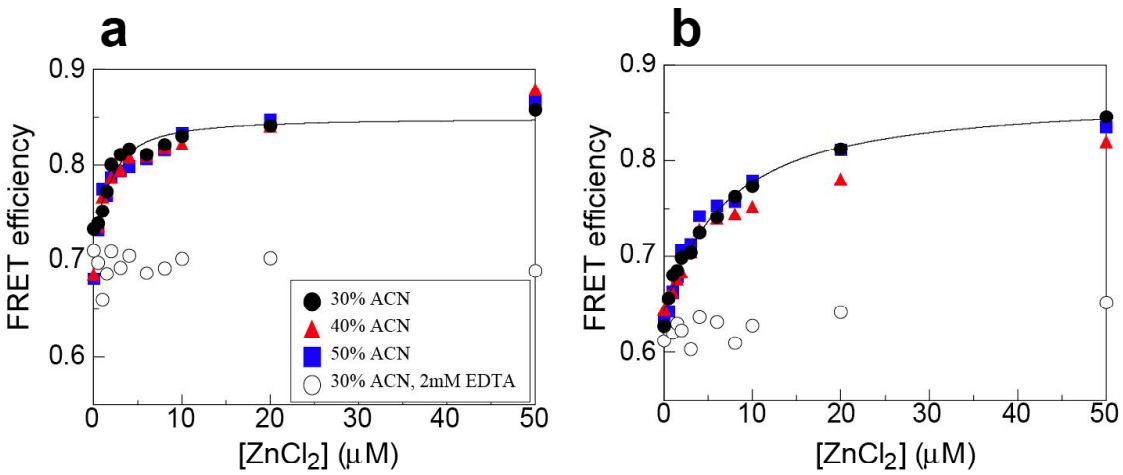

Figure S8. Effect of acetonitrile on the zinc-binding affinities. Two peptoids, (**a**) **2_FQ** and (**b**) **3_FQ** were titrated with $ZnCl_2$ in the presence of three different concentrations of acetonitrile (30, 40 and 50% (v/v) acetonitrile in water). The dissociation constants for zinc are 1.2±0.5 μM (30 %), 1.3±0.7 μM (40 %) and 1.2±0.6 μM (50 %) for **2_FQ**, and 5.8±1.0 μM (30 %), 5.5±1.0 μM (40 %) and 3.6±0.6 μM (50 %) for **3_FQ**.

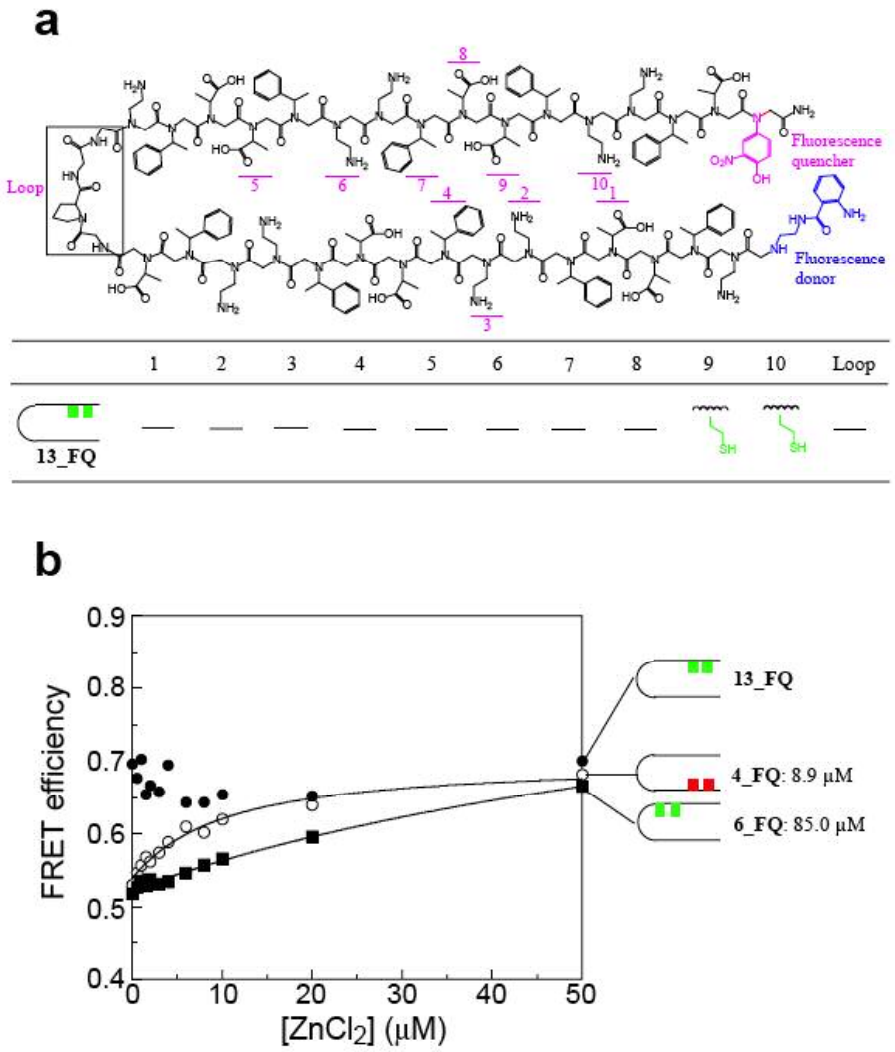

Figure S9. Effect of zinc on the FRET efficiency of the peptoid **13_FQ**. (**a**) The chemical structure of **13_FQ**. (**b**) **13_FQ** was titrated with $ZnCl_2$ in the presence of 30 % acetonitrile. **4_FQ** and **6_FQ** are shown for comparison.

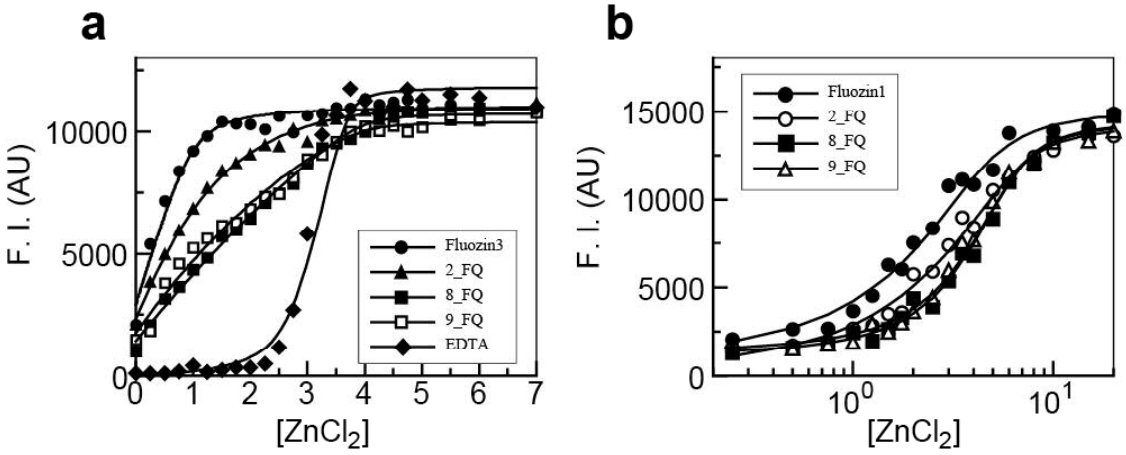

Figure S10. Competition assay for zinc binding between peptoids and Fluozin-3 or Fluozin-1. The wavelength for excitation and emission was 494 and 516 nm, respectively. The data were fitted using DynaFit3 software package from BioKin, Ltd., Massachusetts. The FluoZin-1 and FluoZin-3 has a reported k_d of 8 μM and 15 nM for zinc, respectively (Invitrogen, Corp., CA and *JACS*, 2002, **124**, 776). Under our experimental condition, k_d of FluoZin-1 for zinc was 0.4 μM. For fitting the data, we used 0.4 μM and 15 nM for the zinc-binding dissociation constants of FluoZin-1 and FluoZin-3, respectively. **(a)** The mixture of 1 μM Fluozin-3 and 3 μM peptoids was titrated with $ZnCl_2$ for competition. As a control, Fluozin-3 alone was titrated with $ZnCl_2$ (closed circles). EDTA was also used for this competition. The buffer was 5 mM tris-HCl (pH 7.5) in the absence of acetonitrile. The zinc-binding affinity for **8_FQ** (k_d = 2 x 10^{-8} M) and **9_FQ** (k_d = 3 x 10^{-8} M) is about four times tighter than that for **2_FQ** (k_d = 9 x 10^{-8} M). **(b)** The mixture of 4 μM Fluozin-1 and 2 μM peptoids was titrated with $ZnCl_2$ for competition. The buffer was 5 mM tris-HCl (pH 7.5) with 30 % acetonitrile. As a control, Fluozin-1 alone was titrated with $ZnCl_2$ (closed circles). The zinc-binding affinity for peptoids **8_FQ** (k_d = 6 x 10^{-8} M) and **9_FQ** (k_d = 7 x 10^{-8} M) was one order of magnitude tighter than that of **2_FQ** (k_d = 7 x 10^{-7} M).

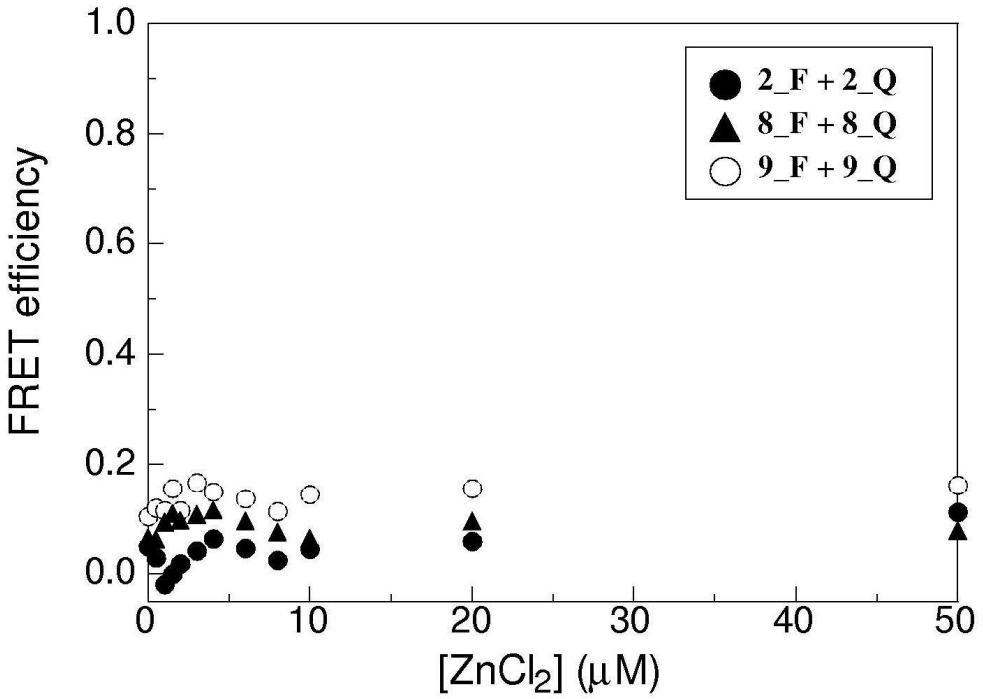

Figure S11. FRET self-association assay for **2_F** + **2_Q**, **8_F** + **8_Q**, and **9_F** + **9_Q**. These peptoids were titrated with $ZnCl_2$ in the presence of 30 % acetonitrile. The fluorescence intensities from reference molecules (**2_F** + **2**, **8_F** + **8**, and **9_F** + **9**) were also measured in order to obtain the FRET efficiency as described in Fig. 2. All the concentration of each individual peptoid is 1 μM.

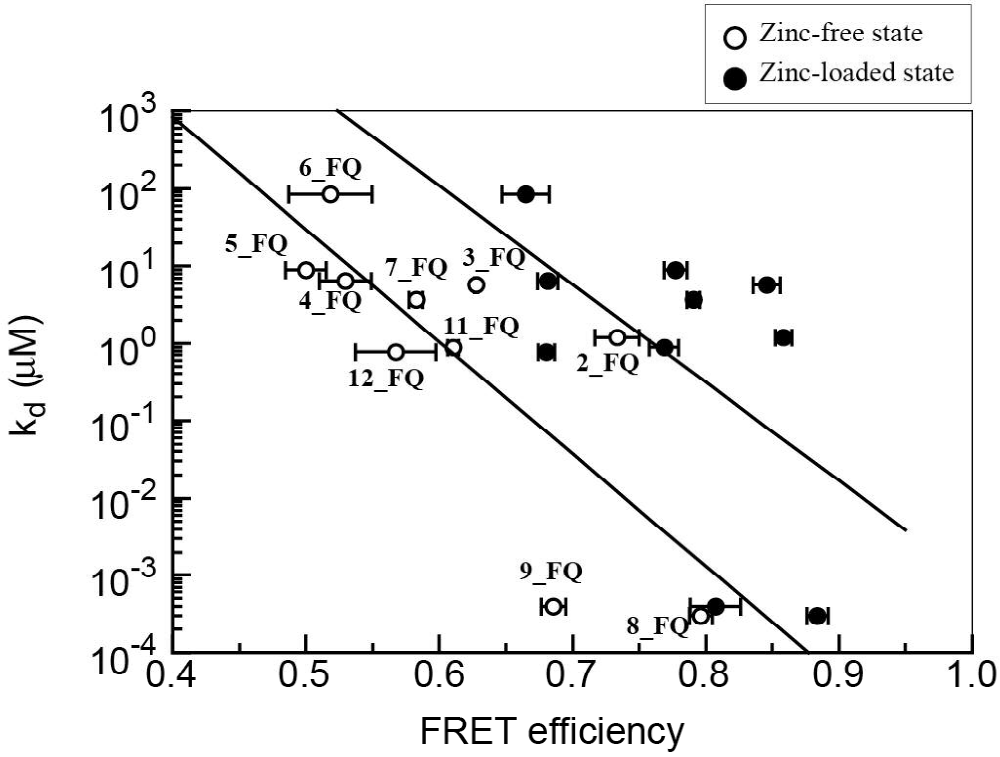

Figure S12. Weak correlation between the FRET efficiency of zinc-free/bound states and the apparent zinc-binding dissociation constants. The logarithm of the zinc-binding dissociation constants for the zinc-free and bound state has weak linear correlation with the folded state of the peptoids. The value of correlation for the goodness-of-fit is 0.77 and 0.55 for the zinc-free and bound state, respectively.

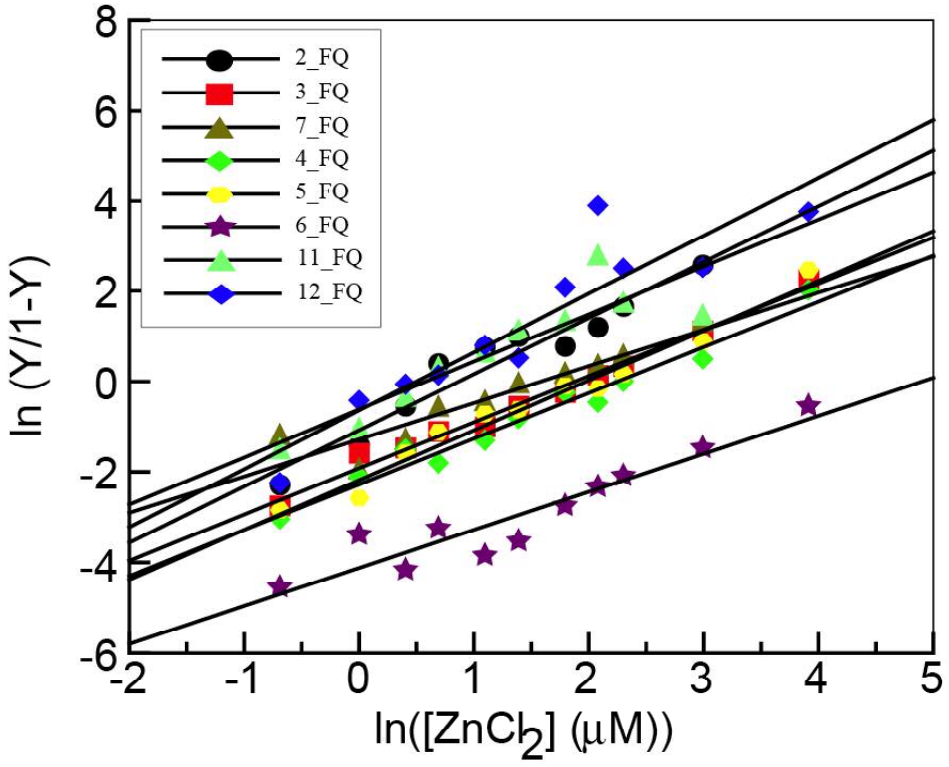

Figure S13. Hill plot for zinc binding. The data presented in Fig. 5 in the main manuscript were used for the Hill plot. Y is a fraction of zinc-binding site filled. The slope of the peptoids shown here was approximately 1. The quality of the data for **8_FQ** and **9_FQ** was not suitable for this Hill plot because the FRET efficiency of **8_FQ** and **9_FQ** in Fig. 5 was saturated above 1 eq. of $ZnCl_2$.

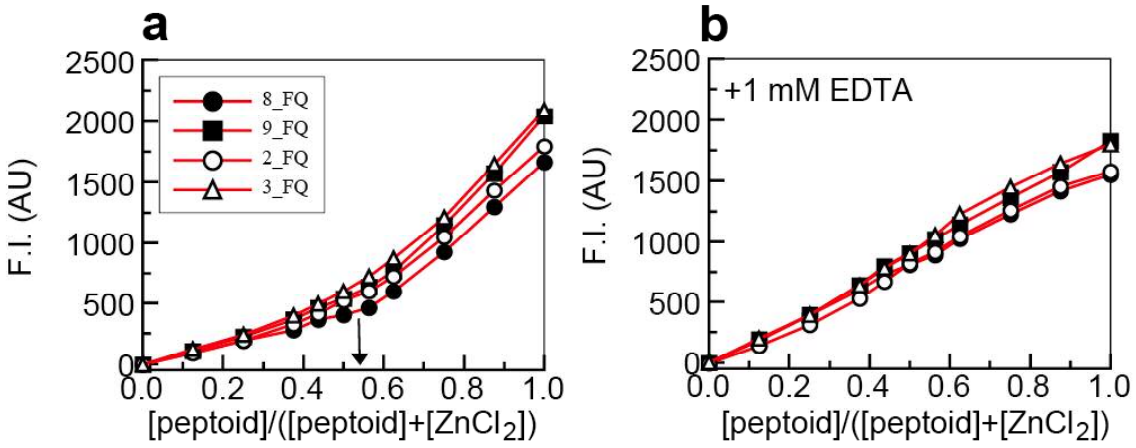

Figure S14. Job plot for zinc binding. The buffer was 5 mM tris-HCl (pH 7.5) with 30 % acetonitrile. The total sum of [peptoid] + [$ZnCl_2$] was 16 μM. **(a)** This plot showed a kink around 0.5 in all of four peptoids (**2_FQ**, **3_FQ**, **8_FQ** and **9_FQ**), indicating that the number of zinc-binding site is 1. **(b)** After excess amount of EDTA (1 mM) was added, the fluorescence intensity increased linearly.

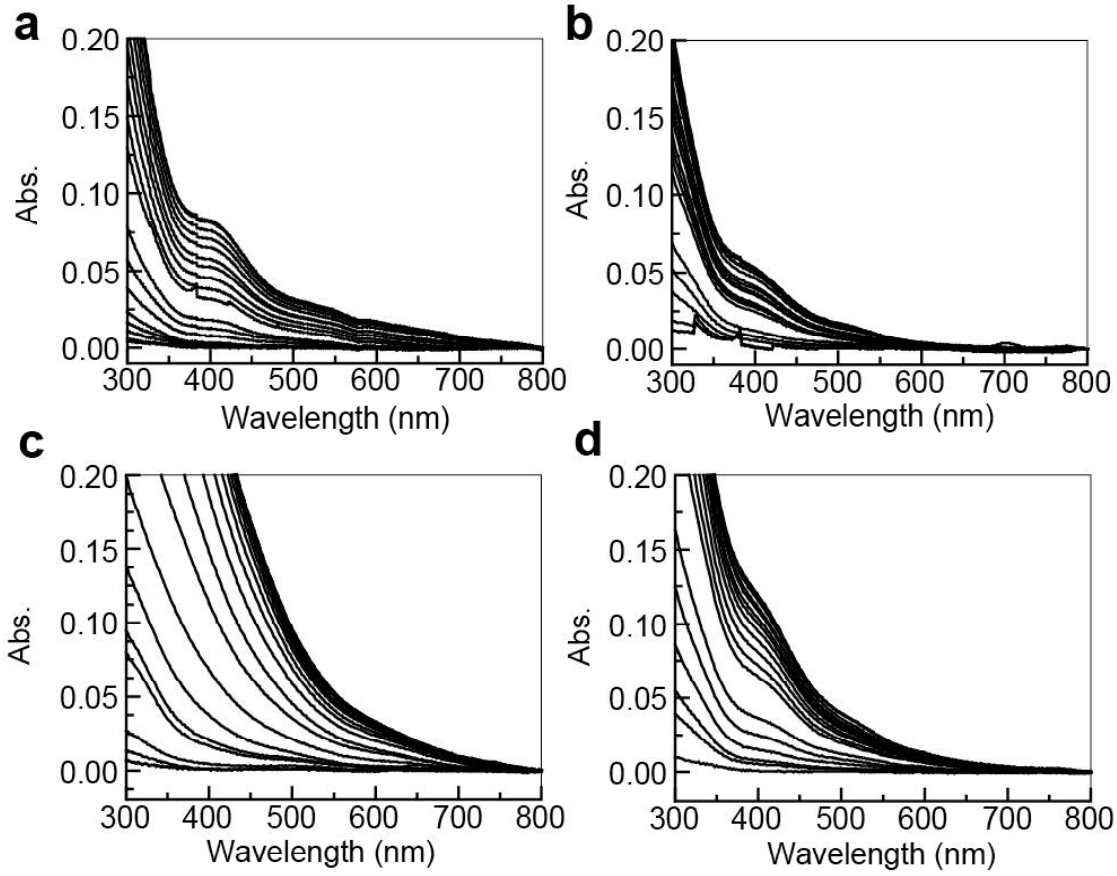

Figure S15. $Co(II)Cl_2$ titration for peptoids **(a) 2**, **(b) 3**, **(c) 8** and **(d) 9**. These peptoids have no fluorescence donor and quencher. 200 μM of peptoids were titrated with concentrated stock of $Co(II)Cl_2$ in the presence of 50 mM tris-HCl (pH 7.5) with 30% acetonitrile.

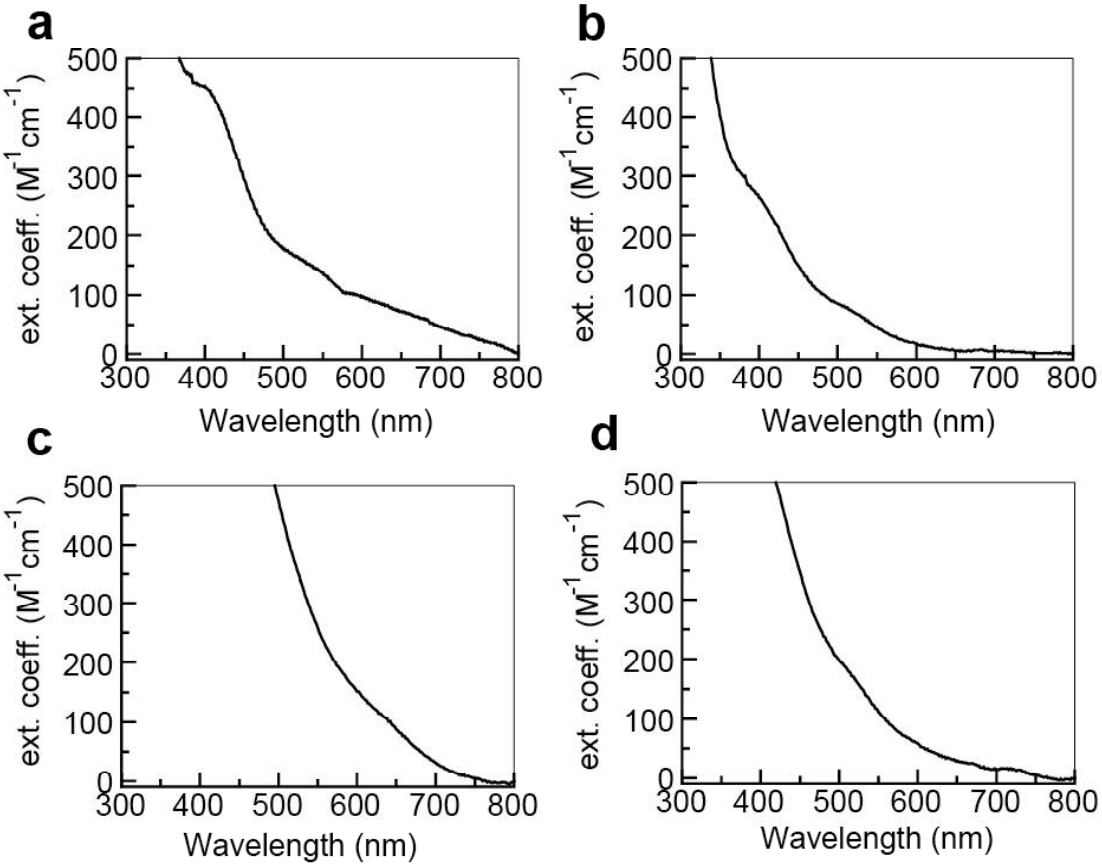

Figure S16. Extinction coefficients for bound Co(II) in peptoids **(a) 2**, **(b) 3**, **(c) 8** and **(d) 9**, indicating a coordination number of 5 or 6.

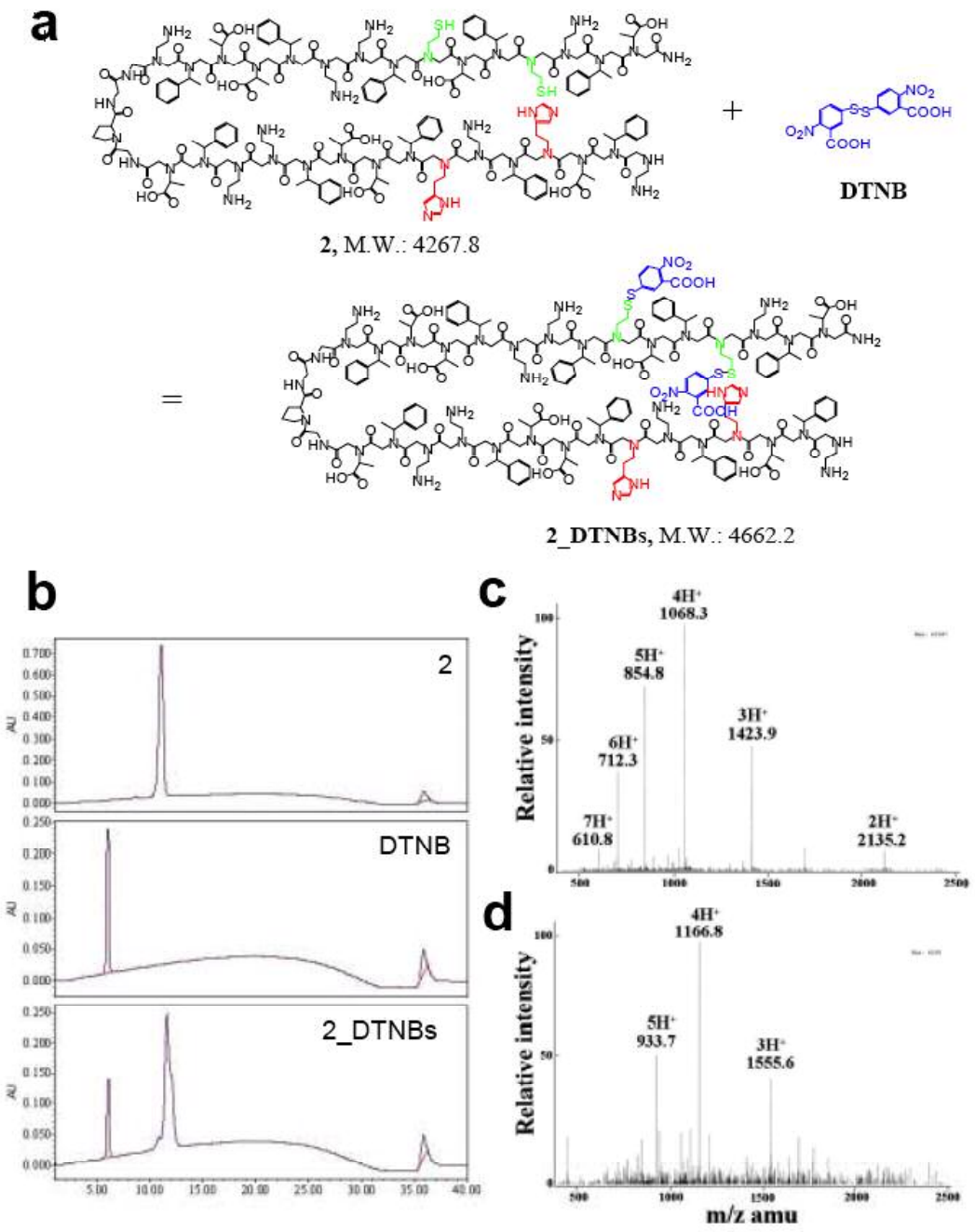

Figure S17. Reaction of the peptoid **2** with DTNB. (**a**) The chemical structure of reactants (peptoid **2** and DTNB) and final product (**2_DTNBs**). (**b**) HPLC profiles for the peptoid **2**, DTNB and the reaction mixture of them. 100 μM of the peptoid was treated with 200 μM of DTNB in 50 mM tris-HCl (pH 7.0) buffer that contains 30 % acetonitrile in water. The mass spectra of the peptoid **2** and the reaction mixture are shown in (**c**) and (**d**), respectively. The final product (**2_DTNBs**) is found as a major species in the reaction mixture.

Amine submonomers. Commercially available amines were purchased from Acros Organics (Morris Plains, NJ), Bachem California, Inc. (Torrance, CA), and Aldrich Chemical Co. (Milwaukee, WI). (*S*)-*N*-(1-phenylethyl)glycine (Nspe), (*S*)-*N*-(1-carboxyethyl)glycine (Nsce), *N*-(2-aminoethyl)glycine (Nae), *N*-(1-mercaptoethyl)glycine (Ncys), *N*-(1-imidazole-ethyl)glycine (Nhis), *N*-(2-nitrophenol)glycine (Nnp) and *N*-(2-anthranilamido-ethyl)glycine (Naae) were derived during peptoid synthesis from the amines *L*(-)-α-methylbenzylamine (Acros), (L)-*O*-t-butyl-alanine-HCL (Bachem), *N*-*t*-BOC-1,2-diaminoethane[1], *S*-tritylaminoethanethiol[2], histamine (Aldrich), 4-amino-2-nitrophenol (Aldrich), and 2-*t*-butyloxycarbonylamino-1-aminoethyl-phenylamide[3], respectively. All other solvents and reagents were obtained from commercial sources and used without further purification.

(1) Krapcho, A. P.; Kuell, C. S. *Synth. Commun.* **1990**, 20, 2559-2564.

(2) Maltese, M. *J. Org. Chem.* **2001**, 66, 7615-7625.

(3) Lee, B. -C.; Zuckermann, R. N.; Dill, K. A. *J. Am. Chem. Soc*. **2005**, 127, 10999-11009.