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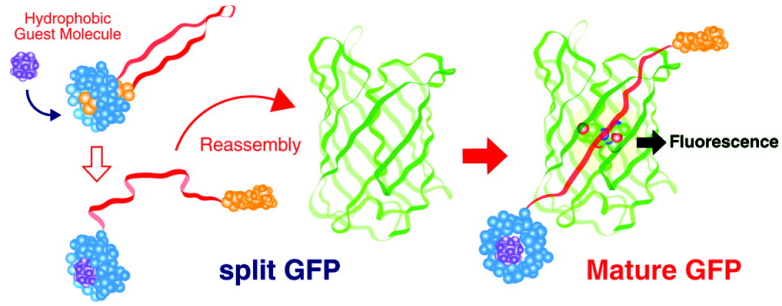

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Supramolecular Control of Split-GFP Reassembly by Conjugation of β-Cyclodextrin and Coumarin Units

Seiji Sakamoto*,† and Kazuaki Kudo

Institute of Industrial Science, University of Tokyo, Komaba 4-6-1, Meguro-ku, Tokyo 153-8505, Japan

Received March 30, 2008; E-mail: sakamoto@tagen.tohoku.ac.jp

Abstract: The design of proteins whose structure and function can be manipulated by binding with specific ligands has been of great interest in the field of protein engineering. Some successful examples of small-molecule-dependent proteins have been reported, but their ligand-binding domains have mainly been limited to those derived from natural proteins. The introduction of synthetic components for ligand responsiveness may expand the versatility of small-molecule-dependent proteins. In this study, we designed and constructed a fragmented green fluorescent protein (split GFP) whose reassembly could be modulated by the non-natural supramolecular interaction. In the design of split GFP, β-cyclodextrin (βCDx) and coumarin units were introduced into a C-terminal fragment (residues 214−230) of split GFP. The C-terminal peptide with βCDx and coumarin, DC-M2-βCDx, which contains both host and guest moieties in the same peptide chain, formed an intramolecular inclusion complex in the absence of exogenous guest molecules. This interaction strongly inhibited reconstitution with the GFP N-terminal fragment (residues 2−214) (GFP 1−10 OPT). However, the addition of suitable guest molecules for βCDx terminated the intramolecular host−guest interaction in the C-terminal peptide, leading to reassembly of the protein fragments and concomitant fluorescence recovery due to the formation of mature GFP. These results successfully demonstrated direct control of protein structure and function by application of synthetic supramolecular interaction to a fragmented protein. The combined system of fragmented protein and synthetic supramolecular elements is expected to be a useful and flexible strategy for regulation of protein structure and function via binding to synthetic ligands.

Introduction

Cyclodextrins (CDx's) have been the subject of much study in the field of supramolecular chemistry due to their ability to bind a variety of organic compounds within their hydrophobic cavities via noncovalent interactions. The hydrophobic nature and size variability of these cavities have so far been utilized, for example, as enzyme mimics, models for protein−substrate binding, solubilizers for water-insoluble substances, and chemosensors.[1] Furthermore, noncovalent interactions between CDx's and suitable guest molecules have been applied in supramolecular control of peptide and protein assembly.[2,3] However, methods for direct control of protein functions using typical supramolecular elements such as CDx's have not been explored sufficiently. The design of proteins whose structure and function can be manipulated by binding with specific ligands has been of great interest in the field of protein engineering due to their potential application as artificial molecular switches which could enable control of biological functions by inputs of small molecules.[4–6] The combination of natural or designed ligand-binding domains with fragmented proteins is expected to be an effective approach to the creation of small-molecule-dependent proteins.[7–12] For instance, Muir and co-workers reported fusion of cleaved intein fragments with FKBP (FK506-binding protein)

† Present address: Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan.

(1) (a) *Comprehensive Supramolecular Chemistry, Vol. 3*; Atwood, J. L., Davies J. E. D., MacNicol, D. D., Vögtle, F., Szejtli, J., Osa, T., Eds.; Pergamon:. Oxford, 1996. (b) Breslow, R.; Dong, S. D. *Chem. Rev.* **1998**, *98*, 1997–2011. (c) Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035–2044. (d) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: Berlin, 1978.

(2) (a) Aizawa, Y.; Sugiura, Y.; Morii, T. *Biochemistry* **1999**, *38*, 1626–1632. (b) Aizawa, Y.; Sugiura, Y.; Ueno, M.; Mori, Y.; Imoto, K.; Makino, K.; Morii, T. *Biochemistry* **1999**, *38*, 4008–4017. (c) Morii, T.; Yamane, J.; Aizawa, Y.; Makino, K.; Sugiura, Y. *J. Am. Chem. Soc.* **1996**, *118*, 10011–10017.

(3) Zhang, L.; Wu, Y.; Brunsveld, L. *Angew. Chem., Int. Ed.* **2007**, *46*, 1798–1802.

(4) (a) Lin, Q.; Barbas, C. F.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 612–613. (b) Guo, Z.; Zhou, D.; Schultz, P. G. *Science* **2000**, *288*, 2042–2045.

(5) Clackson, T. *Curr. Opin. Chem. Biol.* **1997**, *1*, 210–218.

(6) Buskirk, A. R.; Liu, D. R. *Chem. Biol.* **2005**, *12*, 151–161.

(7) (a) Schwartz, E. C.; Saez, L.; Young, M. W.; Muir, T. W. *Nat. Chem. Biol.* **2007**, *3*, 50–54. (b) Mootz, H. D.; Blum, E. S.; Muir, T. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 5189–5192. (c) Mootz, H. D.; Muir, T. W. *J. Am. Chem. Soc.* **2002**, *124*, 9044–9045.

(8) (a) Yuen, C. M.; Rodda, S. J.; Vokes, S. A.; McMahon, A. P.; Liu, D. R. *J. Am. Chem. Soc.* **2006**, *128*, 8939–8946. (b) Buskirk, A. R.; Ong, Y.-C.; Gartner, Z. J.; Liu, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10505–10510.

(9) Skretas, G.; Wood, D. W. *Protein Sci.* **2005**, *14*, 523–532.

(10) Pratt, M. R.; Schwartz, E. C.; Muir, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11209–11214.

(11) (a) Paulmurugan, R.; Gambhir, S. S. *Anal. Chem.* **2007**, *79*, 2346–2353. (b) Paulmurugan, R.; Gambhir, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15883–15888. (c) Paulmurugan, R.; Gambhir, S. S. *Cancer Res.* **2005**, *65*, 7413–7420. (d) Paulmurugan, R.; Massoud, T. F.; Huang, J.; Gambhir, S. S. *Cancer Res.* **2004**, *64*, 2113–2119.

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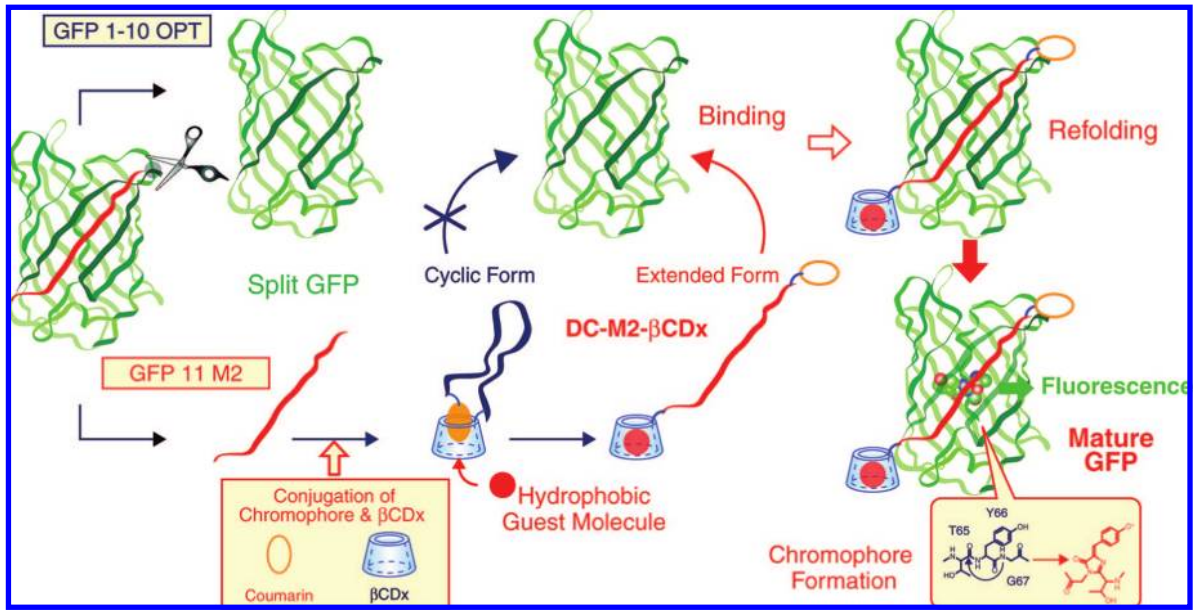

Figure 1. Schematic representation of a split-GFP that reassembles in response to the addition of exogenous guest molecules.

and FRB (FKBP-rapamycin binding domain), creating rapamycin-dependent intein splicing.[7] More recently, Liu and co-workers inserted a ligand-binding domain of an estrogen receptor into an intein and successfully evolved the combined protein in vivo to render small-molecule-dependent functions.[8] In most cases, however, the ligand-responsive domains are limited to those derived or evolved from natural proteins. The introduction of synthetic supramolecular units may result in an increase in the versatility of small-molecule-dependent proteins.[3,12] In this study, we attempted to use the interaction between a CDx host and guest molecules to regulate the reassembly and consequent functional recovery of a fragmented green fluorescent protein (split GFP) (Figure 1). Reconstitution of split GFP has been utilized for analysis of protein−protein interactions[13,14] and direct detection of specific DNA or RNA sequences by ternary complexation.[15–17] To our knowledge, however, control of split-GFP reconstitution by incorporation of synthetic supramolecular elements has not yet been reported. To generate a split GFP that reassembles in response to the addition of exogenous guest molecules, we chose coumarin and β-cyclodextrin (βCDx) as supramolecular elements.[18,19] We envisioned that appending a βCDx host and a coumarin ligand to one of the split-GFP fragments should inhibit protein reassembly due to the formation of an intramolecular inclusion complex. It was expected that the intramolecular host−guest interaction would be terminated by the addition of suitable hydrophobic guest molecules for βCDx, facilitating self-association of the GFP fragments. In this work, we designed and prepared a split GFP in which βCDx and coumarin units were conjugated to the C-terminal fragment of the dissected protein and studied the modulation mechanism of guest-dependent reassembly by means of UV−vis spectroscopy and circular dichroism (CD) and fluorescence measurement. The combined system of fragmented proteins and synthetic supramolecular elements will be a useful and flexible strategy for regulation of protein structure and function.

Results and Discussion

Design and Preparation of Split GFP. In this study, we employed a split GFP developed by Waldo and co-workers as a fragmented protein due to its superior solubility and high expression level in *E. coli* (Figure 2A).[20] Most importantly, the GFP fragments associate spontaneously to form a mature protein, in contrast to other split-GFP systems, most of which require fused interacting domains, coexpression, or corefolding to produce folded and fluorescent GFP. A DNA sequence encoding a GFP N-terminal fragment (residues 2−214) (GFP 1−10 OPT) was prepared by PCR and cloned into pET22b(+) vector. To simplify the process of protein purification, we appended a 6 × His tag to the N-terminal end of GFP 1−10 OPT through a 13-amino-acid linker. The GFP 1−10 OPT protein was expressed in *E. coli* BL21(DE3)pLys at 25 °C under the control of a T7 promoter. The protein was purified by nickel affinity and size-exclusion chromatography.

Among the reported C-terminal fragment sequences of split GFP, we used a variant termed GFP 11 M2 (residues 214−230 of GFP, L221H, F223S, T225N), which showed the lowest complementation efficiency among the examined mutants.[20] We

(12) (a) Grinberg, A. V.; Hu, C.-D.; Kerppola, T. K. *Mol. Cell. Biol.* **2004**, *24*, 4294–4308. (b) Hu, C.-D.; Kerppola, T. K. *Nat. Biotechnol.* **2003**, *21*, 539–545.
(13) (a) Mizuno, T.; Murao, K.; Tanabe, Y.; Oda, M.; Tanaka, T. *J. Am. Chem. Soc.* **2007**, *129*, 11378–11383. (b) Yuzawa, S.; Mizuno, T.; Tanaka, T. *Chem.—Eur. J.* **2006**, *12*, 7345–7352.
(14) (a) Wilson, C. G. M.; Magliery, T. J.; Regan, L. *Nat. Methods* **2004**, *1*, 255–262. (b) Magliery, T. J.; Wilson, C. G. M.; Pan, W.; Mishler, D.; Ghosh, I.; Hamilton, A. D.; Regan, L. *J. Am. Chem. Soc.* **2005**, *127*, 146–157. (c) Ghosh, I.; Hamilton, A. D.; Regan, L. *J. Am. Chem. Soc.* **2000**, *122*, 5658–5659.
(15) (a) Stains, C. I.; Furman, J. L.; Segal, D. J.; Ghosh, I. *J. Am. Chem. Soc.* **2006**, *128*, 9761–9765. (b) Stains, C. I.; Porter, J. R.; Ooi, A. T.; Segal, D. J.; Ghosh, I. *J. Am. Chem. Soc.* **2005**, *127*, 10782–10783.
(16) Ozawa, T.; Natori, Y.; Sato, M.; Umezawa, Y. *Nat. Methods* **2007**, *4*, 413–419.
(17) Valencia-Burton, M.; McCullough, R. M.; Cantor, C. R.; Broude, N. E. *Nat. Methods* **2007**, *4*, 421–427.
(18) Takakusa, H.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *Anal. Chem.* **2001**, *73*, 939–942.
(19) (a) Yana, D.; Takahashi, T.; Mihara, H.; Ueno, A. *Macromol. Rapid Commun.* **2004**, *25*, 577–581. (b) Hossain, M. A.; Mihara, H.; Ueno, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4305–4308.
(20) (a) Cabantous, S.; Waldo, G. S. *Nat. Methods* **2006**, *3*, 845–854. (b) Cabantous, S.; Terwillinger, T. C.; Waldo, G. S. *Nat. Biotechnol.* **2005**, *23*, 102–107.

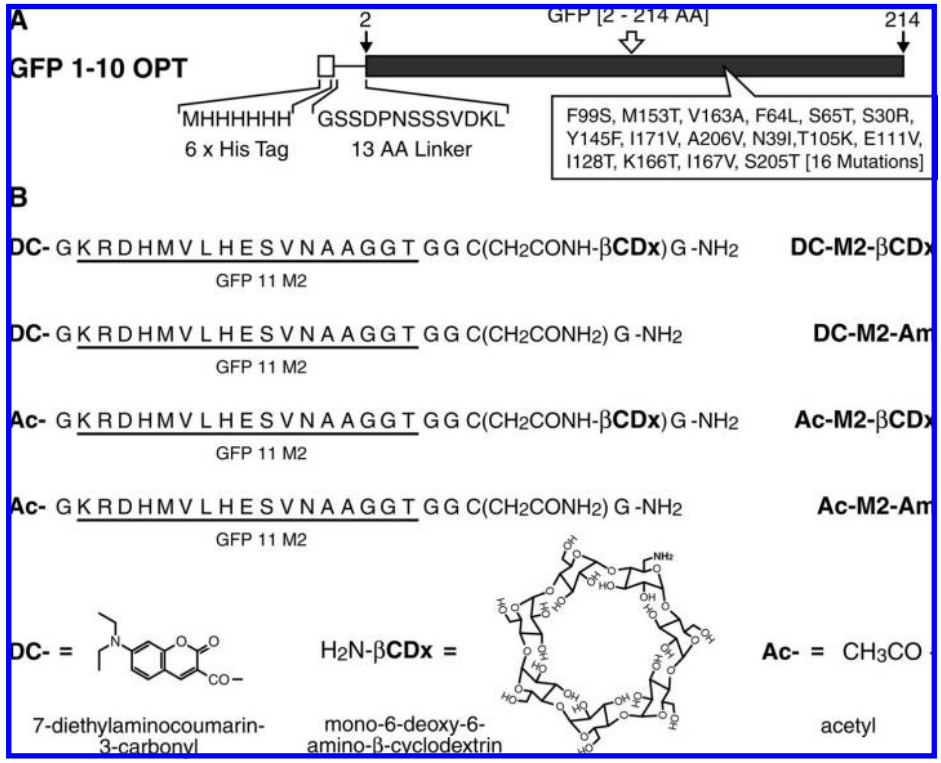

Figure 2. (A) Structure of GFP 1−10 OPT. (B) Structure of designed peptides, DC-M2-βCDx, DC-M2-Am, Ac-M2-βCDx, and Ac-M2-Am.

assumed that its relatively weak interaction with GFP 1−10 OPT would minimize background fluorescence coming from guest-independent reassembly. A fluorescent chromophore, 7-diethylaminocoumarin-3-carboxylate, was conjugated to the N-terminal end of GFP 11 M2 with a Gly residue as a spacer, and mono-6-deoxy-6-bromoacetylamino-β-cyclodextrin was coupled to the side chain of a Cys residue placed at the C-terminal end of the sequence (Figure 2B). The resulting designed peptide, DC-M2-βCDx, contains both host and guest moieties in the same peptide chain. Preliminary experiments revealed that 7-diethylaminocoumarin-3-carboxylic acid bound to βCDx [seven D-(+)-glucopyranose units] in an intermolecular fashion with an affinity constant of $5300 \pm 420\ M^{-1}$, but not to α-cyclodextrin (αCDx) (six units) or γ-cyclodextrin (γCDx) (eight units). Based on the size of the CDx cavity, βCDx should be able to accommodate one coumarin moiety. In addition to the peptide DC-M2-βCDx, we also designed a series of control peptides, DC-M2-Am, Ac-M2-βCDx, and Ac-M2-Am. In DC-M2-Am and Ac-M2-βCDx, the coumarin and βCDx moieties were substituted with acetyl and 2-acetamide groups, respectively, and Ac-M2-Am contained neither a host nor a guest moiety in its sequence. The peptides were prepared by solid-phase peptide synthesis and purified using reversed-phase HPLC (RP-HPLC). All peptides were identified by MALDI-TOF-MS and amino acid analysis.

UV−vis Study of DC-M2-βCDx. The peptides DC-M2-βCDx and DC-M2-Am showed absorption maxima at 424 and 432 nm, respectively, indicating a difference in microenvironmental polarity around the coumarin moiety (Figure 3A). When 1-adamantanol (1-AdOH), which is known to be a typical guest molecule for βCDx, was added in increasing concentrations, the peak wavelength of DC-M2-βCDx shifted gradually to 430 nm with an isosbestic point at 425 nm (Figure 3B). Since the control peptide DC-M2-Am did not show such a peak shift in the presence of guest molecules, it was suggested that the spectral change was due to the interaction between 1-AdOH and the βCDx moiety of DC-M2-βCDx. These results indicate that the N-terminal coumarin group of DC-M2-βCDx forms an intramolecular inclusion complex with the C-terminal βCDx moiety in the absence of competing guest molecules. Intermolecular interaction between coumarin and βCDx in different peptide molecules (i.e., peptide oligomerization or aggregation) was unlikely because the peptide showed a linear relationship between fluorescence intensity and peptide concentration in the range 0.5−25 μM. In addition, DC-M2-βCDx gave a sharp single peak in size-exclusion chromatography, although the apparent MW estimated from the elution volume was slightly greater (1.7 times) than the theoretical value (Figure S1). Since CD measurements (described below) showed that the peptide had a relatively extended conformation, it is reasonable to conclude that DC-M2-βCDx was in monomeric form and the interaction between βCDx and coumarin occurred in an intramolecular fashion under the experimental conditions.

CD Measurements of C-Terminal Peptides. Further insight into the self-inclusion of the coumarin moiety into the βCDx cavity was provided by induced circular dichroism (ICD) spectra in the absorption region of the coumarin moiety. In the absence of guest molecules, DC-M2-βCDx and DC-M2-Am showed different ICD patterns (Figure 3C): DC-M2-βCDx showed a positive ICD band at around 425 nm, whereas DC-M2-Am had a spectrum of opposite sign. The positive ICD signal of DC-M2-βCDx was consistent with axial complexation between coumarin and βCDx, that is, with the long axis of coumarin parallel to the βCDx axis.[18,19b,21] A molecular modeling study using the Insight II program also confirmed that coumarin could be fitted into the βCDx cavity. The negative signal of DC-M2-Am may arise from dipole−dipole coupling of a coumarin transition with $\pi-\pi^*$ and $n-\pi^*$ transitions localized in the

(21) Harata, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375–378.

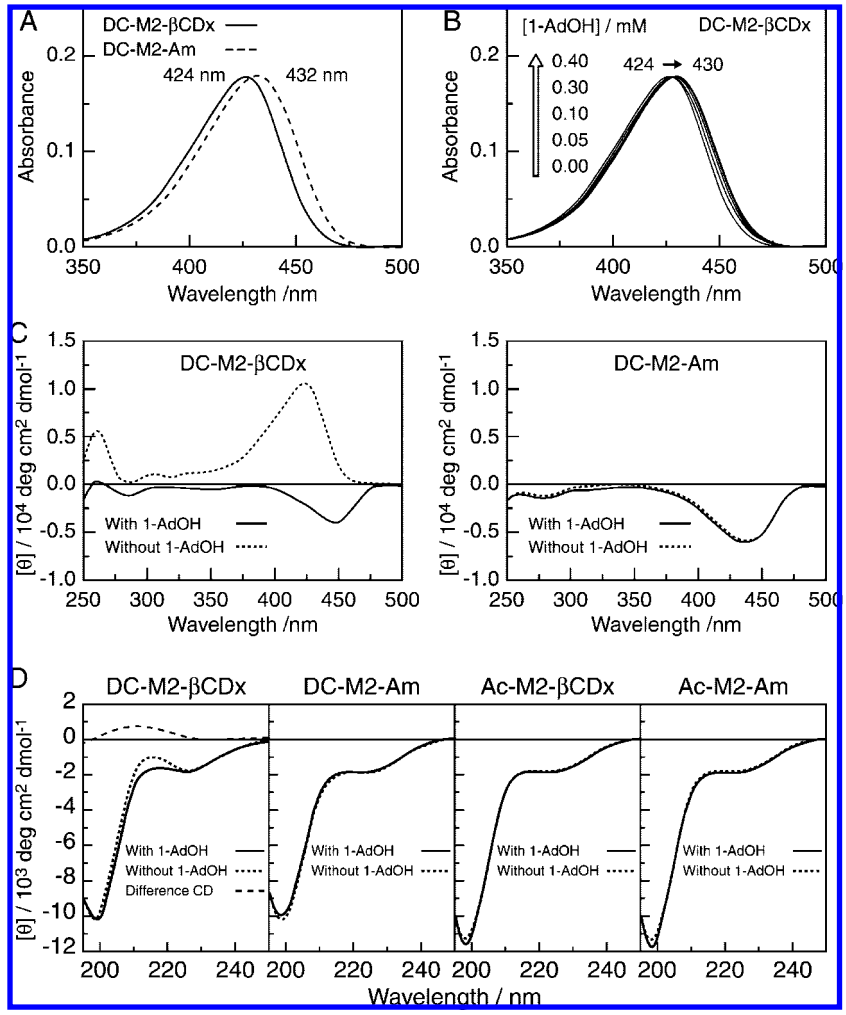

Figure 3. (A) Absorption spectra of DC-M2-βCDx and DC-M2-Am in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT at 25 °C. (B) Absorption spectra of DC-M2-βCDx with increasing 1-AdOH concentration in a buffer (pH 7.4) at 25 °C. [peptide] = 5 μM. (C) CD spectra at the coumarin absorption region of DC-M2-βCDx and DC-M2-Am in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM DTT, and 1% acetonitrile (MeCN) in the presence and absence of 1-AdOH (1 mM) at 25 °C. (D) CD spectra at the amide region of peptides in a buffer in the presence and absence of 1-AdOH (1 mM) at 25 °C. [peptide] = 25 μM.

peptide backbone. Upon addition of 1-AdOH, the ICD spectrum of DC-M2-βCDx changed to a pattern similar to that of DC-M2-Am, with a negative band near 445 nm. This observation also supports the contention that coumarin and βCDx form an intramolecular inclusion complex in DC-M2-βCDx, and the self-inclusion complex collapses upon addition of excess 1-AdOH.

Far-UV CD studies provided information about the secondary structure of the peptides. All of the peptides exhibited CD spectra characteristic of a random-coil dominant conformation (Figure 3D). In the absence of 1-AdOH, however, the CD pattern of DC-M2-βCDx was slightly different from those of the control peptides. DC-M2-βCDx showed a larger mean residue ellipticity than the other peptides near 215 nm despite the fact that all of the peptides possessed the same amino acid sequence. In the case of DC-M2-βCDx, addition of 1-AdOH caused the spectrum to change to a pattern resembling those of the other peptides. The difference CD spectrum, obtained by subtraction of the spectrum of DC-M2-βCDx in the presence of 1-AdOH from the spectrum of DC-M2-βCDx alone, resembles that observed for type II β-turns, with a positive band at approximately 210 nm (Figure 3D).[22] Based on these results, it is most likely that DC-M2-βCDx exists in a circular form due to intramolecular host−guest interaction and that part of the polypeptide chain adopts a β-turn structure.

Fluorescence Study of DC-M2-βCDx. To further examine the interaction between DC-M2-βCDx and 1-AdOH, fluorescence titration experiments were performed in a buffer. When the concentration of 1-AdOH was increased, the emission maximum decreased in intensity and was red-shifted from 465 to 471 nm (Figure S2). This variation in fluorescence indicates that the coumarin moiety of DC-M2-βCDx is excluded from the cavity of βCDx into the aqueous environment for the guest binding.[18] The affinity constant, determined from the change in fluorescence intensity at 465 nm using a single-site binding equation, was 5340 ± 290 M^{-1}. For DC-M2-Am, in contrast, addition of 1-AdOH caused no change in the spectrum, further confirming the existence of intramolecular inclusion complexation between βCDx and the coumarin moiety in DC-M2-βCDx. The affinity constants for DC-M2-βCDx and various guest molecules were also determined and are shown in Figure 4. The affinity constants for (+)-menthol and (−)-menthol were smaller than that for 1-AdOH. Among steroids, DC-M2-βCDx was found to bind strongly to ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA), while showing no affinity for

(22) Brahms, S.; Brahms, J. *J. Mol. Biol.* **1980**, *138*, 149–178.

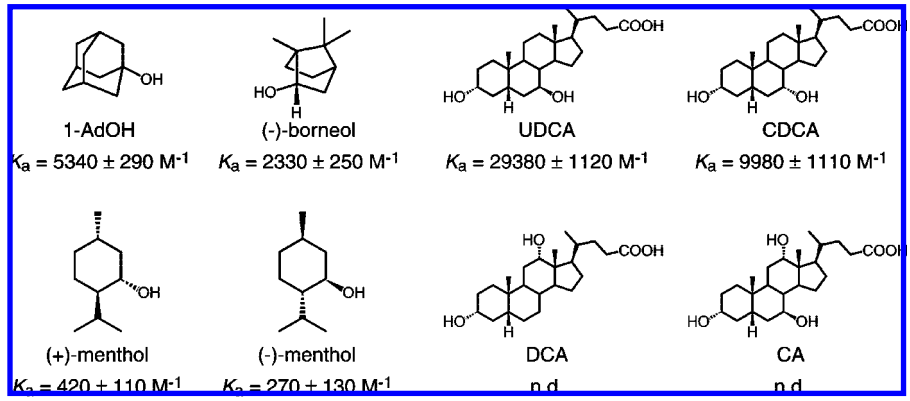

Figure 4. Structures of guest compounds used in this study. The affinity constant (K_a) of DC-M2-βCDx for each guest molecule in a buffer (pH 7.4) is shown below each structure. Not determined (n.d.). The values for DCA and CA could not be determined because of the small changes in the spectra.

deoxycholic acid (DCA) and cholic acid (CA) under the experimental conditions. Because these guest-binding properties are in common with chromophore-conjugated βCDx derivatives,[19,23] it was concluded that the βCDx moiety in the peptide acts as a binding site without losing guest selectivity.

Reconstitution of GFP 1−10 OPT and DC-M2-βCDx in the Presence and Absence of Guest Molecules. To investigate whether the addition of guest molecules affected GFP reassembly, we measured fluorescence spectra after mixing the N- and C-terminal protein fragments. In the presence of 1-AdOH, the fluorescence intensity at 510 nm (mature GFP) increased readily, indicating that DC-M2-βCDx and GFP 1−10 OPT reassembled and then autocatalyzed chromophore formation (Figure 5A and S3).[20] In the absence of 1-AdOH, DC-M2-βCDx and GFP 1−10 OPT showed less effective fluorescence recovery. Figure 5B shows the fluorescence intensities of mixtures with and without 1-AdOH (1.0 mM) after 3 h of incubation at 25 °C. The fluorescence intensity of the GFP 1−10 OPT/DC-M2-βCDx mixture in the presence of 1-AdOH was ca. 3 times greater than that in the absence of 1-AdOH. In the case of the control peptides, the addition of 1-AdOH had no effect on protein reassembly (Figure 5B). Moreover, the reconstitution of GFP 1−10 OPT and DC-M2-βCDx was clearly dependent on the concentration of exogenous 1-AdOH (Figure 6 and S4). Although the reconstitution reaction of split GFP is composed of many steps, including the association of the two fragments, refolding of polypeptide chains, intramolecular annulation of -T65-Y66-G67-, and finally oxidative chromophore formation, the dependence of the change in fluorescence intensity on 1-AdOH concentration was well fitted with a single-site binding equation, yielding an apparent affinity constant of 5790 ± 300 M^{-1}. This value was quite consistent with the affinity constant for DC-M2-βCDx and 1-AdOH determined by the direct fluorescence titration experiment, implying that guest binding in the βCDx cavity of the DC-M2-βCDx peptide is an essential step in the reconstitution reaction.

The efficiency of reconstitution in the presence of various guest molecules was also evaluated based on the fluorescence intensity of GFP 1−10 OPT/DC-M2-βCDx mixtures at a guest concentration of 100 μM (incubation for 3 h at 25 °C) (Figure 7). The order of the observed fluorescence intensities was UDCA > CDCA > 1-AdOH > (−)-borneol > (+)-menthol, (−)-menthol, DCA, CA, which is in agreement with the order of the affinity constants; that is, a guest molecule with a higher affinity for βCDx promotes GFP reconstitution more effectively. On the basis of these observations, it is reasonable to conclude that the intramolecular host−guest interaction in DC-M2-βCDx prevents the reassembly of the C-terminal fragment of split GFP with the N-terminal fragment in the absence of guest molecules and that the addition of suitable guest molecules to DC-M2-βCDx results in a collapse of the intramolecular complex,

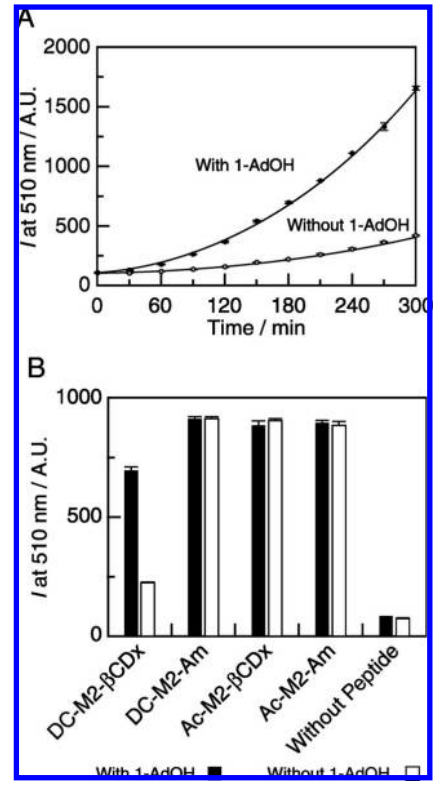

Figure 5. (A) Time courses of fluorescence recovery of the GFP 1−10 OPT/DC-M2-βCDx mixture in the presence and absence of 1-AdOH at 25 °C. *I* denotes fluorescent intensity at the indicated time point. (B) Fluorescence emission intensity (*I*) at 510 nm of the GFP 1−10 OPT/peptide mixture after 3 h incubation at 25 °C in the presence and absence of 1-AdOH. The reconstitution reaction was performed in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 10 mM DTT, and 1% dimethyl sulfoxide (DMSO) at 25 °C (final concentrations; [GFP 1−10 OPT] = 1 μM, [DC-M2-βCDx] = 1 μM, and [1-AdOH] = 1 mM, respectively). The sample (25 μL) was removed from the reaction solution at each time point and diluted into a buffer (225 μL), and the fluorescence spectrum was measured at 25 °C (λ_{ex} = 485 nm). Arbitrary fluorescence units (A.U.).

(23) Matsumura, S.; Sakamoto, S.; Ueno, A.; Mihara, H. *Chem.—Eur. J.* **2000**, *6*, 1781–1788.

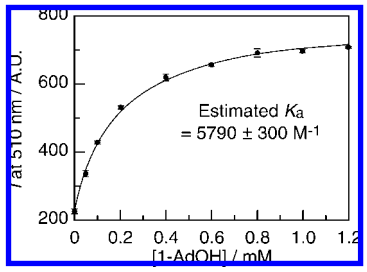

Figure 6. Plot of fluorescence emission intensity (I) at 510 nm of GFP 1−10 OPT/DC-M2-βCDx after 3 h incubation at 25 °C as a function of 1-AdOH concentration. The reconstitution reaction and the fluorescence measurement were performed as in Figure 5 except for the concentration of 1-AdOH. Arbitrary fluorescence units (A.U.).

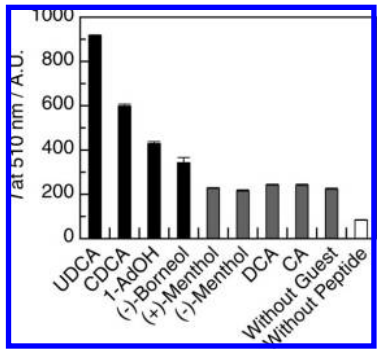

Figure 7. Fluorescence emission intensities (I) at 510 nm of the GFP 1−10 OPT/DC-M2-βCDx mixture after 3 h incubation at 25 °C in the presence of a variety of guest molecules. The reconstitution reaction and the fluorescence measurement were performed as in the case for Figure 5. [Guest] = 100 μM. Arbitrary fluorescence units (A.U.).

leading to more effective protein reassembly and consequent refolding of GFP.

Reconstitution of GFP 1−10 OPT and DC-M2-βCDx in the Presence of CDx's. Based on the hypothesis that the addition of exogenous βCDx would disrupt the intramolecular inclusion complex by capturing the coumarin moiety of DC-M2-βCDx, reconstitution reactions of split GFP in the presence of CDxs were carried out. The addition of βCDx to a solution of GFP 1−10 OPT and DC-M2-βCDx improved reconstitution in a concentration-dependent manner (Figure 8A). Neither αCDx nor γCDx was effective in promoting the reconstitution of GFP 1−10 OPT and DC-M2-βCDx, which suggests that exogenous βCDx specifically interacts with the coumarin moiety of the peptide to disturb the intramolecular host−guest interaction. However, the addition of βCDx appeared to be less effective than the addition of 1-AdOH or UDCA. The fluorescence intensity of the GFP 1−10 OPT/DC-M2-βCDx mixture in the presence of βCDx (10 mM) was only ca. 2 times greater than that in the absence of βCDx (incubation for 3 h at 25 °C). Moreover, the apparent affinity constant, estimated from the observed fluorescence intensities of the reaction mixtures containing various concentrations of βCDx, was 220 ± 40 M^{-1}, which was nearly 25 times less than that for 1-AdOH (5790 ± 300 M^{-1}). This may be due to unfavorable steric interactions between GFP 1−10 OPT and βCDx bound to the coumarin moiety of DC-M2-βCDx. Molecular modeling analysis of mature GFP constituted from GFP 1−10 OPT and DC-M2-βCDx suggested that the coumarin unit was located too close to the protein surface to allow the formation of an intermolecular complex between coumarin and exogenous βCDx. As other evidence supporting the existence of steric hindrance, reconstitution of the control peptide DC-M2-Am was clearly suppressed by the addition of competing βCDx (Figure S5). The influence of the interaction between the added βCDx and the hydrophobic amino acid side chains of the protein fragments seemed to be small, because the addition of βCDx had no effect on the reconstitution of Ac-M2-βCDx or Ac-M2-Am, which lacks a coumarin moiety (Figure S5). Since a single Gly residue was used as a linker to connect the coumarin moiety with the GFP 11 peptide sequence, optimization of the length of the linker will enable more effective control of reassembly through intermolecular interaction with exogenous βCDx.

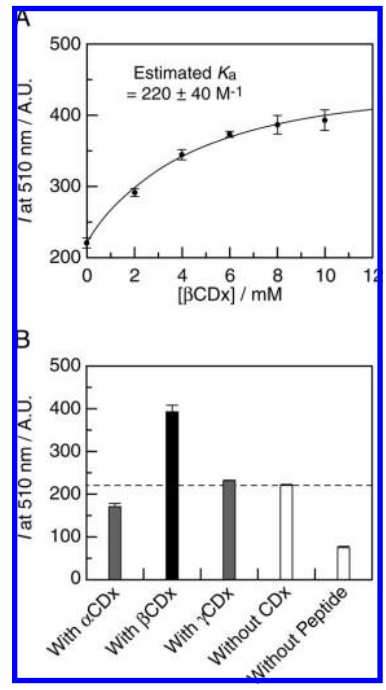

Figure 8. (A) Plot of fluorescence emission intensity (I) at 510 nm of GFP 1−10 OPT/DC-M2-βCDx after 3 h incubation at 25 °C as a function of βCDx concentration. (B) Fluorescence emission intensities at 510 nm of GFP 1−10 OPT/DC-M2-βCDx after incubation at 25 °C for 3 h in the presence of CDxs. The reconstitution reaction and the fluorescence measurement were performed as in the case for Figure 5. [CDx] = 10 mM. Arbitrary fluorescence units (A.U.).

Characterization of Reconstituted GFP. To obtain mature GFP composed of GFP 1−10 OPT and DC-M2-βCDx, the N- and C-terminal fragments were coincubated with 1-AdOH (1.0 mM) at 4 °C for 2 weeks, and the reconstituted GFP 1−10 OPT/DC-M2-βCDx complex was purified using a Superdex 75HR size-exclusion column. Size exclusion chromatography revealed that most of the GFP 1−10 OPT protein existed as a dimer, as reported by Waldo and co-workers.[20] When the reaction mixture was subjected to chromatography, however, the peak due to GFP 1−10 OPT decreased and a new peak appeared at a smaller molecular weight (Figure 9). The UV−vis spectrum of the new component, measured by a photodiode array system, exhibited two absorption bands at 430 and 490 nm, which were assigned to coumarin and the cyclized GFP chromophore, respectively. The ratio of the absorbance at 430 nm to that at 490 nm was 1.29, which is almost the same as the ratio of the absorption coefficients of coumarin and the GFP chromophore (1.17). Additionally, the elution volume of the GFP 1−10 OPT/DC-M2-βCDx complex corresponded to an apparent molecular weight of 25 100, which demonstrated that GFP 1−10 OPT and DC-M2-βCDx assemble to form the mature GFP with 1:1 stoichiometry (theoretical MW = 29 700).

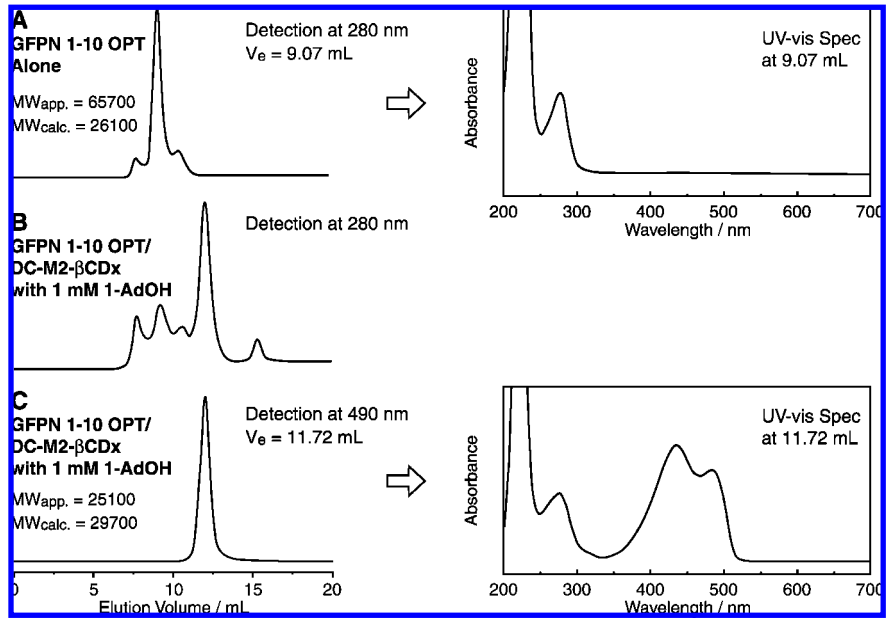

Figure 9. Size-exclusion chromatograms of GFP 1−10 OPT and the GFP 1−10 OPT/DC-M2-βCDx mixture. (A) GFP 1−10 OPT alone; (B) GFP 1−10 OPT/DC-M2-βCDx mixture after 2 weeks of incubation at 4 °C, detection at 280 nm; (C) GFP 1−10 OPT/DC-M2-βCDx mixture after 2 weeks incubation at 4 °C, detection at 490 nm. Column, Superdex 75HR 10/30 (1 cm × 30 cm); Eluent, 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT (pH 7.4); Flow rate, 0.4 mL min^{-1}. The absorption spectra of the main peaks were measured using a Shimadzu SPD10AVP photodiode array UV−vis detector.

The CD spectrum of the isolated GFP 1−10 OPT/DC-M2-βCDx complex, as well as full-length GFP (residues 1−238), revealed signals typical of β-sheet rich proteins, with a negative maximum near 215 nm and a positive maximum near 195 nm (Figure 10A). There were small differences in the intensities of the CD signals of the full-length and reconstituted proteins, which indicated that the GFP 1−10 OPT/DC-M2-βCDx complex adopts a β-barrel conformation without large structural perturbations. In contrast, a mixture of GFP 1−10 OPT and DC-M2-βCDx near time zero exhibited a CD spectrum that differed significantly from that observed for the isolated GFP 1−10 OPT/DC-M2-βCDx complex. The difference CD spectrum for the two states showed a pattern indicating a random-coil structure with a large negative band at around 200 nm, which suggested that GFP 1−10 OPT contains partially disordered regions which are converted to β-sheets in the mature protein. In addition to these results, the fluorescence emission spectrum of the reconstituted complex was indistinguishable from that of full-length GFP when the proteins were excited at 485 nm (Figure 10B). This result confirmed that the reconstituted protein possesses a β-barrel structure which is not significantly altered by the incorporation of βCDx and coumarin moieties.

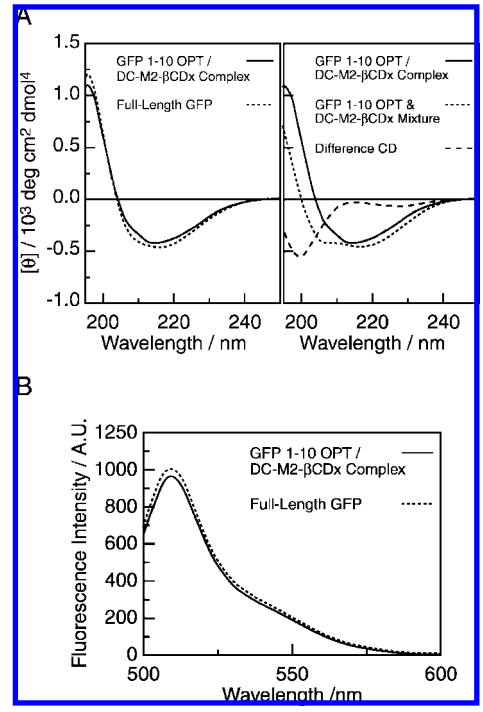

Figure 10. (A) CD spectra at the amide region of GFP 1−10 OPT and the GFP 1−10 OPT/DC-M2-βCDx mixture in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT at 25 °C. [Protein] = 2.5 μM. (B) Fluorescence emission spectra of full-length GFP and the GFP 1−10 OPT/DC-M2-βCDx mixture in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT at 25 °C. [Protein] = 0.5 μM. λ_{ex} = 485 nm. Arbitrary fluorescence units (A.U.).

Conclusion

In summary, we successfully constructed a fragmented GFP system responsive to exogenous hydrophobic molecules, by incorporating synthetic supramolecular elements—a βCDx host and a coumarin ligand—into the C-terminal fragment of split GFP. Detailed characterization of the designed C-terminal peptide demonstrated that the βCDx and coumarin moieties form an intramolecular inclusion complex which prevents self-association of the N- and C-terminal fragments in the absence of competing guests. However, as expected, the addition of suitable guest molecules as external stimuli allowed the reassembly and consequent refolding of GFP by disturbing the intramolecular host−guest interaction in a dose-dependent manner. The reconstitution efficiency of the split GFP was determined based on the affinity constant between the added guest molecule and βCDx; that is, guest molecules with a higher affinity for βCDx are capable of promoting GFP reconstitution more effectively. Thus, this system enables the structure and

function of GFP to be controlled by guest inputs chosen by the researchers. It was also revealed that the reconstituted protein has characteristics similar to those of full-length GFP. This is expected to be quite useful in the development of semiartificial GFP containing non-natural functionalities on its surface, because a variety of non-natural amino acids can be introduced site-selectively by means of the protein reconstitution technique using chemically modified C-terminal fragments.[24]

Possible applications of this strategy include the development of new molecular-sensing devices or biosensors for in vivo and in vitro use. Although the sensitivity may be insufficient, mainly due to the background signal originating from guest-independent reassembly, it may be improved by systematic substitution of an internal ligand and/or amino acid side chains in the split GFP. Furthermore, this strategy can in principle be applied to other fragmented proteins, such as luciferase,[11,25] β-galactosidase,[26] β-lactamase,[27,28] dihydrofolate reductase (DHFR),[29–31] pleckstrin homology (PH) domain,[32] ribonuclease,[33] and ubiquitin.[10,34] In particular, the combination with split enzymes will enable us to gain a further amplified signal by substrate turnover. In addition, since the interaction between the βCDx host and an internal ligand can be replaced with other natural or non-natural interactions such as antibody−antigen, receptor−ligand, DNA−DNA, DNA−PNA, or PNA−PNA, it may be possible to extend this strategy to more general and practical applications. We believe that this new system utilizing synthetic supramolecular elements is a potentially useful method not only for regulation of protein structure and function but also for direct detection of various biological substances.

Experimental Section

General Materials. All chemicals and solvents were of reagent or HPLC grade. All materials were obtained from Sigma-Aldrich unless otherwise noted. Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs. Blend Taq-Plus-DNA polymerase and dNTPs were obtained from Toyobo (Tokyo, Japan). Isopropyl-β-D-(−)-thiogalactopyranoside (IPTG) and DL-dithiothreitol (DTT) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Guest molecules were obtained from Tokyo Chemical Industry (Tokyo, Japan) and used without further purification. Mono-6-deoxy-6-bromoacetylamino-β-cyclodextrin (BrAcNH-βCDx) was synthesized according to a literature procedure.[19b] Amino acid derivatives and reagents for peptide synthesis were purchased from Novabiochem or Watanabe Chemical Co. (Hiroshima, Japan). All peptides were synthesized manually according to a standard solid-phase method by using an Fmoc-strategy (Fmoc, 9-fluorenylmethoxycarbonyl).[35] The peptides were purified and analyzed with RP-HPLC on a Cosmosil 5C18-AR-II column (ϕ 10 mm × 250 mm) (Nacarai Tesque, Kyoto, Japan) and a Wakosil 5C18 analytical column (ϕ 4.6 mm × 150 mm) (Wako Pure Chemical Industries), respectively, using a linear gradient of acetonitrile (MeCN)−0.1% TFA (1.0% min^{-1}) by employing a Shimadzu LC-10ACvp pump equipped with a Shimadzu-SPD10A UV−vis detector. MALDI-TOF-MS measurements were made on a Bruker BIFLEX III mass spectrometer by using 3,5-dimethoxy-4-hydroxycinnamic acid as a matrix. Amino acid analysis was carried out after hydrolysis in 6.0 M HCl at 110 °C for 24 h in a sealed tube, and the subsequent labeling, with phenyl isothiocyanate. Each peptide concentration was determined by quantitative amino acid analysis using Phe as an internal standard.

Peptide Synthesis. The peptides were synthesized by the stepwise elongation of Fmoc-amino acids on Novasyn TGR resin (Novabiochem) according to a reported procedure with Fmoc-AA-OH [Fmoc-Ala-OH·H_2O, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(OBu^t)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Glu(OBu^t)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys-(Boc)-OH, Fmoc-Met-OH, Fmoc-Ser(Bu^t)-OH, Fmoc-Thr(Bu^t)-OH, Fmoc-Val-OH; Pbf, 2,2,4,6-pentamethyldihydrobenzofurane-5-sulfonyl; Trt, trityl; Bu^t, *tert*-butyl; Boc, *tert*-butoxycarbonyl] using 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxy-benzotriazole hydrate (HOBt·H_2O) and diisopropylethylamine (DIEA) as coupling reagents. Each coupling reaction was checked by Kaiser test.[36] For the acetylation of an N-terminus amino group, the Fmoc-deprotected peptide resin was treated with acetic anhydride (10 equiv) and DIEA (5 equiv) in 1-methyl-2-pyrrolidone (NMP) for 20 min. To introduce the coumarin unit, the Fmoc-deprotected peptide resin was reacted with 7-diethylaminocoumarin-3-carboxylic acid (3 equiv), HBTU (3 equiv), HOBt·H_2O (3 equiv), and DIEA (6 equiv) in NMP for 1 h. The protecting groups and the resin were removed by stirring the dried resin for 2 h at room temperature with TFA/*m*-cresol/ethanedithiol/thioanisole (85/6/6/2). The solvent was evaporated, and the residue was solidified with diethyl ether in an ice bath. Crude peptides were purified with RP-HPLC and identified by MALDI-TOF-MS.

The βCDx unit and −2-acetamide group in the peptides were covalently attached on the side chain of Cys by reacting with BrAcNH-βCDx (10 equiv) and 2-iodoacetamide (20 equiv), respectively, in 0.1 M Tris HCl buffer (pH 8.5) at room temperature for 1 h. Peptides were purified with RP-HPLC and identified by MALDI-TOF-MS and amino acid analysis. Total yield; DC-M2-βCDx 25%; DC-M2-Am 18%; Ac-M2-βCDx 22%; Ac-M2-Am 25%; MALDI-TOF-MS; DC-M2-βCDx, *m/z* 3569.5 [$(M + H)^+$] (Calcd = 3570.6); DC-M2-Am, *m/z* 2453.2 [$(M + H)^+$] (Calcd = 2453.7); Ac-M2-βCDx, *m/z* 3368.0 [$(M + H)^+$] (Calcd = 3369.4); Ac-M2-Am, *m/z* 2253.0 [$(M + H)^+$] (Calcd = 2552.5); amino acid analysis of peptides provided satisfactory results.

Cloning and Expression of Proteins. A plasmid encoding the full-length GFP (residue 1−238) containing 16 mutations was constructed with PCR by using a pET24a-GFP_{FR} as a template, which includes the GFP folding-reporter gene (F64L, S65T, F99S, M153T, and V163A).[37,38] Additional mutations (S30R, Y39I,

(24) (a) Hiraoka, T.; Hamachi, I. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 13–15. (b) Hamachi, I.; Eboshi, R.; Watanabe, J.-i.; Shinkai, S. *J. Am. Chem. Soc.* **2000**, *122*, 4530–4531. (c) Hamachi, I.; Yamada, Y.; Matsugi, T.; Shinkai, S. *Chem.—Eur. J.* **1999**, *5*, 1503–1511.

(25) (a) Paulmurugan, R.; Gambhir, S. S. *Anal. Chem.* **2003**, *75*, 1584–1589. (b) Paulmurugan, R.; Umezawa, Y.; Gambhir, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15608–15613.

(26) Rossi, F.; Charlton, C. A.; Blau, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8405–8410.

(27) Galarneau, A.; Primeau, M.; Trudeau, L.-E.; Michnick, S. W. *Nat. Biotechnol.* **2002**, *20*, 619–622.

(28) (a) Porter, J. R.; Stains, C. I.; Segal, D. J.; Ghosh, I. *Anal. Chem.* **2007**, *79*, 6702–6708. (b) Ooi, A. T.; Stains, C. I.; Ghosh, I.; Segal, D. J. *Biochemistry* **2006**, *45*, 3620–3625.

(29) (a) Arndt, K. M.; Pelletier, J. N.; Müller, K. M.; Alber, T.; Michnick, S. W.; Plückthun, A. *J. Mol. Biol.* **2000**, *295*, 627–639. (b) Pelletier, J. N.; Arndt, K. M.; Plückthun, A.; Michnick, S. W. *Nat. Biotechnol.* **1999**, *17*, 683–690.

(30) Pelletier, J. N.; Campbell-Valois, F.-X.; Michnick, S. W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12141–12146.

(31) Phillips, K. J.; Rosenbaum, D. M.; Liu, D. R. *J. Am. Chem. Soc.* **2006**, *128*, 11298–11306.

(32) Sugimoto, K.; Mori, Y.; Makino, K.; Ohkubo, K.; Morii, T. *J. Am. Chem. Soc.* **2003**, *125*, 5000–5004.

(33) Richards, F. M.; Vithayathil, P. J. *J. Biol. Chem.* **1959**, *234*, 1459–1465.

(34) Johnsson, N.; Varshavsky, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10340–10344.

(35) Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis: A Practical Approach*; IRL Press: Oxford, 1989.

(36) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595–598.

(37) Waldo, G. S.; Standish, B. M.; Berendzen, J.; Terwilliger, T. C. *Nat. Biotechnol.* **1999**, *17*, 691–695.

(38) Takahashi, T.; Ohta, K.; Mihara, H. *ChemBioChem* **2007**, *8*, 985–988.

N105K, E111V, I128T, Y145F, K166T, I167V, I171V, S205T, and A206V) were introduced by overlap extension PCR by using appropriate primers. The PCR product was ligated into pET22b(+) vector (Novagen) via the NdeI/XhoI restriction sites to create pET22b-GFP(1−12)-6H. A DNA sequence coding GFP 1−10 OPT was prepared by PCR amplification from the pET22b-GFP(1−12)-6H as a template using primers, 5′-A GAT ATA AAG CTT AGT AAA GGA GAA GAA CTT TTC A-3′ and 5′-T CGA ATT CTC GAG TCA CTT TTC GTT GGG ATC TTT C-3′. The PCR product was ligated into pET22b(+) vector via the *Hin*dIII/XhoI restriction sites. The NdeI/*Bam*HI site of the obtained plasmid was replaced with the DNA fragment ATG CAC CAT CAT CAT CAC CAT GGC TCT TCG coding a 6 × His tag (M H H H H H H G S S), resulting in the construct pET22b-6H-GFP(1−10). Sequences of all constructs were verified by dye-terminator sequencing.

GFP 1−10 OPT and full-length GFP proteins were expressed in BL21(DE3)pLys at 25 °C under the control of the T7 promoter. The proteins were purified with Ni-NTA (Qiagen) and Sephadex G-25 (GE Healthcare) columns. The purity and MW were confirmed by SDS PAGE analysis. The concentrations of GFP 1−10 OPT and full-length GFP were determined by measuring the absorbance using $\varepsilon_{280} = 15\,720$ and $\varepsilon_{490} = 39\,200$, respectively.

Detailed information including the sequences of GFP 1−10 OPT and full-length GFP as well as purification procedures can be found in the Supporting Information.

Size-Exclusion Chromatography. A Superdex 75HR column (1.0 cm × 30 cm) (GE Healthcare) was used, and the chromatographic conditions were as follows: eluent, 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT (pH 7.4); flow rate 0.4 mL min^{-1}. The spectra of peaks were measured using a Shimadzu SPD10AVP photodiode array UV−vis detector. Following proteins were used as standards; bovine serum albumin (67 000), ovalbumin (43 000), chymotrypsinogen A (25 000), and ribonuclease A (13 700).

UV−vis Spectroscopy. UV−vis spectra were measured on a Shimadzu MaltiSpec-1500 spectrophotometer using a quartz cell with a 1.0 cm path length. Peptides were dissolved in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT at a peptide concentration of 5 μM.

CD Measurements. CD spectra were recorded on a JASCO J-725 spectropolarimeter using a quartz cell with a 1.0 mm path length at the amide region (195−250 nm) and 1.0 cm at the absorption region of coumarin (250−500 nm). Peptides were dissolved in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM DTT, and 1% MeCN at a peptide concentration of 25 μM. For the measurements of proteins, proteins were dissolved in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT at a protein concentration of 2.5 μM.

Determination of Affinity Constants for DC-M2-βCDx and Guest Molecules. Fluorescence emission spectra were measured on a Hitachi F-2500 fluorescence spectrophotometer with a 0.5 cm × 0.5 cm quartz cell. Each guest compound was dissolved in dimethyl sulfoxide (DMSO). A peptide in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT was titrated with each guest molecule (peptide concentration of 5 μM, guest stock concentration of 5 mM and 25 mM). After each addition of guest, fluorescence emission spectra (450−550 nm) excited at 425 nm were measured at 25 °C. The decrease of fluorescence intensity at the maximum emission wavelength (465 nm) of the peptide alone with increasing guest concentration was corrected for dilution and fitted by a single-site binding equation (eq 1) using Kaleida Graph (Synergy Software) where P_0 and G_0 represent the initial concentration of peptide and guest, respectively.

$$\Delta I/I_0 = \{(\Delta I_{max}/I_0)/2D_0\}\left[(P_0 + G_0 + 1/K_a) - \{(P_0 + G_0 + 1/K_a)^2 - 4P_0G_0\}^{1/2}\right] \quad (1)$$

ΔI denotes the difference in the fluorescence intensity between the peptide in the absence (I_0) and presence of guest at each concentration (I). When all of the peptide forms the complex, ΔI is equal to ΔI_{max}.

Reconstitution Reaction between GFP 1−10 OPT and DC-M2-βCDx. Fluorescence emission spectra were measured on a Hitachi F-2500 fluorescence spectrophotometer with a 0.5 cm × 0.5 cm quartz cell. GFP 1−10 OPT was dissolved in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 10 mM DTT, and 1% DMSO with and without a guest molecule or CDx. The reconstitution reaction was started by the addition of peptide (final concentrations of protein and peptide were 1 μM). The sample (25 μL) was removed from the reaction solution at the indicated time point and immediately diluted into 225 μL of 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT, and the fluorescence emission spectrum was measured at 25 °C (excitation wavelength = 485 nm).

Preparation of the Reconstituted GFP 1−10 OPT/DC-M2-βCDx Complex. GFP 1−10 OPT (25 μM), DC-M2-βCDx (25 μM), and 1-AdOH (1 mM) were dissolved in 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 50 mM DTT, and 1% DMSO and incubated at 4 °C for 2 weeks. The reconstituted GFP 1−10 OPT/DC-M2-βCDx complex was isolated by a Superdex 75HR size-exclusion column (ϕ 1.0 cm × 30 cm) using 20 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl and 1 mM DTT as an eluent.

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Supporting Information Available: Figures S1−S9 and Table S1 showing additional experimental details; Details of cloning and protein expression. This material is available free of charge via the Internet at http://pubs.acs.org.

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