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Controlling Multiple Fluorescent Signal Output in Cyclic Peptide-Based Supramolecular Systems

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Abstract: A multicomponent equilibrium network based on self-assembling α,γ-cyclic peptides with controlled fluorescence output is described. The network takes advantage of the large association constant of α,γ-cyclic peptides and the controlled formation homo- and heterodimers, making use for the first time of excimer/FRET effects in conjunction for studying complex interaction networks. In addition, we study the Dapoxyl/pyrene FRET pair for the first time.

Introduction

One of the most fundamental and pressing problems in the field of supramolecular chemistry is the control of self-assembly processes through the design of the molecular components[1] and the practical application at the macromolecular level of such supramolecular associations.[2] Self-assembling peptide nanotubes (SPN) have been widely studied as supramolecular model systems and as tools in materials and biological sciences due to their ease of synthesis and modification.[3] In addition, fluorescence techniques have been extensively used in the study of self-assembly processes in macromolecular chemistry,[4] but their application to the study of SPN is still scarce. For example, Ghadiri et al. have recently reported that D,L-α-peptides bearing 1,4,5,8-naphthalenetetracarboxylic acid diimide (NDI) side chains show intermolecular NDI excimer formation upon self-assembly. However, their work was aimed at the development of molecular electronics applications and was not concerned with the detailed study of the equilibria involved or the control of the signal output.[5]

Design and Synthesis

In an approach to novel supramolecular systems, we have recently reported that high-affinity homo- and heterodimerization can be controlled in the context of α,γ-cyclic peptides (α,γ-CPs). This could be done by appropriate design of the peptide sequences involving (1*S*,3*R*)-3-aminocyclohexanecarboxylic acids (γ-**Ach**), (1*S*,3*R*)-3-aminocyclopentanecarboxylic acids (γ-**Acp**), and L-α-amino acids.[6,7] We also found that heterodimeric complexes of these cyclic peptides are more stable than the corresponding homodimers. In this report, we have applied this differential α,γ-CPs homo/heterodimer complex formation in the design of a novel multicomponent equilibrium network of fluorescently derivatized self-assembling α,γ-cyclic peptides that can be brought into different association states, with distinguishable fluorescent output signals, by controlling the concentration of the different α,γ-CPs.[8] This approach might be relevant in the development of molecular systems for information processing based on photoinduced electron or energy transfer.[9] In addition, we also report for the first time the use of pyrene and Dapoxyl as a fluorescence resonance energy transfer (FRET) pair that, due to the unique spectroscopic

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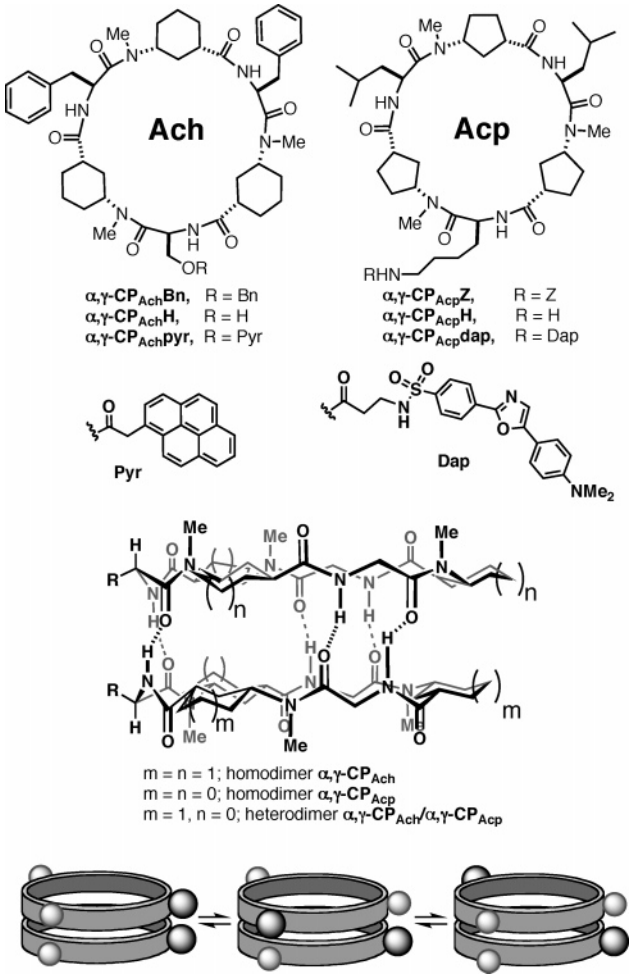
Scheme 1. Cyclopeptides Used in This Study[a]



[a] Ach and Acp peptide series and perspective representation of cyclopeptide dimers showing the hydrogen bonds between both cyclopeptides. Schematic representation of the three nonequivalent dimeric forms that are produced by successive shifts of register between both cyclopeptide rings. Light gray represents nonfluorescent side chains, while dark gray represents fluorescent side chains (either pyrene or Dapoxyl in Ach or Acp cyclic peptides, respectively).

properties of pyrene (i.e., excimer emission), is particularly suited for studying systems involving homo- and heterodimeric species in equilibrium, yielding distinctive fluorescent signals.[10] Although there are examples of pyrene in FRET pairs with other fluorophores such as coumarins or fluorescein,[11] to our knowledge, this is the first time that pyrene excimer formation and FRET processes are combined in the context of complex intermolecular association studies.

The network presented in this paper was formed by different equilibria involving two α,γ-CPs (Scheme 1): cyclo-[L-Ser(1-pyreneacetyl)-D-^{Me}N-γ-Ach-(L-Phe-D-^{Me}N-γ-Ach)$_2$−] (**α,γ-CP$_{Ach}$pyr**) and cyclo-[L-Lys(Dapoxyl)-D-^{Me}N-γ-Acp-(L-Leu-D-^{Me}N-γ-Acp)$_2$−] (**α,γ-CP$_{Acp}$dap**). Both the Acp-based and Ach-based CPs were synthesized as follows. The linear hexapeptides were prepared by a solution-phase method following the strategy previously reported[6,7] and cyclized in dichloromethane in the presence of TBTU. The resulting cyclic peptides were hydrogenated (i.e., balloon pressure) with 10% Pd/C catalyst to provide unprotected cyclic hexapeptides **α,γ-CP$_{Ach}$H** and **α,γ-CP$_{Acp}$H**. The resulting cyclo-[L-Ser-D-^{Me}N-γ-Ach-(L-Phe-D-^{Me}N-γ-Ach)$_2$−] (**α,γ-CP$_{Ach}$H**) was subsequently coupled with 1-pyreneacetic acid in the presence of DIC/DMAP to give **α,γ-CP$_{Ach}$pyr**, while peptide **α,γ-CP$_{Acp}$dap** was prepared by reaction of cyclo-[L-Lys-D-^{Me}N-γ-Acp-(L-Leu-D-^{Me}N-γ-Acp)$_2$−] (**α,γ-CP$_{Acp}$H**) with Dapoxyl 3-sulfonamido propionic acid succinimidyl ester in a 1:4 DMF/$CHCl_3$ solution. The NMR spectra of the resulting cyclic peptides in $CHCl_3$ exhibited clear β-sheet signals, characteristic of α,γ-CP dimeric structures, in which the three nonequivalent dimers (rotamers) are slowly exchanging on the NMR time scale (Scheme 1, bottom).

Steady State Fluorescence Studies

The first equilibrium studied was the homodimerization process of **α,γ-CP$_{Ach}$pyr** (Scheme 2a). Irradiation of dilute solutions of **α,γ-CP$_{Ach}$pyr** (50 nM in $CHCl_3$) generated the typical emission spectrum of pyrene, with two maxima at 377 and 397 nm. Increasing the concentration of **α,γ-CP$_{Ach}$pyr** by addition of successive aliquots of a concentrated stock solution of **α,γ-CP$_{Ach}$pyr** resulted in the appearance of a new band at 472 nm, corresponding to the pyrene excimer emission (Figure 1).[12]

Considering that the formation of excimers is geometrically allowed in only one of the three possible dimers, we used the observed excimer band for the calculation of the homodimerization binding constant of **α,γ-CP$_{Ach}$pyr**, resulting in $K_A = 5.3 \times 10^5$ M^{-1} (Scheme 2a and Figure 1).[13] From previous studies, we expected that the heterodimer formation of **α,γ-CP$_{Ach}$pyr** and any complementary Acp-based CP would take place with much higher affinity than homodimerization of either cyclic peptide.[7] Thus, addition of **α,γ-CP$_{Acp}$Z** competed with the **α,γ-CP$_{Ach}$pyr**/**α,γ-CP$_{Ach}$pyr** homodimer formation by efficiently sequestering **α,γ-CP$_{Ach}$pyr** as a heterodimeric **α,γ-CP$_{Ach}$pyr**/**α,γ-CP$_{Acp}$Z** complex, thus abolishing the excimer emission band (Figure S1 in Supporting Information). This observation supports the model of homodimeric species as responsible for excimer emission.

This preference for heterodimer formation between Acp-based and Ach-based CPs rather than homodimerization was also exploited for the fluorescence resonance energy transfer process between cyclic peptides **α,γ-C$_{Ach}$pyr** and **α,γ-C$_{Acp}$dap**. Irradiation at 340 nm of a 0.1 μM solution of **α,γ-CP$_{Acp}$dap** resulted in a large fluorescence emission band at 500 nm. Addition of successive aliquots of **α,γ-CP$_{Ach}$pyr** over that solution increased the fluorescence emission intensity at 500 nm (Figure S2 in Supporting Information). Given that **α,γ-CP$_{Ach}$pyr** does not emit at 500 nm, and the concentration of the cyclic peptide **α,γ-CP$_{Acp}$dap** is kept constant throughout the titration, the increased

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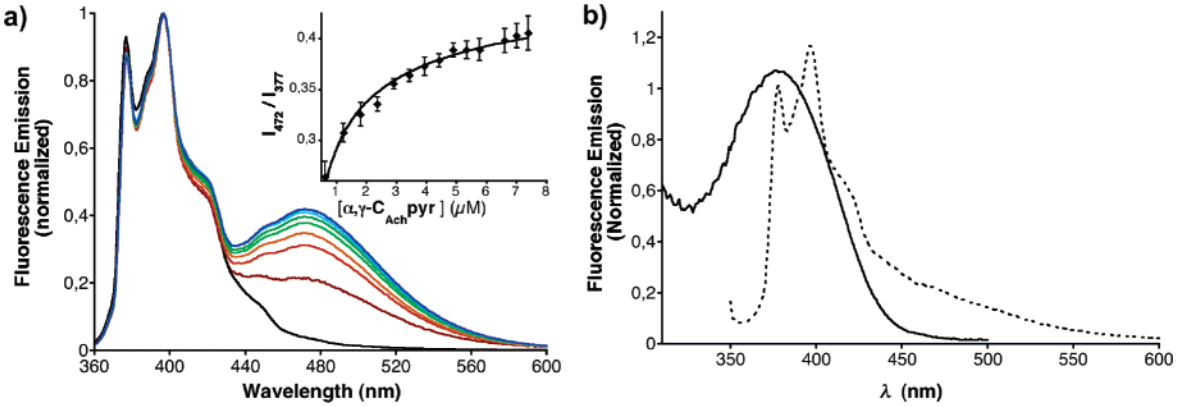
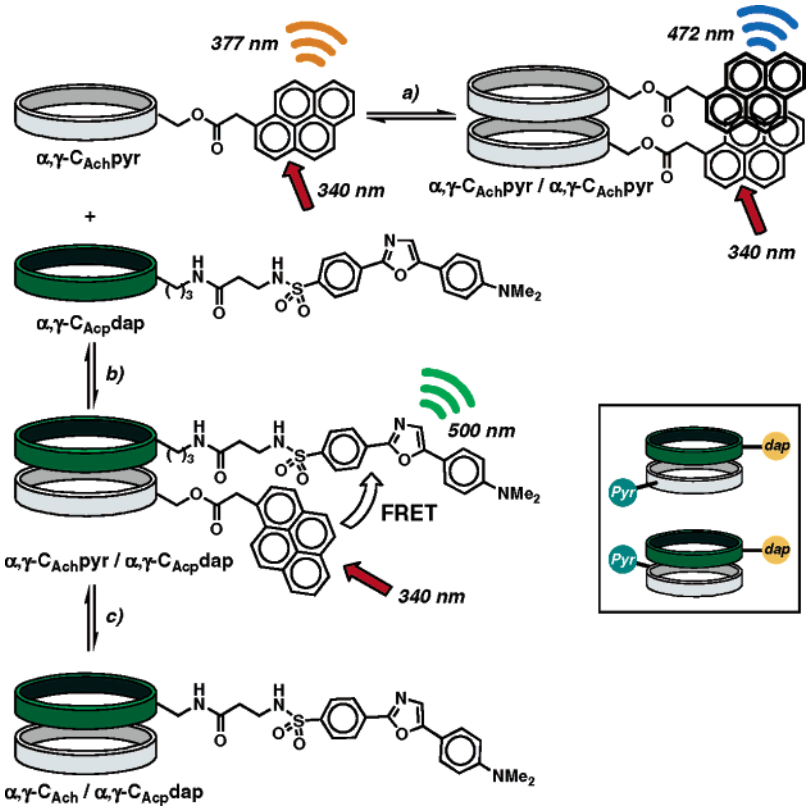


Figure 1. (a) Pyrene fluorescence emission of α,γ-**CP$_{Ach}$pyr** in $CHCl_3$ (340 nm excitation wavelength) from 0.3 μM (brown) to 7.4 μM (dark blue), denoting homodimer formation; 1-pyreneacetic acid (7.4 μM) is also shown (black). Inset shows titration for K_A calculation. (b) Absorption spectrum of Dapoxyl (solid line) showing the overlap with pyrene emission (dashed line). All spectra were normalized for representation.

Scheme 2. Equilibria between α,γ-**CP$_{Ach}$pyr** and α,γ-**CP$_{Acp}$dap** and Signal Outputs Arising from the Different Complexes[a]



[a] Insert: the two other possible α,γ-**CP$_{Ach}$pyr**/α,γ-**CP$_{Acp}$dap** heterodimeric rotamers responsible for the observed FRET.

emission intensity must arise from intramolecular FRET between pyrene and Dapoxyl in the heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** complex (Scheme 2b and Figure 2). This energy transfer is possible because the spectral overlap between Dapoxyl absorption and pyrene emission is almost complete, which ensures efficient transfer between the two fluorophores (Figure 1). Considering the quantum yield of pyrene under these conditions to be 0.03,[14] we calculated from these spectra the Förster radius as 23 Å.

Addition of competitive cyclopeptide α,γ-**CP$_{Ach}$Bn** to the heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** complex solution resulted in an increase in the natural emission of pyrene at 377 and 397 nm and a reduction of the 500 nm signal of Dapoxyl. This observation is consistent with formation of the heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$Bn** and disruption of the supramolecular dimeric species α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** (Scheme 2c and Figure 2). As expected, addition of a 100-fold excess of 1-pyreneacetic acid did not induce any change in the α,γ-**C$_{Acp}$dap** emission intensity, consistent with the absence of the cyclic peptide dimerization unit.

Time-Resolved Fluorescence Studies

These results demanded a full characterization of the dynamic processes taking place using time-resolved fluorescence tech-

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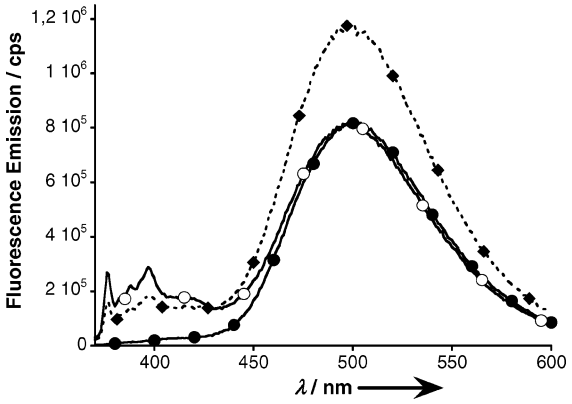

Figure 2. Formation of α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** heterodimer. Fluorescence emission of (a) ● monomeric α,γ-**CP$_{Acp}$dap**; (b) ◆ heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr**; and (c) ○ heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** with excess of α,γ-**CP$_{Ach}$**.

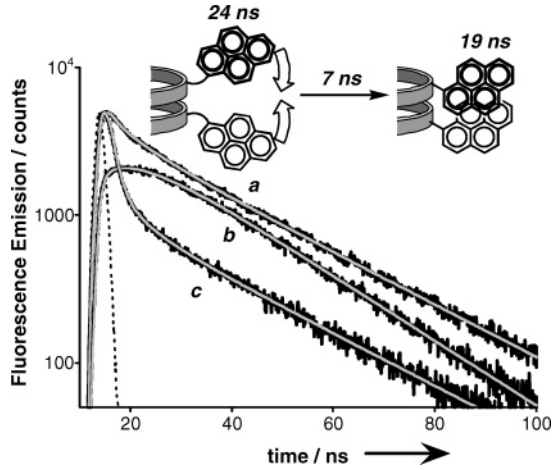

Figure 3. (a) 380 nm emission of 5 μM monomeric α,γ-**CP$_{Ach}$pyr**; (b) 470 nm emission of homodimeric α,γ-**CP$_{Ach}$pyr**; (c) 380 nm emission of heterodimeric α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** (5 μM α,γ-**CP$_{Ach}$pyr** and 1 μM α,γ-**CP$_{Acp}$dap**). Dashed line represents the laser pulse. Gray lines are the best fit to the experimental data.

niques. Irradiation of homodimeric α,γ-**CP$_{Ach}$pyr**/α,γ-**CP$_{Ach}$pyr** resulted in a fluorescence decay that could be fitted to a biexponential model with two different times of 24 and 7 ns (Figure 3). The longer 24 ns time corresponds to the normal pyrene emission decay from the two rotamers where excimer formation is geometrically impossible (Scheme 1); the shorter 7 ns lifetime is assigned to the rotamer with stacked pyrene side chains, which can give rise to excimer emission. Measurements of fluorescence decays at the excimer emission wavelength (470 nm) also showed a biexponential decay (19 and 7 ns). The longer time corresponds to the excimer decay, while the shorter 7 ns decay, having a negative preexponential factor, is assigned to the process of conformational change that gives the required geometrical arrangement for excimer formation (Figure 3).

When the heterodimeric complex α,γ-**CP$_{Acp}$dap**/α,γ-**CP$_{Ach}$pyr** was studied, we were able to observe the expected pyrene decay (24 ns), together with a new fast process with an exponential decay of 1 ns. This lifetime was assigned to the energy transfer process which, together with the previously calculated Förster radius (R_0), allowed us to determine an average pyrene−Dapoxyl distance of 14 Å, with a 96% efficiency in the transfer process using eq 1.

$$r = \frac{R_0}{(\tau_D^0/\tau_{te})^{1/6}} \quad (1)$$

where τ_D^0 is the excited-state lifetime of pyrene in the absence of transfer (24 ns) and τ_{te} is the excited-state lifetime of energy transfer process (1 ns).[15,16] This distance corresponds to the rotamers where pyrene and Dapoxyl are not in proximity. The third rotamer that places both fluorophores in close contact must have an even faster transfer that is not detectable in the nanosecond time scale.

Conclusions

In summary, these studies show the design and characterization of a novel multicomponent network of pyrene and Dapoxyl-derivatized α,γ-CPs that display controlled fluorescent signal output. These studies might be particularly relevant for the development of new biosensor systems, specifically tailored for studying networks involving homo- and heterodimerization processes. In addition, to our knowledge, this is the first time that pyrene excimer formation is used together with FRET to achieve distinct fluorescent signals associated with diverse association states. Finally, we also report for the first time the use of a new FRET pair between pyrene and Dapoxyl characterized by a high transfer efficiency and convenient excitation and emission wavelengths for biological imaging studies.

Experimental Section

Peptide Synthesis. Linear peptides Boc-[L-Phe-D-^{Me}N-γ-Ach]$_2$-L-Ser(Bn)-D-^{Me}N-γ-Ach-OFm and Boc-[L-Leu-D-^{Me}N-γ-Acp]$_2$-L-Lys(Z)-D-^{Me}N-γ-Acp-OFm were prepared following the synthetic strategy previously described.[6,7]

Cyclo-[L-Lys(Z)-D-^{Me}N-γ-Acp-(L-Leu-D-^{Me}N-γ-Acp)$_2$−] (α,γ-CP$_{Acp}$Z). A solution of Boc-[L-Leu-D-^{Me}N-γ-Acp]$_2$-L-Lys(Z)-D-^{Me}N-γ-Acp-OFm (400 mg, 0.35 mmol) in 20% piperidine in DCM (5 mL) was stirred at room temperature for 20 min. After removal of the solvent, the residue was dissolved in DCM (10 mL), and this solution was washed with HCl (5%), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was dissolved in 4 mL of TFA/DCM (1:1) and stirred at room temperature for 15 min. After removal of the solvent, the residue was dried under high vacuum for 3 h and used without further purification. The linear peptide was dissolved in DCM (345 mL) and treated with TBTU (133 mg, 0.41 mmol), followed (dropwise) by DIEA (241 μL, 1.38 mmol) [an additional 1 equiv of TBTU (111 mg, 0.35 mmol) and 4 equiv of DIEA (241 μL, 1.38 mmol) were added when the starting material was detected by HPLC, and the resulting mixture was stirred for 3 h at room temperature to complete the reaction]. After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC, affording 160 mg of α,γ-**CP$_{Acp}$Z** as a white foam [54%, R_t = 16 min (Phenomenex Maxsil-10 semipreparative column, 5−15% MeOH in CH_2Cl_2, 30 min)]. ^{1}H NMR ($CDCl_3$, 500.13 MHz, δ): 8.31−8.11 (overlapping doublets, 3H, NH), 7.41−7.27 (m, 5H, Ar Z), 5.25−4.98 (m, 3.4H, 2 × H_α Leu + H_α Lys + 0.4 × NH), 5.07 (s, 2H, CH_2 Z), 4.96−4.64 (m, 3.6H, 3 × H_γ γ-Acp + 0.6 × NH), 3.25−3.10 (m, 2H, CH_2NH), 3.09−3.00 (overlapping singlets, 9H, NMe γ-Acp), 2.99−2.87 (m, 3H, H_α γ-Acp), 2.30 (m, 3H, CH γ-Acp), 2.09 (m, 3H, CH γ-Acp), 1.96−1.18 (m, 24H, 12 × CH γ-Acp + 2 × CH_2 Leu + 2 × CH Leu + 3 × CH_2

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Lys), 1.04−0.85 (m, 12H, CH_3 Leu). ^{13}C NMR ($CDCl_3$, 125.77 MHz, δ): 175.3 (CO), 173.5 (CO), 156.3 (CO), 136.6 (C), 128.5 (CH), 128.1 (CH), 128.0 (CH), 66.7 (CH_2), 54.7 (CH), 48.2 (CH), 46.9 (CH), 42.4 (CH_2), 42.3 (CH), 40.9 (CH_2), 35.8 (CH_2), 33.1 (CH_2), 29.8 (NCH_3), 29.3 (CH_2), 27.2 (CH_2), 24.8 (CH), 23.1 (CH_3), 22.8 (CH_2), 22.6 (CH_2), 22.4 (CH_3). FTIR (293 K, $CHCl_3$): 3303 (amide A), 3005, 2960, 1662, 1626 (amide I), 1529 (amide II_{II}) cm^{-1}. MS (MALDI-TOF) [*m*/*z* (%)]: 902 ($[M + K]^+$, 10), 886 ($[M + Na]^+$, 40), 864 ($[MH]^+$, 100). HRMS (MALDI-TOF) calcd for $C_{47}H_{74}N_7O_8$ ($[MH]^+$) 864.5593, found 864.5578.

Cyclo-[L-Ser(Bn)-D-^{Me}N-γ-Ach-(L-Phe-D-^{Me}N-γ-Ach)$_2$−] (α,γ-CP$_{Ach}$Bn).[4] Prepared in the same way as **α,γ-CP$_{Acp}$Z** from Boc-{[L-Phe-D-^{Me}N-γ-Ach]$_2$-L-Ser(Bn)-D-^{Me}N-γ-Ach−}OFm (188 mg, 0.21 mmol). Yield after HPLC purification was 125 mg (68%); white solid. ^1H NMR ($CDCl_3$, 250.13 MHz, δ): 8.79−8.24 (overlapping doublets, J = 9.2 Hz, 3H, NH), 7.20 (m, 15H, 2 × Ar Phe + Bn), 5.48−5.21 (m, 3H, 2 × H_α Phe + H_α Ser), 4.70−4.24 (m, 3H, H_γ γ-Ach), 3.77−3.63 (m, 2H, $CH_2\beta$Ser), 3.15−3.01 (m, 6H, 2 × $CH_2\beta$Phe + CH_2Bn), 2.88−2.78 (m, 3H, H_α γ-Ach), 2.51 (s, 9H, NMe γ-Ach), 2.06−1.03 (m, 24H, CH_2 γ-Ach). FTIR (293 K, $CHCl_3$): 3303 (amide A), 2934, 2863, 1664, 1627 (amide I), 1529 (amide II_{II}) cm^{-1}. MS (FAB^+) [*m*/*z* (%)]: 1778 ($[2MH]^+$, 12), 889 ($[MH]^+$, 100). HRMS $[MH]^+$ calcd for $C_{52}H_{69}N_6O_7$ 889.5228, found 889.5219.

Cyclo-[L-Lys-D-^{Me}N-γ-Acp-(L-Leu-D-^{Me}N-γ-Acp)$_2$−] (α,γ-CP$_{Acp}$H). A solution of **α,γ-CP$_{Acp}$Z** (80 mg, 93 μmol) in EtOH (1 mL) was treated with 10% Pd/C (20 mg, 19 μmol) and stirred at room temperature under hydrogen overnight. The resulting mixture was filtered through a celite pad; the residue was washed with ethanol, and the pooled filtrate and washings were concentrated under reduced pressure, affording 62 mg of **α,γ-CP$_{Acp}$H** as a white solid (91%). ^1H NMR (DMSO-d_6, 500.13 MHz, δ): 8.28−8.12 (overlapping doublets, 3H, NH), 7.61 (br s, 2H, NH_2), 4.91−4.52 (m, 6H, 2 × H_α Leu + H_α Lys + 3 × H_γ γ-Acp), 2.92−2.82 (overlapping singlets, 9H, NMe γ-Acp), 2.78−2.69 (m, 2H, CH_2 NH_2), 2.67−2.57 (m, 3H, H_α γ-Acp), 1.98−1.11 (m, 30H, 9 × CH_2 γ-Acp + 2 × CH_2 Leu + 2 × CH Leu + 3 × CH_2 Lys), 0.91−0.81 (m, 12H, CH_3 Leu). MS (MALDI-TOF) [*m*/*z* (%)]: 751 ($[M + Na]^+$, 45), 729 ($[MH]^+$, 100). HRMS (MALDI-TOF) calcd for $C_{39}H_{67}N_7O_6$ ($[MH]^+$) 729.5147, found 729.5100.

Cyclo-[L-Ser-D-^{Me}N-γ-Ach-(L-Phe-D-^{Me}N-γ-Ach)$_2$−] (α,γ-CP$_{Ach}$H).[6d] Prepared in the same way as **α,γ-CP$_{Acp}$H** from **α,γ-CP$_{Acp}$Bn** (40 mg, 45 μmol). Yield after HPLC purification was 36 mg (99%); white solid. ^1H NMR ($CDCl_3$, 750 MHz, δ): 8.81−8.59 (m, 2H, NH), 8.36 (d, J = 8.5 Hz, 0.25H, NH Phe), 8.31 (d, J = 8.7 Hz, 0.25H, NH Phe), 7.92 (d, J = 8.1 Hz, 0.5H, NH Ser), 7.20 (m, 10H, Ar Phe), 5.33 (m, 2.5H, 2 × H_α Phe + 0.5 × H_α Ser), 5.12 (s, 0.5H, H_α Ser−H_α Ser), 4.57 (m, 3H, H_γ γ-Ach), 3.90 and 3.75 (s, 2H, $CH_2\beta$Ser), 3.11−2.92 (m, 7H, 2 × CH_2 βPhe + 3 × H_α γ-Ach), 2.52 and 2.48 (s, 9H, NMe γ-Ach), 2.09−0.98 (m, 24H, CH_2 γ-Ach). FTIR (293 K, $CHCl_3$): 3303 (amide A), 2928, 2857, 1661, 1624 (amide I), 1525 (amide II_{II}) cm^{-1}. MS (FAB^+) [*m*/*z* (%)]: 799 ($[MH]^+$, 100). HRMS $[MH]^+$ calcd for $C_{45}H_{63}N_6O_7$ 799.4758, found 799.4765.

Cyclo-[L-Lys(Dapoxyl)-D-^{Me}N-γ-Acp-(L-Leu-D-^{Me}N-γ-Acp)$_2$−] (α,γ-CP$_{Ach}$dap). A solution of **α,γ-CP$_{Acp}$H** (5.0 mg, 6.9 μmol) in DMF (100 μL) was stirred and sonicated at room temperature for 10 min, and then DIEA (4.8 μL, 27.4 μmol) was added. After 5 min stirring at room temperature, a solution of Dapoxil 3-sulfonamido propionic acid succinimidyl ester (3.5 mg, 6.9 μmol) in $CDCl_3$ (400 μL) was added. After 1 h stirring at room temperature, the resulting mixture was concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (2 mL), washed with HCl (5%) and $NaHCO_3$(sat.), dried over Na_2SO_4, and concentrated under reduced pressure. The resulting crude was purified by preparative thin-layer chromatography (5% MeOH in CH_2Cl_2; two times), giving 6.0 mg of **α,γ-CP$_{Acp}$dap** as a yellow solid [78%, R_f = 0.15 (10% MeOH in CH_2Cl_2)]. ^1H NMR ($CDCl_3$, 750 MHz, δ): 8.22−8.07 (overlapping doublets, 3H, NH), 7.89 (d, J = 8.2 Hz, 2H, Ar Dap), 7.64 (m, 1H, Ar Dap), 7.53 (d, J = 8.6 Hz, 2H, Ar Dap), 7.46 (m, 1H, Ar Dap), 7.05 (s, 1H, Ar Dap), 6.70 (d, J = 8.6 Hz, 2H, Ar Dap), 5.34−5.23 (m, 7H, 2 × H_α Leu + H_α Lys + CH_2 + 2 × NH), 5.16−4.97 (m, 3H, H_γ γ-Acp), 4.74 (m, 2H, CH_2NH), 2.99−2.95 (overlapping singlets, 9H, NMe γ-Acp), 2.99 (overlapping singlets, 6H, NMe Dap), 3.25−3.13 (m, 3H, H_α γ-Acp), 2.54 (m, 2H, CH_2), 2.03−1.02 (m, 30H, 9 × CH_2 γ-Acp + 2 × CH_2 Leu + 2 × CH Leu + 3 × CH_2 Lys), 0.90−0.69 (m, 12H, CH_3 Leu). MS (MALDI-TOF) [*m*/*z* (%)]: 1165 ($[M + K]^+$, 39), 1149 ($[M + Na]^+$, 16), 1127 ($[MH]^+$, 31), 1012 (100). HRMS (MALDI-TOF) calcd for $C_{59}H_{87}N_{10}O_{10}S$ ($[MH]^+$) 1127.6322, found 1127.6331.

Cyclo-[L-Ser(1-pyreneacetyl)-D-^{Me}N-γ-Ach-(L-Phe-D-^{Me}N-γAch)$_2$−] (α,γ-CP$_{Ach}$pyr). A solution of 1-pyreneacetic acid (1.6 mg, 6.3 μmol) in $CDCl_3$ (250 μL) was stirred and sonicated at room temperature for 10 min, and then DIC (1.5 μL, 9.4 μmol), **α,γ-CP$_{Ach}$H** (5.0 mg, 6.3 μmol), and DMAP (1.2 mg, 9.4 μmol) were successively added. After 1 h stirring at room temperature, the solution was washed with HCl (5%) and $NaHCO_3$(sat.), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by HPLC, giving 5.2 mg of **α,γ-CP$_{Ach}$pyr** as a yellow solid [80%, R_t = 27 min (Phenomenex Maxsil-10 semipreparative column, 0−10% MeOH in CH_2Cl_2, 30 min)]. ^1H NMR ($CDCl_3$, 500.13 MHz, δ): 8.63−8.51 (overlapping doublets, 2H, NH), 8.30−7.75 (m, 10H, Pyr + NH), 7.24−7.07 (m, 10H, Ar Phe), 5.45−5.14 (m, 3H, 2 × H_α Phe + H_α Ser), 4.62−3.93 (m, 7H, 3×x H_γ γ-Ach + CH_2 Pyr + $CH_2\beta$Ser), 3.21−2.78 (m, 7H, 2 × CH_2 βPhe + 3 × H_α γ-Ach), 2.65−2.38 (overlapping singlets, 9H, NMe γ-Ach), 1.92−0.68 (m, 24H, CH_2 γ-Ach). MS (MALDI-TOF) [*m*/*z* (%)]: 1079 ($[M + K]^+$, 31), 1063 ($[M + Na]^+$, 100), 1041 ($[MH]^+$, 9). HRMS (MALDI-TOF) calcd for $C_{63}H_{72}N_6NaO_8$ ($[M + Na]^+$) 1063.5304, found 1063.5277.

Time-Resolved Fluorescence. Fluorescence lifetimes were determined by time-correlated single-photon counting on an Edinburgh Instruments CD-900 spectrometer equipped with a hydrogen-filled nanosecond flash lamp. The instrumental response width of the system is 1.0 ns. We measured usually until 10 000 counts were reached in (2 × 10^3 channels). The emission band-pass for the lifetime measurements was usually 20 nm. The experiments were performed at room temperature, and samples were purged with argon prior to measurement.

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Supporting Information Available: Figures S1 (elimination of the excimer emission band of dimeric **α,γ-CP$_{Ach}$pyr** by addition of **CP$_{Acp}$Z**); S2 (addition of **α,γ-CP$_{Ach}$pyr** over a solution of **α,γ-CP$_{Acp}$dap**). Characterization of peptides **α,γ-CP$_{Acp}$Z**, **α,γ-CP$_{Acp}$H**, **α,γ-CP$_{Acp}$dap**, and **α,γ-CP$_{Ach}$pyr**, including NMR (^1H and ^{13}C, NOESY, and/or ROESY), FTIR, and UV spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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