Self-Assembly

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Templated Chromophore Assembly by Dynamic Covalent Bonds

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Abstract: *Through the simultaneous use of three orthogonal dynamic covalent reactions, namely disulfide, boronate, and acyl hydrazone formation, we conceived a facile and versatile protocol to spatially organize tailored chromophores, which absorb in the blue, red, and yellow regions, on a preprogrammed α-helix peptide. This approach allowed the assembly of the dyes in the desired ratio and spacing, as dictated by both the relative positioning and distribution of the recognition units on the peptide scaffold. Steady-state UV/Vis absorption and emission studies suggest an energy transfer from the yellow and red donors to the blue acceptor. A molecular dynamics simulation supports the experimental findings that the helical structure is maintained after the assembly and the three dyes are confined in defined conformational spaces.*

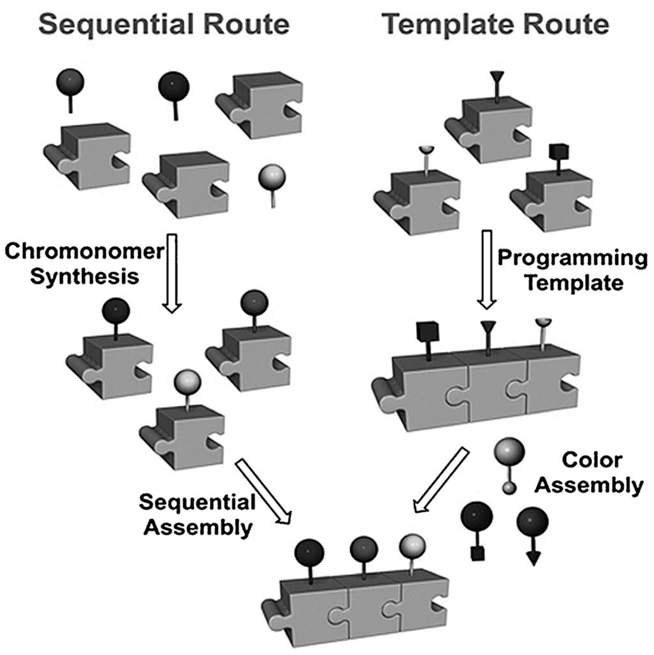

Figure 1. The sequential and templated routes for engineering multichromophoric architectures.

Natural photosynthesis is initiated by the absorption of sunlight by a large number of light-harvesting pigments that funnel the excitation energy to a reaction center where energy conversion takes place. The efficiency of this process relies on a particular organization and ratio of selected chromophores that enables maximum light absorption and energy conversion.[1]

One of the main challenges in mimicking natural antenna systems is to develop high-yielding and easy-to-implement protocols that can lead to the rational assembly of chromophores into spatially organized architectures. To be efficient, these should feature well-defined distances, orientations, and an exact ratio of chromophoric donors to acceptors.[2] In this respect, two approaches are followed: a sequential and a templated route (Figure 1). Following the sequential approach, covalent or noncovalent dendrimers,[3] macrocycles,[4] supramolecular polymers,[5] nanostructures[6] and functional surfaces[7] have been prepared starting from programmed monomeric chromophores. However, these systems are limited by the synthetic accessibility and versatility to tailor different colors, as they are often restricted to a limited number of dyes. On the other hand, natural macromolecules such as peptides,[8] nucleic acids,[9] and artificial polymers[10] have been successfully used as platforms to template the organization of chromophores (Figure 1). In this respect, helical peptide scaffolds appear to be suitable templates as they can be easily programmed with a multitude of receptor sites at given positions to spatially organize chromophores in a specific orientation.[11] We have followed the templated route (Figure 1), and report herein the first simultaneous assembly of a multichromophoric architecture that consists of three tailored chromophores linked through dynamic covalent bonds to an amphiphilic α-helix peptide featuring complementary receptor sites positioned every two pitches. This arrangement imposes a distance of 1.08 nm and a parallel orientation of the dyes in the final architecture.

In the search of recognition processes occurring simultaneously and orthogonally,[12] dynamic covalent bonds are certainly excellent candidates because of their reversible or irreversible nature.[13] The use of three dynamic covalent bonds in concert has been very recently reported,[14] but their exploitation for the simultaneous[15] assembly of multicomponent architectures is unknown. With the aim of developing a fast multicomponent assembly protocol, we have prepared model disulfide–pyridine **1**, bicyclic diol **2**, and hydrazide **3** derivatives and studied their chemoselectivity in simultaneous disulfide exchange, boronate, and acyl hydrazone formation, respectively (Scheme 1). Whereas for the disulfide exchange reaction a classical disulfide–pyridine functionality undergoing fast interchange with phenyl thiol has been used,[16] 1,2-*cis*-diol 7-azanorbornane and 2,6-difluorobenzaldehyde functionalities were selected to accelerate the formation of the linkages and at

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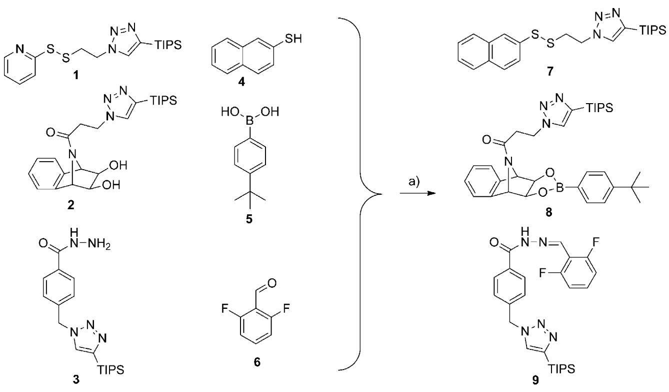


Scheme 1. Simultaneous disulfide interchange, boronate, and acyl hydrazone formation. a) *m*-Phenylene diamine (*m*-PDA), THF, RT, 1 h.

the same time to produce thermodynamically stable boronic esters[17] and hydrazones, respectively. The azanorbornane function was used to anchor a side tether, used for peptide linkage, on the N atom. When operating the three reactions simultaneously (Scheme 1), full conversion of 1,2-*cis* bicyclodiol **2** into *tert*-butylphenylboronate **8**, disulfide pyridine **1** into disulfide naphthalene **7**, and hydrazide **3** into acyl hydrazone **9** was achieved in the presence of a catalytic amount of *m*-PDA,[18] as observed by ^{1}H NMR spectroscopic and ESI-TOF mass spectrometric analyses of the crude material (Figure 2). Purification of the crude material allowed the isolation of the expected products, ultimately confirming their quantitative conversion (see the Supporting Information).

Following these findings, a programmed α-helix peptide, Ac-QLA-**X(disulfide)**-QLAQLA-**X(hydrazide)**-QLAQLA-**X(diol)**-QLA-CONH$_2$, bearing the receptor sites on a modified glycine every (i, $i+7$) residues, was prepared. The amphiphilic trimeric QLA sequence was selected as the main motif. The presence of the hydrophilic glutamine each (i, $i+3$) and (i, $i+4$) brings solubility and helix stability by H-bonding to the alanine/leucine-based motif, two residues well known for their strong tendency to form helices.[19] Three modified glycines were thus synthesized from commercially available amino acid Fmoc-Gly-(Propargyl)-OH through Cu-catalyzed azide–alkyne cycloaddition (Scheme 2 and the Supporting Information). Peptide **16** was synthesized following Fmoc-based solid-phase synthesis that, after disulfide interchange with 2,2′-dipyridyl disulfide (DPS), was transformed into peptide **17** (Scheme 2). In parallel, ethynyl pyrene,[20] perylene diimide (PDI),[21] and naphthalene diimide (NDI)[22] have been selected for their complementary UV/Vis absorption profiles that, covering the blue, yellow and red regions, allow selective excitations in the three spectral zones. In this respect, reactive thiophenol-derived blue NDI (B-NDI) **22**, red PDI (R-PDI) **27** exposing a 2,6-difluoro-L-phenyaldehyde moiety, and phenyl boronic acid derived yellow Py (Y-Py) **30** derivatives were synthesized.

Following a synthetic methodology recently developed by us,[23] AB-type B-NDI **22** was prepared in high yields starting from easily accessible core-substituted monoanhydride **18** (Scheme 2). Sequential condensation reactions in the presence of amine **19** produced monoimide monoanhydride **20**. This compound was transformed into thiol-derived chromophore B-NDI **22** through a condensation and substitution reactions in the presence of amines **21** and *i*PrNH$_2$, respectively, followed by Hg^{II}-assisted cleavage of the thioether group. PDI derivative **27** was synthesized starting from cyclohexyl monoimide **23**, which was subsequently reacted with amine **19** to afford the diimide intermediate, sequentially hydrolyzed into **24**. Final condensation with aniline **25** followed by Suzuki-type cross-coupling with **26** afforded dye **27**. The synthesis of yellow chromophore **30** consisted in a Sonogashira cross-coupling between ethynylpyrene **28** and **29**, followed by an acid-catalyzed hydrolysis of the boronic

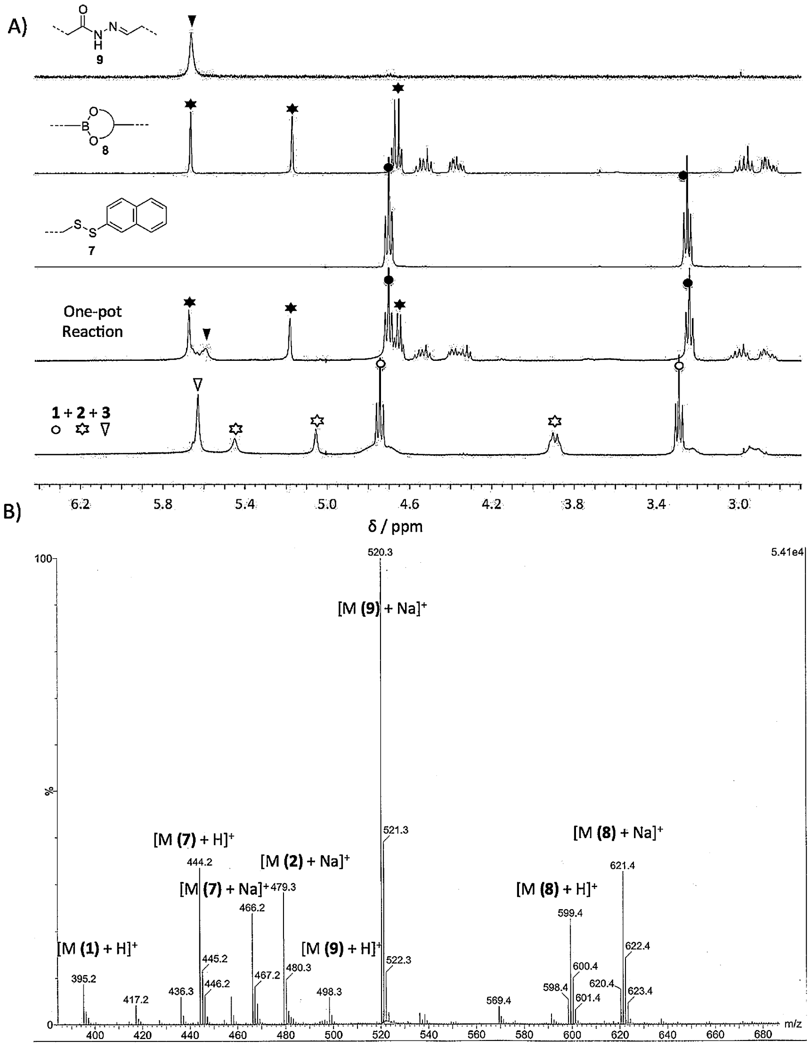
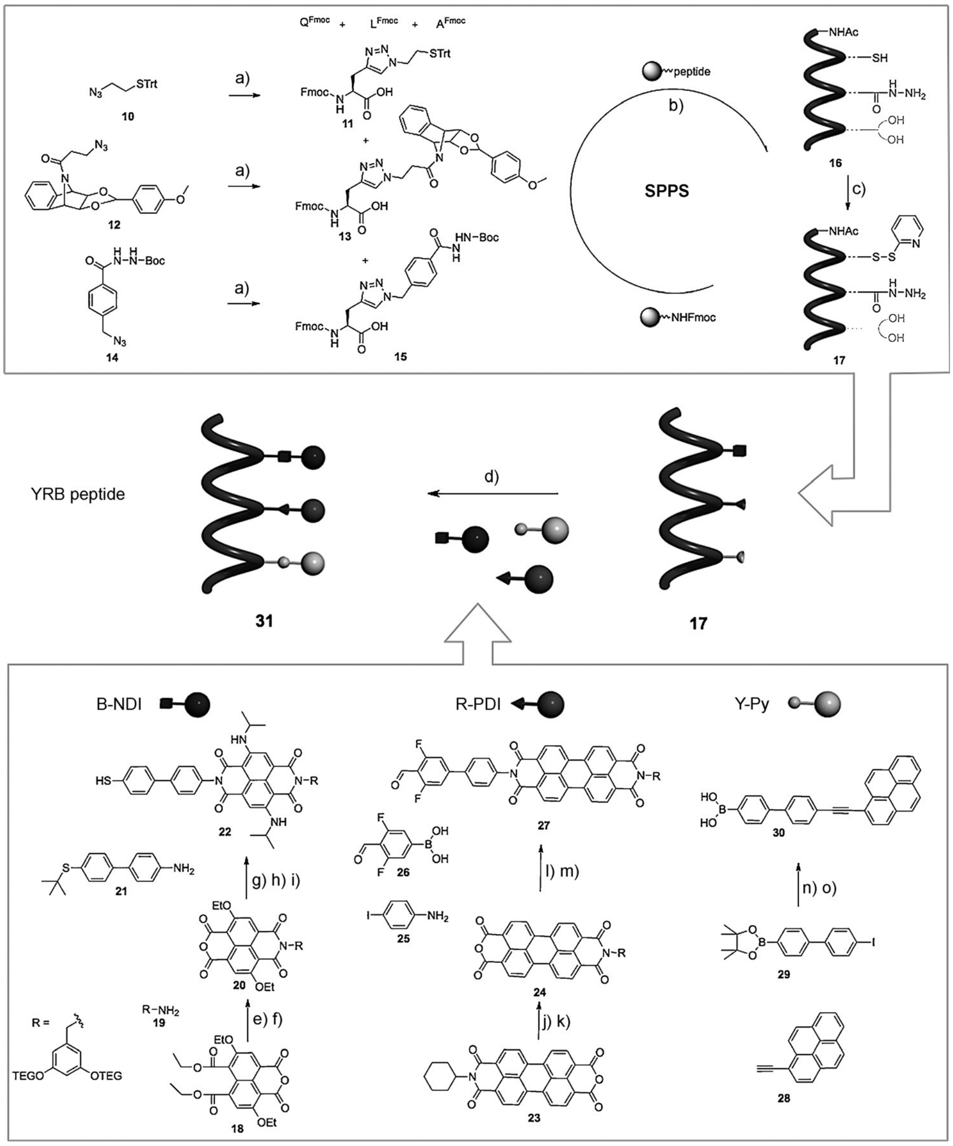


Figure 2. A) Expanded region of ^{1}H NMR spectrum (CDCl$_3$, 25 °C) of 1) physical mixture of **1**, **2**, and **3**; 2) one-pot reaction; 3) disulfide naphthalene **7**; 4) boronate **8**; 5) acyl hydrazone **9**. B) ESI-TOF mass spectrum of the crude material.



Scheme 2. Synthetic approach for preparing YRB peptide **31**. a) Fmoc-Gly(Propargyl)-OH, $CuSO_4{\cdot}5\,H_2O$, sodium ascorbate, DMF/H_2O (4:1), RT, 3 h 76–89%; b) Rink amide MBHA resin, solid-phase peptide synthesis (SPPS; see the Supporting Information); c) 2,2′-DPS, DIEA, DMF, RT, 1 h; d) *m*-PDA, DMF, RT, 4 h; e) **19**, DIEA, dioxane, 101 °C, 2 h, 92%; f) TFA, 73 °C, 24 h, 91%; g) **21**, Et_3N, benzoic acid, DMF, 110 °C, 6 h, 72%; h) *i*$PrNH_2$, 110 °C, 48 h, 91%; i) 1) $Hg(ClO_4)_2$, RT, 30 min, 2) H_2S, RT, 10 min −72%; j) **19**, Im, toluene/DMF (6:1), 110 °C, 16 h, 67%; k) 1) KOH, *i*PrOH, 80 °C, 3 h, 2) AcOH, RT, 15 min; l) **25**, Im, toluene/DMF (6:1), 110 °C, 16 h −78% over 2 steps; m) **26**, K_2CO_3, $[Pd(PPh_3)_4]$, dioxane/H_2O (7:1), 80 °C, 20 h, 95%; n) **28**, CuI, $[Pd(PPh_3)_2Cl_2]$, NEt_3, THF, 40 °C, 6 h, 62%; o) 2 M aq. HCl/THF (1:2), RT, 40 h, 85%. Fmoc = 9-fluorenylmethyloxycarbonyl; DIEA = *N*,*N*-diisopropylethylamine; TFA = trifluoroacetic acid; Im = imidazole.

ester. The three chromophores were then simultaneously assembled into peptide **31** by simply mixing the dyes with **17** at room temperature in the presence of *m*-PDA in anhydrous DMF (Scheme 2). Separation by gel permeation chromatography (GPC) afforded colored YRB-peptide **31** as pure material, whose structure was confirmed by mass spectrometry (see the Supporting Information).

The triad absorbs throughout the entire visible spectrum with distinct peaks at 376, 398, 495, 527, and 612 nm, characteristic of the Y-Py, R-PDI, and B-NDI chromophoric units, respectively (Figure 3 A). Notably, a significant hypochromism of the R-PDI bands is observed, suggesting the formation of H-type complexes.[21a] The energy transfer (ET) process for the triad was investigated using steady-state fluorescence spectroscopy. Excitation measurements (Figure 3 C) clearly indicated that all dyes contribute to the emission of the lowest-energy acceptor unit, B-NDI ($\lambda_{em} = 640$ nm), thus confirming the ET from R-PDI to B-NDI and from Y-Py to B-NDI either by direct or stepwise ET cascade. The quantum yield of the acceptor unit B-NDI ($QY_{B\text{-}NDI}$) within YRB peptide by direct excitation ($\lambda_{exc} = 609$ nm) was determined to be 27%. Upon selective excitation of the donor units R-PDI ($\lambda_{exc} = 495$ nm) and Y-Py ($\lambda_{exc} = 398$ nm), $QY_{B\text{-}NDI}$ were evaluated to be 1.5% and 1.4%, respectively. By calculating the ratio of the $QY_{B\text{-}NDI}$ upon indirect and direct excitation, the energy-transfer efficiency Φ_{ET} from the donor to the acceptor was estimated to be 5% and 5.6% for the Y-Py→B-NDI and R-PDI→B-NDI sensitization, respectively. To validate our hypothesis, Φ_{ET} was determined upon comparison of the absorption and excitation spectra, both normalized at the maximum absorption wavelength of blue chromophore unit B-NDI ($\lambda_{max} = 612$ nm), by using the equation $\Phi_{ET} = Ex_D/A_D$, where Ex_D and A_D are fluorescence intensity and absorption of the donor in the normalized excitation and absorption spectra, respectively. The efficiencies were estimated to be 5% and 5.4% for Y-Py→B-NDI and R-PDI→B-NDI, respectively, which are in agreement with previous experimental measurements. To shed further light on the secondary structure of the YRB peptide, CD studies were conducted to unravel the conformational behavior before and after the chromophore assembly. Whereas the measurements in DMF could not be performed ($\lambda_{cut\text{-}off} = 268$ nm), CD spectra recorded in a solution of H_2O/CF_3CH_2OH (TFE; 2:1; $\lambda_{cut\text{-}off} = 190$ nm) clearly showed the typical peptide-centered peaks (positive band at 192 nm and two negative at 208 and 222 nm) fingerprinting an α-helix secondary structure (see the Supporting Information).

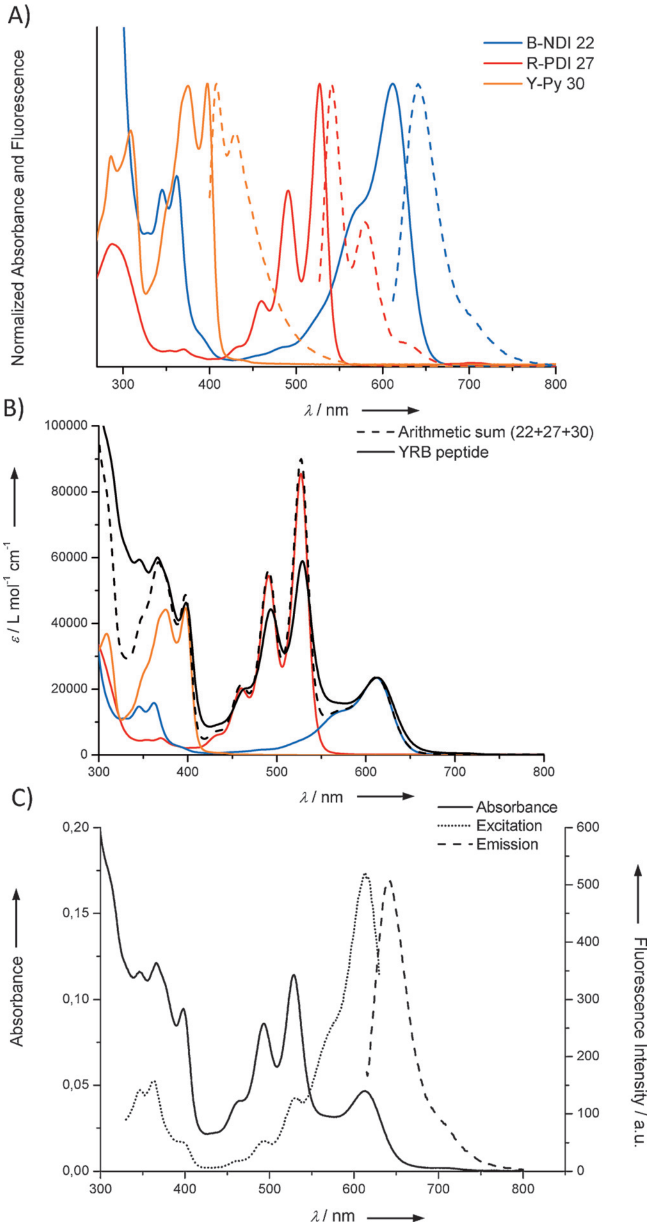


Figure 3. A) Normalized absorption (solid lines) and fluorescence (dashed lines) spectra of B-NDI **22**, R-PDI **27**, and Y-Py **30** in DMF; B) Absorption spectra of B-NDI **22** (blue), R-PDI **27** (red), Y-Py **30** (yellow), their arithmetic sum (-----, **22**+**27**+**30**) and YRB peptide (black) in DMF; C) Absorption (solid line, 2 μM) and emission (long-dashed line, 2 μM, $\lambda_{exc}=612$ nm) and excitation profile ($\lambda_{em}=640$ nm) in DMF (short-dashed line, 2 μM) for YRB peptide.

The α-helix content was estimated to be 82% by the CONTINLL algorithm.[23] Switching the solvent to neat TFE or a H_2O/TFE solution (1:1 and 1:2) did not enhance the inherent helicity of the secondary structure, thus implying that the selected sequence is an ideal helical scaffold. Notably, as both peptides **31** and **17** possess the same inherent helicity, it suggests that the presence of large π-conjugated dyes does not significantly perturb the secondary structure.

On the other hand, the lack of any Cotton effect in the absorption region of the dyes implies the absence of any chiral chromophoric organization, most likely because of the significant distance from the helical template. In TFE, the $QY_{B\text{-}NDI}$ by direct excitation of the acceptor unit ($\lambda_{exc}=620$ nm) collapsed to 6% (see the Supporting Information). Upon excitation of the R-PDI ($\lambda_{exc}=495$ nm) and Y-Py ($\lambda_{exc}=398$ nm) donor units, the $QY_{B\text{-}NDI}$ values were determined to be 0.3% and 0.4%, respectively. As demonstrated by the significant bathochromic shift of NDI-centered absorption band, in this solvent, strong inter- and intramolecular are possibly occurring and favor a significant quenching of the

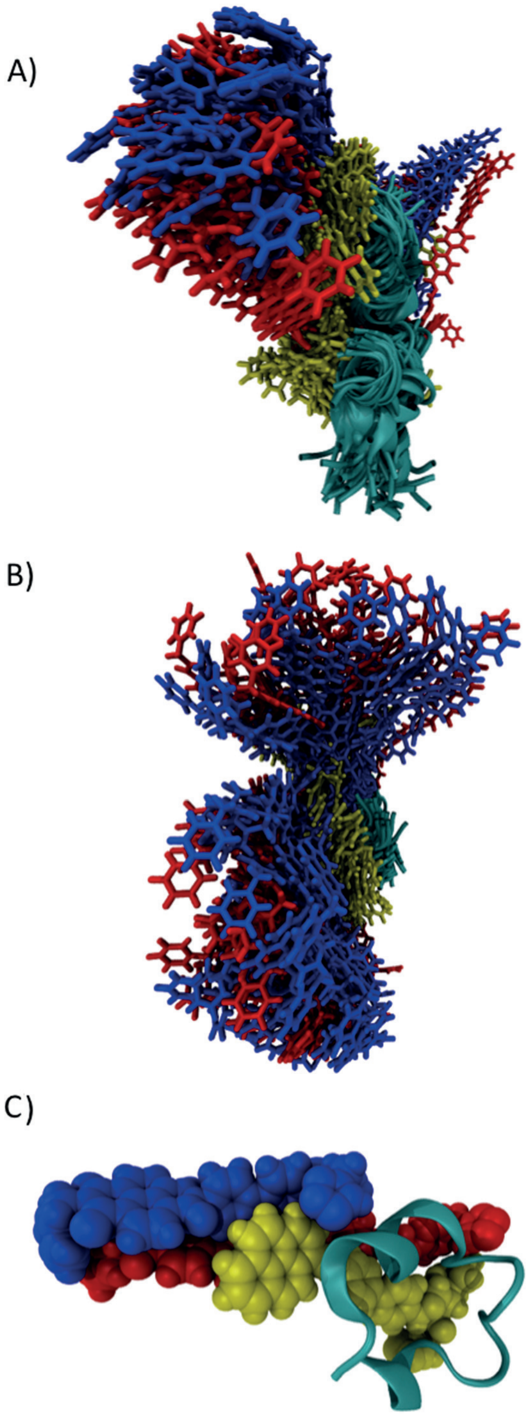


Figure 4. MD simulation (55 ns) of YRB peptide (explicit solvent). A) Side and B) top views of 500 overlaid frames. C) Most stable conformation identified at 53 ns ($E_{tot}=-1001$ kcal mol^{-1}).

radiative emission. Further time-resolved photophysical studies under different conditions are currently under investigation to unravel all possible deactivation pathways, including those arising from aggregation and photoinduced charge transfer phenomena.

MD simulation analysis for the YRB-peptide (Figure 4), clearly showed that the majority of the residues are in the α-helix or 3–10 helix (see the Ramachandran plot in the Supporting Information). The chromophoric units show a different spatial occupancy owing to the conformational flexibility of the linkers and the geometrical constrains imposed by the helical template. As shown in Figure 4, the chromophores occupy three different conformational spaces in which red, blue, and yellow regions are easily discerned. Notably, in the most stable conformation, the red and blue units are facing each other through H-type complexes, thus supporting the hypochromism observed in the UV/Vis spectra.

In summary, we have engineered the first orthogonal multireaction system consisting of simultaneous disulfide exchange, boronate, and acyl hydrazone formation. Exploitation of this methodology allowed the simultaneous organization of tailored blue, red, and yellow chromophores on a preprogrammed α-helix peptide. The energy transfer from the primary donor Y-Py to the acceptor B-NDI, and from the intermediate donor R-PDI to B-NDI was estimated to be around 5 % and 5.5 %, respectively. The proof-of-concept put forward in this paper will allow the bottom-up approach of multichromophoric architectures exhibiting any desired absorbed or emitted colors, enabling unlimited surfing through the color coordinate diagram.

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Keywords: dynamic covalent chemistry · energy transfer · peptides · self-assembly · supramolecular chemistry

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