Molecular Assembly

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# Amplification of Molecular Information through Self-Assembly: Nanofibers Formed from Amino Acids and Cyanine Dyes by Extended Molecular Pairing**

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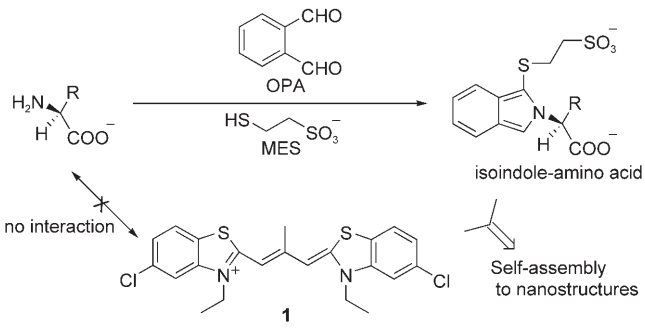
Self-assembly of different chemical species plays a pivotal role in numerous biological functions. For example, chlorophyll molecules in photosynthetic complexes are noncovalently bound to peptide scaffolds that control their energy transfer to the reaction centers.[1] Heterogeneous self-assembly of bicomponent superstructures has also been demonstrated in artificial systems.[2,3] More recently, we devised a molecular pairing technique in which the mixing of adenosine triphosphate (ATP) and a cyanine dye gave exciton-delocalized nanofibers.[4] This study clearly indicates the potential capacity of small biomolecules to serve as building blocks for self-assembly.

In this study we have extended heterogeneous molecular paring to include various amino acids. The molecular structure of the amino acids (for example, chirality and the structure of side chains) is fundamental molecular information in biology. It has been translated to artificial systems by using synthetic receptors,[5] indicator-displacement assays,[6] and fluorescent labeling.[7] To our knowledge, there exists no general strategy to amplify molecular information of the amino acids by using self-assembly techniques. We have developed an in situ premodification technique to link the molecular information and self-assembly process. This technique was inspired by the principle of catalytic antibodies,[8] where small hapten molecules are conjugated to a larger carrier protein in order to be recognized by antibodies formed by an immune response.

The extended molecular pairing approach is shown in Scheme 1. Amino acids are converted into isoindole derivatives (isoindole-amino acids) by reaction with *ortho*-phthalaldehyde (OPA) and alkyl thiols.[9] 2-Mercaptoethanesulfonic acid (MES) was employed beacuse it gives an anionic charge tethered to the isoindole unit. The introduction of aromatic and anionic groups was expected to enhance interactions with cationic molecules. Upon mixing the amino acids, OPA, and MES in water, isoindole-amino acids are immediately formed, which was confirmed by the detection of fluorescence and a new absorption maximum at 333 nm (Figure S1a in the Supporting Information).[10] In the case of isoindole-Lys, two amino groups are both converted into isoindole units, as confirmed by the twofold increase of absorption intensity compared to that of the other amino acids (Figure S1b in the Supporting Information). Cyanine dye **1** was employed as a functional molecular counterpart.[4] The addition of **1** to aqueous isoindole-amino acids resulted in immediate color changes from pink to reddish pink or orange, depending on the chemical structure of the amino acids (Figure 1a). These color changes were not observed when various amino acids and cyanine dye **1** were mixed in the absence of OPA and MES. Therefore, the observed color changes must originate from interactions between the isoindole-amino acids and the dye.




***Scheme 1.*** A schematic representation of the extended molecular pairing technique. Premodification of amino acids promotes the molecular association with the cationic cyanine dye, which develop into hierarchical nanostructures.

Figure 1b compares the absorption spectra of dye **1** mixed with the isoindole-amino acids formed by reaction of OPA, MES, and various amino acids (20 μM). The mixtures without amino acids (OPA + MES/**1**) and isoindole-Arg/**1** gave absorption maxima at 506 and 546 nm. These bands were also observed for **1** in water, and are ascribed to dimeric and monomeric species of **1**, respectively.[4] In contrast, intensities of the bands at 506 and 546 nm are decreased and a new band appeares at 460 nm for the aqueous mixture of isoindole-Ala/**1**. This blue-shifted band is characteristic of parallel-oriented dye molecules (H-aggregates).[11] Interestingly, the intensity of the band at 460 nm is dominant for orange aqueous dispersions of isoindole-Glu/**1**, and is accompanied by a shoulder component at 437 nm. The aqueous mixture of

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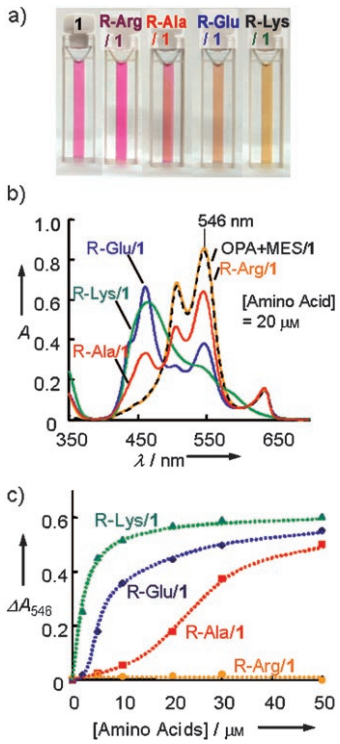


***Figure 1.*** a) Photographs of the mixed solutions of various isoindole-amino acids and dye **1**. b) UV/Vis absorption spectra of the mixed solutions. [**1**] = 10 μM, [amino acid] = 20 μM, [OPA] = 600 μM, [MES] = 600 μM, borate buffer = 20 mM (pH 9) containing 10 vol% methanol. Spectra were measured after 10 min of mixing. c) Dependence of the changes in the intensity of the monomeric species of dye **1** on the concentration of the amino acids. $\Delta A_{546}$ is the absolute variation in the absorbance of the monomer at 546 nm against the aqueous mixture of OPA + MES/**1**. R = isoindole.

isoindole-Lys/**1** showed a broad and blue-shifted absorption band at 460 nm, with a smaller contribution from the monomeric species at 546 nm.

The formation of H-aggregates is dependent on the concentration of amino acids. Figure 1c shows a decrease in the absorbance band at 546 nm as a function of the amino acid concentration (0–50 μM), and this reflects the formation of H-aggregates. In the case of isoindole-Lys, spectrum changes occur immediately at low concentrations, thus indicating a higher affinity to dye **1**. This can be explained by the presence of the two isoindole units in isoindole-Lys. In contrast, isoindole-Glu/**1** and isoindole-Ala/**1** showed a sigmoidal change, which indicates the presence of a critical aggregation concentration between the various isoindole-amino acids and dye **1**. Similar spectrum changes were also observed for the other amino acids (Figure S2 in the Supporting Information). On the other hand, isoindole-Arg showed no appreciable changes in the spectrum, probably because of its bulky, cationic side-chain structure which may suppress the interaction with **1**. It is apparent that the tendency to form H-aggregates of the cyanine dye is highly dependent on the chemical structure of the amino acid used.

To determine the stoichiometry, absorption spectral changes for isoindole-Glu/**1** were analyzed by using the continuous variation method (Figure S3 in the Supporting Information). The bands at 437 and 462 nm associated with the H-aggregate peaks reached maximum intensity at dye molar fractions of 0.80 and 0.83, respectively. These values correspond to molar ratios of isoindole-Glu/**1** of 1:4 and 1:5. The component molecules possess the electronic charges of $(\text{isoindole-Glu})^{3-}$ and $\mathbf{1}^{+}$, and the observed molar ratio indicates that the assembly isoindole-Glu/**1** contains excess dye molecules compared to that expected from electrostatic interactions (namely, isoindole-Glu/**1** = 1:3). This tendency is similar to that observed for molecular pairs of ATP/**1**,[4] which indicates that the self-assembly process is determined by the delicate balance of multiple factors including electrostatic, van der Waals, and aromatic stacking interactions.

As isoindole-amino acids are chiral, it is expected that molecules of dye **1** in the aggregates are organized in a chiral microenvironment. Chiral-induction phenomena have been reported in artificial molecular assemblies such as bilayer membranes[2,3] and liquid crystals.[12] Figure 2 shows circular

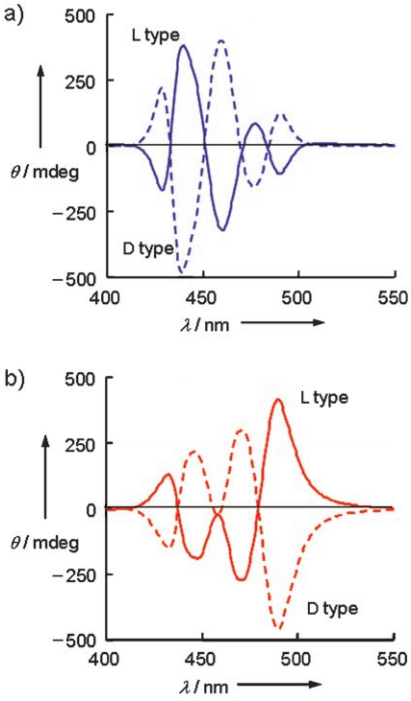


***Figure 2.*** CD spectra of a) isoindole-Glu/**1** and b) isoindole-Ala/**1** for D and L isomers. [**1**] = 10 μM, [amino acid] = 50 μM, [OPA] = 600 μM, [MES] = 600 μM, borate buffer = 20 mM (pH 9) containing 10 vol% methanol. Spectra were measured after 10 min of mixing.

dichroism (CD) spectra recorded for **1** in the presence of either isoindole-Glu or isoindole-Ala. Aqueous solutions of the isoindole-amino acids and dye **1** before mixing never gave CD signals in the visible region. Interestingly, isoindole-Glu/**1** and isoindole-Ala/**1** showed intense induced circular dichroism (ICD) spectra with complex exciton-coupling patterns. The ICD spectra have mirror symmetry with respect to the chirality of the amino acids used. It is noteworthy that any difference in the chemical structure of the side chains and

  

chirality is readily distinguishable from the ICD patterns. The intensity of these visible ICD bands is enhanced by more than 100 times relative to the intensity of the CD spectra of isoindole-amino acids in the ultraviolet region. Therefore, molecular information of amino acids is amplified and translated into UV/Vis and circular dichroism spectroscopic information, through self-assembly.

The formation of abundant nanofibers (width of about 50 nm and length of several μm; Figure 3a,b) were observed for isoindole-Glu/**1** and isoindole-Ala/**1** by transmission electron microscopy (TEM). Neither isoindole-amino acids without dye **1** nor dye **1** alone showed any ordered structures by TEM. These observations are consistent with the exciton-coupled ICD spectra, thus supporting the formation of ordered molecular structures. The observed nanofiber width of 50 nm is larger than the size of each component molecule, which indicates that isoindole-amino acid/dye molecular pairs are organized into bundles in which photoexcitation is delocalized among the oriented dye molecules (Figure S5 in the Supporting Information). Similar nanofibers are also observed for isoindole-Gly/**1** (Figure 3c). On the other hand, small aggregates (about 100 nm) are observed in the case of isoindole-Lys/**1** (Figure 3d). These findings indicate that the morphology of the isoindole-amino acid/**1** molecular pairs is again highly sensitive to the chemical structure of the amino acid side chains.

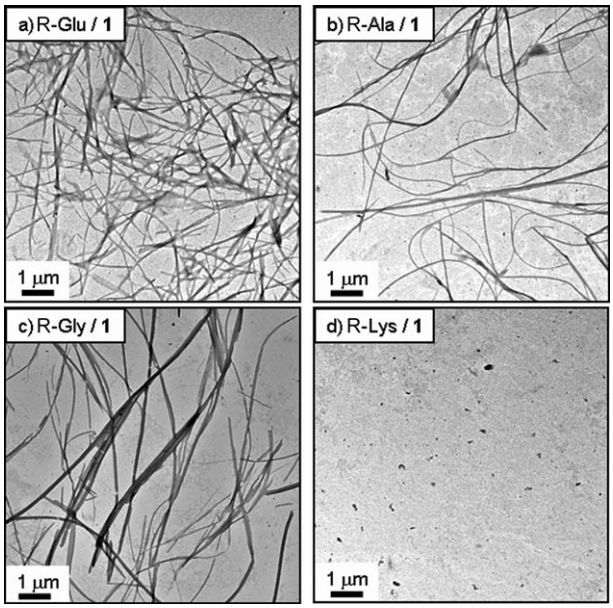


***Figure 3.*** TEM images of the mixtures of isoindole-amino acids and dye **1**. a) isoindole-Glu/**1**, b) isoindole-Ala/**1**, c) isoindole-Gly/**1**, and d) isoindole-Lys/**1**. [**1**] = 10 μM, [amino acid] = 20 μM, [OPA] = 600 μM, [MES] = 600 μM, post stained by uranyl acetate. R = isoindole.

In conclusion, heterogeneous molecular self-assembly has been successfully extended to amino acids by using an in situ premodification strategy. This extended molecular pairing approach enables the molecular information of biomolecular components to be amplified and translated into spectroscopic and morphological information. This approach will be applicable to a wide combination of biomolecules and functional molecules. It not only provides a useful means to self-assemble small biomolecules and their derivatives, but also enables the self-assembly-based processing of molecular information.

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