

## Supporting Information

### ***De novo* designed Hetero-chiral Blue Fluorescent Protein**

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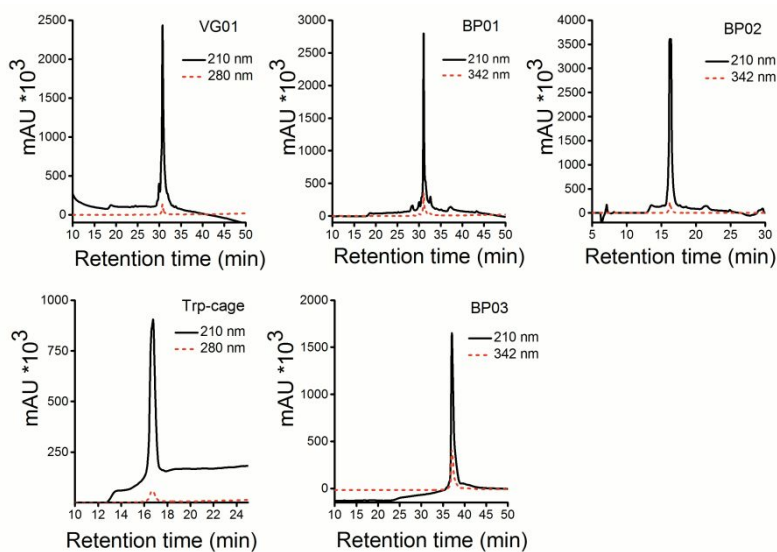
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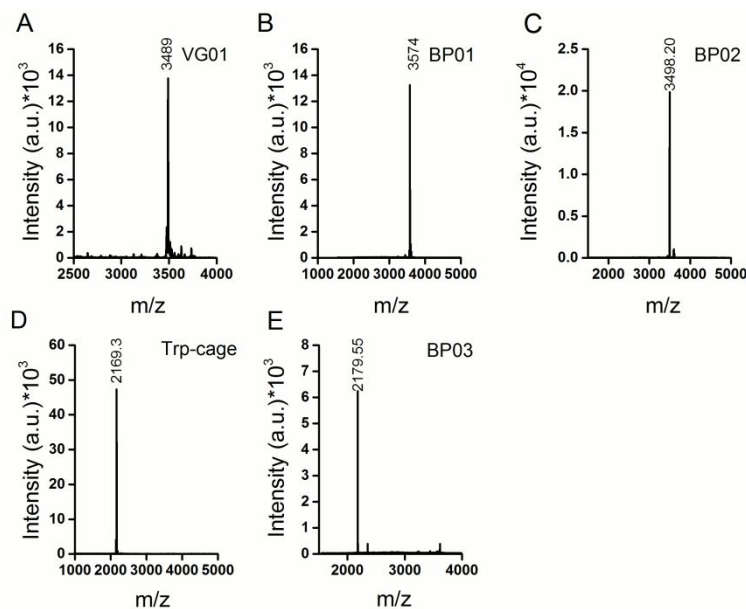
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## Purification and Characterization of Peptides

Synthesized peptides were purified by performing HPLC (Shimadzu, LC 20AD). The HPLC chromatograms of the synthesized peptides are shown in Figure S1. The observed mass was found equivalent to the calculated mass (Figure S2).



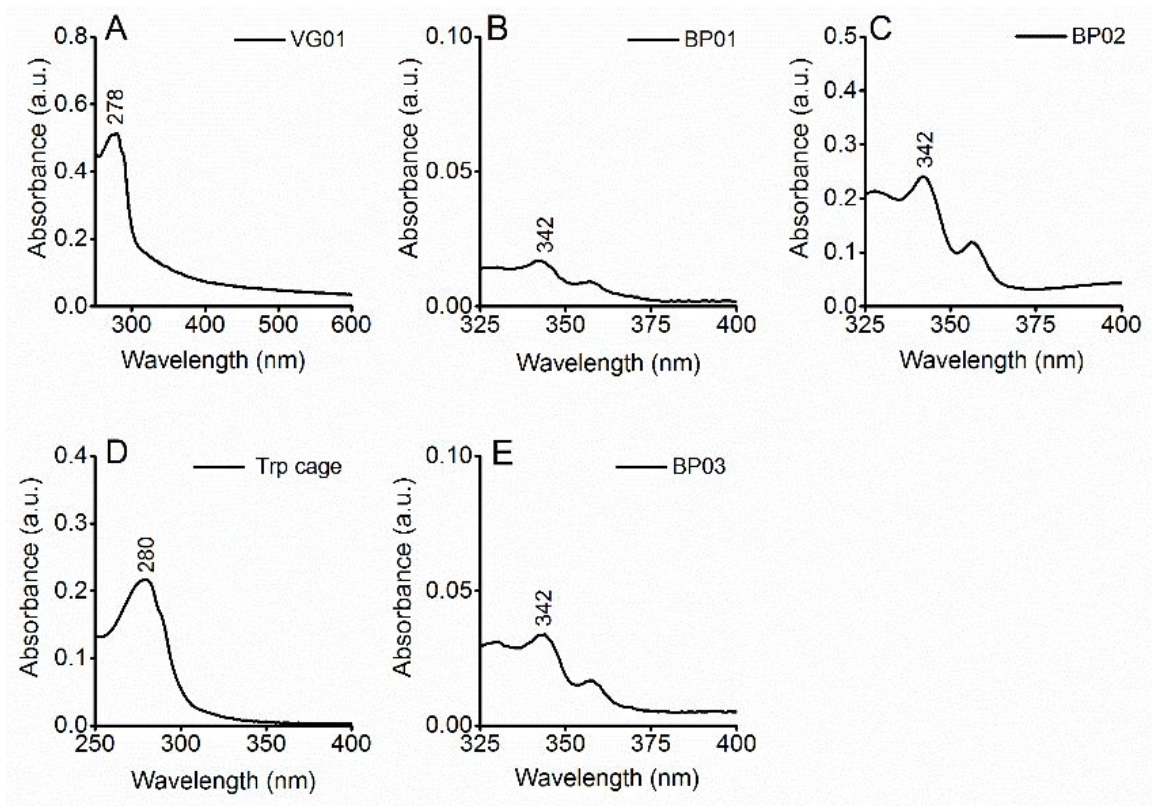
**Figure S1.** HPLC Chromatogram of synthesized peptides. Chromatogram was recorded at 342 nm (for BP01, BP02, and BP03), 280 nm (for Trp-cage and VG01), and 210 nm (for VG01, Trp-cage, BP01, BP02, and BP03).



**Figure S2.** MALDI/TOF chromatogram of synthesized peptides; (A) VG01, (B) BP01, (C) BP02, (D) trp-cage, (E) BP03

## UV-vis Absorption Spectroscopy

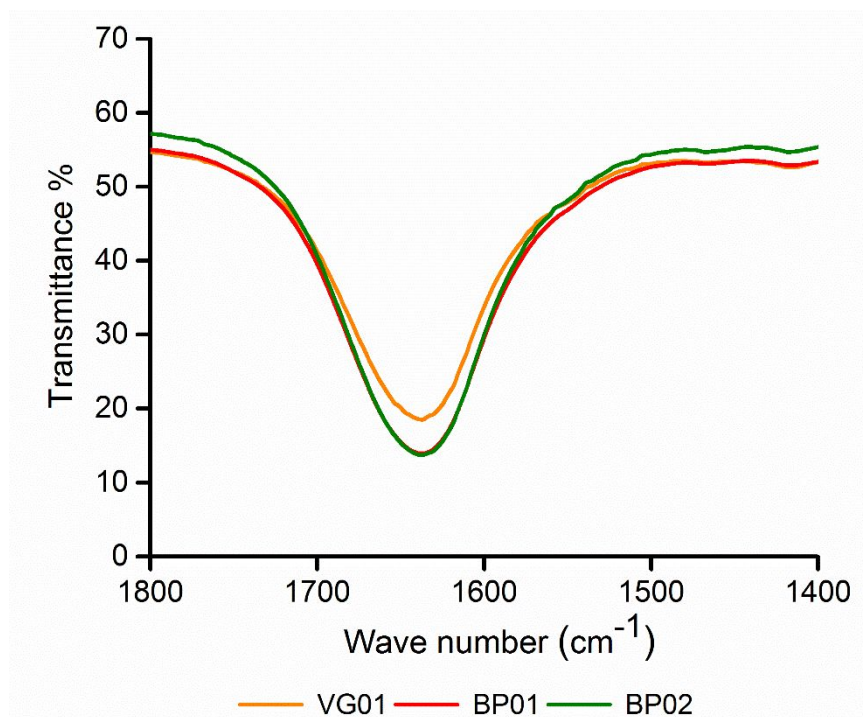
UV-vis absorption spectra were recorded to confirm the presence of AzAla in the designed peptide which showed the absorption peak of peptide BP01, BP02 and BP03 at 280 nm and 342 nm while VG01 and Trp-cage showed at 280 nm (Figure S3).



**Figure S3.** UV-vis absorption spectra of the synthesized peptides. The absorption peaks (A) VG01, and (D) Trp-cage showed absorption peak at 280 nm while of (B) BP01, (C) BP02, and (E) BP03 were observed at 342 nm.

## Secondary Structure Determination

CD spectroscopy was used as a standard method to determine the secondary structures of proteins. The spectra of proteins BP01, BP02, and VG01 shows positive peak at 215 nm with low ellipticity and negative peak at 195 nm suggesting disordered structure (Figure 2D). FT IR spectra of the proteins show absorption in amide-I region (Figure S4).



**Figure S4.** FT IR spectra of the synthesized proteins VG01 (orange), BP01 (red) and BP02 (green). Spectra shows fingerprint region at 1638 cm<sup>-1</sup> which confirms  $\beta$  sheet content of the proteins.