Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Hydrogen Bonding Play a Significant Role in Binding of Coomassie Brilliant Blue-R to Hemoglobin: FT-IR, Fluorescence and Molecular Dynamics Studies

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Fluorescence spectra of free BHG (5mM) and free CBB-R

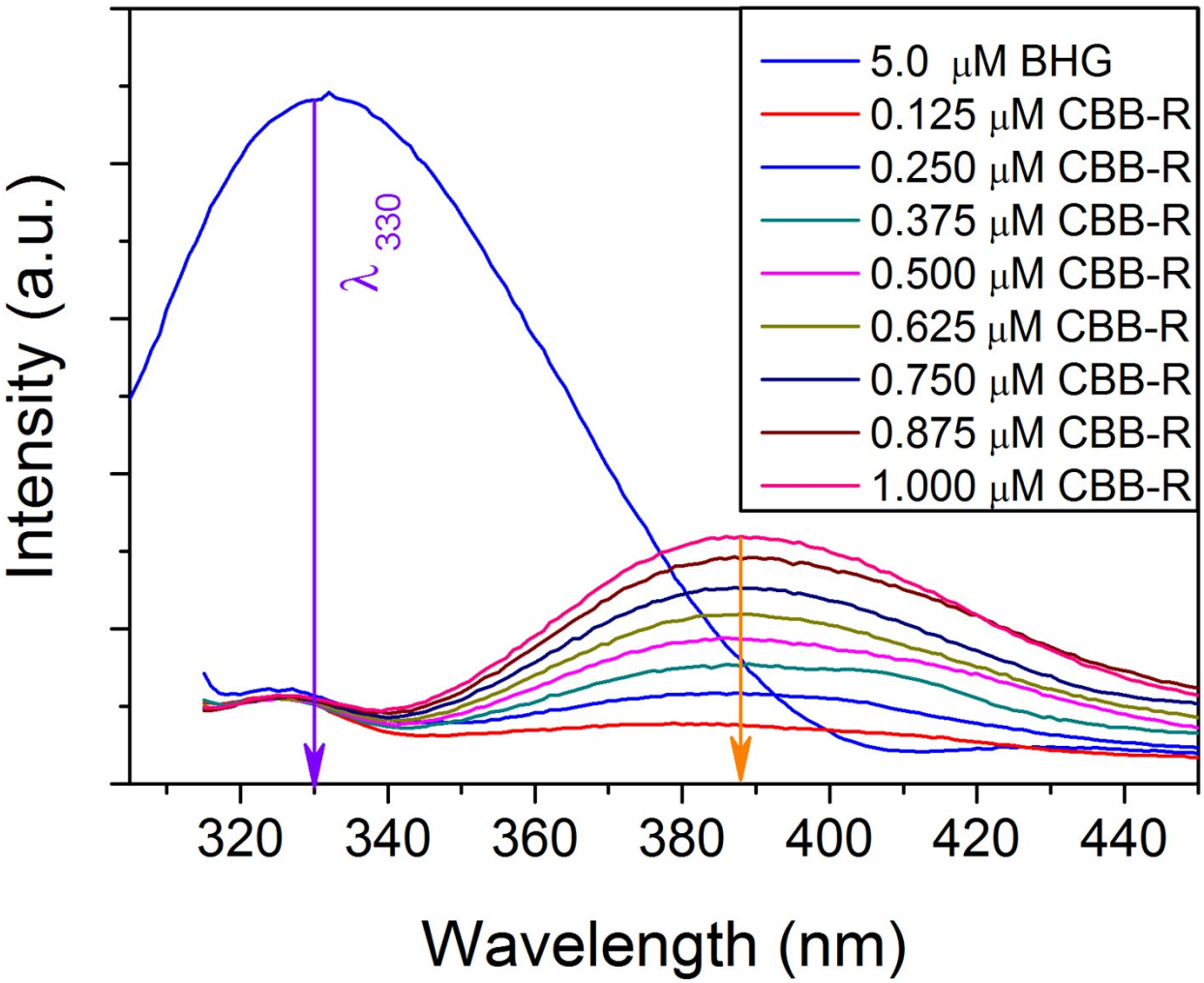

Figure S1: The fluorescence emission spectra of free BHG (5mM) and free CBB-R at different concentration. These concentrations of CBB-R were used for quenching study of BHG. From the above spectra it was clear that the contribution of CBB-R fluorescence in BHG fluorescence at 330 nm was minimum even at maximum concentration of CBB-R.

Normalization and inner-filter correction:

Each and individual fluorescence spectrum of the protein in the presence of different concentration of CBB-R was multiplied with a normalized factor (fluorescence intensity of the protein alone solution at 440 nm divided by fluorescence intensity of the protein/CBB-R complex.) The resultant spectrum was the contribution of CBB-R to the protein/CBB-R complex and it was subtracted from the original spectrum. Then the inner-filter correction was done by using equation 1 (in main manuscript).

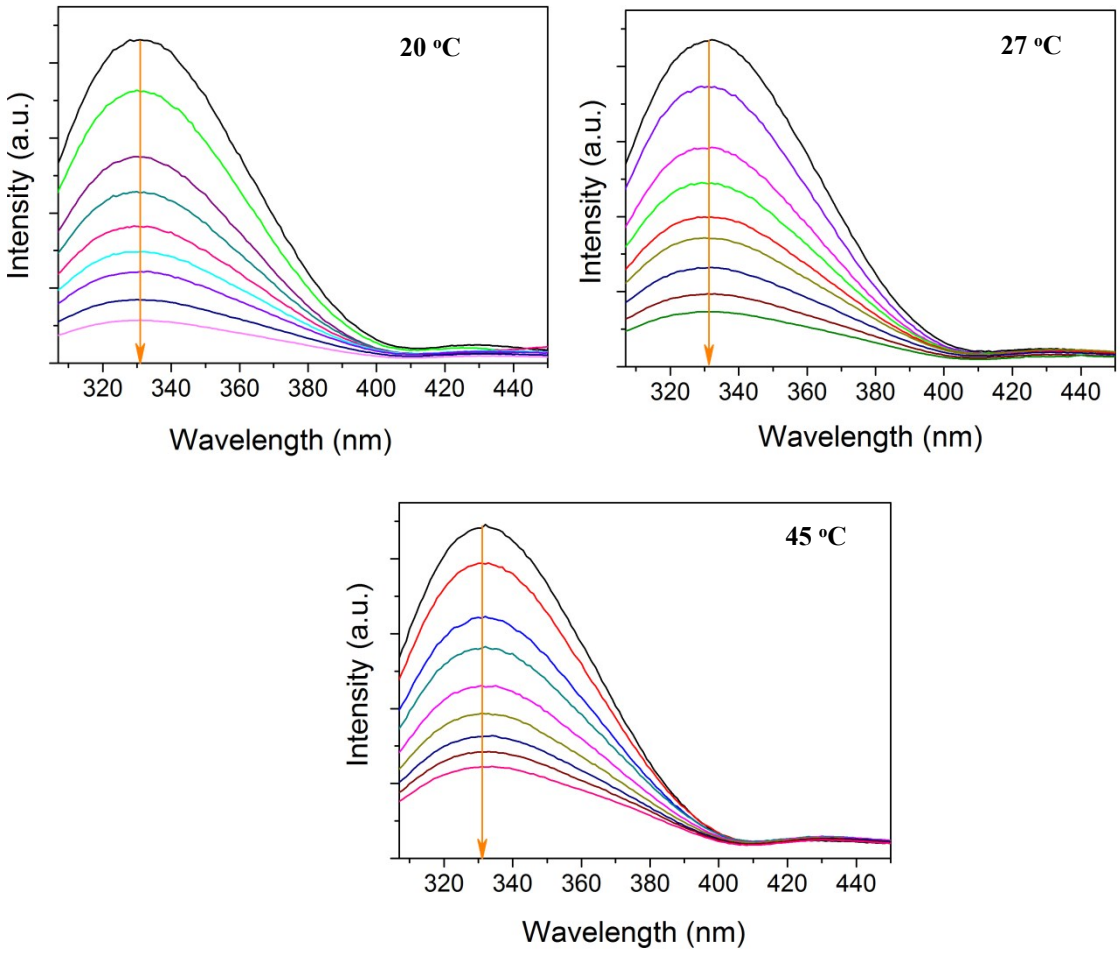

Figure S2: Change of fluorescence spectrum of 5.0 μM BHG solutions upon the addition of different concentration of CBB-R in 10.0 mM phosphate buffer at pH 7.4 at other three different temperatures (20 °C, 27 °C and 45 °C), with the increase of CBB-R concentration the intrinsic fluorescence intensity of BHG decreases. The concentration of CBB-R was varied from 0.125 μM to 2.0 μM. The excited wavelength was (λ_{ex}) 290 nm and the path length of the cell was 10 mm. All spectrums are normalized by using the emission values at 440 nm and inner-filter corrected.

The plot of F_0/F_{corr} against different concentration of CBB-R

The corrected fluorescence data were analyzed by using Stern-volmer equation.

$$\frac{F_0}{F_{corr}} = 1 + K_{SV}[Q]$$

Where F_0 and F_{corr} are the fluorescence intensity of BHG in the absence and presence of CBB-R, respectively. K_{SV} is the stern-volmer constant and [Q] is the concentration of CBB-R in the protein solution. Figure S3 shows the fitted Stern–Volmer plots.

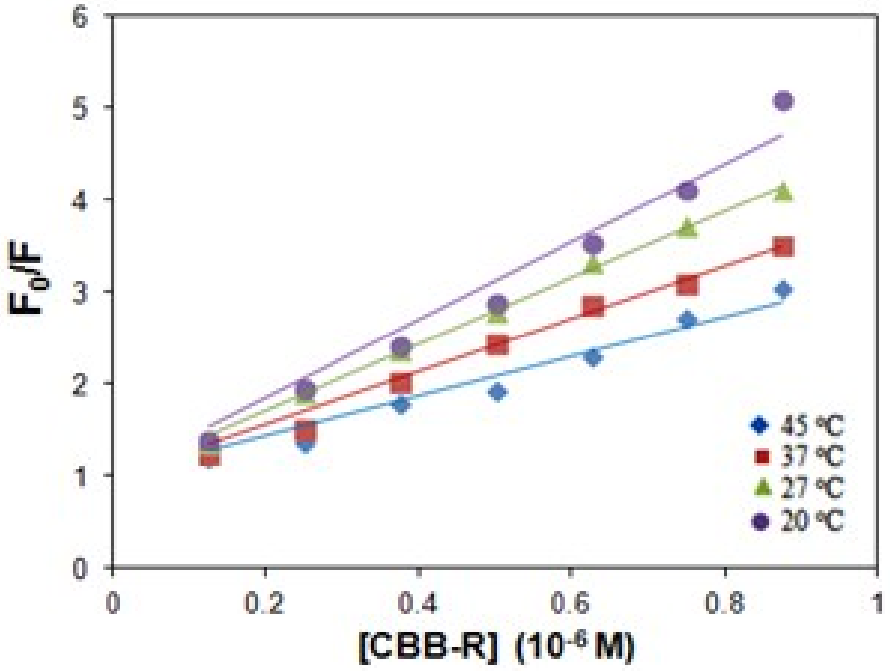

Figure S3: The panel shows the S–V plot obtained using the fluorescence intensity at 330 nm of BHG and in the presence of the different concentrations of CBB-R at four different temperatures.

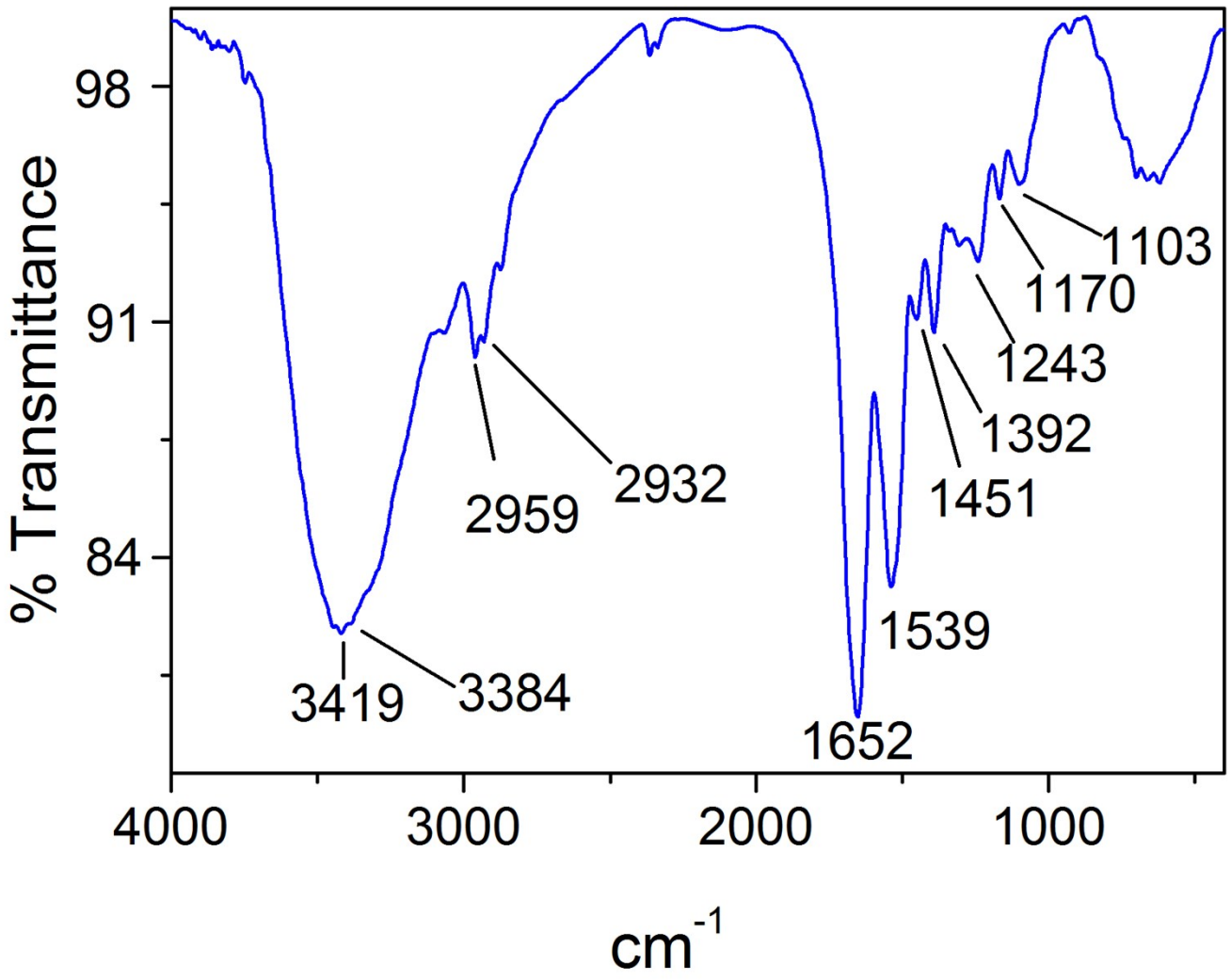

Figure S4: FT-IR spectra of bovine hemoglobin collected from KBr pellet techniques

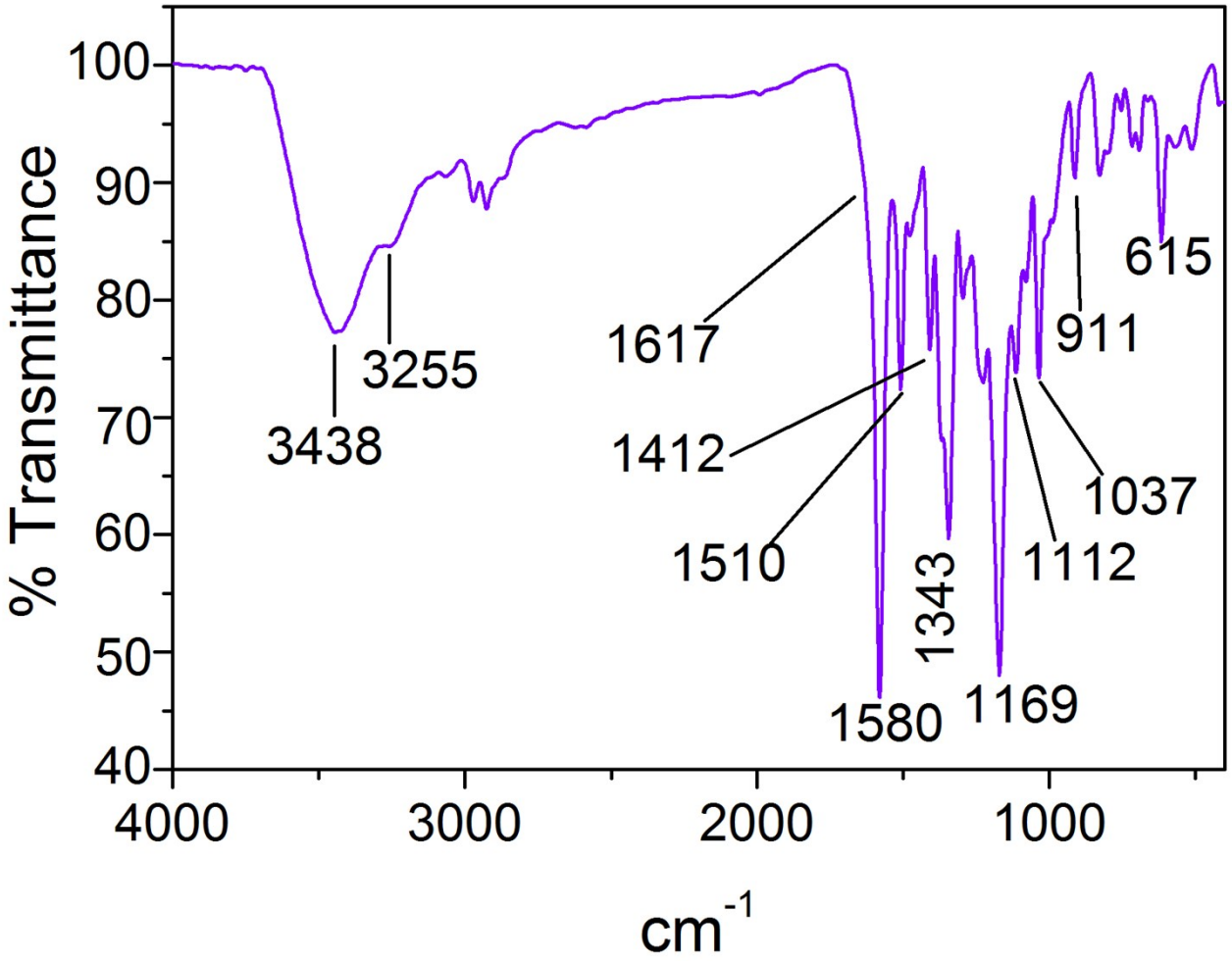

Figure S5: FT-IR spectra of coomassie brilliant blue R (CBB-R). The spectrum was recorded using KBr pellet technique.

Full range FT-IR spectra of BHG and CBB-R in aqueous buffer solutions. Different panels represent different solution conditions. All the samples were prepared in 5mM phosphate buffer at pH 7.4. The instrumental base was corrected with same buffer solution.

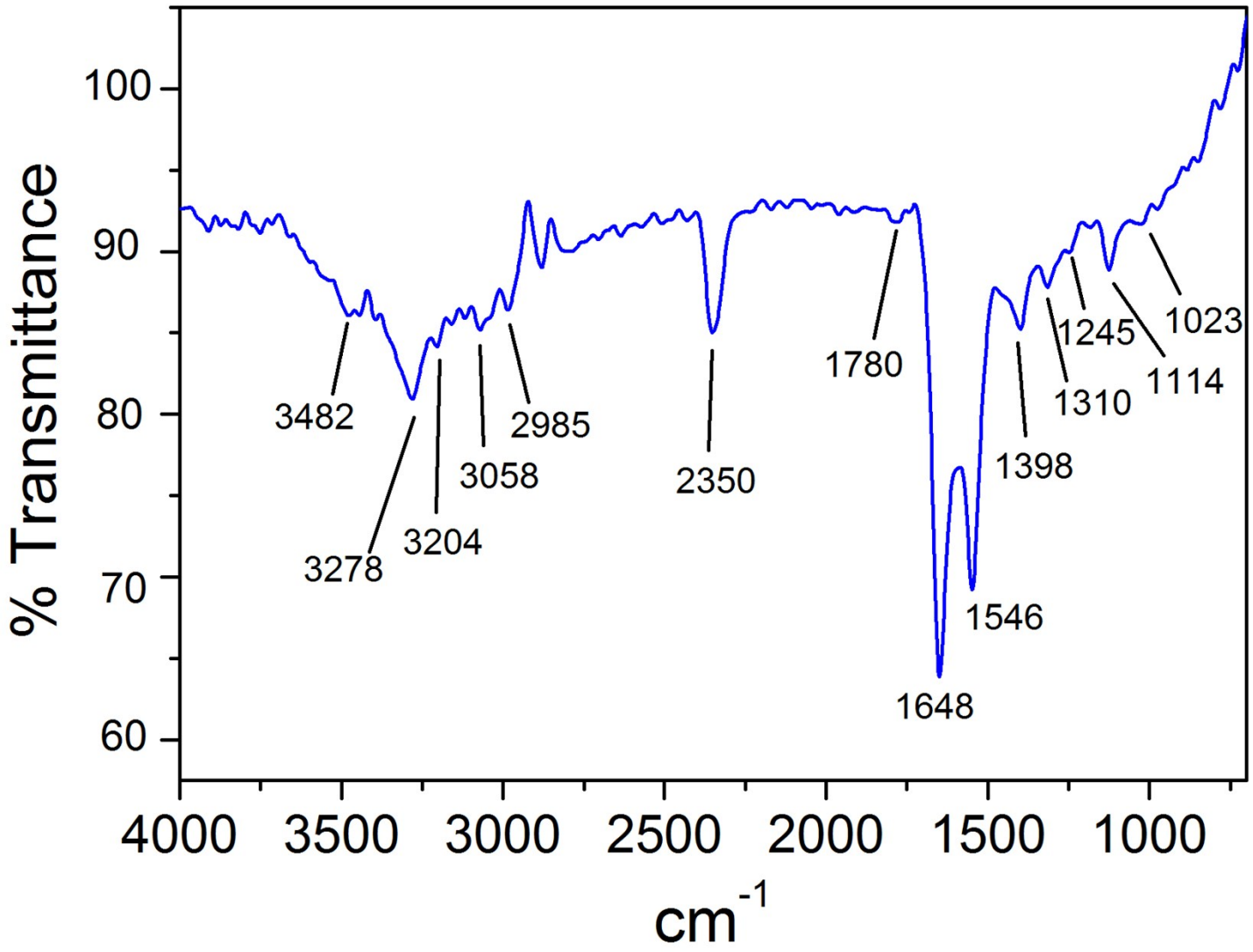

Figure S6: panel A. FT-IR spectra of BHG (10 μM) in aqueous buffer solution.

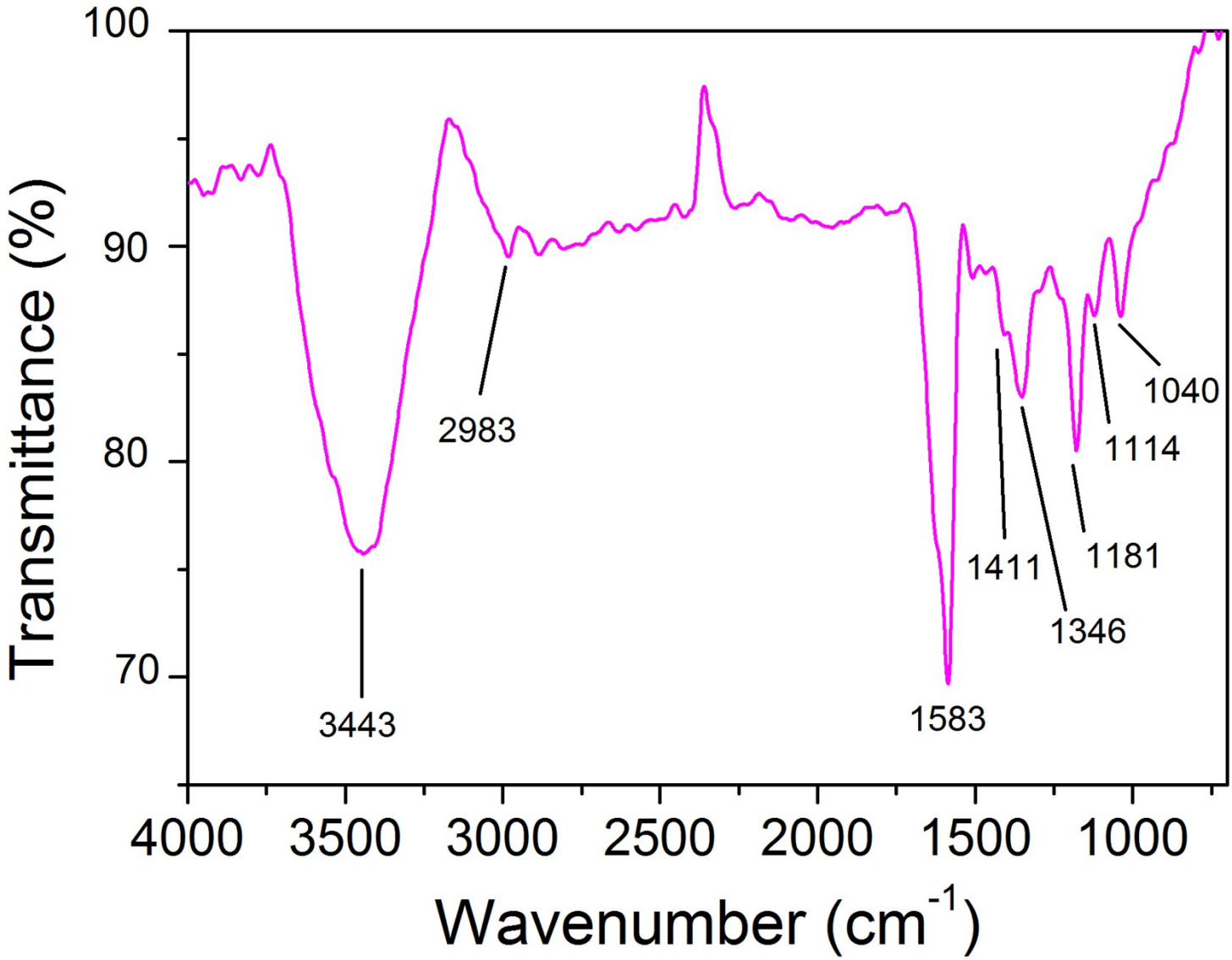

Figure S6: Panel B**.** FT-IR spectra of CBB-R (20.0 μM) in aqueous buffer solution.

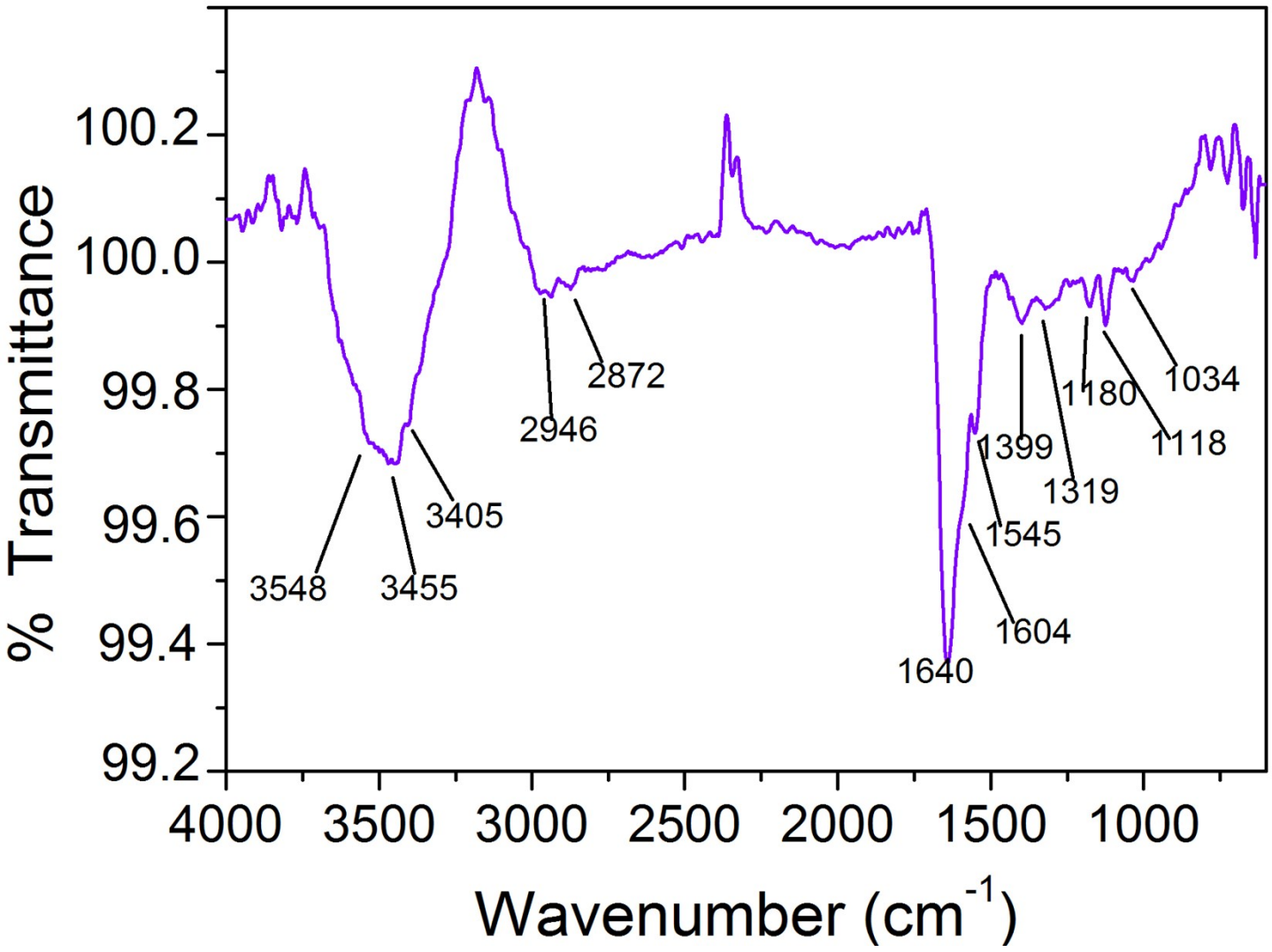

Figure S6: Panel C. FT-IR spectra of BHG (10μM) in the presence of CBB-R (7.5 μM) in aqueous buffer solution.

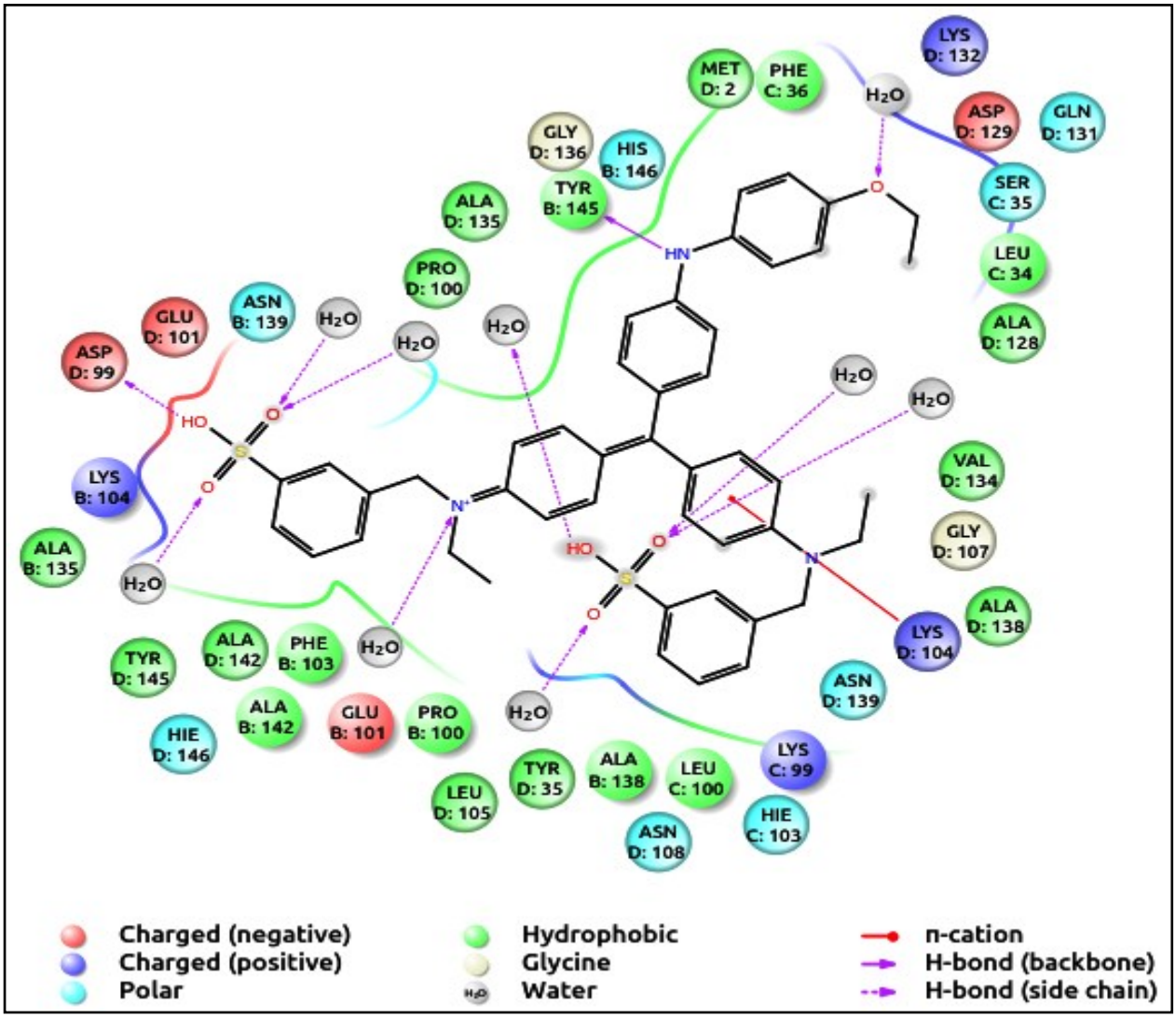

Figure S7: 2D plot of CBB-R with BHG complex observed after 40 ns of MD simulation. The H-bonds between Asp99 of β2 subunit, Tyr145 of β1 subunits, and π-cation interactions between Lys104 of β1 subunits (the notation as A, B, C and D below the three letter code of amino acids indicates α1, β1, α2 and β2 subunit, respectively).

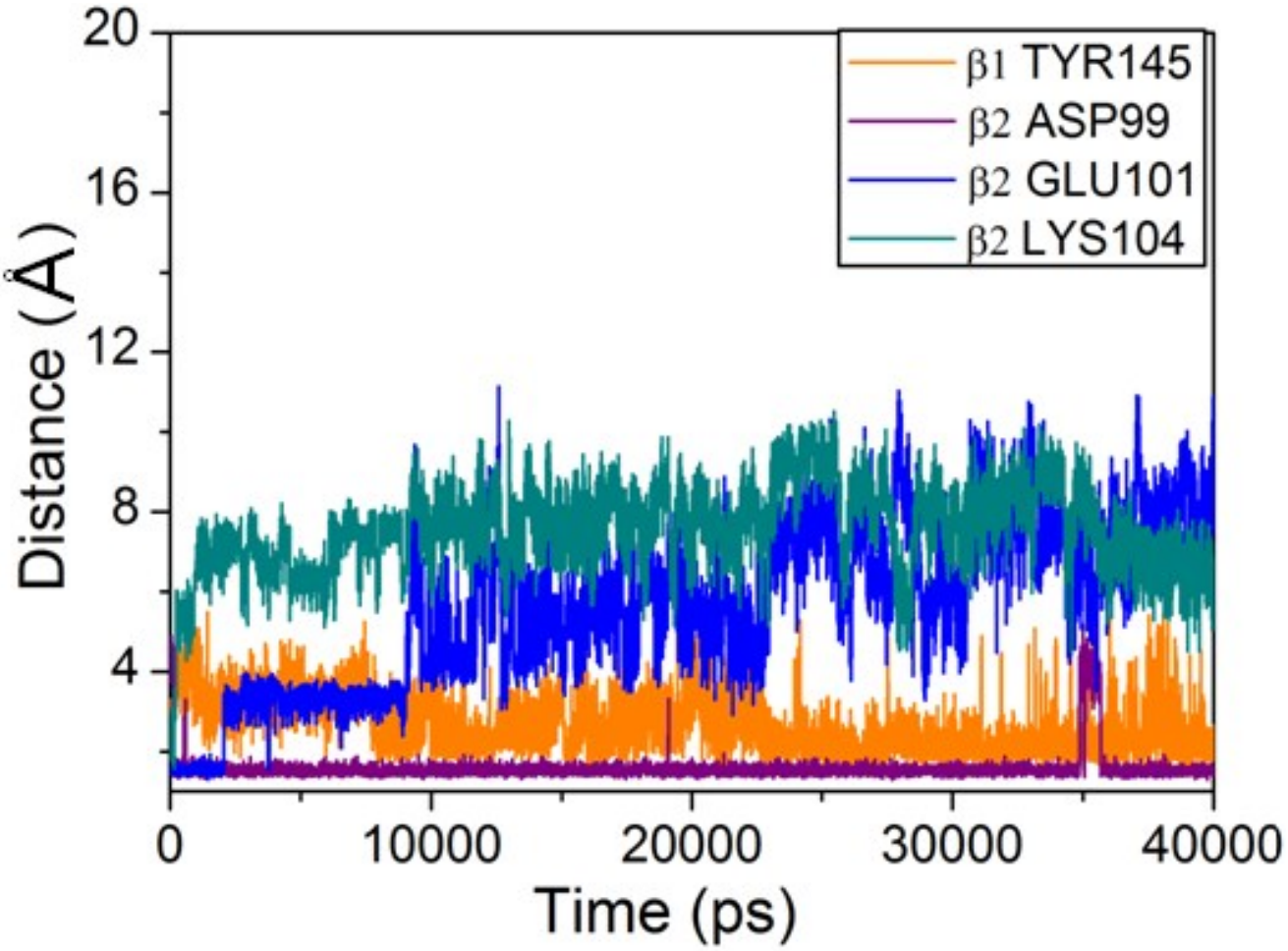

Figure S8: Hydrogen bonding donar-acceptor distances during 40000 ps MD simulation.

Molecular docking of CBB-G with BHG:

The ΔG^0 value of best energy conformer as docked was -26.33 kJ mol^{-1}. The best energy ranked structure revealed that CBB-G located near the cavity formed among the α1, β1 and β2 subunits. It was observed that CBB-G was stabilized by hydrogen bond interaction with Lys104 of β1 subunit, Asp94 and His97 of β2 subunits of the protein. The two π -cation interactions were also observed with Lys104 of β1 subunit.

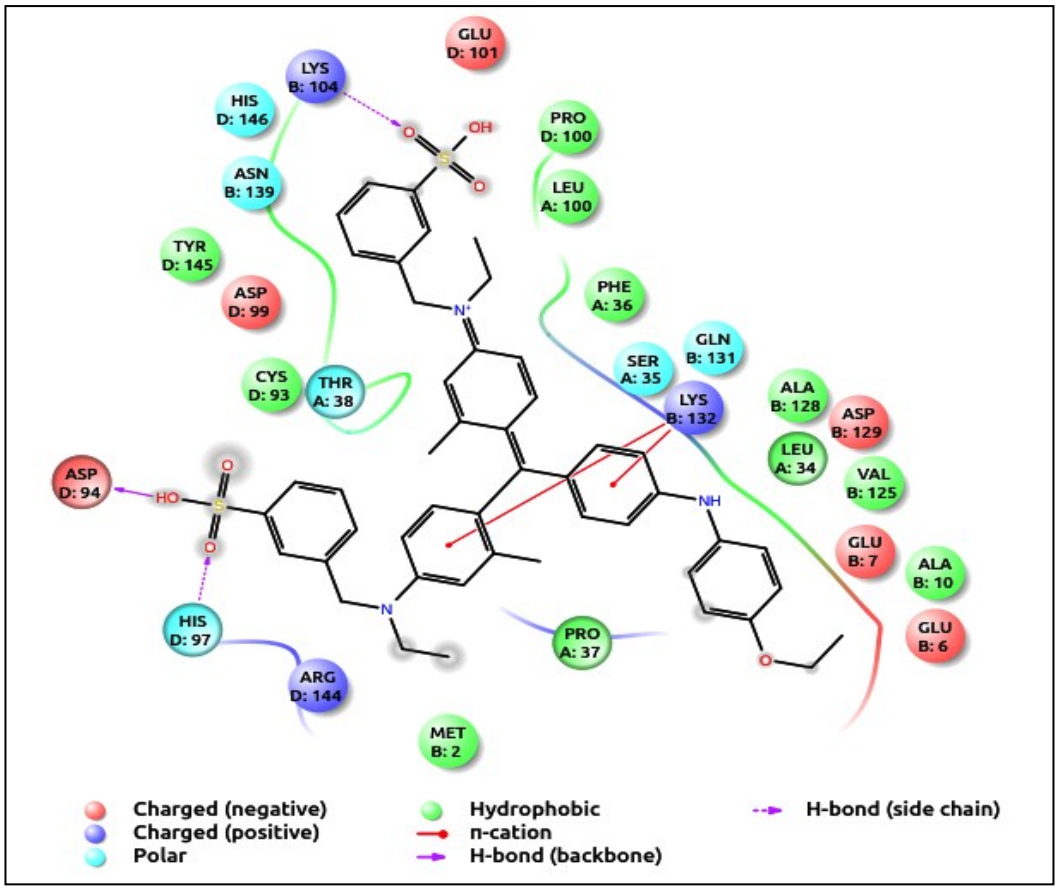

Figure S9: 2D plot of docking conformation observed during docking simulation. The H-bonds between Lys104 of β1 subunit, His97 and Asp94 of β2 subunit, and two π-cation interactions between Lys132 of β1 subunit (the notation as A, B, C and D below the three letter code of amino acids indicates α1, β1, α2 and β2 subunit, respectively).

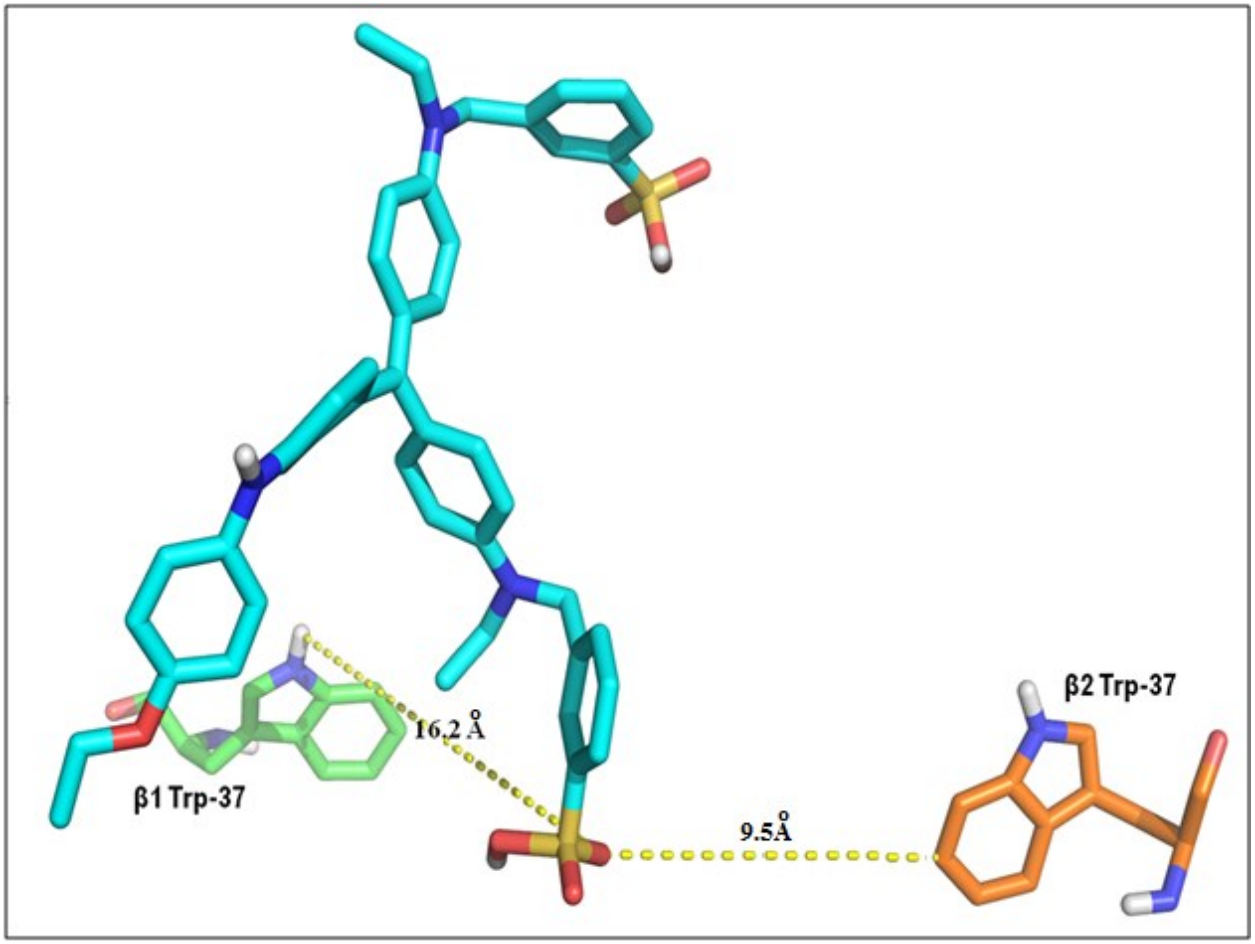

Figure S10: The distance between CBB-R and subunit of β1 Trp37 and β2 Trp37 after 40 ns MD simulation, presented by stick model using pymol.

Time-resolved fluorescence measurements:

Fluorescence lifetime measurement was performed by time correlated single-photon counting (TCSPC) method on FluoroCube-01-NL spectrometer (Horiba Jobin Yvon) using a Laser-Diode has an output of 375 nm, The signals were collected at the magic angle at 54.7^0 and the details of the method are given elsewhere.[1 2] The Excitation wavelength was 290 nm.

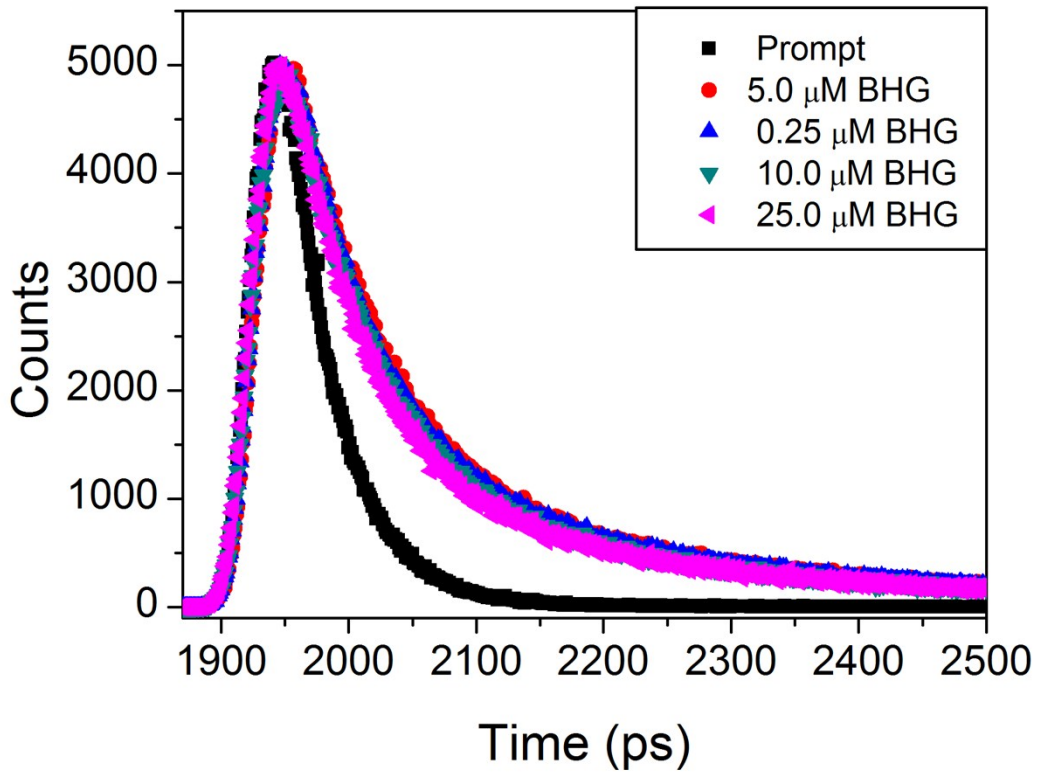

Figure S11: Time resolved fluorescence decay of BHG in solution and in the presence of CBB-R. 'Prompt' is the laser pulse profile.

Table TS1: Fluorescence lifetime of BHG as a function of concentrations of CBB-R

samples	**τ_1(ns)**	**τ_2(ns)**	**τ_3(ns)**	**A_1**	**A_2**	**A_3**	**τ (ns)**	**χ^2**
BHG	**1.2**	**5.4**	**0.08**	**0.05**	**0.01**	**0.94**	**~0.2**	**1.07**
BHG:CBB-R (1:2)	**1.3**	**5.4**	**0.08**	**0.04**	**0.01**	**0.95**	**~0.2**	**1.01**

References:

1. B. K. Paul and N. Guchhait, *J. Phys. Chem. B*, 2011, 115, 10322-10334.
2. W. Peng, F. Ding, Y.-K. Peng and Y. Sun, *Mol. BioSyst.*, 2014, 10, 138-148.