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Supporting Information

Live cell fluorescent stain of bacterial curli and biofilm through supramolecular recognition between bromophenol blue and CsgA

Lixia Zhang, Zhenhua Li, Zhijun Chen*

State Key Laboratory of Supramolecular Structure and Materials, Institute of Theoretical Chemistry, College of Chemistry and International Joint Research Laboratory of Nano-Micro Architecture Chemistry, Jilin University, 2699 Qianjin Street, 130012, Changchun, P. R. China

E-mail: zchen@jlu.edu.cn

EXPERIMENTAL METHODS

Reagents and Chemicals

Yeast extract and tryptone were obtained from Angel Yeast CO., Ltd (Tianjin, P. R. China). Ager, IPTG, GdnHCl, imidazole, EDTA and $(Ni)_2SO_4$ were purchased from Dingguo Changsheng Biotechnology (Beijing, P. R. China). KH_2PO_4 and K_2HPO_4 were obtained from Tianjin Guangfu Technology Development CO., Ltd (Tianjin, P. R. China). Escherichia coli (BL21, DE3) cells were purchased from Agilent Technologies Co., Ltd (Beijing, P. R. China). Normal non-immune goat serum were purchased from Dingguo Changsheng (Beijing, P. R. China). Deionized water (18.0 M Ωcm^{-1}) was purified using Water Purifier (Water Purified, Chengdu, P. R. China).

CsgA Purification

The CsgA with carboxyl-terminally encoded His tag, was subcloned into PET101/D bacterial expression vector. The E. coli strain BL21 (DE3) was transformed with the CsgA encoded plasmid. Protein overexpression were performed according to Chapman at al.[19] A single E. coli colony was inoculated into LB media. The overnight culture was transferred into a large culture flask containing 1L LB media and cultured till the OD_{600} reach 0.6. IPTG (1mM) was then added to the culture for 1-h induction.

The cells were collected via centrifugation for 10 min at 5000×g. The resulting precipitated cells were resuspended in lysis buffer (50 mM Kpi, 8 M GdnHCl, pH 7.2) overnight (12 h) followed by breaking using ultrasound (Beijing, P. R. China).

Following centrifugation for 60 min at 10000×g, the pellet was removed and the supernatant was filtrated through a 0.22-μm polyethersulfone filter. The supernament was mixed with the nickel-nitrilotriacetic acid (Ni-NTA) beads for 3 h at 4 °C before loading to the column. The Ni-NTA column was washed with 10 column volume of buffer A (50 mM Kpi, 8 M GndHCl, 12.5 mM imidazole, pH 7.2) and eluted with 5 column volume of buffer B (50 mM Kpi, 8 M GndHCl, 125 mM imidazole, pH 7.2) and stored at −80 °C.

BPB Fluorescence Assay

The purified CsgA was dialyzed against PBS buffer to remove GndHCl and imidazole. At different time intervals, the samples were taken and mixed with BPB (30 μM) followed by fluorescence measurement using the RF-5301PC fluorescence spectrophotometer (Shimadzu, Japan). Also, a series of different concentration of CsgA fibrils were combined with BPB (30 μM) for quantitative detection. For interference test, 1% serum was added into the test tubes. For selectivity test, CsgA fibrils were replaced by various biological samples such as DNA, BSA, pepsin, papsin, trypsase, CsgA fibrils, RNA and metal ions (30 μM each).

CD Spectroscopy

CsgA fibrillation were recorded on a J-810 CD spectrometer (JASCO, Tokyo, Japan) (190−260 nm) in a quartz cell with a path length of 0.1 cm.

Morphology of CsgA

Confocal fluorescence micro-images (CLSM) were measured on a LSM710 instrument (ZEISS, Germany). Transmission electron microscopy (TEM) images were taken on a Hitachi H-8100 microscope with an acceleration voltage of 200 kV. The samples (3 μL each) were dropped on the copper net and dried before testing. Atomic force microscopy (AFM) images were collected on an SPA 300 atomic force microscope (NSK, Japan). The samples (3 μL each) were dropped on the treated silicon wafer and dried before testing.

Curli Biofilm Formation

LB-BPB plates (control) contain 1×10^{-2} g mL^{-1} peptone, 5×10^{-3} g mL^{-1} yeast extract, 1×10^{-2} g mL^{-1} NaCl, 1.5×10^{-2} g mL^{-1} ager and 50 μM BPB. LB-BPB plates (induction) were supplemented with 1 mM ampicillin and 1×10^{-3} M IPTG. Then the cells were steaked or spotted onto the plates and incubated for 24 h at 37 °C.

Table S1. Determination of CsgA fibers in 1% serum (n=5).

Samples	CsgA fiber added (μM)	CsgA fiber found (μM)	RSD (%)	Recovery (%)
Serum (1%)	10.00	10.06	2.20	100.6
	15.00	14.79	2.16	98.6
	20.00	20.84	3.16	104.2

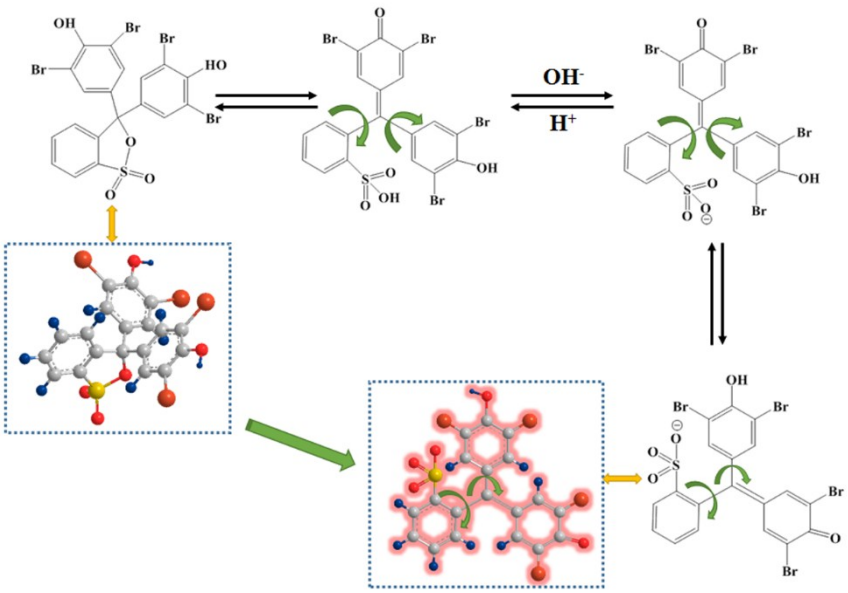

Fig. S1 The possible light up mechanism of BPB after binding to CsgA fibrils.