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## Supramolecular interactions of oxidative stress biomarker glutathione with fluorone black



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### ABSTRACT

Oxidative stress biomarkers, including glutathione (GSH) and related compounds, are involved in a variety of interactions enabling redox potential maintenance in living cells and protection against radicals. Since the oxidative stress is promoting and, in many cases, inducing serious illnesses, monitoring of GSH levels can aid in diagnostics and disease prevention. Herein, we report on the discovery of the formation of a supramolecular ensemble of GSH with fluorone black (9-phenyl fluorone, FB) which is optically active and enables sensitive determination of GSH by resonance elastic light scattering (RELS). We have found that supramolecular interactions of GSH with FB can be probed with spectroscopic, RELS, and electrochemical methods. Our investigations show that RELS intensity for FB solutions increases with GSH concentration while fluorescence emission of FB is not affected, as quenching begins only above 0.8 mM GSH. The UV–Vis difference spectra show a positive peak at 383 nm and a negative peak at 458 nm, indicating a higher-energy absorbing complex in comparison to the non-bonded FB host. Supramolecular interactions of FB with GSH have also been corroborated by electrochemical measurements involving two configurations of FB–GSH ensembles on electrodes: (i) an inverted orientation on Au-coated quartz crystal piezoelectrode (Au@SG–FB), with strong thiolate bonding to gold, and (ii) a non-inverted orientation on glassy carbon electrode (GCE@FB–GS), with weak  $\pi$ – $\pi$  stacking attachment and efficient charge mediation through the ensemble. The formation of a supramolecular ensemble with hydrogen bonding has also been confirmed by quantum mechanical calculations. The discovery of supramolecular FB–GSH ensemble formation enables elucidating the mechanisms of strong RELS responses, changes in UV–Vis absorption spectra, and the electrochemical reactivity. Also, it provides new insights to the understanding of the efficient charge-transfer in redox potential homeostasis which is likely based on an intermediate formation of a similar type of supramolecular ensembles.

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### 1. Introduction

Maintaining redox-potential in a living organism on a proper level is a key challenge and an important activity [1–5] which is necessary to protect cells against damaging radicals and reactive oxygen species (ROS) on one hand, and to enable the immune systems to fight microbial invasion on the other hand. Among biomarkers of the oxidative stress, a tripeptide glutathione (GSH) distinguishes itself with remarkable abilities to neutralize excessive ROS and organic radicals, thus protecting

against their harmful effects on DNA, proteins, and lipids [6–8]. There are many examples of GSH protective roles against toxicants, radicals, and compounds participating in radicals' formation [9–11]. For instance, GSH can protect DNA against damage by toxicants like paraquat [10]. It has been shown that GSH not only neutralizes ROS after they are formed but it can also prevent their formation in a Fenton type  $\text{HO}^{\bullet}$  radical generation induced by catechol and Cu(II) or Fe(III) ions. Herein, we report on the interactions of GSH with a catechol-moiety bearing compound, fluorone black (FB). This compound has been proposed as a redox mediator in sensors for GSH [12], based on the mechanism elucidated earlier for catechol [13]. Since fluorone black is also optically active, we reason that the interactions of GSH with FB could further be probed using spectral analysis and light scattering methods.

There is strong evidence that oxidative stress plays a critical role or contributes to the development of many diseases such as stroke, cardiovascular diseases, diabetes, and Alzheimer's disease [14–16]. It can also contribute to the development of autism in children [17] and to other diseases [1]. In view of the increasing occurrence of these common diseases, the studies of biomarkers of oxidative stress become a new

*Abbreviations:* CV, cyclic voltammetry; DFT, density functional theory; EQCN, electrochemical quartz crystal nanogravimetry; FB, fluorone black; GCE, glassy carbon electrode; GSH, glutathione; LDOS, local density of states; MD, molecular dynamics; QM, quantum mechanics; RELS, resonance elastic light scattering; ROS, reactive oxygen species; SAM, self-assembled monolayer.

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pressing necessity for both the diagnostics and treatment [18–20] and many spectroscopic, electrochemical, and other techniques have been applied for their analysis [11,20–26].

Fluorone black (Fig. 1) is structurally based on a rigid triple ring of xanthene providing pronounced fluorescence properties. Fluorone and other xanthene dyes have found wide applications in the detection of high-valency metal ions [27–30], biothiols [12,13,31,32], sialic acid [33], in screening assays for mitochondrial pore opening/closing [34], and in other fields. They were also utilized in construction of various oligonucleotide molecular beacons [10,22,35] for biomedical diagnostics, imaging and therapy.

Earlier electrochemical studies of Compton and coworkers [13,32] and the Strongin group [12,31] have indicated that FB interacts with GSH, possibly forming substitution compounds at the reactive sites of xanthene triple ring. While the formation of substitution compounds is well documented, it is reasonable to assume that the initial

interactions between FB and GSH result in an intermediate weakly-bound associate in the form of a supramolecular ensemble. Herein, we present the results of investigations of the initial GSH interactions with FB using resonance elastic light scattering (RELS), UV–Vis spectroscopy, fluorescence, electrochemical methods, and DFT calculations to evaluate the predicted formation of supramolecular ensembles FB–GSH. This type of supramolecular interactions is important for biothiols detection methods and may play a key role in redox potential homeostasis and recycling of GSH in biological systems.

## 2. Materials and Methods

### 2.1. Chemicals

9-Phenyl-2,3,7-trihydroxy-6-fluorone (fluorone black, FB,  $C_{19}H_{12}O_5$ ) (Fluka), L-glutathione reduced, minimum 99% (GSH,  $C_{10}H_{17}N_3O_6S$ ) and methanol were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, U.S.A.). Sodium phosphate monobasic dihydrate ( $NaH_2PO_4 \cdot 2H_2O$ ) and sodium phosphate dibasic ( $Na_2HPO_4 \cdot 7H_2O$ ) were obtained from Fisher Scientific Company (Pittsburgh, PA, U.S.A.). All chemicals used for investigations were of analytical grade purity. Solutions were prepared using Milli-Pore Milli-Q deionized water (conductivity  $\sigma = 55$  nS/cm). They were deoxygenated by bubbling with purified argon.

The stock solutions of 100  $\mu$ M fluorone black in methanol and 10 mM glutathione in 20 mM phosphate buffer pH 7.43 were using in these experiments.

### 2.2. Apparatus

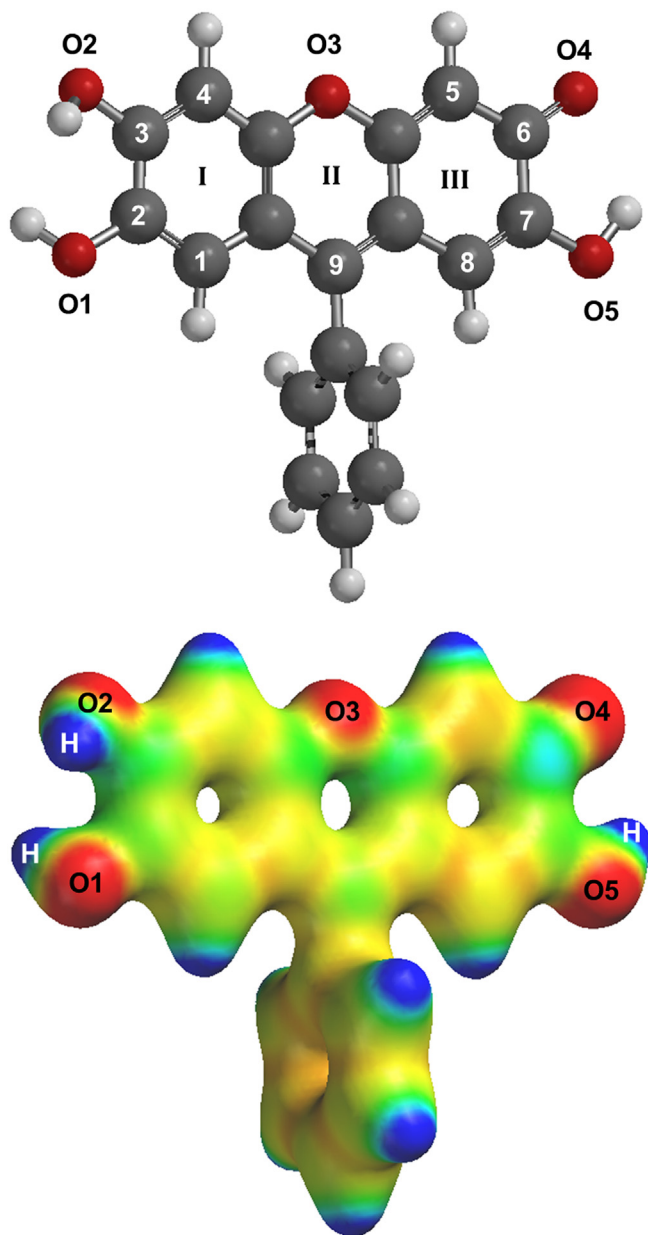
The fluorescence spectra were recorded using LS55 Spectrometer (Perkin Elmer, Waltham, MA, U.S.A.) equipped with 20 kW Xenon light source operating in 8  $\mu$ s pulsing mode. Separate monochromators for the incident beam and the detector beam enabled to use monochromatic radiation with wavelengths from 400 nm to 740 nm. The dual detector system consisted of a photomultiplier tube (PMT) and an avalanche photodiode.

The UV–Vis spectra were recorded using Varian Cary 50 Bio, UV–Visible Spectrophotometer (Varian Inc.) in the range from 200 nm to 600 nm at room temperature.

The voltammetric and nanogravimetric measurements were recorded using Model PS-205B Potentiostat/Galvanostat and Electrochemical Quartz Crystal Nanobalance, Model EQCN-700 (Elchema, U.S.A.) with a Data Logger and Control System, Model DAQ-716v, operating under Voltscan 5.0 data acquisition and processing software. A double-junction saturated (KCl) Ag/AgCl electrode (Elchema) was used as the reference electrode, Pt wire (Elchema) as the counter electrode and gold coated quartz crystal piezoresonators (QC-10Au-PB, Elchema) with resonant frequency of 9.975 MHz or glassy carbon electrode (GCE, Elchema) were used as working electrodes. During all experiments, the electrochemical cell was maintained in a Faraday cage to minimize the electromagnetic noise.

### 2.3. Procedures

The gold electrodes used in electrochemical nanogravimetric experiments were 5 mm dia., concentric disk electrodes deposited over a Ti basal film on 14 mm dia., 0.166 mm thick, quartz crystal resonator wafers. The geometric surface area of Au electrodes was 0.196  $cm^2$  and the roughness factor  $R = 1.3$ . Before the experiments, they were thoroughly washed with acetone and propanol, followed by rinsing with distilled water. Then, each electrode was subjected to potential scanning from  $E = 0$  to +800 mV vs. Ag/AgCl in 0.1 M NaOH with scan rate of 50 mV/s, and then to scanning from  $E = -300$  to +1500 mV in 0.1 M  $H_2SO_4$  solution until stable voltammogram typical for a clean polycrystalline gold electrode was achieved [36]. The electrochemical



**Fig. 1.** Structure of fluorone black (FB) and electron density surface with mapped electrostatic potential, scaled from negative (red) to positive (blue); electron density  $d = 0.08$  au $^{-3}$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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