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Autofluorescence generation and elimination: a lesson from glutaraldehyde

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Experimental Section

1. Reagents

Glutaraldehyde (grade I, 70% in H_2O, specially purified for use as an electron microscopy fixative or other sophisticated use) and diethylenetriamine (ReagentPlus®, 99%) and Triethylenetetramine hydrate (98 %), ethylenediamine (ReagentPlus®, 99%), N-(3-Trimethoxysilylpropyl) diethylenetriamine, N-[3-(Trimethoxysilyl)propyl]ethylenediamine (97 %), histone from calf thymus (Type ll-A, lyophilized powder), L-Ascorbic acid (bioxtra, CRYSTALLINE), CM Cellulose, Phosphate buffered saline, L-glutathionine reduced (>98 %) and hydroquinone were purchased from Sigma-Aldrich and used in as received. Ammonia (28~30 % aqueous solution) was purchased from Samchun chemicals in Korea.

2. Synthesis of fluorophores

All samples were prepared either in PBS or deionized water (18MΩ). The effect of ionic strength on the synthesis of fluorophore was conducted by comparing samples made out of solutions with different ion concentration. In general, glutaraldehyde (20 mM) and other compounds with amine groups, such as diethylenetriamine (9.7 mM), triethylenetetramine (13.7 mM), Trimethoxysilyl propyl) zdiethylenetriamine, N-[3-(Trimethoxysilyl)propyl]ethylenediamine (97%) and histone were mixed in the aqueous solution at pH 12 and stayed in the dark at room temperature for 24 hours. In

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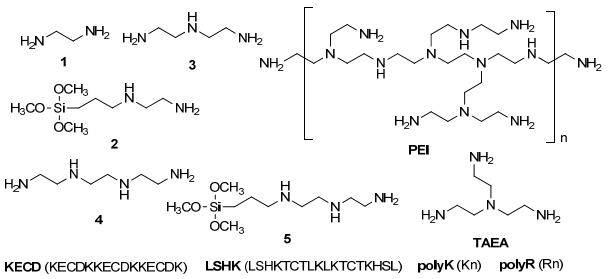
terms of purification, samples were concentrated through lyophilizing (Ilshin Lab, TFD 5505) and then purified with Ion exchange column (CM Cellulose). 90 mM ~ 100 mM NH_3 solution was used as eluent. Samples were further analyzed with HPLC-MS ststem (acetonitrile/water, MSI-MS system (LCQ).

Reducing agents, such as sodium borohydride, L-Ascorbic acid, L-glutathionine reduced, hydroquinone, were added to the sample at a molar ratio of glutaraldehyde and reducing agents at 1:1.

Measuring quantum yield was conducted compared to Rhodamin 6G. Emission spectra and absorption spectra were obtained with QM-40 (Photon Technology International, Inc.) and S-4100 (SCINCO), respectively.

Freeze-and-thaw method was used to degas. Nitrogen gas was refilled after degassing steps to prevent inflow of oxygen from the air, and sample was left at room temperature before taking spectra.

Live cell was fixed by 2 wt % glutaraldehyde dissolved in PBS solution (pH 7, pH 13) and their autofluorescence was measured by confocal microscope (Carl Zeiss LSM710) under 514 nm laser excitation.



Scheme 1S Structures of glutaraldehyde-reactive species

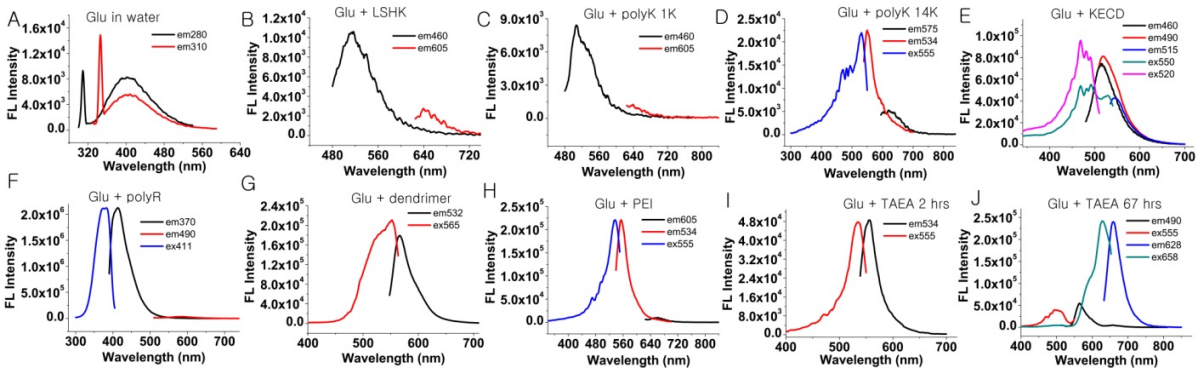


Figure 1S Spectra of 1 mg/mL glutaraldehyde solutions in the presence of 1 mg/mL various organic species. Glu represents glutaraldehyde. "em*xxx*" indicates an emission spectrum at *xxx* nm excitation. Similarly, "ex*xxx*" stands for an excitation spectrum at *xxx* nm detection. A. Aqueous glutaraldehyde solution (λ_{max} 400 nm). The emission is likely due to impurities. B. With peptide LSHK (LSHKTCTLKLKTCTKHSL), showing weak green (λ_{max} 515 nm) and red emission (λ_{max} 645 nm). C. With peptide poly-L-lysine (MW 1 K to 4 K), showing weak green emission (λ_{max} 515 nm). D. With peptide poly-L-lysine (MW 4 K to 15 K), showing weak yellow (λ_{max} 550 nm) and red emission (λ_{max} 625 nm). E. With peptide KECD (KECDKKECDKKECDK), showing medium green (λ_{max} 518 nm) and weak yellow emission (λ_{max} 545 nm). F. With peptide poly-L-arginine (MW 4 K to 15 K), showing strong blue emission (λ_{max} 412 nm). G. With PAMAM dendrimer, ethylenediamine core, generation 2.0, showing medium yellow emission (λ_{max} 567 nm). H. With PEI (Poly(ethyleneimine)) (MW 2 K), showing medium yellow emission(λ_{max} 555 nm). I. With Tris(2-aminoethyl)amine, showing weak yellow emission(λ_{max} 555 nm) in two hours. K. With Tris(2-aminoethyl)amine, showing weak yellow (λ_{max} 565 nm) and medium red emission (λ_{max} 656 nm) in 67 hours.

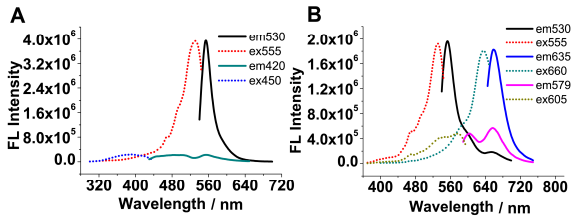


Figure 2S Fluorophore formation between glutaraldehyde and several organics. diethylenetriamine (3) at pH 12 (A) and diethylenetriamine at pH 7 in phosphate buffer (B). "em*xxx*" indicates an emission spectrum at *xxx* nm excitation. Similarly, "ex*xxx*" stands for an excitation spectrum at *xxx* nm detection.

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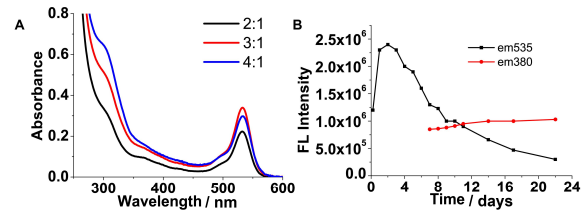

Figure 3S Formation of the yellow between glutaraldehyde and **3**. A. Absorption spectra at varied glutaraldehyde to **3** ratios. B. The yellow emitter decayed but a blue emitter occurred. The intensity plots of the above two emitters versus time.

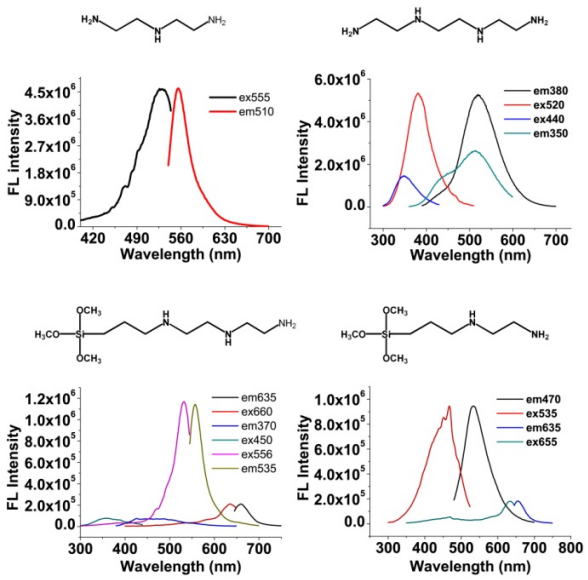

Figure 4S Spectral comparison of products between glutaraldehyde and various ethylenediamine derivatives. "em*xxx*" indicates an emission spectrum at *xxx* nm excitation. Similarly, "ex*xxx*" stands for an excitation spectrum at *xxx* nm detection. Structurally similar amine derivatives, such as **2** and **4** as well as **3** and **5**, yielded similar spectra.

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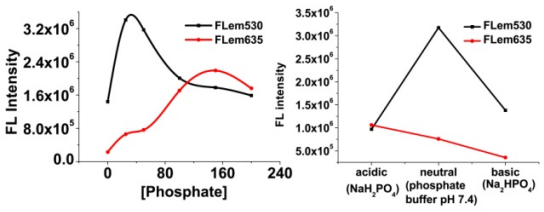

Figure 5S Influence of ionic concentration on the products of the reaction between glutaraldehyde and diethylenetriamine derivatives. "em*xxx*" indicates an emission spectrum at *xxx* nm excitation. Higher ion concentration generally promoted the formation of the red emitter. However, higher pH suppressed the generation of the red emitter.

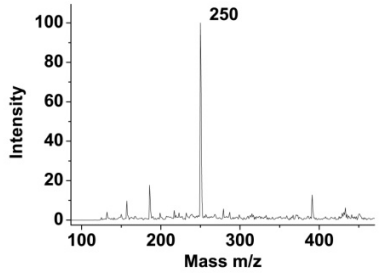

Figure 6S Mass spectrum of the preliminary purified mixture of glutaraldehyde and diethylenetriamine.

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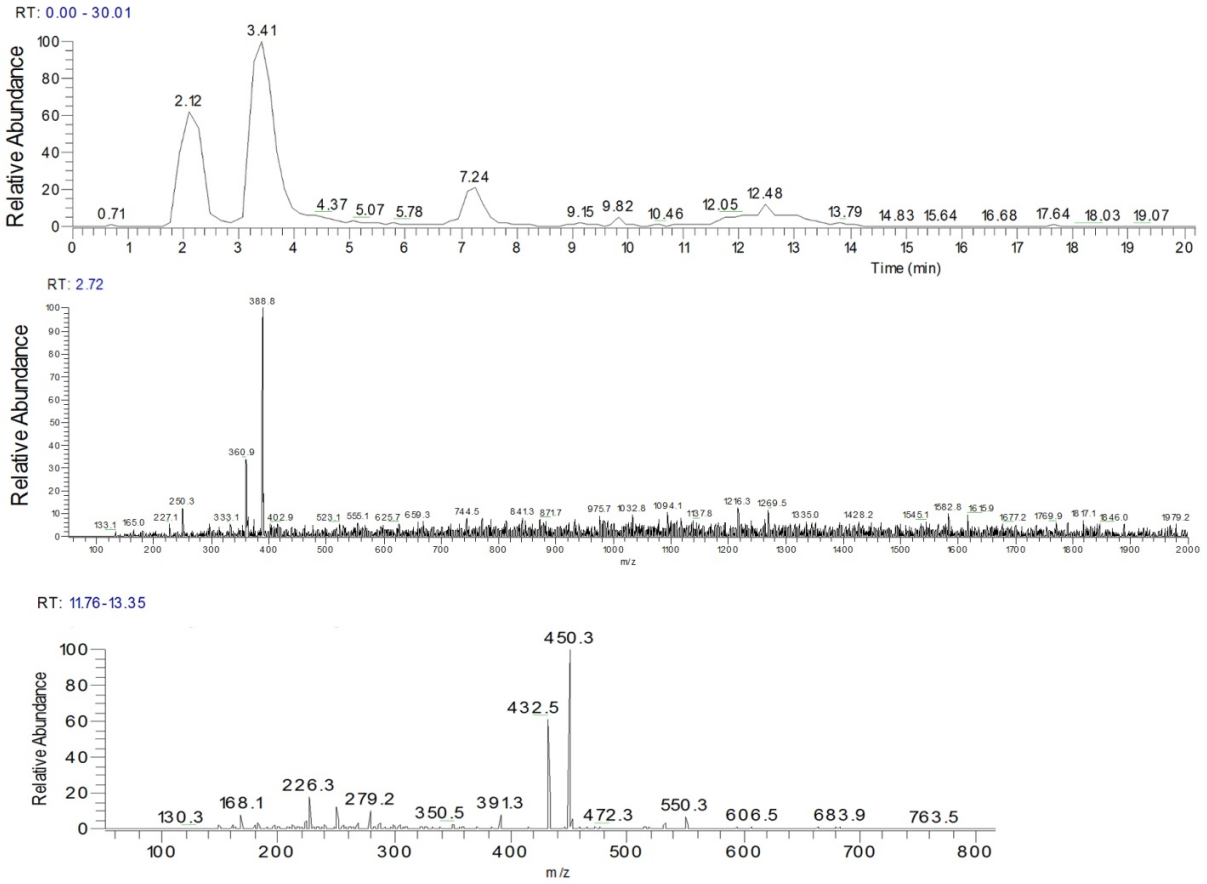

Figure 7S HPLC-Mass spectrum of the ion-exchange column-purified products of glutaraldehyde and diethylenetriamine. The top is the ion abundance spectrum; the middle is the mass at real time 2.72 mim, matching the mass of **10** plus sodium; the bottom is the mass spectrum at real time 11.76 to 13.35, matching the mass of **11** (432.5) and a less dehydrated derivative of **11** (450.3).

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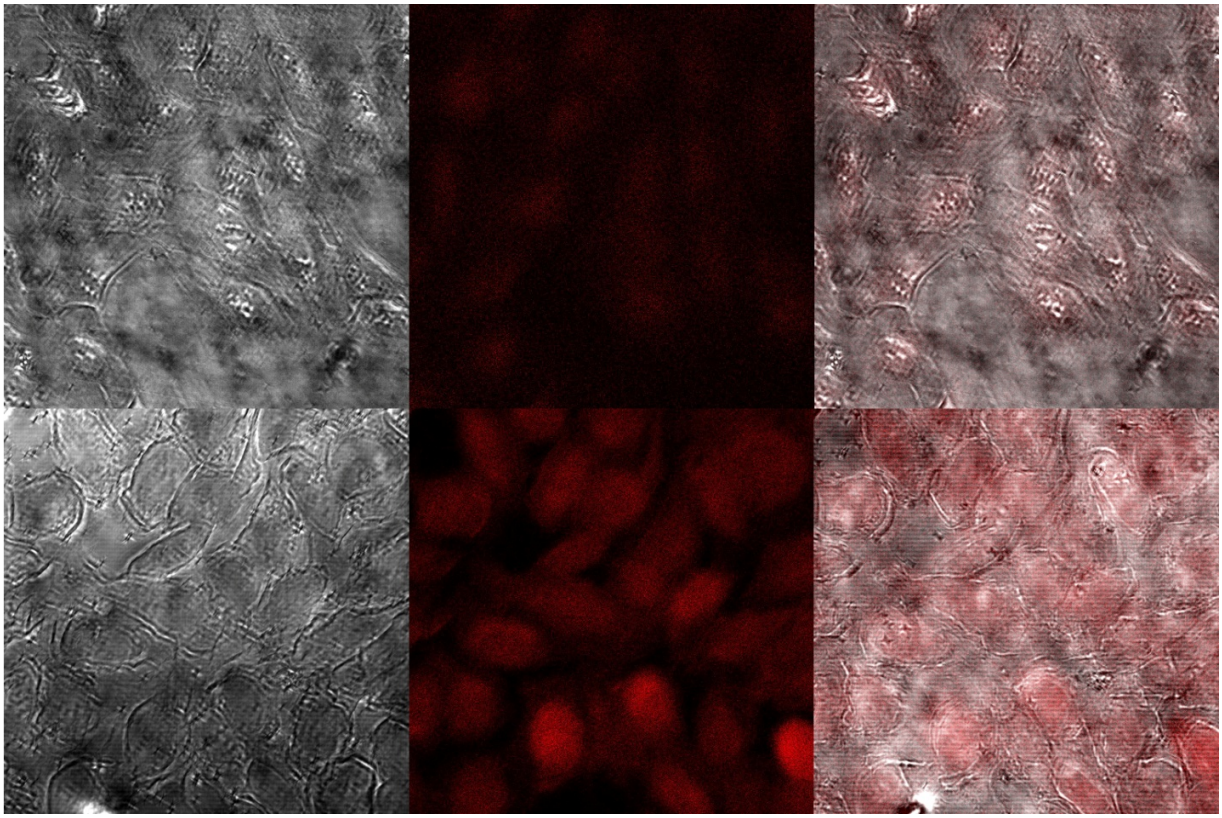

Figure 8S Confocal microscopy images of glutaraldehyde-fixed U2OS cells. The bright field images (left) were merged with fluorescence images (middle) to yield merged images (right). Upper, pH 7; bottom, pH 12.

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