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Heat-initiated detection for reduced glutathione with ^{19}F NMR probes based on modified gold nanoparticles

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ABSTRACT

For detecting reduced glutathione (GSH) with a ^{19}F NMR spectroscopy with time-specificity, we developed the probes based on gold nanoparticles modified with the fluorinated groups via the thermally-cleavable linkers. Before the heating treatment with the probe, the maleimide moiety as a binding site with GSH in the probe is inactivated by cycloaddition of furan. At this silent state, the magnitude of ^{19}F NMR signals from the fluorinated groups was suppressed. By heating for the activation of the probe, the maleimide moiety was produced via retro Diels–Alder reaction, and ^{19}F NMR signals were observed. From this moment, GSH started the reaction with the probe via Michael addition to the maleimide moiety, leading to the observation of the new peak in ^{19}F NMR spectra. Finally, the amounts of GSH were determined from the increase of the magnitude of ^{19}F NMR signals.

Reduced glutathione (GSH) is the buffering biomolecule to maintain the cellular redox homeostasis.[1–3] On the other hand, it has been reported that the GSH concentrations are relatively higher in cancer cells than those in normal cells.[4–6] Thereby, the activity of the tumor can be estimated using GSH as a marker.[7] In addition, reactive oxygen or heavy metal species would be recognized as a stress to the cells, leading to the production of GSH.[1–3] Thus, the GSH-responsive materials are promised to be molecular probes,[8] tumor-targeting prodrugs[9,10] and drug releasing vesicles.[11] In contrast, the cellular distribution of GSH is commonly heterogeneous.[12] Furthermore, the GSH concentrations can be changed during biological events such as apoptosis via the transporting systems.[13] Therefore, the evaluation of the GSH concentrations is of great significance. However, GSH can be found not only in the extracellular matrices but also in the blood.[14,15] Site- and time-specific analytical tools should be required for evaluating the GSH concentration with high accuracy.

By using stimuli-responsive fluorinated materials to the biological molecules and reactions, the information can be gathered from the deep spots inside vital bodies with ^{19}F NMR or MRI. Various kinds of fluorinated compounds have been applied as ^{19}F NMR probes for monitoring biological events such as gene expression,[16] protein distribution,[17] enzymatic reaction,[8,18–23] environmental alteration,[7,24–28] and biological reactions with small molecules.[29–31] In particular, the quantitative detections for enzymatic activities were accomplished by evaluating the changes of signal intensities from the probes in the target reactions.[18,19] However, if the degradation of the probes occurs before reaching to the target spot, background noise levels should increase. Thus, to improve the accuracy in the quantitation of the biological activities, the probe degradation should be suppressed.

Herein, we report simple procedure to detect GSH utilizing a ^{19}F NMR spectroscopy. We synthesized the probes based on the modified gold nanoparticles with fluorinated compounds tethered with thermally-cleavable linkers. By the heating treatment, the linkers were removed, and the active sites in the probes were revealed. Corresponding to the progress of the reaction with GSH, the peak position of chemical shifts in ^{19}F NMR spectra was altered. Finally, we observed the rate changes of the peak area ratios depending on the amount of GSH.

Figure 1 illustrates the structure and the detection mechanism of the nanoparticle-based probe for GSH in this study. The probes consist of three significant components, the gold nanoparticle,[32,33] the heat-cleavable linker, and the signal unit. The trifluoromethyl group is a ^{19}F NMR signal unit, and the recognition to GSH is evaluated by the alteration of the chemical shift in ^{19}F NMR spectra. The reaction yields are also obtained from the peak ratio between before and after recognition. The thermally-cleavable linker is composed of the cycloaddition with maleimide and furan in a Diels–Alder reaction. Before thermal activation, the maleimide group is protected from the nucleophiles such as the thiol group in GSH.[34,35] Under the heating treatment, the retro Diels–Alder reaction proceeds, and the maleimide group should be presented. Subsequently, the Michael addition with the primary thiol group in GSH to the maleimide moiety in the probe could occur.[34]

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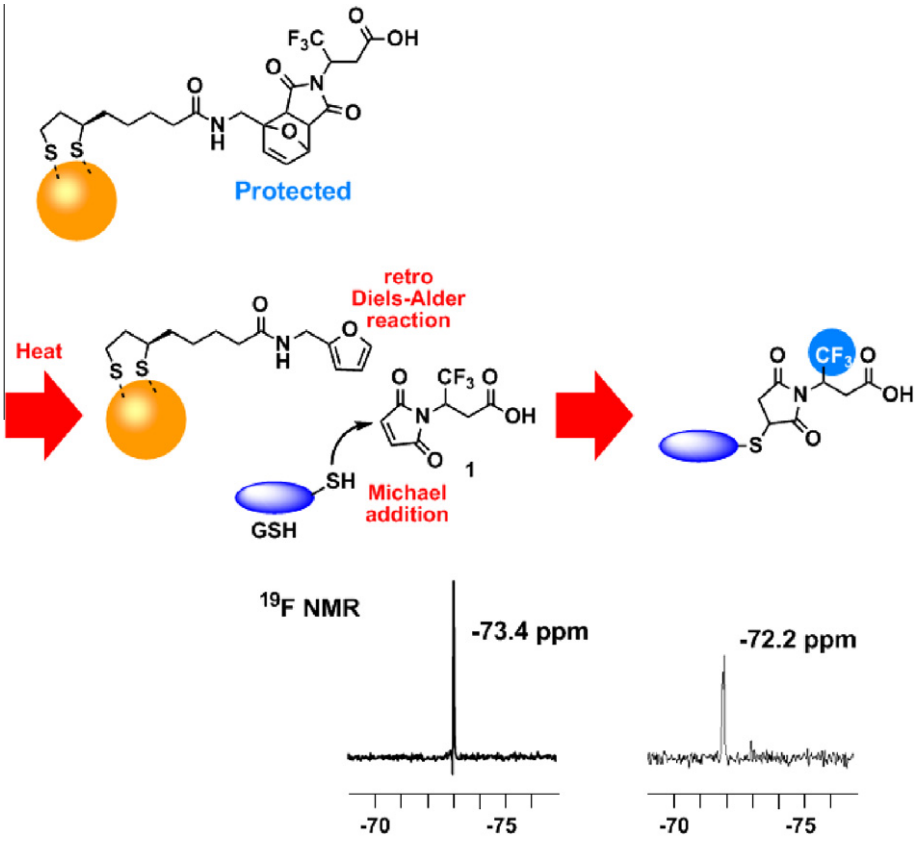
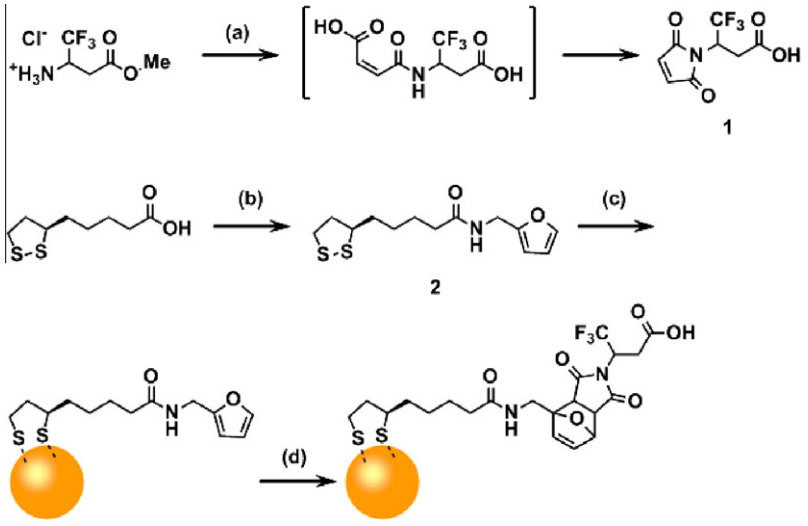


Figure 1. Structure of the linker at the surface of the nanoparticle probes and the proposed scheme of Michael addition for the GSH detection. ^{19}F NMR spectra of **1** were recorded before (left) and after (right) the GSH treatment.

Consequently, the electronic state of the trifluoromethyl group should be changed, resulting in the observation of the chemical-shift alteration in ^{19}F NMR spectra. Gold nanoparticles are convenient scaffolds for delivering the large amount of drugs to the target spots.[36] The local concentration of drug can be readily enhanced by accumulating the bio-active molecules at the surface of the particles and releasing them simultaneously. Moreover, gold nanoparticles can convert the near infrared light energy to heat efficiently.[37] Temperature controls at the local spot can be achieved.

Synthesis of the nanoparticle probes is illustrated in Scheme 1.[38] Methyl 3-amino-4,4,4-trifluorobutyrate hydrochloride which had the trifluoromethyl group at α-carbon of amino group was used as a starting material. We prepared gold nanoparticles (d = 3.4±0.4 nm calculated from TEM image shown in Fig. 2) as a scaffold for constructing probes.[32,33] As shown in Figure 1, **1** was



Scheme 1. Synthesis of the probes. Reagents and conditions: (a) (i) Maleic anhydride, triethylamine, dichloromethane, room temperature; (ii) NaOH aq, room temperature; (iii) sodium acetate, acetic anhydride, 85 °C; (b) furfurylamine, WSC, dichloromethane, room temperature; (c) AuNP, water, room temperature; (d)1, 100 mM phosphate buffer (pH = 7.0), 60 °C.