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Fluorogenic Resveratrol-Confined Graphene Oxide For Economic and Rapid Detection Of Alzheimer's Disease

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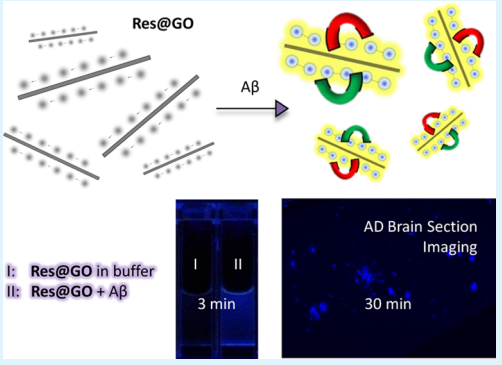
Supporting Information

ABSTRACT: Developing an effective means for the real-time probing of amyloid β (Aβ) that is closely implicated in Alzheimer's disease (AD) could help better understand and monitor the disease. Here we describe an economic approach based on the simple composition of a natural product, resveratrol (Res), with graphene oxide (GO) for the rapid, fluorogenic recognition of Aβ. The **Res@GO** composite has proved specific for Aβ over a range of proteins and ions, and could sensitively capture both Aβ monomers and fibers in a physiological buffer solution within only 3 min. The composite can also fluorescently image amyloid deposits in a mouse brain section within 30 min. This new protocol is much cheaper and more timesaving than the conventional immunofluorescence staining technique employed clinically, providing an economic tool for the concise detection of AD.



KEYWORDS: *graphene, Alzheimer's disease, resveratrol, amyloid β, fluorogenic, detection*

Alzheimer's disease (AD), the most common form of dementia, suffers tens of millions of people per year and is estimated to impact one out of 85 people globally by 2050.[1,2] Unfortunately, the pathogenesis and progression of AD have not been well understood, marking the disease among the most costly social burdens in developed countries.[3,4] Furthermore, the fact that there are currently no treatments available that cure or reverse the progression of the disease implicates that effective means for the better dissection of AD is both challenging and highly desirable.

Amyloid β (Aβ) that is truncated successively by β and γ secretases from the amyloid precursor protein[5] is tightly associated with the pathology of AD; it is the main component of amyloid plaques found in the brains of AD patients.[6] Aβ can form not only tangles of regularly organized fibrillar aggregates (the Aβ fibrils) in the brain but also highly toxic soluble oligomers that are believed to boost the development of AD.[5,7,8] As a result, sensitive monitoring of Aβ of various forms under physiological conditions is of paramount importance towards the elucidation of AD. However, current techniques (e.g., immunofluorescence staining) used in laboratories and hospitals for detection of Aβ in solution and brain sections is expensive and onerous, and thus alternative tools that address these issues should be developed.

Graphene oxide (GO),[9] because of its promising optical properties, good water solubility, low toxicity, and cost-effectiveness, has become a material of choice for the development of bioprobes.[10−17] GO has also proved to be a universal quencher of fluorescence (FL) dyes through the fluorescence resonance energy transfer (FRET) effect.[12,18] A recent study demonstrates that, comparing to carbon nanotubes and gold nanoparticles, GO is more efficient for DNA analyses.[19] We also exemplify that GO may serve as a potent and economic medium in the fabrication of optical or electrochemical composite bioprobes.[20,21]

Given the said interesting aspects of GO, we sought to fabricate a GO-based fluorogenic probe for the rapid and cost-effective determination of Aβ. Resveratrol (Res), a natural product widely found in red wine, has shown neuroprotective effects[22] and could bind specifically with Aβ.[23,24] On the basis of its intrinsic FL, we devised a new probe composite that utilizes Res as both the signaling and recognition molecule. Scheme 1 depicts the design rationale behind which to construct the fluorogenic **Res@GO** (GO confined with Res). Stacking of the fluorescent Res to the surface of GO by, probably, pi-interactions generates a fluorogenic composite that quenches the FL of Res via FRET. While in complexation with an Aβ, the composite will fluoresce because of impeded energy transfer between GO and the photoexcited Res specifically encapsulated by the peptide.

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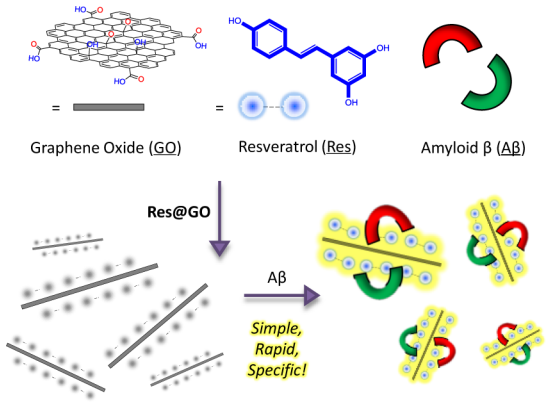
Scheme 1. Structure of Resveratrol (Res) and Graphene Oxide (GO), and Cartoon Depicting the Simple Construction of Res@GO for the Rapid Fluorogenic Probing of Amyloid β (Aβ)



To begin with, the quenching effect of GO for Res (20 μM) was tested. As shown in Figure 1a, increasing GO (0–50 μg/mL) led to gradual FL (excited at 310 nm) decrease of the natural product with a maximum quenching rate of 88.4% (F/F_0, where F is the quenched FL and F_0 the original FL). This is probably caused by the FRET effect between the photo-excited Res and GO, the energy acceptor, upon composition.[12,18,20] Then, we observed that addition of increasing monomeric Aβ42 to the composited **Res@GO** led to rapid (3 min incubation) and sharp FL recovery of the composite in a concentration-dependent manner (Figure 1b). Figure 1c illustrates the fluorogenic response of the composite to Aβ42 (I to II), whereas further addition of congo red (a known strong Aβ binder)[25] competitively quenched the FL (III). This means that the FL "off–on" detection is reversible.

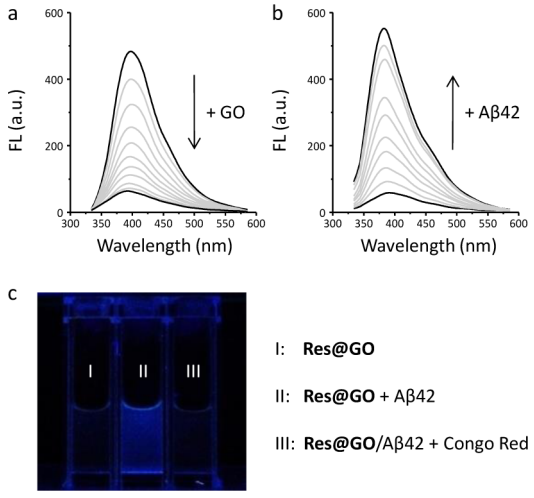


Figure 1. Fluorescence (FL) titration spectra of (a) resveratrol (Res, 20 μM) with increasing graphene oxide (GO, 0–50 μg/mL), and (b) **Res@GO** with increasing monomeric amyloid β42 (Aβ42, 0–200 μM) in phosphate buffered saline (0.2 M, pH 7.4) with excitation at 310 nm. (c) Photographed FL change of **Res@GO** (I) in the presence of Aβ42 (II) and then congo red (III) excited with a portable UV lamp.

To corroborate the stacking between Res and GO, a variety of analytical techniques were employed. UV–vis (Figure 2a) and Fourier transmission infrared (FTIR) spectra (see Figure S2 in the Supporting Information) first showed that **Res@GO** displays peaks characteristic of both the compound and the carbon flake. Typical D band (1355 cm^{-1}) and G band (1600 cm^{-1}) curves were recorded in the Raman spectrum of GO with an I_D/I_G (where I is intensity) ratio of 0.83 (Figure 2b). In contrast, the I_D/I_G ratio of **Res@GO** increased to 0.89 (Figure 2c), suggesting an increased sp^2-hybridization disorder of GO composited with the aromatic Res.[20,21] In addition, the atomic force microscope (AFM) image of bare GO (Figure 2d) showed that the height of which is 0.95 nm, whereas stacking of Res to GO increased the height to 1.2 nm (Figure 2e). Complexation of **Res@GO** with Aβ42 further thickened the composite to 1.8 nm.

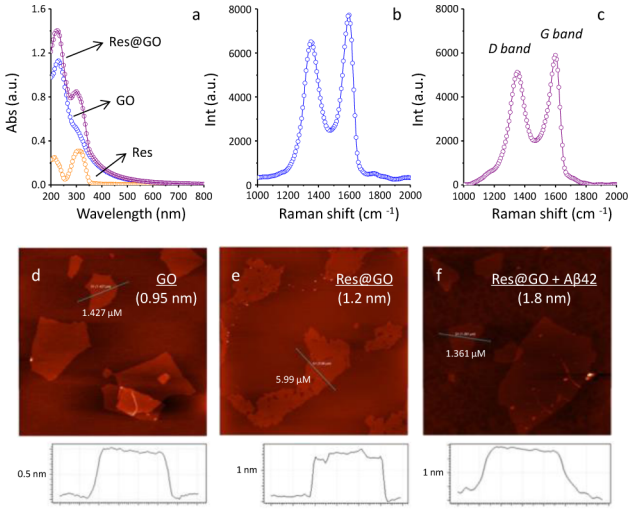


Figure 2. (a) UV–vis spectra of resveratrol (Res), graphene oxide (GO), and **Res@GO**. Raman spectra of (b) GO, and (c) **Res@GO**. Atomic force microscope image of (d) GO, (e) **Res@GO**, and (f) **Res@GO** in complex with amyloid β42 (Aβ42).

These data, together with the FL titration results shown in Figure 1, suggest that the stacked **Res@GO** composite may serve as a fluorogenic probe that can rapidly adhere Aβ42 onto its Res-confined surface, leading to FL enhancement due to the encapsulation of the Res cluster by the peptide. The specificity of **Res@GO** for Aβ was interrogated using a panel of physiological proteins including ribonuclease A (RNaseA), lysosome (Lys), pepsin (pep), and cytochrome c (CyC), and a variety of cations and anions as shown in Figure 3. We determined that all these species hardly fluctuated the FL of the composite, which manifests the good specificity of the concisely constructed **Res@GO** for Aβ42.

Next, as it has been reported that Aβ with 40 and 42 residues are the main components to form insoluble amyloid fibrils that eventually induce necrosis of neuronal cells,[5–8] we tested the recognition of **Res@GO** using both monomeric (m-) and fibril (f-) Aβ40/Aβ42. The Aβ fibrils were produced as indicated in a previous study.[14] As shown in Figure 4, although its response to f-Aβ40 (Figure 4b) is slightly stronger than that to m-Aβ40 (Figure 4a), **Res@GO** showed evidently more increased FL with f-Aβ42 (Figure 4d) than with m-Aβ42 (Figure 4c). This suggests that the higher binding affinity of Res with the Aβ fibrils than the monomers[23] could be accurately interpreted by the composite. Furthermore, good linear ranges as regards the FL alternation in response to the analytes were produced by the composite (see Figure S2 in the Supporting Information); the

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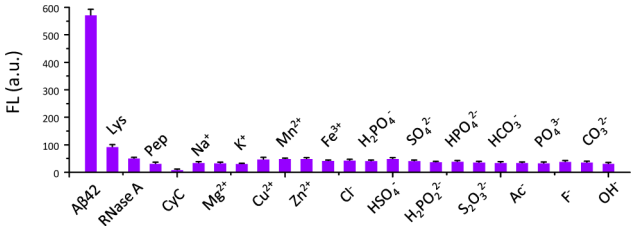

Figure 3. FL change of **Res@GO** (20 μM of resveratrol with 50 μg/mL graphene oxide) in the presence of Aβ42 (200 μM) or a variety of physiological proteins (200 μM) including ribonuclease A (RNase A), lysosome (Lys), pepsin (pep), and cytochrome c (CyC), and ions (10 mM) in phosphate buffered saline (0.2 M, pH 7.4) with excitation at 310 nm.

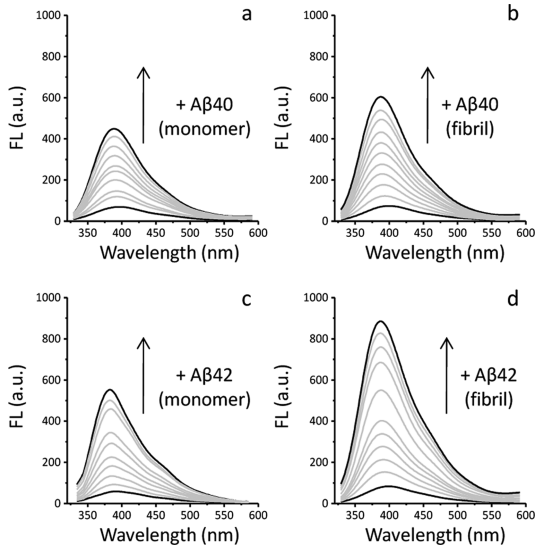

Figure 4. Fluorescence (FL) titration spectra of **Res@GO** (20 μM of resveratrol with 50 μg/mL graphene oxide) with increasing (a) Aβ40 monomer (0–200 μM), (b) Aβ40 fibril (0–200 μM), (c) Aβ42 monomer (0–200 μM), and (d) Aβ42 fibril (0–120 μM) in phosphate buffered saline (0.2 M, pH 7.4) with excitation at 310 nm.

limits of detection for m-Aβ40, m-Aβ42, f-Aβ40, and f-Aβ42 were determined to be 277, 219, 270, and 120 nM, respectively.

Having demonstrated the good sensitivity and selectivity of **Res@GO** for Aβ, we attempted its applicability in imaging amyloid plaques formed in a brain section. Section collected from the brain (Figure 5a) of a transgenic mouse carrying APPswe/PS 1dE9 mutations (APP/PS1 mouse, a kind gift given by prof. Haiyan Zhang of Shanghai Institute of Materia Medica)[26] was used to be imaged by the composite or by an anti-Aβ antibody as a positive control. As the composite will remain at its quenching state unless it binds with Aβ, the conventional rinsing step after treatment with **Res@GO** was envisioned to be unnecessary. Indeed, we observed that after incubation of the section with **Res@GO** for only 30 min, clear FL imaging of the plaques was recorded with almost no background FL observed (Figure 5b). The imaged area was well-correlated to that immunofluorescently stained by the specific anti-Aβ antibody (Figure 5c, d). The composite also showed little toxicity to a human normal cell line (human embryonic kidney 293, HEK293), even with a 5-fold elevated concentration used for imaging (see Figure S3 in the Supporting Information).[27]

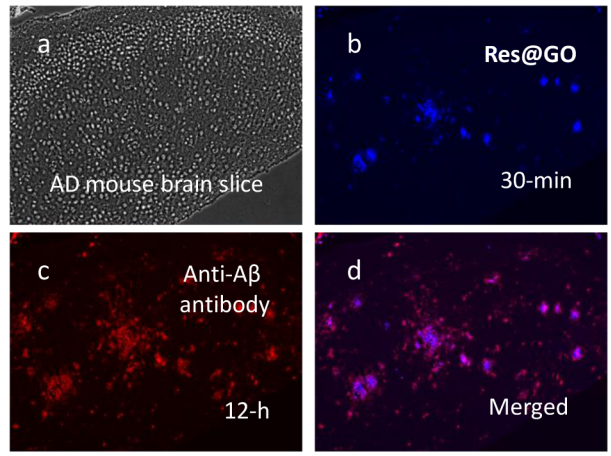

Figure 5. (a) Bright-field image of a mouse brain section with amyloid plaques; fluorescence imaging of the plaques using (b) **Res@GO** (wash-free) and (c) anti-Aβ antibody; (d) the merged fluorescence staining.

We note that, for the brain section imaging, the **Res@GO** composite simply developed here is much more advantageous than the conventional immunofluorescence staining in terms of the following: (1) whereas only ~30 min was required from the treatment of the brain section with **Res@GO** to FL imaging, approximately 12 h was needed for the antibody staining (which includes the onerous blocking processes, primary/secondary antibody labeling, and repeated washing to rinse off residual species); (2) the cost of the conventional technique was estimated to be ~¥1500 per 100 slices (roughly taking into account the price of antibodies), whereas that of the present protocol ~¥0.85 per 100 slices (roughly taking into account the price of GO and Res; details for cost estimation are shown in the Supporting Information). As a consequence, **Res@GO** could become a potentially economic and timesaving tool for both laboratorial and clinical uses.

In summary, we have developed a succinct fluorogenic **Res@GO** composite for the rapid and cost-effective detection of Aβ in solution as well as brain section. This low-cost material might hold the promise to replace antibody-based immunofluorescence staining of clinical samples and serve as a reliable probe for the fast determination of Aβ of various forms. This study also offers valuable insights into the concise dissection of AD.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental section and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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