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A multimodal, β-amyloid-targeted contrast agent†‡

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A multimodal, β-amyloid-targeted contrast agent was synthesized and studied *in vitro*. The agent has a higher relaxivity than a clinically approved contrast agent and interacts with β-amyloid aggregates producing changes in relaxation rate and fluorescence emission.

β-Amyloid plaques are a diagnostic marker of Alzheimer's disease, which is one of the most common neurodegenerative diseases in the world;[1] hence, detection of β-amyloid plaques is important in the diagnosis of, monitoring of treatments for, and research related to Alzheimer's disease. The β-amyloid aggregates that comprise the plaques are often detected using fluorescent dyes or β-amyloid-targeted radiolabels that interact with β-amyloid aggregates.[2–7] However, fluorescence microscopy has limited tissue penetration, and radiolabels for nuclear imaging use harmful ionizing radiation. Magnetic resonance imaging (MRI) is a non-invasive imaging technique with excellent tissue penetration and high spatial resolution (25–100 μm)[8] that has the potential to overcome the limitations of fluorescence microscopy and nuclear imaging with respect to the detection of β-amyloid aggregates.

MRI has been used in several imaging studies of β-amyloid plaques.[9–13] In these studies, both transverse relaxation time (T_2)-weighted and longitudinal relaxation time (T_1)-weighted imaging methods were reported.[9–12] The T_1-weighted methods are more desirable because T_1-weighted imaging can distinguish plaques from hemorrhages and blood vessels unlike T_2-weighted methods. Although T_1-weighted imaging is desirable, it suffers from poor contrast enhancement of plaques leading to a widespread interest in the labelling of plaques with target-specific contrast agents. Examples of β-amyloid-targeting groups that have been reported include β-amyloid peptides,[10] monoclonal antibodies,[11] and β-amyloid-binding dyes.[12] Agents composed of conjugates of these targeting groups are capable of labeling β-amyloid plaques, but possible amyloidogenesis, toxicity, and the large size of these contrast agents limit their usefulness. These limitations reveal the need for new β-amyloid-targeted contrast agents with higher efficiency, smaller size, and non-amyloidogenic and non-toxic properties to make MRI a useful technique in β-amyloid imaging.

Here, we report a β-amyloid-targeted contrast agent for MRI that is more efficient than current clinical contrast agents. To target β-amyloid aggregates, we used a non-toxic small molecule that can be traced with fluorescence microscopy; thus, our agent is multimodal being detectable by both MRI and fluorescence microscopy. We synthesized our multimodal contrast agent using the conjugation strategy for target specific molecules by linking a clinically approved contrast agent to a target-specific moiety.[14] For our target-specific moiety, we chose curcumin, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, because it is non-toxic, interacts with β-amyloid aggregates, is non-amyloidogenic, and inhibits aggregate formation.[2–4] Moreover, curcumin is fluorescent and has been used for imaging β-amyloid plaques with fluorescence microscopy.[2,5] Curcumin conjugates are often synthesized *via* the total synthesis of curcumin derivatives[4,15,16] or the direct reaction of linkers with commercially available curcumin.[16,17] Total synthetic routes are tedious and limited to the synthesis of specific derivatives. Alternatively, the direct reaction of commercially available curcumin provides shorter synthetic routes and has been used to synthesize conjugates with reactive functional groups including alkenes, alcohols, esters, amides, cyanide, carboxylic acids, and azides.[16,17]

To facilitate conjugation to curcumin, we introduced an amine-functionalized linker at one of the phenolic sites of curcumin. To the best of our knowledge, conjugation of the amine group to curcumin has not been reported, and we expect that amine-functionalized curcumin will be a useful intermediate for other conjugations of curcumin because of the facile synthesis and resistance of amine-derived conjugates including amides, thioureas, ureas, and carbamates, to hydrolysis in enzyme-rich biological environments.[18] The phenolic group was selected as a site of conjugation because conjugations to this position do not inhibit the targeting ability of curcumin.[4] Furthermore, the amine

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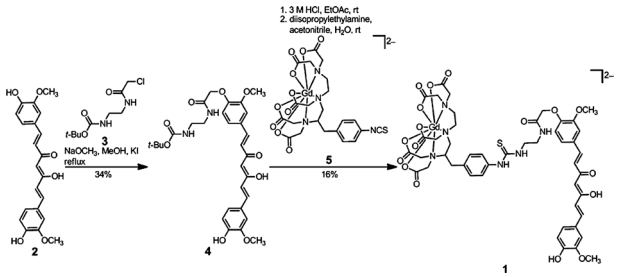

Scheme 1 Synthetic route to curcumin-conjugated contrast agent **1**.

group enabled facile reaction with an isothiocyanate derivative of Gd^{III}diethylenetriaminepentaacetate (Gd^{III}DTPA), which is a clinically approved contrast agent.

We synthesized conjugate **1** *via* the direct reaction of commercially available curcumin with linker **3** (Scheme 1). The reactions were performed in the dark because of the photoinstability of curcumin.[19] The amine group was deprotected with HCl and reacted with isothiocyanate-containing Gd^{III}DTPA, **5**, to obtain curcumin-conjugated contrast agent **1**.

After synthesis, we determined the efficiency of conjugate **1** as a contrast agent for MRI. The longitudinal relaxivity (r_1) of **1** is 13.63 ± 0.03 mM^{-1} s^{-1} [phosphate buffered saline (PBS), pH 7.4, 1.4 T, and 37 °C]. This value is 4.1 times greater than that of clinically approved Gd^{III}DTPA under the same conditions (3.30 ± 0.06 mM^{-1} s^{-1}). The high relaxivity of **1** demonstrates that it is an efficient contrast agent. The efficiency is likely caused by a slowing of the molecular reorientation rate upon conjugation to curcumin and potential aggregation in solution.[20]

To measure the interaction of **1** with β-amyloid aggregates, we measured the T_1 of solutions containing complex **1** in the presence and absence of β-amyloid fibrils that were prepared following published procedures.[6,21] The formation of fibrils (diameters of 200–600 nm) was confirmed with dynamic light scattering and transmission electron microscopy. Different stoichiometries (0, 0.5, 1, and 2 equiv. relative to **1**) of aggregated β-amyloid were incubated with complex **1**, and T_1 values were measured at a clinically relevant field strength (1.4 T) at 37 °C in PBS (pH = 7.4). As a non-binding control, T_1 measurements of β-amyloid aggregates at each concentration were measured in the presence of clinically approved Gd^{III}DTPA, and all measurements were replicated with independently prepared samples. The T_1 of samples containing conjugate **1** that were incubated with 1 or 2 equiv. of β-amyloid aggregates were shorter than the blank of **1** that did not contain β-amyloid aggregates (Student *t* test, 99% confidence interval). However, the Gd^{III}DTPA control did not have different T_1 values at any concentration of β-amyloid aggregates (Student *t* test, 99% confidence interval) with respect to Gd^{III}DTPA in the absence of β-amyloid aggregates.

To quantify the magnitude of the influence of the interaction of fibrils with **1** on relaxation rates, values of $\Delta 1/T_1$ were calculated for samples containing conjugate **1**, the Gd^{III}DTPA control, and PBS (eqn (1)). In eqn (1), $\Delta 1/T_1$ is the change in $1/T_1$ due to the interaction with β-amyloid aggregates; $(1/T_1)_n$ is the relaxation rate in the presence of n equiv. of β-amyloid aggregates where n = 0, 0.5, 1, or 2; and $(1/T_1)_0$ is the relaxation rate in the absence of β-amyloid aggregates.

$$\Delta\frac{1}{T_1} = \left(\frac{1}{T_1}\right)_n - \left(\frac{1}{T_1}\right)_0 \quad (1)$$

A plot of $\Delta 1/T_1$ *vs.* equiv. of β-amyloid aggregates demonstrates that there is an increase in relaxation rate for **1** with increasing amounts of β-amyloid aggregates (Fig. 1). A 67×10^{-3} s^{-1} (9%) change in $1/T_1$ in the presence of 2 equiv. of β-amyloid aggregates was observed compared to in the absence of aggregates. The controls of Gd^{III}DTPA with β-amyloid aggregates and the β-amyloid aggregates alone did not show an increase in relaxation rate as a function of β-amyloid concentration. We expected T_1 values to decrease upon the interaction of contrast agents to macromolecules, like fibrillar aggregates, due to the reduction in tumbling rate based on Solomon–Bloembergen–Morgan theory.[20,22] Hence, a decrease in T_1 values in the presence of β-amyloid aggregates provides evidence of the interaction of conjugate **1** with β-amyloid aggregates.

To explore the multimodal nature of complex **1**, we investigated the effect of β-amyloid aggregates on the fluorescence emission of conjugate **1**. Emission spectra were acquired at the end of each T_1 measurement (Fig. 2), and the fluorescence emission maximum of conjugate **1** (516 nm) was blue shifted 9 nm in the presence of 2 equiv. of β-amyloid aggregates. Furthermore, the fluorescence intensity of conjugate **1** increased by 43% in the presence of 2 equiv. of β-amyloid aggregates relative to **1** in the absence of β-amyloid aggregates. Similar observations have been reported for β-amyloid-specific fluorescent dyes due to their interaction with β-amyloid aggregates,[5,6,12,23] thus agreeing with our measurements and providing further indication of the presence of an interaction of complex **1** with the β-amyloid aggregates. The changes in emission wavelength and intensity make conjugate **1** a multimodal imaging agent for β-amyloid plaques. Importantly, these probes allow for validation of results using orthogonal modalities: The multimodal nature of conjugate **1** enables the validation of MRI results with fluorescence studies.

We have synthesized an efficient, small molecular probe for β-amyloid aggregates from non-toxic curcumin and a derivative of a clinically approved contrast agent for MRI. *In vitro* studies demonstrate that curcumin-conjugated contrast agent **1** interacts

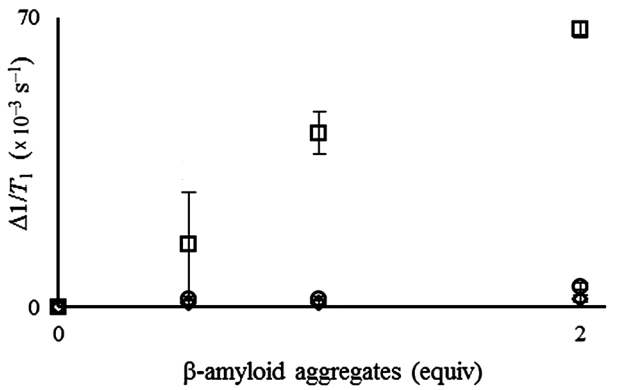

Fig. 1 Plot showing the change in relaxation rate ($\Delta 1/T_1$) as a function of the amount of β-amyloid (βA) aggregates in PBS: 1 + βA (□); Gd^{III}DTPA + βA (○); and βA (◇). Error bars represent standard error of the mean.

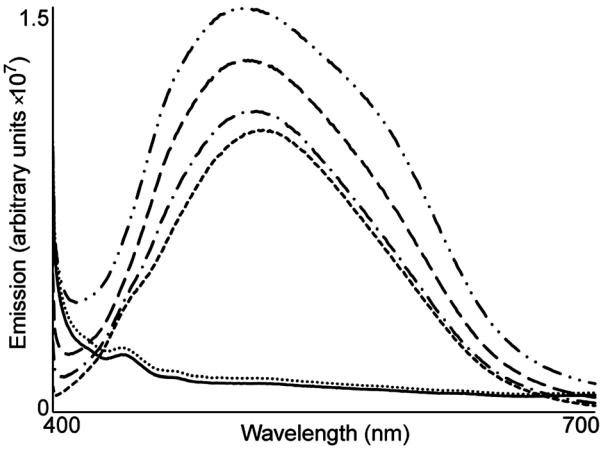

Fig. 2 Emission spectra (λ_{ex} = 385 nm) of **1** and Gd^{III}DTPA incubated with β-amyloid aggregates (βA) in PBS: 2 equiv. βA + **1** (·· —); 1 equiv. βA + **1** (— —); 0.5 equiv. βA + **1** (· —); **1** (--), 2 equiv. βA + Gd^{III}DTPA (···); and βA (—).

with β-amyloid plaques resulting in a shortening of T_1 and change in the wavelength and intensity of fluorescence emission. While future studies related to imaging and pharmacokinetic delivery are necessary to evaluate the utility of this work *in vivo*, the multimodal nature of imaging agent **1** is important for Alzheimer's disease related research. In addition, we expect that the facile synthesis of the curcumin-amine linker will be a powerful tool for the synthesis of other curcumin conjugates that are stable in biological environments.

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