RNA Aptamer Probes as Optical Imaging Agents for the Detection of Amyloid Plaques

Christian T. Farrar1*, Christopher M. William2, Eloise Hudry3, Tadafumi Hashimoto3, Bradley T. Hyman3

1 Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, United States of America, 2 Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, United States of America, 3 Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts, United States of America

Abstract

Optical imaging using multiphoton microscopy and whole body near infrared imaging has become a routine part of biomedical research. However, optical imaging methods rely on the availability of either small molecule reporters or genetically encoded fluorescent proteins, which are challenging and time consuming to develop. While directly labeled antibodies can also be used as imaging agents, antibodies are species specific, can typically not be tagged with multiple fluorescent reporters without interfering with target binding, and are bioactive, almost always eliciting a biological response and thereby influencing the process that is being studied. We examined the possibility of developing highly specific and sensitive optical imaging agents using aptamer technology. We developed a fluorescently tagged anti-Aβ DNA aptamer, β55, which binds amyloid plaques in both ex vivo human Alzheimer’s disease brain tissue and in vivo APP/PS1 transgenic mice. Diffuse β55 positive halos, attributed to oligomeric Aβ, were observed surrounding the methoxy-XO4 positive plaque cores. Dot blots of synthetic Aβ aggregates provide further evidence that β55 binds both fibrillar and non-fibrillar Aβ. The high binding affinity, the ease of probe development, and the ability to incorporate multiple and multimodal imaging reporters suggest that RNA aptamers may have complementary and perhaps advantageous properties compared to conventional optical imaging probes and reporters.

Introduction

An emerging class of novel molecular probes based on RNA or DNA aptamers may offer significant advantages as both therapeutic and diagnostic imaging agents [1–4]. Very large RNA libraries can be rapidly screened to identify sequences that bind a given molecule with high affinity using Selective Evolution of Ligands by EXponential enrichment (SELEX) [5,6]. The affinities and specificities of RNA aptamers are comparable to or even better than those of antibodies [1,7]. Aptamers can fold back into their natural conformation after denaturation and are stable in the reducing environment of the cell [3]. Aptamers are much smaller than antibodies, thereby improving their biodistribution [3,8]. Aptamers can be delivered to cells using viral vectors [4,9]. Aptamers can also be made by chemical synthesis, which allows for tailor-made modifications and avoids biological contamination. Imaging agents can be easily incorporated into aptamers using labeled nucleotides, providing the potential for multiplexing and for tuning reagents to the imaging platform. For example, fluorescently-tagged nucleotides can be used for 2-photon imaging, Cy5-tagged nucleotides for near infrared imaging, 18F-labeled nucleotides for positron emission tomography (PET) imaging, and 19F-labeled nucleotides for 19F magnetic resonance imaging (MRI). Aptamers may also provide powerful tools for developing therapeutic agents [3,4]. Aptamers have even been selected that cross the blood-brain barrier [10]. Finally, aptamers typically have low or no immunogenicity [3,11]. As a test of the idea that aptamers could be used as a new in vivo optical imaging tool, we have investigated the use of a fluorescently labeled anti-Aβ aptamer for imaging both amyloid plaques and oligomeric Aβ in Alzheimer’s disease (AD) mouse models.

Increasing evidence suggests that soluble oligomeric Aβ is synaptotoxic and plays a central role in the early pathogenesis of AD [12,13]. Studies in neuronal cultures and organotypic slices demonstrated that soluble forms of Aβ induce synaptic changes and dendritic spine loss and are toxic to mature central nervous system neurons [14–16]. Shankar et al. demonstrated that Aβ dimers, but not insoluble plaque cores, impaired long-term potentiation, lowered the threshold for induction of long-term depression, and reduced dendritic spine density in normal mouse hippocampus [17]. Similarly, the presence of a 56-kDa Aβ assembly has been correlated with memory loss in Tg2576 transgenic mouse models of AD [18,19], while Tris-buffered saline (TBS) soluble Aβ from Alzheimer’s disease brain has been shown to disrupt the memory of a learned behavior in normal rats [17]. Koffie et al. demonstrated that oligomeric Aβ, present in a halo surrounding dense core plaques, is associated with postsynaptic densities and correlates with excitatory synapse loss near amyloid plaques [20,21]. Finally, recent studies of transgenic mice that co-express mutant forms of amyloid precursor protein (APP) and tau...
have demonstrated that oligomeric Aβ accumulation, but not total amyloid plaque burden, correlates with neuronal loss and inflammatory response [22]. These studies suggest that amyloid plaques serve as reservoirs of oligomeric Aβ and that oligomeric Aβ is synaptotoxic. Given this evidence of a direct role of Aβ oligomers in AD, there is a great need for new reagents capable of detecting not only mature amyloid plaques but also oligomeric forms of Aβ.

A large number of antibodies that recognize a variety of epitopes of different Aβ assemblies, including low molecular weight Aβ oligomers, have been developed and used extensively in ex vivo studies [23–31]. However, the use of antibodies for in vivo studies is complicated by the plaque clearance that is induced by anti-Aβ antibodies [32]. In addition, the incorporation of multiple optical labels, to provide for increased detection sensitivity, typically significantly reduces the antibody binding affinity. A small molecule positron emission tomography (PET) tracer for optical labels, to provide for increased detection sensitivity, recently, a DNA aptamer selected against monomeric Aβ has been used in optical studies of AD transgenic mouse models [33]. Similarly, a small molecule fluorescent derivative of Congo Red, methoxy-XO4, has been extensively used in many clinical studies [33]. Aptamers, here we demonstrate for the first time the ability of an aptamer as an in vivo optical imaging probe.

Materials and Methods

A novel RNA aptamer, 55, generated from a SELEX screen against monomeric Aβ1–40 was previously shown to bind synthetic amyloid fibrils [35]. Similar SELEX screens against covalently stabilized Aβ trimers [36] and against Aβ1–40 conjugated to colloidal gold nanoparticles [37] have been performed. More recently, a DNA aptamer selected against α-synuclein was shown to also bind oligomeric Aβ [38]. However, to date no ex vivo stains of human AD brain tissue or in vivo mouse studies in amyloid-laden transgenic AD models have been performed with aptamer probes. Here we demonstrate for the first time the ability of 55 to bind amyloid plaques in ex vivo human AD brain tissue and in live transgenic mouse models of AD. Furthermore, we demonstrate that 55 binds Aβ oligomers and is able to visualize the oligomeric halo surrounding the dense cores of amyloid plaques. This is the first use of an aptamer as an in vivo optical imaging probe.

Synthesis of Labeled RNA Aptamers

Double-stranded DNA template (132 bp) was synthesized by polymerase chain reaction of the 76 mer 5’ ends of 55 and its reverse complement (Operon Biotechnologies, Huntsville, AL), which contain a 20 bp overlap. The DNA sequence (Table 1) included a 25 mer T7 RNA polymerase primer. Imaging probes were introduced into the aptamer by transcription of the DNA template with T7 RNA polymerase and either biotin-labeled uracil 55 or its reverse complement, 55rc, used as a control probe. Probes were visualized by reacting with avidin binding complex followed by tyramide signal amplification using AlexaFluor 488 tyramide (Invitrogen, Carlsbad, CA). For slides co-stained with Thioflavin-S, AlexaFluor 594 tyramide (Invitrogen, Carlsbad, CA) was used for visualizing 55 positive plaques in the red channel. Slides were coverslipped and imaged on an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan) or a Zeiss LSM 510 confocal microscope (Carl Zeiss MicroImaging, Jena, Germany).

Dot and Western Blot of Synthetic Aβ Aggregates

1.1 μM of synthetic Aβ1–40 and Aβ1–42 (Peptide Institute, Osaka, Japan) were incubated at 37°C for 3 days with gentle shaking. 4 μl each of Aβ1–40 and Aβ1–42 were blotted onto nitrocellulose paper and allowed to dry. The dot blot was then stained with biotinylated 55. 55 was visualized by secondary staining with streptavidin IRDye 700DX (Rockland Immunochemicals, Gilbertsville, PA) and imaged on an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NB). Western blots of the synthetic Aβ reaction mixtures were also performed to identify the Aβ species present in each reaction mixture. Western blots were stained with 6E10 antibody (1:1000), which is reactive to amino acid residues 3–8 of Aβ. 6E10 was visualized by secondary staining with anti-mouse IgG antibody conjugated to IRDye800 (Rockland Immunochemicals, Gilbertsville, PA) and imaged on an Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NB).

In vivo 2-photon Imaging of APP/PS1 Transgenic Mice

In vivo multiphoton images of optical fluorophores were obtained in real time using an Olympus Fluoview 1000 MPE with prechirp optics mounted on an Olympus BX61WI upright microscope. APP/PS1 transgenic mice which coexpress mutant alleles of amyloid precursor protein (APP) and presenilin (strain B6C3-Tg(APPsw, PSEN1dE9)85Dbo/J, Jackson Laboratory, Bar Harbor, ME) [39] were used for all imaging experiments. After craniootomy, 20 μg of fluorescein labeled aptamer probe (either 55 or 55rc) was topically applied to the mouse brain for 30 minutes. The surface of the brain was then flushed with artificial cerebral spinal fluid, followed by placement of the coverslip window. A wax ring was placed on the edges of the coverslip covering the craniootomy window and was filled with distilled water to maximize contact between water and an Olympus 20× dipping objective (numerical aperture, 0.95). A mode-locked titanium/sapphire laser (MaiTai; Spectra-Physics) generated two-photon fluorescence with 800-nm excitation, and
detectors containing three photomultiplier tubes (Hamamatsu)
collected emitted light in the ranges of 380–480, 500–540, and
560–650 nm (4). Average power reaching brain tissue in each
experiment ranged from 20 to 60 mW. Images were acquired
between 100 and 500 μm below the surface of the brain. Images
were acquired approximately 1 and 24 hours after topical
application of the aptamer probe. All animal experiments were
approved by the Massachusetts General Hospital Subcommittee
on Research Animal Care (SRAC protocol # 2004N000092) and
conformed to national and institutional guidelines.

**Determination of Plaque Contrast-to-noise Ratio**

Plaque regions-of-interest (ROI) were determined by thresholding
the in vivo images to signals greater than two standard
deviations above the background signal intensity. The plaque
contrast-to-noise ratio (CNR) was calculated from the difference
between the plaque and background signal-to-noise ratios (SNR).
The gold standard used for the identification and counting of the
number of amyloid plaques was the presence of methoxy-XO4
staining.

**Results**

The predicted secondary structures for the β55 aptamer and a
RNA aptamer synthesized from the reverse complement of the
β55 coding sequence (β55rc), shown in Figure 1, were determined
using the RNAfold Webserver suite of programs (http://rna.tbi.
unicev.ac.at/cgi-bin/RNAfold.cgi) [40,41]. The β55rc aptamer
has a very different predicted secondary structure from that of β55
and was used as a control aptamer probe.

The ability of the β55 aptamer to stain amyloid plaques was
demonstrated by staining frozen-section brain tissue from AD
subjects with biotinylated β55 and β55rc aptamers. While plaques
were clearly visible with the β55 aptamer (Figure 2a), only a few
very faint β55rc positive plaques were observed (Figure 2b).
A tissue section co-stained with β55 and Thioflavin-S showed good
colocalization of β55 and Thioflavin-S for amyloid plaques
(Figure 2c).

Dot blots of synthetic Aβ1–40 and Aβ1–42 that were incubated
at 37°C for 3 days were performed to examine the binding of β55 to
different Aβ species. β55 staining of both Aβ1–40 and Aβ1–42 was
observed (Figure 3a). The increased staining observed for Aβ1–42 is
consistent with the increased propensity of Aβ1–42 to form high
molecular weight (HMW) species relative to Aβ1–40 as shown in
the 6E10 western blot of the synthetic Aβ reactions (Figure 3b),
where no HMW species were observed for Aβ1–40.

Sodium dodecyl sulfate (SDS) western blots of human AD brain
homogenates were stained with both 6E10 monoclonal antibody,
which is reactive to amino acid residues 3–8 of Aβ, and
biotinylated β55 (Figure S1 and File S1). β55 and 6E10 bound
many of the same bands, in particular higher molecular weight
oligomer bands between 20 and 60 kDa. In contrast, no binding of
monomeric Aβ or soluble APP (sAPP) by β55 was observed. While
some caution must be taken in the interpretation of the western
blot given the documented effects of SDS on Aβ oligomer
structure and aggregation [42–46], the colocalization of 6E10 and
β55 bands provides further support for the binding of β55 to Aβ
oligomers.

The ability of β55 to bind to plaques in post mortem tissue and
fibrillar and non-fibrillar Aβ species on dot blots of synthetic Aβ
raised the possibility that the aptamer may label plaques in an
in vivo setting. In vivo 2-photon imaging studies performed in APP/
PS1 transgenic mice, which overexpress mutant alleles of amyloid
precursor protein (APPwe) and presenilin-1 (PS1de9), showed that
fluorescein-labeled β55 binds amyloid plaques in the cerebral
cortex (Figure 4a) and vasculature (Figure 4b). Plaque staining by
β55 was then compared to plaque staining with methoxy-XO4
(Figure 5). While methoxy-XO4 stained only the dense core of
each plaque, β55 stained both the plaque core and a diffuse halo
surrounding the plaque core (Figure 5c,f).

In vivo β55 plaque staining was compared to plaque staining
with the β55rc control aptamer (Figure 6a). Images were acquired
1 and 24 hours after topical application of the aptamer probes in 2
mice for each aptamer. The β55 aptamer detected almost all of the
methoxy-XO4 positive plaques at both 1-hour (96%) and 24-hour
(83%) time points (see Table 2). In contrast, while the β55rc
control aptamer detected 83% of plaques at the one-hour time
point, only a few faint plaques (<30%) were detected after 24
hours. The contrast-to-noise-ratio (CNR) for β55 positive plaques
was significantly greater (p < 0.01) than that for β55rc plaques at
both 1- and 24-hour time points (Table 2 and Figure 6b).

To examine whether β55 induces plaque clearance or
degradation, similar to antibody probes of amyloid plaques,
longitudinal in vivo 2-photon imaging of a 10 month-old APP/PS1
mouse at 1 and 4 days following topical application of β55 was
performed. However, no changes in plaque number or appearance
were observed.

**Discussion**

RNA aptamers have previously been shown to detect synthetic
Aβ fibrils with very high sensitivity [35–37]. These RNA aptamers
were selected in SELEX screens against either Aβ monomers or
low molecular weight Aβ oligomers and may therefore prove to be
excellent probes for detecting not only amyloid plaques, but also
Aβ oligomers. Here we examined the ability of β55, an aptamer
previously selected in a SELEX screen against synthetic Aβ1–40
[35], to bind oligomeric Aβ and amyloid plaques in ex vivo human
AD brain tissue slices and in an in vivo transgenic mouse model of
AD.

As demonstrated in Figure 2, robust staining of amyloid plaques
by β55 was observed in ex vivo human AD brain tissue slices, while
only a few very faint plaques were observed with the β55rc control
aptamer. In addition, costaining with Thioflavin-S demonstrated
that β55-positive plaques colocalize with Thioflavin-S stained
plaques (Figure 2c). Similarly, β55 bound amyloid plaques in the
cortex and vasculature of live APP/PS1 mice (Figure 4) and
colocalized with methoxy-XO4 staining (Figure 5). Interestingly,
β55 stained not only the plaque core, but also labeled a diffuse
core that was not observed with methoxy-
XO4. These data suggest that β55 may bind smaller aggregates of
Aβ surrounding the dense core, including oligomeric Aβ.
To gain more insight into whether β55 binds oligomeric Aβ, dot blots of synthetic Aβ aggregates were performed. Strong β55 staining of Aβ₁₋₄₂ aggregates was observed with fainter staining of Aβ₁₋₄₀ aggregates (Figure 3a). The difference in staining is attributed to the increased propensity of Aβ₁₋₄₂ to form fibrils relative to Aβ₁₋₄₀. This is demonstrated in the 6E10 western blot of the Aβ samples, where a large number of high molecular weight species were observed for Aβ₁₋₄₂, but not Aβ₁₋₄₀ (Figure 3b). The Aβ₁₋₄₀ dot blot staining therefore indicates that β55 binds low molecular weight, non-fibrillar Aβ species. In accord with these findings, Tsukakoshi et al observed binding of a DNA aptamer to oligomeric Aβ₁₋₄₀ [38].

The in vivo optical imaging data indicates that both β55 and β55rc control aptamer bind plaques. This is consistent with previous studies that demonstrated that RNA aptamers in general have a high affinity for amyloidogenic structures. In a SELEX screen for Aβ targeted RNA aptamers, Rahimi et al showed that both the naive SELEX library and the final selected aptamers bound a large number of different amyloidogenic proteins, suggesting that RNA has an intrinsically high affinity for amyloidogenic, β-sheet structures [36]. Further support for the affinity of RNA for amyloidogenic structures is provided by a recent molecular modeling study that observed a very high homology between Aβ and the RNA binding protein AF-Sm1 [47] and by the fact that senile plaques have been found to contain high

![Figure 1. Predicted Secondary Structure of RNA Aptamers. Predicted secondary structure of the β55 (left) and β55rc (right) aptamer probes with base pair probability indicated by the color scale bar. Minimum free energy structures were determined using the RNAfold Webserver suite of programs. doi:10.1371/journal.pone.0089901.g001](#)

![Figure 2. β55 Staining of Amyloid Plaques in Ex Vivo Human AD Brain Tissue. Merged red and green channel confocal images of frozen-section human AD brain tissue stained with biotinylated β55 (a) and β55rc (b). β55 positive plaques (green) were clearly visible, while only a few very faint β55rc positive plaques were observed. Background auto-fluorescence, observed in both red and green channels, is shown in yellow. (c) Fluorescence images of human AD brain tissue costained with biotinylated-β55 (red) and Thioflavin-S (green). β55 colocalized with Thioflavin-S positive plaques. (Scale bars: 50 μm). doi:10.1371/journal.pone.0089901.g002](#)
levels of RNA [49–50]. The affinity of RNA for amyloidogenic structures would explain the in vivo plaque binding observed for the β55rc aptamer that was used as a control in our study. However, while RNA may have some affinity for beta sheet structures, the fact that a significantly increased CNR was observed for β55 plaques compared to β55rc plaques in the in vivo multiphoton images (Figure 6 and Table 2), at both early and late imaging time points, suggests that there are differences in binding affinity with β55 having a significantly greater affinity than β55rc. This is consistent with the fact that only a few very faint, β55rc-positive plaques were visible in the ex vivo human AD brain tissue (Figure 2b). In addition, Ylera measured aptamer dissociation constants (Kₐ) for binding to Aβ using affinity chromatography and while a Kₐ of 29 nM was determined for β55 no Kₐ was measurable for the RNA aptamer pool obtained from the first SELEX cycle [51], again indicating that RNA aptamers do display significant differences in binding affinity.

While the large molecular weight of the β55 aptamer (34 kDa) precludes its crossing the blood-brain barrier (BBB), a recent study by Cheng et al, in which an in vivo SELEX screen for a BBB penetrating aptamer was performed, did find an aptamer (A15) capable of crossing the BBB [10]. While the exact mechanism of the brain uptake of the BBB penetrating aptamer is unclear, given the large molecular weight of the aptamer (23 kDa) the most likely mechanism is by receptor-mediated transcytosis. Studies are currently underway to investigate the possibility of designing a fusion aptamer consisting of BBB penetrating and amyloid binding domains that would allow aptamers to be delivered non-invasively to the brain.

These studies demonstrate the great potential of RNA aptamers as in vivo and ex vivo imaging probes. One third of uracil nucleotides were fluorescein labeled, corresponding to a total of 10 fluoresceins per aptamer, providing high detection sensitivity. The incorporation of multiple fluorescein labels did not interfere with the amyloid binding. The fact that the fluorescein-labeled aptamer still bound plaques with high affinity is likely due to the fact that only a relatively small conserved region of the aptamer is required for binding. A significant fraction of the aptamer consists of sequences required for the selection/amplification process in the SELEX screen. In addition, topical application of β55 did not result in

Figure 3. β55 Staining of Dot Blots of Synthetic Aβ Aggregates. (a) Dot blot of synthetic Aβ1–42 and Aβ1–40 aggregates probed with biotinylated-β55. (b) Western blot of the synthetic Aβ1–42 and Aβ1–40 aggregates probed with 6E10 antibody. The increased staining of Aβ1–42 aggregates in the dot blot relative to Aβ1–40 aggregates is consistent with the greater fibril and high molecular weight oligomer composition of Aβ1–42 aggregates observed in the western blot.
doi:10.1371/journal.pone.0089901.g003

Figure 4. In Vivo Imaging of β55 Positive Amyloid Plaques. In vivo 2-photon microscopy images from an 18 month old APP/PS1 transgenic mouse obtained 1 hour after topical application of fluorescein-labeled β55 (a,b). Texas Red labeled dextran was intravenously injected for visualization of blood vessels. β55 positive plaques and cerebral amyloid angiopathy are clearly visible in the cortex (a) and vasculature (b), respectively. (Scale bars: 20 μm).
doi:10.1371/journal.pone.0089901.g004
Figure 5. Colocalization of β55 and Methoxy-XO4 Positive Amyloid Plaques. In vivo 2-photon microscopy plaque images from a 7 month old APP/PS1 transgenic mouse acquired 1 hour after topical application of fluorescein-labeled β55 (a,d) and 1 day after intraperitoneal injection of methoxy-XO4 (b,e). While methoxy-XO4 stains only the dense core of the plaque, β55 stains both the plaque core and a diffuse halo surrounding the plaque (c,f). (Scale bars: 20 μm).

doi:10.1371/journal.pone.0089901.g005

Figure 6. Contrast-to-Noise Ratio for β55 and β55rc Positive Amyloid Plaques. (a) Representative in vivo 2-photon microscopy images from 7.5 month old APP/PS1 transgenic mice acquired 1 hour (left column) and 24 hours (right column) after topical application of either fluorescein-labeled β55 (top row) or β55rc (bottom row). Most β55 plaques were still visible 24 hours after topical application. In contrast, only a small number of very faint β55rc plaques were still visible after 24 hours. (b) Average plaque contrast-to-noise ratio (CNR) observed 1 hour and 24 hours following topical application of fluorescein-labeled β55 (n = 2) or β55rc (n = 2). β55 positive plaques had a significantly greater CNR than β55rc plaques (p<0.01) at both time points. (Scale bars: 50 μm).

doi:10.1371/journal.pone.0089901.g006
RNA Aptamer Probes for Imaging Amyloid Plaques

Table 2. Contrast-to-noise ratio (CNR) distribution of amyloid plaques observed in 2-photon in vivo images of 7.5 month-old APP/PS1 transgenic mice with β55 and β55rc.

<table>
<thead>
<tr>
<th>β55</th>
<th>β55rc</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNR</td>
<td>Time (hrs)</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Total plaques</td>
<td>99</td>
</tr>
<tr>
<td>Total detected</td>
<td>95</td>
</tr>
<tr>
<td>% Detected</td>
<td>96.0</td>
</tr>
<tr>
<td>Average CNR</td>
<td>3.7 ± 0.2</td>
</tr>
</tbody>
</table>

Conclusions

In summary, the β55 aptamer binds amyloid plaques in both ex vivo human AD brain tissue and in vivo APP/PS1 transgenic mice. Diffuse halos surrounding the methoxy-XO4 positive plaque cores were observed with β55 in vivo, which may represent oligomeric Aβ. The unprotected RNA aptamer appears to be quite stable and detectable for at least 24 hours under in vivo conditions when bound to amyloid plaques. In addition, no detectable tissue response to the aptamer application, such as plaque clearance, was detected. These data suggest that Aβ-targeted aptamers bind oligomeric Aβ and may be useful reagents for imaging both fibrillar and non-fibrillar Aβ. Moreover, these data illustrate the broader principle that RNA aptamers, which are nearly infinitely adaptable using SELEX technologies and that can be labeled with any number of off-the-shelf multimodal imaging agents (fluorophores, biotin, radioisotopes or epitope tags), can be developed with high enough detection sensitivity and selectivity to rival antibodies and small molecules in in vivo and ex vivo imaging studies.

Supporting Information

Figure S1  β55 Staining of Western Blot of Human AD Brain Tissue Extracts. Western blot of human AD brain tissue extracts obtained after sequential treatment with TBS, 2% Triton X-100, 0.5% SDS, and 70% formic acid. The western blot was probed with both 6E10 antibody (green) and biotinylated-β55 (red). β55 binds many of the same bands as 6E10 (yellow asterisks). The green and red asterisks indicate bands unique to 6E10 or β55, respectively. Faint bands at ~8 and 16 kDa were visible in the β55 image (arrows) of the SDS soluble fraction. (TIF)

File S1  Methods and Results for β55 Staining of Western Blot of Human AD Brain Tissue Extracts. (DOCX)

Author Contributions

Conceived and designed the experiments: CTF CMW BTH. Performed the experiments: CTF CMW EH TH. Analyzed the data: CTF CMW TH BTH. Wrote the paper: CTF CMW BTH.

References


