# RNA aptamers as research tools and diagnostic reagents in amyloid disease

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## Introduction

There are more than 30 known human disorders characterised by the self-assembly of proteins or peptides into amyloid fibrils and various oligomeric assemblies. The aggregation of the $\beta_2$-microglobulin ($\beta_2$m) protein (both full length and a truncated variant termed ΔN6) in the joints of patients undergoing long-term haemodialysis, leads to a debilitating, and ultimately fatal, disorder known as Dialysis Related Amyloidosis (DRA). Similarly, the amyloid-β (Aβ) peptide is considered the primary neurotoxic agent in Alzheimer's disease. The peptide accumulates into typical amyloid fibrils that are found deposited in the neuronal plaques associated with the disease. Aβ also forms a number of soluble, oligomeric species that are thought to play important roles in disease progression.

Nucleic acid aptamers are small structured polynucleotide sequences that can be isolated by automated *in vitro* selection from randomised oligonucleotide libraries (Figure 1). The chemical simplicity and adaptability of RNA aptamers make them a promising class of compounds for the diagnosis or therapy of human disease, including amyloidosis. Aptamers have advantages over antibodies as potential therapeutics and diagnostics as they are significantly smaller and are thus able to access epitopes hidden from antibodies. In addition, they do not carry the secondary functional signals of antibodies, such as complement fixation; do not elicit significant immune responses; can be stabilised against nuclease action by several non-toxic chemical modifications; are easily modified to include chromophores, fluorophores, radiolabels or reactive functional groups; and can be amplified *via* PCR. Several aptamer-based drugs are now approved for clinical use. Aptamers thus provide exciting new opportunities for exploitation in diagnosis and disease intervention.

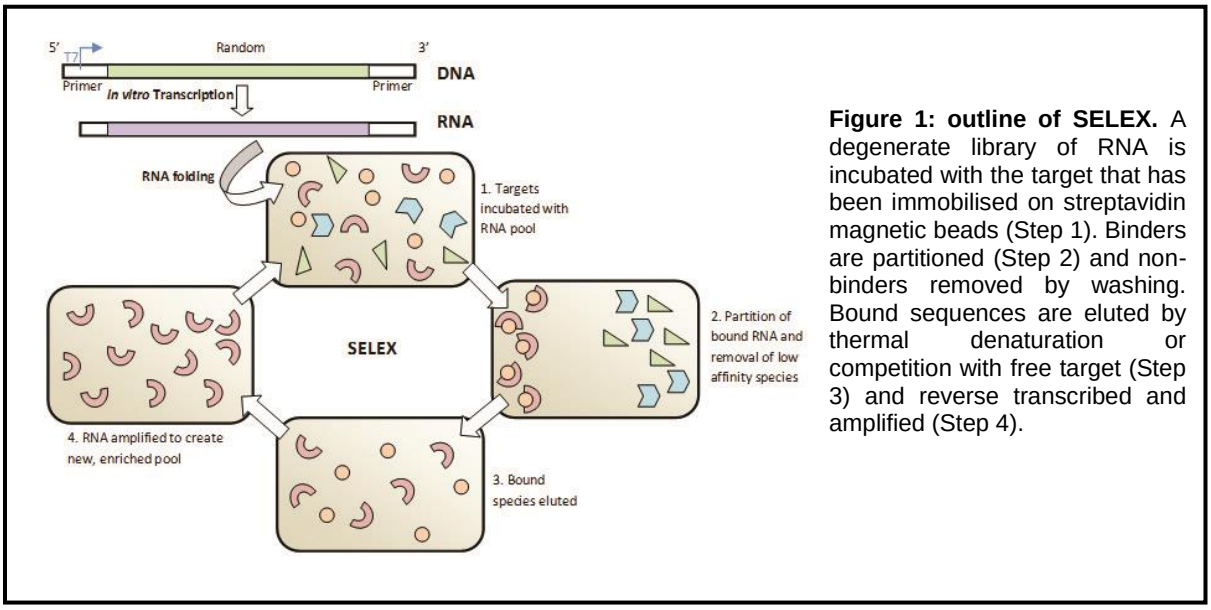


**Figure 1: outline of SELEX.** A degenerate library of RNA is incubated with the target that has been immobilised on streptavidin magnetic beads (Step 1). Binders are partitioned (Step 2) and non-binders removed by washing. Bound sequences are eluted by thermal denaturation or competition with free target (Step 3) and reverse transcribed and amplified (Step 4).

## Searching for an anti- $\beta_2$m aptamer

Modified RNA aptamers were raised against monomeric $\beta_2$m (Figure 2A), monomeric ΔN6 and fibrils formed from these monomers that have three different morphologies, formed under different conditions. Two aptamers were isolated against monomeric $\beta_2$m: B6 and B9. The secondary structure of aptamer B6, as predicted by Mfold, is a 44 nucleotide long stem-loop interrupted by single-stranded bulges. This is the fragment of the full-length aptamer that encompasses the structural motif required for specific recognition of the target ligand and

was determined through enzymatic and chemical cleavage protection assays. The binding affinity of B6 was determined by Surface Plasmon Resonance and tryptophan fluorescence quenching (Figure 2 B): a dissociation constant of 100 nM was found, and B6 had no cross reactivity with monomeric ΔN6.

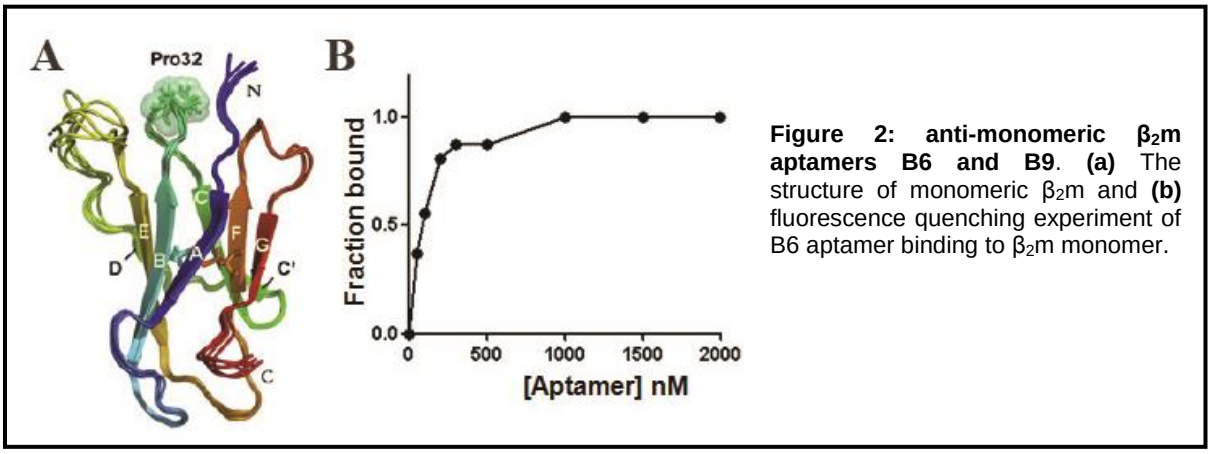


**Figure 2: anti-monomeric $\beta_2$m aptamers B6 and B9**. **(a)** The structure of monomeric $\beta_2$m and **(b)** fluorescence quenching experiment of B6 aptamer binding to $\beta_2$m monomer.

Work to raise aptamers against Aβ targets is ongoing, with aptamer sequences selected against fibrils with different morphology as well as monomeric Aβ. Aptamer binding affinities and epitopes will be determined as performed with $\beta_2$m aptamers. Aptamers will also be tested in a number of i*n vivo* assays, determining their potential as diagnostic probes or therapeutic agents.

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**Collaborators**
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