Supporting materials

FaptaSyme: a Strategy for Converting a Monomer/Oligomer Non-Selective to Oligomer-Selective Aptameric Sensors

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1. Materials and instruments. All oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). DNAse/RNAse free water was purchased from Fisher Scientific and used for all assays including buffers, and for dissolution of oligonucleotides. Concentrations of oligonucleotide were determined based on UV light absorption at 260 nm. Reactions took place in DZ reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 20 mM NaCl, 0.03% Triton X-100, and 1% DMSO) at a final volume of 60 µl. Oligomeric and monomeric α-syn were kindly donated by Han Seok Ko of Johns Hopkins University. Fluorescence quantification occurred using a Perkin-Elmer LS-55 spectrometer (Waltham, MA) or Tecan m200 pro fluorescence plate reader.

2. Table S1. Oligonucleotides used in this study

Name	Sequence 5'→ 3'	Purification
F_Sub	AAG GTT^{FAM} TCC TCg uCCC TGG GCA-BHQ1	HPLC
T-SO504	CAG GGG TGG GCA AAG GGC GGT GGT G	SD
SAptz-1a	CAG GGG TGG GC /iSp9/*A CAA CGA* GAG GAA AC	SD
SAptz-1b	CCA GGG A *GG CTA GCT* /iSp9/GGG CGG TGG TG	SD
FaptaSyme-a	CAG GGG TGG GCA AAG GGC GGT GGT G /iSp18/*A CAA CGA* GAG GAA AC	SD
FaptaSyme-b	CCA GGG A *GG CTA GCT* /iSp18/ CAG GGG TGG GCA AAG GGC GGT GGT G	SD

iSp18-hexaethylene glycol linkers; SD, standard desalting; RNA nucleotides are in low case; **F-sub** binding arms are underlined.

3. Detailed Experimental Procedure

General Fluorescence assay for SDZ sensors. Oligomeric and Monomeric α-syn were diluted in PBS and sonicated at 20 Hz for one minute with 0.5-second pulses. DZa, DZb, and **F-sub** were used at concentrations of 2 nM, 50 nM, and 200 nM, respectively, in 60 µl reactions containing DZ reaction buffer. All reactions were incubated for 18 hr in a 30°C water bath. To perform selectivity analysis, each reaction tube was administered 100 nM of oligomeric α-syn, or monomeric α-syn. Negative control reactions were administered 26 µM of thrombin. To perform LOD analysis, reactions were administered 1 nM, 10 nM, 20 nM, 40 nM, and 100 nM of oligomeric α-syn. Raw fluorescence values were then plotted to establish a calibration curve, from which the LOD was calculated.

4. Figure S1 Design of split aptazyme sensors

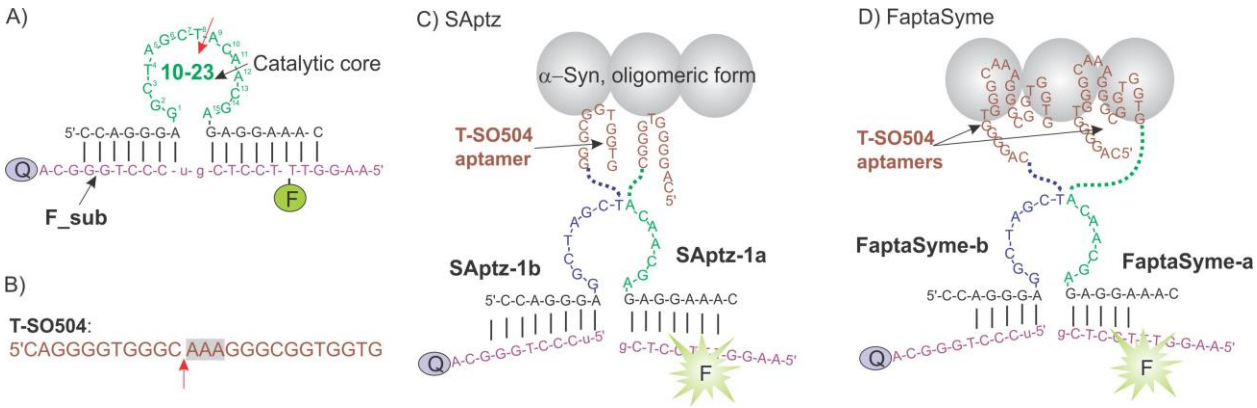

Figure S1. Design of aptazyme sensors for detection of α-syn A) 10-23 deoxyribozyme (DZ) selected by Santoro and Joyce[1] can cleave a fluorophore- and quencher-substrate, as reported by Mokany et al.[2] B) the sequences of T-SO504 aptamer obtained by Tsukakoshi et al.[3] The split sites are shown by the red arrows. Shadowed AAA sequence was not included in the fragment attached to the DZ sensor strands on panel C. C) Split aptamer design. The structure of the split aptamer sensor bound to the oligomeric α-syn. The sensor produces fluorescent signal by cleaving fluorogenic **F-sub**. D) The FaptaSyme design. Two aptamer-containing structures **FaptaSyme-a** and **FaptaSyme-b** bind to α-syn oligomeric in close proximity. The presence of flexible oligoethylene glycol linkers provides sufficient freedom for the DZ fragments to from functional DZ catalytic core upon binding to α-syn.

5. Figure S2. Optimization of FaptaSyme strand concentrations.

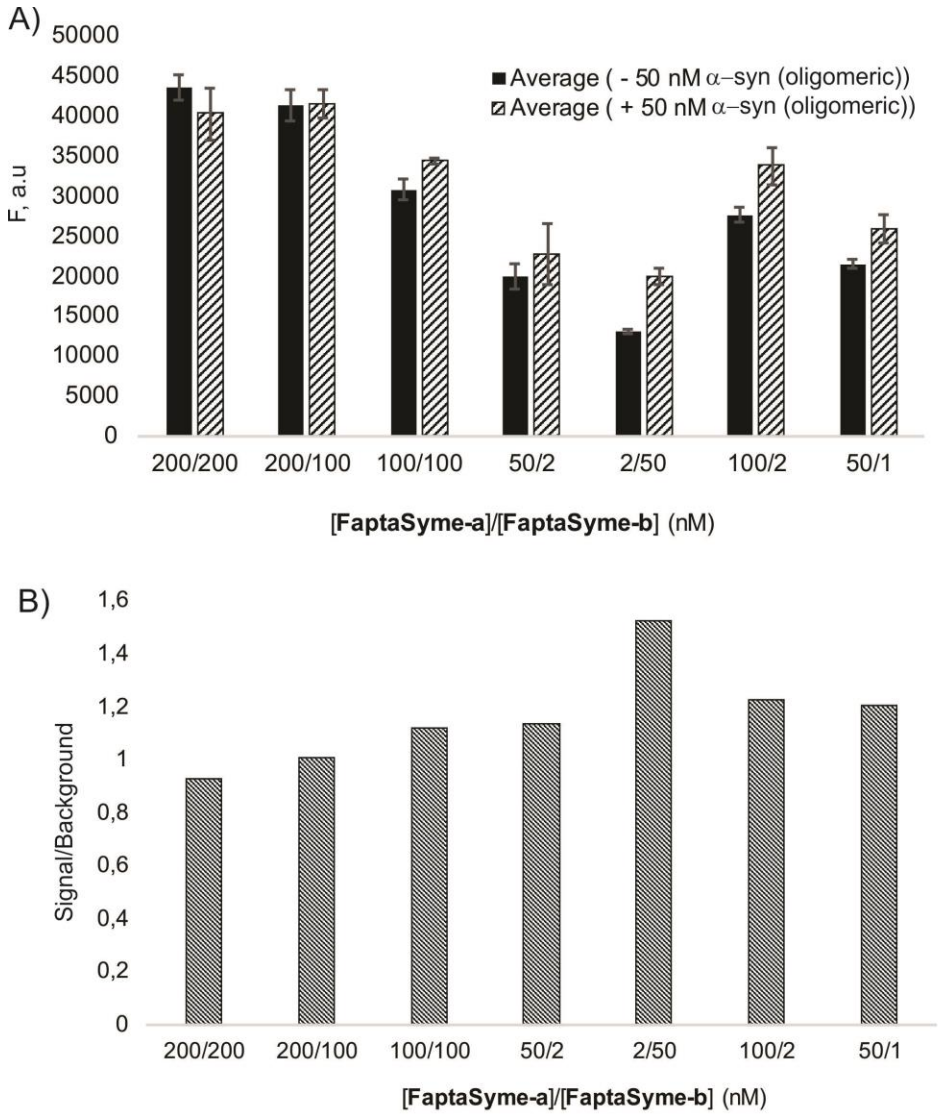

Figure S2. Optimization of **FaptaSyme** strands concentrations for the detection of 50 nM α-syn. A) Samples were incubated in the DZ reaction buffer (50 mM HEPES, pH 7.4, 50 mM $MgCl_2$, 20 mM NaCl, 0.03% Triton X-100, and 1% DMSO) containing different concentrations of **FaptaSyme–a** and **FaptaSyme-b** strands in the absence (black bar) or presence of 50 nM α-syn (cross hatched bars). The fluorescence was measured after 18 hrs of incubation at 30°C. Average data of 3 independent experiments are present together with standard deviation. B) Signal–to-background ratios for **FaptaSyme** depending on the concentrations of the 2 sensor strands calculated from the data shown in panel A). The maximum S/B was detected for 2 nM **FaptaSyme–a** and 50 nM **FaptaSyme–b**, which were used in the following experiments.

6. Figure S3. Split aptamer sensors' performance in a complex sample

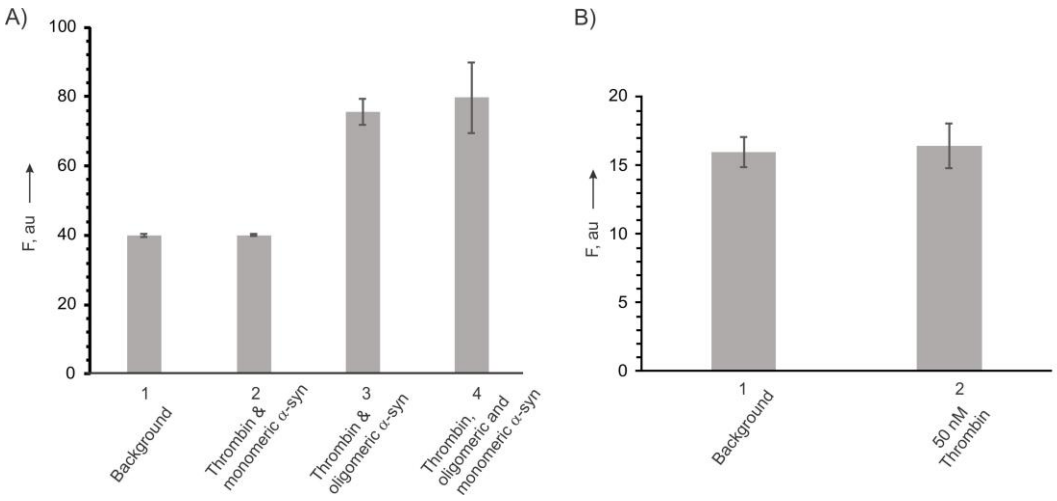

Figure S3. Fluorescent response of the **FaptaSyme** sensor in the presence of thrombin. A) The sensors was incubated in the presence of 100 nM monomeric (2), 100 nM oligomeric (3), or 100 nM monomeric and oligomeric (4) α-syn spiked with 26 μM thrombin. B) Sensor response to the presence of 50 nM thrombin. Reactions were incubated at 30℃ for 18 hours. Fluorescence was quantified using Perkin-Elmer LS55 Luminometer.

7. Figure S4. Time-Dependent Fluorescence Output After 2 and 18 hours of Incubation

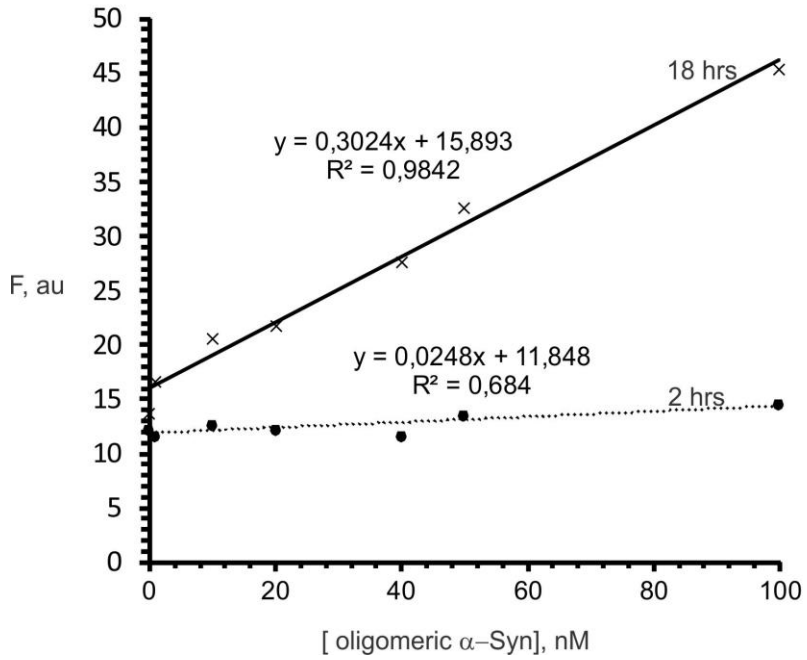

Figure S4. Time-dependent fluorescence output by **FaptaSyme** sensor. Two hour incubation (dashed) produced little resolution compared to eighteen hours incubation (solid), illustrating the enzymatic properties of DZ and the contribution to lowered limits of detection.

8. Figure S5 Limits of detection for SDZ sensors

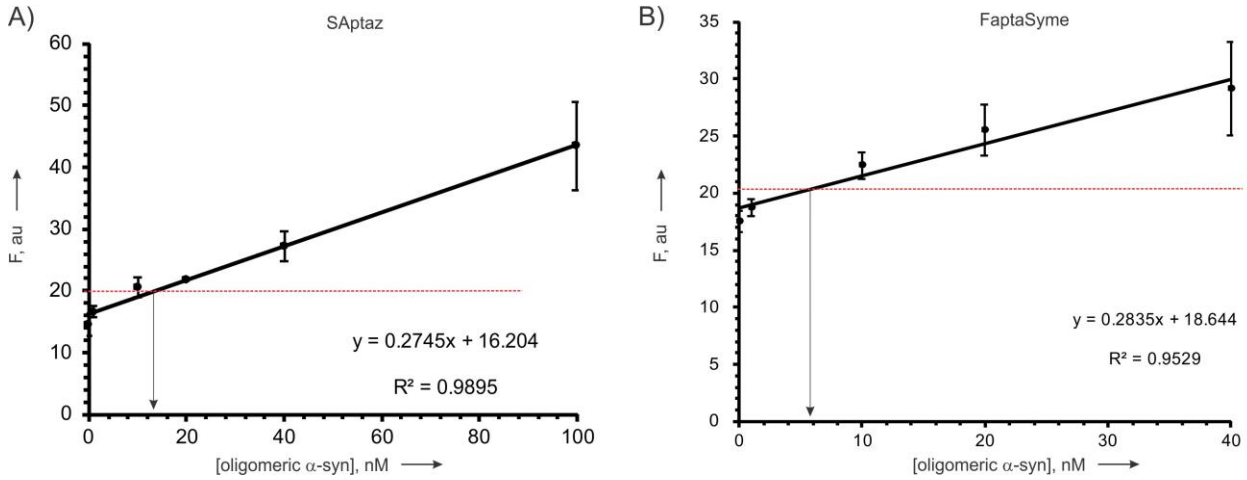

Figure S5. Limit of detection (LOD) for the two classes of split DZ sensors. The LODs were determined based on the concentration dependence shown in panels A and B and were found to be as follows: A) SAptz: 13.4 nM. B) FaptaSyme: 5.6 nM. C. The LOD values were obtained using the equation $\frac{[3\sigma+c]-b}{m}$; where σ is the standard deviation of the 1 nM sample raw a.u., while *c* is the raw a.u. value of the 1 nM sample, *b* is the y-intercept, and *m* is the slope of the line.

9. Reference

1. Santoro S.W., Joyce G. F. Mechanism and utility of an RNA-cleaving DNA enzyme. *Biochemistry*. **1998**, *37*, 13330-13342.

2. Mokany, S. M. Bone, P. E. Young, T. B. Doan and A. V. Todd, MNAzymes, a versatile new class of nucleic acid enzymes that can function as biosensors and molecular switches. *J. Am. Chem. Soc.*, **2010**, *132*, 1051-1059.

3. Tsukakoshi K., Abe K., Sode K., Ikebukuro K., Selection of DNA aptamers that recognize α-synuclein oligomers using a competitive screening method. *Anal Chem.* **2012**, *84*, 5542-5547.