Supporting Information

Barnaby et al. 10.1073/pnas.1409431111

SI Text

Selection of 10% Solutions of FBS. To study the interaction between a spherical nucleic acid nanoparticle conjugate targeting human androgen receptor (SNA-siRNA$_{AR}$) and serum nucleases, we examined the degradation of SNA-siRNA$_{AR}$ and siRNA$_{AR}$ in solutions of 10% FBS. We used FBS as a source of serum ribonucleases, as opposed to buffered solutions of purified ribonucleases, because we wished to investigate the interaction of SNA-siRNA$_{AR}$ with serum typically used to supplement media for mammalian cell culture in vitro (e.g., minimal essential medium supplemented with 10% FBS). Such solutions of FBS contain a complex mixture of proteins, including ribonucleases, and can serve as a crude model for biological media in vivo (i.e., blood).

Degradation of SNA-3 in Solutions of 10% FBS. Incubation of SNA-3 in solutions of FBS at 37 °C led to a decrease of ∼12% in the ratio of noncovalently associated oligonucleotide to gold nanoparticle (AuNP) in 15 min before remaining approximately constant for the following 100 min (Fig. S6*A*). Through polyacrylamide gel electrophoresis (PAGE) and electrospray ionization mass spectrometry (ESI-MS), we observed two products corresponding to the elimination of either one or five nucleotides after 15 min of reaction (Fig. S6*B*, lane v and Fig. S7*C*. Analysis at later time points (≥1 h) revealed the persistence of a single major band (Fig. S6*B*, lanes ii and iv). We established that the length of the major product was 16 nt by resolving a single major band in the analysis of a mixture of the reaction product and a 16-nt standard, derived from the sense oligonucleotide (Fig. S6*B*, lane iii). The identification of the major degradation product was supported by the mass observed through ESI-MS (5,039 amu; Fig. S6*D*), which is in agreement with the predicted and observed mass of the 16-nt analytical standard (Fig. S6*E*).

We analyzed the minor degradation products of SNA-3 by PAGE (minor bands in lanes iv and v of Fig. S6*B*) and by ESI-MS, which revealed a 20-nt fragment (Fig. S7*C*) that we interpreted as the loss of a deoxythymidine (dT) nucleotide from the 3′ terminus of the sense oligonucleotide. This product is probably generated by 3′–5′ exonuclease activity present in solutions of FBS (1, 2), which is likely independent of the endonuclease activity implicated in Fig. S6.

We chose to interpret the ESI-MS measurements in Fig. S6*D* as evidence that the degradation product of the sense oligonucleotide of SNA-3 is a 16-nt fragment generated by the loss of TTUAU (3′–5′) from the 3′ terminus. However, the mass of 5,039 amu does not allow for an unambiguous assignment. An alternative interpretation is that a mass of 5,039 arises from loss of the five DNA nucleotides (GAACT, 3′–5′) from the 5′ terminus of the sense oligonucleotide. This scenario is unlikely, however, because of the absence of a 2′-OH group at the location of the phosphodiester bond that would be cleaved in this scenario. We applied the same logic in the assignment of the degradation product in Fig. 5*C*, where a dT nucleotide could, in theory, be lost from either the 3′ or 5′ end of the sense oligonucleotide. Fig. S5 shows the sequences and masses of all possible degradation products.

Determining the Site of Nuclease-Catalyzed Hydrolysis for the Sense Oligonucleotide of siRNA$_{AR}$ Free in Solutions of 10% FBS. We analyzed the degradation of siRNA$_{AR}$ free in solutions of 10% FBS at 37 °C through the use of siRNA$_{AR}$ where the sense oligonucleotide was biotin labeled at the 5′ terminus (Fig. S3*A*). We recovered biotin-labeled degradation products from the reaction mixture; analysis of these products by denaturing PAGE revealed three major bands (Fig. S3*B*). The appearance of these bands, each with mobility greater than that of the starting material, suggests that the degradation of the sense oligonucleotide of siRNA$_{AR}$ free in solutions of 10% FBS is rapid (complete within 20 s) and generates a different set of products than that observed for siRNA$_{AR}$ when conjugated to AuNPs (Fig. S6*B*). Comparison of the mobilities of these reaction products to those of analytical standards suggests the loss of two, four, and five nucleotides from the 3′ terminus of the sense oligonucleotide (Fig. S3 *C*–*F*). The major degradation product results from the loss of dTdT from the 3′ terminus of the sense oligonucleotide (Fig. S3*C*) and lies in contrast to the major product of degradation observed for the sense oligonucleotide in SNA-3 (loss of five nucleotides).

Furthermore, the degradation of sense oligonucleotide of siRNA$_{AR}$ in serum solution generates two additional products. Analysis of these products in Fig. S3 *D* and *E*, by comparison of the mobilities of these products to those of the 17-nt and 16-nt standards, suggests that these products result from the loss of four and five nucleotides. Analysis of a mixture of the degradation products and of the 15-nt standard, shown in lane iii of Fig. S3*F*, results in a different pattern than that observed in lane i; the appearance of an additional band in lane iii suggests that the mixture of degradation products does not include the 15-nt product.

Although the set of degradation products includes the 16-nt degradation product observed in the degradation of siRNA$_{AR}$ immobilized onto AuNPs in SNA-3, it also contains two additional products not observed in the degradation of siRNA$_{AR}$ in SNA-3. This difference in the pattern of degradation products of the sense oligonucleotide of siRNA$_{AR}$, between that of siRNA$_{AR}$ free in solution and siRNA$_{AR}$ on SNA-3, suggests a difference between the recognition by serum nucleases of siRNA$_{AR}$ free in solution and of siRNA$_{AR}$ immobilized onto AuNPs.

SI Materials and Methods

Preparation of Sterile, RNase-Free Solutions. To prepare sterile solutions of water, 1× PBS (Corning), 5 M sodium chloride (NaCl; Sigma-Aldrich), 10 nM AuNPs, and 10% Tween-20 (Sigma-Aldrich), 1 mL of diethylprocarbonate (DEPC; Sigma-Aldrich) was added to 1 L of the aforementioned solution. The mixture was then shaken at 50 rpm for 2 h at 45 °C before being autoclaved.

RNA Oligonucleotide Synthesis. RNA oligonucleotides were synthesized using 2′-O-[(triisopropylsilyl)oxy] methyl-RNA phosphoramidites (ChemGenes) on a MerMade 6 system (Bioautomation) according to the manufacturer-recommended cleavage and deprotection protocols. All oligonucleotides were purified using reverse-phase high-performance liquid chromatography (RP-HPLC) on a Varian Microsorb C_{18} column (10 μM; 300 × 10 mm) with 0.1 M triethylammonium acetate (TEAA) at pH 7 with a 1% gradient of 100% CH_3CN at a flow rate of 3 mL/min, while monitoring the UV signal of the nucleic acids at 254 nm. After purification, the oligonucleotides were lyophilized, resuspended in sterile water, and stored at −80 °C.

Synthesis of Spherical Nucleic Acid Nanoparticle Conjugates (SNA-siRNA). SNAs were synthesized by modification of previously published methods (3). AuNPs with 13-nm diameter were synthesized by modification of the Frens method, where chloroauric acid was reduced by sodium citrate in aqueous solution (4). To prepare duplex siRNA, the sense oligonucleotide (mRNA sequence

targeted by the siRNA duplex; 150 mM) and AS oligonucleotide (complementary to messenger RNA; 150 mM) were hybridized in a buffer consisting of 30 mM Hepes and 100 mM potassium acetate, pH 7.5 (available from IDT) by first heating the solution to 95 °C for 10 min, then cooling to 37 °C for 60 min while shaking at 350 rpm in a thermomixer (Benchmark Scientific). Duplex siRNA was added to solutions of AuNPs (10 nM) in 0.2% Tween-20 (vol/vol) and 150 mM NaCl (final siRNA concentration of 2 μM). The solution was then sonicated for 30 s and placed on a rotary shaker (Sigma-Aldrich) for 4 h, at which point the NaCl concentration was increased to 350 mM and allowed to shake for 16 h. The solution was then brought to 10 μM in thiolated 5 kDa polyethylene glycol (PEG; Nanocs) and allowed to shake for 4 h. The solution was then centrifuged for 10 min in a swinging bucket rotor at 1,500 × *g* in an Amicon Ultra-15 centrifugal filter unit (50-kDa cutoff; EMD Millipore). The flow-through was removed and the SNAs were washed twice with 0.01% Tween in 1× PBS by centrifugation at 1,500 × *g* for 10 min. The SNAs were then resuspended in 1 mL of 0.01% Tween-20 in 1× PBS and centrifuged in an Eppendorf tube for 25 min at 21,000 × *g*. The supernatant was removed and the pellet was resuspended in 1× PBS. The concentration of SNA was measured by absorbance at 520 nm ($\varepsilon_{520nm} = 2.7 \times 10^8\ M^{-1}\ cm^{-1}$) using a Cary 5000 UV-Vis. The SNAs were stored at 4 °C when not in use.

Measurement of the Ratio of the Noncovalently Associated RNA Oligonucleotide to AuNP. To determine the stoichiometry of the noncovalently associated RNA oligonucleotide to AuNP in a batch of SNA-siRNA, the concentrations of noncovalently associated oligonucleotide and AuNPs were independently analyzed (Fig. S1). To measure siRNA loading on the nanoparticle, Quant-iT OliGreen (Invitrogen) assays against a standard curve were used. To start, 3×10^{-12} mol of SNA-siRNA were resuspended in 100 μL of 8 M urea (Sigma-Aldrich) and heated to 45 °C with shaking for 20 min. The solution was diluted with 0.01% Tween-20 to a final concentration of 4 M urea and centrifuged at 21,000 × *g* for 25 min. A portion of the supernatant (25 μL) was analyzed by mixing with OliGreen reagent and measurement of OliGreen fluorescence ($\lambda_{ex} = 480$ nm) in a 96-well plate (Biotek synergy plate reader). The concentration of the AuNP was measured by resuspending the pellet in 1 mL of DEPC-treated water and measuring the absorbance at $\lambda_{max} = 520$ nm in a Cary-5000 UV-Vis spectrophotometer.

Analysis of the Degradation of the Noncovalently Associated RNA Oligonucleotide of SNA-siRNA in Media. The lifetime of $siRNA_{AR}$ in serum containing media was determined using the method described in Fig. S1. SNAs (20 nM in AuNP concentration) were suspended in a solution of 1× PBS or 1× PBS with 10% (vol/vol) FBS (HyClone) at 37 °C. For reactions in solutions of FBS, an aliquot (150 μL) was removed at time points and mixed with 30 mM SDS (Sigma-Aldrich) to deactivate serum RNases and stop the reaction. The mixture was then centrifuged at 21,000 × *g* for 25 min, at which point the supernatant was removed and the pellet was washed in 0.01% Tween-20 in 1× PBS. After washing, the SNAs were analyzed to determine the ratio of noncovalently associated RNA oligonucleotide to AuNP (with the protocol described above).

Deactivation of RNase A Type Enzymes. A 200-mM stock solution of ribonucleoside vanadyl complex (New England Biolabs) was heated to 65 °C until a green-black clear solution was observed. The solution was cooled to room temperature before addition to 10% FBS in 1× PBS at a final concentration of 10 mM. The procedure produced solutions of FBS with reduced ribonuclease activity in the analysis of the degradation of SNA-siRNA.

Recovery of Noncovalently Associated RNA Oligonucleotide from SNA-siRNA Following Incubation in 10% FBS or 1× PBS. The remaining supernatant consisting of noncovalently associated oligonucleotide in 4 M urea that was not analyzed by fluorescence was run through a size-exclusion column (Sephadex G-25 DNA Grade; GE Healthcare Life Sciences) and eluted with aqueous 0.1 M triethylammonium acetate (TEAA; Sigma-Aldrich). Fractions were collected, lyophilized, and then resuspended in 10 μL of 90% formamide (USB Corporation), 10% 0.1× Tris/borate/EDTA (TBE; Bio-Rad) buffer. The oligonucleotide concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific). The samples were then heated to 95 °C for 10 min to denature the RNA oligonucleotides. After cooling to room temperature, 5 μL of loading dye (a 40× diluted solution of glycerol, Tris, bromophenol blue sodium salt, xylene cyanole, water, pH 8; BioRad) was added to the solution, followed by vortexing and brief centrifugation.

Denaturing 20% Polyacrylamide Gel Electrophoresis. A gel stock was prepared with 12 g of ultra-pure urea (Invitrogen), 12.5 mL of 40% acrylamide/bis acrylamide (BioRad) and 2.5 mL of 10× TBE for a final solution that is 20% acrylamide in 8 M urea. The mixture was then sonicated and degassed for 5 min, at which point it was split into two equal volume fractions. Ammonium persulfate (35 μL of an aqueous solution at a concentration of 100 mg/mL; Sigma-Aldrich electrophoresis grade) was added to the solution followed by the addition of 30 μL of *N,N,N′,N′*-tetramethylethylenediamine (TEMED; BioRad). The solution was stirred and then poured between two 1.5-mm glass plates (BioRad) and allowed to polymerize for 40 min. The gel was then prerun at 200 V for 30 min in 1× TBE, after which the samples were loaded and separated by electrophoresis at 200 V for 2 h. Following electrophoresis, the gel was rinsed with water and stained with 0.02% methylene blue (Sigma-Aldrich) with shaking at 50 rpm for 20 min. Stained gels were rinsed with nanopure water and destained for 3 h in nanopure water before being imaged with an Alpha Innotech FluorChemQ.

Analysis of the Degradation of $siRNA_{AR}$ in Solutions of 10% FBS in PBS. To analyze the degradation of $siRNA_{AR}$ free in solution (i.e., unconjugated to AuNPs), we designed and analyzed two derivatives of $siRNA_{AR}$ independently, one with a biotinylated sense oligonucleotide and the other with a biotinylated antisense (AS) oligonucleotide. Upon incubation of biotin-labeled $siRNA_{AR}$ (10 nmol) in solutions of 10% FBS in PBS for 20 s (37 °C, 0.2 mL), reactions were quenched with the addition of SDS (30 mM), followed by the addition of urea (0.25 g) to dehybridize $siRNA_{AR}$. Quenched reaction mixtures were diluted into solutions of PBS to a final volume of 2.5 mL and added to 0.3 mL of streptavidin-acrylamide beads (Pierce; Streptavidin Plus UltraLink Resin). The slurry was equilibrated for 1 h to allow for the association of biotin-labeled oligonucleotide to the beads. After removal of unbound material from the beads and three washes with PBS (5 mL each), biotin-labeled oligonucleotides were eluted from the beads by the addition of 8 M urea (1.0 mL) and heated to 90 °C for 1 min. Eluates were purified by gel filtration chromatography (GE Healthcare Life Sciences; NAP-25) to obtain samples of biotin-labeled oligonucleotide free of urea and PBS. Biotin-labeled oligonucleotides recovered from reaction mixtures were analyzed by denaturing PAGE (20% acrylamide, 0.5% bis-acrylamide, 8 M urea in TBE buffer).

Electrospray Ionization Mass Spectrometry. Accurate mass data were obtained using an Agilent 6210A LC-TOF mass spectrometer equipped with an Agilent Series 1200 HPLC binary pump and an Agilent Series 1200 autoinjector. RNA oligonucleotides were prepared at a concentration of 0.02 mg/mL in sterile water. A 30-μL siRNA injection was run on a reverse phase cartridge C_{18} column at 45 °C (2.5 μm, 2.1 × 10 mm^2; Waters) with 0.2 M hexafluoroisopropanol (Sigma-Aldrich) and 16 mM triethylamine (Sigma-Aldrich) at pH 8. A gradient of 100% pure HPLC grade methanol (Sigma-Aldrich) at a flow rate of 0.4 mL/min was used,

while monitoring the UV-Vis absorption signal of the nucleic acids at 254 nm. The methanol gradient used is as follows:

Time (minutes)	% B
0	10
1	10
7	25
8	25
8.1	10
15	10

For LC-TOF detection, negative ion mode was used for the acquisition range of 600–3,200 Da. The conditions for the LC-TOF are detailed below:

Gas temperature	340 °C
Gas flow	8 L/min
Nebulizer	40 psi
V capillary	4,200 V
Fragmentor voltage	230 V
Skimmer	64 V
Octapole RF peak	250

For deconvolution of the mass spectra, the parameters are detailed below:

Data from 600–3,200 amu
Adduct: proton loss
Average mass at 75% of peak height
S/N threshold: 5
Minimum number of consecutive charge states: 3.

1. Shaw J-P, Kent K, Bird J, Fishback J, Froehler B (1991) Modified deoxyoligonucleotides stable to exonuclease degradation in serum. *Nucleic Acids Res* 19(4):747–750.
2. Zou Y, Tiller P, Chen IW, Beverly M, Hochman J (2008) Metabolite identification of small interfering RNA duplex by high-resolution accurate mass spectrometry. *Rapid Commun Mass Spectrom* 22(12):1871–1881.
3. Giljohann DA, Seferos DS, Prigodich AE, Patel PC, Mirkin CA (2009) Gene regulation with polyvalent siRNA-nanoparticle conjugates. *J Am Chem Soc* 131(6):2072–2073.
4. Kimling J, et al. (2006) Turkevich method for gold nanoparticle synthesis revisited. *J Phys Chem B* 110(32):15700–15707.

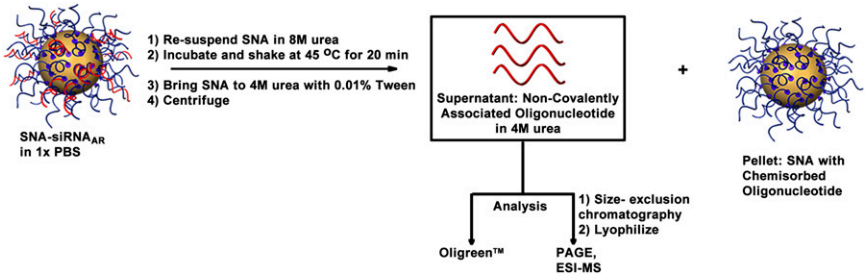

Fig. S1. Removal of the noncovalently associated oligonucleotide from SNA-siRNA$_{AR}$ for analysis by OliGreen, PAGE, and ESI-MS. An SNA in 1× PBS is re-suspended in 8 M urea and incubated at 45 °C while shaking for 20 min to dissociate the noncovalently associated oligonucleotide from the SNA. The SNA is then brought to 4 M urea in 0.1% Tween-20 in water before centrifugation at 21,000 × *g* for 25 min. Centrifugation allows the SNA with the chemisorbed oligonucleotide to be separated from the noncovalently associated oligonucleotide. The supernatant containing the noncovalently associated oligonucleotide in 4 M urea is removed and can be mixed with OliGreen reagent to determine the concentration. For analysis by PAGE and ESI-MS, the supernatant is first run through a size exclusion column to separate the noncovalently associated oligonucleotide from 4 M urea and subsequently lyophilized to reduce the volume. After resuspension in water, the oligonucleotide is ready for analysis.

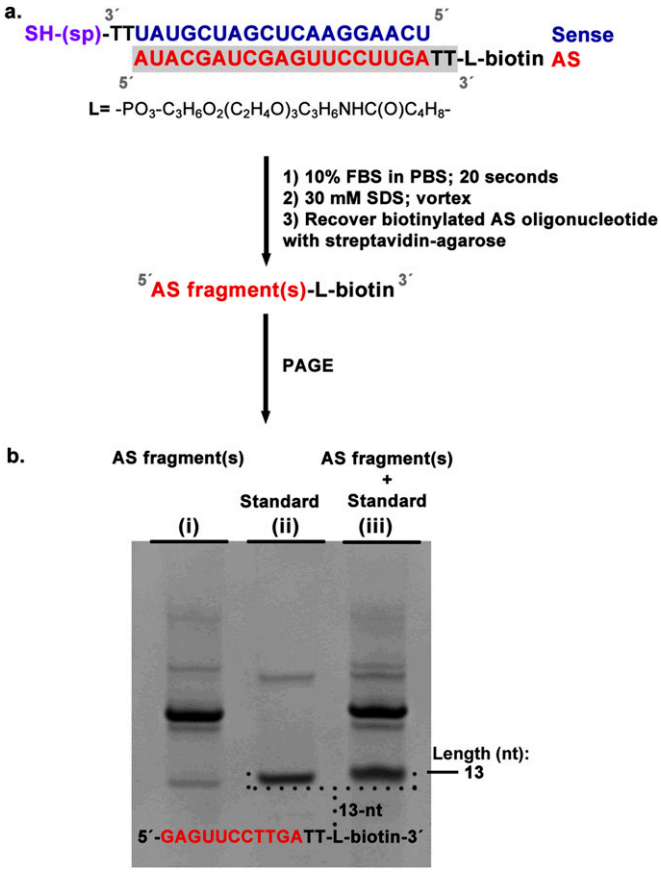

Fig. S2. Analysis of the degradation of AS oligonucleotide of siRNA$_{AR}$ in solutions of 10% FBS in PBS. (*A*) The design of siRNA$_{AR}$ with a 3′-biotinylated AS oligonucleotide and scheme for recovering biotinylated degradation products from reaction mixtures of siRNA$_{AR}$ in solutions of FBS. (*B*) Resolution by PAGE of oligonucleotides recovered from the reaction mixtures of siRNA$_{AR}$ with biotin-labeled AS oligonucleotide and FBS. The stained gel shows the presence of intact AS oligonucleotide and the appearance of a degradation product at lower molecular weight (lane i). Comparison of the mobilities of the degradation product and that of an analytical standard (lanes ii and iii, respectively) indicate that the degradation product is the result of the loss of approximately eight nucleotides from the 5′ terminus of the AS oligonucleotide. These results show that the major degradation product derived from the AS oligonucleotide of siRNA$_{AR}$ free in solutions of 10% FBS is different from that observed for siRNA$_{AR}$ when conjugated to AuNPs (Fig. 6*B*).

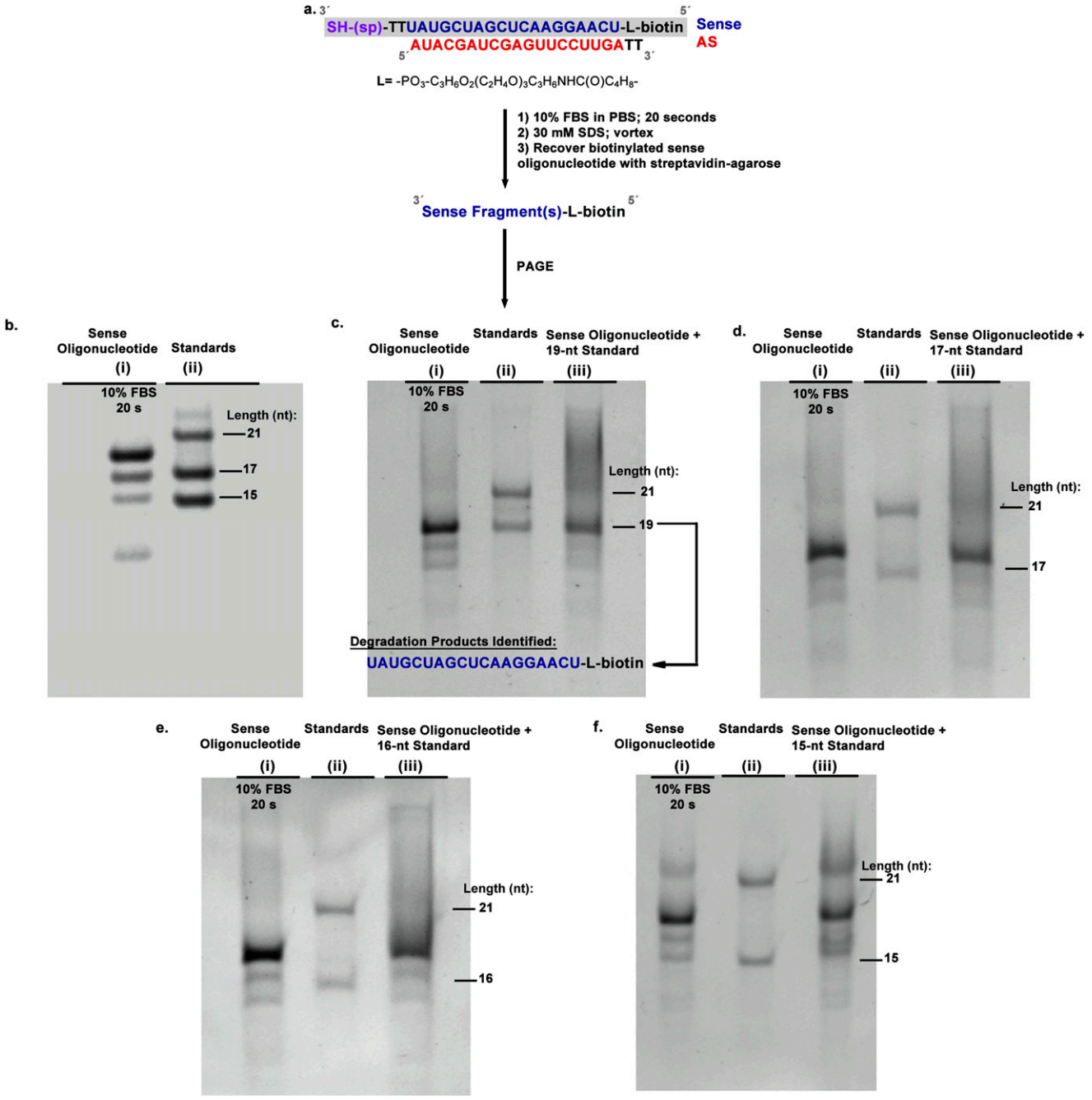

Fig. S3. Analysis of the degradation of the sense oligonucleotide of $siRNA_{AR}$ in solutions of 10% FBS. (*A*) The design of $siRNA_{AR}$ with a 5′-biotinylated sense oligonucleotide and scheme for recovering biotinylated degradation products from reaction mixtures of $siRNA_{AR}$ in solutions of 10% FBS. (*B*) Analysis of degradation products by PAGE, by comparing the mobilities of the degradation products in lane i to those of analytical standards designed by subtracting SH-(sp), which denotes SH-C_3H_6-O-[$(C_2H_4O)_6$-PO_3-]^2, and four or six nucleotides from the 3′ terminus of the sense oligonucleotide that is biotin-labeled at the 5′ terminus (lane ii). (*C*–*F*) Analysis of degradation products by PAGE and the use of truncated analytical standards, designed by the subtraction of 3′-SH-(sp)- and two, four, five, or six nucleotides, respectively. In each gel, lane i resolves the degradation products, lane ii analyzes analytical standards (21-nt starting material and a truncated oligonucleotide), and lane iii analyzes a mixture of the degradation products and analytical standards.

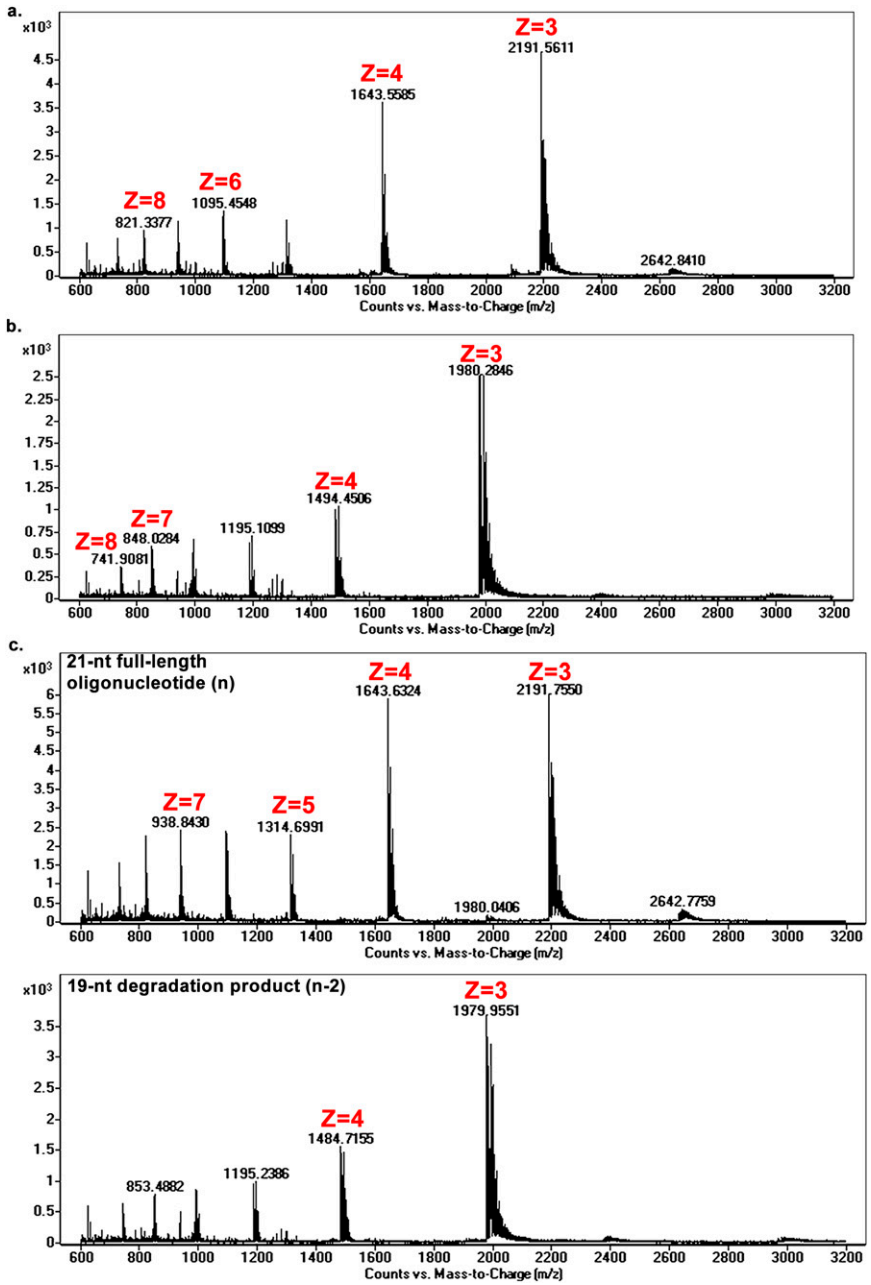

Fig. S4. ESI-MS specta of standards and degradation products for the AS oligonucleotide of SNA-2. (*A*) AS oligonucleotide standard (21 nt); (*B*) 19-nt degradation product for the AS oligonucleotide after 2 h in 10% FBS at 37 °C; (*C*) 21- (*Upper*) and 19-nt (*Lower*) degradation products for the AS oligonucleotide after 15 min in 10% FBS at 37 °C.

Standard/Sample	Name		# of nucleotides (n)	Sequence (5'-3')	Average Mass(AMU)$_{Predicted}$	AverageMass (AMU)$_{Observed}$
Standard: AS Oligonucleotide of SNA-2	n	From 5'-end	21	AUACGAUCGAGUUCCTTGATT	6579.17	6579
	n-1		20	UACGAUCGAGUUCCTTGATT	6249.96	6250
	n-2		19	ACGAUCGAGUUCCTTGATT	5943.79	5944
	n-3		18	CGAUCGAGUUCCTTGATT	5614.58	
	n-4		17	GAUCGAGUUCCTTGATT	5309.39	
	n-5		16	AUCGAGUUCCTTGATT	4964.18	
	n-6		15	UCGAGUUCCTTGATT	4634.91	
	n-1	From 3'-end	20	AUACGAUCGAGUUCCTTGAT	6274.97	
	n-2		19	AUACGAUCGAGUUCCTTGA	5970.78	
	n-3		18	AUACGAUCGAGUUCCTTG	5641.56	
	n-4		17	AUACGAUCGAGUUCCTT	5312.35	
	n-5		16	AUACGAUCGAGUUCCT	5008.15	
	n-6		15	AUACGAUCGAGUUCC	4703.95	
Sample: AS Oligonucleotide from SNA-2	2hr: FBS 1hr: FBS 15min: FBS					5943 (From Z=3 molecular ion at 1980.2846) 6579 (From Z=3 molecular ion at 2192.3053); 5944 6580, 5944
Standard: Sense Oligonucleotide of SNA-3	n	From 3'-end	21	TCAAGGAACUCGAUCGUAUTT	6588.19	6588
	n-1		20	TCAAGGAACUCGAUCGUAUT	6283.99	6284
	n-2		19	TCAAGGAACUCGAUCGUAU	5979.79	5980
	n-3		18	TCAAGGAACUCGAUCGUA	5670.86	
	n-4		17	TCAAGGAACUCGAUCGU	5341.80	
	n-5		16	TCAAGGAACUCGAUCG	5038.20	5039
	n-6		15	TCAAGGAACUCGAUC	4690.73	
	n-1	From 5'-end	20	CAAGGAACUCGAUCGUAUTT	6283.99	
	n-2		19	AAGGAACUCGAUCGUAUTT	5994.80	
	n-3		18	AGGAACUCGAUCGUAUTT	5681.58	
	n-4		17	GGAACUCGAUCGUAUTT	5368.37	
	n-5		16	GAACUCGAUCGUAUTT	5039.15	
	n-6		15	AACUCGAUCGUAUTT	4693.94	
Sample: Sense Oligonucleotide from SNA-3	2hr: FBS 15min: FBS					5038 (From Z=3 molecular ion at 1678.2459) 6589, 6284

Fig. S5. Table of predicted and observed mass using ESI-MS for SNA-2 and SNA-3. Sequences of the analytical standards used to analyze the degradation of SNA-2 and SNA-3 are listed with their sequence and average mass. To establish that the loss of nucleotides occurs at the terminus of the oligonucleotide directed away from the AuNP surface during nuclease-catalyzed hydrolysis (i.e., from the 5′ end of the AS oligonucleotide of SNA-2 and from the 3′ end of the sense oligonucleotide of SNA-3), sequences and the average mass are shown for all possibilities (i.e., the loss of nucleotides from either end of the oligonucleotide). Comparison of the mass of the analytical standards to that of degradation products recovered from SNA-2 and SNA-3 enabled the identification of the degradation products.

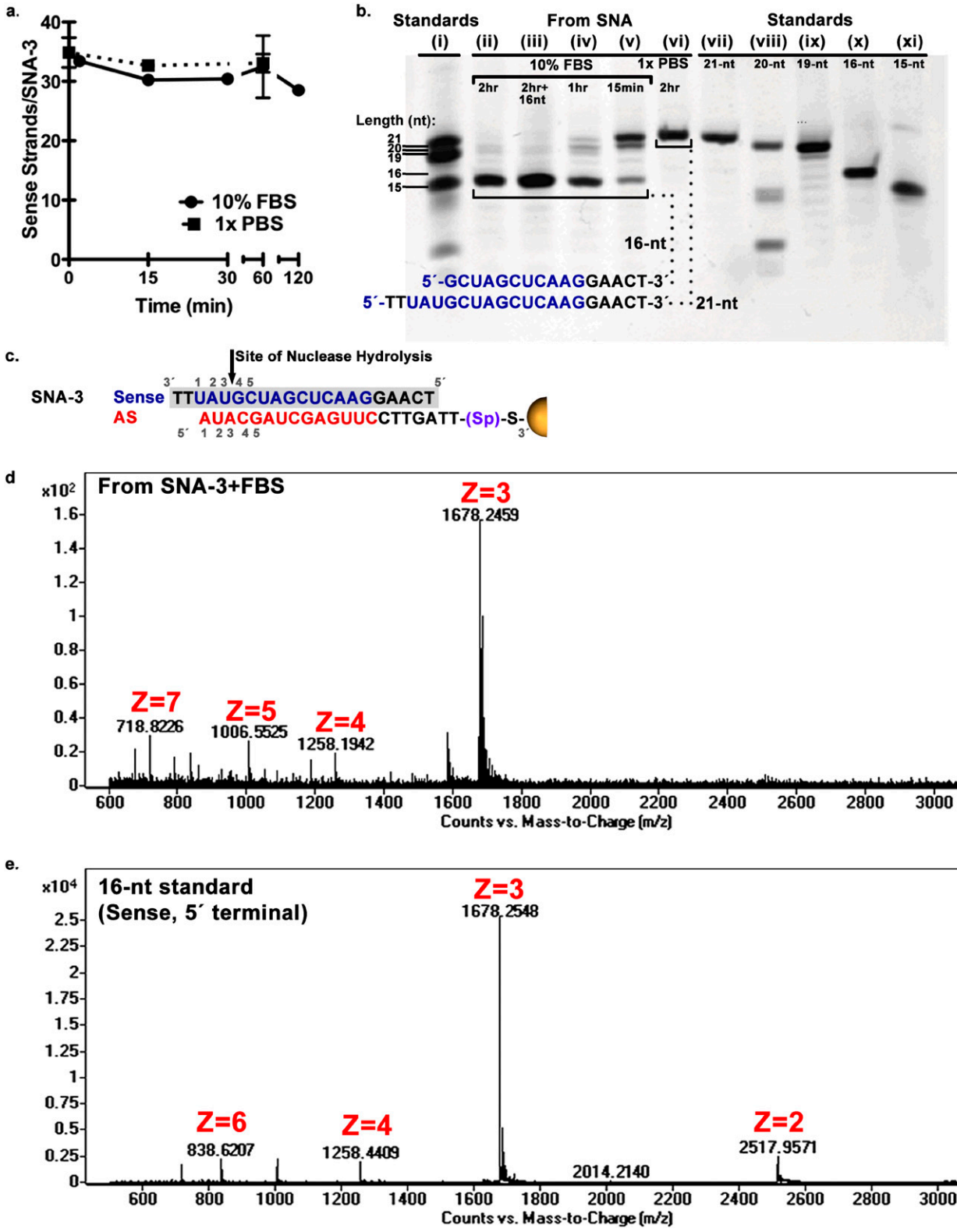

Fig. S6. Determining the site of nuclease-catalyzed hydrolysis for the sense oligonucleotide of SNA-3. (*A*) Plot of the ratio of sense oligonucleotides per SNA-3 versus time using OliGreen fluorescence indicating a loss of approximately five sense oligonucleotides per SNA-3, which corresponds to a loss of about 12% of the oligonucleotides over the course of 2 h in 10% FBS. (*B*) Denaturing polyacrylamide gel of analytical standards and degradation products for the sense oligonucleotide of SNA-3. After incubation of SNA-3 in 10% FBS at 37 °C for 15 min (lane v), three bands are visible, including a band corresponding to the full-length oligonucleotide. After 1 (lane iv) and 2 (lane ii) h in 10% FBS, the full-length oligonucleotide is converted to a single degradation product. To confirm the identity of the degradation product, we coloaded the degradation product after 2 h with the 16-nt analytical standard (lane iii) and observed only one band, which suggests the presence of a single oligonucleotide species. After 2 h in 1× PBS, the sense oligonucleotide remains full length (lane vi). The 21-, 20-,

Legend continued on following page

19-, and 16-nt standards are run together in lane i. (*C*) Proposed location of the site of nuclease-catalyzed hydrolysis for the sense oligonucleotide of SNA-3. (*D*) ESI-MS spectrum, identifying the degradation product for the sense oligonucleotide of SNA-3. The spectrum from the degradation product has a Z = 3 molecular ion at 1678.2459, which corresponds to a molecular weight of 5,035. (*E*) The 16-nt analytical standard of the sense oligonucleotide of SNA-3. Z = 3 molecular ion is observed at 1678.2548, which corresponds to a molecular weight of 5,035.

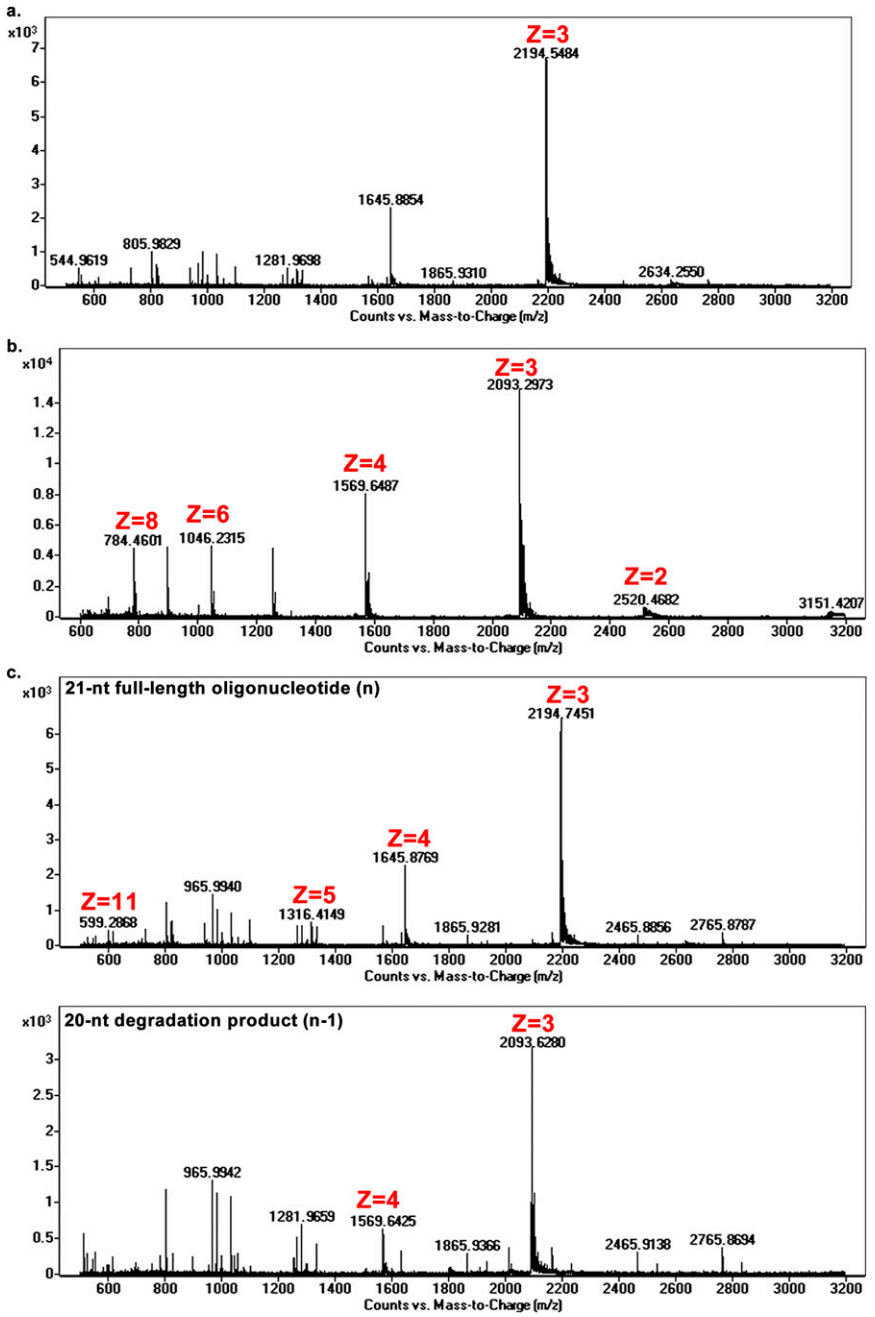

Fig. S7. ESI-MS spectra of standards and degradation products for the sense oligonucleotide of SNA-3. (*A*) Sense oligonucleotide standard (21 nt); (*B*) sense oligonucleotide standard (20 nt); and (*C*) 21-nt full-length oligonucleotide (*Upper*) and 20-nt degradation product (*Lower*) for the sense oligonucleotide of SNA-3 after 15-min incubation in 10% FBS.

Table S1. Analysis of the noncovalently associated oligonucleotides from SNA-siRNA$_{AR}$

Name of SNA	Noncovalently associated oligonucleotides per AuNP (n = 4)
SNA-1	27 ± 1
SNA-1-(2′-H)$_5$	39 ± 2
SNA-1-(2′-OMe)$_5$	48 ± 6
SNA-2	37 ± 4
SNA-3	35 ± 3 (n = 2)
SNA-1-(4, 2)	30 ± 1
SNA-1-(3, 3)	40 ± 5