Supporting Information

Hairpin-Like siRNA-Based Spherical Nucleic Acids

Matthew K. Vasher[1,2], Gokay Yamankurt[2,3], and Chad A. Mirkin[1,2,4,*]

[1]Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States
[2]International Institute for Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States
[3]Interdisciplinary Biological Sciences Graduate Program, Northwestern University, 2205 Tech Drive, Evanston, Illinois 60208, United States
[4]Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208, United States
*Email: chadnano@northwestern.edu

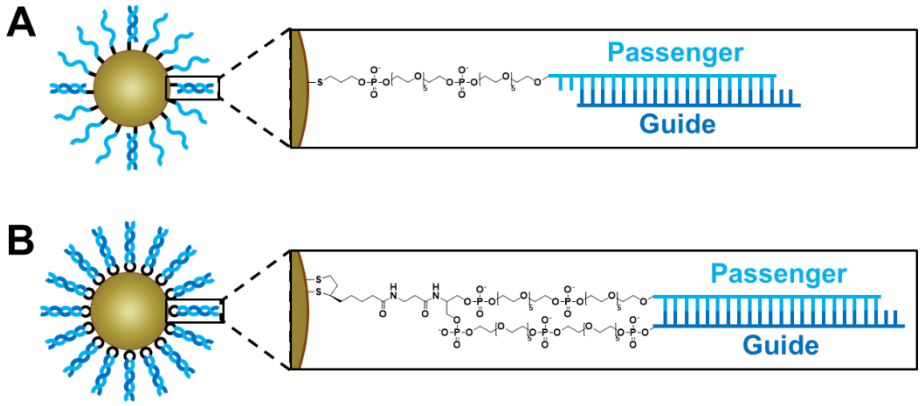

Figure S1. Chemistry of siRNA-SNA Attachment Architectures. (A) Hybridized architecture, in which the passenger strand is attached to the core via a PEG linker with a thiol group. (B) Hairpin-like architecture, in which both strands are attached to the core via a PEG linker with a dithiol serinol group.

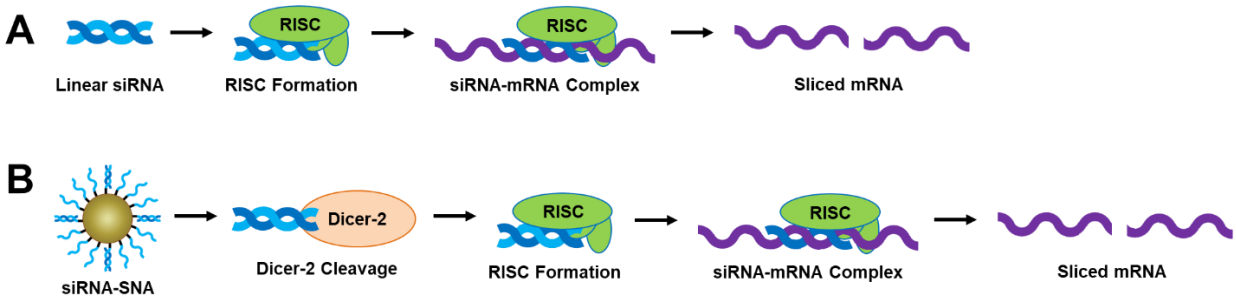

Figure S2. RNAi pathway for linear siRNA and siRNA-SNAs. (A) Linear siRNA is processed by the RISC and hybridizes to complementary mRNA, which is then sliced, resulting in gene silencing. (B) For siRNA-SNA, Dicer-2 cleaves off siRNA duplexes from the SNA. The cleaved siRNA then proceeds through the canonical RNAi pathway.

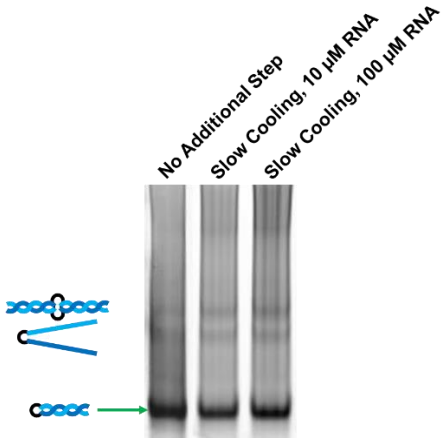

Figure S3. Slow cooling does not increase self-hybridization efficiency. Self-complementary hairpin-like siRNA was heated to 95 °C and then slow-cooled to room temperature in duplex buffer at various concentrations. Upon native PAGE analysis, slow-cooling did not significantly increase the fraction of hairpin-like siRNA that existed in a closed conformation.

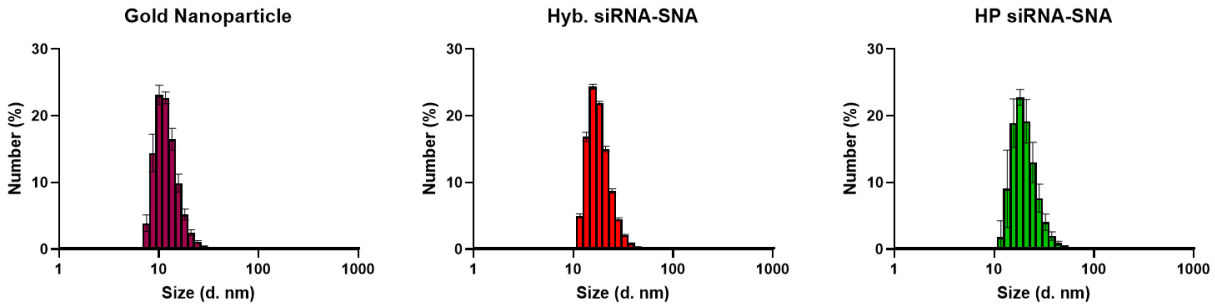

Figure S4. DLS number distribution. The size increase from bare gold nanoparticle to SNAs of both forms indicates the addition of siRNA to the nanoparticle surface and the successful formation of SNAs.

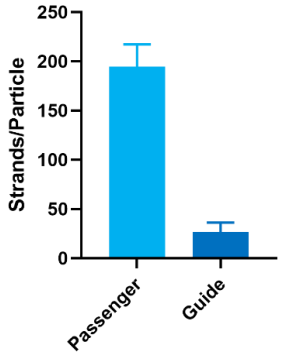

Figure S5. Hybridized siRNA-SNA architecture has low duplex efficiency. In the hybridized siRNA-SNA architecture, very few passenger strands have an attached guide strand, resulting in low duplex efficiency. Since an intact siRNA duplex is necessary for gene silencing, the hybridized architecture limits the amount of active siRNA that can be loaded on each SNA. Error bars are SD of 3 batches of SNAs.

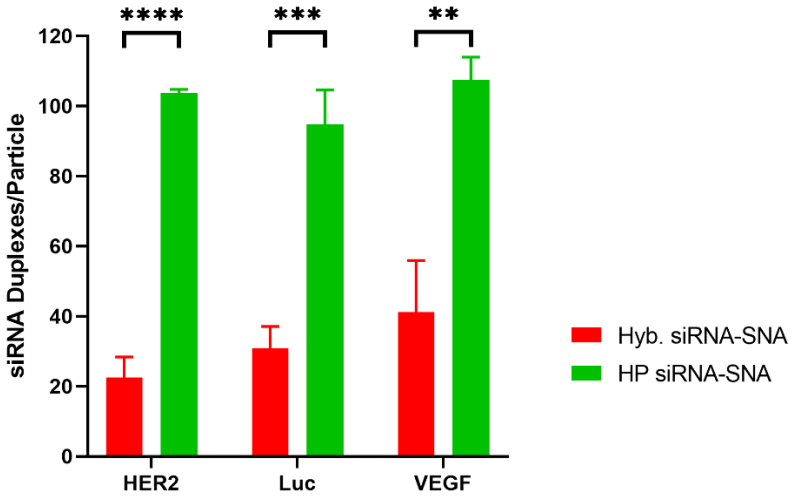

Figure S6. Hairpin-like siRNA architecture increases duplex loading on SNAs for a variety of sequences. Hybridized and hairpin-like siRNA-SNAs with sequences targeting *HER2*, *Luc*, and *VEGF* were synthesized. For all sequences tested, the hairpin-like architecture increased duplex loading. Error bars are SD of 3 batches of SNAs (***P* ≤ 0.01, *****P* ≤ 0.0001).

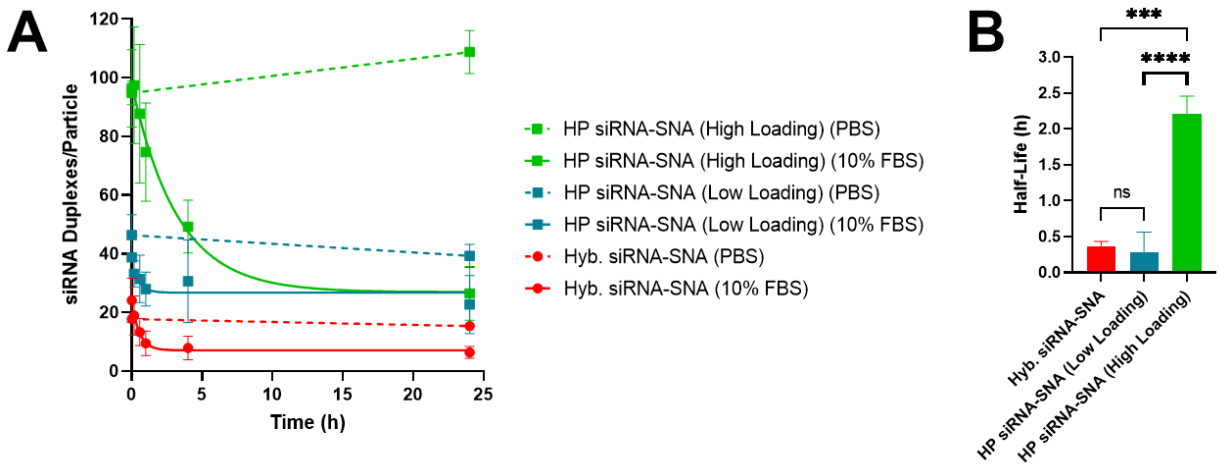

Figure S7. Serum nuclease resistance enhancement of hairpin-like siRNA-SNAs is dependent on duplex loading density. (A) The degradation of hairpin-like siRNA-SNAs with high (96 duplexes/SNA) and low (39 duplexes/SNA) loading was compared with that of hybridized siRNA-SNAs (24 duplexes/SNA) in serum. (B) Half-lives of siRNA-SNAs in serum, derived from curves in (A). Error bars are SD of 3 experimental replicates (ns $P > 0.05$, ***$P \leq 0.001$, ****$P \leq 0.0001$).

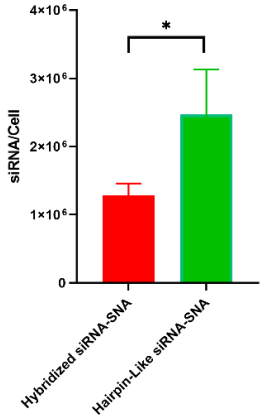

Figure S8. Architecture affects cellular uptake. The cellular uptake of siRNA-SNAs with hairpin-like or hybridized designs was compared. Hairpin-like siRNA-SNAs retain the ability to enter cells without the use of transfection reagents and deliver more siRNA into cells than SNAs with the hybridized architecture. Error bars are SD of 3 biological replicates (*$P \leq 0.05$).

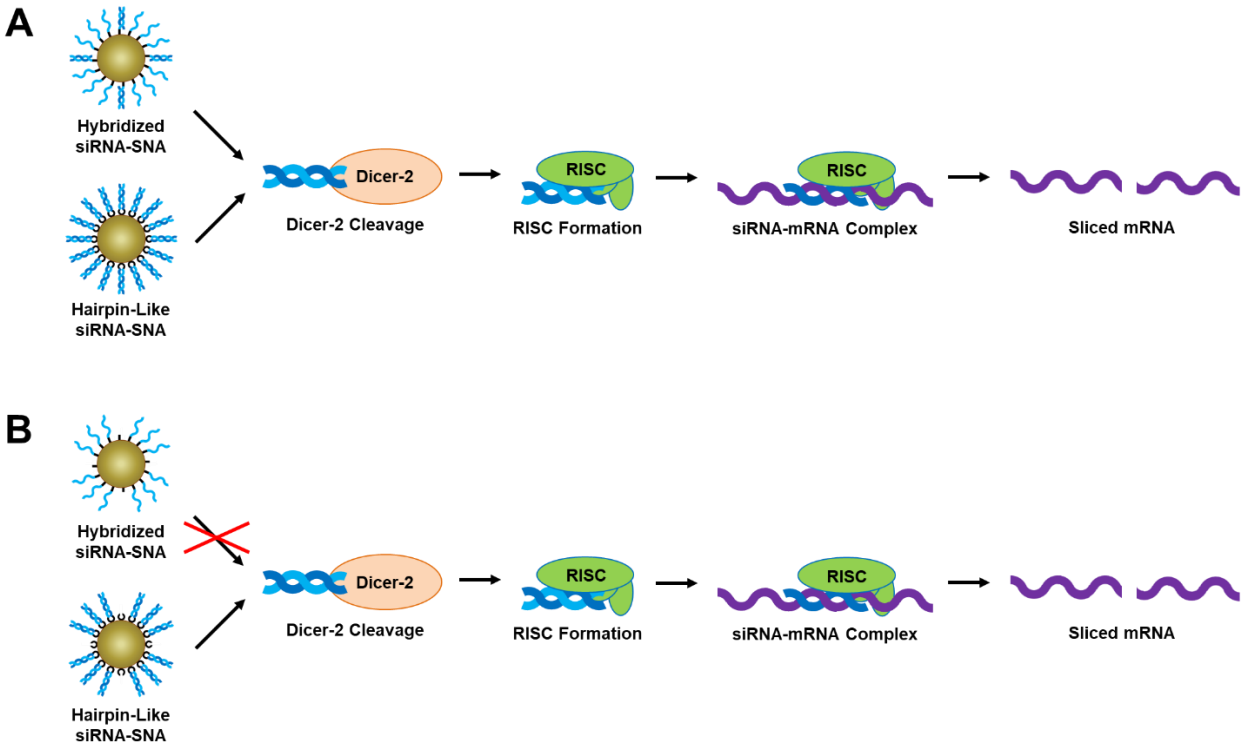

Figure S9. RNAi pathway for hybridized and hairpin-like siRNA-SNA at early and late time points. (A) At early time points, Dicer-2 cleavage releases siRNAs from both architectures of siRNA-SNAs at similar rates, resulting in a similar level of gene silencing. (B) At later time points, hybridized siRNA-SNAs will be depleted of siRNAs first, after which they will no longer sustain a further gene silencing effect. Due to their higher loading, hairpin-like siRNA-SNAs will still have siRNA remaining that can be cleaved by Dicer-2 and processed to sustain a more durable gene silencing effect.

Table S1. Sequences of RNA oligonucleotides used in this study and calculated (calc.) and observed (obs.) molecular weights (MW), as measured by MALDI-TOF. Sp18: spacer-18, SH: thiol modification, DS: dithiol serinol.

Name	Sequence (5' → 3')	Calc. MW (Da)	Obs. MW (Da)
HER2 Passenger	GCUCAUCGCUCACAACCAAUU-$(Sp18)_2$-SH	7509	7425
HER2 Guide	UUGGUUGUGAGCGAUGAGCAC	6769	6806
HER2 Hairpin	GCUCAUCGCUCACAACCAAUU-$(Sp18)_2$-DS-$(Sp18)_2$-AAUUGGUUGUGAGCGAUGAGCAC	15912	15964
Nontargeting (*Luc*) Passenger	CGUACGCGGAAUACUUCGAUU-$(Sp18)_2$-SH	7606	7614
Nontargeting (*Luc*) Guide	UCGAAGUAUUCCGCGUACGUG	6689	6729
Nontargeting (*Luc*) Hairpin	CGUACGCGGAAUACUUCGAUU-$(Sp18)_2$-DS-$(Sp18)_2$-AAUCGAAGUAUUCCGCGUACGUG	15928	15888
VEGF Passenger	ACCUCACCAAAGCCAGCACUU-$(Sp18)_2$-SH	7531	7505
VEGF Guide	GUGCUGGCUUUGGUGAGGUUU	6740	6713
VEGF Hairpin	ACCUCACCAAAGCCAGCACUU-$(Sp18)_2$-DS-$(Sp18)_2$-AAGUGCUGGCUUUGGUGAGGUUU	15904	15876
Noncomplementary 1	GCUCAUCGCUCACAACCAAUU-$(Sp18)_2$-DS-$(Sp18)_2$-GACAAUCCCGACACCCUUAUUAC	15694	15518
Noncomplementary 2 (Hybridized to Noncomplementary 1 for Cocomplementary RNA)	AAUAAGGGUGUCGGGAUUGUC-$(Sp18)_2$-DS-$(Sp18)_2$-AAUUGGUUGUGAGCGAUGAGCAC	16129	16052