Structural basis for sequence-specific recognition of guide and target strands by the *Archaeoglobus fulgidus* Argonaute protein

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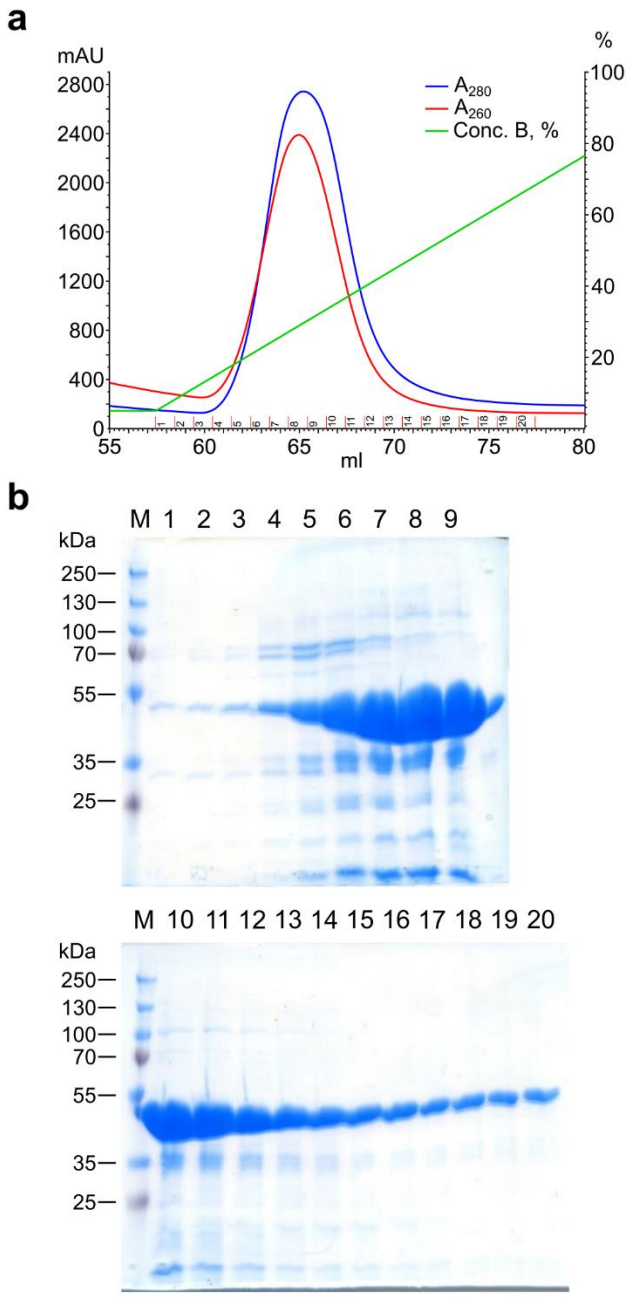
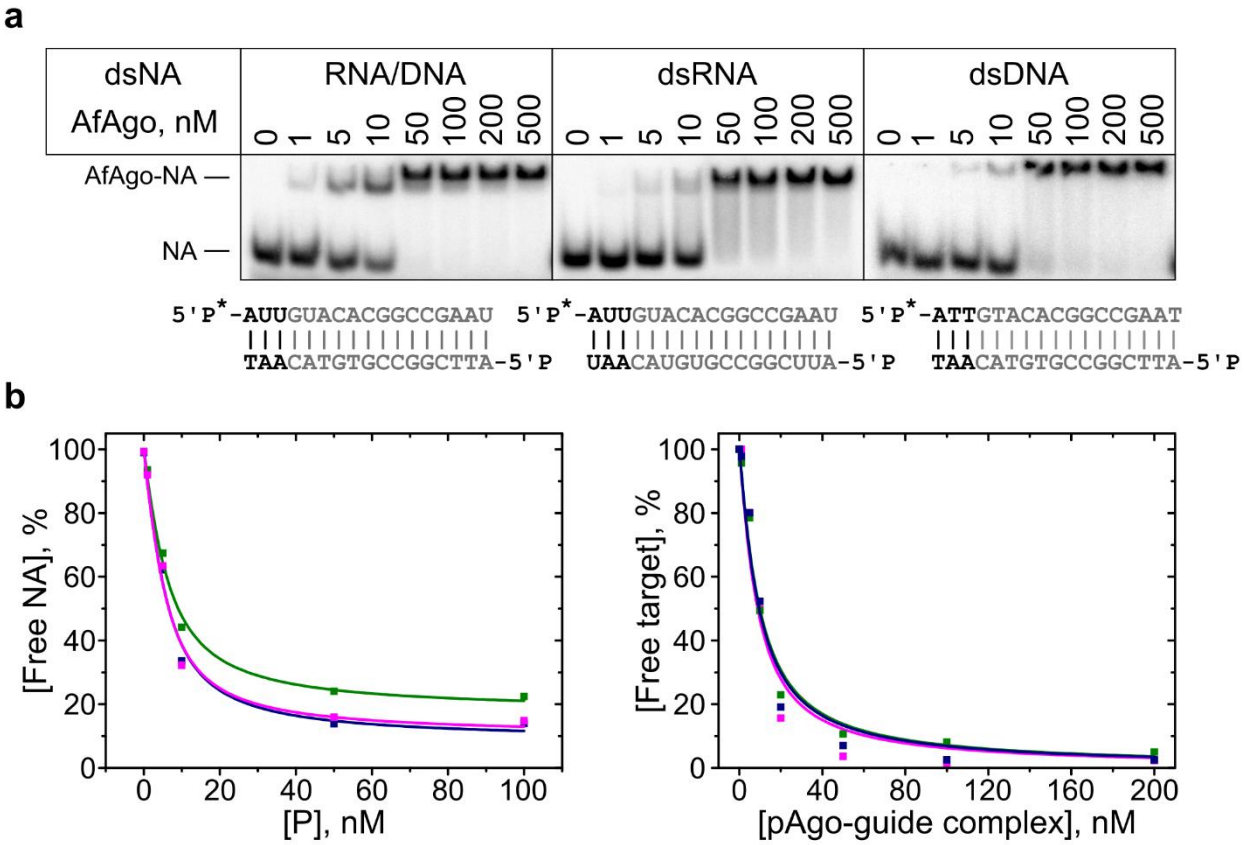
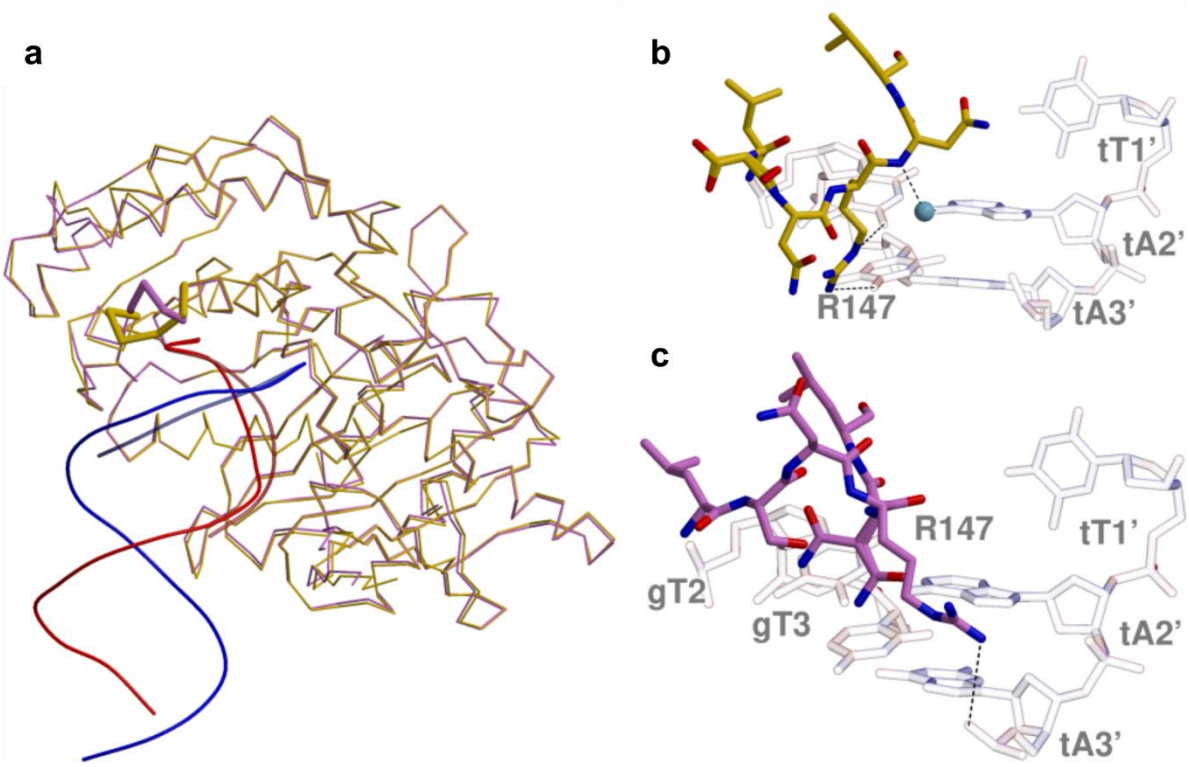
SUPPLEMENTARY INFORMATION



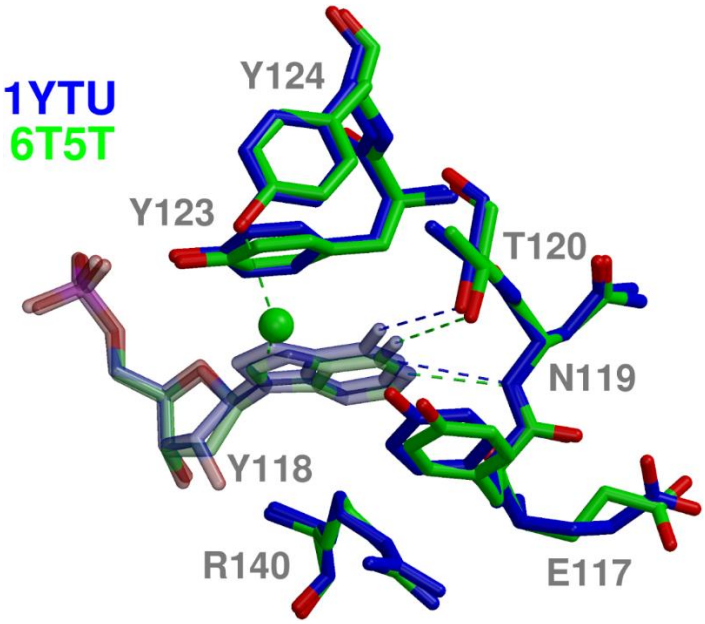
Supplementary Figure S1. Purification of AfAgo through a HisTrap affinity column. (**a**) Chromatogram. A_{280} – blue; A_{260} – red; percentile fraction of elution buffer B – green. Collected fractions are indicated below the curves. (**b**) SDS-PAGE of fractions collected; numbering corresponds to numbers in (**a**). M – mass marker.



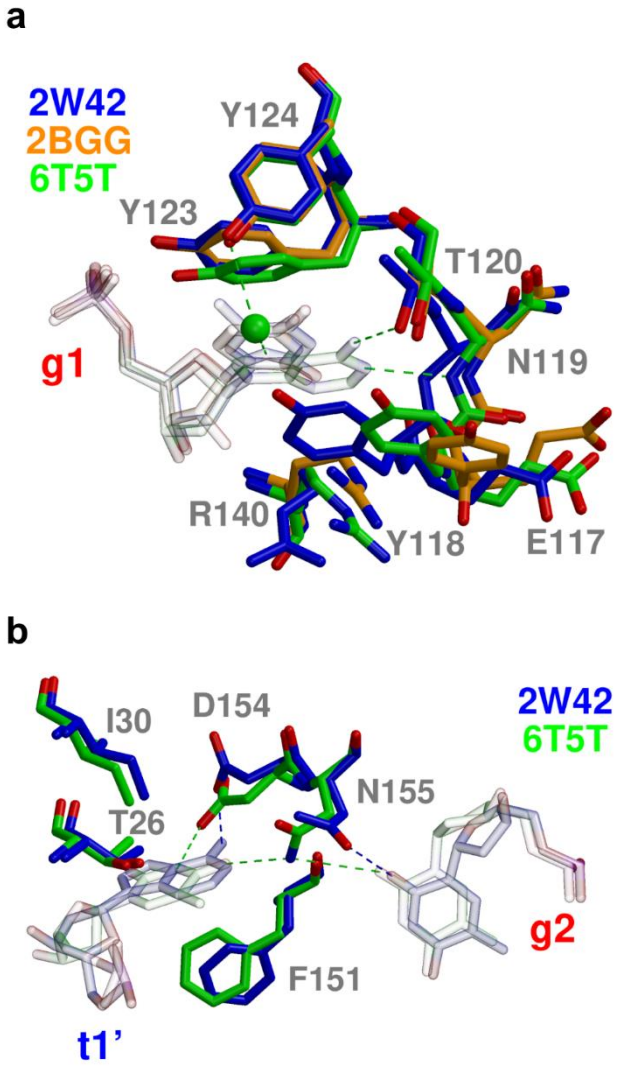
Supplementary Figure S2. (**a**) Double-stranded nucleic acid binding by AfAgo studied by EMSA. AfAgo concentrations are indicated above each lane. Double-stranded nucleic acid sequences and structure are depicted schematically below each gel, with 5′-terminal bases of the guide and 3′-terminal bases relevant to AfAgo base recognition highlighted in black, remaining strands in grey. 5′^{32}P-labelled strand indicated with an asterisk. (**b**) Representative binding fit curves of several independent replicates used to calculate K_d of ssRNA guide binding by AfAgo (left) and of target DNA binding by AfAgo-gRNA complex (right).



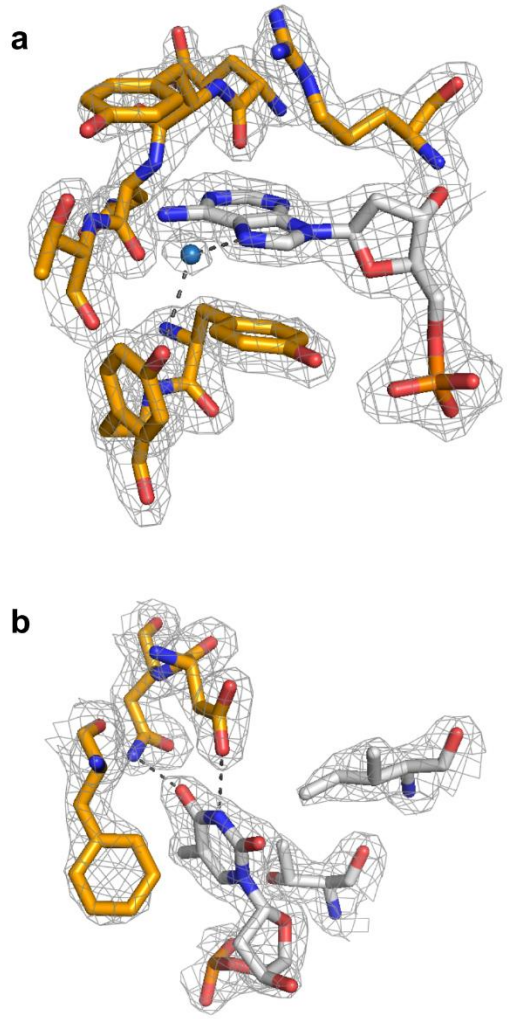
Supplementary Figure S3. Different conformation of the loop 144-149 in crystal structures of AfAgo. (**a**) AfAgo protein chains from the complex with 5′-ATT (PDB ID **6T5T**, yellow) and with 5′-ATC (PDB ID **6XUP chain A**, magenta) are shown as traces. Guide and target DNA strands are red and blue, respectively. (**b**) The conformation of the loop 144-149 in the structure AfAgo-5′ATT, PDB ID **6T5T**. (**c**) Loop 144-149 from the A protein chain in the crystal structure AfAgo-5′ATC, PDB ID **6XUP**.



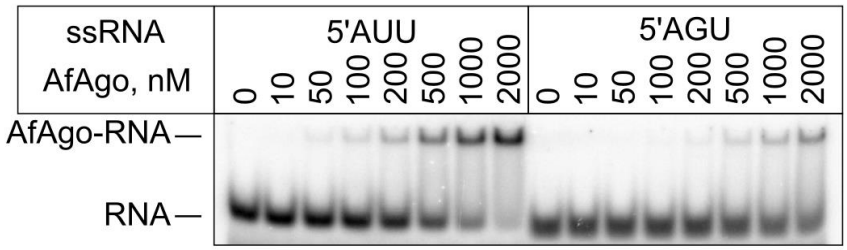
Supplementary Figure S4. Binding of g1A base in 6T5T and 1YTU. The water molecule from 6T5T is shown as a green sphere. H-bonds are shown as dashed lines.



Supplementary Figure S5. (**a)**, Comparison of the first guide base in crystal structures of AfAgo 2W42, 2BGG and 6T5T. (**b)**, Binding of gT2 and t1' bases in the "side" pocket in 2W42 and 6T5T.



Supplementary Figure S6. The electron density is $2F_o$-F_c at 1.6 sigma. (**a)**, gA1 base recognition. (**b**), tT1' base recognition.



Supplementary Figure S7. AfAgo interactions with ssRNA *in vitro* at elevated temperature. EMSA experiments were performed with 5 nM total 5′P-ssRNA, complexes pre-formed at 70 °C, gels were run at room temperature. The general binding affinity of ssRNAs decreased, however the specificity of AfAgo towards a 5′AUU over 5′AGU ssRNA remains.

Supplementary Table S1. Oligonucleotides used in this work.

Name	Sequence, 5′->3′	Application
MZ-952	ATCGTGGCCACGAT	Crystallization; self-complementary
MZ-1288	ATTGTGGCCACAAT	Crystallization; self-complementary
MZ-1289	ATTGTACGTACAAT	Crystallization; self-complementary
MZ-1447	ATTGTACACGGCCGAAT	ssDNA for EMSA
MZ-1455	ATTCGGCCGTGTACAAT	Duplex formation with MZ-1447 and MZ-1480 for EMSA
MZ-1480	AUUGUACACGGCCGAAU	ssRNA for EMSA and gRNA for RNA-guided NA targeting experiments
MZ-1481	AUUCGGCCGUGUACAAU	Duplex formation with MZ-1480 for EMSA
MZ-1556	CGGAAUAUAUGUACAAU	8 nt complementary target RNA
MZ-1557	CGGAAUAUUGGUACCCG	4 nt complementary target RNA
MZ-1560	CGGAATATATGTACAAT	8 nt complementary target DNA
MZ-1561	CGGAATATTGGTACCCG	4 nt complementary target DNA
MZ-1698	GUUGUACACGGCCGAAC	ssRNA for EMSA
MZ-1699	UUUGUACACGGCCGAAA	ssRNA for EMSA
MZ-1706	AUCGUACACGGCCGGAU	ssRNA for EMSA
MZ-1707	AGUGUACACGGCCGACU	ssRNA for EMSA
MZ-1708	CUUGUACACGGCCGAAG	ssRNA for EMSA

Supplementary table S2. Data collection and refinement statistics.

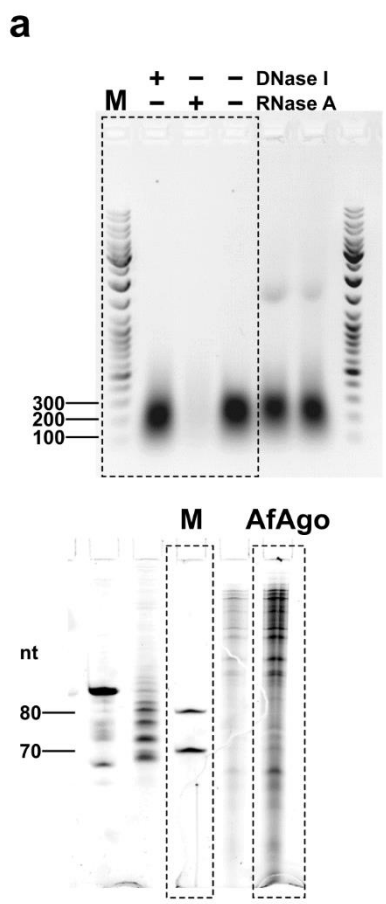
Oligoduplex	**ATC**GTGGCCAC**GAT** |||||||||||||| **TAG**CACCGGTG**CTA**	**ATC**GTGGCCAC**GAT** |||||||||||||| **TAG**CACCGGTG**CTA**	**ATT**GTGGCCAC**AAT** |||||||||||||| **TAA**CACCGGTG**TTA**	**ATT**GTACGTAC**AAT** |||||||||||||| **TAA**CATGCATG**TTA**
Crystallization buffer	50 mM sodium cacodylate (pH 5.5 at 25 °C), 120 mM KCl, 10 mM $MgCl_2$, 7% (w/v) PEG3350, 5% (v/v) glycerol	50 mM sodium cacodylate (pH 6.5 at 25 °C), 40 mM KCl, 10 mM $MgCl_2$, 11% (w/v) PEG3350	50 mM sodium cacodylate (pH 5.5 at 25 °C), 200 mM KCl, 10 mM $MgCl_2$, 5% (w/v) PEG4000, 5% (v/v) glycerol	
Cryo protection buffer	100 mM sodium cacodylate (pH 5.5 at 25 °C), 200 mM KCl, 10 mM $MgCl_2$, 20% PEG3350 (w/v), 10% (v/v) glycerol			100 mM sodium cacodylate (pH 6.5 at 25 °C), 40 mM KCl, 10 mM $MgCl_2$, 20% (w/v) PEG3350, 20% (v/v) glycerol
Data collection statistics				
Space group	P 1	P 1	P 2 21 21	P 2 21 21
Cell constants a, b, c, α, β, γ	a=51.80 Å, b=60.87 Å, c=101.72 Å, α=76.56°, β=75.59°, γ=79.39°	a=51.91 Å, b=61.20 Å, c=103.09 Å, α=98.32°, β=104.96°, γ=100.62°	a=52.10 Å, b=99.55 Å, c=109.90 Å, α=β=γ=90°	a=52.01 Å, b=99.63 Å, c=109.80 Å, α=β=γ=90°
Wavelength, Å	0.97630	0.97630	0.97970	0.97970
X-ray source	PETRA III, EMBL C/O DESY, P14	PETRA III, EMBL C/O DESY, P14	PETRA III, EMBL C/O DESY, P13	PETRA III, EMBL C/O DESY, P13
Unique reflections: overall (outer shell)	83713 (4556)	85105 (12355)	63695 (9192)	53664 (3128)

Resolution range, Å	41.50 - 1.90	54.90 - 1.80	99.52 - 1.70	99.63 - 1.80
Completeness: overall (outer shell), %	91.6 (91.2)	91.5 (90.9)	100 (100)	99.9 (100)
Multiplicity: overall (outer shell)	3.8 (3.9)	3.9 (3.9)	10.3 (10.1)	6.5 (6.7)
I/σ: overall (outer shell)	11.2 (1.6)	8.3 (1.7)	20.8 (2.3)	20.8 (2.6)
Rmerge: overall (outer shell), %	4.9 (82.1)	6.1 (63.8)	6.3 (96.9)	4.4 (74.8)
B-factor from Wilson, $Å^2$	36.4	35.0	30.8	29.6
Refinement statistics				
Resolution range, Å	40.72 - 1.90	46.45 - 1.90	54.95 - 1.70	54.90 - 1.80
Reflections: work (non-anomalous)/test	83692 (9394)	85025 (8390)	63601 (6249)	53695 (5011)
Atom number: protein/solvent	7671 (449)	7175 (495)	4325 (349)	4325 (394)
Rcryst (Rfree), %	18.1 (22.4)	17.7 (22.2)	18.3 (21.6)	18.8 (23.1)
RMSD: bond lengths, Å / bond angles, (°)	0.010 / 0.999	0.012 / 1.104	0.012 / 1.148	0.005 / 0.753
Ramachandran: favoured/allowed/	96.3 / 3.7 / 0	96.6 / 3.4 / 0	98 / 8 / 0	97.24 / 2.51 / 0.25

outliers, %				
Average B-factors: all atoms/ main chain/ side chain/ solvent/ DNA, Å^2	48.0 / 42.5 / 43.9 / 53.0 / 81.2	46.9 / 40.0 / 40.5 / 49.2 / 85.9	42.0 / 33.8 / 39.6 / 46.0 / 74.2	47.0 / 35.3 / 41.3 / 48.6 / 91.1
PDB ID	6XUP	6XU0	6T5T	6TUO

SOURCE DATA

Source data Figure 1A



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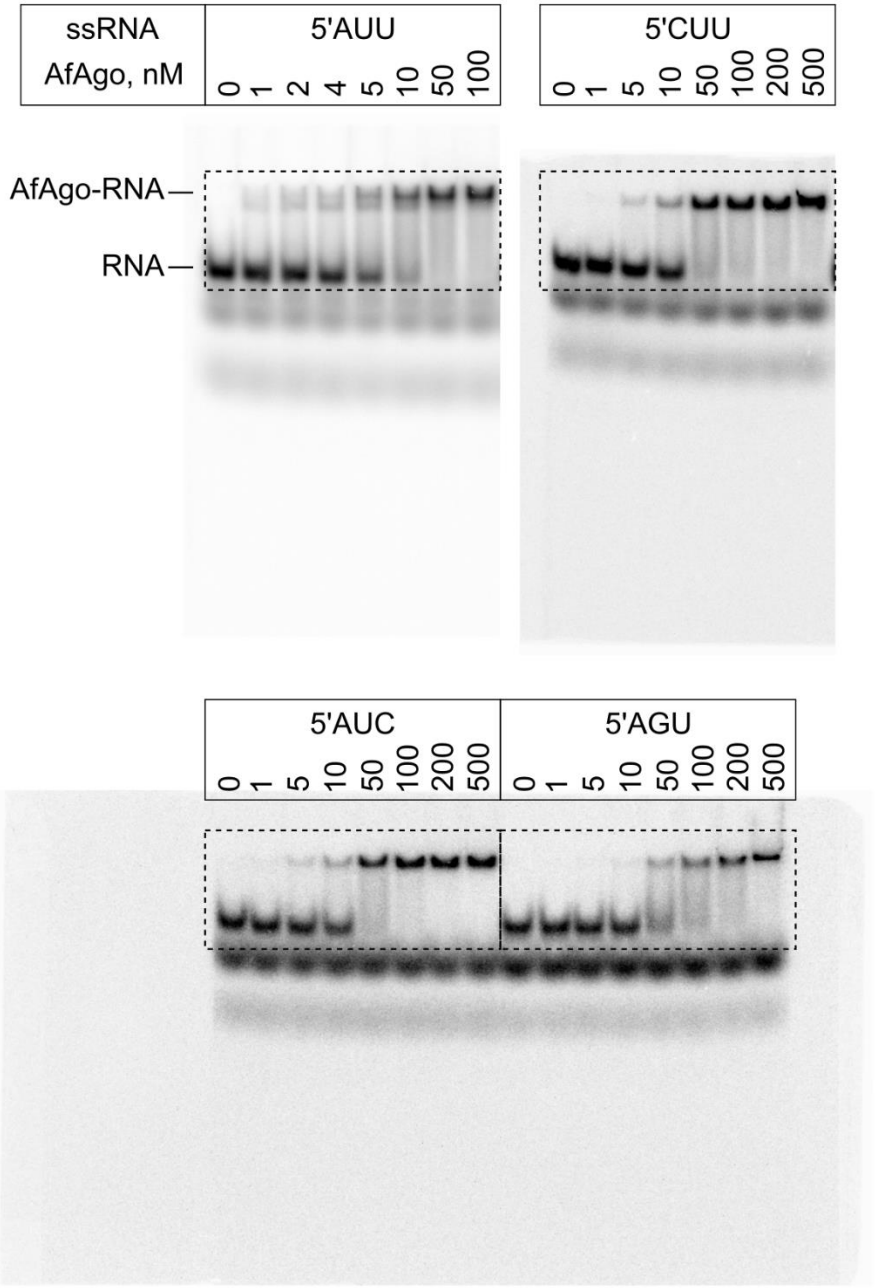

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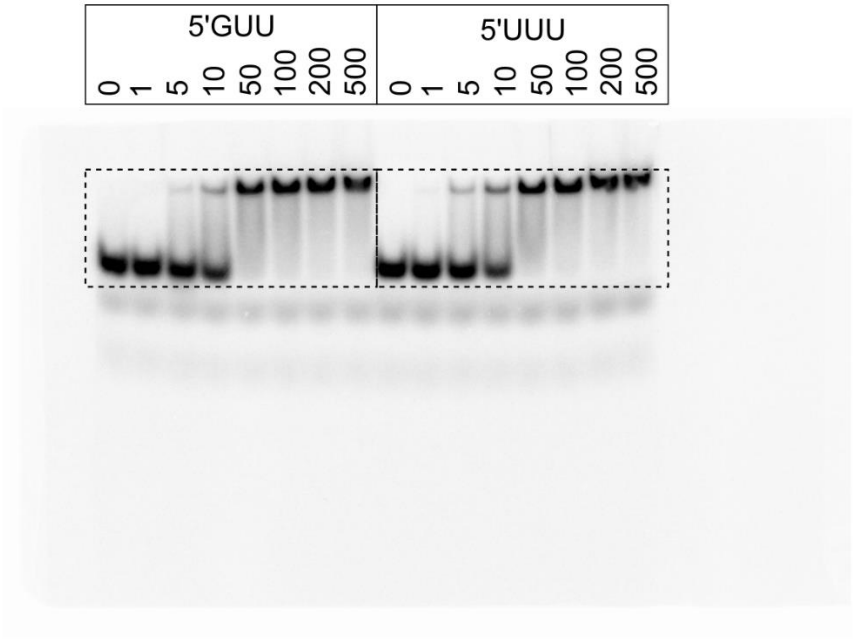

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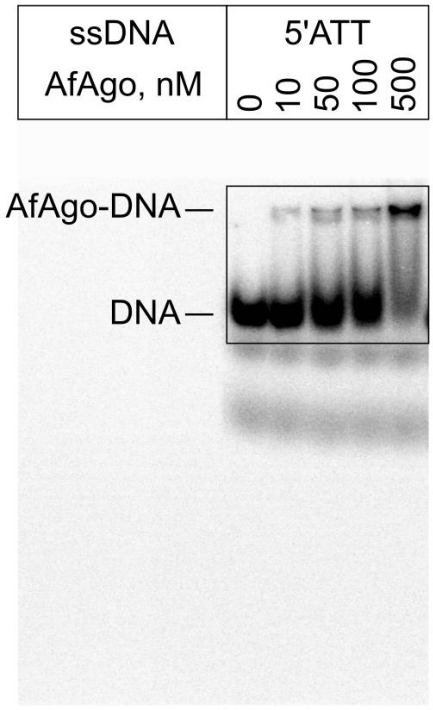

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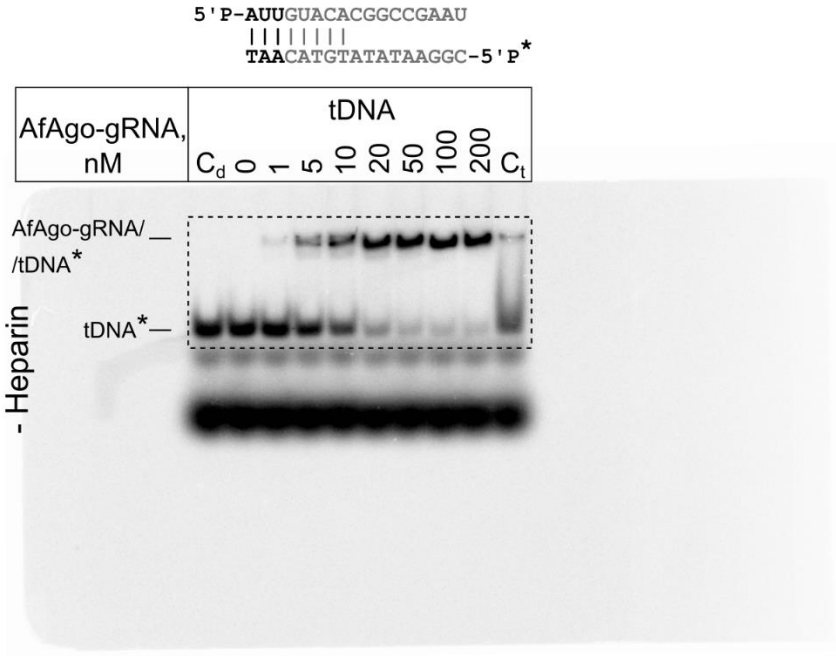

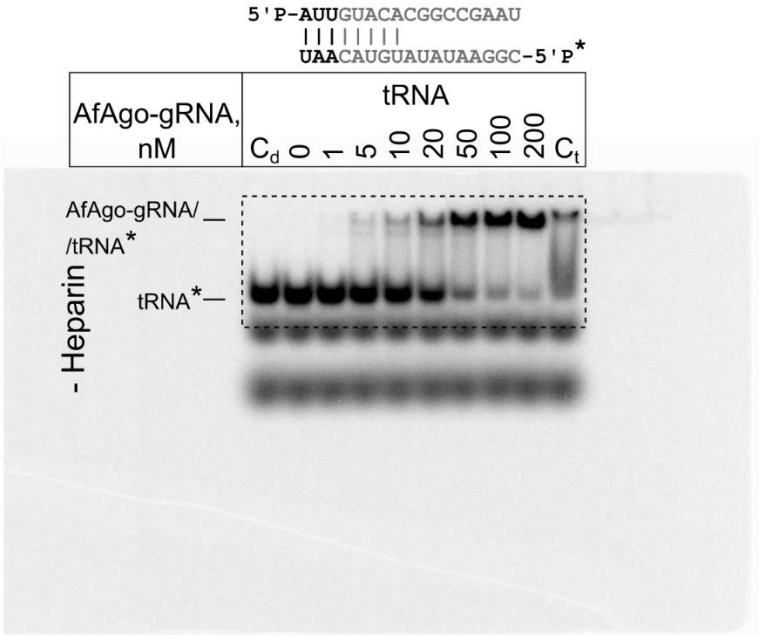

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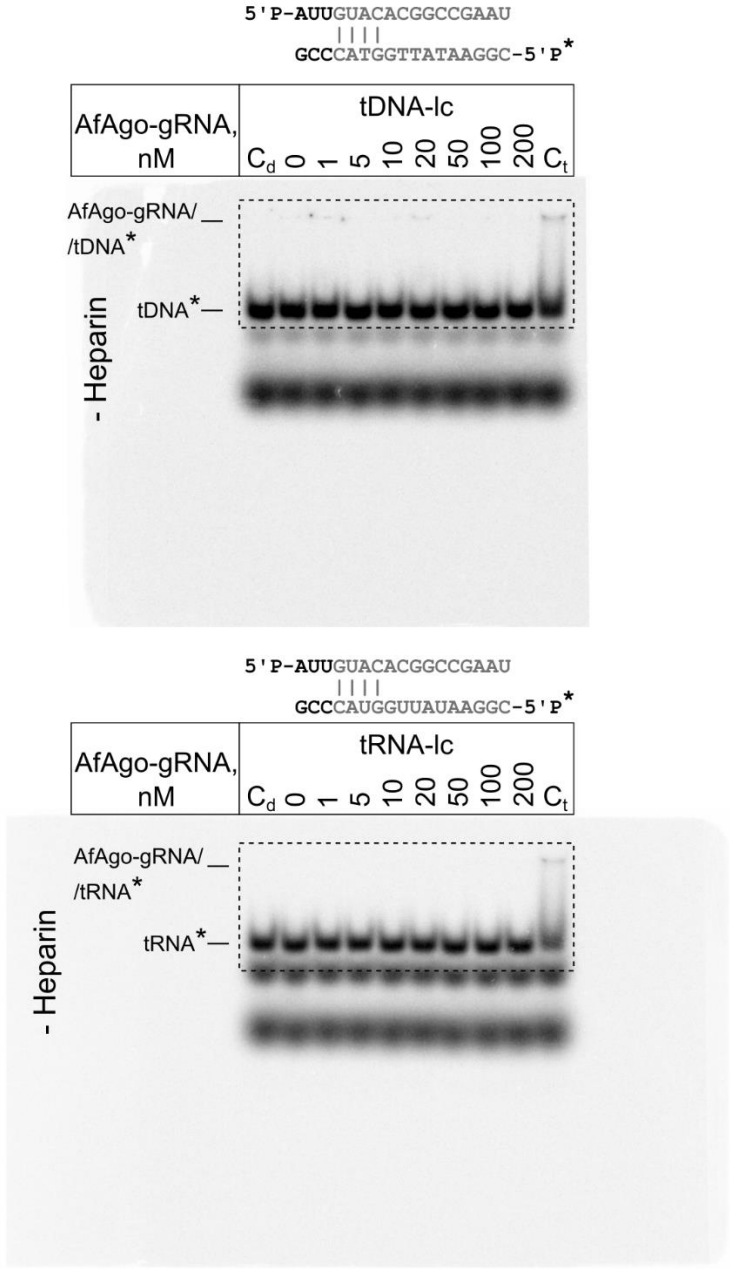

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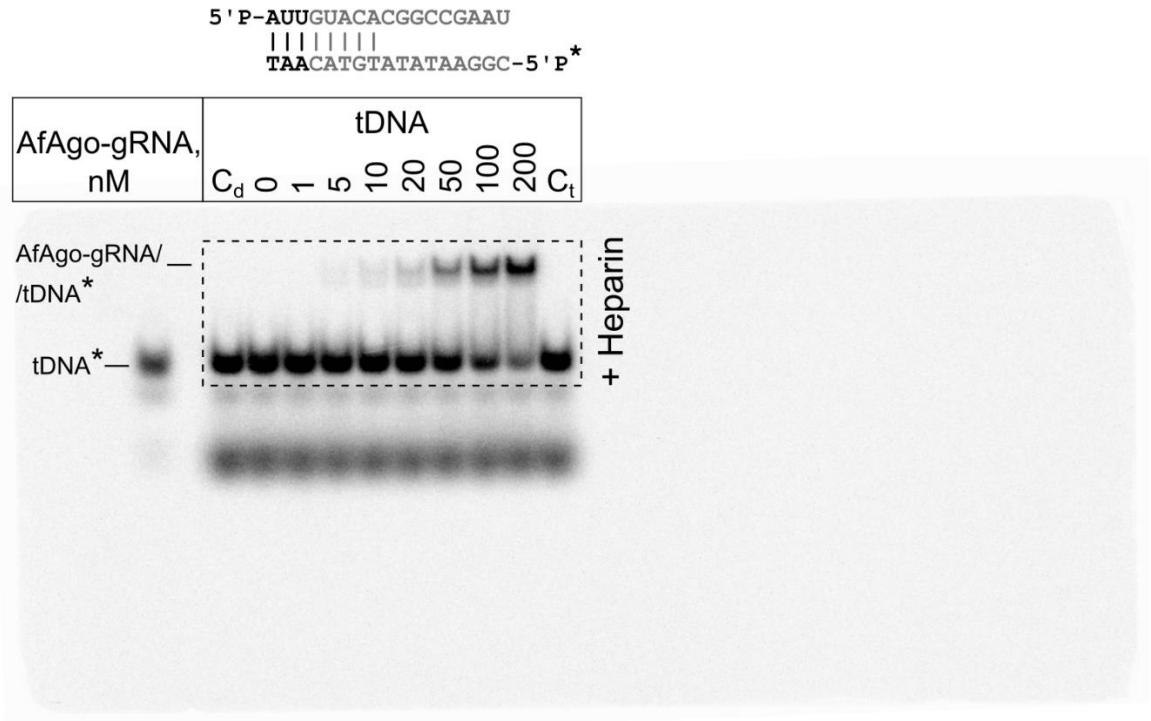

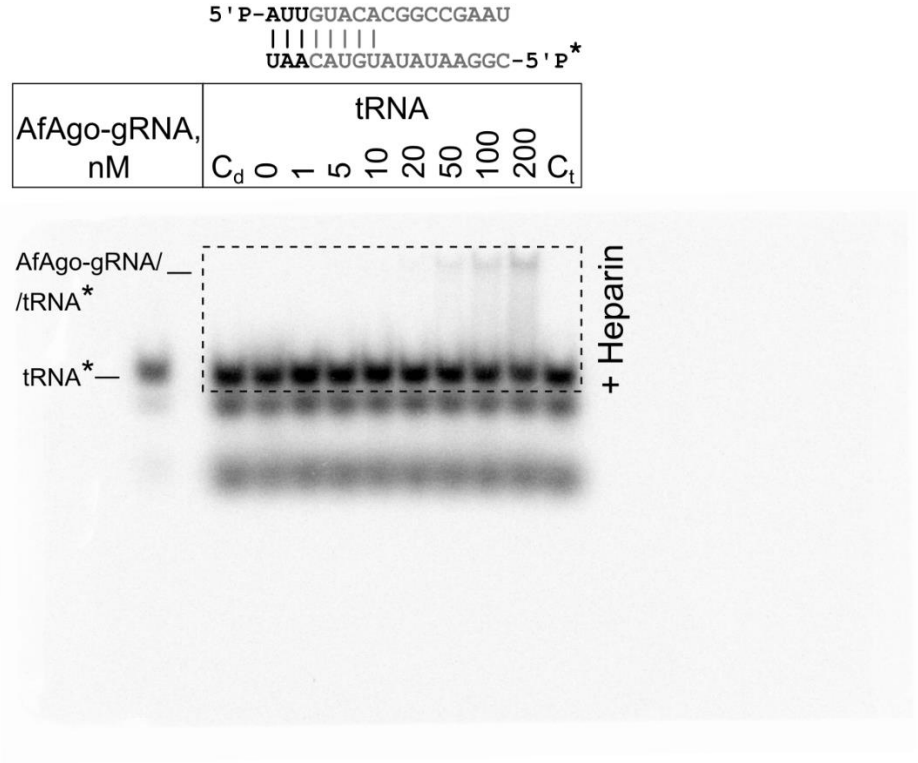

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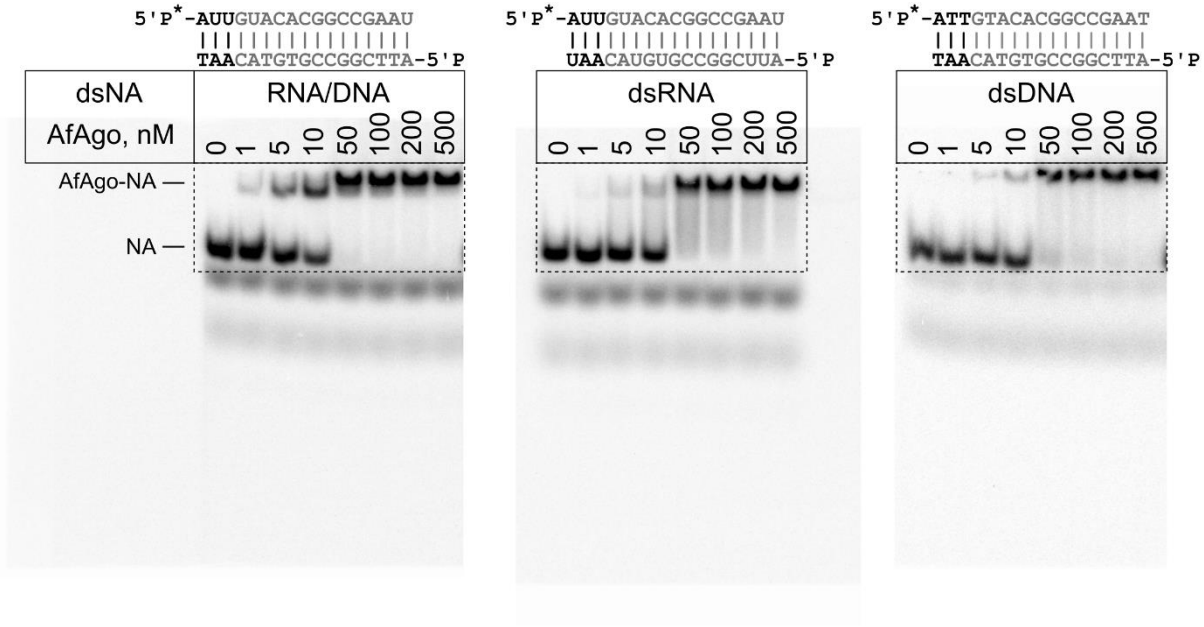

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