

Supplementary Information for

Mn²⁺-Induced Structural Flexibility Enhances the Entire Catalytic Cycle and the Cleavage of Mismatches in Prokaryotic Argonaute Proteins

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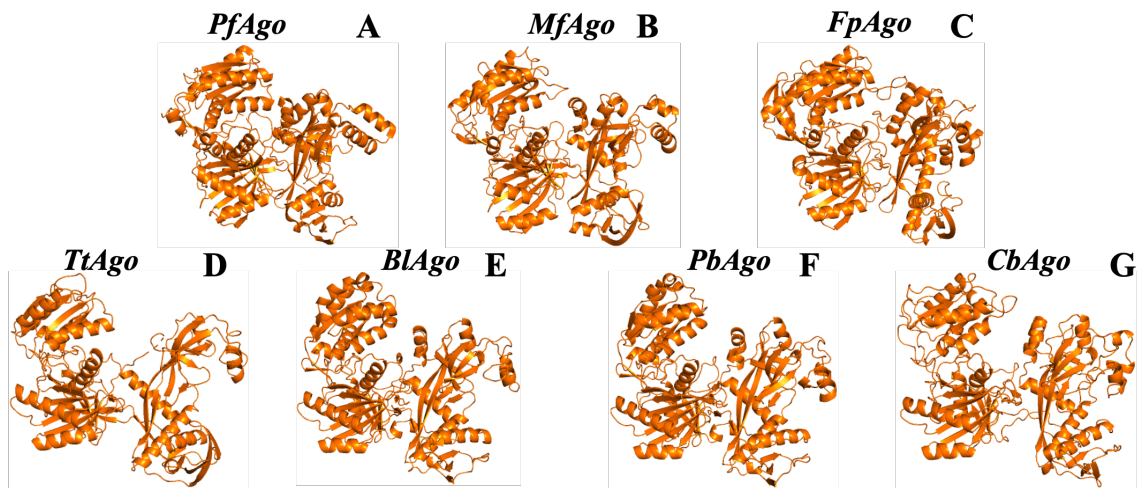


Fig. S1. Atomic structure of (A) *PfAgo*, (B) *MfAgo*, (C) *FpAgo*, (D) *TtAgo*, (E) *BlAgo*, (F) *PbAgo*, and (G) *CbAgo*. The atomic structures of *PfAgo*, *TtAgo*, and *CbAgo* are obtained from the crystal structure PDB ID: 1Z25, 4N47, and 6QZK, respectively. The atomic structures of *MfAgo*, *FpAgo*, *BlAgo*, and *PbAgo* are obtained from AlphaFold2.

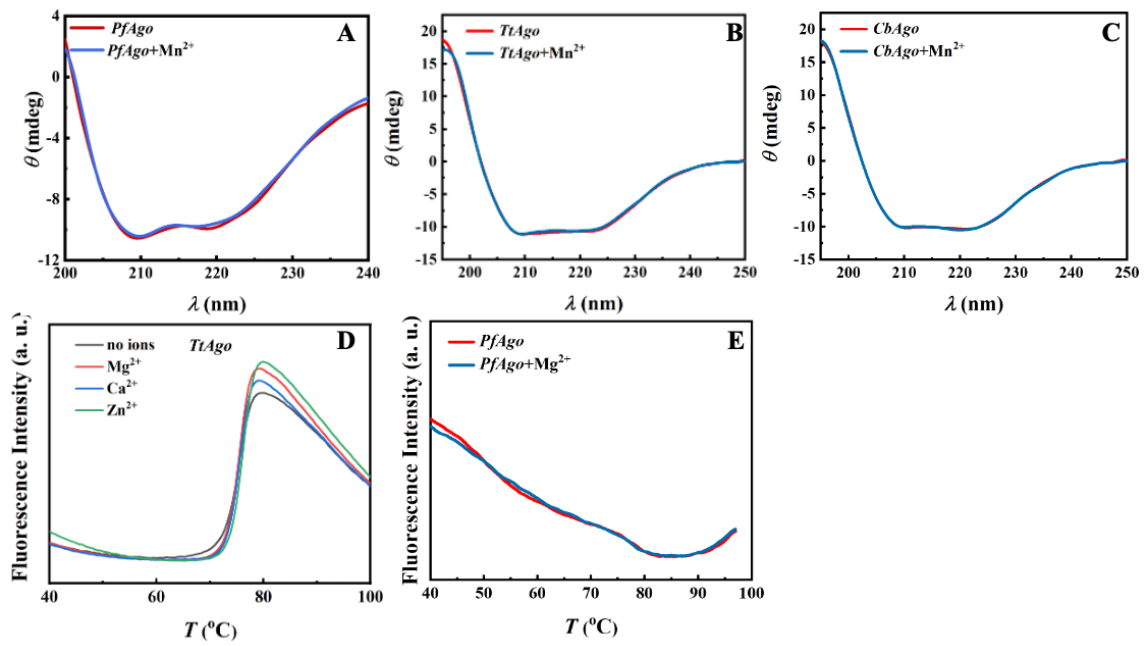


Fig. S2. The CD spectra of (A) *PfAgo*, (B) *TtAgo*, and (C) *CbAgo* incubated with and without Mn^{2+} at their respective physiological temperatures. The DSF of (D) *TtAgo* and (E) *PfAgo* incubated with and without divalent cations.

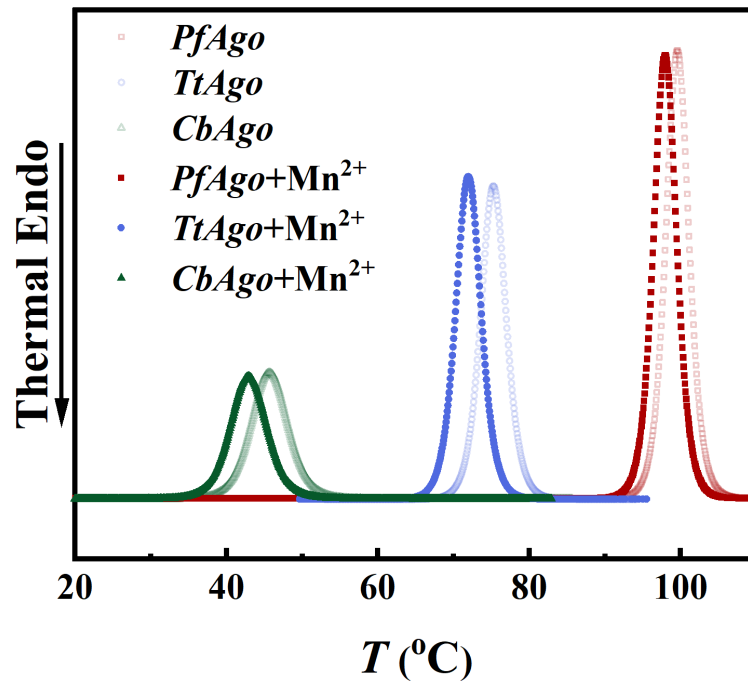


Fig. S3. Thermal denature curves of pAgo proteins incubated with and without Mn²⁺ determined by nanoDSC.

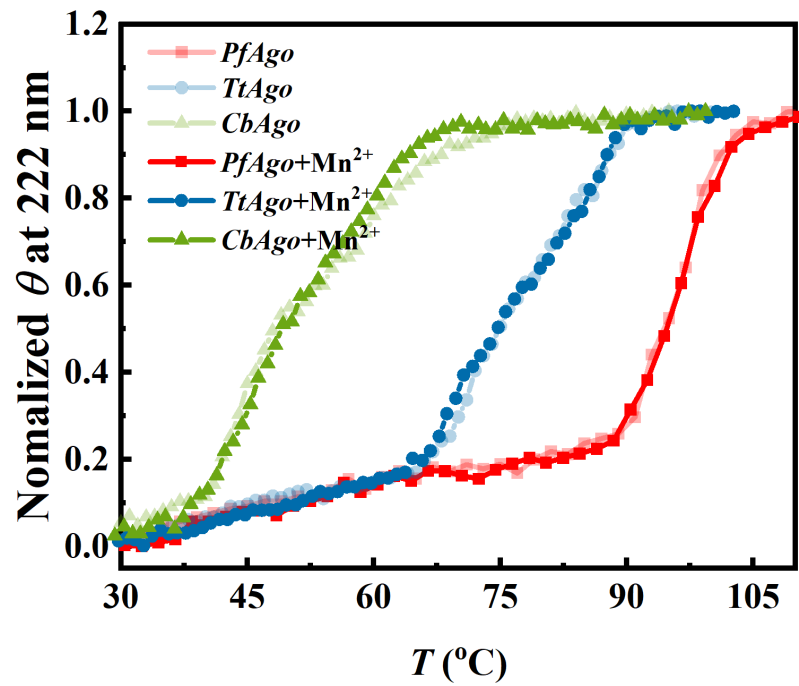


Fig. S4. Thermal denature curves of pAgo proteins incubated with and without Mn²⁺ determined by CD.

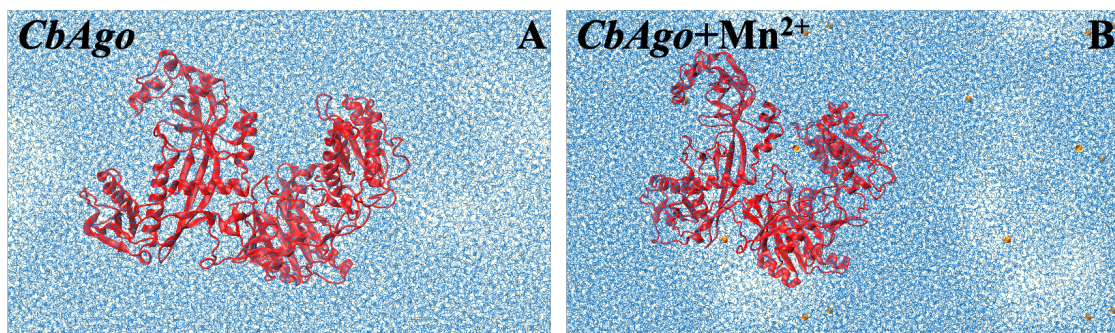


Fig. S5. A snapshot of the MD simulation of *CbAgo* incubated (A) without and (B) with Mn^{2+} in solution. The golden spheres represent Mn^{2+} .

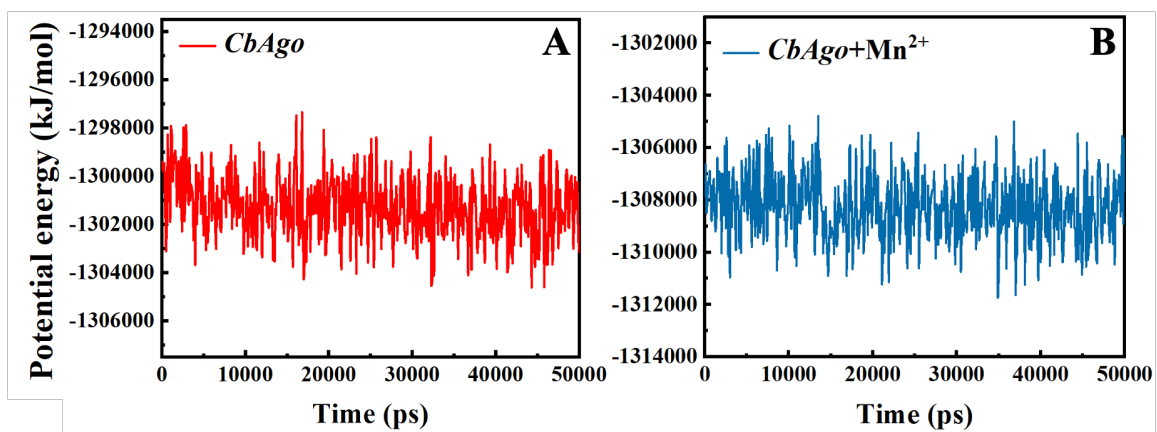


Fig. S6. The potential energy of protein as a function of MD simulation time of *CbAgo* incubated (A) without and (B) with Mn^{2+} . The simulation time is set as 50 ns.

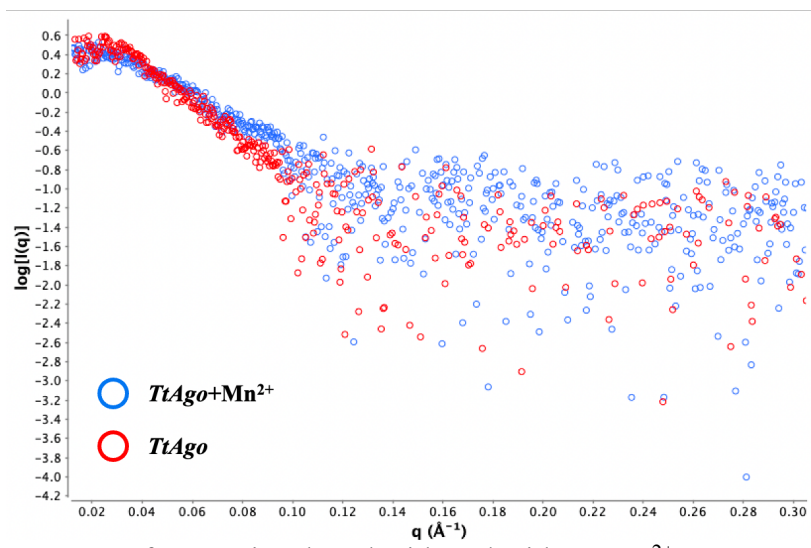


Fig. S7. SAXS curves of *TtAgo* incubated with and without Mn^{2+} .

TtAgo

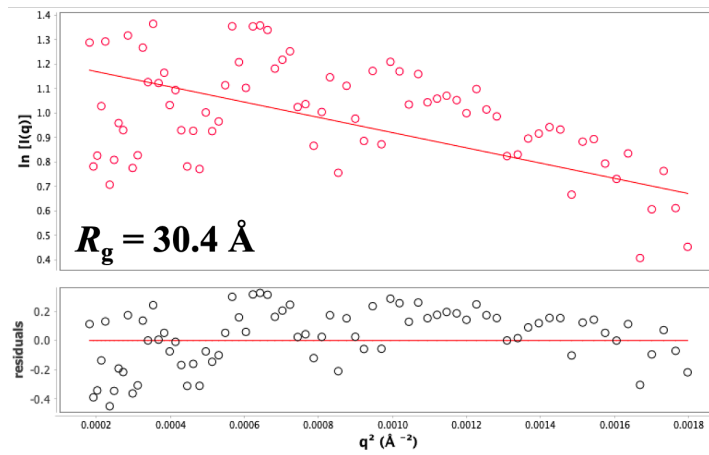


Fig. S8. Guinier plots of *TtAgo*. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

TtAgo+Mn²⁺

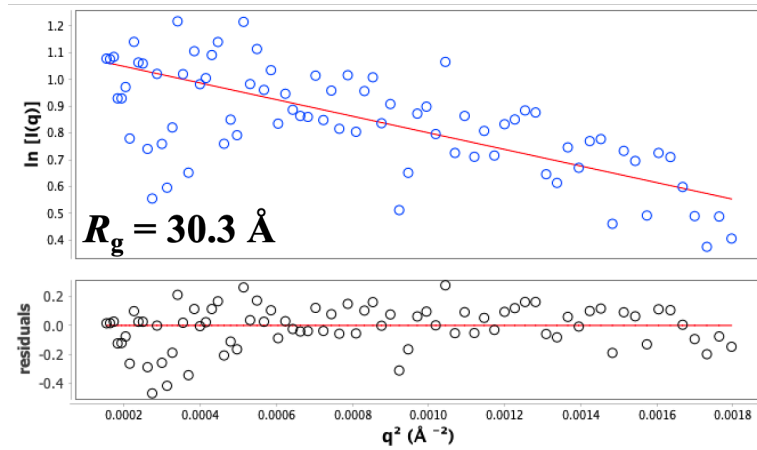


Fig. S9. Guinier plots of *TtAgo* incubated with Mn²⁺. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

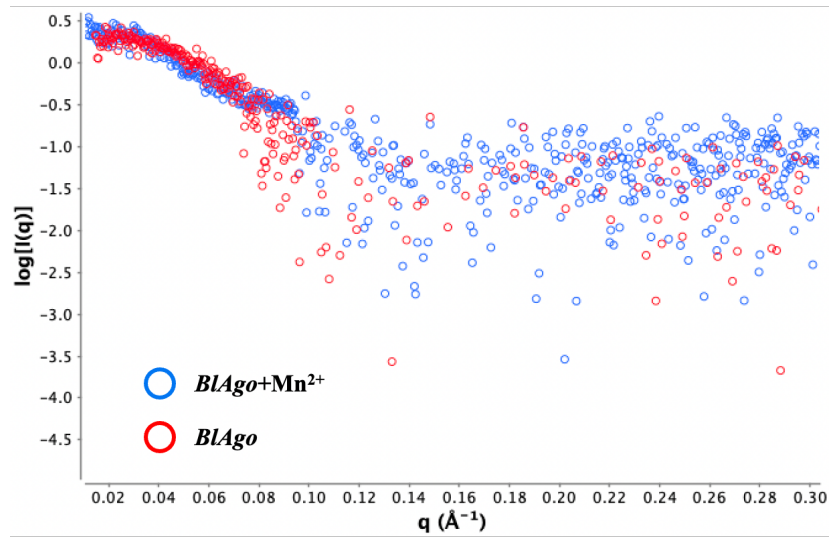


Fig. S10. SAXS curves of *BLAGo* incubated with and without Mn^{2+} .

*BL*AgO

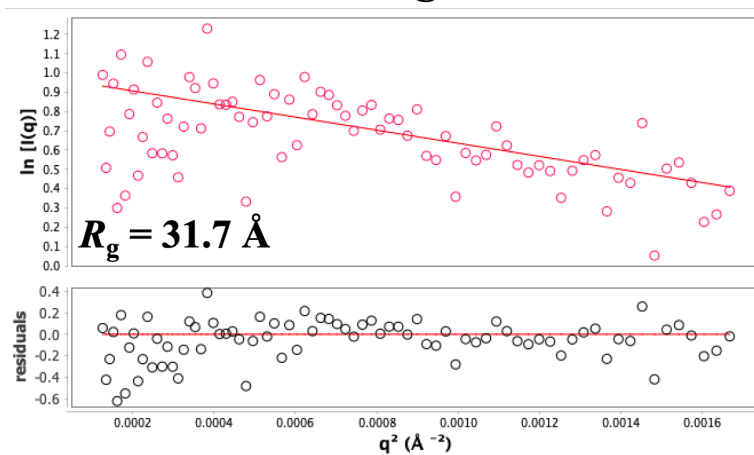


Fig. S11. Guinier plots of *BL*AgO. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

BlAgo+Mn²⁺

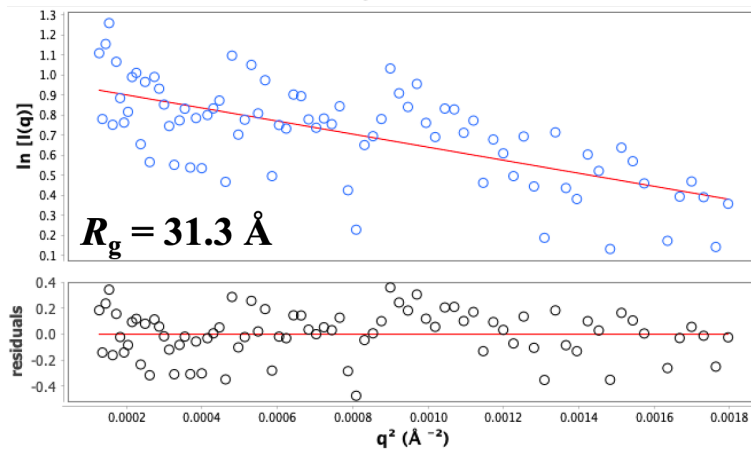


Fig. S12. Guinier plots of *BlAgo* incubated with Mn²⁺. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

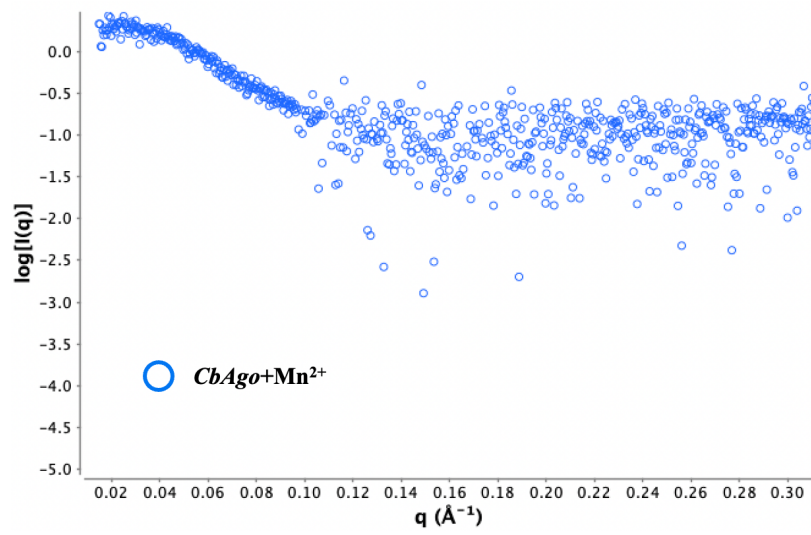


Fig. S13. SAXS curve of *CbAgo* incubated with Mn^{2+} .

CbAgo+Mn²⁺

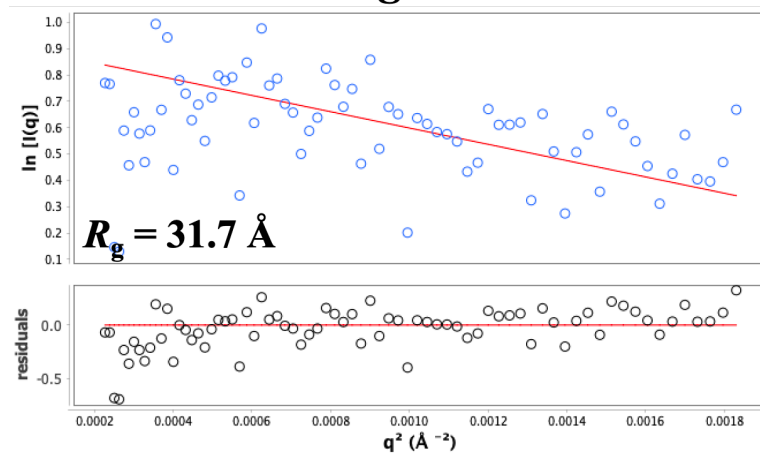


Fig. S14. Guinier plots of *CbAgo* incubated with Mn²⁺. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

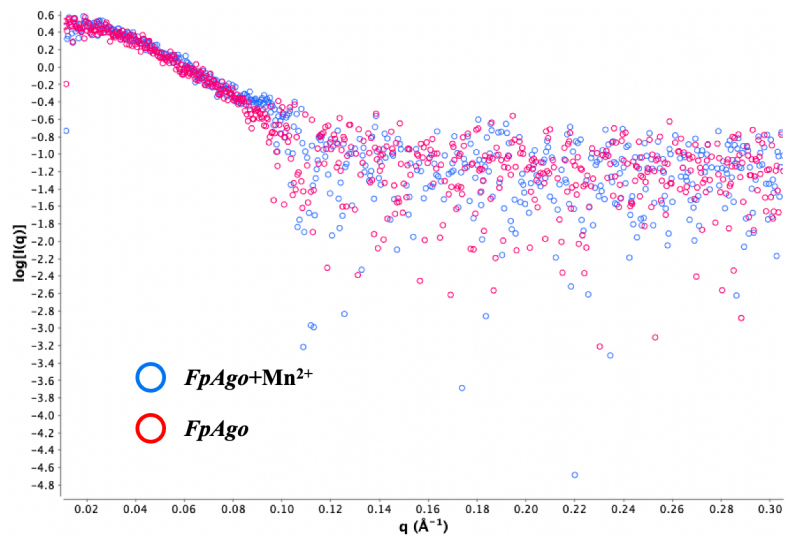


Fig. S15. SAXS curves of *FpAgo* incubated with and without Mn^{2+} .

FpAgo

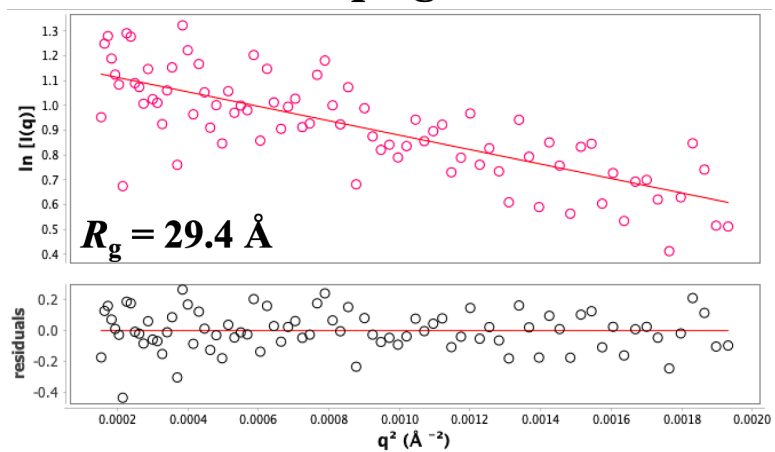


Fig. S16. Guinier plots of *FpAgo*. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

FpAgo+Mn²⁺

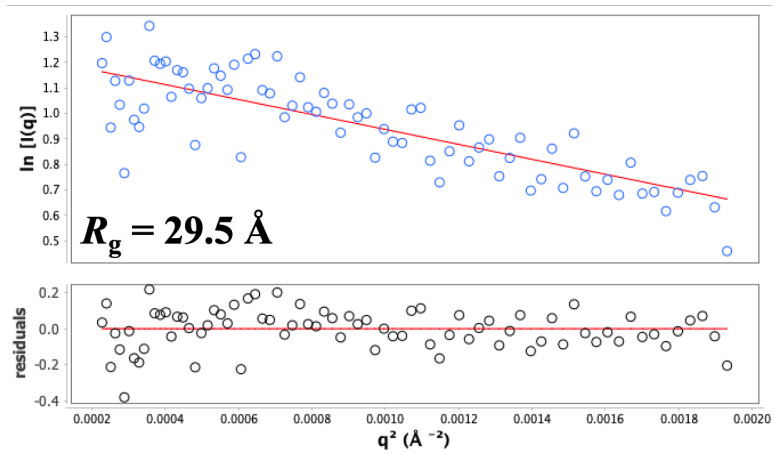


Fig. S17. Guinier plots of *FpAgo* incubated with Mn²⁺. The lower insets show the error-weighted residual difference plots for the Guinier fitting.

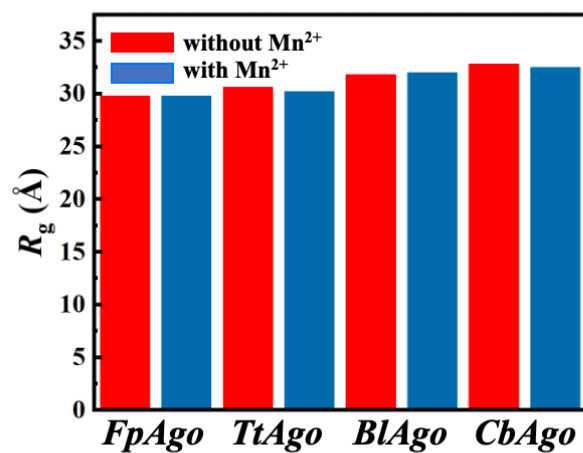


Fig. S18. The radius of gyration (R_g) of *FpAgo*, *TtAgo*, *BLAgo*, and *CbAgo* incubated with and without Mn^{2+} derived from MD simulations.

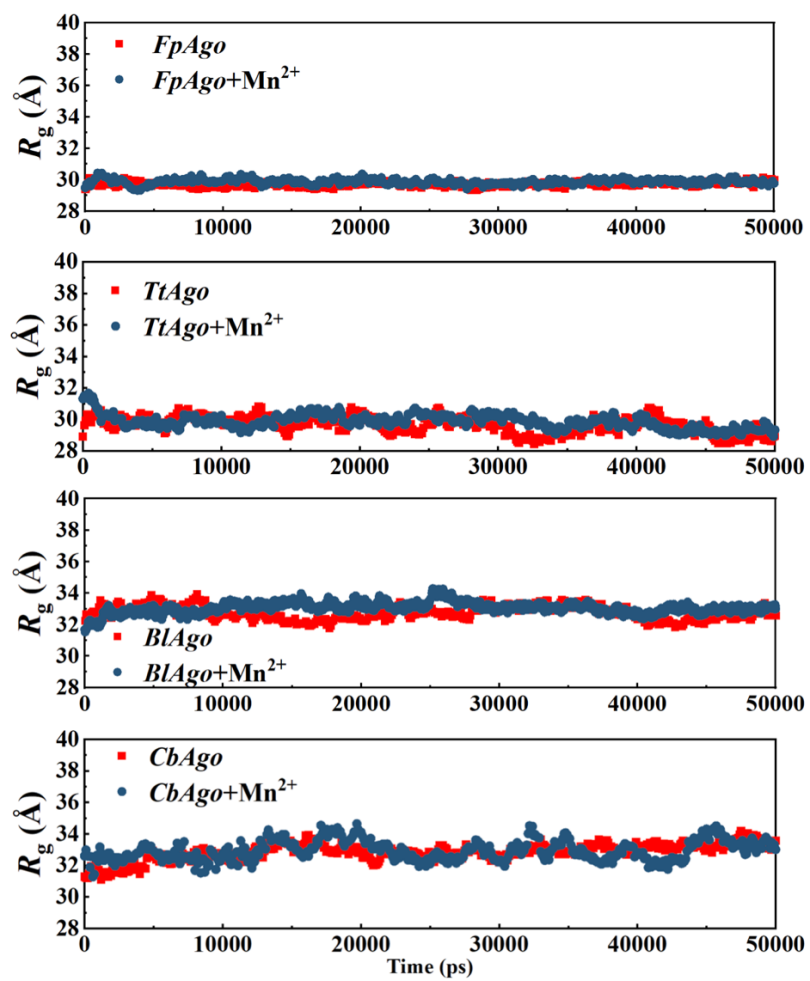


Fig. S19. R_g of pAgo proteins incubated with and without Mn^{2+} as a function of MD simulation time.

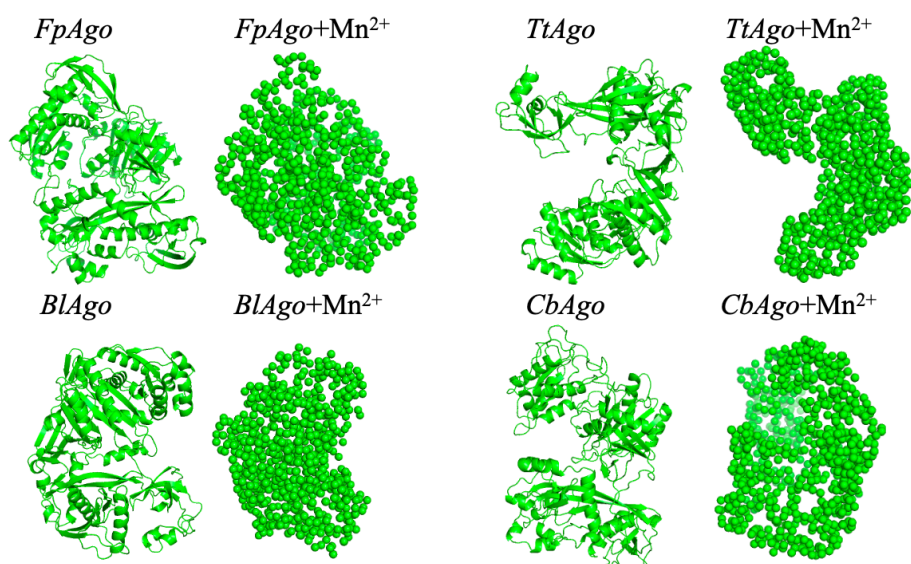


Fig. S20. The *ab initio* structures of (A) *FpAgo*, (B) *TtAgo*, (C) *BlAgo*, and (D) *CbAgo*.

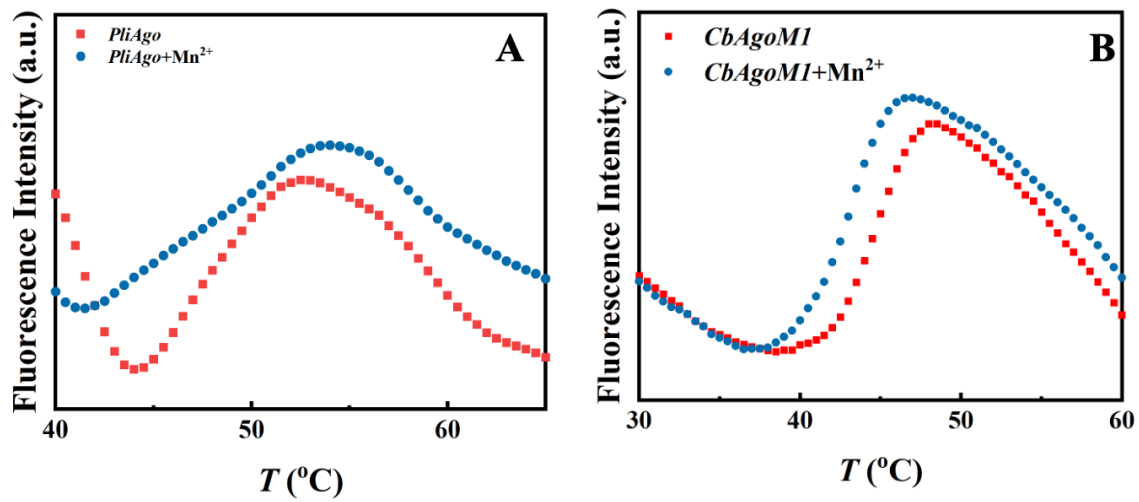


Fig. S21. DSF measurements of (A) *PliAgo* and (B) *CbAgoM1*.

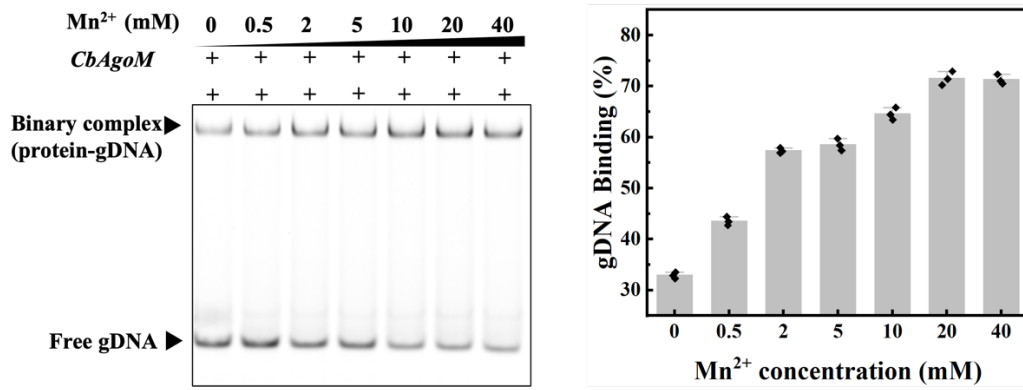


Fig. S22. Electrophoresis mobility shift assay of the binding of pAgo protein to gDNA at different concentrations of Mn^{2+} . Left panel: binding assay with gDNA at different concentrations of Mn^{2+} . Right panel: quantifying the effect of incubating with Mn^{2+} on gDNA binding activity by EMSA.

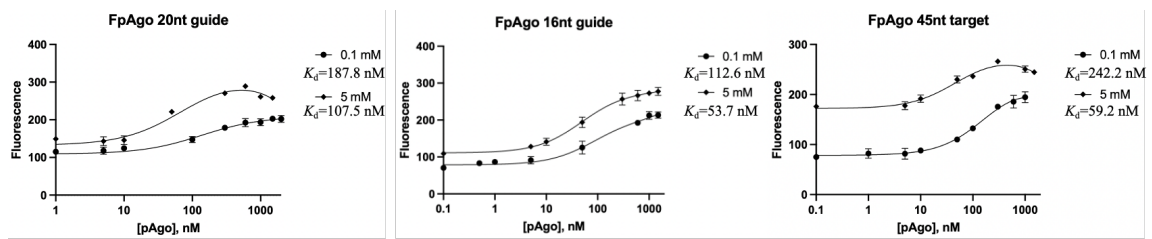


Fig. S23. Fluorescence polarization assay of the tDNA and gDNA of varying lengths at different concentrations of Mn^{2+} . The results from three independent experiments were quantified. Error bars represent the standard deviations of three independent experiments.

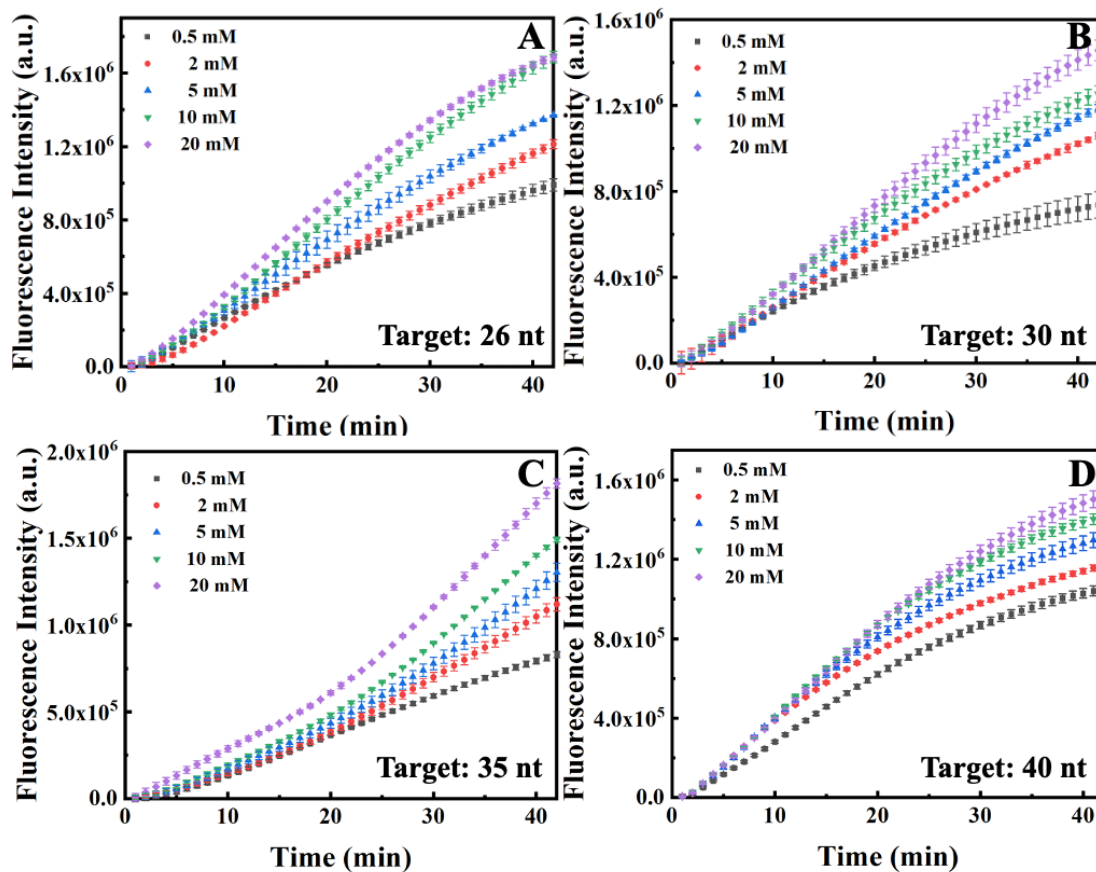


Fig. S24. The role of the Mn²⁺-dependent structural dynamics of pAgo proteins for cleavage target DNA of varying length. Cleavage assays with gDNA and tDNA of varying lengths at different concentrations of Mn²⁺. *PliAgo* preloaded with 21 nt gDNA cleaves (A) 26 nt, (B) 30 nt, (C) 35 nt, and (D) 40 nt tDNA. In all experiments, protein, guide, and target were mixed at a 5:1:1 molar ratio and incubated at 37 °C. The reaction buffer without Mn²⁺ contains 10 mM EDTA. Three samples were used for each experimental condition. The results from three independent experiments were quantified. Error bars represent the standard deviations of three independent experiments. The detailed procedure is presented in the Materials and Methods. The nucleotide sequences of the gDNA and tDNA are presented in Table S4.

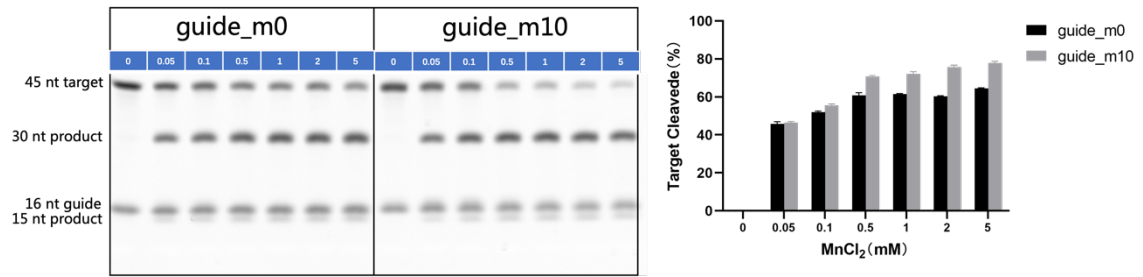


Fig. S25. Left panel: Gel detection results for the designed mismatched gDNA-directed cleavage on tDNA at different concentrations of Mn²⁺. Quantifying the effects of incubating with Mn²⁺ on tDNA cleavage obtained from the left panel. *CbAgo* is used here. The results from three independent experiments were quantified. Error bars represent the standard deviations of three independent experiments.

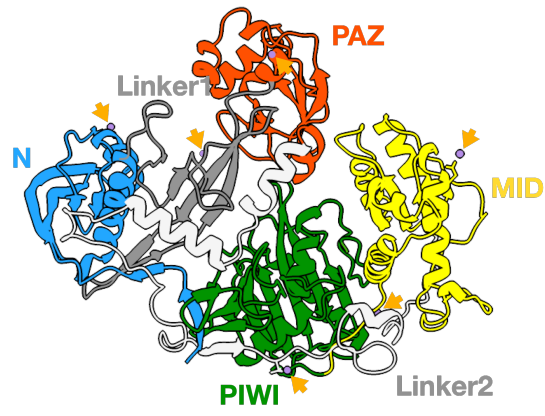


Fig. S26. Locations of Mn²⁺ on protein are obtained from MD simulations. The purple spheres represent Mn²⁺.

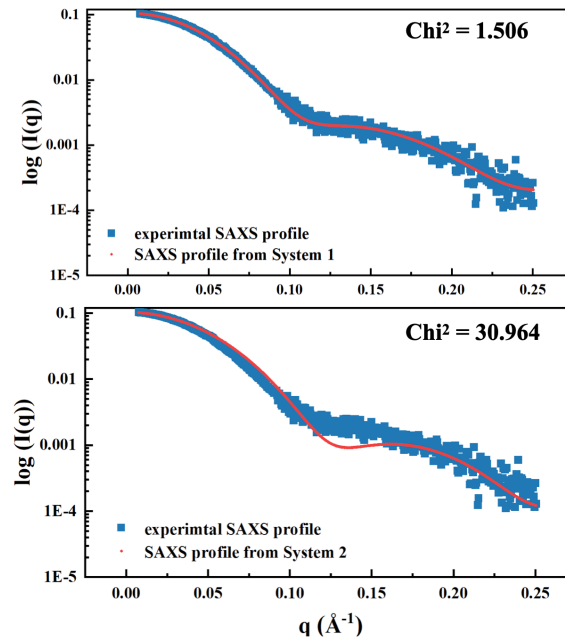


Fig. S27. Comparison between the experimental SAXS profile of *CbAgo* incubated with Mn^{2+} (blue dots) and the scattering profile (red lines) obtained from (upper panel) System 1 and (lower panel) System 2.

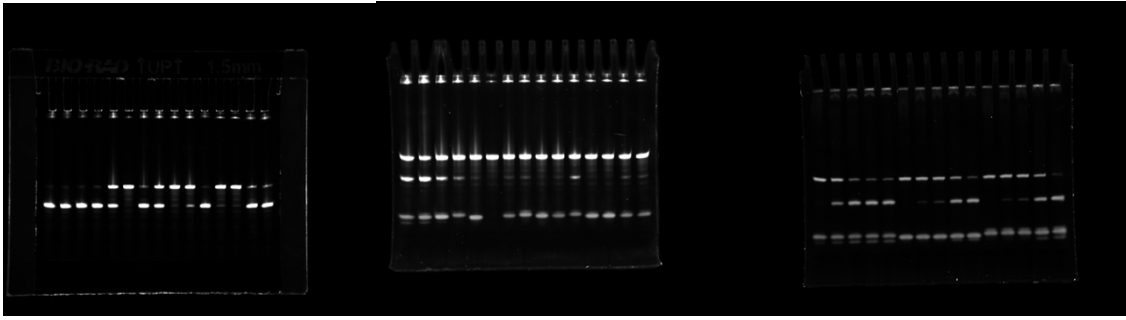


Fig. S28. Left panel: raw image of Fig. 4B (upper panel). Middle panel: raw image of Fig. 4B (lower panel). Right panel: raw image of Fig. 4C (lower panel).

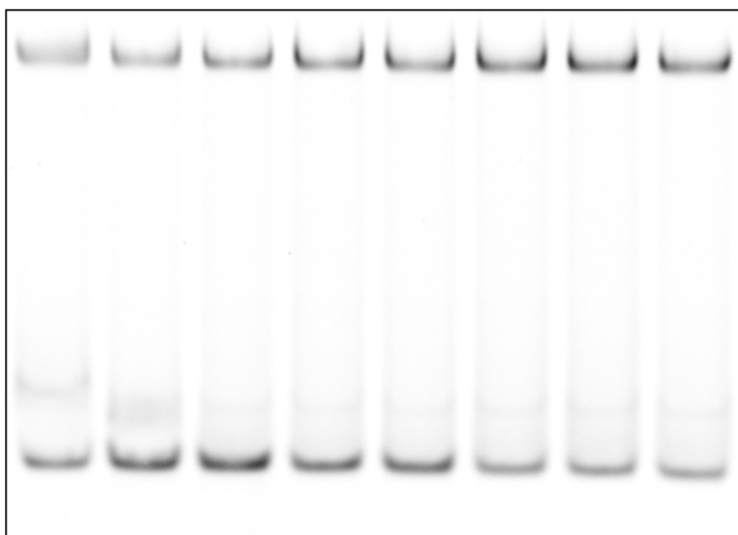


Fig. S29. Raw image of Fig. S22.

Table S1. The relative contents of different secondary structures in pAgo proteins derived from MD simulations, as defined by mass fraction.

Protein	Alpha-helix (%)	Beta-sheet (%)	Turn and loops (%)
<i>CbAgo</i> +Mn ²⁺	29	25	46
<i>CbAgo</i>	29	25	46
<i>TtAgo</i> +Mn ²⁺	31	31	38
<i>TtAgo</i>	31	31	38
<i>FpAgo</i> +Mn ²⁺	33	28	39
<i>FpAgo</i>	33	28	39
<i>BlAgo</i> +Mn ²⁺	30	27	43
<i>BlAgo</i>	30	27	43

Table S2. M_w of *CbAgo*, *TtAgo*, *BlAgo*, and *FpAgo* estimated from SAXS. M_w^{expected} is obtained from the amino acid sequence.

Protein	$M_w^{\text{experimental}}$ (kDa)	M_w^{expected} (kDa)
<i>CbAgo</i>	91	86
<i>CbAgo</i> +Mn ²⁺	88	
<i>TtAgo</i>	78	77
<i>TtAgo</i> +Mn ²⁺	79	
<i>BlAgo</i>	72	80
<i>BlAgo</i> +Mn ²⁺	78	
<i>FpAgo</i>	90	83
<i>FpAgo</i> +Mn ²⁺	84	

Table S3. SAXS data collection and analysis.

	<i>FpAgo</i>	<i>FpAgo</i> + Mn ²⁺	<i>TtAgo</i>	<i>TtAgo</i> + Mn ²⁺	<i>CbAgo</i>	<i>CbAgo</i> + Mn ²⁺	<i>BLAgo</i>	<i>BLAgo</i> + Mn ²⁺
Data acquisition								
Beamline facility	BL19U2-SSRF		BL19U2-SSRF		BL19U2-SSRF		BL19U2-SSRF	
Wavelength (nm)	0.103		0.103		0.103		0.103	
Sample-to-detector distance (m)	3		3		3		3	
Detector	Pilatus 2M		Pilatus 2M		Pilatus 2M		Pilatus 2M	
Overall parameter								
R_g (Å) (from P(r))	2.95	2.95	3.0	3.1	3.20	3.20	3.14	3.17
R_g (Å) (from Guinier)	2.94	2.95	3.04	3.03	3.20	3.17	3.17	3.13
Porod volume estimate (nm ³)	148	150	122	121	185	188	195	192
Oligomeric state	monomer		monomer		monomer		monomer	
Software								
SAXS data integration	<i>ScÅtter</i>		<i>ScÅtter</i>		<i>ScÅtter</i>		<i>ScÅtter</i>	
Ab initio modeling	GASBOR		GASBOR		GASBOR		GASBOR	
Simulated SAXS	CRY SOL		CRY SOL		CRY SOL		CRY SOL	

Table S4. List of nucleic acid sequences for binding and cleavage assay used in this study

Oligonucleotide name	Sequence (5'-3')	Description
gDNA 21 nt	5'P-TGAGGTAGTAGGTTGTATAGT	
tDNA 26 nt	5'FAM-ATATACTATACAACCTACTACCTCGT-3'BHQ1	5'FAM labeled tDNA, 3'BHQ1
tDNA 30 nt	5'FAM-ATATACTATACAACCTACTACCTCGTATAA-3'BHQ1	5'FAM labeled tDNA, 3'BHQ1
tDNA 35 nt	5'FAM-ATATACTATACAACCTACTACCTCGTATAAATTTT-3'BHQ1	5'FAM labeled tDNA, 3'BHQ1
tDNA 40 nt	5'FAM-ATATACTATACAACCTACTACCTCGTATAAATTTTAAAT-3'BHQ1	5'FAM labeled tDNA, 3'BHQ1
tDNA 45 nt	5'FAM-ATATACTATACAACCTACTACCTCGTATAAATTTTAAATAAAT-3'BHQ1	5'FAM labeled tDNA, 3'BHQ1

Table S5. List of amino acid sequences of pAgo proteins used in this study

pAgo proteins	Sequence	Description
<i>CbAgo</i>	<p>MANNLTFEAFEGIGQLNELNFYKYRLIGKGGQIDNVHQAIWSVK YKLQANNFFKPVFVKGEILYSLDELKVIPEFENVEVILDGNIILSI SENTDIYKDVIVFYINNALKNIKDITNYRKYITKNTDEIICKSILTT NLKYQYMKSEKGFKLQRKFKISPVVFRNGKVILYLNCSSDFSTD KSIYEMLNDGLGVVGLQVKNKWTNANGNIFIEKVDNTISDPG TSGKLGQSLIDYYINGNOKYRVEKFTDEDKNAKVIQAKIKNKT YNYIPQALTPVITREYLSHTDKKFSKQIENVIKMDMNYRYQTLK SFVEDIGVIKELNNLHFKNQYYTNFDFMGFESGVLEEPVLMGA NGKIKDKKQIFINGFFKNPKENVKFGVLYPEGCMENAQSIARSIL DFATAGKYNKQENKYISKNLNMNIGFKPSECIFESYKLGDITEYKA TARKLKEHEKVGAVIAVIPDMNELEVENPYNPFKKVWAKLNIPS QMITLKTTEKFNIVDKSGLYYLHNIALNILGKIGGIPWIKDMP GNIDCFIGLAVGTREKGIHFPACSVLFDKYGKLINYYKPTIPQSG EKIAETILQEIFDNVLISYKEENGEYPKNIVIHRAFGSRENIDWYK EYFDKKGKIFNIIIEVKKNIPVKIAKVVGSNICNPIKGSYVLKNDK AFIVTTDIKDGVASPNPLKIEKTYGDVEMKSILEQIYSLSQIHVGS TKSLRLPITTYADKICKAIEYIPQGVVDNRLFFL</p>	Wild type
<i>CbAgoM</i>	<p>MNNLTFEAFEGIGQLNELNFYKYRLIGKGGQIDNVHQAIWSVKY KLQANNFFKPVFVKGEILYSLDELKVIPEFENVEVILDGNIILSIS ENTDIYKDVIVFYINNALKNIKDITNYRKYITKNTDEIICKSILTT NLKYQYMKSEKGFKLQRKFKISPVVFRNGKVILYLNCSSDFSTD KSIYEMLNDGLGVVGLQVKNKWTNANGNIFIEKVDNTISDPG TSGKLGQSLIDYYINGNOKYRVEKFTDEDKNAKVIQAKIKNKT YNYIPQALTPVITREYLSHTDKKFSKQIENVIKMDMNYRYQTLK SFVEDIGVIKELNNLHFKNQYYTNFDFMGFESGVLEEPVLMGA NGKIKDKKQIFINGFFKNPKENVKFGVLYPEGCMENAQSIARSIL DFATAGKYNKQENKYISKNLNMNIGFKPSECIFESYKLGDITEYKA TARKLKEHEKVGAVIAVIPRMNELEVENANPFCKVWAKLNIPS QMIHLKSTEFKFNIVDKSGLYYLHNIALNILGGIGGIPWIKDMP GNIDCFIGLDVGTREKGIHFPACSVLFDKYGKLINYYKPTIPQSG EKIAETILQEIFDNVLISYKEENGEYPKNIVIHVDGFSRENIDWYK EYFDKKGKIFNIIIEVKKNIPVKIAKVVGSNICNPIKGSYVLKNDK AFIVTTDIKDGVASPNPLKIEKTYGDVEMKSILEQIYSLSQIHVGS TKSLRLPITTYADKICKAIEYI</p>	Mutant
<i>PbAgo</i>	<p>MNTPLTHYVLTEWESDTNTNVLHIHLYTLPVRNVFEQHKENG ACFDLRKLNRSLLIDFYDQYIVSWQPIENWGEYTFQHEYRSINP TILAEAILERLLLRTIESVQPKKEIAAGSRKFTWLKAEKVVENI SIHRVIQCDVTVDYAGKISVGFDLNHSYRTNESVYDLMKNSAIF KGDRVIDIYNLHVEFVEISNSTINDSIPELNQSVMNYFTKERKQ AWKVDKLEQSMVVYLKAFNGSRIAYAPAMLQKELTFESLPTN VVRQTSEIFKQANQKIKTLLDEIQKILARTDKIKFNKQKLLVQ QAGYEILELSNPNLQFGKNVTQTQLKYGLDKGGVVASKPLSINL LVPELIDTKLDVINDFNDKLNALSHKWGVPLSILKKSAYRNR PIDFTNPHQLAILLKELTKNLFQELTLVIPEKISGMWYDLVKKEF GGNSSVPTQFITIETLQKANDYILGNLLGLYSKSGIQPWILNSPL SSDCFIGLDVSHEAGRSTGIVQVVGKDGRLSSKANTSNEAGE KIRHETMCQIVYSAIDQYQQHYNERPKHVTFHRDGFCDRELLS LDEVMSLDVQYDMVEIHKTNRRMALTVGKQGWETKPGLCY LKDESAYLIATNPHPRVGTAPIKIHKKGLPIEAIQDIYHLSFM HIGSLLKCRLPITTYADLSSTFFNRQWLPIDSGEALHFV</p>	Wild type
<i>PliAgo</i>	<p>MTLETTLPLEGLEGLTASYQLYAVKGLSGLDETEYHKNVNLV RRLSFSMKAPFVALSRDGEQFIAPPNYVTEFPVDHRVVRAMVK LVPTGEPLNLRFDAADDEYDGLRLRYLDFVLQQPLFANHHLWQ PGSGQPFFHKKPLKRLDDVDLYDGVSVRAAKHPEGGFIVCDA RSKFITHTPIGARADRKRLGKLINRSCLYKMGDHWYQFRIDAVS DWKVGEPSPFEGNVPISLAQQLVRTAGNAAPKSIIDLPEGGAL EYFTSTNERMAPAELCFLIEDTHGRRAAKLQRQTILSPSERRAR</p>	Wild type

	VNGFIRRYLSELNIGGAKLSAGARAHAFFTETHMPPALSFGNGT VLAPDTSKDRFQAMQEYSSMRRTMMLDKKVGFFHQDVFPPT LLLPEVVKKSWGPAFASDFVGTVQELYPAGGYRPEIIEYRDKAY GGGVPGQMKALLEVAERGEIKSGDVLVMLHRINGAPRAQDKL AAMVCNEFEKRFGRVQVIHSDSPGRGYKRIFKNDKPTYVQQR GRGVNIKGYLKGAAALNKVCLGNSRWPVFLRDPLNADVTIGIDV KNNMAVFTMVAEGGRIVRVQRSRSRQREQLLESQVTQVITEML SKELPEIKKQVQRVVIHRDGRAWPAEIAGARKTFADMAESGLIA VDADVSVEVLKSSPAPLRLFSFEEPTQENPKGVINPVLGSWLK LSENDGYICTTGAPLLLQGTADPLHVRKAFGPMAIEDALKDVF DLSCLTWPKPDSMRLPLTIKLCIALFDAAEYDVDVVRFAD GNTGEASA	
<i>BlAgo</i>	MNDFQVLTEWTLDRATEFIIHIYTMPINDLNKSHTESYELVKT RRLNNTKEIVFYEQQIAALTKVENWGHYTRGDYQHRCIKLSVP RERELLQRLLLKVVGARQPKKVYAEGGLFINASPQYKIENINIH EALNLDVSVTQEGLIIVGDFDFTHKLYYKDTLLEFVKRNQIQKGD RVIDPIYHISYVYDEVAPYTVSESSPYLGQSILQYYKNKDWILKK LNTDMSVHVHRNKENKIFPYAPVFLKKECSLSSLDQRIVTKINR VIKLGPNKMTKSIREVQNILSRFEVLRNKNLLVSNQGYKIK YFPVPSLRFKGNVTSKSLKNGLIKGGVVDPRNLELAYFIDPVIK NNTESIEVFMKNLEEKSKQLGVPLQRLKKGRSFYNQLDTQMFS SPNELVLGLKKAKEFNCLTVVITTTQONIDKCYGAIKKEFGGNY DIPTQFVTADTAKEKNDYILLNILLGIYAKASIQSWILKEPLHSDC FIGLDVSHEENRHSTGLVQVVGKDGRLVSSKAMSTIESGERIRD ETMKEIVYEAHSYENQYGYRPHVTFHRDGFRENIDNIEYIL KNLGVLFDYVEVIKSNRRMANFLSKEEGWKTEIGASYLKDDL AYLCSTAPGKQVGMaipVKITQVTGHLTMSAIVSDIFNLSHMHV GSLIKSRLPISTYYADLSSTFFNRGWISSRSNGLQFV	Wild type
<i>TtAgo</i>	MNHLGKTEVFLNRFALRPLNPEELRPWRLEVVLDPGGREEVY PLLAQVARRAGGVTVRMGDGLASWSPPEVLVLEGLTARMGQT YAYRLYPKGRRLDPKDPGERSVLSALARLLQERLRRLEGVW VEGLAVYRREHARGPGWRVLGGAVLDLWVSDSGAFLEVDPA YRILCEMSLEAWLAQGHPLPKRVRNAYDRRTWELLRLGEEDPK ELPLPGGLSLLDYHASKGRLQREGGRVAVADPKDPRKPIPHL TGLLVPVLTLEDLHEEEGSLALSPLWEERRRRRTREIASWIGRRLG LGTPEAVRAQAYRLSIPKLMGRRAVSKPADALRVGFYRAQETAL ALLRLDGAQGWPEFLRRALLRAFGASGASLRLHTLHAHPSQGL AFREALRKAKEEGVQAVLVLTPMAWEDRNRLKALLLREGLPS QILNVPLREEERHRWENALLGLLAKAGLQVVALSGAYPAELAV GFDAGGRESFRFGAACAVGGDGGHLLWTLPEAQAGERIPQEV VWDLLEETLWAFRRKAGRLPSRVLLLRDGRVPQDEFALALEAL AREGIAYDLVSVRKS GGGRVYPVQGRADGLYVPLEDKTFLLLT VHRDFRGTPrPLKLVHEAGDTPLEALAHQIFHLTRLYPASGFAP RLPAPLHLADRLVKEVGRLGIRHLKEVDREKLFFV	Wild type
<i>PfAgo</i>	MKAKVVINLVKINKKIIPDKIYVYRLFNDPEEELQKEGYSIYRLA YENVGIVIDPENLIIATTKLEYEGEPIEPEGEISFSELNDYQSKLV LRLKENGIGEYELSKLLRKRFRKPKTFGDYKVIPVEMSVIKHD EDFYLVIIHQIQSMKTLWELVNKDPKELEEFMTHKENLMLK DIASPLKTVYKPCFEEYTKKPKLDHNQEIVKYWYNYHIERYWN TPEAKLEFYRKFGQVDLKQPAILAKFASKIKKNKNYKIYLLPQL VVPTYNAEQLESVAKIELEYTKLMPEERKELLENILAEVDSII DKSLSEIEVEKIAQELNKRVRDDKGNVSPISQLNVQKSQLLL WTNYSRKYPVILPYEVPEKFRKIREIPMFIILDSGLLADIQNFATN EFRELKSMYYSLAKKYNLAKKARSTNEIGLPFLDFRGKEKVI TEDLNSDKGIIIEVVEQVSSFMKGKELGLAFIAARNKLSSEKFEI KRRLFNLVISQVVNEDTLKNKRDKYDRNRLDLFVRHNLLFQV LSKLGVKYVLDYRFNYDYIIGIDVAPMKRSEGYIGGSAMVFD QGYIRKIVPIKIGEGESVDMNEFFKEMVDKFEFNIKLDNKK ILLRDGRITNNEEGLKYISEMFDIEVVTMDVIKNHPVRAFAN MKMYFNLGGAIYLIPHKLKQAKGTPPIKLAKKRIKNGKVEKQ SITRQDVLDFILTRLNYGSISADMRLPAPVHYAHKFANAIIRNEW KIKEEFLAEGFLYFV	Wild type

<i>MfAgo</i>	MKNLRYKINAYRIKKDYIPKEVYRYRIRSFIEININIYRFVGFYGG VALNQSEFILPYPVENLVLEYDQKDVKLEHIDTLNLEDIENKDK EKAELVRYGYSIYKLPILYKILRDVRESKIINDIRVDPIDFTV KRHNNEYLVDFNHTATVFLKNLWDFVGRDKLKLKLEDYIGKKIIF KPNPKKRYTIKSIEKQNKDIDDIVEHIEYYKWTEEEIKSTFGEI DYTQPIIHCEGIPYFPAPQFCNIVFTMEDLDENTLKDLQSYWRLP NEIKGNIINQIAKKLRFVENEPIELEFIKFNNTPLIVKDENGKPTKI YTTNRLFRWNYDSKSKLYLPYDIPDIKNKTLTTFVLIDENLKNV SGKIKRKYVQMFKNYNKIASKTELPKDFANKWKYFSNNNIRD VIRKIKDEFNEELGFALIIGNRYYENDYYETLKMQLFNLNII SQNI LWENWSKDDNFMNTNLLIQIMGKLGKIFALDAKVNYDYIM GLDSGLGAFKSNRVSGCTVIYDSEGKIRRIQPIDVPSGERIPIHL VVEFLETKT DINMENKNILFLRDGFVQNSEREELKLSKELNSN IEVISIRKNNKYKVFTSDYGIGSIFGNDGIFLPHKTTFGSNPVKLS TWLRFNSGNEEKLKINESIMQLLYDLTKMNYSALYGEGRNLRIP APIHYADKFVKALGKNWKIDEELLKHGFLYFI	Wild type
<i>FpAgo</i>	MMLNIFEVDKNRVEVPQDVYLYKVHLKTLEQRKRDMCIAVLR NSFGYLDVNSFVIYSYKEIRDLPRRIKKYCDLEPTGKVKMNEVD ENVRNALVKTFLRSKIRKDIKKLLKKFKKQQSVGRWTVALDL ERIELVEHNGEILVSNVKNLNISSMINLWDIHERDVNRLKGLCWS PENLNDNRIWFRYIPHLIIEEVEDFEESAKSFILSDVHTGEEFFG WTTEDLKKYPENEYGLSEDAIKKIGNYTQFDSTQPIIKGVTWSG KEYPFLPQHCIPAYNPMLATAEEKRIIEIKINLKNKKDEIIRKIE QLPYLKQPDNIEIKKVQETARLRAKFKVVEVTKGKITKTLSPY EKPVSSTLDLFGWISRIIDGGDIIEICIPDYIPENLSRIKEIEAFLLI ENDLNENERKVGDKLLNDAIYVYNFVRSVCLRCGINIPYLYNK GNRFYFENSKEGIRDIYKRITTSLSGEIGFALIFGKRDNYEDEEGE DSFDYYNPLKSALFRNNILSQNFDVTNYVRGDGKINKNTIKYAV SNIIYNIFGKLGKLVKFFVLEEDVPYDYILGIDVGYGEAYTGKVAGC TTVHDSEGRLRNLIPIEKQNYPSKETARIKALLEEIEQKKKIYNID FENKSILIRDGRINKEEINQLMEFSEERNCRITYIEIRKNIVHQFL VNSSQACYVKIGDYYILKAHNPRIGFPRAIKIARKIVIEGDAWRE SSLTEDDILLIYKLTALNYSTIGRDSNLRIPAPIYYADKLVKALKK GWKFDERFLRYGILYFL	Wild type

Table S6. List of nucleic acid sequences for the designed mismatched gDNA directed cleavage on tDNA

Name	Sequence (5'-3')
45nt DNA target	ATATACTATACAACCTACTACCTCGTATAAATTTTAAATAA ATA-FAM
guide m0	P-TGAGG <u>T</u> AGTAGGTTGT
guide m1	P- <u>A</u> GAGG <u>T</u> AGTAGGTTGT
guide m2	P-TCAGG <u>T</u> AGTAGGTTGT
guide m3	P-TG <u>T</u> G <u>G</u> TAGTAGGTTGT
guide m4	P-TGAC <u>G</u> TAGTAGGTTGT
guide m5	P-TGAG <u>C</u> TAGTAGGTTGT
guide m6	P-TGAGG <u>A</u> AGTAGGTTGT
guide m7	P-TGAGG <u>T</u> TAGTAGGTTGT
guide m8	P-TGAGG <u>T</u> A <u>C</u> TAGGTTGT
guide m9	P-TGAGG <u>T</u> A <u>G</u> AAGGTTGT
guide m10	P-TGAGG <u>T</u> A <u>G</u> T <u>T</u> G <u>G</u> TTGT
guide m11	P-TGAGG <u>T</u> A <u>G</u> T <u>A</u> C <u>G</u> TTGT
guide m12	P-TGAGG <u>T</u> A <u>G</u> T <u>A</u> G <u>C</u> TTGT
guide m13	P-TGAGG <u>T</u> A <u>G</u> T <u>A</u> G <u>G</u> A <u>T</u> GT
guide m14	P-TGAGG <u>T</u> A <u>G</u> T <u>A</u> G <u>G</u> T <u>A</u> G <u>T</u>