Origins of the increased affinity of phosphorothioate-modified therapeutic nucleic acids for proteins

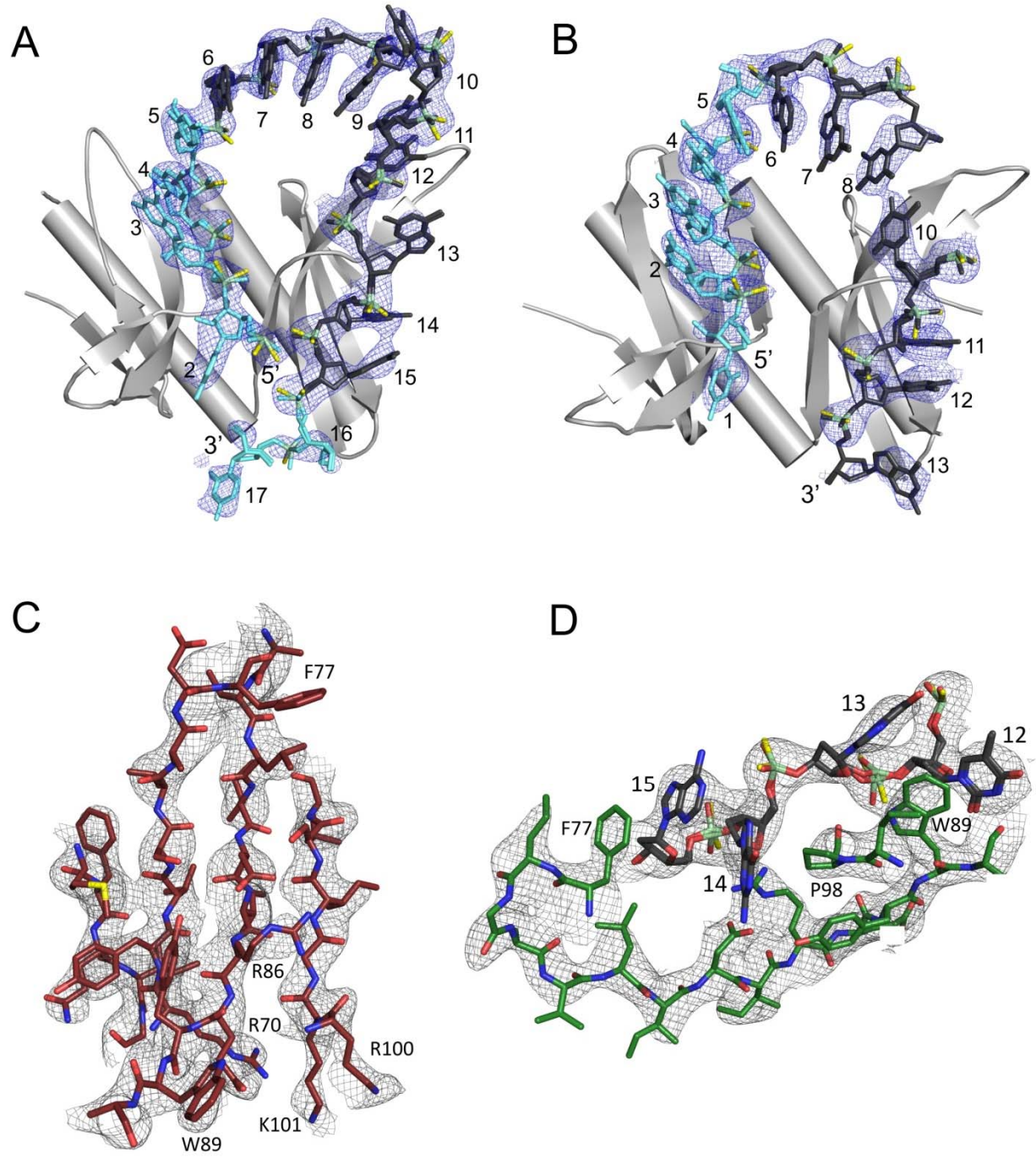
Malwina Hyjek-Składanowska[1], Timothy A. Vickers[2], Agnieszka Napiórkowska[1], Brooke A. Anderson[2], Michael Tanowitz[2], Stanley T Crooke[2], Xue-hai Liang[2], Punit P. Seth[2,*], and Marcin Nowotny[1,3,*]

[1] ProBiostructures, Structural Biology Center, [3] Laboratory of Protein Structure, International Institute of Molecular and Cell Biology, 4 Trojdena St., 02-109 Warsaw, Poland

[2] Ionis Pharmaceuticals, Inc., 2855 Gazelle Court, Carlsbad, CA 92010, USA

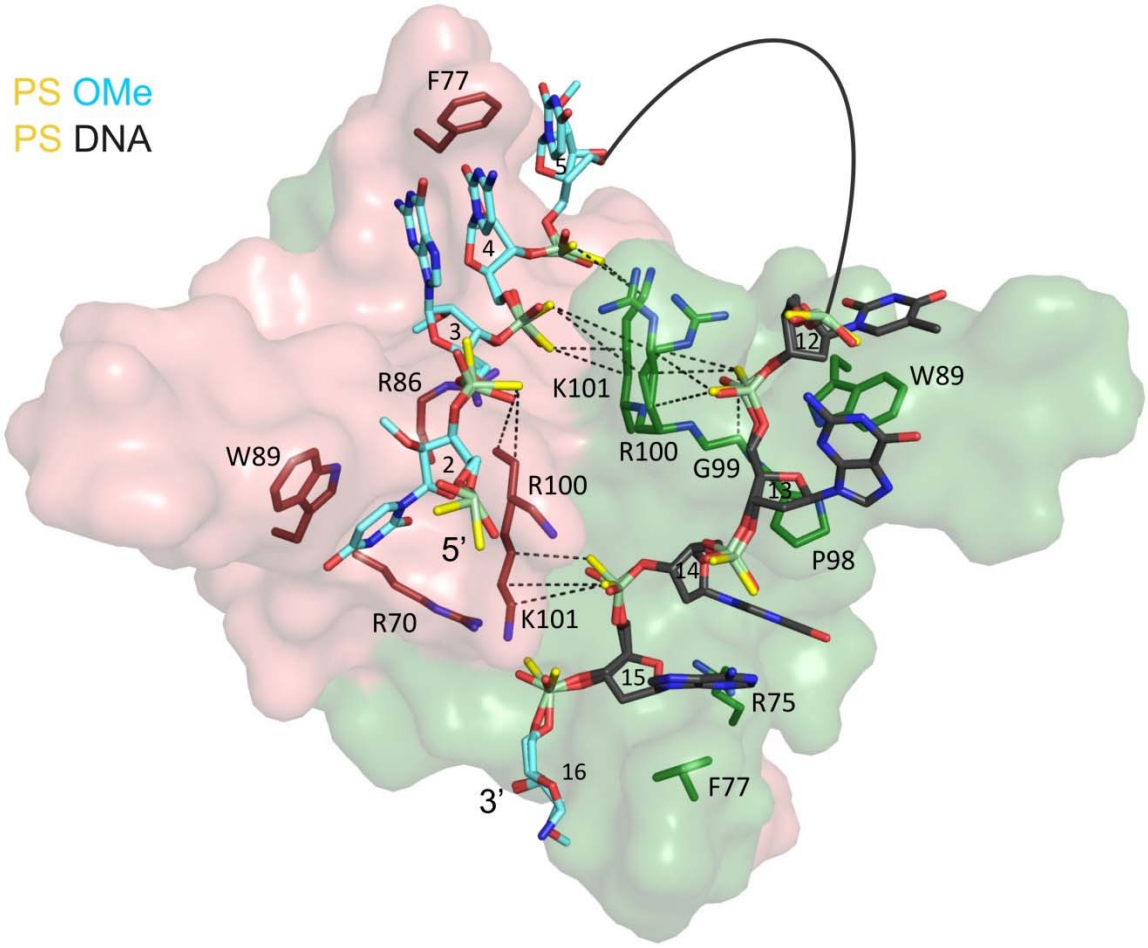
* To whom correspondence should be addressed. e-mail: pseth@ionisph.com, mnowotny@iimcb.gov.pl

SUPPORTING INFORMATION



Supplementary Figure 1. Electron density maps. (**A**, **B**) Simulated annealing composite omit electron density maps contoured at 1.4 (A) and 1.0 (B) σ overlaid on the fragments of structure around the 2′-OMe DNA gapmer PS ASO in complex I (A) and II (B). The PC4 dimers are shown in cartoon and colored in gray. For the stereorandom ASO, the nucleic acid was modelled as alternative conformations of full Rp

and full Sp PS diastereoisomers and both Rp/Sp conformations are shown. 2′-OMe PS nucleotides are shown as aquamarine sticks, PS DNA nucleotides are shown as dark grey sticks and labeled. The phosphorus atoms are shown in pale green and the sulfur atoms are shown as yellow sticks. 2Fo-Fc simulated annealing composite-omit map is shown as blue mesh for the nucleic acid. (**C**, **D**) Simulated annealing composite omit electron density maps contoured at 1.0 σ overlaid on the fragments of structure around the β-sheet (C) and the hydrophobic clamp binding nucleotides 12-15 (D). Amino acids are shown as sticks and color coded (ruby for chain B, forest for chain A). PS DNA nucleotides are shown as dark grey sticks and labeled. Oxygen atoms are shown in red, nitrogen atoms are shown in blue, phosphorus atoms are shown in pale green and sulfur atoms are shown in yellow. 2Fo-Fc simulated annealing composite-omit map is shown as gray mesh.



Supplementary Figure 2. Hydrophobic interactions with PS 2'-OMe DNA gapmer ASO in the binding interface. The PC4 protomers are shown in surface representation. Key residues involved in DNA binding are shown as sticks and labeled. Nucleotides which do not interact with the protein were omitted for clarity and their trajectory is marked with dark grey line. Phosphorus atoms are shown in pale

green, oxygen atoms are shown in red, nitrogen atoms are shown in blue and sulfur atoms are shown in yellow. Hydrophobic interactions are shown as black dotted lines. Please note that atoms NH1 and NH2 of residue Arg100 in chain B were not modelled due to poor electron density. For the stereorandom PS ASO, the backbone is shown for both Rp/Sp diastereoisomers and the nucleosides are shown only for one diastereoisomer (Rp PS) for clarity. 2′-OMe PS nucleotides are shown as aquamarine sticks, PS DNA nucleotides are shown as dark grey sticks.

Supplementary Table 1. Sequences of PCR and site-directed mutagenesis primers used to generate NLuc-PC4 fusions and binding site point mutations. Upper case, plasmid sequence; lower case, mutation.

Plasmid	Primer type	Vector	Forward primer	Reverse primer
pNLuc-PC4	PCR	FN31K	GCAT TCGA C TCGAG C CCT AAA TCA AAG GAA CTT GTT TCT TC	CAT TGA TGA TGC AGT AAG AAA ACT G TAG GAA TTC GCAT TCGA
pNLuc-PC4d60	SDM	NLuc-PC4	GATAACATGTTTCAGATTGGGAAAATGAGGTAC	GCTCGAGCCCGCCAGAAT
R70A	SDM	NLuc-PC4d60	TGGGAAAATGgcgTACGTTAGTGTTC	ATCTGAAACATGTTATCATCTC
R75A	SDM	NLuc-PC4d60	CGTTAGTGTTgcaGATTTTAAAGGCAAAGTG	TACCTCATTTTCCCAATC
F77A	SDM	NLuc-PC4d60	TGTTCGCGATgctAAAGGCAAAG	CTAACGTACCTCATTTTCC
R86A	SDM	NLuc-PC4d60	AATTGATATTgcaGAATATTGGATGGATC	AGCACTTTGCCTTTAAAATC
W89A	SDM	NLuc-PC4d60	TAGAGAATATgcgATGGATCCTGAAG	ATATCAATTAGCACTTTGC
R100A	SDM	NLuc-PC4d60	GAAACCAGGAgcaAAAGGTATTTCTTTAAATC	ATTTCACCTTCAGGATCC
K101A	SDM	NLuc-PC4d60	ACCAGGAAGAgcaGGTATTTCTTTAAATC	TTCATTTCACCTTCAGGATC
S104A	SDM	NLuc-PC4d60	AAAAGGTATTgcaTTAAATCCAGAACAATG	CTTCCTGGTTTCATTTCAC