Supplementary Information to

The first crystal structures of RNA-PNA duplexes and a PNA-PNA duplex containing mismatches – towards anti-sense therapy against TREDs.

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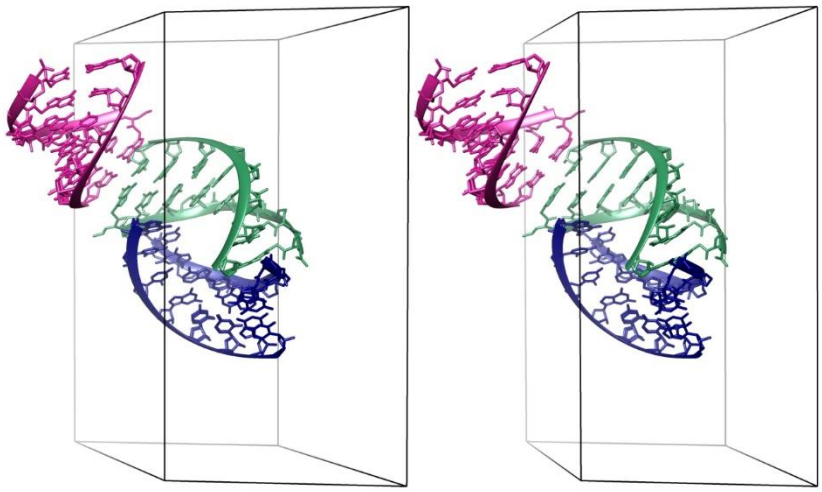
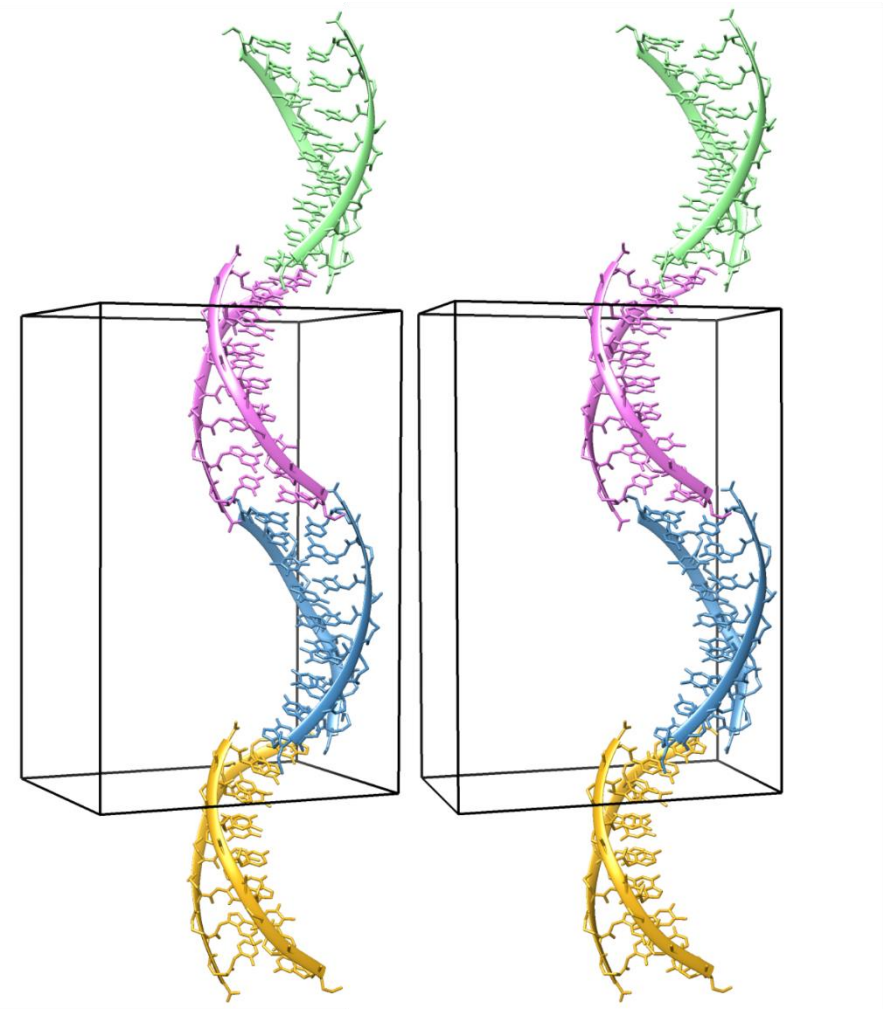
Supplementary Table S1. A comparison of PNA backbone torsion angles in published structures

Helix type	modifications	method	α (°)	β (°)	γ (°)	δ (°)	ε (°)	χ1 (°)	χ2 (°)	χ3 (°)	PDB code	Ref
PNA-PNA	–	X-ray	-108.9 (11.4)* 77.8 (10.1)§	62.3 (7.4)	73.1 (7.7)	92.5 (11.4)	-2.9 (11.6)* 154.5 (10.8)§	2.4 (6.5)	-176.2 (7.7)	79.5 (8.1)	5EMG	This work
PNA-RNA	–	X-ray	-114.3 (6.6) 79.3 (2.7)	70.8 (9.4)	70.4 (8.4)	96.0 (4.6)	-6.1 (9.0) 162.4 (6.8)	4.3 (3.9)	-172.4 (5.5)	83.2 (7.8)	5EME	This work
PNA-RNA	–	X-ray	-111.6 (7.8) 76.5 (2.4)	66.5 (7.4)	73.0 (7.3)	94.4 (5.7)	-5.7 (9.9) 159.2 (7.8)	6.5 (6.2)	-174.4 (4.5)	81.4 (5.0)	5EMF	This work
PNA-RNA	–	NMR	176.9 (24.5)	100.4 (56.4)	116.0 (73.1)	131.5 (82.2)	131.0 (81.8)	-0.9 (19.5)	-155.7 (41.6)	57.0 (23.1)	176D	(1)
PNA-DNA	–	NMR	-112.5 (50.9) 80.9 (30.1)	163.5 (36.9)	132.8 (87.7)	164.5 (56.5)	-24.4 (22.0) 60.3 (32.6)	-2.8 (2.3)	146.1 (42.5)	73.3 (35.0)	1PDT	(2)
PNA-DNA	backbone	X-ray	-112.0 (14.7) 71.7 (6.8)	71.3 (9.5)	67.1 (3.8)	92.3 (8.1)	-0.5 (10.4) 152.3 (6.2)	3.3 (5.6)	-178.5 (3.7)	88.3 (8.9)	1NR8	(3)
PNA-DNA	C-terminus; backbone	X-ray	-39.4 (5.2)	72.1 (7.3)	66.6 (4.1)	88.9 (7.8)	-24.8 (29.2)	5.7 (3.4)	-174.4 (3.7)	92.3 (6.4)	3PA0	(4)
PNA-PNA	–	X-ray	-115.8 (7.5) 82.2 (11.3)	64.3 (5.1)	73.9 (9.1)	96.4 (15.0)	-7.1 (10.4) 151.7 (10.0)	0.2 (14.0)	-174.3 (5.5)	86.8 (6.9)	1PUP	(5)
PNA-PNA	C-terminus	X-ray	-102.4 (20.2) 76.2 (12.9)	74.4 (14.4)	71.2 (8.4)	95.7 (21.3)	-32.4 (39.0) 162.3 (14.0)	-0.9 (5.1)	-171.9 (5.8)	79.0 (6.3)	1QPY	(6)
PNA-PNA	C-terminus; nucleobase	X-ray	-114.4 (9.6) 64.6 (7.0)	73.40 (10.0)	71.8 (5.0)	92.0 (11.0)	-5.0 (16.3) 146.3 (4.0))	3.0 (3.2)	-174.5 (6.3)	85.8 (5.3)	1HZS	(7)
PNA-PNA	C-terminus	X-ray	-110.5 (9.9) 78.2 (26.2)	73.7 (16.2)	73.0 (8.3)	89.6 (12.5)	-10.9 (25.9) 133.1 (30.0)	-1.2 (8.1)	-173.4 (7.6)	97.7 (14.7)	1RRU	(8)
PNA-PNA	C-terminus	X-ray	-112.4 (7.6) 78.1 (2.0)	67.2 (9.1)	69.1 (7.2)	88.5 (8.0)	1.1 (6.3) 154.9 (2.0)	6.8 (3.5)	-178.2 (4.8)	84.3 (5.1)	3MBS	(9)
PNA-PNA	C-terminus; nucleobase	X-ray	-113.6 (8.4) 85.2 (10.9)	65.1 (6.1)	69.9 (5.8)	89.3 (13.1)	5.5 (8.8) 159.4 (12.4)	7.0 (4.0)	-177.6 (3.6)	81.9 (4.5)	3MBU	(9)
PNA-PNA	–	NMR	-116.1 (14.5) 82.1 (2.9)	73.6 (32.0)	90.7 (51.6)	90.9 (23.1)	1.5 (13.7) 158.1 (13.0)	-3.3 (4.4)	-163.3 (17.3)	81.5 (17.5)	2K4G	(10)
PNA-PNA	backbone	NMR	-125.2 (9.8) 62.9 (5.7)	65.5 (14.3)	76.1 (16.5)	99.3 (12.6)	0.6 (13.7) 103.1 (23.3)	1.6 (5.4)	-172.9 (23.8)	93.1 (15.6)	2KVJ	(11)
PNA-PNA-DNA triplex	–	X-ray	-106.3 (8.3)	71.1 (9.8)	68.9 (5.3)	96.2 (24.1)	-2.8 (5.3)	1.2 (1.3)	-171.5 (7.4)	94.8 (9.8)	1PNN	(12)
PNA-PNA tiplex-like	–	X-ray	-100.7 (18.9) 88.1 (26.8)	96.4 (36.5)	77.7 (17.5)	103.7 (28.3)	-18.1 (37.4) 164.4 (12.0)	2.9 (26.5)	179.5 (22.0)	97.1 (19.0)	1XJ9	(13)

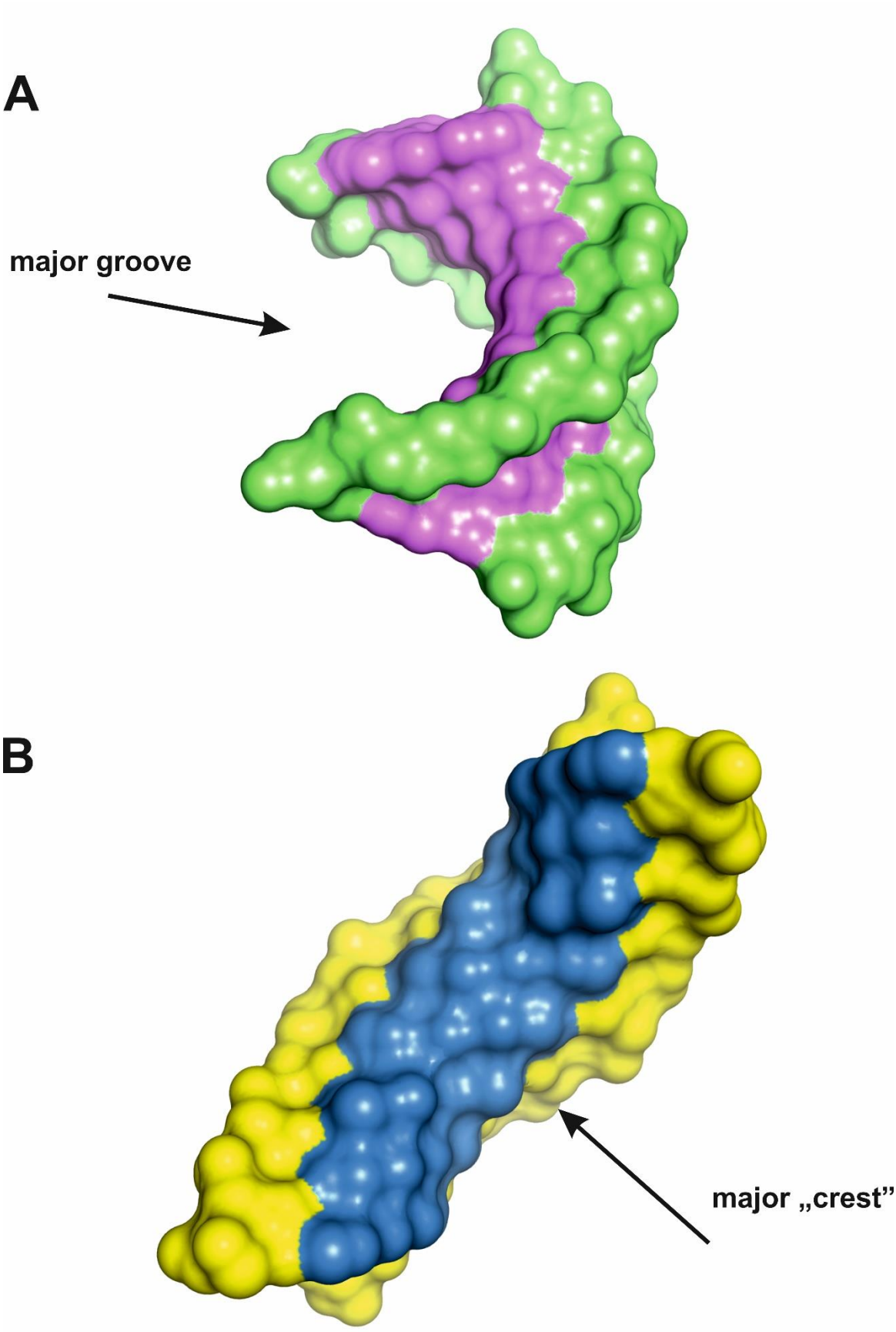
Values for α and ε are calculated according to the peptide bond conformation: * the carbonyl O atom points outwards or § towards the minor groove.



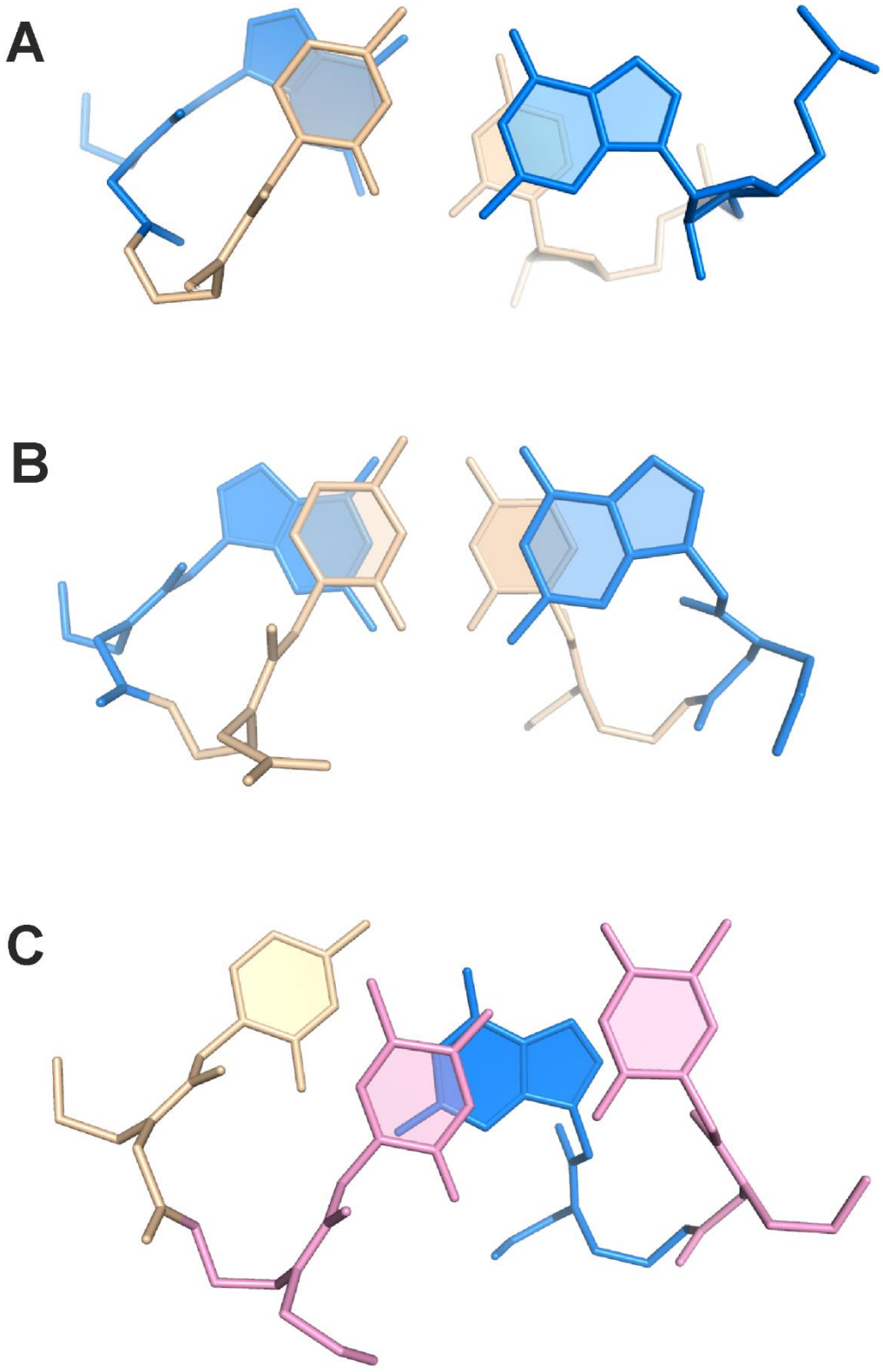
Supplementary Figure S1. Stereo view of packing of RNA-PNA duplexes in the crystal lattice. One end of each duplex makes stacking interactions (seen between the green and violet colored molecules); the other end adjoins the PNA strand of a neighboring molecule (green and navy blue molecules). The unit cell is shown as a black box.



Supplementary Figure S2. Stereo view of the crystal packing of PNA-PNA structure. Right-handed and left-handed PNA-PNA duplexes stack end-to-end to form pseudo-infinite columns. The unit cell is shown as a black box.



Supplementary Figure S3. Surface representations of an RNA-PNA duplex (**A**) and PNA-PNA duplex (**B**), showing in different colors the backbone and base pairs. In PNA-PNA instead of the major groove we observe a crest.



Supplementary Figure S4. Stacking interactions in steps r(GC)/p(GC) (**A**), p(GC)/p(GC) (**B**) and p(CT)/p(TG) (**C**). Guanosine residues are in blue, thymidine residues are pink and cytosine residues yellow.

Supplementary References:

1. Brown, S.C., Thomson, S.A., Veal, J.M. and Davis, D.G. (1994) NMR solution structure of a peptide nucleic acid complexed with RNA. *Science*, **265**, 777-780.
2. Eriksson, M. and Nielsen, P.E. (1996) Solution structure of a peptide nucleic acid-DNA duplex. *Nature structural biology*, **3**, 410-413.
3. Menchise, V., De Simone, G., Tedeschi, T., Corradini, R., Sforza, S., Marchelli, R., Capasso, D., Saviano, M. and Pedone, C. (2003) Insights into peptide nucleic acid (PNA) structural features: the crystal structure of a D-lysine-based chiral PNA-DNA duplex. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 12021-12026.
4. Yeh, J.I., Shivachev, B., Rapireddy, S., Crawford, M.J., Gil, R.R., Du, S., Madrid, M. and Ly, D.H. (2010) Crystal structure of chiral gammaPNA with complementary DNA strand: insights into the stability and specificity of recognition and conformational preorganization. *Journal of the American Chemical Society*, **132**, 10717-10727.
5. Rasmussen, H., Kastrup, J.S., Nielsen, J.N., Nielsen, J.M. and Nielsen, P.E. (1997) Crystal structure of a peptide nucleic acid (PNA) duplex at 1.7 A resolution. *Nature structural biology*, **4**, 98-101.
6. Haaima, G., Rasmussen, H., Schmidt, G., K. Jensen, D., Sandholm Kastrup, J., Wittung Stafshede, P., Norden, B., late) Ole, B. and E. Nielsen, P. (1999) Peptide nucleic acids (PNA) derived from N-(N-methylaminoethyl)glycine. Synthesis, hybridization and structural properties. *New Journal of Chemistry*, **23**, 833-840.
7. Eldrup, Anne B., Nielsen, Bettina B., Haaima, G., Rasmussen, H., Kastrup, Jette S., Christensen, C. and Nielsen, Peter E. (2001) 1,8-Naphthyridin-2(1H)-ones − Novel Bicyclic and Tricyclic Analogues of Thymine in Peptide Nucleic Acids (PNAs). *European Journal of Organic Chemistry*, **2001**, 1781-1790.
8. Rasmussen, H., Liljefors, T., Petersson, B., Nielsen, P.E., Liljefors, T. and Kastrup, J.S. (2004) The influence of a chiral amino acid on the helical handedness of PNA in solution and in crystals. *Journal of biomolecular structure & dynamics*, **21**, 495-502.
9. Yeh, J.I., Pohl, E., Truan, D., He, W., Sheldrick, G.M., Du, S. and Achim, C. (2010) The crystal structure of non-modified and bipyridine-modified PNA duplexes. *Chemistry*, **16**, 11867-11875.
10. He, W., Hatcher, E., Balaeff, A., Beratan, D.N., Gil, R.R., Madrid, M. and Achim, C. (2008) Solution structure of a peptide nucleic acid duplex from NMR data: features and limitations. *Journal of the American Chemical Society*, **130**, 13264-13273.
11. He, W., Crawford, M.J., Rapireddy, S., Madrid, M., Gil, R.R., Ly, D.H. and Achim, C. (2010) The structure of a gamma-modified peptide nucleic acid duplex. *Molecular bioSystems*, **6**, 1619-1629.
12. Betts, L., Josey, J.A., Veal, J.M. and Jordan, S.R. (1995) A nucleic acid triple helix formed by a peptide nucleic acid-DNA complex. *Science*, **270**, 1838-1841.
13. Petersson, B., Nielsen, B.B., Rasmussen, H., Larsen, I.K., Gajhede, M., Nielsen, P.E. and Kastrup, J.S. (2005) Crystal structure of a partly self-complementary peptide nucleic acid (PNA) oligomer showing a duplex-triplex network. *Journal of the American Chemical Society*, **127**, 1424-1430.