Table S1 PNA constructs

PNA	Found	MS Calculated	[§]Purity %
Precursors			
3603	1567	1564	99
3605	1395	1392	99
3791	1370	1367	99
3792	1398	1394	99
3947	1129	1126	99
3948	1276	1273	98
3950	1119	1116	99
3951	1123	1120	95
Templates			
3737	2747	2745	99
3111	2939	2939	99
3956	2768	2770	95
3957	2916	2914	98
3946	2221	2218	98
3949	2384	2381	95
4334	2231	2228	95
4333	2373	2371	98

[§]Purity (%): 98+ corresponds to single peak with slight foot; 95 corresponds to single peak with slight foot and/or shoulder peak

Table S2

Sample no.	PNA no. combination (nomenclature)	No. of bases	PNA sequences		T_m
Pentameric Self-replicating System					
1	3737 (**cd**)+3111 (**ab**)	10+10	Ac-ACTCGGTATC-NH_2	Ac-eg_1-GATACCGAGT-NH_2	76.0
2	3737 (**cd**) +3605 (*a*)	10+5	-do-	H-CGAGT-NH_2	48.1
3	3737 (**cd**) +3603 (*b*)	10+5	-do-	Ac-eg_1-GATAC-OH	44.0
4	3111(**ab**)+3791 (*d*)	10+5	Ac-eg_1-GATACCGAGT-NH_2	H-GTATC-NH_2	40.0
5	3111 (**ab**)+3792 (*c*)	10+5	-do-	Ac-ACTCG-OH	41.0
6	3605 (*a*)+3792 (*c*)	5+5	H-CGAGT-NH_2	Ac-ACTCG-OH	30.0
7	3603 (*b*)+3791 (*d*)	5+5	Ac-eg_1-GATAC-OH	H-GTATC-NH_2	23.0
Tetrameric Self-replicating system					
1	3946 (**mn**)+3949 (**kl**)	8+8	Ac-CTCGGTAT-NH_2	Ac-eg_1-ATACCGAG-NH_2	60.0
2	3946 (**mn**)+3947 (*k*)	8+4	-do-	H-CGAG-NH_2	20.0
3	3946 (**mn**)+3948 (*l*)	8+4	-do-	Ac-eg_1-ATAC-OH	$^+$
4	3949 (**kl**) +3950 (*n*)	8+4	Ac-eg_1-ATACCGAG-NH_2	H-GTAT-NH_2	$^+$
5	3949 (**kl**) +3951 (*m*)	8+4	-do-	H-CTCG-NH_2	$^+$
6	3948 (*l*)+3950 (*n*)	4+4	Ac-eg_1-ATAC-OH	H-GTAT-NH_2	$^+$
7	3947 (*k*)+3951(m)	4+4	H-CGAG-NH_2	H-CTCG-NH_2	$^+$

Table S2 T_m values of smaller precursors in the PNA Self-replication systems.

Duplex concentrations of 1.0 µM in strands were used. All in 50 mM imidazole buffer at pH 7.
$^+$Data could not be evaluated because of incomplete thermal melting curves (too low melting temperature).

Fig. S1

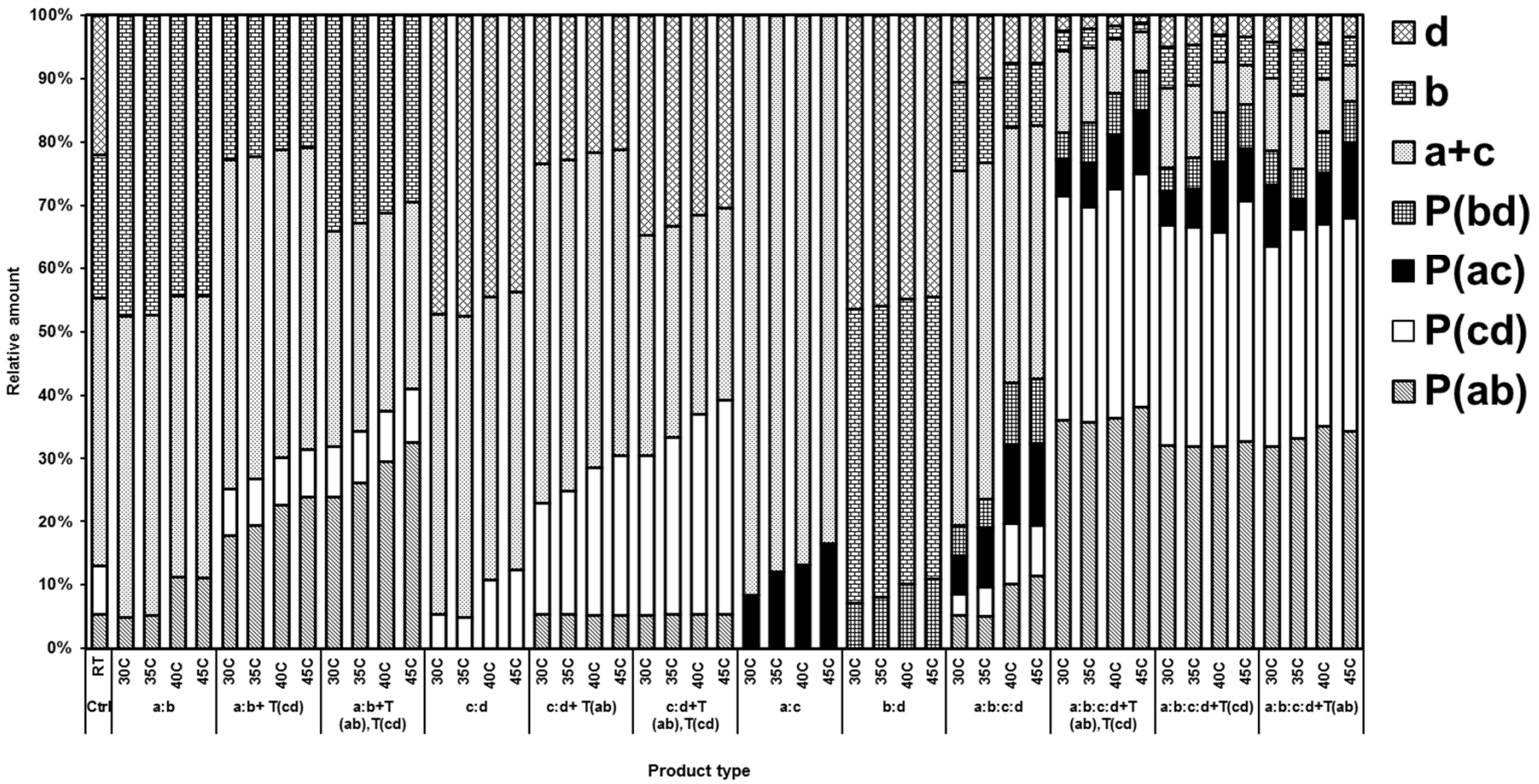

Fig. S1 Matrix experiments demonstrating the effect of temperature on relative amount (%) of formed products in the pentameric PNA cross-catalytic self-replicating system. Reactions were carried out with different combination of two or four PNA precursors (a, b, c and d; concentration 100 μM) both in the absence or presence of one or two chemically synthesized complementary templates ($\mathbf{T_{ab}}$ and $\mathbf{T_{cd}}$; concentration 10 μM) under the same experimental conditions (see Fig. *1B*) at the designated temperature.

Reaction mixture was analyzed through MALDI mass spectrometry and product amounts (%) were calculated related to the internal standard. The experiment should be considered only semi-quantitative as the quantification of the, b, c & d precursors is not as accurate as that of the templates due to their significantly lower mass (around 1000 mw).

Fig. S2

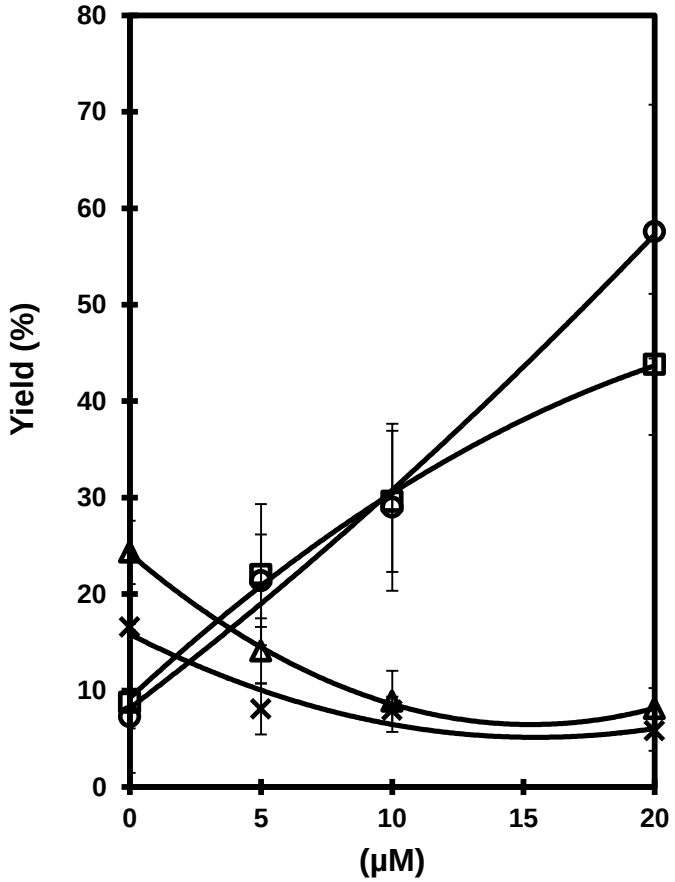

Fig. S2 Template dependence of the product (P_{ab}, P_{cd}, P_{ac} and P_{bd}) **yields in the pentameric PNA, cross-catalytic self- replicating reaction system.** Reactions were carried out with increasing amount (0-20 uM) of both chemically synthesized complementary templates (Tab and Tcd) while fixing the concentration of all four pentameric PNA precursors (a, b, c and d; concentration 100 μM) under the same experimental conditions (see Fig. 1*B*). Reaction mixture was analyzed through MALDI mass spectrometry and the product yields (%) (square) P_{ab}; (circle) P_{cd}; (triangle) P_{ac}; (cross) P_{bd} were calculated related to the internal standard.

Table S3

PNA	mw	5µM	10µM	20µM	40µM
T_{cd}	2744	50	55	44	43
T_{ac}	2768	18	25	12	9.7
T_{bd}	2911	11	3	8	6.2
T_{ab}	2938	32	41	30	28
IS	4126	44	33	8.3	3.3

Table S3. Measured arbitrary mass ion current intensities of PNA templates. Each column represents an experiment containing a mixture of the four template each at the concentration indicated (5, 10, 20 or 40 µM) and a constant concentration of the internal standard (IS).

Table S4

PNA	mw	5µM	10µM	20µM	40µM
T_{cd}	2744	1.14	1.67	5.30	13.03
T_{ac}	2911	0.41	0.76	1.45	2.94
T_{bd}	2768	0.25	0.09	0.96	1.88
T_{ab}	2938	0.73	1.24	3.61	8.48
IS	4126	1	1	1	1

Table S4. Normalization of the ion current data shown in Table S3 relative to the internal standard (IS).

Fig. S3.

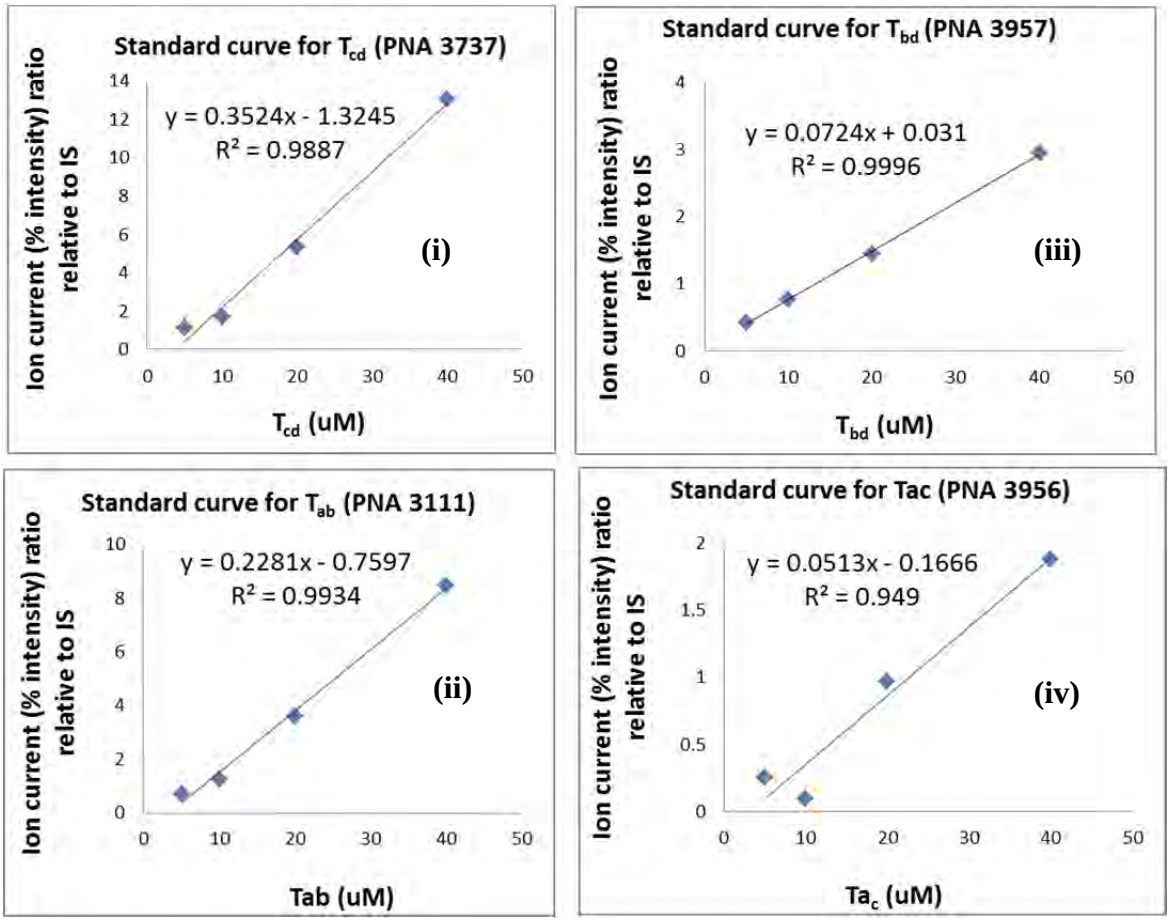

Fig. S3. Standard calibration curve for each template/product (i) $T_{cd,}$ (ii) T_{ab}, (iii) T_{bd} (iv) T_{ac} generated from the data in Table S4.

Table S5

Tab	Time (min)					
	0	10	20	30	60	90
(A)0µM	0	0.00	0.10	0.37	0.69	0.80
(B)5µM	0	0.02	0.59	1.27	2.42	2.78
(C)10µM	0	0.00	2.64	2.64	4.22	4.44
(D)20µM	0	0.31	2.75	3.47	4.46	6.54

Table S5. Example of data from one of the kinetics experiments behind the results of Figure 2A. Ion current (% intensity) for product Pab (PNA3111) relative to the internal standard (IS) are reported in the table at the different time points (0, 10, 20, 30, 60, and 90 min) and using different starting concentration of the template PNA3111 (0, 5, 10, 20 & 20 µM)). The intensity corresponding the starting concentration (at 0 min) of the PNA has been subtracted.

Table S6

T_{ab}	Time (min)					
	0	10	20	30	60	90
(A)0µM	0	0	3.75	4.95	6.34	6.84
(B)5µM	0	3.43	5.93	8.88	13.96	15.51
(C)10µM	0	0	14.90	14.90	21.84	22.82
(D)20µM	0	4.68	15.38	18.56	22.886	32.02

Table S6. Using the results from the standard curve presented in Figure S3 (ii) the data in Table S5 was converted to concentration.

Fig. S4.

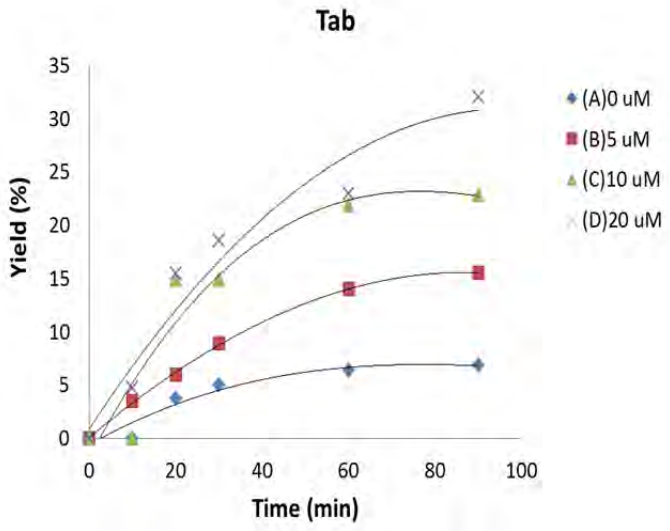

Fig. S4. Graphical plot of the data from Table S6.