Supporting Informations

Calculation of the melting temperature

The melting temperature for the staple strands was calculated by the thermodynamic standard term T_m[°C] = 69.3 +0.41x GC(%) -650/b (S1: red); the term T_m = 81.5 + 16.6 log10 [J+] + 0.41*GC(%) - 500/b (S1: purple) 22-24 and by the nearest neighbor term T_m = [(1000 *dH) /(A + dS + R*ln (C/4))] – 273.15 + 16.6 * log c (K+)25 (S1: green). Depending on the model for the calculation the melting temperatures vary in their position. The thermodynamic standard term[22-24] with an assumed ionic strength of 37.5 mM fits the observed temperature range for the folding of the rectangle roughly. Nether the less the calculations always show a wide spread of the melting temperatures for the different staple strands. Decreasing this temperature spread should lead to a higher folding yield at an optimal temperature and folding process below this temperature.

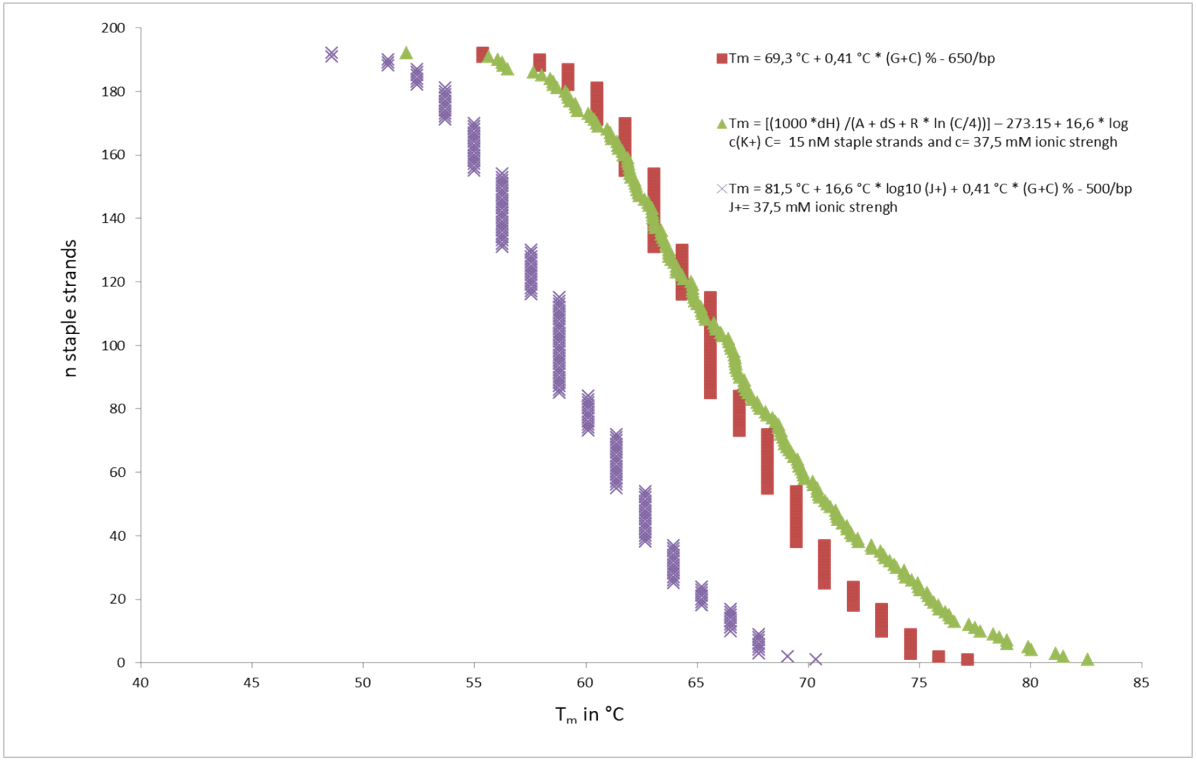

Fig. S1. Plot of the calculated melting temperatures of the staple strands with different methods.

Results for temperature gradient folding

The rectangles were folded with a temperature ramp with a cooling rate of 1 K/min to RT with different starting temperatures (S2). Decreasing the starting temperature also results in a shorter process time, if the cooling rate is constant. Nether the less it was observed that 50% of the rectangles were well shaped with at starting temperature of 50°C. Below this temperature no well-formed rectangles have been observed.

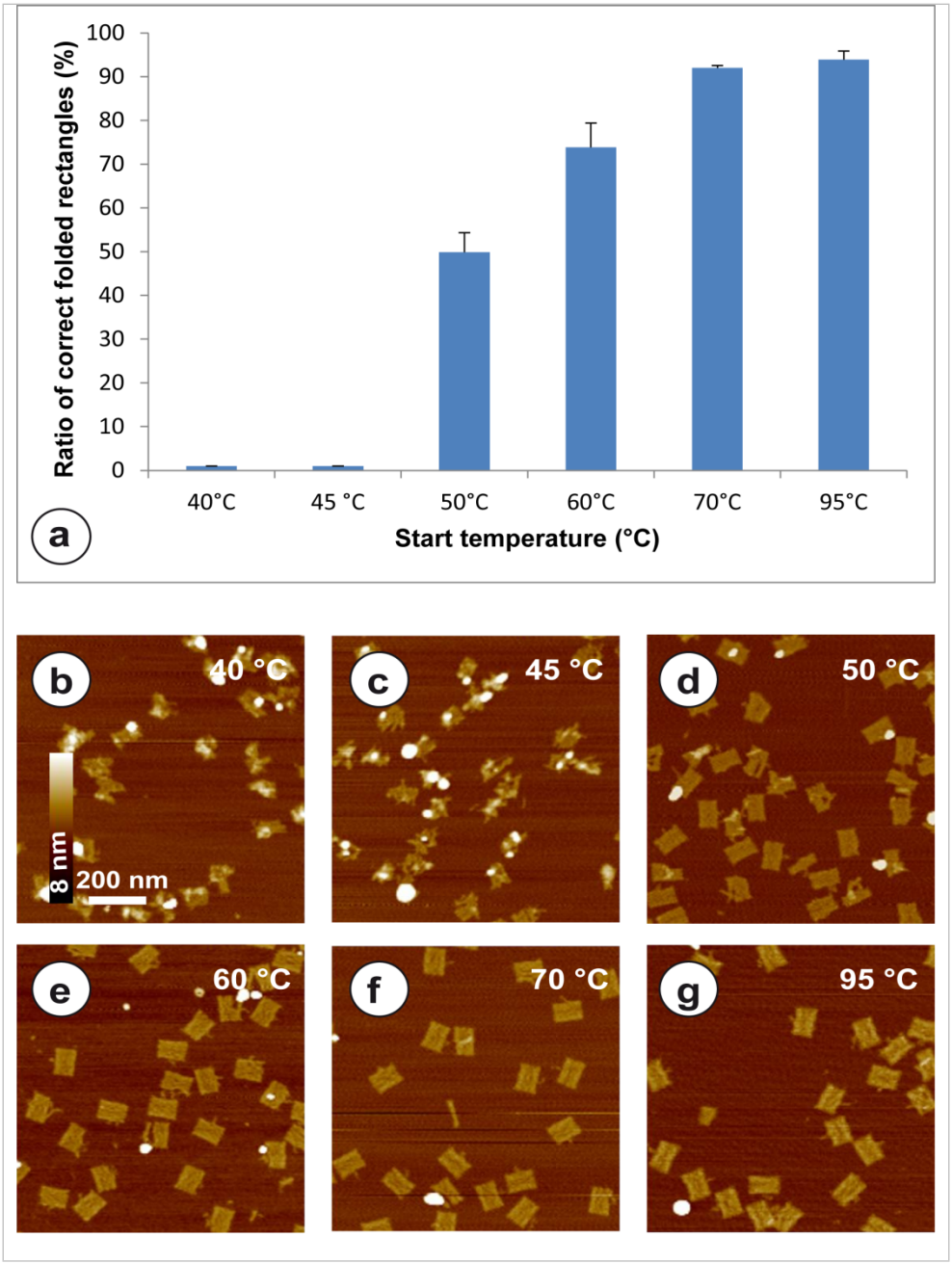

Fig. S2. Folding efficiency of the DNA rectangles with a constant cooling rate of 1 K/min and different starting temperatures without preceding denaturation.

For starting temperatures below 50°C, the hybridization time of 70 min was kept constant resulting in smaller cooling rates (0.28 K or 0.21 per minute). In contrast to the results before a few rectangles were observed for 45°C but not for 40°C (S3).

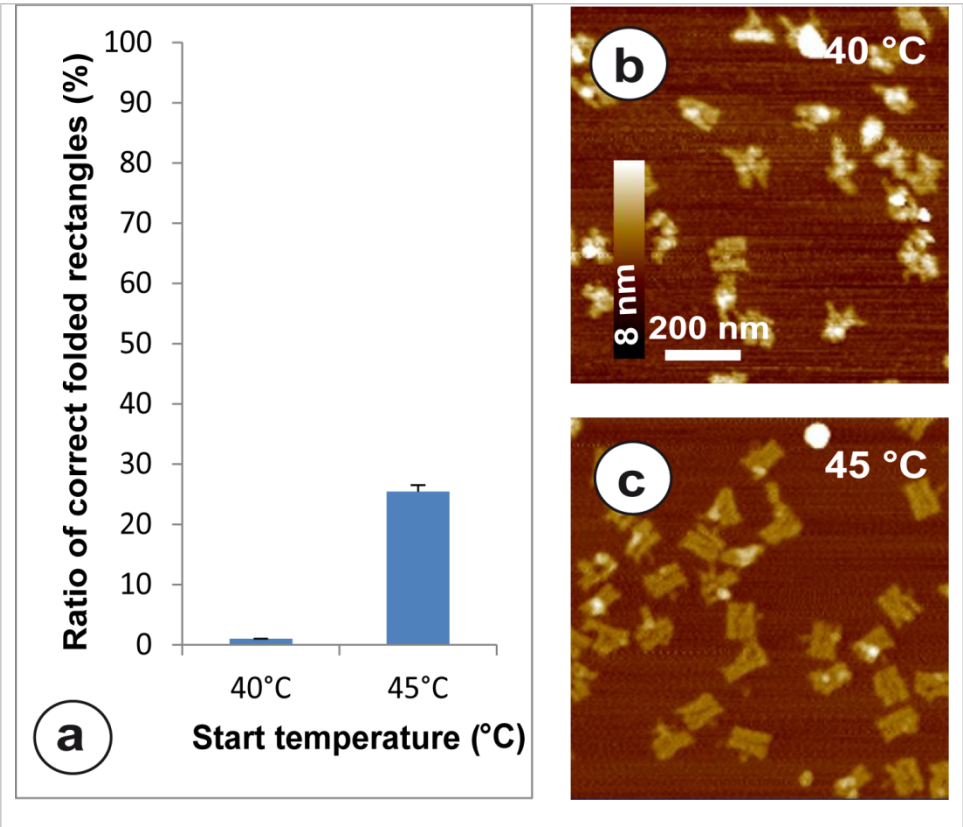

Fig. S3. Folding efficiency of the DNA rectangles with a constant hybridization time of 70 min with a cooling rate of 0.28 K/min for 45 °C and 0.21 K/min for 40 °C without preceding denaturation.

Results for isothermal folding

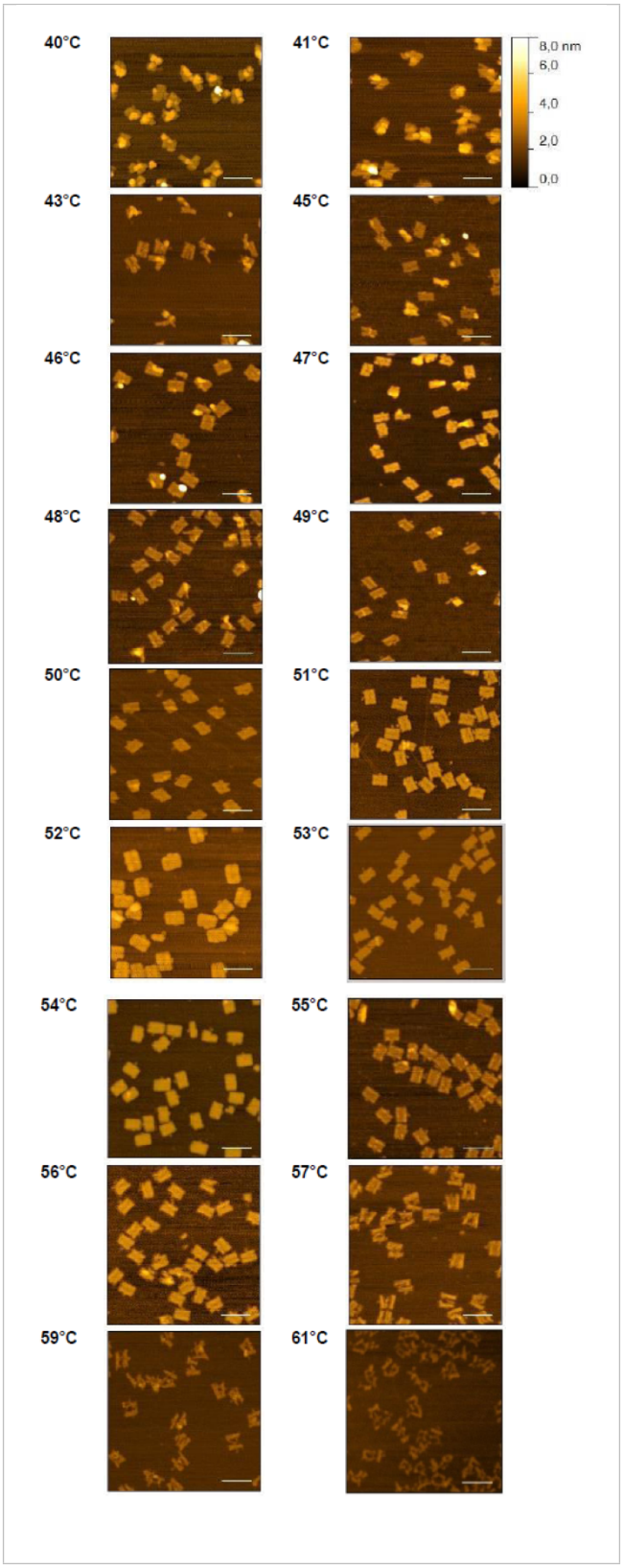

Fig. S4. AFM images of 2D origamis for 1h isothermal folding at the stated temperature (scale bare 200 nm).

Folding using betaine

Some rectangles could be also observed for a folding protocol using 1 M betaine at 37°C with the hybridization time reduced to 1 h. The results vary too much to evaluate this protocol sufficiently to be more than a proof of principle.

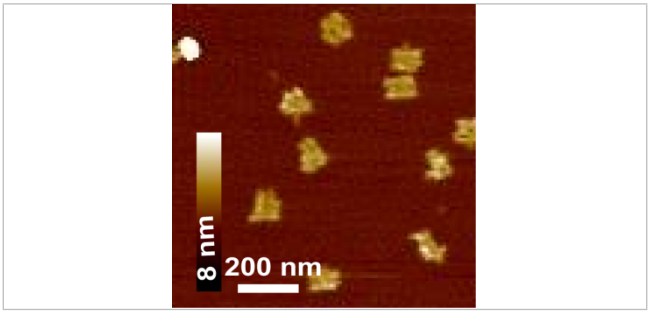

Fig. S5. Folding results using betaine (37°C, 1 h).

The positive effect of betaine for the folding process was also demonstrated for another 2D DNA structure, the DNA Smiley from Rothemund(2006)

The protocol using 1 M betaine at 37° C with the hybridization time of 1 day

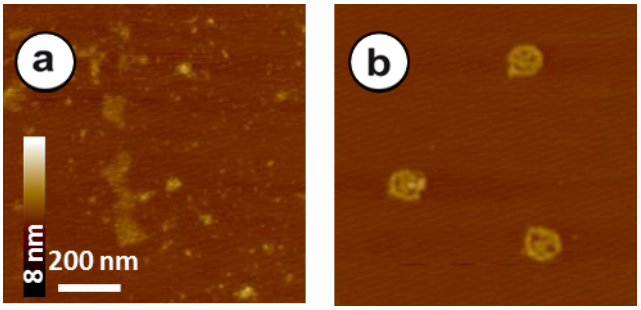

Fig. S6. Effect of the betaine on DNA origami folding efficiency . No thermal denaturing was applied. Temperature (37°C) and time (1 d), the control sample without betaine (a) shows only a low efficiency, which is significantly improved by the addition of betaine (1 M) (b).