Appendix B

Supplementary information for Chapter 3

B.1 Catalytic Dicer substrate formation mechanism based on 5′-toehold hairpins

Prior to designing the conditional Dicer substrate formation mechanism presented in Chapter 2, several mechanisms using a 5′-toehold based hairpins were explored. Figure B.1(a) depicts a schematic representation of such a mechanism (M1). *In vitro* Dicer cleavage assays of strands corresponding to mechanism M1 demonstrate that hairpin C, as well as complexes X_{short}·A and X_{short}·A·B, get degraded by Dicer (Figure B.1(b), no Dicer '−' lanes vs. Dicer '+' lanes). Some degradation is also observed for hairpins A and B. Using 2′-OMe chemical modifications (hairpins A2, B2 and C2) abrogate this pattern such that only the final product B2·C2 is processed (Figure B.1(c), no Dicer '−' lanes vs. Dicer '+' lanes). Using a mechanism with hairpins that is based on 5′-toeholds results in premature exposure of the antisense strand of the siRNA. In complex X_{short}·A·B a single-stranded region of hairpin B is exposed containing the domains 'z*-y*-x*' which comprise the antisense (Figure B.1(a)); this might affect the performance of the mechanism. For this reason, this current version of a 5′-toehold mechanism is not suitable for *in vivo* application.

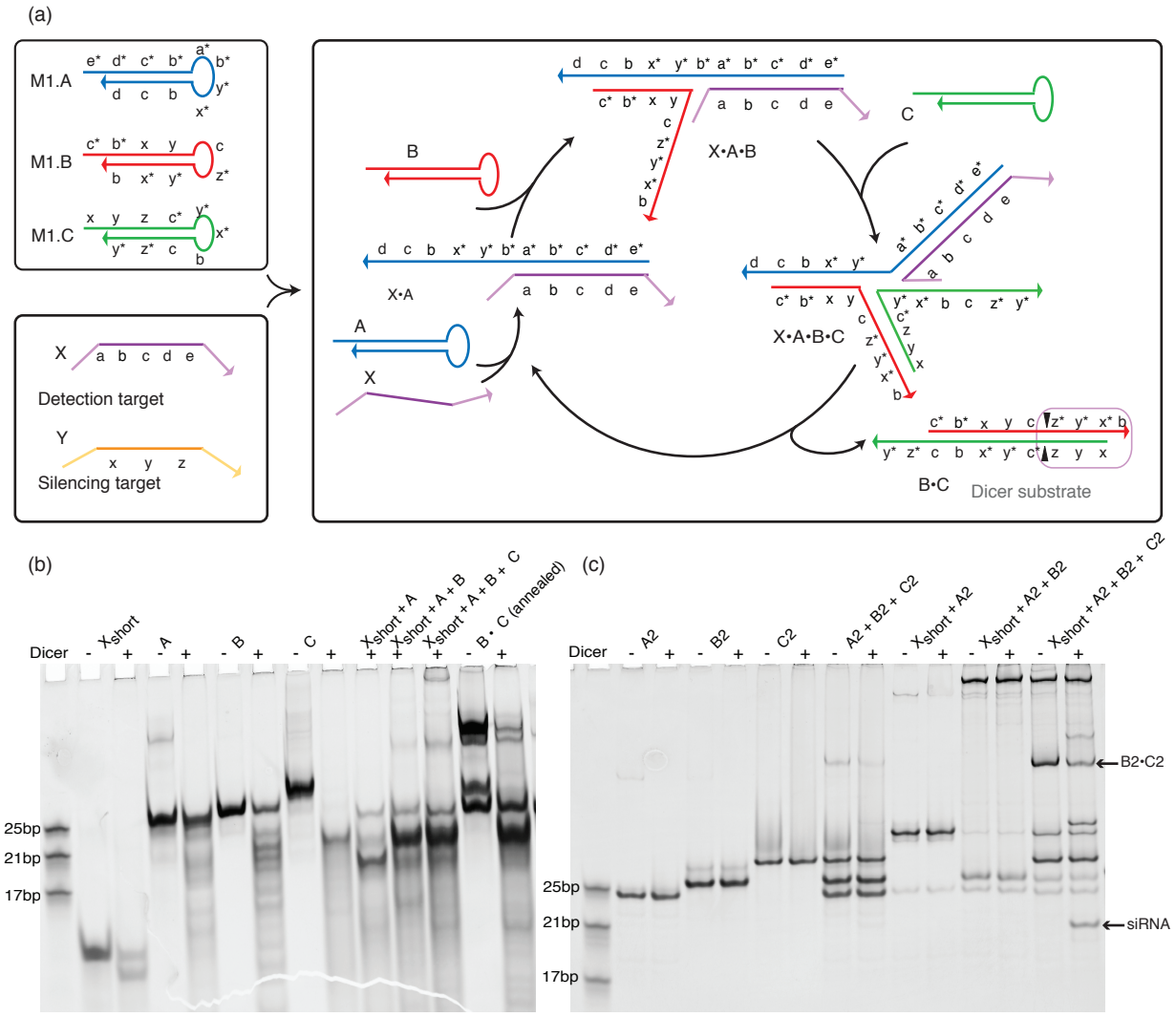

Figure B.1: **Catalytic Dicer substrate formation mechanism based on 5′-toehold hairpins (M1).** (a) Schematic representation of 5′-toehold hairpin based mechanism. (b) *In vitro* Dicer cleavage assay of M1 mechanism using Dicer block it kit (Invitrogen). Dicer reaction was carried out according to the manufacturer. (c) 2′-OMe chemical modifications of M1 mechanism abrogate unwanted Dicer cleavage. Dicer reaction was carried out as described in the Materials and methods section of Chapter 2. For a list of unmodified M1 sequences refer to the materials and methods section of Chapter 3. 2′-OMe-modified sequences are listed in Table B.1

Strand	Sequence
M1.A2	CUCGAUCUCGAACUCGUGGCUGGUCAGCUUGCCGUACACGA GUUCG
M1.B2	CGAACUCGUGUACGGCAAGCUGACCGAGACUUCAGGGUCAG CUUGCCGUACA
M1.C2	UACGGCAAGCUGACCCUGAAGUCUCGGUCAGCUUGCCGUAC ACGAGACUUCAGGGUCAGC

Table B.1: List of M1 sequences modified with 2′-OMe. 2′-OMe modifications are underlined.