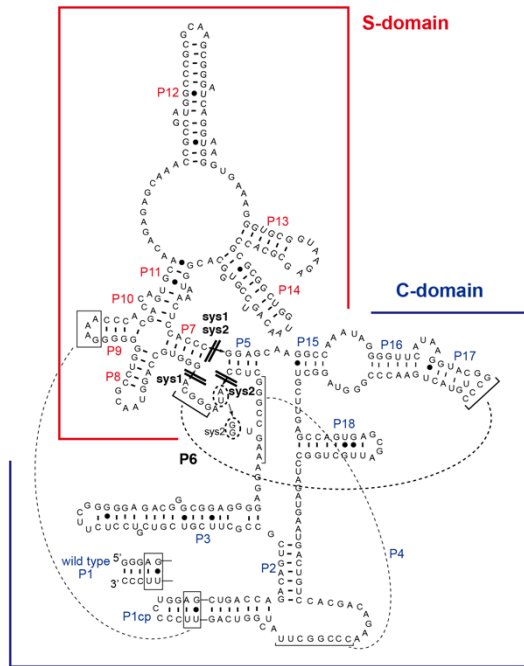


A *E. coli* RNase P ribozyme



B *B. subtilis* RNase P ribozyme

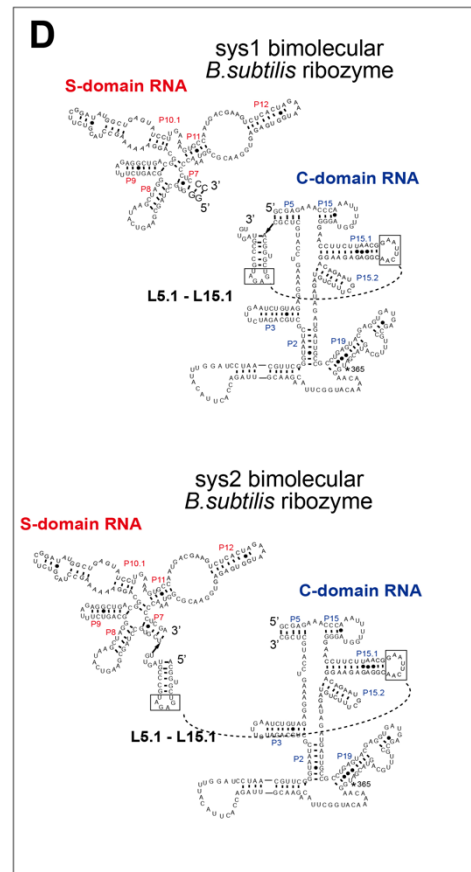
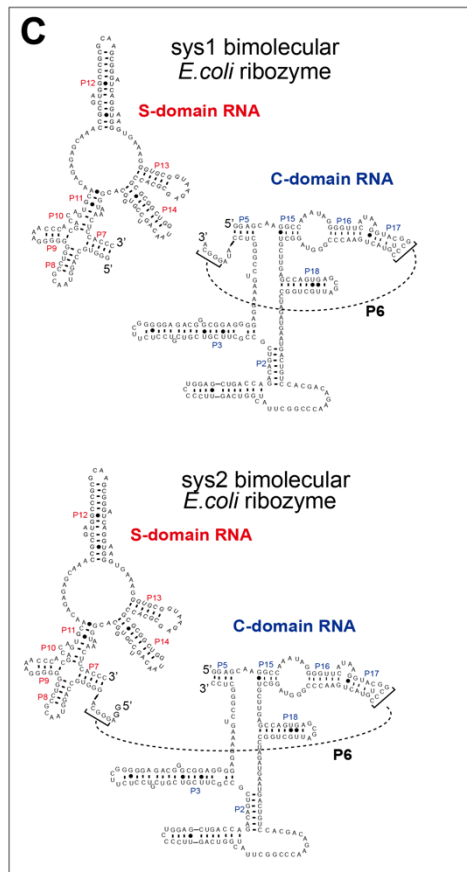
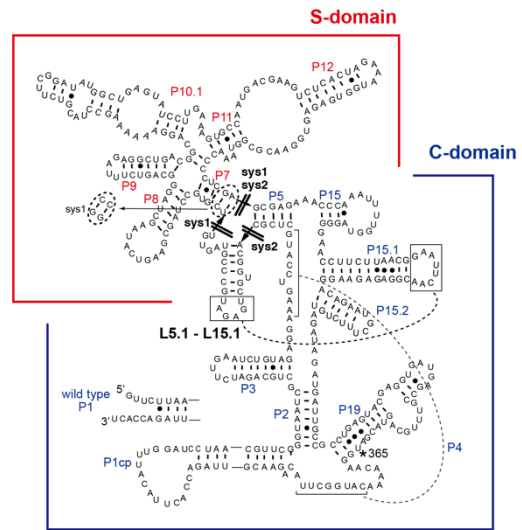


Figure S1

Secondary structures of *E. coli* and *B. subtilis* RNase P ribozymes.

(A, B) Nucleotide sequences and secondary structures of the *E. coli* (A) and *B. subtilis* (B) RNase P ribozymes with circular permutation.

(C) The sys-1 (top) and sys-2 (bottom) bimolecular forms of the *E. coli* RNase P ribozyme.

(D) The sys-1 (top) and sys-2 (bottom) bimolecular forms of the *B. subtilis* RNase P ribozyme.

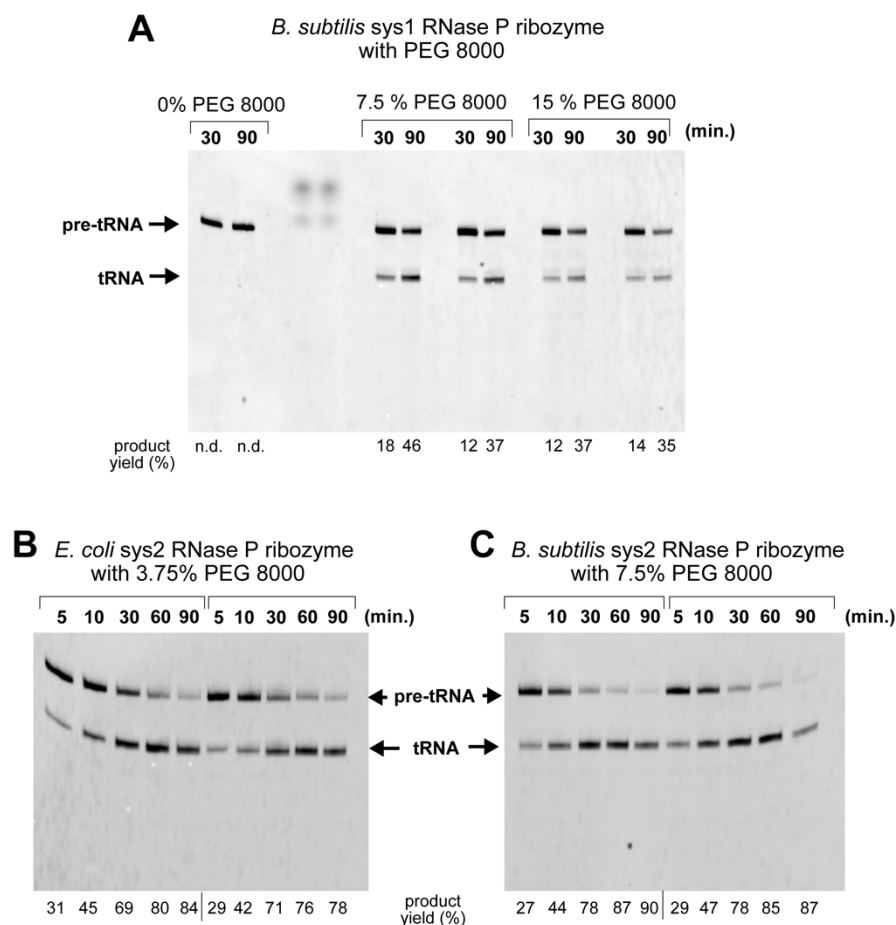
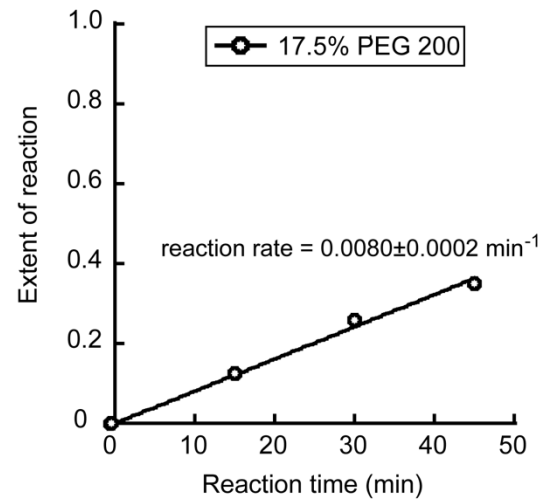
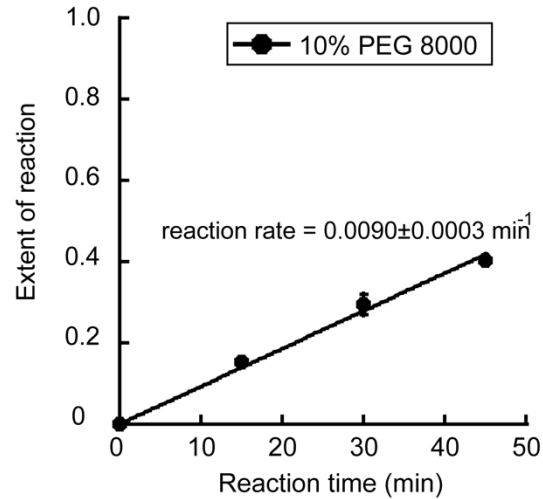


Figure S2

Typical electrophoresis gels to analyze pre-tRNA cleavage reactions catalyzed by bimolecular RNase P ribozymes.

- (A) A denaturing polyacrylamide gel to analyze effects of PEG 8000 on pre-tRNA cleavage reaction catalyzed by the sys1 *B. subtilis* bimolecular RNase P ribozyme. These data were used to prepare figure 2B.
- (B) A denaturing polyacrylamide gel to analyze pre-tRNA cleavage reaction catalyzed by the sys2 *E. coli* bimolecular RNase P ribozyme in the presence of 3.75% PEG 8000. These data were used to prepare figures 4C and S4B.
- (C) A denaturing polyacrylamide gel to analyze pre-tRNA cleavage reaction catalyzed by the sys2 *B. subtilis* bimolecular RNase P ribozyme in the presence of 7.5% PEG 8000. These data were used to prepare figures 5C and S5B.

A*B. subtilis* sys1 RNase P ribozyme with 17.5 % PEG 200**B***B. subtilis* sys1 RNase P ribozyme with 10 % PEG 8000**Figure S3**

Time courses of pre-tRNA cleavage reaction catalyzed by the sys1 *B. subtilis* bimolecular RNase P ribozyme.

- (A) Time course of pre-tRNA cleavage reaction catalyzed by the sys1 *B. subtilis* bimolecular RNase P ribozyme in the presence of 17.5% PEG 200.
- (B) Time course of pre-tRNA cleavage reaction catalyzed by the sys1 *B. subtilis* bimolecular RNase P ribozyme in the presence of 10% PEG 8000.

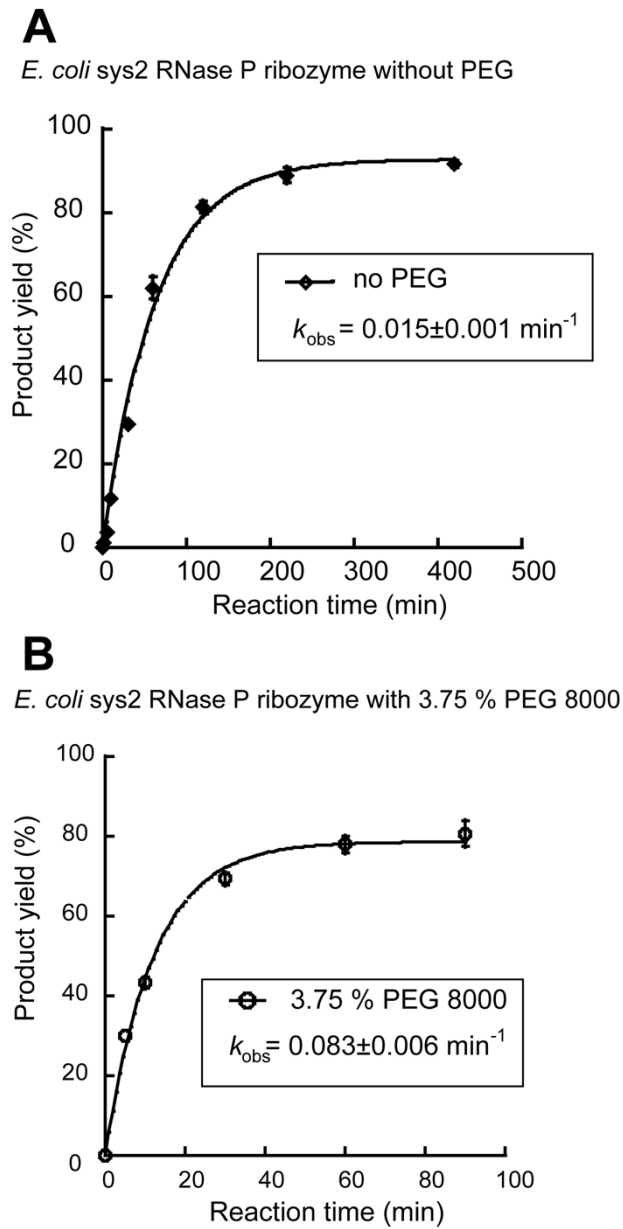
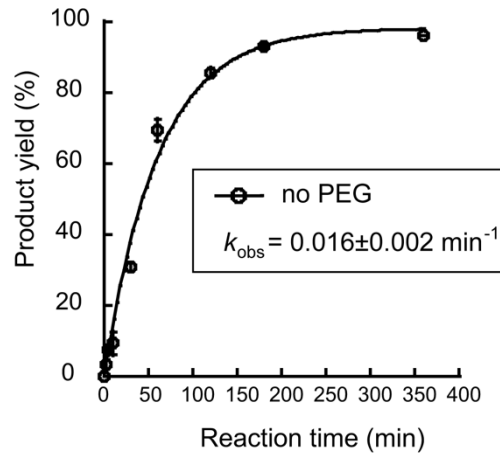
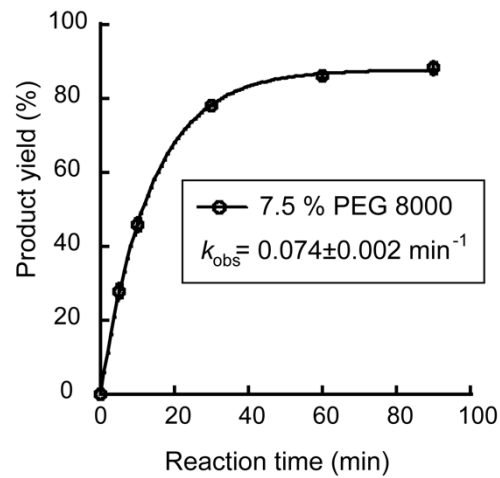


Figure S4

Time courses of pre-tRNA cleavage reaction catalyzed by the sys2 *E. coli* bimolecular RNase P ribozyme.

- (A) Time course of pre-tRNA cleavage reaction catalyzed by the sys2 *E. coli* bimolecular RNase P ribozyme in the absence of PEG.
- (B) Time course of pre-tRNA cleavage reaction catalyzed by the sys1 *E. coli* bimolecular RNase P ribozyme in the presence of 3.75% PEG 8000.

A*B. subtilis* sys2 RNase P ribozyme without PEG**B***B. subtilis* sys2 RNase P ribozyme with 7.5 % PEG 8000**Figure S5**

Time courses of pre-tRNA cleavage reaction catalyzed by the sys2 *B. subtilis* bimolecular RNase P ribozyme.

- (A) Time course of pre-tRNA cleavage reaction catalyzed by the sys2 *B. subtilis* bimolecular RNase P ribozyme in the absence of PEG.
- (B) Time course of pre-tRNA cleavage reaction catalyzed by the sys2 *B. subtilis* bimolecular RNase P ribozyme in the presence of 7.5% PEG 8000.

Table S1 Rate constants of the reactions with the sys2 *E. coli* ribozyme.

Ribozyme	PEG (w/v %)	k_{obs} (min⁻¹)	calculated final product yield (%)
sys2 <i>E. coli</i>	PEG 200 (10%)	0.15 ± 0.014	89
sys2 <i>E. coli</i>	PEG 8000 (3.75%)	0.083 ± 0.007	78
sys2 <i>E. coli</i>	no PEG	0.015 ± 0.001	92

Table S2 Rate constants of the reactions with the sys2 *B. subtilis* ribozyme.

Ribozyme	PEG (w/v %)	k_{obs} (min⁻¹)	calculated final product yield (%)
sys2 <i>B. subtilis</i>	PEG 200 (12.5%)	0.22 ± 0.03	88
sys2 <i>B. subtilis</i>	PEG 8000 (7.5%)	0.074 ± 0.002	87
sys2 <i>B. subtilis</i>	no PEG	0.016 ± 0.002	98