Supplementary Figure legends

S1: Time courses of GFP synthesis reaction with the IVTT used in this study. (A) Time courses of the reaction at 37°C with 0, 0.05, 0.1, 0.2, 0.4, 0.6, and 1 nM DNA encoding GFP. (B) Relationship between rate of the fluorescence increase (correlated with the GFP synthesis rate) and DNA concentration at indicated time points. A linear relationship was seen regardless of the time point. GFP synthesis was carried out using the PCR product amplified using pET-GFPuv5 (34) as a template and T7F and T7R primers. PCR was carried out essentially as described for GAL and GUS.

S2: Time courses of (A) GAL, (B) GAL-snap, (C) GUS, and (D) GUS-snap synthesis reactions with three different DNA concentrations (0.3, 0.1, 0.03 nM). Fluorescence signals began to be seen earlier with higher DNA concentrations. While GAL and GAL-snap showed nearly identical time courses, the difference between GUS and GUS-snap was not negligible. The difference may result in up to 40-fold higher k_2 or 6.3 (= $40^{0.5}$)-fold higher k_1 with GUS compared to GUS-snap. Below, we describe the mathematical basis of these conclusions.

Assuming that GUS synthesis reaction is a fourth-order reaction as described in eq. 4, and defining the time required for the fluorescence signal to reach a value of $F_{\text{threshold}}$ as τ, the relationship between [*DNA*] and τ is written as:

$$Log(\tau) = -\frac{Log(A/F_{threshold})}{12} - \frac{4}{12}Log([DNA])$$

where A is the product of rate constants (see eq. 4). This equation indicates that Log(τ) and Log[*DNA*] have a linear relationship with a slope of –1/3, which we indeed saw with both GUS and GUS-snap. The plot in (E) shows the relationship between Log(τ) and Log[*DNA*] with $F_{\text{threshold}} = 0.1$, and the line shows the fit with a slope of –1/3. The difference in GUS and GUS-snap is the intercept, which was estimated to be 0.13. Thus, the difference between GUS and GUS-snap is only in A, and A is different by $10^{0.13\times 12} \approx 40$-fold. As $A = \frac{k_{cat}k_2k_1^{\,2}k_{t2}^{\,4}k_{t1}^{\,4}}{2\cdot 2\cdot 5\cdot 2\cdot 2\cdot 5\cdot 11\cdot 12}$, the difference in A may be due to k_{t2} or k_{t1}, but when assuming that these are not affected, it results in up to 40-fold higher k_2 or 6.3 (= $40^{0.5}$)-fold higher k_1.

S3: Kinetic analyses of GAL, GAL-snap, GUS, and GUS-snap. Reaction velocity was measured with different substrate concentrations. By fitting the data to the Michaelis-Menten equation, we obtained V_{max} of 0.028, 0.020, 0.086, and 0.077 (min^{-1}) and K_M of 16.4, 15.7, 8.4, and 5.8 μM for GAL, GAL-snap, GUS, and GUS-snap, respectively. To determine the enzymatic rate constants, four enzymes (GAL, GUS, GAL-snap, and GUS-snap) were overproduced using *E. coli* BL21 (DE3) strain and purified by immobilized metal affinity and size exclusion chromatography. Purity was confirmed by SDS-PAGE. Enzyme activity was measured in 70S buffer containing 0.1% BSA and TG-bgal or TG-GlcU at 37°C.

S4: Oligomerization state of GAL-snap and GUS-snap determined by size exclusion chromatography. (A) Analysis of molecular weight standard (purchased from BioRad). Correlation between molecular weight and elution volume of thyroglobulin (670 kDa), bovine γ-globulin (158 kDa), chicken ovalbumin (44 kDa), and equine myoglobin (17 kDa). Detection was performed with excitation and emission wavelengths of 275 and 330 nm, respectively. (B) Estimated and theoretical molecular weights of monomer, dimer, and tetramer of GAL-snap and GUS-snap. (C) Correlation between the concentrations of tetrameric GAL-snap and GUS-snap labeled with Alexa Fluor 488, and the peak height obtained by size exclusion chromatography.

S5: RNase treatment of *in vitro* synthesized GAL-snap and GUS-snap showed that molecules

appearing at the void volume (Vo) were the ribosome-nascent chain complex. Chromatograms of (A,B) GAL-snap and (C,D) GUS-snap detected by (A,C) fluorescence of Alexa Fluor 488 or (B,D) absorbance at 260 nm before and after adding RNase (10 units/mL), and incubated at 37°C for 30 min.

Reference

34. Ito, Y., Suzuki, M., and Husimi, Y. (1999) *Biochem. Biophys. Res. Commun.* **264**, 556-560

Figure S1

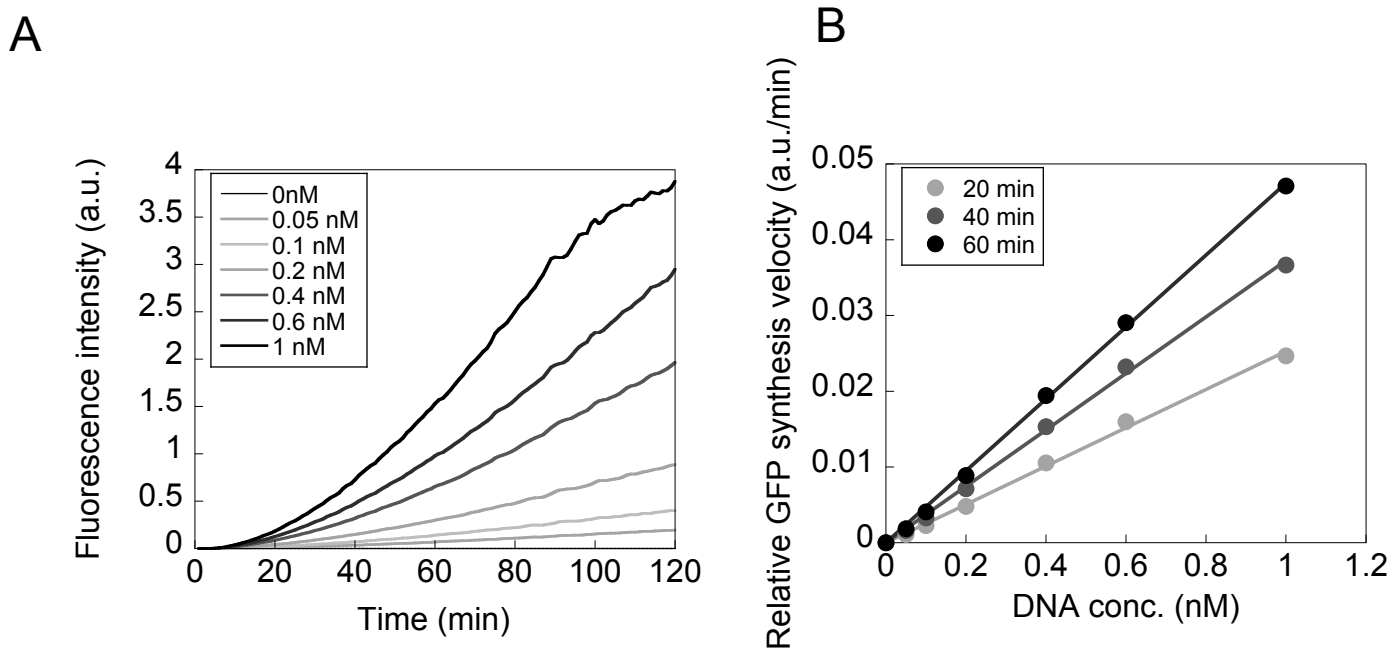

A
Fluorescence intensity (a.u.)
Time (min)
0nM
0.05 nM
0.1 nM
0.2 nM
0.4 nM
0.6 nM
1 nM
0
0.5
1
1.5
2
2.5
3
3.5
4
0
20
40
60
80
100
120
B
Relative GFP synthesis velocity (a.u./min)
DNA conc. (nM)
20 min
40 min
60 min
0
0.01
0.02
0.03
0.04
0.05
0
0.2
0.4
0.6
0.8
1
1.2

Figure S2

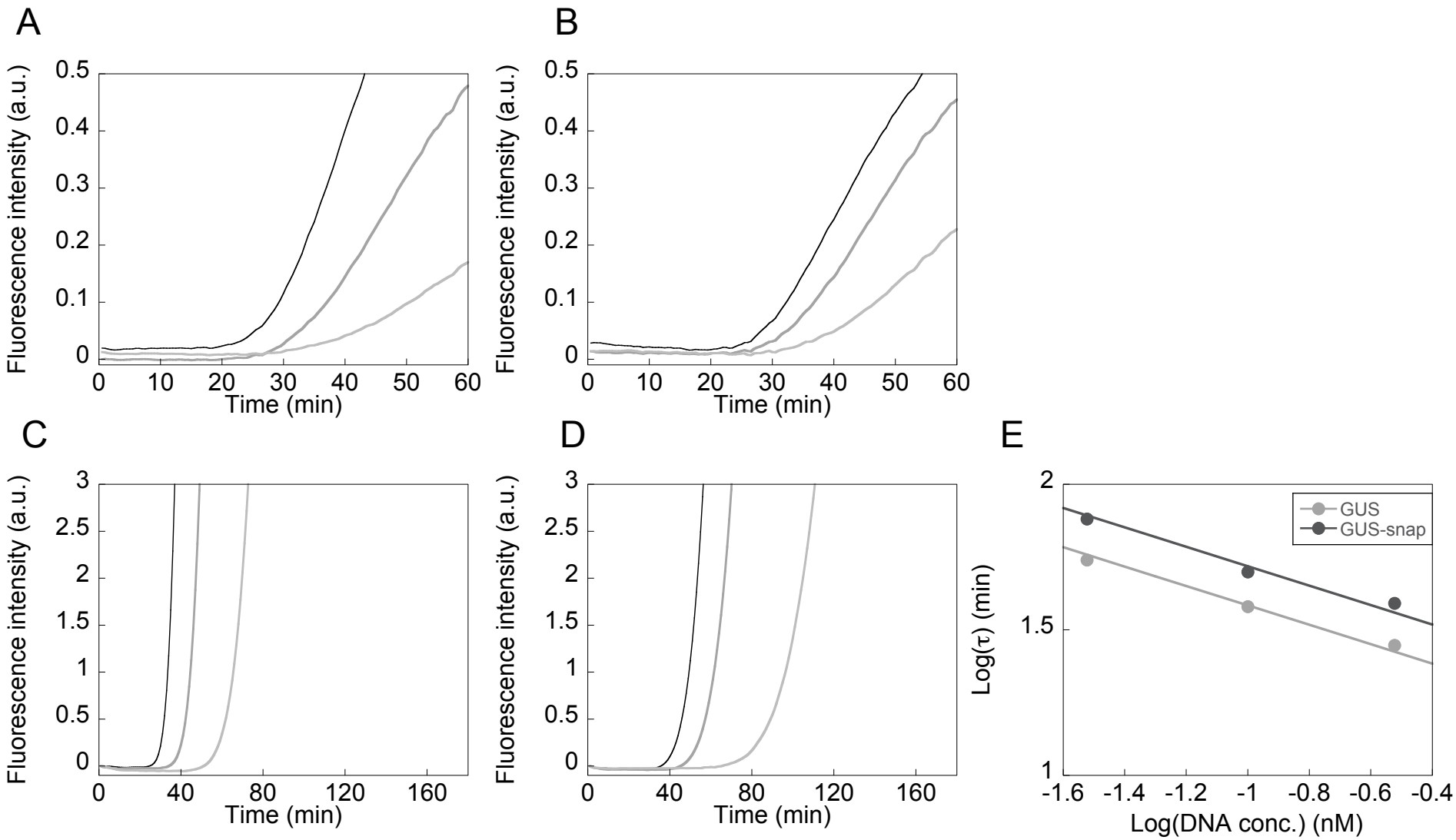

A
B
C
D
E
Fluorescence intensity (a.u.)
Time (min)
Log(τ) (min)
Log(DNA conc.) (nM)
GUS
GUS-snap

Figure S3

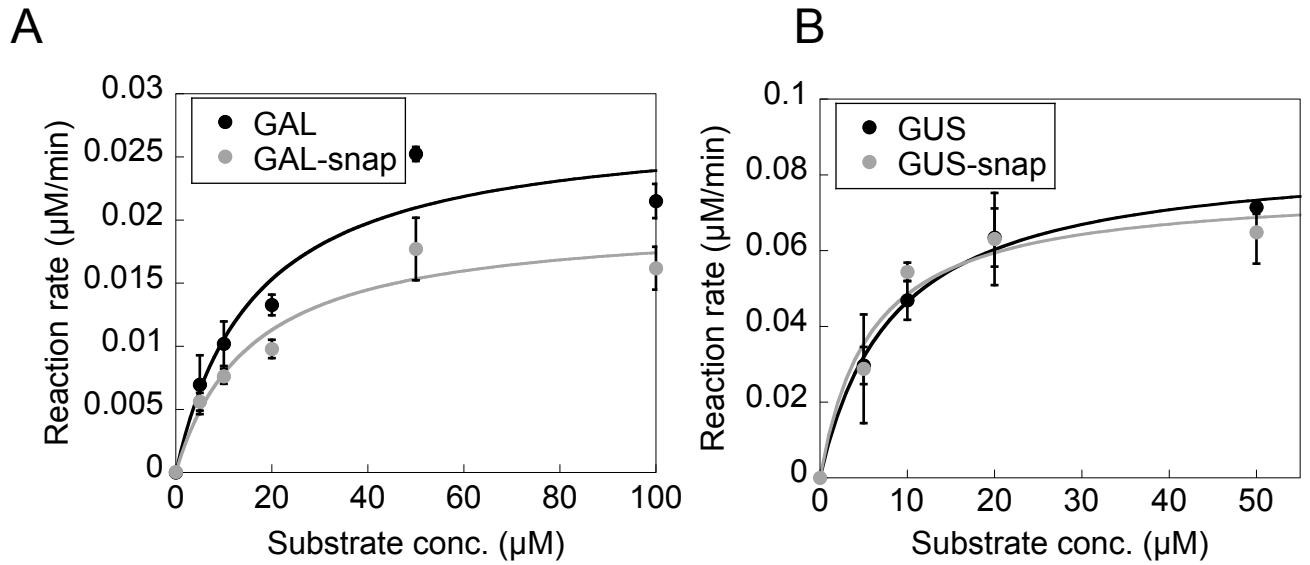

A
B
GAL
GAL-snap
GUS
GUS-snap
Reaction rate (μM/min)
Substrate conc. (μM)
0
0.005
0.01
0.015
0.02
0.025
0.03
0.04
0.06
0.08
0.1
10
30
40
50
60
80
100

Figure S4

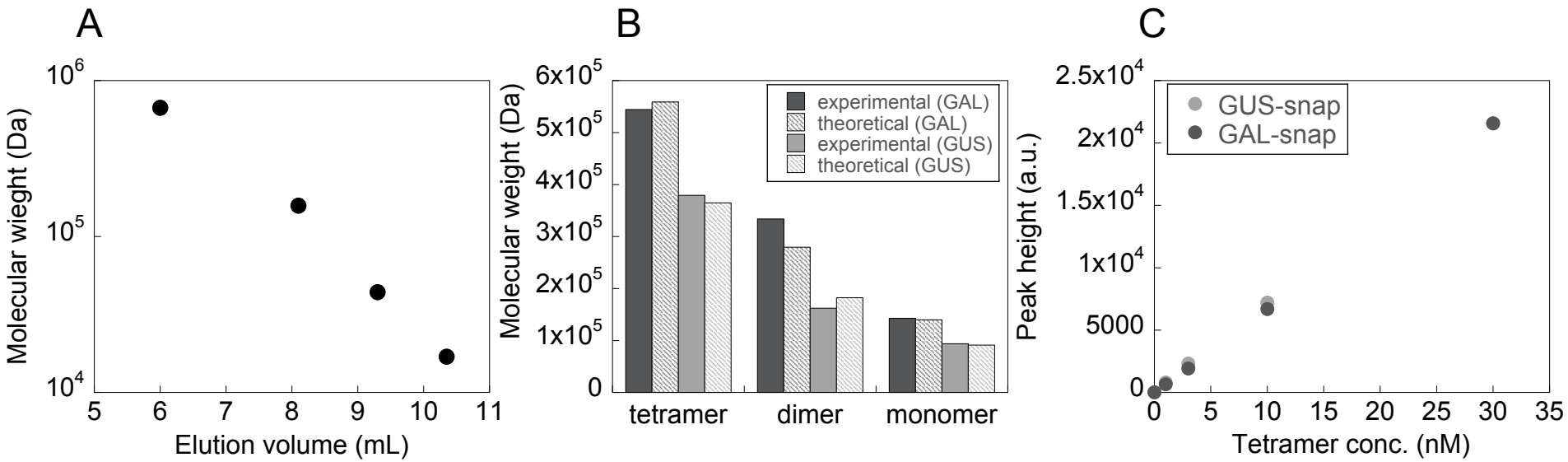
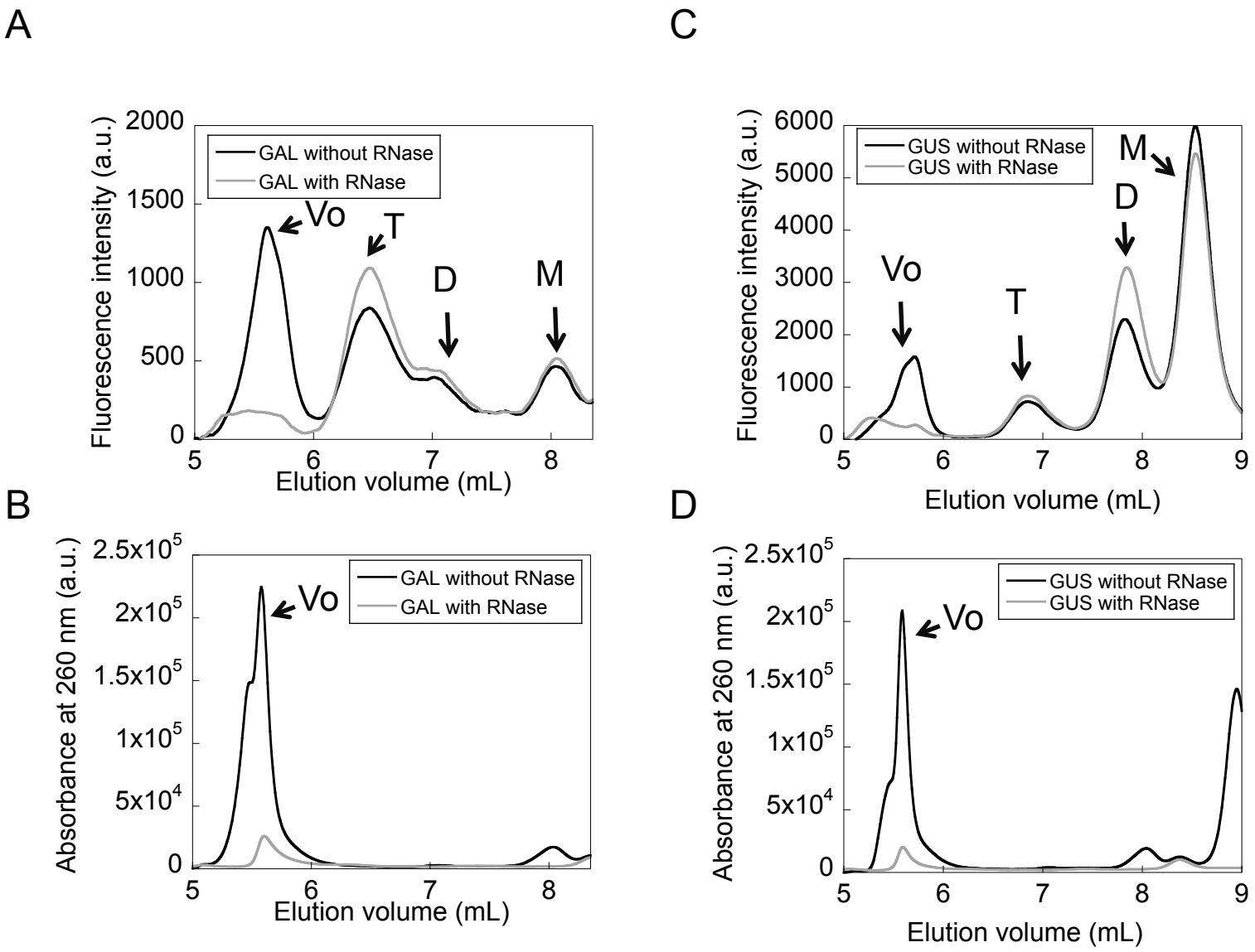

A
Molecular wieght (Da)
10^6
10^5
10^4
5
6
7
8
9
10
11
Elution volume (mL)
B
Molecular weight (Da)
6x10^5
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
experimental (GAL)
theoretical (GAL)
experimental (GUS)
theoretical (GUS)
tetramer
dimer
monomer
C
Peak height (a.u.)
2.5x10^4
2x10^4
1.5x10^4
1x10^4
5000
0
GUS-snap
GAL-snap
0
5
10
15
20
25
30
35
Tetramer conc. (nM)

Figure S5


A
C
B
D
Fluorescence intensity (a.u.)
Absorbance at 260 nm (a.u.)
Elution volume (mL)
GAL without RNase
GAL with RNase
GUS without RNase
GUS with RNase
Vo
T
D
M
2000
1500
1000
500
0
6000
5000
4000
3000
2000
1000
2.5x10^5
2x10^5
1.5x10^5
1x10^5
5x10^4
5
6
7
8
9