# Pre-steady State Kinetics of Initiation of Transcription by T7 RNA Polymerase - A Complete Kinetic Model

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

**SUMMARY:**. In order to begin to understand the mechanism of the initiation of transcription in the model T7 RNA polymerase system, the simplest possible reaction, the synthesis of dinucleotide, has been followed by quench-flow kinetics, and numerical integration of mechanism-specific rate equations has been used to test specific kinetic models. In order to fit the observed time dependence in the pre-steady state kinetics, a model for dinucleotide synthesis is proposed in which rebinding of the dinucleotide to the enzyme-DNA complex (in the case of dinucleotide synthesis, product inhibition, but a productive intermediate in the synthesis of longer products) must be included. Reactions using dinucleotide as a substrate confirm this mechanism and the globally determined rate constant. The rate limiting step leading to product formation shows a substrate dependence consistent with the binding of two substrate GTP molecules, and at saturating levels of GTP, is comparable in magnitude to the product release rate. The rate of product release shows a (positive) correlation with the concentration of GTP, suggesting that the reaction shows (base-specific) substrate activation. The binding of another substrate molecule, presumably via interaction with the triphosphate binding site, likely facilitates displacement of the dinucleotide product from the complex.

In the initiation of transcription, an RNA polymerase must facilitate melting of the DNA at the transcription start site, exposing (at the very least) the first two bases in the template strand. Subsequently, the initiating and elongating nucleotide triphosphates must bind to the template strand positioned at the active site, and formation of the phosphodiester bond ensues. The observation of long transcription products is influenced both by this essential first step, but also by the efficiency and rate at which RNA polymerase escapes the initial abortive cycling phase. Thus, monitoring of long transcription products is not a measure of initial events in transcription.

Previous pre-steady state kinetic studies have characterized the kinetics of synthesis of poly-G RNA (slippage) products up to 5 bases in length and abortive products up to 6 bases in length (Jia & Patel, 1997a; Jia & Patel, 1997b). For optimal promoters and native enzyme at saturating levels of NTP's, at least, polymerization steps beyond the initial step are typically fast enough to be safely ignored. The accumulation of total RNA products shows a distinct burst in the kinetics, which is independent of the order of addition of the enzyme, DNA, and substrate NTP's. These results led to the conclusions that under these conditions binding of RNA polymerase to its promoter is not rate limiting, dinucleotide synthesis or the step immediately preceding it limits the rate of the fast (burst) phase, and product release (or enzyme recycling) is rate limiting in the steady state. These transcription studies agree well with measurements of the kinetics of changes in fluorescence following GTP binding (Jia et al., 1996; Jia & Patel, 1997a).

The use of simple exponential equations to describe even this reaction does not provide mechanistic insight. Variations in the amplitude of the burst phase, for example, don't always fit well to simple models, and have, in fact, led some to propose that the enzyme preparation was only partially active (Woody et al., 1998). The use of integrated rate equations is essential to understanding the initiation process (Jia & Patel, 1997b), but the fitting of such equations becomes increasingly difficult as steps are added to the model.

In the current study, we examine in a simple and well-controlled system the pre-steady state kinetics of dinucleotide synthesis on a template which, in the presence of GTP as the sole substrate, encodes only the dinucleotide pppGpG. This simplest reaction avoids complications (see below) arising from interpretations of kinetic data where multiple RNA products are synthesized. The data are fit to explicit kinetic models, revealing several new features of the mechanism.

## Materials

**RNA polymerase**. T7 RNA polymerase was prepared from *E. coli* strain BL21 carrying the overproducing plasmid pAR1219 (kindly supplied by F. W. Studier), which contains the T7 RNA polymerase gene under the inducible control of *lac*UV5 promoter (Davanloo et al., 1984; Morris et al., 1986). The enzyme was purified and concentration determined ($\varepsilon_{280}=1.4 \times 10^5\ M^{-1}\,cm^{-1}$) as described previously (King et al., 1986). Purity of the enzyme was verified by SDS-PAGE.

**Oligonucleotides**. Oligonucleotides were synthesized by the phosphoramidite method on an Applied Biosystems Expedite 8909 DNA synthesizer. Standard phosphoramidites (CPG Inc.) were coupled normally. Single strands from a 1 µmol scale synthesis were purified trityl-on using an Amberchrom CG-161cd reverse phase resin (TosoHaas Inc) as described (Schick & Martin, 1993). Purity of the oligonucleotides was confirmed by denaturing (urea) gel electrophoresis of 5'–end labeled single strands. Double-stranded

DNA was made by annealing complementary single strands at 90° C and allowing the resulting mixture to cool to room temperature over 2 hours.

**Kinetic assays**. Quench-flow experiments were performed on a KinTek RQF–3 quench-flow apparatus (KinTeK Inc., Austin, TX) at 25°C. The final reaction mixture contained 30 µM HEPES (pH 7.8), 15 mM magnesium acetate, 25 mM potassium glutamate, 0.25 mM EDTA, 0.05%(v/v) TWEEN-20 (Calbiochem, protein grade), variable amounts of GTP (Roche Biochemicals), less than 0.03 µM [$\alpha$-$^{32}$P] GTP (NEN Life Sciences) as a label, 3.0 µM DNA promoter and 5.0 µM T7 RNA polymerase. Reactions were quenched by addition of a 95% formamide, 20 mM EDTA (pH 7.8) gel-loading buffer. Aliquots (3.0 µl) were loaded onto a 7M urea/18% polyacrylamide, sequencing gel. After 2.5 hr electrophoresis at 50 W (2000 V), gels were dried and quantified using a Molecular Dynamics Storm 840 Phosphorimager.

Dinucleotide was synthesized by T7 RNA polymerase under similar conditions, with 0.8 mM GTP, 0.5 µM promoter DNA, and 0.5 µM T7 RNA polymerase at 37°C for 30 min. The resulting mixture was loaded onto a 6M urea/15% polyacrylamide sequencing gel for gel purification. After 6 hr of electrophoresis at 25 W (1000 V), the gel was imaged with X-ray film. The band corresponding to the dinucleotide was excised and soaked in 10 mM Tris (pH 7.8). Eluent was collected and was purified further on a 25 cm C18 Microsorb MV chromatography column (Beckman). The column was run at 1.0 mL min$^{-1}$ in 0.1 M triethylamine-acetic acid buffer pH 7.0, using a 30 min gradient from 0 to 30% (v/v) acetonitrile. The radioactivity-containing peak eluted at 35 min. Fractions were collected, dried down and resuspended in 100 µM HEPES (pH 7.8). Dinucleotide consumption was measured under conditions similar to the above, with 400 µM (each) of ATP, CTP and UTP, and enzyme, DNA, and dinucleotide as indicated in the figures.

## Results

Previous studies have shown that a minimal promoter for T7 RNA polymerase consists of the sequence from position –17 to the initial templating bases (Martin & Coleman, 1987; Rong et al., 1998), and recent crystal structures support earlier conclusions that the essential sequence-dependent interactions occur upstream of position +1 (Chapman & Burgess, 1987; Cheetham et al., 1999; Cheetham & Steitz, 1999; Ikeda et al., 1992; Jorgensen et al., 1991; Li et al., 1996; Újvári & Martin, 1997). While this untranscribed part of the T7 RNA polymerase promoter has a reasonably conserved consensus, the downstream (early message) sequence varies more widely among the seventeen phage promoters (Moffatt et al., 1984). Many studies of transcription in this system have used the phage φ10 promoter, which best represents the consensus sequence, however, this promoter encodes an RNA with an initial sequence of GGGAGA..., and it has been shown previously that in the presence of GTP as the sole substrate, a ladder of poly-G products is synthesized as the result of a non-physiological slippage mechanism (Martin et al., 1988).

Transcription in the presence of GTP as the sole substrate on the following promoter template

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  -17             -1   +5
   |               |    |
5'-TAATACGACTCACTATAGGACT-3'
3'-ATTATGCTGAGTGATATCCTGA-5'
                    ➡
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yields the dinucleotide pppGpG as the sole product, and so avoids complications from "slippage" synthesis of longer products (Martin et al., 1988). Characterization of dinucleotide synthesis in this system presents the simplest assay of initiation and avoids potentially complicating chemical steps which follow initiation.

**Fitting of pre-steady state kinetic data**. The pre-steady state kinetic trace shown in Figure 1 presents the time course of the synthesis of dinucleotide RNA. As demonstrated previously for the synthesis of slippage and abortive products longer than dinucleotide, there is a clear fast initial phase, followed by an approach to a slower steady state kinetic phase (Jia & Patel, 1997a; Jia & Patel, 1997b; Kumar & Patel, 1997). As has been done previously, the data can be fit well by the equation

$$[RNA] = A_{Burst}\left(1 - e^{-k_{fast}t}\right) + k_{ss}t \qquad (1)$$

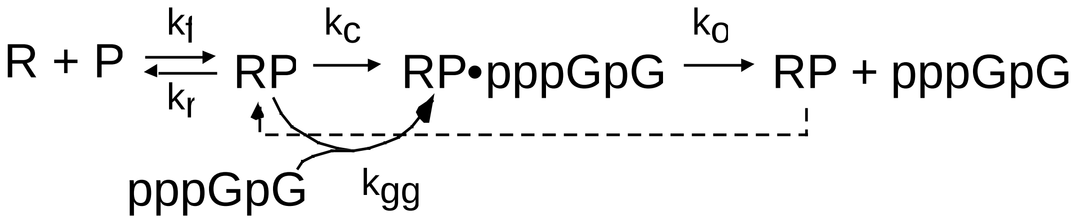
The steady state parameter suggests a unimolecular rate constant of $k_{ss}$/[RP], or 0.23 $s^{-1}$ at 25°C (given that DNA is limiting, at 3 µM). It was previously proposed that the rate determining step in the steady state synthesis of short RNA products is the release of product RNA and/or polymerase recycling, with a first order rate constant of about 0.15 $s^{-1}$ (Jia & Patel, 1997a; Jia & Patel, 1997b).

Equation 1, however, does not derive from a specific kinetic model. In order to more clearly interpret the kinetic profile, we instead fit the data to numerically integrated solutions of simple kinetic models. Recognizing that open complex formation is not rate determining in this system (Jia et al., 1996; Újvári & Martin, 1996), we initially attempted to fit the above data to an equation of the form

$$\begin{tikzcd}
R + P \arrow[r, shift left, "k_f"] & RP \arrow[l, shift left, "k_r"] \arrow[r, "k_c"] & RP\bullet pppGpG \arrow[r, "k_o"] & RP + pppGpG \arrow[lll, dashed, bend left]
\end{tikzcd} \qquad (2)$$

where RNA polymerase (R ) combines with the promoter DNA (P) to form an initial binary complex (RP). Under conditions in which the concentration of GTP does not change substantially during the course of the reaction, the binding of two molecules of GTP and the catalysis of phosphodiester bond formation can be treated as pseudo-first order process (represented by a concentration-dependent $k_c$). The best fit of the data in Figure 1 to the above kinetic mechanism is shown in that figure as a dashed line. The parameters describing binding have been previously determined ($k_f = 126\ \mu M^{-1}\ s^{-1}$ and $k_r = 0.63\ s^{-1}$) and since that reaction is fast and lies far to the right under these conditions, the fit shows only a minor dependence on them (Jia et al., 1996; Újvári & Martin, 1996). Fixing those parameters, the best-fit values for $k_c$ ($2.3 \pm 1.0\ s^{-1}$) and $k_o$ ($0.35 \pm 0.03\ s^{-1}$) are comparable to those obtained previously, $3.5\ s^{-1}$ and $0.15\ s^{-1}$, respectively (Jia & Patel, 1997b). Careful inspection of the curve and the residuals plot, however, shows that equation (2) does not accurately represent the observed data. Similar kinetic models were tried, including models in which the enzyme-DNA complex initially formed must undergo a transition to an activated state. However, these models were unable to provide a fit significantly better than that provided by equation 2.

In considering alternate models, we can exclude from consideration possible product inhibition by accumulated pyrophosphate since the reported $K_i$ for $PP_i$ is 830 µM (Guajardo & Sousa, 1997), however, it is well-known that T7 RNA polymerase can initiate with a dinucleotide as an initial substrate (Jia & Patel, 1997b; Moroney & Piccirilli, 1991), so we reasoned that as the product dinucleotide accumulates, it might serve as a competitive inhibitor of initiation (this results in inhibition only under conditions of dinucleotide synthesis, in the synthesis of longer products such rebinding is productive, but nevertheless cannot be ignored in kinetic treatments). The solid line in Figure 1 shows the best fit of the data to the following equation, which incorporates product inhibition explicitely:



(3)

In this case, at a saturating concentration of GTP (800 µM, see below), the best fit values for $k_c$ ($1.3 \pm 0.2$ $s^{-1}$) and $k_o$ ($1.0 \pm 0.3$ $s^{-1}$) are comparable, and the apparent bimolecular rate constant for product rebinding ($k_{gg}$) is $0.21 \pm 0.07$ $\mu M^{-1}$ $s^{-1}$, such that this step becomes significant within a single turnover (at 3 µM pppGpG, the pseudo-first order rate constant for this step becomes 0.6 $s^{-1}$). The ratio $k_o/k_{gg}$ (4.8 µM) provides a measure of the equilibrium dissocation constant for binding of dinucleotide to the polymerase-promoter complex. This means that the product dinucleotide binds much more tightly than the substrate GTP.

**Direct measurement of dinucleotide consumption.** In order to measure more directly kinetic parameters for the incorporation of dinucleotide described by the parameter $k_{gg}$, we carried out transcription in the presence of ATP, CTP, UTP and the dinucleotide pppGpG (producing a five base runoff transcript). The kinetic curves shown in Figure 2A follow simultaneously the production of the runoff transcript and the consumption of dinucleotide under conditions of low dinucleotide concentration, where the binding represented by $k_{gg}$ is rate limiting. For this reaction, the estimated second order rate constant for dinucleotide consumption ($k_{app}$ divided by the limiting concentration of enzyme-DNA complex, 0.02 µM) is 0.25 $\mu M^{-1}$ $s^{-1}$, and is comparable to the value for $k_{gg}$ (0.21 $\mu M^{-1}$ $s^{-1}$) obtained from the global fit to the data in Figure 1. This result also demonstrates that the $K_m$ for the substrate dinucleotide must be higher than 2 µM, consistent with the above estimate of $K_m$ (5 µM).

Given this estimate for $K_m$, we next carried out the same reaction at saturating concentrations of dinucleotide. The results presented in Figure 2B yield a steady state value for turnover of 0.4-0.5 $s^{-1}$ (depending on the exact steady state concentration of the enzyme-DNA complex). The initial linear rate under these conditions confirms that $K_m$ for dinucleotide is well below 40 µM. Pre-steady state kinetic measurements of this reaction can provide a measure of the rate determining step following dinucleotide synthesis, but preceding product dissociation (Jia & Patel, 1997b).

**Dependence of kinetic parameters on GTP concentration.** In the pre-steady state synthesis of runoff dinucleotide, the time interval studied has been chosen to prevent significant depletion of the substrate pool, in order to justify the use of the pseudo-first order approximation of $k_c$ in the model.

In order to more fully understand the parameters in the mechanistic model, we repeated the kinetic measurements of Figure 1 at a series of GTP concentrations. For each concentration of GTP, a complete new fit was performed, providing the simultaneous best fit values for $k_c$, $k_{gg}$, and $k_o$. For the data at 400, 800, and 2000 µM GTP, this was a reasonable approach, however, the burst phase on the curves obtained at lower concentrations of substrate was insufficiently pronounced to allow all three parameters to be fit independently. In particular, the value for $k_{gg}$ was not well defined ($k_c$ fit well and reproducibly in all cases). Since $k_{gg}$ was ill-defined below 800 µM, but was constant at 800 and 2000 µM GTP, $k_{gg}$ was set to a constant for subsequent fits, as summarized in Table 1.

The step represented by $k_c$ involves at the very least, the binding of two GTP molecules, followed by phosphoryl transfer chemistry, although a conformational change in the complex preceding phosphoryl transfer is thought to be rate-determining at saturating levels of substrate (Jia & Patel, 1997a; Jia & Patel, 1997b). As shown in Figure 4, at sub-saturating concentrations of GTP, the pseudo first order rate constant $k_c$ depends on GTP in a manner which suggests that it reflects the binding of both substrate GTP molecules, as proposed previously (Jia & Patel, 1997a; Jia & Patel, 1997b).

$$k_c = \frac{k_c^{sat}[GTP]^2}{K_d^2 + [GTP]^2} \qquad K_d = \sqrt{K_1 K_2} \tag{4}$$

Fitting the data to the Hill equation, as in the previous work, yields a geometric average for the two GTP dissociation constants of 290 ± 90 µM, comparable to the value of 320 ± 97 µM from the previous study.

The parameter $k_o$, which represents the dissociation of the ternary complex (most likely dinucleotide dissociation, see discussion above), also varies with the concentration of GTP, as shown in Figure 5. The fact that $k_o$ increases with increasing concentration of GTP suggests that GTP binding may play a role in facilitating complex dissociation. Extrapolation to zero GTP concentration yields an inherent value for the dissociation rate of approximately 0.6 $s^{-1}$. In any case, the concentration dependence of this rate complicates simple kinetic analyses.

## Discussion

Transcription is a very complex process, involving numerous steps with (possibly) sequence and context-dependent rates. The system presented here probes the simplest possible reaction which can be carried out by an RNA polymerase: the polymerase-promoter open complex forms, two substrate molecules bind, product dinucleotide is formed and released. Hence, mechanistic analysis of this reaction should be relatively straightforward. Assumptions do not have to be made regarding the rates of intermediate processes and alternate initiation sites (with alternate rates) can be ignored. In fact, translocation of the complex along the DNA is not required for this process.

**A kinetic mechanism for initiation**. Despite the apparent simplicity of this reaction, the current results demonstrate that accurate fitting of the rate data for this process requires inclusion of an additional step, not present in previous related reaction schemes. In the kinetic mechanism represented by equation 3, the polymerase-promoter association ($k_f$ = 50 µ$M^{-1}$ $s^{-1}$) and dissociation ($k_r$ = 0.2 $s^{-1}$) rates have been previously determined (Jia et al., 1996; Újvári & Martin, 1996). At the concentrations used in this study, the association is much faster than the kinetic parameters observed and the equilibrium lies substantially to the right ($K_d$ for complex dissociation is approximately 10 nM). Consequently, variation of these parameters does not dramatically effect the kinetics.

The above model shows product release as the last step, represented by $k_o$, thereby leaving enzyme and DNA associated. Previous results have demonstrated that during turnover on similar templates, recycled complexes show some resistance to challenges by heparin or by another promoter, indicating that a model in which the complex remains associated is more correct (Diaz et al., 1996; Jia & Patel, 1997b). Since rebinding is fast under the current conditions, the nature of this last step does not impact the fits of the current study, however, the ratio of forward synthesis ($k_c$ = 1.2 $s^{-1}$) measured here to binary complex dissociation ($k_r$ = 0.2 $s^{-1}$) determined previously (Újvári

& Martin, 1996) is, in fact, consistent with the above observations and confirms the kinetic mechanism as portrayed in equation 3.

The current results agree reasonably well with previous pre-steady state kinetic quench-flow and stopped-flow measurements in this system (Jia et al., 1996; Jia & Patel, 1997a; Jia & Patel, 1997b). As expected, the apparent first order step represented by $k_c$ shows a saturable dependence on the concentration of GTP consistent with the binding of two substrate GTP molecules with the $K_m$'s (about 1000 µM and 50 µM for the initiating and elongating GTP's, respectively) estimated previously (Jia & Patel, 1997a).

**Rebinding of product dinucleotide**. A new feature of the current model is the product inhibition by substrate dinucleotide. In retrospect, this result should not be surprising, in that it has been previously determined that RNA polymerase can efficiently extend a substrate dinucleotide (Moroney & Piccirilli, 1991). The current results, however, point out that at concentrations of enzyme and DNA used for quench-flow measurements, rebinding of product cannot be ignored at even very short time points. Previous studies have contained up to 10 µM polymerase-promoter complexes, such that after even one turnover, rebinding of 10 µM dinucleotide would occur at an apparent first order rate of 1.7 $s^{-1}$, a rate faster than the maximal rate of dinucleotide synthesis (1.3 $s^{-1}$). In reactions synthesizing an array of abortive products, dinucleotide product grows rapidly at first, but then increases more slowly (data not shown) as it begins to be consumed, as expected by the current model.

This result substantially complicates a recent steady state kinetic study of dinucleotide synthesis, in which the amount of dinucleotide formed in 5 min was followed as a function of the concentration of GTP (Villemain et al., 1997).

The current results show that at low concentrations of GTP (less than micromolar), rebinding of dinucleotide may be safely ignored, but at micromolar or higher concentrations of GTP, product inhibition will very significantly influence the observed concentrations of product dinucleotide.

From the mechanism in equation 3, rebinding of product to the complex competes directly with the binding of the correct substrate. When brought in to the model, however, the observed rate constant for product rebinding, $k_{gg}$, should be independent of GTP concentration. For concentrations of GTP at which $k_{gg}$ can be reliably measured, this is observed, further supporting the current mechanism.

**Complex dissociation is promoted by free nucleoside triphosphate**. Surprisingly, the dissociation of the ternary complex shows a mild, but significant dependence on the concentration of GTP. A model which could explain this result is shown in Figure 6. We know from studies of homologous polymerases that the incoming substrate (elongating NTP) is bound not only by Watson-Crick interactions with the template strand, but also (and very importantly) by interactions with the triphosphate group (Astatke et al., 1995). Following dissociation of the pyrophosphate group, as shown in Figure 6B, the triphosphate binding pocket is available to bind another nucleotide triphosphate. This might allow binding of another substrate molecule while product remains in place. Indeed, this type of behavior has recently been postulated in the T7 RNA polymerase system (Guajardo & Sousa, 1997). However, in the current experiment, the correct substrate (ATP) is not present to drive forward translocation. Instead, GTP can bind at the substrate binding site, competing with the +2 G in the product for binding of the template strand C at position +2, thereby increasing the rate of product release. This model further suggests that the disruption of the ternary complex, represented by the final step in equation (3), is indeed RNA dissociation, at least in the synthesis of dinucleotide, consistent with the studies cited above which conclude that the polymerase and the promoter do not immediately dissociate on recycling . Finally, consistent with this mechanism, inclusion in the reaction of non–templated nucleotide (0.4 mM UTP or 1.6 mM CTP) does not affect the kinetic parameters (data not shown).

In summary, we have presented data for the simplest possible initiation reaction, and have fit that data to the simplest, complete kinetic mechanism. No approximations are made, and intermediate steps are avoided. The results reveal that rebinding of the dinucleotide product cannot be ignored in these measurements, and must be included in future kinetic studies which try to measure subsequent steps, as the enzyme translocates along the DNA template.

## Figure Legends

**Figure 1**: Pre-steady state kinetic reaction profile for dinucleotide synthesis on a template encoding GGACU, in the presence of GTP as the sole substrate. Reaction (at 25° C) contained 3 µM promoter DNA, 5 µM T7 RNA polymerase, and 800 µM GTP, in a buffer of 30 mM HEPES (pH 7.8), 25 mM potassium glutamate, 15 mM $MgCl_2$ 0.05% (v/v) TWEEN-20, 0.25 mM EDTA. Reactions were initiated by preincubating DNA with T7 RNAP, then initiating the reaction by the addition of GTP. The observed product RNA is the sum of free RNA plus any RNA bound in a complex (these cannot be determined independently). The dotted line is the best fit to the exponential function, equation (1), with burst amplitude $A_{burst}$ = 5.2 µM, $k_{fast}$=0.34 $s^{-1}$, and $k_{steady\ state}$ = 0.70 µM $s^{-1}$. The dashed line is the best fit (numerically integrated) to equation (1), with $k_c$=2.3 $s^{-1}$ and $k_{o2}$ = 0.35 $s^{-1}$ ($k_f$ is set to 126 $\mu M^{-1}$ $s^{-1}$ and $k_r$ is set to 0.63 $s^{-1}$). The solid line is the best fit to equation (3) with $k_c$ = 1.3 $s^{-1}$, $k_{o2}$ = 1.0 $s^{-1}$, $k_{gg}$ = 0.21 $s^{-1}$. Residuals for each fit are presented. For equations 2 and 3, numerical integrations were carried out using Euler integration, with a constant step size of 2 msec. Non-linear regression of the integrated function was carried out using the Levenberg-Marquardt approach in the program pro Fit (Quantum Soft).

**Figure 2**. Transcription kinetics using exogenous dinucleotide as a substrate. **A**) Time course of dinucleotide conversion to a five base runoff transcript under conditions of limiting dinucleotide. The solid curves represent best fits with $k_{app}$ = 0.307±0.019 and 0.312±0.018 $min^{-1}$ for the consumption and production curves, respectively. Concentrations of polymerase and promoter DNA were 0.2 and 0.02 µM, respectively, while the initial concentration of dinucleotide was

2.0 µM. **B**) Time course of dinucleotide conversion under a large excess of dinucleotide. Initial rates were determined by linear regression of time points from 0 to 5 min, and fit 4.91±0.11 µM $\text{min}^{-1}$. Concentrations of polymerase and promoter DNA were 0.2 µM each, while the initial concentration of dinucleotide was 40 µM. For both reactions, the reaction conditions were as described in the methods section. The temperature was 25 °C.

**Figure 3**. Production of dinucleotide pppGpG at various concentrations of substrate GTP (given in µM above each data set. Conditions are given in Methods. Concentrations of enzyme and DNA are 5.0 and 3.0 µM, respectively.

**Figure 4**. The dependence of the product formation step on the concentration of substrate GTP. Data presented are from Table 1.

**Figure 5**. The dependence of the fit parameter $k_{off}$ on the concentration of substrate GTP. Data presented are from Table 1.

**Figure 6**. A model for substrate activation of product dissociation.

**Table 1**. Best fit parameters for the kinetic data presented in Figure 3. In the second row of the cells representing 400, 800, and 2000 µM GTP, all three parameters in the model represented by Equation 3 were allowed to vary; the results of those fits are reported in the second row within each cell. For the first rows of these, and for 20, 100, and 200 µM GTP, $k_{gg}$ was forced to remain constant at 0.21 $\mu M^{-1}$ $s^{-1}$ (it is not well-determined at lower concentrations of GTP).

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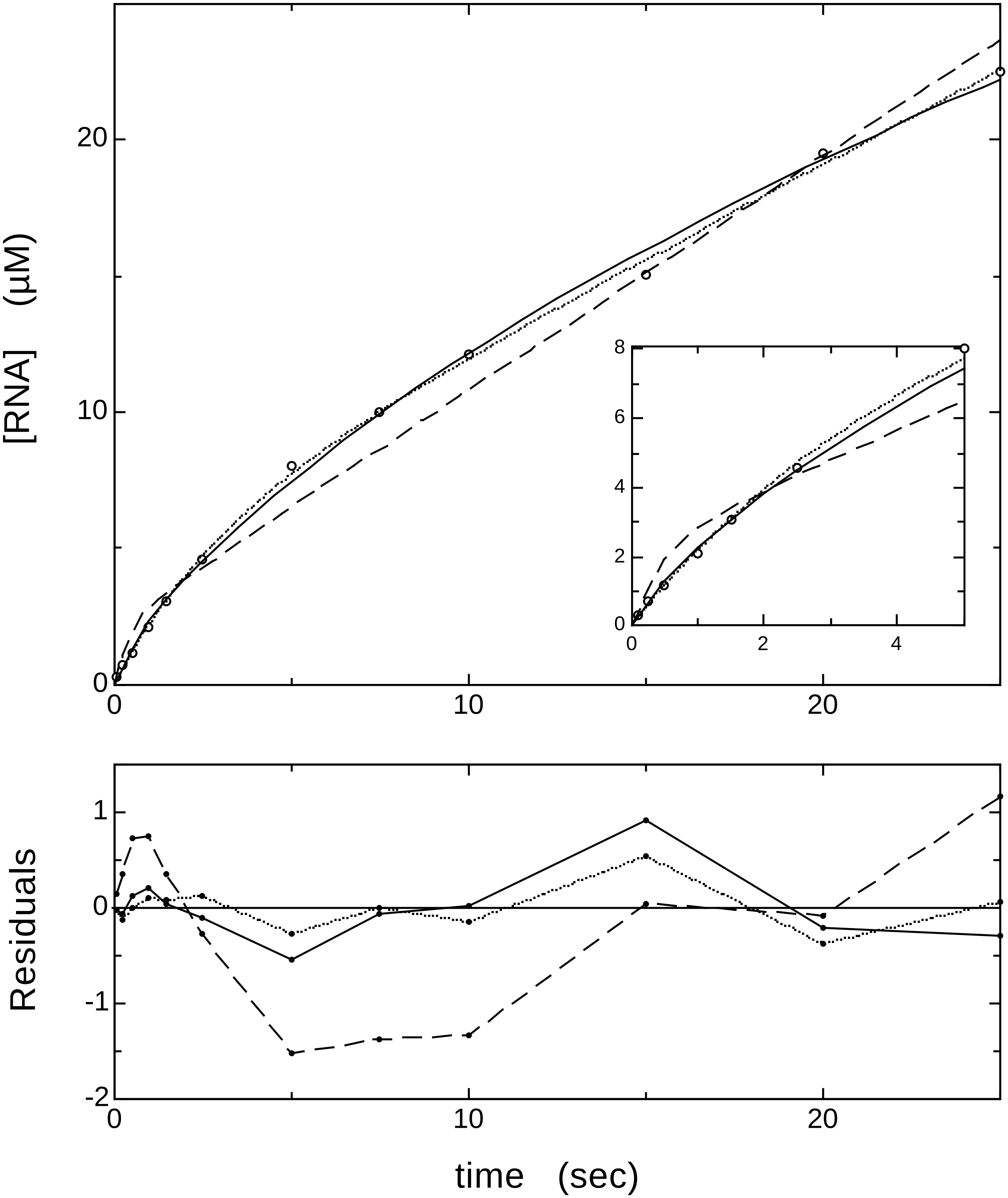
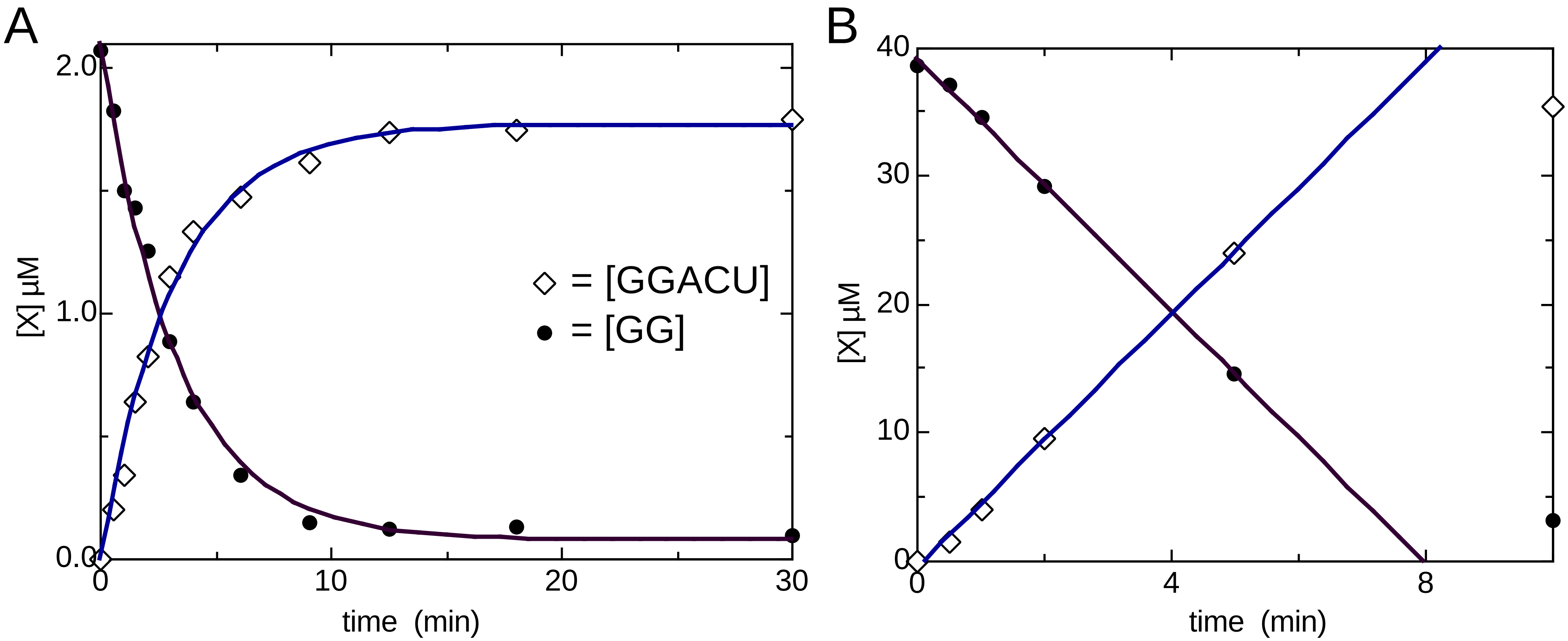
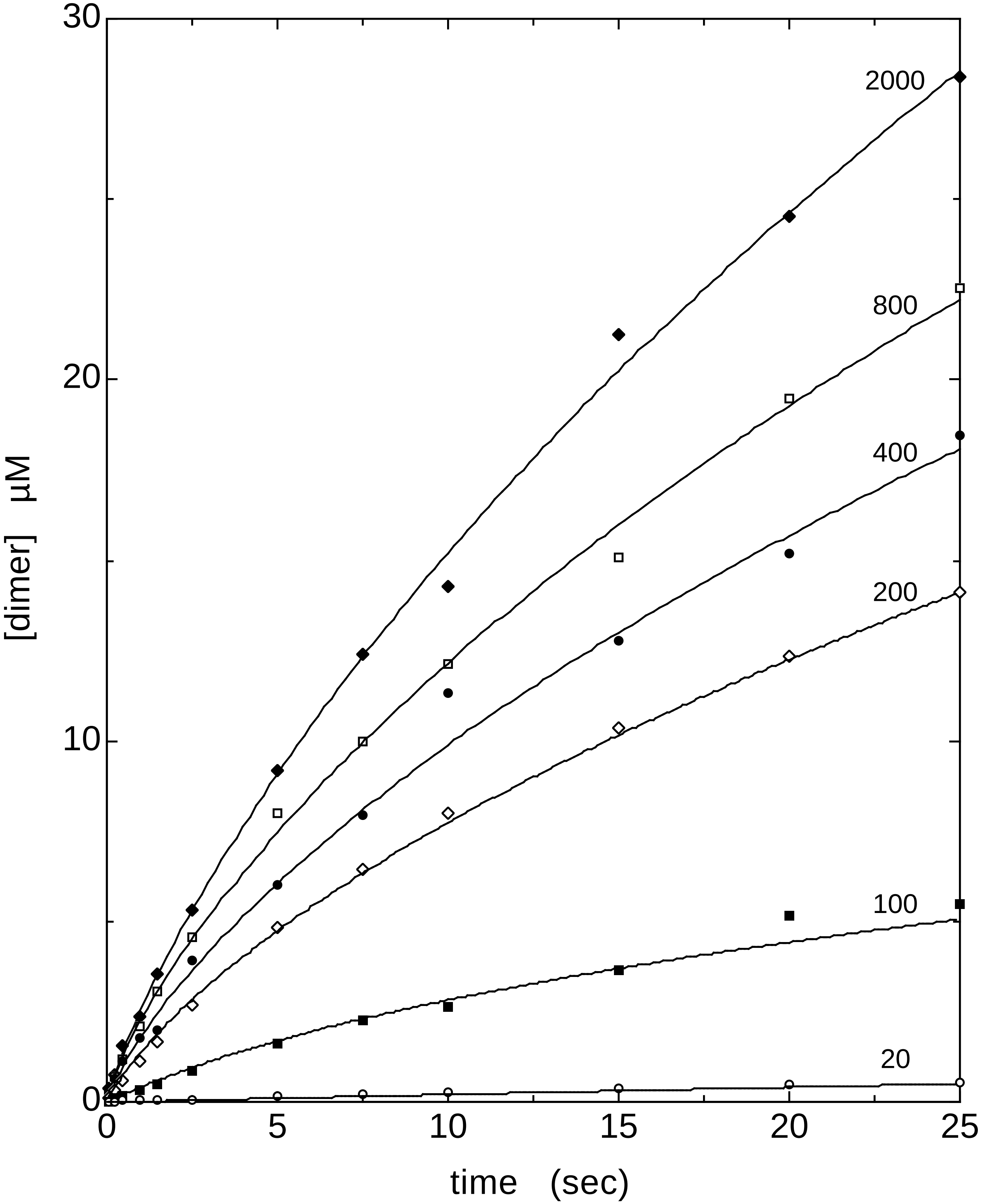
# Table 1

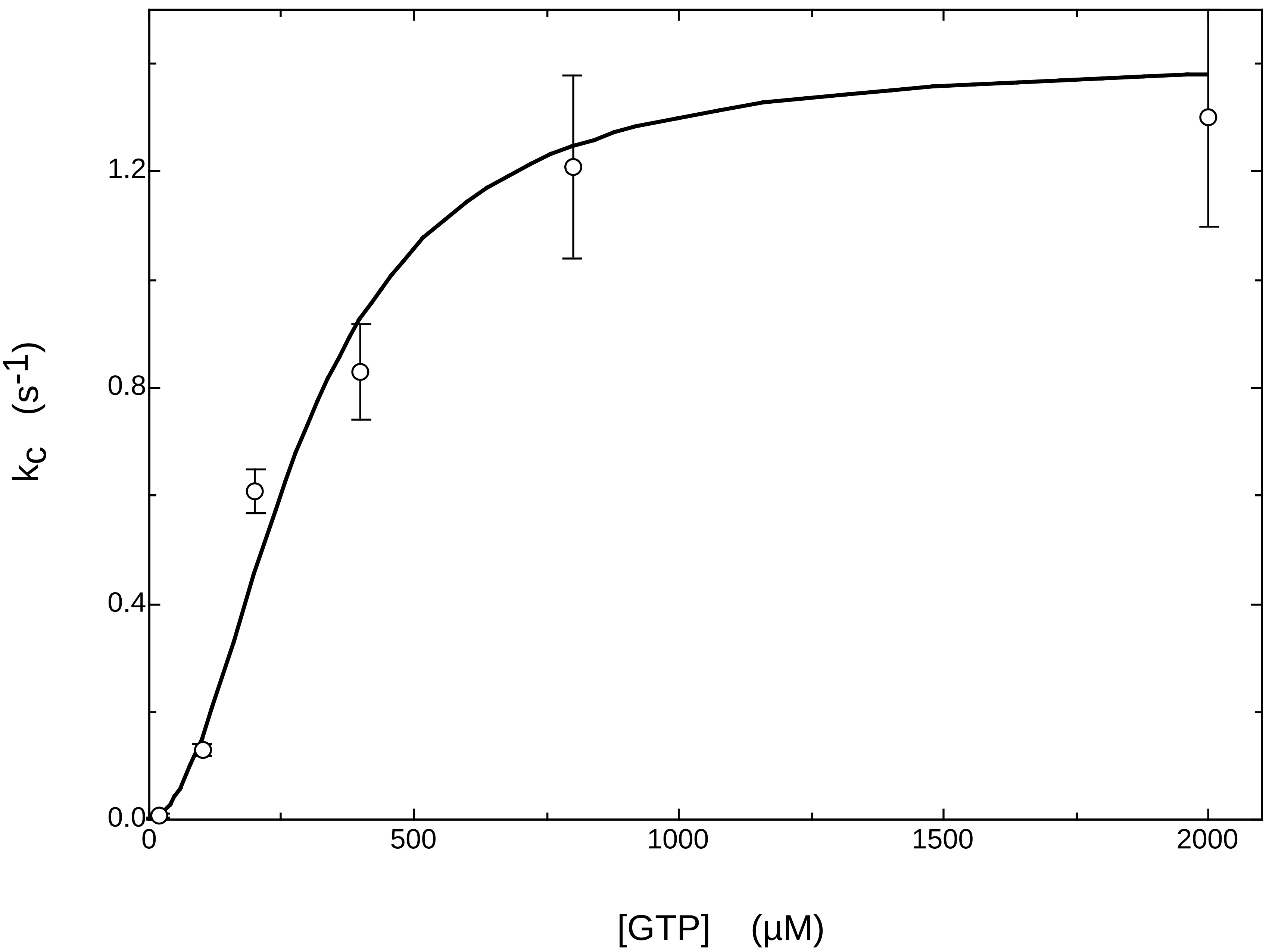
| [GTP] μM | $k_c$ ($s^{-1}$) | $k_{o2}$ ($s^{-1}$) | $k_{gg}$ ($\mu M^{-1}\ s^{-1}$) |
|---|---|---|---|
| 2000 | **1.3** ± 0.2<br>1.3 ± 0.3 | **1.4** ± 0.2<br>1.4 ± 0.7 | 0.21<br>0.18±0.07 |
| 800 | **1.2** ± 0.2<br>1.2 ± 0.2 | **0.91** ± 0.14<br>1.0 ± 0.4 | 0.21<br>0.21±0.01 |
| 400 | **0.83**±0.09<br>0.89±0.13 | **0.84**±0.11<br>0.65±0.20 | 0.21<br>0.13±0.06 |
| 200 | **0.61**±0.04 | **0.68**±0.07 | 0.21 |
| 100 | **0.13**±0.01 | **0.61**±0.18 | 0.21 |
| 20 | **0.0091** | nd | 0.21 |


[RNA] (µM)
0
10
20
0
10
20
8
6
4
2
0
0
2
4
Residuals
1
0
-1
-2
0
10
20
time (sec)

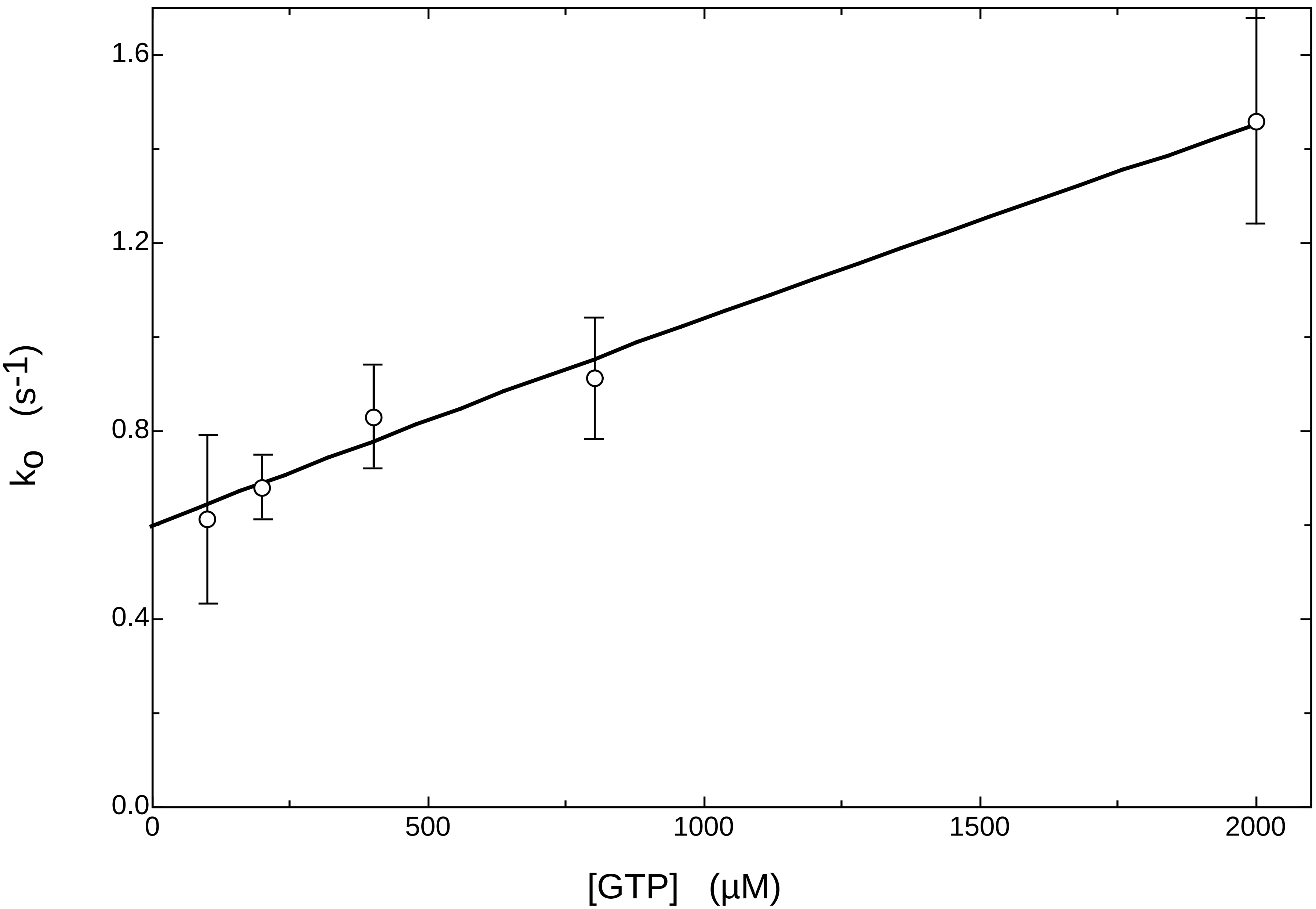

A
2.0
1.0
0.0
[X] µM
0
10
20
30
time (min)
◇ = [GGACU]
● = [GG]
B
40
30
20
10
0
[X] µM
0
4
8
time (min)

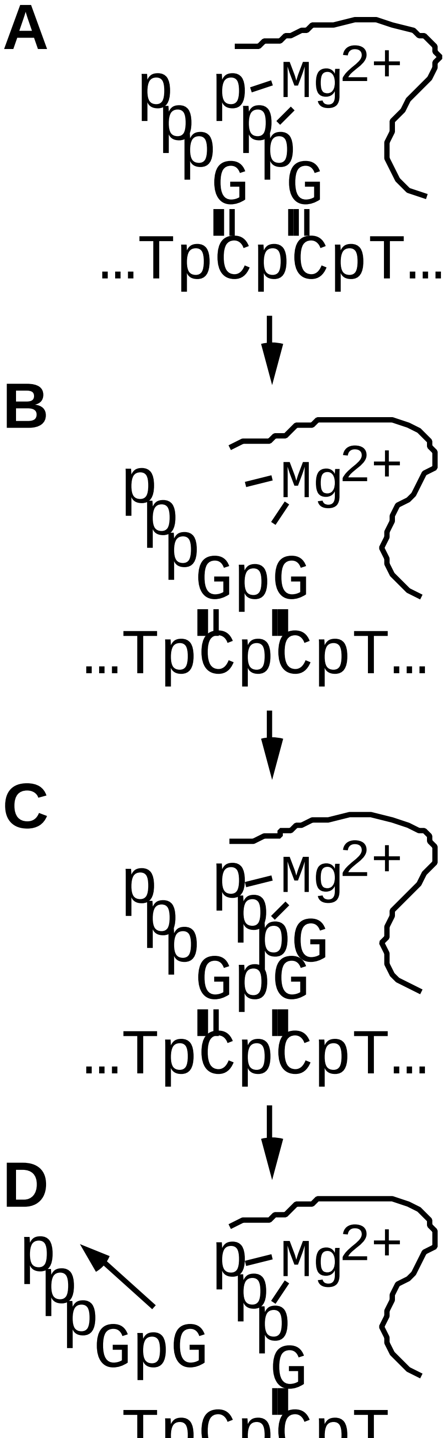


30
20
10
0
0
5
10
15
20
25
[dimer] μM
time (sec)
2000
800
400
200
100
20



k_C (s^-1)
0.0
0.4
0.8
1.2
0
500
1000
1500
2000
[GTP] (µM)


1.6
1.2
0.8
0.4
0.0
0
500
1000
1500
2000
$k_o$ $(s^{-1})$
[GTP] (µM)


A
p p p
p p p
Mg2+
G G
…TpCpCpT…
B
p p p
Mg2+
GpG
…TpCpCpT…
C
p p p
p p pG
Mg2+
GpG
…TpCpCpT…
D
p p pGpG
p p p
Mg2+
G
…TpCpCpT…