Effect of Magnesium Concentration on Immobilized Transcription

Magnesium can alter the flexibility of long DNA molecules, and thus may effect steric inhibition or mass transfer to the bead. To study these effects, 4 hour transcription reactions were run with 2mM total rNTP and ~1.8 ng/μL of DNA (~γ=0.25, β=6.6) at $MgCl_2$ concentrations ranging from 6-24mM. Magnesium binds to rNTPs, so 6-24mM $MgCl_2$ corresponds to 4-22mM free magnesium ions. The result shows that excess magnesium does not change the properties of DNA enough to significantly affect transcription efficiency between the solution and bead immobilized phase (Bonferroni two-tailed t test, $p<0.24$). In fact, excess magnesium decreases overall transcription in both solution and bead-immobilized reactions (figure S1). This is likely due to the increased chlorine counter ion concentration with increasing magnesium concentration. Transcription reactions are highly salt sensitive, and are particularly sensitive to Cl^- ions due to an inhibition of polymerase interaction with the DNA promoter site [8]. Overall, increased magnesium concentration does not effect bead immobilized transcription differently than solution phase transcription, implying that mechanical properties of long bound DNA molecules do not affect reagent and enzyme delivery to the template.

Figure S1: Effect of magnesium concentration on immobilized transcription, where β=6.6 and γ=0.25. There is no significant difference between the effect of excess magnesium on solution phase transcription when compared to bead immobilized transcription ($p<0.24$).