

## “low Dimer Assembly: A built-in Delay Mechanism of Cro Negative Autoregulation”

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Cro’s activity as a transcriptional repressor of bacteriophage lambda is a function of active dimer bound to DNA. *In vitro* dimer assembly is slow and limits the rate at which Cro dimers bind DNA (Jia et al. 2005). Based on Cro’s subunit association rate constant ( $10^4 \text{ M}^{-1} \text{ S}^{-1}$ ) to assemble the nanomolar concentration of dimers needed for half-saturation of an operator site it would take  $10^5$  seconds. In this case, how does Cro accumulate fast enough to actively regulate lambda transcription?

Despite slow accumulation of Cro dimers, DNA binding is fast. In fact, equilibrium dissociation constants predicts that at nanomolar concentrations of subunits necessary for half-saturation of an operator site, most DNA free subunits exist as monomers (Darling et al. 2000). In order to study the dynamics of Cro binding *in vivo* we constructed simplified genetic circuits that have variable Cro genes modified by genetic engineering to change dimerization characteristics. These circuits contain the right promoter ( $P_R$ ) of lambda under the dual control of lac and lambda operators that drive the expression of Cro in tandem with the lac Z reporter gene. Also like lambda, Cro positive circuits are negatively autoregulated by a feedback loop. Here we show that the feed forward assembly of Cro dimers is a built-in delay resulting in a pulse of transcription from the circuit and causes an overshoot of active levels. This pulse of transcription is only noticed at levels below the active concentration of Cro dimers, when monomer levels are increased. If dimer assembly is fast, as is the case for the scCro pre-assembled mutant, there is immediate repression (no delay). The coupling of slow assembly of Cro dimers and negative autoregulation speeds transcriptional response using dimer assembly as a stable tunable parameter.