SSB increases *Escherichia coli* clamp loader lifetime on DNA, and is rapidly remodeled

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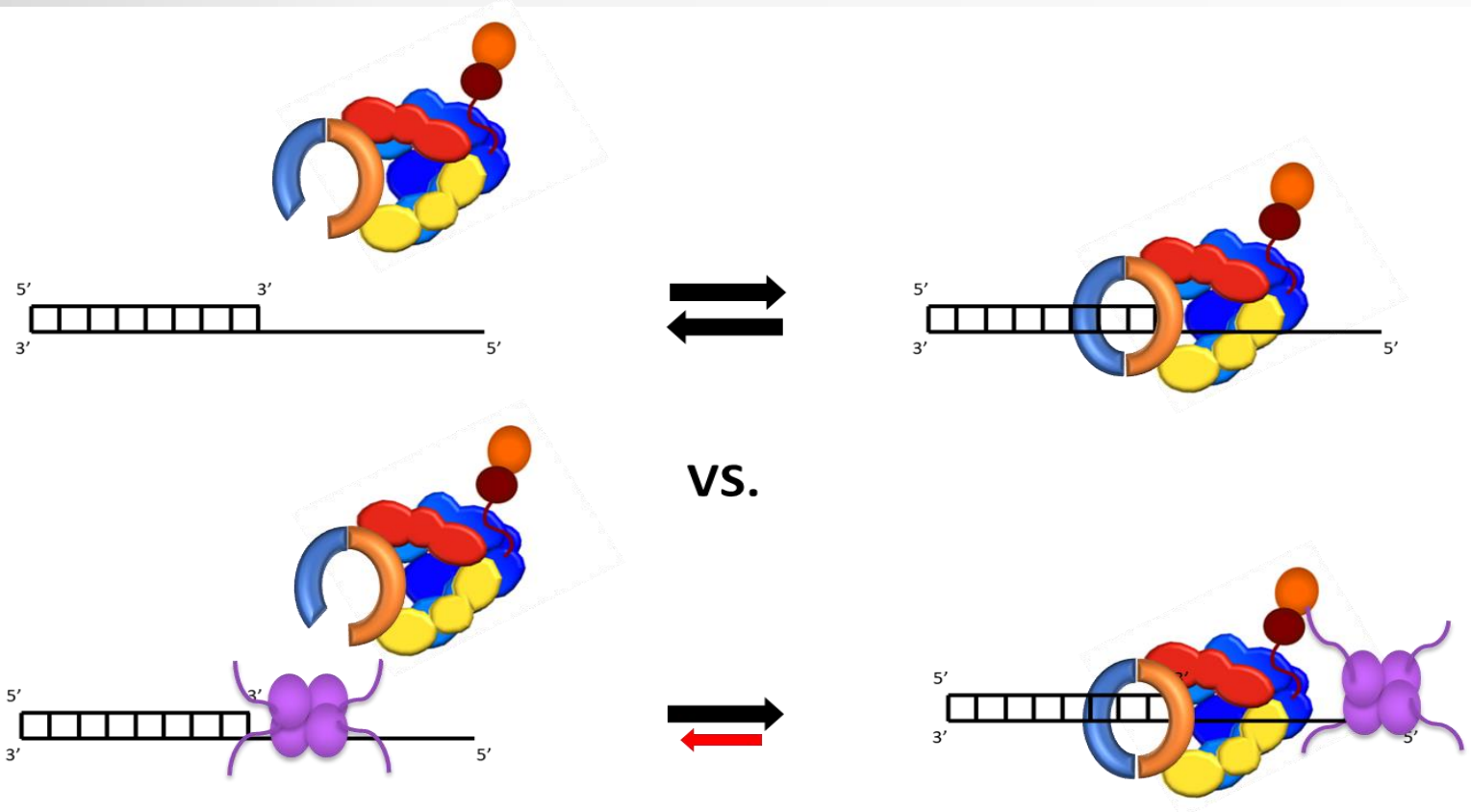
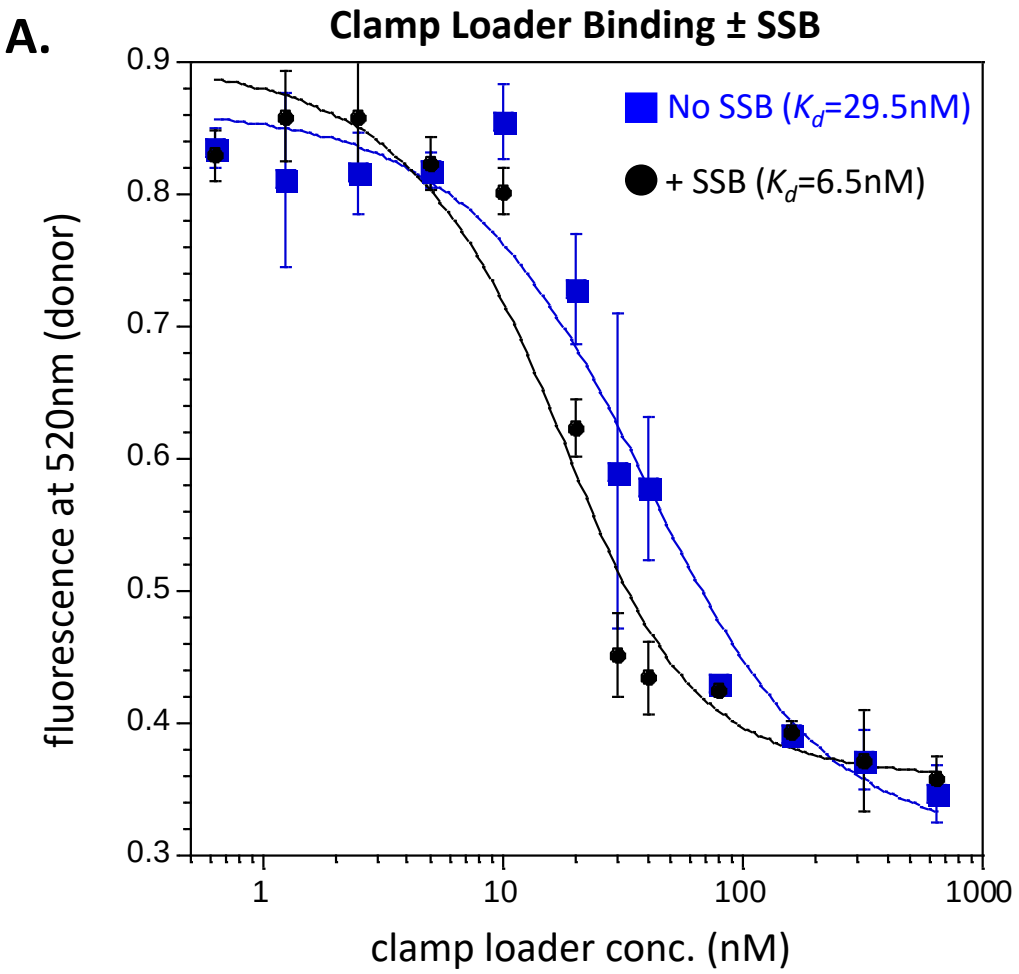


Introduction to *E. coli s*ingle stranded DNA binding protein (SSB) and clamp loader

- **SSB**
  - Avidly binds and protects single stranded DNA from nuclease attack and secondary structure formation
  - Binds many replication proteins via its C terminal tail
  - Acts as an organizer for genome replication/maintenance
- **Clamp loader**
  - Part of DNA Pol III Holoenzyme
  - Uses hydrolysis of ATP to load sliding clamps around DNA
    - Sliding clamps increase processivity of polymerase during replication

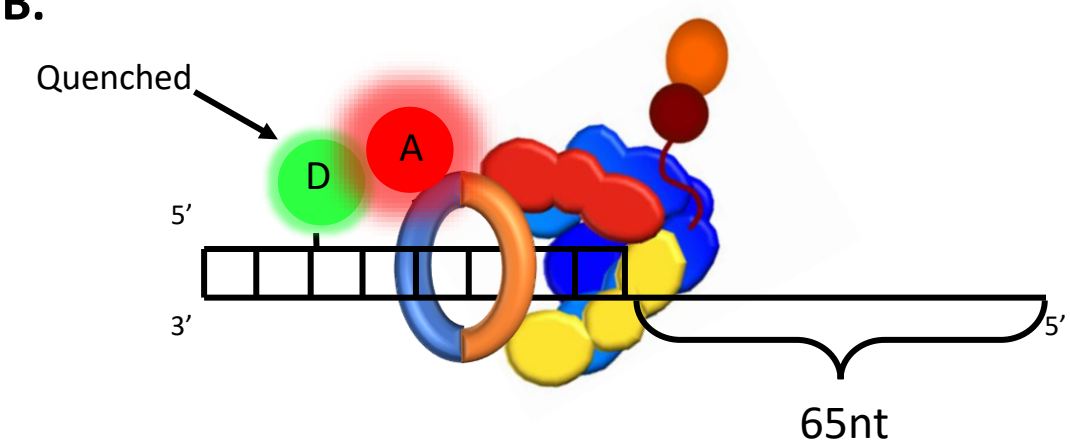
SSB increases clamp loader affinity for DNA

A.



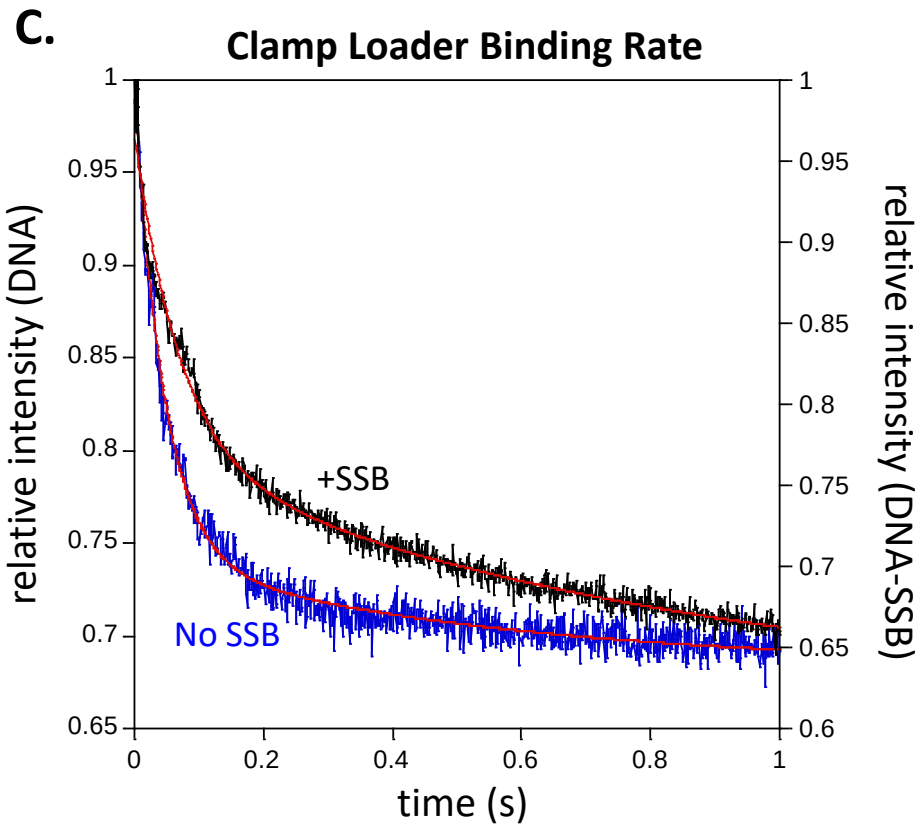
B.

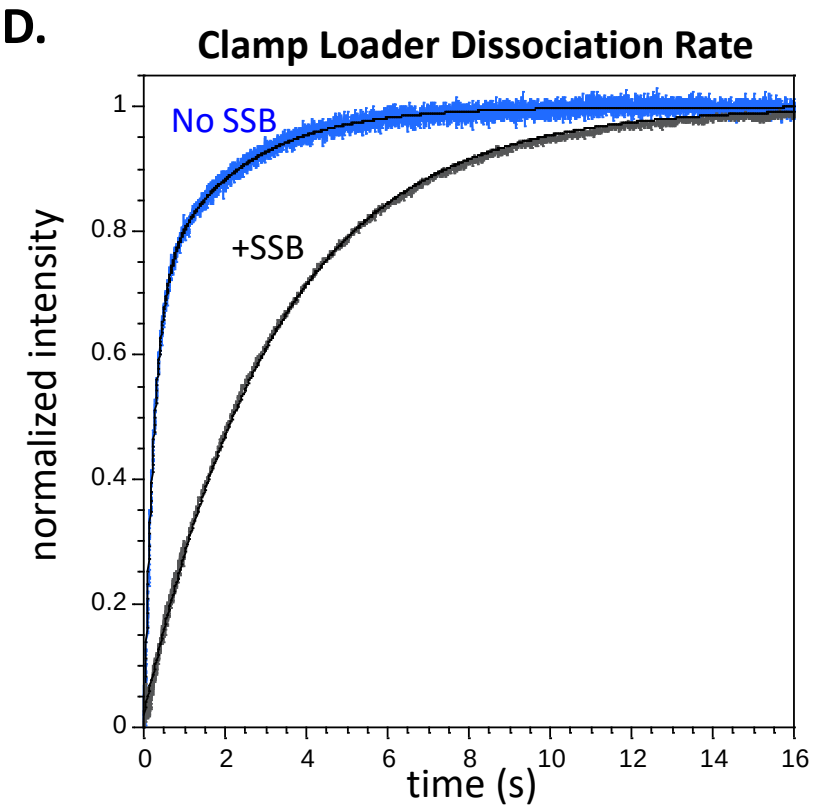
FRET Clamp Loader Binding Assay



- Clamp loader and sliding clamps pre-incubated before addition to DNA
- Fluorescein (donor) on DNA is quenched when in proximity to β sliding clamps labeled with AF647.

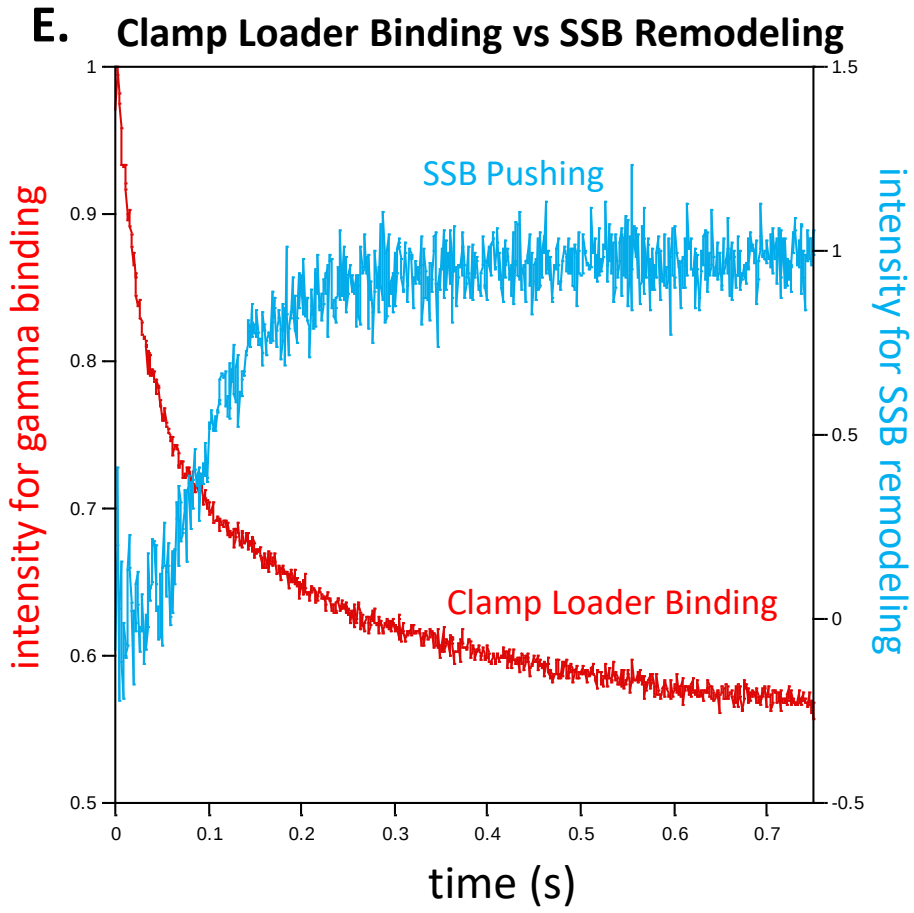
Effects of SSB on clamp loader binding and dissociation rates

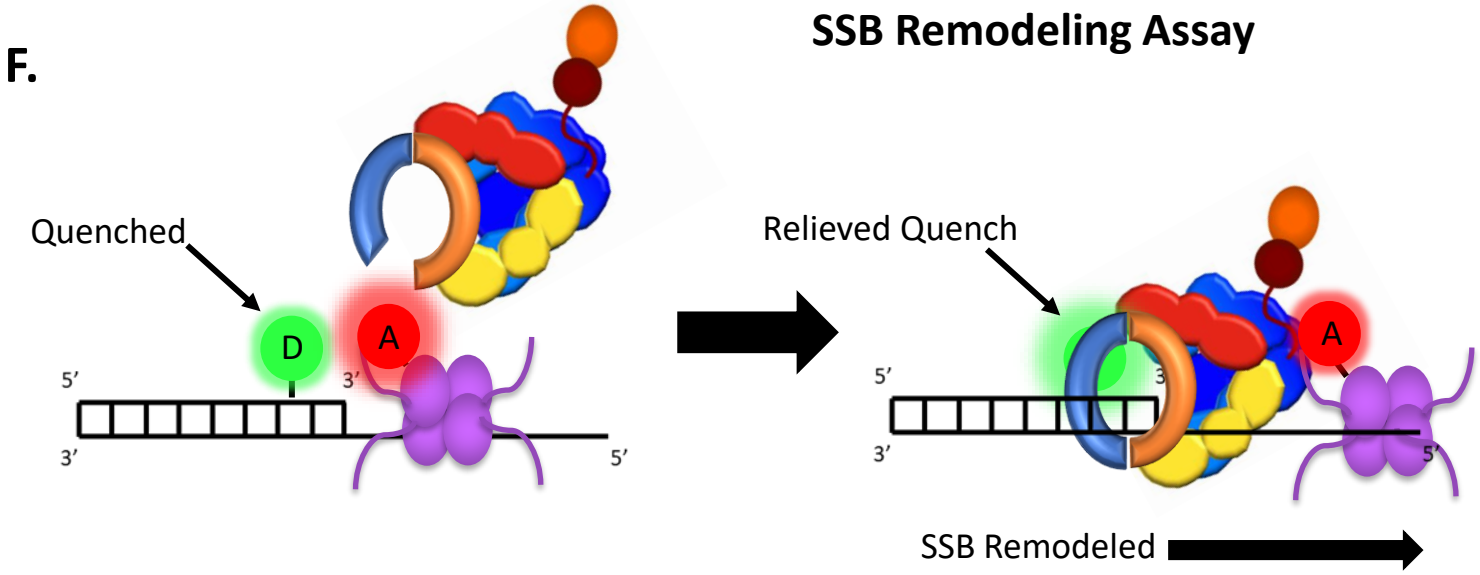




- Using binding assay shown previously, the pre-steady state rates of clamp loader binding and dissociation were measured via stopped flow
- SSB does not speed up clamp loader binding to DNA
- SSB decreases dissociation rate of clamp loader from DNA

SSB remodeling occurs on a similar timescale to clamp loader binding





- Quench of fluorescein (donor) is relieved as SSB remodeled at the 3' end of the duplex
- Clamp loader may actively remodel SSB to gain access to 3' end of duplex

Potential model for clamp closing reaction