# Developing micro-to-milisecond time resolved cryo-EM using caged ATP - GroEL a proof of concept

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**Background:** While time-resolved (TR) cryo-EM offers temporal resolutions spanning microseconds to seconds, its widespread adoption is hindered by significant technical complexity and limited throughput. Streamlining these methodologies is critical for transforming TR-cryo-EM into a versatile tool for capturing the rapid conformational dynamics of diverse biomolecules.

**Objective:** This study aimed to optimize two TR-cryo-EM workflows to achieve resolution across the µs-to-ms range. Using the chaperonin GroEL as a benchmark model, we sought to resolve the under-characterized structural mechanism of initial ATP binding, while simultaneously improving the accessibility and reproducibility of these temporal techniques.

**Methods:** We employed photolytic activation of caged (NPE) ATP via two distinct TR-Cryo-EM approaches: laser-induced melting and rapid revitrification of pre-made grids for microsecond resolution [1], and a custom-built blotting system integrated with an activation lamp for millisecond (ms) timescales [2].

**Results:** We resolved four independent temporal snapshots. The first confirmed the apo-state. The second revealed caged ATP bound within the pocket without inducing global conformational changes, suggesting unproductive binding. The third timepoint showed incomplete uncaging/binding, likely due to the extreme speed of the µs trigger. The final timepoint yielded structures consistent with the ATP-bound states reported by Torino et al. (2023) [3].

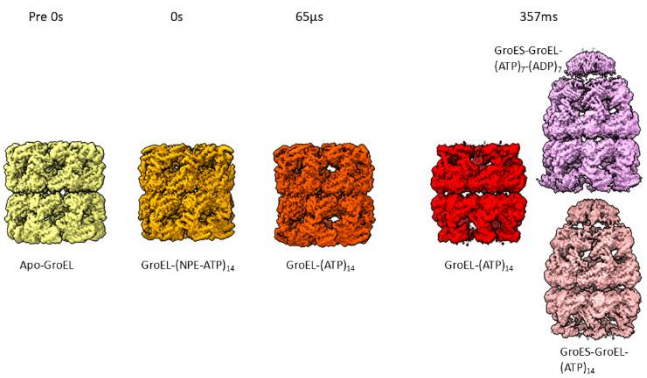


*Figure 1. The various structural GroEL timepoints collected during the study.*

**Conclusion:** Our data suggest that NPE-ATP binds uncooperatively to GroEL, contradicting previously found results [4]. Methodologically, while the µs laser-melting approach faced kinetic limitations for this specific process, the ms-blotter system proved highly reproducible. Further refinements are required to extend the blotter system's utility into the sub-millisecond range.

**References:**

1. Voss, J. M., et al. (2021) 2. Shaikh, T. R., et al. (2009) 3. Torino, S., et al. (2023) 4. von Germar, F. *et al.* (1999)