Kravats et al.'s "Unfolding and translocation pathway of substrate protein controlled by structure in repetitive allosteric cycles of the ClpY ATPase"

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Clp macromachines

- Clp: caseinolytic protease
- Found in all forms of life
- Involved with selective destruction of proteins
- Clp ATPases force protein unfolding and translocation through narrow pores
- Functional ClpATPases are homohexameric assemblies (form in presence of ATP)

The Clp ATPase channel

- Each monomer has one or two AAA domains for binding nucleotides
- Core of hexamer forms a channel with a minimum diameter of 9-15$\mathring{A}$
- Each AAA domain provides a diaphram-forming central loop in the channel
- Loops have a conserved aromatic-hydrophobic dipeptide flanked by glycines
- Loops involved with regulated substrate selection, binding to tags fused to target proteins
- Protein unfolded to pass through channel, often involving ATP hydrolysis
- Mechanical force comes from nucleotide-dependent loop displacement

Possible mechanisms

Paddles cannot generate enough force to match AFM-measured unfolding forces.
What's going on?

- Loop padling action
- Distinct unfolding mechanisms (?) from AFM-based studies
- Constant force + periodic modulation to shift AFM unfolding pathway

This paper

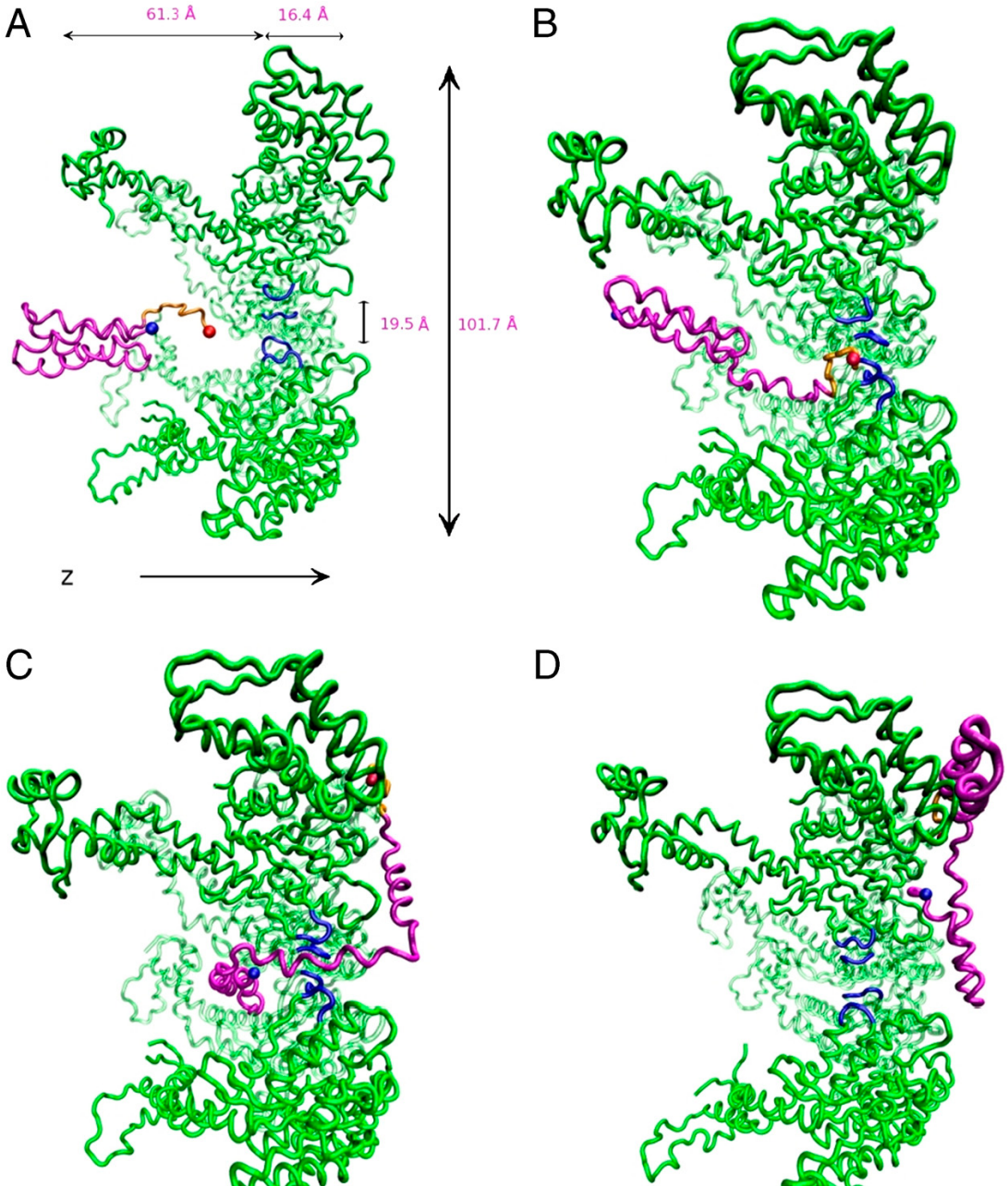
Approach:

- Coarse-grained simulation
- Check each mechanism to estimate timescale

Results:

- Obligatory unfolding intermediate (C-terminal unraveling)
- Non-native substrate conformations stabilized by ClyY at pore entrance
- Weaker loop forces sufficient to unfold and translocate stabilized substrate

Snapshots of unfolding and translocation



Simulating AFM unfolding

- Fix N terminus, pull C terminus at constant velocity
- Identify major pathway (75%) where N-terminal helix unravels first, followed by C-terminal helix and intermediate helicies.
- Simulated peak forces $\sim$ 50-100 pN. (experiment: 25-35 pN)
- Similar pathway to implicit-solvent simulations

Is ATP required?

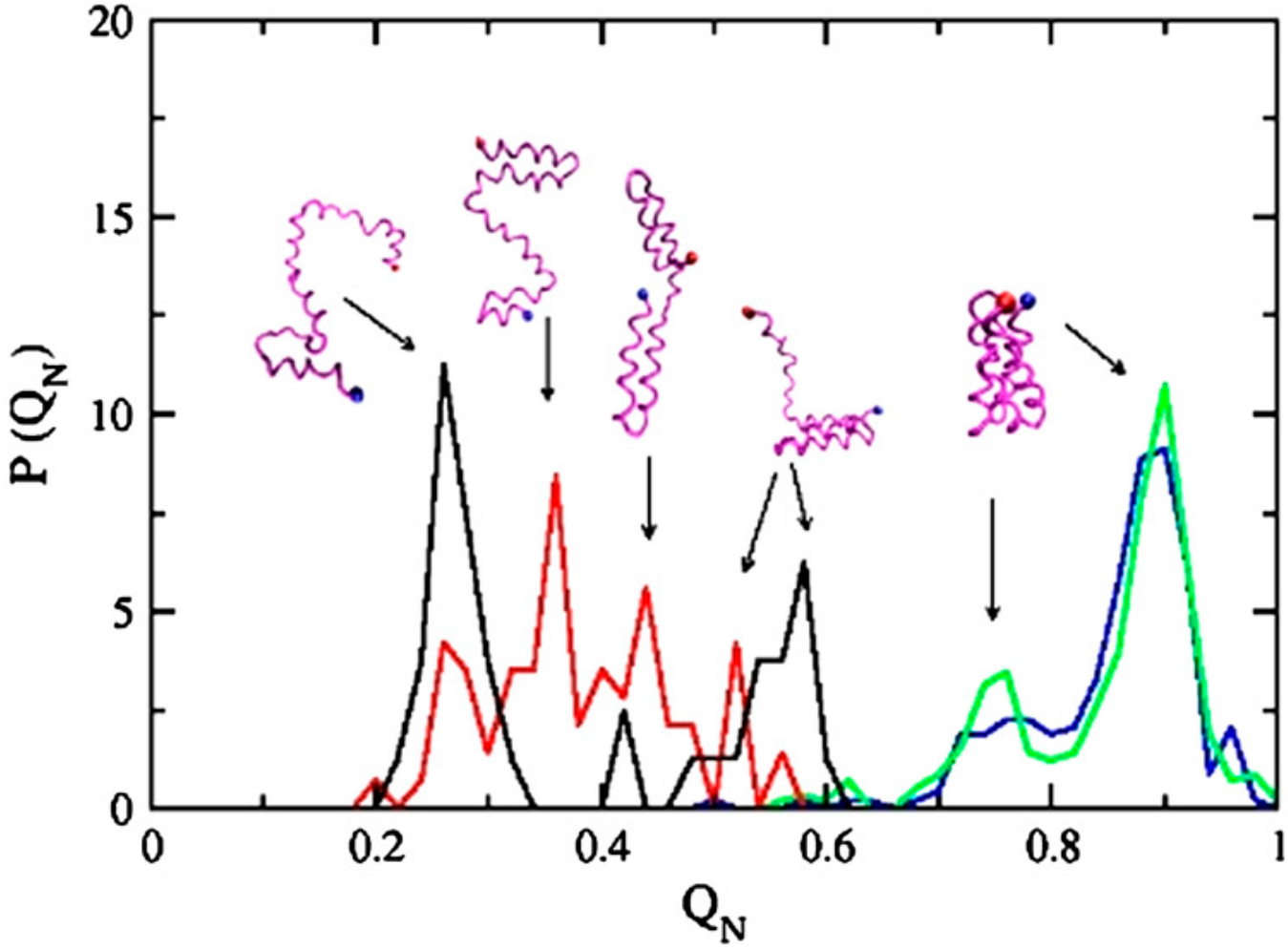
- Presented HBP to nonallosteric, open-state ClpY ring
- SsrA tag binds to central channel loops
- No HBP translocation, HPB retains native structure

ATP-driven pumping

- Simulate ATP pumping by forcing subunit motion (more later)
- Generates periodic force driving substrate
- Brings folded HBP up against channel loops
- Starts unraveling C terminus

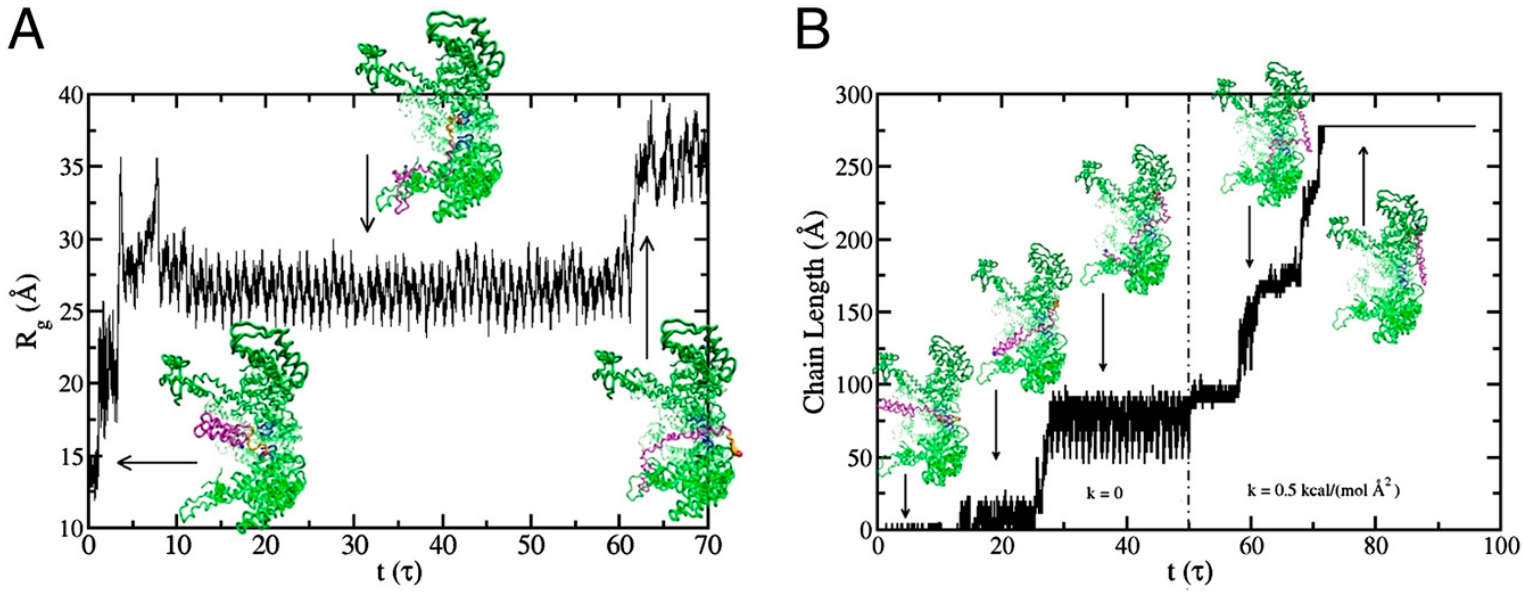
Fraction of native contacts

Blue: after binding, black: after 50 ATP-driven cycles, red: after 50 cycles with ClpQ, green: native

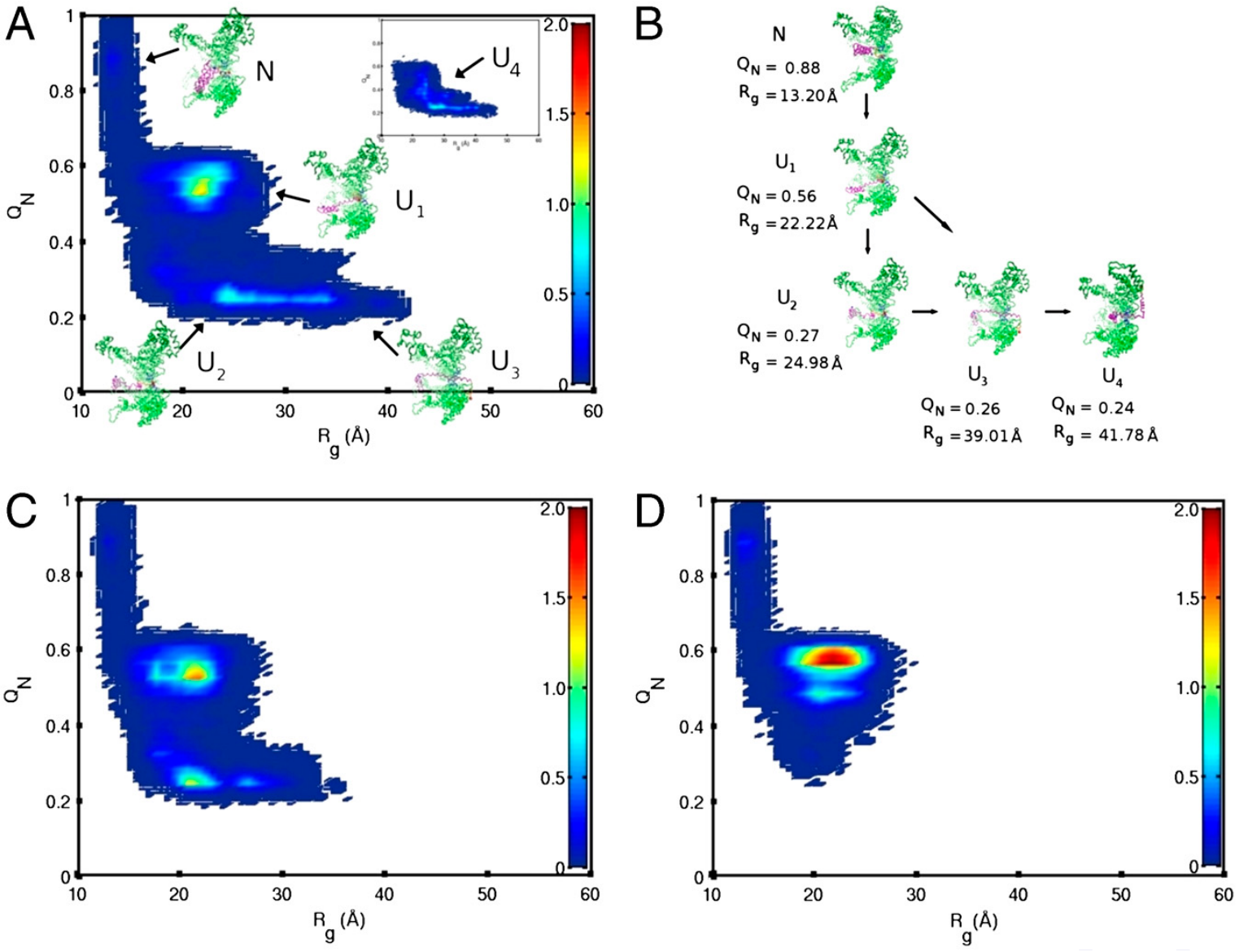


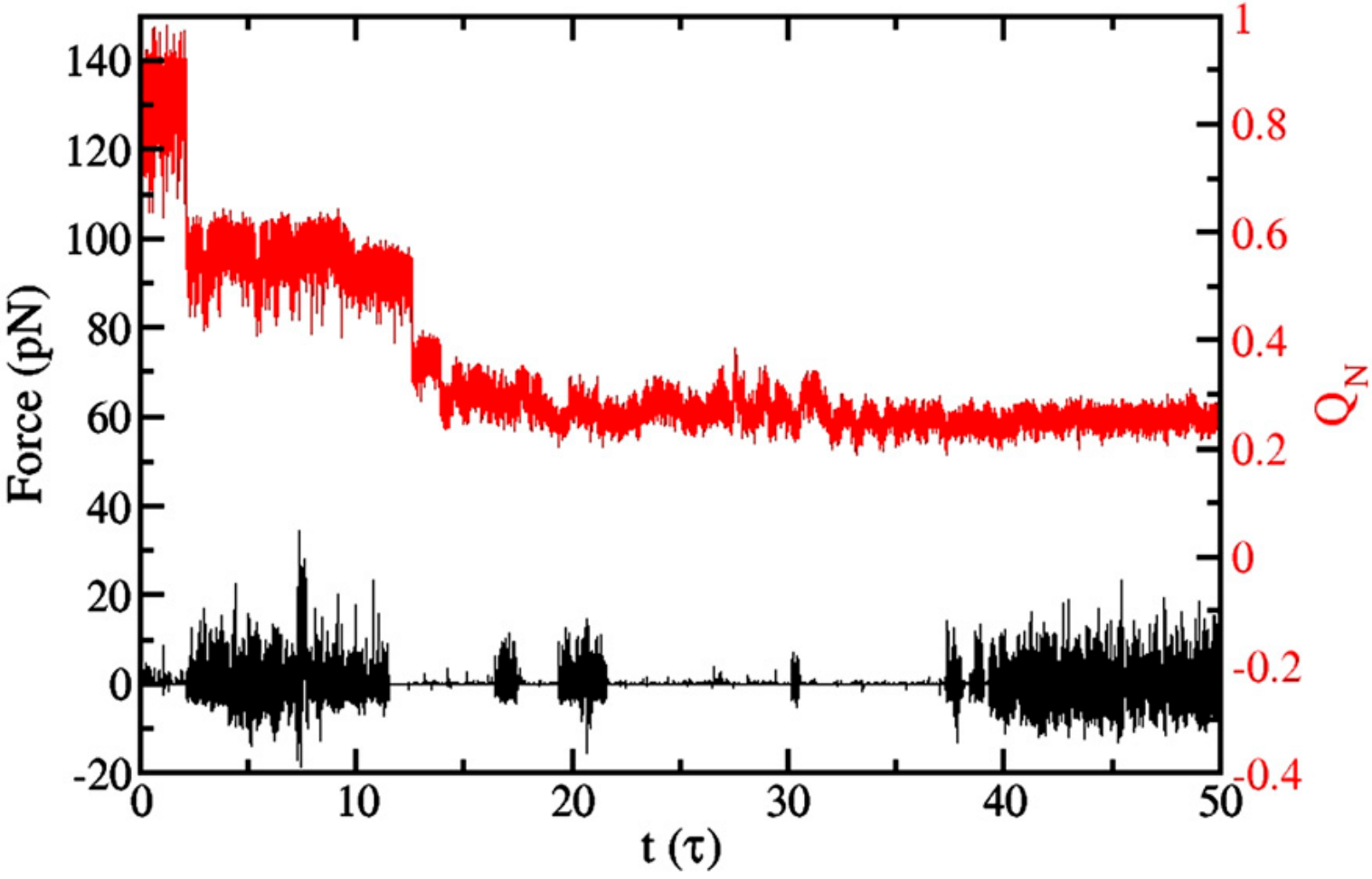
Translocation and radius of gyration

(A) Radius of HBP under ATP-driven ClpY; (B) Length of HBP translocated in the pore



Fraction native contacts and radius of gyration




Force (pN)
140
120
100
80
60
40
20
0
-20
0
10
20
30
40
50
t (τ)
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
Q_N