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Interaction of substrate-mimicking peptides with the AAA+ ATPase ClpB from Escherichia coli


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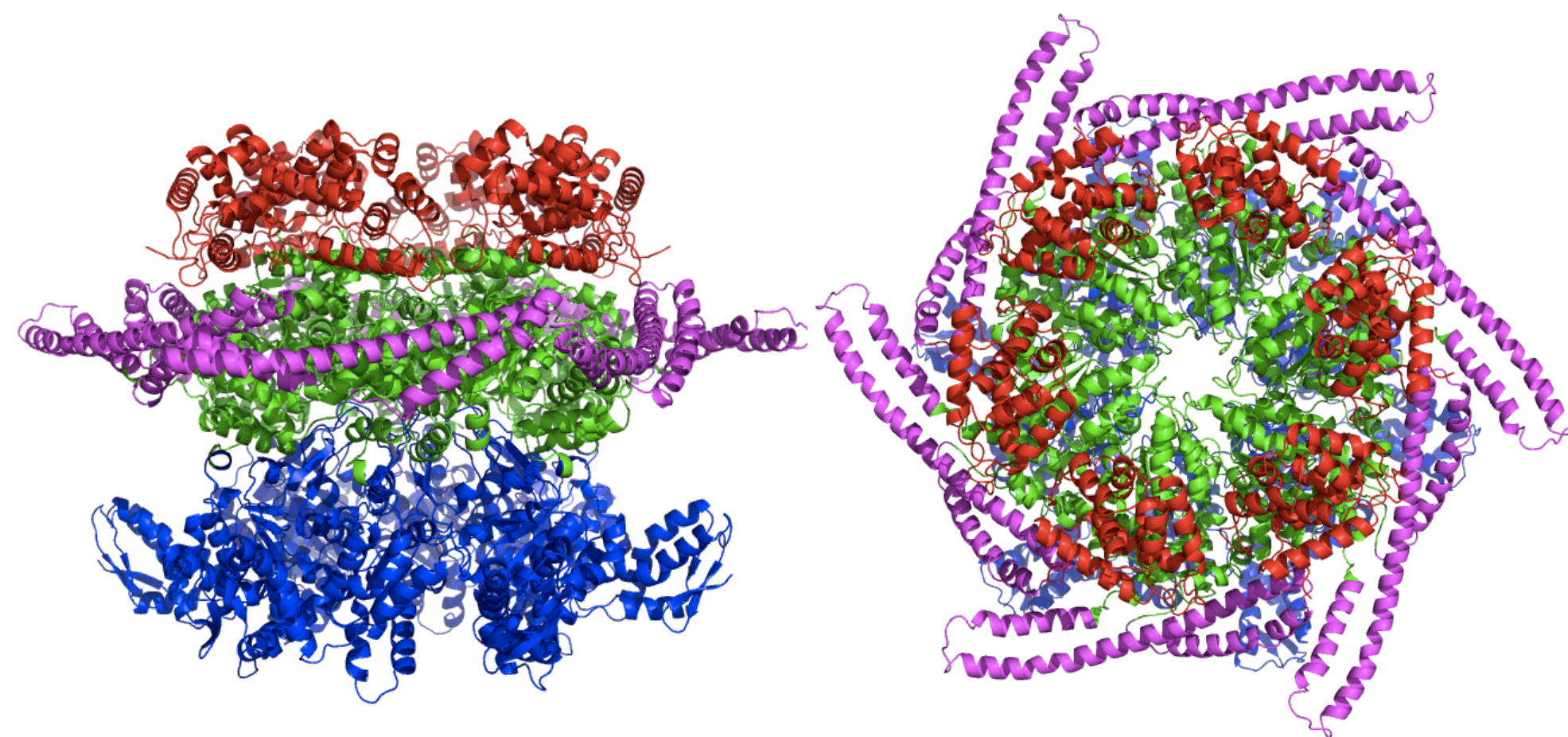
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Interaction of substrate-mimicking peptides with the AAA+ ATPase ClpB from *Escherichia coli*

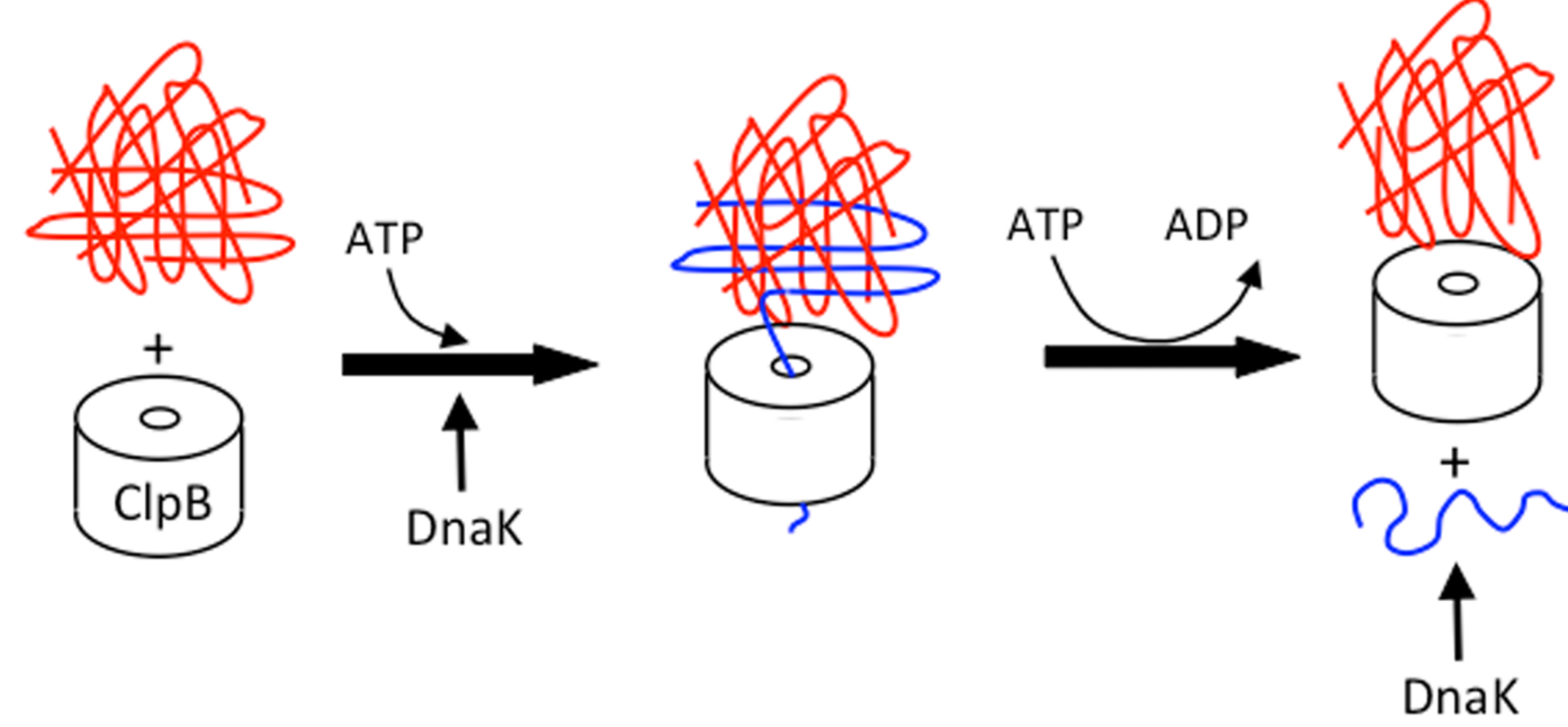
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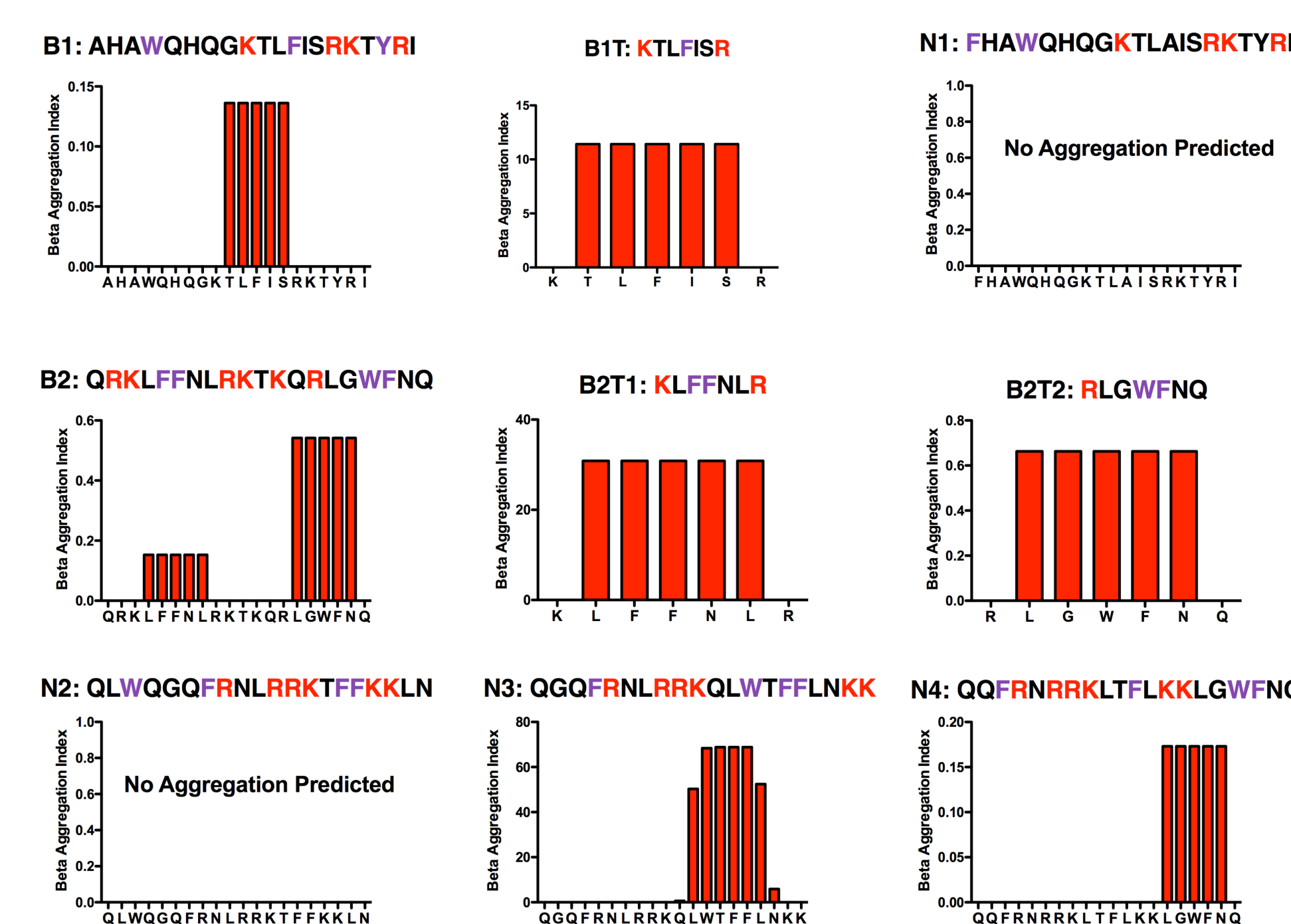
1. ClpB is an energy-dependent protein disaggregase



ClpB reactivates aggregated proteins in cooperation with DnaK. The ClpB monomer contains two AAA+ ATP-binding domains (D1, D2), the coiled-coil domain, and the N-terminal domain. The ClpB-mediated protein disaggregation is linked to translocation of substrates through the central channel in the hexameric ClpB.

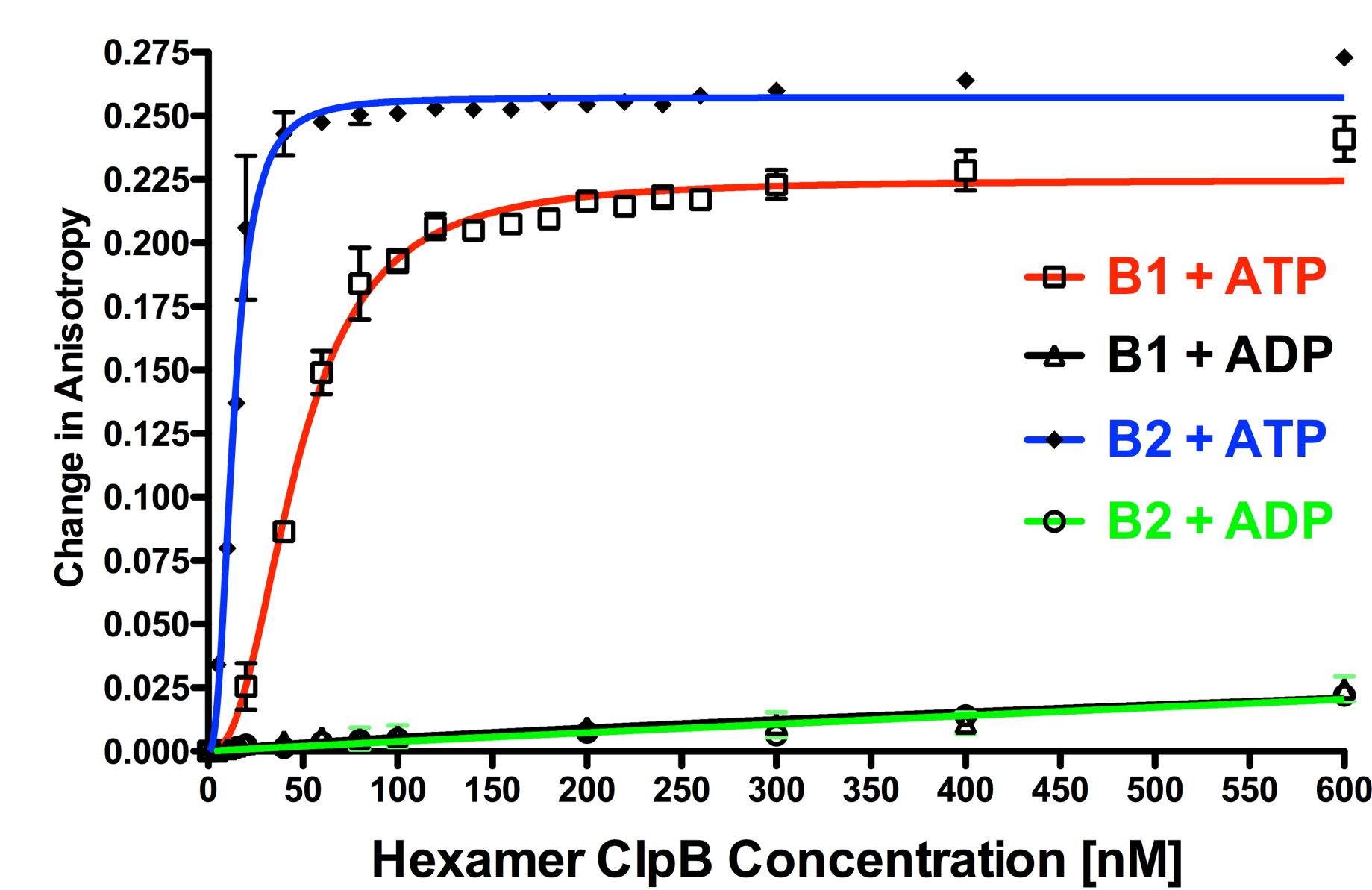


2. Prediction of aggregation-prone segments in ClpB-binding peptides



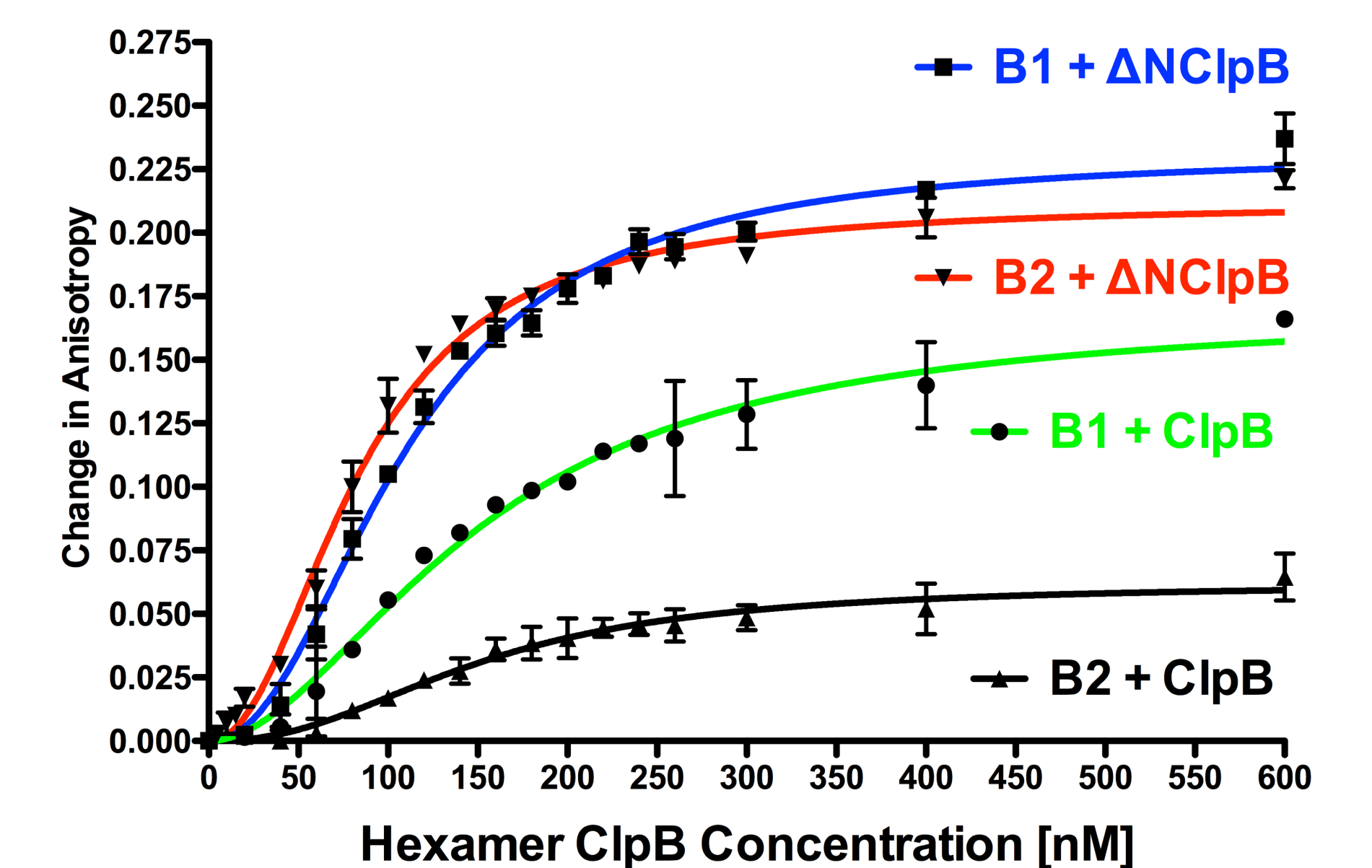
We investigated the peptides B1 and B2, which have been shown to mimic ClpB substrates (1). The TANGO algorithm (2) predicts that B1 and B2 contain discrete aggregation-prone sequence segments. To test the role of the aggregation-prone segments in supporting binding to ClpB, we also produced truncated and scrambled derivatives of B1 and B2. The peptides have been labeled with FITC at their N termini.

3. ATP, but not ADP, stimulates peptide binding to ClpB



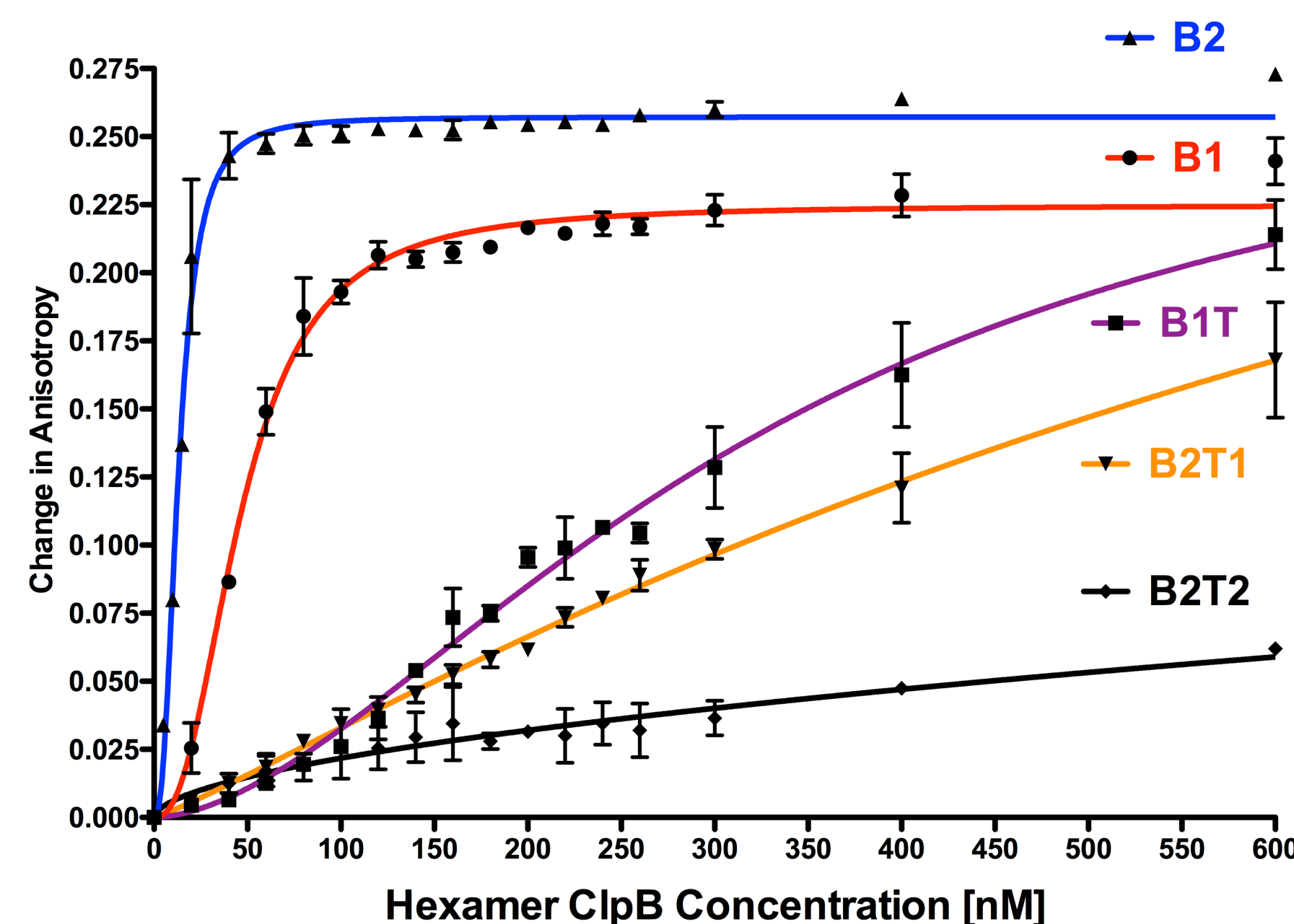
Binding of the peptides to the ClpB-trap variant (3) was associated with an increase in the FITC fluorescence anisotropy. The binding required ATP, which is consistent with the substrate-binding mechanism of the AAA+ ATPases. The peptide binding to ClpB showed positive cooperativity, consistent with the linkage between substrate binding and ClpB self-association into hexamers.

4. The N-terminal domain of ClpB is not required for peptide binding



B1 and B2 bind to wild type ClpB in the presence of ATP_γS. The N-terminally truncated ΔNClpB binds the peptides with a higher affinity than the full-length ClpB.

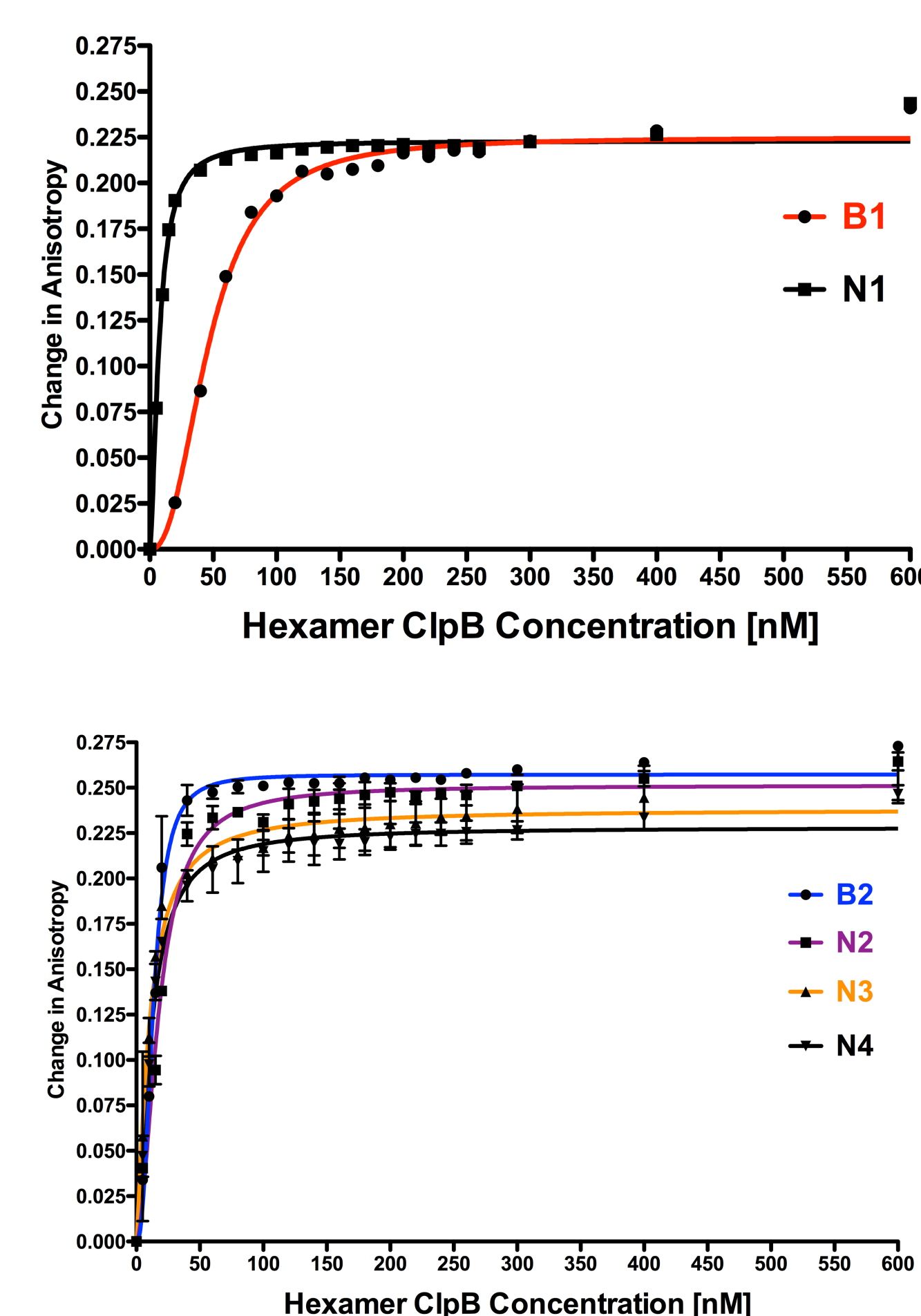
5. Aggregation-prone segments are not sufficient to support the peptide binding to ClpB



Truncated peptides that contain only the aggregation-prone segments in B1 and B2 interact with ClpB with a lower affinity than the full-length B1 and B2.

This experiment was performed using ClpB-trap in the presence of ATP.

6. Aggregation-prone segments are not necessary to support the peptide binding to ClpB



The affinity of the scrambled peptides towards ClpB does not correlate with the presence of aggregation-prone regions. This experiment was performed using ClpB-trap in the presence of ATP.

7. Summary of the ClpB-peptide interaction affinities

Peptide	K_d [nM]	Hill Coefficient
B1	47	2.4
B1T	329	1.7
B2	13.2	2.5
B2T1	912	1.1
B2T2	>1000	0.5
N1	7.3	1.4
N2	17.6	1.7
N3	10.2	0.9
N4	11.5	1.3

All experiments were performed using ClpB-trap in the presence of ATP. The non-linear least-squares fitting was performed using GraphPad Prism software.

8. Conclusions

- We tested the hypothesis that the predicted aggregation-prone segments in peptides mediate the substrate recognition by ClpB. We found that the aggregation-prone segments are neither sufficient nor necessary for the peptide interactions with ClpB.
- Our results suggest that the substrate recognition mechanism of ClpB may rely on global surface properties of aggregated proteins rather than on local sequence motifs.

References:

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- Fernandez-Escamilla et al., Nat. Biotech., **22**, 1302, 2004.
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