

# Supplementary Information for “Tube geometry controls protein cluster conformation and stability on the endoplasmic reticulum surface”

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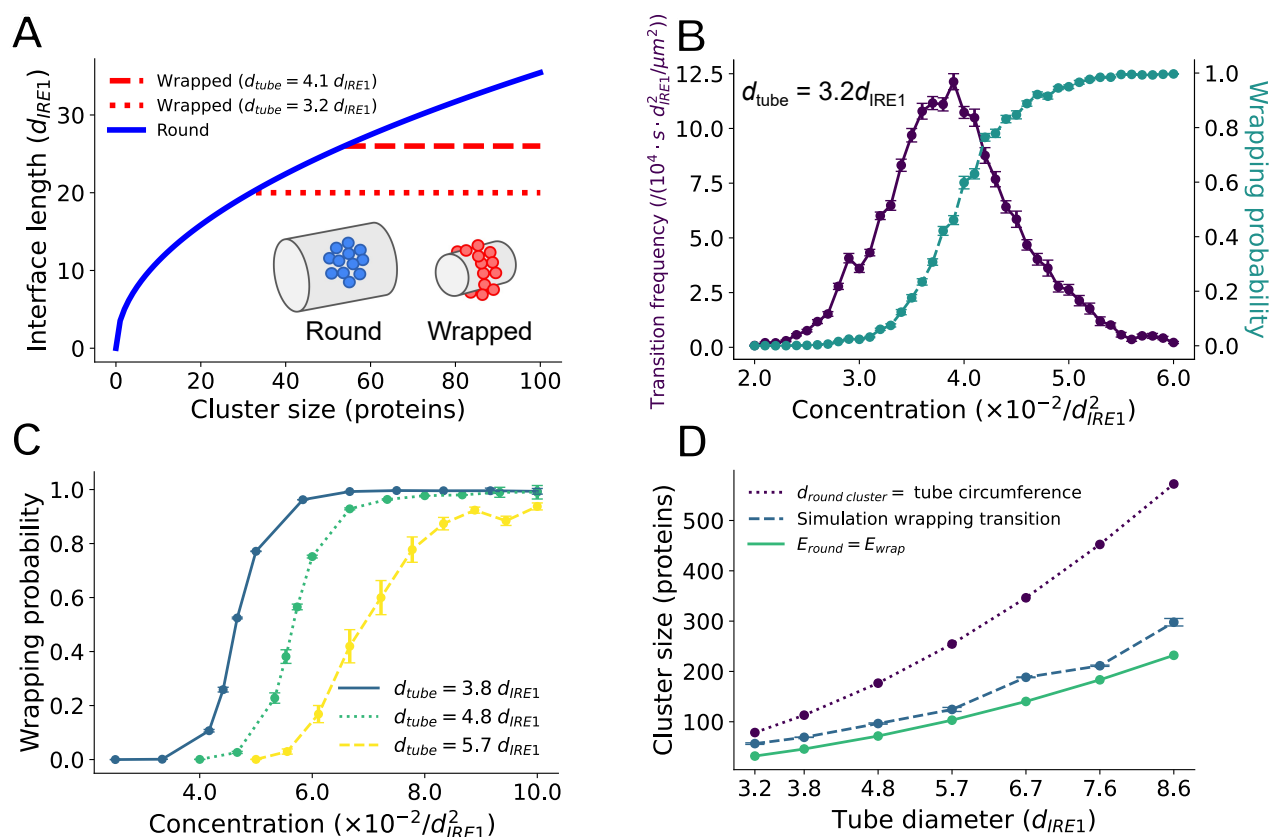


Figure S1: Cluster conformation transitions. (A) Schematics of round (left inset) and wrapped (right inset) cluster conformations. Cluster interface lengths for round (blue solid curve), and wrapped (red dashed and dotted, tube diameters  $4.1 d_{IRE1}$  and  $3.2 d_{IRE1}$ , respectively) clusters on tubes. (B) Frequency of transitions between round and wrapped clusters on a  $3.2 d_{IRE1}$  diameter and  $100 d_{IRE1}$  long closed tube, averaged over 24 runs; and corresponding probability of wrapped cluster conformation. Error bars shown are the standard error.  $J = 3 k_B T$ . (C) Probability of wrapped cluster conformation on a closed tube (no proteins enter or exit) for various protein concentrations, with  $3.8 d_{IRE1}$ ,  $4.8 d_{IRE1}$  and  $5.7 d_{IRE1}$  tube diameter and  $100 d_{IRE1}$  length. Simulations begin with all proteins in a round cluster at tube center.  $J = 3 k_B T$ , average of 24 runs. Error bars shown, the standard error, are larger for larger  $d_{tube}$  due to slower transitions between cluster conformations. (D) Cluster size at which the interface energy of wrapped clusters becomes energetically favored compared to round clusters (solid curve, Eq. 2), cluster size at which 50% of clusters are in a wrapped conformation in simulations over 24 runs from C (dashed curve), and cluster size at which round cluster diameter is equal to tube circumference (dotted curve). For simulation wrapping transition, IRE1 concentration at which wrapping probability is 50% varies with  $d_{tube}$ , as in C. Error bars for simulation wrapping transition are uncertainty for linear fit of data in C near 50% wrapping probability. Tube length is  $100 d_{IRE1}$  and  $J = 3 k_B T$ .

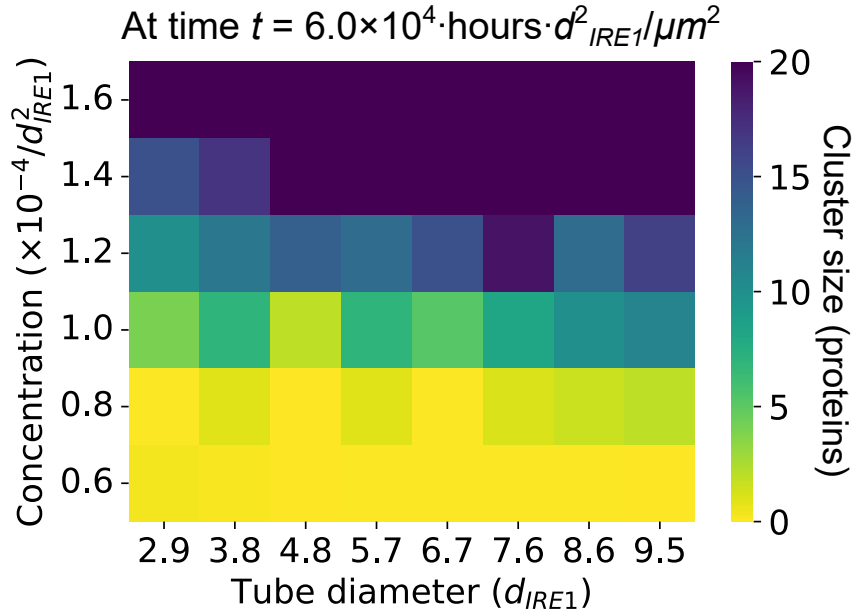


Figure S2: Cluster formation with open boundary conditions. The IRE1 external (to the tube region under consideration) concentration  $c_{\text{ext}}$  and the tube diameter are varied, with color map indicating mean number of proteins in a cluster at a time  $6.0 \times 10^4 \cdot \text{hours} \cdot d_{IRE1}^2 / \mu\text{m}^2$  after initializing with one protein in the tube, averaged over 20 runs with tube length of  $100 d_{IRE1}$  and  $J = 5.3 k_B T$ . Cluster sizes over 20 are indicated as 20. Each tube initially contains a single IRE1 protein, as the maximum diameter and concentration sampled correspond to less than a single IRE1 protein in the tube region. Standard errors of these cluster sizes are shown in Fig. S8F.

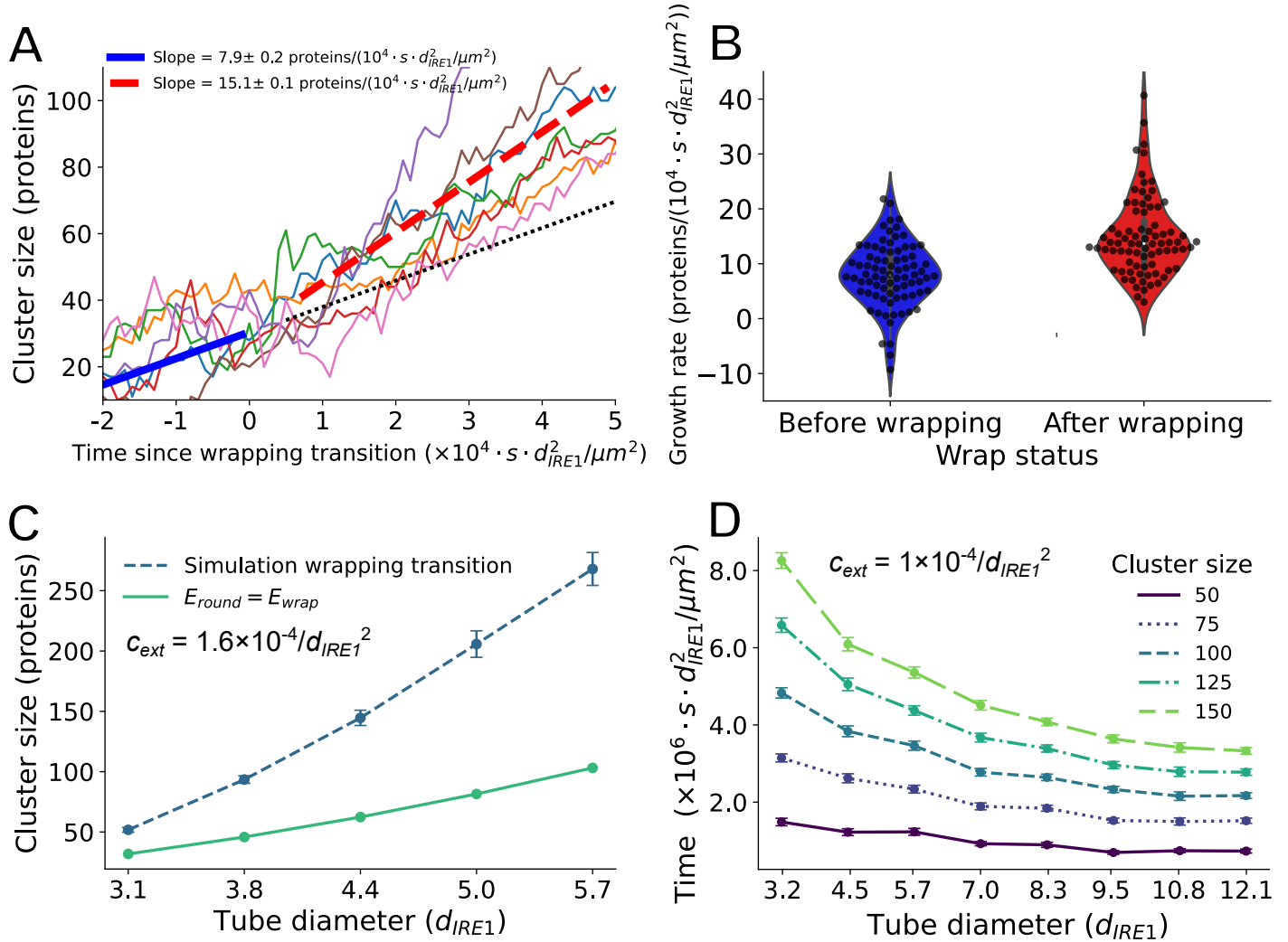


Figure S3: Cluster conformation affects cluster growth rate. (A) Individual cluster growth trajectories (thin lines) vs time, as well as linear fits of 80 cluster size trajectories before (thick solid blue) and after (thick dashed red) round-to-wrapped transition, which is set at  $t = 0$ .  $J = 3 k_B T$ ,  $c_{ext} = 34 \times 10^{-4} / d_{IRE1}^2$ ,  $3.0 d_{IRE1}$  tube diameter, and tube length of  $100 d_{IRE1}$ . (B) Violin plot of the 80 sample cluster growth rates before and after the cluster wrapping transition, with same parameters as (A). The cluster growth rate distribution before the wrapping transition is distinct from the distribution after the transition, with Kolmogorov-Smirnov p-value of approximately  $10^{-7}$ . Before wrapping has a mean growth rate of  $8.0 \pm 0.6$  proteins/ $(10^4 \cdot s \cdot d_{IRE1}^2 / \mu m^2)$  and after wrapping has a mean growth rate of  $14.9 \pm 0.8$  proteins/ $(10^4 \cdot s \cdot d_{IRE1}^2 / \mu m^2)$ , indicating a significant difference in mean growth rates before and after clustering. (C) Cluster size at wrapping transition vs tube diameter, with cluster size at which the interface energy of wrapped clusters becomes energetically favorable (solid curve, Eq. 2) and cluster size at which wrapping transition occurs during cluster growth simulations (dashed curve). Tube length  $100 d_{IRE1}$ ,  $J = 5.3 k_B T$ ,  $c_{ext} = 1.6 \times 10^{-4} / d_{IRE1}^2$ , averaged over 30 samples. (D) Mean time period for a cluster to grow to a specific size (see legend) vs tube diameter.  $J = 5.3 k_B T$ ,  $c_{ext} = 1 \times 10^{-4} / d_{IRE1}^2$ , tube length of  $100 d_{IRE1}$ , averaged over 30 samples with initial cluster size of 30 proteins. Error bars are the standard error in panels C and D.

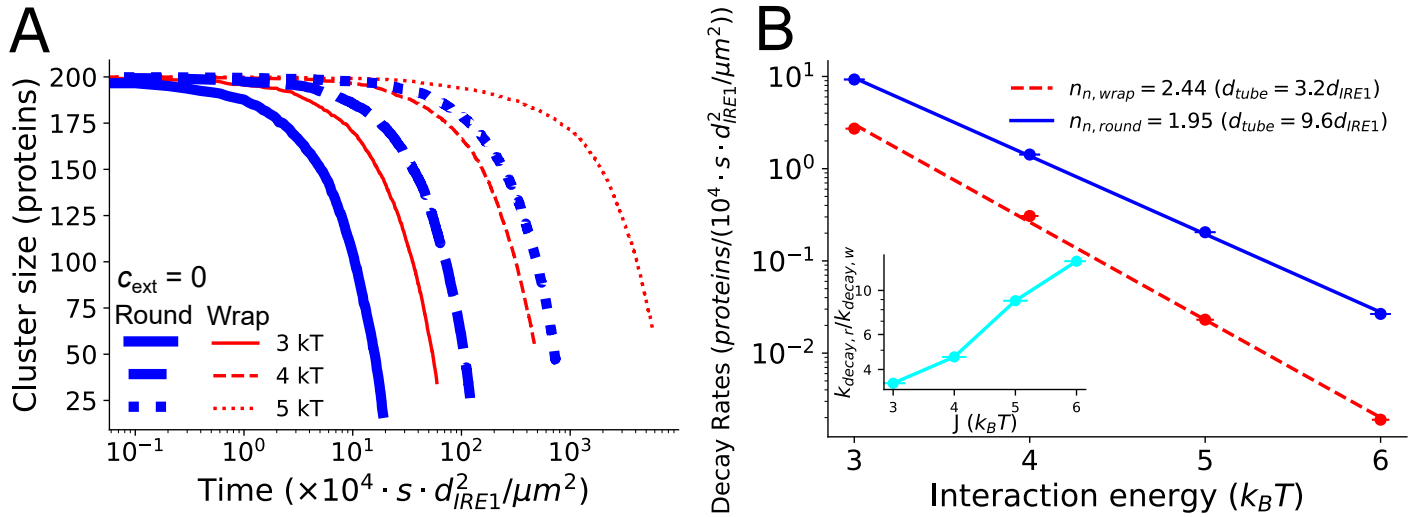


Figure S4: Cluster decay. (A) Mean cluster size vs time with external concentration  $c_{ext} = 0$ . Red (thin) lines are a wrapped cluster on a narrow ( $3.2 d_{IRE1}$  diameter) tube and blue (thick) lines are a round cluster on a wide ( $9.6 d_{IRE1}$  diameter) tube.  $J = 3 k_B T$  (solid curves),  $J = 4 k_B T$  (dashed), and  $J = 5 k_B T$  (dotted). Cluster size is mean over 30 samples, with clusters initially 200 proteins,  $100 d_{IRE1}$  tube length. Error bars (standard error) are too small to be shown. (B) Cluster decay rates from decay curves in (A), which are over 30 samples, clusters initially with 200 proteins, and  $100 d_{IRE1}$  tube length. Indicated  $n_{n,round}$  and  $n_{n,wrap}$  found by linear regression. Inset shows ratio of round cluster (wide tube) decay rate to wrapped cluster (narrow tube) decay rate,  $k_{decay,r}/k_{decay,w}$ .

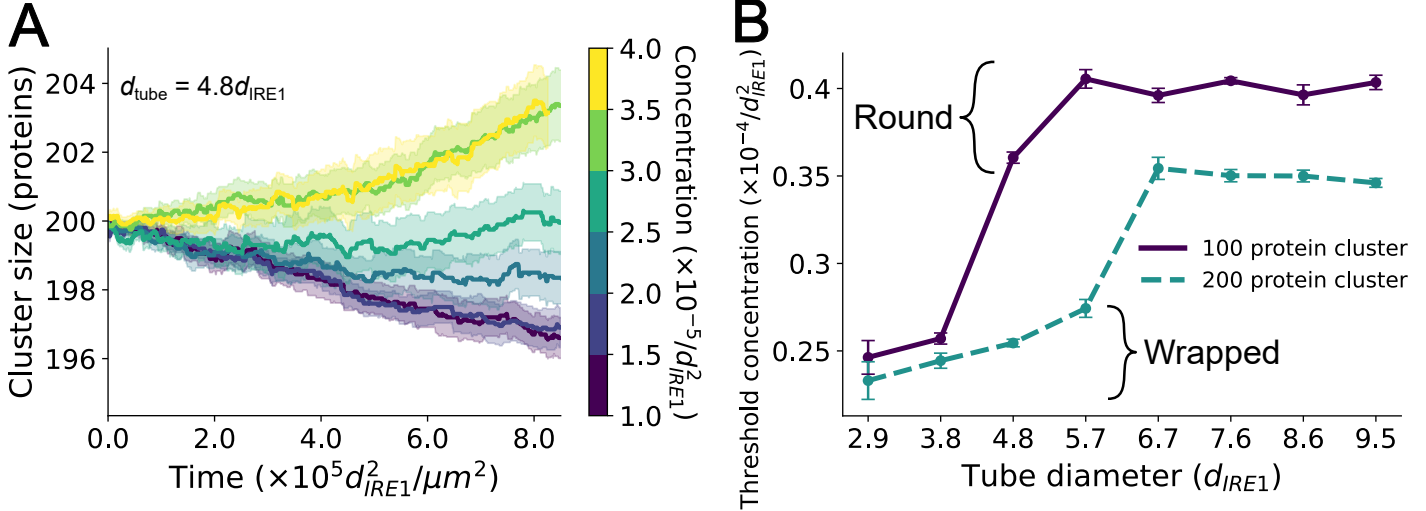


Figure S5: Threshold concentration between cluster growth and decay. (A) Mean trajectories for clusters in tube with different external concentrations  $c_{ext}$  (each curve is a different  $c_{ext}$ ). External concentration  $c_{ext}$  as indicated by color bar. Each concentration averaged over 20 runs. Clusters begin with 200 proteins, and begin as round unless tube is too narrow, in which case the cluster starts wrapped. Translucent region around concentration means represent the standard error. Tube length of  $100 d_{IRE1}$ , tube diameter of  $4.8 d_{IRE1}$ , and  $J = 5.3 k_B T$ . (B) Threshold concentration  $c_0$  between cluster growth and decay as tube diameter is varied. Threshold concentration determined by linear fit of cluster growth rates (averaged over 25 runs) near zero cluster growth. For diameters less than the diameter at which the threshold concentration transitions (suddenly jumps), clusters are wrapped; for diameters greater than the transition, clusters are round. Solid line for initial 100 protein cluster and dashed line for initial 200 protein cluster. Tube length  $100 d_{IRE1}$ ,  $J = 5.3 k_B T$  as in the phase diagram of Fig. S2. Error bars for threshold concentration are uncertainty for concentration at which linear fit of cluster growth rate crosses zero for data as in A.

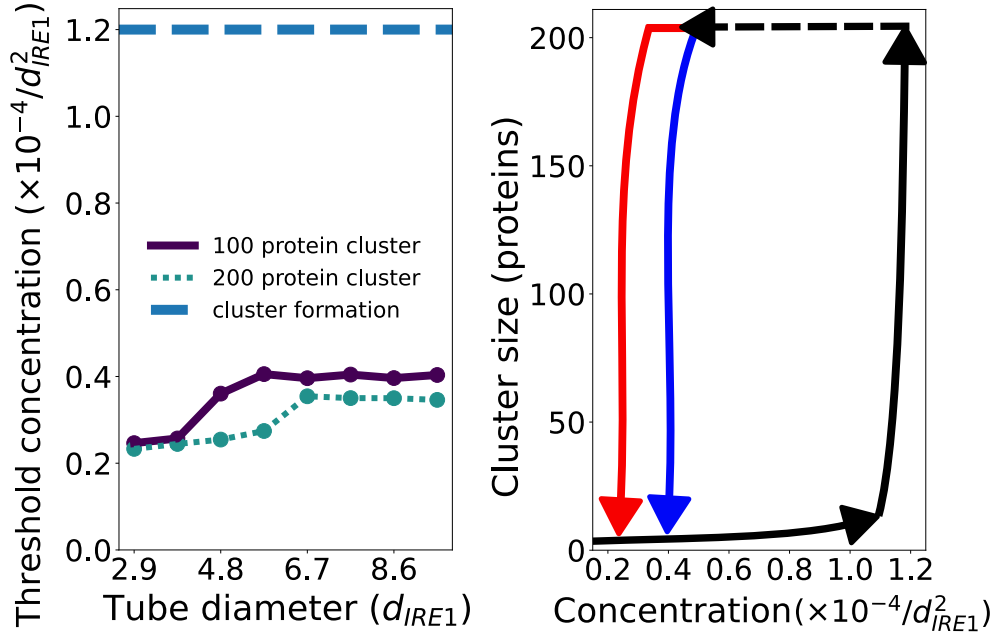


Figure S6: Schematic summary of IRE1 concentrations important for cluster dynamics. Left: Threshold concentrations for cluster formation (dashed light blue line, Fig. S2), decay of 100- and 200-protein clusters (solid and dotted lines, respectively. Data from Fig. S5B). Clusters do not form until a relatively high IRE1 concentration is reached (approximately  $1.2 \times 10^{-4}/d_{IRE1}^2$  in Fig. S2) — as concentration decreases, clusters can grow below this high cluster formation threshold. Clusters will decay on wider tubes at a higher concentration than on narrower tubes, because clusters on narrower tubes are in a wrapped configuration and are more stable, compared to round clusters on wider tubes. Right: Schematic of cluster growth and decay trajectory. Concentration begins near zero, with no cluster. As the concentration increases past the cluster formation threshold, clusters will form and grow (black solid curve). As the concentration begins to decrease, the cluster growth diminishes (dashed black curve). Once the concentration is sufficiently low, first round clusters (blue solid curve) and then wrapped clusters (red solid curve) will decay.  $J = 5.3 k_B T$  for both panels.

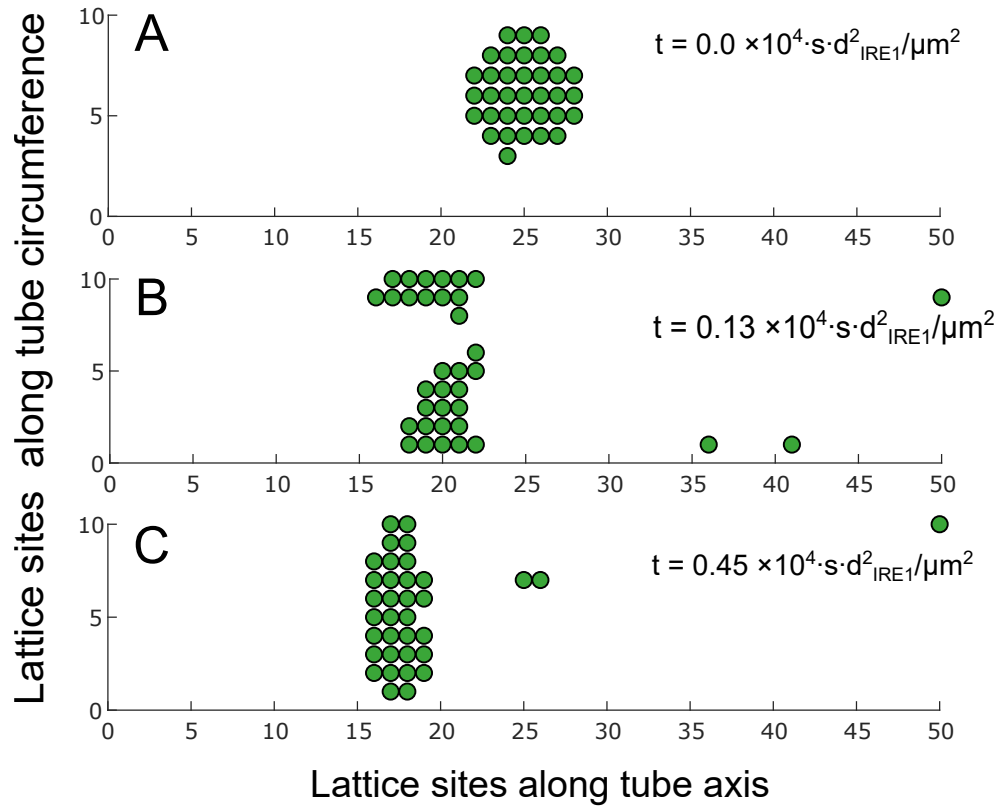


Figure S7: Snapshots of cluster wrapping transition. The lattice has periodic boundary conditions in the direction of the tube circumference. (A) Cluster is in an approximately round state. (B) Cluster is growing long in the direction of the circumference of the tube. (C) The cluster has wrapped around the tube. Closed boundary conditions at tube edges,  $J = 3 k_B T$ , and 35 proteins in approximately  $3.2 d_{IRE1}$  diameter and length  $50 d_{IRE1}$  tube.

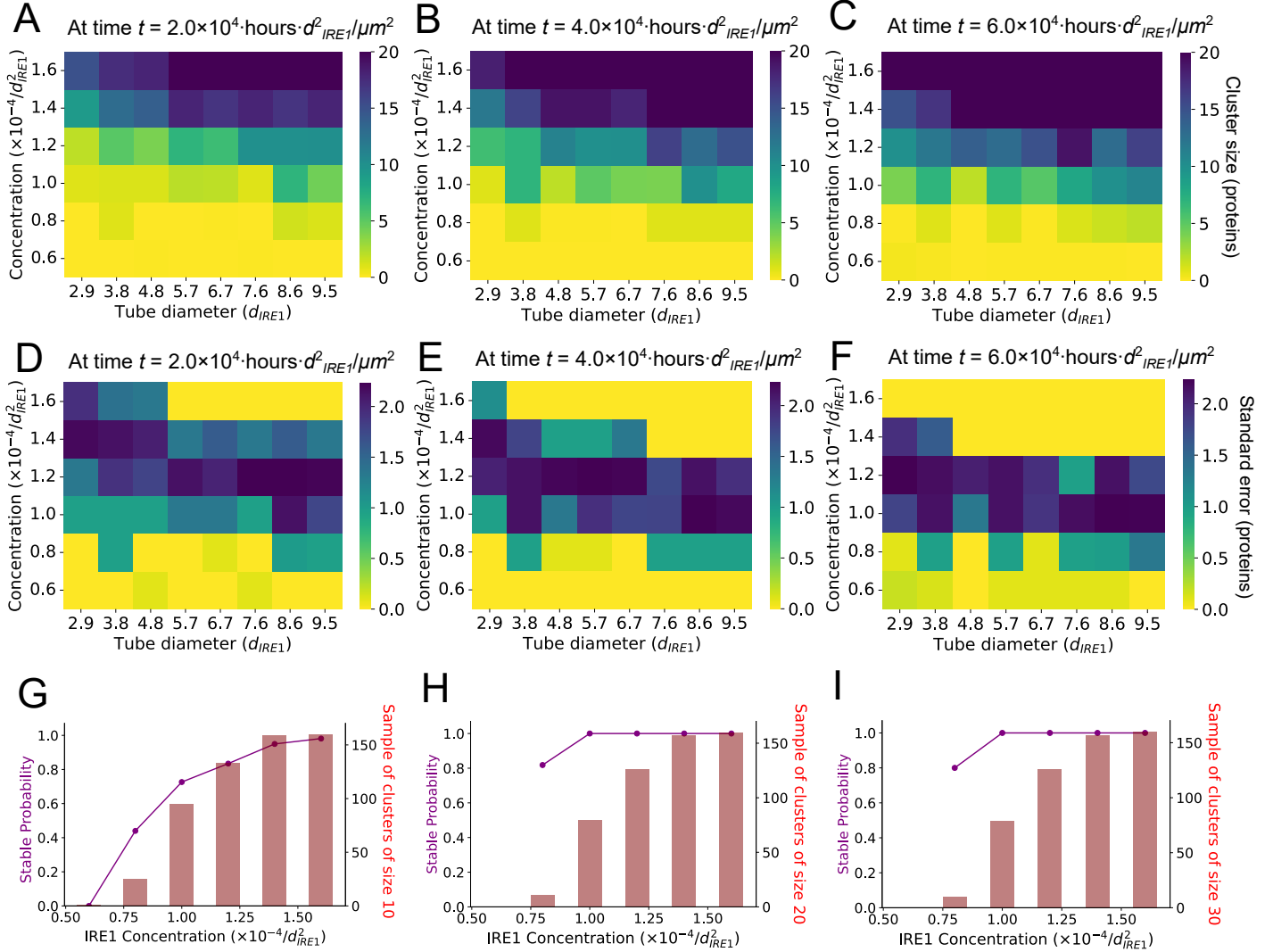


Figure S8: Cluster formation with open boundary conditions. (A,B,C) mean IRE1 cluster size with open boundary conditions at the indicated times. The IRE1 external (to the tube region under consideration) concentration  $c_{ext}$  and the tube diameter are varied, with color map indicating mean number of proteins in a cluster. Clusters of sizes greater than 20 are binned with cluster size 20. (D,E,F) standard error of data in panels, A,B,C, respectively. Clusters of size greater than 20 are treated as size 20 for calculation of standard error. Standard error indicated by color map. Panels A-F are over 20 runs. (G,H,I) Cluster decay probability for clusters of different size with different external IRE1 concentrations  $c_{ext}$ . The left axis (magenta line) is the probability that a cluster of size 10 (G), 20 (H), and 30 (I) will decay. The right axis (red bars) is the number of clusters included in each decay probability calculation. Data for G,H,I aggregates across tube diameter from panel C. Tube length  $100 d_{IRE1}$  and  $J = 5.3 k_B T$  across panels.

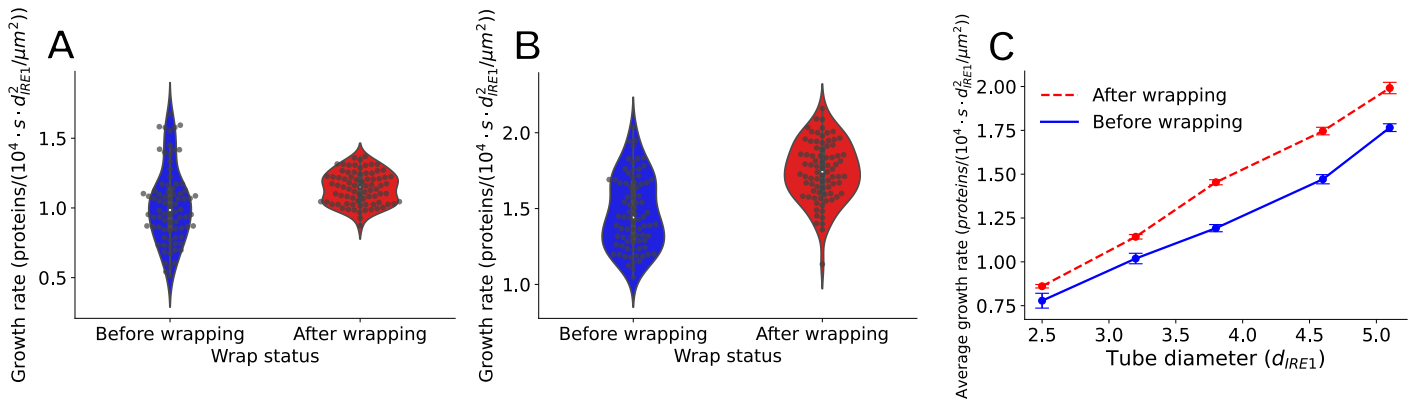


Figure S9: (A,B) Violin plots of the cluster growth rates before and after the cluster wrapping transition. (A) Tube diameter of  $3.2 d_{IRE1}$ . The Kolmogorov-Smirnov (K-S) test returned a p-value of  $4.27 \times 10^{-8}$  between the two distributions. Before wrapping has a mean growth rate of  $1.02 \pm 0.03$  proteins/( $10^4 \cdot s \cdot d_{IRE1}^2/\mu m^2$ ) and after wrapping has a mean growth rate of  $1.14 \pm 0.01$  proteins/( $10^4 \cdot s \cdot d_{IRE1}^2/\mu m^2$ ). (B) Tube diameter of  $4.6 d_{IRE1}$ . The K-S test returned a p-value of  $6.20 \times 10^{-10}$  between the two distributions. Before wrapping has a mean growth rate of  $1.47 \pm 0.03$  proteins/( $10^4 \cdot s \cdot d_{IRE1}^2/\mu m^2$ ) and after wrapping has a mean growth rate of  $1.75 \pm 0.02$  proteins/( $10^4 \cdot s \cdot d_{IRE1}^2/\mu m^2$ ). (C) Average cluster growth rate vs tube diameter before (solid blue curve) and after wrapping (red dashed curve). Error bars are standard error. For all panels,  $J = 5.3 k_B T$  as in Fig. S2, tube length is  $100 d_{IRE1}$ ,  $c_{ext} = 1.0 \times 10^{-4}/d_{IRE1}^2$ , and means averaged over 80 runs.