Clustered bottlenecks in mRNA translation and protein synthesis

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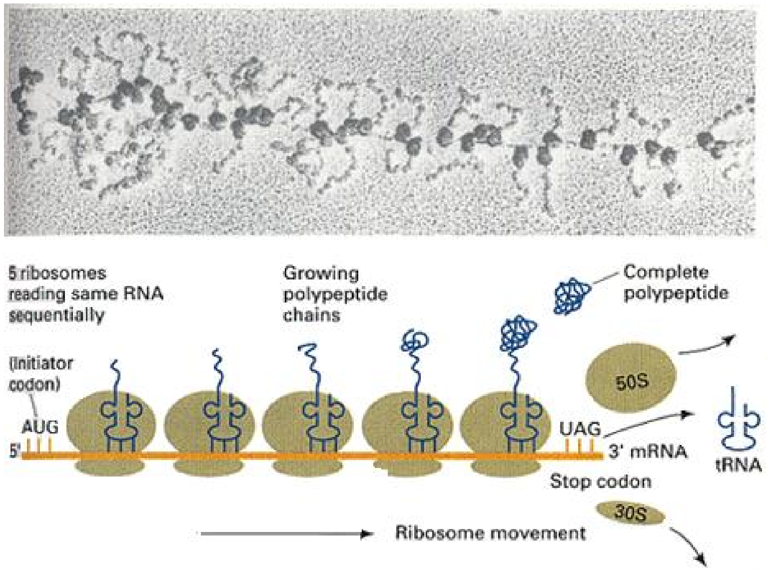
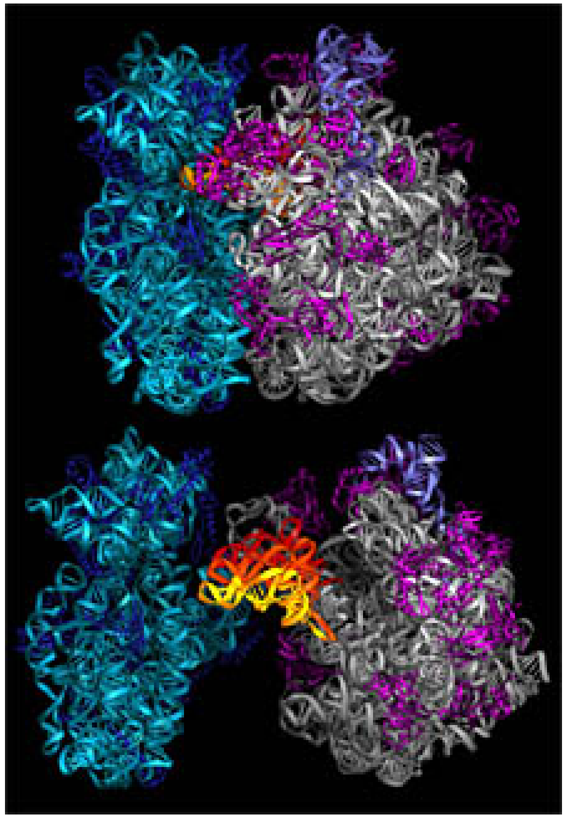
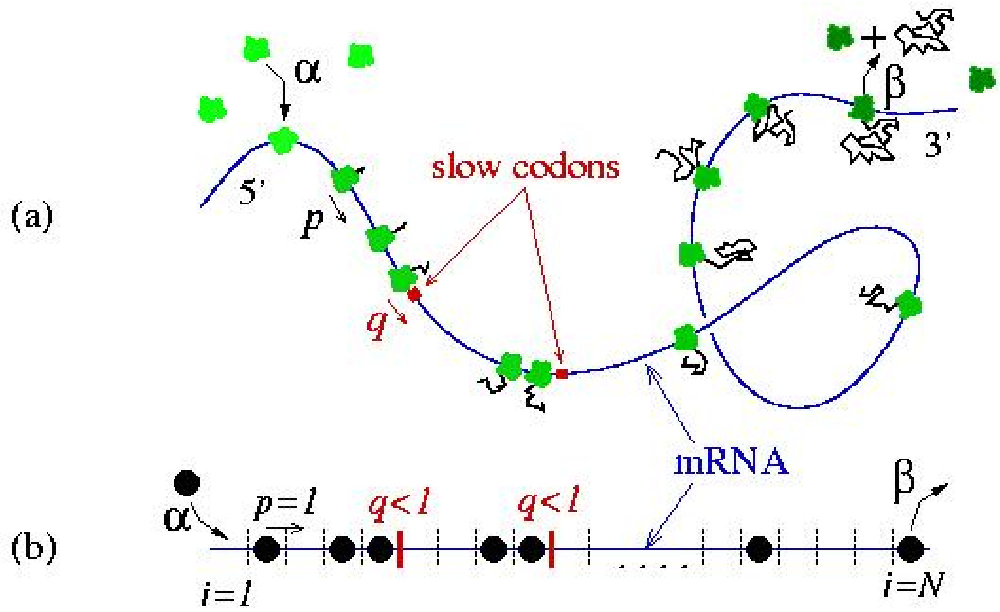
Translation machinery





Ribosome machinery assembles on initiation sites on mRNA and docks tRNA that off-load and amino acids on a growing polypeptide chain

Totally Asymmetric Exclusion (TASEP)



Totally asymmetric exclusion process on N lattice sites $\Leftrightarrow$ mRNA translation

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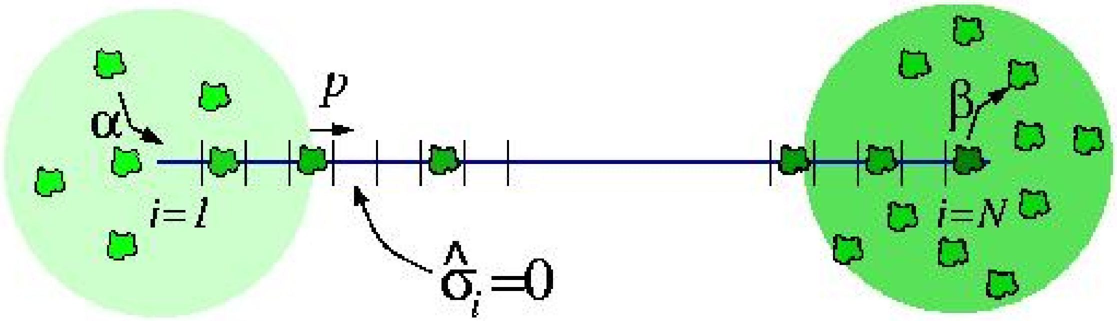

Uniform chain:

$$J_N = p\frac{S_{N-1}(p/\beta) - S_{N-1}(p/\alpha)}{S_N(p/\beta) - S_N(p/\alpha)}, \quad \text{where}$$

$$S_N(x) = \sum_{k=0}^{N-1} \frac{(N-k)(N+k-1)!}{N!k!} x^{N-k+1}$$

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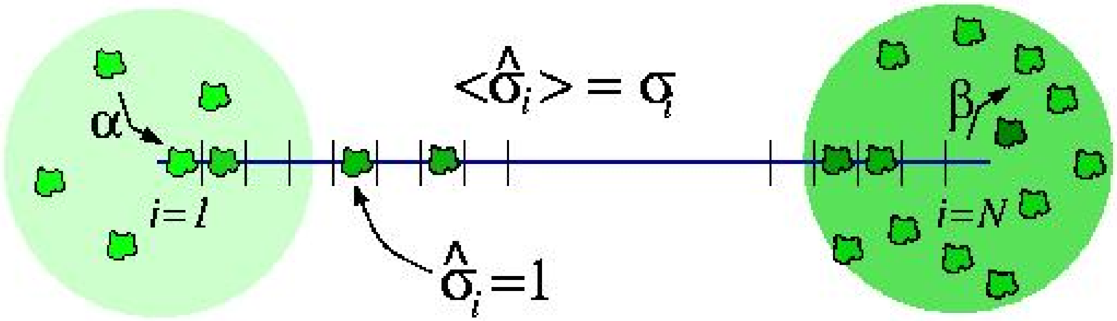

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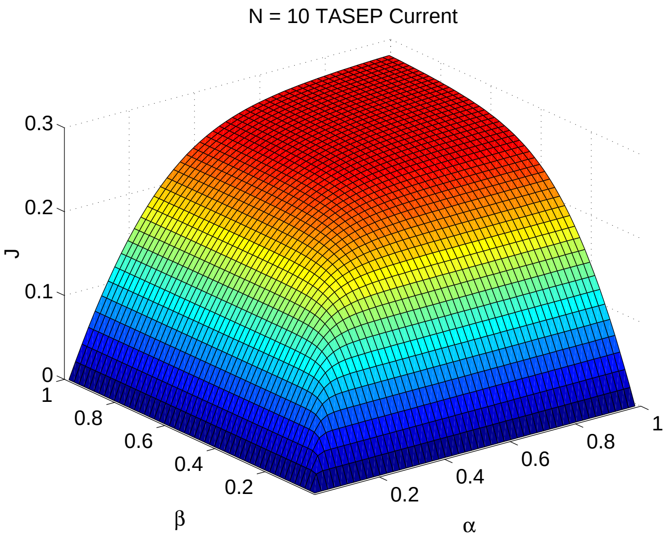
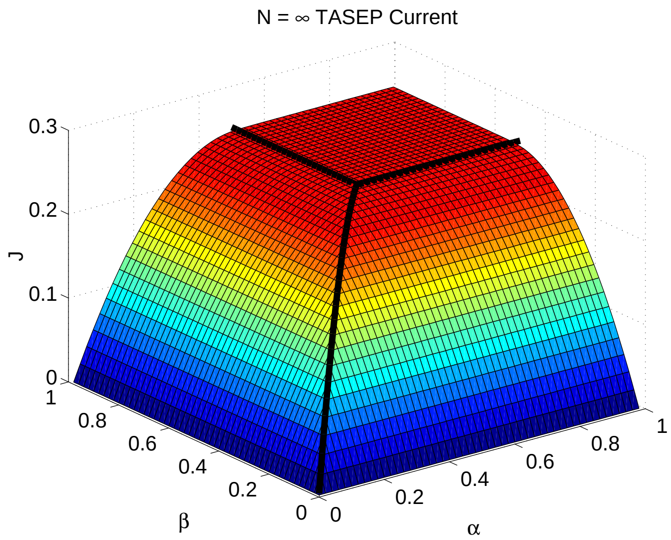




For $N \to \infty$, $p_i \equiv 1$,

$$J(\alpha \leq 1/2, \beta \geq \alpha) \to \alpha(1-\alpha)$$

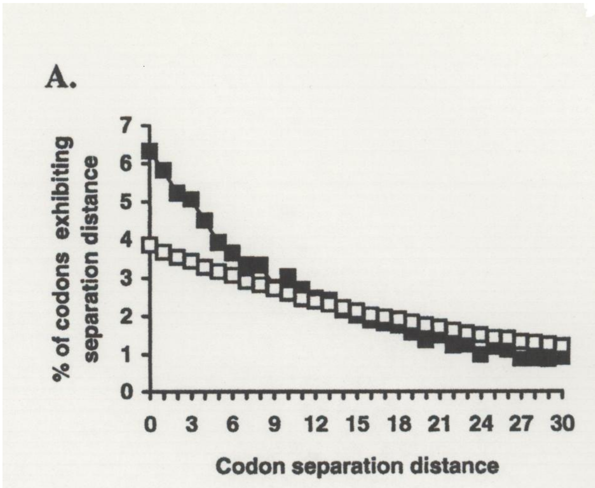
$$J(\beta \leq 1/2, \alpha \geq \beta) \to \beta(1-\beta)$$

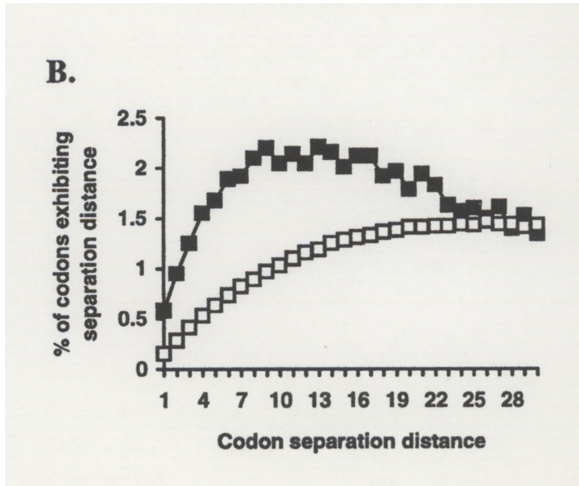
$$J(\alpha, \beta \geq 1/2) \to 1/4$$

Clustered Defects

- Rare codons identified: CTA (Leu), ATA (Ile), ACA (Thr), CCT and CCC (Pro), CGG, AGA, and AGG (Arg)

- Statistically small (2-5) clusters of rare codons occur in *E. coli*, *Drosophilia*, yeast, and primates





Korotkov & Phoenix, FEMS *Microbiol. Letts.* **155**, 63-66, (1997).

Solution of general TASEP

To solve inhomogeneous TASEP, solve $2^N \times 2^N$ master eqn:

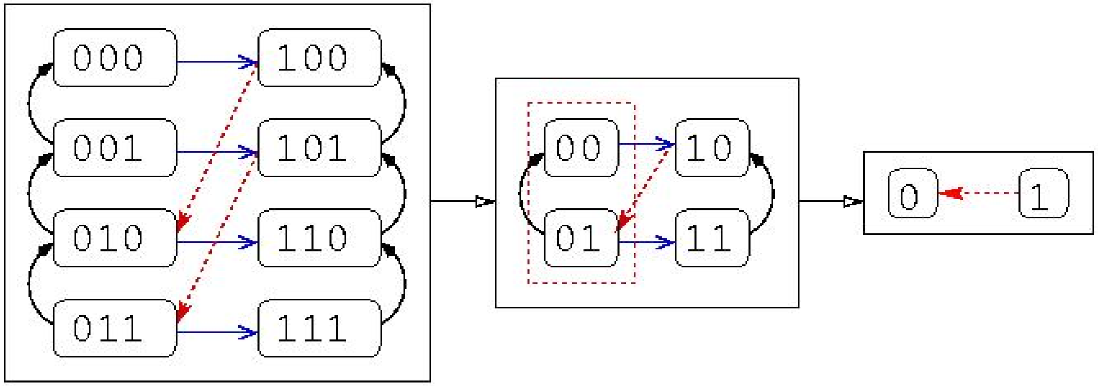
$$\frac{dP_i}{dt} = M_{ij} P_j, \quad \sum_{i=1}^{2^N} P_i = 1.$$

M_{ij} is sparse, but has transition rates scattered throughout

$\Rightarrow$ generate M_{ij} and find eigenvectors to the zero-eigenvalue

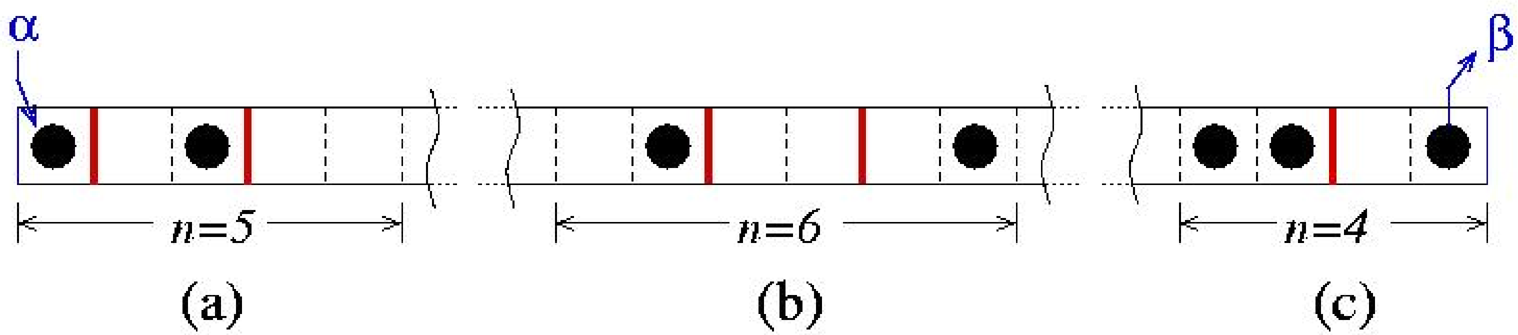
Algorithm to generate band-diagonal matrix

Enumerate states to allow generation of transition matrix:



Algorithm for $3-$site model. Each configuration is associated with a bit pattern, and the state is enumerated with the corresponding decimal value. *I. e.*, since 011 is the binary representation of 3, we label the state with particles occupying the second and third lattice sites as *state 3*.

Finite Segment MFT



(a) Two defects near the initiation site straddled by an $n = 5$ lattice segment.

(b) Two defects in the chain interior, away from the boundaries, $n = 6$.

(c) A single slow defect near the termination end of the chain, $n = 4$.

For n sites, there are 2^n distinct states. Choose $n \ll N$, but large enough to give accurate results.

Finite Segment MFT

- Flux out of rightmost site of segment:

$$J(\sigma_-, \sigma_+, \{p_i\}) = (1 - \sigma_+) \sum_{j=odd} P_j(\sigma_-, \sigma_+, \{p_i\}),$$

where σ_- and σ_+ are densities far to the left and right of defects.

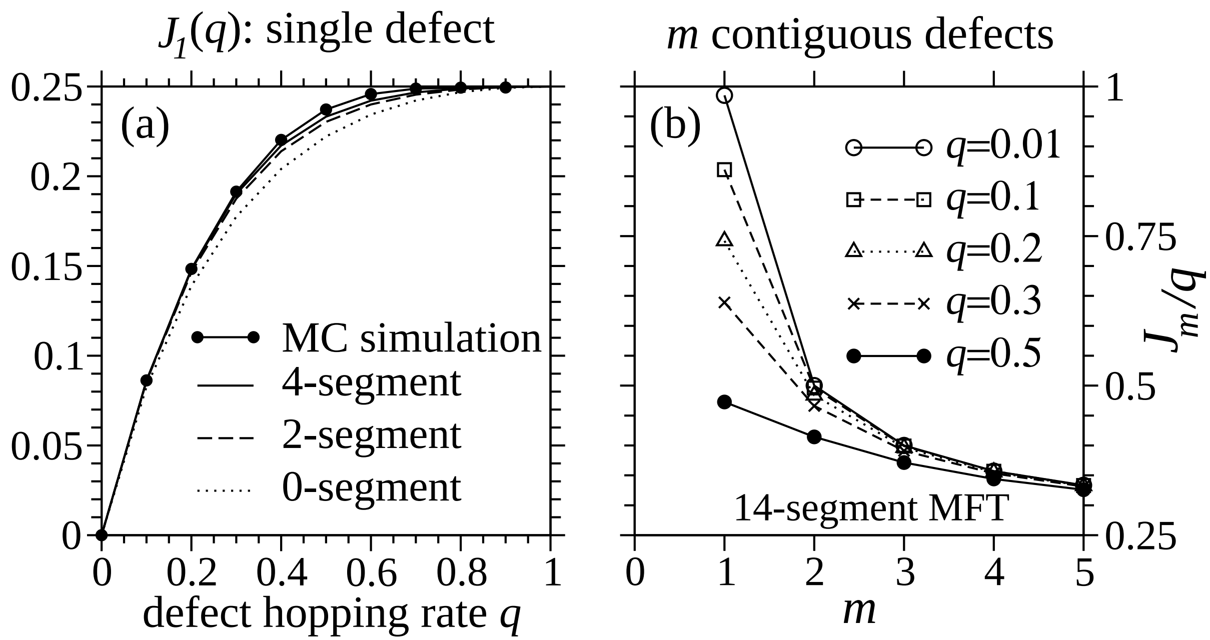
- Current conservation: $J_- = \sigma_-(1 - \sigma_-) = J_+ = \sigma_+(1 - \sigma_+)$
$\Rightarrow \sigma_+ = 1 - \sigma_-$ and

$$\sigma_-(1 - \sigma_-) = J(\sigma_-, 1 - \sigma_-, \{p_i\})$$

- Solve σ_- numerically $\Rightarrow J = \sigma_-(1 - \sigma_-)$.

Finite Segment MFT

For bottlenecks $p_i = q$, all other rates $p_i \equiv 1, \alpha = \beta = 10$



(a) For $n \geq 4$, the FSMFT results are within 2% of those from MC simulations.

(b) Addition of successive, identical defects. The first few defects cause most of the reduction in current.

Asymptotic results

- For strong bottlenecks, $q \ll 1$, a single defect gives

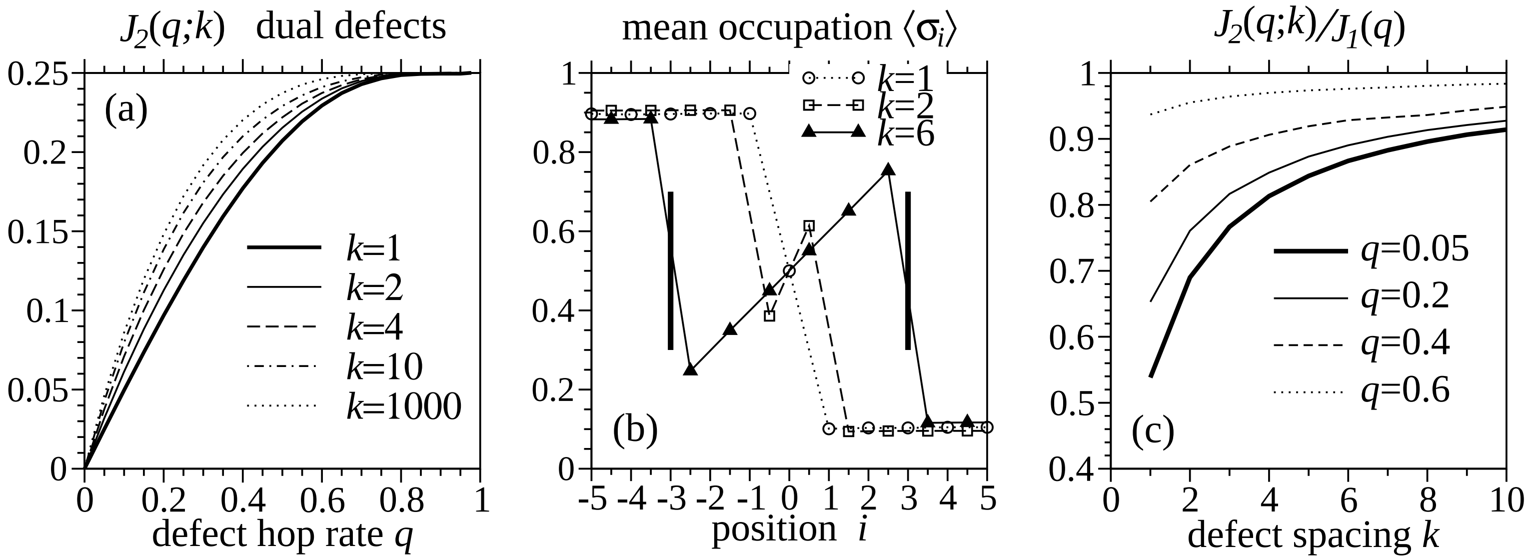
$$J \sim q - 3q^2/2 + \mathcal{O}(q^3)$$

- For m contiguous defects,

$$J_m(q \to 0) \sim \left(\frac{m+1}{4m-2}\right) q + \mathcal{O}(q^2).$$

- For $q \lesssim 0.3$, largest reduction in $J_m(q)$ occurs as $m = 1 \to 2$.

Two $k-$separated defects



(a) Currents maximally suppressed when defects are close.

(b) Densities about the midpoint between two defects ($q = 0.15$) of various spacings k.

(c) Normalized current $J_2(q;k)/J_1(q)$ as a function of separation k.

$$J_2(q;k) \sim \left(\frac{k}{k+1}\right) q + \mathcal{O}(q^2).$$

Randomly spaced defects

The total number of ways m defects can be placed on $N-1$ interior sites, with minimum pair spacing k is

$$Z_k(m,N) = \binom{N-1-(m-1)(k-1)}{m}.$$

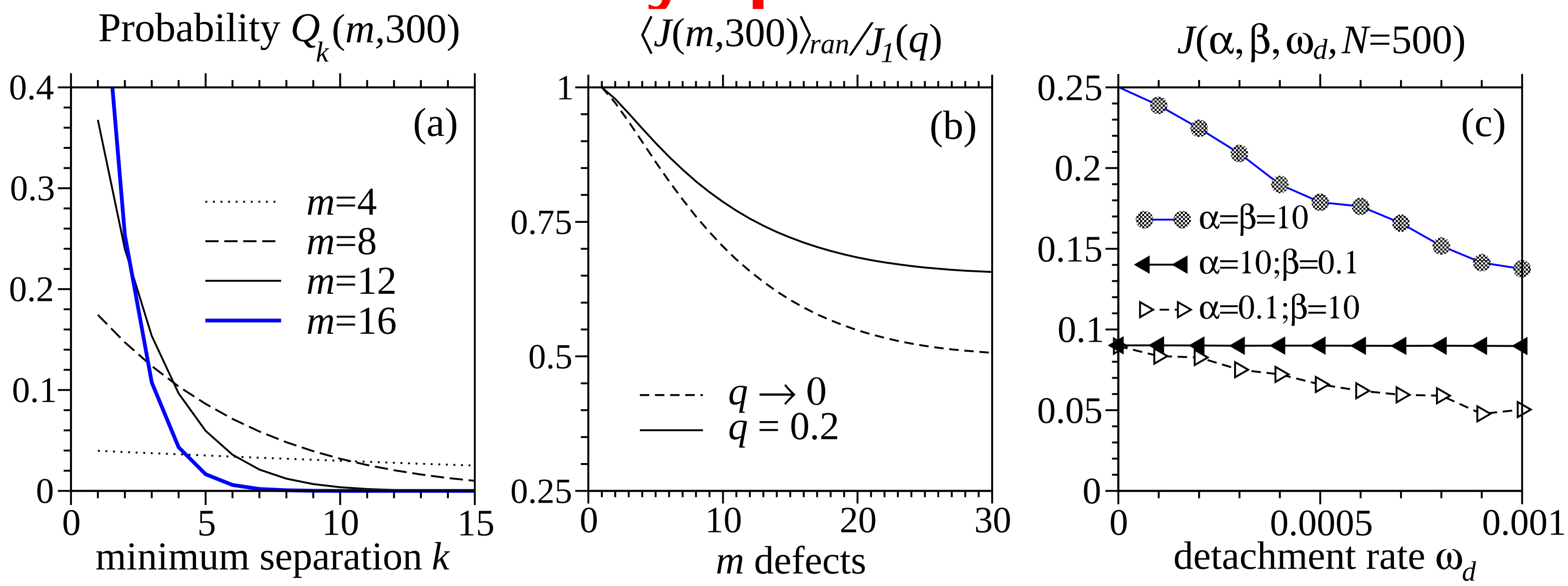
The probability density that the minimum inter-defect spacing equals k is thus

$$Q_k(m,N) = \frac{(Z_k(m,N) - Z_{k+1}(m,N))}{Z_1(m,N)}.$$

Maximum current for any configuration with a minimum defect spacing k is $J_2(q;k)$. Upper bound for the randomness-averaged current is

$$\langle J \rangle_{ran} \leq \sum_{k=1}^{\text{int}\{(N-1)/(m-1)\}} Q_k(m,N) J_2(q;k).$$

Randomly spaced defects



(a) The probability m defects on $N-$site lattice has a mimimum spacing $= k$.

(b) Upper bound for the current. Only pairwise suppression considered. Lower bound for $m \to N$ is $\langle J(m \to N-1)\rangle_{ran} \to q/4$.

(c) Weak detachments ω_d suppress ribosome throughput only in the entry-limited ($\alpha = 0.1, \beta = 10$) and maximal current ($\alpha = \beta = 10$) regimes.

Summary and Conclusions

- Modified TASEP to allow inhomogeneous hopping rates
- Formulated systematic numerical approach for clustered bottlenecks
- Clustering of defects further suppress throughput
- Cooperative effect arises from exclusion interactions

Summary and Future Avenues

- Extensions to particles that occupy d lattice sites (ribosomes $d \sim 10$) *e.g.*, $J = 1/4 \rightarrow 1/(\sqrt{d} + 1)^2$
- Distribution (unequal) bottleneck strengths
- Multiple-scales asymptotics for TASEP with random p_i and detachments