Active sorting of particles and the Gibbs mixing paradox

Cato Sandford,1 Daniel Seeto,2 and Alexander Y. Grosberg1

1Center for Soft Matter Research and Department of Physics, New York University, 726 Broadway, New York, NY 10003, USA
2Stern School of Business, New York University, 44 West 4th Street, New York, NY 10012, USA
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The Gibbs Mixing Paradox is a conceptual touchstone for mixtures in statistical mechanics. While debates over the theoretical subtleties of particle distinguishability continue to this day, we seek to extend the discussion in another direction by considering devices which can only distinguish particles with limited accuracy. We introduce two illustrative models of sorting devices which are designed to separate a binary mixture, but which sometimes make mistakes. In the first model, discrimination between particle types is passive and sorting is driven, while the second model is based on an active proofreading network. We show that the performance of these devices may be enhanced out of equilibrium, and we further probe how the quality of particle sorting is maintained by trade-offs between the time taken and the energy dissipated.

I. INTRODUCTION

The goal of this article is to look from a united point of view at two related issues: the famous Gibbs Mixing Paradox in equilibrium statistical mechanics, and the more practical issue of particle sorting, relevant to a number of non-equilibrium processes in biological cells. We hope to demonstrate that considering these issues together sheds light on both.

The Gibbs Mixing Paradox concerns the curious fact that the change in entropy when separating a mixture of ideal gases into two volumes does not depend on the nature of the gases. (Equally, the entropy change when two different gases mix into a single volume doesn’t depend on the gases in question.) This is illustrated in the cartoon figure 1, where gases are depicted as particles of different colors and shapes: the mixing entropy for two equal sub-volumes is \( \Delta s = k_B \ln 2 \) per particle if the gases are different and \( \Delta s = 0 \) if they are identical. Gibbs himself noted this fact [1–3], though he did not call it a paradox (consistent with the fact that the issue is not even mentioned in some of the best statistical physics textbooks [4]).

The resolution to the Gibbs Mixing Paradox is widely held to be quantum mechanical [5–13] (see also further discussion in [14–16]). However, this point of view conspicuously fails to account for the usefulness and accuracy of statistical mechanics in the context of classical systems, such as colloids or proteins. Such particles are not strictly identical, yet may still be treated as such [17] – a fact compellingly emphasised in recent works [18, 19]. In this context, the unsettling discontinuity of the Gibbs Paradox, between distinguishable and indistinguishable particles, arises from the temptation to regard entropy as an absolute quantifier of a system’s properties, rather than a function of a macrostate [20–23]. In fact, particle identity is merely one component of the macrostate, to be included whenever necessary. This is especially clear for classical particles, which are always distinguishable in principle [24, 25].

Macrostates, the dichotomy between distinguishable and indistinguishable particles, and even entropy itself, are some of the idealisations employed by equilibrium statistical mechanics. We ask to what extent these idealisations are applicable in case of real colloids or proteins: such particles may be so similar that distinguishing them, although possible in principle, may be difficult and prone to mistakes.

For example, suppose we wish to purify a binary mixture of gases into two volumes, as in figure 2. To achieve this, we must have some way of determining the identity of each gas particle. In the cartoon, it is done by sieves on each of the two pistons, which in an ideal scenario are perfectly permeable for one particle species and impermeable for the other. Each piston therefore compresses only one of the two mixed gases and performs work of at least \( k_B T \ln 2 \) per particle (assuming the system is in contact with a thermostat of temperature \( T \)). This situation is ideal in the sense that the macrostate implies that particles can be distinguished, that distinguishing
them does not require any work, and that entropy is a good proxy for the work done to separate them.

Another example, which we shall investigate in some detail, is more reminiscent of a Maxwell demon (sometimes called a “concentration demon” [26]). Using the device depicted in figure 3, we treat particle distinguishability not as a “yes-no” binary, but as a continuous parameter which encapsulates the accuracy of an imperfect sorting device. We investigate in section II the consequences of the device’s mistakes, and how its performance may be improved through the introduction of active (energy consuming) discrimination steps. In section III we consider a different situation in which particles have some pre-existing energetic preference for one of the boxes, such that a certain level of “sorting” will be achieved even in equilibrium. We then show how an active process, similar to kinetic proofreading [27, 28], may be employed to improve the sorting quality.

II. PURELY KINETIC SORTING

A. Sorting Model and an Elementary Example

Our first model is broadly similar to the illustration in figure 2, in that sorting depends on an external driving. We shall first introduce the model with only passive discrimination, and then describe how additional active discrimination improves performance.

Consider a system with two species of particles, \( \mathcal{A} \) and \( \mathcal{A}' \), initially distributed evenly between two equal volumes, labelled 1 and 2. Notation-wise, we denote a particle of \( \mathcal{A} \) in box 1 as \( \mathcal{A}_1 \), etc. We use the same symbols also for the numbers of corresponding particles, so that \( \mathcal{A}_1(t) \) is the number of \( \mathcal{A} \) particles in box 1 at time \( t \), etc. The sorting device is sketched in figure 3. Sorting proceeds through the combination of two processes. First, particles are distinguished (passively) by the two coloured channels, which allow passage to particles of the same colour, so \( \mathcal{A} \)-type particles can move only through the upper channel, and \( \mathcal{A}' \)-type particles through the lower. (We might imagine that the particles are of different shapes, as in figure 2, and place a sieve at each entrance of each channel.) The second process is driving, which is performed by the turbine. Driving is insensitive to the particles’ type, but induces them to flow in a particular direction – toward box 2 in the upper channel and toward box 1 in the lower channel. (The virtue of this setup over figure 2 is that it can be run continuously, but otherwise they operate on the same principle.)

![Figure 2](image1.png)

**FIG. 2.** From left to right: A mixture of gases is sorted into two volumes by compressing with special pistons. The process requires at least \( k_B T \ln 2 \) of work per particle. However, if the pistons do not distinguish the two types of particles, no work is performed.

![Figure 3](image2.png)

**FIG. 3.** Schematic of a sorting device. Two types of particles are contained in two volumes connected by two channels. These channels preferentially pass one type of particle, and are entirely passive. The central turbine indiscriminately pushes particles in the upper channel toward box 2, and those in the lower channel toward box 1 – an action which costs some amount of free energy. The combined action of the channels and the turbine is to reach a sorted steady state, whose purity depends on the relative probability of errors, \( \eta \).

To introduce the problem, let us first consider its most elementary version. Suppose the sorters at the entrances of both channels make no mistakes, and pass through only their “own” particles with rate constant \( K \). Then

\[
\begin{align*}
\dot{\mathcal{A}}_1(t) &= -K \mathcal{A}_1(t) , \quad \mathcal{A}_1(t) + \mathcal{A}_2(t) = N \\
\dot{\mathcal{A}}'_2(t) &= -K \mathcal{A}'_2(t) , \quad \mathcal{A}'_1(t) + \mathcal{A}'_2(t) = N 
\end{align*}
\]

where \( N \) is the total number of particles of either type. This of course results in \( \mathcal{A}_1(t) = \mathcal{A}'_2(t) = \frac{N}{2} e^{-Kt} \), etc. How much work does the turbine perform? To transfer one \( \mathcal{A} \) particle from box 1 to box 2, one needs to perform work which is at least equal to the difference of chemical potentials between two boxes, \( \Delta \mu = k_B T \ln \frac{\mathcal{A}_2(t)}{\mathcal{A}_1(t)} \) (and similarly for primed particles). Since the current through either channel is \( J(t) = -\mathcal{A}_1(t) \), the minimal
overall amount of work is
\[ W = \int_0^\infty J(t) \Delta \mu(t) \, dt = k_B T \int_0^\infty A_1(t) \ln \frac{A_2(t)}{A_1(t)} \, dt . \] (2)

Evaluating this integral yields the familiar answer of \( k_B T \ln 2 \) per particle.

While the channels are intended to allow passage for one type of particle only, the other type may still leak through. We introduce the relative probability of such an error \( \eta \) (to be defined more precisely below), and note that \( \eta \) is a proxy for the distinguishability of the particle types: \( \eta = 0 \) corresponds to zero mistakes and perfect distinguishability, while \( \eta = 1 \) corresponds to perfect indistinguishability.

The possibility of a particle mistakenly going through the wrong channel modifies kinetic equations (1) as follows:
\[ \dot{A}_1(t) = -K A_1(t) + K \eta A_2(t) , \quad \dot{A}_1(t) + \dot{A}_2(t) = N \]
\[ \dot{A}_2(t) = -K A_2(t) + K \eta A_1(t) , \quad \dot{A}_1(t) + \dot{A}_2(t) = N , \] (3)
leading to
\[ \frac{A_1(t)}{N} = \frac{\eta}{1 + \eta} + \left[ \frac{1}{2} - \frac{\eta}{1 + \eta} \right] e^{-K(1+\eta)t} \] (4)
and similarly for primed particles (henceforth we will not repeat symmetrical-looking equations for both species of particles). As expected, when mistakes are possible, each box remains contaminated with incorrect particles.

The issue of work in this case becomes interesting. As long as \( \eta \) is very small, the initial kinetics of separation is almost the same as in the mistake-free system, and the amount of work performed in one channel \( W(t) = k_B T \int_0^t K \dot{A}_1(t') \ln \frac{A_2(t')}{A_1(t')} \, dt' \) increases initially with time \( t \) following very nearly the same schedule. If \( \eta \) is really very small, then the work approaches \( k_B T \ln 2 \) per particle. However, for times larger than \( -\ln \eta/K \), mistakes start to take their toll: some particles return to their original box, going down the gradient of chemical potential, and have to be transported back a second time. Thus the device has to perform work at a steady rate forever to maintain the system’s purity (assuming, of course, that when a particle leaks through the wrong channel it does not return the corresponding energy to the turbine).

Although this model does illustrate some important ideas about particle sorting, it lacks the flexibility to introduce active control of errors. Therefore, we now introduce a more sophisticated model.

B. Kinetic Sorting with Passive Discrimination

The essence of the model is summarised in figure 4. Once again, the two passive channels allow passage of the different particles with rates \( K \) and \( \eta K \ll K \), while the turbine pushes particles in a direction which depends solely on which channel they’re in and not on their type.

We shall assume here that the turbine works at a constant rate, expending a free energy of \( \Delta f \) for every particle it pushes through the channel in the correct direction, and receiving \( \Delta f \) for each particle that pushes it in the incorrect direction. This stands in contrast to the previous model, where the energy cost per particle transported, \( \Delta \mu \), depended on the state of the system. Using \( \Delta f \) rather than \( \Delta \mu \) will simplify our calculations a little. It also means that even when the channels perfectly distinguish the two particle types (\( \eta = 0 \)), there will be imperfect sorting, since particles are allowed to make transitions away from their target box as long as they pay \( \Delta f \) to the turbine. While this consideration changes the details of the results, it doesn’t affect the overall thrust of our findings.

We translate the figure 3 setup into the reaction network in figure 4, which shows the paths a particle of type \( A \) or \( A' \) may take to transition between the boxes. The states \( B \) and \( B' \) denote a particle which has entered one of the channels, with indices \( u, \ell \) indicating whether it is in the upper or lower channel, and indices 1, 2 indicating which side of the channel it’s in.

As in the previous section, transitions between states are governed by kinetic rates. The diffusion-controlled rates \( k_{on} \) and \( k_{off} \) represent respectively the rate at which a particle in one of the boxes enters a channel, and vice-versa. The rates \( K \) and \( K \eta \) represent transitions within the two channels when there is no assistance from the turbine (that is, when \( \Delta f = 0 \)).

Representing this network as a set of linear, coupled, ordinary differential equations, we may easily calculate the steady state, and hence the sorting quality, as a function of \( \Delta f \) and \( \eta \). This is plotted in figure 5, where the sorting quality is parameterised by the entropy change \( \Delta S \) between the maximally unsorted initial state and the maximally (but imperfectly) sorted steady state (see appendix B). As one might expect, sorting is best when \( \eta \) is small, and is poor when the driving is either very low or very high.

C. Kinetic Sorting with Active Discrimination

For a given error \( \eta \) and driving \( \Delta f \), we seek to invest work from some energy reservoir to improve the sorting quality. The obvious solution would be to introduce an additional active sorting mechanism, to shuttle particles in one direction or another depending on their type. This will be explored in more depth in section III; for now we shall focus on a slightly subtler mechanism of active discrimination. By this we mean an active process which is sensitive to particle’s type, but is agnostic about which box it should be assigned to. In other words, the active discrimination adds to the existing structure of the figure 4 network in a way that is symmetrical with respect to the box numbers, but asymmetrical with respect to
Our aim is simply to enhance this discrepancy through a modification of the existing kinetics. A possible implementation of this is shown in figure 6. It extends the figure 4 network with additional kinetic branches in each channel (there is now a “+” branch and a “−” branch), and also provides a route for transitioning between them. The rates in the “−” branches are reduced with respect to the “+” rates by an active process which consumes free energy $\Delta F$ per transition. Furthermore, any particle in the “+” branch is more likely to be jump to the “−” branch than the other way around, meaning that the whole sorting process is slowed. Crucially, the overall rate of jumping between branches in a given channel is different for different particles – this is the discrimination part. For a particle in the correct channel, the jumping rate is small compared to the rate of transition between boxes, so particles in their correct channel will be relatively unperturbed by the extra active process. For a particle in the incorrect channel, however, there will be a substantial flow from the fast “+” branch to the slow “−” branch, which reduces the effective $\eta$. In figure 7, we show that this modification does indeed improve sorting above the $\Delta F = 0$ baseline.

An issue of relevance to real sorting devices is the work required to achieve a given entropy reduction, and the concomitant trade-offs with the time taken to complete the sorting. To answer this question for our system requires some numerical root-finding; but the example shown in figure 8 illustrates the fact that sorting can be completed quickly at the expense of additional work. Unexpectedly, it also demonstrates that strong active proof-reading can in some cases reduce the work required to achieve fast sorting.

It is worth pointing out that, while we use our sorting device to sort a mixed system at the expense of work, it may equally well be run in reverse as an “entropy engine” which produces work from an initially segregated system. Two further regimes are accessible to this model: one where the network simultaneously sorts and produces work, and another where the network consumes work to increase the entropy of the system.
FIG. 6. A modification of the kinetic network in figure 6, which employs additional branches in each channel. The rates in the “−” branches are suppressed by an active process which costs/produces free energy $\Delta F$ per transition. Notations are the same as in figure 6, but for compactness we denote $\delta \equiv e^{\Delta f}$ and $\Delta \equiv e^{\Delta F}$.

III. A HOPFIELD–NINIO SORTER

We now introduce our second model sorting device, which exploits energetic differences between the particle types rather than just kinetic differences: we imagine the particles are distinguished by their energetic preference for one box over another. We shall denote this energetic difference $\Delta F \geq 0$ (see figure 10). This is not to be confused with the active driving of the previous section: here $\Delta F$ quantifies the (passive) distinguishability of the particles, and thus fills the role played by $\eta$ in the previous model.

In equilibrium, their distribution between the boxes would be controlled by the Boltzmann factor $e^{\Delta F}$. We seek to actively improve the quality of sorting above that mandated by the Boltzmann factor.

This is not a new problem. In the early seventies it was known that the accuracy of certain biological pro-
cesses for distinguishing very similar particles (for example different nucleotides during RNA transcription) far exceeds the equilibrium expectation (based on the binding energy between the relevant enzyme and the different substrates). In response, Hopfield and Ninio independently developed dissipative “proofreading” schemes capable of drastically amplifying the existing binding energy difference [27–29]. It is natural for us to apply the Hopfield–Ninio proofreading scheme to our particle sorting problem, when there exists some difference $\Delta F$ in the free energy landscape of the two particle types.

Our model apparatus is sketched in figure 9, and is broadly similar to the earlier figure 3. A sorting device $S$ sits in the single channel connecting the volumes, and may grant or deny passage through the channel depending on the outcome of a sorting process. This process is modelled as the network sketched in figure 10, which has four independent parameters: $\Delta F$ and four rate constants, one of which may be used to set the time-scale (for clarity we leave all the rate constants explicit and with units). When $\Delta F > 0$, the sorting device promotes the accumulation of $A$ in box 2 and $A'$ in box 1, as in section II. Every transition along one of the unidirectional edges dissipates $\Delta F$. Thus, when the kinetic rate $\kappa$ is zero, the network is an equilibrium sorter whose performance is ultimately controlled by the Boltzmann factor $e^{\Delta F}$.

### A. Steady State

The system of nonlinear ODEs which represent this reaction network is written in appendix B. As in the previous model, the steady state is exactly calculable, and we can compute the entropy change for a given set of parameters as before. With reference to equation (B3), we find that we can achieve better-than-equilibrium sorting for any $\Delta F$, provided the kinetic rate of the energy-consuming transition, $\kappa$, is substantially smaller than the other rates, and $k_{\text{off}} \gg k_{\text{on}}$. The improvement is particularly impressive when the particles are highly distinguishable, and the Hopfield–Ninio sorter behaves like an equilibrium sorter with $\Delta F \to 2\Delta F$.

We may also choose kinetic rates that cause us to pay
work for sorting worse than Boltzmann. This is possible when the rate \( \kappa \) is large, and the sorting network encourages particles of both species to bypass the proofreading machinery in both directions via the energy-consuming transitions. These predictions for good and bad sorting regimes are shown to agree with simulations in appendix B.

C. Cost of a Desired Purity

The foregoing findings allow us finally to find the costs to maintain a desired quality of sorting for a given particle distinguishability \( \Delta F \). All the trade-offs we have met so far, between the quality of the sort, the time to reach the steady state, and the work performed in sorting, can be projected onto a diagram such as figure 11. This confirms that high-quality sorting (colours closer to red) needn’t cost more work than low-quality sorting. The key message to take from this diagram, however, is that for a given sorting quality, high speeds cost more work. This finding is robust for other values of \( \Delta F \).

IV. CONCLUSION

The Gibbs Mixing Paradox is traditionally invoked to illustrate the role of particle distinguishability in the macrostates of statistical mechanics. In this paper, we struck a different path, and investigated the performance of devices which can partially distinguish two similar species.
type of particles. When such devices are allowed to dissipate energy, they may achieve sorting efficiencies surpassing (or disappointing) those of their passive, equilibrium counterparts.

Such processes are relevant to a variety of biological systems, whose function depends on maintaining a level of purity with respect to their environment. We may consider organisms’ ability to isolate and expel/metabolise contaminants, or the segregation of sodium and potassium ions across cellular membranes. Our aim was to elucidate the physics which underlies such energy-consuming processes.

For concreteness, we introduced specific models based on two different sorting mechanisms, where the similarity of the particles to be sorted was represented by a single parameter. We showed how the efficacy of sorting was a continuous function of the particle similarity, and that it could be improved with the inclusion of active processes which effectively enhance the particle distinguishability. Furthermore, we found for both models that accurate sorting can be achieved quickly and with very low dissipation for carefully selected model parameters; but any improvement in speed must generally be paid for with more work, and vice-versa.

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Appendix A: Entropy Definition

For both sorting models (sections II and III), the quality of sorting is measured as an entropy change from the initial, maximally-disordered state to the partially-ordered steady state. Since we consider sorting devices which treat particle type $A$ identically to type $A'$ (with box 1 and box 2 switched), we can restrict attention to particle type $A$ alone.

The total entropy per particle is then calculated from the number of $A$ particles in box 1, $A_1$, using the formula

$$\frac{\Delta S}{N} = -\frac{A_1}{N} \ln \left( \frac{A_1}{N} \right) - \frac{N-A_1}{N} \ln \left( \frac{N-A_1}{N} \right) - \ln 2,$$

where $N$ is the total number of particles, and we assume the capacity of the boxes is much larger than that of the sorting device which connects them (so $A_2 \approx N - A_1$).

With this definition, the minimum possible entropy change is zero, and perfect sorting would change the entropy by $-\ln 2$.

Appendix B: Hopfield–Ninio Sorter Steady State

The kinetics for the $A$-type particles in the figure 10 is given by:

$$\dot{A}_1 = -k_{on} \cdot S \cdot A_1 + k_{off} \cdot B_1 + \kappa \cdot B_2$$

$$\dot{B}_1 = +k_{on} \cdot S \cdot A_1 - (k_{off} + K + \kappa \cdot e^{\Delta F}) \cdot B_1 + K \cdot B_2$$

$$\dot{B}_2 = +K \cdot B_1 - (k_{off} \cdot e^{\Delta F} + K + \kappa) \cdot B_2 + k_{on} \cdot S \cdot A_2$$

$$\dot{A}_2 = +\kappa \cdot e^{\Delta F} \cdot B_1 + k_{off} \cdot e^{\Delta F} \cdot B_2 - k_{on} \cdot S \cdot A_2,$$

where $A_1$ denotes the number of particles in box 1, etc. A similar system of equations obtains for the $A'$ particles.

There is an additional constraint, inherited from the Hopfield–Ninio proofreading scheme, that $S$ can only sort a finite number of particles at once. Calling the maximum $S_0$, we have

$$S = S_0 - B_1 - B_2 - B_1' - B_2'.$$

The presence of $S$ in equation (B1) makes the system nonlinear, and also couples the dynamics of the two networks. However, the symmetry between the two types of particles allows us to mostly avoid the complications of coupling.

The steady state occupation of either box can be easily computed by setting all the time derivatives in equations (B1) to zero, and solving algebraically. The result is

$$\frac{A_1^{SS}}{N} = \frac{c_1 + k_{off} e^{\Delta F}}{c_1 + c_2 e^{\Delta F} + k_{off} k e^{2\Delta F}},$$

FIG. 11. Entropy change in colour, plotted against the time and work to reach the steady state. Each point corresponds to a simulation of the network in figure 10 with different kinetic rates. $\Delta F = \ln 10$ is fixed, and the sorting quality tends to increase for smaller vaules of $W_{\text{sort}}$. 
where \( c_1 \equiv k_{off} \kappa + k_{off} K + \kappa K \) and \( c_2 \equiv 2K^2 + k_{off} K + \kappa K \).

Due to the symmetry we imposed on our model, equation (B3) accounts for other steady state densities via \( A_1 = A'_1 = N - A_0 = N - A'_0 \) (see also appendix A). The network discussed here promotes the state \( A_2 \) over \( A_1 \), so good sorting will result in \( A_1 \) close to zero. Note that for \( \Delta F = 0 \), \( A_1^{SS} = N/2 \) as expected.

For an equilibrium sorter (with \( \kappa = 0 \)), equation (B3) becomes \( A_1^{SS}/N = (1 + e^{\Delta F})^{-1} \). This is the correct Boltzmann result, and is naturally independent of kinetic coefficients. As noted in section III A of the main text, equation (B3) tells us that the quality of the active sorter may be much better than an equilibrium sorter when \( \kappa \) is substantially smaller than the other rates, and \( k_{off} \) is simultaneously large enough to support the \( e^{2\Delta F} \) term in the denominator.

From equation (B3), we also observe that the active sorter performs worse than a Boltzmann sorter for certain parameter choices. For instance, if we consider high \( \Delta F \), then when \( \kappa \) is of order \( K \), but \( k_{off} \) is much smaller than \( K/e^{\Delta F} \), we have \( A_1^{SS}/N \sim 1/3 \), which is larger than the equilibrium value for \( \Delta F > \ln 2 \). This is also illustrated in figure 12. In this case the network encourages particles of both species to bypass the proofreading machinery via the energy-consuming transitions, such that we pay for worse sorting. In appendix C we find that the time to perform the sorting may be reduced by sacrificing sort quality (since particles avoid rattling in the heart of the network and are expeditiously transferred). However, this sacrifice is not absolutely necessary, as fast accurate sorting can be achieved with carefully chosen parameters.

To verify equation (B3) and the results of the following sections, we perform stochastic simulations of the figure 10 network (the details are described in appendix E). Figure 12 shows the steady state entropy of the system as a function of the distinguishability parameter \( \Delta F \) for two choices of kinetic rates – corresponding to a better-than-equilibrium active sorter and a worse-than-equilibrium active sorter. We find good agreement with our prediction, and see clearly how the discontinuity of the traditional Gibbs Mixing Paradox is softened, as \( S \) approaches \(-1\) asymptotically as \( \Delta F \to \infty \).

**Appendix C: Hopfield–Ninio Sorting Time**

In section III B, we describe the “intermediate steady state” approximation for calculating the non-linear system’s dynamics. This, along with the “large box” assumption described in appendix A, yields the un-coupled ODE for \( A_1 \):

\[
\dot{A}_1 = \frac{1 - A_1/A_1^{SS}}{\gamma + \delta A_1/N},
\]

(C1)

where \( A_1^{SS} \) is given by equation (B3), and the known constants \( \gamma \) & \( \delta \) are positive, intensive and have units of time.

Equation (C1) can be solved for the (non-invertible) evolution:

\[
t(A_1) = \theta \cdot \left( \frac{1}{2} - \frac{A_1}{N} \right) + \tau \cdot \ln \left( \frac{N/2A_1^{SS} - 1}{A_1/A_1^{SS} - 1} \right),
\]

(C2)

where we’ve used the maximum-entropy initial condition \( A_1(t = 0) = \frac{N}{2} \). Thus there are two effective time-scales, \( \theta \equiv \delta A_1^{SS} \) and \( \tau \equiv A_1^{SS} \left( \gamma + \delta A_1^{SS}/N \right) \) (using the constants introduced in equation (C1)). Both \( \theta \) and \( \tau \) are extensive in the system size \( N \).

The total time to reach the steady state should correspond to evaluating equation (C2) at \( A_1 = A_1^{SS} \); however, the second term diverges to positive infinity at this point, demonstrating that the intermediate steady state approximation is no longer valid when \( A_1 \) is close to its steady state value (and thus the approximation never works when \( \Delta F \) is close to 0). Because of the divergence of the term associated with \( \tau \), it makes sense to provisionally identify \( \tau \) as the dominant timescale in the problem. Note that choosing \( \tau \) as the timescale associated with

![FIG. 12. The steady state entropy \( S \) as a function of the distinguishability parameter \( \Delta F \), compared with the theoretical prediction from equation (B3), and the Boltzmann value. The entropy is quoted in units of \( \ln 2 \), and low values correspond to accurate sorting. Top: a better-than-equilibrium sorter; bottom: a ‘worse-than-equilibrium sorter.](image)
the dominant term in equation (C2) does not necessarily mean that \( \tau \) is greater than \( \theta \).

While the full expression for \( \tau \) in terms of kinetic coefficients and \( \Delta F \) is long and not particularly illuminating, some features are easy to understand. We already noted in the main text that \( \tau (\Delta F \gg 0) \sim \frac{N}{S_0} \frac{1}{k_{\text{off}}} e^{-\gamma k_{\text{off}}} \). In general when \( N \) is large, \( \tau \sim \frac{N}{S_0} \) for any choice of parameters. Another interesting case is \( \kappa = 0 \), which brings us back to a passive Boltzmann sorter. Then the time-scale is

\[
\tau^{\text{eq}} \sim \frac{N}{S_0} \frac{4(K + (K + k_{\text{off}}) e^{\Delta F})}{k_{\text{off}} K (1 + e^{\Delta F})^2}.
\] (C3)

In figure 13, the full prediction for \( \tau \) is plotted alongside the time to reach the steady state measured in simulations (see appendix E).

![Figure 13](image1.png)

**FIG. 13.** The time to reach the steady state as a function of \( \Delta F \) for some choice of kinetic coefficients. The theoretical prediction for the time-scale \( \tau \) is shown as dashed lines.

### Appendix D: Hopfield–Ninio Sorter Dissipation

As stated in section III B, we use the intermediate steady state approximation to find the rate of dissipation \( \dot{W} \) as a function of \( A_1 \):

\[
\dot{W}(A_1) = \frac{\epsilon + \zeta A_1^{SS}}{\gamma + \delta A_1^{SS}},
\] (D1)

where \( \epsilon, \zeta \) are known constants, and \( \gamma, \delta \) are friends from appendix C. Dividing by equation (C1), we get \( d\dot{W}/dA_1 \).

In principle, integrating this from \( A_1 = N/2 \) to \( A_1^{SS} \) gives the total work done to complete the sorting. However, the integral diverges, reflecting the fact that the intermediate steady state approximation fails when \( A_1 \approx A_1^{SS} \).

The work done to achieve \( A_1 \) particles in box 1 is

\[
W(A_1) \approx A_1^{SS} \left( \zeta \left( \frac{1}{2} - \frac{A_1}{N} \right) + \left( \epsilon + \zeta A_1^{SS} \right) \ln \left( \frac{N}{2A_1^{SS}} - 1 \right) \right),
\] (D2)

provided \( A_1^{SS} \ll A_1 \leq N/2 \). We may evaluate this at say \( A_1 = 2A_1^{SS} \) to get an idea of the amount of work done to sort. The full expression is not particularly interesting. But, as noted in the main text, the high-\( \Delta F \) regime yields a work \( W_{\text{sort}}/N \approx \frac{\epsilon}{k_{\text{off}}} \Delta F \), which is zero when \( \kappa = 0 \) (as it should be). It’s perhaps a little surprising that more distinguishable particles require more work to sort, but this is simply because the dissipation of the nonequilibrium steps is commensurately greater.

The full approximate calculation is shown alongside simulation data in figure 14.

![Figure 14](image2.png)

**FIG. 14.** The total work required to reach the steady state: comparison of simulation and the equation (D2) approximation. Kinetic coefficients are the same as for figure 13.

A potentially unwelcome feature of our sorting device is that it continues to consume energy even after the steady state has been reached. Using the full steady state densities in equation (B3), we obtain the rate of dissipation

\[
\dot{W}_R = \frac{\text{const} + k_{\text{off}} \kappa \epsilon \Delta F}{\text{const} + \text{const} \cdot e^{\Delta F} + k_{\text{off}} e^{2\Delta F}},
\] (D3)

where the constants, which have been omitted for compactness, depend on the kinetic rates. If we examine the high-\( \Delta F \) regime of equation (D3), we find again that for a “good sorter” (with \( k_{\text{off}} \) large compared to \( \kappa \)), the dissipation in the steady state is suppressed and falls more quickly with \( \Delta F \).

The simulation data matches the predicted trend, as seen in figure 15. Also visible in both plots is the competition between accurate discrimination reducing number of unnecessary dissipative transitions, and the energy...
cost of each transition: for smaller $\Delta F$ the latter (linear) dominates, while at higher $\Delta F$ the (exponential) discrimination wins and reduces the work cost.

![Figure 15](image1.png)

**FIG. 15.** Dissipation rate in the steady state: comparison of simulation and the equation (D1) approximation. Kinetic coefficients are the same as for figure 13.

**Appendix E: Hopfield–Ninio Sorter Simulations**

The network in figure 10 was simulated using a “time-triggered” stochastic procedure: at each time step, the sorting device decides with some probability whether to “bind” to a new particle if it is unoccupied, or whether to progress an already-bound particle along the reaction chain.

Starting from the maximum-entropy initial condition, the simulation continues until the steady state is reached. See for example figure 16, which shows the value of the entropy as a function of time for some choice of parameters.

![Figure 16](image2.png)

**FIG. 16.** System entropy for the Hopfield–Ninio sorter as a function of time (blue solid line), which converges to and fluctuates around the prediction for steady state value (blue dotted). The free energy difference is $\Delta F = \ln 10$, and the steady state entropy for an equilibrium sorter is shown as a green dashed line, while the onset of the steady state (found algorithmically) is marked with a vertical black solid line.

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