1 Optimal spatial allocation of enzymes as an investment problem

2 Giovanni Giunta,¹ Filipe Tostevin,¹ Sorin Tănase-Nicola,² and Ulrich Gerland^{1,*}

3 ¹*Physics of Complex Biosystems, Physik-Department,*
4 *Technische Universität München, James-Frank-Str. 1, 85748 Garching, Germany*

5 ²*Department of Cell and Molecular Biology, Uppsala University, Sweden*

6 (Dated: December 15, 2021)

Abstract

Given a limited number of molecular components, cells face various allocation problems demanding decisions on how to distribute their resources. For instance, cells decide which enzymes to produce at what quantity, but also where to position them. Here we focus on the spatial allocation problem of how to distribute enzymes such as to maximize the total reaction flux produced by them in a system with given geometry and boundary conditions. So far, such distributions have been studied by computational optimization, but a deeper theoretical understanding was lacking. We derive an optimal allocation principle, which demands that the available enzymes are distributed such that the marginal flux returns at each occupied position are equal. This ‘homogeneous marginal returns criterion’ (HMR criterion) corresponds to a portfolio optimization criterion in a scenario where each investment globally feeds back onto all payoffs. The HMR criterion allows us to analytically understand and characterize a localization-delocalization transition in the optimal enzyme distribution that was previously observed numerically. In particular, our analysis reveals the generality of the transition, and produces a practical test for the optimality of enzyme localization by comparing the reaction flux to the influx of substrate. Based on these results, we devise an additive construction algorithm, which builds up optimal enzyme arrangements systematically rather than by trial and error. Taken together, our results reveal a common principle in allocation problems from biology and economics, which can also serve as a design principle for synthetic biomolecular systems.

* To whom correspondence should be addressed. E-mail: gerland@tum.de

7 INTRODUCTION

Living systems often need to allocate limited resources to different tasks. For instance, this situation arises when a colony of microbes differentiates into different cell types, each specialized to a task that contributes to the growth of the colony as a whole [1]. Cells solve this allocation problem by regulating the splitting ratios between cell types during differentiation. Conceptually similar allocation problems arise on the molecular level, where enzymes need to be allocated to different targets. For instance, a limited number of ribosomes must produce different types of proteins in different ratios to achieve balanced cell growth [2–4]. Accordingly, cells dynamically control these ratios when their environment changes to support different growth rates [5, 6]. Cells also control the spatial localization of enzymes to optimize or regulate metabolic fluxes [7, 8]. These and many other biological examples share the basic characteristics of generic resource allocation problems, in which a given amount of a resource must be distributed among competing alternatives to maximize the expected performance of the system [9].

The optimal allocation of limited resources has been extensively studied in economics. Rules such as Kelly’s criterion [10] and returns-variance analysis [11] are used to determine optimal betting strategies or optimal portfolios [12]. These criteria typically assume that the expected return on a bet only depends on its placement and not on the placement of other bets. Although there are biological systems for which such criteria can be directly applied [13], the treatment needs to be generalized to describe systems, for which the performance depends in coupled, nonlinear ways on the amounts of the resource allocated to each task. Here we show that the problem of allocating enzymes to different spatial positions to optimize the total enzymatic reaction flux has a one-to-one mapping to an investment problem where investments and returns are interdependent. In this context, enzyme molecules represent capital that can be invested at different positions within the system. Each position corresponds to an asset generating a certain expected return in terms of reaction flux. However, the placement of enzymes affects the substrate profile, and thereby the flux return from other enzymes. Hence, the spatial enzyme allocation problem corresponds to a betting problem where the coupling between the returns and the bets is determined by the reaction-diffusion dynamics of the substrate.

We consider a general class of models (Fig. 1), in which the substrate $\mathcal{S}$ can enter an

arbitrarily shaped system at (subsections of) the exterior or interior boundaries, while internal $\mathcal{S}$ is transported via diffusion and possibly also by advection, and $\mathcal{S}$ is lost within the system to competing reactions or at the boundaries due to leakage. The enzymes $\mathcal{E}$ can be freely arranged on the boundaries and within the system. Potential co-localization of $\mathcal{E}$ with other enzymes [8, 14–16] is incorporated in our model to the extent that the substrate influx at the boundaries may be caused by other, boundary-localized enzymes.

A previous numerical study of a minimal one-dimensional model within the class of models of Fig. 1 observed a localization-delocalization transition in the optimal enzyme distribution as a function of a dimensionless reaction-diffusion parameter [17]: When reactions are slow compared to diffusion, localizing all $\mathcal{E}$ at the source boundary is optimal, while a more extended profile with some enzymes also in the interior is optimal for faster reactions. The existence of this transition was subsequently found to be robust with respect to the spatial dimension and specific loss mechanism and reaction kinetics [18]. However, the physical principles governing the optimal configurations and the generality of the transition have remained elusive, and a construction principle for optimal enzyme arrangements is lacking. Within the economics analogy, the localization-delocalization transition corresponds to a transition in investment strategy from investing everything on a single asset to having a diversified portfolio with multiple assets. Can we explain the generality of the transition by analogy with a diversification investment strategy? Moreover, just as Kelly’s criterion [10] and returns-variance analysis [11] provide a set of rules for the optimal partition of bets, can we derive a generalized criterion to construct optimal enzyme arrangements?

We address these open questions with a variational approach, which ultimately reveals the investment strategy underlying optimal enzyme arrangements. The practical significance of our theoretical analysis has three aspects. First, it produces a criterion to determine, for a given experimental situation, whether enzyme localization is optimal. Second, we turn this criterion into an additive construction scheme for optimal enzyme arrangements. This is relevant for synthetic biology [19], where spatial optimization of enzymatic systems is used to boost yields of useful products such as drug molecules [20–22] and biofuels [23, 24]. Third, the conceptual framework underlying our analysis and the economic interpretation that it provides can be transferred to other biological or economical allocation problems. This supports the agenda of rationalizing biological strategies with economic principles such as bet-hedging [25–27], division of labor [28], and Pareto fronts [29].

Figure 1. Schematic representation of the class of models considered in this work. (A) An enzyme $\mathcal{E}$ converts a substrate $\mathcal{S}$ to product $\mathcal{P}$ with a catalytic rate k_{cat} . The enzyme molecules can be freely placed, both within the system and on its interior or exterior membranes. (B-Import) $\mathcal{S}$ is either imported from the exterior or produced within internal compartments. (B-Transport) Within the system, $\mathcal{S}$ is transported diffusively, and possibly also advectively, e.g. by active transport or cytoplasmic streaming. (B-Loss) During transport, $\mathcal{S}$ can also be degraded with rate σ or leak out of the system via a membrane.

RESULTS

Variational approach to the spatial enzyme allocation problem

We consider catalytic reactions where a substrate $\mathcal{S}$ is converted into a product $\mathcal{P}$ by an enzyme $\mathcal{E}$ (Fig. 1A). The substrate can enter the system from the exterior or from an internal compartment, e.g. an organelle (Fig. 1B-Import). Enzymes may be located both in the bulk of the system and at the boundaries where the substrate enters. The former fraction is described by the concentration field $e(\mathbf{r})$, with $\mathbf{r}$ denoting positions inside the system, while $e_S(\mathbf{s})$ is the density of $\mathcal{E}$ on the boundary surface at position $\mathbf{s}$. The concentration of $\mathcal{S}$ at time t is $\rho(\mathbf{r}, t)$. We consider the distribution of $\mathcal{E}$ as stationary and explore all possible spatial arrangements of the enzymes. The rate at which enzymes locally

80 catalyze product formation depends on the substrate concentration $\rho(\mathbf{r}, t)$. We express this
 81 dependence in the general form $k_{\text{cat}}eF[\rho]$, where k_{cat} is the catalytic rate of the enzyme and
 82 $F[\rho]$ is a monotonically-increasing reaction function that depends on the enzyme's reaction
 83 mechanism, e.g., $F[\rho] = \rho/(K_M + \rho)$ for Michaelis-Menten kinetics and $F[\rho] \propto \rho$ for linear
 84 mass-action kinetics.

85 Within the system, the transport of substrate molecules (Fig. 1B-Transport) can be both
 86 stochastic, with a diffusion coefficient D , as well as directed, with a velocity field $\mathbf{v}(\mathbf{r})$,
 87 where the latter could be due to cytoplasmic streaming [30] or cargo-carrying molecular
 88 motors [31]. If the substrate is intrinsically unstable [32] or subject to competing reaction
 89 pathways [16], it decays with rate constant σ (Fig. 1B-Loss). The dynamical interplay of
 90 these processes then follows

$$\partial_t \rho(\mathbf{r}, t) = D \nabla^2 \rho(\mathbf{r}, t) - \nabla \cdot [\rho(\mathbf{r}, t) \mathbf{v}(\mathbf{r})] - \sigma \rho(\mathbf{r}, t) - k_{\text{cat}} e(\mathbf{r}) F[\rho(\mathbf{r}, t)]. \quad (1)$$

91 In the following, we focus on steady-state conditions, $\partial_t \rho(\mathbf{r}, t) = 0$, where $\rho(\mathbf{r}, t) = \rho(\mathbf{r})$. We
 92 consider two types of boundaries for the system. First, boundaries at which substrate enters.
 93 Including reactions of substrate with enzymes that may be located at such a boundary, we
 94 have a boundary condition of the form

$$j(\mathbf{s}) = \left[D \frac{\partial \rho}{\partial \mathbf{n}(\mathbf{s})} - \rho(\mathbf{s}) \mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) \right] + k_{\text{cat}} e_S(\mathbf{s}) F[\rho(\mathbf{s})], \quad (2)$$

95 where $j(\mathbf{s})$ is the influx of $\mathcal{S}$ at position $\mathbf{s}$, $\hat{\mathbf{n}}(\mathbf{s})$ is a unit vector normal to the boundary,
 96 pointing outwards from the system, and $\frac{\partial}{\partial \mathbf{n}(\mathbf{s})}$ represents the magnitude of the normal com-
 97 ponent of the gradient. This condition enforces flux conservation at the boundary: the
 98 influx $j(\mathbf{s})$ must equal the sum of the local transport and reaction flux. We allow for $j(\mathbf{s})$ to
 99 be a discontinuous function of $\mathbf{s}$ as there could be regions on the boundary without influx,
 100 $j(\mathbf{s}) = 0$ (see Appendix A for details on how such discontinuities are treated). The second
 101 kind of boundaries we consider are boundaries at which substrate is lost (Fig. 1B-Loss), e.g.
 102 via leakage through a membrane [33]. For these, we impose absorbing boundary conditions,
 103 $\rho(\mathbf{s}) = 0$. For convenience, we represent these boundary conditions as

$$0 = k(\mathbf{s}) \left\{ j(\mathbf{s}) - \left[D \frac{\partial \rho}{\partial \mathbf{n}(\mathbf{s})} - \rho(\mathbf{s}) \mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) \right] - k_{\text{cat}} e_S(\mathbf{s}) F[\rho(\mathbf{s})] \right\} - h(\mathbf{s}) \rho(\mathbf{s}), \quad (3)$$

104 where $k(\mathbf{s})$ and $h(\mathbf{s})$ are set to represent absorbing boundaries ($k = 0$, $h = 1$) or boundaries
 105 with influx of substrate ($k = 1$, $h = 0$).

The interplay of processes of the type illustrated in Fig. 1 and described by Eqs. 1 to 3 have been demonstrated to generate stable gradients of intracellular components in a variety of biological contexts [34–37]. An immediate consequence of non-uniform substrate profiles is that different spatial enzyme distributions $e(\mathbf{r})$ generate different total reaction fluxes. In cases where the substrate is produced inside organelles, e.g. mitochondria or endoplasmic reticula, the associated enzymes tend to be concentrated in proximity of the organelle membrane but also present in the cytoplasm [38–41]. In contrast, when the substrate is imported into the cytoplasm from the external environment, some of the associated metabolic enzymes are localized to the external membrane [42].

The total reaction flux $J^{\mathcal{P}}$, i.e. the rate at which the whole system produces $\mathcal{P}$, has contributions from enzymes at the boundaries and within the bulk,

$$J^{\mathcal{P}} = \int_S k_{\text{cat}} e_S(\mathbf{s}) F[\rho(\mathbf{s})] d\mathbf{s} + \int_V k_{\text{cat}} e(\mathbf{r}) F[\rho(\mathbf{r})] d\mathbf{r}, \quad (4)$$

where the first integral is over all boundary surfaces S and the second is over the volume V of the system. Given a fixed amount of available enzymes, $E_T = \int_S e_S(\mathbf{s}) d\mathbf{s} + \int_V e(\mathbf{r}) d\mathbf{r}$, how should these enzymes be positioned such as to maximize the rate $J^{\mathcal{P}}$ of product formation?

This optimization problem can be approached by defining a functional of the form $\mathcal{L} = J^{\mathcal{P}} - \sum_i \lambda_i C_i$, where C_i represent different constraints with associated Lagrange multipliers λ_i . For our model, these constraints are that (i) the total amount of enzymes must equal E_T , (ii) $\rho(\mathbf{r})$ and $e(\mathbf{r})$ must jointly satisfy the reaction-diffusion-advection equation, Eq. 1, at each point $\mathbf{r}$, and (iii) $\rho(\mathbf{s})$ and $e_S(\mathbf{s})$ must jointly satisfy the boundary condition, Eq. 3, at each boundary point $\mathbf{s}$. The resulting Lagrangian has the form

$$\begin{aligned} \mathcal{L} = J^{\mathcal{P}} - \lambda_e \left[\int_S e_S(\mathbf{s}) d\mathbf{s} + \int_V e(\mathbf{r}) d\mathbf{r} - E_T \right] \\ + \int_V \lambda_V(\mathbf{r}) \mathcal{D}[e(\mathbf{r}), \rho(\mathbf{r}), \mathbf{r}] d\mathbf{r} + \int_S \lambda_S(\mathbf{s}) \mathcal{B}[\rho(\mathbf{s}), e_S(\mathbf{s}), \mathbf{s}] d\mathbf{s}, \end{aligned} \quad (5)$$

where $\mathcal{D}[e(\mathbf{r}), \rho(\mathbf{r}), \mathbf{r}]$ and $\mathcal{B}[\rho(\mathbf{s}), e_S(\mathbf{s}), \mathbf{s}]$ represent the right-hand side of Eqs. 1 and 3 respectively, and the signs in front of the Lagrange multipliers have been chosen such that they result to be positive. Maximizing $\mathcal{L}$ with respect to $e_S(\mathbf{s})$ and $e(\mathbf{r})$ while simultaneously minimizing with respect to all Lagrange multipliers yields the optimal enzyme arrangements satisfying the constraints.

The optimal enzyme arrangement may consist of both regions with a finite density of enzymes and regions with no enzymes. Indeed, previous numerical studies have shown that

the optimal enzyme distributions are often discontinuous [16–18]. To account for such discontinuities, we split the volume V into different sub-spaces (and similarly for the boundaries S); within each sub-space the enzyme density must be continuous and $e(\mathbf{r}) \geq 0$, but at the interfaces continuity is not required. The number of sub-spaces as well as the shape of the interface between each pair of sub-spaces are then also variables to be optimized (see Appendix A for details and Appendix B, E, F for examples). This is equivalent to applying the Karush-Kuhn-Tucker conditions [43, 44] on the Lagrangian, Eq. 5, while imposing $e(\mathbf{r}) \geq 0$.

We will see below that the variational approach is useful even when it is impossible to solve for the optimal enzyme arrangement analytically. In cases where it is possible to extract the exact functional form of the optimal profile, the problem can usually be reduced to a low-dimensional optimization over a small number of parameters, which is then easily performed numerically (see Appendix E for an example). For systems where the functional form of $e(\mathbf{r})$ cannot be found analytically, such as those with complex geometries, we will describe a scheme for constructing the optimal enzyme arrangement.

Enzyme allocation problem as a betting game

The spatial enzyme allocation problem defined above can be mapped to a betting game. We consider a game that has a single winning outcome out of a set of mutually exclusive events. Each outcome i has a certain probability p_i of being the winning event. We assume that a gambler bets a total amount $B = \sum_i b_i$ with $b_i \geq 0$ denoting the bet on outcome i . If i is the winning event, the gambler wins the amount $\alpha_i b_i$, with $\alpha_i \geq 0$ referred to as the odds of event i . The expected amount of capital won by the gambler is thus $C = \sum_i \alpha_i b_i p_i$. If the expected odds $\alpha_i p_i$ are independent of the bets b_i , the optimal strategy for a given total budget B is to invest everything in the outcome with highest expected odds. However, if the expected odds are coupled to the bets, the optimization of C subject to the budget constraint becomes nontrivial, and diversification of bets may be the optimal strategy.

By comparing the objective function C of the betting game to the reaction flux J^P of our spatial enzyme allocation problem, we see that the set of events $\{i\}$ maps to the set of possible enzyme positions, a bet b_i corresponds to the enzyme concentration $e(\mathbf{r})$ allocated to $\mathbf{r}$, and the fixed budget B corresponds to the total enzyme amount E_T . Moreover, the probability of i being the winning event maps to the probability density for a substrate

163 molecule to bind to an enzyme at position $\mathbf{r}$, $p_i \leftrightarrow F[\rho(\mathbf{r})]$, and the odds α_i correspond to
 164 the catalytic rate k_{cat} .

165 In the enzyme system, part of the substrate is lost via the mechanisms of Fig. 1B-Loss,
 166 which can be included in the betting game via events with finite probability but zero odds.
 167 However, the objective function $C = \sum_i \alpha_i b_i p_i$ needs to be generalized to account for the
 168 nonlinearity and spatial coupling in the enzyme system: The “expected odds” $k_{\text{cat}} F[\rho(\mathbf{r})]$
 169 depend on the “bets” $e(\mathbf{r})$, since the binding between enzymes and substrate at a given
 170 location generates a diminishing returns effect, i.e., the addition of extra enzymes at that
 171 position becomes gradually less efficient. Moreover, the substrate concentrations at different
 172 positions are coupled, due to the substrate diffusion (and possibly advection), such that the
 173 substrate concentration at one position ultimately depends on the enzymes at every position.
 174 This means that the bets placed on one event i also affect the winning probabilities p_j with
 175 $j \neq i$. These effects are captured by making the probabilities p_i a function of the vector of
 176 bets $p_i = f_i(\mathbf{b})$ and adding the feedback from the bets onto the winning probabilities via
 177 constraints in the objective function,

$$C = \sum_i \alpha_i b_i p_i - \sum_i \lambda_i [f_i(\mathbf{b}) - p_i] - \lambda \left(\sum_i b_i - B \right), \quad (6)$$

178 where λ_i are the Lagrange multipliers for the feedback constraint, analogous to $\lambda(\mathbf{r})$ in the
 179 Lagrangian $\mathcal{L}$ of the enzyme system, Eq. 5. The behavior of this generalized betting game
 180 depends on the form of $f_i(\mathbf{b})$. In Appendix H, we show for example that in a simple game
 181 with two possible winning outcomes, a diminishing returns effect ($\partial f_i / \partial b_i < 0$) can result in
 182 an optimal strategy with diversified bets ($b_1, b_2 > 0$).

183 The above mapping illustrates that in order to solve our optimal enzyme allocation prob-
 184 lem, we need to generalize the treatment of betting games to include the coupling between
 185 bets and expected gains. Note that we considered only a single round of the betting game so
 186 far. Classical treatments of betting games, e.g. the one by Kelly [10], often assume that the
 187 gambler iteratively reinvests a certain fraction of the current capital, such that the long-term
 188 capital growth rate must be optimized rather than C . In the enzyme context, this would
 189 correspond to a situation where the product of the enzymatic reaction is used to generate
 190 additional enzymes to be placed in the system. Here, we first treat the stationary enzyme
 191 allocation problem, corresponding to a repetitive game in which the gambler bets the same
 192 total amount B in every round, such that the optimal strategy is the same as the optimal

strategy for a single round. We will later see that our scheme to construct optimal strategies can also be adapted to the case where a constant fraction of the capital is iteratively reinvested (see below and Appendix H).

Optimal allocation principle

We now leverage the above variational approach to solve the enzyme allocation problem. Towards this end, we examine the variation of the Lagrangian, Eq. 5, with respect to the enzyme density,

$$\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})} = \underbrace{k_{\text{cat}} F[\rho(\mathbf{r})] - \lambda_V(\mathbf{r}) k_{\text{cat}} F[\rho(\mathbf{r})]}_{\equiv \frac{dJ^P}{de(\mathbf{r})} \text{ "marginal return"}} - \lambda_e. \quad (7)$$

This variation corresponds to the change in the constrained flux $\mathcal{L}$ as enzymes are added at position $\mathbf{r}$. Three different terms contribute to this change. The first is the increase in flux that would be observed upon adding enzymes at position $\mathbf{r}$ if everything else in the system were to remain unaffected, which corresponds mathematically to $\frac{\delta J^P}{\delta e(\mathbf{r})}$. However, changing $e(\mathbf{r})$ also alters the substrate density profile $\rho(\mathbf{r})$, thereby affecting the rate of reactions at all positions. This coupling is captured by the second term in Eq. 7, where the Lagrange multiplier $\lambda_V(\mathbf{r})$ ensures that $\rho(\mathbf{r})$ and $e(\mathbf{r})$ satisfy the constraint of the reaction-diffusion equation. Thus the sum of the first two terms is the total rate of change of the reaction flux as extra enzymes are added at position $\mathbf{r}$. In the following, we refer to this quantity as the “marginal return” on an investment of enzymes, and denote it by $\frac{dJ^P}{de(\mathbf{r})}$. Finally, the third term, $-\lambda_e = -\frac{\partial \mathcal{L}}{\partial E_T}$, corresponds to a marginal cost of adding extra enzymes into the system. This marginal cost can be interpreted as the reduction in flux from other positions $\mathbf{r}' \neq \mathbf{r}$ in the optimal configuration, as enzymes are moved from these positions to $\mathbf{r}$ in order to satisfy the constraint of constant total enzyme number.

In the optimal enzyme profile $e^*(\mathbf{r})$, we must distinguish between regions where placing enzymes is optimal ($e^*(\mathbf{r})$ finite), and empty regions where placing enzymes is suboptimal ($e^*(\mathbf{r}) = 0$). Wherever $e^*(\mathbf{r}) > 0$, the variation $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}|_{e^*} = 0$. The functional derivative with respect to the surface density $\frac{\delta \mathcal{L}}{\delta e_S(\mathbf{s})}$ has the same form as Eq. 7. Thus the optimal profile follows a ‘homogeneous marginal returns’ criterion (HMR criterion): it is such that at any position with enzymes, the marginal return on placed enzymes equals a constant value, namely $\frac{dJ^P}{de(\mathbf{r})}|_{e^*} = \lambda_e$. By contrast, in empty regions, the marginal flux gain from placing

enzymes is less than the associated marginal cost, $\frac{dJ^P}{de(\mathbf{r})}|_{e^*} < \lambda_e$.

To illustrate this optimal allocation principle, we apply the HMR criterion to the simple case of a linear reaction ($F[\rho] = \rho/K_M$) with no advection or decay ($|\mathbf{v}| = \sigma = 0$) in a one-dimensional domain of size L , with a source of substrate at the left boundary ($x = 0$) and an absorbing boundary on the right (Fig. 2A). This case is exactly solvable within the variational approach, such that we can explicitly see how variations of the enzyme profile affect the marginal returns landscape. Moreover, this case allows us to cross-validate our variational approach with a previous numerical study [17]. As shown in Appendix B, the optimal enzyme profile for this example is

$$e_S^*(0) = \begin{cases} E_T \\ \sqrt{E_T/\alpha} \end{cases} \quad \text{and} \quad e^*(x) = \begin{cases} 0 \\ \frac{E_T}{L} \Theta\left(\frac{x_0 - x}{L}\right) \end{cases} \quad \text{for} \quad \begin{cases} \alpha E_T \leq 1 \\ \alpha E_T > 1 \end{cases}, \quad (8)$$

with $\alpha = Lk_{\text{cat}}/(K_M D)$, the Heaviside step function $\Theta(x)$, and $x_0 = L \left[1 - (\alpha E_T)^{-1/2}\right]$. Eq. 8 shows that the optimal enzyme profile undergoes a localization-delocalization transition from having all enzymes localized at the source, to an extended profile with enzymes also in the interior of the system (Fig. 2B, orange lines). The transition occurs as a function of the dimensionless parameter αE_T , with $\alpha E_T = 1$ marking the transition point.

The analytic solution demonstrates how the optimal enzyme profile arises from a balance between gain and cost. The shape of the marginal returns landscape (Fig. 2B, green lines) changes as the total enzyme amount E_T is increased. Below the transition, the marginal returns $\frac{dJ^P}{de(x)}$ strictly decrease with distance from the source, i.e., the maximal value occurs only at $x = 0$, whereas above the transition the marginal returns take on a constant value over the region $0 \leq x \leq x_0$ where enzymes are present and decreases only for $x > x_0$. The level of this marginal returns plateau corresponds to the marginal cost λ_e of adding extra enzymes into the system (Fig. 2C, middle). This marginal cost decreases with increasing availability of enzymes (increasing E_T). Fig. 2C shows how the shape of the marginal returns landscape changes when the enzyme profile deviates from the optimum. If it deviates by having a larger proportion of enzymes located at the source (Fig. 2C, top), the marginal returns landscape becomes non-monotonic with a peak at a position $x > 0$. The elevated enzyme density in this region depletes the substrate locally, reducing the turnover by each enzyme and diminishing the returns from adding additional enzymes. The peak in the marginal returns landscape implies that a higher flux can be achieved by moving some

Figure 2. Homogeneous Marginal Returns (HMR) criterion for the optimal spatial allocation of enzymes. (A) Illustration of the analytically solvable 1D model with a source of substrate at $x = 0$ and an absorbing boundary at $x = L$. Within the system, the substrate diffuses and can react with the spatially arranged enzymes. (B) Optimal enzyme profile with corresponding marginal return landscape as a function of the total enzyme amounts E_T (the reaction-diffusion parameter is fixed to $\alpha = 10^{-2}$). (C) Different enzyme and substrate profiles (left) with corresponding marginal returns (right) for $E_T = 400$. Top: Localized configuration. The marginal returns landscape is peaked at $x > 0$, indicating that enzymes should be moved away from the source. Middle: Optimal profile with constant marginal return over the region with enzymes (HMR criterion). In the constant region, the marginal return is equal to the marginal cost λ_e of adding extra enzymes into the system. Bottom: Overextended profile. Monotonically decreasing marginal return, indicating that enzymes should be moved towards the source at $x = 0$.

enzymes away from the source. In contrast, if the enzyme profile is overextended (Fig. 2C, bottom), the marginal returns landscape has a maximum at the source, such that the flux can be increased by moving enzymes towards the source.

Note that for the 1D problem analyzed in Fig. 2, the single source at $x = 0$ is always the position with the highest substrate concentration, independently of the enzyme arrangement (see purple lines). Hence an individual enzyme would always optimize its own productivity

at $x = 0$. However, for $\alpha E_T > 1$, the total productivity of the population of enzymes is optimized only when some enzymes are located at positions $x > 0$ with non-maximal substrate concentrations. The optimal profile is thus the result of a bet-hedging strategy: Some productivity of individual enzymes is sacrificed to optimize the reaction flux produced by the entire population of enzymes. How relevant is the transition for biological systems? Given that the ratio k_{cat}/K_M varies by orders of magnitude, 10^2 - 10^9 ($M s$)⁻¹ [45], the corresponding $\alpha = Lk_{\text{cat}}/(K_M D)$ for a system with $L = 1\mu m$, $D = 100\mu m^2/s$ is in the range from 10^{-7} to 1, such that the transition can occur for enzyme numbers E_T^t in the range 1 - 10^7 . For comparison, the range of protein copy numbers in a yeast cell is 1 - 10^6 [46], suggesting that different enzymatic systems cover both sides of the transition.

General condition for transitions in the optimal enzyme arrangement

Transitions in the optimal enzyme arrangement of the above type, from a regime in which enzymes are colocalized with the source of $\mathcal{S}$ to a regime where enzymes are distributed within the system, have been observed in a range of reaction-diffusion models [18]. We therefore asked whether there was an underlying principle determining when such transitions occur, and whether it can be generalized, e.g. to systems with more complex geometries.

Fig. 2 suggests that the marginal returns landscape leads to a general condition for localization-delocalization transitions. For E_T below the transition value, the landscape is strictly decreasing, with the position generating the highest returns coinciding with the source of substrate. As the transition is reached and passed, the landscape becomes flat as positions in the vicinity of the source begin to generate the same returns as the position. This behavior generalizes to higher dimensional systems (see Appendix D): Enzymes should be placed only at the source on the surface and not in the interior, as long as

$$\frac{\partial}{\partial \mathbf{n}(\mathbf{s})} \frac{dJ^P}{de} > 0, \quad (9)$$

corresponding to a negative slope of the marginal returns landscape from the source at $\mathbf{s}$ into the interior of the system, as in Fig. 2B for $E_T < \alpha^{-1}$. By contrast, if Eq. 9 evaluated at $\mathbf{s}$ becomes an equality, the positions adjacent to $\mathbf{s}$ generate the same returns as $\mathbf{s}$, so that the optimal enzyme profile features enzymes both at the boundary $\mathbf{s}$ and in the interior of the system adjacent to $\mathbf{s}$.

Figure 3. The local reaction flux, $j^P(\mathbf{s})$, and net diffusive flux, $j^D(\mathbf{s})$, as a fraction of $\mathcal{S}$ influx, $j(\mathbf{s})$, are plotted for different models against the amount of enzymes in the system, E_T , rescaled by the transition amount, E_T^t . In all cases, $j^D > j^P$ until they coincide at the transition point, as predicted by Eq. 10. When $\nu = 0$, $j^P = j^D = j/2$ at and above the transition. Lines show analytical solutions of the constrained optimization. Points show results of numerical optimizations computed either by solving numerically an analytically-derived optimization condition (Appendix E), or using the construction algorithm. For the two-source model, the two curves show the two unequal sources, each rescaled with the corresponding E_T^t value; see Appendix F and Fig. F.1 for a detailed analysis of this model.

284 The abstract mathematical condition Eq. 9 can be turned into an experimentally mean-
 285 ingful condition (see Appendix D) by expressing it as a comparison between the local net
 286 diffusive flux $j^D(\mathbf{s})$ of $\mathcal{S}$ away from the surface, and the local flux $j^P(\mathbf{s})$ of product formation
 287 due to surface enzymes,

$$j^D(\mathbf{s}) \equiv D \frac{\partial \rho}{\partial \mathbf{n}(\mathbf{s})} > k_{\text{cat}} e_S(\mathbf{s}) F[\rho(\mathbf{s})] \equiv j^P(\mathbf{s}). \quad (10)$$

288 If this inequality holds, no enzymes should be placed in the interior of the system in the
 289 vicinity of $\mathbf{s}$. For low amounts of enzymes, the optimal strategy is to place enzymes at
 290 position $\mathbf{s}$ to counter the diffusive flux $j^D(\mathbf{s})$. This is optimal up to the point at which the
 291 diffusive flux $j^D(\mathbf{s})$ equals the reaction flux $j^P(\mathbf{s})$, which defines the transition point of the

292 localization-delocalization transition.

293 Further combining Eq. 10 with the boundary condition Eq. 2 yields $j^P(\mathbf{s}) < (j(\mathbf{s}) -$
 294 $\rho(\mathbf{s})\mathbf{v}(\mathbf{s}) \cdot \hat{n}(\mathbf{s}))/2$, which simplifies in the case of no advection ($\mathbf{v} = 0$) to $j^P < j(\mathbf{s})/2$. Thus,
 295 in systems with purely diffusive $\mathcal{S}$ transport, a localization-delocalization transition occurs
 296 when the reaction flux equals exactly half the influx of $\mathcal{S}$. Note that Eq. 10 is general,
 297 independent of $\mathbf{v}$ and σ and other details of the system in question. It is required only
 298 that the boundary with influx follows Eq. 2. Other aspects of the model will affect $\rho(\mathbf{r})$,
 299 but not the form of Eq. 10, showing that the diffusive motion of $\mathcal{S}$ and the strength of the
 300 reaction are the crucial determinants of the transition point. To illustrate this generality,
 301 we compared the fluxes $j^D(\mathbf{s})$ and $j^P(\mathbf{s})$ as E_T was varied, in systems with different spatial
 302 dimensions as well as systems with and without decay and advection of $\mathcal{S}$. As shown in
 303 Fig. 3, in all cases both Eq. 10 below the transition value of E_T and equality above the
 304 transition were satisfied.

305 The inequality condition Eq. 10 is a strictly local condition. In systems with multiple or
 306 spatially varying sources of substrate there will in general not be a single global transition
 307 but rather a sequence of local transitions at different positions $\mathbf{s}$ at different E_T levels. We
 308 therefore investigated a system with two unequal sources of $\mathcal{S}$. In such a system the optimal
 309 enzyme profile typically undergoes three transitions (see Fig. F.1). For small E_T , enzymes
 310 accumulate where the influx of $\mathcal{S}$ is the highest. As E_T is increased, the marginal returns
 311 for placing enzymes in the vicinity of the two sources of $\mathcal{S}$ become more similar, until at a
 312 threshold value of E_T they become equal. Above this value of E_T , enzymes accumulate at
 313 both sources in a configuration with two unequal clusters. Increasing E_T further, a second
 314 transition is reached where the optimal enzyme profile begins to extend from the stronger
 315 source into the system. Finally, at yet larger E_T , the optimal enzyme profile begins to
 316 extend also from the weaker source. For the latter two transitions, at which the optimal
 317 enzyme arrangement changes from membrane-localized to extended, we confirmed that the
 318 inequality Eq. 10 held below the transition point and equality above (Fig. 3 green and blue).

319 Geometrical interpretation and analogy to portfolio selection

320 Our optimal enzyme allocation problem has a geometrical interpretation in the space
 321 of (discretized) enzyme configurations $\mathbf{e}$. A component e_i of the vector $\mathbf{e}$ corresponds to

Figure 4. Geometrical analysis of the optimal enzyme configuration in a system with two sites. Dashed black lines show lines of constant $E_T = e_1 + e_2$, while dashed green lines show lines of constant reaction flux J^P . The optimal configuration $\mathbf{e}^* = (e_1^*, e_2^*)$ for each value of E_T forms an optimal trajectory $\mathbf{e}^*(E_T)$ (orange). Green arrows show the marginal returns vector $\frac{dJ^P}{de}$. The tangent vector $\frac{de^*}{dE_T}$ (blue arrows) shows how added enzymes should be optimally partitioned between the two sites.

the amount of enzymes allocated to position i , and the dimension of $\mathbf{e}$ to the number of possible enzyme positions. Hence, the subspace with constant total enzyme amount E_T is the hyperplane intersecting each axis at $e_i = E_T$. Each point in the enzyme configuration space has an associated flux J^P , and the allocation problem amounts to finding the point of maximal flux within the hyperplane.

Fig. 4 illustrates the geometrical problem for the simplest case with only two possible enzyme positions (here, we assume a substrate source at position one, and loss of substrate via one of the loss mechanisms of Fig. 1B). In Fig. 4, E_T hyperplanes are indicated by dashed black lines, while the dashed green curves are lines of constant flux J^P . Since the marginal returns vector $\frac{dJ^P}{de}$ is the flux gradient, the lines of constant flux must be locally perpendicular to it. Adding extra enzymes at any location with nonzero substrate concentration will increase the total reaction flux, implying that the direction of the marginal returns vector always lies in the first quadrant (all components positive). Furthermore, the direction must turn clockwise as we move along a line of constant flux in the direction of increasing e_2 , since the second component of the marginal returns vector must decrease as we allocate a larger fraction of enzymes to position two, while the first component must increase. Hence, the lines of constant flux are convex.

Geometrically, the solution of the optimal allocation problem corresponds to the touching point $\mathbf{e}^*$ between a given E_T line and the J^P line with the largest J^P value that still touches

the E_T line. At small E_T values, the J^P line is steeper than the E_T line, such that $\mathbf{e}^*(E_T)$ lies on the e_1 axis (orange line in Fig. 4). However, at a certain threshold value of E_T , the E_T line becomes tangential to the J^P line. At this point, the marginal returns from position 1 and 2 become equal, i.e., the marginal returns vector points in the (1, 1) direction. When E_T is further increased, the optimal path $\mathbf{e}^*(E_T)$ departs from the e_1 axis and follows the tangent point between the E_T and J^P lines, maintaining equal marginal returns. Note that, due to the convexity of the lines of constant J^P , this tangent point is the global optimum at a given E_T value.

The above geometrical interpretation is somewhat analogous to Markowitz' geometrical analysis of investment portfolio optimization [11]. Markowitz considered the problem of minimizing the variance of a portfolio of investments at a constant level of returns (returns-variance analysis). The space of portfolios, which is determined by the relative amounts invested in different securities, is analogous to the space of enzyme configurations $\mathbf{e}$. Markowitz noted that optimal portfolios are tangent points between lines of constant expected returns and lines of constant variance. One key difference to our case is that the returns are independent of the portfolio choice in Markowitz' case, leading to simple straight lines of constant returns in the space of portfolios. Another key difference is that the variance of the returns has played no role in our analysis so far, since we considered enzymes in a steady-state environment. If, instead, we would consider a fluctuating environment in which, for instance, the substrate source is only available for a finite time T , then the variation of the flux $J^P(t)$ would become relevant. The total amount of product formed during this time, $\mathcal{P}_T$, would be

$$\mathcal{P}_T = \int_0^T J^P(t) dt = \langle J^P \rangle T + \int_0^T \delta J^P(t) dt, \quad (11)$$

where $\delta J^P(t) = J^P(t) - \langle J^P \rangle$ is the time-dependent fluctuation of the flux around its mean value $\langle J^P \rangle$, caused by the stochasticity of the enzymatic reaction and substrate diffusion. The integral over $\delta J^P(t)$ scales as $\sqrt{T}$, due to the central limit theorem, such that its contribution to $\mathcal{P}_T$ becomes negligible in the long time limit. However, for times T comparable to the intrinsic time-scales of the system (associated with the processes of diffusion, reaction, and decay), the contribution of the fluctuations could become sizeable. In such scenarios, a generalization of Markowitz' returns-variance analysis would be more appropriate than an optimization of $\langle J^P \rangle$.

Additive construction of optimal enzyme arrangements

The above geometrical analysis leads us to an additive construction scheme, which generates the trajectory of optimal enzyme configurations as enzymes are added to the system. Generalizing from the example of Fig. 4, we consider a system in which space is discretized into N sites, denoting by $e_i = e(\mathbf{r}_i)$ the density of enzymes at position $\mathbf{r}_i$ and by $\rho_i = \rho(\mathbf{r}_i)$ the corresponding substrate density. For a given total enzyme level E_T , there will be an optimal enzyme configuration $\mathbf{e}^* = (e_1^*, e_2^*, \dots, e_N^*)$ that maximizes J^P on the $(N - 1)$ -dimensional hyperplane defined by $E_T = \sum_{i=1}^N e_i$. As in Fig. 4, the optimal configurations at different E_T values trace out a trajectory $\mathbf{e}^*(E_T)$ in $\mathbf{e}$ space. We seek a procedure to construct $\mathbf{e}^*(E_T)$. We begin with no enzymes and iteratively add enzymes until the target E_T value is reached. According to the principle of homogeneous marginal returns, the largest components of $\frac{dJ^P}{d\mathbf{e}}$ define the sites at which enzymes should be added in the next step. Addition may initially be restricted to a single site, with further “construction sites” being introduced gradually as different E_T thresholds are crossed.

Since enzymes should be added at all positions where the marginal returns take on their maximal value, we must determine how to partition new enzymes between these positions. This partitioning corresponds to the tangent vector $\frac{d\mathbf{e}^*}{dE_T}$ of the optimal trajectory, which can be obtained from the variation of the marginal returns as enzymes are added (see Appendix G 1),

$$\frac{d}{dE_T} \frac{dJ^P}{d\mathbf{e}} = \mathbf{H} \frac{d\mathbf{e}^*}{dE_T} . \quad (12)$$

Here, derivatives $\frac{d}{dE_T}$ are taken tangentially to the line of optimal enzyme configurations and $\mathbf{H}$ is the $N \times N$ Hessian matrix of J^P in the space of enzyme densities, $H_{ij} = \frac{d^2 J^P}{de_i de_j}$. In the subspace of only those sites where enzymes should be added (sites at which $\frac{dJ^P}{de_i} = \lambda_e$), the left hand side of Eq. 12 becomes

$$\frac{d}{dE_T} \frac{dJ^P}{d\mathbf{e}_{(n)}} = \frac{d\lambda_e}{dE_T} \mathbf{1}_{(n)} , \quad (13)$$

where the subscript (n) indicates that we are considering only the n -dimensional subspace of sites with equal returns, yielding

$$\mathbf{H}_{(n)} \frac{d\mathbf{e}_{(n)}^*}{dE_T} = \frac{d\lambda_e}{dE_T} \mathbf{1}_{(n)} . \quad (14)$$

This n -dimensional linear system can be solved for the tangent $\frac{d\mathbf{e}_{(n)}^*}{dE_T}$ to the optimal trajectory

[47]. Taken together, we obtain the following additive construction scheme for the optimal enzyme arrangement:

1. Begin with $\mathbf{e} = 0$.
2. For each site $\mathbf{r}_i$, evaluate the marginal returns $\frac{dJ^P}{de_i}$ for adding enzymes at that position.
3. Identify the subset of positions at which the returns are equal (within numerical tolerance) to the maximal value.
4. For this subset of positions, evaluate the Hessian matrix $\mathbf{H}_{(n)}$ of the flux with respect to the enzyme densities.
5. Solve $\mathbf{H}_{(n)}\delta\mathbf{e}^* = \mathbf{1}$ for $\delta\mathbf{e}^*$, and normalize such that the total change in the amount of enzymes $\sum_i \delta e_i^* = \delta E_T$.
6. Update the optimal enzyme arrangement, $\mathbf{e}^* \rightarrow \mathbf{e}^* + \delta\mathbf{e}^*$.
7. Repeat from step 2 until the target total enzyme amount E_T is reached.

The discretization of the system, which is required for the above construction algorithm, can be chosen using a criterion based on Eq. 10 (see Appendix G 2).

Fig. 5 illustrates the algorithm for a two-dimensional system featuring multiple sources of substrate (dashed rectangles) with uniform influx on their surfaces and an absorbing outer boundary. Fig. 5A compares two optimal enzyme configurations with a different amount of enzymes. For low E_T values, enzymes localize non-uniformly at the boundaries of the sources. For higher E_T values, the optimal enzyme profile extends into the interior of the system. However, the highest densities of enzymes are found in the regions between the sources and the outer boundary of the system, where the gradient of substrate concentration is steepest. A significant fraction of enzymes are thus devoted to substrates that, by the nature of where they are produced, are likely to rapidly diffuse out of the system.

Remarkably, the sites with the highest enzyme densities at small E_T do not necessarily retain the highest densities as E_T is increased. Fig. 5B compares the enzyme densities as a function of E_T at three positions (marked by colored squares in Fig. 5A). The blue location is the first at which enzymes are added. However, although enzymes are added at the green and red positions only later, the densities here become higher. Fig. 5C shows the respective

Figure 5. Optimal enzyme arrangements in a 2-dimensional system with multiple sources of substrate (dashed rectangles), calculated using the construction algorithm. (A) Snapshots of the optimal enzyme distribution at two E_T levels. (B) Optimal enzyme densities and (C) ratios of marginal returns at the positions indicated by colored squares in (A). (D) The optimized reaction flux J_{opt} relative to the flux J_u produced by uniformly distributed enzymes, as a function of E_T .

ratios of the returns of the red and green position to the returns from the blue position. Enzymes are added at the green and red position only when the returns are equal and the ratios are one. At low enzyme amounts, the reaction flux of the optimal configuration is about 3-fold higher than for the uniform configuration, whereas this advantage gradually decreases for higher amounts of enzyme (Fig. 5D).

Compared to the stochastic search algorithms that have previously been used to find the optimal enzyme arrangements in systems with simple geometries [17, 18], the additive construction scheme derived here has several advantages. In terms of computational effort, it generally performs better than stochastic optimization (see Appendix G3), in particular for more complex systems. Moreover, while stochastic optimization proceeds by trial-and-error

and can get stuck in local optima, the construction scheme follows a rational principle. By design, the construction scheme not only generates the optimal enzyme arrangement for the given total enzyme amount E_T , but all optimal configurations for total enzyme amounts up to this level. As seen in the example of Fig. 5, this trajectory of optimal configurations leads to additional insight about the properties of the particular system at hand.

The construction scheme can also be adapted to other allocation problems in which investments and returns are coupled. As long as the resources to be invested follow a linear budget constraint, the adaptation amounts to a modification of the objective function. For instance, as mentioned above, one could consider an objective function penalizing high variance as in Markovitz’ problem [11]. One could also consider problems where the capital of the investor grows exponentially (iterative reinvestment) as in Kelly’s problem [10] or more complex variants [48]. In each case, one needs to compute the marginal returns and the Hessian of the new objective function with respect to the resources invested in the different assets, and determine the optimal allocation of resources as the total budget gradually increases via the construction scheme. In Appendix H, we present a generalization of Kelly’s problem to a case with coupled investments and returns for which the objective function becomes the long-term capital growth rate and the above additive construction scheme can be used.

DISCUSSION

We presented a solution for the problem of optimally allocating enzymes in space, to maximize the reaction flux of an enzymatic reaction. Our solution encompasses a class of systems with potentially complex geometries, in which the substrate enters via internal or external boundaries, is transported via diffusion and possibly advection, and can be lost by leakage or competing reactions (Fig. 1). The solution is based on the concept of a ‘marginal returns landscape’ (Fig. 2), defined as the derivative $\frac{dJ^P}{de(\mathbf{r})}$ of the total reaction flux with respect to the local enzyme density (Eq. 7). The optimal arrangement of enzymes is such that the marginal returns are spatially homogeneous over all positions with enzymes. This criterion of homogeneous marginal returns (HMR) can more generally be applied to betting games where each bet globally feeds back onto all returns (Appendix H). In the enzyme allocation problem, the feedback is generated by enzymes locally depleting their substrate,

465 which affects the global substrate profile.

466 The HMR criterion leads to a general local condition for the occurrence of a localization-
467 delocalization transition in the optimal enzyme arrangement (Eq. 10), which compares the
468 local diffusive flux $j^D(\mathbf{s})$ to the local reaction flux $j^P(\mathbf{s})$ at a source position $\mathbf{s}$: As long
469 as $j^D(\mathbf{s}) > j^P(\mathbf{s})$, localization of enzymes at the source is optimal, while the two fluxes
470 are equal at the transition point and above (Fig. 3). The simplicity of this flux condition
471 makes it practical for application to experimental systems. For instance, for an *in vivo*
472 system in which enzyme localization is observed and the enzyme abundance is quantified,
473 the condition can be used to estimate whether the observed enzyme localization indeed
474 maximizes the reaction flux, or whether it is more likely to play a regulatory role [7, 8]. In
475 this context, it is beneficial that the flux condition is not affected by any advective transport
476 of the substrate, which may be present in the system, e.g. due to cytoplasmic streaming
477 or transport by molecular motors. This independence is due to the deterministic nature of
478 advective transport – the HMR criterion is fundamentally a bet-hedging strategy to deal
479 with the probabilistic nature of diffusive transport.

480 Just as the Kelly criterion [10] and returns-variance analysis [11] provide rules for op-
481 timally placing bets or optimally constructing portfolios, the HMR criterion allowed us to
482 derive an algorithm for constructing optimal enzyme arrangements (Figs. 4 and 5). This
483 algorithm is obtained by analyzing how the marginal returns change at each position as
484 the amount of enzymes in the system is increased (Eq. 14). It constitutes an exact deter-
485 ministic construction scheme, which is applicable to systems with arbitrary geometries and
486 multiple sources. Here, we presented the simplest general form of the algorithm. Various
487 modifications can be made to improve its efficiency for particular systems. Using the sys-
488 tem geometry as a guide, one can limit the sites at which the marginal returns need to be
489 evaluated in each update step. For example, at low E_T values the positions with the highest
490 returns coincide with sources of $\mathcal{S}$. Generally it suffices to compute the marginal returns
491 only at source positions, sites where enzymes are already present, and neighboring sites. One
492 could further speed up the algorithm by enabling larger increments δE_T with higher-order
493 update schemes for $\mathbf{e}^*$.

494 We explicitly considered absorbing and reflective boundaries, also allowing for non-
495 uniform and discontinuous influx profiles. However our results can be extended to systems
496 with partially permeable boundaries, corresponding to $k(\mathbf{s}) = 1$ and $h(\mathbf{s}) = p(\mathbf{s}) > 0$ in

Eq. 3, where $p(\mathbf{s})$ is the permeability. For such systems, the HMR criterion still holds. Similarly, the condition of Eq. 9 still determines transitions in the optimal enzyme profile. However, expressing this condition in term of substrate fluxes will lead to a more complicated expression than Eq. 10. A permeable boundary typically increases the transition threshold E_T^t . For example, in the one-dimensional system of Fig. 2, a non-zero permeability at the origin shifts the threshold value of E_T from α^{-1} to $\alpha^{-1}(1 + p(0)L/D)$.

Our continuous reaction-diffusion model neglects the finite size of enzyme molecules that limits the attainable enzyme density. Imposing a maximal density condition $e(\mathbf{r}) \leq e_{\max}$ at each point [16] could be incorporated into our analytical framework as an additional constraint in the Lagrangian. This would introduce an effective position-dependent cost in Eq. 7, resulting in a marginal returns landscape that is a function of position. The construction algorithm could still be employed with the additional condition that enzymes cannot be added at positions where $e_i = e_{\max}$, such that Eq. 14 would be restricted to positions with sub-maximal enzyme densities.

The construction algorithm for optimal enzyme arrangements appears suited for applications in synthetic biology [19]. The attachment of enzymes to membranes can be controlled by fusing enzymes to transmembrane proteins [49, 50] or by using protein scaffolds [51, 52]. The arrangement of enzymes in the interior of membrane-enclosed systems can be partially controlled with RNA assemblies [53], synthetic protein scaffolds [20], or fusion proteins [16]. Enzyme positioning on surfaces can be controlled with nanometer precision via single-molecule cut-and-paste surface assembly [54, 55], and in 3D by arranging enzymes on DNA scaffolds [56–59]. Combining this arsenal of techniques with the rational construction scheme, one could optimize synthetic bioreactors to produce drug molecules [20–22] or biofuels [23, 24].

MATERIALS AND METHODS

Computation of $\frac{dJ}{de}$ and $\mathbf{H}$ for linear reactions

For a discrete reaction-diffusion system with linear reactions, following the dynamics of Eq. 1, the steady-state equation can be written in matrix form as

$$(\mathbf{D} + \mathbf{V})\boldsymbol{\rho} - \alpha \mathbf{e} \odot \boldsymbol{\rho} - \sigma \boldsymbol{\rho} = \mathbf{A}, \quad (15)$$

Figure 6. Dependency graph for a discrete reaction-diffusion system with linear reaction kinetics.

where $\mathbf{D}$ and $\mathbf{V}$ are the diffusion and advection operator on the lattice respectively, ρ the substrate density and $\mathbf{A}$ the substrate influx vector, while $\odot$ denotes the element-wise (Hadamard) product. The reaction flux J^P is given by the scalar product $J^P = \alpha \mathbf{e} \cdot \rho$. Thus $\mathbf{e}$ enters J^P both directly and through the solution ρ of Eq. 15. These multiple dependencies of J^P on $\mathbf{e}$ are summarized by the dependency graph in Fig. 6, where the matrix $\mathbf{M} = \mathbf{D} + \mathbf{V} - \alpha \text{diag}(\mathbf{e}) - \sigma \text{diag}(\mathbf{1})$ is the operator that applied to ρ gives the substrate source vector $\mathbf{M}\rho = \mathbf{A}$, $\text{diag}(\mathbf{e})$ is the diagonal matrix with diagonal elements given by $\mathbf{e}$ and $\text{diag}(\mathbf{1})$ is the identity matrix.

The marginal returns vector $\frac{dJ^P}{d\mathbf{e}}$ can be evaluated by back-propagating the derivative of the reaction flux with respect to the enzyme vector through the dependency graph. We find that

$$\frac{dJ^P}{d\mathbf{e}} = \frac{\partial J^P}{\partial \mathbf{e}} + \left(\frac{d\rho}{d\mathbf{e}} \right)^t \frac{\partial J^P}{\partial \rho} \quad (16)$$

$$= \frac{\partial J^P}{\partial \mathbf{e}} + \left(\frac{d(\mathbf{M}^{-1}\mathbf{A})}{d\mathbf{M}} \frac{d\mathbf{M}}{d\mathbf{e}} \right)^t \frac{\partial J^P}{\partial \rho}, \quad (17)$$

where $\frac{d\rho}{d\mathbf{e}}$ is a matrix with $(i, j)^{\text{th}}$ element given by $\frac{d\rho_i}{de_j}$, and t denotes the transpose. Each of the derivatives appearing in Eq. 17 has a simple form, which we substitute to obtain the marginal returns vector,

$$\frac{dJ^P}{d\mathbf{e}} = \alpha \rho \odot \left[\mathbf{1} + \alpha (\mathbf{M}^{-1})^t \mathbf{e} \right], \quad (18)$$

where $\mathbf{1}$ is a vector of ones.

By taking a second derivative with respect to $\mathbf{e}$, and again by back-propagating the derivatives, we can determine the Hessian,

$$\mathbf{H} = \alpha^2 \left\{ \left[\mathbf{M}^{-1} \odot (\{\mathbf{1} + \alpha (\mathbf{M}^{-1})^t \mathbf{e}\} \otimes \rho) \right] + \left[\mathbf{M}^{-1} \odot (\{\mathbf{1} + \alpha (\mathbf{M}^{-1})^t \mathbf{e}\} \otimes \rho) \right]^t \right\}, \quad (19)$$

where $\otimes$ indicates the outer product.

Non-linear reactions

For non-linear reaction kinetics the reaction-diffusion equation becomes

$$(\mathbf{D} + \mathbf{V})\boldsymbol{\rho} - \alpha \mathbf{e} \odot \mathbf{F} - \sigma \boldsymbol{\rho} = \mathbf{A}, \quad (20)$$

where $\mathbf{F}$ is the vector with $F_i = F[\rho_i]$. Since $F[\rho]$ is non-linear, it is no longer possible to write the reaction-diffusion equation as a linear system that can be solved for $\boldsymbol{\rho}$. As a result the dependency graph includes a loop, making it inconvenient to back-propagate derivatives. Instead, taking derivatives of the reaction-diffusion equation with respect to $\mathbf{e}$, we can directly solve for

$$\frac{d\boldsymbol{\rho}}{d\mathbf{e}} = \alpha \mathbf{N}^{-1} \text{diag}(\mathbf{F}), \quad (21)$$

where $\mathbf{N} = \mathbf{D} + \mathbf{V} - \alpha \text{diag}(\mathbf{e} \odot \mathbf{F}') - \sigma \text{diag}(\mathbf{1})$ and $F'_i = F'[\rho_i]$. We then take derivatives of $J^P = \alpha \mathbf{e} \cdot \mathbf{F}$ with respect to $\mathbf{e}$, to obtain the marginal returns vector,

$$\begin{aligned} \frac{dJ^P}{d\mathbf{e}} &= \alpha \left[\mathbf{F} + \left(\frac{d\boldsymbol{\rho}}{d\mathbf{e}} \right)^t \{ \mathbf{e} \odot \mathbf{F}' \} \right] \\ &= \alpha \mathbf{F} \odot \left[\mathbf{1} + \alpha (\mathbf{N}^{-1})^t \{ \mathbf{e} \odot \mathbf{F}' \} \right]. \end{aligned} \quad (22)$$

By taking a further derivative of the returns vector with respect to $\mathbf{e}$ we find the components of the Hessian,

$$\begin{aligned} \mathbf{H}_{j,k} &= \alpha^2 \left\{ \mathbf{N}_{j,k}^{-1} \left[\mathbf{F} \otimes (\mathbf{F}' + \alpha (\mathbf{N}^{-1})^t \{ \mathbf{e} \odot \mathbf{F}'^2 \}) \right]_{k,j} \right. \\ &\quad \left. + \mathbf{N}_{k,j}^{-1} \left[\mathbf{F} \otimes (\mathbf{F}' + \alpha (\mathbf{N}^{-1})^t \{ \mathbf{e} \odot \mathbf{F}'^2 \}) \right]_{j,k} \right\} \\ &\quad + \alpha^3 F_j F_k \sum_i \mathbf{N}_{i,j}^{-1} e_i F_i'' \mathbf{N}_{i,k}^{-1} \left[1 + \alpha [(\mathbf{N}^{-1})^t \{ \mathbf{e} \odot \mathbf{F}' \}]_i \right], \end{aligned} \quad (23)$$

which simplifies to Eq. 19 for linear reactions with $F'' = 0$.

ACKNOWLEDGMENTS

This work was supported by the German Research Foundation via SFB1032 “Nanoagents for Spatiotemporal Control of Molecular and Cellular Reactions” and the excellence cluster ORIGINS. G.G. was supported by a DFG Fellowship through the Graduate School of Quantitative Biosciences Munich (QBM).

AUTHOR CONTRIBUTIONS

All authors designed research and developed the theoretical framework. G.G. and F.T. developed computational methods and analyzed data. G.G., F.T., and U.G. wrote the paper.

-
- [1] K. Kumar, R. A. Mella-Herrera, and J. W. Golden, Cold Spring Harb. Perspect. Biol., **2**, a000315 (2010).
- [2] M. Scott, C. W. Gunderson, E. M. Mateescu, Z. Zhang, and T. Hwa, Science, **330**, 1099 (2010).
- [3] C. You, H. Okano, S. Hui, Z. Zhang, M. Kim, C. W. Gunderson, Y.-P. Wang, P. Lenz, D. Yan, and T. Hwa, Nature, **500**, 301 (2013).
- [4] A. Maitra and K. A. Dill, Proc. Natl. Acad. Sci. U.S.A., **112**, 406 (2015).
- [5] M. Y. Pavlov and M. Ehrenberg, Proc. Natl. Acad. Sci. U.S.A., **110**, 20527 (2013).
- [6] D. W. Erickson, S. J. Schink, V. Patsalo, J. R. Williamson, U. Gerland, and T. Hwa, Nature, **551**, 119 (2017).
- [7] D. L. Schmitt and S. An, Biochemistry, **56**, 3184 (2017).
- [8] F. Hinzpeter, F. Tostevin, and U. Gerland, J. R. Soc. Interface, **16**, 20190444 (2019).
- [9] N. Katoh and T. Ibaraki, in *Handbook of Combinatorial Optimization* (Kluwer Academic Publishers, 1998) pp. 159–260.
- [10] J. L. Kelly, Bell Labs Tech. J., **35**, 917 (1956).
- [11] H. Markowitz, J. Finance, **7**, 77 (1952).
- [12] E. O. Thorp, in *Stochastic Optimization Models in Finance* (Elsevier, 1975) pp. 599–619.
- [13] D. Cohen, Journal of Theoretical Biology, **12**, 119 (1966), ISSN 0022-5193.
- [14] P. Bauler, G. Huber, T. Leyh, and J. A. McCammon, J. Phys. Chem. Lett., **1**, 1332 (2010).
- [15] O. Idan and H. Hess, ACS Nano, **7**, 8658 (2013).
- [16] M. Castellana, M. Z. Wilson, Y. Xu, P. Joshi, I. M. Cristea, J. D. Rabinowitz, Z. Gitai, and N. S. Wingreen, Nat. Biotechnol., **32**, 1011–1018 (2014).
- [17] A. Buchner, F. Tostevin, and U. Gerland, Phys. Rev. Lett., **110**, 208104 (2013).
- [18] A. Buchner, F. Tostevin, F. Hinzpeter, and U. Gerland, J. Chem. Phys., **139**, 135101 (2013).

- 585 [19] C. M. Agapakis, P. M. Boyle, and P. A. Silver, *Nat. Chem. Biol.*, **8**, 527 (2012).
- 586 [20] J. E. Dueber, G. C. Wu, G. R. Malmirchegini, T. S. Moon, C. J. Petzold, A. V. Ullal, K. L.
587 Prather, and J. D. Keasling, *Nat. Biotechnol.*, **27**, 753 (2009).
- 588 [21] J. Zhou and B. Xu, *Bioconjugate Chem.*, **26**, 987 (2015).
- 589 [22] J. Li, Y. Kuang, J. Shi, J. Zhou, J. E. Medina, R. Zhou, D. Yuan, C. Yang, H. Wang, Z. Yang,
590 *et al.*, *Angewandte Chemie*, **127**, 13505 (2015).
- 591 [23] S. Moraïs, Y. Barak, J. Caspi, Y. Hadar, R. Lamed, Y. Shoham, D. B. Wilson, and E. A.
592 Bayer, *Appl. Environ. Microbiol.*, **76**, 3787 (2010).
- 593 [24] S.-L. Tsai, J. Oh, S. Singh, R. Chen, and W. Chen, *Appl. Environ. Microbiol.*, **75**, 6087 (2009).
- 594 [25] E. Kussell and S. Leibler, *Science*, **309**, 2075 (2005), ISSN 0036-8075, 1095-9203.
- 595 [26] O. Rivoire and S. Leibler, *J. Stat. Phys.*, **142**, 1124 (2011), ISSN 1572-9613.
- 596 [27] G. Fritz, N. Walker, and U. Gerland, *J. Mol. Biol.*, **431**, 4760 (2019).
- 597 [28] T. Taillefumier, A. Posfai, Y. Meir, and N. S. Wingreen, *Elife*, **6**, e22644 (2017).
- 598 [29] O. Shoval, H. Sheftel, G. Shinar, Y. Hart, O. Ramote, A. Mayo, E. Dekel, K. Kavanagh, and
599 U. Alon, *Science*, **336**, 1157 (2012), ISSN 0036-8075, 1095-9203.
- 600 [30] R. E. Goldstein and J.-W. van de Meent, *Interface Focus*, **5**, 20150030 (2015).
- 601 [31] J. F. Presley, N. B. Cole, T. A. Schroer, K. Hirschberg, K. J. Zaal, and J. Lippincott-Schwartz,
602 *Nature*, **389**, 81 (1997).
- 603 [32] B. Hove-Jensen, K. R. Andersen, M. Kilstrup, J. Martinussen, R. L. Switzer, and M. Wille-
604 moës, *Microbiol. Mol. Biol. Rev.*, **81**, e00040 (2017).
- 605 [33] E. W. Miles, S. Rhee, and D. R. Davies, *J. Biol. Chem.*, **274**, 12193 (1999).
- 606 [34] J. R. Robbins, D. Monack, S. J. McCallum, A. Vegas, E. Pham, M. B. Goldberg, and J. A.
607 Theriot, *Mol. Microbiol.*, **41**, 861 (2001).
- 608 [35] Y. E. Chen, C. Tropini, K. Jonas, C. G. Tsokos, K. C. Huang, and M. T. Laub, *Proc. Natl.*
609 *Acad. Sci. U.S.A.*, **108**, 1052 (2011).
- 610 [36] T. E. Saunders, K. Z. Pan, A. Angel, Y. Guan, J. V. Shah, M. Howard, and F. Chang, *Dev.*
611 *Cell*, **22**, 558 (2012).
- 612 [37] D. Oh, C.-H. Yu, and D. J. Needleman, *Proc. Natl. Acad. Sci. U.S.A.*, **113**, 8729 (2016).
- 613 [38] G. Hrazdina and G. J. Wagner, *Arch. Biochem. Biophys.*, **237**, 88 (1985).
- 614 [39] J. W. Graham, T. C. Williams, M. Morgan, A. R. Fernie, R. G. Ratcliffe, and L. J. Sweetlove,
615 *Plant Cell*, **19**, 3723 (2007).

- 616 [40] J. B. French, S. A. Jones, H. Deng, A. M. Pedley, D. Kim, C. Y. Chan, H. Hu, R. J. Pugh,
617 H. Zhao, Y. Zhang, *et al.*, Science, **351**, 733 (2016).
- 618 [41] T. Laursen, J. Borch, C. Knudsen, K. Bavishi, F. Torta, H. J. Martens, D. Silvestro, N. S.
619 Hatzakis, M. R. Wenk, T. R. Dafforn, *et al.*, Science, **354**, 890 (2016).
- 620 [42] M. E. Campanella, H. Chu, and P. S. Low, Proc. Natl. Acad. Sci. U.S.A., **102**, 2402 (2005).
- 621 [43] H. W. Kuhn and A. W. Tucker, Proceedings of the Second Berkeley Symposium on Mathe-
622 matical Statistics and Probability, 481 (1951), publisher: University of California Press.
- 623 [44] W. Karush, M. Sc. Dissertation. Dept. of Mathematics, Univ. of Chicago (1939).
- 624 [45] A. Bar-Even, E. Noor, Y. Savir, W. Liebermeister, D. Davidi, D. S. Tawfik, and R. Milo,
625 Biochemistry, **50**, 4402 (2011), ISSN 0006-2960.
- 626 [46] B. Ho, A. Baryshnikova, and G. W. Brown, Cell Syst., **6**, 192 (2018).
- 627 [47] In general we do not know $\frac{d\lambda_e}{dE_T}$ *a priori*. However, this scalar prefactor affects only the length,
628 and not the direction, of $\frac{de^*}{dE_T}$. Hence it suffices to treat $\frac{d\lambda_e}{dE_T}$ as an arbitrary constant, and
629 subsequently to rescale the resulting $\frac{de^*}{dE_T}$ to unit length once its direction is known.
- 630 [48] L. Dinis, J. Unterberger, and D. Lacoste, EPL (Europhysics Letters), **131**, 60005 (2020), ISSN
631 0295-5075.
- 632 [49] T. Thomik, I. Wittig, J.-y. Choe, E. Boles, and M. Oreb, Nat. Chem. Biol., **13**, 1158 (2017).
- 633 [50] H. Rabeharindranto, S. Castaño-Cerezo, T. Lautier, L. F. Garcia-Alles, C. Treitz, A. Tholey,
634 and G. Truan, Metab. Eng. Commun., **8**, e00086 (2019).
- 635 [51] S.-L. Tsai, N. A. DaSilva, and W. Chen, ACS Synth. Biol., **2**, 14 (2013).
- 636 [52] J.-L. Lin, J. Zhu, and I. Wheeldon, ACS Synth. Biol., **6**, 1534 (2017).
- 637 [53] C. J. Delebecque, A. B. Lindner, P. A. Silver, and F. A. Aldaye, Science, **333**, 470 (2011).
- 638 [54] S. Kufer, E. Puchner, H. Gump, T. Liedl, and H. Gaub, Science, **319**, 594 (2008).
- 639 [55] K. R. Erlich, S. M. Sedlak, M. A. Jobst, L. F. Milles, and H. E. Gaub, Nanoscale, **11**, 407
640 (2019).
- 641 [56] J. Müller and C. M. Niemeyer, Biochem. Biophys. Res. Commun., **377**, 62 (2008).
- 642 [57] O. I. Wilner, Y. Weizmann, R. Gill, O. Lioubashevski, R. Freeman, and I. Willner, Nat.
643 Nanotechnol., **4**, 249 (2009).
- 644 [58] J. Fu, M. Liu, Y. Liu, N. W. Woodbury, and H. Yan, J. Am. Chem. Soc., **134**, 5516 (2012).
- 645 [59] J. Fu, Y. R. Yang, A. Johnson-Buck, M. Liu, Y. Liu, N. G. Walter, N. W. Woodbury, and
646 H. Yan, Nat. Nanotechnol., **9**, 531 (2014).

Appendix A: General Lagrangian with multiple subspaces

As noted in the main text, our functional Lagrangian formalism assumes smooth, positive enzyme distributions within different subdomains. At the interfaces of such domains, the optimal enzyme distributions may feature discontinuities. For example, there may be a finite enzyme density in one region of the system, and no enzymes (zero density) in another region. These subdomains should be picked in the proximities of the substrate sources. For example if we have patches of sources on the surface domains, for each source patch we would consider a surface subdomain around the patch and a correspondent volume subdomain in proximity of the source. These are the regions where it is optimal to distribute the enzymes as diffusion does not allow for peak of substrate concentration away from the sources. Within each such subdomain i , the substrate and enzyme densities $\rho^{(i)}(\mathbf{r})$ and $e^{(i)}(\mathbf{r})$ are smooth and their local gradients are well defined. However, at the interfaces between such subdomains, $e(\mathbf{r})$ need not be continuous (although $\rho(\mathbf{r})$ still must be). The positions of the interfaces, and therefore the extents of the different domains, should be optimized over and as we have seen in the main text they depend on the E_T considered.

In this formulation, the total reaction flux can be written as a sum of contributions from each surface and boundary domain, and the corresponding reaction-diffusion or boundary condition acts as a constraint that must be satisfied within each domain. In addition, we introduce explicit matching conditions on $\rho(\mathbf{r})$ at the interfaces I . The resulting Lagrangian has the following form:

$$\begin{aligned} \mathcal{L} = & \sum_i \int_{S_i} k_{\text{cat}} e_S^{(i)}(\mathbf{s}) F[\rho^{(i)}(\mathbf{s})] d\mathbf{s} + \sum_j \int_{V_j} k_{\text{cat}} e^{(j)}(\mathbf{r}) F[\rho^{(j)}(\mathbf{r})] d\mathbf{r} \\ & - \lambda_e \left[\sum_i \int_{S_i} e_S^{(i)}(\mathbf{s}) d\mathbf{s} + \sum_j \int_{V_j} e^{(j)}(\mathbf{r}) d\mathbf{r} - E_T \right] \\ & + \sum_j \int_{V_j} \lambda_V^{(j)}(\mathbf{r}) \left\{ D \nabla^2 \rho^{(j)}(\mathbf{r}) - \nabla \cdot [\rho^{(j)}(\mathbf{r}) \mathbf{v}(\mathbf{r})] - \sigma \rho^{(j)}(\mathbf{r}) - k_{\text{cat}} e^{(j)}(\mathbf{r}) F[\rho^{(j)}(\mathbf{r})] \right\} d\mathbf{r} \\ & + \sum_i \int_{S_i} \lambda_S^{(i)}(\mathbf{s}) \left\{ k(\mathbf{s}) \left[j(\mathbf{s}) - D \frac{\partial \rho^{(l)}}{\partial \mathbf{n}(\mathbf{s})} + \rho^{(l)}(\mathbf{s}) \mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) - k_{\text{cat}} e_S^{(i)}(\mathbf{s}) F[\rho^{(l)}(\mathbf{s})] \right] - h(\mathbf{s}) \rho^{(l)}(\mathbf{s}) \right\} d\mathbf{s} \\ & + \sum_k \int_{I_k} \left\{ \lambda_I^{(k)}(\mathbf{r}) [\rho^{(k_1)}(\mathbf{r}) - \rho^{(k_2)}(\mathbf{r})] + \lambda'_I{}^{(k)}(\mathbf{r}) \cdot [\nabla \rho^{(k_1)}(\mathbf{r}) - \nabla \rho^{(k_2)}(\mathbf{r})] \right\} d\mathbf{r}. \end{aligned} \tag{A1}$$

where l represents the index of the volume domain adjacent to the boundary at $\mathbf{s}$, and k_1 ,

k_2 represent the two domains on either side of the interface I_k . Here $\lambda_I(\mathbf{r})$ is a Lagrange multiplier that enforces smoothness of the density profile at each point $\mathbf{r}$ on an interface between two domains, and $\lambda'_I(\mathbf{r})$ is a vector of Lagrangian multipliers where each component enforces smoothness of $\rho(\mathbf{r})$ along one of the system dimensions.

It is convenient to consider first some manipulations of the general Lagrangian, Eq. A1. Using the vector identity $\nabla \cdot (f\mathbf{a}) = \nabla f \cdot \mathbf{a} + f\nabla \cdot \mathbf{a}$ we can rearrange the diffusive and advective transport terms to read

$$\lambda_V(\mathbf{r})\nabla^2\rho(\mathbf{r}) = \nabla \cdot [\lambda_V(\mathbf{r})\nabla\rho(\mathbf{r}) - \rho(\mathbf{r})\nabla\lambda_V(\mathbf{r})] + \rho(\mathbf{r})\nabla^2\lambda_V(\mathbf{r}), \quad (\text{A2})$$

$$\lambda_V(\mathbf{r})\nabla \cdot [\rho(\mathbf{r})\mathbf{v}(\mathbf{r})] = \nabla \cdot [\rho(\mathbf{r})\lambda_V(\mathbf{r})\mathbf{v}(\mathbf{r})] - \rho(\mathbf{r})\mathbf{v}(\mathbf{r}) \cdot \nabla\lambda_V(\mathbf{r}) \quad (\text{A3})$$

Applying Gauss theorem ($\int_V \nabla \cdot \mathbf{a} \, d\mathbf{r} = \oint \mathbf{a} \cdot \hat{\mathbf{n}}(s)ds$), Eq. A1 becomes

$$\begin{aligned} \mathcal{L} = & \sum_i \int_{S_i} k_{\text{cat}} e_S^{(i)}(\mathbf{s}) F[\rho^{(i)}(\mathbf{s})] d\mathbf{s} + \sum_j \int_{V_j} k_{\text{cat}} e^{(j)}(\mathbf{r}) F[\rho^{(j)}(\mathbf{r})] d\mathbf{r} \\ & - \lambda_e \left[\sum_i \int_{S_i} e_S^{(i)}(\mathbf{s}) d\mathbf{s} + \sum_j \int_{V_j} e^{(j)}(\mathbf{r}) d\mathbf{r} - E_T \right] \\ & + \sum_j \int_{V_j} \left\{ \rho^{(j)}(\mathbf{r}) \left[D\nabla^2\lambda_V^{(j)}(\mathbf{r}) + \mathbf{v}(\mathbf{r}) \cdot \nabla\lambda_V^{(j)}(\mathbf{r}) - \sigma\lambda_V^{(j)}(\mathbf{r}) \right] - k_{\text{cat}}\lambda_V^{(j)}(\mathbf{r})e^{(j)}(\mathbf{r})F[\rho^{(j)}(\mathbf{r})] \right\} d\mathbf{r} \\ & + \sum_i \int_{S_i} \lambda_S^{(i)}(\mathbf{s}) \left\{ k(\mathbf{s}) \left[j(\mathbf{s}) - D\frac{\partial\rho^{(l)}}{\partial\mathbf{n}(\mathbf{s})} + \rho^{(l)}(\mathbf{s})\mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) - k_{\text{cat}}e_S^{(i)}(\mathbf{s})F[\rho^{(l)}(\mathbf{s})] \right] - h(\mathbf{s})\rho^{(l)}(\mathbf{s}) \right\} d\mathbf{s} \\ & + \sum_k \int_{I_k} \left\{ \lambda_I^{(k)}(\mathbf{r}) [\rho^{(k_1)}(\mathbf{r}) - \rho^{(k_2)}(\mathbf{r})] + \lambda'_I{}^{(k)}(\mathbf{r}) \cdot [\nabla\rho^{(k_1)}(\mathbf{r}) - \nabla\rho^{(k_2)}(\mathbf{r})] \right\} d\mathbf{r} \\ & + \sum_i \int_{S_i} \left\{ D\lambda_V^{(l)}(\mathbf{s})\frac{\partial\rho^{(l)}}{\partial\mathbf{n}(\mathbf{s})} - D\rho^{(l)}(\mathbf{s})\frac{\partial\lambda_V^{(l)}}{\partial\mathbf{n}(\mathbf{s})} - \lambda_V^{(l)}(\mathbf{s})\rho^{(l)}(\mathbf{s})\mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) \right\} d\mathbf{s} \\ & + \sum_k \int_{I_k} \left\{ D\lambda_V^{(k_1)}(\mathbf{r})\frac{\partial\rho^{(k_1)}}{\partial\mathbf{n}(\mathbf{r})} - D\rho^{(k_1)}(\mathbf{r})\frac{\partial\lambda_V^{(k_1)}}{\partial\mathbf{n}(\mathbf{r})} - D\lambda_V^{(k_2)}(\mathbf{r})\frac{\partial\rho^{(k_2)}}{\partial\mathbf{n}(\mathbf{r})} + D\rho^{(k_2)}(\mathbf{r})\frac{\partial\lambda_V^{(k_2)}}{\partial\mathbf{n}(\mathbf{r})} \right. \\ & \quad \left. - [\lambda_V^{(k_1)}(\mathbf{r})\rho^{(k_1)}(\mathbf{r}) - \lambda_V^{(k_2)}(\mathbf{r})\rho^{(k_2)}(\mathbf{r})] \mathbf{v}(\mathbf{r}) \cdot \hat{\mathbf{n}}(\mathbf{r}) \right\} d\mathbf{r} \end{aligned} \quad (\text{A4})$$

Here the last three lines contain the contributions from the boundary integral.

Appendix B: Optimal enzyme arrangement in a one-dimensional system

In this section we go through in detail the full analytic optimization of the simplest exactly-solvable model. We consider a one-dimensional system with a source of substrate at

one boundary at $x = 0$, ($j(0) = j_0$, $k(0) = 1$, $h(0) = 0$) and an absorbing boundary at $x = L$ ($j(L) = k(L) = 0$, $h(L) = 1$). Further we assume a linear reaction functional, no drift and no instability of $\mathcal{S}$, i.e. $F[\rho] = \rho/K_M$ and $v = \sigma = 0$.

In our formalism we divide the system into two boundary domains (S_1 : $x = 0$ and S_2 : $x = L$) and two “volume” domains (V_1 : $0 \leq x \leq x_0$ and V_2 : $x_0 \leq x \leq L$, with $0 \leq x_0 \leq L$ a parameter to be optimized). For simplicity of presentation we will assume that enzymes can be present at $x = 0$ or in the domain V_1 , while the boundary at $x = L$ and the domain V_2 are free of enzymes. Whilst one can include an arbitrary number of additional domains within the system, we found that such domains turned out to be degenerate with the two specified above. Therefore, we will not include these possibilities here. The effective enzyme profile is therefore of the form $e_S\delta(x) + e(x)\Theta(x_0 - x)$, where $\delta(x)$ is the Dirac delta function and $\Theta(x)$ is the Heaviside function.

The Lagrangian for this specific model is

$$\begin{aligned} \mathcal{L} = & \kappa e_S \rho^{(1)}(0) + \int_0^{x_0} \kappa e(x) \rho^{(1)}(x) dx - \lambda_e \left(e_S + \int_0^{x_0} e(x) dx - E_T \right) \\ & + \int_0^{x_0} \lambda^{(1)}(x) [D\partial_x^2 \rho^{(1)}(x) - \kappa e(x) \rho^{(1)}(x)] dx - \int_{x_0}^L \lambda^{(2)}(x) D\partial_x^2 \rho^{(2)}(x) dx \\ & + \lambda_0 [j_0 + D\partial_x \rho^{(1)}(0) - \kappa e_S \rho^{(1)}(0)] - \lambda_L \rho^{(2)}(L) \\ & + \lambda_{x_0} [\rho^{(1)}(x_0) - \rho^{(2)}(x_0)] + \lambda'_{x_0} [\partial_x \rho^{(1)}(x_0) - \partial_x \rho^{(2)}(x_0)] , \end{aligned} \quad (\text{B1})$$

where we have introduced the catalytic efficiency $\kappa = k_{\text{cat}}/K_M$. Here λ_e is the Lagrange multiplier corresponding to the constraint of having a fixed amount of enzymes E_T ; $\lambda^{(1)}(x)$ and $\lambda^{(2)}(x)$ those corresponding the constrained dynamics of the substrate in the two domains; λ_0 and λ_L to the boundary conditions; and λ_{x_0} and λ'_{x_0} to the continuity and smoothness that must be imposed on $\rho(x)$ at the interface x_0 .

To calculate the optimal enzyme arrangement e_S^* , $e^*(x)$ we must compute the various functional derivatives of $\mathcal{L}$. Let us first consider the derivatives with respect to $e(x)$ and $\rho^{(1)}(x)$. After integrating by parts Eq. B1 (corresponding to the form Eq. A4 of the Lagrangian), we find

$$\frac{\delta \mathcal{L}}{\delta e(x)} = 0 \quad \Leftrightarrow \quad \rho^{(1)}(x) = \frac{\lambda_e}{\kappa[1 - \lambda^{(1)}(x)]} \quad (\text{B2})$$

$$\frac{\delta \mathcal{L}}{\delta \rho^{(1)}(x)} = 0 \quad \Leftrightarrow \quad e(x) = -\frac{D\partial_x^2 \lambda^{(1)}(x)}{\kappa[1 - \lambda^{(1)}(x)]}. \quad (\text{B3})$$

We can compute the derivatives $\partial_x \rho^{(1)}(x)$ and $\partial_x^2 \rho^{(1)}(x)$ starting from the expressions above and we can substitute the results into the reaction-diffusion equation (given also by $\frac{\delta \mathcal{L}}{\delta \lambda^{(1)}(x)} = 0$) to obtain an ordinary differential equation for $\lambda^{(1)}(x)$:

$$\frac{\partial_x^2 \lambda^{(1)}(x)}{\partial_x \lambda^{(1)}(x)} = -\frac{\partial_x \lambda^{(1)}(x)}{1 - \lambda^{(1)}(x)}. \quad (\text{B4})$$

The solution to Eq. B4 can be found by direct integration to be

$$\lambda^{(1)}(x) = 1 - A_1 e^{B_1 x}, \quad (\text{B5})$$

where A_1 and B_1 are constants of integration still to be determined. Using Eqs. B2-B3 we obtain

$$\rho^{(1)}(x) = \frac{\lambda_e}{\kappa A_1} e^{-B_1 x} \quad (\text{B6})$$

$$e(x) = \frac{DB_1^2}{\kappa}, \quad (\text{B7})$$

Equation B7 immediately shows that the enzyme density in the region $0 \leq x < x_0$ is constant, in agreement with previous numerical studies [17, 18].

Evaluating the functional derivative of $\mathcal{L}$ with respect to $\lambda^{(2)}$, we recover the diffusion equation $\partial_x^2 \rho^{(2)}(x) = 0$ in the domain V_2 , from which

$$\rho^{(2)}(x) = A_2 + B_2 x, \quad (\text{B8})$$

where A_2 and B_2 are again constants of integration.

The constants $A_{1,2}$ and $B_{1,2}$ can be evaluated using the boundary conditions at $x = 0$ and $x = L$, and the continuity and smoothness conditions at $x = x_0$. Additionally, the dependence on e_S can be eliminated using $\frac{\delta \mathcal{L}}{\delta \lambda_e} = 0$, which is simply the constraint on the total enzyme number. This leaves us with an expression for the Lagrangian that, since all constraints have been satisfied equals the constrained reaction flux J^P , in terms of the single optimization variable x_0 ,

$$\mathcal{L} = J^P = j_0 \left[1 - \frac{1 - \frac{x_0}{L}}{1 - 2\frac{x_0}{L} + \alpha E_T (1 - \frac{x_0}{L})^2} e^{-\frac{x_0}{L-x_0}} \right] \quad (\text{B9})$$

where we define $\alpha = \kappa L/D$. Finally, we maximize over x_0 to find

$$x_0^* = \begin{cases} 0 & \alpha E_T \leq 1 \\ L \left[1 - (\alpha E_T)^{-1/2} \right] & \alpha E_T > 1 \end{cases}. \quad (\text{B10})$$

By substitution we can then obtain expressions for the enzyme and substrate densities

Regime	e_S^*	$e^*(x)$	$\rho^{(1)}(x)$	$\rho^{(2)}(x)$	$J^{\mathcal{P}^*}$
$\alpha E_T \leq 1$	E_T	0	N/A	$\frac{j_0(L-x)}{D(1+\alpha E_T)}$	$\frac{j_0}{1+(\alpha E_T)^{-1}}$
$\alpha E_T > 1$	$\sqrt{\frac{E_T}{\alpha}}$	$\frac{E_T}{L}$	$\frac{j_0}{2} \frac{L}{D\sqrt{\alpha E_T}} \exp\left[-\sqrt{\alpha E_T} \frac{x}{L}\right]$	$\frac{j_0}{2} \frac{L-x}{D\sqrt{\alpha E_T}} \exp\left[-\sqrt{\alpha E_T} \frac{x_0}{L}\right]$	$j_0 \left(1 - \frac{1}{2} e^{1-\sqrt{\alpha E_T}}\right)$

Table B.1. Optimal enzyme distribution and corresponding substrate profile and flux for a one-dimensional system

Appendix C: Marginal returns landscape

Given a particular enzyme arrangement, the marginal returns as a function of position can be calculated directly by considering the addition of an amount δe of enzymes at position x' , and solving the new modified reaction-diffusion system. Here we present an example of such a calculation. We consider the same one-dimensional system analyzed above, and suppose that we are in the regime $\alpha E_T > 1$ and the enzyme profile is the optimal one of Table B.1. For other enzyme profiles, such as those shown in Fig. 2C of the main text, the calculation proceeds in the same way.

We now have to consider two cases: $0 \leq x' \leq x_0$ and $x_0 < x' \leq L$.

If $0 \leq x' < x_0$, we solve the reaction-diffusion equation in the three domains: $0 \leq x < x'$, $x' < x \leq x_0$, and $x_0 \leq x \leq L$. The resulting density profiles are

$$\rho_{[0,x']}(x) = a_1 e^{\sqrt{\frac{E_T \kappa}{DL}} x} + b_1 e^{-\sqrt{\frac{E_T \kappa}{DL}} x} \quad (C1)$$

$$\rho_{[x',x_0]}(x) = a_2 e^{\sqrt{\frac{E_T \kappa}{DL}} x} + b_2 e^{-\sqrt{\frac{E_T \kappa}{DL}} x} \quad (C2)$$

$$\rho_{[x_0,L]}(x) = a_3 x + b_3. \quad (C3)$$

To fix the constants of integration a_i , b_i , we make use of the boundary conditions at $x = 0$ and $x = L$ as well as matching conditions on ρ at x' and x_0 . Specifically, we require continuity of ρ at x' and x_0 , and smoothness at x_0 ,

$$\rho_{[0,x']}(x') = \rho_{[x',x_0]}(x') \quad (C4)$$

$$\rho_{[x',x_0]}(x_0) = \rho_{[x_0,L]}(x_0) \quad (C5)$$

$$\partial_x \rho_{[x',x_0]}(x_0) = \partial_x \rho_{[x_0,L]}(x_0). \quad (C6)$$

Additionally, conservation of mass at $x = x'$ requires that

$$D \partial_x \rho_{[x',x_0]}(x') - D \partial_x \rho_{[0,x']}(x') = \kappa \delta e \rho_{[x',x_0]}(x'). \quad (C7)$$

723 The total flux J^P is then given by

$$J^P(x', \delta e) = \kappa \left[e_S^* \rho(0) + \delta e \rho(x') + \int_0^{x_0} e^*(x) \rho(x) dx \right], \quad (\text{C8})$$

724 into which we substitute the final density profile $\rho(x)$ as well as e_S^* and $e^*(x)$ from Table B.1.

725 Finally, the marginal returns at position x' can be calculated as

$$\frac{dJ^P}{de(x')} = \lim_{\delta e \rightarrow 0} \frac{\partial J^P}{\partial \delta e} = \frac{j_0}{4} \sqrt{\frac{\alpha}{E_T}} e^{1-\sqrt{\alpha E_T}}. \quad (\text{C9})$$

726 Therefore, for $0 \leq x' \leq x_0^*$ the marginal returns do not depend on x' , and all positions at
727 which we have enzymes give the same returns. It is straightforward to verify that this agrees
728 with the value of λ_e derived from the optimal reaction flux that appears in Table B.1,

$$\lambda_e = \frac{dJ^{P*}}{dE_T} = \frac{j_0}{4} \sqrt{\frac{\alpha}{E_T}} e^{1-\sqrt{\alpha E_T}}. \quad (\text{C10})$$

729 In the case $x_0 \leq x' < L$, the same procedure can be applied, except with the three
730 domains $0 \leq x \leq x_0$, $x_0 \leq x \leq x'$, and $x' \leq x \leq L$. We find that the marginal returns is
731 given by

$$\frac{dJ^P}{de(x')} = \frac{j_0 \alpha}{4} e^{1-\sqrt{\alpha E_T}} \left(1 - \frac{x'}{L} \right) \left(1 + \sqrt{\alpha E_T} \frac{x' - x_0}{L} \right). \quad (\text{C11})$$

732 As expected, this approaches Eq. C9 as $x' \rightarrow x_0^* = L \left[1 - (\alpha E_T)^{-1/2} \right]$.

733 Appendix D: Derivation of the transition condition

734 In this section we derive the inequality condition, Eq. 10 of the main text, that determines
735 whether or not enzymes should be placed in the volume adjacent to a boundary position $\mathbf{s}$.
736 We begin by examining the functional derivative of $\mathcal{L}$ with respect to substrate profiles at
737 the system boundary, $\rho(\mathbf{s})$. In the vicinity of a reflecting boundary, (i.e. $h(\mathbf{s}) = 0$, $k(\mathbf{s}) = 1$),
738 we obtain from Eq. A4

$$\frac{\delta \mathcal{L}}{\delta \rho(\mathbf{s})} = k_{\text{cat}} e_S(\mathbf{s}) \frac{\delta F[\rho]}{\delta \rho} \{1 - \lambda_S(\mathbf{s})\} - D \frac{\partial \lambda_V}{\partial \mathbf{n}(\mathbf{s})} - [\lambda_V(\mathbf{s}) - \lambda_S(\mathbf{s})] \mathbf{v}(\mathbf{s}) \cdot \hat{\mathbf{n}}(\mathbf{s}) = 0, \quad (\text{D1})$$

739 where equality with zero assumes that the enzyme distribution is the optimal one. We can
740 also consider the functional derivative with respect to the gradient of $\rho(\mathbf{r})$ at the boundaries
741 of the system,

$$\frac{\delta \mathcal{L}}{\delta \frac{\partial \rho}{\partial \mathbf{n}(\mathbf{s})}} = D [\lambda_V(\mathbf{s}) - \lambda_S(\mathbf{s})] = 0. \quad (\text{D2})$$

Equation D2 requires $\lambda_V(\mathbf{s}) = \lambda_S(\mathbf{s})$, which simplifies Eq. D1 to

$$k_{\text{cat}}e_S(\mathbf{s})F'[\rho(\mathbf{s})]\{1 - \lambda_V(\mathbf{s})\} - D\frac{\partial\lambda_V}{\partial\mathbf{n}(\mathbf{s})} = 0, \quad (\text{D3})$$

where $F'[\rho(\mathbf{s})] = \frac{\delta F[\rho]}{\delta\rho}$.

We next examine variations of $\mathcal{L}$ with respect to the enzyme densities,

$$\frac{\delta\mathcal{L}}{\delta e_S(\mathbf{s})} = k_{\text{cat}}F[\rho(\mathbf{s})]\{1 - \lambda_S(\mathbf{s})\} - \lambda_e \leq 0 \quad (\text{D4})$$

$$\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = k_{\text{cat}}F[\rho(\mathbf{r})]\{1 - \lambda_V(\mathbf{r})\} - \lambda_e \leq 0. \quad (\text{D5})$$

Both $\lambda_V(\mathbf{r})$ and $\rho(\mathbf{r})$ must be continuous, since they must both satisfy (generally non-linear) diffusion equations. Therefore, it follows from Eq. D5 that $\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})}$ is itself continuous and that we can define the spatial gradient $\nabla\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})}$. At all points where it is optimal to allocate a non-zero density of enzymes (both in the interior of the system or on the boundaries) we have that $\frac{\delta\mathcal{L}}{\delta e_S(\mathbf{s})} = \frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = 0$, implying that $\nabla\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = 0$. In regions where enzymes are not present because it is suboptimal to add them, we get that $\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} < 0$, and the gradient $\nabla\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})}$ will in general be finite. Furthermore, since $\rho(\mathbf{r}) \rightarrow \rho(\mathbf{s})$ and $\lambda_V(\mathbf{r}) \rightarrow \lambda_V(\mathbf{s}) = \lambda_S(\mathbf{s})$ smoothly as $\mathbf{r} \rightarrow \mathbf{s}$, $\nabla\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})}$ must remain well defined as we approach the system boundaries.

If it is optimal to have enzymes at the boundaries of the system but not locally in the interior, $\frac{\delta\mathcal{L}}{\delta e_S(\mathbf{s})} = 0$ and $\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} < 0$ for a small displacement $\mathbf{r} = \mathbf{s} + \mathbf{e}$. In this case, evaluating the gradient of Eq. D5 and taking $\mathbf{r} \rightarrow \mathbf{s}$ we find

$$\frac{\partial}{\partial\mathbf{n}(\mathbf{s})}\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = k_{\text{cat}}\left[F'[\rho(\mathbf{s})](1 - \lambda_V(\mathbf{s}))\frac{\partial\rho}{\partial\mathbf{n}(\mathbf{s})} - F[\rho(\mathbf{s})]\frac{\partial\lambda_V}{\partial\mathbf{n}(\mathbf{s})}\right] > 0. \quad (\text{D6})$$

The inequality Eq. D6, $\frac{\partial}{\partial\mathbf{n}(\mathbf{s})}\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} > 0$, is equivalent to Eq. 9 of the main text, $\frac{\partial}{\partial\mathbf{n}(\mathbf{s})}\frac{dJ^{\mathcal{P}}}{de} > 0$, because $\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = \frac{dJ^{\mathcal{P}}}{de(\mathbf{r})} - \lambda_e$ and λ_e is constant. Hence $\frac{\partial}{\partial\mathbf{n}(\mathbf{s})}\frac{\delta\mathcal{L}}{\delta e(\mathbf{r})} = \frac{\partial}{\partial\mathbf{n}(\mathbf{s})}\frac{dJ^{\mathcal{P}}}{de}$. Substituting for $\frac{\partial\lambda_V}{\partial\mathbf{n}(\mathbf{s})}$ from Eq. D3, this inequality becomes

$$k_{\text{cat}}F'[\rho(\mathbf{s})](1 - \lambda_V(\mathbf{s}))\left\{\frac{\partial\rho}{\partial\mathbf{n}(\mathbf{s})} - \frac{k_{\text{cat}}}{D}e_S(\mathbf{s})F[\rho(\mathbf{s})]\right\} > 0. \quad (\text{D7})$$

Since we assume that $F[\rho]$ is monotonically increasing, $F'[\rho] > 0$. Furthermore, setting Eq. D4 equal to zero implies that $1 - \lambda_V(\mathbf{s}) > 0$, since $\lambda_e > 0$. Therefore, Eq. D7 reduces to

$$D\frac{\partial\rho}{\partial\mathbf{n}(\mathbf{s})} > k_{\text{cat}}e_S(\mathbf{s})F[\rho(\mathbf{s})], \quad (\text{D8})$$

which corresponds to Eq. 10 of the main text.

Appendix E: Reduction of numerical complexity in a one-dimensional system with drift

In this section we demonstrate how, even in cases for which the full analytical solution is not tractable, it is nevertheless possible to use the Lagrangian formalism to express the optimization problem as a single equation, which can then be solved numerically.

We consider a one-dimensional system, similar to that considered in Section B but with the addition of a constant drift v . If $v > 0$ substrate molecules tend to flow away from the source at the origin, whereas for $v < 0$ they tend to flow towards it. The corresponding Lagrangian is

$$\begin{aligned} \mathcal{L} = & \kappa e_S \rho^{(1)}(0) + \int_0^{x_0} \kappa e(x) \rho^{(1)}(x) dx - \lambda_e \left(e_S + \int_0^{x_0} e(x) dx - E_T \right) \\ & + \int_0^{x_0} \lambda^{(1)}(x) \left[D \partial_x^2 \rho^{(1)}(x) - v \partial_x \rho^{(1)}(x) - \kappa e(x) \rho^{(1)}(x) \right] dx \\ & - \int_{x_0}^L \lambda^{(2)}(x) \left[D \partial_x^2 \rho^{(2)}(x) - v \partial_x \rho^{(2)}(x) \right] dx \\ & + \lambda_0 \left[j_0 + D \partial_x \rho^{(1)}(0) - v \rho^{(1)}(0) - \kappa e_S \rho^{(1)}(0) \right] - \lambda_L \rho^{(2)}(L) \\ & + \lambda_{x_0} \left[\rho^{(1)}(x_0) - \rho^{(2)}(x_0) \right] + \lambda'_{x_0} \left[\partial_x \rho^{(1)}(x_0) - \partial_x \rho^{(2)}(x_0) \right]. \end{aligned} \quad (\text{E1})$$

The derivation proceeds in the same way as in Section B. We again use the functional derivatives to find expressions for $e(x)$ and $\rho^{(1)}(x)$ in terms of the Lagrange multiplier $\lambda^{(1)}(x)$,

$$\frac{\delta \mathcal{L}}{\delta e(x)} = 0 \quad \Leftrightarrow \quad \rho^{(1)}(x) = \frac{\lambda_e}{\kappa [1 - \lambda^{(1)}(x)]} \quad (\text{E2})$$

$$\frac{\delta \mathcal{L}}{\delta \rho^{(1)}(x)} = 0 \quad \Leftrightarrow \quad e(x) = - \frac{D \partial_x^2 \lambda^{(1)}(x) + v \partial_x \lambda^{(1)}(x)}{\kappa [1 - \lambda^{(1)}(x)]}. \quad (\text{E3})$$

Surprisingly, when we combine these expressions with the reaction-diffusion equation with drift, we find that the resulting differential equation for $\lambda^{(1)}(x)$ is again exactly Eq. B4, which we can solve to find

$$\lambda^{(1)}(x) = 1 - \tilde{A}_1 e^{\tilde{B}_1 x}, \quad (\text{E4})$$

$$\rho^{(1)}(x) = \frac{\lambda_e}{\kappa \tilde{A}_1} e^{-\tilde{B}_1 x} \quad (\text{E5})$$

$$e(x) = \frac{D \tilde{B}_1^2 + v \tilde{B}_1}{\kappa}. \quad (\text{E6})$$

Notably the optimal enzyme density $e(x)$ is again constant, but now depends on the drift velocity v .

772 In the region with no enzymes, $\rho^{(2)}(x)$ obeys a diffusion equation with drift, $D\partial_x^2\rho^{(2)}(x) -$
773 $v\partial_x\rho^{(2)}(x) = 0$, which has the solution

$$\rho^{(2)}(x) = \tilde{A}_2 + \tilde{B}_2 e^{\text{Pe} x/L}, \quad (\text{E7})$$

774 where $\text{Pe} = vL/D$ is the Péclet number. As before, we evaluate the integration constants
775 $\tilde{A}_{1,2}$, $\tilde{B}_{1,2}$ using the boundary conditions at $x = 0$ and $x = L$, and the continuity and
776 smoothness conditions at x_0 . We again eliminate the dependence on e_S by using the con-
777 straint on the total enzyme number. We are left with an expression for J^P in terms of the
778 single optimization variable x_0 ,

$$\mathcal{L} = J^P = j_0 \left\{ 1 - \frac{\exp\left\{-\text{Pe} \frac{x_0}{L} [e^{\text{Pe}(1-x_0/L)} - 1]^{-1}\right\}}{1 + \frac{\alpha E_T}{\text{Pe}} [1 - e^{-\text{Pe}(1-x_0/L)}] - \text{Pe} \frac{x_0}{L} [e^{\text{Pe}(1-x_0/L)} - 1]^{-1}} \right\}, \quad (\text{E8})$$

779 where α is defined as in Section B. It can be shown that Eq. E8 reduces to Eq. B9 in the
780 limit $\text{Pe} \rightarrow 0$.

781 It is not possible to find the x_0^* that maximizes J^P analytically. However, it is straight-
782 forward to solve $\frac{dJ^P}{dx_0} = 0$ numerically for specified values of the other parameters appearing
783 in Eq. E8. The results for the rescaled reaction flux and the rescaled net diffusive flux at
784 the origin are shown in Fig. 3 of the main text.

785 Appendix F: One-dimensional system with two sources of substrate

786 Here we present an example of a scenario of multiple sources of substrate with different
787 strengths. We considered a one-dimensional system in the domain $0 \leq x \leq L$, with reflecting
788 boundaries at both ends. At $x = 0$ we apply a source of substrate with $j(0) = 1$, and at
789 $x = L$ we apply a source with $j(L) = 0.3$. Instead of at the boundary, loss of substrate
790 occurs via decay with rate σ . We again assume linear reactions, $F[\rho] = \rho/K_M$. For this
791 system we have identified a number of different regimes of the optimal enzyme arrangement
792 as a function of E_T , shown in Fig. F.1A.

793 At the lowest E_T values, the optimal arrangement consists of a single cluster at $x = 0$,
794 the site of strongest substrate influx. As E_T crosses a first transition level, E_T^{t1} , the optimal
795 configuration features clusters of enzymes at both ends of the system: a larger one at $x = 0$
796 (the stronger source) and a smaller one at $x = L$ (the weaker source). In this regime
797 the optimal distribution of enzymes between the two poles can be calculated exactly by

Figure F.1. Transitions in the optimal enzyme arrangement for a one-dimensional system with two sources of substrate. The system is defined by $\sigma = 1$, $\alpha = Lk_{\text{cat}}/(K_M D) = 1$, $v = 0$ and boundary conditions $j(0) = 1$, $k(0) = -1$, $h(0) = 0$ and $j(L) = 0.3$, $k(L) = -1$, $h(L) = 0$. (A) Examples of the optimal enzyme profiles for different values of E_T . (B) Diffusion and reaction fluxes at $x = 0$ and $x = L$, normalized by the local influxes of substrate, are plotted as a function of $(E_T - E_T^t)/E_T^t$. For both poles of the system, we confirm that $j^D > j^P$ when the optimal enzyme distribution consists of only a single boundary cluster at that pole, while $j^D = j^P = j/2$ when the optimal enzyme arrangement extends a finite distance away from the pole. Lines show analytical solutions. Points show results of numerical optimization. Fig. 3 of the main text shows the same data with the fluxes at $x = L$ plotted against $(E_T - E_T^t)/E_T^t$.

solving the reaction-diffusion equation with arbitrary cluster densities e_0 and e_L , and then optimizing over e_0 and e_L subject to the constraint $e_0 + e_L = E_T$. The transition position E_T^{t1} is then the value at which the resulting optimal $e_L^* = 0$. However, since the expressions are large and complex we do not include them here.

As E_T is increased further we reach a second transition at E_T^{t2} , with enzymes being placed within the system in the vicinity of $x = 0$. This transition occurs when the diffusive and reaction fluxes at $x = 0$ become equal, $j^D(0) = j^P(0) = j(0)/2$ (see Fig. F.1B). At the transition value E_T^{t2} , the corresponding fluxes at $x = L$ are not equal and still satisfy the inequality condition Eq. D8 (Eq. 10 of the main text). Therefore, around $x = L$ the optimal configuration is still purely clustered, with no enzymes at $x < L$. The transition threshold E_T^{t2} can be determined exactly by calculating the fluxes $j^D(0)$ and $j^P(0)$ from the full solution of the system with the two optimal polar clusters.

At a larger transition value E_T^{t3} , the diffusive and reactive fluxes at $x = L$ also become equal, $j^D(L) = j^P(L) = j(L)/2$ (see Fig. F.1B). For $E_T > E_T^{t3}$, enzymes are present away from the boundaries at both ends of the system. In the regime $E_T > E_T^{t2}$ it is not possible to solve for the form of the optimal profile $e^*(x)$, or therefore to calculate $j^D(L)$ and $j^P(L)$. The value of E_T^{t3} must therefore be determined numerically.

Finally, if E_T is increased further, the enzyme density profiles extending from the two ends of the system will eventually coalesce, and enzymes will be present at all positions $0 \leq x \leq L$.

It is also possible for these transitions to occur in a different order for different parameter combinations. For example, if $j(L) \ll j(0)$, the order of the first two transitions may be reversed. In this case, the condition $j^D(0) = j^P(0) = j(0)/2$ is satisfied before the transition value E_T^{t1} is reached. The first transition will then be from a single cluster at $x = 0$ to a regime in which there is a finite extension of enzymes from $x = 0$, but no cluster at $x = L$. The appearance of the second cluster at $x = L$ may then occur subsequently, at larger E_T , resulting in an optimal enzyme profile than resembles type III in Fig. F.1A.

Appendix G: Optimal enzyme allocation algorithm for discretized systems

1. Derivation of the enzyme repartition rule

In this section we derive Eqs. 12, 14 of the main text, which lead to the optimal enzyme repartition rule used in step 5 of the construction algorithm. For the implementation of an optimization algorithm, we consider a system with discretized space. In this model, the densities of substrate and enzyme change from functions of position to a discrete set of variables, $\{e_i\} = \{e(\mathbf{r}_i)\}$ for $i = 1..N$, where N is the number of lattice sites. Instead of being a functional of $e(\mathbf{r})$, $\rho(\mathbf{r})$, the corresponding discrete Lagrangian then becomes a function of $\{e_i\}$, $\{\rho_i\}$, and functional derivatives $\frac{\delta \mathcal{L}}{\delta f(\mathbf{r})}$ become normal derivatives $\frac{\partial \mathcal{L}}{\partial f_i}$.

The derivative of $\mathcal{L}$ with respect to the enzyme density has an analogous form as in the continuous system,

$$\frac{\partial \mathcal{L}}{\partial e_i} = k_{\text{cat}} F[\rho_i] \{1 - \lambda_i\} - \lambda_e, \quad (\text{G1})$$

where λ_i is the Lagrange multiplier associated with the discrete reaction-diffusion equation at site i . We again denote the first term above by $\frac{dJ^P}{de_i}$, with the same interpretation as in the continuous case. This quantity is generally easily accessible in any numerical analysis: one simply increases the amount of enzymes at position i by a small amount, solves the resulting modified reaction-diffusion system, and calculates the resulting change in the total reaction flux. In the same way as for the continuous system, for the optimal enzyme arrangement $\{e_i^*\}$ we must have that $\frac{\partial \mathcal{L}}{\partial e_i} = 0$ at all sites i where $e_i^* > 0$, and $\frac{\partial \mathcal{L}}{\partial e_i} \leq 0$ where $e_i^* = 0$.

We now consider the optimal amount of enzymes at site i to be a function of E_T , $e_i^*(E_T)$. That is, there is a line $\mathbf{e}^*(E_T)$ in the space of $\mathbf{e} = (e_1, e_2, \dots, e_N)$, that we can parameterize by the value of E_T , for which the reaction flux $J^P(\mathbf{e}^*(E_T))$ is the highest that can be achieved for that value of E_T . We begin by considering the derivative of $\frac{dJ^P}{de_i}$ with respect to E_T along the line defined by $\mathbf{e}^*(E_T)$, $\frac{d}{dE_T} \frac{dJ^P}{de_i}$. We now expand the total derivative $\frac{d}{dE_T}$ in terms of derivatives with respect to the underlying variables $\{e_i\}$,

$$\frac{d}{dE_T} \frac{dJ^P}{de_i} = \sum_{j=1}^N \frac{de_j}{dE_T} \frac{d}{de_j} \frac{dJ^P}{de_i}. \quad (\text{G2})$$

Note that here $\frac{d}{de}$ takes into account the changes in the flux both due directly to altering the enzyme density, and the resulting change in the substrate density profile $\{\rho_i\}$. Since $\frac{de_j}{dE_T}$ is understood to be in the direction of the line of optimal profiles, it coincides with $\frac{de_j^*}{dE_T}$.

852 Writing the set of N equations Eq. G2 in vector form we have

$$\frac{d}{dE_T} \frac{dJ^P}{de} = \mathbf{H} \frac{de^*}{dE_T}, \quad (\text{G3})$$

853 where $\mathbf{H}$ is the Hessian of J^P , $H_{ij} = \frac{d^2 J^P}{de_j de_i}$.

854 From Eq. G1 we know that if there are $1 \leq n \leq N$ positions at which the optimal enzyme
855 density is non-zero, then

$$\begin{pmatrix} \frac{dJ^P}{de_1} \\ \vdots \\ \frac{dJ^P}{de_n} \\ \frac{dJ^P}{de_{n+1}} \\ \vdots \\ \frac{dJ^P}{de_N} \end{pmatrix} = \lambda_e \begin{pmatrix} 1 \\ \vdots \\ 1 \\ c_{n+1} \\ \vdots \\ c_N \end{pmatrix}, \quad (\text{G4})$$

856 where all c 's lie in the range $0 < c_i < 1$, and we have numbered lattice sites in descending
857 order of marginal returns. Therefore, Eq. G3 becomes

$$\mathbf{H} \frac{de^*}{dE_T} = \frac{d\lambda_e}{dE_T} \begin{pmatrix} 1 \\ \vdots \\ 1 \\ c_{n+1} + \frac{dc_{n+1}}{dE_T} \frac{\lambda_e}{\frac{d\lambda_e}{dE_T}} \\ \vdots \\ c_N + \frac{dc_N}{dE_T} \frac{\lambda_e}{\frac{d\lambda_e}{dE_T}} \end{pmatrix}. \quad (\text{G5})$$

858 In general it is not possible to directly compute the right hand side of Eq. G5, because
859 without knowing the direction of the optimal line it is not possible to calculate the derivatives
860 $\frac{d\lambda_e}{dE_T}$ and $\frac{dc_i}{dE_T}$. However, exploiting the fact that no enzymes should be added to the system at
861 positions where $\frac{dJ^P}{de_i} < \lambda_e$, the vector $\frac{de^*}{dE_T}$ must have zeros at all positions $i > n$. Therefore,
862 the N -dimensional system in Eq. G5 can be split into two parts. Primarily, we have the
863 n -dimensional system

$$\mathbf{H}_{(n)} \frac{de_{(n)}^*}{dE_T} = \frac{d\lambda_e}{dE_T} \mathbf{1}_{(n)}, \quad (\text{G6})$$

864 where the subscript (n) denotes that only the first n positions of the lattice are considered.
865 Importantly, in order to solve Eq. G6 it is not necessary to know $\frac{d\lambda_e}{dE_T}$ in advance. Instead, one
866 can solve for any constant prefactor, and then subsequently use the normalization condition

867 $\sum_{i=1}^n \frac{de_i^*}{dE_T} = 1$ to express $\frac{d\lambda_e}{dE_T}$ in terms of this constant. For self-consistency, the solution to
868 Eq. G6 must also solve the residual $(N - n)$ -dimensional system

$$\begin{pmatrix} \frac{d^2 J^P}{de_1 de_{n+1}} & \frac{d^2 J^P}{de_2 de_{n+1}} & \cdots & \frac{d^2 J^P}{de_n de_{n+1}} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{d^2 J^P}{de_1 de_N} & \frac{d^2 J^P}{de_2 de_N} & \cdots & \frac{d^2 J^P}{de_n de_N} \end{pmatrix} \frac{de_n^*}{dE_T} = \frac{d\lambda_e}{dE_T} \begin{pmatrix} c_{n+1} + \frac{dc_{n+1}}{dE_T} \frac{\lambda_e}{d\lambda_e} \\ \vdots \\ c_N + \frac{dc_N}{dE_T} \frac{\lambda_e}{d\lambda_e} \end{pmatrix}, \quad (\text{G7})$$

869 which can be interpreted as defining the evolution of the marginal returns at positions
870 without enzymes, $\frac{dc_i}{dE_T}$.

871 2. Discretization effects

Figure G.1. Value of the reaction flux at the transition $j^P(\mathbf{s})$ vs the number of lattice sites N . The blue line is the exact solution obtained by iteratively solving the discrete reaction-diffusion system at steady-state, while the red crosses are the result of the construction algorithm for different N values.

872 How large should the number of lattice sites N be to have a good approximation of the
873 continuum limit of the system? In this section we discuss a criterion to find such a value.

874 In the main text we derive a general condition for the transition in the optimal enzyme
875 arrangement from a localized arrangement into an extended profile. The condition (Eq. 10

of the main text), in combination with the boundary condition, at the transition, reads:

$$j^{\mathcal{P}}(\mathbf{s}) = \frac{1}{2}(j(\mathbf{s}) - \rho(\mathbf{s})\mathbf{v}(\mathbf{s}) \cdot \hat{n}(\mathbf{s})), \quad (\text{G8})$$

which becomes $j^{\mathcal{P}}(\mathbf{s}) = j(\mathbf{s})/2$ in case of no advection $\mathbf{v} = 0$. This condition does not depend on the geometry, nor on the loss mechanism considered for the substrate and it applies locally at every position of the system boundaries with influx of substrate. As can be seen in Appendix D, the above condition is equivalent to $\frac{\partial}{\partial \mathbf{n}(\mathbf{s})} \frac{dJ^{\mathcal{P}}}{de} = 0$ and in general is derived by combining different derivatives of the Lagrangian $\mathcal{L}$. The derivatives are going to be affected by the system discretization, implying that Eq. G8 is valid only in the limit $N \rightarrow \infty$.

For example in the case of the discrete version of the system described in Fig. 2 of the main text and solved in Appendix B, C, it can be shown by solving iteratively the reaction-diffusion equation, that the transition happens for $\alpha E_T^t = N/(N - 2)$ and that the reaction flux at the transition is $j^{\mathcal{P}}(0) = j(0)(N - 1)/(2N - 3)$. In the limit of $N \rightarrow \infty$ the two limits are $\alpha E_T^t \rightarrow 1$ and $j^{\mathcal{P}}(0) \rightarrow j(0)/2$, which coincide with the continuum solution, cf. Appendix B. In Fig. G.1 we can see how $j^{\mathcal{P}}(0)$ scales as a function of the number of lattice sites N . In blue is the exact solution, while the red crosses are the result of the construction algorithm for different discretizations. For example, we can see how for $N = 100$ the discrepancy between the continuum limit and the discrete system is approximately 0.51%.

3. Efficiency of the construction algorithm

In this section we compare the computational times of the construction algorithm derived in this work and a stochastic optimization algorithm that has been previously used to find the optimal enzyme arrangements in various systems belonging to the class of systems illustrated in Fig. 1 of the main text [17, 18].

The stochastic optimization algorithm takes an initial uniform enzyme configuration. Then, an iteration of the algorithm consists in taking a random amount of enzymes and moving it from a random site to a different random site. This new enzyme configuration is then the initial configuration for the next optimization step if the reaction flux generated by the new enzyme profile is higher than the previous configuration. The optimization is stopped after a maximum number of iterations of $N_{iter.} = 10^5$, or if the flux does not

increase for $N_{iter.} = 10^4$. For each change in the enzyme arrangement, the steady-state reaction-diffusion equation needs to be solved. The general form of the equation for a discrete system in matrix form is (cf. Material and Methods of the main text):

$$(\mathbf{D} + \mathbf{V})\boldsymbol{\rho} - \alpha \mathbf{e} \odot \mathbf{F} - \sigma \boldsymbol{\rho} = \mathbf{A}, \quad (\text{G9})$$

where $\mathbf{D}$ and $\mathbf{V}$ are the diffusion and advection operator on the lattice respectively, $\boldsymbol{\rho}$ the substrate density, $\mathbf{F}$ is the vector with $F_i = F[\rho_i]$ and $\mathbf{A}$ the substrate influx vector, while $\odot$ denotes the element-wise (Hadamard) product.

For the comparison of the two algorithms, we decided to consider a 1D system with two sources of substrate and linear reactions as described in Appendix F. We consider such a system because we expect the stochastic optimization algorithm to perform at its best in 1D systems with linear reactions. For such systems the diffusion matrix $\mathbf{D}$ becomes a tridiagonal symmetric matrix and Eq. G9 becomes a linear algebraic system that can be solved with the use of a tridiagonal inversion algorithm that is $o(N)$. Since the random selection of sites is $o(N^2)$, we expect the stochastic optimization algorithm with a tridiagonal solver to be $o(N^3)$.

Our construction algorithm is also $o(N^3)$ since the Hessian inversion required in step 5 of the algorithm follows a Cholesky decomposition. Moreover, given the iterative nature of our construction algorithm, we have a prefactor that increases with the target amount of enzymes E_T that we want to reach.

In Fig. G.2A we plot in blue circles the average computational time spent by the stochastic optimization algorithm with a solver for tridiagonal symmetric matrices over 20 repeats and with the green pluses we mark the computational time of the construction algorithm. The standard deviation of the time spent by the stochastic optimization algorithm is not shown as it is smaller than the size of the markers used. We can see that the construction algorithm is faster than the stochastic optimization with tridiagonal solver only for low amounts of E_T . Passed the first transition value E_T^t , the construction algorithm gets up to 10 times slower than the stochastic optimization one with tridiagonal solver. Note, however, that the stochastic optimization algorithm returns the optimal enzyme configuration for a single E_T value, whereas the construction algorithm gives the whole optimal trajectory up to the target value E_T . Moreover, this is the limiting case of a system having a symmetric tridiagonal diffusion operator. In general this is not the case, e.g. higher dimensional systems. In

Figure G.2. Computational time *vs* the total number of enzymes in the system for different algorithms. We mark the computational time spent by the construction algorithm derived in this work (green pluses) and the average time spent by a stochastic optimization algorithm using either a tridiagonal symmetric solver (blue circles) or an LU-decomposition algorithm (red crosses). The system under consideration is a 1D system with two sources of substrate, one for each boundary. In (A) and (B) we consider a number of lattice sites of $N = 100$ and $N = 200$ respectively. The construction algorithm out-competes the stochastic optimization algorithm with LU-decomposition, which is the standard solver used in systems with no specific symmetries and higher dimensions. The construction algorithm can be a factor of 10 slower than the stochastic optimization algorithm with a tridiagonal solver. Such a solver however can be used only for specific systems, e.g. a 1D system with a tridiagonal diffusion operator. The optimizations were conducted on a single core of a Intel(R) Xeon(R) CPU E5-2640 v3. The average time spent by the stochastic optimization is taken over 20 repeats, the standard deviation is not shown as it is smaller than the size of the markers used.

the more general setups, one would typically use an LU-decomposition to solve Eq. G9 for linear reactions in the stochastic optimization algorithm. Such solver is $o(N^3)$, implying that the stochastic optimization algorithm becomes $o(N^5)$. In Fig. G.2A,B the computational time of the stochastic optimization algorithm using a LU-decomposition is plotted with red crosses for $N = 100$ and $N = 200$ respectively. We can see how the construction algorithm out-competes the stochastic optimization one for all E_T values and that the higher is the amount of lattice sites N , the larger is the difference in computational times as expected

Figure G.3. Flux comparison and convergence of the stochastic optimization algorithm vs. the construction algorithm. (A) We plot the relative difference of fluxes $\Delta J/J = 1 - J_{s.o.}/J_{c.a.}$ as a function of the number of iterations taken in the stochastic optimization algorithm for different E_T values and a discretization of $N = 200$. Different colors correspond to different E_T values as given by the points in panel (B). Some of the stochastic optimizations do not converge to $J_{c.a.}$. For example, 16 out of 20 optimization runs get stuck at $\Delta J/J \approx 10^{-5}$ for $E_T = 1$. We plot an example of such trajectory with a black dashed line in the inset of panel (A). The optimization stops before reaching our imposed limit of $N_{iter.} = 10^5$ because the flux does not increase for 10^4 iterations. The shaded areas correspond to the standard deviation of $\Delta J/J$ over 20 repeats. (B) We plot the average and the standard deviation of the number of iterations needed for the stochastic optimization to end $N_{iter.}^{end}$. The stochastic optimization for $N = 200$ gets about 3-times higher than the number of iterations needed for $N = 100$ for $E_T \geq 20$. The $N_{iter.}^{end}$ for the two discretizations get similar as E_T decreases. This is due to the non-convergence of the stochastic optimization at low E_T values for $N = 200$. The stochastic optimization gets stuck at sub-optimal enzyme configurations and it is stopped after 10^4 iterations with no flux increase.

941 from the scaling argument. We therefore expects the construction algorithm to always be
 942 convenient as compared to the stochastic optimization algorithm unless the systems present
 943 simple geometries with specific symmetries. Then the construction algorithm could be slower
 944 by a factor of 10 for large E_T values.

945 The construction optimization algorithm has also the advantage of being exact and certain
 946 to converge to the optimal solution. This is not always the case for the stochastic optimiza-

tion algorithm, which may or may not converge to the global optimum. In Fig. G.3A, we plot the relative difference between the two fluxes $\Delta J/J = 1 - J_{s.o.}/J_{c.a.}$, where $J_{s.o.}$ is the flux obtained via the stochastic optimization and $J_{c.a.}$ the one of the construction algorithm. We plot $\Delta J/J$ for different E_T values as a function of the number of iterations taken in the stochastic optimizations. None of the realizations, both for $N = 100$ and $N = 200$ resulted in negative values of $\Delta J/J$, i.e. $J_{s.o.} > J_{c.a.}$. In the case of $N = 100$, for any E_T value and for all the repeats, $\Delta J/J$ goes to zero. However, in the case of $N = 200$ (Fig. G.3A), the stochastic optimization does not always converge to the flux obtained via the construction algorithm $J_{c.a.}$. For example, in the case of $E_T = 1$, 16 out of 20 repeats do not converge to $J_{c.a.}$ and they get stuck at a value of $\Delta J/J \approx 10^{-5}$. We plot an example of such an optimization trajectory in the inset of Fig. G.3A (black dotted line) in log-scale. For such a trajectory, the stochastic optimization algorithm stops after $N_{iter.} \approx 6 \cdot 10^4$. This is better shown in Fig. G.3B, where we plot the number of iterations needed to end the stochastic optimization, $N_{iter.}^{end}$, for different E_T values. We can see that for a discretization of $N = 200$, $N_{iter.}^{end}$ is about 3-times higher as compared to the iterations needed for $N = 100$ for $E_T \geq 20$. As E_T decreases, the values of $N_{iter.}^{end}$ of the two discretizations get closer to each other. This is because in this regime the stochastic optimization for $N = 200$ do not always converge to the optimal enzyme arrangement. It gets stuck at some sub-optimal configuration, causing the optimization to end prematurely after 10^4 iterations with no flux increase.

Appendix H: Betting games with coupled outcomes and bets

1. Gambler repeatedly betting a fixed amount of resources

In the main text, we show that the optimal enzyme spatial allocation problem can be mapped to a betting game where a gambler bets a fixed amount of resources at every repetition of a game. In case of victory, the gambler wins an amount proportional to the bet b_i on the winning event i , times the odds payed back to the winner α_i , i.e. an amount of money $\alpha_i b_i$. The long-term amount of capital won by the gambler grows arithmetically and is $C = \sum_i \alpha_i b_i p_i$, where p_i is the probability that i is the winning outcome. Betting a fixed amount of resources at every repetition of the game implies that $\sum_i b_i = B$. We can therefore ask: what is the optimal way to repartition the bets b_i under the budget constraint?

976 To answer the question we can look at the maximum of the following Lagrangian

$$\mathcal{C} = \sum_i \alpha_i b_i p_i - \lambda \left(\sum_i b_i - B \right). \quad (\text{H1})$$

977 The Lagrangian is linear with respect to the bets b_i . This implies that the optimal solution
978 is to bet all the budget on the outcome that has the highest expected odds $\alpha_i p_i$. Therefore
979 in this simple scenario the gambler never diversifies the bets placed. By looking at the
980 derivatives of the constrained capital with respect to the bets, we can see how they do not
981 depend on any of the bets b_i and are given by the sum of two terms:

$$\frac{\partial \mathcal{C}}{\partial b_i} = \alpha_i p_i - \lambda. \quad (\text{H2})$$

982 The derivative above can be interpreted as the variation of the constrained capital per extra
983 bet placed. The first term represents the net expected gain $\alpha_i p_i$ per extra bet placed on
984 the outcome i , if no constraint was considered. It is equivalent to the first term present in
985 the derivative $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, Eq. 7 of the main text, corresponding to the increase in reaction flux
986 that would be observed upon adding extra enzymes at position $\mathbf{r}$, if the constraints on the
987 reaction-diffusion dynamics and the enzyme budget are neglected. The second term $-\lambda$ is
988 the marginal cost of adding extra capital and it is analogous to the $-\lambda_e$ term of $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$.

989 What we are missing is an equivalent of the second term of $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, $-\lambda_V(\mathbf{r}) k_{\text{cat}} F[\rho(\mathbf{r})]$, which
990 is generated by the coupling between $e(\mathbf{r})$ and $\rho(\mathbf{r})$ through the reaction-diffusion equation.
991 In fact, in the enzyme spatial allocation problem there is a dependency between the enzymes
992 allocated and the reaction flux generated, i.e. a dependency between the resources invested
993 in the system and the returns obtained. We can therefore ask: what would happen to our
994 betting problem if a feedback between the bets placed b_i and the expected odds $\alpha_i p_i$ is
995 introduced?

996 In our mapping, the bets placed on a certain outcome correspond to the enzymes allocated
997 at a certain position, $b_i \leftrightarrow e(\mathbf{r})$, while the fixed budget corresponds to the total enzyme
998 amount, $B \leftrightarrow E_T$. The probability of i being the winning event maps to the probability of
999 an enzyme being bound with the substrate at a certain position, $p_i \leftrightarrow F[\rho(\mathbf{r})]$, and the odds
1000 correspond to the catalytic rate, $\alpha_i \leftrightarrow k_{\text{cat}}$. Since in our correspondence α_i is constant, we
1001 introduce a feedback between the bets b_i and the expected odds $\alpha_i p_i$ by considering each p_i
1002 to be a function of the vector of bets $p_i = f_i(\mathbf{b})$. Note, however, that a feedback could also
1003 be constructed by having the odds α_i function of the bets $\mathbf{b}$. A new functional $\mathcal{C}_{fb}$ can be

considered:

$$\mathcal{C}_{fb} = \sum_i \alpha_i b_i p_i - \sum_i \lambda_i [f_i(\mathbf{b}) - p_i] - \lambda \left(\sum_i b_i - B \right), \quad (\text{H3})$$

where λ_i are the Lagrange multipliers corresponding to the constraints on the probabilities p_i being functions $f_i(\cdot)$ of the bets placed $\mathbf{b}$. The role played by the λ_i is analogous to the one played by $\lambda(\mathbf{r})$ in the Lagrangian $\mathcal{L}$ Eq. 5 of the main text. When we take derivatives of $\mathcal{C}_{fb}$ with respect to b_i we now have extra terms:

$$\frac{\partial \mathcal{C}_{fb}}{\partial b_i} = \alpha_i p_i - \sum_j \left(\lambda_j \frac{\partial f_j(\mathbf{b})}{\partial b_i} \right) - \lambda \quad (\text{H4})$$

The contribution of the terms in the sum $\sum_j (\cdot)$ comes from the coupling between the bets placed and the returns generated, analogously to the second term present in the derivative $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, Eq. 7 of the main text. The exact form of $f_i(\mathbf{b})$ depends on the details of the model considered for the game played by the gambler.

For an optimal allocation of capital the derivative $\frac{\partial \mathcal{C}_{fb}}{\partial b_i} = 0$, meaning that the sum of the first term with the sum $\sum_j (\cdot)$ equals the marginal cost λ , in a similar way as what we found for the optimal allocation of enzymes by imposing $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})} = 0$. It results optimal to invest up to the point for which the net marginal gain expected from our investment (including the contribution due to the feedback) equals the marginal cost of adding extra resources. Moreover, diversification of bets can be optimal depending on the functional form of $f_i(\cdot)$. For example, let's consider the case of two possible winning outcomes plus a third outcome having nonzero probability of happening but zero odds. In this simple scenario and in the case of bets affecting only the correspondent outcomes, i.e. $\frac{\partial f_i}{\partial b_j} = 0$ with $i \neq j$, the optimal strategy is

$$b_1^* = -\frac{\alpha_1 f_1 - \alpha_2 f_2 - \alpha_2 B \frac{\partial f_2}{\partial b_2}}{\alpha_1 \frac{\partial f_1}{\partial b_1} + \alpha_2 \frac{\partial f_2}{\partial b_2}}, \quad b_2^* = B - b_1^*, \quad b_3^* = 0. \quad (\text{H5})$$

The optimal strategy results in diversified bets (i.e. $b_1^* > 0, b_2^* > 0$), if, e.g., the events have diminishing returns ($\frac{\partial f_i}{\partial b_i} < 0$, with $i = 1, 2$) and at the same time an event has higher expected odds as compared to the other ($\alpha_1 f_1 > \alpha_2 f_2 + \alpha_2 B \frac{\partial f_2}{\partial b_2}$). The term $\alpha_2 B \frac{\partial f_2}{\partial b_2}$ accounts for how the expected odds of the second event change due to the diminishing returns effect. Alternatively one could have diversified bets if the expected odds of an event are lower than the other ($\alpha_1 f_1 < \alpha_2 f_2 + \alpha_2 B \frac{\partial f_2}{\partial b_2}$) and the events present an increasing returns effect for which the probabilities of the events of being the winning ones increase with the amount of bets placed on them ($\frac{\partial f_i}{\partial b_i} > 0$, with $i = 1, 2$). Moreover, if the events behave differently and

for example an event presents diminishing returns and the other increasing returns, we still get optimal diversification of bets as given by Eq. H5.

To see how such optimal diversification strategies vary as a function of the total budget B , one can use the construction algorithm presented in the main text and derived in Appendix G. The algorithm consists in looking at the Hessian matrix of the capital $\mathcal{C}_{fb}$ with respect to the bets placed on different outcomes, as similarly illustrated in the main text for the enzyme spatial allocation problem. The algorithm applies to any game having a linear budget constraint. This means that it can be applied to investment problems where the revenue grows arithmetically as in Eq. H3, but also problems where the revenue grows exponentially as it is the case for example for the famous Kelly problem [10]. There, instead of considering the Hessian matrix of the capital as given by $\mathcal{C}_{fb}$, one would look at the Hessian of the capital growth rate with respect to the bets placed at different outcomes and would optimize for the growth rate as the budget gradually increases.

2. Gambler repeatedly reinvesting a fraction of the resources - Generalization of Kelly's criterion

In this section we generalize Kelly's optimal betting criterion [10] for a gambler that wants to maximize his steady state capital growth rate G by betting on a series of mutually exclusive events. In the original formulation of Kelly, the capital growth rate G has the following form:

$$G = \sum_i p_i \log [k + b_i \alpha_i], \quad (\text{H6})$$

where p_i is the probability for the event i to be the winning one, k is the fraction of the gambler's capital kept, b_i is the fraction of gambler's capital invested on the event i and α_i are the odds, i.e. the money payed back to the gambler in case i is the winning event.

Kelly's aim was to determine the optimal way of partitioning the gambler's capital given that the gambler cannot borrow nor lend money, hence his aim was to determine the optimal set of b_i and k , under the constraint $\sum_i b_i + k = 1$. We can therefore consider the functional

$$\mathcal{G} = \sum_i p_i \log [k + b_i \alpha_i] - \lambda \left[\sum_i b_i + k - 1 \right], \quad (\text{H7})$$

where λ is the Lagrange multiplier associated with the constraint on the gambler's capital.

To find the optimal set of b_i and k we perform the following derivatives:

$$\frac{\partial \mathcal{G}}{\partial b_i} = \frac{p_i \alpha_i}{k + \alpha_i b_i} - \lambda = 0 \quad \text{for } i \in s \quad (\text{H8})$$

$$\frac{\partial \mathcal{G}}{\partial k} = \sum_i \left[\frac{p_i}{k + \alpha_i b_i} \right] - \lambda = 0 \quad (\text{H9})$$

$$\frac{\partial \mathcal{G}}{\partial b_i} = \frac{p_i \alpha_i}{k} - \lambda \leq 0 \quad \text{for } i \in s', \quad (\text{H10})$$

where we indicated with s the set of events for which it is optimal to bet and $b_i > 0$, and with s' the set of events for which betting is suboptimal and $b_i = 0$. The derivatives above can be interpreted as the variation of the constrained capital growth rate $\mathcal{G}$ per extra bet placed [H8](#), [H10](#) or per extra resource kept [H9](#). The first terms of the derivatives are the net marginal increase of G generated by either placing the bets [H8](#), [H10](#) or by keeping an extra amount of resources [H9](#), if no constraint was considered. The $-\lambda$ terms correspond to the marginal cost of adding the extra resources, analogously to the $-\lambda_e$ term present in the derivative $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, Eq. 7 of the main text. Analogously to the enzyme spatial allocation problem, we see that the optimal strategy is to bet on those events $i \in s$ up to the point for which the marginal gain generated equals the marginal cost. Moreover the optimal way of repartitioning the gambler's capital, known as Kelly criterion, can be obtained by solving for the above derivatives, together with the use of the constraint:

$$\lambda = 1 \quad (\text{H11})$$

$$k = \frac{1 - \sum_{i \in s} p_i}{1 - \sum_{i \in s} 1/\alpha_i} \quad (\text{H12})$$

$$b_i = \frac{p_i \alpha_i - k}{\alpha_i} \quad \text{for } i \in s. \quad (\text{H13})$$

1057 To understand how to determine the set s of events for which it is optimal to bet, we refer
1058 the readers to Kelly's original paper [\[10\]](#), here we just say that finding the optimal s is
1059 similar in determining the positions at which is optimal to allocate enzymes in the class of
1060 reaction-diffusion systems considered in the main text.

1061 Again, the variation of the constrained quantity we want to maximize per extra resource
1062 invested, i.e the derivative Eq. [H8](#), is given by the sum of just two terms and it lacks an
1063 equivalent of the second term of $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, $-\lambda_V(\mathbf{r})k_{\text{cat}}F[\rho(\mathbf{r})]$, which is generated by the coupling
1064 between $e(\mathbf{r})$ and $\rho(\mathbf{r})$ through the reaction-diffusion equation. We can imagine to add a
1065 dependency between the probabilities of winning and the bets placed, by assuming that

the probabilities p_i are now functions of the bets placed $p_i = f_i(\mathbf{b})$, where $\mathbf{b}$ is the vector $(b_1, b_2, \dots)$. A new functional $\mathcal{G}$ can be considered:

$$\mathcal{G} = \sum_i p_i \log [k + b_i \alpha_i] - \lambda \left[\sum_i b_i + k - 1 \right] - \sum_i \lambda_i [p_i - f_i(\mathbf{b})] , \quad (\text{H14})$$

where λ_i are the Lagrange multipliers corresponding to the constraints on having the probabilities functions of the bets placed. The role played by the λ_i is analogous to the one played by $\lambda(\mathbf{r})$ in the Lagrangian $\mathcal{L}$ Eq. 5. When we take the derivatives of $\mathcal{G}$ with respect to b_i we now have extra terms:

$$\frac{\partial \mathcal{G}}{\partial b_i} = \frac{p_i \alpha_i}{k + \alpha_i b_i} + \sum_j \left(\lambda_j \frac{\partial f_j(\mathbf{b})}{\partial b_i} \right) - \lambda = 0 \quad (\text{H15})$$

The sum $\sum_j(\cdot)$ comes from the coupling between the bets placed and the returns generated, analogously to the second term present in the derivative $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})}$, Eq. 7 of the main text. The exact form of $f_i(\mathbf{b})$ depends on the details of the model considered for the game played by the gambler.

For an optimal allocation of capital the derivative $\frac{\partial \mathcal{G}}{\partial b_i} = 0$, meaning that the sum of the first term with the sum $\sum_j(\cdot)$ equal the marginal cost λ , in a similar way as what we found for the optimal allocation of enzymes by imposing $\frac{\delta \mathcal{L}}{\delta e(\mathbf{r})} = 0$.

Since the cost of adding extra resources is constant and equals $-\lambda$, we can use a similar construction algorithm as the one presented in the main text to find the optimal gambling strategy. Instead of considering the Hessian of the reaction flux with respect to the addition of enzymes at different positions, one needs to compute the Hessian of the capital growth rate with respect to the bets placed on different outcomes. Once the Hessian is found, one can derive the optimal way of repartitioning the gambler's bet to track the optimal G as the total budget gradually increases in exactly the same way as we did for the enzyme spatial allocation problem.