Enzyme Models

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Response to Shape Emerges in a Complex Biochemical Network and Its Simple Chemical Analogue**

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Although biology is governed by underlying chemical processes, equations of chemical kinetics do not directly predict many properties that emerge in living systems.[1–3] For example, cells and organisms can detect and respond to the shape of objects, as observed in endothelial cell attachment to patterned islands,[4] selective phagocytosis in neutrophils,[5] and insect recognition of shape,[6] such as the outline of flowers.[7] Response to shape in these systems probably occurs at a high level of organization (cytoskeleton or neural networks) with no immediate connection to the underlying chemistry. This leaves one to wonder, what level of chemical complexity does the emergence of unanticipated functions require? Is this emergence unique to living organisms or can it occur in more simple chemical systems and be explained by chemical principles? Herein, we show that response to shape can emerge at the level of a biochemical network. We relied on a previously developed mechanism[8] and an experimental system[9] to examine initiation of coagulation of human blood plasma in vitro. We found that this biochemical network responded to shape, that is, the shape of the patch of stimulus controlled whether clotting was initiated.

To characterize the response of initiation of the blood clotting cascade (initiation) to the shape of a patch presenting a stimulus of clotting, we detected clotting by monitoring both the formation of fibrin and the formation of thrombin.[10] Fibrin was detected by using brightfield microscopy, and thrombin was detected by using fluorescence microscopy to visualize a blue fluorogenic substrate[11] (Figure 1a). Surface patches of tissue factor (TF), an integral membrane protein that stimulates initiation, were patterned by using photolithography. TF was reconstituted[12,13] in phospholipid bilayers[13] containing 0.5 mol% of lipid labeled with a red fluorescent dye. We presented various shapes of the TF patches to human blood plasma in a microfluidic chamber. When comparing patches of different shapes, the area of all patches (and therefore the amount of TF) was kept constant (3.14×10^4 μm^2).

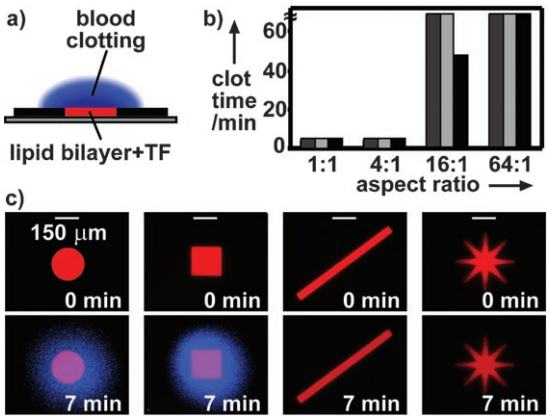

Figure 1. The initiation of clotting of human blood plasma responded to the shape of surface patches of identical area and the amount of a clotting stimulus, TF. a) Side-view drawing showing clotting (blue) on a patch (red) of phospholipid bilayer containing TF. b) Chart quantifying the initiation times of human blood plasma on rectangular patches of varying aspect ratio, measured in triplicate. c) Time-lapse fluorescent micrographs (top view) showing clotting on circular and square-shaped patches but not on narrow-rectangular and star-shaped patches of the same area. All scale bars are 150 μm.

When human blood plasma was exposed to patches containing TF, initiation only occurred on specific shapes. Initiation occurred on circular patches above a critical size, which is consistent with previous results.[9] Initiation on other shapes showed a fascinating trend. Wide rectangles, such as a square (aspect ratio = 1:1), initiated in less than four minutes, whereas narrow rectangles (aspect ratio ≥ 16:1) did not initiate within 48 minutes (Figure 1b,c). From these experiments, it appeared that there is a critical rectangle width necessary to cause initiation (≈ 90 μm for the experiments described above). Interestingly, star-shaped patches were on the border for initiation and initiated in only half of the experiments (7 out of 14). These results imply that the shape of vascular damage, and its orientation relative to the direction of blood flow, may be important in determining whether clotting occurs. Additional work in vitro and in vivo is required to establish whether these implications are valid.

To examine the mechanism behind this response to shape, we used a 3D numerical simulation that considered a simplified reaction–diffusion system. We previously showed that some properties of hemostasis could be explained by a simple modular mechanism.[8,9] In those studies, initiation of clotting displayed a threshold response to the concentration

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of activators in which competition between diffusion and production of activators by spatially localized reactions dictated whether the threshold was reached. By considering this mechanism here, we reproduced the response to shape in numerical simulations. In this simulation, an autocatalytic reaction mixture was in contact with a surface patterned with patches of stimulus of various shapes with the same area (7854 μm^2). These patches of stimulus produced an activator, *C*, which is analogous to the autocatalytic enzymes in the clotting cascade, such as thrombin.[14,15] This simulation reproduced the experimental results seen in human blood plasma (Figure 2). First, to characterize the effects of

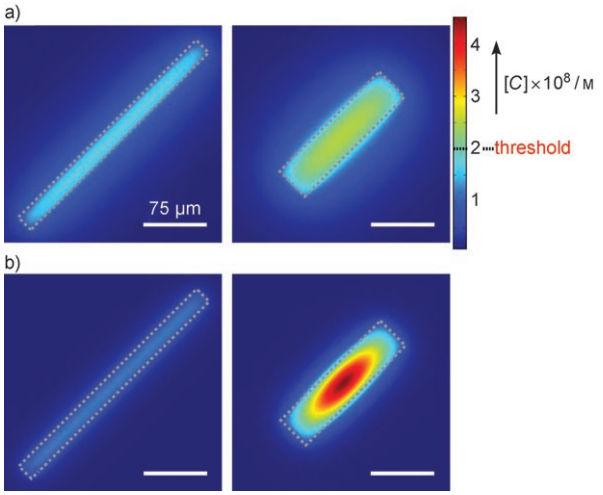

Figure 2. Numerical simulations of a simplified reaction–diffusion system demonstrating a response to shape. a) 2D concentration plots from 3D simulations that considered only diffusion and first-order production of activator from a patch show that the concentration of activator, [*C*], was lower on narrow patches. Diffusive removal of the activator was more effective on the narrow patch (high aspect ratio, blue, left), which maintained [*C*] below the threshold, whereas the maximum concentration of activator *C* on the wide patch (low aspect ratio, yellow, right) was above the threshold concentration of activator. b) When solution-phase reactions corresponding to second-order autocatalytic production and first-order inhibition were also considered in the simulation, diffusion dominated for the narrow patch (left), maintaining the concentration of activator *C* below the threshold. Production dominated for the wide patch (right) and the concentration of activator *C* increased above the threshold and was extensively amplified, resulting in initiation. All scale bars are 75 μm.

diffusion on the concentration of activator, [*C*], on patches of different shapes, we considered only first-order production of the activator from the patch and did not consider reactions in solution (Figure 2a). For wider rectangles (lower aspect ratio), the timescale for diffusion from the center of the patch to outside the patch was longer, generating a higher maximum concentration of *C* on wide patches than narrow patches (high aspect ratio). Second, to investigate how this difference in the concentration of *C* between wide and narrow patches affected initiation of an autocatalytic medium, we added solution-phase reactions to the simulation (Figure 2b). Initiation of this autocatalytic medium had a threshold response to the concentration of *C* as a consequence of two competing reactions in solution: 1) second-order autocatalytic production of an activator and 2) first-order consumption, or inhibition, of the activator (see the rate plot in the Supporting Information). Consideration of these solution-phase reactions amplified small differences in the concentration of *C* between patches, and initiation displayed an all-or-nothing response; the concentration of *C* either increased several orders of magnitude, resulting in initiation, or remained below the threshold concentration of activator *C*, resulting in no initiation. In these simulations, the threshold concentration of activator necessary for initiation was 2×10^{-8} M, which corresponds to the boundary between blue and yellow in Figure 2. For a given set of parameters, rectangles with aspect ratios less than or equal to 4:1 initiated in less than 12 s, whereas rectangles with aspect ratios greater than or equal to 16:1 did not initiate within 1000 s, at which point the system reached a steady state and the simulation was stopped.

If this mechanism for the response to shape is correct, a nonbiological system based on the same chemical principles as the simulation would reproduce the results seen in human blood plasma. We previously developed an experimental, chemical model for hemostasis[8] that reproduced the threshold response to patch area seen in human blood plasma.[9] This model consisted of well-characterized, nonbiological reactions that constitute an autocatalytic system based on inhibition and autocatalytic production of an activator, H^+.[16] In this model, UV light was a stimulus for initiating "clotting". UV light converted the photoacid, 2-nitrobenzaldyhyde, to 2-nitrosobenzoic acid, and "clotting" occurred when the concentration of H^+ reached the threshold level necessary to induce precipitation of alginic acid from alginate, which is indicated by a shift in bromophenol blue to yellow (Figure 3a).

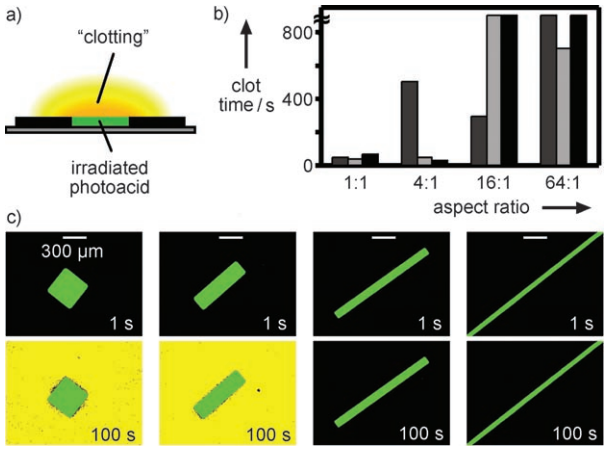

Figure 3. A simplified chemical system constructed to mimic hemostasis responded to the shape of surface patches that present the same areas of a stimulus. a) Side-view drawing showing "clotting" (yellow) on a patch (green) of a photoacid surface irradiated with a UV-light stimulus. b) Chart quantifying the initiation times on rectangular patches, measured in triplicate. c) Time-lapse fluorescent micrographs (top view) showing that "clotting" (yellow) occurred on rectangular patches with a small aspect ratio, such as a square, but not on patches with the same surface area and a large aspect ratio. All scale bars are 300 μm.

As observed in human blood plasma and predicted by simulations, the shape of patches with the same area (1.26×10^4 μm^2) dictated whether or not initiation of this chemical system occurred. Again, initiation was dependent on the aspect ratio of the rectangle (Figure 3b,c) in which wide rectangles initiated and narrow rectangles did not. Interestingly, in contrast to the results in human blood plasma, stars did not initiate in these experiments. This observation was explained by the numerical simulations. Stars produced concentrations of activators close to the threshold, and changing parameters, such as the rate of production from the patch and the diffusion coefficient of the activator, could shift stars from initiating to not initiating, whereas other shapes retained the same response. These results emphasize that simplified models and simulations capture the overall dynamics of complex networks, but experimental measurements are needed to establish the more subtle details of the dynamics. The chemical system described herein provided a strong, yes-or-no response to shape and allowed for an easy readout. However, measuring smaller differences in rates of reactions could provide even finer recognition of intricate shapes and patterns. In theory, increased sensitivity to shape could also be achieved by designing chemical systems in which reaction rates and diffusion coefficients of multiple species can be tuned independently to recognize desired shapes.

Herein, we demonstrated that response to shape can emerge not only at the level of an organism, but also at the more-basic level of a biochemical network. We referred to this phenomenon as "emergent" to emphasize that it could not have been (and was not) easily predicted from current knowledge of hemostasis. Understanding this phenomenon came not from detailed simulations of the individual reactions and the underlying kinetic equations of the network, but rather from recognizing that the dynamics of the clotting network is governed by a threshold response. These results may not be considered surprising—mathematically speaking, a number of nonlinear systems are sensitive to boundary conditions.[17–21] For example, the degree of curvature of an interface can dictate whether or not a chemical wave will propagate out of an opening into bulk solution;[21] the response to shape demonstrated herein must be a related phenomenon. On the other hand, emergence of this dynamics in the ubiquitous and well-characterized blood clotting network, as well as a simple, nonbiological chemical system, implies that unanticipated functions can emerge in systems much less complex than a cell and that rate equations alone may not be sufficient to describe the function of some complex networks. Chemical models and tools of nonlinear chemical dynamics[17,20–23] could become especially useful to understand the emergence of such functions and possibly reproduce them for practical applications. It remains to be seen whether sensitivity to shape is observed in other surface-driven systems that operate with a threshold response, such as T cell activation,[24] and whether this sensitivity can be described within the framework presented herein.

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