Response to shape emerges in a complex biochemical network and its simple chemical analogue

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Experimental Section

Measuring clot times of human blood plasma on patches of tissue factor (TF) of different shapes

To generate patches of clotting stimulus, inert lipid bilayers were pattern by deep UV light and backfilled with vesicles containing reconstituted TF.[1, 2] The inert lipid bilayers contained 97 mol % of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids) and 3 % of a green fluorescent dye (N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt, Invitrogen).[3] A photomask with different shapes of the same surface area (31400 µm² shapes, chrome on quartz mask, Photosciences) was placed over the inert bilayer and irradiated with deep UV light. The patterned bilayer was backfilled with vesicles containing 79.5 mol % of 1,2-dilauroyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids), 20 % of L-α-phosphatidylserine from porcine brain (Avanti Polar Lipids), 0.5 % of a red fluorescent dye (Texas Red® 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, Invitrogen), and TF (EMD Biosciences) (TF:lipid ratio of 1x10⁻⁷). Normal pooled plasma (human) was purchased from George King Bio-Medical, Inc. The plasma was incubated with corn trypsin inhibitor (100µg/mL, EMD Biosciences) to inhibit the factor XII pathway of initiation of clotting.[4] Citrated plasma was recalcified by adding a solution of CaCl₂ containing a thrombin-sensitive fluorescent dye, Boc-Asp(OBzl)-Pro-Arg-MCA, (40 mM CaCl₂, 90 mM NaCl and 0.4 mM Boc-Asp(OBzl)-Pro-Arg-MCA, Peptides International). Recalcified plasma (400 µl) was placed in contact with the patterned bilayer substrate in a microfluidic chamber.[3]

Measuring activation of the non-biological, chemical system on acidic patches of different shapes

The reaction solution in the non-biological, chemical system was composed of equal parts of two precursor aqueous solutions: 1) sodium thiosulfate (Na₂S₂O₃, 0.492mmol, Fisher Scientific), alginic acid (5.8 mg/mL, Sigma), and bromophenol blue (0.425 mM, Acros) with a final pH 7, and 2) sodium chlorite (NaClO₂, 2.99mmol, pH 10.7, Acros).[5] The photoacid-coated substrate
was prepared by spin coating a 20-30 µm thick layer of 2-nitrobenzaldehyde (50% by weight, Adrich) in dimethylsiloxane-ethylene oxide block copolymer (Gelest) onto a siliconized coverslip (Hampton Research). The reaction solution was placed in contact with the photoacid substrate in a microfluidic chamber.\[^3\] UV light (300-400 nm) was irradiated on a photomask (CAD Art Services) and passed through clear patches (125700 µm\(^2\)) to generate H\(^+\) only in specific areas.

**Fabricating the microfluidic chambers**

The microfluidic chamber used in the blood plasma experiment was prepared from poly(dimethylsiloxane) (PDMS) cured on multi-level, machine-milled brass masters. A disposable device was generated with an inner diameter of 13 mm, an outer diameter of 20 mm, and a depth of 1 mm. The microfluidic chamber used in the non-biological, chemical system emperiment consisted of a PDMS gasket with an inner diameter of 10 mm, an outer diameter of 20 mm, and a depth of 1 mm. The gasket was sealed to a siliconized coverslip and to the photoacid-coated substrate.

**Analysis of fluorescence images**

Image analysis was preformed as previously described.\[^3\] The original greyscale fluorescence images were collected and false-colored using MetaMorph\textsuperscript{®} software. For each wavelength, the levels were adjusted to the same values. Images were overlaid using Adobe Photoshop software.

**Numerically simulating initiation of an autocatalytic system on patches of different shapes**

Numerical simulations were performed using a commercial finite element package (Comsol 3.2, Comsol). The simulation was preformed using units of mol/m\(^3\), whereas all values are reported with units converted to mol/dm\(^3\) (M). Three rate equations were utilized in the simulations: (1) production of activator, C, at the surface of the patch, rate = \(k_{\text{patch}}\) (\(k_{\text{patch}} = 4 \times 10^{-8}\) M·s\(^{-1}\)); (2) autocatalytic production of C in solution, rate = \(k_{\text{prod}}\)[C]\(^2\) + \(b\) (\(k_{\text{prod}} = 2 \times 10^7\) M\(^{-1}\)·s\(^{-1}\), \(b = 2 \times 10^{-10}\) M·s\(^{-1}\)); and (3) linear consumption of C in solution, rate = \(-k_{\text{consum}}[C]\) (\(k_{\text{consum}}[C] = 0.2\) s\(^{-1}\)). The initial concentration of C was \(1 \times 10^{-9}\) M, and the diffusion coefficient was \(5 \times 10^{-11}\) m\(^2\)·s\(^{-1}\). Rate constants were based on known values for reactions in the blood clotting network.\[^6\] The
simulation of Figure 2a (main text) considered only Equation (1) while the simulation shown in Figure 2b (main text) considered all three equations.

**Comparing the Response to Shape to the Proposed Rate Plot**

To qualitatively understand why differences in \([C]\) on patches of different shapes would result in differences in initiation of clotting, we analyzed our previously\(^5\) proposed rate plot for hemostasis. The rate plot was based on our proposed mechanism for the kinetics of initiation in hemostasis, which consisted of three interacting modules corresponding to autocatalytic production of C, linear consumption of C, and the generation of a precipitate at high \([C]\) (Figure S1 a). Each module had a corresponding rate equation associated with it, and a key component was that the order of reaction was higher for production than consumption. In this mechanism, initiation of clotting would not occur unless a threshold \([C]\) was obtained, where C represents activators in the clotting cascade, such as factor IIa, factor Xa and many others.\(^7\) The stimulus for these reactions was tissue factor (TF) which, in our experiments, was patterned in surface patches. In our proposed mechanism, the interaction of the production and consumption modules created two steady states in the system, one which was stable and the other unstable. The unstable steady state occurred at the threshold \([C]\). For small increases of \([C]\) that were smaller than the threshold \([C]\), the rate of consumption was greater than the rate of production, and the clotting cascade was not initiated. This was the case for rectangles with aspect ratios greater than 16:1, which were not capable of producing a high enough \([C]\) to initiate (Figure S1 b). When \([C]\) was near the unstable steady state, small perturbations in the system could push it over the threshold for initiation or push the system back below the threshold. This was the case for clotting on star shaped patches, which clotted in only half of the experiments (Figure S1 c). If \([C]\) went above unstable steady state, the rate of production was higher than consumption, which led to the accumulation of C and the initiation of clotting (or precipitation). This was the case for the square, which produced an above-threshold \([C]\) and initiated clotting (Figure S1 d).
**Figure S1.** Correlation between patches of different shapes and the proposed rate plot for initiation in hemostasis. This rate plot was modified from reference 5. a) Schematic representation of a rate plot for the hemostasis network composed of three different modules: autocatalytic production of C, linear consumption of C, and precipitation. If [C] is lower than the unstable steady state (yellow region) clotting will not be initiated. When [C] is near the unstable steady state (orange region), the system is very sensitive to small perturbations in [C] and stochastic fluctuations can push the system below (yellow region) or above (red region) the threshold [C]. When the [C] is above threshold (red region), clotting will be initiated. Surface patches containing TF produced different maximum [C], which affected their ability to initiate clotting. b) Rectangles with aspect ratios of 16:1 produced [C] in the yellow region and did not clot. c) Stars produced [C] in the pink region and initiated clotting in only half of the experiments. c) Squares produced [C] in the red region and initiated clotting.