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Sustained self-organizing pH patterns in hydrogen peroxide driven aqueous redox systems

Supplementary Material

The determination of the effective diffusion coefficient of protons in the presence of the low-mobility proton binding sites of agarose, pH-indicator and polyacrylic acid

In addition to the intentionally added polyacrylic acid, two other low-mobility or immobile agents are able to reversible protonation in our patterning systems and could potentially contribute to the slowing down of the protons: (1) The agarose gel itself with its immobile acid sites and (2) the pH indicators that are indispensable for visualisation but their colour changes result from protonation-deprotonation equilibria.

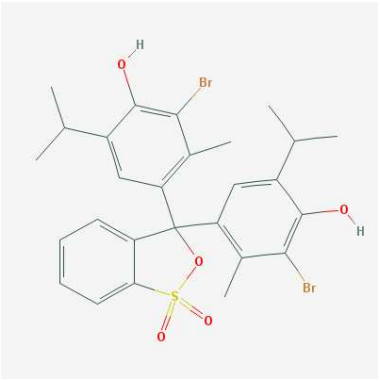
Agarose

Agarose, in principle a neutral gel, in fact always contains small amounts of anionic charges. These are situated on the residues of the agaropectin fraction that remains in the agarose fraction from the raw material agar-agar after purification. Conductometric acid-base titration of a suspension of our low-charge agarose (Fluka 05077) showed 0.2 mM acid functions per weight%.[17] These charged groups can be sulphate half esters of hydroxyle groups, *D*-glucuronic acid (pK_a = 2.95–3.06) and small amounts of pyruvic acid (pK_a = 2.49).[S1] In the further calculations we used the pK_a value of the weakest acid (*D*-glucuronic acid) which provides an upper approximation for the binding of protons to the agarose gel.

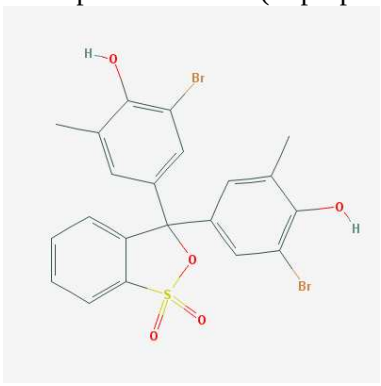
pH-indicators

The pH indicators BTB, BCP (and former BCG in the TuIS reaction[16-17]) used with the different pattern forming reactions belong to the same group of colour indicators, namely sulphonated triphenylmethane dyes. They differ from each other only in isopropyl, methyl or bromo substituents (Chart S1), and have very similar diffusion coefficients 3.27–3.35 × 10^{-6} cm^2s^{-1} in aqueous solution.[S2] This is a factor 4 less than the diffusion coefficient of HSO_3^- (1.2×10^{-5} cm^2s^{-1}).[S3]

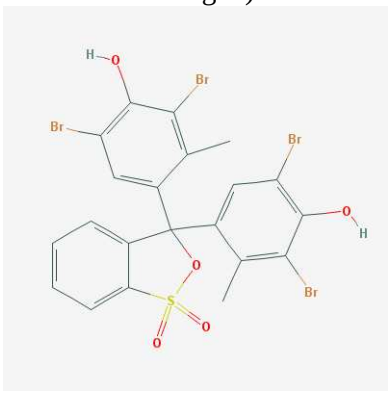
Chart S1 Constitutional formulae of the pH-indicators (http://pubchem.ncbi.nlm.nih.gov)



Bromothymol blue



Bromocresol purple



Bromocresol green

To determine if the agarose gel matrix has an additional slowing down effect on the diffusion of the indicators, we determined the diffusion coefficient of BTB (the blue basic form at pH 9) in 1, 2 and 3 weight% agarose gels. Glass capillaries (inner diameter 2.5 mm) filled with agarose gel were immersed with one end in large amount of BTB solution. This arrangement ensured a constant concentration at the boundary, and snapshots of the raising concentration profiles were taken at given time intervals. In the case of semi-finite diffusion, the concentration profile, as a function of the distance x from the boundary, is described by (R-S1) where concentrations have been expressed by absorbance values.

$$A(x,t) = A_0\left(1 - erf\,\frac{x}{\sqrt{4Dt}}\right) \qquad \text{(R-S1)}$$

$A(x)$ at a given time t was obtained as follows:

$$A(x) = -\lg\frac{I(x)}{T_{gel}\,I_0(x)} \qquad \text{(R-S2)}$$

where $I(x)$ and $I_0(x)$ are the measured light intensities through the capillary and in its close proximity, respectively, and T_{gel} is the transmittance of the gel without dye (89±1%). The samples were illuminated with orange light, and the green channel of the RGB images was used for the analysis. Only the range $A \leq 0.8$ was used for fitting (see Fig. S1) as I at darker (more concentrated) regions could not be determined with enough precision and the absorbance showed deviation from linearity with concentration.

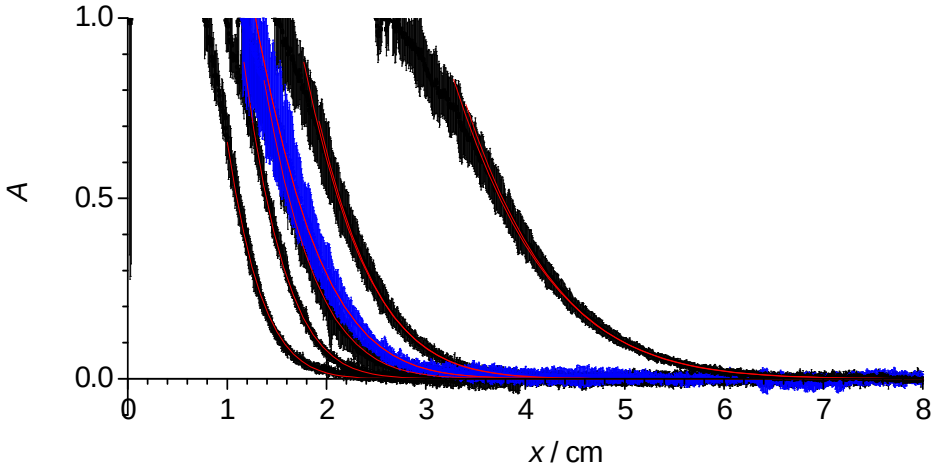


Figure S1 Diffusion of BTB in agarose gel. Fitted $A(x,t)$ curves at t = 16h, 24h, 40h, 48h, and 112h.

We obtained (4.09±0.58) × 10^{-6} cm^2s^{-1} for the diffusion coefficient of BTB in agarose gel which is clearly not lower than the literature values in aqueous solution. No detectable tendency was found between the D_{BTB} values in 1, 2 and 3 weight% agarose gels.

Polyacrylic acid

It is known that polyacids cannot be characterized by a single pK_a value because their acidity decreases with increasing charge density - thus they are more easily protonated at higher degrees of neutralization. We estimated the dependence of pK_a on pH by the linear relationship (R-S3) using the titration curve at ionic strength I=0.2 in Fig.1 and 3 in ref S4.

$$pK_a = 2.83 + 0.444\,pH \qquad \text{(R-S3)}$$

Calculation of the effective diffusion coefficient

According to the above considerations, we use the values in Table S1.

Table S1 Dissociation and diffusion coefficients, and concentrations of the low-mobility proton binding sites used in the calculations

	pK_a	D / cm^{2}s^{-1}	M / g mol^{-1}	HPSF	HPSC
agarose	3.00[a]	0.00	-	0.40 mM [b]	0.30 mM [b]
BTB	7.10	3.31×10^{-6} [c]	624	-	0.054–0.264 mM
BCP	6.25	3.35×10^{-6} [d]	540	0.080 mM	-
BCG	4.66	3.27×10^{-6} [d]	698	-	-
PAA	eq. R-S3	1.50×10^{-7} [e]	~15 000	1.0 mM [f]	0.43 mM [f]

[a] pK_a of *D*-glucuronic acid (upper limit)
[b] 1 weight% agarose (Fluka 05077) gel contains 0.2 mM acid functions
[c] interpolated between BCP and BCG according to the molar masses
[d] from ref. S2
[e] extrapolated value
[f] minimum concentration needed to obtain stationary patterns

The effective diffusion coefficient of protons was calculated by eq.(R-S4).

$$D_{H^+,eff} = \frac{D_{H^+} + D_{HInd}\frac{[HInd]}{[H^+]} + D_{HPAA}\frac{[HPAA]}{[H^+]}}{1 + \frac{[HAgr]}{[H^+]} + \frac{[HInd]}{[H^+]} + \frac{[HPAA]}{[H^+]}} \qquad \text{(R-S4)}$$

Here [HAgr], [HInd] and [HPAA] stand for the equilibrium concentration of the protonated form (functional group) of agarose, colour indicator and polyacrylate, respectively. The agarose term does not appear in the numerator because the diffusion coefficient of the acid functions on the agarose (HAgr) was taken as zero.

With this approximation we intend to describe the conditions in the M state when the HSO_3^- has already been consumed. In the F state, in the presence of large amount of SO_3^{2-}, the protons are mostly bond to this species so that $D_{H,eff} < D(HSO_3^-)$ is ensured.

Distribution of protons among the low-mobility buffers

First we calculated the distribution of protons between the different low-mobility proton binding sites as a function of pH (Fig. S2–S3). Here Σ[HX] stands for the sum $[H^+]+[HAgr]+[HInd]+[HPAA]$.

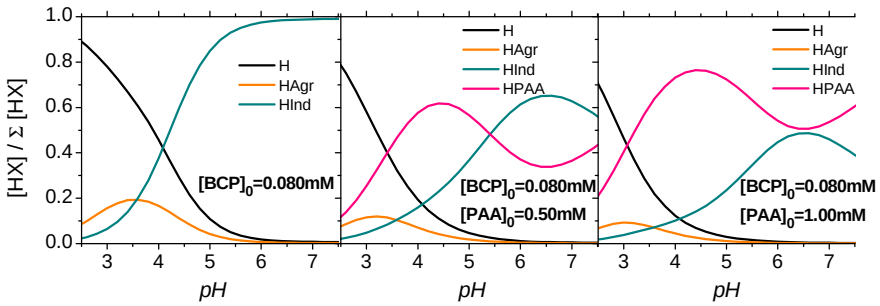


Figure S2 Distribution of protons between the low-mobility proton binding sites agarose, pH-indicator and polyacrylate in the HPSF reaction. $[agaropectine]_0 = 0.40$ mM.

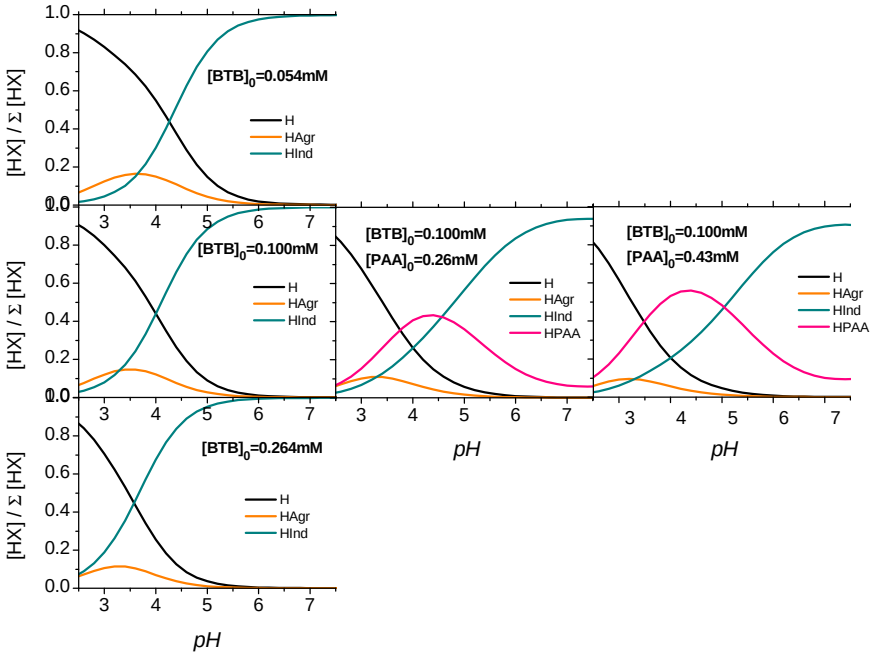


Figure S3 Distribution of protons between the low-mobility proton binding sites agarose, pH-indicator and polyacrylate in the HPSC reaction. $[\text{agaropectine}]_0 = 0.30$ mM.

In Fig. S2 the second extremum (a minimum) appears on the HPAA curve because PAA has increasing pK_a with pH, and finally becomes a weaker acid than BCP. Compared to BTB, PAA is a stronger acid through the whole pH range. Therefore, at higher pH in the HPSC system, protons are bound to BTB rather than to PAA, even if the concentration of PAA is much higher.

The effective diffusion coefficient of protons

Figures S4–S5 show the relative slowing down of protons. The same ratios hold even if the diffusion coefficient of the "free" protons is $D_{H,0} < 9.81\times10^{-5}$ cm^2s^{-1} as a consequence of *e.g.* the electrostatic field of other mobile ions. The introduction of the protonation of HCO_3^- in the calculation has no effect on $D_{H,eff}$ above pH 4.5. Curves for single species represent individual contributions, *i.e.* if that low-mobility agent was present alone in the system.

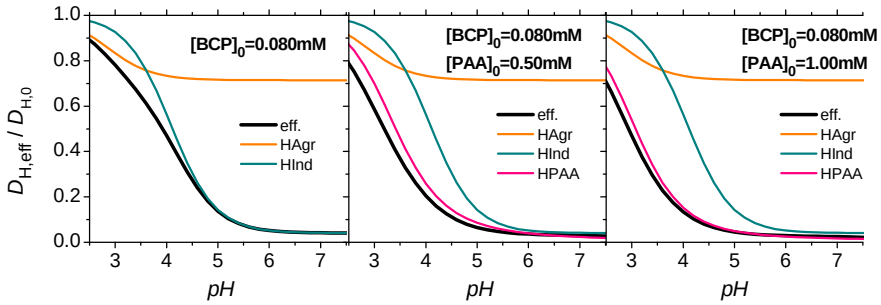


Figure S4 Relative effective diffusion coefficient of protons in the presence of the low-mobility proton binding sites in the HPSF reaction. $[\text{agaropectine}]_0 = 0.40$ mM.

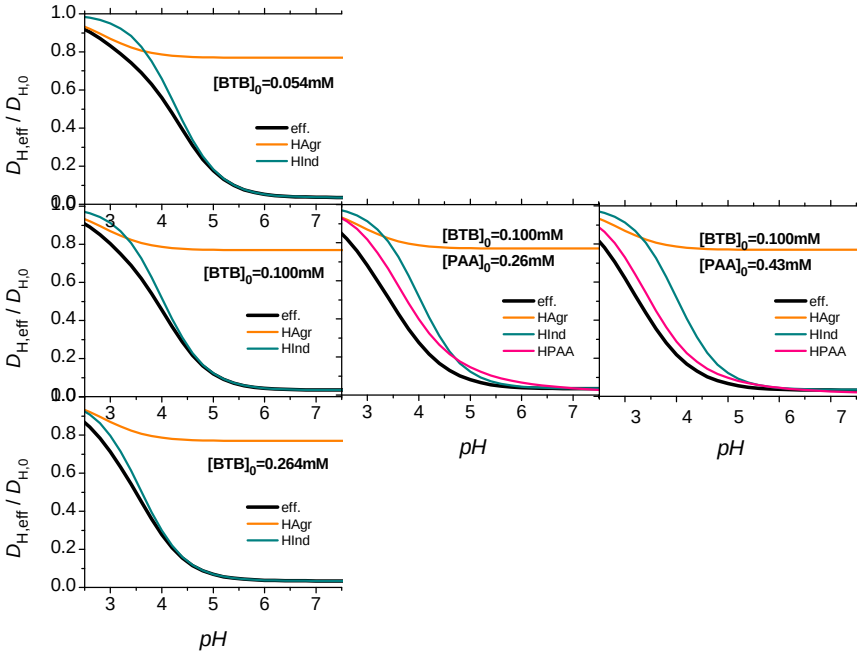


Figure S5 Relative effective diffusion coefficient of protons in the presence of the low-mobility proton binding sites in the HPSC reaction. $[\text{agaropectine}]_0 = 0.30$ mM.

Comparing the effect of the three low-mobility agents, at the concentrations that they are present in the three different patterning reactions, the following observations can be made about the contributions:

- The contribution of agarose can be neglected above pH 4.5. The immobile binding sites of the agarose gel, although almost 5 times more concentrated than $[\text{Ind}]_0$, contribute only below pH 4.5 because of their relatively strong acidity.

- BCP more effectively binds protons than the agaropectine sites and, despite its lower concentration, alone slows down the effective proton diffusion below 10% above pH 5.5.

- In the presence of the amounts of PAA used in the HPSF experiments, mainly the PAA determines the $D_{H,eff}$ curves, and, this way, the domain where $D_{H,eff} / D_{H,0} < 10\%$ can be extended down to pH 4.5–4.0.

Supplementary references

S1 *Schweizerisches Lebensmittelbuch, Chapter 40: Gelier- und Verdickungsmittel.* Eidgenössische Drucksachen- und Materialzentrale, Bern 1993.

S2 D. W. Armstrong, R. A. Menges and S. M. Han *J. Coll. Int. Sci.* 1988, **126**, 239–242.

S3 T. Eriksen, *Chem. Eng. Sci.* 1967, **22**, 727–736.

S4 T. Miyajima, M. Mori, S. I. Ishiguro, K. H. Chung and C. H. Moon *J. Coll. Int. Sci.* 1996, **184,** 279–288.