# Percolation Model for Enzyme Gel Degradation

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We study a model for the gel degradation by an enzyme, where the gel is schematized as a cubic lattice, and the enzyme as a random walker, that cuts the bonds over which it passes. The model undergoes a (reverse) percolation transition, which for low density of enzymes falls in a universality class different from random percolation. In particular, we have measured a gel fraction critical exponent $\beta = 1.0 \pm 0.1$, in excellent agreement with experiments made on the real system.

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The extracellular matrix (ECM) is a gel composed by various proteins, including collagen, elastin, fibronectin, and laminin, connected to form an elastic network that extends macroscopically. This gel is normally impermeable to cell passage, and ensures organ integrity by insulating organs and preventing cell dissemination. Moreover, it is the support of cell adhesion and regulates cell proliferation, differentiation, and locomotion. During specific processes, the ECM can be degraded by a variety of proteolytic enzymes, especially metalloproteinases, that catalyze the hydrolysis of the crosslinks between peptide chains constituting the ECM, increasing its permeability to the passage of cells. This degradation process can at some point solubilize the gel, realizing a reverse "gel-sol" transition, and bringing the ECM to a liquid state, in which cells are no longer confined and can freely diffuse. This solubilizing transition is especially connected with tumor invasiveness, in which some cells access the lymphatic and blood circulation, and disseminate to distant organs (metastasis). In this view, beyond the biochemical processes involved at molecular level, the understanding of the physical mechanisms of the ECM degradation is of great importance.

The passage from a liquid to a gel state is a critical phenomenon [1,2], in which soluble monomers bind to form larger and larger clusters. At some point, when the bond density $p$ becomes grater than some critical threshold $p_c$, an "infinite" cluster extending macroscopically is formed, and the network becomes a solid gel with an elastic response. The reverse transition, in which bonds are removed, and the system goes from a gel to a liquid state, can be clearly described in the same framework. The theory of critical phenomena predicts that, near the transition, the macroscopic quantities describing the system are related to the distance from the transition $(p - p_c)$ by power laws. The average cluster diameter diverges as $|p - p_c|^{-\nu}$, while the weight average mass as $|p - p_c|^{-\gamma}$. The viscosity diverges as $|p - p_c|^{-k}$ below the transition ($p < p_c$), and stays infinite above it, while the gel fraction (the density of the infinite cluster) and the elastic modulus, that are zero below the transition, grow above it, respectively, as $|p - p_c|^{\beta}$ and $|p - p_c|^{t}$. It is important to point out that exponents $\nu$, $\beta$, $\gamma$, etc. are universal, that is they do not depend on the microscopic details of the system, but only on characteristics like the dimensionality of the system, or whether or not there is a long range correlation in the distribution of the bonds.

Recently, a series of interesting experiments has been realized to study the *in vitro* degradation of protein gels by exogenous proteinases, under cell-free conditions [3,4]. In particular, in [3] it was shown that a gel-sol transition adequately describes the degradation of the gel. Two kinds of gel, fibronectin and ECM gel, and three kinds of enzyme, thermolysin, trypsin and proteinase K, were used in various combinations. An enzyme solution was added to a certain quantity of gel, and the solubilized fraction $X_{\mathrm{sol}}(t)$ of peptides was measured as a function of time. The gel-sol transition is reached at some time $t_c$, when $X_{\mathrm{sol}}(t)$ becomes equal to one, or when the gel fraction $X_{\mathrm{gel}}(t) = 1 - X_{\mathrm{sol}}(t)$ becomes zero. With various gel-enzyme combinations, and different enzyme concentrations, it was found that $X_{\mathrm{gel}} \propto |t - t_c|^{\beta}$, with $\beta \simeq 1$, for $t < t_c$. For ECM gel and trypsin for example $\beta = 1.01 \pm 0.03$ [3]. As the critical exponents are extracted from the behavior of the system near the transition, and being the density of bonds a regular function of time around $t_c$, we can make a Taylor expansion and take only the first order term, obtaining $(p - p_c) \propto (t - t_c)$ near the transition. Therefore $X_{\mathrm{gel}} \propto |p - p_c|^{\beta}$, with $\beta \simeq 1$, for $p > p_c$.

This result is quite unexpected, because sol-gel transition is usually well described by random percolation, which is obtained when each bond between two monomers is present with probability $p$, and there is no correlation between different bonds. Random percolation in three dimensions gives a critical exponent $\beta = 0.41$, very different from the one measured in the gel degradation experiments. In Ref. [3] different possible explanations of this discrepancy were proposed.

A possibility, in order to explain the change in the universality class with respect to random percolation, is the presence of a long range correlation in the distribution

 

of nonhydrolyzed bonds [4]. The correlation function is defined as $G(|\mathbf{r}|) = \langle \rho(\mathbf{r}')\rho(\mathbf{r}'+\mathbf{r})\rangle - \langle \rho(\mathbf{r}')\rangle^2$, where $\rho(\mathbf{r})$ is the density of bonds, and the average $\langle\cdot\rangle$ is done over the reference position $\mathbf{r}'$. It was shown by Weinrib and Halperin [5] that if the correlation obeys a power law $G(r) \simeq r^{-a}$ at long distances with $a < d$ (where $d$ is the dimensionality of the system), then the percolation transition falls in a universality class different from the random percolation, in particular, with a correlation length exponent $\nu = 2/a$.

In this Letter, we study a very simple model, which we call "pacman percolation model," in which the protein gel is schematized as a cubic lattice of $N = L^3$ sites, where each site represent an (exavalent) monomer. At time $t = 0$ all the bonds between nearest neighbor monomers are present. One or more enzymes are then introduced in the system in random initial positions. At each step, every enzyme moves from one site to a nearest neighbor site, chosen randomly between the six possible neighbors, and hydrolyzes (deletes) the corresponding bond if not yet hydrolyzed. Periodic boundary conditions are chosen. The site version of this model was studied, in a different context, in Ref. [6]. In Fig. 1(a) it is shown the two-dimensional version of the model, with only one enzyme in the system, after the enzyme has walked around for some time (roughly at the percolation threshold). Note how the remaining nonhydrolyzed bonds are spatially correlated, with respect to a random percolation model [Fig. 1(b)]. At each time step, there will be a distribution of clusters, where two sites belong to the same cluster if there is a path of nonhydrolyzed bond between them. We measure then, as a function of the density $p$ of bonds: a boolean variable equal to one if there is a percolating cluster, to zero otherwise; the size of the percolating cluster, if any; the mean cluster size, that is $\frac{1}{N}\sum_s n_s s^2$, where $n_s$ is the number of clusters of size $s$, and the percolating cluster is excluded from the sum.

We perform the simulation many times, with different starting points and realization of the paths of the enzymes, and average over all the runs the above mentioned quantities. In this way we expect that all the quantities will be translationally invariant [7]. We obtain the percolating probability $\Pi(p, L)$, the percolating cluster density $\rho(p, L)$, and the mean cluster size $\chi(p, L)$ as a function of the bond density and of the size $L$ of the lattice. From these quantities, it is possible to evaluate the percolation density $p_c$ and the critical exponents $\nu$, $\beta$ and $\gamma$ [1]. Plotting the percolating probability $\Pi(p, L)$ as a function of $p$ for different lattice sizes $L$, it is possible to measure the percolation threshold density $p_c$ as the point in which the different curves intersect, for $L \to \infty$. Plotting then $\Pi(p, L)$ as a function of $(p - p_c)L^{1/\nu}$, one can measure the correlation length exponent $\nu$ as the value that gives the best collapse of the curves. The error on the exponent can be defined by looking for the largest interval of $\nu$, such that the curves collapse within the error bars. In the same way, plotting $\rho(p, L)L^{\beta/\nu}$ and $\chi(p, L)L^{-\gamma/\nu}$ as a function of $(p - p_c)L^{1/\nu}$, one can measure the exponents $\beta$ and $\gamma$.

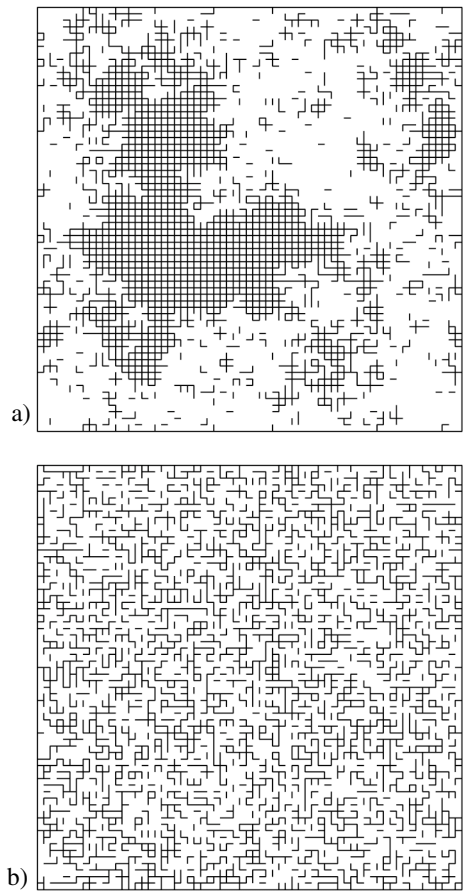


FIG. 1. (a) Pacman percolation model on a square lattice of size $64^2$, with a single enzyme and after 12 000 steps, when the density of nonhydrolyzed bonds is $p = 0.42$. (b) The random percolation model with the same bond density.

We have first studied the "single enzyme" version of the model on the cubic lattice. In Figs. 2(a) and 2(b) we show $\Pi(p, L)$ and $\rho(p, L)$ for lattices of size $30^3$, $40^3$, $50^3$, and $60^3$, together with the data collapses (insets). We find $p_c = 0.139 \pm 0.001$, $\nu = 1.8 \pm 0.1$, $\beta = 1.0 \pm 0.1$. Note that the exponent $\beta$ is in excellent agreement with the experimental results of Ref. [3]. We have also computed $\chi(p, L)$ (not shown) and extracted the exponent $\gamma$, finding $\gamma = 3.5 \pm 0.2$. The exponents $\nu$, $\beta$, and $\gamma$ satisfy well the hyperscaling relation $2\beta + \gamma = \nu d$, expected on general grounds [1]. In Table I the critical exponents found are compared with those of random percolation. These results show that the single enzyme version of the pacman percolation model falls in another universality class with respect to random percolation, which we call "pacman percolation universality class."

We have also verified the relation predicted by Weinrib and Halperin [5] between the exponent $\nu$ and the power

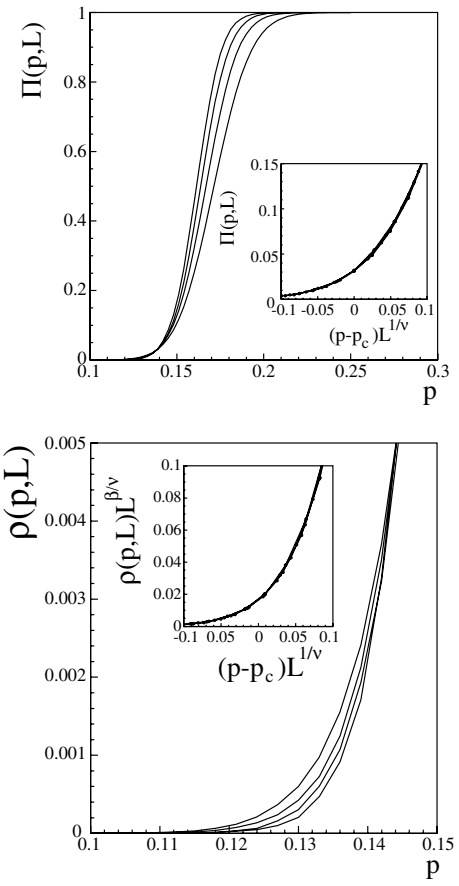


FIG. 2. (a) Percolation probability $\Pi(p, L)$ and (b) density $\rho(p, L)$ of the percolating cluster as a function of the bond density $p$, with a single enzyme and for cubic lattices of size $L = 30$, 40, 50, 60. Insets: data collapses obtained plotting $\Pi(p, L)$ and $\rho(p, L)L^{\beta/\nu}$ $(p - p_c)L^{1/\nu}$, with $\nu = 1.8$ and $\beta = 1.0$.

law governing the decay of correlations, in the single enzyme model. In Fig. 3 the correlation $G(|i - j|) = \langle n_i n_j \rangle - \langle n_i \rangle \langle n_j \rangle$ in the occupation of the bonds $i$ and $j$, where $|i - j|$ is the distance between the centers of the bonds, is shown for a system of size $100^3$ at the percolation threshold $p = 0.139$. The correlations obeys a power law $G(r) \sim r^{-a}$ with $a = 1.15 \pm 0.05$, with an exponential cutoff, presumably due to finite size effects, at distances larger than $r \simeq 30$. The relation $\nu = 2/a$ predicted by Weinrib and Halperin, is quite well verified within the errors. It has been recently argued [4] that for such a model the correlations between bonds should decay as $1/r$, implying $a = 1$ and $\nu = 2$. The prediction, however, is valid only if some conditions are verified, such as long times and large distances. The discrepancy between this prediction and our results may be due to the fact that these asymptotic regimes are not reached in our simulations.

TABLE I. Percolation density and critical indices in the pacman percolation model, and in random percolation, in three dimensions.

| | Pacman percolation | Experiment | Random percolation |
|---|---|---|---|
| $p_c$ | $0.139 \pm 0.001$ | | 0.2488 |
| $\nu$ | $1.8 \pm 0.1$ | | 0.88 |
| $\beta$ | $1.0 \pm 0.1$ | $1.0 \pm 0.1$ | 0.41 |
| $\gamma$ | $3.4 \pm 0.2$ | | 1.80 |
| $\mu$ | $3.5 \pm 0.1$ | | 2.0 |
| $s$ | $1.1 \pm 0.1$ | | 0.73 |

We have then studied the model with a uniform density $\rho_E$ of enzymes. A number $\rho_E L^3$ enzymes are distributed in random positions on the lattice, and at each step every enzyme makes one move. Critical exponents are extracted in the usual way. We found that, as long as the concentration is $\rho_E \leq 0.4$, the effective critical exponents measured are the same of the single enzyme model, that is those of the pacman percolation universality class. For $\rho_E \geq 0.8$ instead we measure exponents in agreement with random percolation. This is expected because, for very high density of enzymes, each bond is cut by a different enzyme, so that there are no correlations between bonds.

From a renormalization point of view, one may expect that the pacman percolation universality class should be relevant only for a density of enzymes vanishing in the thermodynamic limit, such as for the single enzyme model, while for any finite density of enzymes the model should fall in the random percolation universality class. On the other hand, from our results, it comes out that a slow crossover exists, such that for finite but low density of enzymes effective critical exponents are measured in the pacman percolation universality class.

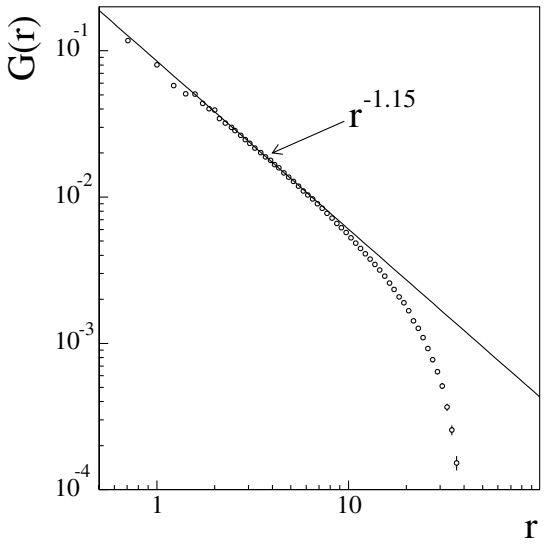


FIG. 3. Spatial correlation $G(r)$ in the occupation of the bonds, with a single enzyme on a cubic lattice of size $L = 100$, near the percolation threshold $p \simeq 0.14$.

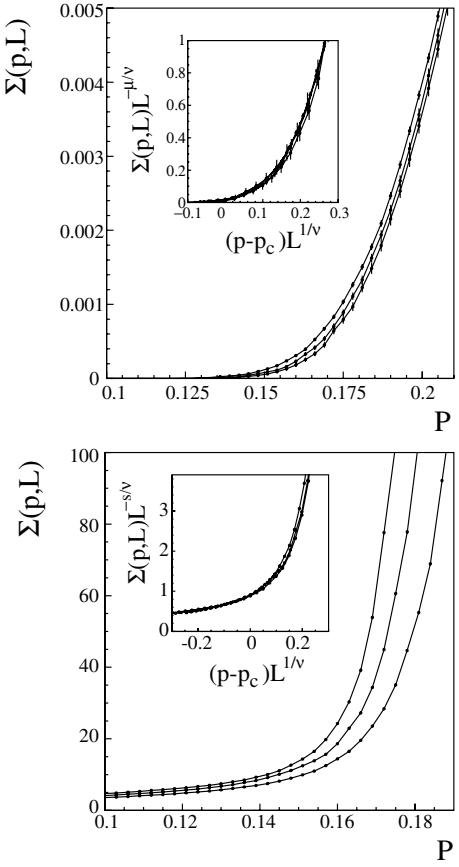


FIG. 4. Conductivity $\Sigma(p, L)$ of (a) the random-resistor network and (b) the conductor-superconductor network as a function of the bond density $p$, with a single enzyme and for the same lattice sizes of Fig. 2. Insets: data collapses obtained plotting $\Sigma(p, L)L^{\mu/\nu}$ versus $(p - p_c)L^{1/\nu}$, with $\nu = 1.8$, $\mu = 3.5$, and $s = 1.1$.

This may be true also for the experimental observations. To compare the results of the model with experiments, one has to express the density of enzymes in the same manner. This can be done expressing the experimental density as $(d_B/d_E)^3$, where $d_E$ is the mean distance between enzymes, and $d_B$ is the mean distance between the crosslinks of the network. The latter can be evaluated by $d_B \simeq (k_B T/G)^{1/3}$, where $G$ is the elastic modulus of the gel (before enzyme degradation), and $T$ the temperature. In the experiments of Ref. [3], $G \simeq 40$ Pa and $T \simeq 300K$, so that $d_B \simeq 40$ nm, while $d_E$ is between 70 and 400 nm. The experimental concentration corresponds therefore to an enzyme density between 0.001 and 0.2, in the density region where the effective critical exponents measured in the simulations are those of the pacman percolation universality class. This confirms the agreement of the value of the exponent $\beta$ measured in the simulations and in the experiments.

To complete our study, we have analyzed the critical exponent $\mu$ and $s$ of the conductivity in the random-resistor and conductor-superconductor networks, that should be in correspondence, respectively, with the exponents $t$ of the elastic modulus, and $k$ of the viscosity [8,9]. In the first case, each present bond of the model is substituted with a resistor of unitary conductance, while absent bonds have zero conductance. The total conductivity $\Sigma$ of the model is then measured as a function of bond density, and it is zero for $p < p_c$, while it grows as $|p - p_c|^{\mu}$ for $p > p_c$. Using finite size scaling as usual [see Fig. 4(a)] we find $\mu = 3.5 \pm 0.1$. In the second case each present bond of the model is substituted with a superconductor of infinite conductance, while absent bonds are substituted with resistors of unitary conductance. In this case the total conductivity $\Sigma$ diverges as $|p - p_c|^{s}$ for $p < p_c$, and stays infinite for $p > p_c$. In this case we find $s = 1.1 \pm 0.1$ [see Fig. 4(b)].

In conclusion, we have used a percolation model to study the degradation process of ECM due to the action of enzymes. Our results show that, for low density of enzymes, our model belongs to a different universality class from random percolation. The change in the critical indices may be due to long range correlation. If the density of enzymes is sufficiently high, the correlation between bonds disappears and there is a crossover to random percolation.

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