Fractal Dimension of Microbead Assemblies Used for Protein Detection

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We use fractal analysis to calculate the protein concentration in a rotating magnetic assembly of microbeads of size 1 μm, which has optimized parameters of sedimentation, binding sites and magnetic volume. We utilize the original Forrest–Witten method, but due to the relatively small number of bead particles, which is of the order of 500, we use a large number of origins and also a large number of algorithm iterations. We find a value of the fractal dimension in the range 1.70–1.90, as a function of the thrombin concentration, which plays the role of binding the microbeads together. This is in good agreement with previous results from magnetorotation studies. The calculation of the fractal dimension using multiple points of reference can be used for any assembly with a relatively small number of particles.

Rotating magnetic microbead assemblies have been recently used[1] as a signal transduction method for protein detection. It is important to examine in detail the shape of such assemblies, which can be used to determine the concentration of a protein, such as thrombin. Concerning biomarkers, such concentrations are in the femtomolar domain. One such method is to estimate the fractal dimension of the microbead assembly and its rotational period.[1] In that work the values of the fractal dimension of several such bead assemblies were calculated using the ImageJ plugin FracLac. Here we refine the calculation of the fractal dimension, by using a greater number of iterations in the algorithm, and using repeatedly a multiplicity of origins.[2] We also describe the stepwise method of calculating these values. The method presented here further supports the published technique.

The calculation of the fractal dimension has been very common for a large variety of systems, ranging practically in all sciences. See review references.[3–6] In order to calculate the fractal dimension of the particle assemblies we follow a standard technique originally proposed by Forrest and Witten.[2] This is done by monitoring the mass of particles present within boxes of progressively increasing size. Figure 1 shows a typical assembly of microbeads made of 1 μm magnetic beads, functionalized with thrombin specific aptamers, after incubation with 2.16 pM of thrombin. The thrombin attachment controls the geometry and fractal dimension of the assembly. As we see here the assembly is not symmetrical, and different realizations of the experiment may have different layouts for the spreading of particles, which is somewhat of a challenge to the determination of the fractal dimension. Thus, we need to first find the center of mass for each realization, by calculating the mean values of the x and y coordinates (x, y). We then construct a square of side $L = 10$, which is centered on (x, y) and we count the number of particles (M) that are contained in this square. We then increase L by 10 and we repeat the same procedure. We do this several times up to $L = 200$. We stop when $L = 200$ because we notice that at this point practically all particles are contained inside this square. Again we plot M vs. L in log-log axes and we find the best fit for the ensuing straight line. We note that when the calculation is based on a single snapshot of the lattice, it is amenable to large local fluctuations in the positions of the particles due to the relatively small number of particles in our samples, which is of the order of ~500 particles. To avoid such problems, we average out the locations of the particles by including a large number of different origins. We do this in the following manner: We take each particle to be the point of origin for the calculation of the fractal dimension and we apply the sandbox method[2]
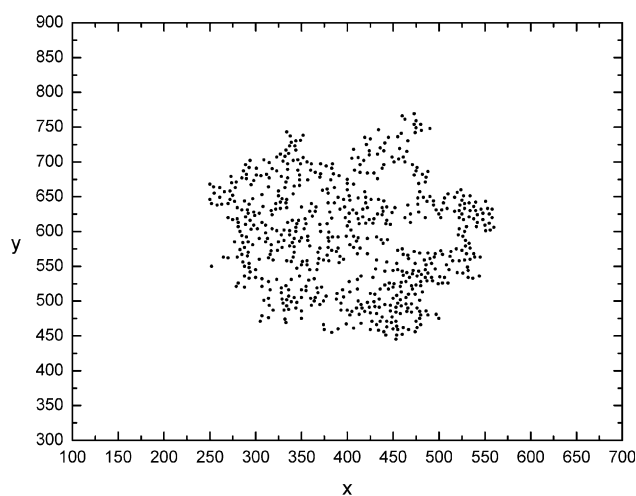
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Figure 1. A two-dimensional projection of a particle assembly for the 2.16 pM thrombin concentration.

for each case separately, except the particles that are too close to the periphery of the aggregation. We then average the results, thus taking into account almost all particles in the sample. The initial value of L is taken to be $L = 4$ and we go up to $L = 40$ with increment $\Delta L = 1$. We repeat the same procedure for 150–650 points of origin in each assembly (depending on how many points are too close to the periphery of the aggregation) and we calculate the mean value of mass for each L. In this method, while the values of L are confined to a short range (4 to 40), the averaging procedure smoothens out all fluctuations.

The data utilized consist of 37 points for each assembly, and are plotted on a double logarithmic plot (M vs. L). We find the best linear fit and then calculate the corresponding slopes, which present an estimate of the fractal dimension. The data were obtained from experimental images[1] and cover 34 different configurations of magnetic beads for 10 different thrombin concentrations. As was originally done,[1] we examine the de-

pendence of the fractal dimension of the assemblies on the thrombin concentration. A sample plot is given in Figure 2. Here we give three sets of data for three different concentrations, each set being an average of four different particle snapshots. The straight lines are linear fits, producing the slopes, as marked. We present the results for all concentrations in Table 1, and give the values of the derived fractal dimension for each concentration.

We now use two different methods for estimating the fractal dimension of the assemblies, for verification purposes. In the first method we use the box counting procedure,[7] and in the second we use the "correlation" fractal dimension estimation, which is a different kind of boxing based on the sum of the squared occupancies of the boxes for several lengths.[8] The results are given in Figure 3 and 4 respectively. In all cases, we used the samples with the largest number of particles. Obviously, for the calculation of the slope of the straight line section we excluded the section of several points parallel to the
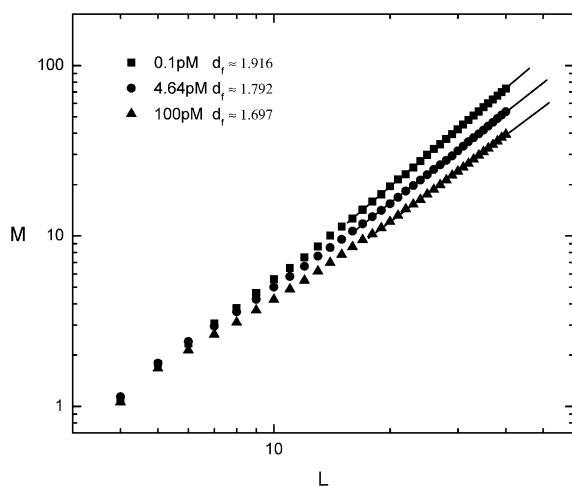

Figure 2. Number of particles M versus lattice size L; log–log plot for three different thrombin concentrations, giving three different fractal dimensions.
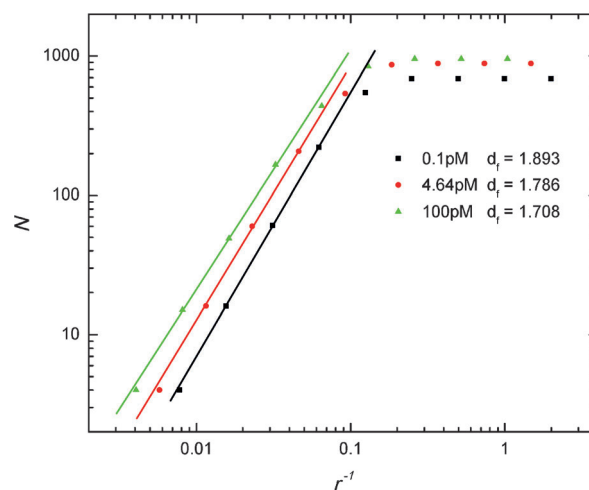

Figure 3. Minimum number of grid cells of length r required to cover the assembly (N) versus r^{-1}. The results were obtained using the box counting method.

Table 1. Dependence of fractal dimension on thrombin concentration. The values given are the mean values for each concentration. Note: the average difference between the original and new values is 1.4 %. Using a t-test for these two data sets, $p = 0.22$.

Thrombin concentration [pM]	Newly calculated fractal dimension[a]	Originally calculated fractal dimension[b]
0.100	1.900 +/− 0.034	1.917 +/− 0.011
0.216	1.880 +/− 0.021	1.904 +/− 0.003
0.464	1.889 +/− 0.023	1.901 +/− 0.020
1.000	1.860 +/− 0.034	1.831 +/− 0.030
2.160	1.817 +/− 0.009	1.761 +/− 0.022
4.640	1.775 +/− 0.028	1.720 +/− 0.013
10.00	1.771 +/− 0.019	1.725 +/− 0.016
21.60	1.687 +/− 0.011	1.685 +/− 0.033
46.40	1.706 +/− 0.047	1.706 +/− 0.017
100.0	1.704 +/− 0.028	1.699 +/− 0.007

[a] The fractal dimension calculated with the multiple origins method.
[b] The fractal dimension calculated with FracLac.
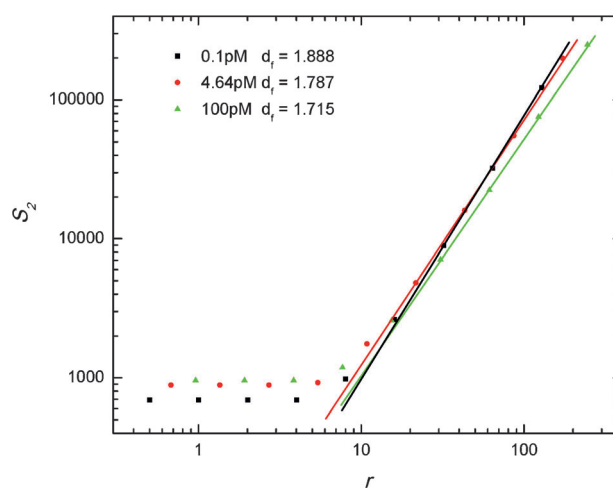
Figure 4. Sum of squared occupancies for a grid of scale r (S_2) versus r. The results were obtained using the method described in reference [8].
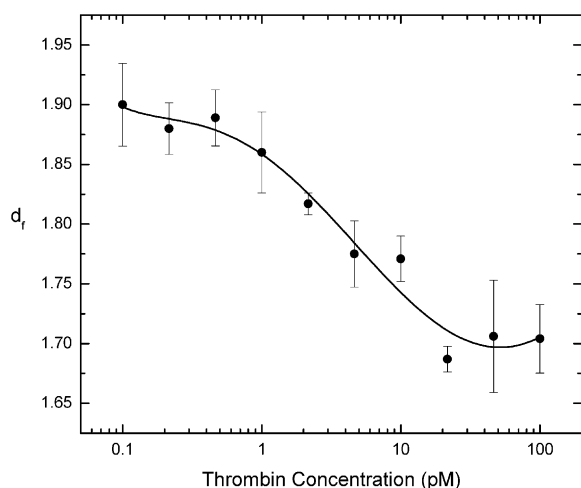
Figure 5. Plot of fractal dimension (d_f) versus thrombin concentration. The line is obtained by using the sigmoidal fit given by the Hill1 sigmoidal function of the Origin software, as follows: $y = y(\text{start}) + [y(\text{end}) - y(\text{start})]*x^n/(k^n + x^n)$, where $y(\text{start}) = 1.900$, $y(\text{end}) = 1.687$, with parameters $k = 3.241$ and $n = 1.224$.

x-axis, and from the remaining points only a few points are used for the calculation of the slope of the straight line section. The results of these two methods are in fairly good agreement with those of our earlier described method, even though they now contain more noise.

Our results agree very well with those originally calculated using the FracLac plugin.[1] Again the fractal dimension decreases from 1.90 to 1.70 as the thrombin concentration increases from 0.1 pM to 100 pM. The errors in the calculations are caused by three main factors: The limited number of points used for the calculation, the small number of configurations used (2–4 for each concentration), the large empty areas at the edges of many configurations that could not be avoided completely. This could be improved in future optimized work.

Figure 5 shows a plot of fractal dimension vs. thrombin concentration. It also shows that the majority of points fall close to the fitted sigmoid line. This is a good indication that the direct method we followed is sufficiently accurate for calculating the fractal dimension of such micro-assemblies.

Summarizing, we applied standard methodologies for estimating the fractal dimensions of non-random assemblies. These refer to our previously published experimental data of thrombin connected microbead assemblies. We find that the resulting values of the fractal dimension decrease monotonically with the thrombin concentration, and are within the range of 1.90 to 1.70. This is in excellent agreement with, and thus verifies previous results.

Acknowledgements

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