Computational Mesoscale Framework for Biological Clustering and Fractal Aggregation†

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Hierarchical clustering due to diffusion and reaction is a widespread occurrence in natural phenomena, displaying fractal behavior with non-integer size scaling. The study of this phenomenon has garnered interest in both biological systems such as morphogenesis and blood clotting, and synthetic systems such as colloids and polymers. The modeling of biological clustering can be difficult, as it can occur on a variety of scales and involve multiple mechanisms, necessitating the use of various methods to capture its behavior. Here, we propose a novel framework, the generalized-mesoscale-clustering (GMC), for the study of complex hierarchical clustering phenomena in biological systems. The GMC framework incorporates the effects of hydrodynamic interactions, bonding, and surface tension, and allows for the analysis of both static and dynamic states of cluster development. The framework is applied to a range of biological clustering mechanisms, with a focus on blood-related clustering from fibrin network formation to platelet aggregation. Our study highlights the importance of a comprehensive characterization of the structural properties of the cluster, including fractal dimension, pore-scale diffusion, initiation time, and consolidation time, in fully understanding the behavior of biological clustering systems. The GMC framework also provides the potential to investigate the temporal evolution and mechanical properties of the clusters by tracking bond density and including hydrodynamic interactions.

1 Introduction

Clustering, gelling, or coagulation are very common phenomena. Clusters are groupings of atoms, molecules, or ions that stick together due to a variety of physical-chemical interactions. They are important in physics, chemistry, biology (i.e. multicellular organisms, blood clotting, bronchi, nervous system morphogenesis, and tumor cells in cancer). Biological clustering is defined as the formation of higher-molecular-mass species as a result of the adhesion of smaller species. Different factors (e.g. species concentration, interactions, chain reactions, etc.) can affect the clustering process leading to specific biological responses or functionality. For blood coagulation, for example, key factors that govern the clustering process have been identified: i) the concentration of biological species; ii) stages of coagulation; iii) characteristic time scales; iv) activation and aggregation of the species; and v) complex interactions including adhesion or linking. The existence of this multiplicity of governing factors poses significant challenges when modeling coagulation. Typical approaches involve the construction of system-specific models targeting narrower aspects of the coagulation cascade. However, comprehensive clustering models suitable for a wide range of factors and non-system specific are still missing.

Cluster fractality, known as fractal dimension df, has developed as a significant research area offering valuable insights into the physical, chemical, and biological attributes of various systems. Computational studies in clustering have predominately centered on investigating the fractal characteristics of percolating clusters. Notable examples of these approaches encompass diffusion-limited-aggregation (DLA), ballistic aggregation (BA), and reaction-limited aggregation processes (RLA). Moreover, the exploration of relevant reaction-diffusion modeling packages has extended to biological systems in recent years, exemplified by tools like SMOLDYN and MesoDyn. Carlock et al. introduced a stochastic scheme that allows the formation of clusters with various morphologies and incorporates an effective interaction or aggregation range. Other numerical investigations using particle-based methods include Monte Carlo, Brownian dynamics, molecular dynamics simulation, and dissipative particle dynamics simulation. These methodologies have helped to reveal some of the fundamental aspects of

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clustering and showed the capability to reproduce diverse cluster fractality and morphology typically observed in aggregation processes. However, limitations on these approaches still exist since they target limited spatial-temporal scales (or even neglect temporal evolution), focusing mostly on steady-state features of the cluster. In general, due to the complexity of the dynamical process, a complete understanding of the factors that determine the evolution and steady state of the clusters remains hidden. Also, they consider a limited number of biomarkers for studying clusters. This issue can be exacerbated when only one descriptor (i.e. $d_f$) is used to rationalize the clustering. In the context of biological clustering, the number of studies that explore a range of particle interactions, initial conditions, system parameters, and spatial/temporal scales is still scarce.

We present a generalized mesoscale clustering (GMC) framework that integrates hydrodynamic interactions, permanent bonding, and surface tension effects to model clustering in biological systems. To address the limitations of current methods in modeling relevant spatial-temporal scales, we employ the smoothed dissipative particle dynamics (SDPD) method. The SDPD method discretizes the fluctuating Navier-Stokes equations and consistently satisfies the First and Second Laws of Thermodynamics and the Fluctuation-Dissipation Theorem. It has been used to study complex fluids, colloid-solvent interactions, colloidal interactions, and blood flow.

The proposed model, which relies on the modified SDPD method, is both versatile and adjustable, unveiling the correlation between physical model parameters and alterations in cluster properties. Furthermore, our method proves to be less computationally demanding than other methods, rendering it suitable for examining the growth kinetics of fractal clusters. We implement surface tension and inter-particle bonding in our SDPD method to activate particles, enabling us to generate clusters with a wide range of fractal dimensions, setting it apart from diffusion-limited aggregation (DLA), ballistic aggregation (BA), and reaction-limited aggregation (RLA) methods which have limited fractal dimension ranges. Our method involves particle bonding and activation exhibiting parallels with (RLA). However, we do not account for explicit reaction kinetics to define the bonding criteria or particle transformation. The potential extension of the proposed methodology to account for system-specific kinetics could be achieved by combining existing reaction-diffusion dynamics models. By manipulating the parameters that govern surface tension and bond formation in our SDPD method, we can modify the physical properties of the system and study the resulting effects on the formation and growth of fractal clusters. Our analysis goes beyond traditional approaches that rely solely on the fractal dimension as a cluster descriptor, and instead, we examine multiple biomarkers to achieve a more comprehensive understanding of clusters. Our results emphasize the critical influence of initial conditions and interparticle interactions on both static and dynamic cluster behavior.

We showcase the GMC framework using a variety of biological clustering mechanisms. To characterize the cluster’s evolution and steady state, we use biomarkers such as the number of bonds, fractal dimension ($d_f$), pore-scale diffusion ($D$), and time scales ($\tau_1$ and $\tau_2$) of initiation and consolidation. Unlike previous models that prescribe the specified fractal dimension values, our model allows for arbitrary values. Our results show that the use of multiple biomarkers is crucial for a correct characterization of the clusters, as aggregates with similar fractal dimensions may have different transport-related and mechanical properties. Our approach offers a unique and versatile way to study the growth and properties of clusters, with potential applications in various fields, including materials science, nanotechnology, and biophysics. The paper details the cluster formation mechanisms, numerical schemes of the GMC framework, and the biomarkers definition, followed by results and discussion.

## 2 Clustering System Definition

We define three stages as fundamental ingredients for a biological clustering process, activation, adhesion, and aggregation. Additionally, we consider that the systems are constituted by three different types of species: passive (P), active (A), and solvent (S). Each species is represented in our methodology as an individual type of particle. In Fig. 1, we could see the schematic of all these stages and particle types. Conceptually, this definition allows for the representation of clustering entities with different states of activity or reactivity. The change from passive to active states is referred to as activation stage. For the initial condition of our system, we place randomly passive particles and solvent particles. We also initiate the clustering of the particles by placing a single (A) particle in the center of the domain. The initial concentration (or volume fraction) of (P) particles is denoted as $\phi_{\text{int}} = (N_p/N_t)$, where $N_p$ and $N_t$ are the numbers of passive and total particles in the system, respectively.

### 2.1 Activation

Activation of the species in the biological clusters can be mediated by complex chemical interactions with smaller molecules and other aggregating species. Activation proceeds at various rates depending on the mechanism, producing either free active or clustered active species. The activation method, probability, and delay have a significant effect on the later growth of the cluster. Moreover, the activation can also determine the porosity, fractality, structure, and strength of the cluster. To model (P) particle activation, we proposed three schemes, denoted self-activation, or clustered active species. The activation method, probability, and delay have a significant effect on the later growth of the cluster. Moreover, the activation can also determine the porosity, fractality, structure, and strength of the cluster. To model (P) particle activation, we proposed three schemes, denoted self-activation, or clustered active species. The activation method, probability, and delay have a significant effect on the later growth of the cluster. Moreover, the activation can also determine the porosity, fractality, structure, and strength of the cluster.
In principle, the activation of a particle can be triggered as soon as the particle is located within the activation-cutoff radius. However, in many physical systems depending on biological conditions, the activation may not take place immediately but with a certain probability or frequency. This translates in an effective delay time (\( \tau_{\text{delay}} \)) between the activation-triggering mechanism and the other existent particle interactions. Unless otherwise stated, in the simulation results presented, \( \tau_{\text{delay}} = 0 \).

Since biological clustering via permanent and transient interactions are quite prevalent in nature. The proposed framework is equipped with energy potentials (see Simulation methods section) to account for both types of driving forces. Depending, on the particular field, permanent interactions are associated with the strong-chemical bond formation and are usually referred to as adhesion. Transient interactions, in contrast, are generally weaker and long-ranged associations that allow for relative particle rearrangements. We describe the modeling of permanent and transient associations in the following sections.

### 2.2 Cluster growth (Aggregation and Adhesion)

In the current study, we define the term aggregation to describe the affinity-driven association of affine particles in a phase separation process. In contrast, we use bonding to refer to the adhesion process made by the Morse-like bonding potentials. It’s important to note that the bonding terminology distinguishes it from the transient nature of affinity-based aggregation. We must note that despite that Morse potentials can account for bond-breaking processes, on the range of parameters investigated here, the established bonds do not break and can be considered permanent associations. Aggregation occurs because the unbalance in the affinity (interfacial tension) of the particles induces the formation of interfaces to minimize the energy of the system. To model the aggregation mechanism in our GMC, we use pair-wise forces that despite that Morse potentials can account for bond-breaking interactions are associated with the strong-chemical bond formation and are usually referred to as adhesion. Transient interactions, in contrast, are generally weaker and long-ranged associations that allow for relative particle rearrangements. We describe the modeling of permanent and transient associations in the following sections.
and consolidation are consecutive stages. A passive (P) particle undergoes initiation by bonding to \( n \) active particles, at which point it becomes active and can undergo consolidation, bonding with up to other \( m \) active particles.

In general, since the connectivity of the network can keep evolving during the consolidation stage, here we define the gelling time \( \tau_G \) as the condition where the rate of the bond formation reaches a steady condition. For practical purposes, in our simulations, we consider that steady condition when the change in the number of bonds between \( 10^4 \) time steps is lower than 0.1%. Therefore, the consolidation stage can in principle continue after gelling occurs. Similar descriptions of \( \tau_G \) can be found in biological clustering where the complete network is not established at the gel point, and new fibers and branch points can form afterward [3].

During both the initiation and consolidation stages, the formed bonds are permanent and possess a constant rigidity. Additionally, there is no distinction between the different types of bonds (bond potential and parameters) formed during these two steps.

For physical systems, the degree of overlapping between stages can be a characteristic indication of the reactivity of (P) particles, and the mechanical evolution of the cluster.

### 2.3 Cluster formation mechanisms

To facilitate the discussion of the results for the different clustering schemes, we introduce the following notation. Initiation and consolidation stages of adhesion are described in terms of the driving force used, bonding \( B(k) \), and aggregation is described as \( A \), where \( k \) denotes the number of bonds threshold for the stage. Thus, a stage where bonding and aggregation occur at the same stage is expressed as \( B(n)A \), whereas a stage with only bonding can be simply represented with \( B(n) \). The whole clustering mechanism can be written in compact notation as \( B(n)A_B(m)A \). Here, \( n \) and \( m \) correspond to the threshold number of bonds in the initiation and consolidation stages, respectively. Given the generality of the framework and the possibility to explore different parameters and cluster mechanisms (type of activation, stages with a varied number of bonds, or use of aggregation), the number of possible combinations to showcase the GMC is significantly large.

We introduce a generalized nodes diagram for the clustering formation process in Fig. 2 depicts the whole path for mechanisms of (a) and (f) at \( \phi_{act} = 30\% \). In this diagram, a series of vertical parallel lines are used to indicate relevant factors such as (P) particle concentration, activation mechanism, bonding number, and aggregation for the different stages. Discrete nodes along each factor indicate the specific value adopted, and the path connecting those nodes fully describes the clustering process.

For simplicity, we will streamline the presentation of the GMC framework and the cluster characterization using 7 representative mechanisms. These mechanisms are chosen to illustrate different aspects of the methodology proposed and highlight the need for complementary biomarkers for cluster analysis. These mechanisms are denoted as:

- a) Proximity Activated (\( Pr \))
- b) Proximity Activated-Aggregation (\( Pr_A \))
- c) Bonding Activation (\( B(3)_B(0) \))
- d) Bonding Activation (\( B(3)_B(2) \))
- e) Bonding Activation (\( B(3)_B(10) \))
- f) Bonding Activation (\( B(10)_B(10) \))
- g) Aggregation-Delay Bonding Activation (\( B(3)_A_B(0) \))

We use "Pr" to denote proximity-activated, whereas "A" indicates the aggregation mediated by larger surface tension between (P) and (S) particles. In SI Fig. S2 we present the corresponding paths for all the mechanisms (a)–(g). Fractal dimension and pore-scale transport analysis are performed for mechanisms (a)–(f), whereas mechanism (g) is defined to explore activation-delay time (\( \tau_{delay} \)). The delay time, denoted as \( \tau_{delay} \), represents the time interval between the triggering of one interaction (e.g., aggregation) and the occurrence of another interaction (e.g., bonding). This delay could result from various factors, such as biological conditions, where reactive time scales are significantly larger, or critical recruitment of constituents is required before adhesion occurs. The sequence of events we described in this type of mechanism (\( B(n)A_B(m) \)) is summarized as follows:

i) Aggregation: Particles come together within the activation-cutoff radius and form aggregates via reversible associations. As the particles aggregate, the bond formation does not occur; ii) Delay time: The simulation proceeds mediated only by aggregation (A) during \( \tau_{delay} \); iii) Bonding: After \( \tau_{delay} \), the bonding conditions are evaluated, and permanent associations take place. To this end, different \( \tau_{delay} \) between bonding events and aggregation are explored. To highlight the pertinence and applicability of the investigated mechanisms,
in Table 1 we give a breakdown of relevant biological examples that are consistent with them.

Table 1 Examples of Different Mechanisms in Biological Processes

<table>
<thead>
<tr>
<th>Different Mechanisms</th>
<th>Physical Examples</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximity Activation</td>
<td>Embryonic development,</td>
<td>[44,55]</td>
</tr>
<tr>
<td></td>
<td>Quorum sensing in bacteria</td>
<td></td>
</tr>
<tr>
<td>Aggregation</td>
<td>Cellular and protein aggregation processes</td>
<td>[56]</td>
</tr>
<tr>
<td>Bonding Activation</td>
<td>Enzymatic Reactions,</td>
<td>[57,58]</td>
</tr>
<tr>
<td></td>
<td>Ligand-receptor interactions</td>
<td></td>
</tr>
<tr>
<td>Aggregation-Bonding</td>
<td>Formation of blood clots,</td>
<td>[50,59,60]</td>
</tr>
<tr>
<td></td>
<td>Formation of amyloid fibrils in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>neurodegenerative diseases</td>
<td></td>
</tr>
<tr>
<td>Bonding Activation-Consolidation</td>
<td>Neural network formation,</td>
<td>[61,62]</td>
</tr>
<tr>
<td></td>
<td>Formation of multicellular</td>
<td></td>
</tr>
<tr>
<td></td>
<td>structures in tissue development,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibrin–thrombin gel</td>
<td></td>
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</tbody>
</table>

In the results section, we investigate how the morphology and other properties of the clusters are determined by different factors that define the system. The factors of interest are: (i) the initial concentration \( \phi_{\text{int}} \) of \( \mathbf{P} \), (ii) the maximum number of bonds \( n \) and \( m \) (during initiation and consolidation), and (iii) the balance between aggregation and adhesion (bonding) phenomena. To explore (i), we focus on medium to large concentrations relevant to many biological applications. In particular, we simulate the range \( \phi_{\text{int}} = [20\%, 30\%, 40\%, 50\%, 60\%, 70\%] \). Regarding (ii), the maximum number of \( n \) and \( m \) bonds are crucial for the connectivity and form of the clusters. Factor (iii), is relevant in the clustering process where the strength of the aggregation and adhesion can vary dynamically depending on physiological conditions.

3 Simulation methods

We now describe the mathematical models that describe the hydrodynamic interactions, aggregation, and adhesion, between the different \( \mathbf{P} \), \( \mathbf{A} \), and \( \mathbf{S} \) particles.

3.1 Mathematical model for hydrodynamic interactions

We describe the mass and momentum balance of our system in terms of the Navier-Stokes [NS] equations. In a Lagrangian reference frame, the mass balance reads \( d\rho / dt = -\rho \nabla \cdot \mathbf{v} \) and the momentum equation is given by

\[
\rho \frac{d\mathbf{v}}{dt} + \nabla p - \eta \nabla^2 \mathbf{v} - (\zeta + \eta / D) \nabla \cdot \mathbf{v} = \mathbf{f}^{\text{external}} \quad \text{for } D = 2, 3
\]

where \( \rho \) is the mass density, \( \mathbf{v} \) is the velocity \( p \) is the pressure, \( \eta \) and \( \zeta \) are the standard shear and bulk viscosities. We discretize the system as a set of \( N \) particles with a volume \( V_i \), such that \( 1/V_i = d_i = \sum_j W(r_{ij}, h) \), being \( d_i \) the density number of particles, \( r_{ij} = |\mathbf{r}_i - \mathbf{r}_j| \), and \( W(r_{ij}, h) \) an interpolating kernel with finite support \( h \) and normalized to one. To discretize the NS equations we also define the positive function \( F_{ij} = -\nabla W(r_{ij}, h) / r_{ij} \). We have the mass and momentum balance using the SDPD method such that consistent thermal fluctuations are incorporated. Using this description, we can model the transport of the different particles in the systems at mesoscales. The equations for the position of the particle is \( d\mathbf{r}_i / dt = \mathbf{v}_i \), whereas the stochastic differential equation of the momentum, can be expressed as

\[
\frac{d\mathbf{v}_i}{dt} = -\frac{1}{\rho_i} \sum_j \left( \frac{p_i}{d_i^2} + \frac{p_j}{d_j^2} \right) F_{ij} + \sum_j \left( \frac{a v_{ij} + b (v_{ij} \cdot \mathbf{e}_{ij}) \mathbf{e}_{ij}}{d_i d_j} \right) F_{ij} + \frac{1}{D} \sum_{k \neq j} (\mathbf{r}_{kj} - \mathbf{r}_{ij}) \cdot \mathbf{e}_{ij}.
\]

where \( v_{ij} = v_i - v_j \), \( e_{ij} = r_{ij} / |r_{ij}| \), \( a \) and \( b \) are friction coefficients related to the shear \( \eta \) and bulk \( \zeta \) viscosities of the fluid through \( a = (D + 2)/\eta - \zeta \) and \( b = (D + 2)(\zeta + \eta / D) \). \( D \) is the dimension of the system. The last term in equation (2), consistently incorporates thermal fluctuations in the momentum balance. A complete description of the terms \( A_{ij}, B_{ij} \), the equation of state used to define the pressure \( p \), and the form of the function \( F_{ij} \) are given in SI Eqs. (S1-S5).

3.2 Mathematical models of aggregation

We incorporate the interfacial tension effects between the different types of particles \( \mathbf{P}, \mathbf{A}, \) and \( \mathbf{S} \) by including in the momentum equation (2) an additional pair-wise force \( F^{\text{int}} \) such that \( F^{\text{int}}_{ij} = -s_{ll} \Phi(r_{ij}) / r_{ij} \) where \( s_{ll} = \begin{cases} \tilde{s}_{kl}, & \text{if } r_i \in \Omega_k \text{ and } r_j \in \Omega_l, \\ \tilde{s}_{kl}, & \text{if } r_i \in \Omega_k \text{ and } r_j \in \Omega_l, \\ \tilde{s}_{ll}, & \text{if } r_i \in \Omega_l \text{ and } r_j \in \Omega_l, \end{cases} \)

where \( k, l = \mathbf{P}, \mathbf{A}, \mathbf{S}, \) and \( \Phi(r_{ij}) \) is a shape factor given by \( \Phi = r_{ij} \left[ -G e^{-\frac{r_{ij}^2}{2\sigma^2}} + e^{-\frac{r_{ij}^2}{2\tilde{a}_{kl}^2}} \right] \), where \( G = 2^{D+1} \), being \( D \) the dimension. The range for repulsive and attractive interactions is defined as \( 2r_a = r_b = \rho_n^{1/D} \), such that a relatively uniform particle distribution is obtained for a given surface tension \( \sigma \). The interaction parameters satisfy \( \tilde{s}_{kl} = s_{ii} = 10^5 \tilde{s}_{kl} \), and the magnitude can be obtained from the surface tension and Particle density of the system as \( \rho_n \)

\[
s_{ll} = \frac{1}{2(1-10^{-3})} \rho_n^2 \frac{\sigma}{|4-D|^{-1} [(4-D)]^{1/4-D} (-G r_a^{D+3} + r_b^{D+3})}
\]

In SI Table. S2 displays the value relating to surface tension \( \sigma \), used in our simulations.

3.3 Mathematical models of adhesion

The mathematical model of adhesion in this work is modeled by Morse bonding potentials as this equation, \( U_{\text{adh}} = D_{\text{Morse}} [1 - e^{-a(r_{ij} - r_{adh})}]^2 \) (6). Among the several molecular potentials, the Morse potential is an ideal and typical anharmonic potential, leading to an adhesion force \( f_{\text{adh}} = -\partial U_{\text{adh}} / \partial r_{ij} = 2D_{\text{Morse}} a e^{-a(r_{ij} - r_{adh})} - e^{-2a(r_{ij} - r_{adh})} \) (7). The characteristic parameters of the Morse potential are shown in SI Table. S2.
3.4 Simulation details

We focus our investigations on two-dimensional systems and consequently reported clusters with characteristic $d_f$ ranging from 1.4±0.01 to 2±0.01. We use a 2D periodic box for simulations. The characteristic parameters of the SDPD method are shown in the SI Table. S1. In general, the selection of a suitable time step size for cluster formation depends on the balance between the activation, $\tau_{act}$, and diffusion, $\tau_{diff}$, time scales. In SDPD, the transport properties of the fluid and temperature define $\tau_{diff}$, and the numerical stability of the momentum equation sets the upper bound for the time step size. Here, we adopt this numerical stability condition to define the time step size. Since the bond formation events are allowed to occur at every time step with a probability of one, the investigated systems exhibit activation time scales in the same order as the diffusional ones $\tau_{act} \leq \tau_{diff}$. For specific systems with $\tau_{act} \ll \tau_{diff}$, a smaller time step size can be adapted to capture the proper activation dynamics. In contrast, for systems with $\tau_{act} \gg \tau_{diff}$ the rate of activation can be reduced by decreasing the bonding probability or the frequency. Investigations for these particular cases are out of the scope of this study and would require specific calibration for real biological systems. To account for the influence of thermal noise and the intrinsic randomness of the clustering process, all the simulations are conducted over ten randomly generated particle positions and velocities, in addition, we computed the standard deviation of all reported results. All the simulations are conducted using a modified version of the open-source software LAMMPS.""
light the main findings. An extended breakdown of the structures obtained and their biomarkers for the whole range of systems are included in the Supplementary Information.

4.1 Effect of concentration and mechanism type on $d_f$

In this section, we compute the value of $d_f$ for various initial concentrations of (P) particles, $\phi_{int}$, and mechanism types. SI Table. S3, summarizes the $d_f$ values, with a maximum standard deviation of ±0.01, for initial concentrations of $\phi_{int} = 30\%, 40\%, 50\%$ and the six mechanisms evaluated. Fig. 3 illustrates the morphology of $\phi_{int} = 40\%$ for different mechanisms, while SI Figs. (S3, S4) for higher and lower concentrations.

In general, we observe that the interplay between passive particle transport, bonding, and agglomeration plays a crucial role in determining the magnitude and sensitivity of $d_f$. We found that $d_f$ increases with the concentration of (P) particles, $\phi_{int}$. For concentrations greater than 50%, $d_f \sim 2$ (indicating space-filling in 2D), and the magnitude of $d_f$ becomes insensitive to variations in clustering mechanisms. This is because the probability for particle encounters scales with the concentration, leading to the rapid formation of clusters that occupy the entire space and reducing the influence of particle-transport effects. The increase in $d_f$ with concentration is attributed to the ability of more particles to diffuse into the internal regions of the forming aggregate before being irreversibly bound and the ability of aggregates to interpenetrate before bonding occurs.

In contrast, at lower concentrations (Fig. 3), both particle-transport and particle interaction effects are relevant in shaping the diversity of $d_f$. Lower values of $d_f$ are observed as the number of bonds increases. Additionally, this reduction is significantly sensitive to bonding during the consolidation stage. Bonding-mediated activation results in the preliminary formation of highly-branched clusters with closed pores. However, as activation proceeds, particle mobility is reduced, limiting their ability to penetrate the internal regions of a growing cluster. As a result, the sensitivity of $d_f$ with the number of bonds ($n$) in the activation stage exhibits a threshold, beyond which further increase in $n$ does not affect the fractality of the sample (see Fig. 3 for mechanisms $a$ and $f$). Further reduction in $d_f$ can be achieved in clustering processes that exhibit a consolidation stage. The reduced mobility due to activation is overcome by local rearrangements of the particles, leading to dense clusters with large closed pores. However, we must note that the sensitivity of $d_f$ with the number of bonds ($m$) during the consolidation stage is limited by steric hindrance.

4.2 Effect of concentration and mechanism type on MSD of (S) particles

This section computes MSD values for various initial concentrations of (P) particles, $\phi_{int}$, and mechanism type. To compute the MSD of (S) particles, we first freeze the cluster particles or (A) particles while freely moving (S) particles for $10^6$ time steps (a long enough period of time). (S) particles do not penetrate the clusters but only diffuse in the existent pores. MSDs are computed as an ensemble average over solvent particles.

4.2.1 Effect of concentration on anomalous diffusion exponent, $\alpha$ and $D_w$

Fig. 4a shows the mean time-averaged MSD of solvent as a function of lag time (log-log scale) for $\phi_{int} = 20\%, 30\%, 40\%, 50\%, 60\%$ and mechanism $c$ ($B(3) \_B(0)$). For comparison, we include the corresponding MSD for pure solvent particles (unconfined). In Fig. 4, normal diffusion for pure fluid ($\phi = 0\%$) exhibit a characteristic slope, $\alpha \sim 1$, whereas for anomalous diffusion $\alpha \leq 1$. In general, we observe that anomalous diffusion emerges at short-to-intermediate time scales, while at longer time scales normal diffusion is restored, reaching the limiting value $D_w$. The transition from anomalous to normal diffusion for each system occurs at the characteristic crossover time $t_{cR2}$.

As expected, we identify that $\alpha_c$, $t_cR2$, and $D_w$ are all functions of cluster concentration. In Table 2, we give a breakdown of the dimensionless diffusion coefficient ($D = D_w/D_0$) and $\alpha$ along with $d_f$ for different concentrations and mechanisms. Where $D_0$ is the diffusion coefficient of the pure solvent. The deviation of MSD from normal diffusion grows as cluster concentration increases, indicating a consistent deviation from Fick’s normal diffusion towards a sub-diffusion. The inside plot in Fig. 4a illustrates the decay in the anomalous diffusion exponent ($\alpha$) as the volume fraction of the cluster increases for mechanism $c$. A similar trend has been identified by previous studies on percolating gels.

Over the range of concentrations investigated (0.0-0.6), we find that the steep decay of $\alpha$ (1 to $\sim 0.6$) is consistent with colloidal-type gels in contrast to polymer gels that exhibit a smaller decay (1 to $\sim 0.9$). We must note, however, that our results correspond to two-dimensional simulations that can significantly preclude the motion of the solvent, and lead to a stronger decay in $\alpha$. Overall, we find that as the concentration increases, the crossover time increases (see solid markers in Fig. 4), whereas $\alpha$ and $D_w$ decreases. The subdiffusive behavior observed is due to the dynamics of particles trapped inside the pores. At high concentrations, a significant portion of particles is confined in closed pores, resulting in restrictions on their motion and oscillations of individual parti-
Table 2 Dimensionless diffusion coefficient ($\bar{D} = D_{\infty}/D_0$) and anomalous $\alpha$ for different initial concentration of (P) particles, $\phi_{int}$ and different mechanisms. The standard deviation of $\alpha$, $\bar{D}$ and $d_f$ are equal to $\pm 0.002$, $\pm 0.004$, and $\pm 0.01$ respectively (maximum values for all mechanisms and concentrations).

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>$\phi_{int} = 30%$</th>
<th>$\phi_{int} = 40%$</th>
<th>$\phi_{int} = 50%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>$D = 0.642 , D = 0.361 , d_f = 1.84$</td>
<td>$\alpha = 0.581 , D = 0.292 , d_f = 1.92$</td>
<td>$\alpha = 0.522 , D = 0.253 , d_f = 1.98$</td>
</tr>
<tr>
<td>$b$</td>
<td>$D = 0.653 , D = 0.448 , d_f = 1.81$</td>
<td>$\alpha = 0.597 , D = 0.312 , d_f = 1.90$</td>
<td>$\alpha = 0.531 , D = 0.270 , d_f = 1.96$</td>
</tr>
<tr>
<td>$c$</td>
<td>$D = 0.704 , D = 0.553 , d_f = 1.65$</td>
<td>$\alpha = 0.649 , D = 0.426 , d_f = 1.77$</td>
<td>$\alpha = 0.591 , D = 0.325 , d_f = 1.87$</td>
</tr>
<tr>
<td>$d$</td>
<td>$D = 0.740 , D = 0.627 , d_f = 1.48$</td>
<td>$\alpha = 0.685 , D = 0.551 , d_f = 1.71$</td>
<td>$\alpha = 0.653 , D = 0.508 , d_f = 1.82$</td>
</tr>
<tr>
<td>$e$</td>
<td>$D = 0.794 , D = 0.781 , d_f = 1.43$</td>
<td>$\alpha = 0.757 , D = 0.680 , d_f = 1.56$</td>
<td>$\alpha = 0.697 , D = 0.624 , d_f = 1.75$</td>
</tr>
<tr>
<td>$f$</td>
<td>$D = 0.795 , D = 0.795 , d_f = 1.43$</td>
<td>$\alpha = 0.752 , D = 0.692 , d_f = 1.56$</td>
<td>$\alpha = 0.713 , D = 0.631 , d_f = 1.75$</td>
</tr>
</tbody>
</table>

Fig. 4 a) Mean time-averaged MSD of solvent as a function of lag time (log-log scale) for $\phi_{int} = 30\%$, 40\%, 50\%, along with pure fluid. Solid markers indicate the range of anomalous diffusion. The inside plot shows the anomalous diffusion exponent ($\alpha$) as the concentration of the cluster increases for mechanism $c$. b) Mean time-averaged MSD of solvent as a function of lag time (log-log scale) for $\phi_{int} = 50\%$, mechanisms $a,b,c,d,e$ and $f$ along with pure fluid. Final cluster morphology is shown for each curve.

4.2.2 Effect of mechanism type on anomalous diffusion exponent, $\alpha$ and $D_{\infty}$.

Fig. 4b shows the mean time-averaged MSD of solvent as a function of lag time (log-log scale) for $\phi_{int} = 50\%$ and six mechanisms to evidence how aggregation, bonding in both stages of initiation and consolidation affect the magnitude and sensitivity of MSD values. In general, we find that $\alpha$, $D_{\infty}$, and $t_{CR2}$ are highly sensitive to the activation type. Values of $\alpha$ and $D_{\infty}$ for Proximity Activated (a) and Proximity Activated-Aggregation (b) mechanisms, are the lowest among all mechanisms, whereas the $t_{CR2}$ value is the highest. These mechanisms are responsible for percolated clusters with reduced pore size. In contrast, when activation occurs via bonding mechanism (c,d), branched clusters with larger pore sizes lead to larger values of $\alpha$ and $D_{\infty}$. Further increase of $\alpha$ and $D_{\infty}$ is identified for the clustering mechanism that involves a consolidation stage (e,f), inducing the local rearrangement of the cluster particles leading to branched and more compact structures with increased pore size.

Our results demonstrate the impact of aggregation and bonding mechanisms on particle transport in (S). Different cluster morphologies result in varying subdiffusive motions. To further understand these findings, we compare $d_f$ values for different cases in Table 2 and observe how some cases have similar $d_f$ but different $\bar{D}$ and $\alpha$ values. For example, mechanism $f$ at $\phi_{int} = 50\%$ has the corresponding values of $\alpha = 0.713 , D = 0.631 , d_f = 1.75$ and mechanism $c$ at $\phi_{int} = 40\%$ has $\alpha = 0.649 , D = 0.426 , d_f = 1.77$. They show almost equal fractal dimensions while the values of $\alpha$ and $\bar{D}$ are significantly different. This highlights the limitations of using $d_f$ as the sole criterion for clustering analysis. We suggest that a combined analysis of $d_f$ and MSD of the percolating fluid (or probes) provides a comprehensive characterization of the cluster. Furthermore, different MSD-related parameters such...
as $\alpha$, $D_{\omega}$, and $\kappa_{CR}$ offer important insights into biological systems, which are not revealed by $d_I$ alone. These parameters have been extensively studied in relation to biological systems.

### 4.3 Effect of concentration and mechanism types on initiation time, $\tau_i$ and gelling time, $\tau_G$

In Table 3 we summarize the measured dimensionless gelling time ($\tau_G$) and dimensionless initiation time ($\tau_i$) for various concentrations and mechanisms. To make the time scales dimensionless, we normalize $\tau_i$ and $\tau_G$ using the diffusive time ($\tau_{diff}$) as $\tilde{\tau}_i = \tau_i/\tau_{diff}$ and $\tilde{\tau}_G = \tau_G/\tau_{diff}$. The diffusive time is calculated as $\tau_{diff} = (h)^2/D_0$, where $h$ is the kernel cutoff radius, which approximates the size of the SDPD fluid particle and $D_0$ is the diffusion coefficient of the pure fluid.

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>Concentrations</th>
<th>$\phi_{out} = 30%$</th>
<th>$\phi_{out} = 40%$</th>
<th>$\phi_{out} = 50%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>$\tilde{\tau}_i = 0.38$</td>
<td>$\tilde{\tau}_i = 0.19$</td>
<td>$\tilde{\tau}_i = 0.05$</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>$\tilde{\tau}_G = 1.63$</td>
<td>$\tilde{\tau}_G = 0.22$</td>
<td>$\tilde{\tau}_G = 0.05$</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>$\tilde{\tau}_i = 1.51$, $\tilde{\tau}_G = 2.55$</td>
<td>$\tilde{\tau}_i = 0.26$, $\tilde{\tau}_G = 1.12$</td>
<td>$\tilde{\tau}_i = 0.04$, $\tilde{\tau}_G = 0.84$</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>$\tilde{\tau}_i = 1.55$, $\tilde{\tau}_G = 6.46$</td>
<td>$\tilde{\tau}_i = 0.11$, $\tilde{\tau}_G = 5.34$</td>
<td>$\tilde{\tau}_i = 0.03$, $\tilde{\tau}_G = 5.11$</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>$\tilde{\tau}_i = 5.12$, $\tilde{\tau}_G = 6.76$</td>
<td>$\tilde{\tau}_i = 1.63$, $\tilde{\tau}_G = 5.35$</td>
<td>$\tilde{\tau}_i = 0.11$, $\tilde{\tau}_G = 5.11$</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 $\tilde{\tau}_i$ and $\tilde{\tau}_G$ (Dimensionless gelling time and initiation time) for different initial concentrations of (P) particles, $\phi_{out}$, and mechanisms. The standard deviation of initiation time $\tau_i$, and $\tau_G$ are equal to $\pm 0.03$.

Our results show that $\tau_i$ and $\tau_G$ have an inverse relationship with concentration, and this trend holds true for all mechanism types. Higher concentrations lead to faster consolidation or gelling due to the increased probability of particle interactions.

Regarding the type of mechanism, we find that the initiation time, $\tilde{\tau}_i$, and gelling time, $\tilde{\tau}_G$ are the lowest for clusters due to the proximity (a) and aggregation (b) mechanisms and the rise in those that activation is driven mainly by the bonding (c,d,e,f). Overall, we observe that the initiation time scale, $\tau_i$, is related to the number of first-stage bonds, whereas the gelling time scale, $\tau_G$, is linked to the number of second-stage bonds and increases with their number. Thus, by adjusting the number of bonds, we can regulate and tune these time scales.

Different gelling mechanisms can have varying kinetics, and this can impact the gelling time. For example, mechanisms that involve the formation of physical bonds, such as hydrogen bonding or van der Waals forces, tend to have slower kinetics and result in longer gelling times. On the other hand, mechanisms that involve chemical reactions, like the cross-linking of polymers, can result in faster gelation due to the rapid formation of chemical bonds. Understanding the relationship between initiation time and activation mechanism is important for comprehending the regulation of biological processes.

### 4.4 Cluster evolution over time

#### 4.4.1 Time evolution of several biomarkers

In this section, we focus our attention on the temporal evolution of the different biomarkers, to illustrate additional dynamical features that can be accounted for in the GMC framework. In Fig. 5 we compare the time evolution of dimensionless values for bond numbers $N_B$, fractal dimension $d_f$, active particle numbers $N_A$, passive particle numbers $N_P$, and the radius of gyration of the cluster $R_G$ for three mechanism types $c$ $(B(3)_A B(0))$, $f$ $(B(10)_B B(10))$, and $g$ $(B(3)A B(0))$, at $\phi_{out} = 40\%$. This allows us to explore the effect of surface tension (aggregation) between mechanisms $c$ and $g$, and the consolidation effect between mechanisms $c$ and $f$. The non-dimensionalization is done using the corresponding steady-state values for each parameter, whereas for $d_f$, we use the dimensionality of the box (2). The dimensionless values are denoted using the upper bar notation. The time scale is normalized using the gelling time, $\tau_G$, of each mechanism.

It can be observed that the fractal dimension $d_f$ changes as the cluster grows in all three mechanisms. Unlike traditional methods that keep the fractality constant during growth, our method shows a change in $d_f$ values over time.

In addition, we see a decreasing trend in (P) particles and an increasing trend in (A) particles, bond numbers, and radius of gyration. Understanding these changes in the fractal dimension, active particle numbers, passive particle numbers, and bond numbers can provide important insights into biological cluster formation and behavior. It could assist us in identifying and curing disorders that are related.

Mechanism $g$ is introduced to investigate the effect of activation delay time on cluster morphology. During this process, there is a delay between activation (bonding) and aggregation events. The activation delay time reflects how the cluster’s evolution changes as aggregation takes precedence over bonding. In Fig. 5, we can observe a step-wise evolution of the biomarkers in mechanism $g$, indicating a sudden activation of passive particles that were previously together as a droplet. Compared to mechanism $c$ in Fig. 5, the fractal dimension of mechanism $g$ is consistently smaller during the whole process, and the gelling time is approximately five times higher. The activation delay time is an important feature that characterizes biological clustering. It refers to the time interval between the start of a clotting event and the beginning of the active phase, during which platelets and clotting factors become activated.

The impact of the consolidation stage (second-stage of bonding) on the time evolution of biomarkers can be seen by comparing mechanisms $c$ and $f$ in Fig. 5 and Fig. 5c. Mechanism $f$ has a continuous formation of bonds, causing the structure of the cluster to continue evolving, even after converting (P) particles into (A) particles. As a result, the $d_f$ value of the cluster decreases over time, even after gelling, and is lower than mechanism $c$. The time gap between initiation and gelling is also larger in mechanism $f$ compared to mechanism $c$, with a gelling time that is roughly 25 times higher.
4.4.2 Time evolution of bond numbers in various mechanisms.

This section compares the time evolution of bond numbers in various mechanisms. The time evolution of dimensionless bond number, $\tilde{N}_B$, for mechanisms $c,d,e,f$ and $\phi_{int} = 40\%$ is shown in SI Fig. S10. Bond numbers are dimensionless with their values in a steady state or gelling state and time values are also made dimensionless using the $\tau_G$ of mechanism $f$. The changing bond number over time demonstrates that our method can alter the system’s kinetic properties over time in a manner similar to real biological systems. The time required to reach the steady state increases as the number of bonds in the second-stage bonding, $m$, increases. This allows us to construct clusters with a wide range of gelling times (depending on activation and aggregation mechanism conditions) and different final cluster morphologies.

Overall, we have shown that the interplay between activation, consolidation, and surface tension effects originate a multiplicity of transitions that mimic naturally occurring clustering processes. However, cluster morphology can be also marginally altered by systematic variation of the parameters in our model. This is the number of bonds allowed per stage or variation on the delay time. Such systematic variation will be typically associated with the specific biological system modeled, and require an appropriate fine-tuning of the model parameters that are out of the scope of this study. However, in SI S3.1, S3.2, and S3.3 we illustrate a preliminary case of study, where parametric variations in the bonding for different stages lead to different quantitative and qualitative results on cluster morphology.

5 Discussions and Conclusion

In this study, we introduce a new method for modeling cluster growth based on the SDPD method, that incorporates hydrodynamics, adhesion-, and aggregation-type interactions between constituents. Our method can be used to study the effects of different characteristic parameters, such as initial concentration, and activation methods (proximity and bonding). Additionally, we could examine the effects of the first-stage bonding, second-stage bonding, and the delay time between the first-stage bonding (activation) and aggregation. This method enables us to link measurable distinctive biomarkers such as fractal dimension ($d_f$), pore-scale transport properties (anomalous diffusion exponent, $\alpha$, and infinite diffusion coefficient, $D_\infty$), and time scales (initiation time, $\tau_i$, and gelling time, $\tau_G$) with microscopic cluster effects.

We summarise the results obtained with the GMC scheme in Fig. 5 for $\phi_{int} = 40\%$ (see SI Figs. S11, S12 for $\phi_{int} = 30\%$ and $\phi_{int} = 50\%$). In Fig. 6, we present a biomarker diagram that displays the variety of clusters that can be generated and their corresponding characteristics. The biomarker diagram helps us to navigate the effects of concentration and clustering paths into the identified biomarkers, along with the cluster morphology. In Fig. 6 we show activation of a passive particle occurs in two ways: 1-Proximity and 2-Bonding. We define two mechanism types $a$ and $b$ for the proximity activation approach, in which mechanism $b$ is combined with aggregation. The bonding approach has five
mechanism types $c - g$ with varying bond numbers during initiation and consolidation. In mechanism $g$, there is also aggregation, which has a $\tau_{\text{delay}}$ between aggregation and activation (first-stage bonding).

The results show how altered particle interactions and initial concentrations lead to distinct changes in cluster morphology and kinetics. As in our study, we incorporate surface tension and inter-particle bonding into the SDPD method, allowing us to generate clusters with diverse microstructure and fractal dimensions in a range of $1.4 \pm 0.01$ to $2.1 \pm 0.01$. By controlling the parameters governing surface tension and bond formation, we are able to investigate the effects of these physical properties on the growth and formation of fractal clusters. Furthermore, it is obvious that the use of a unique measurable biomarker, such as $d_f$, is insufficient to provide a thorough differentiation between clusters and therefore a differentiation between distinct micro-patterns. We study several biomarkers to gain a more thorough knowledge of the clusters, which differs from conventional approaches that just use fractal dimension as a cluster descriptor. We proposed that additional characterization of the mean square displacement of solvent particles (or probes) confined within the clusters provides a better integral characterization of biological clusters. Furthermore, by analyzing the time-dependent diffusion coefficient or anomalous diffusion exponent, we are able to distinguish two physically distinct regimes: the short-time regime and the long-time regime approaching asymptotic Gaussian diffusion. This approach highlighted different biological system features that are not evident from $d_f$ alone. Therefore providing a more comprehensive way to macroscopically access biomarkers related to distinct microscopic features of the clusters.

The introduced GMC has the potential to further study complex cluster mechanisms, such as those related to blood coagulation, and could range from fibrin network formation to platelet aggregation. Future studies will examine the mechanical properties of different cluster configurations, which frequently have biological implications. For example, changes in the mechanical properties of blood clots have been observed in coronary artery disease and venous thrombosis, highlighting the potential of using viscoelastic measurements to improve our understanding of these conditions.

**Author Contributions**

N.M and M.E conceived and supervised the project. E.Z designed the computational experiments and conducted the simulations and data processing. The original draft of the manuscript was written by E.Z and N.M. and all authors contributed to the final version of the manuscript. N.M. developed the numerical implementation. All the authors discussed and analyzed the results. All authors approved the final version of the manuscript.

**Conflicts of interest**

There are no conflicts to declare.

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**Notes and references**

A biomarker diagram is provided step by step regarding the implementation of different mechanisms in our model for $\phi_{\text{sat}} = 40\%$. Biomarkers include $d_f$, $D$, $\alpha$, $\tau_I$ and $\tau_G$. 

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