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Mesoscopic Rigid Body Modelling of the Extracellular Matrix Self-Assembly

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Abstract:
The extracellular matrix (ECM) plays an important role in supporting tissues and organs. It even has a functional role in morphogenesis and differentiation by acting as a source of active molecules (matrikines). Many diseases are linked to dysfunction of ECM components and fragments or changes in their structures. As such it is a prime target for drugs. Because of technological limitations for observations at mesoscopic scales, the precise structural organisation of the ECM is not well-known, with sparse or fuzzy experimental observables. Based on the Unity3D game and physics engines, along with rigid body dynamics, we propose a virtual sandbox to model large biological molecules as dynamic chains of rigid bodies interacting together to gain insight into ECM components behaviour in the mesoscopic range. We have preliminary results showing how parameters such as fibre flexibility or the nature and number of interactions between molecules can induce different structures in the basement membrane. Using the Unity3D game engine and virtual reality headset coupled with haptic controllers, we immerse the user inside the corresponding simulation. Untrained users are able to navigate a complex virtual sandbox crowded with large biomolecules models in a matter of seconds.

Keywords: Interactive simulation, Rigid bodies, Virtual reality

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1 Introduction

Biologists are increasingly studying biological systems using holistic approaches such as genomics and proteomics. This means that a large amount of structural and interaction data becomes available. Interaction proteomics is especially focused on protein-protein interactions. Online databases like the MatrixDB centralises interactions data between proteins of the extracellular matrix (ECM) [1]. While these provide invaluable pieces of information, there is no mean to simulate/visualise these interactions and to test their behaviour in a dynamical process. Although experimental observations methods have made great progress, there are still large portions of the cell or its environment, especially the ECM, that can only be observed indirectly.

The ECM is an ensemble of molecules secreted outside the cells that were first considered as a medium providing structural support to the surrounding cells in tissues before being demonstrated as a reservoir of active molecules triggering biological signalling and functions [2], [3]. ECM composition varies according to multicellular structures and cell types and it plays fundamental roles in normal and pathological conditions by affecting cell adhesion, cell-to-cell communication and cell differentiation [4], [5]. Macromolecules of the ECM are often huge molecular systems [6], with scales lying in the 10^{-8} m–10^{-7} m region. There are constituted of numerous multi-globular domains and patterns, of multi-domains linked with flexible unstructured fragments, with fibrous structures and/or glycosylated molecules [7]. These domains and patterns could adopt numerous transitional conformations and have a great adaptability and flexibility to perform their functions. Most of the three-dimensional structures at the atomic and molecular levels are provided by experimental data from crystallographic and/or NMR experiments, and since few years, with molecular modelling approaches data from Electron Microscopy (EM) or SAXS experiments. These methodologies lead to some structure/function relationships but lack the structure/function/dynamic relationships.

Nicolas Belloy is the corresponding author.

The dynamic behaviour of proteins is investigated using simulation tools such as molecular dynamics (MD) that rely on solving Newton's equations. As researchers get interested in larger and larger systems to access representative biological conditions, they have to rely increasingly on simulations to make sense of partial/blurry experimental data. The advent of MD simulation in biology is probably the single most important tool to have been developed these last decades. It gave researchers unprecedented access to complex systems at molecular scale as computer power went up.

While microseconds timescale computations for 100 to 1000 atoms systems are almost routine, larger simulations on larger timescales still require huge computational resources. Proteins assemble into complexes, like in the ECM, so large that it cannot be simulated in a reasonable amount of time, even on a petascale machine. According to Suzuki et al. [8], nanoseconds timescales become irrelevant for systems upward of 10^7 atoms. For example, a simulation of the viral capsid of the HIV virus was simulated with 64 million atoms for a 100 ns. This required a 13 PetaFlops supercomputer running at 8 ± 2 nanoseconds per day [9].

Thus, there is a need for tools that can run elsewhere than a whole supercomputer and yet be able to simulate and visualise large biological systems on timescales consistent with biological events (protein folding, proteins interaction ...). Coarse-grained MD (CGMD) gives us a hint as to how we can achieve such opposite goals. CGMD derives from MD in the sense that it uses Newtonian physics to simulate the behaviour of molecules. CGMD reduces the degree of liberty of a system by clustering many single atoms into single larger beads thus reducing the computational resources needed [10]. It is multiscale in the sense that it is possible to go from an MD simulation to a CGMD simulation and vice versa.

Molecular visualisation goes alongside simulation as the primary mean for the user to instinctively make sense of large complicated datasets. However, the average molecular visualisation package requires that you memorise hundreds of commands and shortcuts to be efficiently used. Biologists often have to get help from skilled computer users to learn the basics of navigating these software tools. And even then, the end-user often has to practice for a long time to achieve real proficiency. Immersive technologies such as virtual reality headsets free the users from the tedious apprenticeship required by molecular visualisation software. It allows unskilled persons to dive in, move and observe a simulation with minimal (if any) training.

Here, we propose a multiscale approach that uses rigid body dynamics to further decrease the amount of calculation required to simulate large biological systems such as those found in the basement membrane, a layer of ECM in direct contact with cells.

2 Related Works

Numerous initiatives in the field of mesoscale and/or multiscale modelling are worth being mentioned.

CellPACK [11] builds 3D mesoscale models from a list of molecular ingredients provided by the user. These ingredients are treated as rigid bodies that CellPACK tries to pack as efficiently as possible inside a given geometrical shell that serves as a boundary. CellPACK itself does not generate motion or create trajectories. It relies on animation software or uses pre-calculated trajectories computed with other tools.

The Open Dynamics Engine [12] rigid body physics has been used to study biological objects in the micrometre scale. The yeast nucleus has been studied using a simple physics model where each chromosome is modelled as chains of linked rigid bodies [13].

UnityMol [14] is a visualisation tool developed with Unity3D. Unity3D is a cross-platform game engine that is used in many applications outside of video games, mainly serious game but for simulation or data visualisation as well [15], [16]. UnityMol is designed as a proof of concept to advocate the use of technologies developed in the video game industry to boost the performances of scientific visualisation tools. UnityMol uses Unity3D through scripting in C# or with custom shaders. Material shaders, like hyperball [17], are a set of instructions that are computed by the GPU for rendering. Compute shaders are softwares intended to be loaded and computed on the GPU for non-visual rendering tasks. UnityMol has also been used to make applications to explore the potential of augmented/mixed reality associated with tangible models [18].

We think that the technology is now mature enough to try combining mesoscale models with rigid body chains. The main advantage of game engines for interactive visualisation is that it is not necessary to be a skilled computer developer to create efficient advanced graphics. This is managed by the game rendering engine. But, the favour given to speed rather than accuracy is a serious drawback. While this poses no trouble for molecular visualisation, it is best to be aware of this when it comes to physics simulations.

3 From PDB to Immersive Simulation

The basement membrane is composed of molecules such as type IV collagens, laminins, nidogens/entactins, perlecans and integrins. Due to their size, no solved structure at the atomic resolution of most of these principal components exists. However, observables defined as "fuzzy" can be obtained from experiments such as Atomic Force Microscopy (AFM) [19], rotary shadowing and EM [20] or SAXS (Small Angle X-rays Scattering) and cryo-EM data [21]. These do not give precise structures, though they can give us a rough idea of the scale and shapes of these large objects.

3.1 Structural Data

To gather as many information as possible on a macromolecular complex to make an accurate model of it, our main source of 3D structural data is derived from the Protein Data Bank (PDB) (www.rcsb.org) [22] providing atomic coordinates for macromolecules, in a standard human-readable file, the PDB format.

The perlecan molecule is a good example of a relatively simple reconstruction procedure we used because its domains have all been resolved individually and can be found directly in the PDB. In the literature, perlecan is described as being organised into five multi-domains (labelled from I to V), themselves consisting in a succession of domains of known structures. Biologists and biochemists already represent these multi-domain macromolecules as a series of colour schematic drawings as shown in the Figure 1A. Using solved domains of perlecan as shown in Figure 1B, we have built the corresponding macromolecular structure of perlecan as provided in Figure 1C (see description below).

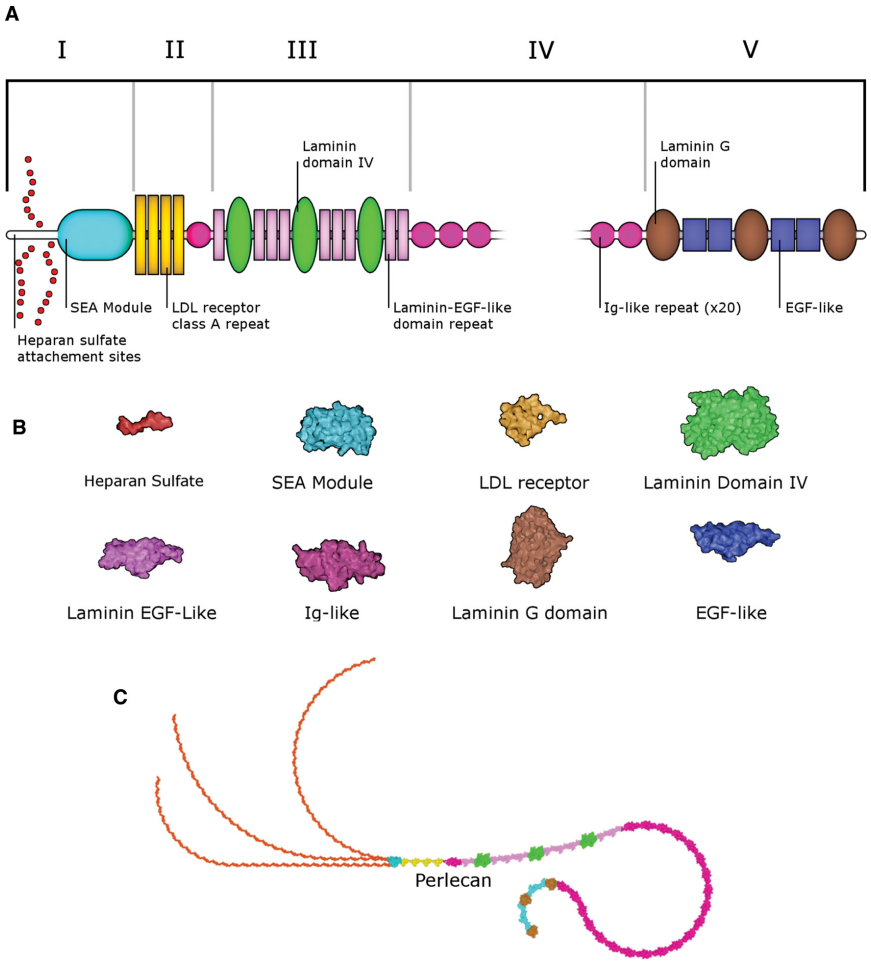

Figure 1: Perlecan domain maps and molecular ingredients.

While direct mesoscale observations exist, as previously mentioned, these are blurry and/or often incomplete but are invaluable when it comes to checking if our model matches up to these observations. Matching our model with AFM measurement of the perlecan support that our model is in qualitatively good agreement with the observation regarding molecular dimensions.

When no obvious 3D structures exist, other techniques must be applied to get that information. *De novo* simulations [23] of the G1 domain of nidogen has provided three candidate structures. One model amongst the best score was chosen as a likely structure to make an accurate representation in terms of scale and proportions. It is worth mentioning that a switch between models would be easy as soon as we get relevant structural or interaction data in the future.

Another approach based on homologous structures permits the building of the laminin triple coil domain, a region missing in the starting model. The small helical part found in the integrin-binding region of laminin-111 (pdb id: 5MC9) bears a great structural similarity with Salmonella coiled coil (pdb id: 2WPQ), a triple coil structure solved and deposited in the PDB.

3.2 Coordinates Preparation

Atoms coordinates found in the PDB are seldom centred around 0 and can make it harder to assemble the imported object together with joints if the pivot point is offset from the real centre of the molecule. Custom python scripts have been used in Blender [24] to manipulate 3D structural data by translating them so that the pivot point is located at the geometrical centre of the molecule. In cylindrical/elongated molecules, when a principal axis is self-evident, we realign them along one of the Cartesian axes. The Y axis is chosen because it is the default "up" vector in Unity's editor. The corrected coordinates are opened with VMD [25] to generate a surface mesh, then exported in a wavefront object file to be used in Unity.

3.3 Unity 3D

In Unity editor, object files are imported as assets that are nothing more than the mesh depicting the surface representation exported from VMD. Then, the proper components that allow a physics engine to properly manage these surface representations are added. We present the different steps to process within an immersive simulation.

3.3.1 Rigid Bodies

The rigid bodies are the essential components to run physics simulations in Unity. Physics engines generally apply forces and torques on the rigid body centre of mass at each step. Without rigid body component, the object is not a part of the simulation at all.

MDs simulations often rely on particles. Atoms are represented as punctual objects that are assigned physical properties which are then used to update the state of a system at each timestep. Rigid bodies have similarities to particles as their motion is ruled by Newtonian equations, but unlike particles, rigid bodies can be of any shape. Consequently, they are not only defined by their position but also by their rotation in space. They are considered as rigid because their shape cannot change, which is another way of reducing the amount of calculation required, compared to soft deformable bodies.

3.3.2 Colliders

Colliders are components that are used to define the shape and spatial occupation of the objects in Unity3D. While physics engines allow for objects of any shapes to be treated as colliders, they still perform best with simple primitives like spheres, cubes or cylinders. Proteins are easily represented by spheres, cylinders or a compound of both (Figure 2).

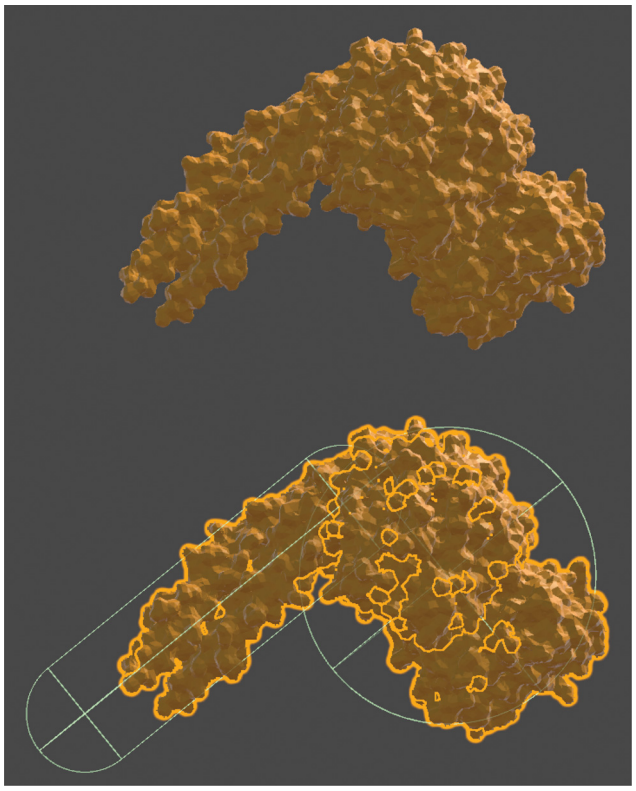

Figure 2: Laminin globular domain compound collider made from a capsule primitive and a sphere primitive collider (bottom).

3.3.3 Joints/Constraints

To create our molecular models, rigid bodies must be linked together using joints. Physics engines define joints as a set of constraints that describe how two rigid bodies move relative to one another. At each step, forces act on the rigid bodies to correct any drifting and minimise distance errors. It is possible to link more than one or two bodies on a single body, or even to circularise a chain which allows for a relative freedom when building models mimicking large macromolecules. Limits can be set for rotational/positional constraints and can be very useful to define how much a joint can bend. This has been used in our simulations to make the collagen model more or less flexible.

3.3.4 Random Forces

Each body of the simulation is subject to random forces using the Langevin equation as presented in [13]. We translated the discretized Langevin function of this study from Python to C# which is one of the scripting languages used in Unity3D. The function generates randomly oriented forces applied to each rigid body of the simulation coupled with a friction term. Generating randomness with computers is not trivial and most existing random number generators are really pseudo-random. According to Unity3D's Random API documentation, the random number generator appears to be based on the system time.

In mesoscopic scale systems for long simulation times, observables such as the molecules location are statistically reproducible. We compared the motion of an integrin molecule, simplified to a 2D diffusion as it is a membrane protein, to a 2D cloud of points generated using Numpy random number generator (Figure 3). After a reasonably long time of simulation the molecule explore the space in a uniform way.

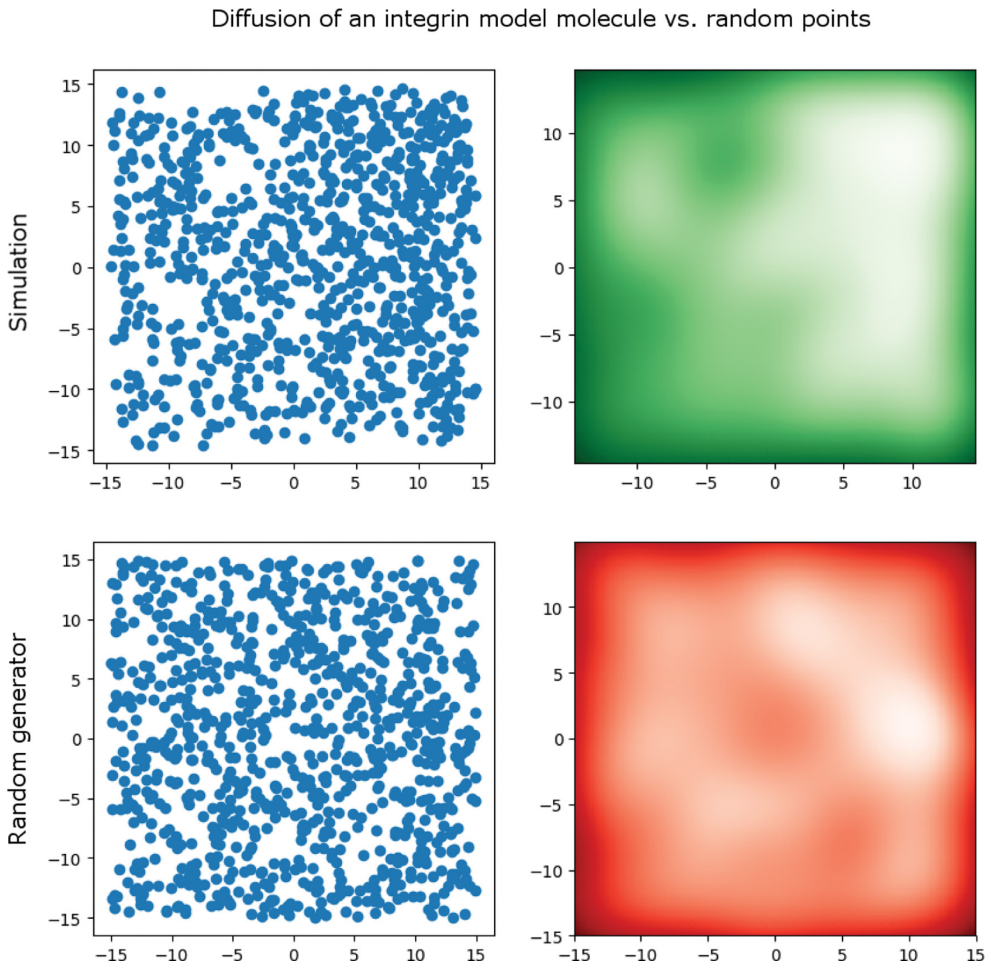

Figure 3: Comparison of the diffusion of an integrin molecule taken from the simulation vs. points generated using Numpy uniform random number generator.

3.3.5 Virtual Reality Toolkit

The Virtual Reality Toolkit (VRTK) is a free third-party asset [26] that makes it easier for developers to create applications in Unity3D interfaced with VR hardware. VRTK allows us to develop for any types of headsets, like the Oculus Rift, Microsoft Hololens or HTC Vive. While Unity3D already support virtual reality, VRTK cuts down the time required to make common VR interactions with ready-made scripts. A relatively new Unity3D user can quickly build a bare-bone VR experience with the ability to move and interact with the simulation. User interaction with the simulation is very similar in concept to steered MDs as described in [27], only instead of using forces, VRTK creates a joint where the virtual representation of the controller meets the rigid body.

3.3.6 Simulation Environment

The thickness of the basement membrane has been measured as being between 30 and 70 nm thick depending on the tissue. For this reason, we set our simulation environment to be a box with the height set at roughly 60 nm. In comparison, the diameter of a globular domain of type-IV collagen is 6 nm (12 nm in the dimer). Width and length are arbitrarily chosen to allow long molecular models to fit in.

The matrix simulation is placed in a virtual glass display on a table, giving something the user can focus on (Figure 4). We implicitly limit the teleport regions in and around the table, avoiding such pitfalls of the user teleporting itself inside the glass display.

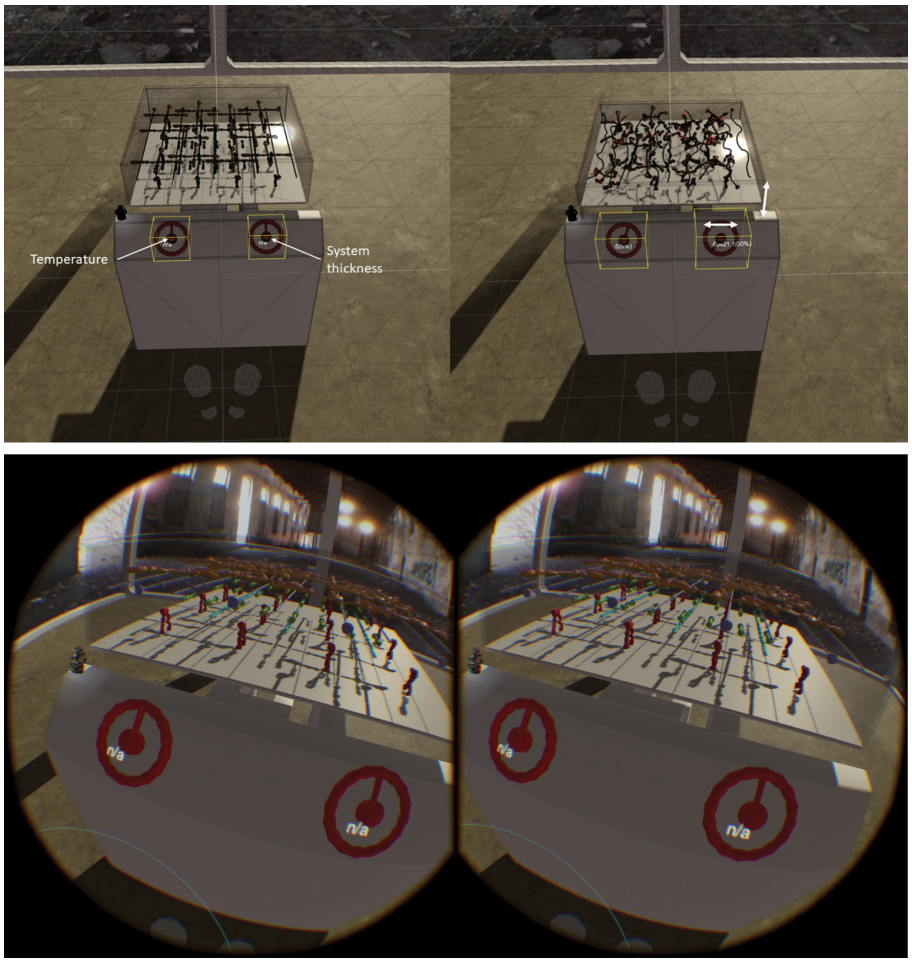

Figure 4: View of the simulation environment from the editor (top) and from the head mounted display (bottom). Top right shows what happens when the dial controlling thickness is interacted with.

The user interactions with the environment are kept simple. The user can grab and move molecules, he can also use two dials near the display to change simulation parameters such as its temperature/speed and thickness (Figure 4 top).

Attention was given to the way the VR controllers are used during demonstration sessions in the lab. Initially, we configured the controllers' buttons to "grip" the simulation's objects when the users pressed the grip button found on the hilt of the Vive controller. After noticing that users could not find the button easily and preferred to press the trigger, the configuration was subsequently changed.

3.4 Hardware

Development machine is configured as follow:
Octo-core Intel Xeon E5-2650 2.20GHz 128Gb RAM and a GeForce GTX 1080.
We used an HTC Vive headset and controllers for VR immersion.
HTC Vive resolution per eye is 1080 × 1200 and refresh rate is 90 Hz.

4 Application: Self Assembly

Numerous biochemistry papers reference the interactions occurring inside the basement membrane between the components that make it up [28], [29], [30], [31]. Surprisingly, there is a gap between the real size of molecular models and the way biologists often depict them in the literature [32] (see Figure 5). By incorporating these pieces of information into our simulations, we are able to describe how they crosslink. Thus, our tool can be used to enhance the sampling of the conformational space: knowing how much a rigid body has moved or turned gives us conformational information that can be transposed back to an all-atom model.

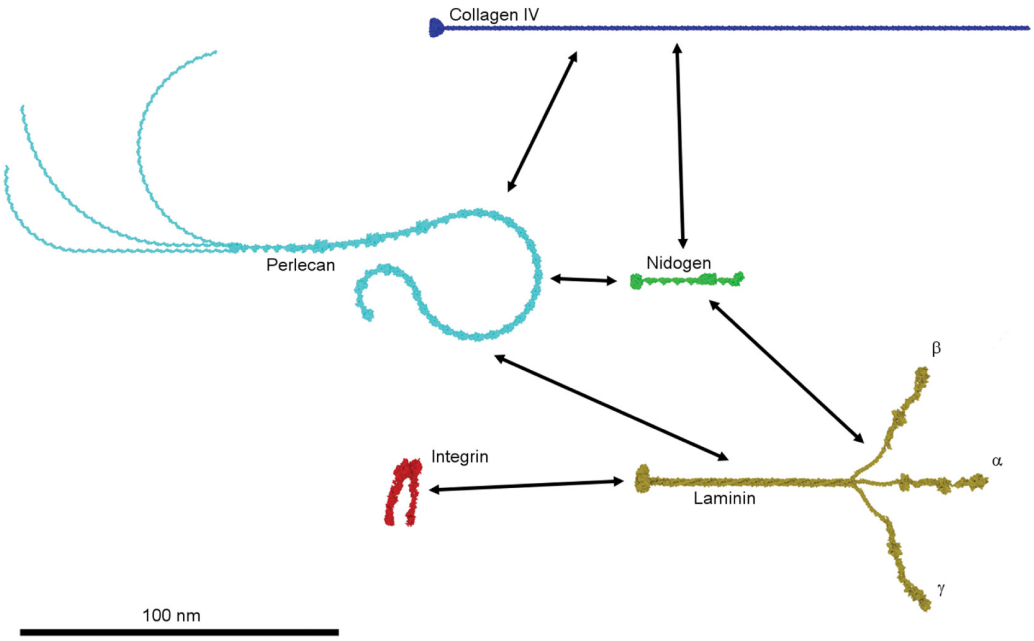

Figure 5: Known interactions between basement membrane components.

Type-IV Collagen is, with laminin, one of the macromolecules that makes up most of the basement membrane [33]. Experimental observations show that its globular domain (NC1) dimerizes and the other end can tetramerize [34], hence creating a network. In our simulation, the stiffness (joint bending amplitude) parameters of the collagen model can be adjusted to test the effects of kinks (Figure 6) along the molecule [35].

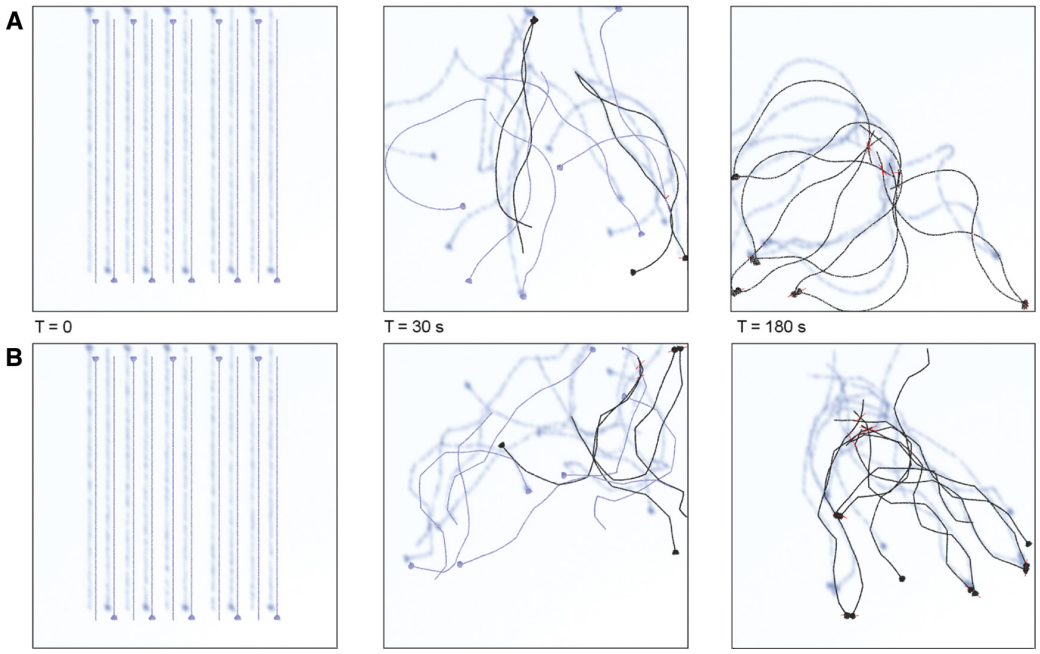

Figure 6: Initial conformation and reticulation of the collagen (black molecules are molecules involved in a network). Simulations of Type IV Collagen without kinks (A) and kinks (B). Time in seconds represent running time in the computer and does not represent real time in the simulation.

Laminin has been proposed as a stabilizing influence on the basement membrane by forming a hexagonal lattice [36]. We have very few information about the flexibility of the **α**, **β**, **γ** arms of laminin. Consequently, the tool developed here could help us to characterise the effects of stiffness variations on binding target research.

Nidogen is known to mediate the interaction between laminin and collagen [37] although the precise nature of its function in the basement membrane is still not well known [29].

Integrins are part of a larger transmembrane complex that goes from the basement membrane to the inside of the cells themselves [38]. As such, they never leave the surface of the membrane. Integrins are known to anchor laminins and play an important role in cell-matrix and cell-cell adhesion [39].

Perlecan interacts with many molecules in and out of the ECM, but the exact mechanism of actions of the perlecan are still not well known [40]. Our simulations suggest that its size and flexibility allow it to wrap around many of the molecules of the basement membrane even without specific interactions.

The aim of the present work is to observe what happens when all these molecules are inside the simulated environment, what sort of structures they can assemble into (Figure 7) and investigate what subtle influence the structures have on this assembling phase. Indeed, many matrix functions (and diseases for that matter) come from changes in molecular interactions that occur upon sequence variants/mutations or changes in post-translational modifications levels that can affect molecules flexibility. The consequences on the ECM of these variations are hard to predict from an experimental point of view and could be investigated with our tools.

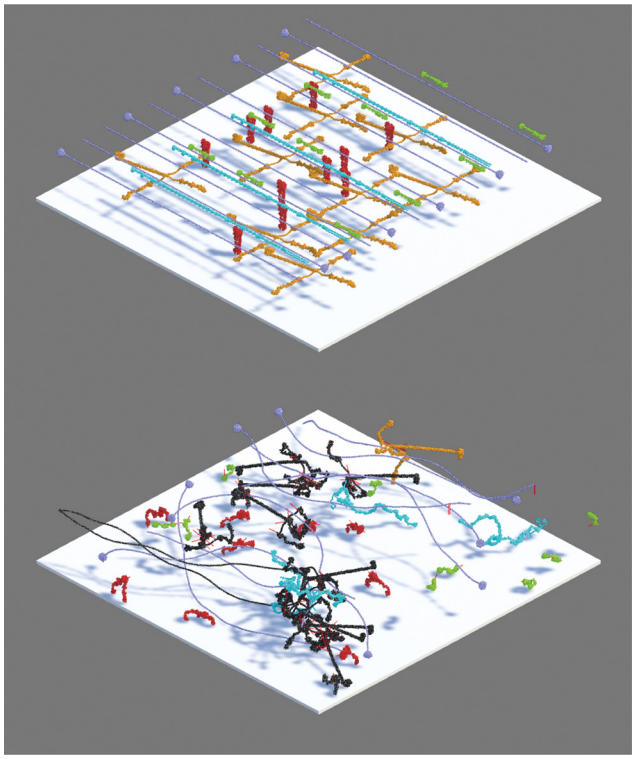

Figure 7: Typical simulation run molecules involved in a network are coloured in black.

5 Discussion

5.1 Rigid Body Models of Mesoscale Objects

While we are still far away from having a basement membrane model, we surmise there is a large unexploited potential in using rigid bodies to model mesoscopic biological systems in general. One improvement would be to have something providing an initial starting conformation that is not fully extended by a prior conformational sampling of the individual ingredients with classical MD methods. In the future, we would like to explore other physics engines that would not be slowed down by a large number of collisions. Such engines, in an interesting twist, simulate rigid bodies as a group of particles [41] allowing for GPU treatment of this physics engine bottleneck. One such engine is PhysX Flex which has just started being implemented by third-party in Unity, sold as uFlex in the asset store.

uFlex is not officially supported by Unity3D and only works on NVIDIA's GPUs so it is not cross-platform, one of the main selling points of Unity3D.

There are also some limitations. At the time we tested, uFlex did not allow the creation of joints between particle-based rigid bodies. So we could not use it to build large mesoscopic macromolecules such as those featured in this paper.

Also, the asset we use in our application for VR interactions, VRTK, does not yet include the ability to interact with the particles managed by Flex, only with PhysX and its rigid bodies and joints constraints.

5.2 Interactivity and Immersion

Virtual reality helps a lot in making the simulation accessible to inexperienced computer users. Instead of memorising keyboard shortcuts, command lines or mouse actions, the user just walks around the simulation in a natural way and look around to change his viewpoint. It also has a modicum of influence on the simulation itself by being able to manipulate the molecules with the controllers. Head mounted displays provide stereoscopic feedback. Depth perception allows users to precisely grab single molecules inside our simulations. Using the interactivity functions implemented in VRTK, we experimented with on the fly joints creation or destruction to link unlink two rigid bodies. This can be used to create or destroy molecular complexes while the simulation runs.

A new user learns intuitively to move in a complex simulation very quickly. The flip side is that it makes it necessary to do some "level design". We have tried many configurations regarding the simulation environment setups. Because we want the simulation to be as simple to use as possible, the user is free to roam in the simulation. By that, we mean, apart from a teleport function, the user simply move around. The VR hardware tracking takes care of proper camera placement in the simulated environment. However, just as in video games, an enjoyable experience means a lot of work is done in the background unbeknownst to the user.

The very first iteration simulated a vast hangar-like environment where the user was surrounded by the simulation. Interestingly, the users kept getting lost by teleporting right into and sometimes outside the "walls" of the simulation.

Subsequent environments forgo the idea of simply immersing the user into the simulation. Instead, we opt for a more familiar type of interaction. Our current approach is similar to how an architectural model is put on a table so the client gets an idea of the overall structure of a complex building and communicate design ideas. Users' feedback has been very positive so far. Even those familiar with the macromolecules used in our simulation find it informative to see them move with correct proportions and scale relative to each other. The biggest negative feedback would be the virtual reality sickness signs experienced by some users. Frame rate lag can lead to nausea while poor adjustment can lead to headaches.

5.3 Performance

In the video game industry, the amount of frame per seconds (FPS) is an indication of comfort for users. Twenty-four FPS is considered as the minimum amount of FPS for an interactive video game experience. Our simulation runs at roughly 90/100 FPS with 1054 rigid bodies (Table 1). We did further stress test on our simulation by doubling and tripling the number of rigid bodies and checking for GPU and CPU usage. It is interesting to note that the number of rigid bodies does not seem to affect GPU performance, it remains under 16 ms (60 FPS). CPU performance, however, takes a dip with each increase in the population of rigid bodies. And this, in turn, affects the actual frame rate as the rendering pauses while computing is done. This is probably due to the way collision detection is handled by the CPU. First is the broad-phase detection [also called sweep and prune (SAP)] where the simulation space is subdivided and the volume searched for potential bounding boxes intersection. Then there is the narrow-phase detection where more time-consuming collision detection algorithms are used on potential collisions tagged by SAP. For compatibility reasons, as of 2017, PhysX is not hardware accelerated on Unity3D, so it relies solely on the CPU, limiting the number of concurrent rigid bodies in interactive simulations. A possible workaround would be to develop our own compute shader which is not a trivial task. We could also turn to other physics engines that are not officially supported by Unity3D but have been interfaced by third-party. One candidate would be Bullet [42] which include a preview of the "all particle" pipeline described in [41] as well as hardware acceleration through OpenCL.

Table 1: Framerate vs. number of rigid bodies in the simulation.

Number of rigid bodies	All atom size	GPU performance (lower is better)	CPU Performance (lower is better)
1054	Roughly 1 M atoms	8 ms (100 FPS)	8 ms
2108	Roughly 2 M	11 ms (90 FPS)	16 ms
3162	Roughly 3 M	11 ms (90 FPS)	30 ms

5.4 ECM Self-Assembly, Structure and Collagen Flexibility

We only use thermal energy in our simulations. Yet we observe that our macromolecules form a network. This might mean that apart from affinity binding between proteins, no other form of energy is required.

Collagen has been observed to form polygonal networks [43]. In our model, we tested various type of flexibility to see the influence this parameter has on the structure formed by the collagen as reduced stiffness can lead to diseases.

Figure 8A shows the structure formed by very stiff collagen molecules. It shows a clear polygonal network formed through aforementioned interactions. Figure 8B is in the middle range of flexibility. The collagen molecules are flexible enough to sample surrounding space but not to the point of clumping together. Figure 8A and B are in agreement with EM observations of the collagen IV network as found in [43]. Figure 8C shows the other extreme, with collagens behaving like freely jointed chains. In that case, there does not seem to be a network in any form, but rather dispersed aggregates. Reduced collagen stiffness has been found to contribute to diseases such as osteogenesis imperfecta [44]. Meanwhile, changes in collagen IV stiffness might change the mechanical properties of the renal basement membrane of people suffering from Alport's syndrome [45].

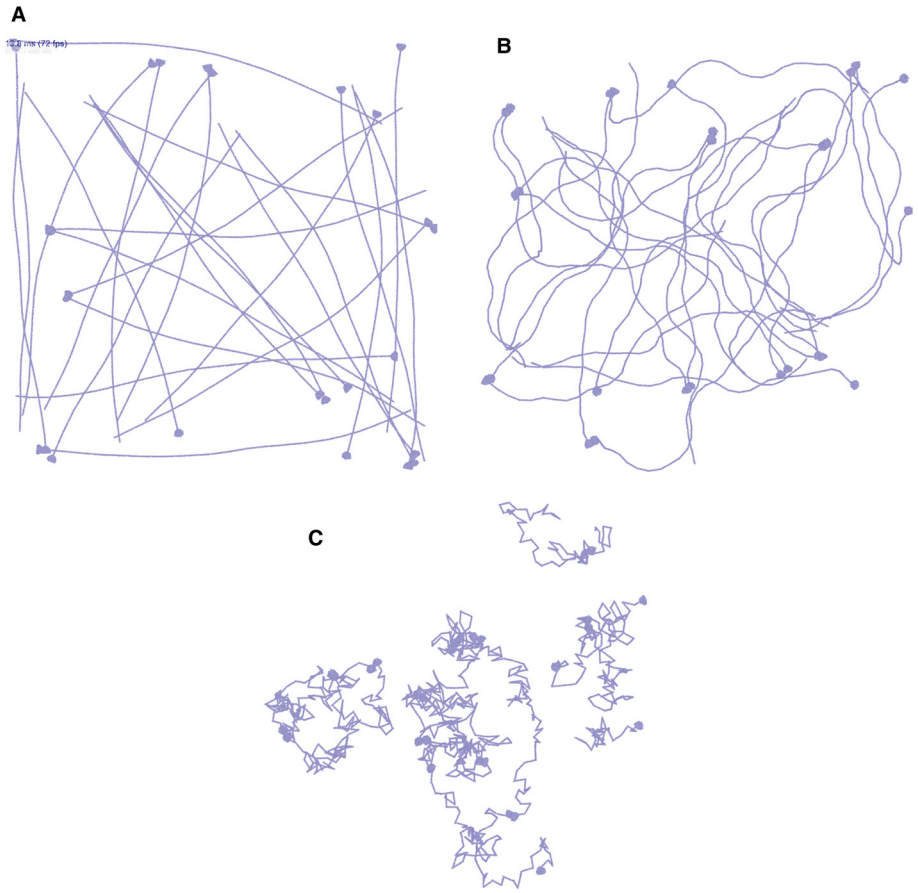

Figure 8: Effect of collagen flexibility on self assembled structure. Top left shows very stiff models of the collagen. Top right shows flexible models. Bottom shows freely jointed chains.

5.5 Conclusions and Future Work

We still have to unleash the true potential of immersive interactive simulation. The difficulty, for the time being, is whether we can implement functionalities in a useful way for the end user. It is closer to what could be described as designing a user experience, which is a concept that is seldom the focus point for scientific visualisation.

We are looking forward to new advances taking advantages of GPU acceleration, such as OpenCL and all particles rigid bodies as they provide the key to the making of interactive simulations that would otherwise stall the desktop computer configuration described in the hardware part of this paper.

In conclusion, we think interactive simulations of mesoscopic biological systems such as the ECM are achievable in a reasonably near future with current technologies.

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