

Molecules and reactions on surfaces

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Surface regions

Previously we learned how to create objects and add them to our model. Now we will learn how to select specific regions on the surface of these objects, and then assign them to our model as the sites for surface molecules and reactions. Follow these steps:

- I. Create an object and assign it to your model
- II. Enter Edit mode – you can select this from the menu bar at the bottom of the screen or hit tab on your keyboard
- III. Click the Face-Select option to triangulate the object
- IV. Select the faces you want to add to your region
- V. Go to Model Objects and under Define Surface regions, click the plus sign
- VI. Enter the region name and click assign.

Tip: To make sure that your region has been correctly assigned to your model, it might be a good idea to hit deselect and select. You should see your region on the object highlighted in orange.

Figure 10 illustrates some of these steps.

Surface classes

Surface classes are used to define properties of surfaces with respect to molecules. For example, we may wish to simulate a model where molecule A can move through a surface but molecule B cannot – imagine two proteins, one capable of moving through the nuclear membrane into the nucleus, and another which is strictly cytosolic. Surface classes allow us to define our boundary conditions.

Figure 11 shows the CellBlender interface for defining a surface class. A surface class is defined by specifying a name, as well as its properties – such as absorptive, reflective, or transparent, with respect to various molecules.

Assign surface classes

Once a surface class has been defined, it must be assigned to a surface region. Figure 12 illustrates the CellBlender interface for doing so. This is a relatively simple panel and all that needs to be entered for each assigned surface class is:

- I. The name of the surface class

- II. The name of the surface region one is assigning the class to
- III. The name of the object on which this region is defined

Figure 10. Defining surface regions in CellBlender. (A) Menu bar with the relevant options to enter Edit Mode and use Face-Select to triangulate the object outlined in red. (B) Triangulated object with all the faces selected (C) Triangulated object with 6 faces selected to form a hexagon (D) CellBlender interface to define and assign the region to the object.

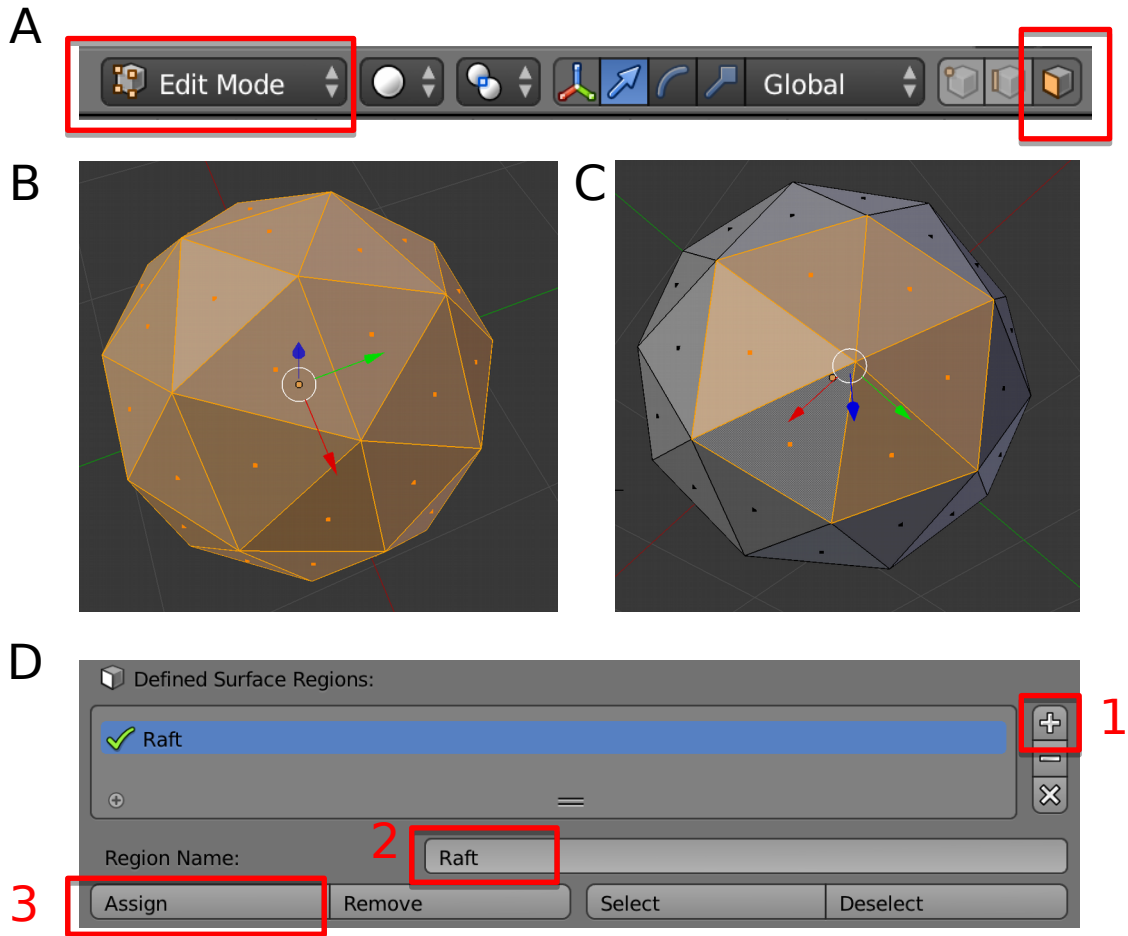


Figure 11. Defining Surface Classes in CellBlender. This figure shows the CellBlender interface for defining a surface class by specifying its name and properties – such as absorptive, reflective, or transparent, with respect to various molecules. In particular, it shows the creation of a surface class named RaftBoundary that is reflective w.r.t molecules with name chol.

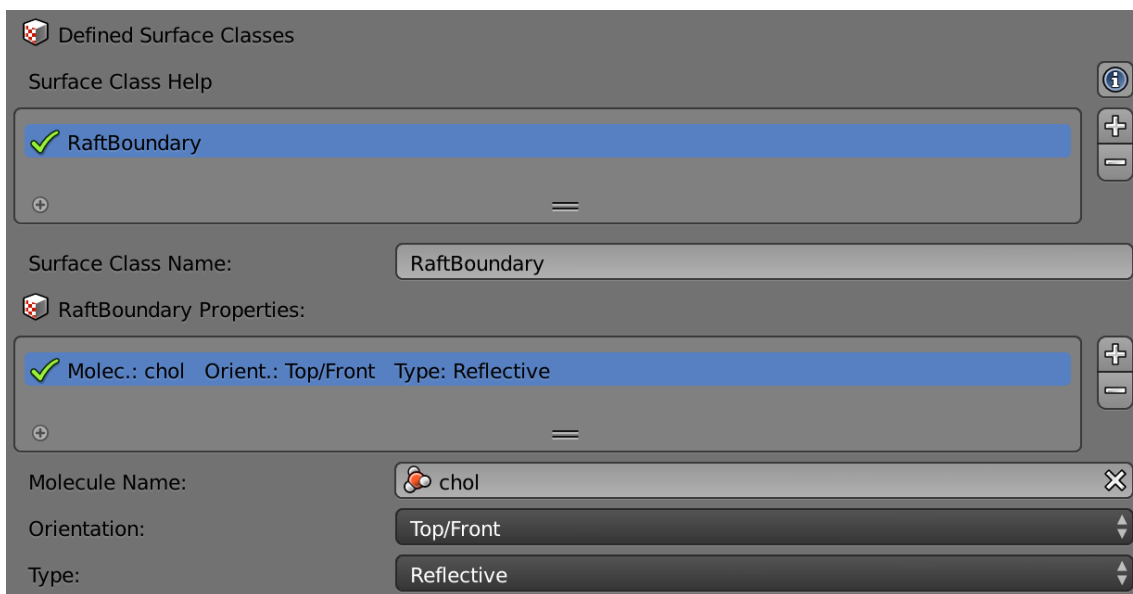
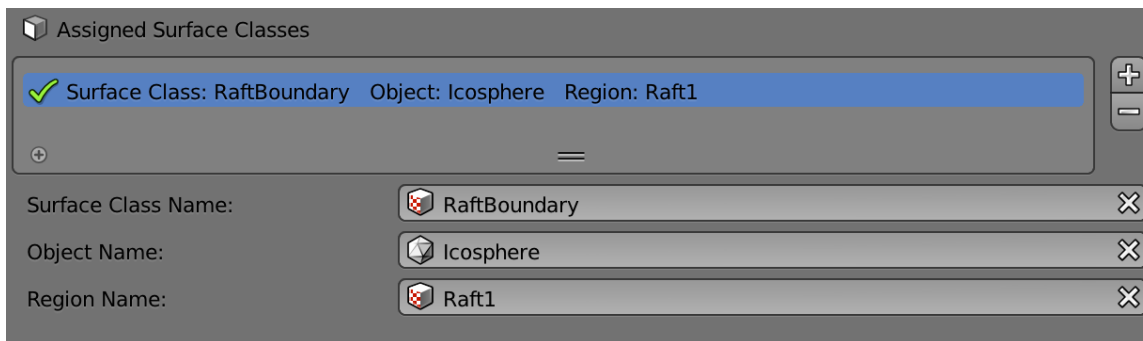


Figure 12. Assigning surface classes to regions in CellBlender. The Surface Class RaftBoundary is assigned to the surface region Raft1 on the object named Icosphere.



Absolute and relative orientations of surface molecules

Surface reactions may proceed with or without absolute orientation. An example of a reaction without absolute orientation is $B' \rightarrow B, [10]$. This implies that molecule B changes its orientation from outward facing to inward facing or vice versa at a rate of 10s^{-1} .

Another example would be $B' \rightarrow B' + A' + C$,

This implies that a molecule B retains its orientation while releasing a molecule A with the same orientation and a molecule C with the opposite orientation.

In both these examples we see that the absolute orientations of the molecules do not matter, and reactions are specified using relative orientations. Sometimes however, we may wish to specify an absolute orientation of a reaction with respect to the surface, for example a ligand might bind to its receptor on the cell surface only if the binding site is on top of the plasma membrane – otherwise the ligand may not be able to see this receptor. We may define such reactions by adding an '@ <surface_class_name>' after our reactants.

For example, the reaction $A' + B' @ surf' \rightarrow C, [1e5]$ implies that surface molecules B present on surface regions identified by the surface class surf, that are aligned with the surface normal, i.e. they have their top domain on the front of the surface, reacts with a molecule A, also on surf and aligned with the surface normal, to produce a molecule C which is aligned against the surface normal, i.e. its top domain is located on the back of the surface. To tie this example to simplified models of possible biological systems, one might imagine two outward facing receptors on the surface of the plasma membrane bind to form a dimer, which is then internalized.

Here is another example similar to the one before, $A' + B, @ surf' \rightarrow C,$
In this case, an outward facing molecule on the surface, binds an inward facing molecule on the surface to create a new inward facing molecule.

Orientation classes

An interesting thing that we can do with surface reactions – both with absolute and relative orientations, is to specify different orientation classes. Molecules across orientation classes can't see each other's orientation. Simply using a different number of commas or apostrophes allows us to specify different classes. For instance ' would be the first orientation class, '' would be the second, ''' would be the third, and so on. The same holds for the commas.

Consider the reaction $A'' + B, \rightarrow B' [1e5]$. Here A is in an orientation class separate from that of B, therefore there is no 'orientation cross talk' between the two. This reaction implies that a molecule of A in any orientation, binds to a molecule of B in some orientation and flips it – since we have only specified relative and not absolute orientations.

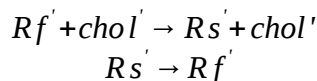
We could also decide to place no geometrical constraints on our surface reaction, in which case, we would specify something like this – $A' + B'' \rightarrow C''' + D''''$. Each molecule is in an orientation class of its own, and cannot see the orientations of the other molecules, therefore A and B can react in any orientation to produce C and D in random orientations. Another way to say that we don't care about the orientation, would be to use a semi colon after the molecule name, e.g. A; or to use both a comma and an apostrophe, since these add up, e.g. A', or A',

Here is another example where we use absolute orientations: $A' + B' @ surf' \rightarrow C,,$

An outward facing molecule of A, binds an outward facing molecule of B – i.e. they are both aligned with the surface normal of surf, to produce a molecule of C in a random orientation with respect to the surface, i.e. top up or top down.

Exercise: A simple model of surface receptors aggregating in lipid rafts

Create a simplified model of receptors that diffuse over the surface of the cell and collide with cholesterol molecules in lipid rafts, as a consequence of which they slow down and form clusters. A simplified reaction scheme for this system could be



Here Rf represents a fast moving receptor, Rs represents a slow moving receptor, and chol represents a cholesterol molecule. The apostrophes are used to indicate the orientation of the surface molecules. This reaction simply says that a fast moving receptor molecule pointing outwards, collides with a cholesterol molecule pointing outwards, and becomes a slow moving receptor molecule, still pointing outwards.

You may use the following steps and refer to the figures for guidance:

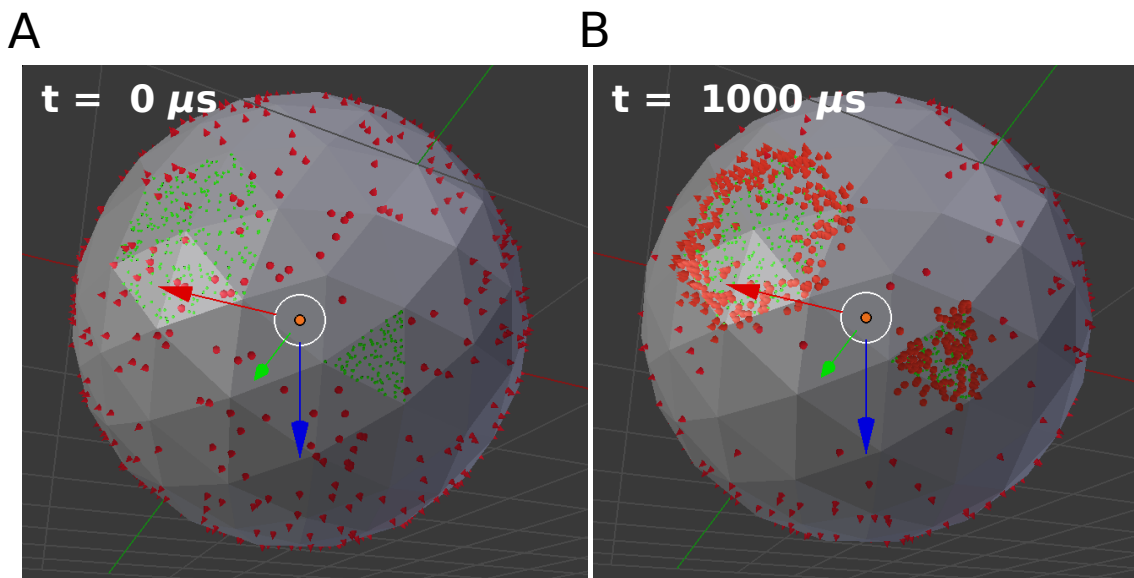
1. Create an icosphere to represent a cell, and add it to your model. Increase the number of subdivisions to 3 to increase the number of triangles and result in a more spherical appearance.
2. Define molecules Rf, Rs and chol, with diffusion coefficients of $1e-6 \text{ cm}^2 \text{ s}^{-1}$, $1e-6 \text{ cm}^2 \text{ s}^{-1}$ and $1e-9 \text{ cm}^2 \text{ s}^{-1}$ respectively.
3. Create a lipid raft named **raft1** by selecting 6 triangles to form a hexagon, and add the region to your model. To make sure that cholesterol molecules in the raft cannot diffuse out, we must make the boundaries reflective to cholesterol. Create a surface class with this property and assign it to the raft.
4. Use the same procedure as in step 2 to create another raft named **raft2**, some distance away from the first comprising just one triangle. Note that you can assign your previously defined surface class to this region as well.
5. Define the reactions specified previously, with rate constant of $1e8$ and $1e4$ for the bimolecular and unimolecular reactions respectively.
6. Release 250 molecules of cholesterol on the bigger lipid raft, 100 molecules of cholesterol on the smaller lipid raft, and 1000 fast receptor molecules distributed throughout the surface.

Note: To specify a specific surface region as a molecule placement site, enter ObjectName[SurfaceRegionName] in the Object/Region field, e.g. Icosphere[raft1]

7. Run your simulation for 1000 iterations at a time step of $1e-5$.
8. Reload the visualization data. Before playing your simulation, go to the molecule display settings and make sure that you can see all your molecules. If you want, you may choose identical settings for Rf and Rs since these are meant to represent the same molecule moving at two different speeds. When you play your simulation you should see the receptors diffusing around, and getting caught near lipid rafts where they slow down and start to cluster. This is another example of a phenomenon that would have been lost in a non-spatial deterministic simulation.

Exercise: You may have noticed that the reaction scheme in our previous model was extremely simplified and perhaps not biologically realistic. Modify the model to include a reaction scheme that is a little more realistic.

Figure 13. Simulation results for the model of surface receptors aggregating in lipid rafts. (A) Snapshot of simulation movie at $t = 0$, showing cholesterol molecules in green sequestered to the regions defined as lipid rafts, and receptor molecules in red distributed homogeneously across the surface of the icosphere representing the cell. (B) Snapshot of simulation movie at $t = 1000 \mu s$ showing the receptor molecules aggregating in the lipid rafts as a consequence of the choice of reaction scheme wherein these molecules collide with cholesterol molecules and slow down.

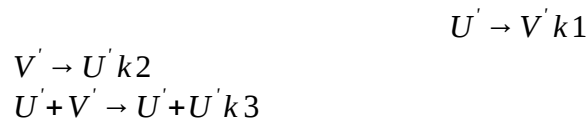


5.5 Extended example: a density dependent switch

Clustering of species is always a good candidate for a spatial model, as there is a clear visible spatial element that would have been lost in an ODE simulation that did not consider diffusion and only reported reaction output. In the previous section, we simulated an example of a mechanism that lead to clustering of receptors on the surface of a cell. Now we shall consider another example of a simple clustering mechanism, adapted from Jilkine et al., “A Density-Dependent Switch Drives Stochastic Clustering and Polarization of Signaling Molecules.” [10]

The model uses a simple positive feedback loop to achieve receptor clustering when the system is diffusion limited. By varying the density of our molecules, we can change whether the system is reaction or diffusion limited, and can achieve qualitatively different behaviors from the system.

The reaction scheme is as follows:



U and V are active and inactive forms of a surface molecule respectively. U is a slow moving molecule with a diffusion coefficient of $1e-10 \text{ cm}^2 \text{ s}^{-1}$, while V is fast, and has a diffusion coefficient of $1e-7 \text{ cm}^2 \text{ s}^{-1}$. U can spontaneously transform into V and vice versa at a rate of 40 s^{-1} and $1e-5 \text{ s}^{-1}$ respectively. In addition a molecule of U may react with a molecule of V to produce two molecules of U, and this bimolecular surface reaction takes place at a rate of $40 \text{ } \mu\text{m}^2 \text{ s}^{-1}$.

Exercise: Define a plane surface of area $16 \text{ } \mu\text{m}^2$ centered at the origin. Release 5 molecules of U and 395 molecules of V on to your surface by defining a release site for each molecule. Define the reaction scheme and parameters as mentioned above, and run your simulation for 1e5 iterations with a time step of 1e-5 seconds. Play your simulation. What do you see?

Hint: You should see the initial U molecules acting like seeds, and clusters forming around them, while the V molecules diffuse across the whole surface.

Exercise: Decrease the area of your plane from $16 \text{ } \mu\text{m}^2$ to $1 \text{ } \mu\text{m}^2$, and rerun your simulation. What do you see?

Hint: When the density is high enough, we should see a more homogeneous distribution of U molecules, as the simulation is no longer diffusion limited with respect to U.

Exercise: Now increase the surface area to $1600 \text{ } \mu\text{m}^2$ and rerun your simulation. You should see the molecule U become extinct.

Thus simply by changing the area in which our molecules are confined, we can alter the system behavior and produce extinction, clustering, and a homogeneous distribution of

molecule U. If you have performed all your steps correctly, your results should resemble the simulation snapshots shown in Figure 13.

Figure 13. Simulation results for the density dependent switch. Density increases from left to right. The top row shows simulation results at $t = 0$, and the bottom row shows simulation results at $t = 1$ s. (A) At low densities the active species goes extinct (B) At intermediate densities there is clustering of the active species (C) At high densities the active species is dominant, and is relatively homogeneously distributed across the plane.

