Simulations of Vesicular Disentanglement

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Abstract

As part of the European Horizon 2020 project ACDC, a chemical compiler is being developed that allows the self-assembly of artificial, three-dimensional, vesicular structures to be first simulated and then translated into reality. This work reports on simulations that shed light on an important aspect: How to disentangle inter-vesicular connections?

Introduction

The scenario to be discussed in this extended abstract is illustrated in Fig.1. Two vesicles are joined by a sticker mechanism based on specific DNA-DNA interactions (Hadorn and Eggenberger Hotz (2010); Hadorn et al. (2012); Schneider et al. (2021)). The goal is to introduce a mechanism that makes this process reversible. The motivation for doing so is to correct possible errors, separate parts from a larger assembly, or create empty spaces in larger clusters Weyland et al. (2020).

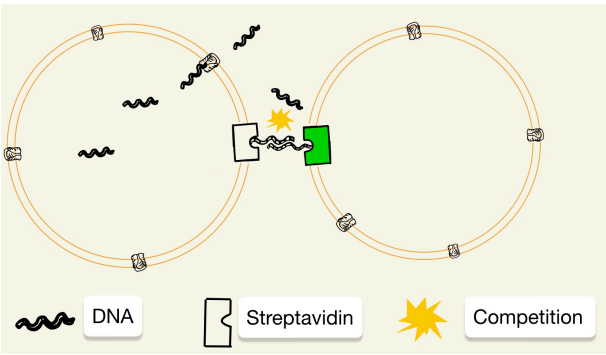

Figure 1: Two vesicles linked with streptavidin-DNA-linkers together. The incorporated pores allow the passage of DNA-strands designed to compete with the already linked ones and liberate the vesicle from its partners.

Therefore, mechanisms have been devised in which DNA tags can leave a vesicle and compete with the pre-existing DNA strands connecting the two vesicles. If the interaction of the exiting DNA is higher than that of the bound DNA, competition occurs which, if strong enough, can free the vesicle from its partners.

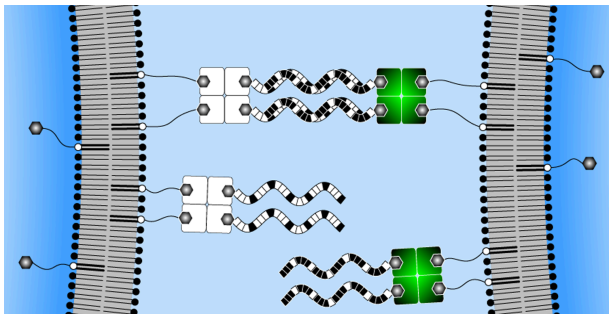

Figure 2: The protein Streptavidin (white and green squares) is anchored to the vesicular membrane with biotinylated poly-ethylen-glycol (PEG), which is linked to phospholipids (amphiphilic molecules building up the membrane). The DNA-strands (wavy lines) are linked to the Streptavidin and can bind to each other by specific DNA-DNA interactions.

The details of the linking mechanism are explained in Fig. 2. Chemicals that interfere with the specific linkers have been developed and the incorporation of them into the vesicles is done by the Pautot-Weitz-Method(Pautot et al. (2003)). The vesicles are equipped with pores that allow competing molecules to diffuse into the environment and compete with the established linkers. Using nucleic acid snippets (DNA, RNA), it is possible to shape the interaction energy between the different vesicles and ensure that the competing free snippet also breaks the existing links.

The following steps were performed to produce a sticker that can be used to selectively bond two vesicles together: Polyethylene glycol (PEG) is a non-toxic polymer with the general molecular formula $C_{2n}H_{4n} + 2O_{n+1}$. PEGs can be anchored in vesicular membranes and have a biotin at their end. The protein streptavidin has a high affinity for

biotin and is used to bind it to biotinylated PEGs, which are anchored in the vesicular membrane. The DNA strands (wavy lines) are linked to the streptavidin molecule. The use of PEG to link the vesicles has the advantage of reducing non-specific binding of the vesicles due to entropic effects. If two vesicles are now equipped with DNA strands that can specifically link to each other via hydrogen bonds, they can attach to each other. In the process, the vesicles deform due to the adhesion forces and a flat adhesion zone is formed. Unbound stickers can diffuse freely on the surface of the vesicles.

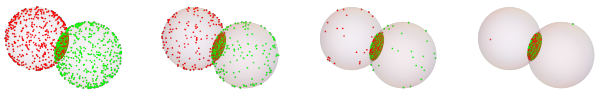

Figure 3: The linkers can diffuse freely in the vesicular membrane until they find a partner of the other vesicle to link and get trapped in the adhesion zone between the two vesicles.

A simulation was developed for the competition of the snippets with already existing links. The conservation of mass and the reactions lead to the following set of equations:

$$\begin{aligned} \frac{OL[t]}{dt} &= k1 * FL[t] * C2[t] - k2 * OL[t] \\ \frac{NL[t]}{dt} &= k3 * FL[t] * C1[t] - k4 * NL[t] \\ FL[t] &= 1.0 - NL[t] - OL[t] \end{aligned}$$

OL is the concentration of DNA-DNA linkers between the bound vesicles. As explained in Fig. 3, the linkers are free to diffuse on the surface of their vesicles until they find a partner on the other vesicles and become trapped between the vesicles. As a result, a linker zone is formed between the vesicles. We assume that all available stickers are in this zone. NL are the linkers where the old linker OL is replaced by the new one. NL is the concentration of successfully replaced linkers by the competing DNA snippet from inside the vesicle. Since the DNA-DNA interactions can also break down, there is also a concentration of free linkers, which we denote by FL. The number of all streptatividin molecules on the two vesicles remains constant and is the sum of $FL + NL + OL = const$. We set this constant to 1.0. [1]

Results and Discussion

The results in Fig. 4 are intuitively clear. The binding energy of the DNA-DNA interaction must be higher for the competing DNA strand than for the old interactions. What may not be so obvious is that the rate of resolution of the DNA-DNA interactions is also important (see Fig. 5): the higher the interaction energy of the old linkers, the longer one has to wait until equilibrium is reached. To obtain a working experiment, one should design the system with a rather weak interaction energy between the DNA-DNAs, just enough to keep the vesicles together and ensure a short waiting time until equilibrium is reached. This reflects an important design mechanism in nature: developmental processes often rely on a large number of weak interactions instead of a few strong ones, which has the advantage that the processes can be easily adjusted because changing a single interaction requires little energy.

[1] All the simulations were performed with Mathematica®, a product of Wolfram Inc.

Acknowledgements

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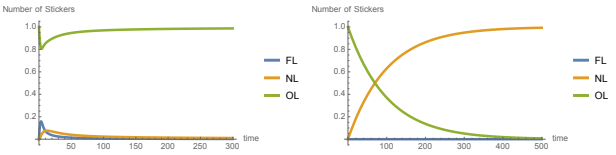

Figure 4: **Left**: The competition is weak (k1 smaller than k3) and the old linker concentration does change only slightly. NL = 0.0106716 OL = 0.987037 FL = 0.00229118 (equilibrium values)). **Right**: The competition is strong (k1 bigger than k3) and the old linkers is displaced by the new one.

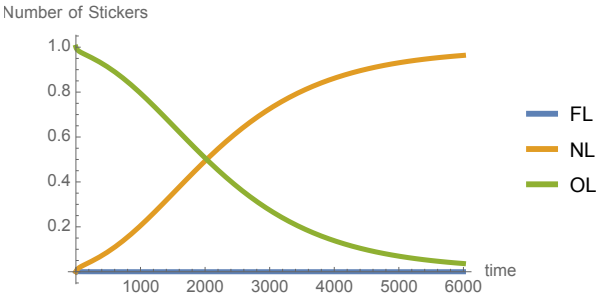

Figure 5: If the binding of the weaker linker is very high, the experimentalist has to wait a long time until equilibrium is reached. k1 = 0.5 k2 = 0.0001 k3 = 0.1 k4 = 0.001 NL = 0.963488 OL = 0.0365123 FL = 1.27385 $\times$ 10^-7 (equilibrium values)

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