Towards tailored communication networks in assemblies of artificial cells

Hadorn, M; Burla, B; Eggenberger Hotz, P

Towards Tailored Communication Networks in Assemblies of Artificial Cells

Maik Hadorn[1], Bo Burla[2,3] and Peter Eggenberger Hotz[1,4]

[1] University of Zurich, Department of Informatics, Artificial Intelligence Laboratory, 8050 Zurich, Switzerland
[2] University of Zurich, Institute of Plant Biology, 8008 Zurich, Switzerland
[3] Global Research Laboratory, 790784 Pohang, South Korea
[4] University of Southern Denmark, The Mærsk Mc-Kinney Møller Institute, Campusvej 55, 5230 Odense M, Denmark
hadorn@ifi.uzh.ch, bburla@botinst.uzh.ch, eggen@mmmi.sdu.dk

Abstract. Living Technology is researching novel IT making strong use of programmable chemical systems. These chemical systems shall finally converge to artificial cells resulting in evolvable complex information systems. We focus on procedural manageability and information processing capabilities of such information systems. Here, we present a novel resource-saving formation, processing, and examination procedure to generate and handle single compartments representing preliminary stages of artificial cells. Its potential is exemplified by testing the influence of different glycerophospholipids on the stability of the compartments. We discuss how the procedure could be used both in evolutionary optimization of self-assembling amphiphilic systems and in engineering tailored communication networks enabling life-like information processing in multicompartment aggregates of programmable composition and spatial configuration.

Keywords: Living Technology, self-assembly, programmability, glycerophospholipids, vesicles, multivesicular aggregates, adhesion plaque, phase transition

1 Introduction

Engineering Living Technology from non-living materials has attracted particular attention in minimal life and complex information systems. As part of the complex systems Future Emerging Technologies initiative, PACE (Programmable Artificial Cell Evolution) was researching novel Information Technology (IT) that makes strong use of life-like properties such as robustness, homeostasis, self-repair, self-assembly, modularity, self-organization, self-reproduction, genetic programmability, evolvability, complex systems design, and bootstrapping complexity. In this context, PACE has created the foundation for a new generation of embedded IT to build evolvable complex information systems using programmable chemical systems that converge to artificial cells [1]. Because experiments were realized both in the laboratory and in

simulation, findings of *in vitro* and *in silico* experiments interacted and lead to essential additions to the evolutionary approach in design of laboratory experimentation [2].

According to the guidelines of PACE, the creation of simple forms of life from scratch in the laboratory should have pursued a bottom-up strategy choosing simple organic compounds of low molecular weight over highly evolved polypeptides. Complexity of an artificial cell featuring all aspects of a living system should have been achieved in an evolutionary process. It is hypothesized that a membrane partitioning internal constituents off the environment might have been one of the minimal requirements for living systems to arise [3, 4]. A lipid membrane represents a formidable barrier to the passage of polar molecules. It organizes biological processes by compartmentalizing them. Compartmentalization enables segregation of specific

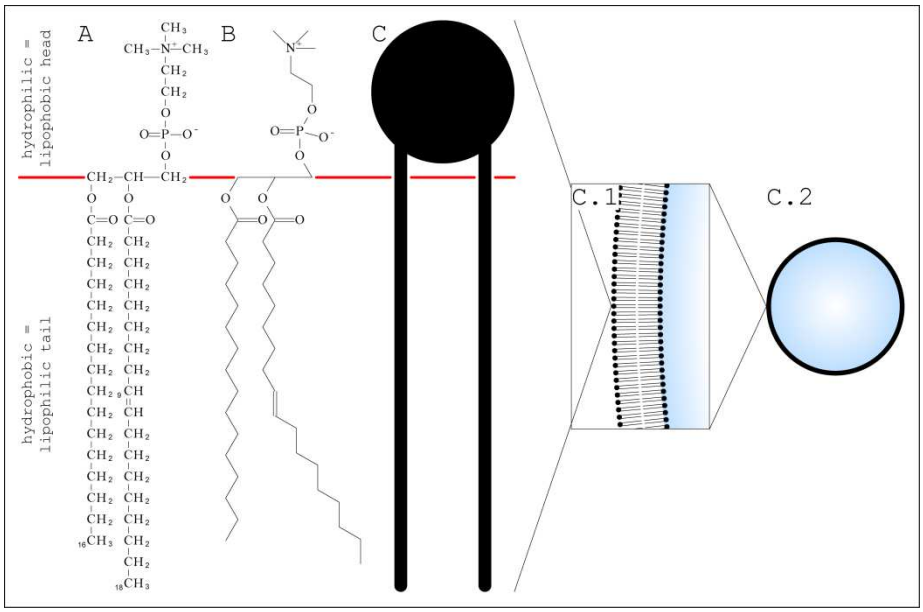

Fig. 1. Structure of glycerophospholipids and bilayer formation. The structure of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (PC(16:0/18:1(Δ9-Cis)) is represented (*A*) as a structural formula, (*B*) as a skeletal formula, and (*C*) as a schematic representation used throughout this publication. Glycerophospholipids are amphiphilic molecules with lipophilic hydrocarbon "tails" and hydrophilic "heads". In PC(16:0/18:1(Δ9-Cis) the headgroup is phosphatidylcholine; a saturated C_{16} palmitic acid (16:0) hydrocarbon chain and a monounsaturated C_{18} oleic acid (18:1) hydrocarbon chain occur at the C1 and C2 position of the glycerophospholipid and constitute the tail. The double bond (monounsaturation) occurs between the C9 and C10 atoms (Δ9) of the oleic acid, has cis configuration, and puts a rigid 30° bend in the hydrocarbon chain. (*C.1*) Phospholipids can form lipid bilayers (membranes) that partition an aqueous compartment off the surrounding medium. (*C.2*) In vesicles an *intra*vesicular fluid (light blue) is separated from the *inter*vesicular medium (white).

chemical reactions for the purposes of increased biochemical efficiency by restricted dissemination and efficient storage of reaction products. This partitioning is not only

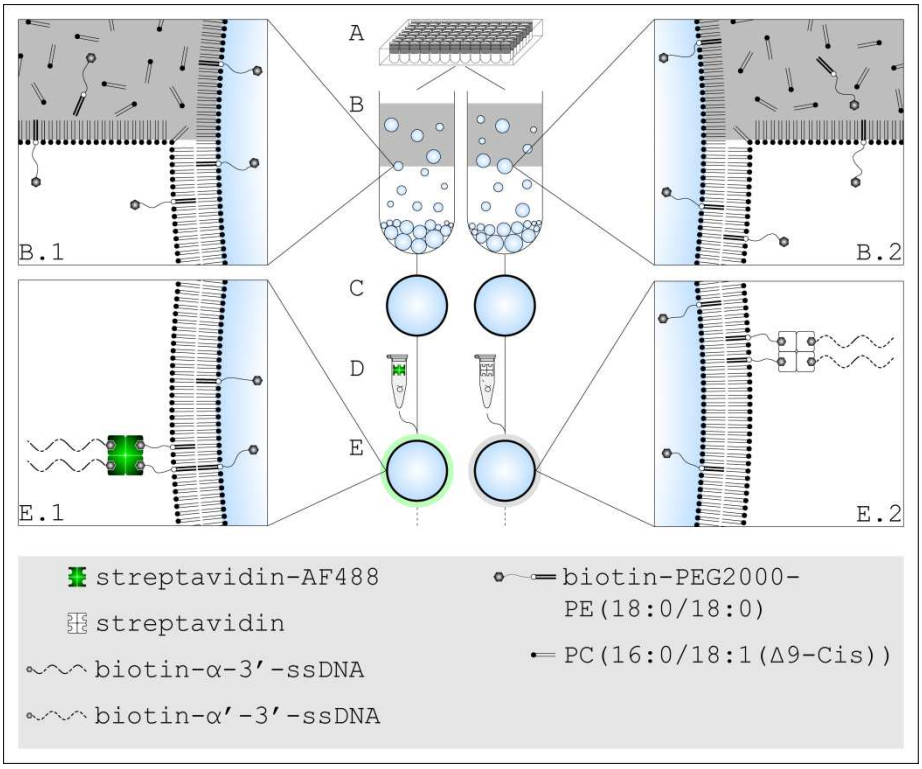

Fig. 2. Schematic representation of the parallel vesicle formation and membrane doping procedure. (*A*) Vesicles are produced in 96-well microtiter plates, providing parallel formation of up to 96 distinct vesicle populations. (*B*) The sample is composed of two parts: water droplets (light blue) in the oil phase (light gray) and the bottom aqueous phase (white), which finally hosts the vesicles. (*B.1*,*B.2*) Due to their amphiphilic character, glycerophospholipids (PC(16:0/18:1(Δ9-Cis)), biotin-PEG2000-PE(18:0/18:0)), solved in mineral oil, stabilize water-oil interfaces by forming monolayers. Two monolayers form a bilayer when a water droplet, induced by centrifugation, passes the interface. Glycerophospholipids are incorporated into the bilayer according to their percentage in the solution. Due to both the density difference of the *intra*- and *inter*vesicular fluid and the geometry of the microplate bottom, vesicles pelletize in the centre of the well (cp. *B*). (*C*) Vesicles remain in the same microtiter plate during formation and membrane doping. (*D*) Vesicle populations become distinct by incubating them with single stranded DNA (ssDNA) of different sequence (α: biotin-TGTACGTCACAACTA-3', α': biotin-TAGTTGTGACGTACA-3') and streptavidin differing in fluorescence labeling (Alexa Fluor 488 conjugate (AF488) or unlabeled). (*E.1*,*E.2*) ssDNA covalently bound to biotin is non-covalently linked to phospholipid-grafted biotinylated polyethylene glycol tethers (biotin-PEG2000-Phosphoethanolamine) using streptavidin as cross-linking agent.

realized between the cell and its environment, but it is even recapitulated within the cell. Vesicles, as an instance of minimality, feature an aqueous compartment partitioned off the surrounding by an impermeable lipid membrane. Like cellular membranes, vesicular membranes consist of amphiphilic phospholipids that link a hydrophilic "head" and a lipophilic "tail" (Fig. 1). Suspended phospholipids can self-assemble to form closed, self-sealing solvent-filled vesicles that are bounded by a two-layered sheet (a bilayer) of 6 nm in width, with all of their tails pointing toward the center of the bilayer. This molecular arrangement excludes water from the center of the sheet and thereby eliminates unfavorable contacts between water and the lipophilic (= hydrophobic) tails. The lipid bilayer provides inherent self-repair characteristics due to lateral mobility of its phospholipids. Wide usage of artificial vesicles is found in analytics [5-9] and synthetics, where their applications include bioreactors [10-12], and drug delivery systems [13-17].

In the laboratory work, we focused on intrinsic information processing capabilities of vesicles and multivesicular assemblies. To achieve compartmentalization we developed methods to self-assemble multivesicular aggregates of programmable composition and spatial configuration composed of distinct vesicle populations that differ in membrane and *intra*vesicular fluid composition. The assembly process of multivesicular aggregates was based on the hybridization of biotinylated single-stranded DNA (ssDNA) with which the vesicles were doped. Doping was realized by anchoring biotinylated ssDNA to biotinylated phospholipids via streptavidin as a cross-linking agent (Fig. 2). The potential of a programmable self-assembly of superstructures with high degrees of complexity [18] has attracted significant attention to nanotechnological applications in the last decade. So far, cross-linkage based on DNA hybridization was proposed to induce self-assembly of complementary monohomophilic vesicles [5, 19] or hard sphere colloids [20-23], to induce programmable fusion of vesicles [5, 24], or to specifically link vesicles to surface supported membranes [5, 25-27]. By introducing a surface doping of distinct populations of ssDNA, as realized in the self-assembly of hard sphere colloids [28, 29], we provide n-arity to the assembly process. As a result, linkage of more than two distinct vesicle populations, as proposed by Chiruvolu *et al.* [30] and already realized for hard sphere colloids [31], becomes feasible.

Concerning procedural manageability in laboratory experimentation, we established a new protocol for *in vitro* vesicle formation and modification. It increases the versatility of the underlying vesicle formation method [11, 32, 33] by introducing microtiter plates and vesicle pelletization (Fig. 2). The potential of the vesicle formation method that provides independent composition control of the *intra*- and *inter*vesicular fluid as well as of the inner and outer bilayer leaflet was exemplified by the production of asymmetric vesicles combining biocompatibility and mechanical endurance in asymmetric vesicles [32]. Here, we exemplify the advantages of the novel protocol by carrying out a high-throughput analysis of constituents affecting vesicle formation and stability. Thereby the effect of nine different glycerophospholipids on vesicle formation was tested. We discuss how this procedure could be used both in evolutionary optimization of self-assembling amphiphilic systems and to realize tai-

lored communication networks in assemblies of artificial cells by programmable localization of glycerophospholipids within the vesicular membrane.

2 Material & Methods

Major technical modifications of the vesicle formation protocol reported in Ref. [32] were: the introduction of (i) 96-well microtiter plates U96 to provide a high-throughput analysis and (ii) a density difference between *intra*- and *inter*vesicular solution to induce vesicle pelletization. For a description of the modified vesicle protocol see Fig. 2. To analyze the effect of different glycerophospholipids on vesicle formation, data on vesicle yield of four times nine equimolar mixtures of 100%-m (m ∈ {100, 50, 10, 1}) PC(16:0/18:1(Δ9-Cis)) and m% PC(x:0/x:0) (x ∈ {12, 14, 16, 18, 24}), PC(y:1(Δ9-Cis)/y:1(Δ9-Cis)) (y ∈ {14, 16, 18}), or PC(24:1(Δ15-Cis)/24:1(Δ15-Cis)) were collected and compared to 100%-m PC(16:0/18:1(Δ9-Cis)) and m% mineral oil (solvent for all glycerophospholipids) as control. Vesicle formation was performed in duplication. Length of circumference of the vesicle pellet is used as a measure of vesicle yield (Fig. 3). Light-microscopy was performed using a Wild M40 inverted microscope equipped with a MikoOkular microscope camera. All camera settings were identical for the recordings. Confocal laser scanning microscopy was performed using an inverted Leica Confocal DMR IRE2 SP2 confocal laser scanning microscope.

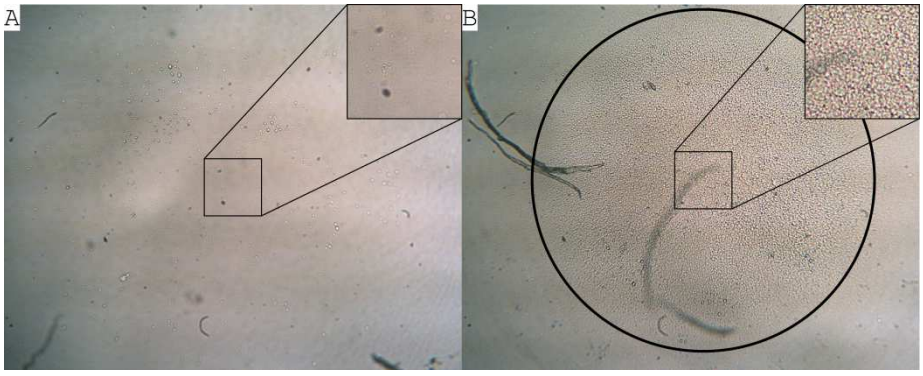

Fig. 3. Measurement of vesicle yield. (*A*) No vesicle pellet emerged for the mixture of 50% PC(24:1(Δ15-Cis)/24:1(Δ15-Cis)) and 50% PC(16:0/18:1(Δ9-Cis)). (*B*) The vesicle yield is expressed by length of circumference of the vesicle pellet (circle) emerged for the mixture 10% PC(24:1(Δ15-Cis)/24:1(Δ15-Cis)) and 90% PC(16:0/18:1(Δ9-Cis)). Fibers represent pollutants not affecting vesicular yield or handling.

3 Results and Discussion

In the literature many examples of artificially produced vesicles are reported, whose membranes are composed of PC(16:0/18:1(Δ9-Cis)) (POPC) exclusively.[1] To vary the intrinsic material properties of membranes we have to be able to alter their phospholipid content. In this study, we realized a high-throughput analysis of glycerophospholipids affecting vesicle formation. All glycerophospholipids differed in length and saturation of their hydrocarbon chains only. In the control experiment, vesicle yield was constant when POPC was present (Fig. 4). Influence of the phospholipids tested at an admixture of one percent was marginal and will not be discussed. Intergroup comparisons (cp. Fig. 4) of saturated and unsaturated glycerophospholipids of equal chain length revealed remarkable differences in vesicle yield. Whereas unsaturated glycerophospholipids of chain length up to 18 carbon atoms both could form vesicles by themselves and did not or just slightly affect the yield at 50 and 10 percent, saturated lipids seem to disturb vesicle formation down to 10 percent of admixture. For a chain length of 24 carbon atoms the situation is diametrical. A small amount of vesicles is found for solutions containing 100 percent saturated PC(24:0/24:0). Whereas vesicle yield is halved at 50 percent for this lipid, vesicle formation is inhibited totally by the unsaturated PC(24:1(Δ15-Cis)/24:1(Δ15-Cis)) up to an admixture of 10 percent (cp. Fig. 3). Intragroup comparison within saturated or unsaturated glycerophospholipids reveals a decrease in vesicle yield depending on chain length (except for PC(24:0/24:0) at 100 percent). Only a limited number of glycerophospholipid is able

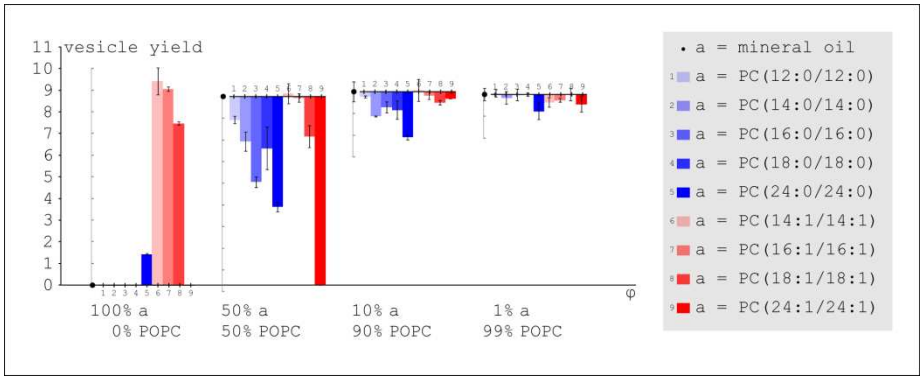

Fig. 4. Effect of different glycerophospholipids on vesicle formation. Vesicle yield of 100%-m (m ∈ {100, 50, 10, 1}) POPC = PC(16:0/18:1(Δ9-Cis)) and m% mineral oil serves as standard (XY (scatter) chart). Deviations from control for the glycerophospholipids tested are presented in absolute values as bar charts. Error bars indicate the standard deviation. The glycerophospholipids tested can be summarized according to the level of saturation of their hydrocarbon chains in two groups (saturated, PC(x:0/x:0) (x ∈ {12, 14, 16, 18, 24}); unsaturated, PC(y:1(Δ9-Cis)/y:1(Δ9-Cis)) (y ∈ {14, 16, 18}), PC(24:1(Δ15-Cis)/24:1(Δ15-Cis))).

[1] In 2008 more than 150 articles were published concerning POPC vesicles or liposomes.

to form vesicles on their own or in cooperation with POPC.

By providing parallelism in vesicle formation, processing and examination (cp. Fig. 2), we not only increased the experimental throughput and the procedural manageability, but we lowered the costs and reduced the amount of contributory factors. This reduction of dimensionality and the parallelism in vesicle formation provided by the novel vesicle formation method presented herein may prove to be useful in evolutionary design of experiments by shortening the search toward the optimality region of the search space [2]. The application of microtiter plates may further enable the automatization in vesicle formation.

By self-assembling multivesicular aggregates of programmable composition and spatial configuration, composed of vesicles differing in membrane and *intra*vesicular fluid composition, an inhomogeneous distribution of information carriers is achieved

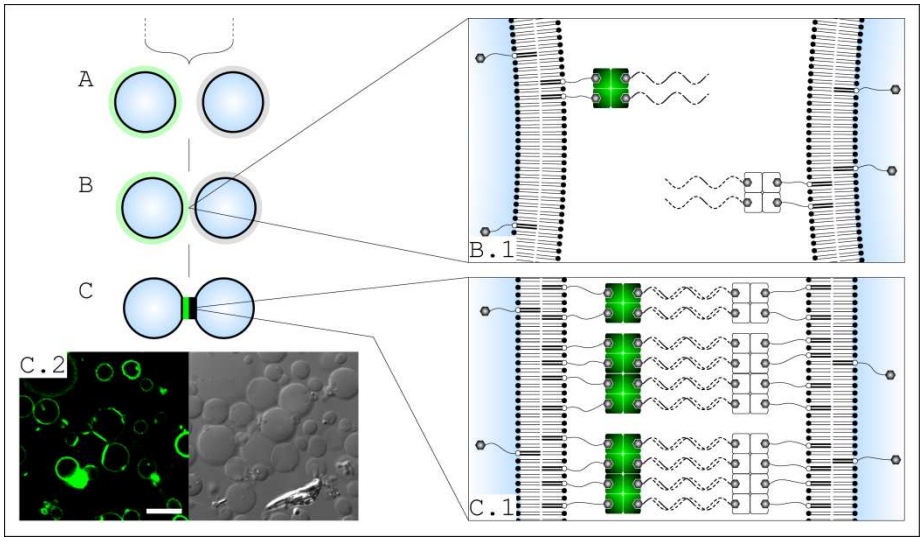

Fig. 5. Schematic representation of the self-assembly process and micrographs of adhesion plaques. (*A*) For vesicle formation, membrane doping and illustration symbols see Fig. 2.Two distinct vesicle populations are merged (brace). (*B*) The lateral distribution of linkers in the lipid membrane is homogeneous. (*B.1*) Vesicles doped with complementary single stranded DNA come into contact. (*C*) Hybridization of DNA strands results in double stranded DNA and induces the assembly process. Due to their lateral mobility, linkers accumulate in the contact zone forming an adhesion plaque – the lateral distribution of linkers in the outer leaflet becomes inhomogeneous. (*C.1*) Biotinylated phospholipids (biotin-PEG2000-PE(18:0/18:0)) colocalize with the linkers. Even though the lateral distribution of phospholipids in the inner leaflet is not affected, the membrane composition *intra*-adhesion-plaque (by accumulation) and *inter*-adhesion-plaque (by depletion) becomes different. (*C.2*) CLSM (confocal laser scanning microscope) and DIC (differential interference contrast) micrograph of a vesicular aggregate. Accumulation and depletion of linkers are clearly visible in the CLSM micrograph. Scale bar represents 10μm.

(Fig. 5). To build complex information systems, the exchange of information carriers between the compartments themselves and/or between the environment and the compartments has to be realized. Biological membranes contain membrane proteins that were evolutionary optimized for catalyzing numerous chemical reactions, mediating the flow of nutrients and wastes, as well as participating in communication across the membrane. The use of such membrane proteins in information exchange was excluded by the guidelines of PACE. Thus, information processing capabilities of multivesicular aggregates had to be realized by exploiting intrinsic material properties of the phospholipid membrane. A key property of the lipid membrane is its phase [34]. Lipid bilayers can undergo phase transitions in which they become a gel-like solid and therefore lose their fluidity. The state depends on temperature; the transition temperature of a bilayer increases with the chain length and the degree of saturation of its fatty acid residues. The phase transitions are triggered externally by changing temperature; a fact exploited in exchanging reactants between the surrounding medium and a vesicular compartment setting up consecutive enzymatic reactions in a single container [35]. A selective exchange of information between compartments of a multivesicular aggregate relying on inherent material properties of phospholipid membranes has not been demonstrated so far. We recognized that the formation of adhesion plaques [36, 37] triggered by the aggregation process results in an inhomogeneous distribution of phospholipids in the lateral dimension of the membrane (Fig. 5). This inhomogeneity in phospholipid distribution causes a difference in phase transition characteristics of membrane portions *intra*- and *inter*-adhesion-plaque. Selective opening of communication channels between the compartments, whereas the membrane portions *inter*-adhesion-plaques are still impermeable, becomes conceivable. By using membrane anchors of different phospholipid composition the adhesion plaques would differ in phase transition characteristics among each other enabling programmable and triggerable communication networks in multivesicular aggregates of programmable composition and spatial configuration.

Realization of such tailored communication networks is hindered by restrictions in commercial availability of lipid anchors to dope vesicular surfaces with ssDNA. The high-throughput analysis presented herein allows for identification of lipid candidates not affecting vesicle formation and differing in phase transition characteristics. In a next step the head group of these candidates could be covalently linked to biotinylated PEG tethers therefore providing anchors for biotinylated ssDNA (via (strept-)avidin).

Acknowledgements. This work was conducted as part of the European Union integrated project PACE (EU-IST-FP6-FET-002035). Maik Hadorn was supported by the Swiss National Foundation Project 200020-118127 Embryogenic Evolution: From Simulations to Robotic Applications. Peter Eggenberger Hotz was partly supported by PACE. Wet laboratory experiments were performed at the Molecular Physiology Laboratory of Professor Enrico Martinoia (Institute of Plant Biology, University of Zurich, Switzerland).

References

1. Rasmussen, S.: Protocells: bridging nonliving and living matter. MIT Press, Cambridge, Massachusetts (2009)
2. Forlin, M., Poli, I., De March, D., Packard, N., Gazzola, G., Serra, R.: Evolutionary experiments for self-assembling amphiphilic systems. Chemometrics Intell. Lab. Syst. 90, 153--160 (2008)
3. Griffiths, G.: Cell evolution and the problem of membrane topology. Nat. Rev. Mol. Cell Biol. 8, 1018--1024 (2007)
4. Israelachvili, J.N., Mitchell, D.J., Ninham, B.W.: Theory of self-assembly of lipid bilayers and vesicles. Biochimica Et Biophysica Acta 470, 185--201 (1977)
5. Chan, Y.H.M., van Lengerich, B., Boxer, S.G.: Effects of linker sequences on vesicle fusion mediated by lipid-anchored DNA oligonucleotides. Proc. Natl. Acad. Sci. U. S. A. 106, 979--984 (2009)
6. Hase, M., Yoshikawa, K.: Structural transition of actin filament in a cell-sized water droplet with a phospholipid membrane. J. Chem. Phys. 124, 104903 (2006)
7. Hotani, H., Nomura, F., Suzuki, Y.: Giant liposomes: from membrane dynamics to cell morphogenesis. Curr. Opin. Colloid Interface Sci. 4, 358--368 (1999)
8. Limozin, L., Roth, A., Sackmann, E.: Microviscoelastic moduli of biomimetic cell envelopes. Phys. Rev. Lett. 95, 178101 (2005)
9. Luisi, P., Walde, P.: Giant vesicles. John Wiley & Sons, Ltd, Chichester (2000)
10. Michel, M., Winterhalter, M., Darbois, L., Hemmerle, J., Voegel, J.C., Schaaf, P., Ball, V.: Giant liposome microreactors for controlled production of calcium phosphate crystals. Langmuir 20, 6127--6133 (2004)
11. Noireaux, V., Libchaber, A.: A vesicle bioreactor as a step toward an artificial cell assembly. Proc. Natl. Acad. Sci. U. S. A. 101, 17669--17674 (2004)
12. Nomura, S., Tsumoto, K., Hamada, T., Akiyoshi, K., Nakatani, Y., Yoshikawa, K.: Gene expression within cell-sized lipid vesicles. Chembiochem 4, 1172--1175 (2003)
13. Abraham, S.A., Waterhouse, D.N., Mayer, L.D., Cullis, P.R., Madden, T.D., Bally, M.B.: The liposomal formulation of doxorubicin. In: Liposomes, Pt E. Elsevier Academic Press Inc, San Diego (2005)
14. Allen, T.M., Cullis, P.R.: Drug delivery systems: Entering the mainstream. Science 303, 1818--1822 (2004)
15. Marjan, J.M.J., Allen, T.M.: Long circulating liposomes: Past, present and future. Biotechnology Advances 14, 151--175 (1996)
16. Tardi, P.G., Boman, N.L., Cullis, P.R.: Liposomal doxorubicin. J. Drug Target. 4, 129--140 (1996)
17. Sengupta, S., Eavarone, D., Capila, I., Zhao, G.L., Watson, N., Kiziltepe, T., Sasisekharan, R.: Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. Nature 436, 568--572 (2005)
18. Licata, N.A., Tkachenko, A.V.: Errorproof programmable self-assembly of DNA-nanoparticle clusters. Physical Review E (Statistical, Nonlinear, and Soft Matter Physics) 74, 041406 (2006)
19. Beales, P.A., Vanderlick, T.K.: Specific binding of different vesicle populations by the hybridization of membrane-anchored DNA. J. Phys. Chem. A 111, 12372--12380 (2007)

20. Biancaniello, P.L., Crocker, J.C., Hammer, D.A., Milam, V.T.: DNA-mediated phase behavior of microsphere suspensions. Langmuir 23, 2688--2693 (2007)
21. Mirkin, C.A., Letsinger, R.L., Mucic, R.C., Storhoff, J.J.: A DNA-based method for rationally assembling nanoparticles into macroscopic materials. Nature 382, 607--609 (1996)
22. Valignat, M.P., Theodoly, O., Crocker, J.C., Russel, W.B., Chaikin, P.M.: Reversible self-assembly and directed assembly of DNA-linked micrometer-sized colloids. Proc. Natl. Acad. Sci. U. S. A. 102, 4225--4229 (2005)
23. Biancaniello, P., Kim, A., Crocker, J.: Colloidal interactions and self-assembly using DNA hybridization. Phys. Rev. Lett. 94, 058302 (2005)
24. Stengel, G., Zahn, R., Hook, F.: DNA-induced programmable fusion of phospholipid vesicles. J. Am. Chem. Soc. 129, 9584--9585 (2007)
25. Benkoski, J.J., Hook, F.: Lateral mobility of tethered vesicle - DNA assemblies. J. Phys. Chem. B 109, 9773--9779 (2005)
26. Yoshina-Ishii, C., Boxer, S.G.: Arrays of mobile tethered vesicles on supported lipid bilayers. J. Am. Chem. Soc. 125, 3696--3697 (2003)
27. Li, F., Pincet, F., Perez, E., Eng, W.S., Melia, T.J., Rothman, J.E., Tareste, D.: Energetics and dynamics of SNAREpin folding across lipid bilayers. Nat. Struct. Mol. Biol. 14, 890--896 (2007)
28. Maye, M.M., Nykypanchuk, D., Cuisinier, M., van der Lelie, D., Gang, O.: Stepwise surface encoding for high-throughput assembly of nanoclusters. Nat. Mater. 8, 388--391 (2009)
29. Prabhu, V.M., Hudson, S.D.: Nanoparticle assembly: DNA provides control. Nat. Mater. 8, 365--366 (2009)
30. Chiruvolu, S., Walker, S., Israelachvili, J., Schmitt, F.J., Leckband, D., Zasadzinski, J.A.: Higher-order self-assembly of vesicles by site-specific binding. Science 264, 1753--1756 (1994)
31. Xu, X.Y., Rosi, N.L., Wang, Y.H., Huo, F.W., Mirkin, C.A.: Asymmetric functionalization of gold nanoparticles with oligonucleotides. J. Am. Chem. Soc. 128, 9286--9287 (2006)
32. Pautot, S., Frisken, B.J., Weitz, D.A.: Engineering asymmetric vesicles. Proc. Natl. Acad. Sci. U. S. A. 100, 10718--10721 (2003)
33. Träuble, H., Grell, E.: Carriers and specificity in membranes. IV. Model vesicles and membranes. The formation of asymmetrical spherical lecithin vesicles. Neurosciences Research Program bulletin 9, 373--380 (1971)
34. Feigenson, G.W.: Phase diagrams and lipid domains in multicomponent lipid bilayer mixtures. Biochim. Biophys. Acta-Biomembr. 1788, 47--52 (2009)
35. Bolinger, P.Y., Stamou, D., Vogel, H.: An integrated self-assembled nanofluidic system for controlled biological chemistries. Angew. Chem.-Int. Edit. 47, 5544--5549 (2008)
36. NopplSimson, D.A., Needham, D.: Avidin-biotin interactions at vesicle surfaces: Adsorption and binding, cross-bridge formation, and lateral interactions. Biophys. J. 70, 1391--1401 (1996)
37. Farbman-Yogev, I., Bohbot-Raviv, Y., Ben-Shaul, A.: A statistical thermodynamic model for cross-bridge mediated condensation of vesicles. J. Phys. Chem. A 102, 9586--9592 (1998)