From droplets and particles to hierarchical spatial organization: nanotechnology challenges for microfluidics

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Abstract: The compartimentation of fluids in the microliter, nanoliter and picoliter range leads recently to many applications of microfluidics in material development, in diagnostics and biological screenings. Droplet-based microfluidics allows the improvement of nanoparticle homogeneity and the tuning of particle properties. It supports combinatorial synthesis of inorganic as well as organic substances and can be applied for the cultivation and screening of bacteria, eucaryotic cells and fish embryos. The well-ordered handling and the addressing of microfluidssegments improves the information transfer between chemical, biological and electronic systems. Despite this remarkable technical progress, there is a particular importance of microfluidics for future nanotechnological solutions. The hierarchical spatial organization of liquids, particles and gels in microfluidics represents a fundamental biomimetic principle which overcomes the limits of planar technology and opens the gate for realizing complex structured threedimensional nanoarchitectures. Recent applications of microstructured fluids in chemistry and biology and concepts for future developments will be discussed.

Keywords: nanotechnology, microfluidics, nanoparticles, segmented flow, concentration spaces, screening, nested phases, hierarchical organization, droplet-based systems

1 Introduction

Fluid compartimentation is a basic principle in living nature. Many cells and cell organelles represent fluid compartments in the volume range between the sub-femtoliter and the nanoliter level. Liquid droplets in the femtoliter and picoliter range are technically used in a lot of different emulsions. Small droplets are essentially for ink-jet printing and in other recent technologies. But, there is a principle difference between the droplets in emulsion on one side and cells and ink-jet droplets on the other side: Emulsions are statistical ensembles of large numbers of droplets with no exact definition of position and speed and no individuality in their composition. In contrast, cells and cell organelles have a life history, can be distinguished one from each other carrying individual information. The droplets of an inkjet printer are information carrying objects, too. They are marked by a certain time of formation and place for their deposition on paper or and other substrate.

The individuality of cells is mainly driven by their molecularbiological components. They control cell metabolism, cellular motion, the communication between cells and their reproduction. Interaction of cells leads to the formation of larger or smaller cell aggregates up to the building of tissues, organs and multicellular organisms. Each multicellular system represents a hierarchy of liquid compartments. Obviously, the compartimenation of liquids by hierarchically organized assemblies is one of the most fundamental principles in the organization of higher life.

Is the liquid compartmentation a suited natural example for technical systems? The developments in the last years in the field of droplet-based microfluidics [1] and on micro segmented-flow [2-4] suggest a formerly unrecognized power of liquid microcompartmentation in technology. It is related to the possibility to generate and manipulate small droplets and the increasing ability to control and change their composition. Meanwhile, micro devices allow to generate and to transport droplets in the lower microliter, in the nanoliter and the upper picoliter range with high reproducibility. The formation of droplets and their transport behavior through microtubes and micro channels is comparatively well understood [5]. The interface properties can be controlled [6. 7]. The droplet-based microfluidics

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allows to describes the motion of liquid inside the droplets, explains the pressure increase and the effect of interface tensions [8-10]. Droplets can be transformed, split and fused [11, 12]. Droplets can be distinguished by their size, shape and composition. The application reaches from analytical purposes in chemistry [11, 14], over the synthesis of inorganic and organic compounds and nanoparticles of dielectric materials [15, 16], semiconductors and metal nanoparticles [17-33] to a wide variety of biological application. Bacteria [21, 22] and eucaryotic cells [23, 24] as well as small multicellular organisms can be cultivated in microdroplets [25-27]. The micro segmented flow is applied beside other microfluidic methods for DNA amplification by PCR [29, 30] as well as for biochemical applications and bioassays [31, 32] and toxicological screenings [33].

In the following, the characterization and high reproducibility of micro fluid segments will be discussed and the application of this technique in nanoparticle synthesis and toxicological investigations as typical examples will be demonstrated. Beside the progress in micro systems for droplet manipulation and the emerging application fields, the technical systems operating micro droplets are still far away from the complex compartimented fluidic systems of living nature. This is the reason why, finally, visions of further technical developments into direction of hierarchically organized microfluidic systems will be discussed.

2 Experimental

The actuation by a syringe pump is the easiest way for generating sequences of micro fluid segments with high reproducibility. These pumps work nearly independently on the counter pressure, what means the volume flow rate can easily be varied over a large dynamic range. Micro fluid segments can be generated either by T or cross junction or on nozzle-like injectors. In our experiments, micro channels, tube and connectors with an internal diameter of 0.5 mm were used preferentially. PTFE tubings supply suited surface properties for segment generation and transport. If a glass or silicon channel is used for the segment generation and processing of aqueous segments, a hydrophobization of the internal surfaces is required in order to keep the surface wetting low.

In principle, chip devices as wells as tube arrangements are suited for segment flow applications for nanoparticle synthesis. The main important aspects are the precise control of flow rates, the regular formation of fluid segments and the suppression of deposition of particles at the inner wall surfaces and the initiation of nucleation. The last mentioned problem is strongly dependent on the surface properties of the channel or tube material. Silicon or glass chips are critical for metal nanoparticle formation due to their hydrophilic surface. It must be hydrophobized, for example by alkylsilanization. These surface films are often not stable enough for long-time experiments due to the oxidative damaging by the reactants. So, the synthesis in PTFE tube or devices made by similar perfluorinated hydrocarbons is much more convenient. A typical arrangement for the synthesis of binary metal nanoparticles is shown in fig. 1. The both reactant solutions for metal application (colloidal solution of gold seeds and silver nitrate solution), the reducing agent (ascorbic acid) and the carrier liquid tetradecane are actuated by four separately controlled syringe pumps. The segments are formed by injection of the aqueous solution containing the gold seeds into the flow of tetradecane. Then, these primary formed segments passes two further injectors for up-taking small liquid portions of the silver nitrate and the ascorbic acid solution. The amount of added reactants is dependent on the frequency of primary segment formation and the pump rates of the both other liquids. A fast mixing inside the fluid segments is supported by a multi-knot structure of the tubes behind the injector positions.

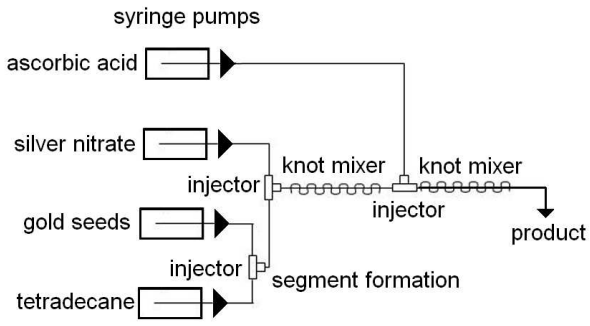

Fig. 1 Experimental arrangement for generation of plasmonic binary Au core/ Ag shell nanoparticles

The fluidic demands for microtoxicological screenings with two or more effectors are different from the requirements for binary nanoparticle formation. Up to six fluid input channels are required. The application of complex composed nutrition solutions and cells makes the repeated feeding of solutions into preformed segments less precisely. This effect is probably due to adsorption of molecules and cells at the walls surfaces and the continuous changes or local fluctuations of the wetting behavior. Therefore, a commercial fluid manifold was applied for segment formation and mixing instead of a serial multi-injector arrangement (fig. 2).

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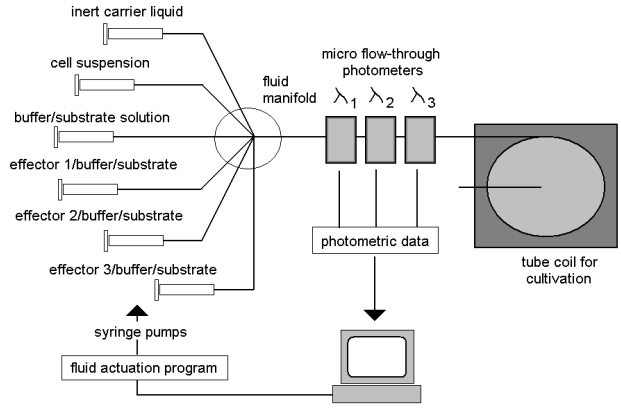

Fig. 2 Experimental arrangement for microtoxicological screenings with bacteria using micro fluid segments

The initial cell suspension, buffer solution and the effector solutions are guided together into the fluid manifold and injected into the carrier liquid (tetradecane or a perfluorinated liquid hydrocarbon). The total of flow rates of all aqueous solutions is kept constant in order to have always the same hydrodynamic conditions for the segment formation. Concentration changes are realized by variation of effector pump rates which are compensated by a complementary change of the flow rate of the buffer solution. The flow rate programs were controlled by PC. The formed micro fluid segment sequences with several hundred up to several thousand well-separated liquid compartments are stored in tube coils and incubated for cell growth. A multi-photometer arrangement allows the process monitoring during the segment formation, for controlling the segment composition and can also be used for the evaluation of cell density after incubation.

3 Experimental examples

Micro fluidics is under focus for the synthesis of high-quality nanomaterials, in general. The synthesis of nanoparticles by micro continuous-flow synthesis using micro fluid segments is a simple, but typical example for the connection between spatial organization in fluids and the formation of well-defined nano-scaled objects. The simple principle of a linear order of droplets in a chain leads to constant and reproducible convection patterns and, therefore, to a suppression of random motion and to steady in mixing and reaction initiation. The fundamental difference between a conventional large reaction vessel and a micro fluidic system is less to see in the volume reduction and the improved conditions for fast transfer of matter and heat. These factors are very important for improving the chemical process engineering and to find new process windows. But, they are not mainly responsible for changing the principle state of organization in the system. In this sense, the introduction of a spatial arrangement and operation of larger groups of liquid portions is the much more fundamental step. This step is marked by the appearance of an “individuality” of the small liquid portions. They are marked by their time of formation, by their position in the sequence and can be distinguished in time and space and- if necessary - in composition from the other liquid portions.

For the synthesis of homogeneous nanoparticles, only a low level of organization is required. The individuality of micro fluid segments is not necessary. Constant size, constant distance, constant composition and constant transport conditions are sufficient for the realization of identical mixing and reaction conditions in all liquid compartments. The high reproducibility of convective and reaction conditions is the key feature for the Segmented Flow Tubular Reactor SFTR [34]. Different nanomaterials could be synthesized with very high homogeneity by this method. This principle was also successfully applied in a down-scaled version for synthesis of nanoparticles in droplets in the nanoliter and sub-nanoliter range. So, quantum dots as well as plasmonic nanoparticles [35] were synthesized in micro continuous flow.

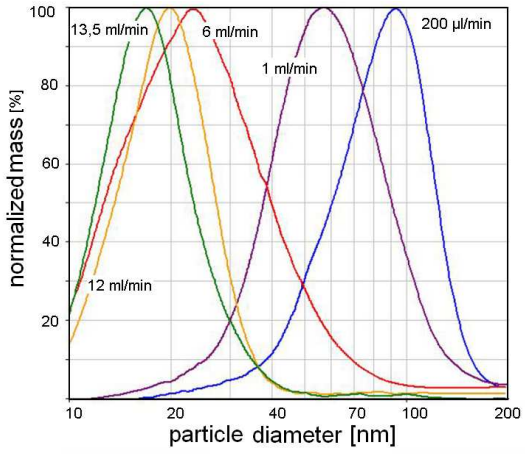

Fig. 3 Size spectra of Au/Ag core/shell nanoparticles generated by micro segmented flow at same reactant concentrations, but different total flow rates

Following features represent the key advantages for micro segmented-flow in nanoparticle synthesis:

- plug flow transport resulting in very narrow residence time distribution
- fast and reproducible mixing by segment-internal convection
- support of fast heat transfer by segment-internal convection

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- suppression of undesired nucleation and particle deposition by decoupling of reaction liquid from the tube wall by the carrier liquid

The reproducible and fast mixing inside the micro fluid segments is of particular importance for the nucleation. The strong effect of mixing on nucleation is reflected by the strong influence of flow rate on the mean size and size distribution of nanoparticles obtained by a segmented-flow synthesis. Plasmonic nanoparticles are of particular interest for labels and biosensors [36-38]. An example for the formation of Au/Ag core/shell particles is shown in fig. 3.

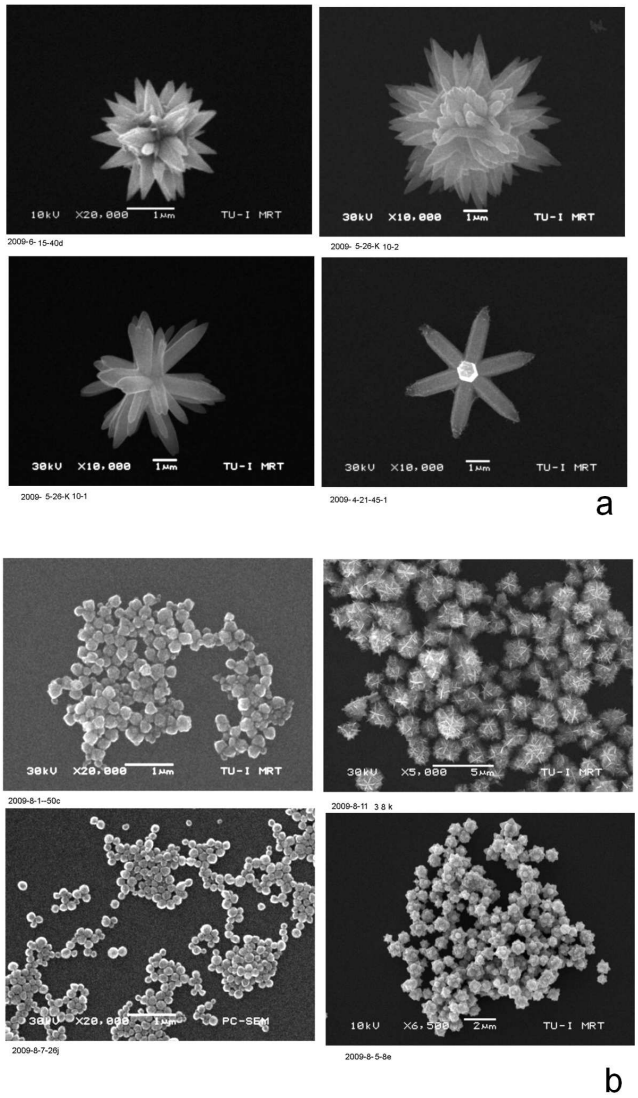

Fig. 4 ZnO micro and nanoparticles obtained from micro segmented flow systems: a) variety of crystal shapes, b) different populations of homogeneous ZnO particles obtained in a hydrothermal continuous-flow synthesis

Zinc oxide represents a material with a very large spectrum of different sizes and shapes of nanoparticles in dependence on the reaction conditions. Fig. 4a gives an idea on the high variability of the material morphology. The application of micro segmented flow leads to particle formation under well-controlled conditions, and a high homogeneity of particles can be obtained. Examples from a hydrothermal synthesis in micro fluid segments under different conditions are shown in fig. 1b.

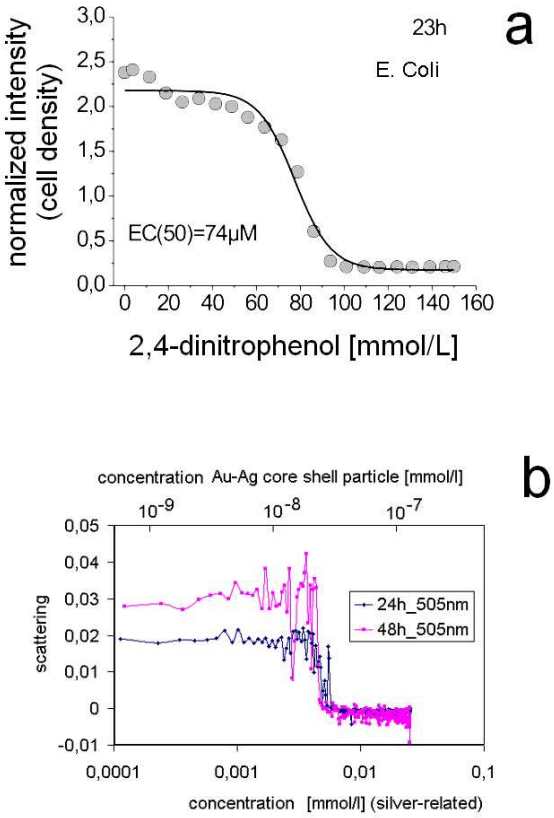

Fig. 5 Microtoxicological screenings: a) example of a dose response function obtained by micro fluid segment technique (effect of 1,4-dinitrophenol on *E. Coli*), b) example of a high-resolved dose/response function in a larger micro fluid segment sequence (effect of gold/silver nanoparticles on *E. Coli*),

The possibility for easy screening of two- or higher-dimensional parameter fields is of large interest for testing of pharmaceuticals and toxicology. The cultivation of cells or mircroorganisms inside the submicroliter fluid compartments allows to combine the effector variation with the cultivation and the monitoring of cell growth. So, it is possible to characterize the cell density inside the fluid segments either by measuring the optical density, by light scattering, by the cellular autofluorescence or by changes in pH or oxygen content. Complete dose/response curves can be determined by a single micro fluid segment sequence with a total volume of less than one milliliter (fig. 5a). The critical concentration (EC(50) = 74 µM for 2,4-dinitrophenol for *E. coli*) found in the microfluidic measurement is in good agreement with the known values. The high number of data points in a single run and the tiny

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concentration steps allow the determination of very high resolved dose/response functions, for example for the effect of silver nanoparticles on bacteria (fig. 5b). In this case, a increase in cell density fluctuations was found if the concentration of the effector was approached to the critical value.

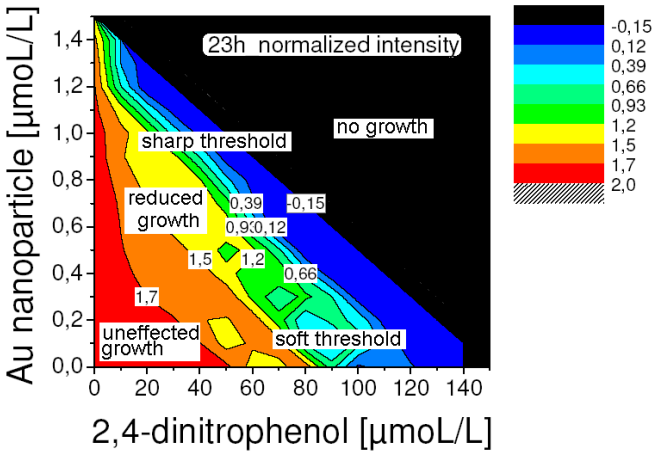

Fig. 6 Combinatorial toxicological effects of gold nanoparticles and a phenolic ancoupler on *E. Coli* reflected by bolographic presentation of dose/response investigations in two-dimensional effector spaces

Although a lot of knowledge on the effect of single substance on cells is available, there is a huge lack of information on the interference between two or more effectors. But, the common action of two or more important substances is the normal case in the environment, for consumption of food, for application of cosmetics and in many medical treatments. The segmented flow technique opens a convenient gate for determination of two-dimensional and higher dose/response functions and for detection of nonlinear combination effects. The method was tested for the effect of a colloidal solution of gold nanoparticles and dinitrophenol on bacteria. It was found that the effect of the noble metal particles must not be neglected. Its strength is strongly related to the concentration of 2,4-dinitrophenol as the second investigated effector (fig. 6). The isobologram shows sharp and weaker transitions for the critical threshold at different combinations of both effectors and illustrate that the dose of a chemical substance or a medical drug can strongly be dependent on the presence of other effectors.

4 Challenges

Micro flow-chemistry can support the solution of several central problems of nanoparticle preparation. This includes the synthesis of particles of high homogeneity and the tuning of particle size (fig. 7).

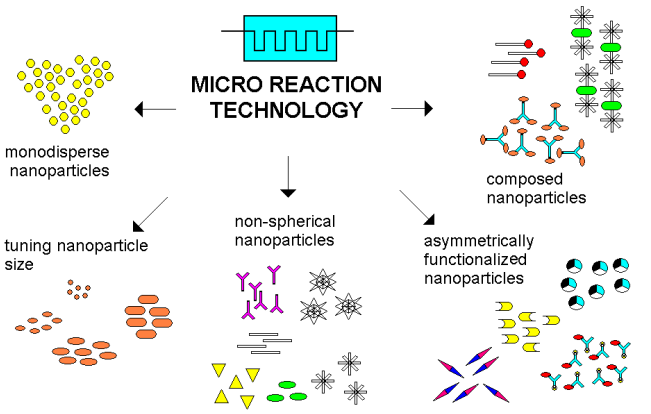

Fig. 7 Tasks of micro fluidics for making nanoparticles

A particular challenge is the preparation of non-spherical nanoparticles with identical shape and size at high yield. Such particles are formed normally by crystal growth under surface-controlled conditions. Beside the chemical composition of the reaction solutions, also the transport conditions and the mechanical stress should be kept constant in order to get a strongly reproducible formation of these particles with complex geometry. The support of formation of particles with asymmetric functionalization is a particular challenge for micro flow chemistry. The generation of particles with two, three or four surface regions with distinguishable surface functions is a precondition for self-assembling nanoparticle architectures. This is one promising strategy for the synthesis of complex composed nanoparticles (fig.7).

The segmented flow technique realizes perfectly the principle of separated reaction compartments and gives an unique possibility for the implementation of multi-step reactions, if the reaction yield in each step can kept close to hundred percent. The well defined conditions for addition of effectors, fast mixing and nucleation should allow the formation of complex composed nanoparticles of high regularity.

The deposition of one material on an other one can proceed either under kinetic or under thermodynamic control. On one hand, micro flow-chemistry allows to realize reactions at high reaction rates, what means short time intervals for achieving the chemical equilibrium and, therefore, conditions close to the thermodynamic equilibrium. On the other hand, reactions can be quenched quickly by addition of chemical quenchers or by rapid cooling. So, the system can be brought rapidly into a far-equilibrium state. The conditions for starting of material deposition and for control by transport or control by kinetics can be optimized if surface reactions can be

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adapted to the desired kind of material deposition. The surface reactions of particles can be switched between a non-selective behavior and a selective behavior controlled by the crystallographic planes of surfaces of the involved particles and the coverage of these planes by ligands.

The most challenging task is to find compromises between the spatial order of micro liquid compartments and flexibility and possibility autonomous actions of them. This problem is related to the question, how and where the information of the individual liquid entities is stored and how the information exchange can proceed. Full autonomy of compartments demands for a complete information storage and communication system. The outer system must cover a part of the tasks of information exchange and operation in case of reduced information storage inside the compartments. In general, architectures of liquid-like hierarchies have to distribute the tasks of organization on the different levels. Therefore, a system-specific optimum between external and internal data storage and data management, between external data transfer systems and direct inter-compartment communication and between a full control by the central unit and a full autonomy of single liquid units has to be found.

In full-determined technical system, the activities of units on all levels are controlled by the highest level. But, the ability of control is subdivided between levels if autonomous activities are allowed. In a strictly organized hierarchy with semi-autonomous units, each level would be responsible for the control of all units of the next lower level. Following functions has to be covered by the master levels inside a hierarchically structured liquid-architecture system (fig. 8):

- formation of liquid subunits
- arranging of liquid subunits
- packaging of liquid subunits
- information and/or matter transfer
- incorporation and release of semi-autonomous subunits
- controlled motion of subunits

Information exchange must be combined with liquid handling operation for these purposes. It can obviously only managed, if phases can be separated and phases can be united, if subunits can be fixed or activated for motion, if interfaces can be switched between closed and open state. These functions demand for support by actuating and sensing micro systems, which are preferably directly incorporated inside the units on the master levels.

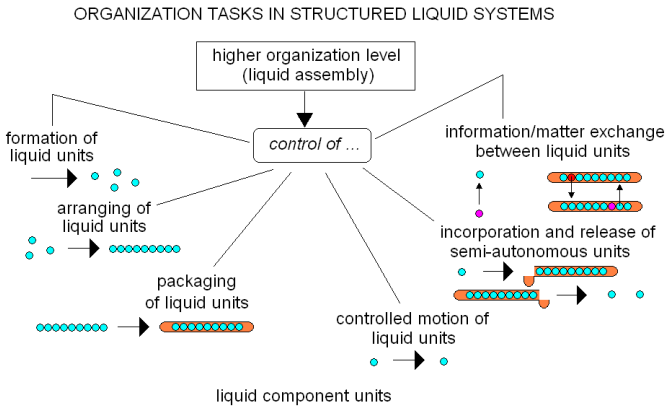

Fig. 8 Tasks for fluidic operations for generation and integration of fluidic subunits at each level of a hierarchical system

The technical development for the realization of such systems could be guided from the recent state of microfluidic generation of nested phase [39, 40] over complex nested phases and incorporated switched phases states, externally controlled systems of operations in hierarchically structured liquid systems (fig. 9a) to hierarchical multi phase systems [41] with semi-autonomous acting subunits (fig. 9b).

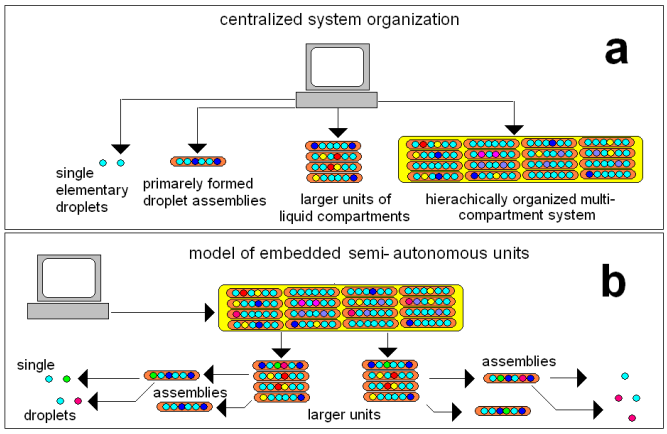

Fig. 9
Vision of fluidic microsystem-internal information transfer and signal transduction: a) directly centralized system organization and communication, b) central system organization with partial autonomy at each level of the hierarchic system structure.

Nanoparticles, chemical modifications of small labels and molecule or nanoparticle converting nano machineries could help to construct phases with individual activities and to connect them with control units. The basic operations therefore would be the writing of information in nano-storages inside compartments and the read-out of such information. One vision is a nano-sized reading and transducer system which would be able to read molecular

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sequences and transmit sequence-related signals to an outside receiver (Fig. 20).

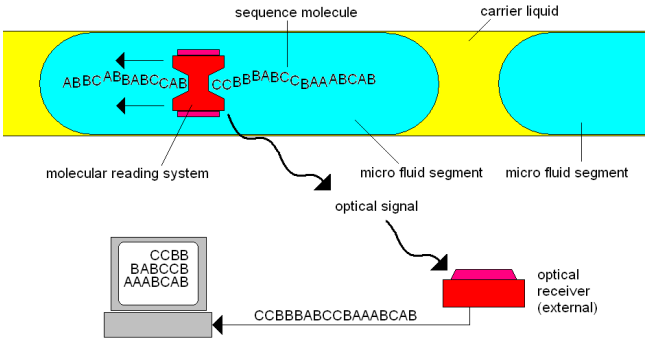

Fig. 10 Vision of a nanotechnical reading system for molecular chains operating inside fluidic micro compartments and transmitting the sequence data to an outside receiver

This receiver could be directly connected with the central unit or could be incorporated in a liquid master unit. The energy for driving such systems should by either supplied by molecular systems inside the operated liquid (similar to chemical energy systems in living cells) or by an energy feeding from outside, for example by light.

5 Conclusions

The micro fluid segment technique is a special part of the droplet-based microfluidics and is well introduced in miniaturized processes in analytical and synthetic chemistry, in diagnostics and biological screenings. Sequences of micro fluid segments can be generated, manipulated and characterized, for example by flow-through photometry or fiber spectrophotometry. The technique offers important advantages for the synthesis of nanoparticles with high homogeneity as it was be shown in case of binary metal nanoparticles and zinc oxid particles. Complex concentration programs can be realized in segment sequences if PC-controlled pumps are applied for the fluid actuation. They can be used for variation of reactant concentration ratios in larger parameter fields and for combinatorial experiments. The technique is particular suited for the determination of high-resolved or two- or higher-dimensional dose/response functions in microtoxicology.

The micro segmented flow technique support visions for realizing challenging tasks in nanoparticle synthesis, reaching from monodispersity over size and shape tuning to composed nanoparticles, asymmetrically functionalized nanoparticles and complex nanoparticle architectures. Beside, simple segments, hierarchically organized liquid microcompartiments could open the way to create and manage molecules, data and particles and could give the possibility to construct guided, semi-autonomous or autonomous nanomachines.

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