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Hierarchical Assemblies of Polymer Particles through Tailored Interfaces and Controllable Interfacial Interactions

Nikunjkumar R. Visaveliya* and Johann Michael Köhler*

Hierarchical assembly architectures of functional polymer particles are promising because of their physicochemical and surface properties for multi-labeling and sensing to catalysis and biomedical applications. While polymer nanoparticles' interior is mainly made up of the cross-linked network, their surface can be tailored with soft, flexible, and responsive molecules and macromolecules as potential support for the controlled particulate assemblies. Molecular surfactants and polyelectrolytes as interfacial agents improve the stability of the nanoparticles whereas swellable and soft shell-like cross-linked polymeric layer at the interface can significantly enhance the uptake of guest nano-constituents during assemblies. Besides, layer-by-layer surface-functionalization holds the ability to provide a high variability in assembly architectures of different interfacial properties. Considering these aspects, various assembly architectures of polymer nanoparticles of tunable size, shapes, morphology, and tailored interfaces together with controllable interfacial interactions are constructed here. The microfluidic-mediated platform has been used for the synthesis of constituents polymer nanoparticles of various structural and interfacial properties, and their assemblies are conducted in batch or flow conditions. The assemblies presented in this progress report is divided into three main categories: cross-linked polymeric network's fusion-based self-assembly, electrostatic-driven assemblies, and assembly formed by encapsulating smaller nanoparticles into larger microparticles.

1. Introduction

Hierarchical assembly of polymeric materials is one of the most promising yet challenging aspects of nanoscience largely because of their swellability and softness.[1–5] Hierarchical materials also add complexity and dramatically increase the opportunities for random errors in assembly due to the high collision rate, high surface energy, and high surface to volume ratio at the nanoscale in the solution phase.[5,6] In general, particulate assembly architectures integrating various active components and accumulate the functions of two or more different domains.[7,8] Not only the combination of the specific functions of different materials' worlds, but also a better particle manipulation and control of their physicochemical, structural, and interfacial properties can be achieved if the assembly strategy properly controlled.[9] Complex coupled functional activities and advanced properties realized by assemblies cannot be harvested by isolated components. In general, nanoscale particles possess a high surface to volume ratio compared to their macro counterparts[10] and hence try to achieve a thermodynamically more stable state with minimizing energy barrier by promoting aggregation or growing in a larger size. On one hand, appropriate control over the surface of nanoparticles needs to be tailored with interfacial agents to achieve high stability as well as avoiding uncontrolled aggregation randomly in the solution phase.[11] Architecting the suitable surface state, on another hand, of nanoparticles with active/functional components that intensify interfacial interactions are required for controlled and specific assemblies.[12–15] Therefore, the interface (as initial and essential contact of interest) of the constituent nano objects of assemblies playing a crucial role in determining the strength of the interfacial interactions to form the preferred nanoscale assembly systems.

Usually, a cross-linking network of polymer nanoparticles is swellable, amorphous, and more or less rigid independent of the stiffness of molecular building units of the density of cross-linking structures and solvent content. Amphiphilic interfacial agents of various types such as molecular surfactants, polyelectrolytes, nonionic polymers, etc.[11] not only stabilize polymer nanoparticles and directing their shapes, but also provide a soft interfacial layer as a host for efficient interaction with guest nanoparticles.[12] Various routes are frequently employed to construct the assemblies based on various interfacial interactions such as via ligand binding affinity, complementary DNA strands, electrostatic interactions, etc.[16–19] The formation of assemblies based on the affinity of the ligand's functional group is very specific and DNA-directed assembling is selective to molecular interactions.[16,18] Alternatively, the formation of assemblies based on electrostatic interactions is widespread, versatile, and uncomplicated because of the strong attraction

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force between oppositely charged surfaces.[17,20–22] On one hand, complete coverage of the surface by charged interfacial agents (with high concentration) is required for the stability of nanoparticles as well as avoiding uncontrolled nanoparticle aggregation. But, an uncontrolled assembling state during post-synthesis electrostatic interactions with oppositely charged nanoparticles in the solution can likely take place with the application of a high concentration of surfactant, on the other hand. Therefore, controlling the assembling specificity while maintaining the size, shape, and stability of the nanoparticles at the same time in case of electrostatic interactions is a challenging task. A systematic and strategical synthesis approach needs to be applied to address this concern where the stability of the isolated nanoparticles and controlled assemblies should be achieved. Second, besides the nanoparticles' post-synthesis electrostatic assembling between oppositely charged nanoparticles, another efficient route to form assemblies is where growing nanoparticles can be self-assembled themselves in a highly controlled manner during a continuous polymerization reaction.[23–25] Here, growing hydrophobic nanoparticles can be self-assembled by minimization of their surface energy if the interfacial layer can be equipped with flexible, movable, and dynamic molecules/macromolecules. The selection of interfacial agents, the amplification of the reaction condition, and the control of the desired level of assembling, however, is very challenging in the case of a continuous self-assembly process. Third, the application of the swellable polymeric materials' layer on the surface of polymer nanoparticles can provide both robustness as well as specificity for the assembly system, but it is difficult to realize them by a single-step process. At the fourth, the layer-by-layer (LBL) assembly approach is highly beneficial because of their ability to switch the surface state and allow tunability of the assembly system.[26–28] Similarly, particulate assembly of nanoparticles with microscale particles[29] at the surface and/or by incorporation of nanoparticles inside microparticles' interior[25,30] provide the multi-scale hierarchical assembly approach[31] and polymer–metal nanoparticulate assemblies reveal hybrid properties.[32]

Generally, a wide range of various assembly systems is reported in recent literature with various preparation techniques. Different assemblies such as spherical nanoparticles assembly, Janus assemblies, patchy assemblies, colloidal molecules, compartmentalized nanoparticles, colloidal assemblies, core–shell micellar assemblies, vesicle assemblies, and polymerization-induced self-assemblies have been realized with better control by various methods.[5,33–42] The interactions between different types of nanoparticle constituents are playing a crucial role during the formation of assemblies.

In this progress report, structural and surface-controlled polymer nanoparticles are prepared through the microfluidics[43–50]-supported syntheses, and their assemblies are obtained in batch processes or flow-driven processes. Various types of assemblies presented here are divided into three main categories as shown in **Figure 1**: i) fusion-based continuous self-assembly realized during the polymerization growth of the nanoparticles in a single-step process, ii) electrostatic-interaction-based assemblies of charged particles, and iii) assembly formation by incorporation of small nanoparticles in the interior and at the surface of microparticles during photopolymerization flow process. The electrostatic assemblies are further divided into various

categories based on direct interaction between surface ligands, interaction through the swellable cross-linked polymeric surface layer, or interfacial interactions via layer-by-layer macromolecular architectures. The progress in the field of hierarchical particulate polymeric assemblies has been phenomenal, and here, in this progress report, only the research progress from the authors' laboratory during the last eight years has been considered.

2. Continuous Self-Assembly

The mechanism hypothesis of the continuous self-assembly of the growing polymer nanoparticles during polymerization in a single-step has been explained here with the analogical concept of biological membrane-fusion. The process of fusions of various components are ubiquitous and specifically in the field of biology.[51] The fusion, as a natural process in biology, is essential for key events in physiology and anatomy ranging from fertilization to various organ developments.[52,53] A biological cell is a building block of life. Until recently, various types of cells were thought to be integral and discrete components of tissues in organs, and their state was determined by cell differentiation. However, now it has been extensively studied that various cells such as stem cells can fuse with other types of cells under certain conditions for functioning the organs and the developments.[51,54,55] As far as fusion is essential, the main interaction site for the fusion of cells in the form of interfacial interaction is the cell membrane. The cell membrane consists of a lipid bilayer where lipid molecules self-assemble by facing head hydrophilic parts outward to extracellular space as well as toward the interior of the cell whereas hydrophobic tails of both layers facing inward to each other forming a lipophilic nano environment which can contain non-polar molecules like cholesterol (essential for fluidity).[56] The membrane fusion is the process whereby two separate lipid bilayers merge to become one. As an impact, membrane fusion is essential for communication between membrane-delineated compartments allowing the exchange of luminal contents or releasing their contents such as hormones and neurotransmitters into the extracellular milieu, or to deposit receptors and transporters to the membrane.[56–58] Homotypic fusion initiates the process to merge similar types of compartments such as endosome-endosome fusion whereas the dissimilar type of compartments (for instance, synaptic vesicle exocytosis) can be fused in case of heterotypic fusion where latter fusion could be of central importance for development, repair of tissues and the pathogenesis of diseases.[54,57] During the membrane fusion process, the very first initiation required is to bring membranes of two fusing cells into proximity. Membrane fusion is an energy-consuming process in which the energy barrier should be lowered to realize fusion.[59,60] In this regard, the specialized protein molecules ("fusogens") directs the fusion of membranes. For instance, myomaker—a muscle-specific protein—and myomerger together confer fusogenic activity to otherwise non-fusogenic cells.[61] Similar to the primary class of fusogenic agents such as proteins and peptides, the other types of fusogens such as small molecules[62] and ions are also promising for the membrane fusion process.[63] Fusogens are not only useful in destabilizing the bilayers and fusing the membranes
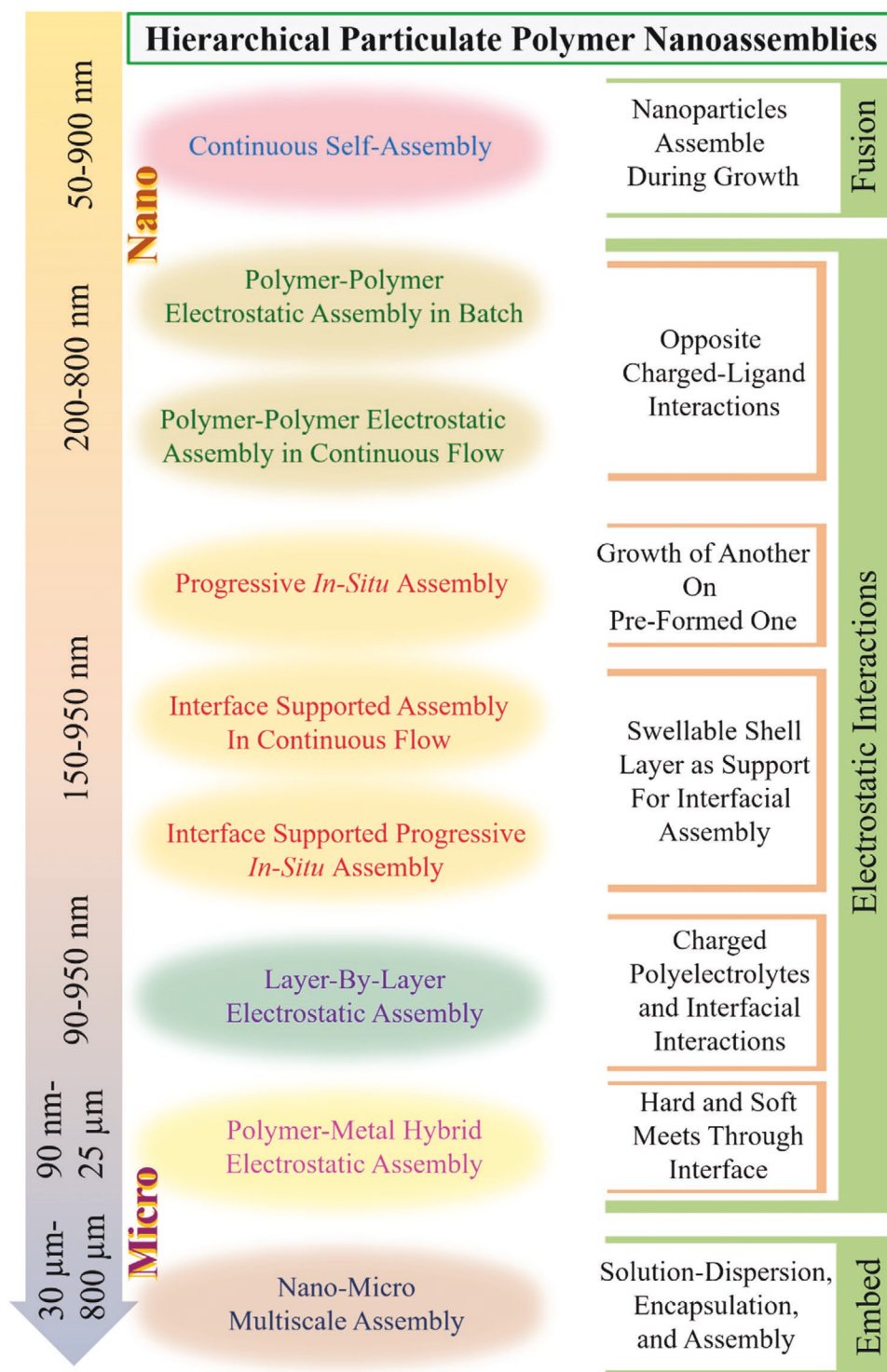
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Figure 1. Overview and hierarchical approach of the polymeric particulate assemblies based on various interfacial interactions explained in this progress report.

by dealing with basic thermodynamics and biophysical energetic requirements but also provide specific directionality during fusion. To begin the process, the outer monolayer initially loosely tethered with fusogens and is later converted into a tightly docked state through which membranes come

closer (dehydrating polar head groups). Once they approach in proximity, the high energy barrier tends to be lowered to initiate the membrane fusion process that promotes hemifusion stalk. Subsequently, it is believed that fusion-pore opening can be realized and later it expands where two membranes merge

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and become one.[64] Overall, these steps of membrane fusion are governed by energy barriers, which need to be overcome to complete fusion.

Considering this natural process of the cell membrane fusion as an analogy, their materials' counterpart[65] through chemical synthesis can be a promising approach for the formation of nanoscale assembly materials which is also helpful for the understanding of the fusion process at the nanoscale in more detail. Therefore, here, a spontaneous self-assembling process has been conducted for the case of assembling polymeric nanoparticles during ongoing polymerization which is termed as "continuous self-assembly" because the assembling has been realized during ongoing continuous polymerization by a single-step synthesis approach. The nanoscale particle has high surface energy[10] compared to their bulk counterpart and polymeric materials are soft;[66] hence strategic synthesis condition is required to achieve an oriented and controlled assembling without uncontrolled aggregation. Particles of polymeric materials preferably adopt a spherical shape as it has the lowest energy barrier.[11] In other words, it is difficult to obtain nonspherical shapes of polymer nanoparticles through the single-step process because polymers are soft, swellable, flexible, and amorphous.[66] Smaller the nanoparticles are, higher is their surface energy;[10] and shapes other than sphere at the same size has even more surface energy.[67] Therefore, the thermodynamically preferred shape for polymer nanoparticles at the nanoscale is a sphere. Despite this situation, however, if two or more spherical nanoparticles can be assembled systematically by combining their cross-linking network during the ongoing polymerization process, it is then possible to create dumbbell as well as further linear shapes as isolated assembly particles. Thus, the controlled assembly process of the growing nanoparticles is highly useful in the formation of shape-controlled polymer nanoparticles in a single-step. As applications, shape-controlled polymer nanoparticles are highly useful in various biomedical and other applications such as drug delivery, phagocytosis, receptor-mediated endocytosis, etc.[68–73]

The formation of polymer nanoparticles via emulsion polymerization is realized inside the micelle-like surface protecting layer of surface-active agents, that is, hydrophobic polymer nanoparticles formed under the amphiphilic surfactant layer whose hydrophilic charged head is floating outward that compatible with an aqueous phase.[11] It can be imagined that if polymer nanoparticles are covered by molecular surfactants of low molecular weight, they are considered as hard multi-charged nanoobjects where nanoparticles often adopt spherical shape. The surface dynamics and interfacial approach is completely changed if molecular surfactants are replaced by charged polyelectrolytes. Charged polyelectrolytes are long-chained multi-charged macromolecules, and nanoparticles can be considered as soft multi-charged nano object if they are covered by polyelectrolytes at the surface.[23] Polyelectrolytes positioned at the oil–water (hydrophobic–hydrophilic) interface of the polymer nanoparticles and protecting as well as controlling the surface state of nanoparticles during the growth process.[74–80] As shown in **Figure 2**A the initial emulsion of both immiscible phases (organic monomer phase and polyelectrolyte dissolved aqueous phase) was realized through microreactor and further polymerization takes place externally at the heating block.[23] Once

the polymerization starts at polymerizing temperature, it can be imagined that the monomer units are continuously added to proceed with the growth of nanoparticles upon movement of the polyelectrolyte that lied at the surface of nanoparticles. Smaller spherical nanoparticles can experience high motion and hence collision in the solution where they try to assemble to grow bigger to minimize the surface energy, movement, and collision rate. Strong motion brings the smaller growing spheres in proximity where long-chained polyelectrolytes controllably move toward the end direction and allow the assembling of two separately growing hydrophobic spheres to become one under the surface layer of polyelectrolytes. Cross-linking threads of both the assembling spheres connect and the dumbbell type shape of the assembly particles can be realized.[23]

Polyelectrolytes are also essential in controlling the surface tension of the nanoparticles. Therefore, the concentration of polyelectrolytes is controlling the size of the growing nanoparticles, on one hand, and decide when the assembling event will be taking place, on the other hand. Early assembling event in case of very high polyelectrolyte concentration where a large number of free monomers are still available in the system during ongoing polymerization which subsequently attaches to the growing cross-linking network until the end of polymerization and finally, they produce the ellipsoidal-shaped polymer nanoparticles. The more highly curved an interface is (that is the case in smaller nanoparticles than in larger counterparts), the more potential to assemble it becomes. Hence, smaller nanoparticles are formed in the case of high polyelectrolyte and they can initiate assembly events at an early stage of polymerization. Dumbbell shape, on the other hand, is the result of the moderate concentration of polyelectrolyte in the aqueous phase (Figure 2B). Not only limited to assembling of two spheres that form ellipsoid and dumbbell but assembling of three, four, and more separately growing spheres can be assembled linearly to form astragal-like or long-necklace type linear nanoparticles depending on various reaction condition (Figure 2C).[23] Zigzag-type assembly nanoparticles can also be produced upon further control over the reaction parameters (Figure 2D).[81] The formation of the systematically controlled linear and branched assembling polymer nanoparticles in a single-step is based on the limited polarizability, partial electrostatic control, moderate repulsion, and hydrophobic interactions.[23,81,82] A systematic tuning in linear assembly and their surface charge has been systematically tuned in case of poly(methyl methacrylate) (PMMA) nanoparticles by using anionic polyelectrolytes polystyrene sodium sulfonate (PSSS) through semi-microfluidic emulsion polymerization (Figure 2F).[23,81–83] While specific proteins—fusogens—plays the main role in cell membrane fusion, the role of polyelectrolytes in the case of assembling polymer nanoparticles is of central importance.[84]

The directionality in the assembling pattern (linear assembly) can be altered by the application of surface-active agents of different characteristics than charged polyelectrolyte PSSS. To investigate such consequences, here, a case of PMMA nanoparticles with flower-type compact assembly has been studied which is the result of non-ionic polymer polyvinylpyrrolidone (PVP) as an interfacial agent. PVP is well soluble in the aqueous phase and has solvation power.[85] PVP helps to form
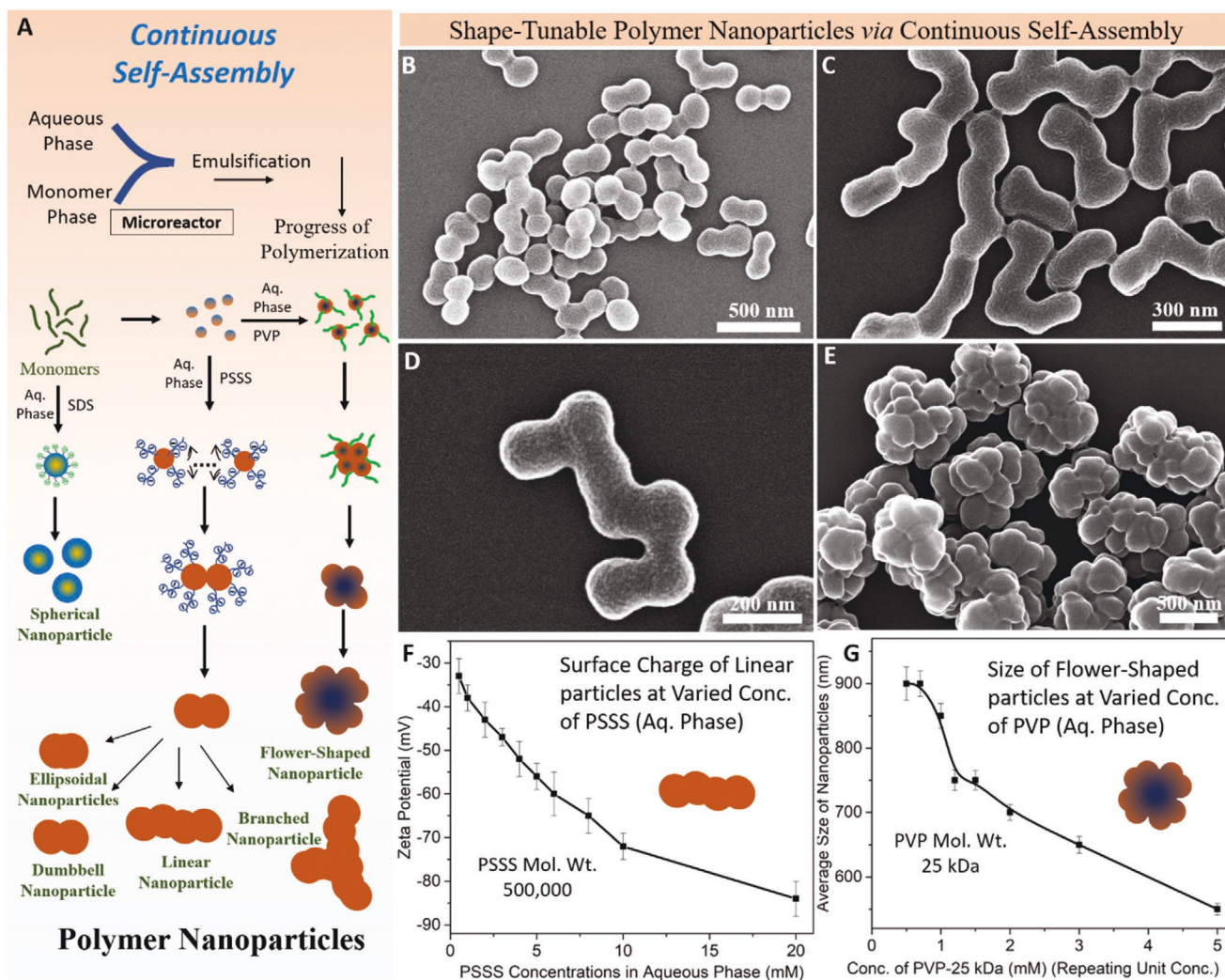
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Figure 2. Shape-tunable polymer nanoparticles in a single-step continuous polymerization process via continuous self-assembly. A) A scheme of the formation of nanoparticles in which various interfacial agents play an important role to control the self-assembly of the growing nanoparticles during ongoing polymerization. B) Dumbbell-shaped poly(methyl methacrylate) (PMMA) nanoparticles formed when 10 mм polystyrene sodium sulfonate (PSSS) of 500 000 molecular weight was used with aqueous to monomer phase flow rate ratio 400/150 µL min⁻¹. C) Linear-chained PMMA nanoparticles formed when 10 mм PSSS of 1 000 000 molecular weight were used with aqueous to monomer phase flow rate ratio 1800/150 µL min⁻¹. D) Zigzag-type PMMA nanoparticles formed when 10 mм PSSS of 500 000 molecular weight were used with aqueous to monomer phase flow rate ratio 1000/150 µL min⁻¹. E) Flower-shaped PMMA nanoparticles formed when 3 mм polyvinylpyrrolidone (PVP) of 25 kDa molecular weight was used with aqueous to monomer phase flow rate ratio 1200/80 µL min⁻¹. F) Graphical results of the surface charge by zeta potential measurements of the linear PMMA nanoparticles obtained by using various concentrations of PSSS in the aqueous phase. G) Graphical results of the average size measurements of the flower-shaped PMMA nanoparticles at various concentrations of PVP 25 kDa in aqueous phase by dynamic light scattering. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[23,81,86] Copyright 2014, American Chemical Society; Copyright 2015, Wiley-VCH; Copyright 2014, American Chemical Society.

the emulsion solution of the monomer phase thereafter polymerization process begins. Initially, it can be believed that PVP macromolecules are attached to the smaller nanoparticles and protect the nanoparticles against uncontrolled aggregation. At an early stage when PVP molecules fully covered the surface of polymer nanoparticles (small spheres), the mobility of the nanoparticles, as well as their solvation ability, are high.[86] Size of the spheres increases upon progress in polymerization where solvation power becomes weak and few of the nearly growing spheres assemble by which primary assembling event can be realized. Once again, a large density of the PVP macromolecules

adheres at the surface of primarily assembled small particles that again enhance the solvation power. Similar to the primary assembling event, further growth of the nanoparticles proceeds where second and third assembling events can be realized during the ongoing continuous polymerization reaction. The assembling events in a highly controlled manned are continued to be realized until the end of the polymerization by consumption of all free monomers. The solvation ability and mobility factors govern the overall assembling events in a continuous manner that finally form flower-type compact assembly nanoparticles (Figure 2E) via single-step continuous polymerization

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(continuous self-assembly). The size of the overall assembly nanoparticles is tunable and that largely depends on the concentration of PVP in the aqueous phase (Figure 2G).[83,86]

The soft interface facilitated by interfacial agents (PSSS and PVP) is a key player to control the interfacial interactions and assembling process in a precise manner during ongoing polymerization that finally forms compact assembly polymer particles (assembly particles with the single cross-linking network).

3. Various Assemblies Driven by Electrostatic Interactions

Interfacial agents of amphiphilic characteristics such as molecular surfactants and polyelectrolytes stabilize the individual nanoparticles from unwanted aggregation in the solution. Furthermore, the electrical charges at the surface of nanoparticles initiate the interfacial interactions with oppositely charged nanoparticles and form the assembly systems. In this broad section, various types of assemblies are explained based on electrostatic interactions.

3.1. Polymer–Polymer Electrostatic Assembly in Batch

The assembling reactions of nanoparticles based on their interfacial interactions can be carried out by traditional chemical reaction setup (usually in a flask with stirring arrangements) is called a "batch assembly." The principal advantage of the batch reaction is where assembly materials can be produced in a larger quantity. Also, batch setup is widely used because it is the simplest as well as non-expensive choice. The initiation is directed by surface interactions when two different types of pre-synthesized polymer nanoparticles of similar or different characteristics begin the assembly process. Whether it is self-assembly or templated assembly, their potency is high at the nanoscale because of the high surface energy compared to their larger lengthscale counterparts.[10] Therefore, there is a high possibility of the realization of random aggregation to lower down the energy barrier at the nanoscale. To avoid that issue of random coagulations, the nanoparticles need to be prepared with appropriate surface functionalities that allow systematic and controlled interactions.[12] On one hand, the formation of assemblies of nanoparticles based on chemical bonds such as amide bond or through dynamic covalent chemistry[18] is chemically selective but could not be efficient because the size of nanoparticles is too big compared to their surface ligand molecules. On the other hand, an efficient assembly system could be formed via complimentary stands of the DNA (oligonucleotide bonds)[16] bonding but the process can be very complex in the case of nanoparticles assemblies. Alternatively, a simple, stronger, and versatile route for nanoparticles' assemblies is via electrostatic interactions.[17,20,21,87] While assembly reaction by electrostatic interactions is spontaneous and strong, but there is a lack of selectivity and specificity because of the strong attraction between oppositely charged nanoparticles in solution. However, by controlling the charge density on the surface of nanoparticles, it is possible to form systematic assemblies without random aggregation.[17]

Here, a model batch assembly process through electrostatic interaction has been conducted between the oppositely charged nanoparticles of flower and spherical shape. As described in above Section 2, the flower-shaped PMMA nanoparticles were synthesized by using PVP as an interfacial agent. Through the mobility and solvation power, the growing nanoparticles' interface (equipped with PVP) initiates the assembly process by controlled aggregation which finally forms flower-shaped PMMA nanoparticles.[86] The flower shape is a result of the multiple aggregation phases during ongoing single-pot polymerization (**Figure 3**A). The size of the obtained nanoparticles is dependent on the PVP concentration used in the aqueous phase.[86] Despite the non-ionic character of the PVP, the zeta potential of the obtained nanoparticles is in the range of lower negative value that is likely due to an enolization of PVP molecules in the aqueous phase after attached to the nanoparticle surface. The zeta potential of the obtained flower-shaped nanoparticles is –11 mV.[86] An SEM image of the obtained flower-shaped PMMA nanoparticles is shown in Figure 3C. Since flower-shaped nanoparticles are anionic, the positively charged PMMA nanoparticles were synthesized for the preparation of electrostatic nanoparticles' assemblies. A sphere is the common shape of nanoparticles in the case of polymeric materials largely due to their lowest surface energy domain. Molecular surfactant is key to protect the nanoparticles against uncontrolled aggregation. Therefore, cationic molecular surfactant cetyltrimethylammonium bromide (CTAB) has been used in the aqueous phase for the formation of PMMA nanoparticles via emulsion polymerization. Due to their amphiphilic nature, surfactant molecules form a micelle-like structure at their critical micelle concentration in an aqueous solution (Figure 3B).[11] At the same time, charged CTAB is a mediator for the efficient emulsion of both immiscible phases (aqueous continuous phase and monomer dispersed phase). Overall, various concentrations of CTAB can control the surface tension of the nanoparticles and allow the formation of nanoparticles of tunable size. For instance, 70 nm sized cationic PMMA nanoparticles were obtained in presence of 1 mM CTAB in aqueous phase.[88] Similarly, size can be systematically increased with a gradual decrease in CTAB concentration where about nanoparticles of 110 and 160 nm diameter were obtained by using 0.1 and 0.01 mM CTAB concentration in the aqueous phase, respectively.[88] While the size of the nanoparticles is dependent on CTAB concentration, the zeta potential of the nanoparticles is also dependent on CTAB concentration. The zeta potential of the PMMA nanoparticles between +9 and +42 mV can be tuned by using various CTAB concentrations in the aqueous phase.[88] The obtained spherical PMMA nanoparticles are shown in Figure 3D.

Anionic flower-shaped nanoparticles and cationic spherical nanoparticles form nanoscale particulate polymer assemblies by electrostatic interaction in batch reaction setup as shown in Figure 3E,F. As electrostatic nanoassemblies are strong, the population of spherical nanoparticles on the surface of flowers can be controlled by tuning the size and hence surface charge of the spherical nanoparticles.[89] Similar to a model batch assembly as shown here, a wide range of different types of nanoparticles can be assembled to combine the diverse properties via batch reaction in a tunable manner.
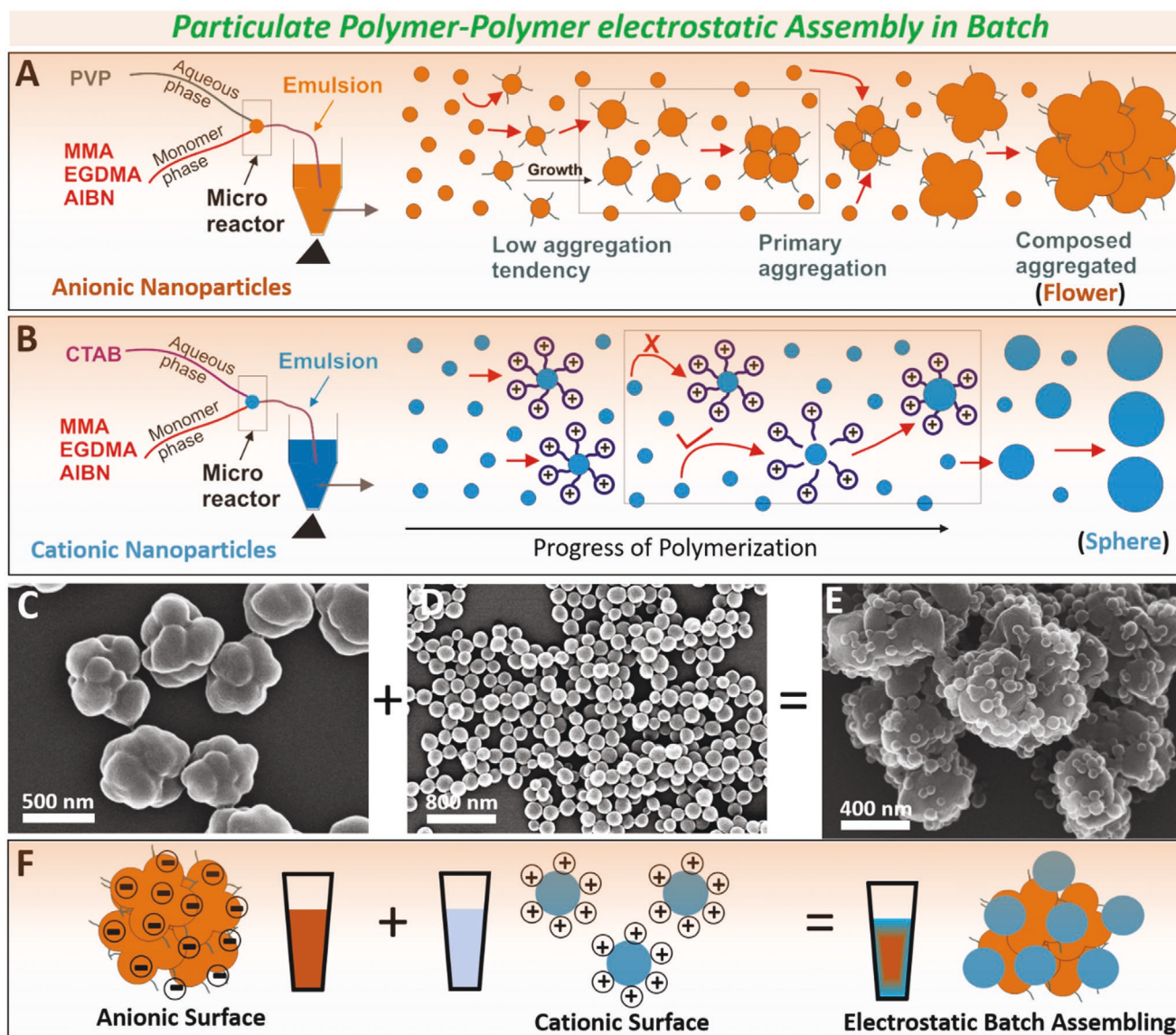
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Figure 3. Electrostatic assembly between two different types of oppositely charged polymer nanoparticles in a batch process. A) A scheme for the formation of flower-shaped PMMA nanoparticles in which PVP is playing the main role for controlling the surface aggregation of the growing nanoparticles in a highly controlled manner during ongoing polymerization in one step through solvation power and mobility factors.[86] B) A scheme for the formation of the spherical nanoparticles in presence of cationic surfactant cetyltrimethylammonium bromide (CTAB) in the aqueous phase.[89] C) SEM image of the flower-shaped PMMA nanoparticles. D) SEM image of the spherical PMMA nanoparticles. E) Flower-sphere electrostatic assembly of anionic flower-shaped nanoparticles and cationic spherical nanoparticles in batch reaction at room temperature with 12 h stirring. F) A cartoon represents the formation of electrostatic assembling nanoparticles. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[86,89] Copyright 2014, American Chemical Society; Copyright 2016, Elsevier Ltd.

3.2. Polymer–Polymer Electrostatic Assembly in Continuous Flow

Electrostatic interaction is a strong interaction[17] where two oppositely charged nano objects can strongly assemble through the interparticle attraction.[90,91] Higher the charge strength, the stronger the assembly can be realized rapidly. In this case, surface charge density (the quantity of charge per unit area), size of nanoparticles, the concentration of particles (dispersion), and dispersion medium (solvent) playing equally important roles during interfacial interactions through surface charges.

Imbalance in the reactants surrounding during the reaction can induce inhomogeneities in interfacial interactions of the nanoparticles. Inhomogeneity in interaction can cause nano assembly with non-uniform distribution of the nanoparticles on the surface of oppositely charged host particles, which is a frequent case in batch syntheses. Inhomogeneous assembly results in a weak outcome during their applications. Therefore, to avoid this concern of imbalance in interfacial interactions, a well-defined reaction strategy needs to be applied to achieve better control and uniformity of the assemblies of polymer particles. In address, the microflow strategy is very

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promising because of its vast range of advantages over the traditional batch syntheses.[46,49,92,44] During flow syntheses, the operation of the mixing of the reactants takes place at a smaller volume (picoliter or nanoliter) that flows in the microscale channel.[92–96] Because the reaction is carrying out in the flow channel of low dimension in a continuous manner, important advantages such as fast heat and mass transfer as well as efficient reactants mixing and robust interfacial interactions can be used.[97] As electrostatic interaction is rapid (time-sensitive), the short interaction time can be crucial for uniform interactions that result in homogeneous assemblies. Also, due to low Reynolds numbers, well-defined and reproducible streaming patterns in microreactor allow control of the integration conditions of nanoparticles resulting in enhanced yield and homogeneity of assembled nanoparticles.[97] The microflow setup with computer-controlled syringe-pumps is very well suited for precise injection of small volumes of one reactant solution into another reactant in microreactors whereby various reactants being efficiently mixed. Also, the design of the microreactor can be fabricated based on different designs that are, in general, either of cross-flow, co-flow, or flow-focusing.[31,98]

At first, the microflow technique is useful for the formation of polymer nanoparticles with low polydispersity by which flower-shaped and spherical polymer nanoparticles were synthesized. Second, the flow arrangement is highly promising for assembling nanoparticles of opposite surface charge in a highly uniform manner without irregular distribution. Here flow assembling between anionic flower-shaped polymer nanoparticles (zeta potential 16 mV)[86] and cationic spherical polymer nanoparticles (zeta potential +23 mV)[99] are performed in the flow setup as shown in **Figure 4**A. In a simple microflow setup (top left of Figure 4A), both cationic and anionic nanoparticles were pumped through nearby two inlets and generated an integrated solution mixture that is flowing through the outlet to the knot mixture microchannel which is immersed in the water bath. Likewise, another equally simple setup has been arranged where both the nanoparticles being mixed through a Y-shaped connector to the knot mixture microchannel which is also immersed in the water bath for temperature-controlled assembly reaction (top right of Figure 4A). In this setup, continuous co-flow streaming in the microchannel (inner diameter 0.5 μm) can provide high surface area to interact both nanoparticles in a uniform manner by which homogeneous distribution of smaller sized spherical nanoparticles can be realized electrostatically on the surface of flower nanoparticles. Based on results, it was observed that the assembling process (electrostatic interaction) becomes faster with increasing temperature. It can be hypothesized that the mobility of the assembling nanoparticles and hence their interactions through collision can be enhanced at high thermal energy.[100] A best-case scenario to obtain the highly homogeneous distribution of spheres on flowers were obtained by using 110 nm-sized spheres (zeta potential +23 mV) and around 550 nm-sized flower-shaped PMMA nanoparticles (zeta potential −16 mV) at 70 °C (Figure 4B).[89] It was also possible to obtain equally uniform nanoassembly by using the same range of charges (zeta potential +23 and −16 mV) of nanoparticles at 90 °C as shown in Figure 4C.[89] Here it can be imagined that the electrostatic repulsion between similar charged spherical nanoparticles is

as important as the electrostatic attraction between spheres and flowers to obtained well-distributed and uniform nanoassembly particles without irregular aggregation. At first, the flow condition provides a homogeneous assembling environment. Second, surface charge density is equally important for creating uniform assemblies even in flow-conditions. A very high surface charge can lead to rather regular distances between attached smaller particles whereas lower charges than threshold level can end up with a low yield of assembling (Figure 4D).[89] Similar to flower-sphere assembly, other types of assembly systems such as sphere-sphere assembly, sphere-dumbbell assembly, etc. can also be generated by flow syntheses setup.

3.3. Progressive In Situ Assembly

To achieve a thermodynamically stable state, smaller nanoparticles that experiencing high Brownian motion in the solution phase often try to assemble in a random manner or an organized pattern if the interface is properly tailored with appropriate functionalities.[100–102] On one hand, the self-assembling of growing nanoparticles as shown in Section 2 can form smaller colloidal molecule-like shaped nanoparticles.[23,103,104] Nanoparticles' post-synthesis assembling, on the other hand, through electrostatic interaction between opposite surface charge can form simple nanoparticulate assembly as shown in Section 3.1.[89] Besides the self-assembly and colloidal electrostatic assembly, a "progressive in situ assembly" is advantageous to achieve better assembling control. The progressive assembly, here, is described in which the newly forming (growing) nanoparticles can be assembled in an in situ manner during the polymerization on the surface of pre-formed polymer nanoparticle of a different type. A basic synthesis arrangement for the progressive assembly is briefly shown in **Figure 5**A. The pre-formed nanoparticles are of flower shape obtained by using PVP in the aqueous phase (Figure 5B). To initiate the progressive assembling reaction, an aqueous suspension of the pre-formed flower-shaped PMMA nanoparticles were inserted in the polymerizing vial before beginning the polymerization of incoming (assembling) polymer nanoparticles. The emulsion polymerization process of the in situ assembling nanoparticles has been conducted through a semi-microfluidic synthesis setup at polymerization temperature (95 °C).[89] As pre-formed flower-shaped nanoparticles are anionic (zeta potential is −11 mV.[86]), the requirement for the newly forming spherical nanoparticles for in situ assembling is the creation of their cationic surface so that efficient electrostatic interaction can be realized. Therefore, the aqueous phase is made up of the cationic surfactant CTAB for emulsification with a new monomer phase as shown in Figure 5A.

In general, a micelle-like arrangement of the CTAB at the surface of nanoparticles drives the formation of spherical nanoparticles which also protect the nanoparticles against aggregation through the same charge repulsion in the normal polymerization process without pre-formed nanoparticles. In contrast, the newly forming nanoparticles' movement and growth kinetics can significantly change when they grow in the
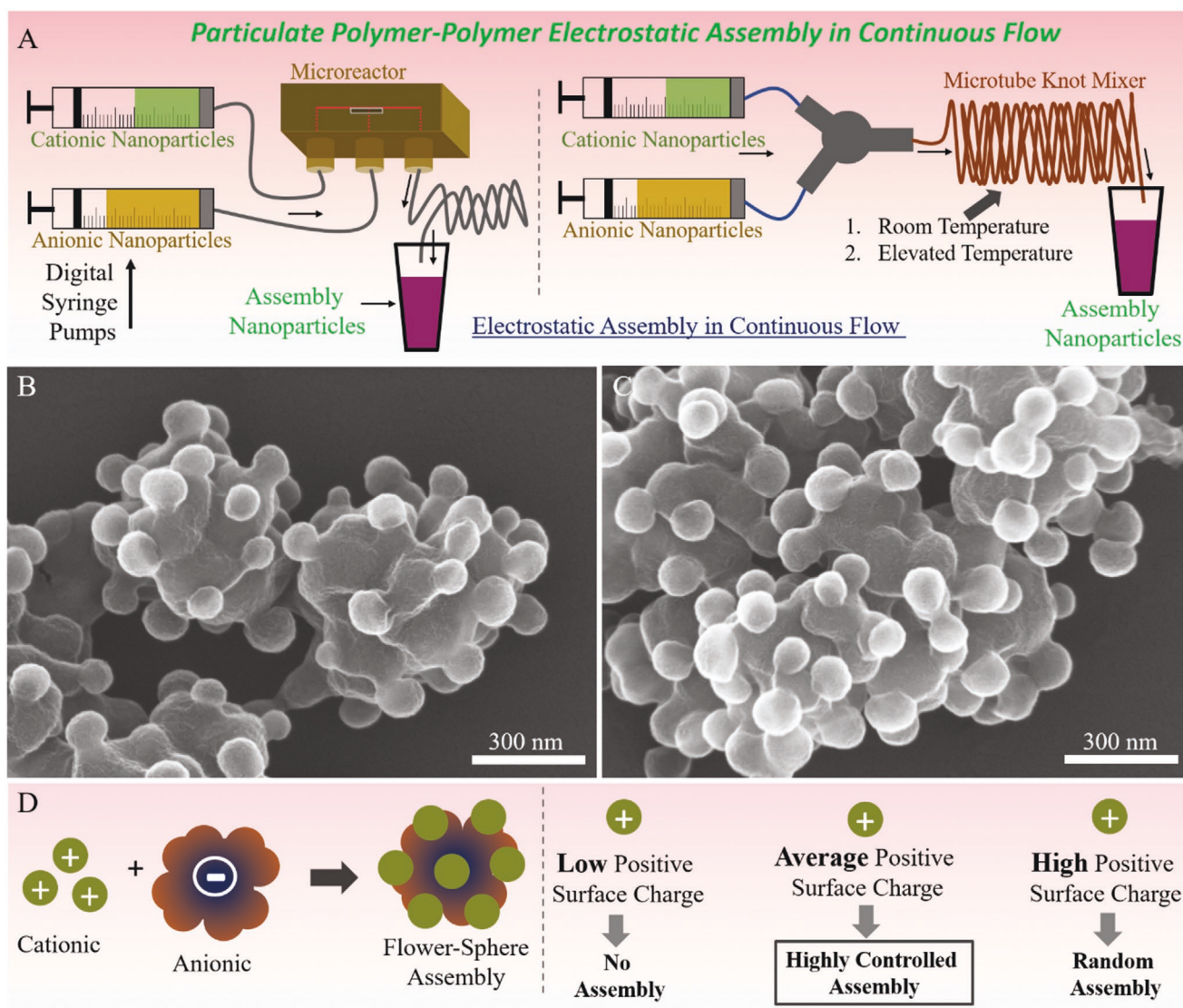
Figure 4. Flow assembly systems where two oppositely charged polymer nanoparticles are electrostatically assembled in a continuous flow manner. A) Schemes for the nanoparticle assembly in the flow reaction: highly efficient nanoparticle mixing takes place in a silicon-chip-based crossflow microreactor (left), and the knot mixer-based flow channel in which two oppositely charged nanoparticles assembles at different temperatures (right). B,C) SEM images of the obtained flower (anionic)-sphere (cationic) nanoassembly particles in knot mixer-based arrangement at temperatures 70 and 90 °C, respectively. D) A cartoon represents the flower-sphere nanoassembly particles where middle ranged cationic charged particles form highly controlled and well-defined electrostatic nanoassembly particles without any undesired aggregations. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[89,112] Copyright 2016, Elsevier Ltd; Copyright 2017, Wiley-VCH.

presence of oppositely charged pre-formed nanoparticles. It is hypothesized, when CTAB-mediated emulsion solution meets anionic flower shape suspension, the cationic head of the CTAB molecules can attach to the surface of flower nanoparticles via electrostatic attractions. Afterward, at polymerization temperature, monomer molecules start to be linked under the micelle-like CTAB surface layer. In this case, it can be imagined that the bottom part of the micelles is attached to the surface of flower particles (electrostatically fixed) and hence only the outer side of the micelle is available to allow the incoming monomer units during ongoing polymerization to realize the growth of spheres on flowers. In this regard, the growth and simultaneously progressively assembling of spherical nanoparticles leads

to smaller sizes compared to their formation in the absence of pre-formed flowers during polymerization (Figure 5C).[89] Here, spherical nanoparticles were obtained with diameters of about 70 and 50 nm by using 1 mM CTAB concentration in the aqueous phase during the polymerization in the absence and presence of pre-formed flower-shaped nanoparticles, respectively.[89] Similarly, about 60 and 70 nm-sized spherical nanoparticles were obtained in the presence of pre-formed nanoparticles during the application of 0.1 and 0.01 mM CTAB in the aqueous phase, respectively. These diameters of nanoparticles at respective CTAB concentrations are much lower than freely forming spheres in absence of pre-formed spheres where 0.1 and 0.01 mM CTAB concentration can able to form
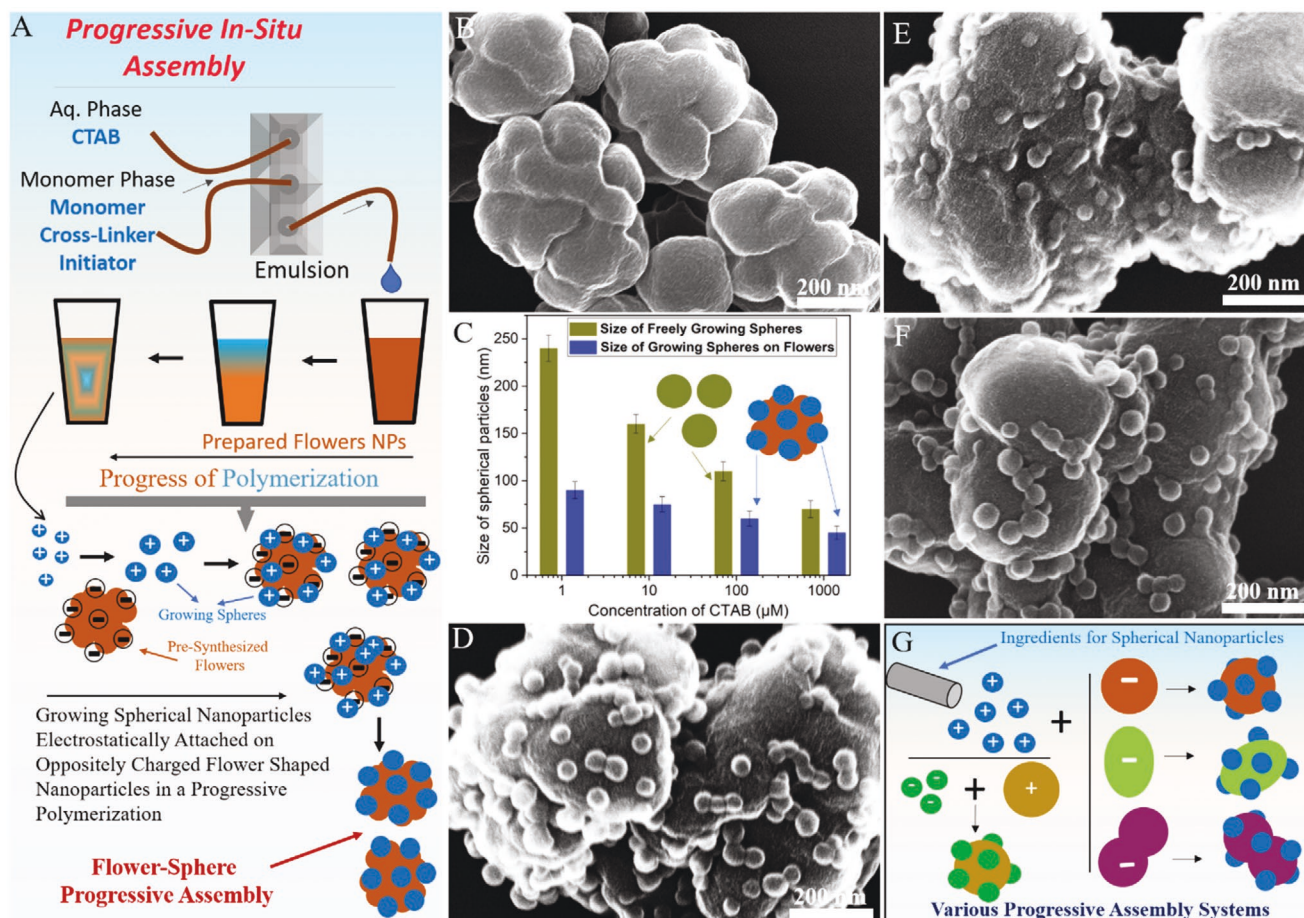
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Figure 5. A concept of the progressive in situ assemblies in which assembly of the growing nanoparticles takes place on the oppositely charged pre-synthesized polymer nanoparticles via electrostatic interaction. A) A scheme represents a brief overview of the assembly of growing spherical polymer nanoparticles (cationic) on the pre-synthesized negatively charged flower-shaped PMMA nanoparticles. B) An SEM image of the pristine flower-shaped PMMA nanoparticles.[86] C) Graphical results of the size of growing spherical nanoparticles in a progressive way in which in situ assembly nanoparticles are smaller in size than the spherical nanoparticles growing separately (without assembly) in the solution. The size of the spherical nanoparticles is tunable concerning the concentration of surfactant CTAB in the aqueous phase. D) An SEM image of the progressive flower-sphere assembly in which spheres are formed and assembled (at 0.1 mм CTAB concentration in aqueous phase) on the flower-shaped nanoparticles prepared by using 3 mм PVP in the aqueous phase. E,F) SEM images of the progressive flower-sphere assembly in which spheres are formed and assembled (at 1 and 0.1 mм CTAB concentration in the aqueous phase, respectively) on the flower-shaped nanoparticles prepared by using 2 mм PVP in the aqueous phase. G) A cartoon shows the possible progressive assembly of spheres on various shaped nanoparticles. The concept and parts of the results are reproduced from the authors' previously published article. Reproduced with permission.[89] Copyright 2016, Elsevier Ltd.

the freely moving spherical nanoparticles of diameter 110 and 160 nm, respectively (Figure 5C).[89] At the end of the polymerization, newly formed spheres are strongly adsorbed on the surface of flowers as shown in Figure 5D. The size of the in situ assembling spheres on the flowers can systematically be tuned by varying the CTAB concertation in the aqueous phase (Figure 5E,F). A comparatively systematic assembling can be achieved via the progressive assembling process (as an advantage) because there is a low chance of uncontrolled aggregation as the anionic surface of flowers is covered by cationic spherical nanoparticles that can repel the further incoming spheres of the same charge. Like flower-sphere progressive assembly, other types of nanoparticle systems can also be obtained such as sphere-sphere, ellipsoidal-sphere, dumbbell-sphere, etc. as shown in Figure 5G.

3.4. Interface Supported Progressive In Situ Assembly

The assembly interactions in case of progressive in situ assemblies between pre-formed and freshly formed nanoparticles are realized through oppositely charged surface ligands at the interface. Surface ligands can protect the similar-charged nanoparticles from aggregation and allow the assembling of oppositely charged forming nanoparticles. In this scenario, if soft and swellable support in form of the intensely charged shell-like cross-linked material layer on the surface of nanoparticles is applied, then the particulate assembly system can be generated with more specificity, selectivity, and strength.[12,80,105] Therefore, the progressive assembly where freshly forming nanoparticles during the progress of polymerization being fixed/strongly assemble electrostatically in the supported shell

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layer at the surface of pre-formed nanoparticles is termed as "supported progressive assembly." Here, a soft interface[14] of the pre-formed nanoparticles can lead to efficient interfacial interaction that is finally able to generate tunable assemblies of polymer particles of different properties.

A multimodal and synergistic treatment approach is efficient for the co-delivery of various drugs of different properties for biomedical applications.[106,107] Therefore, from an application point of view, the hydrophobic–hydrophilic core–shell structure of the polymer nanoparticles is promising for co-delivery of multiple cargos of different therapeutic properties via a single delivery vehicle. In particular, the particles with core–shell structures are useful in efficient, sustained, and triggered release of the drugs of different properties at targeted sites in a sequential manner.[108–110] Besides the biomedical applications, core–shell polymeric nanoparticles are favorable for multi-fluorescence labeling, stimuli-responsive sensing, etc. Polymers are amorphous and their particulate structures are cross-linked. A dispersion of the hydrophobic polymer nanoparticles in the aqueous solution is possible due to the amphiphilic molecular interface. It can be possible to create a hydrophobic core-hydrophilic shell structure through an amphiphilic mediator via a multi-step synthetic approach. In contrast, because of different wetting characteristics, it is a challenge to connect the hydrophilic cross-linking network of the shell with the hydrophobic cross-linking network of core particles directly without any mediator through the single-step process. The formation of the core–shell structures of polymer nanoparticles through a single-step polymerization process is also advantageous because it allows tuning of the interfacial properties via in situ manner such as the thickness of the shell layer, their charge density, the overall size of the nanoparticles, etc. Therefore, here, a single-step semi-microfluidic reaction approach has been applied to generate core–shell polymer nanoparticles as shown in the upper panel of **Figure 6**A. Hydrophilic monomers (cationic diallyl dimethyl ammonium chloride (DADMAC)) plays a dual role as building unit for hydrophilic shell polymeric layer and as interfacial agent for hydrophobic core nanoparticles (PMMA). The core–shell particles are generated by emulsion co-polymerization where initial emulsion of both monomers (hydrophilic DADMAC and hydrophobic MMA) is realized in a microreactor and further polymerization was completed externally at polymerization temperature (95 °C).[111] The concentration of DADMAC can control the overall size of the core–shell nanoparticles and shell thickness during co-polymerization systematically.[111] An SEM image of the soft shell layered core–shell polymer nanoparticles (cationic) is shown in Figure 6B. Similar to cationic, anionic core–shell polymer nanoparticles of tunable size can also be prepared through a single-step process.[112]

For obtaining supported progressive polymer assembly particles, the core–shell particles have been inserted in a vial as pre-formed nanoparticles and further polymerization to grow and in situ assemble spheres on the surface of core–shell particles was conducted through the semi-microfluidic setup as shown in the right panel of Figure 6A. The surface of the core–shell polymer nanoparticles is cationic (zeta potential +35 mV),[111] hence further polymerization needs to be performed based on the anionic interfacial agent for in situ electrostatic assembly. An aqueous phase, therefore, is made up of the sodium dodecyl

sulfate (SDS), an anionic surfactant, which controls the surface dynamics to form anionic spherical nanoparticles. Like progressive assembling, anionic SDS molecules can be attached to the cationic surface of core–shell nanoparticles electrostatically. Here, the hydrophilic shell layer can be imagined as swellable in the aqueous phase. Aqueous suspension of the pre-formed core–shell particles is already available in the vial during the polymerization of in situ assembling spheres at the surface. Therefore, during the swelling state of the shell, the formation of spheres can take place inside the shell layer where anionic spheres are strongly bound and forming the robust nanoscale assembly particles. Supported progressive assembly particles are shown in Figure 6C. The size and population of the assembling spheres on the core–shell surface can systematically be tuned by varying the concentration of SDS in the aqueous phase as well as varying flow rate ratio of the aqueous and monomer phase.[111,112]

3.5. Interface Supported Assembly in Continuous Flow

On one hand, soft surface support (swellable interface) is useful for binding the guest nanoparticles more robustly. On the other hand, flow setup can provide uniform reaction surrounding that creates homogeneous nanoassembly particles. In an integrated approach, a performance of the supported assembly of interfacial active nanoparticles under flow condition is termed, here, as "supported flow assembly." Soft surface layer as a shell with tunable thickness can be applied on the surface of core nanoparticles by hydrophilic–hydrophobic single-step co-polymerization synthesis.[111] The soft shell-layered nanoparticles act as supported nanoparticles for incoming assembly nanoscale particles of opposite surface charge. It can be imagined that the hydrophilic shell layer can be swelled in an aqueous medium where their cross-linking network creates small pores that are suitable to hold smaller sized nanoparticles. A basic flow setup as shown in **Figure 7**A allows the assembly of anionic nanoparticles on the surface of cationic core–shell polymer nanoparticles. At first, spherical polymer nanoparticles of diameter about 65 nm can be adsorbed on the surface of soft layer nanoparticles of bigger size through flow assembly (Figure 7B). Besides, if smaller nanoparticles in the range of about 20 nm are used for assembling, then there is a possibility that smaller nanoparticles can assemble at a much deep level in the porous-like cross-linking network of the shell interior. In this way, a high density of assembling (guest) nanoparticles at the surface as well as in the interior space of the host can be realized. Considering this aspect, smaller sized metal nanoparticles were applied on the swellable soft shell layer of polymer nanoparticles to obtain nanoscale assembly particles under flow conditions.

Polymer–polymer nanoassembly is useful for dual fluorescence labeling applications where each type of polymer nanoparticles can be functionalized with different fluorophores.[111] On the other side, polymer–metal nanoscale assemblies are highly useful for surface-enhanced Raman scattering (SERS) sensing, biosensing, and catalysis application.[111–116] In particular, the porosity of the substrate and high loading of metallic content is highly appreciated for the enhanced sensing outcome.[116] Here, the requirement of the porosity can be met
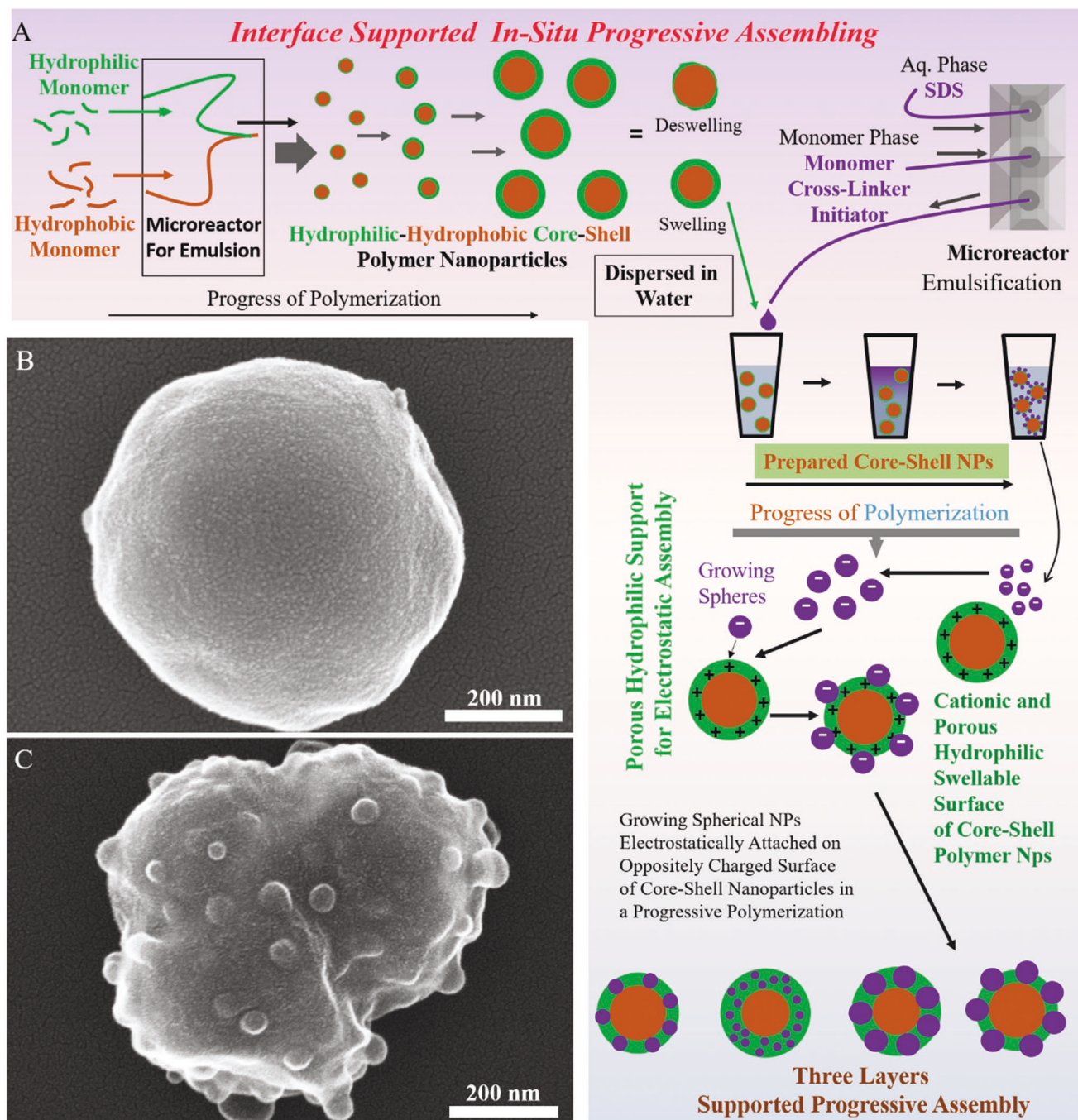
Figure 6. Supported progressive assembly system in which incoming (growing) charged spherical nanoparticles assemble with an oppositely charged supportive swellable surface layer of the polymer nanoparticles progressively during growth. A) A scheme showing the formation of supportive nanoparticles with their core–shell type structure: the core is hydrophobic PMMA and the shell surface layer is hydrophilic polyDADMAC. Further addition of the polymer nanoparticles ingredients to the core–shell nanoparticles allows the formation of three-layered assembling nanoparticles: PMMA core, polyDADMAC shell layer, and surface assembled PMMA nanoparticles on the top.[111] B) SEM image of the core–shell type polymer nanoparticles: swellable surface layer is cationic, soft, and hydrophilic. C) SEM image of the assembly nanoparticles: Growing nanoparticles progressively assemble on the supported core–shell type nanoparticles. The concept and parts of the results are reproduced from the authors' previously published article. Reproduced with permission.[111] Copyright 2015, Wiley-VCH.

by using swellable surface layered polymer nanoparticles and that also allows high loading of the smaller-sized assembling metal nanoparticles. High loading can also generate the roughened metallic surface that leads the efficient analyte interaction

and reveal the enhanced sensing outcome even by using trace concentration of analyte of interest.[111,112] In general, polymer–metal assembly particles are promising for coupled properties[7] where metal nanoparticles are electrically conductive as well as
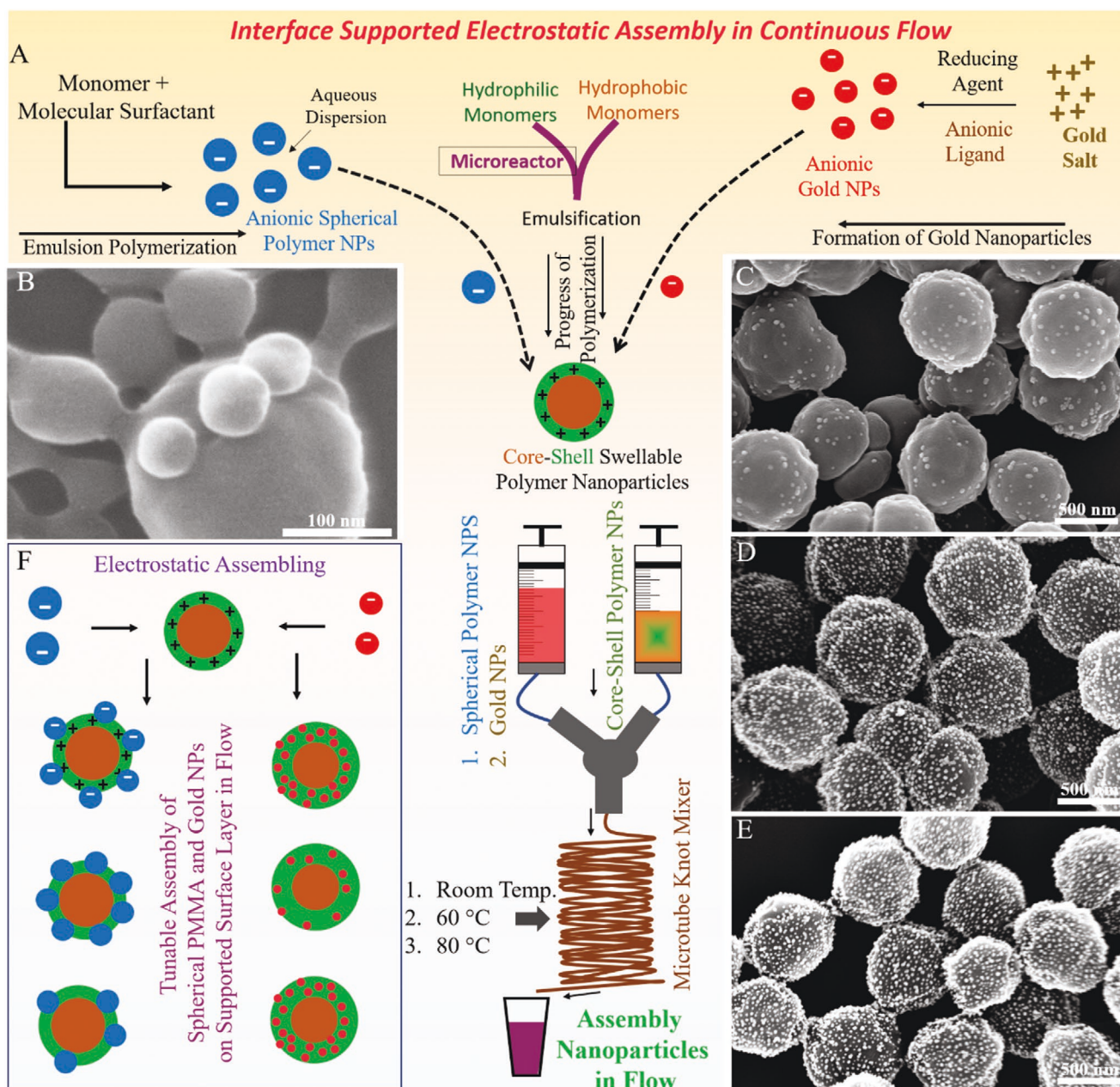
Figure 7. Nanoparticles assembly on the supported swellable layer in a flow setup. A) Schematics overview of the formation of polymer–polymer and polymer–metal nanoassembly particles in which negatively charged PMMA and gold nanoparticles electrostatically assembled on the swellable cationic surface layer of the core–shell polymer nanoparticles in a continuous flow condition. B) SEM image of the SDS-capped PMMA nanoparticles on the cationic surface of core–shell polymer nanoparticles at 80 °C. SEM images of the polymer–metal nanoassembly particles: C,D) 50 and 100 μL of the gold suspension assembled electrostatically on cationic polymer nanoparticles at 60 °C in flow setup, respectively.[111,112] E) SEM image of the polymer–gold nanoassembly particles prepared at room temperature. F) A cartoon for the structures of the assembly nanoparticles. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[111,112] Copyright 2015, Wiley-VCH; Copyright 2017, Wiley-VCH.

plasmonic active[117,118] and polymer nanoparticles are porous, swellable, and flexible support for assembling interactions.[119] For obtaining polymer–metal nanoassemblies, gold nanoparticles were prepared by the citrate method.[120] On the one side, the zeta potential of the gold nanoparticles is −22 mV.[111,120] On the other side, the zeta potential of the utilized core–shell polymer nanoparticles for polymer–metal nanoassembly was

+32 mV.[111] The flow assembly process has been conducted at room temperature where a suitable volume ratio of nanoparticles' suspension (polymer:metal 1:6) allows the complete coverage of the surface of polymer nanoparticles by smaller metal nanoparticles. Also, flow assembling can be performed at various temperatures for the study of the spontaneity of the electrostatic interaction inflow.[100,111,112] Different volume ratios

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of the metal and polymer nanoparticles can systematically tune the loading of metal nanoparticles on the surface of polymer nanoparticles as shown in Figure 7C–E. Overall, with flow synthesis, a systematic tuning in the uniform assembling can be realized in both cases of polymer–polymer and polymer–metal nanoassemblies by varying volume ratio of nanoparticles (between 1:1 and 1:8), the concentration of nanoparticles' suspension (between original suspension and 1/20 dilution), and temperature (between room temperature and 90 °C) (Figure 7F).[111,112]

3.6. Layer-By-Layer Electrostatic Assembly

An interface, where two types of nanoparticles interact and assemble, is an active site that is equipped with reactive functional groups, electrical charges, and responsiveness to external stimuli.[121,122] Usually, nanoparticles' interface is tailored by a molecular interfacial agent that is electrically charged or amphiphilic, flexible in movement, and capable of initiating interfacial interactions with other suitable components.[11] Appropriate tailoring of the surface state of nanoparticles can lead to the specificity, selectivity, and robustness of the overall assembly process to generate the desired nanoscale assembly particles.[88] On one hand, the inherent molecular interface of the nanoparticles is potential where binding of two different types of particulate entities can take place through adsorption with the shortest possible thickness of the molecular layer between two solid particles.[88] The layer-by-layer tailoring on the surface of nanoparticles with multiple molecular layers, on the other hand, can provide the space for more variability and tunable interaction dimensionality for assembly nanoparticles. Multi-layer stacking of functional molecules on nanoparticles' surface by layer-by-layer tailoring can be directed by either covalent, electrostatic, van der Waals, secondary interactions, or weak interactions such as H-bonding, etc.[123,124] Overall, assemblies of the nanoscale particles facilitated by a multi-layered surface-functionalized approach is described here as "layer-by-layer particulate assembly."

Alongside the Langmuir–Blodgett layer[125] as well as self-assembled monolayer deposition,[126] a layer-by-layer deposition at the surface hold promise for various biomedical and catalytic applications.[26,27,127–130] The layer-by-layer functionalization approach is well-studied in the case of the flat surface and film-based substrates.[125,126] Besides 2D multi-layer formation, the layer-by-layer functionalization on the surface of freestanding nanoparticles is promising because of their 3D interfacial ability.[28] Multiple layers at the surface of nanoparticles can also be useful for generating the switchable surface based on chemical functionalities and electrical charging. Multilayers can be applied by amide bond formation (covalent bonding) between attaching molecular polymeric layers. For instance, because of the strong binding affinity, poly-L-lysine and poly-L-glutamic acid link each other with the desired number of layers on the surface.[99,131] On the other hand, a simple, versatile, and efficient layer-by-layer functionalization can be achieved by oppositely charged polyelectrolytes.[127,132–134] Multi-layered polyelectrolytes create soft and swellable interfaces that finally leads to a strong interaction

with oppositely charged nanoparticles to form particles-particles nanoassemblies. Here, multi-layered functionalization by cationic poly(diallyldimethyl ammonium bromide) (poly-DADMAC) and anionic poly(styrene sulfonate sodium salt) (PSSS) has been applied on the surface of PMMA nanoparticles of different size and shape as shown in **Figure 8**A. The anionic surface of flower-shaped PMMA nanoparticles (zeta potential −16 mV) as shown in Figure 8B becomes cationic (zeta potential +43 mV) after applying the polyDADMAC layer.[89] Afterward, the desired numbers of polyelectrolyte layers of opposite surface charge one-by-one can be applied.[89] At the end of multilayered polyelectrolyte functionalization, oppositely charged nanoparticles produce particle-particle nanoscale assemblies. Here, a flower-sphere particulate assembly is formed as shown in Figure 8C. Like flower-sphere, other types of size and shaped polymer nanoparticle assembly can also be obtained by a multi-layered assembling approach. For instance, two different sized anionic spherical nanoparticles were assembled after surface modification of larger spheres with polyDADMAC as shown in Figure 8D. The tunable zeta potential of the nanoparticles is shown in Figure 8E after applying alternatively charged polyelectrolytes.

3.7. Polymer–Metal Hybrid Electrostatic Assembly

As described above that polymer–metal nanoscale assembly particles can combine the properties of two or more different domains. Specifically, it is possible to achieve field enhancement based on plasmon-centric light–matter interactions.[135] Polymers are not interacting with light in a direct means and polymer–polymer assembly is a singular type assembly. Oppositely, when functional polymer nanoparticles assemble with light-interactive components, the synergistic effect can be realized that is not able to harvest by individual components. Therefore, here, a metal–polymer nanoparticulate assembly is described as "hybrid assembly." In the hybrid assemblies, surface plasmon—a collective oscillation of the surface electrons upon interaction with light—is highly useful in a wide range of advanced applications such as photoacoustic effects, photocatalysis, SERS sensing, energy conversion, and nonlinear optical conversion, etc.[135–137] The properties of surface plasmons are strongly related to materials and structures. Particularly, the shape of metal nanoparticles can tune the optical signatures in a dramatic way where smaller sized spherical metal nanoparticles give the intense single optical peak and shape tuned metal nanoparticles can create multiple plasmonic peaks in a single optical spectrum.[138,139] Shape-control or symmetry-breaking of the metal nanoparticles can be achieved by seed-mediated processes or surface directing growth processes.[140]

For obtaining the systematic polymer–metal hybrid assembly, here 1D silver nanorods were synthesized by a self-seeding polyol synthesis (**Figure 9**A).[141] The symmetry breaking during the nucleation and growth can be realized by a surface directing agent (PVP) that passivates the low-energy facets and allow the growth of nanostructure in 1D that finally creates tunable silver nanorods.[141] The obtained silver nanorods are shown in Figure 9B. Zeta potential of the silver nanorods showing negative value (−16 mV).[88] These anionic nanorods are suitable
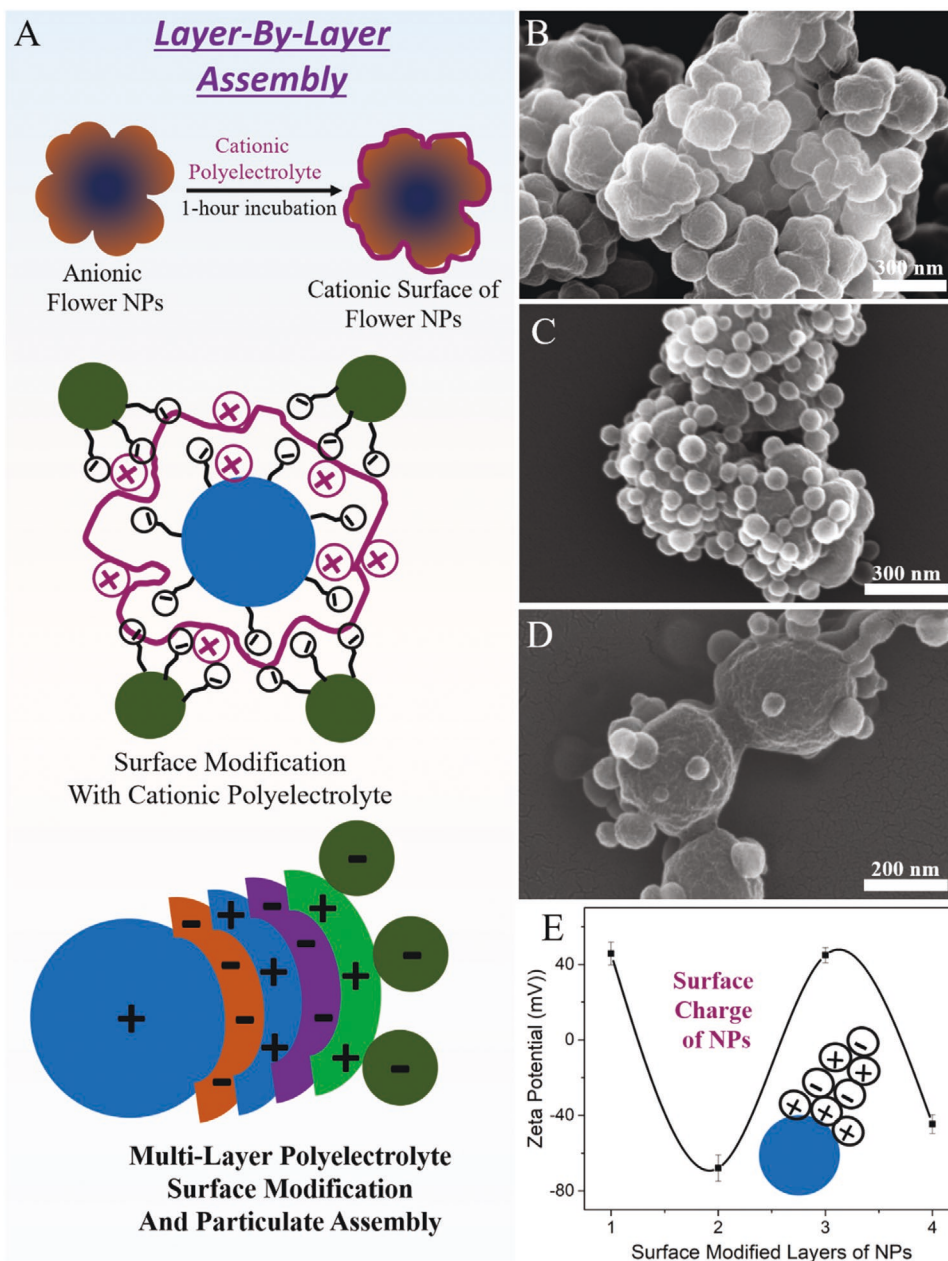
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Figure 8. Layer-by-layer surface modification with alternatively charged polyelectrolytes and electrostatic assembly of the polymeric nanoparticles. A) Concept of the desired number of surface modification with polyelectrolytes and nanoparticle assembly. SEM images: B) surface-modified flower-shaped PMMA nanoparticles, C) flower-sphere nanoassembling particles with one layer of surface modification by cationic polyDADMAC of flower-shaped PMMA nanoparticles, D) sphere-sphere nanoassembly particles with one layer of surface modification by cationic polyDADMAC of spherical PMMA nanoparticles. E) Surface charge characterization of the PMMA nanoparticles by zeta potential after several layers of the surface modification. The concept and parts of the results are reproduced from the authors' previously published article. Reproduced with permission.[89] Copyright 2016, Elsevier Ltd.

to form assemblies with cationic polymer nanoparticles via electrostatic interaction. Therefore, cationic spherical polymer nanoparticles of tunable size and surface charge were obtained by semi-microfluidic synthesis.[88] A model SEM image of cationic polymeric spheres is shown in Figure 9C. The 1D structure of the silver nanorod is suitable to hold the spheres on its surface that can able to form a decorative hybrid assembly. For uniform and systematic assembly, size and surface charge of the polymeric spheres are playing a crucial role. Smaller sized

spheres (around 65 nm) have a high surface charge (+42 mV) and they can electrostatically adsorb everywhere on the surface of silver rods due to the strong attraction between oppositely charged surfaces (Figure 9D). In this scenario, a highly systematic and decorative assembly system can be obtained if the charge density of the assembling nanoparticles can be controlled. Therefore, by using 0.1 mм CTAB in the aqueous phase, 110 nm-sized spherical polymer nanoparticles were synthesized whose surface charge has been appeared +22 mV.[88] The
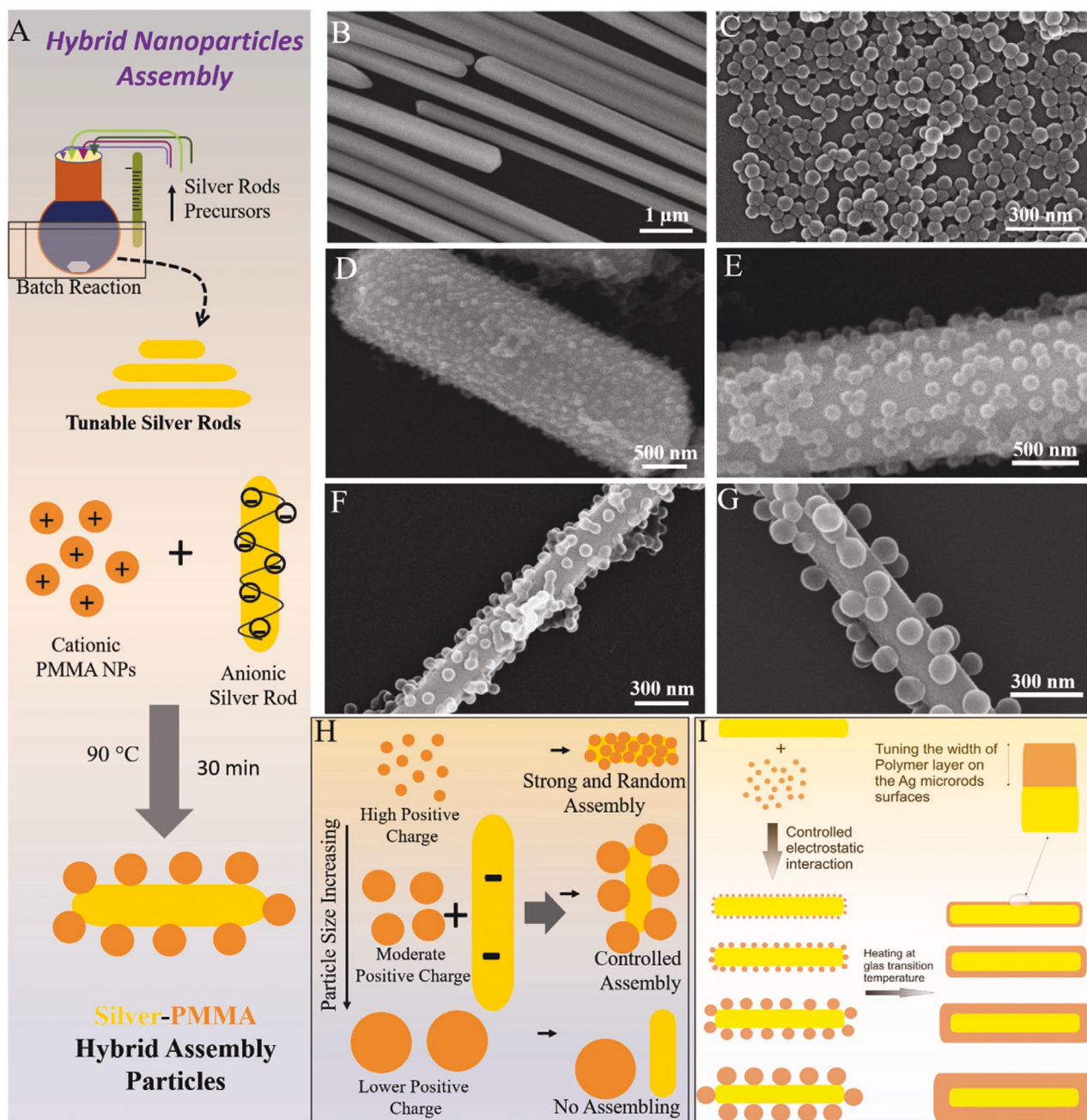
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Figure 9. Metal–polymer hybrid nanoparticles assembly system. A) A scheme for the formation of silver rods via ethylene glycol-based polyol synthesis and their assembly with oppositely charged polymer nanoparticles. SEM images: B) silver rods via polyol synthesis,[141] C) PMMA nanoparticles in a semi-microfluidic process,[88] D) silver-PMMA nanoassembly particles (70 nm-sized PMMA particles prepared by using 1 mM CTAB concentration in aqueous phase),[88] E) silver-PMMA nanoassembly particles at 65 °C for 2-h stirring (110 nm-sized PMMA particles prepared by using 0.1 mM CTAB concentration in aqueous phase), F) silver-PMMA nanoassembly particles at 90 °C for 30 min stirring (70 nm-sized PMMA particles prepared by using 1 mM CTAB concentration in aqueous phase), and G) silver-PMMA nanoassembly particles at 90 °C for 12 min stirring (110 nm-sized PMMA particles prepared by using 0.1 mM CTAB concentration in aqueous phase). H) Schematic overview of silver-PMMA nanoassembly particles with various sizes and surface charge of the PMMA nanoparticles. I) A schematic for the future application to apply a polymeric insulator of tunable surface layer thickness for conducting nanorods-nanowires with insulating templates for electronic devices. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[88,141] Copyright 2013, Wiley-VCH; Copyright 2013, IOP Publishing Ltd.

spheres of 110 nm diameter were highly suitable to arrange on silver rods by keeping a certain distance from adjacent spheres on rods (Figure 9E–G). Hybrid assembly based on controlled surface charge strongly influences by volume ratio, time of reaction, dilution (particle concentration), and temperature (Figure 9H).[88] The systematic arrangement of the polymeric spheres on silver rods in a decorative manner is possibly based on two interactions: the controlled electrostatic attraction

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between spheres and rod, and partial electrostatic repulsion between the same charged spheres on the rods. Because of this relatively uniform distribution of spheres on a rod, it is possible to create a uniform polymeric layer on the silver rod by heating the assembly at or slightly above the glass transition temperature of the polymer (concept shown in Figure 9I). The polymeric insulating layer on the nanoscale conducting silver rod can be useful for smart nanotechnology-equipped electronic devices. Various functionalities such as fluorescence functionality can be introduced in the interior or at the interface of polymer nanoparticles, so that they can be able to create hot spots while adsorbed on silver rods. The generated hot spots in functional hybrid assemblies can be useful in creating enhanced field effect in a synergistic outcome. Like polymer–metal assembly, other types of polymer-based hybrid assemblies can also be generated such as polymer–semiconductor assembly, polymer–dielectric assembly, etc.

4. Nano–Micro Multiscale Assembly

At a lower lengthscale, various atoms are linked by covalent bonding to form molecules, and molecules (monomers) can be cross-linked to form nanoscale polymer particles. From the applications' point of view, the lengthscale is playing a very important function. In the nanoscale domain, for instance, surface area and surface energy are crucial parameters that can be significantly tuned by increasing or decreasing the size of the nanoparticles that directly lead to consequence in application outcomes. While nanoscale polymer particles are prominent for various functional applications such as immunoassays, endocytosis, drug delivery, labeling, etc.,[68,142] their microscale counterparts, on the other hand, show excellent potential for other prominent applications such as tissue regeneration, biosensing, surface-enhanced Raman scattering, cargo carrying, etc.[116,143,144] Assembly of nanoscale particles with microscale counterparts combines the properties and functions of both domains and can be able to generate novel properties that are crucial for advanced applications.

Multiscale assembly particles can be fabricated by various means either through a single-step process or through multistep processes. At first, beginning with smaller nanoparticles, hierarchical assemblies of different lengthscale ranging from lower nanometer up to higher micrometer scale can be formed by multiple nanoparticulate assembling steps. The advantage of the nanoparticle-based hierarchical assembly is that there is a possibility to introduce desired numbers of active functions by energetic components such as molecular reporter, labeling agents, therapeutic cargos, nanosensors, etc. throughout the assembly structures during the step-by-step formation of assemblies.[145–147] Second, multiscale assemblies by using particles of various length scale can be fabricated by applying one type of particles on the surface of others through electrostatic interactions as well as other interfacial interactions.[148] In this case, it is possible to tune the size, shape, and density of the assembling particles on the surface of host particles which is useful for tunable assembly product. A third, a single-step microfluidic formation of the microscale particles and their assemblies with embedded droplets/nanoparticles can be formed by double or triple (multiple) emulsion through droplet-based microfluidics.[143,149–153] Multiple emulsion-based droplet microfluidics forms capsules of different wetting properties and also of tunable functionalities that are promising for biomedical applications. At fourth, systematic distribution of the functional nanoparticles of smaller to larger size throughout the interior of the microparticles that form efficient nano–micro particulate assemblies can be developed by microfluidics with photopolymerization.[30] In this technique, pre-formed functional nanoparticles can be dispersed in the monomer phase where microscale droplets can be solidified by photopolymerization and produce microparticles with uniform distribution of nanoparticles in the interior. All these techniques for the development of multiscale assemblies are efficient, and the details of the fourth technique are provided here as a proof-of-concept. As shown in **Figure 10**A, the formation of PMMA-embedded hydrophilic polyacrylamide microparticles takes place by photopolymerization. Spherical PMMA nanoparticles of a diameter of 110 nm were synthesized by semi-microfluidic emulsion polymerization. For the formation of PMMA nanoparticle-containing polyacrylamide microparticles, nanoparticles were dispersed in an aqueous solution of acrylamide which also contains a photoinitiator and a cross-linker. The size of the microparticles depends on the size of the droplets in a microchannel, that can be controlled by varying the concentration of surfactant in the carrier phase, flow rate ratio, and channel dimension.[31,116,154–159] The formation of polyacrylamide microparticles of a size of about 320 μm is shown in Figure 10B. Different types of size and shape-controlled nanoparticles can be embedded in the microparticles for carious application requirements (Figure 10C–E). By microfluidic photopolymerization, surface fabrication of the microscale particles such as wrinkled and porous surface can be fabricated which has a high potential for some renowned biomedical applications such as tissue regeneration and surface-enhanced Raman scattering based molecular sensing.[160]

5. Summary and Outlook

This progress report is focused on various syntheses strategies to achieve better control over their assembling interactions to form uniform particulate assemblies. Polymers are amorphous or lower crystalline materials, and particulate polymeric structures are cross-linked network. The mechanical strength of the polymeric structures depends on the type and combination of various polymers. The distinct characteristics of the particulate polymers such as softness, flexibility, and responsiveness to surrounding solvents/stimuli are crucial for diverse properties and advanced functions. As the interior of the nanoparticles is relatively soft and cross-linked, it is of specific importance to carry functional molecular as well as small particulate components such as therapeutic cargos, drugs, insulin, proteins, DNA, or RNA, etc. to deliver at targeted sites. Here, for high loading and efficient delivery of the functional components in a sustained manner, the structural aspects of the polymer particles such as size, shape, interior spacing, etc. are playing significant roles. Besides the structural aspects, the most important characteristics of the polymer particles are their interfaces. At first, whether the particulate polymers are hydrophilic or hydrophobic, their first
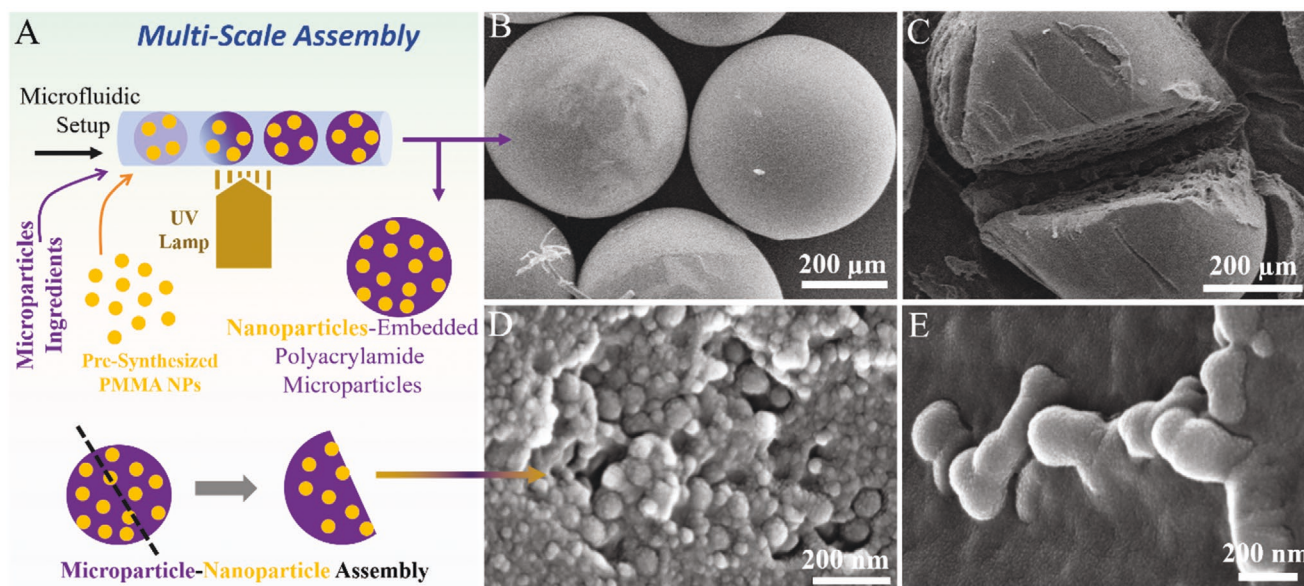
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Figure 10. Multiscale assembly concept. A) A scheme for the formation of the microparticles in a microfluidic setup through photopolymerization, which simultaneously embeds nanoscale PMMA particles. SEM images: B) PMMA nanoparticles-embedded acrylamide microparticles, C) cutting of the microparticles for observing the embedded nanoparticles through imaging, D,E) the higher magnified SEM images in which spherical and linear-shaped PMMA nanoparticles embedded in the interior of the polyacrylamide microparticles, respectively. The concept and parts of the results are reproduced from the authors' previously published articles. Reproduced with permission.[89,116] Copyright 2015, American Chemical Society; Copyright 2016, Elsevier Ltd.

contact with the surrounding medium (solvent) is through the surface. Second, irrespective of the hydrophilicity or hydrophobicity, polymer particles can be capped with amphiphilic molecules that allow to adapt different types of polymeric particles to the surrounding medium and support their dispersity in solution by providing stability against uncontrolled aggregation. A third, the availability of surface ligands at the surface of polymer particles creates a soft interface, on one hand, and stimulates efficient interfacial interactions, on the other hand. By precisely controlling the structural and interfacial aspects of the polymer particles, significant interfacial interactions can be stimulated for polymeric particulate assemblies that present synergistic properties and functions useful for a diverse range of applications. With these perspectives, different assemblies are presented here.

Polyelectrolytes as interfacial agents create a soft interface and direct the growth of polymer nanoparticles, which can be spontaneously self-assembled during the ongoing continuous polymerization process that forms ellipsoidal, dumbbell, and other linear shapes of the final nanoparticles. The soft interface-driven self-assembly of the growing nanoparticles is the result of limited polarizability, partial repulsion, controlled electrostatic interaction, minimizing energy barrier, and hydrophobic interaction. A successful proof-of-concept of PMMA nanoparticles self-assembly presented here, and other polymeric materials (hydrophilic, hydrophobic, decreased amorphousness, increasing semi-crystallinity, co-polymers, etc.) need to be explored for continuous self-assembling/shapes based on microfluidics/semi-microfluidics and interfacial chemistries. Nanoparticle assemblies through electrostatic interactions, on the other hand, through batch processes is a simple and powerful method for large-scale production. Charge density, nanoparticle dispersion (concentration), size of particles, reaction surrounding (solvent),

etc. playing roles during the electrostatic interaction in batch synthesis. Here, assembly takes place at the nanoscale, and the reaction medium is bulk in a batch method that may produce inhomogeneity in the assembly particulate product. There is a need to investigate feasible and novel batch processes, as well as suitable interfacial chemistry, which needs to be tailored to achieve more uniformity in the particulate nanoassemblies.

If a reaction environment can be developed where one type of growing nanoparticles can be formed and simultaneously assembled at the surface of pre-formed nanoparticles progressively during polymerization, then there is a high possibility for the generation of nanoassemblies with a systematic distribution of the assembling nanoparticles. Like flower-sphere progressive assembly presented in this paper, other types of potential nanoparticulate systems can be developed based on size, shape, and surface charge tunability in future endeavors. Also, more specific, selective, and strong assembly particles cab be obtained if soft, swellable, and nanoporous surface support can be systematically tailored to the pre-formed particles. In this way, in situ growing nanoparticles do not attach directly at the surface but entrapped inside the swellable shell layer at the surface that provides robustness in overall assembly architecture. Applying a tunable thick shell layer on the surface of polymer nanoparticles during the one-step process is challenging but owing to their advantage of providing efficient support for the strong assemblies, more development on the soft-shell layers of diverse interfacial properties needs to be investigated. The batch assembly may have a concern of non-uniformity that can be resolved by microfluidic flow arrangement. Microfluidics is advantageous in many ways over their batch counterparts. Microfluidics deals with a small volume (picoliter or nanoliter), fast phase, and mass transfer as well as rapid heat transfer, efficient reactant mixing

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due to high surface area in a microchannel, spontaneous compositions of various reactants for time-sensitive chemical reactions, and reaction in a continuous flow with droplet dynamics. Considering these advantages, microfluidics hold promises for the generation of uniform particulate assemblies. As presented here a model polymeric flower-sphere uniform nanoassembly particles, other systems required to be developed. The supported assembly provides strength and microfluidics provides uniformity; strengthened as well as uniform assemblies can be obtained if flow setup can be used for the supported assemblies. The availability of nanopores on the supported nanoparticles allows entrapping smaller sized light-active nanoparticles with high loading and very useful for efficient sensing applications. The detailed analysis of the sensing outcome in static, as well as sequential manner, needs to be investigated further.

Hybrid assembly combines the properties of two or more different functional nanoscale particles and creates completely novel properties that cannot be able to be harvested by individual components. A wide range of different combinations of the assembling nanoparticles of diverse properties such as silica–gold, silica–alumina, metal–semiconducting, etc. needs to be explored with their systematic distribution of assembling nanoparticles to create decorative nanoassemblies. The layer-by-layer particulate assembly approach, on the other hand, provides a platform for the generation of 3D and hierarchical assembly architecture. A wide range of different functions such as fluorescence functionality, nanosensors, etc. can be linked in multilayers that create multi-functional assembly architectures. Like an approach based on multiple polyelectrolytes presented here as a proof-of-principle, other types of functional molecular, as well as particulates layers, can be applied more systematically. In the end, nanoscale particles of high surface energy assemble through multiple steps to hierarchical particulate assembly structures of micrometer length scale, or nanoscale particles assemble with microscale particles at the surface or embed in the interior to form multiscale assemblies. The surface of the polymer microparticles for their potential assemblies can be fabricated by inducing surface porosity, wrinkles, surface textures, etc. that support additional functionality in the hierarchical architectures.

Conflict of Interest

The authors declare no conflict of interest.

[1] A. H. Gröschel, A. Walther, T. I. Löbling, F. H. Schacher, H. Schmalz, A. H. E. Müller, *Nature* **2013**, *503*, 247.
[2] W. Jiang, Z.-B. Qu, P. Kumar, D. Vecchio, Y. Wang, Y. Ma, J. H. Bahng, K. Bernardino, W. R. Gomes, F. M. Colombari, A. Lozada-Blanco, M. Veksler, E. Marino, A. Simon, C. Murray, S. R. Muniz, A. F. de Moura, N. A. Kotov, *Science* **2020**, *368*, 642.
[3] H. Jin, Y. Zhou, W. Huang, D. Yan, *Langmuir* **2010**, *26*, 14512.
[4] X. Wang, P. Gao, Y. Yang, H. Guo, D. Wu, *Nat. Commun.* **2018**, *9*, 2772.
[5] F. Li, D. P. Josephson, A. Stein, *Angew. Chem., Int. Ed.* **2011**, *50*, 360.
[6] A. Walther, M. Drechsler, S. Rosenfeldt, L. Harnau, M. Ballauff, V. Abetz, A. H. E. Müller, *J. Am. Chem. Soc.* **2009**, *131*, 4720.
[7] A. C. Balazs, T. Emrick, T. P. Russell, *Science* **2006**, *314*, 1107.
[8] N. Farkas, P. V. Scaria, M. C. Woodle, J. A. Dagata, *Sci. Rep.* **2019**, *9*, 1655.
[9] A. Walther, A. H. E. Müller, *Chem. Rev.* **2013**, *113*, 5194.
[10] B. Molleman, T. Hiemstra, *Phys. Chem. Chem. Phys.* **2018**, *20*, 20575.
[11] A. Musyanovych, K. Landfester, *Macromol. Biosci.* **2014**, *14*, 458.
[12] S. Kinge, M. Crego-Calama, D. N. Reinhoudt, *ChemPhysChem* **2008**, *9*, 20.
[13] Y. Jiang, R. Chakroun, P. Gu, A. H. Gröschel, T. P. Russell, *Angew. Chem., Int. Ed.* **2020**, *59*, 12751.
[14] A. Böker, J. He, T. Emrick, T. P. Russell, *Soft Matter* **2007**, *3*, 1231.
[15] H. J. Martin, B. T. White, C. J. Scanlon, T. Saito, M. D. Dadmun, *Soft Matter* **2017**, *13*, 8849.
[16] S. N. Barnaby, R. V. Thaner, M. B. Ross, K. A. Brown, G. C. Schatz, C. A. Mirkin, *J. Am. Chem. Soc.* **2015**, *137*, 13566.
[17] A. M. Kalsin, M. Fialkowski, M. Paszewski, S. K. Smoukov, K. J. M. Bishop, B. A. Grzybowski, *Science* **2006**, *312*, 420.
[18] F. Zhang, Z. Yang, J. Hao, K. Zhao, M. Hua, Y. Yang, J. Wei, *Commun. Chem.* **2019**, *2*, 123.
[19] A.-C. Genix, J. Oberdisse, *Soft Matter* **2018**, *14*, 5161.
[20] F. Nan, F. Han, N. F. Scherer, Z. Yan, *Adv. Mater.* **2018**, *30*, 1803238.
[21] S. Li, B. A. Moosa, J. G. Croissant, N. M. Khashab, *Angew. Chem., Int. Ed.* **2015**, *54*, 6804.
[22] Z. Zhu, N. Xu, Q. Yu, L. Guo, H. Cao, X. Lu, Y. Cai, *Macromol. Rapid Commun.* **2015**, *36*, 1521.
[23] N. Visaveliya, J. M. Kohler, *ACS Appl. Mater. Interfaces* **2014**, *6*, 11254.
[24] Z. Wang, Z. Wang, J. Li, C. Tian, Y. Wang, *Nat. Commun.* **2020**, *11*, 2670.
[25] B. Karagoz, C. Boyer, T. P. Davis, *Macromol. Rapid Commun.* **2014**, *35*, 417.
[26] Y. Yan, M. Björnmalm, F. Caruso, *Chem. Mater.* **2014**, *26*, 452.
[27] Z. Poon, D. Chang, X. Zhao, P. T. Hammond, *ACS Nano* **2011**, *5*, 4284.
[28] C. Jiang, V. V. Tsukruk, *Adv. Mater.* **2006**, *18*, 829.
[29] J. Lee, P. W. Bisso, R. L. Srinivas, J. J. Kim, A. J. Swiston, P. S. Doyle, *Nat. Mater.* **2014**, *13*, 524.
[30] W. Yu, N. Visaveliya, C. A. Serra, J. M. Köhler, S. Ding, M. Bouquey, R. Muller, M. Schmutz, I. Kraus, *Materials* **2019**, *12*, 3921.
[31] C. A. Serra, Z. Chang, *Chem. Eng. Technol.* **2008**, *31*, 1099.
[32] S. Zhang, R. Geryak, J. Geldmeier, S. Kim, V. V. Tsukruk, *Chem. Rev.* **2017**, *117*, 12942.
[33] W. Li, H. Palis, R. Mérindol, J. Majimel, S. Ravaine, E. Duguet, *Chem. Soc. Rev.* **2020**, *49*, 1955.
[34] A. Piloni, A. Walther, M. H. Stenzel, *Polym. Chem.* **2018**, *9*, 4132.
[35] N. J. W. Penfold, J. Yeow, C. Boyer, S. P. Armes, *ACS Macro Lett.* **2019**, *8*, 1029.
[36] N. J. Warren, S. P. Armes, *J. Am. Chem. Soc.* **2014**, *136*, 10174.
[37] C. Huang, D. Quinn, Y. Sadovsky, S. Suresh, K. J. Hsia, *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 2910.
[38] Y. Lu, J. Lin, L. Wang, L. Zhang, C. Cai, *Chem. Rev.* **2020**, *120*, 4111.

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[39] J. Zhang, B. A. Grzybowski, S. Granick, *Langmuir* **2017**, *33*, 6964.

[40] C. Marschelke, O. Diring, A. Synytska, *Nanoscale Adv.* **2019**, *1*, 3715.

[41] Q. Yang, K. Loos, *Polym. Chem.* **2017**, *8*, 641.

[42] A. M. Percebom, L. H. M. Costa, *Adv. Colloid Interface Sci.* **2019**, *269*, 256.

[43] K. Jähnisch, V. Hessel, H. Löwe, M. Baerns, *Angew. Chem., Int. Ed.* **2004**, *43*, 406.

[44] H. Song, D. L. Chen, R. F. Ismagilov, *Angew. Chem., Int. Ed.* **2006**, *45*, 7336.

[45] D. T. Chiu, A. J. deMello, D. Di Carlo, P. S. Doyle, C. Hansen, R. M. Maceiczyk, R. C. R. Wootton, *Chem* **2017**, *2*, 201.

[46] Y. Ding, P. D. Howes, A. J. deMello, *Anal. Chem.* **2020**, *92*, 132.

[47] A. Suea-Ngam, P. D. Howes, M. Srisa-Art, A. J. deMello, *Chem. Commun.* **2019**, *55*, 9895.

[48] M. Ugrinic, A. deMello, T. Y. D. Tang, *Chem* **2019**, *5*, 1727.

[49] A. Günther, K. F. Jensen, *Lab Chip* **2006**, *6*, 1487.

[50] R. L. Hartman, J. P. McMullen, K. F. Jensen, *Angew. Chem., Int. Ed.* **2011**, *50*, 7502.

[51] N. G. Brukman, B. Uygur, B. Podbilewicz, L. V. Chernomordik, *J. Cell Biol.* **2019**, *218*, 1436.

[52] J. M. Hernández, B. Podbilewicz, *Development* **2017**, *144*, 4481.

[53] M. J. Petrany, D. P. Millay, *Trends Cell Biol.* **2019**, *29*, 964.

[54] B. M. Ogle, M. Cascalho, J. L. Platt, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 567.

[55] Q.-L. Ying, J. Nichols, E. P. Evans, A. G. Smith, *Nature* **2002**, *416*, 545.

[56] W. Qiu, Z. Fu, G. G. Xu, R. A. Grassucci, Y. Zhang, J. Frank, W. A. Hendrickson, Y. Guo, *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 12985.

[57] S. Martens, H. T. McMahon, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 543.

[58] S. V. Gadhiya, C. Hu, W. W. Harding, *Tetrahedron Lett.* **2016**, *57*, 2090.

[59] J. H. Kim, Y. Ren, W. P. Ng, S. Li, S. Son, Y.-S. Kee, S. Zhang, G. Zhang, D. A. Fletcher, D. N. Robinson, E. H. Chen, *Dev. Cell* **2015**, *32*, 561.

[60] J. Pérez-Vargas, T. Krey, C. Valansi, O. Avinoam, A. Haouz, M. Jamin, H. Raveh-Barak, B. Podbilewicz, F. A. Rey, *Cell* **2014**, *157*, 407.

[61] M. E. Quinn, Q. Goh, M. Kurosaka, D. G. Gamage, M. J. Petrany, V. Prasad, D. P. Millay, *Nat. Commun.* **2017**, *8*, 15665.

[62] Y. Zhao, K. Thorkelsson, A. J. Mastroianni, T. Schilling, J. M. Luther, B. J. Rancatore, K. Matsunaga, H. Jinnai, Y. Wu, D. Poulsen, J. M. J. Fréchet, A. Paul Alivisatos, T. Xu, *Nat. Mater.* **2009**, *8*, 979.

[63] S. Mondal Roy, M. Sarkar, *J. Lipids* **2011**, *2011*, 528784.

[64] F. S. Cohen, G. B. Melikyan, *J. Membr. Biol.* **2004**, *199*, 1.

[65] X. Chen, J. Li, *Mater. Chem. Front.* **2020**, *4*, 750.

[66] X. Liu, Y. Yang, M. W. Urban, *Macromol. Rapid Commun.* **2017**, *38*, 1700030.

[67] S. Sacanna, M. Korpics, K. Rodriguez, L. Colón-Meléndez, S.-H. Kim, D. J. Pine, G.-R. Yi, *Nat. Commun.* **2013**, *4*, 1688.

[68] O. C. Farokhzad, R. Langer, *ACS Nano* **2009**, *3*, 16.

[69] L. E. Euliss, J. A. DuPont, S. Gratton, J. M. DeSimone, *Chem. Soc. Rev.* **2006**, *35*, 1095.

[70] P. Kolhar, A. C. Anselmo, V. Gupta, K. Pant, B. Prabhakarpandian, E. Ruoslahti, S. Mitragotri, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 10753.

[71] J.-M. Williford, J. L. Santos, R. Shyam, H.-Q. Mao, *Biomater. Sci.* **2015**, *3*, 894.

[72] J. A. Champion, S. Mitragotri, *Pharm. Res.* **2009**, *26*, 244.

[73] J. A. Champion, S. Mitragotri, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 4930.

[74] D. K. Beaman, E. J. Robertson, G. L. Richmond, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 3226.

[75] B. V. Parakhonskiy, M. F. Bedard, T. V. Bukreeva, G. B. Sukhorukov, H. Möhwald, A. G. Skirtach, *J. Phys. Chem. C* **2010**, *114*, 1996.

[76] S. Saraf, C. J. Neal, S. Das, S. Barkam, R. McCormack, S. Seal, *ACS Appl. Mater. Interfaces* **2014**, *6*, 5472.

[77] F. M. Gilles, F. M. Boubeta, O. Azzaroni, I. Szleifer, M. Tagliazucchi, *J. Phys. Chem. C* **2018**, *122*, 6669.

[78] R. J. Nap, S. H. Park, I. Szleifer, *J. Polym. Sci., Part B: Polym. Phys.* **2014**, *52*, 1689.

[79] J. Zhou, S. Moya, L. Ma, C. Gao, J. Shen, *Macromol. Biosci.* **2009**, *9*, 326.

[80] M. Husemann, D. Mecerreyes, C. J. Hawker, J. L. Hedrick, R. Shah, N. L. Abbott, *Angew. Chem., Int. Ed.* **1999**, *38*, 647.

[81] N. Visaveliya, J. M. Kohler, *Macromol. Chem. Phys.* **2015**, *216*, 1212.

[82] J. M. Koehler, N. Visaveliya, A. Knauer, *Nanotechnol. Rev.* **2014**, *3*, 553.

[83] N. R. Visaveliya, J. M. Köhler, *Biomacromolecules* **2018**, *19*, 1047.

[84] N. Visaveliya, A. Knauer, J. M. Köhler, *Macromol. Chem. Phys.* **2017**, *218*, 1600371.

[85] K. M. Koczkur, S. Mourdikoudis, L. Polavarapu, S. E. Skrabalak, *Dalton Trans.* **2015**, *44*, 17883.

[86] N. Visaveliya, J. M. Kohler, *Langmuir* **2014**, *30*, 12180.

[87] R. M. Parker, J. Zhang, Y. Zheng, R. J. Coulston, C. A. Smith, A. R. Salmon, Z. Yu, O. A. Scherman, C. Abell, *Adv. Funct. Mater.* **2015**, *25*, 4091.

[88] N. Visaveliya, S. Li, J. M. Köhler, *Part. Part. Syst. Charact.* **2013**, *30*, 614.

[89] N. Visaveliya, A. Knauer, W. Yu, C. A. Serra, J. M. Kohler, *Eur. Polym. J.* **2016**, *80*, 256.

[90] Y. J. Min, M. Akbulut, K. Kristiansen, Y. Golan, J. Israelachvili, *Nat. Mater.* **2008**, *7*, 527.

[91] K. J. M. Bishop, C. E. Wilmer, S. Soh, B. A. Grzybowski, *Small* **2009**, *5*, 1600.

[92] D. Dendukuri, P. S. Doyle, *Adv. Mater.* **2009**, *21*, 4071.

[93] J. I. Park, A. Saffari, S. Kumar, A. Günther, E. Kumacheva, *Ann. Rev. Mater. Res.* **2010**, *40*, 415.

[94] M. Seo, Z. Nie, S. Xu, M. Mok, P. C. Lewis, R. Graham, E. Kumacheva, *Langmuir* **2005**, *21*, 11614.

[95] T. S. Kaminski, P. Garstecki, *Chem. Soc. Rev.* **2017**, *46*, 6210.

[96] P. Jankowski, R. Kutaszewicz, D. Ogończyk, P. Garstecki, *J. Flow Chem.* **2020**, *10*, 397.

[97] J. M. Köhler, S. Li, A. Knauer, *Chem. Eng. Technol.* **2013**, *36*, 887.

[98] J. K. Nunes, S. H. Tsai, J. Wan, H. A. Stone, *J. Phys. D: Appl. Phys.* **2013**, *46*, 114002.

[99] N. Visaveliya, C. Hoffmann, A. Gross, E. Tauscher, U. Ritter, J. M. Koehler, *Nanotechnol. Rev.* **2016**, *5*, 259.

[100] R. Prasher, P. Bhattacharya, P. E. Phelan, *Phys. Rev. Lett.* **2005**, *94*, 025901.

[101] L. A. Renna, C. J. Boyle, T. S. Gehan, D. Venkataraman, *Macromolecules* **2015**, *48*, 6353.

[102] B. Uma, T. N. Swaminathan, R. Radhakrishnan, D. M. Eckmann, P. S. Ayyaswamy, *Phys. Fluids* **2011**, *23*, 073602.

[103] S. Sacanna, W. T. M. Irvine, P. M. Chaikin, D. J. Pine, *Nature* **2010**, *464*, 575.

[104] Y. Wang, Y. Wang, D. R. Breed, V. N. Manoharan, L. Feng, A. D. Hollingsworth, M. Weck, D. J. Pine, *Nature* **2012**, *491*, 51.

[105] R. J. Amir, S. Zhong, D. J. Pochan, C. J. Hawker, *J. Am. Chem. Soc.* **2009**, *131*, 13949.

[106] B. Jang, H. Kwon, P. Katila, S. J. Lee, H. Lee, *Adv. Drug Delivery Rev.* **2016**, *98*, 113.

[107] S. Naderinezhad, G. Amoabediny, F. Haghiralsadat, *RSC Adv.* **2017**, *7*, 30008.

[108] Y. Cao, B. Wang, Y. Wang, D. Lou, *RSC Adv.* **2014**, *4*, 30430.

[109] S. Deshpande, S. Sharma, V. Koul, N. Singh, *ACS Omega* **2017**, *2*, 6455.

[110] I. U. Khan, L. Stolch, C. A. Serra, N. Anton, R. Akasov, T. F. Vandamme, *Int. J. Pharm.* **2015**, *478*, 78.

[111] N. Visaveliya, J. M. Köhler, *Small* **2015**, *11*, 6435.

[112] N. R. Visaveliya, X. Li, A. Knauer, B. L. V. Prasad, J. M. Köhler, *Macromol. Chem. Phys.* **2017**, *218*, 1700261.

[113] X. Li, N. Visaveliya, L. Hafermann, G. A. Gross, A. Knauer, J. M. Köhler, *Chem. Eng. J.* **2017**, *326*, 1058.

[114] X. Li, N. R. Visaveliya, L. Hafermann, A. Knauer, S. Schneider, J. M. Köhler, *ACS Appl. Nano Mater.* **2018**, *1*, 6398.

[115] N. Visaveliya, S. Lenke, A. Gross, J. M. Kohler, *Chem. Eng. Technol.* **2015**, *38*, 1144.

[116] N. Visaveliya, S. Lenke, J. M. Kohler, *ACS Appl. Mater. Interfaces* **2015**, *7*, 10742.

[117] C. Noguez, *J. Phys. Chem. C* **2007**, *111*, 3806.

[118] B. S. Hoener, S. R. Kirchner, T. S. Heiderscheit, S. S. E. Collins, W.-S. Chang, S. Link, C. F. Landes, *Chem* **2018**, *4*, 1560.

[119] W. W. Y. Lee, C. P. McCoy, R. F. Donnelly, S. E. J. Bell, *Anal. Chim. Acta* **2016**, *912*, 111.

[120] H. Al-Johani, E. Abou-Hamad, A. Jedidi, C. M. Widdifield, J. Viger-Gravel, S. S. Sangaru, D. Gajan, D. H. Anjum, S. Ould-Chikh, M. N. Hedhili, A. Gurinov, M. J. Kelly, M. E.l Eter, L. Cavallo, L. Emsley, J.-M. Basset, *Nat. Chem.* **2017**, *9*, 890.

[121] D. F. Moyano, V. M. Rotello, *Langmuir* **2011**, *27*, 10376.

[122] M. A. C. Stuart, W. T. S. Huck, J. Genzer, M. Muller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, *Nat. Mater.* **2010**, *9*, 101.

[123] J. Borges, J. F. Mano, *Chem. Rev.* **2014**, *114*, 8883.

[124] K. E. Sapsford, W. R. Algar, L. Berti, K. B. Gemmill, B. J. Casey, E. Oh, M. H. Stewart, I. L. Medintz, *Chem. Rev.* **2013**, *113*, 1904.

[125] L. Xu, A. R. Tetreault, H. H. Khaligh, I. A. Goldthorpe, S. D. Wettig, M. A. Pope, *Langmuir* **2019**, *35*, 51.

[126] A. Ulman, *Chem. Rev.* **1996**, *96*, 1533.

[127] Z. Tang, Y. Wang, P. Podsiadlo, N. A. Kotov, *Adv. Mater.* **2006**, *18*, 3203.

[128] D. Alkekhia, P. T. Hammond, A. Shukla, *Ann. Rev. Biomed. Eng.* **2020**, *22*, 1.

[129] N. Boehnke, S. Correa, L. Hao, W. Wang, J. P. Straehla, S. N. Bhatia, P. T. Hammond, *Angew. Chem., Int. Ed.* **2020**, *59*, 2776.

[130] Y. Liu, M. G. Williams, T. J. Miller, A. V. Teplyakov, *Thin Solid Films* **2016**, *598*, 16.

[131] M. Szczęch, K. Szczepanowicz, *Nanomaterials* **2020**, *10*, 496.

[132] G. Decher, *Science* **1997**, *277*, 1232.

[133] Y. Lvov, G. Decher, H. Moehwald, *Langmuir* **1993**, *9*, 481.

[134] G. B. Sukhorukov, E. Donath, H. Lichtenfeld, E. Knippel, M. Knippel, A. Budde, H. Möhwald, *Colloids Surf., A* **1998**, *137*, 253.

[135] H. Yu, Y. Peng, Y. Yang, Z.-Y. Li, *NPJ Comput. Mater.* **2019**, *5*, 45.

[136] S. C. Warren, E. Thimsen, *Energy Environ. Sci.* **2012**, *5*, 5133.

[137] N. J. Halas, S. Lal, W.-S. Chang, S. Link, P. Nordlander, *Chem. Rev.* **2011**, *111*, 3913.

[138] A. L. González, C. Noguez, J. Beránek, A. S. Barnard, *J. Phys. Chem. C* **2014**, *118*, 9128.

[139] Y. Xia, X. Xia, H.-C. Peng, *J. Am. Chem. Soc.* **2015**, *137*, 7947.

[140] Y. Xia, Y. Xiong, B. Lim, S. E. Skrabalak, *Angew. Chem., Int. Ed.* **2009**, *48*, 60.

[141] N. Visaveliya, J. M. Kohler, *Nanotechnology* **2013**, 24.

[142] A. Albanese, P. S. Tang, W. C. W. Chan, *Ann. Rev. Biomed. Eng.* **2012**, *14*, 1.

[143] G. C. Le Goff, R. L. Srinivas, W. A. Hill, P. S. Doyle, *Eur. Polym. J.* **2015**, *72*, 386.

[144] M. B. Oliveira, J. F. Mano, *Biotechnol. Prog.* **2011**, *27*, 897.

[145] Y.-C. Lin, C.-Y. Chen, H.-L. Chen, T. Hashimoto, S.-A. Chen, Y.-C. Li, *J. Chem. Phys.* **2015**, *142*, 214905.

[146] A. Restuccia, D. T. Seroski, K. L. Kelley, C. S. O'Bryan, J. J. Kurian, K. R. Knox, S. A. Farhadi, T. E. Angelini, G. A. Hudalla, *Commun. Chem.* **2019**, *2*, 53.

[147] Z. Sun, P. Li, S. Xu, Z.-Y. Li, Y. Nomura, Z. Li, X. Liu, S. Zhang, *J. Am. Chem. Soc.* **2020**, *142*, 10833.

[148] W. K. Tan, Y. Araki, A. Yokoi, G. Kawamura, A. Matsuda, H. Muto, *Nanoscale Res. Lett.* **2019**, *14*, 297.

[149] W. Li, L. Zhang, X. Ge, B. Xu, W. Zhang, L. Qu, C.-H. Choi, J. Xu, A. Zhang, H. Lee, D. A. Weitz, *Chem. Soc. Rev.* **2018**, *47*, 5646.

[150] W. Wang, M.-J. Zhang, L.-Y. Chu, *Acc. Chem. Res.* **2014**, *47*, 373.

[151] C.-H. Choi, H. Lee, A. Abbaspourrad, J. H. Kim, J. Fan, M. Caggioni, C. Wesner, T. Zhu, D. A. Weitz, *Adv. Mater.* **2016**, *28*, 3340.

[152] S. S. Datta, A. Abbaspourrad, E. Amstad, J. Fan, S.-H. Kim, M. Romanowsky, H. C. Shum, B. Sun, A. S. Utada, M. Windbergs, S. Zhou, D. A. Weitz, *Adv. Mater.* **2014**, *26*, 2205.

[153] G. T. Vladisavljević, R. Al Nuumani, S. A. Nabavi, *Micromachines* **2017**, *8*, 75.

[154] J. M. Köhler, A. März, J. Popp, A. Knauer, I. Kraus, J. Faerber, C. Serra, *Anal. Chem.* **2012**, *85*, 313.

[155] N. Visaveliya, J. M. Kohler, *J. Mater. Chem. C* **2015**, *3*, 844.

[156] Z. Chang, C. A. Serra, M. Bouquey, L. Prat, G. Hadziioannou, *Lab Chip* **2009**, *9*, 3007.

[157] C. A. Serra, I. U. Khan, Z. Q. Chang, M. Bouquey, R. Muller, I. Kraus, M. Schmutz, T. Vandamme, N. Anton, C. Ohm, R. Zentel, A. Knauer, M. Kohler, *J. Flow Chem.* **2013**, *3*, 66.

[158] S. Gadhiya, S. Madapa, T. Kurtzman, I. L. Alberts, S. Ramsey, N.-K. Pillarsetty, T. Kalidindi, W. W. Harding, *Bioorg. Med. Chem.* **2016**, *24*, 2060.

[159] S. Gadhiya, S. Ponnala, W. W. Harding, *Tetrahedron* **2015**, *71*, 1227.

[160] N. R. Visaveliya, C. W. Leishman, K. Ng, N. Yehya, N. Tobar, D. M. Eisele, J. M. Köhler, *Adv. Mater. Interfaces* **2017**, *4*, 1700929.

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