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# Polymer–Aptamer Hybrid Emulsion Templating Yields Bioresponsive Nanocapsules

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**This article describes the synthesis of a DNA–polymer, being the nucleotide sequence an aptamer selected in vitro to target specifically the immunoglobulin E (IgE) protein, an allergy biomarker. Subsequent to coupling to poly(2-alkyl-2-oxazoline) with *N*-Boc protected amino acid side chains, the resulting amphiphilic DNA–polymer hybrid composed of the water-soluble DNA fragment grafted to the hydrophobic polymer segment can be regarded as a high molecular weight analogue of a surfactant. It is demonstrated that the copolymer–aptamer stabilizes efficiently submicrometer size oil-in-water and water-in-oil emulsions, by dynamic light scattering, microscopy, and reflectometry. Particularly interesting is that the aptamer remains functional after coupling to a polymer backbone, stabilization of the emulsion droplets, and locking of the structure subsequent to cross-linking polymerization. The resulting nanocapsules still target specifically the IgE protein. The biological-stimulus responsiveness of the structures is of high potential for future developments of carriers for sustained and targeted delivery.**

## 1. Introduction

DNA–copolymers composed of either water-soluble or hydrophobic polymer segments of various compositions are nowadays available through both organic and molecular biotechnology routes.[1] Amphiphilic DNA–polymer can self-assemble in aqueous solution into micelles of various shapes such as spherical core–shell micelles[1a,b] or vesicles,[1c] which are closed spherical copolymer shells. Sizes in the sub-micrometer range could be achieved by this mechanism of microphase separation.[1] Of particular interest is Watson–Crick base pairing between complementary nucleotide sequences, which assemble to form a double helix through hybridization to enable further either structure manipulations such as stimulation of morphological transitions from spherical to rod-like micelles,[1a,b] immobilization of self-assembled structures on surfaces[2] as well as chemical functionalization using a labeled complementary sequence to target cell surface receptors for instance.[1a,b]

Besides hybridization, nucleotide sequences might however interact specifically with a target moiety. We ourselves observed a positive response of bacteria to surface-tethered nucleotide sequences and self-assembled structures thereof.[2] Although no specificity of the nucleotide sequences towards *Escherichia Coli* could be expected, the bacteria produced curli, which are organelles of adhesion expressed upon interaction with surfaces coated with nucleotide sequences.[2a] Aptamers however are synthetic single stranded nucleotide sequences that undergo remarkable molecular recognition properties.[3] Binding affinities are comparable to those achieved with antibodies due to an efficient in vitro combinatorial strategy of selection called systematic evolution of ligands by exponential enrichment (SELEX), a major advantage over production of antibodies.[3a] The specific and complex 3D shape of aptamers, which are short single-stranded nucleotide sequences (ssDNA or RNA) enables binding with high specificity to a wide variety of targets from single molecules to complex mixtures or whole organisms. We therefore describe herein the grafting of the aptamer against the immunoglobulin E (IgE), an allergy biomarker, to a hydrophobic polymer segment. Anti-IgE aptamers have been shown to block interactions with the IgE receptor with high affinity to inhibit IgE-mediated serotonin release from cells in tissue culture.[3a] Anti IgE-apatmers might therefore prove to be useful for blocking local inflammatory responses mediated by IgE. With future biomedical applications in mind and the possibility to use methods developed in polymer science to investigate the system under consideration, we thus report in here the grafting of the IgE-aptamer to a poly(2-alkyl-2-oxazoline) with *N*-Boc protected amino acid and alkenyl side chains. This synthetic macromolecule is a bioinspired amphiphilic copolymer with a structural relation to polypeptides, which therefore reveals high potential for biomedical applications.[4] The emulsification methodology to prepare both aqueous and liquid oil cores enables the facile encapsulation of various water soluble or hydrophobic active agents with efficient applicability in various fields like pharmacy, food and cosmetics.[5] Of particular interest is the combination of the emulsification process with

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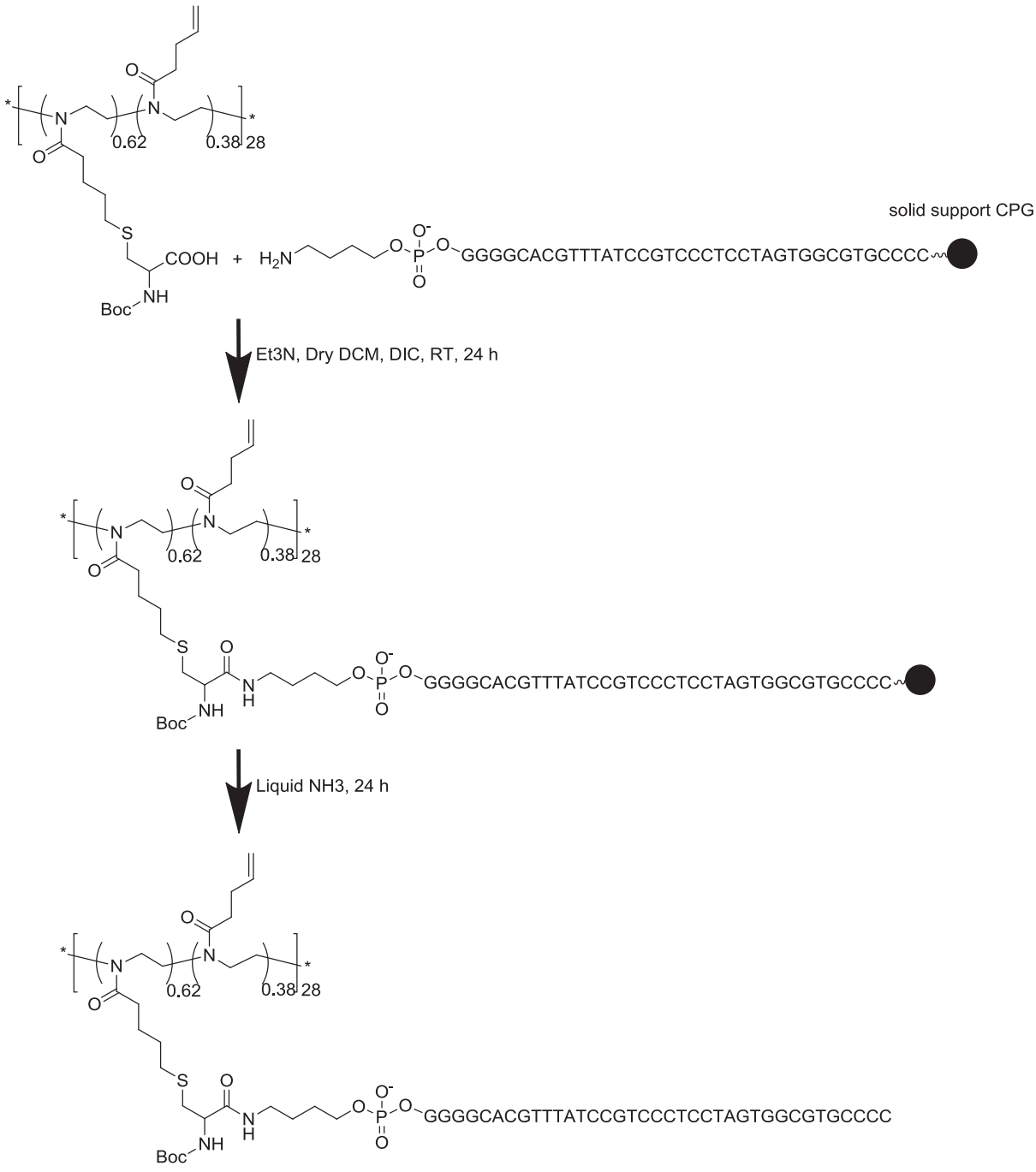


**Scheme 1.** Synthesis of the PBOX–aptamer hybrid by solid phase synthesis.

the self-assembly of copolymer to generate variety of structures with tunable morphology as well as size by controlling factors such as confinement effects, interfacial tension and others.[5f] Towards this end, pickering emulsions have been prepared using ferritin-polymer conjugates that can undergo UV cross-linkage stabilization to enable the transfer of the soft protein-polymer capsules to media of equal polarity as the inside of the capsules.[6] Monodisperse poly(dopamine) polymer capsules could be prepared by one-step interfacial polymerization on dimethyldiethoxysilane emulsion droplets, being the template removed in a subsequent step.[7] Nucleotide sequences have been used for capsules preparation based on DNA hybridization as well.[8] To infer targeting properties to particles or capsules prepared by emulsification, such structures were functionalized with antibodies to target cancer cells.[9] In this context, the polyoxazoline–aptamer hybrid described herein is an amphiphilic grafted copolymer, which enables the stabilization of sub-micrometer size emulsions and the preparation

of biological-stimulus responsive nanocapsules. According to the definition given the recent review from Delcea et al.,[5b] the system described in here could indeed enable specific targeting owing to IgE biding in a ligand-receptor recognition mode.

## 2. Results and Discussion

The IgE-aptamer polymer hybrid has been synthesized by solid phase synthesis according to chemistry routes published previously.[1] Briefly, poly(2-alkyl-2-oxazoline) with pendant *N*-Boc protected amino acid and alkenyl side chains (PBOX/BocCys) ($^1$H NMR in Figure S1) was grafted to the 37 nucleotide-long aptamer sequence (5′-GGGGCACGTTTATCCGTCCCTC-CTAGTGGCGTGCCCC-3′), which is modified at the 5′ end with an amine group by a hexyl spacer and linked to the controlled pore glass (CpG) phosphoramidite solid support at the 3′ end (**Scheme 1**). The carboxylic acid group at the PBOX/BocCys was first activated by *N,N*′-diisopropylcarbodiimide (DIC) to react with the aptamer. After the coupling reaction, the resin is washed thoroughly with DCM and DMF, filtered out prior to cleavage with liquid ammonia to yield the final product further purified by dialysis (MWCO 14 kDa) and finally lyophilized.

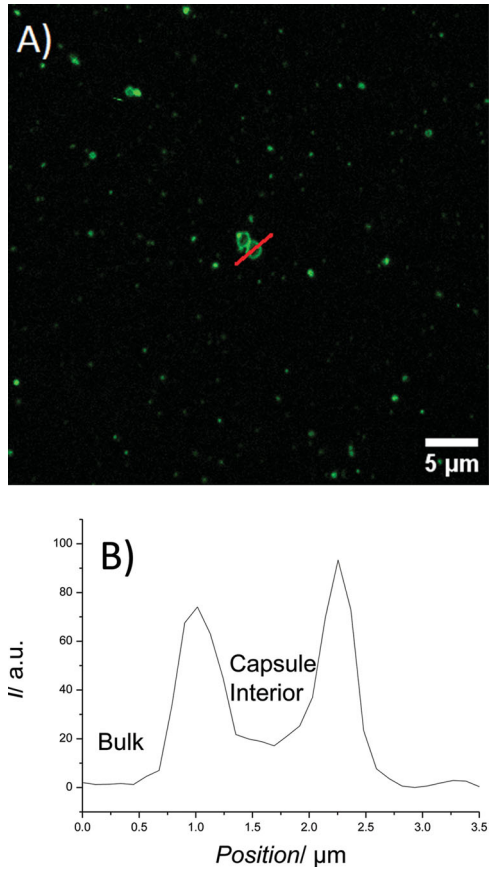


**Figure 1.** Confocal laser scanning microscopy of the A) Oil-in-water emulsion stabilized by the PBOX–aptamer, incubated with FITC labeled IgE and B) corresponding fluorescence intensity profile, which evidences that the IgE protein is located at the rim of the droplet.

Analyses by analytical ultracentrifugation (AUC sedimentation-velocity, Figure S2) support the success of the solid phase synthesis route, in particular that of the purification stage. UV–vis spectroscopy (Figure S3) indicates that every polymer chain carries in average two aptamer sequences, hence the PBOX–aptamer is a macromolecule of ~30.2 kDa with a hydrophilic weight fraction of 75%. It is worth being mentioned that, due to the high hydrophilic weight fraction, the PBOX–DNA does not exhibit temperature sensitivity, as indicated by the absence of turbidity change in the temperature range of 20–60 °C.

Although of high hydrophilic weight fraction, the PBOX–aptamer is composed of a hydrophobic PBOX backbone along which are grafted the water soluble aptamers. The

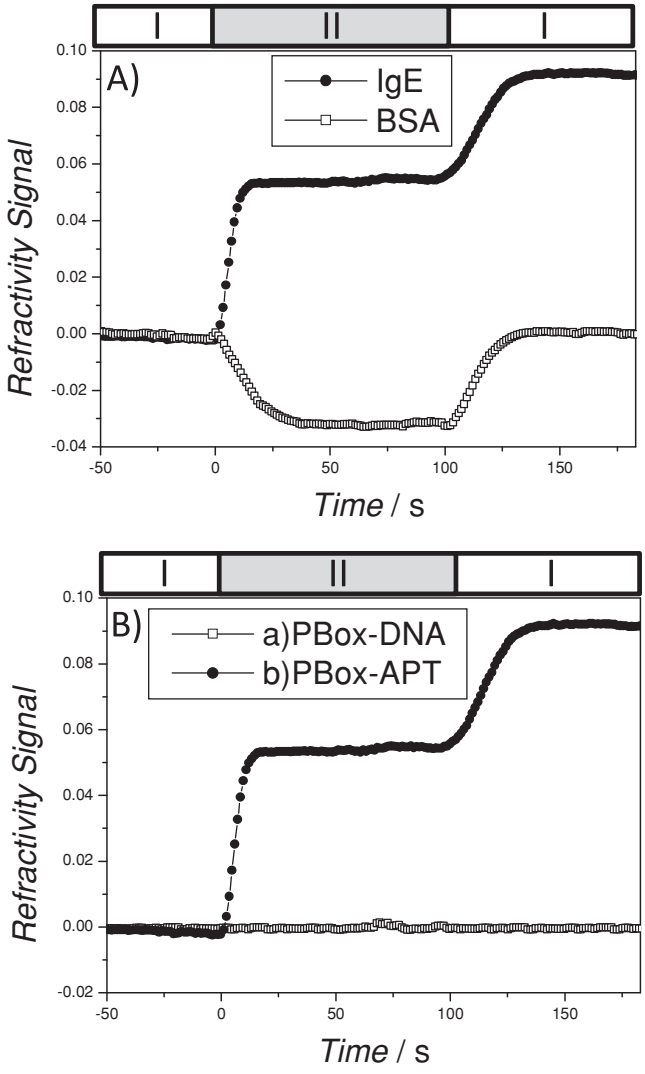


**Figure 2.** Representative time course of the refractive index as measured by reflectometry upon adsorption of the PBOX–aptamer stabilized emulsion adsorption on A) silica modified with □ = BSA (non-target protein of the aptamer), • = IgE aptamer target. B) Adsorption of emulsion droplets on IgE modified silica stabilized by PBOX–DNA (□; the sequence is not specific to the IgE protein), and PBOX–aptamer (•). The surface is initially flushed with a 15 mM NaCl buffer solution (I). At time zero, a 10 times diluted filtered emulsion solution in 15 mM NaCl is introduced (II) and eventually, the surface is again flushed with the buffer solution (solution I).

macromolecule can thus be regarded as a high molecular weight analogue of a surfactant with the major advantage of being constituted of a bioinspired biocompatible synthetic polymer segment coupled to a biological stimulus-responsive nucleotide sequence. The surface activity of the PBOX–aptamer could indeed be assessed by monitoring surface pressure isotherms (Figure S4). This surface activity of the PBOX-aptamer thus enables the stabilization of both oil-in-water (o/w) and water in oil (w/o) emulsions to design biological stimulus responsive nanocapsules.

100 µL of a 10% dodecane solution of the copolymer-aptamer was added dropwise to 20 mL of water and subsequently exposed to ultrasounds (ultrasonic probe, time 5 min, amplitude 35%) in order to break the continuous phase. The resulting droplets were imaged by confocal laser scanning microscopy (CLSM) subsequent to encapsulation of a hydrophobic dye (Nile red) dissolved in dodecane along with the PBOX-aptamer (Figure S5). Imaging demonstrates the formation of oil-in-water droplets. The emulsion was further filtered through 0.2 µm pore-size LCR filter membranes to decrease both the size and the size distribution, to subsequently perform dynamic light scattering (DLS) in order to quantify the size and the stability of the emulsion prior and subsequent to stabilization with the PBOX-aptamer. The emulsion is stable in time as assessed by DLS. An aggregation rate of $-1.2 \times 10^{-19}$ $m^3s^{-1}$ clearly evidences that the 100 nm average size droplets are stabilized by the PBOX-aptamer hybrid. At high ionic strength (1 M KCl) this aggregation rate is of $9.4 \times 10^{-20}$ $m^3s^{-1}$ to be compared to the $1.2 \times 10^{-17}$ $m^3s^{-1}$ theoretical value of the fast or diffusion controlled aggregation of an unstable, rapidly aggregating system.[10] Stability is further supported by the value of the surface charge. A zeta potential value of –56.5 mV is suitable to induce repulsion between the negatively charged copolymer stabilized capsules and prevent aggregation.[11]

At this stage, the question that is raised is whether this aptamer remains functional subsequent to coupling to the polymer and emulsification. Since the chosen aptamer has been selected against immunoglobulin E (IgE), a first set of experiments were conducted with fluorescently labeled proteins. Oil-in-water emulsions were therefore at first stabilized with the copolymer–aptamer and incubated for one hour with fluorescein isocyanate (FITC) labeled IgE prior to CLSM (**Figure 1**, Figure S6A and B). As can be observed, IgE is localized at the rim of the dodecane droplets in water. However, in the case of water-in-oil emulsion, the aptamer points towards the inner core of the structure (Figure S6C), which is fluorescent subsequent to binding to the FITC-labeled IgE protein.

These primary results evidence that the aptamer remains functional subsequent to coupling to the polymer and engagement in the stabilization of the emulsion. Nevertheless, efficiency of binding might be hindered by intermolecular interactions owing to steric hindrance upon organization at the interface. To overcome this limitation, we further resorted to a method we established to achieve optimal functionality of surface tethered nucleotide sequences.[12] Briefly, the PBOX–aptamer is incubated with its target, the IgE protein, prior to emulsion stabilization. Fluorescence of the outer rim of the droplets could also be observed by CLSM (Figure S6D).

To assess the specificity of binding of the PBOX–aptamer engaged in the emulsification process, reflectometry was eventually combined to atomic force microscopy (AFM) imaging. Emulsions stabilized with either the PBOX–aptamer or a non-specific PBOX–DNA were incubated with a surface modified with either IgE or BSA. As can be seen on the time course monitoring of the adsorption of the emulsion (**Figure 2**A), mass increase is detected when the emulsion is stabilized by the PBOX–aptamer and the surface-coated with IgE up to an average value of 1.01 mg $m^{-2}$ whereas on a BSA coating, no adsorption occurs (Figure 2A). Similarly, with a nucleotide sequence which has no specificity of binding to IgE, no adsorption could be detected (Figure 2B). This specificity of binding has been further assessed by AFM. As can be observed in **Figure 3**, the PBOX–aptamer stabilized emulsions could be located on a surface coated with the IgE protein whereas no structures could be detected when the emulsion is stabilized with a PBOX–DNA that has no specificity to IgE (Figure S7). Imaging by AFM further evidences the robustness of the emulsion stabilization by

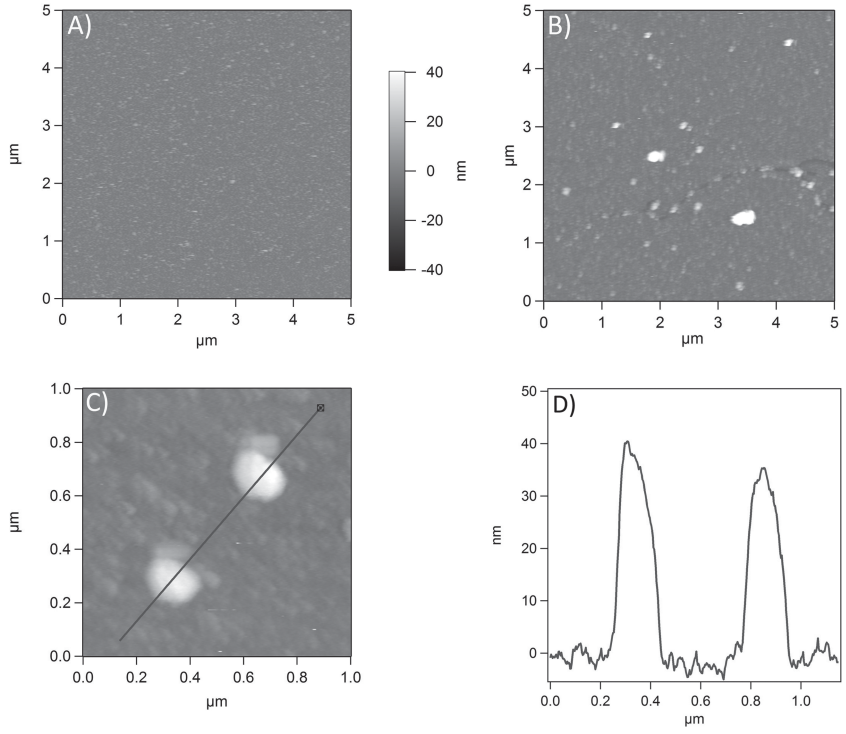


**Figure 3.** Adsorption of the PBOX–aptamer-stabilized emulsion as observed by AFM. A) IgE-coated silica, B) PBOX–aptamer-stabilized emulsion adsorption on IgE modified silica, C) soft emulsion droplets of the PBOX–aptamer are stable. D) Corresponding height profile (same height scale for all micrographs).

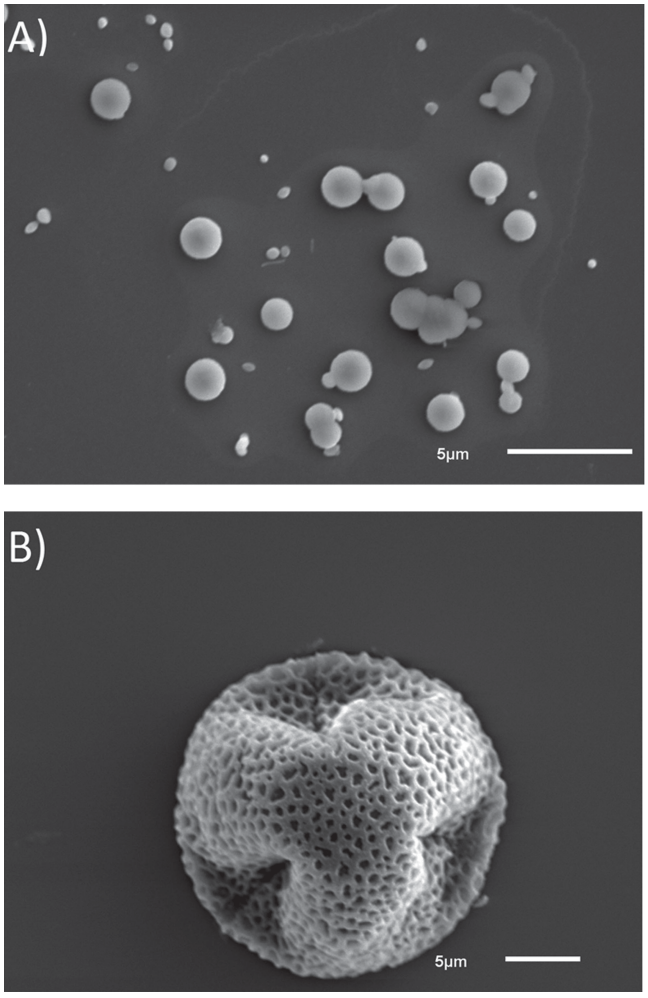


**Figure 4.** SEM images of PBOX–aptamer capsules after cross-linking by irradiation with UV light. A) Cross-linkage is performed subsequent to filtration. B) Large porous structures are observed (owing to the organic solvent evaporation in the vacuum of SEM) when no extrusion is performed to reduce the size and fusion of the droplets might occur.

the PBOX–aptamer. The droplets collapse without disruption as evidenced by a 40 nm height as compared to the 100 nm size of the structure in solution as quantified by DLS (Figure S8). Although specific interaction occurs between the surface and the droplets, the emulsion droplets immobilized on the surface shrink but are not disrupted (Figure 3B,C).

Further stabilization could be achieved by UV-irradiation of the cross-linkable pendent groups which were not modified with cysteine for subsequent grafting to the aptamer sequences. 11 vinyl pendant groups are therefore available in average per PBOX backbone for an eventual cross-linking polymerization step. As can be seen in **Figure 4**A and **Figure 5**, this final stabilization step enables imaging by both scanning and transmission electron microscopy (SEM and TEM, respectively) which is otherwise not possible with the pristine soft emulsion. Since this cross-linking step occurs at the oil water interface, the function of the aptamer is not affected. The cross-linked structures could be located on a surface modified with the IgE aptamer target (**Figure 6**), which indicates that the aptamer remains functional and binds to the

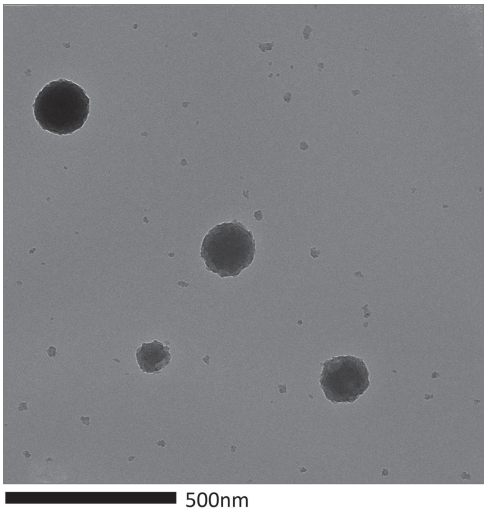


**Figure 5.** TEM image of cross-linked PBOX–aptamer spheres (filtered before cross-linking polymerization).

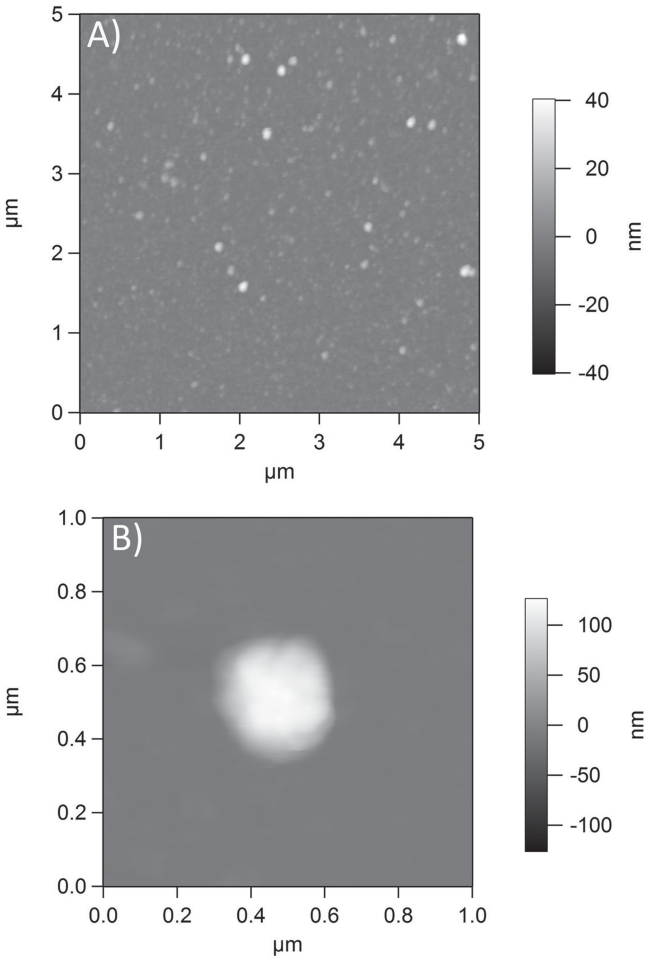


**Figure 6.** Adsorption of the cross-linked emulsion on IgE coated silica. A) 5 × 5 μm, B) 1 × 1 μm micrographs obtained by AFM.

protein subsequent to sequential grafting to the polymer, emulsion stabilization and cross-linkage to achieve stable biological stimulus responsive capsules. In vivo, this stabilization is crucial since it would slow down the degradation of the capsules by nucleases and proteinases, which would leave either structure of small size or polymer backbones of low molecular weight that could be cleared out by the renal system. Cross-linkage might occur between the droplets, inducing their fusion into large microspheres at the surface of which one can observe porosity probably induced by the evaporation of the organic solvent (Figure 4B).

## 3. Conclusions

Coupling of the IgE aptamer to PBOX has been conducted through solid phase synthesis. The resulting amphiphilic copolymer-aptamer efficiently stabilizes oil-in-water and water-in-oil emulsions. Further stabilization could be demonstrated subsequent to an UV induced crosslinking polymerization step. Engagement of the aptamer in the emulsion stabilization does not hinder its specificity of binding to its target, which paves the wave for further developments of capsules for sustained and targeted delivery through the synergistic combination of site specific aptamer recognition of the encapsulated load. The quantification of the aptamer binding kinetics and affinity to its target subsequent to polymer coupling and emulsion stabilization is currently under investigation.

## 4. Experimental Section

*Materials*: Dichloromethane (DCM) (extra dry, 99.8%), *N,N*-dimethylformamide (DMF) (99.8%), sodium chloride (>99.5%), triethylamine (99%) were purchased from Acros Organics (Geel, Belgium). *N,N′*-diisopropylocarbodiimide (>98%), dialysis tube (molecular weight cut-off, MWCO 14 kDa), dimethoxy(methyl)octyl silane were supplied by Sigma Aldrich (Buchs, Switzerland). The ammonia solution 35% was purchased from Fisher Scientific SA (Wohlen, Switzerland). Dodecane (99%) was provided by Alfa Aesar (Zürich, Switzerland). 5′-GGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCC-3′ (5′-G11C14A3T9–3′,"Aptamer") and 5′-CTCTCTCTCTTT-3′ (5′-$C_5T_{7-}$3′,"DNA") modified at the 5′ end through a $C_6$ hexyl spacer with an amine group was purchased from Microsynth Laboratory (Balgash, Switzerland). Immunoglobulin E (IgE) labelled with FITC and albumin from bovine serum (BSA) were supplied by Invitrogen (Zug, Switzerland). Poly(ethylene imine) (PEI, nominally 40 000 g $mol^{-1}$) was purchased from Polysciences (Eppelheim, Germany). LCR 0.22 µm pore size syringe filters were purchased from Merck Millipore (Zug, Switzerland). Zeta cells were provided by Instrumat SA (Lausanne, Switzerland). Silicon wafers single side polished, orientation N/Phos <100>, (625 ± 25) µm thickness, 1–50 Ohm cm resistivity were purchased from Silchem (Freiburg, Germany).

Poly[2-(3-butenyl)-2-oxazoline] (PBOX) was prepared by cationic ring-opening isomerization polymerization of 2-(3-butenyl)-2-oxazoline as described in the literature.[4a] The isolated PBOX sample had a number-average molecular weight ($M_n$) of 3.5 kDa (corresponding to an average number of 28 repeat units) ($^1$H NMR) and a dispersity index ($M_w/M_n$) of 1.05 (SEC). *N*-(*tert*-butyloxycarbonyl)-L-cysteine (BocCys) (Merck) was then partially added to the alkenyl groups of PBOX via thiol-ene photochemical reaction.[4a] $^1$H NMR analysis (Figure S1) of the purified product, PBOX/BocCys, revealed that 62% of the unsaturated units were functionalized with the *N*-protected amino acid (~17 units per chain) and 38% remained unreacted (~11 units); see the chemical structure in Scheme 1 (top left).

The syntheses of PBOX–aptamer and PBOX–DNA are described in the Supporting Information. Milli-Q water was used in all cases.

*Analytical Instrumentation*: $^1$H NMR measurements were carried out at room temperature using a Bruker DPX-400 spectrometer operating at 400.1 MHz. Reflectometer Abbemat WR MW(Anton Paar, Germany) was used to determine the refractive index increment (dn/dc) of the PBOX-aptamer. UV-Vis spectra were registered on a Perkin Elmer Lambda 900 in a quartz cuvette of 1 cm path length for all measurements. Size exclusion chromatography (SEC) with simultaneous UV and RI detection was performed in *N*-methyl-2-pyrrolidon (NMP + 0.5 wt% LiBr) at +70 °C, flow rate: 0.8 mL $min^{-1}$, column set: two 300 × 8 $mm^2$ PSS-GRAM (7 µm spherical polyester particles) columns with porosities of $10^2$ and $10^3$ Å. Calibration was done with polystyrene standards. Analytical ultracentrifugation (AUC) was performed on an Optima XL-I ultracentrifuge (Beckmann-Coulter, Palo Alto, CA) equipped with Rayleigh interference optics. Sedimentation-velocity experiments were done with 1 mg $mL^{-1}$ solutions of polymer in water/ethanol 1:1 (v/v) at a rotational speed of 50 krpm at 25 °C. Hydrodynamic sizes (diameter) of the emulsion droplets were assessed by dynamic light scattering (DLS) at 25 °C using a Zetasizer Nano Series from Malvern Instruments with the backscattered angle detection at 173° in optically homogeneous square polystyrene cells. The zeta potential of emulsion droplets was measured by the micro electrophoretic method using a Malvern Zetasizer Nano ZS apparatus. Surface pressure isotherms were conducted on an R&K Langmuir Through (Freiburg, Germany). For this measurement, a 1 mg $mL^{-1}$ copolymer solution in chloroform was prepared. All measurements were carried out at 292 K.

*Emulsion Preparation*: 4 mg of PBOX-aptamer was dissolved in 100 µL of dodecane and left under stirring for 24 h. Subsequently, the copolymer solution was added to 20 mL of a 15 mM NaCl solution and exposed to ultrasounds in order to break the organic phase (Branson Sonifier Digital 450, Zurich, Switzerland) during 2 min, amplitude 35%, max temp; 40 °C, impulse on; 1 s, impulse off; 1 s. The resulting emulsion was filtered through 0.2 µm pore size LCR non-protein binding filters in order to decrease the size and size distribution prior to the determination of the hydrodynamic radius and zeta potential of the droplets.

*Reflectometry*: 5 × $10^{-3}$ g $L^{-1}$ protein (IgE) solution was prepared in 15 mM NaCl buffer solution. All substrates (silicon pieces with dimensions of 1.2 × 0.5 cm) were cleaned in a 3:1 solution of concentrated sulphuric acid and 30% hydrogen peroxide for 30 min, washed with Milli-Q water and dried under nitrogen. Afterwards, the precise thickness of the native silica layer (average thickness = 1.2 nm) was determined by null ellipsometry (Multiskop, Optrel, Berlin, Germany).[13] Subsequently, to induce physical adsorption of proteins on the substrates, plates were placed in an IgE solution for 24 h. Later on, substrates were extensively washed with a buffer solution (15 mM NaCl aqueous solution) in order to remove the excess of protein. Subsequently, ellipsometry was again performed to measure the thickness of the adsorbed protein layer (average thickness = 3.82 nm). In order to determine the thickness of the protein layer, a fixed value of refractive index was used, equal to the $SiO_2$ refractive index. Binding of the PBOX-aptamer stabilized emulsion to protein was quantitatively assessed by reflectometry measurements, conducted on a home-built fixed-angle reflectometer which allows determination of the adsorbed dry mass.[13,14] The average sensitivity value was equal to 0.054 $m^2$ $mg^{-1}$ with a dn/dc value of the PBOX-aptamer of 0.075 mL $g^{-1}$. In brief, after IgE adsorption, the silicon, plate was placed in the cell and the system washed with a buffer solution (solution I). Subsequent to signal stabilization, the solution of filtered dodecane emulsion stabilized by the PBOX-aptamer copolymer (solution II) was pumped into the cell and the change of refractivity signal (which is proportional to the adsorbed mass) was measured. After signal stabilization, buffer solution was again pumped into the cell (solution I), in order to verify if mass desorption would occur. The refractivity signal indeed increased due to the high refractive index difference between the emulsion and buffer solutions. The real adsorption value is thus obtained after buffer washing. Detailed description of the set up and analysis is given elsewhere.[13,14] Blank experiments, with substrates modified with BSA instead of the IgE protein and dodecane emulsion stabilized

by PBOX-$C_5T_7$ instead of PBOX-aptamer, were conducted in the same manner. All solutions were degassed under vacuum.

*Atomic Force Microscopy (AFM)*: Samples for AFM were prepared by the same procedure as for reflectometry. However, in order to adsorb emulsion droplets, substrates were dipped in the emulsion solution for 5 min and subsequently gently washed with the buffer solution. AFM images were acquired with a Cypher AFM (Asylum Research, Santa Barbara, CA) operating in the AC-mode. Biolever mini AC40TS cantilevers (Olympus, Japan) with a nominal tip radius smaller than 9 nm were used for recording images in liquid. These probes have spring constants of around 0.1 N $m^{-1}$ and resonance frequencies in liquid of around 25 kHz. The free oscillation amplitude (FOA) and the scan rate were set to 40 nm and 4.88 Hz respectively. The set point used varied between 60 and 70% of FOA. The data acquisition and analysis were operated with the Asylum Research software, delivered with the AFM instrument.

*Scanning Electron Microscopy (SEM)*: A 10 µL of the fresh crosslinked emulsion solution was dried at room temperature on silicon wafers. Dried samples were coated with gold for 20 s in a Jeol JFC-1200 Fine coater. Subsequently, SEM images were acquired on a Jeol 6510LV microscope, equipped with a tungsten filament gun, operating at WD 10.6 mm and 10 kV.

*Transmission Electron Microscope (TEM)*: For TEM, 5 µL of fresh sample was placed on a carbon coated 400-mesh copper grid. After sample drying, the sample was imaged without staining directly with a Tecnai G2 electron microscope operating at 120 kV.

*Confocal Laser Scanning Microscopy (CLSM)*: In order to visualize protein interaction with oil-in-water emulsions stabilized by the PBOX-aptamer, 250 µL of FITC-IgE solution was directly added to 250 µL of the original non filtered PBOX-aptamer emulsion (100 times diluted), stirred on vortex and co-incubated for 1 h at 4 °C (Scheme S1A). To enable imaging, immobilization of the emulsion droplets on glass slides was ensured by modification of the glass surface with positively charged poly(ethylene imine) (PEI) (5 $10^{-3}$ g $L^{-1}$) since the outer layer of droplets is composed of the negatively charged aptamer. In brief, the solution of PEI was spread on the microscopy glass slide and left for adsorption for 5 min. Afterwards, the glass surface was extensively washed with Milli-Q water and 10 µL of the PBOX-aptamer stabilized emulsion incubated with FITC-IgE was spread on the glass slide, covered by the cover glass and observed by microscopy. In order to observe reverse emulsions, a 5% solution of PBOX-aptamer copolymer was prepared in a protein solution and incubated at 4 °C for 1 h. Subsequently, the mixture was added to 2 mL of dodecane and stirred vigorously (1500 rpm) to induce emulsification. In this case, the hydrophobic PBOX is located at the outer rim of the droplet (Scheme S1B). To enable immobilization of the droplets, microscopic glass slides were silanized with dimethoxy(methyl) octyl silane. Briefly, glass slides along with 100 µL of dimethoxy(methyl) octyl silane were kept in a petri dish for 3 h under vacuum. Consecutively 10 µL of the reverse emulsion with FITC-IgE was spread on a modified glass slide, covered with a cover glass and imaged under a Zeiss LSM 700 confocal microscope (with laser excitation at 488 nm, lens 100 × 1.4NA).

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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