Description of the main research directions investigated by the institute

Macromolecular science is highly interdisciplinary. The best results are typically achieved by combination of approaches from different scientific fields and by using various complementary experimental methods. The Institute benefits from synergy of wide range of complementary expertise with experimental or theoretical methods all gathered under one roof. With approximately 120 scientists (including 40 post-doctoral researchers) and 40 PhD students, specialising in subjects ranging from synthetic chemistry, through physical chemistry of macromolecular systems to polymer physics and material processing on one hand and biochemistry and biology on the other, one can always find an expert in a particular field to get an advice or to start a highly qualified collaboration on a project.

The research programme of the IMC can be divided into five main research directions, which are reflected in the organisational structure that comprises five Centres of Polymer Science, namely:

Supramolecular Structures and Self-Assembling Processes (SUPRAMOL)
Biomacromolecular and Bioanalogous Systems (BIOMOL)
Polymer Materials and Technologies (MATER)
Structure and Dynamics of Macromolecules (STRUCTURE)
Polymers for Optoelectronic and Energy Applications (OPTOEL)

Research of each of these five Centres is described in the sections that follow.

Supramolecular Structures and Self-Assembling Processes

The investigation of supramolecular and self-associating polymer systems focuses both on the design and synthesis of polymers exhibiting self-association as well as on the physico-chemical description of self-associative processes and structural characterisation of supramolecular systems. Supramolecular organisation in polymers ranks among the key issues in modern polymer science, and it forms the knowledge basis for other research programmes of the Institute, especially for those directed to nanostructured polymer materials on the one edge of the research spectrum and to biomacromolecular polymer systems for nanomedicine and diagnostics on the other edge.

Physics and physical chemistry in this research area cover supramolecular structures in systems containing homopolymers, block and statistical copolymers either in bulk or in solution or in gel as well as dispersions of polymeric nanoparticles. Special attention is paid to the processes of phase separation, association, complexation and to the formation of micelles, nanoparticles, nanocapsules – self-organisation processes on microscopic, mesoscopic and macroscopic levels are all included. Investigation of the structure and dynamics involves description of a) the kinetics during phase-separation, micellization, intermolecular association and dissociation, and complexation; b) structure evolution; c) responsiveness to external stimuli, such as temperature, concentration or pH or combinations thereof. The aim of this Centre is to master the process of self-organisation in polymers and copolymers to obtain such nanostructured materials and formulations that can be used for nanotechnology or developed for biomedical applications.

In the following paragraphs, several examples of outstanding research results obtained in the evaluated period are reported. A more comprehensive description with literature references is presented in Section 3-4.

Very interesting, from the self-assembly point of view, are triblock copolymers, in which different nature of the three blocks may bring such structural features to the nanoassemblies that are not achievable by any other means. Triphilic polymers have hydrophilic, hydrophobic and perfluorinated blocks, which are immiscible with each other and are highly relevant for

applications. We focused on *triphilic* poly(2-oxazoline) triblock copolymers with high fluorine content to progress toward our aim of developing ^{19}F magnetic resonance imaging (MRI) contrast agents. These polymers were shown to have high potential for future development of ^{19}F MRI contrast agents. We developed a simple approach, in which commercially available perfluoroalkyl precursors are used as perfluorinated ”blocks” and the structures and shapes of the nano-assemblies are governed by the length of the perfluoroalkyl chain. Single-layer and multi-layer vesicles as well as rod-like micelles were prepared in a controlled manner in aqueous solutions (Fig. 1) (ASEP (ID 492559), ASEP (ID 472329)).

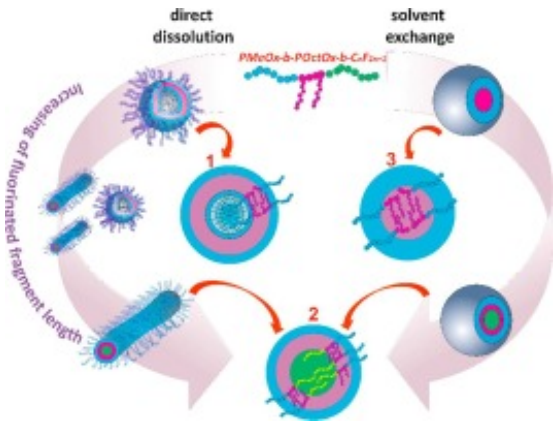


Fig. 1. Supramolecular structures self-assembled from triphilic polymers in aqueous milieu.

Injectable polymer depots may be constructed in the tissue with the depot-forming polymer being itself biologically active and not serving only as a carrier of bioactive cargo. We described a whole family of thermoresponsive hybrid biodegradable peptidoglycan-like polymers, β-glucan-graft-poly(2-isopropyl-2-oxazoline-co-2-butyl-2-oxazoline)s, that can be used for depot-formation. A conceptually new bimodal immunoradiotherapy treatment using these polymers was demonstrated *in vivo*. Complete inhibition of tumour growth was observed and about half of the mice treated were completely cured. Thus, a considerable synergistic effect of using immunoradiotherapy was achieved when compared to separate use of either immunotherapy or radiotherapy (ASEP (ID 491333), ASEP (ID 480890)).

Cells in inflamed tissues, cells under hypoxia and cancer cells overproduce reactive oxygen species (ROS) – concentrations of ROS under these conditions are orders of magnitude higher than in healthy cells. In fact, ROS can be used as partly intracellular and partly extracellular trigger, since ROS are also secreted extracellularly. We developed a new drug-delivery system of polymer nanoparticles (NPs) bearing pinacol-type boronic ester and alkyne moieties, which display self-immolative polymer degradation triggered by the presence of ROS. The NPs specifically release their drug cargo under such concentrations of ROS that are commonly found in the intracellular environment of certain tumours and of inflamed tissues and exhibit thus significant cytotoxicity to cancer cells compared to their non-ROS-responsive counterparts (ASEP (ID 458295), ASEP (ID 507295))

We also developed a biodegradable, biocompatible nanoparticle system for the delivery of the antituberculotic antibiotic rifampicin. Mycobacterium tuberculosis, the aetiological agent of tuberculosis, is an intracellular pathogen of alveolar macrophages. These cells avidly take up nanoparticles making the use of nanotherapeutics ideal for the treatment of such infections. Our nanoparticles contain a Förster resonance energy transfer (FRET) sensor that allows real-time assessment of drug release not only *in vitro*, but also in living macrophages where the mycobacteria typically reside as hard-to-kill intracellular parasites. The fluorophore also enables *in situ* monitoring of the enzymatic nanoparticle (Fig.2).

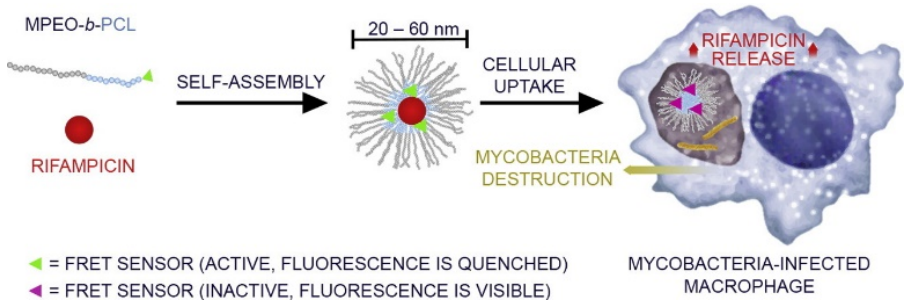


Fig. 2. System for the delivery of the antituberculotic antibiotic rifampicin with a built-in drug release and nanoparticle degradation fluorescence sensor.

Biomacromolecular and Bioanalogous Systems (Therapeutics + Tissue Engineering)

This Centre's research deals with the development of polymer systems for human medicine and for better quality of life. The Centre specialises in the development of materials suitable for the delivery of active molecules, such as drugs and diagnostic dyes, alongside the development of polymer materials for tissue engineering. This involves the design, synthesis, and physico-chemical and biological evaluation of polymer nanomedicines, tailor-made for targeted drug delivery, diagnostics, vaccination, and theranostics. Controlled syntheses, novel polymerisation techniques, self-assembly principles, and tailored design are employed in the preparation of such nanomedicines. Furthermore, the Centre's research is also focused on functional polymer surfaces, on biosensing based on sophisticated polymer architectures, polymer micro- and nano-particles, and on tissue engineering 3D scaffolds with the main effort to create polymer surfaces with controlled interactions. These surfaces range from those that are protein-repulsive to bioactive ones, which exhibit selective interactions with proteins and cells mediated by surface-bound biomimetic ligands.

Several examples of outstanding research results obtained are introduced below. A more comprehensive description is presented in the Appendices devoted to the BIOMOL – Therapeutics and BIOMOL – Tissue Engineering teams.

Advanced biodegradable polymer-based nanomedicines with tuneable structure and properties. We thoroughly investigated the relationship between polymer drug carrier structure and its suitability as a drug delivery system (DDS). We focused on the optimisation of polymer carrier architecture and size to maximise the therapeutic outcome. We developed an advanced process for their simple and robust synthesis by employing controlled polymerization of hydrophilic copolymers and by reduction of the number of synthetic steps (Fig.3). We showed the high potential of diblock or star-shaped polymer-drug conjugates in the treatment of various poorly treatable tumours *in vivo*.

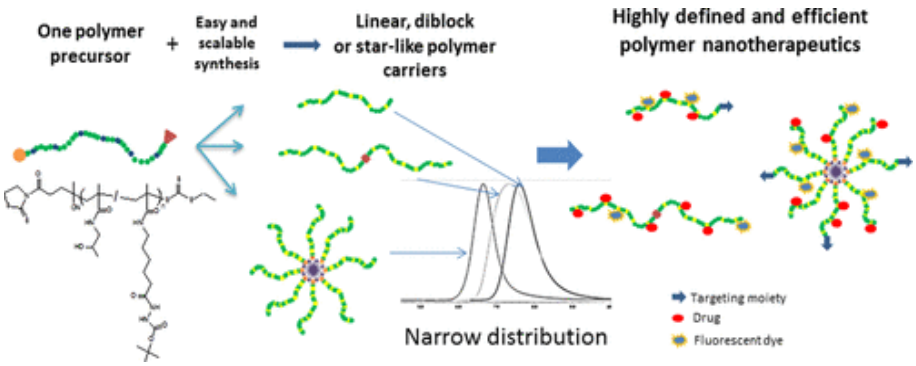


Fig. 3. Schematic description of new synthetic approach for advanced nanomedicine.

Polymer nanomedicines to overcome the multi-drug resistance (MDR). MDR is a common cause of failure in chemotherapy for malignant diseases. We showed that tumour-targeted micelle-forming amphiphilic block copolymers based on polypropylene glycol enable enormous tumour accumulation and consequent inhibition of MDR driven by the micelle and by the delivery of the cytotoxic payload.

Polymer theranostics. Simultaneous treatment and diagnostic pre-observation or observation are required for the future active nanomedicines. The employment of fluorescence imaging or positron emission tomography (PET) using polymer nanomedicines containing both the therapeutic molecule and diagnostic label enables the real-time monitoring of the treatment. We described a novel polymer platform suitable for efficient diagnostics and theranostics based on ^{89}Zr-, or fluorescently labelled nanomedicines. Afterward, the theranostic potential of the platform was verified during simultaneous patient-derived xenografts (PDX) treatment and non-invasive imaging using nanomedicine with reduced side effects (Fig.4).

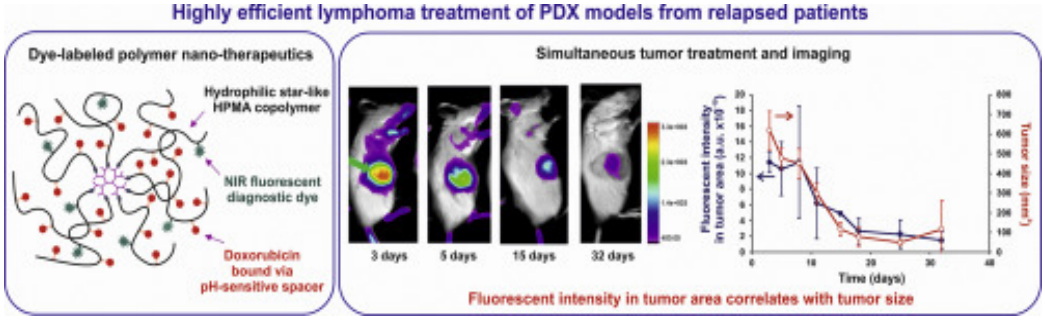


Fig. 4. Theranostics feature of polymer-doxorubicin-fluorescent dye nanosystem in the treatment of PDX.

Tailored biomimetic surfaces. The biomimetic surfaces were constructed as well-defined surface brushes with polymer chains, which are capable of suppressing non-specific material/biological media interactions. Protein-repulsive polymer chains capable of reducing or completely suppressing the protein adsorption from biological media (blood plasma, urine, *etc.*) were achieved by self-assembly of amphiphilic copolymers, by the formation of stable nanoparticles, by chemical end-tethering of polymer chains, and by surface-initiated controlled polymerizations. A new type of surface-initiated photoinduced single-electron transfer living radical polymerization (SET-LRP, Fig. 5) was developed to synthesise and to pattern polymer brushes. The developments are used directly for the fabrication of biosensing platforms for detection of various pathogens. A nanobiosensing platform based on porous magnetic microspheres that are efficient in capturing and pre-concentration was developed for the detection of Alzheimer's disease biomarkers. Similarly, copolymer brushes were synthesised on microparticles to scavenge lipopolysaccharides and prevent sepsis in hemofiltration.

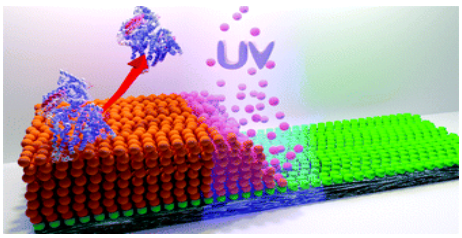


Fig. 5. Introducing surface-initiated photoinduced single-electron transfer living radical polymerisation.

Upconverting nanomaterials. New upconverting nanocrystals and nanoparticles (UNCNPS) were developed as prospective agents for near-infrared photothermal/photodynamic and single-photon emission computed tomography cancer theranostics. The decoration of the surface of UNCNPS with cell adhesive and cell-penetrating peptides enabled the selective targeting and imaging of specific tumour phenotypes. The developed UNCNPS were utilised as sensing platforms based on nanopaper and single-molecule immunoassays.

3D scaffolds. Micro-resolved 3D mesostructures were fabricated by employing a direct laser writing to a novel functional photoresist based on the radical coupling reaction of thiols and alkynes. Post-modification reactions based on thiol-Michael addition and copper-catalysed azide-alkyne cycloaddition that employed residual thiols and alkynes rendered the surface of various scaffolds bioactive. The required bioactivity of the designed pouch was introduced through LbL (layer by layer) approaches to bioengineer a pre-vascularised cavity. Assemblies of thin-film silk fibroin hydrogels were optimised for the encapsulation of living Langherhans islets. Various bioactive fibrous scaffolds were synthesised and their potential in bio-applications was proven.

Polymer Materials and Technologies

The scientific activities pursued in the MATER Centre can be divided into four partially overlapping and complementary research areas.

The first one covers investigations on structure-properties relationships in heterogeneous polymer materials. Blends and composites are important classes of polymer materials with heterogeneous phase structure with a broad field of application. Both theoretical and experimental studies were performed in this area. The former were concerned with the effect of rheological properties of blend components and of the presence of the block copolymer at the interface. The latter were focused on biodegradable systems based on polylactic acid and polycaprolactone or starch-based materials. These investigations led also to two patents, one of them is commercialised already. The effect of filler geometry on structure and properties of polymer composites was studied intensively as well.

The second research area involves advanced approaches in polymer chemistry and characterisation. In the MATER Centre, the microwave-assisted chemical processes based on the efficient heat transfer achieved by dielectric heating are investigated continuously. Microwave-assisted recycling technologies for polyurethanes, polyesters and polycarbonates were studied using reagents from renewable sources. Another application of the microwave energy is the microwave-assisted polymerisation. The influence of ionic liquid-modified layered double hydroxide on polymerisation of ε-caprolactone under microwave irradiation was investigated and the process was patented successfully. Advanced characterisation techniques including microindentation or evaluation of stabiliser efficiency were employed in studies focused on supramolecular structure of ultra-high molecular weight polyethylene for total joint replacements and light stabilisation of polyolefins with natural phenolic antioxidants.

Soft polymer materials responsive to external stimuli stand in focus of the next research area. Thermosensitive poly(*N*-isopropylacrylamide) based hydrogels are a typical example of these materials. As these materials with covalently cross-linked network structures are fragile and possess a low degree of swelling at higher crosslinking densities, their mechanical properties have to be improved for practical applications. Thus, the effect of the reinforcing laponite clay on the gel formation and structure evolution was investigated. Furthermore, the gel properties were modified by introducing porosity using cryopolymerisation and/or by incorporating of nano-silica, starch or electrically conducting particles. The other soft materials under study were self-healing polymers based on systems with reversible covalent bonds or with physical supramolecular structures. The former case is represented by reversible Diels–Alder type networks, which were prepared from furan and maleimide monomers of different structure and

functionality and the latter by aliphatic polycarbonate-based polyurethanes showing a high degree of ordering and strong superstructures that can undergo order–disorder transitions.

The fourth research area includes investigations on high performance thermosets with tailored properties. Special attention was paid to systems containing inorganic silica phase. The control over the interface between organic and inorganic phase due to incorporation of ionic liquids was studied. Ionic liquids were also evaluated with respect to their ability to self-catalyse reactions between carboxyl groups and epoxy rings. The control of the interface was studied also in systems of epoxy matrix and titanate nanotubes. Finally, siloxane polymers as precursors for ceramic foams were prepared *via* simultaneous curing and foaming of liquid methylsiloxane resins using ethanol as a blowing agent. Mechanically very strong foams were prepared *via* pyrolysis of polysiloxane composites containing epoxy powder as sacrificial filler.

Structure and Dynamics of Macromolecules

The scientific program of the STUCTURE Centre focuses on one of the most important unsolved problems in current chemistry, which is: "*The ability to accurately predict the structure of complex molecular and macromolecular systems and to relate the molecular and supramolecular structures to properties and reactivity*." Consequently, the research in the STUCTURE Centre deals with the development and applications of advanced techniques of high-performance spectroscopy to unveil the structure of functional materials down to atomic resolution level and to describe the intermolecular interactions and dynamics in these materials. In this regard, we systematically develop and explore various combinations of advanced techniques of NMR spectroscopy, vibrational spectroscopy, and X-ray diffraction with DFT calculations and data processing with the aim to formulate robust and reliable strategies of structure analysis that are applicable in academic as well as industrial laboratories. This way, through the synergistic interplay between the measurements, calculations and the statistical analysis, we want to develop and optimise an integrated approach providing otherwise unavailable structural information for complex multicomponent systems in various physical states (liquids, solids, gels). The STUCTURE Centre also represents a “research & service” unit, which provides internal and external services to other centres at the IMC and to external partners. Therefore, to cover analysis of the most important materials currently developed at the IMC, and to simultaneously lead fundamental research, three main research directions were defined. The research of the STRUCTURE Centre is thus focused on the structure analysis of:

- advanced multicomponent pharmaceutical solids;
- amphidynamic networks and complexes for energy applications;
- framework aluminosilicates and metalorganic frameworks.

Of these directions, the highest priority research of the Centre STRUCURE lies in the development and advanced application of *NMR crystallography for finding key structural motifs and dynamics phenomena governing physicochemical properties of active pharmaceutical ingredients and materials*. Consequently, since 2016, we have systematically developed methodology for complete determination of crystal structure without the assistance of diffraction data exclusively from NMR parameters. In this regard, the concept of NMR crystallography represents a unique protocol of an *ab initio* determination of the crystal structure based on the combination of solid-state NMR spectroscopy (ss-NMR), computer crystal structure prediction (CSP) and density function theory (DFT) chemical shift calculations. However, the widespread application of this approach still requires further extension. The problem of structure determination at atomic resolution level is particularly urgent for structural studies of advanced generation of multicomponent polycrystalline micro-/nano-sized solids, which not only represent promising drug-delivery systems, but also constitute materials with hierarchical architecture executing multiple functions. Presence of multiple crystalline components, however, results in complicated diffraction patterns, the conversion of which into

the refined crystal structures is practically unattainable. In this regard, we extended the above-mentioned concept of NMR crystallography by utilising domain-selective 2D solid-state NMR techniques which made it possible to monitor the atomic-resolved structure of polycrystalline multicomponent systems whose domains are incorporated in a crystalline matrix. Specifically, without the assistance of diffraction data and using exclusively NMR parameters in combination with the crystal structure prediction, we determined the complete 3D structure of individual components in a micro-/nano-composite consisting of the crystallites of a drug, low-molecular weight excipient and crystallites of polymer matrix. This research thus demonstrates the synergy effects of the proposed combination of several experimental and computational procedures, which considerably extends the NMR crystallography approach into the area of intricate mixtures and nanostructured composites in micro- and nano-sized forms. [*Macromolecules* **2018**, *51/14*, 5364-5374]

Structure analysis of *advanced polymer composites and energy related materials* represent another scientifically and industrially important research topic of the Centre STRUCTURE. Clean energy, global warming, emission reduction — terms currently frequently used and often introduced in a variety of meanings and contexts. Effective utilisation of solar and wind energy, and increased use of electric vehicles and portable electronics raises the demand for electric-energy conversion and storage devices. Our effort is thus also focused on the synthesis, optimisation and precise structural characterisation of innovative inorganic and hybrid functional materials exhibiting promising electric and ionic conductivity and other end-use properties.

Since 2019, we have been collaborating extensively with the researchers from the Department of Material Chemistry, Ångström Advanced Battery Centre, Uppsala University (Prof. Daniel Brandell) and Prof. Feng Gao (Division of Biomolecular and Organic Electronics, Linköping University). Our joint research is focused on the development of advanced polymeric electrolytes and energy related materials for solar cells. Although this collaboration is relatively new, we have already reached several significant outcomes that have already been published in prestigious scientific journals. Specifically, this cooperation has recently resulted in the development of perovskite-molecule composite thin films for efficient and stable light-emitting diodes. The developed perovskite-molecule composite thin films consist of *in-situ* formed high-quality perovskite nanocrystals embedded in the electron-transport molecular matrix, which controls nucleation process of perovskites, leading to perovskite light-emitting diodes (PeLEDs) with a peak external quantum efficiency of 17.3% and half-lifetime of approximately 100 hours. This work thus provided an effective strategy for deep understanding of efficient and stable PeLEDs from both material and device perspectives. [*Nature Communications*. 11/1 (2020), 891_1-891_9]

Polymers for Optoelectronic and Energy Applications

Research of this Centre is focused on the development of advanced polymers with superior charge or ionic transport, with photoelectrical sensitivity, and with energy storage or conversion capability, relying on close cooperation of chemists, material scientists and physicists. Synthetic procedures are designed or refined for obtaining conducting, mostly conjugated, polymers with targeted properties for applications in organic memory, light-emitting and photovoltaic devices, organic printable electronics, polymer electrodes and supercapacitors, solid state electrolytes and polymer membranes for fuel cells and water electrolysis. Photophysical experimental and theoretical studies are carried out to improve the understanding of underlying phenomena and to provide feedback for creating new paradigms and materials optimisation.

In the following, several examples of outstanding research results obtained in the evaluated period are described. A more comprehensive discussion with literature references is presented in the Section 3-4 devoted to the OPTOEL Centre.

Conducting polymers are favourable candidates for electrodes in supercapacitors that can revolutionise the field of energy storage. Such polymers should possess good electrical conductivity and high specific surface area. The OPTOEL team contributed to this effort by inventing synthesis of novel polymer materials, dubbed conducting cryogels. These cryogels based on polyaniline supported with poly(vinyl alcohol) were successfully prepared by a facile one-step cryo-copolymerisation approach. The pore sizes, conductivity and mechanical properties of cryogel can be easily tuned by the ratio between polyaniline and poly(vinyl alcohol) [ASEP (ID 471010)]. Such electroactive, porous and free-standing conducting macroporous polymer materials were found suitable for the conversion to aerogels by freeze-drying procedure and, subsequently, to nitrogen-containing carbogels by the carbonisation in an inert atmosphere. Their morphology and chemical composition, such as carbon-to-nitrogen ratio, can be varied to a large extent and reliably controlled already during the initial phase of the preparation, *i.e.* by adjusting the conditions during the synthesis of (poly(aniline-*co*-*p*-phenylenediamine)/poly(vinyl alcohol) copolymer cryogel [ASEP (ID 522109)]. The macroporous morphology was preserved after carbonisation but, at the same time, the specific surface area increased from 12 to 680 m^2g^{-1} after carbonisation at 500 °C in an inert atmosphere. By subsequent thermal treatment in an inert atmosphere, the charge storage mechanism of the aerogel can be converted from faradaic process (pseudocapacitor), to prevailing electric double-layer formation (electrostatic mechanism) [ASEP (ID 500356)].

Donor-acceptor (D-A) copolymers were extensively studied as a third generation of semiconducting polymers with application potential as active materials for photonics and optoelectronics. D-A copolymers composed of various combinations of electron donors (9,9-bis(alkyl)fluorene, 2,5-dialkyl- or 2,5-dialkyloxy- substituted 1,4-phenylene, bithiophene and carbazole derivatives) and electron acceptors (4,6-bis(3′-alkylthiophen-2′-yl)thieno[3,4-*c*][1,2,5]thiadiazole or *N,N′*-dialkylperylene-3,4,9,10-tetracarboxydiimide) bearing various side chain combinations were designed and synthesised. Effects of backbone composition and the alkyl side chain combinations on their photophysical and electrochemical properties were elucidated, and their potential applications were shown. Some of these new copolymers exhibited optical absorption covering the entire visible spectral region, extended even to near-infrared, and showed interesting electrochromic behaviour with fast response times. [ASEP (ID 506545), ASEP (ID 512150)] Such copolymers are promising for the use as optical and electrochromic (EC) materials having great potential in applications such as displays, sensors, EC devices, optical communications, and smart windows for promising energy saving technology, which can dynamically control the amount of solar heat and light entering a building in response to changes of indoor temperature and light conditions.

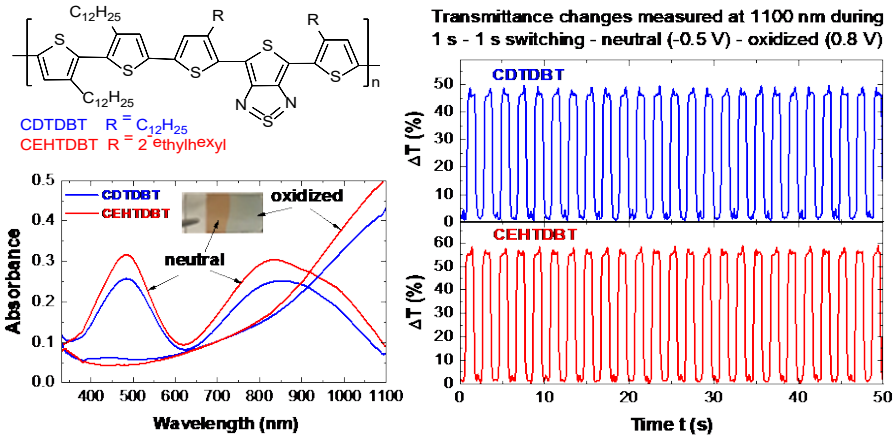


Fig. 6. Absorption and electrochromic switching in D-A copolymers composed of 3,3′-didodecyl-2,2′-bithiophene donor and 4,6-bis(3′-alkylthiophen-2′-yl)thieno[3,4-c][1,2,5]thiadiazole acceptor.

Using time-resolved absorption optical spectroscopy, the relaxation processes in oligomers and metallo-supramolecular polymers (dynamers) composed of α,ω–bis (terpyridyl) oligothiophene were characterised. The effect of coplanarity disturbed by solubilization side groups on the excitation energy decay was elucidated [ASEP (ID 445857)]. The supramolecular structure of polymer thin films was found favourable for effective transformation of singlet excitons to triplets. The process, dubbed singlet fission, yields two triplet excitons from a single photon absorbed in an organic compound. This phenomenon has been considered as a promising way to overcome the Shockley–Queisser power efficiency limit of solar cells. Our discovery thus extends the materials base for solar cells with higher energy conversion efficiency [ASEP (ID 477332)].

Research activity and characterisation of the main scientific results

Activity of centre SUPRAMOL in the evaluation period is represented by 208 publications and was concentrated on four topics related to synthesis, characterisation, sensitivity to external stimuli and application of supramolecular self-associated polymer systems. Essential achievements are documented by selected results as detailed below.

Some results listed in this section appear also in the section describing selected results of the Institute.

In the rest of this section **NP** stands for **nanoparticle**.

Topic 1 – Development of synthetic procedures for the preparation of functional polymers with desired properties

For the development of advanced self-assembled systems, we had to master or develop a portfolio of tailored procedures for special polymer synthesis. Within these studies, the research team of SUPRAMOL was responsible for synthesis of the polymers, their characterisation by instrumental physico-chemical techniques and, where relevant, for radiochemical studies.

Aqueous copper(0)-mediated reversible-deactivation radical polymerization (Cu-RDRP) of *N*-(2-hydroxypropyl)methacrylamide, a monomer with important biomedical applications, was optimised and used for the construction of the poly[*N*-(2-hydroxypropyl)]methacrylamide-based copolymeric carrier having the anti-cancer drug doxorubicin (DOX) conjugated through a pH-sensitive hydrazone linker. ASEP (ID 500627) Contribution of SUPRAMOL 80 % with corresponding and first author who provided system concept, synthesis and characterisation of polymers, designed the study, performed all the polymerization experiments, and wrote the manuscript.

Two new mannan conjugates, containing a gadolinium complex and a fluorescent probe, one based only on polysaccharide and the other one comprising polysaccharide with poly(2-methyl-2-oxazoline) grafts, were prepared and simultaneously visualized *in vitro* and *in vivo* by magnetic resonance and fluorescence imaging. We showed, that mannan-based conjugates possess exceptional features for multimodal imaging because of their biocompatibility, biodegradability and self-targeting properties. When used to detect lymph nodes, the polymers showed better imaging properties than a commercially available contrast agent**.** ASEP (ID 489337) Contribution of SUPRAMOL 70% with corresponding author (M.Hrubý) and first author (M.Rabyk); system concept, synthesis and physico-chemical characterisation of mannan-based conjugates.

The $Mn_2(CO)_{10}$-photomediated activation of perfluoro alkyl iodides under visible light was applied in the initiation of the polymerization of neopentyl styrene sulfonate (NeoSS) and of vinylidene fluoride (VDF) (Fig. 1) providing simpler, cleaner, excellently defined and powerful way towards the synthesis of well-defined sulfonated and fluorinated block copolymers for fuel cell membranes. ASEP (ID 444813) Contribution of SUPRAMOL 60 % corresponding and first author; synthesis of polymers.

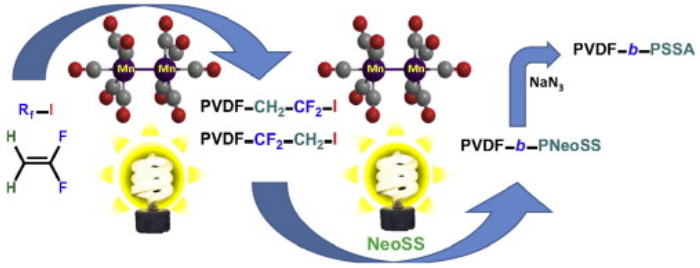


Figure. 1 Scheme of the $Mn_2(CO)_{10}$-photomediated activation of perfluoro alkyl iodides under visible light in the initiation of the polymerization of NeoSS and VDF.

An important task for biomedical use is the choice of appropriate polymer material, while head-to-head comparisons of different materials for the particular application are often missing in the literature. We prepared two analogous pH-responsive hydrazone-based DOX delivery systems, one poly(2-ethyl-2-oxazoline)-based and one PHPMA-based. *In vitro* and *in vivo* anti-cancer properties of these conjugates were critically compared. Considering the synthetic advantages of poly(2-alkyl-2-oxazoline)s, the presented study demonstrates their potential as a versatile alternative to well-known PEO- or PHPMA-based materials for construction of drug delivery systems. ASEP (ID 478902) Contribution of SUPRAMOL 70 % with corresponding and first author; concept of the study, synthesis and physico-chemical characterisation of polymers before and after irradiation.

We have studied polymer scavengers of reactive oxygen species (ROS) for wound and gastrointestinal pathologies healing. To fine-tune ROS-scavenging activity, we synthesised a series of model linear copolymers of 2-hydroxyethyl methacrylate (HEMA) and a sterically hindered amine derivative [*N*-(2,2,6,6-tetramethyl-piperidin-4-yl)methacrylamide (HAS)]. These copolymers compared very favourably to established low-molecular weight antioxidant standards (BHA and dexpanthenol). ASEP (ID 447407) Contribution of SUPRAMOL 90 % with corresponding and first author: system concept and design, synthesis and characterisation of the polymers and polymeric films, evaluation of the antioxidant properties.

Energetic ions represent an important tool for the creation of controlled structural defects in solid nanomaterials, *e.g.* in fluorescent nanodiamonds to be polymer-coated for biomedical applications. However, the current preparative irradiation techniques in accelerators show significant limitations in scaling-up, because only very thin layers of nanoparticles can be efficiently and homogeneously irradiated. We have designed an easily scalable and remarkably cost-effective method for rapid irradiation of nanomaterials by light ions formed homogeneously in situ by a nuclear reaction. The target nanoparticles are embedded in B_2O_3 and placed in a neutron flux. Neutrons captured by ^{10}B generate an isotropic flux of energetic α particles and $^{7}Li^{+}$ ions that uniformly irradiates the embedded nanoparticles. We produced tens of grams of fluorescent nanodiamonds in an unusually short, approximately 30-minute irradiation session. Our method (patent pending) thus increased current preparative yields by a factor of 10^2–10^3. We envision that our technique will enable a dramatic increase in the production of ion-irradiated nanoparticles, facilitating their use in various applications.
Such core–shell nanoparticles based on reactor-produced fluorescent nanodiamonds and coated with a biocompatible *N*-(2-hydroxypropyl)methacrylamide copolymer shell (Fig. 2) were applied for background-free near-infrared imaging of cancer cells. The particles showed excellent colloidal stability in buffers and culture media. After conjugation with a cyclic RGD peptide they selectively targeted integrin αvβ3 receptors on glioblastoma cells with high internalization efficacy. ASEP (ID 498523), ASEP (ID 443136) Contribution of SUPRAMOL: One of two corresponding authors (M. Hrubý); developing the system concept, part of the preparation and analysis of the polymer and nanoparticle samples. The authors from SUPRAMOL provided the key principle of the procedure, and thus made possible the development of this unique procedure.

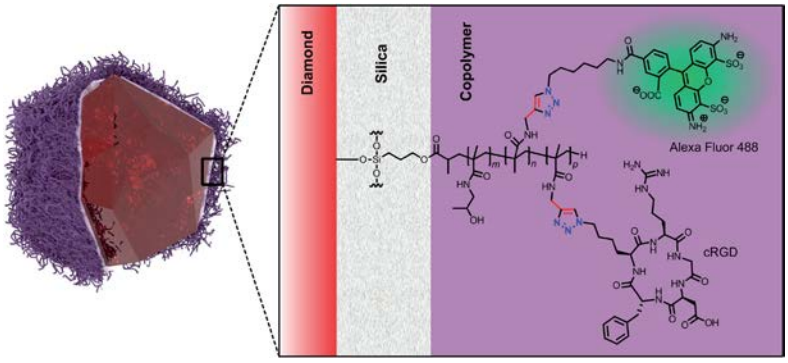


Figure 2. Schematic structure of the fluorescent nanodiamond crystal coated with a biocompatible methacrylamide copolymer.

Topic 2 – Investigating internal structure and advanced instrumental characterisation of self-assembled polymer species

The beauty of self-assembled supramolecular polymer systems brings extraordinary benefits to various applications. To elucidate the ways how polymers self-assemble, and in order to have deep insight into internal structure of such nanoassemblies, we have performed a number of studies. The contribution of workers from SUPRAMOL centre is a part of the synthesis of polymers and more importantly the whole polymer physics and physico-chemical part.

We synthesised and studied aqueous solution properties of novel amphiphilic PCL-*b*-PHPMA diblock copolymers possessing stable nitroxyl radicals covalently conjugated to the hydrophobic poly(ε-caprolactone) (PCL) block. EPR spectroscopy was applied to investigate the dynamics of the polymer chains before and after micellization. These novel NPs should find applications in drug delivery systems and for the treatment of oxidative stress injuries. ASEP (ID 462673) Contribution of SUPRAMOL 60 % with corresponding and first author (S. Petrova) and second corresponding author (S. K. Filippov); system concept, synthesis of polymers, physico-chemical characterisation of formulations, EPR studies.

Poly(2-methyl-2-oxazoline)-*b*-poly[*N*-(2,2-difluoroethyl)acrylamide] self-assembled NP show great potential in biological applications as, *e.g.*, ^{19}F MRI tumour diagnostic agents. We elucidated internal structure of the nanoparticles formed by temperature-driven self-assembly of the above-mentioned diblock copolymer by various methods. The ability of nanoparticles to provide usable ^{19}F NMR signal was also demonstrated. ASEP (ID 509826) Contribution of SUPRAMOL 75 % with corresponding author (M. Hrubý) and first author (D. Babuka); system concept, polymers synthesis and characterisation, self-assembly study.

Very interesting from the self-assembly point of view are such triblock copolymers, where different nature of the three blocks may bring structural features to the nanoassemblies not achievable by other means. Triphilic polymers have hydrophilic, hydrophobic and perfluorinated blocks which are immiscible with each other and are highly application-relevant. We focused on *triphilic* poly(2-oxazoline) triblock copolymers with high fluorine content toward our future aim of developing ^{19}F magnetic resonance imaging (MRI) contrast agents. These polymers were shown to have high potential for future development of ^{19}F MRI contrast agents. ASEP (ID 492559) Contribution of SUPRAMOL 50 % with corresponding author (S. K. Filippov) and first author (L. I. Kaberov); system concept, synthesis of monomers and part of polymers, physico-chemical characterisation of self-assemblies.

An easier approach is to use commercially available perfluoroalkyl precursors as perfluorinated ”blocks“. With such approach, the structures and shapes of the nanostructures are controlled

by the length of the perfluoroalkyl chain. Single-layer and multi-layer vesicles as well as rod-like micelles were prepared in aqueous solutions (Fig. 3). ASEP (ID 472329) Contribution of SUPRAMOL 50 % -– corresponding author (S. K. Filippov) and first author (L. I. Kaberov); system concept, synthesis and characterisation of polymers, self-assembly study.

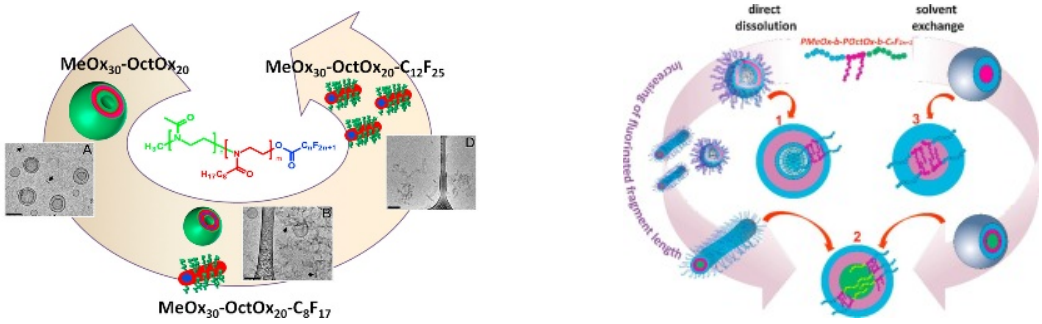


Figure. 3. Supramolecular structures self-assembled from triphilic polymers in aqueous milieu.

Amphiphilic ***gradient*** copolymers are copolymers, where the blocks gradually pass from one to another. These copolymers also self-assemble in selective solvents, but, in a uniquely different way than the corresponding block copolymers. We studied in detail the self-assembly properties of these, up to now, undeservedly highly understudied materials. We have proven that poly(2-methyl-2-oxazoline-*grad*-2-phenyl-2-oxazoline) forms hollow ”bitterball“ micelles with high drug loading capacity. ASEP (ID 476710) Contribution of SUPRAMOL 35 % with corresponding and first author (S. K. Filippov); part of polymer synthesis and characterisation of polymers, preparation of micelles, structural study.

Even more advanced structures can be formed from block-gradient copolymers where one block is a homopolymer [poly(2-dimethylaminoethyl acrylate)] and the other block is gradient copolymer [poly(2-dimethylaminoethyl acrylate-*grad*-styrene)]. Such micelles may be simultaneously pH- and thermoresponsive (see Fig. 4). ASEP (ID 491337) Contribution of SUPRAMOL 50 % with first author (M. Rabyk) and corresponding author (P. Štěpánek); synthesis of polymers, physico-chemical characterisation of polymers, scattering studies.

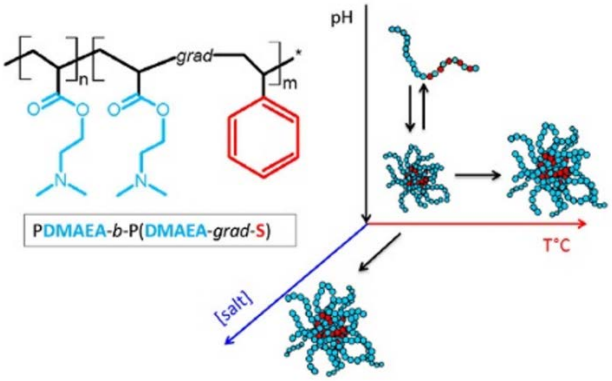


Fig. 4. Self-assembly of poly(2-dimethylaminoethyl acrylate)-*b*-poly(2-dimethylamino ethyl acrylate-*grad*-styrene) in aqueous milieu as a function of pH and temperature.

An important feature of devices, which should fulfil their tasks in the organism, is ***nonbiofouling properties***, *i.e*. repellent properties against biomolecules the adhesion of which may lead to opsonisation and unfavourable biological behaviour. Here, we utilized advanced techniques for studying protein adsorption.

We synthesized well-defined polymer brushes, which represent an effective surface modification, to resist the adhesion of blood and its components. Poly[oligo(ethylene glycol)methylether methacrylate] (poly(MeOEGMA)), poly(hydroxyethyl methacrylate) (poly(HEMA)), poly[*N*-(2-hydroxypropyl) methacrylamide] (poly(HPMA)), and poly(carboxybetaine acrylamide) (poly(CBAA)) brushes were grown by surface initiated atom transfer radical polymerization (SI-ATRP). All brushes decreased the fouling from blood plasma by over 95% and prevented the adhesion of platelets, erythrocytes, and leukocytes as evidenced by SPR and SEM measurements. ASEP (ID 443947) Contribution of SUPRAMOL 40 % with first author (F. Surman): synthesis of commercially non-available monomers, preparation of polymer brushes and characterisation of their physico-chemical properties.

We also revised the current understanding of the protein corona that is created on the surface of nanoparticles in blood plasma after an intravenous injection. We tested *in vitro* and *in vivo* nanoparticles that have a proven therapeutic outcome and are based on two types of biocompatible amphiphilic copolymers: PCL-*b*-PHPMA, and a statistical HPMA copolymer bearing cholesterol moieties. It was found that individual proteins and plasma have very low binding affinity to nanoparticles. ASEP (ID 488750) Contribution of SUPRAMOL 60%: corresponding and first author (D. Klepac) and second corresponding author (S. Filippov); system concept, polymer labelling with spin probe, preparation of nanoparticles, physico-chemical characterisation of nanoparticles.

Solution behaviour of polymers in body fluids containing thousands of proteins may be very different from that in water or buffer due to polymer-protein interactions and is extremely tricky to study. Florescence correlation spectroscopy (FCS) offers a solution to this difficulty as it ”sees“ only the fluorescently labelled species in a complex environment. We developed a new mathematical procedure for the determination from FCS of diffusion coefficient distributions applicable to polydisperse polymers or nanoparticles and tested it on both simulated FCS correlation functions and real experimental data. ASEP (ID 489135) Contribution of SUPRAMOL 100 %.

Topic 3 – Advanced polymer nanostructured materials

Within these studies, the research team of SUPRAMOL was responsible for the synthesis of materials, their characterisation by instrumental physico-chemical techniques and, where relevant, for radiochemical studies – and most importantly for designing new nanostructured materials.

Most of the work performed in SUPRAMOL is related to organic polymer micelles and nanoparticles. However, we also work on polymeric structures containing inorganic core. We developed multimodal probes, which can be simultaneously visualized by multiple imaging modalities, that enable tracking the cellular uptake in organisms, intracellular fate, biodistribution and elimination of labelled moieties. We synthesised crystalline WO_3 and $CaWO_4$ doped with Eu^{3+} or Tb^{3+} nanoparticles coated with polysaccharides, that constitute a versatile easy-to-construct modular toolbox for multimodal imaging. ASEP (ID 480995) Contribution of SUPRAMOL 50 % with corresponding and first author (V. Lobaz); system concept, synthesis of precursors, preparation of nanoparticles, physico-chemical characterisation of nanoparticles.

Inorganic nanoparticles can be also used as functional part of nanocomposites. We reported a novel method for the preparation of polymer nanocomposites containing *in situ* exfoliated graphene nanoplatelets. Gas permeability measurements on the polymer composites were also carried out, confirming that the initial graphene nanoplatelets were present in an exfoliated form. ASEP (ID 476829) Contribution of SUPRAMOL 50 % with corresponding and first author: design, synthesis and characterisation of the nanocomposites and related polymer nanocomposite membranes.

Freezing polymer solutions with subsequent lyophilization usually leads to microporous materials. However, in some cases – as for the polysaccharide glycogen (also present in humans), a self-associated microfibrous net can be formed. We described a conceptually new, microfibrous biodegradable functional material prepared from glycogen, which showed great potential as direct-contact dressing and/or interface material for wound healing *via* solvent-free method and also allowing preparation of thick layers. Crosslinking of the samples was performed by microtron β-irradiation. Biological testing showed, that these highly porous, hydrophilic, readily functionalisable materials were completely nontoxic to cells growing in their presence. The fibres were gradually degraded in the presence of cells. ASEP (ID 460861) Contribution of SUPRAMOL 60 % with corresponding author (M. Hrubý) and first author: system concept, modification of glycogen, preparation of nanofibres.

Wilson's disease is a genetic disorder that causes excessive accumulation of copper in the body, leading to toxic damage. The current treatment causes burdensome side effects. We invented microparticle biopolymer carriers that are based on microcrystalline cellulose and crosslinked chitosan containing a highly specific copper chelator based on 8-hydroxyquinoline. The chelators scavenge copper ions released from food during digestion and those present in secretions in the gastrointestinal tract, and do not exhibit any side effects. The technology was patented (European Patent EP 3 370 735 B1) and discussions with European strategic partners are under way. ASEP (ID 486034) Contribution of SUPRAMOL 60 % with corresponding and first author (M. Vetrík) and second corresponding author (M. Hrubý); system concept, synthesis of polymers, preparation of formulations, their physico-chemical characterisation, metal adsorption studies, radiochemistry.

Tailored polymersomes represent highly defined drug delivery carriers. Microfluidics preparation technique, for which we built a well-equipped facility, enables a precise setting of the size and architecture of these nanostructures. The extracellular and subcellular compartments are characterized by specific pH levels, that can be modified by pathophysiological states such as cancer. We have engineered anticancer antibiotic doxorubicin (DOX)-loaded pH-responsive quasi-monodisperse assemblies that encourage the use of such advanced pH-responsive platforms to target damaged cells while preserving healthy environments during systemic circulation. ASEP (ID 505875) Contribution of SUPRAMOL 75% with corresponding author and first author (L.C. Albuquerque) and second corresponding author (E. Jäger); system concept, synthesis of polymers, preparation of nanoparticles on microfluidic chip, drug loading study

Topic 4 – Exploitation of external stimuli-triggered polymer self-assembly and disassembly as the core activity of SUPRAMOL

Within these studies, the research team of SUPRAMOL was responsible for synthesis of the materials, their physico-chemical characterisation by instrumental physico-chemical techniques and, where relevant, for radiochemical studies. This is the essential core activity of SUPRAMOL that stems from the previous supporting activities.

We have divided these results into two partial topics according to the main stimulus to which the studied systems are responsive and according to whether this stimulus is primarily localized in the organism extracellularly (Topic 4A – temperature change) or inside the cells (Topic 4B – pH change, the presence of reactive oxygen species – ROS – and intracellular enzymes).

*Topic 4A – Responsivity to **extracellular** systemic stimuli – temperature-responsive systems*

We described a new approach to depot drug delivery in which a copolymer poly[*N*-isopropyl acrylamide-*co*-*N*-(3-imidazolylpropyl)methacrylamide] (PNIPAM-*co*-ImPM) is used for a new

formulation strategy that provides controlled and sustained release of an incorporated drug. A dual sensitivity was imparted to the polymer. The NIPAM units exhibit a thermo-sensitive behaviour while the ImPM units are pH-sensitive. A depot is formed at the application site upon simultaneously heating to body temperature and increasing the pH from 5 to the physiological value of 7.4. This prevents precipitation of the polymer in the injection needle. An *in vivo* experiment using the polymer with the drug paliperidone showed excellent release results of the drug from a depot. ASEP (ID 462640) Contribution of SUPRAMOL 70 % with first author and corresponding author (M. Hrubý); system concept, synthesis of polymers, physicochemical characterisation of polymers and their stimuli-responsive behaviour in aqueous milieu.

Polymer depots may be constructed in the way that the depot-forming polymer is itself biologically active and is not only a carrier. We described a family of thermoresponsive hybrid biodegradable peptidoglycan-like polymers, β-glucan-*graft*-poly(2-isopropyl-2-oxazoline-*co*-2-butyl-2-oxazoline)s. ASEP (ID 491333) Contribution of SUPRAMOL 60 % with corresponding author (M. Hrubý) and first author (L. Loukotová): system concept, synthesis and modification of polymers, physico-chemical characterisation of the prepared constructs including temperature-dependent solution behaviour.

A conceptually new bimodal immunoradiotherapy treatment was demonstrated *in vivo* with these polymers. Complete inhibition of tumour growth was observed and about half of the mice were completely cured. Thus, a considerable synergistic effect of using immunoradiotherapy was achieved compared to separately using immunotherapy or radiotherapy (Fig. 5). ASEP (ID 480890) Contribution of SUPRAMOL 60 % with corresponding author (M. Hrubý) and first author (L. Loukotová); concept of the system, synthesis of polymers, preparation of formulations, physico-chemical characterisation of formulations including stimuli-responsive solution behaviour.

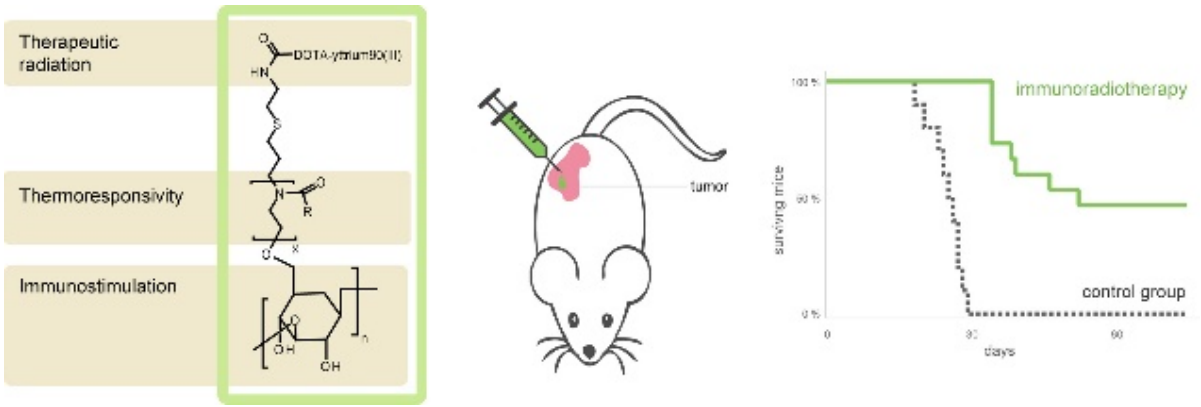


Figure. 5. Synergistic radioimmunotherapy with radiolabelled β-glucan-*graft*-poly(2-isopropyl-2-oxazoline-*co*-2-butyl-2-oxazoline)s.

Replacement of β-glucan in the construction of such polymers by an algal polysaccharide κ-carrageenan, also possessing potent immunostimulating properties makes possible the construction of multiresponsive hybrid biocompatible systems of κ-carrageenan-*graft*-poly(2-isopropyl-2-oxazoline-*co*-2-butyl-2-oxazoline)s with a unique combination of responsiveness to external stimuli, forming a gel at lower temperatures due to upper critical solution temperature (UCST) of the polysaccharide part, a clear solution at room temperature and cloudy nanophase-separated dispersion at elevated temperatures due to lower critical solution temperature (LCST) of the polyoxazoline part. The developed constructs also retain unique property of κ -carrageenan, the K^+-responsivity (potassium-induced gelation) providing to the

system additional important advantage as it may be injected as fully soluble sodium salt while at potassium-containing biological environment the K^+-responsivity facilitates (nano)phase separation. ASEP (ID 500339) Contribution of SUPRAMOL 75 % with corresponding author (M. Hrubý) and first author (L. Loukotová); system concept, synthesis of polymers, preparation and characterisation of self-assemblies, their stimuli-responsive self-assembly behaviour.

The thermoresponsive nanoparticle core can be constructed in the way that it is solid at room temperature and it melts in hyperthermic tissues such as tumours thus triggering bioactive cargo release. We developed fully biodegradable/metabolizable nanosystem based on polymer surfactant-stabilised thermoresponsive solid lipid nanoparticles with non-covalently bound photosensitizer temoporfin (T-SLNP). The system was proven to be significantly more efficient in *in vivo* murine models than the commercial formulation. ASEP (ID 462810) Contribution of SUPRAMOL 50 % with corresponding author, M. Hrubý, who was also the co-supervisor of the first author; concept of the system, synthesis of precursors, preparation of formulations, physico-chemical characterisation of formulations.

*Topic 4B – Responsivity to **intracellular** stimuli – pH change, presence of reactive oxygen species (ROS) and intracellular enzymes*

In the organism, the pH of most tissues is maintained at 7.4. However, after internalization into the cells, the endosome is acidified to pH below 5, which can be used for triggering a cargo release.

The potential of self-assembled nanoparticles (NPs) containing the fine-tuneable pH-responsive hydrophobic poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) core and the protein repelling hydrophilic poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA) shell for *in vitro* cytostatic activity has been explored on cancer cells. In the PHPMA-*b*-PDPA nanoparticles on decreasing pH the hydrophobic inner PDPA block becomes protonated (hydrophilized) resulting in a fast disassembly and release of the chemotherapeutic drug. ASEP (ID 445417) Contribution of SUPRAMOL 50 % with corresponding and first author (A. Jäger) and second corresponding author (E. Jäger); system concept, synthesis of polymers, self-assembly in solution, preparation of formulations, physico-chemical characterisation of polymers and formulations.

Reactive oxygen species (ROS) represent a trigger useable for cells in inflamed tissues; hypoxia and cancer overproduce concentrations of ROS orders of magnitude higher than healthy cells. In fact, this is partly intracellular and partly extracellular trigger since ROS are also secreted extracellularly.

We developed a new drug-delivery system of polymer nanoparticles (NPs) bearing pinacol-type boronic ester and alkyne moieties displaying triggered self-immolative polymer degradation in the presence of ROS. The NPs specifically release their drug cargo under concentrations of ROS that are commonly found in the intracellular environment of certain tumours and of inflamed tissues and exhibit significant cytotoxicity to cancer cells compared to their non-ROS-responsive counterparts. ASEP (ID 458295), Contribution of SUPRAMOL: first and corresponding author (E. Jäger); system concept, synthesis of polymers, preparation of nanoparticles, physico-chemical characterisation of nanoparticles studies on their *in vitro* degradation. ASEP (ID 507295) Contribution of SUPRAMOL 45 %; system concept, synthesis of polymers, preparation and characterisation of nanoparticles.

Polyesters of suitable structure are enzymatically degradable by intracellular enzymes, which can also trigger bioactive cargo release.

Mycobacterium tuberculosis, the etiologic agent of tuberculosis, is an intracellular pathogen of alveolar macrophages. These cells avidly take up nanoparticles making the use of nanotherapeutics ideal for the treatment of such infections. We have developed a biodegradable, biocompatible system for the delivery of the antituberculotic antibiotic

rifampicin. The nanoparticles contain a Förster resonance energy transfer (FRET) sensor that allows real-time assessment of drug release not only *in vitro*, but also in living macrophages where the mycobacteria typically reside as hard-to-kill intracellular parasites. The fluorophore also enables *in situ* monitoring of the enzymatic nanoparticle (Fig.6). ASEP (ID 470718) Contribution of SUPRAMOL 60 % with corresponding author (M. Hrubý) and first author (J. Trousil); system concept, synthesis of polymers, preparation and characterisation of nanoparticles, drug loading.

The polymeric nanoparticles uptake, consequent organelle targeting and intracellular degradation were shown to be highly dependent on the nanoparticles' physicochemical properties. We show that the nanoparticles are efficiently taken up by macrophages and are able to effectively neutralize the persisting bacilli. We demonstrate, using a zebrafish model of tuberculosis, that the nanoparticles are significantly more efficient compared to a free form of rifampicin. ASEP (ID 503810) Contribution of SUPRAMOL 50 % with corresponding and first author (J. Trousil) and second corresponding author (M. Hrubý); concept of the system, synthesis of polymers, preparation and characterisation of nanoparticles, drug loading.

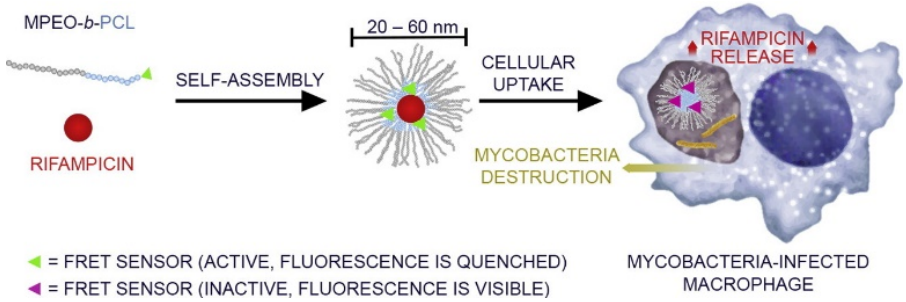


Figure. 6. System for the delivery of the antituberculotic antibiotic rifampicin with a built-in drug release and nanoparticle degradation fluorescence sensor.

As a step beyond the Food and Drug Administration approved polyesters (*e.g.*, poly-ε-caprolactone) for the delivery of the anticancer drug paclitaxel (PTX), we prepared a polymer, poly(ethylene oxide monomethyl ether)-*block*-poly(propylene succinate) (mPEO-*b*-PPS). PTX was loaded to the NPs, and its *in vitro* release was evaluated in different cell models and compared with commercial PTX formulations. The mPEO-*b*-PPS copolymers display lower toxicity, drug solubilization efficacy and enzymatic degradability after uptake into the cells and a general superior efficacy of the NPs compared with commercial PTX formulations. ASEP (ID 491038) Contribution of SUPRAMOL 50 % with corresponding and first author (A. Jäger) and second corresponding author (E. Jäger); system concept, synthesis of polymers including fluorescent labelling, preparation and characterisation of nanoparticles, drug loading.

Research activity and characterization of the main scientific results

The following summary of the main results is organized in sections, each representing the achievements of the specified tasks followed long-term by Tissue Engineering team of the center Biomacromolecular and Bioanalogous Systems. The specified tasks are indicated by section headings. References to publications are given by their respective ASEP number, which links them directly to the complete information on-line.

Functional Polymer Surfaces

The research within this sub-direction builds on our continuous effort to create bioactive polymer surfaces with controlled interactions: these surfaces range from those that are protein-repulsive to bioactive ones, which exhibit selective interactions with proteins and cells mediated by surface-bound biomimetic ligands. The biomimetic surfaces were constructed primarily as well-defined surface brushes with polymer chains, which are capable of suppressing non-specific material or non-specific biological media interactions. Protein-repulsive polymer chains capable of reducing or completely suppressing the protein adsorption from biological media (blood plasma, blood serum, saliva, urine, etc.) were performed by one of the following approaches: self-assembly of amphiphilic poly(L-lactide)-b-poly(ethylene oxide), PLLA-b-PEO, copolymers (ASEP (ID 474915)); formation of stable nanoparticles with a kinetically frozen PLLA core (ASEP (ID 461624)); chemical end-tethering of PEO and polyoxazoline chains (ASEP (ID 508134)) (**Figure 1**); and surface-initiated controlled radical polymerizations under ATRP conditions.

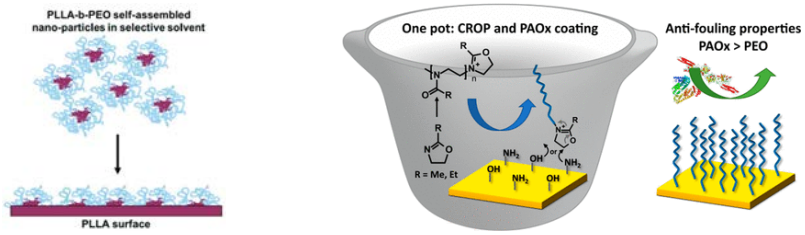


Figure 1. Self-assembly of amphiphilic poly(L-lactide)-b-poly(ethylene oxide) copolymers and chemical end-tethering of living polyoxazoline chains as routes for obtaining antifouling surfaces.

We developed a new type of surface-initiated photoinduced single-electron transfer living radical polymerization (SET-LRP) utilizing extremely low copper catalyst concentrations to synthetize and to pattern over surfaces acrylate (ASEP (ID 447651)), methacrylate (ASEP (ID 465611)), and methactrylamide brushes of poly[N-(2-hydroxypropyl) methacrylamide] (PHPMA) (ASEP (ID 445125)) (**Figure 2**).

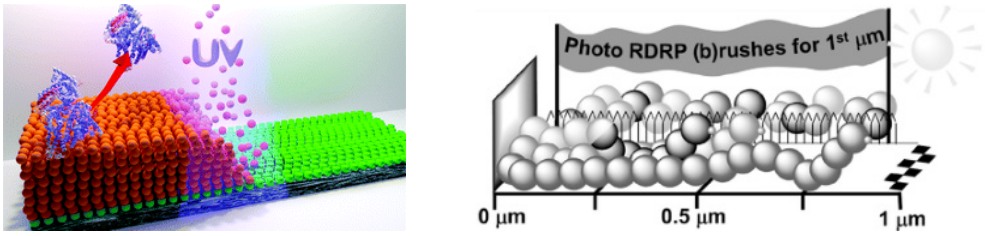


Figure 2. Introducing surface-initiated photoinduced single-electron transfer living radical polymerization (SET-LRP) utilizing extremely low copper catalyst concentrations to obtain thick acrylate, methacrylate and methacrylamide brushes.

Uniquely, the surface-initiated controlled radical polymerizations enabled the synthesis of random and block copolymer hierarchical brush structures and together with the possibility of modifying the living ends of the polymer chains gave the opportunity for further post-modification of the polymer brushes (ASEP (ID 474248)) to attain bioactive, biomimetic and/or bioresponsive properties (**Figure 3**). The chemical composition and profile of the synthesized brushes and their co-polymer counterparts were assessed by GAATR-FTIR, depth profiling and angle resolved X-Ray photoelectron spectroscopy, time-of-flight secondary ion mass spectroscopy and neutron reflectivity (ASEP (ID 471012), **Figure 4**).

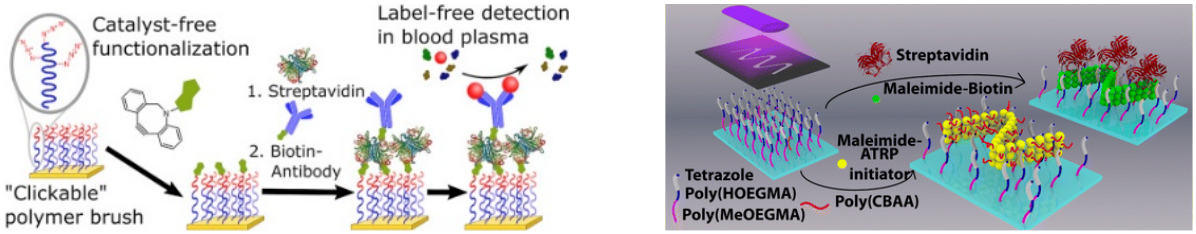


Figure 3. Chemical routes to attain bioactive, biomimetic and/or bioresponsive polymer brushes via catalyst-free modification of the distal chain ends (left) and adopting a photopatterning approach for the synthesis and subsequent modification of hierarchical polymer brushes (right).

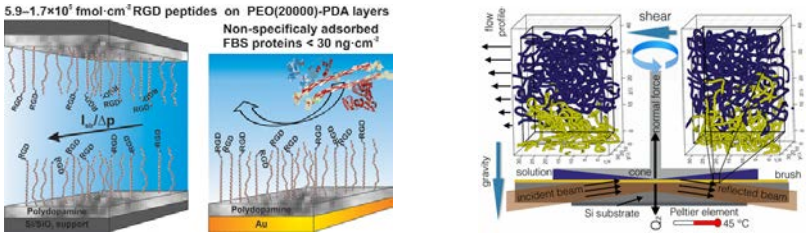


Figure 4. Proving the polymer brush structure via advanced techniques such as micro-slit (left) and neutron reflectivity (right) techniques.

The unique functionable co-polymer brush based on poly(carboxybetaine methacrylamide) (PCBMAA) and PHPMA managed to completely suppress the non-specific adsorption from blood, i.e. from the most demanding biological medium when non-specific adsorption is in question (ASEP (ID 479511), ASEP (ID 460886), ASEP (ID 482530)). The various polymer brush systems were further rigorously tested for their anti-trombogenic activity (ASEP (ID 458015)) and their anti-adhesion properties when exposed to bacterial pathogens (ASEP (ID 446174)) (**Figure 5**).

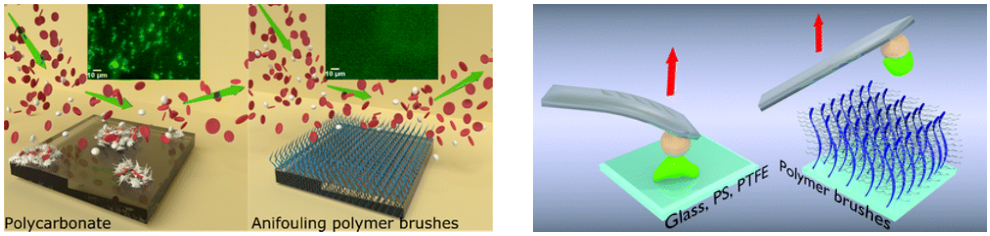


Figure 5. Polymer brush systems show anti-trombogenic activity (left) and resist the adhesion of bacterial pathogens (right).

Bioactivity was introduced to the antifouling polymer brushes via various highly specific photo-triggered and “click” chemistry reactions (Cu catalyzed and strain-promoted alkyne–azide cycloaddition reactions) giving the opportunity for spatial patterning of bioactive molecules over the polymer brush surface (ASEP (ID 445126), ASEP (ID 473863), **Figure 6**). We utilized electrokinetic micro-slit measurements and data predictions to verify the brush structure of the end-tethered bioactive polymer chains and prove that the biofunctionalization

utilizing biomimetic bioactive peptide sequences does not deteriorate the initially observed protein repellent properties of the brushes (ASEP (ID 489813)).

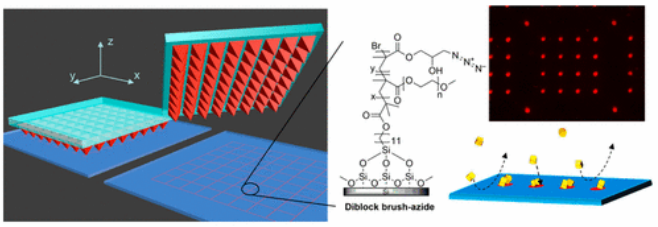


Figure 6. Spatial patterning of bioactive molecules on polymer brush surface via dip pen lithography.

A different rationale relies on the tendency of biomolecules to adsorb onto various surfaces. We utilized this and developed a method of cross-linking albumin/heparin layer-by-layer (LbL) assemblies. The LbL assemblies enabled loading and release of basic fibroblast growth factor, leading to proliferation of surface adherent human endothelial cells (ASEP (ID 473967)). Similarly to the concept exploiting the physisorption of proteins, we exploite the ability of catechol based compounds to create surface confluent layers and thus to form LbL cyclic catechol-based multifunctional layers (ASEP (ID 470754)) and to create re-codable molecular printboards for highly specific host-guest interactions (ASEP (ID 447659)) (**Figure 7**).

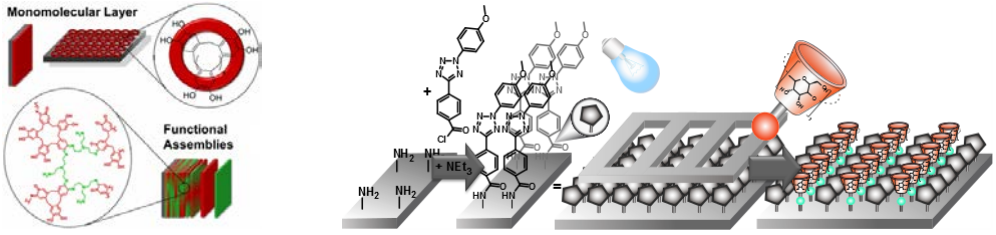


Figure 7. Layer-by-layer cyclic catechol based multifunctional layers (left) and the creation of re-codable molecular printboards for highly specific host guest interactions (right).

The knowledge obtained within the center of Biomacromolecular and Bioanalogous Systemsby its Tissue Engineering team on physico-chemical characterization of surfaces and materials was applied to unraveling the peculiarities in long-term stability in/of hybrid perovskite layers and solar cells. On the basis of XPS spectroscopy we have identified lead halide residue as a source of defects which impedes the efficacy of perovskite based solar cells ASEP (ID 520317).

Biosensing based on developed polymer architectures

The advantages provided by the controlled polymerization, the well-defined structure and chemical properties, the site-specific post-polymerization modification reactions for incorporation of various bioactive molecules and the high specificity of the developed surface modifications that were established within the work package ***Functional Polymer Surfaces*** were used directly for creation of biosensing platforms based on surface plasmon resonance (SPR) detection principles. The ability to withstand, and even completely cancel-out the fouling of the polymer brushes from complex media while bearing biorecognition elements was utilized for SPR detection of food-borne bacterial patogens in homogenized foods (such as hamburger, lettuce, cucumber and milk) (ASEP (ID 467359)), aflatoxin M1 (AFM1) in milk (ASEP (ID 460418)), hepatitis B antibodies in clinical saliva (ASEP (ID 472674)) and clinical serum samples (ASEP (ID 459400)). The developed affinity SPR biosensors could be regenerated and repeatedly utilized for further testing without loss of functionality, thus proving their effectiveness and potential in medical diagnostics.

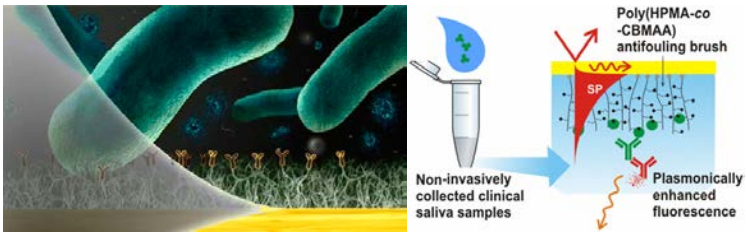


Figure 8. SPR detection and biosensing based on developed non-fouling polymer architectures.

Polymer Micro-/Nano-particles

Controlling the surface properties, architecture, chemical reactivity, non-fouling properties and physico-chemical characteristics in general, is also a crucial issue in the design and development of nanomedicine applications. A crucial segment of nanomedicine is represented by polymer micro- and nano-particles, which push further the versatility, efficacy, effectiveness and limits of detection originally established for biosensing that was based on transducer principles (such as SPR, quartz crystal-microbalance, optical waveguide, etc.) and in addition, give the opportunity for labeling, tracking, drug delivery, therapeutics, etc. The principles developed within the work-package of ***Functional Polymer Surfaces*** were directly implemented in the design of bioactive polymer architectures for micro- and nano-particles.

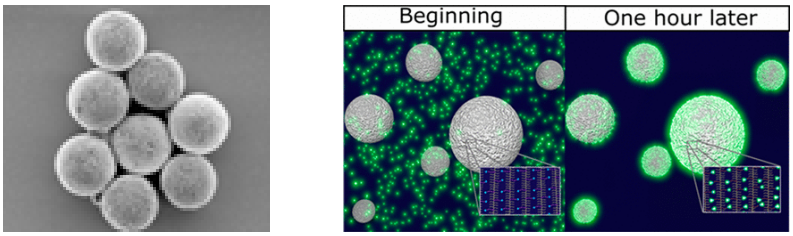


Figure 9. Developed monodisperse microspheres are efficient capturing and pre-concentrating platforms for detection of Alzheimer's disease, for scavenging lipopolysaccharides and for prevention of sepsis during hemofiltration.

A nanobiosensing platform based on porous magnetic microspheres (pMM) that are efficient in capturing and pre-concentration was developed for detection of Alzheimer's disease (AD) biomarkers. These pMMs prepared by a multistep swelling polymerization combined with iron oxide precipitation afford carboxyl functional groups suitable for immobilization of antibodies on the particle surface and thus allow an enhanced efficiency in the capturing of AD biomarkers from clinical serum samples (ASEP (ID 441435) , **Figure 9**). However, similarly to the developed planar biosensing platforms, the minimization of nonspecific protein adsorption is a crucial step in the development of bioactive polymer-based micro- and nano-particles. We prepared monodisperse poly(glycidyl methacrylate) (PGMA) microspheres by dispersion polymerization and rendered their surfaces biocompatible via reversible addition-fragmentation chain transfer (RAFT) polymerization of [3-(methacryloylamino) propyl]dimethyl(3-sulfopropyl)ammonium hydroxide (SBMA). The short PSBMA brushes all over the microsphere surface provided low-fouling (ASEP (ID 473652)). As in the case of the planar substrates, we utilized SET-LRP to synthesize P(HPMA-ran-CBMAA) copolymer brushes on microparticles. These non-fouling brushes were functionalized with polymyxin B to specifically scavenge lipopolysaccharides and thus prevent sepsis in hemofiltration (ASEP (ID 501316), **Figure 9**).

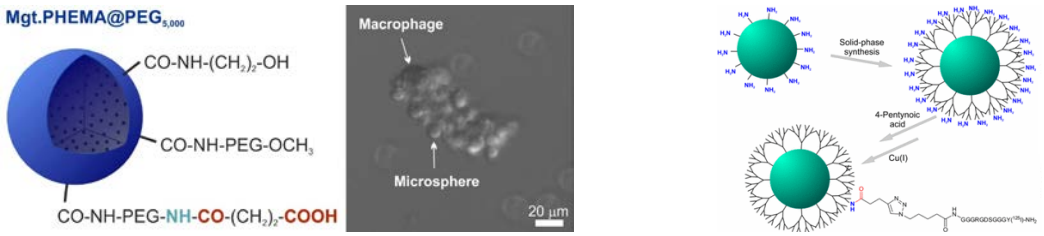


Figure 10. Various types of polymer-based magnetic micro-particles utilized in drug delivery systems and monitoring of the efficiency of phagocytosis (left). Antifouling and at the same time bioactive character of the surface of magnetic microparticles is attained by a controlled growth of 3rd level peptide dendrimers (right).

Various types of polymer-based micro-particles, composed mainly of poly(glycidyl methacrylate-co-ethylene dimethacrylate) [P(GMA-EDMA)], can be made magnetic by precipitation of maghemite. Thus introduced magnetic properties and the developed biomimetic peroxidize-like activity in the pH range between 4 and 6 at temperature ~37 °C, represent a highly sensitive sensor component that can be potentially useful in enzyme-based immunoassays (ASEP (ID 501381)). Biocompatible magnetic polymer microspheres with reactive functional groups that could withstand nonspecific protein adsorption from biological media were synthetized utilizing a magnetic poly(2-hydroxyethyl methacrylate) (mgt.PHEMA) core encapsulated by various shells consisting of anti-fouling PEO brushes of tunable chain lengths. The attained biocompatibility and inertness towards engulfing by macrophages (cells that commonly eliminate foreign microbodies appearing in organisms) of such mgt.PHEMA-PEO microparticles proved their suitability for future applications in drug-delivery systems and in monitoring of the phagocytosis efficiency (ASEP (ID 476589), **Figure 10**). The antifouling and at the same time bioactive character of the surface of magnetic microparticles was attained by a controlled growth of 3rd level peptide dendrimer generations composed of Ser-Lys-Ser/Lys-Ser/Lys-Ser by peptide chemistry methods. The introduction of bioactive peptide moieties was achieved by a highly selective azide–alkyne cycloaddition reaction utilizing ^{125}I-radiolabeled azidopentanoyl-GGGRGDSGGGY(^{125}I)-NH_2 peptide. We thus demonstrated, that the non-fouling bioactive dendrimer-modified particles are suitable for separation of peptides and other biomolecules, diagnostics, mimetics, and vaccine synthesis (ASEP (ID 471738), **Figure 10)**.

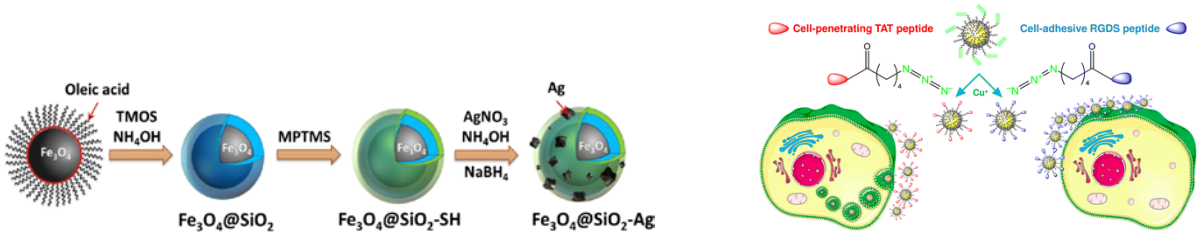


Figure 11. Coating of nanoparticles can be tuned to target bacterial infections (left) or monitor individual internalization steps and mechanisms of the NP uptake in tumor tissue (right).

An effective way of restraining, inhibiting and even cancelling out the invasion caused by pathogen bacteria such as Staphylococcus aureus and Escherichia coli was achieved by synthesizing monodisperse based Fe3O4@SiO2-Ag nanoparticles. The strong antibacterial properties of these core@shell inorganic nanoparticles makes them a strong candidate for targeting bacterial infections and for disinfection of medical instruments, water purification and food packaging (ASEP (ID 507831), **Figure 11**).

The LbL concepts can be introduced to magnetic nanoparticles to guide the interaction with tumor cells by molecular mechanisms, thus mimicking the infection machinery of certain viruses and potentially precisely targeting the tumor tissue. The resulting magnetic PLL-glycoconjugates facilitated the NP uptake in human glioma and HeLa cells enabled monitoring

of the individual internalization steps and mechanisms of the uptake (ASEP (ID 488486), **Figure 11**).

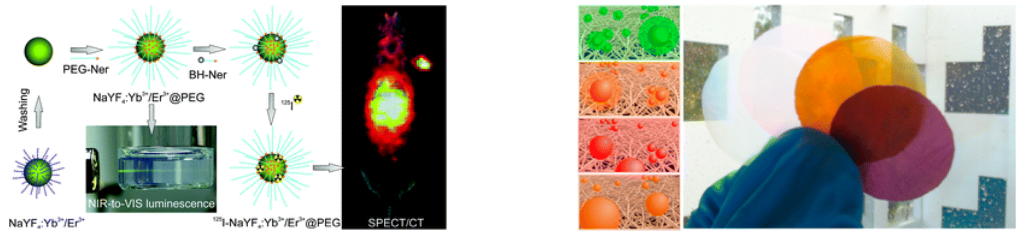


Figure 12. Upconverting nanocrystals – from basic physicochemical studies to near-infrared photothermal/photodynamic and single-photon emission computed tomography cancer theranostics (left) and platforms combining nanopaper (right).

Upconverting nanocrystals (UCNC) have recently been subjected to intensive investigation due to their interesting optical properties and high potential for practical applications. Despite the attention paid to these materials, their very low quantum yield is still an important issue. In order to break through this limitation, understanding of the emission intensity is crucial. Therefore, we investigated the influence of percolation phenomena on the limitation of the emission intensity from $NaYF^4:Yb^{3+},Er^{3+}$ nanocrystals. We proposed a numerical model and supported it experimentally at the single nanocrystal level, explaining the influence of Yb^{3+} concentration on the optical properties of UCNC (**Figure 12**). Moreover, based on the experimental and numerical results, we clarified the existence of the optimal Yb^{3+} concentration in the core architecture (ASEP (ID 496957)). Based on these initial findings we synthesized monodisperse upconversion $NaYF^4:Yb^{3+},Er^{3+}$ NP's. An anti-fouling shell was achieved via "grafting to" reactions utilizing PEO chains bearing neridronate groups. In vitro cytotoxicity and biodistribution in living organisms have proven that UCNP's are prospective agents for near-infrared photothermal/photodynamic and single-photon emission computed tomography/computed tomography cancer theranostics (ASEP (ID 481234), **Figure 12)**. The decoration of the surface of upconversion $NaYF^4:Yb^{3+},Er^{3+}$ NP's with the cell adhesive and cell penetrating azidopentanoyl-GGGRGDSGGGY-NH2 (RGDS) and azidopentanoyl-GGGRKKRRQRRR-NH2 (TAT) via Cu(I)-catalyzed alkyne–azide cycloaddition enabled the selective targeting and imaging of the specific tumor phenotypes (ASEP (ID 461624)). The developed upconversion $NaYF^4:Yb^{3+},Er^{3+}$ NP's were utilized as sensing platforms based on nanopaper (ASEP (ID 446153), **Figure 12**) and single-molecule (digital) immunoassays. The UCNPs provided sub-femtomolar concentration range (LOD: 23 fg/mL, 800 aM) and showed excellent correlation with more traditional electrochemiluminescence methods (ASEP (ID 507894)).

The multidisciplinary knowledge obtained within sub-center Tissue Engineering of center Biomacromolecular and Bioanalogous Systems that is spanning polymer chemistry, particle synthesis and formulation of targeting and delivery systems was utilized in design of NP's, which were used for suppression of the immune response of non-human primates and adult pigs (ASEP (ID 519437), ASEP (ID 522159)).

Tissue Engineering 3D Scaffolds

The work on the biomaterial surfaces and models provided the knowledge basis for the implementation of the developed concepts in the design, optimization and application of various 3D scaffolds in-for tissue engineering.

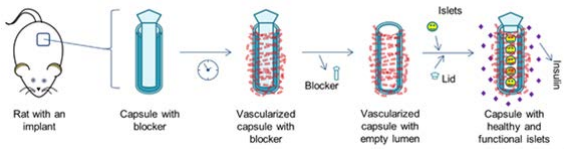


Figure 13. Heparin/pro-angiogenic growth factors route for encapsulation of living Langer islets.

Micro-resolved 3D mesostructures were fabricated by employing a direct laser writing to a novel functional photoresist based on radical coupling reaction of thiols and alkynes. Post-modification reactions based on thiol-Michael addition and copper-catalyzed azide alkyne cycloaddition that employed residual thiols and alkynes rendered the surface of the mesostructures bioactive. The necessary bioactivity of poly(lactide-co-caprolactone) pouch was introduced through the previously established LbL approaches (stated in work-package ***Functional Polymer Surfaces****), which are based on heparin and* pro-angiogenic factor, thus a pre-vascularized cavity is bioengineered (ASEP (ID 522100), **Figure 13**). Assemblies of thin film silk fibroin hydrogels and porous foams were optimized for the encapsulation of living Langer islets, various cells and/or for the controlled release of both hydrophilic and hydrophobic drugs (ASEP (ID 457077)). Building on previous experience of planar substrates, we used robust polydopamine coatings for anchoring of antifouling polymer brushes on the surface of biodegradable poly(ε-caprolactone) nanofibers (ASEP (ID 454747)). The antifouling brushes managed to withstand the adhesion of mouse embryonic fibroblasts thus paving the way towards bioactive nanofiber 3D materials without the need of surface activation enabling thus the preservation of the material's mechanical properties. Similarly, thin polymer-brush/chitosan hydrogels were synthesized as a route for attaining better hemocompatibility of implant coatings (ASEP (ID 475191)).

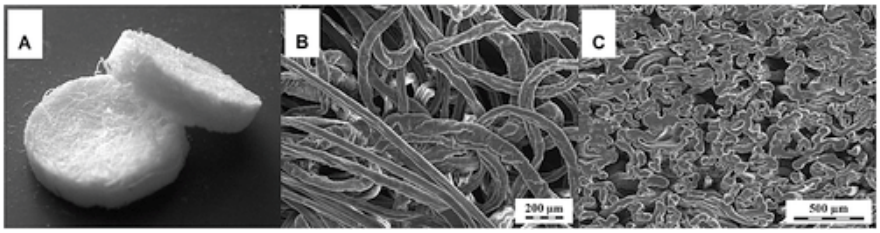


Figure 14. Poly(gamma-benzyl-l-glutamate) (PBLG) fibrous scaffolds bearing bioresponsive RGD peptide sequence.

We performed bioactive fibrous scaffolds by the bioresponsive RGD peptide sequence on the surface of poly(gamma-benzyl-l-glutamate) (PBLG) mesh with defined pore size. The optimized bioactivity of the fibrous scaffold, in terms of precisely controlled peptide surface concentration, induced the differentiation of human tooth germ stem cells (HTGSCs) into chondrocytes (ASEP (ID 472812), **Figure 14**).

The engineering of soft tissues demands high porosity, high pore interconnection and high swelling ratios. These prerequisites are fulfilled by covalently cross-linked gels with permanent pores. These gels were formed under cryogenic conditions by free-radical copolymerization of poly[N5-(2-hydroxyethyl)-l-glutamine-stat-N5-(2-methacryloyl-oxy-ethyl)-l-glutamine] (PHEG-MA) with HEMA and, optionally, N-propargyl acrylamide (PrAAm) as minor comonomers. The cryogels can be designed as enzymatically degradable and can further be biodiversified by post-polymerization reactions for the incorporation of biomimetic peptides and at the same time form biofunctional and biodegradable gels (ASEP (ID 450082), ASEP (ID 483972), **Figure 15**).

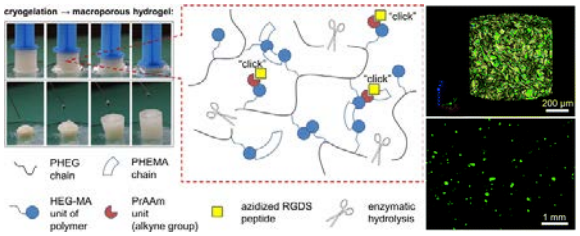


Figure 15. Enzymatically degradable biofunctional cryogels.

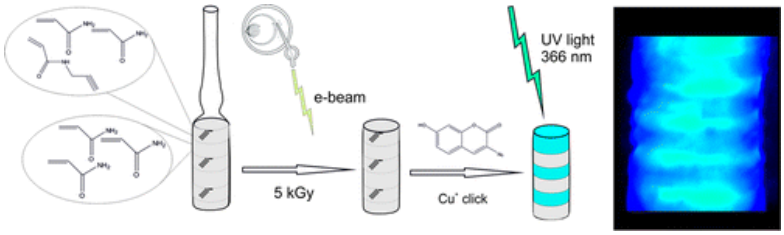


Figure 16. Stratification and tailoring of cryogel properties.

While the cryogelation process offers the possibility for the creation of porous materials of high chemical, mechanical and structural uniformity, it lacks the possibility to tailor these properties. The ability to tailor mechanical properties and architecture is crucial in creating macroporous hydrogel scaffolds for tissue engineering. We achieved this via regulation of the dose of irradiation by an electron beam and we succeeded in precise modification of the pore size and stiffness of acrylamide-based cryogels bearing biomimetic peptide sequences. We envisage the approach will be the key to the future preparation of complex structured hydrogel-based scaffolds to mimic the extracellular microenvironment in a wide range of applications (ASEP (ID 443231)).

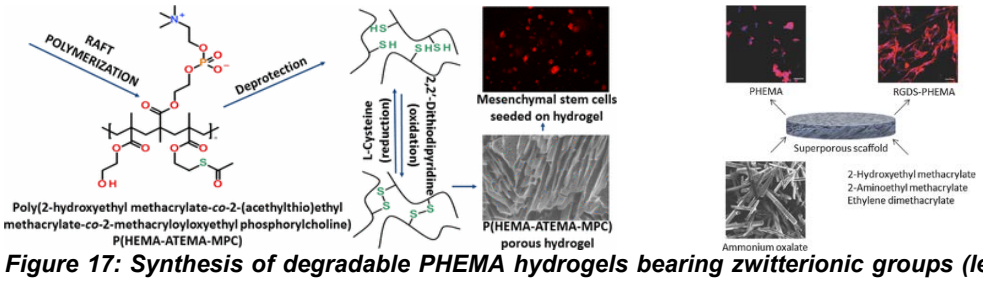


Figure 17: Synthesis of degradable PHEMA hydrogels bearing zwitterionic groups (left) and bioactive peptide sequences of RGDS and SIKVAVS (right). The bioactive hydrogels support the proliferation of human mesenchymal stem cells and human fetal neural stem cells.

Additional control of pore orientation can be obtained by utilization of various porogens, such as needle-like sodium acetate or ammonium oxalate crystals. Degradable PHEMA hydrogels were prepared from a linear copolymer of HEMA and 2-(acethylthio)ethyl methacrylate (ATEMA), zwitterionic 2-methacryloyloxyethyl phosphorylcholine or 2-aminoethyl methacrylate (AEMA). The obtained hydrogels were evaluated as a possible support for human mesenchymal stem cells (MSCs). The presence of amino group of AEMA was used to immobilize RGDS and SIKVAVS bioactive peptide sequences to boost the adhesion and proliferation of human mesenchymal stem cells and human fetal neural stem cells (ASEP (ID 473652), ASEP (ID 465604), **Figure 17**).

Nondegradable monolithic polymer gels can be utilized as permanent multifunctional implants which mimic the original function of the lost organ or tissue without the need for activation of the self-regeneration tendency of the human organism. A mimetic of human vocal cord was designed on the basis of continuous and non-porous PHEMA gel. The method developed for tuning the vocal cord substitution as described in the invention is based on/lies in the fact, that the basic frequency of the human vocal fold is determined therefore, an initial prestressing of the artificial vocal fold is created by setting-up a distance of setting frames and enclosing the artificial vocal folds. Ultimately, we achieved to attain similarity of vibration characteristics of the artificial and human vocal cord (ASEP (ID 460330)).

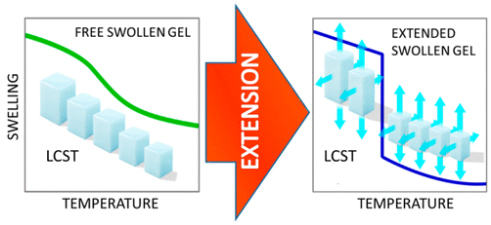


Figure 18. Theoretical studies on volume phase transitions in polymer gels.

Further, we theoretically approached the phenomena occurring in gel formation and we studied the possibility of induction of volume phase transitions in various polymer gels by external stress aiming thus to optimize their responsiveness to stimuli, to strengthen their response, and to model their properties (ASEP (ID 502281), **Figure 18**).

To summarize, within the past period, the **BIOMOL-Tissue Engineering team published over 200 impacted papers and filed 8 patent applications**, from which 54 were selected here for the evaluation. We aimed to present the high-quality outputs in recognized international peer-reviewed journals. Generally, the team members are inclined to publish **complete scientific results in highly impacted journals**, instead of dividing them into several papers with partial results. Thus, **the quality and soundness are strongly preferred over quantity**.

Research activity and characterization of the main scientific results

Within the past period, the **BIOMOL-Therapeutics team published 179 papers** and contributions **and filed 7 patent applications**, from which 50 are selected here for evaluation. We aimed to present our high-quality data in recognized international peer-reviewed journals. Generally, the team members preferred to publish **complete scientific results in highly impacted journals**, instead of partitioning them into several papers. Thus, **the quality was strongly preferred over quantity**. The following overview of the main results is organized in sections – each representing the tasks solved on a long-term basis in the center BIOMOL, the sections are identified here by bold headings. References to publications of results are given by their respective ASEP number, which links directly to full information on-line. In some aspects, the division of the results of the center BIOMOL to therapeutics and tissue engineering is not unambiguous and accurate.
In general, the results can be divided into three main research areas.

1. Water-soluble and micellar systems for drug delivery and vaccination

The first research area is dealing with the application of water-soluble and micellar polymer systems within biomedical research, toward the development of **nanomedicines for anti-tumor therapy or vaccination or intended as nano-diagnostics**. The field of anti-tumor nanomedicines is highly competitive, progressive and developing rapidly, thus to provide the state-of-art in this field, we published a highly comprehensive review of the knowledge in the drug delivery field ASEP (ID 459357). Indeed, nanomedicines represent a fresh impetus in the fight against cancer due to their selectivity and effectivity. To emphasize a particular task within this research area, the results are further divided into three sub-sections focusing on **novel nanomedicines, on targeting and theranostics, and on gene delivery and vaccines**.

1.1 Novel nanomedicines

Novel structures

In the past period, we have thoroughly investigated the relationship between polymer structure and its suitability as DDS with the emphasis on simple linear, or advanced biodegradable di-block and star-shaped polymer systems. While the molecular weight of linear polymer precursors is below the limit of renal threshold, which limits their long-term circulation in the body, the molecular weight of advanced systems is above this limit enabling their prolonged blood clearance as well as the removal of the system from the body after releasing its cargo in a tumor. We have developed **advanced process for their simple and robust synthesis** [ASEP (ID 494320)]. This new approach, Fig.1, significantly reduces the number of required synthetic steps. Within the synthesis employing the **controlled Reversible Addition Fragmentation Chain Transfer (RAFT) polymerization**, we also developed a new procedure for the selective deprotection of *tert*-butoxycarbonyl group-protected hydrazide and amine groups on hydrophilic *N*-(2-hydroxypropyl)methacrylamide based copolymers (pHPMA), including the one-pot removal of polymer end groups.

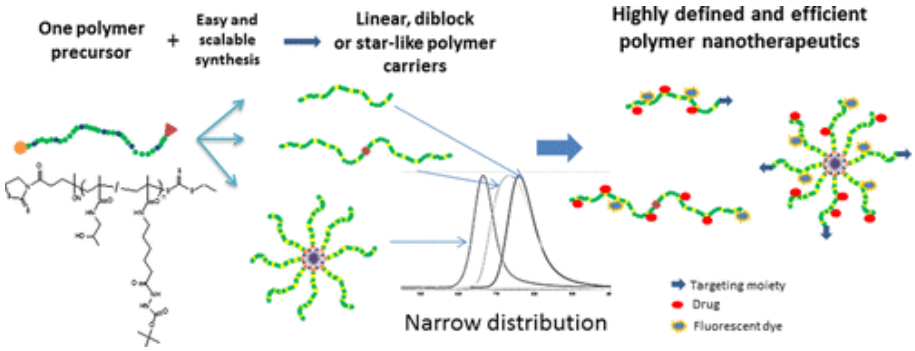


Fig. 1. Schematic description of new synthetic approach for advanced nanomedicines

Moreover, the following investigation enabled us to determine **structure-dependent toxicity, pharmacokinetics, and anti-tumor activity of developed pHPMA nanomedicines** in the treatment of solid tumors and leukemias. The star-shaped nanomedicines showed almost four times lower maximal tolerated dose (MTD), which was compensated by more pronounced anti-tumor activity. We postulated **clear benefit of star nanomedicines** in the treatment of dissipated leukemias, whereas the treatment of solid tumor was comparable for all nanomedicines during equitoxic dosing ASEP (ID 463915).

Di-block and simple linear nanomedicines containing pirarubicin linked via pH-sensitive spacer were thoroughly compared regarding the tumor accumulation, toxicity, and therapeutic effect. The biodegradable di-block conjugate showed both better tumor delivery and prolonged plasma half-life. In addition, **the di-block conjugate had better antitumor effect** than the linear conjugate **without any apparent toxicity** in S180 tumor model ASEP (ID 475079). In a similar way, the linear and star-like pHPMA nanomedicine containing pirarubicin was compared for pharmacokinetics and antitumor activity in tumor-bearing mice ASEP (ID 442367). Because of the larger size, the tumor AUC 5 h – 72 h of star conjugate was 3.3 times higher than that of linear. More importantly, released free pirarubicin was retained selectively in the tumor tissue for at least up to 72 h after administration of the star polymer conjugate. We found that **the star nanomedicine exhibited a superior antitumor effect to linear one** in tumor-bearing mice, most probably due to their different molecular size. In our comparative study of *in vitro* and *in vivo* behavior of star and linear nanomedicines, we concluded that the star conjugate exhibited enhanced therapeutic efficacy.

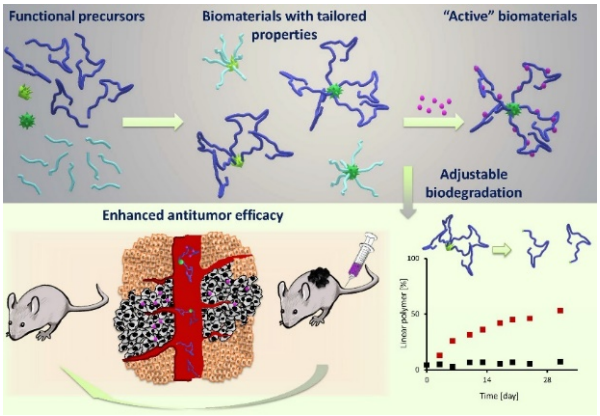


Fig. 2. Schematic description of new synthetic approach for advanced biodegradable star nanomedicines with tunable structure and properties.

To obtain **star nanomedicines with controlled structure,** a novel RAFT polymerization technique was employed. The water-soluble star polymer carriers were prepared by the grafting of poly(amidoamine) dendrimers by hetero-telechelic pHPMA. The well-defined star copolymers showing low dispersity below 1.2 were obtained and model anticancer drug, doxorubicin, was bound to the star polymer through a linkage enabling pH-controlled drug activation in the tumor. Moreover, the ends of polymer arms of the star copolymer carrier enabled a one-point attachment of targeting ligands and/or a labeling moiety. The tailor-made structure of the star polymers **should facilitate the synthesis of targeted nanomedicines**, even polymer theranostics, for simultaneous tumor drug delivery and imaging. ASEP (ID 444687). Additionally, controlled synthesis, physico-chemical and biological characteristics of novel **biodegradable star-shaped copolymers** intended for advanced drug delivery was described. These biocompatible star copolymers were synthesized by grafting monodispersed semitelechelic linear pHPMA onto 2,2-bis(hydroxymethyl)propionic acid (bisMPA) polyester dendritic cores, Fig 2. The **size of developed star copolymers could be tuned to a large extent** and could be adjusted to a given purpose by proper selection of dendritic core type and generation and by considering the copolymer size and polymer-to-core molar ratio. The hydrolytic degradation of the star copolymers was proven to show spontaneous hydrolysis. Finally, it was shown that the therapy with the biodegradable star conjugate with attached doxorubicin strongly suppresses the tumor growth in mice and is fully curative in most of the

treated animals at a dose corresponding approximately to one-fourth of MTD value. They **showed superior efficacy and tumor accumulation** over the previously prepared star nanomedicines ASEP (ID 522066).

Polymer micelles

Polymer micelles are important DDS showing high passive accumulation in solid tumors due to their supramolecular structure formed by self-assembly of amphiphilic copolymers. Often, the sampling of micelles for application is complicated, thus we focused on the synthesis of **thermoresponsive amphiphilic diblock conjugates** with cancerostatic drug pirarubicin. Here, the polymer drug conjugate was fully soluble in PBS buffer at room temperature, but formed micelles spontaneously at body temperature, thus it facilitated sample application. All studied conjugates demonstrated the ability to efficiently penetrate the cell membrane and release a drug in the intracellular environment of model cancer cell lines. It was demonstrated, that the **micelle-forming conjugates have a great potential to become efficacious *in vivo* pharmaceuticals** ASEP (ID 447192). Another drawback consists of often undesired accumulation of amphiphilic polymer carriers in various organs, e.g. liver. Thus, we focused on preparation of **biodegradable micellar DDS enabling a disintegration of the supramolecular micellar structure** after the release of drug cargo in tumor tissue and facilitating the removal of carrier from the body. We synthesized micelle-forming polymer conjugates with a biologically active derivative of betulinic acid and proved micelles disintegration after drug release ASEP (ID 465442). Also, we compared various doxorubicin-containing polymer conjugates bearing highly hydrophobic cholesterol-derived compound bound by pH-sensitive spacers that differed in the rate of hydrolysis, Fig. 3. We described not only the micelles disintegration, but we also proved, that **the blood-stream stability of micellar polymer-drug conjugates is important for their efficient solid tumor accumulation and high anti-tumor activity** ASEP (ID 492820).

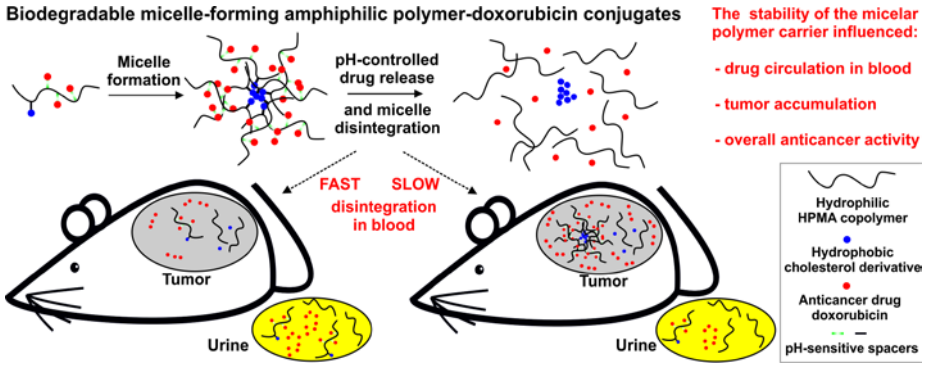


Fig. 3. Schematic description of the influence of the micellar stability on its efficacy.

Novel drug-containing nanomedicines

We have developed novel **polymer nanomedicines containing anti-cancer drug pirarubicin** and extensively evaluated its potential during the anti-cancer treatment. The structure of the polymer nanomedicine led to a pronounced cellular uptake of pirarubicin versus that of other commercial anthracyclines ASEP (ID 466564), Fig. 4. Moreover, **superior penetration and cytotoxicity** of these pHPMA-pirarubicin nanomedicines **in tumor cell spheroid** ASEP (ID 507279) was observed which further demonstrated the efficacy of this polymeric drug. Indeed, enhanced efficacy of the pHPMA-pirarubicin nanomedicine was proved also *in vivo* on animal models and in human compassionate use, see below.
Similarly, we have thoroughly investigated novel star polymer nanomedicines carrying highly potent and commercially exploitable anti-cancer drug docetaxel ASEP (ID 439488). The **star polymer conjugates with docetaxel** attached via tumor stimuli-sensitive linkages were **designed for the improved biodistribution and enhanced tumor accumulation** which led to high anti-tumor activity and low systemic toxicity. Such behavior enabled us to investigate

the structure-to-treatment efficacy relationship of the combination treatment of advanced tumors with nanomedicines carrying doxorubicin and docetaxel both bound to the carrier via stimuli sensitive spacers ASEP (ID 473875).

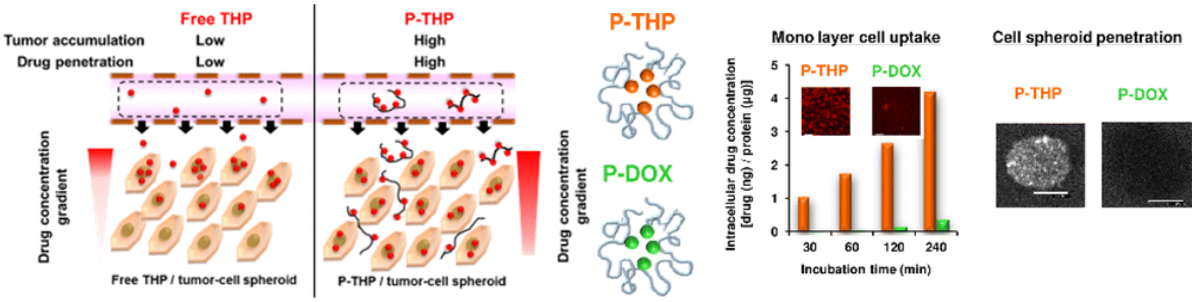


Fig. 4. Superior anti-cancer efficacy of the developed polymer-pirarubicin nanomedicines

Polymer nanomedicines to overcome the multi-drug resistance

Multidrug resistance (MDR) is a common cause of failure in chemotherapy for malignant diseases. The resistance of cancer cells to current chemotherapy is associated with the overexpression of at least two ATP-dependent efflux pumps, P-glycoprotein (P-gp; MDR1) and multidrug resistance-associated protein 1 (MRP1), both belonging to a superfamily of ATP-binding cassette (ABC) transporters. **Two different approaches were employed to target the MDR inhibition** – **either polymer inhibitor** was investigated, or a **delivery system for small molecule MDR inhibitor** was introduced. Tumor-targeted micelle-forming amphiphilic block copolymers based on polypropylene glycol (PPG)-co-pHPMA were designed and synthesized ASEP (ID 466229). The block copolymers take advantage of the micellar structure, which enables enormous tumor accumulation, and consequent inhibition of MDR driven by the structure and by the delivery of the payload. Moreover, the structure-to-MDR activity relationship was studied and the design of the di-block polymer was optimized, ASEP (ID 510951), to obtain both high inhibition activity and cytostatic activity, Fig 5. Interestingly, the polymer micelles were loaded with 5 – 40 wt.% of free PPG, which showed increased P-gp inhibition in comparison to the unloaded micelles and increased the inhibition potency of the systems.

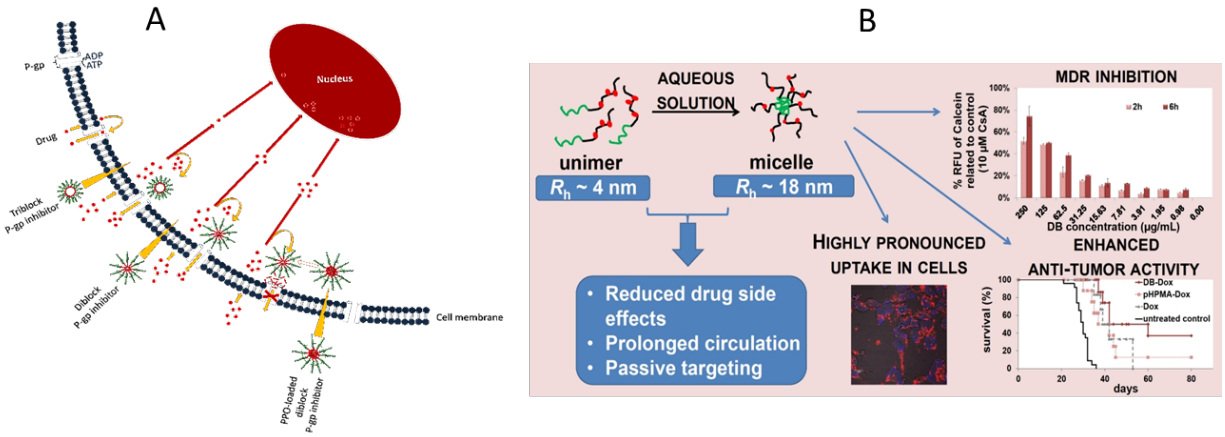


Fig. 5. Schematic description of the structure-to-MDR inhibition relationship (A) of PPG-pHPMA diblock copolymer systems and MDR activity (B).

Secondly, the DDS containing derivatives of low molecular weight MDR inhibitor ritonavir or reversin 205 were synthesized and evaluated for their MDR-inhibition potency. pHPMA nanomedicine bearing the 5-methyl-4-oxohexanoic acid ritonavir ester or reversin 205 showed profound optimized release kinetics of both inhibitors and were employed for the treatment of various MDR cell lines *in vitro* or *in vivo*, using either lymphoma and colorectal ASEP (ID 473557) or neuroblastoma cell lines ASEP (ID 459478). We documented the beneficial effects of P-gp inhibitors in combination with anthracyclines using various methods, e.g. microscopy, flow cytometry, or cell cytotoxicity assay. These nanomedicines were able to enhance the treatment efficacy of resistant lymphoma and colorectal solid tumors *in vivo.* **Nanomedicines with ritonavir derivative exhibited cell-penetrating properties** as well as enhanced co-localization in mitochondria **enabling intracellular targeting of drugs** ASEP (ID 466223).

Thorough study of developed nanomedicines with biological system

Although new and new DDS are still being presented, the study of their behavior in body fluids is generally underestimated. **Undesired interaction with plasma proteins can limit the use of such DDS** based on the opsonization and removal by RES. An experimental verification of such interaction is a crucial parameter, nevertheless, its determination is not trivial. In collaboration with physicists abroad, we proved, using polymer-carriers-bearing probes for electron paramagnetic resonance (ESR), that our **amphiphilic pHPMA-based nanoparticles did not possess hard protein corona**, Fig. 6., ASEP (ID 488750). Also, synchrotron small-angle X-ray scattering (SAXS) and isothermal titration calorimetry (ITC) were employed to investigate the morphology of nanoparticles with or without doxorubicin and their structural changes due to interactions with human serum albumin ASEP (ID 486438). Based on our experimental data, **we proposed a general rule for the future design of polymer drug delivery systems**: By using a small amount of a highly hydrophobic component along with a high fraction of the hydrophilic component, the latter can prevent the undesirable interactions between the drug-delivering nanoparticles and the blood proteins. The excellent biocompatibility of developed amphiphilic copolymers was unequivocally proven.

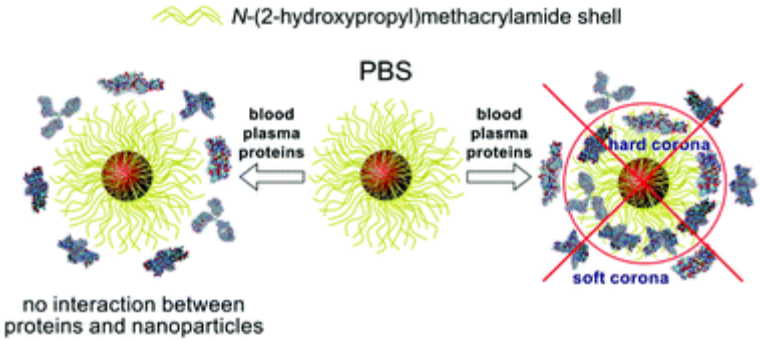


Fig. 6. Possible interaction of DDS with blood plasma proteins.

In conclusion, various novel polymer biomaterials were developed for highly effective drug delivery. They comprised advanced properties that were based on the simultaneous inhibition of MDR, excellent biocompatibility, controlled activation using tumor-associated-stimuli, the formation of supramolecular structures, and tailored polymer architectures.

1.2 Targeting and theranostics

The EPR-effect augmentation

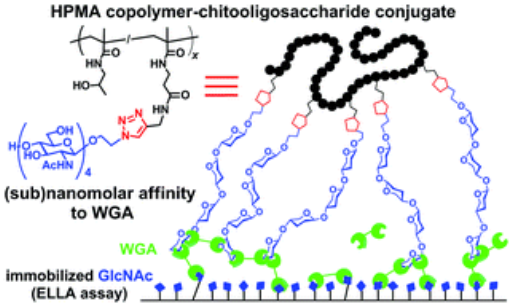
Polymer nanomedicines intended for the treatment of solid tumors are designed to be accumulated in the tumor via the Enhanced Permeation and Retention (EPR) Effect. The EPR effect is highly pronounced in the rapidly growing mice tumor models, nevertheless, the naturally occurring human tumors show a less pronounced effect based on the occluded or embolized tumor blood vessels. Thus, **we aimed to augment the EPR effect to improve tumor accumulation of nanomedicine**. We exploited vascular mediators, nitric oxide (NO) generators nitroglycerin, hydroxyurea, and L-arginine. Use of different nanosized drugs significantly increased delivery of nanomedicines (1.5–2 times) to different solid tumor models, as well as markedly improved antitumor activity (two- to three-fold), was proven. Moreover, in a murine autochthonous azoxymethane-induced colon cancer model, NO donors markedly improved the therapeutic effects of the linear polymer conjugate with pirarubicin even after one injection. These findings strongly suggest the potential **usefulness of NO donors as the EPR effect enhancers to improve the therapeutic efficacy of nanomedicines** ASEP (ID 497718). NO-donor based enhancement of the EPR effect was applied also during the human compassionate treatment, ASEP (ID 456866), where the NO-donor effect was exploited in the therapy of metastatic prostate cancer using polymer-pirarubicin nanomedicine.

Similarly, we focused on another vascular mediator involved in the EPR effect, carbon monoxide (CO). Two CO-generating agents were utilized to generate CO selectively in solid tumors, which resulted in the increase of the EPR effect and a two- to three-fold increased tumor accumulation of nanomedicines. **Consequently, the combination of CO generators with anticancer nanomedicines resulted in an increased anticancer effect in the different transplanted solid tumor models** ASEP (ID 506415).

To further increase the EPR effect during the treatment, we have designed and developed **polymeric NO donors, which were able to potentiate the treatment of experimental solid tumors** by increasing drug accumulation in the tumor tissue ASEP (ID 481666). A significant increase in accumulation was achieved by the binding of the chemical precursor of NO, based on an organic nitrate, to a series of pHPMA. The polymer NO donors were shown to overcome the drawbacks related to low-molecular-weight NO-releasing compounds, namely systemic toxicity, lack of site-specificity, and fast blood clearance.

Actively targeted polymer drug delivery systems, drug-free therapeutics

The recognition of highly specific interactions between DDS and target antigens on treated cells is highly important issue within the field of active targeting of DDS. Besides antibodies, as highly complex targeting units, also other targeting ligands, antibody fragments, oligosaccharides and oligopeptides, are under development with the aim to find a suitable replacement for antibodies. We successfully developed **tailored linking strategy of the recombinant single chain fragment** (scFv) of GD2 antibody to the polymer conjugate via a noncovalent specific interaction, employing bungarotoxin - bungarotoxin binding peptide interaction, preventing the loss of activity of the scFv fragment. We proved the suitability of the conjugation method for the design of highly therapeutically active delivery systems ASEP (ID 499714). Further on, we investigated the potential of oligosaccharides for the nanomedicine targeting to the lectin antigens. First, the proof of principle was verified using tailored hydrophilic glycopolymers with chitooligosaccharides distributed along the polymer chain that demonstrated excellent affinity to wheat germ agglutinin, Fig. 7. The **binding affinities in the low nanomolar and subnanomolar** ranges place the prepared glycoconjugates among the best known wheat germ agglutinin ligands ASEP (ID 474869). Further on, we moved to therapeutically interesting target – human lectin galectin-3 connected with tumor progression in the body. We observed and clarified the relation between the affinity of hydrophilic polymers decorated by glycan ligand to galectin-3 and the polymer carrier structure, i.e. glycan



distribution and content or linker between polymer and glycan. We proved **excellent affinity of such polymers to galectin-3 in nanomolar scale** ASEP (ID 496863). Last but not least, we have observed additional feature of the developed glycoconjugates.

Fig. 7. Schematic description of the interaction between glycopolymers and wheat germ agglutinin.

Polymer theranostics

Simultaneous treatment and diagnostic observation are required for the future active nanomedicines. Observation of the treatment efficacy during the treatment would give oncologists real information about the therapy outcome. Moreover, proper pre-diagnostics of the nanomedicine accumulation effectiveness could be also determined by the novel platform described below. The combination of fluorescence imaging, magnetic resonance imaging, or positron emission tomography (PET) using polymer nanomedicines containing both the active therapeutic molecule and diagnostic label enables the real-time monitoring of the treatment outcome ASEP (ID 456934). We described a **novel polymer platform suitable for efficient diagnostics and potential theranostics** based on ^{89}Zr-, or fluorescently labeled pHPMA conjugates. Moreover, the feasibility of two imaging techniques, fluorescence imaging, and PET, was compared using labeled polymer conjugates. Both methods gave comparable results, thus showing the enhanced diagnostic potential of the prepared polymer–dye or polymer–chelator–^{89}Zr constructs. Therefore, pHPMA with low dispersity and a molecular weight near the limit of renal filtration can be used as highly efficient polymer carrier of tumor-targeted therapeutics or diagnostics with minimal side effects ASEP (ID 476747). The theranostic potential of the developed platform was afterward confirmed during simultaneous patient-derived xenografts (PDX) anti-lymphoma treatment and non-invasive imaging using polymer-doxorubicin-fluorescent dye nanomedicine, long-circulating systems with reduced side effects, Fig. 8. Polymer theranostics were designed as stimuli-sensitive systems in which the anti-cancer drug doxorubicin was bound to the hydrophilic copolymers via tumor pH-activatable hydrazone linkage. We repeatably proved the significantly enhanced anti-lymphoma efficacy of the polymer-doxorubicin conjugates when compared to equally toxic doses of conventional free or liposomal doxorubicin. Favorable pharmacokinetics for carried drug and labeled polymer carrier was observed, showing predominant uptake of the drug and polymer itself by the tumor mass. Importantly, **we observed a promising diagnostic potential of fluorescently labeled polymer prodrugs.** Dynamically analyzed fluorescence intensity over subcutaneously xenografted lymphomas closely corresponded to changes in the lymphoma tumor volumes, thereby enabling a non-invasive assessment of treatment ASEP (ID 493938).

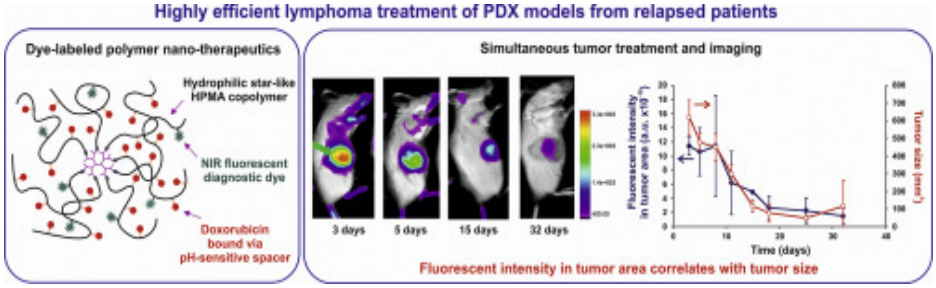


Fig. 8. Theranostics feature of polymer-doxorubicin-fluorescent dye nanosystem in the treatment of PDX.

Antibody mimetics

Monoclonal antibodies are indispensable tools for biomedicine and anticancer therapy, they are also thoroughly studied as possible targeting units within the field of nanomedicine. Nevertheless, their use is compromised by high production costs, limited stability, and difficulty chemical modification. We **designed and developed synthetic polymer constructs capable of replacing antibodies** in biomedical applications such as ELISA, flow cytometry and immunocytochemistry.

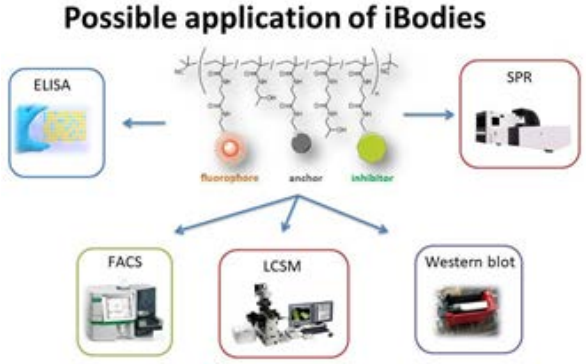


Fig. 9. Possible application of the developed polymer construct iBodies.

The constructs, named "iBodies", consist of the HPMA copolymer decorated with low-molecular-weight compounds that function as targeting ligands, affinity anchors, and imaging probes. We developed specific conjugates targeting several proteins with known ligands and **used these iBodies for enzyme inhibition, protein isolation, immobilization, quantification, and live-cell imaging**, Fig. 9. Our data indicate that this highly modular and versatile polymer system can be used to produce inexpensive and stable antibody substitutes directed toward virtually any protein of interest with a known ligand ASEP (ID 458826).

Based on the huge marketing applicability, we filed and **invention, which provides a synthetic macromolecular conjugate** for selective interaction with proteins, comprising a synthetic copolymer, a minimum of one binding group, one further group selected from an affinity tag and an imaging probe. This macromolecular construct protected by a patent is suitable particularly for isolation, immobilization, and visualization of proteins ASEP (ID 518008). Similarly, we patented an invention that describes iBodies construct intended for visualization and separation of proteins and/or cells ASEP (ID 518012).

In conclusion, we designed and synthesized novel polymer constructs showing the ability to serve as targeted DDS or antibody mimetics, and thus employing their potential in drug delivery and biotechnology. Even more, the drug-free nature of glycopolymers showed a high potential for future exploitation.

1.3 Gene delivery and vaccines

Gene delivery vectors

Besides low molecular weight drugs, we aimed in the past period also for drug delivery of the genes. We reported novel **pH-reversibly surface-shielded polyplexes** with enhanced gene transfer activity upon systemic administration. A four-arm-structured sequence-defined cationic oligomer was designed and synthesized on solid-phase, containing additional lysine residues not only for improved pDNA polyplex stability but also for providing attachment points for subsequent polyplex functionalization with amine-reactive shielding hydrophilic polymers, PEG or pHPMA, enabling shielding of the surface of polyplexes. *In vitro* transfection studies revealed higher gene expression by the polyplexes with the acid-labile shield as compared to their irreversibly shielded counterparts. *In vivo* intravenous **administration of pHPMA-modified polyplexes in a tumor mouse model mediated enhanced gene expression** in the subcutaneous tumor and reduced undesirable expression in the liver ASEP (ID 460354).

However, these agents are limited when delivered intravenously due to their rapid clearance from the bloodstream and poor passage from the bloodstream into target tumors. We described a **novel stealthing strategy** which addresses both these limitations and we thereby demonstrated, that both the passive and mechanically-mediated tumor accumulation of the model nanomedicine adenovirus (Ad) can be substantially enhanced. We described a novel **hybrid nanomedicine based on an adenovirus vector coated with Au nanoparticles modified with PEG** for delivery of genetic material to tumors. In addition, we showed its accumulation by the tumor can be substantially enhanced by ultrasound. We were able to conclude that our stealthing strategy, in combination with mechanically-mediated tumor accumulation, could enhance the clinical utility of intravenously delivered therapeutic genes to the target tissue. This stealthing and density-increasing technology could ultimately enhance clinical utility of intravenously delivered nanomedicines including viruses and liposomes, ASEP (ID 444229).

Polymer vaccines

Although vaccines that mediate protection through antibodies are in routine clinical use, vaccines that generate robust and durable T-cell immunity are still needed for protection against certain infections, e.g. tuberculosis, and as therapies for cancer. One means of improving T-cell immunity by vaccines is through rational choice of adjuvants formulated with defined protein or peptide antigens. Some of the most effective adjuvants for promoting T-cell immunity are Toll-like receptor agonists (TLRa), which stimulate distinct populations of antigen-presenting cells (APCs) to present antigen, express co-stimulatory molecules and produce selective cytokines that drive T-cell responses. We investigated how **delivery of low-molecular-weight adjuvants TLR-7/8a on biocompatible polymer scaffolds** of different size, structure and composition **can modulate innate immune activation *in vivo* to enhance T-cell immunity to protein antigens**. We showed, that particle formation by polymer—TLR-7/8a was the most important factor for restricting adjuvant distribution and prolonging activity in draining lymph nodes. The improved pharmacokinetic profile by particulate polymer—TLR-7/8a was also associated with reduced morbidity and enhanced vaccine immunogenicity for inducing antibodies and T-cell immunity ASEP (ID 450872). We provided insights as to how physicochemical properties of macromolecular conjugates of TLR-7/8a, including **polymer chain structure, composition and hydrodynamic behavior, can be chemically tuned to alter the magnitude and quality of innate and adaptive CD8 T cell immunity**. Attachment of TLR-7/8a to any of the polymer compositions resulted in a nearly 10-fold reduction in systemic cytokines (toxicity). Overall, this work has important implications for the development of vaccines for inducing CD8 T-cells for the treatment of infectious diseases and cancer ASEP (ID 501308). Moreover, we dealt with the development of a **new polymer-based vaccine platform for the delivery of HIV-1** glycopeptide immunogens to target immune responses to specific broadly neutralizing Ab epitopes. The data show that a dense array of HIV-1 immunogens together with T-helper epitopes and TLRa on our unique star-shaped polymer platform effectively engages B cells and other APCs and elicits high titer Ab in mice and NHP, making these systems promising immunological tools to prevent HIV-1 infection (ASEP (ID 506417). We described a generalizable approach for co-delivering peptide antigens and adjuvants in nanoparticles for inducing anticancer T-cell immunity. This approach provided precise loading of diverse peptide neoantigens linked to TLR-7/8 agonist in nanoparticles, which increased uptake by and activation of APCs that promote T-cell immunity. ASEP (ID 522069)

In conclusion, polymer–TLRa conjugates represent a versatile class of adjuvants that can be tuned to achieve the optimal innate immune activity required for eliciting antibody and T-cell immunity for applications in preventive and therapeutic vaccines for infections and tumors.

2. Magnetic nanoparticles for treatment of cancer

Innovative nanotechnology aims to develop particles that are small, uniform in size, smart, and do not cause side effects. Moreover, magnetic nanoparticles became excellent tool as theranostic constructs that enable highly effective treatment of various cancer diseases. New types of **surface-modified uniform magnetic nanoparticles** were developed by the team using thermal decomposition and/or precipitation of iron precursors. To make the particles dispersible in aqueous buffers, their surface was modified with α-carboxyl-ω-bis(ethane-2,1-diyl)phosphonic acid-terminated poly(3-O-methacryloyl-α-D-glucopyranose) ASEP (ID 458269) or PEG-neridronate. **The particles exhibited high relaxivity in MR relaxometry measurement**. Cytotoxicity of the particles was thoroughly evaluated in terms of proliferative activity of T-lymphocytes and T-dependent B-cell response, phagocytic activity of monocytes and granulocytes, and respiratory burst of phagocytes ASEP (ID 506160). Uniformly sized PEG-neridronate-modified magnetic nanoparticles exhibited a prolonged blood circulation and biodistribution in mouse preclinical model depending on the particle size ASEP (ID 507245). The **particles proved their great potential in cancer diagnostics** (contrast agent in MRI) **and therapy**. Poly(*N,N*-dimethylacrylamide)-modified iron oxide nanoparticles exhibited increased antitumor activity in model rats with inoculated mammary gland carcinosarcoma, which correlated with Fe^{2+} release from the particles. We also prepared uniform **antibacterial silver-conjugated magnetic nanoparticles** that combined both magnetic separation/targetability and biocidal activity in single entity; such particles are useful for disinfection of waste waters. The particles were coated with silica shell using a water-in-oil reverse microemulsion, involving hydrolysis and condensation of tetramethyl orthosilicate and modification with (3-mercaptopropyl)trimethoxysilane. Finally, the Fe_3O_4@SiO_2-SH nanoparticles were decorated with silver nanoclusters formed by reduction of silver nitrate with $NaBH_4$. **Antibacterial properties were proven against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria** cultivated on Luria agar plates or in Luria broth ASEP (ID 507831).

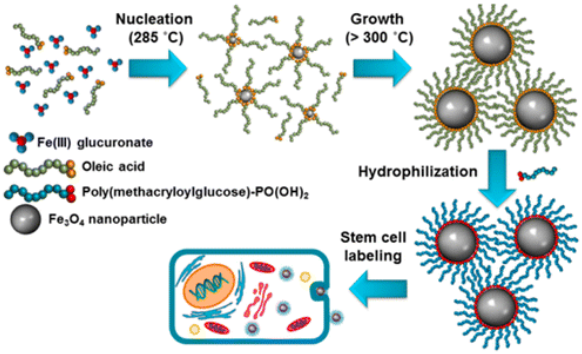


Fig. 10 Schematic description of preparation of surface-modified monodisperse/uniform magnetic nanoparticles.

3. Hydrogel implant for local treatment of retinoblastoma

Currently, chemotherapy is the most common treatment for oncological diseases. Recently, **nanofibrous materials as local drug delivery systems have attracted much attention**. They have considerable potential in the treatment of various cancers as they can provide a high concentration of the drug at the target site for a prolonged time, thereby lowering total exposure and adverse effects ASEP (ID500259). A **new hydrogel implant for local delivery of anticancer agents into the eye globe via diffusion through the sclera was designed and tested**. The implant as a two-layered construct was composed of inner hydrophilic drug reservoir and outer hydrophobic layer directing drug distribution and protecting surrounding vascularized tissue against cytotoxicity. We designed constructs, optimized chemical composition and fabrication methodology, which controlled the drug release characteristics, and realized the implant preparation in clean room environment. The hydrogel implant was

proposed as a reservoir of the chemotherapeutic agent and the outer hydrophobic layer from 2-ethoxyethyl methacrylate (EOEMA) acting as a barrier to protect the surrounding vascularized tissue against cytotoxicity of the delivered chemotherapeutics, topotecan and vincristine ASEP (ID505096).

Moreover, the **nanofibrous constructs for local application of hydrophobic immunosuppressant cyclosporine A and anticancer drug paclitaxel were investigated**. The polymeric matrices based on poly(D,L-lactide) were electrospun with addition of amphiphilic polyethylene glycols of various molecular weight to moderate the release of hydrophobic agents. We managed the research, suggested the composition of the nanofibrous materials, characterized the morphology and drug release kinetics, which revealed the impact of various effects on the drug release profile ASEP (ID500120).

Research activity and characterisation of the main scientific results

The scientific activities pursued in the MATER centre can be divided into four research areas. This section summarizes the main results obtained in these partially overlapping and complementary topics.

1. Structure-properties relationships in heterogeneous polymer materials

Blends and composites are important classes of polymer materials with heterogeneous phase structure with a broad field of application. End-use properties of immiscible polymer blends are strongly affected by their phase structure, which is formed during their melt processing. Therefore, a reliable description of the phase structure evolution in immiscible polymer blends during their melt mixing is a necessary condition for tailoring their properties. Flow field in mixing devices is complex and position-dependent even for the steady flow of homogeneous materials. The phase structure evolution in immiscible polymer blends was studied from the theoretical point of view taking into account rheological properties of blend components [ASEP (ID 504684)] and considering the presence of the block copolymer compatibilizer at the interface [ASEP (ID 491735)]. Simple theoretical model was developed showing that concentration of a block copolymer at the interface increases with increasing chain length of copolymers or homopolymers. Copolymers with block lengths comparable to chain length of the blend components reduce interfacial tension most efficiently. The analysis showed, that the distribution of the copolymer between the interface and the bulk phases changes in a broad range depending on the system parameters [ASEP (ID 491735)].

Precise determination of particle size distribution in polymer blends with a complex morphology is a necessary prerequisite for correct determination of structure-properties relationships. Novel approach for a quantitative evaluation of morphology in droplet-matrix polymer blends was proposed using a new program MDISTR. The calculations were based on a linear combination of standard particle size distributions from at least two sets of micrographs with different magnifications, different locations within the sample and precisely defined statistical weights [ASEP (ID 439731)].

Experimental studies on polymer blends were also focused on biodegradable systems based on polylactic acid (PLA) and polycaprolactone (PCL). The role of rheological properties of individual components was studied. It was found, that by precise control of dispersed particle size due to processing conditions and viscosity of components, biodegradable polymer blends with high toughness and stiffness could be obtained — they are promising materials for medical applications, in particular for bone tissue engineering [ASEP (ID 470664)].

A complex study was performed on the effect of anisotropic fillers on the structure and properties of PLA/PCL blends [ASEP (ID 457893), ASEP (ID 473963) , ASEP (ID 489984)]. The flow behaviour of the polymer blends was altered considerably by addition of the nanofillers. Thus, phase structures resulting in enhanced mechanical performance of the blends were obtained. Halloysite nanotubes were used for preparation of a composite based on PCL matrix with in situ formed PLA microfibers, which could not be prepared from unmodified components. The application of halloysite with relatively large lateral dimensions in the PCL/PLA mixture substantially improved stability of extrusion and enabled subsequent melt drawing [ASEP (ID 457893)]. This concept was further investigated by using graphite nanoplatelets (GNP). It was found, that with the use of GNP particles one can tailor structure and resulting mechanical properties of the PLA/PCL blends efficiently [ASEP (ID 473963)]. With this knowledge, materials with PLA matrix reinforced by PCL microfibers were successfully prepared. By melt spinning of these materials, fibres and threads with wide range of parameters including biodegradability and with unique structures with high potential for biomedical applications could be obtained [ASEP (ID 489984)].

High potential of graphite nanoplatelets for preparation of polymer/polymer composites with in situ generated fibrous reinforcement was successfully tested also in polyethylene/polyamide systems. Using GNP, more rigid and stronger material with enhanced toughness in

comparison with unmodified blends was obtained. It was shown that GNP affect interface parameters, thermodynamic and kinetic aspects of dynamic phase behaviour, including the formation of self-assembled rigid/elastic complex structures [ASEP (ID 488610)].
Starch-based materials are another group of biodegradable systems, which were investigated by MATER researchers. The investigations were focused on preparation of plastic materials with controlled biodegradability and optimized mechanical performance. Structure and property control was performed by specifically defined starch esterification and plasticization processes using proper additives [ASEP (ID 509690)]. In the framework of these studies, a biodegradable scent device to prevent access of animals was developed and commercialized. The final product is protected by patent CZ307421 and it has been successfully used to combat swine fever in the Czech Republic. The starch-based materials were investigated also for medicinal applications. This research led to an invention of a thermoplastic biodegradable composition containing antibiotics, which can be used as an insert for treatment and prevention of local infections in orthopedy and surgery. The material itself and the method of its preparation is protected by patent CZ307056.
The structure and its changes induced by defined mechanical deformation and its effect on rheology and electrical conductivity of polymer composites containing carbonic fillers were studied by simultaneous electrical-rheological measurements. This work presented first systematic study concerning the electrical behaviour of composites with anisotropic microfiller under deformation in the molten state. It was found, that the electrical conductivity of composites with carbon microfibres reacts very sensitively to the mechanical deformation [ASEP (ID 486432)].
Sufficient dispersion of nanofiller particles is a key parameter necessary for effective control of nanocomposite properties. Ionic liquids were tested as delaminating agents for layered double hydroxides (LDH). LDH with intercalated ionic liquid anions were synthesized by a direct co-precipitation method in the presence of ionic liquid and subsequently used as functional nanofillers for in-situ preparation of poly(butylene adipate-co-terephthalate) nanocomposites. The prepared nanocomposite films showed improved water vapor permeability and mechanical properties making them promising candidates for food packaging applications [ASEP (ID 505101)].
All the above-mentioned studies were designed by the members of the MATER centre. They prepared the materials and they were responsible for a majority of characterization techniques used. A part of the techniques employed were performed in cooperation with other research centres of the Institute or other national or international research institutions.

2. Advanced approaches in polymer chemistry and characterization
Microwave-assisted chemical processes are based on the efficient heat transfer achieved by dielectric heating, which is dependent on the ability of the solvent or reagent to absorb microwave energy. The use of microwave energy has drawn a significant attention in the past two decades with new and innovative applications in polymer chemistry and material science. In the MATER centre the microwave-assisted recycling technologies for polyurethanes, polyesters and polycarbonates are investigated continuously. Medium-chain glycerides of coconut oil were used as solvolysis reagents for microwave-enhanced conversion of polycarbonate into recycled polyols. The important accelerating effect of microwave irradiation on kinetics of polycarbonate solvolysis was observed. The developed recycling process provided a mixture of low-molecular weight polyols applicable for synthesis of novel polyurethanes [ASEP (ID 466784)]. This was proven in a subsequent study, in which polycarbonate and polyurethane scraps from end-of-life vehicles were converted into liquid recycled polyols. The obtained polyols were used for preparation of low-density rigid polyurethane foams. This approach utilizes the renewable coconut oil-derived reagent and provides a sustainable recycling solution for two major plastics from automotive waste. Both of the recycled polyols obtained can be applied up to 50 wt % as virgin polyol replacement in low-density rigid PUR foams with potential application as a thermal insulating material in the building industry [ASEP (ID 475988)].

Another application of the microwave energy in polymer science is the microwave-assisted polymerization. An influence of ionic liquid-modified layered double hydroxide (LDH) on polymerization of ε-caprolactone under microwave irradiation was investigated. Surprisingly, no additional catalysts or initiators were needed in the presence of LDH and the ring-opening polymerization of ε-caprolactone proceeds quantitatively. The developed process is fast, environmentally-friendly (solvent-free) and adaptable to various polymer matrices, since a broad variety of ionic liquid anions might be applied [ASEP (ID 462149)]. The process is protected by Czech patent CZ307421 and patent application WO2017076379A1.
Supramolecular structure of ultra-high molecular weight polyethylene (UHMWPE) for total joint replacements and its modification by high-energy irradiation is another topic at the boundary between fundamental and applied research pursued in the centre in the long term. Microindentation hardness testing was applied to UHMWPEs that were highly-crosslinked at different irradiation conditions. The values of microhardness, microcreep and microplasticity from these experiments were in excellent agreement with the changes of UHMWPE structure characterized by spectroscopic and calorimetric methods. Statistical evaluation of the results, the agreement with theoretical predictions and the comparison with previous studies on similar systems demonstrated that micro-indentation is a reliable and sensitive method of UHMWPE characterization [ASEP (ID 439729)].
Polymers lose their material properties during outdoor exposure due to photodegradation processes initiated by photooxidation. Thus, polymer stabilization is a key factor for their application. In cooperation with the SUPRAMOL centre, investigations into light stabilization of polyolefins using accelerated weathering technique were performed. It was confirmed that natural phenolic antioxidant, vitamin E, in particular its most active component α-tocopherol, can exhibit pro-oxidant activity. The pro-oxidant activity of vitamin E manifests itself during the exposure of stabilized polymers to UV/visible light. Similar effect was observed also for synthetic phenolic antioxidant, Irganox®1010. Therefore, vitamin E should not be used for food packaging applications as recommended elsewhere [ASEP (ID 505869)].
All the above-mentioned studies, unless mentioned otherwise, were designed by the members of the MATER centre. They prepared the materials and they were responsible for a majority of characterization techniques used. A part of the techniques employed were performed in cooperation with other research centres of the Institute or other national or international research institutions.

3. Soft polymer materials responsive to external stimuli
Polymer hydrogels are attracting increasing attention as very promising soft materials due to their ability to undergo significant changes in volume in response to external stimuli. Thermosensitive poly(N-iso-propylacrylamide) (PNIPA)-based hydrogels are a typical example of these materials. However, the PNIPA hydrogels, with covalently cross-linked network structures, are fragile materials and possess a low degree of swelling at higher crosslinking densities. Therefore, for practical applications, the mechanical properties of such soft materials should be improved. The effect of the reinforcing laponite clay on the gel formation and structure evolution in PNIPA was investigated. Particularly, significant effect of the redox initiating system on gel formation and structure was established. This finding implies the possibility of controlling the chain length between crosslinks independently of the crosslinking density, that is determined by the clay content. The mechanism of gel formation was refined using experimental results [ASEP (ID 451682)]. The mechanical properties of the PNIPA-clay gels were efficiently controlled by the initiating conditions of the polymerization. The initiating system provided a very efficient tool for tuning mechanical properties of the gel within a broad range. Additionally, the mechanical properties of the gels were described while applying the theory of elasticity, which includes physical interactions such as nanofiller effect of clay or crosslinking by entanglements [ASEP (ID 458840)].
The gel response is generally accelerated by introducing porosity using various techniques. Freezing cryopolymerization is a simple and efficient method for preparation of macroporous gels. The cryopolymerization of the PNIPA/clay system was studied and the mechanism of

formation of the macroporous nanocomposite cryogel was determined. The phase and the molecular structure during two simultaneously proceeding processes, i.e. cryostructuration and cryopolymerization, were determined. The relative rates of these processes govern the final gel structure, morphology and properties. The gel porosity depends on the cooling rate and the relative rates of cryostructuration and polymerization [ASEP (ID 470837)].
In order to enhance mechanical properties of PNIPA hydrogels, porous and non-porous poly(N-isopropylacrylamide) hydrogels reinforced by nano-silica and starch particles were prepared. Starch intercalation highly improved the extensibility of the materials. The elongation at break increased 2-fold in porous samples and 3–6-fold in non-porous ones. The porous gels displayed an ultra-fast and symmetrical temperature-responsiveness, the bulk ones a very fast one-way deswelling. Interface interactions of the matrix/fillers type played a key role in the response rate. The porous gels could be used as soft actuators, the bulk ones as drug release systems [ASEP (ID 471497)].
Incorporation of electrically conducting fillers can broaden functionality of responsive hydrogels. These gels are of interest due to their ability to behave like a temperature-sensitive and conductive element (e.g. temperature-triggered switch) or as a mechanical actuator triggered by induction or ohmic heating. Electrically conductive super-porous hydrogels gels with very fast and symmetrical volume response to temperature jumps were prepared using sub-micrometre-sized polyaniline particles and nano-silica as fillers. A two-step impregnation route was developed for selective polyaniline deposition in pore walls. The gels offer potential applications as actuators or switches, which can be triggered directly by heat, or by electrical current or light, which also cause heating [ASEP (ID 469851)].
Dually temperature- and pH-sensitive super-porous PNIPA/nano-silica hybrid hydrogels were prepared by incorporation of sodium methacrylate comonomer. These hydrogels combined the ultra-fast and symmetric response to temperature as well as the fast pH-induced deswelling, although the pH-induced re-swelling was relatively slow. It is the porosity of gels that makes the ultra-fast response to stimuli possible (5.5 s for 70% in both directions in response to temperature). The rate of pH-response was found to be always slower than in the case of the temperature response. The vast difference in the rates of pH- and temperature response can be explained by different mechanisms of stimulus propagation, i.e. by the difference in momentum transfer for temperature and in diffusion for pH [ASEP (ID 508103)].
Self-healing polymers belong to the category of advanced materials that were investigated intensively in the last decades. The intrinsic self-healing procedure is based on autonomous or on the stimuli-responsive reversible crosslinking resulting in reconstruction of the damaged molecular structure. The intrinsic self-healing could be attained in systems containing reversible covalent bonds or in systems with physical supramolecular structures. The former case is represented by reversible Diels–Alder type networks, which were prepared from furan and maleimide monomers of different structure and functionality. The structure of the maleimides was found to affect the thermodynamics and kinetics of the Diels-Alder reaction very strongly as well as it was found to affect the gelation processes, stability and crosslinking density. The design of a self-healing reversible network with the optimum structure was proposed [ASEP (ID 505741)]. The latter case, i.e. the self-healing polymers possessing physical supramolecular structures was investigated as well. The synthesized aliphatic polycarbonate-based polyurethanes (PCPU) show a high degree of ordering and strong superstructures that can undergo order–disorder transitions. Moreover, the presence of thermally stable entanglement network and their excellent mechanical properties make the PCPU suitable as a strong self-healing polymer. The damaged polymer was heated above the disorder temperature of hard segment domains, thus chain mobility was increased for interdiffusion to take place and to heal a crack, while the entanglement network kept the shape of the material. The efficient and relatively fast healing and restoration of the original structure and mechanical properties of a polymer were checked by microscopy and by tensile testing [ASEP (ID 494318)].
Soft thermoplastic polyurethanes have been used in medicine for several decades now. They are applied as robust biomaterials that are stable long-term and are suitable for fabrication of

biomedical devices and implants. However, current needs require the development of efficient biodegradable or bioresorbable materials with short or limited lifetimes. Thus, hydrolytic stability of novel polycarbonate-based polyurethane elastomers was tested in physiologically simulated conditions. Elastomeric all-aliphatic polyurethane films made from polycarbonate-based macrodiol, diisocyanate-1,6-hexane and butane-1,4-diol featured typical thermoplastic character accompanied with outstanding mechanical and with suitable thermal properties. The properties kept practically unchanged for films that were immersed in the model physiological environment for a period of up to 12 months. They can be practically used, e.g., as strong and durable topcoats in (bio)medical applications [ASEP (ID 444278)]. To control biodegradation of prepared systems, lactide linkers were introduced into the polyurethane structure. The hydrolytic degradation of aliphatic polyurethane films containing D,L-lactide-based linker was tested in phosphate-buffered saline (PBS) at 37 °C up to 12 months. Tensile testing, DSC, SEM, AFM, FTIR and WAXS analyses characterized the raw and PBS-treated films. Studied films can be used either as fairly stable high-performance elastomers for short-term applications (up to 3 months) or as degradable materials, when the time of exposure to the physiology-mimicking conditions is sufficient [ASEP (ID 456111)].
All the above-mentioned studies were designed by the members of the MATER centre. They prepared the materials and they were responsible for a majority of characterization techniques used. A part of the techniques employed were performed in cooperation with other research centres of the Institute or other national or international research institutions.

4. High performance thermosets with tailored properties

Thermosets based on epoxy matrix and dispersed silica domains of various shapes and architectures have been the topic of numerous studies in recent years. These investigations are driven by the outstanding properties of inorganic silica phase which, through the combination of hardness and thermal and chemical stability with toughness, flexibility, and easy organic phase processability, allow tailoring of the final material properties.
The control over the interface between organic and inorganic phase due to incorporation of ionic liquids was studied. High performance shape memory epoxy–silica nanocomposites were prepared by in situ generation of nanosilica in the epoxy matrix using a non-aqueous sol–gel process under catalytic action of ionic liquids. The study contributed to the better understanding of the shape memory (SM) behaviour of polymers. The recovery stress, as a crucial SM property, was found to be governed by the material toughness while the efficiency of the SM performance was controlled by the morphology and viscoelasticity of the polymer [ASEP (ID 453622)].
The next work described the solvent-free sol−gel synthesis of silica-based precursors in the presence of 1-butyl-3-methylimidazolium-based ionic liquids (IL) containing two different anions: chloride and methanesulfonate. The IL-driven mechanisms were investigated using experimental characterizations and a theoretical computational DFT method. The application of ionic liquid−silica precursors as reinforcing additives into epoxy network led to an improvement in the organic/inorganic interphase interactions. The systems prepared showed unaltered optical transparency, reduced UV absorption tendency and improved thermo-oxidative stability, which made them multifunctional additives for epoxy networks [ASEP (ID 474629)].
Brønsted-acidic imidazolium ionic liquids were also evaluated with respect to their ability to self-catalyse reactions between carboxyl groups and epoxy rings. These mono-, bi-, and tetra-functionalized ionic liquids with carboxyl groups acted as all-in-one reaction systems for material synthesis or modification, with the potential of producing a broad range of epoxy-based materials via metal catalyst-free coupling reactions. This study provided tools for the preparation of a number of formulations between epoxides and carboxylates using an ionic liquid platform. This platform is expected to be useful for reactions from small molecules coupling to polymerization and cross-linking processes that fulfil the principles of green chemistry, i.e. prevention of waste and atom economy (water is the only residue), low toxicity

reactants and products, solvent-free, energy efficient and metal-catalyst-free one-pot reaction [ASEP (ID 512147)].
The control of the interface was the focus in systems of epoxy matrix and titanate nanotubes. Nanotube surface treatment is crucial in preparation of high-quality nanomaterials for advanced applications. In this study we provided an environmentally friendly and practical route for the design of high-performance composites using the surface chemistry of titanate nanotubes to enhance the interfacial nanotube−epoxy bonding. This study demonstrated that titanate nanotubes functionalized with fully aqueous-based protocols are promising alternatives to carbon nanotubes in epoxy composites [ASEP (ID 492561)].
Ceramic foams enjoy a considerable research interest due to a wide variety of potential applications, like heat-resistant energy-absorbing materials, thermal and fire insulation, light-weight and refractory structural panels, 3D reinforcing structures for polymer and metal composites, but also as catalyst support or as biological implants. The most popular synthetic path to Si-O-C foams is the pyrolysis of siloxane precursor polymers. Polysiloxane foams as precursors to ceramic foams were prepared via simultaneous cure and foaming of liquid methylsiloxane resins, using ethanol as blowing additive of the boiling-solvent-type, and aqueous ammonia as catalyst. Bubble nucleation was done by mechanical stirring and siloxane surfactants were added. After pyrolysis at 1000°C, ceramic foams with macro-porosities of 79–91% were obtained [ASEP (ID 447628)].
The chemistry of pyrolysis of polymethylsiloxane resins into the valuable refractory Si-O-C glass was further optimized. It was shown that dimethylsiloxane units, which contribute to higher weight losses during the pyrolytic transformation to Si-O-C since they eliminate and mostly evaporate, additionally also make possible micro-creep and hence stress relaxation in the pyrolyzed samples and, thus, they prevent cracking, which occurs at low dimethylsiloxane contents. Optimal contents are between 15 and 25 mol% of dimethylsiloxane [ASEP (ID 461612)].
Mechanically very strong Si-O-C foams were prepared via pyrolysis of polysiloxane composites containing 20–70 wt% of epoxy powder as sacrificial filler, which quantitatively degraded during pyrolysis. Si-O-C foams with hierarchical macro-porosities of 35–69% were obtained. Precursor shape was well-preserved and large samples were easily prepared. The strongest foam was obtained at 56% porosity [ASEP (ID 443898)].
All the studies concerning epoxy systems were designed by the members of the MATER centre. They prepared the materials and they were responsible for a majority of characterization techniques used. A part of the techniques employed were performed in cooperation with other research centres of the Institute or other national or international research institutions. The studies concerned with ceramic foams were done in cooperation with the Institute of Rock Structure and Mechanics, Czech Academy of Sciences. The MATER researchers were responsible for preparation of the precursors for the pyrolysis experiments and for morphological characterization of the pyrolysis products.

Research activity and characterization of the main scientific results

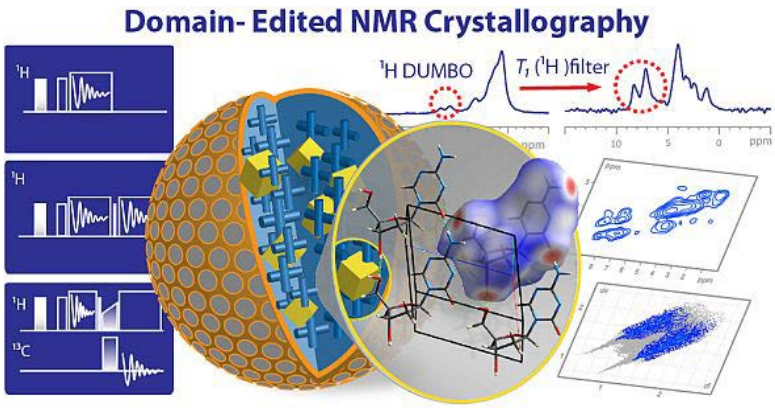
As mentioned above, the scientific program of the STUCTURE center generally focuses on one of the most important unsolved problems in current chemistry, which is: "*the ability to accurately predict the structure of complex molecular and macromolecular systems and to relate the molecular and supramolecular structures to properties and reactivity.*" Consequently, the research in the STUCTURE center deals with the development and applications of advanced techniques of high-performance spectroscopy to unveil the structure of functional materials down to atomic resolution level and to describe the intermolecular interactions and dynamics in these materials. In this regard, we systematically develop and explore various combinations of advanced techniques of NMR spectroscopy, vibrational spectroscopy, and X-ray diffraction with DFT calculations and data processing with the aim to formulate robust and reliable strategies of structure analysis that are applicable in academic as well as industrial laboratories. This way, through the synergistic interplay between the measurements, calculations and the statistical analysis, we want to formulate and optimize an integrated approach providing otherwise unavailable structural information for complex multicomponent systems in various physical states (liquids, solids, gels). In order to cover the most important topics investigated at the IMC as well as to follow scientific plans formulated in 2015, the research of the STRUCTURE center focuses predominantly on the following topics:

- structure analysis of advanced multicomponent pharmaceutical solids including hybrid nanostructured drug-delivery systems (e.g. *liquisolid* mesoporous silica nanoparticles);
- structure analysis of amphidynamic polymeric composites, porous networks and complexes including polymer electrolytes for energy applications; and
- structure analysis of framework aluminosilicates (zeolites) and metalorganic frameworks (MOFs).

NMR crystallography of active pharmaceutical ingredients and materials: This topic represents the whole field of structure analysis of advanced multicomponent pharmaceutical liquids and solids including hybrid nanostructured drug-delivery systems such as *liquisolid* mesoporous silica nanoparticles, complex polymeric microbeads containing active substances, polymeric dispersions and/or various novel forms of active pharmaceutical ingredients. Bear in mind, that besides the sophisticated synthesis, the development of a next generation of advanced pharmaceuticals requires detailed insight into their molecular and crystal structure. In this context, NMR crystallography as a complex of various computational-experimental approaches with its potential to probe structure of solids at atomic-resolution level seems to be nearly a universal method. At the *Joint Laboratory of Solid-State NMR Spectroscopy* and in close cooperation with universities and pharmaceutical companies, we are developing advanced approaches of NMR crystallography, which allows efficient and reliable monitoring of manufacture of pharmaceutical products, easy identification of polymorphic impurities as well as precise description of crystal structures of complex active pharmaceutical ingredients.

Since 2016, we have systematically optimized methodology for complete determination of crystal structure without the assistance of diffraction data exclusively from NMR parameters. This effort is given by the fact that the crystal structure determination remains a challenge, when diffraction data are not available. In this regard, the concept of NMR crystallography, representing a unique protocol of an ab initio determination of the crystal structure based on the combination of solid-state NMR spectroscopy (ss-NMR), computer crystal structure prediction (CSP) and density function theory (DFT) chemical shift calculations, exhibits remarkable potential. However, the widespread application of this approach still requires an experimental verification and validation, especially considering the fact, that only few examples have been presented in the scientific literature so far. Therefore, in the first step of our research, we focused on the complete determination of 3D crystal structure of active compounds without the assistance of diffraction data using exclusively NMR parameters in combination with the crystal structure prediction. In this regard, we discussed the previously unconsidered influence of long-range molecular packing symmetry on NMR parameters and subsequent selection of the correct crystal structure. For this purpose, we extended the previously introduced approach of powder NMR crystallography by simulating entire 2D ^{1}H-^{13}C HETCOR and ^{1}H-^{1}H DQ/SQ NMR correlation spectra. This way, the large set of automatically predicted/generated NMR-consistent candidates was significantly narrowed down and the correct crystal structure could be unambiguously identified. Moreover, we were able to discriminate between the predicted crystal structures in which the molecular conformations and short-range arrangements were basically identical and whose differences in global molecular packing were generated only by different symmetry operations. Overall, the proposed procedure thus allows the description of differences in the long-range symmetry of molecular packings, to which ss-NMR spectroscopy is otherwise less sensitive, and expands the capabilities of NMR crystallography to predicting supramolecular structures. [*Crystal Growth & Design*. **2016**, *16/12*, 7102-7111]

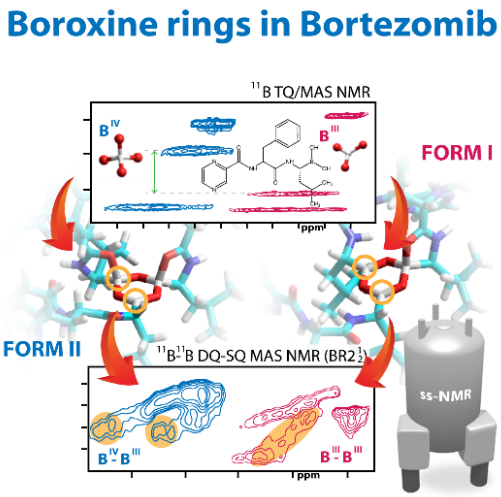
The problem of structure determination at atomic resolution level when diffraction data are not available is particularly urgent for structural studies of advanced generation of polycrystalline micro-/nano-sized solids, which represent not only promising drug-delivery systems but also constitute materials with hierarchical architecture executing multiple functions. Presence of multiple crystalline components, however, results in complicated diffraction patterns, the conversion of which into the refined crystal structures is practically unattainable. In this regard, the above-mentioned concept of NMR crystallography exhibits remarkable potential. Therefore, we explored the combination of various domain-selective 2D solid-state NMR techniques and DFT calculations to formulate an extended experimental/computational strategy for monitoring the atomic-resolved structure of polycrystalline multicomponent systems whose domains are incorporated in the crystalline matrix. Specifically, we focused on the complete 3D structure determination of each component in polycrystalline micro-/nano-composite microbeads. We found, that when applying this procedure, it is clearly important to preselect a representative set of crystal structure predictions to be subjected to DFT optimization and statistical evaluation. This way, even intricate hierarchical structure of novel microbeads formulations of decitabine can be efficiently probed. Specifically, by applying domain-edited ss-NMR experimental techniques, i.e. on the basis of differences in nuclear-spin relaxation, individual constituents of polycrystalline micro-/nano-composite microbeads could be spectroscopically discriminated. Consequently, the presence of a crystalline polymeric matrix that is



accompanied by the partly immobilized amorphous phase, microcrystallites of an active compound and nanoscaled crystallites of low-molecular-weight excipient was revealed. Complete 3D structure of both types of dispersed crystallites was then successfully predicted, determined and verified. Regarding the future perspectives of this approach, we found out that it is clearly important to preselect the representative set of CSPs, which will be involved in the DFT optimization and statistical evaluation. As a nearly a countless number of structural models can be generated, a method allowing reliable preselection to narrow down the search is of particular interest. To avoid the problem of losing suitable models, we are currently working on a procedure based on a systematic search through a set of models created from experimentally determined specific interatomic distances (distance restraints). As the obtained results also opened a route toward the structure refinement of synthetic polymers with a limited amount of spectroscopic data available, finding a procedure for the reliable generation of a representative set of CSPs of synthetic polymers is of paramount importance. This research thus demonstrates the synergy effects of the proposed combination of several experimental and computational procedures, which considerably extends the NMR crystallography approach into the area of intricate mixtures and nanostructured composites in micro- and nano-sized forms. [*Macromolecules* **2018**, *51/14*, 5364-5374]

The above-mentioned extended protocol, however, still can fail for really large molecular and crystallographic systems such as oligopeptides, which exhibit nearly countless number of insufficiently resolved NMR resonances. To overcome this limitation, the cooperation between NMR crystallography approach and X-ray powder diffraction analysis seems to be the most promising way. The determination of crystal structures from powder X-ray diffraction (PXRD) data by using direct-space methods is, however; significantly limited by the degrees of conformational freedom (DOF). This limit currently lies between 30 and 40 DOF. Consequently, novel approaches are continuously developed to allow an increase in DOF while keeping computational time realistic. Therefore, in parallel with the development of NMR crystallography approaches, we focused on development of reliable PXRD approaches to determine crystal structures of *crystallographicaly* large systems. In this regard, the long-term colaboration and concentrated effort of 4 academic institutions from the Czech Republic together with scientists from Teva Czech Industries grouped around the European Synchrotron Radiation Facility (ESRF) led to the successful development of an experimental-computational strategy allowing crystal structure determination from powder X-ray diffraction data of very complex systems. Specifically, we achieved this by combining the restraints extracted from the Cambridge Structural Database (CSD), optimized simulated annealing (SA) parameters, and parallel code execution, which were complemented by DFT-D calculations and analysis of solid-state NMR (ss-NMR) parameters. This way, the crystal structure of selexipag, a drug for the treatment of pulmonary arterial hypertension (PAH) was unambiguously determined. With 38 DOF, this structure is currently one of the most complex organic molecular structures that was actually solved *ab initio* from powder diffraction data. The ability to accurately describe all processes that occur in the manufacture and formulation of drugs thus opens a more straightforward way to optimize advanced highly active pharmaceutically active substances. [*Crystal Growth & Design* 19/8 (2019), 4625-4631]

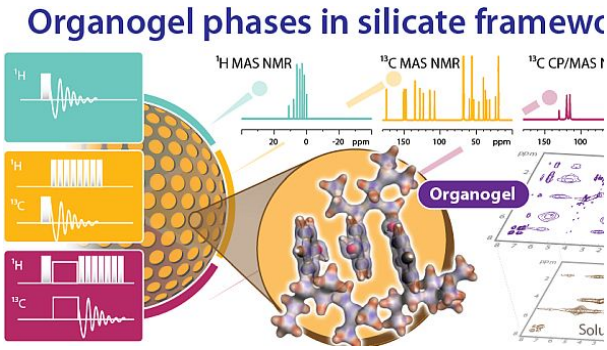
Peptide derivatives of boronic acid and their unique structure: Boron-containing compounds have long been recognized as potentially active pharmaceutical ingredients. As recent investigations have resulted in the discovery of many promising pharmaceuticals exhibiting anticancer and antibacterial activity, the research of peptidic derivatives with boronic acid fragment is rapidly gaining in intensity. Uncertainties in the structure determination of these peptide analogues, however, represent an obstacle in understanding their role in living organisms and thus also in development of the next generation of anticancer drugs. Therefore, for full exploitation of these drugs we focused on the development of experimental-computational strategy allowing atomic-resolution structure determination of complex boronic acid derivatives. In this regard, crystalline bortezomib, a proteasome inhibitor approved for the treatment of multiple myeloma, represents a unique combination of a complex unpredictable supramolecular solid-state structure with an extremely high multilateral pharmaceutical activity. To unveil its complicated solid-state structure resulting from the complex pathway of reversible covalent interactions, we explored various 2D ^{11}B-^{11}B solid-state NMR correlation spectroscopic techniques supported by DFT calculations to formulate a reliable tool for monitoring the assembly of boronic acid residues in the solid state. This way, we discovered and described quite unique and previously unreported boroxine structures of bortezomib polymorphs exhibiting secondary coordination of boron atoms. The self-condensation of bortezomib molecules was clearly confirmed, and different local constitutions of boroxine motifs were unveiled. The ^{11}B NMR parameters responded sensitively to subtle changes in local geometries, which were reliably interpreted and directly visualized by the DFT calculations. A uniform 2.6 Å distance in bortezomib ^{11}B-^{11}B spin pairs was conclusively identified by the through-space ^{11}B-^{11}B double-quantum (DQ) coherence build-ups, whereas distinct 2D ^{11}B-^{11}B DQ correlation patterns revealed unique boroxine structures. The boroxine rings were found to be internally stabilized through transformation of the trigonal boron sites toward tetrahedral geometry, when the secondary five-membered rings were formed. These structural motifs were also found in amorphous phase. The efficient strategy for exploring the assembly of boronic acid derivatives in the solid state where no crystallographic data were available (crystalline or amorphous) was thus developed, the nature of bortezomib polymorphism disclosed, and the previously unknown bortezomib boroxine structures clearly described. [*Physical Chemistry Chemical Physics.* 19/1 (2017), 487-495]



This approach utilizing solid-state ^{11}B NMR spectroscopy was further optimized when describing the crystal structures of two non-solvated polymorphs of ixazomib citrate directly from synchrotron powder diffraction data, which was a challenging task because the two molecules in the asymmetric unit cell exhibited 32 degrees of conformation freedom (DOF), which pushed the limits of current solution procedures. In this regard, we used a novel two-step Rietveld refinement based on DFT-D restraints to improve information quality derived from powder diffraction data to be comparable with that of single-crystal solutions. The previously developed protocol of NMR crystallography was applied to verify the crystal structures, and the importance of exploitation of ^{11}B NMR parameters for the solution of unknown structures was demonstrated. Quite surprisingly, we found, that evolution of ^{11}B-^{11}B double-quantum coherences allowed probing of interatomic distances up to 7 Å! In this context we also explored various computational methods to establish reliable protocol to calculate ^{11}B NMR parameters with high precision and accuracy. Our approach was based on DFT-GIPAW methods. These findings thus opened new windows for structure determination of complex boron-containing organic solids. Overall, we again presented an integrated approach that applied several

techniques in conjunction to provide otherwise unavailable structural information. [*Crystal Growth & Design*. 18/6 (2018), 3616-3625, *Chemical Physics Letters*. 655–656, 2016, 66-70]

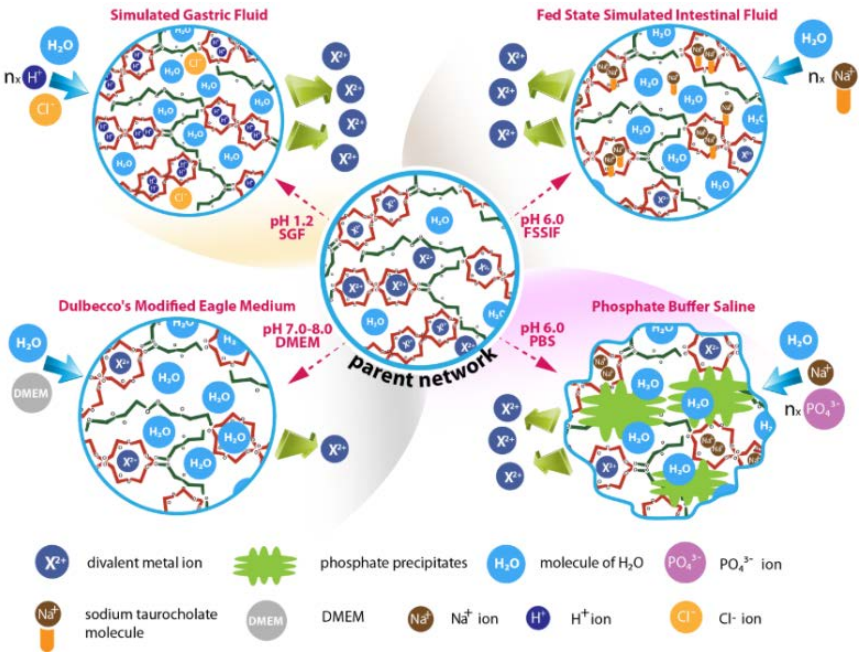
Hybrid materials and nanomaterials for biomedical and pharmaceutical applications: In general, constant efforts to synthesize organic compounds have resulted in discovery of a range of novel, highly efficient drugs. In many cases, these newly discovered chemical entities exhibit unfavorable physicochemical properties. Consequently, their administration requires sophisticated formulations. In this context, *liquisolid* drug-delivery systems based on framework materials represent a highly promising alternative. Although pharmaceutical research is continuously gaining momentum, many obstacles remain that prevent rapid launch of these pharmaceuticals into clinical practice. Among these challenges, predicting the structure of the organic phases in these "framework formulations" is particularly difficult and important to resolve. This problem arises because these systems naturally exist at the borderline between solids and liquids, for which high-quality diffraction data are inherently unavailable; organic phases become hidden for X-ray analyisis "*behind the crystalline walls of framework delivery systems"*; additionally, a range of local arrangements can be expected, including crystalline, amorphous, and partially ordered protocrystalline and organogel phases. However, a comprehensive methodology suitable to explore such *liquisolid* systems at atomic resolution was still absent. In collaborations with pharmaceutical companies (TEVA and Raciopharm), we developed computational-experimental approaches, which provide insight into these complex multicomponent systems. In this regard, we extensively explored structure of a range of *liquisolid* systems, the composition of which was systematically varied using various active compounds, ordinarily available solvents and a range of silica carriers. This way we covered majority of possible arrangements. Consequently, we formulated a general, easy-to-implement strategy for mapping the structure of organic phases integrated in mesoporous silica devices. This approach is based on a few straightforward solid-state NMR techniques, has no limitations regarding concentrations of the active compounds and enables straightforward discrimination of various organic constitutions. Among a range of typical arrangements of the active compounds and solvent molecules (crystalline, amorphous, liquid etc.), a unique, previously unknown organogel phases were unveiled and clearly identified. Moreover, with an aid of 2D ^{1}H-^{1}H MAS NMR correlation spectroscopy and high-level quantum-chemical calculations this uncommon low-molecular-weight organogel phases, existing exclusively in the porous system of the silica carrier, were described in detail. Overall, the proposed experimental approach allows clear discrimination of a variety of local structures of active compounds loaded in mesoporous silica drug-delivery devices in reasonably short time, and is thus applicable in advancement of novel drug-delivery systems in pharmaceutical industry. [*Molecular Pharmaceutics*. 14/6 (2017), 2070–2078]



Diversity of pharmaceutical formulations is large and strongly depends on demand for the most suitable administration of active compounds and their bioavailability. Mucoadhesive buccal films are an innovative way to efficiently deliver the active compounds to specific inflammatory sites in the oral cavity. As mucoadhesive buccal films are complex multicomponent and multiphase systems, traditional experimental approaches involving structural analysis are often not as useful for their studies. Therefore, we developed an alternative experimental strategy for comprehensive characterization of the structures of these films allowing us to probe the structure of the systems at nanometer scale. The potential applications and reliability of the proposed strategy, which is based on domain-selective ss-NMR spectroscopy, were demonstrated using novel mucoadhesive buccal films consisting of ciclopirox olamine, glycerol and poly(ethylene oxide) designed for the local treatment of oral candidiasis. By using the

abovementioned strategy, two distinct types of CPX/PEO mucoadhesive buccal films were distinguished. At low API loading, a nano-heterogeneous solid solution of CPX molecularly dispersed in an amorphous PEO matrix was created, whereas at high API loading, a pseudo-co-crystalline system containing CPX:2-aminoethanol nanocrystals incorporated into the interlamellar space of a crystalline PEO matrix was revealed. These structural differences were found to be closely related to the mechanical and physicochemical properties. Moreover, the molecular mechanisms controlling the structural and physicochemical properties of these mucoadhesive buccal films were unveiled. Therefore, we believe that detailed structural characterization is a fundamental prerequisite not only for the rational design of these pharmaceutical solids but also for the protection of intellectual property. [*Molecular Pharmaceutics*. 13/5 (2016), 1551-1563].

Structure of polysaccharides and their interactions: Following extensive development of advanced biomaterials, we also deal with the structure and dynamics of natural polysaccharides such as cellulose, starch, alginates, chitosan and glucans. Specifically, alginates (ALGs), naturally occurring biopolymers obtained from brown sea algae, are currently finding an increasing number of applications in many areas of human life. They are widely used in the delivery of many bioactive agents, in tissue engineering for cell transplantation, in regenerative medicine, and in other modern medical techniques, including the assembly of advanced medical devices. The applications of ALGs are, however, much wider and go beyond the field of biomedicine — for instance use of materials for capturing toxic metal ions such as Pb^{2+} and Cd^{2+} from waste water is very promising. Therefore, we focused on comprehensive investigation of the atomic-resolution structure and dynamics of polyvalent ion-cross-linked alginate gels. By applying advanced ss-NMR spectroscopy techniques, we verified the homogeneous distribution of the cross-linking ions in the alginate gels and the high degree of ion exchange. We also established, that the two-component character of the alginate gels arises from the concentration fluctuations of residual water molecules that are preferentially localized along polymer chains containing abundant mannuronic acid (M) residues.

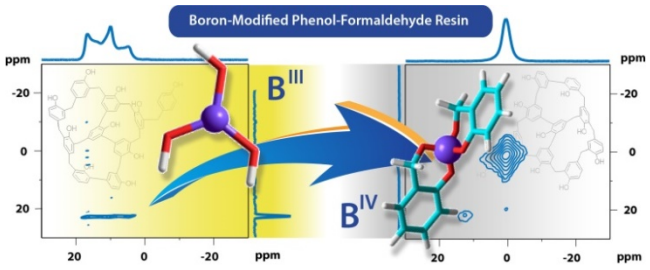


These hydrated M-rich blocks tend to self-aggregate into sub-nanometer domains. The resulting coexistence of two types of alginate chains differing in segmental dynamics was revealed by $^{1}H–^{13}C$ dipolar profile analysis. Next, the ^{13}C CP/MAS NMR spectra indicated that the alginate polymer microstructure was strongly dependent on the type of cross-linking ion. The observed disordering of the alginate chains was exclusively attributed to the M residues, whereas the structurally well-defined gels all contained guluronic acid (G) residues. Therefore, a key role of the units in M-rich blocks as mediators promoting the self-assembly of alginate

chains was experimentally confirmed. Finally, combining 2D ^{27}Al 3Q/MAS NMR spectroscopy with density functional theory (DFT) calculations provided previously unreported insight into the structure of the Al^{3+} cross-linking centers. Subsequently, we focused on investigation of interactions in alginate gels and of their structural transformations in physiological environments. In this regard, we prepared a set of ALG gels cross-linked by various ions and monitored their structural changes at different media simulating gastric and intestinal fluids and cellular environments. As demonstrated on the scheme above, depending on the environment, ALG chains and networks adopted different forms, such as acidic (hydro)gels stabilized by strong hydrogen bonds, and/or weakly cross-linked Na/H-gels. Simultaneously, the exchanged polyvalent ions extensively interacted with the environment even forming in some cases insoluble phosphate microdomains directly deposited in the ALG bead matrix. The extent of the transformations and incorporation of secondary phases into the alginate beads followed the size and electronegativity of the cross-linking ions. The applied combination of various macroscopic and biological tests with multinuclear ss-NMR revealed a complex pathway of alginate beads transformations in physiological environments. [*Biomacromolecules.* 18/8 (2017), 2478-2488, *Biomacromolecules.* 20/11 (2019), 4158-4170]

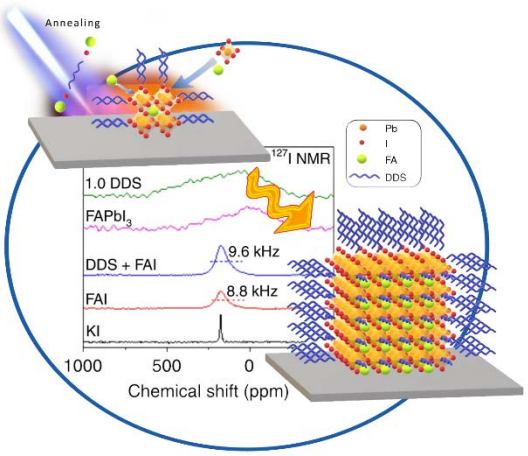
Advanced polymer composites and energy related materials: Clean energy, global warming, emission reduction — terms currently frequently used and often introduced in a variety of meanings and contexts. Effective utilization of solar and wind energy, and increased use of electric vehicles and portable electronics raises the demand for electric-energy conversion and storage devices. Our effort is thus also focused on the synthesis, optimization and precise structural characterization of innovative inorganic and hybrid functional materials exhibiting promising electric and ionic conductivity and other end-use properties.

Novel soft magnetic composites, that are comprised of spherical FeSi powder covered by chemically modified phenolic resins, demonstrate a remarkable combination of mechanical, electric and magnetic properties, and are typical example of such innovative polymer-based composites. Although boronic acid had been long in use as a crosslinking agent in the synthesis of phenol-formaldehyde systems, mechanism of its incorporation into the network was not thoroughly described. Therefore, we performed a detailed structural study of ammonium catalyzed boron-modified phenol-formaldehyde systems, including their thermally induced transformation. To describe the structure of the system in detail, we applied the quite recently developed advanced ^{11}B-^{11}B double-quantum MAS NMR correlation technique combined with quantum chemical DFT geometry optimizations and ^{11}B nuclear shielding calculations. These experiments revealed that boric acid is completely transformed to tetragonal mono-diol and di-diol spirocyclic borate anions, uniformly distributed in the polymer matrix. All the obtained experimental data were in an agreement with DFT geometry optimizations and ^{11}B nuclear shielding calculations performed for the set of predicted structures and reference compounds. Hence, we were able to conclude, that the application of advanced ^{11}B solid-state NMR experiments combined with quantum chemical calculations helped us to successfully disclose the arrangement of the boron units in the investigated systems. Moreover, we believe that this study can help to further clarify the mechanisms behind the formation of boron-modified phenol-formaldehyde systems and aid other researchers in the on-going quest towards new satisfactory soft magnetic composites. [*Macromolecules*. 48/14 (2015), 4874-4881]



Since 2019, we have been collaborating extensively with the researchers from the Department of Material Chemistry, Ångström Advanced Battery Centre, Uppsala University (Prof. Daniel Brandell) and Prof. Feng Gao (Division of Biomolecular and Organic Electronics, Linköping

University). Our joint research is focused on the development of advanced polymeric electrolytes and energy related materials for solar cells. Although this collaboration is relatively new, we have already reached several significant outcomes that have already been published in prestigious scientific journals. Specifically, implementation of Li metal anodes has the potential of substantially boosting the energy density of current Li-ion battery technology. However, this concept suffers greatly from fast fading of performance largely due to substantial volume change during cycling and from the poor stability of the solid electrolyte interphase (SEI). Fluoroethylene carbonate (FEC) is widely acknowledged as an effective electrolyte additive for improvement of the cycling performance of batteries, that consist of electrode materials that undergo large volume changes during cycling such as Li metal. In our joint study, we found, that while FEC is able to form a robust SEI on the electrode, it also deteriorates the shelf life of electrolytes containing LiPF6. The degradation mechanism of LiPF6 in FEC solutions was unraveled by liquid- and solid-state NMR. Specifically, traces of water residues induce the hydrolysis of LiPF6, releasing HF and PF5 which further trigger ring-opening of FEC and its subsequent polymerization. Moisture scavenger additives, such as lithium 4,5-dicyano-2-(trifluoromethyl)imidazole, are able to delay the degradation reaction as well as improve the cycling stability of LiNi1/3Mn1/3Co1/3O2/Li lithium metal batteries at 55 °C. [*ACS Applied Energy Materials*. 2/7 (2019), 4925-4935]



Similarly, our cooperation with Prof. Feng Gao (Division of Biomolecular and Organic Electronics, Linköping University) has recently resulted in the development of perovskite-molecule composite thin films for efficient and stable light-emitting diodes. Although perovskite light-emitting diodes (PeLEDs) experienced significant progress, there were only scattered reports of PeLEDs with both high efficiency and long operational stability, which called for additional strategies to address this challenge. We developed perovskite-molecule composite thin films for efficient and stable PeLEDs. The perovskite-molecule composite thin films consist of in-situ formed high-quality perovskite nanocrystals embedded in the electron-transport molecular matrix, which controls nucleation process of perovskites, leading to PeLEDs with a peak external quantum efficiency of 17.3% and half-lifetime of approximately 100 h. In addition, we found that the device degradation mechanism at high driving voltages was different from that at low driving voltages. This work thus provided an effective strategy for and deep understanding of efficient and stable PeLEDs from both material and device perspectives. [*Nature Communications*. 11/1 (2020), 891_1-891_9]

Polymers as well as metal-organic frameworks and covalent organic frameworks due to their structural variability and well-defined porous architecture are predetermined to be explored as the materials suitable for developing Li-battery (LiBs) electrodes, all-solid-state electrolytes and fuel cell ion-conducting membranes. In close cooperation with the department of Hybrid and Inorganic Materials for Energy Conversion, a series of new zirconium and titanium phosphates-organophosphonates, in which the organophosphonate moiety is functionalized with a sulfo group, was prepared by a topotactic reaction involving the gamma modification of zirconium or titanium hydrogen phosphate with 2-bis(phosphonomethyl)amino-ethan-1-sulfonic acid (H4TDP). The way how the topotactic reaction proceeds and how the grafted organophosphonate groups are bonded to the layers of the host structure were suggested on the basis of the solid-state NMR data. [*Inorganic Chemistry*. 59/1 (2020), 505-513].

Advanced catalysts, inorganic framework materials and ultra-wide-line NMR spectroscopy: Chemical production is a key industrial activity of current civilization. The vast majority of industrial chemical manufacturing utilizes a variety of catalytic processes.

Therefore, in order to achieve the most environmentally friendly chemical production, it is necessary to develop the most selective, most energy efficient processes. In collaboration with the JHIPC we focus on structural studies of zeolites and related framework materials - highly important heterogeneous catalysts.

Our extensive collaboration first focused on investigating the structure of catalytic centers, i.e. the framework aluminum Lewis sites and perturbed aluminum atoms in zeolites with the chabasite (CHA) and ferrierite (FER) topology. Besides Brønsted SiOHAl acid sites, also framework AlFR Lewis acid sites are often found in their protonated H-forms. The formation of AlFR Lewis sites in zeolites is a key issue regarding their selectivity in acid-catalyzed reactions. Therefore, we focused on structural investigation of the local structures of AlFR Lewis sites in dehydrated zeolites and their precursors — "perturbed" AlFR atoms in hydrated zeolites. For such study, we used combination of high-resolution MAS NMR and FTIR spectroscopy and DFT/MM calculations. We found, that perturbed framework Al atoms corresponded to $(SiO)_3AlOH$ groups and were characterized by a broad ^{27}Al NMR resonance (δ_i=59–62 ppm, C_Q=5 MHz) with a shoulder at 40 ppm in the ^{27}Al MAS NMR spectrum. We supposed, that dehydroxylation of $(SiO)_3AlOH$ that occurred at mild temperatures led to the formation of AlFR Lewis sites tri-coordinated to the zeolite framework. Al atoms of these $(SiO)_3Al$ Lewis sites exhibited an extremely broad ^{27}Al NMR resonance (δ_i≈67 ppm, C_Q>20 MHz). Our study thus indicated, that tri-coordinated framework (FR) $(SiO)_3Al$ atoms served as electron-pair-acceptor AlFR Lewis sites in zeolites with the CHA and FER topology. These sites could be formed by the dehydroxylation of AlOH atoms tri-coordinated to the framework. Both AlOH and AlFR Lewis species had broad ^{27}Al NMR resonances, but they were differentiated on the basis of their δ_i, C_Q, and η values. [*Angew. Chem. Int. Ed.* 2015, *54*, 541 –545]

Structural characterization of tri-coordinated sites with planar geometry is, however, extremely demanding and encounters hardware limitations, particularly when the expected quadrupolar broadening exceeds 25-30 MHz. Therefore, we turned our attention on finding friendly ss-NMR technique to obtain the required spectroscopic and structural parameters. In this regard we investigated a range of organoaluminum compounds (Lewis acids and Lewis adducts) considerably differing in their chemical constitution. For such description, we optimized an experimental/computational strategy based on solid-state NMR crystallographic approach. In particular, we focused on the precise measurement and subsequent quantum-chemical analysis of many different ^{27}Al NMR resonances in the broad range of quadrupolar coupling constants from 1 to 50 MHz. In this way, we resolved spectroscopically six different products in the resultant polycrystalline mixture. Interestingly, in some cases the recorded ^{27}Al solid-state NMR spectra showed unexpected quadrupolar coupling constant values reaching up to ca. 30 MHz, which were attributed to tetra-coordinated aluminum species (Lewis adducts with trigonal pyramidal geometry). The cause of this unusual behavior was explored by analyzing the natural bond orbitals and complexation energies. Our findings thus provided a viable approach for the direct identification of Lewis acids and Lewis adducts, in multicomponent organoaluminum compounds as well as in zeolites featuring catalytically active trigonal (Al^{III}) and strongly perturbed Al^{IV} sites. [*Inorganic Chemistry*. 57/12 (2018), 7428-7437]

In parallel, our research continued in an attempt to optimize efficient strategies of NMR crystallography for the detailed characterization of monovalent cations in inorganic matrixes. Specifically, we focused on Li^+ siting and the local structure of Li^+ sites in ferrierites. In this regard, we utilized techniques of ^7Li–^7Li correlation MAS NMR spectroscopy. The obtained experimental data were subsequently interpreted using periodic DFT calculations including molecular dynamics conformational sampling of Li^+ sites. This way we obtained a detailed view into the siting of Li^+ at exchangeable positions of ferrierites and the local structure of these Li^+ sites. The developed approach thus can be in general applied to Li^+ ions in other zeolites and various crystalline matrixes with large unit cells and a low concentration of Li^+ and also to other NMR-active cations without a significant limitation of their concentration. [*Chem. Commun.*, 2015, *51*, 8962-8965]

Research activity and characterisation of the main scientific results

Research activities of the team can be presented in three main directions: conducting polymers, polymers for organic electronics and photonics, including polymers for energy conversion, and polymers for energy storage. The most important results of this mutually interconnected research achieved during the evaluated period are listed below. Some results listed in this section are also shown in the respective section describing selected results of the Institute.

I. Conducting polymers

The research activities were focused on achieving increased electrical conductivity of polypyrrole and polyaniline by optimisation of their nanostructures using various organic and inorganic templates during their polymerisation. Although the synthesis is based on well-established oxidative polymerisation, it was discovered that when some organic molecules are added during the polymerisation or the polymerisation takes place in the presence of inorganic nanoparticles, better organised molecular structures can be obtained with superior values of the electrical conductivity.

Result I.1: Polypyrrole with improved conductivity

In our several recent papers, the close correlation of polypyrrole conductivity and its nanostructure was shown. We found that conductivity of polypyrrole was increased by around two orders of magnitude after addition of anionic organic dyes (methyl orange) to the polymerisation medium. Based on the morphological studies, we attributed this increase to the preferential formation of the nanotubular form of polypyrrole. On the other hand, when ethyl orange was admixed instead to the reaction mixture, polypyrrole with globular morphology was formed but the conductivity was still improved, reaching the value of 27 S cm^{-1}. The marked difference in morphology is explained by the ability of methyl orange salt to act as a solid template for the nanotubular growth of polypyrrole under acidic conditions, in contrast to ethyl orange. The latter dye, however, acts similarly to surfactants, which yields smaller but still marked increase in polymer conductivity even in its globular form. [ASEP (ID 474353), ASEP (ID 471844)]. The above principle was successfully applied in the preparation of functional conductive fabrics. Together with company “INOTEX”, a partner from the industrially directed project within Center of Competence financed by the Technology Agency of the Czech Republic, we developed a procedure for coating cotton with nanotubular polypyrrole [ASEP (ID 446891)]. The fabric was used in a functional conductive textile with antistatic and non-flammable properties. The method was protected by a Utility Model as an intellectual property [Bober, P., Josefík, F., Kubáč, L., Martinková, L., Marek, J.: Functional conductive flexible fabric with antistatic and non-flammable properties. 2019, Utility Model No. 33188].

Our team brought the idea, performed the synthesis and electrical characterisation. Optical spectroscopy was performed outside the team.

Result I.2: Conducting cryogels

Until recently, composites of conducting polymers were prepared mostly as powders, and their insolubility caused their poor processability, while their infusibility limited their practical applications. To overcome this problem, synthesis of new soft polymer materials, dubbed conducting cryogels, was invented by our team. Polyaniline cryogels were produced when the polymerization of aniline took place in a frozen medium containing a supporting water-soluble polymer, such as poly(vinyl alcohol) [ASEP (ID 471010)]. Conducting polymers in the combination with supporting polymers can afford the hydrogel/cryogel with desired properties, such as high electrical conductivity, but also with enhanced mechanical strength.

OPTOEL team brought the idea, performed synthesis and partially also electrical characterisation. Other characterisation was contributed by Wood K Plus, GmbH, Linz, Austria, and by Charles University in Prague.

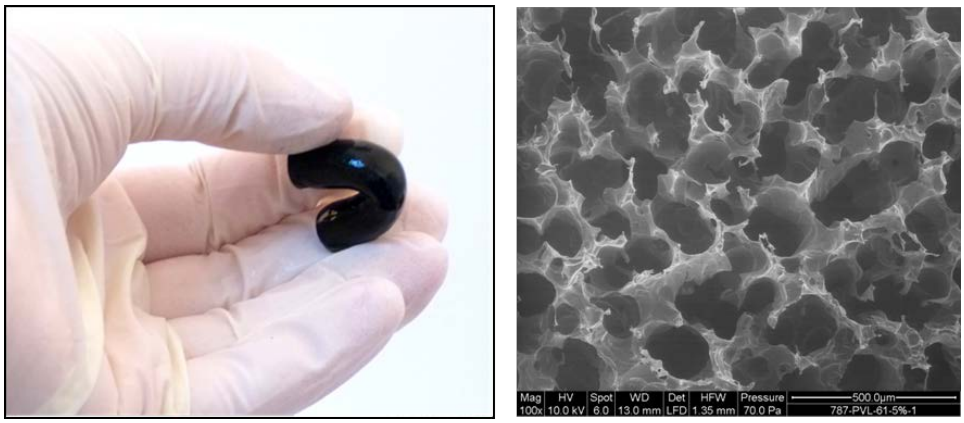


Figure I.1: Conducting polyaniline cryogel

II. Polymers for organic electronics and energy conversion

Result II.1: Semiconducting thienothiadiazole-based donor-acceptor copolymers

Low-bandgap donor-acceptor (D–A) copolymers belong to the third-generation semiconducting polymers that are extensively studied due to their potential as active materials for photonic and electronic applications. Series of D–A copolymers with the backbones consisting of various combinations of electron-donating and electron-accepting units, and derivatised with various side chain combinations were designed, prepared and their optical absorption, photo- and electro-luminescence, spectroelectrochemical and photoelectrical properties were studied in solutions, thin films, polymer blends and/or hybrid systems. The optical and electrical properties were correlated with the copolymer backbone and side chain chemical structure and molecular weight [ASEP (ID 440636), ASEP (ID 465301), ASEP (ID 474405), ASEP (ID 474645), ASEP (ID 506545), ASEP (ID 512150), ASEP (ID 475915)].

In the first series, the alkyl substituted 4,6-di(thiophen-2′-yl)thieno[3,4-c][1,2,5]thiadiazole (T) unit, which had not been widely explored in D–A copolymers previously, was used as an electron-acceptor building block. We demonstrated that the thienothiadiazole-based D–A copolymers with various electron-donor blocks and side chain combinations (Fig. II.1) possess interesting optoelectronic and electrochromic properties, which are influenced by the donor strength of the electron-donor unit and the nature and length of the alkyl chains attached to the thiophenes of T unit.

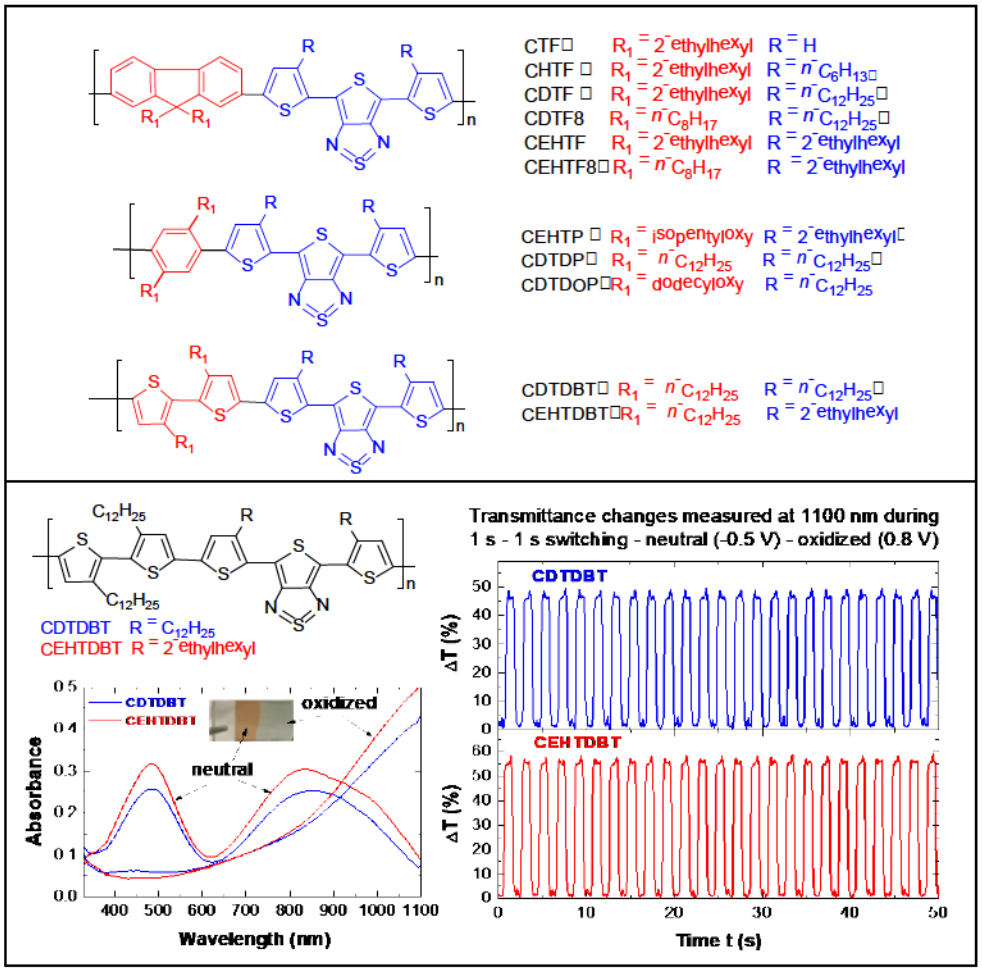


Figure II.1: Chemical structures of D–A copolymers with 4,6-di(thiophen-2′-yl)thieno[3,4-*c*][1,2,5]thiadiazole (T) acceptor units and an example of electrochromic switching.

Selected copolymers were tested as active layers in photovoltaic devices [ASEP (ID 440636), ASEP (ID 465301), ASEP (ID 474405), ASEP (ID 474645), ASEP (ID 506545)]. Correlations between device performance and copolymer structure were elucidated. The devices based on copolymers with linear side chains on T unit exhibited better performance than those based on copolymers with branched chains [ASEP (ID 440636), ASEP (ID 465301),ASEP (ID 474405)]. Detailed study of thin films made of these copolymers and their blends with fullerene [60]PCBM used as active layers in bulk heterojunction solar cells was performed by means of UV–vis absorption spectroscopy and Raman microspectroscopy and provided information on polymer molecule planarity, homogeneity of blends and interactions that occur in polymer thin films and affect the device performance [ASEP (ID 474645)]. Effects of alkyl or alkyloxy side chains in CDTDP and CDTDOP copolymers were shown. Both copolymers exhibited interesting electrochromism. Optical switching with faster response times were found for CDTDOP, which exhibited also interesting thermochromic effect [ASEP (ID 474405)]. New copolymers CDTDBT and CEHTDBT showed optical absorption covering the entire visible spectrum and extending up to near infrared, and electrochromic switching with fast response time, which is favourable for many electrochromic applications (Fig. II.1) [ASEP (ID 506545)]. For the series of D–A copolymers with substituted T units, we revealed, that the response times are affected by the nature of the donor units and not by the nature of the sidechains attached to T unit.

The work was done within OPTOEL team besides IR and Raman characterization contributed by another team at the IMC.

Result II.2: Perylene tetracarboxydiimide based D–A copolymers

Perylene-3,4,9,10-tetracarboxydiimide (PDI) derivatives and PDI-based polymers are of interest due to many potential applications in photonics and electronics. For the application of PDI-based polymers, it is important to understand how their optical and electrochemical properties can be tuned by various donors or via attachment of different side chains to the perylene core or to the donor units. We focused on the D–A copolymers composed of confirmed 1,7-regioisomers of *N,N′*-dialkylperylene-3,4,9,10-tetracarboxydiimide electron-acceptor and three different electron-donor units (9,9-dioctylfluorene or 9-alkylcarbazoles), which were successfully prepared and their properties studied (Fig. II.2.) [ASEP (ID 512150)]. They exhibit very good thermal and oxidation stability and interesting photophysical, electrochemical and spectroelectrochemical properties. Effects of the side chains were shown and their potential applications in photonics tested. The results of electrochemical and spectroelectrochemical studies indicated their possible use for sensing, where photoluminescence quenching in the reduced copolymer solution was demonstrated.

The work was performed entirely within the OPTOEL team.

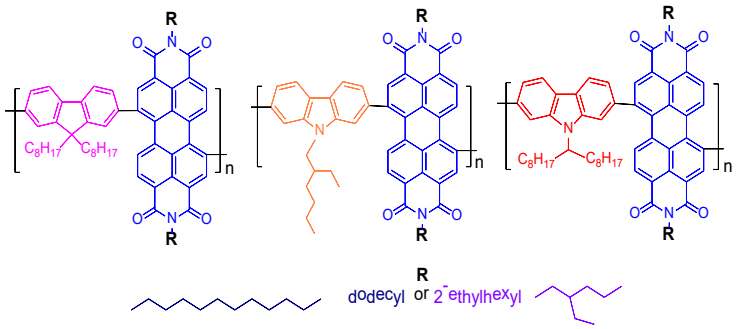


Figure II.2. Perylene-based copolymers with various side chains.

Result II.3: Polymer synthetic routes, molecular parameters and properties relationship

Alternating electroluminescent copolymers, poly(9,9-dihexadecylfluorene-2,7-diyl-alt-2,2′-bithiophene-5,5′-diyl)s, were synthesized using two different routes of Suzuki coupling and microwave-assisted or in high boiling point solvents conventional heating (Fig. II.3). Copolymers with various molecular weights (MW) were obtained depending on the synthetic conditions, and their photophysical, electrochemical and electroluminescent properties were investigated. Impact of MW and of the side chains length on the properties were revealed. [ASEP (ID 484316)].

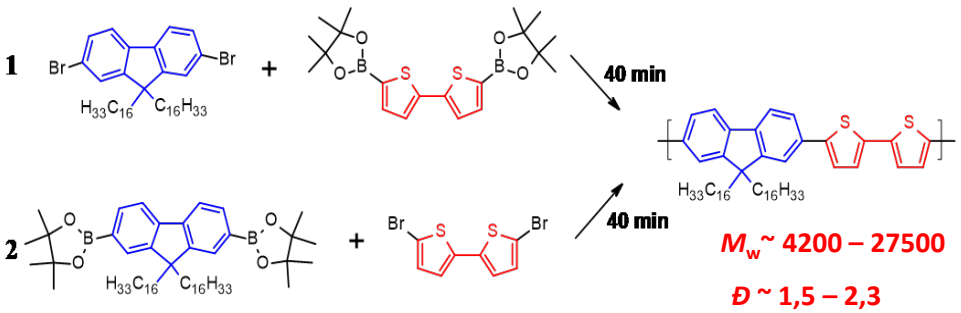


Figure II. 3: Two routes of the synthesis of poly(9,9-dihexadecylfluorene-2,7-diyl-*alt*-2,2′-bithiophene-5,5′-diyl) via Suzuki coupling.

Result II.4: Effects of building block and side chain combinations in poly[(arylene ethynylene)-*alt*-(arylene vinylene)]s

Series of copolymers with poly[(arylene ethynylene)-*alt*-(arylene vinylene)] backbone possessing various combinations of building blocks and side chains were studied with the aim to get information about correlation between the structure and properties, which is important for many photonic applications. The combinatorial effects of side chain configuration in series of poly(*p*-phenylene-ethynylene)-*alt*-(*p*-phenylene-vinylene)s with symmetrical and dissymmetrical configurations (partial or total) of octyloxy and/or octadecyloxy chains at the phenylene-ethynylene and/or phenylene-vinylene segments, respectively, on photophysical, electrochemical and electroluminescent properties were shown [ASEP (ID 455639)]. Contrary to solutions, the influence of various combinations of side chains was more pronounced in thin films, whose properties depended on molecular weight and film thickness as well.

For the first time, large polycyclic aromatic building blocks based on anthanthrone and anthanthrene were employed as the building blocks for the synthesis of copolymers based on poly-[(arylene ethynylene)-*alt*-(arylene vinylene)]s backbone (Fig. II.4 right) [ASEP (ID 481359)]. The effects of side chains and also solvents on photophysical, electrochemical, electroluminescent and photovoltaic properties were revealed. The properties were not only dominated by the polycyclic chromophore, but were also influenced by the nature of the side chains on the phenylene-vinylene unit.

All photophysical, electrochemical and electroluminescent studies were performed and polymers partially characterised by OPTOEL team. The polymers were synthesized by partners abroad.

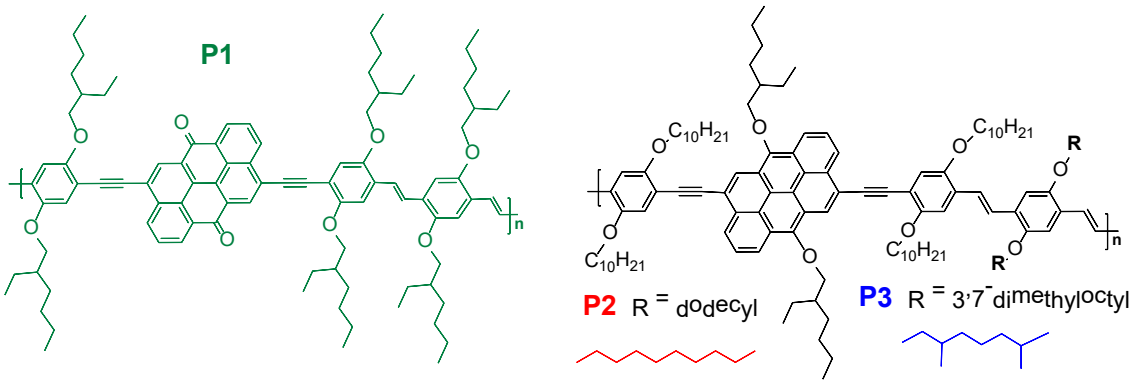


Figure II.4: Chemical structure of anthanthrone and anthanthrene-based poly[(arylene ethynylene)-*alt*-(arylene vinylene)]s under study.

Result II.5: Plasmonic composites

The work on plasmonic composites revealed the processes that occur at early stage after mixing a hydrosol of negatively charged borate-stabilized silver nanoparticles with a solution of cationic regioregular polythiophene and that influence aggregate formation. Three populations of species were found within milliseconds upon mixing the components at various ratios, which differ in the mean size, extent of polymer coating, and temporal stability. The morphology of aggregates was correlated with plasmonic properties [ASEP (ID 453063)]. Using ultrafast transient optical absorption spectroscopy, we found that an ultrashort laser pulse absorbed by the array of metal nanoparticles attached to the polymer semiconductor surface produced a propagating heat wave. We observed, that the diffusivity of excitons – mobile excited species generated in the semiconductor by the same laser pulse – was increased by the wavefront of this heat wave. This effect is interesting for the development of polymer light sensors [ASEP (ID 474036)].

The idea, is from the OPTOEL team, which prepared composites and performed optical study. Other teams from the IMC performed dynamic light scattering and small-angle X-ray scattering. The cationic conjugated polymer was obtained from the Charles University in Prague.

Result II.6: Supramolecular polymers for singlet fission

Using time-resolved absorption spectroscopy, the relaxation processes in oligomers and dynamers based on α,ω–bis (terpyridyl) oligothiophene were characterized. The effect of coplanarity on the excitation energy decay was observed, influenced by side solubilization groups [ASEP (ID 445857)]. We were the first who observed the effect called singlet fission in metallo-supramolecular polymer thin films. Singlet fission is a phenomenon in which two triplet excited states are created from a single photon absorbed in an organic compound. Since this phenomenon has been considered as a possible way to overcome the Shockley–Queisser efficiency limit of solar cells, our discovery extended the material base for the solar cells with higher energy conversion efficiency [ASEP (ID 477332)].

All the photophysical studies and the discovery of singlet fission effect were accomplished by members of the OPTOEL team, crystallography was contributed by another teamat the IMC, and an estimated 20 % contribution represents synthesis of materials at Charles University in Prague.

Result II.7: Charge carrier transport in polymer semiconductors

We elaborated a molecular-scale model of charge carrier transport in polymer semiconductors, which combines the quantum mechanical description of the on-chain charge transport states with a semi-classical solution of the inter-chain charge hopping. Unlike previous models, our model takes into account local structural anisotropy of conjugated polymers. The obtained results explain the experimentally observed mobility degradation in organic field-effect transistors (OFET) at high gate voltage [ASEP (ID 472995)].

We contributed to the methodology of determination of charge carrier mobility from measured electrical characteristics of OFETs. We have shown that the method of calculation of charge mobility in thin film OFETs recommended by the IEEE Standard does not yield relevant numbers due to inappropriate assumptions adopted. Instead, we analytically derived exact relations that describe better the real physical mechanisms in the

OFET [ASEP (ID 485523)]. We developed a new methodology for determining the mobility of charge carriers from the characteristics of OFET, which is especially suitable for polycrystalline polymer semiconductors. Our procedure is based on the analytical solution of the drift-diffusion equation and, in contrast to the standards recommended by the IEEE, accounts for the concentration dependence of the mobility of charge carriers. This methodology can also be combined with the molecular-scale models of mobility *(vide supra)* taking into account the specific properties of conjugated polymeric materials.

We also elaborated a new theoretical model of the charge carrier transport in oligomeric nanobrush layers, which takes into consideration the anisotropy of conjugated chains. We found a significantly increased value of the hole mobility for low values of the gate voltage. The conclusions of this model contribute to the theoretical background for a novel vertical OFET architecture [ASEP (ID 501759))].

We also derived a new method for determination of time-dependent diffusion coefficient of photoexcited species from their collision rates measured by time-resolved optical absorption spectroscopy. [ASEP (ID 494710)] This approach was applied for the analysis of time courses of transient absorption acquired on regioregular poly(3-hexylthiophene) thin films, frequently used in organic solar cells and can be applied for both neutral (excitons) and charged (polarons) species [ASEP (ID 490819)].

The main work and the ideas originated from the OPTOEL team with some contribution from Polish and Taiwanese partners, each of them less than 5 % of the total work on the topic.

III. Polymers for energy storage

The team contributed to the solution of highly topical problem related to zero-carbon energetics by solving one of the crucial tasks — storage of energy from intermittent renewable sources. The activity covered three possible ways of energy storage: batteries, hydrogen fuel cells, and supercapacitors. The team focus was directed particularly towards the development of new materials for electrodes and membranes.

Result III.1: New carbons for energy conversion and storage.

Development and characterisation of electroactive materials with specific redox behaviour have recently become the main focus of the team. For this purpose, several new carbon materials were invented by our team via transformation of conducting polymers.

PANI cryogels with poly(vinyl alcohol) were recently prepared, and further converted to corresponding carbogels by carbonization at 500–600 °C in inert atmosphere [ASEP (ID 500356)].The preparation of nitrogen-containing carbon derivatives by carbonization of freeze-dried conducting polymer cryogels increased the specific surface area of such materials up to four orders of magnitude. It reached the value of 931 $m^2\ g^{-1}$ for carbonized poly(p-phenylenediamine) cryogel, which is larger than that for its carbonized powder analogue [ASEP (ID 522109)].

Polypyrrole nanotubes were carbonized in inert atmosphere to nitrogen-enriched carbon nanotubes, which were subsequently coated with 20 wt% of polypyrrole prepared with different morphologies: globular, nanotubular or nanofibrillar. Morphology of the coating proved to have a marked effect on the capacitive performance. All three coating forms were transformed in the presence of products of oxygen reduction, most probably hydrogen peroxide, in the first ten cycles [ASEP (ID 504696)]. Change of the materials activity with respect to different reactions makes them a multipurpose material enabling fine tuning of their activity for specific applications, in alkaline fuel cells or supercapacitors.

Additionally, phosphorous and nitrogen-containing carbons were developed. Such simultaneous introduction of nitrogen and phosphorus atoms into carbonaceous materials leads to the surface structures exceptionally suitable for the charge storage as noble metal-free catalysts for the oxygen reduction reaction [ASEP (ID 475503)].

Novel nanostructured carbonaceous systems were prepared, in which cellulose fibres were coated with polyaniline and carbonized in inert atmosphere. The carbon core originating from cellulose was thus coated with a nitrogen-containing carbon shell produced from polyaniline. Such composite was not electrically conductive, but had a specific surface area 495 m^2 g^{-1} [ASEP (ID 463921)].

The ideas and synthesis came from the OPTOEL team. Material characterisation was contributed by other teams of the IMC and within the cooperation with University of Belgrade (Serbia), Kompetenzzentrum Holz GmbH (Austria), Polymer Institute SAS (Slovakia) and Harvard University (USA).

Result III.2. Ion exchange membranes

Membranes represent the key component in batteries and fuel cells. We developed a series of ion exchange polymers and used them for preparation of membranes and catalyst binders. Contrary to currently used diaphragms for water electrolysis (AEM WE) that use binder and membranes as hydroxide conducting material, we used a different approach, in which new materials based on quaternised poly(phenylene oxide), PPO, (Figure III.1) were used as a binder and solid electrolyte. We discovered that the cells assembled with membranes made of such material show higher efficiency than common commercial alkaline electrolysers. We showed a unique and simple procedure for the preparation of anion exchange binders for electrodes in alkaline water electrolysers based on commercial PPO. Use of such binders instead of currently used ones (PTFE) brings an advantage of higher thermodynamic efficiency and the possibility to use low concentration KOH electrolyte or even neat water. Besides that, the binders prepared by us are processable from environmentally friendly solvents like ethanol. The binders are stable up to 50 °C [ASEP (ID 439495)].

Another example of ion exchange membrane material developed by OPTOEL is a block copolymer polystyrene-*block*-poly(ethylene-ran-butylene)-*block*-polystyrene (PSEBS), which was chloro-methylated by an innovative indirect method (Fig. III.1). Such prepared membranes were quaternised by 1,4-diazabicyclo[2.2.2]octane. Relatively high ion conductivity of quaternised membrane (75 mS cm^{-1}) and IEC = 0.76 mmol g^{-1} at 30 °C was achieved.

Alkaline water electrolysis has an advantage over acidic electrolysis, because abundant and cheap electrocatalysts may be used. On the other hand, industrial alkaline water electrolysers use diaphragm and gap between electrodes, which increases ohmic losses. To minimize the ohmic losses, highly concentrated (30 %) KOH is used as an electrolyte. With PSEBS DABCO membranes and binder, low concentration of KOH or even water can be used. Chemical stability of these membranes is sufficient up to 50 °C.

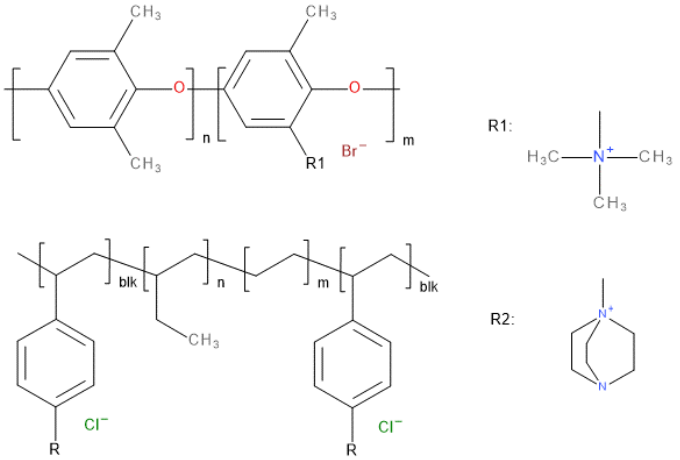


Figure III.1: Top — Trimethylamine-quaternised poly(2,6-dimethyl-1,4-phenylene oxide); bottom — block copolymer polystyrene-*block*-poly(ethylene-ran-butylene)-*block*-polystyrene (PSEBS) quaternised with trimethylamine and 1,4-diazabicyclo[2.2.2]octane.

Utilization of anion exchange membranes in electrolysers is conditioned by stability of functional group. One of the most stable anion exchange group in alkaline environment is benzyltrimethylammonium group. We prepared trimethylamine-quaternised block copolymer (PSEBS TMA,). This polymer utilizes the same chloromethylated precursor as PSEBS quaternised by 1,4-diazabicyclo[2.2.2]octane. Ion conductivity of membrane prepared from this polymer was found to be 56 mS cm^{-1} at 30 °C and 79 mS cm^{-1} at 70 °C. Laboratory electrolyser with trimethylamine-quaternised block copolymer PSEBS and binder of the same material exhibited no significant degradation and no loss of performance during one-month operation.

Result III.3. Application of anion exchange membrane in microbial fuel cell

Electrochemical and microbiological insights into the use of 1,4-diazabicyclo[2.2.2]octane-functionalized anion exchange membrane in microbial fuel cell was shown in a benchmarking study on Nafion. Our anion-exchange membranes based on DABCO functionalized polystyrene-*block*-poly(ethylene-ran-butylene)-*block*-polystyrene (PSEBS) were used in microbial fuel cell and proved, that this membrane outperformed benchmark membrane Nafion in electrochemical parameters. Two polymeric membrane separators (a proton exchange membrane (PEM), Nafion; and an anion-exchange membrane (AEM), 1,4-diazabicyclo[2.2.2]octane (DABCO)-functionalized PSEBS) deployed in microbial fuel cells (MFCs) were comparatively assessed. The performances of MFCs according to membrane type were evaluated by biological and electrochemical techniques, employing metagenomics, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). It was found that the anodic biofilms of MFCs, irrespective of the type of membrane, were dominated by *Geobacter sulfurreducens* (37 and 50 % for AEM-MFC and PEM-MFC, respectively), a well-known electrochemically active species. Furthermore, the AEM-MFC reflected a significantly lower internal resistance (145 Ω) compared to PEM-MFC (339 Ω) and produced higher maximal current densities and energy yields at all substrate (acetate) concentrations. The CV measurements implied diffusion limitations in the MFCs, which were supported by EIS. In addition, the PEM and AEM characterisations revealed that in both cases, the ion exchange capacity, ionic conductivity and oxygen mass transport features were altered considerably during 39 days of the MFCs operation. [ASEP (ID 518951), ASEP (ID 439495), Patent Application PV 2020-481].

The work on the anion-exchange membranes was a collaborative research with University of Chemistry and Technology in Prague. Synthesis of polymers, preparation of membranes and their characterisation were performed within the team. Swelling tests and NMR characterisation were completed by other teams at the IMC, alkaline stability, IEC, IC determination and electrolyser tests at University of Chemistry and Technology, Prague, MFCs experiments were performed at University of Pannonia, Hungary.

Result III.4. Interpenetrating polyaniline/polyphenylene oxide membrane for gas separation

Composite membranes based on polyphenylene oxide (PPO), polyaniline (PANI) separation layer and interpenetrating PANI/PPO layer were prepared. It was demonstrated for the first time that the interpenetrating layer of PPO and PANI plays a crucial role in the transport and separation of gases. The preparation of such composite membranes is very simple and inexpensive. The membranes simultaneously possess good transport properties and excellent separation coefficients for industrially interesting gases, such as $O_2/N_2 > 13$, $H_2/CO_2 > 8$, $CO_2/CH_4 > 110$, $H_2/CH_4 > 1000$ and $H_2/N_2 > 450$. Importantly, the composite membranes exhibit a superior long-term stability: no observable changes in the transport properties and separation coefficients were detected after 14 months of operation. [European Patent EP2858739-B1(granted)]. The work was contributed entirely by members of the OPTOEL team.

Result III.5. Conducting polymers for supercapacitors

The team focused on the electrochemical characterisation of conducting polymers (particularly polyaniline and PEDOT) as active material for pseudocapacitors. It was shown by us that hydrogen bonding played a crucial role in the charge propagation and storage [ASEP (ID 442968)]. Moreover, we isolated and studied individual polyaniline chains for the first time and compared them with bulk polyaniline. We demonstrated, that the interaction between polyaniline chains and their assembly into nanofibrils had a crucial impact on electro- and physical−chemical behaviour of PANI. The mechanism of the redox process for individual PANI chains and PANI nanofibrils is proposed by us. The specific capacitance of 1050 F g^{-1} for three-electrode configuration and 450 F g^{-1} for two-electrode configuration was obtained [ASEP (ID 488904)]. The unique pseudocapacitor performance of polyaniline was investigated and the results were reported in and impacted journal. PANI/CC electrodes with various mass loadings of PANI were prepared as high-rate charge/discharge pseudocapacitors. The maximum areal capacitance was obtained for the highest mass loading of PANI with a value of 2450 mF cm^{-2}, corresponding to an areal energy density of 265 mWh cm^{-2} and areal power density of 48 mW cm^{-2}. The maximum specific capacitance was obtained for the lowest mass loading of PANI with a value of 1120 F g^{-1}, corresponding to a specific energy of 147 Wh kg^{-1} and specific power of 19 kW kg^{-1} [ASEP (ID 481355)]. The work was contributed almost entirely by members of the OPTOEL team, electron microscopy, XPS spectra and photoluminescence were done outside the team.