WP5: Bio-sensors

Research strategy and methodology

The main goal of the WP5 is to explore and find new principles leading to the construction of diamond nanosensors based on optical and quantum detection technologies. We will focus on creation of analytical tools for imaging of biologically important parameters in living cells with ultrahigh spatiotemporal resolution using optically detected magnetic resonance. The primary research strategy involves 1) optimization of NV center creation in nanodiamonds using isotropic irradiation with high energy particles and 2) development of robust nanointerfaces on nanodiamonds ensuring biocompatibility for vast spectrum of biological systems using bioorthogonally reactive hydrophilic polymers (Fig. 13). These interfaces will bear recognition elements for sensing of biomolecular analytes (nucleic acids, proteins) and presence of radicals. New types of antifouling *poly*(glycerol)-based polymers and approaches for their modifications will be also developed.

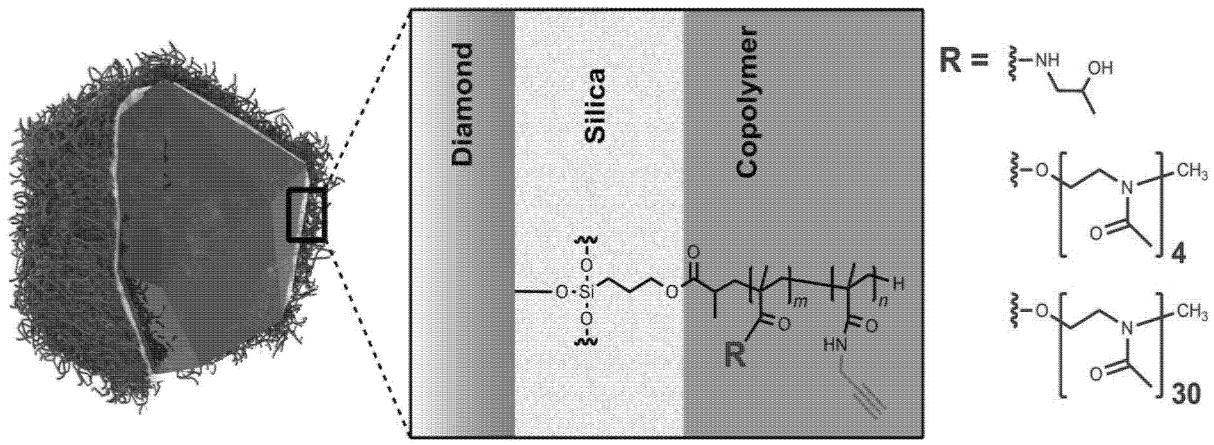

Fig. 13: Poly[N-(2-hydroxypropyl) methacrylamide] and two brush-like poly(2-methyl-2-oxazoline)-based co-polymers grafted from a nanodiamond particle. The chains contain also alkyne groups for rapid and highly bioorthogonal "click" conjugation chemistry suitable for aqueous environments.

An unprecedented approach proposed here is use of such hybrid structures for development of targeted intracellular nanothermometers. We will design and prepare novel *thermoresponsive (TR) architectures* on nanodiamonds operating in completely *reversible* manner. These shells will contain either spin labels (stable radicals or Gd^{3+}-complexes) embedded in TR copolymers or magnetic nanoparticles (MNPs) will serve as an alternative spin bath with several orders of magnitude larger response on a different time scale (Fig. 14). In order to ensure untainted coherent rotation of the MNP giant classical spin at ambient conditions, known as the superparamagnetic response, the particle diameter will be tuned below 8 nm. In order to inspect the role of characteristic relaxation time of the spin bath and mutual coupling of the spin species, a heterogeneous spin bath will be maintained with the help of carefully designed core-shell spin structures. The most suitable candidates were identified among spinel ferrites with relatively soft magnetic properties and approved biocompatibility: $Mn_xFe_{2-x}O_4$; x = 0 – 1. The MNPs with narrow size distribution and tunable crystallinity will be synthesized using decomposition of metal oleates in hydrothermal conditions or decomposition in high boiling solvents. The resulting MNPs are stabilized with oleic acid coordinated to the MNP surface. The oleic acid will be exchanged for PEG-hydrazide and the MNPs of interest will be attached *via* hydrazone ligation to the aldehyde-modified nanothermometer surface.

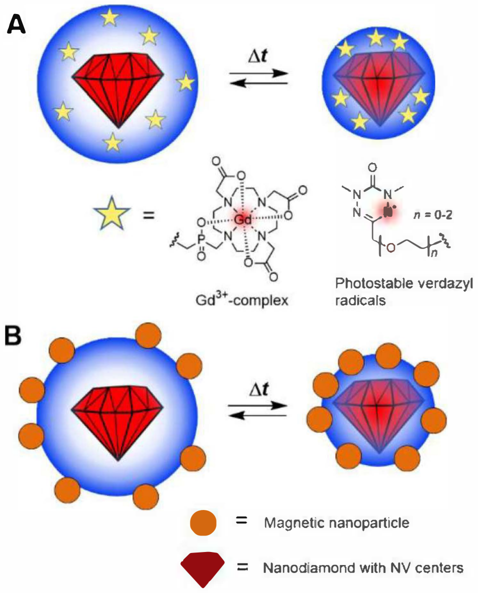

Fig. 14: Structure of hybrid temperature nanosensors based on readout of changes in magnetically active thermoresponsive polymer coating. A) Crosslinked shell loaded with spin labels, B) Magnetic nanoparticles installed on surface of thermoresponsive polymer.

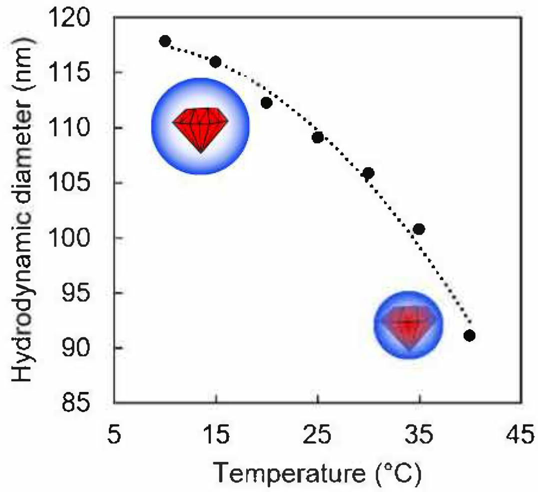

Fig. 15: Nanodiamonds coated with a thermoresponsive polymer show monotonous decrease with increasing temperature (unpublished results).

In our preliminary experiments, we confirmed that such shell can be prepared and the required TR behavior covers the biologically relevant temperature range (Fig. 15). We will synthesize and attach to the best TR nanoparticles cell penetrating peptides (TAT, Arg9) and organelle-targeting peptides/moieties for localized and addressable intracellular temperature imaging. As an internal reference, other nanodiamonds bearing Si-V centers will be coupled with the hybrid nanothermometers. The optically detected magnetic resonance measurements will be performed in collaboration with Prof. Joerg Wrachtrup, University of Stuttgart, Germany, who is actively collaborating with Dr. Cigler.

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Objectives

- To optimize the creation of NV centers in nanodiamonds for construction of nanosensors.
- To formulate a functional diamond nanointerface for *in vitro* and *in vivo* sensors with extreme spatiotemporal resolution.
- To develop intracellular nanosensors based on nanodiamonds and spin entities.

Activities

A5.1.: Pushing detection limitations of nanodiamonds via intrinsic and extrinsic modifications
Duration: M1 – M48

Duration	Task description	Groups involved
M1-M36	Preparation of vacancies in nanodiamonds using new isotropic approaches. The techniques will involve irradiation with high energy electrons in microtron and irradiation of composite materials containing nanodiamonds and ^{10}B with neutrons in nuclear reactor. The neutron capture generates high energy alpha particles from ^{10}B, which leads to formation of vacancies. Alternative nuclear reactions will be explored as well.	UOCHB
M6-M24	Optimization of sample work up (annealing, oxidation), isolation of fractions of very small nanodiamonds (<10 nm) and their purification. Characterization of these particles using TEM, NTA, XRD, FTIR, and XPS.	UOCHB, UFCH, MFF UK
M12-M48	Mass production of nanodiamonds (grams to tens of grams) with high concentration of NV centers using optimal irradiation and annealing conditions.	UOCHB
M1-M24	Surface treatment of nanodiamonds avoiding surface diamond lattice damage and formation of spin noise. Insertion of carbon monoxide to carbocations generated in acidic environment (Koch reaction) followed by hydrolysis and formation of carboxylic acids will be investigated. Characterization using EPR, FTRI and NTA.	UOCHB
M18-M42	Creation of dual probes with internal reference (SiV center) and reporter (NV center). Chemical conjugation of detonation nanodiamonds with SiV centers with HPHT nanodiamonds bearing NV centers. Development of dual sp^3-sp^2 linking chemistry for connection of HPHT and detonation diamonds.	UOCHB, OZM, UFCH, MFF UK

M1-M36	Development of one-step robust polymer coating procedures in environments ensuring colloidal stability of HPHT and detonation nanodiamonds. Novel types of epoxide derivatives containing azide and cationic moieties will be synthesized and copolymerized with glycidol from diamond surface using solvent-free approach. Dense dendritic coatings on nanodiamonds will be analyzed and tested for biocompatibility and ability for bioconjugation.	UOCHB, OZM
Milestone MS5.1 v M24: Identified and confirmed pathway to optimized nanodiamonds with color centers for sensing.		
Deliverable D5.1 v M26: Summary report describing preparation of optimized nanodiamonds with color centers for sensing.		
A5.2.: Polymer interfaces for nanodiamond sensors for *in vitro* and *in vivo* applications Duration: M1 – M48		
Duration	**Task description**	**Groups involved**
M1-M24	Development of thermoresponsive polymer coating on nanodiamonds. Optimization of composition addressing the proper fraction of reactive groups for further modification. Characterization of thermoresponsive behavior using NTA, QELS, TEM, fluorescence spectroscopy. Comparison with other materials (SiC).	UOCHB, UFCH, MFF UK
M12-M48	Controlled growth of ultrathin polymer layers on diamond surfaces for construction of sensitive sensors. Optimization of radical polymerization for diamond surfaces. Decoration of created polymers with biotags (biotin, His-tag).	UOCHB
M12-M36	Covalent (click chemistry) and non-covalent conjugation (biotin, His-tag, electrostatic) of biomolecules (DNA, proteins) to both thermoresponsive and non-responsive polymers coatings. Analysis of conjugation yields, quantification of biomolecules, ability to bind ligands/targets.	UOCHB
M1-M24	Synthesis of photocleavable linkers for remote manipulation of sensors in cells. Optimization of coumarin-based linkers for attachment to polymers and biomolecules. Analysis and optimization of photocleavage conditions using lasers.	UOCHB
M12 – M48	New copper-free bioconjugation strategies for polymer-coated nanodiamonds: tetrazine – *trans*-cyclooctene click reaction. Installation of *trans*-cyclooctene moieties on nanodiamonds and kinetic analysis of the course of bioconjugation with tetrazine-modified biomolecules. Fluorogenic click reactions on diamonds using	UOCHB

	fluorescent Bodipy dyes.	
Milestone MS5.2 in M24: Developed synthetic approach to nanodiamonds with thermoresponsive coating.		
Deliverable D5.2 in M26: Summary report describing synthetic approach to nanodiamonds with thermoresponsive coating.		
A5.3. Intracellular nanosensors with a spin component **Duration: M1 – M36**		
Duration	**Task description**	**Groups involved**
M1-M24	Synthesis and surface modification of magnetic nanoparticles for construction of hybrid quantum sensors from nanodiamonds. Their structural and magnetic characterization (TEM, SEM, XRD, FTIR, thermogravimetry, volume and local magnetometery).	UFCH, MFF UK, UOCHB
M1-M24	Synthesis of new compounds containing verdazyl stable radical on a flexible hydrophillic linker. Attachment of these molecules to polymer-coated nanodiamonds with NV centers. Quantification and characterization of the conjugates using EPR, TEM and thermogravimetry.	UOCHB
M21-M36	Chemical conjugation of magnetic nanoparticles and Gd^{3+}-complexes with thermoresponsive nanodiamonds. Optimization of response magnitude, colloidal stability and loading of magnetic nanoparticles/Gd^{3+}-complexes. Structural and magnetic characterization.	UFCH, MFF UK, UOCHB
M1-M36	Manipulation with magnetic nanoconjugates using magnetic field. Orientational movement, magnetically controlled delivery.	UFCH, MFF UK
M34-M36	Creation of intracellular ultrasensitive temperature sensors from nanodiamonds with thermoresponsive polymers bearing magnetic nanoparticles. Intracellular targeting of these sensors to points of interest (mitochondria, perinuclear space, endoplasmatic reticulum) using designed targeted moieties (triphenylphosphonium cation, peptides).	UFCH, MFF UK, UOCHB
M30 – M48	Stimulation of magnetic nanoconjugates in high-frequency magnetic field in order to promote alternative functionalization and self-assembly pathways and determine heating performance of the magnetic nanoconjugates.	MFF UK
Milestone MS5.3 in M36: Developed preparation of magnetic and spin labels for installation on nanodiamond sensors.		
Deliverable D5.3 in M38: Protocol for preparation of magnetic and spin labels.		
Milestone		

Nr.	Month	Description
MS5.1	M24	Identified and confirmed pathway to optimized nanodiamonds with color centers for sensing.
MS5.2	M24	Developed synthetic approach to nanodiamonds with thermoresponsive coating.
MS5.3	M36	Developed preparation of magnetic and spin labels for installation on nanodiamond sensors.
Deliverables		
Nr.	**Month**	**Description**
D5.1	M26	Summary report describing preparation of optimized nanodiamonds with color centers for sensing.
D5.2	M26	Summary report describing synthetic approach to nanodiamonds with thermoresponsive coating.
D5.3	M38	Protocol for preparation of magnetic and spin labels.

WP5 GANTT Chart																	
	2018	2019				2020				2021				2022			
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