Nanoparticles as energy donors and acceptors in bionanohybrid systems*

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The bionanohybrids are the junctions of at least two objects of different origin: abiotic and biotic. The abiotic part is a nanoparticle (often a fluorescent quantum dot), the biotical one may be a protein (especially fluorescent one or redox-active one), nucleic acid, carbohydrate as well as a simple organic molecule. When such a junction undergoes illumination, the energy transfer between the partners is possible. The nanoparticles, depending on their characteristics, may be donors, acceptors or mediators of the energy transfer. In most cases, the mechanism of the transfer is the Förster resonance energy transfer (FRET) or the electron transfer (ET). Here, we reviewed the newest achievements in the field with special attention paid to those bionanohybrids which allow FRET or ET. Such nanohybrids are important not only for exploration of the mechanism of the partner interaction but mainly for working out nanobiodevices for biosensing and nanotools for modern therapies.

THE BIONANOHYBRIDS AND POSSIBLE MECHANISMS OF ENERGY TRANSFER

The bionanohybrids are the nanoscale junctions of at least two objects of a different origin. One partner in a hybrid is biological, while the second one is abiotic. Nanoscale means that the objects are smaller than 100 nm in at least one of their dimensions. For biological word, it may include virtually all possible types of molecules. The most used are proteins, but there are also sugar- and oligonucleotide-based nanohybrids. The abiotic partners are nanoparticles or broadly – nanomaterials. The most interesting case here are semiconductor quantum dots, especially colloidal ones. There are also carbon-based nanomaterials and nanoparticles of other metals, like silver or gold. The nanoparticles are characterised in more detail in the following paragraph. The aim of this review was to characterize the bionanohybrids that function with energy transfer between the partners. We can distinguish two mechanisms of such a transfer – the resonance energy transfer, with main example of Förster resonance energy transfer (FRET) (Jones & Bradshaw, 2019), also known as electronic energy transfer, and redox process of electron transfer (ET), or charge transfer process with electron/hole exchange. For every type of energy transfer, one molecule is a donor, while the second one is an acceptor. In more complicated cases, there may be mediators, playing both the role of an acceptor and a donor. In general, the energy transfer is possible when the energy of the lowest unoccupied molecular orbital (LUMO) of the donor is higher than the energy level of LUMO of the acceptor. It can be illustrated in a simple way with so-called Jablonski diagram (Fig. 1). In FRET, there is no exchange of electron/hole between the donor and acceptor. The excited electrons recombine to their ground state with the emission of a virtual photon, which is transferred to the acceptor, causing its excitation. Dipole-dipole couplings between donor and acceptor are facilitating the process. FRET efficiency depends on the distance between the donor and acceptor. For each FRET pair, there is a specific distance for 50% transfer efficiency, called R_0. This value is calculated from the dipole orientation factor of the donor and acceptor, the refractive index of the medium, fluorescence quantum yield of the donor and the spectral overlap between the donor emission and acceptor absorption spectra. The last requirement is simply the representation of compatibilities of orbitals energy levels. The FRET efficiency may be also analysed based on the changes in the donor fluorescence quantum yield. The equations and more detailed analysis may be found elsewhere (Lakowicz, 2007). The energy transfer types, related to FRET, are bioluminescence resonance energy transfer (BRET) and chemiluminescence resonance energy transfer (CRET). The base of the mechanism is the same as in FRET, the only difference is the origin of the donor excitation – the event of photon absorption in FRET or the excitation due to chemical (CRET) or biochemical (BRET) transformations. In the basic description, formulated by Förster and allowing FRET to be a molecular ruler, the efficiency decreases with 6th power of distance (R^6). Based on the quantum electrodynamics (QED), the description of this energy transfer contains also the dependency on R^2, dominating in the long-range, and R^4, for intermediate distances (Jones & Bradshaw, 2019). FRET pairs might be also moderately influenced by plasmonic nanoparticles (Bohlen *et al.,* 2019). Plas-
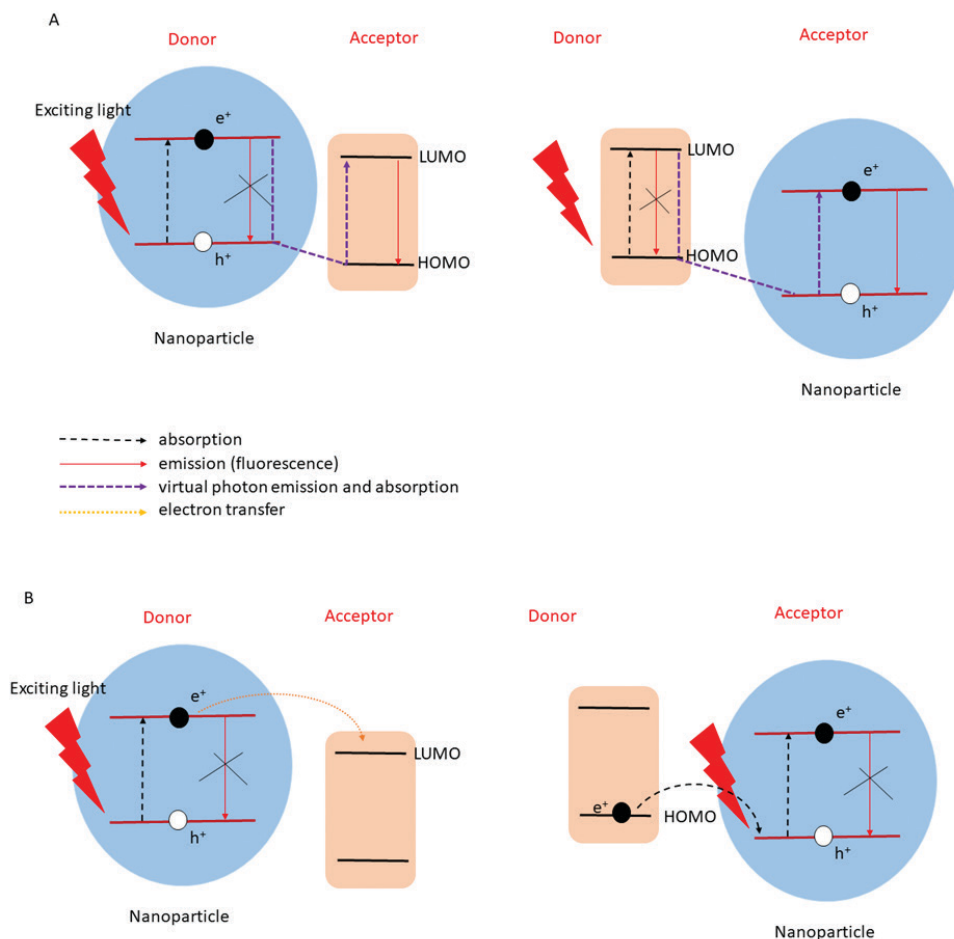
Figure 1. A comparison of FRET mechanism (A) and ET mechanism (B) between nanoparticles and biological molecules, being donors or acceptors.
Based on (Hassan *et al.,* 2018; Lakowicz, 2007).

mon resonance energy transfer (PRET) is distinguished as a separate resonance energy transfer, due to the lack of fluorescence properties of the energy donor. Metallic nanoparticles are known to be excellent PRET examples. The principle of PRET is the oscillation of free electron, spatially confined within the physical boundaries of a nanoparticle (Liu *et al.,* 2007a). This energy might be transferred to the acceptor molecule *via* dipole-dipole interactions, with a requirement that frequencies of donor and acceptor are matched (Cao *et al.,* 2017). This basically means that plasmon resonance peak (measured as the maximum of optical density) of metallic nanoparticle and the absorption spectrum of the donor must overlap. The efficiency of PRET decreases with R^4. Illuminated plasmonic nanoparticles may transfer the energy also via near field coupling and hot electron transfer. In general, those processes may be ascribed to nanosurface energy transfer (NSET) (Erwin *et al.,* 2016).

Electron transfer between the molecules is a phenomenon which lies at the root of many life processes. In the simplest possible version it occurs between two molecules: a donor, giving out the electron, and an acceptor, receiving an electron. So the reaction occurs automatically with the change of the oxidation state – the donor is oxidized while the acceptor is reduced. The rates of electron transfer are described by Marcus theory (Marcus, 1956; Arnaut *et al.,* 2006). The distance for the electron transfer to occur is much lower than the FRET range. However, it is quite often that electron transfer oc-

curs over long distances, but with multiple steps. Those might include classic redox centres, but e.g. in proteins also amino acid side chains or even backbones might be involved. Also, the electron transfer may occur over long distances in DNA molecule, *via* π-stacks (Fukui & Tanaka, 1998).

The direction of electron transfer is defined by the midpoint potential (E_m) of redox centres. E_m of redox-active molecule is directly correlated to the energy of valence orbital and the energy gap. In nature, electron transfer and redox reaction are the basis of photosynthesis, mitochondrial respiration and many enzymatic reactions, catalysed by oxidoreductases. In the special case of photoinduced electron transfer (pET), the electron donor is excited after absorption of light, as it happens in FRET, but the energy is transferred by electron hopping between molecules. As a result, the donor contains a hole, which needs to be filled for the process to continue. In nature, the hole filling is realized e.g. by water splitting by oxygen evolving system, being a part of photosystem II (PSII). In artificial assays, the sacrificial electron donors (e.g. ascorbate) may be added if the longer performance is needed.

Macroscopically, both types of energy transfer may manifest by similar changes in fluorescent properties of the donor nanoparticles – the quenching of fluorescence intensity as well as a decrease of fluorescence lifetime. The difference manifests when the nanoparticle is on the acceptor side; the consequence of FRET is the enhance-

ment of fluorescence intensity and increase of fluorescence lifetime. ET leads to quenching of fluorescence of the acceptor nanoparticles. Specific examples are described below.

NANOPARTICLES USED FOR CONSTRUCTING BIONANOHYBRIDS

Bionanohybrid-constructing kit contains a broad range of nanoparticles: semiconductor quantum dots (QDs), carbon-based nanoparticles including carbon nanodots (CDs) and graphene quantum dots (GQDs), metal nanoparticles, particularly colloidal gold and silver (AuNPs and AgNPs, respectively) and upconverting nanoparticles (UCNPs). In the next paragraphs, we briefly introduced them, referencing to more comprehensive reviews.

QDs are fluorescent nanocrystals of semiconductors optionally coated with high-band gap material (the most popular chemistry is CdSe core with ZnS shell) and capped with various organic ligands determining their colloidal features (Medintz *et al.,* 2005; Blanco-Canosa *et al.,* 2014)). Due to a quantum confinement effect, they exhibit size- and material-tuned emission with a broad absorption band (Michalet *et al.,* 2005; Resch-Genger *et al.,* 2008). The heavy metal QDs biotoxicity encouraged the development of fluorescent carbon-based materials. Different synthesis strategies provide a structural continuum of nanoparticles from graphene flake-like GQDs (including graphene oxide), through crystalline graphite particles, to amorphous CDs (Zheng & Wu, 2017; Chen *et al.,* 2019a). Their fluorescence emission, frequently excitation wavelength-dependent, may be regulated by heteroatom doping and sp^2/sp^3 carbon hybridization ratio.

The optical and electronic properties of metal nanoparticles origin from the localized surface plasmon resonance phenomenon – collective oscillating modes of conduction electrons, responsible for light absorption and resonance energy transfer (Zhan *et al.,* 2018; Loiseau *et al.,* 2019). The size and morphology of AuNPs and AgNPs determine the light absorption and scattering processes and the role of energy donor/acceptor in bionanosystems (Swierczewska *et al.,* 2011).

UCNPs consist of lanthanides trivalent cations doping a transparent host nanocrystals, e.g. $NaYF_4$:Yb, Y_2O_2S:Er (Tu *et al.,* 2015). They exhibit photon upconversion – the process of sequential low energy-photons absorption followed by anti-Stokes emission of a single photon of higher energy. The energy upconversion can be realized by different mechanisms and controlled by doping and core-shell processing (Zhou *et al.,* 2015).

NANOBIOHYBRIDS WITH RESONANCE ENERGY TRANSFER

In terms of luminescence energy transfer, the biological components of bionanohybrid systems can be utilized in two different ways: as an additional constituent besides the FRET donor-acceptor pair or directly as a participant of the energy transfer (Medintz *et al.,* 2003; Dennis & Bao, 2008). In most of examples, the first approach is in use and polypeptide or oligonucleotide is covalently linked to a low molecular weight organic dye or interacts with it in a receptor-ligand manner. Historically, the first examples of nanoparticle bioconjugates were based on the simple architectures (Willard *et al.,* 2001; Dubertret *et al.,* 2001). The energy transfer occurs between the nanoparticle and a small fluorophore and the biomolecule itself is not directly involved. In-

stead, it acts as a mechanical scaffold or a spacer ensuring the assembly and the desired separation distances in the artificial complex (Pons *et al.,* 2007; Kim *et al.,* 2019). The simple bionanohybrid devices may also benefit from features specific for biological chemistry such as ligand recognition by proteins or hybridization of nucleic acids (Patolsky *et al.,* 2003; Liu *et al.,* 2007b; Field *et al.,* 2018).

The relatively large and reactive surface of nanoparticles, enabling the conjugation to multiple copies of macromolecules, allowed to move beyond the conventional paired energy donor-acceptor configuration and to design networks of fluorophores and quenchers with more than one steps of energy transfer. In this chapter, we reviewed the recent advances in the development of biomolecules and nanoparticles conjugates, playing a role in chemical sensing, logical gating or enzymatic catalysis. Moreover, in contrast to labeling the biological component with an organic dye, we focused on the examples of systems which utilize intrinsically fluorescent biomolecules, mainly proteins, as adequate donors and acceptors of energy for nanoparticles. The so-created bionanohybrids may be used not only in applicative purpose but also provide a basic knowledge of photochemical and structural properties of proteins and nanomaterials.

QUANTUM DOTS – FRET RELAYS AND LIGHT-HARVESTING COMPLEXES

By far, QDs are nanoparticles which are the most often utilized in the construction of FRET systems, especially in the new classes of fluorescent nanohybrids. Among them are FRET cascade systems where electronic excitation energy is sequentially forwarded through the relay of fluorophores and 'one donor-one acceptor' scheme is abolished. Multistep photonic wires based on CdSe/ZnS QDs and up to four cyanine dyes were created on the DNA scaffold (Boeneman *et al.,* 2010; Spillmann *et al.,* 2013). They utilized a remarkable feature of nucleic acids, which is the hybridization of complementary strands that facilitates a very precise spatial regulation of the fluorophores arrangement. These FRET cascades proceed from the central nanocrystal to the terminal acceptor over distances of around 15–18 nm. Optimization of spacing between the subsequent fluorophores as a function of Förster distance and increasing the number of DNA wires linked to QD allowed the energy transfer to reach 10% of the overall efficiency (Spillmann *et al.,* 2013). To sum up, the relay in these examples extends the range of energy transfer and allows for a wavelength shift of the resulting emission.

The capability of natural systems, which are light-harvesting complexes of photosynthetic organisms, to perform sequential energy transfer, was also examined in the QDs bionanohybrids. CdSe/ZnS QDs, coated with negatively charged dihydrolipoic acid, interacted noncovalently and played the role of energy donor for the phycobiliprotein antenna of cyanobacterium *Acaryochloris marina* (Schmitt *et al.,* 2012; Schmitt *et al.,* 2011). This rod-shaped complex is formed by four linearly arranged hexameric units, three phycocyanin homohexamers and one phycocyanin-allophycocyanin heterohexamer, containing multiple copies of phycobilintetrapyrrole chromophores. The energy transfer is cold-sensitive and efficient by virtue of a high number of phycobilin acceptors per one antenna. The comparison of different cyanobacterial trimeric complexes, phycoerythrin, phycocyanin and allophycocyanin, showed different FRET efficiencies and dependency on QD diameter (Schmitt *et al.,* 2012). What interesting, FRET rates

did not correlate with J/R^6 parameter of Förster equation for FRET pairs with different QDs, which suggests that additional factors are crucial for the efficient energy transfer and the binding mode of phycobiliproteins can depend on QD size. In partial contradiction to the above, FRET between phycoerythrin-streptavidin conjugate and biotin-coated CdSe/ZnS QDs was reported to be agreeable to the Förster dipole-dipole formalism, with an efficiency that varied with the degree of spectral overlap and the distance between the donor and acceptor (Medintz *et al.* 2009). The large FRET efficiency of 60% resulted from the structure of the multichromophor phycoerythrine, containing multistep-FRET channels between the central QD and the phycobilin dyes distributed within the protein core. Taking into account the distance (counted from single steps in the multistep chain) between the donor and the final acceptor (from 10 to 12.5 nm, depending on the phycoerythrin trimer position to the nanocrystal), the phycoerythrin-QD bioconjugate is a very efficient photonic nanowire, in comparison to the DNA-scaffolded systems (Spillmann *et al.*, 2013). Closer investigation of FRET between allophycocyanin and QDs demonstrated a significant increase in energy transfer efficiency after monomerization of allophycocyanin trimers by high temperature or sodium thiocyanate chaotropic conditions (Karpulevich *et al.*, 2016). Moreover, covalent linking and stabilization of bionanohybrid resulted in even further enhancement of allophycocyanin fluorescence upon excitation of QDs.

Similarly to their excitation relaying function in photosynthesis, light-harvesting complexes may also serve as energy donors for nanocrystal acceptors. Plant light-harvesting complex II (LHCII) trimer was found to be particularly interesting in this context due to its spontaneous self-organization and high extinction coefficient (Barros & Kühlbrandt, 2009). The exact pigments system of LHCII consists of multiple copies of chlorophyll *a* and *b* and various carotenoids. LHCII from *Pisum sativum* was reported to efficiently transfer the excitation energy to the CdTe/CdSe/ZnS QDs with emission maximum at 760 nm after electrostatic attachment (Werwie *et al.*, 2012). The increase in the light-energy utilization was particularly effective in the red region of the visible spectrum where QDs absorption is low. The overall absorptivity of the system was further improved by conjugation of LHCII to Alexa Fluor 555 dye absorbing in the green spectral domain where the window of transparency of chlorophyll is located.

In the opposition to the last paragraph, QDs can also play a role of light-harvesting particles as it was shown for the reaction center of the purple bacteria *Rhodobacter sphaeroides* (Nabiev *et al.*, 2010). This protein heterotrimer comprises two branches of chromophores, active and inactive, each consisting of bacteriochlorophyll, bacteriopheophytin and quinone molecules, connected to the special pair of bacteriochlorophylls. In the noncovalent complex, the excitation energy of CdSe/ZnS QDs with peak emission at 570 nm is relayed through the reaction center and accepted by the special pair. Then, instead of splitting into an electron and a hole, in experimental conditions, the exciton decays with 910 nm emission. The energy transfer was efficient also in the assemblies of QDs and reaction center complexes in the lecithin liposomes and thin solid films (Lukashev *et al.*, 2016).

QUANTUM DOTS – CONCENTRIC FRET AND TIME-GATING

Another convention followed in simple fluorescent bionanoconjugates which was broken in the recent approaches is the rule of a single luminescent output signal. Although multiplexed FRET in this configuration is still possible, it requires multiple donors of energy and consequently usually more than one excitation wavelength. The main benefit of systems with more than one output may be the possibility of simultaneous detection of complex targets as well as the possibility to gain more insight into the single steps of multistep molecular processes. To obtain systems with more than one output signal, the advantage of the multivalence of QD surface was utilized. CdSe/ZnS QDs with peak emission at 520 nm were conjugated to two types of peptides labeled with Alexa Fluor 555 or Alexa Fluor 647 dyes (Algar *et al.*, 2012). The single QD type was initial energy donor for both of the dyes, which were a FRET pair themselves. Hence, when QD was excited, 647 nm-fluorophore was the final acceptor due to, firstly, direct energy transfer from QD and, secondly, through the FRET relay with 555 nm-dye as the intermediate donor. The individual or simultaneous activities of trypsin and chymotrypsin, which recognize the proteolytic cleavage sites on the different peptides, could be assessed by comparison of 555/520 and 647/520 fluorescence intensity ratios. The same conjugate was used for sensing and imaging the diffusion of proteases in the glass capillary (Wu & Algar, 2015). This co-called concentric FRET scheme was additionally extended by employing three molecular fluorophores, linked to the QD either by peptides with different protease recognition sites or oligonucleotides with different restriction sites (Massey *et al.*, 2017; Hu *et al.*, 2019).

The potency of concentric FRET architectures as energy donors was shown in the triple system of CdSe/ZnS QD with 517 nm emission peak, enhanced yellow fluorescent protein (EYFP) and Atto647 dye (Lu *et al.*, 2008). The fluorophores were assembled by DNA oligonucleotides hybridization which allowed for precise control of their separation. The interesting observation was that dominant QD-EYFP-Atto647 pathway was significantly more efficient than direct QD-Atto647 path in terms of energy transfer to the Atto647 terminal acceptor. This power of QD-EYFP conjugate as a FRET donor was assigned to the combined advantages of QDs (multivalence and spectrally broad absorption) and EYFP (high quantum yield).

The biological components of hybrid systems provide the possibility of light generation by chemiluminescence or, in the nomenclature preferred by some, bioluminescence. These self-illuminating systems do not require the source of excitation since the light emission is derived from the chemical reaction, not from the absorption of light. The source of chemiluminescence most popular in biochemical applications, luciferase enzyme (Thorne *et al.*, 2010), was utilized in the bionanoconjugates based on BRET process. Catalyzing the oxidation of species-specific substrates (termed generically as luciferins) by molecular oxygen, luciferase may be a donor of luminescent energy directly for a QD. The BRET-FRET systems, consisting of luciferase-QD-red fluorescent protein (RFP) linear relay or luciferase-QD and molecular fluorophores concentric array, were designed (Alam *et al.*, 2013; Samanta *et al.*, 2015). Employing firefly luciferin producing bioluminescence at 547 nm and QDs absorbing at higher wavelengths enabled the direct BRET from luciferase to RFP and demonstrated the concept of transparent QD scaffold (Alam *et al.*, 2013). The luciferase-QD BRET hybrids found practical utility in DNA sequence-sensing and tissue imaging (Kumar *et al.*, 2011; Kamkaew *et al.*, 2016).

Multiplexing of signal in concentric FRET bionano-systems, especially significant in the context of sensing, may be achieved not only by differentiation of the signal wavelengths but also by separation of different emission lifetimes. The extremely long-lived fluorescent terbium complex was used as an energy donor in concentric assembly with CdSe/ZnS QD and Alexa Fluor 647 dye (Algar *et al.*, 2012). The UV light flash excited both Tb complex and QD and two FRET processes could be observed: the first QD-Alexa Fluor 647 path in the first nanosecond period and the second Tb-QD-Alexa Fluor 647 transfer in the millisecond time window. The monitoring of fluorescence emission of the same wavelength with the microsecond time gate allowed distinguishing these two pathways which was utilized in the duplex assays for protease activity and oligonucleotides hybridization. Another approach was taken in miRNA hybridization sensor consisting of two oligonucleotide probes conjugated to a terbium complex and coupled with QD using different QD-Tb separation distances (Qiu *et al.*, 2017a). Thus, the rate of FRET varied between the two probes which was reflected in different decay times. The distinction of signals was possible due to the fluorescence acquisition in two time windows.

QUANTUM DOTS AND FLUORESCENT PROTEINS CONJUGATES AS SENSORS AND MOLECULAR MACHINES

Combining QDs with biological elements results in systems with the attributes of both components: excellent optical properties of semiconductor nanocrystals and protein- or nucleic acid-based molecular recognition. Thus, the QD bionanoconjugates offer variable sensing strategies of applicative importance with FRET-induced emission as the detectable output (Medintz *et al.*, 2003). In this section, we omitted the simple ligand-receptor detection approaches in favor of more advanced or unconventional sensing strategies.

Fluorescent proteins are particularly valuable in QD bionanosensors design. The example of QD complex with protein dimer associated by heterodimerizing, coiled-coil forming α-helices, presented in our recent paper, demonstrated the most significant advantages of fluorescent proteins: the intrinsic emission without the requirement of dye-labeling, the ability of genetic recombination and one-step selfassembly to QDs (Sztatelman *et al.*, 2019). The other instances include green fluorescent protein (GFP) containing trypsin cleavage site (Suzuki *et al.*, 2008) and mCherry protein with N-terminal His-tag followed by caspase-3 substrate sequence (Boeneman *et al.*, 2009), coupled with CdSe/ZnS QDs. In both sensors, the proteolytic activity was determined by the decrease of QD-protein FRET signal. In the latter example, His-tag electrostatic interaction with nanocrystal surface allowed self-assembly by simple mixing of the two components and the fluorescence monitoring provided kinetic data and sensitive, quantitative detection.

The biocompatibility of proteins facilitates the use of bionanoconjugates as sensors of various physical and chemical quantities *in vivo*. The attribute of some GFP-family proteins, pH-sensitivity, was utilized in the FRET probe comprising QD and mOrange protein or its mutant (Dennis *et al.*, 2012). While under acidic conditions the energy transfer to mOrange acceptor was reported minimal, it was dramatically and reversibly enhanced with a pH increase. This sensor could measure pH in the range of 6–8. After the delivery of the sensor to the cells, the temporal and spatial tracking of intracellular pH during endocytosis and translocation to the cytosol was demonstrated using fluorescence microscopy. A two-component voltage sensor was developed for imaging of another cellular quality, the electric potential of the plasma membrane (Chen *et al.*, 2019b). Glutathione-capped QDs were used to label neural cells to serve as a FRET donor for lipophilic anionic fluorophore, dipicrilamine, which localizes at the positively charged site of the polarized membrane. The translocation of dipicrilamine within the lipid bilayer in response to the membrane potential changes the separation distance between FRET pair components which can be observed as the emission wavelength shift.

Beside sensing functions, the QD bionanohybrids may be engineered as photonic energy converters. QDs were shown to be FRET donors for bacteriorhodopsin of extremophile archaeon *Halobacterium salinas* (Rakovich *et al.*, 2010). This retinal-containing proton pump forms trimers arranged in a semicrystalline lattice in the outer membrane of archaea, known as the purple membrane. Photon absorption triggers the isomerization of all-trans-retinal (peak absorption at 570 nm) to the 13-cis form, inducing the conformational changes of the polypeptide chain and the transport of a proton. The photocycle of bacteriorhodopsin occurs through a series of spectrally different intermediates (Li *et al.*, 2018b). The energy transfer from QD donor significantly increases the rate of light-driven proton-pumping by bacteriorhodopsin. The efficiency of energy conversion and chemiosmotic potential formation was demonstrated by the incorporation of the bionanohybrid into the proteoliposomes (Rakovich *et al.*, 2010). For bacteriorhodopsin, QDs play a role of light-harvesters, either in the UV range of spectrum or in the near-infrared region, in the latter case by virtue of the two-photon excitation process (do not confuse with photon upconversion) which allows upconversion of the photonic energy and its transfer to the bacteriorhodopsin acceptor (Krivenkov *et al.*, 2019).

UPCONVERTING NANOPARTICLES – BIOSENSING AND NIR RADIATION HARVESTING

UCNPs emitting NIR-induced fluorescence are of great interest as biocompatible components of nanosensors. NIR excitation light localizes in biologically transparent spectrum window, exhibits deep tissue penetration and allows to avoid cell phototoxicity and high background autofluorescence in the complex environment. UCNPs, with main emission maximum at 543 nm, capped with ssDNA probes labeled with TAMRA dye and BHQ1 quencher were used for quantification and imaging of miRNA intracellular localization (Yang *et al.*, 2019). After hybridization with miRNA target, complementary ssDNA probes detached from UCNP surface which resulted in an increase of 980 nm-excited visible range fluorescence of UCNP. Dual acceptors enhanced quenching efficiency of UCNP and improved sensitivity of the assay. Another miRNA sensor comprised UCNPs assembled on silica beads and conjugated to molecular beacons probes labeled with FAM energy acceptor (Li *et al.*, 2019). Optical tweezers instrument allowed immobilization of the beads and precise imaging conditions.

An interesting example of bimodal UCNP nanosensor demonstrated the versatile possibility of UCNP emission tuning (Wilhelm *et al.*, 2014). Single wavelength-excited UCNPs displayed emission spectrum with two bands (360 nm and 475 nm) which perfectly overlapped with

the absorption peaks of NADH and FAD coenzymes, respectively. The simultaneous monitoring of both co-enzymes redox state was possible in the complex enzymatic reactions. Nonetheless, given the lack of nanoparticle lifetime changes and the average UCNP-coenzymes separation in solution exceeding any possible Förster distance, it was concluded that FAD or NADH did not act as energy acceptors but as emission screeners which resulted in an inner filter effect.

Upconversion of NIR radiation makes UCNPs promising candidates to play a role of light-harvesters facilitating the practical usage of the low-energy region of the light spectrum. After the incorporation into the bacteriorhodopsin-containing thin films of purple membranes, UCNPs may serve as energy donors (Lu et al., 2016). Two types of UCNPs with green and blue emission maxima accelerated particular transition steps of bacteriorhodopsin photocycle and profiling the green/blue emission ratio enabled to precisely control the proton pumping activity.

CARBON NANOPARTICLES – IMPACT ON BIOMOLECULES CONFORMATION AND DNA-BASED NANOPROBES

The luminescent energy transfer process was used to reveal interaction mechanisms of CDs with two primary biopolymers – proteins and DNA. Ruthenium-doped CDs, coated with amine groups, were shown to form noncovalent complexes with bovine and human serum albumins (BSA and HSA, respectively) and to perturb their secondary structure (Maity et al., 2019). The efficiency of FRET between tryptophan residues as donors and CDs acceptors was substantially higher for BSA which was assigned to the more rigid structure of BSA, in comparison to HSA, which in turn increases the stability of BSA-CD complex. Although molecular modeling confirmed the localization of tryptophan residues of both albumin proteins within a similar range from the CD surface, lower or equivalent to Förster distance, the fluctuations of polypeptide backbone were regarded to disturb the energy transfer more significantly in the case of HSA complex.

Nitrogen-doped CDs, capped with positively charged spermin, were also indicated to bind and induce a conformational change of DNA double helix (Feng et al., 2013). The binding target of spermin-coated carbon dots is the DNA helix major groove region, preferentially GC-rich sequences, which was shown by a competition assay with methyl green dye, major groove binder. The interaction with spermin-capped carbon dot triggered the transition of DNA structure from canonical right-handed B-form to left-handed Z-form, which is a transient structure induced by DNA-binding proteins and various molecular processes involving DNA, e.g. transcription and supercoiling. Such transition process was further investigated as the framework for the construction of several photonic logic gates. They were based on spermin-capped CDs, fluorescent DNA-intercalating agent - ethidium bromide, and iodide ion as a potent quencher. Different combinations of those components resulted in various FRET and quenching processes, which in consequence led to various output fluorescence signals.

The doping of CDs with other elements may result in their dual-emissive characteristics and the improvement of FRET donor quality. Plant biomaterial-derived CDs, exhibiting excitation wavelength-independent emission maxima at 483 nm and 682 nm, were examined as light-harvesters for photosystem complexes (Li et al., 2018a). Due to blue and red emission bands, overlapping with chlorophyll absorption spectrum, CDs after the introduction to isolated chloroplast and intact leaves significantly accelerated the rate of photosynthesis. Unchanged lifetimes of CDs and chloroplasts indicated, though, that it was the reabsorption of CD-emitted light by chlorophyll that was responsible for the effect.

Despite repulsive electrostatic forces between negative charges, DNA-coupled graphene oxide-based nanoparticles are another class of bionanohybrids utilized as prototypic sensors. The reversible adsorption of ssDNA onto the graphene oxide surface is likely an effective outcome of several attractive forces: π-π stacking, dehydration effect and hydrogen bonding (Paul et al., 2016). Hence, graphene oxide nanoparticles are extensively investigated as FRET quenchers in pair with adsorbed ssDNA fluorophore-labeled probes. As FRET bionanosensors, where detection is based on the cleavage or desorption of ssDNA and the increase of fluorescent signal, they were employed for exonuclease activity quantification (Liu et al., 2019), copper ions sensing by DNAzyme autocatalysis (Ding et al., 2018) or aptamer-dependent detection of toxins (Zhu et al., 2015).

METAL NANOPARTICLES – PRET, NSET AND CHEMICAL NOSES

The dependence of PRET on size and morphology of metal nanoparticle and on the PRET donor-acceptor distance separation was indicated in several studies of energy transfer between AuNPs or AgNPs and light-harvesting complexes. After the deposition of a thin film of the cyanobacterial trimer of photosystem I (PSI) from Synechococcus leopoliensis on the AgNP-coated glass, the increase of PSI fluorescence was observed (Kim et al., 2011). The PRET-induced enhancement zones were particularly localized near the aggregates of AgNPs. Surprisingly, the opposite results were obtained for covalent conjugates of AuNPs and AgNPs formed in solution by metal ions reduction in the presence of PSI (Carmeli et al., 2010). Both types of nanoparticles efficiently quenched the PSI fluorescence and enhanced light absorption on the entire range of PSI absorption spectrum, not only in the region of plasmon resonance wavelengths. This effect of absorption increase was assigned to the high dielectric constant near the metal surface. The inconsistency in terms of PRET-induced fluorescence quenching/enhancement in the both foregoing studies, despite the similar nanoparticle diameter (~10–20 nm), could potentially result from a different orientation of bionanohybrid components i.e. separate layers of PSI and AuNPs versus exceptionally close and direct contact after in situ synthesis of the nanoparticles (Kim et al., 2011).

Similarly, the differences in the direction of energy transfer were shown for phycobiliproteins. The monolayers of several types of phycoerythrin, allophycocyanin and phycocyanin were immobilized on the glass coated with silver islands (100–500 nm diameter and 60 nm height) which resulted in the increase of the phycobiliproteins emission (Chowdhury et al., 2007). In the contradiction, phycoerythrin after mixing in solution with gold or silver nanoparticles (10 nm diameter), exhibited strong adsorption onto the metal surface and decrease of its fluorescence (Saraswat et al., 2011). It was proposed, that this discrepancy between the foregoing studies is a result of alternative energy transfer mechanisms. Differ-

ent metal nanoparticles size can decide about their role as donors or acceptors of the energy (Swierczewska *et al.,* 2011). In the first study, PRET from silver islands increased the emission of phycobiliproteins. In the latter case, the NSET mechanism of energy transfer from phycoerythrin to the metal surface could take place.

An interesting example of spatial gating of energy transfer and modulation of its efficiency was demonstrated on citric acid-coated AuNPs capped with survivin protein (Stobiecka & Chalupa, 2015). As a result of FRET to the metal nanoparticle, the strong quenching of fluorescein isothiocyanate (FITC) was observed after mixing and FITC being several nanometers away from the gold surface. The total coverage of metal with survivin resulted in the partial fluorescence recovery due to hindrance for efficient energy transfer. The modulation of protein coverage allowed for regulation of efficiency of FRET from FITC to AuNP. The quantification of quenching efficiency in the dependence on protein concentration was proposed as a novel method for the determination of binding constants between proteins and nanoparticles.

The efficient PRET resulting in quenching of fluorescent proteins by metal nanoparticles was utilized in the multiplex chemical sensing method designed by Rotello's group (You *et al.,* 2007). The assay principle consists of competitive displacement of fluorescent proteins or dye-labeled polymers from the metal nanoparticle by target molecules and the determination of fluorescence recovery. For various physicochemical properties (e.g. hydrophobicity, size, chemical affinities, electric charge) of the particular compound, the displacement capability differs. Using the array of AuNPs with different capping ligands complexed with various fluorescent proteins, it is possible to obtain target-specific pattern of fluorescence signals. So-performed fingerprinting was used for protein sensing (You *et al.,* 2007; De *et al.,* 2009; Xu *et al.,* 2014), thiol compounds distinguishing (Kapur *et al.,* 2017) and identifying anticancer drugs according to the protein profile on the cell surface (Rana *et al.,* 2015). This 'chemical nose' combination of fluorescent bionanohybrid complexes loosely mimics the function of olfactory receptors in term of cross-reactivity and multivariate output.

THE NANOHYBRIDS WITH ELECTRON TRANSFER

Nanoparticles can be a source of (photo)electrons for biological reactions or a sink to electrons, generated during redox processes. The efficiency of such transfer is connected to E_m of QDs and proteins (see e.g. (Grzyb *et al.,* 2015)). The basic version of ET from QDs to an acceptor is the photoinduced formation of reactive oxygen species (ROS), which is one of the therapeutic strategies with application of nanoparticles. ROS production upon illumination was shown e.g. for CdSe QDs (Kauffer *et al.,* 2014), CdTe QDs (Lovric *et al.,* 2005), InPZnS QDs (Chibli *et al.,* 2011) as well as GQDs (Ristic *et al.,* 2014), Cd (Christensen *et al.,* 2011) and AgNPs (Avalos *et al.,* 2014). When oxygen is removed or excluded from the process, the ET from/to QDs becomes more interesting, with electron transferred to enzyme cofactors and other redox mediators.

ET BETWEEN NANOPARTICLES AND REDOX BIOMOLECULES

The most-used protein for electron transfer studies with various nanoparticles is probably glucose oxidase (GOx). GOx catalyses the oxidation of glucose to gluconolactone, with the formation of hydrogen peroxide (H_2O_2). This reactive oxygen form is a potential defence of fungi against microbes. The enzymatic reaction is possible due to the presence of flavin adenine dinucleotide cofactor, FAD, which gets reduced to FADH2 during glucose oxidation. Reoxidation of FAD demands the use of oxygen (Wilson & Turner, 1992). This reaction is also used in enzymatic assays for oxygen removal, in tandem with catalase neutralizing H_2O_2 (see e.g. (Uppoor & Niebergall, 1996)). In 2006, Protein Data Bank made it their molecule of the month because of this enzyme's enormous significance for biotechnological industries (Goodsell, 2006). GOx is basically one of the simplest tools for detection of glucose in blood samples for diabetes diagnosis and monitoring (Cash & Clark, 2010). The same enzyme is also used in the food industry, for oxygen removal from fruits (e.g. to prevent apple puree browning (Parpinello *et al.,* 2002)) or for glucose reduction in wine (Pickering *et al.,* 1998). In combination with nanoparticles, the enzymatic performance of GOx may be influenced by electron transfer from nanoparticle to FAD with formation of FADH2 (inhibition of GOx), or to the nanoparticle, helping to re-oxidase $FADH_2$ to FAD. Indirect influence is also possible, with some redox-active mediators. We can then distinguish direct electron transfer (dET) and indirect electron transfer (iET). dET from graphene to GOx inhibited its biosensing activity which in principle was caused by already-mentioned FAD reduction to $FADH_2$ (Liang *et al.,* 2015). CdSe/CdS quantum dots were shown to be excellent direct electron donors for GOx in electrochemical studies (Huang *et al.,* 2008). Also, dET between multi-walled carbon nanotubes and GOx (Zhao *et al.,* 2010), as well as carbon nitride nanosheets and GOx (Tian *et al.,* 2019) was shown. In GOx conjugates with Mn-doped ZnS QDs, iET plays a role in biosensing, with nanoparticles providing photoinduced electrons for H_2O_2 reduction and O_2 restoring. When an electron is transferred from QDs, the fluorescence is quenched, which is the direct analytical signal (Wu *et al.,* 2010). The same idea was applied using tungsten sulfide (WS_2) quantum dots (Duan *et al.,* 2019). iET between CdS QDs and alkaline phosphatase was achieved with the mediation of 4-aminophenol, which is a product of enzymatic hydrolysis of p-aminophenol phosphate (Khalid *et al.,* 2011). Thiocholine, being a product of enzymatic decomposition of acetylthiocholine by acetylcholine esterase, was reduced by the same QD type (Pardo-Yissar *et al.,* 2003). Another mediator between CdSe/ZnS QDs and an enzyme, glucose dehydrogenase, was NADH (Schubert *et al.,* 2009). In most of the cited biosensors, QDs are also immobilized on an electrode, which enables the measurement of generated photocurrent (Lisdat *et al.,* 2013). dET occurred between multiwalled carbon nanotubes, decorated by gold nanoparticles, and the enzyme laccase (Lalaoui *et al.,* 2016). This enzyme contains a redox center with four copper ions (Solomon *et al.,* 1996), which enables the electron transfer. Our study showed the possibility of dET to the redox center of ferredoxin (Fd) (Grzyb *et al.,* 2015). Fd is a non-enzymatic protein, involved in several processes where it serves as an electron donor or mediator in redox pathways. Its redox cofactor, called iron-sulfur cluster, consists of two iron and two sulphur atoms, coordinated by four cysteine residues of the protein. Another iron-containing redox cofactor is heme, a prosthetic group of cytochromes, myoglobin and hemoglobins. dET between CdTe and cytochrome c (CytC) was shown for stable QD-CytC conjugates (Ger-

hards *et al.,* 2008) and for the transient complex between nanoparticle and protein (Grzyb *et al.,* 2015). Our study showed that photoinduced electrons may be donated from QD conduction band as well as from defects, especially the surface ones (Darżynkiewicz *et al.,* 2017). dET from CdTe to myoglobin resulted in deoxygenation of heme, which under CO atmosphere led to CO binding to the iron atom (Onoda *et al.,* 2012). FAD, mentioned already in the description of GOx, is a common cofactor of redox-active enzymes. It is also a prosthetic group of ferredoxin:NADP$^+$ oxidoreductase (FNR), an enzyme catalyzing NADPH production during the light-dependent phase of photosynthesis. dET was observed for the covalent junction of CdSe QD and FNR (Brown *et al.,* 2016). In this assay, dET resulted in FAD reduction but also in hole formation on the QD, which was then filled by the electron from ascorbic acid (Brown *et al.,* 2016). Several other enzymes were conjugated to QDs, which resulted in altered enzyme kinetics (Breger *et al.,* 2016). For example, in our FNR-CdSe/ZnS QDs covalent junction, dET was not proved, but the enzyme-substrate complex formation was easier, most probably due to the specific interaction with charged QD surface (Szczepaniak *et al.,* 2013).

Phosphorescent Mn-doped ZnS QDs were conjugated to cetyltrimethylammonium bromide (CAB) an electron acceptor and also DNA intercalator. Without DNA in the mixture, photoinduced electrons are transferred to CAB and QDs emission is quenched. Restore of the phosphorescence is a signal of analyte presence (Miao *et al.,* 2014). The same QDs, this time in junction with boronic-acid-substituted viologens, work as glucose detectors. This is possible due to the condensation of glucose with boronic acid into a tetrahedral anionic borate, which causes its detachment from the QDs surface (Miao *et al.,* 2016). Folic acid (FA) was an electron donor for CdS/ZnS QDs, coated with polyethyleneimine, and this system was used for detection of FA receptors over HeLa cells (Zhang *et al.,* 2012). While FA was assembled onto the QD surface, the QD fluorescence was quenched. Release of FA due to its higher affinity to cellular receptor resulted in restoring of QD emission.

CARBON NANOPARTICLES AS EXCELLENT ET DONORS AND ACCEPTORS

CDs, being simply clusters of carbon atoms, were shown to be excellent electron donors and acceptors (Wang *et al.,* 2009). Fluorescence of 4.2 nm carbon QDs, with PEGylated surface, was quenched by nitrotoluene derivatives, which are model electron acceptors. The same nanoparticles, under illumination, were able to reduce anionic silver, acting as the electron donors. The CDs emission was also quenched by electron acceptor, N,N-diethylaniline (Wang *et al.,* 2009). Graphene QDs, with the ability to donate photoelectrons to curcumin, were applied in a probe for Apo e4 DNA. After hybridization of the probe to the target oligonucleotide, the electron transfer rate was reduced, which was measured by the change in curcumin luminescence (Mars *et al.,* 2018).

METAL NANOCLUSTERS AS A SOURCE OF ELECTRONS

Photoinduced electrons may be generated by illumination of silver nanoclusters. In the study on DNA/AgNPs, photoelectrons were used for the reduction of hemin assembled with an oligonucleotide (G-quadruplex) (Zhang *et al.,* 2013). ET manifested by quenching

of nanoclusters fluorescence as well as significant shortening of fluorescence lifetime. The transfer rate was dependent on the distance between the donor and acceptor, and also the base pairs were involved in it by the π-stacking mechanism. When this design was enriched with specific aptamers against ATP, simple biosensor specifically detecting this nucleotide was obtained (Zhang *et al.,* 2013). In a similar approach from the same group, CdTe/CdSe QDs, also conjugated to an oligonucleotide, were a source of photogenerated electrons for hemin acceptor. With aptamers specific for lysozyme, the design was used for this enzyme detection (Qiu *et al.,* 2017b).

NANOPARTICLES IN ET-DEPENDENT POTENTIAL SENSING

Efros et al. considered electron transfer-based sensing of the electric field in neurons by QDs (Efros *et al.,* 2018). In such an approach, QDs located outside the neuron membrane should be connected by some molecular wire to an electric field-sensitive electron donor or acceptor. Bioconjugates of CdSe/CdS/ZnS or CdSe/CdS/CdZnS/ZnS QDs and C-60 fullerene, connected *via* peptide linker, were successfully used for monitoring depolarization in HeLa cells (Nag *et al.,* 2017). Decrease of luminescence upon depolarization occurred due to a preferential attraction of photoexcited electrons of QDs to C60 acceptor, while at resting membrane potential (–70 mV) electrons recombined with holes of the excited nanoparticles (Nag *et al.,* 2017). It is, however, known that even without electron transfer, the strong electric field may measurably quench QD luminescence, most probably due to the quantum-confined Stark effect. The observed changes are especially strong in small nanoparticles (radius < 2.5 nm) which is related to the rate and yield of Auger recombination in such structures (Efros *et al.,* 2018). The proof of that concept is e.g. a study with ZnS/CdSe seeded nanorods sensing voltage of the HEK cells membranes (Park *et al.,* 2019). The nanorods fluorescence quantum yield was correlated to the membrane potential. The emission intensity was higher at the native potential of –101.4 mV and decreased when ionophorevalinomycin was applied (Efros *et al.,* 2018; Nag *et al.,* 2017; Park *et al.,* 2019).

PHOTOSYNTHESIS AND ARTIFICIAL PHOTOSYNTHESIS WITH NANOPARTICLE-DEPENDENT ET

Nanohybrids of QDs and photosynthetic apparatus need special attention because of their possible role in creating artificial photosynthetic systems. Photosynthetic membranes contain three types of redox pigment-protein complexes (photosystem I, photosystem II and cytochrome b6f complex), lipophilic redox mediator (plastoquinone) and water-soluble redox proteins (copper-containing plastocyanin and mentioned earlier ferredoxin and FNR). There are also non-redox active antennae of light-harvesting complexes, whose FRET-based interaction was described in the previous paragraphs. When considering possible donors or acceptors for ET, we have several candidates, depending on E_m of the nanoparticle and redox-active biological molecule. Figure 2 compares E_m of selected QDs with E_m of photosynthetic redox centers. Simple dET is possible between QDs and quinones. Although plastoquinone has not been tested for ET with QDs yet, there are available studies on its analogues. 1,4-benzoquinone molecule (pBQ) is the simple model for plastoquinone. Illuminated PbS QDs were a source of photoinduced electrons, transferred at picosecond times-
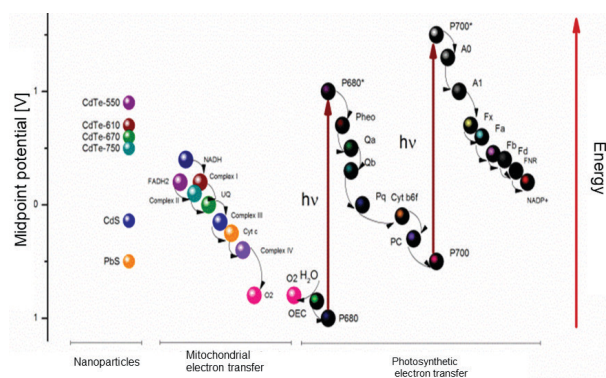
Figure 2. A comparison of the midpoint potential of selected nanoparticles and following steps of the mitochondrial and photosynthetic electron transfer chains.
It does not include Q-cycle and other possibilities of cyclic electron transfer. UQ – ubiquinone, Cyt *c* – cytochrome *c*, OEC – oxygen evolving complex, Pheo – pheophytin, QaiQb – quinones QaiQb in photosystem II, PQ – plastoquinone, Cyt b6f – cytochrome b6f complex; A0, A1, Fx, Fa, Fb – following redox centres in photosystem I; PC – plastocyanin, Fd – ferredoxin, FNR – ferredoxin:NADP+ oxidoreductase. The figure was prepared on the basis of (Blankenship, 2013; Douce, 2012; Grzyb *et al.*, 2015; Rossetti *et al.*, 1983; Cuharuc *et al.*, 2012).

cale to covalently bound pBQ (Aruda *et al.*, 2015; Knowles *et al.*, 2012). The same molecules in solution were also reduced, just at a longer (nanosecond) timescale (Knowles *et al.*, 2012). Donation of the electrons from hydroquinone, a reduced version of pBQ, to QDs is also possible (Mo *et al.*, 2018). With properly selected QDs it is then possible to demonstrate quinone cycles occurring in photosynthetic membranes. Ubiquinone, which is mitochondrial structural and functional analogue of plastoquinone, was used in assembly with CdSe/ZnS QD as a sensor of mitochondrial Complex I function (Ma *et al.*, 2013). Mentioned earlier transfer to Fd is a part of the ET between QDs and the photosynthetic apparatus. Semiconductor QDs may be also included in the photoinduced multielectron transfer chains (Pannwitz & Wenger, 2019).

Most of the studies of QDs-photosystems assemblies focused on FRET type energy transfer in those systems. It is worth to remember that, although not yet shown in the studies, in specific conditions QDs may not only be the antennae but also donors of an electron to the redox steps (QA, QB sites in PSII, A0, A1, Fx, Fa, Fb sites in PSII).

An interesting example, from the artificial photosynthesis point of view, is photoinduced electron transfer occurring in the conjugate of QD to pyridine derivative, 4,4'-bipyridium. This chemical in its reduced form is then able to reduce CO_2 to methanol which is an interesting alternative for assimilation of atmospheric CO_2 (Chen *et al.* 2016). This reaction may be considered as some kind of artificial photosynthesis, although not leading to carbohydrate synthesis. The challenge of CO_2 reduction was also addressed with the nanohybrid of ZnSe QDs and a phosphonic acid-functionalised Ni(cyclam) catalyst (Neri *et al.*, 2015). Photoinduced ET from CdSe/CdS QDs to platinum nanoparticles was shown to be the way to enhance catalytic hydrogen evolution (Li *et al.*, 2017).

COEXISTENCE OF FRET AND ET ROUTES IN BIONANOHYBRIDS

Due to physical and chemical properties of nanoparticles, there is no formal restriction to only one type of

energy transfer if acceptors specific to fluorescence energy transfer or electron transfer are present. The examples described above clearly showed that the same types of QDs can be both donors and acceptors for resonance energy transfer and for electron transfer. There are also studies, although rather sparse, where both types of transfers were involved. In the simple system with rhodamine, organic fluorophore, adsorbed on the QD surface, both FRET and electron transfer was present. Energy transfer manifested here by quenching QD fluorescence. The main pathway (84%) could be ascribed to resonance energy transfer (Boulesbaa *et al.*, 2009). Concurrent quenching of CdSe/ZnS QDs fluorescence was observed by simultaneously attached fluorescent probe Cys and redox probe, ruthenium (II) phenanthroline (Algar *et al.*, 2016).

The example of QD involved in FRET and ET was presented in a study where LHCII was shown to function as antennae for QDs (CdTe/CdSe/ZnS) which in the following reaction were reducing methyl viologen (MV) (Werwie *et al.*, 2018). In the presence of QDs, the MV photoreduction rate was increased at least twofold. In the cited paper, tris(2-carboxyethyl)phosphine/dihydrolipoic acid system was suggested to work as electron donor/hole regenerating system. Electron transfer component was as fast as 200 ps, faster than FRET component (500 ps) (Werwie *et al.*, 2018). QDs were also used as antennae for bacteriorhodopsin in the hybrid system for photovoltaic applications (Renugopalakrishnan *et al.*, 2014). Nanodevices for hydrogen evolution were constructed on the base of bacteriorhodopsin photosensitizing TiO_2 nanoparticles by FRET mechanism with subsequent transfer of the electron from TiO_2 to the connected platinum cluster and converting H^+ to H_2. This resulted in a hole on TiO_2, which was filled by decomposition of methanol to water and carbon dioxide (Wang *et al.*, 2017).

Worth to mention example of both FRET and electron transfer is a self-assembled cascade of QDs, varying in size. Such a cascade was built on TiO_2 electrode, which resulted in charge transfer detection by current measurement. Upon illumination, the excitons were transferred by QD of increasing size, to end up in electron donation to the electrode (Goodman *et al.*, 2015).

STRUCTURAL CHANGES WITHIN HYBRID PARTNERS

Despite the mentioned changes in function, the formation of the junction between proteins and nanoparticles, especially semiconductor ones, may influence the structure of the biological element of the hybrid. The rationale for that is a known fact about protein denaturation in contact with abiotic surfaces. Proteins may bind nanoparticles nonspecifically, in that way forming a corona (Bhunia *et al.*, 2013), which results in at least partial denaturation of the bound proteins. This is due to a strong interaction of some amino acids with heavy metal and transition metal ions, like cadmium, lead or zinc. Charged surfaces may disturb protein stability by attracting charged amino acid residues. To some extent, this is also true for hydrophobic surfaces interacting with hydrophobic patches of proteins. Molecular dynamics simulation dealt with this problem. Several papers showed small deformations of selected proteins near the surfaces of mica, zinc oxide, zinc sulfide and gold (Nawrocki & Cieplak, 2013; Nawrocki & Cieplak, 2014b; Nawrocki & Cieplak, 2014a; Starzyk & Cieplak, 2011). On the other hand, strong specific contact may be desired, to form

protecting cover for nanoparticle or to allow distance-dependent energy transfer. Strong, quasi-specific contact is quite often realized by binding of positively charged protein tag (e.g. polyhistidine tail (Ipe & Niemeyer, 2006; Ipe *et al.*, 2006; Sapsford *et al.*, 2009)). Such binding may be really strong ($K_d \sim 10^{-9}$M) (Sztatelman *et al.* 2019) and also provides a stable orientation of protein versus QD surface which is especially important for the efficiency of resonance energy transfer. The problem appears (Ipe & Niemeyer, 2006; Ipe *et al.*, 2006; Sapsford *et al.*, 2009; Sztatelman *et al.*, 2019) when the protein contains other positively charged patches. Another way to obtain specific orientation is to use more specific interaction, e.g. biotinylated proteins bind to nanoparticles decorated with streptavidin (Nehilla *et al.*, 2005). Usually binding with tag preserves the secondary and tertiary structure of the protein (Ji *et al.*, 2005) but results in a greater distance between the donor and acceptor thus decreasing the rate of energy transfer.

It is also possible to form a protein embrace for QDs using longer peptides and proteins (Dąbrowska *et al.*, 2016). We found in a recent study that α-helical proteins (PUF) may perform better in such a job than β-sheet ones (LRR) (Kopeć *et al.*, 2019; Dąbrowska *et al.*, 2016). The secondary structure of helical proteins was only slightly disturbed, the tertiary structure had to be imposed though due to the adaptation to QD geometry. Fluorescence properties of QDs in such a hybrid were mostly preserved (Antoniak *et al.*, 2019). Short hydrophobic helical peptides were used as additional functionalization, addressing QDs to the cell membrane (Nag *et al.*, 2017).

CONCLUSIONS

Here we described, by several examples, the junctions of biological molecules and abiotic nanomaterials. The energy transfer in such junctions is possible by resonance energy transfer or by the electron transfer mechanism. Several types of the nanoparticle may function as donors or acceptors in both mechanisms: semiconductor quantum dots, other metallic nanoparticles as well as carbon nanomaterials. The biological partners may be proteins with fluorescent cofactors or redox cofactors, as well as simpler organic molecules with appropriate properties. Also, a switch between the first and the second mechanism may take place at the nanoparticles. Several described nanobiohybrids function as sensors of specific molecules or of the change in the environmental conditions (pH, temperature). Some of them may be considered as nanobiodevices for alternative energy sources. For sure there is a huge demand for further studies to fully understand the functioning of such conjunctions in more nature-like conditions, especially under the availability of both FRET and ET partners, in oxidised and reduced versions.

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