Big Signals from Small Particles: the Regulation of Cell Signaling Pathways by Nanoparticles

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1. INTRODUCTION

"Nanoscience" is recognized as an emerging science of objects that have at least one dimension in ranging from a few nanometers to less than 100 nanometers.[1] Through the manipulation of organic and inorganic materials at the atomic level, novel materials can be prepared with different thermal, optical, electrical and mechanical properties, compared to the bulk state of the same materials. Nanoscale entities are abundant in biological systems and include diverse entities such as proteins, small molecule drugs, metabolites, viruses, and antibodies. In the last 20 years, there has been a rapid expansion in the number of engineered nanosystems that have been developed for biological and medical applications.[2] The field of nanotechnology is based on the convergence of technical disciplines such as physics, chemistry, engineering and computer sciences, cell biology and neuroscience.[3] Nanotechnology concerns the design, preparation, characterization and applications of materials where at least one dimension is on the nanometer scale. Engineered nanodevices are finding an ever-expanding range of applications by versatile modifications of their properties. These involve modifications of the shape, size, surface, and chemical properties. For instance, the surface of nanomaterials can be tailored to a desired use, e.g. in order to improve the biocompatibility of implantable materials or through the attachment of receptors for targeted analyte binding or enhanced adhesion to biological structures.[2]

2. MEDICAL APPLICATIONS OF NANOMATERIALS

Medical applications show particular promise, where nanostructures are engineered to serve as devices for sensing, targeting, delivering and imaging biological structures.[4] The nanoscale offers a number of advantages. For applications in medicine and in biology, nanosystems can be designed to interact with cells and tissues at a molecular level with a high degree of functional specificity. Several good reviews[5] describe some general applications of nanotechnology to medicine and biology. Such applications include:

- Novel drug delivery systems (specifically for the blood brain barrier in some cases) using nanoparticles (NPs) [2,6] or highly porous self-assembling bilayer tubule systems[7] to improve drug bioavailability.
- Functionalized dendrimers as molecular building blocks for gene therapy agents or as magnetic resonance imaging (MRI) contrast agents.[8]
- NPs with different optical properties as imaging agents, e.g. gold NPs whose color changes depending on their size, fluorescent quantum dots (QDs), or super-paramagnetic iron oxide NPs (SPIONs).[3c]
- Membranes for the separation of low weight organic compounds from aqueous solutions
- Biomimetic self-assembling molecular motors[9], such as the flagella of bacteria[9], or the mechanical forces produced by RNA polymerase during protein transcription[10]

2.1. The use of nanomaterials in diagnostic applications

The development of nanometer sized particles for diagnostics has given rise to an import new area of medicine.[3c,11] This large area ranges from common techniques such as X-ray imaging, blood cell counting, plasma analysis, drug level measurement, anatomic imaging by computer tomography (CT) or MRI to histological inspection of tissue samples with specialized techniques such as protein assays, gene expression assays or receptor expression studies.[12] Targeted molecular imaging is important for a wide range of diagnostic purposes such as localization of inflammations or tumor tissue, and visualization of the vascular structure. Due to their small size, NP based imaging and contrast agents are not quickly cleared from the body, which prolongs the time available for imaging and monitoring of a biological structure or an organ.[13] Non invasive detection at the single cell level may be possible with nanocarriers loaded with contrast agent and functionalized with biological receptors specific to the targeted cells.[13] Nanoscale semi-conductor materials, like quantum dots (QD), can be used in optical biosensors.[3c,14] In comparison with traditional organic fluorophores, QD are more photostable, have a greater quantum efficiency and much narrower emission spectra.[15] These properties facilitate the development of highly sensitive optical biosensors. Thanks to the development of instrumentation with very high spatial resolution, e.g. atomic force microscopy (AFM), small-angle neutron scattering (SANS), ultrasmall-angle X-ray scattering (SAXS), quasi-elastic neutron scattering (QENS), nuclear magnetic resonance (NMR) spectroscopy, and nuclear resonance reaction analysis (NRRA), it is possible to determine the structure of nano-objects and to measure their physical and chemical properties. Atomic scale modeling by *ab initio*, molecular dynamics (MD), and energy

minimization techniques has offered new insights and is shedding light onto the processes occurring at the nanoscale.[16] A main goal of nanomedicine is the creation of multi-task NPs for the enhanced diagnosis, monitoring and treatment of human diseases. However, a main obstacle remains the poor mechanistic understanding of the interaction of nano-structures with biological systems, in particular their potential toxicity.[17]

3. THE INTERACTION OF NANOMATERIALS WITH LIVING CELLS

Cell interactions with NP are strongly dependent on the properties of the nano-systems, i.e. their chemical composition, size, morphology, surface charge, and functionalization (Fig. 1, 2F). When introduced into biological systems, NP can interact with a wide range of biological molecules including serum proteins, glycoproteins, cell surface proteins, carbohydrates, salts and metabolites. These interactions may profoundly alter the properties of the NPs and produce very distinct responses in different organisms.[18] Proteins bound to NPs can undergo structural changes including denaturation.[19] The consequences of these interactions can be NP agglomeration caused by the aggregation of the denatured proteins, which can have deleterious consequences. Such aggregation has been reported to trigger activation of the complement system resulting in phagocytic cell capture of the aggregates, adherence of aggregated material to blood vessel walls followed by platelet and thrombin activation with tissue injury, anaphylaxis and finally death.[20] On the other hand, Cherukuri *et al.*[21] showed that blood proteins displaced surfactant coatings on single-walled carbon nanotubes (SWCNTs) within seconds in the blood but no sign of toxicity was observed. In fact, some studies showed that interactions of NPs with proteins can facilitate NP biocompatibility.[22]

Many studies have highlighted that interactions between NP and cells, *in vivo* or *in vitro*, can cause a wide range of biological responses. Cells exposed to NPs *in vitro* show changes in the regulation of specific cellular proteins (fibronectin, cadherin or specific enzymes), changes in the cell cycle and the appearance of inflammatory and apoptotic markers.[18,23] Further, NPs cause cell damage[17a,22,24], formation of reactive oxygen species (ROS)[2,24f,25], increase production of specific cytokines[18,25a,26], and abnormalities in cell-adhesion[23b]. However, several NPs showed no apparent damage to biological cells or tissues.[27] Thus, the key in developing NPs with maximum biocompatibility is carefully designed experimental studies that systematically evaluate the interaction of NPs with living systems. The approach for investigating interactions of NPs with cells *in vitro* offers a quick and mechanistically accessible way to study cellular responses to specific NP doses, exposure times, and intracellular NP fates with specific cell types. These tests allow biochemical, ultra-structural, and proteomic analysis of specific types of cells during NP exposure, which is not possible within complex living organisms. Therefore, *in vitro* testing is more accurate and appropriate to study specific variables and cellular responses. Of course, these studies only can capture responses that are specific for certain cell types and even specific states of cells. This heterogeneity is a likely reason for discrepant findings. Panessa-Warren et al.[19a] summarized several studies on carbon based nanomaterials, on quantum dots and on gold NPs. They concluded that many studies have led to dissimilar conclusions: in some studies, these nanostructures were found biocompatible while in others they were found cytotoxic. Of course, the challenge is to

uncover the reasons for these discrepancies and eventually arrive at a reconciled view on the effects of nano-structures on biological systems.

3.1. The role of physico-chemical NP properties in the interaction between NPs and biological systems

The relation of physicochemical properties of NPs and biological response is based on a plethora of physicochemical influences on the interface between nanomaterials and biological systems.[23a] Many studies aimed at correlating NP properties such as size, shape, chemical functionality, surface charge, and composition with biomolecular signaling, biological kinetics, transportation, and toxicity in both cell culture and animal experiments (Fig. 1,2F).[28] However, it has proven difficult to draw general conclusions regarding the impact of size, shape, and surface chemistry-dependent interactions on biological systems. In addition, neither NP properties nor biological process can be viewed independently and only testing the combination of various interfaces will allow the development of predictive relationships between structure and activity that are determined by NP properties.[23a]

In general, NPs are produced by chemical synthesis followed by coatings with polymers, drugs, fluorophores, peptides, proteins, or oligonucleotides before they are administered *in vitro* or *in vivo*. [28] Therefore, not only the engineered geometry of the 'naked' NP, but also the chemical surface property and the ligand density of the nanomaterial strongly influence the interaction between NP-bound ligands and cellular receptors. NP size in combination with the ligand density over a specific curvature will contribute to the

overall effect of the NP. The ligand's binding affinity can increase proportionally to the size of a NP due to a higher protein density on the NP surface [29]. However, unexpected results were observed for different ligands. While the intercellular adhesion molecule I (ICAM-I) is usually not known to trigger endocytosis, ICAM-I-coated NPs were internalized by the cells.[30] In another study, Unfried et al. described 14 nm carbon NPs that can induce cell proliferation of lung epithelial cells via interaction and activation with EGFR and β1-integrins (Fig4).[31] Using specific inhibitors it was demonstrated that NP-induced proliferation was mediated by the activation of two kinases, phosphoinisitide-3 kinase (PI3K) and Akt. In addition, protein ligands conjugated to the NP surface might experience conformational changes including denaturation. It was demonstrated that lysozyme conjugated to gold NPs denatures and interacts with other lysozyme molecules to form protein-NP aggregates.[32] Another example is fibrinogen as a ligand on polyacrylic acid–coated gold NPs, which unfolds when bound to the NP. As a result, the denatured fibrinogen binds to the integrin receptor Mac-1 and leads to inflammation.[33] In conclusion, the denaturation of a conjugated protein can affect binding to its receptor, increase nonspecific interactions or provoke inflammation [28].

Of particular interest for designing safe and efficacious NPs is an understanding of the relationship between the physico-chemical properties of NPs and their biological effects. Some of these rules are beginning to emerge, and reports on correlations between NP cytotoxicity and their physico-chemical properties have increased.[3a,13,16b,17b,22c,24c,d,34]. For instance, Horie *et al.*[35] have described relationships between physico-chemical NP properties and the biological responses they evoke. They suggested a five step *in vitro*

protocol for the evaluation of the effects of NPs on cells: (i) preadsorption of the NPs with growth medium, in particular with serum which contains essential growth factors and hormones that may be depleted by adsorption to NPs; (ii) measurement of elements, such as metals, ions or proteins, released from NPs; (iii); confirmation of the stability of NPs in the growth medium dispersion; (iv) determination of the formation and effects of secondary particles that form through aggregation, coating with serum proteins and salts; and (v) observation of the cellular uptake of the NPs. Mailander and Landfester[36] studied the cytotoxicity of NP by incubating the cells for 4-24 h with NP and cytotoxicity was evaluated by 7-Aminoactinomycin D (7-AAD) uptake. 7-AAD allows distinguishing live, apoptotic, and dead cells by FACS analysis.

3.2. NP uptake into cells

Several uptake ways into cells have been described for small molecules and macromolecules like proteins (Fig.2) .[37] As particles range from a few to several hundred nanometers in size, uptake conditions and mechanisms involve several possible mechanisms such as pinocytosis, nonspecific endocytosis, receptor-mediated endocytosis.[38] Phagocytosis has been discussed for uptake of larger particles (Fig. 2A). Additionally, the particle surface charge has an impact on uptake mechanisms, as modifications of the NP surface with positive or negative charges enhance cellular uptake. This effect can be “titrated” by using a series of NPs with a range of densities of effective side groups. Macropinocytosis seems to be an important mechanism for positively charged NPs as demonstrated by the strong inhibition of the uptake of positively charged NPs by 5-(*N*-ethyl-*N*-isopropyl) amiloride.[39] The microtubule network

and cyclooxygenases are also involved in uptake of positively charged NPs as uptake was hindered in the presence of nocodazole and indomethacin, respectively. About 20 % of the endocytosis of positively charged particles is inhibited by chlorpromazine.[39] Clathrin-coated pits (Fig. 2C) only play a minor role in the uptake of positively charged NPs, and have no effect on the endocytosis of negatively charged NPs. It can be concluded that depending on the surface charge of the NPs differences in uptake and intracellular trafficking of the endosomes may occur. Interestingly, negatively charged NPs were less inhibited by a dynamin inhibitor pointing toward the possibility that a hitherto unidentified dynamin-independent process may contribute to the uptake of negatively charged NPs.[36] Furthermore, cellular uptake can be enhanced by transfection agents. Jing et al. compared seven different, commercially available transfection agents based on dendrimer, lipid, and polyethylenimine formulations and their ability to aid NP uptake. [40] By contrast, NP uptake can by decreased by polyethylene glycol (PEG) if uptake is not favored for an application.

3.2.1. The role of size and shape on NP uptake

NPs are usually sized between one and several hundred nanometers in diameter. Looking at the interaction with living matter, a 'rule of thumb' seemed to emerge, that NPs less than 100 nm diameter can enter cells. NPs smaller than 40 nm are capable of entering the nucleus, while NPs smaller than 35 nm can cross the blood–brain barrier [41]. However, different NPs below a certain size can enter a variety of cells by different processes. These include phagocytosis, the cellular process, where the cell membrane internalizes solids by forming vesicles that pinch off as invaginations of the membrane (Fig. 2A). In

addition, pinocytic processes, i.e. vesicular internalization of liquids, such as macropinocytosis (Fig. 2B), clathrin-mediated (Fig. 2C) and caveolin-mediated (Fig. 2D) processes are likely to be involved in NP uptake.

Rejman and co-workers investigated the internalization of differently sized latex beads (50-500 nm) in non-phagocytic cells, showing that particles as large as 500 nm were internalized by the cells [42]. Furthermore, the mechanism by which such beads were internalized, and their subsequent intracellular routing, was strongly dependent on particle size. While beads smaller than 200 nm were internalized by clathrin-coated pits (Fig. 2C), larger beads were taken up by caveolae-mediated internalization (Fig. 2D). Jian and co-workers could show that different sizes (2-100 nm) of colloidal gold NPs (GNPs) conjugated with Herceptin induce different cellular responses.[29] While the binding affinity of Herceptin to the ErbB2 receptor is 10^{-10} M in solution, 10 nm NPs or 70 nm NPs had affinities of 5.5×10^{-12} M and 1.5×10^{-13} M, respectively. These results indicate that the ligand's binding affinity increases proportionally to the size of a NP due to a higher protein density on the NP surface. However, surprisingly, 40 nm and 50 nm NPs demonstrated the greatest effect on downstream signaling events, suggesting additional factors to be considered for this effect. Several reports suggest that 50 nm seems to be the optimal diameter in order to maximize the rate of uptake and intracellular concentrations of gold NPs, silica NPs, single-walled carbon nanotubes, and quantum dots, in certain mammalian cells [43]. In addition, ligand density seems to be optimal as well for NP diameters of 30-50 nm. Smaller NPs might have less ligand-to-receptor interactions than

do larger NPs per particle, which impacts significantly on size-dependent membrane wrapping und cellular uptake.

Not only size seems to matter, but also the physical shape of the NPs has an influence on cellular uptake. Gratton and co-workers demonstrated that rod-like particles (monodisperse hydrogel particles) show the highest uptake in Hela cells, followed by spheres, cylinders, and cubes [44]. In addition, the results suggest that clathrin-mediated and caveolae-mediated endocytosis and, to a much lesser extent, macropinocytosis are involved with the internalization (Fig. 2). For particles smaller than 100 nm, other studies suggest that spheres show a significant advantage over gold nanorods.[43a,45] In comparison to spherical particles, rod-shaped NPs present two different dimensions to the cell surface as the short and long axis will interact differently with cell surface receptors.[43a] These non-spherical, asymmetrical NPs may provide additional options in presenting ligands to the target receptors.[28] Taken together, cellular uptake of NPs is not only regulated by NP size and shape but also in combination with different cellular uptake mechanisms. Discrepancies in the recent literature suggest that the size 'rule of thumb' seems to be an oversimplified representation of the actual scenario. It is likely that different cell types with different uptake mechanisms, even for the same NPs, react differently to NP exposure. From a technical point of view, the NP uptake very likely depends on the cell being assayed due to individual cell phenotypes. Factors such as varying levels of the target receptor, membrane fluidity, cell cycle will affect the interaction with NPs. Therefore, NPs studies should be expanded to include both primary cells and immortalized cell lines growing under different cell culture configurations (monolayer,

3D environment) in order to identify broad-scope design parameters for NP cell interactions.

3.2.2. The role of surface charge and protein corona on NP uptake

In addition to size, shape, and ligand composition, the surface charge of NPs is considered an important factor for nano-bio interactions (Fig. 2F). For endocytosis, positively charged NPs will show a higher internalization than neutral or negatively charged NPs. The negative charge of cell membranes facilitates anchoring of positively charged NPs to the cell surface, thus favoring endocytosis.[46] For modified NP surfaces with carboxyl and amino side groups, a clear correlation of surface charge and endocytosis has been shown for several cell lines[36,47] In general, positively charged particles independently of the material type seem to induce cell death[41,48], although this correlation is not fully understood and multiple entry pathways might be responsible.[49 42] However, also negatively charged NPs were shown to have enhanced uptake as compared to non-charged NPs. This phenomenon might be explained by NP interactions with other proteins. In general, negatively charged NPs seem to cause local gelation of membranes, whereas positively charged NPs induce fluidity [50].

In addition, the configuration of biological membranes, transmembrane receptors, and underlying signaling machinery influence NP-induced effects. Parameters such as membrane composition, membrane domains like rafts, and extracellular matrix proteins may have significant impact on NP-induced signaling.[23a,51] At this point it is crucial to note that NPs do not arrive ‘naked’ at the cell. Independent of surface charge and ligand

composition, the NP surface is in contact with the biological milieu, such as growth medium, salts, metabolites and serum proteins. Due to this dynamic exchange with biomolecules, the NP surface becomes quickly covered by multiple biomolecules and proteins, called corona.[52] The dynamic composition of this corona affects the way cells interact with, recognize and process the NPs. This knowledge is crucial, when NPs are used *in vivo*. While neutrally charged NPs have the longest half-life in blood, positively charged NPs are cleared most quickly from the blood and were shown to cause several complications such as hemolysis and platelet aggregation.[28] The protein corona seems to be a key factor for these discrepancies, as different surface curvatures and charges attract different proteins (e.g. immunoglobulins, lipoproteins, and coagulation factors) to form the corona.[52c]

Thus, *in vitro* studies give important information on mechanisms in the evaluation of NP uptake and toxicity. The induction of oxidative stress, apoptosis, production of cytokines, and cell death were observed with many kinds of NPs *in vitro* (Fig. 3). This information contributes to the prediction and evaluation of *in vivo* toxicity. However, inconsistencies between the results from the same NPs are frequently reported.[22c,24f] In part this is due to the lack of thorough physico-chemical characterization of NPs. A better understanding of the interactions between cells and NPs is becoming increasingly important as cellular therapies are very promising for regenerative medicine, especially repair of tissue function after organ damage.[53] Nanomaterials could influence the fate of differentiation of these cells or enable the detection of migration and homing of cells. To track the migration and homing of transplanted cells[13], techniques like bioluminescence[54],

radioactive substrates[55], near-infrared fluorescence[56], and labeling with magnetic resonance imaging (MRI) contrast agents[13,57] are applied in small animal studies. Of these, only labeling with radioactive agents and MRI contrast agents are suitable for studies in humans.

3.3. Activation of signal processing pathways by NPs

The promising capability of nanoscale object materials for activation of cellular signaling pathways has two major outcomes - controllable and uncontrollable biological responses. The controllable biological responses are very appealing for medical communities. In this case, the artificial control of signaling pathways by NPs leads to the induction of a desired cellular phenotype change such as cell shape, cytoskeletal organization, and cell fate.[58] In contrast to these very promising applications of NPs in controllable activation of cellular signaling pathways, catastrophic outcomes may be expected for uncontrollable activation of signaling pathways by NPs. Recently, it has been confirmed that NPs can cause DNA damage to cells cultured below a cellular barrier without crossing this barrier.[59] The outcome, which includes DNA damage without significant cell death, is different from that observed in cells subjected to direct exposure to NPs. The indirect damage is exerted by purine nucleotide second messengers and intercellular communication within the barrier cells through gap junctions that causes the production of oxygen radicals in the mitochondria.[60]

The ever increasing development of NPs with various physicochemical properties for different industrial applications has greatly enhanced human exposure to nanomaterials.

This exposure can be deliberate, such as in applications where NPs are used as imaging agents or for drug delivery, and unintentional, e.g. through NPs pollution of the environment by industrial production.[34b,61] In the latter case the risk of uncontrolled entrance of NPs into living organisms is already becoming a serious issue. To control this potentially dangerous risk it is essential to develop safety guidelines and health regulations based on a deep understanding of the various and complex effects of NPs on organisms.

3.3.1. Interaction of NPs with cell membrane receptors

There is increasing evidence that NPs are not only able of passive interaction with living cells and cell membranes, but that they can interact with membrane receptors thereby actively and specifically modulating signal transduction pathways (Fig. 4).[46] These interactions are determined by the size and surface chemistry of the NPs and the protein corona[23a] assembled on the surface. These interactions are of major importance since they have biomedical and toxicological relevance.

The interactions of NPs with cell membrane receptors were described in several studies. Among others, Unfried et al. described carbon NPs that can induce cell proliferation of lung epithelial cells via interaction with and activation of the epidermal growth factor receptor (EGFR) and β1-integrins (Fig. 4).[31] Integrins are transmembrane proteins that mediate cell adhesion and the communication between cells and their environment. As such they play key roles in cellular signalling, and their activation by extracellular ligands elicits biological processes such as angiogenesis, differentiation and migration.[62] Using

specific inhibitors it was demonstrated that NP-induced proliferation is due to EGFR and β1-integrin mediated activation of PI3K and Akt.[31] In an earlier study, Sydlik et al. could show that ultrafine carbon particles activate EGFR in lung epithelial cells subsequently leading in parallel to apoptotic events as well as proliferation.[63] While the kinase activity of EGFR was found essential for both processes, β1-integrin-mediated activation of ERK was instrumental for proliferation, whereas apoptosis was mediated by activation of c-Jun. In other studies, it was demonstrated, that in human bronchial epithelial cells, exposure to PM2.5 particles (i.e. particles with a diameter of 2.5 micrometers or less) or diesel exhaust particles activated the EGFR and subsequent ERK-mediated amphiregulin expression and secretion.[64] Such an autocrine loop might reflect a mechanism for sustaining the proinflammatory response. Interestingly, secretion of amphiregulin could be inhibited by the antioxidant N-acetyl cysteine but not by a neutralizing anti-EGFR, indicating that the transactivation of the EGFR occurred via the production of reactive oxygen species (ROS). Our own results show that small negatively charged superparamagnetic iron oxide NPs (snSPIONs) can activate the EGFR and downstream ERK and Akt signaling independent of ROS production.[65]. Mutation of Ras enhanced the response, and snSPIONs could support the proliferation of Ras transformed breast epithelial cells with a similar potency as EGF. As Ras is mutated in 20-30 % of all human cancers[66], this result suggests that NPs potentially could stimulate the growth of tumors with Ras mutations. It is not clear yet how exactly snSPIONs stimulate the EGFR, but they may activate it directly by receptor crosslinking. In a similar vein, Huang et al. could demonstrate that gold NPs coated with dinitrophenyl at a controlled density were capable

of binding and cross-linking IgE-Fc epsilon receptors subsequently leading to degranulation of rat basophilic leukemia cells and the release of chemical mediators.[67]

In a recent study, Bhabra et al. asked whether a cellular barrier, i.e. a confluent layer of cells, could protect human fibroblasts from DNA damage induced by cobalt-chromium NPs (29.5 nm) when placed between the NPs and the responder cells.[59a] Surprisingly, the NPs could cause DNA damage across this biological barrier without translocation of the NPs. This response was mediated by cell-to-cell signaling within the barrier through the gap junctions. In a subsequent study the same group demonstrated that the indirect DNA damage depends on the thickness of the biological barrier, and that the signaling through gap junction proteins is followed by the generation of mitochondrial ROS.[60] This demonstration of indirect adverse effects of NPs raises a potential concern when applying NPs *in vivo*, as tissues behind cellular barriers might be affected indirectly.

3.3.2. The role of oxidative stress and ROS in NP induced signaling

During the last years, NP-induced toxicity has become one of the most investigated topics in toxicology, mainly through studies on fine particle exposure. A number of nanomaterials cause oxidative stress through the accumulation of reactive oxygen species (ROS) and the cell's inability to detoxify and repair the damage resulting from the oxidation of DNA, proteins and lipids (Fig. 3).[23a] ROS include superoxide anion radicals ($\cdot O_2^-$), hydroxyl radicals ($\cdot OH$), and hydrogen peroxide (H_2O_2). After passing the cell membrane NPs in the cytoplasm can elicit several biological stress responses including the disruption of mitochondrial function, activation of the oxidative stress-mediated

signaling cascade, cell cycle arrest, apoptosis, and inflammatory responses (Fig. 3).[59a,68] Even more severe, hydrophilic titanium oxide NPs were shown to have an oncogenic potential by converting benign mouse fibrosarcoma cells into malignant cells through the generation of ROS.[69] Several NPs were shown to induce high ROS levels upon exposure. TiO_2 (30 nm) and silver (15 nm) NPs seem to induce the highest ROS levels[70], while NP size does not to appear to be significant.

So far, NP toxicity was mainly described through the induction of oxidative stress, inflammation and apoptosis as cellular responses.[18] Cell death highly depends on NP concentration, duration of the exposure and the cellular system investigated and both, programmed cell death (apoptosis) as well as necrosis, were observed as an outcome [63,71]. Interestingly, the chemical nature of the NP seems to highly influence the pathways usage involved in apoptosis. Comparing toxic effects of carbon black with TiO_2 NPs, Hussain et al. could show, that both NP formulations induce apoptosis in bronchial epithelial cells [72]. However, while carbon black NPs induce apoptosis by a ROS-dependent mitochondrial pathway, TiO_2 NPs induce cell death through lysosomal membrane destabilization and lipid peroxidation.

However, ROS also play important roles as intercellular second messengers and activators of specific pathways.[73] In human microvascular endothelial cells, iron NPs were shown to enhance cell permeability through the production of ROS and stabilization of microtubules.[74] Several studies have demonstrated that NP-mediated ROS can induce or mediate the activation of the Mitogen-activated protein kinase (MAPK) pathways (Fig.

3), which consist of the growth factor-regulated extracellular signal-related kinases (ERKs), and the stress-activated MAPKs, c-Jun NH2-terminal kinases (JNKs) and p38 MAPKs [75]. However, the exact mechanisms by which ROS can activate the MAPK pathways are not well defined. One way is via activation of growth factor receptors as demonstrated by Gyoton et al. in several cell types.[76] Another mode of action is through direct oxidative modification of the kinases that are involved in the MAPK signaling cascade. ASK-1, a redox-sensitive kinase upstream of JNK and p38, binds to reduced thioredoxin in non-stressed cells. Upon an oxidative stress thioredoxin becomes oxidized and disassociates from ASK-1 leading to the oligomerization and activation of ASK-1and subsequent activation of JNK and p38 pathways.[77] Another mechanism for ROS-mediated MAPK activation is through inactivation and degradation of the MAPK phosphatases (MPK) that maintain MAPKs in an inactive state. Among other studies, intracellular H_2O_2 accumulation was shown to inactivate MKPs via oxidation of their catalytic cysteine, which leads to sustained activation of JNK pathway.[78]

NP-mediated activation of MAPK often influences gene transcription through redox-sensitive transcription factors, such as NFκB and Nrf-2 (Fig. 3). Nrf-2 is known to induce the expression of various genes encoding for several antioxidant enzymes such as haeme-oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO1).[79] Amara et al. could demonstrate that Diesel exhaust particles activate ERK1/2 which, combined with activation of the NADPH oxidase analog NOX4, modulate the expression and activity of the matrix metalloprotease (MMP)-1.[80] As a recent example, cerium oxide NPs were shown to elevate ROS levels leading to a strong induction of HO-1 via the p38-Nrf-2

signaling pathway in human bronchial epithelial cells (Beas-2B).[81] Interestingly, in another study, exposure of cells with silver NPs decreased nuclear Nrf-2 expression, its translocation into nucleus, and the transcriptional activity of Nrf-2 [82]. Upstream, silver NPs attenuated both active forms of ERK and Akt protein expression, resulting in suppression of Nrf-2 and decrease of 8-Oxoguanine DNA glycosylase 1 (OGG1) expression. OGG1 is a DNA repair enzyme involved in repairing silver NP-induced DNA damage.

Comfort et al. evaluated the effects of silver NPs (10 nm), gold NPs, and iron oxide NPs (SPION) on EGFR-mediated signaling in the human epithelial cell line A431[83] All NPs used in this study altered the signaling processes, but interestingly, the metallic composition seems to determine the mechanism. While silver NPs induced high quantities of ROS and attenuated Akt and ERK activation, gold NPs decreased EGF-dependent Akt and ERK activation. SPIONs were shown to strongly alter EGF-mediated gene transcription, thus influencing cell proliferation, migration, and receptor expression (Fig. 4).[83]

As components of the MAPK signaling cascades are deregulated in a variety of human cancers, targeting these components through NP-mediated therapies is a logical step. Several selective inhibitors of MAPK signaling pathways are now available. The best developed are inhibitors of MEK[84], the kinase which activates ERK. Basu et al. demonstrated that polylactic acid glycolic acid (PLGA)-based NPs which were conjugated with the selective MEK inhibitor PD98059, were taken up by cancer cells by

endocytosis resulting in the inhibition of the ERK pathway.[85] Furthermore, NP-mediated ERK inhibition suppressed the proliferation of melanoma and lung carcinoma cells and induced apoptosis *in vitro*, while *in vivo* administration inhibited tumor growth in melanoma-bearing mice and enhanced the antitumor efficacy of cisplatin chemotherapy. In human neutrophils, titanium dioxide NPs markedly activated the MAPKs p38 and ERK1/2, thereby activating the neutrophils and leading to changes in cellular morphology.[86] Interestingly, after prolonged exposure, TiO_2 inhibited neutrophil apoptosis in a concentration-dependent manner and induced the secretion of several cytokines and chemokines including IL-8. MAPKs feature a multitude of substrates, e.g. ERK alone has more than 150 known substrates.[87] Therefore, it is no surprise that MAPK activation can have widespread consequences for cellular physiology and induce profound biological responses. However, apart from ROS mediated mechanisms it is still unclear how NPs activate MAPKs. Peuschel et al. could show that Src family kinases might represent a potential link between carbon NP-mediated receptor activation and subsequent MAPK signaling (Fig. 4).[88]

3.3.3. NPs induce cell death pathways

Several NP formulations are known to induce oxidative stress through elevation of ROS levels [23a]. If the cell cannot counteract these situations, NPs can induce cell death as was shown in a variety of *in vitro* systems. Cell death highly depends on NP concentration, duration of the exposure and the cellular system investigated and both, programmed cell death (apoptosis) as well as necrosis, were observed as an outcome [63,71]. Interestingly, the chemical nature of the NP seems to highly influence the pathways usage involved in

apoptosis. Comparing toxic effects of carbon black with TiO_2 NPs, Hussain et al. could show, that both NP formulations induce apoptosis in bronchial epithelial cells. [72] However, while carbon black NPs induce apoptosis by a ROS-dependent mitochondrial pathway, TiO_2 NPs induce cell death through lysosomal membrane destabilization and lipid peroxidation.

3.3.4. Activation of mechano-transduction receptors by magnetic NPs

One very appealing application of NPs is the activation of cell signaling pathways by magnetic NPs (Fig. 5). Paramagnetic NPs are coated with specific ligands that enable them to bind to specific membrane receptors on the cell surface.[89] By applying an external magnetic field the magnetic NPs can apply nanoscale forces on the cellular receptors. This mechanical stimulation of the receptors allows for the activation of underlying signaling pathways by mechano-transduction, i.e. the process by which cells convert physical force into a biochemical signals.

4. MAGNETIC NPs AND CELL SIGNALING

Magnetic NPs can be tailored in order to have specific dimensions and magnetic properties in combination with unique surface coatings and ligands (Fig. 5A). Very common are iron oxide particles which can be fairly simply synthesized by coprecipitation from iron salts, but also other magnetic materials like cobalt or nickel are used.[90] Importantly, NP size does not seem to impact on the magnetization at the nanoscale. Thus, the force which an external magnetic field exerts on the particle can range from 10^{-12} to 10^{-9} Newton.[89b,91] Several formulations are commercially available

and via reactive functional groups ligands of choice can be chemically attached. Due to the usually short-term application of magnetic NPs when probing extracellular receptors, cytotoxic side effects can often be avoided.

4.1. Controlled activation of cell surface receptors by magnetic NPs

Cells are exposed to ligand-conjugated magnetic NPs, which bind to specific targets on the surface (Fig. 5B). Magnetic fields can deliver nanoscale forces to the NPs and their bound receptors. This mechanical stimulation was shown to activate specific cellular signaling pathways (mechanotransduction pathways) and is applied through magnetic twisting (Fig. 5C), pulling or clustering of particles (Fig. 5D) and several technique platforms are available. While, so-called magnetic tweezers allow for pulling NPs by gradients in a magnetic field, magnetic twisting cytometry generates a mechanical torque at the particle-cell interface by applying a field in a direction perpendicular to the magnetic dipole of the particle [89a,92]. The applied torque drives the magnetic particle to twist or roll on a cell's surface. However, because the particle is physically restrained by binding to its receptor, the rolling action produces a shearing force at the cellular receptor that affects downstream signaling (Fig. 5B).

4.1.1. Activation of signal transduction receptors by magnetic NPs

One very appealing application of NPs is the activation of cell signaling pathways by magnetic NPs. Paramagnetic NPs are coated with specific ligands that enable them to bind to specific membrane receptors on the cell surface.[89] By applying an external magnetic field the magnetic NPs can apply nanoscale forces on the cellular receptors

(Fig. 5B). For instance, the magnetic aggregation of receptors through the NP-ligand interaction was used to activate immunological responses in mast cells. SPIONs in combination with IgE antibodies were applied for the clustering of FcεRI receptors.[93] This mechanical stimulation of the receptors allows for the activation of underlying signaling pathways by mechano-transduction, i.e. the process by which cells convert physical force into a biochemical signals (Fig. 5B).

4.1.2. Activation of mechanotransduction receptors and mechano-sensitive signaling pathways by magnetic NPs

Using magnetic NPs in combination with specific conjugated ligands, a series of pathways was uncovered which regulate mechanotransduction. Probably, among the best studied molecular transducers of mechanical force are integrins. Integrins are transmembrane receptors, which mediate the attachment between a cell and the surrounding tissues by binding other cell surfaces and components of the extracellular matrix. Integrins are obligate heterodimers consisting of a set of alpha and beta subunits that connect membrane surface sensors and the cell's internal cytoskeleton. In addition to a structural role they also transduce external signals and play a crucial role in regulating cellular shape, motility, differentiation, and cell cycle events. [94] Several studies successfully used magnetic NPs conjugated with known integrin interactors to induce mechanotranduction (Fig. 5B,C,E,F). Glogauer et al. used collagen-conjugated NPs, which bind focal adhesion-associated proteins such as talin, vinculin, α2-integrin and β-actin.[95] When force was applied to the collagen beads, force-mediated actin assembly was mediated by calcium ions and tyrosine-phosphorylation of paxillin thus resulting in

changes in membrane rigidity (Fig. 5E). In rabbit ventricular myocytes stretching of β1-integrin by specific antibody-coupled beads was shown to activate an outwardly rectifying chloride current, and these events were regulated by focal adhesion kinase (FAK) and c-Src (Fig. 5F).[96] Data suggest that integrin stretching may contribute to a mechano-electric feedback in the heart by modulating electrical activity. In a vascular endothelial cell model, integrin-linked magnetic beads were used to demonstrate that the transduction of local mechanical forces into biological signals is mediated through the activation of focal adhesion sites.[97] Again, tyrosine phosphorylation of c-Src was important downstream for force-induced translocation, and signals were transduced rapidly via the pre-stressed cytoskeleton.[98] In another study, Hu et al. asked how mechanical stress applied from the outside of the cell is transmitted within the cytoplasm of an adherent cell.[99] Magnetic twisting of integrins in combination with labeling of intracellular components revealed that mechanical forces are transferred across discrete cytoskeletal elements over long distances through the cytoplasm.

Besides integrins, other receptors were successfully targeted by magnetic twisting or tweezers including the urokinase receptor[100], E-selectin[101], E-cadherin[102], and VE-cadherin.[103] Furthermore, magnetic tweezers were applied to study stretch-activated ion channels.[92b,104] Moreover, receptor-mediated artificial triggering of cell growth in pre-angiogenesis, which is a vital process both for the growth and development of blood vessels and for tumor metastasis stage[105], has been successfully controlled by magnetic antibody-conjugated NPs binding the Tie2 receptor.[106]

In conclusion, by applying magnetic NPs in combination with specific ligands it is possible to manipulate and control cellular functions via an external magnetic field. The specific activation of single receptor species led to the discovery of mechano-sensitive mechanisms, and several crucial signaling pathways were identified. Nanomagnetic regulation has tremendous applications not only *in vitro* but also in a clinical setting, as remote manipulation of cellular receptors in specific tissues might be a realistic option.

4.2. Pathway activation by magnetic NP induced hyperthermia

During hyperthermia therapy the body is exposed to elevated temperatures in order to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiation and anti-cancer drugs. This type of medical treatment is based on the idea that tumor tissue has difficulty dissipating heat due to its less organized vascularization compared to normal tissue. Therefore, hyperthermia causes tumor cells to undergo apoptosis, while healthy tissues can more easily maintain a normal temperature leaving them less affected by the treatment.[107] Furthermore, tumor cells may become more susceptible to ionizing radiation therapy or to certain chemotherapy drugs. Thus, hyperthermia treatment is usually administered together with other cancer treatment modalities. Several clinical trials have shown beneficial effects of this combination.[107] Three types of administration are distinguished: *whole-body hyperthermia*, where the whole body is heated to 39-41°C in order to treat metastatic malignancies; *regional hyperthermia*, where parts of the body, e.g. limbs, whole tissues, are heated; and *local hyperthermia*, which heats a very small area, usually the tumor itself. The heat is usually

created by microwave, radiofrequency, ultrasound energy or using magnetic hyperthermia.

Initially, magnetic particles were applied by direct injection into the tumor mass after which the tumor is exposed to an alternating magnetic field.[108,34a] Later, magnetic NPs were conjugated with tumor specific antibodies for actively targeting the particles to the tumor. Here, particles are selectively ingested by the tumor cells, thereby increasing the NP's retention in the tumor region.[109] Magnetic hyperthermia is based on the fact that magnetic NPs, when subjected to an alternating magnetic field, produce heat. For treatment of malignancies, magnetic NPs are put inside a tumor and the whole patient is placed in an alternating magnetic field of well-chosen amplitude and frequency, resulting in elevated temperatures in the tumor. The details of this procedure are well described in other reviews[13,34a,89c,107,110], so here we want to briefly discuss the underlying biological consequences und cell signaling pathways.

4.2.1. The molecular targets of hyperthermia: heat shock proteins and p53

Increasing temperature affects several cellular targets including cell membranes, cytoskeletal components, biosynthesis, apoptosis and DNA repair.[111] One of the best-studied consequences is the induction of heat shock proteins. Hyperthermia triggers adaptive challenges in the tumor cells. One obvious adaptation mechanism is the induction of the heat-shock response and expression of heat shock proteins (HSPs). HSPs are a family of proteins that were initially identified as stress proteins mediating resistance to physical stresses such as elevated temperatures in all cellular organisms.[112]

After exposure to elevated temperatures, HSP levels are rapidly elevated and confer a temperature resistant phenotype. HSPs function as molecular chaperones, prevent aggregation of their substrates, and act as inhibitors for components of the apoptotic machinery. Interestingly, the expression of many HSPs is increased in tumor cells. HSPs promote tumor growth by inhibiting apoptosis (Hsp27, Hsp70) and by promoting autonomous growth (Hsp90), and inducing resistance to chemotherapy and hyperthermia [112-113]. Interestingly, HSPs together with MHC class I molecules are also involved in the presentation of antigens, influencing on the immunogenicity of tumor cells.[114] Therefore, the induction of HSPs through local hyperthermia seems to be a double-sided sword. One the one hand, HSPs may have beneficial effects on tumor growth, one the other hand tumors might become more immunogenic for the immune system, which might enhance tumor rejection or the effect of drug therapies.

The tumor suppressor p53, also known as "the guardian of the genome", regulates DNA repair, cell cycle, apoptosis among other processes, thereby preventing malignant degeneration of normal cells. In about half of all tumors, the p53 gene is mutated or lost allowing malignant changes in the cancer progress at a high frequency.[115] Several studies reported that heat treatment depresses the DNA repair of radiation-induced DNA strand breaks and thymine lesions[111a,116] suggesting that the synergistic effects of hyperthermia on radiation-induced cell killing is induced mainly through the inhibition of DNA repair mechanisms.[117] In the context of hyperthermia, the heat- and chemosensitivity of several malignancies are dependent on the p53 status of the cells and correlate with induction of apoptosis in those cells.[118] The synergistic hyperthermic enhancement of radiosensitivity

was demonstrated for tumors expressing wild-type p53 cells, but not in mutated p53.[119] Thus, the *p53* gene status of cancer cells may be useful as a predictive assay for the effectiveness of local hyperthermia therapy in combination with radiation.

5. CONCLUSIONS

The existing data provide tantalizing evidence for the unprecedented opportunities nano-materials offer for the monitoring and manipulation of biological systems. In order to deliver these promises we need to fully understand how nano-materials interact with biological systems. In this respect, we are currently only scratching at the surface. When considering this question we have to distinguish between the effects of the nano-materials themselves, the effects induced by their cargo, and possible combinatorial effects arising from blending the two. There are many general questions arising, e.g. about the biodistribution, clearance, degradation and long-term fate of nano-materials in the body. In the context of cell signaling, we will need to identify the signal transduction pathways that respond to nano-materials and elucidate how they are engaged by nano-materials. The main attention has been focused on the role of ROS and stress activated signaling, and the engagement of cell surface receptors. However, rather little is known about the molecular mechanisms that mediate the functional interactions between nano-materials and signaling networks. For instance, as nano-materials are synthetic, man-made structures, a pertinent question is whether this interaction is similar or fundamentally different from known interactions between cells and physiological ligands. Cells have evolved highly intricate means to sense environmental cues and conditions. The binding of growth factors, cytokines and hormones to specific receptors can elicit very specific responses that coordinate the function of different cell types, tissues and organs to orchestrate homeostasis in multicellular organisms. Cells also have learned how to sense unspecific environmental stimuli, such as heat, radiation or other stresses[120], which do not have specific and dedicated receptors. In this case, specificity largely is achieved by the

combinatorial integration of many in itself unspecific effects. It is not unlikely that the cellular responses to nano-materials are computed in a similar combinatorial way.

The use of modern transcriptomics and proteomics technologies have greatly advanced[121] our capacities to identify the components of signal transduction networks and decipher the network connections. Extending these approaches to systematically study the effects of nano-materials on cellular signal transduction networks will reveal important information about the nature and specificity of the functional interactions between nano-materials and cells. This knowledge will facilitate the purposeful design of nano-materials and lay the foundation for the controlled manipulation of biological systems through nano-materials.

FIGURES

Figure 1. Classes of nanoparticles. Metals (e.g. gold, silver, and iron), metal oxides (e.g. zinc oxide, titanium oxide, and nickel oxide), magnetic (e.g. superparamagnetic iron oxide), polymers (e.g. polyvinyl alcohol and polylactic-*co*-glycolic acid), semiconductors (e.g. boron nitride), carbon based (e.g. C60 Fluorine), and multi classes (e.g. silver coated SPIONs with polymeric gap) are the main employed NPs for biomedical applications.

Figure 2. Cellular uptake pathways for nanoparticles. Several routes were discovered for cellular NP uptake, including phagocytosis **(A)**, macropinocytosis **(B)**, clathrin-mediated endocytosis **(C)**, caveolin-mediated endocytosis **(D)**, and non-clathrin, and non-caveolin-mediated endocytosis. **(A)** During phagocytosis, NP are engulfed by the cell membrane involving pseudopodia and intracellular actin filaments to form an internal early phagosome. Subsequently, the phagosome is processed to form the matured, late phagosome and lysosome. **(B)** For macropinocytotic processes, NP together with extracellular fluid are transferred into the cell by forming an invagination around them at the plasma membrane involving actin filaments action. Once inside the cell, these macropinosomes are processed to form lysosomes. **(C)** Clathrin-mediated endocytosis, also called receptor-mediated endocytosis, internalizes molecules and NP by inward budding of specific plasma membrane regions called clathrin-coated pits. The process is usually mediated by membrane-bound receptors specific to the molecules being internalized. Upon internalization, clathrin-coated vesicles are processed via early and late endosomes and lysosomes. **(D)** Caveolin-mediated uptake of NP is mediated by specific plasma membrane regions called caveolae. These membrane buds are rich in

cholesterol, glycolipids, and the cholesterol-binding protein caveolin, which are found only on a subset of cells and process particle internalization. Particles from the caveosomes can be transferred either into the cytoplasm or endoplasmatic reticulum. **(E)** Non-clathrin, non-caveolin-mediated endocytosis refers to the particle internalization process, where neither clathrin or caveolin are involved. Again, internalized particles are matured to become early and late endosomes. **(F)** Physico-chemical parameters of NP influencing cellular uptake.

Nanoparticles are depicted as red circles, the approx. NP size limit for each uptake mechanism is shown in bold/red. PM – plasma membrane, NP – nanoparticle.

Figure 3. Nanoparticle-mediated activation of oxidative stress signaling. On the cell surface or upon endocytosis, several NP formulations were shown to trigger the production of reactive oxygen species (ROS). Elevated levels of ROS lead to the activation of the cellular stress-dependent signaling pathways mediated by MAPK activation (ERK, JNK, p38) and subsequent transcription factor activation (NF-kB, Nrf2, etc.). Ultimately, these transcription factors result in altered gene expression to produce phase II and antioxidant enzymes allowing the cell to adapt to the changed intracellular milieu. In addition to these pathways, elevated ROS levels also lead to direct damage of organelles such as mitochondria and DNA fragmentation in the nucleus, resulting in cell cycle arrest, apoptosis, and inflammatory response.

Nanoparticles are depicted as red circles, ROS as black stars.

Figure 4. Nanoparticle-mediated activation of receptor signaling. Several NP formulations were shown to interact with cellular receptors such as the EGF receptor (EGFR) and integrins inducing cellular phenotypes like proliferation, apoptosis, differentiation, and migration. In lung epithelial cells, NP interact with both EGFR and integrins leading to cell proliferation via activation of PI3K and AKT. NP were shown to activate the EGFR leading in parallel to apoptosis and proliferation and oncogenic Ras mutations might influence these effects. Interestingly, while integrin-mediated activation of ERK was instrumental for proliferation, apoptosis was mediated via activation of JNK. In addition, NP (PM2.5) are able to bind the EGFR to activate the MAPK signaling cascade. Activation of ERK lead to the expression and secretion of the epidermal growth factor amphiregulin, thus forming an autocrine loop, which might be instrumental for sustained inflammatory responses.

Figure 5. Applications of magnetic nanoparticles and mechanosensitive signaling pathways. (A) Magnetic NP can be conjugated with different reactive surface groups, agents for imaging, drugs, antibodies, and other ligands or biomolecules. **(B)** In order to activate mechanosensitive signaling pathways, cells are exposed to ligand-conjugated magnetic NPs, which bind to specific targets on the surface. Magnetic tweezers allow for pulling NPs by gradients in a magnetic field. **(C)** Magnetic twisting generates a mechanical torque at the particle-cell interface, which drives the magnetic particle to twist or roll on a cell's surface. Because the particle is physically restrained by binding to its receptor, the rolling action produces a shearing force at the cellular receptor that affects downstream signaling. **(D)** Magnetic clustering can be applied to aggregate

cellular receptors thus activating downstream signaling events. In mast cells, magnetic NP were used to induce clustering of IgE-bound FcεRI receptors and subsequent activation of immunological responses. **(E)** Collagen-coated magnetic NP were used to activate focal adhesion complexes including integrin, talin, activated paxillin and actin cytoskeleton components, thus regulating membrane rigidity. **(F)** In myocytes, antibody-coated magnetic NP were used to stretch integrin receptors in order to regulate an outwardly rectifying chloride current. These events were mediated by focal adhesion kinase (FAK) and c-Src.

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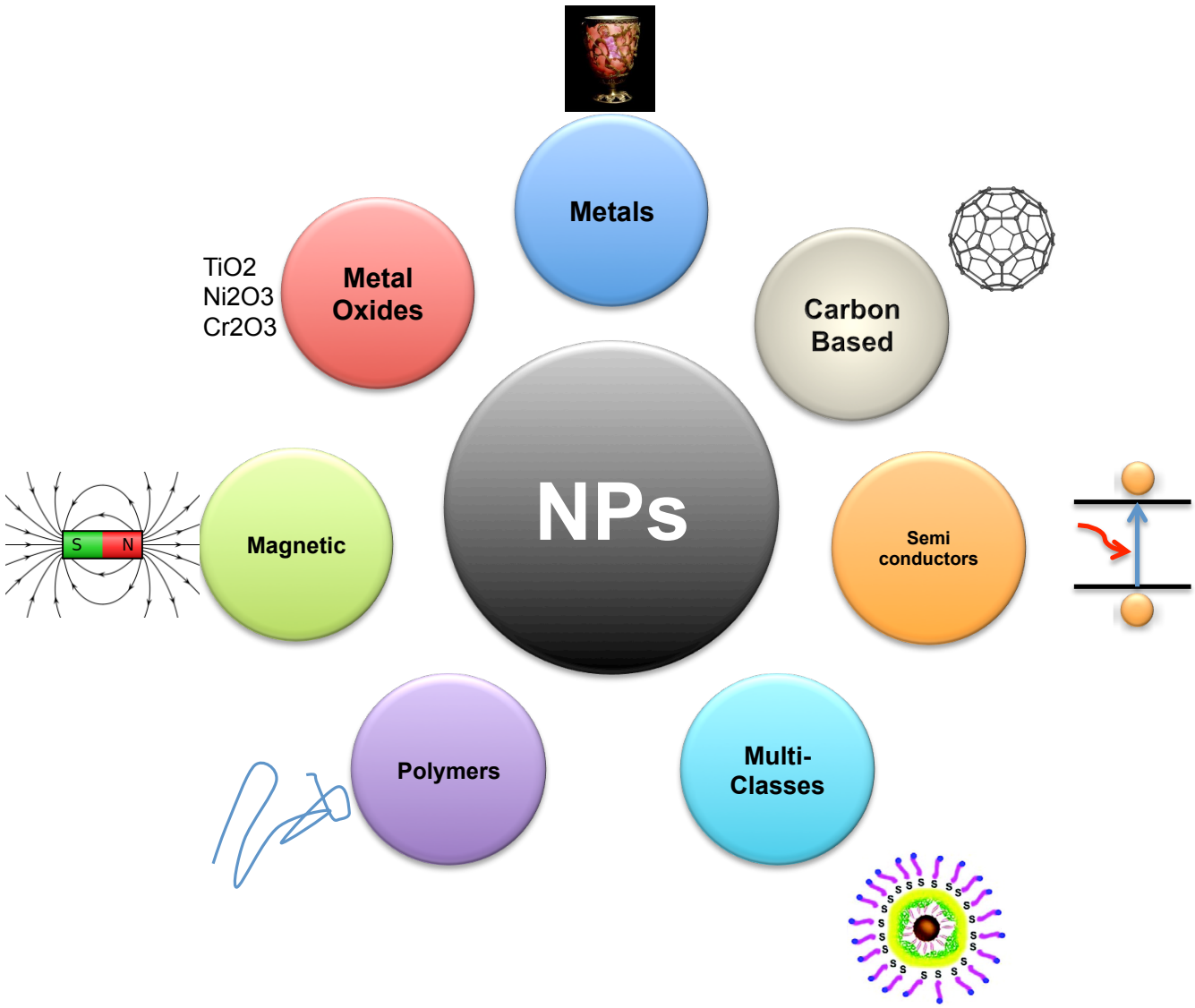
Having obtained a MD from the University of Vienna, Austria, Walter Kolch worked in academia, research institutions and pharmaceutical industry. Currently, he is Director of Systems Biology Ireland and the Conway Institute of Biomolecular & Biomedical Research at University College Dublin, Ireland. His research interests developed from the study of oncogenes to include signal transduction, proteomics and systems biology with a view to understand how biochemical signalling networks specify biological behaviour.

Dr. Jens Rauch graduated from the University of Leipzig, Germany in Biochemistry in 2001. In 2002, he started his PhD in the Clinical Cooperation Group Molecular Oncology at the University of Munich, Germany, where he investigated new tumour markers for head and neck carcinomas using novel proteomic approaches. After receiving his PhD in biochemistry in 2006, Jens moved to the Beatson Institute for Cancer Research, Glasgow, UK as a post-doctoral researcher. Here, he investigated cellular signalling pathways using proteomics. In 2009, he joined Systems Biology Ireland, Dublin, Ireland, studying signalling complexes in combination with systems biology approaches.

Dr. Sophie Laurent was born in 1967. Her studies were performed at the University of Mons-Hainaut (Belgium) where she received her PhD in Chemistry in 1993. She joined then Prof R.N. Muller's team and was involved in the development (synthesis and physicochemical characterization) of paramagnetic Gd complexes and super paramagnetic iron oxide nanoparticles as contrast agents for MRI. She is currently working on the vectorization of contrast agents for molecular imaging. She is lecturer and co-author around 120 publications and more than 200 communications in international meetings.

Dr. Morteza Mahmoudi obtained his PhD in 2009 from Sharif University of Technology with specialization on the cytotoxicity of superparamagnetic iron oxide nanoparticles. He is Director of NanoBio Interaction Laboratory at Tehran University of Medical Sciences (http://www.biospion.com). His current research involves the "ignored" parameters in nanotoxicological approaches.

Figure 1: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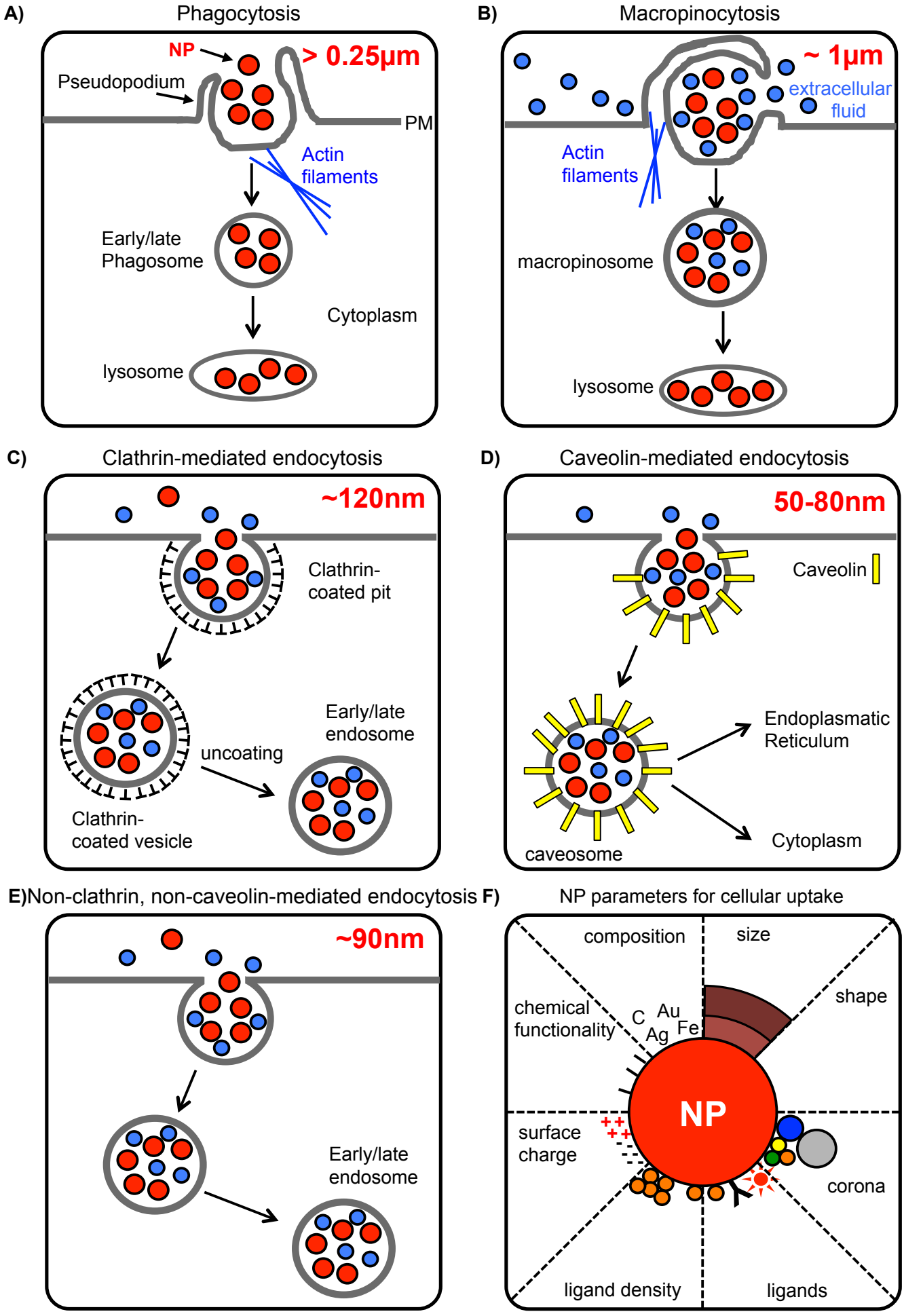
Semi conductors
Polymers
Multi-Classes

Figure 2


A)
Phagocytosis
NP
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PM
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Cytoplasm
lysosome
B)
Macropinocytosis
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extracellular fluid
Actin filaments
macropinosome
lysosome
C)
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Clathrin-coated pit
Early/late endosome
uncoating
Clathrin-coated vesicle
D)
Caveolin-mediated endocytosis
50-80nm
Caveolin
Endoplasmatic Reticulum
Cytoplasm
caveosome
E)Non-clathrin, non-caveolin-mediated endocytosis
~90nm
Early/late endosome
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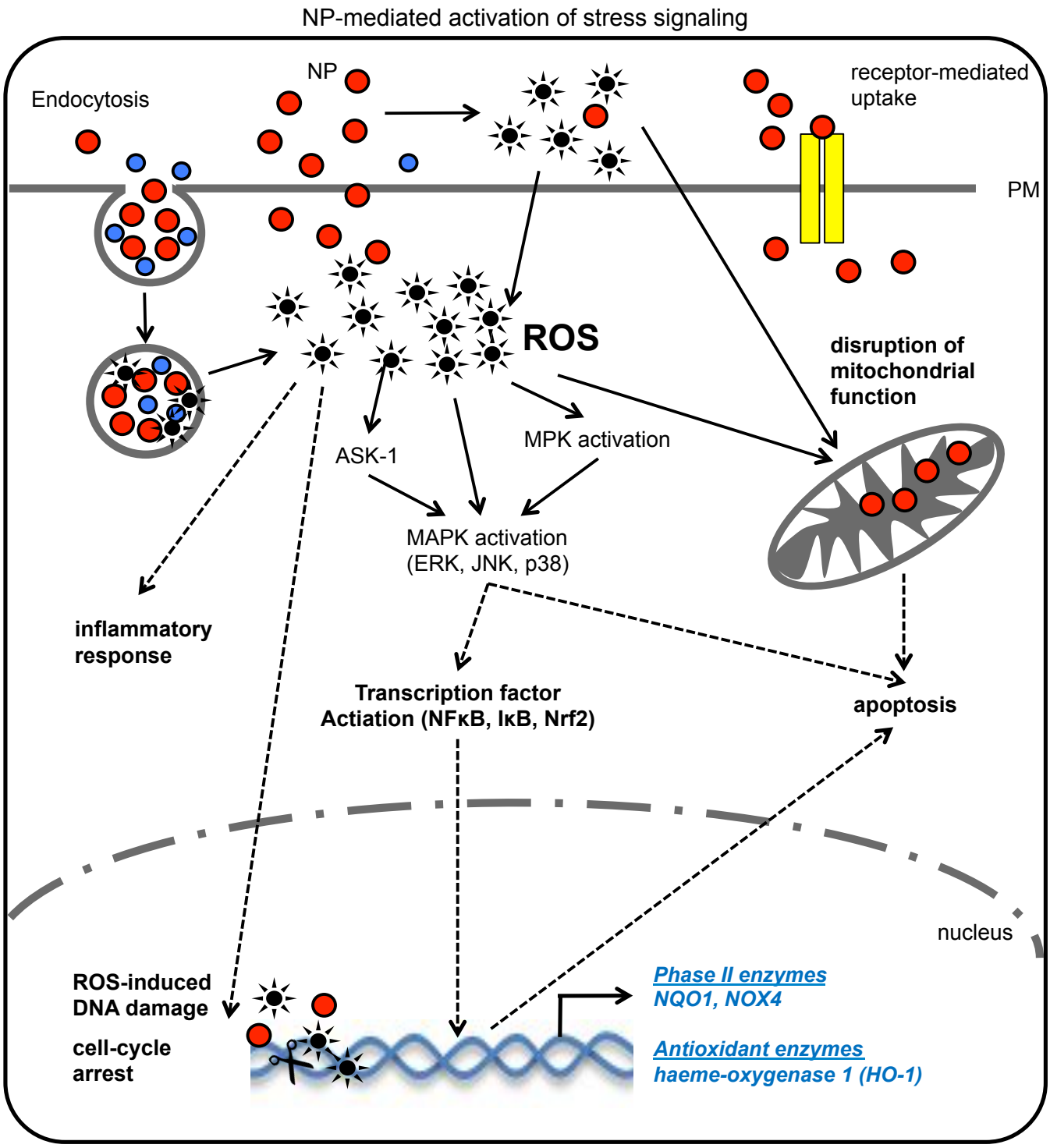
ligand density
ligands

Figure 3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Endocytosis
NP
receptor-mediated uptake
PM
ROS
disruption of mitochondrial function
ASK-1
MPK activation
MAPK activation (ERK, JNK, p38)
inflammatory response
Transcription factor Actiation (NFκB, IκB, Nrf2)
apoptosis
nucleus
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cell-cycle arrest
Phase II enzymes NQO1, NOX4
Antioxidant enzymes haeme-oxygenase 1 (HO-1)

Figure 4

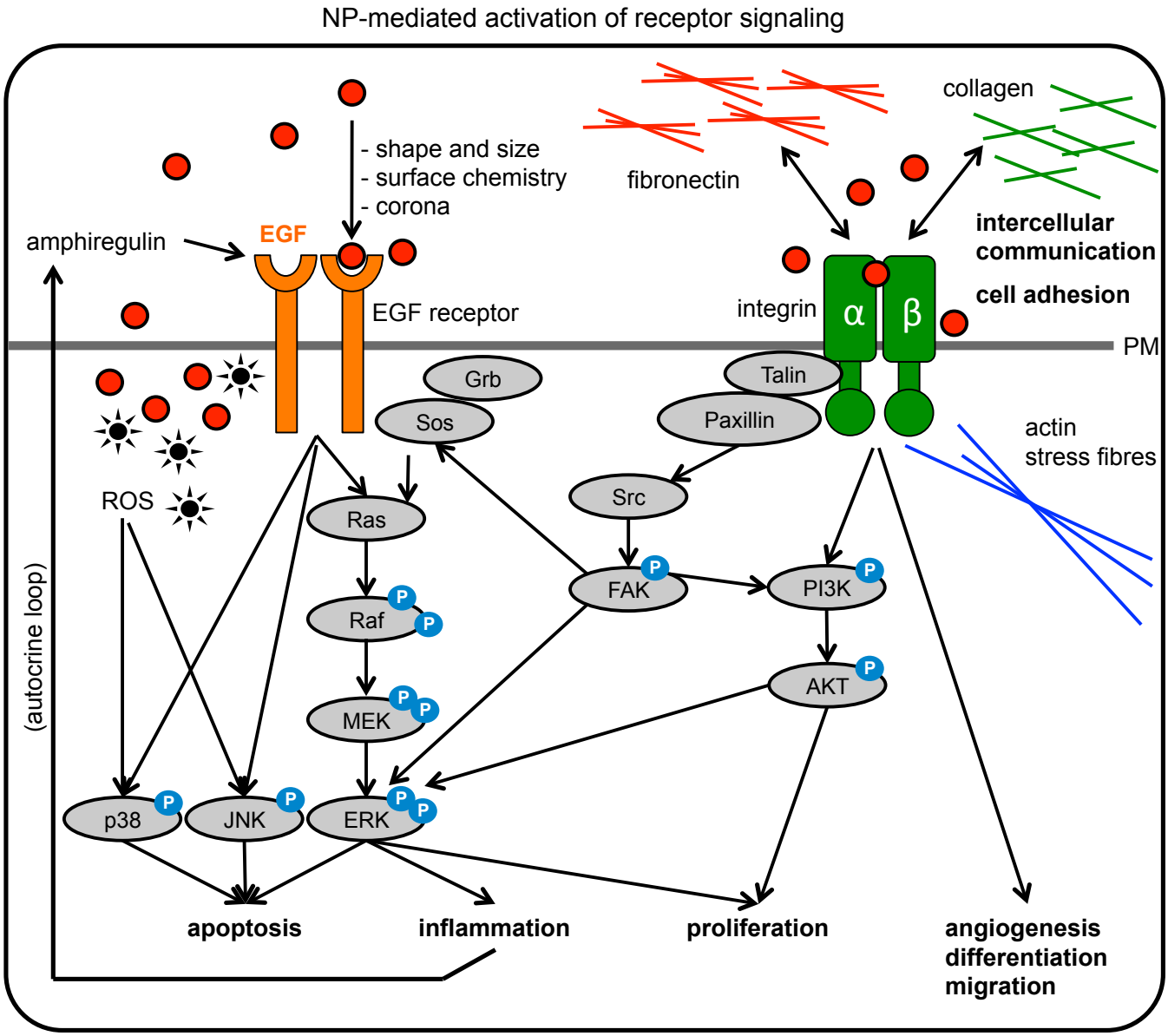
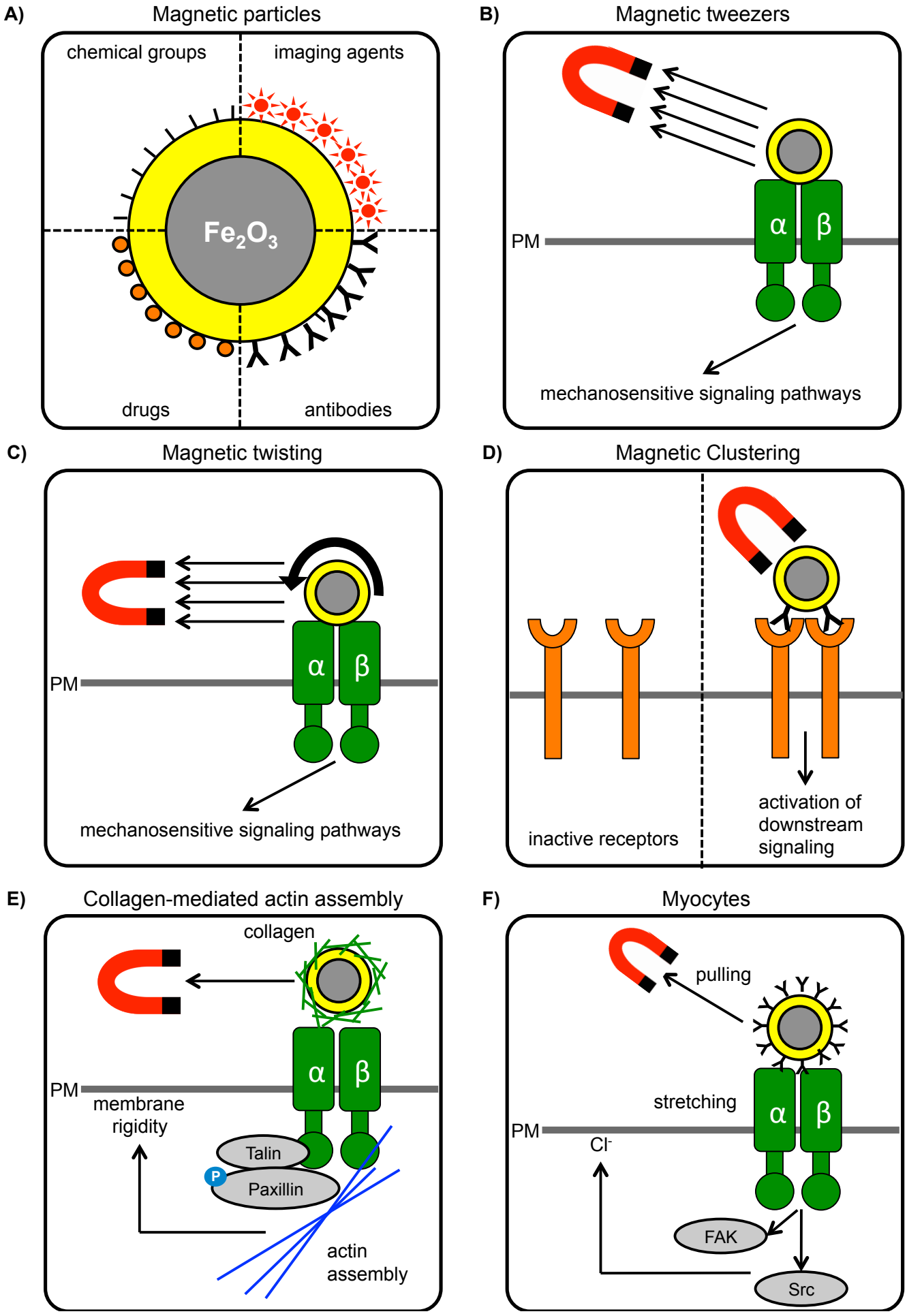
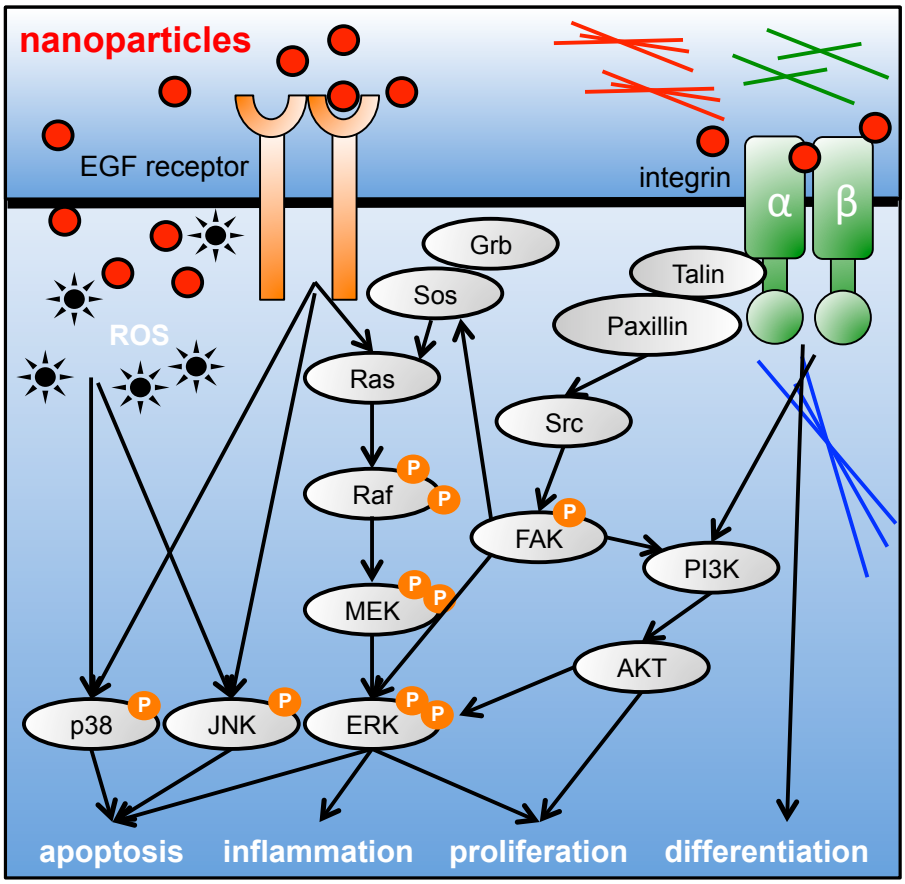

NP-mediated activation of receptor signaling
- shape and size
- surface chemistry
- corona
amphiregulin
EGF
EGF receptor
ROS
(autocrine loop)
Grb
Sos
Ras
Raf
MEK
p38
JNK
ERK
fibronectin
collagen
intercellular communication
cell adhesion
integrin
α
β
PM
Talin
Paxillin
Src
FAK
PI3K
AKT
actin stress fibres
apoptosis
inflammation
proliferation
angiogenesis differentiation migration

Figure 5


A)
Magnetic particles
chemical groups
imaging agents
Fe_2O_3
drugs
antibodies
B)
Magnetic tweezers
PM
α
β
mechanosensitive signaling pathways
C)
Magnetic twisting
PM
α
β
mechanosensitive signaling pathways
D)
Magnetic Clustering
inactive receptors
activation of downstream signaling
E)
Collagen-mediated actin assembly
collagen
PM
membrane rigidity
α
β
Talin
P
Paxillin
actin assembly
F)
Myocytes
pulling
stretching
PM
Cl-
α
β
FAK
Src

Coverart


nanoparticles
EGF receptor
integrin
α
β
ROS
Grb
Sos
Talin
Paxillin
Ras
Src
Raf
FAK
PI3K
MEK
AKT
p38
JNK
ERK
P
apoptosis
inflammation
proliferation
differentiation