What Is Unique About Nanomedicine? The Significance of the Mesoscale

George Khushf and Ronald A. Siegel

I. Introduction

In prominent funding and policy statements, a particle with at least one dimension in the 1-300 nm size range must have novel physicochemical properties to count as a "nanoparticle." Size is thus only one factor. Novelty of a particle's properties is also essential to its "nano" classification.[1] When particles in this size range are introduced into living systems, they often interact with their host in novel ways that require some modification of existing methods and models used by pharmaceutical scientists and toxicologists for assessing their efficacy and safety.[2] It is not clear, however, whether the novelty of the intended physicochemical properties is in any way related to the novel behavior of those particles when their toxicity is evaluated. In fact, when considering toxicity, much of the concern about nanoparticles relates to the unanticipated or poorly understood interactions. Nor is it clear whether either kind of novelty implies that there are any novel challenges for regulators or clinical researchers who must determine whether nanoparticles are safe and effective when they are used as therapeutic agents in humans.

In this article, we provide a framework for distinguishing between these different kinds of novelty and for understanding the specific kinds of challenges that nanoparticles pose for regulators evaluating their use as therapeutic agents in human subjects. We first discuss areas of overlap and differences between efforts to understand and intervene at the nanoscale in the physical and biomedical sciences. In both cases, nanoparticles may exhibit properties in common with larger and smaller constructs of similar composition, or they may exhibit novel or "emergent" properties. In both the physical and biological sciences, much of the novelty associated with nanoscience is due to their intermediate, or "meso," size. Thus, if nanoparticle-based medicines raise unique challenges for regulators, this must involve a kind of novelty that is differ-

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ent from both the unanticipated side effects that are already managed by the infrastructure associated with toxicological analysis and clinical trials, and a novelty that is different from the intended emergent physicochemical properties that are of interest to the physical scientists who synthesize and characterize those particles before they are introduced into living systems. We argue that regulatory challenges associated with evaluating nanotherapeutic agents arise from the way they interface with a biological mesoscale. As a result of their mesoscale biological characteristics, there is a qualitative change in the rates of unanticipated interaction effects; and methods, instruments, and models used by regulators for evaluating such particles are insufficient, and must be extended before the proposed therapeutic agents can be properly evaluated. This requires that research be directed toward stabilizing the infrastructure used to evaluate the new technology.

We argue that regulatory challenges associated with evaluating nanotherapeutic agents arise from the way they interface with a biological mesoscale. As a result of their mesoscale biological characteristics, there is a qualitative change in the rates of unanticipated interaction effects; and methods, instruments, and models used by regulators for evaluating such particles are insufficient, and must be extended before the proposed therapeutic agents can be properly evaluated. This requires that research be directed toward stabilizing the infrastructure used to evaluate the new technology.

While the above-mentioned considerations show that there are novel challenges associated with nanotherapeutic agents, these problems are not just found with nanoparticles. They are similar to problems that have been raised for protein-based drugs, therapeutic uses of nucleic acids, and microsphere-based injectable drug depots. In all these cases, standard assays, methods, models, and explanatory frameworks cannot be applied in the way they would be applied for a conventional drug. Instead, evaluation of the therapeutic agent must simultaneously be coupled with an evaluation and extension of conventional techniques used for assessing them.[3] Novel regulatory challenges relate to the way mesoscale therapeutic agents require this expansion of the repertoire of tools that are used to evaluate them at both the preclinical and clinical stages.

II. Definitions of Terms

In the introduction the terms "nano," "meso," and "novel" have been used. For the purposes of this article, "nano" is taken to represent a size range that is agreed to by convention, e.g., 1-300 nm. "Meso" refers to properties that exist or emerge between lower and upper scales, the latter two having well worked out and roughly independent logics of explanation. These logics, and their domains of validity, will be discipline specific. We will distinguish physicochemical and biological accounts of nanoscience by means of such discipline specific characteristics of the upper and lower domains.

Nano-objects may or may not exhibit meso properties, depending on context. For example, the meso character of nanoparticles may not be manifested until they are exposed to a biological environment. With these definitions in mind, "novelty" may refer to properties that emerge at the mesoscale, or to methods of physical, chemical, or biological analysis that are needed to investigate these properties.

III. Similarities and Differences between Nanoscale Research in the Physical and Biomedical Life Sciences

Many research disciplines have extensive interest in the nanoscale. This common concern has provided justification for broad initiatives such as that the U.S. National Nanotechnology Initiative (NNI). Nanoscience is interdisciplinary, and work in one discipline must draw on knowledge, tools, skills, and platforms associated with others. But there are also some subtle, yet important differences between nanoscale research in the physical sciences and associated engineering fields, on one hand, and the biomedical and life sciences, on the other.[4] As a result of these differences, it is not clear whether novelty of a nanoparticle's physicochemical properties, as judged from the perspective of one synthesizing the particle, translates into any novel opportunities or challenges, when that particle is introduced into a biological system. This second, biologically relevant novelty is the one that matters

in any assessment of the challenges associated with human subjects research with nanomedical products.

In the physical sciences, interest in the nanoscale arises from the capacity to exploit meso-level principles to generate products with novel properties.[5] Here something like a quantum dot is representative: by controlling the size of the particle, one can determine optical properties such as color or the ability to locally generate heat when illuminated by a laser. What makes the nanoscale distinctive depends on the way quantum phenomena can be exploited. On the bottom end of the nanoscale, we have the scale of atoms and molecules, and understanding requires quantum principles or rules derived from those principles. At the upper end where "nano" grades over to "micro," there is the kind of averaging, aggregate behavior characteristic of the classical and bulk level domain, where fundamental properties are mostly independent of size and shape. In between these, we have a complex space where increased understanding and control might be used to generate novel properties. Here phenomena are too complex to model from *ab initio*, quantum principles, but quantum mechanics determines properties in ways that may vary critically with change in particle size or shape. New alliances of experimental and theoretical work are then needed to stabilize and manage this middle level, meso domain, and an interdisciplinary language of complexity, systems, and control is used to align top-down design with bottom-up self-assembly.

"Nano" is important because of the way this scale bridges quantum phenomena (on the low end) and classical phenomena (on the high end). But here it is important to recognize that size alone does not determine what is of interest about nano. All chemistry might, on a loose interpretation, be regarded as "nano" if we simply extended the scale associated with the term "nano" down to molecular dimensions. In conventional chemistry, however, products arise by mass action, measured in moles of particles, and are usually generated by macro-level manipulations of such quantities. Classical tools such as chemical thermodynamics and kinetics enable precise predictions and descriptions. In nanoscale research, a more coarse grained control of this meso range is used to generate particles and systems having "emergent properties" that depend on mesoscale principles, which are not always as well worked out. In sum, for physical sciences and associated engineering practices, the nanoscale is of interest because of the special characteristics of this mesoscale bridging of quantum and classical, with the associated problems of complexity and control. An "emergent" or "novel" property associated with this scale depends on this bridging and complexity.[6]

In the biomedical arena, nanoscience is also considered important, but for more complex reasons. On one hand, "nano" might mean use of particles, techniques, or technological platforms that in some way exploit the mesoscale. Here, the life sciences would presuppose the same meaning and importance of nano arising in the physical sciences; for example, one might use a quantum dot to tag and track some element of interest in a biological process. From the perspective of the biologist, however, the physical mesoscale basis of the property of a quantum dot does not matter. All that matters is, for example, the range of color selection, stability and reliability of optical property, and so on.[7] From the biologist's perspective, synthesis can be turfed to the chemist, and the quantum dot can be treated as a black box or as a tool for investigation, provided the quantum dot is coated in such a way that it is inert or interacts favorably with the intended host biological milieu.

One prominent meaning of "nano" in biology depends on just this kind of "application" of this science and technology to problems of interest to biologists. However, additionally, "nano" is considered to be of importance because it is at that scale that we investigate and control biological phenomena at levels of organization that are difficult to access either at the molecular or "bulk" level.[8] This scale characterizes the most fundamental genetic and metabolic processes of living systems: for example, it is the scale of functional elements of nucleic acids, proteins and protein complexes, traffic between subcellular compartments and across cell boundaries, and so on. Here the nanoscale is of interest because of the way it coincides with a scale of importance for understanding and interfacing with biological systems, not because of the way that quantum-based novelty might be exploited.

This biological nanoscale also has a meso character. When we speak of a "mesoscale," we have a middle region where two distinct logics of explanation intersect.[9] On the low end of this biological mesoscale, elements are understood in terms of their biochemical and biophysical interactions. Conventional *drugs* are generally understood according to such a biochemical logic — for example, in terms of molecular structure, biokinetics, solubility in water, binding affinity, and so on. On the high end of the biological mesoscale, a different hierarchical and functional logic of explanation is dominant. Within a cell, organelles make specific contributions to the ongoing dynamic of cellular life. Cells, in turn, jointly function as parts of tissues that perform specific functions within organ systems. *Medical devices* interface with and are understood in

terms of such functional interactions at the high end, such as a stent placed within an artery to assure ongoing blood flow. Between these biochemical regimes of explanation on the low end and the hierarchically functional regimes on the high end — and roughly corresponding to the nanoscale — we find mid-level structures such as proteins, nucleic acids, the complex lipid assemblies associated with membranes, and "molecular machines" consisting of numerous proteins and sometimes nucleic acids, which act in concert to perform specific cellular functions.[10]

Nanomedical products or interventions may exhibit either physical or biological "meso" properties, or both, and these two domains need to be studied separately. For example, the optical properties of a quantum dot arise as a physical mesoscale phenomenon. A biomedical application might exploit this physicochemical novelty for some purpose. However, this novelty of a particle's property, as judged from the perspective of the physical scientist, does not necessarily introduce any novel problems in a biomedical use of such a particle. Any biomedical novelty would relate to the way the quantum dot interfaces with the biological mesoscale. It is thus logically possible that nanoparticles with novel physicochemical properties might be used in biological systems without introducing novel problems for one who needs to evaluate their health and safety. A physicochemical novelty would concern the physicochemical mesoscale (with its bridging of quantum and classical logics), while a biological novelty would concern the biological mesoscale (with its bridging of the biochemical and hierarchically functional logics). A key question thus concerns whether there are special challenges and opportunities associated with medical uses of nanoparticles whose size is associated with novel physicochemical properties. How might novelty in one arena relate to novelty in the other? Is there a reason to think that a nanoparticle will interface with biological systems in ways that raise novel challenges for the regulatory frameworks we use for assessing medical interventions?

A key question thus concerns whether there are special challenges and opportunities associated with medical uses of nanoparticles whose size is associated with novel physicochemical properties. How might novelty in one arena relate to novelty in the other? Is there a reason to think that a nanoparticle will interface with biological systems in ways that raise novel challenges for the regulatory frameworks we use for assessing medical interventions?

Proteins are representative of biological mesoscale structures.[11] Like drugs, proteins can be understood biochemically. Proteins fold into compact structures in a way that is governed by interactions between chemical groups integral to the amino acids that make up their polypeptide chains, and they interact biochemically with other molecules; for example, as enzymes necessary for metabolic processes. Proteins must also be understood within the complex informational and functional networks associated with a biological system. Polypeptide chains are produced by a complex machinery that transcribes and is governed by information encoded in nucleic acid base pair sequences, and they often interact with other elements according to complex lock-and-key logics, contributing to the ongoing dynamic of cellular life. Further, precise protein structure, function, metabolism, and aggregation into larger complexes essential for mechanical and signaling functions, are controlled by post-transcriptional modifications that occur either immediately following biosynthesis, or as the result of ongoing biochemical processes, which depend in turn on the cellular "state," the latter being determined in part by the concentrations and post-translational states of the same or other proteins. An understanding of this biological mesoscale thus involves a complex bridging of lower level biochemical and higher level functional logics that is analogous with, but not equivalent to, the bridging between quantum and classical logics associated with the physicochemical mesoscale.[12]

In this article, we focus on a subset of this general question. Specifically, we consider whether there are novel challenges associated with assessing the toxic effects of nanoparticles that might be used in nanomedical interventions. To what degree might nanoparticles with "novel" or "emergent" physicochemical properties generate "novel" or "emergent" effects of concern in toxicological analysis? The first sense of novelty and emergence is of an intrinsic kind, and relates to the particle in isolation, or to the particle in a specific relation to some target system. That is the intended or designed property, and it is associ-

ated with the presupposed mechanism of the particle's action. The second sense of novelty is relational, and concerns possible interactions including unanticipated biological side effects associated with use of a nanoparticle formulation. Are there reasons for suspecting that novel intended properties might generate a qualitatively different likelihood of unanticipated, possibly harmful interaction effects, or that such particles might interact with biological systems in complex ways that are difficult to track by our current regulatory schemes for drugs and devices? Might a nanoparticle be both drug-like and device-like, and thus have novel properties arising from its biological mesoscale? If so, can we provide greater clarity regarding the reasons why nanoparticles introduce new challenges and greater concerns about toxicity?

IV. Assumptions Integral to a Clinical Trial of a New Drug

When assessing whether nanoparticles might introduce novel challenges for regulatory systems used to assess them in human subjects, we need to first appreciate how certain kinds of novelty are already accounted for and managed by means of the methods, instruments, and models used by toxicologists, epidemiologists, and clinical researchers involved in the design of preclinical testing and clinical trials associated with a new therapeutic agent.

We begin by noting that the chance that a newly discovered molecular entity will make it to market is commonly estimated to be about 1 in 10,000. The reasons for this are complex,[13] but a common explanation runs something like this: at early stages some mechanism of action might be suggested by a refined understanding of some pathology, for example, by considering some gene product found only in a pathological population, understanding how it generates some kind of system failure at the molecular scale, and then engineering some product that compensates for that failure. Following initial screening, searching, and chemical modification of lead compounds, an agent of interest is identified, and further drug development involves a relatively rational effort to exploit the agent or mechanism identified as promising. The pathway from this early stage to a successful, commercial product next involves a systematic process to preserve the isolated causal agency through the processes of synthesis, *in vitro*, and nonhuman *in vivo* testing ("preclinical") human clinical trials, and, if success is achieved though all these stages, then approval is granted.

When moving from the hypersimplified simulations or *in vitro* laboratory contexts to real world products and biological systems, failures arise at multiple levels. Simple problems such as poor insolubility or poor stability, or unsatisfactory absorption, distribution, metabolism and elimination (ADME) problems may render the drug unusable without developing expensive specialized delivery systems.[14] It may also turn out that agents or mechanisms presumed effective were only apparently so, and that increased study and testing shows a more complex mechanism of action. Alternatively, an expected effect may be much weaker, or there might be a host of unanticipated interaction effects with other systems that prevent delivery of an agent to needed site of action. All of the problems might be summarized under a general category of "biological complexity." Preclinical testing is designed in such a way as to yield evidence that the presumed mechanisms of action are preserved in circumstances that approximate contexts of application.[15] Then, in clinical trials, there is an effort to completely abstract away all assumptions about the mechanisms of action and isolate whether or not an agent, i.e., the drug candidate, has beneficial effects that can be measured in terms of direct benefit to the individual treated by the agent. The ideal study design — the randomized, double-blind controlled clinical trial — is configured in such a way that any biases about how the agent works will not distort assessment of whether it does, in fact, have a beneficial causal effect.

Similar assumptions about complexity cover potential negative effects of some pharmaceutical agent, but here there is a subtle yet important asymmetry. Clinical trials are not designed to look for unanticipated beneficial effects. Only occasionally can such an effect be noticed, say by reports from trial subjects (Viagra being a famous case of serendipitous discovery of an unexpected, unsought beneficial effect). While the exact mechanism of a beneficial effect might not be understood, as was the case for aspirin until relatively recently, the kind of benefit in question is specified at the outset, e.g., to control pain, and the whole process of drug development involves a systematic effort to isolate, preserve, and enhance that benefit. The impact of biological complexity, and thus the primary concern of the clinical trial, relates to an unanticipated loss or attenuation of the expected beneficial effect. But negative effects might be of an anticipated or unanticipated kind. Anticipated negative effects, such as a known toxic effect associated with some general category of drug, can be studied in much the same way the beneficial effect might be studied. Such anticipated effects might be enhanced or diminished by the same factors that would influence the beneficial effect. However, beyond these, there is a reasonable basis for expecting other toxic effects that may arise from some side interaction with the complex biochemical

machinery integral to life. To put this in a somewhat paradoxical way, we could say that there is a reasonable expectation of unexpected negative effects.[16] The phase I trial is then oriented toward assessing whether any negative effects do, in fact, arise. In studying efficacy, trial design starts with an anticipated benefit and is oriented toward ruling out the null hypothesis. But for toxicity, especially as related to unanticipated effects, no such study design is possible. Here novel effects arise from unanticipated interactions between the pharmaceutical agent and the complex, biochemical machinery of the living system.

The reasons for this asymmetry between negative and positive effects is roughly the same as the reason in evolution for expecting that some random mutation will have a deleterious, rather than beneficial effect on the phenotype. This is why most such mutations are rapidly eliminated from the gene pool. Only very rarely does the happy accident arise: in fact, this is so rare that those designing clinical trials do not look for it. But the unhappy accident has a much higher incidence, and thus needs to be carefully considered in clinical investigation. These unanticipated negative effects can be strong enough that they counteract a beneficial effect. Even in cases where the overall risk profile of a drug is positive, the unwanted side effects may need to be managed, and they indicate a pervasive challenge in both development and use of nearly all drugs. Subsequent efforts at refining a drug may be explicitly targeted toward diminishing these negative side effects, and a whole industry strategy is oriented toward extending patent protection by such means.

From the above considerations, it is clear that accidental (unanticipated as opposed to anticipated) negative effects associated with some drug need to be considered independently, and they need to be understood and managed in the testing process by different principles and strategies than those used for anticipated beneficial and/or deleterious effects. Toxicology has emerged as the field for understanding and managing anticipated and unanticipated negative effects. Toxicology is thus already oriented toward managing certain kinds of novelty, e.g., that novelty associated with the unanticipated, negative effects. Clinical trials, and the epidemiological and biostatistical sciences informing their design, then involve a complex balancing between the partially independent strands related to beneficial and deleterious effects and their associated causes.

When evaluating whether a nanoparticle is in some way novel or raises novel challenges when introduced in a human subject, we need to carefully distinguish between the kinds of novelty that are already anticipated and managed by means of clinical trials and those other kinds of novelty that might in some way make it more difficult to understand what happens to that particle. Any new pharmaceutical is novel by definition, and the purpose of a clinical trial, besides evaluating effectiveness, is to ascertain whether there are any unanticipated, emergent side effects. If nanoparticles raise novel concerns, it will be because they do not behave like other drugs in such a way that effort needs to be directed toward developing the general infrastructure for managing them, and not just to the normal application of those tools for the study of the specific new therapeutic agent in question.

V. On the Infrastructure Associated with Conventional Drugs

When a new candidate drug is being considered, preclinical or clinical studies presume that it is sufficiently like other drugs that have been tested over time, so established instruments and methods can be applied. Where specific positive or negative effects are expected, methods of study can be oriented toward measuring them and determining their likelihood. This all takes place against a background that assumes the overall rate of unexpected effects is unaltered, i.e., the rate of relevant unknown unknowns remains constant and is reflected in the complex host of concepts, models, and methods for understanding and managing both expected and unexpected effects. Thus, while toxicology is oriented toward understanding and managing certain kinds of novelty arising from unanticipated or anticipated interactions between an agent and its biological host, this is not an open-ended, fully general capacity. It depends on very specific tools, concepts, methods, and model systems.[17]

The infrastructure for preclinical and clinical testing of a new drug has resulted from a long sequence of iterative adjustments and refinements encoding collective social wisdom regarding how best to manage uncertainty associated with a new drug. Integral to these systems of evaluation are taxonomies of drug type, mechanism of action, target tissue, and so on. Experience gained over time is reflected in many aspects of protocols that may be poorly understood by any single person using a given protocol or model. What is important is that a protocol can be effectively used without understanding all of the reasons why a specific protocol has taken a chosen form. Implicit in this collective machinery is a time tested, realistic expectation about the background rates of unanticipated effects, their kinds, and how these should be managed.[18] This includes a variety of concepts, models, and methods for assessing whether some unanticipated effect has arisen, and, if so, how it should be

measured, the time frame for discerning whether it has arisen, and where to look for it.

A host of technologies are available for carrying out the various tests that are needed. Few appreciate the extent of background infrastructure that is necessary for quality assurance and quality control (QA/QC) of a drug that undergoes preclinical evaluation, clinical trials, and ultimate release into the market.[19] This infrastructure includes constantly improving analytical techniques, facility of digital record keeping, and experience that permits highly complex methodologies to be carried out, step by step, by well-trained personnel. Drug company employees must sign off, at every stage, on processes and analyses during development and production of products. This system is meant not only to satisfy regulatory authorities, but also to provide a means for pinpointing the source of problems as they arise. It operates both before the drug is marketed and afterwards in order to ensure the safety of drug products.

A long line of basic research preceded development of the analytical technologies used in drug discovery and development.[20] For example, nuclear magnetic resonance (NMR), a primary tool for identifying and verifying chemical structure, was invented in the 1940s. Mass spectrometry (MS), used to identify metabolites derived from parent drugs, has been around for a century. These and other analytical techniques have over the years exponentially expanded their capabilities, speed, and precision. High performance liquid chromatography (HPLC), which permits efficient separation of molecular species for analysis, came into widespread use in the 1970s with the advent of well-specified column packing materials and fluid control systems, although various other kinds of chromatography had been available for decades. Recent advances in microfluidics, robotics, and reproducible cell culture have enabled rapid, high-throughput screening and analysis of drug candidates. All of these infrastructural QA/QC methodologies took time to develop. Now that they are in place, however, they set the standard that is demanded by regulatory agencies such as FDA, and also by units that exchange materials and information along research, development, and production supply chains.

An inherent advantage of pharmaceuticals is that all drug molecules with a given designation have the same molecular structure (with some exceptions usually involving isomers). Purity, stability, and lot-to-lot consistency can be assessed by QA/QC methodologies at the point of manufacture and thereafter, and drug and metabolite concentrations can be followed in blood over time after the drug is administered. Information regarding a drug's pharmacokinetics (PK: where the drug goes in the body and how long it stays in different regions) and metabolite profiles, the drug's binding affinity to critical tissues and its solubility in water and other solvents, along with its physical and chemical stability during storage, is routinely culled in animal models before clinical trials of a drug product are initiated. Where possible, pharmacodynamic (PD) markers reflecting drug effect are also collected. The availability of increasingly user-friendly software packages permits analysis and statistical characterization of PK/PD data. These packages are also useful in clinical trials, where analyses are carried out at both the individual and population levels.[21]

If the molecular structure of a therapeutic agent and its purity, stability, lot-to-lot consistency, and so on, could not be fully assessed by the methods and instruments available, and in the manner now assumed by QA/QC infrastructure, then this would raise novel challenges for assessing the safety and efficacy of that agent.[21] The difficulties associated with assessments of such novel agents can be seen in other areas besides what is now called "nanomedicine" (although these would be in the biological meso region associated with the nanoscale). In the 1970s genetic engineering led to the promise of numerous protein-based therapeutics. Few products emerged initially, however, due in part to a lack of precise means to characterize these more complex molecules. Roughly over the past 35 years, however, advances in preparation, purification and analytical techniques, and understanding of protein pharmacokinetics and pharmacodynamics have led to an acceleration in introduction of protein-based drugs into the market.

Protein drugs are inherently more complex than smaller molecular weight drugs, and this has led to both problems and opportunities. Proteins are long chains of amino acids that typically fold into relatively rigid shapes, which determine their activity. In the body, proteins are subject to biochemical modifications that alter their surface charge, shape, and solubility, and their tendency to denature (lose the shape required for activity) and form aggregates. Denaturation and aggregation also occur during storage or passage through a catheter. Thus, formulation of a protein in its common physiological form may have distinct disadvantages. For example, genetically engineered human insulin tends to denature and form aggregates, which can clog catheters, and it also tends to be slow acting. However, a slight genetically engineered alteration of the amino acid sequence leads to a much more stable and fast acting, nonaggregating form of insulin, which is now available for use in pumps. Development of such a modified product required a rigorous demonstration that alterations in

amino acid sequence would not lead to an immune response. The jump from small molecule to protein thus entails a significant increase in complexity.[23]

The difficulties associated with these mesoscale therapeutic interventions are also illustrated by efforts to therapeutically use nucleic acids such as genes (DNA), antisense oligonucleotides, and short interfering RNA's (siRNA's), which are regarded as highly promising alternatives to traditional drug therapies. Like proteins, these are polymer chains consisting of common building blocks whose sequence codes their effect. The sequences are precise, and they potentially work with the natural quality control systems associated with nucleic acid replication, transcription, translation, and post-translational modification. Since these therapeutics act at the level of fundamental molecular biology, they have the potential to exert extremely powerful effects. However, their introduction into the market has been delayed by several factors. While genes can be readily delivered into cells using viruses, the threat of immunogenicity of the viral coat is always present, and there is considerable research into vectors consisting of more immunotransparent materials.[24] Delivery of antisense oligos and siRNA's into target cells is difficult due to their size and charge, and many proposed nanomedicines are being investigated to solve these problems. Moreover, the potential for "off-target" effects (i.e., toxicities) must be considered. Before the promise of these new therapeutics can be realized, efforts must directed toward better understanding and hopefully mitigating these novel off-target effects. While some aspects of the QA/QC may be relatively straightforward for "naked" nucleic acids due to their precise structures and sequences, standards for characterizing nanodelivery systems for these agents, which are typically complexes of the nucleic acids and specially chosen polymers,[25] need to be developed as part of the QA/QC/regulatory infrastructure. Similarly, monitoring for off-target effects is expected to be of great importance during clinical trials.

What makes protein and nucleic acid-based therapeutic agents novel from a regulatory perspective relates to the research effort that must be directed toward stabilizing the QA/QC/regulatory infrastructure for evaluating those agents. One cannot simply "apply" existing tools and methods in the manner associated with a conventional drug.

VI. On the Infrastructure Associated with Conventional Devices

From a regulatory perspective, therapeutic introduction of objects into the human host comes under three general headings: drugs and devices, and biologicals. Thus far, we have been assuming that use of nanoparticles as a therapeutic agent would come under the "drug" category, although we saw that such a category is stretched with some new mesoscale agents. In this section, we consider the second general heading: devices.[26] Generally, a device is something that is large enough to manipulate and place in the body, with the typical expectation that it will remain in place. This class includes implants for bone and joint repair, cardiac pacemakers, implantable drug pumps, and drug-coated coronary stents. We also include in this category injectable microparticle depot formulations that degrade and release drug over prescribed periods of time.

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Devices of this kind are produced by engineering techniques, and QA/QC measures derived in part from those that are already in place for non-biomedical technologies. For example, mechanical tests and electron microscopy can be used to analyze both computer chips and the artificial hips. Macroscale biomedical devices must also prove rugged in the biological host environment, which provides extra challenges.

An illustrative example is provided by drug eluting cardiac stents, which have seen increasing use over the past decade in postsurgical management of heart attacks.[27] Following removal of the coronary artery occlusion that cause the heart attack, a wire mesh stent, surrounding a small balloon, is placed in the artery by means of an externally guided catheter. Inflation of the balloon expands the wire mesh, which ratchets into place, holding the coronary artery open. The balloon is then withdrawn. Films contain-

ing powerful antiproliferative drugs are coated onto the wire mesh, and slow release of the drugs over time and directly into the arterial wall prevents regrowth of tissue around the stent, or restenosis, which can lead to secondary occlusions.

Production of drug eluting stents is complex, involving drawing and knitting very thin metal wires into a precise meshwork, polishing the metal, and uniformly coating drug-containing films onto specific parts of the wire surfaces.[28] The wires must be strong enough to resist collapse in the coronary artery over the patient's lifetime. Conversely, they should not damage the host tissue. The coating should control drug release in a predictable way, and the coating material, if it sheds from the surface, must do so in a way that is nontoxic. Various mechanical tests, chemical analyses, and observations under the microscope can be used to characterize all steps of stent production, and changes in stent properties can be monitored following implantation into animals. By these means, useful information can be gathered before conducting clinical trials in humans.

While the drug eluting stent already is somewhat of a hybrid between drug and device, it still has one of the important characteristics of most "macrodevices": once inserted, it remains in the same place. A coronary stent should not migrate from its point of implantation. The "locality" of an implanted macroscale device permits the developer at least the initial luxury of focusing on the device's immediate tissue neighborhood when searching for adverse effects. Local inflammatory and foreign body responses can be monitored by histology, and surface alterations aimed at mitigating inflammation can be attempted. The main point is that for such devices, the biointerface is localized and is readily observable in the preclinical setting. Of course, secondary physiological reactions also need to be considered, ultimately.

Just as the "drug" category is being stretched by novel, macromolecular agents, so too is the "device" category being stretched by hybrid, or combination products. We already see this with the drug-eluting stent. When it is introduced into an artery, there are issues of dispersion throughout the body of stent components, for example, due to inadvertent drug or polymer film release into the bloodstream. While they can be viewed as potential hazards, these possibilities are not integral to the mechanism by which the device is intended to act. On the other hand, microsphere-based injectable drug depots, with drug dispersed in degradable polymer matrices, are programmed to degrade into nontoxic products and release encapsulated drugs into the body over prolonged periods. Here degradation is inherent to mechanism. Whether a drug delivery device is designed to intentionally degrade, or if degradation is unintended, the identity and disposition of degradation products over time and their interactions with the body, along with disposition of the drug, need to be well understood. This requirement adds complexities at the QA/QC/regulatory levels,[29] which are similar to some of the complexities associated with nanomedicines to be described below.

VII. Between Drugs and Devices: Nanomedicines and the Biological Mesoscale

While a biochemically specific "drug" and a macroscale "device" are vastly different in size, developers of both classes of biomedical products can tap into vast, mature, analytical, and quality-control infrastructures, along with knowledge bases that enable proper questions to be asked at all stages of development. There is also a high degree of standardization and reproducibility, from molecule to molecule and from device to device. As already indicated, methodologies and infrastructures are still being developed for macromolecular agents such as proteins and nucleic acids (which stretch the "drug" category) or microsphere-based injectable drug depots (which stretch the "device" category). Because of their relative sizes compared to conventional molecules and devices, we see them as transitional, on either end, to a "mesoscale" domain, in which nanomedicines reside. The difficulties in characterizing and regulating these transitional classes of therapeutics presage similar challenges for nanomedicine.

Some caution is needed when equating the biological mesoscale (as the middle level between biochemical and cell/tissue/organ logics) and the nanoscale (as region of spatial resolution between 1-300 nm). Objects in this nanoscale have significant variation. Some objects in this range are subsumable under the "drug" category, and can be synthesized, measured, and controlled without development of new methods or instruments. Some of these "nanoparticles" were introduced into biomedical vernacular before the term "nano" was coined. Most of these products, such as micelles, liposomes and emulsions, consist of oils and lipids, and are closely related to endogenous structures such as bile salts, chylomicrons, and vesicles that are already present in the body, and their metabolism makes use of common biological pathways. There are other nanostructures, e.g., surface coatings on materials, that are subsumable under the QA/QC mechanisms associated with the "device" category. Additionally, there are attributes of nanotherapeutic agents that are important when considering their interaction within a biological system, yet are not related to scale alone; for example, particle shape, porosity, bulk, and

surface composition. However, all things being equal, nanoparticles have at least three general properties that lead to mesoscale interactions with biosystems.

First, the extremely large surface to volume ratio of nanoparticles is such that surfaces, whose properties may differ substantially from those of a particles interior, take on a dominating role. Increased bioavailability and reactivity are properties that make such particles attractive for therapeutic applications, but without proper care, deleterious surface controlled reactions may occur. In addition, adsorption of molecular components such as proteins and lipids to nanoparticle surfaces, which may vary from place to place and from time to time, may lead to wide variations in nanoparticle functionality (see below). Conversely, the availability of a large total surface area may affect the conformation and stability of adsorbed proteins, which may trigger adverse reactions. There is thus a direct relation between the size scale of particles and anticipated rates of unanticipated interactions with other particles and structures.

Second, nanoparticles will, upon injection, disperse throughout the vascular space and even may gain entry to certain body spaces like the interior of solid tumors, which are inaccessible to micron-sized particles. The extent of access, routes of transport, and sites of accumulation often depend more on nanoparticle size and surface properties than on characteristics of the particle's interior or "bulk."[30]

Third, a nanoproduct is actually an ensemble of elements, i.e., nanoparticles, whose composition, size, and shape can only be specified statistically. While average size, shape and composition can be specified, often there will be unavoidable variability from nanoparticle to nanoparticle. Unlike molecular entities such as conventional drugs, whose structure and purity can be evaluated by a number of well established methods, heterogeneity is a fact of life for most nanosystems, and new means for quality assurance and control of variation are needed to promote uniformity of a specific product within and between batches or lots.[31] The importance of variation on functionality and risks associated with nanoproducts may depend on the nature of the product (liposome *versus* biodegradable nanosphere *versus* gold nanoshell, etc.). We are at an early stage in understanding the nature of the "nano-bio" interaction, and extensions of the quality assurance/quality control infrastructure will likely be required before their risk profile in a human host can be properly evaluated. In our view, this is what makes nanomedicines novel from a regulatory perspective.

Just as mesoscale physical objects such as quantum dots are interesting since they have both classical and quantum characteristics, mesoscale nanomedicines have properties in common with both drugs and macroscale devices. Like drugs, they are dispersed following systemic administration, and they are subject to metabolism and elimination. Like macroscale devices, they are large enough that a biointerface develops between particle and host environment. However, subtle differences lurk beneath these apparent similarities.

Unlike a drug dosage in which all molecules are identical in structure, there will be unavoidable fluctuations of numbers of atoms or molecules contained, and hence differences in size and shape, of nanoparticles derived from the same lot, or between nanoparticles derived from different lots.[31] These fluctuations can be controlled within certain statistical limits, but they probably cannot be completely eliminated, at least with present manufacturing technologies. The importance of such variations may differ between classes of mesoscale products. In some cases, uncontrolled differences in size might lead to significantly different pathways of transport, metabolic degradation, and bioaccumulation. As a result of uncertainties associated with such variability, exploratory experimentation may be needed to determine which methods, tissue, instruments, and so on, may be required for *in vivo* study of risk associated with therapeutic use of such an agent.[33]

As nanoparticles disperse throughout tissues along individualized trajectories, each one may create its own biointerface, or "corona," of adsorbed proteins, and it seems likely that no two particles will have exactly the same corona, just as no two snowflakes are believed to have the same shape. Further, the corona of each particle is likely to be dynamic, with certain proteins sticking tightly while others attach to and detach from the particle surface as the particle's environment changes.[34] The total biointerfacial area will be extremely large, and on a per unit mass basis will increase inversely proportional to average particle diameter. Great efforts have been made to mask nanoparticle surfaces with a shell layer consisting of hydrophilic polymers or lipid mimics, which discourage protein adsorption. During metabolic breakdown of nanoparticles, this shell will be stripped off, however, in processes that again may depend on the nanoparticle's location. The precise breakdown pathways of individual nanoparticles within an administered dose, or ensemble, are thus expected to be widely variable.

Variations in initial conditions, and biointerface and metabolic trajectories of mesoscale particles introduce, we believe, a new element of complexity to the analysis of nanomedicines compared to conventional drugs and implanted devices. Their rates

of unanticipated interactions, and the nature of those interactions, may be very different from those of conventional drugs. A qualitative shift in the rate and character of the "unknown unknowns" may require changes in the way uncertainty associated with these novelties are managed; for example, it makes it more difficult to determine what is needed for preclinical testing and what kind of data is sufficient for transitioning to clinical trials. In ways similar to gene therapies, many nanoparticles will interface in complex ways with the informational and functional machinery of biosystems, and thus cannot just be considered in terms of their chemical structure and biochemical interactions. Like devices, they may evoke immune system responses, either directly or by means of their "coronas." Unlike most devices, they do not stay put in one place, and thus pose new problems for identifying where they are and what they are doing.

These problems may be more or less serious for certain kinds of mesoscale nanomedicines. Particles that degrade into common metabolites, or metabolites that are nontoxic and readily excreted, are likely to pose the fewest problems. On the other hand, mesoscale particles consisting of foreign materials, a fraction of which persist in the body for months or years, may be the most problematic. Persistence issues are, of course, not unique to mesoscale particles. Most macroscale devices are designed to persist. In fact, the ability of such devices to withstand challenges provided by the host is a quality measure. On the other hand, the best drugs tend to be those that do not persist, since buildup of drug in tissues not associated with the drug's effect often leads to toxicity, and usually drug effect is only desired for a prescribed time period. Here there are tensions between the properties of a good device and a good drug. Which combinations of such properties make good mesoscale agents is still unclear. The answer to this question in the case of a specific agent will partly depend on additional research that is oriented toward stabilizing the QA/QC infrastructure.

VIII. Conclusion

By highlighting the mesoscale character of nanotherapeutic agents, we have accounted for why they raise novel challenges for the regulatory structures used to evaluate them. We have also made clear why these novel challenges are not unique to nanomedicines: the challenges they pose will be similar to those posed by other hybrid extensions of the drug and device categories — for example, protein-based agents and nucleic acids, biochemically active surfaces, and microsphere-based injectable drug depots. Also, by highlighting the mesoscale interactions rather than size alone, we have provided a basis for identifying those nanoparticles that are unlikely to raise novel regulatory challenges. These will be the class of agents that can be assimilated to existing quality assurance/quality control mechanisms, i.e., those that can be well characterized in terms of molecular structure, whose biokinetics and metabolic pathways are well understood, and so on. Finally, by highlighting the mesoscale character of nanotherapeutic agents, we have shown how the regulatory challenges they pose are intertwined with, and may also be advanced by the same omics, systems, and epigenetic research trajectories that enable a better understanding and control of biological complexity at this most fundamental scale of biology. Here we can recognize an important similarity between the physicochemical and biological mesoscales: in both areas, we need to integrate top-down and bottom-up strategies, theory and experiment, and methods and instruments from a wide range of disciplines to stabilize these complex, middle-level regions. This mesoscale research is as vital for stabilizing the regulatory infrastructure as it is for development of the promising new therapeutic agents that must be evaluated by that infrastructure.

We can recognize an important similarity between the physicochemical and biological mesoscales: in both areas, we need to integrate top-down and bottom-up strategies, theory and experiment, and methods and instruments from a wide range of disciplines to stabilize these complex, middle-level regions. This mesoscale research is as vital for stabilizing the regulatory infrastructure as it is for development of the promising new therapeutic agents that must be evaluated by that infrastructure.

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Nanotherapeutics: Building Research Ethics and Oversight" (S. M. Wolf, PI; J. McCullough, R. Hall, J. P. Kahn, Co-Is). The contents of this article are solely the responsibility of the authors and do not necessarily represent the views of NIH or NHGRI.

References

1. The definition provided by the National Nanotechnology Initiative (NNI) is representative: "Nanotechnology is the understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nanometers, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. Matter such as gases, liquids, and solids can exhibit unusual physical, chemical, and biological properties at the nanoscale, differing in important ways from the properties of bulk materials and single atoms or molecules." This wording is from the NNI website (http://www.nano.gov/nanotech-101/what) (last visited November 1, 2012), and variants of it are in many NNI policy documents. Note how this definition concerns both the size (1-100 nm) and the novelty of phenomena/applications. The size range used by NNI is controversial. Ledet and Mandel argue that "[f]or most pharmaceutical applications, nanoparticles are defined as having a size up to 1,000 nm." G. Ledet and T. K. Mandal, "Nanomedicine: Emerging Therapeutics for the 21st century," *U.S. Pharmacist* 37, no. 3, *Oncology* supp. (2012): 7-11. Many of the meso-level, biological phenomena we reference in this essay occur at roughly 1-300nm, so we will use this range. However, it should also be kept in mind that the properties of nanomaterials may extend to their aggregated forms, which may have larger dimensions. See M. A. Hamburg, "FDA's Approach to Regulation of Products of Nanotechnology," *Science* 336, no. 6079 (2012): 299-300.
2. Summary of the novel challenges nanoparticles pose for toxicology can be found in: G. Oberdorster et al., "Principles for Characterizing the Potential Human Health Effects from Exposure to Nanomaterials: Elements of a Screening Strategy," *Particle and Fibre Toxicology* 2, no. 8 (2005), *available at* <http://www.particleandfibretoxicology.com/content/2/1/8> (last visited November 17, 2012); P. Borm et al., "The Potential Risks of Nanomaterials: A Review Carried Out for ECETOC," *Particle and Fibre Toxicology* 3, no. 11 (2006), *available at* <http://www.particleandfibretoxicology.com/content/3/1/11> (last visited November 17, 2012); G. Oberdorster, "Safety Assessment for Nanotechnology and Nanomedicine: Concepts of Nanotoxicology," *Journal of Internal Medicine* 267, no. 1 (2009): 89-105; B. Fadeel amd A. E. Garcia-Benenett, "Better Safe than Sorry: Understanding the Toxicological Properties of Inorganic Nanoparticles Manufactured for Biomedical Applications," *Advanced Drug Delivery Reviews* 62, no. 3 (2010): 362-374; A. Elsaesser and C. V. Howard, "Tociology of Nanoparticles," *Advanced Drug Delivery Reviews* 64, no. 12 (2012): 129-137. A more general review of phenomena occurring at the interface between nanoparticle and biological host, at several levels of integration, is provided by A. E. Nel at al., "Understanding Biophysicochemical Interactions at the Nano-Bio Interface," *Nature Materials* 8 (2009): 543-557.
3. Whether protein-based drugs and therapeutic uses of nucleic acids raise problems, and, if so, what these are, has always been controversial. The best studied example relates to "gene therapies," with the special oversight associated with the Recombinant DNA Advisory Committee (RAC). When considering the things that make gene transfer novel from the perspectives of clinical trials, Nancy King highlights "those characteristics that produce decision-making challenges" ("RAC Oversight of Gene Transfer Research: A Model Worth Extending," *Journal of Law, Medicine & Ethics* 30, no. 3 [2002]: 381-389). Among these are the complexity of gene therapies, the lack of good animal models, and the uncertainty engendered by these. When considering questions of "special scrutiny," Carol Levine and colleagues highlight research projects which "are, in some morally relevant sense, 'outliers,' presenting novel or ethically challenging questions, situations, and strategies or a challenge to the status quo." (C. Levine et al., "'Special Scrutiny': A Targeted Form of Research Protocol Review," *Annals of Internal Medicine* 140, no. 3 [2004]: 220-223). Two of their three criteria focus on the problems we consider in this essay. Their first criterion relates to research that "involves initial experiences of translating new scientific advances to studies in humans, especially when the intervention is novel, irreversible, or both." Their third criterion concerns research protocols that raise "ethical questions about research design or implementation for which there is no consensus or there are conflicting or ambiguous guidelines." In the essays of King and Levine et al., the primary concern is with the issues we consider: namely, with those kinds of interventions that require innovation/research related to the infrastructure that is used to evaluate the products. We have attempted to disentangle that primary element from the others, and make it the crucial one when considering what makes a therapeutic agent novel from a regulatory perspective. An extensive discussion of the questions of special oversight is found in the winter 2009 issue of *Journal of Law, Medicine & Ethics*, edited by Susan Wolf, Gurumurthy Ramachandran, Jennifer Kuzma, and Jordan Paradise.
4. G. Silva – "Neuroscience Nanotechnology: Progress, Opportunities, Challenges," *Nature Reviews* 7, no. 1 (2006): 65-74 – distinguishes between the "intrinsic novelty" of nanoparticles, such as the size-dependent wavelength of light emitted by a quantum dot (QD) – and the "extrinsic novelty" that arises when the QD is functionalized and used in a biological system to perform a specific function. According to Silva's contrast, physical scientists are concerned with the intrinsic novel properties, while life scientists and clinical researchers are concerned with the extrinsic novelty arising from use of those particles and their functional interaction with biological systems. For an overview of the diverse ways nanoscience is defined in the physical versus life sciences, see G. Khushf, "The Ethics of Nano-Neuro Convergence," in *Oxford Handbook of Neuroethics* (Oxford: Oxford University Press, 2011): 467-492, esp. at 468-472.
5. In a more detailed review, we would need to distinguish between general-, network-, and complexity-based accounts of the meso-scale and those accounts that are discipline specific. In discipline-specific definitions of the mesoscale, the etymological meaning of "meso" is prominent (deriving from the Greek word for "middle"). This scale is between two different scales of analysis, each with roughly independent logics of explanation. In condensed matter physics, for example, the mesoscale characterizes a region between the atomic scale, where quantum principles of explanation are needed, and a bulk level, classical scale. In meteorology, the meso-scale is between a microscale and storm-scale cumulus systems (on the low end) and synoptic scale systems (on the high end). So, in similar ways, we could find meso regions of importance in a host of other areas. Discipline specific accounts of meso thus are distinguished by the regions and logics of analysis that characterize their lower and upper scale. We will distinguish physicochemical and biological accounts of nanoscience by means of such discipline specific characterization of the upper and lower domains. Beyond these discipline specific accounts of the meso-scale, there are also general accounts that are informed by complexity and network analysis. In one prominent account that has informed nanoscience in many different disciplinary areas, George Whitesides and colleagues follows complexity theorists: "[t]he distinctive properties of meso-scale systems arise when the characteristic length of a process of interest, such as a ballistic movement of an electron, excitation of a collective resonance by light, diffusion of a redox-active molecule close to an electrode, or an attachment and spreading of a eukaryotic cell, is similar to a dimension of a structure in (or on) which it occurs. These processes involve interactions with small localized ensembles of atoms and molecules." (A. Kumar, N. Abbott, E. Kim, H. Biebuyck, and G. Whitesides, "Patterned Self-Assembled Monolayers and Meso-Scale Phenomena," *Accounts of Chemical Research* 28, no. 5 [1995]:

219-226. Even when highlighting this general definition, Whitesides et al. highlight the middle level, bridging character of this scale: "Meso-scale systems bridge the molecular and macroscopic." As a result of the complexity and nonlinear character of the interactions that constitute this middle scale, research practices need to integrate theoretical and experimental techniques from high and low scales. Meso-scale work will thus involve a convergence of top-down and bottom-up strategies. For an example of how a general, network-based account of the meso-scale can work with a discipline specific account in ecology, see E. Estrada, "Characterization of Topological Keystone Species Local, Global and 'Meso-Scale' Centralities in Food Webs," *Ecological Complexity* 4, nos. 1-2 (2007): 48-57.

6. When characterizing the nanoscale as bridging the quantum and classical domains, we use a discipline specific account that is perhaps too dominated by the physics of the particle, especially as worked out in areas like condensed matter physics. In some areas of nano-chemistry, emphasis would still fall on the "intrinsic" novelty of the systems, but there is much greater interest in the kind of collective, multi-particle systems discussed by Whiteside and colleagues (see note 5). A good example of chemical meso-systems can be found in the self-assembly of lipid bilayers (for a representative early account, see O. G. Mouritsent and K. Jorgensen, "Micro-, Nano- and Meso-Scale Heterogeneity of Lipid Bilayers and Its Influence on Macroscopic Membrane Properties," *Molecular Membrane Biology* 12, no. 1 [1995]: 15-20). These structures provide a valuable bridgework between the physics of the meso-scale and a biological meso-scale. For an account of how these lipid assemblies may relate to higher order, functionally organized dynamics of cells, see S. Mayor and M. Rao, "Rafts: Scale-Dependent and Active Lipid Organization at the Cell Surface," *Traffic* 5 (2004): 231-240.
7. The way biologists black box the intrinsic novelty and focus on functional uses of quantum dots can be seen in W. Chan and S. Nie, "Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection," *Science* 281, no. 5385 (1998): 2016-18.
8. When considering nanomedicine and other areas of nanobio, it is difficult to find carefully developed definitions, and some definitions have odd characteristics that can only be understood in terms of disciplinary specific uses of language. The European Science Foundation (ESF) defined nanomedicine as "the science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving, and of preserving and improving health, using molecular tools and molecular knowledge of the human body" (ESF, *Nanomedicine An ESF – European Medical Research Councils (EMRC) Forward Look Report* (2004), Strasbourg Cedex, France). In an editorial on how his journal will view nanomedicine, Thomas Webster observed that many medical researchers find the European definition odd, since they (and molecular biologists generally) have long been focusing on molecular interactions (T. Webster, "Nanomedicine: What's in a Definition?", *International Journal of Nanomedicine* 1, no. 2 (2006): 115-116). But this criticism misses something important about medical history: namely, that many view modern , scientific medicine as arising with an appreciation of anatomy and physiology at a gross scale, and they view progress as moving down scale from organ systems and organs to tissue, and from tissue to cellular functions and histology. The "molecular revolution" designates the final advance in precision and shift in the organization of medical knowledge, where disease processes are understood at their most basic, i.e., molecular level. Nanoscience is then viewed as enabling this advance in both the understanding and interface with biosystems at the sub-cellular level. Webster contrasts the ESF definition with the US NIH Roadmap definition, where nanomedicine is defined as "an offshoot of nanotechnology, [which] refers to highly specific medical interventions at the molecular scale for curing disease or repairing damaged tissues." Following the U.S. NNI, Webster wishes to emphasize the novelty of properties. However, it is not clear how such novelty manifests in medicine. Webster tries get at this by emphasizing "significantly changed medical events," but he tells us nothing about how such novelty is to be identified. Since this novelty is supposed to distinguish nanomedical approaches from other molecular approaches, the emphasis falls upon a middle range between conventional molecular approaches (such as one might find with development and use of a conventional drug) and a higher-scale, functional interface (such as one finds when understanding and interfacing with cells, tissues, and organs as one might find with a device).
9. See *supra* note 5.
10. The biological mesoscale might also be taken to include the network of interactions among proteins, nucleic acids and membrane elements that provide the basis for genetic control of cellular behavior.
11. While proteins are representative of meso-scale, biological structures, they are not usually identified as nanoparticles. As McNeil notes, "particles such as DNA, bacteriophage, and monoclonal antibodies (mAb) may have nanometer-sized dimensions but would not be considered examples of nano*technology*" (emphasis added; S. McNeil, "Nanotechnology for the Biologist," *Journal of Leukocyte Biology* 78, no. 3 [September 2005]: 585-594). Nanoparticles are *artificial*, i.e., *technology*. McNeil's narrowing of the meaning of nanotechnology so it only covers artificial particles does reflect common conventions. Following this convention, we could define the biological mesoscale in such a way that it corresponds to the region of nano-scale size (approximately 1-300 nm), and has middle level properties that require bridging physical and biochemical logics (on the low end) and hierarchically functional logics (on the high end, and associated with cellular, tissue, organ, and organ system functions of an organism). This mesoscale includes both natural structures (e.g., proteins and nucleic acids) and artificial structures (nanoparticles). Classification of modified natural proteins such as artificial insulin is tricky. Whether or not we view artificial insulin as a nanoparticle, it is a meso-scale particle.
12. "Systems Biology" attempts to understand the function, regulation and interactions of networks of cellular components including proteins, nucleic acids, lipids, and small molecule mediators. This field is in its infancy, and tends to be very mathematical (c.f. U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits* (Boca Raton: Chapman and Hall/CRC, 2007). A basic introduction to protein structure, modification, function, metabolism, and aggregation into mechanically functional structures and signaling complexes can be found in B. Alberts et al., *Molecular Biology of the Cell*, 5th ed. (New York: Garland Science, 2008).
13. We focus on novelty arising from complexity and the role this plays in thwarting rational drug design. The issues we consider are general and would apply to all drugs, whether they involve novel targets or not. But even within the conventional drug category, a distinction could be drawn between conventional and novel drug targets, and many of the challenges we consider with meso-level agents would also arise with drugs that target novel targets. For a discussion of these challenges associated with novel targets, see P. Ma and R. Zemmel, "Value of Novelty?" *Nature Reviews Drug Discovery* 1, no. 8 (August 2002): 571-572; E. Butcher, "Can Cell Systems Biology Rescue Drug Discovery?" *Nature Reviews Drug Discovery* 4, no. 6 (June 2005): 461-467; and M. Hopkins et al., "The Myth of the Biotech Revolution: An Assessment of Technological, Clinical and Organizational Change," *Research Policy* 36, no. 4 (2007): 566-589. Each of these essays note that research oriented toward novel targets has not lead to expected returns and profit, and that the primary challenge associated with such novelty relates to health and safety.
14. Review of the need for ADME studies for nanomaterials can be found in B. Zolnik and N. Sadrieh, "Regulatory Perspective on the Importance of ADME Assessment of Nanoscale Materials Containing Drugs," *Advanced Drug Delivery Reviews* 61, no. 6 (2009): 422-427. Many of these problems are not surprising in view of the multiple and often conflicting constraints that need to be met by drugs. For example, it is extremely desirable for drug molecules to bind tightly and specifically to specific

receptors at the cell surface or inside the cell. As the binding sites tend to be very hydrophobic, drugs that bind tightly will also tend to be very hydrophobic and hence exhibit poor solubility in water, which is the majority component of most biological fluids. The polarity (hydrophilicity versus hydrophobicity) of a drug also affects its absorption and distribution. A major task for medicinal chemists is to alter potent "lead" drug candidates so that their absorption, distribution, metabolism, and toxicological profiles are acceptable. A useful discussion of the process of elimination or modification of drug candidates, leading to successful products, is provided by J. F. Pritchard et al., "Making Better Drugs: Decision Gates in Non Clinical Drug Development," *Nature Reviews Drug Discovery* 2, no. 7 (2003) 542-553.

15. The transition from in vitro, exploratory studies to in vivo studies with animals involves an increase in complexity that can roughly serve as a model for the increase in complexity associated with the transition from all pre-clinical studies (in vitro and in vivo) to clinical trials with humans. A nice review of the way ADME questions motivate the need for in vivo studies with nanoparticles can be found in H. Fischer and W. Chan, "Nanotoxicity: The Growing Need for In Vivo Study," *Current Opinion in Biotechnology* 18, no. 6 (2007): 565-571.
16. *Id.*
17. The difficulties associated with an open-ended ascertainment of novelty can be seen at every stage of the process that moves from in vitro to in vivo animal models, and finally to clinical trials. A nice illustration of the problems can be found in the reporting of adverse events with clinical trials. When conducting clinical trials, clinicians are to report "adverse events." But what kinds of events are relevant to the trial? And who can make sense of these events? IRBs are often overwhelmed with such information. To provide assistance in addressing these questions, the FDA and other agencies have put together a guidance document: U.S. Department of Health and Human Services, FDA, OC, CDER, CBER, CDRH, and OGCP, *Guidelines for Clinical Investigators, Sponsors, and IRBs Adverse Event Reporting to IRBs – Improving Human Subject Protection*, January 2009, *available at* <http://www.fda.gov/downloads/ RegulatoryInformation/Guidances/UCM126572.pdf> (last visited November 5, 2012). The guidelines make clear how any adverse event must be situated within a complex context, and how ascertainment and proper interpretation of that event depends on specific tools, concepts, methods, and model systems.
18. "Inductive risk" concerns the risk of mistaken inference from a given knowledge base. Any science involves a complex weighting of evidence that is reflected in assumptions that inform any set of experimental practices. When non-epistemic consequences are managed by means of those practices, a qualitative transition in the background rates of unanticipated interactions/harms can lead to a reappraisal of the way uncertainty is managed. This reappraisal is motivated by both scientific and ethical considerations. For a nice review of the issues related to inductive risk, see H. Douglas, "Inductive Risk and Values in Science," *Philosophy of Science* 67 (December 2000): 559-579.
19. For a review of the methods, instruments, and models integral to assuring quality of pharmaceuticals, see the International Conference on Harmonization of Technical Requirements for Registration of Pharmceuticals for Human Use (ICH). Their guidelines can be found at: <www.ich.org/products/guidelines.html> (last visited November 5, 2012).
20. A basic overview of some QA/QC considerations can be found in L. Yo, "Pharmaceutical Quality by Design: Process Development, Understanding, and Control," *Pharmaceutical Research* 25, no. 4 (2008): 781-791.
21. A standard reference for PK and PD in M. Rowland and T. N. Tozer, *Clinical Pharmacokinetics and Pharmacodynamics*, 4th ed. (Baltimore: Kluwer/Lippincott Williams and Wilkins, 2011).
22. An extensive literature now shows that nanoparticles are novel in just this way, i.e., they cannot be assessed by methods and instruments that are currently available, and thus require research efforts that are directed toward the QA/QC infrastructure itself. Representative essay documenting this need for infrastructure innovation include: H. Fischer and W. Chan, "Nanotoxicity: The Growing Need for In Vivo Study," *Current Opinion in Biotechnology* 18, no. 6 (2007): 565-571; D. Warheit, "How Meaningful Are the Results of Nanotoxicity Studies in the Absence of Adequate Material Characterization?" *Toxicological Sciences* 101, no. 2 (2008): 183-185; G. Oberdorster, "Safety Assessment for Nanotechnology and Nanomedicine: Concepts of Nanotoxicology," *Journal of Internal Medicine* 267, no. 1 (2009): 89-105; C. F. Jones and D. W. Grainger, "In Vitro Assessments of Nanomaterial Toxicity," *Advanced Drug Delivery* 61, no. 6 (2009): 438-456; P. R. Gil et al., "Correlating Physico-Chemical with Toxicological Properties of Nanoparticles: The Present and the Future," *ACS Nano* 4, no. 10 (2010): 5527-5531.
23. See, for example, A. K. Banga, *Therapeutic Peptdies and Proteins. Formulation, Processing and Delivery Systems*, 2nd ed. (Boca Raton: Taylor and Francis, 2006).
24. See, for example, R. I. Mahato, ed., *Biomaterials for Delivery and Targeting of Proteins and Nucleic Acids* (Boca Raton: CRC Press, 2005).
25. A recent special issue of Accounts of Chemical Research, edited by C. F. Meares and M. Yokoyama, is devoted to gene silencing and delivery nanotechnologies. See also M. S. Shim and Y. J. Kim, "Stimuli-Responsive Polymers and Nanomaterials for Gene Delivery and Imaging Applications," *Advanced Drug Delivery Reviews* 64, no. 11 (2012): 1046-1069.
26. In this essay, we ignore biologicals since they represent a somewhat orthogonal classification. Biologicals are typically, but not always, derived from plant or animal (including human) sources, which is irrelevant to the present discussion. On the other hand, many biologicals do share some of the unusual properties that we find for mesoscale therapeutics, including complexity and inherent variability.
27. For a comparison between nano-based therapeutics and drug-eluting stents, see G. Agich, "Drug-Eluting Stents: Some Lessons for the Ethics of Medical Nanotechnology (Abstract)," *Journal of Long-Term Effects of Medical Implants* 18, no. 1 (2008): 6.
28. Excellent introductory and technical discussions of drug eluting stent technologies are provided in *Advanced Drug Delivery Reviews* 58, no. 3 (2006), edited by H. M. Burt and W. L. Hunter.
29. In 1990, Mark Chasin and Robert Langer published an edited book titled *Biodegradable Polymers as Drug Delivery Systems*, vol. 45 (Dekker, New York: Drugs and the Pharmaceutical Sciences): at Chapter 1. H. Lewis wrote "Unfortunately, investigators seeking advanced drug delivery systems are severely limited in candidate polymeric materials as evidenced by the relatively small number of systems described in this text." This situation has scarcely changed since then. The need to pass stringent regulatory requirements and fears of potential liability [see J. Kohn, "Biomaterials science at a crossroads: Are current product liability laws in the United States hampering innovations and the development of safer medical implants?," Pharmaceutical Research 13 (1996) 815-819] has likely slowed the introduction of new biodegradable and other implantable materials into the market.
30. Much attention has been focused towards the so-called enhanced permeability and retention (EPR) effect, which leads to increased relative partitioning of drugs associated with nanoparticles into tumors, compared to "free" drugs. The extent to which EPR provides benefits in cancer therapy has been the subject of debate. See P. Ruenraraengsak et al., "Nanosystem Drug Targeting: Facing Up to Complex Realities," *Journal of Controlled Release* 141, no. 3 (2010): 265-276; and Y. H. Bae and K. Park, "Targeted Drug Delivery to Tumors: Myths, Reality, Possibility," *Journal of Controlled Release* 153, no. 3 (2011): 198-2005.
31. The effects that the presence of dilute impurities during synthesis can have on a nanomaterial product, are reported by L.

K. Wolf, "Sweating the Small Stuff," *Chemical and Engineering News* 90, no. 22 (2012): 48-50.

32. The difficulties associated with analysis and validation of size uniformity are described by M. Gaumet et al., "Nanoparticles for Drug Delivery: The Need for Precision in Reporting Particle Size Parameters," *European Journal of Pharmaceutics and Biopharmaceutics* 69, no. 1 (2008): 1-9. This statement is not meant to imply that drug products are absolutely uniform. For example, a typical tablet contains the active ingredient (drug) along with excipients such as binders, bulking agents, and other molecules used to mask the bitter taste of the drug. Tablets are usually made by mixing powders of these ingredients are compressing the mixture in a die. Depending on granule size and uniformity of the powder mixture, there will be some variability in the amount of drug incorporated in each tablet. What can be said, however, is that each drug molecule is the same (save the possibilities of isomerization or chemical conversion during storage) to any degree of specified purity. We believe that the "inherent" variability of nanoparticles is more fundamental, for reasons set forth in this essay.
33. K. Elliott, "Varieties of Exploratory Experimentation in Nanotoxicology," *History and Philosophy of Life Sciences* 29, no. 3 (2007): 311-334.
34. M. D. Monopoli et al., "Physical-Chemical Aspects of Protein Corona: Relevance to In Vitro and In Vivo Impacts of Nanoparticles," *Journal of the American Chemical Society* 133, no. 8 (2011): 2525-2534; P. Aggarwal et al., "Nanoparticle Interaction with Plasma Proteins as it Relates to Particle Biodistribution, Biocompatibility, and Therapeutic Efficacy," *Advanced Drug Delivery Reviews* 61, no. 6 (2012): 428-437; S. Dufort et al., "Physicochemical Parameters That Guide Nanoparticle Fate Also Dictate Rules for Their Molecular Evolution," *Advanced Drug Delivery Reviews* 64, no. 2 (2012): 179-189.