Part One:
Nanomedicine: The Next Waves of Medical Innovations

Nanotechnology, Volume 5: Nanomedicine. Edited by Viola Vogel
Copyright © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
ISBN: 978-3-527-31736-3

1
Introduction

Viola Vogel

1.1
Great Hopes and Expectations are Colliding with Wild Hype and Some Fantasies

What is nanomedicine? Will nanomedicine indeed help to cure major diseases and live up to the great hopes and expectations? What innovations are on the horizon and how can sound predictions be distinguished from wild hype and plain fantasy? What are realistic timescales in which the public might benefit from their ongoing investments?

When first exploring whether nanotechnology might reshape the future ways of diagnosing and treating diseases, the National Institutes of Health stated in the report of their very first nanoscience and nanotechnology workshop in 2000 (http://www.becon.nih.gov/nanotechsympreport.pdf. Bioengineering Consortium):

> *Every once in a while, a new field of science and technology emerges that enables the development of a new generation of scientific and technological approaches. Nanotechnology holds such promise.*

Our macroscopic bodies and tissues are highly structured at smaller and smaller length scales, with each length scale having its own secrets as to how life-supporting tasks are mastered. While we can still touch and feel our organs, they are all composed of cells which are a little less than one million times smaller and only visible under the light microscope (microscopic). Zooming further into the cell, about one thousand times, we find the nanoscale molecular machineries that drive and control the cellular biochemistry, and thereby distinguish living systems from dead matter. Faced with a rest of new technologies that has enabled researchers to visualize and manipulate atoms and molecules, as well as to engineer new materials and devices at this tiny length scale [1], major think tanks have begun since the late 1990's to evaluate the future potential of nanotechnology [2], and later at the interface to medicine [3–11]. These efforts were paralleled by a rapid worldwide increase in funding and research activities since 2000. The offset of a gold rush into the 'nano', by which the world of the very small is currently discovered, will surely also lead to splendid new entrepreneurial opportunities. Progress impacting on human health came much faster than expected.

Nanotechnology, Volume 5: Nanomedicine. Edited by Viola Vogel
Copyright © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
ISBN: 978-3-527-31736-3

1.2
The First Medical Applications are Coming to the Patients' Bedside

The public most commonly associates nanomedicine with engineered nanoparticles in the context of drug delivery devices or advanced medical imagine applications. Novel is that the molecules which are coassembled into nanoparticles can nowadays carry many different chemical functionalities. It has thereby become feasible to integrate multiple tasks into drug delivery device, from targeting specific tissues to releasing drugs, from enhancing contrast to probing their environment. How is this all done? First of all, the nanoparticles are loaded with drugs. The particles might then carry molecules on their surfaces that bind with great specificity to complementary molecules that are unique to cancers or to other diseased tissues, as reviewed later in this volume [12, 13]. For example, by using antibody– antigen recognition such engineered nanoparticles can be accumulated in the targeted tissues rather than being distributed over the entire body. Local accumulation greatly enhances the efficiency of drugs and reduces any unintended adverse side effects that might otherwise harm other organs. The selectivity by which disease can be treated by using engineered nanoparticles is thus in stark contrast to how conventional drugs operate; as conventional drugs lack the capacity to target specific tissues, they are distributed much more uniformly over the entire body, and must therefore be administered at much higher doses. Beyond tissue targeting, the nanoparticles might further be engineered to absorb therapeutic radiation, which might heat them up when they have reached the diseased tissues to damage the local tissue, either by heat or by the release of drugs [11, 12, 14]. Alternatively, the nanoparticles might hold on to drugs by bonds that can be locally cleaved by enzymes or be broken by light or radiation, thereby releasing the drug under the control of a physician (as reviewed elsewhere in this volume [12, 13]). The goal here is to design new strategies to inflict damage only to the aberrant cells, while leaving the surrounding tissues unharmed. This multifunctional integration of many different diagnostic and therapeutic tasks in single particles thereby enables applications that go far beyond those of conventional drugs.

Engineered nanoparticles can also change the future of medical imaging, as they enable us to combine structural imaging with spatially resolved diagnostics and interventions. Only eight years after wondering whether nanotechnology will revolutionize medicine, the US National Cancer Institute (NCI) stated that [15]:

> *Nanodevices are used in detecting cancer at its earliest stages, pinpointing its location within the body, delivering anticancer drugs specifically to malignant cells, and determining if these drugs are killing malignant cells.*

Increasingly sophisticated medical imaging technologies continue to revolutionize medicine. X-ray imaging, which was later complemented by ultrasound, positron emission tomography (PET) and magnetic resonance imaging (MRI), opened the possibility to visualize *nonintrusively* first bones, and then the inner organs, of our bodies. The objectives now are to obtain images of the structure of organs, at much higher resolution, together with spatially resolved biochemical information which is reflective of how well cells and organs function. This includes probing noninvasively whether certain organs produce the hormones and enzymes at normal rates, and whether other metabolic activities might deviate from the norm. Major advances are about to come from the usage of nanoparticles that are engineered to serve both, as drug delivery systems and to enhance the contrast in ultrasound, PET and MRI images (for a review, see Ref. [16] and elsewhere in this volume [12, 13]). Enhancing the contrast and spatial resolution of images will enable the detection of cancers and other structural abnormalities of organs at much earlier stages, which in turn will enhance the chances of an effective therapy. Such multitasking approaches might also one day substitute for a variety of surgical interventions. Today, many books and articles have been published discussing the various medical applications of such engineered nanoparticles, while the pharmaceutical industry continues to invest heavily in their development (for reviews, see Refs [6, 15–22]).

1.3
Major Advances in Medicine Have Always been Driven by New Technologies

During the past few decades, the deciphering of the molecular origins of many diseases has had a most profound impact on improving human health. One historical step was the deciphering of the first protein structure in 1958 [23]. This opened a new era in medicinal chemistry, as drugs could since then be designed in a rational manner – that is, drugs that fit tightly into essential binding pockets thereby regulating protein and DNA functions. The invention of how to harness DNA polymerase in order to amplify genetic material in the test tube – which we now know as the polymerase chain reaction (PCR) [24] – then opened the field of molecular biology during the 1980s. PCR also enabled targeted genetic alterations of cells to identify the functional roles of many proteins, and this in turn led to the discovery that cell signaling pathways of many interacting proteins existed, and could be altered by diseases. The explosion of knowledge into how cell behavior is controlled by biochemical factors opened the door to target drugs to very specific players in cell signaling pathways. This also led to a host of new biotechnology start-up companies, the first of which became profitable only around 2000.

The next major breakthrough came with the solving of the human genome in 2001 [25–29]. Access to a complete genetic inventory of more than 30 000 proteins in our body, combined with high-resolution structures for many of them, enables a far more systematic search for correlations between genetic abnormalities and diseases. Finally, various diseases could for the first time be traced to inherited point mutations of proteins. In achieving this, much insight was gained into the regulatory roles of these proteins in cell signaling and disease development [30]. This includes recognizing genetic predispositions to various cancers [31], as well as to inherited syndromes where larger sets of seemingly uncorrelated symptoms could finally be explained by the malfunctioning of particular proteins or cell signaling pathways [32–38], including ion channel diseases [39–42].

1.4

Nanotechnologies Foster an Explosion of New Quantitative Information How Biological Nanosystems Work

Far less noticed by the general public are the next approaching waves of medical innovations, made possible by an explosion of new quantitative information how biological systems work.

The ultimate goal is to achieve an understanding of all the structural and dynamic processes by which the molecular players work with each other in living cells and coregulate cellular functions. Driven by the many technologies that have emerged from the physical, chemical, biological and engineering sciences, to visualize (see elsewhere in this volume [43, 44]) and manipulate the nanoworld, numerous discoveries are currently being made (as highlighted in later chapters [43–51]). These findings result from the new capabilities to create, analyze and manipulate nanostructures, as well as to probe their nanochemistry, nanomechanics and other properties within living and manmade systems. New technologies will continuously be developed that can interrogate biological samples with unprecedented temporal and spatial resolution [52]. Novel computational technologies have, furthermore, been developed to simulate cellular machineries in action with atomic precision [53]. New engineering design principles and technologies will be derived from deciphering how natural systems are engineered and how they master all the complex tasks that enable life. Take the natural machineries apart, and then learn how to reassemble their components (as exemplified here in Chapter 8 for molecular motors [48]).

How effectively will these novel insights into the biological nanoworld are recognized in their clinical significance, and translated into addressing grand medical challenges? This defines the time that it takes for the emergence of a next generation of diagnostic and therapeutic tools. As these insights change the way we think about the inner workings of cells and cell-made materials, totally new ways of treating diseases will emerge. As described in detail elsewhere in this volume, new developments are already under way of how to probe and control cellular activities [45, 47, 49–51, 54, 55]. This implicates the emergence of new methodologies of how to correct tissue and organ malfunctions. Clearly, we need to know exactly how each disease is associated with defects in the cellular machinery before medication can be rationally designed to effectively cure them.

Since every one of the new (nano)analytical techniques has the potency of revealing something never seen before, a plethora of opportunities can be envisioned. Their realization, however, hinges on the scientists' ability to recognize the physiological and medical relevance of their discoveries. This can best be accomplished in the framework of interdisciplinary efforts aimed at learning from each other what the new technologies can provide, and how this knowledge can be effectively translated to address major clinical challenges. Exploring exactly how these novel insights into the nanoworld will impact medicine has been the goal of many recent workshops [3–11, 56–58]). This stimulated the creation of the NIH

Roadmap Initiative in Nanomedicine [57, 58], and is the major focus of this volume.

1.5
Insights Gained from Quantifying how the Cellular Machinery Works will lead to Totally New Ways of Diagnosing and Treating Disease

Which are some of the central medical fields that will be impacted? Despite these stunning scientific advances, and the successful suppression or even eradication of a variety of infectious diseases during the past 100 years, the goal has not yet been reached of having medication at hand to truly cure many of the diseases that currently kill the largest fraction of humans per year, including cancers, cardiovascular diseases and AIDS. Much of the current medication against these diseases fights symptoms or inhibits their progression, often inflicting considerable side effects. Unfortunately, however, much of the medication can slow down but it cannot *reverse* disease progression in any major way – all of which contributes to healthcare becoming unaffordable, even in the richest nations of the world. For instance, intense cancer research over the past decades has revealed that the malignancy of cancer cells progresses with the gradual accumulation of genetic alterations [12, 50, 59–65]. Yet, little remains known as to how cancer stem cells form, in the first place, and about the basic mechanisms that trigger the initiation of their differentiation into more malignant cancer cells after having remained dormant, sometimes for decades [66–69]. While much has been learned in the past about the molecular players and their interactions, the above-mentioned shortcomings in translating certain advances in molecular and cell biology into more effective medication reflect substantial gaps in our knowledge of how all these components within the cells work in the framework of an integrated system. How can so many molecular players be tightly coordinated in a crowded cellular environment to generate predictable cell and tissue responses? Whilst lipid membranes create barriers that enclose the inner volumes of cells and control which molecules enter and leave (among other tasks), it is the proteins that are the 'workhorses' that enable most cell functions. In fact, some proteins function as motors that ultimately allow cells to move, as discussed in different contexts in the following chapters [46, 48, 50]. Other proteins transcribe and translate genetic information, and efforts to visualize these in cells have been summarized in Chapter 6 [45]. Yet other sets of proteins are responsible for the cell signaling through which all metabolic functions are enabled, orchestrated and regulated. But what are the underlying rules by which they play and interact together to regulate diverse cell functions? How do cells sense their environments, integrate that information, and translate it to ultimately adjust their metabolic functions if needed? Can this knowledge help to develop interventions which could possibly reverse pathogenic cells such that they performed their normal tasks again? Deciphering how all of these processes are regulated by the physical and biochemical microenvironment of cells is key to addressing various biomedical challenges with new perceptions, and is described as one of the major foci in this volume. But, how can this be accomplished?

1.6
Engineering Cell Functions with Nanoscale Precision

The engineering of nanoenvironments, nanoprobes and nanomanipulators, together with novel modalities to visualize phenomena at this tiny scale, have already led to the discovery of many unexpected mechanisms of how cellular nanoparts function, and how they cooperate synergistically when integrated into larger complex systems [43, 46–51]. Today, nanotechnology tools are particularly well suited to explore and quantify the physical aspects of biology, thereby complementing the tool chests of biochemists, molecular biologists and geneticists. Such nanotechnology approaches could already reveal that not only biochemical factors but also mechanical aspects as well as the micro- and nanoscale features of a cell's microenvironment, play pivotal roles in regulating their fate. The insights and implications thereof are described in chapters 9 to 14 [47, 49–51].

These discoveries are particularly relevant since most of our biomedical knowledge of how cells function has been derived in the past from the study of cells cultured on flat polymer surfaces (Petri dishes or on multi-welled plates). Cells in a more tissue-like environment, however, often show a vastly different behavior [70–75] (and chapters 9 to 14). With a still poorly understood cell signaling response system, cells in tissues thus 'see' and 'feel' an environment that is poorly mimicked by the common cell culturing conditions or scaffolds used in tissue engineering. Nanotechnologies will thus be pivotal to deciphering how cells sense and integrate a broad set of cues that regulate cell fate, from the moment that a sperm fertilizes an egg, to sustained, normal tissue functions. Moreover, these dependencies must be known in order to develop far more efficient drugs and treatment methods. However, ultimately it is the combination of many different technologies – some of which may originate from the physical sciences and others from biology – that must be combined to understand and quantify biology. Unfortunately, today an insufficient number of research workers are trained to perform these tasks [76].

1.7
Advancing Regenerative Medicine Therapies

Virtually any disease that results from malfunctioning, damaged or failing tissues may be potentially cured through regenerative medicine therapies, as was recently stated in the first NIH report on Regenerative Medicine [4]. But, how will nanotechnology make a difference? The repair – or ultimately replacement – of diseased organs, from larger bone segments to the spinal cord, or from the kidneys to the heart, still poses major challenges as discussed in chapters 9 to 14 [47, 49, 50, 54, 55, 77]. The current need for organ transplants surpasses the availability of suitable donor organs by at least an order of magnitude, and the patients who finally receive an organ transplant must receive immune suppressant drugs for the rest of their life. Thus, one goal will be to apply the mounting insights into how cells work, and how their functions are controlled by matrix interactions, to design alternate therapies that stimulate regenerative healing processes of previously irreparable organs. In a most promising approach, some molecules have been designed that can self-assemble in the body into provisional matrices [55]. If these are injected shortly after injury, they help to repair spinal cord injuries and heart tissues damaged by an infarction. And if such strategies do not work, then another possibility might be to seed the patient's cells or stem cells into engineered biohybrid matrices to grow simple tissues *ex vivo* – that is, in the laboratory – and later implant them to support or regenerate failing organ functions [51, 54]. This could provide new ways of treating diabetes, liver and kidney failures, cardiovascular and many other diseases, or of replacing or repairing organs damaged in accidents or removed during surgery. Learning how to control the differentiation of stem cells in engineered matrices is therefore central to advancing our technical abilities in tissue engineering and regenerative medicine, and the challenges ahead as discussed in chapters 9 to 14 [50, 51, 54]. Nanofibers thereby mimic much better the fibrous nature of extracellular matrices [54], and the nanoscale patterning of ligands can control cell activation [49], including the activation of cells that play central roles in the immune response system (for a review, see [49]). In summary, the insights derived with the help of nanotechnology will enable the engineering of tissue-mimetic scaffolds that better control and regulate tissue function and repair. Improving human health will thus critically hinge upon translating nanotechnology-derived insights about cellular and tissue functions into novel diagnostic and therapeutic technologies.

1.8
Many More Relevant Medical Fields Will be Innovated by Nanotechnologies

Whilst the major focus of this volume is to outline the biomedical implications derived from revealing the underpinning mechanisms of how human cells function, it should also be mentioned that fascinating developments that are prone to alter medicine are being made in equally relevant other biomedical sectors. Future ways to treat infection will change when the underpinning mechanisms of how microbial systems function are deciphered, and how they interact with our cells and tissues. Many beautiful discoveries have already been made that will help us for example to interfere more effectively with the sophisticated machinery that bacteria have evolved to target, adhere and infect cells and tissues. Nanotechnology tools have revealed much about the function of the nano-engines that bacteria and other microbes have evolved for their movement [78–80], how bacteria adhere to surfaces [81–83], and how microbes infect other organisms [81–85]. Equally important when combating infection is an ability to exploit micro- and nanofabricated tools in order to understand the language by which microorganisms communicate with each other [86, 87] and how their inner machineries function [88–90]. A satisfying understanding of how a machine works can only be reached when we are capable of reassembling it from its components. It is thus crucial to learn how these machineries can be reassembled *ex vivo*, potentially even in nonbiological environments, as this should open the door to many technical and medical applications [84, 91–93]. Today, we have only just started along the route to combining the natural and synthetic worlds, with the community seeking how bacteria might be used as 'delivery men' for nanocargoes [94], or in manmade devices to move fluids and objects [95, 96].

Finally, microfabricated devices with integrated nanosensors, nanomonitors and nanoreporters – all of which are intrinsic to a technology sector enabled by (micro/nano)biotechnology – will surely also lead to changes in medical practice. In the case of chemotherapies and many other drugs, it is well known that they may function well in some patients, but fail in others. It is feasable that this 'one-size-fits-all' approach might soon be replaced by a more patient-specific system. *Personalized medicine* refers to the use of genetic and other screening methods to determine the unique predisposition of a patient to a disease, and the likelihood of them responding to particular drugs and treatments [30, 97–100]. Cheap diagnostic systems that can automatically conduct measurements on small gas or fluid volumes, such as human breath or blood, will furthermore enable patients to be tested rapidly, without the need to send samples to costly medical laboratories. Needless to say, portable integrated technologies that will allow the testing and treatment of patients on the spot (point-of-care) will save many lives, and are urgently needed to improve human health in the Third World [101–104].

Faced with major challenges in human healthcare, an understanding what each of the many nanotechnologies can do – and how they each can best contribute to address the major challenges ahead – is crucial to drive innovation forwards. An improved awareness of how new technologies will help to unravel underpinning mechanisms of disease is crucial to setting realistic expectations and timescales, as well as to prepare for the innovations to come.

Since ultimately, thriving towards providing access to efficient and affordable healthcare, by improving upon technology, is not just an intellectual luxury, but our responsibility.

Acknowledgments

Many of the thoughts about the future of nanomedicine were seeded during exciting discussions with Drs Eleni Kousvelari (NIH) and Jeff Schloss (NIH), as well as with Dr Mike Roco (NSF), when preparing for the interagency workshop on Nanobiotechnology [9]. The many inspirations and contributions from colleagues and friends worldwide are gratefully acknowledged, several of whom have contributed chapters here, as well as from my students and collaborators and the many authors whose articles and conference talks have left long-lasting impressions.

References

1 Nalwa, H.S. (2004) *Encyclopedia of Nanoscience and Nanotechnology*, American Scientific Publishers.

2 Roco, M.C. (2003) National Nanotechnology Initiative: From Vision to Implementation. http://www.nano.gov/nni11600/sld001.htm.

3 National Institutes of Health (2000): Nanoscience and Nanotechnology: Shaping Biomedical Research. Organized by the Bioengineering Consortium. Available at http://www.becon.nih.gov/nanotechsympreport.pdf.

4 National Institutes of Health (2003) 2020: A New Vision – A Future for Regenerative Medicine, U.S. Department of Health and Human Services. Available at http://www.hhs.gov/reference/newfuture.shtml

5 National Institutes of Health (2003) Nanotechnology in Heart, Lung, Blood, and Sleep Medicine. http://www.nhlbi.nih.gov/meetings/nano_sum.htm.

6 Duncan, R. (2005) Nanomedicine. Forward Looking Report. http://www.esf.org/publication/214/Nanomedicine.pdf, European Science Foundation (ESF)-European Medical Research Council (EMRC). Report Number 2-912049-52-0.

7 NIBIB/DOE (2005) Workshop on Biomedical Applications of Nanotechnology. Organized by the National Institute of Biomedical Imaging and BioEngineering, Department of Energy, USA. Available at http://www.capconcorp.com/nibibdoenanotech/

8 Vogel, V. and Baird, B. (2005) 'BioNanotechnology'. National Nanotechnology Initiative Grand Challenge Workshop Report. Available at http://nano.gov/nni_nanobiotechnology_rpt.pdf.

9 European-Technology-Platform (2006) Nanomedicine: Nanotechnology for Health. Available at ftp://ftp.cordis.europa.eu/pub/nanotechnology/docs/nanomedicine_bat_en.pdf

10 Nantional Institute of Health (2008) Roadmap Initiative in Nanomedicine. Available at http://nihroadmap.nih.gov/nanomedicine/index.

11 Schmid, G., Brune, H., Ernst, H., Grünwald, W., Grunwald, A., Hofmann, H., Janich, P., Krug, H., Mayor, M., Rathgeber, W., Simon, U., Vogel, V. and Wyrwa, D. (2006) *Nanotechnology. Assessment and Perspectives*, Springer Verlag.

12 Godin, B., Serda, R.E., Sakamoto, J., Decuzzi, P. and Ferrari, M. (2009) Nanoparticles for cancer detection and therapy, in *Nanomedicine* (ed. V. Vogel), Ch. 3, Wiley-VCH, Weinheim.

13 Wallace, K.D., Hughes, M.S., Marsh, J.N., Caruthers, S.D., Lanza, G.M. and Wickline, S.A. (2009) From i*n vivo* ultrasound and MRI imaging to therapy: Contrast agents based on target-specific nanoparticles, in *Nanomedicine* (ed. V. Vogel), Ch. 2, Wiley-VCH, Weinheim.

14 Johannsen, M., Gneveckow, U., Thiesen, B., Taymoorian, K., Cho, C.H., Waldofner, N., Scholz, R., Jordan, A., Loening, S.A. and Wust, P. (2007) Thermotherapy of prostate cancer using magnetic nanoparticles: feasibility, imaging, and three-dimensional temperature distribution. *European Urology*, **52** (6), 1653–1661.

15 National Cancer Institute (2008). Alliance for Nanotechnology in Cancer. http://nano.cancer.gov/resource_center/tech_backgrounder.asp.

16 Phelps, M.E. (2004) *PET: Molecular Imaging and Its Biological Applications*, Springer.

17 National Cancer Institute (2004) Cancer Nanotechnology. Going Small for Big Advances, NIH.

18 Jain, K.K. (2007) Applications of nanobiotechnology in clinical diagnostics. *Clinical Chemistry*, **53** (11), 2002–2009.

19 Nie, S., Xing, Y., Kim, G.J. and Simons, J.W. (2007) Nanotechnology applications in cancer. *Annual Review of Biomedical Engineering*, **9**, 257–288.

20 Kumar, C.S.S.R. (ed.) (2008) *Nanomaterials for Cancer Diagnosis*, Nanotechnologies for the Life Sciences, Wiley-VCH, Weinheim.

21 Kumar, C.S.S.R. (ed.) (2008) *Nanomaterials for Cancer Therapy*. Nanotechnologies for the Life Sciences, Wiley-VCH, Weinheim.

22 Kumar, C.S.S.R. (ed.) (2008) *Nanomaterials for Medical Diagnosis and*

Therapy Nanotechnologies for the Life Sciences, Wiley-VCH, Weinheim.

23 Perutz, M.F. (1962) X-ray analysis of haemoglobin. Nobel Lecture.

24 Mullis, K.B. (1993) The Polymerase Chain Reaction. Nobel Lecture.

25 Cheung, V.G., Nowak, N., Jang, W., Kirsch, I.R., Zhao, S., Chen, X.N., Furey, T.S., Kim, U.J., Kuo, W.L., Olivier, M., Conroy, J., Kasprzyk, A., Massa, H., Yonescu, R., Sait, S., Thoreen, C., Snijders, A., Lemyre, E., Bailey, J.A., Bruzel, A., Burrill, W.D., Clegg, S.M., Collins, S., Dhami, P., Friedman, C., Han, C.S., Herrick, S., Lee, J., Ligon, A.H., Lowry, S., Morley, M., Narasimhan, S., Osoegawa, K., Peng, Z., Plajzer-Frick, I., Quade, B.J., Scott, D., Sirotkin, K., Thorpe, A.A., Gray, J.W., Hudson, J., Pinkel, D., Ried, T., Rowen, L., Shen-Ong, G.L., Strausberg, R.L., Birney, E., Callen, D.F., Cheng, J.F., Cox, D.R., Doggett, N.A., Carter, N.P., Eichler, E.E., Haussler, D., Korenberg, J.R., Morton, C.C., Albertson, D., Schuler, G., de Jong, P.J. and Trask, B.J. (2001) Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* **409** (6822), 953–958.

26 Helmuth, L. (2001) Genome research: map of the human genome 3.0. *Science*, **293** (5530), 583–585.

27 McPherson, J.D., Marra, M., Hillier, L., Waterston, R.H., Chinwalla, A., Wallis, J., Sekhon, M., Wylie, K., Mardis, E.R., Wilson, R.K., Fulton, R., Kucaba, T.A., Wagner-McPherson, C., Barbazuk, W.B., Gregory, S.G., Humphray, S.J., French, L., Evans, R.S., Bethel, G., Whittaker, A., Holden, J.L., McCann, O.T., Dunham, A., Soderlund, C., Scott, C.E., Bentley, D.R., Schuler, G., Chen, H.C., Jang, W., Green, E.D., Idol, J.R., Maduro, V.V., Montgomery, K.T., Lee, E., Miller, A., Emerling, S., Kucherlapati, Gibbs, R., Scherer, S., Gorrell, J.H., Sodergren, E., Clerc-Blankenburg, K., Tabor, P., Naylor, S., Garcia, D., de Jong, P.J., Catanese, J.J., Nowak, N., Osoegawa, K., Qin, S., Rowen, L., Madan, A., Dors, M., Hood, L., Trask, B., Friedman, C., Massa, H., Cheung, V.G., Kirsch, I.R., Reid, T., Yonescu, R., Weissenbach, J., Bruls, T., Heilig, R., Branscomb, E., Olsen, A., Doggett, N., Cheng, J.F., Hawkins, T., Myers, R.M., Shang, J., Ramirez, L., Schmutz, J., Velasquez, O., Dixon, K., Stone, N.E., Cox, D.R., Haussler, D., Kent, W.J., Furey, T., Rogic, S., Kennedy, S., Jones, S., Rosenthal, A., Wen, G., Schilhabel, M., Gloeckner, G., Nyakatura, G., Siebert, R., Schlegelberger, B., Korenberg, J., Chen, X.N., Fujiyama, A., Hattori, M., Toyoda, A., Yada, T., Park, H.S., Sakaki, Y., Shimizu, N., Asakawa, S., Kawasaki, K., Sasaki, T., Shintani, A., Shimizu, A., Shibuya, K., Kudoh, J., Minoshima S., Ramser J., Seranski P., Hoff C., Poustka A., Reinhardt R. and Lehrach H. (2001) A physical map of the human genome. *Nature* **409** (6822), 934–941.

28 Olivier, M., Aggarwal, A., Allen, J., Almendras, A.A., Bajorek, E.S., Beasley, E.M., Brady, S.D., Bushard, J.M., Bustos, V.I., Chu, A., Chung, T.R., De Witte, A., Denys, M.E., Dominguez, R., Fang, N.Y., Foster, B.D., Freudenberg, R.W., Hadley, D., Hamilton, L.R., Jeffrey, T.J., Kelly, L., Lazzeroni, L., Levy, M.R., Lewis, S.C., Liu, X., Lopez, F.J., Louie, B., Marquis, J.P., Martinez, R.A., Matsuura, M.K., Misherghi, N.S., Norton, J.A., Olshen, A., Perkins, S.M., Perou, A.J., Piercy, C., Piercy, M., Qin, F., Reif, T., Sheppard, K., Shokoohi, V., Smick, G.A., Sun, W.L., Stewart, E.A., Fernando, J., Tejeda, J., Tran, N.M., Trejo, T., Vo, N.T., Yan, S.C., Zierten, D.L., Zhao, S., Sachidanandam, R., Trask, B.J., Myers, R.M. and Cox, D.R. (2001) A high-resolution radiation hybrid map of the human genome draft sequence. *Science*, **291** (5507), 1298–1302.

29 Reich, D.E., Cargill, M., Bolk, S., Ireland, J., Sabeti, P.C., Richter, D.J., Lavery, T., Kouyoumjian, R., Farhadian, S.F., Ward, R. and Lander, E.S. (2001) Linkage disequilibrium in the human genome. *Nature*, **411** (6834), 199–204.

30 Ideker, T. and Sharan, R. (2008) Protein networks in disease. *Genome Research*, **18** (4), 644–652.

31 King, M.C., Marks, J.H. and Mandell, J.B. (2003) Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science*, **302** (5645), 643–646.

32 Lin, M.T. and Beal, M.F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, **443** (7113), 787–795.

33 Kato, T. (2007) Molecular genetics of bipolar disorder and depression. *Psychiatry and Clinical Neurosciences*, **61** (1), 3–19.

34 Madsen, E. and Gitlin, J.D. (2007) Copper and iron disorders of the brain. *Annual Review of Neuroscience*, **30**, 317–337.

35 Oti, M. and Brunner, H.G. (2007) The modular nature of genetic diseases. *Clinical Genetics*, **71** (1), 1–11.

36 Abrahams, B.S. and Geschwind, D.H. (2008) Advances in autism genetics: on the threshold of a new neurobiology. *Nature Reviews. Genetics*, **9** (5), 341–355.

37 Ferrell, R.E. and Finegold, D.N. (2008) Research perspectives in inherited lymphatic disease: an update. *Annals of the New York Academy of Sciences*, **1131**, 134–139.

38 Judge, D.P. and Dietz, H.C. (2008) Therapy of Marfan syndrome. *Annual Review of Medicine*, **59**, 43–59.

39 Weinreich, F. and Jentsch, T.J. (2000) Neurological diseases caused by ion-channel mutations. *Current Opinion in Neurobiology*, **10** (3), 409–415.

40 Wilde, A.A. and van den Berg, M.P. (2005) Ten years of genes in inherited arrhythmia syndromes: an example of what we have learned from patients, electrocardiograms, and computers. *Journal of Electrocardiology*, **38** (4 Suppl), 145–149.

41 Fiske, J.L., Fomin, V.P., Brown, M.L., Duncan, R.L. and Sikes, R.A. (2006) Voltage-sensitive ion channels and cancer. *Cancer Metastasis Reviews*, **25** (3), 493–500.

42 Terrenoire, C., Simhaee, D. and Kass, R.S. (2007) Role of sodium channels in propagation in heart muscle: how subtle genetic alterations result in major arrhythmic disorders. *Journal of Cardiovascular Electrophysiology*, **18** (8), 900–905.

43 Baker, M.L., Marsh, M.P. and Chiu, W. (2009) Electron cryo-microscopy of molecular nanomachines and cells, in *Nanomedicine* (ed. V. Vogel), Ch. 4, Wiley-VCH, Weinheim.

44 Kukura, P., Renn, A. and Sandoghdar, V. (2009) Pushing optical microscopy to the limit: from single-molecule fluorescence microscopy to label-free detection and tracking of biological nano-objects, in *Nanomedicine* (ed. V. Vogel), Ch. 5, Wiley-VCH, Weinheim.

45 Bao, G., Santangelo, P., Nitin, N. and Rhee, W.-J. (2009) Nanostructured probes for *in vivo* gene detection, in *Nanomedicine* (ed. V. Vogel), Ch. 6, Wiley-VCH, Weinheim.

46 Applegate, K.T., Yang, G. and Danuser, G. (2009) High-content analysis of cytoskeleton functions by fluorescent speckle microscopy, in *Nanomedicine* (ed. V. Vogel), Ch. 7, Wiley-VCH, Weinheim.

47 Fratzl, P., Gupta, H.S., Roschger, P. and Klaushofer, K. (2009) Bone nanostructure and its relevance for mechanical performance, disease and treatment, in *Nanomedicine* (ed. V. Vogel), Ch. 12, Wiley-VCH, Weinheim.

48 Goel, A. and Vogel, V. (2009) Transport, assembly and proof-reading: harnessing the engineering principles of biological nanomotors, in *Nanomedicine* (ed. V. Vogel), Ch. 8, Wiley-VCH, Weinheim.

49 Dunlop, I.E., Dustin, M.L. and Spatz, J.P. (2009) The Micro- and Nanoscale

Architecture of the Immunological Synapse, in *Nanomedicine* (ed. V. Vogel), Ch. 11, Wiley-VCH, Weinheim.

50 Vogel, V. and Sheetz, M.P. (2009) Mechanical Forces Matter in Health and Disease: From Cancer to Tissue Engineering, in *Nanomedicine* (ed. V. Vogel), Ch. 9, Wiley-VCH, Weinheim.

51 Rehfeldt, F., Engler, A.J. and Discher, D.E. (2009) Stem Cells and Nanomedicine: Nanomechanics of the Microenvironment, in *Nanomedicine* (ed. V. Vogel), Ch. 10, Wiley-VCH, Weinheim.

52 Shorokhov, D. and Zewail, A.H. (2008) 4D electron imaging: principles and perspectives. *Physical Chemistry Chemical Physics*, **10** (20), 2879–2893.

53 Sotomayor, M. and Schulten, K. (2007) Single-molecule experiments in vitro and *in silico*. *Science*, **316** (5828), 1144–1148.

54 Khademhosseini, A., Rajalingam, B., Jinno, S. and Langer, R. (2009) Nanoengineered Systems for Tissue Engineering and Regeneration, in *Nanomedicine* (ed. V. Vogel), Ch. 13, Wiley-VCH, Weinheim.

55 Capito, R.M., Mata, A. and Stupp, S.I. (2009) Self-Assembling Peptide-Based Nanostructures for Regenerative Medicine, in *Nanomedicine* (ed. V. Vogel), Ch. 14, Wiley-VCH, Weinheim.

56 Alper, M.D. and Stupp, S.I. (2003) Biomolecular Materials. Basic Energy Sciences Advisory Committee to the Office of Science (DOE) Report http://www.sc.doe.gov/bes/besac/BiomolecularMaterialsReport.pdf.

57 NIH (2008) Roadmap Initiative in Nanomedicine. http://nihroadmap.nih.gov/nanomedicine/index.

58 NIH (2008) US National Cancer Institute (NCI), Alliance for Nanotechnology in Cancer.

59 Sekido, Y., Fong, K.M. and Minna, J.D. (2003) Molecular genetics of lung cancer. *Annual Review of Medicine*, **54**, 73–87.

60 Ishikawa, T., Zhang, S.S., Qin, X., Takahashi, Y., Oda, H., Nakatsuru, Y. and Ide, F. (2004) DNA repair and cancer: lessons from mutant mouse models. *Cancer Science*, **95** (2), 112–117.

61 Sogn, J.A., Anton-Culver, H. and Singer, D.S. (2005) Meeting report: NCI think tanks in cancer biology. *Cancer Research*, **65** (20), 9117–9120.

62 Makrantonaki, E. and Zouboulis, C.C. (2007) Molecular mechanisms of skin aging: state of the art. *Annals of the New York Academy of Sciences*, **1119**, 40–50.

63 Wren, B.G. (2007) The origin of breast cancer. *Menopause (New York, NY)*, **14** (6), 1060–1068.

64 Frey, A.B. and Monu, N. (2008) Signaling defects in anti-tumor T cells. *Immunological Reviews*, **222**, 192–205.

65 Savage, S.A. and Alter, B.P. (2008) The role of telomere biology in bone marrow failure and other disorders. *Mechanisms of Ageing and Development*, **129** (1–2), 35–47.

66 Indraccolo, S., Favaro, E. and Amadori, A. (2006) Dormant tumors awaken by a short-term angiogenic burst: the spike hypothesis. *Cell Cycle (Georgetown, Tex)*, **5** (16), 1751–1755.

67 Townson, J.L. and Chambers, A.F. (2006) Dormancy of solitary metastatic cells. *Cell Cycle (Georgetown, Tex)*, **5** (16), 1744–1750.

68 Vessella, R.L., Pantel, K. and Mohla, S. (2007) Tumor cell dormancy: an NCI workshop report. *Cancer Biology & Therapy*, **6** (9), 1496–1504.

69 Riethdorf, S. and Pantel, K. (2008) Disseminated tumor cells in bone marrow and circulating tumor cells in blood of breast cancer patients: current state of detection and characterization. *Pathobiology*, **75** (2), 140–148.

70 Goodman, S.L., Sims, P.A., Albrecht, R.M. (1996) Three-dimensional extracellular matrix textured biomaterials. *Biomaterials*, **17** (21), 2087–2095.

71 Friedl, P. and Brocker, E.B. (2000) The biology of cell locomotion within three-dimensional extracellular matrix. *Cellular and Molecular Life Sciences*, **57** (1), 41–64.

72 Cukierman, E., Pankov, R., Stevens, D.R. and Yamada, K.M. (2001) Taking cell-matrix adhesions to the third dimension. *Science*, **294** (5547), 1708–1712.

73 Grinnell, F. (2003) Fibroblast biology in three-dimensional collagen matrices. *Trends in Cell Biology*, **13** (5), 264–269.

74 Li, S., Moon, J.J., Miao, H., Jin, G., Chen, B.P., Yuan, S., Hu, Y., Usami, S. and Chien, S. (2003) Signal transduction in matrix contraction and the migration of vascular smooth muscle cells in three-dimensional matrix. *Journal of Vascular Research*, **40** (4), 378–388.

75 Larsen, M., Artym, V.V., Green, J.A. and Yamada, K.M. (2006) The matrix reorganized: extracellular matrix remodeling and integrin signalling. *Current Opinion in Cell Biology*, **18** (5), 463–471.

76 Stryer, L. (2003) Bio2010: Transforming Undergraduate Education for Future Research Biologists. National Research Council Report website.

77 Ratner, B.D. and Bryant, S.J. (2004) Biomaterials: where we have been and where we are going. *Annual Review of Biomedical Engineering*, **6**, 41–75.

78 Berg, H.C. (2003) The rotary motor of bacterial flagella. *Annual Review of Biochemistry*, **72**, 19–54.

79 Weibel, D.B., Diluzio, W.R. and Whitesides, G.M. (2007) Microfabrication meets microbiology. *Nature Reviews Microbiology*, **5** (3), 209–218.

80 Jarrell, K.F. and McBride, M.J. (2008) The surprisingly diverse ways that prokaryotes move. *Nature Reviews Microbiology*, **6** (6), 466–476.

81 Sokurenko, E., Vogel, V. and Thomas, W.E. (2008) Catch bond mechanism of force-enhanced adhesion: counter-intuitive, elusive but . . . widespread? *Cell Host & Microbe, October.*

82 Thomas, W.E., Vogel, V. and Sokurenko, E. (2008) Biophysics of catch bonds. *Annual Review of Biophysics*, **37**, 399–416.

83 Biais, N., Ladoux, B., Higashi, D., So, M. and Sheetz, M. (2008) Cooperative retraction of bundled type IV pili enables nanonewton force generation. *PLoS Biology*, **6** (4), e87.

84 Fletcher, D.A. and Theriot, J.A. (2004) An introduction to cell motility for the physical scientist. *Physical Biology*, **1** (1–2), T1–T10.

85 Brandenburg, B. and Zhuang, X. (2007) Virus trafficking – learning from single-virus tracking. *Nature Reviews Microbiology*, **5** (3), 197–208.

86 Balagadde, F.K., Song, H., Ozaki, J., Collins, C.H., Barnet, M., Arnold, F.H., Quake, S.R. and You, L. (2008) A synthetic *Escherichia coli* predator-prey ecosystem. *Molecular Systems Biology*, **4**, 187.

87 Welch, M., Mikkelsen, H., Swatton, J.E., Smith, D., Thomas, G.L., Glansdorp, F.G. and Spring, D.R. (2005) Cell-cell communication in Gram-negative bacteria. *Molecular BioSystems*, **1** (3), 196–202.

88 Xie, X.S., Choi, P.J., Li, G.W., Lee, N.K. and Lia, G. (2008) Single-molecule approach to molecular biology in living bacterial cells. *Annual Review of Biophysics*, **37**, 417–444.

89 Johansson, M., Lovmar, M. and Ehrenberg, M. (2008) Rate and accuracy of bacterial protein synthesis revisited. *Current Opinion in Microbiology*, **11** (2), 141–147.

90 Zorrilla, S., Lillo, M.P., Chaix, D., Margeat, E., Royer, C.A. and Declerck, N. (2008) Investigating transcriptional regulation by fluorescence spectroscopy, from traditional methods to state-of-the-art single-molecule approaches. *Annals of the New York Academy of Sciences*, **1130**, 44–51.

91 Forero, M., Thomas, W., Bland, C., Nilsson, L., Sokurenko, E. and Vogel, V. (2004) A catch-bond based nano-adhesive sensitive to shear stress. *Nano Letters*, **4**, 1593–1597.

92 Sleytr, U.B., Huber, C., Ilk, N., Pum, D., Schuster, B. and Egelseer, E.M. (2007) S-layers as a tool kit for nanobiotechnological applications. *FEMS Microbiology Letters*, **267** (2), 131–144.

93 Chevance, F.F. and Hughes, K.T. (2008) Coordinating assembly of a bacterial macromolecular machine. *Nature Reviews Microbiology*, **6** (6), 455–465.

94 Diao, J.J., Hua, D., Lin, J., Teng, H.H. and Chen, D. (2005) Nanoparticle delivery by controlled bacteria. *Journal of Nanoscience and Nanotechnology*, **5** (10), 1749–1751.

95 Darnton, N., Turner, L., Breuer, K. and Berg, H.C. (2004) Moving fluid with bacterial carpets. *Biophysical Journal*, **86** (3), 1863–1870.

96 Hiratsuka, Y., Miyata, M., Tada, T. and Uyeda, T.Q. (2006) A microrotary motor powered by bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, **103** (37), 13618–13623.

97 Turner, S.T., Schwartz, G.L. and Boerwinkle, E. (2007) Personalized medicine for high blood pressure. *Hypertension*, **50** (1), 1–5.

98 Katsanis, S.H., Javitt, G. and Hudson, K. (2008) Public health. A case study of personalized medicine. *Science*, **320** (5872), 53–54.

99 Zhong, J.F., Chen, Y., Marcus, J.S., Scherer, A., Quake, S.R., Taylor, C.R. and Weiner, L.P. (2008) A microfluidic processor for gene expression profiling of single human embryonic stem cells. *Lab on a Chip*, **8** (1), 68–74.

100 van't Veer, L.J. and Bernards, R. (2008) Enabling personalized cancer medicine through analysis of gene-expression patterns. *Nature*, **452** (7187), 564–570.

101 Toner, M. and Irimia, D. (2005) Blood-on-a-chip. *Annual Review of Biomedical Engineering*, **7**, 77–103.

102 Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M.R. and Weigl, B.H. (2006) Microfluidic diagnostic technologies for global public health. *Nature*, **442** (7101), 412–418.

103 Park, J.Y. and Kricka, L.J. (2007) Prospects for nano- and microtechnologies in clinical point-of-care testing. *Lab on a Chip*, **7** (5), 547–549.

104 Phillips, K.A., Liang, S.Y. and Van Bebber, S. (2008) Challenges to the translation of genomic information into clinical practice and health policy: Utilization, preferences and economic value. *Current Opinion in Molecular Therapeutics*, **10** (3), 260–266.