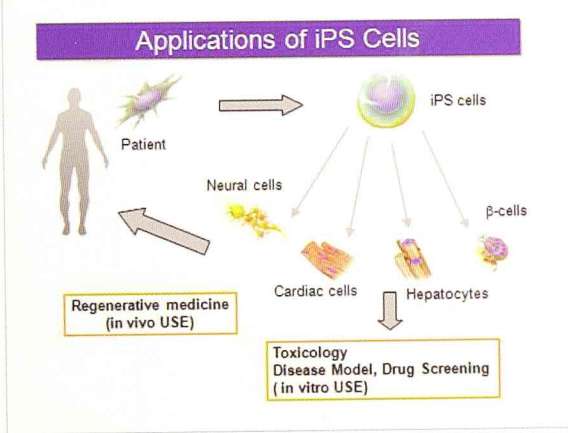
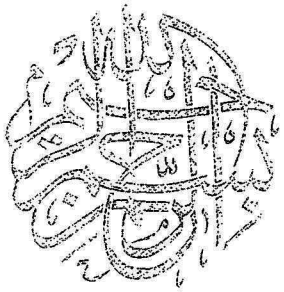
جائزة الملك فيصل العالمية
King Faisal International Prize



THE KING FAISAL MEMORIAL ARTICLES IN MEDICINE AND SCIENCE XI

THE 2011 KING FAISAL
INTERNATIONAL PRIZE



Custodian of the Two Holy Mosques

King Abd Allah Ibn Abdul Aziz Al-Saud

Patron of the King Faisal Foundation

HRH Prince Sultan Ibn Abd Al-Aziz

Crown Prince, Deputy Premier
Minister of Defense and Aviation and
Inspector General

HRH Prince Nayef Ibn Abd Al-Aziz Al-Saud

Second Deputy Premier and

Minister of Interior

Contents

	Page No.
Introduction	11
2011 King Faisal International Prize Award in Medicine	13
The Derivation of Human Embryonic and Human Induced Pluripotent Stem Cell Lines By James Thomos	15
The Discovery of Induced Pluripotent Stem (iPS) Cells By Shinya Yamanaka	25
2011 King Faisal International Prize Award in Science	39
The Science of "In-between" Sizes By George Whitesides	41
My Life with LIF: A Personal Account of Developing Laser-Induced Fluorescence By Richard Zare	49

INTRODUCTION

The King Faisal Foundation continues the traditions of Arabic and Islamic philanthropy, as they were revitalized in modern times by King Faisal. The life and work of the late King Faisal bin Abd Al-Aziz, son of Saudi Arabia's founder and the Kingdom's third monarch, were commemorated by his eight sons through the establishment of the Foundation in 1976, the year following his death. Of the many philanthropic activities of the Foundation, the inception of King Faisal International Prizes for Medicine in 1981 and for Science in 1982 will be of particular interest to the reader of this book. These prizes were modeled on prizes for Service to Islam, Islamic Studies and Arabic Literature which were established in 1977. At present, the Prize in each of the five categories consists of a certificate summarizing the laureate's work that is hand-written in Diwani calligraphy; a commemorative 24-carat, 200 gram gold medal, uniquely cast for each Prize and bearing the likeness of the late King Faisal; and a cash endowment of SR750,000 (US$200,000). Co-winners in any category share the monetary award. The Prizes are awarded during a ceremony in Riyadh, Saudi Arabia, under the auspices of the Custodian of the Two Holy Mosques, the King of Saudi Arabia.

Nominations for the Prizes are accepted from academic institutions, research centers, professional organizations and other learned circles worldwide, as well as from previous laureates. After preselection by expert reviewers, the short-listed works are submitted for further, detailed evaluation by carefully selected international referees. Autonomous, international specialist selection committees are then convened at the headquarters of the King Faisal Foundation in Riyadh each year in January to make the final decisions. The selections are based solely on merit, earning the King Faisal International Prize the distinction of being among the most prestigious of international awards to physicians and scientists who have made exceptionally outstanding advances which benefit all of humanity.

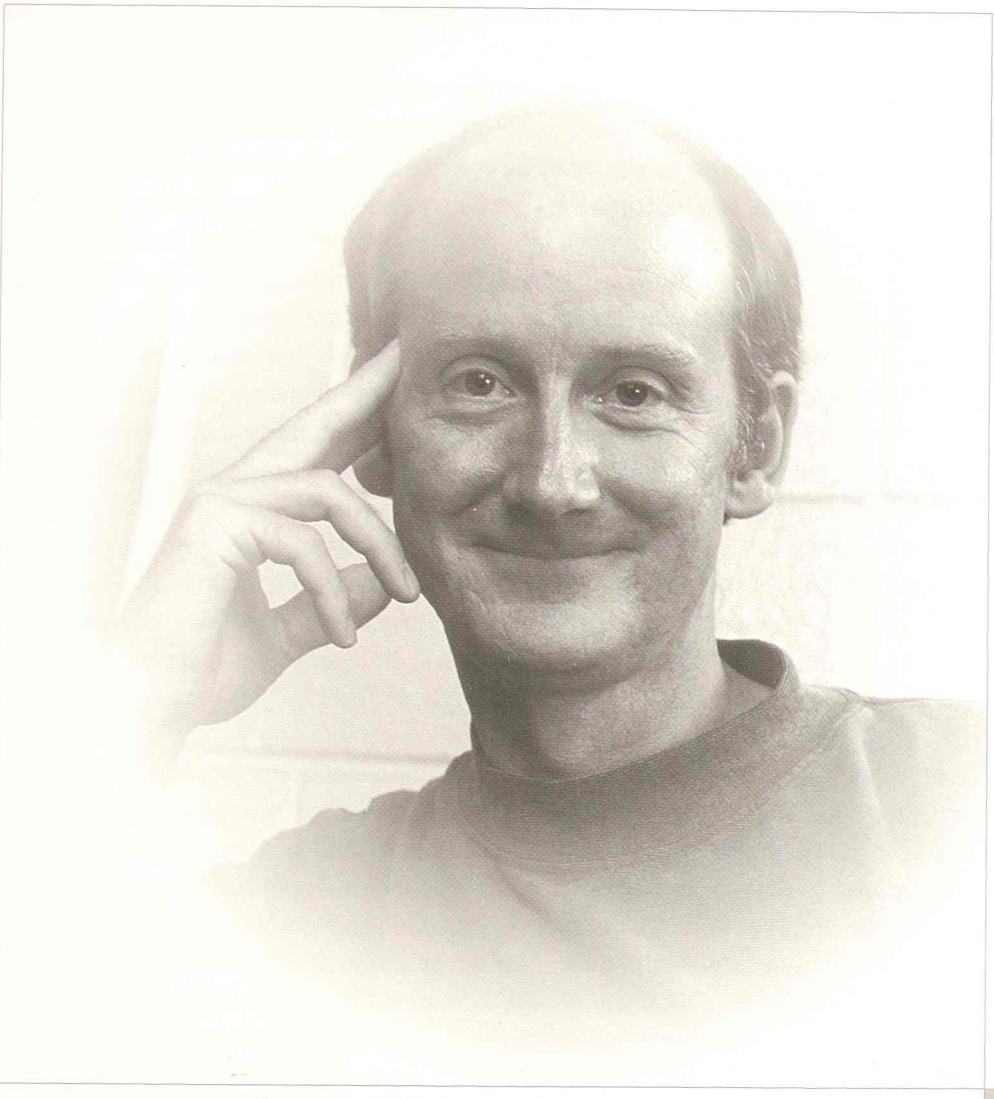
(Excerpt from Introduction to 'Articles in Medicine and Science 1"
by H.R.H. Khaled Al Faisal, Chairman of the Prize Board and Director General of King Faisal Foundation).

WINNERS OF THE 2011 KING FAISAL INTERNATIONAL PRIZE FOR MEDICINE

The King Faisal International Prize for Medicine (Stem Cell Therapy) for the year 1432H - 2011G has been awarded jointly to: Professor James A. Thomson (USA) and **Professor Shinya Yamanaka** (Japan) for their pioneering and seminal stem cell research.

Professor James Thomson is the Director of Regenerative Biology at Morgridge Institute for Research and Professor at the University of Wisconsin. Having succeeded in isolating stem cells from non-human primates in 1995 and from human embryos in 1998, he made – in 2007 – the groundbreaking discovery that induced pluripotent stem cells (iPS) could be generated from human somatic cells. This important breakthrough has revitalized interest in stem cell biology, with many laboratories re-investigating the possible use of these cells in the modeling and treatment of human diseases.

Professor Shinya Yamanaka is Senior Investigator in stem cell biology of the Gladstone Institute of Cardiovascular Diseases, Director of the Center for Induced Pluripotent Stem Cell Research and Application (CiRA) and professor at Kyoto University, Japan and the University of California in San Francisco, USA. Professor Yamanaka achieved a major breakthrough in 2006 when he was able to generate induced pluripotent stem cells from mouse adult fibroblasts. In 2007, he succeeded, independently of Professor Thomson, in reprogramming human adult skin cells into iPS cells.



The Derivation of Human Embryonic and Human Induced Pluripotent Stem Cell Lines

James Thomson[1]

Human embryonic (ES) stem cells and induced pluripotent stem (iPS) cells capture the imagination; after proliferating for months or even for years in the laboratory, they maintain the ability to form any cell type that makes up the body (1-3). These remarkable cells thus give scientists and physicians unprecedented access to all the basic building blocks of the human body. The proliferative and developmental potential of human ES and iPS cells promises an unlimited supply of differentiated cells for basic research, for drug discovery, and for transplantation therapies for diseases ranging from blindness to heart disease to leukemia (4).

Human embryonic stem (ES) cells and human induced pluripotent stem (iPS) cells both trace their origins to cancer research initiated in the 1950s conducted on a then obscure tumor, the teratocarcinoma. These germ cell-derived tumors are composed of undifferentiated embryonal carcinoma (EC) stem cells and differentiated derivatives that can include a variety of tissue types. Stevens's discovery that a strain of 129 mice had a high incidence of testicular teratocarcinomas made these tumors routinely amenable to experimental analysis for the first time (5). Tissue culture conditions allowing the propagation of EC cells in vitro were then developed in the early 1970s (6, 7). Research showed that EC cells exhibit antigen and protein expression similar to the cells present in the inner cell mass (ICM) of the early preimplantation embryo (8-14), thus suggesting that EC cells are the malignant counterpart of the pluripotent cells present in the ICM (15, 16). Some EC cell lines are able to contribute to various differentiated cell types (that is, to a variety of cells forming the body) in chimeras with normal embryos (17-20), but most EC cell lines have limited developmental potential and contribute poorly to chimeric mice, probably reflecting genetic changes acquired during teratocarcinoma formation (21-24). In 1964, Kleinsmith and Pierce demonstrated that a single EC cell was capable of both self-renewal and multilineage differentiation, a demonstration that provided the intellectual foundation for all subsequent pluripotent stem cell work and which preceded the recent interest in cancer stem cells by several decades.

Early mouse embryos, when transferred to other non-uterine sites, can develop into teratocarcinomas that include pluripotent stem cells (25, 26). In fact, these transplantation experiments result in teratocarcinomas at high frequencies, even in strains that do not spontaneously have elevated incidence of germ cell tumors. These results naturally led to the search for culture conditions that would allow the in vitro derivation of pluripotent stem cells directly from the embryo, without the intermediate need to form teratocarcinomas in vivo. As a result, in 1981, two groups independently described the derivation of pluripotent embryonic stem (ES) cell lines directly from the ICM of mouse blastocysts (27, 28). ES

[1]Director of Regenerative Biology at Morgridge Institute for Research and Professor at the Department of Anatomy in the University of Wisconsin

cell cultures derived from a single cell could differentiate into a wide variety of cell types, or could form teratocarcinomas when injected into mice (28). These normal cells could also contribute to chimeras at a higher frequency than EC cells, providing a practical way to introduce modifications to the mouse germ line, and thus revolutionizing the study of gene function in mammals (*29*).

In 1978 the first baby was born from an embryo fertilized in vitro and, without this event, the derivation of human ES cells would not have been possible (30). Although there were attempts to derive human ES cells as early as the 1980s, species-specific differences and suboptimal human embryo culture media delayed their successful isolation. Indeed, by the early 1990's, numerous attempts to derive ES cells from a variety of non-rodent species had all failed. At that time it was not yet clear whether the ability to derive ES cells reflected some unique biology of rodents or whether it would ever be possible to derive such cell lines from other species. In the mid 1990s, our group derived ES cell lines from two non-human primates, the rhesus monkey and the common marmoset (31, 32), establishing for the first time that ES cell lines could indeed be derived from non-rodent species. The prior experience gained with primate ES cell culture allowed my group to derive the first human ES cells in 1998 (1). These human ES cells had normal karyotypes and, even after prolonged undifferentiated proliferation, maintained the developmental potential to contribute to advanced derivatives of all three germ layers. We found that primate ES, human ES, and human EC cells resemble each other closely, but differed substantially in morphology, gene expression profiles, and growth factor dependence from mouse ES cells. Indeed, it is currently thought that mouse ES and human ES cells actually resemble different pluripotent cells of the early embryo, with human ES cell resembling a slightly later cell type, the primitive ectoderm, which explains why entirely different growth factors are needed to grow mouse and human ES cells.

In contrast to mouse ES cells, fibroblast growth factor (FGF) signaling has a central importance in the self-renewal of human ES cells. We found that basic FGF (bFGF) allows the clonal growth of human ES cells on fibroblasts in the presence of a commercially available serum replacement (33). At higher concentrations, bFGF allows feeder independent growth of human ES cells cultured in the same serum replacement (34-36). The mechanism through which these high concentrations of bFGF exert their functions is incompletely known, although one of the bFGF's roles is suppression of BMP signaling (36). Serum and the serum replacement most widely used for human ES cell culture (33) have significant BMP-like activity, which is sufficient to induce differentiation of human ES cells, and conditioning this medium on fibroblasts

reduces this activity (36). At moderate concentrations of bFGF (40 ng/ml), the addition of noggin or other inhibitors of BMP signaling significantly decreases background differentiation of human ES cells. At higher concentrations (100 ng/ml), bFGF itself suppresses BMP signaling in human ES cells to levels comparable to those observed in fibroblast-conditioned medium, and the addition of noggin is no longer needed for feeder independent growth (36, 37). Suppression of BMP activity by itself is insufficient to maintain human ES cells (36), thus bFGF must be serving additional signaling functions. Although FGF signaling appears to have a central role in the self-renewal of human ES cells, other pathways have also been implicated. When combined with low to moderate levels of FGFs, TGFb/Activin/Nodal signaling has a positive effect on the undifferentiated proliferation of human ES cells (38-41), and inhibition of this pathway leads to differentiation (40, 41). We have recently described completely defined media containing both FGF and TGFb that support both the culture and derivation of human ES cells without feeder layers (42, 43). In this culture media, removal of FGF has a strong effect on both cell numbers and the percentage of undifferentiated cells sustained in the culture; removal of TGFb has only a very mild effect that is observable after several passages. Note that these human ES cell culture conditions cause the rapid differentiation of mouse ES cells, and that defined culture conditions used for mouse ES cell culture cause the rapid differentiation of human ES cells.

The cloning of Dolly in 1997 (*44*) preceded the derivation of human ES cells by only a year, and the two events became very much linked in the public's imagination. The proposed connection between Dolly and human ES cells was that somatic cell nuclear transfer (SCNT) would provide a method for making ES cell lines genetically matched to a specific patient, and thus avoid immune rejection. However, the limited availability of human oocytes, the inefficiencies of somatic cell nuclear transfer in mammals, and the resulting unfavorable economics made it inherently unlikely that this approach would ever enjoy widespread therapeutic use, even without the ethical controversy surrounding the procedure. The enduring message of Dolly is not, however, that sheep or people could be cloned, or that ES cell lines could be generated by SCNT, but that the differentiated state is fully reversible in mammals. It is no coincidence that a few years after the cloning of Dolly there was a proliferation of reports that suggested adult cells were far more plastic than previously imagined. Although the extent of adult cell plasticity remains controversial, and some of the early results remain dubious at best, some of the "plasticity" initially reported was clearly due to cell fusion, an event that mixes the cytoplasm between two

cells. Thus, both Dolly and cell fusion experiments suggested that there were unidentified cytoplasmic transacting factors that could reprogram cells to a different cellular state.

Both Dolly and the cell fusion studies led to a search for trans-acting factors that could accomplish reprogramming in the absence of somatic cell nuclear transfer. We had previously shown that human ES cells can reprogram myeloid precursors through cell fusion (45). Therefore, to identify candidate reprogramming factors, we used lentiviral transduction (46) to screen combinations of genes with enriched expression in human ES cells relative to myeloid precursors. Using homologous recombination, we generated an OCT4 knock-in cell line in which EGFP and neomycin phosphotransferase expression reflect an active endogenous Oct4 promoter, a gene specific to pluripotent cells (47). Using this cell line to score for reprogramming events, we identified an initial pool of 14 genes that directed reprogramming of adherent cells obtained from human ES cell-derived CD45+ hematopoietic cells (45, 48, 49) to pluripotent cells exhibiting the basic properties of ES cells. In early June 2006, our lab conducted the first successful transfection of these 14 genes resulting in human iPS cells. As we began to sort through these intial genes to determine which was essential, Dr. Yamanaka's group published in August 2006 the first successful combinatorial screen for genes capable of reprogramming mouse fibroblast to a pluripotent state (50). His group found that four transcription factors (Oct4, Sox2, c-myc, and Klf4) were sufficient to reprogram mouse fibroblasts to cells termed induced pluripotent stem (iPS), cells that very closely resemble embryonic stem (ES) cells (50-54). Because of the much longer cell cycle time of human cells, our combinatorial screen took much longer to complete. However, since Dr. Yamanaka's results were similarly delayed, in his case because of technical difficulties in applying his mouse reprogramming results to human cells (3), his human iPS cell results and ours were ultimately published simultaneously. We identified OCT4, SOX2, NANOG, and LIN28 as sufficient to reprogram human cells (55).

It is important to mention that the successful derivation of iPS cells depended critically on the previous decade of human ES cell research, as the conditions developed for human ES cell culture (e.g., high bFGF conditions) and approaches for genetic manipulation of human ES cells (e.g., lentiviral transduction and homologous recombination) were essential to the successful isolation of human iPS cells. For example, in Dr. Yamanaka's work, mouse ES cell culture conditions failed to support the isolation of human iPS cells, and it was only when he used human ES cell culture conditions (33) that he succeeded

in isolating human iPS cells. In our case, our genetic screen for reprogramming factors was carried out entirely on human ES cell derivatives in human ES cell culture conditions (2). Although human iPS cells meet all the defining criteria originally proposed for human ES cells (without being derived from embryos (*1*)), there is increasing evidence suggesting that ES and iPS cells differ in subtle ways (*56*). These differences include epigenetic "memories" of tissue of origin, iPS cell-specific DNA methylation patterns, and variability in developmental potential, though it is not yet clear how clinically relevant these differences are. Therefore, one of the ongoing goals of human ES cell research is to provide a "gold standard" with which iPS cells can be compared, both to better understand the limitiations of iPS cells and to improve them. In brief, though the derivation of iPS cells has the remarkable potential to finally resolve the controversy surrounding human ES cells, the need for human ES cell research is not yet over.

Caption: Microscopic view of a colony of original human embryonic stem cell lines from James Thomson's lab. These cells, which arise at the earliest stages of development, are blank slate cells capable of differentiating into any of the 220 types of cells or tissues in the human body. They can provide access to tissue and

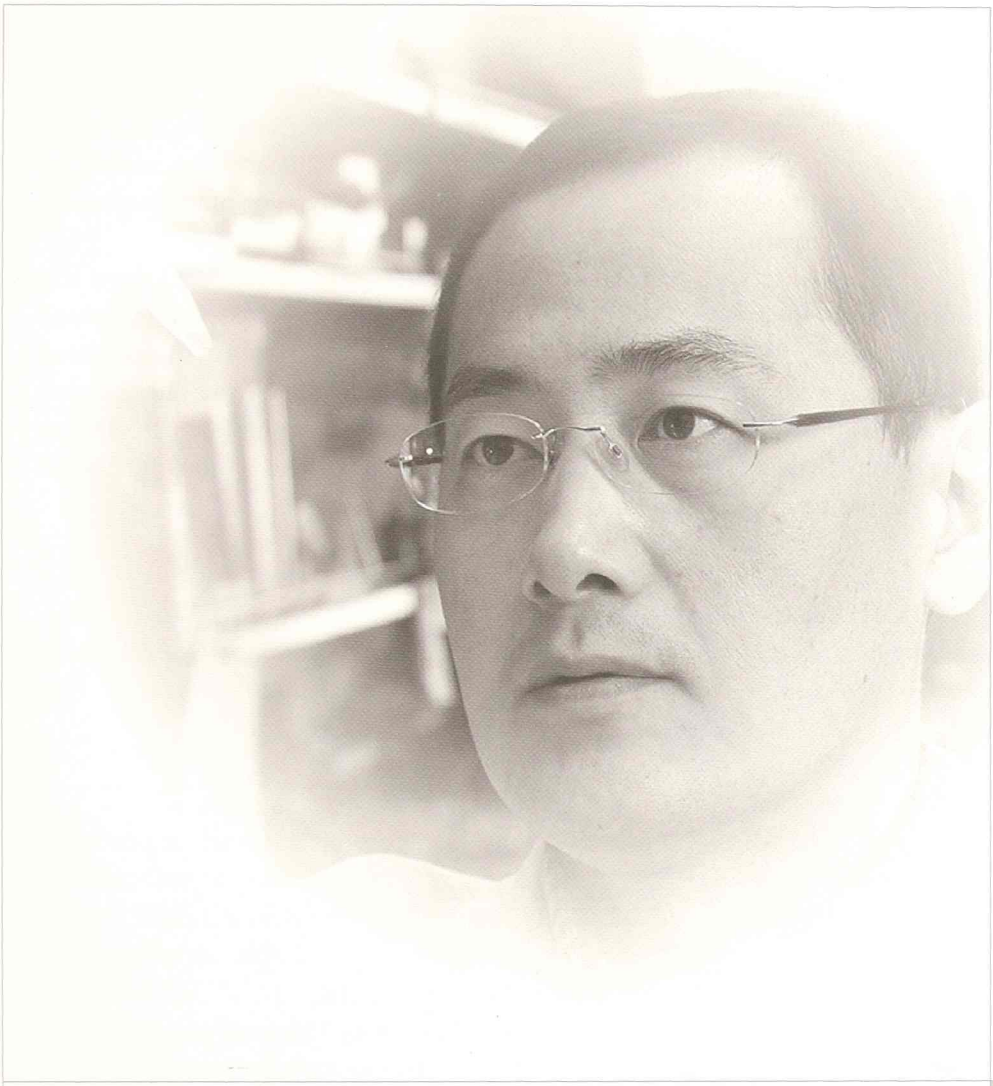
cells for basic research and potential therapies for many types of disease. Thomson directed the research group that reported the first isolation of embryonic stem cell lines from a nonhuman primate in 1995, work that led his group to the first successful isolation of human embryonic stem cell lines in 1998.
Photo by: Jeff Miller & UW-Madison University Communications
Date: 2005

References

1. J. A. Thomson *et al.*, *Science* **282**, 1145 (Nov 6, 1998).
2. J. Yu, Vodyanik, M, Smuga-Otto, K, Antosiewicz-Bourget, J, Frane J, Tian, S, Nie, J, Jonsdottir, GA, Ruotti, V, Stewart, R, Slukvin, II, Thomson, JA. , *Science* **21**, 1917 (2007).
3. K. Takahashi *et al.*, *Cell*, (Nov 20, 2007).
4. J. S. Odorico, D. S. Kaufman, J. A. Thomson, *Stem Cells* **19**, 193 (2001).
5. L. C. Stevens, C. C. Little, *Proc. Natl. Acad.Sci. USA* **40**, 1080 (1954).
6. B. W. Kahan, B. Ephrussi, *J Natl Cancer Inst* **44**, 1015 (May, 1970).
7. M. J. Evans, *J Embryol Exp Morphol* **28**, 163 (Aug, 1972).
8. P. G. Calarco, C. L. Banka, *Biol Reprod* **20**, 699 (May, 1979).
9. D. Solter, B. B. Knowles, *Proc Natl Acad Sci U S A* **75**, 5565 (Nov, 1978).
10. G. Gachelin, R. Kemler, F. Kelly, F. Jacob, *Dev Biol* **57**, 199 (May, 1977).
11. P. M. Comoglio, M. Bertini, G. Forni, *Immunology* **29**, 353 (Aug, 1975).
12. J. V. Klavins, R. Mesa-Tejada, M. Weiss, *Nat New Biol* **234**, 153 (Dec 1, 1971).
13. C. C. Howe, R. Gmur, D. Solter, *Dev Biol* **74**, 351 (Feb, 1980).
14. J. K. Henderson *et al.*, *Stem Cells* **20**, 329 (2002).
15. G. R. Martin, *Science* **209**, 768 (Aug 15, 1980).
16. J. Rossant, V. E. Papaioannou, *Cell Differ* **15**, 155 (Dec, 1984).
17. K. Illmensee, B. Mintz, *Proc Natl Acad Sci U S A* **73**, 549 (Feb, 1976).
18. V. E. Papaioannou, M. W. McBurney, R. L. Gardner, M. J. Evans, *Nature* **258**, 70 (Nov 6, 1975).
19. R. L. Brinster, *J Exp Med* **140**, 1049 (Oct 1, 1974).
20. B. Mintz, K. Illmensee, *Proc Natl Acad Sci U S A* **72**, 3585 (Sep, 1975).
21. D. L. Bronson *et al.*, *Cancer Res* **40**, 2500 (Jul, 1980).
22. M. W. McBurney, *J Cell Physiol* **89**, 441 (Nov, 1976).
23. N. B. Atkin, M. C. Baker, R. Robinson, S. E. Gaze, *Eur J Cancer* **10**,

144 (Mar, 1974).
24. J. Zeuthen *et al.*, *Int J Cancer* **25**, 19 (Jan 15, 1980).
25. L. C. Stevens, *Dev Biol* **21**, 364 (Mar, 1970).
26. D. Solter, N. Skreb, I. Damjanov, *Nature* **227**, 503 (Aug 1, 1970).
27. M. J. Evans, M. H. Kaufman, *Nature* **292**, 154 (Jul 9, 1981).
28. G. R. Martin, *Proc Natl Acad Sci U S A* **78**, 7634 (Dec, 1981).
29. A. Bradley, M. Evans, M. H. Kaufman, E. Robertson, *Nature* **309**, 255 (May 17-23, 1984).
30. P. C. Steptoe, R. G. Edwards, *Lancet* **2**, 366 (Aug 12, 1978).
31. J. A. Thomson *et al.*, *Proc Natl Acad Sci U S A* **92**, 7844 (Aug 15, 1995).
32. J. A. Thomson *et al.*, *Biol Reprod* **55**, 254 (Aug, 1996).
33. M. Amit *et al.*, *Dev Biol* **227**, 271 (Nov 15, 2000).
34. L. Wang, L. Li, P. Menendez, C. Cerdan, M. Bhatia, *Blood* **105**, 4598 (Jun 15, 2005).
35. C. Xu *et al.*, *Stem Cells* **23**, 315 (Mar, 2005).
36. R. H. Xu *et al.*, *Nat Methods* **2**, 185 (Mar, 2005).
37. M. E. Levenstein *et al.*, *Stem Cells* **24**, 568 (Mar, 2006).
38. M. Amit, C. Shariki, V. Margulets, J. Itskovitz-Eldor, *Biol Reprod* **70**, 837 (Mar, 2004).
39. G. M. Beattie *et al.*, *Stem Cells* **23**, 489 (Apr, 2005).
40. L. Vallier, M. Alexander, R. A. Pedersen, *J Cell Sci* **118**, 4495 (Oct 1, 2005).
41. D. James, A. J. Levine, D. Besser, A. Hemmati-Brivanlou, *Development* **132**, 1273 (Mar, 2005).
42. T. E. Ludwig *et al.*, *Nat Methods* **3**, 637 (Aug, 2006).
43. T. E. Ludwig *et al.*, *Nat Biotechnol* **24**, 185 (Feb, 2006).
44. I. Wilmut, A. E. Schnieke, J. McWhir, A. J. Kind, K. H. Campbell, Nature 385, 810 (Feb 27, 1997).
45. J. Yu, M. A. Vodyanik, P. He, Slukvin, II, J. A. Thomson, Stem Cells 24, 168 (Jan, 2006).
46. Y. Ma, A. Ramezani, R. Lewis, R. G. Hawley, J. A. Thomson, Stem Cells 21, 111 (2003).
47. T. P. Zwaka, J. A. Thomson, Nat Biotechnol 21, 319 (Mar, 2003).
48. M. A. Vodyanik, J. A. Bork, J. A. Thomson, Slukvin, II, Blood 105, 617 (Jan 15, 2005).
49. M. A. Vodyanik, J. A. Thomson, Slukvin, II, Blood 108, 2095 (Sep 15, 2006).
50. K. Takahashi, S. Yamanaka, Cell 126, 663 (Aug 25, 2006).
51. K. Okita, T. Ichisaka, S. Yamanaka, Nature, (Jun 6, 2007).
52. A. Meissner, M. Wernig, R. Jaenisch, Nat Biotechnol, (Aug 27, 2007).

53. M. Wernig et al., Nature, (Jun 6, 2007).
54. N. Maherali et al., Cell Stem Cell, 55 (Jul, 2007).
55. J. Yu et al., Science, (in press).
56. R. Lister, Pelizzola, M, Kida, YS, Hawkins, RD, Nery, JR, Hon, G, Antosiewicz-Bourget, J, O'Malley, R, Castanon, R, Klugman, S, Downes, M, Yu, R, Stewart, R, Ren, B, Thomson, JA, Evans, RM, Ecker, JR., Nature, (2011).



The Discovery of Induced Pluripotent Stem (iPS) Cells

Shinya Yamanaka[2]

When I started working on cellular reprogramming about a decade ago, many research teams around the world were trying to differentiate embryonic stem (ES) cells into various functional cells. In contrast, as a young, ambitious scientist, I set my laboratory's goal of generating new type of pluripotent stem cells from differentiated cells such as skin cells.

ES cells are generated by isolating embryos from fertilized eggs and culturing them for a few weeks. This new type of stem cells was first generated in mice in 1981[1,2]. ES cells exhibit two definitive properties. These cells have abilities to grow robustly and to differentiate into any type of the lineages that give rise to the cells of the adult body, which is referred to as pluripotency. Having pluripotency means that ES cells can be differentiated into various functional cells, such as neurons and cardiac muscle cells.

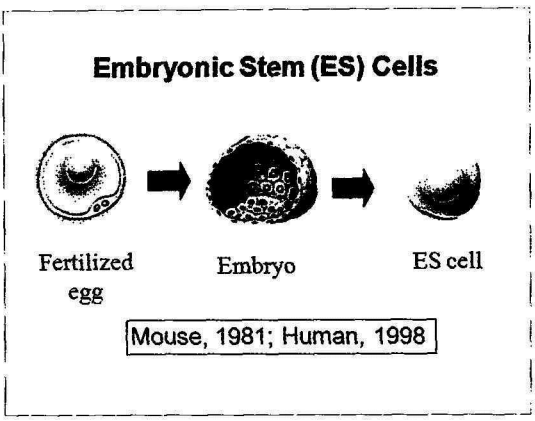


Figure 1. Embryonic Stem Cells.
ES cells are generated by removing cells from a 6 to 7-day-old embryo and growing them in culture. Mouse ES cells were isolated in 1981 and human ES cells in 1998.

When I was working as a postdoctoral fellow at the Gladstone Institute of Cardiovascular Disease in San Francisco, the U.S., from 1993 to 1996, I discovered a gene called *NAT1*[3]. Studying the functions of this gene, I found that *NAT1* is essential to maintain pluripotency in ES cells[4]. This made me interested in ES cell research. In 1998, Professor James Thomson of the University of Wisconsin in the U.S. announced that his research team had successfully generated human ES cells[5]. This achievement was heralded as opening up the possibility of cell transplantation therapies to treat otherwise intractable diseases, encouraging me to continue studying ES cells.

[2]Center for iPS Cell Research and Application (CiRA)
Kyoto University
Kyoto, Japan

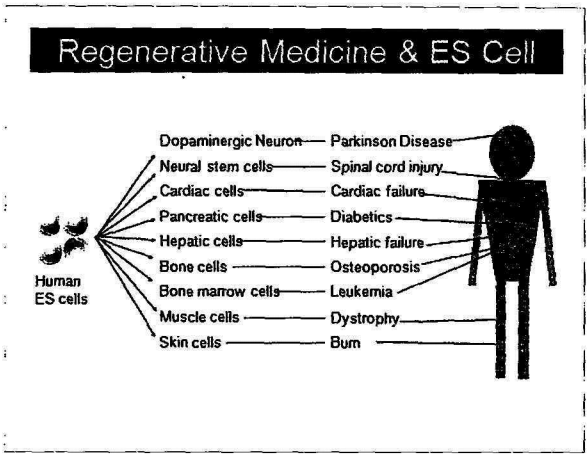


Figure 2. Medical Applications of
ES Cells. ES cells can be derived into any type of differentiated cells in a body. ES cell-derived functional cells may be used for regenerative medicine such as cell transplantation to cure various diseases.

ES cells have enormous potential for medical and pharmaceutical applications. However, the clinical application of ES cells faces two major hurdles. One is that immune rejection is likely to occur after functional cells derived from ES cells are transplanted. The second is due to the ethical issues surrounding the use of human embryos to generate ES cells.

A challenge to generate a new type of pluripotent cell

To bypass such issues, I thought that generating ES-like cells from human somatic cells by direct reprogramming technology would be an optimum solution, and my laboratory started working on a research with the final goal of establishing a new type of pluripotent stem cell from differentiated cells, in a sense turning back the clock on somatic cells to restore them to pluripotency.

The concept of cellular reprogramming was in fact established many years ago. In 1962, Sir John Gurdon reported the generation of frog offspring by transferring tadpole intestinal cell nuclei into enucleated eggs from the African clawed frog, *Xenopus laevis*[6]. Dolly the sheep, reported in 1997[7], was another example, as was a 2001 report showing that thymocytes acquire pluripotency upon electrofusion with mouse ES cells[8]. These experiments clearly showed that eggs and ES cells contain pluripotency-inducing factors in somatic cells.

My first hypothesis was that those factors that maintain pluripotency in mouse ES cells might be used to induce pluripotency in somatic cells. During the early stage of the research, we comprehensively investigated factors that play important roles in the maintenance of ES cell identity – genes that are expressed specifically in mouse ES cells[9-15] by taking advantage of Expressed Sequence Tag (EST) data bases, including a mouse cDNA library. We had identified 24 such factors by 2004 and tried to narrow down which among them was capable of reprogramming.

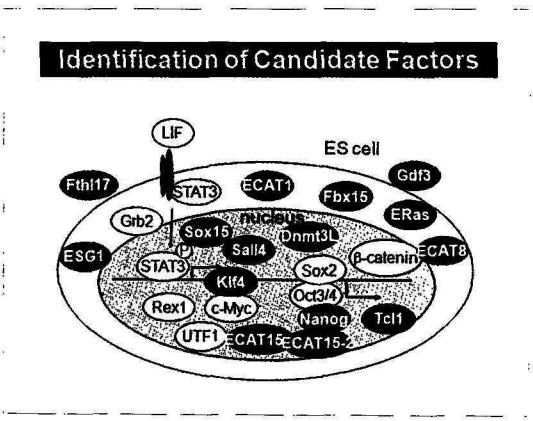


Figure 3. Identifying pluripotency-inducing Factors.
Genes that are expressed in mouse ES cells were candidate factors to reprogram somatic cells into a pluripotent state.

We had observed that when all 24 factors were introduced together into somatic cells, typical ES-like cell colonies appeared. To determine which of the 24 candidates are critical, we repeated the experiment by removing each factor from the 24-factor combination in a process of elimination. When the factor removed was essential to the generation of ES-like cells, we saw no ES-like cells appearing. Finally, we found four transcription factors indispensable for inducing pluripotent cells.

The discovery of Induced Pluripotent Stem Cells

In 2006, we reported that embryonic-like stem cells could be induced by introducing the four factors – *Oct3/4, Sox2, Klf4*, and *c-Myc* – into mouse fibroblasts, or skin cells, via retroviral vectors[16]. These ES-like cells showed

rapid proliferation and differentiated into various somatic cell lineages. The cells also expressed several ES cell marker genes, such as *Oct3/4*, *ERas*, and *Esg1*. When injected subcutaneously into nude mice, they differentiated into various cell types characteristic of all three germ layers, such as gut-like epithelium, cartilage, skeletal muscle, and neural tissue, demonstrating their pluripotency. We named the new cells "induced pluripotent stem cells," or iPS cells.

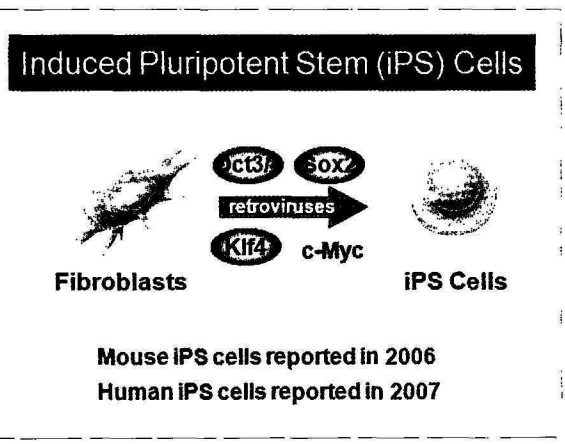


Figure 4. Generation of Induced pluripotent stem cells.
iPS cells were generated by introducing 4 genes – Oct34/, Sox2, Klf4 and c-Myc - into somatic cells via retroviral vectors. Later we found that iPS cells can be generated with 3 factors, excluding c-Myc.

As our final goal is to make the cells usable in the clinic, we worked on development of protocols for inducing pluripotency in human cells. In 2007, we reported the generation of human iPS cells[17], simultaneously with an independent report by Dr. Thomson's group[18]. These first reports were followed in close succession by reports from other labs as well[19].

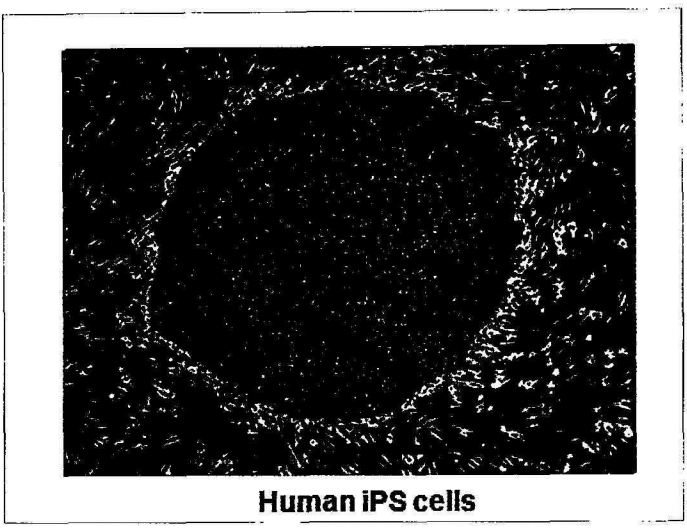


Figure 5. Human iPS cell colony.
An iPS cell colony induced from human fibroblasts was generated on feeder cells, and each colony is comprised of hundreds of iPS cells.

iPS cells can be generated in a relatively easily reproducible manner. First, physicians conduct biopsies to obtain tiny amounts of skin cells, or fibroblasts. The fibroblasts are cultured in a petri dish for two to three weeks, and then the four genes are introduced into the fibroblasts. After culturing the cells for a few weeks, iPS cell colonies emerge. Each iPS cell colony comprises some several hundred iPS cells. iPS cells grow rapidly and, like ES cells, can be differentiated into any functional cell type in the body. iPS cell- derived cardiac muscle cells, for example, show the same synchronized pulsing as beating heart muscle in the body.

The Potential of iPS Cells

The potential of iPS cell technology is tremendous. As iPS cells can in principle be steered to differentiate into any kind of cell, the prospect of patient-specific iPS cells has raised great hope for future medical applications, such as understanding pathogenesis, drug screening, and toxicology, as well as the development of regenerative medical approaches, such as cell transplantation. In iPS-based cell therapies, various types of somatic cells derived from pluripotent stem cells may one day be used to repair tissues damaged through disease or injury. Years of research and rigorously-designed clinical studies will be required

to determine whether these applications are safe and effective.

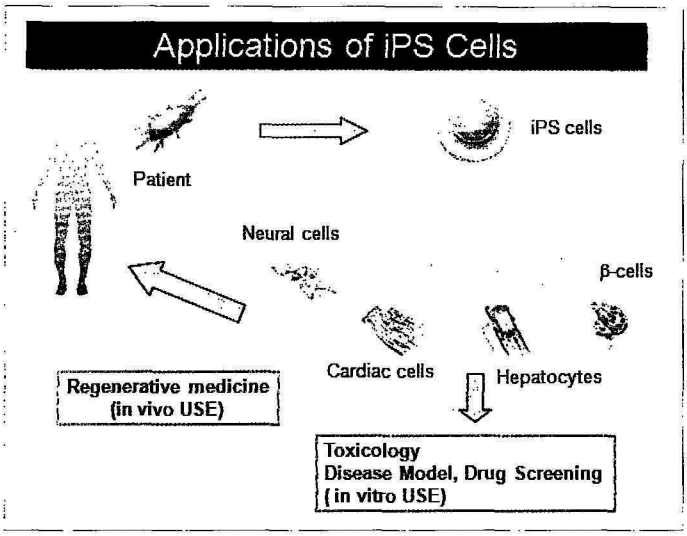


Figure 6. Various medical and pharmaceutical applications of iPS cells. Like ES cells, iPS cells can be derived into various functional cells. The resulting cells may be used for various applications, including toxicology testing, drug screening, disease modeling and cell transplantation therapy.

Many scientists around the world are conducting research aimed at the development of clinical applications using iPS cells. The therapeutic effects of mouse iPS cells have so far been reported in animal models of sickle cell anemia, Parkinson's disease, hemophilia A, and spinal cord injury[20-23]. The Center for Regenerative Medicine in Barcelona, Spain, reported in 2009 that human iPS cells were effective to treat animal models of Fanconi's anemia[24].

Toxicology testing and disease modeling

iPS cell technology can also be used for drug or toxicology screens *in vitro* and for creating disease models in culture, which are regarded as comparatively shorter-term goals than the development of applications in regenerative medicine.

For example, liver cells generated from individuals with different cytochrome p450 enzymes would be of value for predicting the liver toxicity of new drugs. The disorder long QT syndrome (LQTS) is caused by mutations in genes involved in generating cardiac action potentials resulting in lethal arrhythmias. LQTS can also be induced by certain drugs in sensitive individuals.

By generating beating cardiac myocytes from iPS cells derived from these sensitive individuals, drug candidates could be tested *in vitro*.

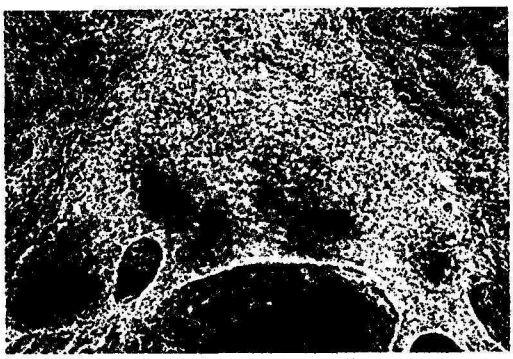


Figure 7. Cardiac muscle cells derived from human iPS cells.
iPS cell-derived cardiac myocytes are used to study the development and course of diseases in ways never before possible. Some companies market hiPSC-derived cardiac myocytes for toxicology testing.

Generating *in vitro* disease models using iPS cell technology may also prove useful in elucidating mechanisms of disease pathogenesis. Many groups have already generated iPS cells from patients with various neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Parkinson's disease, a variety of genetic diseases with either Mendelian or complex inheritance, and spinal muscular atrophy (SMA)[25-27].

An important challenge is how to recapitulate disease in cells derived from patient-specific iPS cells. In genetically inherited diseases, specific pathologies may be easier to model. Indeed, motor neurons generated from iPS cells derived from a SMA patient exhibit selective deficits compared to those generated from iPS cells derived from the patient's healthy mother. However, in many neurodegenerative diseases such as ALS, it takes years for symptoms to develop in patients.

ALS is a progressive, fatal neurodegenerative disease caused by the degeneration of motor neurons that control muscle movement. Effective therapies

for the disease have yet to be found, due in part to the lack of good disease models. If researchers use iPS cell technology, iPS cells generated from patients with ALS are derived into motor neurons that have the same DNA as the patient's. If the symptoms of the disease can be recapitulated in the cells, it may be possible to use them to elucidate the mechanism of ALS and screen drug compounds that may be effective to the disease.

Hurdles to be overcome

The technology is still in its infancy, and the development of clinical applications using iPS cells faces many obstacles - some similar to those facing ES **cells, and others that are unique. Common obstacles to realize cell therapies are that** we have to develop methods to differentiate ES/iPS cells into any functional cells we need and to transplant them into patients. The most challenging common hurdle is teratoma formation. Even a small number of undifferentiated cells can result in the formation of teratomas, a form of tumor. Another key goal is to induce differentiation of human ES/iPS cells into required cell types while leaving few undifferentiated cells behind.

One unique hurdle to be overcome before iPS cells can be used in the clinic is primarily related to the induced reprogramming of somatic cells. We need a reliable evaluation of whether nuclear reprogramming for each iPS cell is complete. Aberrant reprogramming may result in impaired ability to differentiate, and may increase the risk of teratoma formation after directed differentiation. Another important challenge is to develop simple, yet sensitive and reliable, methods to evaluate the effectiveness and safety of the many iPS cell clones and subclones generated by many different protocols.

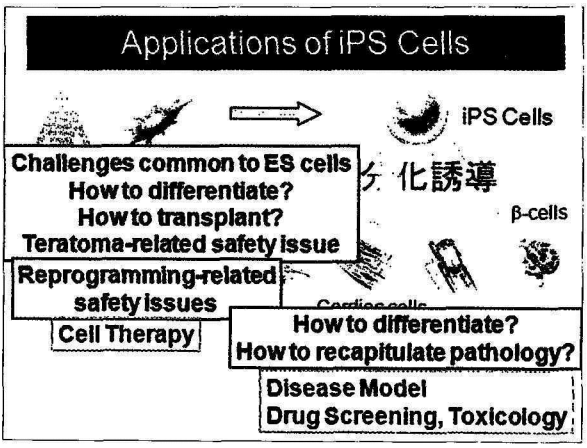


Figure 8. Challenges of iPS cells.
Many hurdles must be overcome before iPS cells are used in cell transplantation therapy.

Despite all these obstacles and challenges, iPS cells offer enormous and unprecedented potential for medical applications. With students and colleagues working in my labs and many other researchers around the world, we are making progress in iPS cell research to overcome the hurdles. It may take many years to reach the final goal, but I sincerely hope that iPS cell technology will contribute to the development of new cures for people suffering from various diseases and injuries.

References

1. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A. 1981; 78(12): 7634-7638.
2. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981; 292 (5819): 154-156.
3. Yamanaka S, Balestra ME, Ferrell LD, Fan J, Arnold KS, Taylor S, Taylor JM, Innerarity TL. Apolipoprotein B mRNA-editing protein induces hepatocellular carcinoma and dysplasia in transgenic animals.Proc Natl Acad Sci U S A. 1995; 92 (18): 8483-8487.
4. Yamanaka S, Poksay KS, Arnold KS, Innerarity TL. A novel translational

repressor mRNA is edited extensively in livers containing tumors caused by the transgene expression of the apoB mRNA-editing enzyme. Genes Dev. 1997; 11(3): 321-333.

5. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282(5391): 1145-1147.
6. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J.Embryol .Exp. Morphol. 1962; 10: 622-640.
7. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997; 385(6619):810-813.
8. Tada M, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. Curr Biol. 2001; 11(19): 1553-1558.
9. Tokuzawa Y, Kaiho E, Maruyama M, Takahashi K, Mitsui K, Maeda M, Niwa H, Yamanaka S. Fbx15 is a novel target of Oct3/4 but is dispensable for embryonic stem cell self-renewal and mouse development. Mol Cell Biol. 2003 Apr;23(8):2699-2708.
10. Takahashi K, Mitsui K, Yamanaka S. Role of ERas in promoting tumour-like properties in mouse embryonic stem cells. Nature. 2003 May 29; 423(6939): 541-545.
11. Mitsui K, Tokuzawa Y, Itoh H, Segawa K, Murakami M, Takahashi K, Maruyama M, Maeda M, Yamanaka S. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. Cell. 2003 May 30; 113(5): 631-642.
12. Maruyama M, Ichisaka T, Nakagawa M, Yamanaka S. Differential roles for Sox15 and Sox2 in transcriptional control in mouse embryonic stem cells. J Biol Chem. 2005; 280(26): 24371-24379.
13. Amano H, Itakura K, Maruyama M, Ichisaka T, Nakagawa M, Yamanaka S. Identification and targeted disruption of the mouse gene encoding ESG1 (PH34/ECAT2/DPPA5). BMC Dev Biol. 2006; 6: 11.
14. Tsubooka N, Ichisaka T, Okita K, Takahashi K, Nakagawa M, Yamanaka S. Roles of Sall4 in the generation of pluripotent stem cells from blastocysts and fibroblasts. Genes Cells. 2009; 14(6): 683-694.
15. Imamura M, Miura K, Iwabuchi K, Ichisaka T, Nakagawa M, Lee J, Kanatsu-Shinohara M, Shinohara T, Yamanaka S. Transcriptional repression and DNA hypermethylation of a small set of ES cell marker genes in male

germline stem cells. BMC Dev Biol. 2006; 6:34.

16. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25; 126(4): 663-676.
17. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131(5): 861-872.
18. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 18(5858): 1917-1920.
19. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008; 451(7175): 141-146.
20. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007; 318(5858): 1920-1923.
21. Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, Broccoli V, Constantine-Paton M, Isacson O, Jaenisch R. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A. 2008 Apr 15; 105(15): 5856-5861.
22. Xu D, Alipio Z, Fink LM, Adcock DM, Yang J, Ward DC, Ma Y. Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. Proc Natl Acad Sci U S A. 2009; 106(3): 808-813.
23. Tsuji O, Miura K, Okada Y, Fujiyoshi K, Mukaino M, Nagoshi N, Kitamura K, Kumagai G, Nishino M, Tomisato S, Higashi H, Nagai T, Katoh H, Kohda K, Matsuzaki Y, Yuzaki M, Ikeda E, Toyama Y, Nakamura M, Yamanaka S, Okano H. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci U S A. 2010; 107(28): 12704-12709.
24. Raya A, Rodríguez-Pizà I, Guenechea G, Vassena R, Navarro S, Barrero MJ, Consiglio A, Castellà M, Río P, Sleep E, González F, Tiscornia G, Garreta E, Aasen T, Veiga A, Verma IM, Surrallés J, Bueren J, Izpisúa Belmonte JC. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Nature 2009; 460(7251): 53-59.
25. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung

W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 2008; 321(5893): 1218-1221.

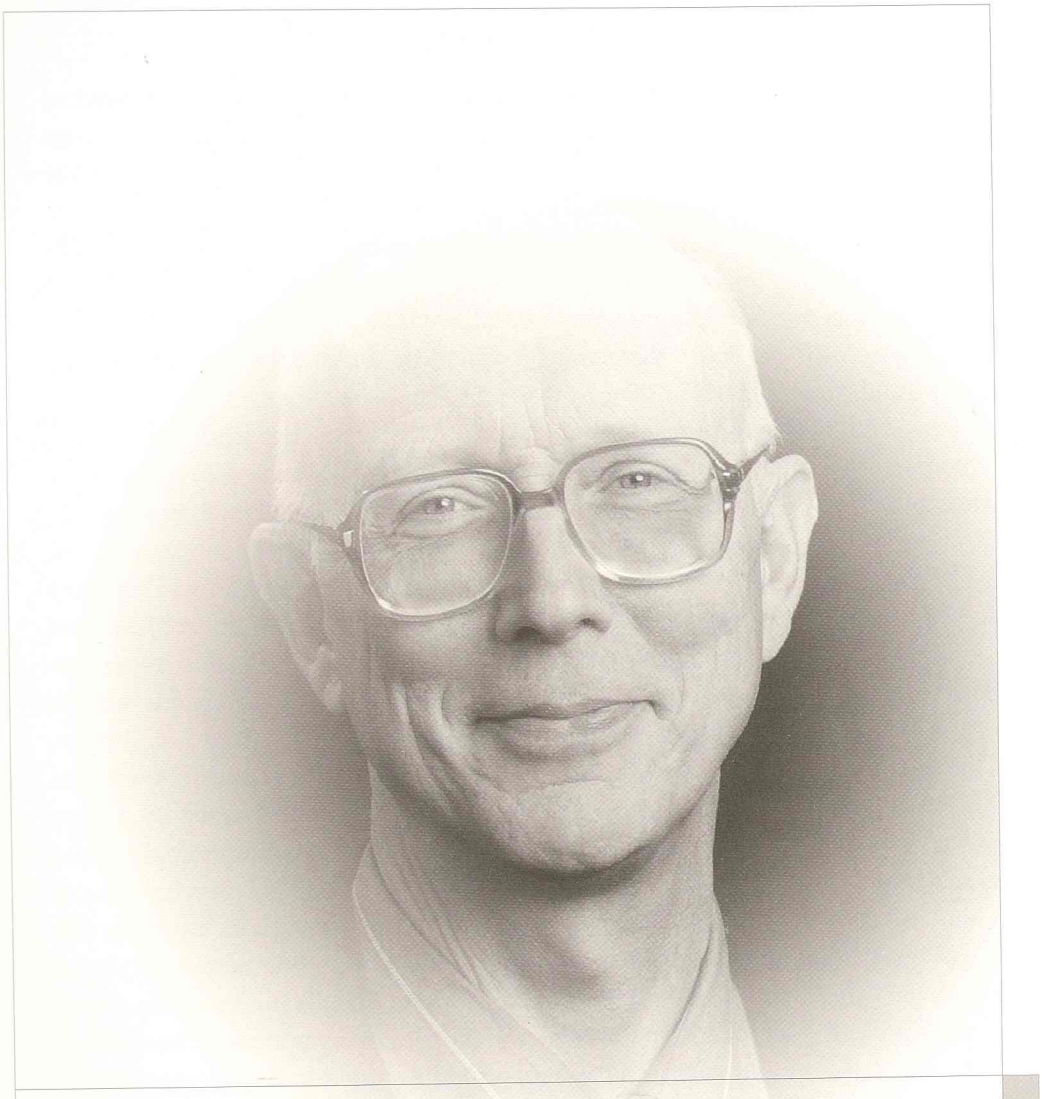
26. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 2009; 136(5): 964-977.
27. Ebert AD, Yu J, Rose FF Jr, Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 2009; 457(7227): 277-280.

WINNERS OF THE 2011 KING FAISAL INTERNATIONAL PRIZE FOR SCIENCE

The King Faisal International Prize for Science **(Chemistry)** for the year 1432H - 2011G has been awarded jointly to: Professor George Whitesides (USA) and Professor Richard Zare (USA).

Professor Whitesides has revolutionized the field of self assembly using molecular scale synthesis to control the macroscopic properties of surfaces. This and his work on soft lithography, where he developed practical methods to mold complex patterns on surfaces, is characterized by its relevance to diverse fields such as molecular electronics, material science and biology. Professor Whitesides has recognized and developed connections between nanoscience and biological systems, leading to new paradigms for drug design, which may enable new and inexpensive approaches to bioscience and medical diagnostics.

Professor Zare is recognized for his fundamental contribution to the understanding of molecular dynamics and chemical reactions. He developed the extremely sensitive technique of laser induced fluorescence and pioneered its application in many fields ranging from analytical chemistry and molecular biology to astrophysics (composition of interstellar media).



The Science of "In-between" Sizes

George M. Whitesides[3]

Prolog. Imagine that you are a tourist traveling through the landscape of science and technology, and, further, that you are on the special tour that views the things made by scientists and engineers laid out by size. At one end of the tour are the very large things: cities and transportation systems. Then somewhat smaller ones: buildings and automobiles. Even smaller things are watches, and then needles and fine thread. All are made from what we call materials – concrete, steel, glass, and organic polymers. These objects are all familiar, and all are visible to the naked eye. They are fabricated and assembled by engineers using cutting and forging and stamping and joining.

At the other end of the tour are the smallest things, especially molecules: structures assembled from atoms, and collections of atoms. Chemists make molecules. The techniques they use to do so are highly developed, but entirely different from the techniques used by carpenters to build houses.

And what about sizes that are in-between the very large and the very small? What about the part of the tour between large, familiar, visible objects, and molecules – equally important, but so small as to be invisible? This region has been, for science and for your tour bus, at best a bit of bumpy dirt road in what is otherwise a smooth highway, and at worst a canyon over which there is no bridge. This region of sizes has been one of the least explored parts of physical science.

The Problem. So, there is a problem: how does one assemble matter, by design, from components that are bigger than atoms, but smaller than macroscopic pieces of metal and ceramic?

This "in-between" region of sizes is more important technologically than one might think. It is, for example, the range of sizes important in micro-

and nano-electronics. It is also the region of sizes of many structures in nature—from organelles in living cells to the light-reflective structures
on the wings of butterflies. It is critical for micromachines of the sort used as accelerometers in automotive airbags, and for understanding how blood flows in capillaries.

We—I and my then-colleagues—set out some 30 years ago to explore what could be done with synthesis (or fabrication) in this intermediate region of sizes, and we have had a most entertaining – and also useful – time of it. Our initial objective was to find methods to make new structures in this size range easily, and particularly to make them inexpensively, since the costs of the methods that were then available to fabricate such structures were prohibitive. (Between then and now, the costs have gotten much worse, as the sizes required for electronics have gotten much smaller and the devices required to fabricate them much

[3] Woodford L. and Ann A. Flowers University Professor Department of Chemistry & Chemical Biology,Harvard University

more complicated.) As we have proceeded, however, we have been less and less concerned just with size, and more concerned with function. Structure is nice, but function is nicer.

Background: Components, Forces, Processes. To understand the story I have to tell, one needs to know just a few words: "components," "forces," and "assembly."

Imagine a necklace. The beads and the string are components. They are the pieces from which the thing is made. The beads are held together by the string. If you try to pull the beads apart, the string resists, and the necklace does not break apart. That resistance is the force that enables the assembly to be stable in use. The process by which the necklace was assembled is simply the stringing of the beads on the string. It is an example of what I will call "directed" assembly: that is, a person or machine determines how the components are assembled.

It would also be possible to make an object in a different way that looked very much like a necklace. If one takes a handful of small, magnetized metal spheres and shakes them together, they will often form strings and circles and spheres. In this instance the components put themselves together—that is, they "self-assemble"—based on magnetic forces. Pulling these necklaces apart is easy: the magnetic forces are relatively weak.

Self-assembly is also ubiquitous in nature—from crystals to animals—but the principles that guide it are less well understood than those used in better-developed areas of engineering, such as the organic synthesis of drugs, or the fabrication of computer microprocessors.

Both directed assembly, and self-assembly, are important in the fabrication of intermediate-sized things. Directed assembly is the basis for electronics: it (in the form of photolithography—a technique of extraordinary elegance) can make almost unbelievably complex structures with 40-nm features, but such assembly requires devices and technologies that are extraordinarily expensive. Self-assembly is usually used to make simpler structures, but it is much less expensive. Much of the intellectual interest in self-assembly stems, however, not from its potential for saving cost, but from the fact that the living cell—a structure that is even more complicated than a computer chip—is entirely self – assembled. In the cell, there is no robot that places the molecules in their correct orientation. Life puts itself together.

Examples of New Science and Technology. I'd like to give you a few examples of synthesis and fabrication (the two words are used in very similar ways) to make structures ranging in size from molecules to the wires used in microelectronics

devices. I would also like to give an example of a use of the concepts from this kind of synthesis to make a larger system with important function.

"Top-Down"—Molding (and Photolithography) for Nanoelectronics. Probably the most highly developed technology ever developed by humans is that which is used in micro- (and now nano-)electronics. This technology is based on what is essentially a very highly refined camera, and the patterns of wires and devices in the microprocessor of a cell phone (for example) are the superposition (transferred into metal and silicon) of a series of pictures made by the camera showing how the functional structures (transistors and capacitors and wires) should be laid out. This technology is unbelievably sophisticated, and correspondingly expensive: a single camera of this type may cost more than 100 million dollars, and many of them are required to build a single microprocessor. There is a clear motivation to try to develop methods to make small structures less expensively.

We and others (notably Grant Willson and Steve Chou) had developed one of the oldest of technologies – molding – for this purpose. A little to our surprise, it works very well—in fact, better than expected. In this type of molding, the uncertainty in the dimensions of the structure being formed are set—not by the fuzziness of light (as in photolithography)—but by how closely the material being molded can fit itself (that is, self assemble) to the walls of the mold. Although in theory that number should be approximately the size of an atom, in practical technology, experiments seldom work out exactly as theory predicts. Here, surprisingly, they seem to. Simple molding of organic polymers seems to be able to replicate features on the mold with dimensions less than 1 nm. (I say "seems" because the tools that are available to measure shapes and dimensions on this scale are still limited, and statements about size are often interpretations that contain a fair amount of guesswork.) So, molding provides an inexpensive alternative to the very expensive techniques of photolithography. Although nano-molding is still early in its development, it is clear that it will be used for making optical structures, and may even find a place in the sophisticated world of nano-electronics.

"Bottom-Up"—Water, Molecular Recognition, and the Design of Drugs. We have now looked at an example of "top-down" assembly. An example from the other end of the spectrum of sizes – from the "bottom up" world that makes aggregates of molecules from individual molecules – is motivated by another important problem: that is, molecular recognition. "Life" – at its most reductionist – is a large collection of organic reactions that take place through the action of a set of catalysts – proteins called enzymes – that select from among the many

molecules present in the cell, and cause reactions between those that should react at any given time in the cycle of the cell as lives and divides. We don't understand how life works: in particular we do not understand how a collection of reactions and molecules (which are individually *not* alive) become the cell (which *is* alive). A step along the way is to understand the smaller problem of how the catalysts – the proteins called enzymes – work; that understanding, in turn, requires understanding molecular recognition: the selective association of two molecules, based somehow on their shape, using relatively weak forces.

Molecular recognition is both one of the most fundamental chemical processes in life, and also crucial to improving the efficiency of the processes by which drugs are made, since a "drug" is simply a molecule that interacts selectively with one of the functional components (a catalyst, a signaling molecule, a transporter) in the cell.

The historically honored metaphor for molecular recognition has been the "lock and key." The idea is that a part of one molecule has a shape that is complementary to a part of the shape of the second: that is, one fits into the other, like a key into a lock. This metaphor has the charm of familiarity and simplicity. Unfortunately, it also seems to be wrong in very fundamental ways. Molecular theory developed by Rossky, Berne, Dill, Chandler, and others has suggested for some years that molecular recognition in water is (in a subtle way) more about the water than about the molecules of protein and drug themselves. Experiment is now beginning to catch up to theory, and our experiments confirm at least some aspects of the theoretical predictions. Molecular recognition now seems to be not so much a reflection of a close complementarity between the shapes of the surfaces of the interacting protein and drug molecules as it is the "shape" of the collection of water molecules that must be displaced when they come together. That is, the protein and the "drug" stick together not because (or not entirely because) their surfaces are perfectly complementary in shape, but rather because when they fit together, the "key" squeezes energetically unfavorable water out of the cavity of the "lock". Understanding the details of this process requires diving into the subject of thermodynamics, and discussing enthalpy and entropy, and probably no one would be happy if I were to do so. Suffice it to say that considering the water in this way provides a new – and possibly useful – way of thinking about molecular self-assembly as a contributor to molecular recognition.

"Top-Down" plus "Bottom-Up" Contact Printing: Low-Cost Fabrication for Consumer Electronics. A third example of synthesis and fabrication in the "in-between" world combines top-down and bottom-up methods. This process involves printing (exactly analogous to the stamping that once was used to

mark messages "confidential"). A soft rubber stamp is inked with the solution of molecules; the stamp comes in contact with the substrate (for example, a thin, very flat film of metallic gold), and the molecules transfer to this substrate. What is remarkable is that when they do so, they further assemble themselves into a highly ordered, semi-crystalline sheet structure exactly one molecule thick). So, the stamp provides a top-down pattern; molecular self-assembly provides a bottom-up order.

These structures – commonly called self-assembled monolayers, or SAMs-- are increasingly widely used in areas such as consumer electronics and bioengineering in processes that provide structures that are, perhaps, only moderately small, but quite inexpensive. They are being developed, for example, in devices used for harvesting solar energy, in displays, and in devices for clinical diagnostics.

A Mélange: From Water Striders to Zero-Cost Diagnostics. As a last example, I wish briefly to show how ideas in these kinds of syntheses-- ideas based on self-assembly and soft lithography—can be used with larger – scale objects to provide new functions. I start with the fact that many insects—for example, water-striders—are able to rest on the surface of water, supported by its surface tension. Interfacial forces are particularly important with water, and underlie phenomena such as, for example, the spreading of spilled coffee on a tablecloth or piece of paper. A variant of soft lithography makes it possible to control the spreading, and to confine the moving liquid between lines on a piece of paper. These lines – a kind of channel in the paper – can then serve to guide the spreading of water (not in coffee, but in urine or blood) to zones containing chemicals that reveal something of the nature of the molecules dissolved in the fluid. This simple idea – using paper (patterned using very low cost technologies)—to carry out biochemical analysis is the basis for a useful method for biomedical analysis variously called "paper diagnostics" or "zero-cost diagnostics." These systems are particularly attractive for use as clinical diagnostics in developing economies, where cost is crucial; they may also eventually be important in developed economies, where cost *control* is now equally crucial.

Conclusion. In this tour from "large" to "small," I have spent most of the time at the stop labeled "in-between." This region of sizes – intermediate between nanoscopic molecules and macroscopic houses – is one of the most important in technology, and also one containing many scientific phenomena that have been unexplored, and were often inaccessible.

I, my colleagues and students, and many other scientists and engineers are

convinced that it is a region of sizes that will generate surprises, new knowledge, and new uses for that knowledge—that is, new science and new technology. I am most pleased to be able to share it with you.

Leading References.

"Nanoskiving: A New Method to Produce Arrays of Nanostructures," Xu, Q., Rioux, R. M., Dickey, M. D., and Whitesides, G. M. *Acc. Chem. Res.* **2008**, *41*, 1566-1577.

"Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices," Martinez, A. W., Phillips, S. T., Carillho, E., and Whitesides, G.M., *Anal. Chem.*, **2009**, *82*, 3-10.

"The Self-assembly of Thiolates on Metals as a Form of Nanotechnology," Love, J.C., Estroff, L.A., Kriebel, J.K., Nuzzo, R.G., and Whitesides, G.M., *Chem. Rev.*, **2005**, *105*, 1171-1196.

"Rapid Prototyping of Microfluidics Systems in Poly(dimethylsiloxane)" Duffy D.C., McDonald, J.C., Schueller, O.J.A., and Whitesides, G.M., *Anal. Chem.*, **1998**, *70*, 4974-4984.

"Self-Assembly at All Scales," Whitesides, G.M., and Grzybowski, B., *Science*, **2002**, *295*, 2418-2421.

"Unconventional Methods for Fabricating and Patterning Nanostructures," Xia, Y.N., Rogers, J.A., Paul, K.E., and Whitesides, G.M., *Chem. Rev.*, **1999**, *99*, 1823-1848.

"The Origins and the Future of Microfluidics," Whitesides, G.M., *Nature*, **2006**, *442*, 368-373.

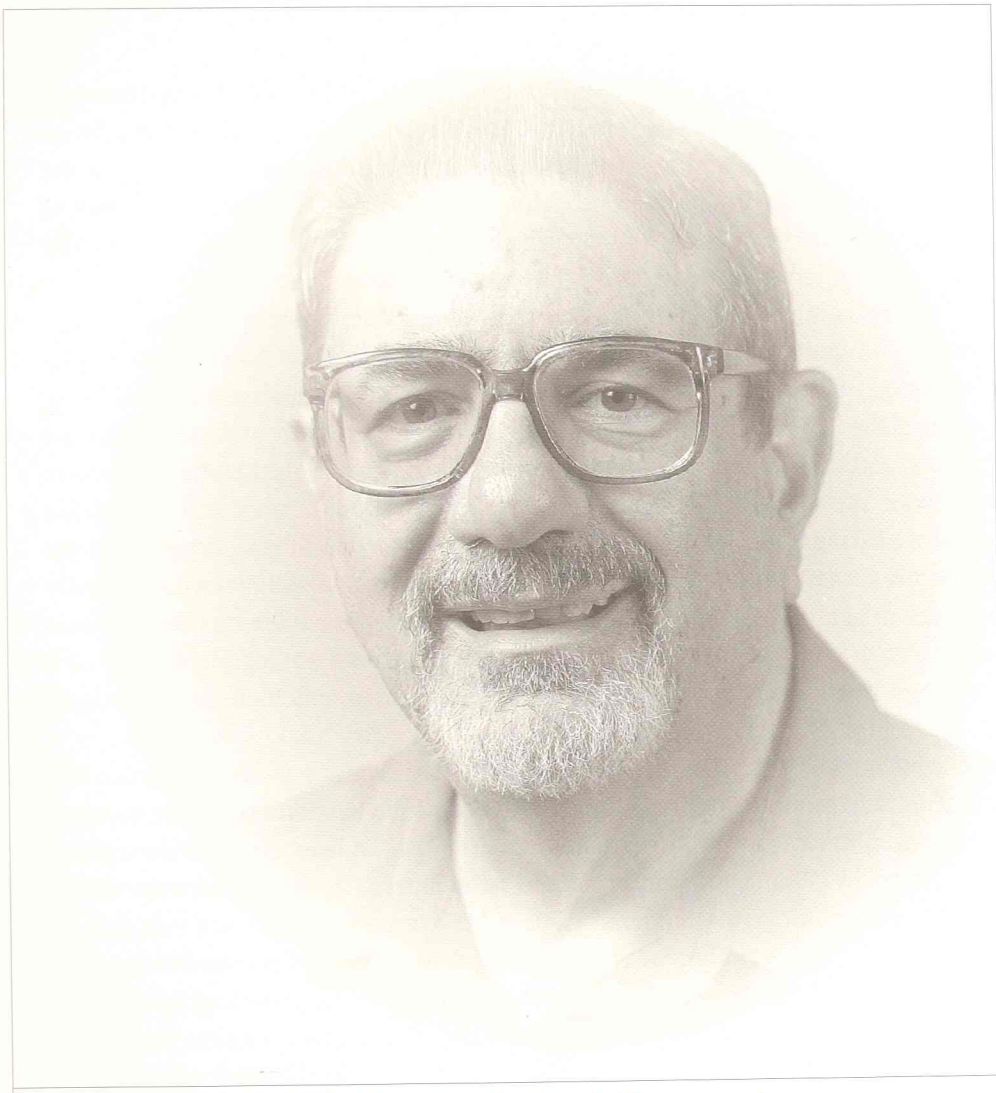
"Soft Lithography in Biology and Biochemistry," Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X., and Ingber, D.E., *Ann. Rev. Biomed. Eng.*, **2001**, *3*, 335-373.

"Making Things by Self-Assembly," Boncheva, M., and Whitesides, G.M., *MRS Bull.*, **2005**, *30*, 736-742.

"New Approaches to Nanofabrication: Molding, Printing, and Other Techniques," Gates, B.D., Xu, Q., Stewart, M., Ryan, D., Willson, C.G., and Whitesides, G.M., *Chem. Rev.*, **2005**, *105*, 1171-1196.

"Organic Materials Science," Whitesides, G.M., *MRS Bull.*, **2002**, *27*, 56-65.

"Beyond Molecules: Self-Assembly of Mesoscopic and Macroscopic Components," Whitesides, G.M., and Boncheva, M., *Proc. Nat. Acad. Sci. USA*, **2002**, *116*, 8571-8577,



My Life with LIF: A Personal Account of Developing Laser-Induced Fluorescence

Richard Zare 4

My story begins with the birth of the laser. On May 16, 1960, Theodore Harold "Ted" Maiman demonstrated the first laser (the word is actually an acronym for "Light Amplification by Stimulated Emission of Radiation") at the Hughes Research Laboratories in Malibu, California. This feat was achieved with a synthetic ruby-crystal rod measuring 1 cm by 1.5 cm. It produced a pulsed beam of deep-red light that was brighter than a million suns.

The concept of the laser goes back to Albert Einstein in the early 20th century but the 1958 groundbreaking paper, "Infrared and Optical Masers", by Charles H. Townes and Arthur L. Schawlow at Bell Telephone Laboratories, inspired the laser's development. The paper proposed an extension into the infrared and visible regions for what had already been achieved in the microwave region of the electromagnetic spectrum. Gordon Gould, who was a graduate student of Townes at Columbia University, also came up with important concepts and coined the term "laser". Unlike the light from a flashlight, which rapidly spreads in shape, the beam of the laser stays tightly bunched together. A laser beam can travel for miles before its spread becomes comparable to that of a flashlight beam going across a room. This property in which all the light waves oscillate in phase is called coherence.

The world learned of this coherent light source at a press conference Ted Maiman held on July 7, 1960 in New York City. But what good could the inventor of the laser imagine for this device? Maiman stated, "A laser is a solution seeking a problem." [1] This sentiment was repeatedly uttered by many experts in the physics community who pondered its possible uses.

In 1960, I was a junior (third-year undergraduate) at Harvard University, pursuing a double major in chemistry and physics. I was spending the summer in my home town, Cleveland, Ohio, working for Clevite Corporation, measuring the intensity of scattered X-rays from a

single crystal of cadmium selenide to learn about the structure of this semiconductor material. This work would lead to my first scientific publication.[2] When Maiman's press release appeared, we avidly discussed around the lunch table what might come from the laser. We were sure that the world would be profoundly changed by it but at the time, we had not imagined how ubiquitous the laser would become. These days, the laser has a multitude of applications, from bar-code readers in supermarkets to devices that help reattach detached retinas. Maiman's ruby laser produced millisecond pulses of light and could not be fired at a rapid rate. The next big advance came on December 12, 1960, when Ali Javan, William R. Bennett, and Donald R. Herriott demonstrated at Bell Telephone

[4]Chair and Professor of Chemistry, Stanford University

Laboratories the first continuously working (cw) laser.[3] It contained helium and neon gases and was called the He-Ne laser. Unlike Maimam's laser which was pumped by a flashtube, the He-Ne laser was pumped by an electrical discharge inside the gas mixture. The electrical energy was turned into coherent radiation. At the time, lasers were quite exotic and only a few existed. I thought of the idea of using a laser to excite molecules and cause them to fluoresce when I was a beginning faculty member of the Department of Chemistry and the Department of Physics and Astrophysics of the University of Colorado (both without tenure) as well as a member of what was then called the Joint Institute for Laboratory Astrophysics, later renamed simply JILA, in Boulder, Colorado. One of my Harvard professors told me that this idea would never work because based on what was found in Gerhard Herzberg's encyclopedic treatises on molecular spectroscopy, many molecules fall apart when electronically excited. I was crestfallen by this off-hand comment but I felt that the pessimism was too severe and was determined to try it anyway.

First, I had to build my own He-Ne laser. With the help of the JILA staff, this was easily accomplished in about three months. Then, I needed to find a molecule that I could put into the gas phase to absorb the coherent red light from the He-Ne laser. The first laser-induced fluorescence (LIF) experiments were successfully carried out on the potassium dimer molecule, K_2, which was made by heating potassium metal in an evacuated glass cylinder fitted with good windows to let the laser beam in and out. Hot potassium discolors normal glass so a special glass cell needed to be made. The first full report[4] of this experiment was published in 1968. I fell in love with the fluorescence streak which, when dispersed in a spectrograph, showed transitions from the electronically excited vibrational-rotational level pumped by the He-Ne laser to the different vibrational-rotational levels of the ground electronic state of K_2. In this set-up, we were able to observe high vibrational-rotational levels of the molecule that had never been seen before, thus greatly extending our knowledge of the forces that pulled together two potassium atoms as a function of separation distance. I had picked this molecule because I was familiar with it from my graduate studies in Prof. Dudley Herschbach's research group, first at the University of California at Berkeley, then at Harvard University. The dimer was actually a thorn in the side of many of my fellow graduate students as it was an unwanted and unwelcomed fellow traveler in atomic beams of potassium in their crossed-beam reactive scattering studies. . .

Soon thereafter, the advent of the argon ion laser made many more sharp lines of a single color available as monochromatic cw excitation sources. But the

field really took off with the invention of the tunable dye laser by Mary Spaeth at the Hughes Aircraft Company, which allowed experimentalists to dial up whatever color was desired. Credit for the invention of the dye laser also needs to go to Peter P. Sorokin at IBM, Yorktown Heights, New York and Fritz P. Schäfer, Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany.
LIF has so many advantages. It gives a bright signal against a dark background. This feature would later become the basis for ultrasensitive chemical analysis, enabling detection limits to be pushed to that of a single molecule. LIF permits preparation of a well-defined excited state whose properties—radiative and collisional—can be studied in great detail. It allows probing of molecules in extremely hostile environments, such as flames, arcs, and sparks. LIF can also be used in other amazing ways, such as sorting cells one at a time, prospecting for petroleum leaks in the ocean floor, and distinguishing between cancerous and noncancerous tissue in a patient. But I am getting ahead of the story here.

When I moved to Columbia University in 1970, I had the idea that LIF could be used to separate the isotopes of elements. All atoms of an element have the same number of protons in the nucleus, which defines the name of the element. But atoms of any particular element can differ by weight because they contain different numbers of neutrons. These variants in neutron numbers are called isotopes. For example, the element uranium consists of the more common isotope U-238 that has 92 protons and 146 neutrons with a natural abundance of about 99.28%. But it also has the rarer isotope U-235 of 92 protons and 143 neutrons (basically, three less neutrons) with a natural abundance of only about 0.72%. Much attention is attached to this lighter isotope of uranium because it sustains a nuclear fission chain reaction and so is the material of nuclear reactors and nuclear bombs. Because of the differing weights of the isotopes, their absorption spectra are distinctly different, allowing a laser beam of the correct wavelength (color) to excite atoms or molecules containing one specific isotope and not the other.

I thought that this idea was an important one and even tried to patent it. But I was told it would only be valuable to the U.S. government who would not pay anything for it so nothing was done. Nevertheless, we did write papers describing one particular technique for laser isotope separation.[5,6] It also led me to write a nontechnical article on this topic for the popular science magazine, Scientific American.[7] I was pleased that this work led me to become a consultant to both Los Alamos National Laboratory and Lawrence Livermore National Laboratory. Both of these government research laboratories were soon locked in an intense competition to determine which one would come up with a viable laser-based

separation procedure for the isotopes of uranium. This situation became a real challenge for I had to make sure that each group trusted me and I did not divulge the progress of one group to the other.

In the process of writing the article for Scientific American, I had the pleasure of working with the great photographer, Fritz Goro. Figure 1 depicts an illustration we made showing what happens when the green beam from an argon ion laser passes through an evacuated glass

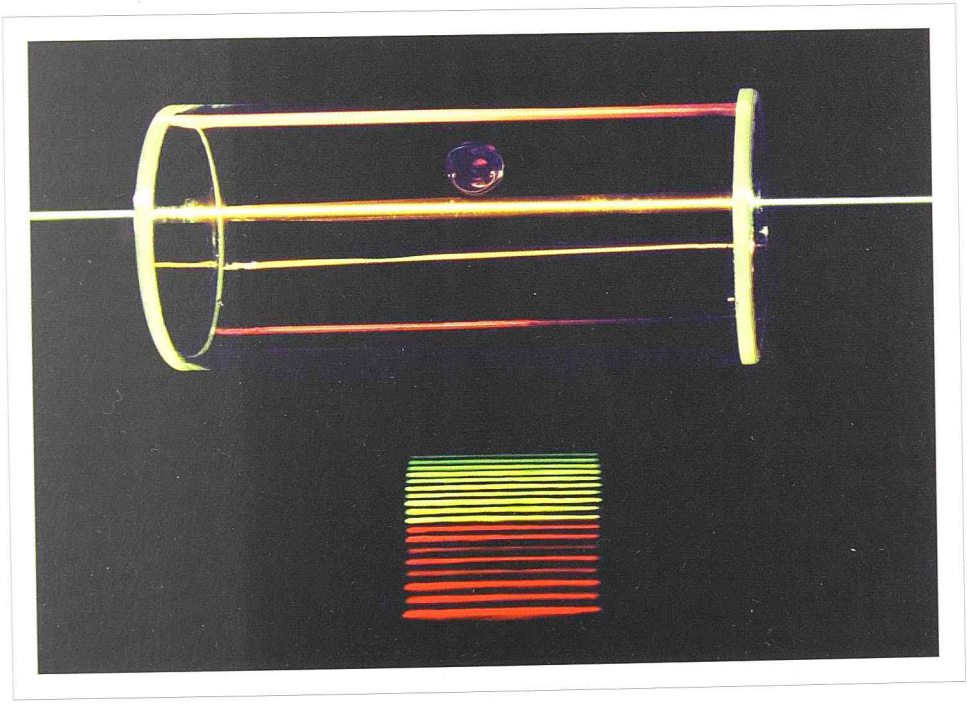


Figure 1. *Photograph of laser induced fluorescence of iodine vapor taken by Fritz Goro and the author in the author's laboratory at Columbia University, 1977. A green laser beam enters an evacuated glass cell containing iodine vapor, causing a yellow streak of fluorescence to appear. Below the glass cell, the light from the yellow fluorescence streak strikes a reflective diffraction grating where it is dispersed into its different colors, each corresponding to a particular molecular transition from the pumped vibrational-rotational state of the excited I_2 molecule to one of the vibrational-rotational levels of the I_2 molecule in its ground electronic state. The appearance of the yellow color in the undispersed fluorescence is the sum of the different colors of the various molecular transitions, weighted by the intensity of each transition.*

cell containing iodine (I_2) vapor. The fluorescence is clearly visible, as shown by the yellow streak inside the glass cell. We placed a diffraction grating beneath the cell. The grating allowed us to catch the reflection of the yellow streak and disperse it into the colors of the different I_2 molecule transitions as the molecule re-radiated its excitation as it fell from the specific vibrational-rotational (v',J') level of the excited state to the various different vibrational-rotational (v'', J'') levels of the ground state. The intensity of each transition is governed by the Franck-Condon principle and the $\Delta J = J' - J'' = \pm 1$ dipole selection rules. There is so much more that can be learned from a careful study of Figure 1.

Close examination shows that the yellow streak is wider than the green laser beam. The explanation is that the pumped I_2 molecules remain in their excited state for such a long time that the random motion of the molecules causes the image of the fluorescence to spread in space. Careful measurements of this spread leads to an estimate of the radiative lifetime of the excited I_2 molecule on the order of a microsecond, that is, one-millionth of a second. If some gas is admitted to the evacuated cell, the spread shrinks because of collisions between I_2 molecules and the gas atoms or molecules. The collisions stop the excited iodine molecules from flying in straight lines. Depending on the gas, the intensity of the lines decreases because of quenching of the fluorescence caused by energy transfer between the collision partners. New lines appear on the grating because some collisions populate new (v',J') levels of the excited I_2 molecule. From such studies, much information can be obtained about the reactive and inelastic collisions that excited molecules undergo.

I need to emphasize the sensitivity of LIF and its ability to detect molecules under extreme conditions. Most stable molecules have an even number of electrons, paired together to make bonds between the atoms of the molecule. In chemical reactions, transient intermediates are often formed having an odd number of electrons, called radicals. Although their existence is often fleeting, radicals play a critical role on diverse areas of chemistry, such as atmospheric chemistry, polymer chemistry, and even human biochemistry. LIF has been able to capture the presence of these elusive entities before they disappear. According to a review[8] published in 2002, the following species, most of which are radicals, have been observed in flames using LIF: H, C, O, C_2, CH, OH, CO, C_3, HCO, CH_2, C_2H, C_2O, CH_2O, HCCO, CH_3O, $C_2H_2O_2$, C_3H_5 (allyl), C_7H_7 (benzyl), N, CN, NH, NO, NS, NH_2, HCN,HNO, NCO, NCN, NO_2, NH_3, CCl, CF, CHF, CF_2, CF_2O, PO, S_2, SH, SO, SIO, and SO_2. By now, the list has grown longer.

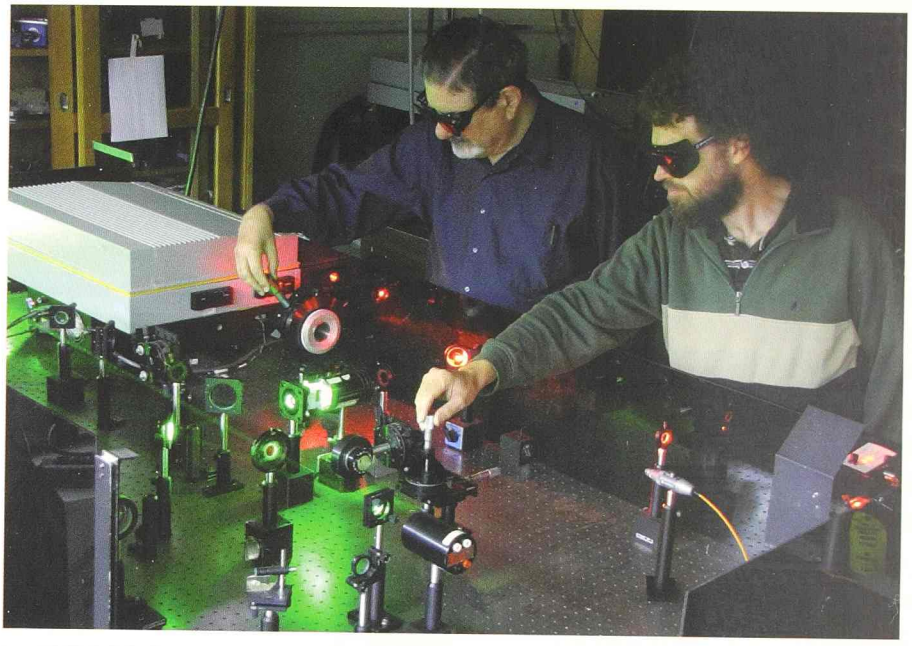
It was an easy step to turn LIF to the detection of nascent reaction products under single-collision conditions. This was achieved in 1972.[9] In

1977, we found that converting molecules to molecular ions by a state-specific resonance-enhanced multiphoton ionization (REMPI) process was even more sensitive. [10] It has predominantly become the method of choice for understanding fundamental elementary reactions, such as the simplest bimolecular reaction, H + D_2 ® HD + D. I studied this reaction system for many years in great detail after moving to Stanford University in 1977. Other elaborations involved multi-laser experiments: One laser operating in the infrared prepared reagents in specific vibrational-rotational level, another laser generated fast atoms in laser photolysis as one of the reagents, and a final laser system detected the reaction products in a state-specific manner using either LIF or REMPI.[11]

A key turning point occurred in 1976 when I attended a national meeting of the American Chemical Society. A U.S. Department of Agriculture chemist, Dr. Larry Seitz, searching for a particular session, walked into the wrong room at the huge ACS meeting. I was giving a presentation in that room on the benefits and promises of LIF. During the question and answer session, Seitz asked me whether I could detect aflatoxins. I did not know what aflatoxins were but ignorance has never stopped me from making up answers. I told him that if aflatoxins fluoresced and entered the gas phase, I could easily detect them Seitz seemed quite excited by this answer and explained how this fungal metabolite was a potent carcinogen that needed to be screened for in the food supply, especially in moldy nuts and grains that had been stored between the time of harvesting and the time of human consumption.

It turns out in subsequent correspondence with Dr. Seitz, aflatoxins decompose when they are heated so it really is difficult to do the gas-phase experiment I first had in mind. I began to think about the possibility of doing LIF on compounds in liquids. Dr. Gerald Diebold, who is now a professor at Brown University, was at that time a postdoctoral research associate in my group. He was willing to boldly go where others had not gone before so together, we did the first LIF experiment on aflatoxins . We used liquid chromatography to separate different aflatoxin molecules in a mixture and then applied LIF as a detector.[12]

Once I moved in that direction, a whole new world of applications appeared. It was a classic case of once you have a hammer; everything begins to look like a nail. Let me describe one notable example. I started developing LIF as a detection method for capillary electrophoresis (CE) following a visit to Prof. James W. Jorgenson's laboratory at the University of North Carolina where he showed me the remarkable separation power of this new technique. A strong electric field is applied across the length of the glass capillary that is filled with a liquid, causing ions to move at different speeds along the capillary. The current



generated produced much heat, but the small cross section of the capillary, only thousandths of a centimeter across, dissipated the heat by conduction through the capillary walls with great effectiveness, allowing different bands of ionic species that traveled the length of the capillary to be resolved with almost no broadening. Jorgenson had been using absorption spectroscopy as a CE detector but the short path length of the capillary greatly limited the sensitivity. To me, the solution to this problem was obvious. The ability to focus laser light into tiny volumes and its extraordinary sensitivity made LIF an ideal detection method. The first demonstration of its power was achieved in 1985.[13] This work naturally led to commercialization by Beckman-Coulter in their PACE system[14]. For many years, this commercial success supplied my research group with substantial financial support until the patent expired.

CE separations with LIF detection led to one of the greatest achievements of the end of the 20th century: the sequencing of the human genome. I feel very proud to have played a part in this advance, which has revolutionized our understanding of biology and offers so many hopeful possibilities for future medical treatments.

The exquisite sensitivity of LIF also had another consequence. In

the 1990s, with my postdoctoral research associate, Shuming Nie, currently a professor at Emory University, and my graduate student, Daniel Chiu, now faculty at the University of Washington, we were able to detect single molecules in room-temperature liquids using LIF.[15] This had been previously done in cryogenic matrices, first by my Stanford colleague, W. E. Moerner. Since then, the field of single-molecule spectroscopy has exploded with all types of biological applications. In particular, it now possible to count the copy numbers of proteins in a single cell.[16]

Today, LIF has become globally popular. This gives me much pleasure because I have shared its success with other people. Now that I am a bit older, I have met young students who explain to me what LIF is, how it works, and why it facilitates what they are investigating. They are blissfully unaware that I am the originator of this technique. This situation is wryly amusing but sobering. It allows me to assess the worth of what I have done and how we all take for granted the struggles of others who came before us.

References

1. D. Martin, *New York Times* Obituary, May 11, 2007.
2. R. N. Zare, W. R. Cook, Jr., and L. R. Shiozawa, "X-Ray Correlation of the A-B Layer Order of Cadmium Selenide with the Sign of the Polar Axis," *Nature* **189**, 217-219 (1961).
3. A. Javan, W. R. Bennett, and D R. Herriott, "Population Inversion and Continuous Optical Maser Oscillation in a Gas Discharge Containing a He-Ne Mixture," Phys. Rev. Lett. **63**, 106-110 (1961).
4. W. J. Tango, J. K. Link, and R. N. Zare, "Spectroscopy of K_2 Using Laser-Induced Fluorescence," *J. Chem. Phys.* **49**, 4264-4268 (1968).
5. S. Datta, R. W. Anderson, and R. N. Zare, "Laser Isotope Separation Using an Intracavity Absorption Technique," J. Chem. Phys. **63**, 5503-5505 (1975).
6. D. D.-S. Liu, S. Datta, and R. N. Zare, "Laser Separation of Chlorine Isotopes. The Photochemical Reaction of Electronically Excited Iodine Monochloride with Halogenated Olefins," J. Am. Chem. Soc. **97**, 2557-2558 (1975).
7. R. N. Zare, "Laser Separation of Isotopes," Sci. Am. **236**, 86-98 (1977).
8. K. C. Smyth and D. R. Crosley, "Detection of Minor Species with Laser Techniques," Applied Combustion Diagnostics, K. Kohse-Höinghaus and J. B. Jeffries , Eds.. Taylor & Francis, London (2002) pp. 9 -68.

9. A. Schultz, H. W. Cruse, and R. N. Zare, "Laser-Induced Fluorescence: A Method to Measure the Internal State Distribution of Reaction Products," *J. Chem. Phys.* **57**, 1354-1355 (1972).
10. D. L. Feldman, R. K. Lengel, and R. N. Zare, "Multiphoton Ionization: A Method for Characterizing Molecular Beams and Beam Reaction Products," *Chem. Phys. Lett.* **52**, 413-417 (1977).
11. J. G. Pruett and R. N. Zare, "State-to-State Reaction Rates: Ba + HF(v=0,1) ® BaF(v=0-12) + H," J. Chem. Phys. **64**, 1774-1783 (1976).
12. G. J. Diebold and R. N. Zare, "Laser Fluorimetry: Subpicogram Detection of Aflatoxins using High-Pressure Liquid Chromatography," Science **196**, 1439-1441 (1977).
13. E. Gassmann, J. E. Kuo, and R. N. Zare, "Electrokinetic Separation of Chiral Compounds," Science **230**, 813-814 (1985).
14. V. P. Burolla, S. L. Pentoney, Jr., and R. N. Zare, "High-Performance Capillary Electrophoresis," American Biotech. Lab. **7**, 20-26 (1989).
15. S. Nie, D. T. Chiu, and R. N. Zare, "Probing Individual Molecules with Confocal Fluorescence Microscopy," Science **266**, 1018-1021 (1994).
16. B. Huang, H. Wu, D. Bhaya, A. Grossman, S. Granier, B. K. Kobilka, and R. N. Zare, "Counting Low-Copy-Number Proteins in a Single Cell," Science **315**, 81-84 (2007).