FORESIGHT INSTITUTE

2018 Atomic Precision for Healthspan and Longevity Competition

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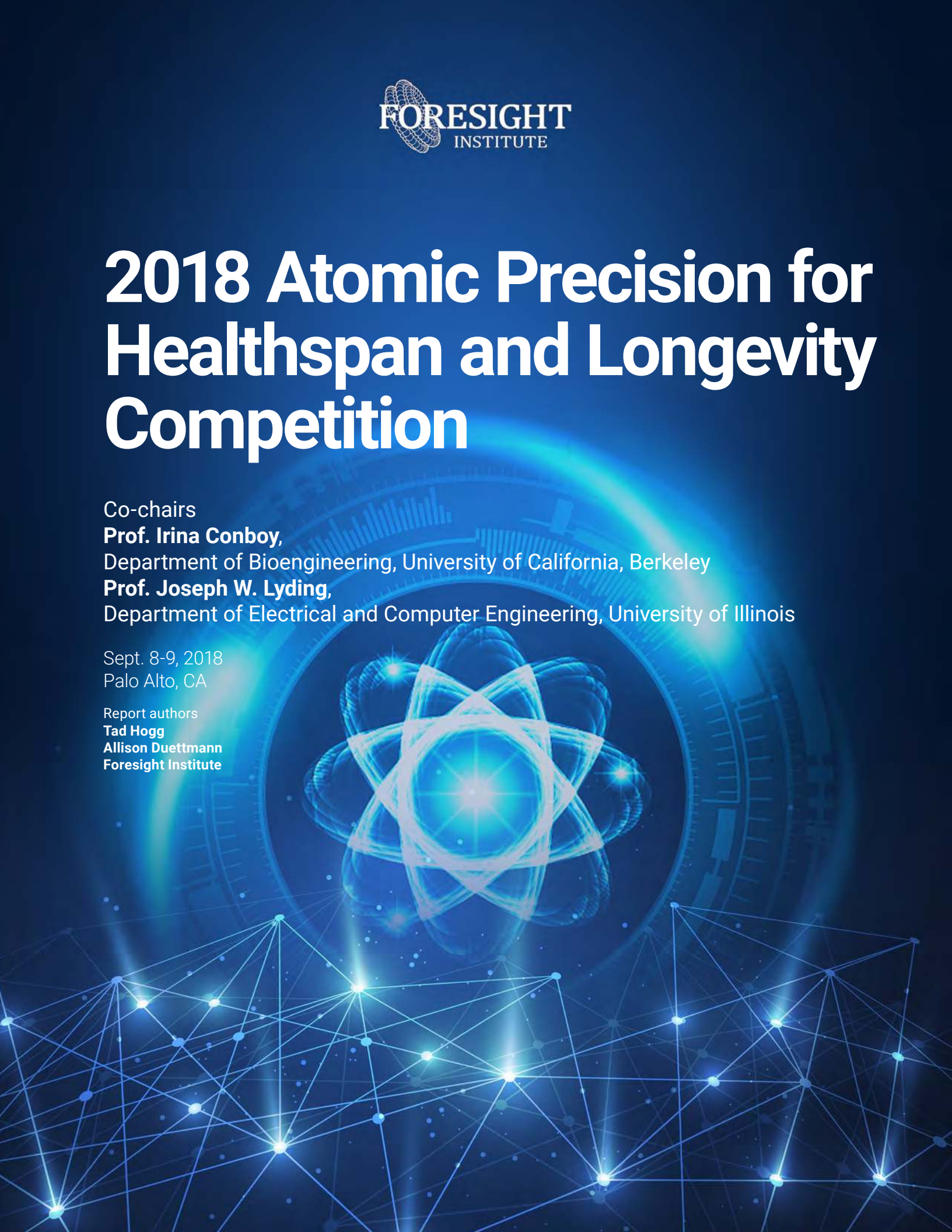

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About Foresight

The Foresight Institute steers emerging and world-shaping technologies for beneficial purposes and has done so for more than 30 years. It is our mission to spark innovation across multidisciplinary fields such as synthetic biology, artificial intelligence, and molecular nanotechnology. We serve as a nexus for innovation to catalyze research, reward excellence, restrain recklessness, and create community aimed at the long-term flourishing of life and the biosphere.

Foresight Team

- **Steve Burgess**: President, Foresight Institute
- **Allison Duettmann**: Co-Facilitator, Foresight Institute
- **Christine L. Peterson**: Co-Founder, Past President of Foresight Institute
- **Lou Viquerat**: Co-Facilitator, Foresight Institute
- **Marcia Seidler**: Events Coordinator, Foresight Institute

Participant List

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Eric Altman	Yale Univ, Dept. of Chem & Environ Eng
Jeremy Barton	Nanofactory
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Martin Edelstein	Covalent / AguaVia
Steve Fowkes	Nanopolymer Systems
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Hanadie Yousef	Juvena Therapeutics

Executive Summary

The aging world population is increasing the human and economic burden of age-related diseases such as cancer and dementia. This is despite the significant resources devoted to developing treatments for these diseases. An alternative to focusing on individual diseases is to slow or reverse aging. Because aging involves damage to cells throughout the body at molecular scales, effective treatment of aging will require broad interventions at this scale. Pharmaceuticals are one approach, but are limited in their precision and capabilities. Overcoming these limitations requires more sophisticated treatment options, such as a variety of complex machines small enough to repair individual cells. Atomically precise manufacturing has the potential to produce such machines in the large quantities necessary for effective treatments and do so at low cost.

This competition continued a series of competitions to identify opportunities for collaborative projects in using atomic precision to increase longevity. It started with an overview of methods and the relevant scientific fields, and was followed by exploratory discussions in small groups and the development of collaborative project proposals that were evaluated by an expert jury. We summarize the highlights in this report and in the video above. To learn more about our competitions, please reach out.

Toward a long and healthy life for all of us,

Allison Duettmann
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Atomic Precision for Longevity Competition Summary

See the video

Introduction

Goals, Methodology, Processes

Christine Peterson opens the technical competition by explaining Foresight's methods and processes and how to get the most out of them.

Foresight's Technical Competitions: Goals, Methodology, Processes - Christine Peterson

See the video

The Plasticity of the Age

Irina Conboy described aging as a regulated process, in contrast to the conventional view of aging as damage inevitably accumulated over time from external factors. An example of a different aging regulation is the difference between squirrels, with 30-year life spans, and rats, with 3-year life spans, in spite of those animals having similar metabolic rates, environments, and foods.

Plasticity of the Age - Irina Conboy See the video

This change in our understanding of aging has analogies in other medical conditions. For example, centuries ago infections were not treatable. Unless the body's immune system could clear the infection, infections would continue

to get worse. With the development of antibiotics, such infections were often treatable. Moreover, in some cases, infections are now preventable through vaccination. A more recent example is the change in our understanding of the process of cellular differentiation. Until fairly recently, the conventional wisdom was that differentiated cells cannot go back to their undifferentiated state. Now we know it is possible to reverse this process, converting differentiated adult cells back to stem cells.

The effect of aging at a cellular level varies with the type of cell. Most cells are fully differentiated. They accumulate damage and have inactive telomerase. Stem cells, on the other hand, accumulate damage at a much lower rate, can activate telomerase as needed, and remain young even in old individuals. They can create new young tissue. Senescent cells have considerable damage to their DNA and internal structures, and are resistant to apoptosis. Such cells accumulate with time.

We are currently learning how to reverse aging, in contrast to the conventional view of aging as an irreversible decline. Important evidence for this viewpoint comes from parabiosis experiments, which show that extrinsic cues in blood can quickly rejuvenate old tissue or age young tissue.

Recent experiments indicate this effect is not mainly due directly to factors in blood. Instead, the changes are largely due to an old animal sharing use of a young animal's organs. In particular, the benefit appears to arise from restoring cell-niche signaling to stem cells. In old animals, stem cells are inhibited by signals in their microenvironments. Young blood can reverse these signals.

These experiments lead to a paradigm shift on aging away from the view of aging as an irreversible accumulation of damage. Instead, systemic and local niches reversibly inhibit stem cells in old tissue. This observation motivates developing methods to filter old blood to remove stem-cell inhibiting factors, and identifying proteins in young blood capable of rejuvenating old tissue. There is not a single "silver bullet" protein to reverse aging. Instead, many proteins from young animals can rejuvenate old animals by altering signal networks for stem cells.

In conclusion, tissue stem cells are numerous and functional enough in old mammals to regenerate tissue but their function is inhibited by factors that increase over time. Thus, boosting the regenerative responses of stem cells could likely attenuate and even reverse aging. This requires identifying and modifying the signals stem cells receive from surrounding cells. Machines able to measure and adjust such signals on the scale of individual cells could enable such treatments.

Atomic Precision for Longevity

David Forrest described the goals of the Department of Energy program for atomically precise manufacturing. Such manufacturing aims to produce products with every atom at its designed position and with desired bonds to other atoms. This capability will lead to a dramatic increase in our ability to make, manipulate, and react to molecules. There is no size restriction on the products of atomically precise manufacturing, beyond what is feasible with available technology.

Currently, we have atomic precision from chemistry assembling molecules and atom-by-atom positional assembly with atomic force microscopes. These approaches are limited to creating structures with a small number of atoms. The goal for atomically precise manufacturing is to extend this precision to much larger structures, and with a faster manufacturing rate.

Introduction

Atomically precise manufacturing contrasts with other terms commonly thought to have similar meanings:

- "nanotechnology": the National Nanotechnology Initiative defines this field based on size: nanometer-scale products;
- "nanomedicine": the application of nanotechnology to medicine;
- "precision nanomedicine": nanomedicine treatments that account for individual variability in genes, environment, and lifestyle, not "precision" referring to treatment devices;
- "pharmaceuticals": drug molecules.

Leveraging Atomic Precision for Longevity - David Forrest

See the video

Current nanomedicine mainly involves functionalized nanoparticles. These are useful technologies but are not atomically precise. Drug molecules are atomically precise. But their manufacture can only produce molecules with relatively small numbers of atoms, which limits their capabilities. For example, drug molecules are too small to include complex molecular machines, such as computers that could precisely determine the circumstances under which the drug should act to avoid damaging healthy cells.

While nanoparticles and drugs are useful, atomically precise manufacturing could produce far more complex microscopic machines able to:

- repair cells at the molecular level to address aging-associated damage that natural repair processes do not handle;
- remove undesirable structures without harming other nearby cells, e.g., plaques in Alzheimer's;
- filter chemicals to remove toxins from the body; and
- improve natural immune systems, e.g., for better or faster recognition of infections.

Importantly, a fully developed capability for atomically precise manufacturing should be able to produce the large numbers of such machines required for useful interventions and at low per-unit cost.

Creating and assembling molecular building blocks into larger structures can achieve atomically precise manufacturing. Chemical synthesis can already make a wide variety of molecules that are useful building blocks. These include DNA origami and engineered proteins. These individual molecules are atomically precise. The challenge is assembling these small atomically precise building blocks into larger structures while maintaining atomic precision. In particular, this assembly must avoid unwanted side reactions. Two approaches to assembling building blocks with atomic precision are self-assembly and positional assembly.

Self-assembly involves making parts that order themselves precisely without defects and form chemical bonds. An example of simple self-assembly is the creation of atomically precise, single-molecule thick membranes. These are highly selective and non-fouling. Applications of such membranes include water purification (Covalent LLC) and hydrocarbon separation (Chris Schafmeister's lab).

Self-assembly can also create far more complex atomically precise structures, as demonstrated by biological organisms. Examples include the assembly of bacteria flagella motors and the T4 virus.

Positional-assembly directly positions individual atoms and molecules to create bonds at precise locations. Unlike the random motions of self-assembly, positional control allows bonding at only desired locations even if there are other highly reactive sites close to that location. Two approaches to positional assembly are a scanning probe microscope with nanoscale tips, and molecular machine systems with controlled movement. The latter can fabricate structures layer by layer, providing an atomically precise version of 3D printing.

The Department of Energy's Advanced Manufacturing Office program for atomically precise manufacturing supports several projects on probe tip-based positional assembly with a budget of about $18 million. With 2D positional assembly, these projects are creating novel nanoelectronic devices (Zyvex) and a MEMS STM platform for high-speed lithography with atomic accuracy (U. of Texas at Dallas). A 3D assembly project is mechanosynthetic 3D extraction of atoms to sharpen atomically precise scanning probe microscope tips (UCLA).

An alternative to tip-based positional assembly is DNA origami. This can produce molecular machines for controlled 2D patterning. In this project, stepper motors self-assembled from DNA are externally controlled to produce precise motion. This provides accurate controlled writing on the surface (Dana-Farber Cancer Institute).

Biology illustrates the potential to improve positional assembly by the use of more elaborate molecular machines, operating in parallel to produce large quantities of atomically precise products. For example, ribosome assemblers, operating in parallel within bone marrow cells produce 1014 hemoglobin molecules per second.
Ribosomes are programmable molecular assemblers of precise amino acid sequences as specified by instructions encoded in RNA. Bacteria cells each have around 20,000 ribosomes and mammal cells about 10 million, giving a great deal of parallel operation even within a single cell.

The development of atomically precise manufacturing will improve on these methods to mechanically guide molecules to desired locations for bonding. This will enable the complex molecular machines required for atomically precise repair of cells. For example, natural DNA repair enzymes do not completely repair DNA damage. These incomplete repairs contribute to aging. Manufactured molecular machines could more thoroughly repair DNA because such machines could incorporate a wide variety of proven error correction and repair capabilities not found in biological organisms.

In addition to the substantial technical challenges in realizing atomically precise manufacturing, improving public perception is an important task. This is due to many media reports on medical nano-scale technologies already in use or demonstrated in laboratories. Sometimes these reports refer to existing technologies, such as drug delivery with nanoparticles or DNA-origami, as "nanorobots". This feeds the perception that medical nanorobots are already here, suggesting there is no need for funding development of atomically precise manufacturing to obtain such robots.

Furthermore, there is considerable public skepticism of the prospect of reversing aging. Thus, while longevity applications are a significant application for future atomically precise manufacturing, this is not currently perceived as a useful justification for its development. Instead, proponents find themselves constrained to focus on applications more directly accepted as feasible and relevant to a funder's mission.

Needs and Capabilities

Previous competitions in this series identified areas requiring further research and development to apply atomically precise manufacturing to medicine, including treating aging. Discussions at the 2018 workshop continued to emphasize these areas as well: the need to further develop atomically precise manufacturing and identify the biomarkers we need to detect and manipulate to significantly improve treatment options.

The lack of quantitative knowledge of age-related biomarkers, and which are causally related to aging, was a recognized limitation during the project proposal discussions. Specifically, we need to determine what to detect, at what level of sensitivity, and from what types of cells. How do measurements of DNA, RNA, proteins, and other biomarkers in individual cells relate to a person's health status? This requires not only improved identification of markers, but also large data analysis to evaluate the implications of these markers and how they change with time naturally and during treatments.

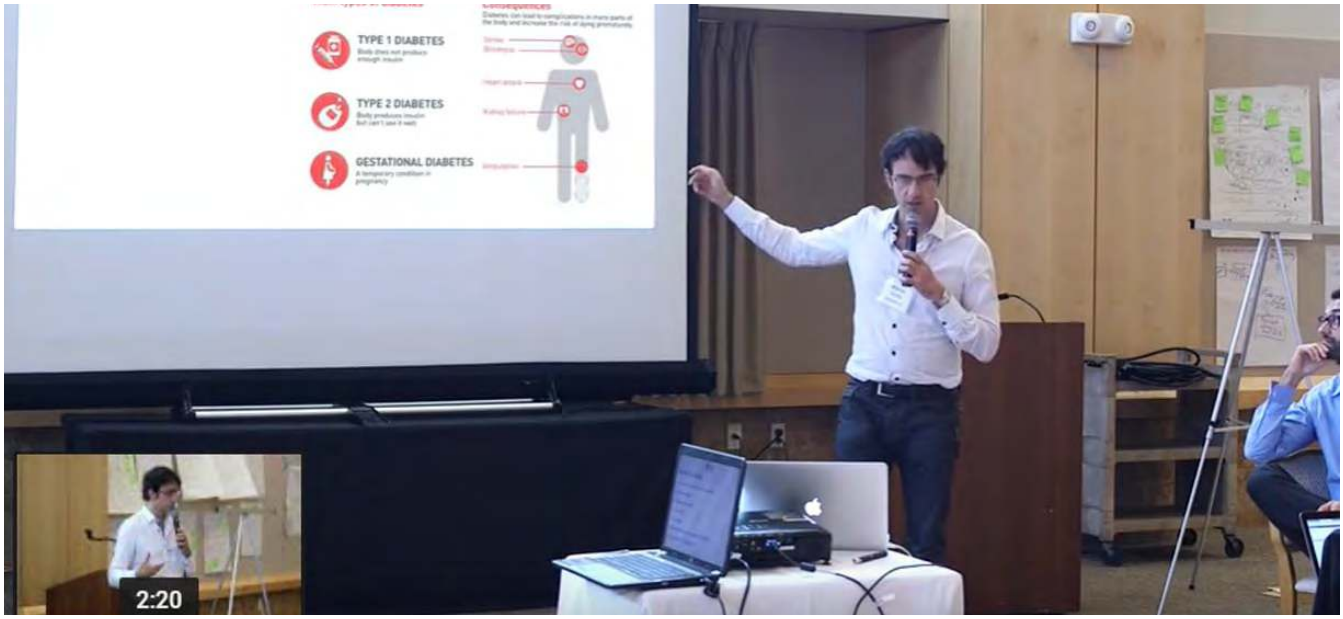
Quantitative estimates are important both for developing treatments and for indicating the required capabilities to aim for when designing atomically precise devices. To aid their use in engineering design, quantitative biomarker information needs to be easily available to engineers. An example of such an effort is the Bionumbers.org project at Harvard that collects quantitative biological information from the scientific literature in a convenient form.

One area identified at the 2017 workshop was proving a quantitative measure of all proteins in a sample (blood or tissue), similar to today's ability to measure gene expression. As described below, one of the project proposals in this 2018 workshop specifically addressed this need.

Project Proposals

The judges selected *SymBioWatch* as the best application of atomic precision to longevity. All workshop participants also voted on the projects and P*roteinReader* received the most votes for the "People's Choice Award".

SymBioWatch: **Implant to Continually Measure Health Status**



SymBioWatch: a Wearable for Longevity - Marco Quarta

See the video

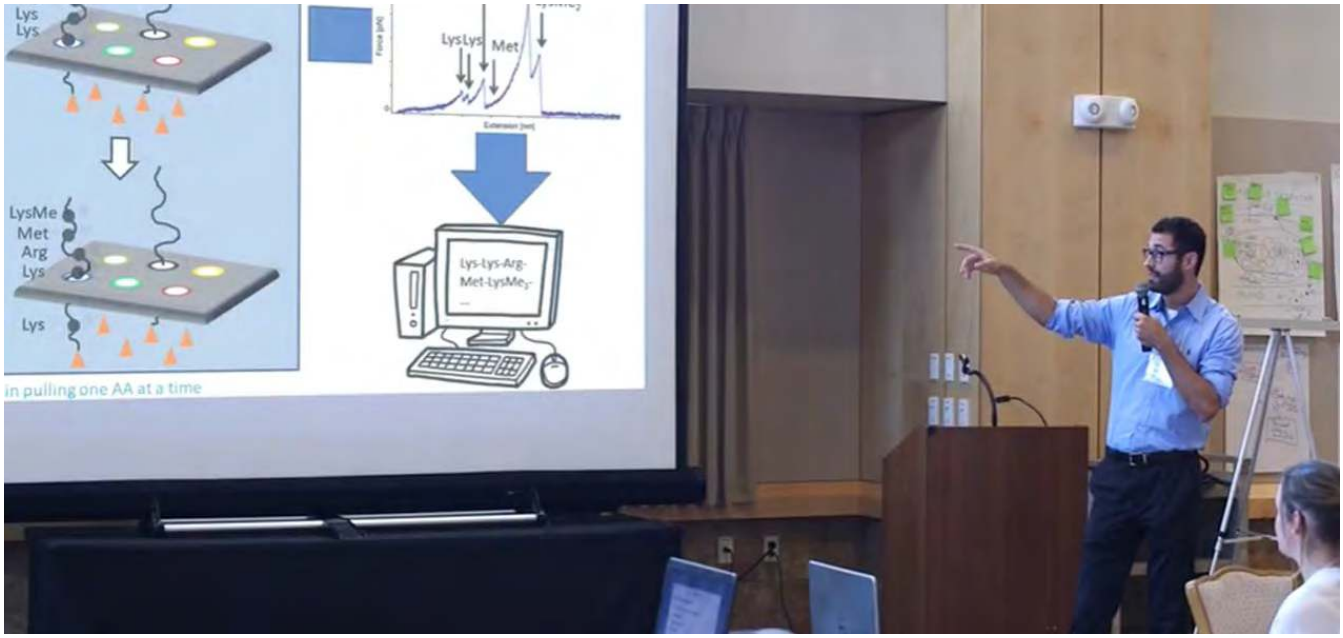
Often disease is not addressed in early stages due to a lack of real time information on body processes. To prevent diseases and promote longer healthspans this project will create an implantable, high-precision sensor to monitor the body continuously and deliver actionable information in real time. This could allow people to adjust diet, lifestyle, and

drug dosage to prevent diseases or allow early treatment as advised by their physician. The first applications of this monitor will be for the early detection of diabetes and breast cancer biomarkers, due to their widespread occurrence and well-characterized markers. Longer term, the monitor could measure biomarkers related to healthspan and longevity.

The proposed device consists of a small microfluidic lab-on-chip with a tiny inlet from a nearby blood vessel. The device collects and sorts cells, lysing them to provide material for analysis. The device uses a nanopore membrane for protein sorting and plasmonic detection of chemicals. The technology in this proposal has been tested, validated, and published. But the components have not been integrated, miniaturized, and optimized for in vivo detection. This integration will require evaluating the compatibility of different techniques and their sensitivity to the biomarkers involved in specific applications.

This project will give each user a personalized health profile that recommends nutrition, sleep, exercise, and medications to promote healthspan and longevity. Beyond providing health information for individual users and their physicians, aggregation of the data from millions of people will improve the predictive capabilities from the measured data and hence improve the personalized recommendations.

Protein Reader: **Tool for High-Throughput Single Protein Sequencing**



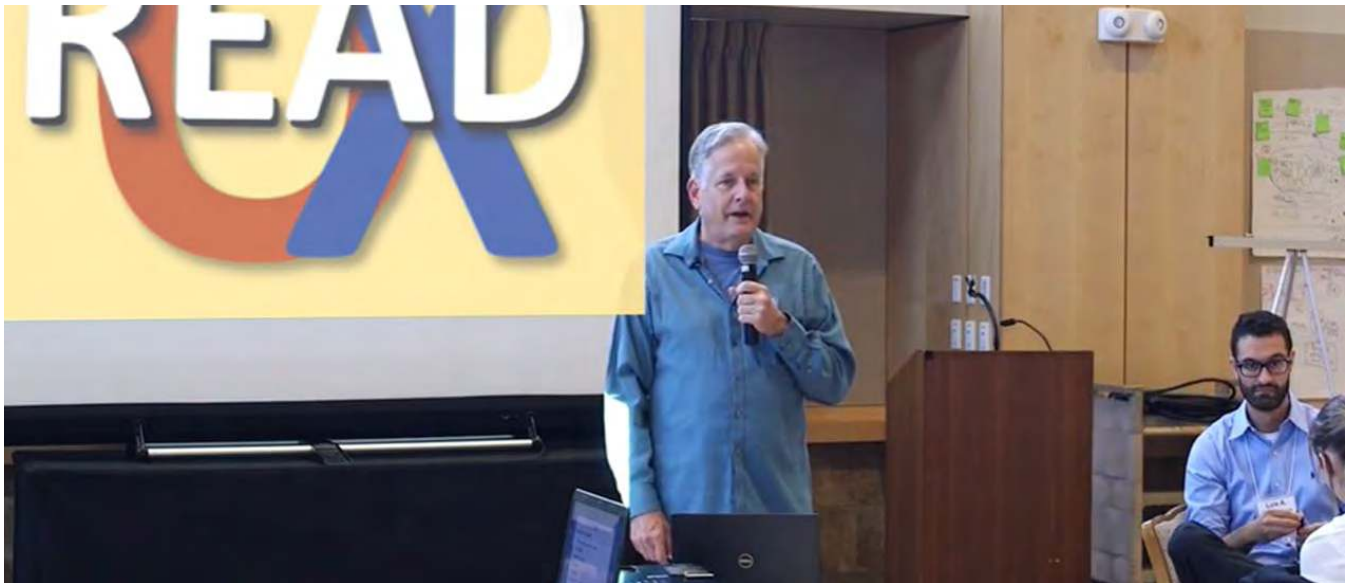
Protein Reader for Longevity - Luis A. Ruis-Pestana

See the video

We could improve our understanding of age-related changes with information on the activity of individual cells and how they change as we age. While gene studies are useful, measuring the proteins actually active in cells at various times will give a more precise evaluation of cell activity. Specifically, determining the sequence of an individual protein molecule with single amino acid precision could detect post-translational modifications in the sequence. This would be useful for the early detection of aging biomarkers.

This project provides access to the proteome with single molecule precision by using atomic force microscopes to pull denatured proteins through pores in a surface. Atomically precise manufacturing will allow creating pores with functional modifications to distinguish amino acids based on their charges and whether or not they are hydrophobic. Measuring force and displacement while pulling will identify the sequence of amino acids. Using a large array of pores and individually controlled atomic force microscope tips will process many proteins in parallel.

ReadOx: **Using Nanotechnologies to Quantify Subcellular Processes**



ReadOX: Mitochondrial Probing for Longevity - Steve Fowkes

See the video

Detailed quantitative evaluation of functions inside cells could aid health management, disease treatment, and rejuvenation therapies. This project will provide such measurements of the metabolism of a sample of mitochondria within cells. To do so, the project will build a device small enough to interact with a single cell and extend probes into some of the cell's mitochondria.

These probes will determine the redox status of chemicals that characterize mitochondria energy production. These measurements will identify the functional status of the mitochondria. Since their energy production capabilities decline with age, these detailed, individual measurements will be useful to monitor the effectiveness of treatments aimed at slowing or reversing aging.

Conclusion

The three project proposals developed at the workshop illustrate promising possibilities of applying atomically precise devices to improve medicine, including treating aging.

These projects are primarily concerned with developing tools to better monitor health. The projects could benefit from collaborators with more quantitative biological knowledge. Specifically, what markers should these tools target that would be beneficial and not already measured with current technology? This information includes the concentrations of these markers, and those of possible confounding markers for individual cells rather than averages over large populations of cells. This information is necessary to quantitatively evaluate project performance. Such evaluation will improve our ability to understand aging mechanisms and intervene in that process. For example, is it feasible to detect the desired markers with tiny sensors? Or will the devices require sophisticated computation to distinguish measured values from noise, such as provided by machine learning?

We encourage further research on the quantitative evaluation of the biological mechanisms involved in aging, such as signaling mechanisms in stem cell niches. This will identify the type of hardware necessary to measure and intervene in aging. In particular, this will distinguish aging treatments that require atomic precision from those that could be adequately treated with less precise and less capable devices, such as functionalized nanoparticles. For instance, atomic precision is necessary for surface antibody binders. Such quantitative information could help develop a wide variety of longevity applications of atomically precise manufacturing.

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