Joshua N. Leonard

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Education

- **University of California, Berkeley** Berkeley, CA
  Doctor of Philosophy in Chemical Engineering (May 2006)
  Overall GPA: 3.9/4.0
- **Haas School of Business, UC Berkeley** Berkeley, CA
  Management of Technology Certificate (May 2005)
- **Stanford University** Stanford, CA
  Bachelor of Science in Chemical Engineering (June 2000)
  Overall GPA: 3.7/4.0

Research and Employment Experience

Postdoctoral Fellowship: Modulating innate immunity via Toll-like receptors
National Cancer Institute, Experimental Immunology Branch **Bethesda, MD**
June 2006 – Present: The innate immune system, which is hard-wired into an individual at birth, plays a vital role in the establishment and maintenance of overall immunity. Consequently, innate immune functions have been implicated in both advantageous responses, such as the rapid detection and isolation of invading microorganisms, and in deleterious responses, such as autoimmune diseases including arthritis and lupus. We seek to better understand the structure and function of a group of innate biosensor proteins, called Toll-like receptors (TLRs), that recognize specific molecular patterns associated with infections and play a key role in detecting microbial incursions and directing an appropriate overall immune response. We recently (and for the first time) elucidated the mechanism by which a TLR (TLR3) binds to its ligand (viral double-stranded RNA) to form a complex that initiates immune activation. Ongoing research includes atomic-scale structural analysis of these complexes and investigations into the intracellular trafficking and crosstalk between TLRs. These studies should guide research into the mechanisms and functions of other TLRs and should aid in the development of treatments that modulate immune responses in a specific and controlled manner.

Doctoral Research: Designing Antiviral Gene Therapies to Suppress HIV Infections
University of California, Berkeley **Berkeley, CA**
Aug 2000 – May 2006: Current treatments for HIV infections are often confounded by the rapid evolution of drug-resistant viral strains. A promising development was the discovery RNA interference (RNAi), a powerful technique for specifically inhibiting the expression of genes. Although RNAi treatments that inhibit HIV in the lab were identified, later reports indicated that HIV can evolve resistance to many of these. We sought to identify RNAi treatment strategies that prevent or delay the emergence of resistant viral strains. I engineered a gene delivery vector that stably induces protective RNAi against HIV, and different treatments based on this vector were tested for their ability to suppress HIV in cell culture models. In particular, one inhibitor we developed successfully suppressed the evolution of resistant

viruses. Surprisingly, we later observed that HIV can modulate its network of genetic regulation in order to *compensate* for this inhibition, without directly evolving resistance to the inhibitor. This represents a novel form of viral evolution in response to RNAi, and our analysis identified several routes by which this escape mechanism may be suppressed. To guide this work, I also developed a novel stochastic simulation of the process of HIV replication and evolution, which incorporates known biological details and reaction-network kinetics, and predictions made with this model were later elegantly validated by our experimental work.

Since the clinical application of antiviral RNAi gene delivery depends on the effective production of gene delivery vectors, I also worked to address a challenge in the production of robust vectors based on adeno-associated virus (AAV). Efficient large-scale manufacturing of AAV vectors typically utilizes a separate helper virus, which must be inactivated. Previous approaches used high temperatures, but this also results in a 50% loss of AAV vectors. We developed and patented a novel process that uses high hydrostatic pressure to accomplish helper virus inactivation without any loss of AAV vector. This research also provided insights into the structure and function of AAV vectors.

Engineering Policy Intern
AIChE, W.I.S.E Program **Washington, DC**
Summer 1999: I conducted policy analysis and produced a position statement (see *Publications and Patents*) on behalf of the American Institute of Chemical Engineers (AIChE) that advocated a modified plan to establish an institute at the NIH for biological engineering. This document was distributed as background material at congressional information sessions, hosted by AIChE. Our recommendations were incorporated into a revised bill and facilitated passage of Public Law 106-580, 106th Congress, the "National Institute of Biomedical Imaging and Bioengineering Establishment Act".

Ligand Registrar
Symyx Technologies **Santa Clara, CA**
Feb 1999-June 1999: I coordinated information tracking for combinatorial chemistry applications. I built and administered an internal information system for dynamically tracking structures and properties of novel compounds, allowing synthetic chemists to document, share, and design new molecules.

Technical Development Engineering Intern
Edison Enterprises - Edison Source **City of Industry, CA**
Summer 1998: Engineered and implemented energy-saving modifications to large commercial structures. I devised and implemented a novel method for data collection and interpretation, resulting in significant cost savings. I also designed and built a system for rapidly and inexpensively assembling on-site data using optical reader technology and wrote an internal software package.

Teaching Experience

Graduate Student Instructor **University of California, Berkeley**
Spring 2003: [Undergraduate] Chemical Kinetics and Reaction Engineering
Received Departmental Award for Outstanding Graduate Student Instructor
Spring 2002: [Graduate] Transport Processes
One of four core courses in the graduate chemical engineering curriculum

Teaching Assistant **Stanford University**
Spring 2000: [Undergraduate] Introduction to Chemical Engineering

Publications and Patents

- **Leonard JN**, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, Segal DM. The TLR3 signaling complex forms by cooperative receptor dimerization. PNAS. (in press)
- **Leonard JN**, Bell JK, Segal DM. Predicting TLR structures and characterizing ligand binding. Methods in Molecular Biology. (in press)
- **Leonard JN**, Shah P, Schaffer DV. HIV escapes from RNA interference directed at TAR by a novel compensatory mechanism. (submitted to Cell Host and Microbe)
- Ricci C, Pastukh V, **Leonard JN**, Wilson G, Schaffer DV, Schaffer SW. Mitochondrial DNA damage triggers mitochondrial-superoxide generation and apoptosis. (under review)
- **Leonard JN**, Ferstl P, Delgado A, Schaffer DV. (2007). Enhanced preparation of adeno-associated viral vectors by using high hydrostatic pressure to selectively inactivate helper adenovirus. Biotechnology and Bioengineering *97*, 1170-9. (Featured in a spotlight in this issue)
- Zhang *et al*. (2007). TLR3 deficiency in patients with herpes simplex encephalitis. Science *317*, 1522-7. (I am 21st of 33 co-authors)
- Ashton RS, Peltier J, Fasano CA, O'neill A, **Leonard J**, Temple S, Schaffer DV, Kane R. (2007). High-throughput screening of gene function in stem cells using clonal microarrays. Stem Cells *25*, 2928-35.
- **Leonard JN** and Schaffer DV. (2006). Antiviral RNAi Therapy: Emerging Approaches for Hitting a Moving Target. Gene Therapy *13*, 532-40.
- Schaffer DV and **Leonard JN**. METHODS FOR PURIFYING ADENO-ASSOCIATED VIRUS. US Provisional patent. (pending, filed March 2005).
- **Leonard JN** and Schaffer DV. (2005). Computational Design of Antiviral RNA Interference Strategies that Resist Human Immunodeficiency Virus Escape. Journal of Virology *79*, 1645-1654.
- **Leonard JN**. (1999). United States bioengineering research programs: a critical analysis. WISE Journal of Engineering and Public Policy *3*. (online publication: http://www.wise-intern.org/)

Achievements and Awards

- NIH Cancer Research Training Award Postdoctoral Fellowship, 2006
- BaCaTeC (Bavaria California Technology Center) Grant for Collaborative Research, "High Pressure Inactivation Kinetics of Adeno-Associated Virus", 2004
- AIChE Annual Meeting - Food, Pharmaceutical and Bioengineering Division Student Poster Award (First Prize), 2003
- Departmental Award for Outstanding Graduate Student Instructor, 2003
- Atofina Chemicals Graduate Fellowship, 2001

Leadership and Professional Service

- Session Co-Chair, Self-Assembled Biomaterials I, AIChE Annual Meeting, 2007
- Berkeley Chemical Engineering Graduate Student Advisory Committee (GSAC), 2000-2003
- Stanford AIChE Student Chapter Leadership Council, 1999-2000

Conference Presentations

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- **Leonard JN**, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, Segal DM. "Activating innate immunity through Toll-Like Receptors: mechanism of signal induction is elucidated by quantifying receptor-ligand complex formation." Oral presentation, AIChE Annual Meeting, 2007.
- **Leonard JN**, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, Segal DM. "TLR3 is activated by cooperative receptor dimerization." Poster and selected oral presentation, NIH Immunology Retreat, 2007.
- **Leonard JN**, Ghirlando R, Askins J, Bell JK, Margulies DH, Davies DR, Segal DM. "Identification of the TLR3 signaling complex." Poster presentation, Pattern Recognition Receptors in Human Disease (Biochemical Society Focused Meeting), 2007.
- **Leonard JN** and Schaffer DV. "HIV's evolution of resistance to antiviral gene therapy is predictable and utilizes novel cooperative mechanisms." Oral presentation, AIChE Annual Meeting, 2006.
- **Leonard JN**, Ferstl P, Delgado A, Schaffer DV. "Purification of AAV gene therapy vectors by selectively inactivating helper adenovirus using high hydrostatic pressure." Oral presentation, AIChE Annual Meeting, 2005.
- **Leonard JN** and Schaffer DV "Computational design of RNA interference gene therapy strategies to treat HIV-1 infections and block viral escape." Oral presentation, AIChE Annual Meeting, 2005.
- **Leonard JN** and Schaffer, DV. "Therapeutic design principles for achieving long-term suppression of HIV-1 with RNA interference". Poster presentation, American Society of Gene Therapy (ASGT) Annual Meeting 2005.
- **Leonard JN**, Ferstl P, Delgado A, Schaffer DV. "Selective inactivation of helper adenovirus with high hydrostatic pressure for AAV-2 vector production." Poster presentation, ASGT Annual Meeting 2005.
- **Leonard JN** and Schaffer, DV. "Complex adaptive behavior in HIV evolution of resistance to RNA interference." Oral presentation, AIChE Annual Meeting, 2004.
- **Leonard JN** and Schaffer, DV. "Towards antiviral RNA interference strategies that resist HIV escape". Poster presentation, ASGT Annual Meeting 2004.
- **Leonard JN** and Schaffer, DV. "Anti-HIV RNA interference strategies that resist viral escape." Oral presentation, American Chemical Society (ACS) Annual Meeting 2004.
- **Leonard JN** and Schaffer, DV. "Retroviral evolution of resistance to RNA interference." Poster presentation, AIChE Annual Meeting, 2003.
- **Leonard JN** and Schaffer, DV. "Viral evolution of resistance to RNA Interference." Oral presentation, Biomedical Engineering Society (BMES) Annual Meeting, 2003.
- **Leonard JN**. "United States bioengineering research programs: a critical analysis." Invited oral presentation for special session at the AIChE Annual Meeting, 1999.