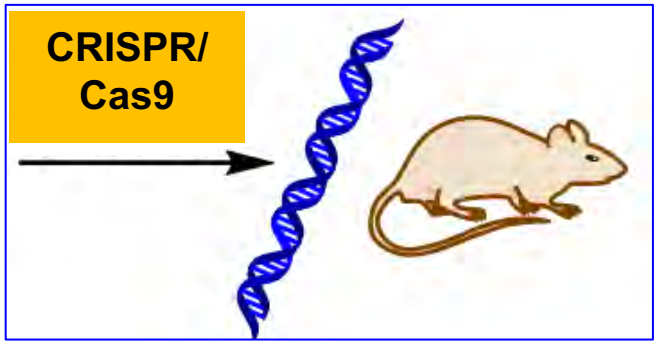
Baylor College of Medicine

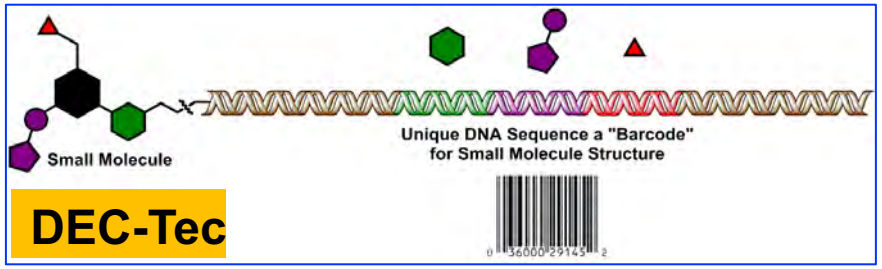
Being (re)productive as a physician scientist

Martin M. Matzuk, MD, PhD

Center for Drug Discovery
Baylor College of Medicine



Aim 1: Apply CRISPR/Cas9 to mechanistically discern the key reproductive tract-specific proteins and their pathways required for spermatogenesis, sperm function, and/or fertilization.



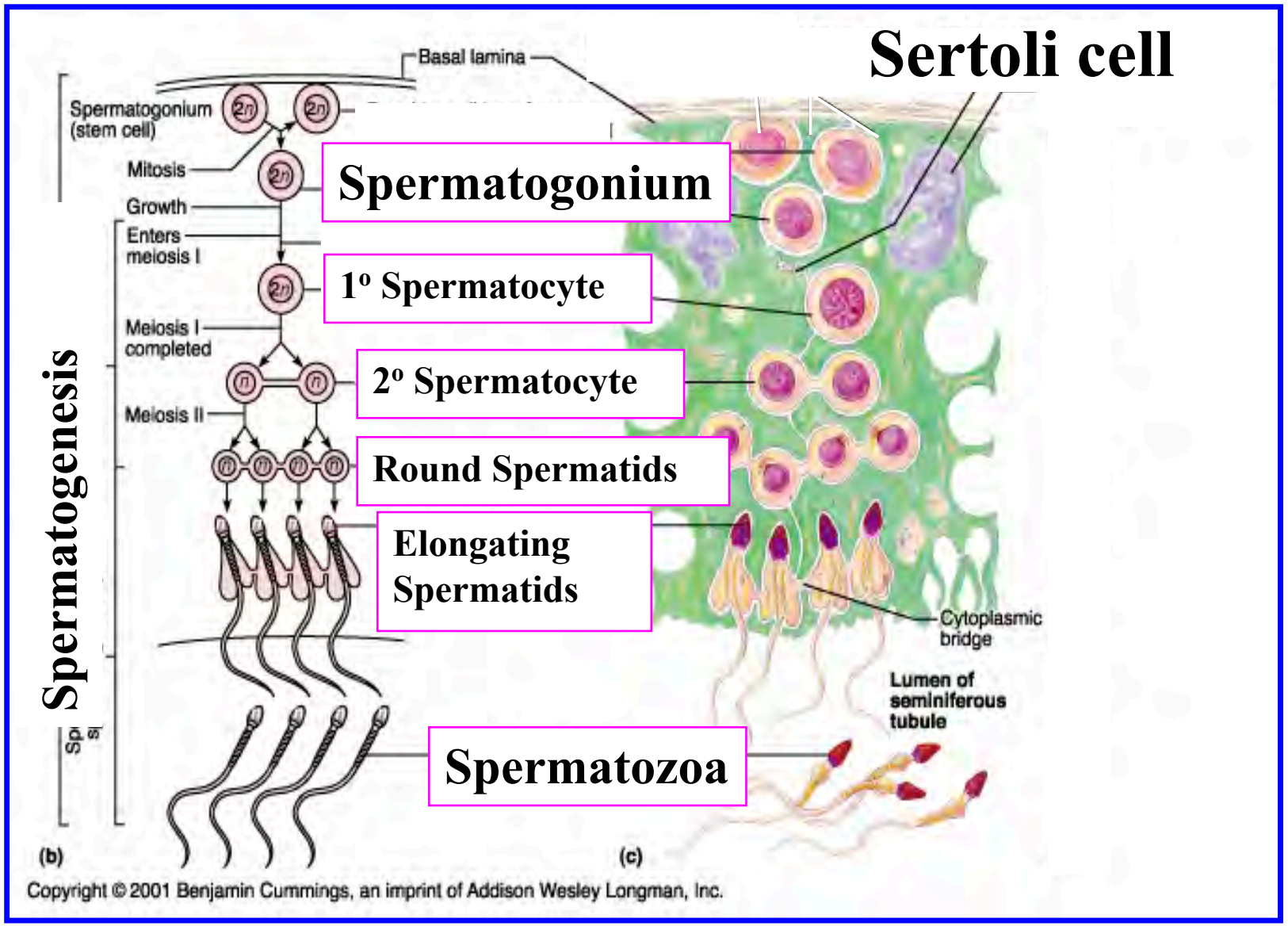
Aim 2: Use DEC-Tec to identify small-molecule probes and preclinical candidates to target druggable proteins essential for spermatogenesis, sperm function, and/or fertilization.

Aim 3: Evaluate *in vitro* efficacy and *in vivo* contraceptive effects of drug-like inhibitors.

Path to Houston

Male Infertility and Contraception

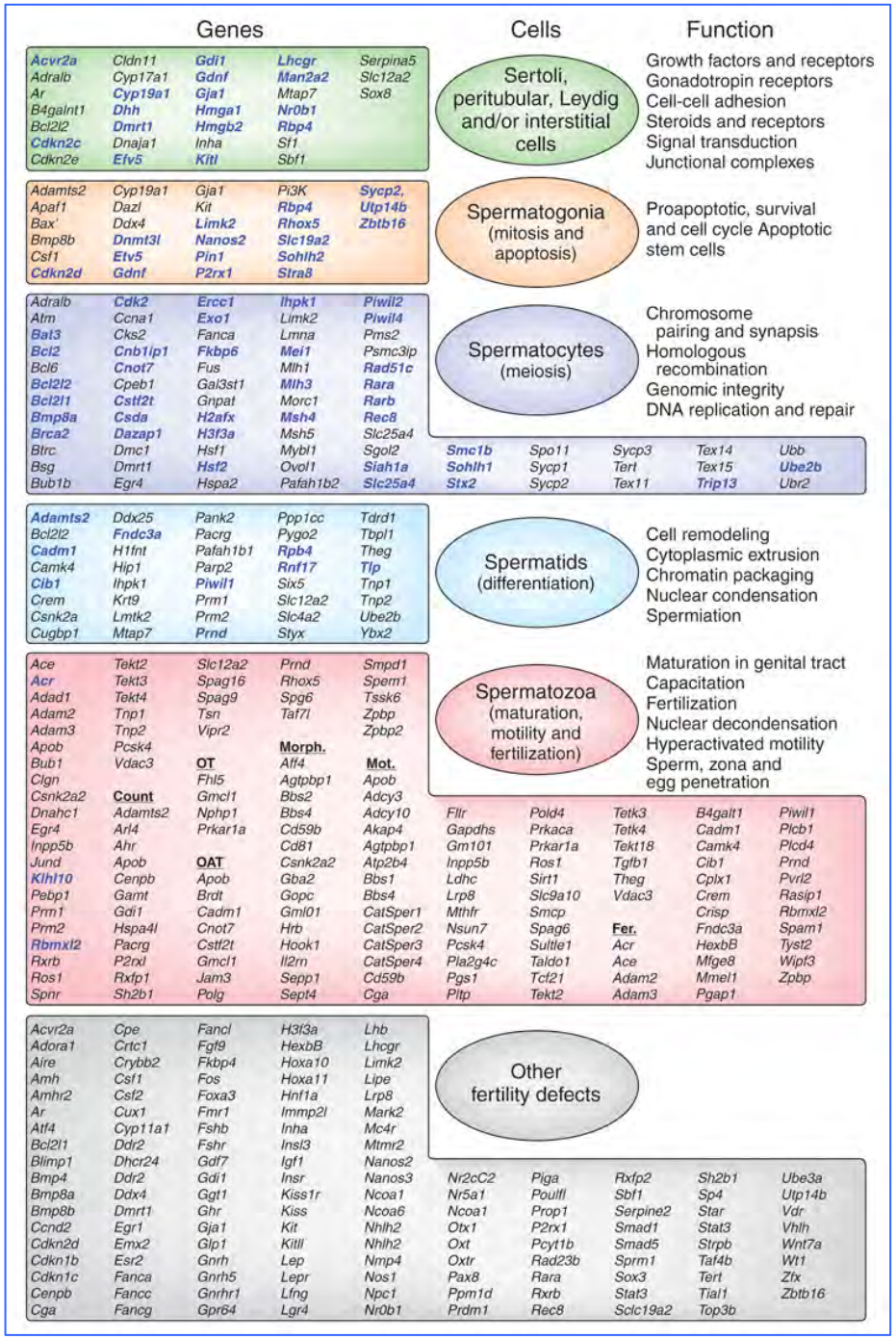
Spermatogenesis is an amazing biological process



Male Introduction

- ~4% of genes expressed in male germ cells (Schultz, Hamra, and Garbers, *PNAS* 2003)
- Mutations in >1000 genes could cause infertility in men
- 100's of candidate germ cell-specific targets for novel contraceptives for men (*i.e.*, where KOs have a phenotype of infertility)

Matzuk & Lamb
Nature Medicine
(2008)



Our first collaborative paper that includes CRISPR/Cas9

(*PNAS* July 12, 2016)

Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice

Haruhiko Miyata[a,1], Julio M. Castaneda[b,c,d,1], Yoshitaka Fujihara[a,1], Zhifeng Yu[b,e,1], Denise Archambeault[b,c,d], Ayako Isotani[f], Daiji Kiyozumi[f], Maya L. Kriseman[b,d], Daisuke Mashiko[a,g,2], Takafumi Matsumura[a,h], Ryan Matzuk[b], Masashi Mori[a], Taichi Noda[a], Asami Oji[a,h], Masaru Okabe[a], Renata Prunskaite-Hyyrylainen[b,d,i], Ramiro Ramirez-Solis[j], Yuhkoh Satouh[a], Qian Zhang[a,3], Masahito Ikawa[a,f,g,h,4], and Martin M. Matzuk[b,c,d,e,k,l,4]

[a]Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 5650871, Japan; [b]Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX 77030; [c]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030; [d]Center for Reproductive Medicine, Baylor College of Medicine, Houston, TX 77030; [e]Center for Drug Discovery, Baylor College of Medicine, Houston, TX 77030; [f]Immunology Frontier Research Center, Osaka University, Suita, Osaka 5650871, Japan; [g]Graduate School of Medicine, Osaka University, Suita, Osaka 5650871, Japan; [h]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 5650871, Japan; [i]Faculty of Biochemistry and Molecular Medicine, University of Oulu, FI-90014 Oulu, Finland; [j]Wellcome Trust Sanger Institute, Hinxton CB10 1SA, United Kingdom; [k]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; and [l]Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030

Strategy to characterize these mice

Create mutants with CRISPR/Cas9

Fertility Defect ↓

(Aim 1) Define defect

↓

(Aim 2) Define molecular mechanism of action and signaling pathway

Fertile ↘

Halt analysis; publish results

A. Use CRISPR/Cas9 to insert tags into alleles for *in vivo* protein localization
B. Generate antibodies if necessary
C. Perform proteomics from pull down using tagged proteins
D. Perform additional *in vitro* and *in vivo* functional studies

↓

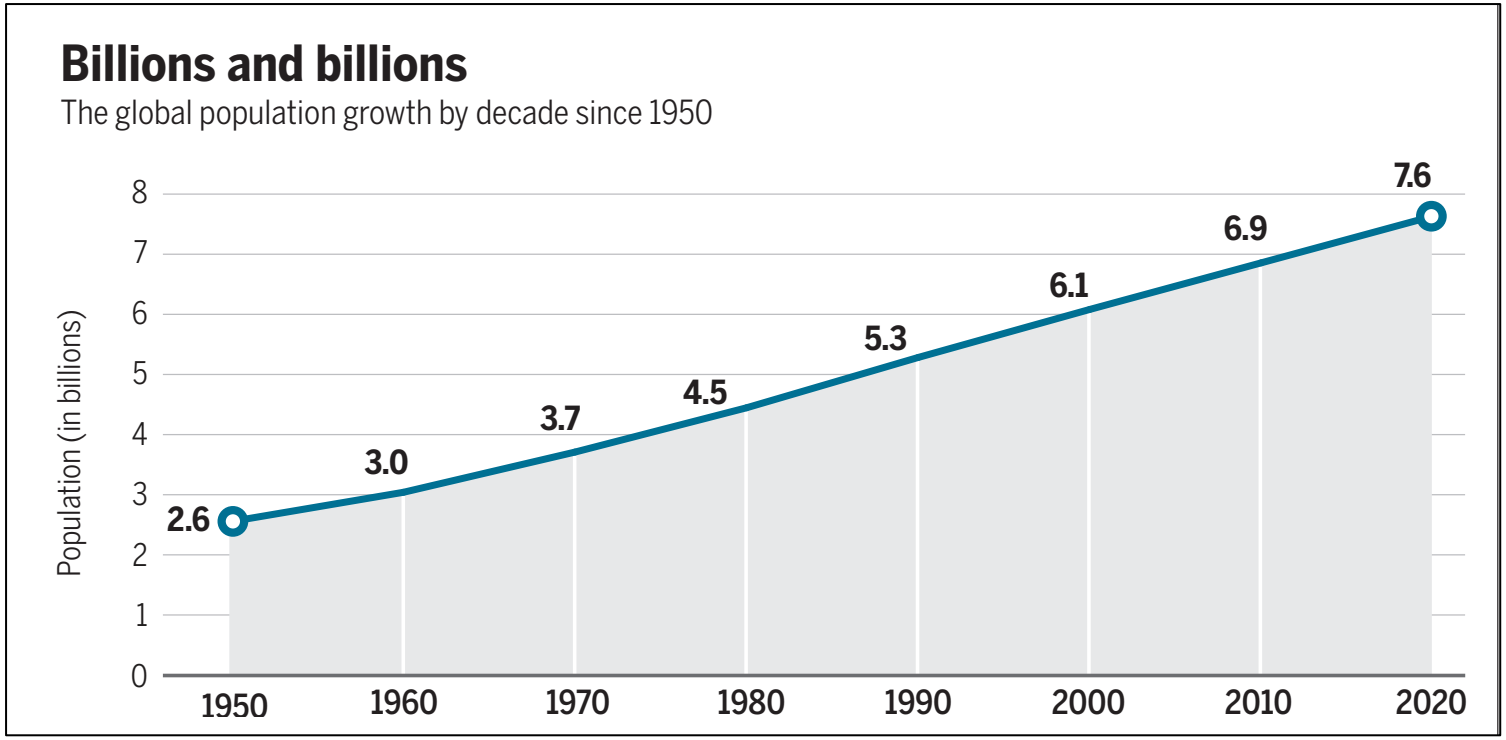
Complete analysis; publish findings

↓ **Sterile mutants (Contraceptive targets)**

A. Generate recombinant proteins
B. Perform drug screen

Our population continues to grow…
7.7 billion in the world today

(Net gain of one person every 16 seconds)



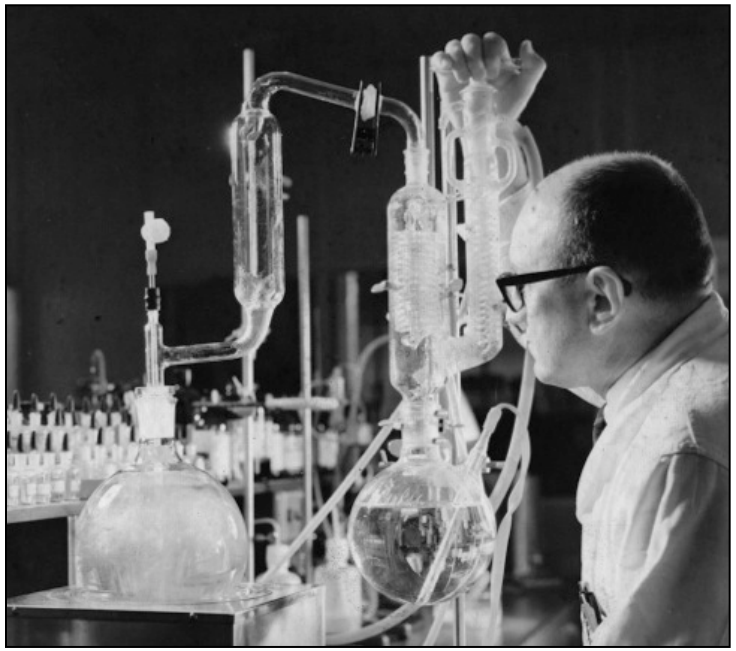
Castaneda and Matzuk, *Science* 2015

Contraception

- 1 million U.S. teenagers get pregnant yearly, 50% end in abortion, and the costs to US taxpayers is ~$10 billion per year
- There are still no oral contraceptives for men

Center for Drug Discovery (CDD)

https://www.bcm.edu/research/centers/drug-discovery



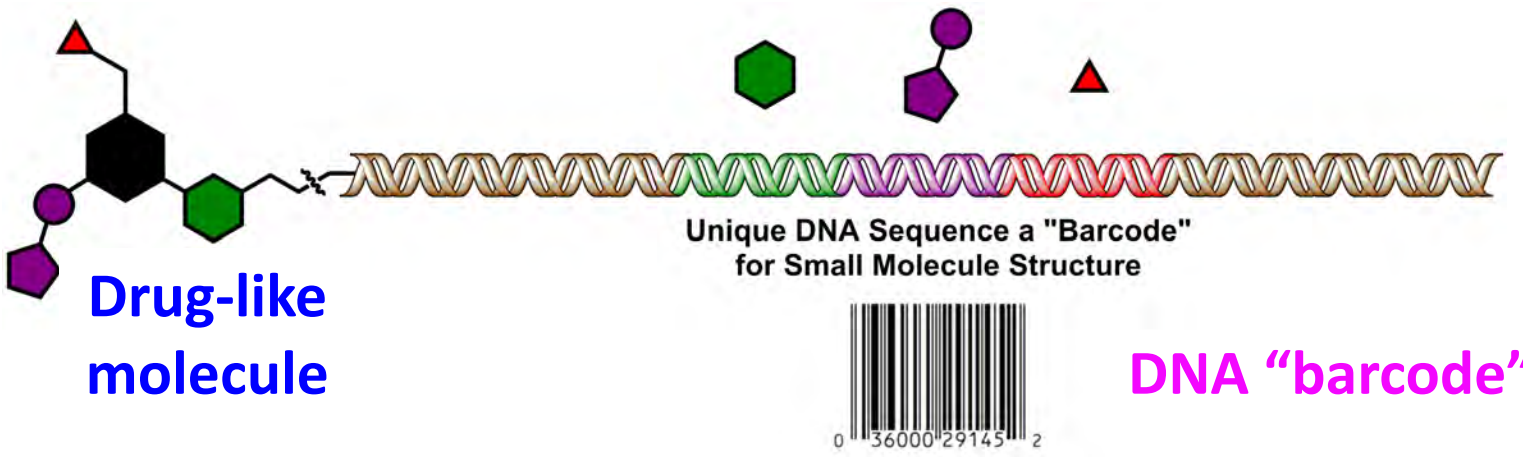
Mission: To develop small molecule probes, preclinical candidates, and drugs for researchers and clinicians in Texas and beyond.

Creating unique compound collections for screening

HTS collections often yield this…

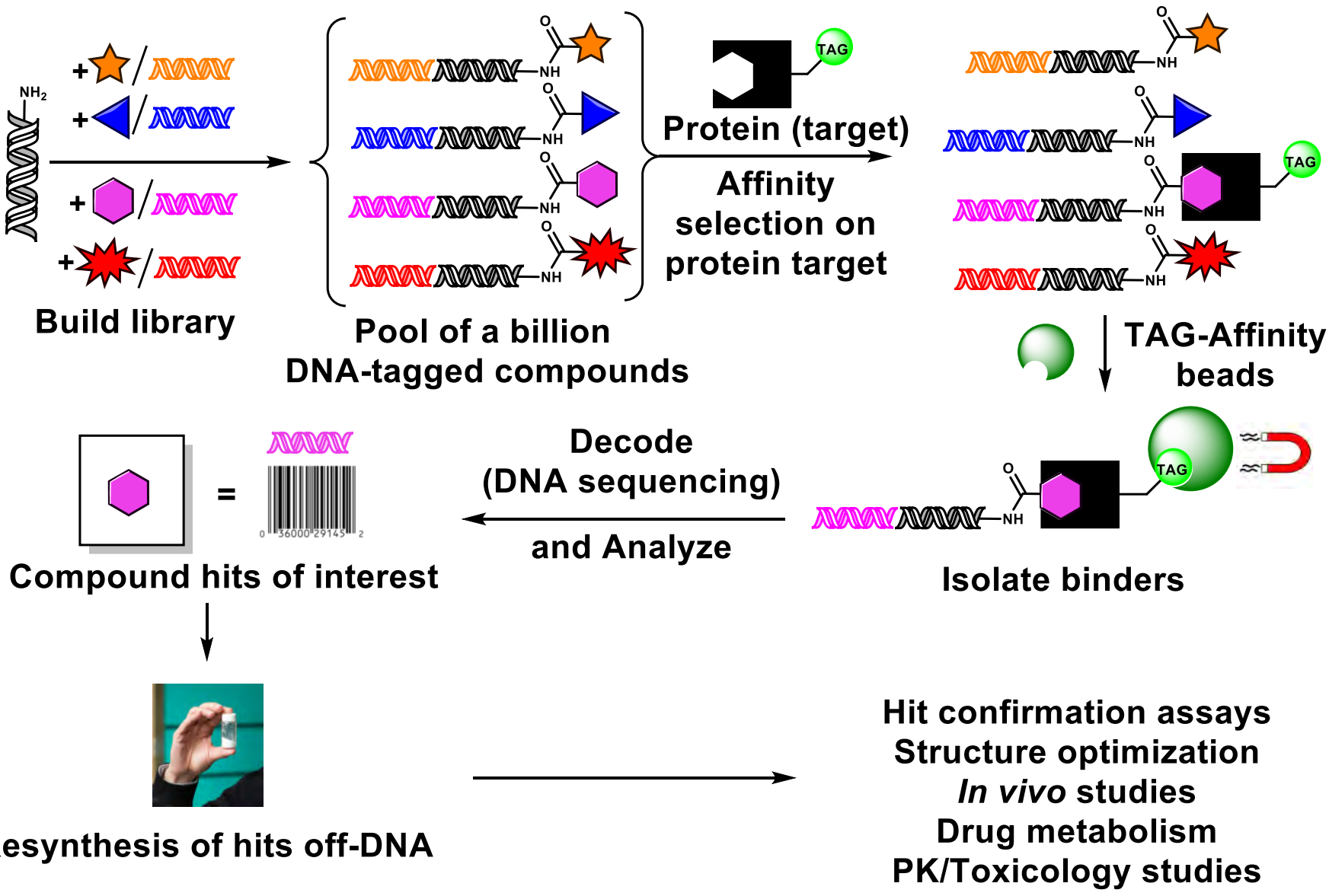
Effective drug discovery requires this…

DNA-Encoded Chemistry Technology (DEC-Tec)



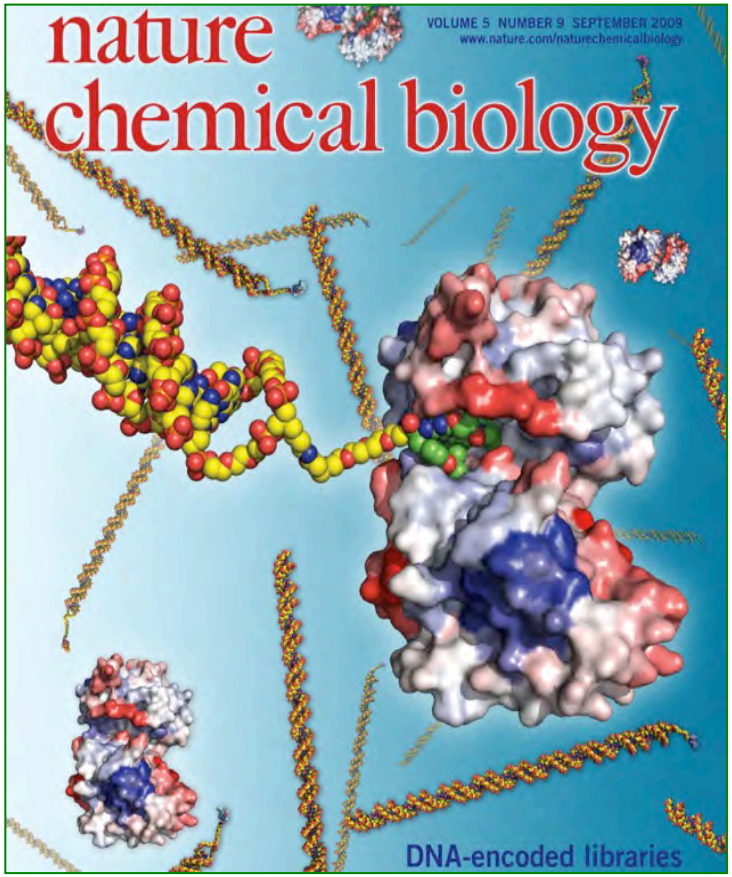
- Synthesize millions of **drug-like molecules** via combinatorial chemistry
- Unique **DNA "barcodes"** enable screens of complex mixtures
- Screen pooled compounds for binding affinity, and then sequence DNA
- Enables wider, cheaper screens than High-Throughput Screening (HTS)

The DEC-Tec Process



DNA-Encoded Chemistry Technology (DEC-Tec)

- Billions of small molecules are screened simultaneously against drug target protein



Clark et al. 2009

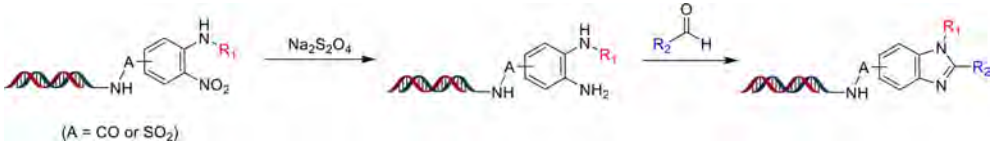
- Like finding a drug-like needle in a billion compound haystack

CDD@BCM’s contributions to DEC-Tec

On-DNA Chemistry Development

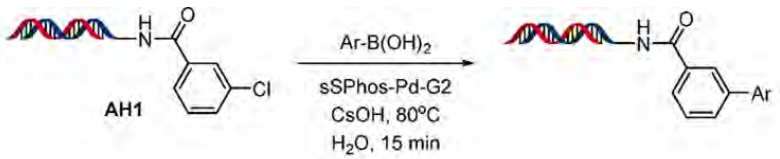
Benzimidazoles

Du et al. *Bioconjugate Chem.* 2017, 28, 10, 2575.



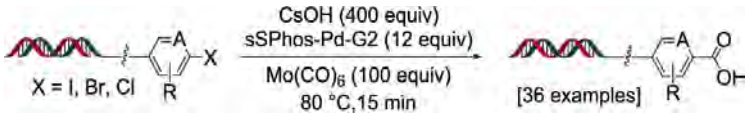
Suzuki-Miyaura

Li et al. *Bioconjugate Chem.* 2018, 29, 11, 3841.



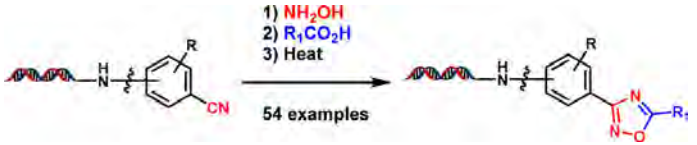
Hydroxycarbonylation

Li et al. *Bioconjugate Chem.* 2019, 30, 8, 2209.



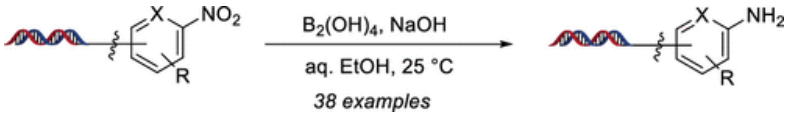
Oxadiazoles

Du et al. *Bioconjugate Chem.* 2019, 30, 5, 1304.



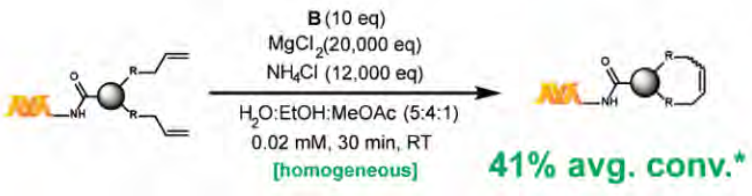
Nitro Reduction

Du et al. *Org. Lett.* 2019, 21, 7, 2194.



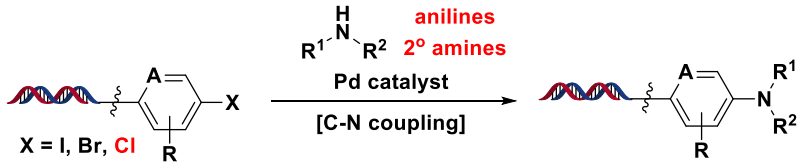
RCM

Monty et al. *ACS Comb. Sci.* 2020 Online



C-N Coupling

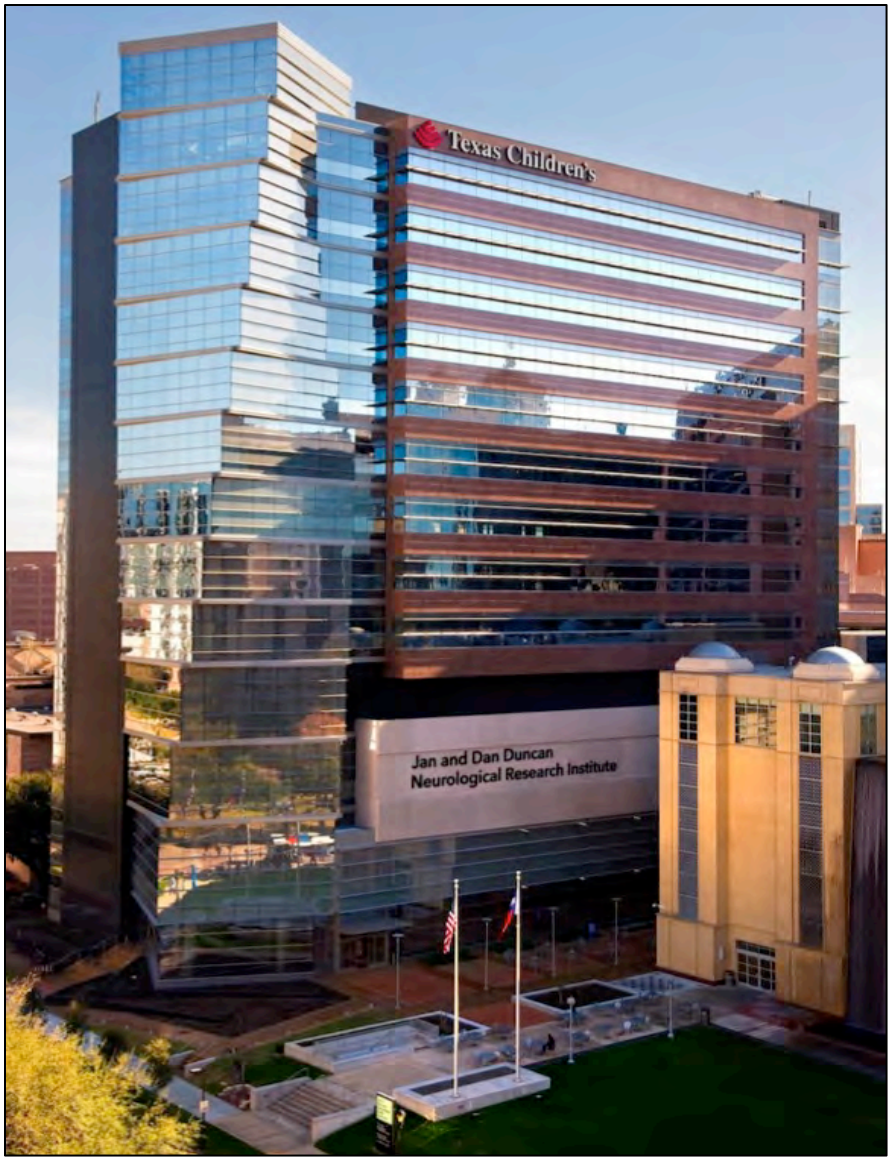
Chen et al. *In Review*



>40 DEC-Tec libraries and >4 *billion* unique compounds synthesized

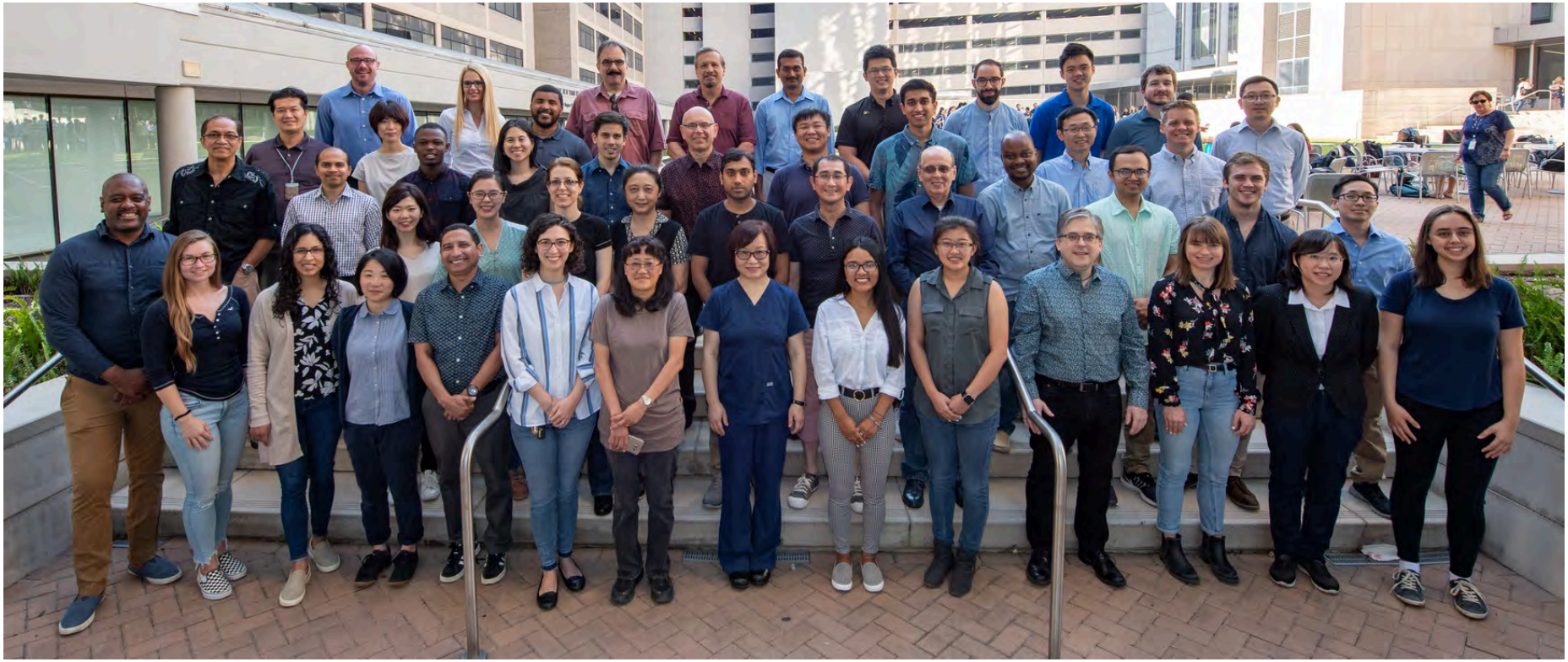
- Libraries may be screened as a pool and at scales for thousands of screens
- Diverse library structures
- Many have produced assay validated hits
- Designed with SAR in mind
- Many libraries utilize internally developed BCM chemical conditions
- **CDD has a team of geneticists, biologists, cheminformaticians, chemists, and crystallographers for validation, DEC-Tec screens, and further workup of small molecule hits and leads.**

NRI centrally located on the TMC Campus: Suite for NMRs & CDD home



- Bruker 600MHz and 800MHz NMRs for drug discovery, structural biology, and metabolomics (2nd floor of NRI)
- 6th floor of NRI developed for the Center for Drug Discovery

Our CDD Team…*a multidisciplinary group of students, postdocs, staff scientists, and faculty*



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CPRIT
CANCER PREVENTION & RESEARCH INSTITUTE OF TEXAS

NATIONAL INSTITUTES OF HEALTH

BILL & MELINDA GATES foundation

10 Points of Advice for Trainees (and New Faculty)

Points 1-3 – Work Ethic

- Work hard at the bench – you cannot get *lucky* during your PhD or post-doc or faculty years if you do not perform experiments and work hard

  (Do not let emails, texts, and internet surfing control your life and prevent you from your goals)

- Work a few hours on Saturday and Sunday when it is quiet, and there are fewer distractions (fewer emails, phone calls, etc. – this will allow you to complete studies from Friday and start or think about studies for Monday

- Bring your work to completion (i.e., a paper)

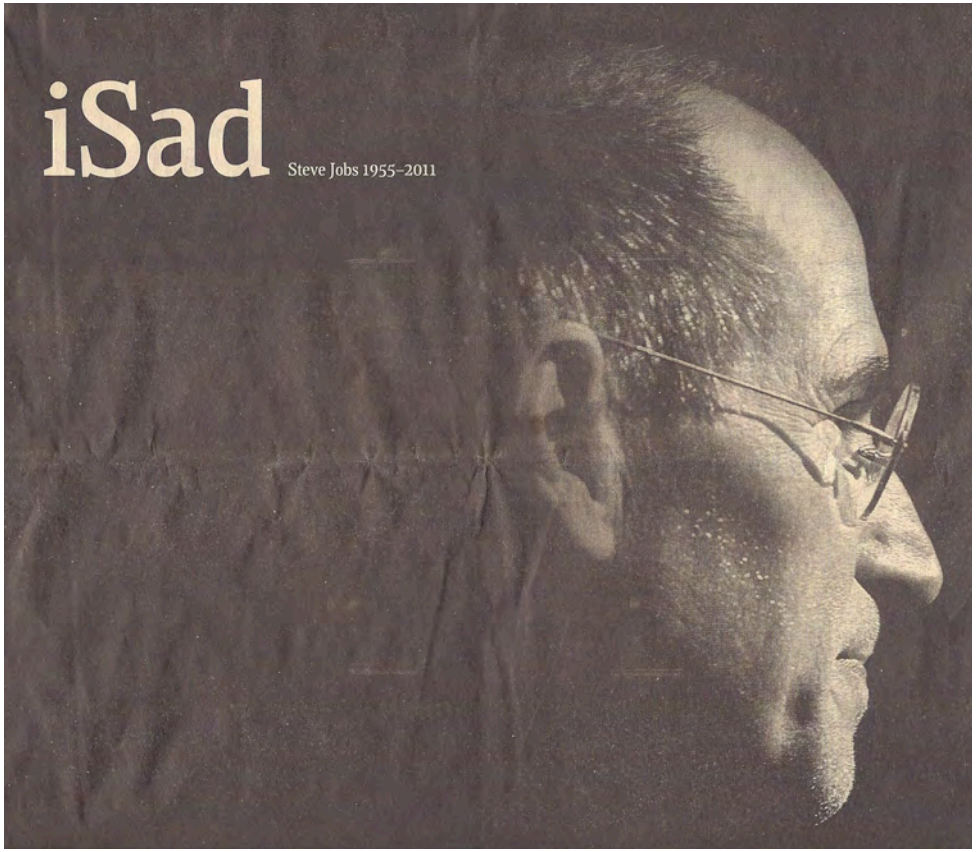
Points 4, 5, and 6 – Risk, Creativity, and Perseverance

- Take risks and think outside the box

- Find your niche – you do not have to do the same thing as everyone else – Novelty helps get your paper published in a top tier journal

- Not all papers and grants get accepted/funded on the first or second try

Message to trainees



"Your time is limited, so don't waste it living someone else's life."

"Don't let the noise of others' opinions drown out your own inner voice."

"And most important, have the courage to follow your heart and intuition."

In 1994, I submitted an NIH grant to generate a knockout of GDF9.

One critique said "There is no known function of GDF9. Why do a KO?"

In 1995, we resubmitted the NIH grant showing that GDF9 KO mice were infertile, and the grant is funded through the present and received a MERIT award in 2001. Our GDF9 KO paper in *Nature* in 1996 is my most cited work.

達磨 *daruma – Perseverance and Great Luck!*

Point 7 - Collaborate

- Seek out experts with whom to collaborate

I Have Been Lucky to Publish with Great Colleagues and Trainees…

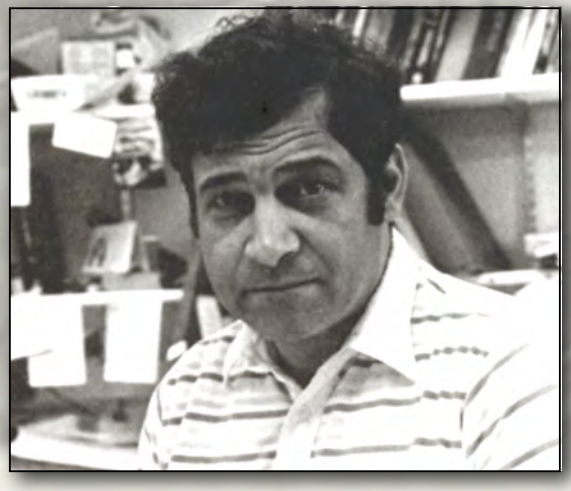
Raj Kumar	36
Kathy Burns	29
Stephanie Pangas	21
Wei Yan	17
Qinglei Li	16
Aleks Rajkovic	13
Shannon Hawkins	11
Franco DeMayo	16
John Eppig	15
Chad Creighton	9
Dorrie Lamb	7

Point 8 - Mentorship

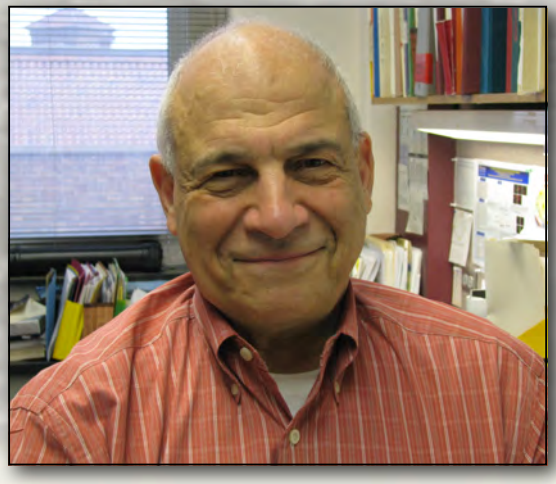
❖ Stay in touch with your mentors and do not burn bridges – your mentors can still give you advice and help you at all stages of your career (including writing LOR)

Edward Mallinckrodt Department of Pharmacology to Developmental Biology

100 Years of Innovative Science and Excellence in Mentoring



Washington University in St. Louis
School of Medicine



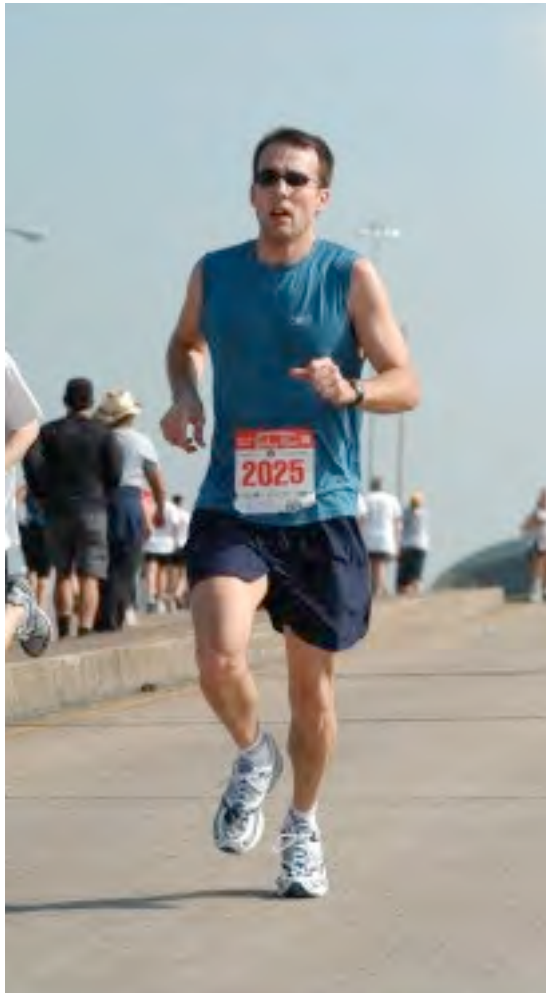
Dr. Irving Boime

Point 9 – EXERCISE

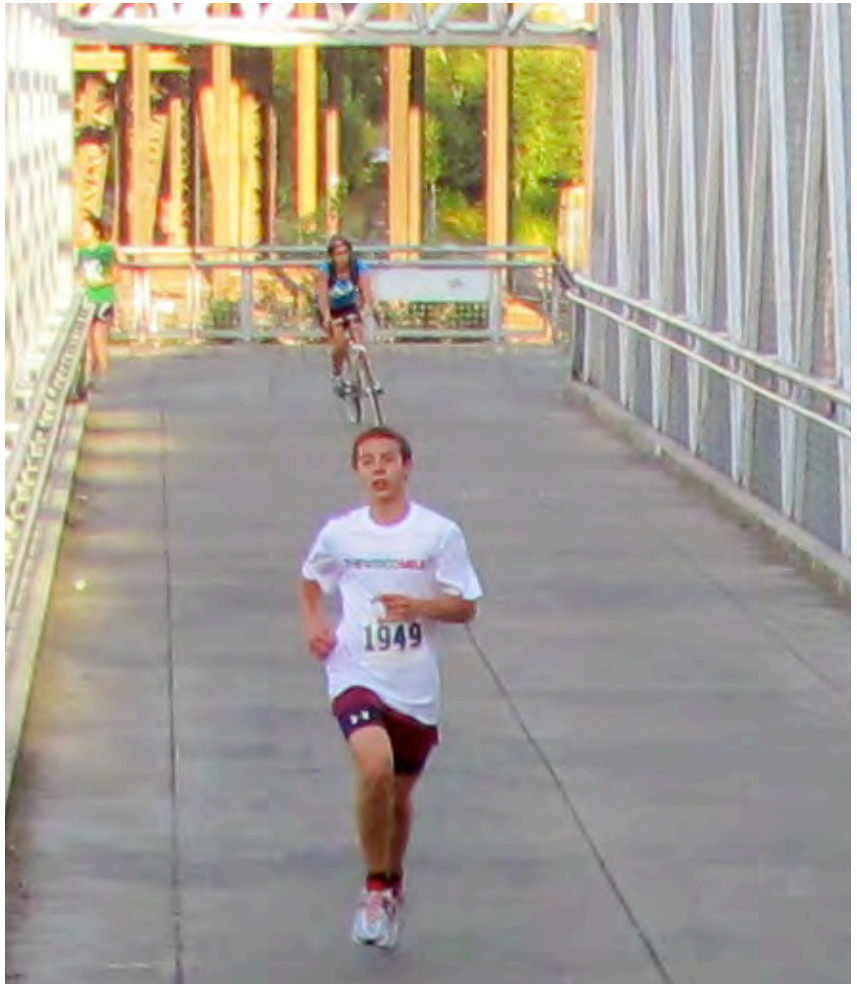
- Exercise – your quality of life will be better and you can keep up with (and *sometimes* out swim!) your children

Exercise

Mark Edson
Former MSTP student



Ryan Matzuk
SSR 5K - 2011 PORTLAND



Points 10 – Play hard and have fun

- Play hard – forget about your work for awhile and clear your head for a little while

- Do something with family or friends every day – **it is easy to become consumed with your work**

My Trainees LOVE All-You-Can-Eat Buffets

Travels since arriving in Houston
>170 symposia in 27 countries