Introduction: A Refresher on Why Drug Discovery is Hard

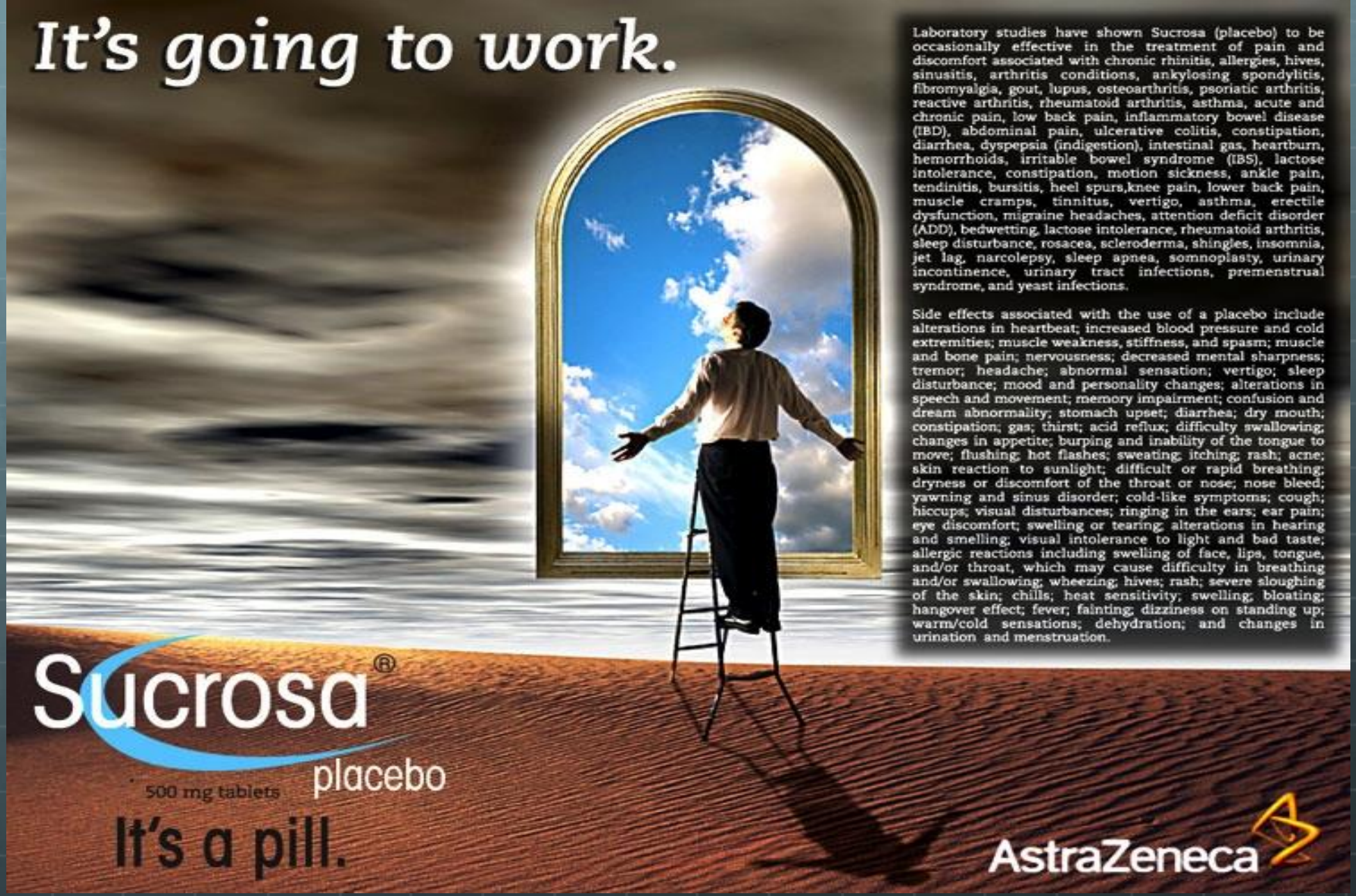
Mt. Katahdin, Maine, "the knife edge trail"
3'wide with 800'drop on either side

LACK OF INFORMATION

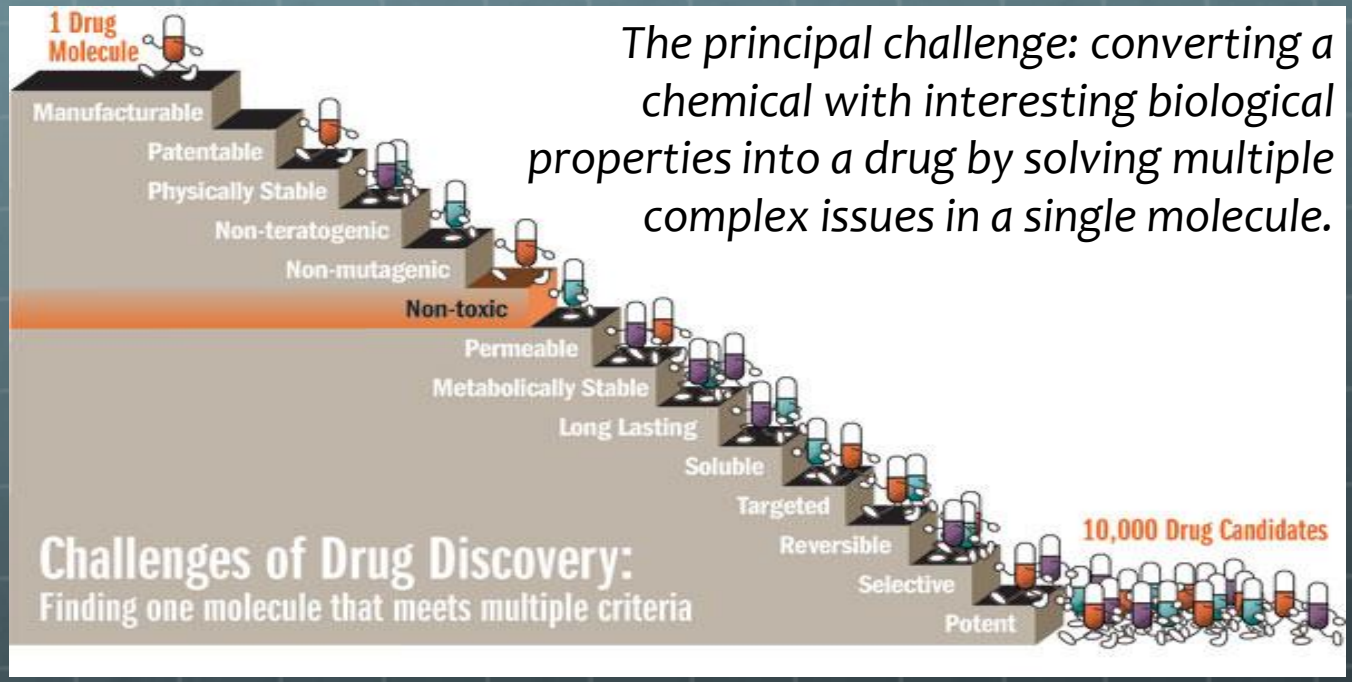
- Wrong molecule
- Wrong delivery approach
- Wrong target
- Wrong indication

- Harder problems
- Higher hurdles
- Poor processes / mgmt
- FUD (M&A; Wall Street)

"It can blow at any seam"



6. Deciding: Data Overload, Fear, Uncertainty, Bias, the whole mess



The principal challenge: converting a chemical with interesting biological properties into a drug by solving multiple complex issues in a single molecule.

HTS
Target binding
Target counter-screening
Thermodynamics
Kinetics
Cell primary
Cell tox
Solubility (multiple)
logP
CaCo-2 (bidirectional)
Transporters (many)
CYP inhibition
CYP induction
Protein binding
Liver microsomes & S9
hERG
ADME (i.v.)
ADME (oral)
Hepatocyte stability
Metabolite ID
Etc, etc

But .. Data ≠ Knowledge … All these measurements approximate the human condition

How Do You Know You're Done?

"Every design balances--connects--dozens of values, like a conceptual mobile, and the weights of those values, their relative utility or attractiveness, are changing constantly."

Photograph of mobile by Alexander Calder removed due to copyright restrictions.

Fred Hapgood, Up the Infinite Corridor : MIT and the Technical Imagination

It's Not All Chance: *Behaviors* of Great Teams

DOING WHAT MATTERS

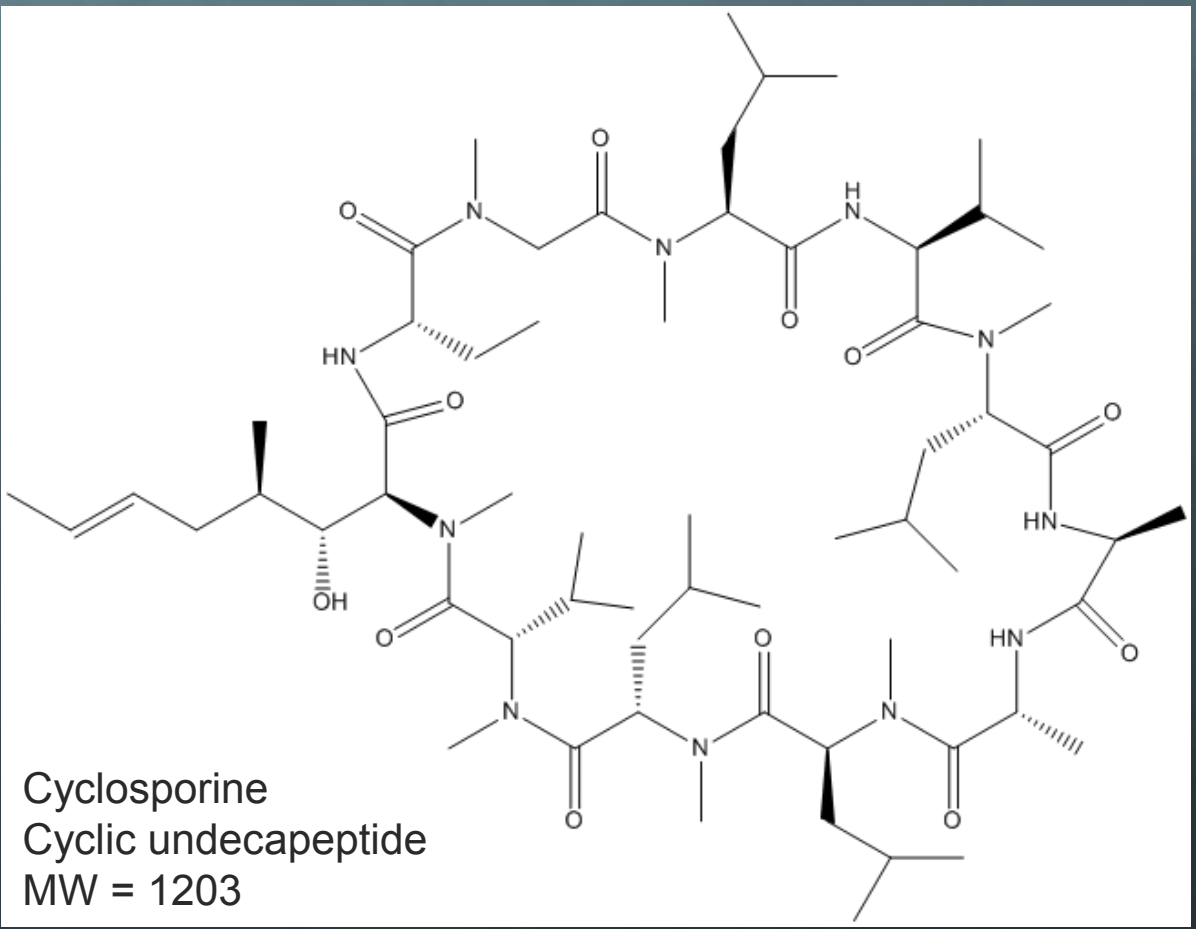
- Focus on patient needs
- Relentlessly solve high-value problems
- Curate the world's relevant knowledge
- Learn how to interpret complex data
- Pay attention to details
- Develop appropriate validated readouts and use wisely
- Generate PK data early & often
- Validate targets

MINDSET

- Challenge assumptions & be open to surprises
- Urgency
- Demonstrate resilience and ignore clues to quit
- Communicate in all directions
- Have a champion at senior levels
- Take chances
- Be practical

Rule-Breakers

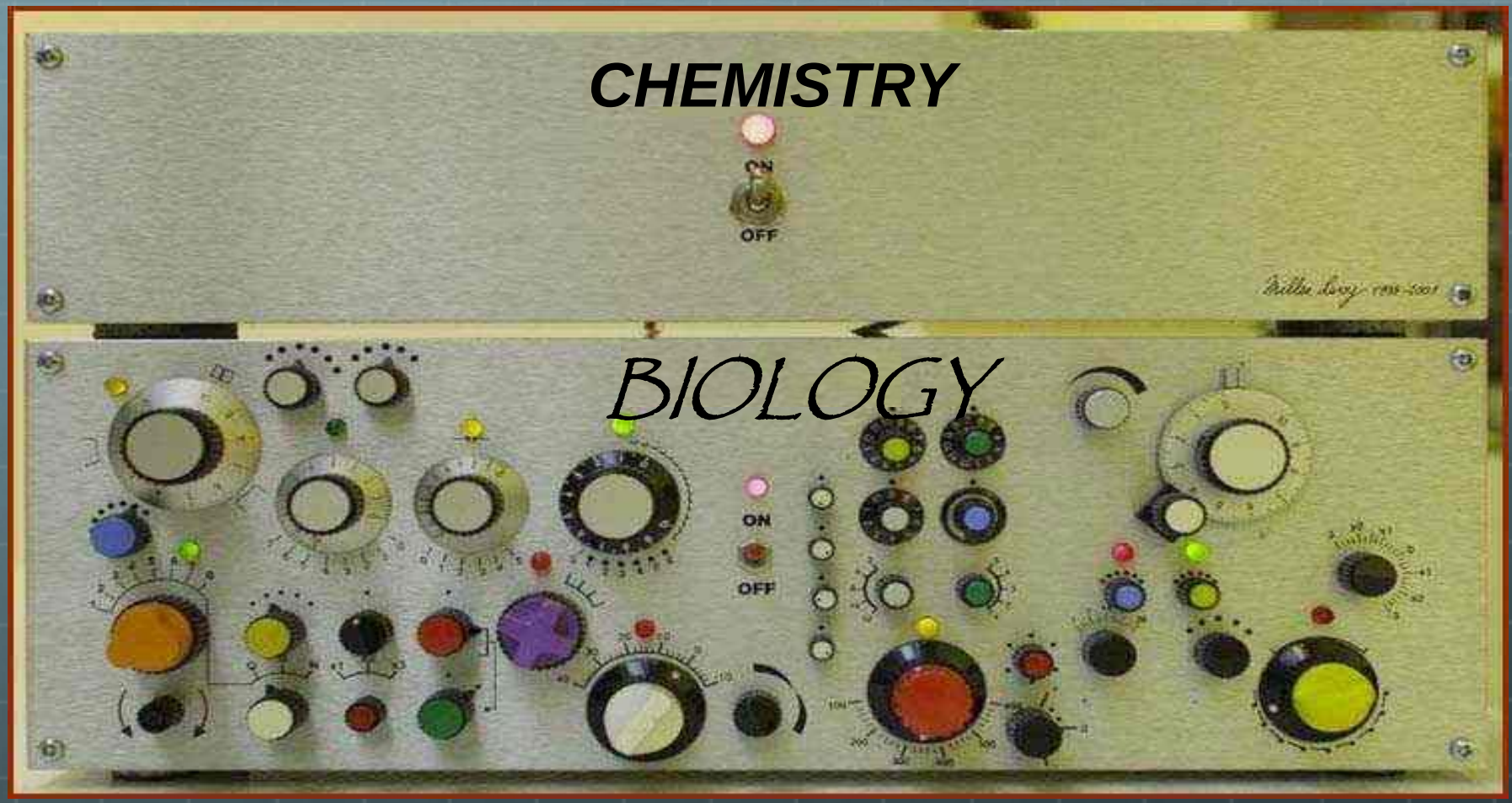
- Most oral drugs follow certain "rules" – MW <500, logP <5, and so forth.
- But there are many drugs that break these rules …



Cyclosporine
Cyclic undecapeptide
MW = 1203

- … and many molecules that follow the rules are not drugs.
- We don't understand the exceptions (in either direction).
- ***Conclusion: there are some trends and patterns, dimly understood at best -- not actually "rules" at all …***

7. Picking & Validating Targets



- Rate-limiting step in R&D is *usually* confusion about basic biology
  - Drug→Target→Network→Disease→Patient

Target Discovery & Validation (1 of 2)

- Target validation requires:
  1. Demonstration critical involvement in a disease process;
  2. Modulating the target is likely to have a therapeutic effect.

- Validating a molecular target in vitro usually precedes the validation of the therapeutic concept in vivo; together this defines its clinical potential.

- Validation involves studies in intact animals or cell-based models that can provide information about the integrative response of an organism to a pharmacological intervention.

Rydzewski; Garman

Target Discovery & Validation (2 of 2)

- Functional genomics: broadly, systematic analysis of gene activity in healthy vs. diseased organisms/organs/ tissues/cells.
- Includes: large-scale exploration of gene function including the analysis of regulatory networks, biochemical pathways, protein-protein interactions, and the effects of gene or functional knockouts or up-regulation/gain-of-function.
- Goal: to determine disease mechanisms & identify disease genes / markers. These results will indicate therapeutic strategies for the development of novel therapeutics.
- Requires model systems (animal and cell) as well as high-throughput data of various kinds (well covered in this dep't).

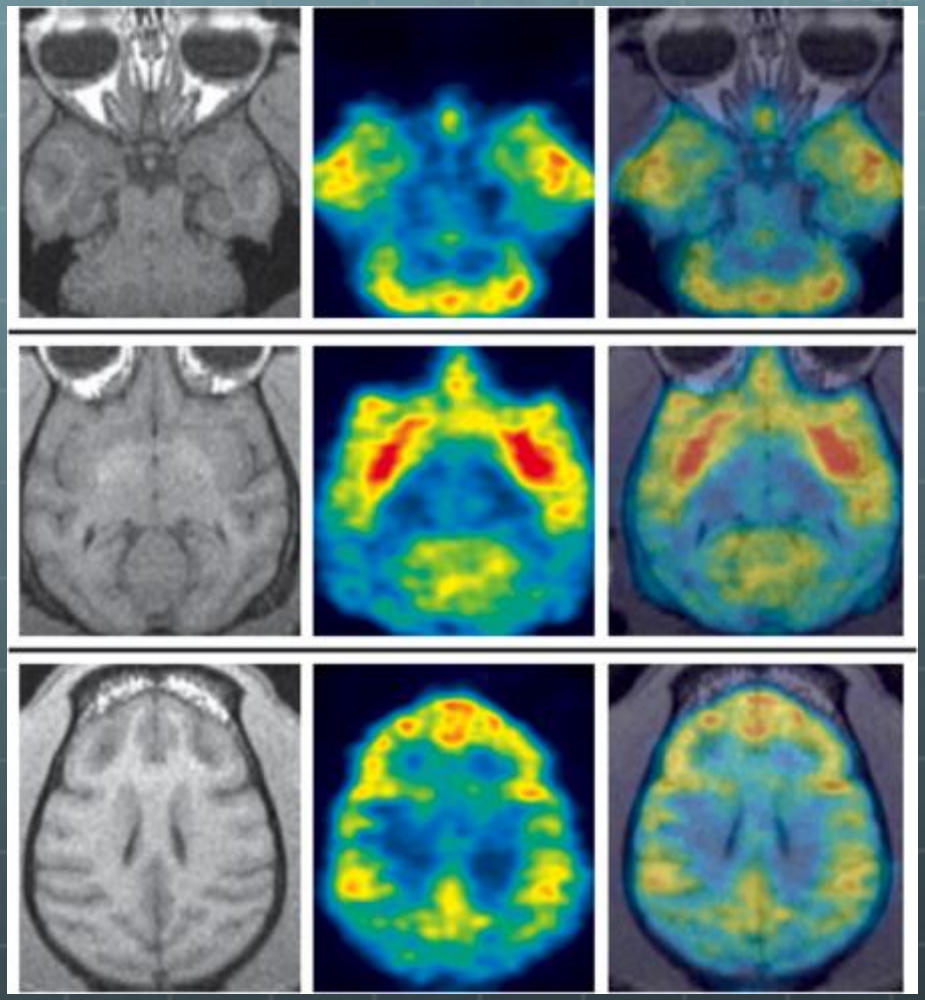
Scott Garman

Classes of Biomarkers

- Class 1 – Target occupancy
  - Gives evidence the drug occupies the target
  - Example – PET radioligand to measure D_2 receptor occupancy in schizophrenia
- Class 2 – Target-Based
  - Changes downstream from target occupancy specific to target
  - Example – Elevated norepinephrine (NE) metabolite levels in the presence of an NE uptake (NET) inhibitor
- Class 3 – Disease-Based
  - Linked to biomarkers found previously in patients w. disease
  - Example – Proteins expressed in plasma in schizophrenia patients vs. controls

PET Ligand for the CB-1 Receptor

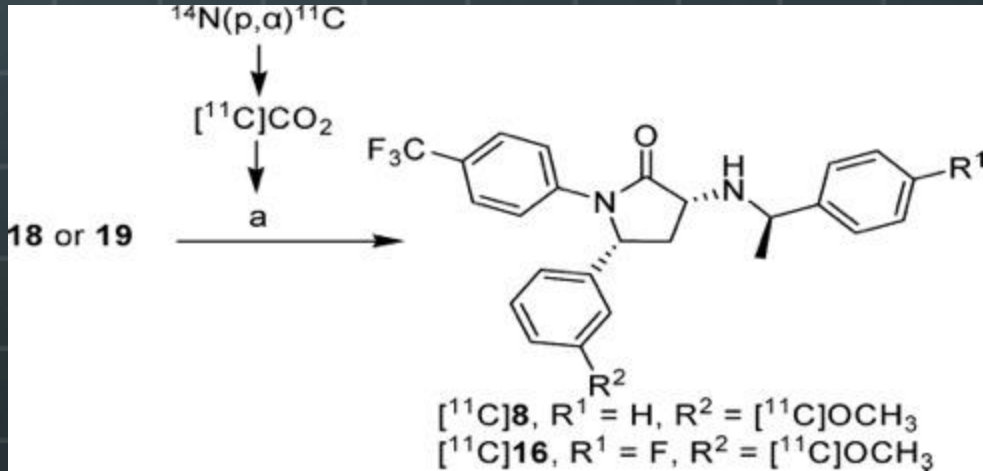
Class 1 Biomarker



Courtesy of American College of Neuropsychopharmacology. Used with permission.
Source: Yasuno, Fumihiko, Amira K. Brown, et al. "The PET Radioligand [^{11}C] MePPEP Binds Reversibly and with High Specific Signal to Cannabinoid CB_1 Receptors in Nonhuman Primate Brain." *Neuropsychopharmacology* 33, no. 2 (2008): 259-69.

Neuropsychopharmacology (2008) **33,** 259–269

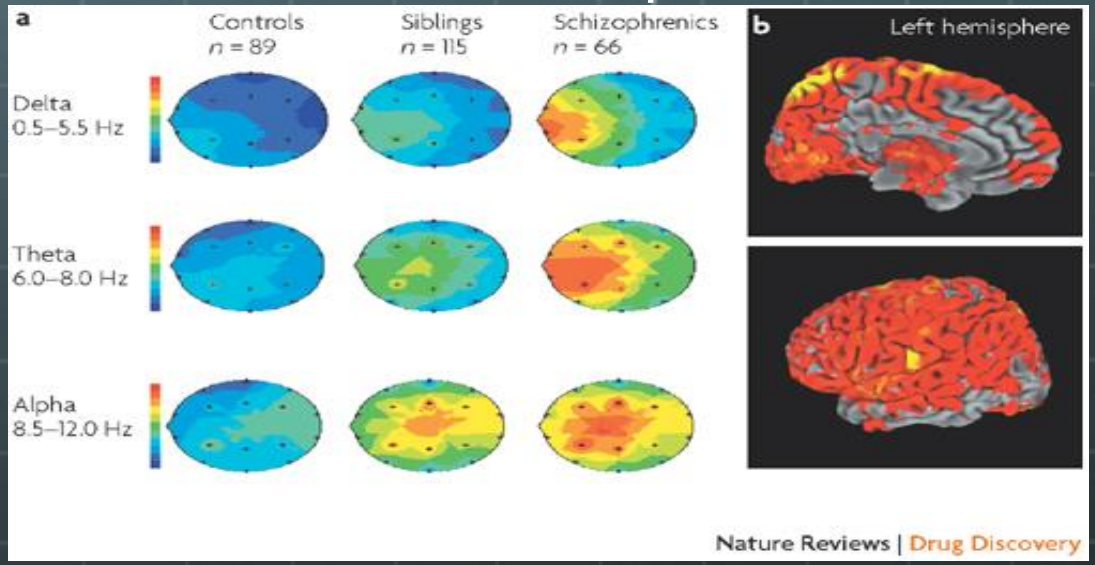
- Pre-clinical study in rhesus shows CB-1 receptor occupancy & displacement by candidate CB-1 antagonist
- Results of clinical study confirmed CB-1 receptor occupancy in humans
- PET ligand preparation and validation is resource-intensive



Schizophrenia as an Example

Class 3 Biomarker

- Several disease-based (class 3) biomarkers available:
  - Prepulse inhibition – responds to clozapine
  - P50 gating – linked to DA, 5HT, NMDA challenges
  - Auditory P300 – also abnormal in AD, BPD, ADHD
  - Mismatch negativity – no response to clozapine
  - Smooth eye pursuits – clozapine worsens
- Both EEG and fMRI / BOLD monitoring

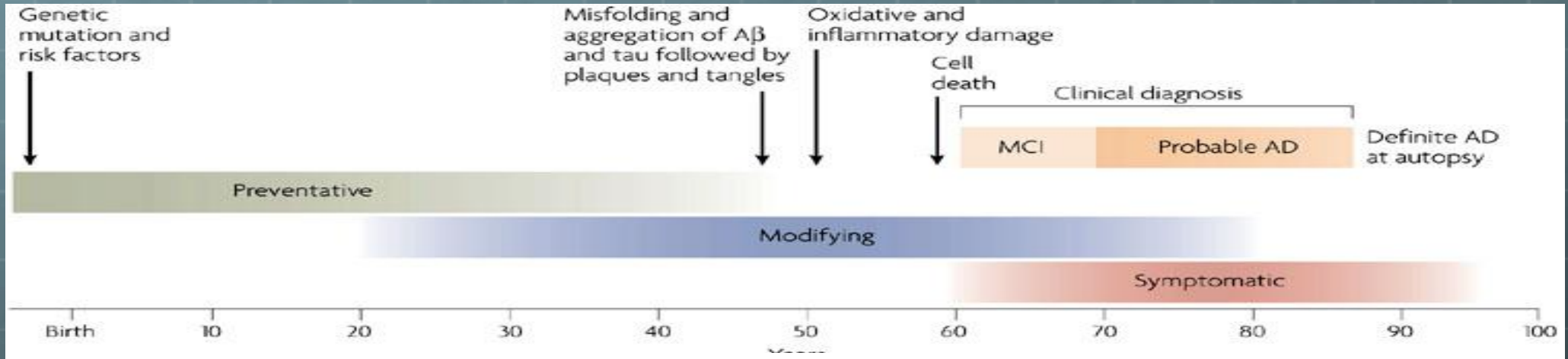


Cortical response variability is increased in schizophrenic patients relative to controls and clinically unaffected siblings – by EEG, BOLD

Nature Reviews Drug Discovery 7, 68-83 (January 2008)

Source: Avitt, Daniel C., Kevin M. Spencer, et al. "Neurophysiological Biomarkers for Drug Development in Schizophrenia." *Nature Reviews Drug Discovery* 7, no. 1 (2008): 68-83.

Alzheimer's Disease: Example of Critical Need for Better Biomarkers



Figuring out new biomarkers for complex diseases like Alzheimer's is just as great a contribution as discovering a new medicine – if not greater -- because it enables a whole field to progress far more rapidly.

- AD typically develops over many years, and clinical trials for a preventative agent would be prohibitively expensive
- Potential class 3 (disease progression) biomarkers include:
  - CSF phospho-tau and b-amyloid (Ab_{1-42})
  - CSF, plasma and urine isoprostanes (inflammatory)

8. Through a Glass, Darkly

Flying Blind

Medicine today is *reactive* (we treat sick people) & *myopic* (we have few tools to measure disease)

"The dirty little secret about medicine is that we physicians make decisions all the time based on woefully incomplete information."

-- Paul Yock, MD, Stanford

"Flying Blind," The Economist, 16 April 2009

The Need for Long-Term Thinking

- From target discovery to the first approved drug against that target typically takes 30+ years.
  - Exceptions: HIV-PR (1985 →1997), CFTR (1989 → 2012)
- A research project started now will not produce an approved drug until 2025-2030.
- How should these incredibly long timelines influence our thinking?
  - Must consider many kinds of change -- demographics, novel therapies, technologies, basic science, etc.

"Everyone overestimates how much progress will be made in the next 2 years and underestimates how much will be made in the next 10."

-- Bill Gates

"P4" Medicine

Medicine today is ***reactive*** (we treat sick people) & ***myopic*** (we have few tools to measure disease) …

Leroy Hood, inventor of protein sequencer, protein synthesizer, DNA synthesizer, ink-jet DNA synthesizer, & automated DNA sequencer

… Medicine will gradually become ***personalized, predictive, preventative, and participatory.***

- Diagnostics & omics → individualized care
- Risk factors understood
- Diseases eliminated before symptomatic
- Each of us bears greater responsibility for our health

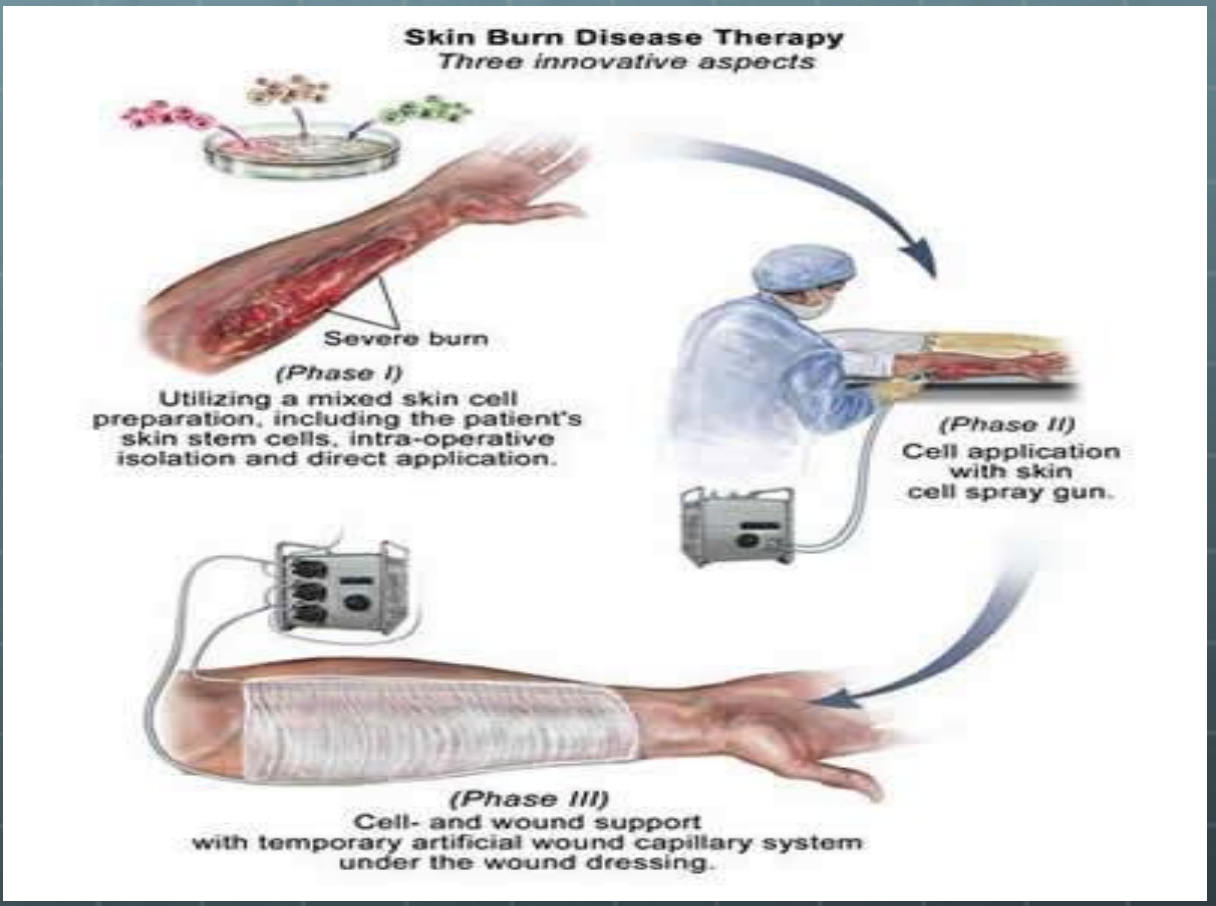
Some Coming Disruptions: Medicine circa 2030

- P4 medicine
- Widespread use of “regenerative” therapies
- Man - machine interface
- Therapy involves multiple diverse elements - not just “drugs”
- Far earlier and more accurate diagnosis; “ubiquitous self”
- Drugs against far more diverse targets (“undruggable”)
- Tests for disease progression and response to therapy
- Higher drug safety bar; better predictors of safety
- Reimbursement for results
- Routine & rational polypharmacy
- Preventative medicine; “neutraceuticals”
- Diverse, precisely targeted drug delivery mechanisms
- “Open source” pharmas

What Will "Therapy" Mean?

- An *assembly of interlinked components* to manage the *entire disease life cycle*
  - Drugs + regenerative approaches + devices + diagnostics + social network
  - Participatory: at-home tests show benefits of therapy & motivate patients
- Focused R&D programs based on selected *subsets of patients* – therapy is personalized, predictive, and preventative
  - Each "disease" will be understood to be many related but distinct afflictions
  - Biomarkers, diagnostics, and devices support patient selection & trial design

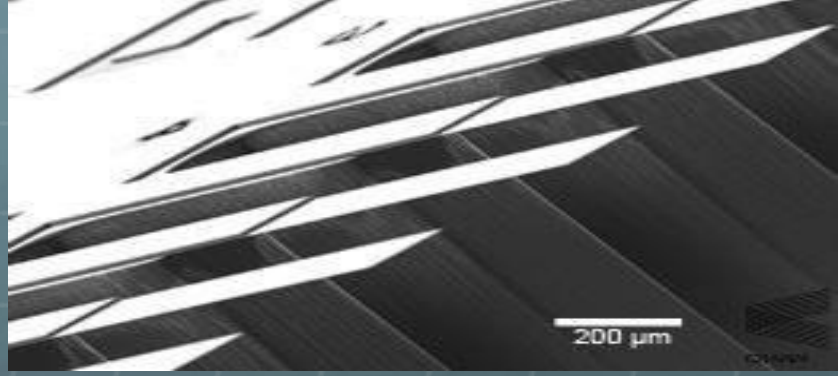
Regenerative Therapies: Bioreactors and Skin Guns



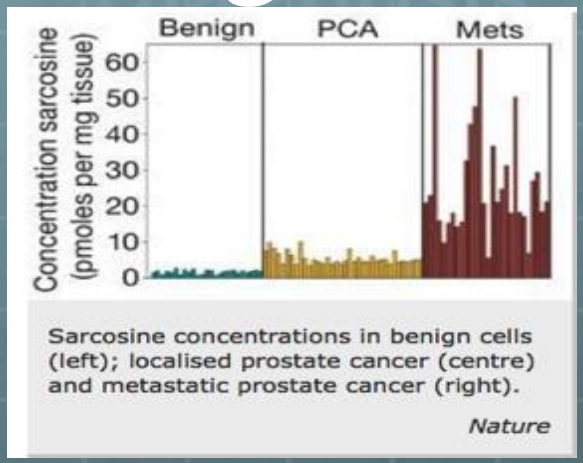
Joerg Gerlach, McGowan Institute for Regenerative Medicine (Pittsburgh)

Far Earlier Diagnosis

Quantitative time-resolved measurement of membrane protein–ligand interactions using microcantilever array sensors.



Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Braun, Thomas, Murali Krishna Ghatkesar, et al. "Quantitative Time-Resolved Measurement of Membrane Protein–Ligand Interactions using Microcantilever Array Sensors." *Nature Nanotechnology* 4, no. 3 (2009): 179-85.



Sarcosine levels predict severity of prostate cancer.

Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Sreekumar, Arun, Laila M. Poisson, et al. "Metabolomic Profiles Delineate Potential Role for arcosine in Prostate Cancer Progression." *Nature* 457, no. 7231 (2009): 910-4.

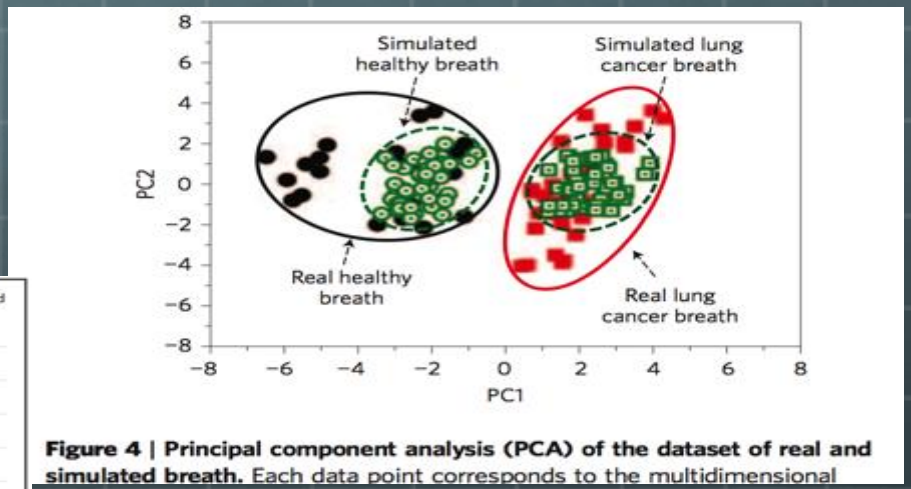
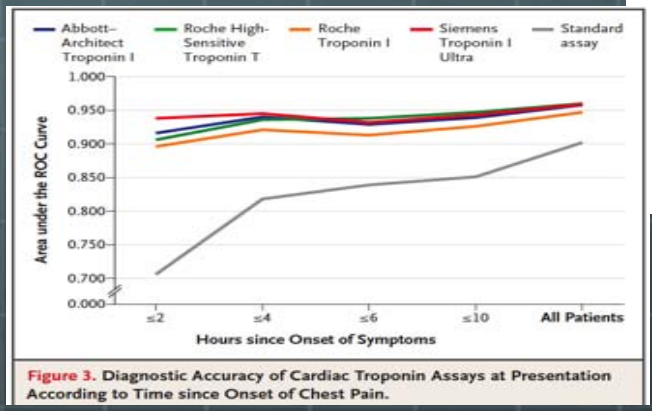


Figure 4 | Principal component analysis (PCA) of the dataset of real and simulated breath. Each data point corresponds to the multidimensional

Courtesy of Macmillan Publishers Limited. Used with permission.
Source: Peng, Gang, Ulrike Tisch, et al. "Diagnosing Lung Cancer in Exhaled Breath using Gold Nanoparticles." *Nature Nanotechnology* 4, no. 10 (2009): 669-73.



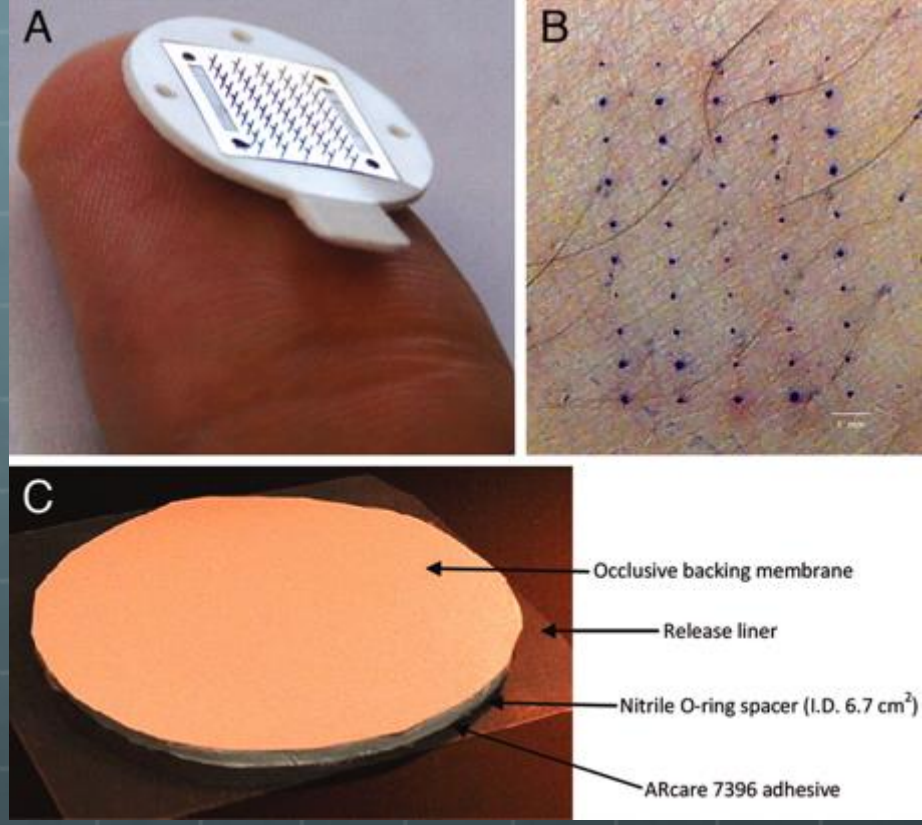
Source: Reichlin, Tobias, Willibald Hochholzer, et al. "Early Diagnosis of Myocardial Infarction with Sensitive Cardiac Troponin Assays." *New England Journal of Medicine* 361, no. 9 (2009): 858-67.

Troponin predicts MI.

Microscopic Needles: Painless Transdermal Delivery

University of Kentucky College of Pharmacy and the Georgia Institute of Technology

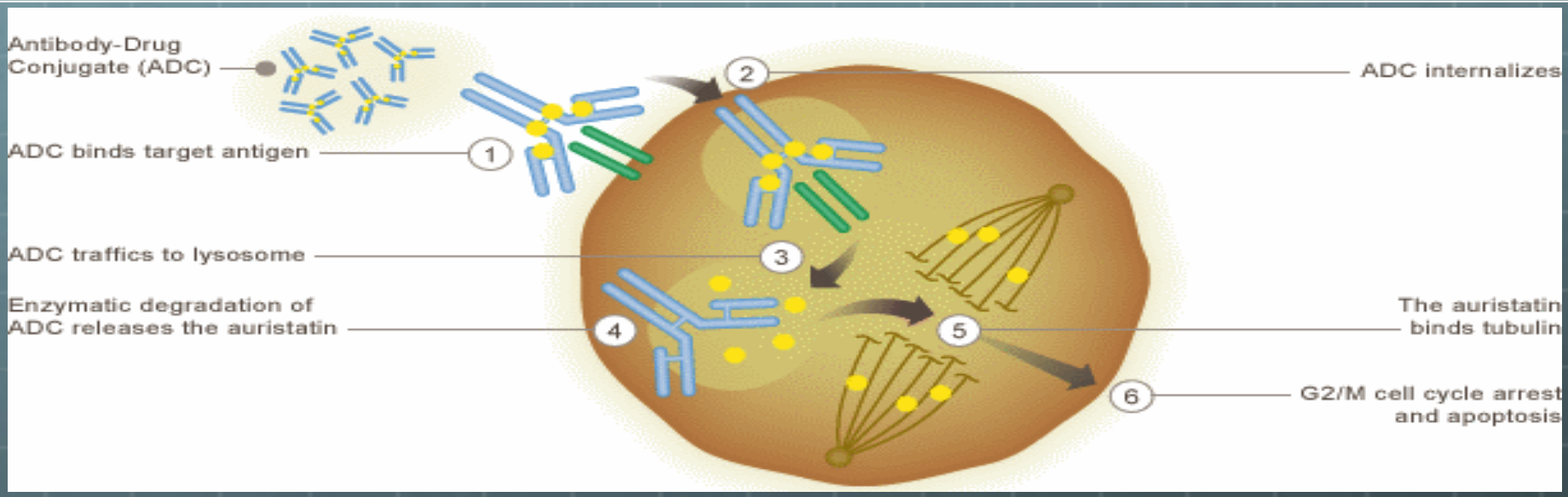
Transdermal drug delivery has proven successful in a number of applications, including pain management, congestive heart failure and hormone replacement … but existing systems can only be used for a narrow range of compounds that easily pass through the skin.

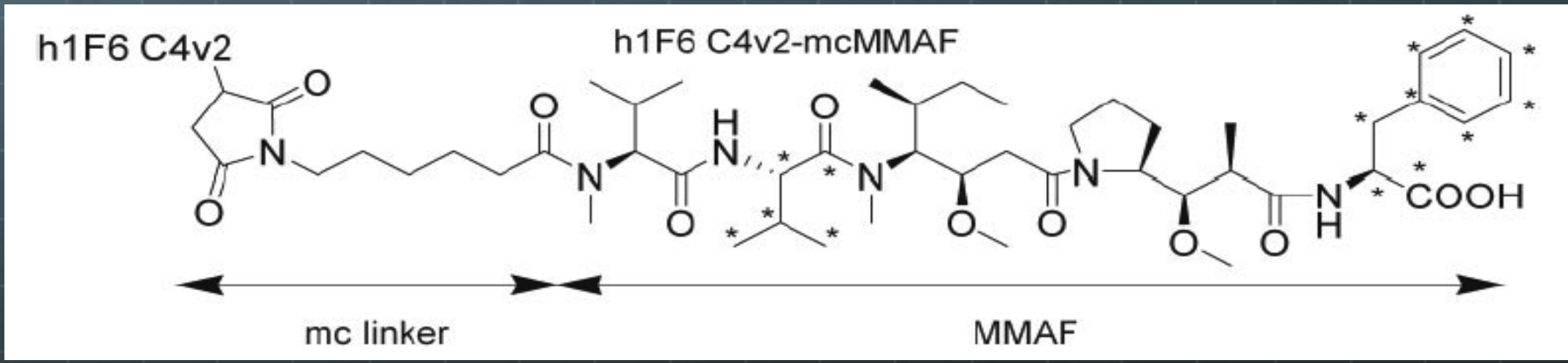


Courtesy of the National Academy of Sciences. Used with permission. Source: Wermeling, Daniel P., Stan L. Banks, et al. "Microneedles Permit Transdermal Delivery of a Skin-Impermeant Medication to Humans." *Proceedings of the National Academy of Sciences* 105, no. 6 (2008): 2058-63.

New work: painlessly punch a series of microscopic holes in the outer layer of skin via pressing and removing a thumb-sized patch containing 50 stainless steel microneedles each about 620 microns -- about 1/40th of an inch -- in length. Next, gel containing naltrexone (a drug used for opiate and alcohol addiction) was applied to the prepared area, which was then covered by a protective dressing. Blood levels stayed constant for ~48 hours. Doses were 4X lower than oral administration and there were 10X lowered production of metabolites.

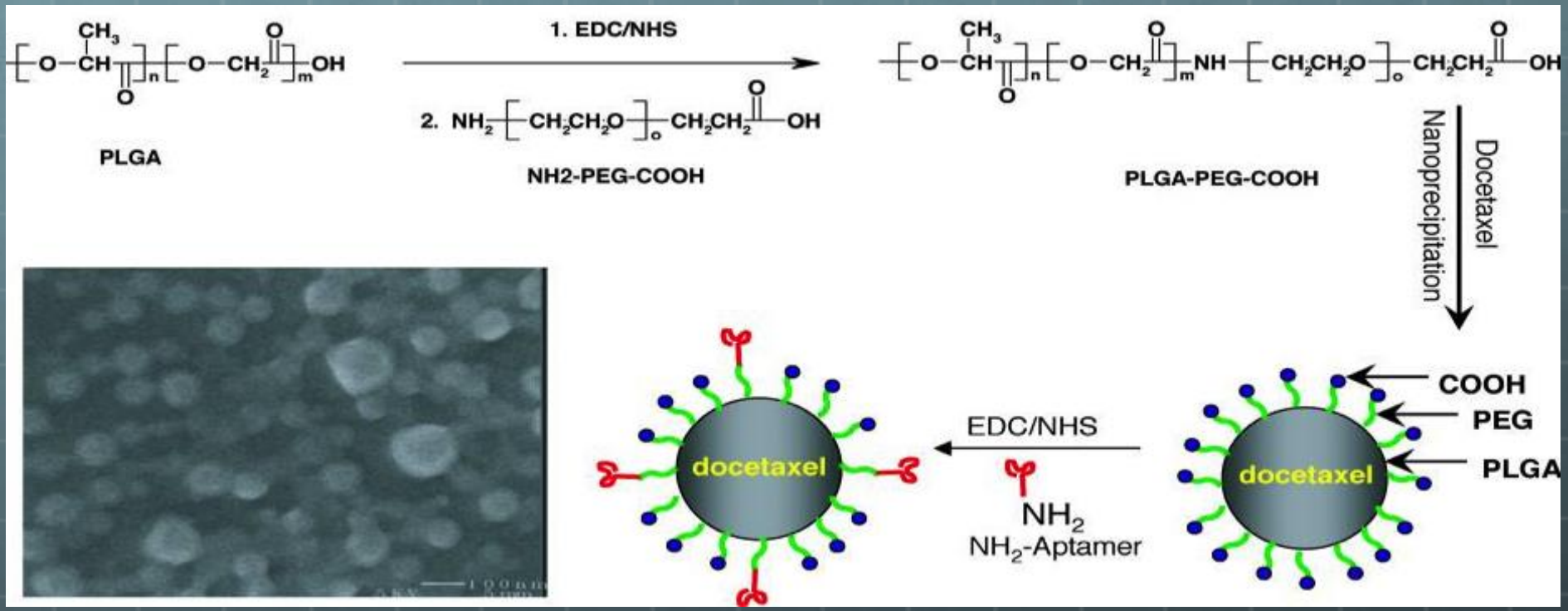
Two Drugs in One Package





The Pharmacologic Basis for Antibody-Auristatin Conjugate Activity.
Alley et al, Seattle Genetics. J. Pharmacol. Exper. Therapeut. 2009, 330, 932.

Nanoparticle - Aptamer Bioconjugates



Courtesy of the National Academy of Sciences. Used with permission.
Source: Farokhzad, Omid C., Jianjun Cheng, et al. "Targeted Nanoparticle-Aptamer Bioconjugates for Cancer Chemotherapy in Vivo." *Proceedings of the National Academy of Sciences* 103, no. 16 (2006): 6315-20.

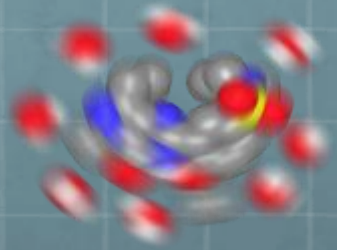
- Docetaxel-encapsulated nanoparticles formulated with biocompatible & bio-degradable copolymer; surface functionalized with fluoropyrimidine RNA aptamers that recognize extracellular domain of prostate-specific membrane antigen. These bioconjugates:
  - Get taken up more effectively into prostate cancer cells
  - Exhibit reduced toxicity in vivo as measured by mean body weight loss
  - Exhibit significantly enhanced efficacy in vivo: tumor reduction & survival

Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. PNAS 2006, 103, 6320.

Route	Description
Oral (PO)	Swallowed by mouth
Intravenous (IV)	Injected directly into the bloodstream as bolus or infusion
Subcutaneous (SC)	Injected under the skin
Transdermal	Applied as a patch or other device and transported through the skin
Topical	Applied onto the skin
Intramuscular (IM)	Injected into the muscle
Epidural	Injected into the epidural (outermost) space in the spinal cord
Suppository	Placed in the rectum
Intranasal	Sprayed into the nose
Buccal	Held between cheek and gum until dissolved
Sublingual	Held under the tongue until dissolved
Intraperitoneal (IP)	Injected within the peritoneal cavity (abdomen)
Intra-arterial	Injected into an artery
Intracerebral	Injected directly into the brain
Intravitreal	Injected into the eye
Intrathecal	Injected into the spinal cord

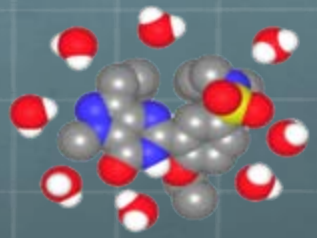
9. The Age of Biological Engineering?

Thermodynamic Decomposition of Ligand/Protein Binding



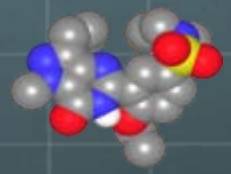
Solvated Ligand

ΔG(1)



Solvated Ligand in Bioactive Conformation

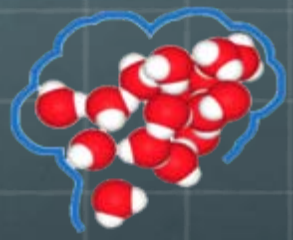
ΔG(3)



Desolvated Ligand

Solvated Apo Protein

ΔG(2)

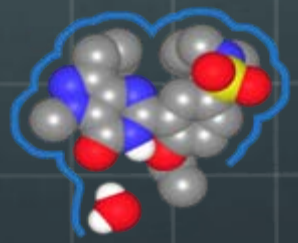


Solvated Protein in Ligand-Induced Conformation

ΔG(4)

Ligand-Induced Desolvated Protein Binding Site

ΔG(5)



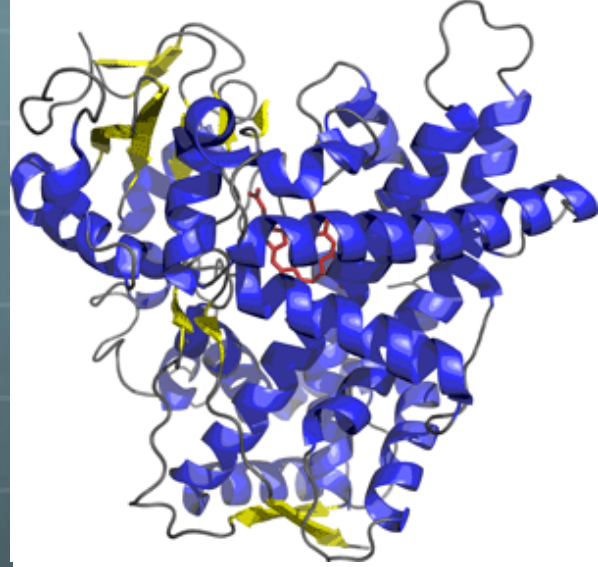
Protein / Ligand /Water Complex

$$\Delta G_{bind} = \sum_{i=1}^{5} \Delta G(i)$$

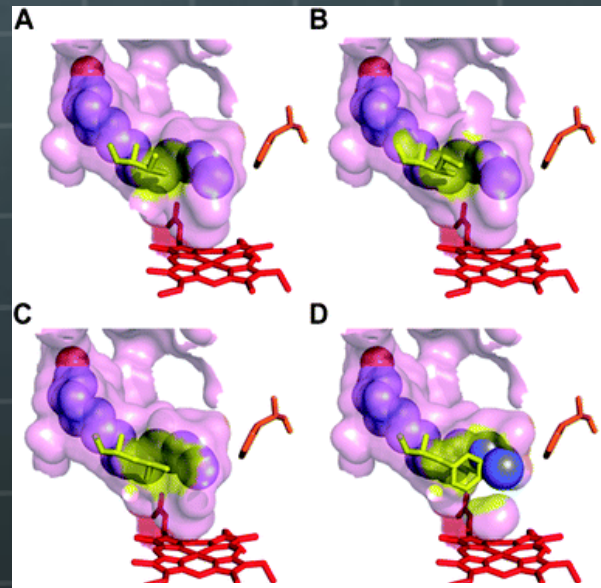
Source: Schrödinger

Atomic-Level Understanding of How to Optimize "Drug-likeness" ?

- All of the challenges we have discussed – metabolism, interaction with transporters, cell permeability – can in principle be understood at the molecular level.
- The first glimmers of success are starting to be seen, e.g. in the use of xray data for P450 enzymes to model both P450 inhibition and oxidative metabolism.
- High-throughput assays are also getting better, leading to larger datasets from which to begin understanding the trends.



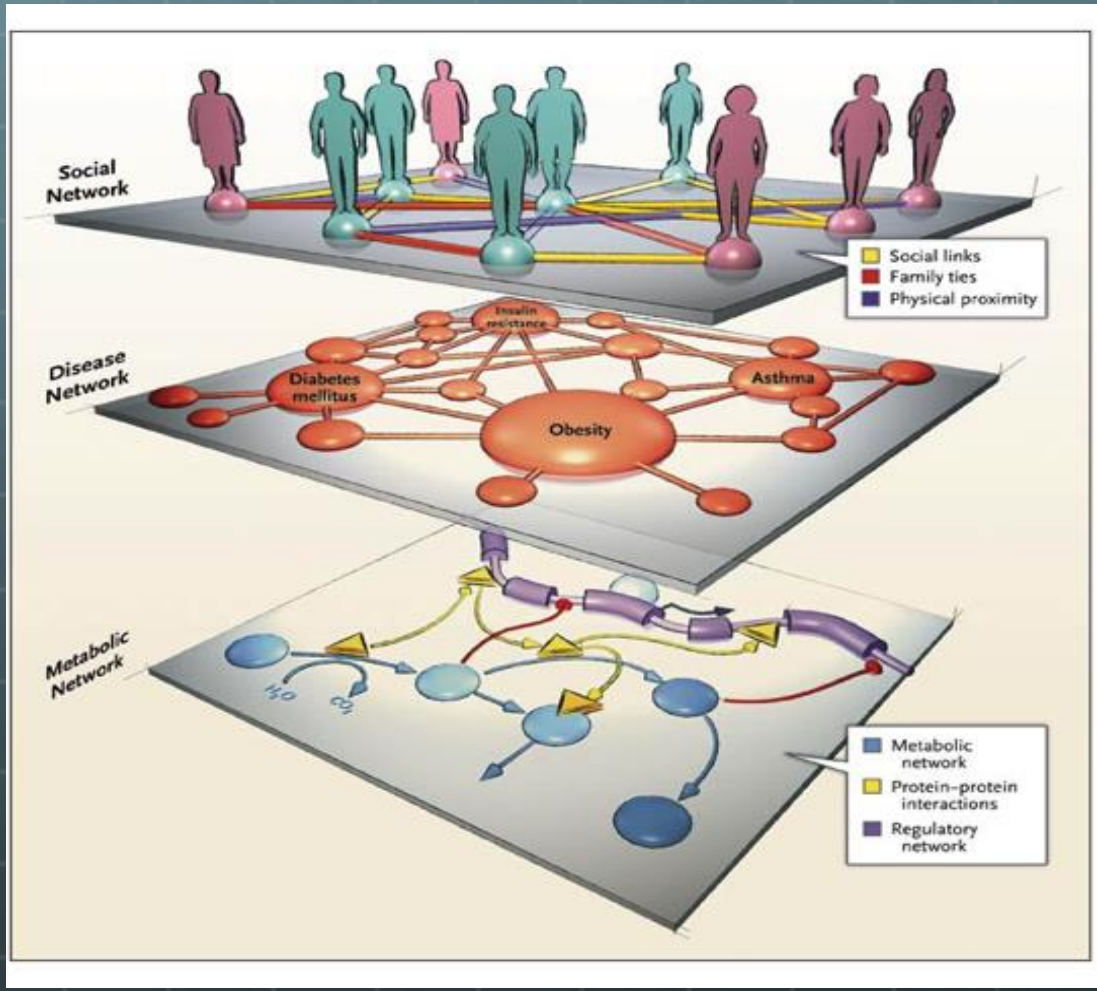
X-ray crystal structure of cytochrome P450 2E1



Modeling palmitic acid in a bacterial P450 enzyme

Network Medicine

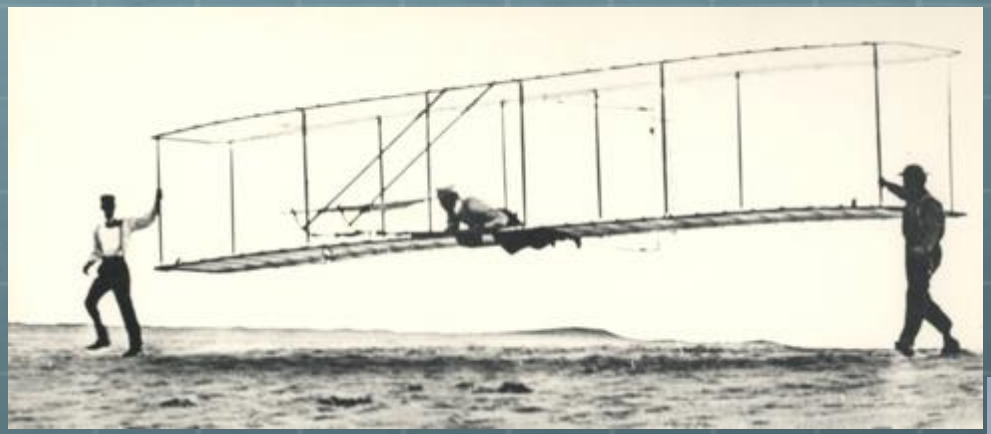
- Cellular function caused by network modules consisting of many interlinked factors.
- Disease = breakdown of these complex functional modules.
- Drugs can affect multiple cellular networks.
- Surprising connections between diseases forces us to rethink the way in which we classify and separate them.



Source: Barabási, Albert-László. "Network Medicine—from Obesity to the "Diseasome"." *New England Journal of Medicine* 357, no. 4 (2007): 404-7.

Network Medicine - From Obesity to the Diseasome. Albert-László Barabási, NEJM 357;4 (2007)

It's Been Done Before



10 October 1902, Kill Devil Hills, NC

Courtesy of Brianski on wikipedia. Photograph is in the public domain.

27 April 2005, Toulouse, France

"High performance computing has fundamentally changed the way Boeing designs flight vehicles"

-- Michael Garrett, Director of Boeing's Airplane Performance Division

- 1980: tested 77 wings for 767
- 2005: tested 11 wings for 787

"wind tunnel results matched CFD predictions"

Figures removed due to copyright restrictions.

Noise reducing chevrons designed entirely in silico

Courtesy of Martin Hartland on flickr. License: CC-BY-NC-SA.

20.201 Mechanisms of Drug Actions

Fall 2013