Discussion Topics for Workshop

Please add your discussion ideas

General

- What will future (10-15 years) bioengineered systems look like?
- Comparison to design in other engineering fields helpful at all? Or do the distinct properties of biology dictate a different approach?
- How much energy does a single cell have?

Robustness and control design

- There is an information problem where parameters aren't known, even in the case where we have complete information, how hard is it to design a circuit with specified behavior out of unknown parameters?
- Can we investigate fundamental limits of control when applied to biological systems?
- How to evaluate robustness properties across different (control) designs architectures on an equal ground?
- How to evaluate/compare robustness of designs in a way that considers multiple performance metrics/objectives of relevance?
- What is the cost to the cell of controllers? Can we work with controllers at low copy numbers?

Predictable behavior

- When does behavior/function need to be *quantitatively* predictable, and how good do quantitative predictions (i.e. engineering methods) need to be?
- How to develop synthetic tunable/adaptive biological systems (e.g. tunable expression systems etc)
- What kind of experimental progress needs to be made (what needs to be measured, how and when) in order to characterize the components/circuits/host for predictable operation?
- Can we characterize (demystify) context enough to enable predictive translation of designs across implementations (cell types, cell states, delivery methods)?
- How do we combine multiple predictive models (e.g. of transcription rate, translation rate, mRNA decay rate, transcriptional regulation, translation regulation, metabolic fluxes, etc) to predict system-level dynamics (stochastic, deterministic)?

Scalability

- Scalable design process within a single cell, how to?
- Scaling design problem from small scale chemostats or microfluidics to large scale chemostats etc and how to treat the problem computationally (what models to use? How to treat parameters on different scales etc)
- Scaling up the design of genetic circuits with many regulators -- how do we improve the reliability of genetic parts so that all function correctly? How do we avoid genetic context effects (ie, undesired changes in function) when combining parts together?

Modularity

- How should modularity be used for design?
    - Should components be insulated from context?
    - Should one design everything as a whole system, perhaps using evolution approaches?
    - Could we establish new connection rules that take context into account?
- Can we improve modularity by physically separating (insulating) molecular reactions in synthetic organelles?
    - What are the key design principles in designing dynamic organelles that exchange information?
    - Can we build synthetic organelles with controlled in-flow and out-flow of components?
    - How do we obtain the emergence of dynamic liquid organelles with controlled properties from constituents with minimal but scalable networking/binding rules
- Modularity, do we really want it? Modularity vs constrained structure (see Mustafa's talk) – not clear separation between controller, sensor, plant. Modularity is convenient but do we really need it?

Standards

- Units of measure...this is an "old" topic but that it is still unsolved...each cell or cell type has different maximal capacity implying that probably relative units of measure are needed in biological systems...this directly impacts the practical applicability of control theory to molecular biology

Multi-cellular systems

- Engineering multicellular consortia beyond two populations: how?
- Finding optimal ways of splitting workload across populations
- Embedded and external growth and ratiometric control strategies

- Can one engineered cell (type) control a complex multicellular system (e.g., a local immune network)? What information is needed to identify suitable control handles/functions and/or evaluate whether such a feasible strategy exists? How could we design an engineered subpopulation to control greater emergent population dynamics?

Merging model-based with data-driven approaches

- What role can machine learning play in synthetic biology?
- How do we combine biophysical models with machine learning to improve parameter estimation, utilizing large datasets?
- Can control strategies based on reinforcement learning be used in a synthetic biological context?

Control at the interface with specific use cases:

- Metabolic engineering
- Synthetic microbial consortia
- Computer-based control of cell populations
- In situ sensing and delivery for healthcare
- Multi-agent (e.g., multi-cell) populations

(Realtime) notes from talks -- to be used in group discussions

Domitilla

Applications

- Short term: detection, materials
- Medium term: targeted drug delivery, microbiome
- Long term: Engineered living materials, reprogrammed patient-specific cells

Modular design, but failures

Grand challenges:

- Robustness
- Genetic stability
- Managing complexity/time
- Cell/cell variability

- Predictability
- Multicellular machines
- Standards

Systems and control theory

- Feedback loops can maintain modularity
- Modify dynamics with feedback
- Managing uncertainty

Robustness to disturbances

Orthogonal parts do not imply orthogonal functions

Logic gates fail due to internal resource sharing

Negative feedback regulation neutralizes dCas9 competition

Hana El Samad

Treat biological molecules as distinct

Engineering with de novo designed proteins

DegronLOCKR

For feedback systems, need to perform the right comparison

Inject an appropriate signal in an open loop control
Need to equalize open and closed loops

CoRa (control ratio)
For quantifying perfect adaptation
Delay of degradation due to transcription factor still active

Incoherent feedforward circuit

Mustafa Khammash

Cybergenetics

Maximal robust perfect adaptation

Setpoint encoding reactions

Independent on all but two parameters. These encode the setpoint

Modularity vs Constrained Structure
    Do we need modularity?
    Intertwine sensor/computation/controller

Dealing with stochastic behavior

Spectral theory for controller design

The cost of molecular control
    Embrace control at low copy numbers

Speeding up the design-build-test cycle
    Cell-in-the-loop
    Controller in the computer
    Target network: stochastic transcription
    Cyberloop

Eduardo Sontag

Implicit feedback (retroactivity)
Resource sharing
Predicting feedback interconnection

Steady state vs. dynamic retroactivity

Insulation costs

Formulation of context phenotypes
    Different responses RNAp /= ribosomes

Steady-state response to step inputs ("I/O characteristic")
    Feedback: from open loop measurements to predicting closed loop characteristics

Relevant for I/O Monotone Systems
Non-monotone - can be decomposed into monotone components

Context effects can affect behavior in unexpected manners

Monotone components as building blocks

Large circuits toxic → distributed computing

Jeff Hasty

Major issues
- Intracellular noise
- Unknown interactions between circuit components
- Complex coupling with host

To address intracellular noise
- Synchronize cells

Unknown interactions:
- Build control circuits
- Need to quantify impact on host (e.g. RNAseq)
- Genome-scale transcriptional dynamics and environmental sensing

Distribute gene circuits across different cells
- Use quorum sensing (converts an analog population signal into a switch-like response)
- A grand challenge for engineered ecologies is exponential growth of cells
- Lingchong You: population control where lysis rate is proportional to population size
- Matt Bennett: two strain dual-feedback network
- Stabilization of small ecologies using lysis of different constituent strains
- Three strains: use bacterial colicins for strains to control each other
- Spatial distribution matters
- Mutations and mutant escape important to consider -- and depend also on population sizes and other experimental setup factors

Ophelia Venturelli

Microbiomes impact every environment on earth
- Various mechanistic modes of microbial interactions (e.g. resource competition, …)

- Complex feedback loops

Control microbial communities bottom-up or top-down

- Challenges: ecological and evolutionary stability, identifying influential and precise control knobs, …

Application: defined consortia with synthetically modified organisms

- Microbial fermentation end products are associated with human health and disease
- Relevant molecules: lactate, acetate, succinate, and butyrate
- Pairwise vs. higher order species interactions -- predict system interactions from pairwise interactions
  - Predicting complex communities at this point requires high order analysis, because pairwise analysis was not sufficient
- Use 25-member synthetic human gut microbiome
- Use machine learning to understand and guide
- Species presence/absence

(Overarching question: pairwise interactions intra-cellularly, pairwise interactions inter-cellularly -- how can these be used to predict higher order system behavior. What are the differences intra-cellularly and inter-cellularly for system prediction?)

Synthetic communities to understand mechanisms of community level functions

Diego Oyarzun

Towards dynamic metabolic engineering

Sense and control expression

Can create feedback regulation via metabolic control (e.g. inhibition)

Multi-objective optimization for control design

David Ross

NIST Cellular Engineering Group goal to provide foundation of measurements to support control and design

Need quantitative specification

Genetic sensors with quantitatively designed I/O

Large library of variants - 10^5 to 10^7

In silico selection and forward engineering

Elisa Franco

Synthetic DNA and RNA nanotechnology
Scalable RNA circuits

- RNA devices may be lower burden
- RNA sensors with fluorogenic reporters
- Composability of RNA circuits. Small transcription activating RNAs (STAR)
- Regulators that are being depleted. Can use compensation to support sustained system behavior.

Artificial liquid organelles made with DNA and RNA

- No proteins
- DNA and RNA interactions to create structures. Programmable dynamic DNA liquids.
- RNA liquids
- Need methods to control liquids to grow/dissolve, carry cargo, and host reactions

Brian Munsky
Optimal microscopy to quantify fluctuations
Observe dynamics and localization of transcription, translation, phosphorylation in single cells, realtime
Bursty behavior
Simple discrete stochastic models are sufficient to quantitatively reproduce observations
But single cell experiments are expensive, noisy, …
Many experimental design considerations
Design minimal and best number of experiments to gain most amount of information
    Fisher Information Matrix
Evaluate the effects of microscopy distortions

Enoch Yeung
Identifying data-driven genetic targets for biological control
Data driven models capture whole system response

- Varied condition space to excite system models
- Perform time-series RNAseq
- Use least squares optimization to estimate a model
- Can data driven models predict genetic markers that control cell fitness?
    - E.g. vary carbon and nitrogen levels and quantify levels of fitness
    - Identify some casualties
- Simulation to rank genetic targets for engineering fitness

Andreas Gyorgy

Tunable Ligand Inducible Plasmid (inducible plasmid copy number)

- Tuning, Probing, Controlling

PCN is typically hard-coded or requires multiple plasmids
To encode on a single plasmid: Separated positive feedback and negative feedback
Can then be used to probe resource competition

Neda Bagheri
Bridging the gap between in vitro and in vivo research with agent based models
ARCADE integrates models at many scales

- Single cells as agents (with rules based state changes)
- Cell actions are anchored to signaling and metabolic states
- Cell agents follow rules governing transitions between cell states
- Then, incorporate vasculature. Vascular function is a function of cell population and can also be remodeled by cell population → bilateral regulation
- Cell morphology can also have significant impact

Need quantitative metrics to quantify emergence
Need methods for rule identification, parameter estimation, and model validation
Hybrid models that integrate machine/deep learning with ABMs

Josh Leonard
Many new extracellular cell sensors
Barriers to acheiving robust performance: knowledge, variations, burden
Design parts that are inherenly robust (to expression leve/ratio)?
Tuning split protein reconstituion (SPORT)
Poly-transfection to sample many expression levels
Composbale Mammalian Elements of Transcription (zinc finger proteins) (COMET)
Composable designs yield circuit that work across topologies, implementations, and level of complexity
Selecting circuits that are more likely to work across a larger fraction of gene expression levels

Howard Salis
Scalable genetic system design
Generated a 100-part genetic system, including 20 sgRNAs
Used redundancy to target specific downregulation of several promoters
Improving evolutionary robustness

- Repetitive DNA causes genetic systems to break (repetition includes promoter sequences or RBS sequences)
- Non-repetitive parts calculator

Improving modularity and predictability

- Promoters are not modular genetic parts. Sequence around promoters has significant effects.
- Thermodynamic model to predict transcription rates
- Designed 14,026 promoters to systematically perturb interactions
- Can predict transcription rates and transcription start sites
- Create ML model for the promoter
- The promoter calculator

Improving systematic design

- Operon calculator

Leo Bleris

Mammalian synthetic biology and control

- Computations, genome editing, control/robustness, network theory

Delivery

- CRISPR-based delivery vehicle
- Control residence time and amplitude

Circuit characterization

- Noise analysis
- Transcriptionally uncoupled, translationally uncoupled, post-translationally uncoupled
- Intrinsic and extrinsic noise
- Significant amount of noise at the transcriptional level

Security

- PUF - a physical entity which provides a measurable output that can be used as a unique and irreproducible identifier for the artifact

Xioajun Tian

Understanding, predicting, and control of circuit-host interactions

EMT is controlled by cascading bistable switches

Topology-dependent interference of synthetic gene circuit function by growth feedback

Emergent damped oscillation induced by nutrient-modulating growth feedback

Winner-takes-all resource competition redirects cascading cell fate transitions

Coupling shared and tunable negative competitive regulation against WTA resource competition

Double-edged role of resource competition in gene expression noise and control

Chris Myers

Design of asynchronous genetic circuits

Glitches in the design of logic circuits

- Safe operations: want to avoid hazards

Burse-mode transitions
Logic hazards

- Redesign logic to be free of logic hazards
- Use redundant logic

Asynchronous genetic circuit design
Stochastic nature of genetic circuits though may lead some level of tolerance

Xiao Wang
Controllability of multiple gen networks
Order of induction impacts states that can be achieved
Fast high-dimensional bifurcation analysis with incomplete/indefinite information
Construction of high dimensional densely connected gene networks will need to employ recombinations of different regulatory mechanisms (e.g. transcription, translation, epigenetics, metabolic)

Marcella Gomez
Approaches and challenges in control of complex biological processes
Program the extracellular environment to adapt with cellular response

- Closing the loop, e.g. with sensor/actuators using bioelectronics
- Modulate pH levels
- Guide stem cell development
- How do you integrate sensor information to drive actuation

Application to complex biological processes: wound healing

- Stages: Homeostasis, inflammatory, proliferative, remodel
- Objective: Reduce wound healing time by 50%
- Compress the time that it takes to go thru the various processes
- There are underlying biological delays
- Somewhat analogous to perfect adaptation circuits
- Building connections between system elements
- Hierarchical model as a foundation for control

Challenges

- Guarantee safety
- Data driven
- Reachable states are known
- System response is highly variable and can change
- Infer dynamic relationships across spatiotemporal scales
-