National Institute of Biomedical Imaging and Bioengineering

2024 SYNTHETIC BIOLOGY CONSORTIUM MEETING

ABSTRACT BOOK

2024 SYNTHETIC BIOLOGY CONSORTIUM MEETING

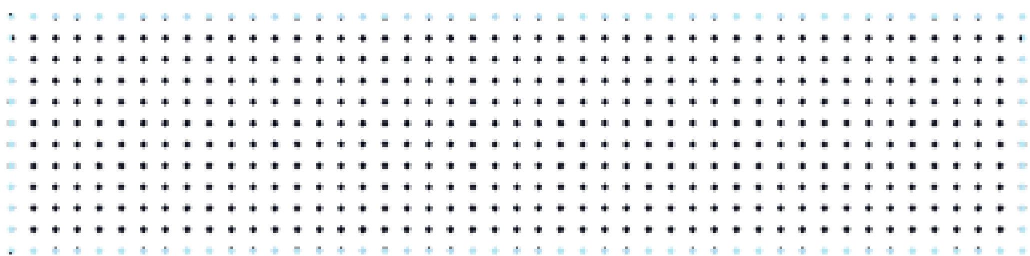


TABLE OF CONTENTS

1. DEVELOPMENT OF A GENE-TRANSFER-RESISTANT AND BIOCONTAINED NEXT-GENERATION BACTERIAL HOST FOR CONTROLLED DRUG DELIVERY 3
Akos Nyerges
Harvard Medical School

2. ENGINEERING A MULTIMODAL VACCINATION STRATEGY FOR COLD TUMORS USING MULTI-INPUT miRNA CLASSIFIER CIRCUITS ENCODED ON SELF-AMPLYING RNA 4
Fabio Caliendo
Massachusetts Institute of Technology

3. DISCOVERY AND OPTIMIZATION OF NOVEL COMPLEMENT COMPONENT 5A RECEPTOR 1 ANTAGONISTIC ANTIBODIES USING MACHINE LEARNING APPROACHES 5
Simuel D Stimple
Twist Bioscience

4. PREPARING FOR DISEASE OUTBREAKS WITH RAPID CELL-FREE DRUG SCREENS 6
Peter Andrew Carr
RTX BBN Technologies

5. ENGINEERING GENETIC TOOLS TO CONTROL INDIVIDUAL MICROBES AND MICROBIOTA WITHOUT ANTIBIOTIC RESISTANCE GENES AT A SINGLE STRAIN LEVEL 8
Tae Seok Moon
J. Craig Venter Institute

6. GENE EXPRESSION TUNING VIA SYNTHETIC GENE CIRCUITS REVEALS A NONMONOTONE INVASION LANDSCAPE OF METASTASIS ACTIVA-TOR BACH1 10
Yimeng Wan
Stony Brook University

DEVELOPMENT OF A GENE-TRANSFER-RESISTANT AND BIOCONTAINED NEXT-GENERATION BACTERIAL HOST FOR CONTROLLED DRUG DELIVERY

Akos Nyerges Ph.D., Harvard Medical School
email: akos_nyerges@hms.harvard.edu, website: https://engineeringbio.science

BACKGROUND: Synthetic biology transformed our ability to rationally reprogram cells and viruses and use such engineered biosystems, instead of small molecule drugs or biologics, as novel therapeutics. However, the use of these therapeutics necessitates the development of tight biocontainment methods that not only prevent unwanted proliferation but also the release of engineered genetic information. Engineering the genetic code of living organisms offers a solution. We recently demonstrated that - using rational genetic code engineering - we can 1.) achieve escapefree biocontainment, 2.) prevent horizontal gene transfer, and 3.) provide resistance to all natural viruses [1]. In this project, we translate this novel technology into bacterial hosts for safe, controlled drug production within the human gut.

METHODS: By repurposing genetic codons to encode nonstandard amino acids (nsAAs) not available in Nature or the human body and addicting growth-essential proteins to require such nsAAs for folding using rational protein engineering, escape- free biocontainment is possible (i.e., an escape rate under 1000× below the NIH standard of <1 escapee/10^8 cell that remains stable for at least 100 days [1, 2]). Our results showed that additional codons can be repurposed in such nsAA-dependent cells to confer a genetic firewall that prevents transgene escape and viral infections [1]. However, existing methods utilize clinically untested, potentially toxic nsAAs with unfavorable properties (poor solubility, light sensitivity, toxicity), preventing therapeutic use. Furthermore, in our first study [1], our genetic-code-based firewall resulted in reduced cell fitness, indicating collateral effects. We are solving this challenge by establishing biocontainment based on an orally bioavailable, safe nsAA that displays suitable pharmacokinetics and by generating high-fitness genetic firewalls in a synthetic E. coli strain using genome engineering and multiomics- & adaptive laboratory evolution-assisted troubleshooting.

RESULTS: Using multiomics, we discovered the rules responsible for the fitness-decreasing effects of synonymous genome recoding and developed a data-driven multi-omics-based genome construction workflow that troubleshoots synthetic genomes and engineered genetic codes [3]. We showed that synonymous recoding induces transcriptional noise, including new antisense RNAs, leading to drastic transcriptome and proteome perturbation in genome-recoded organisms. Using adaptive laboratory evolution and rational genome editing based on these newly discovered effects, we construct a high-fitness firewalled and biocontained bacterial strain that relies on a synthetic, amino-acid-swapped genetic code [1]. In parallel with strain construction, we also developed a computational protein engineering workflow enabling the rapid generation of nsAA-addicted protein variants for genetic biocontainment.

CONCLUSIONS: The combination of genetic-code-based biocontainment and horizontal gene transfer prevention in this project will enable long-term, safe therapeutic applications, including noninvasive drug production inside the human body, without the unwanted proliferation of such living therapeutics or their genetic information outside patients. In follow-up work, we will develop living therapeutic candidates and platform strains for the discovery and deployment of peptide and protein drugs with an expanded amino acid alphabet.

REFERENCES:

1. Nyerges A et al. A swapped genetic code prevents viral infections and gene transfer, (2023) Nature (PMCID: PMC10151025)
2. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules – April 2024. https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf
3. Nyerges A et al. Synthetic genomes unveil the effects of synonymous recoding, (2024) BioRxiv (PMCID: PMC11195188)

Acknowledgements:
The authors acknowledge an NIH/NIBIB K99 Award (Award number 1K99EB035165, PI: Akos Nyerges) as the source of funding for this research.

ENGINEERING A MULTIMODAL VACCINATION STRATEGY FOR COLD TUMORS USING MULTI-INPUT miRNA CLASSIFIER CIRCUITS ENCODED ON SELF-AMPLYING RNA

Dr. Fabio Caliendo, Massachusetts Institute of Technology
email: fabiocal@mit.edu

Background: Although cancer vaccination has shown efficacy in treating highly inflamed tumors, it remains ineffective against "cold tumors," which are characterized by limited T-cell infiltration, essential for identifying and eliminating cancer cells [1]. Thus, there is a pressing need to develop next-generation vaccines tailored to treat these tumors. To address this challenge, we are developing a multimodal vaccination strategy that is capable of triggering cancer cell death and subsequent release of tumor-associated antigens (TAAs) within the tumor microenvironment (TME) while simultaneously inducing the polarization of tumor-resident macrophages toward a pro-inflammatory phenotype, thereby promoting local T-cell recruitment and activation.

METHODS: To achieve this goal, we devised the use of multi-input cell classifier circuits encoded on self-amplifying (replicon) RNA to selectively activate distinct genetic programs in cancer cells and intratumoral macrophages upon vaccination. These multi-input cell classifier circuits utilize miRNA sensing modules, which detect the activity of specific miRNAs in recipient cells and, in response, enable the expression of a genetic payload only in the desired cell type [2]. To regulate the expression of genetic payloads, miRNA sensing modules were inserted into the 3'UTR of replicon RNA. By strategically positioning these modules, we aim to achieve precise, cell-specific control of genetic payloads based on the endogenous miRNA activity within the recipient cells.

RESULTS: We demonstrated that miRNA sensing modules inserted at the 3'UTR of replicon molecules effectively regulate the expression of the genetic payload. Additionally, we showed that we can achieve either coordinated or independent regulation of two genetic payloads by strategically positioning these miRNA sensing modules between two expression units encoded on a single replicon RNA molecule. Building on these findings, we developed a miRNA-high sensor circuit that can be delivered on a single replicon RNA molecule. This circuit activates the expression of the output payload only upon detecting the activity of a desired miRNA in the recipient cell.

CONCLUSIONS: Our results indicate the feasibility of encoding multi-input cell-classifier circuits on replicon RNA for cell-specific expression of distinct genetic programs in different cell types within the tumor. Building on these results, we plan to assemble and evaluate a complete multi-input cell-classifier circuit, starting with in vitro experiments before progressing to in vivo vaccination studies in relevant murine tumor models.

REFERENCES:

1. Paston et al. Frontiers in Immunology. 12:627932, 2021.
2. Wroblewska et al. Nature Biotechnology. 33, pages 839–841, 2015.

DISCOVERY AND OPTIMIZATION OF NOVEL COMPLEMENT COMPONENT 5A RECEPTOR 1 ANTAGONISTIC ANTIBODIES USING MACHINE LEARNING APPROACHES

Dr. Samuel D Stimple, Twist Bioscience
email: sstimple@twistbioscience.com

BACKGROUND: G-Protein Coupled Receptors (GPCR) remain one of the most attractive but more challenging therapeutic targets for antibody discovery. Complement component 5a receptor 1 (C5aR1) is a GPCR of interest with potential therapeutic applications in immuno- oncology and inflammation. This GPCR is involved in the complement cascade and in innate immunity. Stimulation of C5aR1 by its ligand, C5a, promotes tumorigenesis by suppressing T-cell mediated anti-tumor activity while blockade of C5aR signaling has been shown to attenuate tumor growth.

METHODS: In a combined approach with Macromoltek, we leverage Twist’s DNA synthesis and library assembly technology with structure-based machine learning to design a targeted anti-C5aR discovery library to identify several highly potent and selective lead antibodies against C5aR.

RESULTS: Several of the candidates identified from this study are also shown to have functionally antagonistic effects on C5aR1-mediated signaling in cell-based assays.

CONCLUSIONS: This work demonstrates how the combination of highly specific DNA synthesis, coupled with informed design, can be paired to successfully prosecute challenging targets for novel therapeutic discovery.

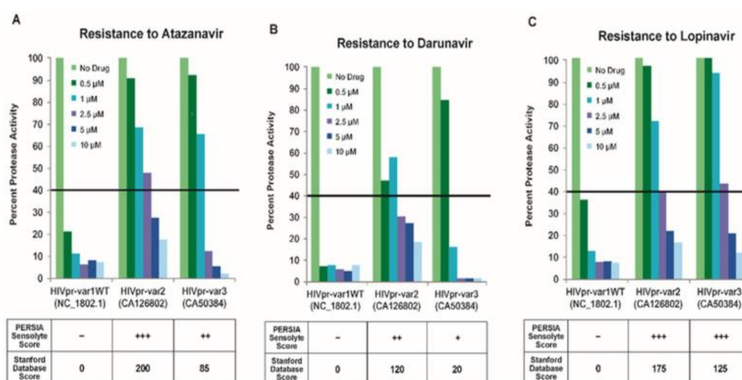
PREPARING FOR DISEASE OUTBREAKS WITH RAPID CELL-FREE DRUG SCREENS

Dr Peter Andrew Carr, RTX BBN Technologies
email: peter.a.carr@rtx.com

Background: The PERSIA (PURExpress-ReAsH-Spinach In-vitro Analysis) cell-free measurement technique was designed to combine simultaneous measurements of RNA transcription, protein translation, and enzymatic activity using a simple cell-free fluorescent assay. Applied to potential drug targets (e.g. viral proteins) this method can be used to determine the effects of candidate drugs, for both repurposing and larger-scale screening. PERSIA is especially adaptable for examining large numbers of genetic variants, such as potentially drug- resistant strains. We consider how this approach can be adapted and scaled to prepare for future disease outbreaks.

METHODS: PERSIA has been used to assess HIV-1 protease activity with multiple inhibitory drugs, and for multiple drug-resistant variants of the protease [1]. Synthetic linear DNA constructs encoding protease variants were used directly from the manufacturer, with no cloning necessary. These variants expressed the Spinach RNA tag and FlAsH protein tag. Cell-free expression of these constructs in concert with the DFHBI and ReAsH binding-dependent fluorescent dyes allowed tracking of RNA and protein levels in real-time [2]. Three different HIV-1 clinically-relevant protease inhibitor drugs were used to create activity inhibition profiles and compared to known values (HIV Drug Resistance Database).

RESULTS: The PERSIA approach demonstrated the capability to rapidly assay drug inhibition of HIV-1 protease in the presence and absence of multiple drugs. Results were comparable to those from clinically-approved assays (Figure 1).



A	HIVpr-var1WT (NC_1802.1)	HIVpr-var2 (CA126802)	HIVpr-var3 (CA50384)
PERSIA Sensolyte Score	-	+++	++
Stanford Database Score	0	200	85

B	HIVpr-var1WT (NC_1802.1)	HIVpr-var2 (CA126802)	HIVpr-var3 (CA50384)
PERSIA Sensolyte Score	-	++	+
Stanford Database Score	0	120	20

C	HIVpr-var1WT (NC_1802.1)	HIVpr-var2 (CA126802)	HIVpr-var3 (CA50384)
PERSIA Sensolyte Score	-	+++	+++
Stanford Database Score	0	175	125

Figure 1

PERSIA analysis of clinical HIV protease variants. HIV Drug Resistance Database scores included for comparison. Wild-type and known resistant mutants were analyzed in the presence of HIV antiviral drugs targeting the protease (A) Atazanavir, (B) Darunavir, and (C) Lopinavir. From Wick et al.

CONCLUSIONS: With a demonstrated ability to assay drug-target activity, we now seek to apply a similar approach to preparing for future pandemics. A dedicated, prepared facility would be poised to rapidly leverage the availability of new viral sequences, synthesize the genetic material of the targets of interest, and safely employ that material to assay candidate drugs [3]. In the early stages of a pandemic, a great deal of time could be saved in determining and approving new treatments, potentially sparing many lives.

REFERENCES:

1. S. Wick et al. (2019) ACS Synth. Biol. 8(5):1010-1025.
2. S. Wick and P.A. Carr (2022) Methods Mol. Biol. 2433:169-181.
3. P.A. Carr et al. (2020) Lincoln Laboratory Journal 24(1):213-233.

Acknowledgements:
I would like to thank Scott Wick (MIT Lincoln Laboratory) for his work in co-developing the PERSIA method. This work was supported by NIH under grant number P50 GM098792.

ENGINEERING GENETIC TOOLS TO CONTROL INDIVIDUAL MICROBES AND MICROBIOTA WITHOUT ANTIBIOTIC RESISTANCE GENES AT A SINGLE STRAIN LEVEL

Prof Tae Seok Moon, J. Craig Venter Institute
email: tsmoon7@gmail.com, website: https://www.linkedin.com/in/tae-seok-moon-12515b23

BACKGROUND: Microbial biocontainment is essential for engineering safe living therapeutics [1, 2]. However, the genetic stability of biocontainment circuits is a challenge. Kill switches are among the most difficult circuits to maintain due to the evolution of escape mutants. We engineered two CRISPR-based, chemical- or temperature-inducible kill switches in the probiotic Escherichia coli Nissle and demonstrated mutationally robust biocontainment [3].

METHODS: In this presentation, we will discuss our machine learning-based microbiota engineering tools that are useful to manipulate microbiota and kill pathogens at a single strain level [4]. Specifically, we will discuss the development and validation of a novel computational program, ssCRISPR, which designs strain-specific CRISPR guide RNAs (gRNAs) that can be utilized to modify complex consortia.

RESULTS: As a proof of concept, we applied the program to two novel applications: the isolation of specific microbes from consortia through plasmid transformations and the removal of specific microbes from consortia through liposome- packaged CRISPR antimicrobials. Additionally, we will discuss antibiotic resistance gene-free plasmid systems that prevent antibiotic resistance spread via horizontal gene transfer [5-8].

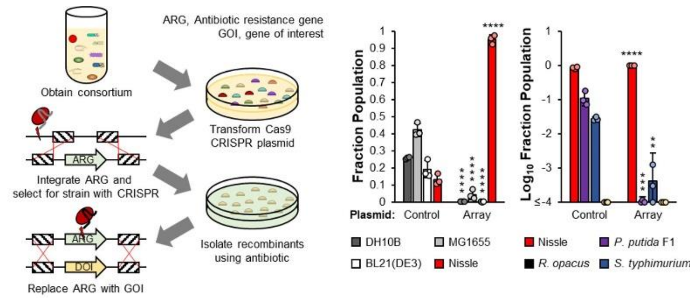


Figure 1

Figure 1. Strain-specific isolation of microbes from environments. Machine learning-based algorithm guides gRNA design to specifically target only one microbe in the consortium. Nissle was isolated from both synthetic consortia.

CONCLUSIONS: This new technology has vast implications in designing strain-specific antimicrobials and combating the growing concern of antibiotic- and bacteriocide-resistant microbes.

REFERENCES:

1. TS Moon, Probiotic and microbiota engineering for practical applications, Current Opinion in Food Science. https://doi.org/10.1016/j.cofs.2024.101130 (2024)
2. Y Ma, A Manna and TS Moon. Advances in engineering genetic circuits for microbial biocontainment. Current Opinion in Systems Biology. doi.org/10.1016/j.coisb.2023.100483 (2023)
3. AG Rottinghaus, A Ferreiro, SRS Fishbein, G Dantas and TS Moon. Genetically stable CRISPR-based kill switches for engineered microbes. Nature Communications. 13, 672 (2022)
4. AG Rottinghaus, S. Vo and TS Moon. Computational design of CRISPR guide RNAs to enable strain-specific control of microbial consortia. PNAS. 120, e2213154120 (2023)
5. TS Moon. SynMADE: Synthetic Microbiota Across Diverse Ecosystems. Trends in Biotechnology. 40, 1405-1414 (2022)
6. TS Moon. EBRC: Enhancing Bioeconomy through Research and Communication. New Biotechnology. 78, 150–152 (2023)
7. TS Moon. SynHEAL: Synthesis of Health Equity, Advancement, and Leadership. ACS Synth. Biol. 12, 1583–1585 (2023)
8. MB Amrofell, S Rengarajan, S Vo, ESR Tovar, L LoBello, G Dantas and TS Moon. Engineering E. coli strains using antibiotic resistance gene-free plasmids. Cell Reports Methods. https://www.cell.com/cell-reports-methods/fulltext/S2667-2375(23)00348-X (2024)

Acknowledgements:

This work was supported by the National Science Foundation [DGE-1745038 to M.B.A.], the National Center for Complementary and Integrative Health of the National Institutes of Health [R01 AT009741 to T.S.M and G.D.], the United States Department of Agriculture [2020-33522032319 to T.S.M.], and the U.S. Environmental Protection Agency [84020501 to T.S.M.]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. This work is dedicated to the memory of Steven T. Vo.

GENE EXPRESSION TUNING VIA SYNTHETIC GENE CIRCUITS REVEALS A NONMONOTONE INVASION LANDSCAPE OF METASTASIS ACTIVA-TOR BACH1

Dr. Yiming Wan, Stony Brook University
email: Yiming.wan@stonybrook.edu
website: https://scholar.google.com/citations?view_op=list_works&hl=en&hl=en&user=1P8MUGEAAAAJ

BACKGROUND: Targeting genetic regulators to limit or even prevent cancer metastasis has long been investigated and evaluated as cancer treatments. Yet only a handful candidates proved to be properly effective though still with unknown side-effects. One of the key reasons is related to the insufficient understanding of the quantitative correlations between regulator level and its phenotypic consequence, leaving a big knowledge gap between research and clinical development. As an example, BTB and CNC Homology 1 (BACH1) has demonstrated to be a widely expressed metastasis activator besides its many other roles as a master transcriptional factor. It has drawn accumulating attention to be considered as a cancer therapy especially in triple negative breast cancer (TNBC), yet its quantitative characteristics in metastasis regulation is still poorly understood. As a step towards quantitatively unraveling BACH1's role as a metastasis regulator in TNBC, here through the strategic combination between synthetic gene circuit and precise cell engineering, we develop a generally applicable, two-step genome engineering pipeline and apply it into the investigation of BACH1 invasion landscape.

METHODS: We use HEK293 cell type for cell engineering development and validation and MDA-MB-231 cell type as the TNBC cell model to investigate the BACH1 invasion landscape. qRT-PCR experiments in this study are mostly done using TaqMan® probes; BACH1 antibodies for immunofluorescence and Western-blot are available through Santa Cruz Biotechnology, CAT#sc-271211. Boyden chamber invasion assay is used to evaluate the cell invasiveness in vitro. Flow cytometry measurement is conducted regularly using BD LSRFortessa flow cytometer with High Throughput Sampler (HTS) (Becton, Dickinson and Company). And all the imaging acquisition are using a Nikon Eclipse Ti-E inverted microscope.

RESULTS: In order to precisely control the expression of the target gene, we applied negative-feedback (NF) gene circuit enabling continuous tuning of GFP-BACH1 fusion or GFP protein expression with minimum population variance. To build up the cell models for quantitative studies, we first generated and verified engineered HEK293 and MDA-MB-231 cells with single-copy FRT Landing-Pad (LP) insert into AAVS1 safe harbor site using CRISPR/Cas9. Then individual synthetic gene circuit was exchanged into the exact LP site using recombinase-medicated cassette exchange (RMCE). Upon vali- dation of every model, we investigated the circuit performances from both transcriptional and translational level using flow cytometry, qRT-PCR, immunofluorescence and Western-blot. We were able to show that BACH1 level was dose- dependently increasing according to the input of chemical inducer (doxycycline), and population variance was reasona- bly low but with distinct differences between monoclonals (Fig1A-B). Next, we explored the cell invasiveness changes with incremental BACH1 level and found a nonmonotone landscape, which was further verified through testing the BACH1 level changes in the invaded subpopulation via flow cytometry. Two MB231 clones with similar expression mean but different variance was tested simultaneously and nosier clone showed higher invasiveness at high BACH1 level (Fig1C). Further, through the analysis of clinical RNA-seq dataset from TCGA, we identified one of the downstream targets of BACH1 – RKIP could be highly related and proved its significant correlation with such nonmonotone invasion landscape via qRT-PCR (Fig1D). This nonmonotone RKIP regulation was also confirmed in HEK293 cell models, making it potentially universal.

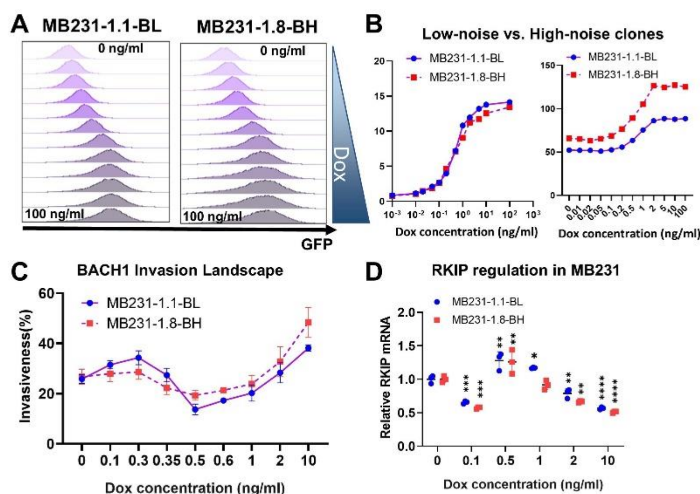


Figure 1

Fig. 1 (A) Representative dose-responses of fluorescence intensity histograms from low-noise (BL) and high-noise (BH) MB231 BACH1 clones. (B) Quantitation of expression mean and variance of BL and BH. (C) Invasion landscape and (D) RKIP dose-responsive regulation in both BL and BH.

CONCLUSIONS: In this study, we developed a generally applicable synthetic biological approach to perform rigorous quantitative studies elucidating genotype-to-phenotype correlation in vitro. We characterized the performances in two cell types and in many monoclonals demonstrating the robustness of the system as well as the unique opportunities for expression variance studies. Further by applying these models, we unraveled the complexity of BACH1's role as metastasis regulator by un-covering a nonmonotone invasion landscape and pointed out a potential corresponding effector – RKIP. More in depth mechanism studies will have to be taken to reveal more effectors and interactions that can comprehensively explain this phenomenon. This study, at the same time, highlights the importance of quantitative understanding of the onco-targets before we boldly head into clinical trials and development.

REFERENCES:

1. Wan, Y. et al. Nonmonotone invasion landscape by noise-aware control of metastasis activator levels. Nat Chem Biol 19, 887-899, doi:10.1038/s41589-023-01344-z (2023).

Acknowledgements:
This research was supported by the National Institutes of Health, NIGMS MIRA Program (R35 GM122561), and by the Laufer Center for Physical and Quantitative Biology and Stony Brook BME department.