Team Meeting #2

Good and Not-So-Good Ideas

Presentation Outline

Each person presents general topic area X

- Good Ideas within topic area X
- Bad Ideas within topic area X

Topic Areas

- Applications of MAGE - Ian Choi
- Adaptations of DNA walkers - In Young Cho
- DNA Origami - Wes
- DNA Delivery - Val
- Modifying cell DNA - Jacob
- Proof of concepts of In Vivo Ideas - Gina

Applications of MAGE - Good Ideas

- Say that RNA scaffolds can be used to increase H2 production. Can we take DNA that can be transcribed into RNA scaffolds and insert it into the genome of a bacteria? After one cycle of mage, take only high-producing bacteria that successfully made scaffold.
- Repeat process until bacteria creates most efficient scaffold possible on its own via evolution
  - Very interesting idea, though possibly very complicated to pull off.

Applications of MAGE

Commentary by Adam:

Keep in mind MAGE only works in e. coli so far. They're working on translating to other systems but that requires an analog of the lambda red system. Also most microbes are not as easily transform-able by electroporation as e. coli and grow/recover much more slowly. So rather than specific applications of Mage in other organisms the area to focus on would be tools to enable Mage to happen at all there!

So, DNA recombination in other systems. Efficient DNA delivery to other cell types. Getting dna across cell membranes! Ways to reduce the number of cycles. Good selections. Things like that.

For h2 production, can you think of a good automated selection for improving that? Could be interesting to take this system and try to improve it by in-vivo directed evolution using Mage as you suggest. We'd then sequence the DNA to see what worked.

I like the polymerase + cargo / displacement which is currently in the random category. That's not a category in the end. Got to reduce these in # and give more in depth analysis of a few, whether "good" or "bad". :-)

Applications of MAGE - Good Ideas

- Right now, bacteria is becoming ever-more resistant to conventional desanitization methods. We also know phage can be made to destroy bacteria. As the bacterial strains evolve in hospitals, can we put them into MAGE and select for phage that can kill them? In other words, can we evolve phage in tandem with resistant bacteria? That way we would never lose the war against resistant microbes.
  - Probably dangerous for us? Maybe use more benign resistant bugs?
  - Maybe can backfire with evolving phage?

Applications of MAGE - Bad Ideas

- Use MAGE to accelerate evolution of certain bacteria found in "skinnier" rat microbiomes.
  - Simple in concept
  - Maybe too simple?
  - Need animal training?
  - Application of known process, maybe not original research

Applications of MAGE - Bad Ideas

- Some bacteria introduce different chemicals based on whether or not the host is having a stress-response. Can we induce the bacteria to introduce some kind of fat-burning chemical associated with stress-response and put it through MAGE?
  - Maybe too complicated?
  - Again, we need animal models

Applications of MAGE - Bad Ideas

- What happens when you accelerate evolution of the microbiome found in humans/rats?
  - Interesting concept
  - Simple
  - Maybe need animal training?

Applications of MAGE - Bad Ideas

- What happens if you put synthetic life into MAGE?
  - Where do we get synthetic life?
  - From Venter?

Applications of MAGE - Bad Ideas

- How do bacteria evolve to the continual reintroduction of H2-accelerating scaffolds? Does it adapt to become more efficient?
  - Probably very labor intensive due to continual introduction of scaffolds

Random Ideas for Quick Review

- Douglas et al. mentions that fluorescent signals are dampened or hidden inside a DNA box. Can't that be an easy indicator for whether or not construction was successful?
- Or say if the box closes, the latch is composed of two halves of GFP. When the latch folds over, the GFP works and fluorescence is viewed
- The scaffolds mentioned for H2 production are not natural. Can we mimic natural "scaffolds" found in the most efficient organisms?

Random Ideas Continued

- Use DNA scaffolds to somehow test microbiome interactions?
- Papers mention difficulties of getting bacteria to remain in one area for drug delivery. Why not use magnetics?
- Liposomes - are they useful with DNA origami?
- Stem cells + DNA origami?
- If you have a polymerase running along DNA, can we use DNA displacement to force the polymerase to "drop" and pick up cargo? <-- ADAM LIKES THIS ONE

Random Ideas (even more)

- Polymerase stops when it reads a certain DNA sequence. Can we exploit that to make robots stop in place? Or do they use stop transcription factors?
- Pathogen filter out of DNA?
- DNA popup assembly?
- Using Brownian motion to power robots

Random Ideas (even even more)

- From the paper on biocompatible artificial DNA linker:
  - Use in MAGE?!
  - Use the linker in DNA origami and scaffolds?
  - Why can't we synthesize a linkage using the natural phosphate and oxygens found in normal links?
  - Why not use repair enzymes used for attaching okazaki fragments?
  - Make a DNA scaffold with enzymes attached to it to make it a veritable "assembly line"

Adaptations of DNA Walkers

- Main Goal - increase the speed and overall movement capabilities of DNA walkers (as taken from RNAP)
- Form sequences of interconnected DNA templates to provide a longer path for the walker
  - Overcome the ~100 bp limit for DNA walker platforms
  - How do we interconnect the strands?
    - Have a sequence of zigzagging DNA strands with modified SBP-His-RNAP complex advanced beyond the promoter. Pass the DNA cargo from strand to strand
    - Use click chemistry to bind the strands together

Adaptations of DNA Walkers

- Manipulate terminator sequences in bacterial genes to create longer templates
  - Higher processivity, since all NTPs can be added at once
  - Greater template length + possibly cheaper manufacturing costs
  - Accuracy of termination?

Adaptations of DNA Walkers

- Bind DNA to microtubules to facilitate in-vivo cargo movement
  - Biotinylate a length of microtubule to act as "glue"
  - Coat surface of microtubule with nickel (?) to bind DNA template in-vitro
  - Use same mechanism as Pomerantz paper to test in-vitro movement of cargo along microtubule
  - In-vivo
    - Need to either sequester the complex or develop mechanisms where NTP exposure does not need to be controlled

Adaptations of DNA Walkers

- Modifications on cargo
  - Attach DNA box as cargo on DNA walkers
    - Allow controlled transport of molecules other than nucleic acids
- Size incompatibility between cargo and RNAP motor?
  - Have hanging strands from each of the vertices of DNA box, or biotinylate the vertices directly and transport cargo in a cargo "tube"

Adaptations of DNA Walkers

- Link RNAPs together to create a cargo train
  - Ideas for linkage strands?
  - Limits on sequence variability and movement control, since RNAPs will be on different nucleotides

DNA Origami Related

- How do these origami structures last when inside the body
  - "Show surprising stability" (Pinheiro et al, 2011)
- Effect of Geometry
  - Shape and structure alter length in vivo
  - DNA vs. RNA benefits
- Negative Signal/Nano Pathway
  - With use of restriction enzymes

DNA Delivery

- magnets!
- attach magnets to nanostructures so that you can use another magnet to localize the nanostructures around, say, a tumor
- previous work with magnets and DNA origami? http://www.mendeley.com/research/dna-origami-selfassembly...

DNA Delivery

- using magnets to activate nanorobot?
- using multiple magnets or moving them around to maneuver the nanorobots around
  - like magnetic tracks!

- bringing together two different structures?

DNA Delivery

- Logic gates!
- for example: AND --> two hinges on a lid, each unlocked by a different particle
- extrapolating:
  - OR --> can be triggered by two different particles, only need one to activate
  - XOR --> activated when one binds to it, deactivated if both bind?

http://www.sciencemag.org/content/335/6070/831.full

DNA Delivery

- apply magnetic and logic gate techniques as steps within the cascade of boxes proposed by last year's team

Working In Vivo - Past work

- DNA nanostructures that are long single strands CAN be amplified with polymerase in vivo
  - Shih's 1.7 kb octahedron, Li's self-assembled tetrahedral

Main challenges of converting in vivo (Hanying Li, et al)

- Degradation by plasma nucleases
- Uptake and distribution to non-targeted tissues
- Poor permeability

Proof of Concepts In Vivo

- Challenge: poor permeability
  - negatively charged DNA cannot penetrate negative cell membrane
- Solution:
  - use cell-penetrating peptides (Holm T, et. al)
  - DNA-RNA aptamers (oligonucleic acid or peptide molecules that bind to specific target molecule)
  - folate (used to facilitate transport of Cy3 into cancer cells)

Proof of Concepts In Vivo

- Expanding on last year's project and testing feasibility of opening sphere and inserting box in model organisms
  - Need to check whether DNA origami would be rejected (biocompatability)
  - Would it form the same shape as in vitro? How to ensure it does if not? (intracellular assembly)

Proof of Concepts In Vivo

- Make analogous structures with RNA and analyzing whether DNA-RNA hybrids are more effective than just DNA complexes
  - Easier to form single strands of RNA than DNA, which is easier to fold into necessary nanostructure
- Seung Hyeon Ko, Min Su, Chuan Zhang, et. al proved self-assembly of RNA can be signaled by DNA
  - DNA-RNA hybrids are more stable thermodynamically but in this study, found it to degrade faster than just DNA complexes

Proof of Concepts In Vivo

- Challenge: Degradation by plasma nucleases
- Solution: Measure half-life of nano-structure and predict what causes different degradation rates
  - difficult to do since need to test in vivo to get accurate results - which is our first big issue in the first place
  - too simple...just measuring degradation rates?

Modifying Cell DNA - Bad Idea

Gene therapy has been around for decades

- heat-shocking E. coli
- virus/retrovirus
- naked plasmid DNA injection

Modifying Cell DNA + Self-assembly
Good Idea?

Incorporate DNA into genome --> transcribe into mRNA --> self-assemble

Challenges

- keep mRNA from being translated/degraded
- get mRNA out of the nucleus (eukaryotes)

Interact with cellular components

- e.g. microtubule walker

Inserting DNA + Self-assembly
Good Idea?

Insert DNA via retrovirus --> self-assemble

Challenges

- prevent integration into genome
  - no integrase in Pre-Integration Complex

Interact with cellular components

- e.g. microtubule walker