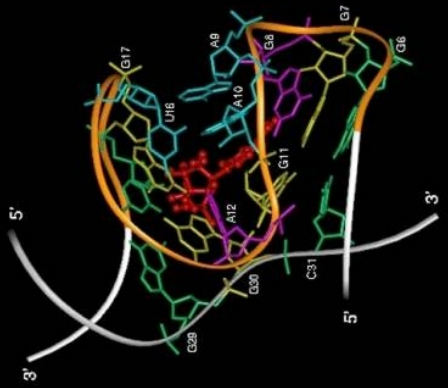
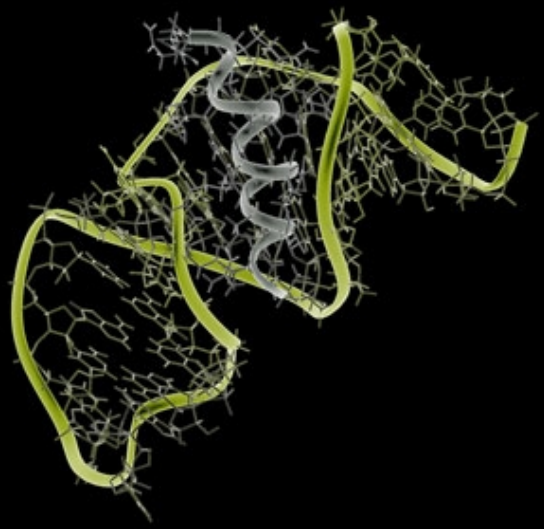
Drug Discovery with SELEX

Systematic Evolution of Ligands by Exponential Enrichment





Linyi Gao

Oligonucleotides—RNA or DNA

- Trillions of possibilities, most never seen…

Nucleotides (*n*)	Number of possible sequences (4^n)
5	1,024
10	1,048,576
15	1,073,741,824
20	1,099,511,627,776
25	**1,125,899,906,842,624**
40	1,208,925,819,614,629,174,706,176

- Could a few of these be very special?

Aptamers—Chemical Antibodies

- Specific DNA or RNA sequences
  - Synthetic, unnatural, 30-40 random nucleotides
- Specific sequence ⟍ 3D structure ⟍ tight binding to target, e.g.
  - Toxins, virus-infected cells, cancer cells, etc.
- Disrupt protein interactions ⟍ treat disease
- Aptamers are very rare
- How can we identify & make

Don’t Analyze, Just Look

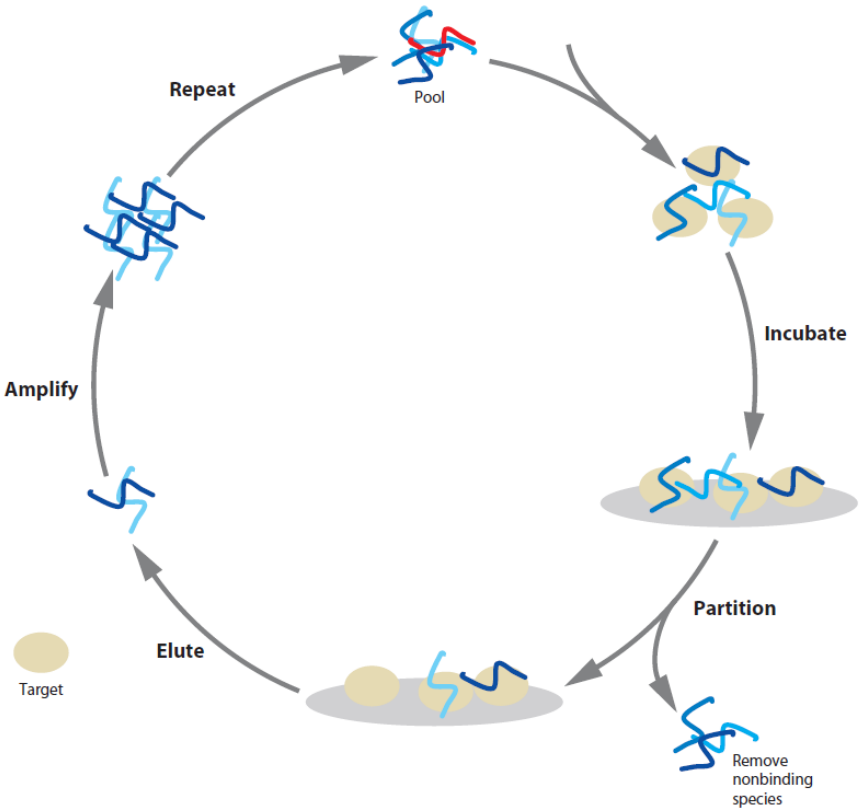
SELEX—Screening Made Possible

- Aptamers are hard to see but easy to screen:

Issue	Macroscopic (Legos)	Biochemical (A,T,C,G)
Time 	Slow, time-consuming to build	Fast—we can use the most efficient builders (RNA pol., DNA pol.)
Money & Resources	$$$ Expensive, wasteful	Free! And abundant (A, T, C, G)
Space	Big—need space for storage	Small—trillions in a test tube
	⤡Not possible	⤡SELEX!

The SELEX Cycle (1990)

- 1. Synthesize random RNA library (1012 to 1015 aptamers)
- 2. Incubate with target protein *in vitro*
- 3. Wash away unbound RNAs
- 4. Collect bound RNAs
- 5. Amplify (PCR, etc.)



A Video Animation from JoVE:
http://www.jove.com/index/Details.stp?ID

Aptamers as Drugs

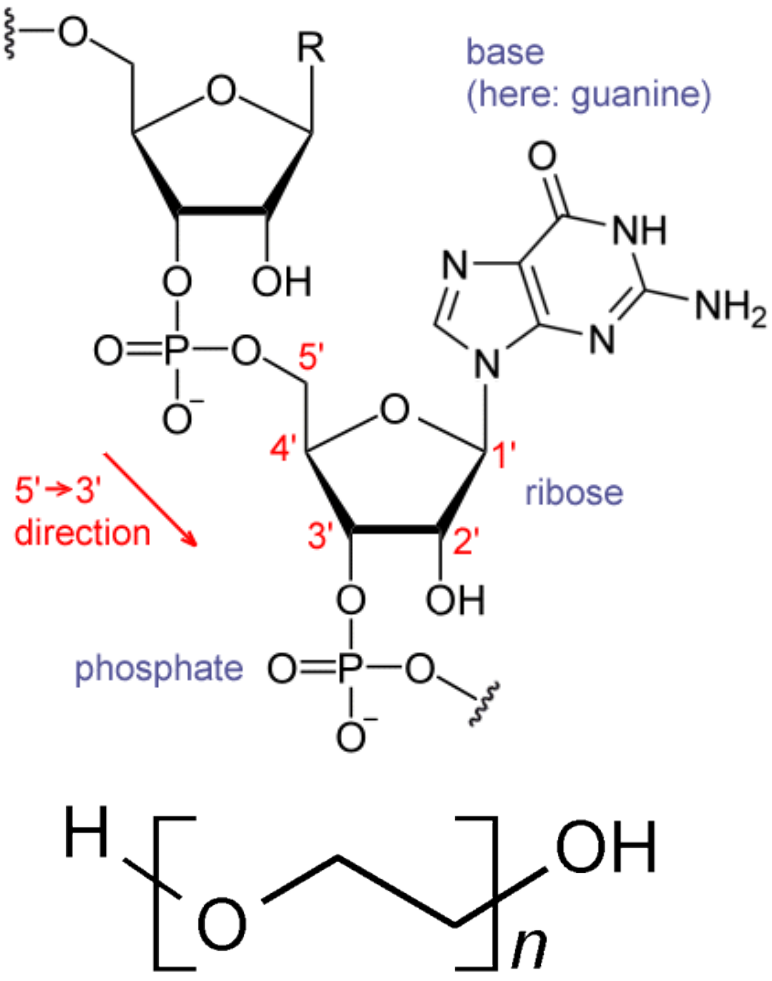
- Goal: Long-term, chronic administration as a treatment for disease
- Disrupt crucial protein interactions
- Toxicity: generally non-toxic, non-immunogenic
- Concerns to be assessed:
  - Nonspecific off-target protein interactions
  - Anticoagulation

Aptamers as Drugs—Concerns

- 1. They are degraded
  - Metabolic instability, degradation by nucleases
- 2. They flush out of the body
  - Rapid elimination of non-protein-bound oligonucleotides through urine
- 3. They don't stay where you want them to
  - Rapid distribution from the plasma & interstitial fluid into the tissues

Improving the Aptamers

- Chemical modifications —better retention in body
  - 2′ —OCH_3 or 2′ —F to increase stability (use mutated RNA polymerase)
  - PEG linkage for (passes through kidneys slower)
  - Other-site specific modifications

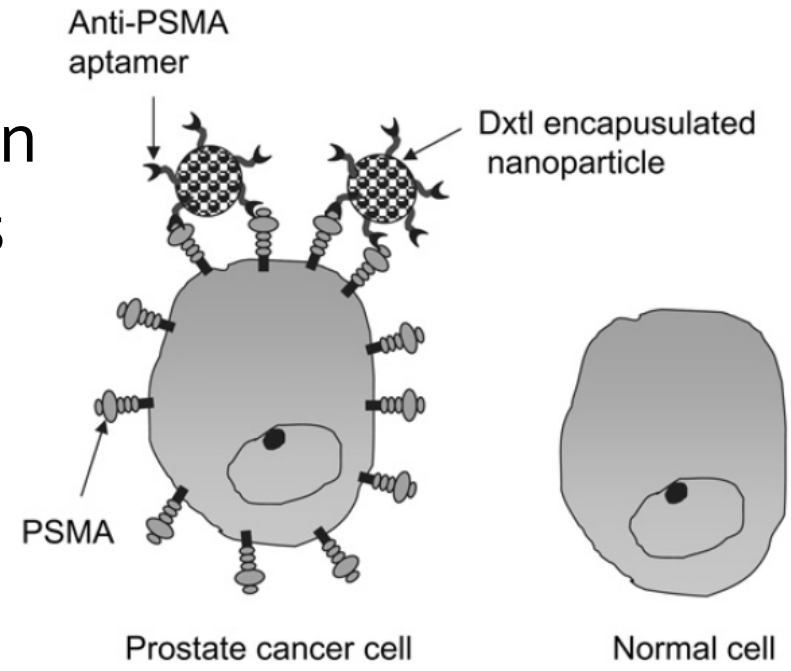


Aptamers as Drugs—Macugen®

- Treats neovascular age-related macular degeneration (AMD)
  - Binds to vascular endothelial growth factor (VEGF) 165
- By injection only (into eye)
- Only currently marketed aptamer drug (2004)

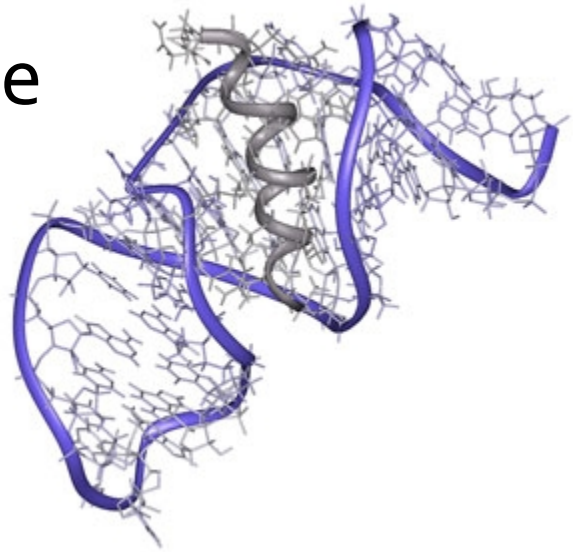
Variations and Applications

- Cell-SELEX—aptamers screened with whole cells
  - Identify differences between cancer and non-cancer cells (e.g. membrane receptors)
  - Affinity vs. selectivity
- Templates for rational drug design
- Diagnostic and biosensing potential (e.g. imaging)



Conclusion

- Promising as a class of drugs; much more development in the future (Bouchard et al. 2010)
  - Next-generation aptamers
- More clinical familiarity with aptamers needed
- The SELEX process is a great idea that takes advantage of biochemical properties and tools



Literature Referenced

- Barbas AS, White RR. The development and testing of aptamers for cancer. *Curr Opin Investig Drugs* 2009; **10**:572-578.
- Bouchard PR, Hutabarat RM, Thompson KM. Discovery and development of therapeutic aptamers. *Annu Rev Pharmacol Toxicol.* 2010; **50**:237–257.
- Khati M. The future of aptamers in medicine. *J Clin Pathol* 2010; **63**: 480-487.
- Ng EW, Shima DT, Calias P, Cunningham ET

Photo Credits

- Photo of dsDNA: http://voices.washingtonpost.com/blog-post/101
- Front slide aptamer: http://aptamer.icmb.utexas.edu/images/aptamer
- Front slide aptamer 2: http://www.biochemistry.ucla.edu/biochem/Facu
- RNA structure: http://upload.wikimedia.org/wikipedia/commons
- Sand sifting through hand: