Module Overview

Day	Lecture	Lab
1	Introduction	DNA library synthesis (PCR)
2	SELEX I: Building a Library	DNA library purification (agarose gel electrophoresis)
3	SELEX II: Selecting RNA with target functionality	RNA library synthesis (*In vitro* transcription = IVT)
4	SELEX III: Technical advances & problem-solving	RNA purification and heme affinity selection
5	Characterizing aptamers	RNA to DNA by RT-PCR
6	Introduction to porphyrins: chemistry & biology	Post-selection IVT Journal Club 1
7	Aptamer applications in biology & technology	Aptamer binding assay
8	Aptamers as therapeutics	Journal Club 2

Therapeutic Aptamers

20.109 Lecture 8

3 March, 2011

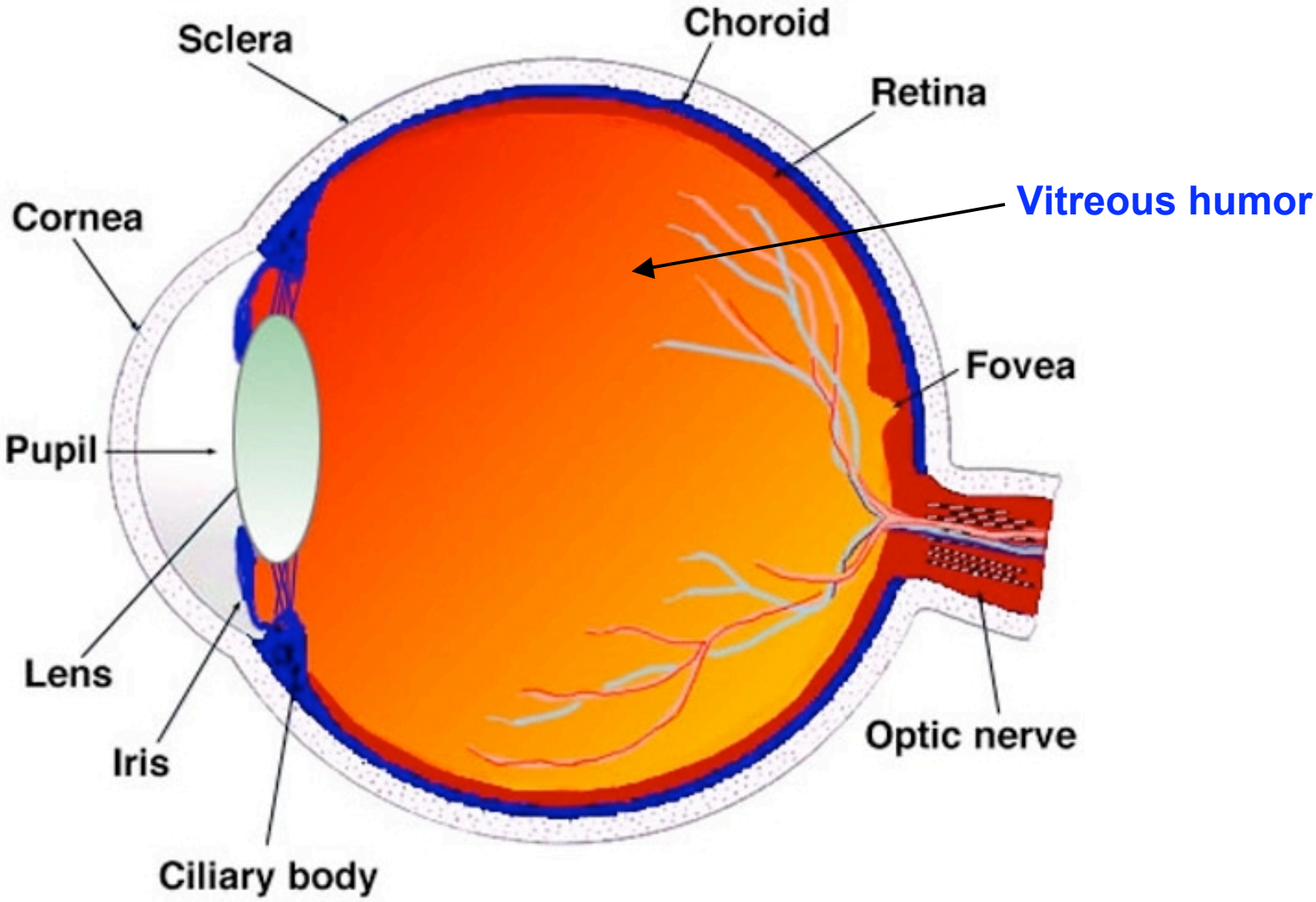
Today's Objectives

- Aptamers developed for therapeutic purposes:
  - Focus on one disease process
  - Gain an appreciation for:
    - Defining the problem you are addressing on several different levels:
      - Organism
      - Anatomical
      - Molecular
    - Developing solutions based on understanding disease mechanism
    - Some challenges in translating your molecular level solutions (aptamer) into efficacious drugs (in people)

Age-related macular degeneration (AMD)

- Disease affecting the eye
  - Most common cause of *irreversible* vision loss in the developed world
  - 8 million in the U.S. are affected
  - Typically, incomplete vision loss
    - Non-life threatening
    - Enough to impair independence in daily living activities

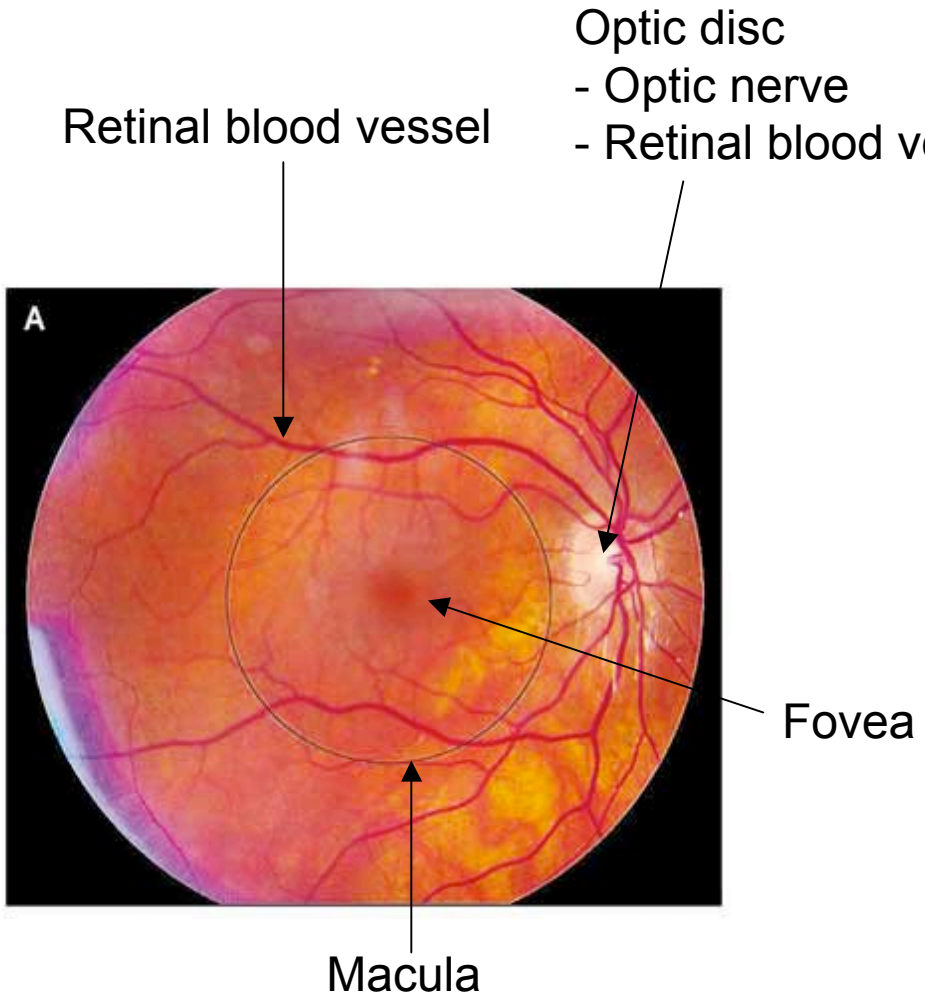
Anatomy of the eye



Gross Anatomy of the Eye, 2007 Update (NCBI Bookshelf)

Anatomy of the eye

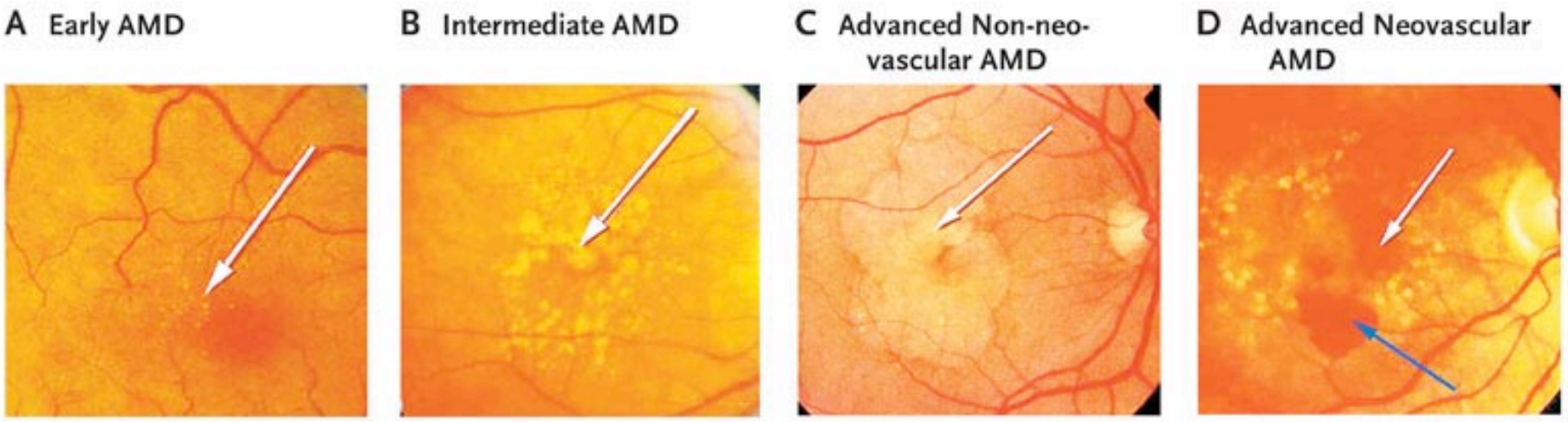
- **Retina**
  - Light sensitive part of the eye
- **Macula**
  - Highest density of light sensitive receptors in this region
    - Highest visual acuity
- **Fovea**
  - Cones concentrated here
  - Most light entering the eye is focused here
    - Color vision
    - High acuity vision

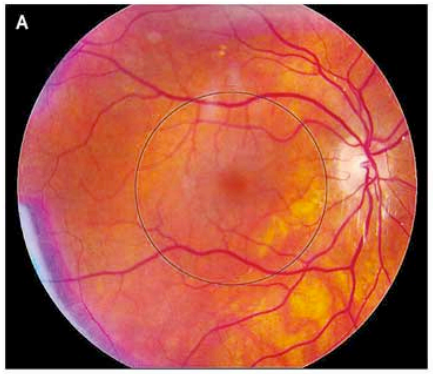


Normal Retinal image

Jager *et al*, ***N. Eng. J. Med***; 358:2606-17 (2008)

Observed retinal changes during AMD





Normal fundus

- Yellow-white deposits (drusen) appear in the macula
- Enlarged as the disease progresses
- New-blood vessels may develop (neovascularization)
  - Retinal hemorrhages may occur

Jager *et al*, ***N. Eng. J. Med***; 358:2606-17 (2008)

Consequences of these retinal changes

- Macula function disrupted
  - Region of highest visual acuity is damaged
  - Central vision can be severely affected

Normal vision
– Intact macula

Severe macular degeneration
– Central vision impaired
– Blurry vision
– Peripheral vision typically spared

Jager *et al*, ***N. Eng. J. Med***; 358:2606-17 (2008)

Age-related macular degeneration (AMD)

- **Risk factors**
  - Age (primary risk factor)
    - "Middle aged" -- 2% risk
    - ≥ 75 years -- 30% risk
  - Smoking
  - Obesity
  - Race (highest in caucasians)
  - Family history
- **Two sub-types of AMD**
  - **Dry AMD**
    - No neovascularization
    - Large drusen deposits
  - **Wet AMD**
    - Neovascularization present
    - Sub-retinal hemorrhages possible
    - ~10-15% of all AMD cases
    - Responsible for ≥ 80 % severe vision loss due to AMD!

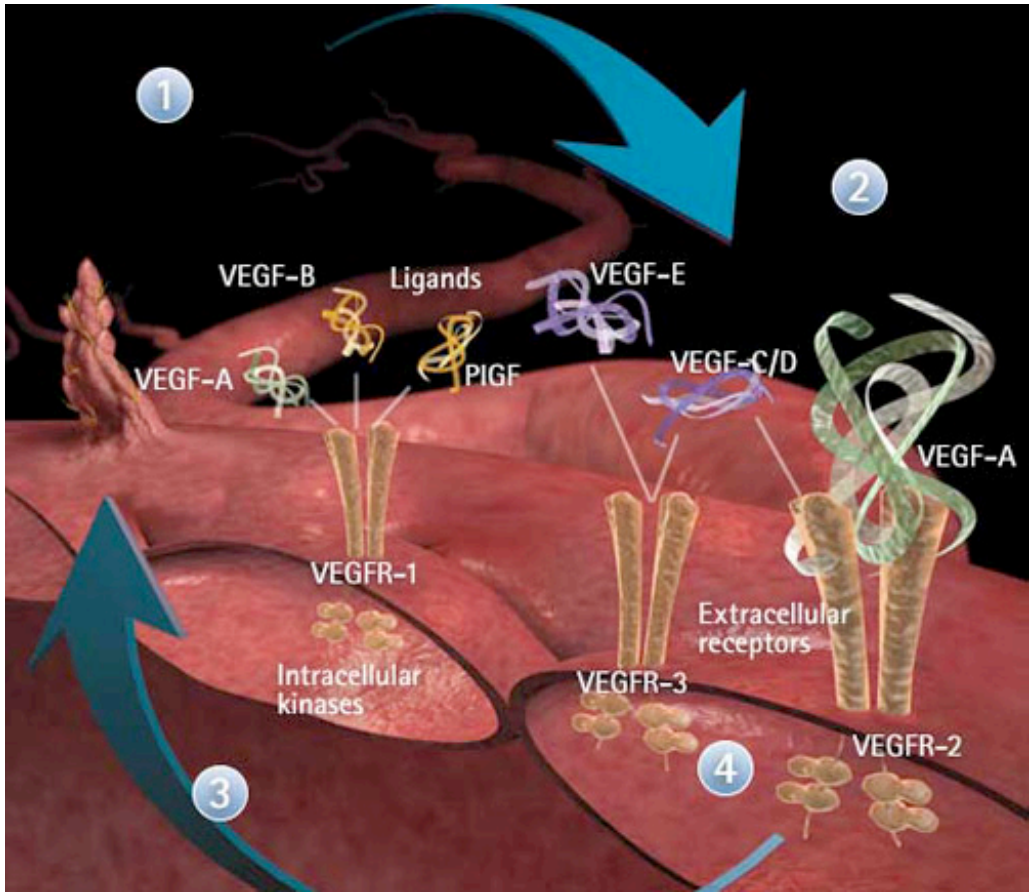
Disease pathophysiology

- Not fully understood

- **Wet AMD**:
  - High Vascular Endothelial cell Growth Factor (VEGF) levels present in the eye

VEGF

- Impacts endothelial cell function
  - Endothelial cell = special cell type lining the interior of all blood vessels
  - Endothelial cells in all blood vessels respond to VEGF
- VEGF affects endothelial cell:
  - Proliferation
  - Differentiation
  - Permeability



http://www.researchvegf.com/researchvegf/index.m (Genentech)

Disease pathophysiology

- Not fully understood

- **Wet AMD**:
  - High Vascular Endothelial cell Growth Factor (VEGF) levels present in the eye

  - VEGF is *pro-angiogenic* (promotes new blood vessel growth)

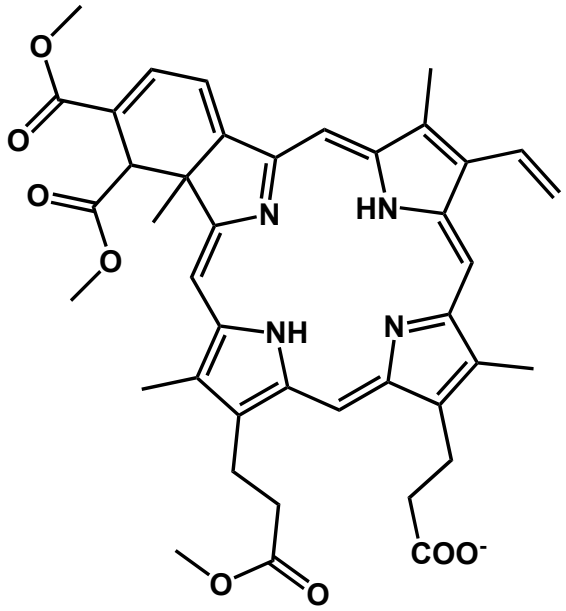
    - New blood vessels are more fragile

    - Leakiness/rupture leads to hemorrhage & vision loss

  - *What are some possible approaches to treating wet AMD based on this information?*

Treatment options for Wet AMD

- **Photodynamic therapy**
  - **Aimed at directly treating new blood vessel formation**
  - Photosensitizer drug injected systemically (entire body exposed)
  - Local irradiation of macula with red light
    - Verteporphin photo-activated in the presence of light and O_2 will produce reactive oxygen species (ROS)
    - ROS are toxic to nearby endothelial cells



Verteporfin

Treatment options for Wet AMD

- **Photo-coagulation therapy**
  - **Laser used to target new blood vessels growing in the macula**
  - Does not prevent/slow disease progression
  - Risks:
    - Irreversible damage to surrounding retina
    - Further deterioration of visual acuity

Treatment options for Wet AMD

- **Anti-VEGF therapy**

Hypothesis:

- Elevated ocular VEGF levels are directly responsible (at least in part) to increased new blood vessel formation

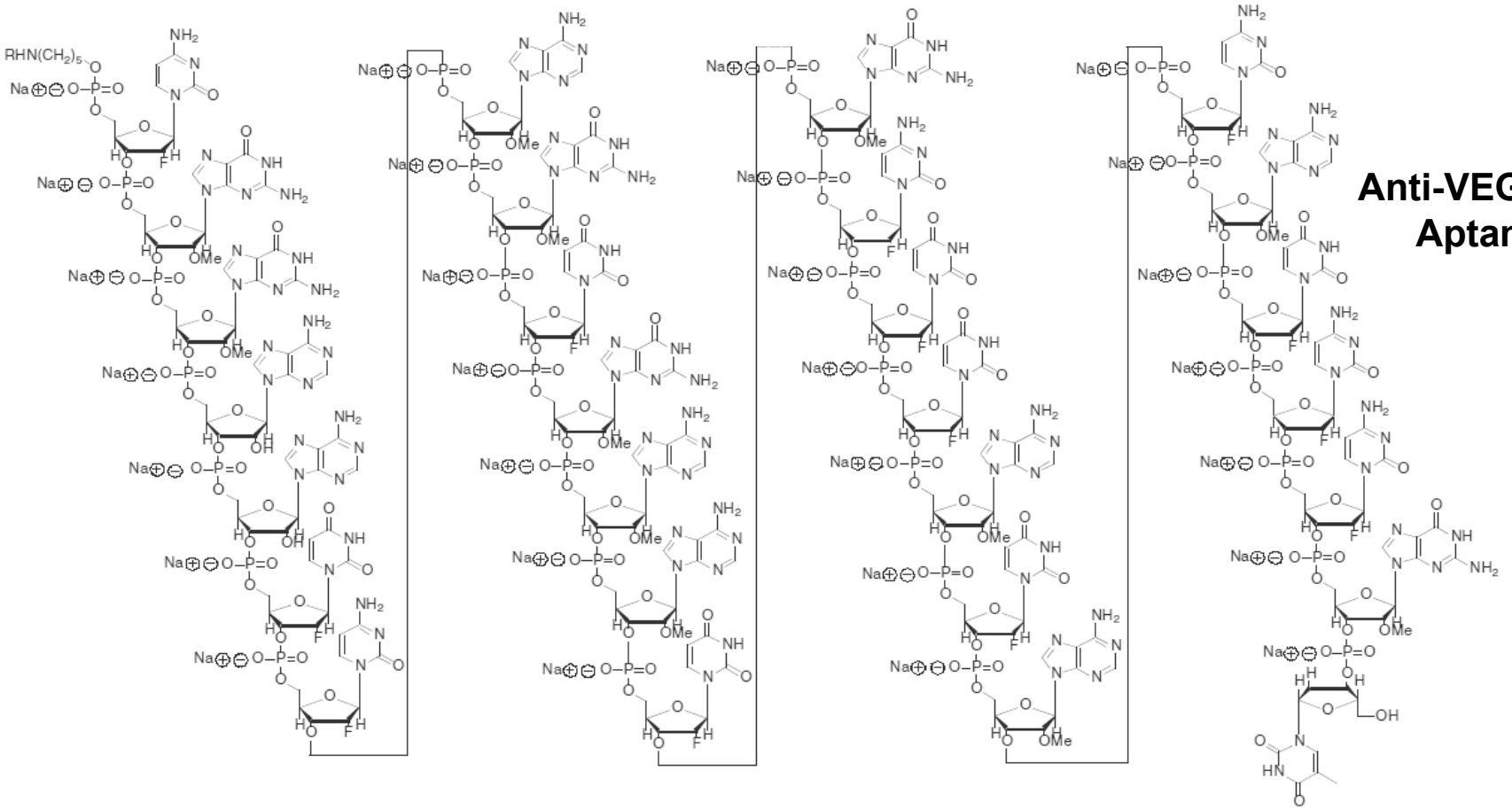
- Inhibiting VEGF activity can significantly reduce new blood vessel growth

  - Slows rate of vision loss by reducing retinal hemorrhages

  - Note: Treatment impacts Wet AMD only!

Treatment options for Wet AMD

- **Anti-VEGF therapy**
  - First FDA approved anti-VEGF drug to treat wet AMD: Pegaptanib, sodium (Macugen)
    - Approved: 2004



Anti-VEGF RNA Aptamer!

NDA 21-756

Developing aptamers as therapeutics

- What are some significant challenges to overcome in developing RNA aptamers as therapeutic agents?
  - **Stability**
    - Nucleases
    - Chemical (e.g. metal catalyzed)
  - Clearance
    - Drug must accumulate to a therapeutic level
    - But not achieve a toxic level
    - Minimize dosing frequency
  - Delivery method/bioavailability
    - Oral, intravenous, etc.

Nuclease activity spectrum

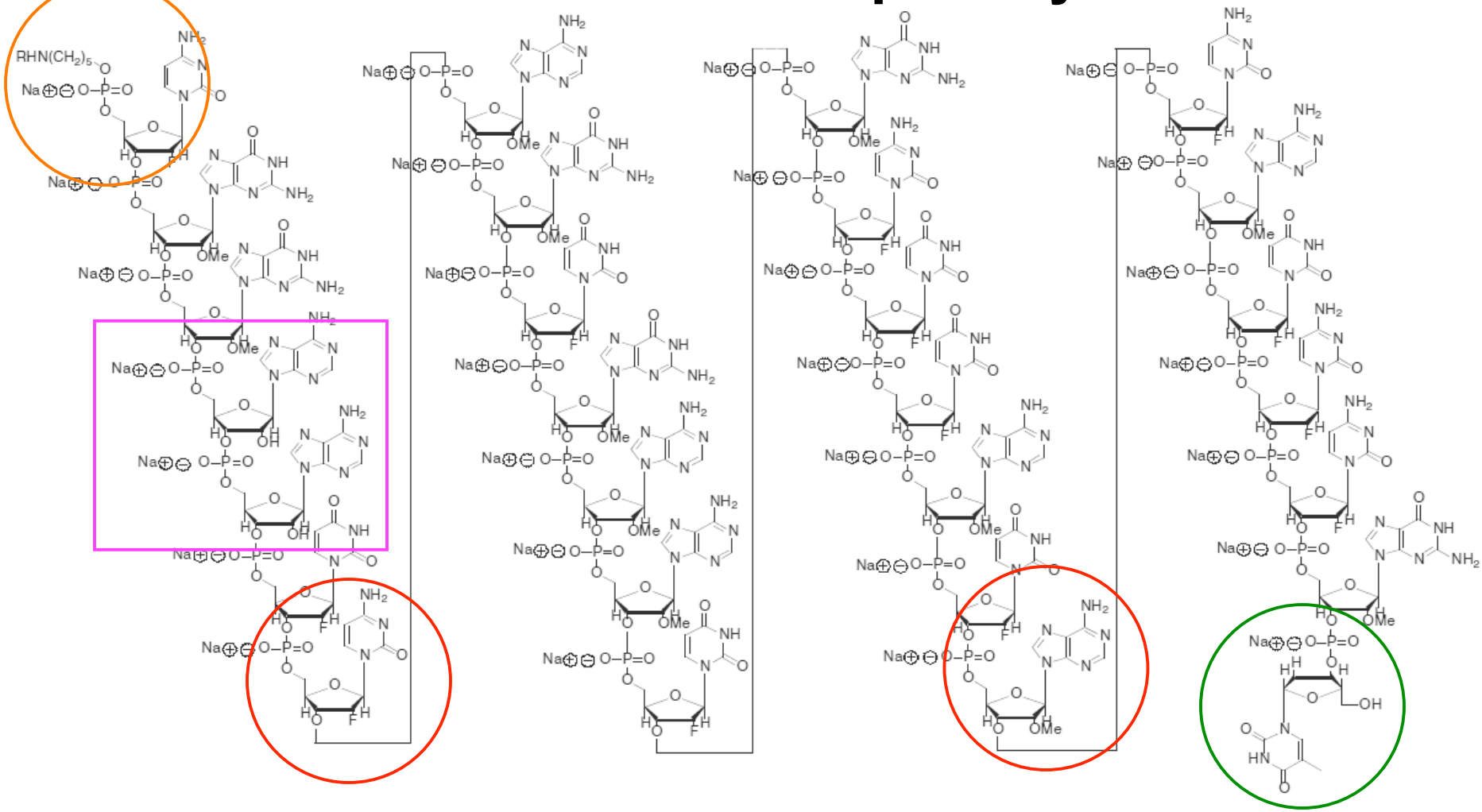
- Nucleases can be categorized broadly as:

- **Endonucleases**
  - Cleave internal phosphodiester bonds anywhere within a nucleic acid polymer

- **Exonucleases**
  - Cleave phosphodiester bond
  - But only at or near a free terminus
  - Two types:
    - 5’-exonuclease
    - 3’-exonuclease

Anti-VEGF aptamer modifications reducing nuclease susceptibility

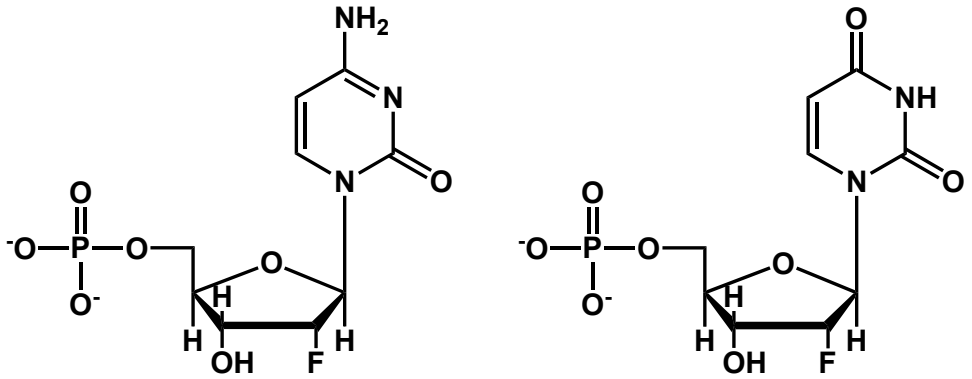
5’-terminus



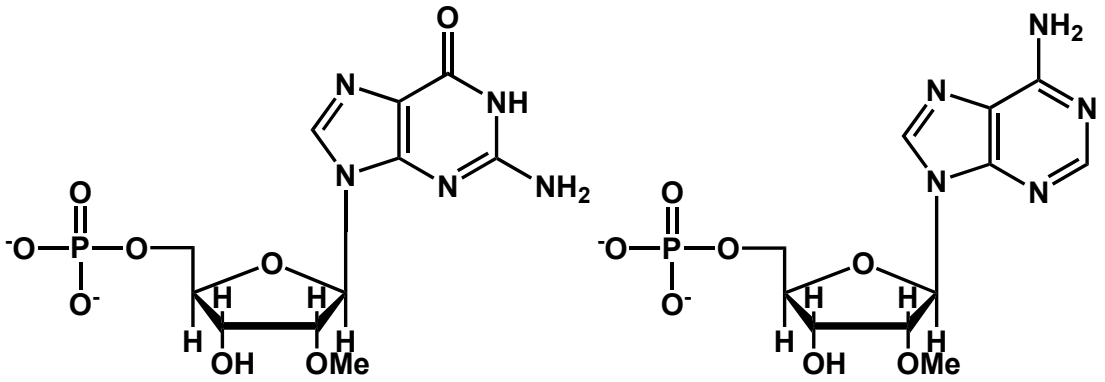
3’-terminus

NDA 21-756

Nucleotide sugar modifications



2’-fluoropyrimidines

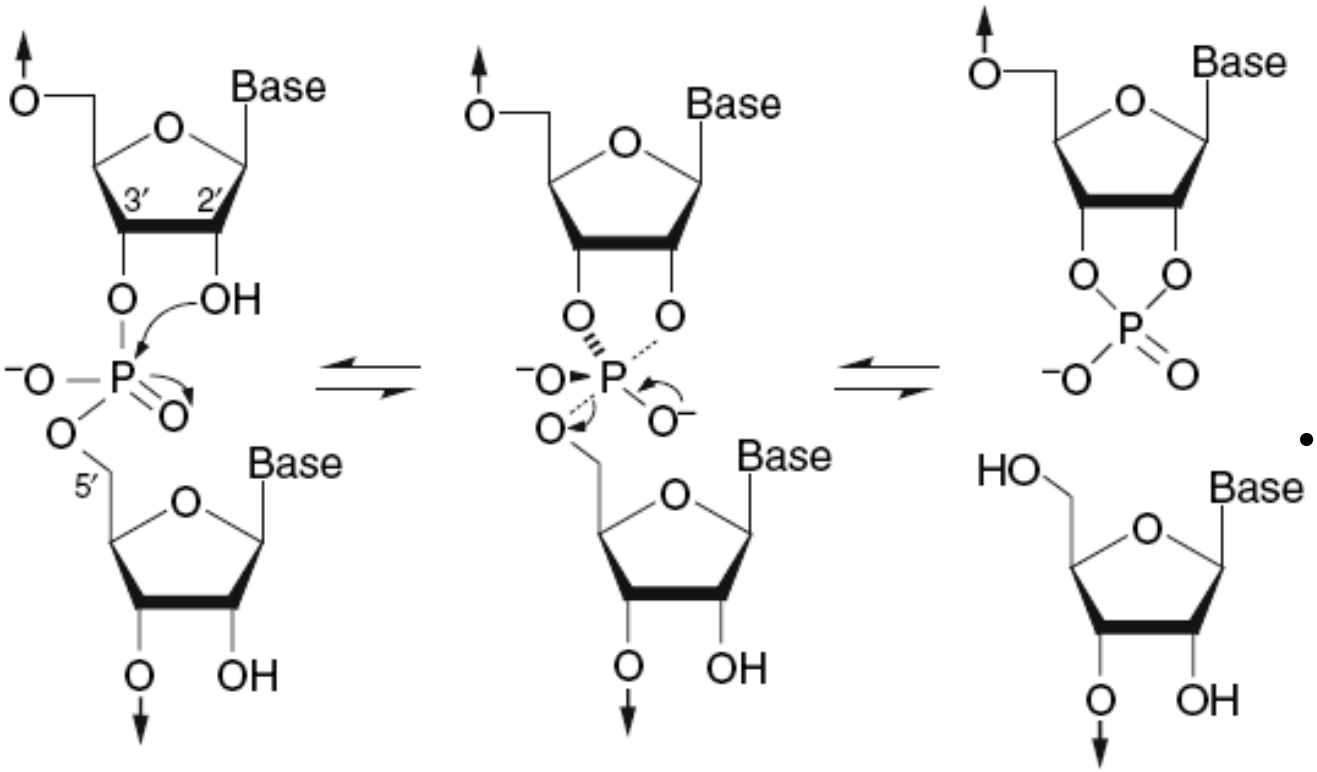


2’-methoxypurines

- RNA is significantly stabilized by introducing 2’-sugar modified nucleotides:
  - Fluoro group
  - Methoxy group
- Impart stabilization against:
  - Endonucleases
  - Spontaneous cleavage
  - Metal-catalyzed (chemical) cleavage
- *What mechanism(s) can you propose to explain this?*

Sugar modified nucleotides and stability

Spontaneous cleavage

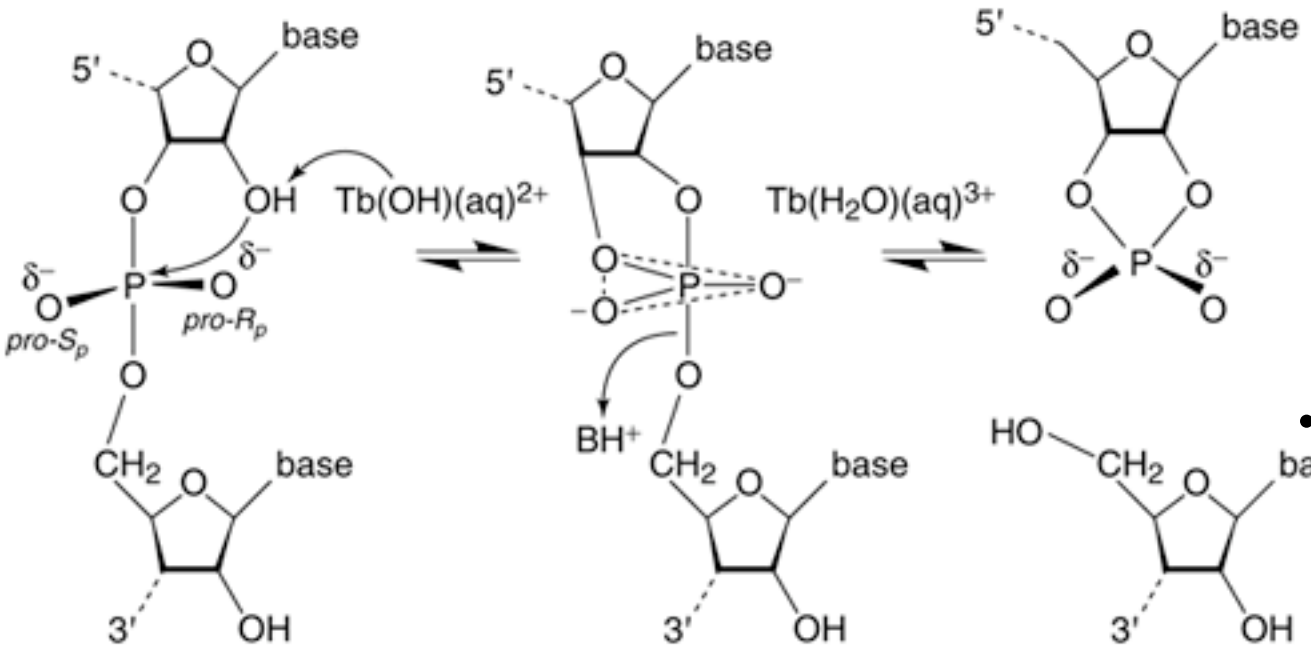


- 2’-OH group has sufficient nucleophilicity to initiate intramolecular reaction that leads to phosphodiester bond cleavage
- Fluorine is highly electronegative
  - **Poor nucleophile**
  - F does not attack the phosphate group
  - Cleavage reduced

Regulski, E.E. and Breaker R.R., *Methods in Molecular Biology*, 419:53-67 (2008)

Sugar modified nucleotides and stability

Metal ion-dependent cleavage chemistry



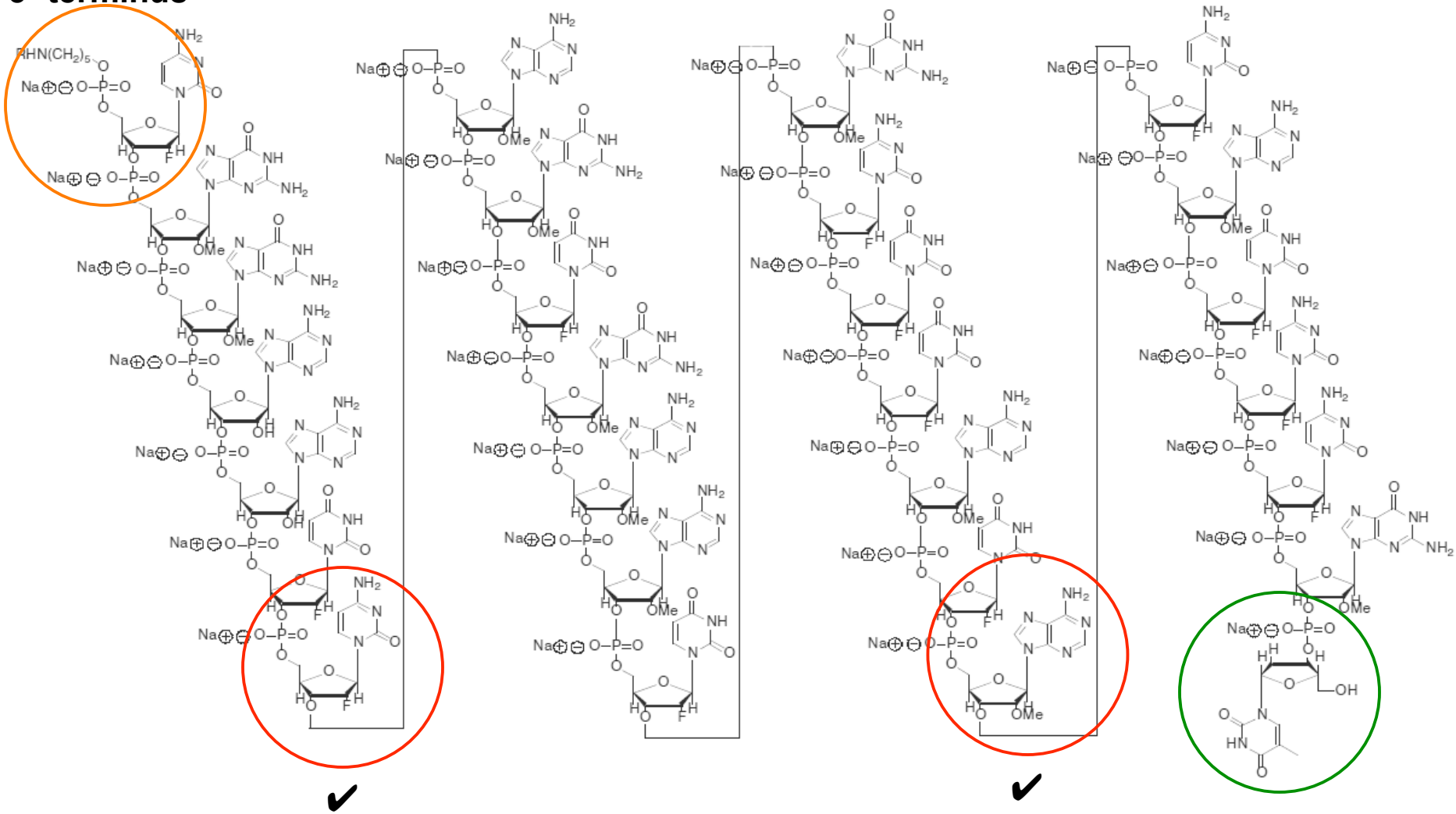
- Some metals can help deprotonate the 2'-OH group in normal RNA
  - Accelerate cleavage reaction
- Both the 2'-F and 2'-OMe derivatives prevent this chemistry
  - Impart stability to RNA

Same factors contribute to imparting resistance to RNases

Harris D.A. and Walter N.G., *Current Protocols in Nucleic Acid Chemistry*, Unit 6.8 (2003)

Modified backbone linkages

5’-terminus

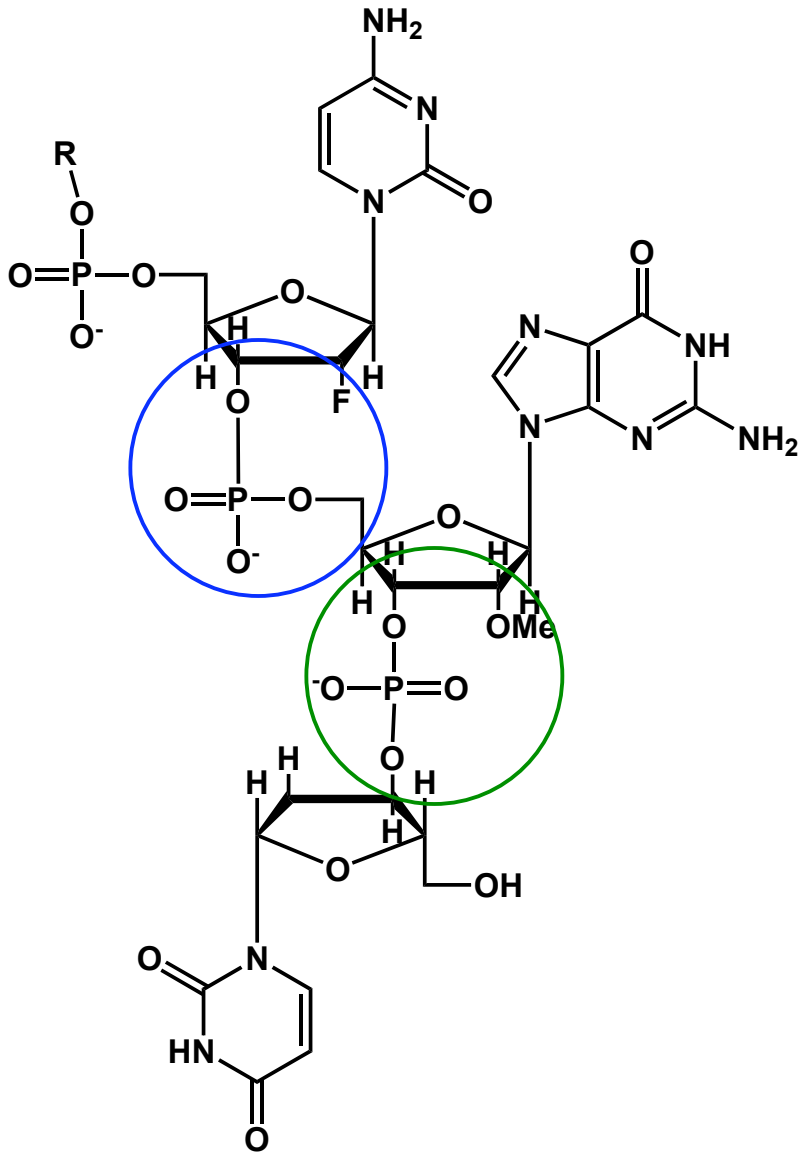


Anti-VEGF RNA Aptamer!

NDA 21-756

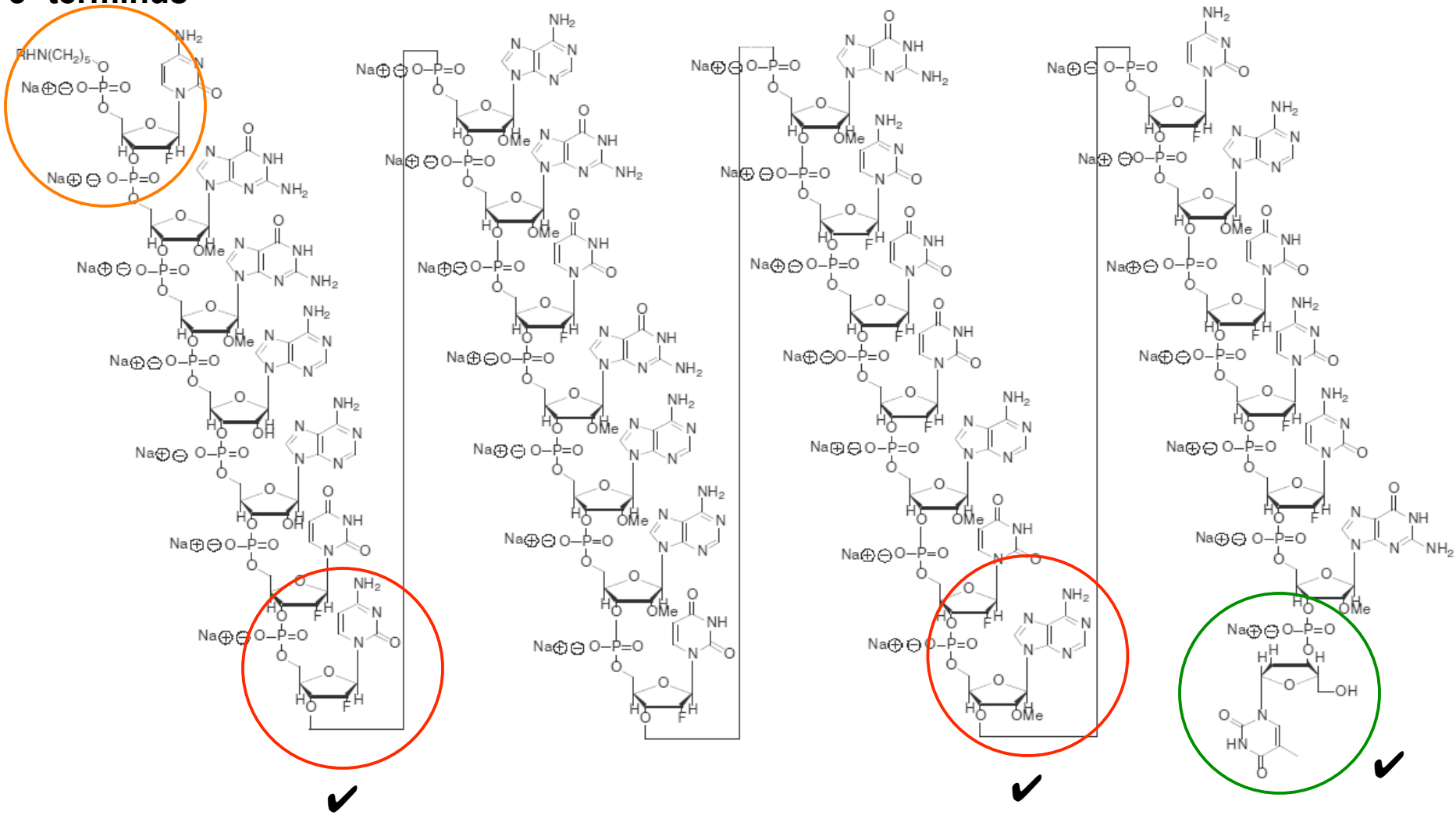
Modified backbone linkages

- Typical linkage in DNA or RNA
  - 3’-5’ phosphodiester linkage
- Notice the presence of new linkage at the 3’-end of the aptamer
  - 3’-3’ phosphodiester linkage
- Provides significant resistance against 3’-**exonucleases**
  - Major nuclease activity present in serum



Modified backbone linkages

5’-terminus



Anti-VEGF RNA Aptamer!

NDA 21-756

Modified 5'-terminus

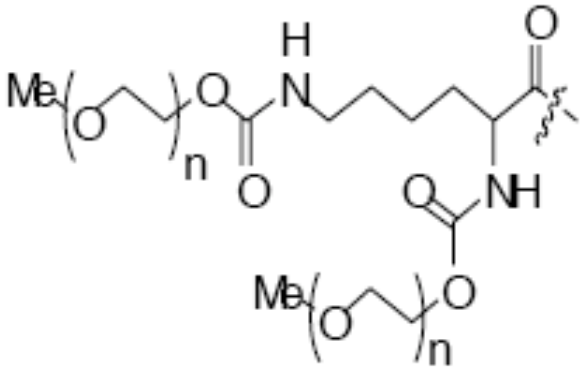
- ***What does this accomplish?***

- Reduced susceptibility to 5'-exonucleases

- Reduced clearance
  - Increased size of PEG-conjugated aptamer

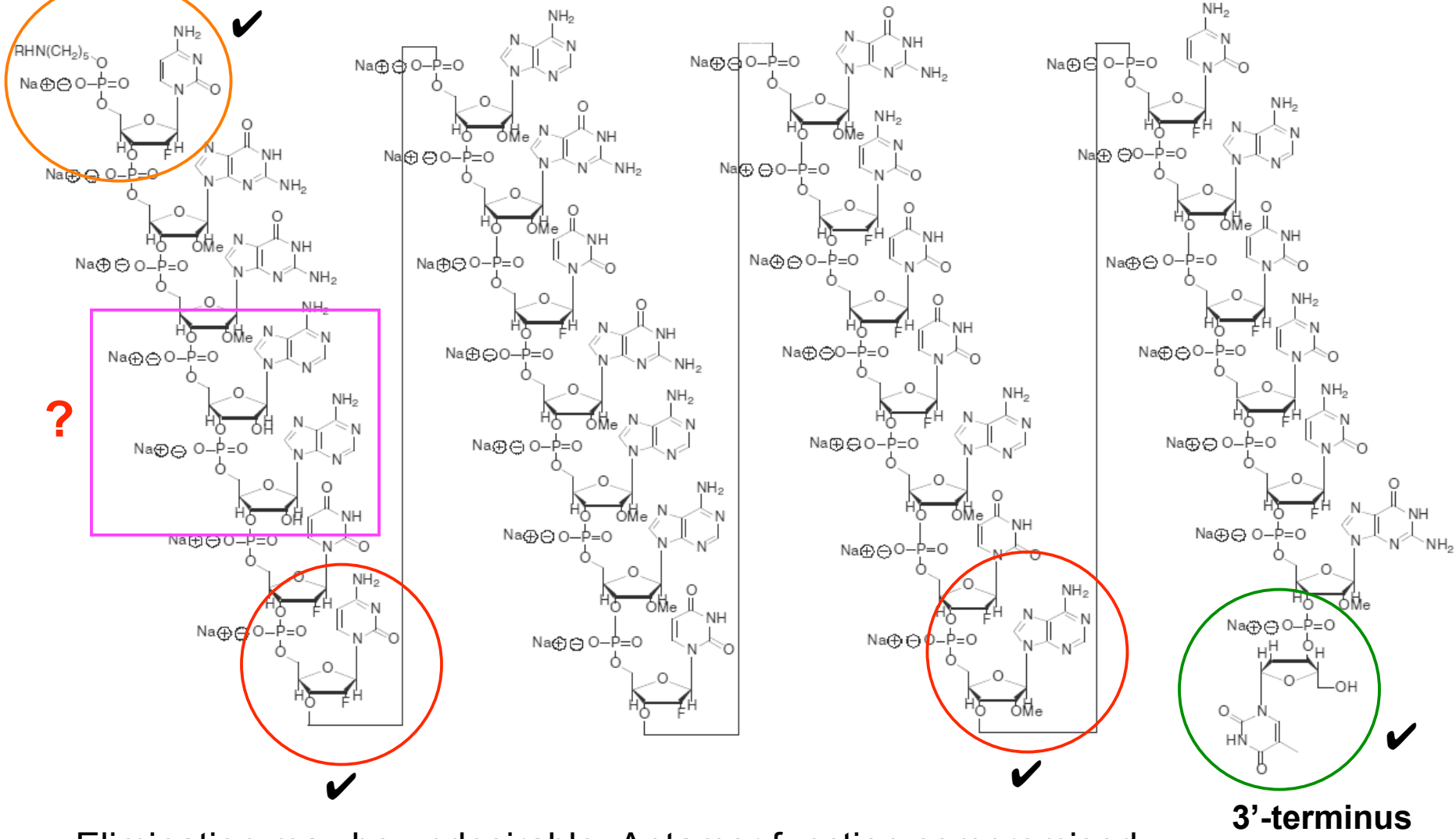
  - Pegaptanib, sodium molecular weight ~ 50 KDa

  - Aptamer alone: ~ 10 KDa



and n is approximately 450.

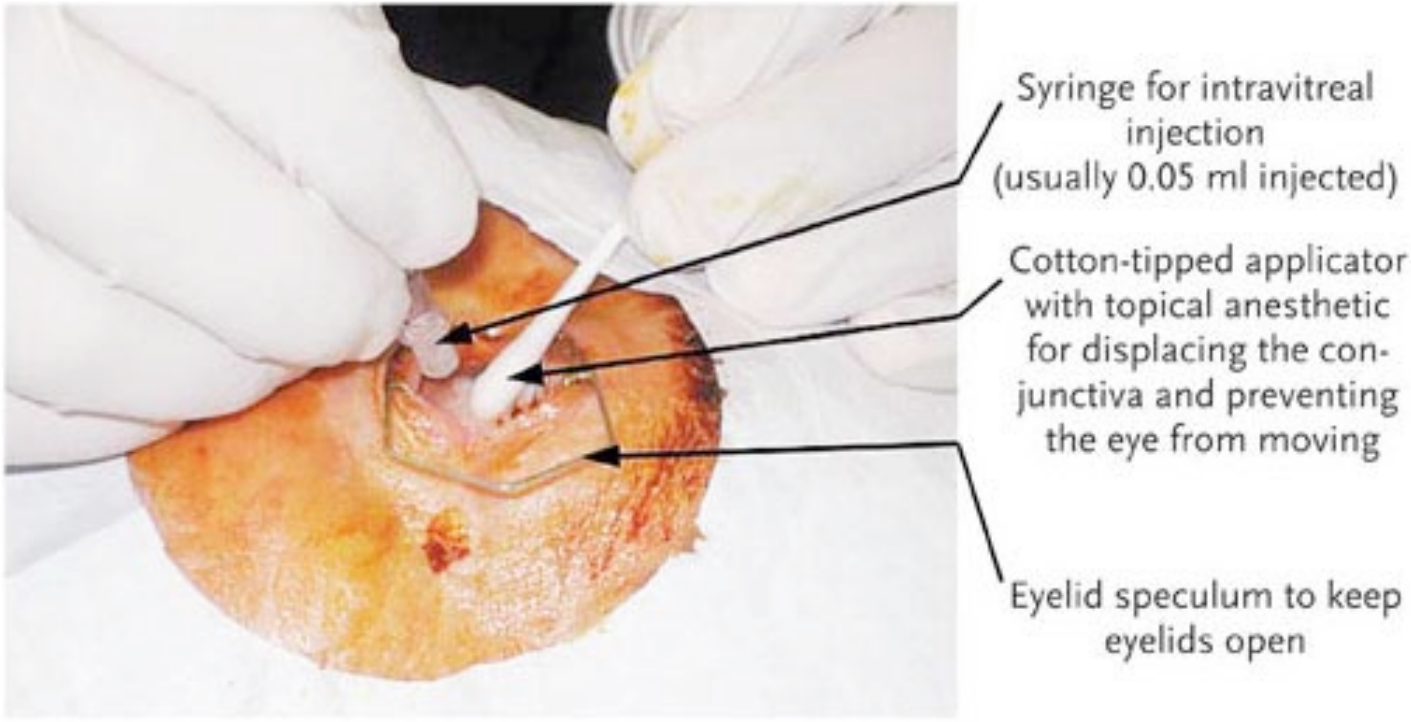
Why not eliminate all sites from which aptamer degradation may occur?



- Elimination may be undesirable: Aptamer function compromised
- Intentional: Modulates aptamer's lifetime within patient (pharmacokinetics)

How do you deliver aptamer drugs to the retina

- **Anti-VEGF therapy**
  - Cannot be delivered systemically (e.g. intravenously)
    - Pro-thrombotic
  - Poor oral bioavailability
  - *Injected directly into the vitreous humor of the eye!*



Jager *et al*, ***N. Eng. J. Med***; 358:2606-17 (2008)

Summary

- Aptamers have been successfully developed as therapeutics
  - Other aptamers in different stages of drug testing trials include:
    - Anti-clotting agents
    - Anti-cancer agents

- Several factors must be specifically addressed to achieve this:
  - Stability to nuclease-mediated degradation
  - Bioavailability
  - Pharmacokinetics
  - Delivery to target interaction site
  - Cost