

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 (<i>In vitro</i> 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
8 March, 2012

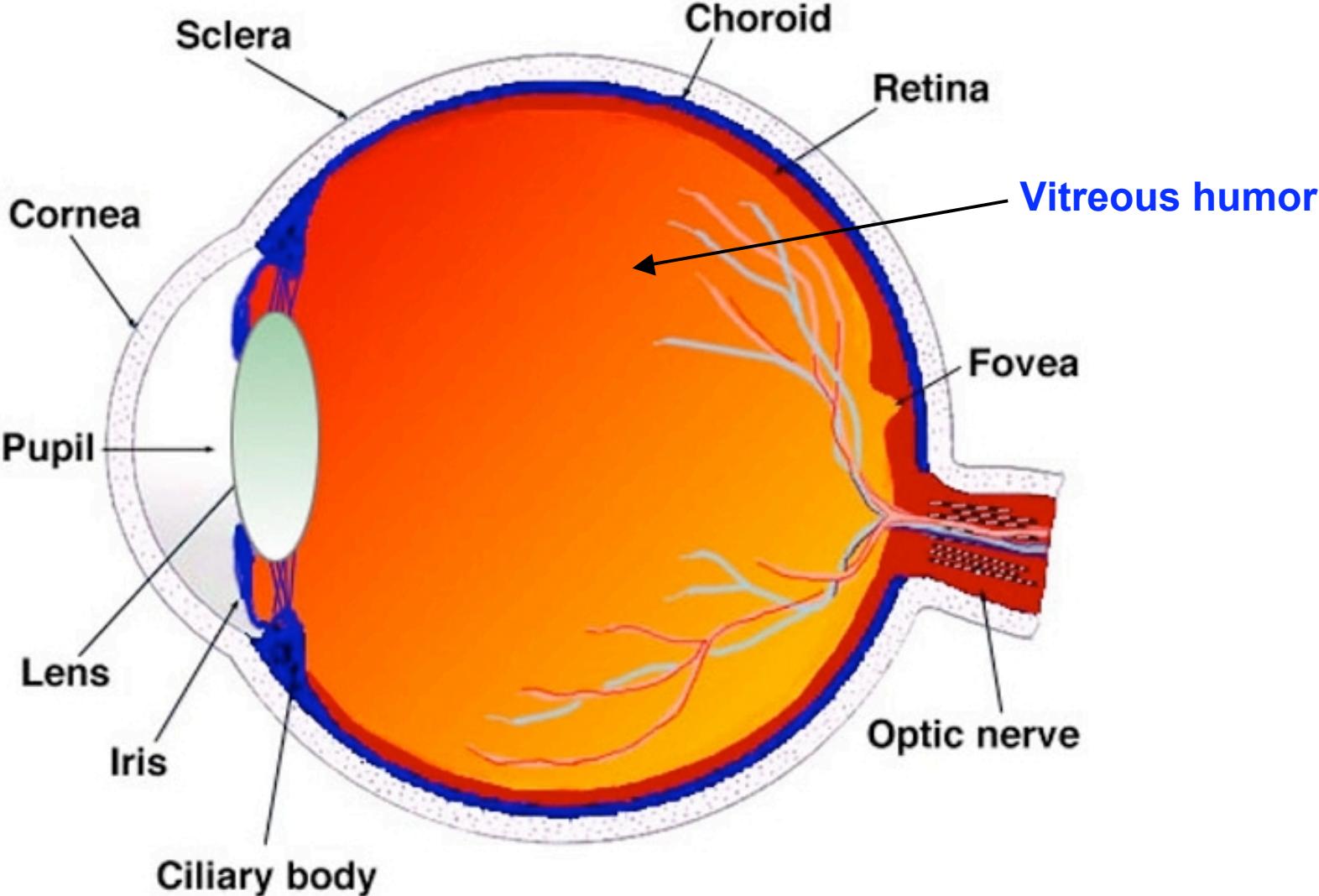
Today's Objectives

- 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



Anatomy of the eye

- **Retina**

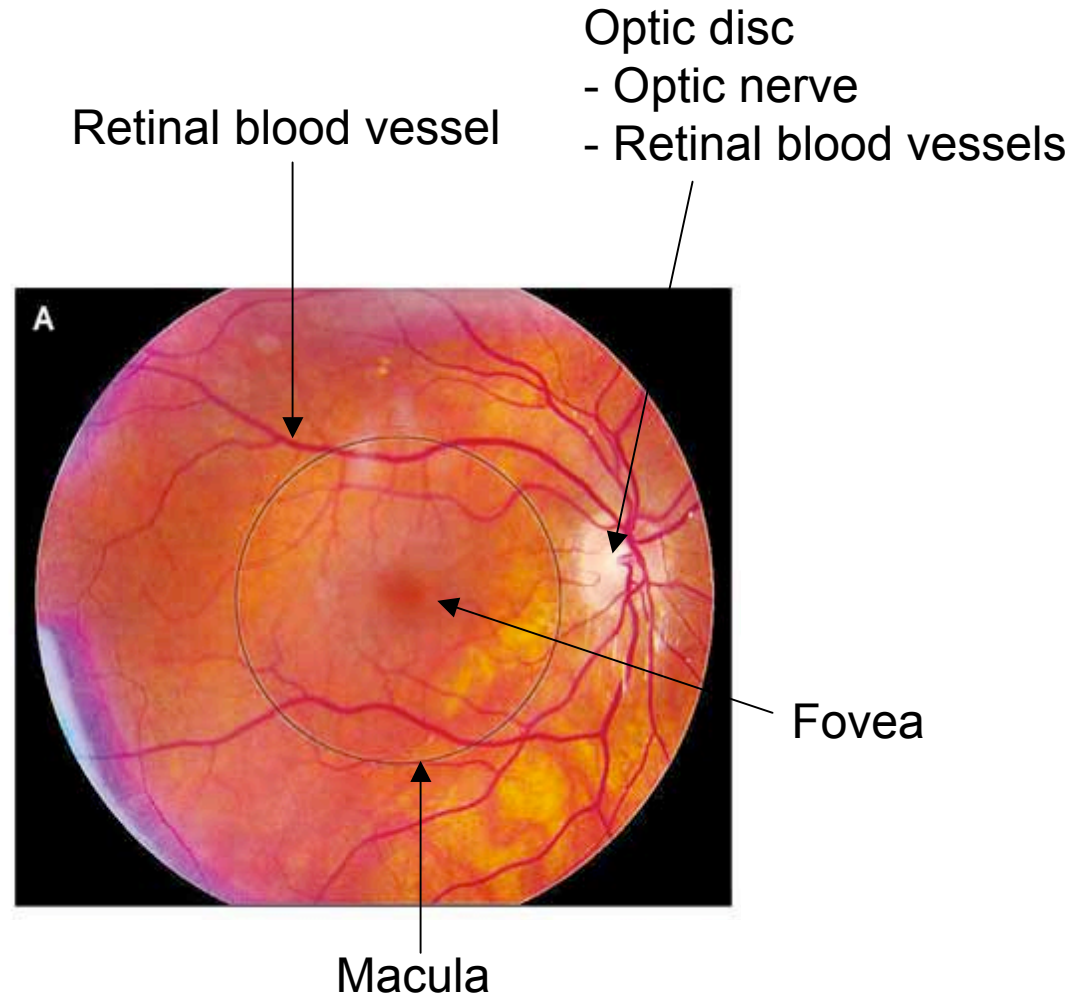
- Light sensitive part of the eye

- **Macula**

- Highest density of light sensitive receptors in this region
 - Highest visual acuity

- **Fovea**

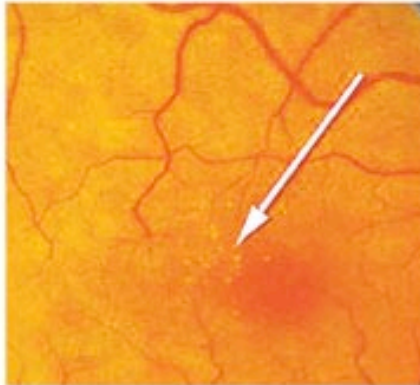
- Cones concentrated here
- Most light entering the eye is focused here
 - Color vision
 - High acuity vision



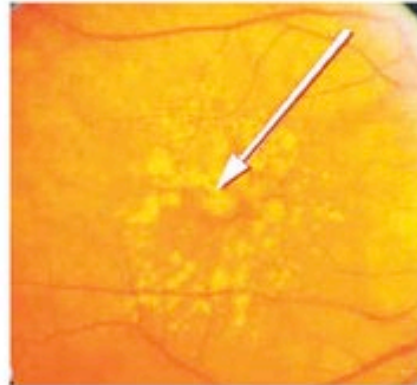
Normal Retinal image

Observed retinal changes during AMD

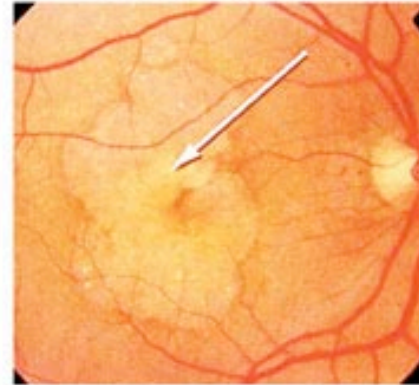
A Early AMD



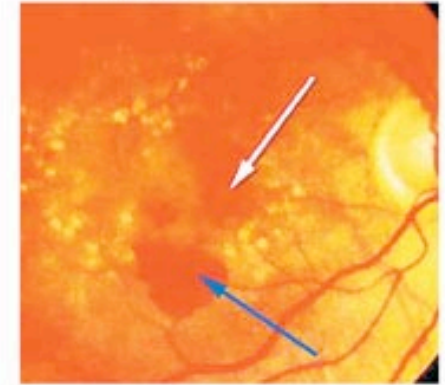
B Intermediate AMD



C Advanced Non-neovascular AMD



D Advanced Neovascular 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

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

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**

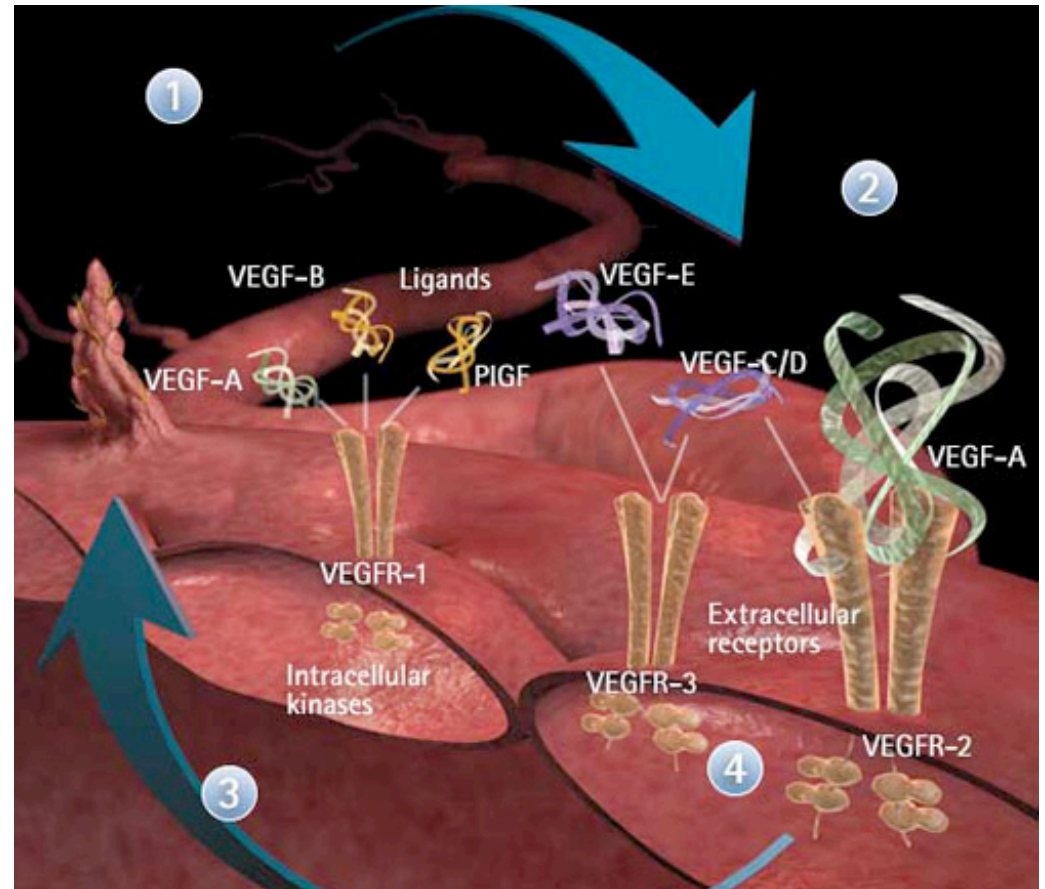
- Neovascularization present
- Sub-retinal hemorrhages possible
- ~10-15% of all AMD cases
- Responsible for ≥ 80 % severe vision loss due to AMD!

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



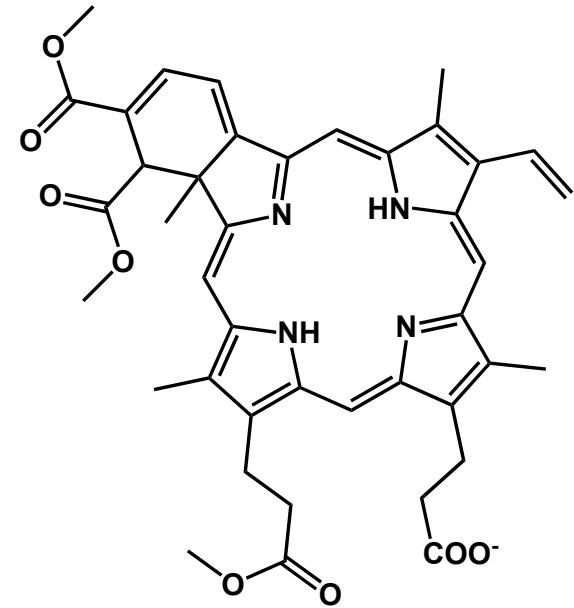
Disease pathophysiology

- 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



Verteporphin

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



Showing treatment with the laser on the eye

Treatment options for Wet AMD

- **Anti-VEGF therapy**

Hypothesis:

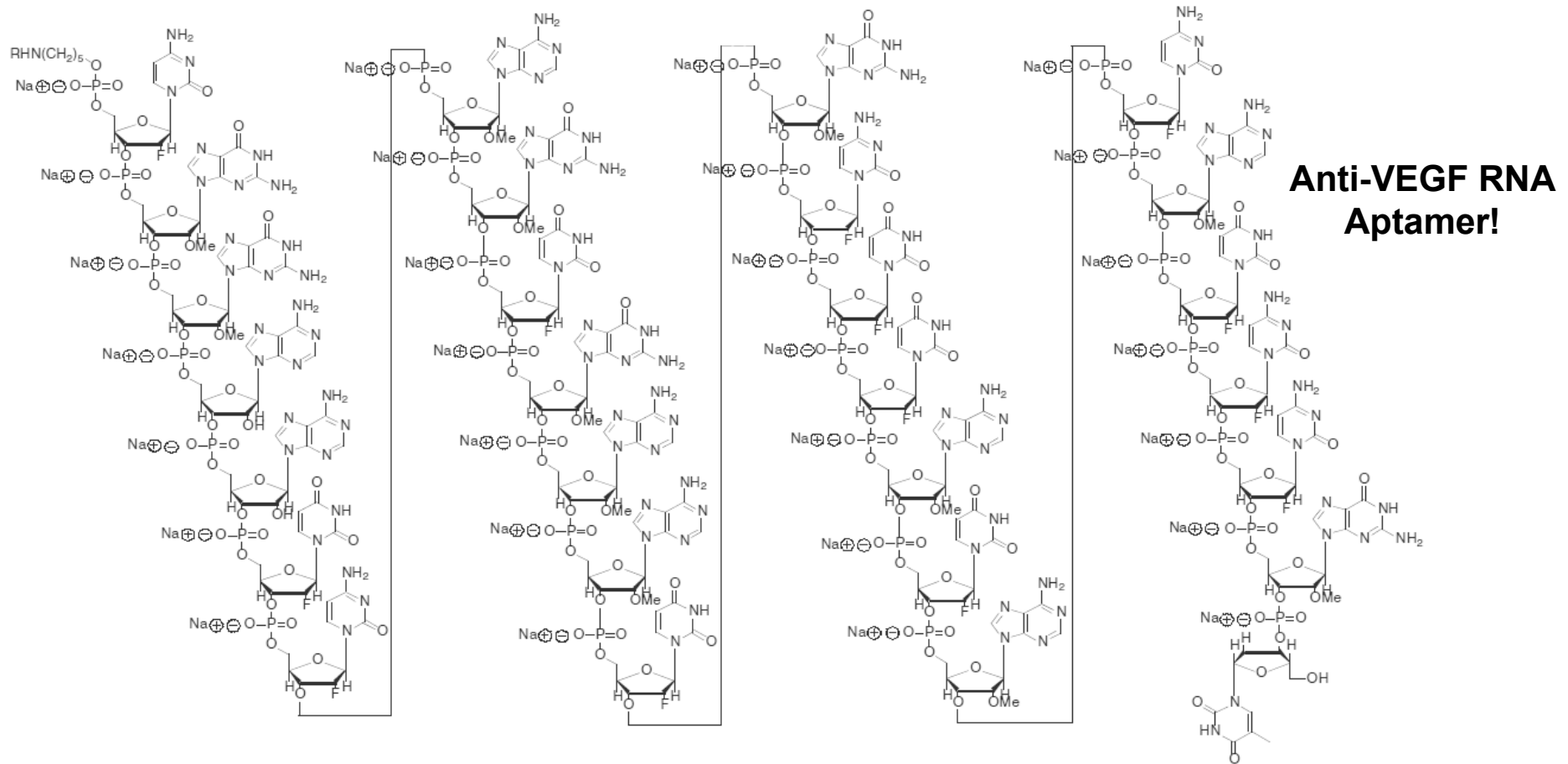
- Elevated ocular VEGF levels are directly responsible (at least in part) to increased new blood vessel formation
- Inhibiting VEGF activity can significantly reduce new blood vessel growth
 - Slows rate of vision loss by reducing retinal hemorrhages
 - Note: Treatment impacts Wet AMD only!

Treatment options for Wet AMD

- **Anti-VEGF therapy**

- First FDA approved anti-VEGF drug to treat wet AMD:
Pegaptanib, sodium (Macugen)

- Approved: 2004



Developing aptamers as therapeutics

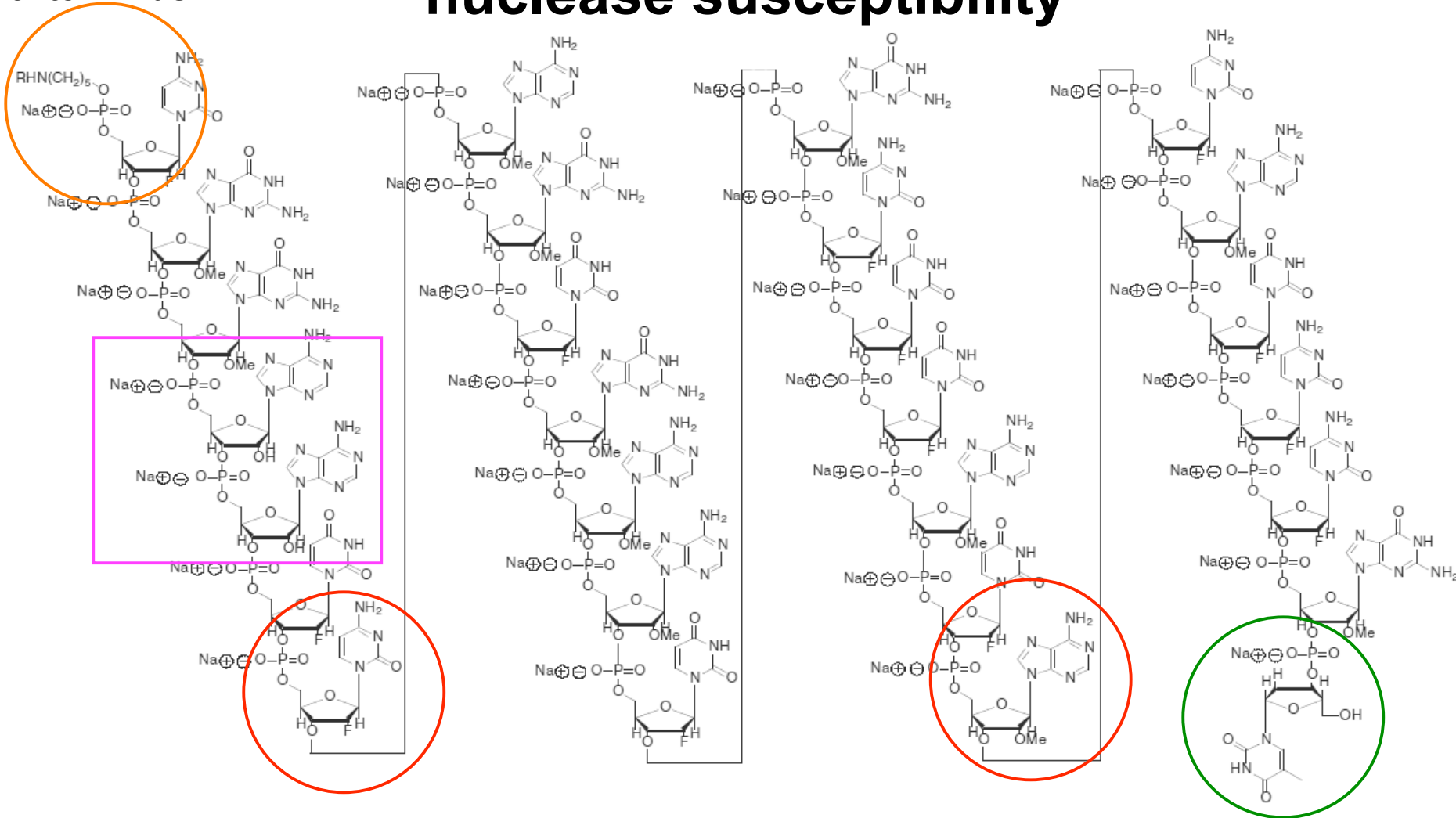
- What are some significant challenges to overcome in developing RNA aptamers as therapeutic agents?
 - **Stability**
 - Nucleases
 - Chemical (e.g. metal catalyzed)
 - Clearance
 - Drug must accumulate to a therapeutic level
 - But not achieve a toxic level
 - Minimize dosing frequency
 - Delivery method/bioavailability
 - 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 a free terminus
 - Two types:
 - 5'-exonuclease
 - 3'-exonuclease

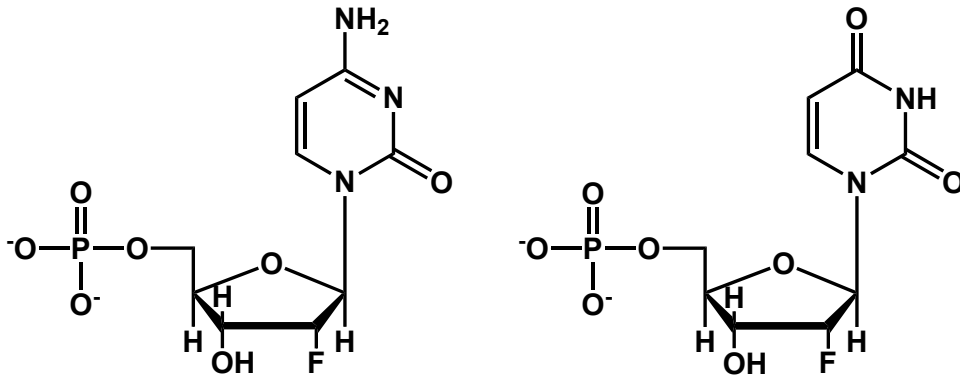
Anti-VEGF aptamer modifications reducing nuclease susceptibility

5'-terminus

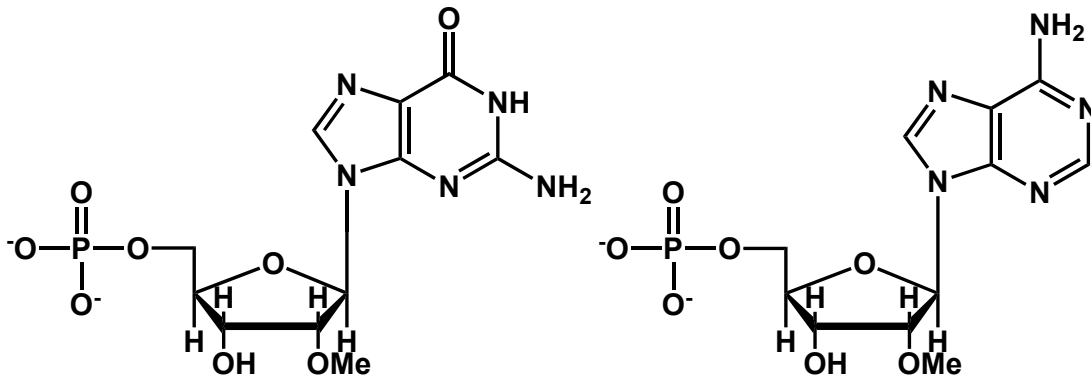


3'-terminus

Nucleotide sugar modifications



2'-fluoropyrimidines

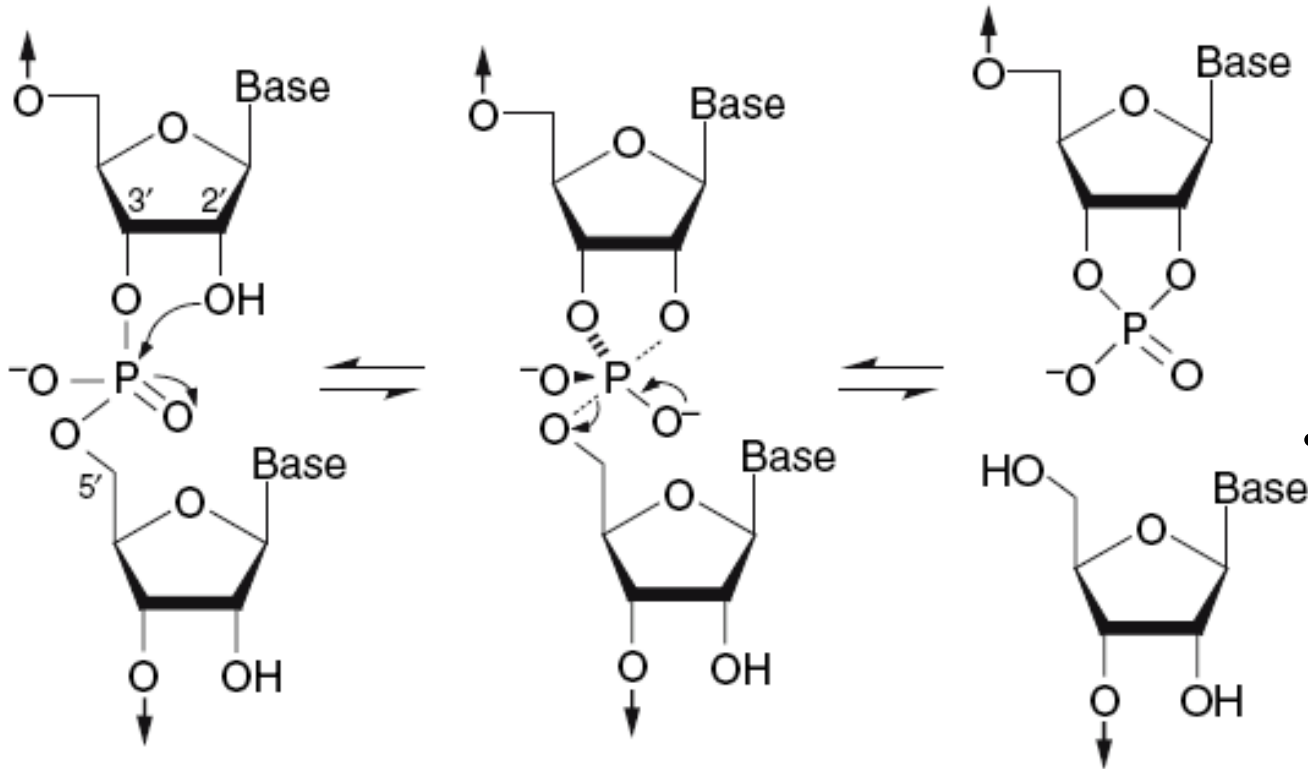


2'-methoxypurines

- 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

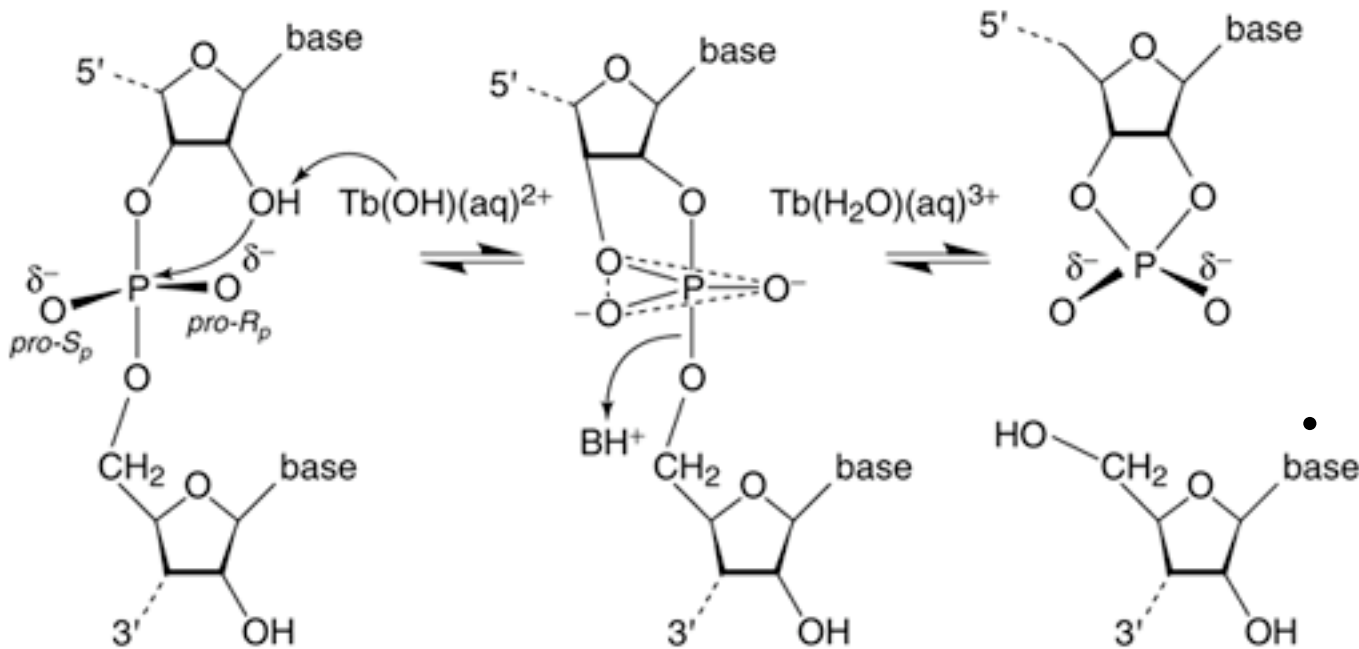


- 2'-OH group has sufficient nucleophilicity to initiate intramolecular reaction that leads to phosphodiester bond cleavage

- Fluorine is highly electronegative
 - **Poor nucleophile**
 - F does not attack the phosphate group
 - Cleavage reduced

Sugar modified nucleotides and stability

Metal ion-dependent cleavage chemistry

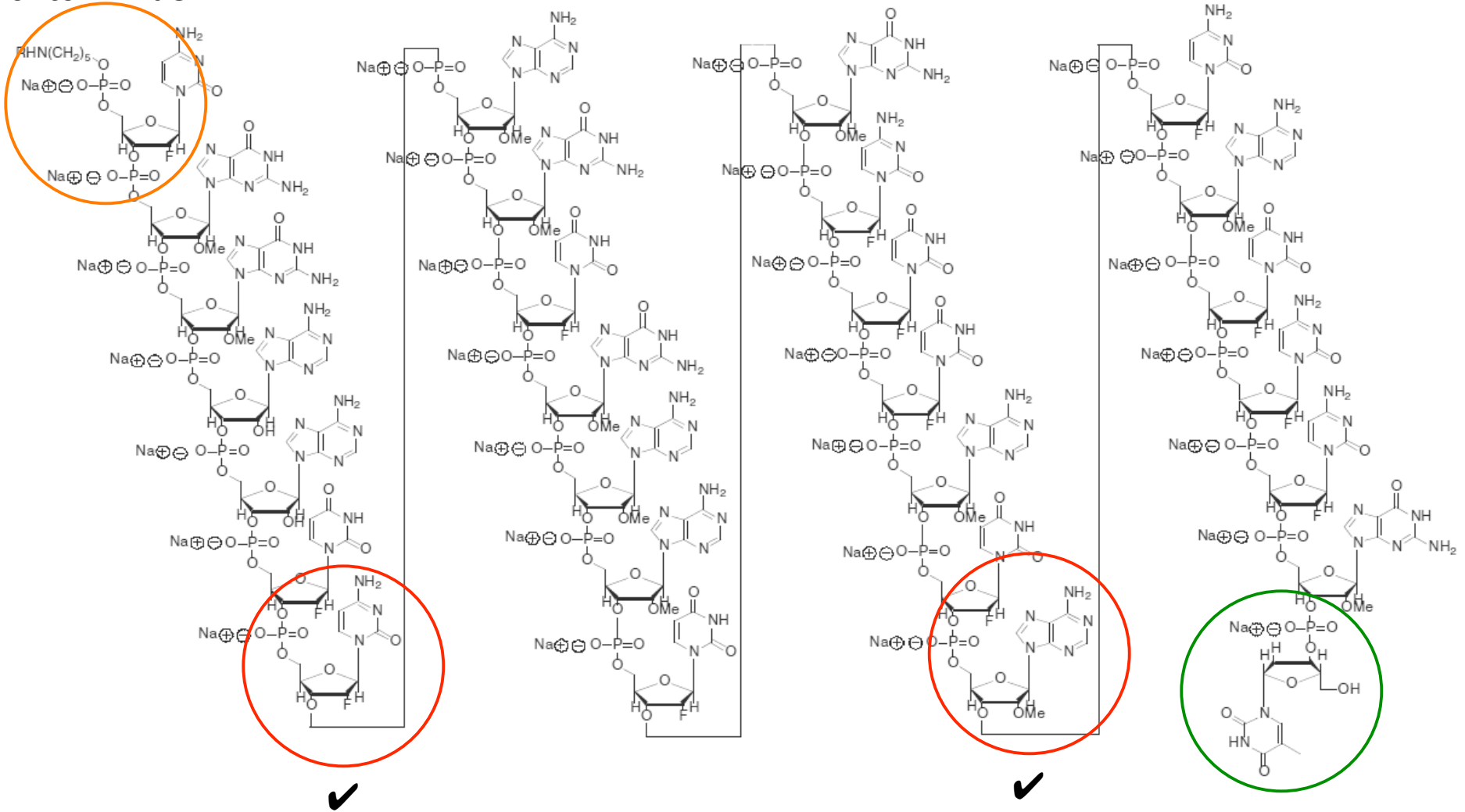


- Some metals can help deprotonate the 2'-OH group in normal RNA
 - Accelerate cleavage reaction
- Both the 2'-F and 2'-OMe derivatives prevent this chemistry
 - Impart stability to RNA

Same factors contribute to imparting resistance to RNases

Modified backbone linkages

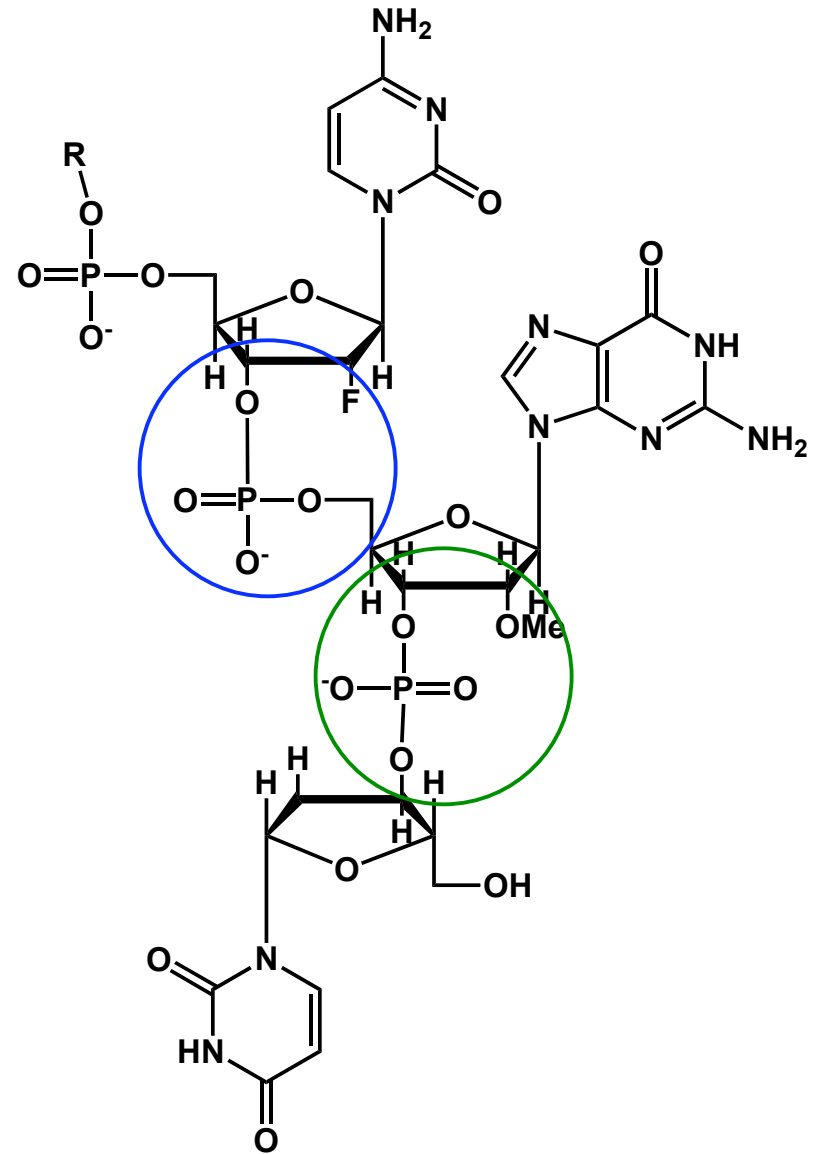
5'-terminus



**Anti-VEGF RNA
Aptamer!**

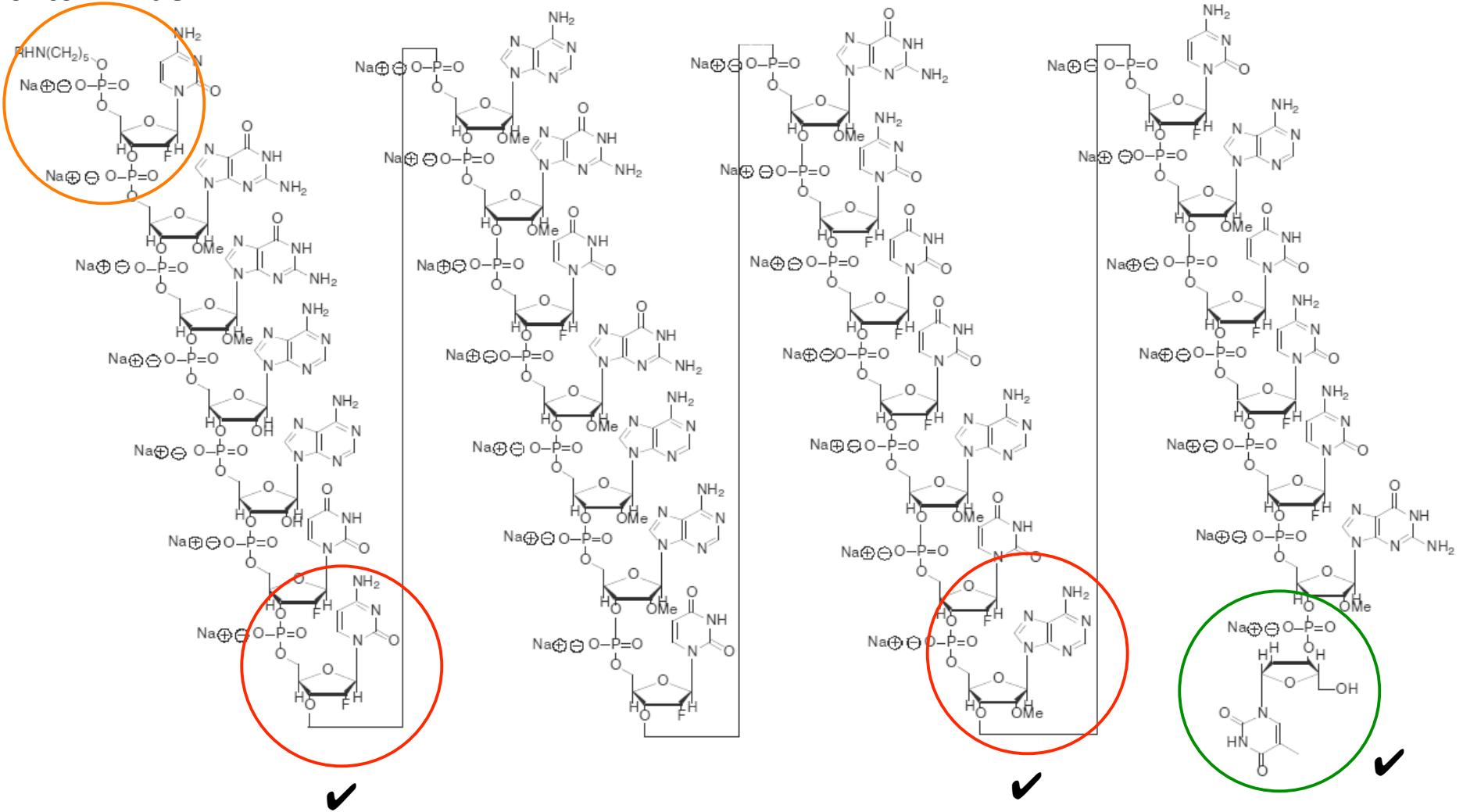
Modified backbone linkages

- 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

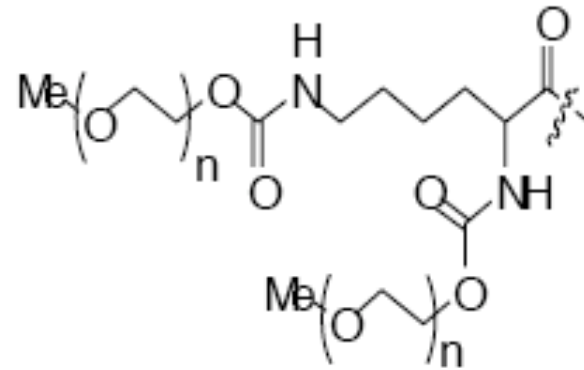
5'-terminus



Anti-VEGF RNA
Aptamer!

Modified 5'-terminus

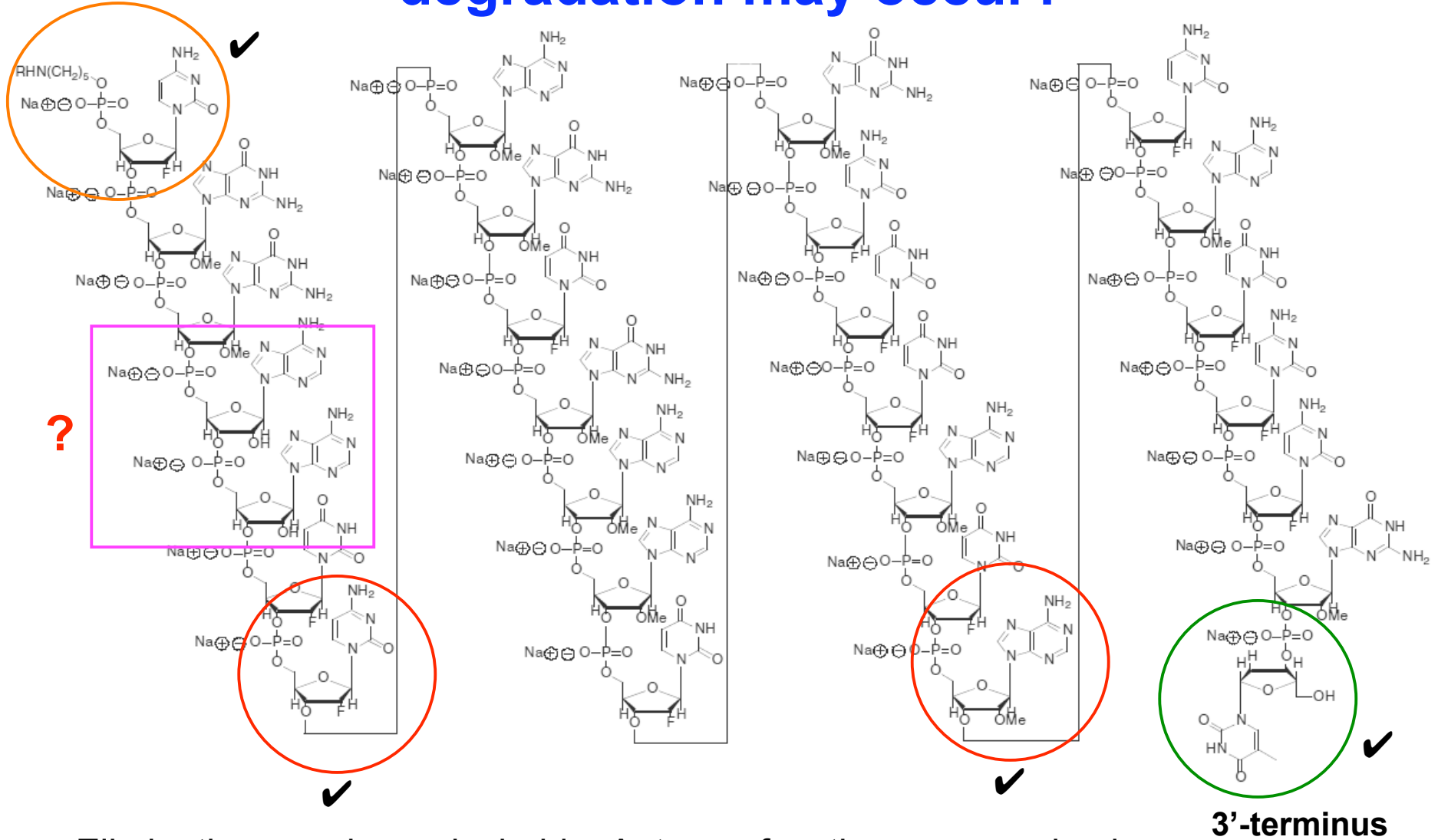
- *What does this accomplish?*
- Reduced susceptibility to 5'-exonucleases
- Reduced clearance
 - Increased size of PEG-conjugated aptamer
 - Pegaptanib, sodium molecular weight ~ 50 KDa
 - Aptamer alone: ~ 10 KDa



and n is approximately 450.

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

5'-terminus



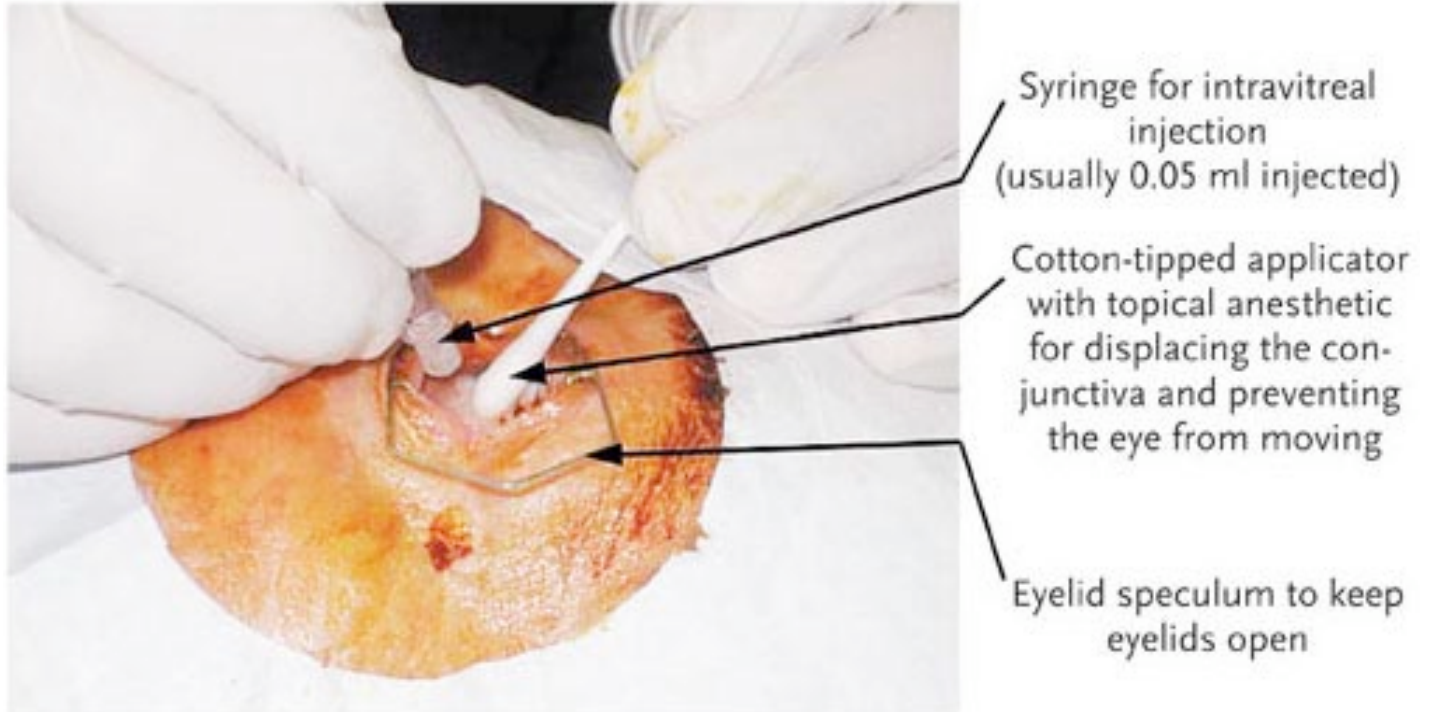
3'-terminus

- 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!*



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