Seminar:
DNA Hybridization Detection

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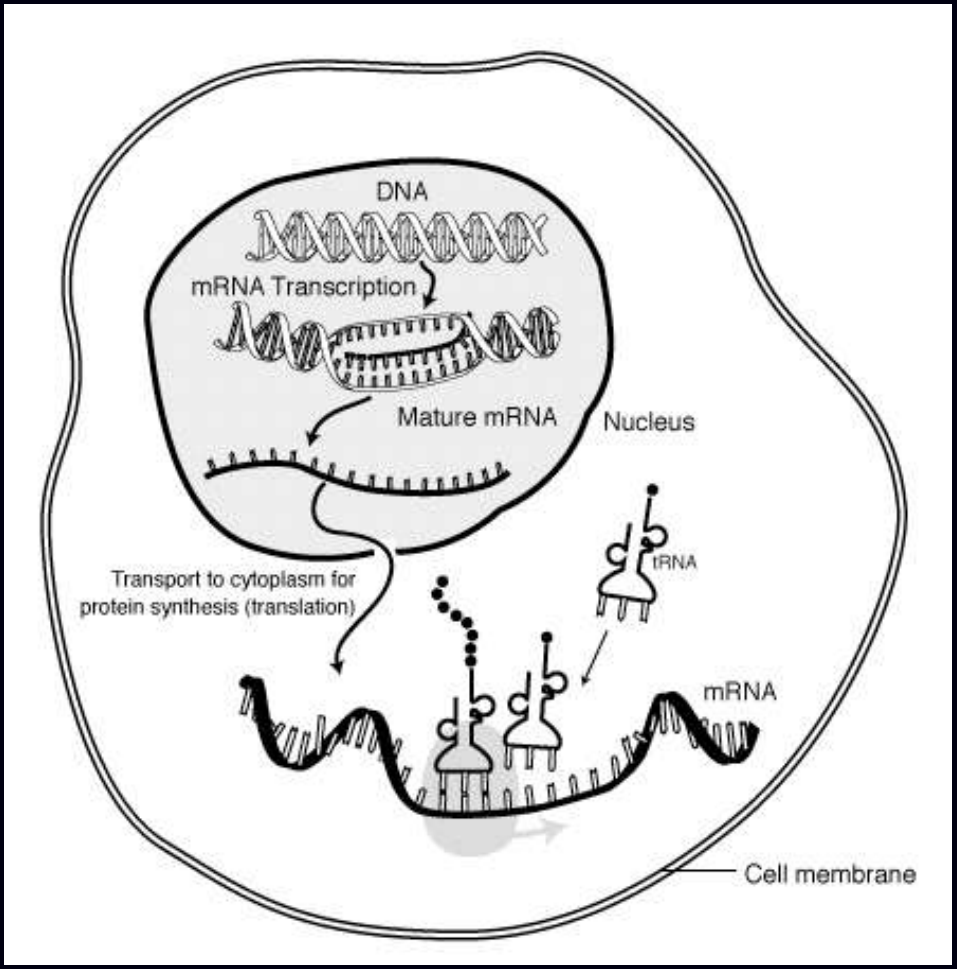
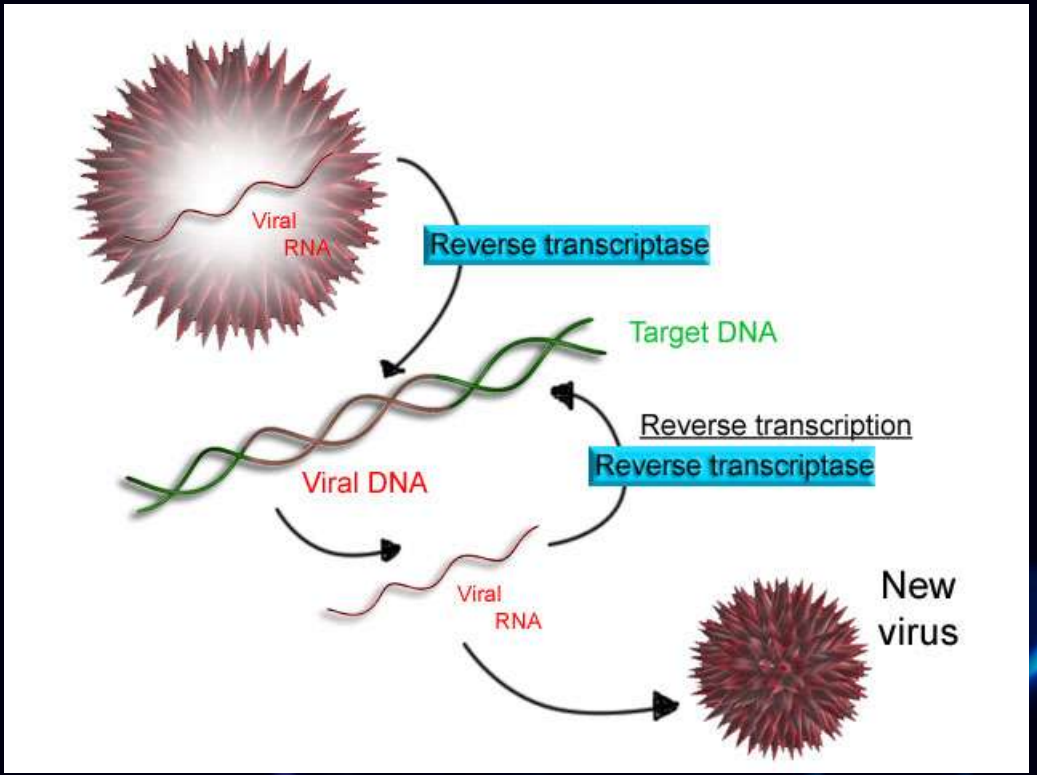
Outline

- Ruthenium project
  - General principles
  - Literature review
  - Experimental data
  - Future prospects
- Conclusion

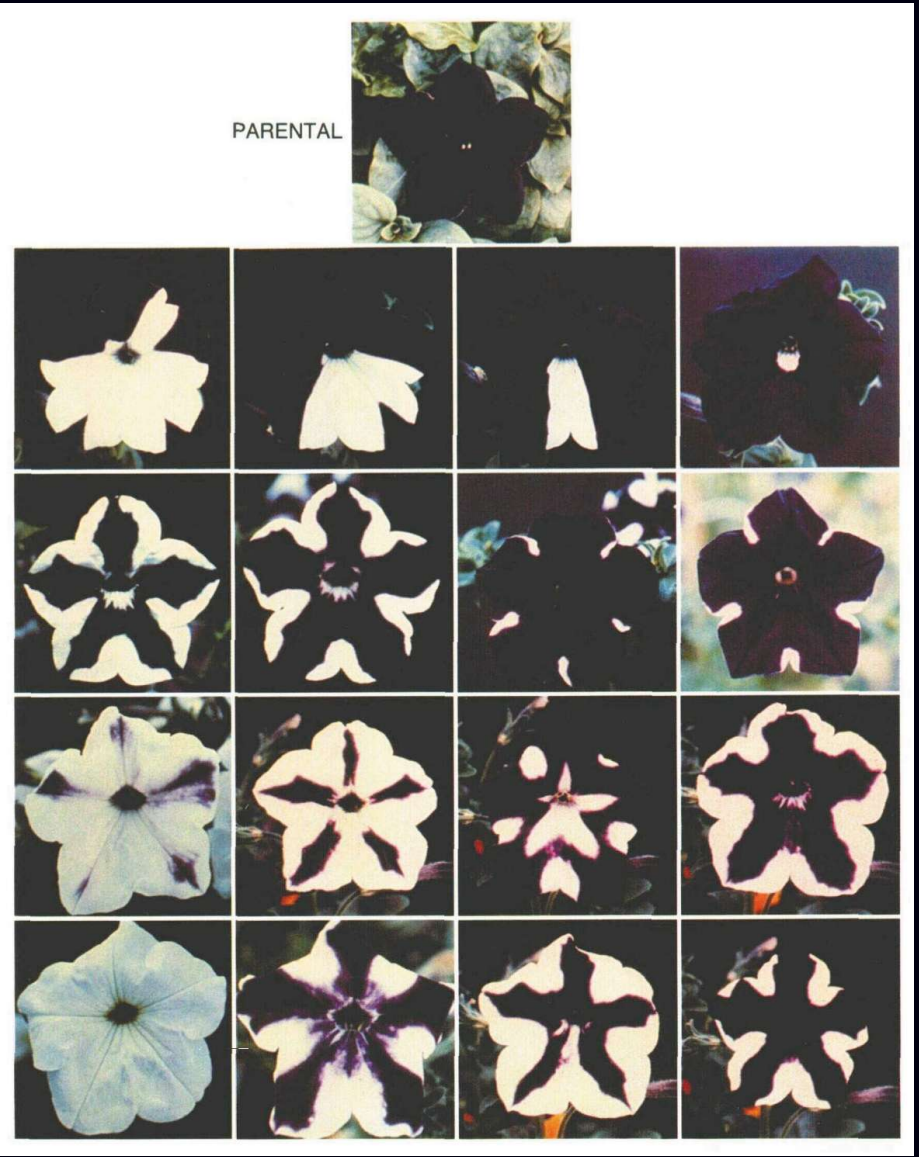
Role of messenger RNA in biological systems [5]

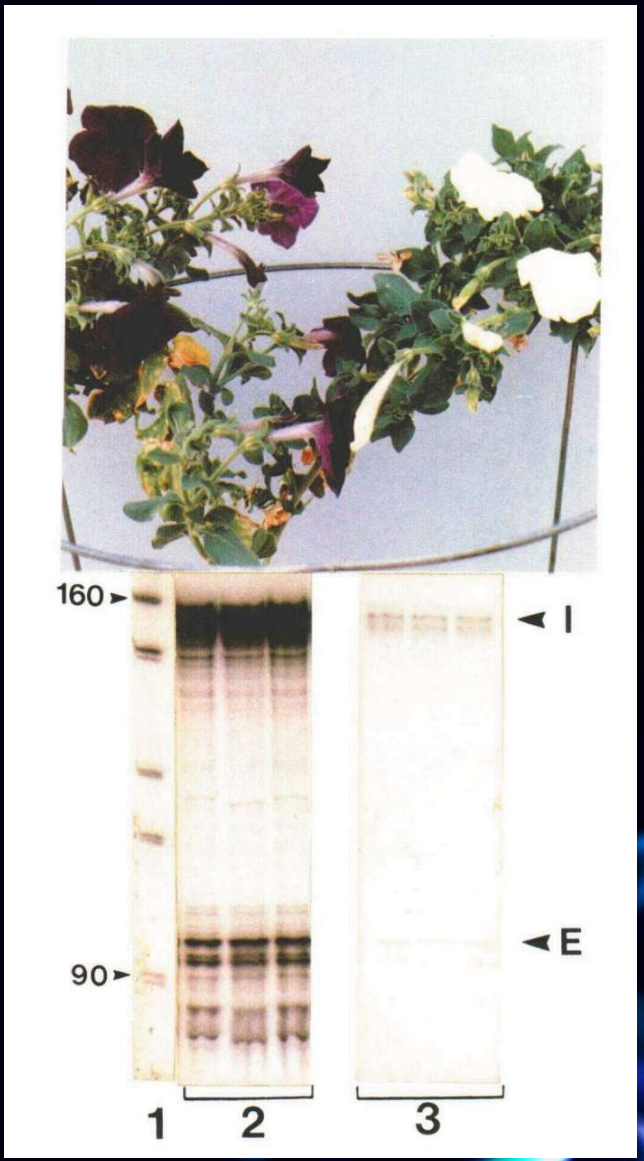




Transcription versus reverse transcription

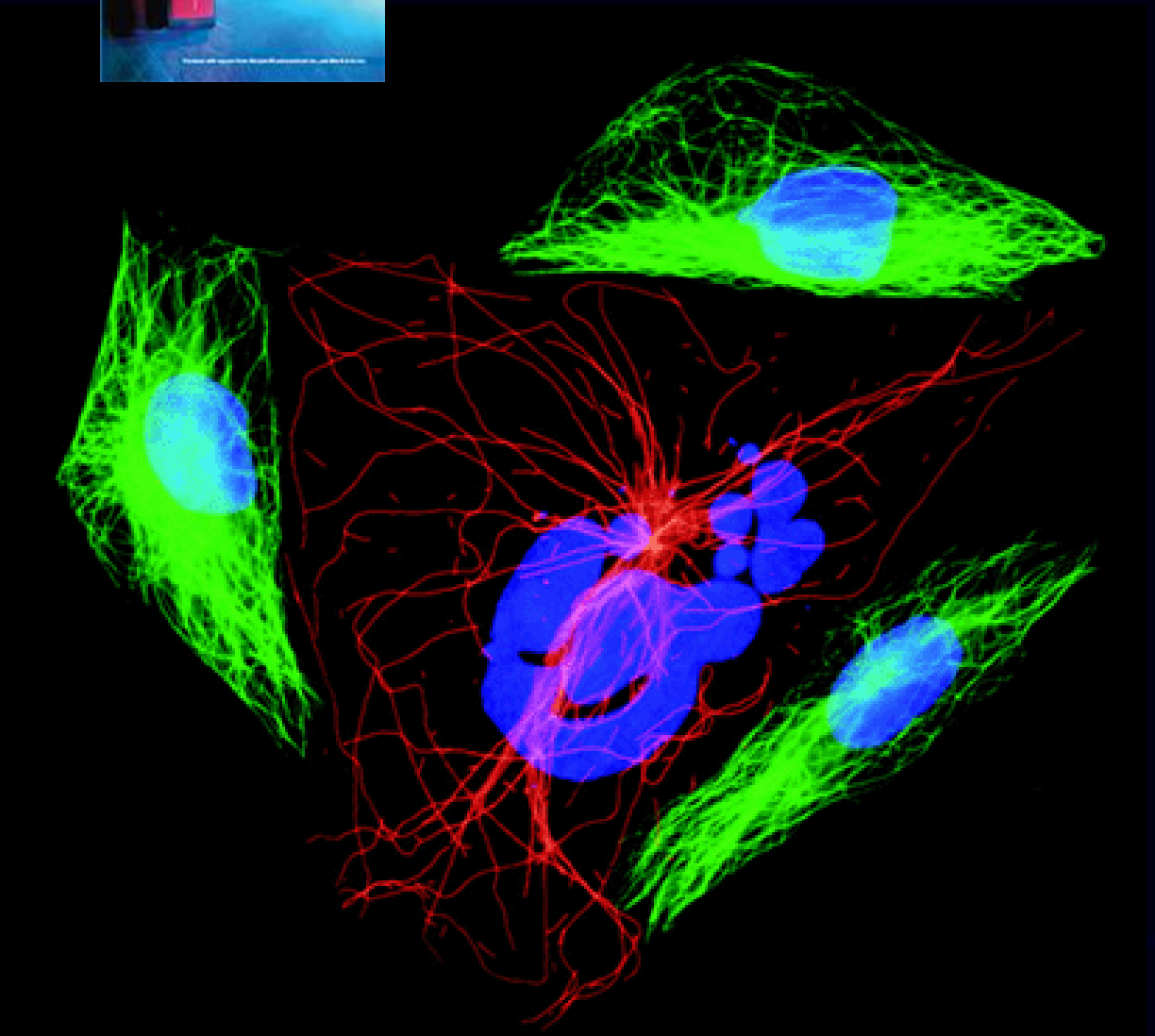
Discovery of RNA interference (RNAi) [4]





Messenger RNA for purple color inserted in petunia seeds

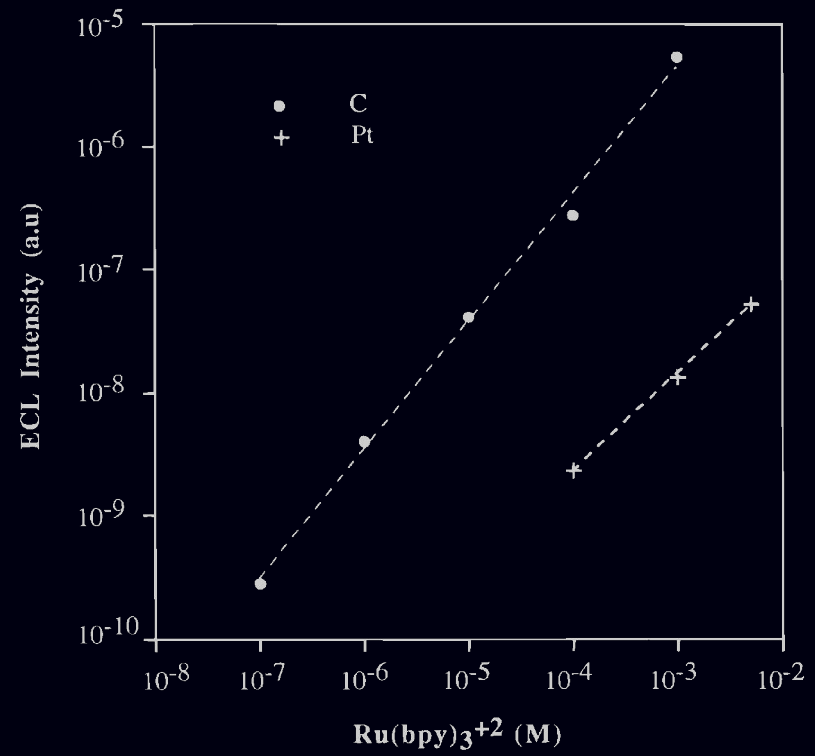
Promise of RNA interference (RNAi)

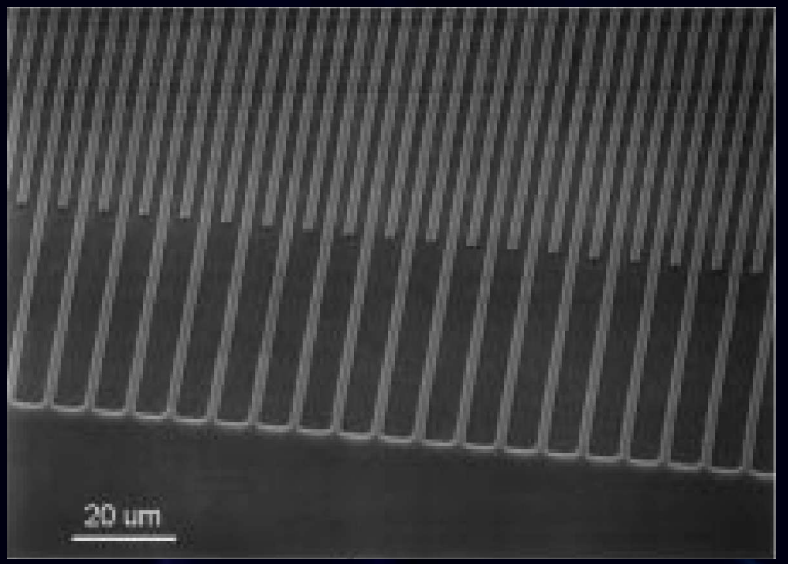


Revolutionary drug therapy allows one to shut off genes in hereditary diseases:

- Diabetes
- Alzheimers
- Breast Cancer
- Depression

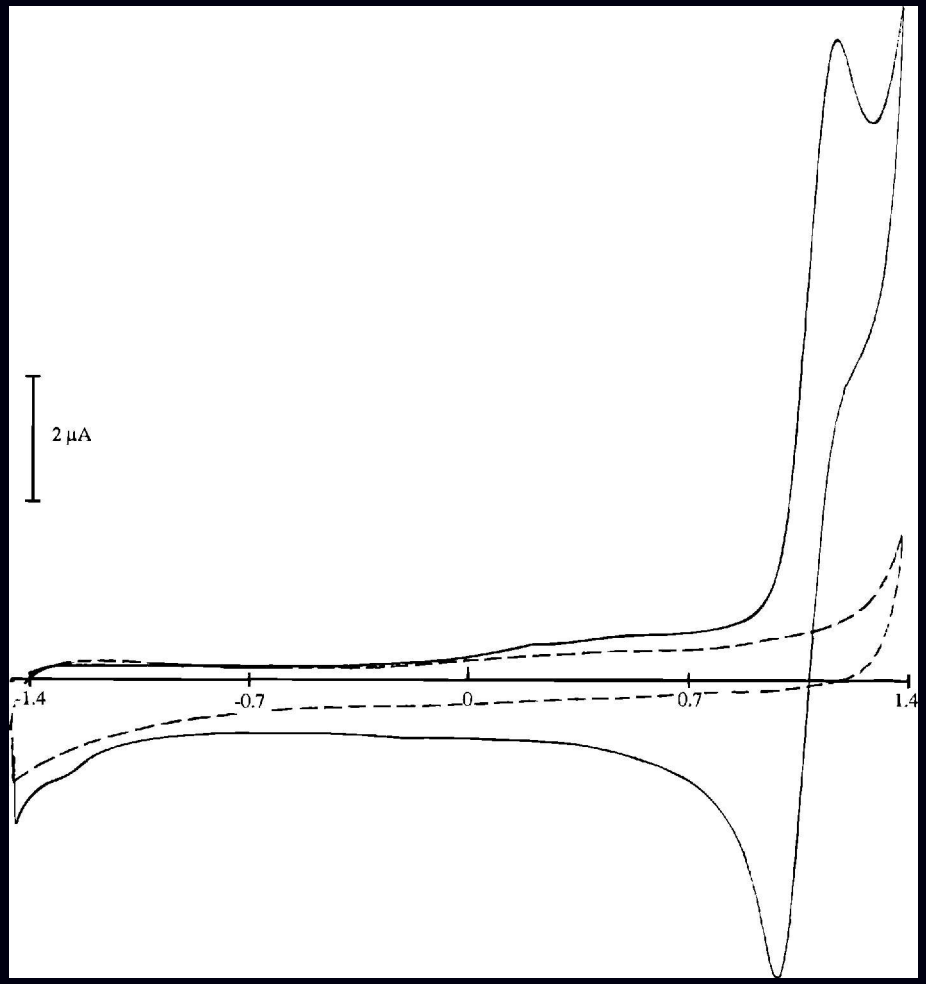
ElectroChemiLuminescence of $Ru(bpy)_3^{+2}$ [1]

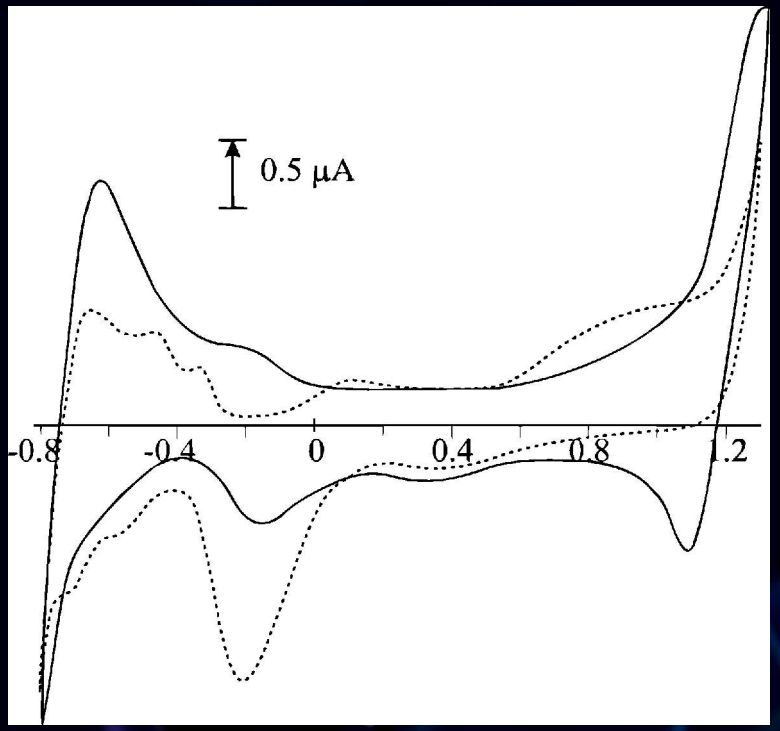




$$Ru(bpy)_3^{+3} + Ru(bpy)_3^{+1} = 2\ Ru(bpy)_3^{+2} + h\nu\ 620\ nm$$

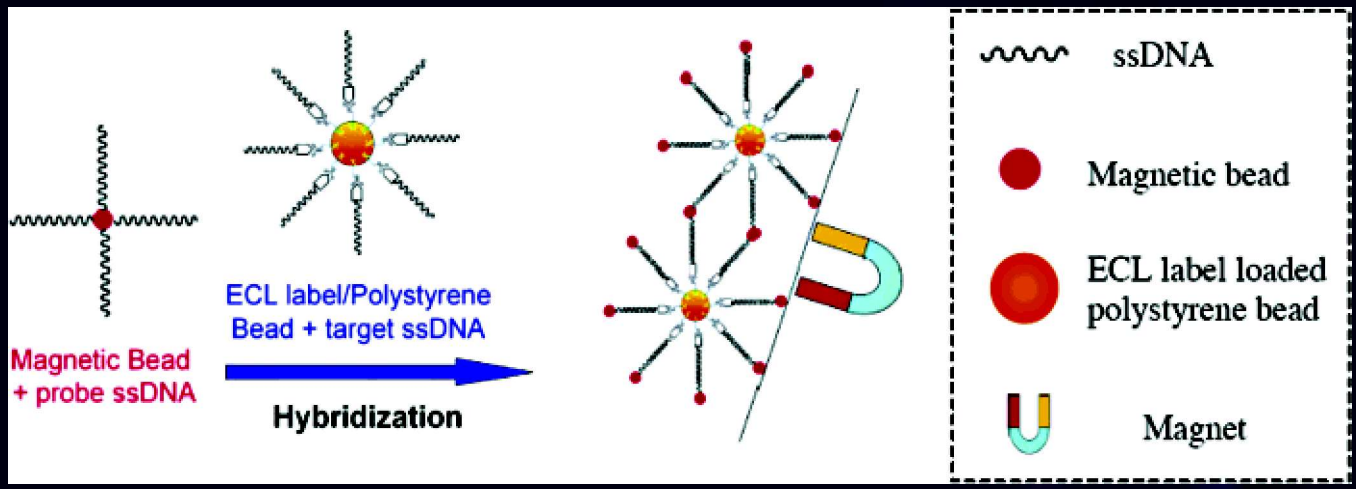
Catalytic effects on cyclic voltammograms of $Ru(bpy)_3^{+2}$ [1]





Glassy Carbon versus Platinum material

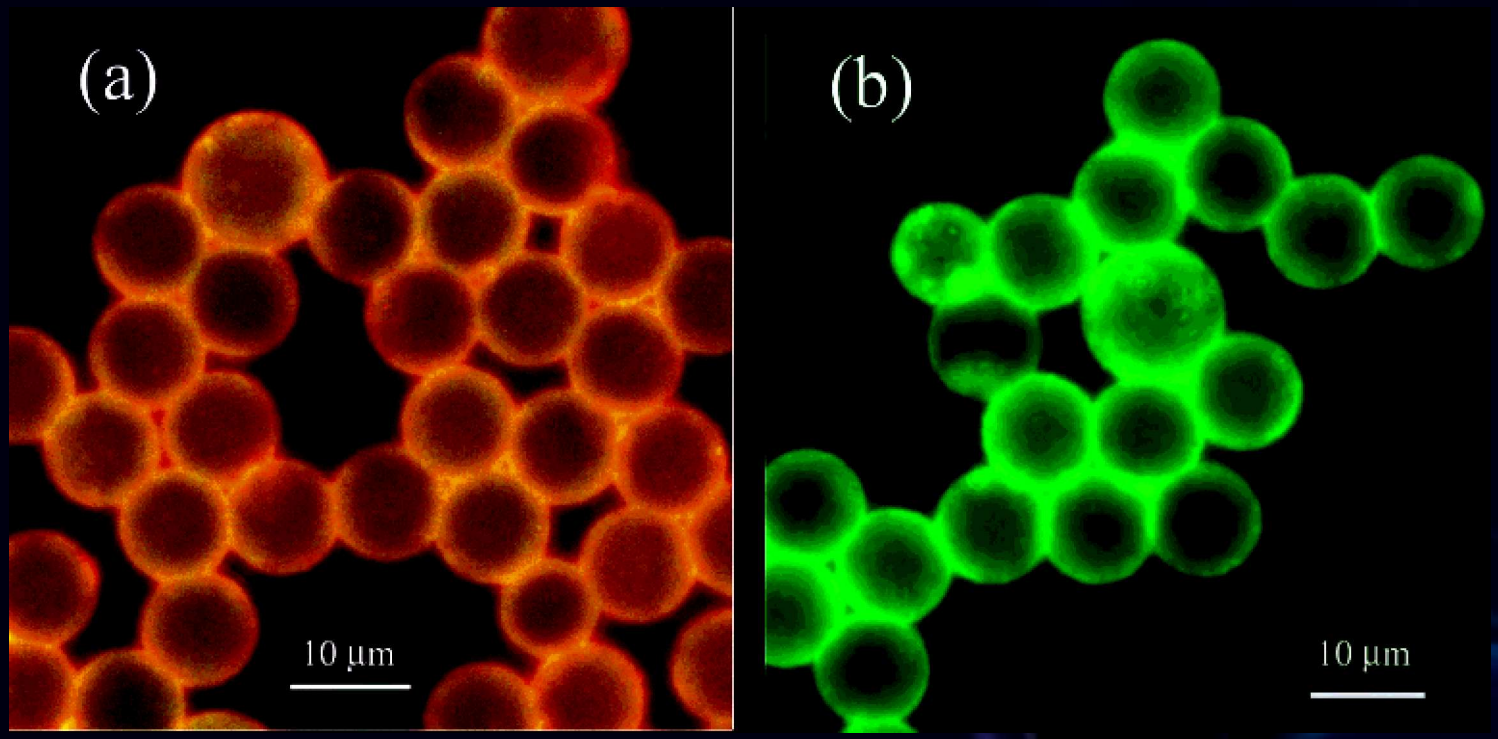
How DNA labeling works [2]



General methodology:

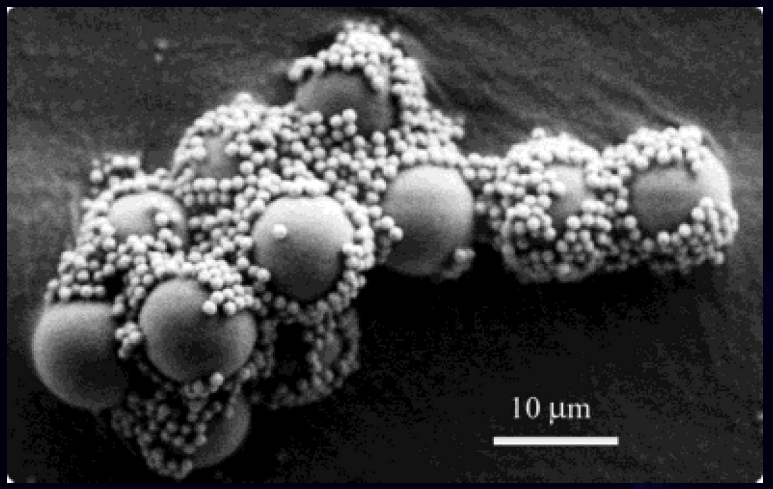
1) Attach probe ssDNA to magnetic bead
2) Attach target ssDNA to polystyrene bead
3) Hybridize DNA strands in aqueous media
4) Isolate hybridized pairs with magnet
5) Perform ECL in acetonitrile

Fluorescence of polystyrene beads [2]



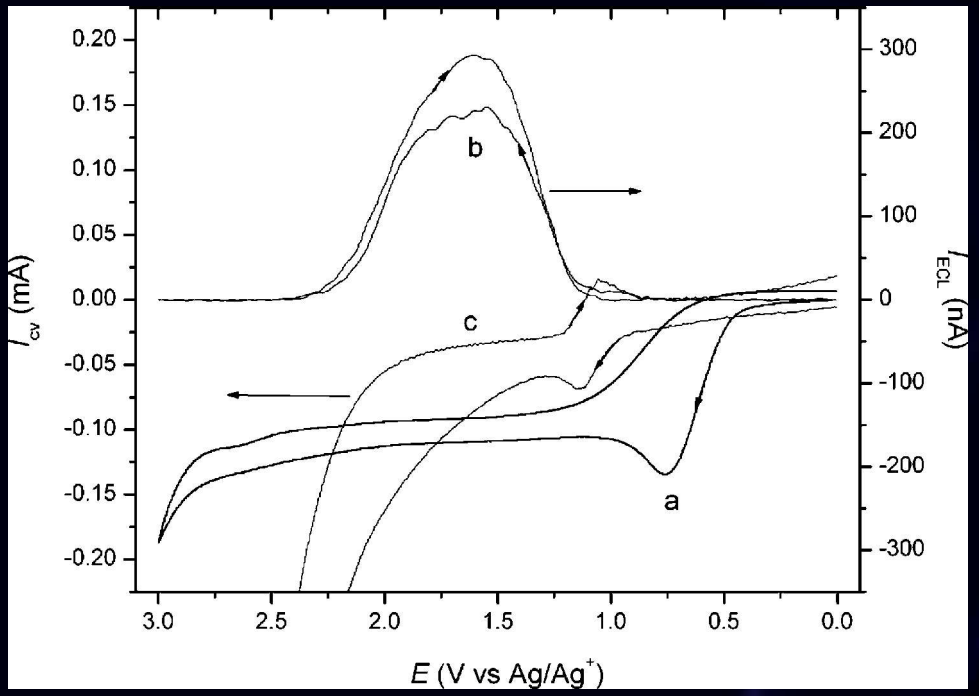
(a) $Ru(bpy)_3^{2+}$; (b) $Ru(bpy)_3^{2+}$ + avidin

Scanning electron image [2]



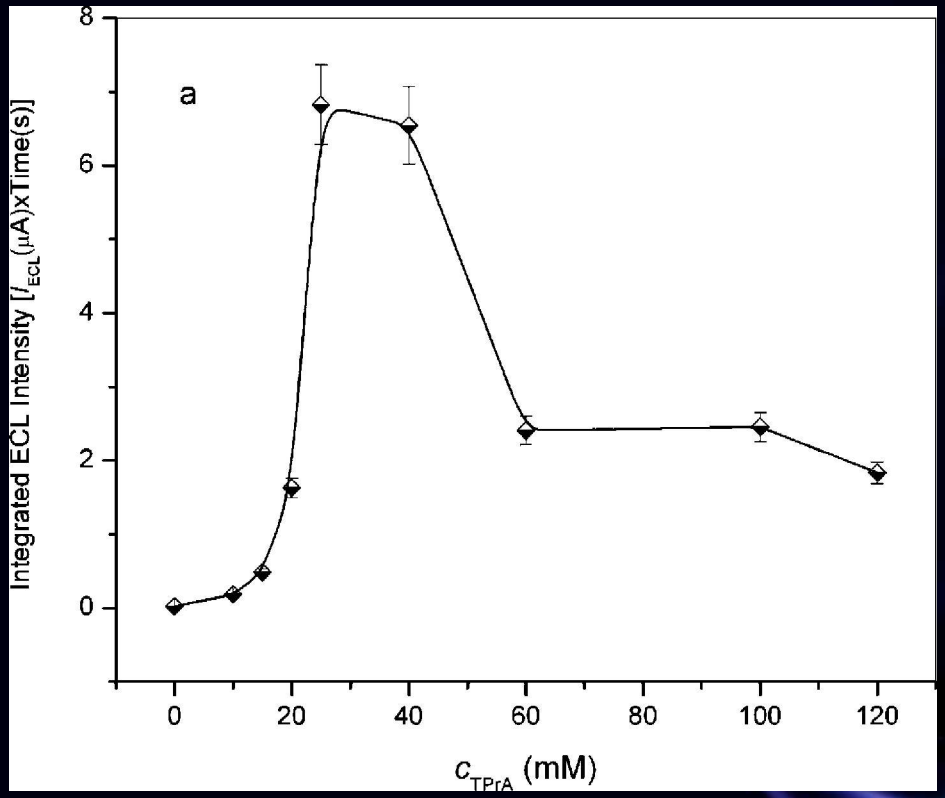
Polystyrene balls surrounded by magnetic beads after hybridization

Electrochemical behavior [2]



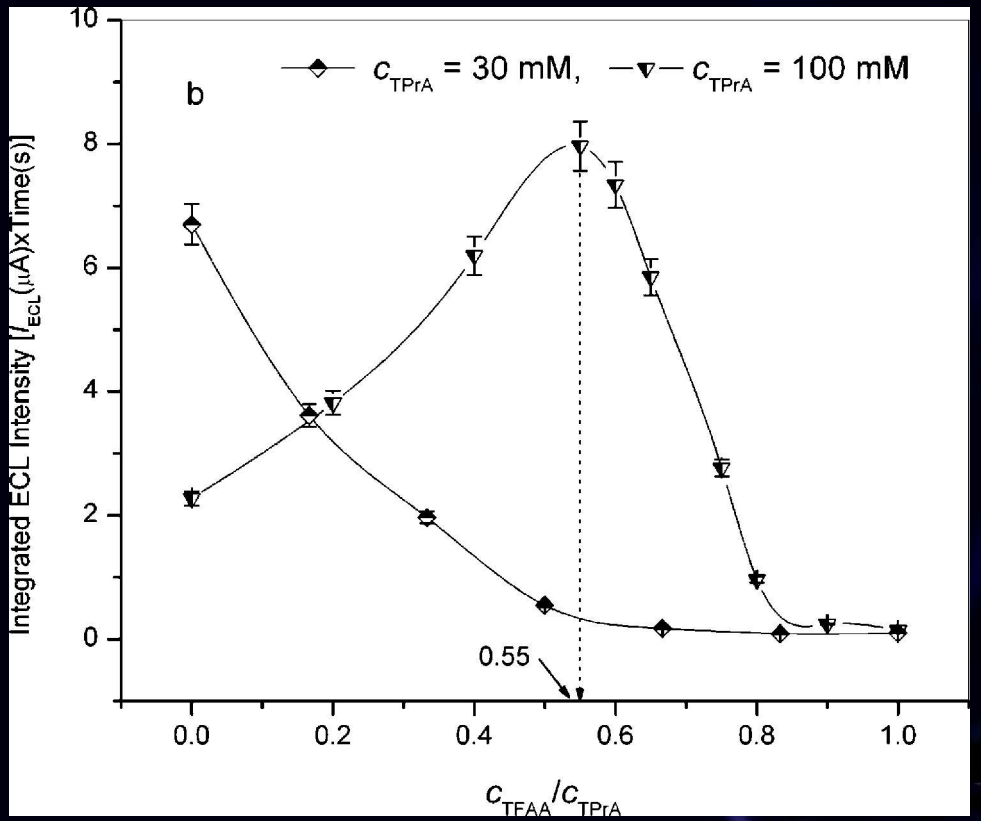
(a) CV of $Ru(bpy)_3^{2+}$ + acetylnitrile + salt + tripropyl amine; (b) ECL response; (c) CV in the absence of tripropyl amine [I x 10]

ECL intensity as a function of tripropyl amine concentration [2]



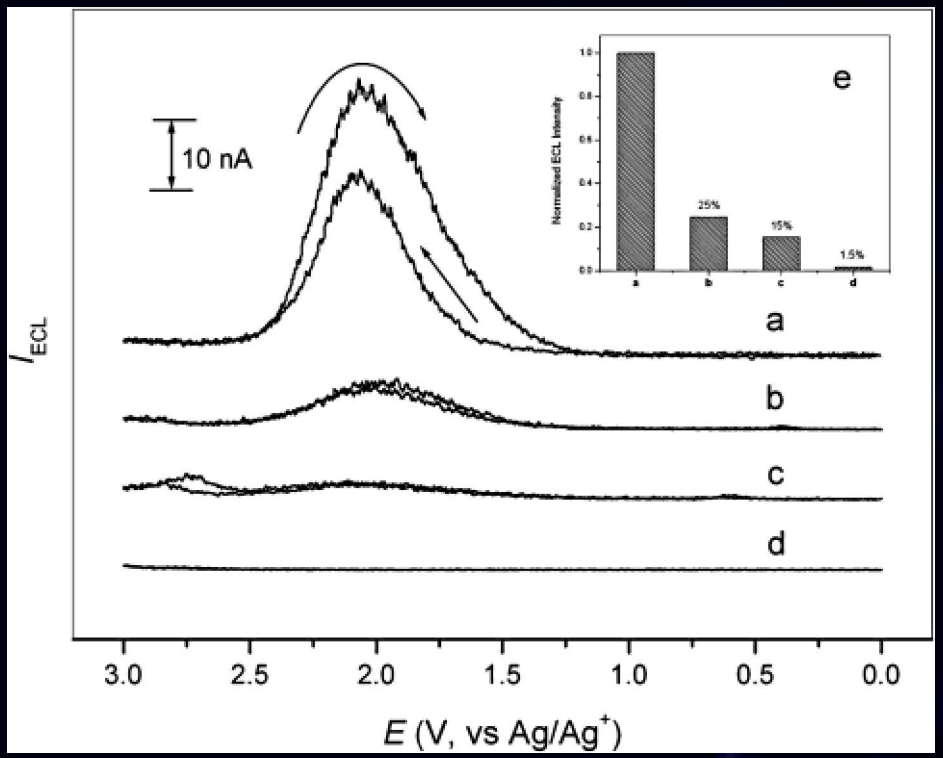
$Ru(bpy)_3^{2+}$, acetylnitrile, and salt present

ECL intensity as a function of trifluoroacetic acid addition: pH optimization [2]



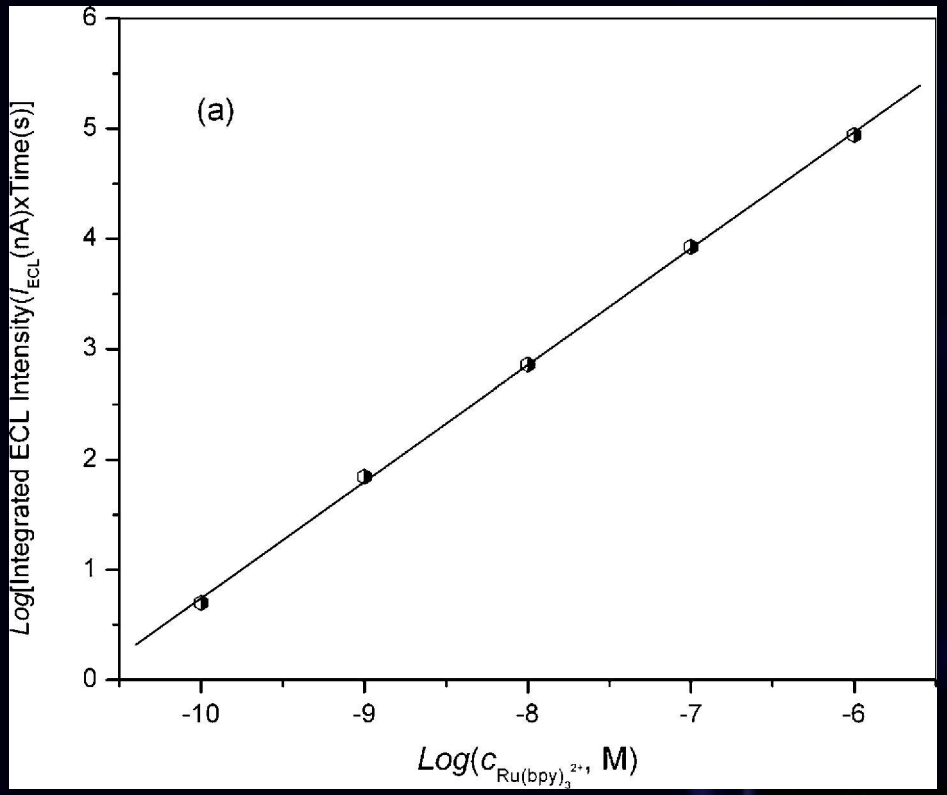
$Ru(bpy)_3^{2+}$, acetylnitrile, salt, and tripropyl amine present

Eradication of tripropyl amine ECL [2]



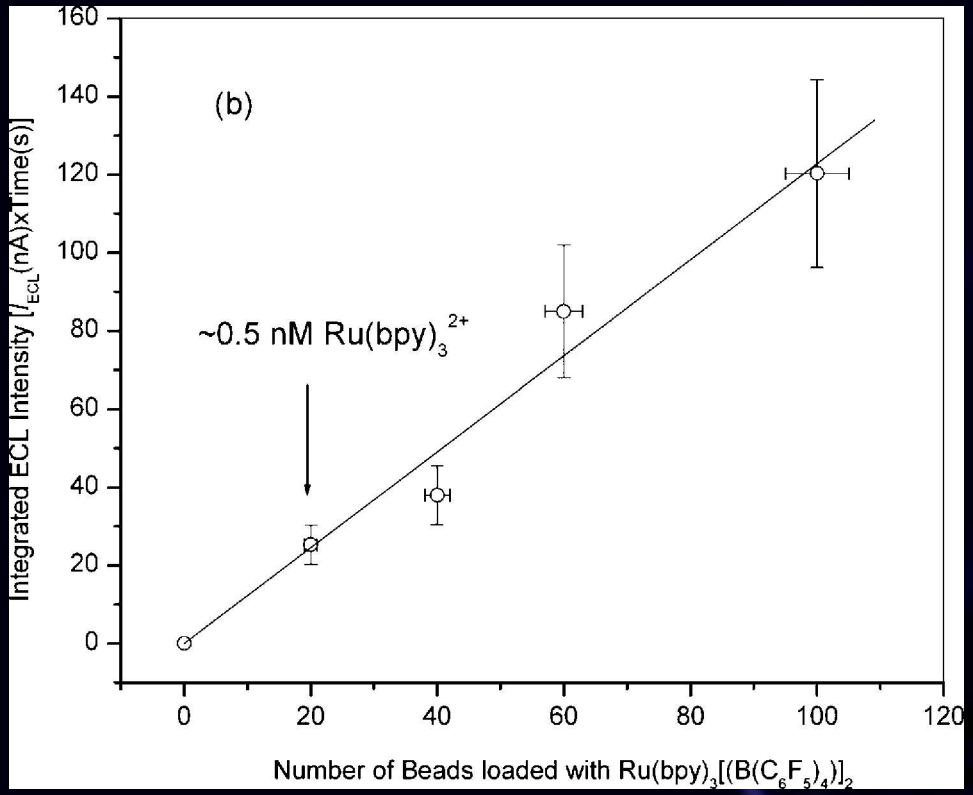
(a) Tripropyl amine + salt + acetonitrile; (b) Add trifluoroacetic acid; (c) Add 1vol% water; (d) Salt + acetonitrile alone

ECL intensity in relation to $Ru(bpy)_3^{2+}$ concentration [2]



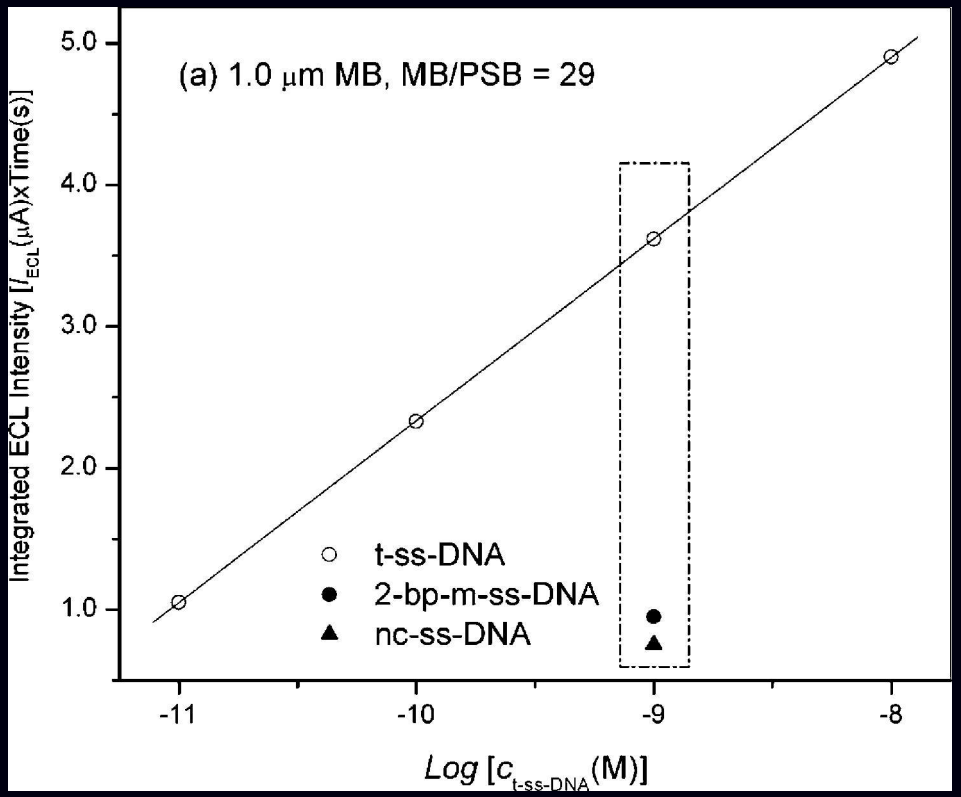
Tripropyl amine, trifluoroacetic acid, salt, acetonitrile, and water present

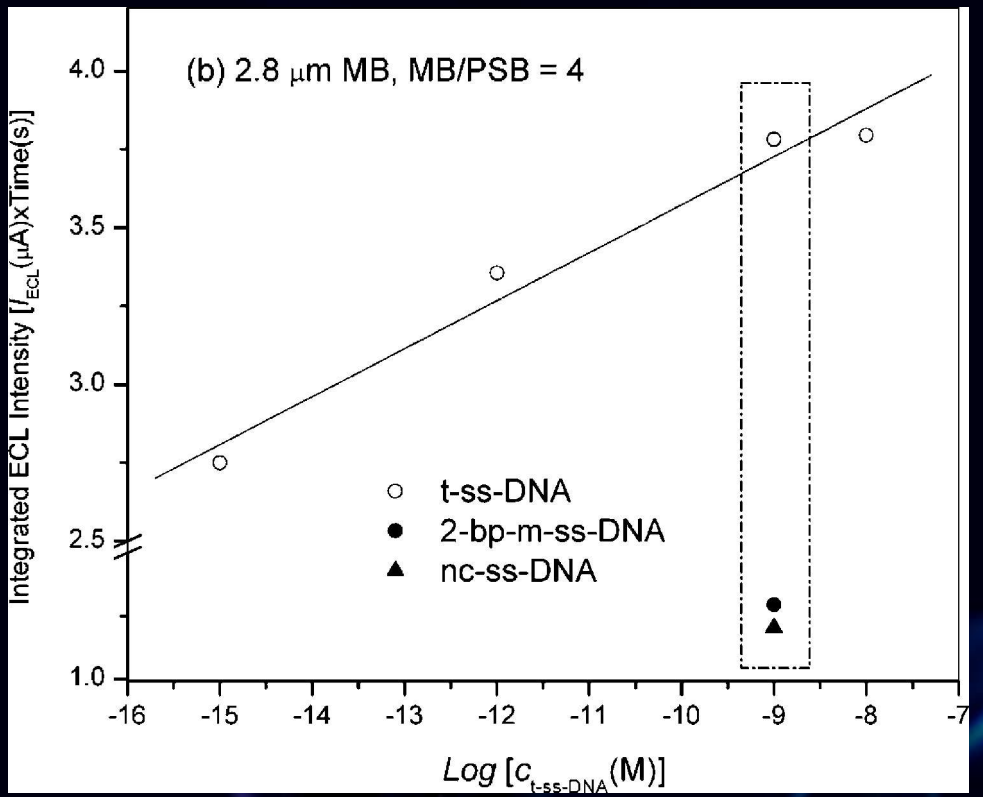
ECL intensity as a function of polystyrene ball quantity [2]



Ru(bpy)$_3^{2+}$ labeling

ECL detection of DNA hybridization [2]





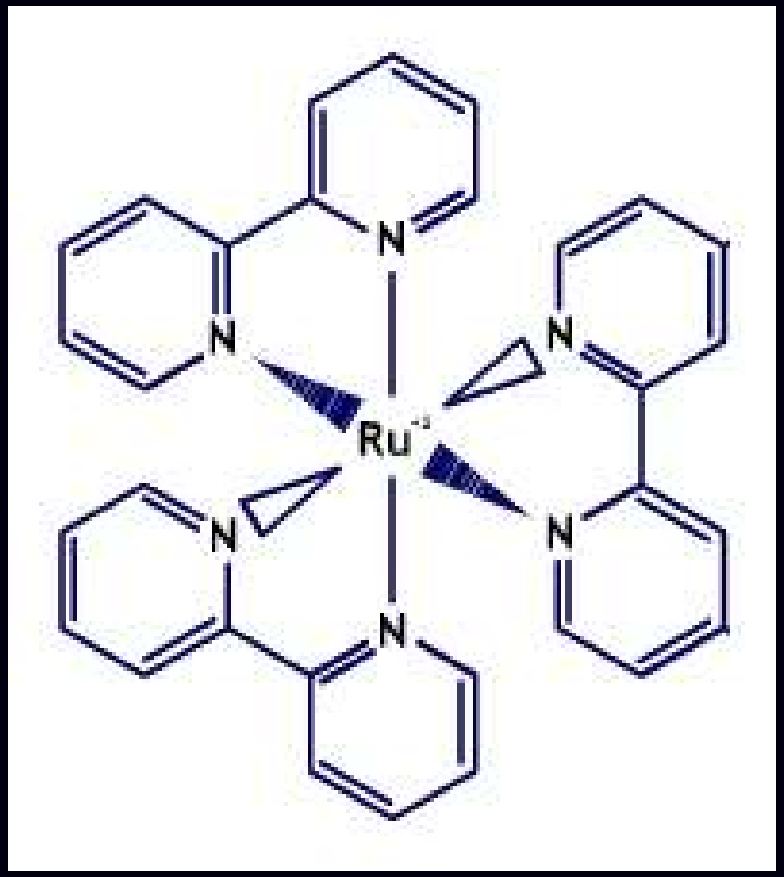
(a) Small magnetic beads and high population ratio;

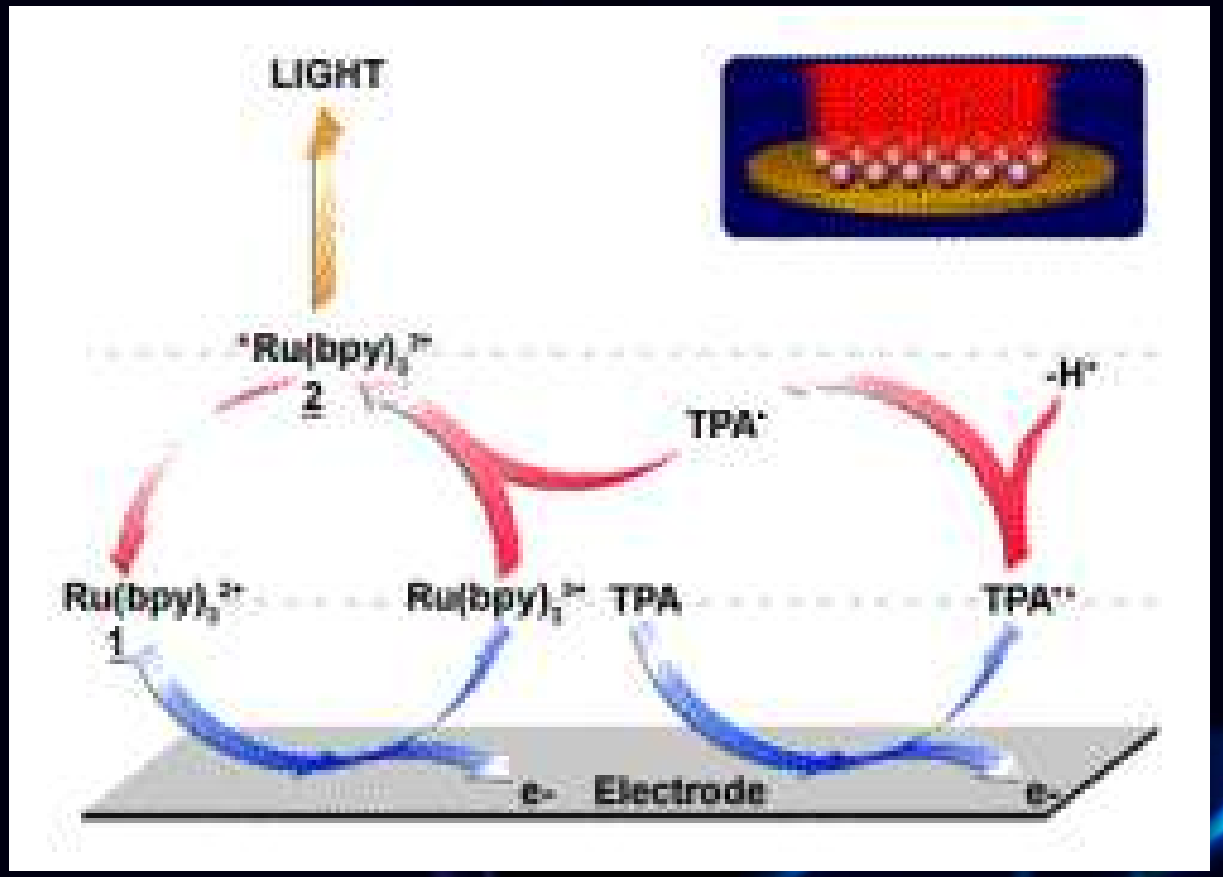
(b) More favorable magnetic bead size and population ratio

Advantages over competing ECL techniques: [2]

- Dual phases: aqueous DNA coupling in combination with acetonitrile analysis.
- High selectivity/ low detection limit: starting as low as 1.0 fM concentration for target DNA.
- Unprecedented stability: once $Ru(bpy)_3^{2+}$ label is released into acetonitrile, ECL measurement can be performed repeatedly.

Chemical processes involved in typical ECL systems [3]



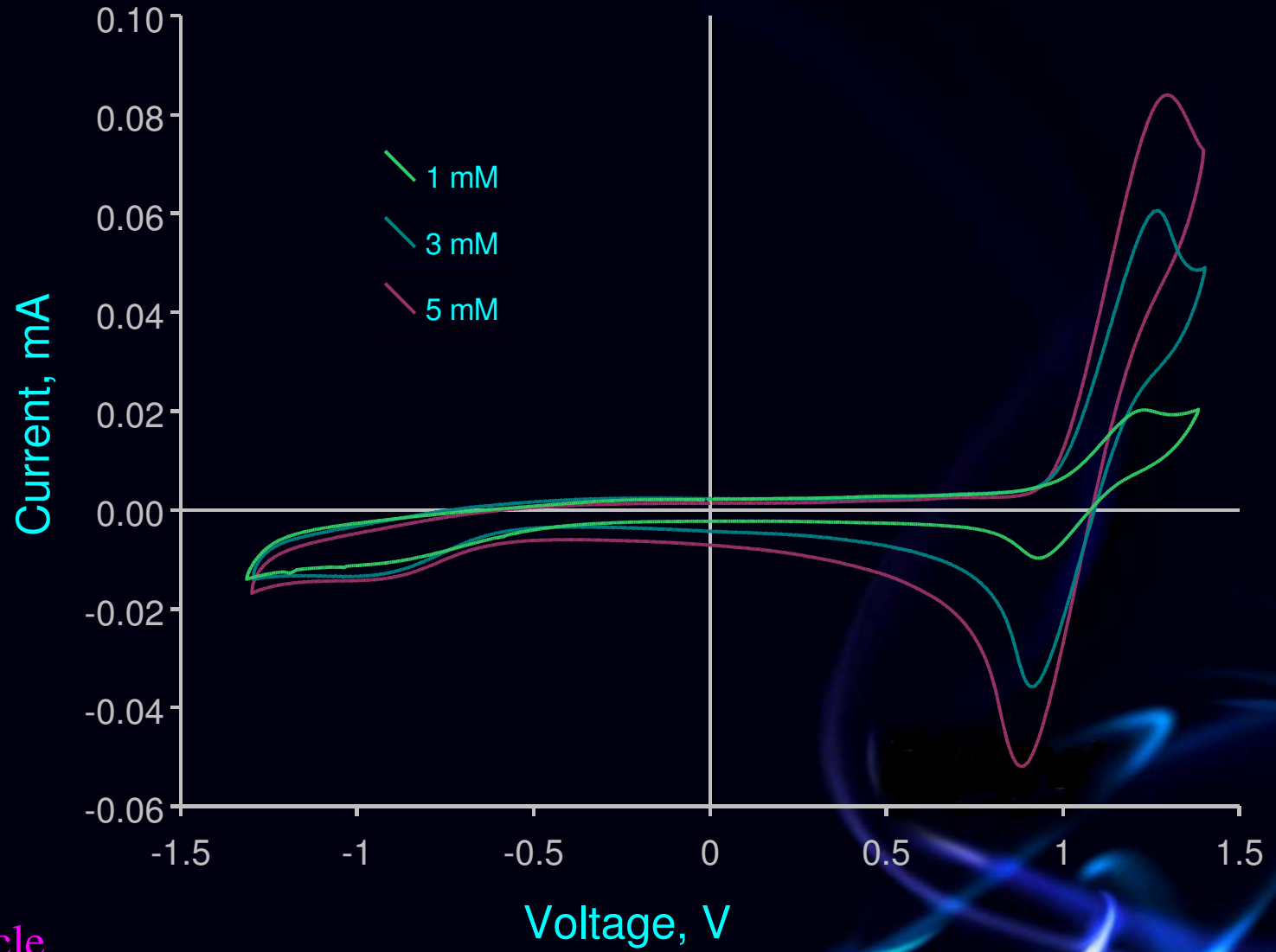


$Ru(bpy)_3^{+3} + TPA^{\bullet} = Ru(bpy)_3^{+2} + DPA + propyl\ aldehyde + h\nu\ 620\ nm$

where: TPA = tripropyl amine

DPA = dipropyl amine

Cyclic voltammogram of $Ru(bpy)_3^{2+}$

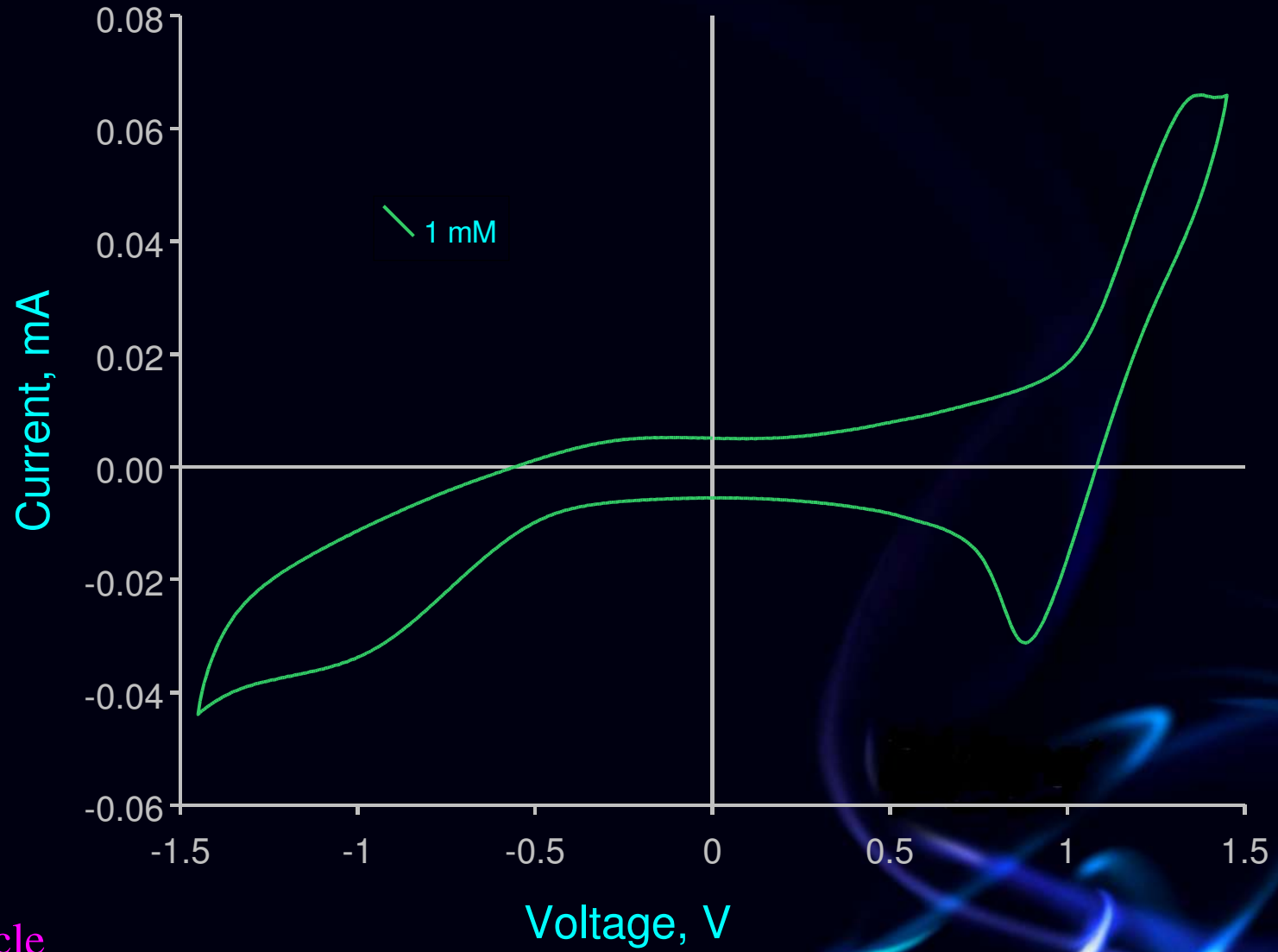


2nd Cycle

Scanrate= 200 mV/s

Interdigitated glassy carbon electrode

Cyclic voltammogram of $Ru(bpy)_3^{2+}$



2nd Cycle

Scanrate= 200 mV/s

Glassy carbon film on Si electrode

Research Frontiers:

- Carbon rod or Pt electrode in acetonitrile using pulses.
- Rotating electrode in acetonitrile to find lifetime of $Ru(bpy)_3^{+1}$.
- Try vertically patterned electrodes rather than interdigitated configuration.

Vertically integrated electrodes

1. Deposit PECVD Si_3N_4 on Carbon Coated Wafers

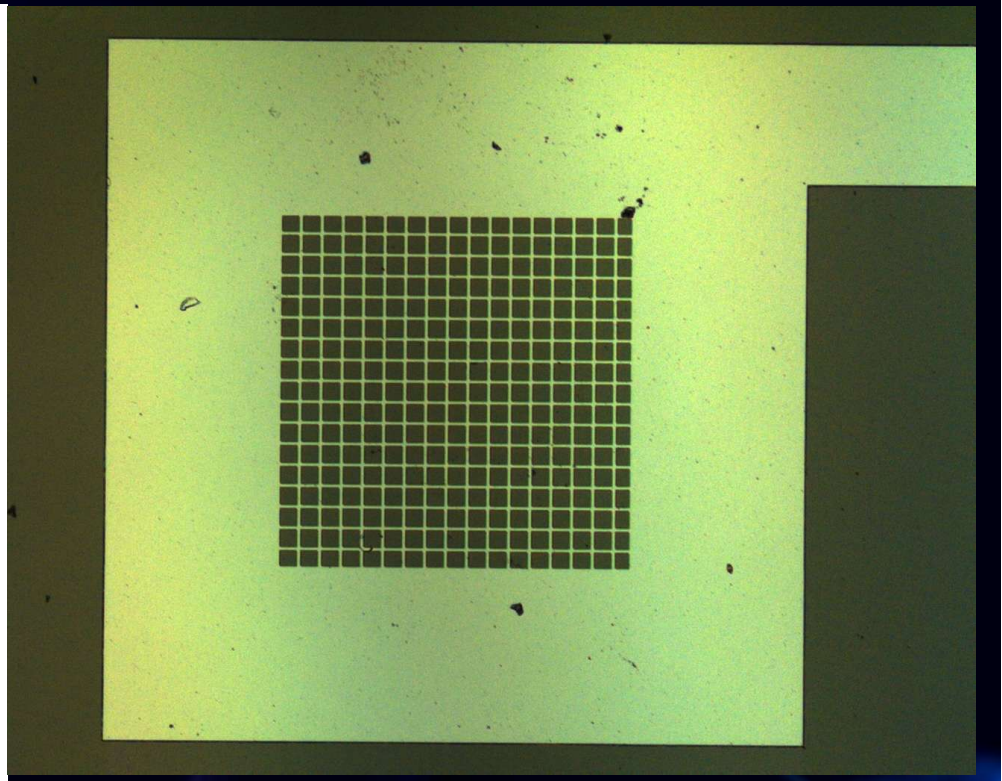
Note: Silicon wafer are coated with oxide/nitride before Carbon deposition

2. Spin/Pattern Photoresist

3. Evaporate Platinum

4. Strip Resist

5. Etch Si_3N_4 using a SF_6 plasma



Deposition steps and top view of vertical matrix

Conclusions:

- Whole new drug development scheme on the horizon which identifies genes attributed to disease, then shuts off expression with messenger RNA.
- Relative to its rival fluorescence, ECL eliminates the excitation light required, enhancing sensitivity and reducing cost.
- With the base pair selectivity ECL offers, DNA matching could evolve into sequencing.

References

[1] G. C. Fiaccabrino, et al., *Analytical Chemistry*, **70**, 4157 (1998).

[2] W. Miao and A. J. Bard, *Analytical Chemistry*, **76**, 5379 (2004).

[3] J. K. Leland and M. J. Powell, *Journal of Electrochemistry Society*, **137**, 3127 (1990).

[4] C. Napoli, C. Lemieux, and R. Jorgensen, *Plant Cell*, **2**, 279 (1990).

[5] http://en.wikipedia.org