
UNDERSTANDING Ag-DNA FLUOROPHORES

and their prospects for

DNA NANOTECHNOLOGY

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DNA nanotechnology

BENEFITS

- + self-assembling
- + programmable
- + high-resolution
- + low-symmetry
- +
- +
- +

BARRIERS

- yield
- stability
- coupling
-
-
-
-



DNA nanotechnology

BENEFITS

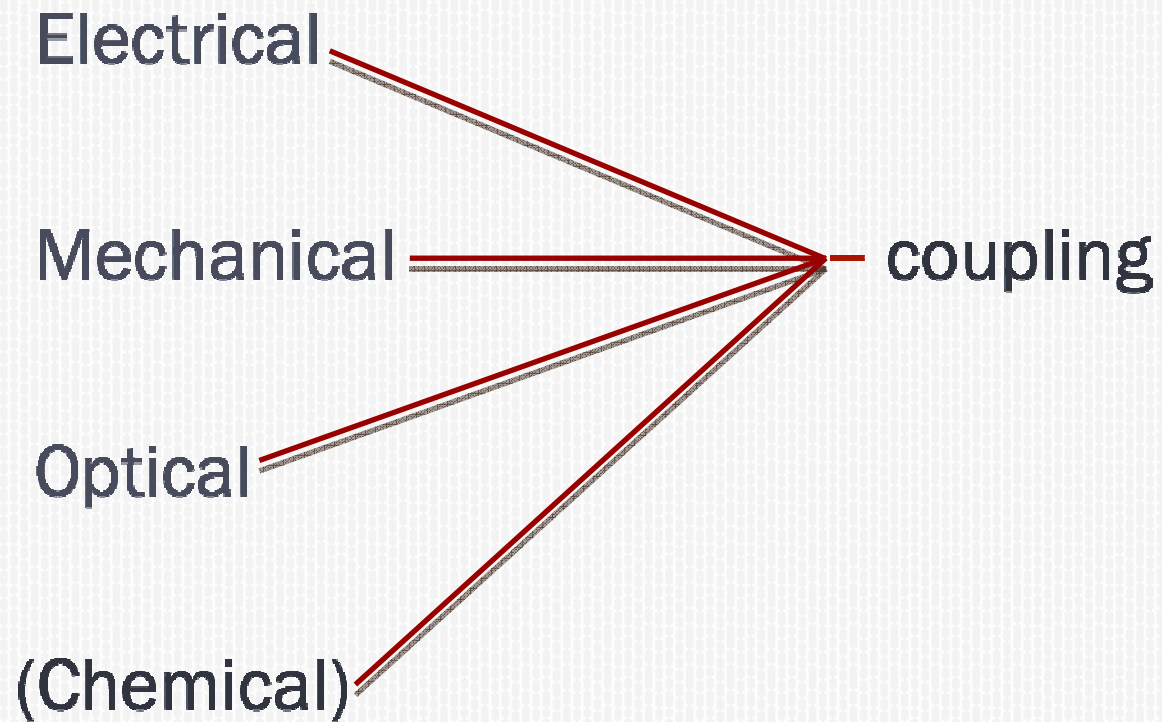
- + self-assembling
- + programmable
- + high-resolution
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- +
- +
- +

BARRIERS

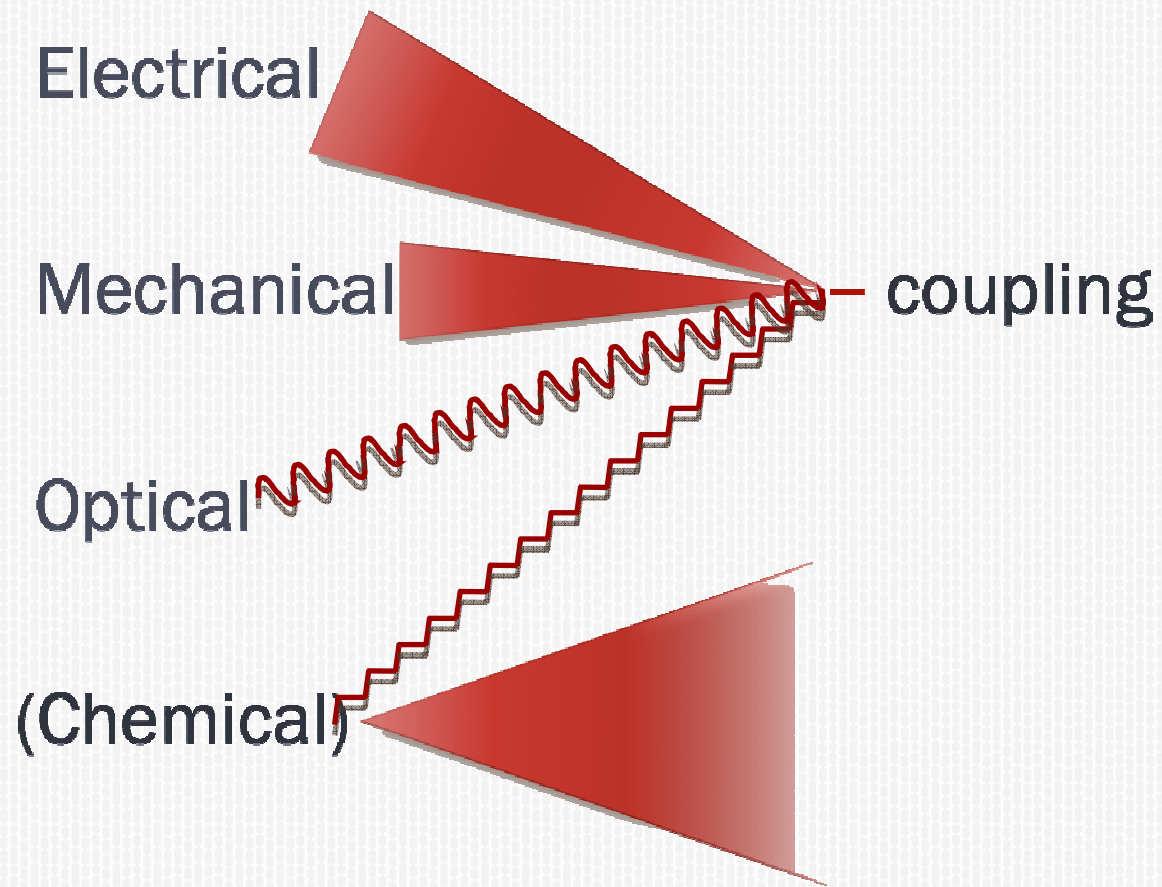
- yield
- stability
- **coupling**
-
-
-



DNA nanotechnology



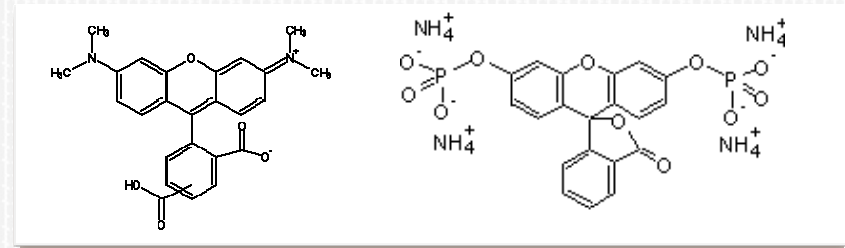
DNA nanotechnology



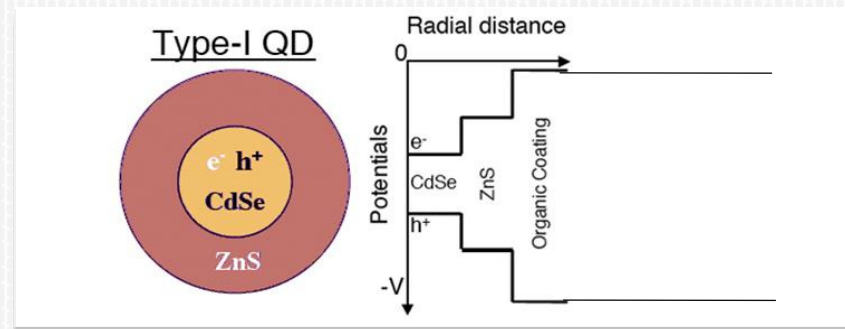
DNA nanotechnology – optical coupling

✘ candidate input/output devices:

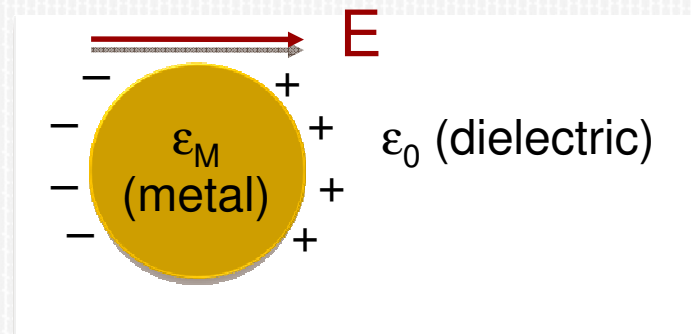
+ Molecular Fluorophores



+ Quantum Dots



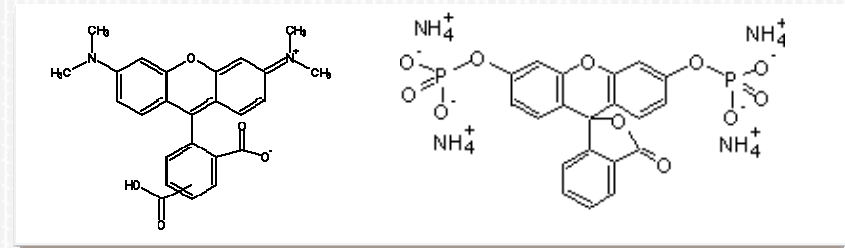
+ Metal Nanoparticles



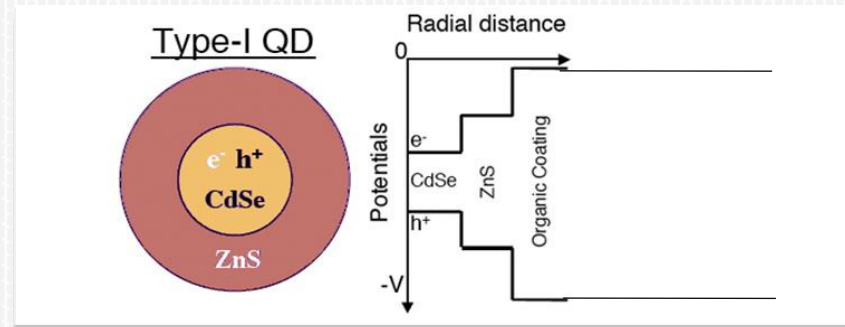
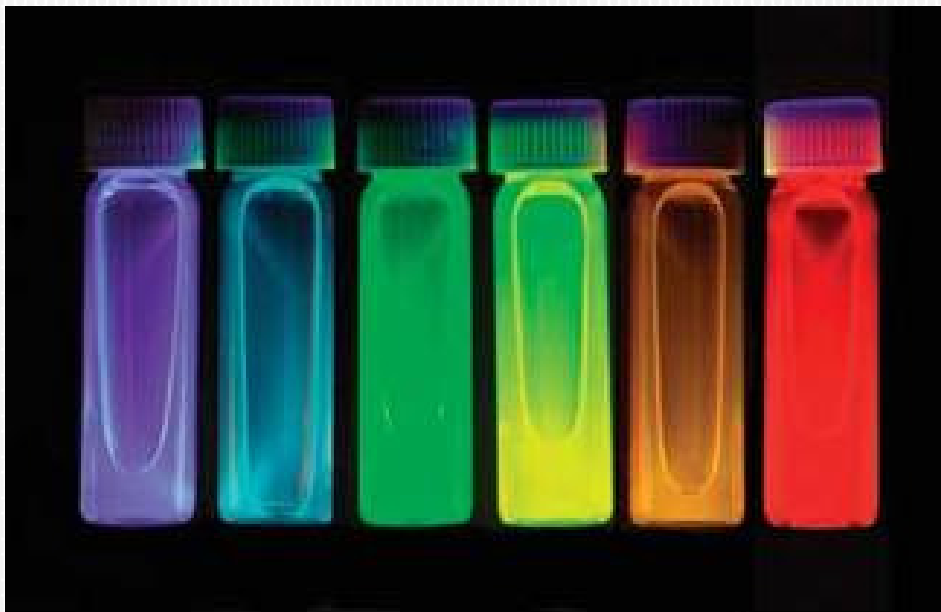
DNA nanotechnology – optical coupling

✘ candidate input/output devices:

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DNA nanotechnology – optical coupling

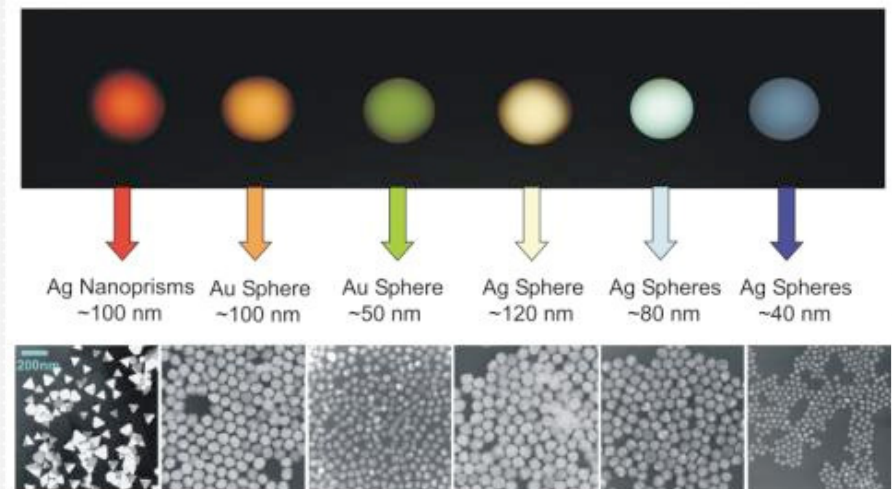
✘ candidate input/output devices:

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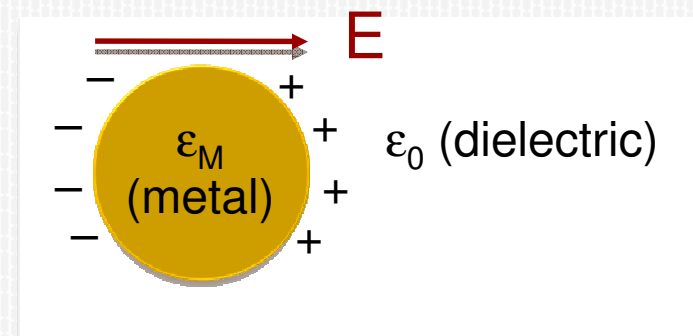
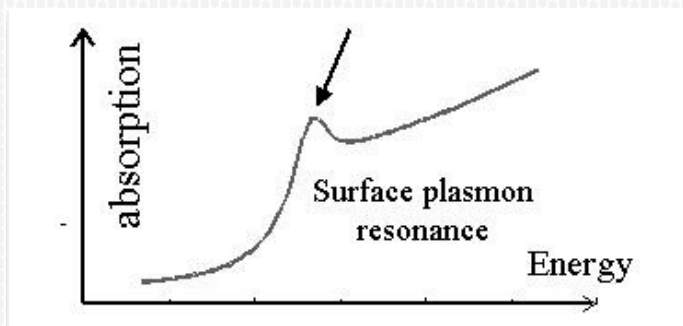
+ Metal Nanoparticles

Rayleigh Light-Scattering of Nanocrystals:
Shape, Size, and Composition Matter



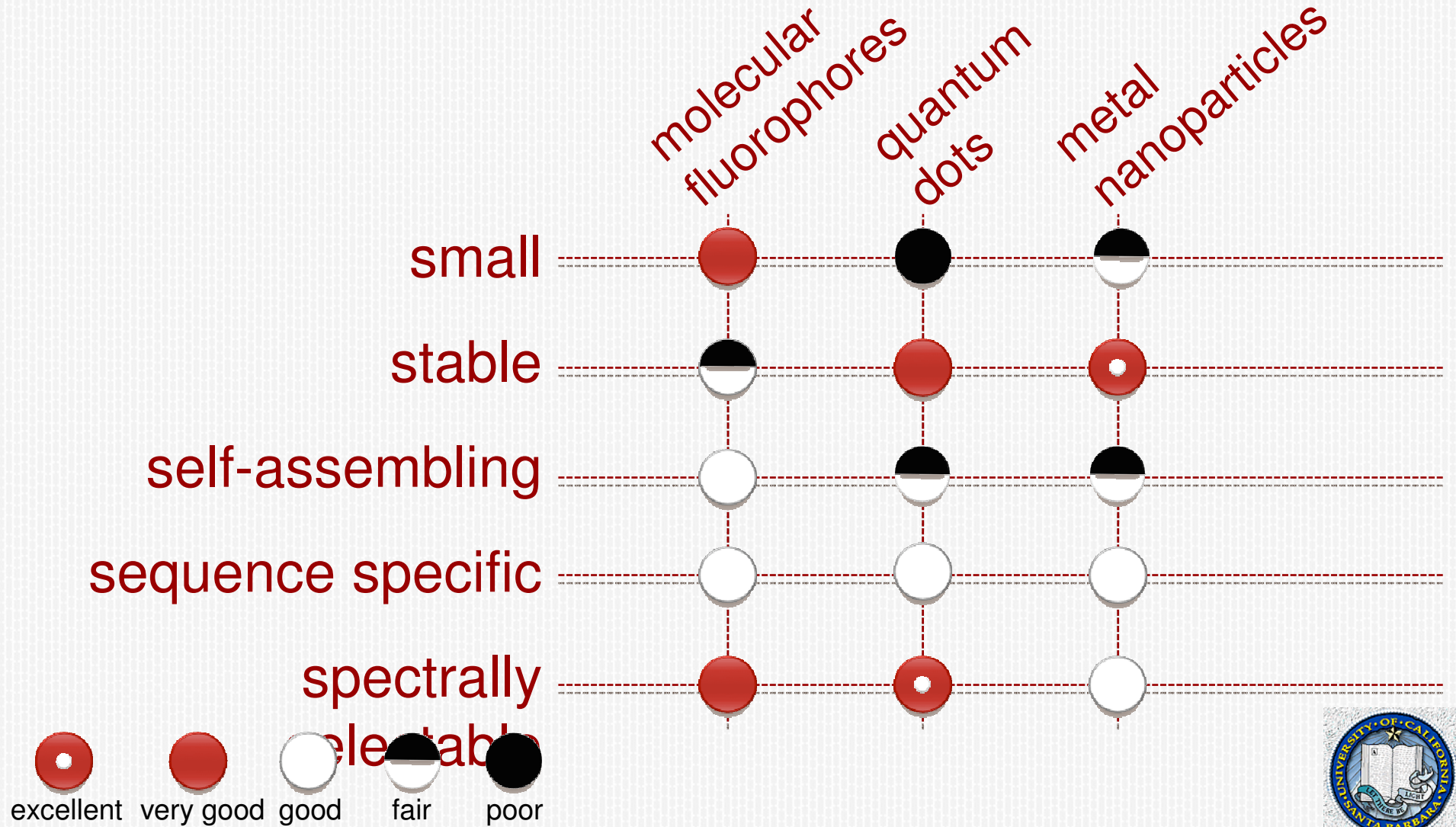
* The scale bar is the same for all the images.

mirkingroup@northwestern.edu

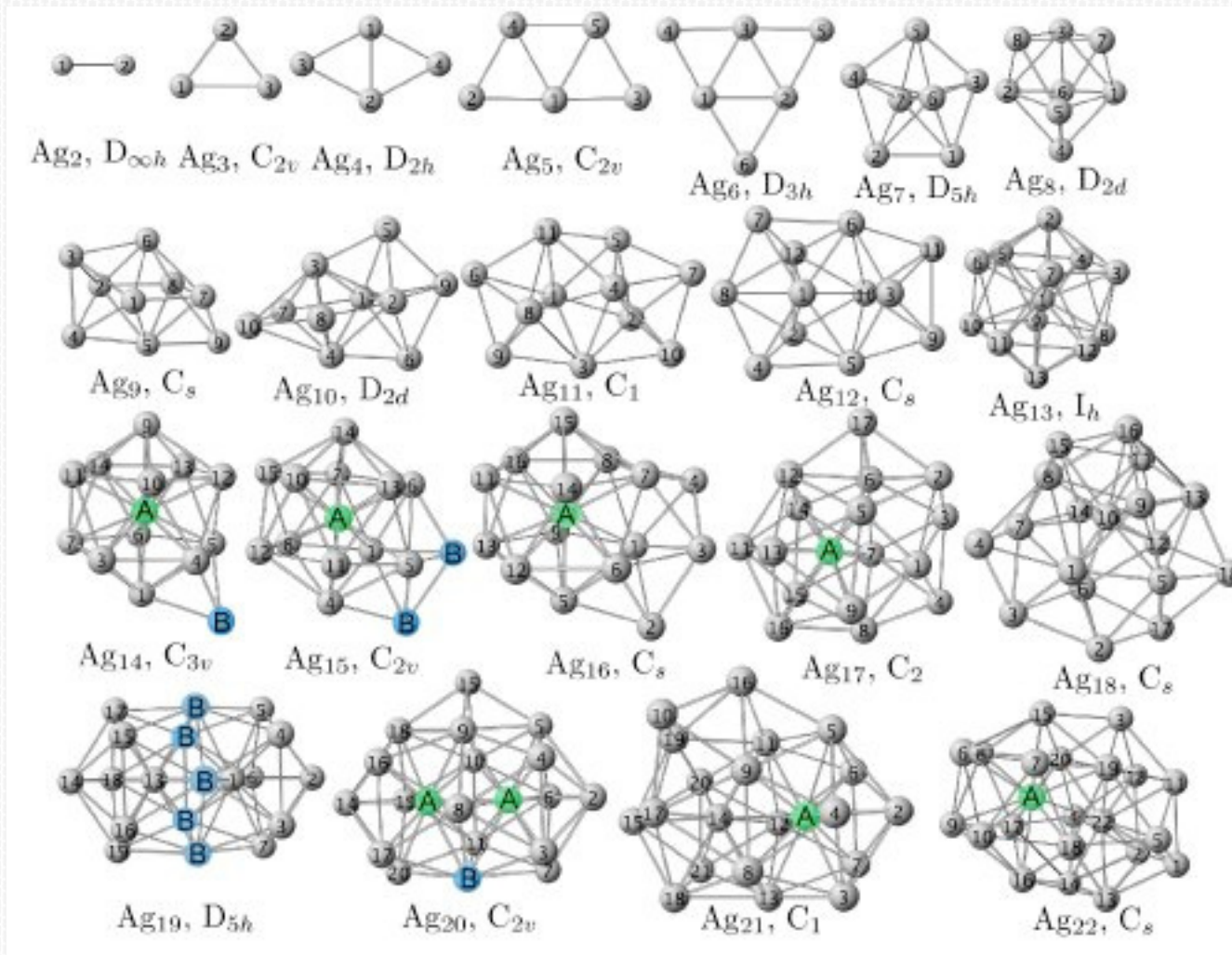


DNA nanotechnology – optical coupling

✘ the ideal input/output device would be:



Ag-atom clusters (a.k.a. superatoms)

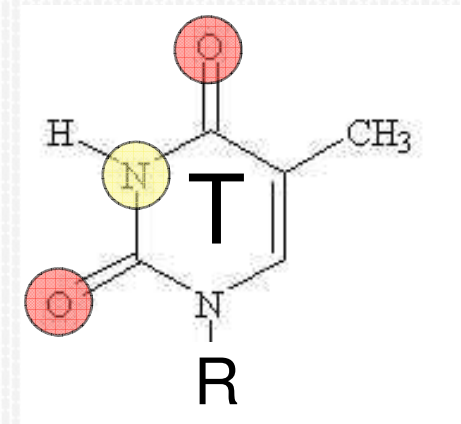
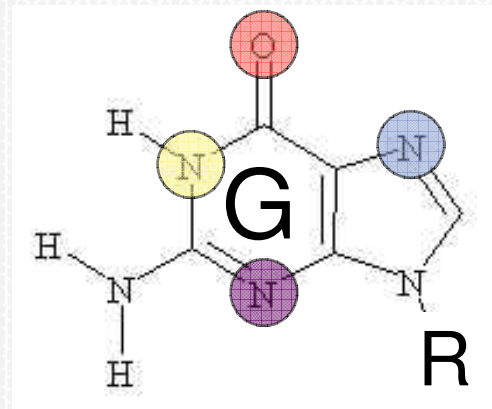
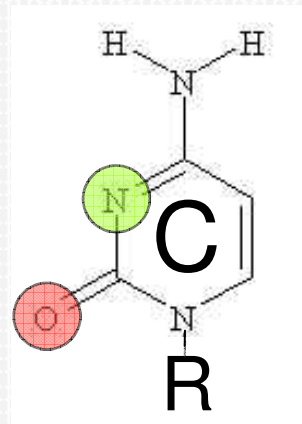
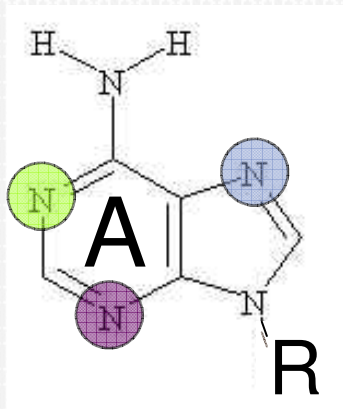


Ag-DNA binding

× Ag^{+1}

+ binds to bases (not phosphates)

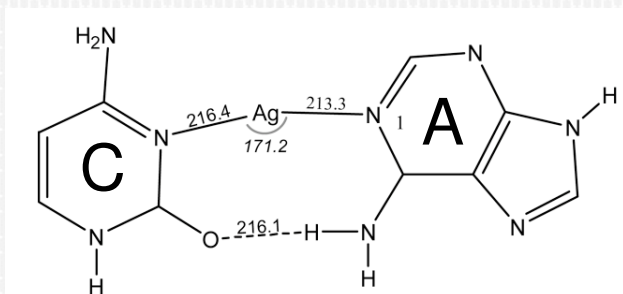
+ prefers ssDNA to dsDNA



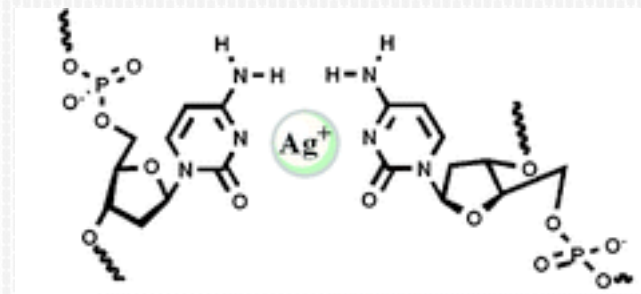
Ag-DNA binding

× Ag^{+1}

- + binds to bases (not phosphates)
- + prefers ssDNA to dsDNA
- + stabilizes C-A and C-C mispairs



M. Schreiber and L. González,
J Comp Chem 28: 2299–2308, 2007



A.Ono, et al., Chem Comm 28: 4825–4827, 2008



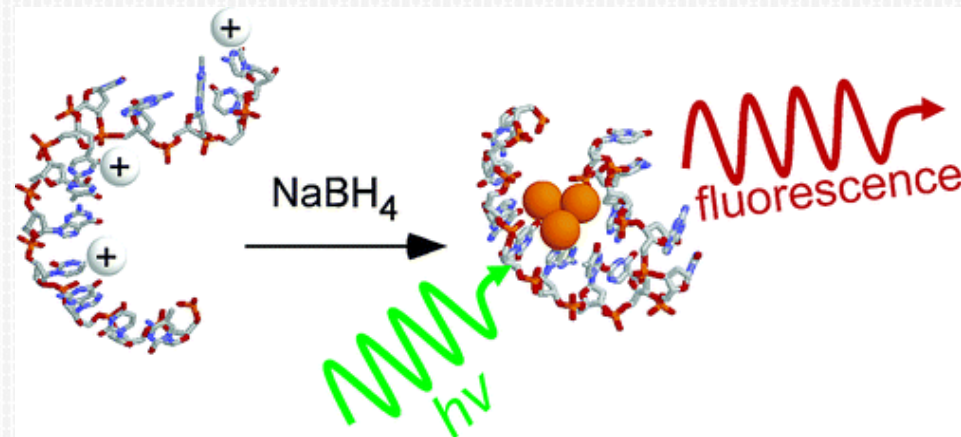
Ag-DNA binding

× Ag(I)

- + binds to bases (not phosphates)
- + prefers ssDNA to dsDNA
- + stabilizes C-A and C-C mispairs

× DNA-Templated Ag-nanocluster Formation

Jeffrey T. Petty, Jie Zheng, Nicholas V. Hud, and Robert M. Dickson
J. Am. Chem. Soc., 126: 5207-5212 (2004)



Ag-DNA fluorescence

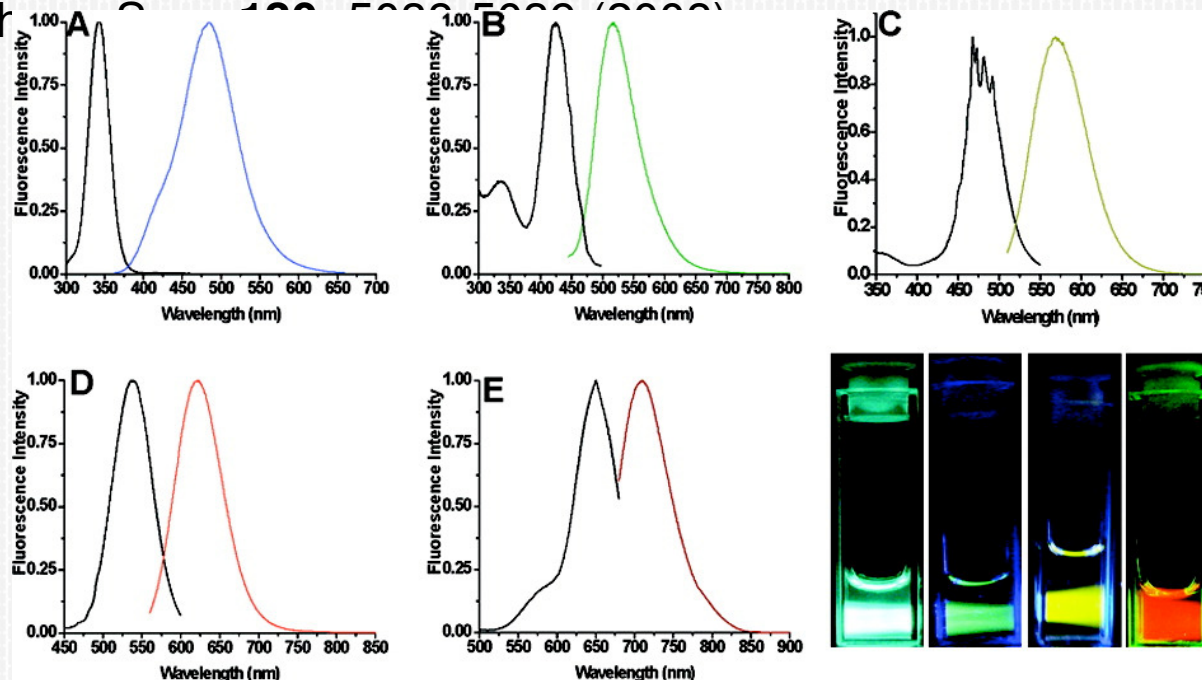
Oligonucleotide-Stabilized Ag-nanocluster Fluorophores

C.I. Richards, S. Choi, J.Hsiang, Y.Antoku, T. Vosch, A. Bongiorno, Y. Tzeng Robert M. Dickson

large random sample
of 12 base sequences

emission ranging from
blue to NIR

Quantum Yields <34%

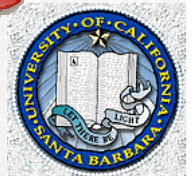
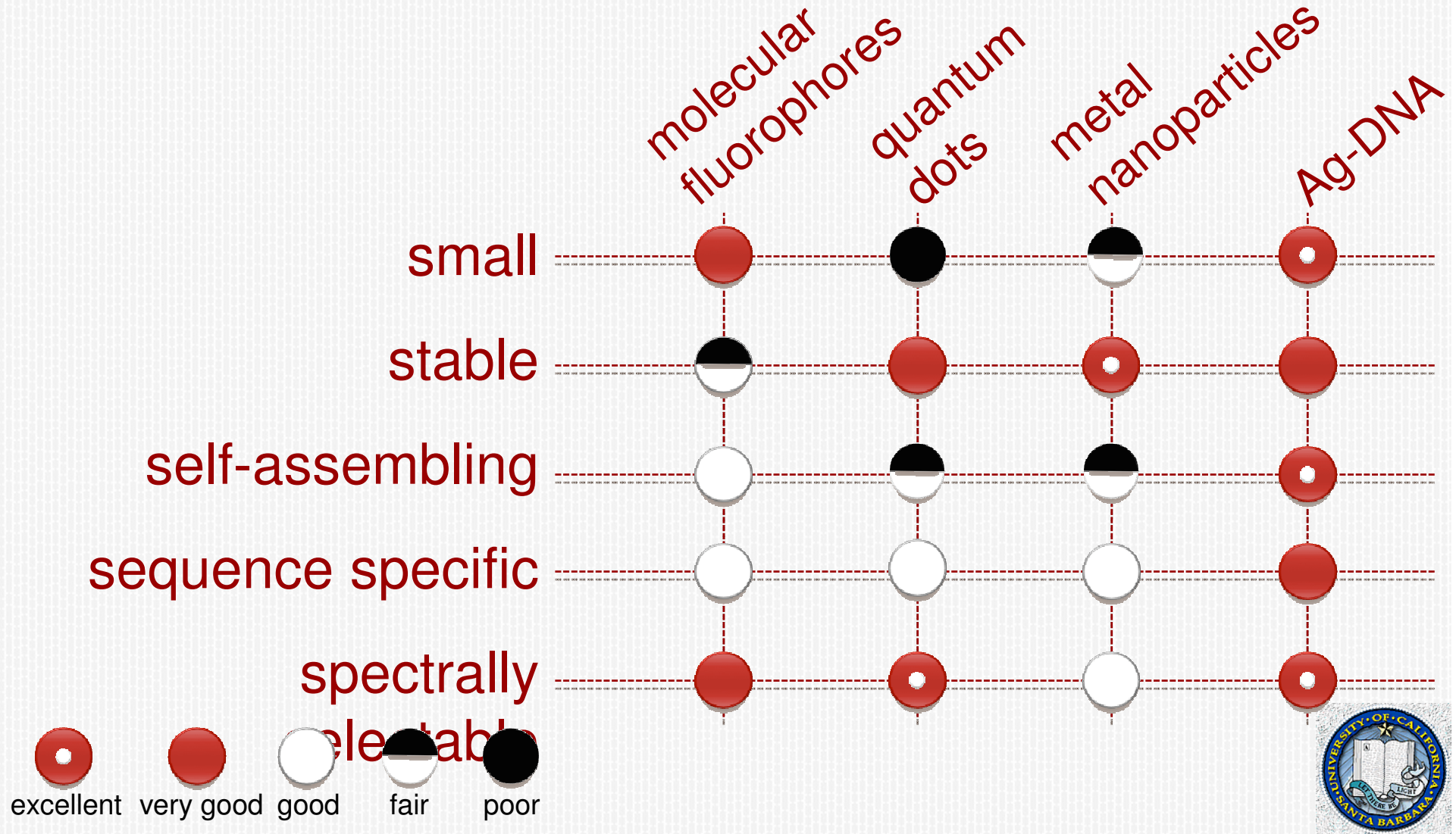


Potential for optimizing QY, chemical stability, photostability



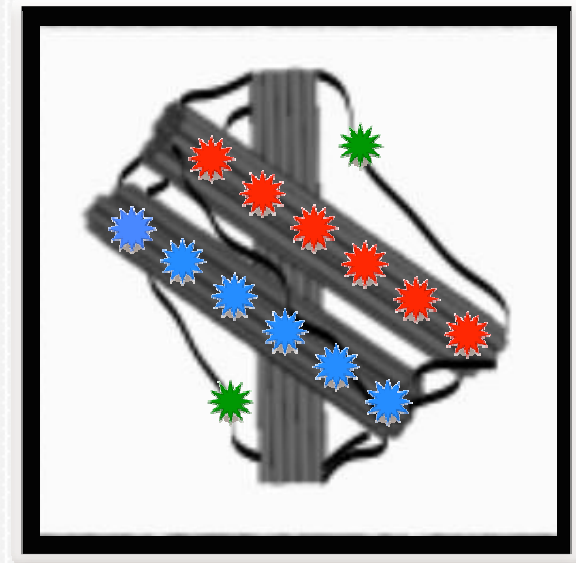
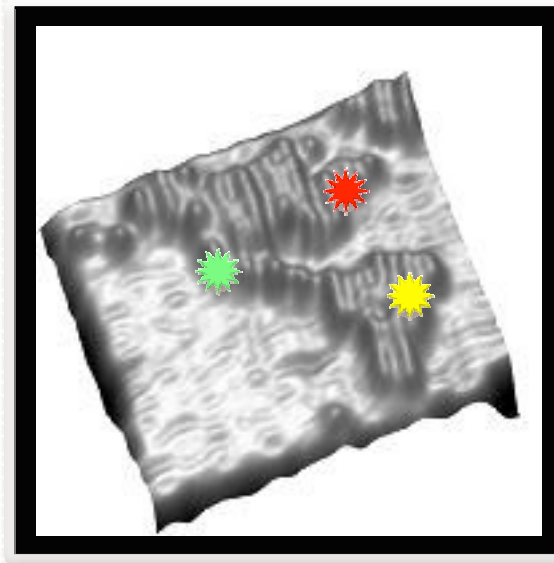
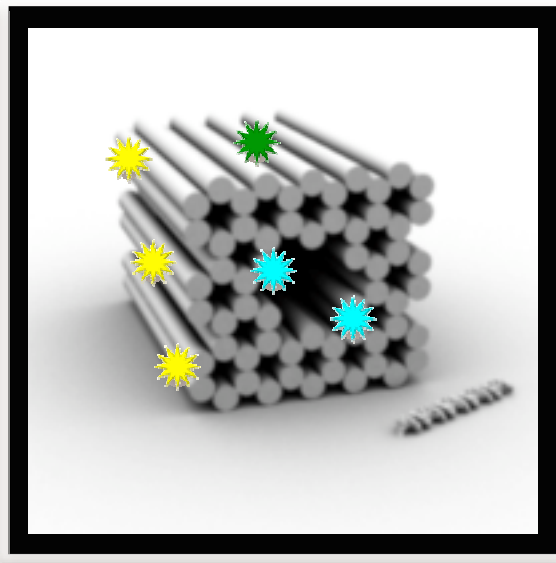
DNA nanotechnology – optical coupling

✖ the ideal input/output device would be:



VISION

- ✘ use sequence design to place different Ag-DNA fluors with nanometer precision on DNA nano-structures in a single synthesis step.



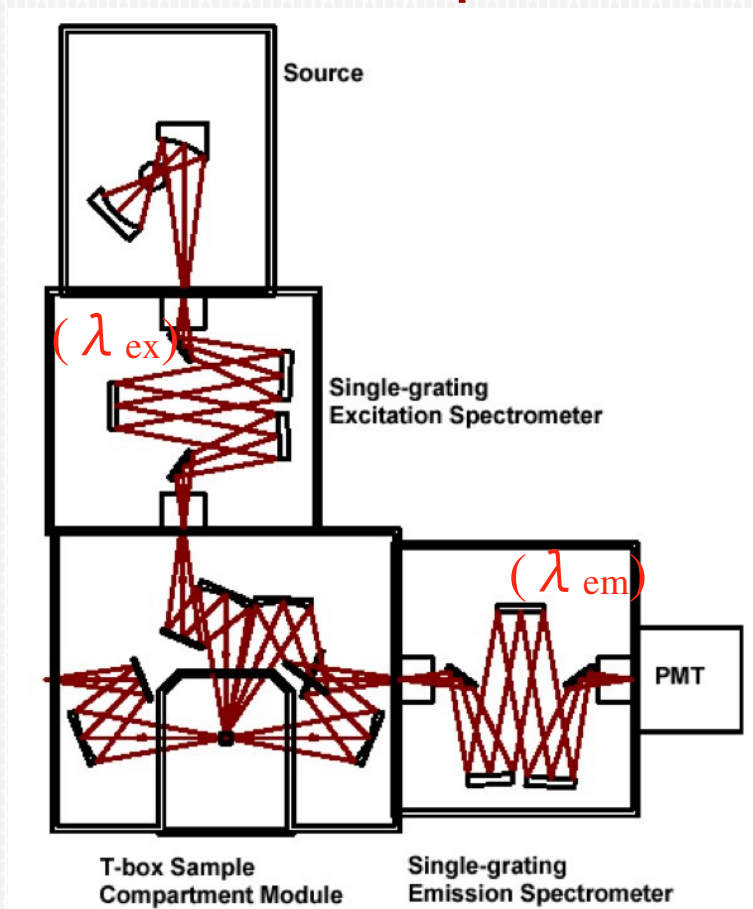
How to get from here to there?

- ✗ understand **fluorescence** = $f(\text{sequence})$
 - + *does dsDNA/2° structure yield, alter or prevent fluorescence?*
- ✗ optimize synthesis
 - + *chemical yield limits complexity of final assembly*
- ✗ develop techniques
 - + *for handling, storing, determining structure*
- ✗ demonstrate compatibility
 - + *is TAE+10mM Mg⁺⁺ inert?*
- ✗ demonstrate utility
 - + *can Ag-DNA fluorescence report on local environmental changes?*

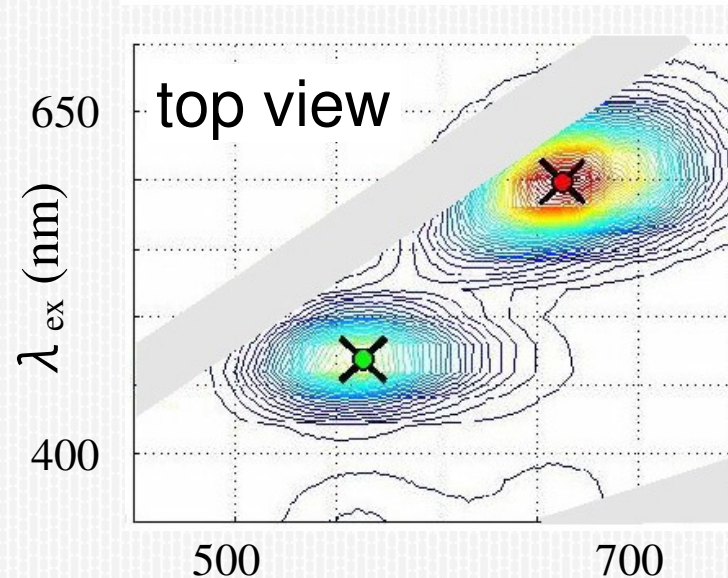
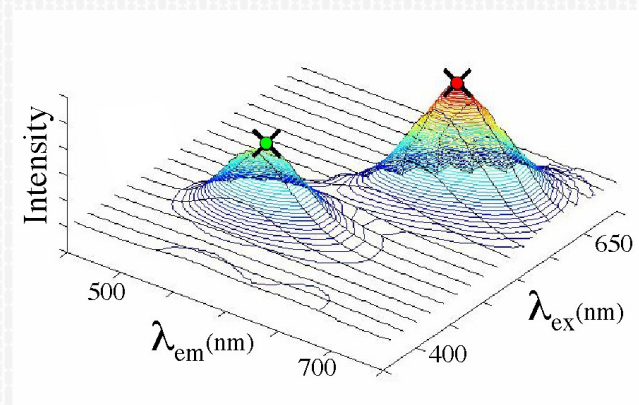


FLUORESCENCE SPECTROSCOPY

Set up

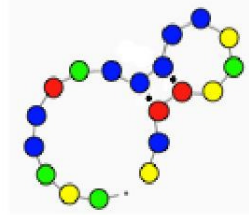


Typical measurement

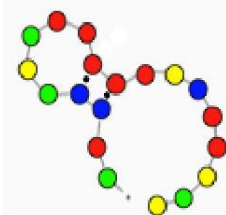


FLUORESCENCE REQUIRES ssDNA

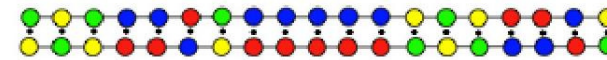
A = yellow
C = blue
G = red
T = green



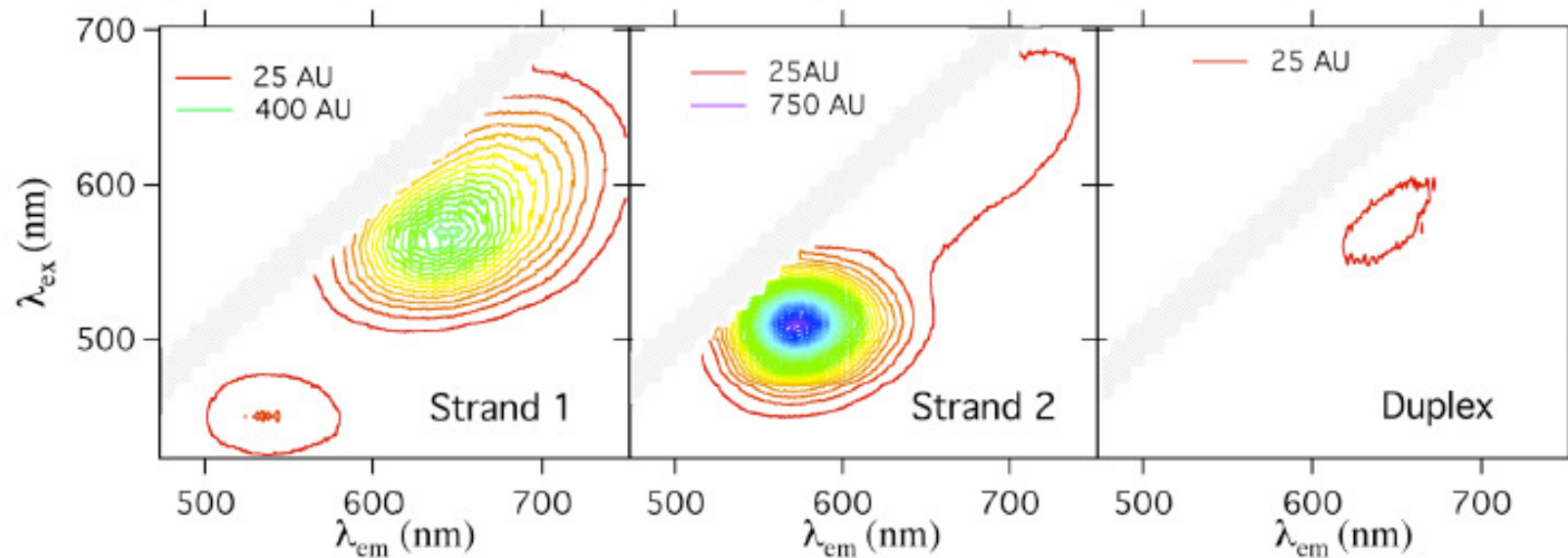
Strand 1
 $T_M = 25\text{ °C}$



Strand 2
 $T_M = 36\text{ °C}$



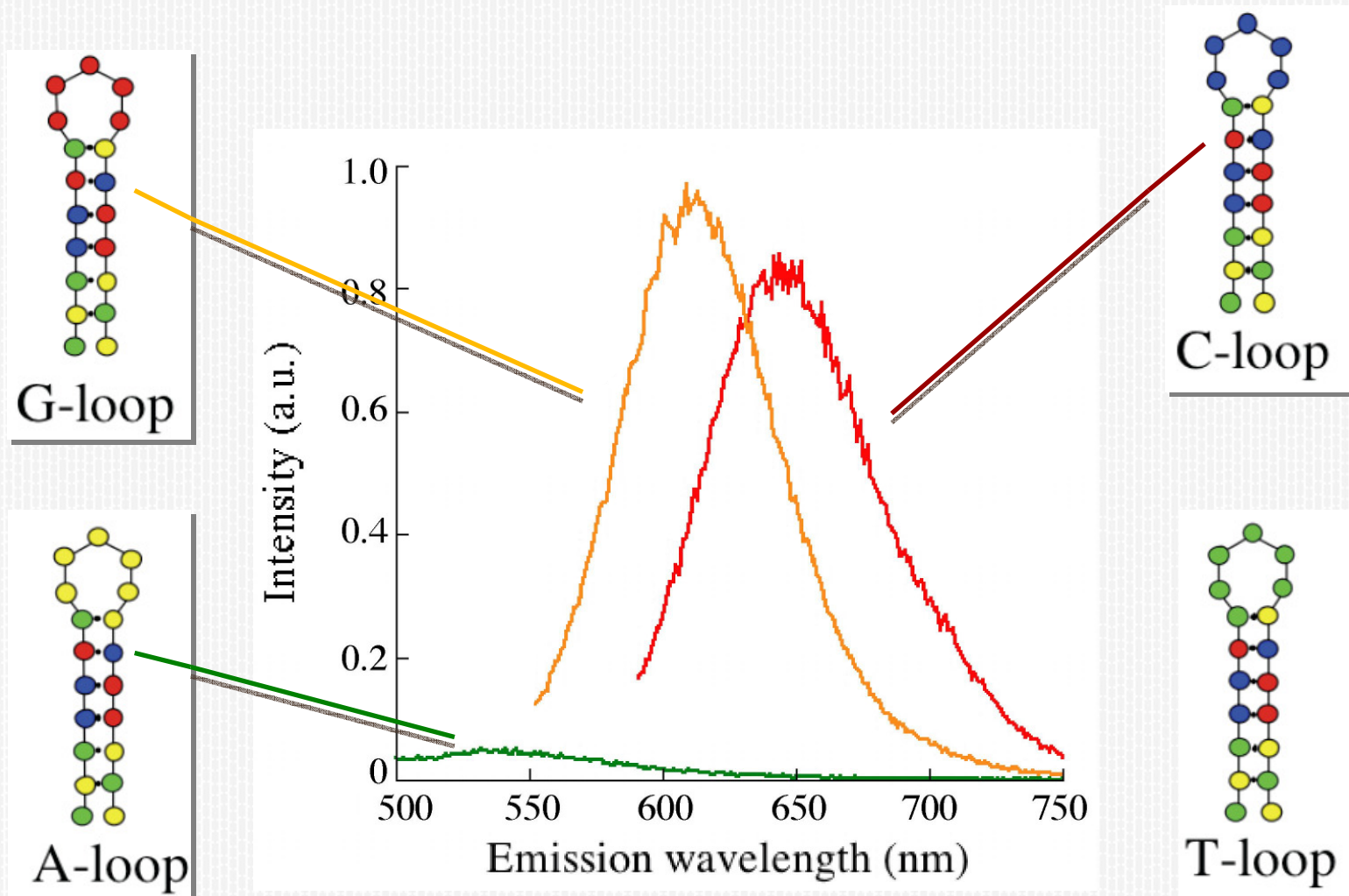
Duplex
 $T_M = 62\text{ °C}$



E.G. Gwinn, et al. **Advanced Materials**, 20:279-283
(2008)



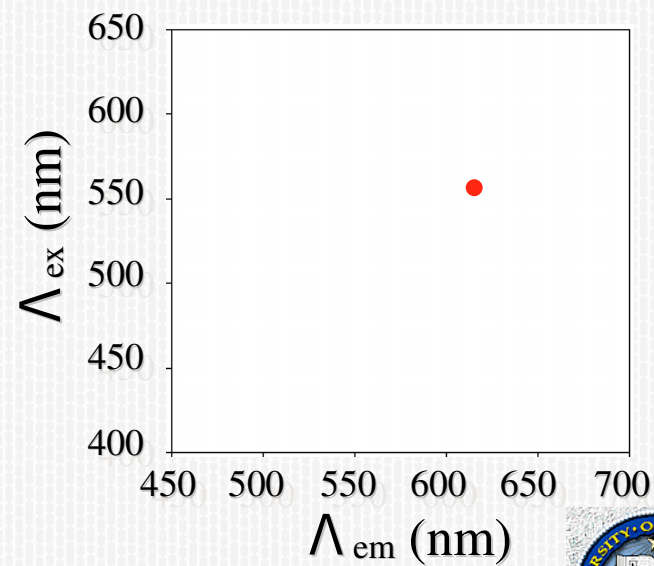
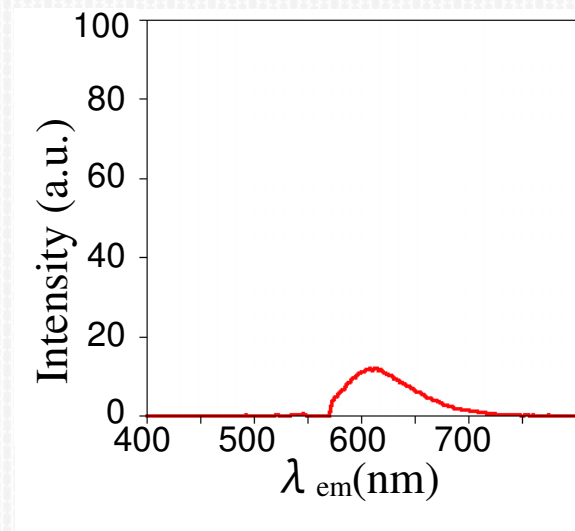
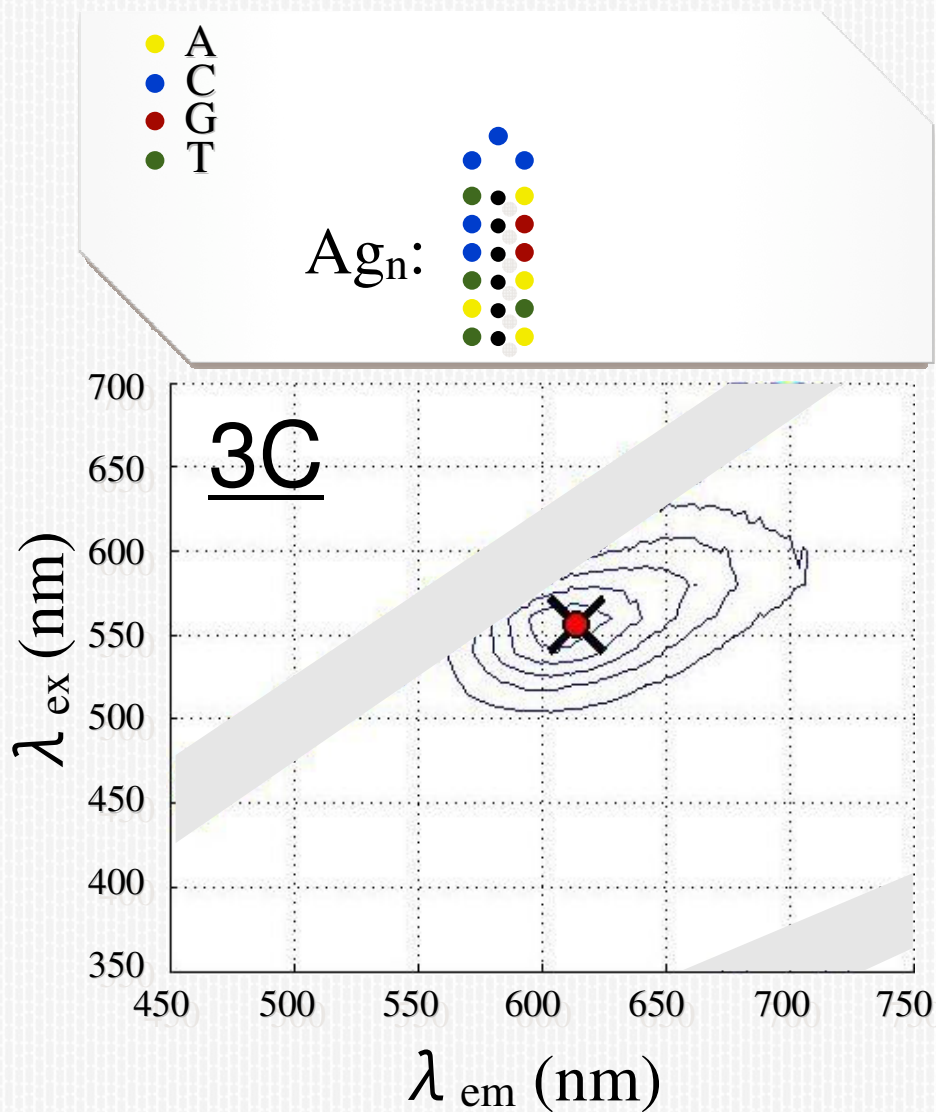
FLUORESCENCE IS Nucleotide Dependant



E.G. Gwinn, et al. **Advanced Materials**, 20:279-283 (2008)



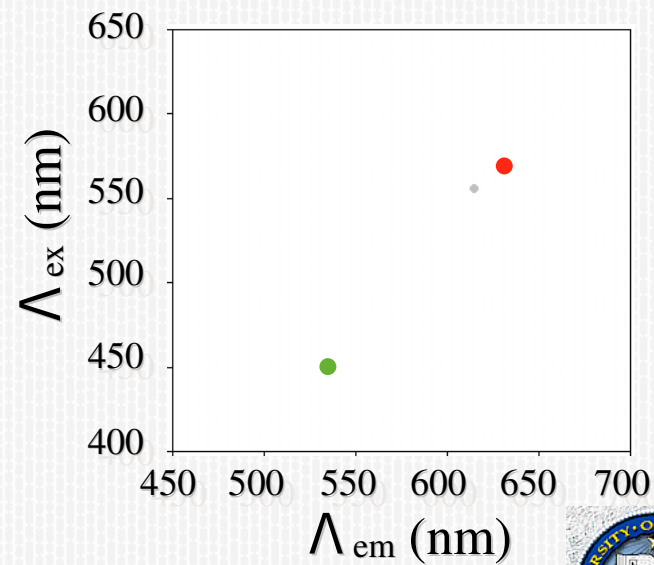
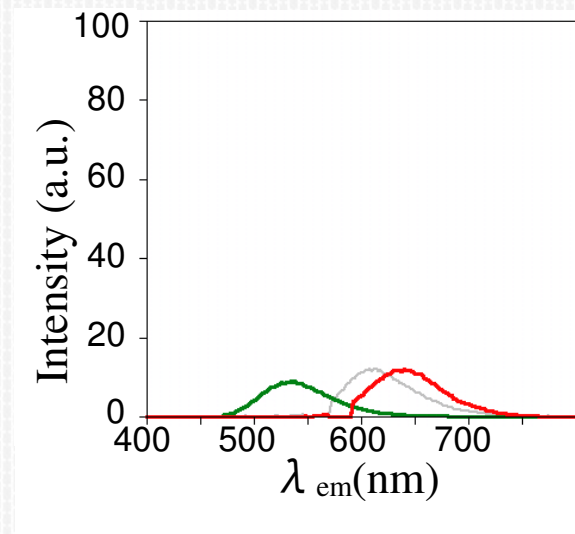
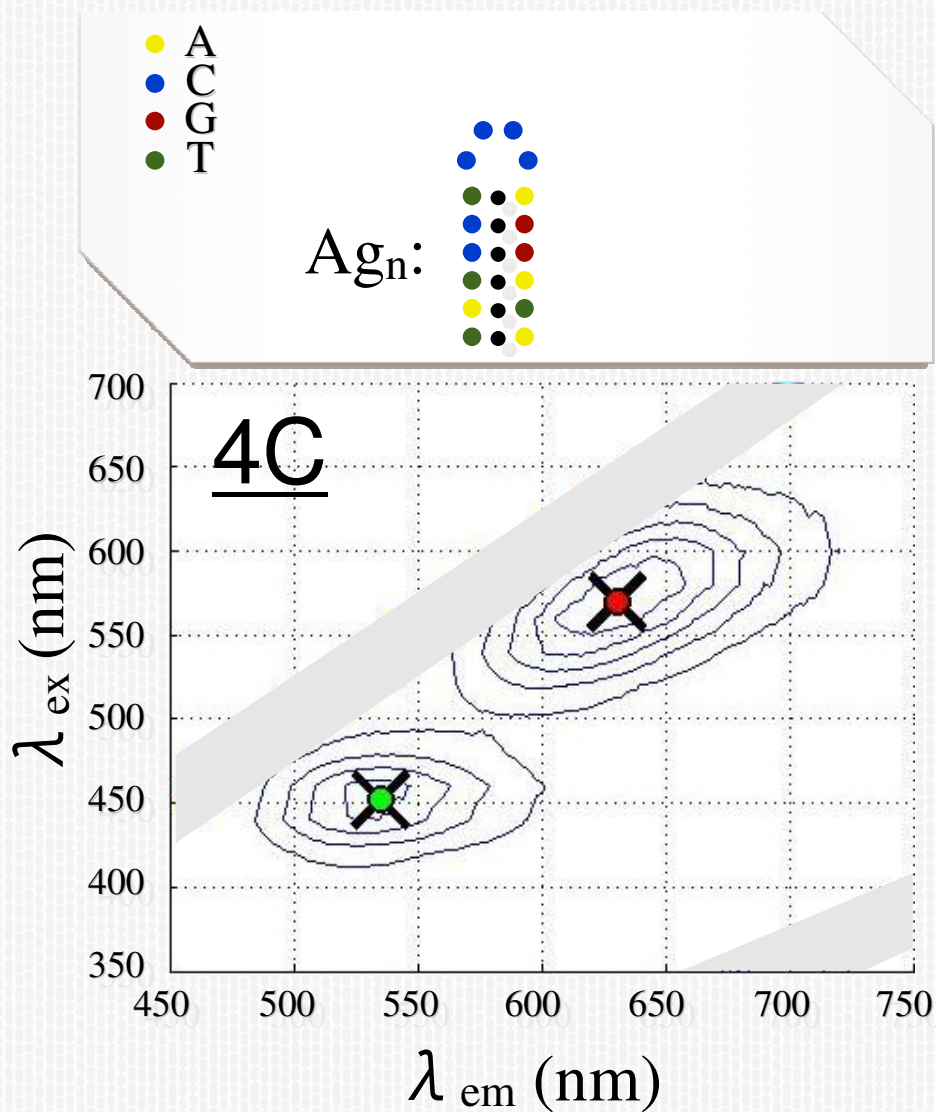
FLUORESCENCE IS Structure Dependant



P.R. O'Neill, et al. **J Phys Chem C**, 113:4229-4233 (2009)



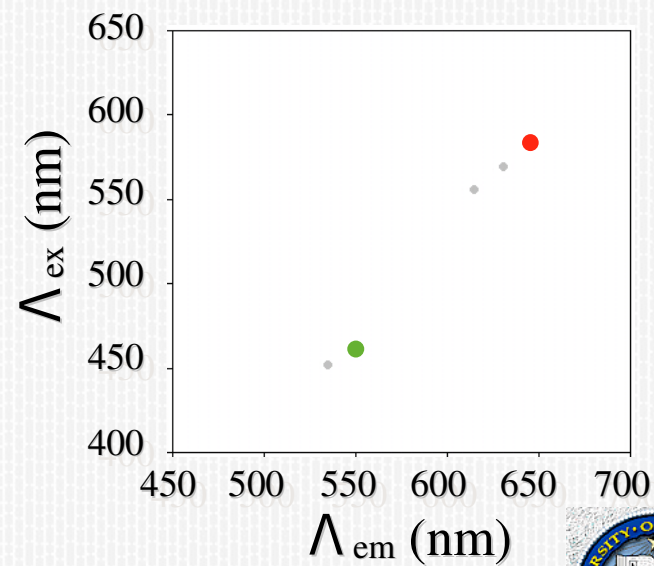
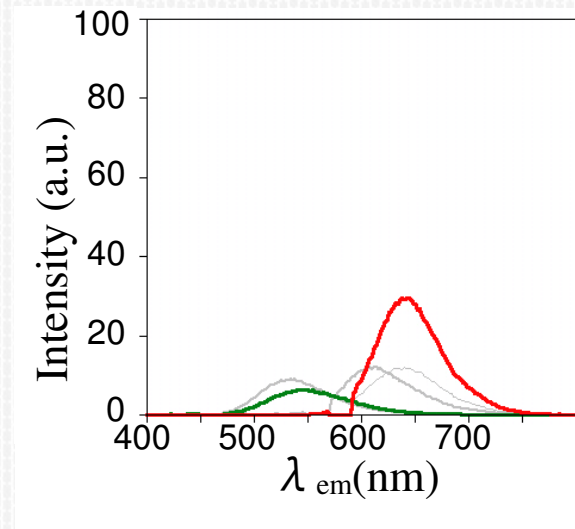
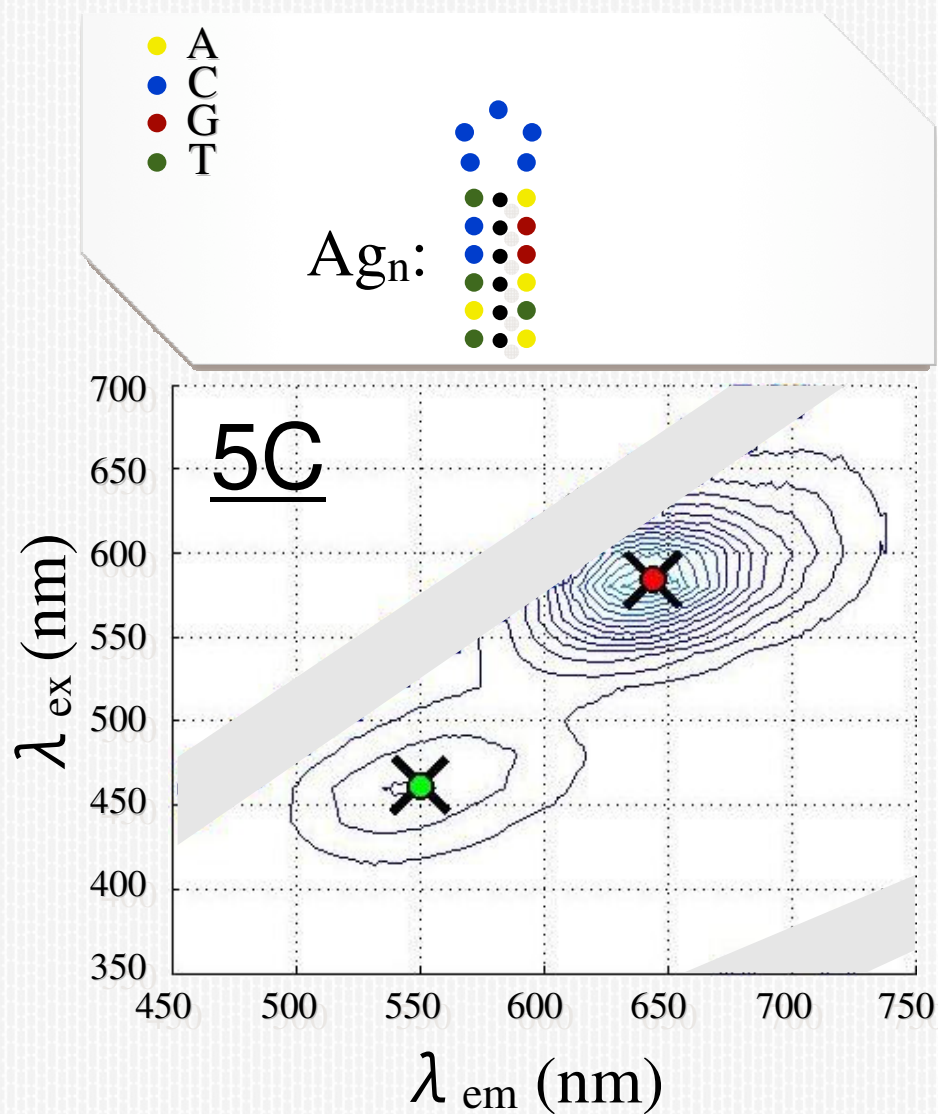
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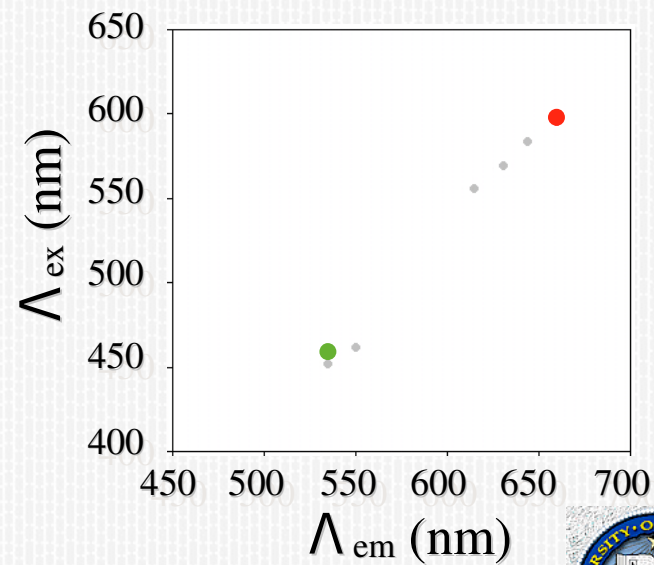
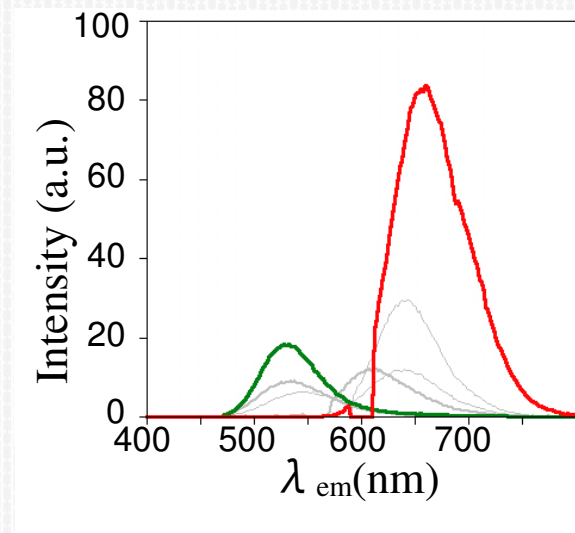
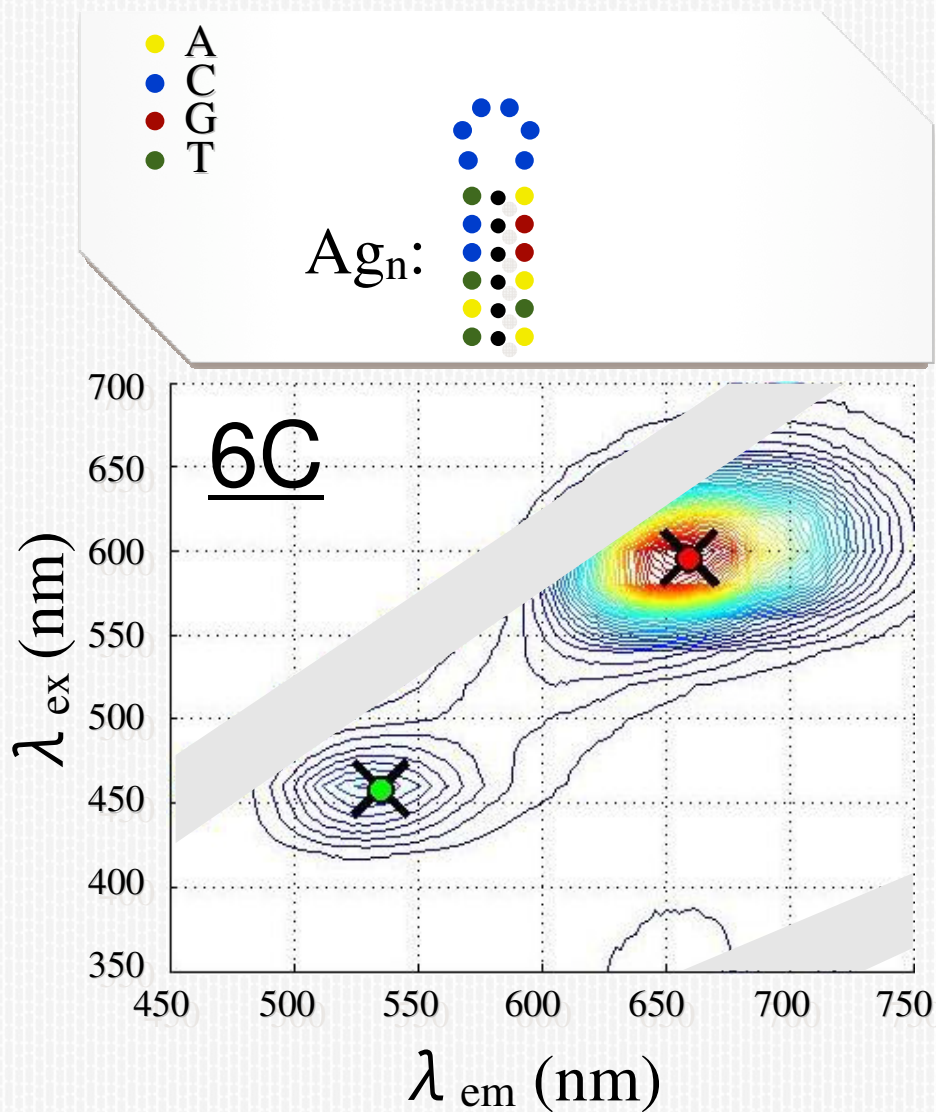
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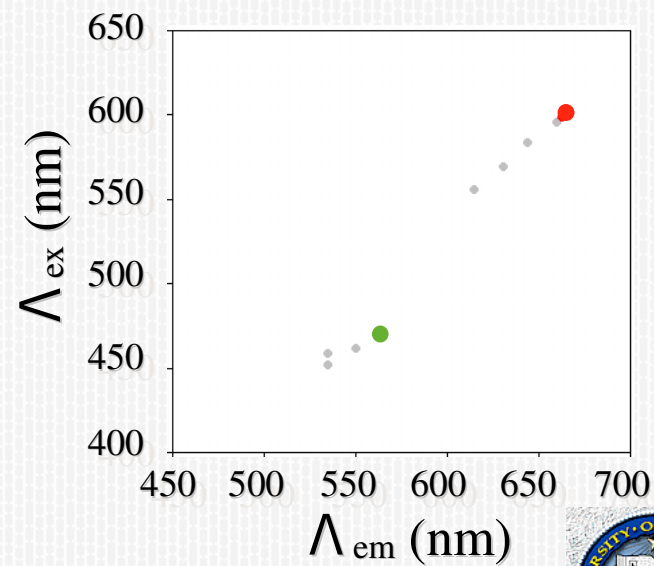
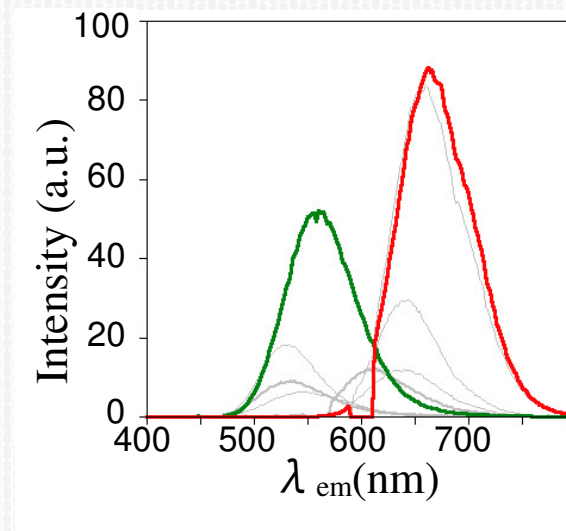
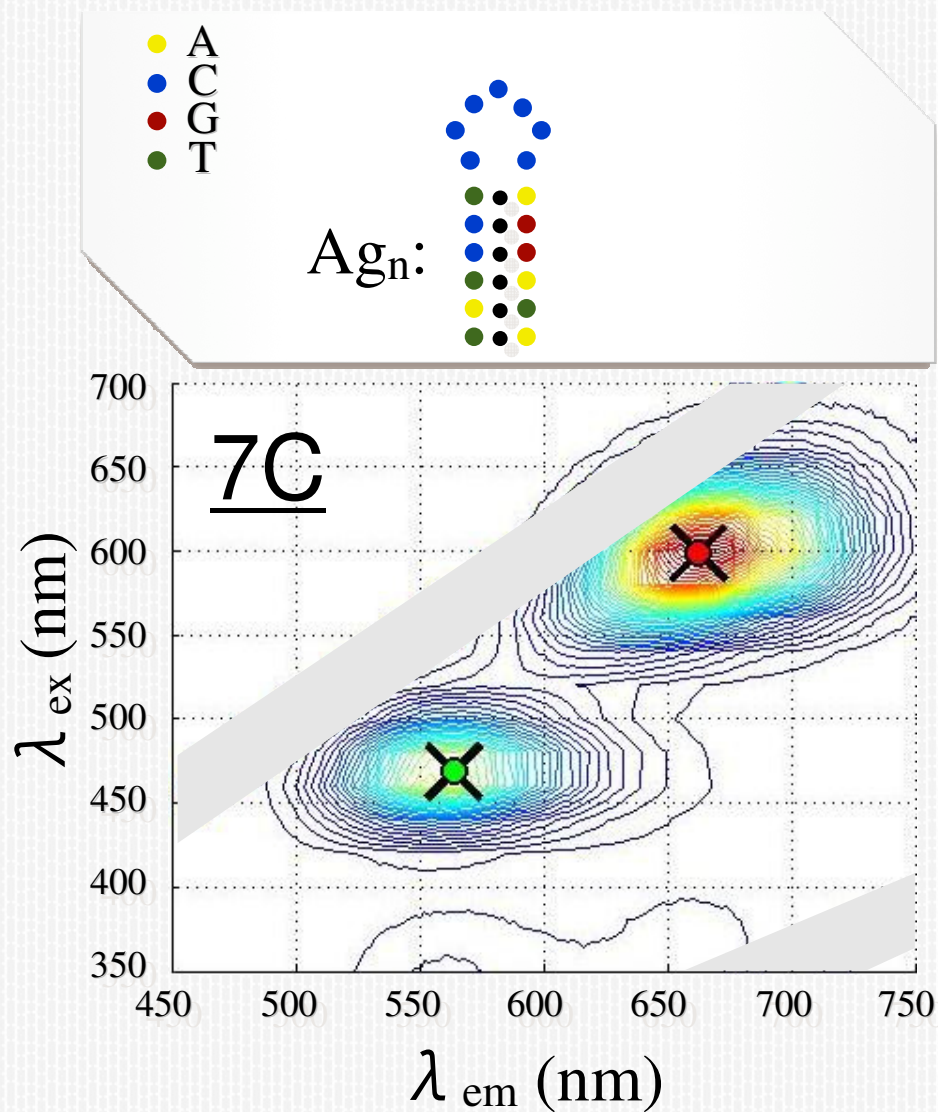
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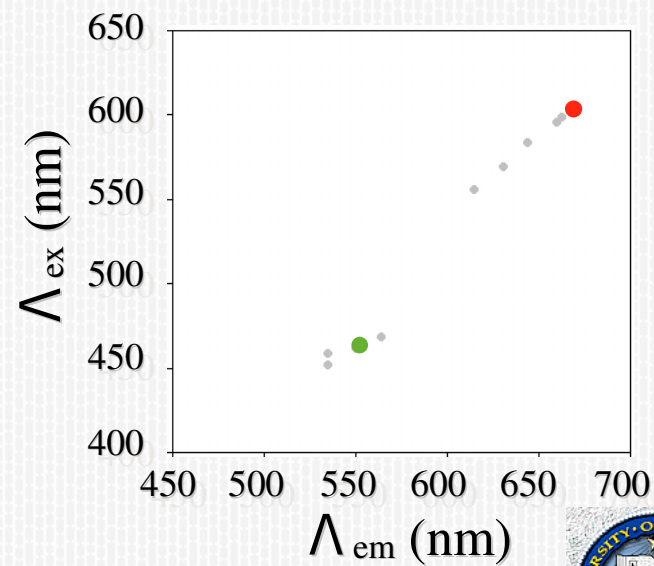
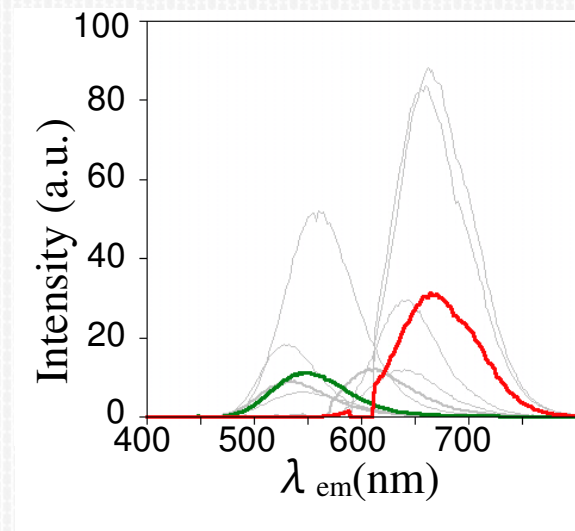
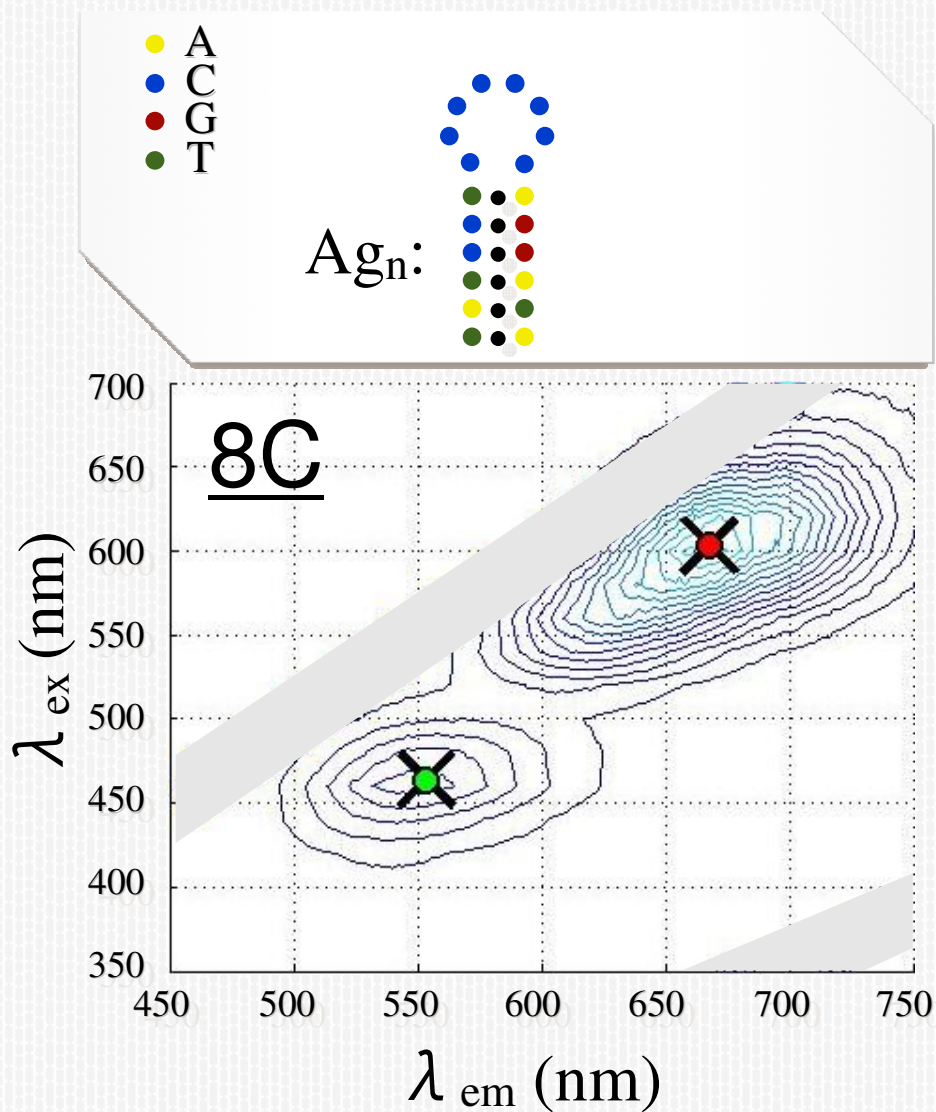
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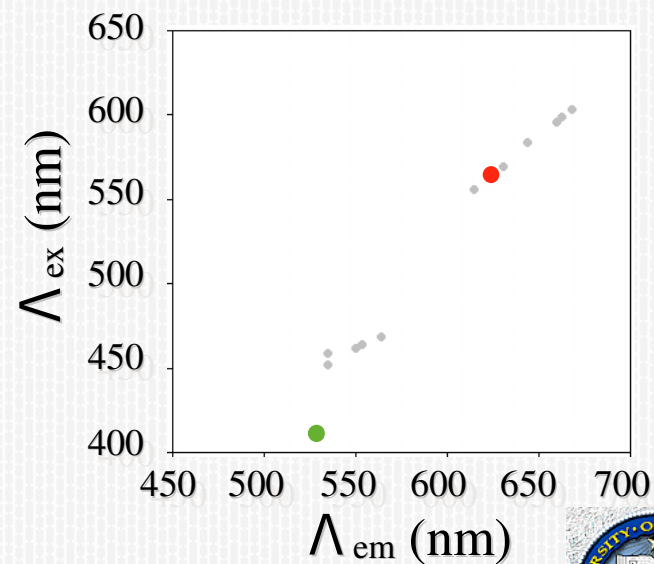
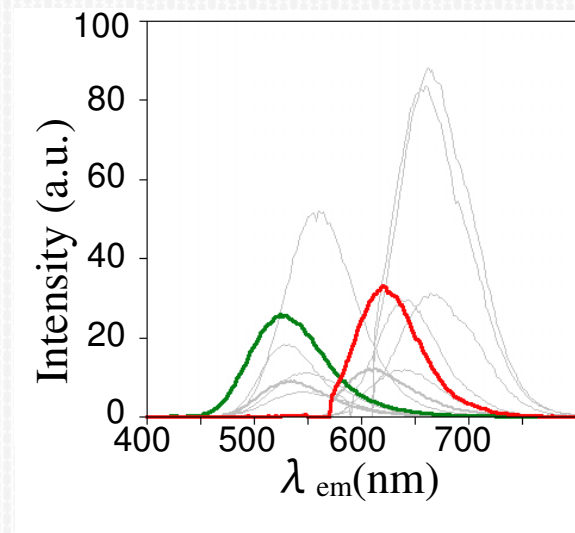
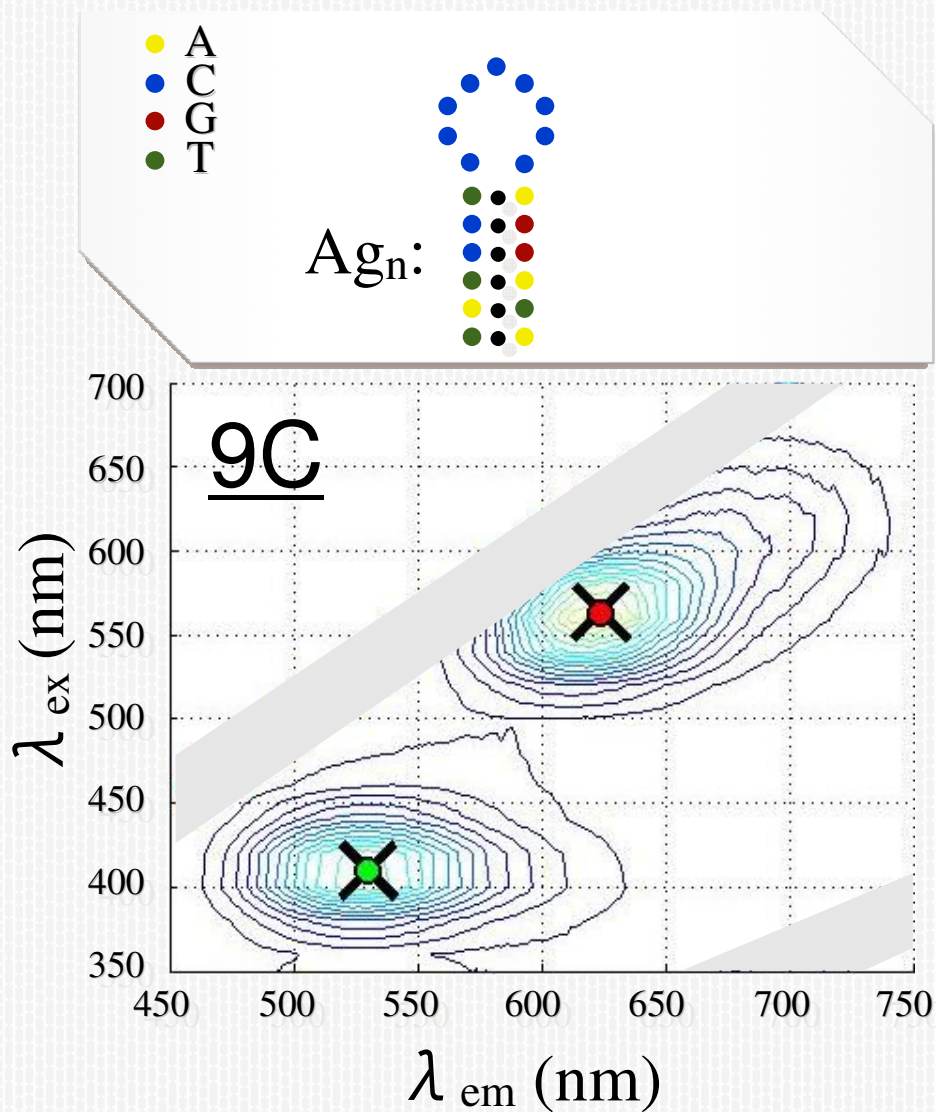
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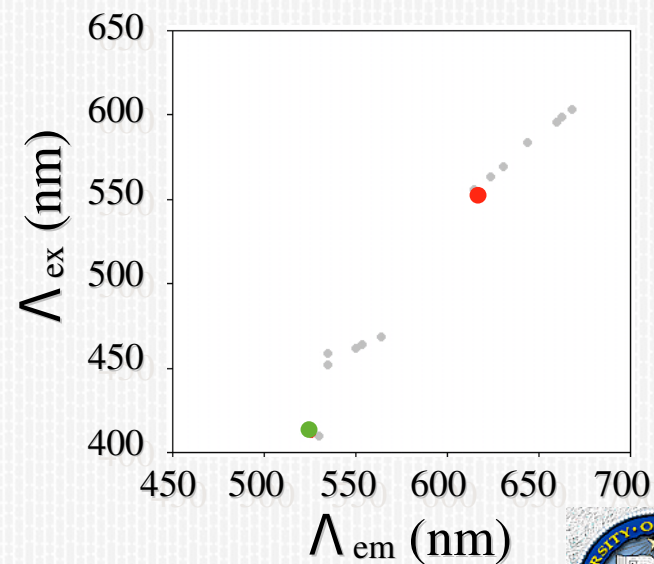
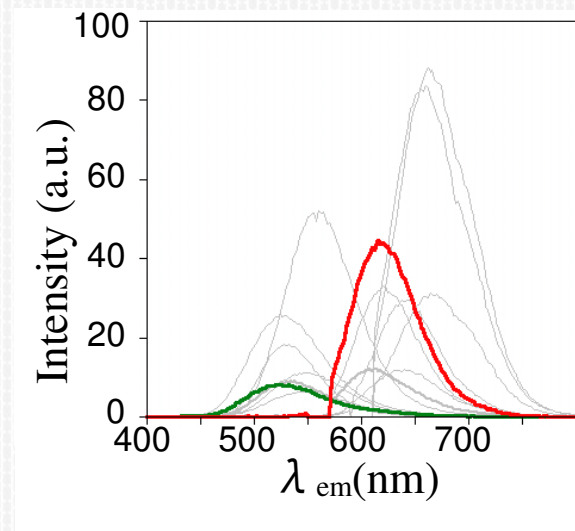
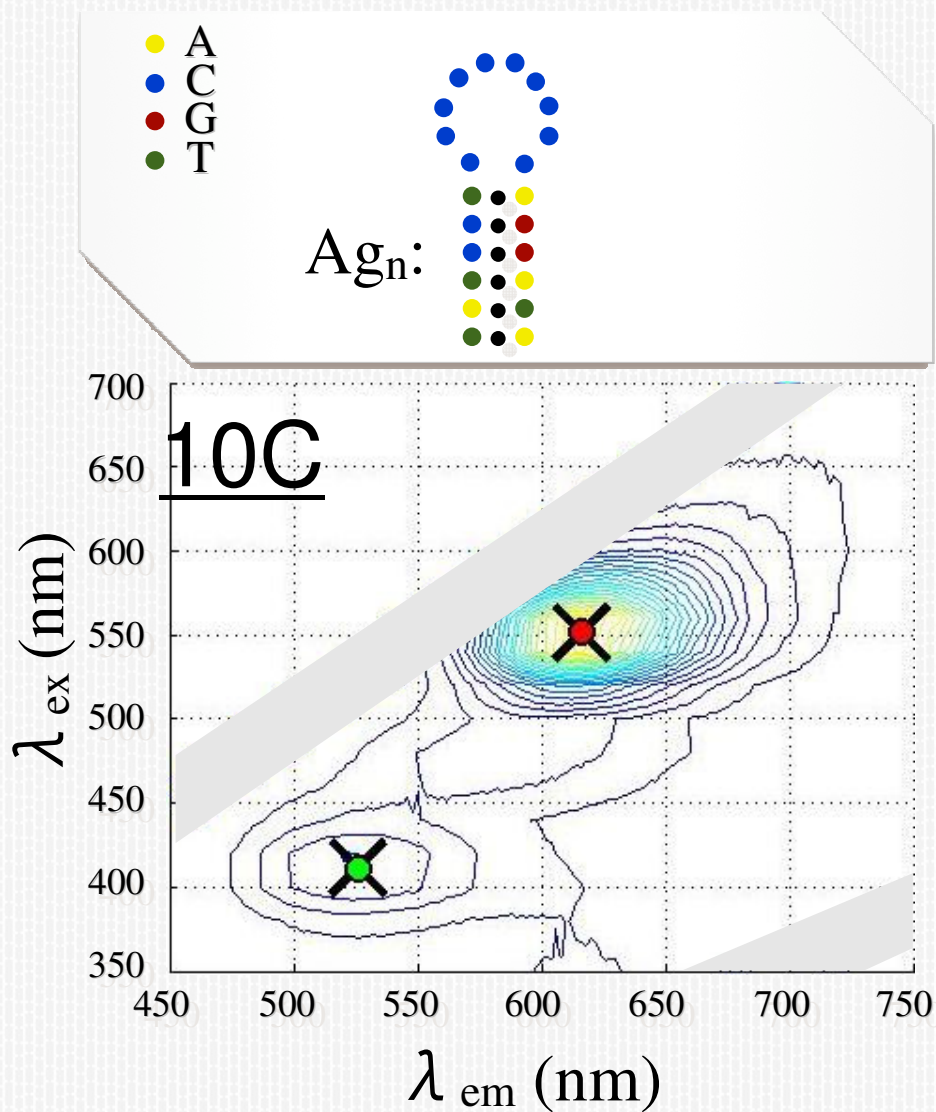
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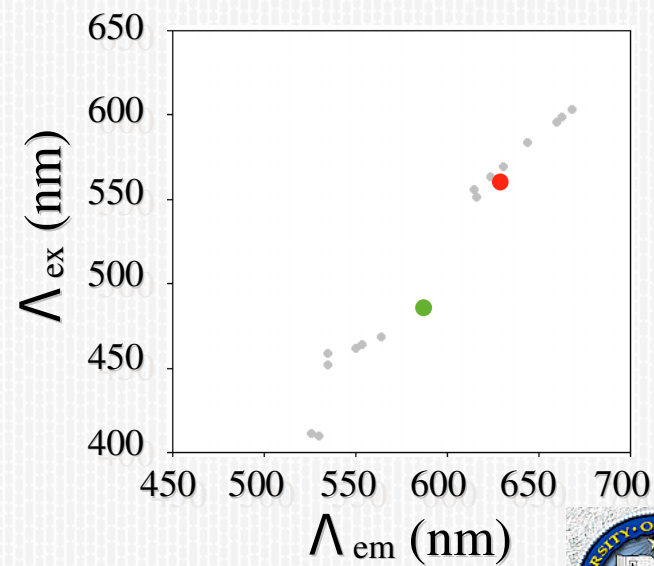
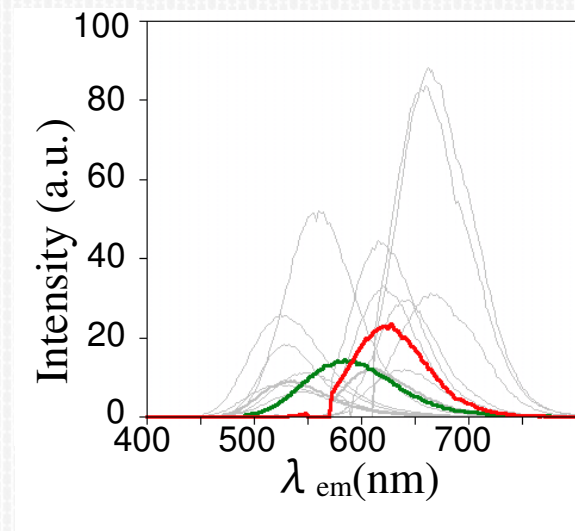
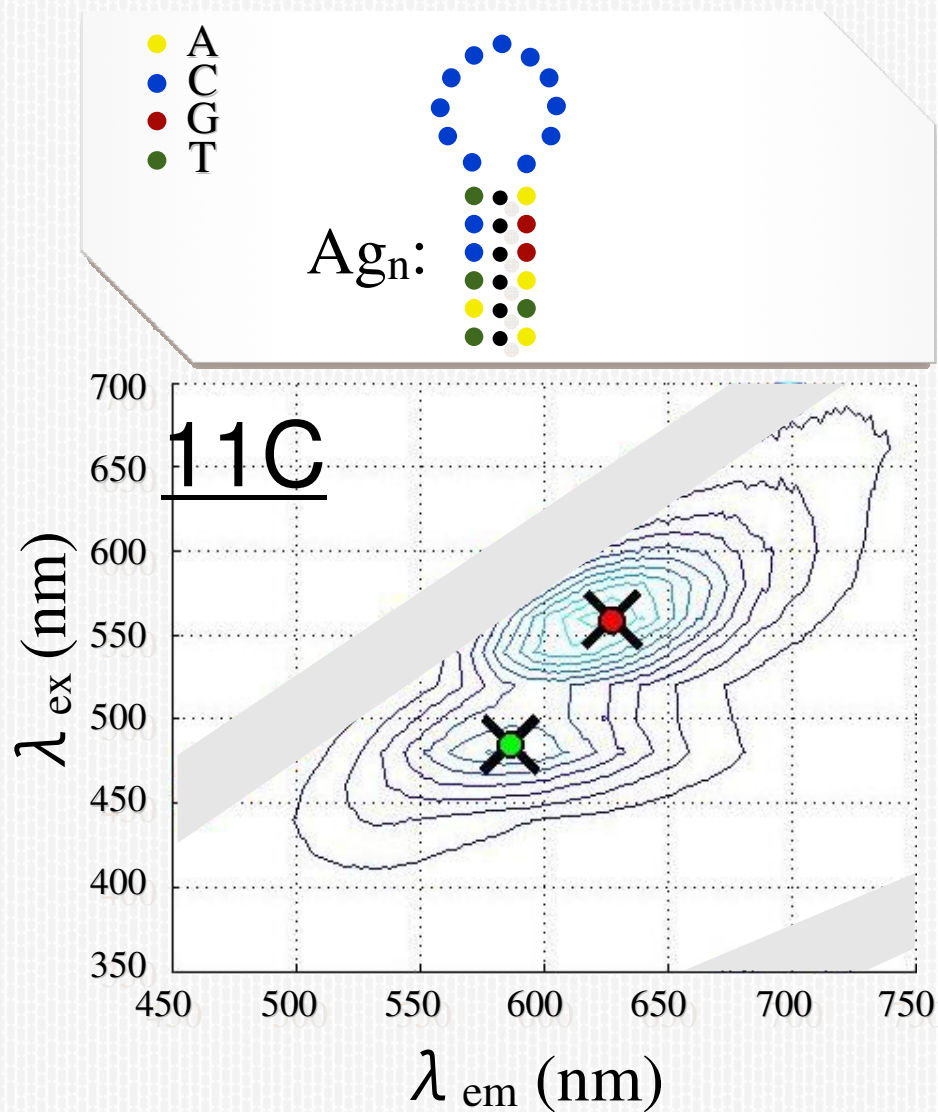
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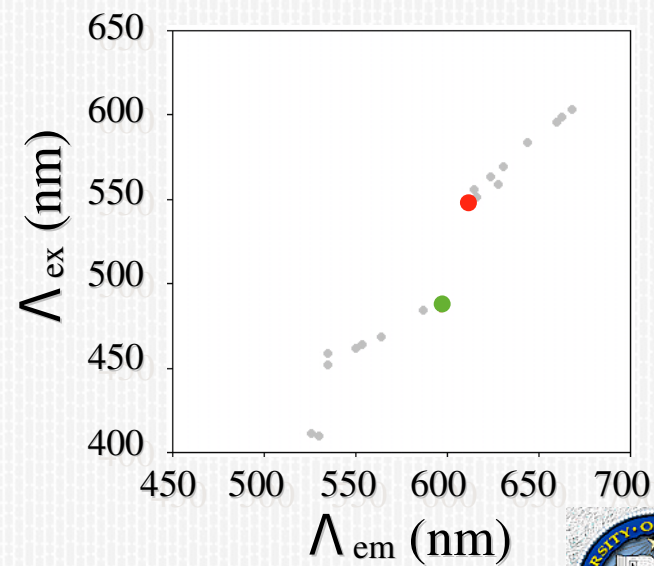
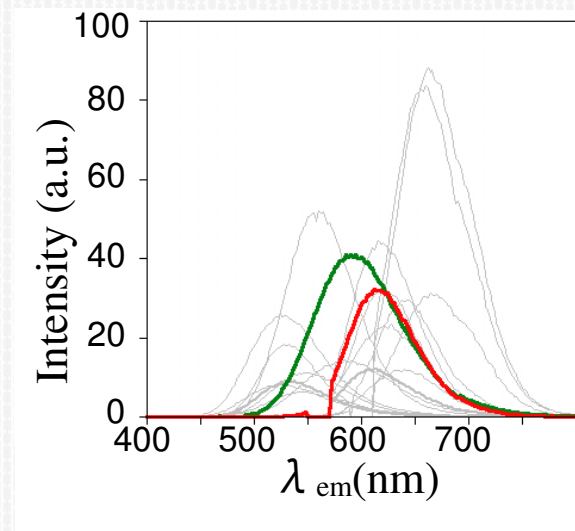
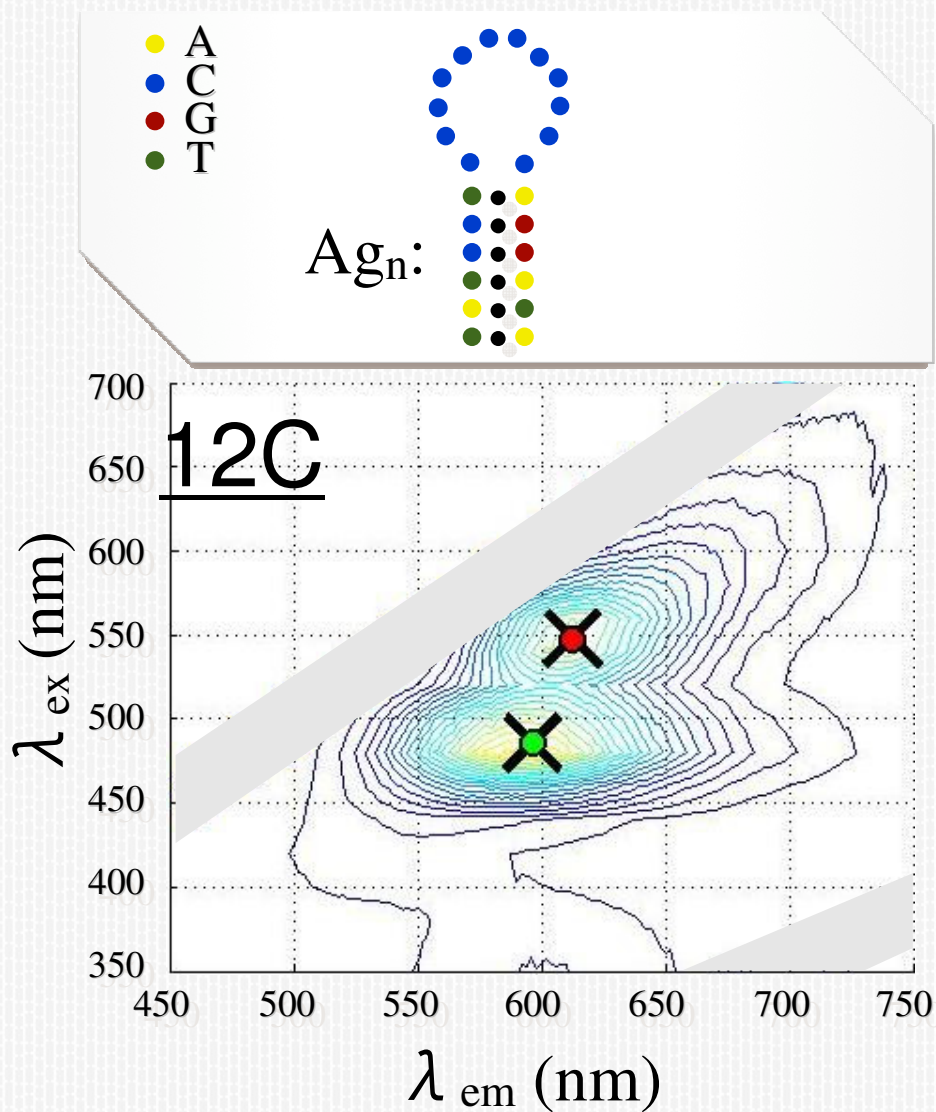
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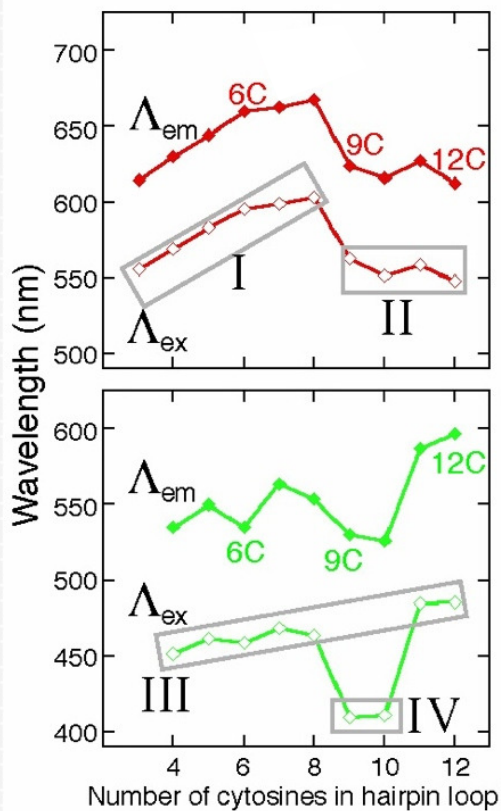
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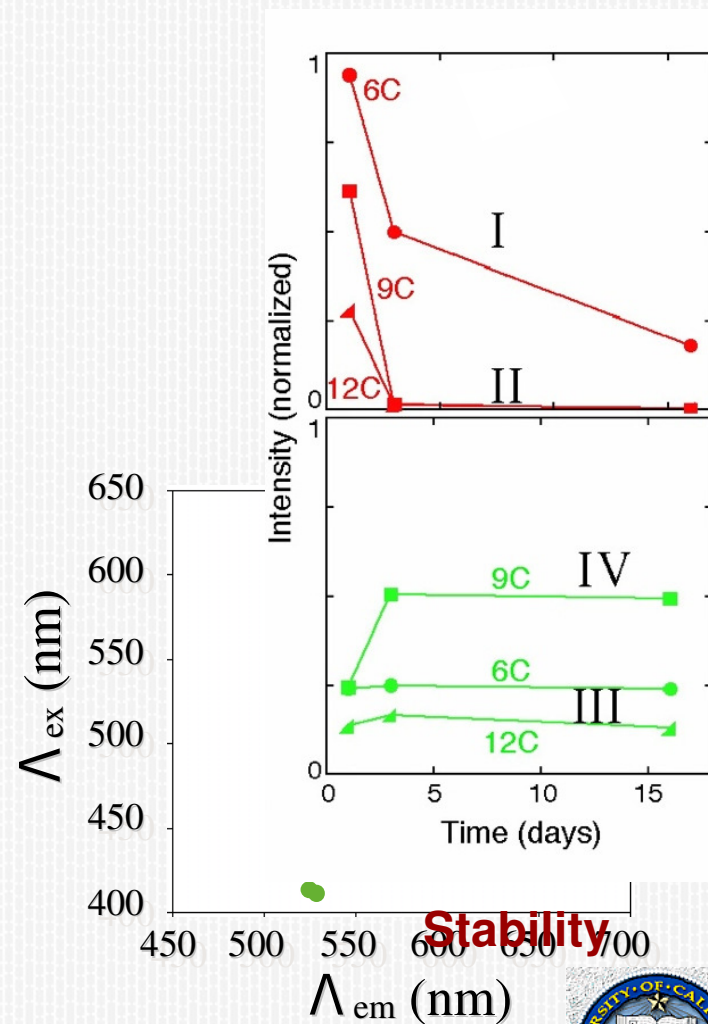
P.R. O'Neill, et al. **J Phys Chem C**, 113:4229-4233 (2009)



Structure study reveals 4 types of Ag-DNA fluors



Spectral Peak

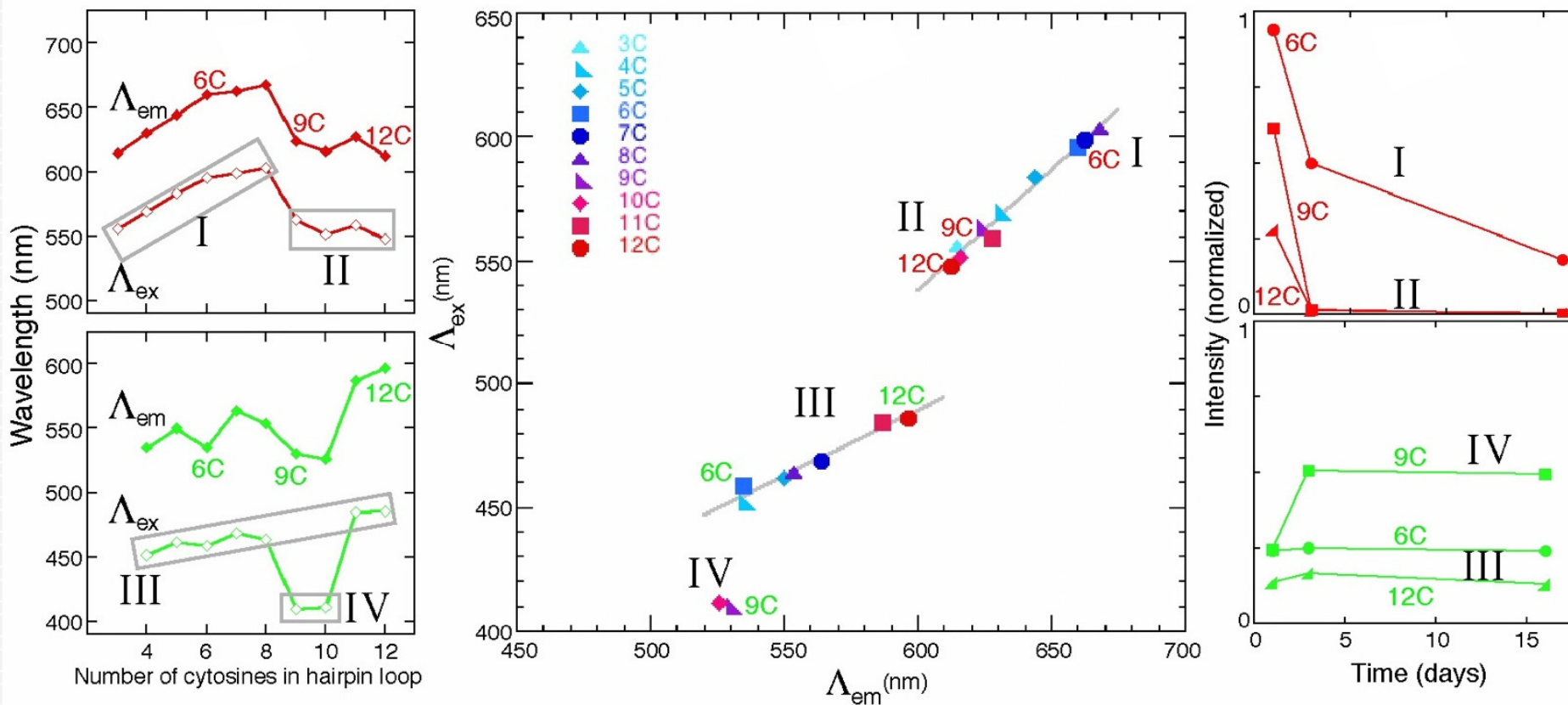


Stability

P.R. O'Neill, et al. *J Phys Chem C*, 113:4229-4233 (2009)



Structure study reveals 4 types of Ag-DNA fluors



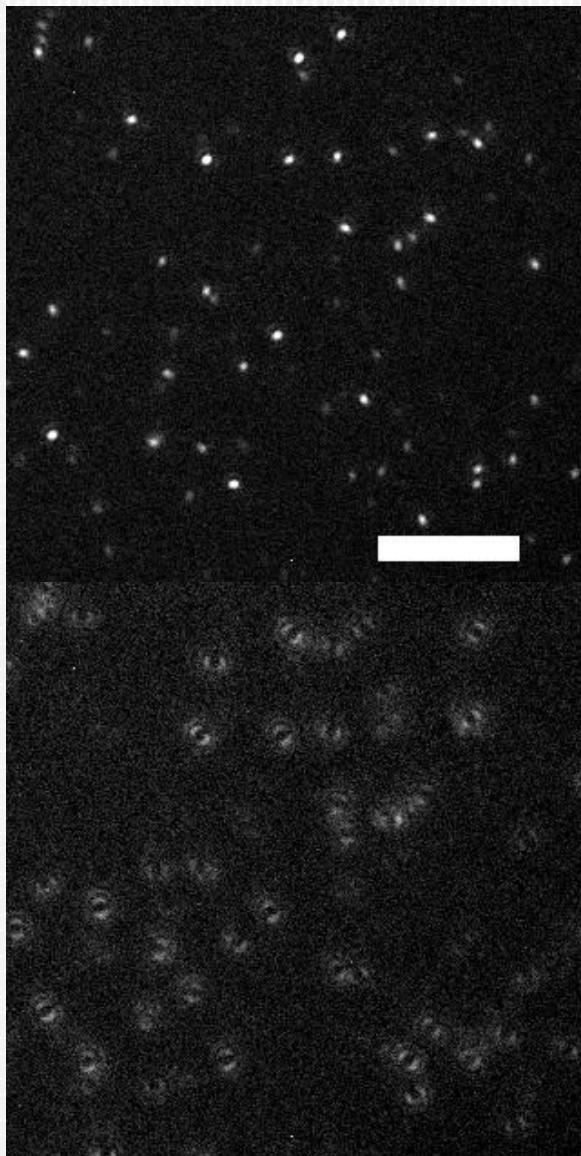
Spectral Peak

Stability

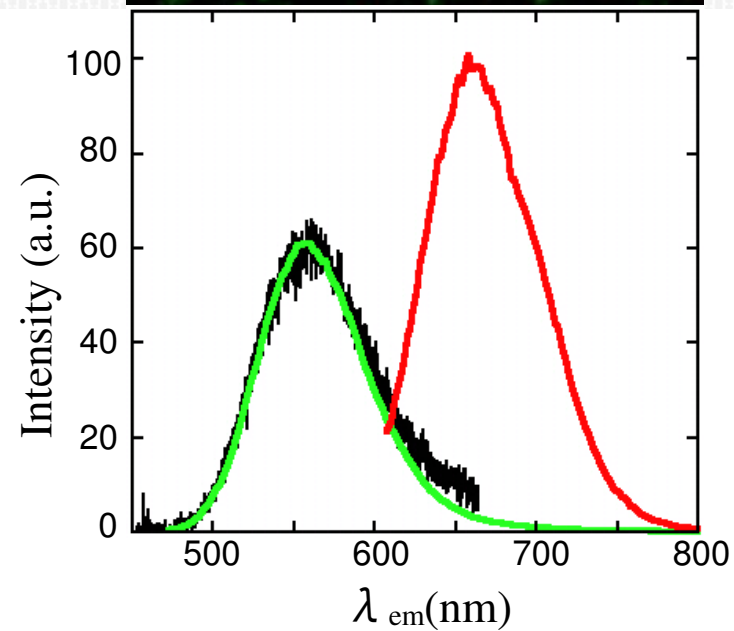
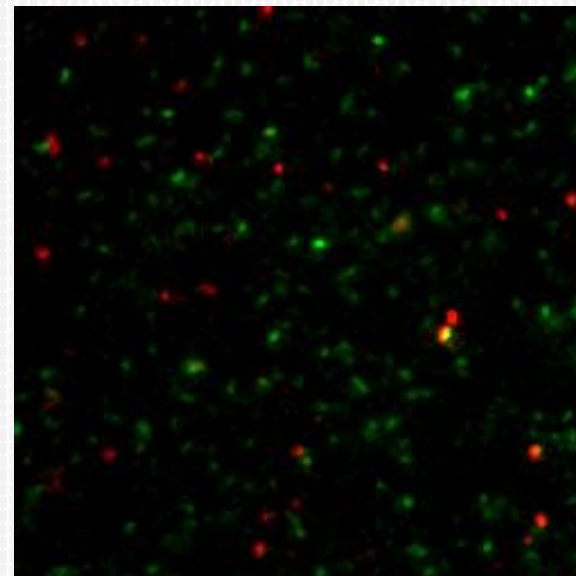
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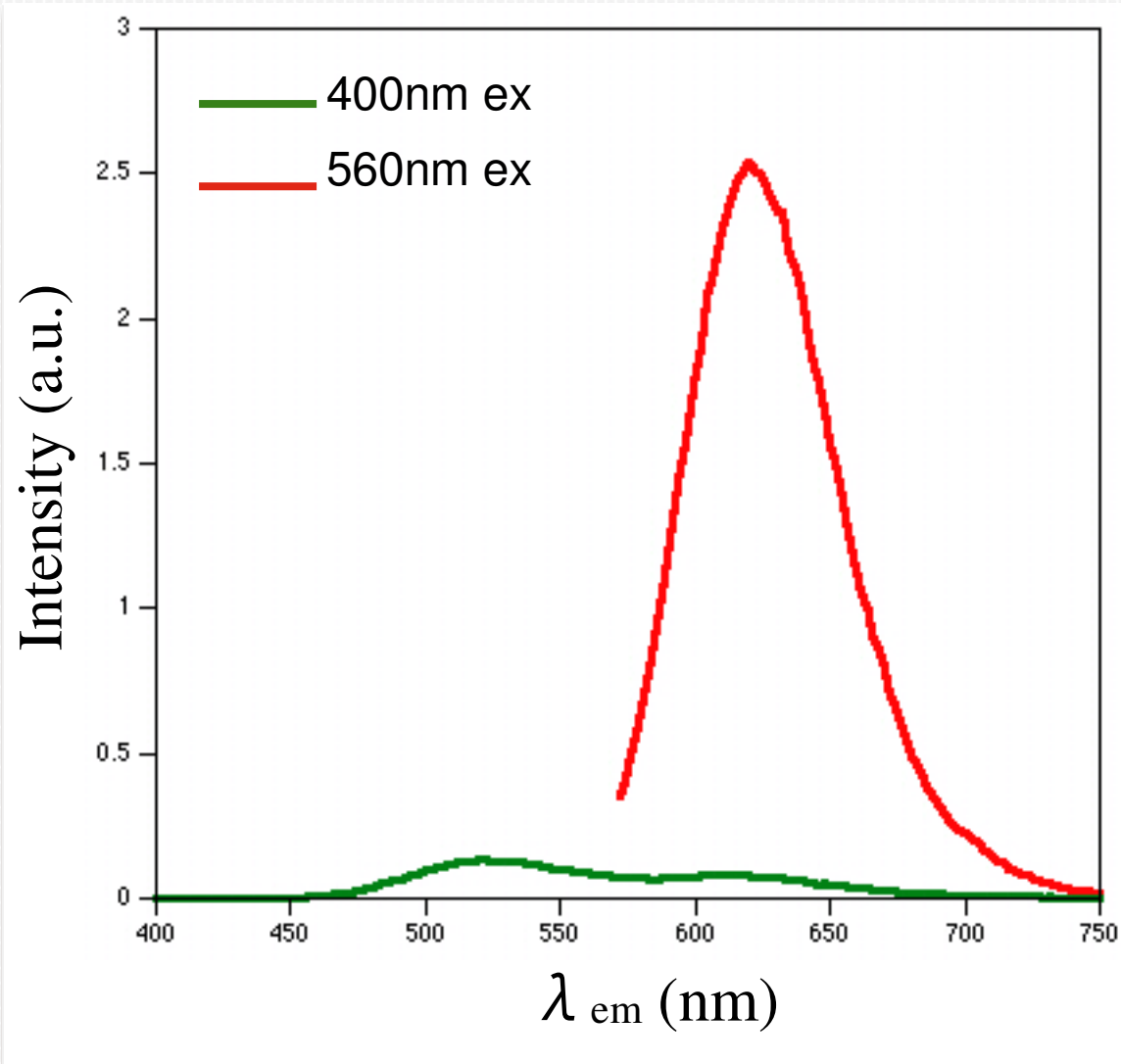
Different STATES or Different SPECIES?



as per: M. Bohmer and J. Enderlein,
J. Opt. Soc. Am. B, 20:554 (2003)



TIME SERIES

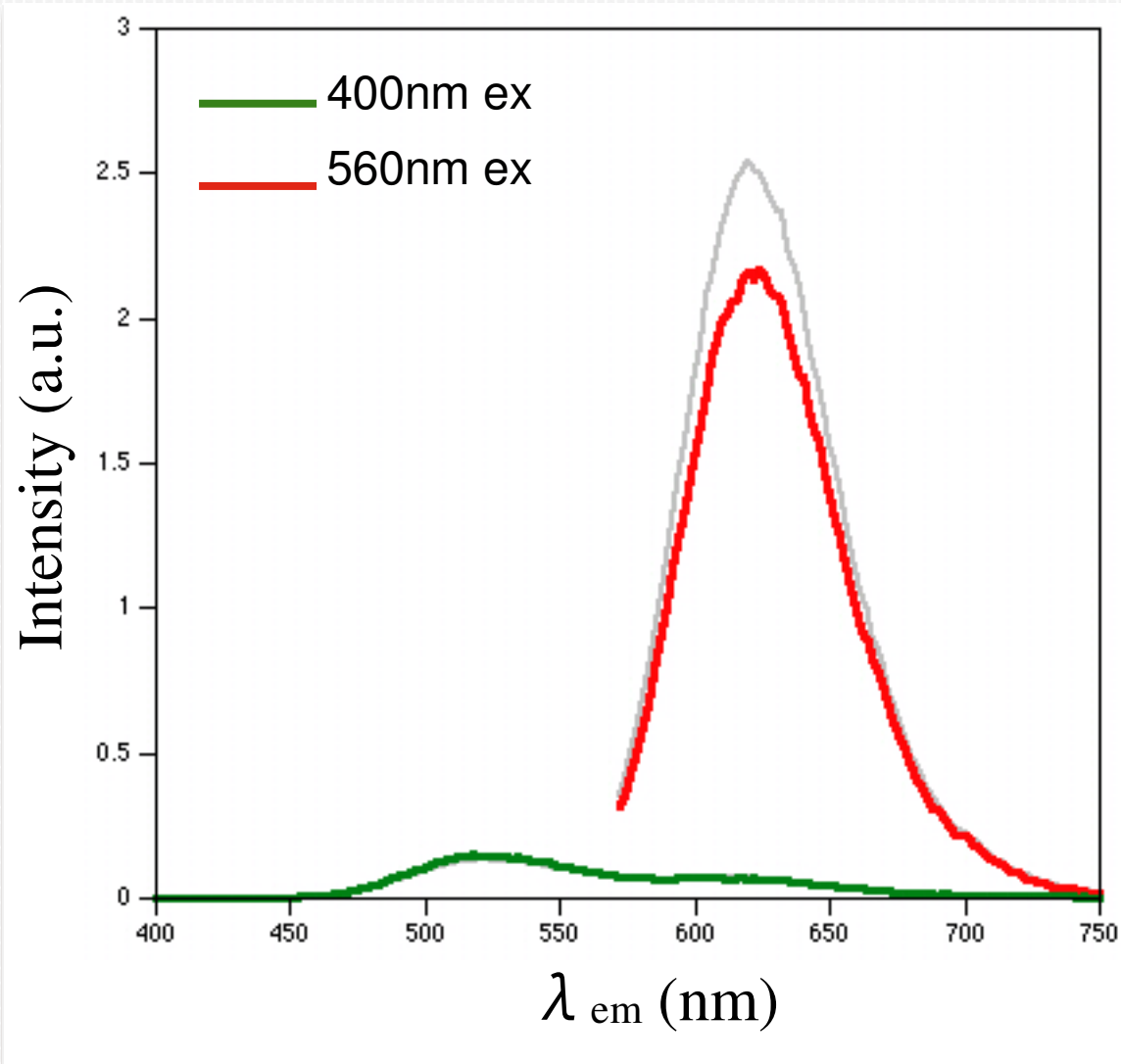


9C-hairpin loop Ag-clusters

time after reduction:
0h05



TIME SERIES

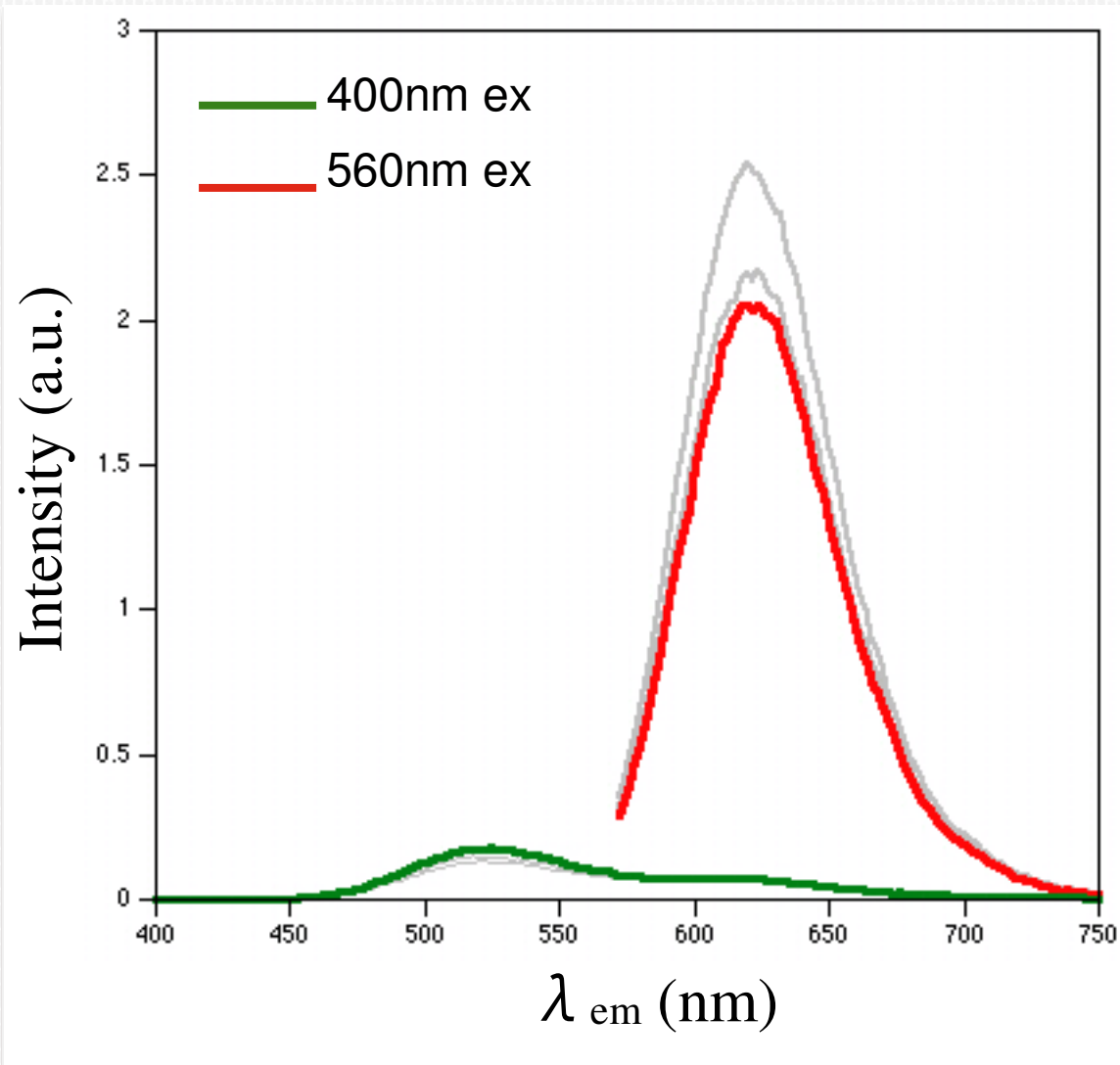


9C-hairpin loop Ag-clusters

time after reduction:
0h30



TIME SERIES

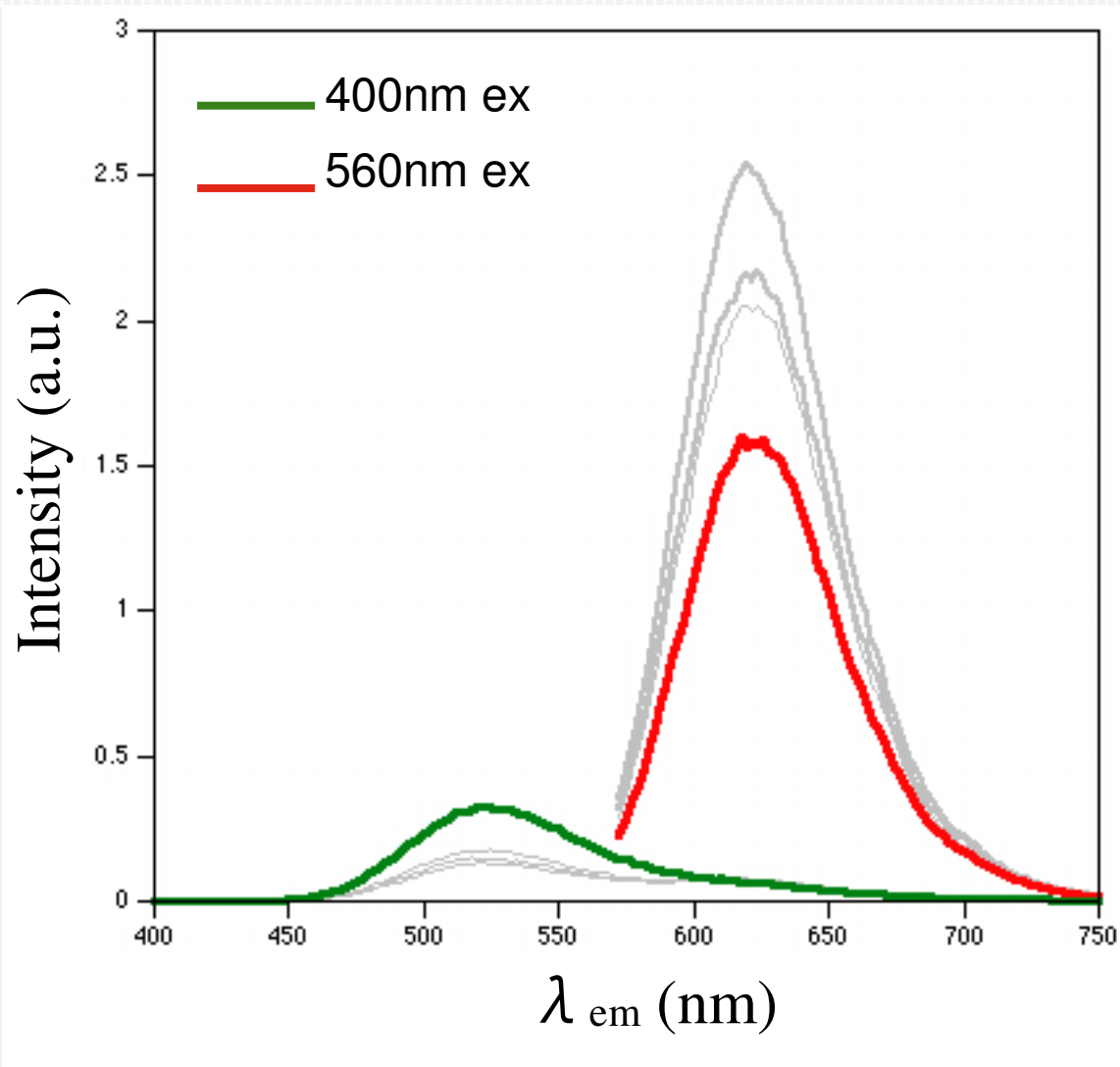


9C-hairpin loop Ag-clusters

time after reduction:
1 h00



TIME SERIES

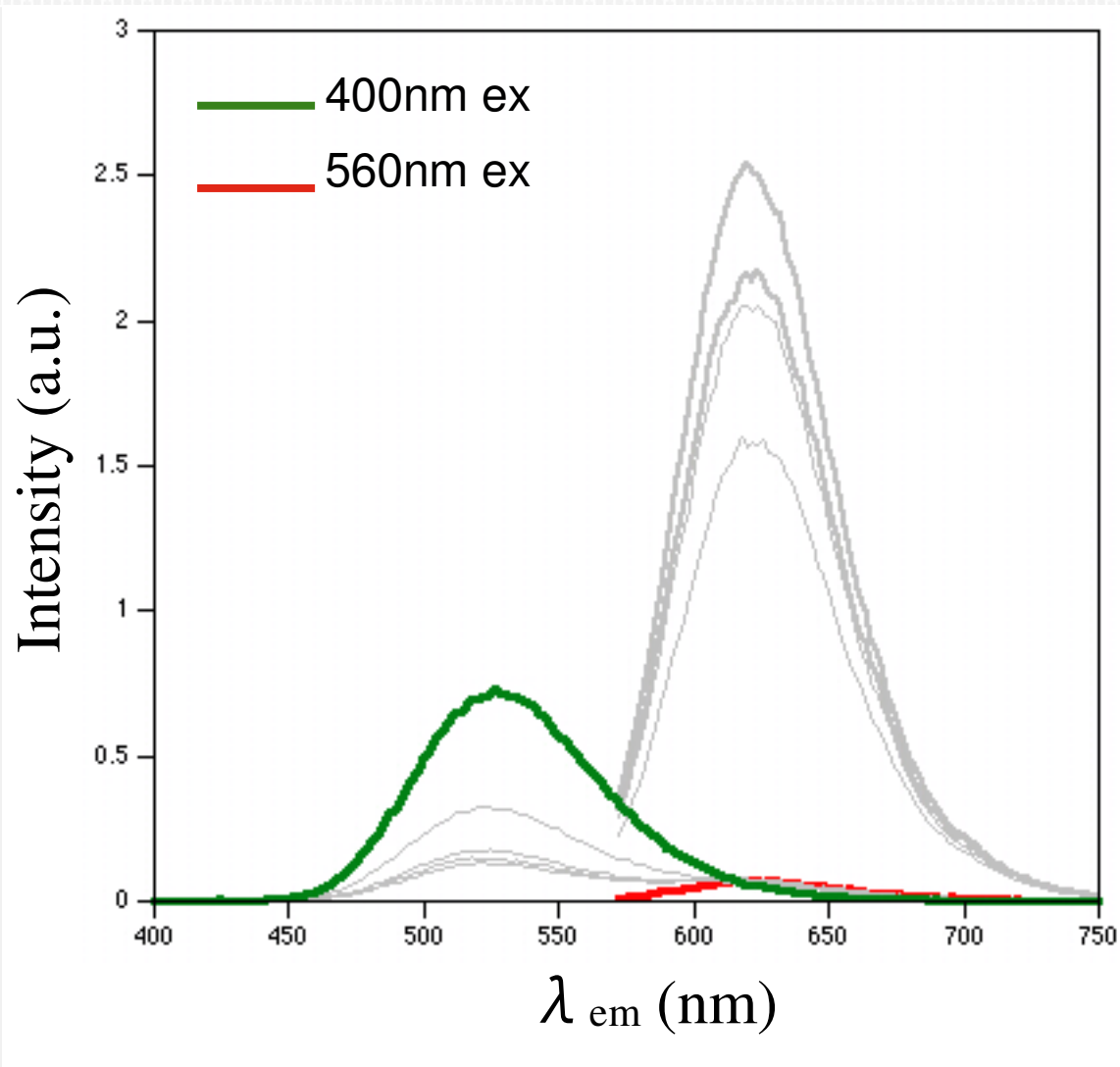


9C-hairpin loop Ag-clusters

time after reduction:
5h00



TIME SERIES

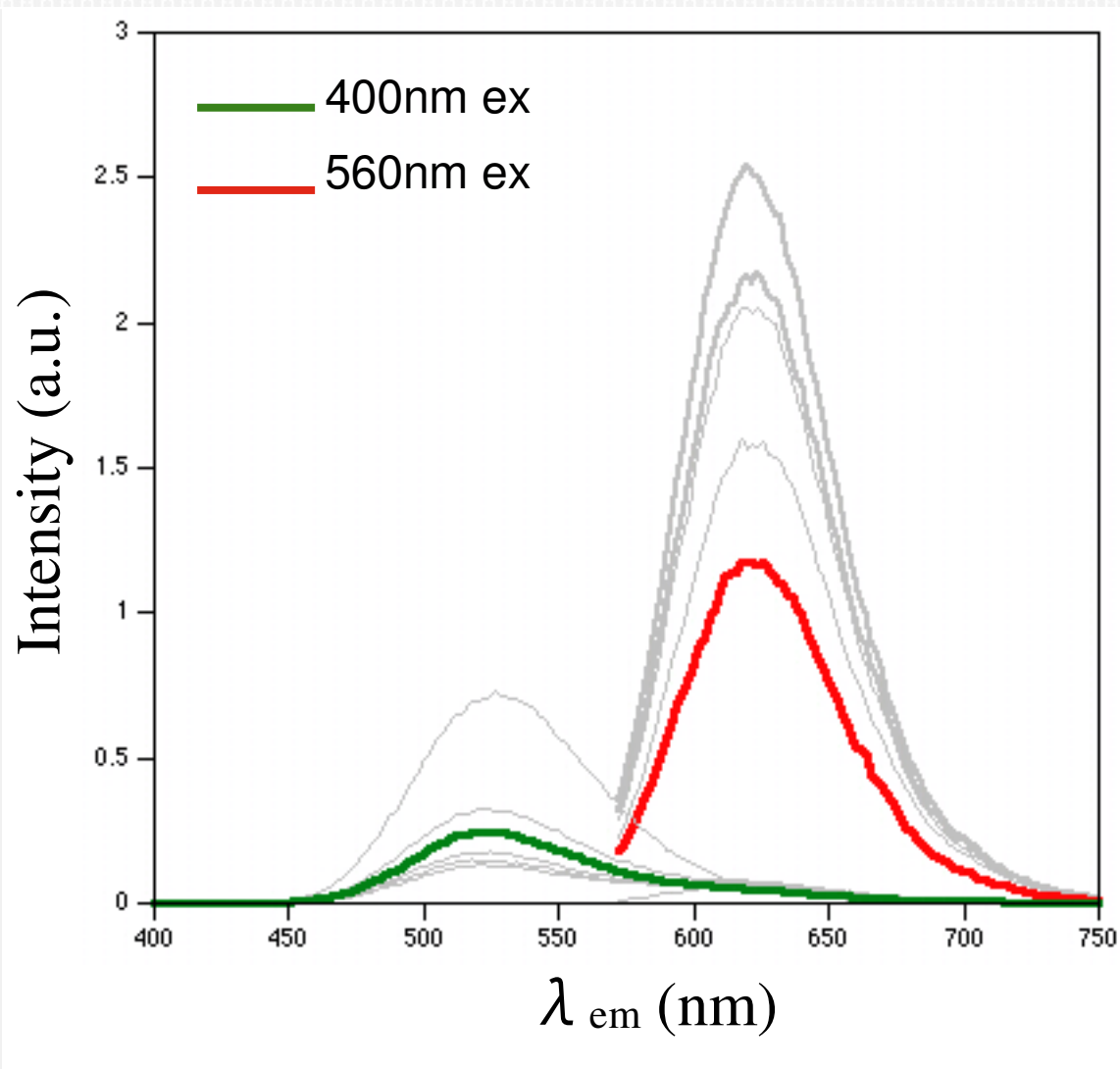


9C-hairpin loop Ag-clusters

time after reduction:
22h00



TIME SERIES

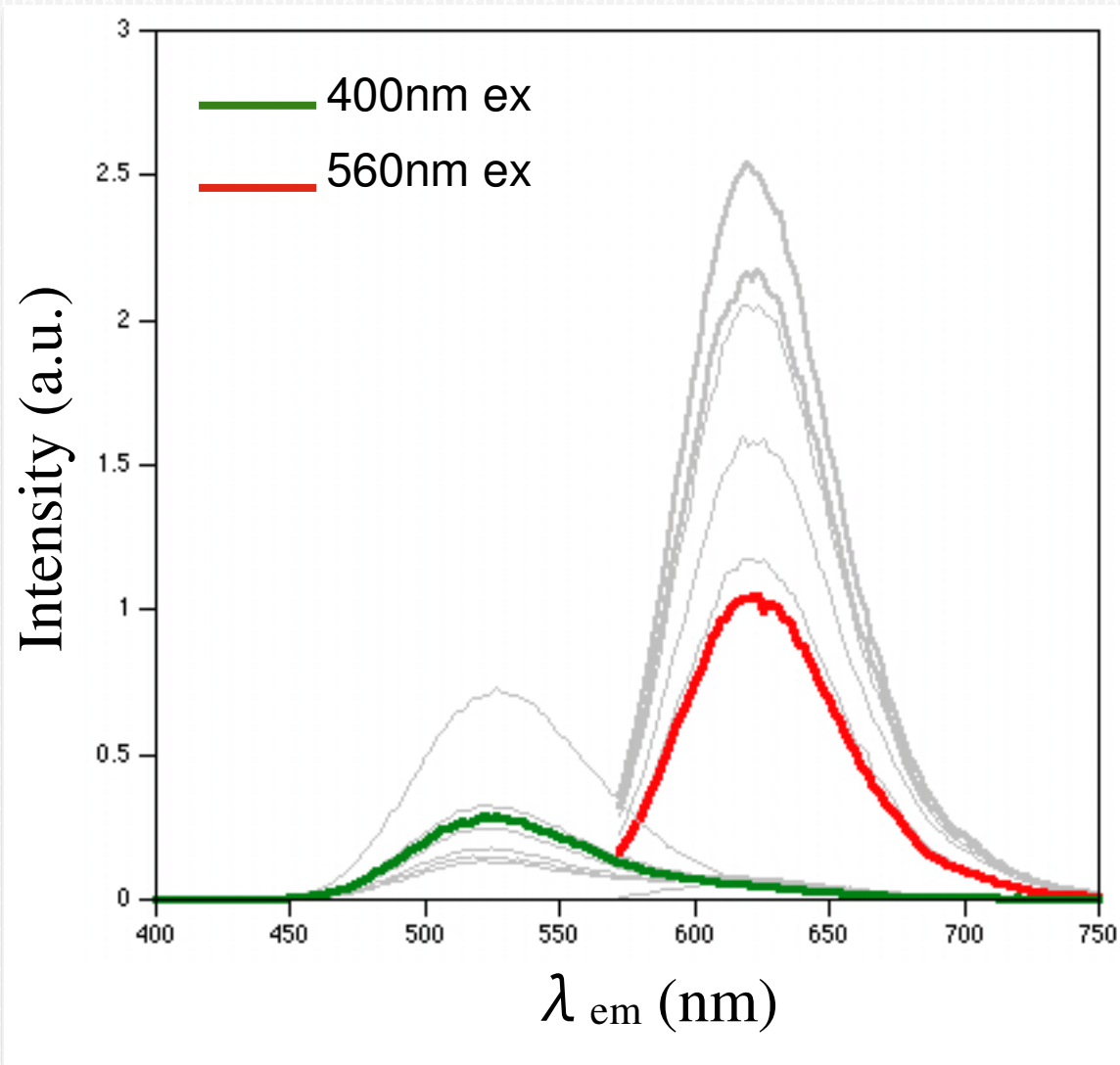


9C-hairpin loop Ag-clusters

time after 2nd
reduction:
0h05



TIME SERIES

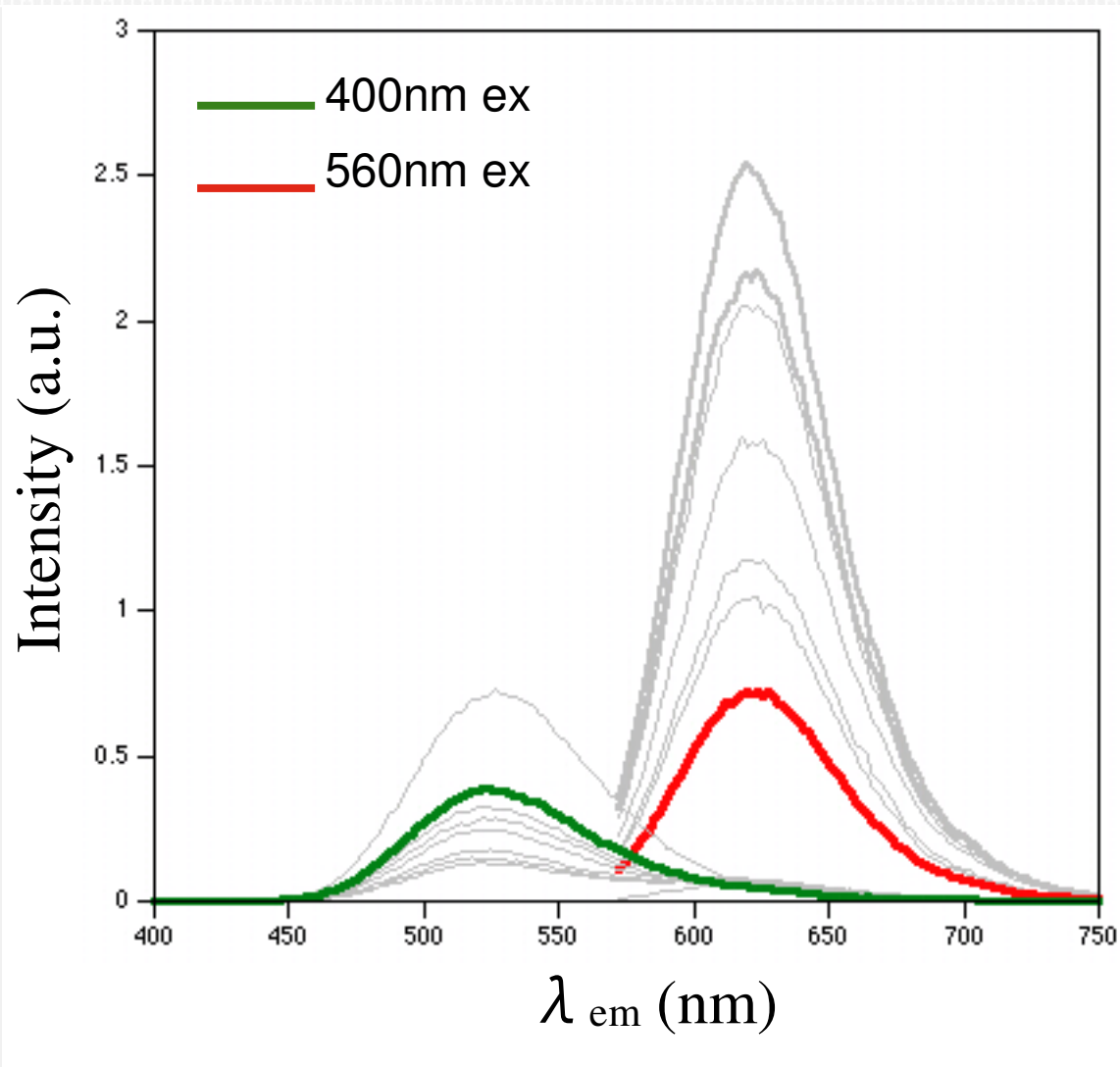


9C-hairpin loop Ag-clusters

time after 2nd
reduction:
0h30



TIME SERIES



9C-hairpin loop Ag-clusters

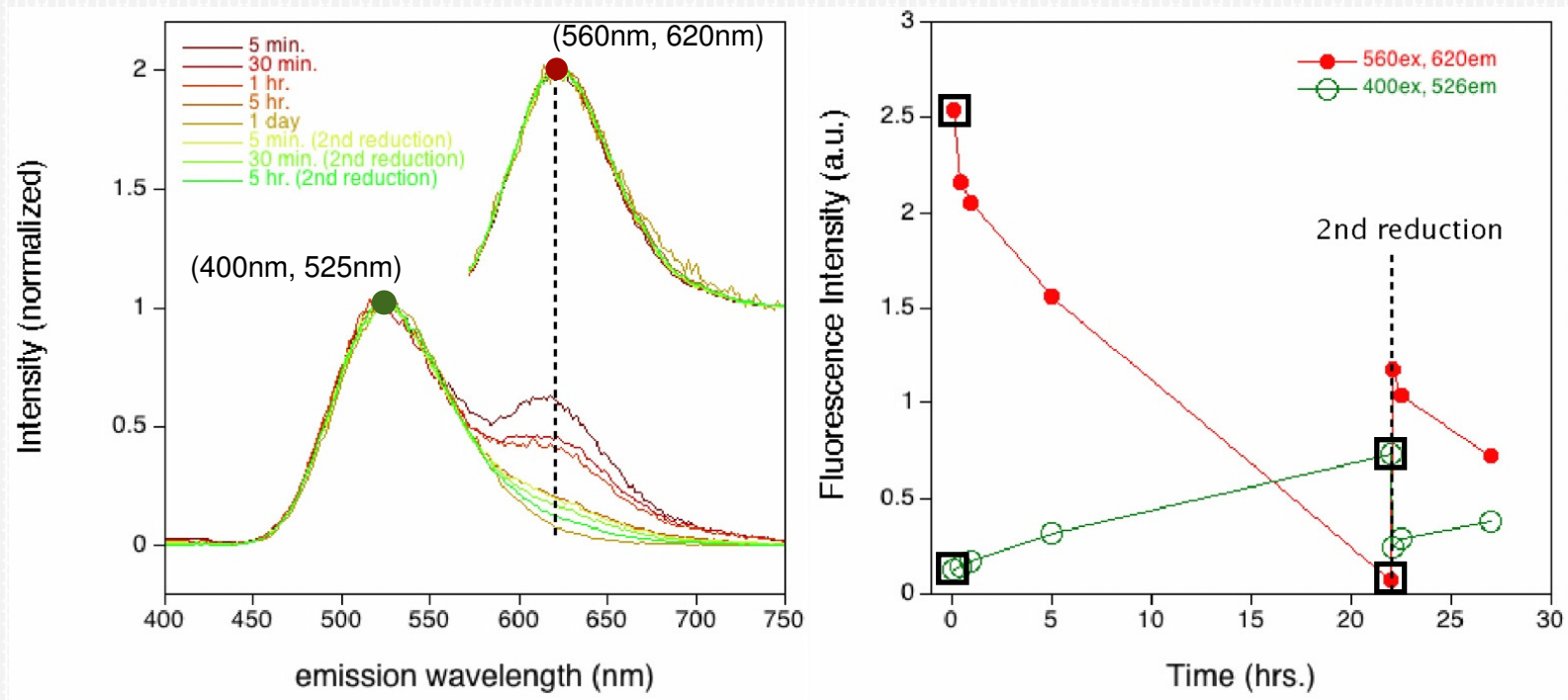
time after 2nd
reduction:
5h00



TIME SERIES – compiled and normalized

The normalized emission spectra suggest that:

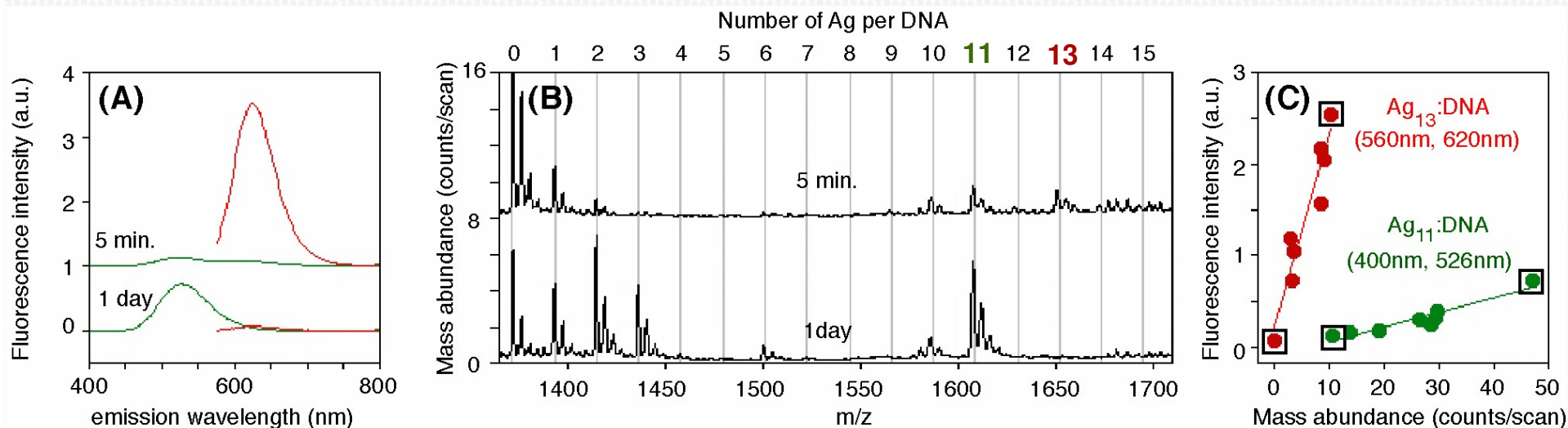
- intensity_(400nm, 525nm) can be attributed to a single fluorescent species
- intensity_(560nm, 620nm) corresponds to a second fluorescent species



CORRELATE FLUOR & MASS SPECTRA

$\text{Ag}_{11}:\text{DNA}_{9\text{C-hairpin}}$ \propto green fluorescence

$\text{Ag}_{13}:\text{DNA}_{9\text{C-hairpin}}$ \propto red fluorescence



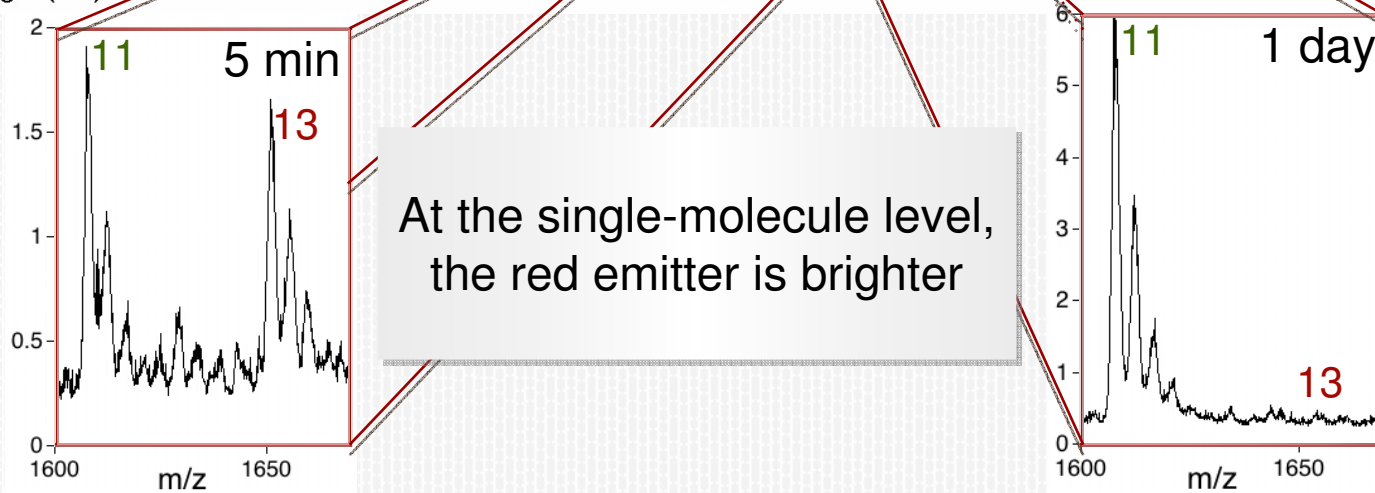
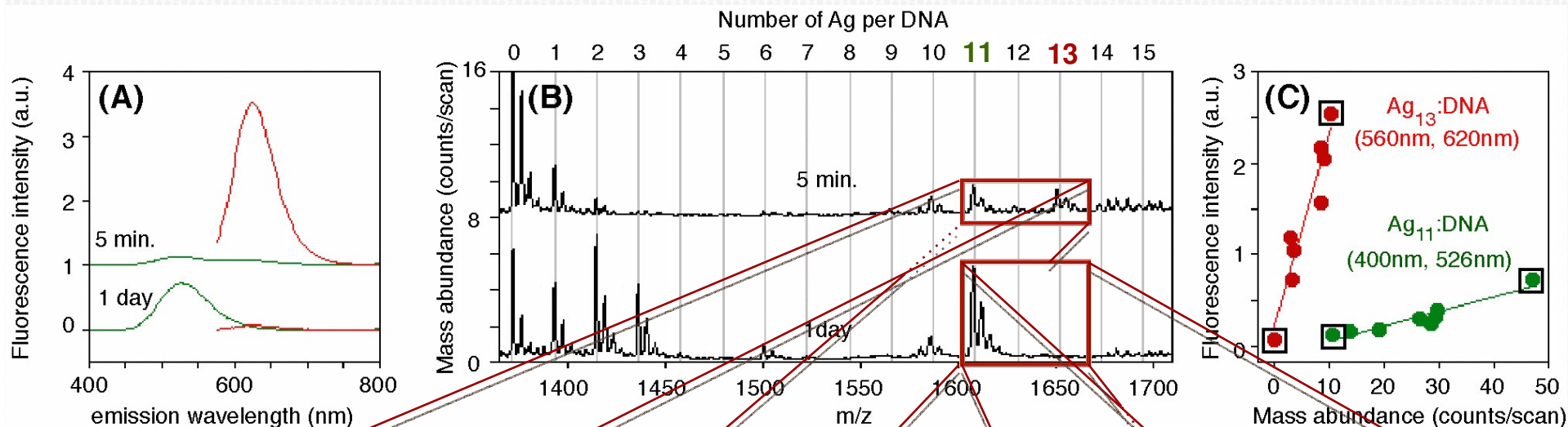
P.R. O'Neill, et al. *J Phys Chem C*, 113:4229-4233
(2009)



CORRELATED FLUOR & MASS SPECTRA

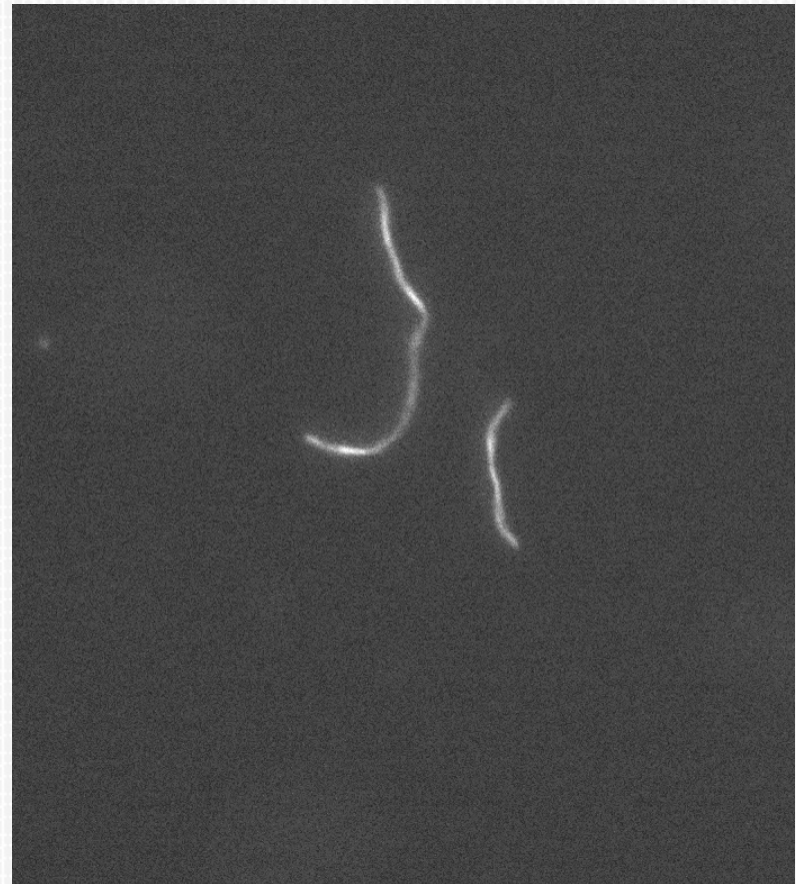
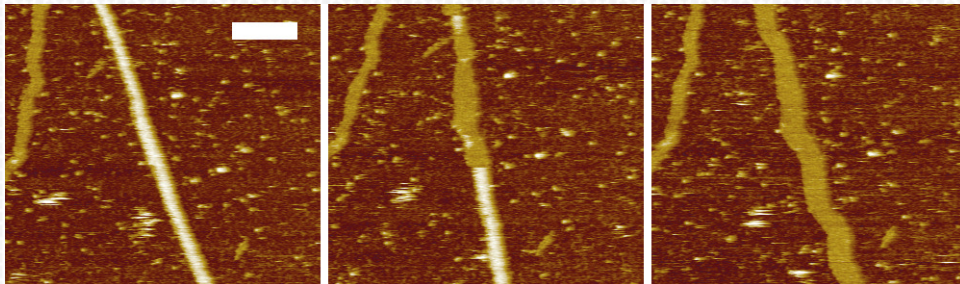
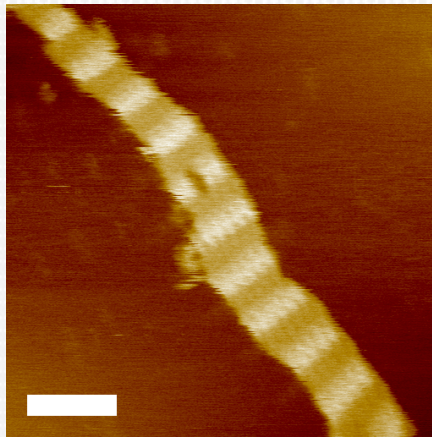
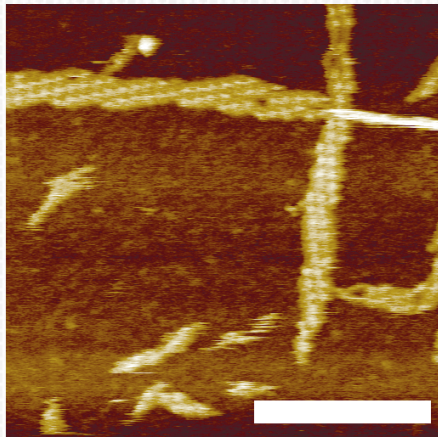
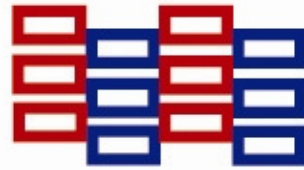
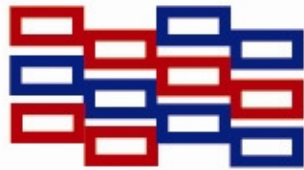
$\text{Ag}_{11}:\text{DNA}_{9\text{C-hairpin}} \propto$ green fluorescence

$\text{Ag}_{13}:\text{DNA}_{9\text{C-hairpin}} \propto$ red fluorescence



Ag-DNA and DNA NANOSTRUCTURES

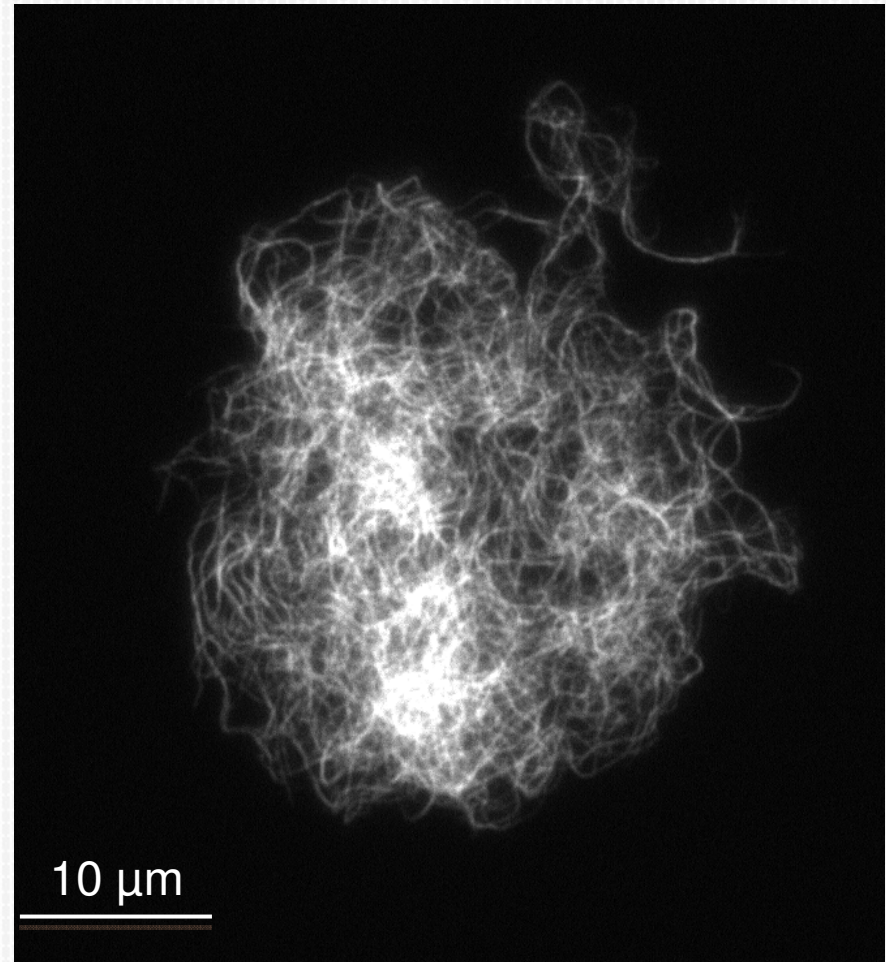
× Tiled DNA-Nanotubes



Ag-DNA and DNA NANOSTRUCTURES

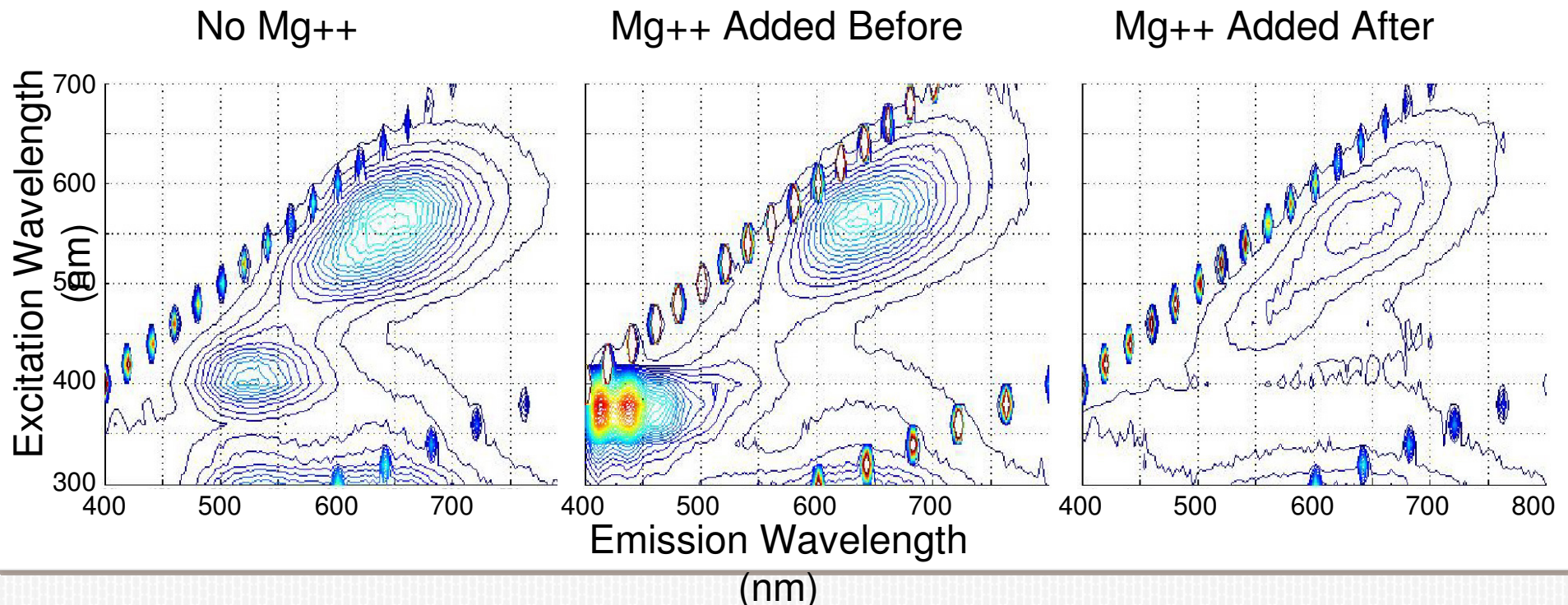
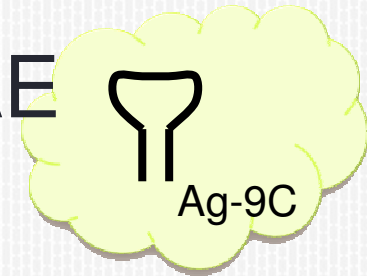
× Tiled DNA-Nanotubes with Ag-DNA

- fluorescent 😊
tangled 😞
- 1 hairpin per tile
- low [DNA] complicates fluorimetry
- tangling due to
 - Ag⁺
 - hairpins



Ag-DNA and DNA NANOSTRUCTURES

✘ Synthesizing Ag-DNA_{9C-hairpin} in TAE

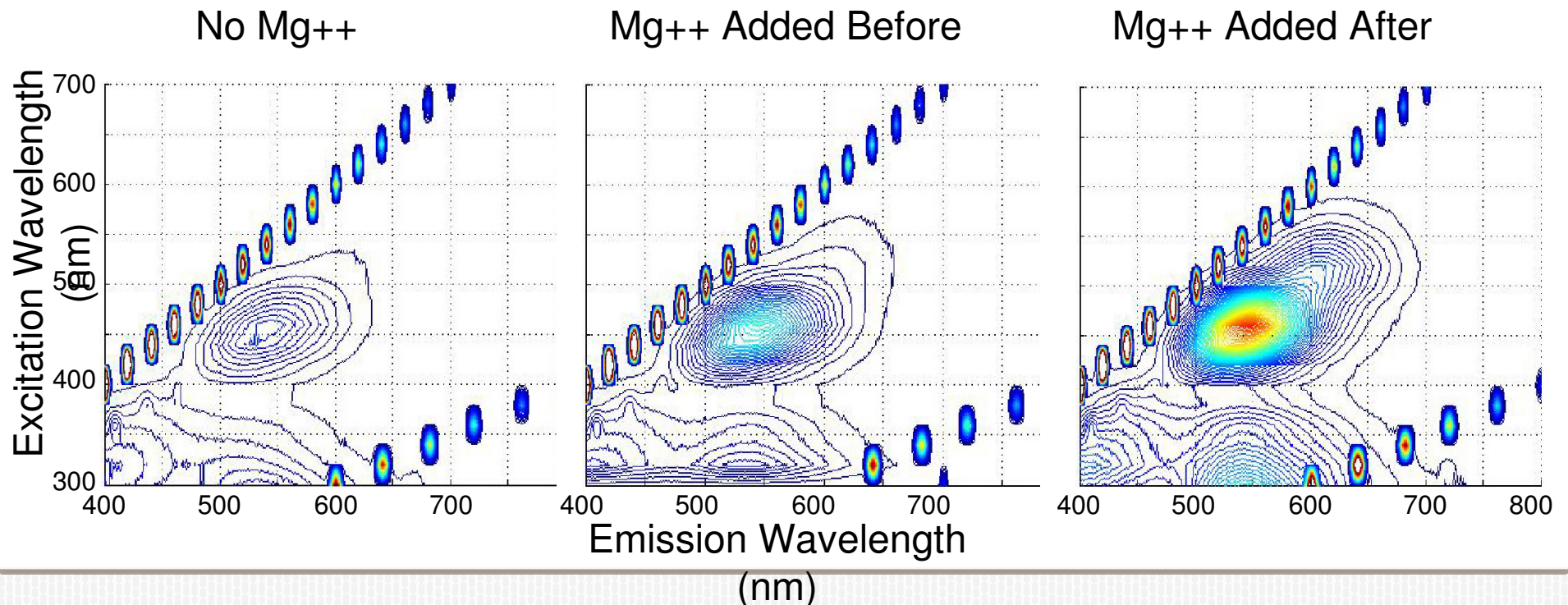
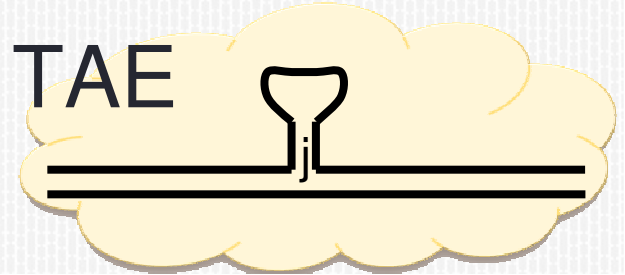


✘ same peaks, altered & diminished by Mg⁺⁺



Ag-DNA and DNA NANOSTRUCTURES

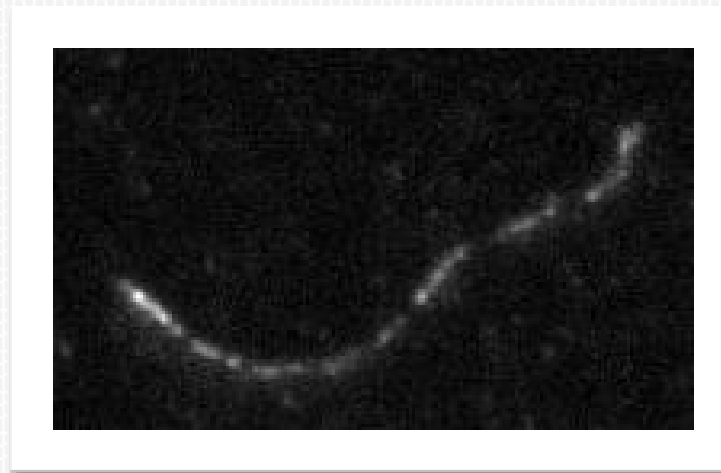
✘ Synthesizing Ag-DNA_{9C-j-SE1} in TAE



✘ stable spectrum, enhanced by Mg⁺⁺



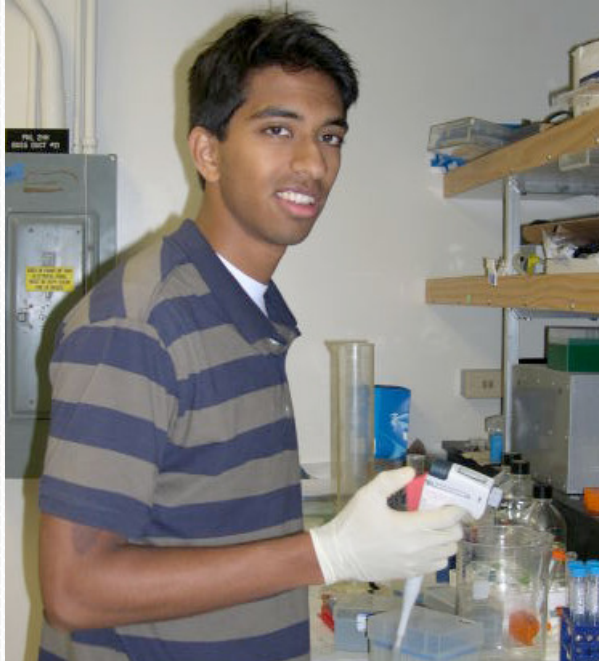
Ag-DNA are compatible with DNA tilings



5 μm



ACKNOWLEDGEMENTS



Kishore Padmaraju



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CHE-0848375



Patrick O'Neill



Kevin Young



Lourdes Velazquez



DGE-0440576
DMR-0611539





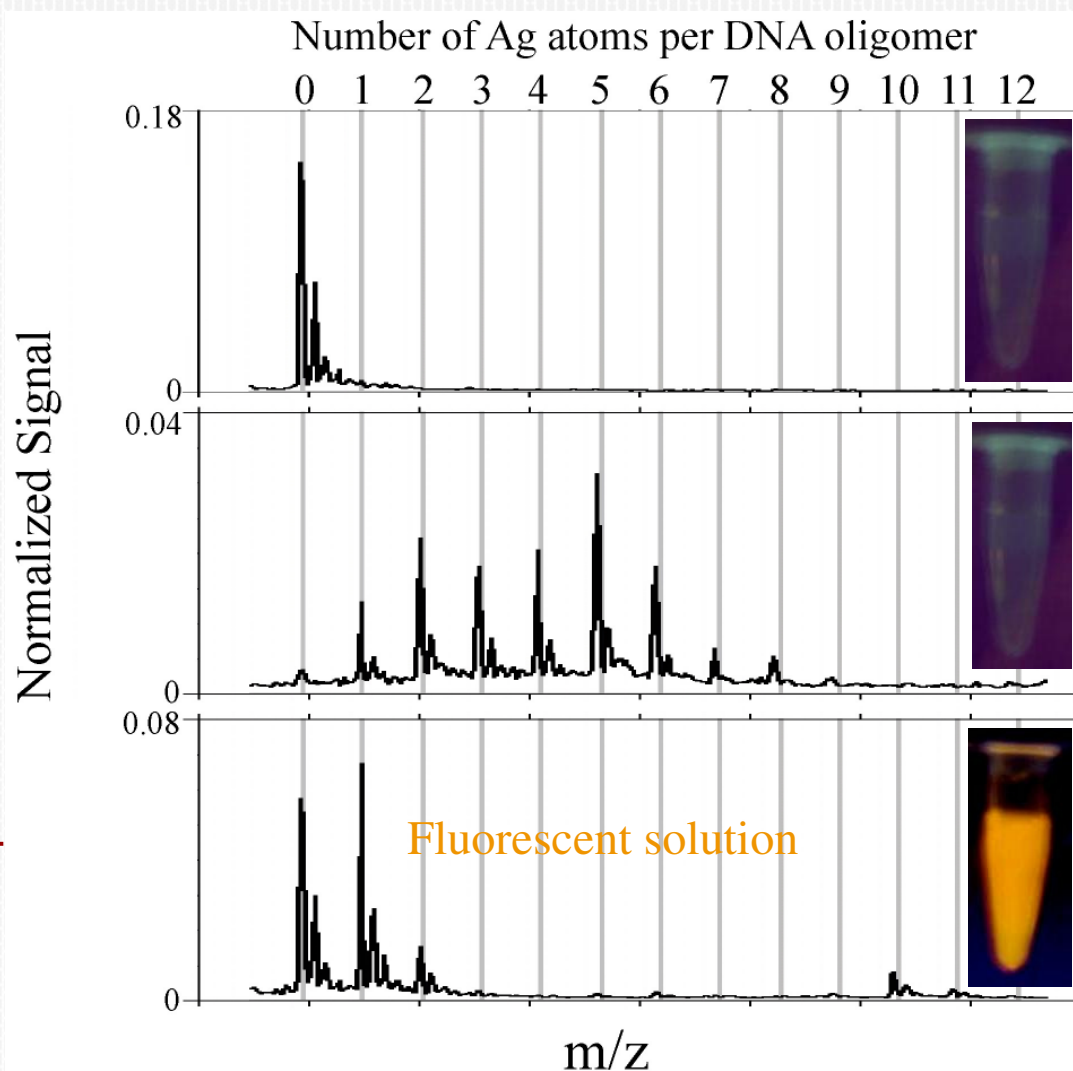
UCSB

FLUOROPHORE SYNTHESIS

DNA in
 NH_4Ac Solution

Add AgNO_3
 Ag^+ ions bind to
DNA

Add NaBH_4
 $\text{Ag}^+ + \text{BH}_4^- \rightarrow \text{Ag}$



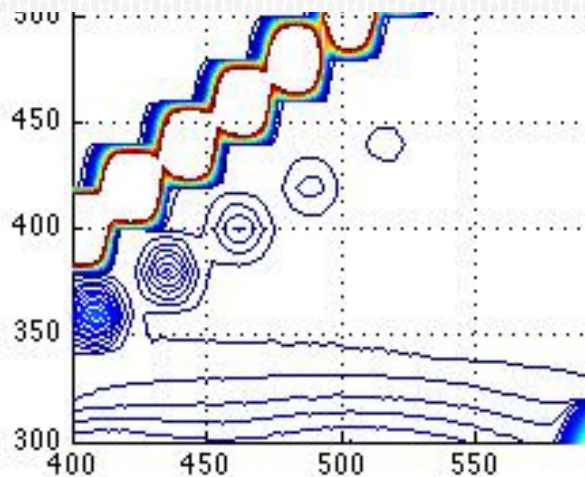
Ag^+ is "reduced" (gains an electron)



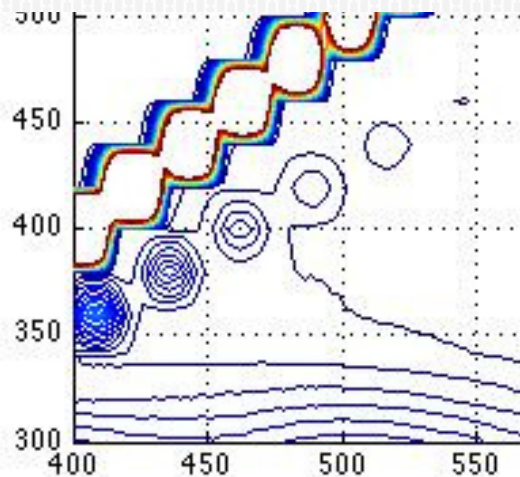
FLOURIMETRY AT LOW [DNA] ($\sim 1\mu\text{M}$)

- False Peaks!
- True peak represents fluorescence

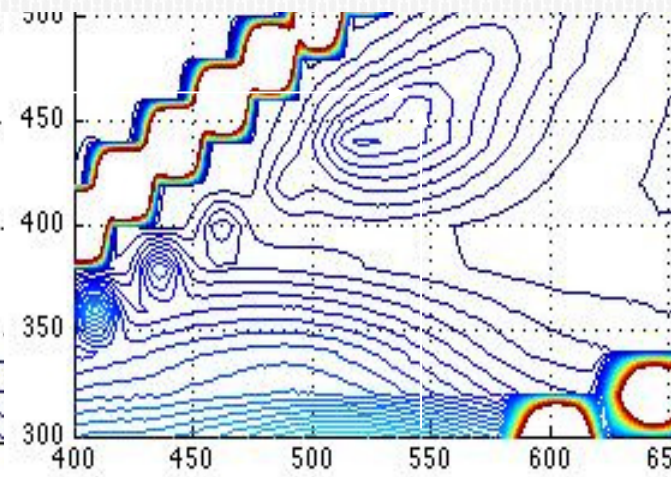
Water



Buffer

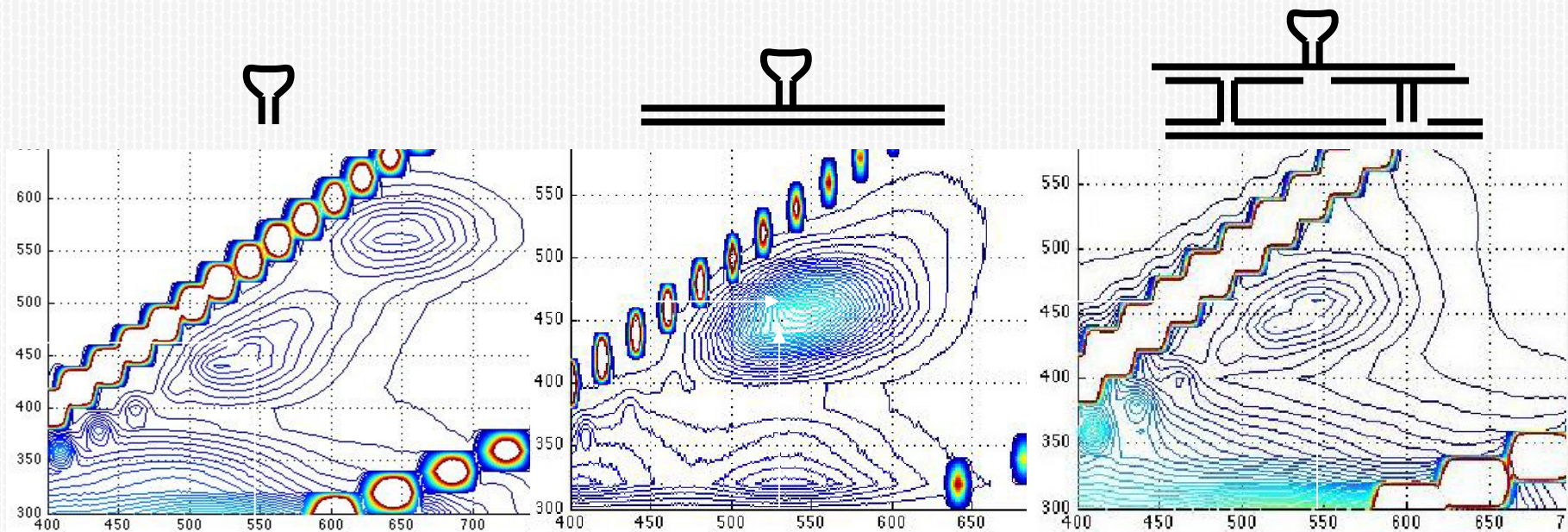


Y



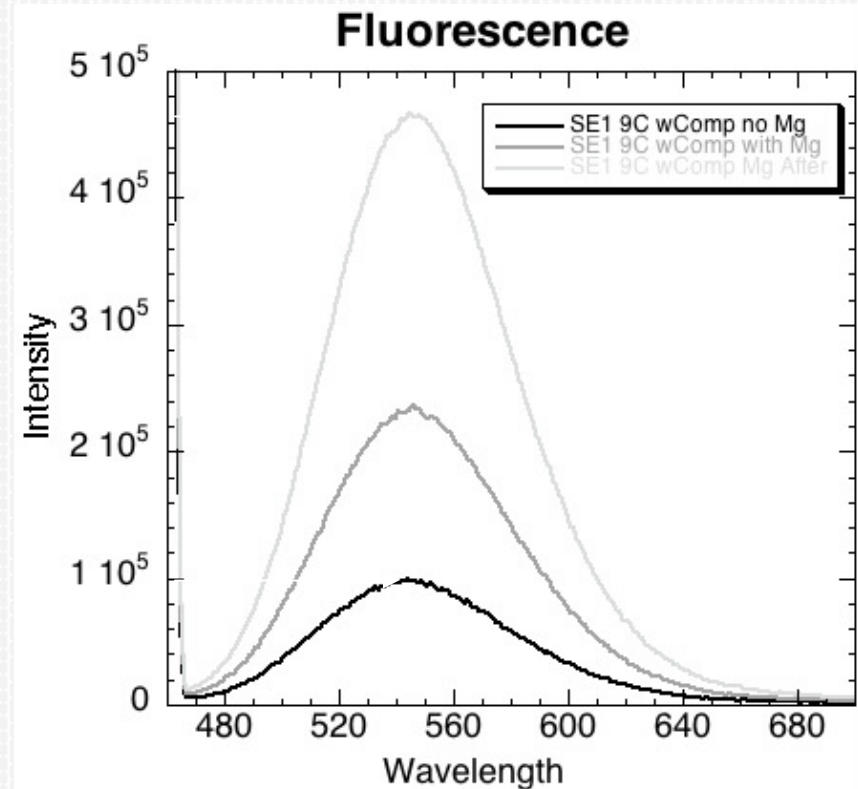
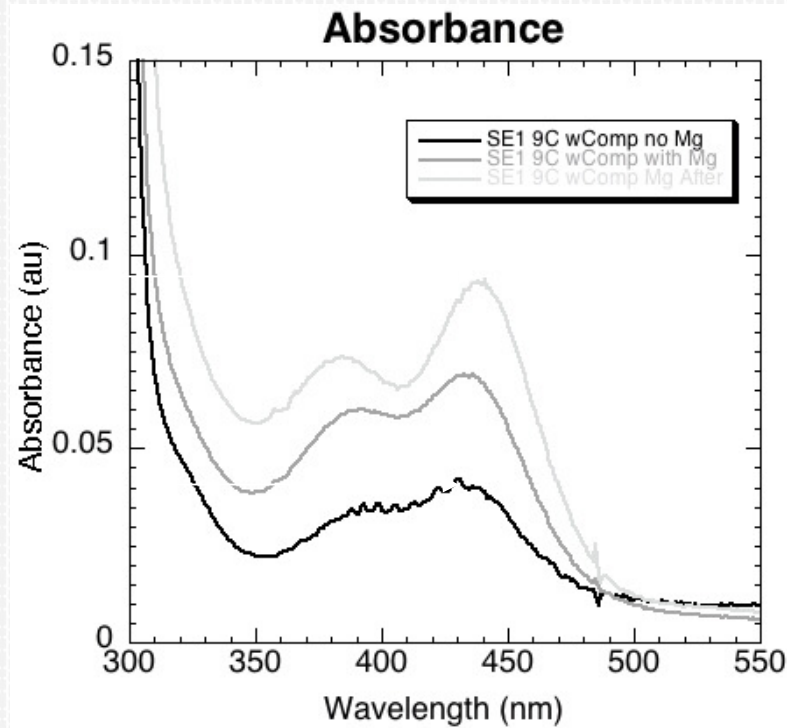
FLUORIMETRY AT LOW [DNA] ($\sim 1\mu\text{M}$)

- Shared Stable Species
- Different time evolution and species stability



WHAT IS MG'S EFFECT?

× SE1-9C with Compliment



AMBER FOLDING

