Quantitative Investigation of Compartmentalized Dynamics of ErbB2 Targeting Gold Nanorods in Live Cells by Single Molecule Spectroscopy

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Paper presentation by,

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❖ Background

- Endocytic trafficking of targeted nanoparticles involving receptor-mediated internalization, transport, and subsequent breakdown inside living cells is a complex and dynamic process.

- Understanding the diffusion dynamics and receptor uptake mechanisms of nanoparticles and nanocarriers in cancer cells at single particle level is crucial for effective design of multifunctional nanostructures for imaging, targeting, and therapy.

- Recent advances in understanding endocytic mechanisms have used quantum dots (QDs) and nanoparticle-based approaches to study localization, cellular uptake, and intracellular fate.

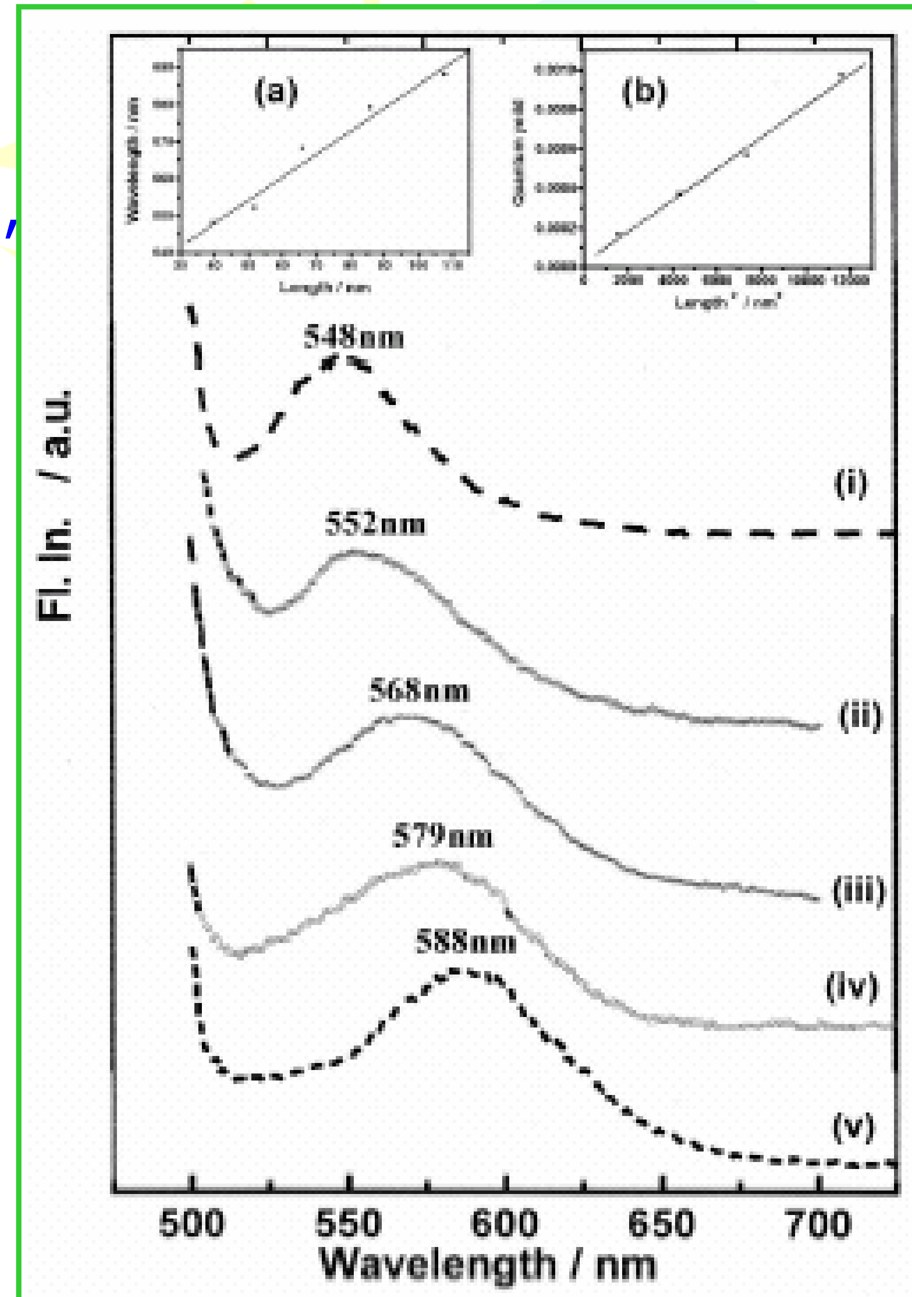
❖ Gold Nanorods

- Ease in control of its size, shape, composition, structure, optical tunability, and nontoxicity to mammalian cells.

- The fluorescence emission of GNRs is millions of times higher than that of bulk gold.

- Enhanced single photon and multiphoton luminescence of GNRs was observed years ago.(CPL, *317, 2000, 517–52*)

Aspect ratio – (i)=2.0, (ii)=2.6,(iii)=3.3, (iv)=4.3, (v)=5.4



❖ Work plan

1. Preparation and characterization of Herceptin conjugated, amine modified GNR
2. Preparation of SK-BR-3 cell lines with cell organelle markers.
3. Live cell imaging to study subcellular localization of GNR
4. Study of compartmentalized dynamics of H-GNR by single molecule spectroscopy.

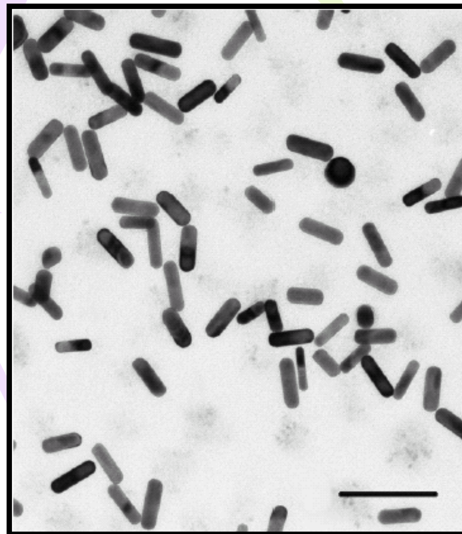
❖ Preparation of CTAB capped GNR

CTAB (0.2 M, 5 mL) + $HAuCl_4$ (0.5 mM, 5mL) + $NaBH_4$ (10 mM, 0.6 mL) $\xrightarrow{5h}$ Seed solution

CTAB (0.2 M, 50 mL) + HAuCl4 (1 mM, 50 mL) + $AgNO_3$ (4 mM, 0.3mL) Gentle mixing

700 µL Ascorbic acid added →

Seed solution added →

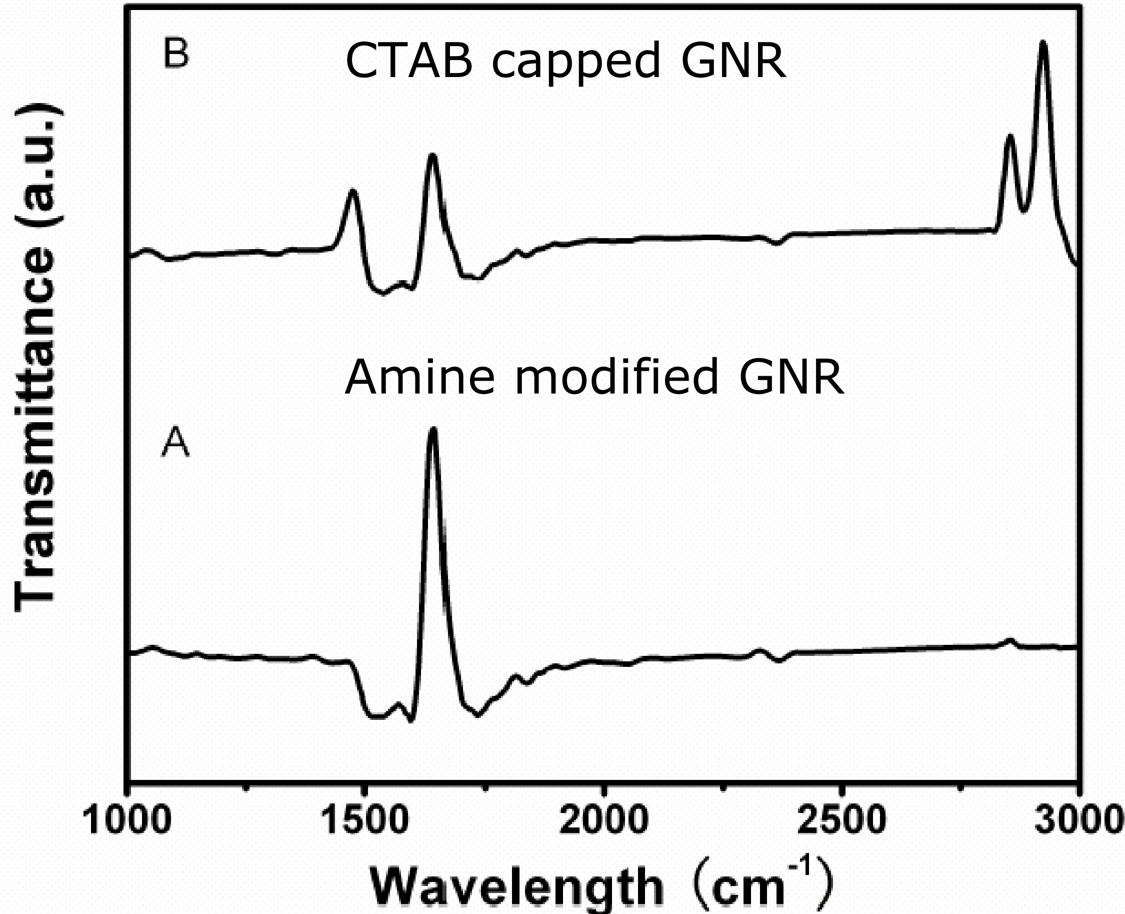


CTAB capped GNR(mixture kept undisturbed for 6 hours)

❖ Preparation of amine modified GNR

Au@CTAB GNR + $NH_2CH_2CH_2SSCH_2CH_2NH_2 \cdot 2HCl$
(10 mL) (30 Mm, 1mL)

Sonication
60ºC
6 hours



Washed and resuspended in 5 mM CTAB (final conc.100nM)

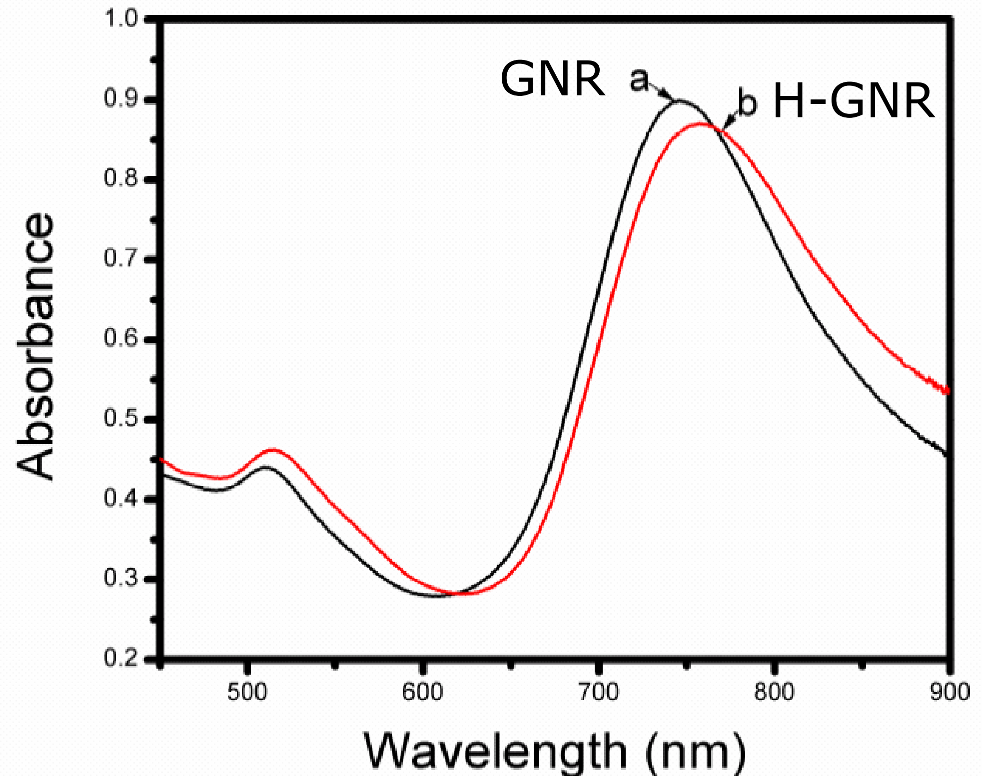
❖Herceptin conjugation to GNR

A = 10 mL amine modified GNR dispersed in 0.01 M PBS (PBS, NaCl 1.38 M, KCl, 0.0027 M, Tween-20 0.05%, pH 7.4) containing 5% glutaraldehyde for about 1 h.

B = A is washed and incubated with Herceptin (FDA approved recombinant humanized antibody) for 12 h at 4°C.

Washed and kept at 4°C in PBS 7.4 pH

Herceptin attached to a single GNR was estimated as 89±13



❖ Preparation of SK-BR-3 cell lines with cell organelle markers

Different markers were used to mark cell organelles,

Endosomes - YFP-RhoB
Golgi network - YFP-Golgi
(Cells were transfected using 1 μL Lipofectamine 2000)

Lysosome – Lysotracker probes
Five nanomolar LysoTracker probes were added and incubated with cells for 30 min to stain the lysosome in live cells.

GNR Excitation - 636 nm
Emission – 737 nm

Weak signal on 465 excitation, emits at 500-540 nm

❖ Live cell imaging to study uptake of H-GNR

1. SK-BR-3 cells were grown in RPMI-1640 (ATCC) media supplemented with 10% fetal bovine serum.

2. Cells were seeded onto sterilized No. l coverslip and placed inside a 6-well plate.

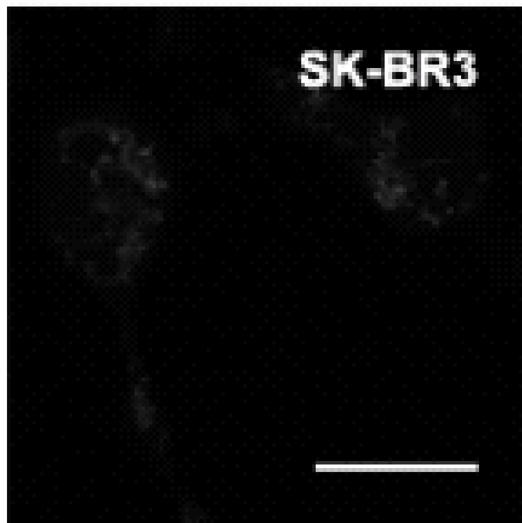
3. After the cells reached 80% confluence, they were incubated with GNRs or H-GNRs of the same concentration (500 μL, 1 nM) at 37 °C for 1 h and washed with PBS three times.

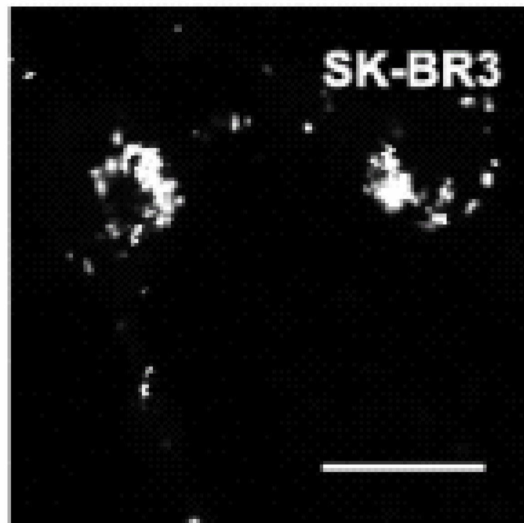
4. The culture was kept active, and the cells were taken out from the incubator for measurements at different time points (1 and 6 h) when needed.

❖ Live cell imaging to study uptake of H-GNR

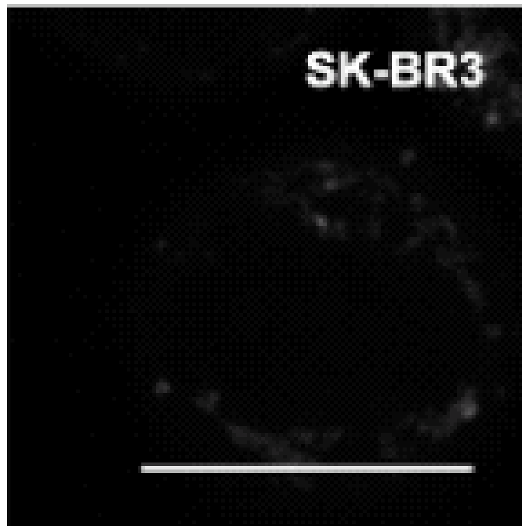
Excitation 465 nm 636 nm

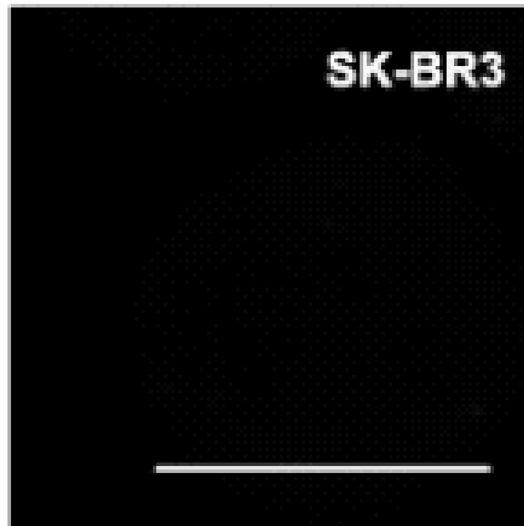
H-GNRs





GNRs

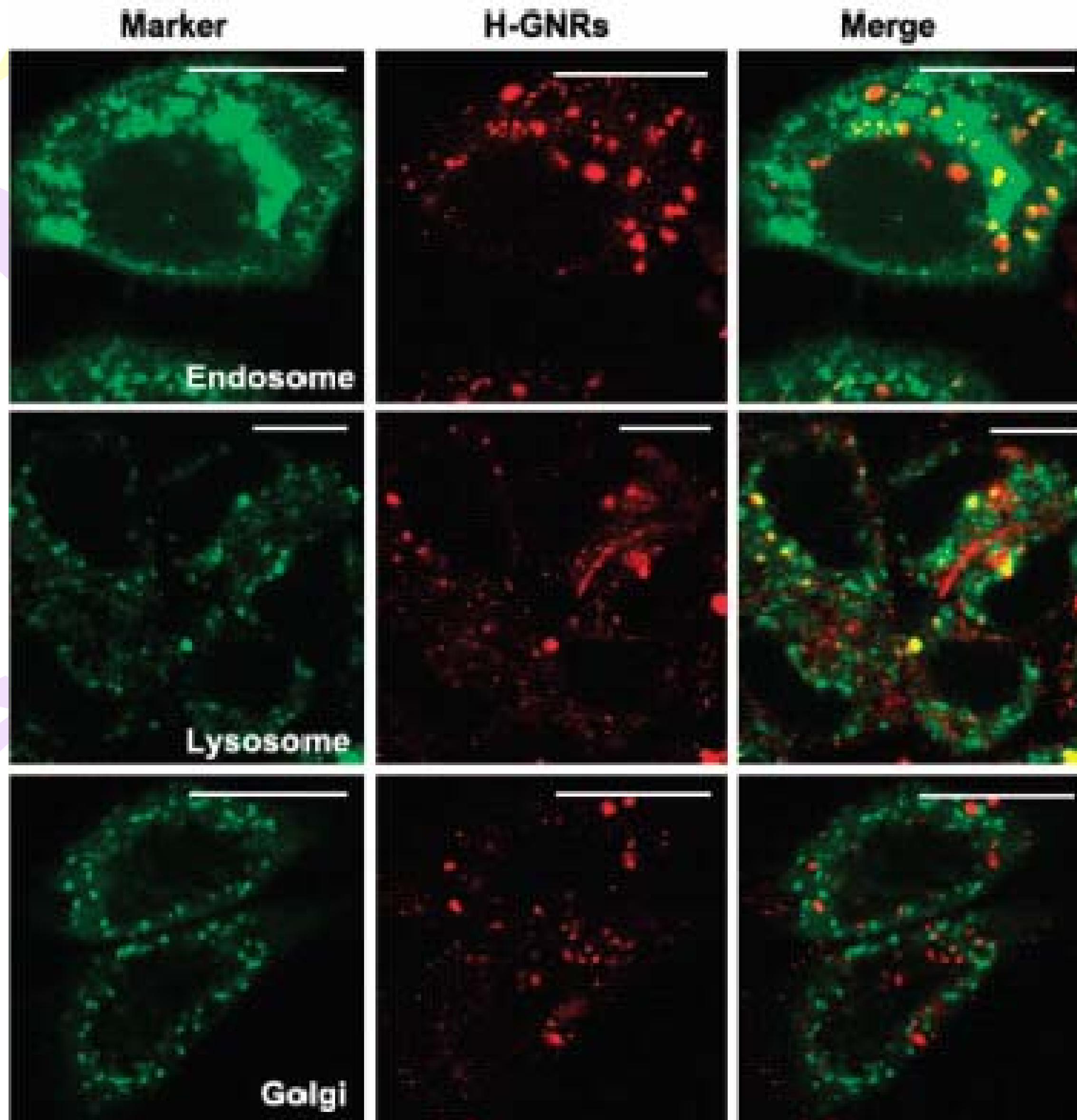




Scale bars 15 μm

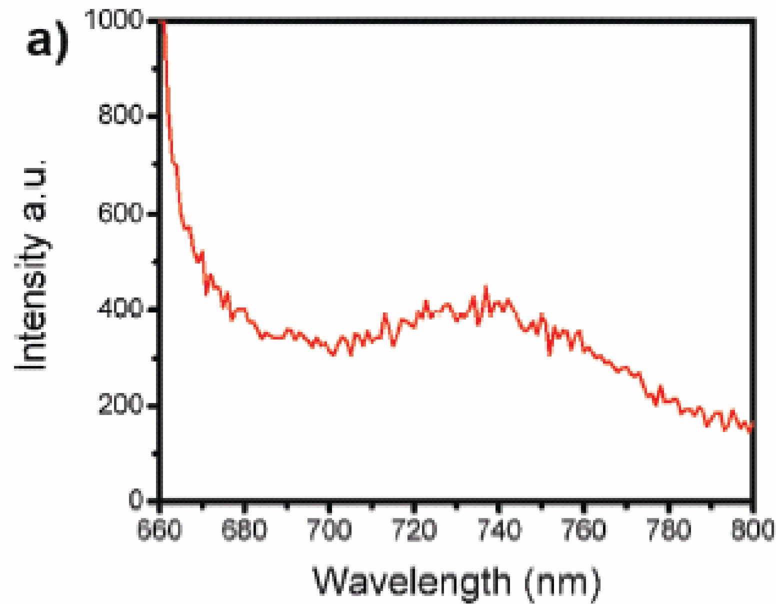
❖ Live cell imaging to study uptake of H-GNR

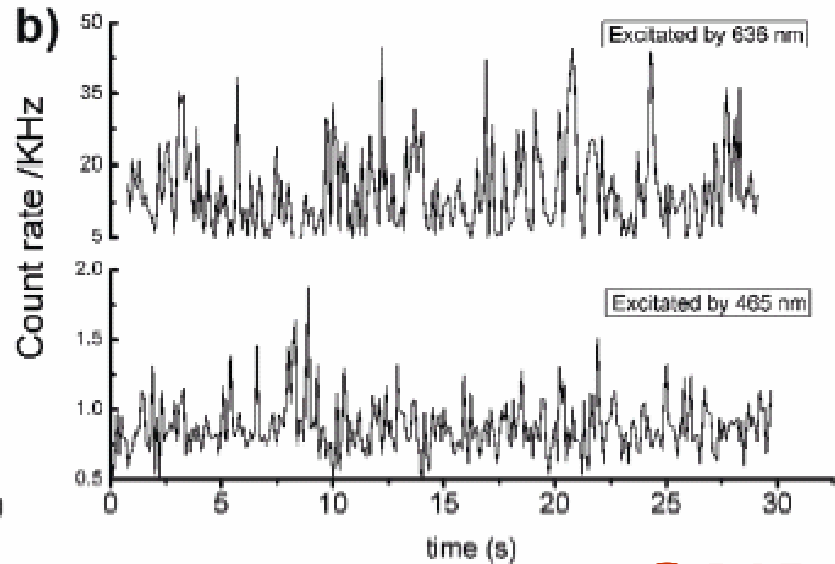
Colocalization => **YELLOW** color



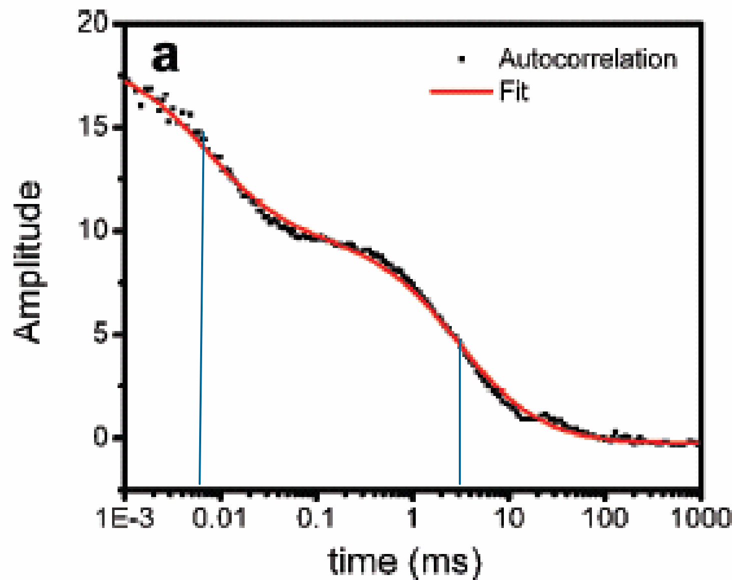
Scale bars 13 μm

❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy





GNR



$$G(\tau) = \frac{< \delta F(\tau) \times \delta F(t+\tau) >}{< F(t) >^2}$$

$$\delta F(t) = F(t) - < F(t) >$$

❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy

$$D = \frac{\omega^2}{4\tau_D}$$

ω = radius of LASER beam size

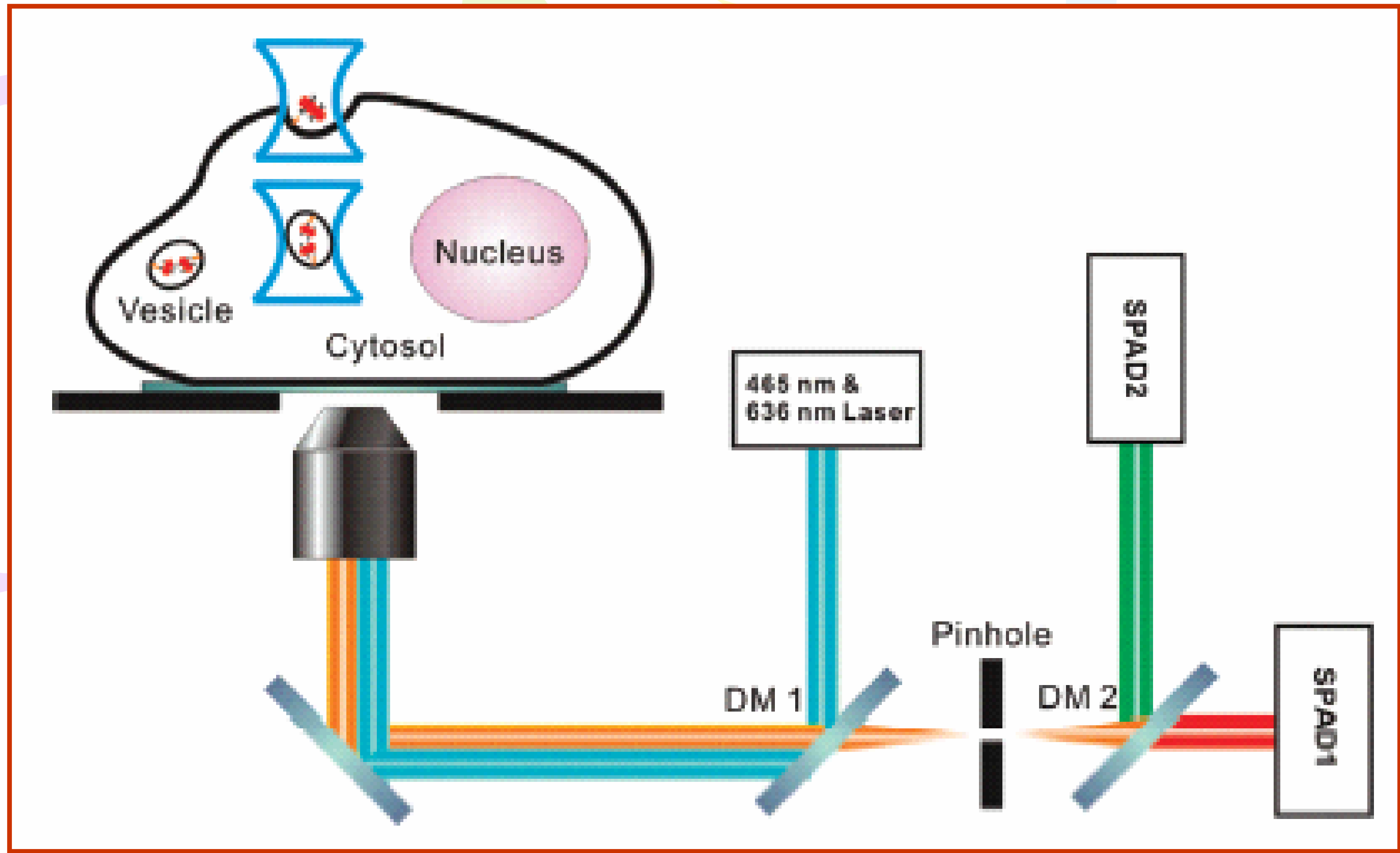
τ_D = Diffusion time

D confirmed by Stokes-Einstein Equation $D = \frac{kT}{(6\Pi\eta\gamma)}$

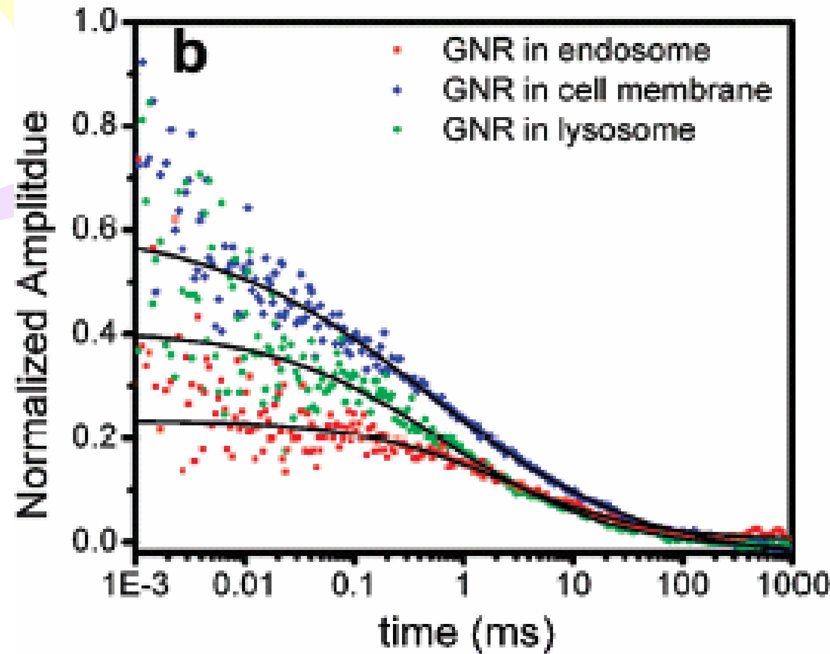
Curve was fitted with,

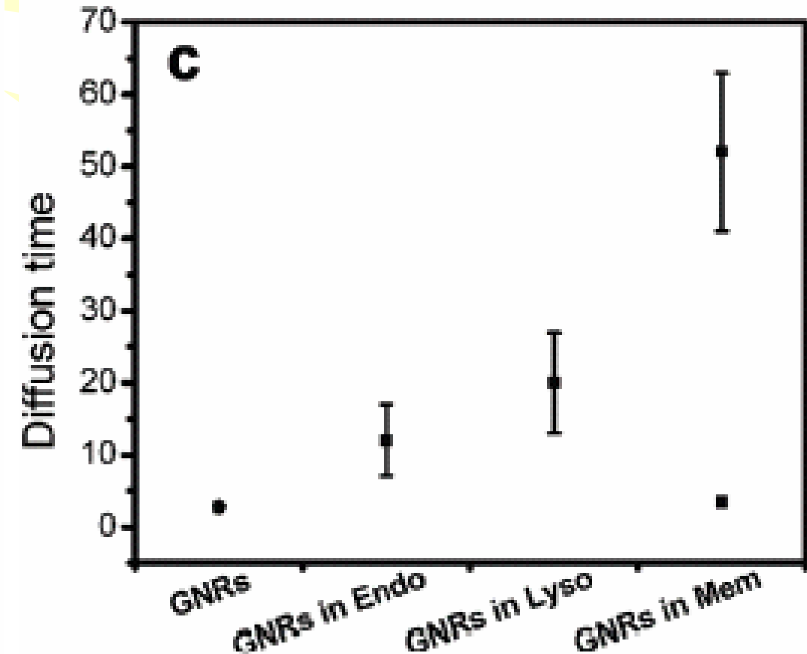
$$G(\tau) = \sum \frac{1}{N_i}\left(1 + \frac{\tau}{\tau_{D_i}}\right)^{-1}\left(1 + \frac{\tau}{\tau_{D_i}.k^2}\right)^{-1/2}$$

❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy



❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy





Equations fitted with,

$$G(\tau) = \frac{1}{<N>}\left((1-y).\left(1+\frac{\tau}{\tau_d^{free}}\right)^{-1}\left(1+\frac{\tau}{\tau_d^{free}k^2}\right)^{-\frac{1}{2}} + y\left(1+\frac{\tau}{\tau_d^{bound}}\right)^{-1}\right)$$

$$G(\tau) = \int \alpha_i \left(1+\frac{\tau}{\tau_{D_i}}\right)^{-1}\left(1+k^2\frac{\tau}{\tau_{D_i}}\right)^{-1/2} d\tau_D$$

For endosome and lysosome

❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy

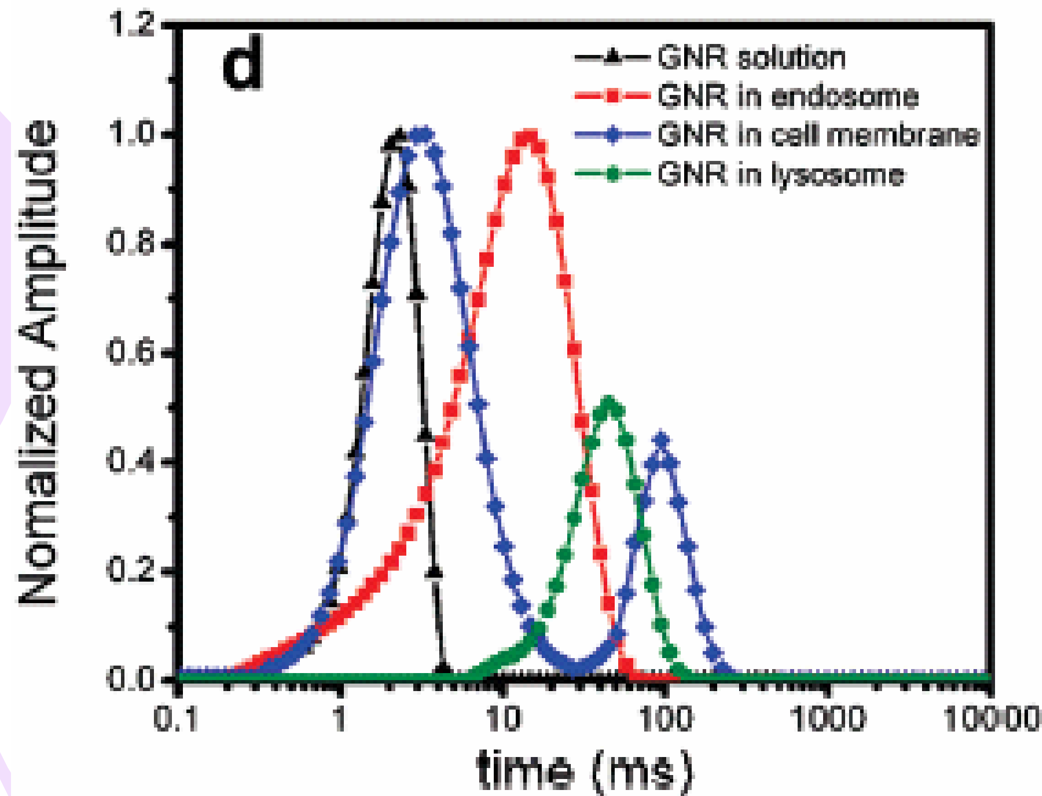
By τ_D found from FCS and Stokes Einstein equations, hydrodynamic radii were found,

endosome = 150 nm
lysosome = 280 nm

Viscosity assumed as 1.4 times viscosity of water.

❖ Compartmentalized dynamics of H-GNR by single molecule spectroscopy

MEMFCS was used to analyse FCS autocorrelation data, which gives contineous distribution of diffusion time.



$$G(\tau) = \int \alpha_i \left(1 + \frac{\tau}{\tau_{D_i}}\right)^{-1} \left(1 + k^2 \frac{\tau}{\tau_{D_i}}\right)^{-1/2} d\tau_D$$

❖ Quantitative investigation of GNR

The average number (N) of molecules in detection volume was determined (at 1 h and 6 h interval) and averaged for 25 cells

$$G(0) = 1/N = 1/Cv_{eff}$$

Where,

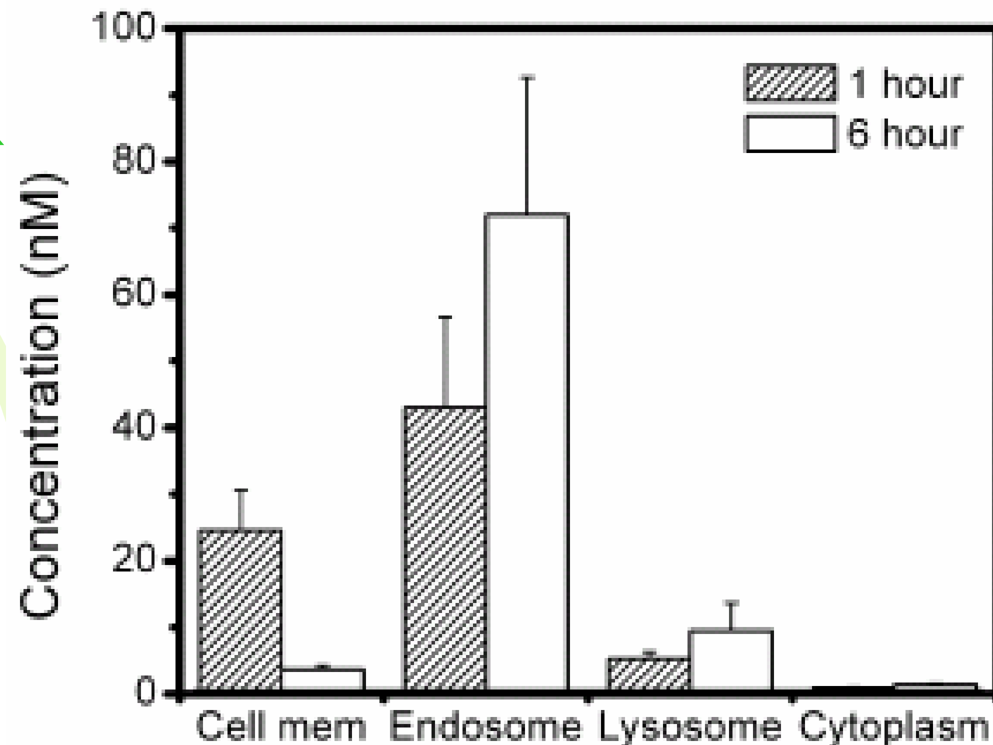
C = concentration of H-GNR

G(0) = autocorrelation at time t=0

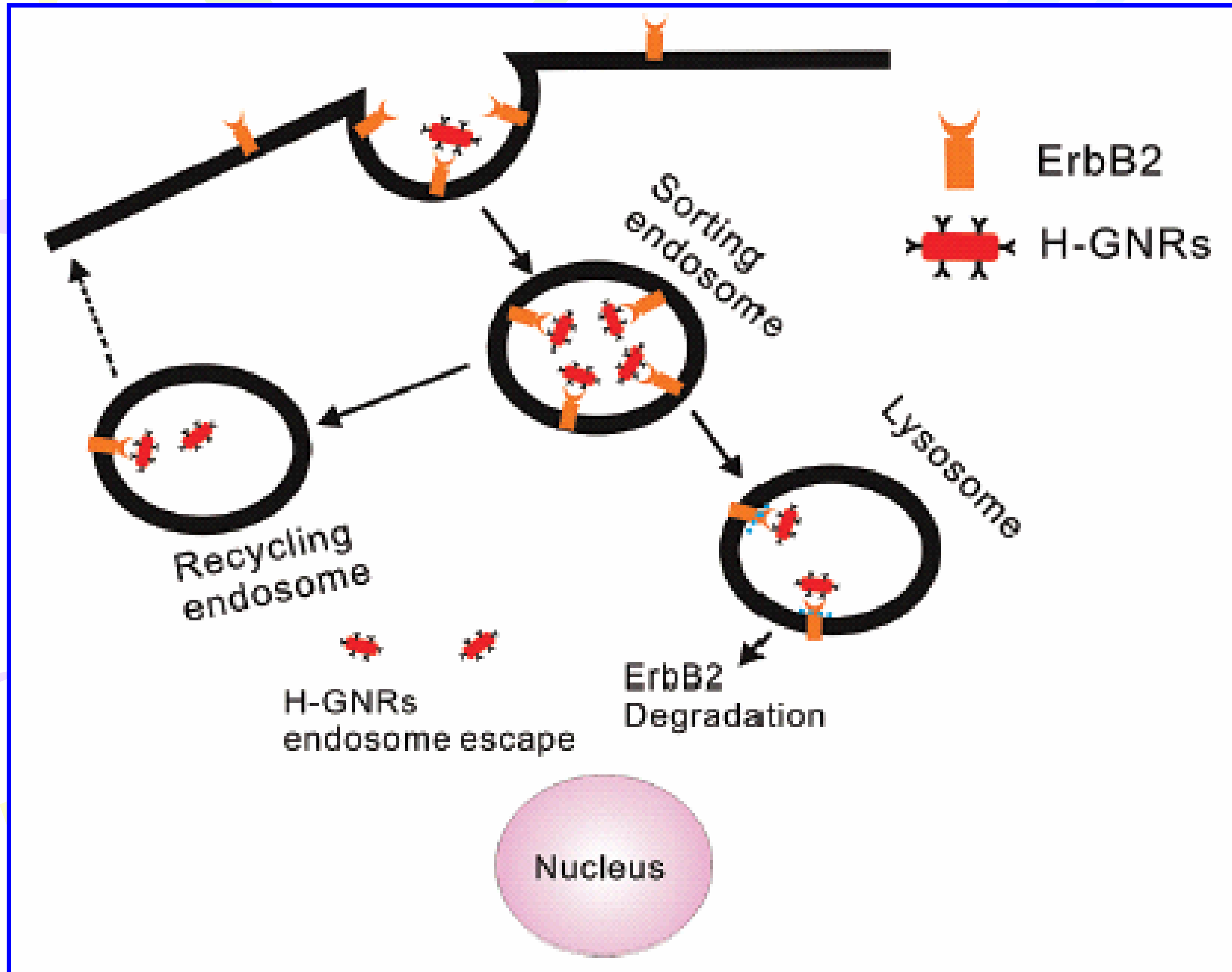
$V_{eff} = \pi^{3/2}\,\omega^2 z$

ω= radius of laser beam

z = axial beam size



❖ Conclusion



Thank you