Paper presentation

Continuous imaging of plasmon rulers in live cells reveals early-stage caspase-3 activation at the single-molecule level

Young-wook Jun[a], Sassan Sheikholeslami[a,1], Daniel R. Hostetter[b,1], Cheryl Tajon[b,c], Charles S. Craikb,[c], and A. Paul Alivisatos[a,2]

[a]Department of Chemistry, University of California and Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [b]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158; and [c]Graduate Program in Chemistry and Chemical Biology, University of California, San Francisco, CA 94158

PNAS October 20, 2009 vol. 106 no. 42 17735-17740.

By
Kamalesh
26-03-2011

Introduction

- Plasmon rulers are comprised of peptide-linked gold nanoparticle satellites around a core particle which highly enhance the signal intensity required for single-molecule detection.

- Plasmon rulers are useful for in vivo studies to observe very long trajectories of single biomolecules in live cells.

- Single molecule imaging has enabled the exploration of biomolecular dynamics and has revealed processes at work that are lost by extrapolation of ensemble assays.

Case selected for study -

- Caspase-dependent apoptotic signaling was selected as a case study which is highly related to various autoimmune diseases and cancer.

- Activation of caspase-3 through the apoptotic signaling pathway can take from several minutes to hours.

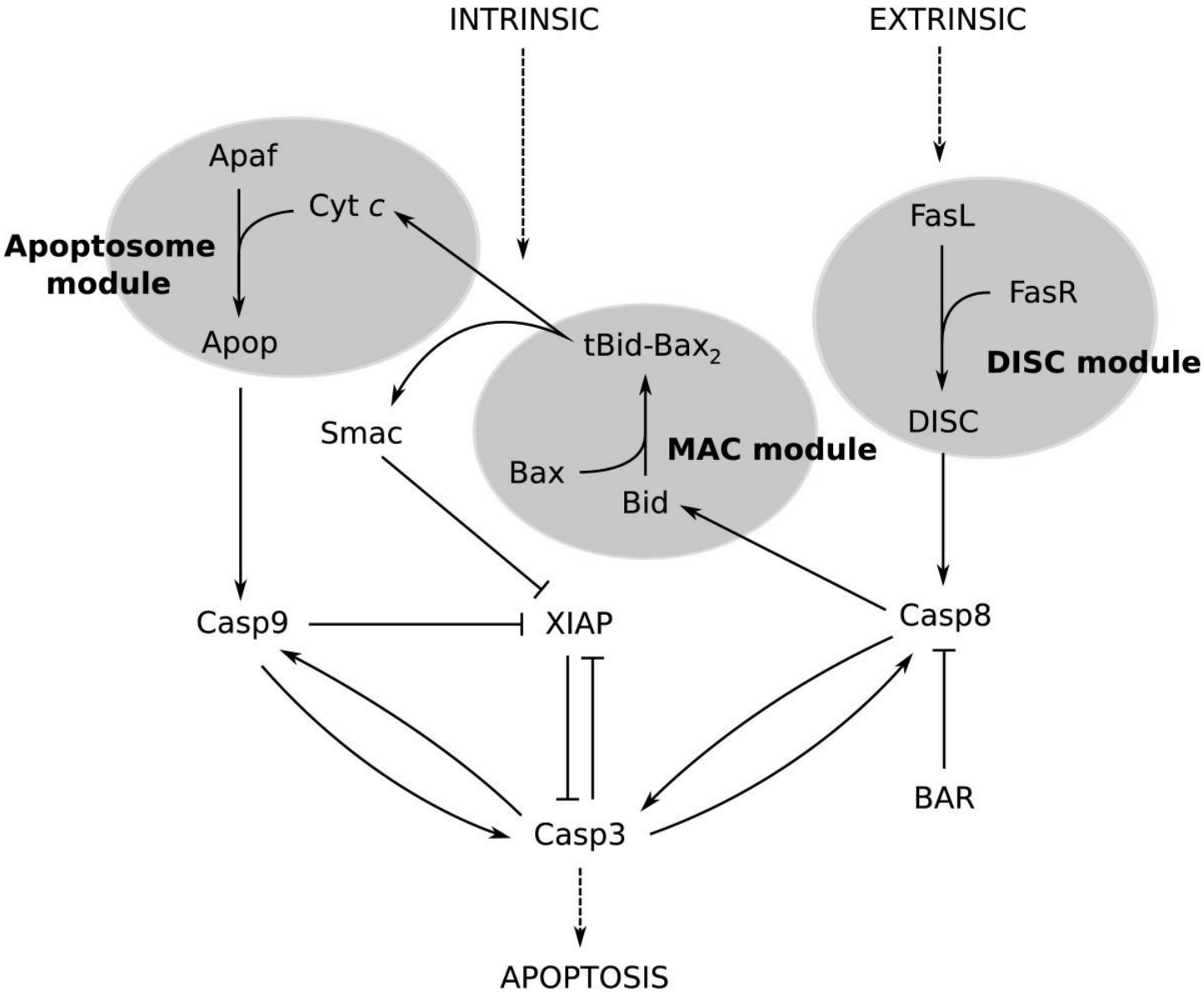
- The long time-scale of these signaling events has made them difficult to measure continuously at the single-molecule level with established techniques.

Caspase 3

- The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family.

- Caspase 3 has its typical role in apoptosis, where it is responsible for chromatin condensation and DNA fragmentation.

Caspase 3


INTRINSIC
EXTRINSIC
Apaf
Cyt c
Apoptosome module
Apop
FasL
FasR
DISC module
DISC
tBid-Bax2
Smac
Bax
MAC module
Bid
Casp9
XIAP
Casp8
BAR
Casp3
APOPTOSIS

Crown Nanoparticle Plasmon Rulers

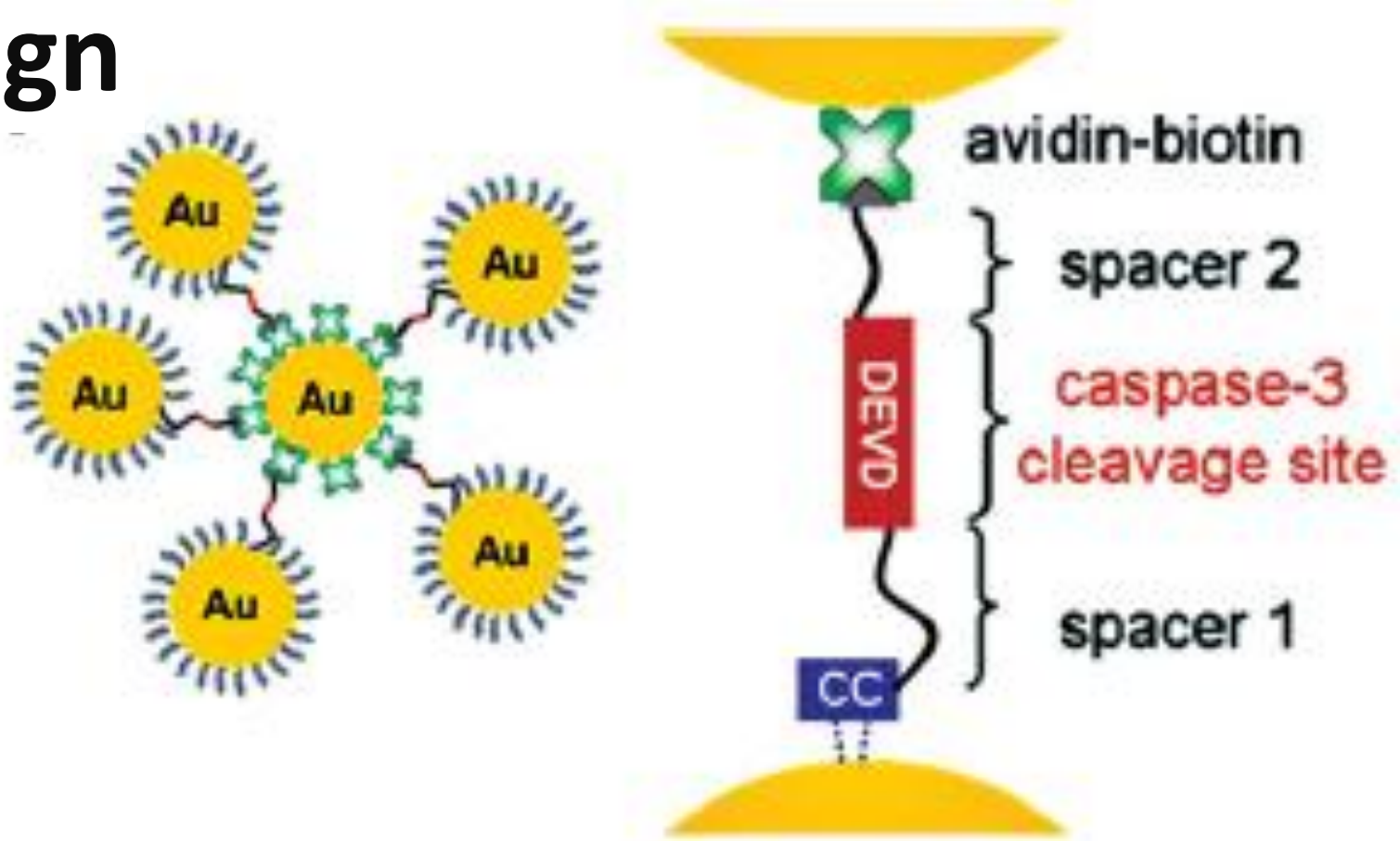
- Light-scattering intensity, for a pair of particles in close proximity (within one diameter) is significantly greater than that of 2 separate particles.

- This applies to assemblies where the particle is surrounded by several others.

- Crown nanoparticle assembly with a core 40-nm particle surrounded by 5 others scatters light ~44X more intensely than a single particle, along with a substantial spectral red shift (75 nm).

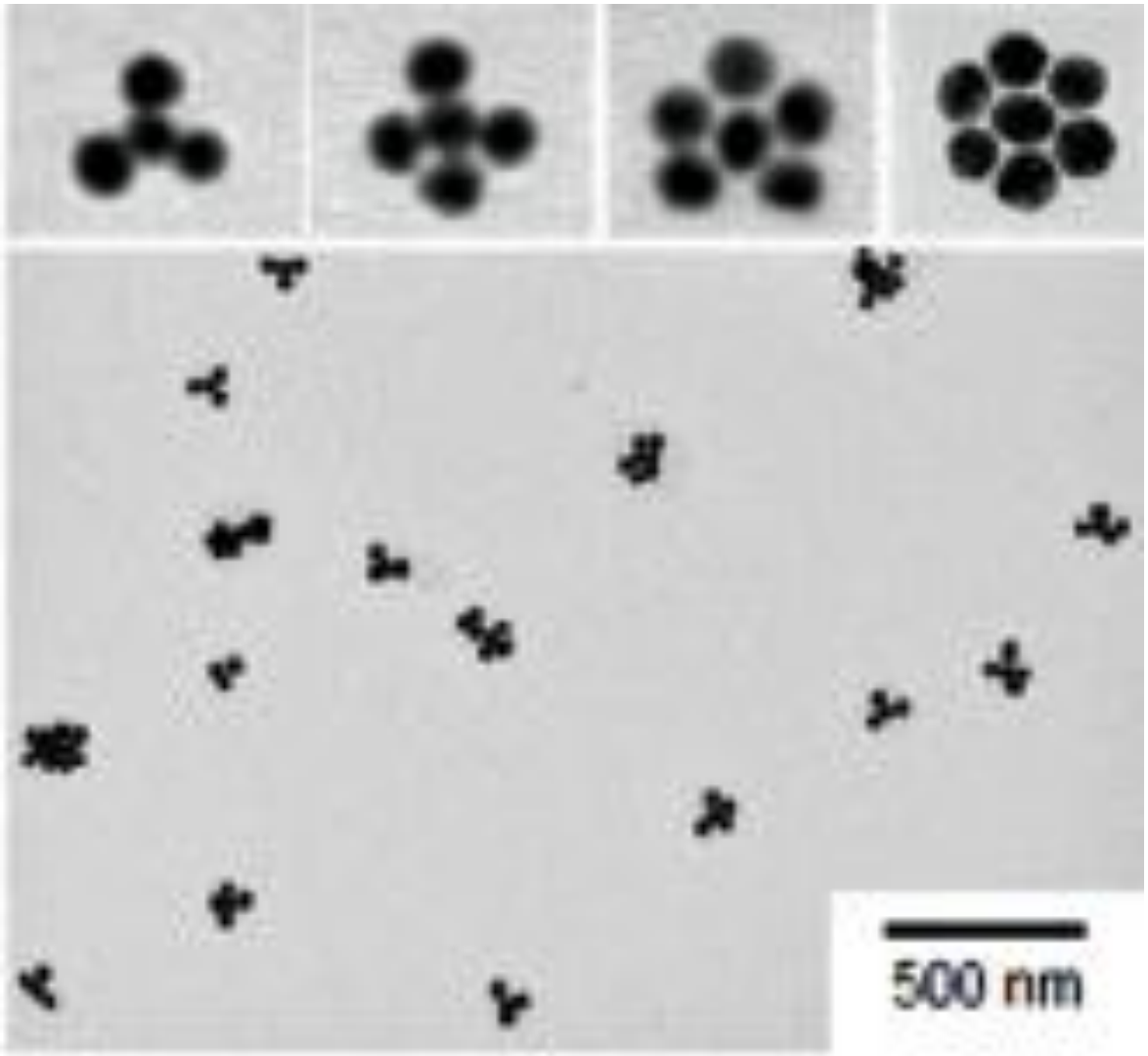
Crown Nanoparticle Plasmon Rulers

Design



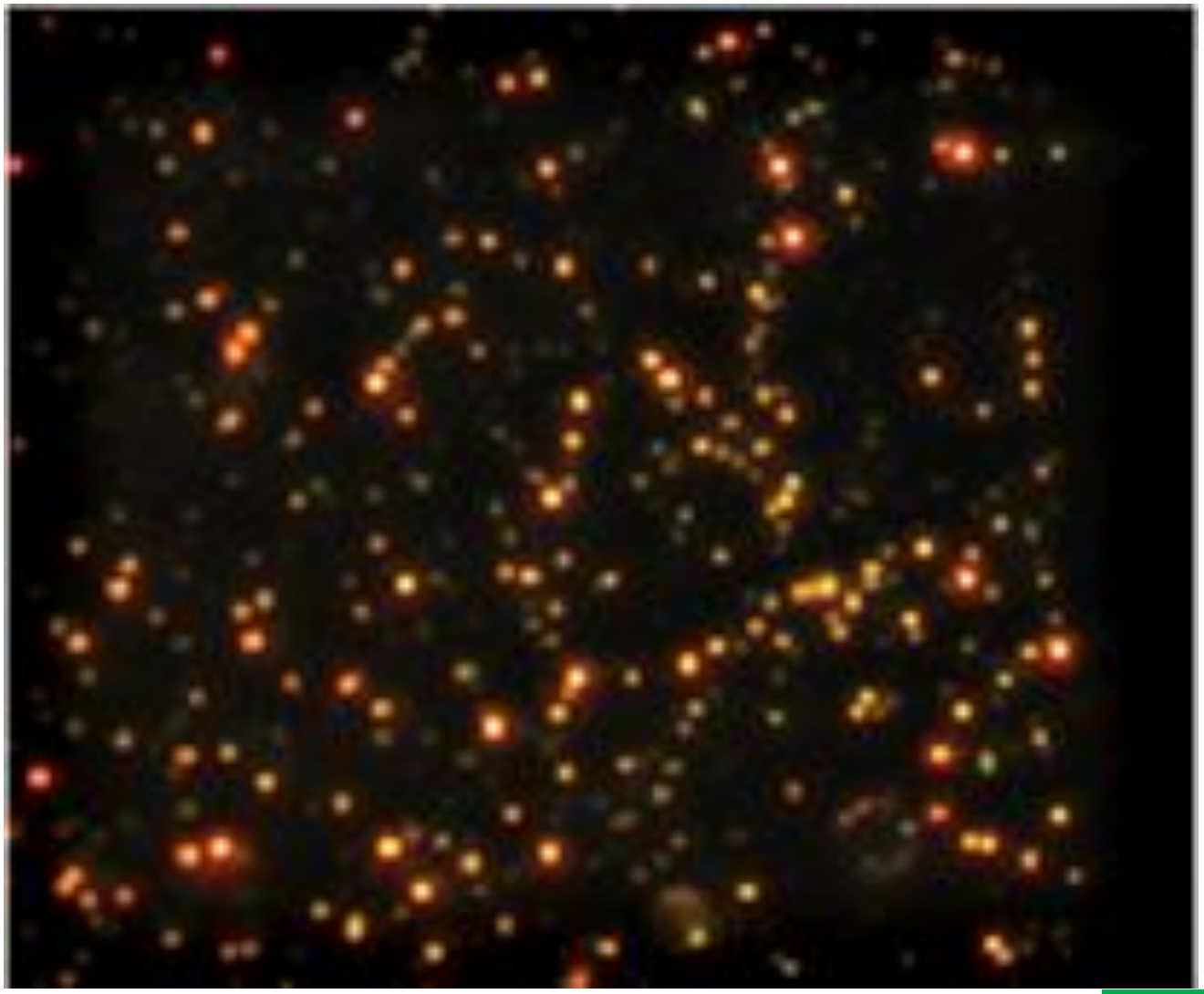
Crown Nanoparticle Plasmon Rulers

TEM



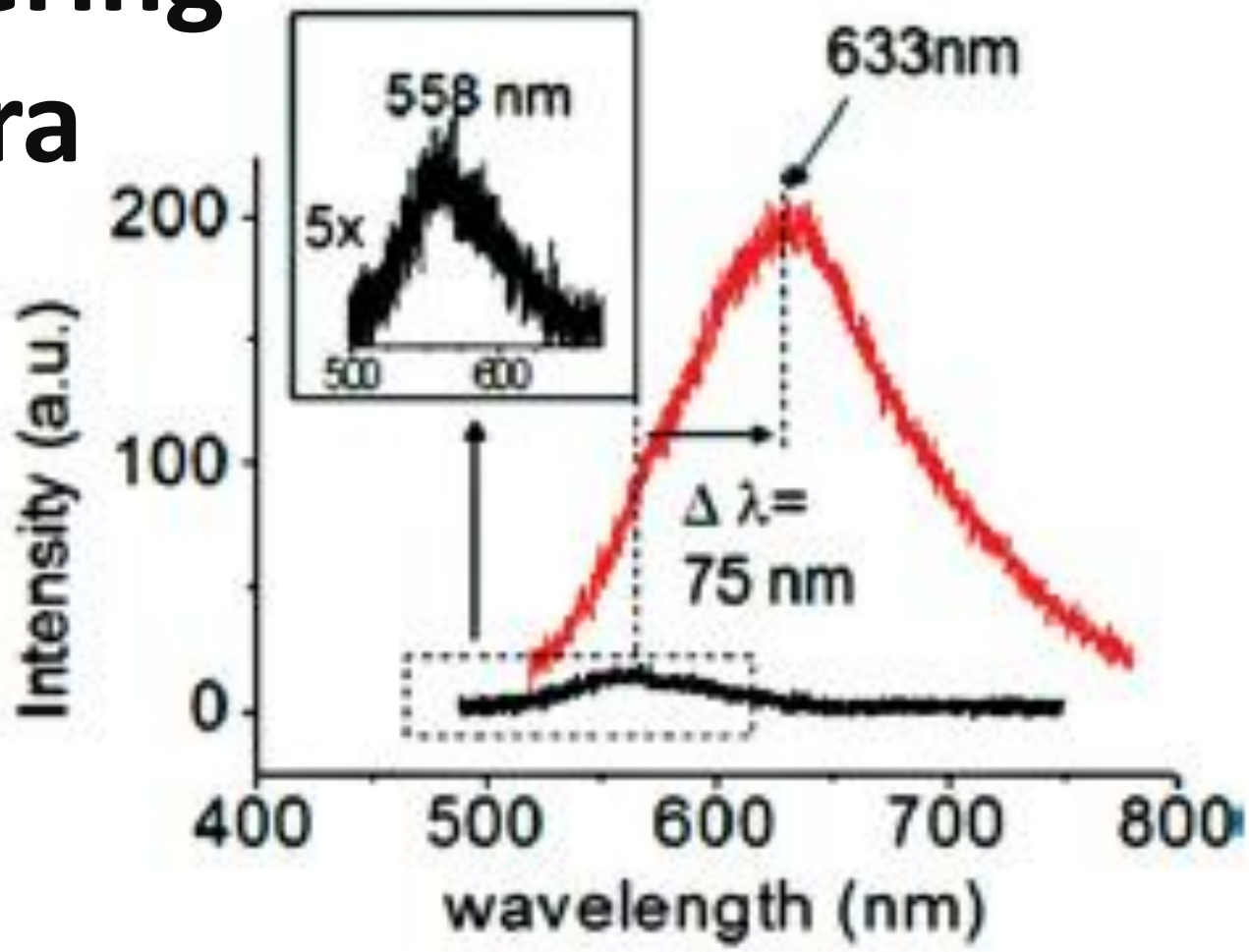
Crown Nanoparticle Plasmon Rulers

Scattering image



Crown Nanoparticle Plasmon Rulers

Scattering spectra



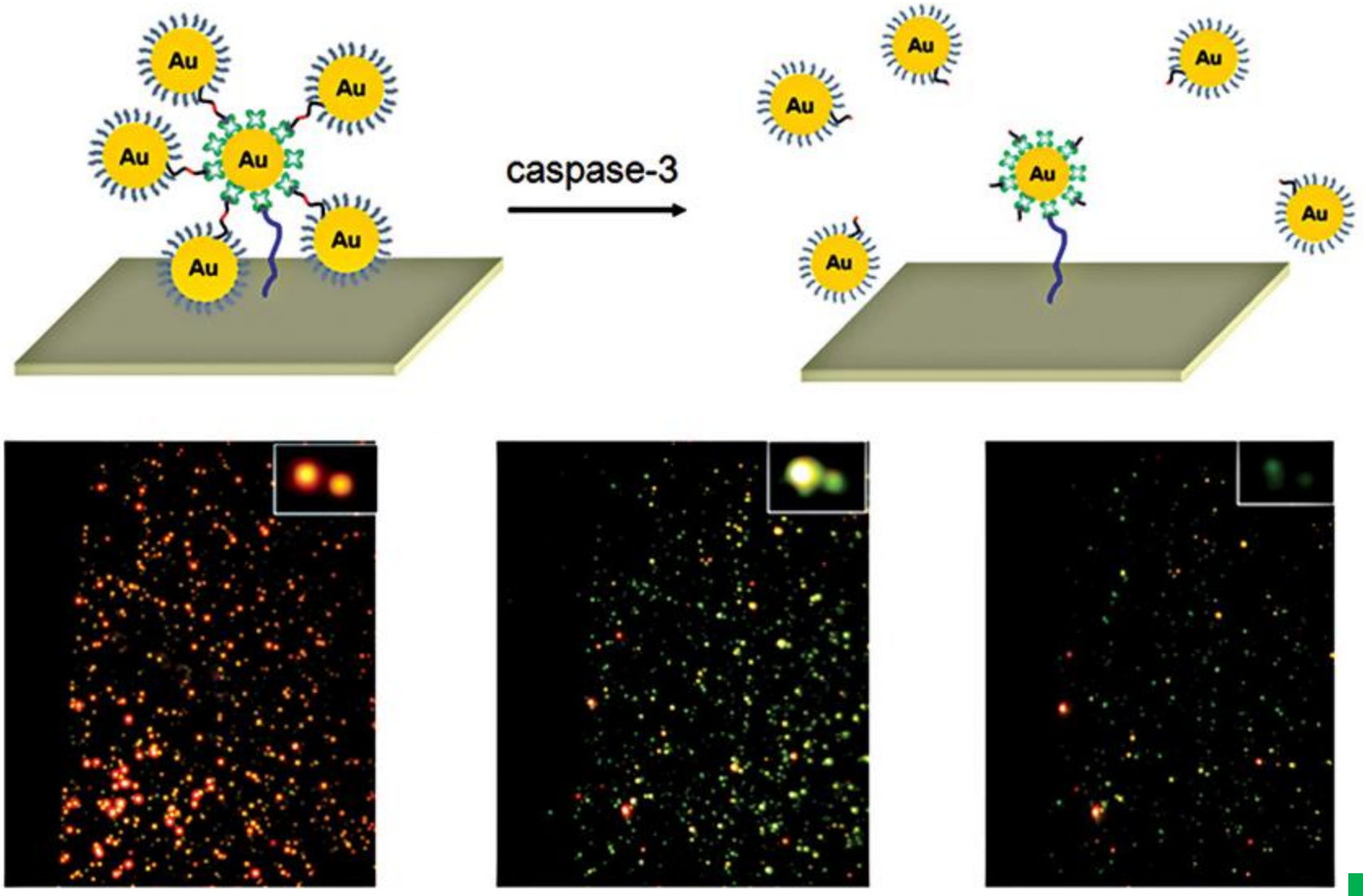
In Vitro Studies -

- The NeutrAvidinfunctionalized crown nanoparticles were immobilized on the biotinylated surface of a glass flow chamber.

- The scattering color and intensity were monitored under a dark-field microscope with a 100-watt tungsten lamp for illumination.

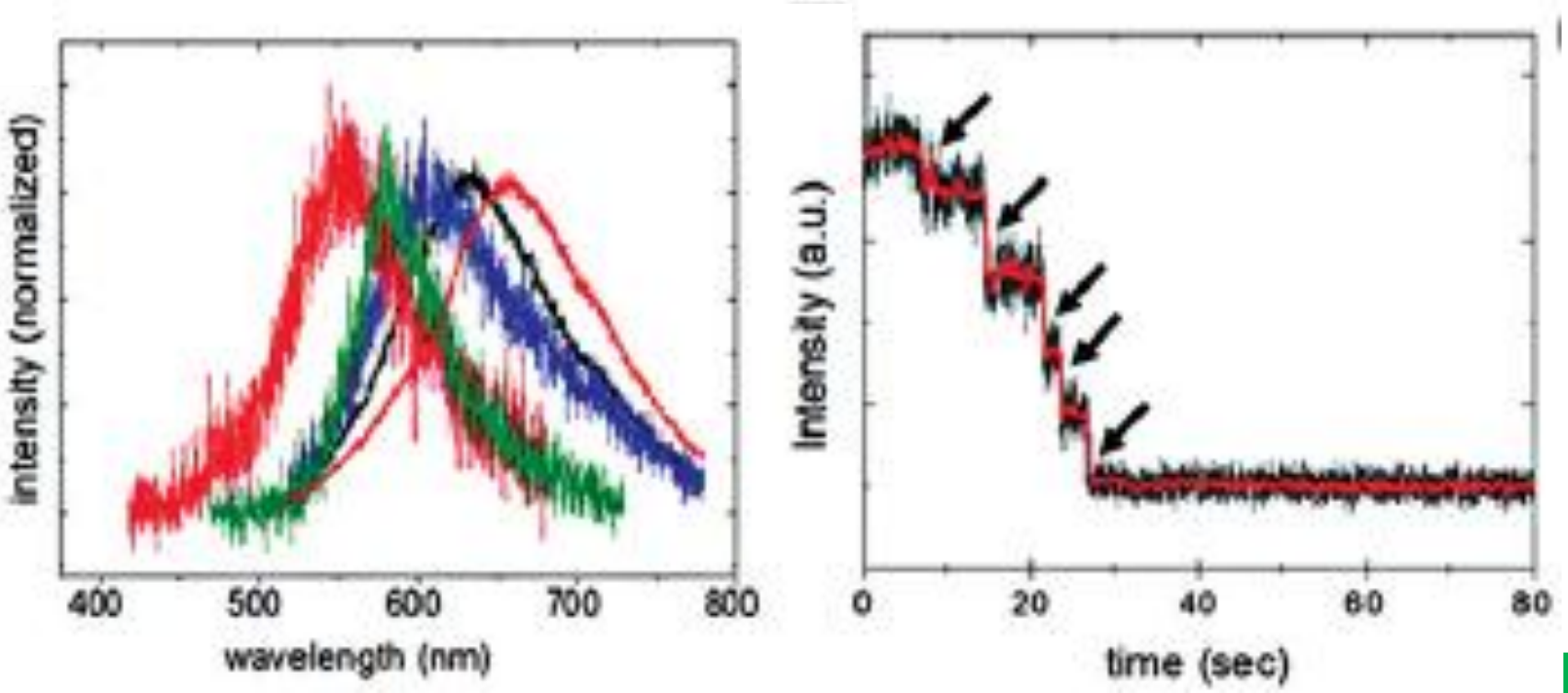
- Upon addition of caspase-3 (250 ng/mL), initial intense red colored spots gradually turned into yellow and then dim green spots as time elapsed.

In Vitro Studies -



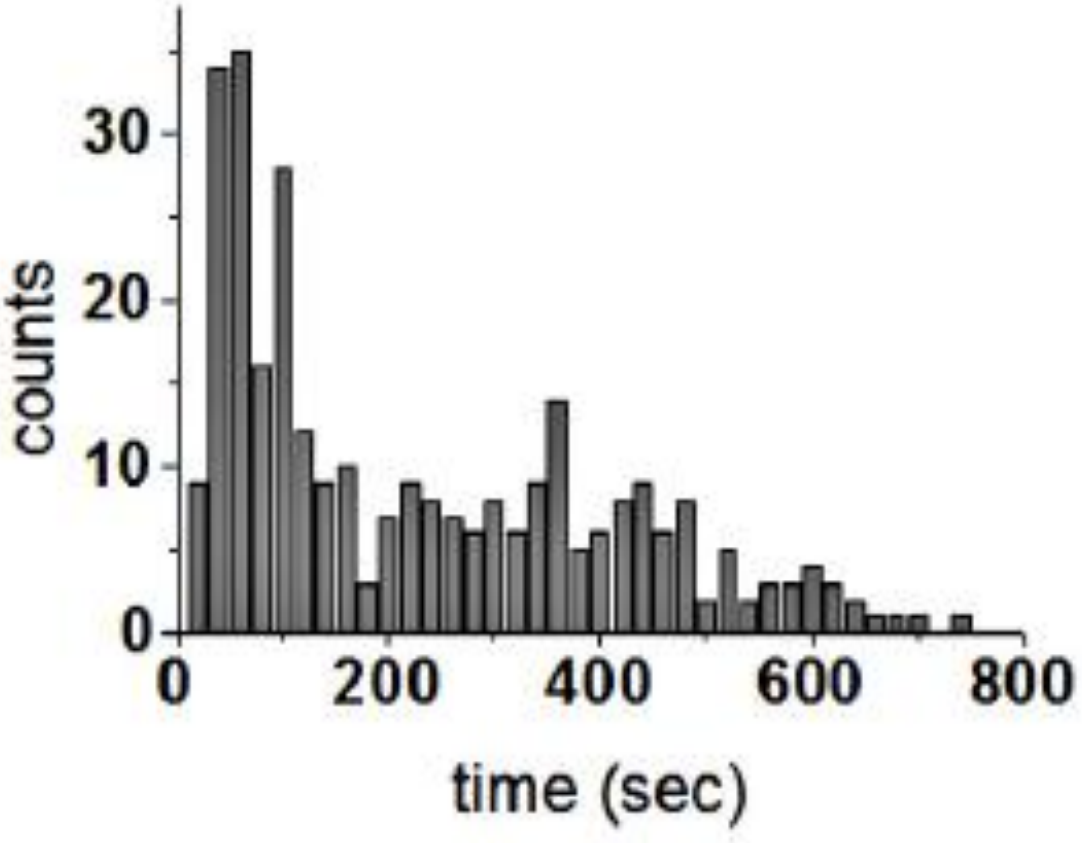
In Vitro Studies -

- Single-particle trajectories of the scattering intensity recorded by electron-multiplying chargecoupled device.
- There observed a stepwise decrease in the scattering intensity corresponding to each individual proteolytic event.



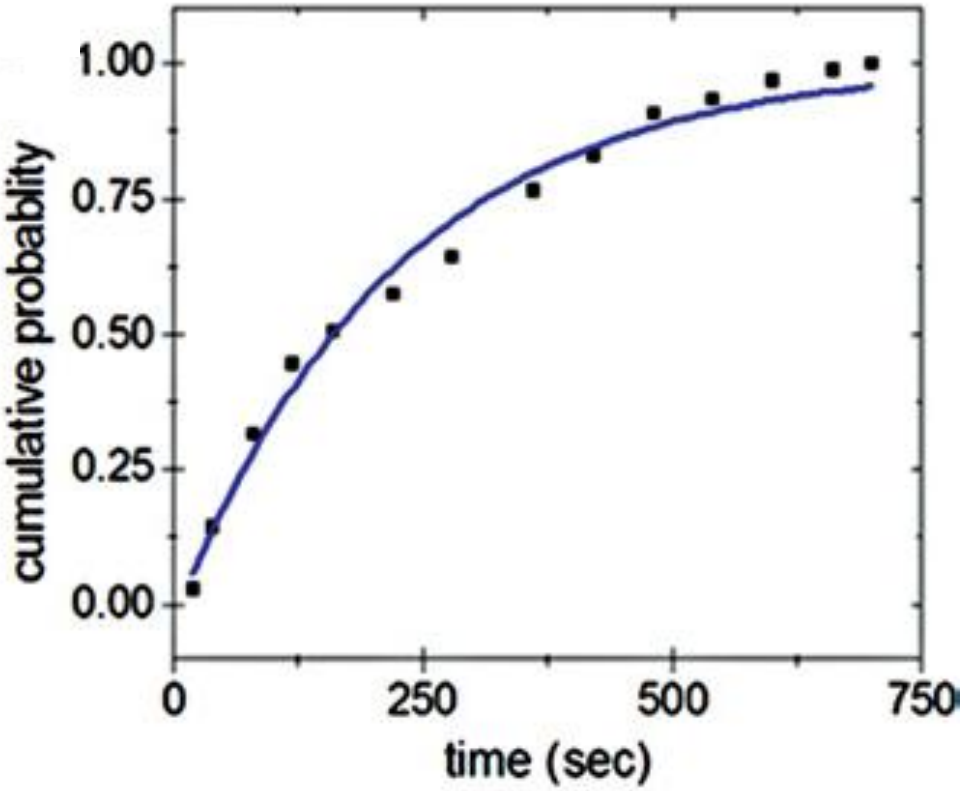
In Vitro Studies -

- To analyze the kinetics of cleavage by caspase-3, each proteolytic event was counted (n = 300) and plotted as a function of time.



In Vitro Studies -

- The cumulative probability was calculated by dividing the number of cleavage events up to a given time, by the total number of cleavage events observed during the experimental time-course.



In Vitro Studies -

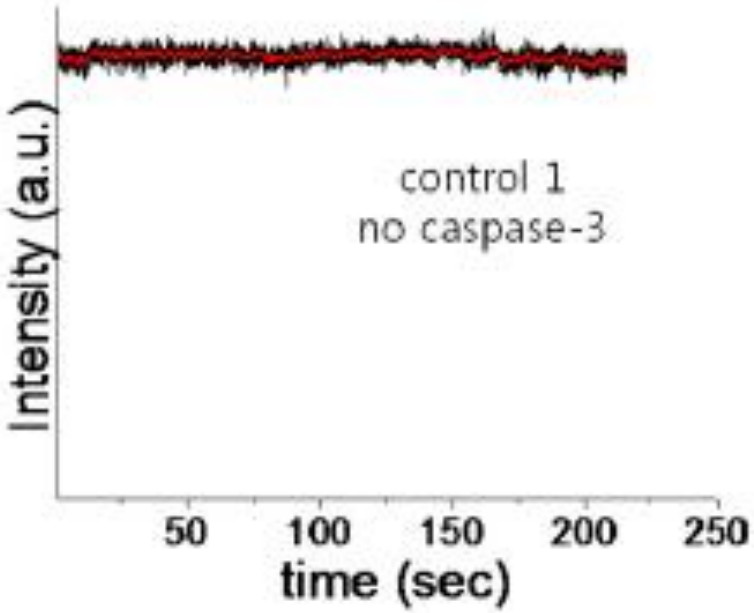
- The findings of this in vitro experiment fit well to a first-order kinetic model, with a kinetic rate constant (k) of 0.0046/sec.

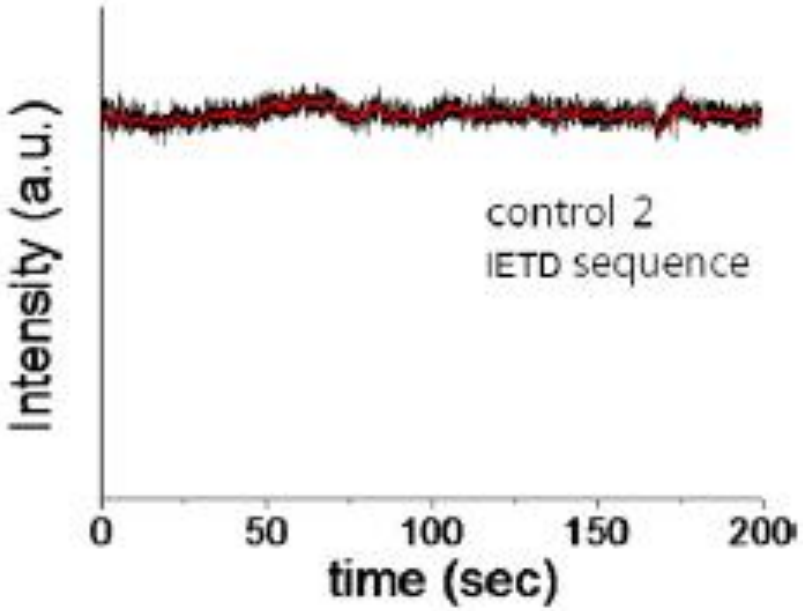
- Using literature values for Km and the rate constant derived from this work, the catalytic kinetic constant (kcat) was calculated to 6.17/sec.

- This value falls within the range of previously reported kcat values (2.4-8.2/sec) obtained in ensemble studies.

In Vitro Studies -

- Control experiments in absence of caspase 3 and with different peptide sequence.





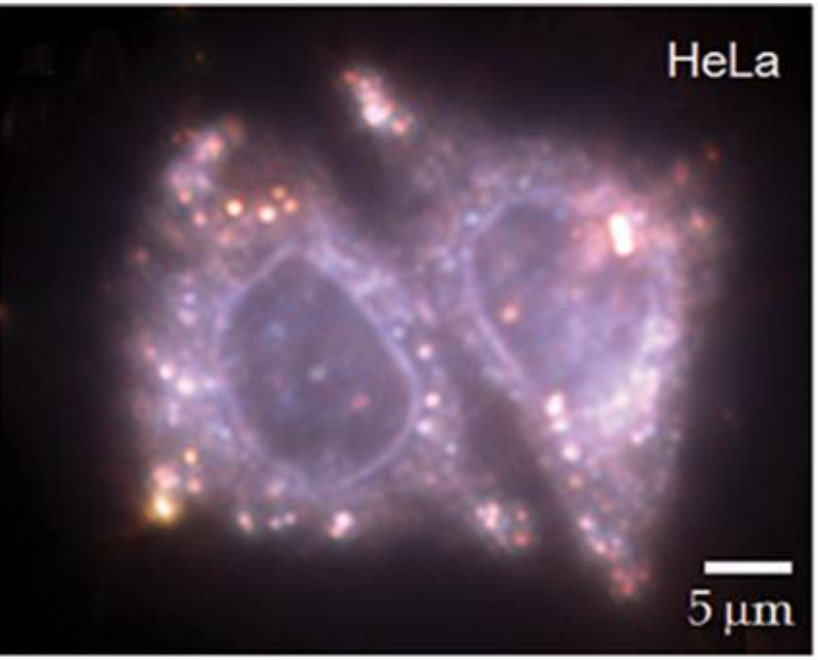
Intracellular Delivery of Plasmon Rulers -

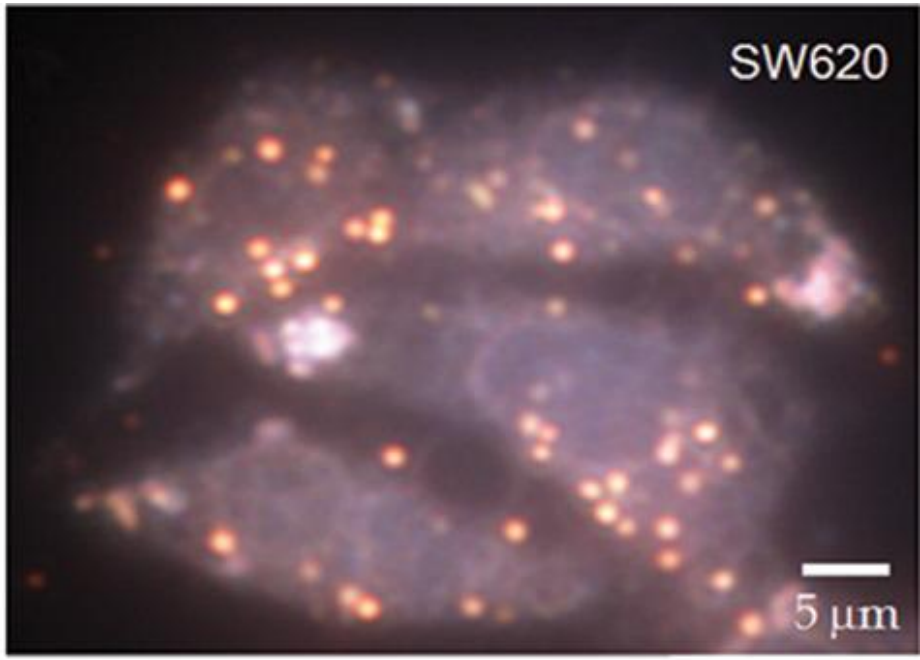
- Crown nanoparticle were conjugated with a biotinylated form of the cell penetration peptide, TAT.

- Either HeLa or SW620 cells were incubated with the TAT-modified crown nanoparticle plasmon rulers for 12 h.

- The background scattering from the cell details its boundary, and the bright red spots inside suggest successful delivery of the plasmon rulers.

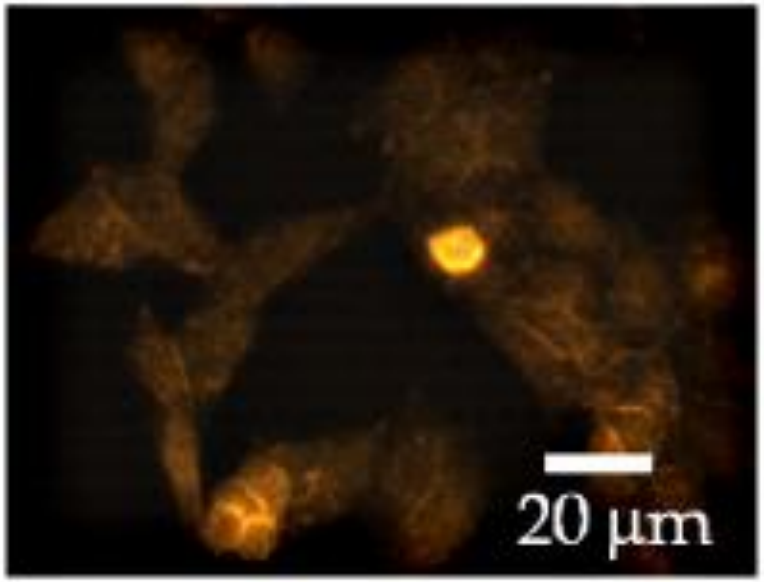
Intracellular Delivery of Plasmon Rulers -



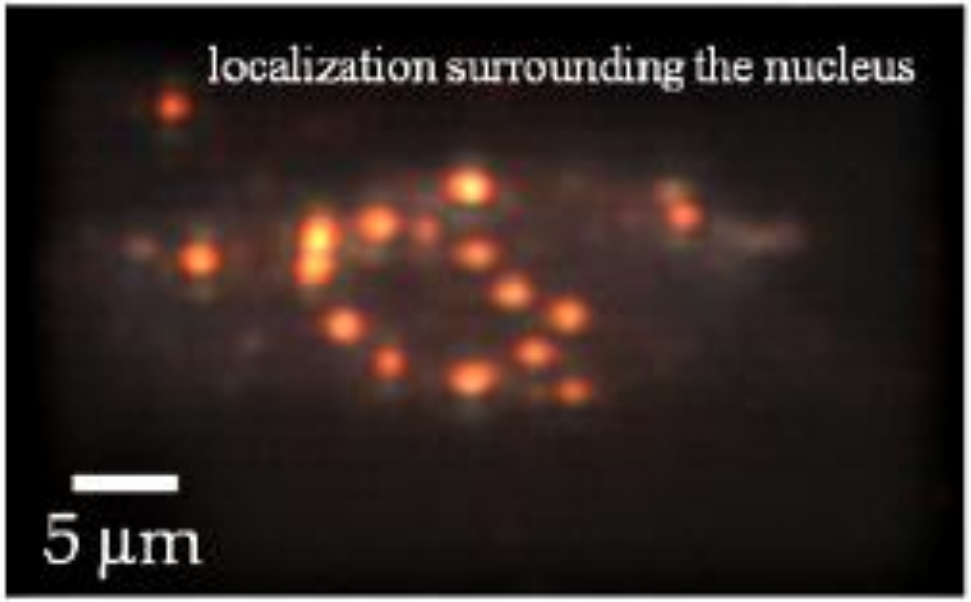


Intracellular Delivery of Plasmon Rulers -

a control:
no biotin-TAT

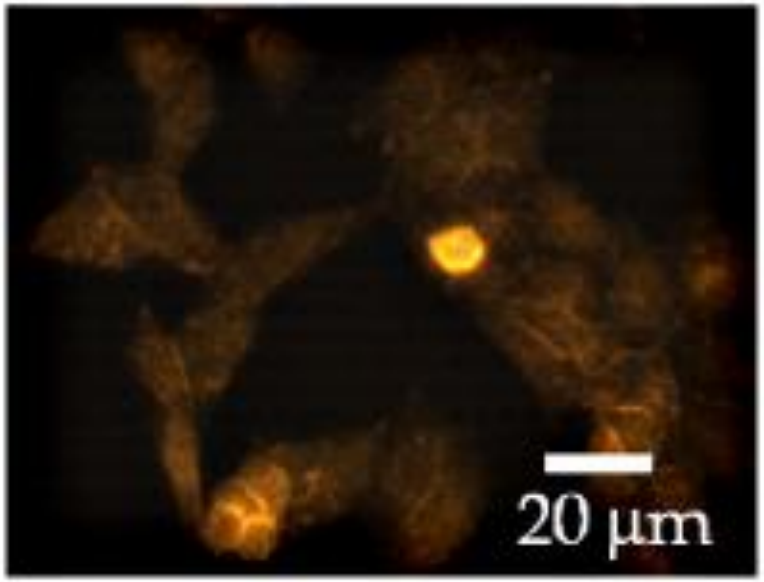


b biotin-TAT-NP
treated sw620 cells

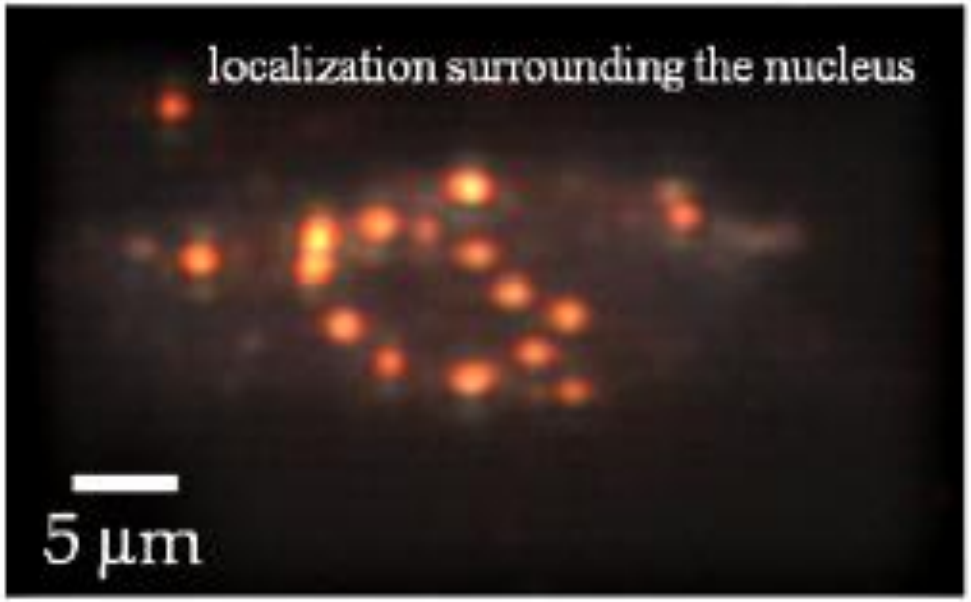


Intracellular Delivery of Plasmon Rulers -

a control:
no biotin-TAT



b biotin-TAT-NP
treated sw620 cells



Intracellular Distribution of Plasmon Rulers -

- TAT peptides release a wide range of cargos, such as gold nanoparticles, from endosomal compartments following endocytosis.

- The nanoparticles that remain endosomally trapped will eventually fuse with lysosomes, which are not accessible to caspase-3.

- The apoptotic inducer used in this study has been shown to permeabilize lysosomal membranes, facilitating the release of proapoptotic cathepsins.

Ensemble Assay of Caspase-3 Activity in SW620

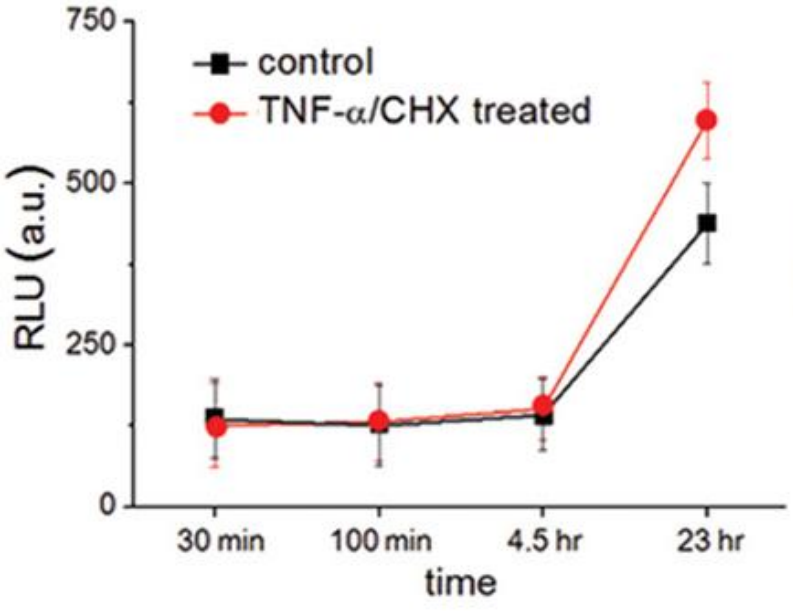
- At first caspase-3 activity in cells was measured via conventional noncontinuous fluorescence-based ensemble techniques.

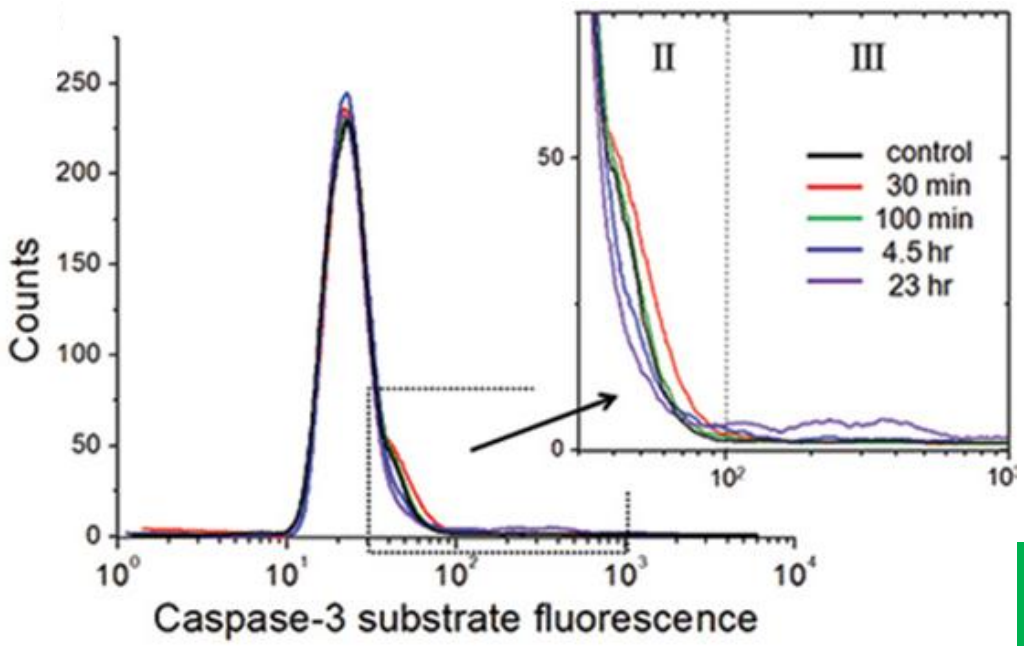
- SW620 cells were treated with the apoptotic inducers, tumor necrosis factor-α (TNF-α), and cycloheximide (CHX).

- SW620 cells were chosen because caspase-7, the major competitor for the DEVD sequence, is absent in SW620 cells at the mRNA level.

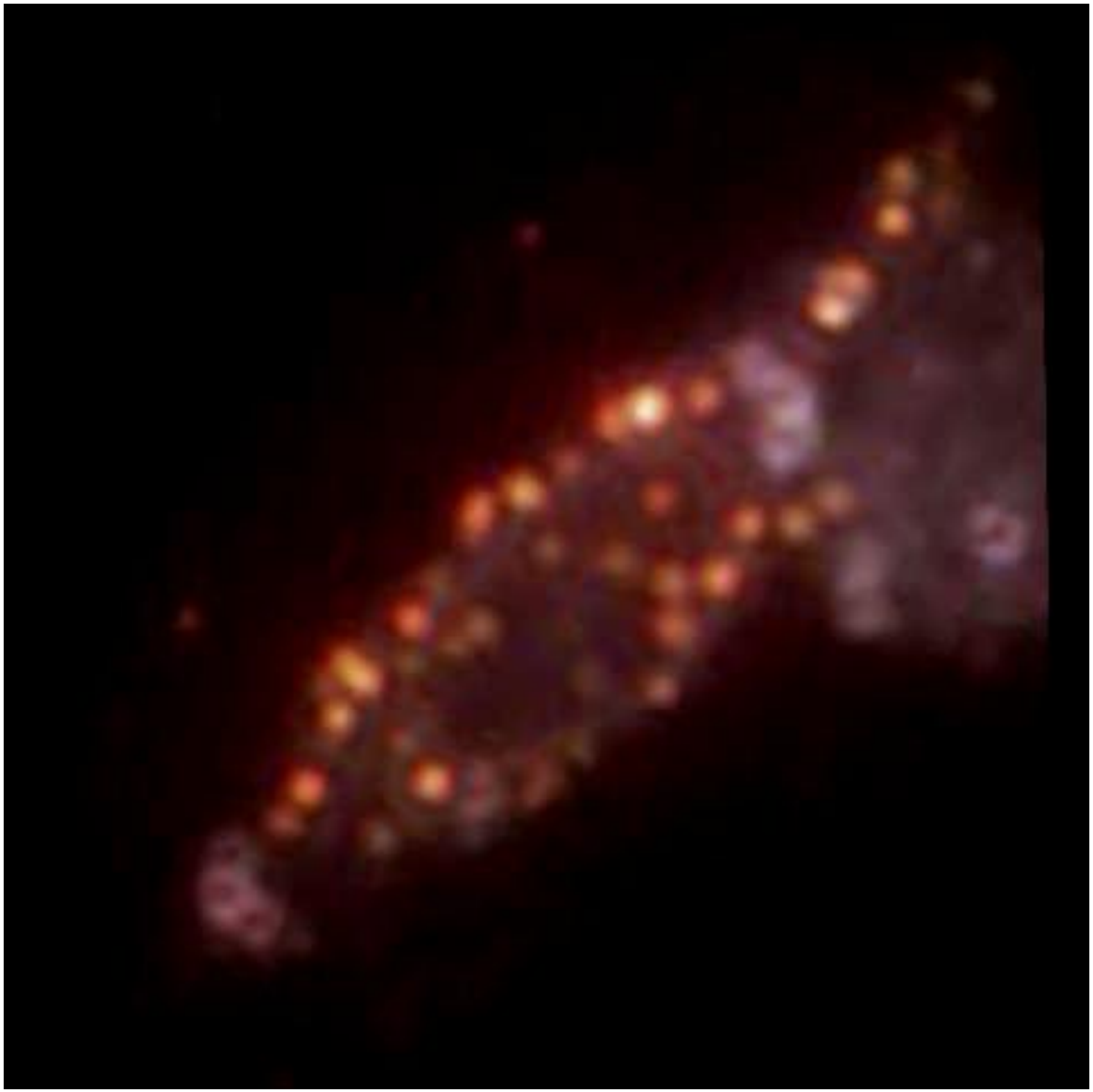
Ensemble Assay of Caspase-3 Activity in SW620

- SW620 cells are resistant to death receptor-induced apoptosis and exhibit low levels of caspase-3 activity upon addition of death receptor agonists.
- Low levels of caspase-3 activity was studied by luminescent assay and flow cytometry.

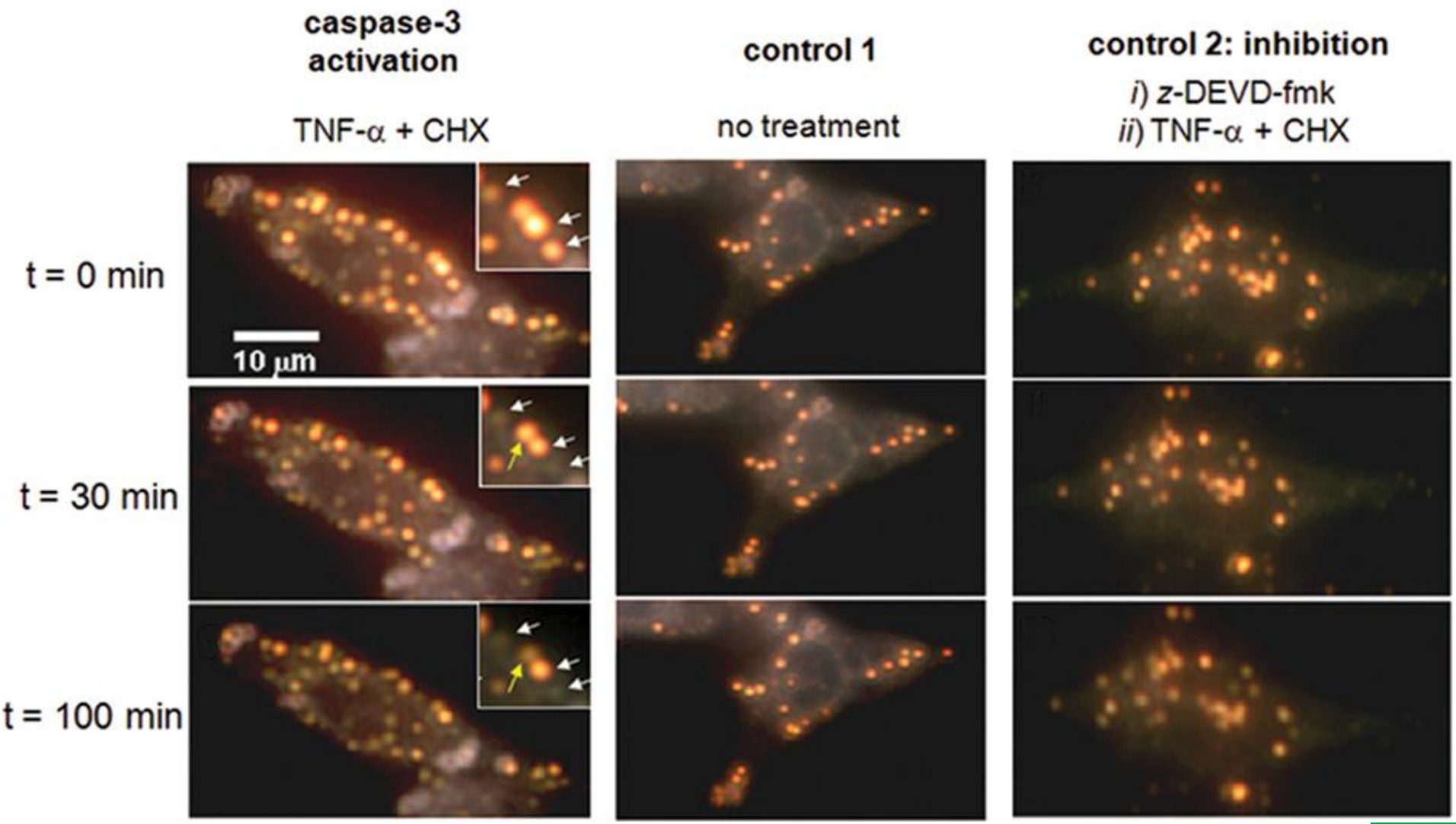




Imaging of Caspase-3 Activation

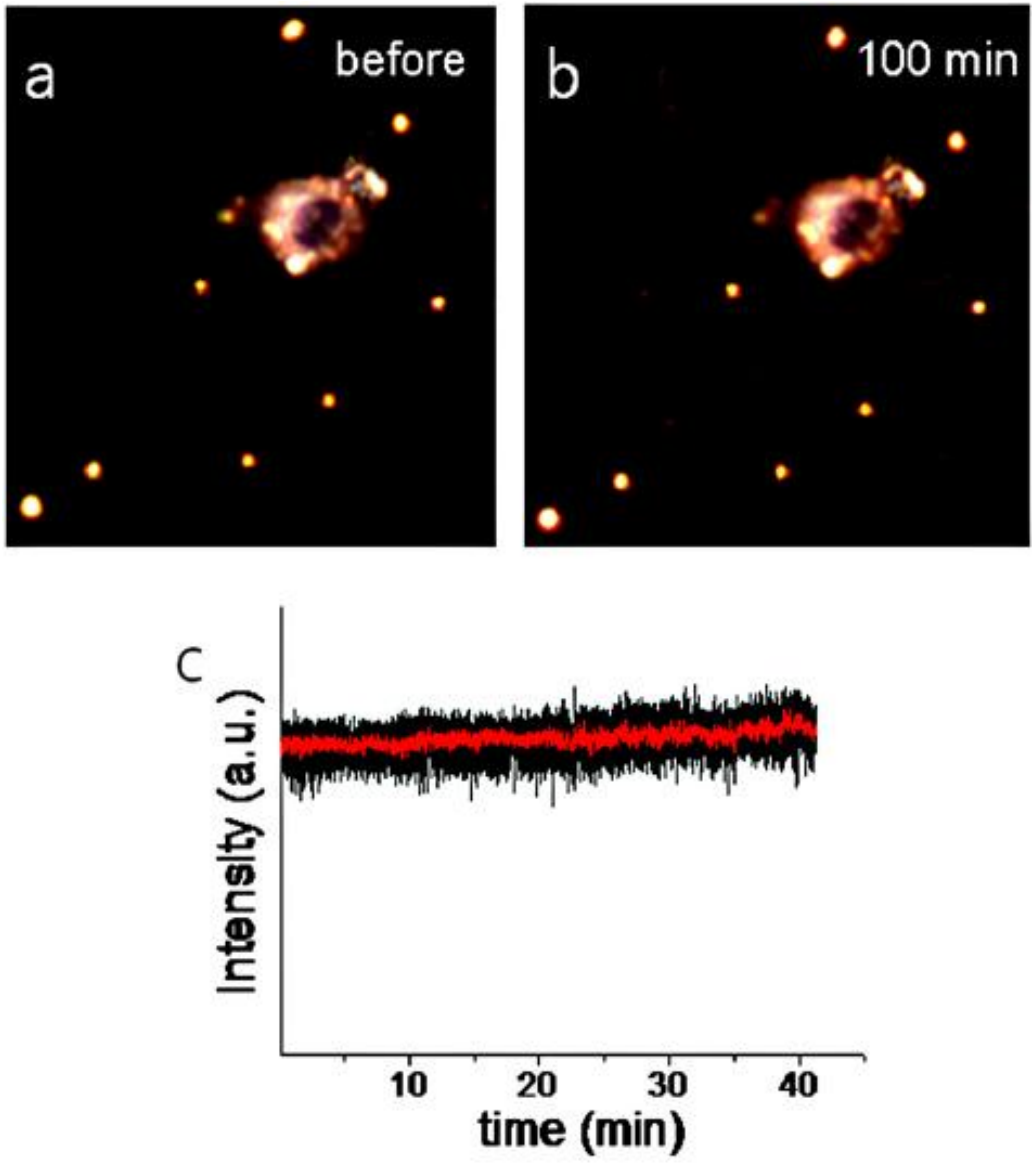


Imaging of Caspase-3 Activation



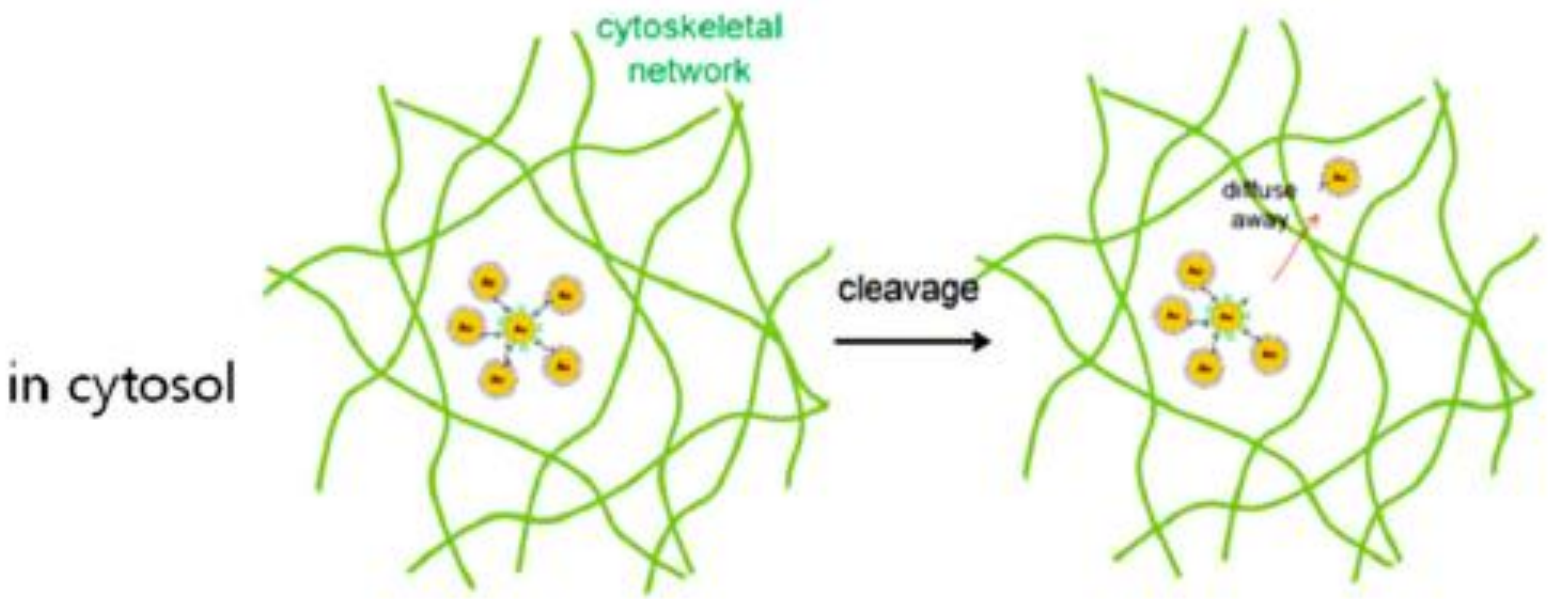
Extracellular plasom rulers -

Control



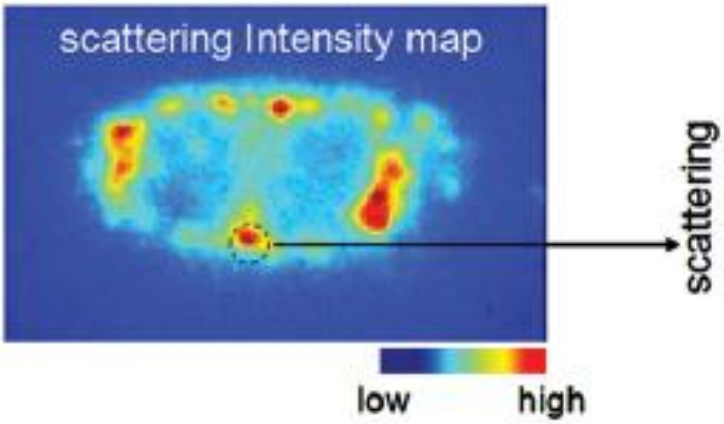
Imaging of Caspase-3 Activation

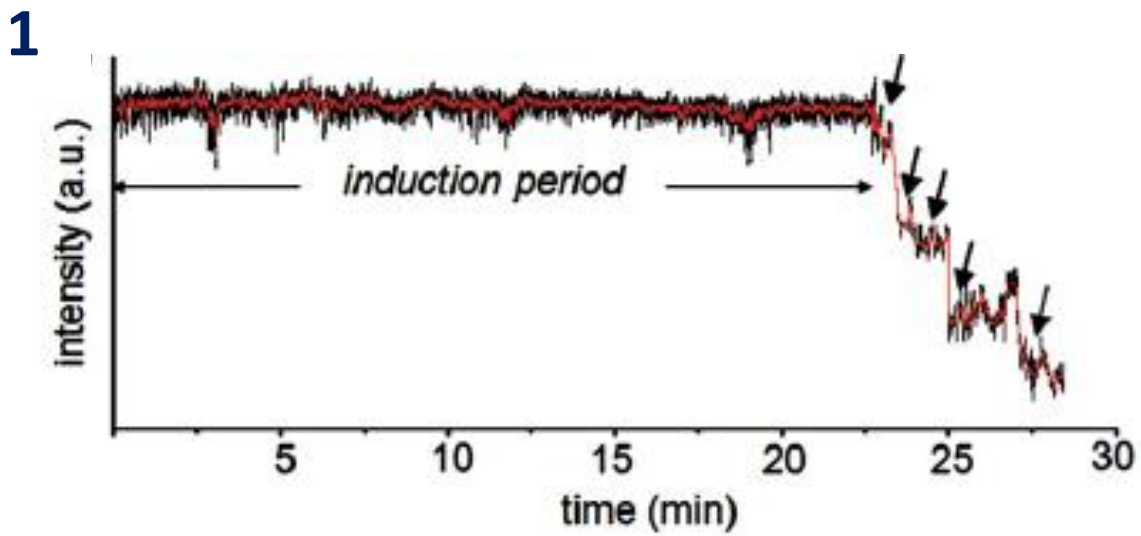
- Unlike small molecular probes, the movement of crown plasmon rulers is minimal during the time course.



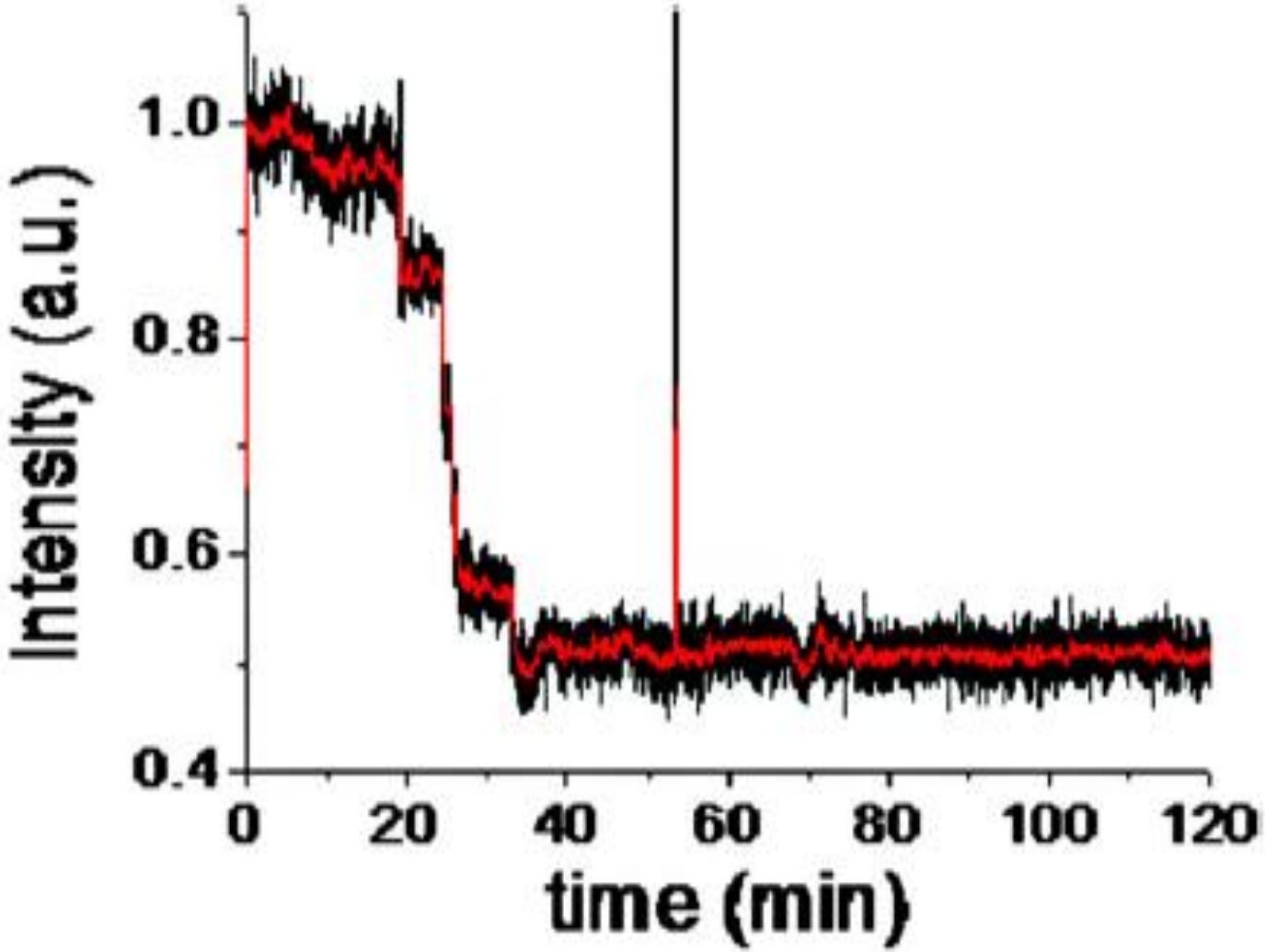
Imaging of Caspase-3 Activation

- Scattering intensity map of a whole cell labeled with crown nanoparticles and a representative time trace of a single crown nanoparticle probe upon addition of the apoptotic inducers.

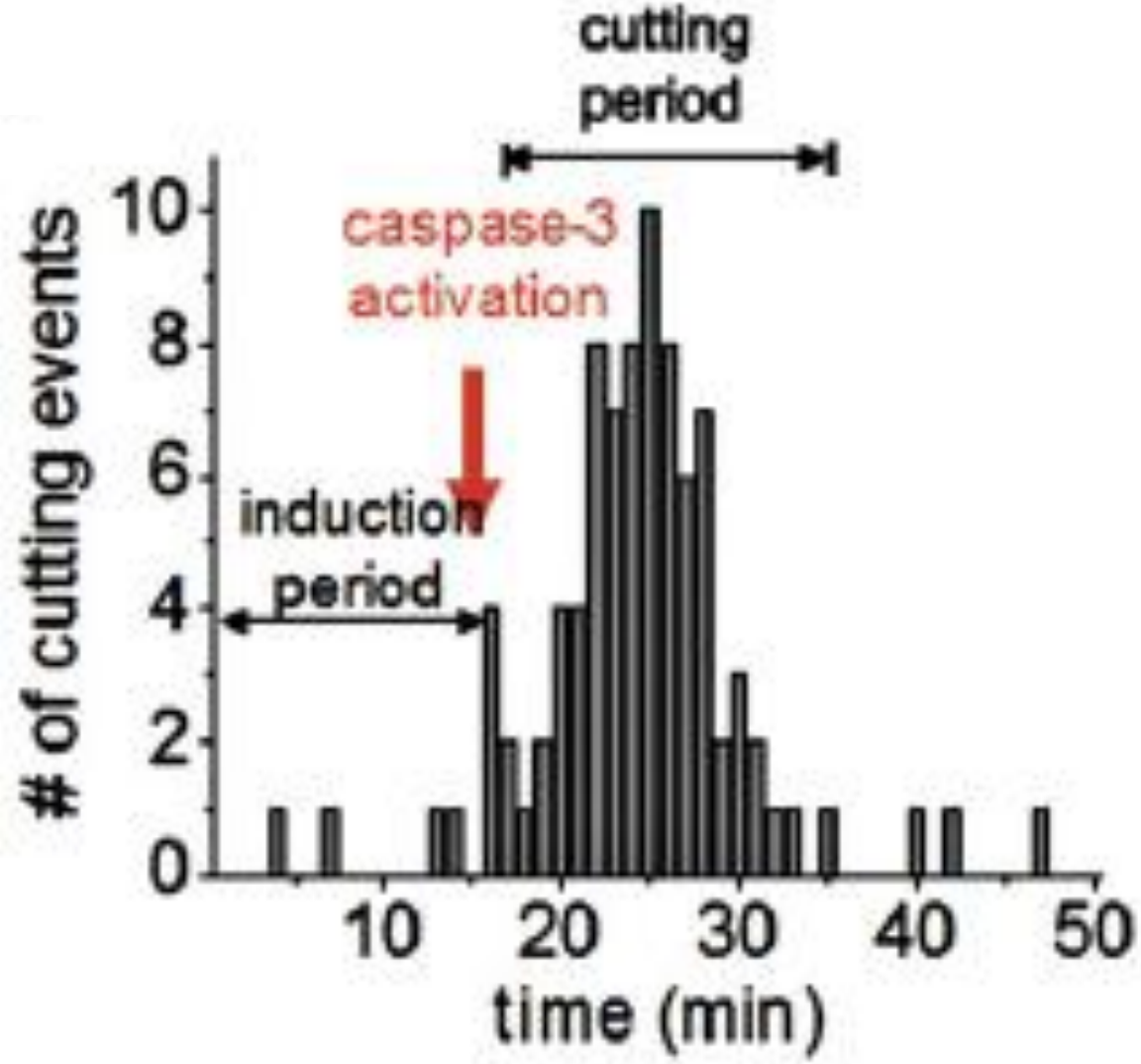




Imaging of Caspase-3 Activation

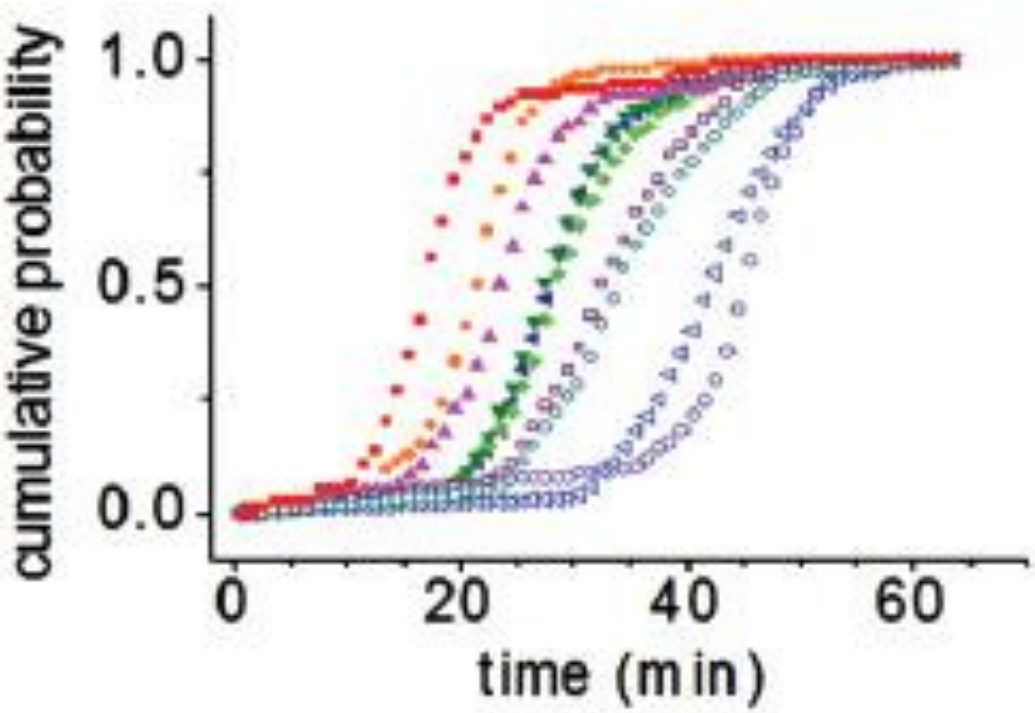


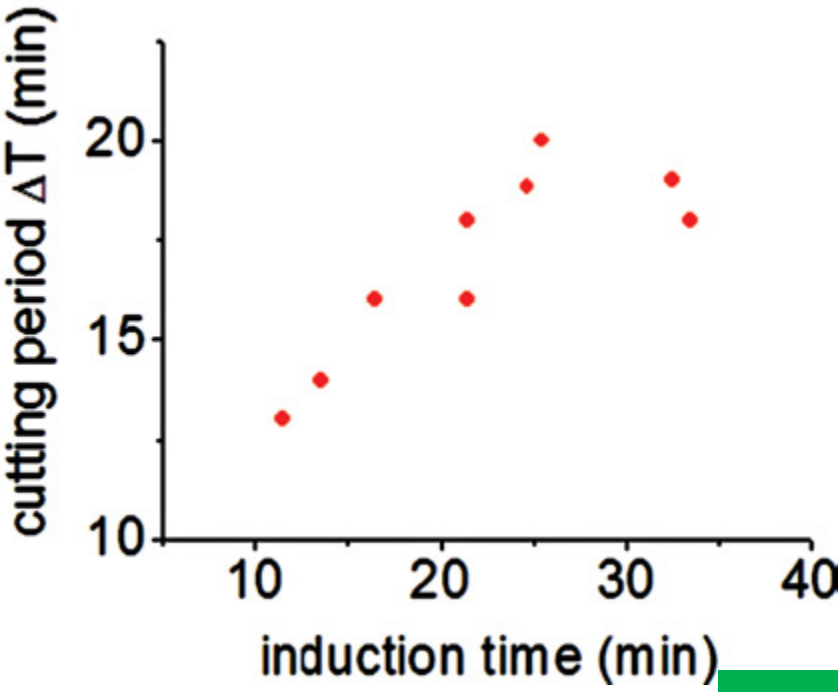
Imaging of Caspase-3 Activation



Imaging of Caspase-3 Activation

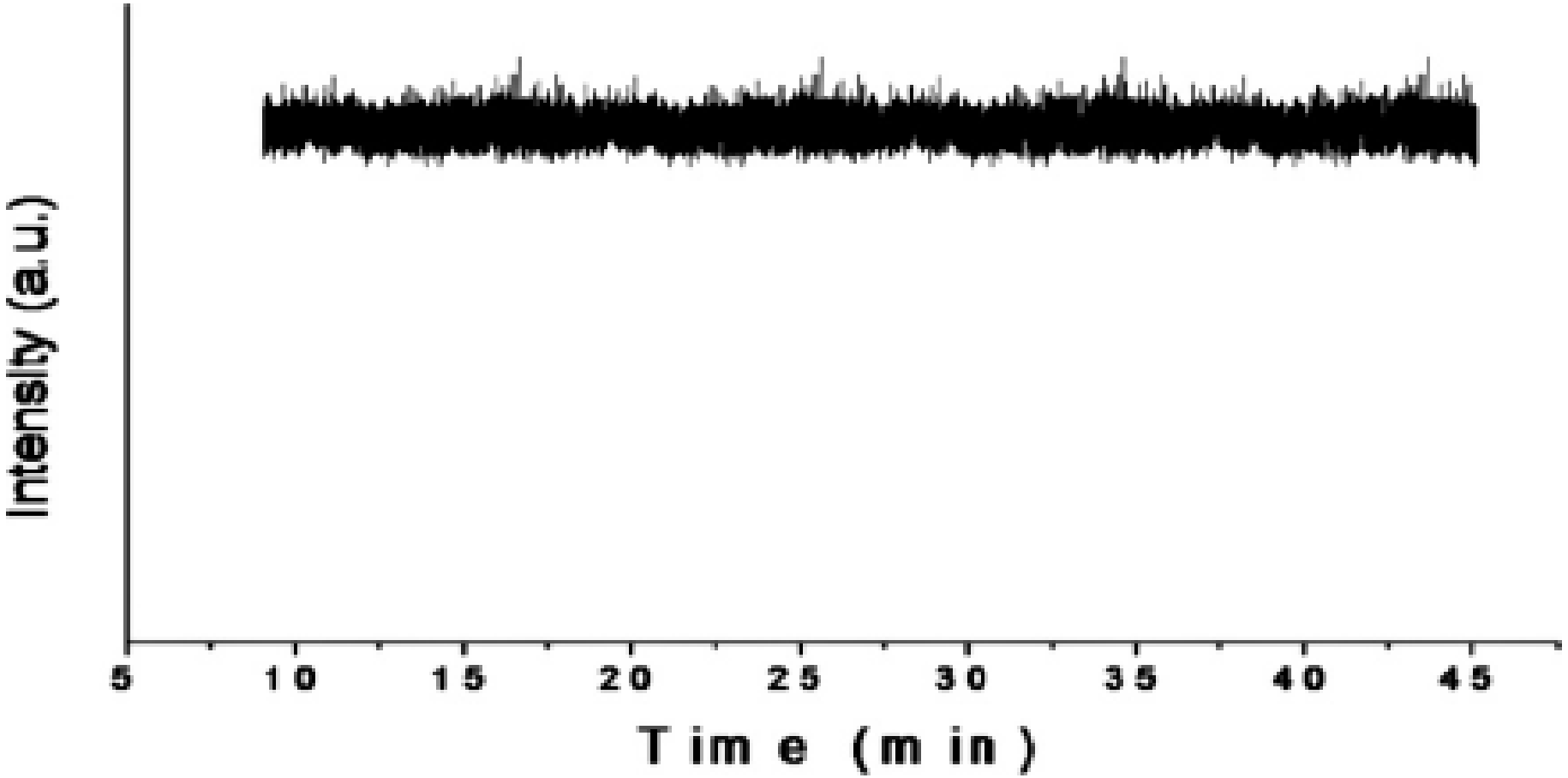
- Trajectory of a single crown nanoparticles located inside vehicle treated SW620 cells.Differences in induction times across cells in the same population reflect cell-by-cell heterogeneity against caspase-3 activation.





Imaging of Caspase-3 Activation

Trajectory for particle Inside vesicle



Conclusions

- Crown nanoparticle plasmon rulers can be used for continuous observation of caspase-3 activity.

- Caspase-3 activation kinetics were successfully analyzed at the single-molecule level.

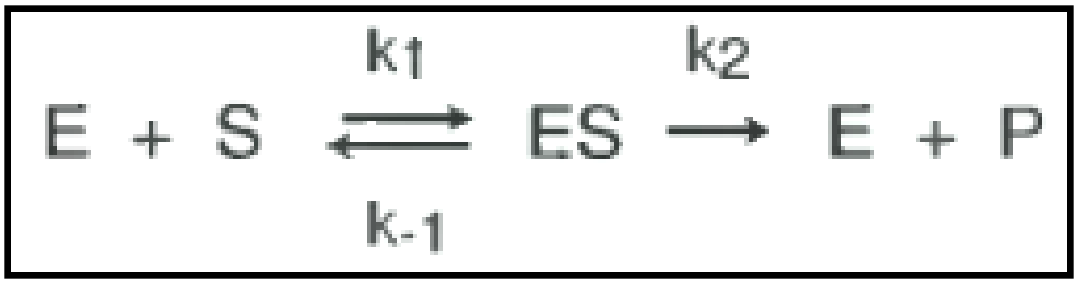
- Unlike small molecular probes, the movement of crown plasmon rulers is minimal during the time course.

- Variability in the cutting period and induction time measurements across cells in the same population reflect the heterogeneity in the resistance of cells to caspase-3 activation.

Thank you

Michaelis-Menten Equation

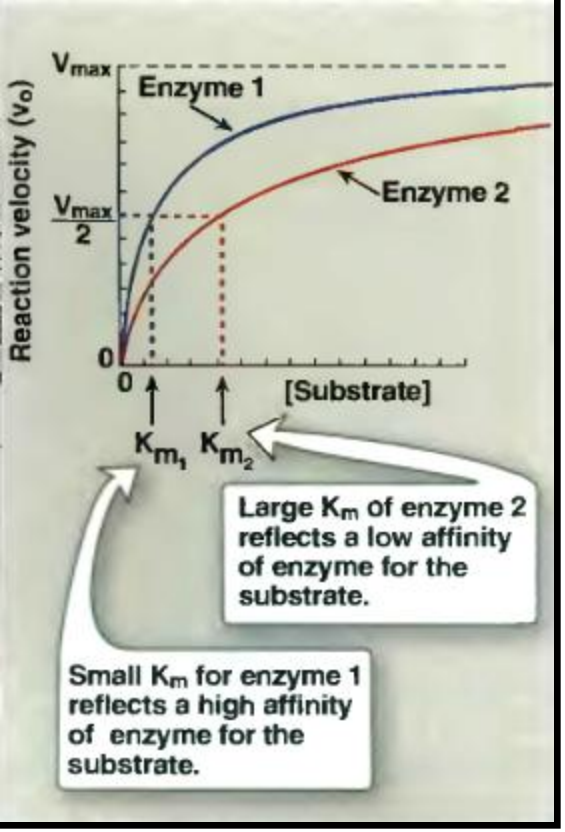
Michaelis-Menten proposed a reaction model, in which enzyme reacts with substrate reversibly to form ES and yield product and enzyme.



$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

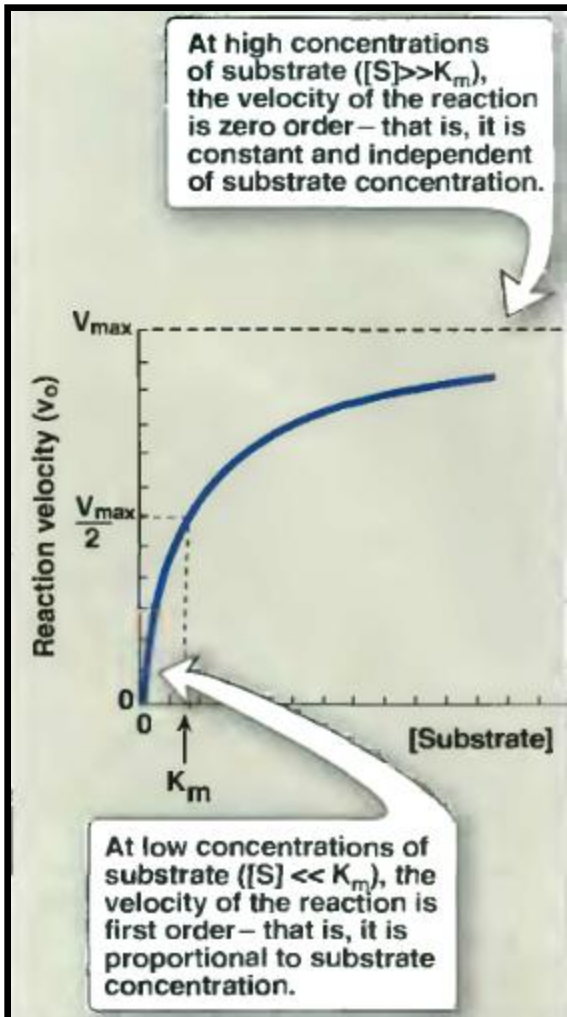
This equation tells how reaction velocity varies with substrate concentration. Here Michaelis constant $K_m = (k_{-1} + k_2)/ k_1$, V_0 = initial velocity. Assumptions in Michaelis-Menten rate equation

1. [S] >>[E]
2. **Steady state assumption** [ES] = constant, rate of formation of ES is equal to rate of breakdown.
3. Only initial velocity is used in analysis of enzyme reactions.



Michaelis-Menten kinetics

- K_m = [S] at which reaction velocity = $V_{max}/2$, and it reflects the affinity of enzyme for substrate
- At all [S], the rate of reaction is directly proportional to [E]



- At lower [S] velocity of reaction is approx. proportional to [S](**first order reaction**) and at higher [S] it is constant => **zero order reaction**