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ARTICLES

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Watching conformational- and photodynamics of single fluorescent proteins in solution

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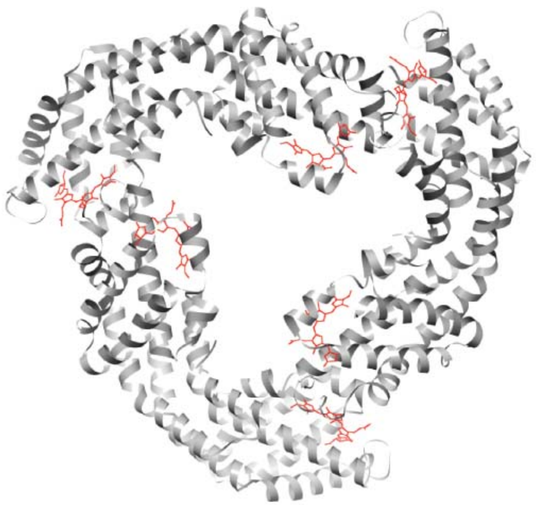
Observing the dynamics of single biomolecules over prolonged time periods is difficult to achieve without significantly altering the molecule through immobilization. It can, however, be accomplished using the anti-Brownian electrokinetic trap, which allows extended investigation of solution-phase biomolecules—without immobilization—through real-time electrokinetic feedback. Here we apply the trap to study an important photosynthetic antenna protein, allophycocyanin. The technique allows the observation of single molecules of solution-phase allophycocyanin for more than one second. We observe a complex relationship between fluorescence intensity and lifetime that cannot be explained by simple static kinetic models. Light-induced conformational changes are shown to occur and evidence is obtained for fluctuations in the spontaneous emission lifetime, which is typically assumed to be constant. Our methods provide a new window into the dynamics of fluorescent proteins and the observations are relevant for the interpretation of *in vivo* single-molecule imaging experiments, bacterial photosynthetic regulation and biomaterials for solar energy harvesting.



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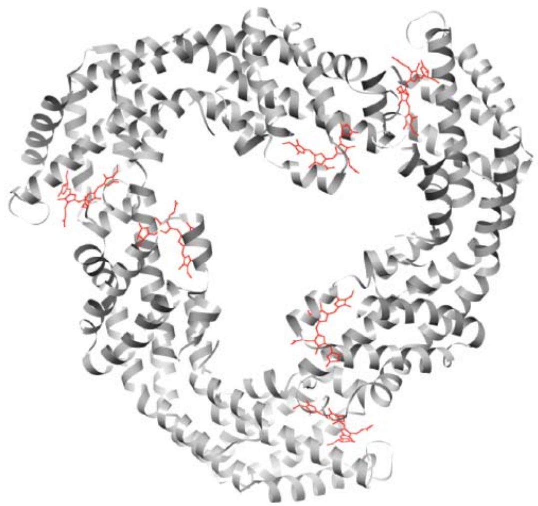
Soft Matter Optical Spectroscopy

Allophycocyanin (APC) fluorescence protein

Disk-like trimer $(\alpha\beta)_3$
11 nm diameter and 3 nm thick



Single Molecule Spectroscopy in the Solution-phase

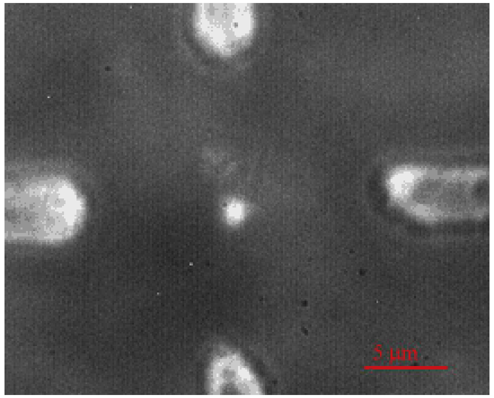
1. Optical Tweezers Method

   Restricted size (100nm to 1μm)

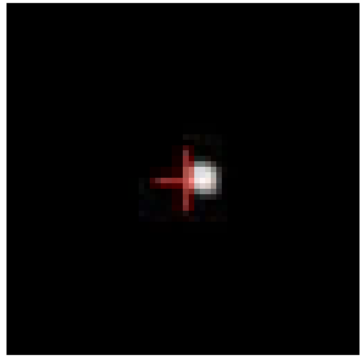
2. Surface-attachment chemistry

   Are surface-immobilized molecules same as their free-solution state?

-> Anti-Brownian electrokinetic (ABEL) Trap method



ABEL (anti-Brownian electrokinetic) Trap



Suppressing Brownian motion of individual biomolecules in solution

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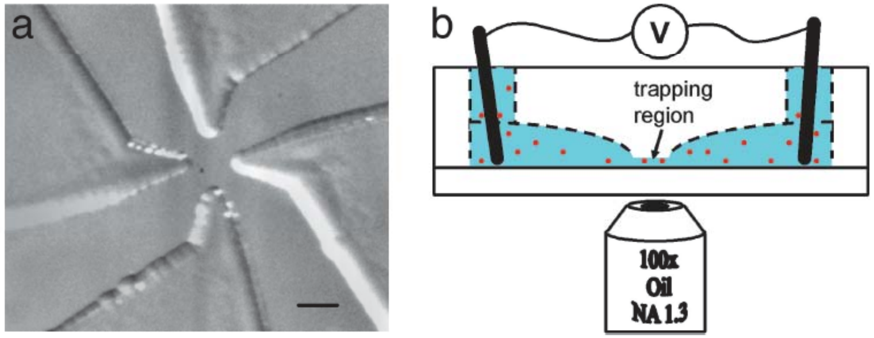
Single biomolecules in free solution have long been of interest for detailed study by optical methods, but Brownian motion prevents the observation of one single molecule for extended periods. We have used an anti-Brownian electrokinetic (ABEL) trap to trap individual protein molecules in free solution, under ambient conditions, without requiring any attachment to beads or surfaces. We also demonstrate trapping and manipulation of single virus particles, lipid vesicles, and fluorescent semiconductor nanocrystals.

anti-Brownian electrokinetic trap | electrophoresis | feedback | single molecule | trapping



Apply Electric Potential Make

1. *Charged particles are directly acted.*
2. *Electroosmotic flow*

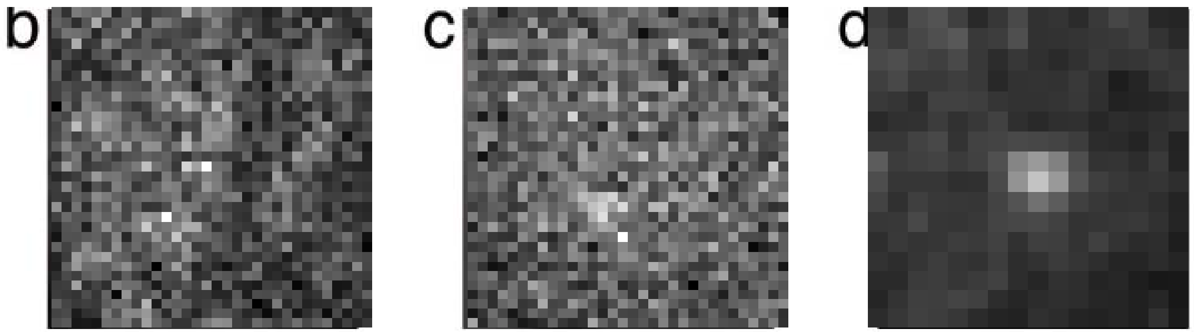
ABEL (anti-Brownian electrokinetic) Trap

Electrokinetic forces is very strong
-> Trapping frequency is determined by latency of the feedback loop

$$k_{eff,ABEL} \propto \eta a$$

$$k_{eff,o.tweezer} \propto a^3$$

ABEL could trap smaller fluorescent than optical tweezers

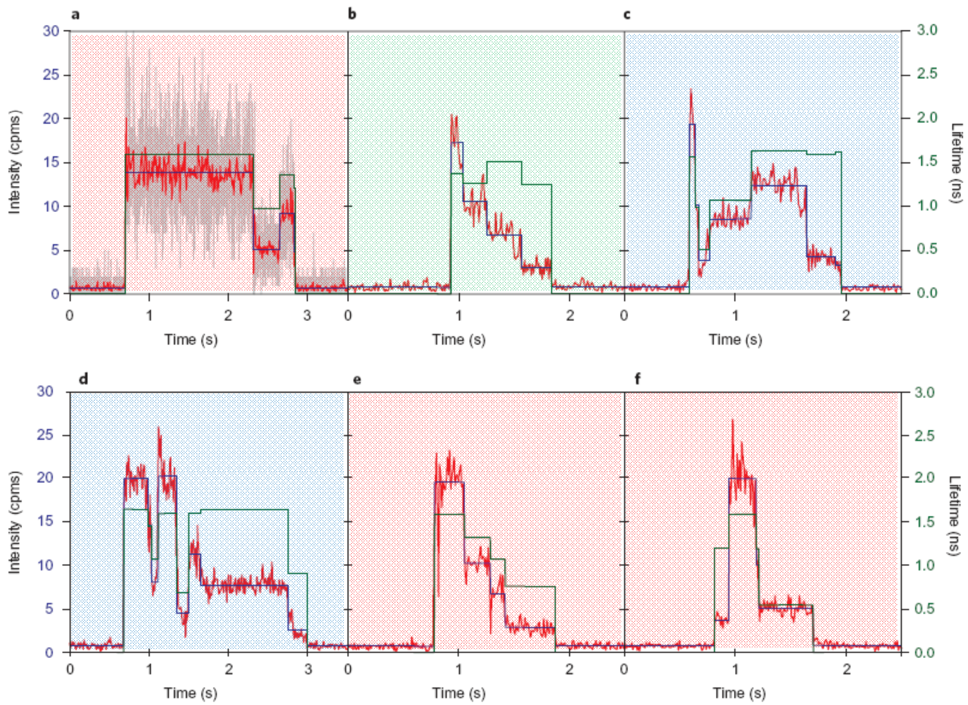


TMV (Virus)
300nm x 15nm

Lipid vesicle

Single CdSe QD

Results 1.



Correlated

Anti-correlated

Non-correlated

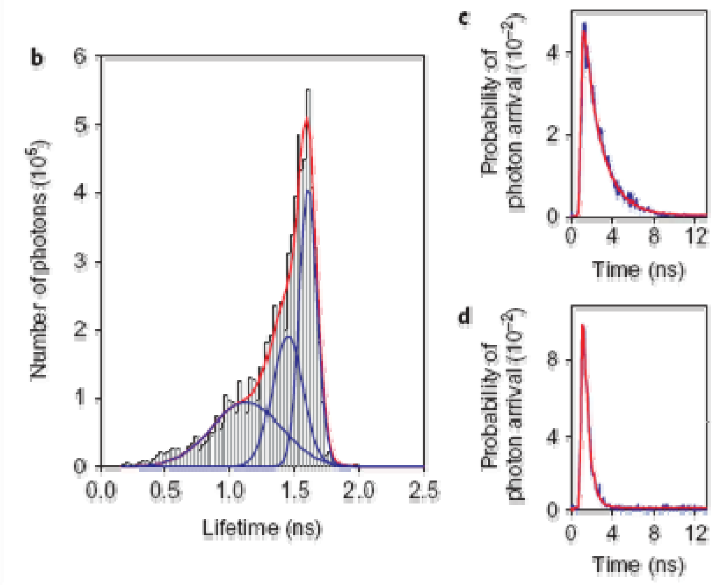
Red : fluorescence intensity binned 10ms

Blue : average intensity from intervals defines as charge-point-findling algorithm

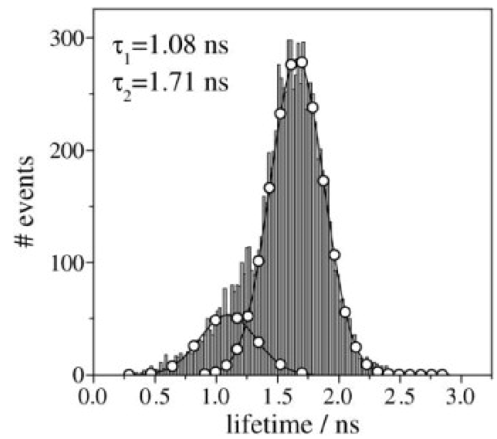
Green : lifetimes from above intervals

Multiple intensity plateaus

Results 2.



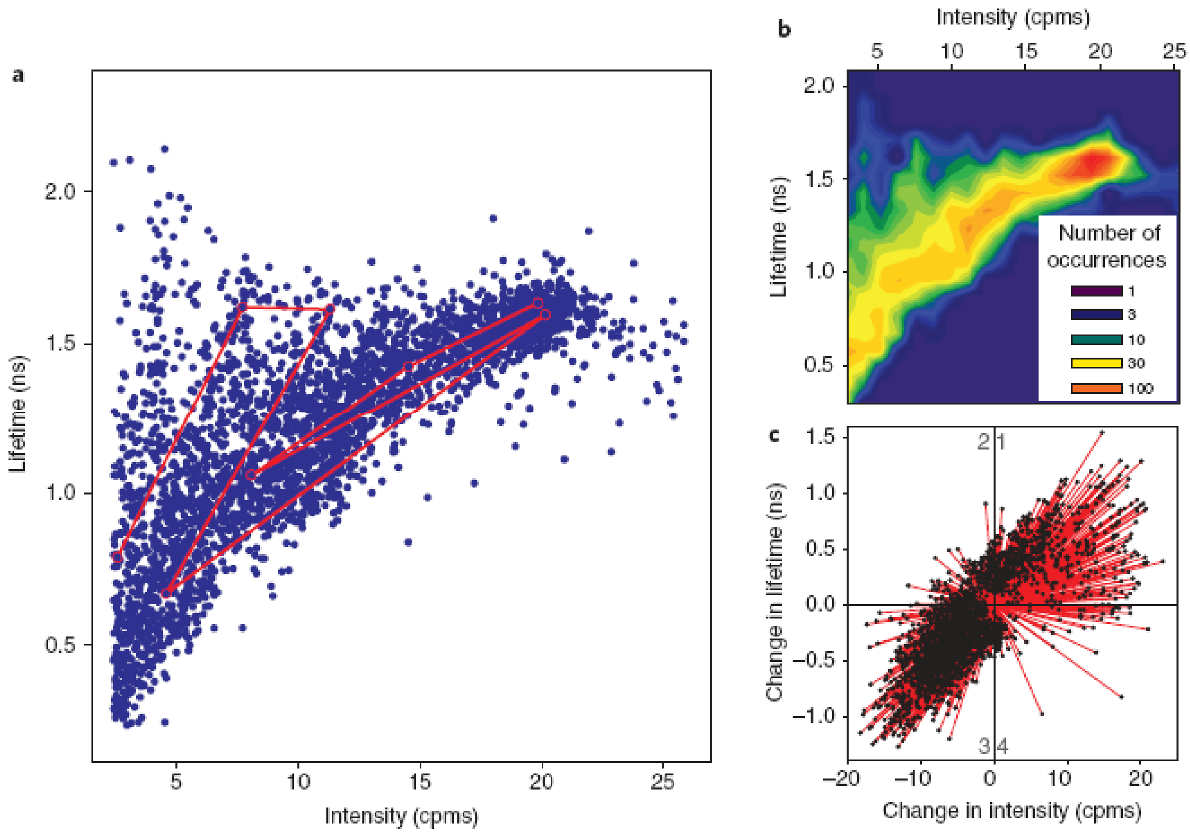
FWHM ~ 0.13ns



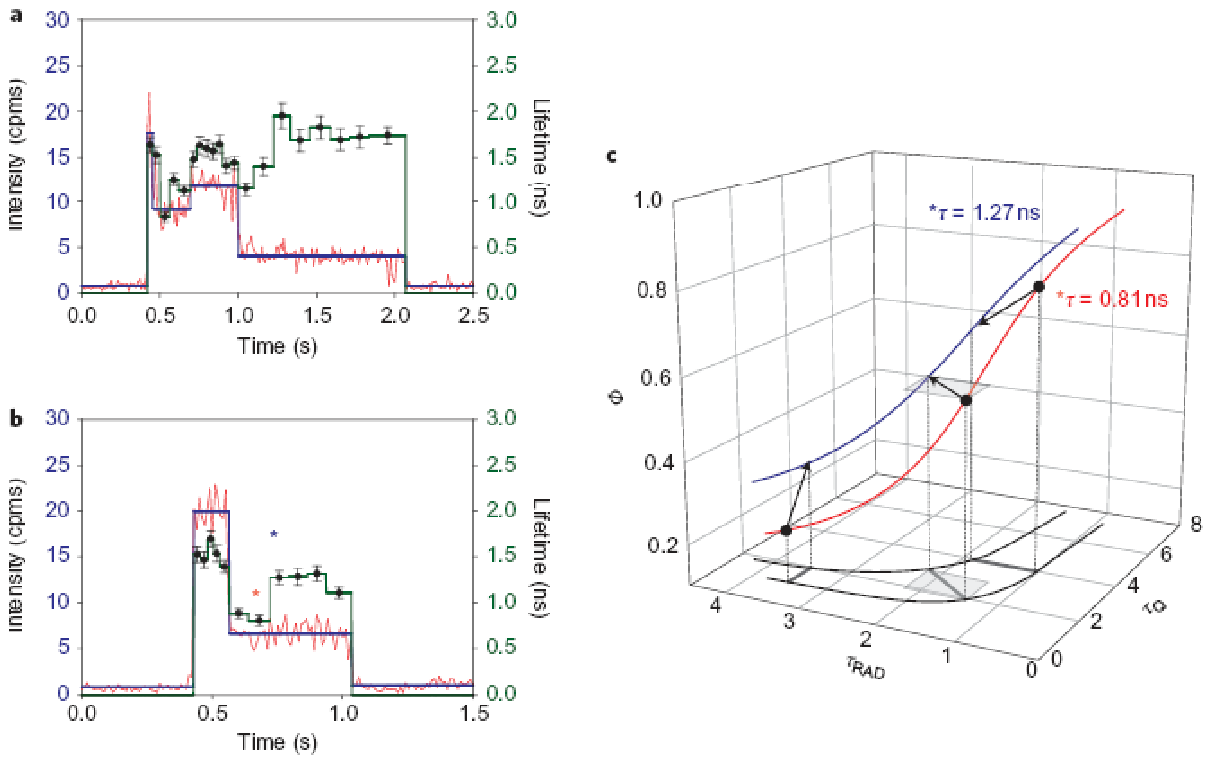
Same molecule in PVA
FWHM ~ 0.55ns
Loos *et al.* Biophys. J.

Immobilization has a tangible effect on the photodynamics of APC
Contribute to inhomogeneous broadening

Results 3.

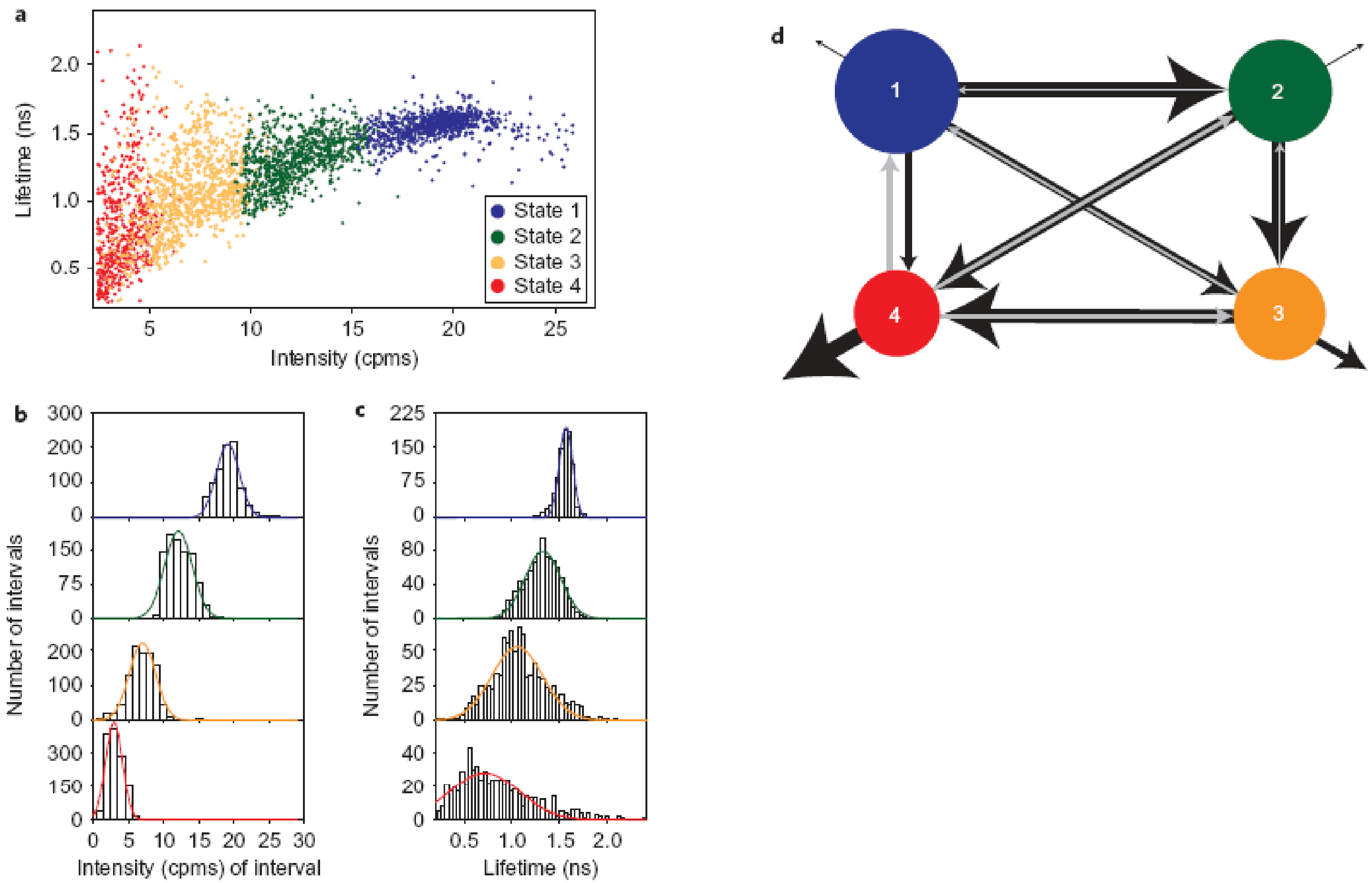

a
2.0
1.5
1.0
0.5
Lifetime (ns)
5
10
15
20
25
Intensity (cpms)
b
Intensity (cpms)
5
10
15
20
25
2.0
1.5
1.0
0.5
Lifetime (ns)
Number of occurrences
1
3
10
30
100
c
1.5
1.0
0.5
0.0
−0.5
−1.0
Change in lifetime (ns)
2
1
3
4
−20
−10
0
10
20
Change in intensity (cpms)

Results 4.



a
b
c
Intensity (cpms)
Lifetime (ns)
Time (s)
*τ = 1.27 ns
*τ = 0.81 ns
Φ
τRAD
τQ

Results 5.



a
Lifetime (ns)
Intensity (cpms)
State 1
State 2
State 3
State 4
b
Number of intervals
Intensity (cpms) of interval
c
Number of intervals
Lifetime (ns)
d
1
2
3
4