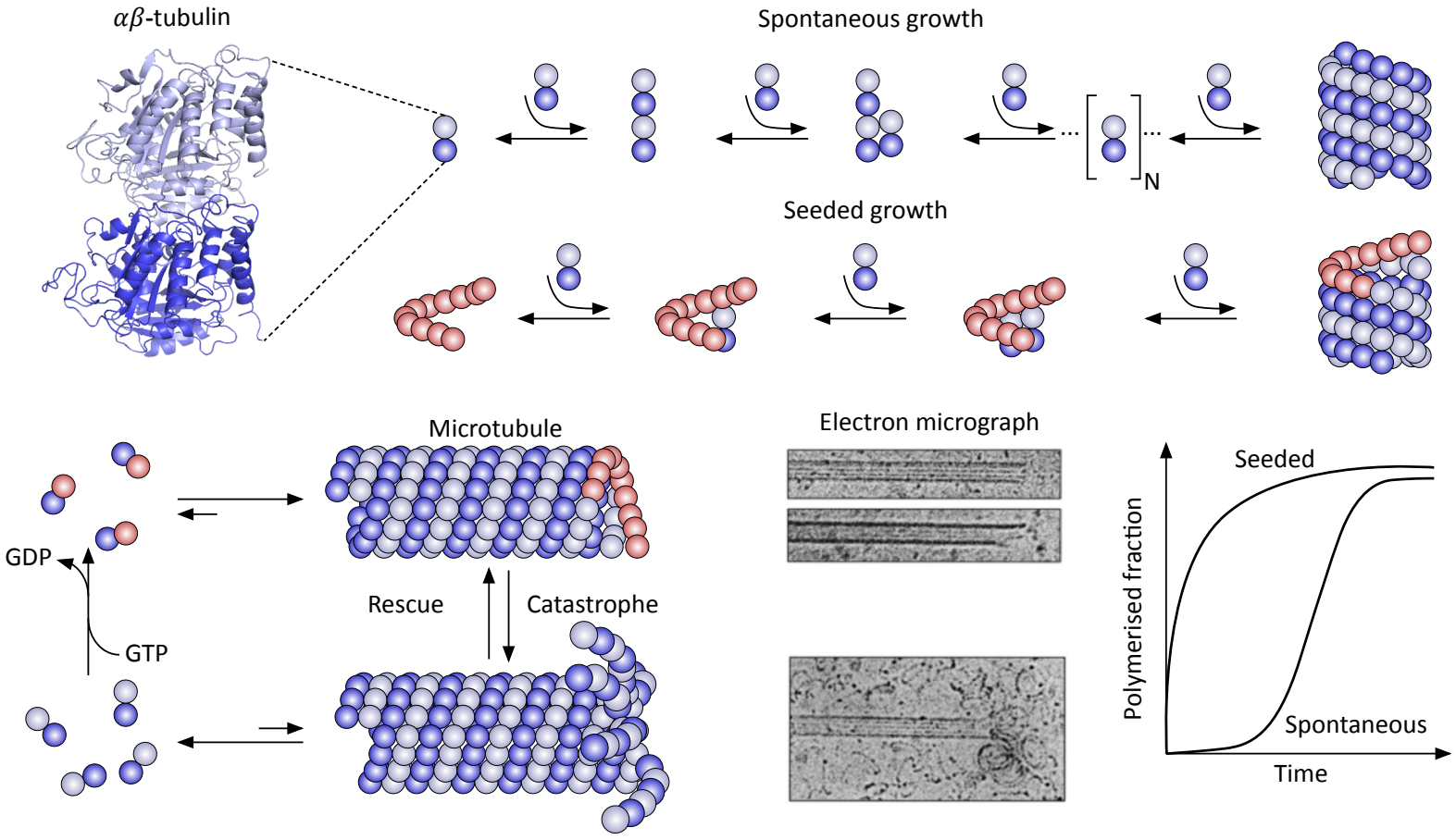
Label-free single molecule imaging with interferometric scattering microscopy

Philipp Kukura

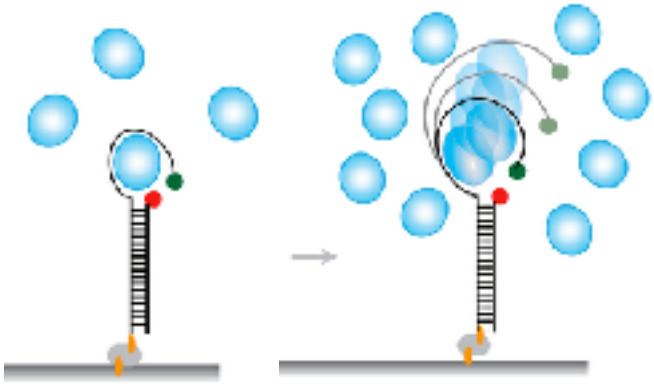
Physical and Theoretical Chemistry Laboratory, University of Oxford

How proteins work most of the time

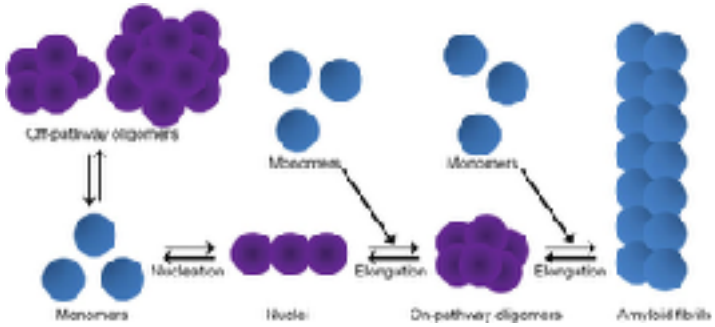


Some examples

Nucleation



Kim et al. Mol. Cell (2016)



Lorenzen et al. Essays Biochem (2014)

Microscopic to single molecule dynamics

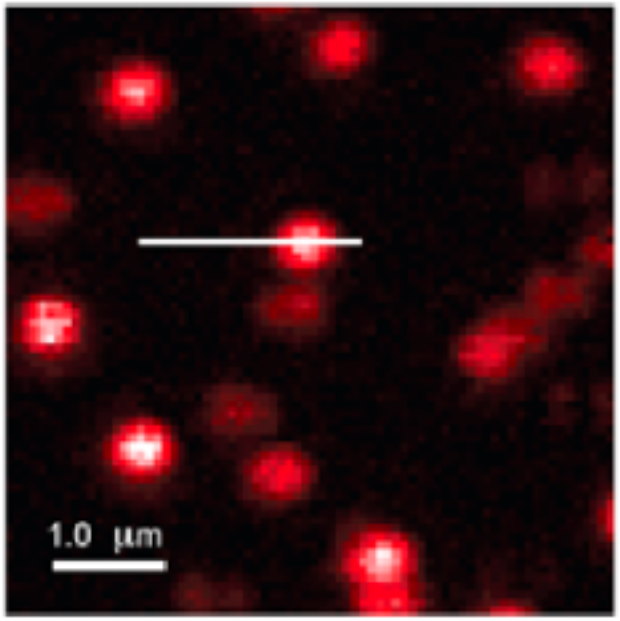
Zakharov et al. BPJ (2015)

We would need to be able to see single molecules the way they are depicted here:

Dynamically, in the presence of many others and with nm precision

State-of-the-art in seeing small things

Single molecule fluorescence



Contrast, specificity, background rejection

Single molecule (ion) scattering?

PHYSICAL REVIEW
LETTERS

VOLUME 70 | 5 APRIL 1993 | NUMBER 14

Young's Interference Experiment with Light Scattered from Two Atoms

U. Eichmann,[a] J. C. Bergquist, J. J. Bollinger, J. M. Gilligan, W. M. Itano, and D. J. Wineland
National Institute of Standards and Technology, Boulder, Colorado 80303

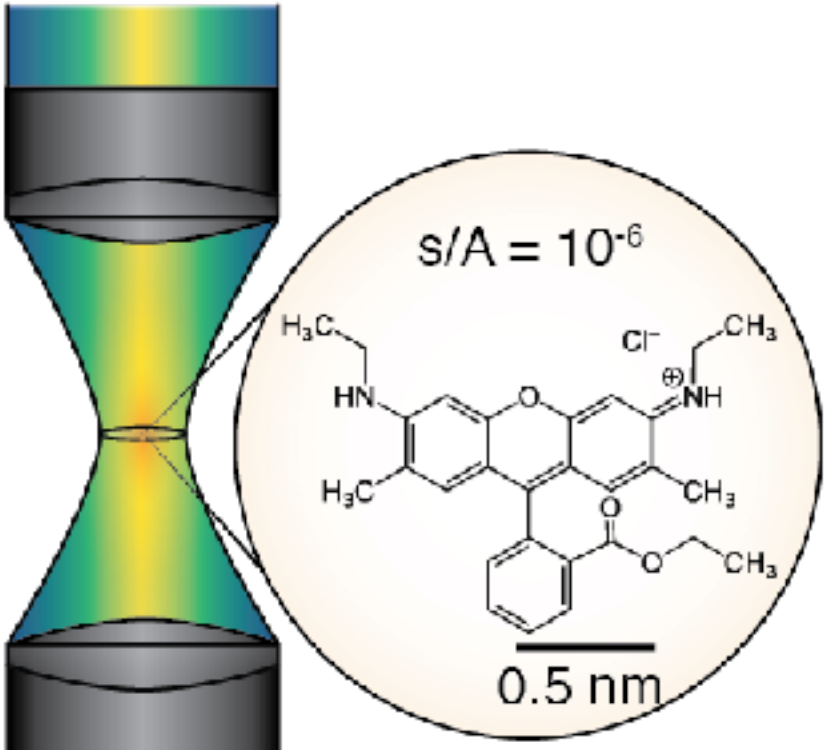
M. G. Raizen
Department of Physics, University of Texas, Austin, Texas 78712
(Received 18 December 1992)

Scattering just as good?

The fundamental problem: molecular size vs. wavelength

300 years ago

Still true today

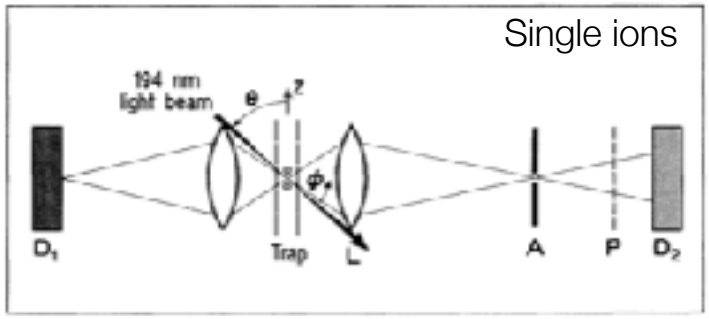


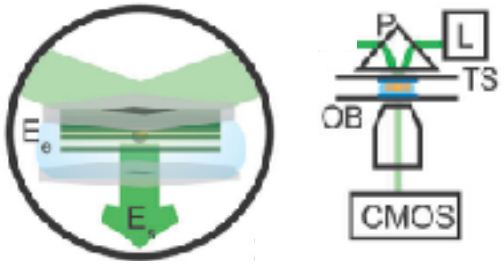
While there are techniques that can **resolve** better than the diffraction limit…

The diffraction limit still holds in terms of focusing

Light scattering

Dark-field imaging

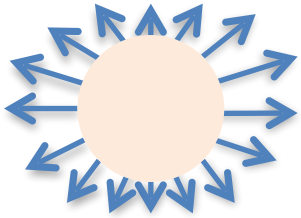


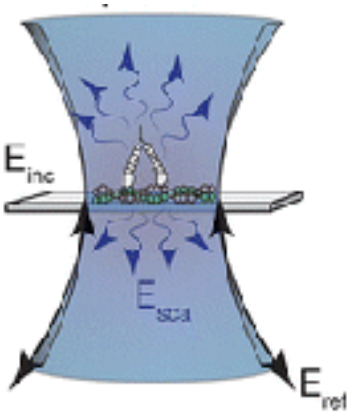


Detection limit: ~20 nm gold

Scattering cross section

$$\sigma = |s|^2 \propto V^2 \left| n_m \frac{n_p - n_m}{n_p + 2n_m} \right|^2$$





Strong size dependence

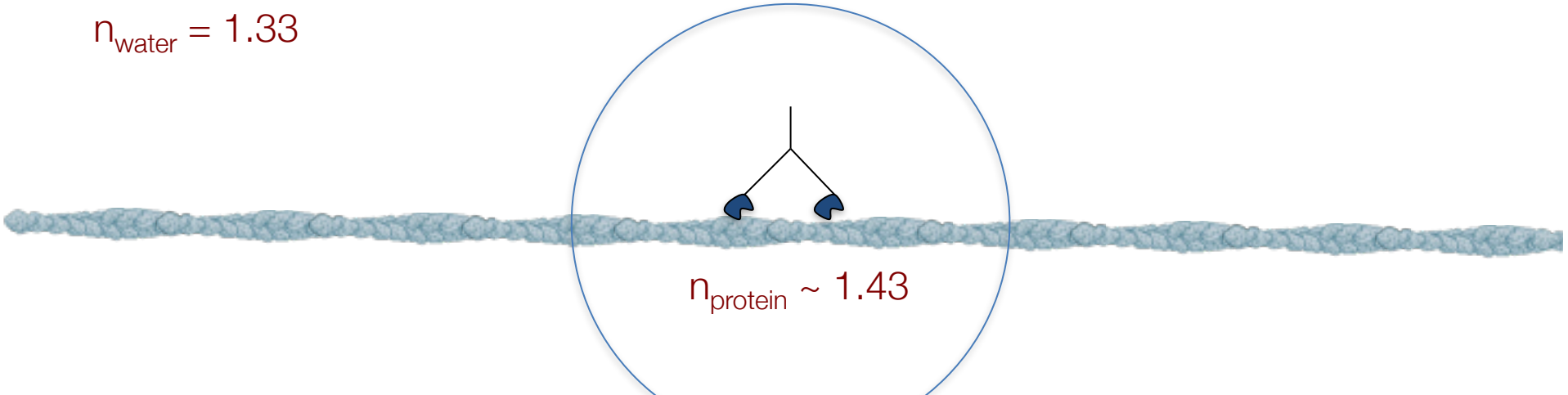
BUT $\sigma_{20\text{ nm gold}} \sim 10^6\ \sigma_{protein}$

Thinking about it differently

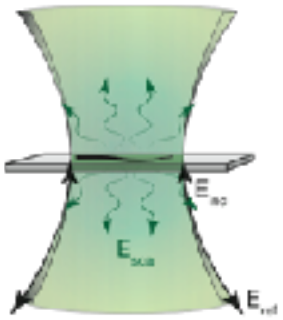
We can all see this:

So why not this?

$n_{water} = 1.33$



Interferometric scattering microscopy (iSCAT)



Combine scattered & reflected light:

$$|E_s + E_r|^2 = |E_i|^2 \left[r^2 + |s|^2 - 2r|s| \sin\phi\right]$$

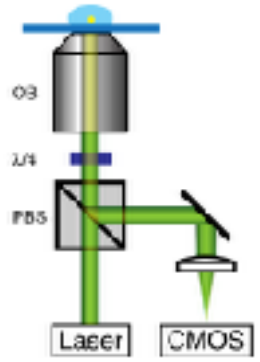
Vasicek (1961) Opt. Spectrosc. 11, 128
Curtis (1964) J. Cell Biol. 20, 199

Signal for a small scatterer:

$$\text{Contrast} = 1 - \frac{2|s| \sin\phi}{r}$$

High sensitivity: weaker size dependence (V vs V^2)

Lindfors, *et al.* (2004) PRL , 93, 037401
Kukura, *et al.* (2010) JPCL, 1, 3323



Shot-noise limited SNR

$$\frac{\text{Contrast}}{\text{Background fluctuations}} = \text{Contrast}\sqrt{N}$$

Simultaneous high localisation precision and time-resolution

Kukura, *et al*, (2009). Nat. Methods, 6, 923

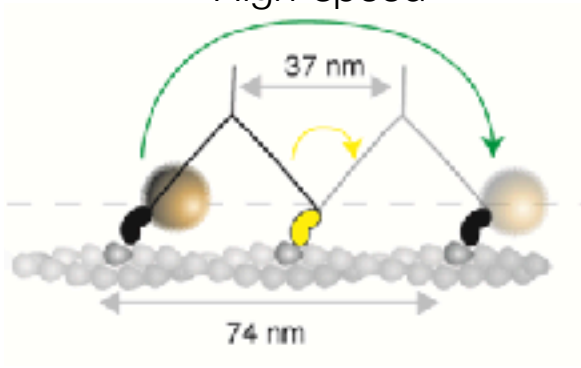
For ideal SNR: suggests ‘arbitrary’ speed and sensitivity

Ortega-Arroyo and Kukura, PCCP (2012) 15625
Ortega-Arroyo and Kukura, Nat. Photonics (2016) 11

You can also go for precision instead of speed

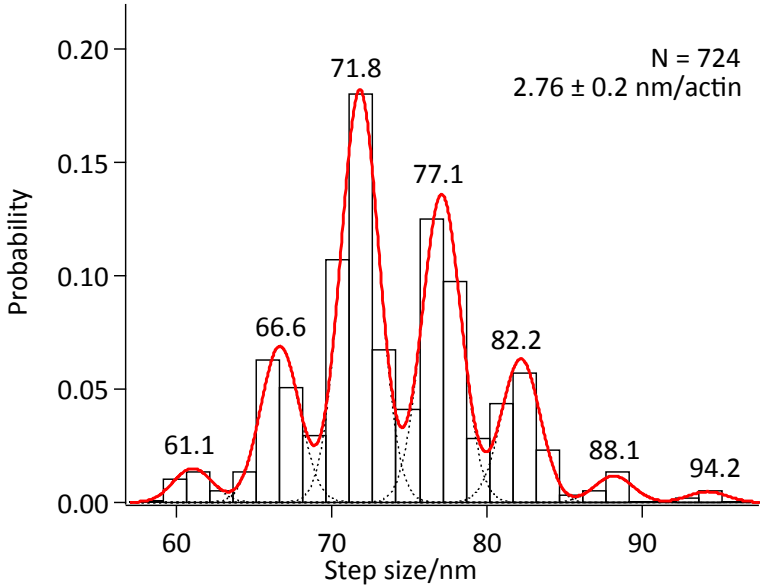
Myosin 5

High-speed



Andrecka et al. eLife (2015)

High precision



100 Hz with <0.5 nm precision

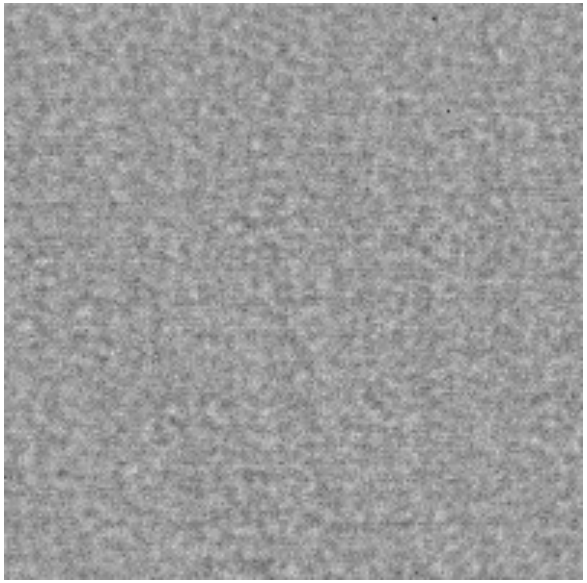
Andrecka et al. Meth. Enzym. (2016) 517

You cannot only see gold particles: you can see anything

Scattering cross section is given by

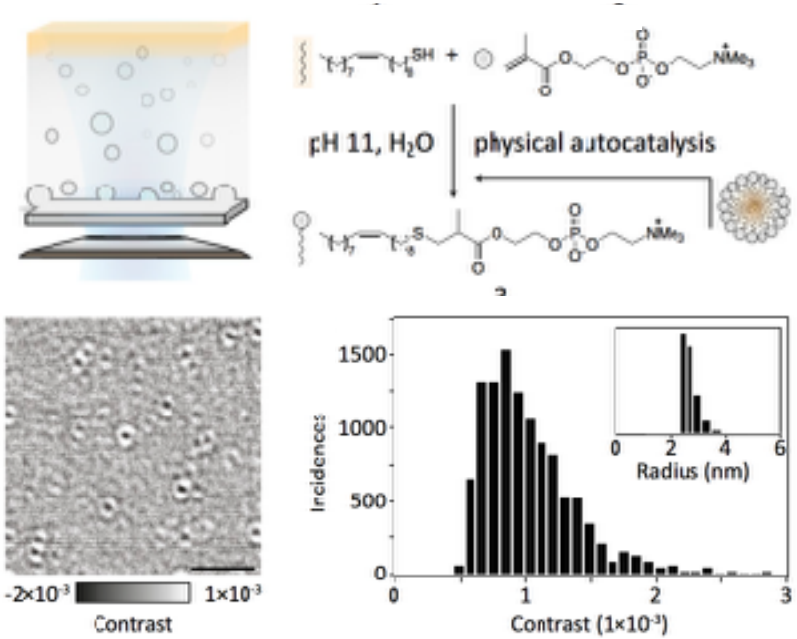
$$\sigma = |s|^2 \propto V^2 \left| n_m \frac{n_p - n_m}{n_p + 2n_m} \right|^2$$

Lipid rafts



deWit et al PNAS (2015)

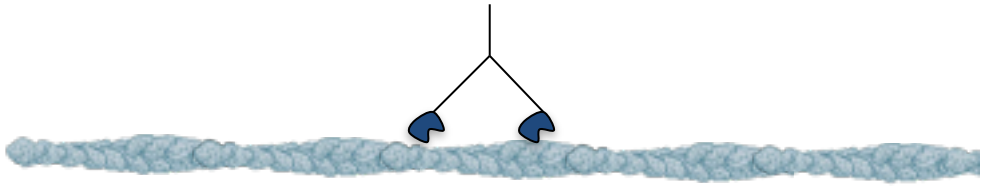
Micelles and vesicles



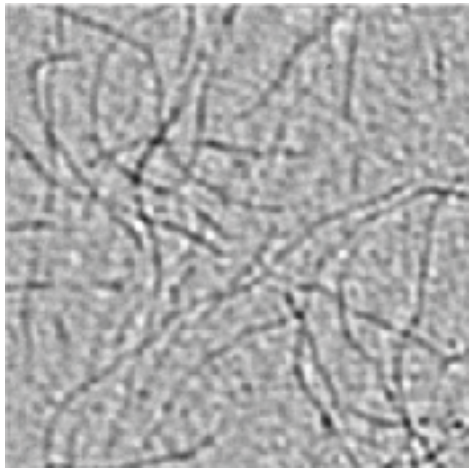
Ortega-Arroyo et al PNAS (2015)

From micelles to single molecules

Myosin 5 bound to actin



Actin filaments on glass



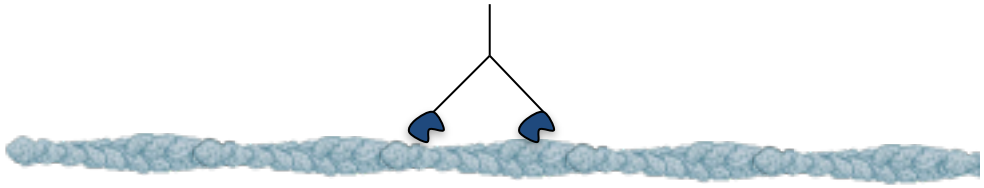
0.98 1.02

Normalised reflected intensity

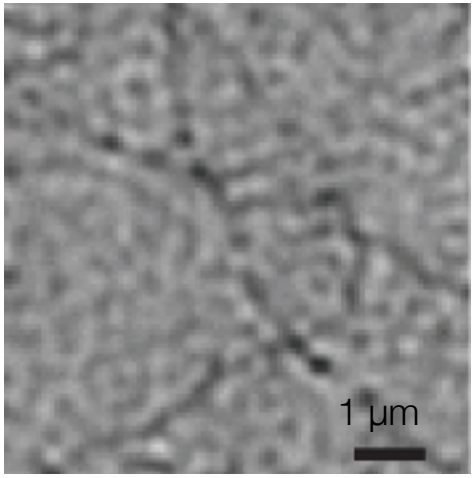
But with this much background, how do I see the molecule I am interested in?

Where is Odlaw?

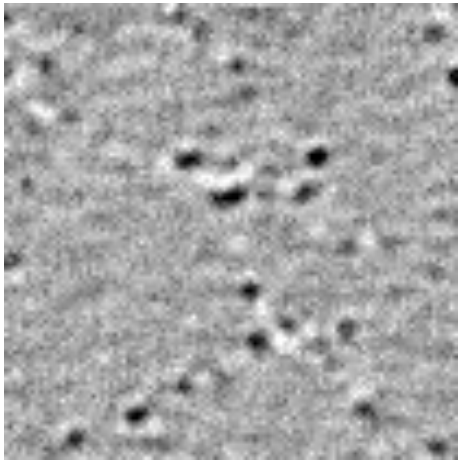
Myosin 5 binds and walks



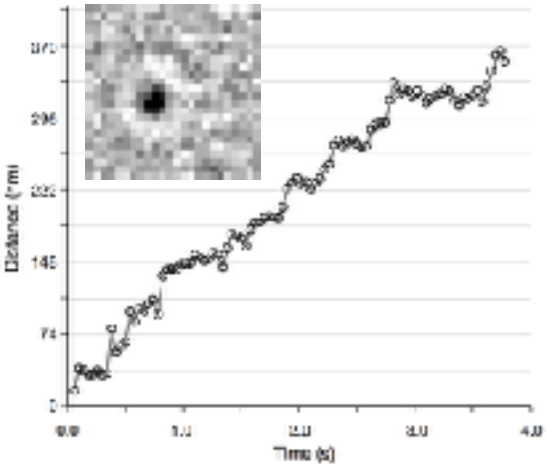
Actin on glass



Background subtracted



Nanometric tracking



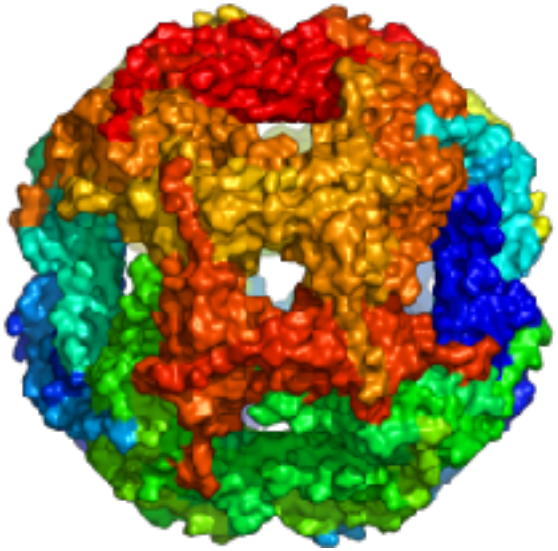
Ortega-Arroyo et al Nano Letters (2014) 2065

All-optical, label-free detection of a single, unlabelled protein

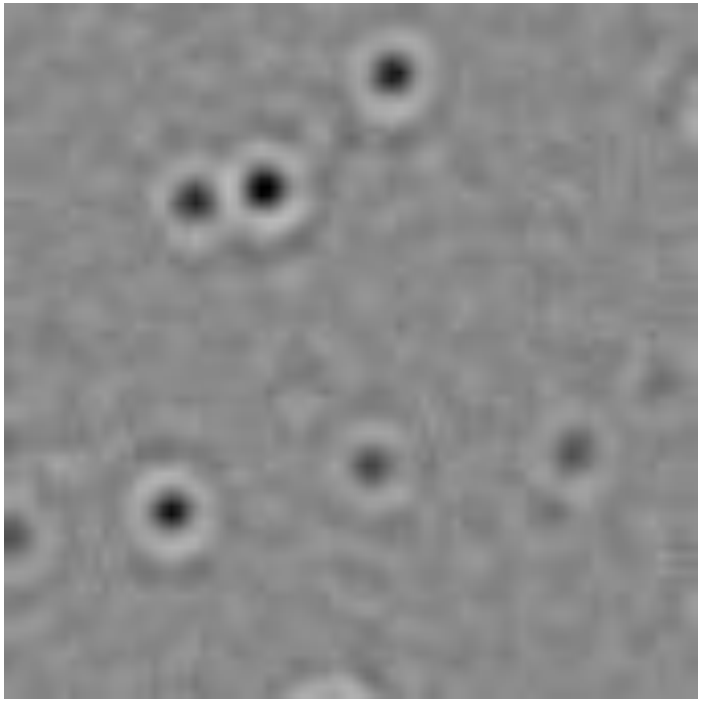
Really becoming shot noise limited

Two years later: Non-specific binding of a similar-sized protein to a bare glass surface

HSP 16.5



396 kDa



Real time (effective 6 Hz), 50 kW/cm^2, 240 nM

Cole et al. ACS Photonics 2017 4: 211 - 216

Let's think about this a little more carefully

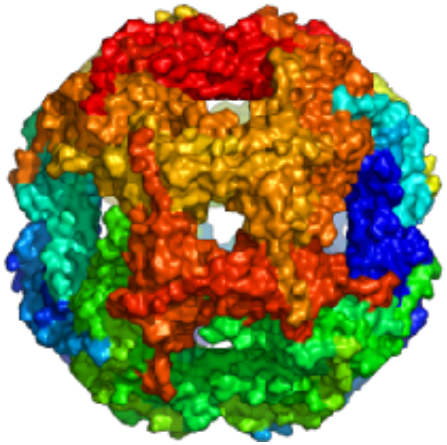
Proteins are made of amino acids

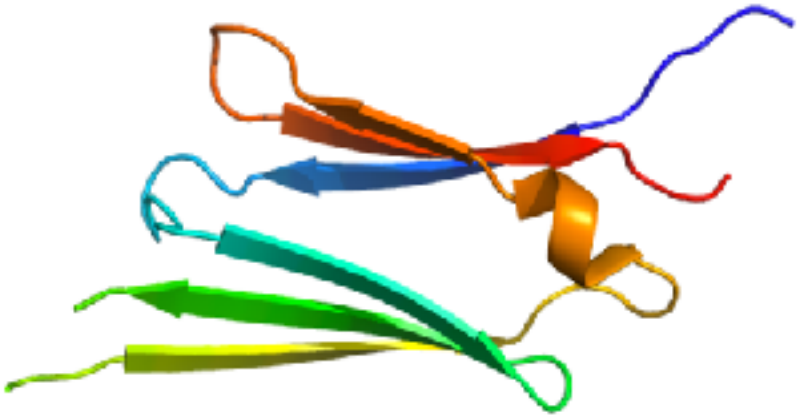
Which all have the same polarisabilities

Which means the signal should be

PROPORTIONAL TO MASS

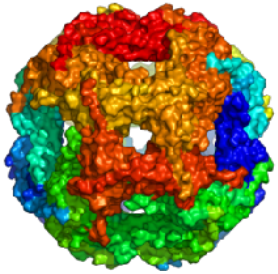
$$\sigma = |s|^2 \propto V^2 \left| n_m \frac{n_p - n_m}{n_p + 2n_m} \right|^2$$



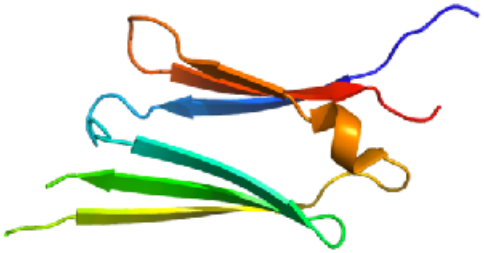


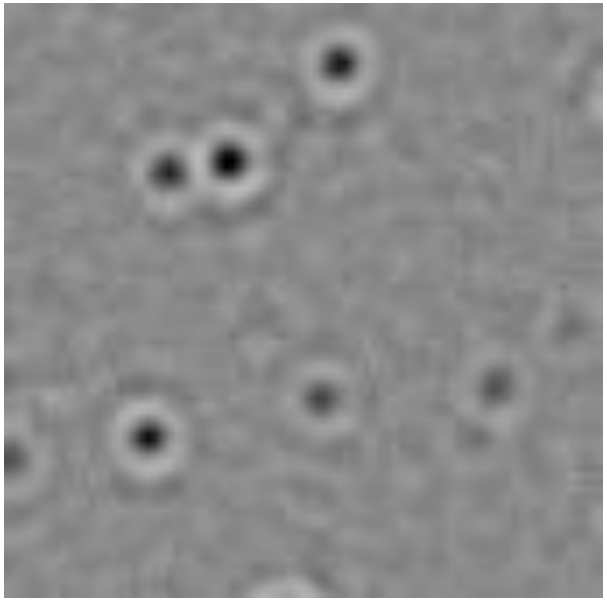
From mono-disperse to poly-disperse

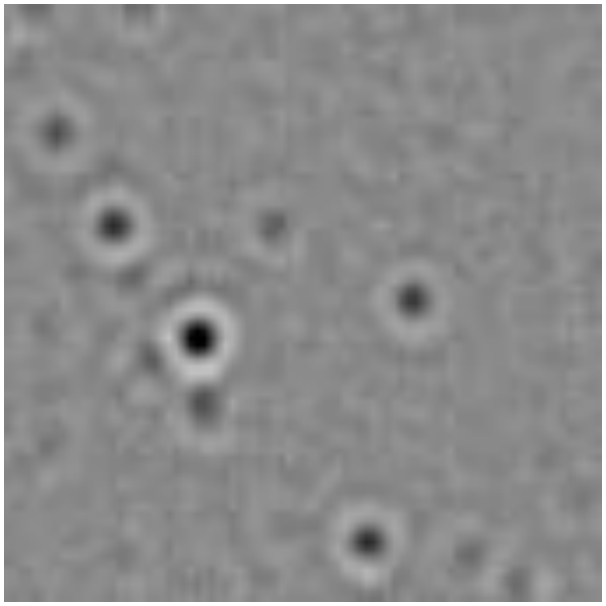
HSP 16.5: Stable 24mer



HSP 27: Highly dynamic oligomer



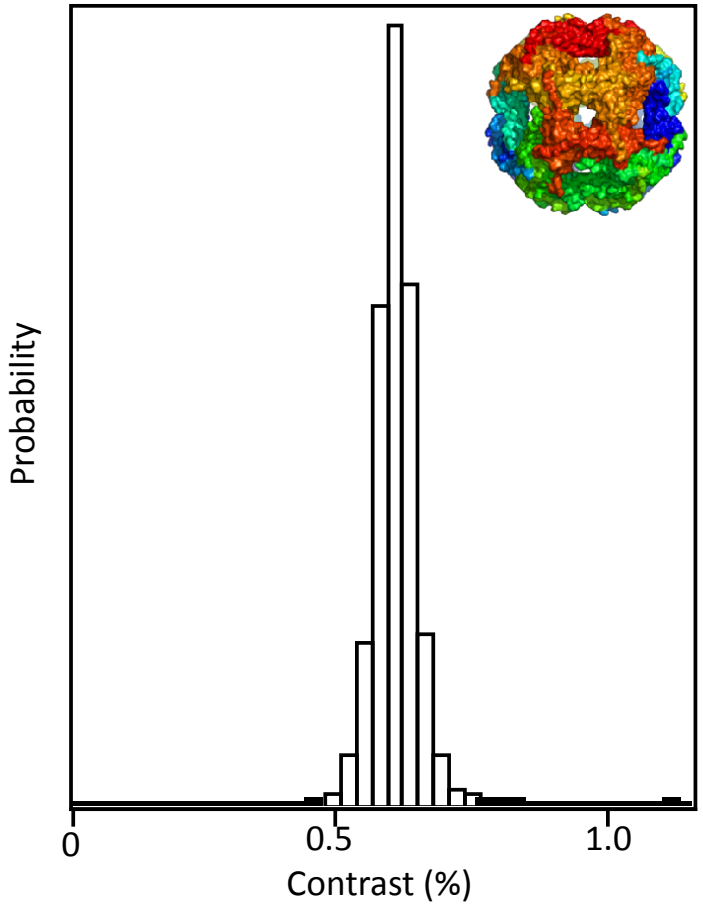




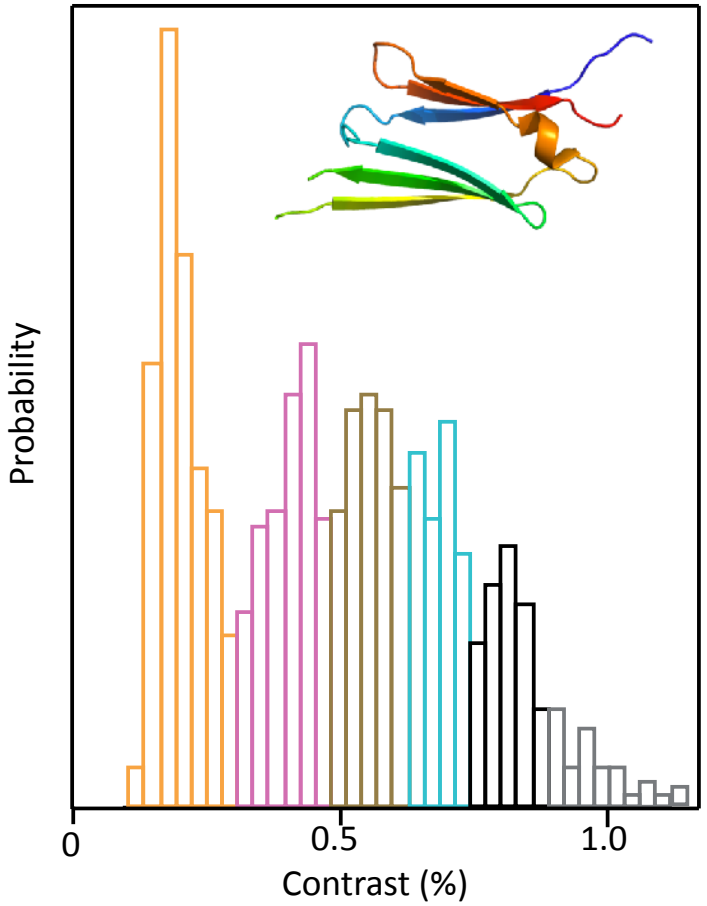
Real time (effective 6 Hz), 50 kW/cm^2, [monomer] = 240 nM

From mono-disperse to poly-disperse

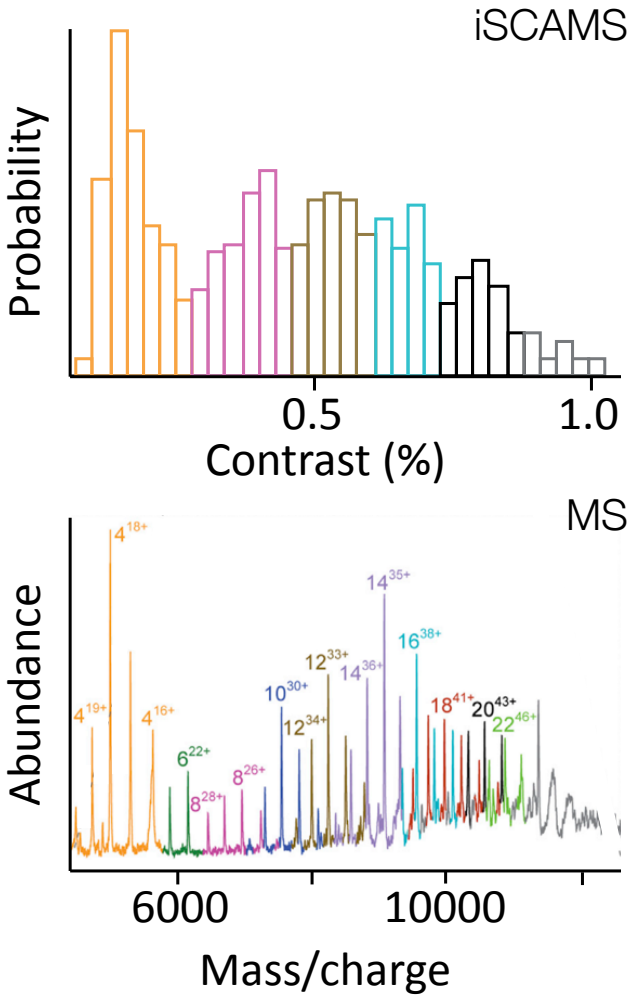
HSP 16.5: Stable 24mer



HSP 27: Highly dynamic oligomer

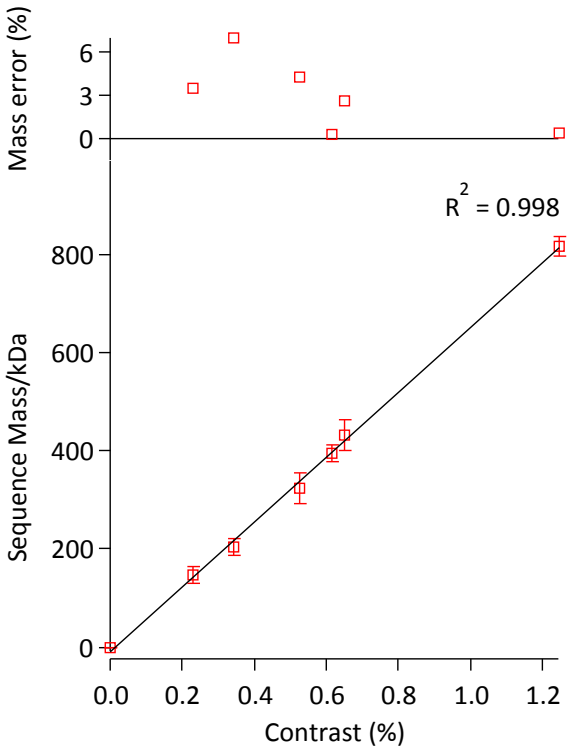


From monodisperse to polydisperse

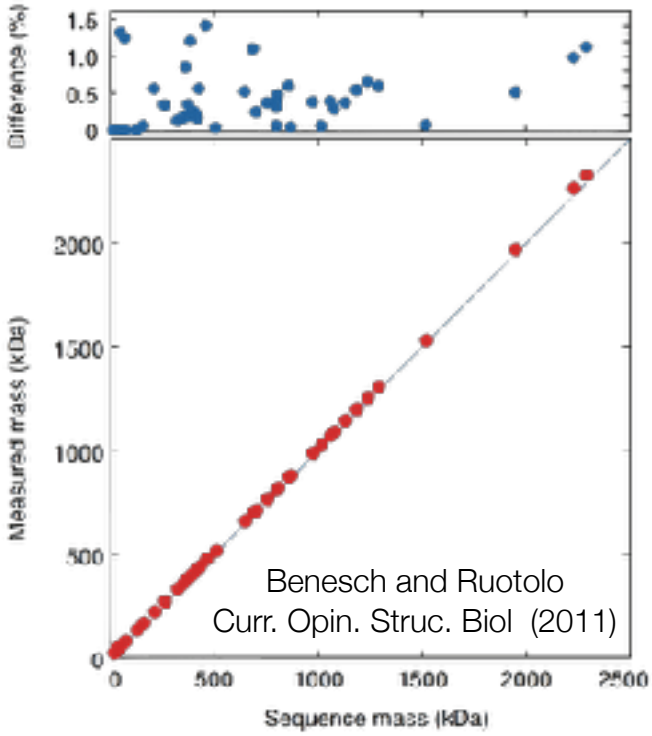


Interferometric scattering mass spectrometry (iSCAMS)?

iSCAMS



Native mass spectrometry



Comparable mass accuracy (<5%)

Protein-drug interactions

NATURE MEDICINE | SPOONFUL OF MEDICINE

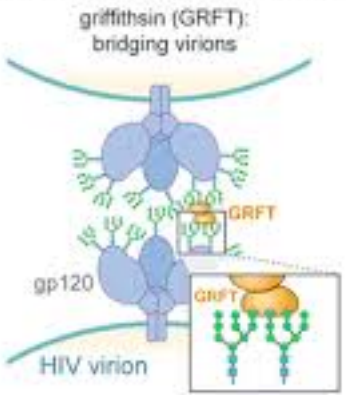
The Daily Dose – HIV protection goes bananas

15 Mar 2010 | 12:00 EDT | Posted by Christian Torres | Category: Daily Dose

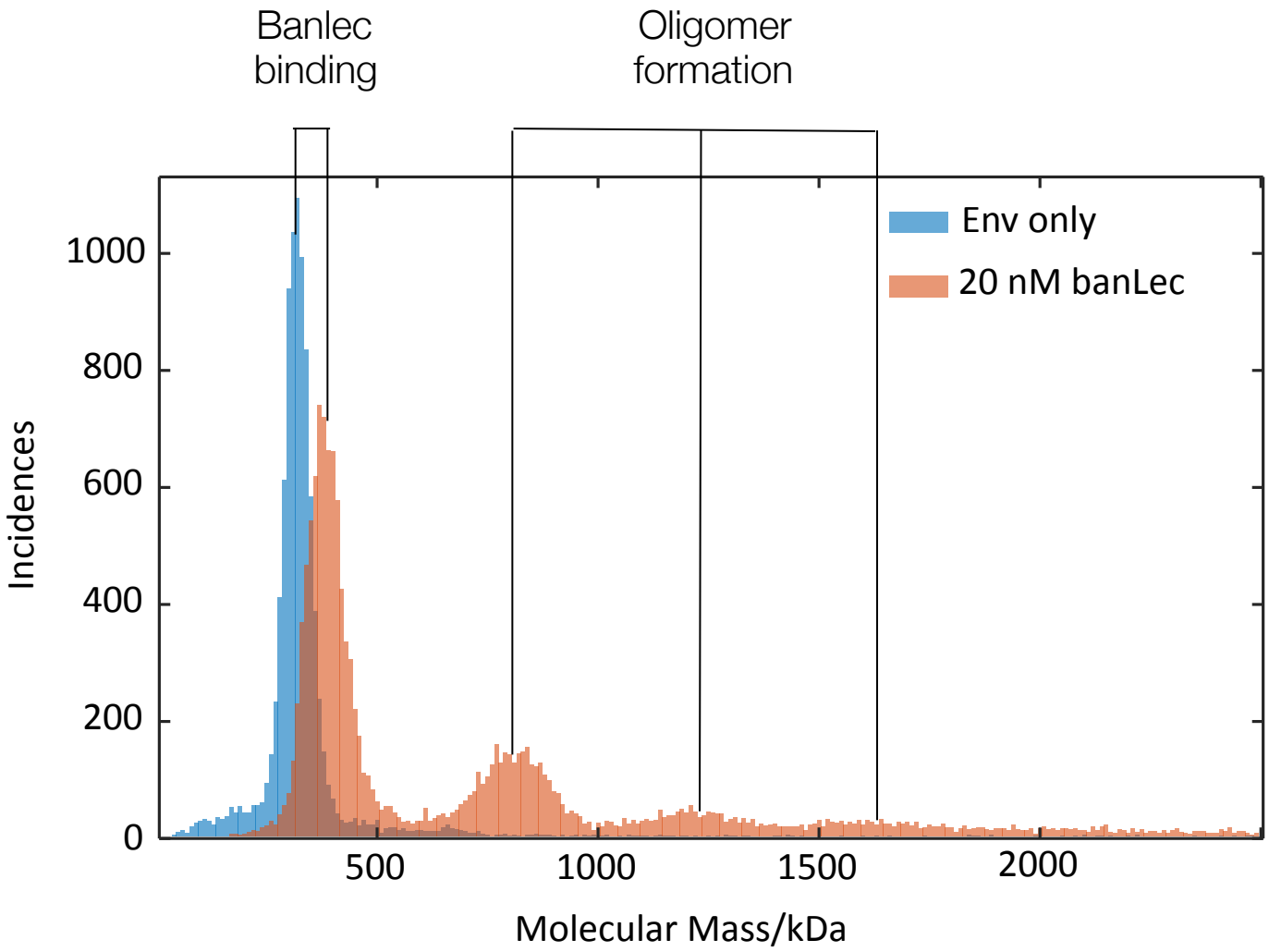
— Bananas, already a beacon of safe sex practices, might have a new role in the fight against HIV. According to researchers, the fruit's sugar-binding proteins called lectins, which attach to molecules on the outside of the HIV virus, are comparable to two current anti-HIV drugs in blocking the virus from infecting cells. Banana lectin (BanLec for short) would be cheaper to produce and less likely to create selection for resistance than existing meds, the study authors say.

Antiviral non-human lectins:

griffithsin (GRFT):
bridging virions

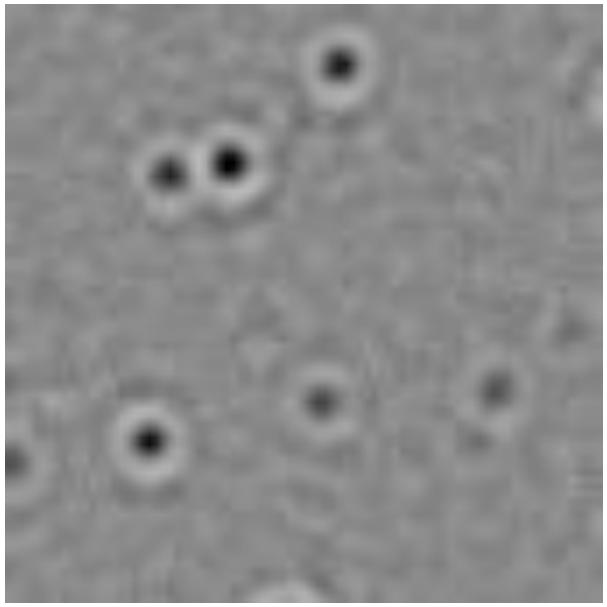


Direct observation of drug-induced protein aggregation



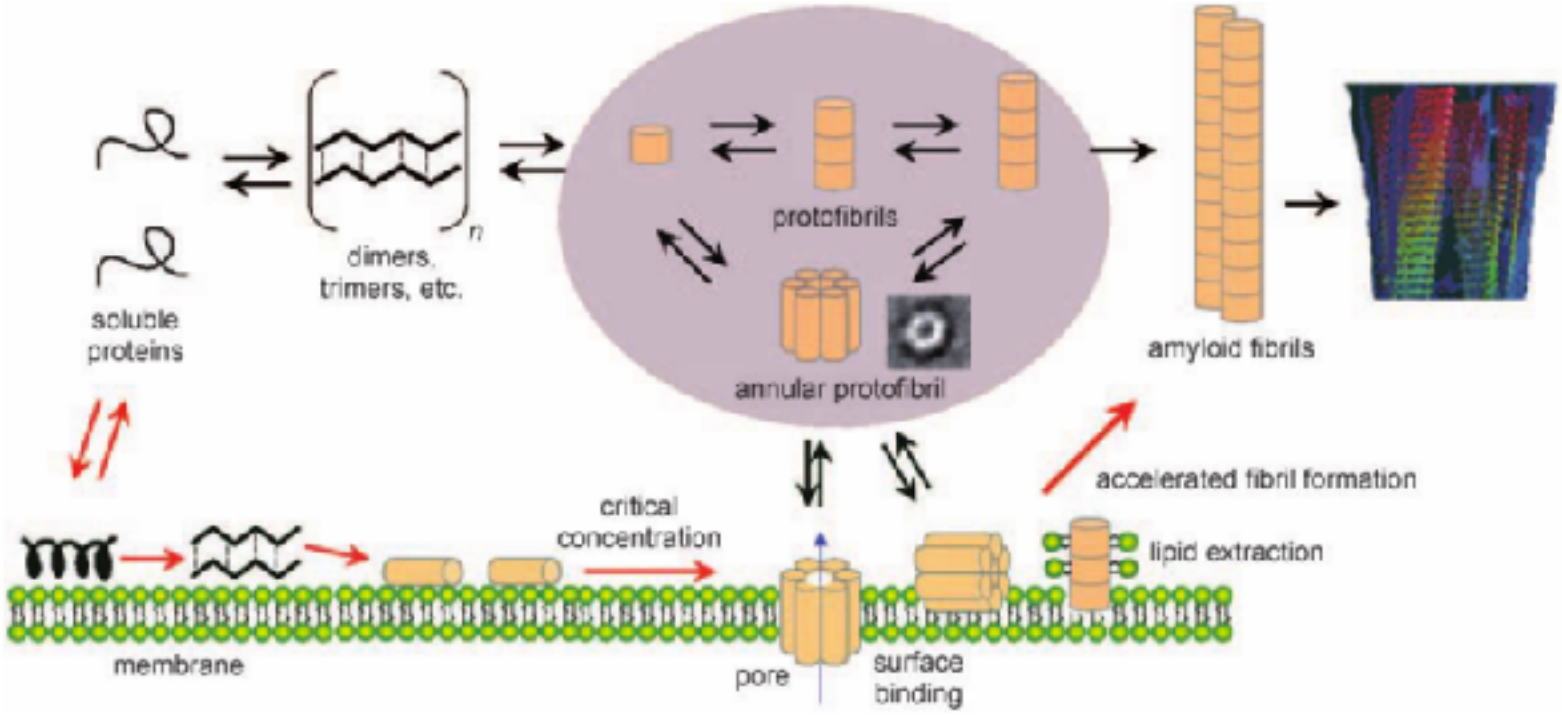
From mass spectrometry to mass imaging

Same approach to super-resolution as PALM/ STORM is valid here as well



150 ms exposure time, 3.5 nm localisation precision

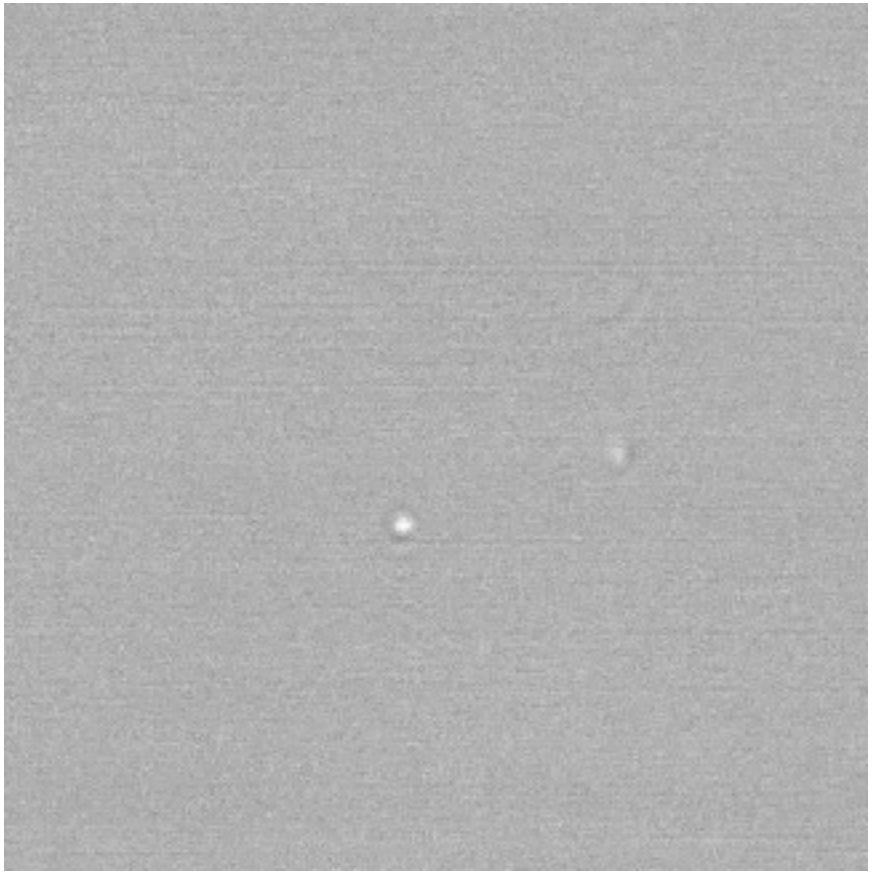
Example 1: Synuclein aggregation on membranes



Butterfield et al Angew. Chemie (2010)

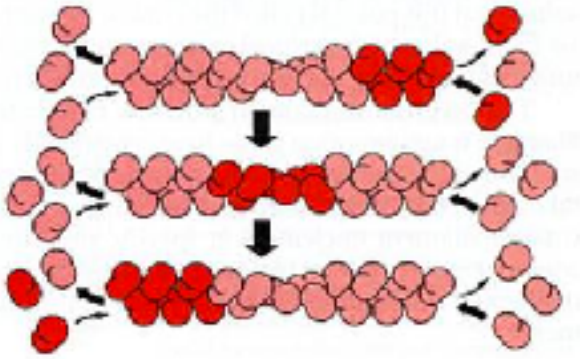
Example 1: α–Synuclein aggregation on membranes

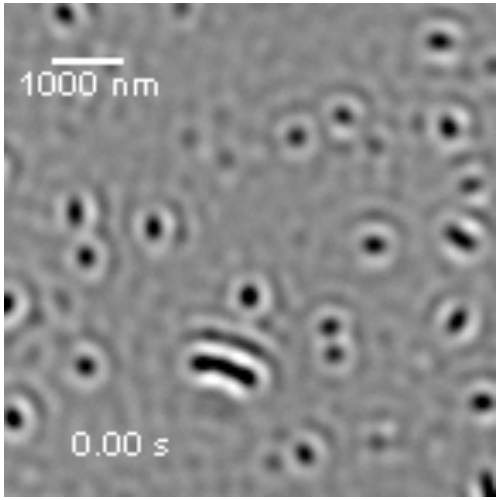
Real-time imaging



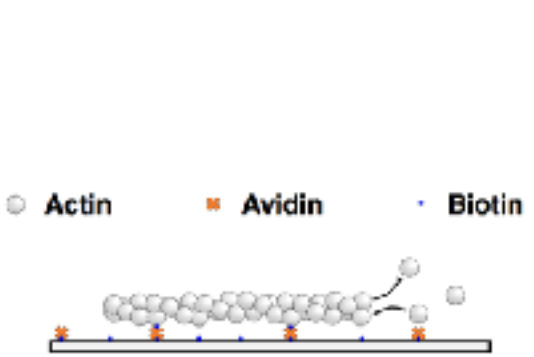
DOPC/DOPS membrane, 50 μM α-synuclein, 35 fps

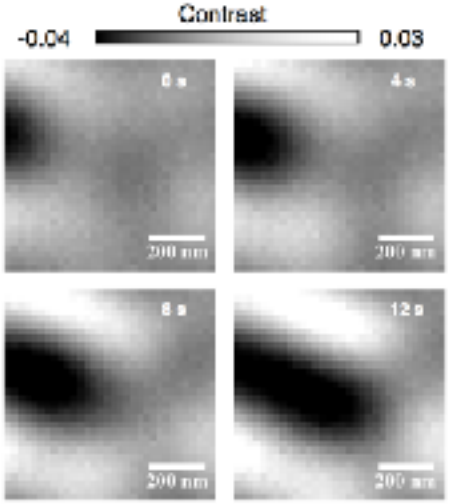
Example 2: actin polymerisation

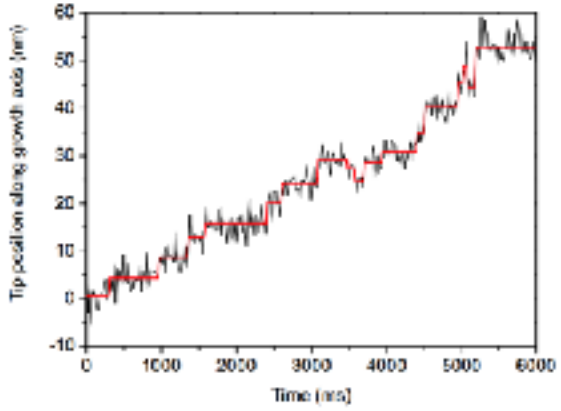


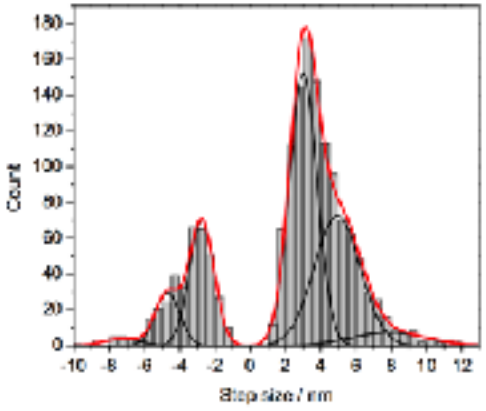


Example 2: actin polymerisation









Justin Benesch

Weston Struwe

Jim Sellers & Harry Takagi

Cedric Eichmann & Phil Selenko

‘GANDAM’

Gavin Young

D**an**iel Cole

A**dam** Fineberg

Joanna Andrecka

Nicolas Hundt

EPSRC

Engineering and Physical Sciences Research Council

erc

MARIE CURIE ACTIONS