# A brownian dynamics interpretation of membrane protein clustering

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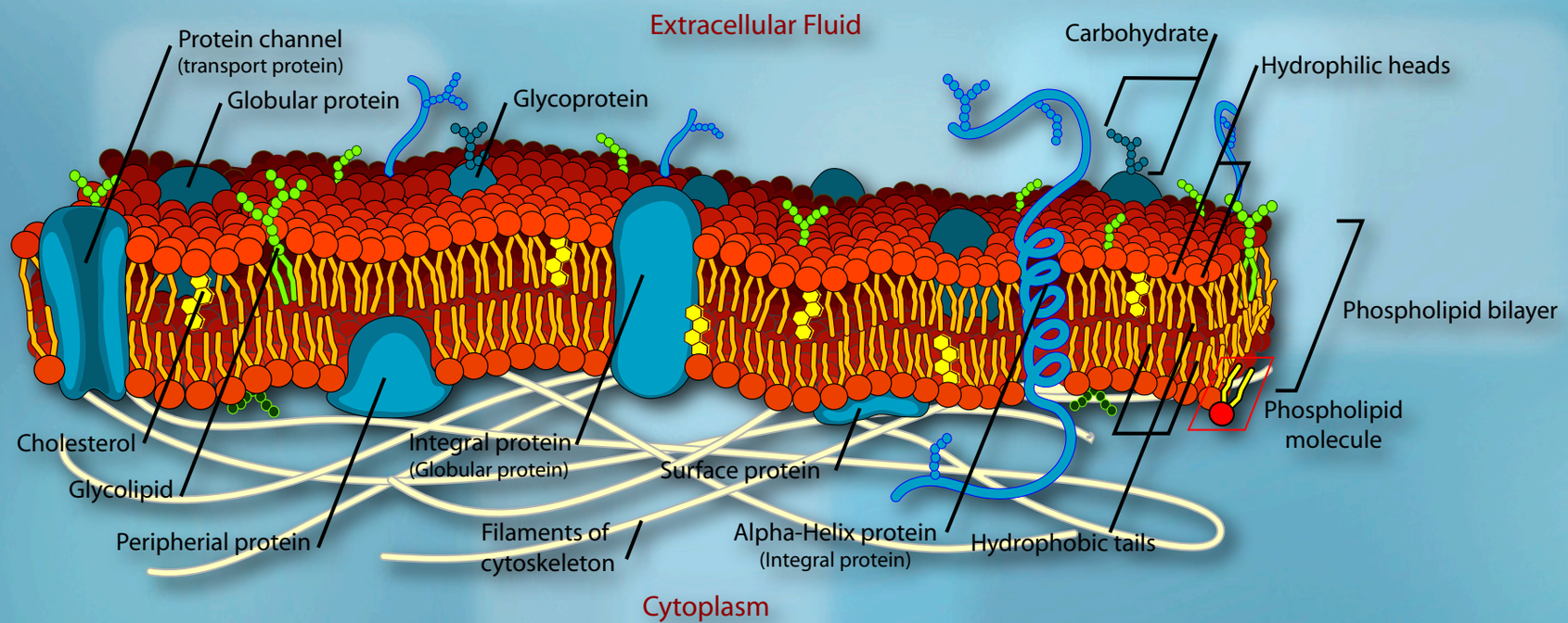
# Questions & motivation

- fluid mosaic model of cell membrane (Singer, Nicolson 1972): individual proteins diffuse freely in a sea of lipids
- however, most membrane proteins are organized in **clusters**
- no satisfactory **explanation**! (subplasmalemmal fences that form compartment boundaries?)
- cluster formation is likely functionally important since syntaxin clusters represent sites for docking & fusion of vesicles
- physical principles underlying most membrane protein clusters poorly understood
- **explain the experimentally accessible properties of the syntaxin-1 (Sx1) clusters**

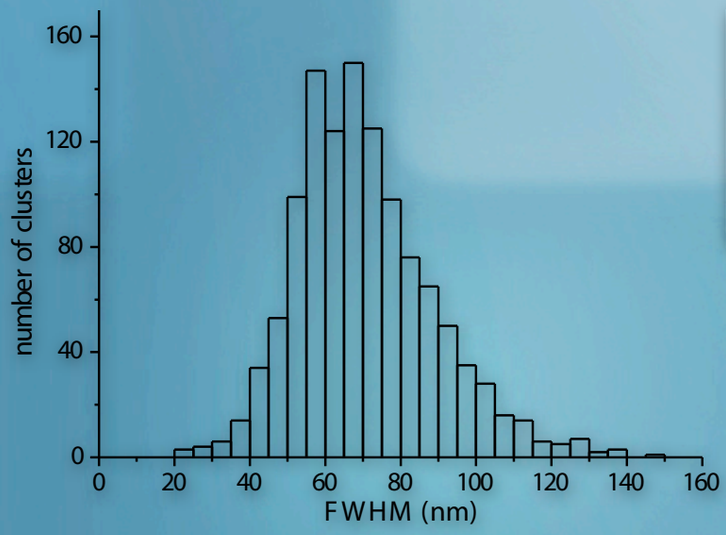
SNARE protein involved in membrane fusion

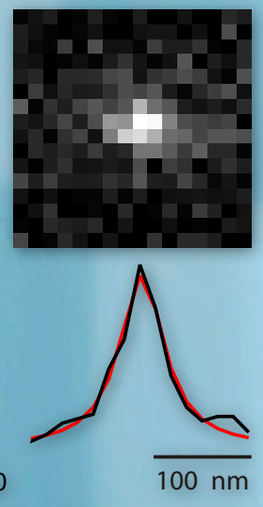


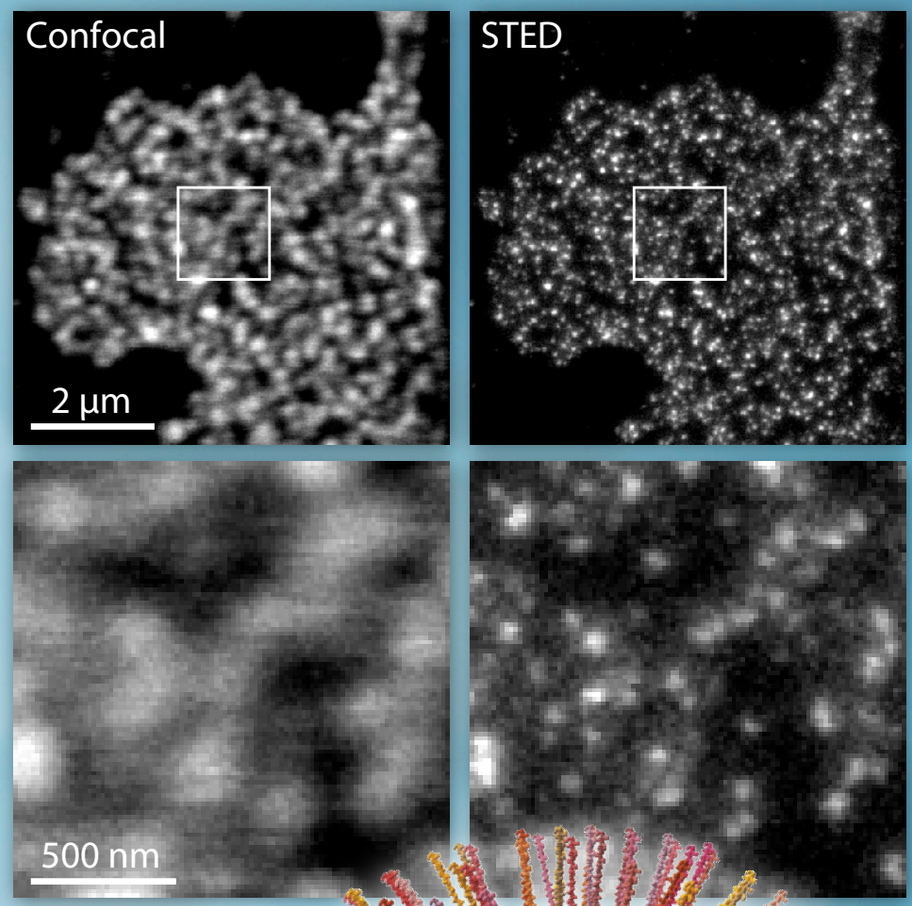
# Experiments 1: cluster density and cluster diameter

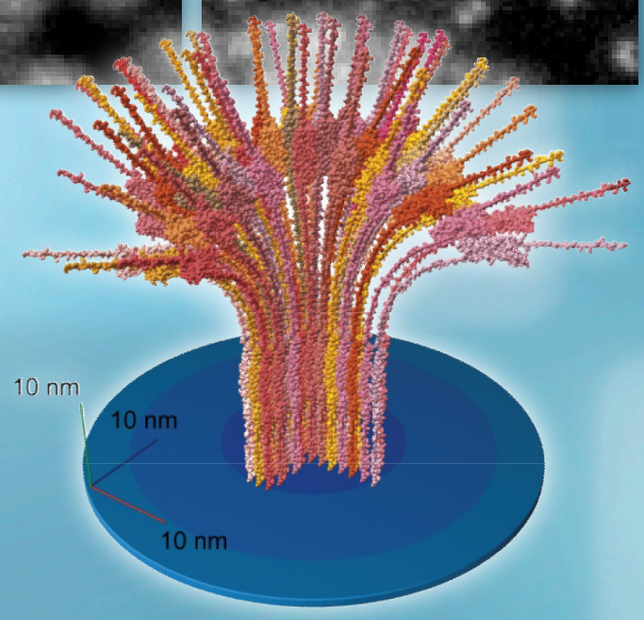
- STED microscopy on plasma sheets yields **density of 19.6(5.7) clusters / µm²**
- average cell surface area is 460 µm²
  ⇒ 9000 clusters per cell
- quantitative immunoblotting: 830 000 Sx1 per cell
  ⇒ **max. 90 Sx1 per cluster** (if all Sx1 in clusters)
- STED: **average cluster diameter 50-60 nm**
  ⇒ dense package
- overexpression ⇒ more clusters

Histogram of measured Sx1 cluster size



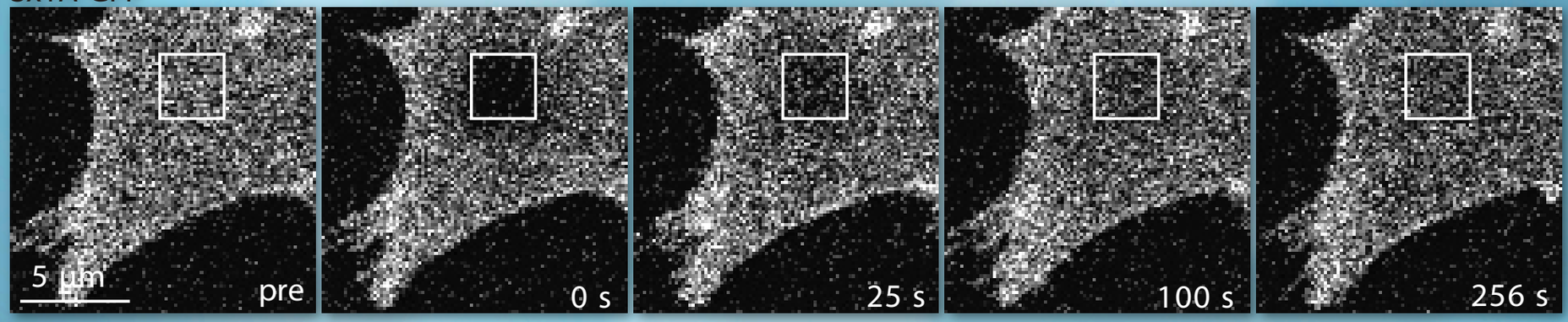



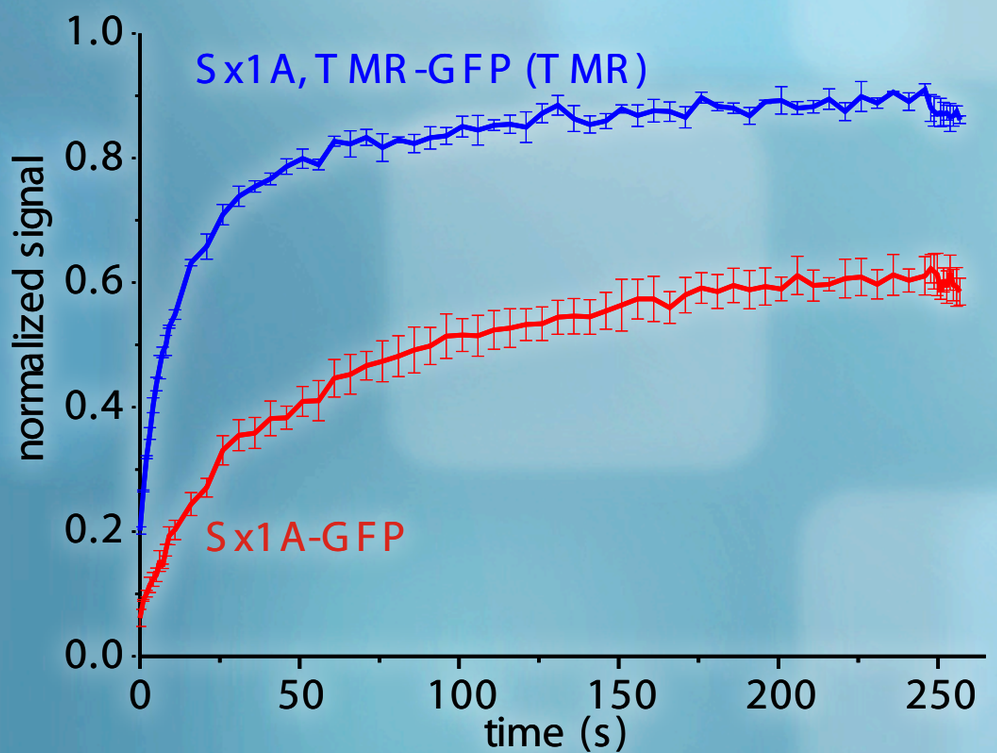
# Experiments 2: syntaxin mobility

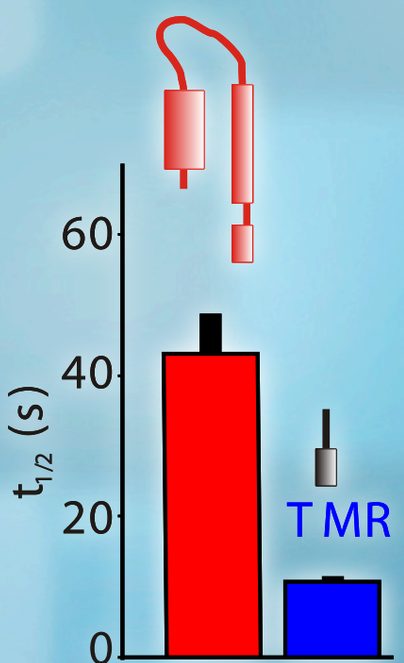
- FRAP measurements with green fluorescent protein (GFP)–labeled Sx1 overall mobility

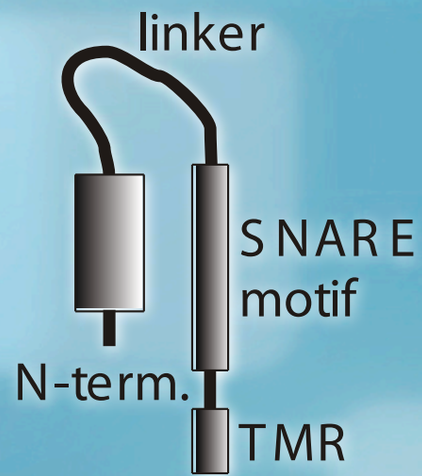
Sx1A-GFP




- recovery half times $t_{1/2}$ range from 40–60 s, much longer than expected for a freely diffusing protein with a single transmembrane region (TMR)

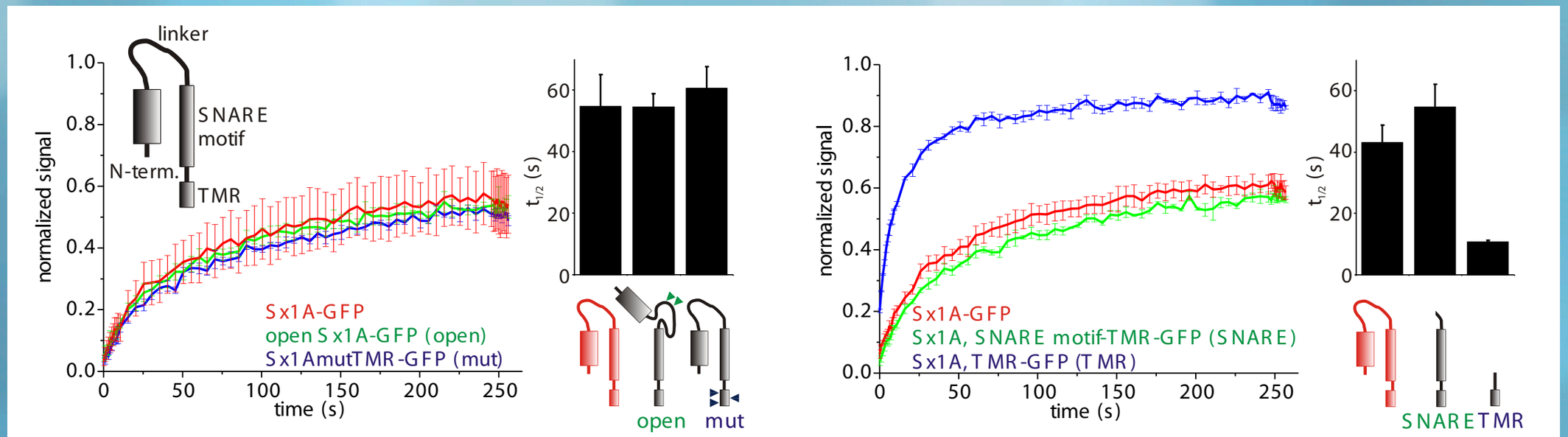


# Experiments 3: which region of Sx1 is responsible for mobility restriction?

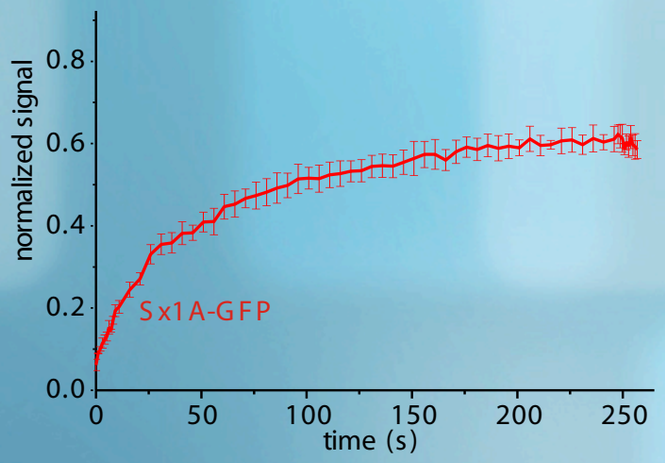
- study mutants and deletion constructs of Sx1
  C-term TMR – SNARE – linker – N-term domain

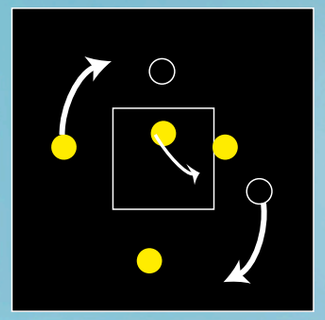



- SNARE motifs are responsible for reduced syntaxin mobility
  (whereas closed conformation plays no role)
- reduced mobility is caused by the assembly of syntaxin into clusters

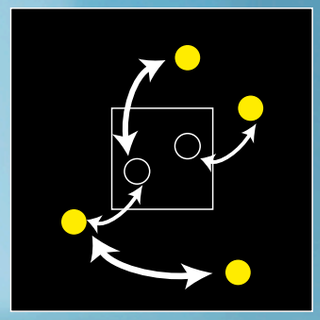
# Experiments 4 – molecule exchange or whole cluster diffusion?

- FRAP recovery du to ...



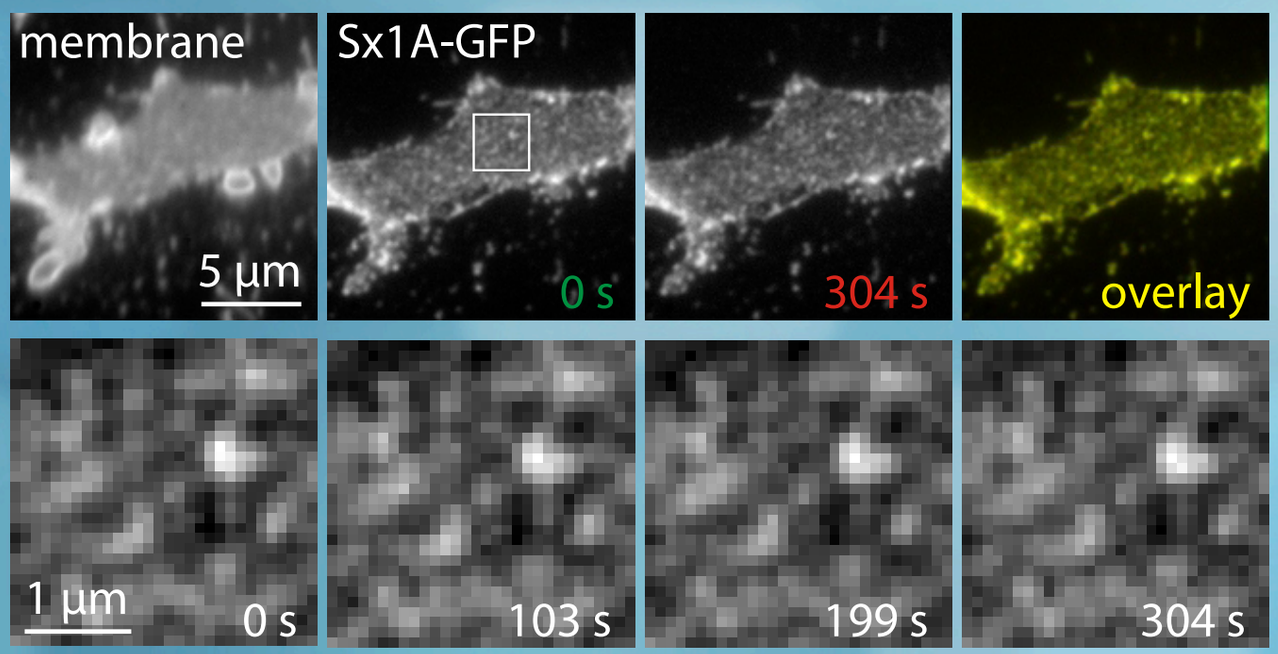



❶ diffusion of entire clusters?



❷ exchange of individual molecules?

- syntaxin spots do not change over minutes
  ⇒ Clusters are immobile. ⇒ Equilibrium of free and clustered syntaxins.



# The BD model

- **can size and dynamics of the syntaxin clusters be explained by simple physical principles?**
  (rather than by elaborate layers of biological regulation)
- MD not feasible, since we eventually need 250 s trajectories to compare with the FRAP experiments
- no need to describe in detail the collisions of syntaxins with surrounding lipids, consider them as a heat bath only
- MD becomes BD (Newtons eq. of motion ⇒ Langevin):
- Position Langevin eq. (Lax 1966, Zwanzig 1969):

$$\frac{d\mathbf{r}}{dt} = D\mathbf{F}(t) + \frac{d\mathbf{r}_S}{dt}$$

positions **r**, diffusion coefficient D, random velocity process d**r**$_S$/dt

- Recursive update of molecular positions with algorithm by Ermak (1975):

$$\mathbf{r}_i(t+\Delta t) = \mathbf{r}_i(t) + D\Delta t\mathbf{F}_i(t) + \xi\sqrt{2D\Delta t}$$

random number drawn
from a 2d normal distribution
with variance 1

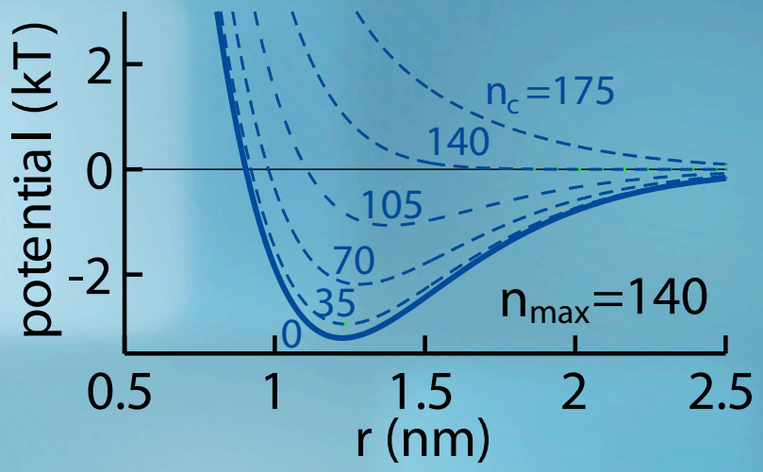
- lateral diffusion coefficient of TMR construct $D$ = 0.075(25) μm$^2$ / s
  determined from half time of recovery according to Ficz et al. (2005)

# The BD model

- diffusion on a 2d plane, periodic boundary conditions
- interaction potential between individual molecules i, j at distance $r = r_{ij}$ :

$$V(r_{ij}) = E_1 \cdot e^{-r^2/(2\sigma^2)} - E_2 \cdot f_s \cdot e^{-r^2/\{2(2\sigma)^2\}}$$

(1) (2) and (3)




  - (1) mutal repulsion of strength $E_1$ and range σ that prohibits molecules to run into each other (Pauli repulsion)
  - (2) effective attraction of strength $E_2$ and range 2σ between the molecules, mediated by the SNARE motifs
  - (3) $f_s$ steric hindrance due to crowding $f_s = 1 - \frac{n_c}{n_{max}}$

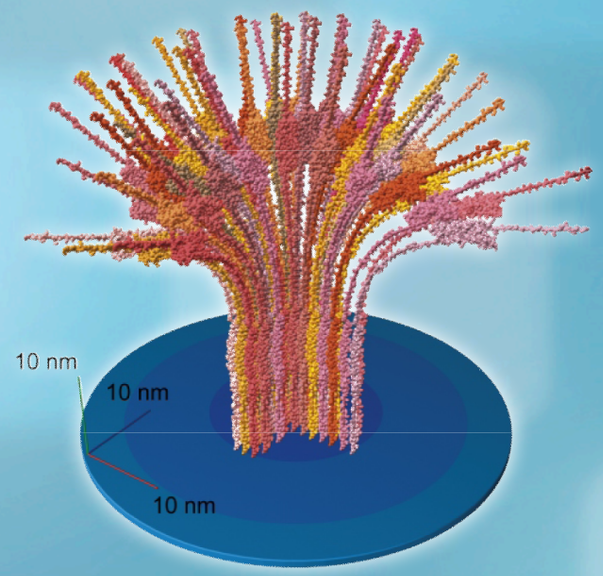
- force

$$\vec{F}(\vec{r}_i) = -\nabla_i U(\vec{r}_1, \ldots \vec{r}_n)$$

$$U = \Sigma_{i<j} V(r_{ij})$$

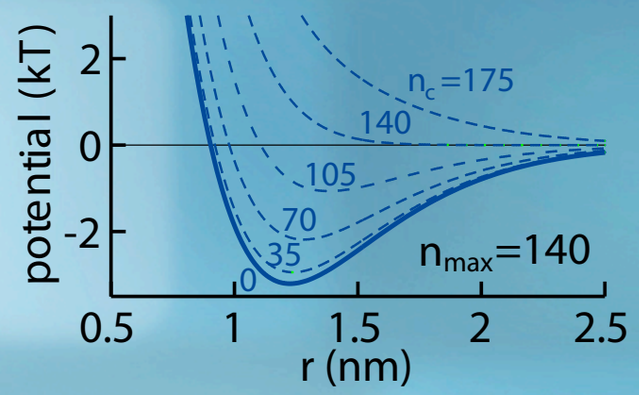
- σ is chosen such that an approximate area per TM helix of 1.5 $nm^2$ is obtained (Takamori et al. 2006)

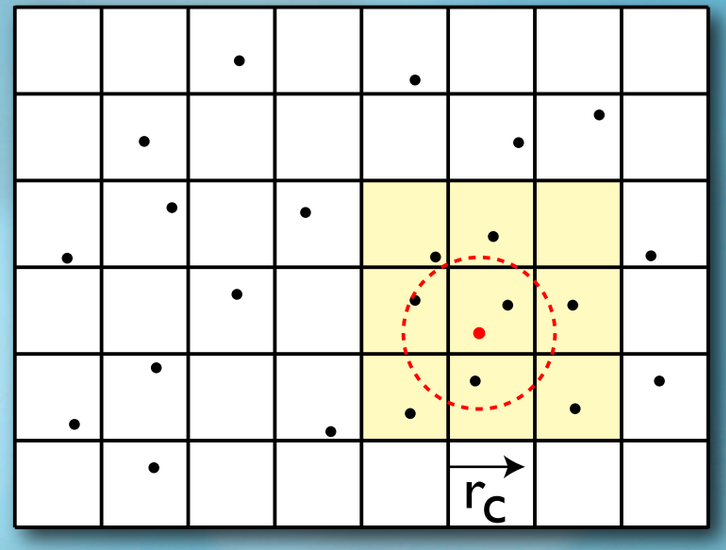
$$min(V) = \sqrt{1.5}\ \text{nm}$$



# Global algorithm

- generate starting configuration
- calculate forces
  - make neighbourlists (every 10th step)
  - detect clusters (every 10th step)
  - calculate forces for particles within cutoff radius
- update positions
- calculate fluorescence recovery
- output
  - $\mathbf{r}_i = (x, y)_i$, bleached?
  - potential energy
  - FRAP signal intensity
  - cluster size distribution

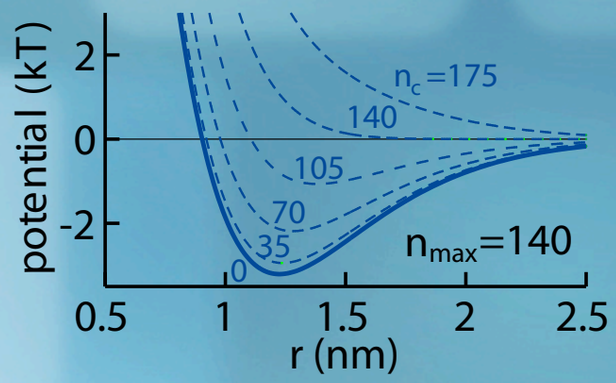


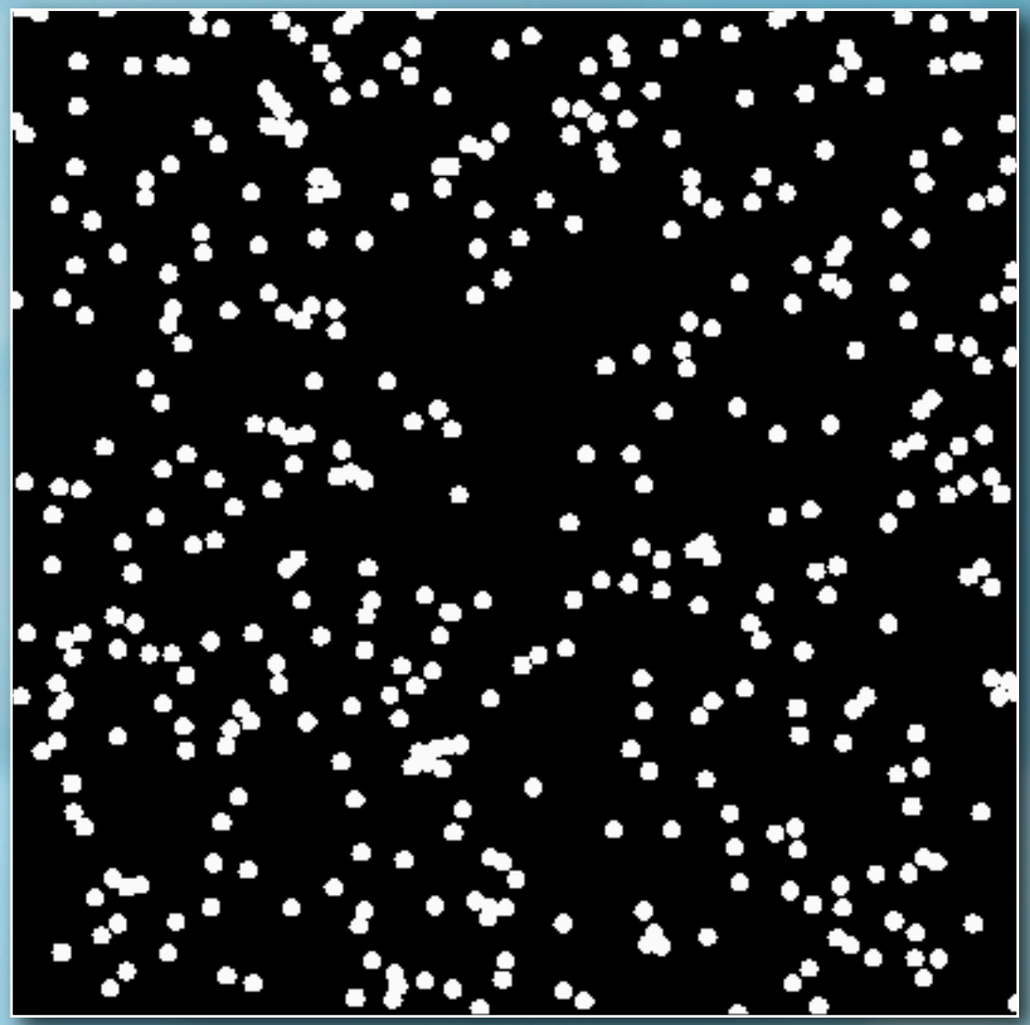




gridded neighbour searching
with cutoff >3.5 nm

# Cluster detection

- needed for cluster penalty term $$f_s = 1 - \frac{n_c}{n_{max}}$$
- if distance d between 2 molecules < $r_d$ =1.5 nm they belong to same cluster
- HOW TO AUTOMATICALLY DETECT THAT?
- if 2 molecules belong to same cluster, they must be in same neighbourlist, since $r_d < r_{cutoff}$

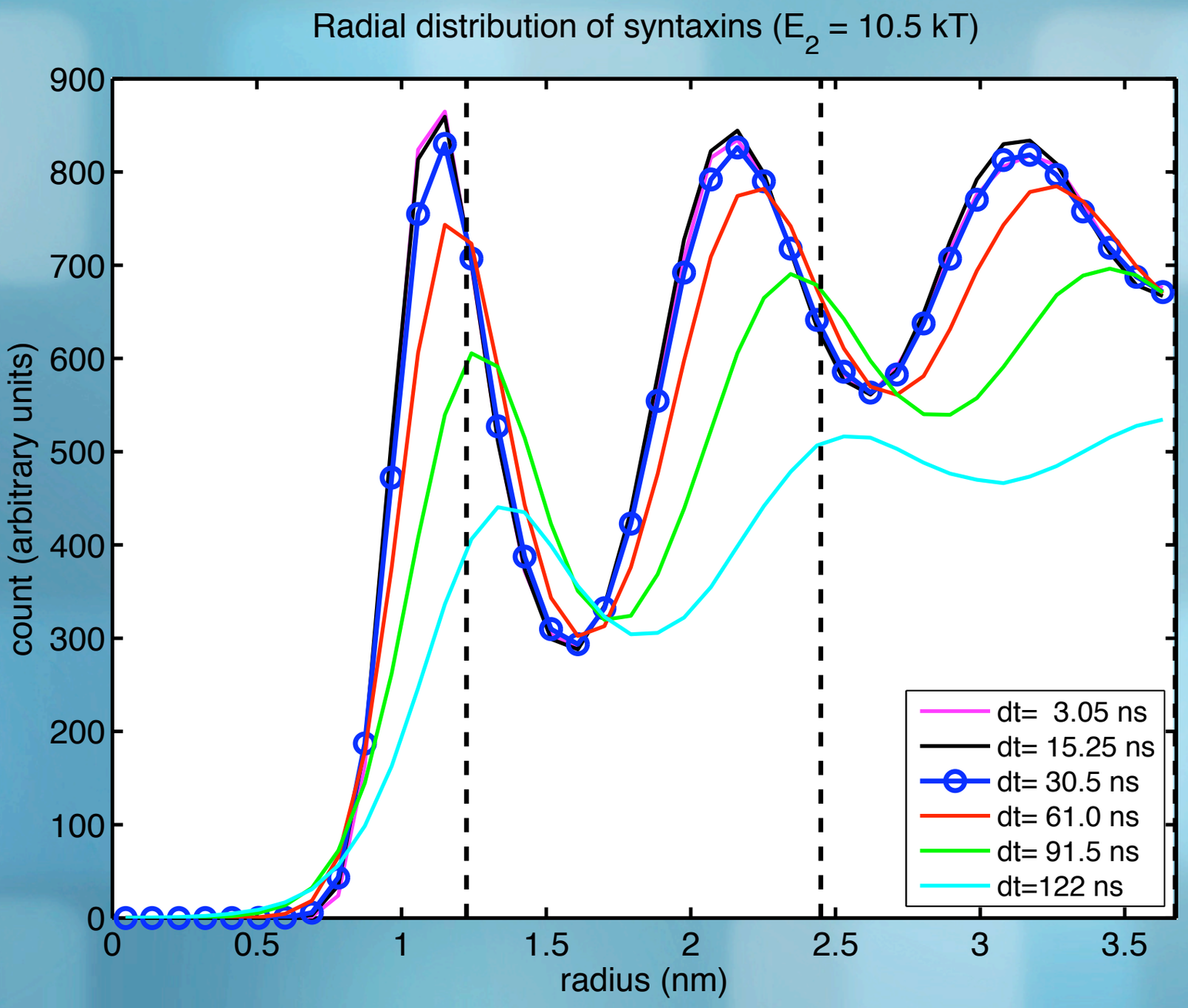



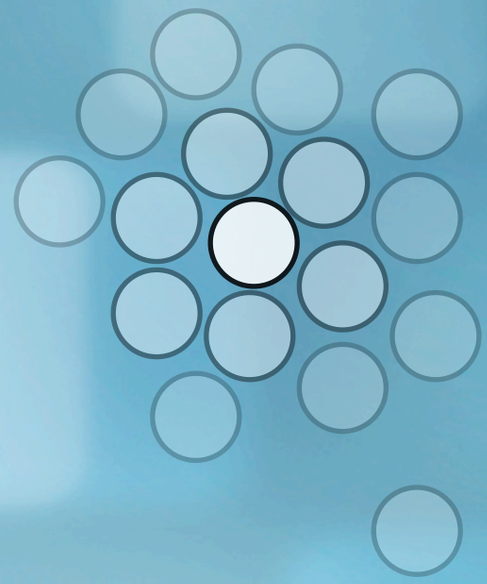



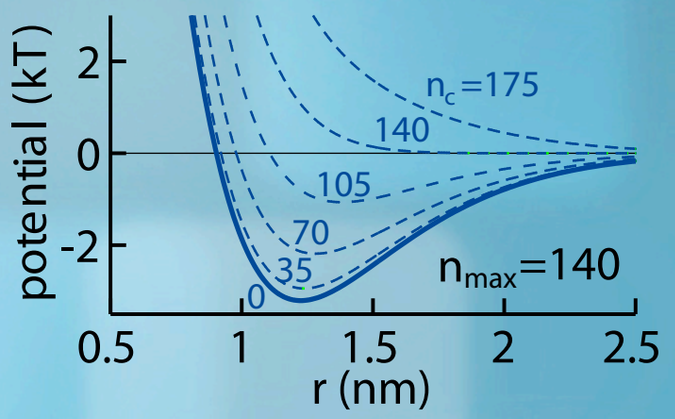
*snapshots every 250 steps*

- go through all molecules
- (if not already assigned to a cluster), build the network that connects all molecules with a mutual distance of less than 1.5 nm;
  mark each of those molecules with a unique cluster-number.

# Time step length





# Parallel force calculation

- fastest CPUs (roc) need ≈3 days for 80M time steps (250 s)
- **icc** ./synsim.c -O3 **-openmp** -o synsim.x

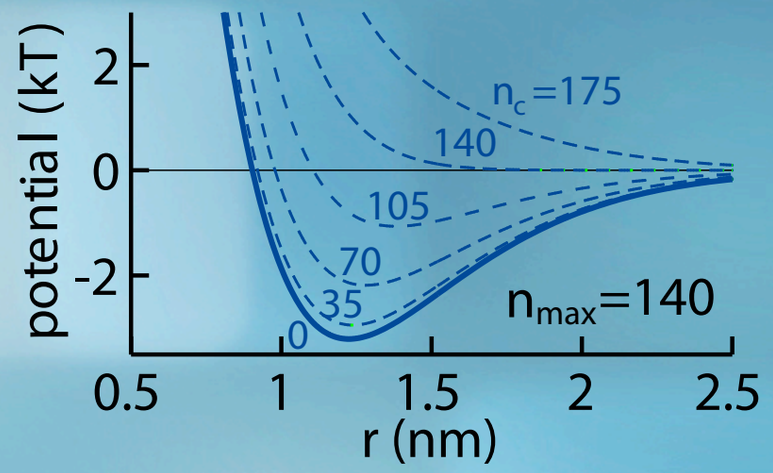
```
#include <omp.h>

...

/*****************************************************************************/
/*  start omp parallel region                                                */
/*****************************************************************************/
#pragma omp parallel for \
  default(none) \
  shared(signal, dvdx, dvdy, stderr) \
  firstprivate(bleachedarr, cluster_size, cluster_no, numneighbours, ind, x, y) \
  private(xoffset, yoffset, signalindex, xdum, ydum, xblock, yblock, blockindex, \
    j, jsize, ijsize, isize, neighbour_no, index, r2, dx, dy, fac, a, \
     exp1, exp2) \
  reduction(+ : etot) \
   schedule(static)

/* loop over molecules */
for (i = 0; i < nsyntaxins; i++)
{
    (calculate potential & force)
    ...
}
```

Benchmark on Kea:

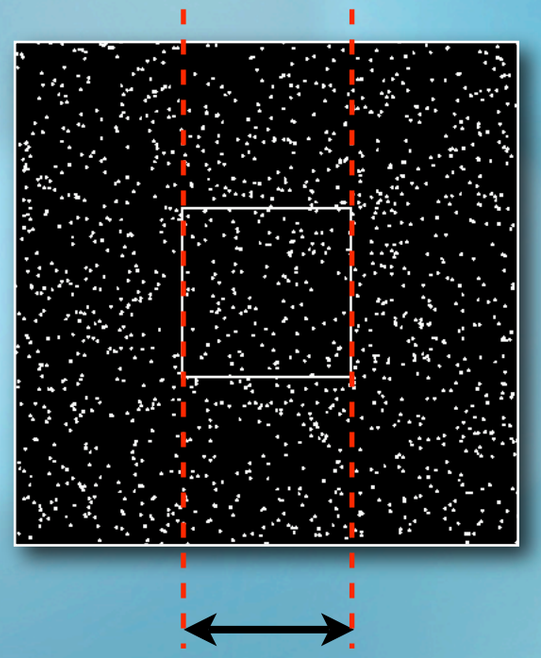
| CPUs | time/s | speedup | scaling | |
|---|---|---|---|---|
| 1 | 165.1 | 1.00 | 1.00 | serial code |
| 1 | 170.6 | 0.97 | 0.97 | threaded code |
| 2 | 93.4 | 1.77 | 0.88 | |
| 4 | 54.0 | 3.06 | 0.76 | |

# Simulation protocol

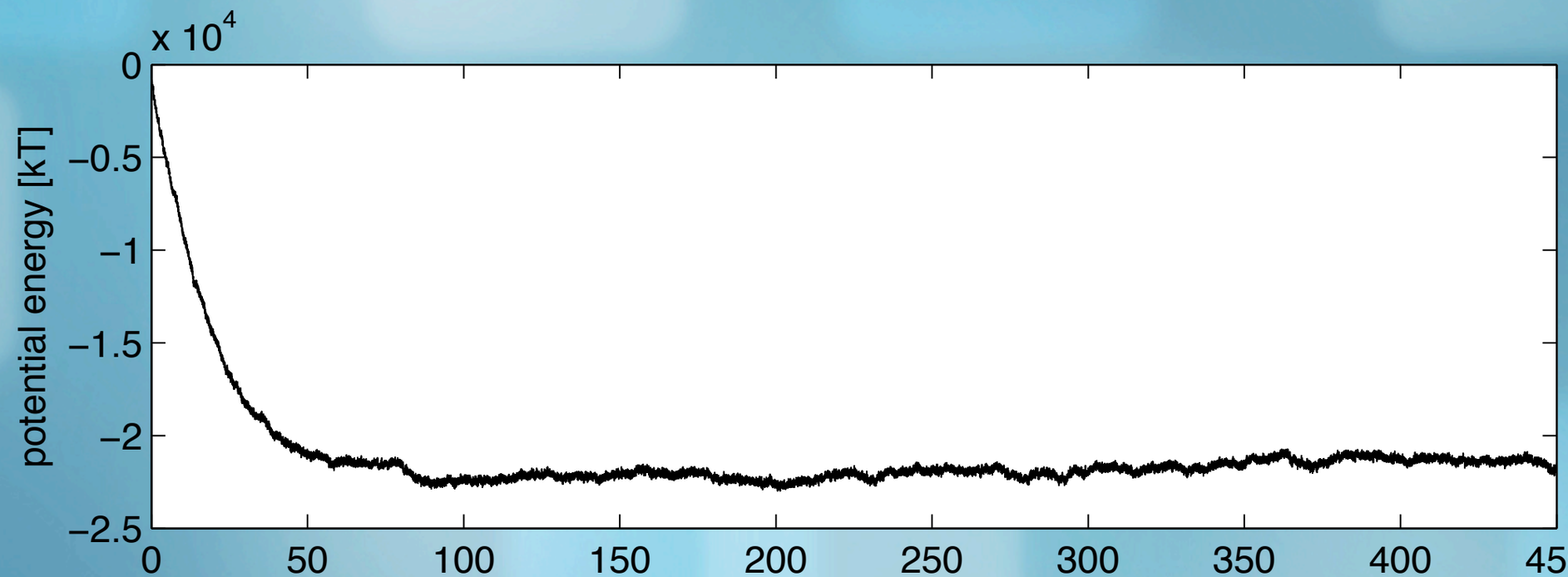
- **make a parameter study:**
  vary $E_2$ and $n_{max}$
  - $E_2$ ⇒ depth of well
  - $n_{max}$ ⇒ at which cluster size force becomes repulsive

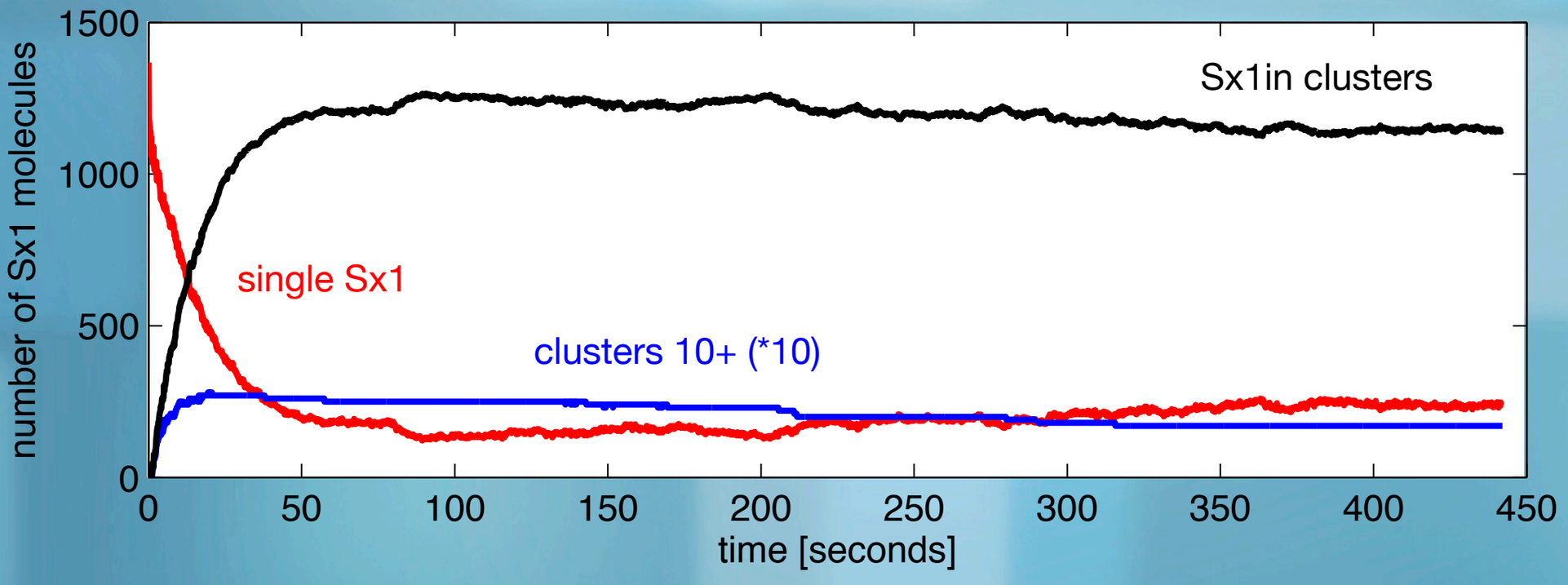



- start with 1391 random positions $\mathbf{r}_i$
- iterate until **equilibrated** (potential energy)
- **if** cluster density matches exp. density of 19.6 (5.7) clusters / $\mu m^2$
  - bleach (simulate FRAP experiment)
  - record 250 s of fluorescence recovery
  - now match experimental FRAP curve
    - extract number of molecules per cluster, fraction of free molecules

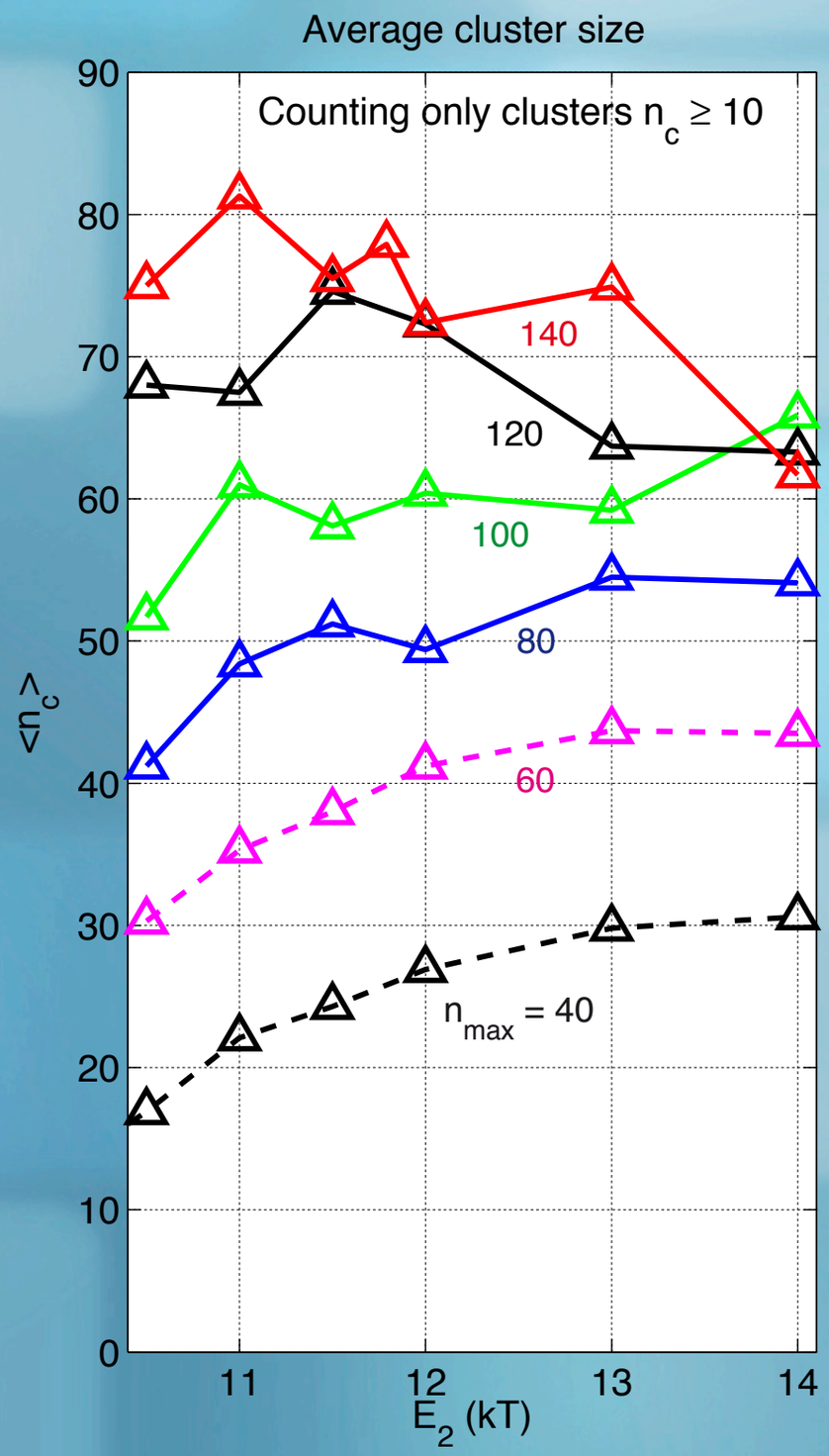


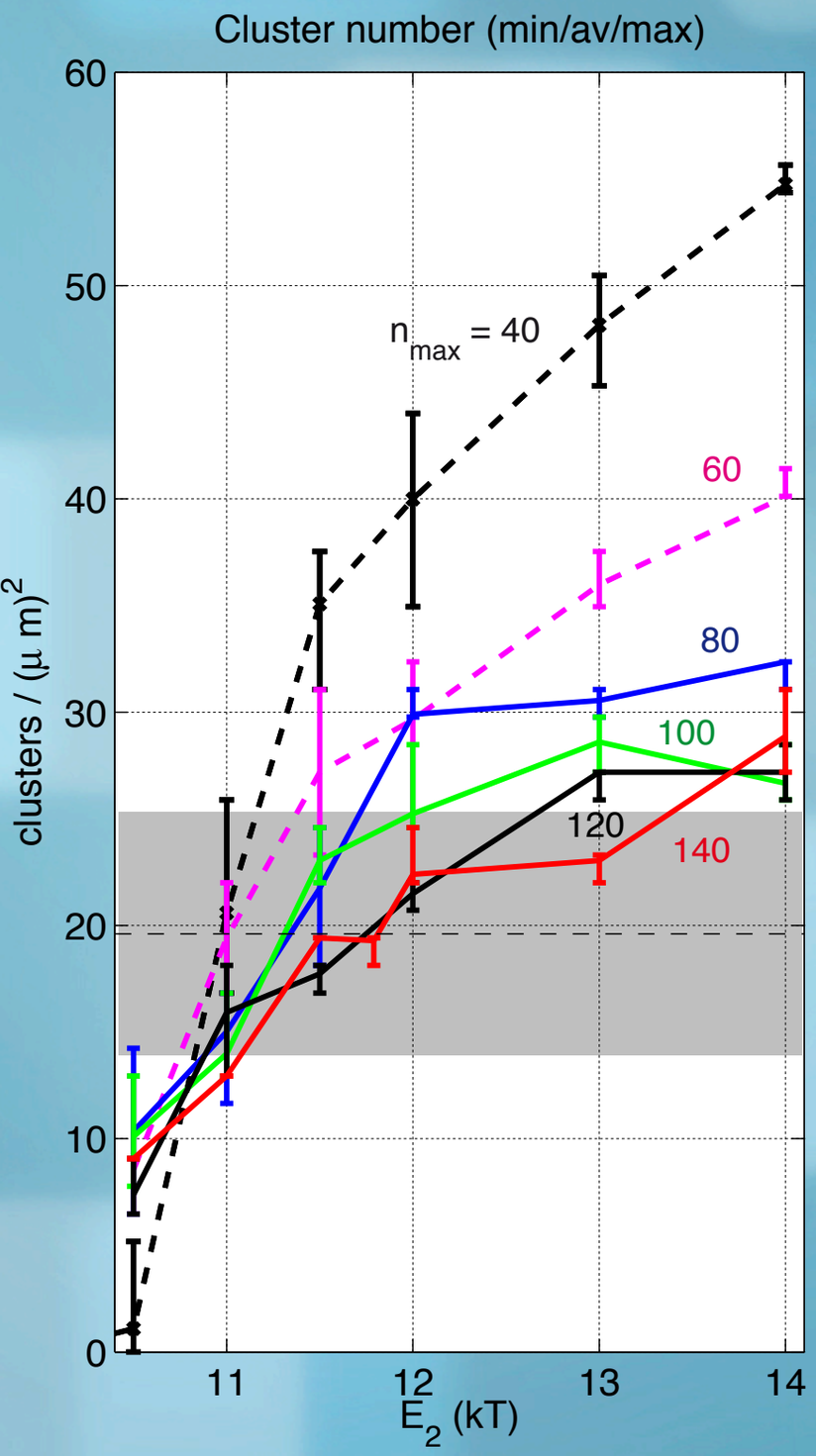
# Equilibration



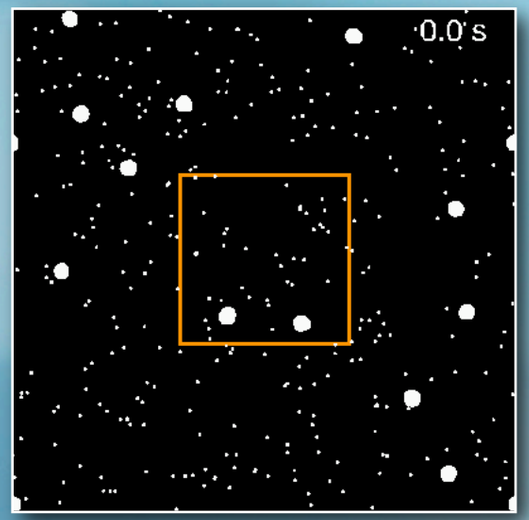
# Parameter study

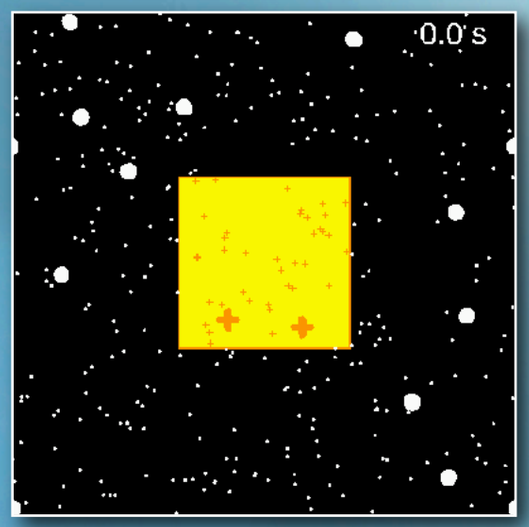


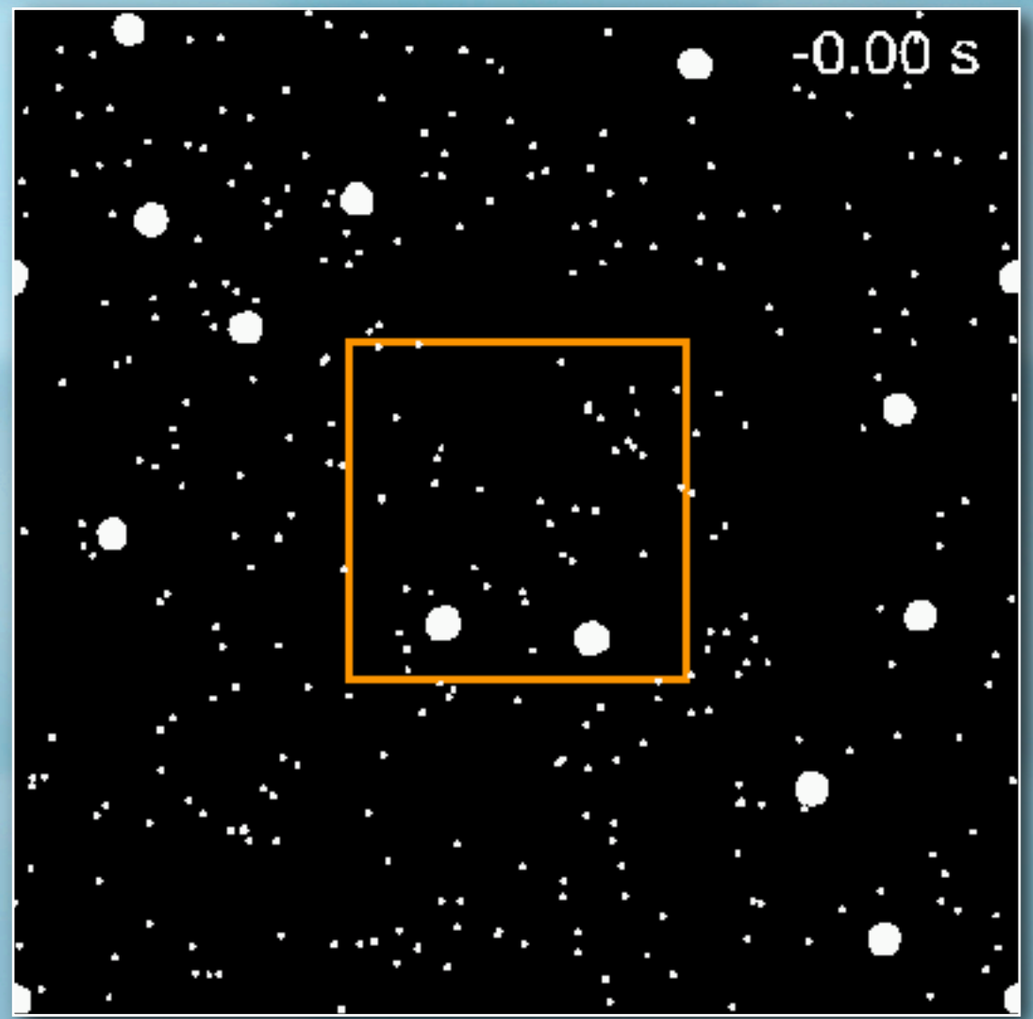



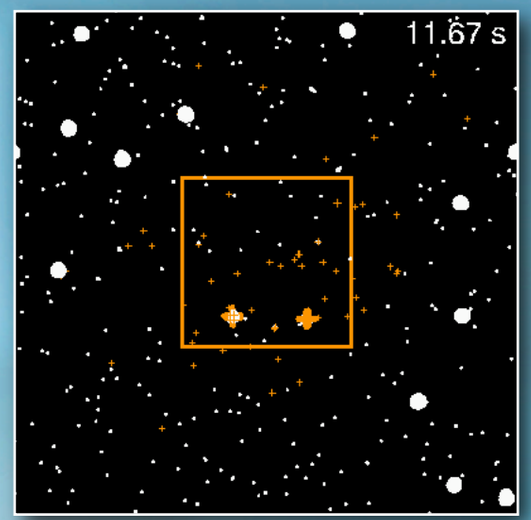

} experimental density of 19.6 (5.7) clusters/ $\mu m^2$

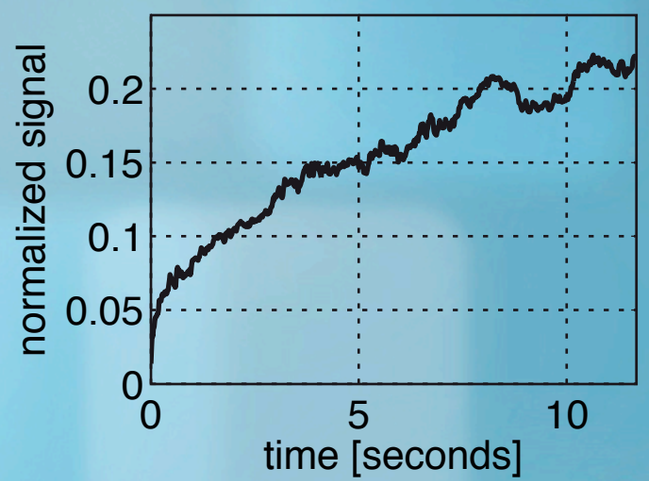
# Example 0-11s



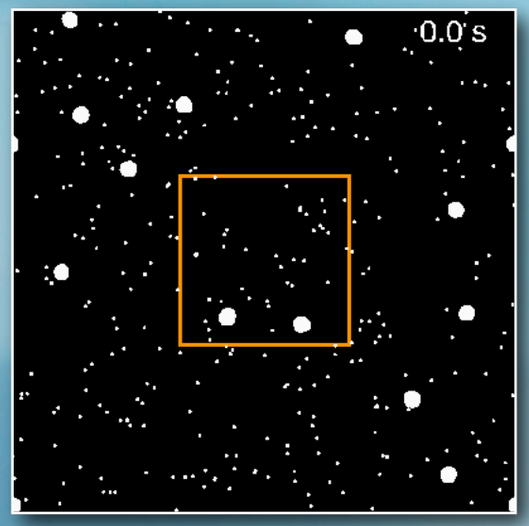



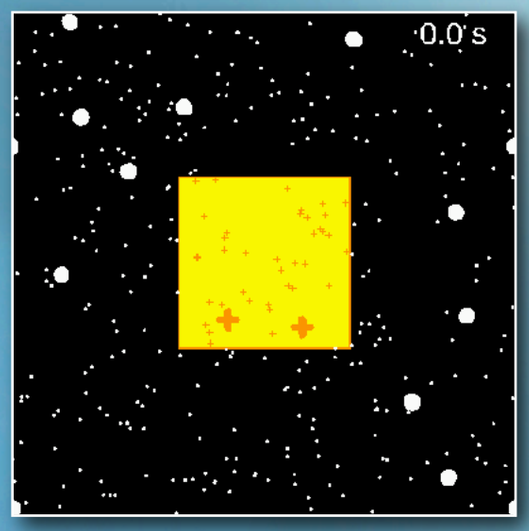



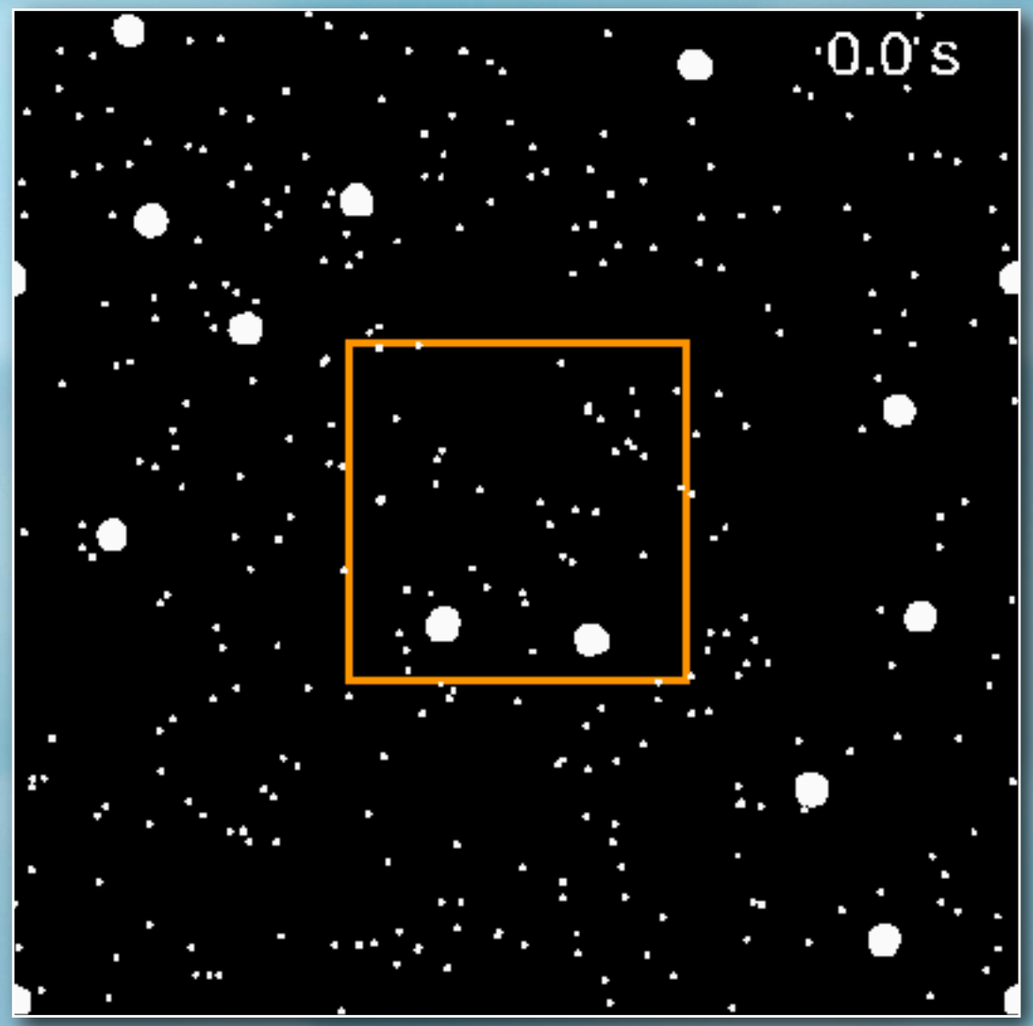



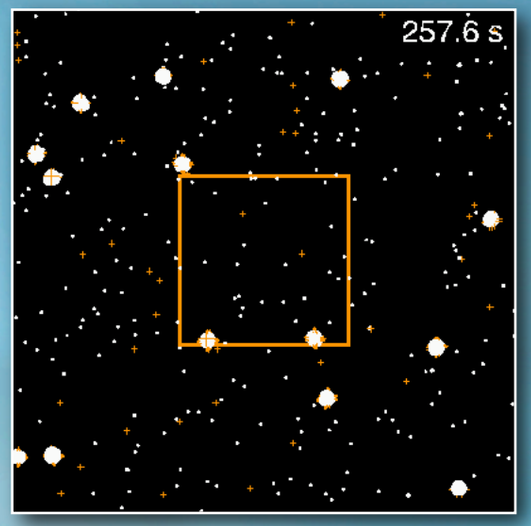

- experiment measures intensity of white molecules in central area
- bleached molecules (orange) are invisible

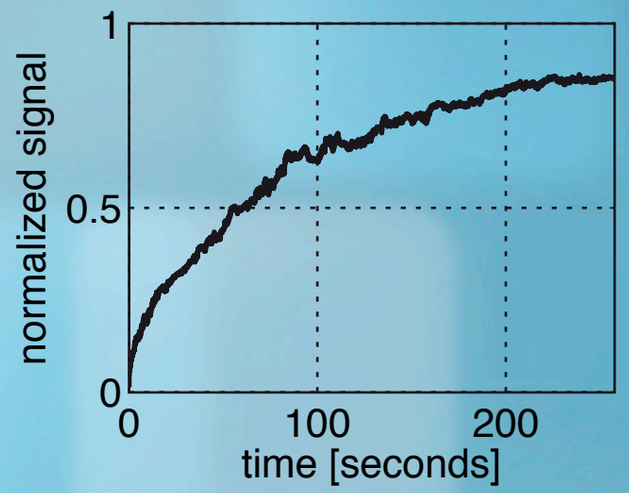
# Example 0-257 s




- experiment measures intensity of white molecules in central area
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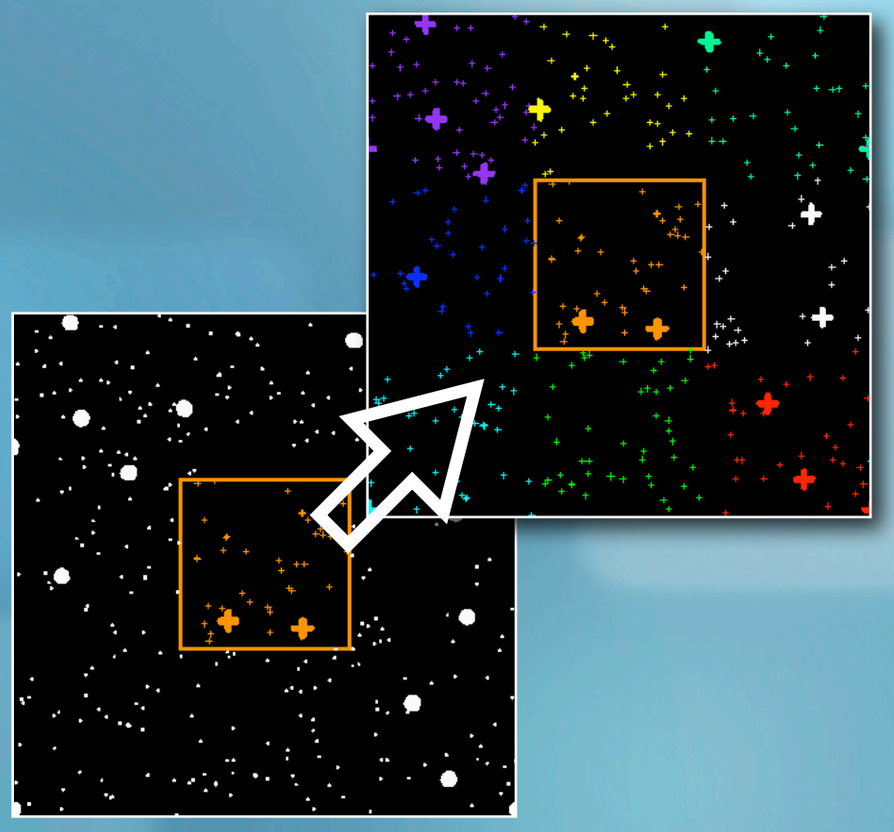
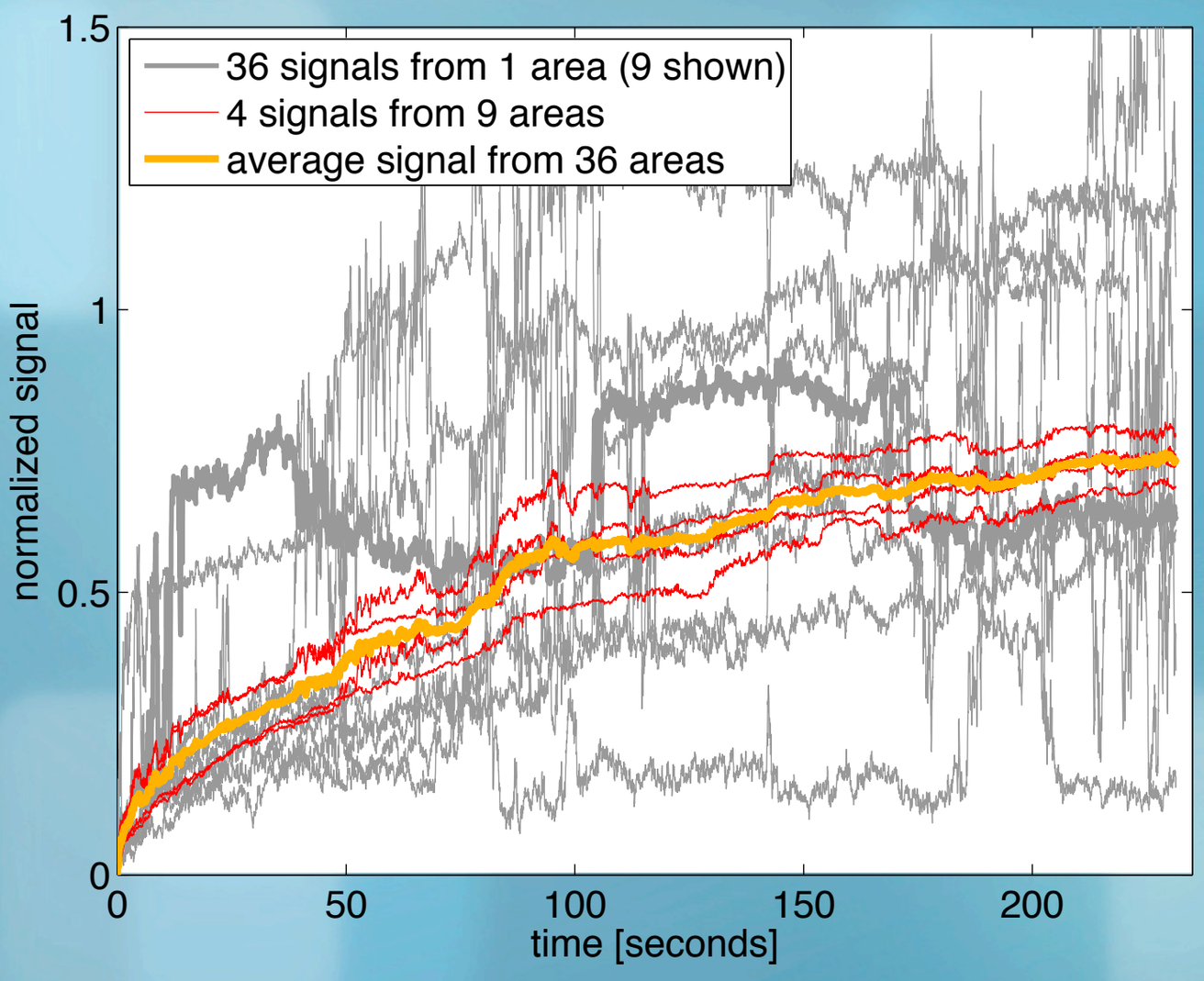
# Simulation of FRAP data

- each unbleached molecule in the middle area adds one count to the intensity each time step
- bleach central area
- bleach 9 areas:
  assign each syntaxin a number from 1-9 depending on where it is at bleach time
- displace by half a bleach box size in x, y, x&y directions
- ⇒ altogether 36 bleach areas

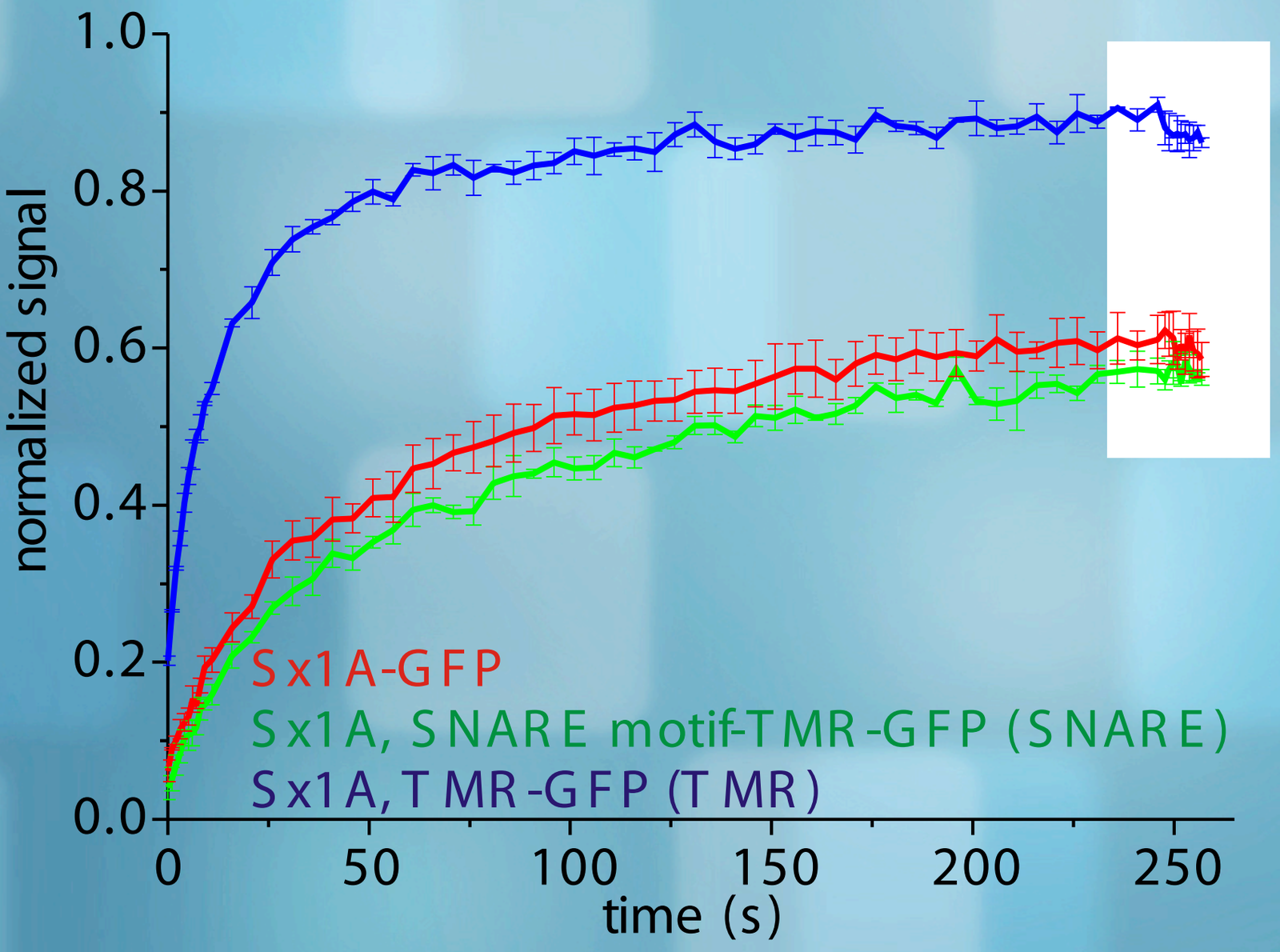




# Image acquisition correction

- FRAP experiments: bleach fraction s of molecules during each image acquisition

$$s = 1 - (I_a^{exp} - I_b^{exp})^{1/10} \approx 0.005$$



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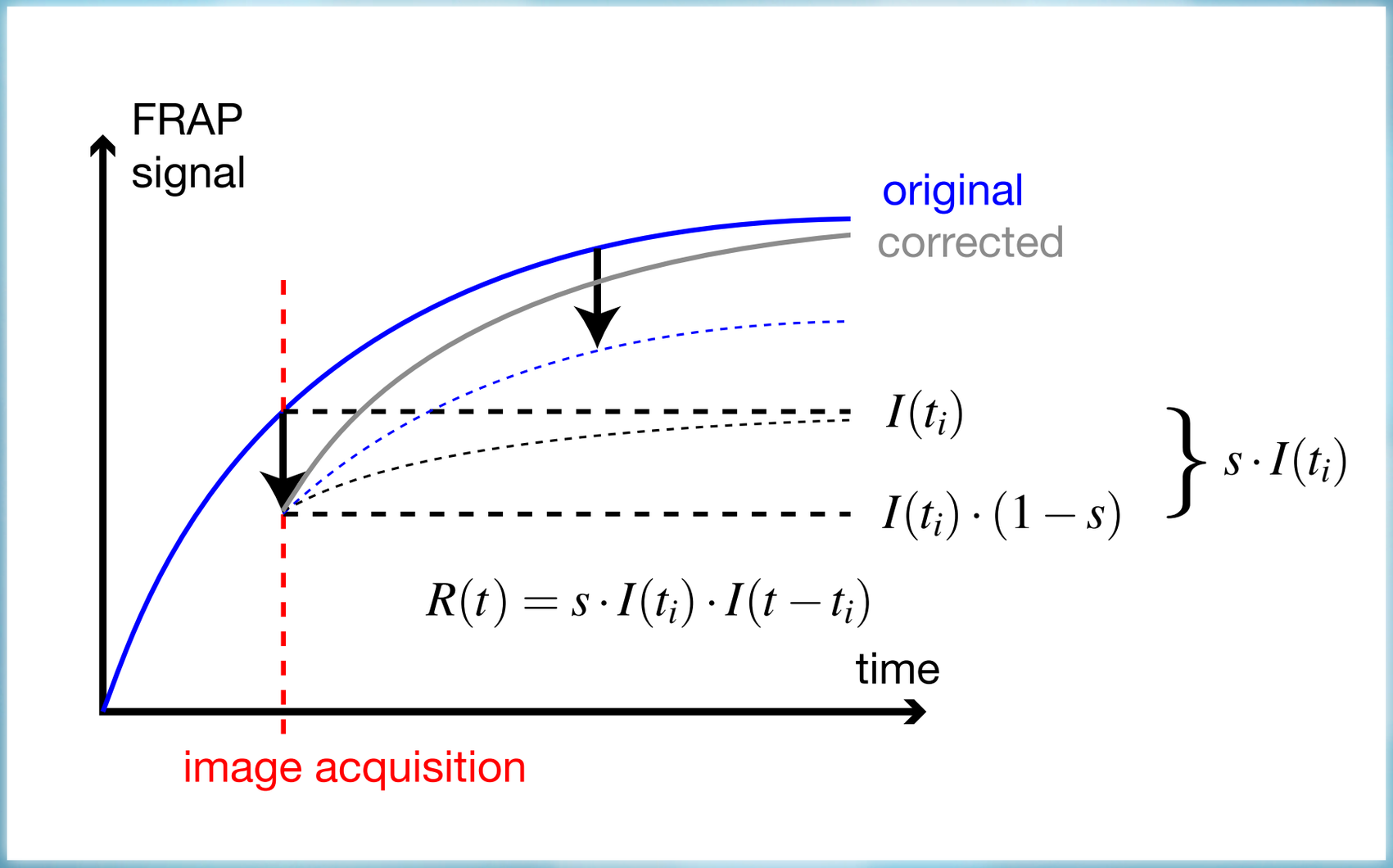
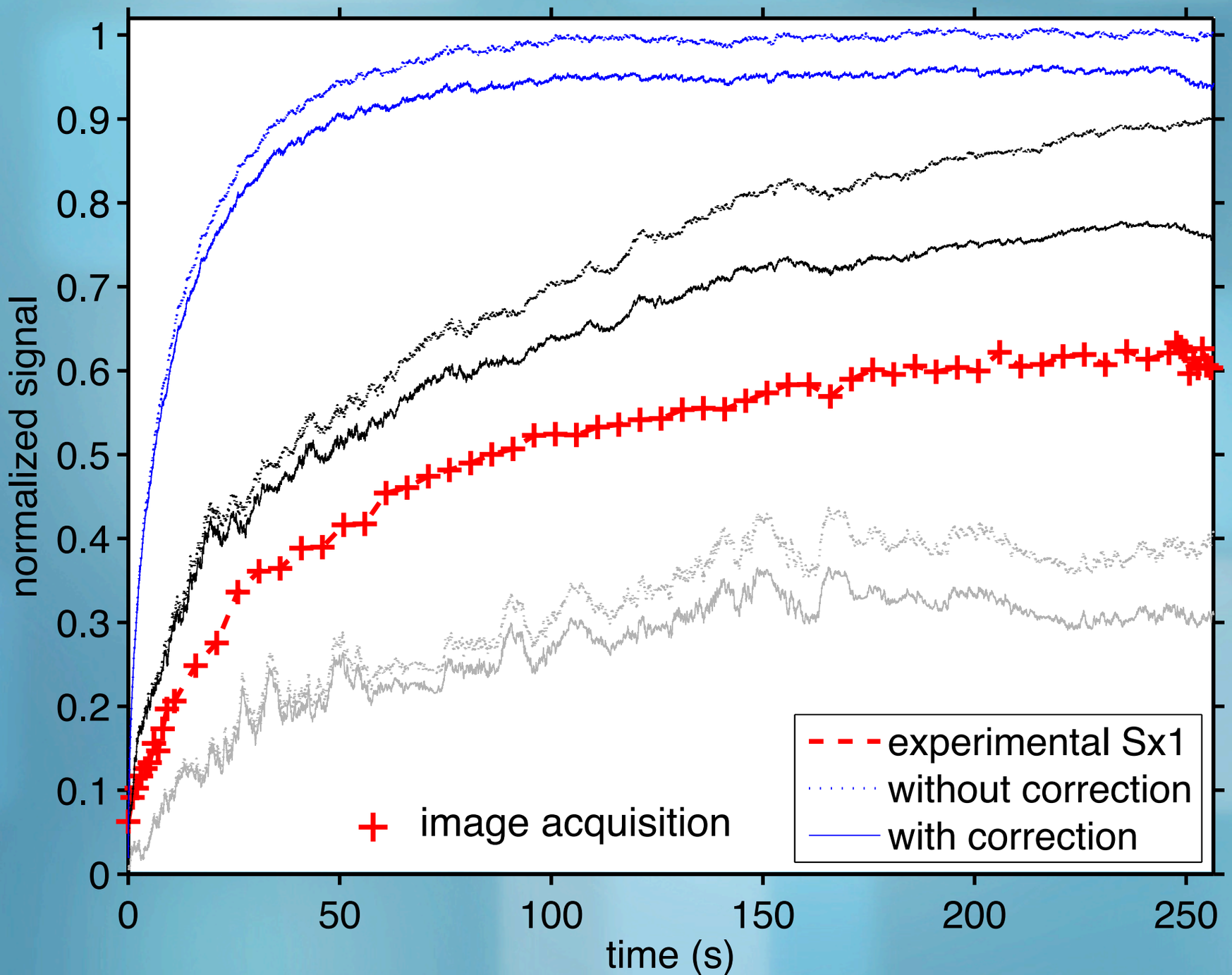


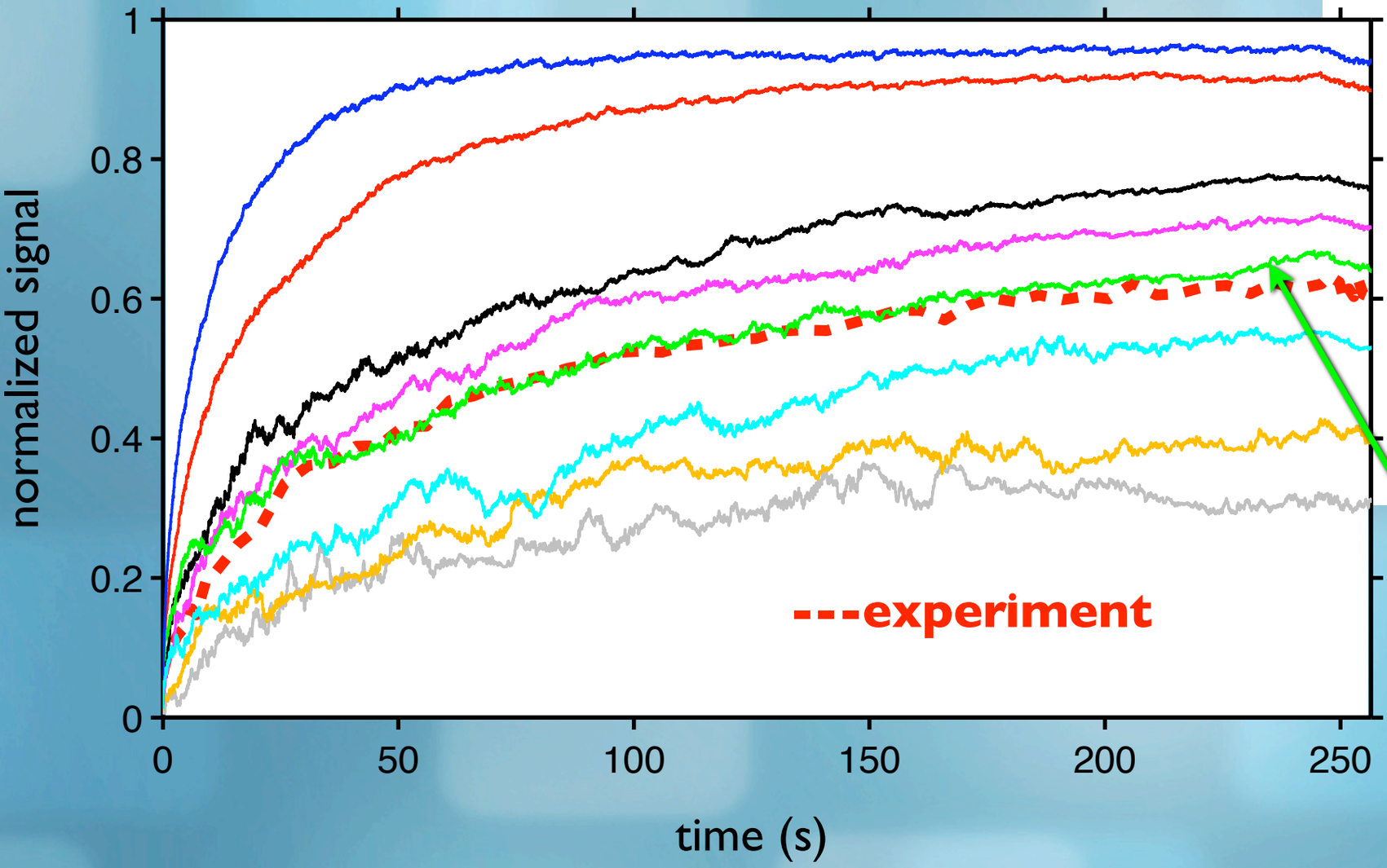

- include this bleaching effect in the simulated curves by simulating 68 data acquisitions

# Image acquisition correction



# Simulated fluorescence recovery

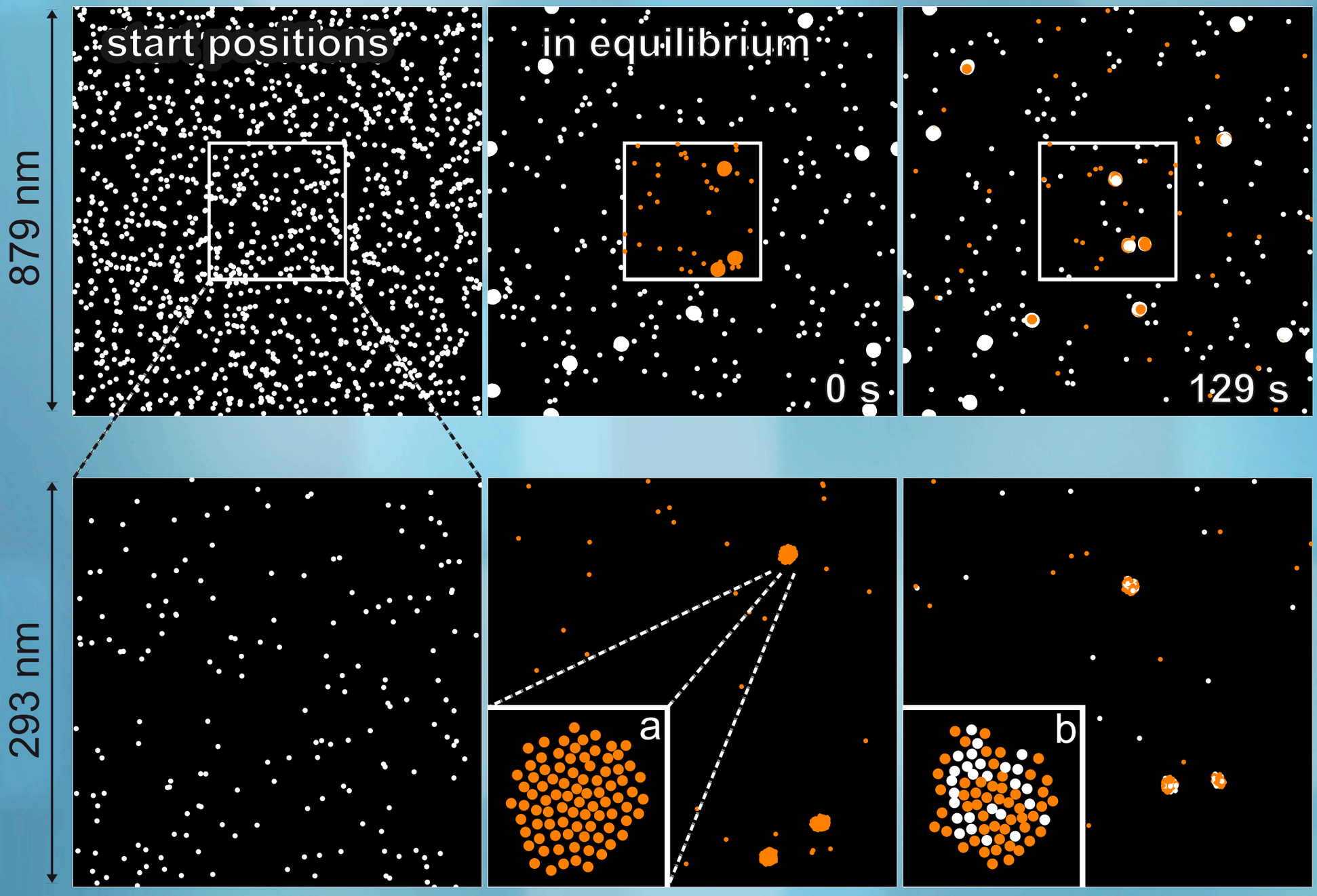
for simulations that match experimentally
observed cluster density




| free Sx1 | $n_{max}$ | $E_2$ |
|---|---|---|
| 100% | ---- | 0.0 |
| 55% | 60 | 11.0 |
| 21% | 120 | 11.5 |
| 18% | 140 | 11.5 |
| 16% | 140 | 11.8 |
| 10% | 140 | 12.5 |
| 4% | 140 | 13.0 |
| 1% | 140 | 14.0 |

- simulated curves are average of 2 trajectories each
- 1:10 scale ⇒ $10^2$ x faster diffusion and FRAP recovery

# Snapshots



# Conclusions

- experimental data on composition and dynamics of Sx1 clusters can be explained by simple physical principles

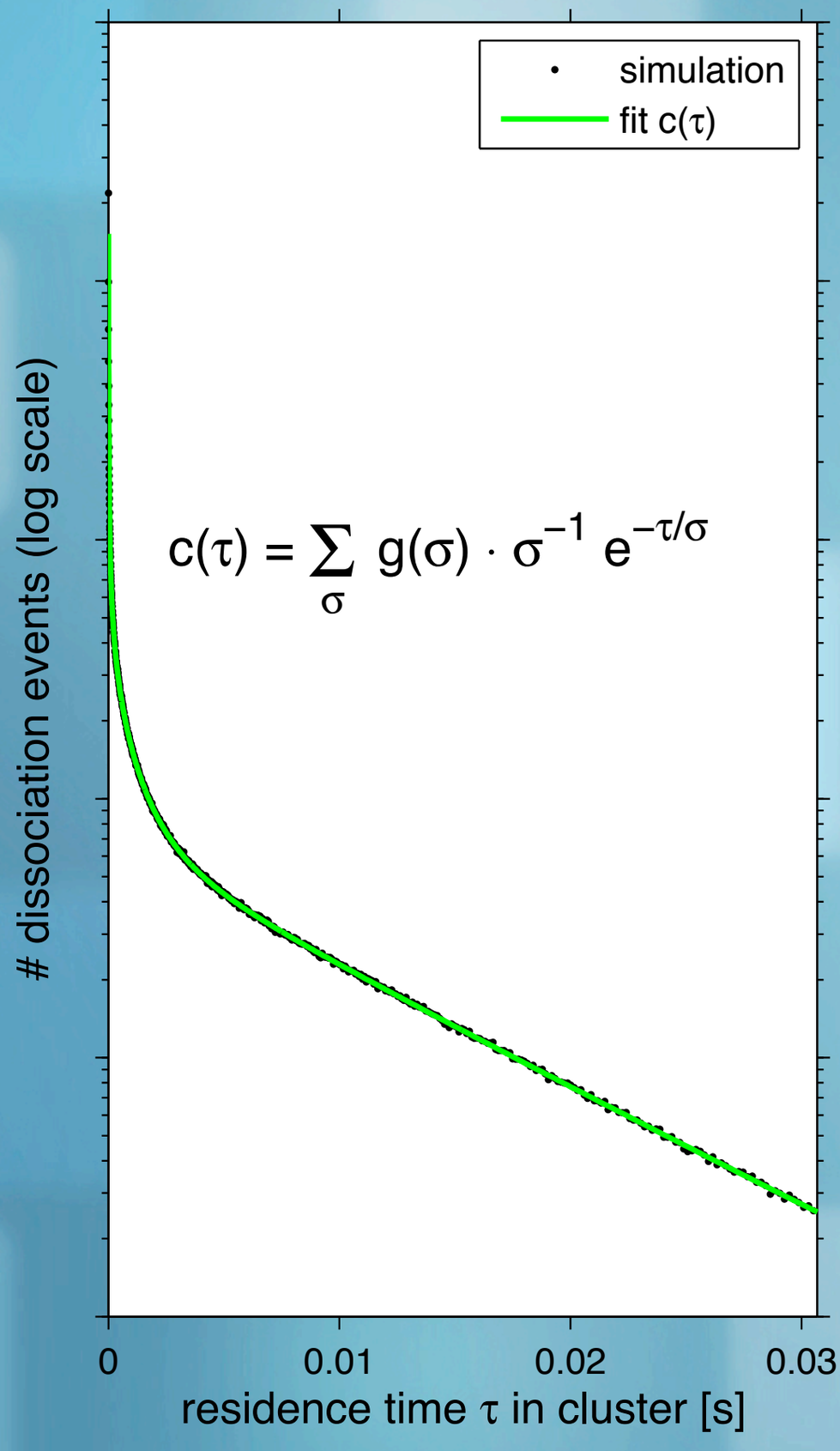
protein-protein attraction ↔ steric hindrance

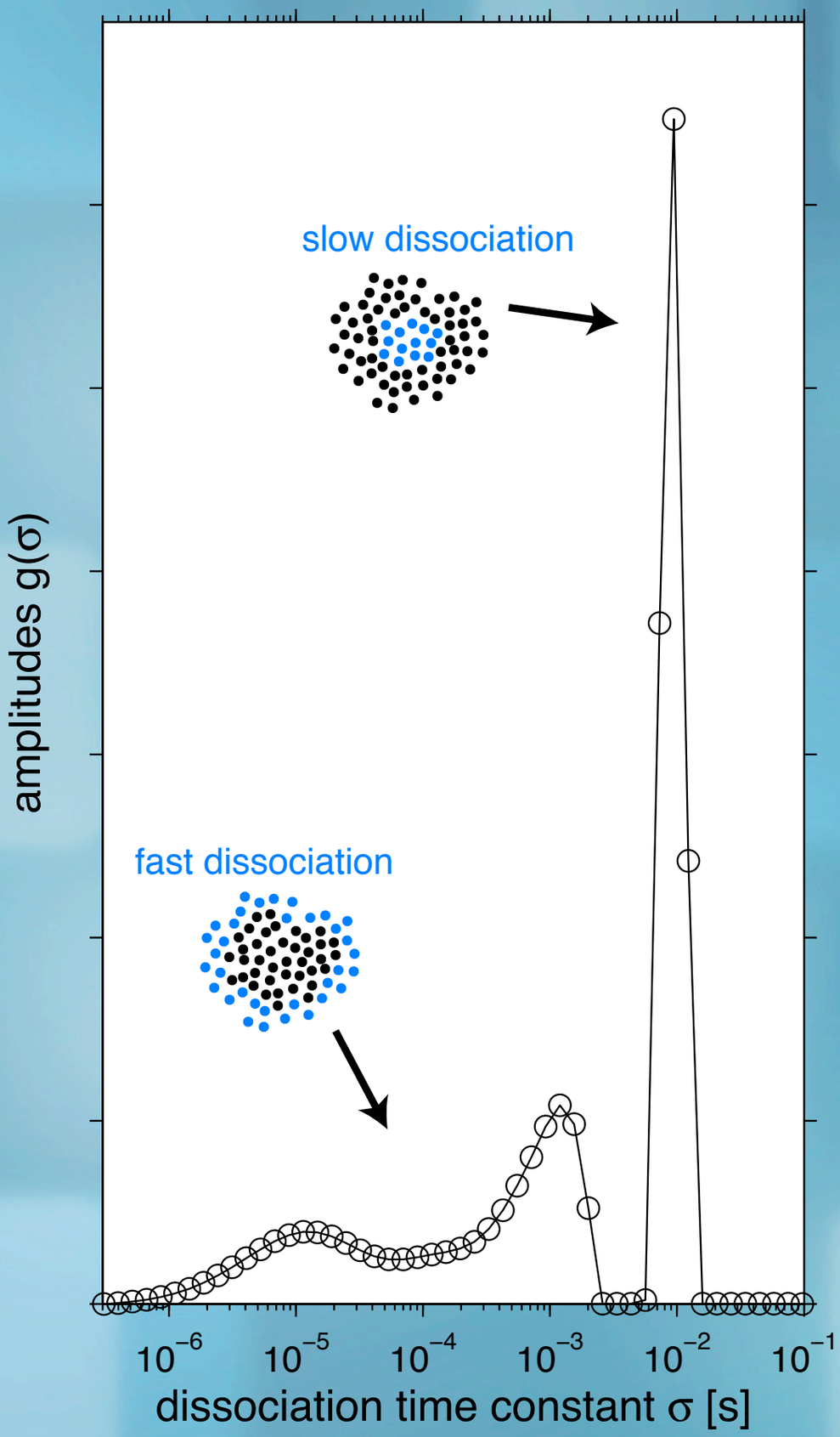
  - Sx1 molecules are quasi-immobile when in clusters
  - a fraction of ≈16% of the molecules diffuse freely between the clusters which contain 70–80 Sx1

- Clustering via self-assembly likely applies to a variety of membrane protein clusters
- presented a framework with syntaxin-1 as an example

# Acknowledgments


- Jochen Sieber & Thorsten Lang – experiments, experiments, ...
- Helmut Grubmüller – Idea & model layout
- 10500 – thanks to all of you!



simulation
fit c(τ)
$c(\tau) = \sum_{\sigma} g(\sigma) \cdot \sigma^{-1} e^{-\tau/\sigma}$
# dissociation events (log scale)
0
0.01
0.02
0.03
residence time τ in cluster [s]


slow dissociation
fast dissociation
amplitudes g(σ)
$10^{-6}$
$10^{-5}$
$10^{-4}$
$10^{-3}$
$10^{-2}$
$10^{-1}$
dissociation time constant σ [s]