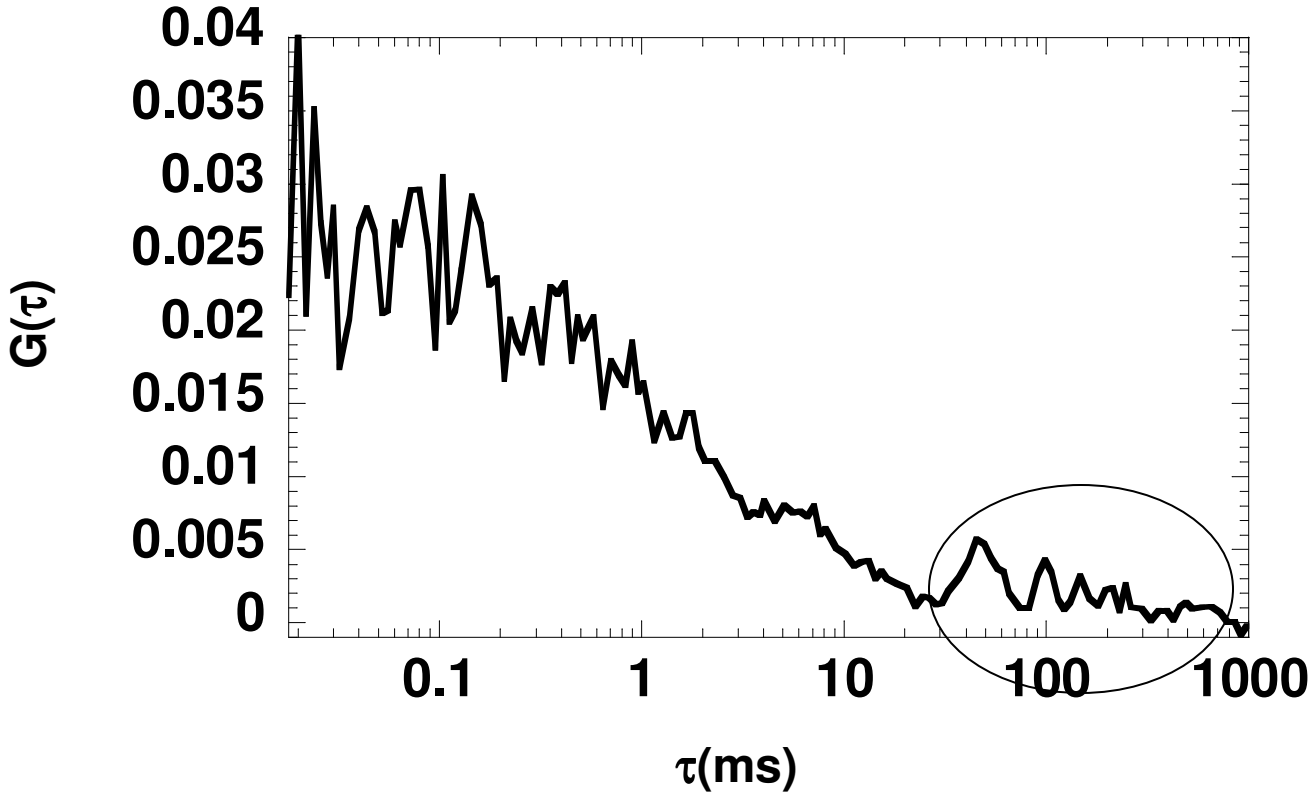
Figure S1



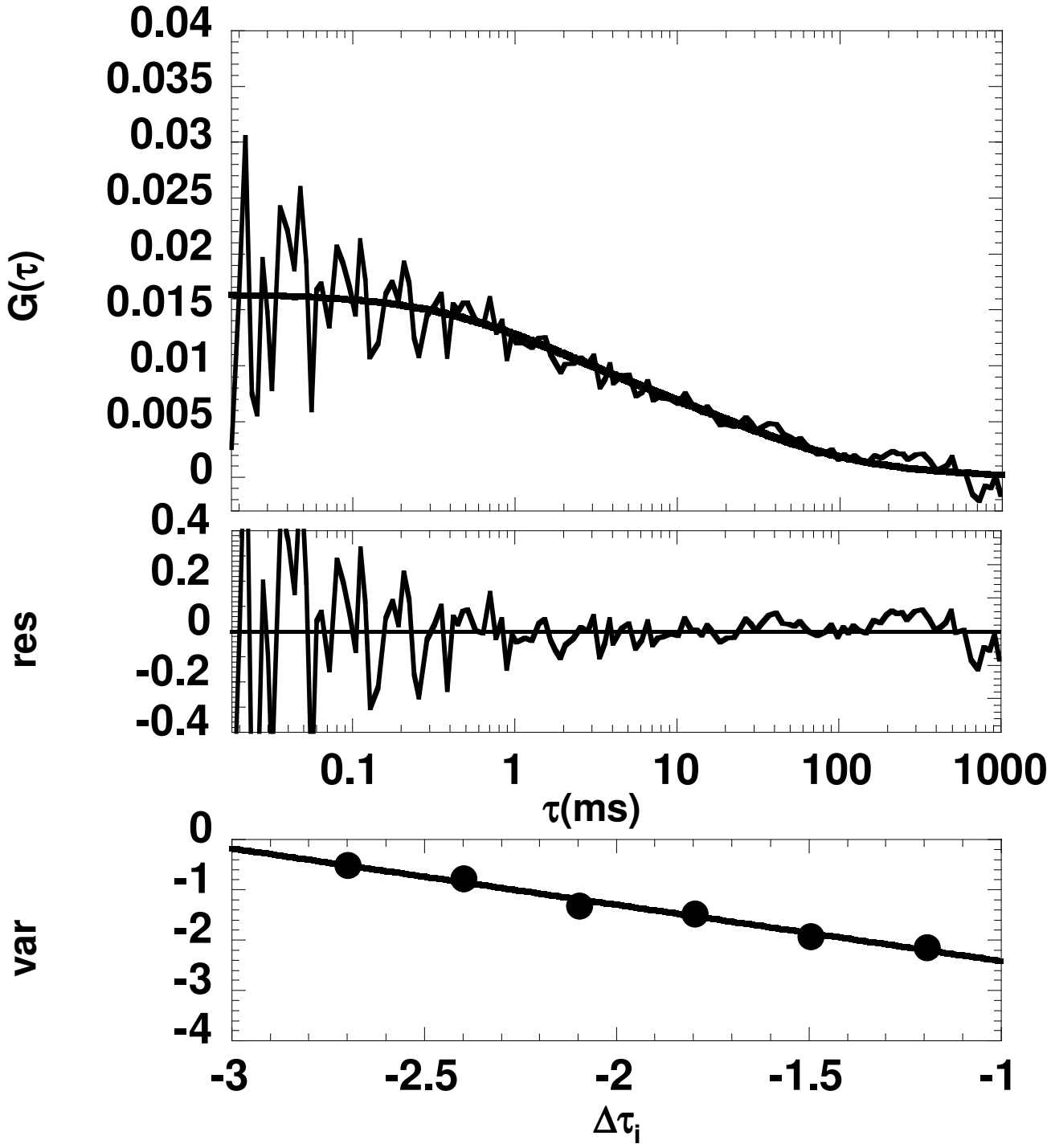

0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
G(τ)
0.4
0.2
0
-0.2
-0.4
res
0.1
1
10
100
1000
τ(ms)
0
-1
-2
-3
-4
var
-3
-2.5
-2
-1.5
-1
Δτi

Figure S2A

Figure S2B

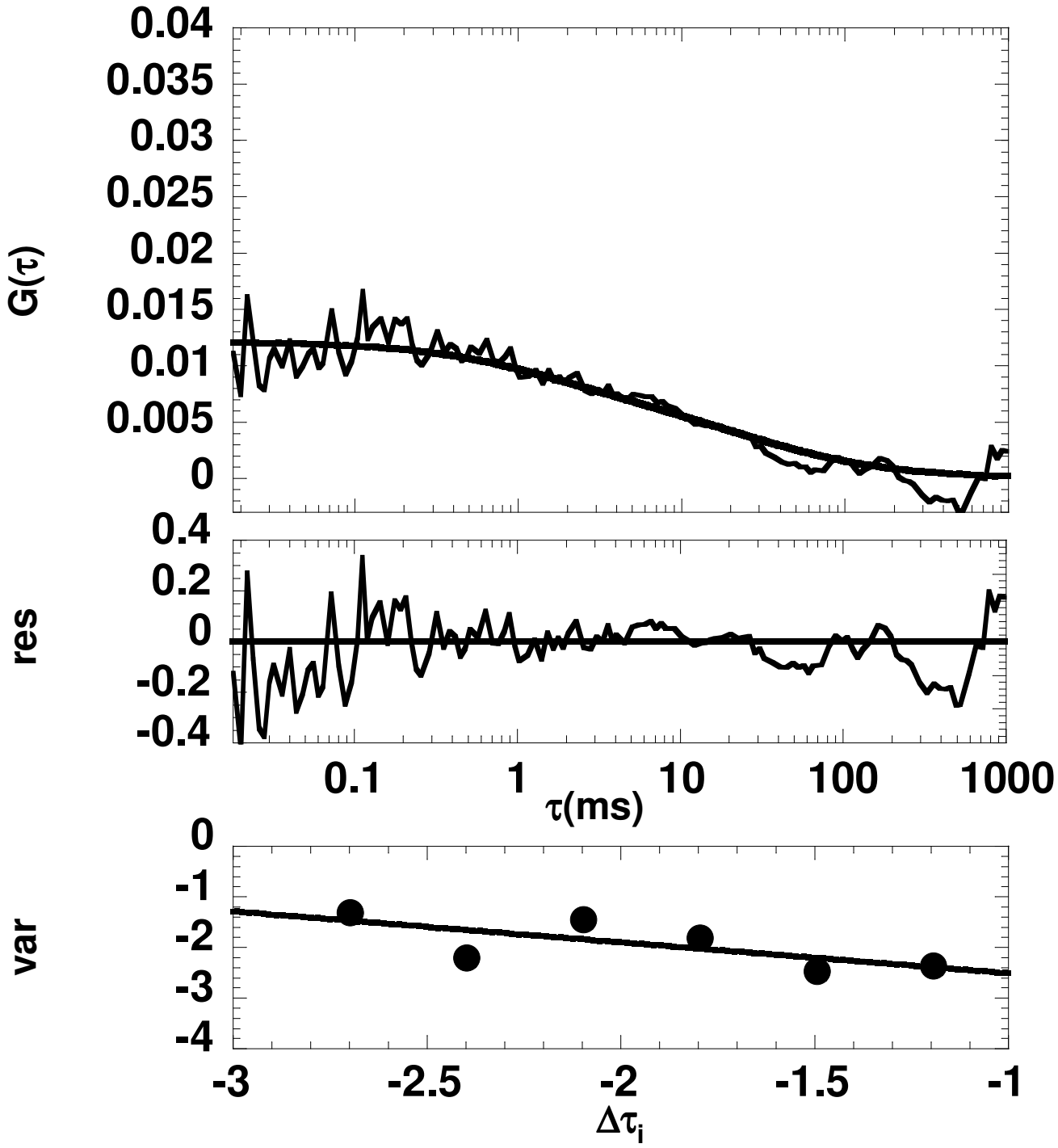

0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
G(τ)
0.4
0.2
0
-0.2
-0.4
res
0.1
1
10
100
1000
τ(ms)
0
-1
-2
-3
-4
var
-3
-2.5
-2
-1.5
-1
Δτi

Figure S2C

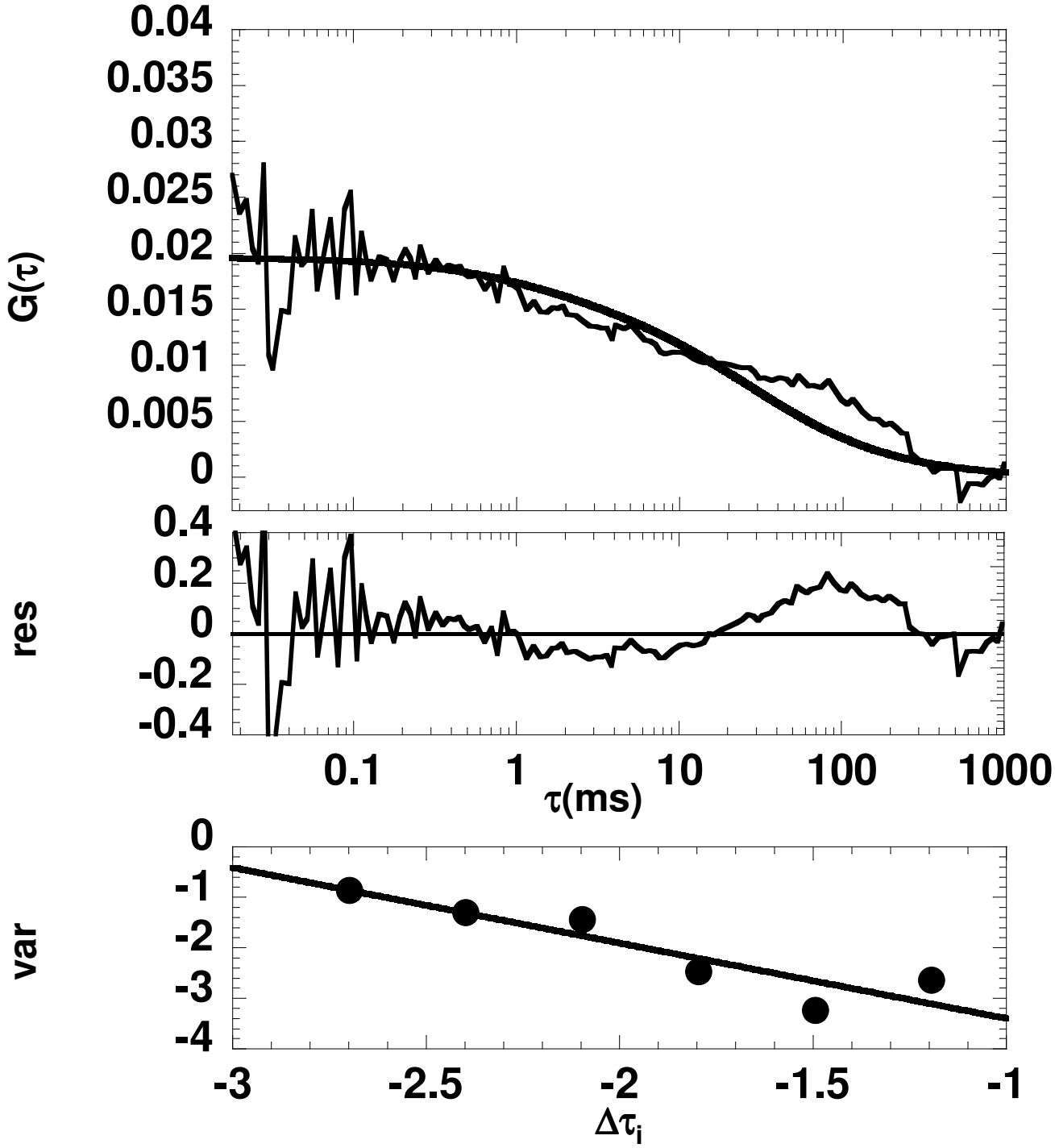

0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
G(τ)
0.4
0.2
0
-0.2
-0.4
res
0.1
1
10
100
1000
τ(ms)
0
-1
-2
-3
-4
var
-3
-2.5
-2
-1.5
-1
Δτi

Figure S2D

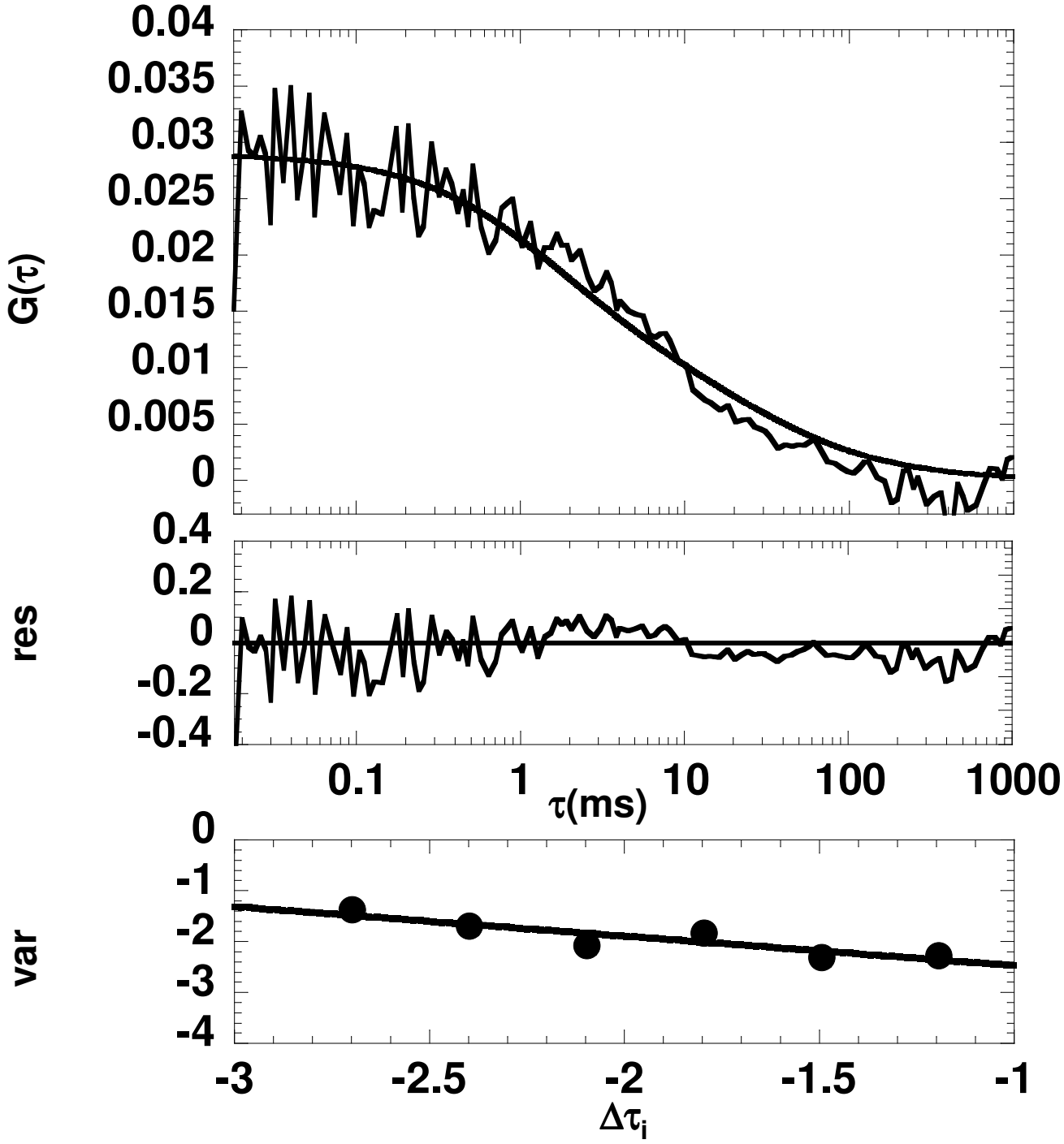

0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
G(τ)
0.4
0.2
0
-0.2
-0.4
res
0.1
1
10
100
1000
τ(ms)
0
-1
-2
-3
-4
var
-3
-2.5
-2
-1.5
-1
Δτi

Figure S3

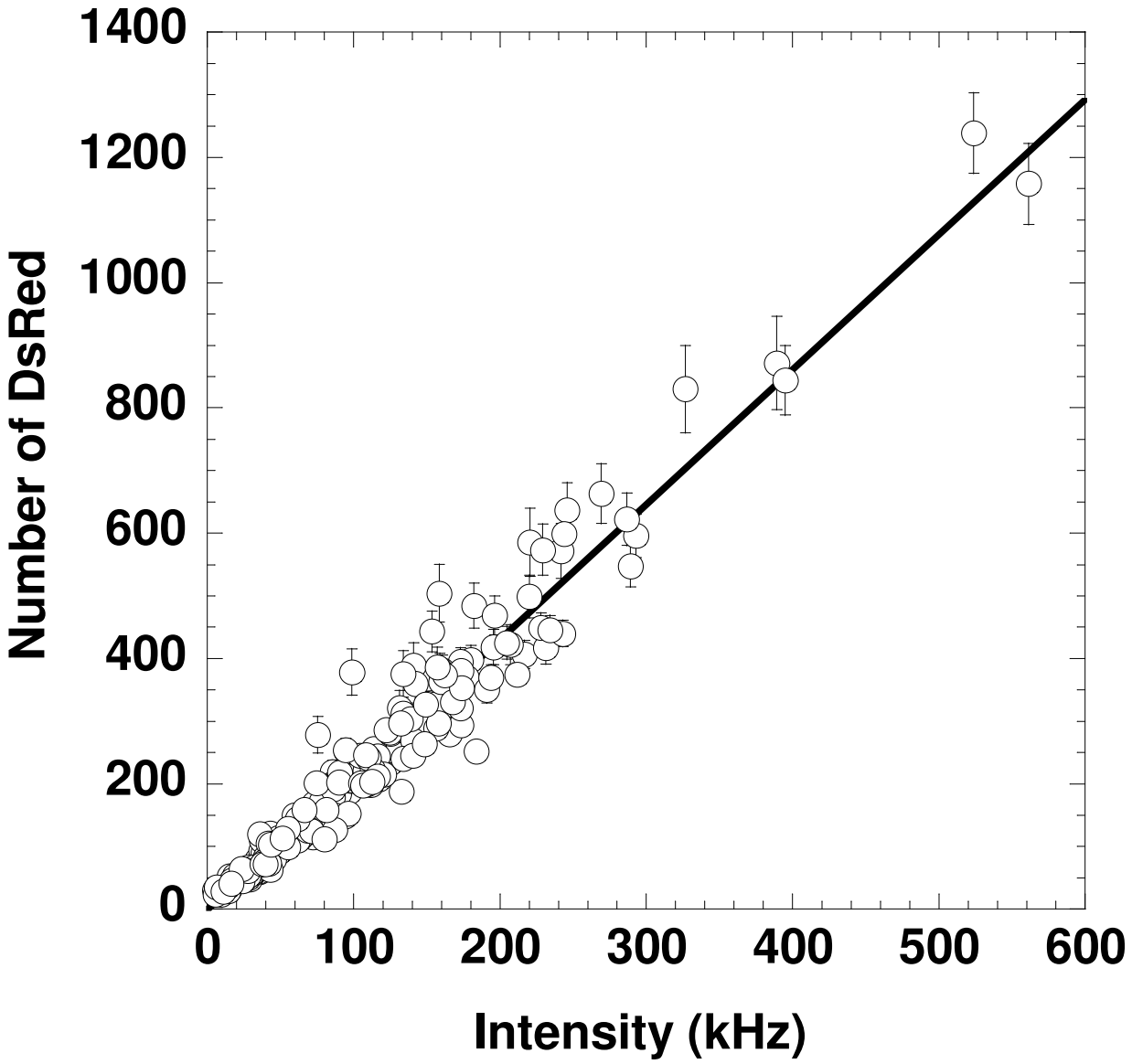

Number of DsRed
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
Intensity (kHz)

Figure S4

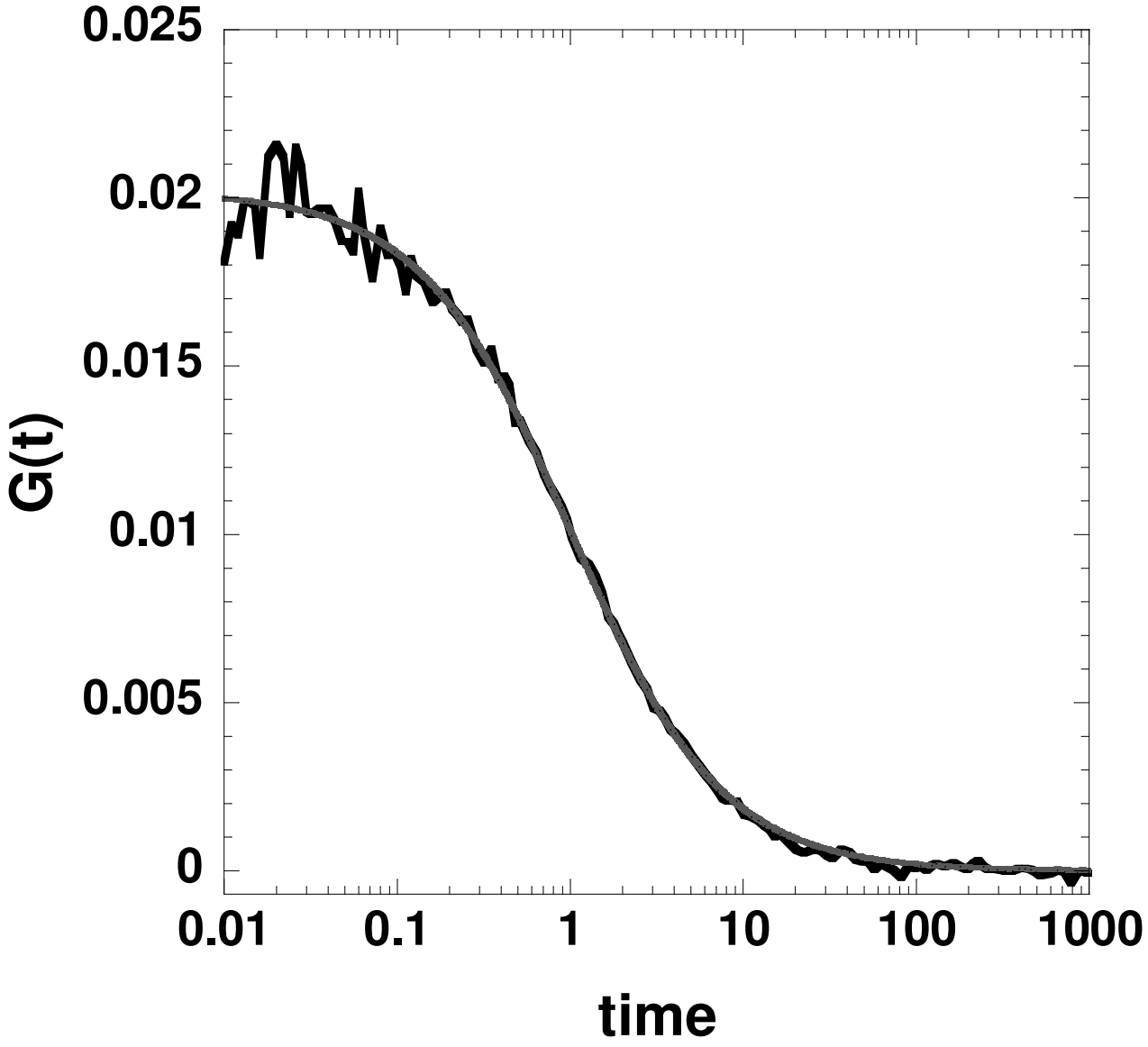


Figure S1 Example of a typical autocorrelation curve when the position of the detection volume on the cell is not well aligned. The circle on the right of the graph shows the characteristic shape of the G(t) curve when cells were not properly positioned.

Figure S2 Noise analysis of autocorrelation curves. Typical FCS autocorrelation curves that passed (**A**) or failed (**B,C,D**) the selection criteria discussed in Materials and Methods. On each figure, the top panel shows an autocorrelation curve. The data is fitted with the two-component function $G(\tau)$. The middle panel represents a plot of the residuals of the fit. The bottom panel depicts the value for the variance of the signal dwell time as a function of log of the dwell time $\Delta\tau(i)$. The noise analysis of each autocorrelation curve relies on three parameters, whose values determine if a curve is kept or rejected. The values of the residuals (middle panels) define two parameters for the behavior at long time scales: 1) value of power spectrum at frequency zero (psf_0), 2) variance of residuals at times between 4.5 ms and 524 ms (psf_var). The third parameter determines the distance to the linear fit of the variance of residuals at short time scales, (max_d).
A. Parameter values: psf_0=0.02; psf_var=0.065; max_d=0.08. All parameters are within the empirically established limits: psf_0<0.03, psf_var<0.2, max_d<0.4 **B.** Parameter values: psf_0=0.087; psf_var=0.45; max_d=0.46 and fails to pass for all three parameters. **C.** Parameter values: psf_0=0.16; psf_var=0.67; max_d=0.32 and fails to pass because psf_var>0.2. **D.** Parameter values: psf_0=0.076; psf_var=0.17; max_d=0.2 and fails to pass because the psf_0>0.03.

Figure S3. Number of DsRed molecules versus intensity calibration curve. The red channel data for all the cells were analyzed using $G(t) = \frac{1}{N}\left(\frac{1}{(1+t/\zeta_{free})}\right)$, and the ones which resulted in good quality fits were plotted against the mean intensity measured across the 3s acquisition interval. The linear fit (forced through 0) of the data gives a slope of 2.15 DsRed tetramers per 1kHz of measured signal intensity. The error bars represent uncertainties in the fit parameters of G(t).

Figure S4. FCS determination of the diffusion of polystyrene beads. The autocorrelation function G(t) for 44nm diameter polystyrene beads. Fluorescence data was acquired during an interval of 30s. We focus the laser beam close to the surface of the glass coverslip as is the case for FCS measurements on bacteria. Grey line represents

the fit function $G(t) = \frac{1}{N}\left(\frac{1}{(1+t/\zeta_{free})}\right)$. We perform ten distinct measurements and determine the diffusion constant of the beads to be $\zeta \sim 1.01 \pm 0.05$ ms.