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# Supplementary material to

# The effect of localisation imprecision on quantification of the directionality of motion for single particle tracking applications

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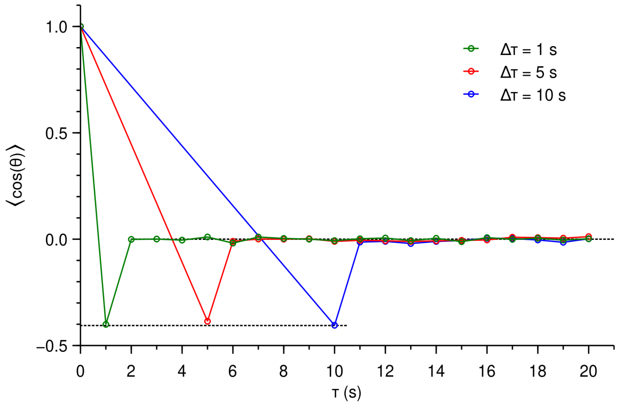


**Figure S1. Average cosθ *vs* time for stationary particles.** Cosine of the angle θ between displacements as a function of the time *τ* separating the displacements averaged over time. Different timescales *Δτ* were used to define the displacements and these were chosen much longer compared to those shown in Fig. 3b. The results have been averaged over all particles. (Dotted lines) Predicted values according to the theory developed in the main text for successive displacements [$\langle\cos\theta\rangle \approx -0.406$; Equation (5) with $\rho$ = -½] and displacements separated in time from each other [$\langle\cos\theta\rangle = 0$; Equation (5) with $\rho = 0$], respectively.

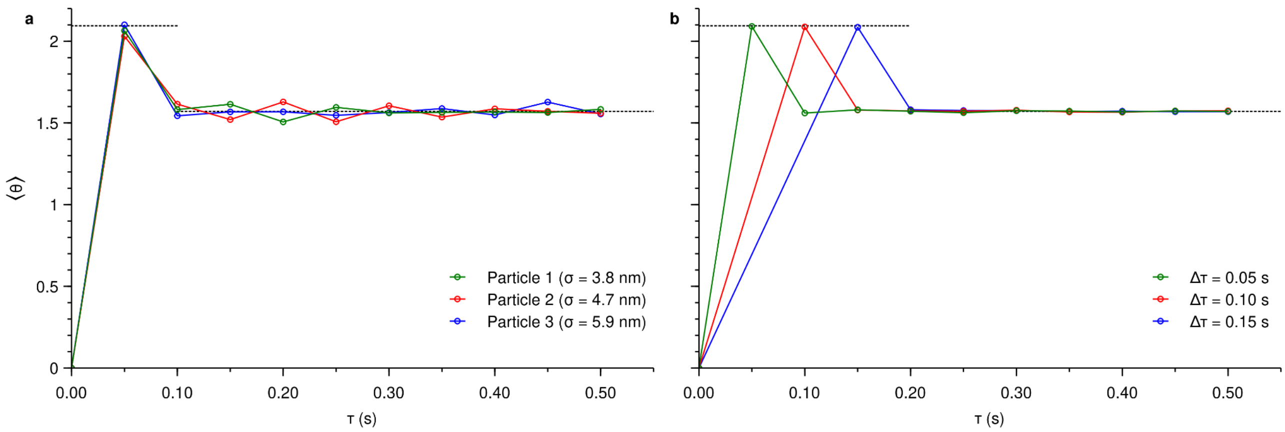


**Figure S2. Average θ *vs* time for stationary particles.** The angle θ between displacements as a function of the time $\tau$ separating the displacements, averaged over time. **a,** Displacements defined for the shortest sampled time ($\Delta\tau$ = 0.05 s, the time between frames; Fig. 2a). Successive displacements ($\tau = \Delta\tau$) thus correspond to $\tau$ = 0.05 s, while displacements separated in time from each other ($\tau > \Delta\tau$) correspond to $\tau$ = 0.10 s, 0.15 s *etc*. Results are shown for three different particles with different localisation imprecisions σ. **b,** Different timescales $\Delta\tau$ used to define the displacements. $\Delta\tau$ = 0.05 s corresponds to the time between frames (as in panel a; Fig. 2a), while $\Delta\tau$ = 0.10 s corresponds to displacements defined over two frames (Fig. 2b) *etc.* The results have been averaged over all particles.

To compare this to the theory developed in the main text, we note that from Equation (4) of the main text one can show [Equation (41) of the Supplementary Information "Explicit mathematical derivations"] that the average angle is given by

$$\langle\theta\rangle = \pi - \arccos(-\rho). \qquad \text{(S1)}$$

For successive displacements ($\tau = \Delta\tau$) we have (see main text) $\rho$ = -½ and find $\langle\theta\rangle = 2\pi/3$, while for displacements separated in time from each other ($\tau > \Delta\tau$) we have $\rho = 0$ and thus $\langle\theta\rangle = \pi/2$. Both of these results agree excellently with our experimental observations (dotted lines and data symbols). Furthermore, the time-dependence, that is, the fact that we find different results for successive and displacements separated in time, but otherwise no dependence on time, also agrees perfectly with experiments, as does the independence on the localisation imprecision σ.

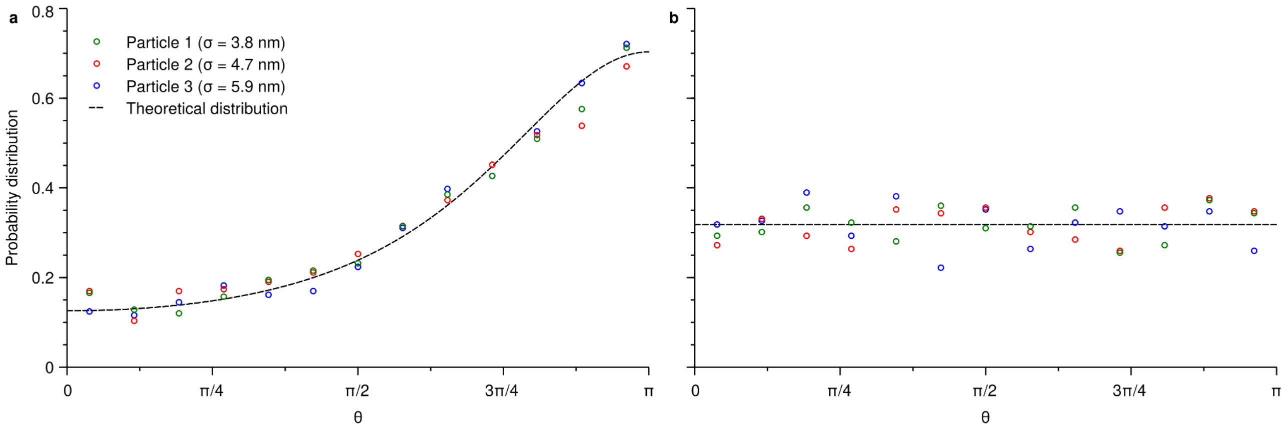


**Figure S3. Distribution of the angle between displacements for some exemplar stationary particles.** The displacements were defined for the shortest sampled time ($\Delta\tau$ = 0.05 s, the time between frames; Fig. 2a). See Fig. 4 for similar results averaged over many particles. Angle θ between **a,** successive displacements ($\tau$ = $\Delta\tau$ = 0.05 s); and **b,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 0.10 s). (Dashed lines) Predicted distributions according to the theory developed in the main text for successive displacements [Equation (4) with $\rho$ = -½; panel a] and displacements separated in time from each other [Equation (4) with $\rho$ = 0; panel b].

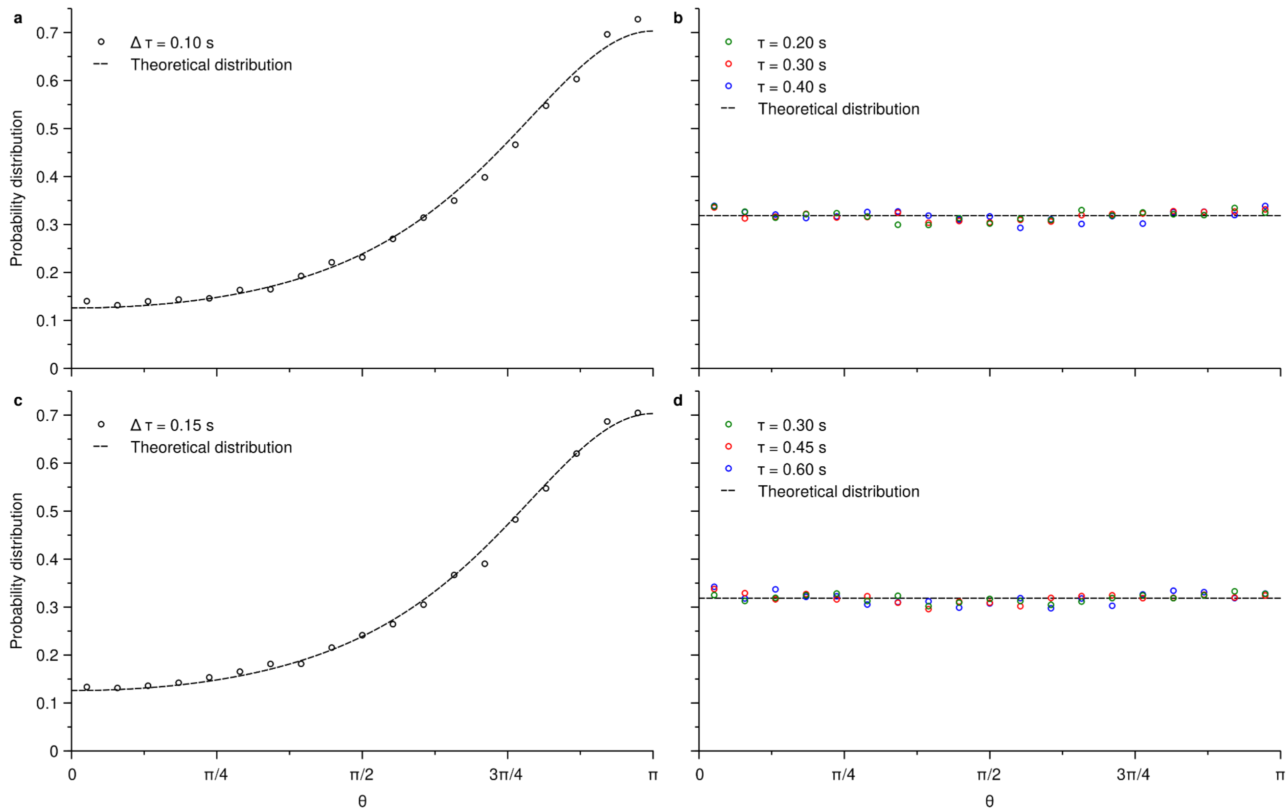


**Figure S4. Distribution of the angle between displacements for stationary particles.** The displacements were defined for **a–b,** $\Delta\tau$ = 0.10 s and **c–d,** $\Delta\tau$ = 0.15 s. Data from all particles have here been pooled together. Angle θ between **a,** successive displacements ($\tau = \Delta\tau$ = 0.10 s); **b,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 0.20 s, $3\Delta\tau$ = 0.30 s and $4\Delta\tau$ = 0.40 s); **c,** successive displacements ($\tau = \Delta\tau$ = 0.15 s); and **d,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 0.30 s, $3\Delta\tau$ = 0.45 s and $4\Delta\tau$ = 0.60 s). (Dashed lines) Predicted distributions according to the theory developed in the main text for successive displacements [Equation (4) with $\rho$ = -½; panel a,c] and displacements separated in time from each other [Equation (4) with $\rho$ = 0; panel b,d].

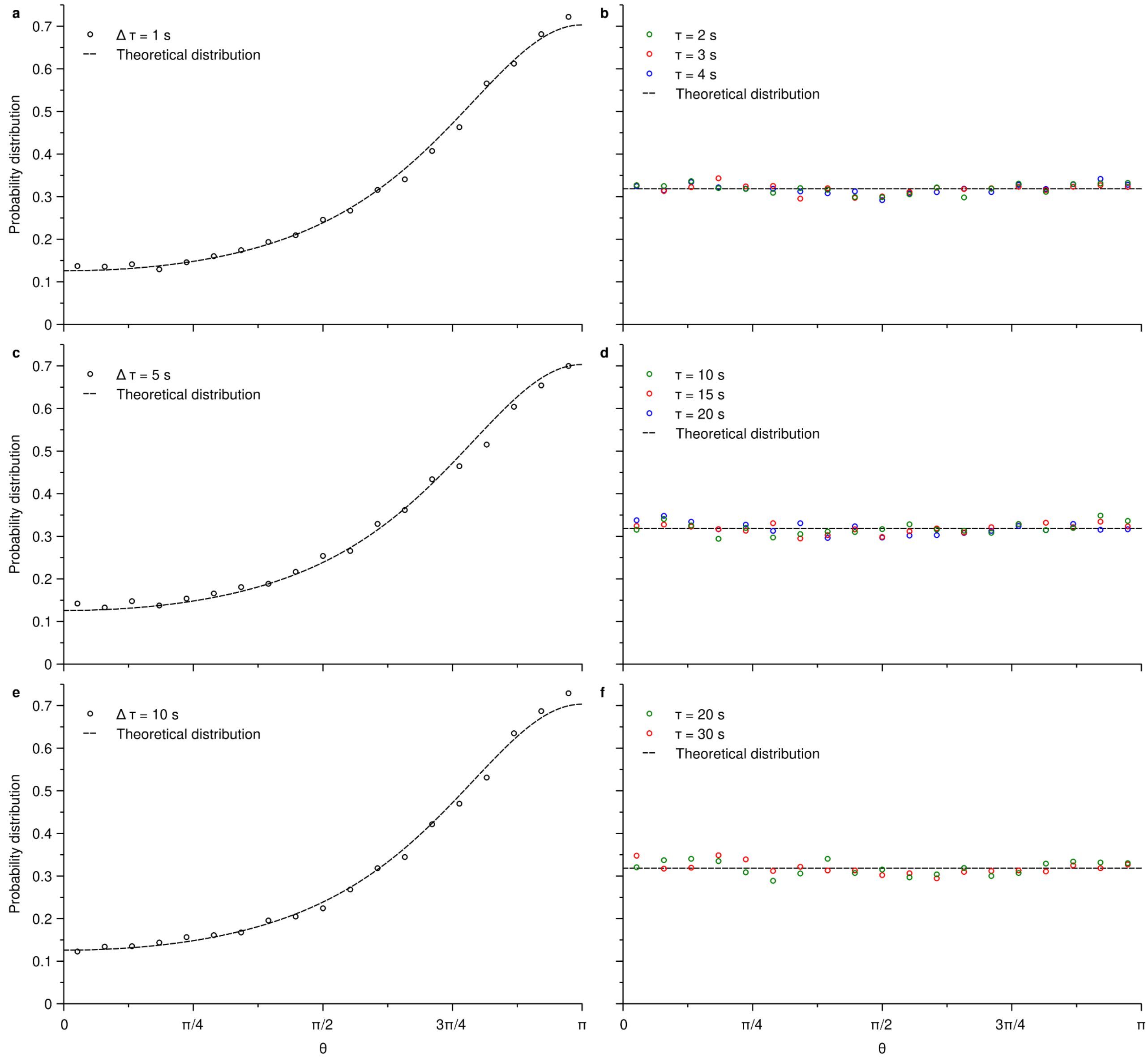


**Figure S5. Distribution of the angle between displacements for stationary particles.** The displacements were defined for **a–b,** $\Delta\tau$ = 1 s, **c–d,** $\Delta\tau$ = 5 s and **e–f,** $\Delta\tau$ = 10 s, much longer compared to those shown in Fig. 4. Data from all particles have here been pooled together. Angle θ between **a,** successive displacements ($\tau = \Delta\tau$ = 1 s); **b,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 2 s, $3\Delta\tau$ = 3 s and $4\Delta\tau$ = 4 s); **c,** successive displacements ($\tau = \Delta\tau$ = 5 s); **d,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 10 s, $3\Delta\tau$ = 15 s and $4\Delta\tau$ = 20 s); **e,** successive displacements ($\tau = \Delta\tau$ = 10 s); and **f,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 30 s, $3\Delta\tau$ = 30 s). (Dashed lines) Predicted distributions according to the theory developed in the main text for successive displacements [Equation (4) with $\rho$ = -½; panel a,c,e] and displacements separated in time from each other [Equation (4) with $\rho$ = 0; panel b,d,e].

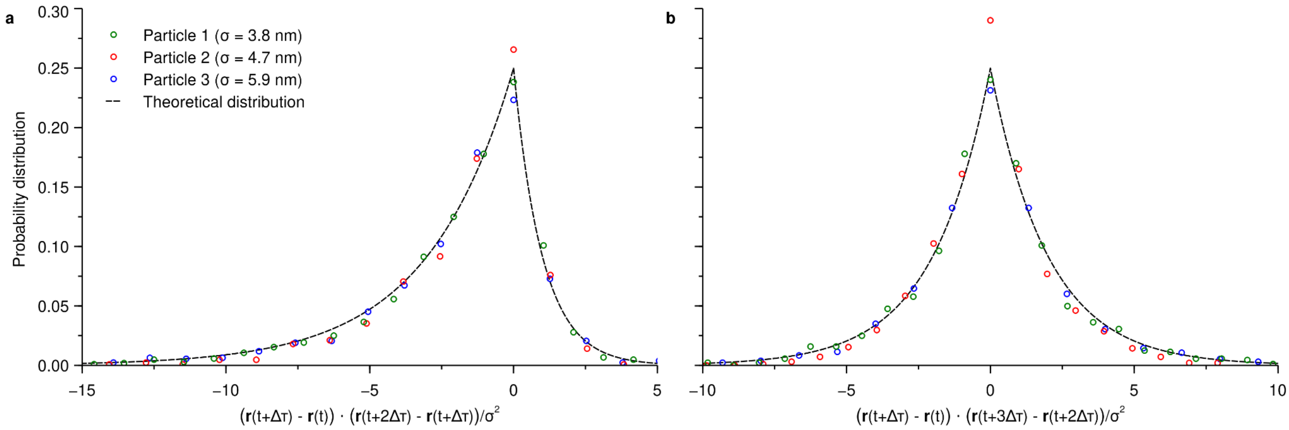


**Figure S6. Distribution of scalar product [Equation (3)] for some exemplar stationary particles.** The displacements were defined for the shortest sampled time ($\Delta\tau$ = 0.05 s, the time between frames; Fig. 2a). The results are for three different exemplar particles with different localisation imprecisions σ; see Fig. 6c–d for similar results averaged over many particles. The scalar product was normalised by the localisation imprecision squared $\sigma^2$ of each individual particle. Scalar product between **a,** successive displacements ($\tau = \Delta\tau$ = 0.05 s); and **b,** displacements separated in time from each other ($\tau = 2\Delta\tau$ = 0.10 s). (Dashed lines) Predicted distributions according to the theory developed in the main text for successive displacements [Equation (7); panel a] and displacements separated in time from each other [Equation (8); panel b], respectively.

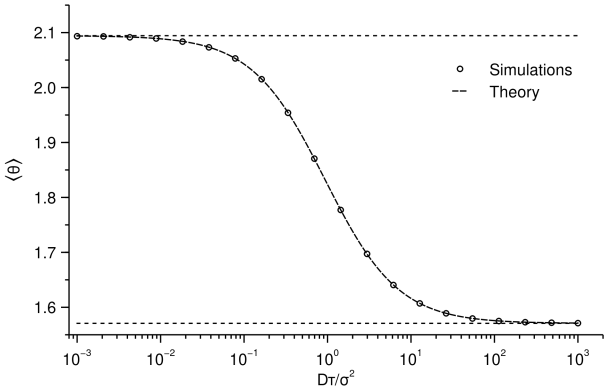


**Figure S7. Average θ *vs* time for particles undergoing Brownian motion in the presence of localisation imprecision.** See Fig. 9 for the equivalent results for the cosine of the angle cosθ. Only successive displacements were considered. Results are presented as a function of the ratio of the diffusional length $\sqrt{(D\tau)}$ to the localisation imprecision $\sigma$. (Symbols) Results from simple numerical simulations. (Dashed line) Prediction according to theory [Equation (S1) with $\rho$ from Equation (9) in the main text]. (Dotted lines) Limiting values: When $D\tau/\sigma^2 = 0$, we have $\langle\theta\rangle = 2\pi/3 \approx 2.09$ and when $D\tau/\sigma^2 = \infty$ we have $\langle\theta\rangle = \pi/2 \approx 1.57$. Note the log-scale.

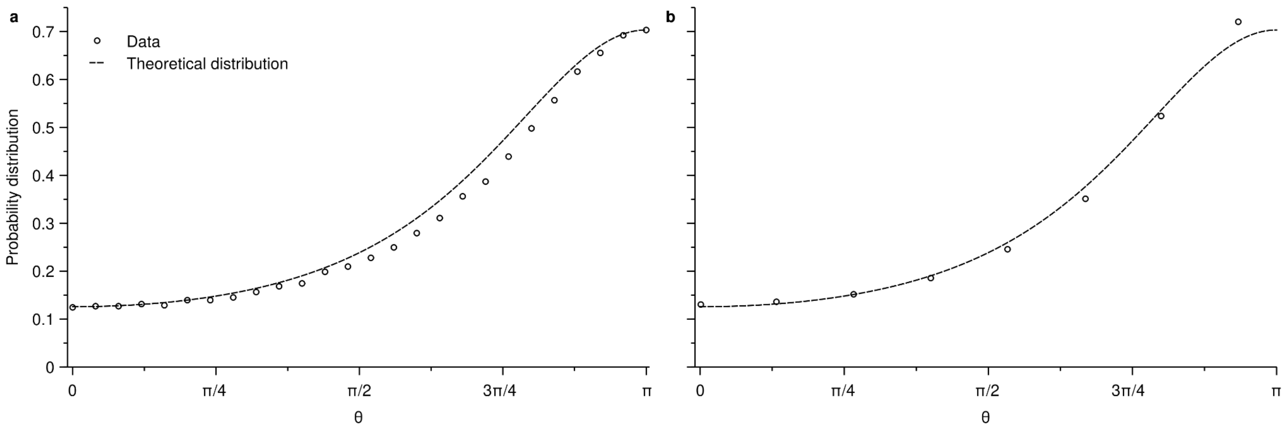


**Figure S8. Distribution of the angle between displacements reproduced from previous literature. a,** Raupach *et al.* assessed the directionality of immobilised micron-sized polystyrene beads (their Fig. 7);[1] **b,** Lenormand *et al.* did the same for micron-sized ferrimagnetic beads (their online supplement 2, panel a; we used the data for 0.08 s).[2] (Symbols) These literature results after resampling to ignore the sign of the angle (both of these works considered the signed angle, whereas the present work does not). (Dashed line) Predicted distributions according to the theory developed here for successive displacements [Equation (4) with $\rho$ = -½]. Compare with Fig. 4a for our results.

# Supplementary References


1 C. Raupach, D. P. Zitterbart, C. T. Mierke, C. Metzner, F. A. Müller and B. Fabry, *Phys. Rev. E*, 2007, **76**, 011918.

2 G. Lenormand, J. Chopin, P. Bursac, J. J. Fredberg and J. P. Butler, *Biochem. Biophys. Res. Commun.*, 2007, **360**, 797–801.