Supplementary figures legends

Figure s1. Parameters calculated by Chemotaxis$_{V1}$. Spermatozoa that immediately experience an ascending chemoattractant gradient (∇C) at the end of the UV irradiation period (ϕ_{UVend}) were classified as ASC, and spermatozoa that immediately experience a descending chemoattractant gradient were classified as DESC.

Figure s2. Characteristic motility changes in single spermatozoa. Left panel: Single sperm trajectories 3 s before (dashed lines) and 3 s after (solid lines) a 200 ms UV irradiation pulse (purple lines) of sperm resuspended in ASW with 10 nM of CS (A) or in ASW additionally containing 30 μM of NFA (B). The gray circle indicates the center of the irradiated area. Right panel: Path curvature κ of the same spermatozoa 3s before stimulation (dashed lines) and 3 s after stimulation (solid lines). The colored circles indicate the onset of individual [Ca^{2+}]i fluctuations (red, when the flagellum becomes visible), individual turns (blue, calculated automatically by Chemotaxis$_{V1}$), or individual straighter swimming episodes (green, calculated automatically by Chemotaxis$_{V1}$).

Figure s3. Modeling the speract gradient dynamics based on the UV light profile. A) Radial distribution of the UV light scattered at the glass-liquid interface generated via an optical fiber coupled to a xenon lamp (purple). The speract gradient was generated by 200 ms of UV irradiation of the swimming plane containing 10 nM of CS. The dynamics of the chemoattractant gradient was computed as $f(x,t) = \frac{1}{\sqrt{2\pi}\sigma} e^{-x/2\sigma^2}$; where $\sigma^2 = 4D(t - t_0)$, and D ≈ 300 μm^2 s^{-1} is the diffusion coefficient of a peptide of 10 amino acids. The solid line (t = 0) corresponds to a Gaussian distribution fitted to the UV light profile considering a mean = 0 and a σ = 90 μm, and illustrates the putative normalized shape of the instantaneously-generated speract gradient. The other lines illustrate the shape of the simulated speract gradient after 1, 2, and 3 seconds (t = 1, t = 2 and t = 3 s). B) Simulated temporal changes in the speract concentration at each 10 radial μm point from the center of the speract gradient. The gray shading indicates the spatio-temporal limit, where the speract concentration barely changes, which promotes chemotaxis of *L. pictus* spermatozoa.

Figure s4. NFA does not affect the mechanism that retards the onset of turning events until reaching a descending phase of the speract gradient

Spermatozoa exposed to the speract gradient alone, Speract (A-C) or in the presence of NFA, Speract + NFA (D-F). A, D) Relative sperm position with respect to the speract gradient at the end of the UV irradiation period (ϕ_{UVend}) vs the delay to the onset of the first turning event (τ_{T1}). B, E) Average delay for the onset of the first turn (τ_{T1}) of ASC (gray) or DESC (black) spermatozoa (*p < 001, Wilcoxon - test, n≥9). C, F) Circular distribution of the relative sperm positioning on the speract gradient at the onset of the first turning event (ϕ_{T1}) or at the onset of the subsequent turning events (ϕ_T), n ≥ 35.

Figure s5. NFA does not alter the interval between $[Ca^{2+}]i$ fluctuations, nor the interval between turning events

Each box contains 50% of single events, the inner lines indicate the median and the error bars delimit the 95% outliers.

Figure s6. The relative positioning of a spermatozoon on a chemoattractant gradient

Given a spermatozoon that is swimming counter-clockwise in the reference axes (x, y) centered at the source of a chemoattractant concentration gradient (C), let $\vec{P}(t)$ be its position and $\vec{D}(t)$ its direction with respect to the chemoattractant gradient center, and $\alpha(t) = arccos\frac{\vec{P}\cdot\vec{D}}{|P||D|}$. We say that this spermatozoon is swimming in the ascending phase of C if $\alpha > \pi / 2$ (cyan and purple shading); conversely it is swimming in the descending phase of C if $\alpha < \pi / 2$ (red and green shading). Furthermore, if $\alpha = \pi / 2$ the spermatozoon is crossing the nearest point (black circle) or the farthest point (white circle) to the center of C. To further discriminate the sperm behavior close to the center of C (purple and red shading) from its behavior close to the farthest point to the center of C (green and cyan shading) we derived the ϕ angle ($\phi(\alpha):[0, 2\pi)$). Values of ϕ between 0 and π identify a spermatozoon as swimming in the descending phase of C (red and green shading), and ϕ values between π and 2π identify it as swimming in the ascending phase of C (cyan and purple shading). When $\phi = 0$ the spermatozoon is crossing the nearest point to the center of C, conversely when $\phi = \pi$ it is crossing the farthest point to the center of C.

Movies

Movie s1. Typical motility and Ca^{2+} responses of *L. pictus* spermatozoa swimming in ASW containing 10 nM caged speract 3 s before and 20 s after 200 ms UV irradiation. An optic fiber of 4 mm internal diameter was used for the UV light path to generate the speract gradient. Real time: 30 frames s^{-1}, 40× objective.

Movie s2. Typical motility and Ca^{2+} responses of *L. pictus* spermatozoa swimming in ASW containing 10 nM caged speract and 30 μM of niflumic acid 3 s before and 20 s after 200 ms UV irradiation. An optic fiber of 4 mm internal diameter was used for the UV light path to generate the speract gradient. Real time: 30 frames s^{-1}, 40× objective.

Figure Supplementary 1

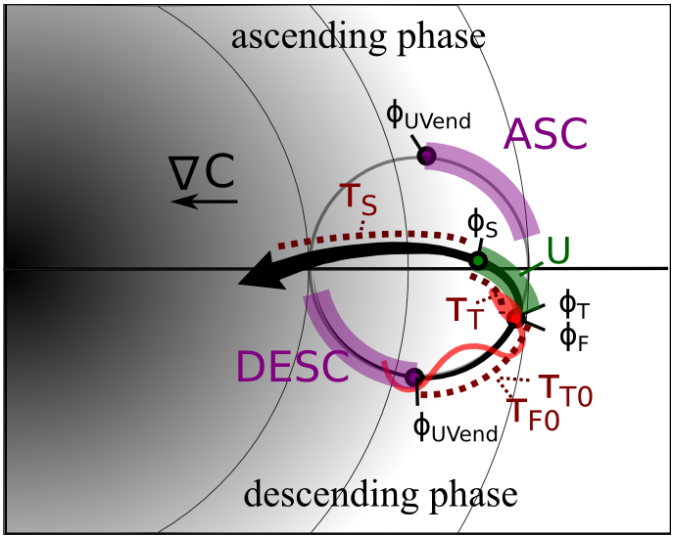


Figure Supplementary 2

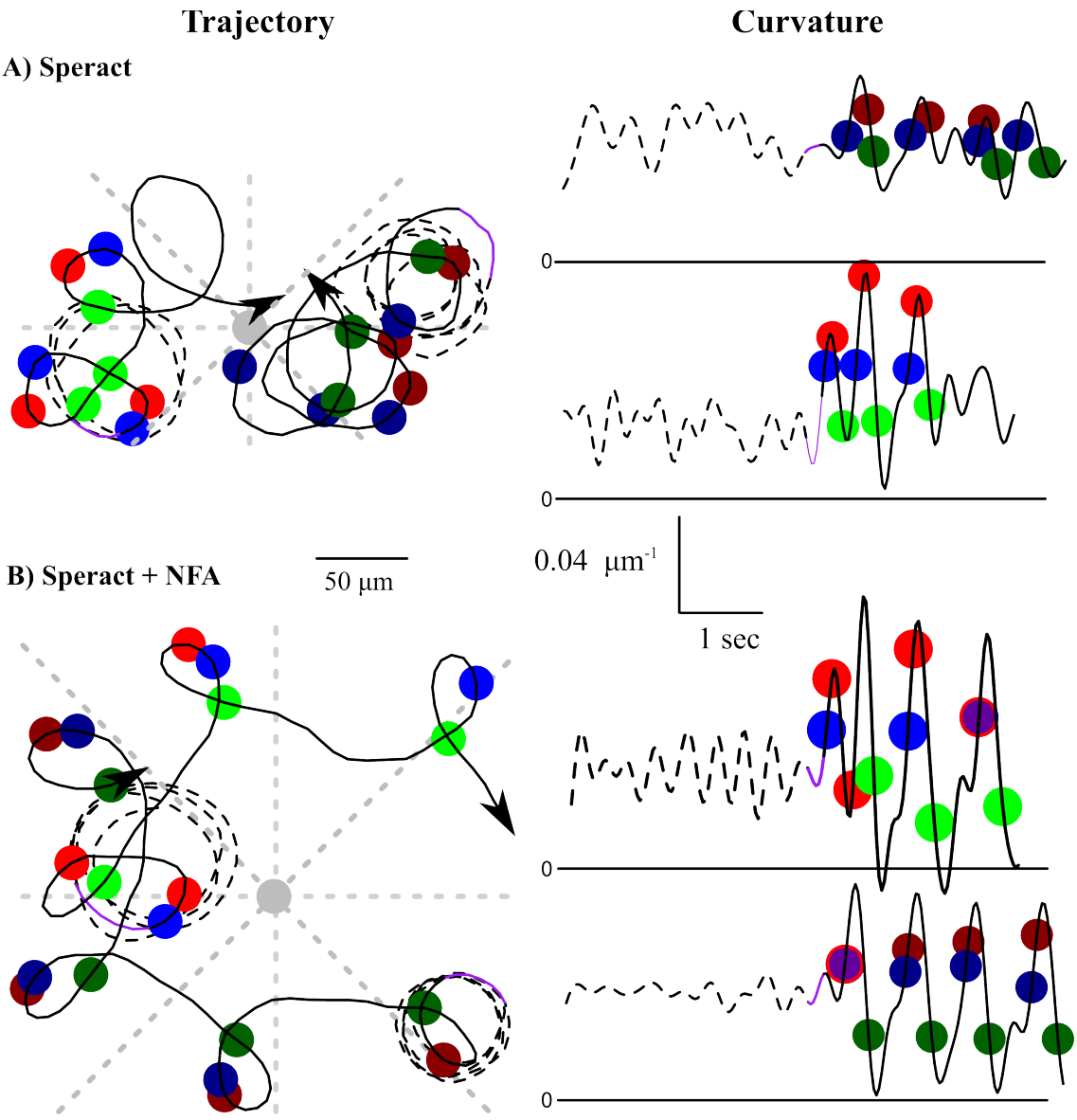


Figure Supplementary 3

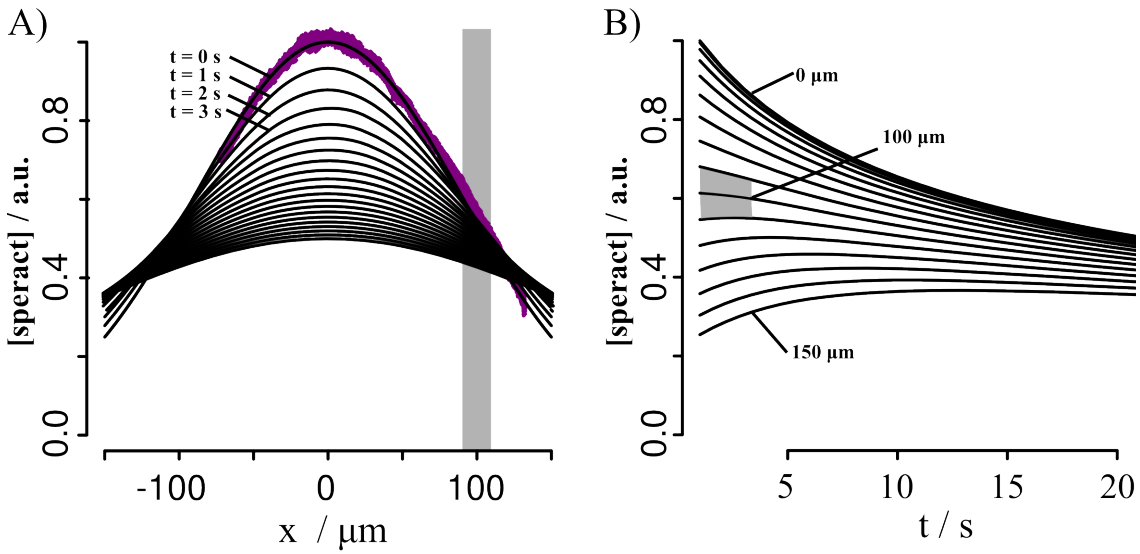
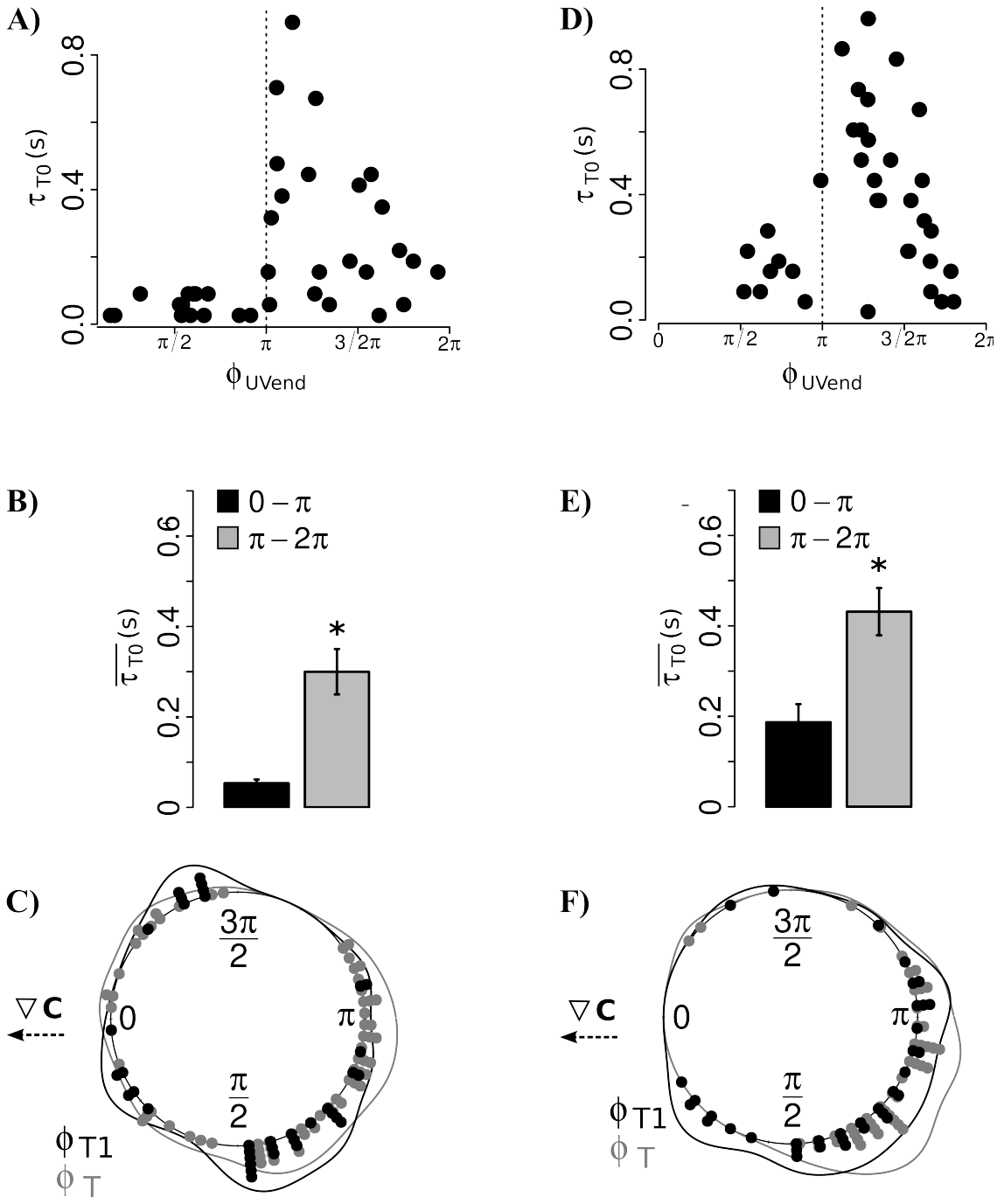


Figure Supplementary 4



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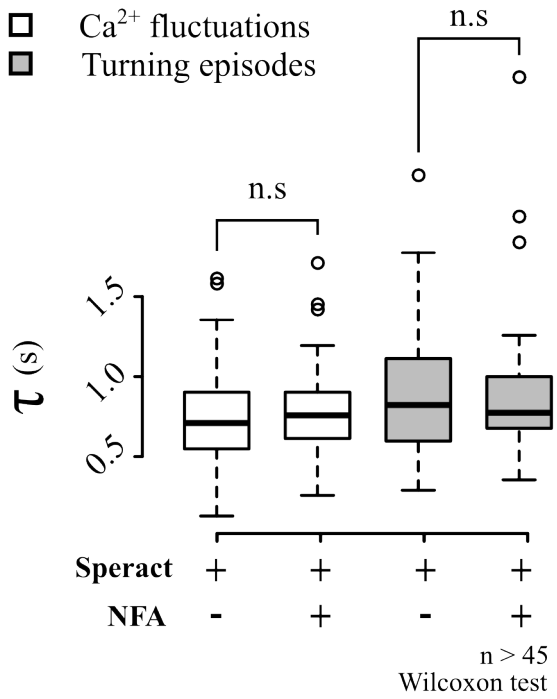
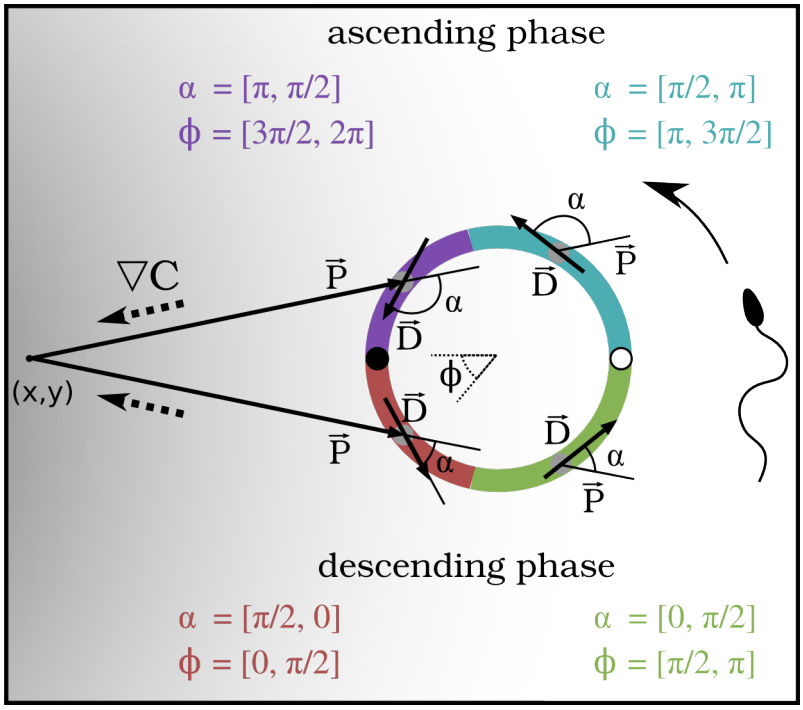


Figure Supplementary 6

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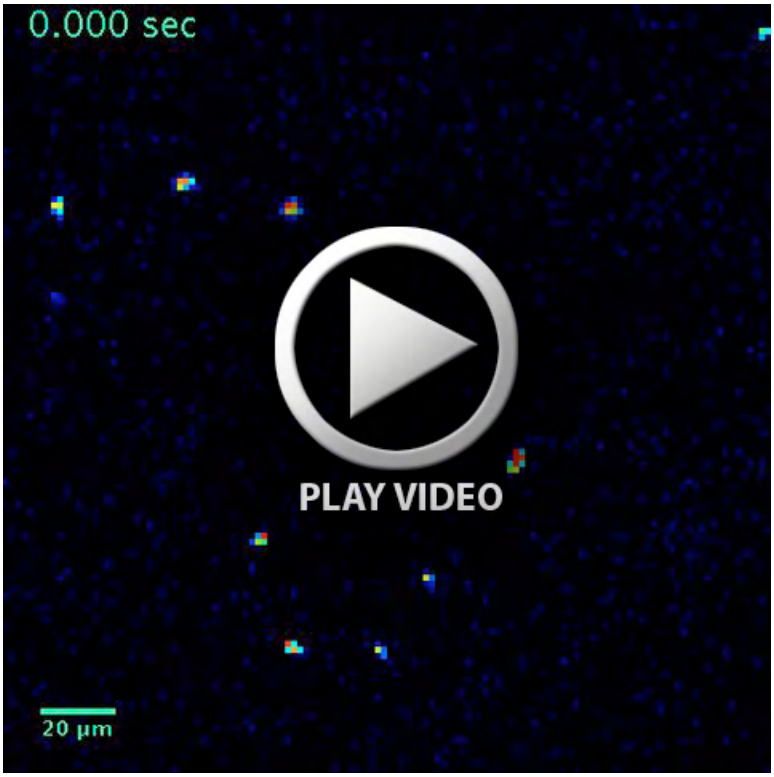
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