Supplementary Information for
“Tangling of Tethered Swimmers: Interactions between Two Nematodes”

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EXPERIMENTAL DETAILS

Wild-type worms (N2) were acquired from the Caenorhabditis Genetics Center and cultivated in an incubator according to standard methods on Escherichia coli (OP50) nematode growth media (NGM) plates at 20°C [1]. Young adult and adult worms were picked from the plates into a droplet of M9 buffer sitting on a thin cover slip. A chamber was then built around the drop with a second cover slip and two (2 mm thick) spacers on both sides. The chamber was filled with more buffer, placed on top of an inverted microscope, and two micropipettes, attached to XYZ translational stages, were finally positioned into the chamber, as shown in Fig. 1. By attaching thin tubes to the back ends of the pipettes and applying suction with syringes, worms were captured by their tails and carefully brought to the middle of the chamber. By capturing the worms as they were swimming at (and in the plane of) the bottom glass slide, both of the worms could be ensured to initially swim in the same plane. Furthermore, by applying a high enough suction to the tails, vast rotation in the pipettes could be avoided.

![FIG. 1. Schematic diagram of the experimental setup as seen from above.](image)

Optical microscopy images were captured at 56 fps for around 3 minutes per worm distance, corresponding to more than 10 000 images per experiment. With an average worm swimming frequency of $f = 2.1 \pm 0.2$ Hz, hundreds of swimming cycles were monitored for each separation distance. The pipettes were made to be very flexible, so that the forces produced by the swimming worms would cause the thin glass capillaries to deflect. The deflections of the pipettes were obtained with cross-correlation image analysis using MATLAB (MathWorks). An example data set is shown in Fig. 2, where the pipette deflections are plotted as a function of time. In states of normal swimming, the pipettes can be used as force sensors to probe the viscous forces experienced by the microswimmers (see Ref. [2]). When an entanglement occurs, the pipettes are pulled together and these clear deflections above that of normal swimming (dashed lines) were used to recognize all entanglements in the long experiments.

![FIG. 2. Pipette deflection as a function of time. During normal swimming, the pipettes follow the undulatory swimming patterns of the worms. The large peaks correspond to entanglements between the worms, pulling the pipettes above the peaks (dashed lines) set by normal swimming.](image)

An entanglement had to, per definition, last longer than at least 8 frames (0.14 s), since tangling events shorter than this did not represent an actual tangle, but occurred when the worms were touching as they swam past each other in slightly different planes. The number and lifetimes of real tangles were then analyzed.

The worms were seen to undergo small changes in their amplitude and/or swimming axis during the experiment. For the analysis, the maximum swimming amplitude was...
considered since a critical $d/A$ ratio was desired.

The vertical error bars in Fig. 4 in the main manuscript represent the standard error of the mean of the entanglement probability at different times of the experiment, propagated together with the standard deviation of the swimming frequencies of both worms and the precision of the analysis. The horizontal error bars were propagated based on the standard deviation of the worm lengths as well as the error in worm separation.