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Flagella as a Novel Alignment Media for the Measurement of Residual Dipolar Couplings in Proteins

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Supporting Information

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S1. Step-wise protocol for flagella isolation (adapted from Craige et al., *Curr. Protoc. Cell Biol., 2013*)

1. *Chlamydomonas reinhardtii* cells were grown in continuous light in TAP-medium to mid-log phase (4-6 million cells/mL).
2. Pellet and wash the cells.
3. Place concentrated cells in a small beaker and stir rapidly using a stir bar and magnetic stirrer. While monitoring the pH of the cell suspension with a pH meter, rapidly decrease the pH from 7.0 to 4.5 by drop-wise addition of 0.5 N acetic acid.
4. After 60 sec, neutralize the pH to 7.0 by drop-wise addition of 0.5 M KOH.
5. Transfer the cells into 50 ml conical tubes (30 to 35 ml of cell suspension/tube)
6. Centrifuge for 10 min at 1800 × g, at 4°C, to remove cell bodies.
7. Collect the supernatant using a 25 ml serological pippet. Sweep the sides of the tubes to collect any flagella that are stuck to the sides. Transfer the supernatant into clean 50 ml conical tubes.
8. Using a 10 ml serological pippet, underlay the flagellar suspension with 9 ml ice-cold 10 mM HEPES (pH 7.4) -25% sucrose.
9. Centrifuge for 10 min at 2800 rpm (2400 × g), at 4°C. Collect the supernatant down to the sucrose interface using a 25 ml serological pippet; sweep the sides of the tube to collect adherent flagella. Transfer the supernatant into 50 ml round-bottom polycarbonate tubes.
10. Concentrate the flagella by centrifuging for 20 min at 30,000 × g, at 4°C. Carefully pour off the supernatant. The flagellar pellet was seen as white substance with little to no green color in it.

Figure S2

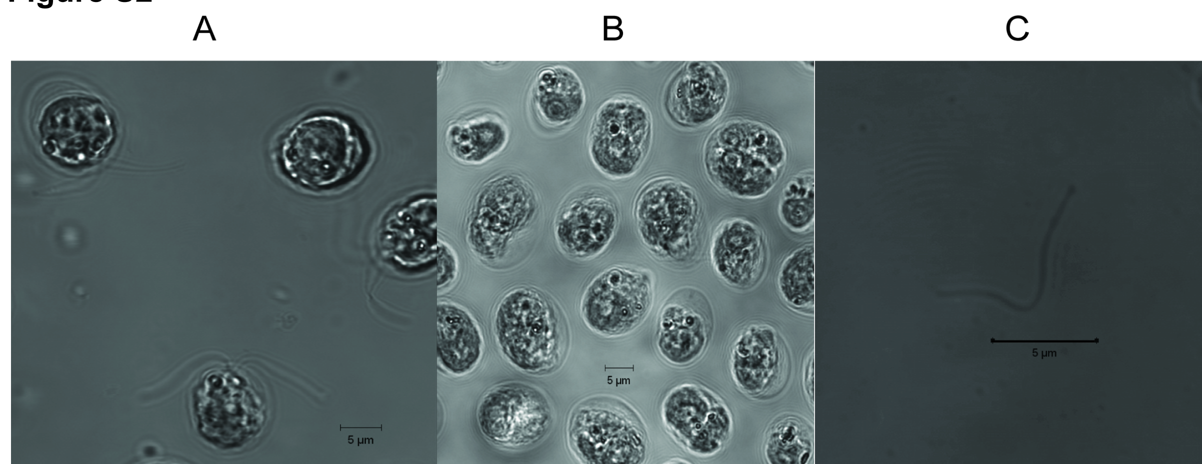

Figure S2. Confocal images of the *C. reinhardtii* cells and purified flagella. **(A)** *C. reinhardtii* cells with flagella, **(B)** suspension of *C. reinhardtii* cells after deflagellation by pH shock and **(C)** purified flagella isolated by low-pH sucrose method. Single flagellum is shown. Bar represents 5 µm.

Figure S3

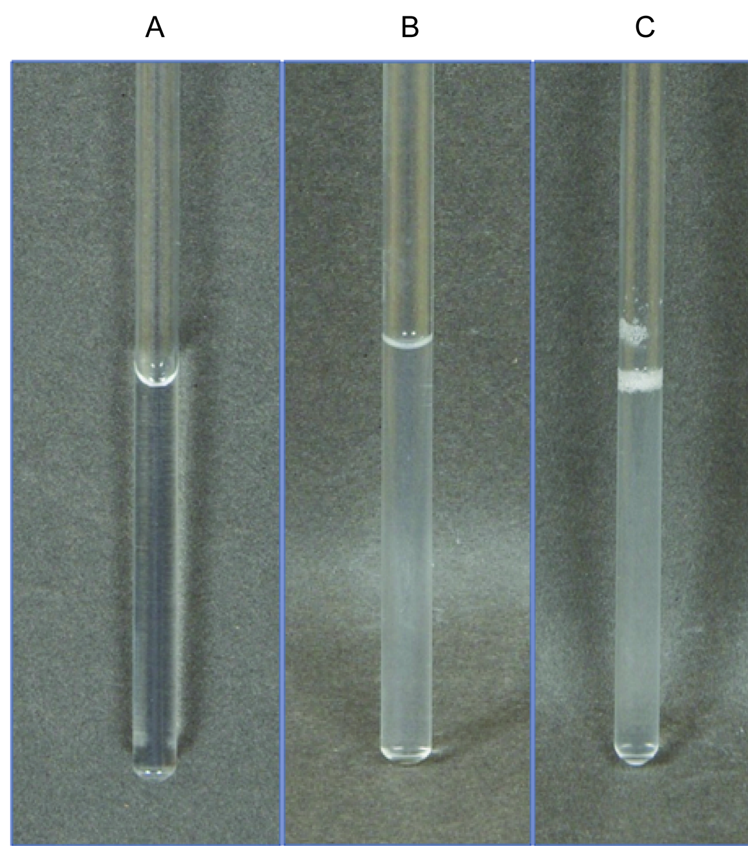

Figure S3. Photographs of protein solution at 25 °C (A) without flagella (B) with 6.0 mg/mL flagella and (C) with 12.0 mg/mL flagella.

Table S4. Alignment tensors.

S. No	Medium	α^a	β^a	γ^a	$D_a{}^b$	R^c	$\chi^{2\,d}$
1	DMPC/DHPC	49.80	33.43	39.44	-9.15	0.170	2.36
2	Flagella	43.69	54.39	28.57	-7.38	0.127	5.76

[a] Euler angles define the orientation of the alignment frame relative to the PDB frame of ubiquitin (PDB ID: 1UBQ).
[b] D_a in Hz.
[c] Rhombicity (R) is $0 \leq R \leq 2/3$.
[d] χ^2 is the value with respect to the X-ray structure 1UBQ (excluding the fourmobile residues 73 – 76 of the C-terminal tail).

In order to check the quality of RDCs obtained for ubiquitin aligned in flagella, the experimentally measured RDCs were compared with the calculated RDCs for ubiquitin. In this endeavour, we first calculated Euler angles, alignment tensor (Da) and rhombicity (R) using experimental RDCs and structural coordinates of ubiquitin. The calculated alignment tensor (Da) and rhombicity (R) for ubiquitin corresponding to the measured RDCs were -7.38 Hz and 0.127, respectively (See Table 1 below). These values were then used to calculate RDCs for ubiquitin using the following equation.

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$D_{NH}(\theta, \phi) = D_a^{NH}\{(3\cos^2\theta - 1) + 1.5 * R * (\sin^2\theta * \cos 2\phi)\}$

The correlation between measured and calculated RDCs for ubiquitin aligned in flagella is shown in Figure 3D (main text). A good correlation supports the quality of the data.

By using the same protocol as described above the alignment tensor (Da) and rhombicity (R) for ubiquitin aligned in an admixture of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dicaproyl-sn-glycero-3-phospho-cholinebicelles (DHPC) this medium were -9.15 Hz and 0.170 respectively (see Table S4).

Thus, the RDCs measured by using flagella as the new alignment media is comparable with the DMPC/DHPC alignment medium.

Figure S5

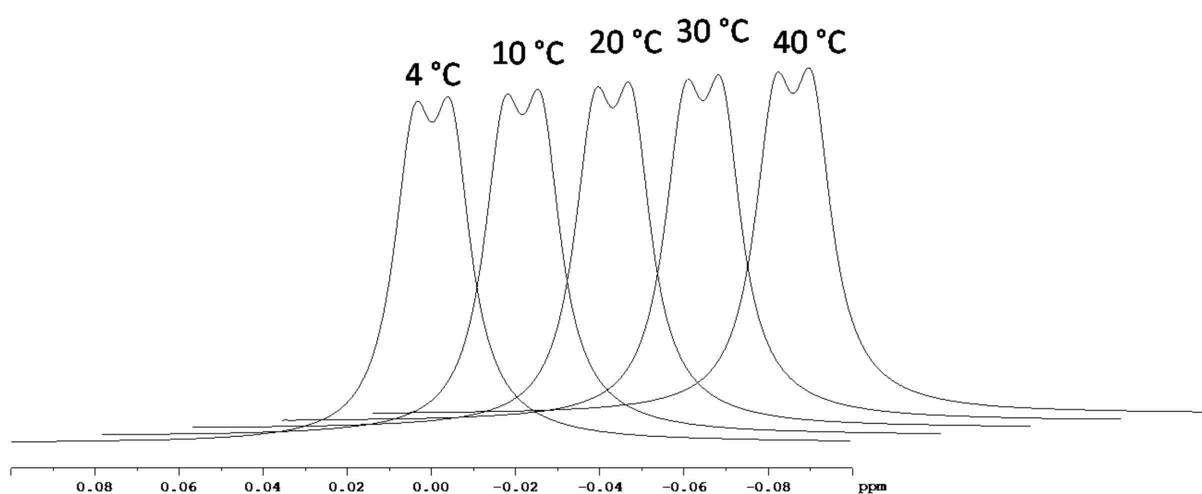

Figure S5. Stacked plots showing 1D ^{2}H spectra of ^{2}H2O in the presence of 6 mg/mL flagella at different temperature.

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Figure S6

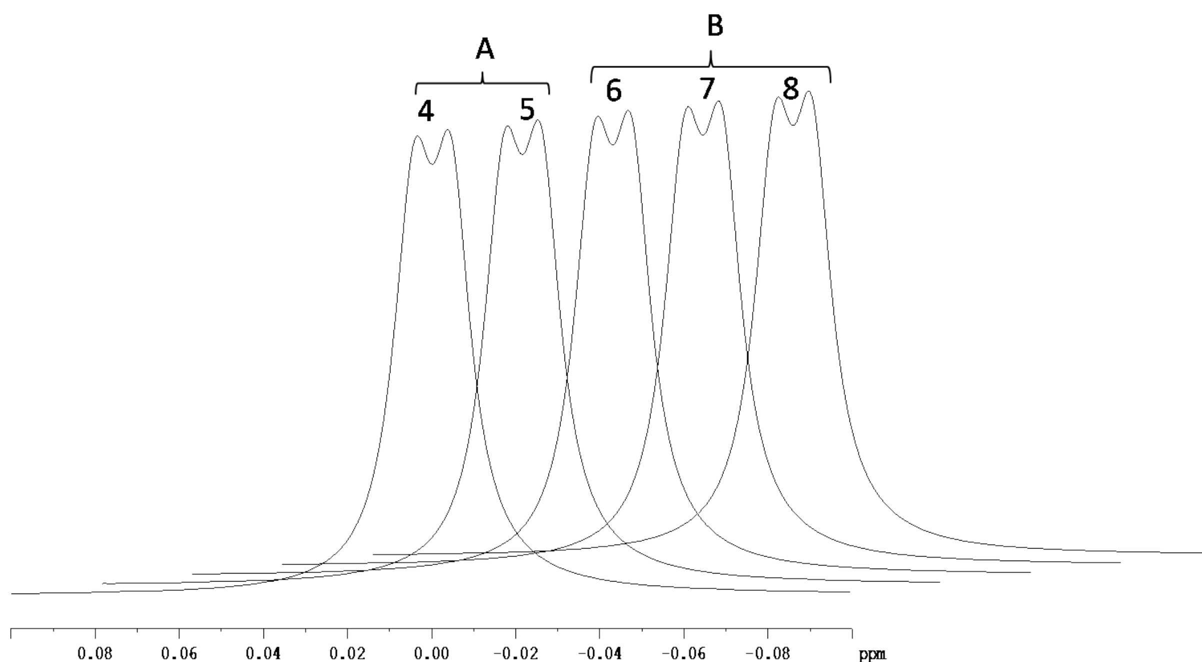

Figure S6. Stacked plots showing 1D ^2H spectra of ^2H2O in the presence of 6 mg/mL flagella at different conditions, **(A)** after soaking in 20 mM acetic acid (pH 4.0 or 5.0). **(B)** after soaking in 50 mM phosphate buffer, pH 6 or 7 or 8, 100 mM Dodecylphosphocholine (DPC).