Supplementary Information

Controlling the kinetics of interaction between microtubules and kinesins over a wide temperature range using the deep-sea osmolyte trimethylamine *N*-oxide

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SUPPLEMENTARY NOTES:

About Fig. 1:

In the analysis of number of microtubules (MTs) per field of view, the size of the field of view was 237.6×281.6 μm^2. In Fig. 1C, various TMAO concentrations are indicated in the legends. Each line in Fig. 1C is a fit to the equation $N_t = N_0 * exp(-k_{off} * t)$, where '$N_t$', '$N_0$', '$k_{off}$', and '$t$' represents number of MTs at any time, initial number of MTs, detachment rate constant of MTs from the motility assay substrate, and time respectively. R^2 values of fitting were 0.9443, 0.9859, 0.9696, 0.9956, and 0.9919 for 0 mM, 1000 mM, 1200 mM, 1500 mM and 2000 mM TMAO respectively. Detachment rate constant of MTs from motility assay substrate for different TMAO concentrations in motility assay, shown in Fig. 1D, were determined from the fitting of the data shown in Fig. 1C.

About Fig. 4:

In Fig. 4C, various TMAO concentrations are indicated in the legends and velocity of MTs was measured after 5 min after increasing the temperature. The data were fitted to a polynomial function, $v = a + b1 \times T + b2 \times T^2$, where '$v$' is velocity of MTs, '$T$' is temperature in Kelvin, 'a' is intercept, 'b1' and 'b2' are fit parameters. The values of 'a', 'b1', 'b2' and 'R^2' for fitting of data are provided in Table S3. In the inset of Fig. 4C, each line is a fit to equation $\ln v = C - (\frac{m}{T})$, where '$v$' is velocity of MTs in nm/s, 'C' is the intercept, 'm' is the slope from which activation energy was calculated, 'T' is temperature in Kelvin. R^2 values for the fitting were 0.9676, 0.9562, 0.9335, 0.9626 for 0 mM, 200 mM, 600 mM and 1000 mM TMAO respectively. In Fig. 4D, the data were fitted to a polynomial function

$E_a = 27 - 0.005C + 0.00002C^2$, where E_a is the activation energy, '*C*' is the concentration of TMAO.

MATERIALS AND METHODS

Chemicals and buffers

TMAO was purchased from Sigma-Aldrich and used without further purification. TMAO solution was prepared using BRB80 buffer (80 mM PIPES, 1 mM $MgCl_2$, and 1 mM EGTA). The pH of BRB80 buffer was adjusted to 6.8 using KOH.

Purification, labelling of tubulin and preparation of MTs

Tubulin was purified from fresh porcine brain according to a previous report using high-concentration PIPES buffer (1 M PIPES, 20 mM EGTA, 10 mM $MgCl_2$; pH adjusted to 6.8 using KOH).[1] Atto550-labelled tubulin (RT) was prepared using Atto550 NHS ester (ATTO-TEC, Gmbh) according to a standard technique.[2] The labeling ratio of fluorescence dye to tubulin was ~1.0 as determined from absorbance of tubulin at 280 nm and fluorescence dye at 554 nm. MTs were prepared by polymerizing a mixture of RT and non-labelled tubulin (WT) (RT:WT = 1:1; final tubulin concentration = 40 μM). 4.0 μL of a mixture of RT and WT was mixed with 1 μL of GTP-premix (5 mM GTP, 20 mM $MgCl_2$, 25% DMSO in BRB80) and incubated at 37 °C for 30 min. After polymerization the MTs were stabilized using paclitaxel (50 μM paclitaxel in DMSO).

Expression and purification of kinesin

Green fluorescent protein (GFP)-fused recombinant kinesin-1 construct consisting of the first 465 amino acid residue of human kinesin-1 (K465), an N-terminal histidine tag, and a C-terminal Avidin-tag was used to propel MTs in the in vitro motility assay. The expression and purification of the kinesin (K465) were done as described in a previously published report.[3]

In vitro motility assay

A flowcell with dimensions of 9×2×0.1 mm^3 (L×W×H) was assembled from two cover glasses (9×18) mm^2 and (40×50) mm^2 (MATSUNAMI) using double-sided tape as a spacer. First, the flow cell was filled with 5 μL of 1 mg mL^{-1} streptavidin solution (Sigma-Aldrich, S4762) and incubated for 5 min. The flowcell was then washed with wash buffer (80 mM PIPES, 1 mM EGTA, 1 mM $MgCl_2$ and ~0.5 mg mL^{-1} casein; pH 6.8). Next, 5 μL of K465 solution (800 nM) was introduced into the streptavidin coated flowcell. The flowcell was incubated for 5 min to allow the binding of kinesins to the glass surface through interaction with streptavidin. After washing the flowcell with 10 μL of wash buffer, 10 μL of MT solution (200 nM, paclitaxel stabilized GTP-MTs) was introduced and incubated for 5 min, which was followed by washing with 10 μL of wash buffer. Finally, motility of MTs was initiated by applying 5 μL of motility buffer containing 5 mM ATP. In case of the experiments where TMAO was used, 5 μL of motility buffer containing 5 mM ATP and TMAO of prescribed concentrations was infused into the flowcell. The flow cell was placed inside the chamber of a heater (Tokai Hit., Co., Ltd., Japan) and humid nitrogen gas was passed through the chamber. The heater was mounted on the stage of a fluorescence microscope. The temperature of the motility assay was controlled using the heater. The stage was allowed to settle for 5 min at all temperatures, followed by monitoring of the MTs until the MTs were detached from the motility assay substrate.

Microscopy image capture and data analysis

Samples were illuminated with a 100 W mercury lamp and visualized by epi-fluorescence microscope (Eclipse Ti; Nikon) equipped with an oil-coupled Plan Apo 60×1.40 objective (Nikon). A filter block with UV-cut specification (TRITC: EX540/25, DM565, BA606/55;

GFP-HQ: EX455-485, DM495, BA500-545; Nikon) was used in the optical path of the microscope that allowed visualization of MTs eliminating the UV part of radiation and minimized the harmful effect of UV radiation on samples. Images were captured using a cooled CMOS camera (Neo CMOS; Andor) connected to a PC. To capture images of MTs for several minutes, ND4 filter (25% transmittance) were inserted into the illuminating light path of the fluorescence microscope to avoid photobleaching. All movies and images captured by the epi-fluorescence microscope were analyzed using an image analysis software (ImageJ 1.46r). Velocity of the gliding MTs were measured using the ImageJ plugin MTrackJ. Average velocity of MTs in different assay conditions were estimated from fitting of the velocity data according to the equation of Gaussian distribution. Statistical analyses were performed using 'Origin 2019'. Two-tailed Student's t-test was used to compare two groups of data where applicable.

REFERENCES

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2. J. Peloquin, Y. Komarova and G. Borisy, Conjugation of fluorophores to tubulin, *Nat. Methods*, 2005, **2**, 299.

3. K. Fujimoto, M. Kitamura, M. Yokokawa, I. Kanno, H. Kotera and R. Yokokawa, Colocalization of quantum dots by reactive molecules carried by motor proteins on polarized microtubule arrays, *ACS Nano*, 2012, **7**, 447.

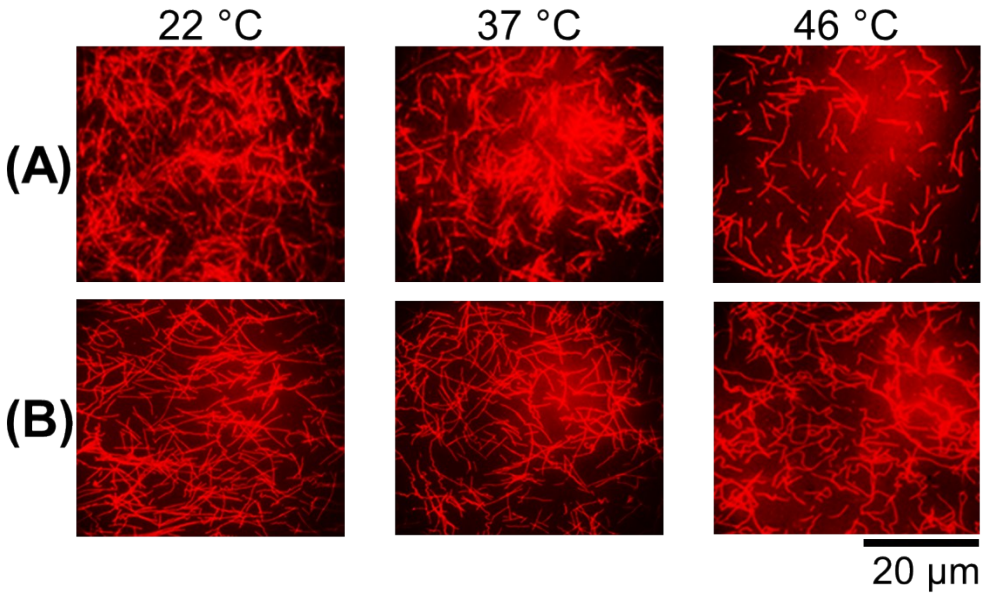

Figure S1. Representative fluorescence microscopy images show detachment of MTs and change in MT density upon increasing the temperature of an in vitro motility assay in the absence of TMAO (A). In the presence of 200 mM TMAO (B) the detachment of MTs was suppressed. For each temperature, the image was captured 30 min after the temperature was raised.

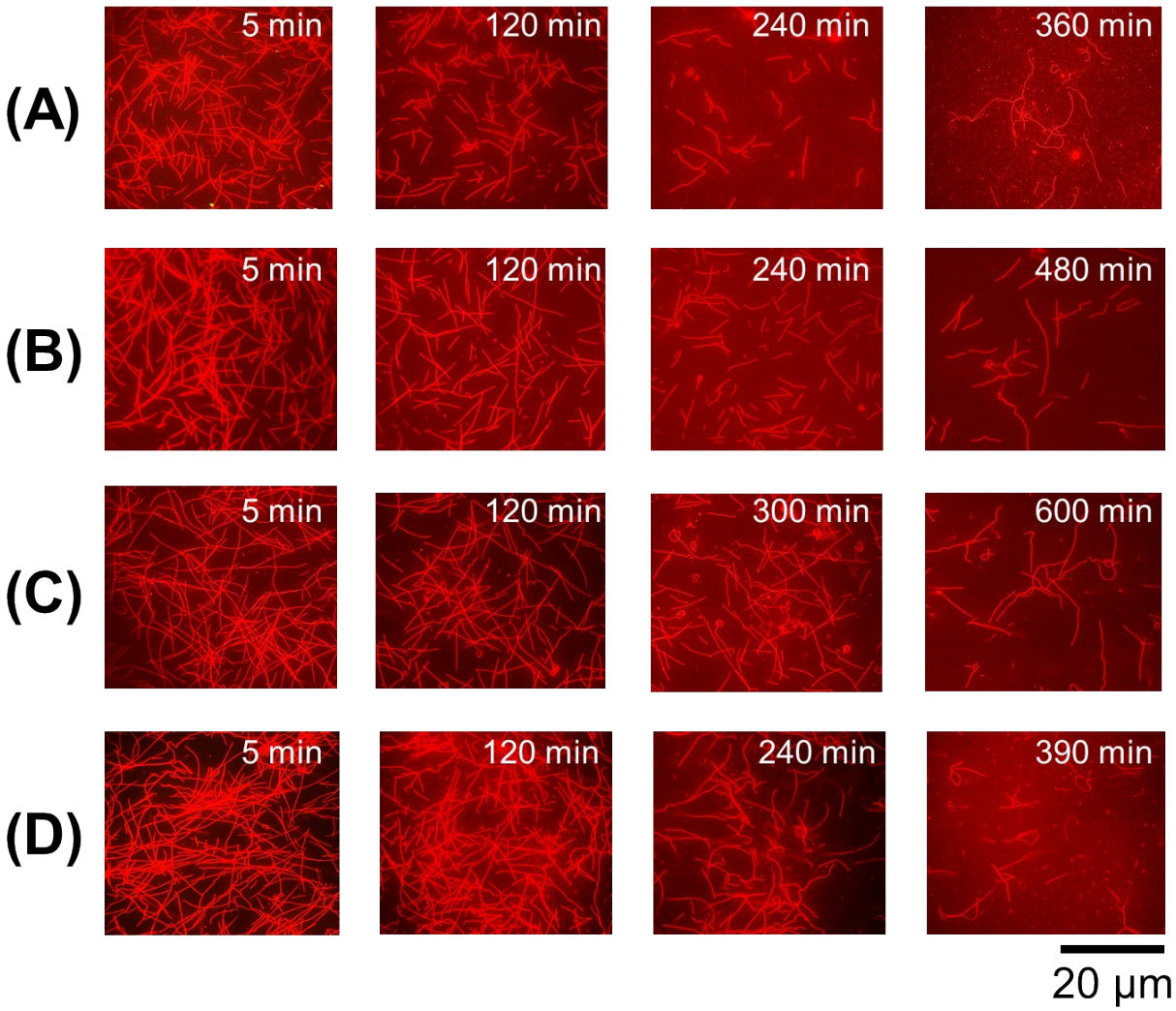

Figure S2. Representative time-lapse fluorescence microscopy images show change in MT density with time in motility assays performed at 32 °C. The concentration of TMAO was (A) 0 mM, (B) 200 mM, (C) 600 mM and (D) 1000 mM.

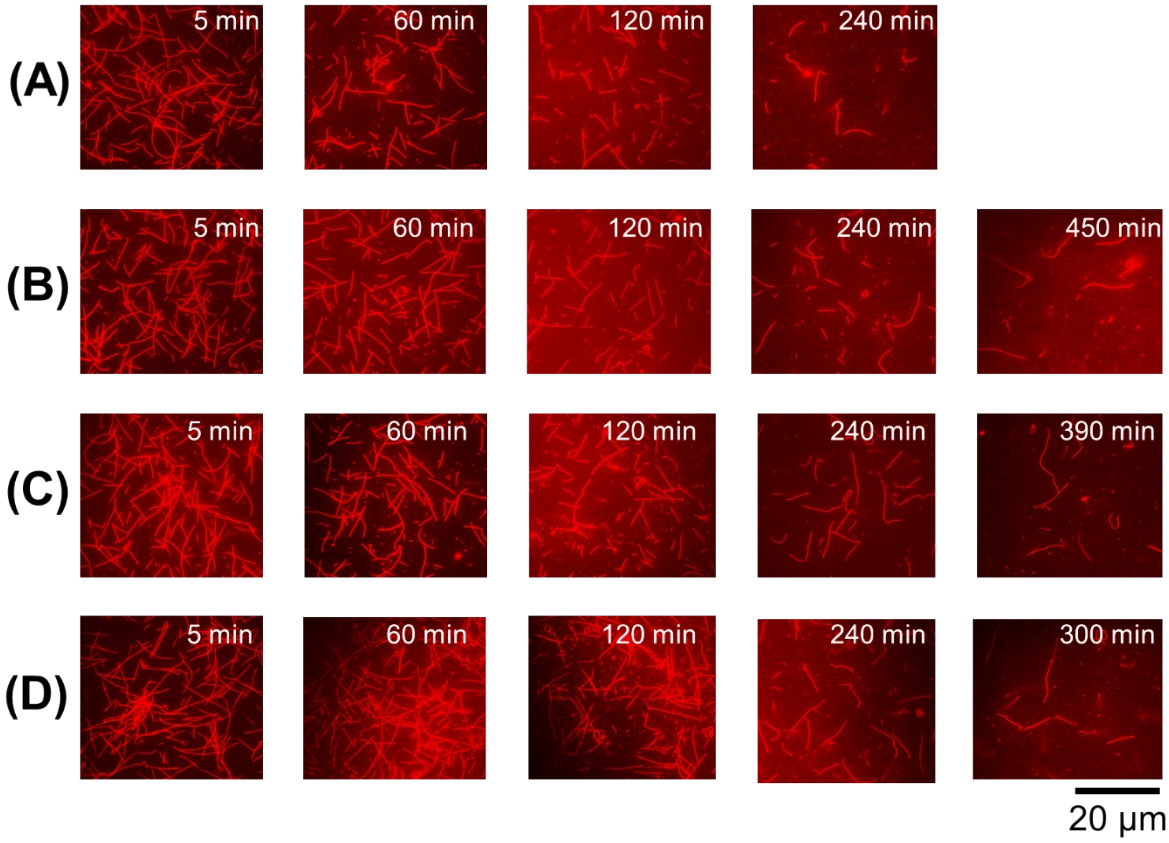

Figure S3. Representative time-lapse fluorescence microscopy images show change in MT density with time in motility assays performed at 37 °C. The concentration of TMAO was (A) 0 mM, (B) 200 mM, (C) 600 mM and (D) 1000 mM.

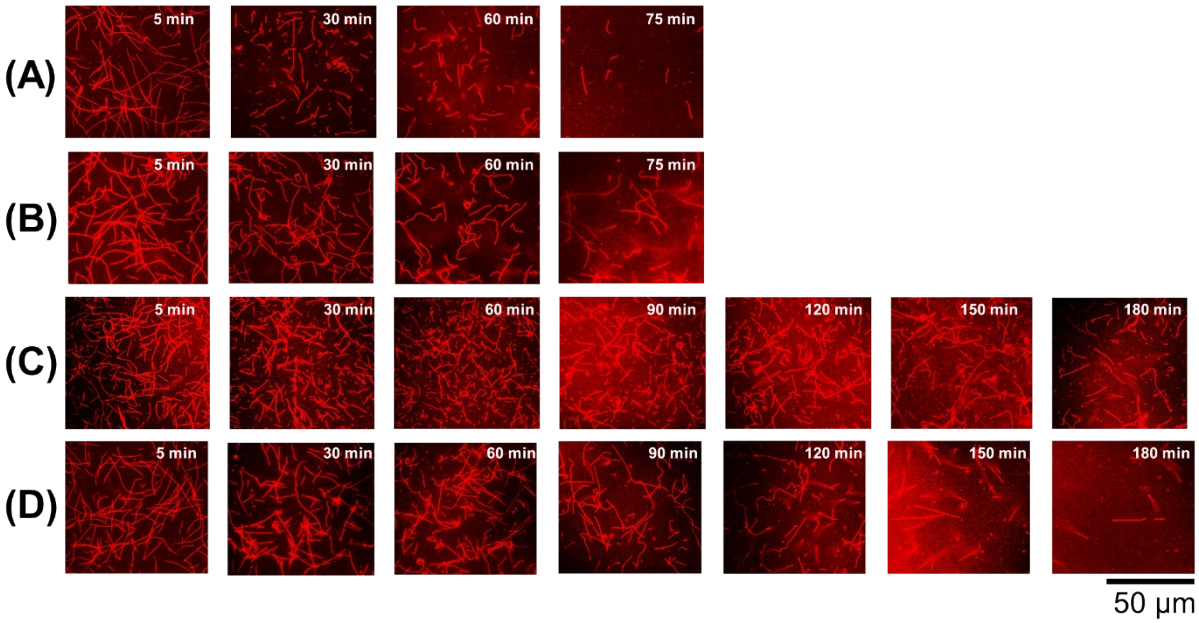

Figure S4. Representative time-lapse fluorescence microscopy images show change in MT density with time in motility assays performed at 46 °C. The concentration of TMAO was (A) 0 mM, (B) 200 mM, (C) 600 mM and (D) 1000 mM.

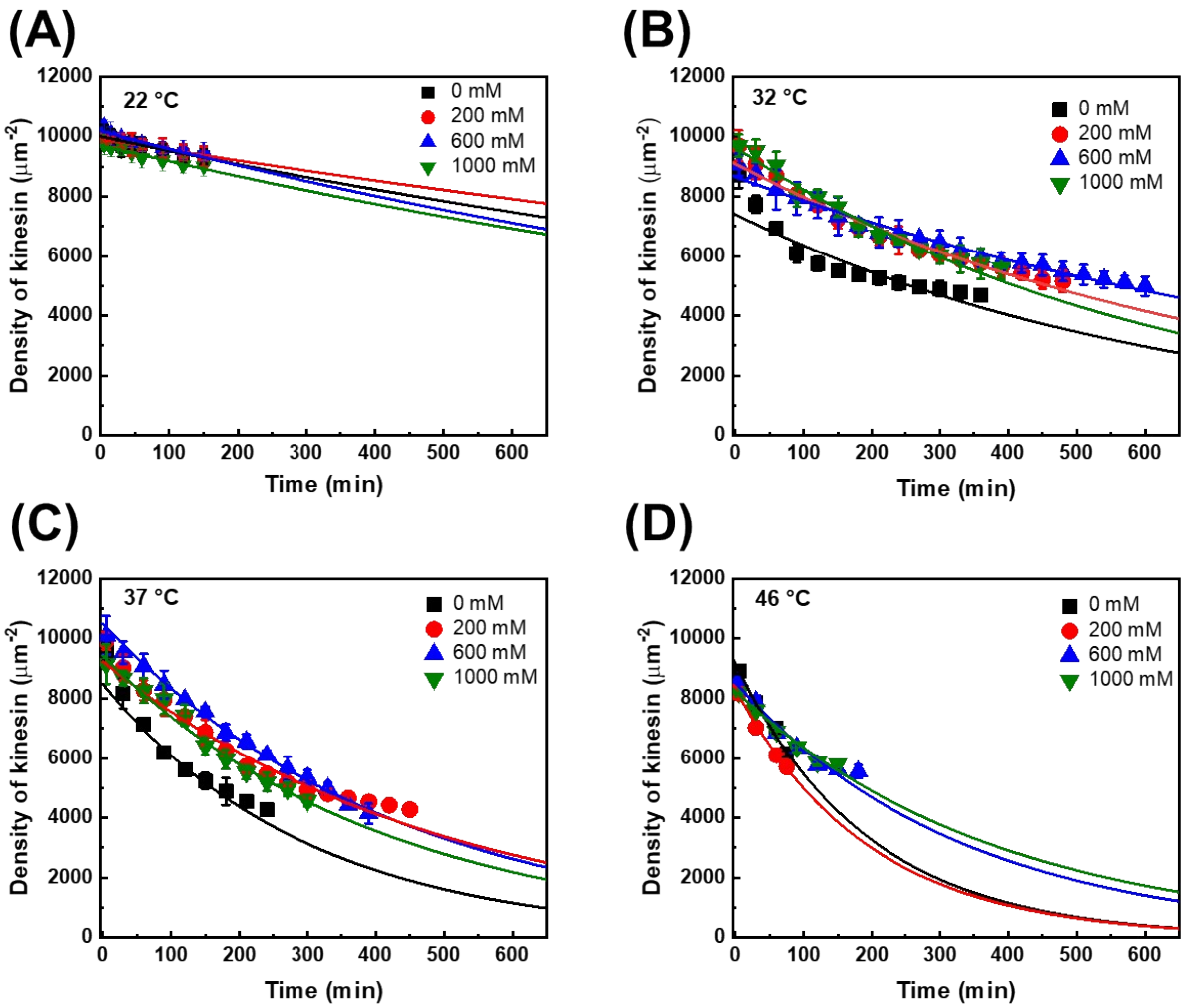

Figure S5. Change in kinesin density with time on the substrate of in vitro motility assay performed at 22 °C, 32 °C, 37 °C and 46 °C in the presence of different TMAO concentrations as indicated by the legends. Each line is a fit to the equation $D_t = D_0 * exp(-k_{off} * t)$, where '$D_t$', '$D_0$', '$k_{off}$', and '$t$' represents density of kinesins at any time, initial density of kinesins, detachment rate constants of kinesins per µm^2 area of the motility assay substrate, and time respectively. Error bars: standard deviation.

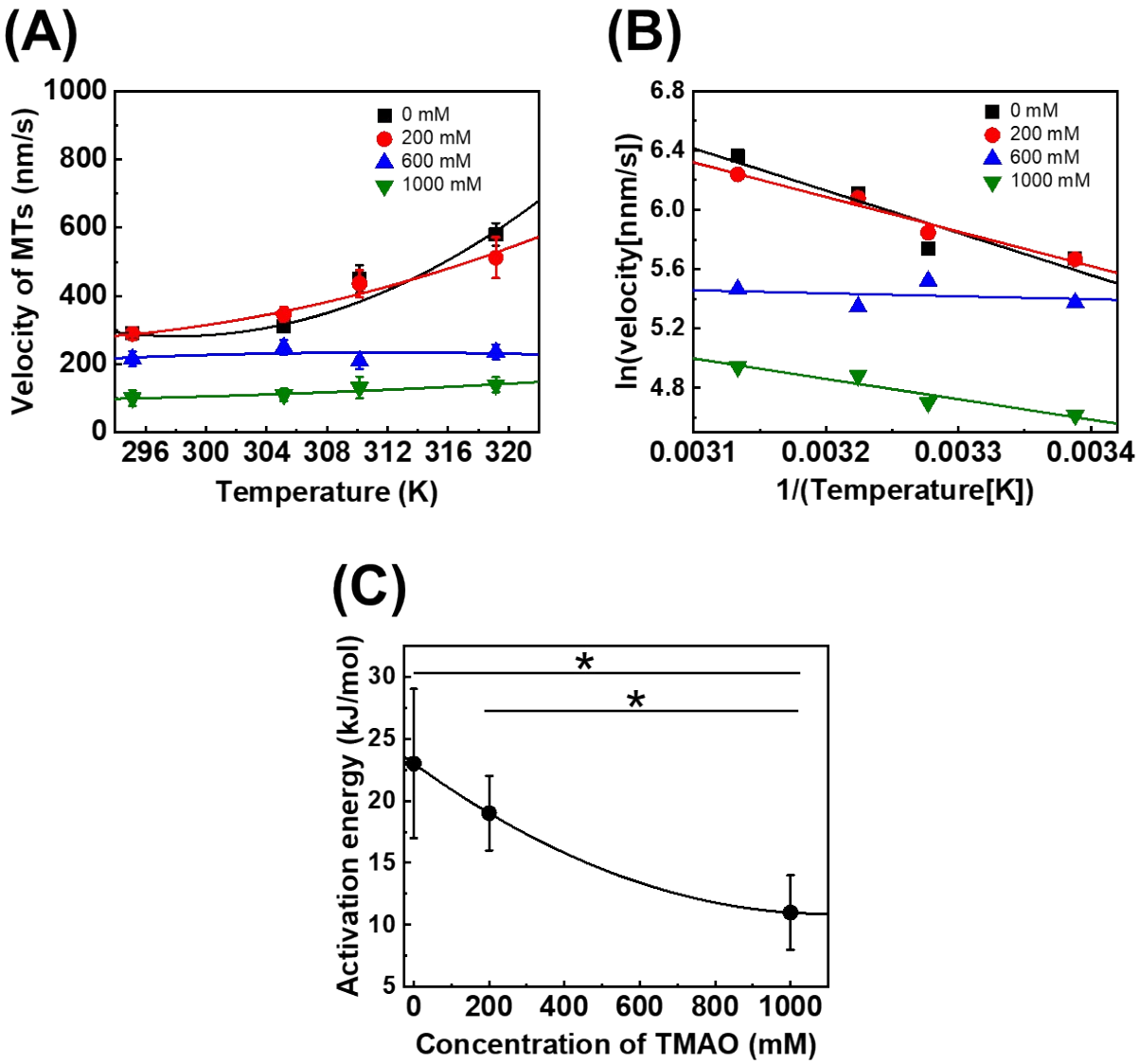
(A)
Velocity of MTs (nm/s)
1000
800
600
400
200
0
296 300 304 308 312 316 320
Temperature (K)
0 mM
200 mM
600 mM
1000 mM
(B)
ln(velocity[nnm/s])
6.8
6.4
6.0
5.6
5.2
4.8
0.0031 0.0032 0.0033 0.0034
1/(Temperature[K])
0 mM
200 mM
600 mM
1000 mM
(C)
Activation energy (kJ/mol)
30
25
20
15
10
5
0 200 400 600 800 1000
Concentration of TMAO (mM)
*
*

Figure S6. (A) Dependence of MT gliding velocity on temperature at different TMAO concentrations indicated by the legends. The velocity data used here were obtained from measurement at the time just before the impairment of the motility assay, i.e. the velocity data correspond to the last data point for each condition shown in Figure 3. The data were fitted to a polynomial function $v = a + b1 * T + b2 * T^2$, where '$v$' is velocity of MTs, '$T$' is temperature in Kelvin, 'a' is intercept, 'b1' and 'b2' are fit parameters. The values of 'a', 'b1', 'b2' and 'R^2' for fitting are provided in Table-2 of supporting information. (B) Corresponding Arrhenius plot of the data shown in (A). Each line is a fit to equation $\ln v = C - (\frac{m}{T})$, where '$v$' is velocity of MTs in nm/s, 'C' is the intercept, 'm' is the slope from which activation energy was calculated, 'T' is temperature in kelvin. R^2 values for the fitting of data were 0.8743, 0.9671, 0.0709, 0.9101 for 0 mM, 200 mM, 600 mM and 1000 mM TMAO respectively. (C) Change in activation energy for the motility of MTs on kinesins upon changing the concentration of TMAO in the motility assay. The data were fitted to a polynomial function $E_a = 23 - 0.022C + 0.00001C^2$, where E_a is the activation energy, 'C' is the concentration of TMAO. Significant difference between the data sets was observed at $P<0.001$ indicated by *. The activation energy for 600 mM TMAO was not considered here since velocity data for 600 mM TMAO did not show temperature dependence according to the Arrhenius equation. Error bars: standard deviation.

Table S1. MT detachment rate constants for different temperatures in the presence of 0 mM, 200 mM, 600 mM and 1000 mM TMAO. The rate constants were estimated from the fitting of experimental data as shown in Figure 2 (A)- 2(D).

Temperature (°C)	**Concentration of TMAO (mM)**	**Rate constant (min^{-1})**
22	0	0.007
	200	0.007
	600	0.0065
	1000	0.007

32	0	0.0155
	200	0.013
	600	0.010
	1000	0.014
37	0	0.025
	200	0.015
	600	0.018
	1000	0.017
46	0	0.08
	200	0.06
	600	0.03
	1000	0.04

Table S2. Fitting parameters and goodness of fitting of the data for temperature dependence of velocity of MTs as shown in Figure 4C.

Concentration of TMAO (mM)	**a**	**b1**	**b2**	**R^2**
0	49677	-337	0.57518	0.99952
200	53619	-362	0.61567	0.99991
600	51207	-345	0.58568	0.98934
1000	50547	-340	0.57624	0.99991

Table S3. Fitting parameters and goodness of fitting of the data for temperature dependence of velocity of MTs as shown in Figure S6 (A).

Concentration of TMAO (mM)	a	b1	b2	R^2
0	62089.24	-414.65	0.695	0.9576
200	19336.06	-133.44	0.233	0.9623
600	-4920.98	33.07	-0.053	0.1231
1000	2294.74	-15.89	0.028	0.9294