



## Supplementary Material

# MPTHub: An Open-Source Software for Characterizing the Transport of Particles in Biorelevant Media

Leandro Gabriel <sup>1,2,3,†</sup>, Helena Almeida <sup>1,2,4,†</sup>, Marta Avelar <sup>1,2,3,4</sup>, Bruno Sarmento <sup>1,2,5</sup> and José das Neves <sup>1,2,5,\*</sup>

<sup>1</sup> i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal; lassig@protonmail.com (L.G.); helena.almeida@i3s.up.pt (H.A.); mavelar@i3s.up.pt (M.A.); bruno.sarmiento@i3s.up.pt (B.S.)

<sup>2</sup> INEB—Instituto de Engenharia Biomédica, Universidade do Porto, 4200-135 Porto, Portugal

<sup>3</sup> FEUP—Faculdade de Engenharia, Universidade do Porto, 4200-465 Porto, Portugal

<sup>4</sup> ICBAS—Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050-313 Porto, Portugal

<sup>5</sup> IUCS—Instituto Universitário de Ciências da Saúde, CESPU, 4585-116 Gandra, Portugal

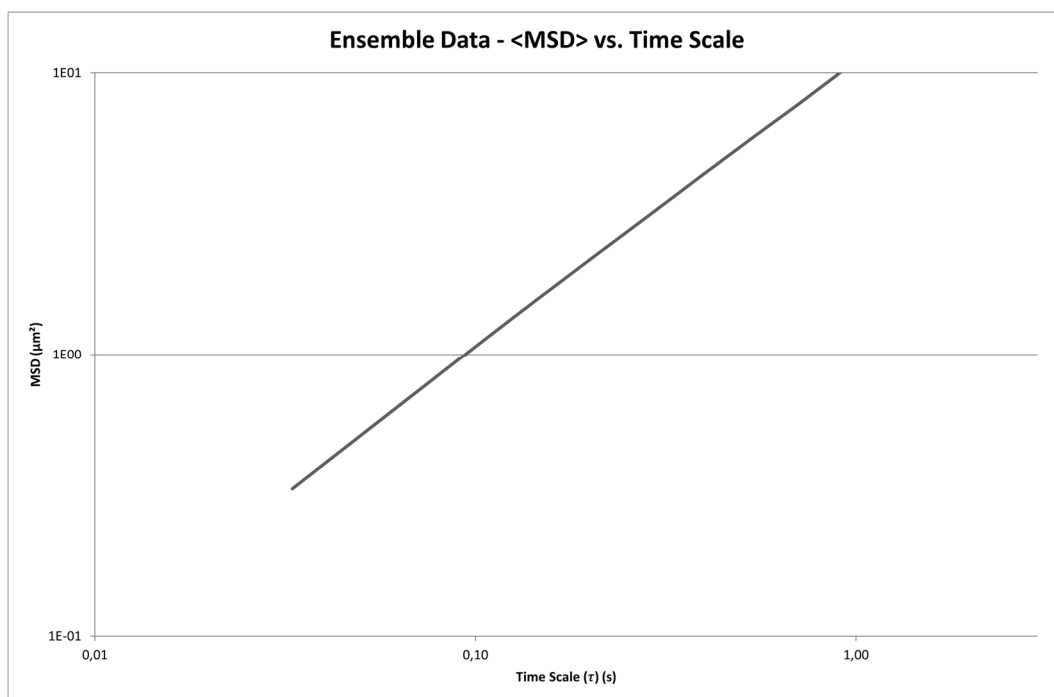
\* Correspondence: j.dasneves@i3s.up.pt; Tel.: +351-220-408-800

† These authors contributed equally to this work.

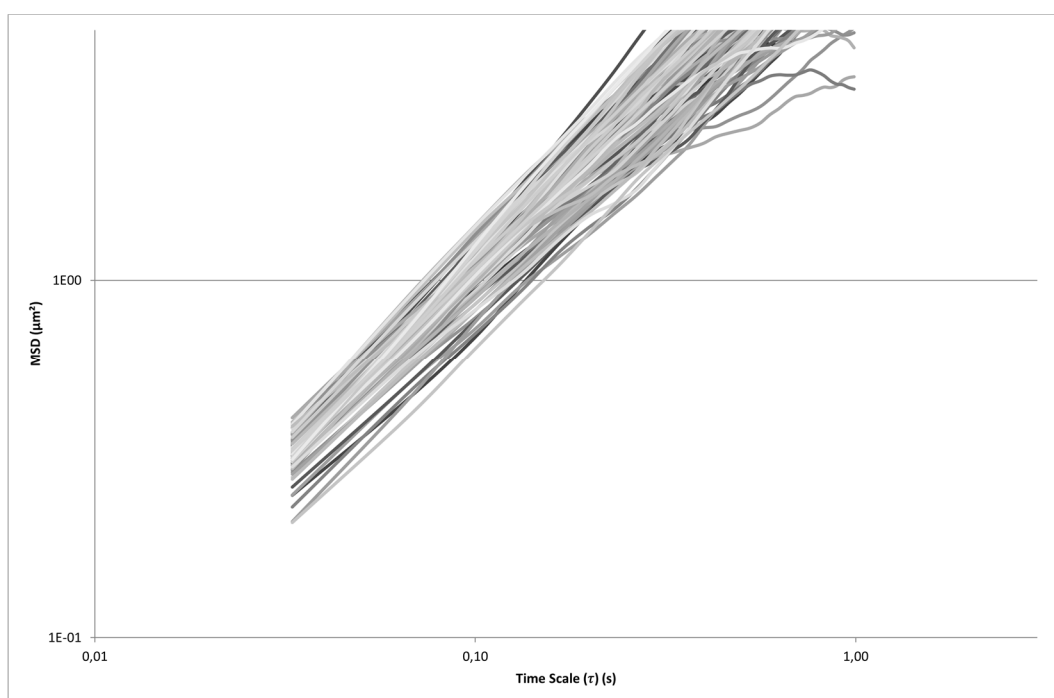
**Table S1.** Details of the three computers used for testing the performance of MPTHub.

Computer	Properties
#1	Intel® Core™ i7-1165G7 processor (12M Cache, 2.80 GHz), 16 GB RAM
#2	Intel® Core™ i7-4500U processor (4M Cache, 2.40 GHz), 16 GB RAM
#3	Intel® Core™ i3-7020U processor (3M Cache, 2.30 GHz), 4 GB RAM

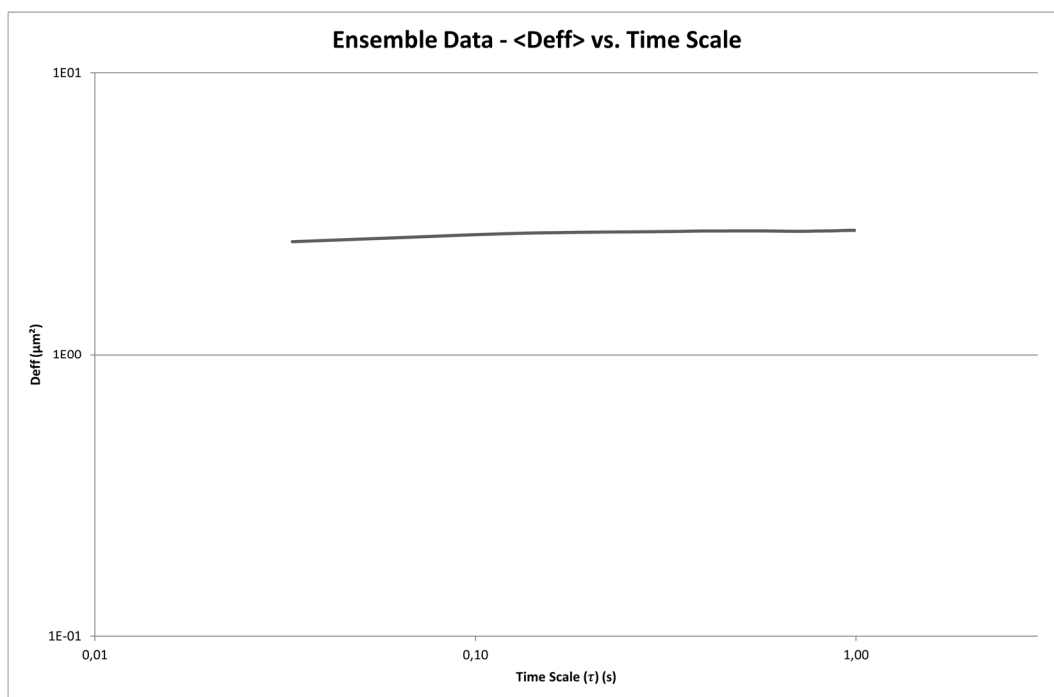
	A	B	C	D	E	F	G	
1								
2	Timescale (τ) (s)	MSD 1 (μm <sup>2</sup> )	MSD 2 (μm <sup>2</sup> )	MSD 3 (μm <sup>2</sup> )	MSD 4 (μm <sup>2</sup> )	MSD 5 (μm <sup>2</sup> )	MSD 6 (μm <sup>2</sup> )	MSD
3	0,033	0,3595162	0,335247965	0,358997906	0,321676024	0,333997556	0,346448755	0,324
4	0,066	0,748341197	0,711585943	0,805129822	0,663219512	0,739098928	0,696488509	0,700
5	0,099	1,120268097	1,174051432	1,304841505	0,921620463	1,121483404	1,091246289	1,082
6	0,132	1,469545014	1,622128414	1,852732019	1,169742825	1,502705168	1,509443649	1,435
7	0,165	1,766896682	2,115902296	2,444736938	1,445253865	1,923771135	1,929793572	1,81
8	0,198	2,051934254	2,575221646	3,10054382	1,741620877	2,342187519	2,372116057	2,145
9	0,231	2,386773102	3,061268762	3,822138155	2,060132619	2,830352796	2,805928233	2,534
10	0,264	2,658738134	3,526845485	4,650600635	2,367288709	3,340736842	3,214870477	2,95
11	0,297	2,958861547	4,01410966	5,510904781	2,69034476	3,831873943	3,625852242	3,291
12	0,33	3,247820116	4,489055587	6,452327422	3,045685978	4,386212756	4,001671855	3,587
13	0,363	3,581053401	4,951982046	7,454170951	3,409032259	4,904573731	4,363976118	3,847
14	0,396	3,899347909	5,388378578	8,51775556	3,806257712	5,429963842	4,739145238	4,128
15	0,429	4,227369462	5,815310817	9,589483376	4,188656076	6,008060872	5,100205105	4,425
16	0,462	4,566077196	6,272755398	10,6501328	4,588058194	6,624934629	5,481468795	4,763
17	0,495	4,919439938	6,703528742	11,68843967	5,00981054	7,215740261	5,873351624	5,082
18	0,528	5,267429448	7,163502192	12,78841263	5,461927387	7,903729865	6,283843286	5,382
19	0,561	5,570356636	7,629477234	13,94870602	5,815024209	8,63011697	6,643446367	5,676
20	0,594	5,80779689	8,134563816	15,08818778	6,206112829	9,399158759	7,045882493	6,035
21	0,627	6,055795755	8,678496593	16,24581773	6,547470114	10,17977257	7,4744327	6,390
22	0,66	6,37880983	9,26087019	17,42309556	6,822020101	10,99886098	7,937936049	6,738
23	0,693	6,660967315	9,788877614	18,52471306	7,147855432	11,8297804	8,422138196	7,098
24	0,726	7,017850546	10,38580921	19,74687562	7,476905524	12,66147328	8,934702678	7,512
25	0,759	7,345536493	11,03392893	21,00784292	7,78728221	13,47645427	9,418716461	7,894
26	0,792	7,624468151	11,71663269	22,24751252	8,064465729	14,3568269	9,888562771	8,255
27	0,825	7,903714939	12,41822614	23,43156142	8,277424859	15,32329787	10,30654166	8,601
28	0,858	8,197189283	13,10474539	24,54026547	8,413704862	16,35429489	10,72432633	8,925
29	0,891	8,50455386	13,78673335	25,65307447	8,537760083	17,42761174	11,00350056	9,271
30	0,924	8,81196796	14,46984122	26,76618347	8,657760083	18,54072365	11,28350056	9,617
31	0,957	9,11938003	15,15294899	27,87929248	8,777760083	19,65383556	11,56350056	9,963
32	0,99	9,4267921	15,8360561	28,99240149	8,897760083	20,76694647	11,84350056	10,309
33	1,023	9,73420417	16,51916321	30,1055105	9,017760083	21,88005738	12,12350056	10,655
34	1,056	10,04161624	17,20227032	31,21861951	9,137760083	22,99316829	12,40350056	11,001
35	1,089	10,34902831	17,88537743	32,33172852	9,257760083	24,1062792	12,68350056	11,347
36	1,122	10,65644038	18,56848454	33,44483753	9,377760083	25,21939011	12,96350056	11,693
37	1,155	10,96385245	19,25159165	34,55794654	9,497760083	26,33250102	13,24350056	12,039
38	1,188	11,27126452	19,93469876	35,67105555	9,617760083	27,44561193	13,52350056	12,385
39	1,221	11,57867659	20,61780587	36,78416456	9,737760083	28,55872284	13,80350056	12,731
40	1,254	11,88608866	21,30091298	37,89727357	9,857760083	29,67183375	14,08350056	13,077
41	1,287	12,19350073	21,98402009	39,01038258	9,977760083	30,78494466	14,36350056	13,423
42	1,32	12,5009128	22,6671272	40,12349159	10,097760083	31,89805557	14,64350056	13,769
43	1,353	12,80832487	23,35023431	41,2366006	10,217760083	33,01116648	14,92350056	14,115
44	1,386	13,11573694	24,03334142	42,34970961	10,337760083	34,12427739	15,20350056	14,461
45	1,419	13,42314901	24,71644853	43,46281862	10,457760083	35,2373883	15,48350056	14,807
46	1,452	13,73056108	25,40000000	44,57592763	10,577760083	36,35049921	15,76350056	15,153
47	1,485	14,03797315	26,08310711	45,68903664	10,697760083	37,46361012	16,04350056	15,499
48	1,518	14,34538522	26,76621422	46,80214565	10,817760083	38,57672103	16,32350056	15,845
49	1,551	14,65279729	27,44932133	47,91525466	10,937760083	39,68983194	16,60350056	16,191
50	1,584	14,96020936	28,13242844	49,02836367	11,057760083	40,80294285	16,88350056	16,537
51	1,617	15,26762143	28,81553555	50,14147268	11,177760083	41,91605376	17,16350056	16,883
52	1,65	15,5750335	29,49864266	51,25458169	11,297760083	43,02916467	17,44350056	17,229
53	1,683	15,88244557	30,18174977	52,3676907	11,417760083	44,14227558	17,72350056	17,575
54	1,716	16,18985764	30,86485688	53,48079971	11,537760083	45,25538649	18,00350056	17,921
55	1,749	16,49726971	31,54796399	54,59390872	11,657760083	46,3684974	18,28350056	18,267
56	1,782	16,80468178	32,2310711	55,70701773	11,777760083	47,48160831	18,56350056	18,613
57	1,815	17,11209385	32,91417821	56,82012674	11,897760083	48,59471922	18,84350056	18,959
58	1,848	17,41950592	33,59728532	57,93323575	12,017760083	49,70783013	19,12350056	19,305
59	1,881	17,72691799	34,28039243	59,04634476	12,137760083	50,82094104	19,40350056	19,651
60	1,914	18,03433006	34,96349954	60,15945377	12,257760083	51,93405195	19,68350056	20,000
61	1,947	18,34174213	35,64660665	61,27256278	12,377760083	53,04716286	19,96350056	20,346
62	1,98	18,6491542	36,32971376	62,38567179	12,497760083	54,16027377	20,24350056	20,692
63	2,013	18,95656627	37,01282087	63,4987808	12,617760083	55,27338468	20,52350056	21,038
64	2,046	19,26397834	37,69592798	64,61188981	12,737760083	56,38649559	20,80350056	21,384
65	2,079	19,57139041	38,37903509	65,72500082	12,857760083	57,4996065	21,08350056	21,730
66	2,112	19,87880248	39,0621422	66,83811183	12,977760083	58,61271741	21,36350056	22,076
67	2,145	20,18621455	39,74524931	67,94722284	13,097760083	59,72582832	21,64350056	22,422
68	2,178	20,49362662	40,42835642	69,05633385	13,217760083	60,83893923	21,92350056	22,768
69	2,211	20,80103869	41,11146353	70,16544486	13,337760083	61,95205014	22,20350056	23,114
70	2,244	21,10845076	41,79457064	71,27455587	13,457760083	63,06516105	22,48350056	23,460
71	2,277	21,41586283	42,47767775	72,38366688	13,577760083	64,17827196	22,76350056	23,806
72	2,31	21,7232749	43,16078486	73,49277789	13,697760083	65,29138287	23,04350056	24,152
73	2,343	22,03068697	43,84389197	74,6018889	13,817760083	66,40449378	23,32350056	24,498
74	2,376	22,33809904	44,52700008	75,71100001	13,937760083	67,51760469	23,60350056	24,844
75	2,409	22,64551111	45,21010719	76,82011102	14,057760083	68,6307156	23,88350056	25,190
76	2,442	22,95292318	45,8932143	77,92922203	14,177760083	69,74382651	24,16350056	25,536
77	2,475	23,26033525	46,57632141	79,03833304	14,297760083	70,85693742	24,44350056	25,882
78	2,508	23,56774732	47,25942852	80,14744405	14,417760083	71,97004833	24,72350056	26,228
79	2,541	23,87515939	47,94253563	81,25655506	14,537760083	73,08315924	25,00350056	26,574
80	2,574	24,18257146	48,62564274	82,36566607	14,657760083	74,19627015	25,28350056	26,920
81	2,607	24,48998353	49,30874985	83,47477708	14,777760083	75,30938106	25,56350056	27,266
82	2,64	24,7973956	49,99185696	84,58388809	14,897760083	76,42249197	25,84350056	27,612
83	2,673	25,10480767	50,67496407	85,69300001	15,017760083	77,53560288	26,12350056	27,958
84	2,706	25,41221974	51,35807118	86,80211102	15,137760083	78,64871379	26,40350056	28,304
85	2,739	25,71963181	52,04117829	87,91122203	15,257760083	79,7618247	26,68350056	28,650
86	2,772	26,02704388	52,7242854	89,02033304	15,377760083	80,87493561	26,96350056	28,996
87	2,805	26,33445595	53,40739251	90,12944405	15,497760083	81,98804652	27,24350056	29,342
88	2,838	26,64186802	54,09050002	91,23855506	15,617760083	83,10115743	27,52350056	29,688
89	2,871	26,94928009	54,77360713	92,34766607	15,737760083	84,21426834	27,80350056	30,03



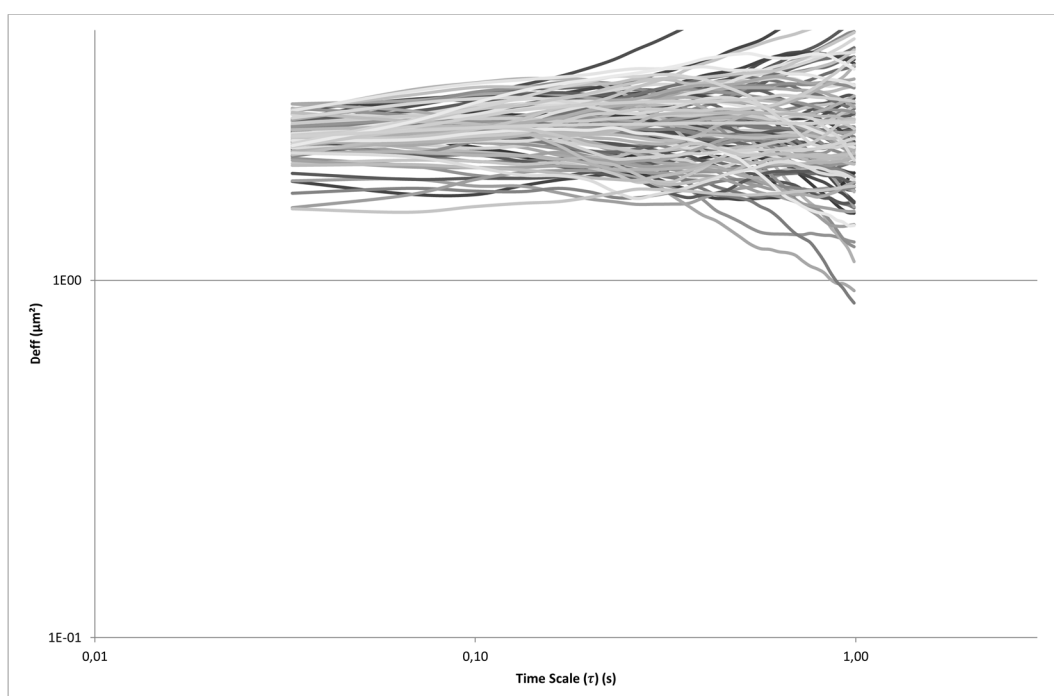
**Figure S2.** Example of a <MSD> vs. time scale graph generated in the 'Individual Particle Analysis' .xlsx file. Please note the logarithmic scales in both axes.



**Figure S3.** Example of a MSD vs. time scale graph generated in the 'Individual Particle Analysis' .xlsx file. Please note the logarithmic scales in both axes.



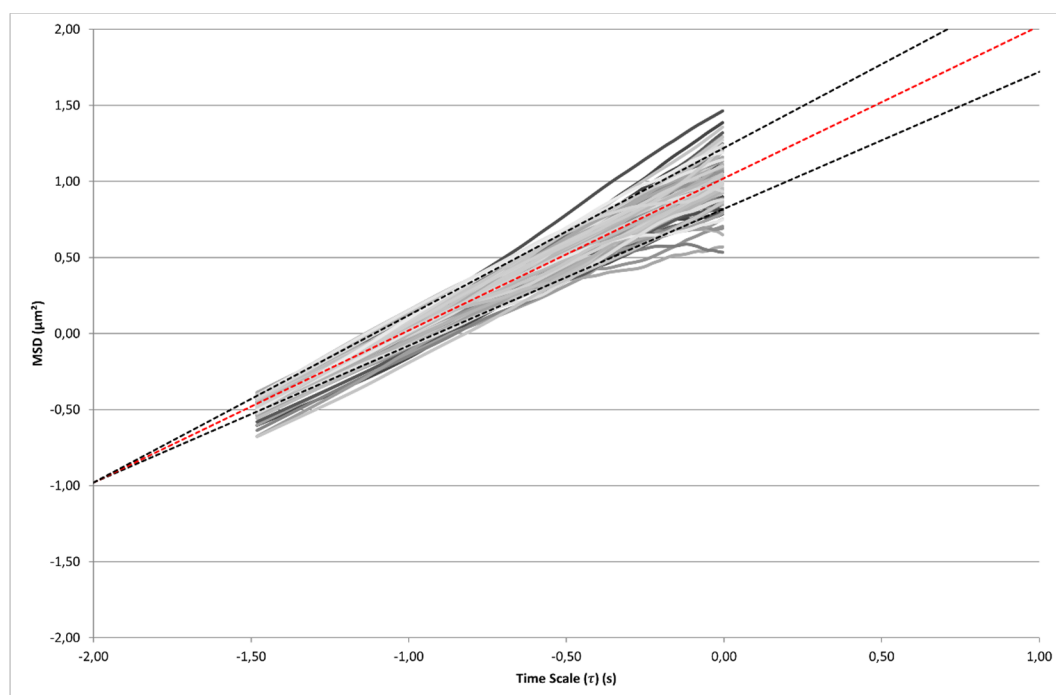
**Figure S4.** Example of a  $\langle D_{\text{eff}} \rangle$  vs. time scale graph generated in the 'Individual Particle Analysis' .xlsx file. Please note the logarithmic scales in both axes.



**Figure S5.** Example of a  $D_{\text{eff}}$  vs. time scale graph generated in the 'Individual Particle Analysis' .xlsx file. Please note the logarithmic scales in both axes.

	A	B	C	D	E	F	G	
1								
2	Timescale ( $\tau$ ) (s)	MSD-LOG 1 ( $\mu\text{m}^2$ )	MSD-LOG 2 ( $\mu\text{m}^2$ )	MSD-LOG 3 ( $\mu\text{m}^2$ )	MSD-LOG 4 ( $\mu\text{m}^2$ )	MSD-LOG 5 ( $\mu\text{m}^2$ )	MSD-LOG 6 ( $\mu\text{m}^2$ )	MSD-LOG 7 ( $\mu\text{m}^2$ )
3	-1,48148606	-0,444281536	-0,474633850	-0,444908085	-0,492581307	-0,476256710	-0,460360994	-0,444281536
4	-1,180456064	-0,125900346	-0,147772640	-0,094134087	-0,178342705	-0,131297428	-0,157086044	-0,125900346
5	-1,004364805	0,049321968	0,069687123	0,115557763	-0,035447891	0,049792851	0,037922780	0,049321968
6	-0,879426069	0,167182893	0,210085232	0,267812607	0,068090390	0,176873780	0,178816905	0,167182893
7	-0,782516056	0,247211155	0,325495610	0,388232134	0,159944140	0,284153404	0,285510855	0,247211155
8	-0,70333481	0,312163441	0,410814614	0,491437874	0,240953622	0,369621662	0,375135933	0,312163441
9	-0,63638802	0,377811135	0,485901460	0,582306381	0,313895178	0,451840573	0,448076559	0,377811135
10	-0,578396073	0,424675565	0,547386433	0,667509047	0,374251227	0,523842267	0,507163480	0,424675565
11	-0,527243551	0,471124644	0,603589233	0,741222907	0,429807937	0,583411214	0,559410102	0,471124644
12	-0,48148606	0,511591967	0,652154983	0,809716397	0,483685124	0,642089694	0,602241473	0,511591967
13	-0,440093375	0,554010797	0,694779061	0,872399348	0,532631111	0,690601268	0,639882365	0,554010797
14	-0,402304814	0,590991986	0,731458101	0,930325173	0,580498190	0,734796938	0,675700018	0,590991986
15	-0,367542708	0,626070206	0,764572932	0,981795211	0,622074703	0,778734324	0,707587642	0,626070206
16	-0,335358024	0,659543250	0,797458353	1,027355023	0,661628918	0,821181597	0,738896946	0,659543250
17	-0,305394801	0,691915663	0,826303476	1,067756539	0,699821302	0,858280892	0,768886002	0,691915663
18	-0,277366077	0,721598727	0,855125398	1,106816641	0,737345922	0,897832088	0,798225346	0,721598727
19	-0,251037139	0,745883001	0,882494781	1,144533921	0,764551527	0,936016682	0,822393433	0,745883001
20	-0,226213555	0,764011420	0,910334271	1,178637081	0,792819667	0,973088985	0,847935396	0,764011420
21	-0,202732459	0,782171219	0,938444497	1,210741576	0,816073525	1,007738075	0,873578236	0,782171219
22	-0,180456064	0,804739655	0,966651797	1,241125319	0,833912995	1,041347713	0,899707596	0,804739655
23	-0,159266765	0,823537303	0,990732899	1,267751490	0,854175760	1,072976683	0,925422363	0,823537303
24	-0,139063379	0,846204115	1,016440340	1,295498391	0,873721893	1,102484243	0,951080105	0,846204115
25	-0,119758224	0,866023521	1,042730182	1,322381461	0,891385914	1,129575642	0,973991723	0,866023521
26	-0,101274818	0,882209555	1,068802815	1,347281460	0,906575601	1,157058464	0,995133175	0,882209555
27	-0,083546051	0,897831268	1,094059564	1,369801230	0,917895247	1,185352244	1,013112963	0,897831268
28	-0,066512712	0,913664964	1,117428588	1,389879257	0,924987274	1,213631824	1,030370021	0,913664964
29	-0,050113306	0,929651176	1,137763371	1,407146558	0,930824084	1,242482087	1,045072485	0,929651176

**Figure S6.** Example of generated ‘Transport Mode Characterization’ .xlsx file spreadsheet presenting raw analysis data after processing of input CSV files.



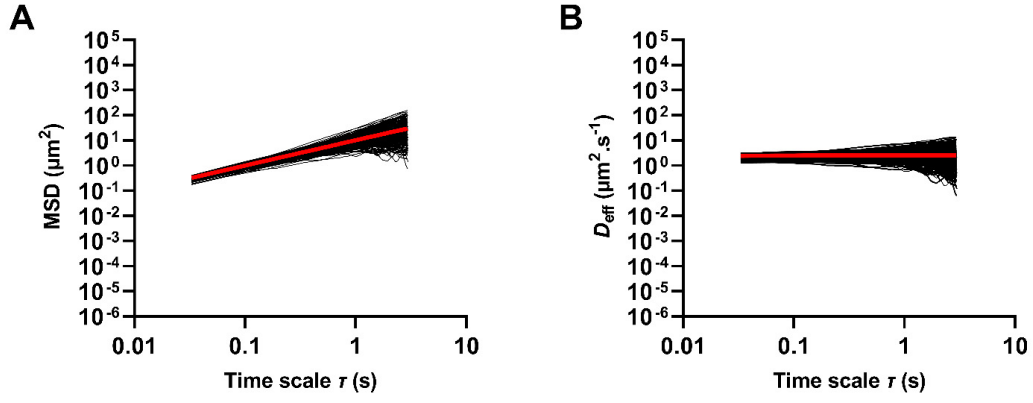
**Figure S7.** Example of a MSD vs. time scale graph generated in the ‘Transport Mode Characterization’ .xlsx file. Dashed lines presenting different slopes (0.9, 1.0 and 1.1) are included for guidance. Please note the linear scales in both axes.

	A	B	C	D	E	F
1	Slopes	Slopes (Excel)	R <sup>2</sup> (Excel)	Transport Mode	Slope	Count
2	0,946003222	0,946003222	0,999286258	Immobile	0,0-0,199	0
3	1,113317046	1,113317046	0,999057535	Sub-diffusive	0,2-0,899	19
4	1,340696417	1,340696417	0,998223359	Diffusive	0,9-1,199	76
5	1,028013443	1,028013442	0,99645975	Active	1,2+	7
6	1,225965352	1,225965352	0,994306885			
7	1,044505004	1,044505004	0,999415628			
8	0,990424520	0,99042452	0,998715094	<slope> =	0,997907128	
9	0,936409573	0,936409573	0,996765191	N =	102	
10	0,926102028	0,926102028	0,992990995	STD =	0,134222422	
11	0,820440377	0,820440377	0,979635679			
12	1,014209825	1,014209824	0,993315373			
13	0,802135139	0,802135139	0,989021957			
14	0,978573239	0,978573239	0,997081521			
15	1,040239918	1,040239918	0,992301314			
16	1,083384612	1,083384612	0,998257775			
17	0,946599831	0,946599831	0,996841926			
18	1,132902333	1,132902333	0,997483036			
19	1,061521906	1,061521906	0,991163249			
20	0,880342849	0,88034285	0,929800207			
21	1,255023774	1,255023774	0,99123038			
22	1,124853122	1,124853122	0,9941365720			

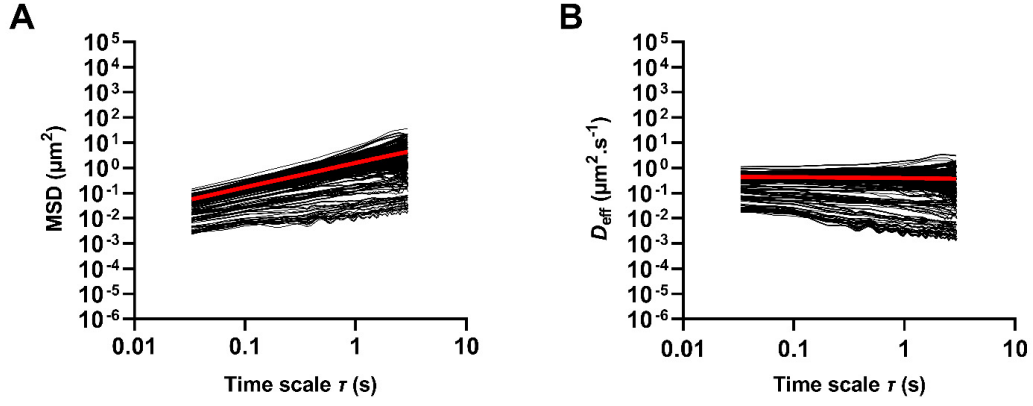
**Figure S8.** Example of a spreadsheet containing individual and ensemble data summary in the 'Transport Mode Characterization' .xlsx file.

	A	B	C	D	E	F	G	H	I	J
1	$D = \frac{k_B T}{6\pi \eta r}$						Timestamp	D <sub>eff</sub>	D <sub>eff</sub>	
2							(s)	(μm <sup>2</sup> ·s <sup>-1</sup> )	(m <sup>2</sup> ·s <sup>-1</sup> )	D <sub>W</sub> / D <sub>eff</sub>
3							0,99000	0,003471341	3,47134E-15	628,3169
4										
5	D <sub>W</sub> (m <sup>2</sup> s <sup>-1</sup> )	2,18110E-12	=>	D <sub>W</sub> (μm <sup>2</sup> s <sup>-1</sup> )	2,1811					
6	K <sub>B</sub> (m <sup>2</sup> kg s <sup>-2</sup> K <sup>-1</sup> )	13,80649E-24								
7	T (K)	298,15	<=	T (°C)	25					
8	Pi	3,1415927								
9	H <sub>2</sub> O viscosity (Pa.s)	0,0008900								
10	Radius (m)	0,0000001	<=	Diameter (nm)	225					
11	NOTE: Pa.s = kg m <sup>-1</sup> s <sup>-1</sup>									
12										
13	Input cells	These cells are used to input known values								
14	Info cells	Informative cells to help understand the formula								
15	Intermediate cells	These are cells used for the calculation. Must not be changed!								
16	Output cells	Desired results are in these cells.								
17										
18										
19										

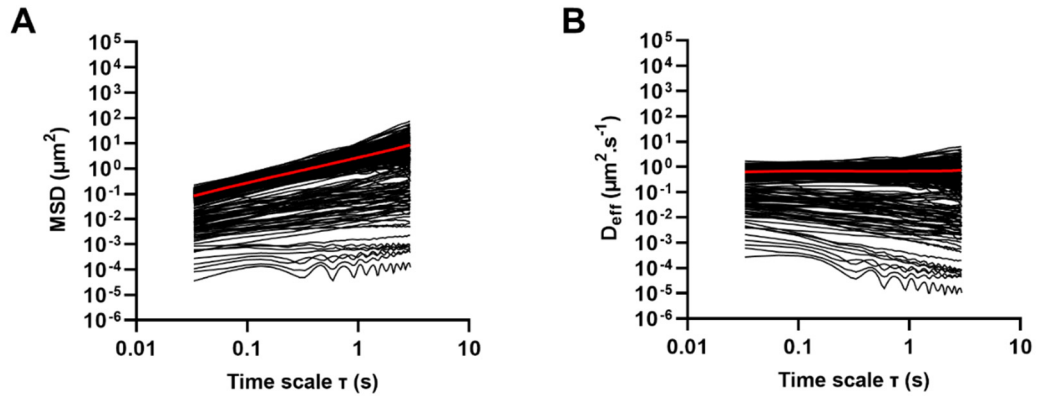
**Figure S9.** Example of generated 'Stokes-Einstein Calculations' .xlsx file spreadsheet generated after processing of input CSV files.



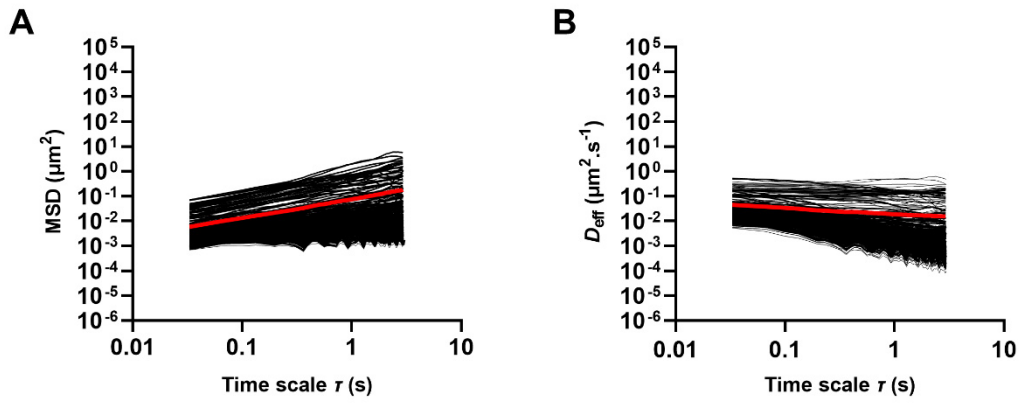
**Figure S10.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 200 nm COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in water as a function of time scale. Ensemble values are also presented in red in both graphs.



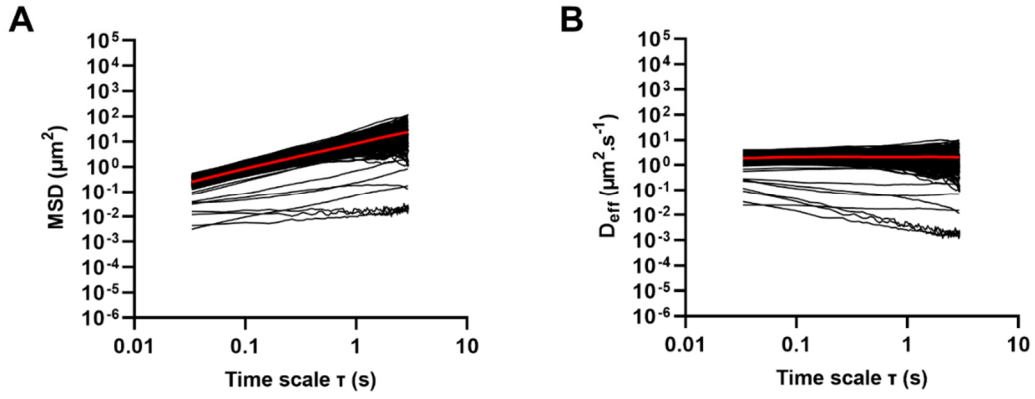
**Figure S11.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 200 nm COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in mucus surrogate containing 3% mucin as a function of time scale. Ensemble values are also presented in red in both graphs.



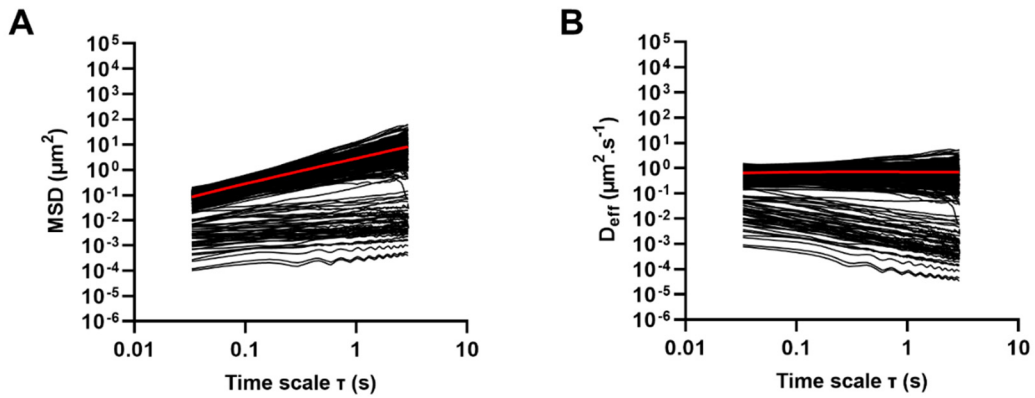
**Figure S12.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 100 nm COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in mucus surrogate containing 3% mucin as a function of time scale. Ensemble values are also presented in red in both graphs.



**Figure S13.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 100 nm COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in mucus surrogate containing 5% mucin as a function of time scale. Ensemble values are also presented in red in both graphs.



**Figure S14.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 100 nm PEG-modified COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in mucus surrogate containing 3% mucin as a function of time scale. Ensemble values are also presented in red in both graphs.



**Figure S15.** Individual (A) MSD and (B)  $D_{\text{eff}}$  of 100 nm PEG-modified COOH-PS NPs ( $\geq 100$  tracked particles per experiment;  $n = 3$ ) in mucus surrogate containing 5% mucin as a function of time scale. Ensemble values are also presented in red in both graphs.