Supplemental Materials

Molecular Biology of the Cell

Sakato-Antoku *et al*.

Supplementary Information for

Phyloproteomics Reveals Conserved Patterns of Axonemal Dynein Methylation Across the Motile Ciliated Eukaryotes

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A

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Chlamydomonas          LTTLSRACRHPALMRQLPPGQ---PACRLYVWEDSPEGLARHVLLAAVLLDGGVPAAQRG
Volvox                 LTTICRATRHTLLQQQQQP-------VHLVVWEDSPEGLARHVLLLAVLLDGSLLPRDRA
Crassostrea            LTTLARSYRYRKKK------------FHFYILENALELYARDMLFLSLSLETPKRMGLQE
Homo                   LRTLSRAKFWPRRR------------FNFFVLENNLEAVARHMLIFSLALEEPEKMGLQE
Mus                    LRTLARAALWPLRS------------FNFYVLENNLEAVARHMLIFSLALEEPEKMGLQE
Xenopus                LKTICQASRWPHRK------------LKFFIIESDLELLARHMLFLSLALEHPEQMGLQE
Strongylocentrotus     LTTIARAWRHRKRK------------LKFYITEGNLELYVRQLLLLHLLLEPPREMGLQE
Danio                  LKTITGLTHS--DT------------LHVWVIENSMEVIARQLLLLYISLLPPDKMSVHK
Batrachochytrium       IKTAGRAWKHSSRQ------------IHFHVIEPQSSLLARHMLLLSILFDPLDDIGIQV
Drosophila             IKTLAKRYTHRIRP-----------KLNIYLLDGCAEIEARNMLLLGVALEDPESFNLVS
Tetrahymena            LKTIADNCISNKDSQK----I---KKLNIYVYEKQKESFCRWLLLLQILQTTSLSFRERV
Thalassiosira          LKTLADAVIERGSLDDGADSYSQSQHLRFHIVEEHTEILARHFLLLHTFFDESVPIKQRS
Trypanosoma            FRTLSSLRVKGSLTSSGESVDP---TWHFYFYEPNLRVHARHLFFLKWLLDSTFSLDELE
Giardia                FANICDIVRAVKGGKQ----------CRVFLYERHAHLLCKLIVKLILLLLIGQGTDVNS
                       : .                        .. . :       : ..
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B

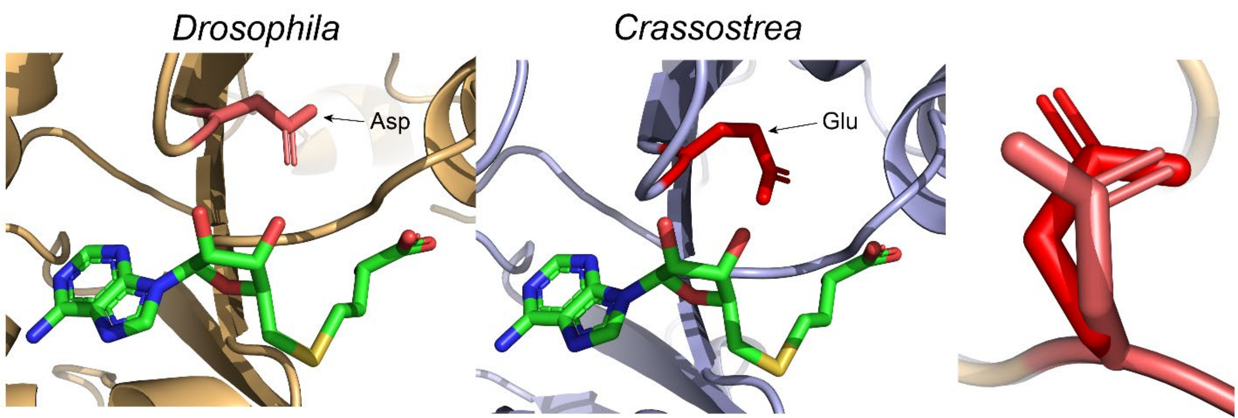

Figure S1 Comparison of the DNAAF3 Region Containing the Key Active Site Acidic Residue Across Motile Ciliated Eukaryotes

A) BLASTP alignment of the DNAAF3 regions from a broad array of motile ciliated eukaryotes containing the essential active site acid residue that coordinates the hydroxyls of SAM. Throughout eukaryotic phylogeny from *Homo* to *Giardia* this residue is a Glu (**green**), with the only identified exception being *Drosophila* in which it is replaced by Asp (**brown**). Intriguingly, no methylated dynein proteins were identified in this organism (see Table S2) raising the possibility that the E→D change leads to altered enzymatic properties. B) AlphaFold 3 structural models for the active sites of *Drosophila* (light orange) and *Crassostrea* (light blue) DNAAF3 orthologs. The key acidic residue side chains are shown – Asp (colored deep salmon) in *Drosophila* and Glu (colored red) in *Crassostrea*. The position of S-adenosylhomocysteine (colored by element: C, green; N, blue, O, red; S, yellow) was obtained by individually overlaying the DNAAF3 structural models with the crystal structure of the AprA methyltransferase (PDB 6D6Y) using the command *cealign* within PyMOL. An overlay showing the relative orientation of these acidic residues from the two DNAAF3 AlphaFold 3 models is shown at left.

Table S1 **Motile Ciliated Eukaryotes Examined in this Study**

Species	Description	Abbreviation Used
Ceratopteris richardii	Water fern (Tracheophyte)	$C_{e}r$
Chlamydomonas reinhardtii	Green alga (Chlorophyte)	Cr
Ciona intestinalis	Sea squirt (Ascidian)	Ci
Crassostrea gigas	Pacific oyster (Mollusk)	Cg
Drosophila melanogaster	Fruit fly (Dipteran)	Dm
Drosophila willistoni	Fruit fly (Dipteran)	Dw
Hemicentrotus pulcherrimus	Sea urchin (Echinoderm)	Hp
Mnemiopsis leidyi	Comb jelly (Ctenophore)	Ml
Oncorhynchus mykiss	Rainbow trout (Actinopterygian)	Om
Rattus norvegicus	Norway rat (Mammal)	Rn
Takifugu rubripes	Pufferfish (Actinopterygian)	Tr
Tetrahymena thermophila	Ciliate (Alveolate)	Tt
Trypanosoma brucei	Kinetoplastid (Excavate)	Tb

Table S2 **Numeric Data for Dynein Heavy Chain Abundance in *pf22* and CC-125 Cilia**

Dynein HC	Cre #	Dynein	*pf22* Cilia Total Spectra	*pf22* Cilia Sequence Coverage x/n residues (%)	CC-125 Cilia Total Spectra	CC-125 Cilia Sequence Coverage x/n residues (%)	Spectral Count Ratio *pf22*:WT
DHC1	Cre12.g484250	I1/f 1alpha	591	2863/4626 (62%)	840	3614/4626 (78%)	0.703
DHC2	Cre09.g392282	Dynein d	410	2151/3478 (62%)	609	2691/3478 (77%)	0.673
DHC3	Cre06.g265950	Minor dynein	38	397/5593 (7%)	243	2805/5593 (50%)	0.156
DHC4	Cre02.g107350	Minor dynein	59	650/4906 (13%)	364	3027/4906 (62%)	0.162
DHC5	Cre02.g107050	Dynein b	24	203/4185 (5%)	571	3036/4185 (73%)	0.042
DHC6	Cre05.g244250	Dynein a	199	1627/4006 (41%)	553	3061/4006 (76%)	0.359
DHC7	Cre14.g627576	Dynein g	486	2743/4191 (65%)	717	3281/4191 (78%)	0.677
DHC8	Cre16.g685450	Dynein e	20	194/4209 (5%)	692	3235/4209 (77%)	0.028
DHC9	Cre02.g141606	Dynein c	16	83/4152 (2%)	819	3377/4152 (81%)	0.019
DHC10	Cre14.g624950	I1/f 1beta	609	3139/4513 (70%)	873	3690/4513 (82%)	0.697
DHC11	Cre12.g555950	Minor dynein	23	228/4757 (5%)	345	2953/4757 (62%)	0.066
DHC12	Cre06.g297850	Minor dynein	156	1665/6150 (27%)	184	2270/6150 (37%)	0.847
DHC13	Cre03.g145127	OAD* alpha	236	1867/4503 (41%)	1622	3956/4503 (88%)	0.145
DHC14	Cre09.g403800	OAD beta	260	2051/4568 (45%)	1675	4043/4568 (89%)	0.155
DHC15	Cre11.g476050	OAD gamma	338	2271/4501 (50%)	1613	3942/4501 (88%)	0.209
DHC16	Cre06.g250300	IFT* dynein	767	3052/4333 (70%)	739	3445/4333 (80%)	1.037

* Abbreviations: IFT, intraflagellar transport; OAD, outer arm dynein

Table S3 **Mass Spectrometry Sequence Coverage and Abundance of Methylated Sites**

Organism	Source	Dynein Heavy Chain Designation‡	Sequence Coverage Residues x/n (%)	MS/MS Spectra Total	MS/MS Spectra Unique	Peptides Unique	Methylated Residues n (Residue number) Arginine Mono-Methyl	Arginine Di-Methyl	Lysine Mono-Methyl	Lysine Di-Methyl	Lysine Tri-Methyl
***Ceratopteris richardii*@@** (Water Fern)	Spermato-zoids Sample #1	Ceric_20G075500 mIAD (= Cr DHC8)	111/4153 (3%)	12	9	9	---	---	---	---	---
		Ceric_03G083100 mIAD (= Cr DHC6)	120/4178 (3%)	12	9	9	---	---	---	---	---
		Ceric_25G055400 IAD 1α (= Cr DHC1)	177/4556 (4%)	16	15	15	---	---	---	---	---
		Ceric_25G028800 mIAD (= Cr DHC2)	311/4052 (8%)	31	27	27	---	---	---	---	---
		Ceric_22G060000 IAD 1b (= Cr DHC10) partial	125/2814 (4%)	12	10	10	---	---	---	---	---
		Ceric_29G018300 mIAD (= Cr DHC7) partial	176/3375 (5%)	20	15	15	---	---	---	---	---
	Spermato-zoids Sample #2	Ceric_20G075500 mIAD (= Cr DHC8)	456/4153 (11%)	51	47	46	---	---	---	---	---
		Ceric_03G083100 mIAD (= Cr DHC6)	459/4178 (11%)	53	44	44	---	---	---	---	---
		Ceric_25G055400 IAD 1α (= Cr DHC1)	483/4556 (11%)	56	48	47	---	---	---	---	---
		Ceric_25G028800 mIAD (= Cr DHC2)	646/4052 (16%)	74	69	65	---	---	---	---	---

		Ceric_22G060000 IAD 1b (= Cr DHC10) partial	284/2814 (10%)	34	28	28	---	---	---	---	---
		Ceric_29G018300 mIAD (= Cr DHC7) partial	320/3375 (9%)	40	34	33	---	---	---	---	---
Chlamydomonas reinhardtii[##] (Chlorophyte Alga)	Cilia	DHC1 (IAD 1a)	3232/4626 (70%)	562	398	279	2 (R_{1069}, R_{1284})	1 (R_{666})	4 (K_{1682}, K_{2582}, K_{2951}, K_{3983})	---	1(K_{1371})
		DHC2 (mIAD)	2236/3478 (64%)	446	285	187	5 (R_{81}, R_{1541}, R_{1594}, R_{2090}, R_{2629})	1 (R_{87})	---	---	---
		DHC3 (minor mIAD)	409/5593 (7%)	38	24	24	---	---	---	---	---
		DHC4 (minor mIAD)	516/4906 (11%)	49	28	28	1 (R_{4510})	---	1 (K_{3552})	---	---
		DHC5 (mIAD)	2484/4185 (59%)	414	271	179	3 (R_{1495}, R_{3704}, R_{4140})	1 (R_{2753})	3 (K_{2106}, K_{2924}, K_{3001})	---	---
		DHC6 (mIAD)	2651/4006 (66%)	369	285	200	3 (R_{134}, R_{1664}, R_{3847})	---	2 (K_{2940}, K_{3148})	---	---
		DHC7 (mIAD)	2914/4191 (70%)	515	363	246	6 (R_{436}, R_{1383}, R_{2267}, R_{2327}, R_{3576}, R_{3769})	---	4 (K_{2089}, K_{2366}, K_{3721}, K_{4144})	---	---
		DHC8 (mIAD)	2714/4209 (64%)	434	307	217	6 (R_{1470}, R_{1507}, R_{1523}, R_{1883}, R_{4126}, R_{4164})	---	3 (K_{1104}, K_{1201}, K_{2967})	---	---
		DHC9 (mIAD)	2993/4152 (72%)	589	405	252	5 (R_{540}, R_{1374}, R_{3020}, R_{3028}, R_{4062})	---	6 (K_{1174}, K_{2789}, K_{3222}, K_{3639}, K_{3699}, K_{3773})	---	---

	DHC10 (IAD 1b)	3315/4513 (73%)	615	428	284	5 (R_{524}, R_{2193}, R_{2832}, R_{3694}, R_{3699})	---	5 (K_{1541}, K_{1709}, K_{1878}, K_{3283}, K_{4059})	1 (K_{2453})	2 (K_{2459}, K_{3193})
	DHC11 (minor mIAD)	497/4757 (10%)	44	29	28	1 (R_{4})	2 (R_{12}, R_{2303})	2 (K_{2291}, K_{4274})	---	1 (K_{3626})
	DHC12 (minor mIAD)	247/6150 (4%)	22	16	16	---	---	---	---	---
	DHC13 (OAD α)$^{++}$	3441/4503 (76%)	1131	553	311	23 (R_{18}, R_{38}, R_{647}, R_{791}, R_{1119}, R_{1168}, R_{1418},R_{1702}, R_{1777}, R_{2111}, R_{2500}, R_{3139}, R_{3654}, R_{3655}, R_{3678}, R_{3720}, R_{3756}, R_{3816}, R_{3878}, R_{4022}, R_{4029}, R_{4068}, R_{4426})	1 (R_{1945})	8 (K_{110}, K_{253}, K_{1436}, K_{1437}, K_{1791}, K_{2840}, K_{3044}, K_{3065})	1 (K_{3072})	3 (K_{3066}, K_{3439}, K_{3445})
	DHC14 (OAD β)$^{++}$	3771/4568 (83%)	1251	633	366	9 (R_{1621}, R_{1975}, R_{2332}, R_{2488}, R_{2710}, R_{2763}, R_{3202}, R_{3467}, R_{3650}, R_{4285})	1 (R_{3959})	13 (K_{296}, K_{527}, K_{756}, K_{1909}, K_{1989}, K_{2149}, K_{2208}, K_{2401}, K_{2491}, K_{2998}, K_{3223}, K_{3635}, K_{4114})	8 (K_{653}, K_{990}, K_{1319}, K_{2606}, K_{3169}, K_{3230}, K_{3296}, K_{3876})	3 (K_{2634}, K_{3224}, K_{3230})
	DHC15 (OAD γ)$^{++}$	3590/4501 (80%)	1250	621	358	7 (R_{213}, R_{1317}, R_{1373}, R_{1904}, R_{2713}, R_{3678}, R_{4424})	---	6 (K_{1533}, K_{1707}, K_{1744}, K_{2952}, K_{2997}, K_{4228})	1 (K_{292})	1 (K_{3079})

		DHC16 (IFT)	3078/4333 (71%)	615	426	291	3 (R_{1585}, R_{2164}, R_{2685})	---	4 (K_{1776}, K_{2006}, K_{2340}, K_{4231})	---	1 (K_{3438})
Ciona intestinalis (Sea Squirt)	Sperm Flagella	Ci_18292 DNAH8 (OAD = Cr γ)	2634/4239 (62%)	991	390	266	1 (R_{3366})	---	2 (K_{137}, K_{3025})	---	---
		Ci_11920 DNAH9 (OAD = Cr β) C-terminal region missing	2061/3522 (59%)	645	266	191	1 (R_{3445})	---	2 (K_{853}, K_{2099})	1 (K_{2102})	1 (K_{2099})
Crassostrea gigas (Pacific Oyster)	Sperm Flagella	XP_034301736 DNAH8 (OAD = Cr γ)	2933/4648 (63%)	950	391	283	2 (R_{893}, R_{3506})	---	1 (K_{995})	---	---
		XP_011428027 DNAH9 (OAD = Cr β)	2768/4465 (62%)	887	412	302	3 (R_{246}, R_{4020}, R_{4240})	---	1 (K_{4361})	---	---
		XP_034298943 DNAH10 (IAD = Cr 1α)	2596/4668 (56%)	522	328	260	---	---	1 (K_{2949})	---	---
		XP_034301924 DNAH7 (mIAD)	2122/4022 (53%)	477	n/a†	n/a	---	---	3 (K_{1572}, K_{2301}, K_{3397})	---	---
		XP_034330723 DNAH2 (IAD = Cr 1β)	2422/4554 (60%)	474	308	239	1 (R_{843})	---	---	---	---
		XP_034334098 DNAH1 (mIAD)	2212/4211 (52%)	433	278	212	2 (R_{132}, R_{1239})	---	2 (K_{792}, K_{2842}	---	---
		XP_034321313 DNAH6 (mIAD)	2148/4220 (51%)	394	n/a	n/a	---	---	---	---	---
		XP_034306561 DNAH12 (mIAD)	2014/4030 (50%)	352	n/a	n/a	---	---	---	---	---
		XP_011412296 DNAH3 (mIAD)	1850/4073 (45%)	343	211	168	1 (R_{2832})	---	2 (K_{18}, K_{2908})	---	---

Drosophila melanogaster[**] (Fruit Fly)	Seminal Vesicles Sample #1	Kl-3 (OAD = Cr γ)	1560/4593 (34%)	248	176	160	---	---	---	---	---
		Kl-5 (OAD = Cr β)	1986/4559 (44%)	339	211	185	---	---	---	---	---
		DHC98D (IAD = Cr 1α)	942/5080 (19%)	107	91	86	---	---	---	---	---
		Kl-2 (IAD = Cr 1β)	815/4459 (18%)	107	79	77	---	---	---	---	---
		DHC64C (cytoplasmic = Hs DYNC1H1)	850/4638 (18%)	83	74	73	---	---	---	---	---
		DNAH3 (mIAD = Cr DHC5) [††]	806/4385 (18%)	94	73	70	---	---	---	---	---
		DHC16F (mIAD = Cr DHC7) [††]	967/4981 (24%)	113	95	93	---	---	---	---	---
		DHC36C (mIAD = Cr DHC6) [††]	958/4024 (24%)	120	98	91	---	---	---	---	---
		DHC62B (mIAD = Cr DHC9) [††]	767/3964 (19%)	87	69	66	---	---	---	---	---
	Seminal Vesicles Sample #2	Kl-3 (OAD = Cr γ)	2019/4593 (46%)	434	278	210	---	---	---	---	---
		Kl-5 (OAD = Cr β)	2244/4559 (49%)	503	317	228	---	---	---	---	---
		DHC98D (IAD = Cr 1α)	1432/5080 (28%)	184	157	134	---	---	---	---	---
		Kl-2 (IAD = Cr 1β)	1113/4459 (25%)	146	125	110	---	---	---	---	---
		DHC64C (cytoplasmic = Hs DYNC1H1)	1592/4638 (34%)	182	n/a	138	---	---	---	---	---
		DNAH3 (mIAD = Cr DHC5) [††]	1514/4385 (35%)	213	169	141	---	---	---	---	---

		DHC16F (mIAD = Cr DHC7) ††	1428/4081 (35%)	205	167	132	---	---	---	---	---
		DHC36C (mIAD = Cr DHC6) ††	1683/4024 (42%)	298	215	159	---	---	---	---	---
		DHC62B (mIAD = Cr DHC9) ††	1332/3964 (34%)	188	148	120	---	----	---	---	---
Drosophila willistoni (Fruit Fly)	Seminal Vesicles	Kl-3 (OAD = Cr γ)	671/4594 (14%)	148	84	67	---	---	1 (K_{127})	---	---
		Kl-5 (OAD = Cr β)	2285/4562 (50%)	596	349	246	---	1 (R_{799})	1 (K_{3413})	---	---
		DNAH3	1586/4382 (36%)	253	180	153	---	---	---	---	---
		DNAH6	1498/4027 (37%)	225	163	142	---	---	---	---	---
		DNAH7	1375/4029 (34%)	226	159	129	---	---	---	---	---
		DNAH10	1122/4567 (24%)	161	120	106	---	---	---	---	---
		DNAH12	1456/3967 (37%)	204	153	204	---	---	---	---	---
		B4MMX1 (cytoplasmic = Hs DYNC1H1)	2061/4655 (44%)	311	4†	3†	---	---	---	---	---
				Total spectra	Unique spectra	Unique peptides					
Hemicentrotus pulcherrimus (Sea Urchin)	Sperm Flagella	HPU_11785 β HC (OAD = Cr β)	2465/4076 (60%)	805	369	251	4 (R_{394}, R_{3192}, R_{3714}, R_{3851})	---	5 (K_{389}, K_{443}, K_{1111}, K_{1341}, K_{2911})	---	---
		HPU_12543 DNAH7 (mIAD)	2352/3926 (60%)	564	302	206	1 (R_{805})	---	2 (K_{577}, K_{2664})	---	---

		HPU_07454[@] α HC DNAH8 (OAD = Cr γ)	1314/2408 (55%)	385	163	117	3 (R_{1087}, R_{1518}, R_{2014})	---	4 (K_{1085}, K_{1490}, K_{1541}, K_{2375})	---	---
		HPU_14295[@] α HC DNAH5 (OAD = Cr γ)	166/2265 (7%)	54	8	6	1 (R_{3167})	---	---	---	---
		HPU_06524 DNAH6 (mIAD)	2101/4236 (50%)	387	256	193	2 (R_{2337}, R_{2662})	---	2 (K_{852}, K_{1725})	---	---
		HPU_18186[+] DNAH12 (mIAD)	1911/3683 (52%)	369	233	173	---	1 (R_{2656})	2 (K_{270}, K_{1595})	---	---
		HPU_13585 DNAH2 (IAD = Cr 1β)	1818/3780 (48%)	362	220	167	---	---	2 (K_{2147}, K_{2996})	---	---
		HPU_17685 DNAH10 (IAD = Cr 1α) partial	1236/2469 (50%)	255	158	128	---	---	---	---	---
Mnemiopsis leidyi (Ctenophore)	Comb Plates	ML07114a (OAD = Cr γ)	1420/4505 (32%)	192	156	145	1 (R_{3172})	---	---	---	---
		ML002216a (OAD = Cr β) partial	1063/3176 (34%)	147	129	111	---	---	---	---	---
		ML053015a (mIAD = Cr DHC9?)	993/3999 (25%)	110	98	94	---	---	---	---	---
		ML23952a (mIAD = Cr DHC9?)	636/4054 (16%)	64	55	55	---	---	---	---	---
		ML329912a (mIAD = Cr DHC9?)	615/3846 (16%)	63	55	53	---	---	---	---	---
		ML011724a (mIAD = Cr DHC7) partial	139/900 (15%)	18	15	14	---	---	---	---	---
		ML34752a (mIAD = Cr DHC2) partial	33/1077 (3%)	3	3	3	---	---	---	---	---
		ML019112a (IFT) partial	22/2556 (1%)	2	2	2	---	---	---	---	---

		ML03391a (IAD = Cr 1α) partial	504/2564 (20%)	57	54	52	---	---	---	---	---
		ML14857a (IAD = Cr 1β) partial	430/2191 (20%)	48	41	41	---	---	---	---	---
Oncorhynchus mykiss (Rainbow Trout)	Sperm Axonemes	DNAH1 (mIAD)	1672/4208 (40%)	269	184	153	---	---	---	---	---
		DNAH2 (IAD = Cr 1β)	1221/3930 (31%)	172	125	109	1 (R_{3473})	1 (R_{3478})	1 (K_{1142})	---	---
		DNAH3 (mIAD)	1412/4063 (35%)	236	154	130	---	---	---	---	---
		DNAH6 (mIAD)	1094/3611 (30%)	195	110	95	---	---	---	---	---
		DNAH7 (mIAD)	1549/4013 (39%)	290	172	140	---	---	1 (K_{2785})	---	---
		DNAH8 (OAD = Cr γ)	2049/4362 (47%)	523	264	215	---	---	1 (K_{2872})	---	---
		DNAH10 (IAD = Cr 1α)	1607/4629 (35%)	249	179	160	---	---	---	---	---
		DNAH12 (mIAD)	1263/3964 (32%)	219	145	125	---	---	1 (K_{2731})	---	---
		DNAH14 (mIAD)	237/4450 (5%)	30	20	20	---	---	---	---	---
		DNAH17 (OAD = Cr β)	1993/4460 (45%)	605	286	227	1 (R_{3435})	---	---	---	---
Rattus norvegicus (Norway Rat)	Tracheal Cilia	DNAH1 (mIAD)	2526/4250 (59%)	516	338	231	---	---	---	---	---
		DNAH2 (IAD = Cr 1β)	2388/4508 (53%)	500	359	228	1 (R_{3432})	---	1 (K_{3157})	---	---
		DNAH3	1910/4069	366	245	169	---	---	1 (K_{150})	---	---

		(mIAD)	(47%)								
		DNAH5 (OAD = Cr γ)	2798/4621 (61%)	975	512	293	1 (R_{2873})	---	1 (K_{2856})	---	---
		DNAH6 (mIAD)	2311/4144 (56%)	549	356	231	2 (R_{2166}, R_{2172})	---	---	---	---
		DNAH7 (mIAD)	2677/4023 (67%)	700	419	243	---	---	2 (K_{1665}, K_{2904})	---	---
		DNAH9 (OAD = Cr β)	2850/4410 (65%)	972	521	295	1 (R_{3865})	---	5 (K_{1335}, K_{2868}, K_{3142}, K_{3539}, K_{3833})	---	---
		DNAH10 (IAD = Cr 1α)	2550/4592 (56%)	594	393	262	---	---	---	---	---
		DNAH11 (OAD = Cr β)	2748/4487 (61%)	702	410	269	1 (R_{3172})	---	3 (K_{677}, K_{1682}, K_{3180})	---	---
		DNAH12 (mIAD)	2086/3960 (53%)	407	279	196	---	---	2 (K_{2595}, K_{2804})	---	---
		DNAH17 (OAD = Cr β)	240/4459 (5%)	84	1	1	---	---	---	---	---
		DYNC2H1 (IFT)	2550/4306 (59%)	434	320	241	1 (R_{1306})	---	---	---	---
		DYNC1H1 (Cytoplasmic)	2441/4646 (53%)	407	328	236	---	---	---	---	---
Takifugu rubripes (Pufferfish) ‡‡	Sperm Axonemes Sample #1	Tr_188439 DNAH3 (mIAD)	1991/4013 (50%)	841	303	222	---	1 (R_{3181})	1 (K_{965})	---	---
		Tr_742129 DNAH10 (IAD = Cr 1α)	1349/4228 (32%)	218	107	91	---	---	---	---	---
		Tr_728981 DNAH12 (mIAD)	1246/3581 (35%)	203	140	120	---	---	---	---	---

		Tr_732194 DNAH2 (IAD = Cr 1β)	1115/4685 (24%)	159	125	114	---	---	---	---	---
	Sperm Axonemes Sample #2	Tr_188439 DNAH3 (mIAD)	3023/4013 (75%)	2031	752	343	5 (R_{318}, R_{607}, R_{1801}, R_{1996}, R_{2920})	1 (R_{3181})	14 (K_{965}, K_{1264}, K_{1360}, K_{1647}, K_{1985}, K_{2025}, K_{2300}, K_{2516}, K_{2696}, K_{2853}, K_{2921}, K_{3004}, K_{3680}, K_{3969})	1 (K_{3553})	---
		Tr_742129 DNAH10 (IAD = Cr 1α)	2271/4228 (54%)	576	287	167	2 (R_{1366}, R_{3166})	---	6 (K_{336}, K_{1195}, K_{1356}, K_{1398}, K_{1891}, K_{2804})	---	---
		Tr_728981 DNAH12 (mIAD)	2076/3581 (58%)	540	365	203	---	---	1 (K_{2025})	---	---
		Tr_732194 DNAH2 (IAD = Cr 1β)	2269/4685 (48%)	575	410	234	2 (R_{865}, R_{1940})	---	7 (K_{317}, K_{1207}, K_{1656}, K_{2608}, K_{3002}, K_{3298}, K_{3732})	---	---
Tetrahymena thermophila (Alveolate/Ciliate)	Purified Dynein[$]	TTHERM_01276420 DYH3 (OAD α = Cr γ)	3701/4620 (80%)	1371	620	412	1 (R_{585})	2 (R_{585}, R_{2818})	1 (K_{2269})	2 (K_{311}, K_{1629})	---
		TTHERM_00499300 DYH4 (OAD β = Cr β)	3384/4595 (74%)	1284	562	380	4 (R_{960}, R_{1577}, R_{3075}, R_{4026})	---	4 (K_{749}, K_{1020}, K_{1032}, K_{3198})	3 (K_{1020}, K_{2216}, K_{3962})	---
		TTHERM_00486600 DYH5 (OAD γ = Cr α)	3075/4168 (74%)	1112	479	314	2 (R_{1205}, R_{2909})	1 (R_{1205})	2 (K_{437}, K_{2016})	1 (K_{963})	---
		TTHERM_00688470 DYH6 (IAD = Cr 1α)	3037/4383 (69%)	704	420	313	---	---	---	---	---

		TTHERM_00912290 DYH7 (IAD = Cr 1β)	3066/4805 (64%)	698	434	309	---	---	2 (K_{3253}, K_{3550})	---	---
		TTHERM_001151438 DYH21 (mIAD)*	1815/3911 (46%)	216	159	135	---	---	---	---	---
		TTHERM_00252430 DYH11 (mIAD)	997/4257 (23%)	101	77	72	---	---	---	---	---
		TTHERM_00193520 DYH24 (mIAD)	1036/4126 (25%)	108	85	74	---	---	---	1 (K_{3289})	---
		TTHERM_00774810 DYH25 (mIAD; not full-length)	958/2425 (40%)	104	87	73	---	---	---	---	1 ($K_{????}$)#
		TTHERM_00565600 DYH22 (mIAD)	506/4564 (11%)	58	36	35	---	---	---	---	---
Trypanosoma brucei (Excavate/Kineto-plastid)	Flagella	Tb927.4.560 (IFT = Cr DHC16)	25/4232 (1%)	2	2	2	1 (R_{2779})	---	---	---	1 (K_{2782})
		Tb927.11.3250 (OAD = Cr β)	3747/4658 (80%)	1461	791	412	14 (R_{499}, R_{908}, R_{943}, R_{959}, R_{997}, R_{1017}, R_{1774}, R_{1861}, R_{2040}, R_{2780}, R_{2877}, R_{3532}, R_{4431}, R_{4589})	---	7 (K_{81}, K_{223}, K_{394}, K_{1374}, K_{1550}, K_{2165}, K_{2239})	2 (K_{1041}, K_{4451})	1 (K_{1580})
		Tb927.3.930 (OAD = Cr γ)	3595/4639 (77%)	1498	811	410	3 (R_{859}, R_{2803}, R_{3766})	1 (R_{281})	6 (K_{379}, K_{416}, K_{884}, K_{977}, K_{1855}, K_{2460})	1 (K_{3223})	---
		Tb927.8.3250 (IAD = Cr 1β)	3289/4674 (70%)	707	527	317	2 (R_{143}, R_{329})	---	2 (K_{37}, K_{2192})	---	---
		Tb927.4.870 (IAD = Cr 1α)	3384/4599 (74%)	756	561	322	2 (R_{1696}, R_{3942})	---	1 (K_{1679})	---	---

	Tb927.2.5270 (mIAD = Cr DHC9)	3241/4246 (76%)	700	542	309	1 (R_{2506})	---	1 (K_{240})	---	---
	Tb927.11.11220 (mIAD = Cr DHC7)	3096/4242 (73%)	712	503	277	4 (R_{623}, R_{698}, R_{1442}, R_{2250})	---	4 (K_{1521}, K_{2780}, K_{2938}, K_{3965})	---	---
	Tb11.10.5350 (mIAD = Cr DHC9)	3033/4142 (73%)	623	480	293	1 (R_{2775})	---	2 (K_{2092}, K_{4127})	---	---
	Tb927.11.8160 (mIAD = Cr DHC9/DHC2)	2935/4152 (71%)	620	458	269	---	---	2 (K_{3143}, K_{4107})	---	---
	Tb927.7.920 (mIAD = Cr DHC7/ DHC9)	2922/4112 (71%)	641	472	258	3 (R_{700}, R_{3276}, R_{3799})	---	1 (K_{2735})	---	---

[$] Abbreviations: IAD, inner arm dynein I1/f; IFT, intraflagellar transport; mIAD, monomeric inner arm dynein; OAD, outer arm dynein.

[‡] The equivalence of various HCs to those of *Chlamydomonas reinhardtii* (Cr) and/or *Homo sapiens* (Hs) is indicated where orthology is clear *e.g*. (OAD α = Cr γ).

[*] This *Tetrahymena* axonemal inner arm dynein heavy chain (DYH21; (Wilkes et al., 2008)) has been misannotated in many sequence database entries as DYH1 which in *Tetrahymena* is the heavy chain of canonical cytoplasmic dynein.

[#] The available sequence for *Tetrahymena* DYH25 is not full length and starts just before the Walker A P-loop of AAA2. The K_{me3} residue identified is 345 residues C-terminal of the Walker A P-loop in AAA4 within the tryptic peptide (K)PQIDNLK$_{me3}$TMK.

[@] Both available *Hemicentrotus* α heavy chain isoform sequences (DNAH5 and DNAH8) are incomplete. DNAH8 is truncated at the N-terminus and terminates in AAA4 while the DNAH5 sequence starts after the Walker A P-loop of AAA1. All methylated basic residues identified in *Hemicentrotus* are conserved in the sea urchin *Strongylocentrotus purpuratus* and therefore the residue numbers from the equivalent heavy chains of that organism are shown.

[+] The available *Hemicentrotus* sequence for DNAH12 is missing ~150 residues including the Walker A and B motifs of AAA1.

[†] Not available as the Scaffold viewer confuses the number of unique spectra/peptides due to two very closely related isoforms.

[††] Assignment of *Drosophila* monomeric inner arm dynein heavy chains to their *Chlamydomonas* orthologs is from (Zur Lage et al., 2019).

** Although no methylated sites were identified in *Drosophila* dynein heavy chains, methylation was readily found on other proteins present in the same gel band including, for example, two trimethylated myosin heavy chain peptides.

@@ Mono-, di-, and tri-methylation was identified on several non-dynein *Ceratopteris* proteins including a phospholipid-translocating ATPase, a carbohydrate-binding protein, and a potassium transporter.

Chlamydomonas data are from (King et al., 2024; Sakato-Antoku et al., 2024). The indicated sequence coverage derives from tryptic digests and does not include overlapping coverage from endoproteinase Asp-N digests.

‡‡ No outer arm dynein heavy chain-derived peptides were identified in two independent samples of *Takifugu* sperm flagella axonemes.

++ Outer arm dynein heavy chain residues identified as methylated in axoneme-derived samples but absent in one or more detergent-soluble ciliary membrane plus matrix samples are highlighted in yellow.

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