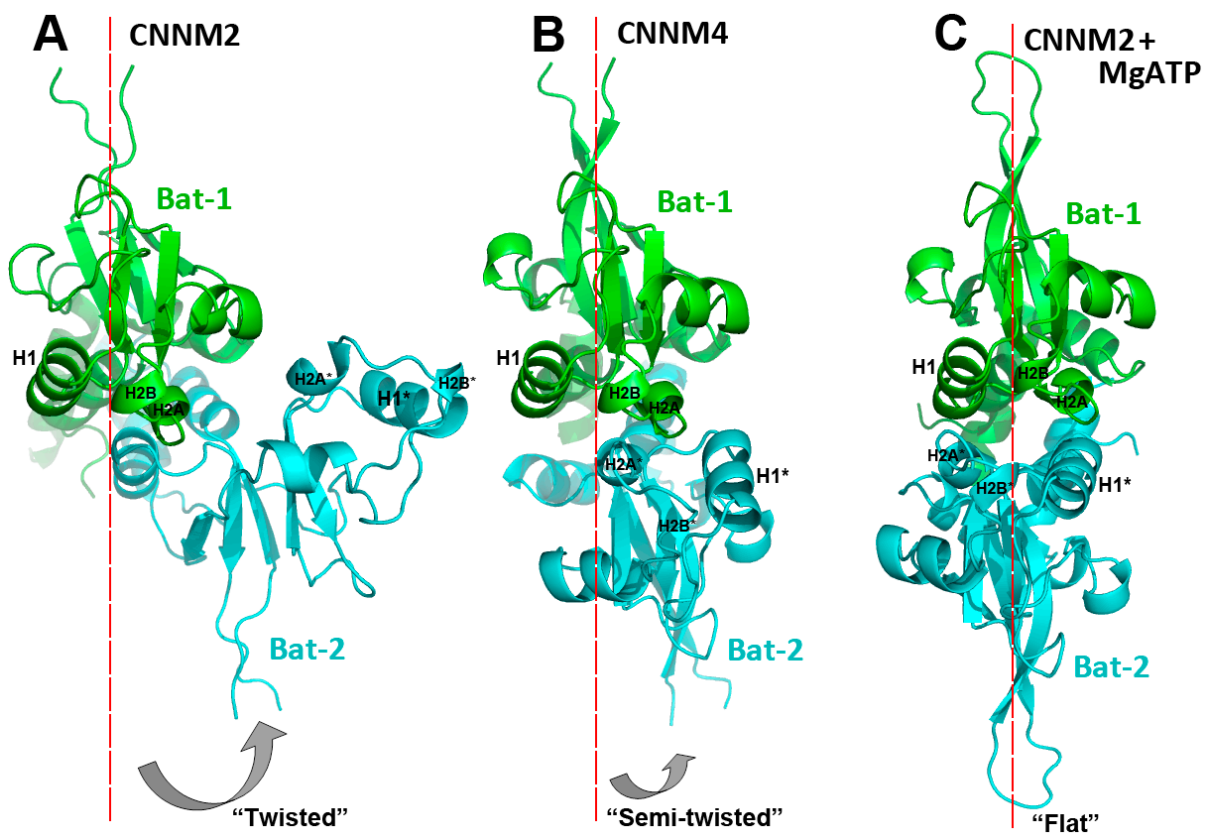


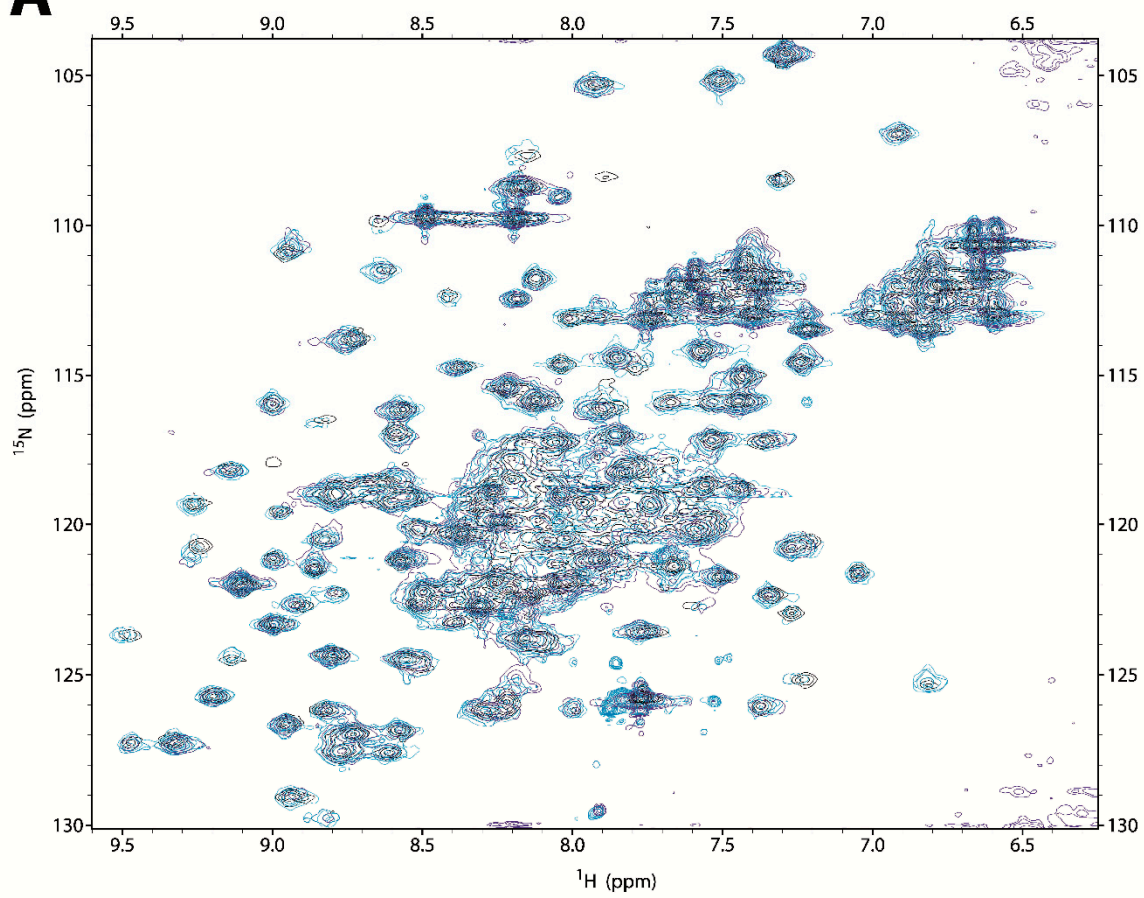
Supplementary Information

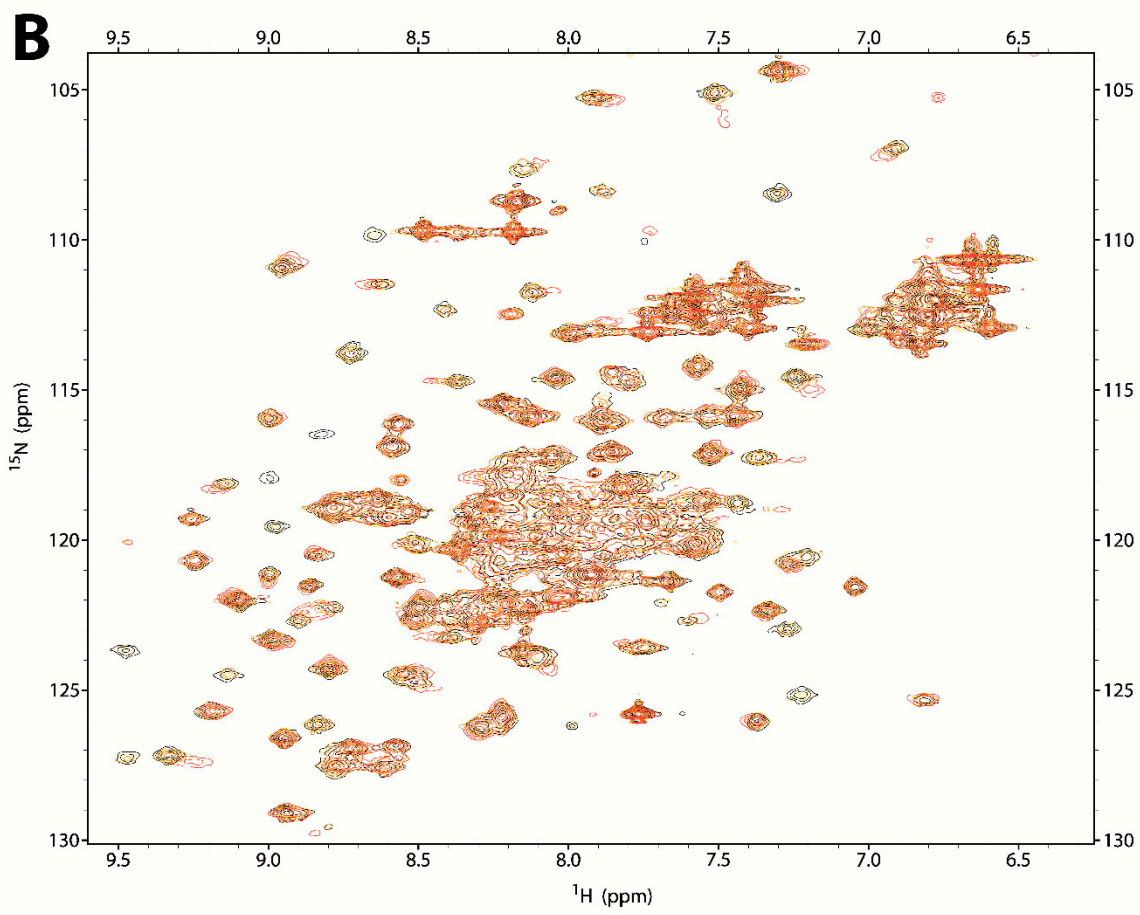
	HA2	β2	β6	H4	
CNNM1	432 V L T P L G D C F M 441	459 S G Y T R I P 465	549 G I V T L E D I I E E 559		
CNNM2	449 V M T P L R D C F M 458	476 S G Y T R I P 482	565 G I V T L E D V I E E 575		
CNNM3	317 V L T P L E D C F M 326	344 S G H T R I P 370	433 G L V T L E D V I E E 443		
CNNM4	376 I M T Q L Q D C F M 385	403 S G Y T R I P 409	492 G L V T L E D V I E E 502		
SA0657	221 I M V P R T Q M I T 230	247 H Q F T R Y P 253	326 G I L T M E D I L E E 336		
CorB	192 I M V P R N E I I G 201	218 S P H G R I V 224	299 G L V T V E D I L E E 309		
CorC	72 I M I P R S Q M I T 81	98 S A H S R F P 104	177 G L V T I E D I L E L 187		

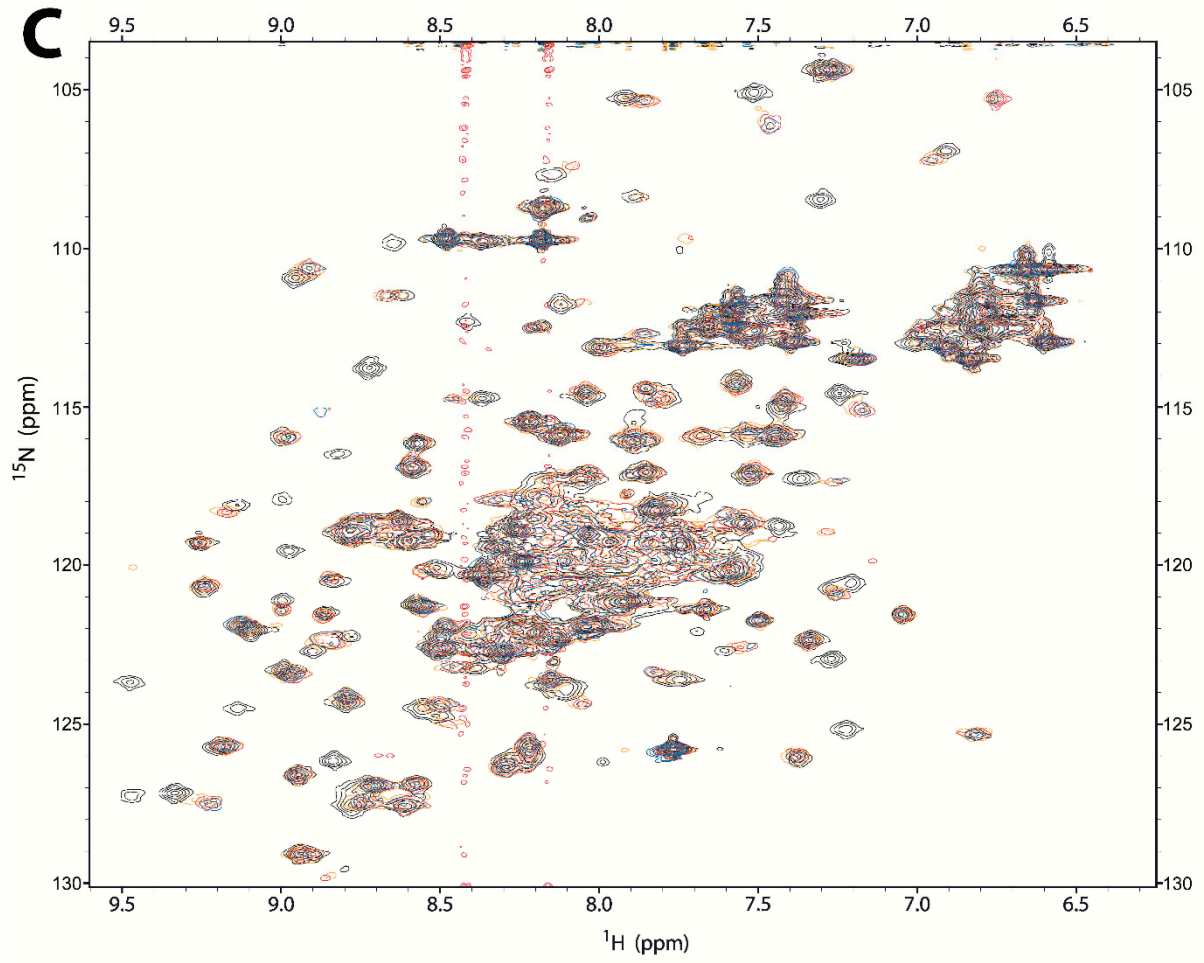
Supplementary Figure S1. Sequence alignment of the three structural blocks that conforms site S2 in the CNNMs and in bacterial proteins SA0657, CorB and CorC (Uniprot codes A0A0H3JL60, A0A0H3NER8 and P0A2L3 respectively). The conserved acidic cluster (which prevents binding of ATP in the absence of Mg²⁺ in CNNMs [5]) is highlighted with a red rectangle. Secondary structure elements are represented above the alignment.

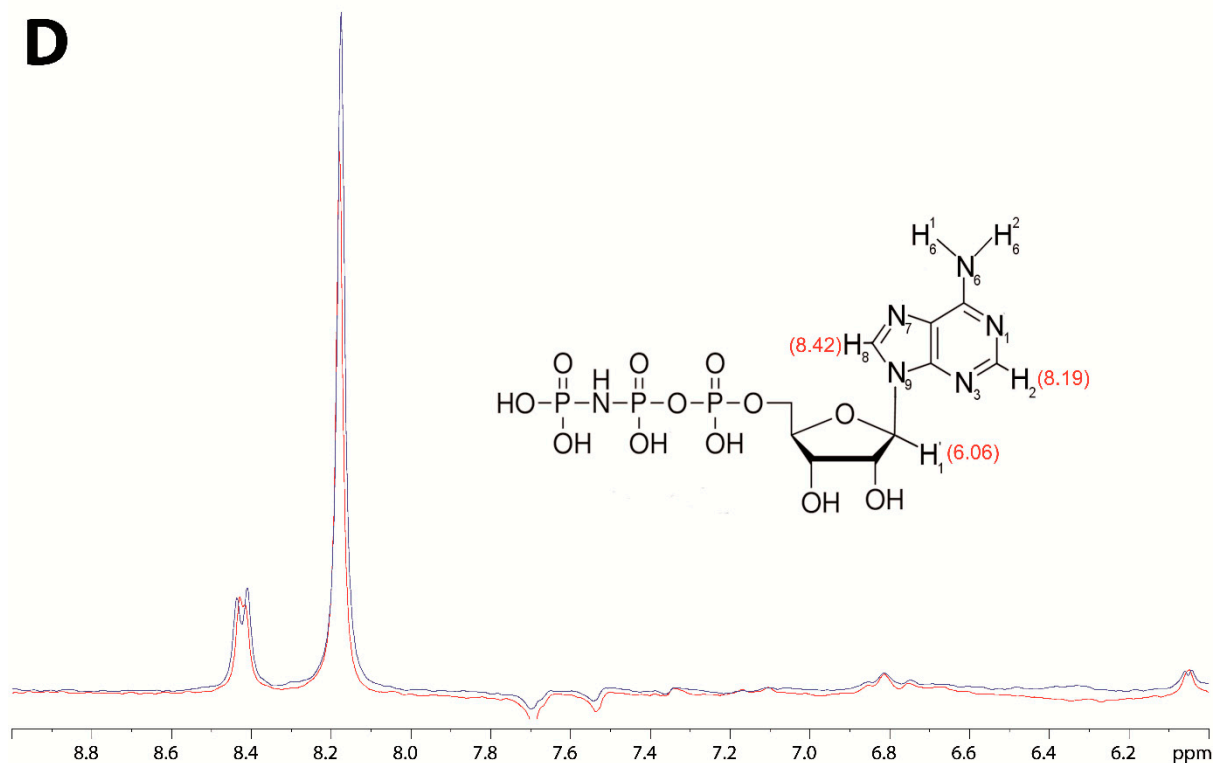


Supplementary Figure S2. Structure of the CBS module in CNNMs in three distinct conformations. The two Bateman modules are shown in *cyan* and *green*. CNNM2 adopts two limiting conformations: (A) a *twisted* disk in the absence of MgATP, and (C) a *flat* disk in the presence of bound MgATP. The plane intersecting the central β-sheets of one CBS module is represented by a *red* line to emphasise the *flat* to *twisted* conformational transition. (B) In the crystals, CNNM4 forms a *semi-twisted* disk in the absence of MgATP as an intermediate between conformations (A) and (C)

A

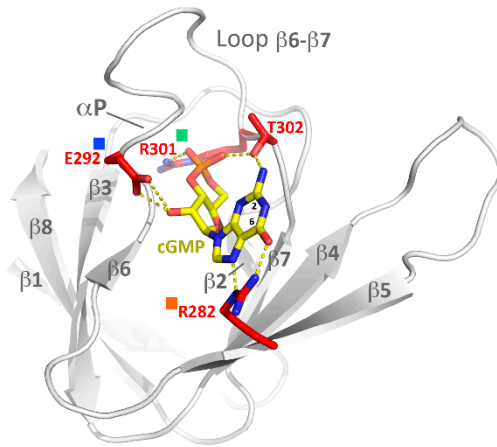




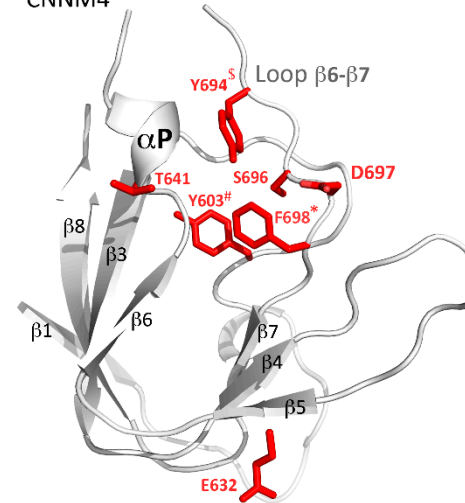


Supplementary Figure S3. (A) Ligand binding by hCNNM4_{BAT}. (A – C) Observation via 2D ^{15}N HSQC NMR spectra of the protein (100 μM). The complete spectral region is shown (corresponding zoom regions in Figure 2). **(A)** Effect of Mg^{2+} addition to a final concentration of 0 mM (*black*), 10 mM (*light blue*), 20 mM (*blue*), and 40 mM (*dark blue*). **(B)** Effect of ADPNP addition to a final concentration of 0 mM (*black*), 570 μM (*yellow*), 1.14 mM (*orange*), and 5.7 mM (*red*). **(C)** Effect of combined Mg^{2+} and ADPNP addition: 0 mM Mg^{2+} plus 0 mM ADPNP (*black*), 0 mM Mg^{2+} plus 5.7 mM ADPNP (*orange*), 0 mM Mg^{2+} plus 18 mM ADPNP (*red*), and 10 mM Mg^{2+} plus 5.7 mM ADPNP (*blue*). Spurious t_1 noise at ^1H frequencies of ca. 8.2 ppm and 8.4 ppm derive from protons H_2 and H_8 in the purine ring of ADPNP. **(D)** Observation via 1D $^1\text{H}_{\text{protein}} \rightarrow ^1\text{H}_{\text{ligand}}$ Saturation Transfer Difference (STD) spectra of the ligand ADPNP (5.7 mM; 300 μM hCNNM4_{BAT}). The superposed STD spectra in the absence (*red*) and presence (*black*) of Mg^{2+} (10 mM MgCl_2) confirm ADPNP binding in both cases, where the stronger STD signals in the presence of Mg^{2+} indicate positive cooperativity of Mg^{2+} and ADPNP binding. The molecular structure and assigned ^1H signals of ADPNP are shown in the inset.

hPKG1-beta (4Z07)



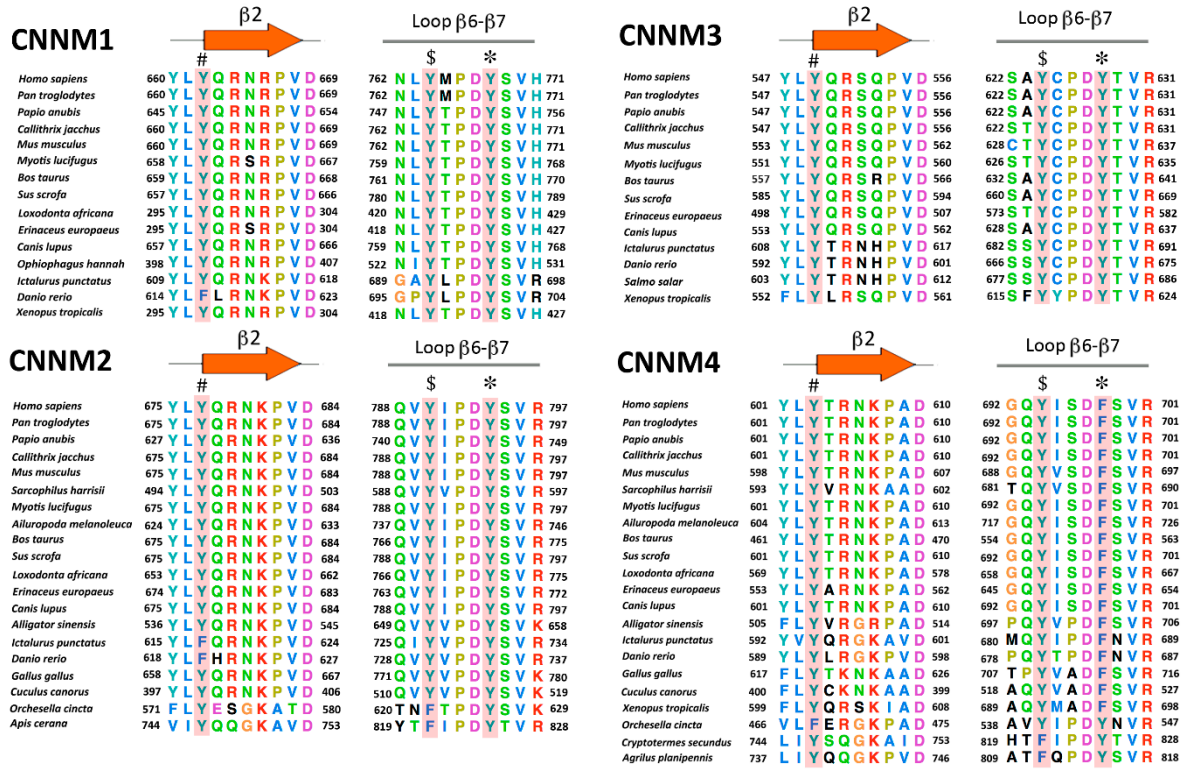
CNNM4



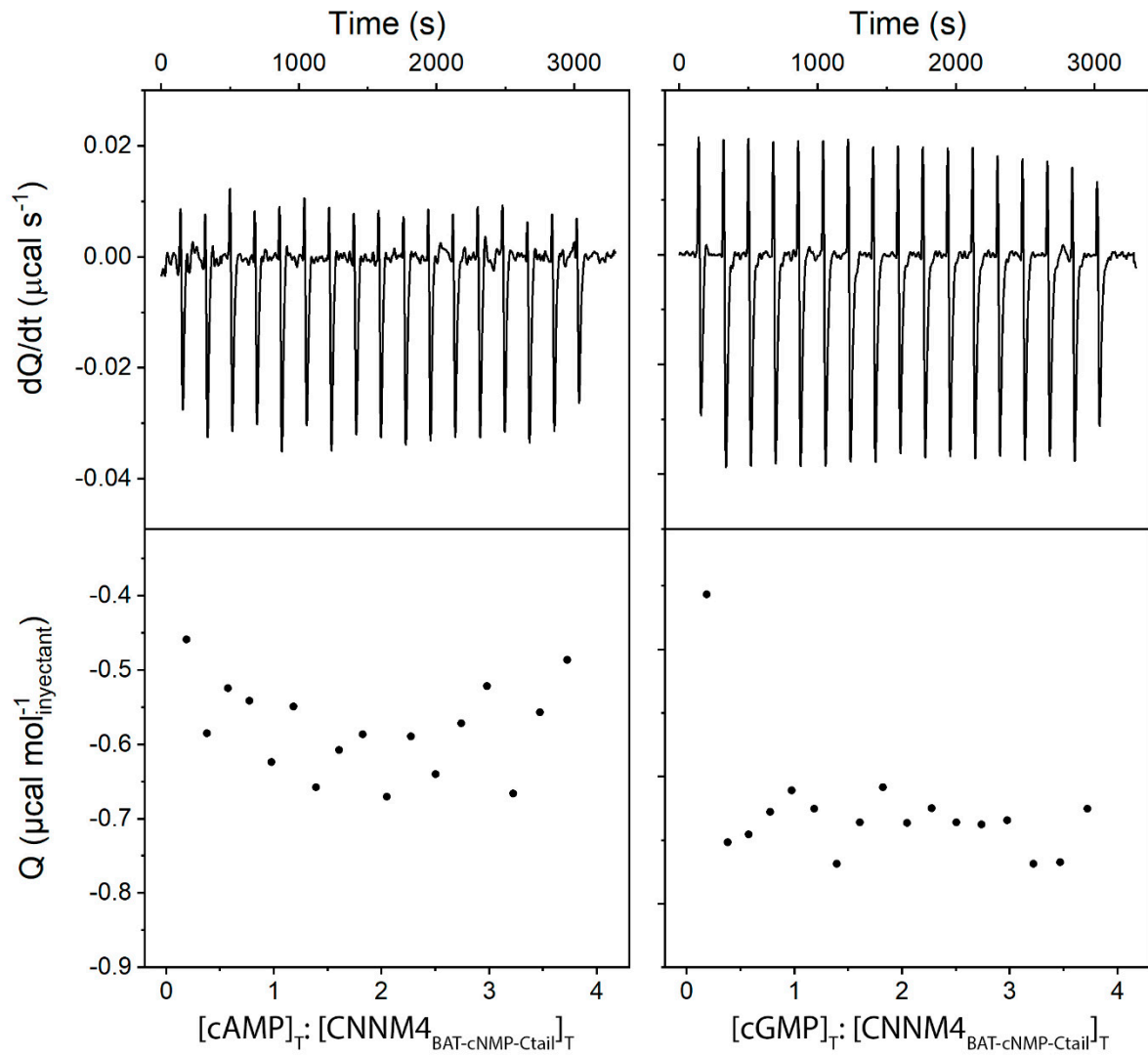
	β2		β5		β6		αP	Loop β6-β7								
	#							\$	*							
- hCNNM4	601	Y L V Y T A	605	628	N M K T F F G	634	636	F S Y Y	639	640	G T M A L T S	646	694	Y I S D F S	V	700
- mKCNH1	607	L I Y Y H A	611	633	E V V A I L G	639	641	G D V F	644	645	G D V F W K L	651	655	A D S C A N	V	661
- hKCNH1	607	L I Y Y H A	611	633	E V V A I L G	639	641	G D V F	644	645	G D V F W K L	651	655	A D S C A N	V	661
- hKCNH2	768	L V V H A	772	794	V V V A I L G	800	802	N D I F	805	806	G L P L N L Y	812	817	K T S N G D	V	822
- hKCNH3	608	Y L I H Q	612	634	T V L A I L G	640	642	G D L I	645	646	G C E L P R R	652	657	K A N A D	V	662
- hKCNH4	582	Y L L R R	586	608	M V L A I L G	614	616	G D L I	619	620	G A D I P L P	626	640	I K T S A D	V	646
- hKCNH5	576	L I Y H A	580	602	E V V A I L G	608	610	G D V F	613	614	G D I F W K E	620	624	A H A C A N	V	630
- hKCNH6	619	L V H L	623	646	V V V A I L G	652	654	N D I F	657	658	G E P V S L H	664	669	K S S A D	V	674
- hKCNH7	771	L V H C	775	797	I V V A I L G	803	805	N D I F	808	809	G E M V H L Y	815	820	K S N A D	V	825
- hKCNH8	577	L L R Q	581	603	M V L A I L G	609	611	G D L I	614	615	G A N L S I K	621	626	K T N A D	V	631
cAMP hHCN1	501	Y I I K F	505	527	S K F M K I I	533	535	G S Y F	538	539	G I I C L L T	545	548	R R T A S	V	553
cAMP hHCN2	569	Y I I K F	573	596	N K E M K L S	602	604	G S Y F	607	608	G E I C L L T	614	617	R R T A S	V	622
cAMP hHCN3	454	L V V R E	458	480	A R D T R L T	486	488	C S Y F	491	492	G E I C L L T	498	501	R R T A S	V	506
cAMP hHCN4	621	Y I I K F	625	647	N K I K I A	653	655	G S Y F	658	659	G I I C L L I	665	668	R R T A S	V	673
cAMP hCNGA1	505	Y I C R K	509	533	T Q F V V L S	539	541	G S Y F	544	545	G E I S I L N	551	559	N R R T A N	V	565
cAMP hCNGA2	480	Y I C R K	484	508	T Q Y A L L S	514	516	C S Y F	519	520	G E I S I L N	526	534	N R R T A N	V	540
cAMP hCNGA3	508	Y I C R K	512	536	T Q F V V L S	542	544	C S Y F	547	548	G L I S I L N	554	562	N R R T A N	V	568
cAMP hCNGA4	374	Y V C R K	378	402	T Q Y A V I G	408	410	G L Y F	413	414	G E I S I I N	420	428	N R R T A N	V	434
cAMP hCNGB1	988	Y V C R K	992	1017	S V L V L Q	1023	1025	C S V F	1028	1029	G E I S L L A	1035	1039	N R R T A N	V	1045
cAMP hCNGB3	550	F V C R K	554	579	K V L V L T K	585	587	G S V F	590	591	G E I S L L A	597	602	N R R T A N	V	608
cAMP hPKAI	281	K I V V Q	285	313	V F V G R I G	319	321	S D Y F	324	325	G I I A L L M	331	333	R P R A T V	V	339
cAMP hPKAII	302	Q I I A Q	306	339	V E I A R C S	345	347	G Q Y F	350	351	G E L A L V T	357	359	K P R A A S	V	365
cAMP hPKGI	247	Y I I R Q	251	279	V F L R T L G	285	287	G D W F	290	291	G E K A L Q G	297	299	D V H R T A N	V	305
cAMP hPKGII	312	Y I I R P	316	344	G I I K T L G	350	352	G E Y F	355	356	G L N A L I S	362	364	D V R T A N	V	370
cAMP hRAPGEF2	162	I V L H D	166	188	G K A E I L G	194	196	G N S F	199	200	G V S P L M D	206	209	Y M K G V M R	V	215
cAMP hRAPGEF4	383	V L F R D	387	410	G V V C L L H	416	418	G D D F	421	422	G K L A L V N	428	431	P H A S I	V	436
cAMP hRAPGEF6	307	I I L L D	311	333	C K V E N L F	339	341	C N S F	344	345	G I T P T L D	351	354	Y M H G I V R	V	360
hSLC9C1	896	H I F E E	900	929	M V F S C R G	935	949	G I I I	952	952	G I I N C L L	958	960	E P M K Y S A	V	966
hSLC9C2	893	T I C A G	897	926	N Q R C D F G	932	944	G D I I	947	948	G E L S C L I	954	956	R L I E Y Y S	V	962
hPLP6_CN81	212	Y F A A P	216	243	C Y V X E V V	249	251	C D S V	254	255	N S L L S I L	261	270	P Q R T V T V	V	276
hPLP6_CN82	529	I I A V Q	533	561	V C I I V A Q	567	569	C I I V	572	573	G Q I A V L T	579	581	E P L I F I L	V	587
hPLP6_CN83	646	A L R G Q	650	677	E L V G E Y G	683	685	C D S L	688	689	G V V L A L I	695	697	Q P R A L T V	V	703
hPLP7_CN81	171	H V F R P	175	201	V V V X E V L	207	210	C D S V	213	214	H S L L S I L	220	229	P Y K T V S V	V	235
hPLP7_CN82	485	V S A R D	489	517	C L T L L A	523	525	C L M V	528	529	G Q L A V L T	535	537	E P L I F T V	V	543
hPLP7_CN83	602	A I Y A Q	606	633	R I A G E Y G	639	641	G D L V	644	645	G V V E T L T	651	653	D A R A T V	V	659

Supplementary Figure S4: Comparison of CNNM4_{cnMP} with further CNBD or CNBDH domains. (Top)

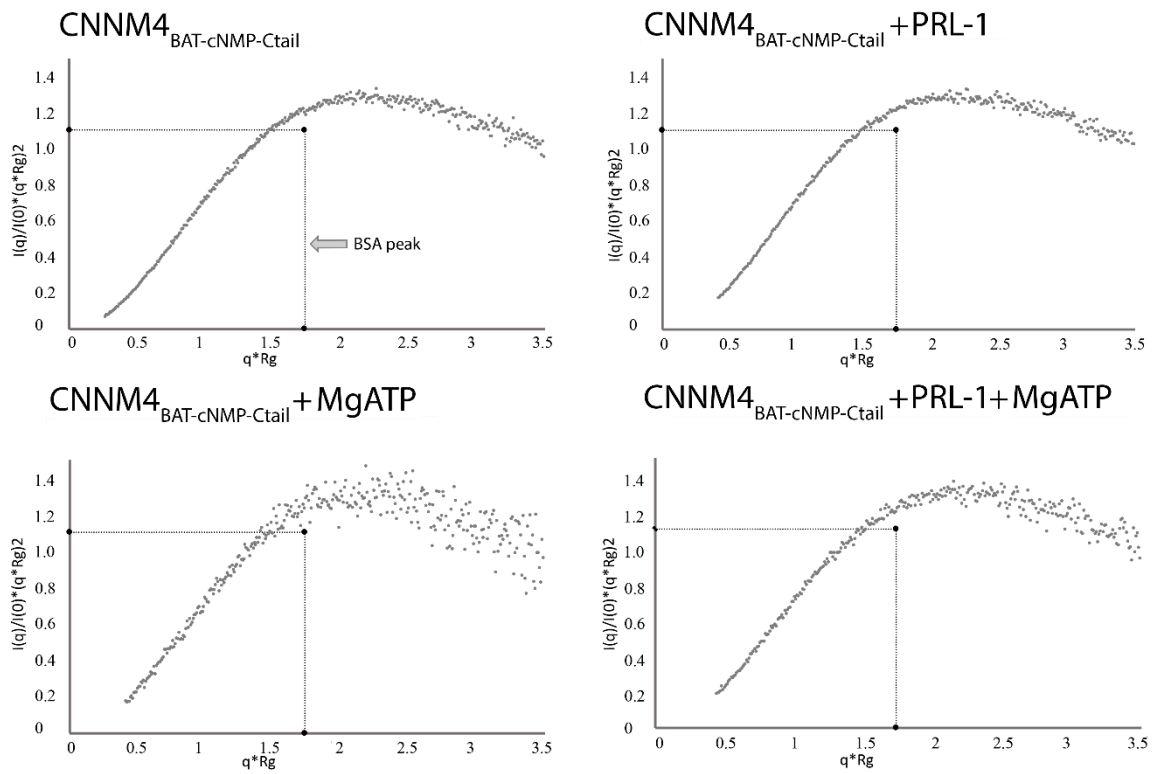
Ribbon and stick representation of the β -roll in the CNB domain of PKG-1 (left; PDB ID 4Z07) and CNNM4_{cnMP} (right; PDB ID 6G52). Residues involved in cGMP (yellow) interaction are highlighted in red. CNNM4_{cnMP} neither conserves these residues nor provides for sufficient space to host a cNMP ligand since the cavity is occupied by bulky residues. CNNM4 residues T641, S696, D697, and E632 are located at positions equivalent to PKG-1 residues E292, R301, T302, and R282, respectively (Bottom). Sequence alignment of hCNNM4 vs human CNBD and CNBDH containing proteins. The location of key residues involved in cNMP binding and their analogs in CNNM4_{cnMP} are highlighted by colored symbols above the alignment (below the secondary structure elements). The known cNMP ligands are indicated in the first column, where known absence of cNMP binding is marked by (-). The Uniprot codes for the analyzed proteins are: hCNNM4, Q6P4Q7; MmKCNH1, Q60603; hKCNH1, O95259; hKCNH2, Q12809; hKCNH3, Q9ULD8; hKCNH4, Q9UQ05; hKCNH5, Q8NCM2; hKCNH6, Q9H252; hKCNH7, Q9NS40; hKCNH8, Q96L42; hHCN1, O60741; hHCN2, Q9UL51; hHCN3, Q9P1Z3; hHCN4, Q9Y3Q4; hCNGA1, P29973; hCNGA2, Q16280; hCNGA3, Q16281; hCNGA4, Q8IV77; hCNGB1, Q14028; hCNGB3, Q9NQW8; hPKAI, P10644; hPKAII, P31323; hPKGI, Q13976; hPKGII, Q13237; hRAPGEF2, Q9Y4G8; hRAPGEF4, Q9EQZ6; hRAPGEF6, Q8TEU7; hSLC9C1, Q4G0N8; hSLC9C2, Q5TAH2; hPLP6, Q8IY17; hPLP7, Q6ZV29



Supplementary Figure S5: Sequence alignment of CNNM proteins from different species. The indicated bulky hydrophobic residues Y603 (#), Y694 (\$) and F698 (*) that occupy the main cavity of the β -roll in CNNM4_{CNMP} are highly conserved in all CNNMs from different species, from insects to mammals, while not all organisms encode all four CNNM members. Residue numbers and secondary elements are indicated. The Uniprot codes for the analyzed proteins are: *Hs*_{CNNM1}, Q9NRU3; *Pt*_{CNNM1}, H2Q2E3; *Pac*_{CNNM1}, A0A096P643; *Cjc*_{CNNM1}, F7I103; *Mmc*_{CNNM1}, Q0GA42; *Mlc*_{CNNM1}, G1P8Z7; *Bt*_{CNNM1}, F1MD84; *Ssc*_{CNNM1}, F1S8W6; *Lac*_{CNNM1}, G3TYT9; *Eec*_{CNNM1}, A0A1S3AFW3; *Clc*_{CNNM1}, F1PMJ7; *Ohc*_{CNNM1}, V8NPD9; *Ipc*_{CNNM1}, A0A2D0QLR5; *Drc*_{CNNM1}, A0A0G2KKC2; *Xtc*_{CNNM1}, F6WMC5; *Hs*_{CNNM2}, Q9H8M5; *Pt*_{CNNM2}, K7CZV7; *Pac*_{CNNM2}, A0A096P6G5; *Cjc*_{CNNM2}, U3E1C9; *Mmc*_{CNNM2}, Q3TWN3; *Shc*_{CNNM2}, G3X2G0; *Mlc*_{CNNM2}, G1Q8B9; *Amc*_{CNNM2}, G1MHQ7; *Btc*_{CNNM2}, E1BIL3; *Ssc*_{CNNM2}, A0A287AM25; *Lac*_{CNNM2}, G3UIL2; *Eec*_{CNNM2}, A0A1S3WKT5; *Clc*_{CNNM2}, E2RJ19; *Asc*_{CNNM2}, A0A1U7RW72; *Ipc*_{CNNM2}, A0A2D0QNG1; *Drc*_{CNNM2}, A2ATX7; *Ggc*_{CNNM2}, A0A1D5PIB9; *Ccc*_{CNNM2}, A0A091G624; *Occ*_{CNNM2}, A0A1D2NLQ6; *Acc*_{CNNM2}, A0A2A3EE16; *Hs*_{CNNM3}, Q8NE01; *Pt*_{CNNM3}, A0A2I3TCA8; *Pac*_{CNNM3}, A0A096NGS2; *Cjc*_{CNNM3}, U3DC74; *Mmc*_{CNNM3}, Q32NY4; *Mlc*_{CNNM3}, G1PS60; *Bt*_{CNNM3}, F1N293; *Ssc*_{CNNM3}, F1STC7; *Eec*_{CNNM3}, A0A1S3AJ95; *Clc*_{CNNM3}, E2RGW9; *Ipc*_{CNNM3}, A0A2D0SQK4; *Drc*_{CNNM3}, E7F3M2; *Ssc*_{CNNM3}, A0A1S3KYV5; *Xtc*_{CNNM3}, F7B785; *Hs*_{CNNM4}, Q6P4Q7; *Pt*_{CNNM4}, H2QID4; *Pac*_{CNNM4}, A0A096NGR9; *Cjc*_{CNNM4}, F7I1N7; *Mmc*_{CNNM4}, Q69ZF7; *Sjc*_{CNNM4}, G3WZR3; *Mlc*_{CNNM4}, G1QFN; *Amc*_{CNNM4}, G1LFK1; *Bt*_{CNNM4}, F1MK24; *Ssc*_{CNNM4}, F1STC6; *Lac*_{CNNM4}, G3TEW1; *Eec*_{CNNM4}, A0A1S3AIV6; *Clc*_{CNNM4}, F1PJ05; *Asc*_{CNNM4}, A0A1U7S4Z7; *Ipc*_{CNNM4}, W5UIQ1; *Drc*_{CNNM4}, F1Q7I7; *Ggc*_{CNNM4}, A0A1D5P3M6; *Ccc*_{CNNM4}, A0A091H8N3; *Xtc*_{CNNM4}, A0JPA0; *Occ*_{CNNM4}, A0A1D2NLL8; *Csc*_{CNNM4}, A0A2J7RP8; *Ap*_{CNNM4}, A0A1W4XF17.



Supplementary Figure S6. ITC data from cAMP (left) or cGMP (right) titration to CNNM4_{BAT-cNMP-Ctail}. Upper panels show the thermogram, lower panels the mixing isotherm.



Supplementary Figure S7. Dimensionless Kratky plots of SAXS data for CNNM4_{BAT-cNMP-Ctail} (*left*) in the absence (*above*) and presence (*below*) of MgATP, and for its complex with PRL-1 (*right*). In all cases, the plots indicate a well folded and elongated molecule. The pertaining BSA reference peak is indicated by a black dot.

Supplementary Movie S1: SAXS derived solution structure of CNNM4_{BAT-cNMP-Ctail} in the absence of MgATP. The associated Bateman modules are shown in *orange* and *red*, their connected cNMP binding domains in *dark* and *light blue*, and the interdomain linkers (added with CORAL) in *dark* and *light green*, respectively. In the absence of ATP, the Bateman modules associate to form a *twisted* disk shaped CBS module. The two main cavities formed between connected CBS1 and CBS2 motives are named S1 and S2, where S2 provides the MgATP binding site. The CBS module is located above the cNMP domain dimer, with their CBS1 motives inserting into the main cleft formed between both cNMP monomers. Helix H0 links the Bateman module to the preceding DUF21 transmembrane region at the distal end. The disordered α terminal tail (following the cNMP binding domain) is not shown.

Supplementary Movie S2: SAXS derived solution structure of the complete intracellular region, CNNM4_{BAT-cNMP-Ctail}, in the presence of MgATP. Naming and colouring as in movie S1. MgATP binding causes the Bateman modules to shift into a *flat* conformation of the CBS module by sliding of their CBS1 ends within the cleft formed by the cNMP domain dimer.

Supplementary Movie S3: SAXS derived solution structure of the complex formed by CNNM4_{BAT-cNMP-Ctail} with PRL-1 in the presence of MgATP (indicated by a label). Naming and colouring as for movie S1, with PRL-1 shown in *yellow*. The location of the long loop of the cNMP domain (not visible in the crystals) is indicated.

Supplementary Movie S4. Model of the twisted-to-flat conformational change induced by MgATP in CNNM4. Binding of MgATP at the Bateman modules (colored in *red* and *orange*) triggers a rotation of their CBS motifs that transforms the disk shaped CBS module from a twisted to a flat conformation. The CBS1 motifs from the Bateman modules insert into the large cleft formed between the rigidly dimerized cNMP domains (in *dark* and *light blue*, respectively), which restricts their sliding during the conformational change. The linkers connecting both intracellular domains as well as the C-tail following the cNMP domain are omitted for clarity.