

Supplementary Materials for
**An Atypical CNG Channel Activated by a Single cGMP Molecule
Controls Sperm Chemotaxis**

Wolfgang Bönigk, Astrid Loogen, Reinhard Seifert, Nachiket Kashikar, Clementine Klemm, Eberhard Krause, Volker Hagen, Elisabeth Kremmer, Timo Strünker, U. Benjamin Kaupp*

*To whom correspondence should be addressed. E-mail: u.b.kaupp@caesar.de

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A

MAQRTVSLLELEPETGGSTDRATISSQPQACFLNPQNSRMRAWETLIIISILTTFSLDLF - 60
 LAAFDSQVLHLWIIYTCDIMFLTDVVMRFFLGYMKDGLISDSNKIKLHYLRTTFPLDL - 120
 FTILPTDLLALVYPGLGSKNIWKVATFRFANRLFRTRLRINNYFDNRESKLSNTALLRT - 180
 LKYTVLAAMVIHSTACGWYFACTGWHGSEPPRCHNGSWAKSFDLGDMDQAKPGVSYVI - 240
 ALYWSVATATSTGYGDISAVNEKEKWFSIAMLLGIVVFFGMILGGMASMLTNFDGQRAR - 300
 YTHRFNVIKDSLDRDQSVEEELQKQVIGYIEYLWVRKKGVTDESLINALPLTFHAEVSLSG - 360
 NKFILDKAPMFQGLSDGFLRMLSLEIKPSLYLPRQTIADRNEICHDMYFIQRGEIEILSA - 420
 EDDDTPIERLNHGKLFGEVSLIFSMRPTNRIRAAAHCDDLVLVDKADLQNVLHHYPEVAKK - 480
 LQQIAEQRCDAAGLLEDRVRAGEGII PASKSLSRHATPQTKRDFPINSYEAFDMEDVDSE - 540
 WVTVHRGDVMMRTRGRQLQILWDDIQSFFTDVVMPSNRFKATWELLVVLVTIVIAFLYPY - 600
 CASFIAANQNRSQSPDPNEERVQENLILLLTMLYLMDFVLVIDIVVRLRTAVSTPNGTFKD - 660
 FHYIRDYVRSWGFVADVLAAILPLDLFCLLFPVGPKRTHALYLLALNRLIKCWRVPNYLQ - 720
 NLEQNLEVNIGTIRFFKFVIYISLLSHWCSCWLWYLAACPSGSARGTPAPAVSDPTAAVAN - 780
 PVPWHEYIISLYWAAATMTSTGYGDISAHSTIGRAIALGAMLVGLLLYGYCLSSIAATLA - 840
 NSDAPRVGFQEKLFQVQEFMKRHNLSPLDQQRVINYLSTLVFRRHRGEAIPGGKRIMHDMP - 900
 IQLQQDIAYQDVEATLSKVPLFKGCDANFLRMLALKLHVFI FMPGDVVVYQGDMGREMYF - 960
 IRRGTCEVLSKDGKHMVSNIGPSQYFGEVGLIFGDYRTATVRAASYCELLMLKRADLDDV - 1020
 LKHFPLIEKQFSDAAKNQGHLELRDASKQKRDEPMVAGTVLQPEIQDVEEEDMSAYPEA - 1080
 TSLRPVDISVKVFDTRSEDYTEPFQAISLPSRLVSKLLMSRSFIPSGTCFKRWEAIRVAV - 1140
 AILAAFAVTLQAAFLHMNIGLWVVNYTLDVICFVDMYLKFHTAFYNENNVLVTHPLSTAK - 1200
 HYLRTNFLIDVLACFPTELIAYAVIGHLSPESIHVYALVRINRLQLYRVPLAFNYLESD - 1260
 VQNLTGNIIRMMKFFFFYMVLFIHMLSCLWYMNACPPVFTFPI SNTDFQDLILTNYHRCKNG - 1320
 SWTSHDNTFNLTITAQYLTSMYWASATGASVGYGDIHAKNISEMILALFSMIVGIVFFG - 1380
 YIIASVAASLANADPHRARYQEKLTAIKRYLKDQGVKSLAQHIDVYYNYMWRTRGVPEP - 1440
 DSLFDGLPLALKADVSLNLYQGMINKVPLFHNTEIGFQKMLAMCIKPVYYLNKEYIVRKH - 1500
 DFGKEMFFIHRGLVEVVEDSGSIVFDTMQSGRFFGEISLVFSCPRTASIRAENNVDMFVL - 1560
 TKEDLDEVLTHYPSIKAQIYTVAEERISAVRKRKAKVQASNTPNNSNNNSIKGSSINSI - 1620
 EKSENSTASASSAGVPPSSSSTAESAAPARPDSSQSTATAATRNSQGQGESTSVSSAVATT - 1680
 PTSTIPPASPTPTPTPTQENANSNANNNTGSGESSGNNNNQGGNASSQPDSTPATDG - 1740
 ASATPHVPTRYPAFFCCVRRDKNYLEALLHANRFVLPNPSVVRNLARLTCILAVATSWT - 1800
 IMYQAFYVQVQSTPFLIFSYLECECVFIFEIYIKFHVSCADEYGALEKDFTKIYNTYLRKWS - 1860
 GFTQDFVPTVPIELLALCFSGETLFPVLSFLRFRQLLRFVRVVSQFFDRWECELNINVLIV - 1920
 RLIKFFVLLIIHILFASIWYTIACPLSVCHPGSWADSLNYNGSDPYIFYRYCDTIYWAV - 1980
 ATLSTGYGDIHAYSVPEIVFASVMVFGKLLFGWVLGNIASLANAESGRVSYEERLAA - 2040
 VKDQMKDMRLSSKLRNRVISYFDYVWARNKIDQSNLFRDAPFCLQTDLGLNVCGDHLLR - 2100
 VSLFQEADESFRHRLSMLKPVLFMPSDLIVRQGDVGDMEYFISRGVVEEMEVSNSRVA - 2160
 RILESGEFFDDINLLYDVPRTSFKARTHVDVKSLSVRDLRSVLEQYPNVEAQIRRIGKE - 2220
 LYGDYAASINAPEHFPLRQTVV - 2242

B

ApCNGK1	GFLRMLSLEIKPSLYLPRQTIADRNEICHDMYFIQRGEIEILSAEDDDTP	-	426			
ApCNGK2	NFLRMLLALKLHVFI FMPGDVVVYQDGMGREMYFIRRGTCCEVLS-KDGKHV	-	976			
ApCNGK3	GFQKMLAMCIKPVYYLNKEYIVRKHDFGKEMFFIHRGLVEVVS-EDGSIV	-	1524			
ApCNGK4	SFHRALSMLKPVLFMPSDLIVRQGDVGDDEMYFISRGVVEEMVNSNSRV	-	2159			
bCNGA1	GLLVELVLKLPQVYSPGDYICKKGDIGREMYIIKEGKLAVVA-DDGITQ	-	532			
rCNGA2	GLLVELVLKLRPQVFS PGDYICKKGDIGREMYIIKEGKLAVVA-DDGVTQ	-	511			
SpHCN1	NFVTRVVTLLLEFEVFPADYVIQEGTFGDRMFFIQQGIVDIIMSDGVIAT	-	601			
mHCN2	NFVTAMLTKLKFVFPQGDYIIREGTIGKKMYFIQHGVSVL-TKGNKEM	-	572			
m1CNG	AVLVEIVRALRARTVPAGAVICRIGEPGDRMFFVVEGVS SVATPNP----	-	287			
	α A	β 1	β 2	β 3	β 4	
ApCNGK1	IERLNHGKLFGEVSLI-----FSMPRTNRIRAAAHCDDLVLVDKADLQNV	-	470			
ApCNGK2	MSNIGPSQYFGEVGLI-----FGDYRTATVRAASYCELLMLKRALDDV	-	1020			
ApCNGK3	FDTMQSGRFFGEISLV-----FSCPRTASIRAENNVDMFVLTKELDDEV	-	1568			
ApCNGK4	ARILESGEFFDDINLL-----YDVPRRT-SFKARTHVDVKLSLVRDLTSV	-	2203			
bCNGA1	FVVLSDGSYFGEISILNIKGSKAGNRRTANIKSIGYSDLFCLSKDDLMEA	-	581			
rCNGA2	YALLSAGSCFGEISILNIKGSKMGNRRTANIRSLGYSDFCLSKDDLMEA	-	561			
SpHCN1	--SLSDGSYFGEICLLTRE-----RRVASVKCETYCTLFSLSVQHFNQV	-	643			
mHCN2	--KLSDSYFGEICLLTRG-----RRTASVRADTYCRLYSLSDVDFNEV	-	614			
m1CNG	-VELGPGAFFGEMALIS-----GEPRSATVSAATTVSLLSLHSADFQML	-	330			
	β 5	β 6	β 7	β 8	α B	
ApCNGK1	LHHYPEVAKKLQQIAEQRCDAAGLLE	-	496			
ApCNGK2	LKHFLPIEKQFSDAAKNQGHRLRELRD	-	1046			
ApCNGK3	LTHYPSIKAQIYTVAEERISAVRKRS	-	1594			
ApCNGK4	LEQYPNVEAQIRRIGKELYGDYAASI	-	2229			
bCNGA1	LTEYPDAAKGMLEEKQKQILMKDGLLD	-	608			
rCNGA2	VTEYPDAAKVVLEERGRQILMKEGLLD	-	587			
SpHCN1	LDEFPAMRKTMEEIAVRRLTRIGKES	-	669			
mHCN2	LEEYPMRRAFETVAIDRLDRIGKKN	-	640			
m1CNG	CSSSPEIAEIFRKTALERRGAAASA	-	355			
	α B	α C				

Fig. S1: Primary structure of the CNGK channel. (A) Peptides determined by mass spectrometry are shown as bars above the sequence. Red bars: peptides identified at around 50 to 70 kD; blue bars: peptides identified at around 170 kD. (B) Comparison of the cyclic nucleotide-binding domains of repeats 1-4 with those of CNG channels (bCNGA1, NP_776703; rCNGA2, NP_037060), HCN channels (SpHCN1, NP_999729; mHCN2, NP_032252), and a cyclic nucleotide-gated K⁺ channel from *Mesorhizobium loti* (m1CNG, NP_104392). Amino-acid residues that are important for the structure of the cNMP-binding domains and for the binding of the ligand are indicated by red rhombs and black circles. Alpha helices (α A-C) and beta sheets (β 1-8) of the secondary structure are shown below the sequences.

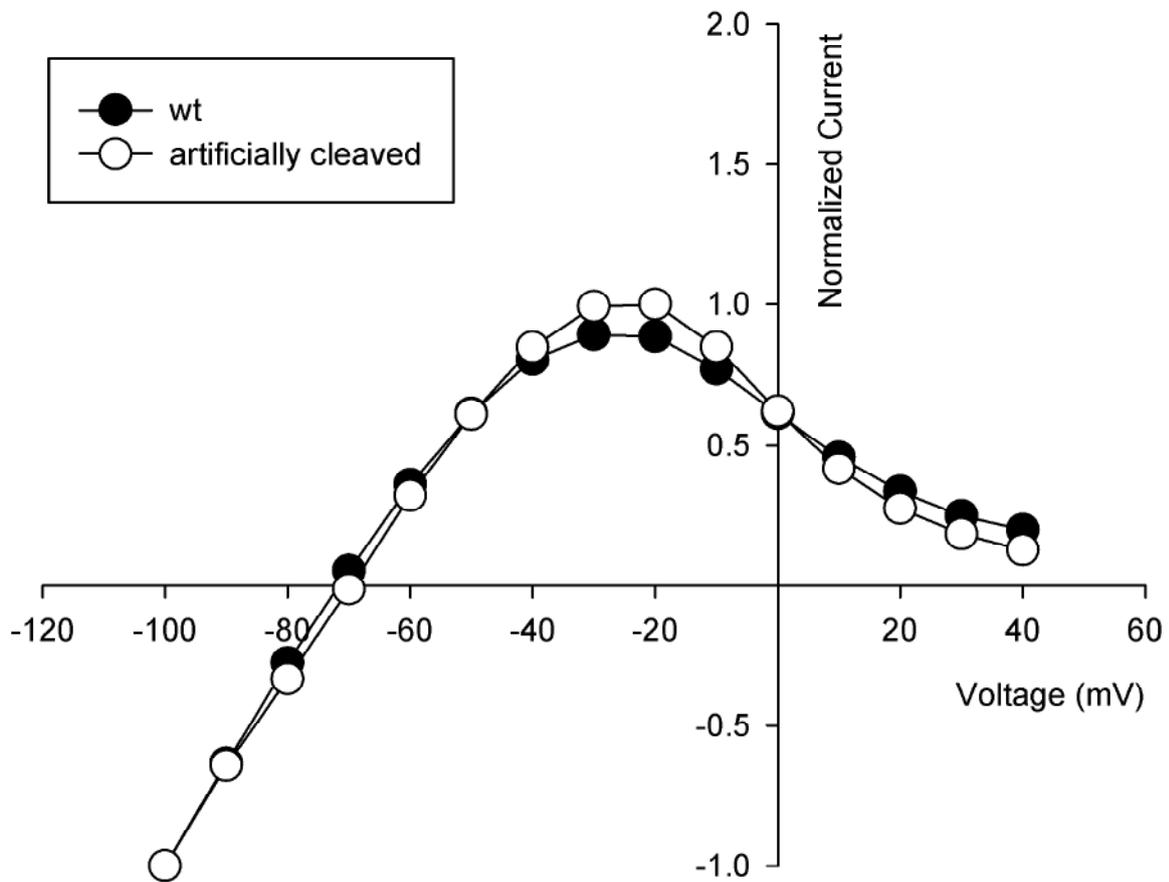


Fig. S2: Comparison of currents from wild-type and artificially cleaved CNGK channels. Current-voltage (IV) relation of mean whole-cell currents of wild-type channels (filled circles) and channels that formed during coexpression of constructs coding for the repeats 1-3 and repeat 4 (open circles). The pipette solution consisted of (in mM): KCl 140, EGTA 0.1, HEPES 10, cGMP 0.01, pH 7.4 (KOH).

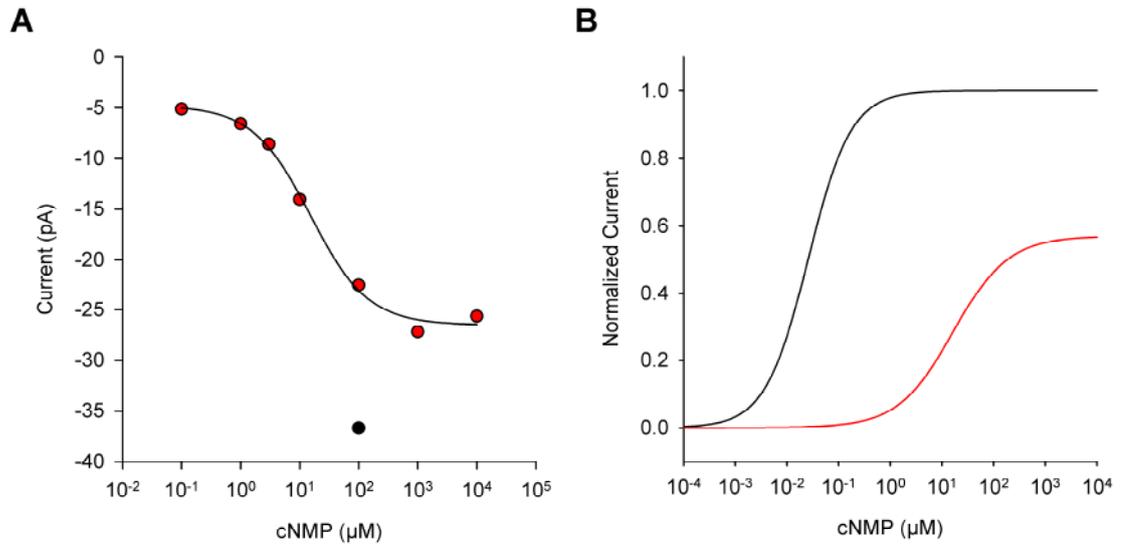


Fig. S3: Activation of CNGK channels by cAMP.

(A) Mean currents (at -80 mV) of an inside out patch activated by various concentrations of cAMP (red circles) or 100 μM cGMP (black circle). The solid line was calculated with the Hill equation $I = (I_{\max} - I_{\min}) * c^n / (c^n + K_{1/2}^n) + I_{\min}$, with the following parameters: $K_{1/2} = 15.35 \mu\text{M}$, $n = 0.88$, $I_{\max} = -26.5 \text{ pA}$, $I_{\min} = -4.8 \text{ pA}$. (B) Normalized mean activation curve of cGMP (black line) and cAMP (red line) calculated using mean $K_{1/2}$ and Hill coefficients (see text). Currents were normalized to the maximal currents in the presence of saturating concentrations of cGMP.