

Correlation between activity and domain complementation in adenylyl cyclase demonstrated with a novel FRET sensor
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Molecular Pharmacology

Supplementary Figure 1- Sequence and domain organization of SYNAC

Amino Acid Sequence of SYNAC

MG**TG**MQNEYCYRLDFLWKNKFKKEREEIETMENLNRVLLLENVLPAAHVAEHFLARSLKN
EELYHQSYDCVCMFASIPDFKEFYTESDVNKEGLECLRLLNEIIADFDDLLSKPKFSGVEK
IKTIGSTYMAATGLSAVPSQEHSQEPERQYMHIGTMVEFAFALVGKLDAINKHSFNDFKLR
VGINHGPIAGVIGAQPQYDIWGNTVNVASRMDSTGVLDKIQVTEETSLVLQTLGYTCTC
RGIINVKGKGLDKTYFVNTEMSRSLSQSNVAS**GSGTSGSG**VSKGEELFTGVVPILVELDGDV
NGHKFSVSGEGEGDATYGKLT**LKFI**CTTGKLPVPWPTLVTTFTWGVQCFARYPDHMKQH
DFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLE
YNAISDNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLST
QSKLSKDPNEKRDHMLLEFVTAAGITLGMDELY**KEFGSGGSG**ENLYFQ**GGSG**EEEEKKK
EEEEKKQKEEQERLAKEEAERKQKEEQERLAKEEAERKQKEEEERKQKEEEERKQKEEE
ERKLKEEQERKAAEEKKAKEEAERKAKEEQERKAAEEERKKKEEEERLERERKEREEQEK
KAKEEAERIAKLEAEKKAEEERKAKEEEERKAKEEEERKKKEEQERLAKEEAAERKAAE
EKKAKEEQERKEKEEAERKQR**GSGGSG****GAP**VSKGEELFTGVVPILVELDGDVNGHKFSV
GEGEGDATYGKLT**LKFI**CTTGKLPVPWPTLVTTFTGYGLMCFARYPDHMKQHDFFKSAMP
EGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNV
YIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKDP
NEKRDHMLLEFVTAAGITLGMDELY**GSGGSG****GAP**MEMKADINAKQEDMMFHKIYIQKH
DNVSILFADIEGFTSLASQCTAQELVMTLNELFARFDKLAENHCLRIKILGDCYYCVSGLP
EARADHAHCCVEMGMDMIEAISLVREVTGVNVNMRVGIHSGRVHCGVLGLRQWQFDVW
SNDVTLANHMEAGGKAGRIHITKATLNYLNGDYEVPEPGCGGERNAYLKEHSIETFLILRCT
QKRKEEKAMIAKMN**RQRTNSI****VDGSGGSG**VSKGEELFTGVVPILVELDGDVNGHKFSVSG
EGEGDATYGKLT**LKFI**CTTGKLPVPWPTLVTTFTGYGLMCFARYPDHMKQHDFFKSAMP
GYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVY
IMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKDP
NEKRDHMLLEFVTAAGITLGMDELY**GSG****DYKDDDK***

Nucleotide Sequence of SYNAC

Methionine-Glycine

Atg gga

AgeI

aCCGGT

ACII-C2a (*H. sapiens*, aa 823-1083)

ATGCAGAATGAATATTACTGTAGGTTAGACTTCTTATGGAAGAACAATTCAAAAAAGA
GCGGGAGGAGATAGAGACCATGGAGAACCTGAACCGCGTGCTGCTGGAGAACGTGCTTCC
CGGCACGTGGCTGAGCACTTCTGGCCAGGAGCCTGAAGAATGAGGAGCTATAACCACCA
GTCCTATGACTGCGTCTGTGTCATGTTTGCCTCCATTCCGGATTTCAAAGAATTTTATAC
AGAATCCGACGTGAACAAGGAGGGCTTGAATGCCTTCGGCTCCTGAACGAGATCATCGC
TGACTTTGATGATCTTCTTTCCAAGCCAAAATTCAGTGGAGTTGAAAAGATTAAGACCA
TTGGCAGCACATACATGGCAGCAACAGGTCTGAGCGCTGTGCCAGCCAGGAGCACTCCC
AGGAGCCCAGCGGCAGTACATGCACATTGGCACCATGGTGGAGTTTGTCTTTGCCCTGG
TAGGGAAGCTGGATGCCATCAACAAGCACTCCTTCAACGACTTCAAATTGCGAGTGGGTA
TTAACCATGGACCTGTGATAGCTGGTGTGATTGGAGCTCAGAAGCCACAATATGATATC
TGGGGCAACACTGTCAATGTGGCCAGTAGGATGGACAGCACCGGAGTCCTGGACAAAAT
ACAGGTTACCGAGGAGACGAGCCTCGTCCTGCAGACCCTCGGATACACGTGCACCTGTCC
AGGAATAATCAACGTGAAAGGAAAGGGGACCTGAAGACGTACTTTGTAAACACAGAAA
TGTC AAGTCCCTTCCCAGAGCAACGTGGCATCC

Glycine-Serine-Glycine linker

GGAAGCGGA

SpeI

actagt

Gly-Ser-Gly linker

GGAAGCGGA

mCerulean

gtgagcaagggcgaggagctgtcaccggggtggtgcccatcctggtcgagctggacggcgacgtaaaccggccacaag
ttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccaccggcaagc
tgcccgtgccctggcccacctcgtgaccacctgacctggggcgtgcagtgttcgcccgctaccccgaccatgaag
cagcacgacttcttaagtcgccatgccgaaggctacgtccaggagcgcaccatcttctcaaggacgacggcaacta
caagaccgcgcccaggtgaagttcgagggcgacacctggtgaaccgcatcgagctgaagggcatcgacttcaagga
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gaacggcatcaaggccaactcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccactaccagca
gaacacccccatcggcgacggccccgtgctgctgcccacaaccactacctgagcaccagtcgaagctgagcaagac
cccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgcccgccgggatcactctcgcatggacgagctgta
caAG

EcoRI
GAATTC

Gly-Ser-Gly x2
GGAAGCGGAGGAAGCGGA

TEV Protease site
GAAAACCTGTATTTTCAG

Gly-Gly-Ser-Gly linker
GGCGGAAGCGGA

ER/K linker (30 nm)
GAAGAGGAAGAGAAGAAGAAAGAAGAGGAAGAAAAGAAACAAAAAGAAGAACAAGAAA
GACTTGCAAAAGAAGAGGCAGAGAGAAAACAAAAAGAAGAACAAGAAAGACTTGCAAA
AGAAGAGGCAGAGAGAAAACAAAAGGAGGAAGAAGAGAGAAAACAAAAGGAAGAAGAA
GAGAGAAAACAAAAGGAGGAAGAAGAAGAAAATTAAAGGAGGAACAAGAAAGAAAAG
CTGCAGAAGAAAAGAAAGCTAAAGAAGAAGCTGAGAGAAAGGCTAAAGAAGAACAAGA
AAGGAAAGCTGAAGAAGAGAGAAAAGAAGAAGAAGAGGAAGAAGACTTGAAAGAGAA
AGAAAAGAGAGAGAAGAACAAGAAAAGAAAGCCAAAGAAGAGGCAGAGAGAATTGCAA
AGTTAGAGGCTGAAAAGAAGGCAGAAAGAAGAAAAGAAAAGCCAAAGAAGAAGAAGAGAG
AAAAGCCAAAGAAGAAGAGGAAAGAAAGAAGAAAAGAGGAGCAAGAAAGACTTGCAAAA
GAAAAGGAAGAAGCAGAAAGAAAAGCTGCAGAGGAAAAGAAAAGCTAAAGAAGAACAAG
AAAGAAAAGAAAAGGAAGAAGCAGAAAGAAAACAAAGA

Gly-Ser-Gly linker x2
GGCTCTGGCGGCTCTGGC

AscI Base added for frame shift
GGCGCGCC C

mCitrine
gtgagcaagggcgaggagctgttcaccggggtggtgccatcctggtcgagctggacggcgacgtaaaccggccacaag
ttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccaccggcaagc
tgcccgtgccctggcccaccctcgtgaccacctcggctacggcctgatgtgcttcgccgctaccccgaccatgaagc
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aagaccgcgccgaggtgaagttcgagggcgacacctggtgaaccgcatcgagctgaagggcatcgacttaaggag
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aacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcgagcgtgcagctcgccgaccactaccgag
aacaccccatcggcgacggccccgtgctgctgccgacaaccactacctgagctaccagtccaaactgagcaaagacc
ccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgggatcactctcgcatggacgagctgtac
aag

Gly-Ser-Gly linker x2
ggcagtggtgatca

Ascl Base added for frame shift
ggCGCGCC C

ACV-C1a (*H. sapiens*, aa 442-667)

ATGGAGATGAAAGCAGACATCAACGCCAAGCAGGAGGATATGATGTTCCATAAGATTTA
CATCCAGAAACATGACAACGTGAGCATCCTGTTTGCTGACATCGAGGGCTTCACCAGCCT
GGCGTCCCAGTGCACACTGCACAGGAAGTGGTCATGACCCTCAACGAGCTCTTCGCCCGCTT
TGACAAGCTGGCCGAGAGAATCACTGTTTACGTATTAAGATCCTTGGGGATTGTTATT
ACTGCGTCTCGGGGCTGCCTGAAGCAAGGGCTGACCACGCCACTGCTGTGTGGAGATGG
GCATGGACATGATCGAGGCCATCTCGTTGGTCCGGGAGGTGACAGGGGTGAACGTGAAC
ATGCGTGTGGGAATTCACAGCGGGCGAGTACACTGCGGTGTCTTGGTCTCAGGAAGTGG
CAGTTCGACGTCTGGTCTAACGATGTACGCTAGCCAACCACATGGAGGCTGGCGGCAAG
GCAGGACGCATCCACATCACCAGGCTACACTCAACTACCTGAATGGGGACTACGAGGTG
GAGCCAGGCTGTGGGGCGAGCGCAACGCCTACCTCAAGGAGCACAGTATCGAGACCTTC
CTCATCCTGCGCTGCACCCAGAAGCGGAAAGAAGAGAAGGCCATGATCGCCAAGATGAAC
CGCCAGAGAACCAACTCCATC

Sall
GTCGAC

Gly-Ser-Gly linker x2
ggaagcgggGGCTCTGGC

mCitrine

gtgagcaagggcgaggagctgtcaccggggtggtgcccacctcctggtcgagctggacggcgacgtaaaccggccacaag
ttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgaccctgaagttcatctgcaccaccggcaagc
tgcccgctgccctggcccaccctcgtgaccaccttcggtacggcctgatgtgcttcgccgctaccccgaccacatgaagc
agcagcacttctcaagtccgcatgcccgaaggctacgtccaggagcgcaccatcttctcaaggacgacggcaactac
aagaccgcgccgaggtgaagttcgagggcgacaccctggtgaaccgcatcgagctgaagggcatgacttcaaggag
gacggcaacatcctggggcacaagctggagtacaactacaacgccaacaacgtctatatcatggccgacaagcagaag
aacggcatcaaggtgaactcaagatccgccacaacatcgaggacggcagcgtgcagctcggcaccactaccagcag
aacaccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagctaccagtccaaactgagcaaagacc
ccaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgccgggatcactctcggcatggacgagctgtac
aag

Gly-Ser-Gly linker
ggaagcggg

FLAG tag
GACTACAAGGACGATGACGACAAG

Stop NotI
TGA GCggccgc

Supplementary Figure 1- Sequence and domain organization of SYNAC

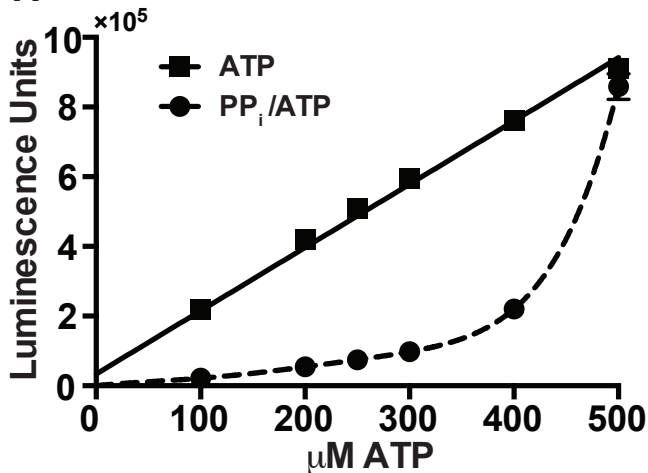
Amino acid and DNA sequences of SYNAC. Colors of domains correlate between the DNA sequence and the protein sequence. Briefly, the methionine and stop codons are uncolored, restriction sites used for cloning are colored red, the adenylyl cyclase II c2 domain is colored cyan, glycine-serine-glycine linkers are magenta, mCerulean is navy blue, the TEV site is colored grey, the ER/K linker is colored dark teal, mCitrine is yellow, adenylyl cyclase V c1 domain is colored green, and the FLAG tag is colored brown.

SUPPLEMENTAL FIGURE 2

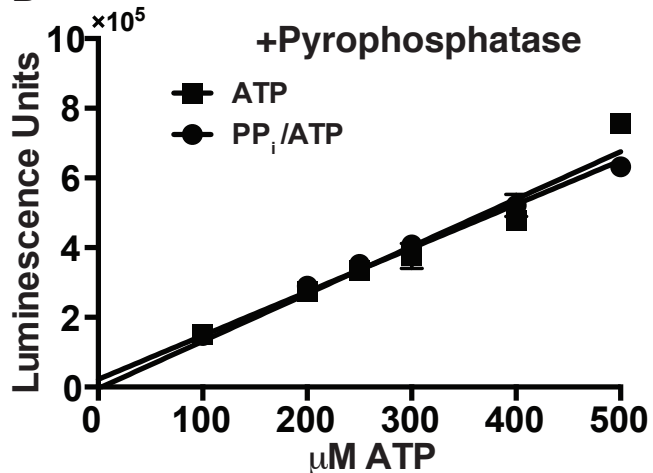
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A



B



Supplementary Fig. 2. Luciferase inhibition by pyrophosphate.

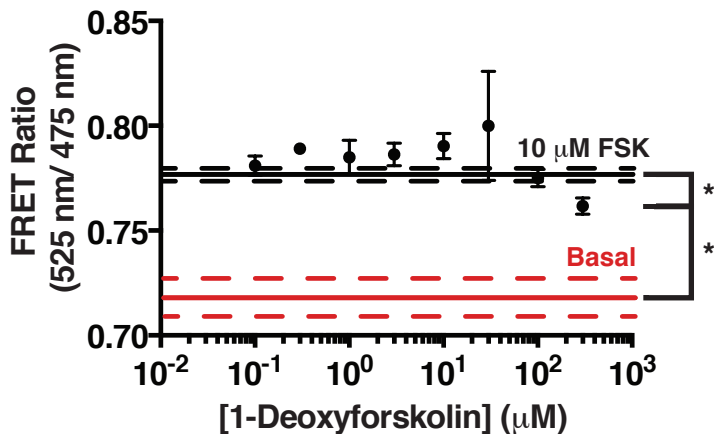
(A) Pyrophosphate inhibits luciferase activity. Squares (solid line) show luminescence in terms of ATP concentration. Circles (dashed line) show luminescence in terms of ATP with a balance of pyrophosphate concentration up to 500 μM (300 μM ATP has 200 μM PP_i ; 100 μM ATP has 400 μM PP_i , etc). This simulates ATP metabolism by cyclase (ATP is metabolized by cyclase into AMP and PP_i). (B) The same reaction conditions in (a), but treated with pyrophosphatase in the reaction (see Methods). Data is representative

SUPPLEMENTARY FIGURE 3

Correlation between activity and domain complementation in adenylyl cyclase demonstrated with a novel FRET sensor

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Supplementary Fig. 3. FRET readout of competitive inhibition of SYNAC by 1-deoxyforskolin.

FRET readout of SYNAC stimulated with 10 μM FSK in the presence of increasing concentrations of 1-deoxyforskolin. Red line is basal SYNAC activity and black line is 10 μM FSK stimulated SYNAC. The 300 μM deoxyforskolin point is significantly different from both basal and stimulated (Student's t-test, * = p<0.05). Dashed lines indicate error bars (+/- SEM) for their respective colored lines. Data is mean ± SEM of three independent repeats