Supplementary Information: Development of a multi-enzyme cascade for 2'3'-cGAMP synthesis from nucleosides

Martin Becker [b], Isabel Nowak [b], Katharina Hildebrand [b], Stephan Lütz [b] and Katrin Rosenthal *[a]

[a] Prof. Dr. K. Rosenthal
School of Science
Constructor University
Campus Ring 1, 28759 Bremen (Germany)
E-mail: krosenthal@constructor.university

[b] M. Becker, Isabel Nowak, Katharina Hildebrand, Prof. Dr. S. Lütz
Department of Biochemical and Chemical Engineering
Chair for Bioprocess Engineering, TU Dortmund University
Emil-Figge-Straße 66, 44227 Dortmund (Germany)

Content

1. Experimental data

1.1 Specific t*hsc*GAS activity

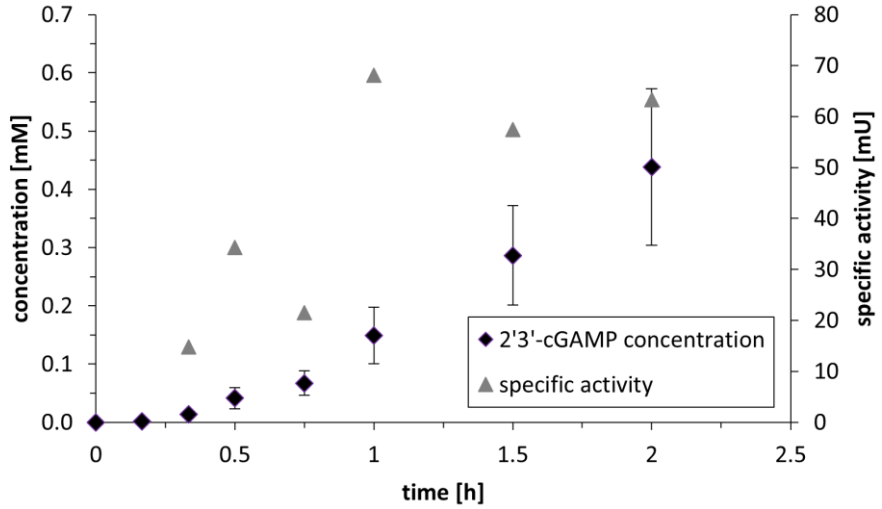

Figure S1: Determination of the specific thscGAS activity for the 2'3'-cGAMP formation from guanosine

Progress of 2'3'-cGAMP-formation within the first 2 h using 0.08 mg/mL t*hsc*GAS in 1.5 mL Eppendorf tubes at 37 °C in biological triplicates. Samples were taken after 10, 20, 30, 45, 60, 90 and 120 min and analyzed by HPLC.

The specific enzyme activity of 43.0 ± 13.7 mU/mg was calculated from the mean activity between the measuring points in the range between 20 min and 2 h.

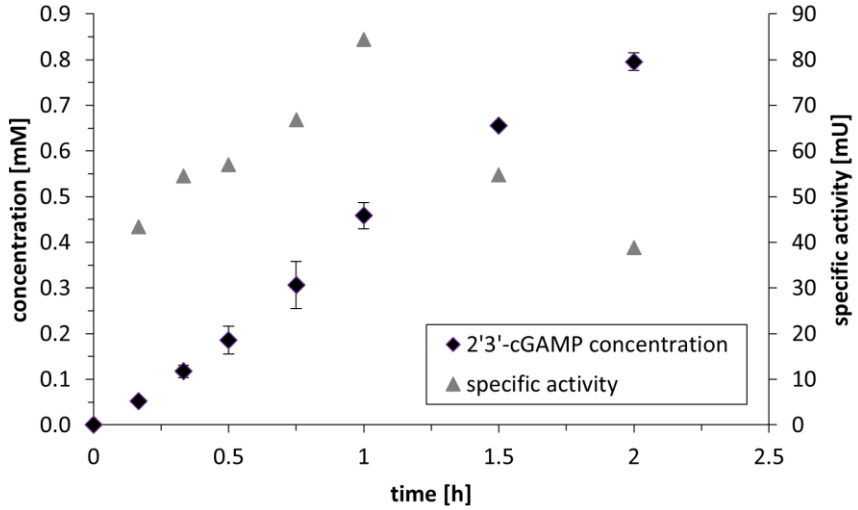

Figure S2: Determination of the specific thscGAS activity for the 2'3'-cGAMP formation from adenosine and guanosine

Progress of 2'3'-cGAMP formation within the first 2 h using 0.12 mg/mL t*hsc*GAS in 1.5 mL Eppendorf tubes at 37 °C in biological triplicates. Samples were taken after 10, 20, 30, 45, 60, 90 and 120 min and analyzed by HPLC.

The specific enzyme activity of 50.0 ± 2.2 mU/mg was calculated from the mean activity between the measuring points in the range between 0 min and 2 h.

1.2 Temperature stability of ATP and GTP

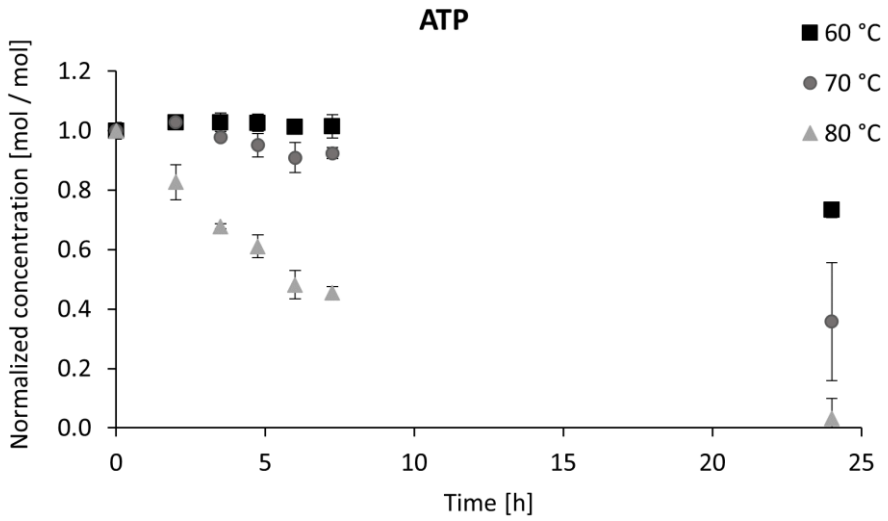

Figure S3: Temperature stability of ATP at 60 °C, 70 °, 80 °C.

In 1.5 mL Eppendorf tubes, 1 mL of 5 mM ATP in 50 mM TRIS-HCl, 40 mM $MgCl_2 \cdot 6H_2O$ activity buffer was incubated in duplicates at 60 °C, 70 °C, and 80 °C. Samples were taken after 0, 2, 3.5, 4.75, 6, 7.25 and 24 h and analyzed by HPLC. The measured concentrations were normalized to the 0 h sample.

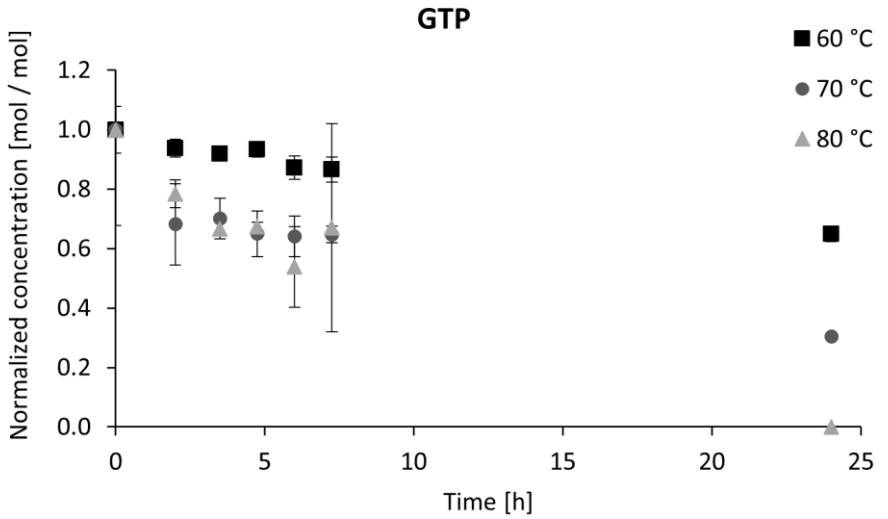

Figure S4: Temperature stability of GTP at 60 °C, 70 °, 80 °C.

In 1.5 mL Eppendorf tubes, 1 mL of 5 mM GTP in 50 mM TRIS-HCl, 40 mM $MgCl_2 \cdot 6H_2O$ activity buffer was incubated in duplicates at 60 °C, 70 °C, and 80 °C. Samples were taken after 0, 2, 3.5, 4.75, 6, 7.25 and 24 h and analyzed by HPLC. The measured concentrations were normalized to the 0 h sample.

1.3 Turnover numbers (TONs)

Table S1: TONs of the Guanosine Cascade

TONs were calculated for the indicated reaction conditions from the enzyme concentration and the difference between the initial and final product concentration of the assays. In the case of class III PPK2s, the transfer of a single phosphate group was considered separately.

Enzyme	TON
***Mj*NK**	1,751
***Ch*PPK2 + *Eb*PPK2**	953
***ths*cGAS**	847

Table S2: TONs of the Nucleoside Cascade

TONs were calculated for the indicated reaction conditions from the enzyme concentration and the difference between the initial and final product concentration of the assays. In the case of class III PPK2s, the transfer of a single phosphate group was considered separately. In the case of *Ch*PPK2, step 2 was not considered as no activity was observed. Only the first reaction step was considered for the *Sc*ADK and only steps three and four for the *Mj*NK, as the corresponding substrates were consumed after these steps. For the calculations of *Eb*PPK2 and *Ch*PPK2 in the last two reaction steps, the consumption of AMP and GMP and the formation of 2'3'-cGAMP were considered.

Enzyme	TON
***Sc*ADK**	7,365
***Ch*PPK2 step 1**	10,458
***Mj*NK**	527
***Ch*PPK2 + *Eb*PPK2 in step 3 and step 4**	6,558
***ths*cGAS**	672

2. SDS gels

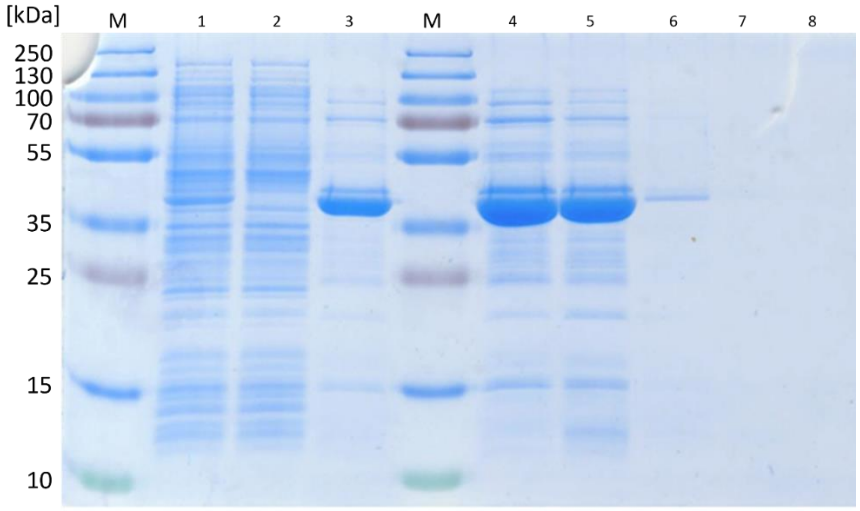

Figure S5: SDS-PAGE to verify protein purification using *Sc*ADK (37 kDa) as an example.

Order of lanes: lane M: PageRuler Prestained Protein Ladder #26619, lane 1: filtered lysate after cell disruption, lane 2: IMAC flow-through, lane 3: purified enzyme after buffer exchange, lane 4: IMAC fraction 1, lane 5: IMAC fraction 2, lane 6: IMAC fraction 3, lane 7: IMAC fraction 4, lane 8: IMAC fraction 5.

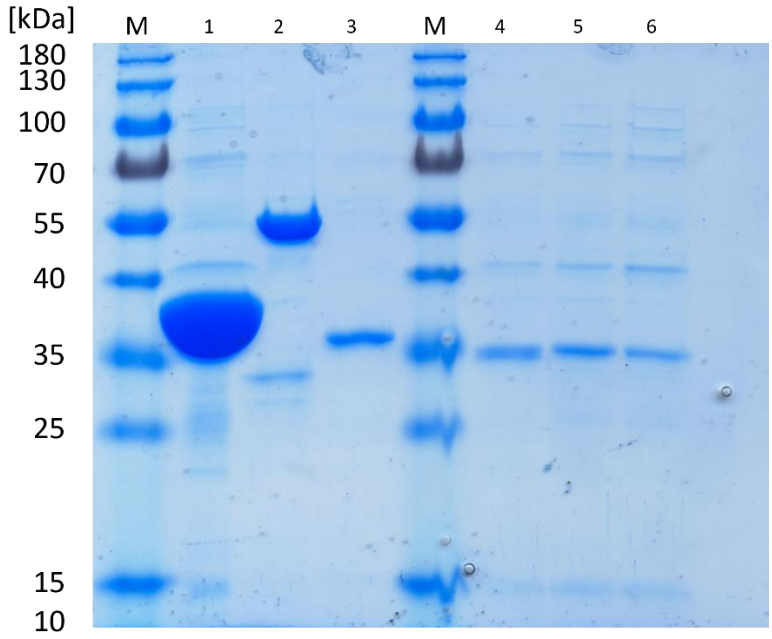

Figure S6: SDS-PAGE of purified enzymes used for enzyme screening.

Order of lanes: lane M: PageRuler Prestained Protein Ladder #26616, lane 1: *Sc*ADK (37 kDA), lane 2: *Aj*PPK2 (58 kDa), lane 3: *Sm*PPK2 (37 kDa), lane 4: *Mr*PPK2 (32 kDa), lane 5: *Mj*NK 2 (34 kDa), lane 6: *Eb*PPK2 (35 kDa).

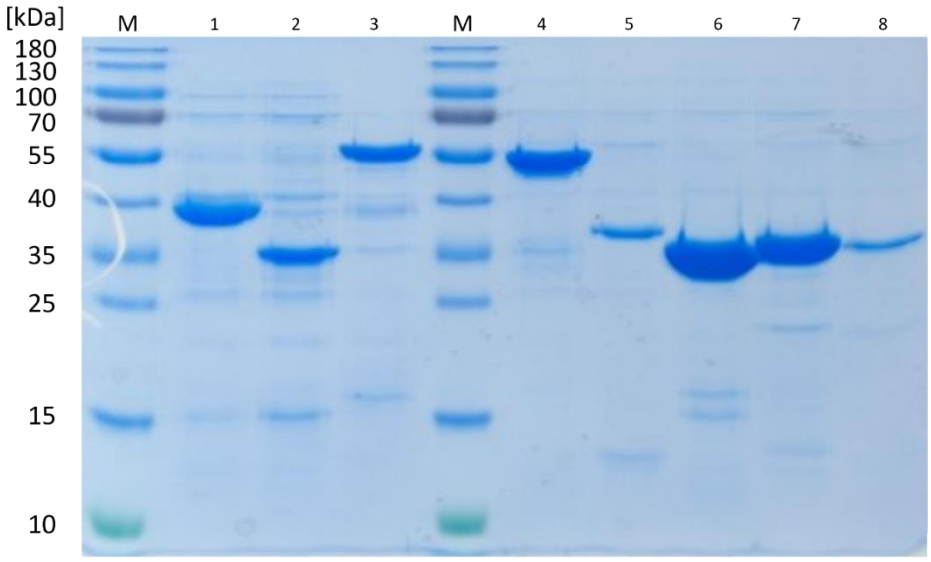

Figure S7: SDS-PAGE of all purified proteins used for the development of the enzyme cascades. The purified *Ch*PPK2 was additionally used for enzyme screening.

Order of lanes: lane M: PageRuler Prestained Protein Ladder #26616, lane 1: *Sc*ADK (37 kDA), lane 2: *Mj*NK (34 kDa), lane 3: *ths*cGAS (56 kDa), lane 4: *Aj*PPK2 (58 kDa), lane 5: *Sm*PPK2 (37 kDa), lane 6: *Eb*PPK2 (35 kDa), lane 7: *Ch*PPK2 (37 kDa), lane 8: *Mr*PPK2 (32 kDa).

3. HPLC and LC-MS measurements

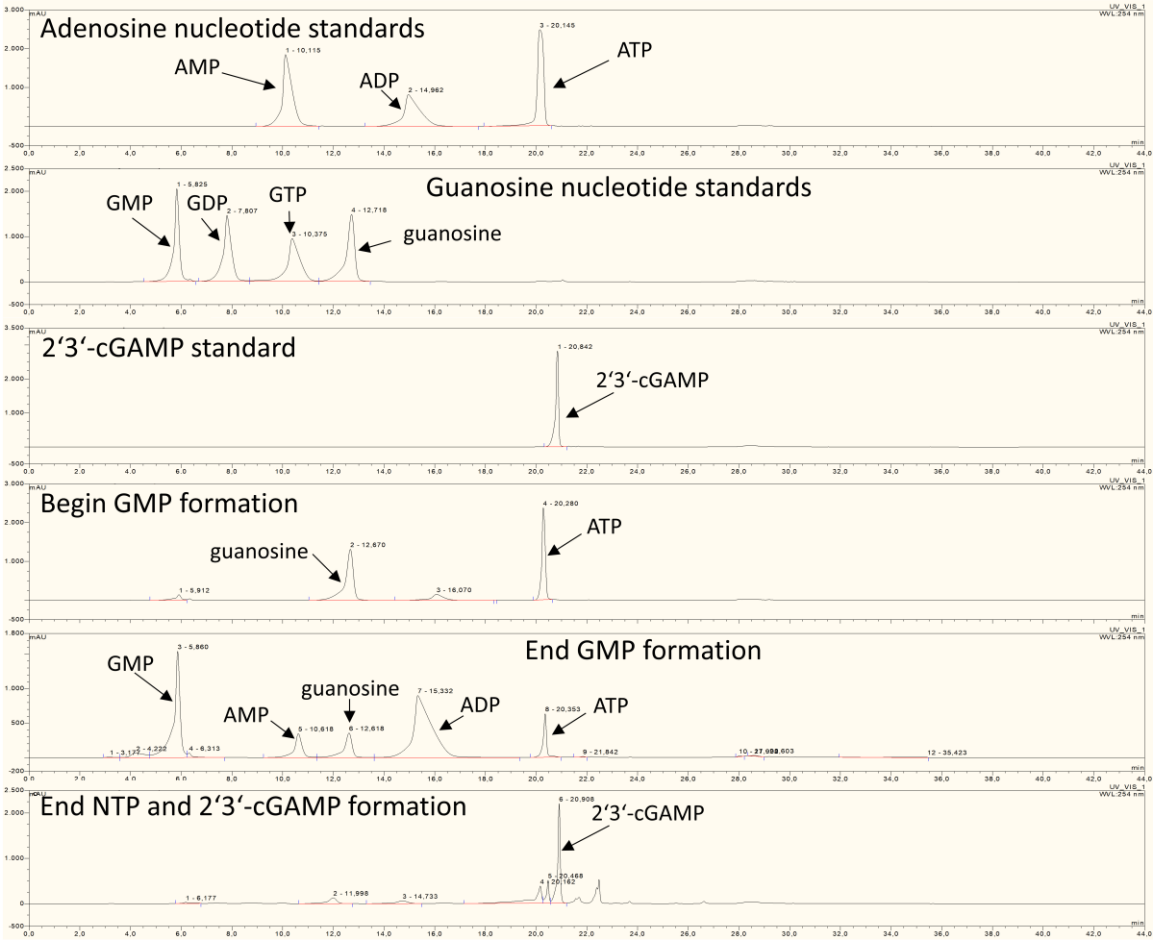

Figure S8: Illustrative HPLC chromatograms of standards and samples for 2’3’-cGAMP formation from guanosine.

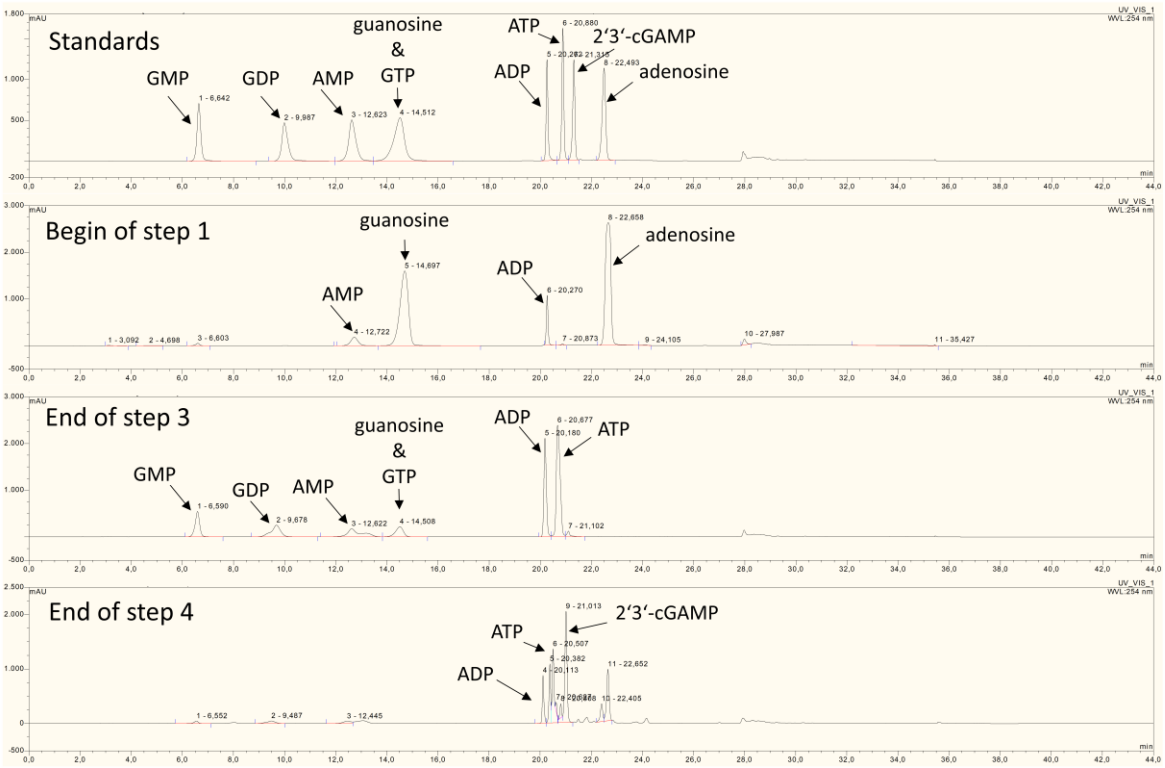

Figure S9: Illustrative HPLC chromatograms of standards and samples for 2’3’-cGAMP formation from nucleosides.

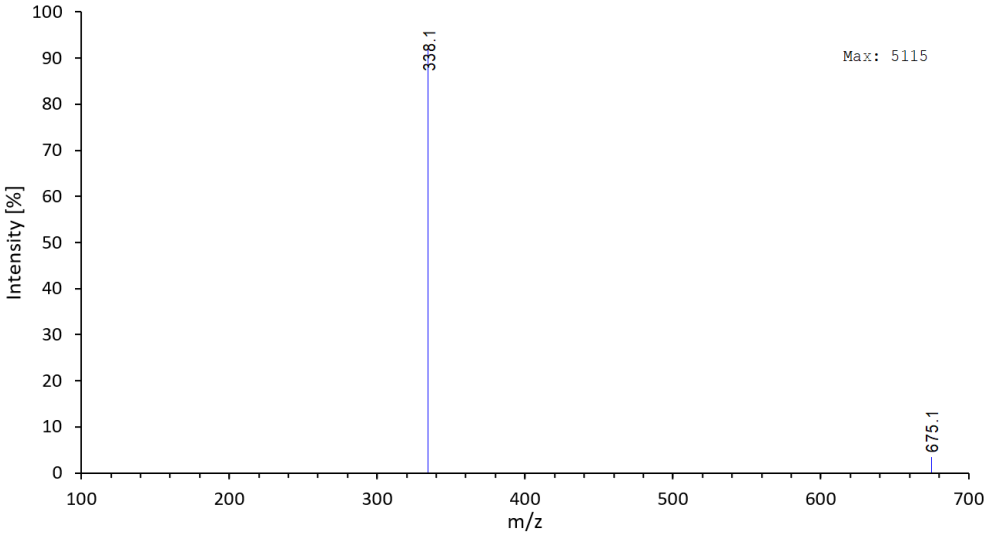

Figure S10: LC-MS SIM measurements of 2'3'-cGAMP formation from nucleosides after 24 h reaction.

The mass 675 m/z was extracted. The signal with mass 675 m/z corresponds to single ionized 2'3'-cGAMP and the signal at 338 to double ionized 2'3'-cGAMP.

4. DNA sequences

***Sc*ADK:**

UniProt: P47143

ATGACCGCACCATTGGTAGTATTGGGTAACCCACTTTTAGATTTCCAAGCCGACGTCACGGCTGAATACCTGGC
CAAGTATTCTCTAAAGGAAAACGACGCAATTTTGGTCGATGCCAAATCAGGCGATGCTAAGATGGCTATTTTTG
ACGAGCTCTTACAGATGCCAGAAACAAAGCTTGTTGCAGGTGGTGCTGCTCAAAACACTGCTAGAGGGGCAG
CATACGTTTTGGGCGCCGGCCAGGTGGTGTACTTCGGTTCCGTCGGTAAGGACAAGTTCAGCGAGAGATTGCT
TAACGAAAACGAAAAAGCTGGTGTCAAGTCTATGTACCAAGTTCAAAATGATATTGGTACCGGTAAGTGTGCC
GCATTAATCACTGGCCATAACCGGTCCTTGGTCACTGACTTGGGTGCTGCCAATTTCTTTACTCCAGACCACTTG
GACAAGCATTGGGACTTGGTCGAAGCAGCTAAGCTCTTCTACATCGGTGGTTTCCACTTGACCGTGTCTCCAGA
CGCTATCGTTAAGTTGGGCCAACATGCTAAAGAGAACAGCAAACCTTTCGTGTTGAACTTTAGTGCTCCTTTCA
TTCCTCATGTCTTCAAAGACGCATTGGCCAGAGTTTTGCCTTATGCTACCGTCATCATCGCTAACGAATCGGAG
GCCGAAGCCTTTTGCGACGCCTTCCAATTAGACTGTGCCAACACTGATTTGGAAGCTATTGCTCAAAGAATTGT
CAAGGACTCTCCAGTTGAAAAGACTGTCATCTTCACCCACGGTGTCGAACCAACAGTGGTCGTGTCCTCCAAG
GGTACCAGCACATATCCAGTCAAACCTTTGGACTCTTCTAAGATCGTCGACACCAACGGTGCTGGTGACGCCTT
CGCTGGTGGCTTTATGGCTGGGTTGACTAAAGGTGAAGATTTGGAAACCTCTATTGACATGGGTCAATGGCTA
GCTGCTTTGTCTATTCAAGAAGTTGGTCCCTCTTACCCTTCCGAAAAAATATCTTACTCTAAA

***Mj*NK:**

UniProt: Q57849

ATGGGTGGTAAAATGGAGAAGATTACATGTGTAGGGCATACTGCCCTTGATTATATCTTCAATGTAGAAAAAT
TTCCAGAACCGAATACTTCAATTCAAATTCCTTCGGCGAGAAAGTATTATGGTGGAGCAGCGGCAAATACAGC

AGTGGGAATAAAAAAACTTGGTGTTAATTCAGAGCTTTTATCATGTGTTGGCTATGATTTTAAAAATAGTGGAT
ATGAGAGGTATTTAAAGAATTTGGATATAAATATTTCTAAACTTTACTATTCTGAAGAAGAAGAAACACCAAAG
GCATGGATATTTACAGACAAGGATAACAATCAGATAACTTTCTTTTTATGGGGAGCTGCTAAGCATTATAAGGA
ACTAAACCCACCAAACTTCAATACAGAAATTGTCCATATAGCCACTGGAGACCCAGAGTTCAACTTAAAATGTG
CAAAAAAAGCTTATGGAAACAATTTGGTCTCTTTCGACCCCGGACAGGACTTACCTCAATACTCAAAAGAAATG
TTGTTGGAAATTATTGAGCATACAAACTTTTTATTTATGAATAAACATGAATTTGAGAGAGCATCTAATTTATTA
AATTTTGAAATTGATGATTATTTAGAGAGGGTTGATGCCCTTATAGTAACAAAAGGTTCTAAGGGTA GTGTAAT
ATACACTAAAGATAAAAAAATAGAAATTCCTTGTATTAAAGCAGGGAAAGTTATAGACCCAACAGGTGCTGGA
GACAGCTATAGAGCTGGATTTTTATCTGCCTATGTCAAAGGGTATGATTTAGAGAAATGTGGTTTAATTGGTGC
TGCAACTGCCTCATTTGTTGTTGAGGCAAAGGGATGCCAAACTAATTTACCAACATGGGATAAGGTTGTTGAG
AGATTAGAAAAACACCGAATATAA

***Aj*PPK2:**

UniProt: Q83XD3

ATGGACACCGAAACCATTGCAAGCGCAGTTCTGAATGAAGAACAGCTGAGCCTGGATCTGATTGAAGCACAGT
ATGCACTGATGAATACCCGTGATCAGAGCAATGCAAAAAGCCTGGTTATTCTGGTTAGCGGTATTGAACTGGC
AGGTAAAGGTGAAGCAGTTAAACAGCTGCGTGAATGGGTTGATCCGCGTTTTCTGTATGTTAAAGCAGATCCG
CCTCACCTGTTTAATCTGAAACAGCCGTTTTGGCAGCCGTATACCCGTTTTGTTCCTGCCGAAGGTCAGATTATG
GTTTGGTTTGGTAATTGGTATGGTGATCTGCTGGCAACCGCAATGCATGCAAGCAAACCGCTGGATGATACCC
TGTTTGATGAATATGTTAGCAACATGCGTGCCTTTGAACAGGATCTGAAAAATAACAATGTGGATGTGCTGAA
AGTGTGGTTTGATCTGAGCTGGAAAAGCCTGCAGAAACGTCTGGATGACATGGATCCGAGCGAAGTTCATTG
GCATAAACTGCATGGTCTGGATTGGCGTAACAAAAAACAGTATGATACGCTGCAAAAACTGCGTACCCGCTTT
ACCGATGATTGGCAGATTATTGATGGTGAAGATGAAGATCTGCGCAACCATAATTTTGCACAGGCAATTCTGA
CCGCACTGCGTCATTGTCCGGAACATGAAAAAAAAGCAGCACTGAAATGGCAGCAGGCACCGATTCCGGATAT
TCTGACCCAGTTTGAAGTTCCGCAGGCAGAAGATGCAAACTATAAAAGCGAACTGAAAAAACTGACCAAACAG
GTTGCAGATGCAATGCGTTGTGATGATCGTAAAGTTGTGATTGCGTTTGAAGGTATGGATGCAGCCGGTAAAG
GCGGTGCAATTAAACGTATTGTTAAAAAACTGGATCCGCGTGAGTATGAGATTCATACCATTGCAGCACCGGA
AAAATATGAACTGCGTCGTCCGTATCTGTGGCGTTTTTGGAGCAAACTGCAGAGTGATGATATCACCATTTTTG
ATCGTACCTGGTATGGTCGTGTTCTGGTTGAACGTGTTGAAGGTTTTGCAACCGAAGTTGAATGGCAGCGTGC
CTATGCAGAAATTAATCGCTTTGAAAAAAATCTGAGCAGCAGCCAGACCGTGCTGATCAAATTTTGGCTGGCA
ATTGATAAAGATGAACAGGCAGCACGTTTTAAAGCACGTGAAAGCACACCGCATAAACGCTTTAAAATCACCG
AAGAGGATTGGCGCAATCGTGATAAATGGGATGATTATCTGAAAGCCGCAGCAGATATGTTTGCACATACCGA
TACCAGCTATGCACCGTGGTATATTATCAGCACCAATGATAAACAGCAAGCCCGTATTGAAGTTCTGCGTGCAA
TCCTGAAACAACTGAAAGCGGATCGTGATACCGATTAA

***Sm*PPK2:**

UniProt: Q92SA6

ATGGACACCGAAACCATTGCAAGCGCAGTTCTGAATGAAGAACAGCTGAGCCTGGATCTGATTGAAGCACAGT
ATGCACTGATGAATACCCGTGATCAGAGCAATGCAAAAAGCCTGGTTATTCTGGTTAGCGGTATTGAACTGGC
AGGTAAAGGTGAAGCAGTTAAACAGCTGCGTGAATGGGTTGATCCGCGTTTTCTGTATGTTAAAGCAGATCCG
CCTCACCTGTTTAATCTGAAACAGCCGTTTTGGCAGCCGTATACCCGTTTTGTTCCTGCCGAAGGTCAGATTATG
GTTTGGTTTGGTAATTGGTATGGTGATCTGCTGGCAACCGCAATGCATGCAAGCAAACCGCTGGATGATACCC
TGTTTGATGAATATGTTAGCAACATGCGTGCCTTTGAACAGGATCTGAAAAATAACAATGTGGATGTGCTGAA
AGTGTGGTTTGATCTGAGCTGGAAAAGCCTGCAGAAACGTCTGGATGACATGGATCCGAGCGAAGTTCATTG
GCATAAACTGCATGGTCTGGATTGGCGTAACAAAAAACAGTATGATACGCTGCAAAAACTGCGTACCCGCTTT
ACCGATGATTGGCAGATTATTGATGGTGAAGATGAAGATCTGCGCAACCATAATTTTGCACAGGCAATTCTGA
CCGCACTGCGTCATTGTCCGGAACATGAAAAAAAAGCAGCACTGAAATGGCAGCAGGCACCGATTCCGGATAT

TCTGACCCAGTTTGAAGTTCCGCAGGCAGAAGATGCAAACTATAAAAGCGAACTGAAAAAACTGACCAAACAG
GTTGCAGATGCAATGCGTTGTGATGATCGTAAAGTTGTGATTGCGTTTGAAGGTATGGATGCAGCCGGTAAAG
GCGGTGCAATTAAACGTATTGTTAAAAAACTGGATCCGCGTGAGTATGAGATTCATACCATTGCAGCACCGGA
AAAATATGAACTGCGTCGTCCGTATCTGTGGCGTTTTTGGAGCAAACTGCAGAGTGATGATATCACCATTTTTG
ATCGTACCTGGTATGGTCGTGTTCTGGTTGAACGTGTTGAAGGTTTTGCAACCGAAGTTGAATGGCAGCGTGC
CTATGCAGAAATTAATCGCTTTGAAAAAAATCTGAGCAGCAGCCAGACCGTGCTGATCAAATTTTGGCTGGCA
ATTGATAAAGATGAACAGGCAGCACGTTTTAAAGCACGTGAAAGCACACCGCATAAACGCTTTAAAATCACCG
AAGAGGATTGGCGCAATCGTGATAAATGGGATGATTATCTGAAAGCCGCAGCAGATATGTTTGCACATACCGA
TACCAGCTATGCACCGTGGTATATTATCAGCACCAATGATAAACAGCAAGCCCGTATTGAAGTTCTGCGTGCAA
TCCTGAAACAACTGAAAGCGGATCGTGATACCGATTAA

***Mr*PPK2:**

UniProt: A0A806DL21

ATGGGGTTCTGCAGCATAGAATTCCTGATGGGAGCCCAGATGAAAAAATACCGCGTTCAACCGGATGGTCGCT
TTGAACTAAAGCGCTTCGATCCCGACGACACCAGCGCCTTTGAGGGGGGCAAGCAAGCGGCCCTGGAAGCCC
TGGCTGTGCTCAACAGGCGTTTGGAGAAGCTGCAAGAGCTGCTGTATGCGGAAGGCCAGCACAAGGTACTGG
TGGTGTTGCAGGCCATGGATGCGGGCGGCAAGGATGGCACCATCCGGGTGGTTTTCGACGGGGTAAACCCCA
GCGGGGTGCGCGTGGCCAGTTTTGGTGTGCCCACCGAGCAGGAGCTGGCCCGCGACTACCTCTGGCGGGTGC
ACCAGCAGGTGCCCCGCAAGGGTGAGCTGGTGATTTTCAACCGCTCCCACTACGAGGACGTGCTGGTGGTGC
GGGTTAAAAACCTGGTGCCCCAACAGGTTTGGCAGAAGCGCTACCGCCACATCCGCGAGTTCGAGCGCATGCT
GGCCGATGAGGGAACCACCATCCTCAAATTCTTCCTGCATATCTCCAAAGACGAGCAGCGCCAGCGGTTGCAG
GAGCGCTTAGATAACCCCGAGAAGCGCTGGAAATTTCGTATGGGCGACCTCGAGGATCGCCGGCTTTGGGAC
AGGTATCAAGAGGCCTATGAAGCAGCCATCCGCGAGACCAGCACCGAGTATGCCCCCTGGTATGTCATTCCGG
CCAACAAGAACTGGTACCGCAACTGGCTGGTGAGCCACATCCTGGTAGAAACCCTGGAGGGCTTGGCGATGC
AGTACCCCCAGCCCGAAACAGCCTCGGAGAAGATTGTGATCGAGTAG

***Eb*PPK2:**

UniProt: A0A3D5XRJ5

ATGATTAATATCTATAAAATCGATAAACTGAACAACTTCAACCTGAACAACCACAAAACCGATGATTATAGCCT
GTGCAAAGATAAAGATACCGCACTGGAACTGACCCAGAAAAACATTCAGAAAATCTATGATTATCAGCAGAAG
CTGTACGCCGAAAAGAAAGAAGGTCTGATTATTGCATTTCAGGCAATGGATGCAGCAGGTAAAGATGGCACC
ATTCGTGAAGTTCTGAAAGCACTGGCACCGCAGGGTGTTCATGAAAAACCGTTTAAAAGCCCGAGCAGCACCG
AACTGGCACATGATTATCTGTGGCGTGTTCATAATGCAGTTCCGGAAAAAGGTGAAATCACCATTTTTAACCGC
AGCCACTATGAAGATGTGCTGATTGGTAAAGTGAAAGAACTGTACAAGTTCCAGAATAAAGCCGATCGCATTG
ATGAAAACACCGTGGTTGATAATCGCTATGAGGATATTCGCAACTTCGAGAAATATCTGTATAATAATAGCGTG
CGCATCATCAAAATCTTTCTGAACGTTAGCAAGAAAGAACAGGCAGAACGTTTTCTGAGCCGTATTGAAGAAC
CGGAAAAGAACTGGAAATTTAGCGATAGCGATTTTGAGGAACGTGTGTATTGGGATAAATATCAGCAGGCAT
TTGAAGATGCCATTAATGCAACCAGCACCAAAGATTGTCCGTGGTATGTTGTTCCGGCAGATCGTAAATGGTAT
ATGCGTTATGTTGTGAGCGAAATCGTTGTGAAAACCCTGGAAGAAATGAATCCGAAATATCCGACCGTTACCA
AAGAAACACTGGAACGTTTTGAAGGTTATCGTACCAAACTGCTGGAAGAGTATAACTATGATCTGGATACCAT
TCGTCCGATCGAGAAATGA

***Ch*PPK2:**

UniProt: A0A6N4SMB5 (codon optimized)

ATGGCAACCGATTTTAGCAAACTGAGCAAATATGTTGAAACCCTGCGTGTTAAACCGAAACAGAGCATTGATC
TGAAAAAAGATTTCGACACCGACTACGATCATAAAATGCTGACCAAAGAAGAAGGCGAAGAACTGCTGAATCT
GGGTATTTCAAAACTGAGCGAGATCCAAGAAAAACTGTATGCAAGCGGCACCAAAAGCGTTCTGATTGTTTTT

CAGGCAATGGATGCAGCAGGTAAAGATGGCACCGTTAAACATATTATGACCGGTCTGAATCCGCAGGGTGTT
AAAGTTACCAGCTTTAAAGTTCCGAGCAAAATCGAACTGAGCCATGATTATCTGTGGCGTCATTATGTTGCACT
GCCTGCAACCGGTGAAATTGGTATCTTTAATCGTAGCCACTATGAAAATGTTCTGGTTACCCGTGTTCATCCGG
AATATCTGCTGAGCGAACAGACCAGCGGTGTTACCGCAATTGAACAGGTTAATCAGAAATTCTGGGATAAACG
CTTTCAGCAGATCAACAACTTTGAACAGCATATTAGCGAAAACGGCACCATTGTGCTGAAATTCTTTCTGCATG
TGAGCAAGAAAGAGCAGAAAAAACGCTTTATTGAACGCATCGAACTGGACACCAAAAACTGGAAATTTAGCA
CAGGCGATCTGAAAGAACGTGCACATTGGAAAGATTATCGCAACGCCTATGAAGATATGCTGGCAAATACCAG
CACCAAACAGGCACCGTGGTTTGTTATTCCGGCAGATGATAAATGGTTTACCCGTCTGCTGATTGCCGAAATTA
TCTGTACCGAACTGGAAAAACTGAATCTGACCTTTCCGACCGTTAGCCTGGAACAGAAAGCAGAGCTGGAAAA
AGCAAAAGCAGAACTGGTTGCCGAAAAAAGCAGCGATTAA

SUMO t*hsc*GAS

UniProt: SUMO: Q12306; human cGAS: Q8N884

ATGTCGGACTCAGAAGTCAATCAAGAAGCTAAGCCAGAGGTCAAGCCAGAAGTCAAGCCTGAGACTCACATC
AATTTAAAGGTGTCCGATGGATCTTCAGAGATCTTCTTCAAGATCAAAAAGACCACTCCTTTAAGAAGGCTGAT
GGAAGCGTTCGCTAAAAGACAGGGTAAGGAAATGGACTCCTTAAGATTCTTGTACGACGGTATTAGAATTCAA
GCTGATCAGACCCCTGAAGATTTGGACATGGAGGATAACGATATTATTGAGGCTCACAGAGAACAGATTGGT
GGTATGGGGGCCTCGAAGCTCCGGGCGGTTTTGGAGAAGTTGAAGCTCAGCCGCGATGATATCTCCACGGCG
GCGGGGATGGTGAAAGGGGTTGTGGACCACCTGCTGCTCAGACTGAAGTGCGACTCCGCGTTCAGAGGCGTC
GGGCTGCTGAACACCGGGAGCTACTATGAGCACGTGAAGATTTCTGCACCTAATGAATTTGATGTCATGTTTA
AACTGGAAGTCCCCAGAATTCAACTAGAAGAATATTCCAACACTCGTGCATATTACTTTGTGAAATTTAAAAGA
AATCCGAAAGAAAATCCTCTGAGTCAGTTTTTAGAAGGTGAAATATTATCAGCTTCTAAGATGCTGTCAAAGTT
TAGGAAAATCATTAAGGAAGAAATTAACGACATTAAAGATACAGATGTCATCATGAAGAGGAAAAGAGGAGG
GAGCCCTGCTGTAACACTTCTTATTAGTGAAAAAATATCTGTGGATATAACCCTGGCTTTGGAATCAAAAAGTA
GCTGGCCTGCTAGCACCCAAGAAGGCCTGCGCATTCAAAACTGGCTTTCAGCAAAAGTTAGGAAGCAACTACG
ACTAAAGCCATTTTACCTTGTACCCAAGCATGCAAAGGAAGGAAATGGTTTCCAAGAAGAAACATGGCGGCTA
TCCTTCTCTCACATCGAAAAGGAAATTTTGAACAATCATGGAAAATCTAAAACGTGCTGTGAAAACAAAGAAG
AGAAATGTTGCAGGAAAGATTGTTTAAAACTAATGAAATACCTTTTAGAACAGCTGAAAGAAAGGTTTAAAGA
CAAAAAACATCTGGATAAATTCTCTTCTTATCATGTGAAAACTGCCTTCTTTCACGTATGTACCCAGAACCCTCA
AGACAGTCAGTGGGACCGCAAAGACCTGGGCCTCTGCTTTGATAACTGCGTGACATACTTTCTTCAGTGCCTCA
GGACAGAAAAACTTGAGAATTATTTTATTCCTGAATTCAATCTATTCTCTAGCAACTTAATTGACAAAAGAAGTA
AGGAATTTCTGACAAAGCAAATTGAATATGAAAGAAACAATGAGTTTCCAGTTTTTGATGAATTTTGATAA