ENGINEERED GUANYLATE KINASE VARIANT ENZYMES

[0001] The present application claims priority to US Prov. Pat. Appln. Ser. No. 63/170,004, filed April 2, 2021, which is incorporated by reference in its entirety, for all purposes.

FIELD OF THE INVENTION

[0002] The present invention provides engineered guanylate kinase (GK) enzymes, polypeptides having GK activity, and polynucleotides encoding these enzymes, as well as vectors and host cells comprising these polynucleotides and polypeptides. Methods for producing GK enzymes are also provided. The present invention further provides compositions comprising the GK enzymes and methods of using the engineered GK enzymes. The present invention finds particular use in the production of pharmaceutical compounds.

REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0003] The official copy of the Sequence Listing is submitted concurrently with the specification as an ASCII formatted text file via EFS-Web, with a file name of "CX2-202WO1_ST25.txt", a creation date of March 29, 2022, and a size of 824 kilobytes. The Sequence Listing filed via EFS-Web is part of the specification and incorporated in its entirety by reference herein.

BACKGROUND OF THE INVENTION

[0004] The Stimulator of Interferon Genes (STING) pathway, which regulates the body's immune response to foreign DNA, has emerged as an important target for cancer therapies. In particular, cyclic dinucleotides that are known to activate STING are attractive targets to activate or enhance the innate immune response. Cyclic dinucleotides may be generated enzymatically by cyclization of nucleoside triphosphate substrates. cGAMP, a cyclic dinucleotide, is known to function as a second messenger that stimulates innate immunity through the endoplasmic reticulum sensor STING. cGAMP activation of STING was demonstrated to have an anti-tumor effect in mice through induction of production of interferon and stimulation of dendritic cells (Li et al., Sci. Rep. 6:19049 [2016]) cGAMP is produced in response to double-stranded DNA (dsDNA) by cyclic GMP-AMP synthase (cGAS) (Gao et al., Cell. 153:1094–1107 [2013]), which functions as a cellular sensor of foreign nucleic acids. cGAS activity requires binding of dsDNA, with single-stranded DNA (ssDNA) or RNA ligands producing only weak or no activity, respectively. Binding to dsDNA causes conformational changes in cGAS to induce the active enzyme state (Kranzusch et al., Cell Rep. 3(5): 1362–1368 [2013]).

[0005] Several cGAS crystal structures have been generated, including murine and human cGAS. These crystal structures reveal several conserved domains, including a nucleotidyltransferase core, a zinc finger domain, and a C-terminal domain (Gao et al., Cell. 153(5): 1094-1107 [2013]; Kranzusch et al., Cell Rep. 3(5): 1362–1368 [2013]). In addition to bound dsDNA, cGAS requires a divalent metal cation (typically

[0013] In some additional embodiments, the present invention provides engineered guanylate kinases, wherein the engineered guanylate kinases comprise polypeptide sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO: 4 and/or 46. In some embodiments, the engineered guanylate kinase comprises a variant engineered guanylate kinase set forth in SEQ ID NO: 4 and/or SEQ ID NO: 46.

[0014] The present invention also provides engineered guanylate kinases wherein the engineered guanylate kinases comprise polypeptide sequences that are at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered guanylate kinase variant set forth in the even numbered sequences of SEQ ID NOs: 4-552.

[0015] The present invention further provides engineered guanylate kinases, wherein said engineered guanylate kinases comprise at least one improved property compared to wild-type *Branchiostoma floridae* guanylate kinase. In some embodiments, the improved property comprises improved activity on a substrate. In some further embodiments, the substrate comprises Sp-3'F-3'-deoxyguanosine-5'-(1-thio)-monophosphate (F-thioGMP or compound **(6)**) and/or Sp-2'F-ara-adenosine-5'-(1-thio)-triphosphate (F-thioATP or compound **(3)**). In some additional embodiments, the improved property comprises improved production of compound Sp-3'F-3'-deoxyguanosine-5'-(1-thio)-diphosphate (F-thioGDP or compound **(7)**). In some additional embodiments, the improved property comprises improved production of compound Sp-3'F-3'-deoxyguanosine-5'-(1-thio)-triphosphate (F-thioGTP or compound **(2)**) when combined in a reaction with acetate kinase. In yet some additional embodiments, the engineered guanylate kinase is purified. The present invention also provides compositions comprising at least one engineered guanylate kinase provided herein.

[0016] The present invention also provides polynucleotide sequences encoding at least one engineered guanylate kinase provided herein. In some embodiments, the polynucleotide sequence encoding at least one engineered guanylate kinase comprises a polynucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOs: 3 and/or 45. In some embodiments, the polynucleotide sequence encoding at least one engineered guanylate kinase, comprises a polynucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOs: 3 and/or 45, wherein the polynucleotide sequence of said engineered guanylate kinase comprises at least one substitution at one or more positions. In some further embodiments, the polynucleotide sequence encoding at least one engineered guanylate kinase or a functional fragment thereof comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOs: 3 and/or 45. In yet some additional embodiments, the polynucleotide sequence is operably linked to a control sequence. In some further embodiments, the polynucleotide sequence is codon optimized. In still some additional embodiments, the polynucleotide sequence comprises a polynucleotide sequence set forth in the odd numbered sequences of SEQ ID NOs: 3-551.

[0028] Similarly, “comprise,” “comprises,” “comprising” “include,” “includes,” and “including” are interchangeable and not intended to be limiting. Thus, as used herein, the term “comprising” and its cognates are used in their inclusive sense (*i.e.*, equivalent to the term “including” and its corresponding cognates).

[0029] It is to be further understood that where descriptions of various embodiments use the term “comprising,” those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language “consisting essentially of” or “consisting of.”

[0030] As used herein, the term “about” means an acceptable error for a particular value. In some instances, “about” means within 0.05%, 0.5%, 1.0%, or 2.0%, of a given value range. In some instances, “about” means within 1, 2, 3, or 4 standard deviations of a given value.

[0031] As used herein, “EC” number refers to the Enzyme Nomenclature of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). The IUBMB biochemical classification is a numerical classification system for enzymes based on the chemical reactions they catalyze.

[0032] As used herein, “ATCC” refers to the American Type Culture Collection whose biorepository collection includes genes and strains.

[0033] As used herein, “NCBI” refers to National Center for Biological Information and the sequence databases provided therein.

[0034] As used herein, “guanylate kinase” (“GK”) enzymes are enzymes that catalyze the interconversion of GDP and GMP using ATP as a phosphate group donor. GK enzymes may also catalyze phosphoryl transfer of modified guanosine nucleosides, including thiolated or fluorinated nucleosides. An example of this is the conversion of Sp-3’F-3’-deoxyguanosine-5’-(1-thio)-monophosphate (F-thioGMP or compound **(6)**) to Sp-3’F-3’-deoxyguanosine-5’-(1-thio)-diphosphate (F-thioGDP or compound **(7)**) using Sp-2’F-ara-adenosine-5’-(1-thio)-triphosphate (F-thioATP or compound **(3)**) as the phosphate donor with acetyl phosphate and acetate kinase (AcK) as the recycling system. GK enzymes may be naturally occurring, including the wild-type GK enzyme of *Branchiostoma floridae* or other guanylate kinases found in humans, bacteria, fungi, plants or other species, or the GK enzymes may be engineered polypeptides generated by human manipulation.

[0035] “Protein,” “polypeptide,” and “peptide” are used interchangeably herein to denote a polymer of at least two amino acids covalently linked by an amide bond, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Included within this definition are D- and L-amino acids, and mixtures of D- and L-amino acids, as well as polymers comprising D- and L-amino acids, and mixtures of D- and L-amino acids.

[0036] “Amino acids” are referred to herein by either their commonly known three-letter symbols or by the one-letter symbols recommended by IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single letter codes.

DNA sequence (e.g., transgene) to drive expression in a host cell, and in some embodiments, also comprises a transcription terminator sequence.

[0117] As used herein, the term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, and post-translational modification. In some embodiments, the term also encompasses secretion of the polypeptide from a cell.

[0118] As used herein, the term “produces” refers to the production of proteins and/or other compounds by cells. It is intended that the term encompass any step involved in the production of polypeptides including, but not limited to, transcription, post-transcriptional modification, translation, and post-translational modification. In some embodiments, the term also encompasses secretion of the polypeptide from a cell.

[0119] As used herein, an amino acid or nucleotide sequence (e.g., a promoter sequence, signal peptide, terminator sequence, etc.) is "heterologous" to another sequence with which it is operably linked if the two sequences are not associated in nature. For example, a “heterologous polynucleotide” is any polynucleotide that is introduced into a host cell by laboratory techniques, and includes polynucleotides that are removed from a host cell, subjected to laboratory manipulation, and then reintroduced into a host cell.

[0120] As used herein, the terms “host cell” and “host strain” refer to suitable hosts for expression vectors comprising DNA provided herein (e.g., the polynucleotides encoding the guanylate kinase variants). In some embodiments, the host cells are prokaryotic or eukaryotic cells that have been transformed or transfected with vectors constructed using recombinant DNA techniques as known in the art.

[0121] The term “analogue” means a polypeptide having more than 70% sequence identity but less than 100% sequence identity (e.g., more than 75%, 78%, 80%, 83%, 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity) with a reference polypeptide. In some embodiments, analogues mean polypeptides that contain one or more non-naturally occurring amino acid residues including, but not limited, to homoarginine, ornithine and norvaline, as well as naturally occurring amino acids. In some embodiments, analogs also include one or more D-amino acid residues and non-peptide linkages between two or more amino acid residues. The term “analogue,” when used in reference to a chemical structure or compound, refers to a similar chemical structure or compound, wherein one or more substituents or chemical groups have been substituted as compared to the reference chemical structure or compound.

[0122] The term “effective amount” means an amount sufficient to produce the desired result. One of general skill in the art may determine the effective amount by using routine experimentation.

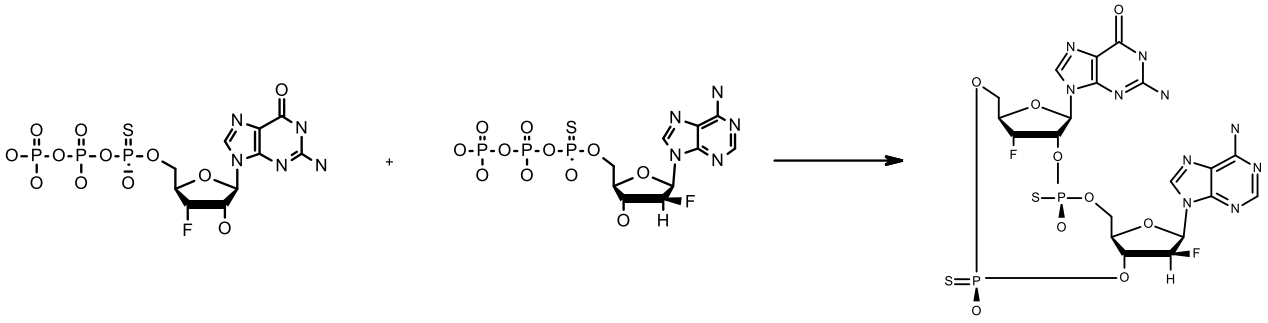
[0123] The terms “isolated” and “purified” are used to refer to a molecule (e.g., an isolated nucleic acid, polypeptide, etc.) or other component that is removed from at least one other component with which it is

[00136] In some embodiments, the engineered enzymes produce nucleoside diphosphates. In some embodiments, the nucleoside diphosphates are substrates for an acetate kinase that produces nucleoside triphosphates. In some embodiments, the engineered enzymes produce nucleoside diphosphates as precursor compounds to nucleoside triphosphates. In some embodiments, the present invention provides enzymes that are useful in producing compounds for the *in vitro* enzymatic synthesis of the non-natural nucleoside diphosphate of compound **(7)**. In some embodiments, the present invention provides enzymes that are useful in producing compound **(7)**, which is converted through *in vitro* enzymatic synthesis to the non-natural cyclic dinucleotide of compound **(1).**

[00137] Nonnatural derivatives of cGAMP, including compound **(1)**, are being investigated in cancer therapy. cGAMP, a cyclic dinucleotide, is known to function as a second messenger that stimulates innate immunity through the endoplasmic reticulum sensor Stimulator of Interferon Genes (STING). cGAMP activation of STING was demonstrated to have an anti-tumor effect in mice through induction of production of interferon and stimulation of dendritic cells (Li et al., Sci. Rep. 6:19049 [2016]) cGAMP is produced in response to double-stranded DNA (dsDNA) by cyclic GMP-AMP synthase (cGAS) (Gao et al., Cell. 153:1094–1107 [2013]), which functions as a cellular sensor of foreign nucleic acids. cGAS activity requires binding of dsDNA, with single-stranded (ssDNA) or RNA ligands producing only weak or no activity, respectively. Binding to dsDNA induces conformational changes in cGAS to the active enzyme state (Kranzusch et al., Cell Rep. 3(5): 1362–1368 [2013]).

[00138] Several cGAS crystal structures have been generated, including murine and human cGAS. These crystal structures reveal several conserved domains, including a nucleotidyltransferase core, a zinc finger domain, and a C-terminal domain (Gao et al., Cell. 153(5): 1094-1107 [2013]; Kranzusch et al., Cell Rep. 3(5): 1362–1368 [2013]). In addition to bound dsDNA, cGAS requires a divalent metal cation (typically Mn^{2+} or Mg^{2+}) for activity. cGAS is capable of producing several diastereomeric forms of cGAMP, depending on whether (s) or (r) nucleoside triphosphates are used as substrates. The desired diastereomeric form is (r,r) (compound **(1)**), which is produced by cGAS conversion of Sp-3'Fluoro-3'-deoxyguanosine-5'-(1-thio)-triphosphate (F-thioGTP or compound **(2)**) and Sp-2'Fluoro-ara-adenosine-5'-1-thio-triphosphate (F-thioATP or compound **(3)**).

Scheme 1: Production of Compound (1) by cGAS



hosts into which they are to be introduced. In some embodiments, the various nucleic acid and control sequences described herein are joined together to produce recombinant expression vectors which include one or more convenient restriction sites to allow for insertion or substitution of the nucleic acid sequence encoding the enzyme polypeptide at such sites. Alternatively, in some embodiments, the nucleic acid sequence of the present invention is expressed by inserting the nucleic acid sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In some embodiments involving the creation of the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

[0189] The recombinant expression vector may be any suitable vector (e.g., a plasmid or virus), that can be conveniently subjected to recombinant DNA procedures and bring about the expression of the enzyme polynucleotide sequence. The choice of the vector typically depends on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

[0190] In some embodiments, the expression vector is an autonomously replicating vector (i.e., a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, such as a plasmid, an extra-chromosomal element, a minichromosome, or an artificial chromosome). The vector may contain any means for assuring self-replication. In some alternative embodiments, the vector is one in which, when introduced into the host cell, it is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, in some embodiments, a single vector or plasmid, or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, and/or a transposon is utilized.

[0191] In some embodiments, the expression vector contains one or more selectable markers, which permit easy selection of transformed cells. A "selectable marker" is a gene, the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Examples of bacterial selectable markers include, but are not limited to the dal genes from Bacillus subtilis or Bacillus licheniformis, or markers, which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance. Suitable markers for yeast host cells include, but are not limited to ADE2, HIS3, LEU2, LYS2, MET3, TRP1, and URA3. Selectable markers for use in filamentous fungal host cells include, but are not limited to, amdS (acetamidase; e.g., from *A. nidulans* or *A. orzyae*), argB (ornithine carbamoyltransferases), bar (phosphinothricin acetyltransferase; e.g., from *S. hygroscopicus*), hph (hygromycin phosphotransferase), niaD (nitrate reductase), pyrG (orotidine-5'-phosphate decarboxylase; e.g., from *A. nidulans* or *A. orzyae*), sC (sulfate adenyltransferase), and trpC (anthranilate synthase), as well as equivalents thereof.

[0192] In another aspect, the present invention provides a host cell comprising at least one polynucleotide encoding at least one engineered enzyme polypeptide of the present invention, the polynucleotide(s) being operatively linked to one or more control sequences for expression of the engineered enzyme(s) in the host cell. Host cells suitable for use in expressing the polypeptides

[0255] Directed evolution began with the polynucleotide set forth in SEQ ID NO: 3. Libraries of engineered polypeptides were generated using various well-known techniques (e.g., saturation mutagenesis, recombination of previously identified beneficial amino acid differences) and screened using HTP assay and analysis methods that measured the polypeptides ability to facilitate the recycling of ATP via ADP and acetyl phosphate (as measured via the conversion of F-thioGMP **(6)** substrate to F-thioGTP **(2)** product under ATP limited conditions).

[0256] The enzyme assay was carried out in 96-well deep-well (2mL) plates, in 100 μL total volume/well, which included 10 μL HTP enzyme lysate and 90 μL of a master mix containing 55 mM F-thioGMP **(6)**, 5mM F-thioATP **(3)**, 200mM acetyl phosphate, 30mM $MgCl_2$, 8g/L WT acetate kinase from *Thermotoga maritima,* and MilliQ water. The reactions were performed by first aliquoting 90 μL of a master mix containing 55 mM F-thioGMP **(6)**, 5mM F-thioATP **(3)**, 200mM acetyl phosphate, 30mM $MgCl_2$, 8g/L WT acetate kinase, and MilliQ water to each well. The reactions were initiated by using 10 μL HTP lysate. The reaction plate was heat-sealed and shaken at 500 rpm at 25 °C for 3 hours.

[0257] After incubation (~3 hours), 400μL 10mM Tris, pH 7.5 were mixed with the samples. For chiral analysis, HTP reactions were quenched with 1:1 volume equivalent of acetonitrile and shaken for 5mins on tabletop shakers before centrifugation at 4,000rpm for 15mins at 4°C to remove solids. The quenched reactions were diluted by transferring 10 μL of the supernatant into 990 μL of water in a new 96-well deep-well plate. Then, 150 μL of the diluted quenched reaction were transferred into a 96-well Co-star round bottom plate for UPLC analysis, using the method described below in Table 2-3.

[0258] Hit variants were grown in 250-mL shakeflasks and enzyme powders generated. The activity of the enzyme powders was evaluated at 0.15 – 24.0 wt% of the SF Powder, 21 g/L F-thioGMP **(6)**, 5mM F-thioATP **(3)**, 30mM $MgCl_2$, 200mM acetyl phosphate, and 8g/L AcK. 100μL of the reaction mix were incubated at 25-60ºC for 3 hours, with shaking at 500 rpm, using a similar assay as described above.

Table 2-1. Guanylate Kinase Variant Activity Relative to SEQ ID NO: 4

SEQ ID NO: (nt/aa)	Amino Acid Differences (Relative to SEQ ID NO: 4)	Conversion Fold Improvement (Relative to SEQ ID NO: 4)[1]
5/6	V42R	+++
7/8	V42C	+++
9/10	V42T	++
11/12	V42A	++
13/14	N115T	++
15/16	A150E	+
17/18	K144A	+
19/20	K153L	+
21/22	K144G	+

Table 2-1. Guanylate Kinase Variant Activity Relative to SEQ ID NO: 4		
SEQ ID NO: (nt/aa)	**Amino Acid Differences (Relative to SEQ ID NO: 4)**	**Conversion Fold Improvement (Relative to SEQ ID NO: 4)[1]**
23/24	S41C	+
25/26	T176L	+
27/28	K153S	+
29/30	S41V	+
31/32	S83A	+
33/34	A157L	+
35/36	V116M	+
37/38	L77Y	+
39/40	N115S	+
41/42	S142N	+
43/44	H60Y	+
[1] Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4 and defined as follows: "+" 1.2 to 2.00, "++" > 2.00, "+++" > 2.70		

Table 2-2. Guanylate Kinase Variant Activity Relative to SEQ ID NO: 4		
SEQ ID NO: (nt/aa)	**Amino Acid Differences (Relative to SEQ ID NO: 4)**	**Conversion Fold Improvement (Relative to SEQ ID NO: 4)[1]**
45/46	F82H/N115T/K144G/K193D/M200G	+++
47/48	F82H/N115T/V116W/K144G/K193E/M200G	+++
49/50	F82H/N115T/K193D	+++
51/52	F82H/V116W/K144G/K193S	+++
53/54	F82H/N100C/N115T/V116W/K144G	++
55/56	F82H/V116W/K144G/N160E	++
57/58	F82H/N115T/V116W/Q172D	++
59/60	F82H/N115T/N160E	++
61/62	N115T/K144G/N160E	++
63/64	F82H/N115T/N160W	++
65/66	F82H/N100P/K144G/N160W/M200G	++
67/68	F82H/N115T/V116W	++
69/70	N115T/K144G/K193E	++

Table 2-2. Guanylate Kinase Variant Activity Relative to SEQ ID NO: 4		
SEQ ID NO: (nt/aa)	**Amino Acid Differences (Relative to SEQ ID NO: 4)**	**Conversion Fold Improvement (Relative to SEQ ID NO: 4)[1]**
71/72	F82H/N100C/K144G/K193D	++
73/74	F82H/K144G/K193S	++
75/76	F82H/N100P/N115T/V116W/N160E/K193D	++
77/78	N115T/K144G	++
79/80	F82H/K144G	+
81/82	F82H/N100C/V116W/N160E	+
83/84	F82H/N100C/V116W/M200G	+
85/86	F82H/V116W/K193D	+
87/88	F82H/V116W	+
89/90	F82H/N160E/K193E/M200G	+
91/92	F82H/K193E	+
93/94	F82H/K193D	+
95/96	F82H/Q172D	+
97/98	F82H/N100P/M200G/G203A	+
99/100	F82H/M200G	+
101/102	F82H/N100C	+
103/104	F82H/K193D/G203A	+
105/106	F82H	+
107/108	K91M/K144G	+
109/110	F82H/K193S/G203A	+
111/112	F82H/N160W/Q172D	+
113/114	N115T/M200G	+
115/116	N115T/K193D	+
117/118	F82H/N160E/D179H	+
119/120	N115T/K193E	+
121/122	N115T	+
123/124	N100C/K144G/K193D	+
125/126	K144G/M200G	+
127/128	K144G/K193S/M200G	+

Table 2-2. Guanylate Kinase Variant Activity Relative to SEQ ID NO: 4		
SEQ ID NO: (nt/aa)	**Amino Acid Differences (Relative to SEQ ID NO: 4)**	**Conversion Fold Improvement (Relative to SEQ ID NO: 4)[1]**
129/130	N100C/K144G/Q172D	+
131/132	K144G	+
133/134	K144G/K193S	+
[1] Levels of increased activity were determined relative to the reference polypeptide of SEQ ID NO: 4 and defined as follows: "+" 1.00 to 3.00, "++" > 3.00, "+++" > 5.00		

Table 2-3. UPLC Parameters	
Instrument	**Thermo Fisher UltiMate 3000**
Column	Waters Xselect Peptide HSS T3 XP 2.5µm 2.1x100mm column
Mobile Phase	Gradient (A: 100 mM Triethylammonium acetate in water pH 5.2; B: acetonitrile Time(min) %B 0.00 5.0 1.50 5.0 2.00 30.0 2.10 5.0 2.75 5.0
Flow Rate	1.2 mL/min
Run time	2.75 min
Peak Retention Times	F-thioGMP **(6)** substrate observed at 0.489min F-thioGDP **(7)** product observed at 0.599min Sp-3'F-thioGTP **(2)** product observed at 0.852min Rp-3'F-thioGTP product observed at 1.127min
Column Temperature	45°C
Injection Volume	5 µL
UV Detection	254 nm

EXAMPLE 3

Evolution and Screening of Engineered Polypeptides Derived from SEQ ID NO: 46 for Improved Guanylate Kinase (GK) Activity

[0259] The engineered polynucleotide (SEQ ID NO: 45) encoding the polypeptide with guanylate kinase activity of SEQ ID NO: 46 was used to generate the engineered polypeptides of Table 3-1 and 3-2. These polypeptides displayed improved the guanylate kinase activity under the desired conditions (e.g., the