strain and plasmid	description	source or reference
E. coli JM109	cloned host	laboratory stock
B. subtilis 168	expression host	laboratory stock
B. subtilis 168/pMA5-*gsj*	*B. subtilis* 168 derivative with pMA5-*gsj*, Kanr	this work
B. subtilis 168/pMA5-*agl*	*B. subtilis* 168 derivative with pMA5-*agl*, Kanr	this work
B. subtilis 168/pMA5-SpyTag-AGL-SpyCatcher	*B. subtilis* 168 derivative with pMA5-SpyTag-AGL-SpyCatcher, Kanr	this work
B. subtilis 168/pMA5-SnoopTag-AGL-SnoopCatcher	*B. subtilis* 168 derivative with pMA5-SnoopTag-AGL-SnoopCatcher, Kanr	this work
B. subtilis 168/pMA5-SdyTag-AGL-SdyCatcher	*B. subtilis* 168 derivative with pMA5-SdyTag-AGL-SdyCatcher, Kanr	this work
B. subtilis 168/pMA5- RIAD-AGL-RIDD	B. *subtilis* 168 derivative with pMA5-RIAD-AGL-RIDD, Kanr	this work
pMA5-*gsj*	Recombinant vector containing *gsj*	this work
pMA5-*agl*	Recombinant vector containing *agl*	this work
pMA5-SpyTag-AGL-SpyCatcher	Recombinant vector SpyTag-*agl*-SpyCatcher	this work
pMA5-SnoopTag-AGL-SnoopCatcher	Recombinant vector SnoopTag-*agl*-SnoopCatcher	this work
pMA5-SdyTag-AGL-SdyCatcher	Recombinant vector SdyTag-*agl*-SdyCatcher	this work
pMA5- RIAD-AGL-RIDD	Recombinant vector RIAD-*agl*-RIDD	this work

TABLE S2. Primers used to construct the cyclized proteins

Primer	sequence (5′→3′)
Spy-F	GGTCGATGCATATAAACCCACCAAAGGTGGTGGTGGTTCAGG TGGTGGTGGTTCAGGTGGTGGTGGTTCACTGTATCAGAAAACG AGCGAAAAAATTG
Spy-R	ACTTTTTGCATTCTACAAACTGCATAACTTTAGATGTGCGCATC GCCTT
Snoop-F	AAACTGGGCGATATTGAATTTATTAAAGTGAACAAAGGTGGT GGTGGTTCAGGTGGTGGTGGTTCAGGTGGTGGTGGTTGTGGTC TGTATCAGAAAACGAGCGAA
Snoop-R	TTTGCATTCTACAAACTGCATAACTTTATTTCGGCGGTATCGGT TCATT
Sdy-F	GACCCGATCGTTATGATCGATAACGATAAACCCATCACCGGT GGTGGTGGTTCAGGTGGTGGTGGTTCAGGTGGTGGTGGTTGTG GTCTGTATCAGAAA T
Sdy-R	TTGCATTCTACAAACTGCATAACTTTAGGAATCCACCCAGATC TGACC
AD-F	ACATATGCATCATCATCATCATCACGGTGGTGGTGGCTCCGGC GGTGGCGGTTCTGGTGGTGGTGGCTCCCTGGAACAGTATGCA AATCA
AD-R	GCATTCTACAAACTGCATAACTTTATTTGGCTTCTTCTTTTTCC AGACG
SpyK11A-F	TATGGTCGATGCATATGCACCCACCAAA
SpyK11A-R	TTTGGTGGGTGCATATGCATCGACCATA

TABLE S3. Amino acid sequence of cyclized protein

Protein	sequence (N-terminal → C-terminal)
SpyTag-AGL-SpyCather	MAHIVMVDAYKPTKGGGGSGGGGSGGGGSLYQKTSEKIVVRNEGKKLEL RVLGDKIINVFVSNKEEKRKDTIAIERKEYDTPEFSISDELESILIETNSLKVKI NKNDLSVSFLDKNGNIINEDYNGGAKFNETDVRCYKKLREDHFYGFGEKA GYLDKKGERLEMWNTDEFMTHNQTTKLLYESYPFFIGMNDYHTYGIFLDN SFRSFFDMGQESQEYYFFGAYGGQMNYYFIYGEDIKEVVENYTYLTGRISL PPLWVLGNQQSRYSYTPQERVLEVAKTFREKDIPCDVIYLDIDYMEGYRVF TWNKETFKNHKEMLKQLKEMGFKVVTIVDPGVKRDYDYHVYREGIEKGY FVKDKYGITYVGKVWPGEACFPDFLQEEVRYWWGEKHREFINDGIDGIWN DMNEPAVFETPTKTMPEDNIHILDGEKVLHKEAHNVYANYMAMATRDGFL RIRPNERPFVLTRAAFSGIQRYAAMWTGDNRSLYEHLLMMMPMLMNIGLS GQPFVGADVGGFEGDCHEELFIRWIEAAVFTPFLRVHSAIGTKDQEPWSFG KRAEDISRKYIKMRYELLPYLYDLFYIASQKGYPIMRPLVFEYQKDENTHKI YDEFMFGEGLLVAPVYLPSKERREVYLPEGIWYDYWTGKGFKGKNYYLV

	DAPIEVIPLFVKEGGILLKQQPQSFIGEKKLEELTVEIYKGKEGHYLHYEDD GKSFDYTKGVYNLFDISFCYKEGRMDIKFDKIHFGYDKGVKKYKFIFKNFD DIKEIKINGEKVEKESCEIELGGGGSGGGGSGGGGSVDTLSGLSSEQGQSGD MTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYL YPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHI
SnoopTag-AGL-SnoopCather	MKLGDIEFIKVNKQHPDYPDIYGAIDQNHGGGGSGGGGSGGGGSLYQKTS EKIVVRNEGKKLELRVLGDKIINVFVSNKEEKRKDTIAIERKEYDTPEFSISD ELESILIETNSLKVKINKNDLSVSFLDKNGNIINEDYNGGAKFNETDVRCYK KLREDHFYGFGEKAGYLDKKGERLEMWNTDEFMTHNQTTKLLYESYPFFI GMNDYHTYGIFLDNSFRSFFDMGQESQEYYFFGAYGGQMNYYFIYGEDIK EVVENYTYLTGRISLPPLWVLGNQQSRYSYTPQERVLEVAKTFREKDIPCDV IYLDIDYMEGYRVFTWNKETFKNHKEMLKQLKEMGFKVVTIVDPGVKRD YDYHVYREGIEKGYFVKDKYGITYVGKVWPGEACFPDFLQEEVRYWWGE KHREFINDGIDGIWNDMNEPAVFETPTKTMPEDNIHILDGEKVLHKEAHNV YANYMAMATRDGFLRIRPNERPFVLTRAAFSGIQRYAAMWTGDNRSLYEH LLMMMPMLMNIGLSGQPFVGADVGGFEGDCHEELFIRWIEAAVFTPFLRV HSAIGTKDQEPWSFGKRAEDISRKYIKMRYELLPYLYDLFYIASQKGYPIMR PLVFEYQKDENTHKIYDEFMFGEGLLVAPVYLPSKERREVYLPEGIWYDYW TGKGFKGKNYYLVDAPIEVIPLFVKEGGILLKQQPQSFIGEKKLEELTVEIYK GKEGHYLHYEDDGKSFDYTKGVYNLFDISFCYKEGRMDIKFDKIHFGYDK GVKKYKFIFKNFDDIKEIKINGEKVEKESCEIELGGGGSGGGGSGGGGSKPL RGAVFSLQKQHPDYPDIYGAIDQKTYQNVRTGEDGKLTFKNLSDGKYRL FENSEPAGYKPVQNKPIVAFQIVNGEVRDVTSIVPQDIPATYEFTNGKHYITN EPIPPK
SdyTag-AGL-SdyCather	MDPIVMIDNDKPITGGGGSGGGGSGGGGSLYQKTSEKIVVRNEGKKLELRV LGDKIINVFVSNKEEKRKDTIAIERKEYDTPEFSISDELESILIETNSLKVKINK NDLSVSFLDKNGNIINEDYNGGAKFNETDVRCYKKLREDHFYGFGEKAGY LDKKGERLEMWNTDEFMTHNQTTKLLYESYPFFIGMNDYHTYGIFLDNSF RSFFDMGQESQEYYFFGAYGGQMNYYFIYGEDIKEVVENYTYLTGRISLPP LWVLGNQQSRYSYTPQERVLEVAKTFREKDIPCDVIYLDIDYMEGYRVFTW NKETFKNHKEMLKQLKEMGFKVVTIVDPGVKRDYDYHVYREGIEKGYFV KDKYGITYVGKVWPGEACFPDFLQEEVRYWWGEKHREFINDGIDGIWND MNEPAVFETPTKTMPEDNIHILDGEKVLHKEAHNVYANYMAMATRDGFLR IRPNERPFVLTRAAFSGIQRYAAMWTGDNRSLYEHLLMMMPMLMNIGLSG QPFVGADVGGFEGDCHEELFIRWIEAAVFTPFLRVHSAIGTKDQEPWSFGK RAEDISRKYIKMRYELLPYLYDLFYIASQKGYPIMRPLVFEYQKDENTHKIY DEFMFGEGLLVAPVYLPSKERREVYLPEGIWYDYWTGKGFKGKNYYLVD APIEVIPLFVKEGGILLKQQPQSFIGEKKLEELTVEIYKGKEGHYLHYEDDG KSFDYTKGVYNLFDISFCYKEGRMDIKFDKIHFGYDKGVKKYKFIFKNFDD IKEIKINGEKVEKESCEIELGGGGSGGGGSGGGGSLSGETGQSGNTTIEEDST THVNKFSKRDANGKELAGAMIELRNLSGQTIQSWISDGTVKVFYLMPGTY QFVETAAPEGYELAAPITFTIDEKGQIWVDS

Different colors represent different parts of the protein: Tag (green), yellow (3×GGGS),
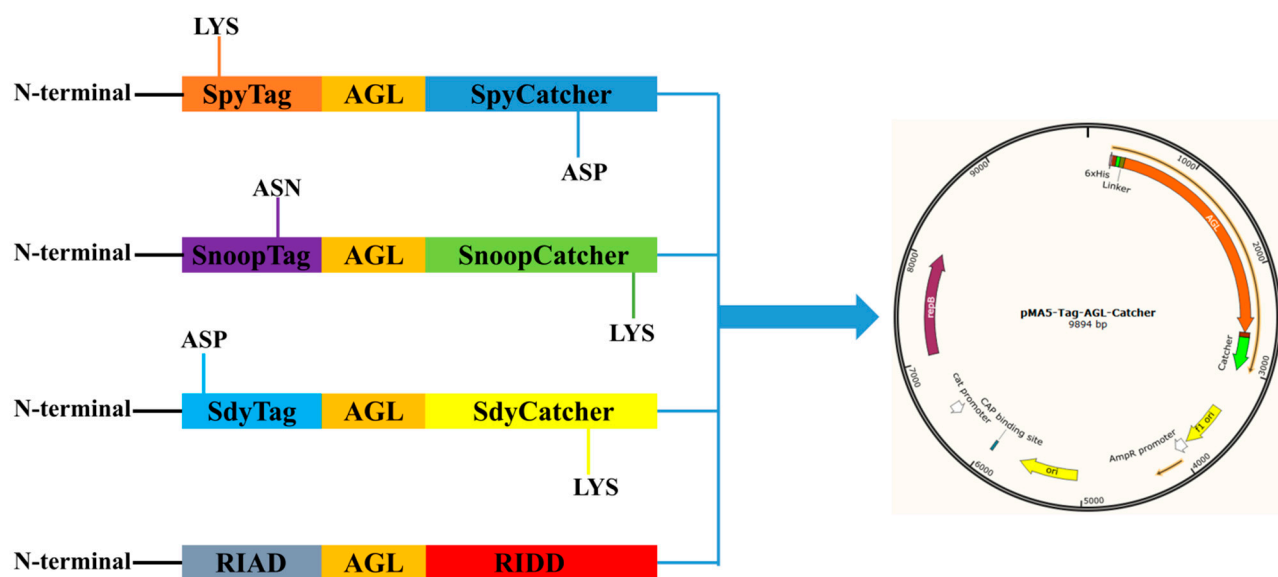red (AGL), blue (catcher), purple (location of the isopeptide bond).

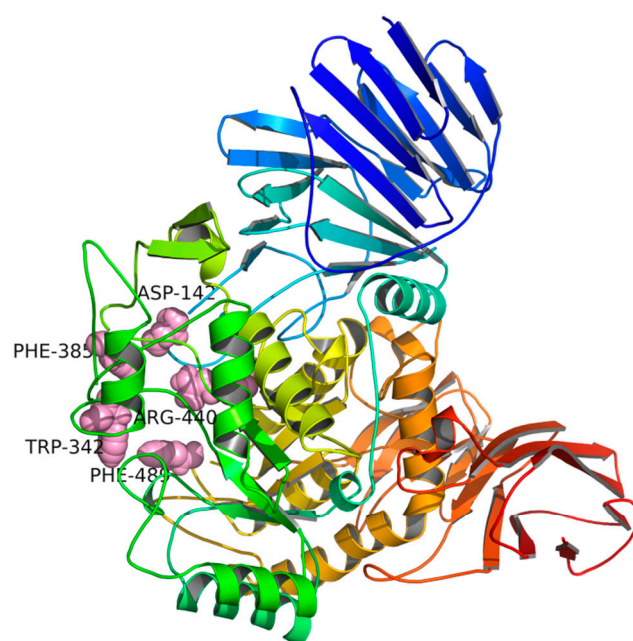**Figure S1**. Construction of recombinant plasmids for cyclized AGL.

Figure S2. Three-dimensional structure of wild-type AGL (the pink spheres highlight the enzyme active sites).
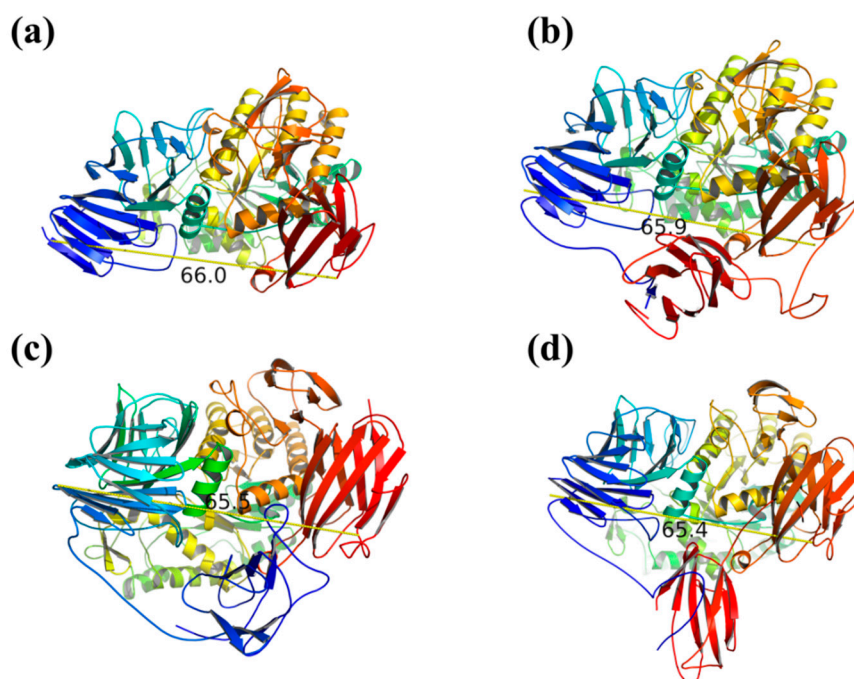
Figure S3. The distance between the terminals. (a) wild-type AGL, (b) SpyTag-AGL-SpyCatcher, (c) SnoopTag-AGL-SnoopCatcher, (d) SdyTag-AGL-SdyCatcher
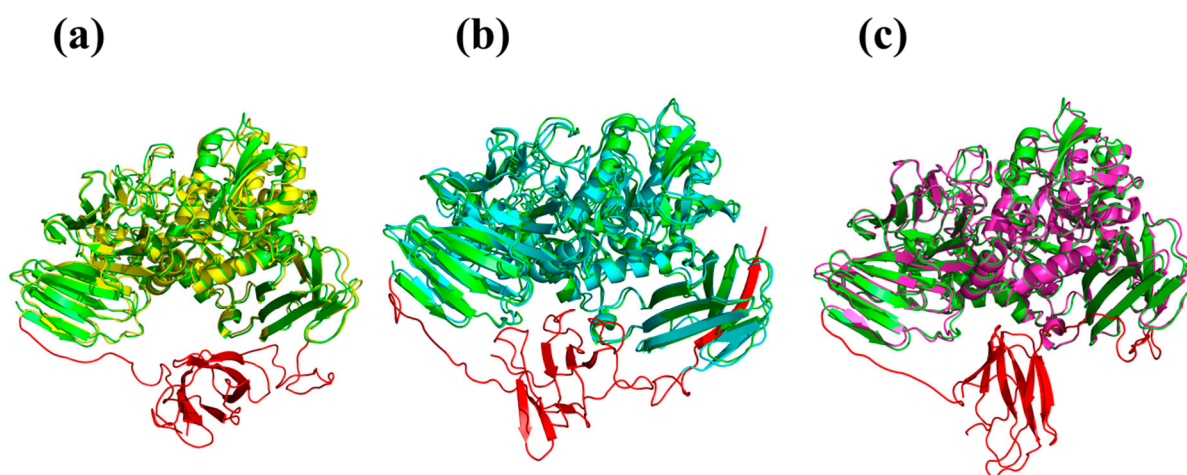

Figure S4. Structural alignment of cyclized AGL with wild-type. The 3D structure of wild-type (green) was aligned with SpyTag-AGL-SpyCatcher (yellow), SnoopTag-AGL-SnoopCatcher (blue) and SdyTag-AGL-SdyCatcher (purple) respectively. Also, the non-overlapping part (red) represents Catcher/Tag system.