

# Generalized Approach towards Secretion-Based Protein Production via Neutralization of Secretion-Preventing Cationic Substrate Residues (Supplementary Text S1, Table S1, and Table S2)

Hyunjong Byun <sup>1</sup>, Jiyeon Park <sup>2</sup>, Benedict U. Fabia <sup>2</sup>, Joshua Bingwa <sup>2</sup>, Mihn Hieu Nguyen <sup>2</sup>, Haeshin Lee <sup>3</sup>, and Jung Hoon Ahn <sup>1,2\*</sup>

1 Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea

2 Department of Chemistry and Biology, Korea Science Academy of Korea Advanced Institute of Science and Technology, Busan 47162, Republic of Korea

3 Department of Chemistry, Korea Advanced Institute of Science and Technology, Daejeon 34141, Republic of Korea

\* Correspondence: (J.H.A.) hoony@kaist.ac.kr; Tel.: (+82)10-8826-6109; (H.L.) haeshin@kaist.ac.kr; Tel.: (+82)10-8974-5141

## Text S1. Sequences of proteins in FASTA format

### Color code for enzyme sites and polypeptide features

Multiple cloning site (MCS):

XbaI: tctaga, SR NdeI: catatg, HM KpnI: ggtacc, GT

NheI: gctac, AS SacI: gagtc, EL

Factor Xa cleavage site: IEGR

LARD3 signal peptide:

GSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVG  
ADTVLSFGADSVTLVGVGLGGLWSEGVVLIS

Polyhistidine tag (His-tag): [HHHHHH](#)

>**TliA, wild type** (as a reference)

MGVFDYKNLGTEASKTLFADATAITLYHNLDNGFAVGYQQHGLGLPATLVGALLGSTDSQGVIPGIPWNP  
DSEKAALDAVHAAGWTPISASALGYGGKVDARGTFFGEKAGYTTAQAEVLGKYDDAGKLLEIGIGFRGTSGPRE  
SLITDSIGDLVSDLALAALGPKDYAKNYAGEAFGGLKTVDYAGAHGLSGKDVLVSGHSLGGLAVNSMADLSTS  
KWAGFYKDANYLAYASPTQSAGDKVLNIGYENDPVFRALDGSTFNSSLGVHDKAHESTTDNIVSFNDHYASTL  
WNVLVLPFSIANLSTWVSHLPSAYGDGMTRVLESGFYEQMTRDSTIIIVANLSDPARANTWQDLNRNAEPHTGNTF  
IIIGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKA  
VGADTVLSFGADSVTLVGVGLGGLWSEGVVLIS

>**TliA, expressed in pDART plasmid** (this is used for computational analysis) [14]

MSRMGVFDYKNLGTEASKTLFADATAITLYHNLDNGFAVGYQQHGLGLPATLVGALLGSTDSQGVIPGIP  
WNPDSEKAALDAVHAAGWTPISASALGYGGKVDARGTFFGEKAGYTTAQAEVLGKYDDAGKLLEIGIGFRGTSG  
PRESLITDSIGDLVSDLALAALGPKDYAKNYAGEAFGGLKTVDYAGAHGLSGKDVLVSGHSLGGLAVNSMADL  
STSKWAGFYKDANYLAYASPTQSAGDKVLNIGYENDPVFRALDGSTFNSSLGVHDKAHESTTDNIVSFNDHYA  
STLWNVLVLPFSIANLSTWVSHLPSAYGDGMTRVLESGFYEQMTRDSTIIIVANLSDPARANTWQDLNRNAEPHTG  
NTFIIGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDH  
AKAVGADTVLSFGADSVTLVGVGLGGLWSEGVVLISELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHN

TFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**NKC-TliA:** NKC is marked cyan. [14]

MSRHMGTAPKAMKLLKKLLQKKGIGSMGVFDYKNLGTEASKTLFADATAITLYTYHNLDNGFAVGYQQHGLGLGLPATLVGALLGSTDSQGVIPGIPWNPDSEKAALDAVHAAGWTPISASALGYGGKVARGTFFGEKAGYTTAQAEVLGKYDDAGKLEIGIGFRGTSGPRESLITDSIGDLVSDLLAALGPKDYAKNYAGEAFGGLKTVADYAGAHGLSGKDVLSGHSLGGLAVNSMADLSTKWAGFYKDANYLAYASPTQSAGDKVLNIGYENDPVFRALDGSTFNLSSLGVHDKAHESTTDNIVSFNDHYASTLWNVLPSIANLSTWVSHLPSAYGDGMTRVLESGFYEQMTRDSTIIVANLSDPARANTWQDLMRNEAEPHTGNTFIIGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**CTP-TliA:** CTP is marked cyan. [14]

MSMRGS~~HHHHH~~GMASMTGGQQMGRDLYDDDDKDRWGSMYGRRARRRRRSMAGTGGMGVF DYKNLGTEASKTLFADATAITLYTYHNLDNGFAVGYQQHGLGLPATLVGALLGSTDSQGVIPGIPWNPDSEKAALDAVHAAGWTPISASALGYGGKVARGTFFGEKAGYTTAQAEVLGKYDDAGKLEIGIGFRGTSGPRESLITDSIGDLVSDLLAALGPKDYAKNYAGEAFGGLKTVADYAGAHGLSGKDVLSGHSLGGLAVNSMADLSTKWAGFYKDANYLAYASPTQSAGDKVLNIGYENDPVFRALDGSTFNLLSLGVHDKAHESTTDNIVSFNDHYASTLWNVLPSIANLSTWVSHLPSAYGDGMTRVLESGFYEQMTRDSTIIVANLSDPARANTWQDLMRNEAEPHTGNTFIIGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**Glutathione S-transferase, wild type (GST)** [15]

MSR~~HHHHH~~HGGPPYTITYFPVVRCEAMRMLADQDQSKEEVVTMETWPPLKPSCLFRQLPKFQDGDLTLYQS NAILRHLGRSFGLYGKDQKEAALVDMVNDGVEDLRCKYATLIYTNYEAGKEKYVKELPEHLKPFETLLSQNQGGQAFVVGSKISFADYNLLRIHQVLPSCDAFPILLSAYVARLSARPKIKAFLASPEHVNRPINGNGKQELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**Glutathione S-transferase, negatively supercharged (GST(-20))** [15]

MSR~~HHHHH~~HGGPPYTITYFPVVRCEAMRMLADQDQSWEVVMTMETWPPLKPSCLFRQLPKFQDGDLTLYQS NAILRHLGRSFGLYGEDEEEAALVDMVNDGVEDLRCKYATLIYTDYEAGKEEYVEELPEHLKPFETLLSENEGGEAFVVGSEISFADYNLLRIHQVLPSCDAFPILLSAYVARLSARPEIEAFLASPEHVDRPINGNGKQELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**Glutathione S-transferase, positively supercharged (GST(+19))** – designed by us

MSR~~HHHHH~~HGGPPYTITYFPVVRCEAMRMLADQKQSKEEVVTMKTWPPLKPSCLFRQLPKFQDGKLTLYQS NAILRHLGRSFGLYGKDQKEAALVDMVNDGVEDLRCKYATLIYTNYKAGKQKYVKELPKHLKPFETLLSKNKGKKAFVVGSKISFADYNLLRIHQVLPSCDAFPILLSAYVARLSARPKIKAFLASPEHVKRPINGNGKQELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**Streptavidin, wild-type (SAv)** [15]

MSR~~HHHHH~~HGGAEGITGTWYNQLGSTFIVTAGADGALTGTYESAVGNAESRYVLTGRYDSAPATDGS GTALGWTVAWKNNYRNAHSATTWSGQYVGGAEARINTQWLTSgtTEANAWKSTLVGHDTFTKVKPSAASELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

>**Streptavidin, negatively supercharged (SAv(-10))** [15]

MSRHHHHHHGGAEGITGTWYNQLGSTFIVTAGADGALTGYESAVGDAESEYVLTGRYDSAPATDGSHTALGW  
TVAWKNDYENAHSAATTWSQYVGGAEARINTQWLLSGTTEADAWKSTLVGHDTFTKVEPSAASELIEGRGSDG  
NDLIQGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDRLDHAKAVGADTV  
LSFGADSVTLVGVGLGGLWSEGVLIS

>**Streptavidin, positively supercharged (SAv(+13))** [15]

MSRHHHHHHGAKAGITGTWYNQLGSTFIVTAGAKGALTGYESAVGNAKSRYVLTGRYDSAPATKGSGTALGW  
TVAWKNKYRNAHSATTWSQYVGGAKARINTQWLLSGTTKAKAWKSTLVGHDTFTKVKPSAASELIEGRGSDG  
NDLIQGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDRLDHAKAVGADTV  
LSFGADSVTLVGVGLGGLWSEGVLIS

>**Cutinase (Cuti)** [14]

MSRHHHHHAPTSNPAQELEARQLGRTRDDLINGNSASCADVIFIYARGSTETGNLGLGPSIASNLESAFGK  
DGVWIQGVGGAYRATLGDNALPRGTSSAAIREMLGLFQQANTKCPDATLIAGGYSQGAALAAASIEDLDSAIRD  
KIAAGTVLFGYTKNLQNRRIPNYPADRTKVFCNTGDLVCTGSLIVAAPHLAYGPDARGPAPEFLIEKVRAVRGS  
ALEELIEGRGSDGNDLIQGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTD  
LRDHAKAVGADTVLSFGADSVTLVGVGLGGLWSEGVLIS

>**Cutinase, negatively supercharged (Cuti(-))**

MSRHHHHHAPTSNPAQELEARQLGETTRDDLD**I**DGSASCADVIFIYARGSTETGNLGLGPSIASNLESAFGE  
DGVWIQGVGGAY**E**ATLGDNALPRGTSSAA**E**EMLGLFQQANTKCPDATLIAGGYSQGAALAAASIEDLDSAIRD  
KIAAGTVLFGYTKNL**E**NEGRIPNYPADRTKVFCNTGDLVCTGSLIVAAPHLAYGPDA**E**GPAPEFLIEKVRAVRGS  
ALEELIEGRGSDGNDLIQGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTD  
LRDHAKAVGADTVLSFGADSVTLVGVGLGGLWSEGVLIS

>**Chitinase (Chi)** [14]

MSRHHHHHANSPEQSQKIVGYFPSWGVYGRNYQVADIDASKLTHLNAYAFADICWNGKHGNPSTHPDNPNKQTW  
NCESGVPLQNKEVPNGTTLVGEWPADVTKSYPGSGTTWEDCDKYARCGNFGELKRLAKYPHLKIISVGWT  
WSNRFSDMAADEKTRKVFAESTVAFLRAYGFDGVLDWEYPGVETIPPGSYRPEDKQNFLLQDVRNALNKAG  
AEDGKQYLLTIASGASRRYADHTELKKISQILDWINIMTYDFHGGWEATSNHNAALYKDPNDPAANTNFYVDGA  
INVYTNEGVPDKLVLGVFYGRGWKSCGKENNGQYQPCPKPGSDGKLASKGTWDDYSTGDTGVYDYGDLAANYV  
NKNGFVRYWNNTAKPVLYNATTGTFISYDDNESMKYKTDSIKTKGLSGAMFWELSGDCRTSPKYSCKGPKL  
TLVKELLGGPINQKDTEPPTNVNIVTNKNSNSVQLNWTASTDNVGVTYEITAGEEKWSTTNSITIKNLKP  
NTEYKFSIIAKDAAGNKSQPTALT**V**KTDEANMTPPDGNGTATFSVTSNWGSGYNFSII**I**KKNNGTNPIKNWKEF  
DYSGNLTQWVDSKISSKTNHYVITNAGWNGEIPPGGSITIGGAGTGNPAEELLNAVISENELIEGRGSDGNDLI  
QGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDRLDHAKAVGADTVLSFG  
ADSVTLVGVGLGGLWSEGVLIS

>**Chitinase, negatively supercharged (Chi(-))**

MSRHHHHHANSPEQSQKIVGYFPSWGVYGRDYQVADIDASKLTHLNAYAFADICWNGEHGNPSTHPDNPNEQTW  
NCEESGVPLQNKEVPNGTTLVGEWPADVTKSYPGSGTTWEDCDEYARCGNFGELKRLKAEPHLKIISVGWT  
WSNRFSDMAADEETREVFAESTVAFL**E**AYGFDGVLDWEYPGVETIPPGSYRPEDKQNFLLQDVRNALNEAG  
AEDGEQYLLTIASGASRRYADHTELKKISQILDWINIMTYDFHGGWEATSNHNAALYKDPNDPAANTNFYVDGA  
INVYTNEGVPDKLVLGVFYGRGWKSCGENDGQYQPCPKPGSDGKLASEGTWDDYSTGDTGVYDYGDLAANYV  
DEDGFVRYWNNTAKPVLYNATTGTFISYDDNESMKYKTDSIKTKGLSGAMFWELSGDCRTSPEYSCCKGPKL  
TLVKELLGGPIDEKDTEPPTNVNIVTNKNSNSVQLNWTASTDNVGVTYEITAGEEKWSTTNSITIKNLKP  
NTEYEFSIIAKDAAGNKSQPTALT**V**KTDEADMTPPDGNGTATFSVTSNWGSGYNFSII**E**DDGTNPIKNWKEF  
DYSGNLTQWVDSKISSETNNHYVITNAGWNGEIPPGGSITIGGAGTGDPAEELLNAVISEDELIEGRGSDGNDLI  
QGGKGADFIETGGKGNDTIRDNSGHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDRLDHAKAVGADTVLSFG

ADSVTLVGVLGGLWSEGVLIS

**>MelC2 tyrosinase (MelC2)**

MSSMTVRKNQASLTAEKRRFVAALLELKRTGRYDAFVTTNAFILGDTDNGERTGHRSPSFLPWHRRFLLEFE  
RALQSVVASVALPYWDWSADRSTRSSLWAPDFLGGTGRSRDGQVMMDGPFAASAGNWPIINV  
GVSELPTRAEVDSVLAMATYDMAPWNNSGSDGFRNHLEGWRGVNLHNRVHVWVGGQMATGVSPNDPVFWLHHAYI  
DKLWAWEQRRHPSSPYLPGGTPNVVDLNEMKPWNDDTPAALLDHTRHYTFDVASELIEGRGSDGNDLIQGGK  
GADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSV  
TLVGVLGGLWSEGVLIS

**>MelC2 tyrosinase, negatively supercharged extensively (MelC2(-40))**

MSRHHHHHHHTVRENQASLTAEKEEVVAALLELKETGEYDAFVTTNAFILGDTDDGERGHRSPSFLPWHRRF  
LLEFEEALQSVDASVALPYWDWSADRSTESSLWAPDFLGGTGESEDGQVMMDGPFAASAGDWPIDEVDFGETFLR  
RALGAGVSELPTAEVDSVLAMATYDMAPWNNSGSDGFRNHLEGWRGVNLHNRVHVWVGGQMATGVSPNDPVFWL  
HHAYIDKLWAWEQEEHPSSPYLPGGTPDVVDLDETMEPWDDTPAALLDTEHYTFDVELIEGRGSDGNDLIQ  
GGKGADFI~~E~~GGKGNDTIRDN~~S~~G~~H~~NTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGA  
DSVTLVGVGLGGLWSEGVLIS

**>MelC2 tyrosinase, negatively supercharged defensively (MelC2(-20))**

MSRHHHHHHHTVRKNQASLTAEKERFVAALLELKETGRYDAFVTTNAFILGDTDDGERGHRSPSFLPWHRRF  
LLEFEEALQSVDASVALPYWDWSADRSTESSLWAPDFLGGTGESEDGQVMMDGPFAASAGNWPIINV  
RALGAGVSELPTAEVDSVLAMATYDMAPWNNSGSDGFRNHLEGWRGVNLHNRVHVWVGGQMATGVSPNDPVFWL  
HHAYIDKLWAWEQERHPSSPYLPGGTPDVVDLDETMKPWNDDTPAALLDTEHYTFDVELIEGRGSDGNDLIQ  
GGKGADFI~~E~~GGKGNDTIRDN~~S~~G~~H~~NTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGA  
DSVTLVGVGLGGLWSEGVLIS

**>MelC2 tyrosinase, selectively superneutralized (MelC2(Q))**

MSRHHHHHHHTVRQNQASLTAEKRQFVAALLELKQTGRYDAFVTTNAFILGDTDNGERTGHRSPSFLPWHRRF  
LLEFEQALQSVDASVALPYWDWSADQSTQSSLWAPDFLGGTGRSQDGQVMMDGPFAASAGNWPIINVQVDGQTFLR  
RALGAGVSELPTQAEVDSVLAMATYDMAPWNNSGSDGFRNHLEGWRGVNLHNRVHVWVGGQMATGVSPNDPVFWL  
HHAYIDKLWAWEQQQHPSSPYLPGGTPNVVDLNEMQPWNDDTPAALLDTEEHYTFDVELIEGRGSDGNDLIQ  
GGKGADFI~~E~~GGKGNDTIRDN~~S~~G~~H~~NTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGA  
DSVTLVGVGLGGLWSEGVLIS

**>M37 lipase (M37) [14]**

MSRHMSYTKEQLMLAFSYMSYYGITHTGSAKKNAELILKKMKEALKTWKPFQEDDWEVVWGPavyTMPFTIFND  
AMMYVIQKKGAEGEYVIAIRGTPVSISDWLFNDFMVSAMKKWPYASVEGRILKISESTS~~Y~~GLKTLQKLKP~~K~~SH  
IPGENKTILQFLNEKIGPEGKAKICVTGHSKGGALSSTLALWLKDIQGVKLSQNI~~I~~STIPFAGPTAGNADFAD  
YFDDCLGDQCTRIANSLDIVPYAWNTSLKKLKSIYISEQASVKPLLYQRALIRAMIAETKGKKYKQIKAETPP  
LEGNINPILIEYLVQAAYQHVVGYPELMGMMDDIPLTDIFEDAIAGLLLEHHHHHHGTASELIEGRGSDGNDLI  
QGGKGADFI~~E~~GGKGNDTIRDN~~S~~G~~H~~NTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFG  
ADSVTLVGVLGGLWSEGVLIS

**>M37 lipase, negatively supercharged extensively (M37(-23))**

MSRHHHHHHHSYTKEQLMLAFSYMSYYGITHTGSAKENAE~~L~~ILEKMKEALETWEPFQEDDWEVVWGPavyTMPFT  
IFNDAMMYVIQKEGAEGEYVIAIRGTPVSISDWLFNDFMVSAMKEWPYASVEGEILKISESTS~~Y~~GLKTLQELK  
PKSHIPGEDK TILEFLNEKIGPEGEAKICVTGHSKGGALSSTLALWLKDIQGV~~E~~LED~~I~~STIPFAGPTAGNA  
DFADYFDDCLGDQCTRIANSLDIVPYAWNTDSLEELKSIYISEEAS~~V~~PE~~L~~LYQRALIEAMIAETEGKEYKQIKA  
ETPPLE~~G~~DINPILIEYLVQAAYQHVVGYPELMGMMDDIPLTDIFEDAIAGLL~~E~~LIEGRGSDGNDLIQGGKGADF  
IEGGKGNDTIRDN~~S~~G~~H~~NTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVG

VGLGLWSEGV LIS

>M37 lipase, negatively supercharged defensively (M37(-14))

MSRHHHHHSYTKEQLMLAFSYMSYYGITHTGSAKKNAELILEKMKEALETWEPFQEDDWEVVWGP AVY TMPFT  
IFNDAMMYVIQKEGAEGEYVIAIRGTNPVSISDWLFNDFMVSAMKKWPYASVEGRILKISESTS YGLKTLQELK  
PKSHIPGEDKTILQFLNEKIGPEGEAKICVTGHSKGGALSSTLALWLKDIQGVELSQQDIDISTIPFAGPTAGNA  
DFADYFDDCLGDQC TRIANSLDIVPYAWNTNSLKKL KSIYISEQASVKPLLYQRALIRAMIAETKGKKYKQIKA  
ETPPLEG NINPILIEYLVQAAYQHVVGYPELMGMMDIPLTDIFEDAIAGLLELIEGRGSDGNLIQGGKGADF  
IEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTD LRDHAKAVGADTVLSFGADSVTLVG  
VGLGLWSEGV LIS

>M37 lipase, selectively superneutralized (M37(Q))

MSRHHHHHSYTKEQLMLAFSYMSYYGITHTGSAKQNAELILQKMKEALQTWKPFQEDDWEVVWGP AVY TMPFT  
IFNDAMMYVIQKQGAEGEYVIAIRGTNPVSISDWLFNDFMVSAMQQWPYASVEGRILQISESTS YGLKTLQQLQ  
PQSHIPGENQTILQFLNEKIGPEGQAQICVTGHSKGGALSSTLALWLKDIQGVQLSQNIDISTIPFAGPTAGNA  
DFADYFDDCLGDQC TRIANSLDIVPYAWNTNSLQQL KSIYISEQASVKPLLYQQALI QAMIAETQQQYKQIKA  
ETPPLEG NINPILIEYLVQAAYQHVVGYPELMGMMDIPLTDIFEDAIAGLLELIEGRGSDGNLIQGGKGADF  
IEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTD LRDHAKAVGADTVLSFGADSVTLVG  
VGLGLWSEGV LIS

>M37 lipase, randomly mutated (design of M37(var))

MSRHHHHHSYTKEQLMLAFSYMSYYGITHTGSAKeNAELILEKMKEALETWEPFQEDDWEVVWGP AVY TMPFT  
IFNDAMMYVIQKEGAEGEYVIAIRGTNPVSISDWLFNDFMVSAMKeWPYASVEGeILKISESTS YGLKTLQELK  
PKSHIPGEDKTILZFLNEKIGPEGEAKICVTGHSKGGALSSTLALWLKDIQGVELSZDIDISTIPFAGPTAGNA  
DFADYFDDCLGDQC TRIANSLDIVPYAWNTBSLeelKSIYISEZASVePLLYQRALIeAMIAETeGKeYKQIKA  
ETPPLEG B INPILIEYLVQAAYQHVVGYPELMGMMDIPLTDIFEDAIAGLLELIEGRGSDGNLIQGGKGADF  
IEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTD LRDHAKAVGADTVLSFGADSVTLVG  
VGLGLWSEGV LIS

# e indicates “Lys or Glu”, or “K or E”, which is encoded by codon RAG.

# B indicates “Asn or Asp”, or “N or D”, which is encoded by codon RAC.

# Z indicates “Gln or Glu”, or “Q or E”, which is encoded by codon SAG.

>M37 lipase, randomly mutated and activity-based screened (M37(var))

MSRHHHHHSYTKEQLMLAFSYMSYYGITHTGSAKKNAELILEKMKEALETWEPFQEDDWEVVWGP AVY TMPFT  
IFNDAMMYVIQKEGAEGEYVIAIRGTNPVSISDWLFNDFMVSAMKKWPYASVEGKVLIKISESTS YGLKTLQELK  
PKSHIPGEDKTILQFLNEKIGPEGEAKICVTGHSKGGALSSTLALWLKDIQGVELS EDIDISTIPFAGPTAGNA  
DFADYFDDCLGDQC TRIANSLDIVPYAWNTNSLKE LKSIYISEEASVKPLLYQRALI EAMIAETEGKEYKQIKA  
ETPPLEG NINPILIEYLVQAAYQHVVGYPELMGMMDIPLTDIFEDAIAGLLELIEGRGSDGNLIQGGKGADF  
IEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTD LRDHAKAVGADTVLSFGADSVTLVG  
VGLGLWSEGV LIS

>Green fluorescent protein (GFP) [14]

MSRMSKGEE LFTGVVPILVELGDVNGHKFSVSGEGEGDATYKLTLKFICTTGKLP VPWPTLVTTFSYGVQCF  
SRYPDHMKRHDFFKS AMPEGYVQERTISFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNY  
NSHNVYITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMV  
LLE FVTAA GITHGMDELIEGRGSDGNLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTD  
RLVFQGADGSTD LRDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

>-30 Negatively supercharged GFP (GFP(-30)) [14, 15]

MSRMGHHHHGGASKGEELFDGVVPILVELGDVNGHEFSVRGEGE DATEGEELTLKFICTTGELPV PWPTLV

TTLTYGVQCFSDYPDHMDQHDFFKSAMPEGYVQERTISFKDDGYKTRAEVKFEGDTLVNRIELKGIDFKEDGN  
ILGHKLEYNFNSHDVYITADKQENGIAEFEIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDDHYLSTESALSK  
DPNEDRDHMVLLEFVTAAGIDHGMDELYKELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSG  
HFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**>Transforming growth factor  $\beta$  (TGF $\beta$ )**

MSRHHHHHMSRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKIHEPKGYHANFCLGPCPYIWSLDTQYSKVL  
ALYNQHNPAGASAAPCCVPQALEPLPIVYYVGRKPKEQLSNMIVRSCKSELIEGRGSDGNDLIQGGKGADFIE  
GGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVG VG  
LGGLWSEGV LIS

**>Transforming growth factor  $\beta$ , LCD-based supercharged (TGF $\beta$ (-))**

MSRHHHHHMSRALDTNYCFSSTEENCCVRQLYIDFREDLGWKIHEPKGYHANFCLGPCPYIWSLDTQYSKVL  
ALYNQHNPAGASAAPCCVPQALEPLPIVYYVGRDPKVEQLSNMIVDSCKSELIEGRGSDGNDLIQGGKGADFIE  
GGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVG VG  
LGGLWSEGV LIS

**>Tumor necrosis factor  $\beta$  (TNF $\beta$ )**

MSRHHHHHMLPGVGLTPSAAQTARQHPKMHLAHLSTLKPAAHЛИGDP SKQNSLLWRANTDRAFLQDGFSLSNN S  
LLVPTSGIYFVYSQVVFSGKAYSPKATSSPLYLAHEVQLFSSQYPFHVPLSSQKMVYVPLQEPWLHSMYHGAA  
FQLTQGDQLSTHTDGIPHLVLS P STVFFGAFALELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNT F  
LFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**>Tumor necrosis factor  $\beta$ , LCD-based supercharged ((TNF $\beta$ (-))**

MSRHHHHHMLPGVGLTPSAAQTAQQHPQMHLAHLSTLKPAAHЛИGDP STQNSLLWRANTDRAFLQDGFSLSNN S  
LLVPTSGIYFVYSQVVFSGEAYSPEATSSPLYLAHEVQLFSSQYPFHVPLSSQKMVYVPLQEPWLHSMYHGAA  
FQLTQGDQLSTHTDGIPHLVLS P STVFFGAFALELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNT F  
LFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**>Fibroblast growth factor 1 (FGF1)**

MSRHHHHHMFNLPPGNYKKPKLLYCSNGGHFLRILPDGTVDGTRDRSDQHQIQLQLSAESVGEVYIKSTETGQY  
LAMTDGLLYGSQTPNEECLFLERLEENHYNTYISKKHAEKNWVGLKKNGSCKRGPRTHYQKAILFLPLPV S  
SDELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDL  
RDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**> Fibroblast growth factor 1, LCD-based supercharged (FGF1(-))**

MSRHHHHHMFNLPPGNYQQPKLLYCSNGGHFLRILPDGTVDGTRDDSDQHQIQLQLSAESVGEVYIKSTETGQY  
LAMTDGLLYGSQTPNEECLFLERLEENHYNTYISQEHAEQNWVGLKQNGSCKDGPRTHYQKAILFLPLPV S  
SDELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDL  
RDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**>SARS-CoV-2 spike protein N-terminal domain (NTD)**

MSRHHHHHVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHSGTNGTKRFD  
NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKCEFCNDPFLGVYYHKNNKSWME  
SEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVD  
LPIGINITRFQTLALHRSYLTQGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTTDAVDCALDPLSETKCT  
LKSELIEGRGSDGNDLIQGGKGADFIEGGKGNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTD  
LRDHAKAVGADTVLSFGADSVTLVGVLGLLWSEGV LIS

**>SARS-CoV-2 spike protein N-terminal domain, LCD under +2 (NTD(LCD≤2))**

MSRHHHHHVNLTTTQLPPAYTNSFRGVVYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHSGTNGTDRFD  
NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYH~~DNN~~ESWME  
SEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFENIDGYFKIYS~~D~~HTPINLV~~R~~DLPQGFSALEPLVD  
LPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYVGYLQ~~P~~RTFLL~~E~~YNENG~~T~~DAVDCALDPLSET~~K~~C  
LKSELIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTD  
LRDHAKAVGADTVLSFGADSVTLVGVLGLWSEGVLIS

**>SARS-CoV-2 spike protein N-terminal domain, LCD under +1 (NTD(LCD≤1))**

MSRHHHHHVNLTTTQLPPAYTNSFTDGVVYPDKVFDSSVLHSTQDLFLPFFSNVTWFHAIHSGTNGTDRFD  
NPVLPFNDGVYFASTEKSNIIRGWIFGTTLDS~~T~~QSL~~L~~IVNNATNVVIKVCEFQFCNDPFLGVYYH~~KNN~~SWME  
SEFDVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFDNIDGYFKIYS~~K~~HTPINLV~~D~~DLPQGFSALEPLVD  
LPIGINITRFQTLLALH~~D~~SYLTPGDSSSGWTAGAAAYVGYLQ~~P~~RTFLL~~D~~YNENG~~T~~DAVDCALDPLSET~~K~~C  
LKSELIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTD  
LRDHAKAVGADTVLSFGADSVTLVGVLGLWSEGVLIS

**>SARS-CoV-2 spike protein receptor binding domain (RBD)**

MSRHHHHHPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSP~~T~~KLNDLCFTN  
VYADSFVIRGDEV~~R~~QIAPGQTGKIAD~~Y~~NKLPDDFTGC~~VIA~~W~~N~~NNLDSKVGGNNYLYRLFRKSNLKF~~E~~RDI  
STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG~~Y~~QPYRVV~~V~~LSFELLHAPELIEGRGSDGNDLIQGGKGAD  
FIEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLV  
GVGLGLWSEGVLIS

**>SARS-CoV-2 spike protein receptor binding domain, LCD under +2 (RBD(LCD≤2))**

MSRHHHHHPNITNLCPFGEVFNATRFASVYAWNRDDISNCVADYSVLYNSASFSTFKCYGVSP~~T~~KLNDLCFTN  
VYADSFVIRGDEV~~R~~QIAPGQTGKIAD~~Y~~NKLPDDFTGC~~VIA~~W~~N~~NNLDSKVGGNNYLYRLFRDSNLEP~~F~~ERDI  
STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG~~Y~~QPYRVV~~V~~LSFELLHAPELIEGRGSDGNDLIQGGKGAD  
FIEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLV  
GVGLGLWSEGVLIS

**>SARS-CoV-2 spike protein receptor binding domain, LCD under +1 (RBD(LCD≤1))**

MSRHHHHHPNITNLCPFGEVFNATRFASVYAWNRDDISNCVADYSVLYNSASFSTFKCYGVSP~~T~~KLNDLCFTN  
VYADSFVIRGDEV~~R~~QIAPGQTGKIAD~~Y~~NKLPDDFTGC~~VIA~~W~~N~~NNLDSKVGGNNYLYRLFDDSNLDP~~F~~ERDI  
STEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVG~~Y~~QPYRVV~~V~~LSFELLHAPELIEGRGSDGNDLIQGGKGAD  
FIEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLV  
GVGLGLWSEGVLIS

**>pDART Translation Structure [14]**

MSRHMGTA~~S~~ELIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTDRLVFQG  
ADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLWSEGVLIS

**>pBR05 Translation Structure**

MSRHMGTA~~S~~ELRRRRRGIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTD  
RLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLWSEGVLIS

**>pBR10 Translation Structure [14]**

MSRHMGTA~~S~~ELRRRRRRRGIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTD  
RLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLVGVLGLWSEGVLIS

**>pBK05 Translation Structure**

MSRHMGTA~~S~~ELKKKKGIEGRGSDGNDLIQGGKGAD~~F~~IEGGKGNDT~~I~~RDN~~S~~GHNTLFSGHFGQDRIIGYQPTD

RLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**>pBK10 Translation Structure**

MSRHMGTASELKKKKKKKKKGIEGRGSDGNLIQGGKGADFIEGGKNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**>pBRD10 Translation Structure**

MSRHMGTASELRRDRDRDRDRDRDRDGIEGRGSDGNLIQGGKGADFIEGGKNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**>pBRN10 Translation Structure**

MSRHMGTASELRNRNRNRNRNRNRNRNGIEGRGSDGNLIQGGKGADFIEGGKNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**>pBKD10 Translation Structure**

MSRHMGTASELKDKDKDKDKDKDKDGIEGRGSDGNLIQGGKGADFIEGGKNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**>pBKN10 Translation Structure**

MSRHMGTASELKNKNKNKNKNKNKNKGIEGRGSDGNLIQGGKGADFIEGGKNDTIRDNSGHNTFLFSGHFGQDRIIGYQPTDRLVFQGADGSTDLRDHAKAVGADTVLSFGADSVTLGVGLGGLWSEGVLIS

**Table S1.** Primers used to construct the plasmids for the positively charged patch experiments.

| Name       | Sequence  | Features  |
|------------|---|---|
| F-Arg10    | <u>TGGGTACCGCTAGC GAGCTC</u> CGTCGCCGACGGC<br>GTCGCCGACGGCGTCGC             | Complementary region, SacI site, insert sequence    |
| R-Arg10    | <u>CCTCGTCCTTCAATGCC</u> GCGACGCCGTCGGCGAC<br>GCCGTCGGCGACG                 | Complementary region, frame-keeper, insert sequence |
| F-Arg05    | <u>TGGGTACCGCTAGC GAGCTC</u> CGTCGCCGACGGC<br>GT                            | Complementary region, SacI site, insert sequence    |
| R-Arg05    | <u>CCTCGTCCTTCAATGCC</u> ACGCCGTCGGCGACG                                    | Complementary region, frame-keeper, insert sequence |
| F-Lys10    | <u>TGGGTACCGCTAGC GAGCTC</u> AAGAAAAAGAAA<br>AAGAAGAAAAAGAAAAAG             | Complementary region, SacI site, insert sequence    |
| R-Lys10    | <u>CCTCGTCCTTCAATGCC</u> CTTTTCTTTCTTCTT<br>TCTTTTCTT                       | Complementary region, frame-keeper, insert sequence |
| F-Lys05    | <u>TGGGTACCGCTAGC GAGCTC</u> AAGAAAAAGAAA<br>AAG                            | Complementary region, SacI site, insert sequence    |
| R-Lys05    | <u>CCTCGTCCTTCAATGCC</u> CTTTTCTTTCTT<br>CTTTTCTTCTT                        | Complementary region, frame-keeper, insert sequence |
| F-ArgAsp10 | <u>TGGGTACCGCTAGC GAGCTC</u> CGTGATCGCGACC<br>GAGATCGGGATCGTGACCGCGATCGAGAT | Complementary region, SacI site, insert sequence    |
| R-ArgAsp10 | <u>CCTCGTCCTTCAATGCC</u> GTCCCGTCACGGTCCC<br>GATCTCGATCGCGGTACCGATCCCG      | Complementary region, frame-keeper, insert sequence |
| F-ArgAsn10 | <u>TGGGTACCGCTAGC GAGCTC</u> CGTAATCGCAACC<br>GAAATCGGAATCGTAACCGCAATCGAAAT | Complementary region, SacI site, insert sequence    |
| R-ArgAsn10 | <u>CCTCGTCCTTCAATGCC</u> GTTCCGGTTACGGTTCCG<br>ATTTCGATTGCGGTTACGGATTCCG    | Complementary region, frame-keeper, insert sequence |
| F-LysAsp10 | <u>TGGGTACCGCTAGC GAGCTC</u> AAGGATAAGGAC<br>AAAGATAAAAGACAAGGACAAGACAAAGAT | Complementary region, SacI site, insert sequence    |
| R-LysAsp10 | <u>CCTCGTCCTTCAATGCC</u> ATCCTGTCTTATCCTT<br>ATCTTGTCTTGTCTTGTCTT           | Complementary region, frame-keeper, insert sequence |
| F-LysAsn10 | <u>TGGGTACCGCTAGC GAGCTC</u> AAGAATAAGAAC<br>AAAATAAAAACAAGAACAAAAACAAAAAT  | Complementary region, SacI site, insert sequence    |
| R-LysAsn10 | <u>CCTCGTCCTTCAATGCC</u> ATTTTGTCTTGTCTT<br>TTTTTATTGTTCTTATTCTT            | Complementary region, frame-keeper, insert sequence |
| F-LysAsp03 | <u>TGGGTACCGCTAGC GAGCTC</u> AAGGATAAAAGAC<br>AAG GA                        | Complementary region, SacI site, insert sequence    |
| R-LysAsp03 | <u>TTGTCCTTATCCTTGT</u> CTTGTCTTATCCTT GAG<br>CT                            | Complementary region, frame-keeper, insert sequence |
| F-LysAsn03 | <u>TGGGTACCGCTAGC GAGCTC</u> AAGAATAAAAAT<br>AAA AA                         | Complementary region, SacI site, insert sequence    |
| R-LysAsn03 | <u>TTGTTCTTATTCTTGT</u> TTTATTCTTATTCTT GAGC<br>T                           | Complementary region, frame-keeper, insert sequence |

**Table S2.** List of genes studied in this experiment.

| Abbrev.         | Full name                                       | Source                                    | Source type                |
|-----------------|---|---|----------------------------|
| TliA            | Thermostable lipase A                           | <i>Pseudomonas fluorescens</i> SIK-W1     | Genomic DNA                |
| NKC-TliA        | NKC-TliA  | [14, 42]                                  | Constructed                |
| CTP-TliA        | CTP-TliA  | [14, 43]                                  | Constructed                |
| GST             | Glutathione S-transferase                       | <i>Sus scrofa domesticus</i>              | Plasmid                    |
| GST(-20)        | GST, -20 negatively supercharged                | [15]                                      | Synthesized                |
| GST(+19)        | GST, +19 positively supercharged                | Manually supercharged                     | Synthesized                |
| SAv             | Streptavidin                                    | <i>Streptomyces avidinii</i>              | Plasmid                    |
| SAv(-10)        | SAv, -10 negatively supercharged                | [15]                                      | Synthesized                |
| SAv(+13)        | SAv, +13 positively supercharged                | [15]                                      | Synthesized                |
| Cuti            | Cutinase  | <i>Nectria haematococca</i> , [14]        | Plasmid                    |
| Cuti(-)         | Cutinase, negatively supercharged               | AvNAPSA supercharging, threshold = 100    | Synthesized                |
| Chi             | Chitinase                                       | <i>Bacillus thuringensis</i> , [14]       | Plasmid                    |
| Chi(-)          | Chitinase, negatively supercharged              | AvNAPSA supercharging, threshold = 100    | Synthesized                |
| MelC2           | MelC2 Tyrosinase                                | <i>Streptomyces antibioticus</i>          | Plasmid                    |
| MelC2(-40)      | MelC2, -40 negatively supercharged              | AvNAPSA supercharging, threshold = 120    | Synthesized                |
| MelC2(-20)      | MelC2, -20 negatively supercharged              | AvNAPSA supercharging, threshold = 100    | Synthesized                |
| MelC2(Q)        | MelC2, selectively superneutralized             | Manually superneutralized                 | Synthesized                |
| M37             | M37 lipase                                      | <i>Photobacterium lipolyticum</i> , [14]  | Genomic DNA                |
| M37(-23)        | M37, -23 negatively supercharged                | AvNAPSA supercharging, threshold = 100    | Synthesized                |
| M37(-14)        | M37 lipase, -14 negatively supercharged         | AvNAPSA supercharging, threshold = 90     | Synthesized                |
| M37(Q)          | M37, selectively superneutralized               | Manually superneutralized                 | Synthesized                |
| M37(var)        | M37, randomly mutated and screened              | Random mutation and activity screening    | Synthesized via mixed-base |
| TGF $\beta$     | Transforming growth factor beta                 | <i>Homo sapiens</i> [44]                  | Synthesized                |
| TGF $\beta$ (-) | TGF $\beta$ , LCD-based negatively supercharged | Linear charge density-based supercharging | Synthesized                |
| TNF $\beta$     | Tumor necrosis factor beta                      | <i>Homo sapiens</i> [45]                  | Synthesized                |
| TNF $\beta$ (-) | TNF $\beta$ , LCD-based negatively supercharged | Linear charge density-based supercharging | Synthesized                |
| FGF1            | Fibroblast growth factor beta                   | <i>Homo sapiens</i> [46]                  | Synthesized                |
| FGF1(-)         | FGF1, LCD-based negatively supercharged         | Linear charge density-based supercharging | Synthesized                |