Supplementary Information for "Functional Proteins from a Random-Sequence Library"

Nature, v410, 715

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Generalized Random Library sequence (DNA)

TTCTAATACGACTCACTATAGGGACAATTACTATTTACAATTACAATGGACTACAAAGACGACGACGATAAGAAGACTYACTGZ(XYZ)$_{18}$YACTGZ(XYZ)$_{18}$YACTGZ(XYZ)$_{18}$YACTGZ(XYZ)$_{18}$YACTGG

Average nucleotide composition of random parts of Random Library

	X	Y	Z
A	35	33	0
T	20	29	22
G	27	21	49
C	18	17	29

(%)

Generalized Random Library sequence (Protein)

MDYKDDDDKKT(Random)$_{81}$WSASCHHHHHHMGMSGS

Average amino acid composition of random part of Random Library (Protein)

Ala	4.1	Leu	7.4
Arg	6.8	Lys	5.1
Asn	7.5	Met	4.5
Asp	5.5	Phe	2.8
Cys	4.6	Pro	2.8
Gln	2.6	Ser	6.6
Glu	4.0	Thr	5.4
Gly	5.3	Trp	4.4
His	3.8	Tyr	5.0
Ile	4.8	Val	7.1

(%)

Selected clones from round 8

Family A

08-05M**N**YKDDDDKKTHWYTNSGFAMTSLRFMMIKWYNWWHDQRHRNIRHHRAMAPRN

CRIQAITPTHGHDLPQSFEDWR**R**DYRYNRDKTMAKGYQPWSASCHHHHHHMGMSGS

08-07MDYKDDDDKKTHWYTNSGFAMTSLRFMMIKWY**D**WWHDQRHRNIRHHRAMAPRN

CRIQAITPTHGHDLPQSFEDWRWDYRYNRDKTMAKGYQPWSASCHHHHHHMGMSGS

08-09MDYKDDDDKKTHWYTNSGFAMTSLRFMMIKW**H**NWWHDQRHRNIRHHRAMAPRN

CRIQAITP**A**HGHDLPQSFEDWRWDYRYNRDKTMAKGYQPWSASCHHHHHHMGMSGS

08-48MDYKDDDDKKTHWYTNSGFAMTSLRFMMIKWYNWWHDQRHRNIRHHRAMAPRN

CRIQAITPTHGHD**P**PQSFEDWRWDYRYNRDKTMAK**D**YQPWSASCHHHHHHMGMSGS

Family B

08-01MDYKDDDDKKTNCHQKRIYRVKPCVICKVAPRDWW**M**ENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-04MDYKDDDDKKTNWHQ**R**RIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CIN**S**GDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-08MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CIN**N**GDDTYYGHDDWLMYTDCKEFSNTYH**D**LGRLPDEDRWSASCHHHHHHMGMSGS

08-10MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDC**E**EFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-12MDYKDDDDKKTNCHQKRIYRVKPCVICKVAPRDWWVEN**G**HLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNL**D**RLPDEDRWSASCHHHHHHMGMSGS

08-13MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-14MDYKDDDD**N**KTNWHQKRIYRVKPCVICKVAPRDWWVEN**K**HLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-15MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-18MDYKDDDDKKTNWHQ**E**RIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

C**V**NYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-21MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CIN**N**GDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-23MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENR**R**LRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-45MDYKDDDDKKTNWHQKRIYR**M**KPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWL**I**YTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-46MDYKDDDDKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

08-47MDYKDDDGKKTNWHQKRIYRVKPCVICKVAPRDWWVENRHLRIYTMCKTCFSN

CINYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRWSASCHHHHHHMGMSGS

Family C

08-11MDYKD**V**DDKKTHCDK**T**VSVDMTFRVRNMKVAKDCWSVVVWTKRSNYFSGRQLH

CDSWHHYNSRRFGTETKLAYWELPKWKWKINNTHAINIHWSASCHHHHHHMGMSGS

08-17MDYKD**I**DDKKTHCDKAVSVDMTFRVRNMKVAKDCWSVVVWTKRSNYF**N**GRQLH

CDSWHHYNSRRFGTETKLAYWELPKWKWKINNTHAINIHWSASCHHHHHHMGMSGS

08-19MDYKD**I**DDKKTHCDKAVS**I**DMTFRVRNMKVAKDCWSVVVWTKRSNYFSGRQLH

SDSWHHYNSRRFGTETKLAYWELPKWKWKINNTHAINIHWSASCHHHHHHMGMSGS

08-06MDYKD**V**DDKKTHCDKAVSVDMTFRVRNMKVAKDCWSVVVWTKRSNYFSGRQLH

CDSWHHYNSRRFGTETKLAYWELPKWKWKINNTHAINIHWSASCHHHHHHMGMSGS

Family D

08-20MDYKDDDDKKT**Y**WHALVTYNKTLSYRLATKFTDWWNLDPPRNMQTKVSELNLH

WLKSGGKGTQKAHSINEISNWVHQHELSDKSMRLHSKVRWSASCHHHHHHMGMSGS

Selected clones from round 18

18-01MDYKDDDDKKTNWQKRIY**Q**VRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

VDYGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-02MDYKDDDDKKTNWQKR**V**YR**A**RPCVICKVAPRDWRV**V**NRHLRIYNMCKTCFSNS

IN**H**GDDTY**H**GH**N**DWLMYTDC**E**EFS**S**T**C**HNLGR**Q**PDEDRHWSASCHHHHHHMGMSGS

18-03MDYKDDDDKKT**Y**WQKRIYRVRPCVICKVAPRDWRV**K**N**G**HLRIYNMCKTCFSNS

I**KC**GDDTYYGHDDWL**IH**TDCK**D**FSNTY**L**NLGRLPDE**E**RHWSASCHHHHHHMGMSGS

18-04MDYKDDDDKKTNWQKR**V**YRVRPCV**V**CK**E**APRDWRV**KD**RHLRIYNMCKTCFSNS

INYGDDT**H**YGHDDWLMYTDCK**G**FSNTYHN**PS**RLPDEDRHWSASCHHHHHHMGMSGS

18-05MDYKDDDDKKTNWQKRIYRV**G**PCVICKVAPRDWRVENRHLRIY**T**MCKTCFSNS

I**Y**YGD**N**TY**H**GH**E**DWLMYTD**S**KEFSNTYHN**Q**GRLPD**V**DRHWSASCHHHHHHMGMSGS

18-06MDYKDDDDKKTNWQKRIYRV**K**PCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

IN**N**GDDTY**H**GHDDWLMYTDCKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-07MDYKDDDDKKINWQKR**T**YRVRPCVICKVAPRDWRV**V**NRHLRIYNMCKTCFSNS

INYGDDTYYGHDDWLMYTDCKE**Y**SNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-08MDYKDDDDKKTNWQKR**F**YRVRPCVICKVAPRDWRV**K**N**G**HLRIYNMCKTCFSNS

I**K**YGDDTYYGHDDWLMYTDCKEFSNTYHNLGRLP**N**EDRHWSASCHHHHHHMGMSGS

18-09MDYKDDDDKKTNWQKR**F**YRV**K**PCV**F**CKVAPRDWRVEN**G**HLRIYNMCKTCFSNS

LN**N**GDDT**H**YGHDDWLMYTDCKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-10MDYKDDDDKKTNWQKRIYRVRPCV**R**CKVAPRDWRVENRHLRIYNMCKTC**Y**SNS

INYGDDTYYGH**E**DWLLYTDC**E**EFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-11MDYKDDDDK**E**T**S**W**H**K**L**M**Y**Q**V**RPCVICKVAPRDWRVENRHLRIY**T**MCKTCF**N**NS

VNYGDDT**HH**GH**N**DWLMY**A**DC**N**EF**T**NT**CR**NL**A**RLPDEDRHWSASCHHHHHHMGMSGS

18-12MDYKDDDDKKTN**R**QK**L**I**F**RV**K**PCVICKVAPRDW**Q**VEN**G**HLRIYNMCKTCF**I**NS

IN**N**GDDTY**H**GHDDWLM**H**TDC**T**EFSNTYHNLGRLP**G**EDRHWSASCHHHHHHMGMSGS

18-13M**N**YKDDD**N**KKTNWQ**N**RI**N**RVRPCVICKVAPRDW**C**V**K**N**G**HLRIYNMCK**S**CFS**DC**

INYGDDT**H**YGH**E**DWLMYTDCKEFSNTYHNLGR**I**P**EK**DRHWSASCHHHHHHMGMSGS

18-14M**AT**KDDDDKKTN**R**QKRI**F**RV**K**PCVICKVAPRDWRV**R**N**G**HLRIYNMCKTCFSNS

INYGDDTYYGHDD**R**LMYTDC**M**EFSNTYHNLG**K**LPDEDRHWSASCHHHHHHMGMSGS

18-15MDYKDDDDKKTNW**L**KRIYRV**K**PCV**N**CKVAPRDWRV**K**NRHLRI**H**NMCKTC**Y**SNS

VNYGDDTYYGHDDWLMYTDC**E**EFSNTY**P**NLG**S**LPDEDRHWSASCHHHHHHMGMSGS

18-16MDYKDDD**V**KKTNWQKRIYRVRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

IN**N**GDDTYYGHDDWLMYTD**S**KEFS**Y**TYHNLG**WQ**P**V**EDRHWSASCHHHHHHMGMSGS

18-17MDYKDDDDKKTNWQKR**T**YRVRPCVICKVAPRDWRV**K**NRHLRIYNMCKTCFSNS

INYG**E**DTYYGH**E**DWLMYTDC**E**EFS**K**TYHNLGRLP**G**EDRHWSASCHHHHHHMGMSGS

18-18MDYKDDDDKKTNWQKRIYRV**K**PCV**N**CKVAPRDWRV**K**NRHLRIYNMCKTC**Y**SNS

VNYGDDTYYGHDDWLMYTDC**E**EFSN**S**YHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-19MDYKDDDDKKTNW**L**KRIYRVRPCV**K**CKVAPR**N**W**K**V**K**N**K**HLRIYNMCKTCF**N**NS

I**DI**GDDTY**H**GHDDWLMY**A**D**S**KE**I**SNTYHNLGRLP**N**ED**K**HWSASCHHHHHHMGMSGS

18-20MDYKDDDDKKTNWQKRIYRV**G**PCVICKVAPRDWRVEN**G**HLRIYNMCKTCF**G**NS

IN**N**GDDT**NF**GHDDWLMYTDCKEFSNTYH**H**LG**G**LPDEDRHWSASCHHHHHHMGMSGS

18-21MDYKDDDDKKTNWQKRI**H**RV**G**PCVICKVAPRDWRV**R**NRHLRIYNMCKTCFSNS

I**K**YGDDTYYGHDDWLMYTDCKEFS**D**TYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-22MDYKDDDDKKTN**R**QKRIYRVRPCVICKVAPRDWRVENRHLR**V**YNMCKTCFSNS

I**H**YGDDTY**H**GHDDWL**LH**TDCKEFSNTYH**Q**LGR**M**PDE**A**RHWSASCHHHHHHMGMSGS

18-23MDYKD**Y**DDKKTNWQKRI**C**RV**K**PCVICKVAPRDWRV**K**NRHLRIYNMCKTCFSNS

I**K**YGDDTYYGHDDWLM**N**TDCKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-24MDYKDDDDKKTN**R**Q**E**R**LC**RVRPCV**F**CKVAPRDWRVEN**K**HLRIYNMCKTCFSNS

I**K**YGDDTY**H**GHDDWLMYTDCKEFSNTYHNL**D**RLPDEDRHWSASCHHHHHHMGMSGS

18-25MDYKDDDDKKTNWQKRIYRVRPCVICKVAPRDWRV**K**NRHLRIYNMCK**S**CFSNS

IN**N**GDDTYYGHDDWLMYT**N**C**E**EFS**S**TYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-26MDYKDDDDKKTN**R**QKR**LF**RVRPCVICKVAPRDWRVEN**G**HLRIYNMCKTCFSNS

I**SN**GDDTY**F**GH**E**D**R**L**I**Y**S**DCKEFS**K**T**N**HN**P**GRLPD**V**D**K**HWSASCHHHHHHMGMSGS

18-27MDYKDDDDKKTNWQKR**N**Y**W**VRPCVICK**E**APRDWRVENRHLRIYNMCKTCFSNS

I**KS**GDDTYYGHDDWLMYTDCKEFS**DR**YHNL**A**RLP**Y**EDRHWSASCHHHHHHMGMSGS

18-29MDYKDDDDKKTNWQKR**N**YRVRPCVIC**R**VAPRDWRV**K**N**G**HLRIYNMCKTCFSNS

INYGDDTYYGHDDWLMYTD**SE**EFSNTYHNL**D**RLPD**G**DRHWSASCHHHHHHMGMSGS

18-30MDYKDDDDKKTNWQKPIYRVRPCVICKVAPRDWRV**K**NRR**N**LRIYNMCKTCFS**D**S

I**K**YGDDT**FH**GHDD**R**LM**F**TD**S**KEFSNTYH**DQ**GR**Q**PDEDRHWSASCHHHHHHMGMSGS

18-31MDYKDDDDKKTNWQKRIYRVRPCVICK**E**APRDWRV**K**N**G**HLRIYNMCKTCFSNS

INYGDDTY**H**GHDDWL**I**Y**K**DCKEFSN**M**YHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-32MDYKDDDDKKTNWQKRIYRV**K**PCVICKVAPR**N**WRVENRHLRIYNMCKTC**Y**SNS

INYGDDTY**H**GHDDWLMYT**G**CKEFSNTYHNLGRLPDE**V**RHWSASCHHHHHHMGMSGS

18-33M**N**YKDDDDKKTNWQKHI**L**RVRPCV**V**CKVAPRDWRV**K**N**K**HLRIYNMCKTCFSNS

IN**C**GDDT**H**YGH**K**DWL**I**YTDCKE**SS**K**T**YH**D**LGRLPDEDRHWSASCHHHHHHMGMSGS

18-34MDYK**G**DDDKKTN**R**QKRIYR**A**RPCVICKVAPRDWRVE**K**RHLRIYNMCKTC**YN**NS

INY**E**DDTY**H**GHDD**LG**MYTDCKEFSNTYH**D**LGRLPDED**K**HWSASCHHHHHHMGMSGS

18-37MDYKDDDDKK**P**NW**L**KR**NH**RVRPC**M**ICKVAPRDWRVEN**G**HLRIY**T**MCKTCF**G**NS

INYGDDT**HH**GH**E**D**L**W**M**N**T**DCKE**Y**S**YA**YHNLGRLP**H**EDRHWSASCHHHHHHMGMSGS

18-38MDYKDDDDKKTNW**K**KRIY**Q**VRPCV**N**CKVAPRDWRVENRHLR**V**YNMC**R**TCFSNS

INYGDDT**F**YGHDDWL**LH**TDCK**Q**FSNTYHNLGR**P**PDEDRHWSASCHHHHHHMGMSGS

18-39MDYKDDDDKKTNW**L**KRIYRVRPCV**V**CKVAPRDWR**LK**N**G**HLRIYNMCKTCFSNS

TN**N**GDDTYYGHDDWLM**N**TDCKEFSN**S**YHNLGRLPDEDR**A**WSASCHHHHHHMGMSGS

18-41MD**N**KDDDDKKTNWQK**CF**YRVRPCV**V**CK**A**APRDWRVENR**R**LRIYNMCKTC**Y**SNS

IN**F**GDDT**H**YGHDDWLMY**S**D**S**KEFSNTYHNLGR**P**PDE**E**RHWSASCHHHHHHMGMSGS

18-44MDYKDDDDKKTNWQKRIY**QMK**PCV**V**CKVAPRDWRV**K**NRHLRIYNMCKTCFSNS

INYGDD**S**YYGHDDWLMNT**NS**KEF**Y**NTYHNLGRL**S**D**A**DRHWSASCHHHHHHMGMSGS

18-46MDYKDDDDKKTN**R**QKRIYRVRPCVICKVAP**Q**DWRVENR**R**LRIYNMCKTCFSNS

INYGDDT**H**YGH**V**DWLM**DM**D**S**KEFSNTYHNLGRLP**V**EDRHWSASCHHHHHHMGMSGS

18-47MDYKDDDDKKTNWQKRIYRVRPCV**V**CK**E**APRDWR**MVD**RHLRIYNMCKTCFSNS

NNYGDDTYYGHDD**R**L**L**YTDCKE**S**SNTYHN**P**G**G**LPDEDRHWSASCHHHHHHMGMSGS

18-48MDYKDDDDKKTNWQKRIYRVRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

I**K**YGDDTY**H**GHDDWLM**N**TDCK**V**FSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-51MDYKDDDDKK**A**NWQK**H**IYRVRPCVICKVAPRDW**WM**EN**G**HLRIY**T**MCKTCFSNS

IN**N**GDDTY**H**GH**E**DWLMY**K**DCKEFS**S**TYHNLGRLP**V**EDRHWSASCHHHHHHMGMSGS

18-52MDYKDDD**Y**KKTNWQK**P**I**F**R**A**RPCV**K**CKVAPRDW**W**VENRHLRIYNMCKTCF**N**NS

INYGDDTYYGHDDWL**V**YTDCKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-54MDYKDDDDKKTNWQKRIYRVRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

INHGDDTYFGHDDWLMYTDRKEFSNTYYNLGRLPGEDRHWSASCHHHHHHMGMSGS

18-56MDYKDDDDKKTNWQKHIYRVRPCVRCKVAPRDWRVENGHLRIYNMCKTCFSNS

INYGDDTNYGHDDWPLYTDSKEFSNTYHNLDRPPDEDRHWSASCHHHHHHMGMSGS

18-57MDYKDDNDKMTNRQKRIYRVRPCVVCKVAPRDWRVENRHLRIYNMCKTCFSDS

IKYRDDTHHGHDDWLMYTDCMEFSNTYHNLGWLPDEDRHWSASCHHHHHHMGMSGS

18-60MDYKDDDDKKTNWLKRNYRVKPCVNCKVAPRDWRVKNRHLRIYNMCKTCYSNS

INYGDDTYYGHDDWLMYTDCEEFSNTYHNRGRLPDEDRHWSASCHHHHHHMGMSGS

18-61MDYKDDDDKKTNWRKRIYRVRPCVICKVAPRDWRVEDSHLRIYNMCKTCFSNS

INYGDDTYFGHEDWLMYTDCKEFSNTYHNLDRLPDENRHWSASCHHHHHHMGMSGS

18-62MDYKDDDDKKTNWQKRIYRVRPCVKCKVAPRDWRVENSHLRIYNMCKTCFSNS

INYGDDTHYGHVDWVMYTDCMKFSNTYHNLSRLPDENRHWSASCHHHHHHMGMSGS

18-63MDYKDDDDKKAYWQERIYRVKPCVICKVAPRDWRVKNRHLRIYNMCKSCFSNS

IIYGDDTYHGHDDWLMYTDCKEFFNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-64MDYKDDDDKKTYWQKRIYRVKPCVICKEAPRDWRVKNRHLRIYNMCKTCFSNS

VNIGDDTYHGHDDWLDEYDCKEFSNTCHNLGRLPGEDKHWSASCHHHHHHMGMSGS

18-65MDYKDDDDKKTYWQKRIYRVRPCVVCKVAPRDWRVENRHLRIYNMCKTCFSNS

INYGDDTHYGHDDWLMNTDCKEFSNTYHNPGKLPDEDRHWSASCHHHHHHMGMSGS

18-66MDYKDDDDKKTNWQKSIYREKPCVVCKVAPRDWRVENGHLRIYNMCKTCYSNS

INYGDDTYYGHDDWLMYKDCKEFSNTYHYQGRLPEEDRHWSASCHHHHHHMGMSGS

18-67MDYKDDDDKKTNWQKRIYRVRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

INNGDDTYHGHDDWLLYTDRKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-68MDYKDDDDKKTNWQKRIYRVRPCVICKVAPRDWRVENRHLRIYNMCKTCFSNS

INYGDDTFYGHDDWLMYTDSKEFSNTYHNLGRLPDEDRHWSASCHHHHHHMGMSGS

18-72MDYKDDDDKKTIRQKRIYRVRPCVNCKVAPRDWRVENRHLRIYNMCKTCFSNS

INYRDDTYYGHDDWLIYTDCKEFSNTYHNLGRLPDKDRHWSASCHHHHHHMGMSGS

Fuller description of protocol for a round of selection:

***In vitro* selection and amplification**

RNA was produced from the DNA library with T7 RNA polymerase and mRNA-displayed proteins were generated as previously described[8,9]. The linker, which connects the RNA 3'-terminus to the protein C-terminus, had the following sequence: $(dA)_{21}$(triethylene glycol phosphate ester)$_3$dAdCdCPuromycin (made using reagents from Glen Research, Sterling, VA). A 10 ml translation (400 nM template, Red Nova Rabbit Reticulocyte Lysate (Novagen, Madison, WI), according to the manufacturer's instructions with 85 mM additional KCl, 0.85 mM additional $Mg(OAc)_2$ and 25 nM ^{35}S-methionine) incubated at 30°C for one hour yielded $7x10^{13}$ mRNA-displayed proteins after high-salt incubation (600 mM KCl, 25 mM $MgCl_2$). The translation mixture was then diluted ten-fold into oligo(dT)cellulose binding buffer (1 M KCl, 100 mM Tris(hydroxymethyl) amino methane, 0.25% w/v Triton X-100, pH 8.0) and this mixture was incubated with 2 mg/ml oligo(dT)cellulose (Pharmacia, Piscataway, NJ) for fifteen minutes at 4°C with rotation. The oligo(dT)cellulose was washed on a chromatography column (Bio-Rad, Hercules, CA) with the same oligo(dT)cellulose binding buffer and then eluted with deionized water. The eluate was mixed with 2x Ni-NTA binding buffer (1x is 6 M guanidinium chloride, 0.5 M NaCl, 100 mM sodium phosphate, 10 mM Tris(hydroxymethyl)amino methane, 10 mM 2-mercaptoethanol, 0.25% w/v Triton X-100, pH 8.0) and then incubated with Ni-NTA agarose (Qiagen, Valencia, CA) for one

hour at 4°C with rotation. The Ni-NTA agarose was then washed with Ni-NTA first wash buffer (8 M urea, 0.5 M NaCl, 100 mM sodium phosphate, 10 mM Tris(hydroxymethyl)amino methane, 10 mM 2-mercaptoethanol, 0.25% w/v Triton X-100, pH 6.3) and then with a gradient of increasing amounts of Ni-NTA second wash buffer (0.5 M NaCl, 10 mM Tris(hydroxymethyl)amino methane, 10 mM 2-mercaptoethanol, 0.25% w/v Triton X-100, pH 8.0), and then was eluted with Ni-NTA elution buffer (0.25 M imidazole, 0.5 M NaCl, 10 mM Tris(hydroxymethyl) amino methane, 10 mM 2-mercaptoethanol, 0.25% w/v Triton X-100, pH 8.0) for one hour at 4°C with rotation. EDTA was added to the eluate to give a concentration of 5 mM. The buffer was exchanged into Reverse Transcription buffer (50 mM Tris(hydroxymethyl) amino methane, 75 mM KCl, 3 mM $MgCl_2$ pH 8.3) on a gel filtration column (Pharmacia, Piscataway, NJ). The mRNA-displayed proteins were then reverse transcribed with Superscript II (Gibco BRL, Rockville, MD) at 42°C for 30 minutes without a heat denaturation step. This sample was then exchanged into selection binding buffer (400 mM KCl, 20 mM HEPES, 4 mM $MgCl_2$, 0.1mM EDTA, 2 mM glutathione, 1 mM glutathione disulfide, 0.25% w/v Triton X-100, pH 7.4) on a gel filtration column (Pharmacia, Piscataway, NJ), and then incubated with 100 µl of ATP-agarose (ATP attached via C8, 9 atom linker, cyanogen bromide activated cross-linked 4% beaded agarose (Sigma, St. Louis, MO)) at 4°C for one hour on a chromatography column (Bio-Rad, Hercules, CA). The column was then washed with 50 column volumes of selection binding buffer at 4°C for one hour and eluted with 12 column volumes of selection elution buffer (as selection binding buffer, but with an additional 5 mM ATP and 4.8 mM $MgCl_2$, pH readjusted to 7.4) at 4°C for one hour. The eluted fraction was then brought to 0.1 M in NaOH, hydrolyzed at 90°C for 10 minutes, exchanged into deionized water on two successive gel filtration columns and amplified using PCR. Every round was assayed by SDS-PAGE to ensure that mRNA degradation had not occurred, and by scintillation counting of the ^{35}S-methionine labelled proteins to measure the efficiencies of the various steps. These data were then used to determine the number of purified individual protein sequences introduced into the round one selection step as $6x10^{12}$ based on the proportion of total methionine incorporated into the mRNA-displayed proteins, and the efficiency of each of the subsequent purification steps.

This procedure was repeated for 18 rounds except that in rounds 10, 11 and 12 the PCR amplification was substituted by a mutagenic PCR amplification with an average mutagenic rate of 3.7% at the amino acid level. In rounds 14, 15 and 16 the amplification cycles were preceded by two ATP-agarose selection steps, and in rounds 17 and 18 the amplification cycles were preceded by three ATP-agarose selection steps. With successive selection steps the eluted fraction was exchanged into deionized water on a gel filtration column and purified on a denaturing Ni-NTA column and reverse transcribed as described above before being incubated with the ATP-agarose for the subsequent selection step. Also, the volume of the translation reaction was reduced to 1 ml except for round 1 in which it was 10 ml, and rounds 2, 10, 11 and 12 in which it was 5 ml. In the rounds of selection preceding the initial mutagenic amplification, incubation with a butyl agarose pre-column (Sigma, St. Louis, MO) was employed with the flowthrough being used for incubation with the ATP-affinity column.

K_d by spin-filtration

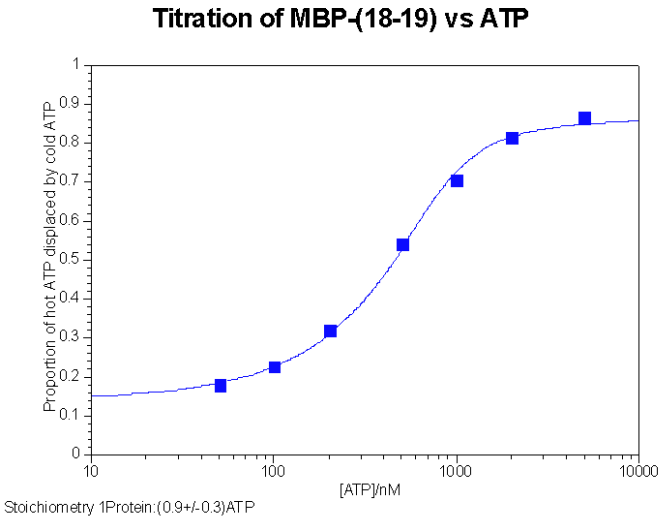
Purified MBP-fusion proteins were exchanged into selection binding buffer by gel filtration and mixed with □-^{32}P-ATP. These samples were incubated at 4°C for 30 minutes. 200 µl samples were then placed into Microcon-30 spin ultrafiltration devices (Millipore, Bedford, MA) and spun at 10,000g for 30 seconds with this filtrate being discarded, spinning at 10,000g for a further 45 seconds yielded a subsequent filtrate which, along with the unfiltered sample, were assayed by scintillation counting. This method was adapted from[14]. In competition experiments, the competitor was added after the incubation step, and then the solution was incubated for an extra 30 minutes at 4°C. Data were treated as above (K_d by Equilibrium Dialysis) and according to the method of Wang and von Hippel[15] to measure the stoichiometry of the interaction.

K_d by equilibrium dialysis

Purified MBP-fusion proteins were exchanged into selection binding buffer by gel filtration and 150 µl aliquots were placed on one side of a 14-16 kDa MWCO dialysis membrane in a Hoefer Scientific Instruments model EMD101B. Equal volumes of the same buffer containing diluted □-^{32}P-ATP were placed on the other side of the membrane. After 24 hours at 4°C, samples were removed from each side of the membrane and assayed by scintillation counting. These data were fitted to $y=c(x/(x+K_d))$, where x is the protein concentration, y the proportion of counts bound by the protein and c is the proportion of counts that are able to be bound by the protein, by an iterative algorithm (Deltagraph) to give the K_d. Competition experiments against unlabelled ATP or ATP analogues were performed similarly.

Back titration of hot ATP against cold ATP to give stoichiometry of interaction

This data was fitted to y = b+c((ANK+PK+1)



y=1.420544E-1+(7.212891E-1*((((x/1000000000)*1.083896E+0*K)+(R*K)+1)-((((((x/1000000000)*1.083896E+0*K)+(R*K)+1)^2)-(4*(x/1000000000)*1.083896E+0*R*K*K))^0.5))/(2*R*K))
R^2 = 9.974595E-1

$-((ANK+PK+1)^2-(4ANPK^2))^{0.5})/2PK$

b is proportion of counts not passing through membrane, an iteratively fitted constant

c is proportion of counts bindable, an iteratively fitted constant

A is the ATP concentration

K is the association constant

P is the active protein concentration

N is the stoichiometry, an iteratively fitted constant