

Fragment-Based Discovery of a Regulatory Site in Thioredoxin Glutathione Reductase Acting as “Doorstop” for NADPH Entry

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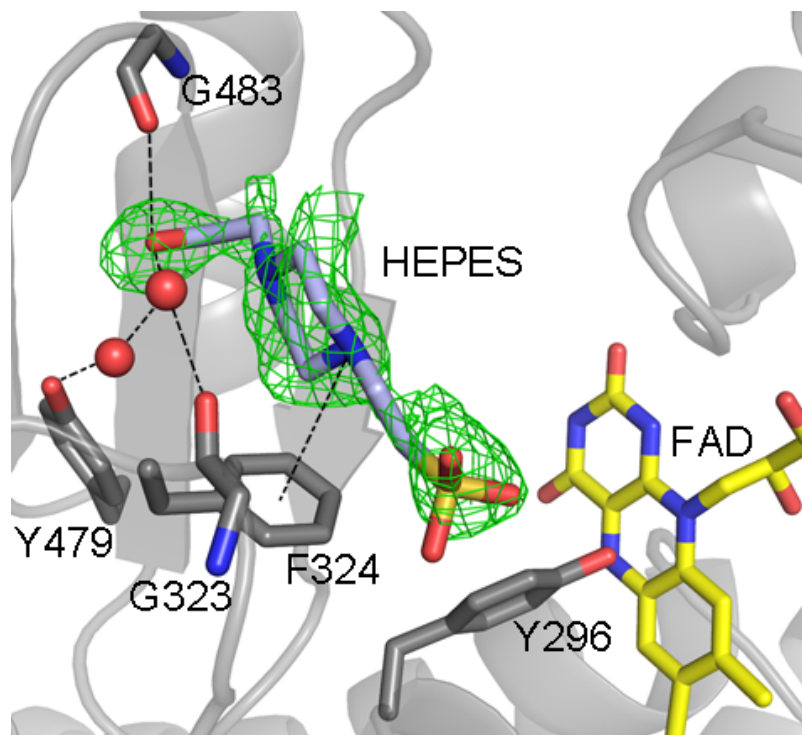
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Supplementary Table 1. Summary of data reduction and refinement statistics

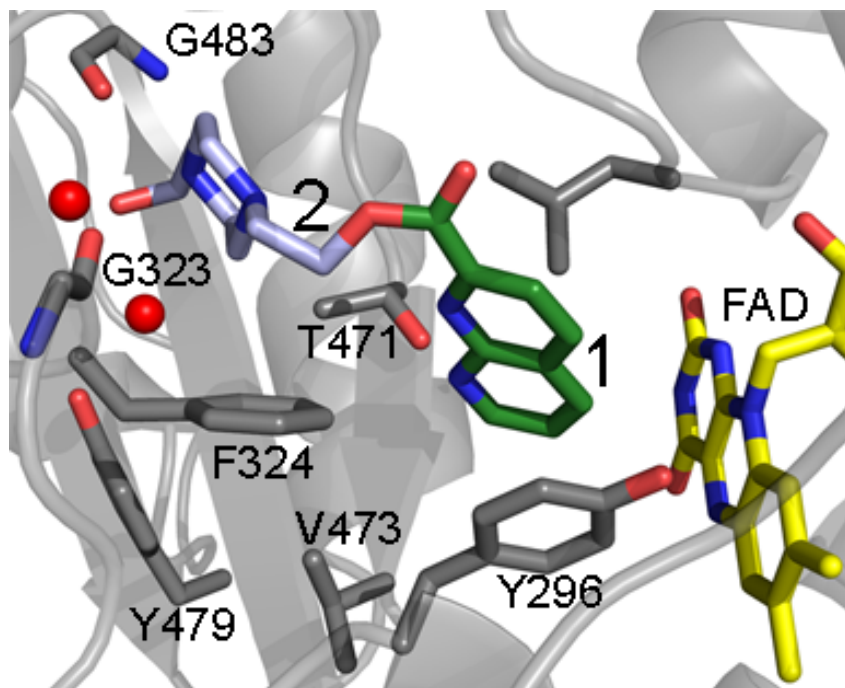
Compound	1 1,8-Naphthyridine-2-carboxylic acid	2 1,4-Bis(2-hydroxyethyl)piperazine	3 2-[4-(4-aminobutyl)piperazin-1-yl]ethanol	4 HEPES
PDB code	6FP4	6FMZ	6FMU	6FTC
Data reduction statistics				
Space group	C2	C2	C2	C2
Unit cell dimensions (Å)	142.31 101.84 58.78 $\beta=112.78$	142.90 102.74 59.135 $\beta=113.12$	143.25 102.21 58.66 $\beta=112.97$	143.93 102.64 61.65 $\beta=113.08$
Resolution range (Å)	40-2.5(2.6-2.5)	41-1.8 (1.84-1.80)	40-1.8 (1.84-1.80)	40-1.8(1.84-1.8)
mean I/sigma(I)	12.4(1.8)	11.4(2.3)	16.7(2.4)	13.1(2.2)
Completeness (%)	99.5(97.2)	99.8(99.5)	99.5(96.9)	99.9(99.9)
CC1/2	99.8(75.3)	99.8(75.0)	99.9(81.4)	99.8(0.70)
Rmerge	6.7(69.8)%	6.7(49.0)	6.6(70.3)%	6.6(55.0)
Number of unique reflections	26602(2937)	72511(4256)	71565 (4144)	76141(4492)
REFINEMENT STATISTICS				
Resolution range (Å)	40-2.5(2.59-2.5)	40.3-1.80(1.82-1.80)	37-1.8(1.82-1.80)	38.0-1.8(1.82-1.8)
No. reflections	26580(2446)	72508(2565)	71553(2659)	76137(2550)
R/Rfree	19.5/22.92(30.5-34.3)	14.9/17.8(0.23/0.25)	0.18/0.15(0.29/0.24)	17.5/19.5(24.2/27.5)
Mean B-factor	62.0	31.0	35.0	30.0
RMS bond lengths deviations	0.004	0.009	0.01	0.005
RMS bond angles deviation	0.685	1.071	1.08	0.854
RAMACHANDRAN (%)				
Favored	94.5	97.5	97.5	97.6
Allowed	5.5	2.5	2.5	2.4
Disallowed	0	0	0	0

*Values given in parentheses refer to highest resolution shell.

Supplementary figure 1. Crystal structure of SmTGR-HEPES complex. HEPES is shown in light blue sticks with the relative $F_o - F_c$ electron density map contoured at 3σ and shown in green, FAD in yellow and the interacting side chain residues are shown in grey sticks. Water molecules interacting with the hydroxyethyl moiety and with N1 of the piperazine ring are displayed as red spheres. The interaction array for the HEPE moiety is the same observed for 1,4-Bis(2-hydroxyethyl)piperazine and 2-[4-(4-aminobutyl)piperazin-1-yl]ethanol showed in Figure 2 of the main text.



Supplementary figure 2. The putative chimeric compound combining the structural features of 1,4-bis(2-hydroxyethyl)piperazine (compound **2**, in light blue sticks) and 1,8-naphthyridine-2 carboxylate (compound **1**, in green sticks) found in the corresponding x-ray structures.



Supplementary figure 3

Partial sequence alignment between the known drug-targets belonging to the FAD/NAD linked reductase family. The analysis is limited to the side-chain residues contributing to the doorstep pocket in SmTGR as found by the Cast-p calculation (Dundas et al., 2006, see main text). The residues contributing with their side chains are those considered in the sequence alignment where they are highlighted in yellow: FAD, K162, Y296, E300, L321, R322, G323, F324, D325, A436, G437, P439, Q440, L441, S467, V469, A470, T471, T472, V473, Y479, A481, C482, G483, L484, S485, D488, H538, I570*, P572* (residue numbering is according to the SmTGR sequence; the underlined residues are those contributing to the doorstep pocket with their side chains; the asterisks indicate the residue belonging to the other subunit). The proteins considered in the sequence alignment are those known to be validated or potential drug targets in human diseases (see the references relative to each pdb ID at the end of the sequence alignment) and whose 3D structure is known. The pdb IDs of each protein are indicated in parenthesis. SmTGR: Thioredoxin glutathione reductase from *Schistosoma mansoni*; SjtGR: Thioredoxin glutathione reductase from *Schistosoma japonicum*; hTrxR: thioredoxin reductase isoform I from *Homo sapiens*; pfTrxR: thioredoxin reductase from *Plasmodium falciparum*; hsGR: glutathione reductase from *Homo sapiens*; ypGR: glutathione reductase from *Yersinia pestis*; PfGR: glutathione reductase from *Plasmodium falciparum*; LiTryR: Trypanothione reductase *Leishmania infantum*; TbTryR: Trypanothione reductase from *Trypanosoma brucei*; TcTryR: Trypanothione reductase from *Trypanosoma cruzi*; BhGR: Glutathione reductase from *Bartonella henselae*. When a residue is not conserved with respect to SmTGR, the green color is used. Side chain residues within 5 Å which are involved in the recognition of compound **1-4** are indicated by a “\$” and “#” symbols, respectively.

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SmTGR (2V6O)      P K K L M H Q A G L L S H A L E - - - - - D A E - H F G W S L D R - S K I S H N W S T M V E G V Q S H I G S L - N W G Y
SjtGR (4LA1)      P K K L M H Q A G L L S H S L E - - - - - D A Q - H F G W S L D K - S E I S H D W S T M V E G V Q S H I G S L - N W G Y
HsTrxR (2J3N)     P K K L M H Q A A L L G Q A L Q - - - - - D S R - N Y G W K V E E - T - V K H D W D R M I E A V Q N H I G S L - N W G Y
PfTrxR (3DH9)     P K K L M H Y A G H M G S I F K L - - - - - D S K - A Y G W K F D N - L - - K H D W K K L V T T V Q S H I R S L - N F S Y
HsGR (3DJG)       P K K V M W N T A V H S E F M H D - - - - - H A - - D Y G F P S C E - G K F - - N W R V I K E K R D A Y V S R L - N A I Y
YpGR (5VDN)       P K K V M W H A A Q I A E A I H L - - - - - Y G P - D Y G F D T T V - N H F - - D W K K L I A N R T A Y I D R I - H Q S Y
PfGR (1ONF)       P K K I M F N A A S V H D I L E N - - - - - S R - H Y G F D T - - - - K F S F N L P L L V E R R D K Y I Q R L - N N I Y
LiTryR (2JK6)     P K K L M V T G A Q Y M D L I R E - - - - - S G - G F G W E M D R - E S L C P N W K T L I A A K N K V V N S I - N E S Y
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TbTryR (2WOI)
TcTryR (1AOG)
BhGR (300H)

PKKLMVTGAQYMDHLRE-----SA-GFGWEFDG-SSVKANWKKLIAAKNEAVLDI-NKSY
PKKLMVTGAQYMEHLRE-----SA-GFGWEFDR-TTLRAEWKNLIAVKDEAVLNI-NKSY
PKKLYFYASQYAQEFK-----SI-GFGWKYAD-PIF--NWEKLVAANKKEISRL-EGLY

SmTGR (2V6O)
SjtGR (4LA1)
HsTrxR (2J3N)
PfTrxR(3DH9)
HsGR (3DJG)
YpGR (5VDN)
PfGR (1ONF)
LiTryR (2JK6)
TbTryR (2WOI)
TcTryR (1AOG)
BhGR (300H)

KVALRDN-QVTYL-NAKGRLLS-----PHEVQITDKN--QKVS-----TITGNKIILA
KVSLRDN-AVTYL-NARGMLLS-----PHEVQITEKN--KKVS-----TITGNKIILA
RVALREK-KVVEE-NAYGQFIG----PHRIKATNNK--GKEK-----IYSAERFLIA
MTGLRSS-KVKYI-NGLAKLKD----KNTVSYYLKGDLSKEE-----TVTGYKYLIA
QNNLTKS-HIEII-RGHAAFTSDP--KPTIE--VSG-----KKYTAPHILIA
ERGLGNN-KVDVI-QGFARFVD---AHTVE--VNG-----ETITADHILIA
RQNLKSD-KVDLY-EGTASFLS----ENRILIKGTKDNNKDNGLPL-NEEILEGRNILIA
KSMFADTEGLSFH-MGFGALQD---AHTVVVRKSEDPHSDV-----LETLDTEYLIA
EGMFNDTEGLDFF-LGWGSLES----KNVVVVRETADPKSAV-----KERLOADHILLA
DEMFRDTEGLEFF-LGWGSLES----KNVVNVRESADPASAV-----KERLETEHILLA
REGLQNS-NVHIY-ESRAVFVD---EHTLELSVTG-----ERISAEKILIA

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SmTGR (2V6O)
SjtGR (4LA1)
HsTrxR (2J3N)
PfTrxR(3DH9)
HsGR (3DJG)
YpGR (5VDN)
PfGR (1ONF)
LiTryR (2JK6)
TbTryR (2WOI)
TcTryR (1AOG)
BhGR (300H)

TGERPKYP---EIPG--AVEYGITSDDLFF----SLPY-FPGKTLVIGASYVALEECAGFLA
TGERPKYP---EIPG--AIEYGITSDDLFF----SLPY-FPGKTLVVGASYVALKACAGFLA 1
TGERPRYL---GIPG--DKEYCISDDLFF----SLPY-CPGKTLVVGASYVALEECAGFLA
TGCRPHIPD--DVEG--AKELSITSDDIF----SLKK-DPGKTLVVGASYVALEECAGFLN
TGGMPSTPHESQIPG--A-SLGITS DGFF----QLEE-LPGRSVIVGAGYIAVEMAGILS
TGGRPSHP---DIPG--A-EYGIDS DGFF----ELDE-MPKRVAVVGAGYIAVEIAGVLN
VGNKPVFPP---VKG--I-ENTISSDEFF----NIKE-S-KKIGIVGSGYIAVELINVIK
TGSWPTRL---GVPG--D-EFCITSNEAF----YLED-APKRMLCVGGGYIAVEFAGIFN
TGSWPQMP---AIPG--I-EHCISSNEAF----YLPE-PPRRVLTVGGGFISVEFAGIFN 1
SGSWPHMP---NIPG--I-EHCISSNEAF----YLPE-PPRRVLTVGGGFISVEFAGIFN 1
TGAKIVSN--SAIKG--S-DLCLTSNEIF----DLEK-LPKSIVIVGGGYIGVEFANIFH

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SmTGR (2V6O)
SjtGR (4LA1)
HsTrxR (2J3N)
PfTrxR(3DH9)
HsGR (3DJG)

SL---GGDVTVMVRSI-LL-RGFDQQMAEKVGDYMEH-HGVKFAKL-CVPDEIKQLKVVD
SL---GGDVTVMVRSI-LL-RGFDQQMAEKVGDYMEH-HGVKFAKL-CVPDEITQLKPVD
GI---GLDVTVMVRSI-LL-RGFDQDMANKIGEHEMEE-HGIKFIRO-FVPIKVEQI----
SL---GYDVTVAVRSI-VL-RGFDQOCAVKVLYMEE-QGVMFKNG-ILPKKLTQMDD--
AL---GSKTSLMIRHDKVL-RSFDMSMISTNCTEELN-AGVEVLKF-SQVKEVKKT---- 1

YpGR (5VDN) GL---GTETHLFVRKHAPL-RTFDPLIVETLLEVMNT-EGPKLHTE-SVPKAVIKN---- 1
 PfGR (1ONF) RL---GIDSYIFARGNRIL-RKFDESVINVLENDMKK-NNINIVTF-ADVVEIKKV---- 1
 LiTryR (2JK6) GYKPCGGYVDLCYRGDLIL-RGFDTEVRKSLTKQLGA-NGIRVRTN-LNPTKITKN----
 TbTryR (2WOI) AYKPPGGKVTLCYRNNLIL-RGFDETIREEVTKQLTA-NGIEIMTN-ENPAKVSLN----
 TcTryR (1AOG) AYKPKDGQVTLCYRGEMIL-RGFDHTLREELTKQLTA-NGIQILTK-ENPAKVELN----
 BhGR (3O0H) GL---GVKTLLHRGDLIL-RNFDYDLRQLLDAMVA-KGISIIYE-ATVSQVQST---- 1

SmTGR (2V60) TENNKPGLLLVKGHYT-D-GKKFEEE-FETVIFAVGREPQLSKVLCETVGVKL-DKNGRV
 SjtGR (4LA1) TENNKPGLLLVKGHYT-D-GKKFEEE-FETVIFAVGREPQLSKLNCEAVGVKL-DKNGRV
 HsTrxR (2J3N) -EAGTPGRLRVVAQSTNS-EEIEGE-YNTVMLAIGRDACTRKIGLETVGVKINEKTGKI
 PfTrxR(3DH9) -----KILVEF-----SDKTSEL-YDTVLYAIGRKGIDGLNLESNMNVNKSNNKI
 HsGR (3DJG) -LSGLEVSMVTAVPGRLP-VMTMIPD-VDCLLWAIGRVPNTKDLNKLGIQTD-DKGHI
 YpGR (5VDN) -ADG-----SLTLQLE-NGTEVT--VDHLIWAIGREPATDNLNLSVTGVKTN-DKGYI
 PfGR (1ONF) -SDKN-----LSIHLS-DGRIYEH-FDHVIYCVGRSPDTENLKLEKLNVTN-NN-YI
 LiTryR (2JK6) -EDG-----SNHVHFN-DGTEED--YDQVMLAIGRVPRSQALQLDKAGVRTG-KNGAV
 TbTryR (2WOI) -TDG-----SKHVTFE-SGKTLD--VDVMMMAIGRIPRTNDLQLGNGVVKLT-PKGGV
 TcTryR (1AOG) -ADG-----SKSVTFE-SGKKMD--FDLVMMAIGRSPRTKDLQIQNAGV-MI-KNGGV
 BhGR (3O0H) -ENCY-----NVVLT-NGQTIC--ADRVMLATGRVPNTTGLGLERAGVKVN-EFGAV

SmTGR (2V60) VCTDDEQTTVSNVYIAIGDI-----NAGKPQLT 439
 SjtGR (4LA1) VCSDDDEQTTVSNYIAIGDI-----NAGKPQLT \$ \$
 HsTrxR (2J3N) PVTDEEQTNVPYIYAIGDI-----LEDKVELT 4
 PfTrxR(3DH9) IADHLSCTNIPSIFAVGDV-----AENVPELA 3
 HsGR (3DJG) IVDEFQNTNVKGIYAVGD-----VCGKALLT 3
 YpGR (5VDN) EVDKFQNTNVKGIYAVGD-----NTGVVELT 3
 PfGR (1ONF) VVDENQRTSVNNIYAVGDCCMVKKSKEIEDLNLLKLYNEERYLNKKENVTEDI FYNVQLT 3
 LiTryR (2JK6) QVDAYSKTSVDNIYAIGD-----VTNRVMLT 4
 TbTryR (2WOI) QVDEFSRTNVPNIYAIGD-----ITDRLMLT 4
 TcTryR (1AOG) QVDEYSRTNVSNIYAIGD-----VTNRVMLT 4
 BhGR (3O0H) VVDEKMTTNVSHIWAIVGD-----VTGHIQLT 3

SmTGR (2V60) PVAIQAGRYLARRL-FAGATELT-DYSNVA-TTVFTPLEYGACGLSEEDAIEKYGDK--- 469
 SjtGR (4LA1) PVAIHAGRYLARRL-FAGATELT-DYSNVA-TTVFTPLEYGACGLSEEDAIEKYGDN--- \$ \$ #

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HsTrxR (2J3N)          PVAIQAGRLLAQLR-YAGSTVKC-DYENVP-TTVFTPLEYGACGLSEEKAVEKFGEE--- 2
PfTrxR(3DH9)         PVAIKAGEILARRL-FKDSDEIM-DYSYIP-TSIYTPIEYGACGYSEEKAYELYGKS--- 3
HsGR (3DJG)          PVAIAAGRKLHRL-FEYKEDSKLDYNNIP-TVVFSHPPIGTVGLTEDEAIHKYGIE--- 6
YpGR (5VDN)          PVAVAAGRRLSERL-FNNKPDEHLDYNNIP-TVVFSHPPIGTIGLTEPOAREKFGDD--- 5
PfGR (1ONF)          PVAINAGRLLADRL-FLKKTRKT-NYKLIPTVIFSHPPIGTIGLSEEAIIQIYGKE--- 6
LiTryR (2JK6)        PVAINEGAAFVETV-FGGKPRAT-DHTKVA-CAVFSIPPPIGTTCGMTEEEAAKNYE----- 5
TbTryR (2WOI)        PVAINEGAALVDTV-FGNKPRKT-DHTRVA-SAVFSIPPPIGTTCGLIEEVAAKEFE----- 6
TcTryR (1AOG)        PVAINAAAALVDTV-FGTTPRKT-DHTRVA-SAVFSIPPPIGTTCGLIEEVASKRYE----- 6
BhGR (3O0H)          PVAIHDAMCFVKNA-FENTSTTP-DYDLIT-TAVFSQPEIGTVGLSEEDALHRYK----- 6

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SmTGR (2V6O)          DIEVYHSNFK-----PLEWTVAHRE-----DNVCYMKLVCRKSDNM
SjtGR (4LA1)          DIEVYHSHFK-----PLEWTVAHRE-----DNVCYMKLVCRI SDNM
hsTrxR(2J3N)         NIEVYHSYFW-----PLEWTIPSRD-----NNKCYAKIICNTKDNE
pfTrxR(3DH9)         NVEVFLQEFN-----NLEISAVHRQKH IRAQKDEYDL DV SSTCLAKLVCLKNEDN
hsGR (3DJG)          NVKTYSTSFT-----PMYHAVTKRK-----TKCVMKMVCA-NKEE
ypGR (5VDN)          QVKVYTSSTF-----AMYSAVTQHR-----QPCRMLKLCV-GAEE
PfGR (1ONF)          NVKIYESKFT-----NLFFSVYDIEPELK-----EKTYLKLVCV-GKDE
LiTryR (2JK6)        TVAVYASSFT-----PLMHNISGSK---H-----KEFMIRIITN-ESNG
TbTryR (2WOI)        KVAVYMSSFT-----PLMHNISGSK---Y-----KKFVAKIVTN-HSDG
TcTryR (1AOG)        VVAVYLSSTF-----PLMHKVS GSK---Y-----KTFVAKIITN-HSDG
BhGR (3O0H)          RVEIYRTVFR-----PMRNVLSGSP-----EKMFMLVVD-GESR

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SmTGR (2V6O)          RVLGLHVLGPNAGEITQGYAVAIKMGATKADF--DRTIGHPTCSETFTTLHVTKKSGVS
SjtGR (4LA1)          RVLGLHVLGPNAGEITQGYAVAIKMGATKEDF--DRTIGHPTCSETFTTLHVTKRSGGS
HsTrxR (2J3N)         RVVGFHVLGPNAGEVTQGF AAALKCGLTKKQL--DSTIGHPVCAEVFTTLSVTKRSGAS
PfTrxR(3DH9)         RVIGFHYVGP NAGEVTQGMALALRLKVKKKDF--DNCIGHPTDAESFMNLFVTISSGLS
HsGR (3DJG)          KVVGIHMQGLGCDEMLQGF AVAVKMGATKADF--DNTVAIHPTSSEELVTLR-----
YpGR (5VDN)          KIVGIHGIGFGMDEILQGF AVAMKMGATKKDF--DNTVAIHPTAAEEFVTMR-----
PfGR (1ONF)          LIKGLHIIGLNADEIVQGF AVALKMNATKKDF--DETIPHPTAAEEFLTLQPWMK----
LiTryR (2JK6)        EVLGVHMLGDSAPEIIQSVGICMKMGAKISDF--HSTIGVHPTSAAEELCSMRTPAYFYES 1
TbTryR (2WOI)        TVLGVHLLGDGAPEIIQAVGVCLRLNAKISDF--YNTIGVHPTSAAEELCSMRTPSYYYVK 1
TcTryR (1AOG)        TVLGVHLLGDNAPEIIQIGIGICLKLNAKISDF--YNTIGVHPTSAAEELCSMRTPSYYYVK 1
BhGR (3O0H)          IVVGAHVLGENAGEIAQLIGISLKGKLT KDIF--DKTMAVHPTMSEELVTMYKPSYVYEN 1

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Supplementary references relative to Supplementary Fig. 3.

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