

SUPPLEMENTARY ONLINE DATA High-resolution NMR studies of structure and dynamics of human ERp27 indicate extensive interdomain flexibility

Nader T. AMIN^{*1}, A. Katrine WALLIS^{*2}, Stephen A. WELLS^{†3}, Michelle L. ROWE[‡], Richard A. WILLIAMSON[‡], Mark J. HOWARD^{‡4} and Robert B. FREEDMAN^{*4}

*School of Life Sciences, University of Warwick, Coventry CV4 7AL, U.K., †Department of Physics, University of Warwick, Coventry CV4 7AL, U.K., and ‡School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, U.K.



Figure S1 ModelFree analysis of ¹⁵N-NMR relaxation data of the ERp27 b domain

Data were collected at 25 °C and 14.1 T. Histograms illustrate the changes across the sequence of (**A**) order parameter S^2 , (**B**) internal correlation time τ_e and (**C**) rate of chemical exchange broadening R_{ex} . A schematic diagram of the domain secondary structure as solved by NMR is shown above each plot. Cylinders represent α -halices and arrows represent β -strands.

- ² Present address: Faculty of Health and Life Sciences, Coventry University, Richard Crossman Building, Coventry CV1 5FB, U.K.
- ³ Present address: Department of Physics, University of Bath, Claverton Down, Bath BA2 7AY, U.K.
- ⁴ Correspondence may be addressed to either of these authors (email m.j.howard@kent.ac.uk or r.b.freedman@warwick.ac.uk).
- The structural co-ordinates for the b domain of human ERp27 reported will appear in the PDB under accession code 2L4C.

¹ Present address: Astex Therapeutics, 436 Cambridge Science Park, Cambridge CB4 0QA, U.K.





A closed circle represents a residue with an amide proton which is still present in the 2D $^{15}N^{-1}H$ spectrum after incubation in $^{2}H_{2}O$ for 300 s. An open circle represents an amide proton still present in the 2D $^{15}N^{-1}H$ spectrum after exchange to $^{2}H_{2}O$ for which there is ambiguity over the assignment. The sequential proton–proton NOE connectivities ($d_{\alpha N}$, d_{NN} and $d_{\beta N}$) are represented by thick and thin bars. These correspond to strong and weak NOE intensities respectively. The medium-range proton–proton NOE connectivities ($d_{\alpha N}$, $d_{n N}$, $d_{\alpha \beta}(i, i + 3)$, $d_{\alpha n}(i, i + 4)$, $d_{\alpha n}(i, i + 2)$ and $d_{\alpha n}(i, i + 2)$] are represented by lines connecting the residues whose protons are correlated. $^{1}H\alpha$ and $^{13}C\alpha$ chemical-shift deviations from random coil values, as determined by CSI are plotted and are labelled as $\Delta \delta$ $^{1}H\alpha$ and $\Delta \delta$ $^{13}C\alpha$ respectively, with units on the *y*-axis of p.p.m. Positive values represent shifts to lower field. The CSI and TALOS secondary structure predictions and the DSSP-CONT secondary structure assignment are represented by arrows and ribbons. Arrows represent β -sheets and ribbons represent α -helices. The Figure was generated using CCPN Analysis.

ERp27_H_sapiens	EPTWLTD · VPAAMEFIAA.TE · VAVIGFFQDLEIPAVPILHSMVQKFPGV <mark>SFGIS</mark> TD · · ·	•							
PDI_H_sapiens	AATTLPD-GAAAESLVES-SE-VAVIGFFKDVESDSAKQFLQAAEAIDDIPFGITSN	•							
PDIp_H_sapiens	SAMRLED · EAAAQALIGG · RD · LVVIGFFQDLQD · · · EDVATFLALAQDA · · · LDMTFGLTDR · · ·								
ERp57_H_sapiens	· SVPLRT·EEEFKKFTSD·KD·ASIVGFFDDSFS···EAHSEFLKAASNLR··DNYRFAHTNV···	•							
PDILT_H_sapiens	KAFLFNS. SEQVAEFVIS. RP. LVIVGFFQDLEE EVAELFYDVIKDF PELTFGVITI	•							
ERp72_R_norvegicus	• SKEILT • LKQVQEFLKDGDD • VVILGVFQGVGD • • PGYLQYQDAANTLR • • EDYKFHHTFS • • •	•							
cCSQ_H_sapiens	· VEIISS·KLEVQAFERI·EDVIKLIGFFKSEDS···EVYKAFEEAAEHFQ··PVIKFFATFD···	•							
ERP44_H_sapiens	• PIQE IRDLAEITTLDRS• KR•• NIIGYF EQKDS••• DNYRVF ERVANIUH•• DDCAFLSAFG•••	•							
ERp 29_H_sapiens	GALPLDT · VTFYKVIPKS · K <mark>F · VLVKFDT</mark> QYPYG · · · EKQDEFKRLAENS <mark>ASSDDLLVAEV</mark> GISDY	G							
PDI_S_cerevisiae	AVAVVAD · LPAYLANETF · VT · PVIVQSGK · IDA · · · DFNATFYSMANKHF · · NDYDFVSAENAD ·	•							
TRX_H_sapiens	M <mark>VKQI</mark> ES <mark>KTAFQEALDA</mark> AGDK· <mark>LVVVDFS</mark> ATW <mark>CGPC</mark> KMIKPFFHSLSEKY···SN <mark>VIFLEVD</mark> VDDC	•							
	TRX active								
	site motif								
ERp27_H_sapiens	site motif •••• <mark>Sevlth</mark> yni•••tgnt <mark>iclfr</mark> lv•••••DN <mark>eqlnl</mark> e•• Dedi •esid <mark>atklsrfiein</mark> sl								
ERp27_H_sapiens PDI_H_sapiens	site motif •••• <mark>SEVLTH</mark> YNI•••TGNT <mark>ICLFR</mark> LV•••••DNEQLNLE•• <mark>DEDI</mark> •ESID <mark>ATKLSRFIEIN</mark> SL •••• <mark>SDVFSK</mark> YQL•••DKD <mark>GVVLF</mark> KKF••••••DEG <mark>RNN</mark> FE••••••GEVT <mark>KENLLDFIKHN</mark> QL								
ERp27_H_sapiens PDI_H_sapiens PDIp_H_sapiens	site motif •••• SEVLTHYNI•••TGNTICLFRLV•••••DNEQLNLE••DEDI •••• SDVFSKYQL•••DKDGVVLFKKF•••••DEGRNNFE••••••GEVTKENLDFIKHNQL ••••PRLFQQFGL•••TKDTVVLFKKF••••••DEGRADFP••VDEE•LGLDLGDLSRFLVTHSM								
ERp27_H_sapiens PD[_H_sapiens PD p_H_sapiens ERp57_H_sapiens	site motif SEVLTHYNITGNTICLFRLVDNEQLNLEDEDI.ESIDATKLSRFIEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFPVDEE.LGLDLGDLSRFLVTHSM SELVNEYDDNGEGIILFRPSHLTNKFEDKTVAYTEQKMTSGKIKKFIQENIF								
ERp27_H_sapiens PD[_H_sapiens PD[p_H_sapiens ERp57_H_sapiens PDILT_H_sapiens	site motif SEVLTHYNITGNTICLFRLVDNEQLNLE.DEDI.ESIDATKLSRFIEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVT <u>KENLLDFIKHN</u> QL PRLFQQFGLTKDTVVLFKKFDEGRADFP.VDEE.LGLDLGDLSRFLVTHSM ESLVNEY DDNG <mark>EGIILF</mark> RPSHLTNKFEDK <mark>TVA</mark> YTEQKMT <mark>SGKIKKFIQEN</mark> IF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT								
ERp 27_H_sapiens PDLH_sapiens PDIp_H_sapiens ERp 57_H_sapiens PDILT_H_sapiens ERp 72_R_norvegicus	site motif SEVLTHYNITGNTICLFRLVDNEQLNLEDEDI.ESIDATKLSRFIEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFPVDEE.LGLDLGDLSRFLVTHSM SELVNEYDDNGEGIILFRPSHLTNKFEDKTVAYTEQKMTSGKIKKFIQENIF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT 								
ERp 27_H_sapiens PDL_H_sapiens PDIp_H_sapiens ERp 57_H_sapiens PDILT_H_sapiens ERp 72_R_norvegicus sCSQ_H_sapiens	site motif SEVLTHYNITGNTICLFRLVDNEQLNLE. DEDI .ESIDATKLSRFTEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFP.VDEE.LGLDLGDLSRFLVTHSM ESLVNEY DDNGEGIILFRPSHLTNKFEDKTVAYTEQKMT <u>SGKTKKFTQEN</u> IF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT GNVIGRFHVSLGKLVLMQPEKFQSKYEPRMHVMDVQGSTEASATKDYVKHAL KGVAKKLSLKMNEVDFYEPFMDEPIAIPNKPYTEEELVEFVKEHQR								
ERp 27_H_sapiens PDL_H_sapiens PDlp_H_sapiens ERp 57_H_sapiens PDLT_H_sapiens ERp 72_R_norvegicus sCSQ_H_sapiens ERP 44_H_sapiens	site motif SEVLTHYNITGNTICLFRLVDNEQLNLE. DEDI -ESIDATKLSRFTEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFP.VDEE.LGLDLGDLSRFLVTHSM ESLVNEYDDNGEGIILFRPSHLTNKFEDKTVAYTEQKMTSGKIKKFIQENIF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT TEIAKFLKVSLGKLVLMQPEKFQSKYEPRMHVMDVQGSTEASATKDYVVKHAL KGVAKKLSLKMNEVDFYEPFMDEPIAIPNKPYTEEELVEFVKEHQR DVSKPERYSGDNIYKPPGHSAPDMVYLGAMTNFDVTYNWIQDKV								
ERp 27_H_sapiens PDL_H_sapiens PDID_H_sapiens ERp 57_H_sapiens PDILT_H_sapiens ERP 72_R_norvegicus sCSQ_H_sapiens ERP 44_H_sapiens ERP 44_H_sapiens	site motif SEVLTHYNITGNTICLFRLVDNEQLNLE. DEDI .ESIDATKLSRFTEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFP.VDEE.LGLDLGDLSRFLVTHSM SELVNEYDDNGEGIILFRPSHLTNKFEDKTVAYTEQKMTSGKIKKFIQENIF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT TEIAKFLKVSLGKLVLMQPEKFQSKYEPRMHVMDVQGSTEASAIKDYVVKHAL KGVAKKLSLKMNEVDFYEPFMDEPIAIPNKPYTEELVEFVKEHQK DVSKPERYSGDNIIYKPPGHSAPDMVYLGAMTNFDVTYNWIQDKGV DKLNMELSEKYKLDKESYPYLFRDGDFENPVPYTGAVKVGAIQRWLKQQGV								
ERp 27_H_sapiens PDLH_sapiens PDIP_H_sapiens ERp 57_H_sapiens PDLLT_H_sapiens ERp 72_R_norvegicus sCSQ_H_sapiens ERP 44_H_sapiens ERp 29_H_sapiens PDL_S_cere visiae	site motif SEVLTHYNITGNTICLFRLVDNEQLNLE. DEDI .ESIDATKLSRFTEINSL SDVFSKYQLDKDGVVLFKKFDEGRNNFEGEVTKENLLDFIKHNQL PRLFQQFGLTKDTVVLFKKFDEGRADFP.VDEE.LGLDLGDLSRFLVTHSM ESLVNEYDDNGEGIILFRPSHLTNKFEDKTVAYTEQKMTSGKIKKFIQENIF GNVIGRFHVTLDSVLVFKKGKIVNRQKLINDSTNKQEL.NRVIKQHLTDFVIEYNT IETAKFLKVSLGKLVLMQPEKFQSKYEPRMHVMDVQGSTEASATKDYVVKHAL KGVAKKLSLKMNEVDFYEPFMDEPIAIPNKPYTEEELVEFVKEHQR DVSKPERYSGDNIJYKPPGHSAPDMVYLGAMTNFDVTYNWIQDKQ DKLNMELSEKYKLDKESYPYFLFRDGDFENPVPYTGAVKVGAIQRWLKQGQV DJFKLSIYLPSAMDEPVVYNGKKADIADADVFEKWLQVEAL								

Figure S3 Multiple sequence alignment between ERp27 b and selected homologous proteins

Sequences of human Trx and various **b** domains of the PDI family are shown. The alignment was generated using ClustalW and modified to take into account known secondary structure. Secondary structure elements were defined using DSSP. Strands are shown in yellow and helices are shown in red. The conserved *cis*-proline residues in the *cis*-proline loop of Trx and ERp29 are boxed in blue. The conserved *cis*-proline in the loop between α 2 to β 3 of calsequestrin is also boxed in blue. The active site CGPC motif of Trx is boxed in black. *H_sapiens, Homo sapiens; R_norvegicus, Rattus norvegicus; S_cerevisiae, Saccharomyces cerevisiae.*

	β1	al	β2	α.2	β3
	39	49	59	69 79	
H_sapiens	EPTWLT	DVPAAMEFIA	AATEVAVIGFFQD	LEIPAVPILHSMVQK	FPGVSFGIS
P_pygmaeus	EPTWLA	DVPAAMEFI	AATEVAVIGFFQD	LEIPAVPILHSMVQK	FPGVSFGIS
P_troglodytes	EPTWLA	DVPAAMEFI	AATEVAVIGFFQD	LEILAVPILHSMVQK	FPGVSFGIS
M mulatta	EPTWLA	DVPAAMEFI	AATEVAVVGFFQD	LEIPAVSILRSMVQK	FPDVSFGIS
C_jacchus	EPTWLT	DVPAAMEFIA	AATEVAVIGFFQD	LEIPAVSILHSMVEK	FPGVSFGIS
M_murinus	EPTWLT	DVPAAVEFI	AAAEVAVLGFFQD	LEIPAVSVFRSMVQK	YPDVPFGIS
P_vampyrus	EPIWLT	DVPAAMEFI	AAAEVAVIGFFQD	LETPAVSVFHSLVQK	FQDVSFGIS
O_gamettii	EPVWLP	DVPAAMEYI	TAAEVAVIGFFQD	LEIPAVPIFRSMVQK	HQDVSFGIS
C_familiaris	KPVMLT	DVQATMEFI	AVAEVAVIGFFQD	LEIPAVSLFHSMVQK	FQDVSFGIS
C_porcellus	GPVWLT	NVPAAMEFIA	AAVEVAVIGFFQD	LEIPIVPAFHSMVQE	FPDVSFGIS
M_lucifugus	EPVRLT	GVPAAMEFI	AAAEVAVIGFFQD	LEIPAVPVFHGVVQK	FQDVSFGIS
E_telfairi	EPVWLT	DVPTAVEFI	AAAEVAVIGFFQD	LEIPAVSVFHSMVKQ	FQDVAFGVS
P_capensis	EPVWLT	DVPAAVEFI	AAAEVAVIGFFQD	LEIPAVSVFQSMVQI	FQEVSFGVS
T_truncatus	EPVWLT	DVQAAMEFV	AAAEVTVIGFFQD	LEVPAVSVLHSVVKD	FQDVSFGIS
B_taurus	EPMRLT	DVQAAMEFI	AAAEVAVIGFFQD	SEVPAVSLIHSVVQN	FQDVSFGIS
E_caballus	EAVWLT	DVPAAMEFIA	AAAEVAVIGFFQD	LEIPAVSIFRSMAQD	FQDVSFGIS
E_europaeus	EPVRLT	DVPATMELI	A G A E V T A I G F F Q D	LEIPAVPIFHSMVQK	FQDVSFGIS
S_araneus	EPTKLT	DVPAAKKFI	FAAEVAVIGFFQD	LEIPVVPMLHSMVQK	FPDVPVGIS
M_musculus	EPIWLT	DVPATVELIA	AAAEVAVIGFFQD	LEIPIVSVFRSMARQ	FQDVSFGIS
R_norvegicus	EPRWLT	DIPATVELIA	AAAEVAVIGFFQD	LEIPIVSIFRSMAQQ	FQDVSFGIS
O_princeps	ELTWLT	DVPAAMAFI	AAAEVALVGFFQD	LELPVVSIFRDMVQN	FQDVSFGIS
A_carolinensis	PVLLE	DVAATEAFI	NAAEVVVVGFFKD	SERPEVSEFLIMVQN	IPEVPFALA
O_anatinus	AAERLG	DVPAAEAFV	A G A E V A V <mark>V</mark> G F F Q D	PEEPAASEFLGVARS	IGDVSFALS
G_gallus	KLVLLN	DIADVEAFI	SG A E V A V <mark>V G F F Q</mark> E	PESPEVSQLRLVAAR	IPEVPFALS
O_latipes	SILKLN	DTKAVEAFVI	SAEVVIIGFLEG	EESHGYKELVAASKK	VDSVPAAVC
G_aculeatus	VLPRLT	DTKAAEAFI	SAEVVVIGFLEG	EESPGYQQLVSAAGR	VHS <mark>V</mark> PVA <u>V</u> C
T_nigroviridis	SPVRLT	DAKAAEAFV	SAEVAVIGFLES	EESRGYQELVAAAKR	VDSVPVAIC

	α3		β4	β5 α4	a5	
	_	99	109	119	129	139
H_sapiens	TDSEVLTH	YNITGNTI	CLFRLVDN	EQLNLEDED	ESIDATKLSR	FIEINSL
P_pygmaeus	TDSEVLTH	IYNITGNTI	CLFRLVDN	EQLNLEDED	IESIDATKLSRI	FIEINSL
P_troglodytes	IDSEVLTH	IYN I TGNT I	CLFRLVDN	EQLNLEDED	IESIDATKLSRI	FIEINSL
M_mulatta	TDSEVLIH	IYNITGNTI	CLFRLVDN	EQLNLEDED	TESIDATKLSRI	FIEINSL
C_jacchus	TDSEVLTH	IYN I TGNT I	CLFRLVDN	EQLNLEDED	TAGIDAAKLSNI	FIEINSL
M_murinus	TDSGVLAH	IYNVTGNSI	SLFRLVDN	EQLDLEGED	ESIDETKLSRI	FIEINSL
P_vampyrus	TDSKVLAH	YNVTGNTI	SLFRLVDN	EQLDLDSED	IESIDATKLSRI	FIEINSL
0_gamettii	NDSEVLSH	IYSI TGNSI	SLFRLVDN	EQLDLEDEV	IESIDATKLSHI	FIEINSL
C_familiaris	TDSEVLGH	YNITGNTI	SLFRLVDN	KKLDLESKY	IESIDATKLSRI	FIEINSL
C_porcellus	NDSEVFSH	IYNITGNTI	SLFRLVDN	EQLKLEGEEI	ESIDATKLSH	FISINNL
M_lucifugus	TDPEVLAH	IYNITENTI	SLFRLVDN	EQLNLESEA	IENIDDTRLSRI	FIEINSL
E_telfairi	TNSEVLTH	IYNITGNTI	SLFRLVDN	EQLHLDSEG	IENIDDTKLSRI	FIEINSL
P_capensis	TDSEVLRH	IYNI TGNTI	SLFRLVDN	QQLNLESRD	ERIDDARLSRI	FIGTRSL
T_truncatus	TASEVLAH	I Y S V T G N T I	SLFRLVDN	KQLDLKSED	IESMDATKLSHI	FIQRNNL
B_taurus	TASEVLAY	YNITGNTI	SLFRLVDN	KQLDLKGED	ESMDATKLSR	FIESNNL
E_caballus	TDSEVLAH	YNVTGNAI	FLFRLVDN	KQLDLKNED	IENIDATKLSLI	FIETNNL
E_europaeus	TDPEVLSH	YNITGNTI	VLFRVVDN	EQLYLASEE	QNIDDAKLSRI	FIEIHSL
S_araneus	TDSEVLAH	FNVTENTI	SLFRQVDG	EQLKLESED	IESIDEAKLSRI	FIEINKL
M_musculus	NHSEVLTH	IYNVTSNSI	CLFRLVDD	QQLHLNAED	IENLDAAKLSRI	FIHVNNL
R_norvegicus	NHSEVLTH	IYNVTSNSI	CLFRLVDN	KQLRLDAED	IDDLDAAKLSRI	FIHLNNL
O_princeps	NNSDVLTH	IYN I TGNT I	SLFRLADN	EQVNLEGEV	IESLNADKLSH	FIEAHNL
A_carolinensis	NGSQVLSH	IYNVTRNT I	SLFRMVDN	KRQDVEIED	I KGLDANKLSRI	VRANEL
O_anatinus	SSQEVLAH	IYNITENTV	/ S L F R Q V D D	KRLDMKIPD	EKKIDATRMSRI	FIEMNKL
G_gallus	SSSAVRE	YGVTAATL	TLFRRADK	DRRDLNMDS	EE - IDADKMSRI	FIRINEL
O_latipes	TEKEVWAA	YSVSSDTI	TLFRKADD	QKKNLVLSE	LQKLDVDGLVN	FIATNEI
G_aculeatus	THKEVWSH	YALSSDTI	ALFRKADN	HQENLVIAT	TKKLEADGLVN	ISVNEV
T_nigroviridis	TVKEVWAD	YNISSDTI	ILFRKADR	HQEKLDLAA	AKKIDADGLVNI	TINEV

Figure S4 Sequence conservation between species in the b domain of ERp27

ERp27 **b** sequences from 27 different species were aligned using ClustalW. Secondary structure is indicated by cylinders for *α*-helices and arrows for *β*-strands. Sequence conservation is denoted by colour coding: light blue for >40 % identity, medium blue for >60 % identity and dark blue for >80 % identity. Residue numbering above the alignment is for human ERp27. *A_carolinensis, Anolis carolinensis; B_taurus, Bos taurus; C_familiaris, Canis familiaris; C_jacchus, Callithrix jacchus; C_porcellus, Cavia porcellus; E_caballus, Equus caballus; E_europaeus, Euonymus europaeus; E_telfairi, Echinops telfairi; G_aculeatus, Gasterosteus aculeatus; G_gallus, Gallus gallus; H_sapiens, Homo sapiens; M_lucifugus, Myotis lucifugus; M_mulatta, Macaca mulatta; M_murinus, Microcebus murinus; M_musculus, Mus musculus; O_anatinus, Ornithorhynchus anatinus; O_gamettii, Otolemur gamettii; O_latipes, Orizyas latipes; O_princeps, Ochotona princeps; P_capensis, Procavia capensis; P_pygmaeus, Pongo pygmaeus; P_troglodytes; P_vampyrus, Pteropus vampyrus; R_norvegicus, Rattus norvegicus; S_araneus, Sorex araneus; T_nigroviridis, Tetraodon nigroviridis; T_truncatus, Tupsiops truncatus.*



Figure S5 Comparison of spectra of b domain and full-length ERp27

Overlay of the ${}^{15}N{}^{-1}H$ HSQC spectra for the **b** domain of ERp27 (black) and full-length ERp27 (red). Residues with minimal shifts greater than 1 S.D. from the mean for residues 39–135 (0.0333 + 0.0292 p.p.m.) are labelled.

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