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## **Supporting Material**

## Sequence determinants of compaction in intrinsically disordered proteins

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## Supporting Material for 'Sequence determinants of compaction in intrinsically disordered proteins'

Name	# of	<b>R</b> <sub>h</sub>	pН	Reference	Sequence	
A. $PFG$ -NMK $h$	<i>neasuremen</i>	14 26	74	(1)	DAEFRHDSGYEVHHOKLVFFAEDVGSNKGAIIGLMVG	
Abeta(1-40)	40	14.50	7.4	(1)	GVV	
SBD	61	25.6	6	(2)	GSMMSASSQSPNPNNPAEYCSTIPPLEYCSTIPPLQQAQ ASGALSSPPPTVMVPVGVLKHP	
CTL9-I98A	92	21.7	6.6	(3)	AAEELANAKKLKEQLEKLTVTIPAKAGEGGRLFGSITS	
					KQAAESLQAQHGLKLDKRKIELADAIRALGYTNVPVK	
Udm2 APD	07	25.7	6	(4)		
Hum2-ADD	21	23.7	0	(4)	SVEFEVESLDSEDYSLSEEGQELSDEDDEVYQVTVYQA	
					GESDTDSFEEDPEISLADYWK	
Sm1	105	23.4	7	(5)	MQNSQDYFYAQNRCQQQQAPSTLRTVTMAEFRRVPLP	
					PMAEVPMLSTQNSMGSSASASASSLEMWEKDLEERLN SIDHDMNNINKEGSGELKSMENOGKVEEMDE	
prothymosin	110	22.7	7	(6)	MSDAAVDTSSEITTKDI KEKKEVVEEAENGRDAPANG	
alpha	110	55.7	/	(0)	NANEENGEQEADNEVDEEEEEGGEEEEEEGDGEEE	
aipna					DGDEDEEAESATGKRAAEDDEDDDVDTKKQKTDEDD	
TC1	112	26.5	5	(7)	HHHHHHMKAKRSHQAIIMSTSLRVSPSIHGYHFDTASR	
					KKAVGNIFENTDQESLERLFRNSGDKKAEERAKIIFAID	
alaha	140	20.2	74	(9)		
	140	28.2	7.4	(8)	GVLYVGSKTKEGVVHGVATVAEKTKEOVTNVGGAVV	
synuclein					TGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAP	
					QEGILEDMPVDPDNEAYEMPSEEGYQDYEPEA	
CFTR R	189	32	6.8	(9)	GAMESAERRNSILTETLHRFSLEGDAPVSWTETKKQSF	
region					KQTGEFGEKRKNSILNPINSIRKFSIVQKTPLQMNGIEED	
-					SULVERKLSLVPDSEQGEAILPRISVISVISVISVISVISVISVISVISVISVISVISVISVI	
					LDIYSRRLSOETGLEISEEINEEDLKECLFDDME	
Tau K45	198	45	6.5	(10)	MSSPGSPGTPGSRSRTPSLPTPPTREPKKVAVVRTPPKS	
		-			PSSAKSRLQTAPVPMPDLKNVKSKIGSTENLKHQPGGG	
					KVQIINKKLDLSNVQSKCGSKDNIKHVPGGGSVQIVYK	
					PVDLSKVISKCGSLGNIHHKPGGGQVEVKSEKLDFKD RVOSKIGSI DNITHVPGGGNKKIFTHKI TERENAKAKT	
					DHGAEIVY	
RYBP	234	39.5	7	(11)	HHHHHHMTMGDKKSPTRPKRQAKPAADEGFWDCSVC	
	_				TFRNSAEAFKCSICDVRKGTSTRKPRINSQLVAQQVAQ	
					QYATPPPPKKEKKEKVEKQDKEKPEKDKEISPSVTKKN	
					INKKI KPKSDILKDPPSEANSIQSANA I I KI SEINHI SK PRI KNVDRSTAOOLAVTVGNVTVIITDFKFKTRSSSTSS	
					STVTSSAGSEQONOSSSGSESTDKGSSRSSTPKGDMSA	
					VNDESF	
3D7-6H	237	34.3	7	(12)	MIKNESKYSNTFINNAYNMSIRRSMAESKPSTGAGGSA	
MSP2					GGSAGGSAGGSAGGSAGGSAGGSAGSGDGNGADAEGSSSTP	
					ATTTTKTTTTTTNDAEASTSTSSENPNHKNAETNP KGKGEVOEPNOANKETONNSNVOODSOTKSNVPPTO	
					DADTKSPTAQPEQAENSAPTAEQTESPELQSAPENKGT	
					GQHGHMHGSRNNHPQNTSDSQKECTDGNKENCGAAT	
					SLLNNSSNHHHHHH	
B. SEC measur	ements	1	1			
EHD-L16A	61	20.1	7	(13)	TNDEKRPRTAFSSEQAARLKREFNENRYLTERRRQQLS	
<b>52 TAD</b>	72	22.0	75	(14)	SELULINEAUINIW FUNKKAKIKK MEEDOSDOSVEDDI SOETESDI WKI I DENNVI SDI DSOA	
poo-TAD	15	23.8	1.5	(14)	MDDLMLSPDDIEQWFTEDPGPDEAPRMPEAAPRV	
p57-ID	73	24	7	(15)	XXTSACRSLFGPVDHEELSRELQARLAELNAEDQNRW	
r - ·				()	DYDFQQDMPLRGPGRLQWTEVDSDSVPAFYRETVQV	

**Table S1.** List of intrinsically disordered proteins with  $R_h$  measurements.

PDE-gamma	87	24.8	7.5	(16)	MNLEPPKAEIRSATRVMGGPVTPRKGPPKFKQRQTRQF KSKPPKKGVQGFGDDIPGMEGLGTDITVICPWEAFNHL ELHELAQYGII
Vmw65	89	28	8	(17)	GSAGHTRRLSTAPPTDVSLGDELHLDGEDVAMAHADA LDDFDLDMLGDGDSPGPGFTPHDSAPYGALDMADFEF EQMFTDALGIDEYGG
LJIDP1	94	24.52	7	(18)	MARSFTNIKAISALVAEEFSNSLARRGYAATAQSAGRV GASMSGKMGSTKSGEEKAAAREKVSWVPDPVTGYYK PENIKEIDVAELRSAVLGKN
Mlph(147- 240)	97	28	7.5	(19)	XXXGGGGSEPSLEEGNGDSEQTDEDGDLDTEARDQPL NSKKKKRLLSFRDVDFEEDSDHLVQPCSQTLGLSSVPE SAHSLQSLSGEPYSEDTTSLEP
TyrRS(D1)	107	21	7	(20)	MALFSGDIANLTAAEIEQGFKDVPSFVHEGGDVPLVEL LVSAGISPSKRQAREDIQNGAIYVNGERLQDVGAILTAE HRLEGRFTVIRRGKKKYYLIRYALEHHHHHH
ASR1	115	27.4	7	(21)	MEEEKHHHHHLFHHKDKAEEGPVDYEKEIKHHKHLE QIGKLGTVAAGAYALHEKHEAKKDPEHAHKHKIEEEI AAAAAVGAGGFAFHEHHEKKDAKKEEKKKLRGDTTIS SKLLF
Nup116	126	25.2	6.8	(22)	GSRRASVGSGALFGAKPASGGLFGQSAGSKAFGMNTN PTGTTGGLFGQTNQQQSGGGLFGQQQNSNAGGLFGQN NQSQNQSGLFGQQNSSNAFGQPQQQGGLFGSKPAGGL FGQQQGASTHHHHHH
Cad136	136	28.1	7	(23)	RLEQYTSAVVGNKAAKPAKPAASDLPVPAEGVRNIKS MWEKGNVFSSPGGTGTPNKETAGLKVGVSSRINEWLT KTPEGNKSPAPKPSDLRPGDVSGKRNLWEKQSVEKPA ASSSKVTATGKKSETNGLRQFEKEP
NTAIL	139	27	7	(24)	MRGSHHHHHHXXXHTTEDKISRAVGPRQAQVSFLHG DQSENELPRLGGKEDRRVKQSRGEARESYRETGPSRAS DARAAHLPTGTPLDIDTASESSQDPQDSRRSADALLRL OAMAGISEEOGSDTDTPIVYNDRNLLD
NL3-cyt	140	28.3	8.5	(25)	MGSSHHHHHHHSSGLVPRGSHMAYRKDKRRQEPLRQPS PQRGAGAPELGAAPEEELAALQLGPTHHECEAGPPHDT LRLTALPDYTLTLRRSPDDIPLMTPNTITMIPNSLVGLQ TLHPYNTFAAGFNSTGLPHSHSTTRV
ShB-C	146	32.9	8	(26)	MXXGQHMKKSSLSESSSDMMDLDDGVESTPGLTETHP GRSAVAPFLGAQQQQQQPVASSLSMSIDKQLQHPLQQ LTQTQLYQQQQQQQQQQQGFKQQQQQTQQQLQQQ QSHTINASAAAATSGSGSSGLTMRHNNALAVSIETDV
Fos-AD	168	35	7	(27)	GSHMSVASLDLTGGLPEVATPESEEAFTLPLLNDPEPKP SVEPVKSISSMELKTEPFDDFLFPASSRPSGSETARSVPD MDLSGSFYAADWEPLHSGSLGMGPMATELEPLCTPVV TCTPSCTAYTSSFVFTYPEADSFPSCAAAHRKGSSSNEP SSDSLSSPTLLAL
HIF1-alpha 530	170	38.3	7.2	(28)	XEFKLELVEKLFAEDTEAKNPFSTQDTDLDLEMLAPYI PMDDDFQLRSFDQLSPLESSSASPESASPQSTVTVFQQT QIQEPTANATTTTATTDELKTVTKDRMEDIKILIASPSPT HIHKETTSATSSPYRDTQSRTASPNRAGKGVIEQTEKSH PRSPNVLSVALSQR
HIF1-alpha 403	202	44.3	7.2	(28)	XAAGDTIISLDFGSNDTETDDQQLEEVPLYNDVMLPSP NEKLQNINLAMSPLPTAETPKPLRSSADPALNQEVALK LEPNPESLELSFTMPQIQDQTPSPSDGSTRQSSPEPNSPS EYCFYVDSDMVNEFKLELVEKLFAEDTEAKNPFSTQD TDLDLEMLAPYIPMDDDFQLRSFDQLSPLESSSASPESA SPQSTVTVFQ
Securin	204	39.7	7.2	(29)	XXMATLIYVDKENGEPGTRVVAKDGLKLGSGPSIKAL DGRSQVSTPRFGKTFDAPPALPKATRKALGTVNRATEK SVKTKGPLKQKQPSFSAKKMTEKTVKAKSSVPASDDA YPEIEKFFPFNPLDFESFDLPEEHQIAHLPLSGVPLMILD EERELEKLFQLGPPSPVKMPSPPWESNLLQSPSSILSTLD VELPPVCCDIDI
Gliotactin	215	33.4	7.4	(30)	XXXXMWRNAKRQSDRFYDEDVFINGEGLEPEQDTRG VDNAHMVTNHHALRSRDNIYEYRDSPSTKTLASKAHT DTTSLRSPSSLAMTQKSSSQASLKSGISLKETNGHLVKQ SERAATPRSQQNGSTAKVASPPVEEKRLLQPLSSTPVT QLQAEPAKRVPTAASVSGSSRSTTPVPSARSTTTHTTTA TLSSQPAAQPRRTHLVEGVPHHHHHH
Mlph(147-	260	49	7.5	(19)	XXXGGGGSEPSLEEGNGDSEQTDEDGDLDTEARDQPL NSKKKKRLLSFRDVDFEEDSDHLVOPCSOTLGLSSVPE

403)		SAHSLQSLSGEPYSEDTTSLEPEGLEETGARALGCRPSP
/		EVQPCSPLPSGEDAHAELDSPAASCKSAFGTTAMPGTD
		DVRGKHLPSQYLADVDTSDEDSIQGPRAASQHSKRRA
		RTVPETQILELNKRMSAVEHLLVHLENTVLPPSAQEPT
		VETHPSADTEEETLRRRLEELTSNISGSSTSSE

Name	# of	<b>R</b> <sub>h</sub>	Reference	
	residues			
bovine pancreatic trypsin inhibitor	58	15.8	(31)	
SH3 domain of PI3 kinase	90	18.6	(31)	
horse heart cytochrome c	104	17.8	(31)	
hen lysozyme	129	20.5	(31)	
horse myoglobin	153	21.2	(31)	
bovine alpha-lactalbumin	123	18.8	(31)	
bovine pancreatic ribonuclease A	124	19	(31)	
sperm whale apomyoglobin	153	20.9	(31)	
ubiquitin	76	16.5	(32)	
(apo)cytochrome C	104	18.5	(32)	
alpha-lactalbumin	123	18.5	(32)	
tumor supressor, p16	156	20	(32)	
(apo)myoglobin	154	20.9	(32)	
beta-lactoglobulin	162	22	(32)	
sarcoplasmic calcium binding	174	21.5	(32)	
adenylate kinase	194	21.9	(32)	
tryptophan synthase	268	24.2	(32)	
beta-lactamase	257	23.7	(32)	
carbonic anydrase B	260	23.3	(32)	
RTEM beta-lactamase	263	24.5	(32)	

**Table S2.** List of folded proteins with  $R_h$  measurements.

Name	# of	<b>R</b> <sub>h</sub>	Reference
	residues		
lysozyme(49-64), 8 M urea, pH 2	16	10.4	(31)
D3(17-37), 8 M urea	22	13.3	(31)
D3(2-38), 8 M urea	32	15.5	(31)
PI3 SH3, 3.5 M GdnHCl	90	27.5	(31)
lysozyme, 8 M urea, pH 2, reduced	129	34.6	(31)
triosephosphate isomerase, 2.5 GdnHCl	247	49.7	(31)
reduced bovine pancreatic ribonuclease	124	31.4	(31)
A, 6 M GdnHCl			
ubiquitin, 8 M urea	76	24.6	(32)
ribonuclease A, 8 M urea	124	32.4	(32)
lysozyme, 8 M urea	129	33.1	(32)
myoglobin, 8 M urea	153	35.1	(32)
beta-lactoglobulin, 8 M urea	162	37.8	(32)
chymotrypsinogen, 8 M urea	245	45	(32)
carbonic anhydrase B, 8 M urea	260	47.8	(32)
beta-lactamase, 8 M urea	257	48.9	(32)
albebetin, 6 M GdnHCl	73	24.7	(32)
beta-lactamse, 6 M GdnHCl	257	52	(32)
carbonic anhydrase B, 8 M urea	260	52	(32)
ubiquitin, 6 M GdnHCl	76	25.8	(32)
prothymosin alpha, 6 M GdnHCl	110	31.4	(32)
ribonuclease A, 6 M GdnHCl	124	32.8	(32)
alpha-lactalbumin, 6 M GdnHCl	123	31.8	(32)
alpha-synuclein, 6 M GdnHCl	140	34.3	(32)
tumor suppressor, p16, 6 M GdnHCl	156	37.1	(32)
dihydrofolate reductase, 6 M GdnHCl	158	37.6	(32)
beta-lactoglobulin, 6 M GdnHCl	162	37	(32)
adenlyate kinase, 6 M GdnHCl	193	42.1	(32)

**Table S3.** List of chemically denatured proteins with  $R_h$  measurements.

## References

- 1. Danielsson, J., J. Jarvet, P. Damberg, and A. Graslund. 2002. Translational diffusion measured by PFG-NMR on full length and fragments of the Alzheimer's Abeta(1-40) peptide. Magn Reson Chem 40:S89-97.
- 2. Chong, P. A., B. Ozdamar, J. L. Wrana, and J. D. Forman-Kay. 2004. Disorder in a target for the smad2 mad homology 2 domain and its implications for binding and specificity. J Biol Chem 279:40707-40714.
- 3. Li, Y., B. Shan, and D. P. Raleigh. 2007. The cold denatured state is compact but expands at low temperatures: hydrodynamic properties of the cold denatured state of the C-terminal domain of L9. J Mol Biol 368:256-262.
- Sivakolundu, S. G., A. Nourse, S. Moshiach, B. Bothner, C. Ashley, J. Satumba, J. Lahti, and R. W. Kriwacki. 2008. Intrinsically unstructured domains of Arf and Hdm2 form bimolecular oligomeric structures in vitro and in vivo. J Mol Biol 384:240-254.
- Danielsson, J., L. Liljedahl, E. Barany-Wallje, P. Sonderby, L. H. Kristensen, M. A. Martinez-Yamout, H. J. Dyson, P. E. Wright, F. M. Poulsen, L. Maler, A. Graslund, and B. B. Kragelund. 2008. The intrinsically disordered RNR inhibitor Sml1 is a dynamic dimer. Biochemistry 47:13428-13437.
- 6. Yi, S., B. L. Boys, A. Brickenden, L. Konermann, and W.-Y. Choy. 2007. Effects of zinc binding on the structure and dynamics of the intrinsically disordered protein prothymosin alpha: evidence for metalation as an entropic switch Biochemistry 46:13120-13130.
- 7. Gall, C., H. Xu, A. Brickenden, X. Ai, and W. Y. Choy. 2007. The intrinsically disordered TC-1 interacts with Chibby via regions with high helical propensity. Protein Sci 16:2510-2518.
- Paleologou, K. E., A. W. Schmid, C. C. Rospigliosi, H.-Y. Kim, G. R. Lamberto, R. A. Fredenburg, P. T. Lansbury, C. O. Fernandez, D. Eliezer, M. Zweckstetter, and H. A. Lashuel. 2008. Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of alpha-synuclein. J Biol Chem 283:16895-16905.
- 9. Baker, J. M. R. 2009. Structural Characterization and Interactons of the CFTR Regulatory Region (PhD Thesis). Department of Biochemistry. University of Toronto, Toronto.
- Soragni, A., B. Zambelli, M. D. Mukrasch, J. Biernat, S. Jeganathan, C. Griesinger, S. Ciurli, E. Mandelkow, and M. Zweckstetter. 2008. Structural characterization of binding of Cu(II) to tau protein. Biochemistry 47:10841-10851.
- 11. Neira, J. L., M. Roman-Trufero, L. M. Contreras, J. Prieto, G. Singh, F. N. Barrera, M. L. Renart, and M. Vidal. 2009. The transcriptional repressor RYBP is a natively unfolded protein which folds upon binding to DNA. Biochemistry 48:1348-1360.
- 12. Zhang, X., M. A. Perugini, S. Yao, C. G. Adda, V. J. Murphy, A. Low, R. F. Anders, and R. S. Norton. 2008. Solution conformation, backbone dynamics and lipid interactions of the intrinsically unstructured malaria surface protein MSP2. J Mol Biol 379:105-121.
- 13. Mayor, U., J. G. Grossmann, N. W. Foster, S. M. Freund, and A. R. Fersht. 2003. The denatured state of Engrailed Homeodomain under denaturing and native conditions. J Mol Biol 333:977-991.
- 14. Lowry, D. F., A. Stancik, R. M. Shrestha, and G. W. Daughdrill. 2008. Modeling the accessible conformations of the intrinsically unstructured transactivation domain of p53. Proteins 71:587-598.
- 15. Adkins, J. N., and K. J. Lumb. 2002. Intrinsic structural disorder and sequence features of the cell cycle inhibitor p57Kip2. Proteins 46:1-7.
- Uversky, V. N., S. E. Permyakov, V. E. Zagranichny, I. L. Rodionov, A. L. Fink, A. M. Cherskaya, and E. A. Permyakov. 2002. Effect of zinc and temperature on the conformation of the gamma subunit of retinal phosphodiesterase: a natively unfolded protein. Journal of Proteome Research 1:149-159.

- 17. Donaldson, L., and J. P. Capone. 1992. Purification and characterization of the carboxyl-terminal transactivation domain of Vmw65 from herpes simplex virus type 1. J Biol Chem 267:1411-1414.
- Haaning, S., S. Radutoiu, S. Hoffmann, J. Dittmer, L. Giehm, D. E. Otzen, and J. Stougaard. 2008. An unusual intrinsically disordered protein from the model legume Lotus japonicus stabilizes proteins in vitro. J Biol Chem 283:31142-31152.
- 19. Geething, N. C., and J. A. Spudich. 2007. Identification of a minimal myosin Va binding site within an intrinsically unstructured domain of melanophilin. J Biol Chem 282:21518-21528.
- 20. Guez, V., S. Nair, A. Chaffotte, and H. Bedouelle. 2000. The anticodon-binding domain of tyrosyl-tRNA synthetase: state of folding and origin of the crystallographic disorder. Biochemistry 39:1739-1747.
- 21. Goldgur, Y., S. Rom, R. Ghirlando, D. Shkolnik, N. Shadrin, Z. Konrad, and D. Bar-Zvi. 2007. Desiccation and zinc binding induce transition of tomato abscisic acid stress ripening 1, a water stress- and salt stress-regulated plant-specific protein, from unfolded to folded state. Plant Physiol 143:617-628.
- 22. Krishnan, V. V., E. Y. Lau, J. Yamada, D. P. Denning, S. S. Patel, M. E. Colvin, and M. F. Rexach. 2008. Intramolecular cohesion of coils mediated by phenylalanine--glycine motifs in the natively unfolded domain of a nucleoporin. PLoS Comput Biol 4:e1000145.
- 23. Permyakov, S. E., I. S. Millett, S. Doniach, E. A. Permyakov, and V. N. Uversky. 2003. Natively unfolded C-terminal domain of caldesmon remains substantially unstructured after the effective binding to calmodulin. Proteins 53:855-Na.
- 24. Longhi, S., V. Receveur-Brechot, D. Karlin, K. Johansson, H. Darbon, D. Bhella, R. Yeo, S. Finet, and B. Canard. 2003. The C-terminal domain of the measles virus nucleoprotein is intrinsically disordered and folds upon binding to the C-terminal moiety of the phosphoprotein. J Biol Chem 278:18638-18648.
- Paz, A., T. Zeev-Ben-Mordehai, M. Lundqvist, E. Sherman, E. Mylonas, L. Weiner, G. Haran, D. I. Svergun, F. A. Mulder, J. L. Sussman, and I. Silman. 2008. Biophysical characterization of the unstructured cytoplasmic domain of the human neuronal adhesion protein neuroligin 3. Biophys J 95:1928-1944.
- 26. Magidovich, E., I. Orr, D. Fass, U. Abdu, and O. Yifrach. 2007. Intrinsic disorder in the Cterminal domain of the Shaker voltage-activated K+ channel modulates its interaction with scaffold proteins. Proc Natl Acad Sci U S A 104:13022-13027.
- 27. Campbell, K. M., A. R. Terrell, P. J. Laybourn, and K. J. Lumb. 2000. Intrinsic structural disorder of the C-terminal activation domain from the bZIP transcription factor Fos. Biochemistry 39:2708-2713.
- 28. Sanchez-Puig, N., D. B. Veprintsev, and A. R. Fersht. 2005. Binding of natively unfolded HIF-1alpha ODD domain to p53. Mol Cell 17:11-21.
- 29. Sanchez-Puig, N., D. B. Veprintsev, and A. R. Fersht. 2005. Human full-length Securin is a natively unfolded protein. Protein Sci 14:1410-1418.
- 30. Zeev-Ben-Mordehai, T., E. H. Rydberg, A. Solomon, L. Toker, V. J. Auld, I. Silman, S. Botti, and J. L. Sussman. 2003. The intracellular domain of the Drosophila cholinesterase-like neural adhesion protein, gliotactin, is natively unfolded. Proteins 53:758-767.
- Wilkins, D. K., S. B. Grimshaw, V. Receveur, C. M. Dobson, J. A. Jones, and L. J. Smith. 1999. Hydrodynamic radii of native and denatured proteins measured by pulse field gradient NMR techniques. Biochemistry 38:16424-16431.
- 32. Tcherkasskaya, O., E. A. Davidson, and V. N. Uversky. 2003. Biophysical constraints for protein structure prediction. J Proteome Res 2:37-42.