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Supporting Material

Thermo- and mesostabilizing protein interactions identified by temperature dependent statistical potentials

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Appendix S1 – Amino acid groups

Due to the limited number of proteins of known structure and melting temperature, the frequencies that are derived from our datasets are not always statistically significant, especially for infrequent pairs. We therefore decided to consider, in addition to the 20 amino acids, several groups of similar amino acids, as given in Table S3. To compute meaningful frequencies and potentials, we need to define the spatial distances *d* between groups of amino acids. Therefore, when computing the distances *d* between C_μ pseudoatoms, we took into account the radii of the amino acids they contain[(*)], and shifted all distances towards smaller distances by subtracting the difference in radii between the amino acids considered and the smallest amino acids in the groups. More precisely, consider the two groups **v** and **w** which contain the residues V_α (α=0,…, m) and W_β (β=0,…, n), and denote V_0 and W_0 the smallest amino acids of their respective groups. The normalized distance *d* that enters in the frequencies and potentials are the distances between the C_μ atoms of the residues V_α and W_β, minus the radii of V_α and W_β, plus the radii of V_0 and W_0. When computing the distances *d* between C_ν pseudoatoms, this normalization was only performed in the groups of small and aliphatic residues, where the C_μ and C_ν are identical. We computed distance potentials between pairs of groups, between single amino acids and groups, and between pairs of amino acids.

[*] Levitt M. 1976. A simplified representation of protein conformations for rapid simulation of protein folding. J. Mol. Biol. 104:59-107.

Appendix S2 – Statistical criterion to identify differences between potentials

To analyze the temperature dependence of the interactions, we compared the potentials derived from the subsets of mesostable and thermostable proteins. If these potentials are sufficiently different, we conclude that the interactions they represent depend on the melting temperature. Two criteria were used to evaluate if potentials are sufficiently different; they are illustrated in Fig. S1. The first criterion is based on the area of the surface between the two curves representing the potentials as a function of the interresidue distance, over the whole distance range considered (between the centers of the two extreme distance bins: from 3.25Å to 8.65Å). We give this surface area a positive sign when the curve computed from the thermostable set is below that of the mesostable set, and a negative sign otherwise. This surface area is denoted A_{total}.

The second criterion focuses on the differences around the minima of the potentials. The minima are first identified on the potential derived from the reference dataset by an automatic procedure: an energy minimum is detected in bin *b* if the energy value is higher in the two flanking bins *b*-1 and *b*+1. The distance range over which the difference in surface area is computed is defined by considering the two intersection points, at the right and left hand sides of the minimum, between the two potentials derived from the mesoresistant and thermoresistant subsets, as illustrated in Fig. S1. If no intersection point is found, the limits are the edges of the complete distance range (3.25 Å and 8.65 Å). We then compute the area, denoted A_{min}, of the surface contained between the curves of the mesoresistant and thermoresistant potentials, limited by these two intersection points. Note that there may be several minima in each potential; in this case the criterion is applied separately to each minimum.

To evaluate the significance of the differences between the potentials, we created 1000 random pairs of protein subsets. Each pair was generated by randomly assigning 83 of the 166 proteins to one subset and the 83 others to the other subset. The random pairs of subsets so created were kept only if their average melting temperatures differ by less than 3°C. We also excluded the proteins whose sequence is too similar (≥25%) to another protein of the subset, as explained above for the reference set.

To estimate whether the differences observed in a given potential are statistically significant and may be related to differences in $\overline{T}_m$, we computed the percentages P_{total} and P_{min} of the 1000 random pairs of subsets for which the surface areas A_{total} and A_{min} are larger than or equal to the corresponding surface areas obtained from the mesostable and thermostable protein sets. If P_{total} is lower than 10% and P_{min} lower than 20%, the difference between the potentials extracted from mesostable and thermostable proteins was considered as statistically significant. These values were lowered to 5% and 10%, respectively, for the potentials involving two groups of amino acids.

Table S1 – Characteristics of the datasets after refinement

			pH		*# residues*	
Data sets	*# proteins*	$\overline{T}_m$	*<pH>*	σ	*<# residues>*	σ
Mesostable subset	65	51.8	6.8	1.2	224.4	124.1
Reference set	115	65.3	6.8	1.2	208.7	121.8
Thermostable subset	65	79.1	6.9	1.3	182.2	114.2

Table S2 – Amino acid composition in each dataset

Residue name	Mesostable subset *(%)*	Reference set *(%)*	Thermostable subset *(%)*
A	8.44	8.44	7.93
R	3.97	4.2	5.02
N	5.17	4.99	4.79
D	6.4	6.45	6.37
C	1.1	1.34	1.62
Q	4.25	4.03	3.49
E	5.67	5.68	6.62
G	8.21	8.25	7.99
H	1.96	2.05	2.13
I	4.9	5.1	5.44
L	8.92	8.36	8.02
K	6.21	6.15	6.5
M	1.69	1.81	2
F	4.04	3.93	4.03
P	4.49	4.47	4.31
S	6.78	6.58	6.01
T	6.09	5.85	5.25
W	1.55	1.58	1.69
Y	3.61	4.04	4.22
V	6.55	6.69	6.6

Table S3 – Characteristics of the complete protein dataset

PDB[*] code	T_m (σ)[†] (°C)	pH	Protein name	Resolution (Å)	Host organism	Number of residues	Reference(s)
1fga	39.45	6.60	Fibroblast growth factor	2.20	*Human (homo sapiens)*	124	BIOCHEMISTRY 39, 7153-7158 (2000);
1hml	39.50	7.00	Alpha lactalbumin	1.70	*Human (homo sapiens)*	123	BIOCHEMISTRY 28, 8568-8576 (1989);
1rg8	40.25 (0.45)	7.30	Acidic fibroblast growth factor	1.10	*Human (homo sapiens)*	141	PROTEIN ENG DES SEL 17, 603-611 (2004);
1ihb	41.53	7.50	Cyclin-dependent kinase inhibitor	1.95	*Human (homo sapiens)*	156	J BIOL CHEM 277, 48827-48833 (2002);
1blc	41.60	7.50	Beta lactamase	2.20	*Staphylococcus aureus*	257	BIOCHEMISTRY 33, 116-125 (1994);
1d0b	42.10	7.50	Internalin b	1.86	*Listeria monocytogenes*	207	J MOL BIOL 337, 453-461 (2004);
1chk	43.20	7.00	Chitosanase	2.40	*Streptomyces lividans*	238	BIOCHIM BIOPHYS ACTA 1429, 365-376 (1999);
1p3j	43.30	7.40	Adenylate kinase	1.90	*Bacillus subtilis*	212	J BIOL CHEM 279, 28202-28208 (2004);
1aqh	44.00	7.20	Alpha-amylase	2.00	*Alteromonas haloplanctis*	448	J BIOL CHEM 276 25791-25796 (2001);
1rtp	45.80	7.40	Parvalbumin	2.00	*Bos taurus*	109	FEBS LETT 442, 241-245 (1999);
1ttq	46.00	7.80	Tryptophan synthase alpha subunit	2.00	*Salmonella typhimurium*	256	BIOCHEM BIOPHYS RES COMMUN 151, 672-678 (1988);
1orc	46.40	7.00	Cro repressor	1.54	*Bacteriophage lambda*	64	FEBS LETT 289 201-204 (1991);
1efc	46.50	7.60	Ef-tu elongation factor	2.02	*Escherichia coli*	386	PROTEIN SCI 13, 89-99 (2004);
2ci2	46.60	6.30	Chymotrypsin inhibitor	2.00	*Hordeum vulgare*	65	BIOCHEMISTRY 34, 1695-1701 (1995);
1bu7	47.00	7.40	Cytochrome p450	1.65	*Bacillus megaterium*	455	J BIOL CHEM 278, 608-616 (2003);
1s3g	47.60	7.40	Adenylate kinase	2.25	*Bacillus globisporus*	217	J BIOL CHEM 279, 28202-28208 (2004);
1aky	47.70	7.50	Adenylate kinase	1.96	*Saccharomyces cerevisiae*	218	EUR J BIOCHEM 231, 405-413 (1995);
1mve	48.00	7.00	Truncated glucanase	1.70	*Fibrobacter succinogenes*	238	BIOCHEMISTRY 44, 9197-9205 (2005);
1lni	48.16 (0.61)	7.00	Ribonuclease sa	1.00	*Streptomyces aureofaciens*	96	BIOCHEMISTRY 37, 16192-16200 (1998);PROTEIN SCI 8, 1843-1849 (1999);J BIOL CHEM 278, 31790-31795 (2003);PROTEIN SCI 12, 2367-2373 (2003);J MOL BIOL 354, 967-978 (2005);
1oa3	49.20	8.00	Cel12a	1.70	*Hypocrea schweinitzii*	217	PROTEIN SCI 12, 848-860 (2003);
1rn1	49.55 (1.25)	7.00	Ribonuclease t1	1.84	*Aspergillus oryzae*	104	J BIOL CHEM 264, 11621-11625 (1989);;
2cpp	50.15	7.40	Cytochrome p450	1.63	*Pseudomonas putida*	405	BIOCHEMISTRY 45, 12715-12722 (2006);
1rx4	50.20	7.80	Dihydrofolate reductase	2.20	*Escherichia coli*	159	J BIOL CHEM 282, 9420-9429 (2007);
1kfw	50.50	7.50	Chitinase	1.74	*Arthrobacter sp.*	435	PROTEIN ENG 16, 497-503 (2003);
1cpm	50.80	6.00	Glucanase	2.00	*Paenibacillus macerans*	214	PROTEIN SCI 5, 2255-2265 (1996);
1pii	51.00	7.40	Anthranilate isomerase	2.00	*Escherichia coli*	452	BIOCHEMISTRY 33, 6350-6355 (1994);
1bmc	51.03	7.00	Beta lactamase	2.50	*Bacillus cereus*	213	BIOCHEMISTRY 31, 6603-6607 (1992);
1wlg	51.10	7.00	Flagellar hook protein	1.80	*Salmonella typhimurium*	293	J MOL BIOL 251, 520-532 (1995);
1ayf	51.39	8.50	Adrenodoxin	1.85	*Bos taurus*	102	PROTEIN SCI 5, 1890-1897 (1996);
1ank	51.80	7.20	Adenylate kinase	2.00	*Escherichia coli*	214	J BIOL CHEM 266, 23654-23659 (1991);
1bd8	51.90	7.50	Cyclin-dependent kinase inhibitor p19	1.80	*Human (homo sapiens)*	156	J MOL BIOL 315 447-457 (2002);
2fal	52.00	7.00	Myoglobin	1.80	*Sea hare (aplysia limacina)*	146	J MOL BIOL 297, 1231-1244 (2000);
1ew4	52.07 (1.29)	7.00	Frataxin cyay	1.40	*Escherichia coli*	106	J MOL BIOL 336, 203-212 (2004);BIOCHEMISTRY 43, 6511-6518 (2004);
9rnt	52.10	7.00	Rnase t1	1.50	*Aspergillus oryzae*	104	BIOCHEMISTRY 33, 3312 (1994);
1am7	52.30	7.00	Lysozyme	2.30	*Bacteriophage lambda*	150	FEBS LETT 460, 442-446 (1999);
1avr	52.50	8.00	Annexin v	2.30	*Human (homo sapiens)*	317	BIOCHEMISTRY 36, 1657-1668 (1997);
1h12	52.60	7.50	Xylanase 8	1.20	*Pseudoalteromonas haloplanktis*	404	J BIOL CHEM 277, 35133-35139 (2002);
2fx5	53.00	5.80	Lipase	1.80	*Pseudomonas mendocina*	258	PROTEIN SCI 15, 1915-1927 (2006) PMID: 16823035;
2nvh	53.00	3.00	Interleukin 1 beta	1.53	*Human (homo sapiens)*	153	BIOCHEMISTRY 33, 9327-9332 (1994);
2rn2	53.10	5.50	Ribonuclease hi	1.48	*Escherichia coli*	155	EUR J BIOCHEM 220, 623-631 (1994);
5pep	53.10 (0.50)	8.00	Pepsin	2.34	*Porcine (sus scrofa)*	326	BIOCHEMISTRY 39, 4182-4190 (2000);
1ey0	53.15 (0.15)	7.00	Staphylococcal nuclease	1.60	*Staphylococcus aureus*	136	PROTEIN SCI 4, 2545-2558 (1995);J MOL BIOL 303, 125-130 (2000);
1abe	53.57	7.40	Arabinose binding protein	1.70	*Escherichia coli*	305	J BIOL CHEM 258, 13193-13198 (1983);
1rro	53.60	7.40	Beta-parvalbumin	1.30	*Rat (rattus rattus)*	108	FEBS LETT 442, 241-245 (1999);
1yea	53.63 (0.90)	6.00	Iso-2 cytochrome c	1.90	*Saccharomyces cerevisiae*	112	BIOCHEMISTRY 33, 9209-9219 (1994);BIOCHEMISTRY 35, 1995-2007 (1996);PROTEIN SCI 9, 536-543 (2000);
1e21	53.70	5.00	Ribonuclease a	1.90	*Human (homo sapiens)*	119	PROTEIN ENG 15, 887-893 (2002);

2acy	53.80	5.50	Acylphosphatase	1.80	*Bos taurus*	98	PROTEINS 62, 64-79 (2006);
1csp	53.80	7.00	Cold shock protein	2.50	*Bacillus subtilis*	67	J MOL BIOL 347, 1063-1076 (2005);
1hxn	53.90	7.40	Hemopexin	1.80	*Oryctolagus cuniculus*	209	BIOCHEMISTRY 32, 7216-7222 (1993);
1bni	54.00	7.00	Barnase	2.10	*Bacillus amyloliquefaciens*	108	BIOCHEMISTRY 34, 5224-5233 (1995);
1h8v	54.50	8.00	Cel12a	1.90	*Trichoderma reesei*	217	PROTEIN SCI 12, 2782-2793 (2003);
1ke4	54.60	6.80	Beta lactamase	1.72	*Escherichia coli*	357	PROTEIN SCI 8, 1816-1824 (1999);
1oxa	55.00	7.40	Cytochrome p450	2.10	*Saccarapolyspora erythraea*	403	J BIOL CHEM 278, 608-616 (2003);
3pgk	56.15	7.00	Phosphoglycerate kinase	2.50	*Saccharomyces cerevisiae*	415	BIOCHEMISTRY 28, 813-818 (1989);
1hfz	56.20	7.40	Alpha lactalbumin	2.30	*Bos taurus*	123	PROTEIN ENG 12, 581-587 (1999);
2rbi	56.50	6.20	Binase	2.20	*Bacillus intermedius*	108	FEBS LETT 445, 384-388 (1999);
1mjc	56.94 (0.99)	7.00	Cold shock protein	2.00	*Escherichia coli*	69	PROTEIN SCI 9, 387-394 (2000);PROTEIN SCI 10 2028-2036 (2001);
3sil	57.00	7.00	Sialidase	1.05	*Salmonella typhimurium*	381	PROTEIN ENG 14 891-896 (2001);
1ten	57.10	5.00	Tenascin	1.80	*Human (homo sapiens)*	90	BIOCHEMISTRY 37, 8071-8079 (1998);
1lmb	57.20	7.00	Lambda repressor	1.70	*Phage lambda*	80	BIOCHEMISTRY 41, 5359-5374 (2002);
1ftg	57.35 (0.05)	7.00	Apoflavodoxin	2.00	*Anabaena PCC7119*	168	PROTEIN SCI 5, 1376-1388 (1996);
3psg	57.50	7.10	Pepsinogen	1.65	*Porcine (sus scrofa)*	365	J MOL BIOL 152 445-464 (1981);
1tca	57.70	7.00	Lipase b	1.55	*Candida antarctica*	317	PROTEIN ENG 16, 599-605 (2003);
1pmk	57.80	7.00	Plasminogen kringle 4 domain	2.25	*Human (homo sapiens)*	78	BIOCHEMISTRY 32, 8799-8806 (1993);
1v0s	57.80	5.50	Phospholipase d	1.75	*Streptomyces septatus*	495	BIOCHIM BIOPHYS ACTA 1696, 75-82 (2004);
1qlp	58.87 (0.66)	7.80	Alpha1-antitrypsin	2.00	*Human (homo sapiens)*	372	J MOL BIOL 313 1161-1169 (2001);BIOCHEMISTRY 41, 4575-4581 (2002);J MOL BIOL 325, 581-589 (2003);
1avu	59.00	7.00	Kunitz type soybean trypsin inhibitor (sti)	2.30	*Glycine max*	172	BIOCHEMISTRY 41, 5359-5374 (2002);
2cab	59.00	6.10	Carbonic hydrase b	2.00	*Human (homo sapiens)*	256	BIOCHEMISTRY 44, 5258-5266 (2005);
2a01	59.20 (0.75)	7.40	Apolipoprotein a-i	2.40	*Human (homo sapiens)*	243	J MOL BIOL 378, 264-272 (2008);BIOCHEMISTRY 39, 15910-15919 (2000);BIOCHEMISTRY 41, 10529-10539 (2002);BIOCHEMISTRY 45, 1242-1254 (2006);
1wq5	59.50	7.20	Tryptophan synthase alpha subunit	2.30	*Escherichia coli*	258	ARCH BIOCHEM BIOPHYS 292, 34-41 (1992);
1ekg	60.00	7.00	Frataxin	1.80	*Human (homo sapiens)*	119	BIOCHEMISTRY 43, 6511-6518 (2004);
1ycc	60.00	6.00	Iso-1 cytochrome c	1.23	*Saccharomyces cerevisiae*	108	PROTEIN SCI 8, 2645-2654 (1999);
1rhg	60.95	6.90	Granulocyte colony stimulating factor	2.20	*Human (homo sapiens)*	145	BIOCHEMISTRY 41, 6422-6431 (2002);
1akd	61.00	7.40	Cytochrome p450	1.80	*Pseudomonas putida*	405	J BIOL CHEM 278, 608-616 (2003);
1w2p	61.20	7.00	Xylanase 10	1.45	*Cellvibrio japonicus*	346	J MOL BIOL 279, 54369-54379 (2004);
1xyp	61.40	5.00	Xylanase ii	1.50	*Trichoderma reesei*	189	J BIOTECHNOL 108, 137-143 (2004);
1esf	61.40	7.00	Enterotoxin a	1.90	*Staphylococcus aureus*	229	J BIOL CHEM 275, 1665-1672 (2000);
6taa	62.00	7.00	Taka-amylase	2.10	*Aspergillus oryzae*	476	BIOCHEMISTRY 26, 4063-4068 (1987);
1shg	62.00	7.00	Sh3 spectrin	1.80	*Escherichia coli*	57	J MOL BIOL 357, 1592-1604 (2006) PMID: 16487539;
1qwo	62.50	5.00	Phytase atcc	1.50	*Aspergillus fumigatus*	435	PROT ENG 13, 49-57 (2000);
1rtb	62.80	7.10	Ribonuclease a	2.50	*Bos taurus*	124	BIOCHEMISTRY 45, 10795-10806 (2006);
1poh	63.40	7.00	Histidine-containing phosphocarrier protein	2.00	*Escherichia coli*	85	J MOL BIOL 286, 1609-1619 (1999);
3rn3	63.60	7.00	Rnase a	1.45	*Bos taurus*	124	PROTEIN SCI 8, 832-840 (1999);
1tpk	64.30	4.50	Tissue plasminogen activator	2.40	*Human (homo sapiens)*	88	BIOCHEMISTRY 28, 4047-4054 (1989);
1bk7	64.40	7.50	Rnase mc1	1.75	*Momordica charantia (seeds bitter)*	190	BIOSCI BIOTECHNOL BIOCHEM 68, 1748-1757 (2004);
1fna	64.40	7.40	Fibronectin (10th type iii module)	1.8	*Human (homo sapiens)*	91	J MOL BIOL 217, 563-575 (1991);
3mbp	64.60	7.00	Maltose-binding protein	1.70	*EScherichia coli*	370	PROTEINS 53, 863-871 (2003);
1wdn	64.65 (1.55)	8.30	Glutamine-binding protein	1.94	*Escherichia coli*	223	PROTEINS 58, 80-87 (2005);PROTEINS 58, 80-87 (2005);
2lzm	64.85 (0.15)	6.50	Lysozyme	1.70	*Escherichia coli*	164	NATURE 334, 406-410 (1988);PROC NATL ACAD SCI U S A 85, 401-405 (1988);
1lz1	64.90	2.70	Lysozyme	1.50	*Human (homo sapiens)*	130	J MOL BIOL 254, 62-76 (1995);
1sup	65.00	7.00	Subtilisin bpn	1.60	*Bacillus amyloliquefaciens*	275	J BIOL CHEM 277, 27553-27558 (2002);
4blm	65.00	7.00	Beta lactamase	2.00	*Bacillus licheniformis*	256	BIOCHEMISTRY 29, 5797-5806 (1990);
1tgn	65.50 (0.50)	4.20	Trypsinogen	1.65	*Bos taurus*	222	J MOL BIOL 247, 701-716 (1995);
1ppi	65.60	7.20	Alpha-amylase	2.20	*Porcine (sus scrofa)*	496	J BIOL CHEM 276 25791-25796 (2001);
1jae	65.90	7.20	Alpha-amylase	1.65	*Tenebrio molitor*	470	BIOCHEMISTRY 38, 4613-4619 (1999);

1g6n	66.40	7.00	Catabolite activator protein	2.1	*Escherichia coli*	200	BIOCHEMISTRY 27, 5257-5261 (1988);
1oa4	66.80	8.00	Cel12a	1.50	*Streptomyces sp. 11ag8*	222	PROTEIN SCI 12, 848-860 (2003);
1gwy	67.00	7.50	Sticholysin	1.71	*Stoichactis helianthus*	175	FEBS LETT 575, 14-18 (2004);
1cyo	67.10 (0.44)	7.00	Cytochrome b5	1.50	*Bos taurus*	88	PROTEIN ENG 10, 575-581 (1997);BIOCHEMISTRY 38, 11961-11972 (1999);BIOPHYS CHEM 83, 3-17 (2000);PROTEIN ENG 6, 953-964 (1993);FEBS LETT 314, 419-424 (19992);
1cea	67.60	7.40	Plasminogen (kringle 1 domain)	2.10	*Human (homo sapiens)*	80	BIOCHEMISTRY 30, 1948-1957 (1991);
2hip	67.70	7.00	High-potential iron sulfur protein (hipip)iso-i	2.50	*Ectothiorhodospira halophila*	71	PROTEIN SCI 4, 2562-2572 (1995);
4tln	68.00	7.50	Thermolysin	2.30	*Bacillus thermoproteolyticus*	316	BIOCHEMISTRY 35, 3477-3486 (1996);
1osa	68.54	7.40	Calmodulin	1.68	*Paramecium*	148	PROTEINS 50, 381-391 (2003);
1olr	68.70	8.00	Cel12a	1.20	*Humicola grisea*	223	PROTEIN SCI 12, 2782-2793 (2003);
1znj	68.70	7.40	Insulin	2.00	*Human (homo sapiens)*	30	BIOCHEMISTRY 44, 11171-11177 (2005);BIOCHEMISTRY 45, 4014-4024 (2006);
1rbp	68.90	7.40	Serum retinol binding protein	2.00	*Human (homo sapiens)*	175	PROTEIN SCI 10 2301-2316 (2001);
1col	69.00	7.00	Colicin a	2.4	*Escherichia coli*	197	J BIOL CHEM 268, 1553-1557 (1993);
1ji3	70.00	7.00	Lipase l1	2.20	*Bacillus stearothermophilus*	388	BIOSCI BIOTECHNOL BIOCHEM 64, 280-286 (2000);
1uw3	70.00	7.50	Prion protein	2.04	*Ovis aries*	106	BIOCHEMISTRY 41, 11017-11024 (2002);
1a70	70.00	7.00	Ferredoxin	1.7	*Spinacia oleracea*	97	BIOCHEMISTRY 42, 1354-1364 (2003);
2imm	70.20	7.00	Immunoglobulin	2.00	*Mus musculus*	114	BIOCHEMISTRY 30, 6922-6929 (1991);
1smd	70.30	7.20	Alpha-amylase	1.60	*Human (homo sapiens)*	495	BIOCHEMISTRY 38, 4613-4619 (1999);
1cec	70.40	7.20	Cellulase c	2.15	*Clostridium thermocellum*	331	BIOPHYS CHEM 132, 229-241 (2002);
1hmk	70.78 (0.47)	7.50	Alpha-lactalbumin	2.00	*Capra hircus*	121	PROTEINS 60, 118-130 (2005);PROTEINS 60, 118-130 (2005);PROTEINS 60, 118-130 (2005);PROTEINS 60, 118-130 (2005);PROTEINS 60, 118-130 (2005);
1pca	71.00	7.50	Carboxypeptidase a	2.00	*Porcine (sus scrofa)*	403	EUR J BIOCHEM 176, 225-230 (1988);
1ag6	71.00	7.00	Plastocyanin	1.60	*Spinacia oleracea*	99	BIOCHEMISTRY 42, 10301-10310 (2003);
4gcr	72.00	6.80	Gamma-crystallin	1.47	*Bos taurus*	174	J BIOL CHEM 268, 18119-18127 (1993);
1tml	72.37 (0.17)	6.80	Cellulase e2	1.80	*Thermomonospora fusca*	286	BIOCHEMISTRY 38, 2570-2576 (1999);
2hpr	73.00	7.00	Hpr protein	2.00	*Bacillus subtilis*	87	BIOCHEMISTRY 45, 4084-4092 (2006);
1jbk	73.00	7.50	Cplb	1.80	*Escherichia coli*	189	PROTEIN SCI 11, 1192-1198 (2002);
1zip	74.50	7.20	Adenylate kinase	1.85	*Bacillus stearothermophilus*	217	BIOCHEMISTRY 31, 3038-3043 (1992);
4lyz	74.80	7.00	Lysozyme	2.00	*Gallus gallus*	129	PROTEINS 40, 49-57 (2000);
1svn	75.50	9.00	Savinase	1.40	*Bacillus lentus*	269	PROT ENG 17, 149-156 (2004);
1fvk	76.77	7.00	Dsba	1.7	*Escherichia coli*	188	PROTEIN SCI 8, 106-112 (1999);
1c9o	76.90	7.00	Cold shock protein	1.17	*Bacillus caldolyticus*	66	NAT STRUCT BIOL 7, 380-383 (2000);J MOL BIOL 319, 541-554 (2002);PROTEIN ENG DES SEL 19, 355-358 (2006) PMID: 16720692;
1aye	77.00	7.00	Ada2h	1.80	*Human (homo sapiens)*	401	BIOCHEMISTRY 41, 5359-5374 (2002);
1poo	77.60	7.00	Phytase	2.10	*Bacillus amyloliquefaciens*	353	NATURE 7, 147-153 (2000);
1ymb	78.30	7.00	Myoglobin	1.90	*Equus caballus*	153	BIOCHEMISTRY 40 5075-5080 (2001);
1ova	78.40	7.00	Ovalbumin	1.95	*Gallus gallus*	385	PROTEIN SCI 12, 2693-2703 (2003);
1gtg	79.40	5.50	Kumamolisin	2.3	*Bacillus novosp. mn-32*	357	BIOCHIM BIOPHYS ACTA 1764, 364-371 (2006);
1pgx	79.40	5.40	B2 of protein g	1.50	*Streptococcus*	70	BIOCHEMISTRY 31 3597 (1992);
1shf	80.10	8.00	Fyn tyrosine kinase (sh3 domain)	1.9	*Human (homo sapiens)*	59	BIOCHEMISTRY 37, 16172-16182 (1998);J MOL BIOL 333, 641-655 (2003);
1e65	81.00	7.03	Azurin	1.85	*Pseudomonas aeruginosa*	128	J PHYS CHEM 99 14864-14870 (1995);
1hrc	81.60	6.00	Cytochrome c	1.90	*Equus caballus*	104	BIOCHIM BIOPHYS ACTA 1646, 49-56 (2003);
1hgu	82.00	5.50	Human growth hormone	2.50	*Human (homo sapiens)*	187	PROTEIN SCI 11, 1452-1461 (2002);
4mbn	82.20	9.60	Myoglobin	2.00	*Physeter catodon*	153	PROTEIN SCI 2, 1099-1105 (1993);
1bvc	82.20	9.60	Myoglobin	1.5	*Cetacea sperm*	153	PROTEIN SCI 2, 1099-1105 (1993);
9pap	83.00	5.60	Papain	1.65	*Papaya (carica papaya)*	212	INT J BIOL MACROMOL 41, 383-390 (2007);
2ocj	84.80	7.00	Tumor suppressor p53	2.05	*Human (homo sapiens)*	194	BIOCHEMISTRY 34, 5309-5316 (1995);
1io2	85.20	9.00	Ribonuclease hii	2.00	*Thermococcus kodakaraensis*	213	BIOCHEMISTRY 45, 12673-12679 (2006);
2ovo	85.20	4.51	Ovomucoid third domain	1.50	*Silver pheasant (lophura nycthemera)*	56	BIOCHEMISTRY 34, 4724-4732 (1995);

2trx	85.46 (1.85)	7.00	Thioredoxin	1.68	*Escherichia coli*	108	BIOCHEMISTRY 34, 2148-2152 (1995);BIOCHEMISTRY 26, 1406-1411 (1987);BIOCHEMISTRY 31, 4901-4907 (1992);BIOCHEMISTRY 32, 7526-7530 (1993);BIOCHEMISTRY 40 10047-10053 (2001);
1cse	85.83	7.00	Eglin c	1.2	*Hirudo medicinalis*	63	BIOPHYS CHEM 55, 247-252 (1995);
1iv7	85.85	7.00	Single chain monellin (scm)	1.82	*Dioscoreophyllum cumminisii*	96	J BIOL CHEM 276, 19624-19630 (2001);
1bsq	86.00	7.00	Beta-lactoglobulin type b	2.22	*Bos taurus*	162	INT J BIOL MACROMOL 38, 9-17 (2006);
1ppn	86.20	3.90	Proteinase	1.6	*Carica papaya*	212	EUR J BIOCHEM 214, 129-134 (1993);
1onc	87.80	7.00	Onconase	1.70	*Rana pipiens*	103	BIOCHEMISTRY 39, 8711-8718 (2000);
1n97	88.00	7.40	Cytochrome p450	1.80	*Thermus thermophilus*	385	J BIOL CHEM 278, 608-616 (2003);
1pga	88.00	7.20	Protein g	2.07	*Streptococcus*	56	BIOCHEMISTRY 39, 965-977 (2000);
1i4n	90.30	7.50	Indole glycerol phosphate synthase	2.50	*Thermotoga maritima*	251	J MOL BIOL 288, 753-763 (1999);
1azp	90.70	7.00	Sac7d	1.60	*Sulfolobus acidocaldarius*	66	BIOCHEMISTRY 43, 2840-2853 (2004);BIOCHEMISTRY 44, 915-925 (2005);
1f4t	91.00	7.40	Cytochrome p450	1.93	*Sulfolobus solfataricus*	367	J BIOL CHEM 278, 608-616 (2003);
1ubq	91.00	4.00	Ubiquitin	1.80	*Human (homo sapiens)*	76	PROTEINS 18, 246-253 (1994);
1h7m	93.65 (0.15)	7.40	Ribosomal protein l30e	1.96	*Thermococcus celer*	98	PROTEIN SCI 12, 1483-1495 (2003);J MOL BIOL 348, 419-431 (2005);
1jji	99.00	7.50	Esterase	2.20	*Archaeoglobus fulgidus*	311	BIOCHEMISTRY 41 1364-1371 (2002);
2bjd	100.80	5.50	Acylphosphatase	1.27	*Sulfolobus solfataricus*	90	PROTEINS 62, 64-79 (2006);
1tmy	100.85	6.00	Chey	1.90	*Thermotoga maritima*	118	BIOCHEMISTRY 40 13107-13113 (2001);
1bli	101.00	7.40	Alpha-amylase	1.90	*Bacillus licheniformis*	481	BIOCHEMISTRY 43, 9589-9599(2004);
1r0f	104.00	7.50	Rubredoxin	1.60	*Clostridium pasteurianum*	53	PROTEINS 57, 118-127 (2004);
5pti	104.00	7.00	Trypsin inhibitor	1.00	*Bos taurus*	58	BIOCHEMISTRY 36, 5323-5335 (1997);
2a3m	108.00	7.60	Cytochrome g20c3	1.50	*Desulfovibrio desulfuricans*	107	J MOL BIOL 358, 1-14 (2006);
2d0s	108.00	5.00	Cytochrome c 552	2.20	*Hydrogenophilus thermoluteolus*	79	BIOCHEMISTRY 45, 6115-6123 (2006);
451c	109.00	7.00	Cytochrome c551	1.6	*Pseudomonas aeruginosa*	82	J AM CHEM SOC 126, 14684-14685 (2004);
1w2i	111.50	7.40	Acylphosphatase	1.50	*Pyrococcus horikoshii*	90	BIOCHEMISTRY 44, 4601-4611 (2005);
2cth	121.60	7.60	Cytochrome c3	1.67	*Desulfovibrio vulgaris*	107	BIOCHEMISTRY 38, 33-41 (1999);
1brf	143.80	7.00	Rubredoxin	1.62	*Pyrococcus furiosus*	53	PROTEINS 57, 118-127 (2004);

[*] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000;28:235-242.

[†] The standard deviation σ of the melting temperature T_m is computed from different results obtained in experiments performed under the same conditions.

Table S4 – List of proteins in each refined subset

Mesostable protein subset	Reference set	Thermostable protein subset
1oa3	1ppi	1smd
1h12	1h12	1cec
1qwo	1qwo	2trx
1bni	1e65	1e65
2acy	2hip	4gcr
1ten	2rbi	2hip
1lni	4blm	2cth
3sil	1azp	1shf
1csp	2acy	1pga
1mve	1lni	4blm
1rhg	3sil	1azp
1aqh	1mjc	1tmy
1oxa	1poh	1aye
1ey0	1rro	1cse
1shg	1io2	1hrc
1rtp	1cpm	1gwy
1qlp	1rhg	1fvk
1pmk	4tln	2hpr
1orc	6taa	451c
2rn2	1oxa	1io2
1ke4	1ey0	4tln
1rn1	1tml	1a70
2fx5	1wdn	5pti
1esf	1shg	1ova
1bmc	9pap	1tml
1kfw	1qlp	1onc
2nvh	1orc	1f4t
1w2p	2rn2	3mbp
1abe	1ke4	1wdn
1e21	1poo	1ppn
2cpp	1jbk	1jji
1d0b	2imm	1cea
5pep	1ubq	1cyo
2cab	1fna	1poo
1ew4	2ocj	1hgu
1wlg	9rnt	2lzm
1blc	1bmc	1n97
1hxn	1esf	1jbk
1lmb	2fx5	1rbp
2fal	1oa4	4lyz
1tca	1kfw	1iv7
1fga	2nvh	2imm
1poh	1bli	1bvc
1v0s	1ag6	1ubq
1rx4	1w2p	1fna
1bu7	1bk7	2ocj
2a01	1ji3	1g6n
1p3j	1osa	1olr
1wq5	1abe	1bli
1am7	1tgn	1ag6
1ayf	3rn3	1col
3pgk	2bjd	1bsq
1avr	1h7m	1zip
1ftg	2ovo	1bk7
1efc	1akd	1ji3
1ekg	1cec	1osa
1ttq	2trx	1brf
1ihb	1d0b	1i4n
1xyp	4gcr	1c9o

Mesostable protein subset (continued)	Reference set (continued)	Thermostable protein subset (continued)
1hml	2a3m	1uw3
1avu	1pgx	1tgn
2ci2	3psg	1znj
1yea	2cab	1w2i
1chk	1ew4	1h7m
1pii	1wlg	2ovo
	1tmy	
	1lmb	
	1pca	
	1sup	
	1hxn	
	1fvk	
	1gwy	
	2d0s	
	1tca	
	1rg8	
	1a70	
	1v0s	
	5pti	
	1rx4	
	1bu7	
	3mbp	
	2a01	
	1jji	
	1tpk	
	1cyo	
	1zip	
	1ayf	
	1am7	
	1wq5	
	1hgu	
	2lzm	
	3pgk	
	1rbp	
	1avr	
	1ftg	
	1iv7	
	1ekg	
	2fal	
	1efc	
	1g6n	
	1onc	
	1col	
	1bsq	
	1ttq	
	1bd8	
	1lz1	
	1xyp	
	1r0f	
	2ci2	
	1uw3	
	1avu	
	1znj	
	1ycc	
	1pii	
	1chk	

Table S5 – Amino acid groups

Type	Amino acids
Aliphatic	AILV
Small	AG
Charged +	KR
Charged -	DE
Aromatic	FWY
Noncharged polar	NQST

Table S6 – Details on all pairs of amino acids and amino acid groups satisfying our statistical significance criteria

Thermostabilizing interactions (C_μ - C_μ potentials)																						
Type of interactions	Interaction	Sign*	1st statistical significance criterion		2nd statistical significance criterion†																	
					1st minimum			2nd minimum			3rd minimum			4th minimum			5th minimum			6th minimum		
			A_{total}	P_{total}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}
Salt bridges	DE - KR	-	4.40	0.70	3.95	5.77	0.60	6.35	5.77	0.60	7.45	5.77	0.60		/			/			/	
	D - KR	-	3.22	5.50	3.85	3.45	1.10	6.25	3.45	3.10		/			/			/			/	
	E - KR	-	4.67	1.40	4.45	4.69	1.10	6.95	-0.02	98.90		/			/			/			/	
	DE - R	-	5.16	2.40	5.75	5.97	3.40	7.15	5.97	3.10	7.45	5.97	3.10		/			/			/	
	D - R	-	4.58	8.60	3.95	0.15	82.30	5.35	3.26	4.60	5.95	-0.00	99.90	6.15	1.52	32.90		/			/	
	E - R	-	5.02	2.60	4.45	4.57	1.10	5.85	4.57	1.10	6.45	0.61	55.40	7.75	2.53	13.30		/			/	
	E - H	-	-0.07	96.30	5.25	0.35	71.60		/			/			/			/			/	
Cation-π interactions	KR - FWY	-	4.31	3.70	3.75	1.96	3.10	5.95	4.88	2.10	7.35	4.88	2.10	8.55	4.88	1.80		/			/	
	R - FWY	-	4.22	6.40	3.65	N.D.	N.D.	6.05	4.82	3.00		/			/			/			/	
	KR - W	-	3.05	3.60	4.95	N.D.	N.D.	6.25	5.60	1.90	7.55	5.60	1.90		/			/			/	
	KR - Y	-	5.37	3.60	6.25	8.12	1.20	7.25	8.12	1.20		/			/			/			/	
	K - F	+	1.16	16.00	4.35	N.D.	N.D.		/			/			/			/			/	
	K - Y	-	3.72	8.90	3.95	N.D.	N.D.	5.15	4.34	3.10		/			/			/			/	
	R - W	-	0.60	1.80	6.25	0.60	7.30		/			/			/			/			/	
	R - Y	-	5.12	0.50	5.95	6.86	0.80	7.35	6.86	0.70		/			/			/			/	
	H - F	-	0.86	2.60	5.15	1.99	0.80		/			/			/			/			/	
Aromatic interactions	F - FWY	-	2.76	8.00	5.45	2.00	7.50	6.15	2.67	12.50		/			/			/		/	/	/
	F - W	-	4.17	1.50	6.45	4.30	2.20	7.15	4.30	1.90		/			/			/		/	/	/
Negatively charged Y or W	DE - W	-	4.26	4.10	4.45	7.03	0.60	5.65	7.03	1.60	6.75	7.03	2.60	7.55	7.03	2.60	8.35	7.03	2.40		/	
	DE - Y	-	4.58	3.30	4.65	8.06	1.20	6.55	8.06	1.20	7.15	8.06	1.20	7.75	8.06	1.20		/			/	
	D - W	-	0.63	6.00	5.45	2.17	1.40		/			/			/			/			/	
	D - Y	-	3.30	8.50	5.45	1.80	19.10	6.75	5.49	0.70	7.45	5.49	0.80	7.65	5.49	0.80	7.85	5.49	0.80	8.05	5.49	0.80
Small - charged	AG - KR	+	3.99	1.20	5.25	3.91	0.70	7.65	1.44	14.80		/			/			/			/	
	G - KR	-	3.72	3.10	4.65	2.43	4.40	7.75	2.40	5.80		/			/			/			/	
	AG - R	-	6.37	0.40	3.65	8.65	0.60	4.75	8.65	0.70	6.65	8.65	0.70		/			/			/	
	G - H	-	1.54	9.10	4.55	N.D.	N.D.	6.55	3.34	8.10	7.35	3.34	11.30		/			/			/	
	G - R	-	5.00	2.80	4.95	8.20	0.50	5.55	8.20	0.50	6.35	8.20	0.50	6.95	8.20	0.50	7.75	8.20	0.50		/	
	AG - E	-	3.78	8.20	7.05	2.84	6.80	7.95	0.37	65.70		/			/			/			/	
	A - E	+	3.33	0.80	6.55	5.73	0.30	7.35	5.73	0.40		/			/			/			/	
Cysteine - uncharged	C - AILV	-	5.24	9.70	4.25	8.62	5.70	6.75	8.62	5.70	7.45	8.62	5.70		/			/			/	
	C - AG	-	3.56	15.80	7.25	5.56	18.00		/			/			/			/			/	
	C - G	-	0.71	2.40	5.15	N.D.	N.D.	6.85	2.99	6.30		/			/			/			/	
	C - FWY	-	6.85	0.40	6.05	13.04	0.20	8.25	13.04	0.20		/			/			/			/	
	C - NQST	-	6.29	7.80	3.85	N.D.	N.D.	5.05	3.04	14.40	5.75	3.29	9.60	6.85	3.29	9.70		/			/	
Isoleucine - hydrophobic or small	I - AILV	-	3.48	1.00	4.25	4.81	2.10	7.65	4.81	2.10		/			/			/			/	
	I - I	-	6.11	0.20	4.95	10.17	0.10	5.65	10.17	0.10	6.55	10.17	0.10	8.45	10.17	0.10		/			/	
	I - AG	-	3.94	2.60	5.65	-0.41	48.60	5.85	-0.41	47.30		/			/			/			/	
	I - A	-	5.66	0.10	4.65	5.05	<0.1	7.65	2.55	1.70		/			/			/			/	
	I - FWY	-	2.40	10.90	4.25	-0.17	83.70		/			/			/			/			/	
	I - W	-	2.98	5.60	6.75	7.00	0.70		/			/			/			/			/	
	I - Y	-	4.29	3.70	4.45	0.95	37.10	5.95	5.38	4.60	8.15	5.38	4.50		/			/			/	
Methionine - charged, aromatic or small	M - KR	+	1.73	3.70	4.95	N.D.	N.D.		/			/			/			/			/	
	M - DE	-	2.04	2.20	6.35	-0.17	50.00		/			/			/			/			/	
	M - FWY	-	3.96	7.60	6.05	3.98	7.00	7.95	1.93	6.30		/			/			/			/	
	M - Y	-	2.02	6.60	6.35	2.30	2.20		/			/			/			/			/	
	M - A	-	4.67	1.10	5.45	3.98	1.70	6.65	3.98	1.90	7.75	3.98	2.10	8.45	0.25	70.20		/			/	
Others	Y - AILV	-	3.24	5.70	4.25	-0.01	99.40		/			/			/			/			/	
	Y - V	-	3.66	9.30	4.15	6.35	0.60	5.95	6.35	1.80	7.95	6.35	1.70		/			/			/	
	R - KR	+	2.42	6.20	5.05	0.82	11.10	5.25	-0.12	64.90		/			/			/			/	
	E - N	-	0.80	59.30	4.25	0.03	70.60	5.85	-0.15	86.40		/			/			/			/	
	R - N	+	1.89	2.10	5.85	-0.08	81.70	6.55	4.39	1.40	7.35	4.39	1.50		/			/			/	
	V - KR	+	3.27	9.40	4.85	-0.08	93.70	5.75	3.69	3.60		/			/			/			/	
	F - P	-	2.94	7.90	4.05	N.D.	N.D.	6.35	1.77	10.40	7.45	1.77	8.80		/			/			/	
	N - P	+	2.00	5.00	4.45	N.D.	N.D.	5.85	2.94	1.40		/			/			/			/	
	T - Y	-	3.12	10.10	5.25	2.21	10.40	6.65	1.22	41.70	7.15	1.22	41.10		/			/			/	

Thermostabilizing interactions (C_v - C_v potentials)

Type of interactions	Interaction	Sign*	1st statistical significance criterion		2nd statistical significance criterion†																	
					1st minimum			2nd minimum			3rd minimum			4th minimum			5th minimum			6th minimum		
			A_{total}	P_{total}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}
Salt bridges	DE - KR	+	3.99	1.70	6.65	3.99	1.20	7.65	0.91	24.20		/			/			/			/	
	D - KR	-	3.53	5.60	6.65	0.47	50.40	8.25	0.89	20.00		/			/			/			/	
	E - KR	+	4.66	6.10	5.65	-0.00	99.70	6.65	3.54	8.50		/			/			/			/	
	DE - R	-	5.04	4.60	3.85	5.07	2.40	6.55	5.07	2.40	8.35	1.08	27.00		/			/			/	
	D - R	-	4.94	5.50	3.85	3.21	6.30	6.45	1.73	32.30	8.25	1.12	26.20		/			/			/	
	E - R	-	5.59	2.50	3.95	1.51	18.60	5.65	6.33	1.70	6.65	6.33	1.80	7.45	6.33	1.90	8.35	6.33	1.60	8.55	6.33	1.60
	E - H	-	2.39	2.70	4.15	2.39	3.70		/			/			/			/			/	
Cation-π interactions	KR - FWY	-	4.58	2.80	3.85	1.42	14.90	4.75	5.34	1.30	6.05	5.34	1.60	7.75	5.34	1.60		/			/	
	R - FWY	-	3.83	8.40	3.75	0.76	34.50	4.75	3.37	4.20	6.15	3.37	5.00	6.35	3.37	4.90	7.65	1.69	16.30		/	
	KR - W	-	2.41	2.90	5.75	-0.09	90.30		/			/			/			/			/	
	KR - Y	-	4.77	3.50	4.65	7.32	1.20	6.35	7.32	1.30	7.85	7.32	1.30		/			/			/	
	K - F	+	1.89	0.30	6.05	2.45	0.70	6.95	2.45	0.80		/			/			/			/	
	K - Y	-	1.77	30.90	4.65	1.16	18.90	6.25	-0.13	94.00	7.05	3.48	8.40		/			/			/	
	R - W	-	<0.1	9.30		/			/			/			/			/			/	
	R - Y	-	4.35	1.50	4.65	6.60	0.30	5.85	6.60	0.70	6.35	6.60	0.70	7.75	6.60	0.70		/			/	
	H - F	-	0.74	15.00	5.35	1.33	11.50		/			/			/			/			/	
Aromatic interactions	F - FWY	-	2.59	8.20	5.85	2.96	9.40		/			/			/			/			/	
	F - W	-	4.37	2.30	6.15	4.41	4.20	6.85	4.41	4.00	8.35	0.53	57.20		/			/			/	
Negatively charged - Y or W	DE - W	-	4.09	1.20	5.35	-0.07	96.30	5.75	4.02	0.50	6.65	4.02	0.70		/			/			/	
	DE - Y	-	3.17	6.10	5.35	1.80	10.90	6.65	4.72	1.00	7.55	4.72	1.10		/			/			/	
	D - W	-	0.63	26.50	5.35	2.42	4.80	6.65	2.42	4.50	7.35	N.D.	N.D.		/			/			/	
	D - Y	-	2.26	22.80	5.25	1.82	25.30	6.75	4.41	3.60	7.65	4.41	4.40		/			/			/	
Small - charged	AG - KR	-	3.72	1.40	3.75	3.86	0.20	5.45	3.86	0.30	7.05	-0.00	99.80		/			/			/	
	G - KR	+	4.84	1.10	3.85	5.29	<0.1	5.45	5.29	<0.1	7.35	1.29	20.20	8.35	1.29	16.60		/			/	
	AG - R	-	4.82	4.00	3.65	5.13	0.60	6.05	-0.45	50.10	6.95	1.46	24.70		/			/			/	
	G - H	-	1.41	5.80	5.95	1.41	6.10	7.35	1.61	25.70		/			/			/			/	
	G - R	-	4.69	5.70	3.75	N.D.	N.D.	5.85	5.82	0.40	6.95	2.12	12.00		/			/			/	
	AG - E	-	2.67	17.00	3.85	3.05	1.70	5.35	-0.16	83.30	6.65	2.43	4.80	7.45	2.43	6.50		/			/	
	A - E	+	1.21	30.10	4.25	N.D.	N.D.		/			/			/			/			/	
Cysteine - uncharged	C - AILV	-	4.84	11.70	4.15	0.14	87.40	7.05	4.86	7.20	7.55	1.26	44.30		/			/			/	
	C - AG	-	3.32	7.80	5.05	N.D.	N.D.	6.15	6.41	8.50	7.25	6.41	9.10		/			/			/	
	C - G	-	1.31	5.60	6.15	5.89	2.40	7.25	5.89	2.60	7.45	5.89	2.40	7.65	5.89	2.60		/			/	
	C - FWY	-	6.59	0.80	4.35	N.D.	N.D.	5.15	12.03	0.40	6.65	12.03	0.40		/			/			/	
	C - NQST	-	4.15	12.10	5.15	3.59	6.30	6.55	-0.48	58.30		/			/			/			/	
Isoleucine - hydrophobic or small	I - AILV	-	3.64	0.80	4.25	3.64	0.90	7.55	1.09	18.20		/			/			/			/	
	I - I	-	6.12	0.20	4.95	10.15	0.10	5.15	10.15	0.10	5.65	10.15	0.10	6.55	10.15	0.10	8.45	10.15	0.10		/	
	I - AG	-	4.04	1.90	4.25	4.33	0.60	7.25	1.88	6.10	8.05	1.88	6.10		/			/			/	
	I - A	-	5.57	0.10	4.65	4.96	<0.1	7.65	2.51	1.80		/			/			/			/	
	I - FWY	-	2.89	4.10	4.35	0.55	45.00	5.95	4.11	1.20	6.55	4.11	1.20	8.15	4.11	1.20		/			/	
	I - W	-	3.28	4.20	4.85	N.D.	N.D.		/			/			/			/			/	
	I - Y	-	4.27	3.80	4.45	0.92	39.00	5.95	5.37	4.30		/			/			/			/	
Methionine - charged, aromatic or small	M - KR	+	1.05	1.20	6.25	N.D.	N.D.	6.85	2.53	4.00		/			/			/			/	
	M - DE	+	1.46	3.50	4.65	3.23	1.60	6.55	3.23	<0.1	8.55	3.23	4.90		/			/			/	
	M - FWY	-	4.28	6.30	4.35	3.45	5.10	6.45	1.11	32.40		/			/			/			/	
	M - Y	-	2.08	6.10	6.25	2.21	2.60		/			/			/			/			/	
	M - A	-	4.68	1.20	4.15	N.D.	N.D.	5.45	4.09	1.30	6.65	4.09	1.60	7.75	4.09	1.70		/			/	
Others	Y - AILV	-	3.51	3.50	4.35	-0.01	98.90		/			/			/			/			/	
	Y - V	-	3.53	10.10	4.25	6.12	0.90	5.95	6.12	2.10	7.25	6.12	2.10	7.95	6.12	1.90		/			/	
	R - KR	+	2.09	15.20		/			/			/			/			/			/	
	E - N	-	2.87	4.10	3.95	N.D.	N.D.	4.15	0.68	6.00		/			/			/			/	
	R - N	+	0.16	75.80	4.65	N.D.	N.D.	5.75	2.52	3.40	7.45	2.52	10.40		/			/			/	
	V - KR	+	1.12	58.90	4.15	N.D.	N.D.	5.05	1.40	28.30		/			/			/			/	
	F - P	-	3.79	3.10	4.15	N.D.	N.D.	5.65	4.46	3.00	6.35	4.46	3.00		/			/			/	
	N - P	+	0.94	26.50	4.95	N.D.	N.D.	5.75	1.17	19.70		/			/			/			/	
	T - Y	-	3.35	3.90	4.85	3.35	3.20		/			/			/			/			/	

Mesostabilizing interactions (C_μ - C_μ potentials)																
Types of Interactions	Interaction	Sign*	1st statistical significance criterion		2nd statistical significance criterion†											
					1st minimum			2nd minimum			3rd minimum			4th minimum		
			A_{total}	P_{total}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}
Aliphatic or small - noncharged polar	AILV - NQST	-	-3.16	1.00	4.35	-4.38	0.50	7.15	-4.38	0.50		/			/	
	AG - NQST	-	-5.26	0.30	3.65	-7.75	0.10	7.35	-7.75	0.10		/			/	
	A - NQST	-	-4.81	1.60	3.75	-6.60	1.40	7.05	-6.60	1.40	7.75	-6.60	1.40		/	
	G - NQST	-	-5.31	0.80	3.75	-8.72	0.10	6.85	-8.72	0.10		/			/	
	L - NQST	-	-4.59	0.30	4.25	-6.24	0.40	5.85	-6.24	0.40	7.35	-6.24	0.40	7.65	-6.24	0.40
	AILV - N	-	-3.83	3.20	4.15	-3.91	2.70	7.45	-0.82	31.00		/			/	
	AILV - Q	-	-3.49	7.80	4.15	-1.94	3.40	7.65	-3.21	8.50		/			/	
	AG - S	-	-5.11	4.80	3.65	-6.55	3.30	7.15	-6.55	4.00	7.85	-6.55	3.90		/	
	AILV - T	-	-3.17	3.50	4.25	-4.18	3.20	7.35	-4.18	3.30	/	/	/		/	
	AG - T	-	-7.42	0.10	3.75	-3.05	0.90	6.95	-7.91	<0.1	7.65	-7.91	<0.1		/	
	A - T	-	-6.00	1.50	4.05	-8.30	0.30	7.25	-8.30	0.50	8.25	-8.30	0.50		/	
	G - S	-	-6.07	2.60	6.75	-8.75	1.40	7.35	-8.75	1.40	8.25	-8.75	1.10		/	
	G - T	-	-4.42	5.40	4.65	-2.38	12.60	5.95	0.48	74.10		/			/	
Noncharged polar - noncharged polar	NQST - NQST	-	-6.62	0.10	7.05	-9.60	0.10		/			/			/	
	N - NQST	-	-8.12	<0.1	3.85	0.26	46.30	5.75	-11.90	<0.1		/			/	
	Q - NQST	-	-10.11	<0.1	4.05	-14.23	<0.1	7.45	-14.23	<0.1		/			/	
	S - NQST	-	-5.53	4.80	7.45	-7.83	2.70		/			/			/	
	T - NQST	-	-3.89	6.30	3.75	0.92	17.90		/			/			/	
	S - N	-	-4.09	14.90	3.95	N.D.	N.D.	5.65	-7.25	2.50		/			/	
Negatively charged - noncharged polar	DE - NQST	-	-2.90	4.50	3.65	-4.18	0.90		/			/			/	
	D - NQST	-	-5.79	0.20	3.55	-6.39	0.30	7.35	-6.39	0.40	7.55	-6.39	0.40		/	
	DE - T	+	-5.92	1.00	5.45	-7.15	0.50	6.95	-7.15	0.50	7.95	-7.15	0.50		/	
Small - small	G - AG	-	-5.68	2.10	3.65	-5.79	1.60	7.35	-1.14	35.20		/			/	
	A - G	-	-5.21	5.10	5.15	-5.65	1.40		/			/			/	
Leucine - other	L - FWY	-	-2.47	12.50	4.35	-3.06	4.30	6.15	-3.06	4.70		/			/	
	L - F	-	-4.27	5.90	4.45	-4.76	7.90	6.15	-4.76	8.10		/			/	
	L - D	+	-3.49	8.60	4.85	-3.81	5.70	6.25	-3.81	6.30		/			/	
	L - G	-	-4.30	1.90	5.15	-3.10	1.80	6.55	-3.62	1.90	7.75	-3.62	1.90		/	
Negatively charged - F	DE - F	+	-3.52	9.30	5.15	-3.96	1.50		/			/			/	
	E - F	+	-1.61	24.60	5.45	-1.61	28.00		/			/			/	

Mesostabilizing interactions (C_v - C_v potentials)																
Types of Interactions	Interaction	Sign*	1st statistical significance criterion		2nd statistical significance criterion†											
					1st minimum			2nd minimum			3rd minimum			4th minimum		
			A_{total}	P_{total}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}	*d*	A_{min}	P_{min}
Aliphatic or small - noncharged polar	AILV - NQST	-	-4.32	<0.1	4.15	-5.72	0.20	7.55	-5.72	0.20		/			/	
	AG - NQST	-	-5.53	0.20	3.35	-1.20	0.50	7.25	-7.19	<0.1		/			/	
	A - NQST	-	-5.40	1.70	3.55	-7.95	0.80	5.65	-7.95	0.80	7.75	-7.95	0.80	8.05	-7.95	0.80
	G - NQST	-	-5.31	1.30	7.05	-7.66	0.50		/			/			/	
	L - NQST	-	-4.24	1.20	4.45	-6.06	0.50	5.55	-6.06	0.50	7.85	-6.06	0.50		/	
	AILV - N	-	-3.57	5.90	4.15	-4.39	4.00	7.65	-4.39	4.60		/			/	
	AILV - Q	-	-4.19	5.10	4.15	-4.07	1.90	7.65	-3.93	0.10		/			/	
	AG - S	-	-5.18	5.80	7.55	-7.08	1.90		/			/			/	
	AILV - T	-	-3.05	7.50	3.95	-3.07	5.60	6.95	-3.07	5.00		/			/	
	AG - T	-	-7.24	0.40	5.15	-9.98	<0.1	7.05	-9.98	<0.1	7.85	-9.98	<0.1		/	
	A - T	-	-5.96	1.20	4.75	N.D.	N.D.	5.55	-8.41	0.30	6.35	-8.41	0.30	7.35	-8.41	0.30
	G - S	-	-4.51	13.50	5.35	-8.10	1.10	6.85	-8.10	1.10	7.75	-8.10	1.10		/	
	G - T	-	-3.75	9.40	5.35	0.20	86.10	7.15	-8.19	<0.1		/			/	
Noncharged polar - noncharged polar	NQST - NQST	+	-7.12	0.10	7.45	-10.08	0.10		/			/			/	
	N - NQST	-	-7.09	0.80	3.35	0.43	61.20	5.35	-11.57	<0.1	5.95	-11.57	<0.1		/	
	Q - NQST	-	-9.71	<0.1	3.35	N.D.	N.D.	5.65	-8.27	<0.1	6.75	-8.27	<0.1		/	
	S - NQST	-	-7.24	1.20	7.45	-9.83	0.60		/			/			/	
	T - NQST	-	-3.52	20.60	5.25	-6.08	1.50	7.05	-6.08	1.50	8.15	-6.08	1.50		/	
	S - N	-	-4.96	5.90	3.45	N.D.	N.D.	5.75	-6.36	2.60		/			/	
Negatively charged - noncharged polar	DE - NQST	+	-2.97	5.30	6.55	0.03	93.40		/			/			/	
	D - NQST	+	-6.31	0.20	5.75	-4.61	2.60	6.55	-4.61	2.60	7.55	-4.61	2.50		/	
	DE - T	+	-4.59	3.20	5.15	-3.51	2.80	6.05	-3.51	4.10	6.95	-3.51	3.50	7.75	-1.06	24.30
Small - small	G - AG	-	-5.75	2.30	3.75	-5.88	1.60	7.35	-1.17	34.30		/			/	
	A - G	-	-5.39	4.50	3.85	-5.76	1.10	5.15	-5.76	1.10		/			/	
Leucine - other	L - FWY	-	-3.16	4.50	4.55	-1.82	3.70	6.05	-1.34	17.50		/			/	
	L - F	-	-3.59	10.80	4.55	-4.27	11.50	5.95	-4.27	11.80	8.55	-0.47	39.60		/	
	L - D	+	-3.07	12.60	4.85	-1.71	21.00	6.55	-1.40	30.10	8.45	-0.86	30.90		/	
	L - G	-	-4.31	1.90	5.15	-3.12	1.80	6.55	-3.64	1.80	7.75	-3.64	1.80		/	
Negatively charged - F	DE - F	+	-2.60	16.00	5.75	-3.53	0.80		/			/			/	
	E - F	+	-2.26	9.20	4.55	N.D.	N.D.		/			/			/	

* The sign is negative (positive) if the interaction is attractive (repulsive) at the global minimum of the potential derived from the reference set.

† The symbol '/' means that no supplementary minima is detected on the potential derived from our reference set. The symbol 'N.D.' means that the minimum detected on the potential derived from our reference set was not detected in one of the two subsets of different thermostability.

Table S7 – Details on linear correlations between folding free energies ΔW and melting temperatures T_m in eight protein families

Protein family	PDB code*	Sequence identity (%)[†]	T_m (°C)	ΔW_{ref} (kcal/mol)	ΔW_{thermo} - ΔW_{meso} (kcal/mol)
Acyclo-phosphatase	2acy	100	53.80	-8.93	0.68
	2bjd	20	100.80	-13.94	-4.28
	1w2i	29	111.50	-13.30	-3.66
	r(T_m,ΔW) [‡]			**-0.96**	**-0.96**
Adenylate kinase	1p3j	100	43.30	-28.84	-0.76
	1s3g	67	47.60	-34.76	0.21
	1aky	42	47.70	-25.69	-2.01
	1ank	48	51.80	-34.44	-5.65
	1zip	74	74.50	-35.12	-6.45
	r(T_m,ΔW) [‡]			**-0.54**	**-0.78**
α-amylase	1aqh	100	44.00	-60.67	-3.70
	1ppi	46	65.60	-75.38	-16.23
	1jae	41	65.90	-78.79	-14.36
	1smd	44	70.30	-77.22	-22.95
	r(T_m,ΔW) [‡]			**-0.97**	**-0.95**
Endoglucanase 12	1oa3	100	49.20	-24.69	7.98
	1h8v	93	54.50	-20.21	3.04
	1oa4	31	66.80	-36.45	-0.20
	1olr	47	68.70	-22.29	-11.01
	r(T_m,ΔW) [‡]			**-0.41**	**-0.88**
Cold shock protein	1csp	100	53.80	-8.49	0.26
	1mjc	59	56.70	-8.05	-0.17
	1c9o	83	76.90	-6.62	-1.30
	r(T_m,ΔW) [‡]			**+0.99**	**-0.99**
Cytochrome P450	1bu7	100	47.00	-72.00	6.95
	1oxa	13	55.00	-75.66	9.51
	1akd	10	61.00	-80.91	-4.96
	1n97	20	88.00	-75.08	-3.22
	1f4t	17	91.00	-67.37	-9.06
	r(T_m,ΔW) [‡]			**+0.40**	**-0.80**
Lysozyme	1am7	100	52.30	-15.23	0.80
	2lzm	16	64.85	-28.52	-1.39
	1lz1	20	64.90	-41.01	-2.73
	4lyz	2	74.80	-28.80	-4.51
	r(T_m,ΔW) [‡]			**-0.58**	**-0.97**
Myoglobin	2fal	100	52.00	-34.13	0.37
	1ymb	21	78.30	-17.72	-3.12
	1bvc	21	82.20	-19.87	-7.85
	r(T_m,ΔW) [‡]			**+0.97**	**-0.88**

[*] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res 2000;28:235-242.

[†] Sequence identities computed with respect to the first protein of the family.

[‡] Linear correlation coefficient computed between the melting temperatures T_m and either ΔW_{ref} or ΔW_{thermo}-ΔW_{meso}.

Figure S1 – Illustration of the two statistical criteria used to identify the differences between the potentials derived from the two protein subsets, in the case of the D-R interaction

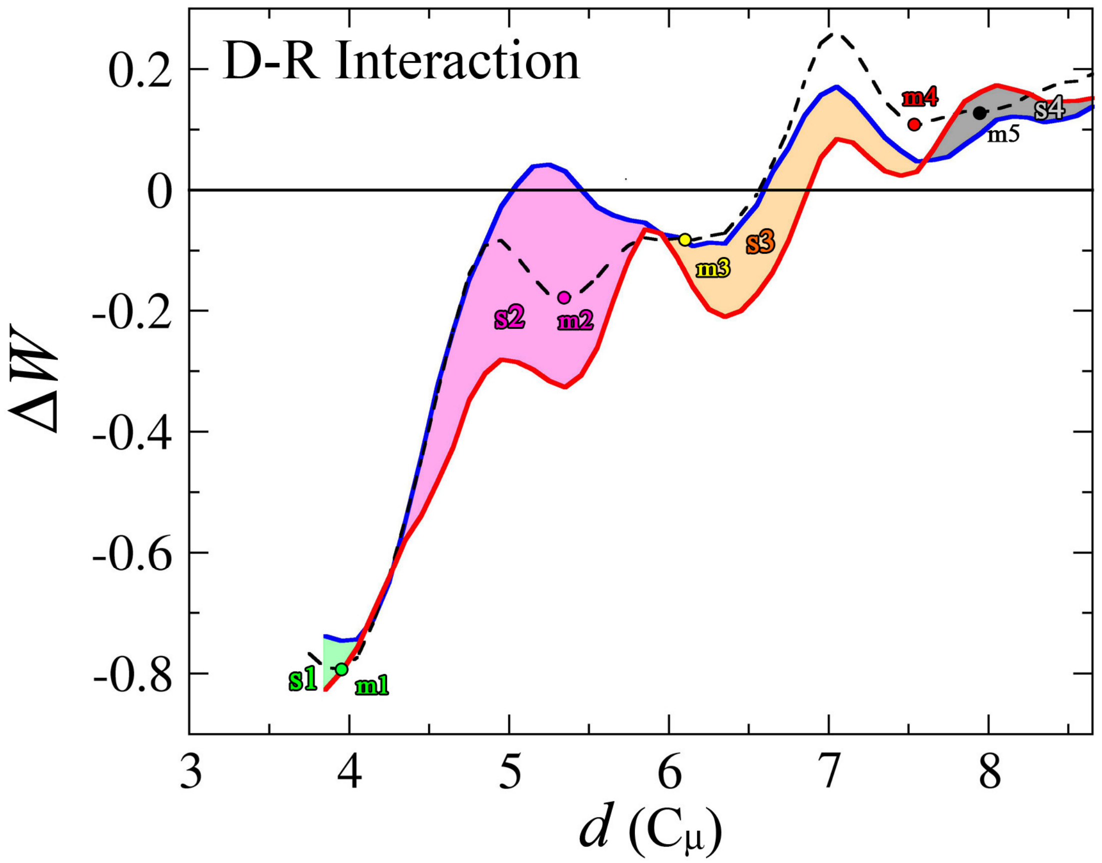
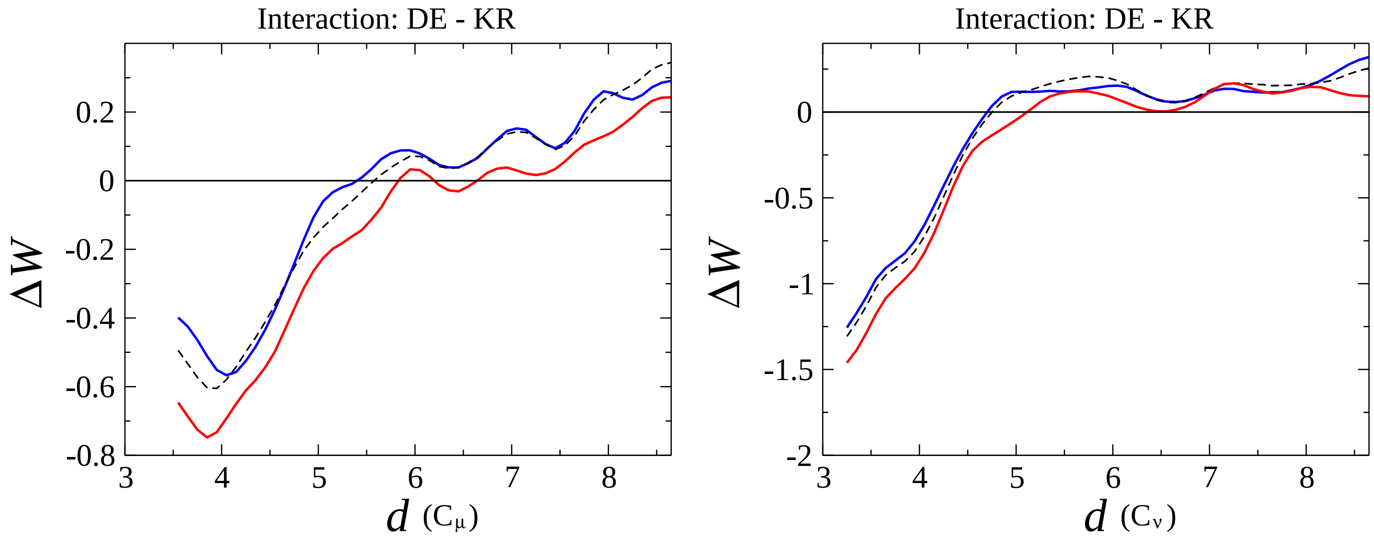


Figure S1 – Illustration of the two statistical criteria used to identify the differences between the potentials derived from the two protein subsets, in the case of the D-R interaction. The first criterion is related to the surface area s1+s2+s3-s4 between the curves representing the potentials derived from the thermoresistant (red line) and mesoresistant (blue line) protein subsets; a negative sign is given when the former curve is above the latter. The second criterion is related to the surface areas (in different colors) between the curves, around each minimum (m1-m5, colored circles). The minima are defined on the basis of the potential derived from the reference protein set (dashed line). In this example, m3 and m4 share the same surface area. Plots designed with XmGrace[38].

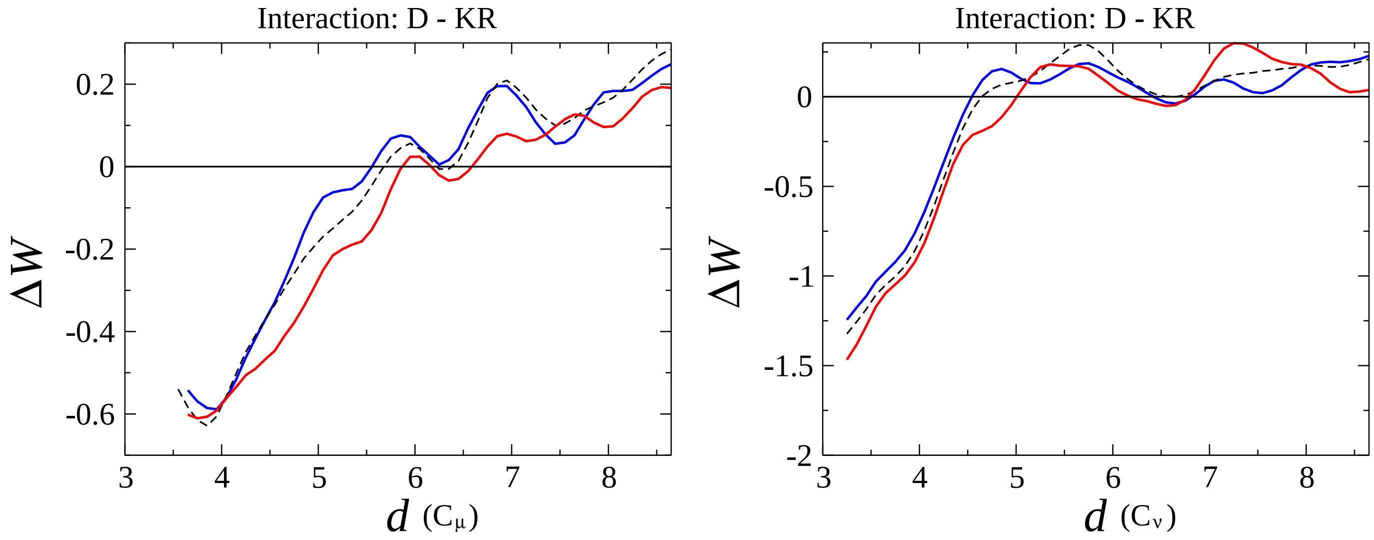
Figures S2-S56 - Thermostabilizing interaction potentials

Folding free energy contribution ΔW (kcal/mol) as a function of the distance d between side chain descriptors for the thermoresistant protein subset (red), the mesoresistant one (blue), and the reference set (dashed line). Left plots are based on C_μ descriptors and right plots on the C_ν descriptors.

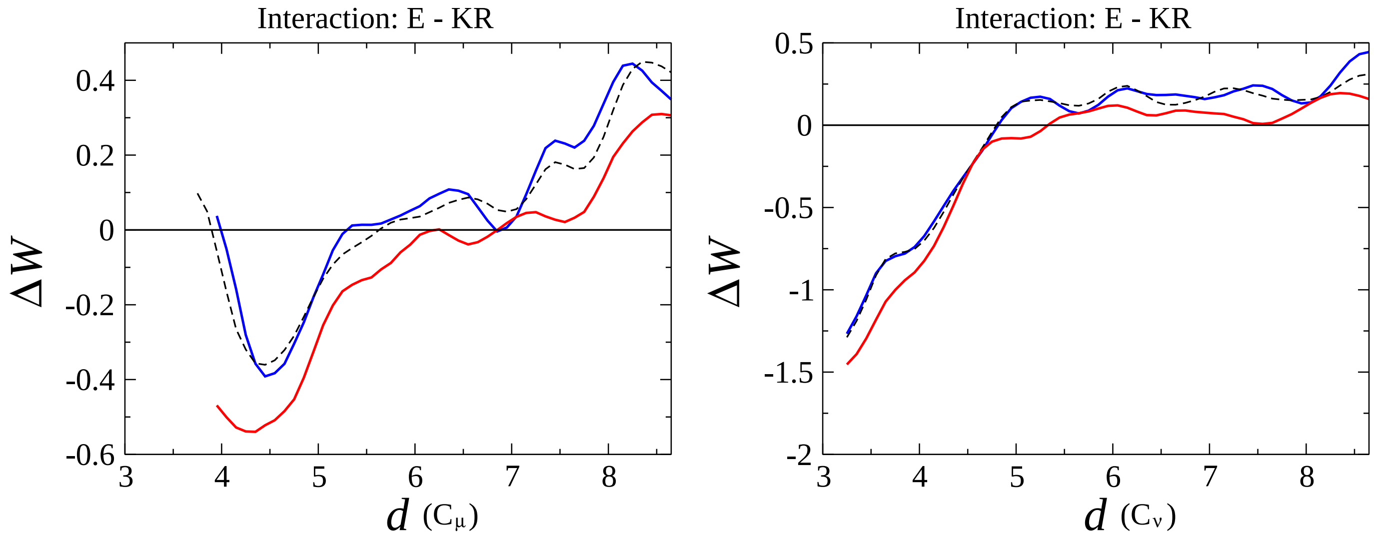
S2)



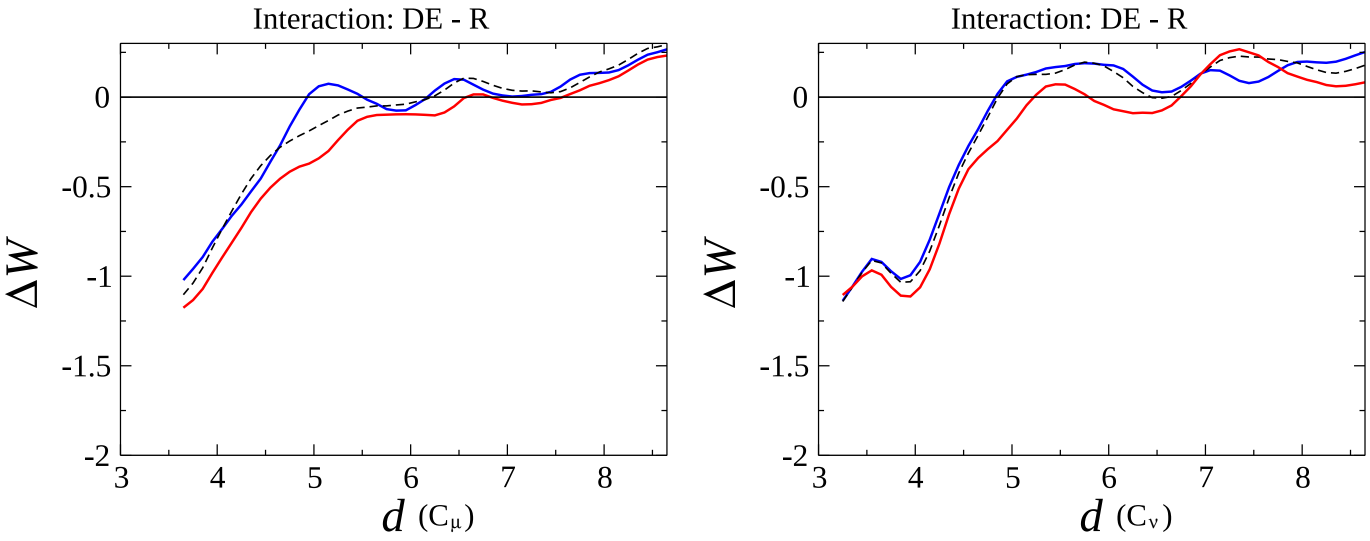
S3)



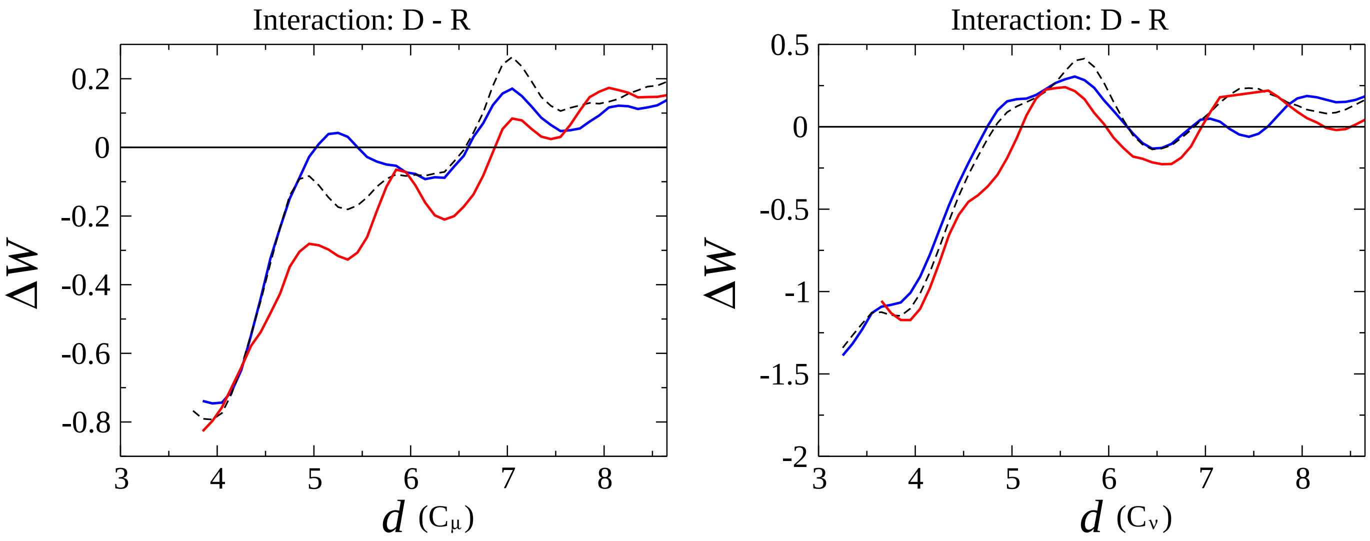
S4)



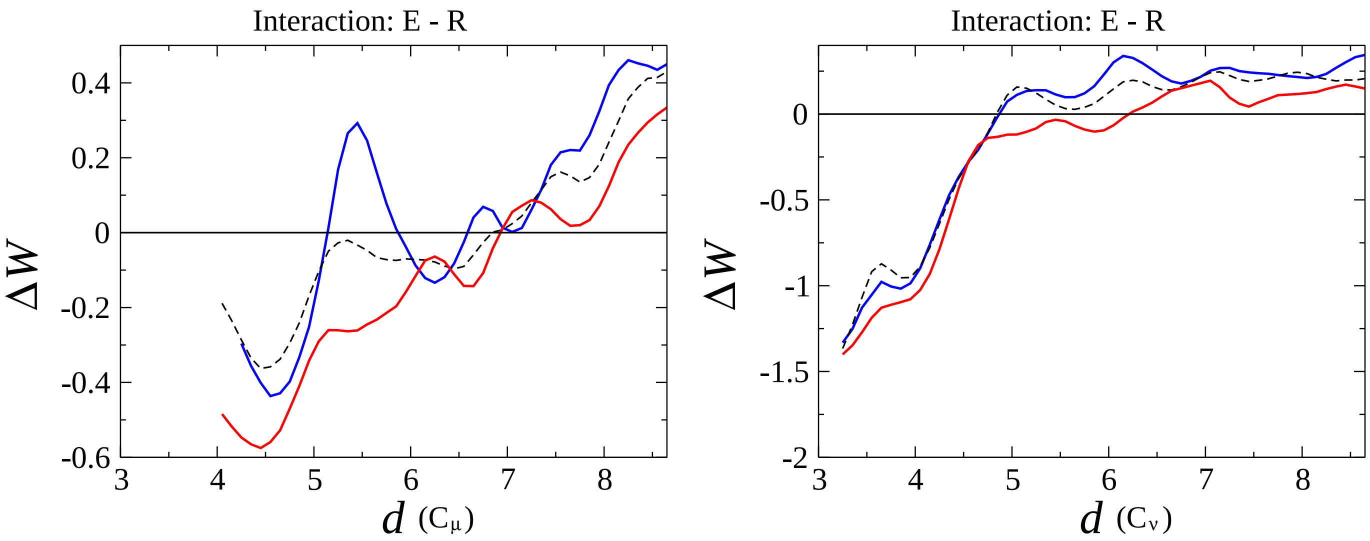
S5)


Interaction: DE - R
Interaction: DE - R
ΔW
ΔW
d (Cμ)
d (Cν)
0
-0.5
-1
-1.5
-2
3
4
5
6
7
8

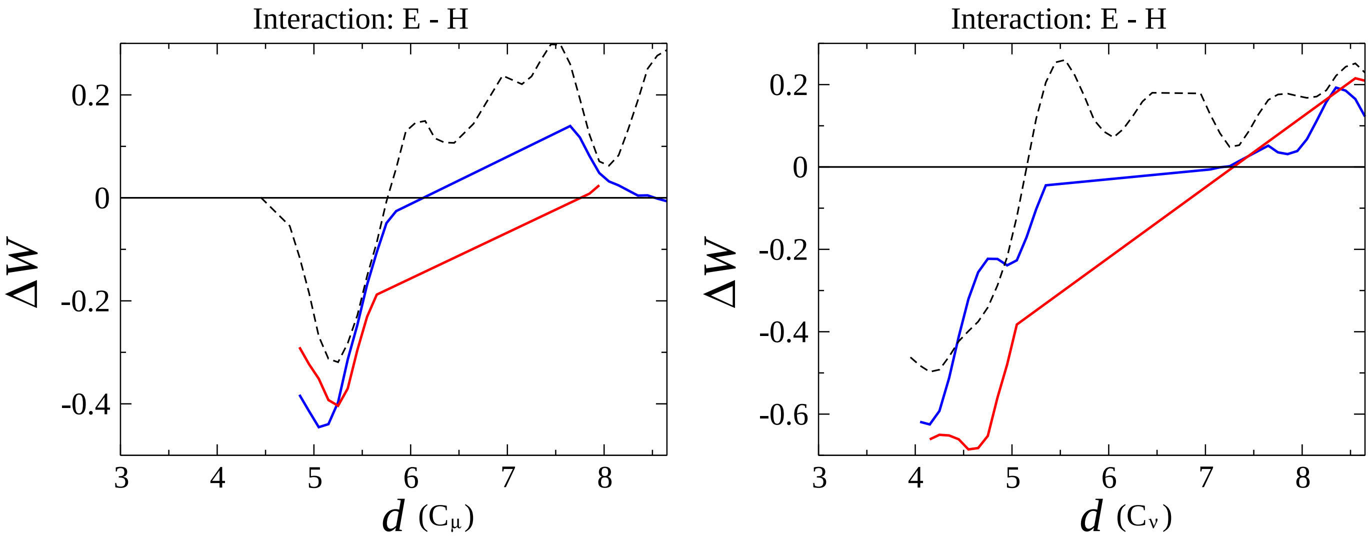
S6)


Interaction: D - R
Interaction: D - R
ΔW
ΔW
d (Cμ)
d (Cν)
0.2
0
-0.2
-0.4
-0.6
-0.8
0.5
0
-0.5
-1
-1.5
-2
3
4
5
6
7
8

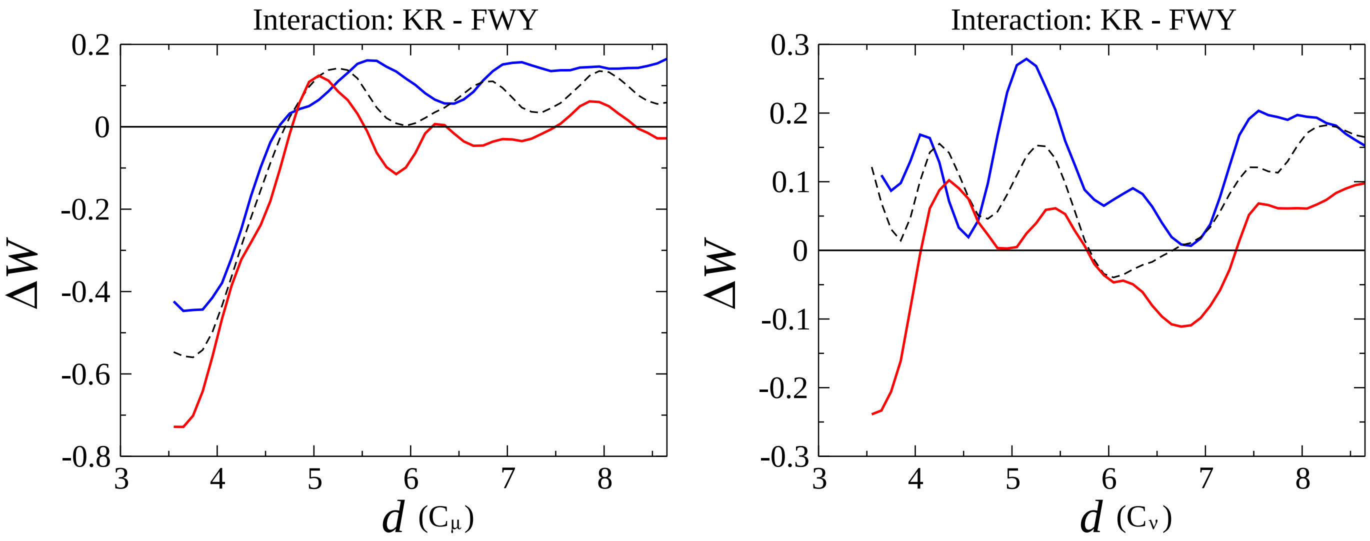
S7)


Interaction: E - R
Interaction: E - R
ΔW
ΔW
d (Cμ)
d (Cν)
0.4
0.2
0
-0.2
-0.4
-0.6
0
-0.5
-1
-1.5
-2
3
4
5
6
7
8

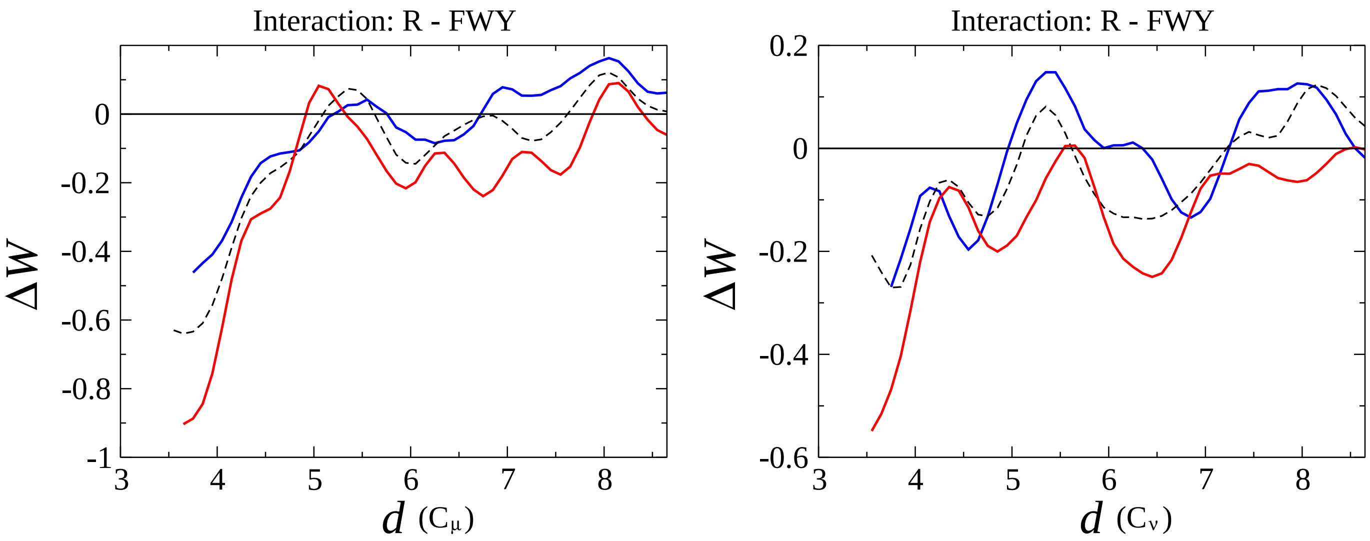
S8)


Interaction: E - H
ΔW
d (Cμ)
Interaction: E - H
ΔW
d (Cν)

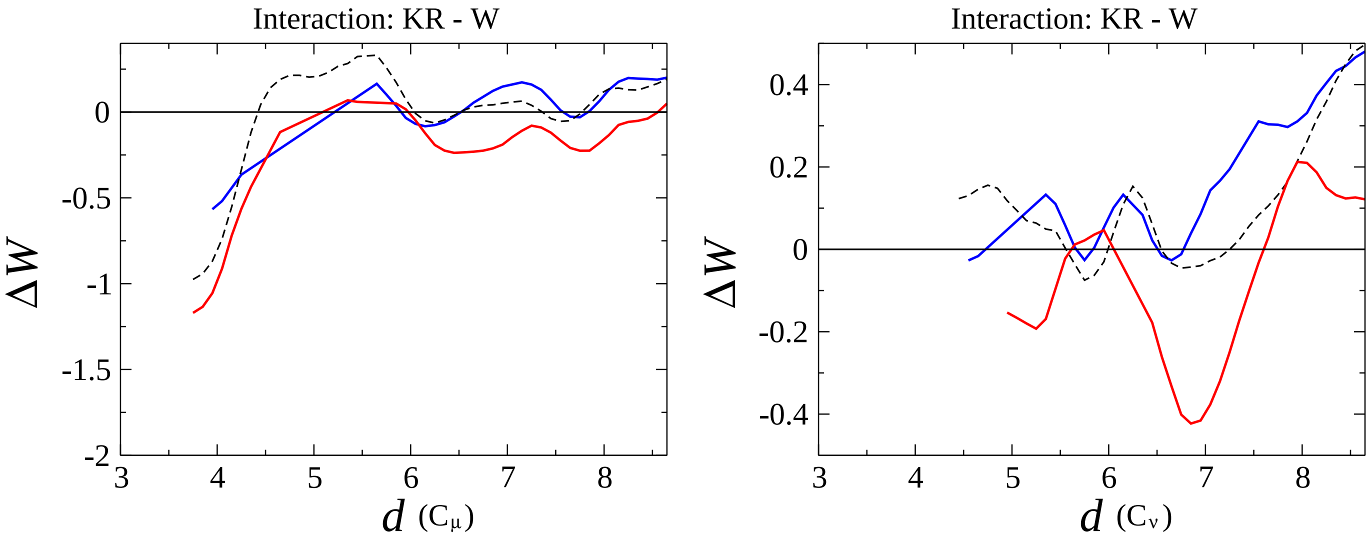
S9)


Interaction: KR - FWY
ΔW
d (Cμ)
Interaction: KR - FWY
ΔW
d (Cν)

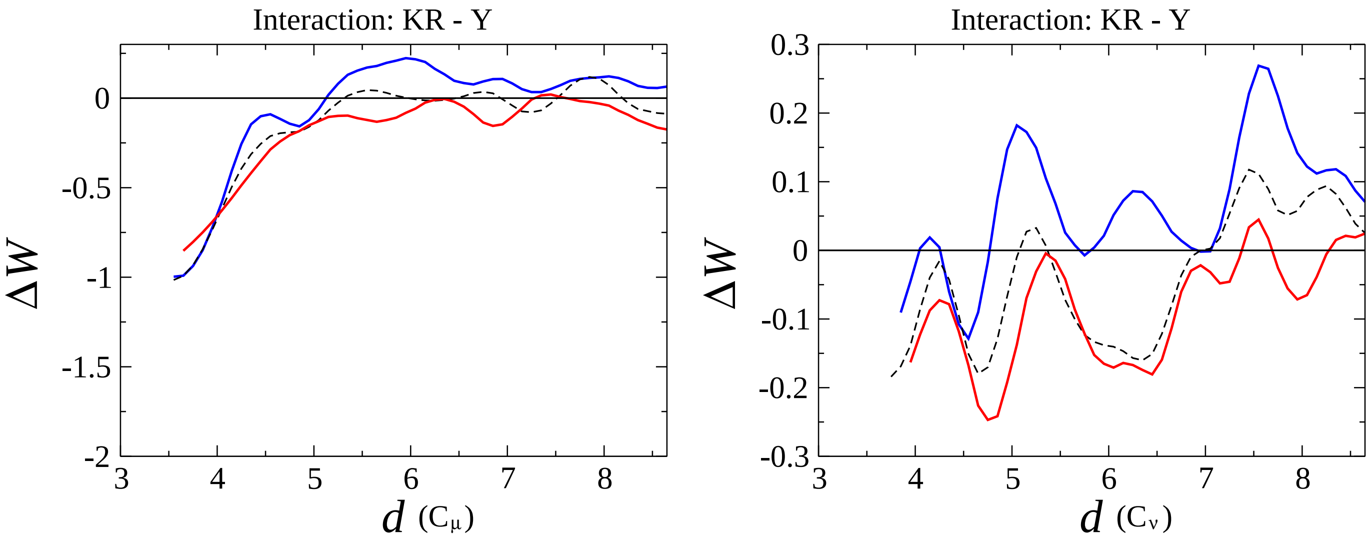
S10)


Interaction: R - FWY
ΔW
d (Cμ)
Interaction: R - FWY
ΔW
d (Cν)

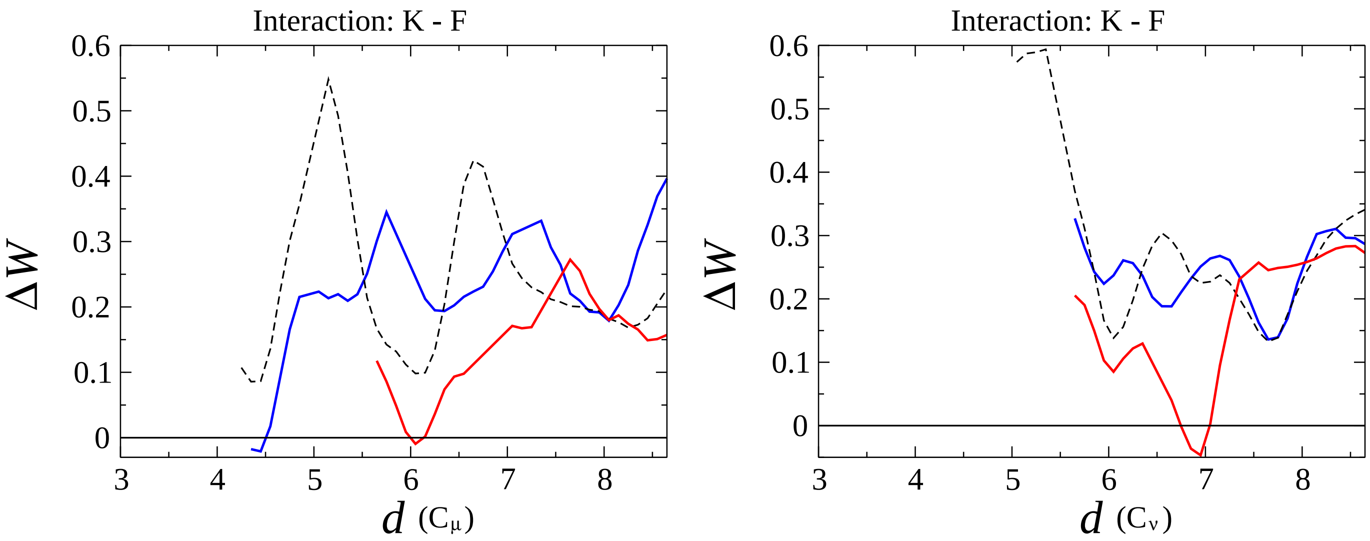
S11)


Interaction: KR - W
ΔW
d (Cμ)
Interaction: KR - W
ΔW
d (Cν)

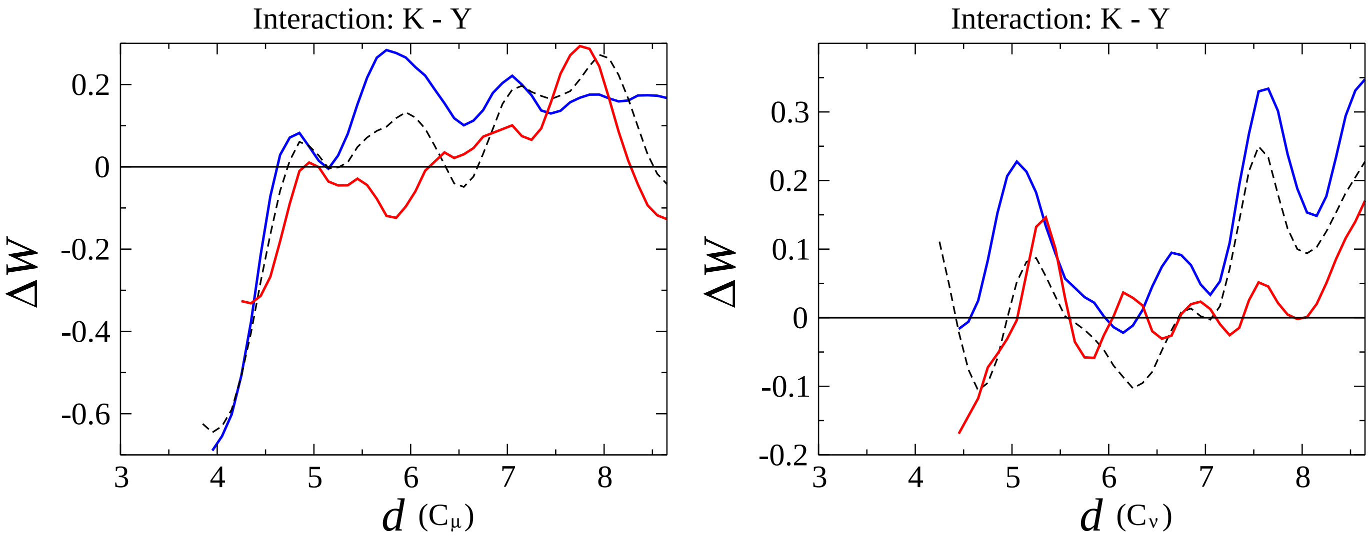
S12)


Interaction: KR - Y
ΔW
d (Cμ)
Interaction: KR - Y
ΔW
d (Cν)

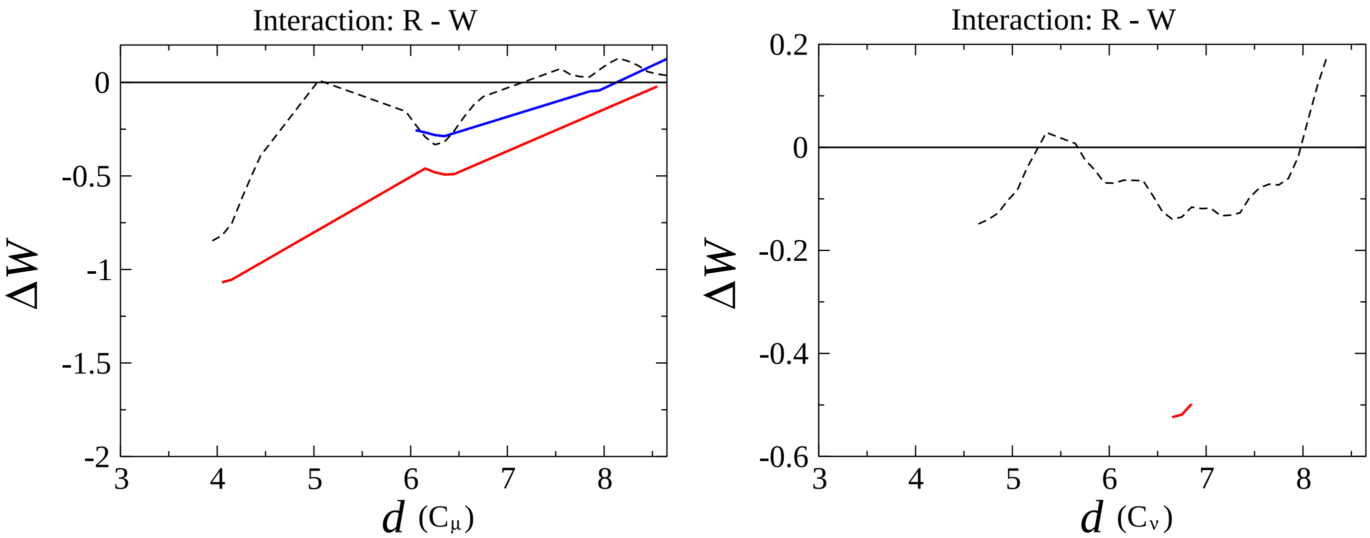
S13)


Interaction: K - F
ΔW
d (Cμ)
Interaction: K - F
ΔW
d (Cν)

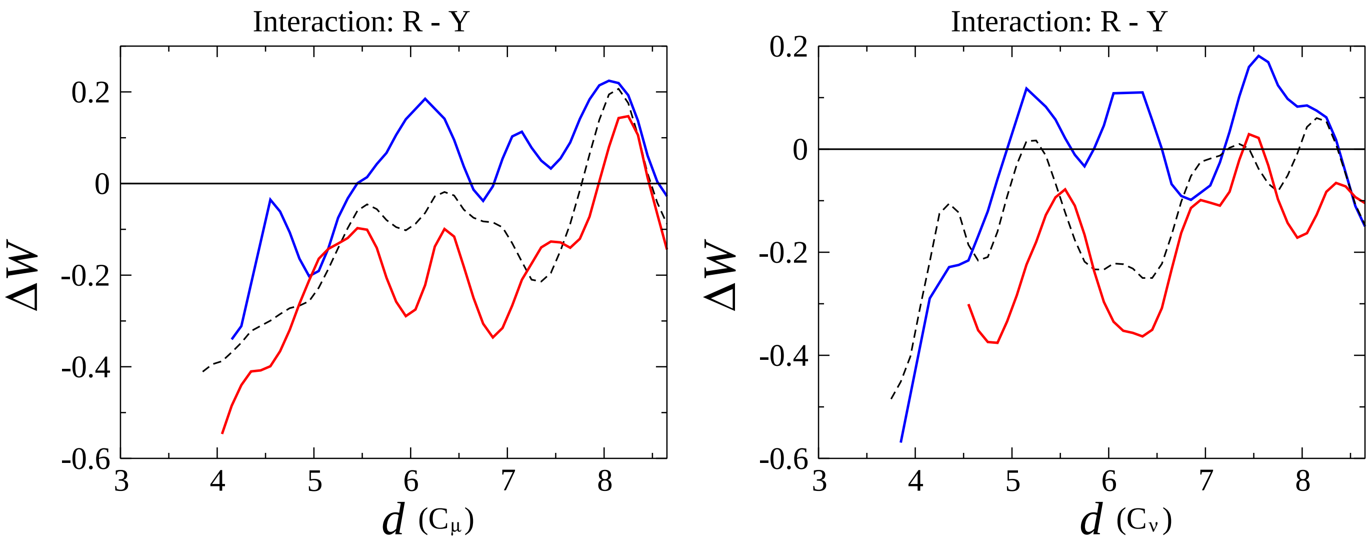
S14)


Interaction: K - Y
ΔW
d (Cμ)
Interaction: K - Y
ΔW
d (Cν)

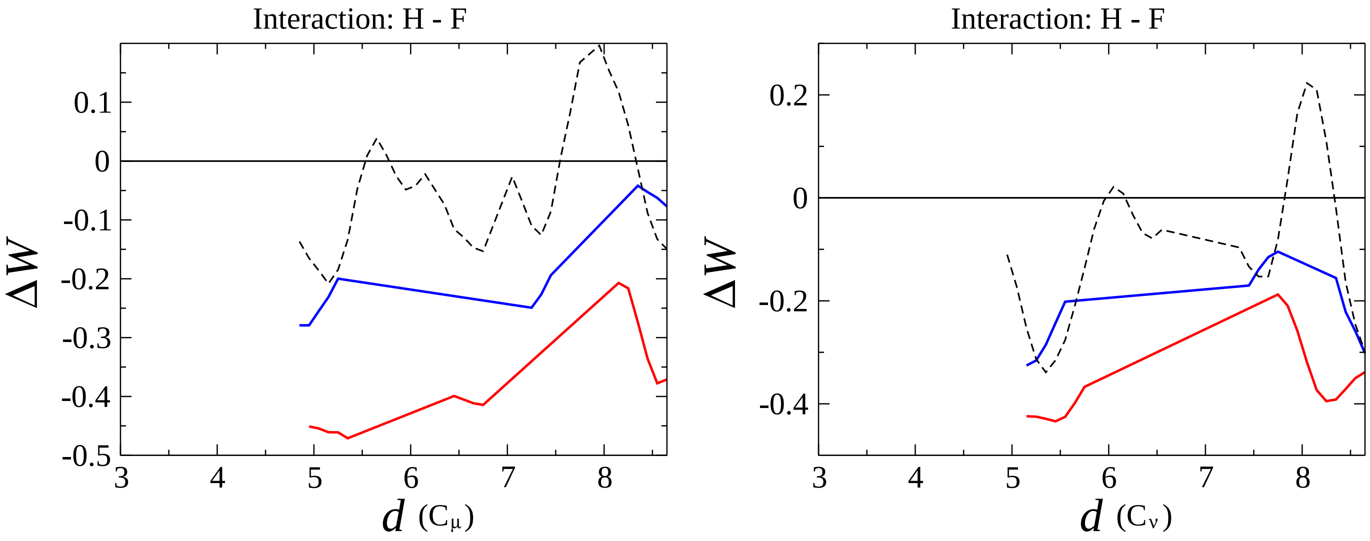
S15)


Interaction: R - W
ΔW
d (Cμ)
Interaction: R - W
ΔW
d (Cν)

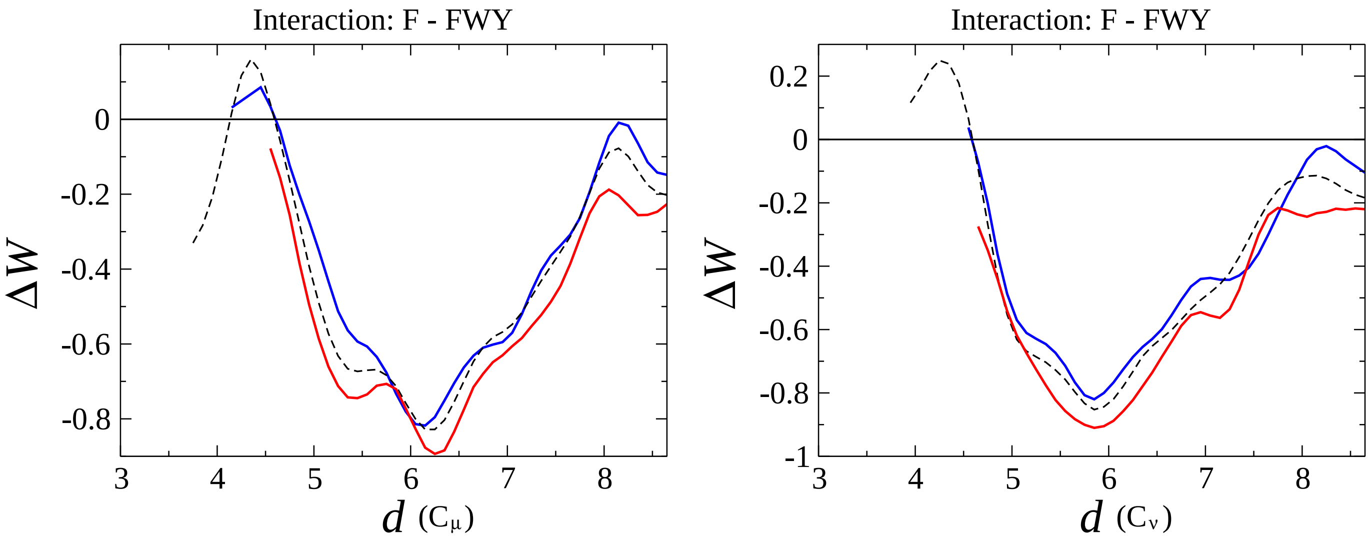
S16)


Interaction: R - Y
ΔW
d (Cμ)
Interaction: R - Y
ΔW
d (Cν)

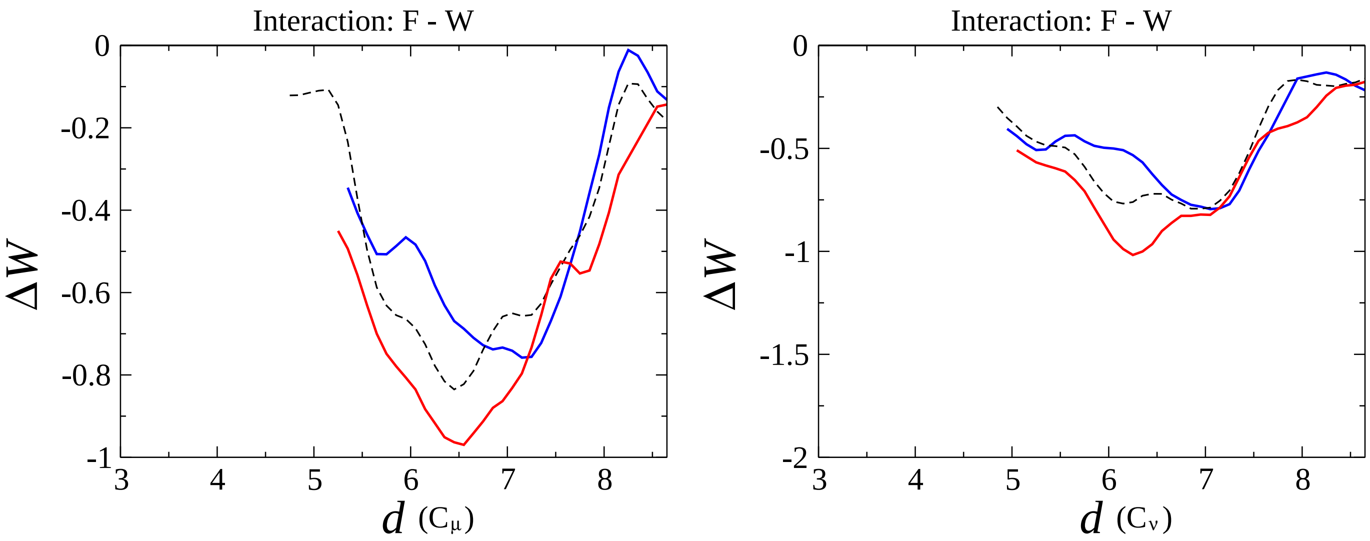
S17)


Interaction: H - F
ΔW
d (Cμ)
Interaction: H - F
ΔW
d (Cν)

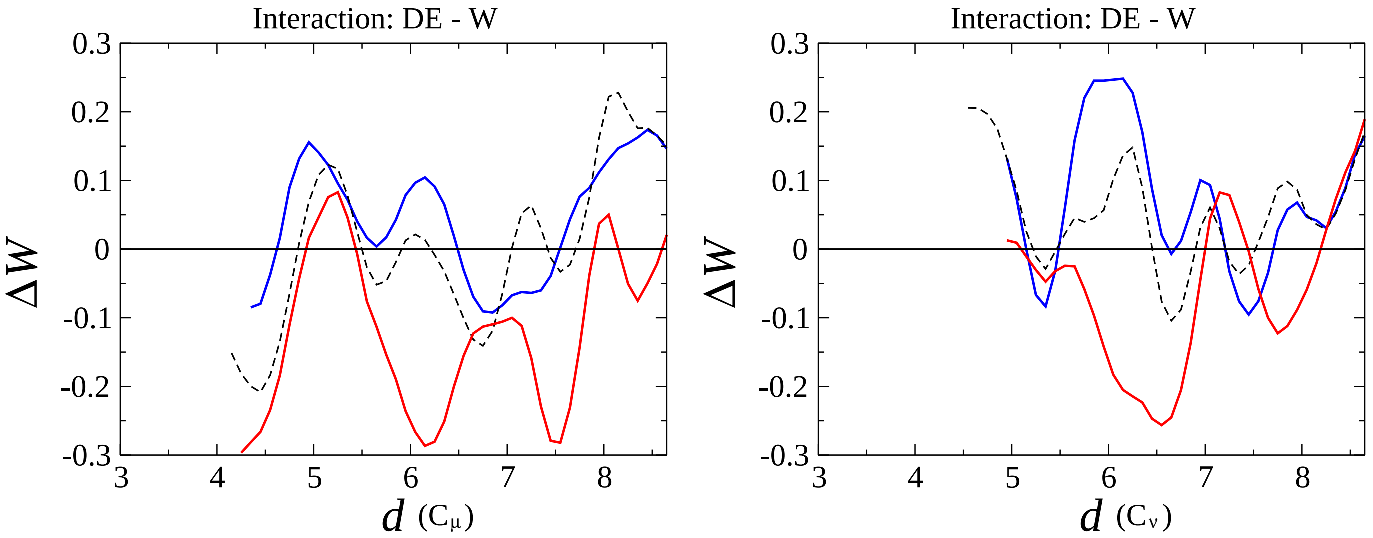
S18)


Interaction: F - FWY
ΔW
d (Cμ)
Interaction: F - FWY
ΔW
d (Cν)

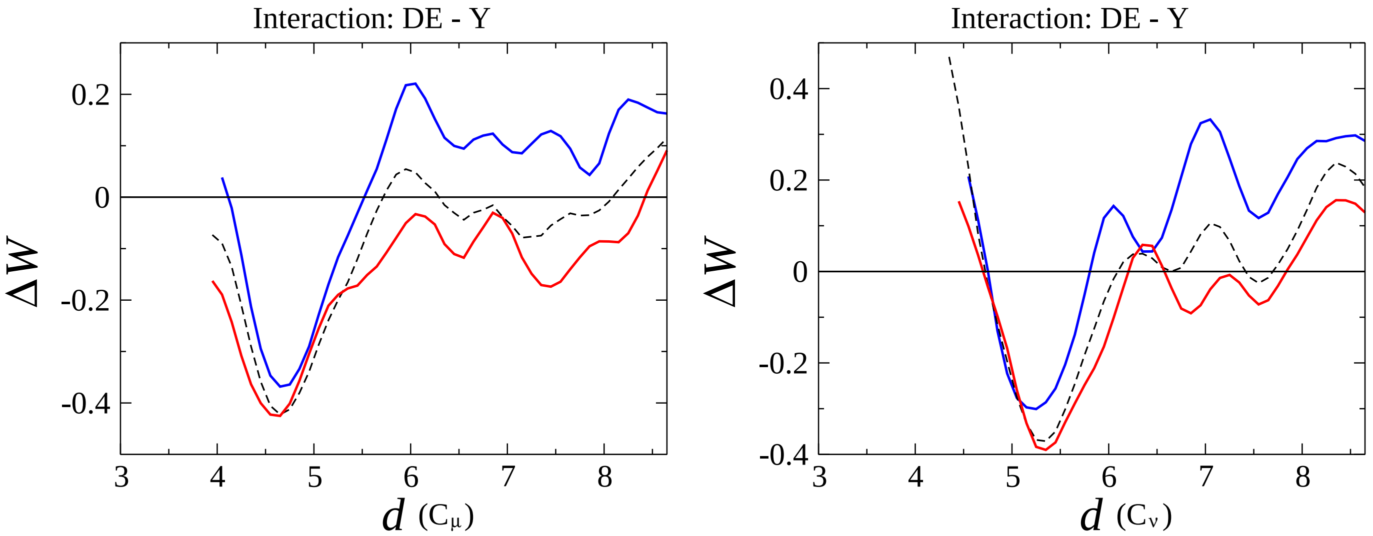
S19)


Interaction: F - W
ΔW
d (Cμ)
Interaction: F - W
ΔW
d (Cν)

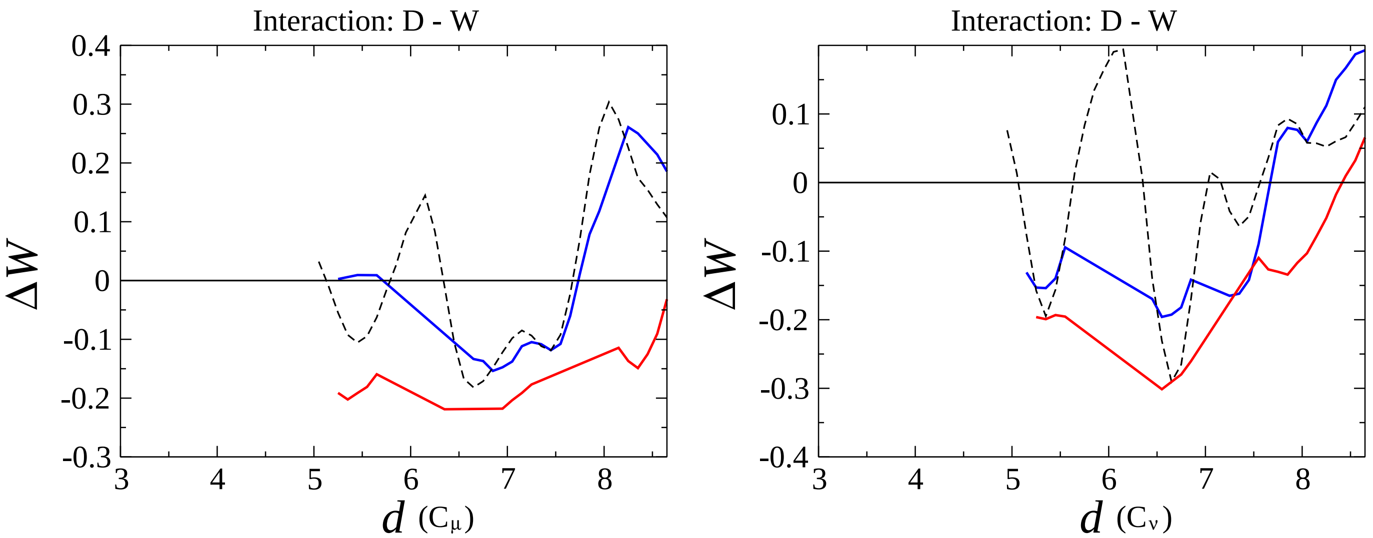
S20)


Interaction: DE - W
ΔW
d (Cμ)
Interaction: DE - W
ΔW
d (Cν)

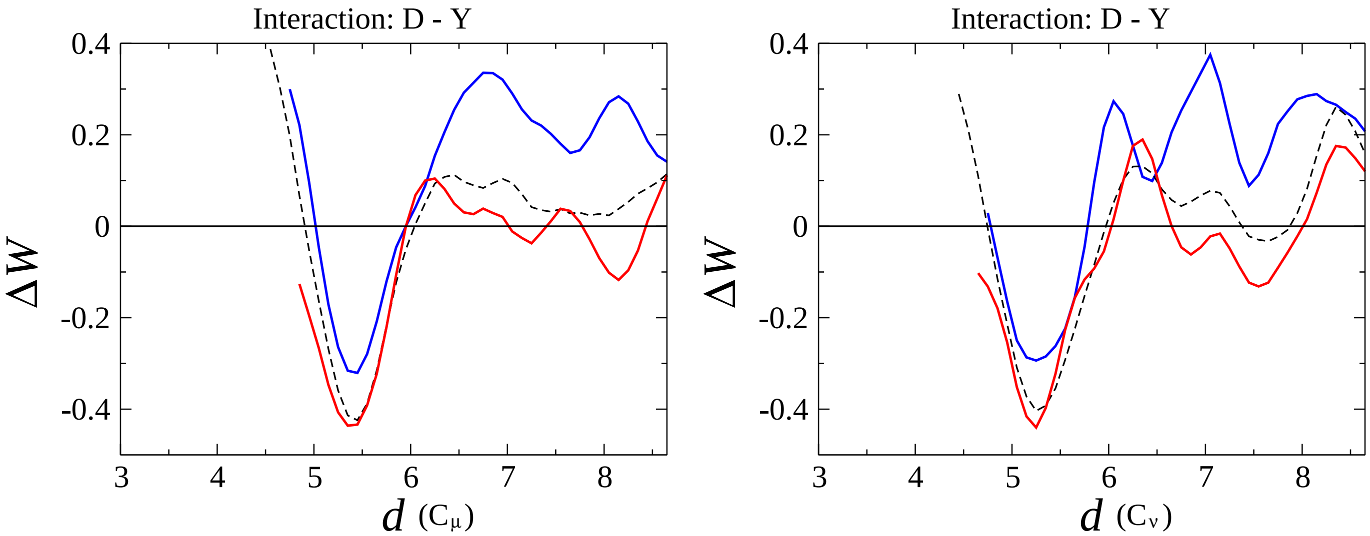
S21)


Interaction: DE - Y
ΔW
d (Cμ)
Interaction: DE - Y
ΔW
d (Cν)

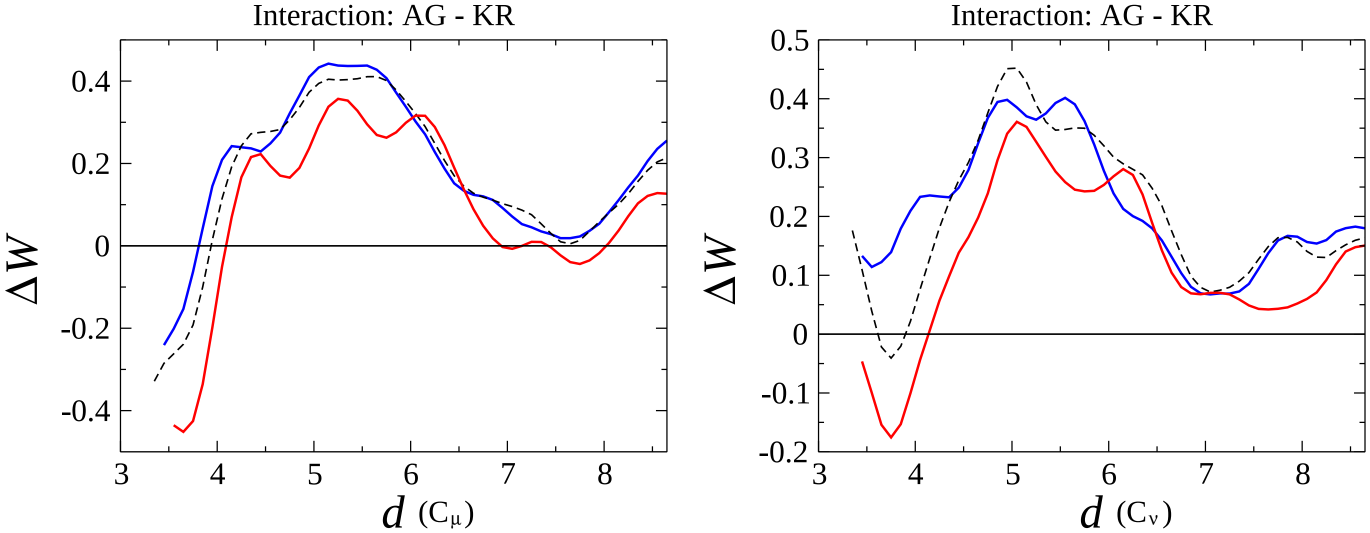
S22)


Interaction: D - W
ΔW
d (Cμ)
Interaction: D - W
ΔW
d (Cν)

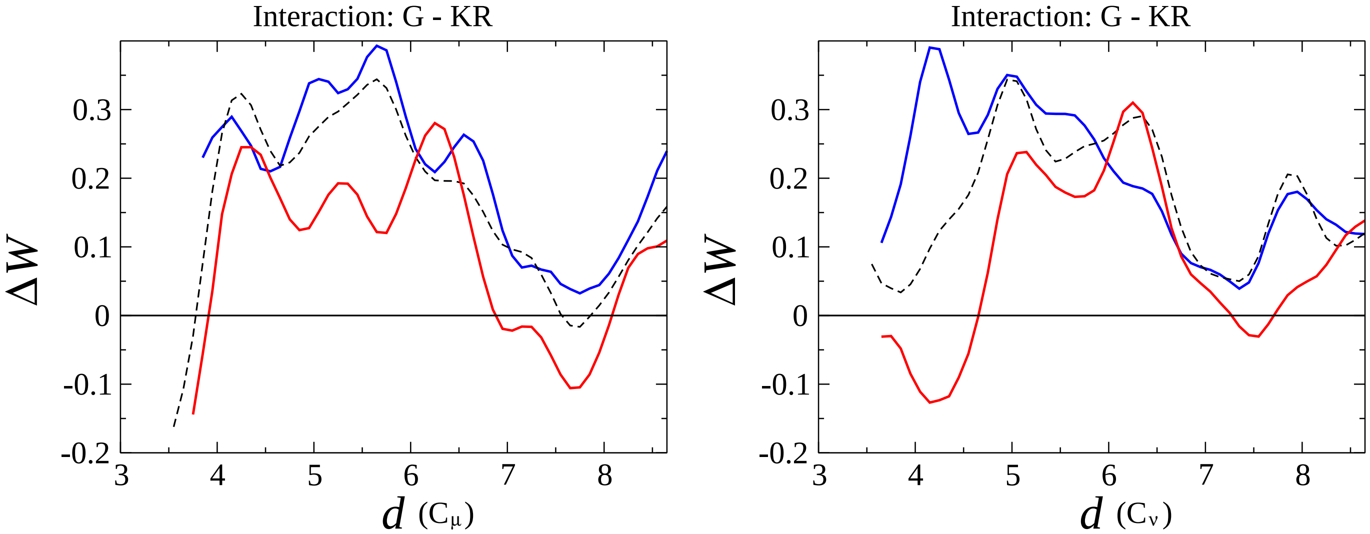
S23)


Interaction: D - Y
ΔW
d (Cμ)
Interaction: D - Y
ΔW
d (Cν)

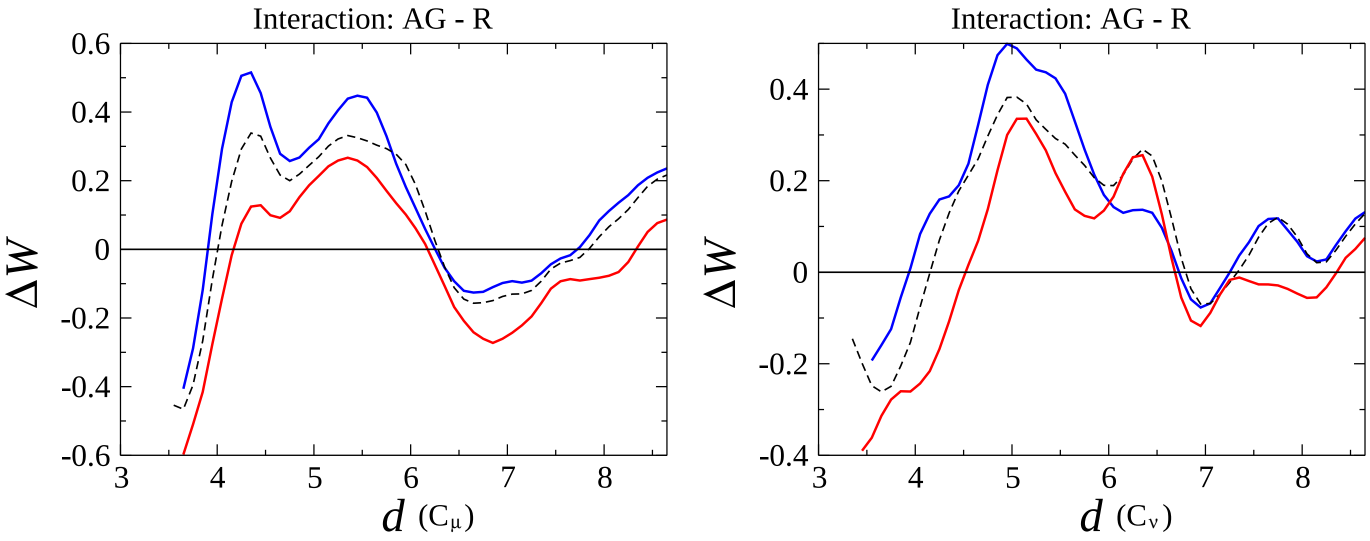
S24)


Interaction: AG - KR
ΔW
d (Cμ)
Interaction: AG - KR
ΔW
d (Cν)

S25)

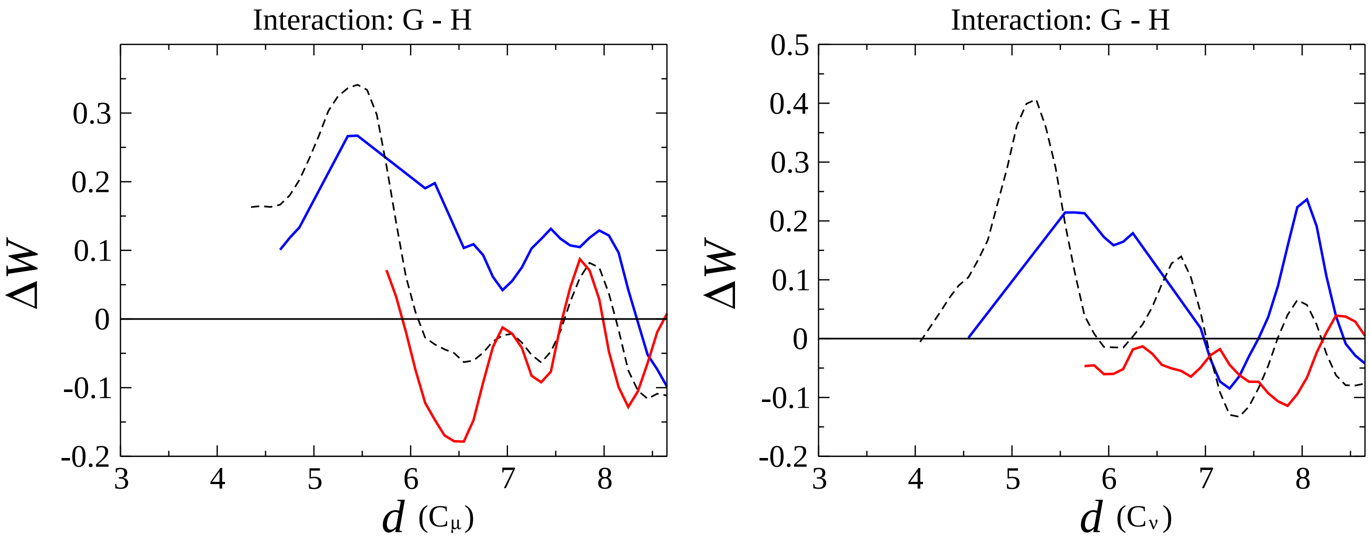

Interaction: G - KR
ΔW
d (Cμ)
Interaction: G - KR
ΔW
d (Cν)

S26)



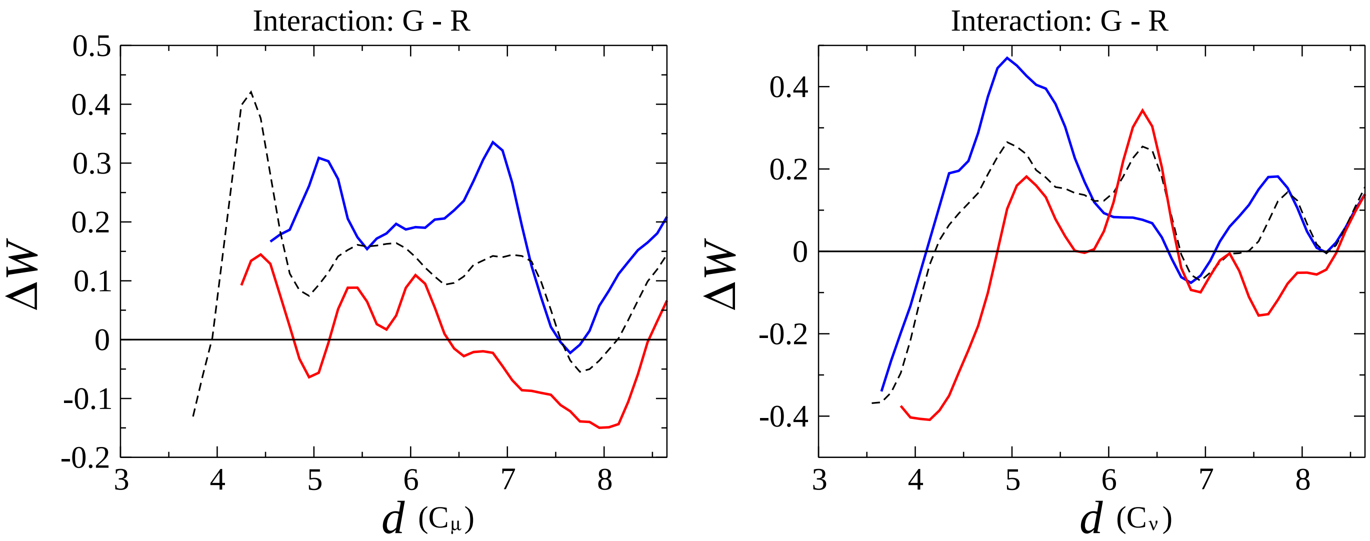
Interaction: AG - R
ΔW
d (Cμ)
Interaction: AG - R
ΔW
d (Cν)

S27)



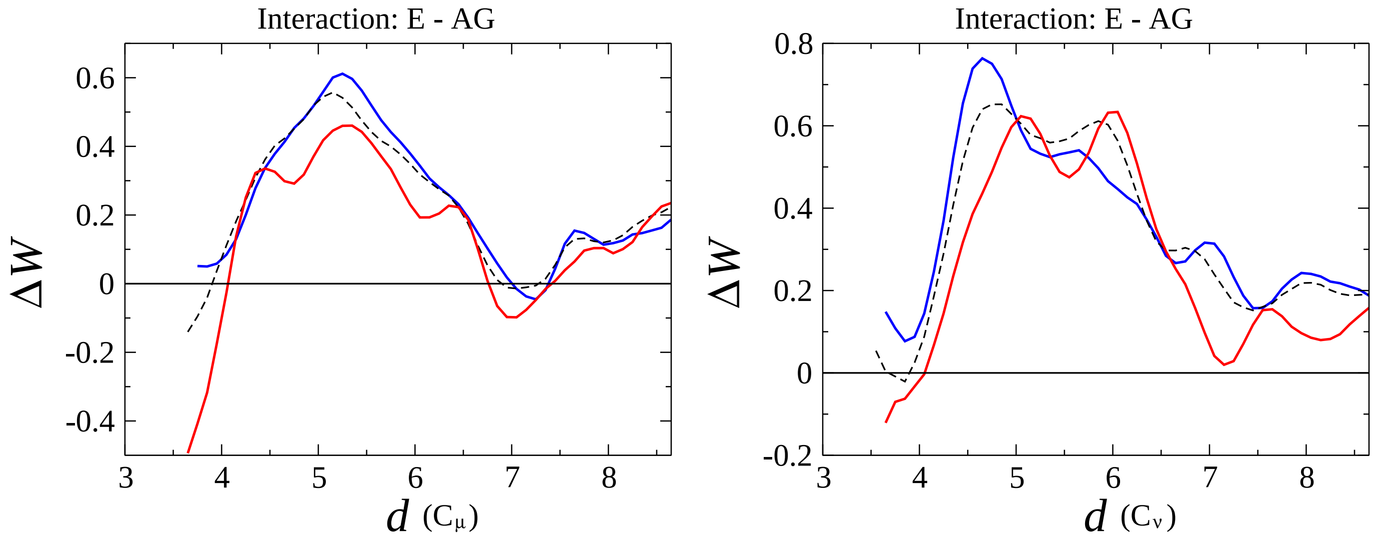
Interaction: G - H
ΔW
d (Cμ)
Interaction: G - H
ΔW
d (Cν)

S28)



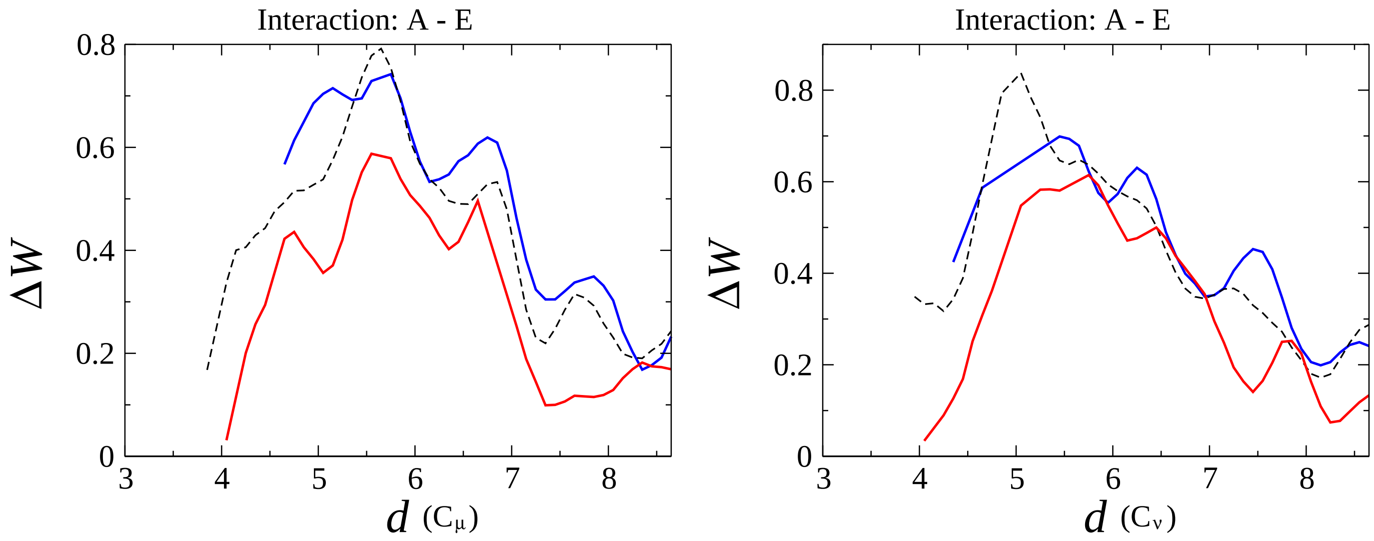
Interaction: G - R
ΔW
d (Cμ)
Interaction: G - R
ΔW
d (Cν)

S29)



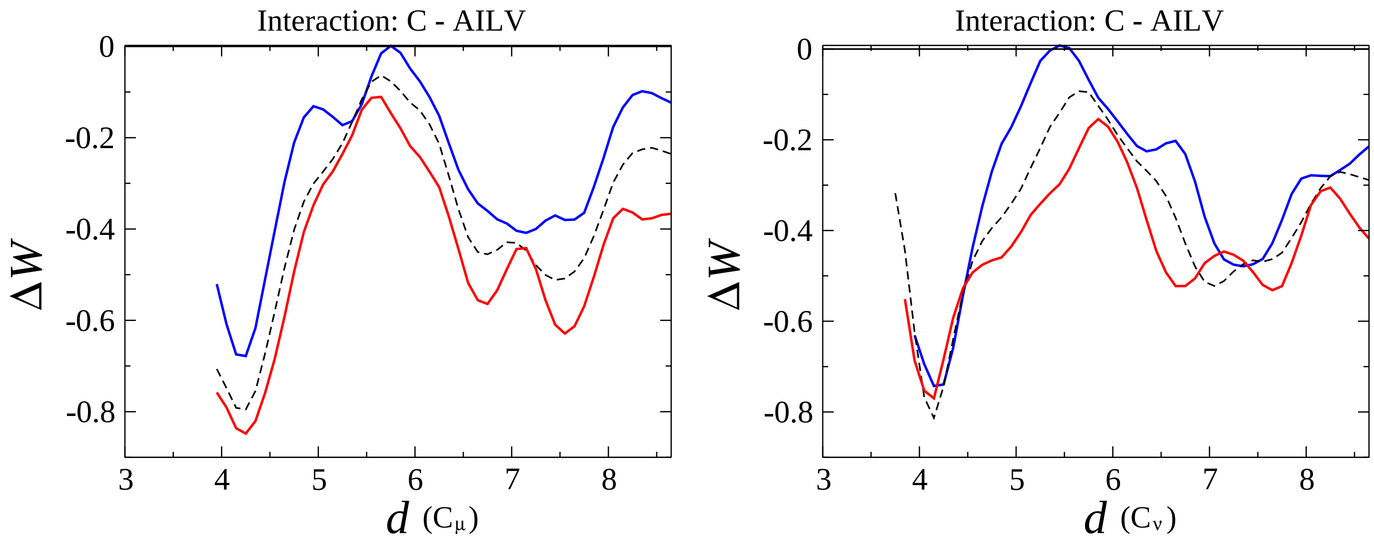
Interaction: E - AG
ΔW
d (Cμ)
Interaction: E - AG
ΔW
d (Cν)

S30)



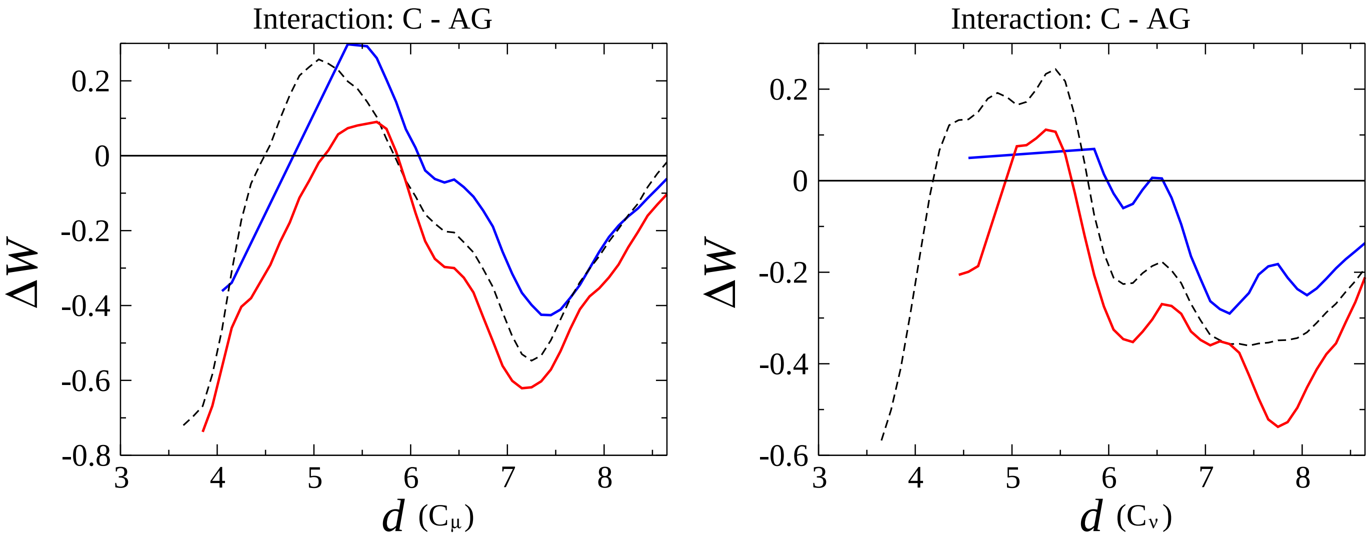
Interaction: A - E
ΔW
d (Cμ)
Interaction: A - E
ΔW
d (Cν)

S31)

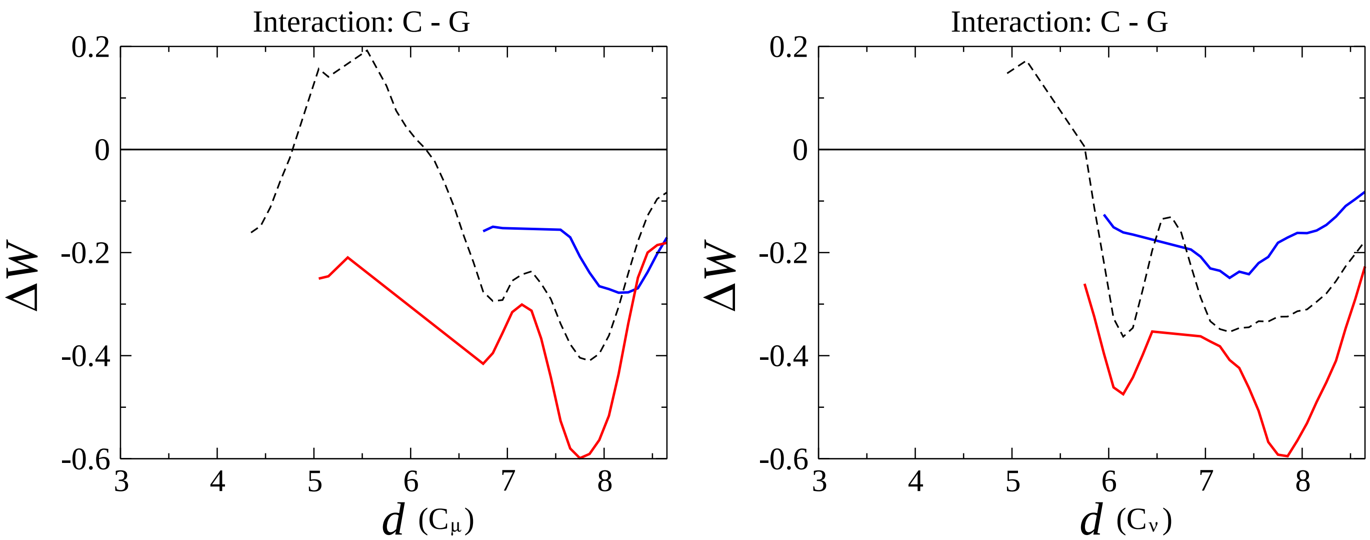


Interaction: C - AILV
ΔW
d (Cμ)
Interaction: C - AILV
ΔW
d (Cν)

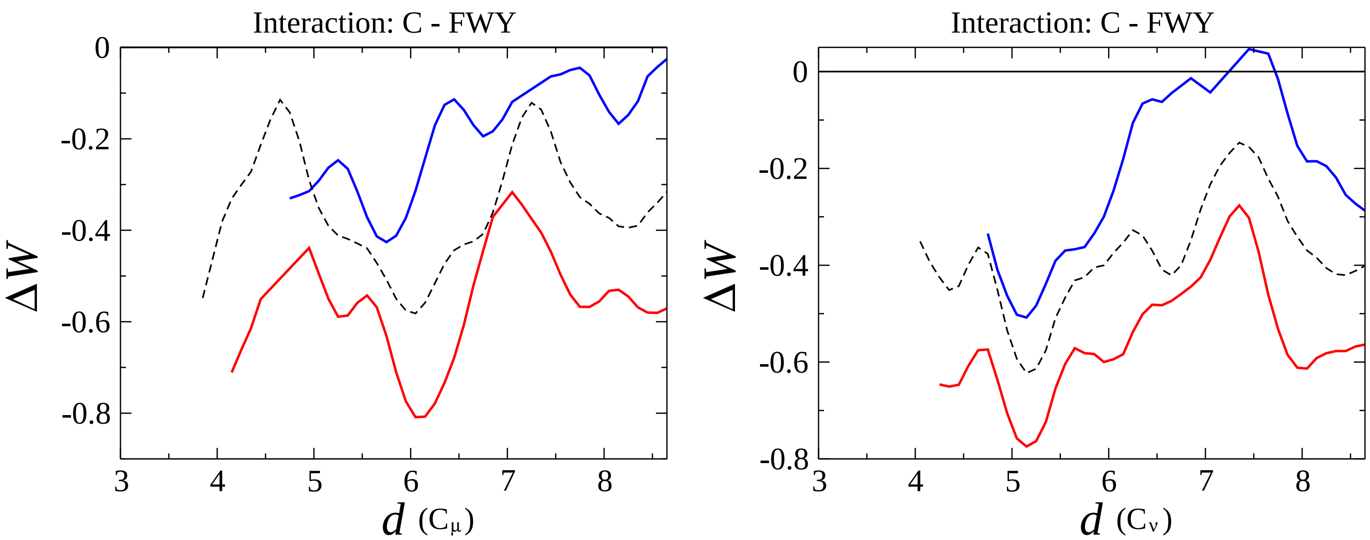
S32)


Interaction: C - AG
ΔW
0.2
0
-0.2
-0.4
-0.6
-0.8
3
4
5
6
7
8
d (Cμ)
Interaction: C - AG
ΔW
0.2
0
-0.2
-0.4
-0.6
d (Cν)

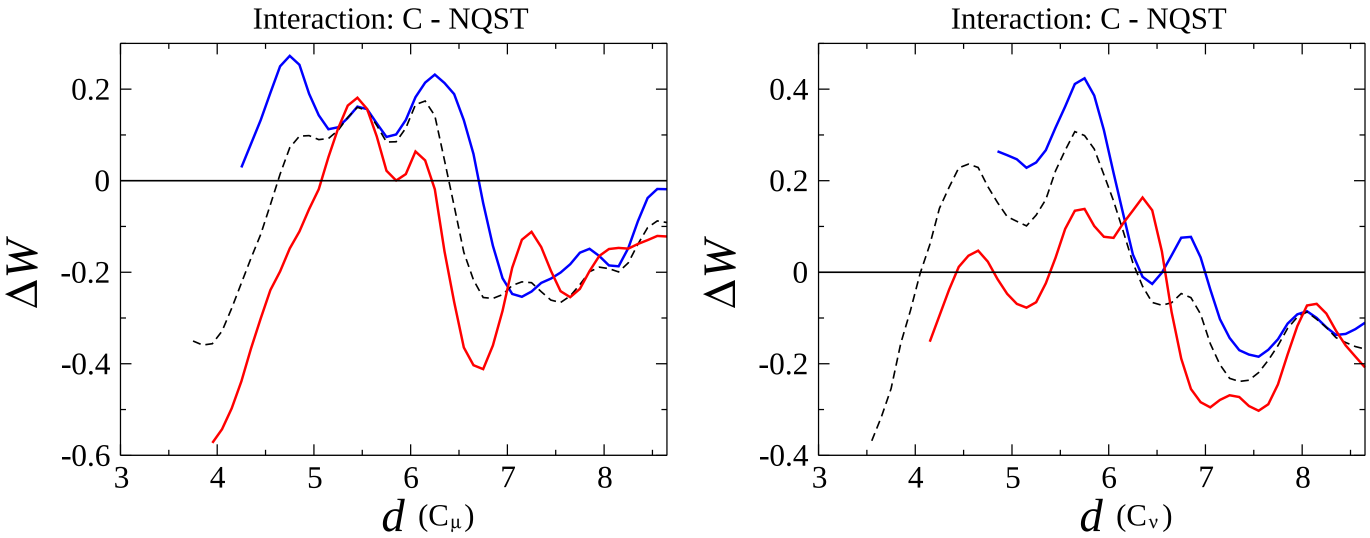
S33)


Interaction: C - G
ΔW
0.2
0
-0.2
-0.4
-0.6
3
4
5
6
7
8
d (Cμ)
Interaction: C - G
ΔW
d (Cν)

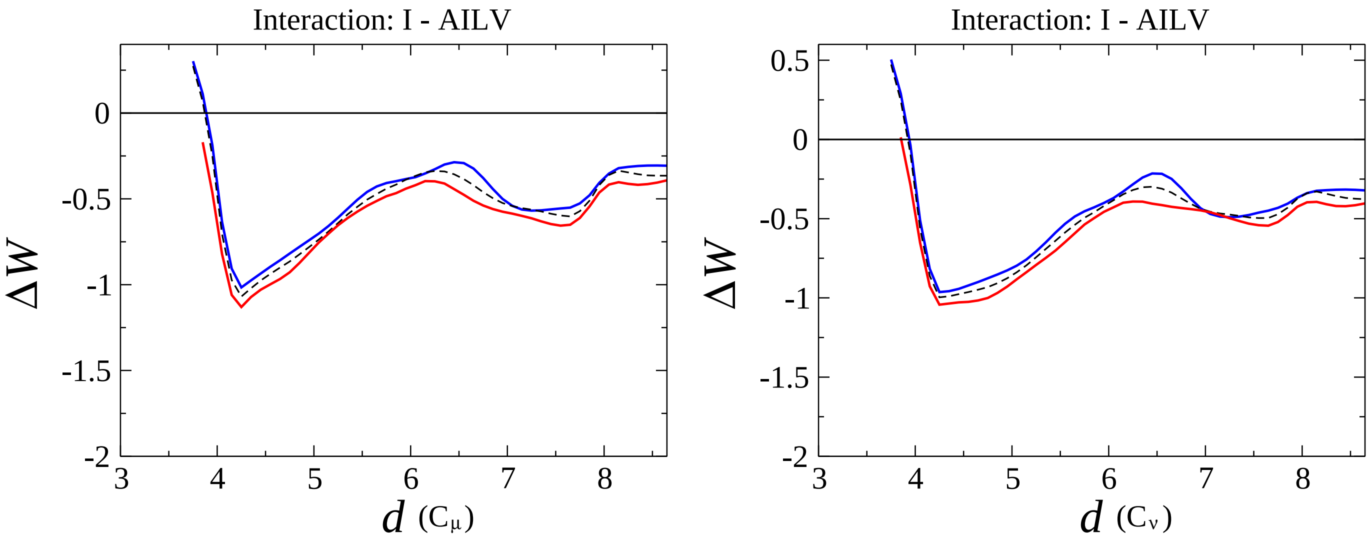
S34)


Interaction: C - FWY
ΔW
0
-0.2
-0.4
-0.6
-0.8
3
4
5
6
7
8
d (Cμ)
Interaction: C - FWY
ΔW
d (Cν)

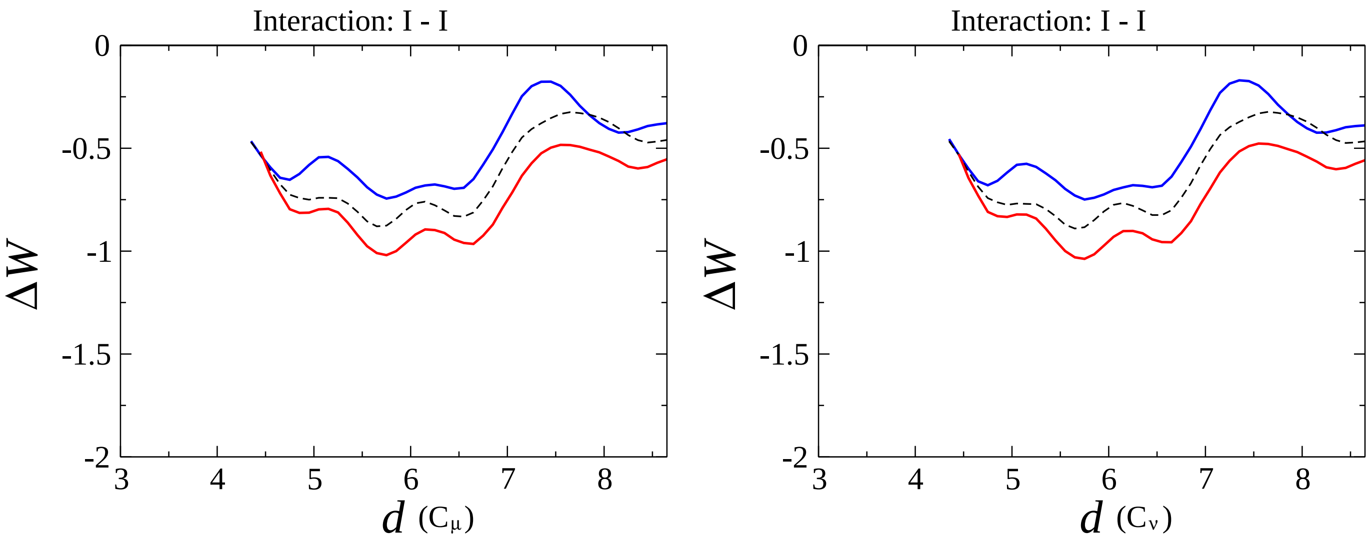
S35)


Interaction: C - NQST
ΔW
d (Cμ)
Interaction: C - NQST
ΔW
d (Cν)

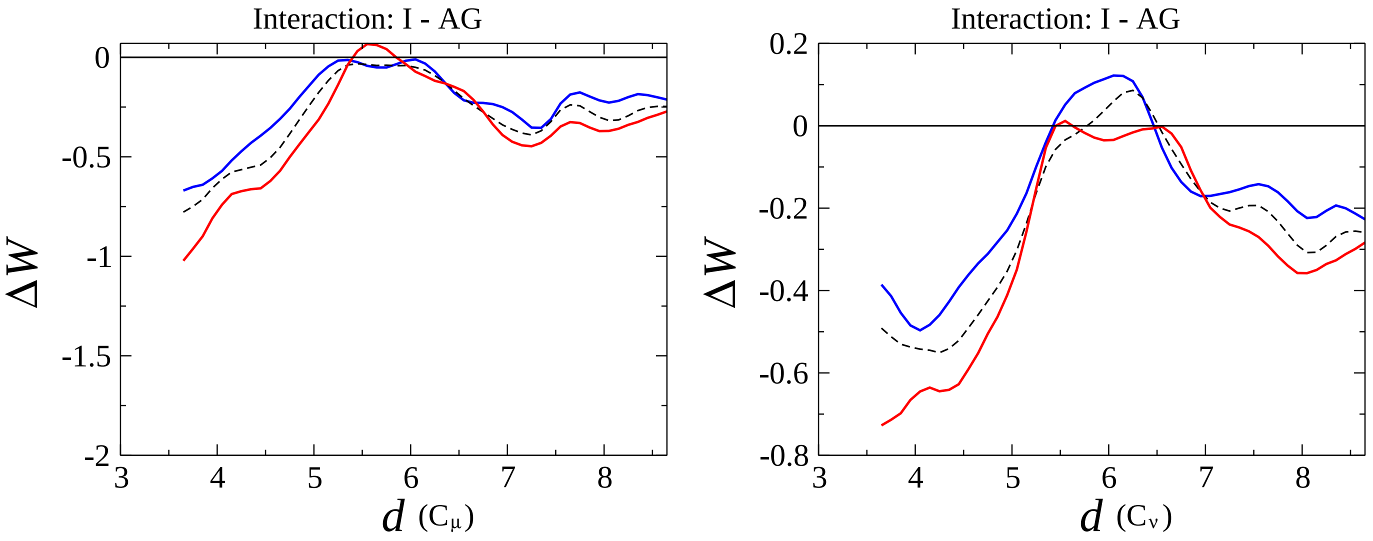
S36)


Interaction: I - AILV
ΔW
d (Cμ)
Interaction: I - AILV
ΔW
d (Cν)

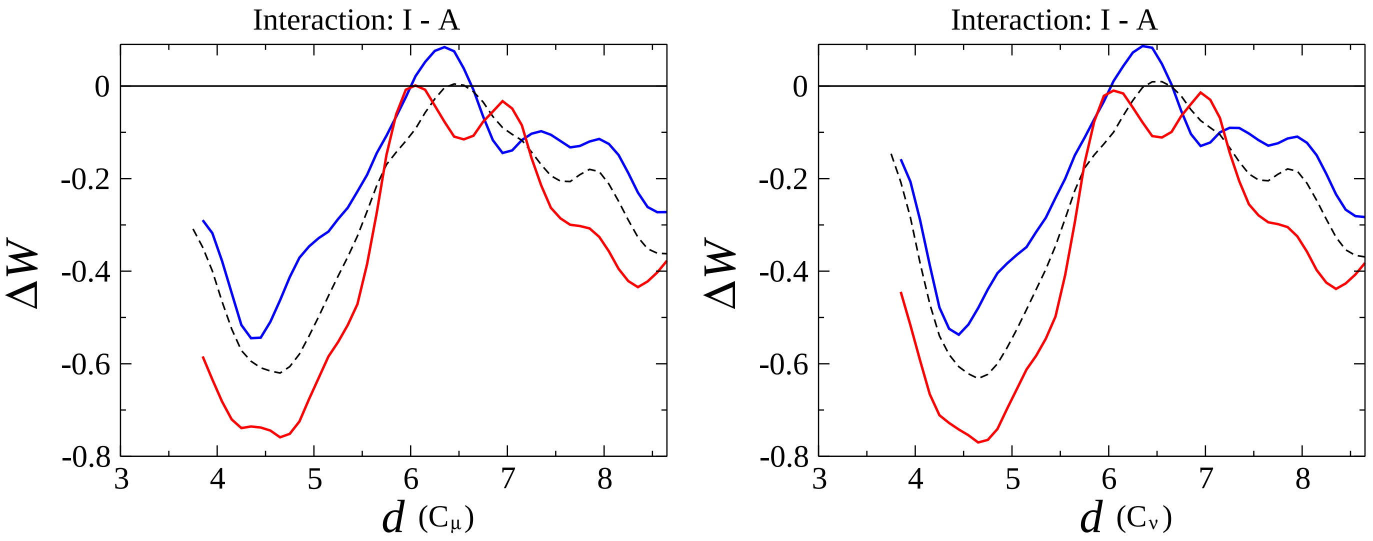
S37)


Interaction: I - I
ΔW
d (Cμ)
Interaction: I - I
ΔW
d (Cν)

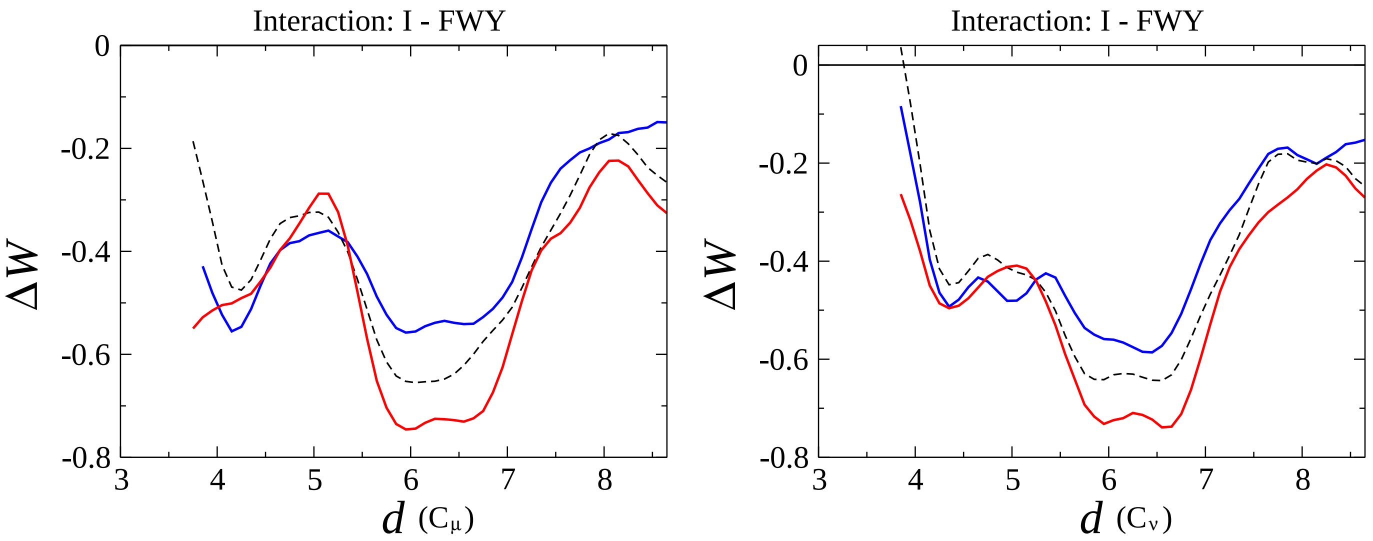
S38)


Interaction: I - AG
ΔW
d (Cμ)
Interaction: I - AG
ΔW
d (Cν)

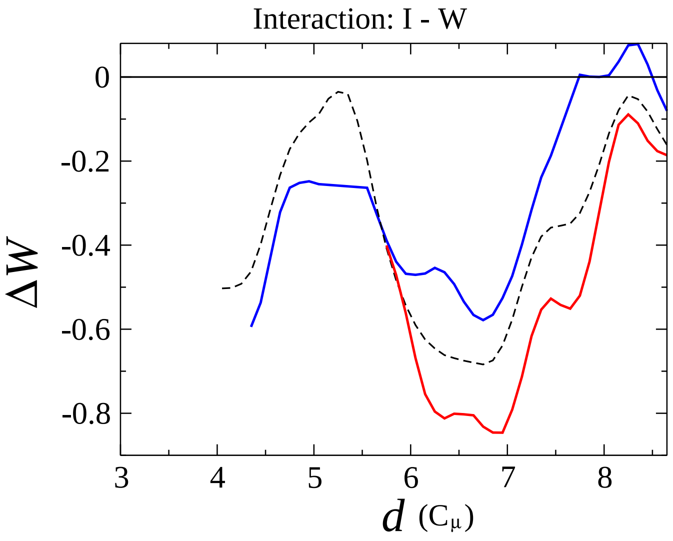
S39)

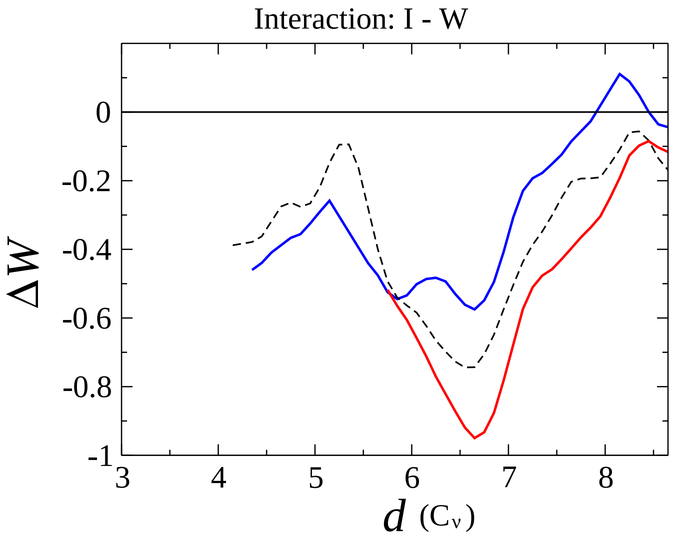

Interaction: I - A
ΔW
d (Cμ)
Interaction: I - A
ΔW
d (Cν)

S40)

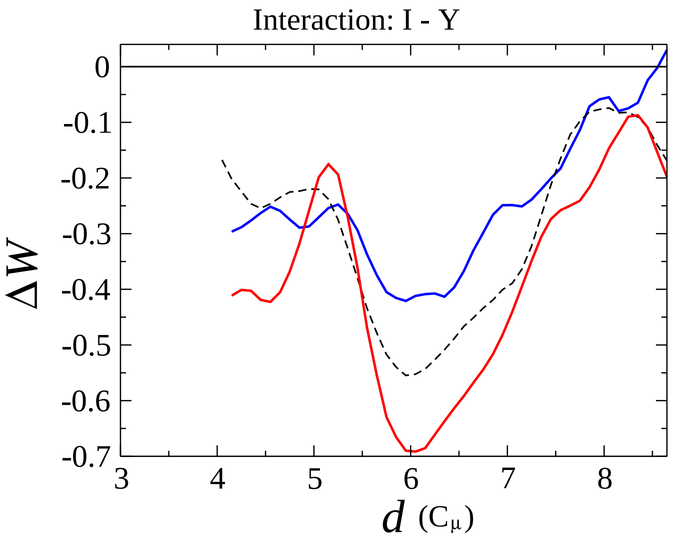

Interaction: I - FWY
ΔW
d (Cμ)
Interaction: I - FWY
ΔW
d (Cν)

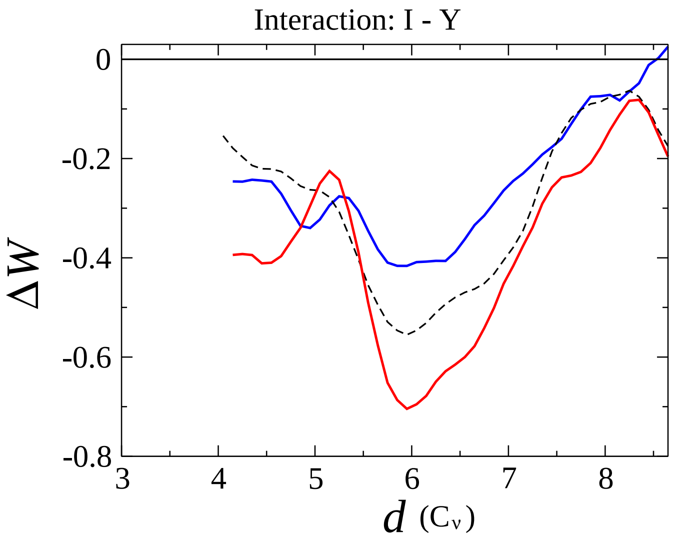
S41)


Interaction: I - W
0
-0.2
-0.4
-0.6
-0.8
ΔW
3 4 5 6 7 8
d (Cμ)

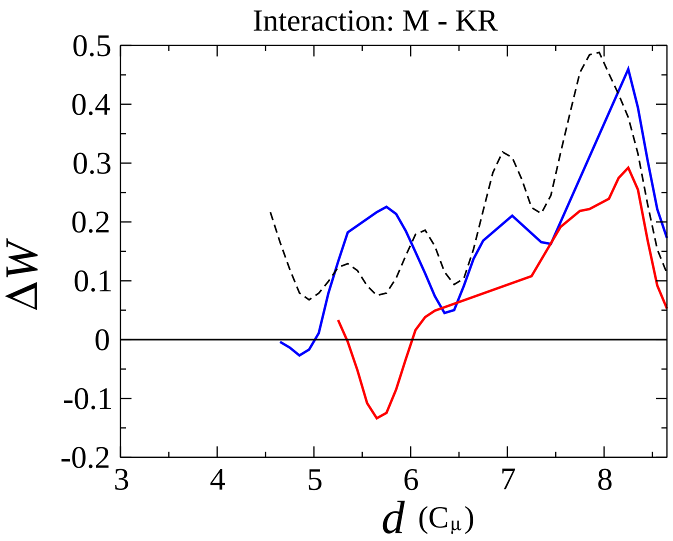

Interaction: I - W
0
-0.2
-0.4
-0.6
-0.8
-1
ΔW
3 4 5 6 7 8
d (Cν)

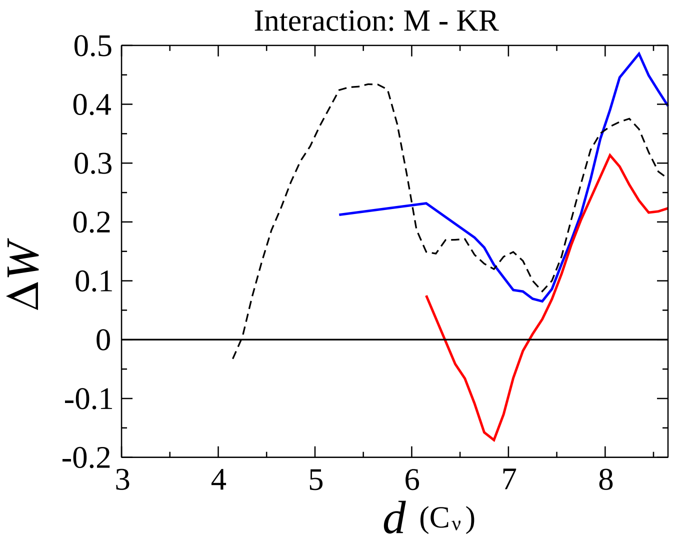
S42)


Interaction: I - Y
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
ΔW
3 4 5 6 7 8
d (Cμ)

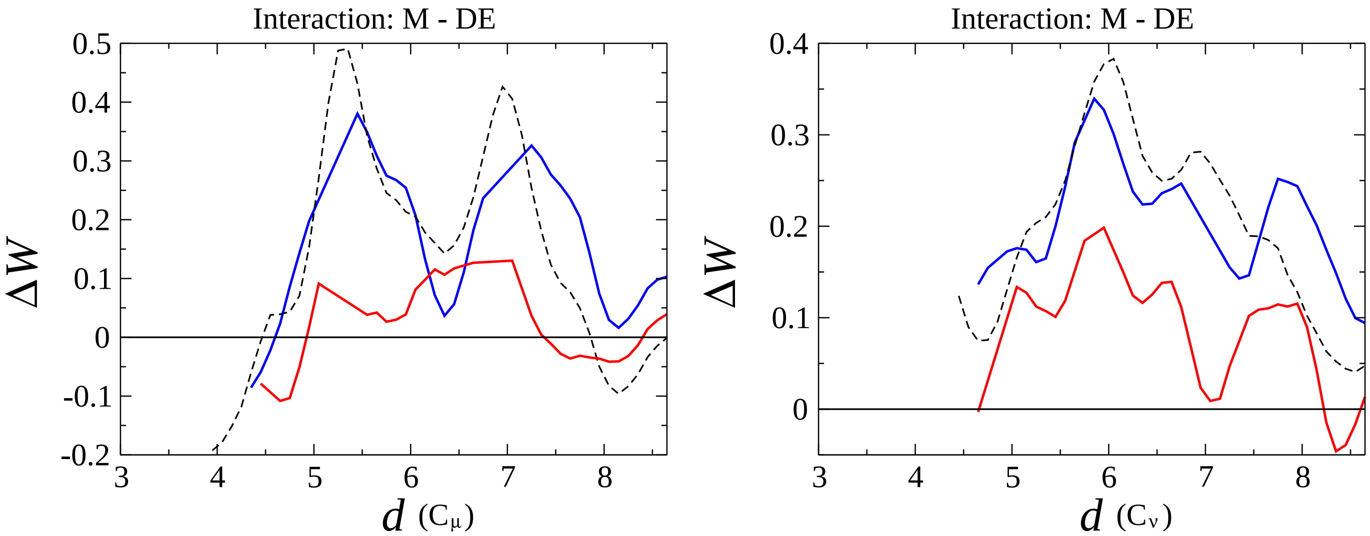

Interaction: I - Y
0
-0.2
-0.4
-0.6
-0.8
ΔW
3 4 5 6 7 8
d (Cν)

S43)

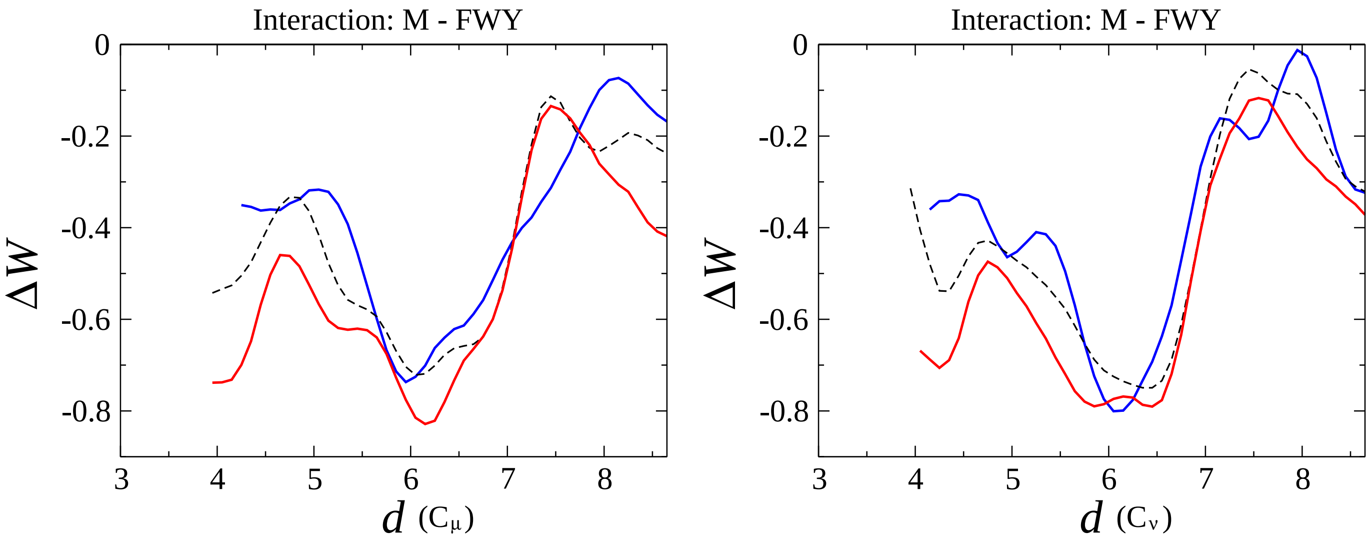

Interaction: M - KR
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
ΔW
3 4 5 6 7 8
d (Cμ)


Interaction: M - KR
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
ΔW
3 4 5 6 7 8
d (Cν)

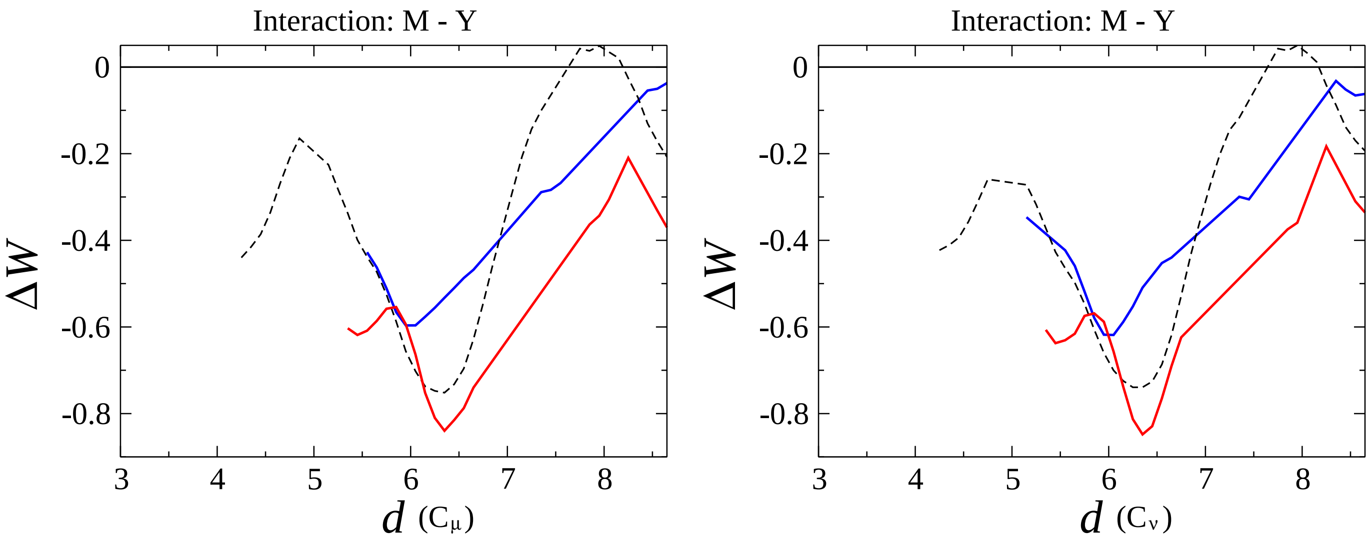
S44)


Interaction: M - DE
ΔW
d (Cμ)
Interaction: M - DE
ΔW
d (Cν)

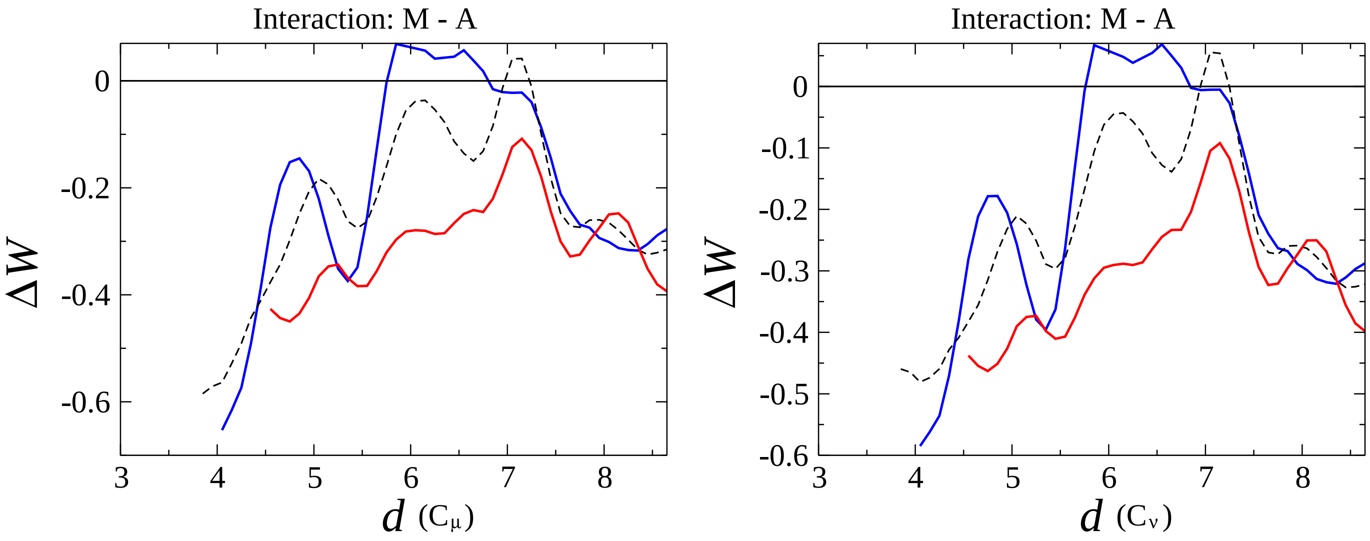
S45)


Interaction: M - FWY
ΔW
d (Cμ)
Interaction: M - FWY
ΔW
d (Cν)

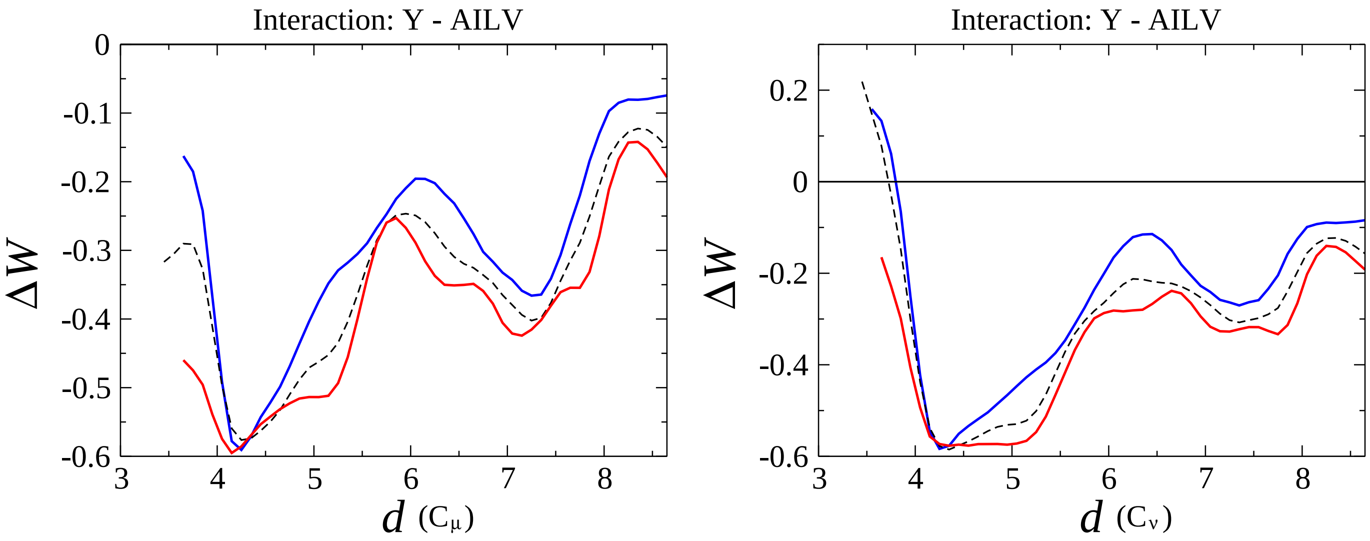
S46)


Interaction: M - Y
ΔW
d (Cμ)
Interaction: M - Y
ΔW
d (Cν)

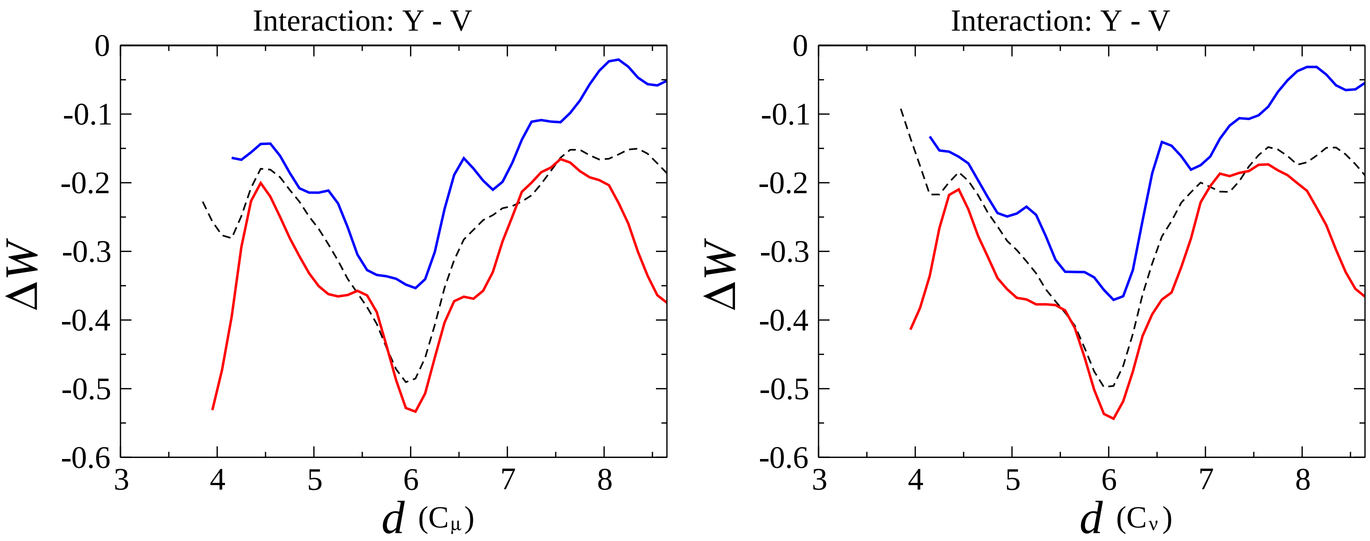
S47)


Interaction: M - A
ΔW
d (Cμ)
Interaction: M - A
ΔW
d (Cν)

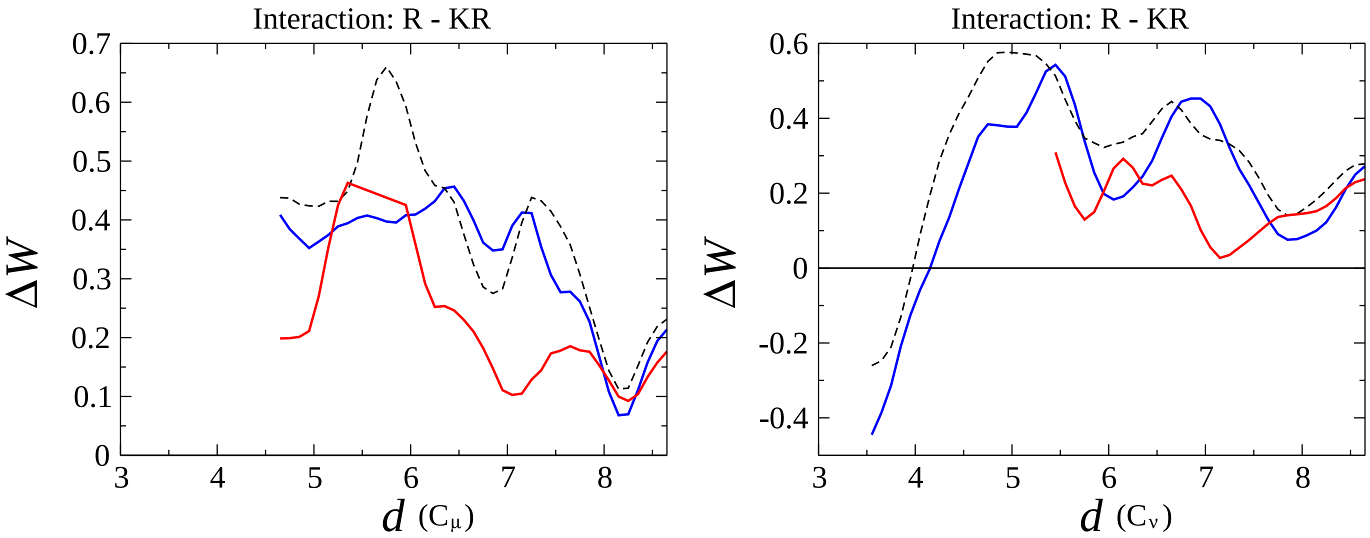
S48)


Interaction: Y - AILV
ΔW
d (Cμ)
Interaction: Y - AILV
ΔW
d (Cν)

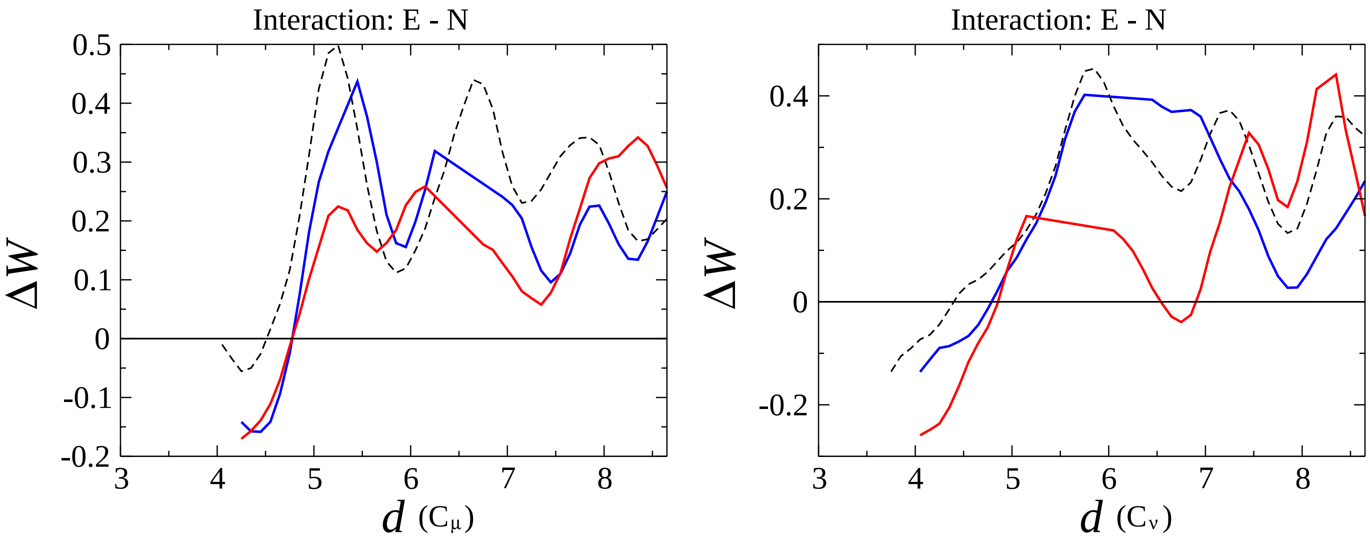
S49)


Interaction: Y - V
ΔW
d (Cμ)
Interaction: Y - V
ΔW
d (Cν)

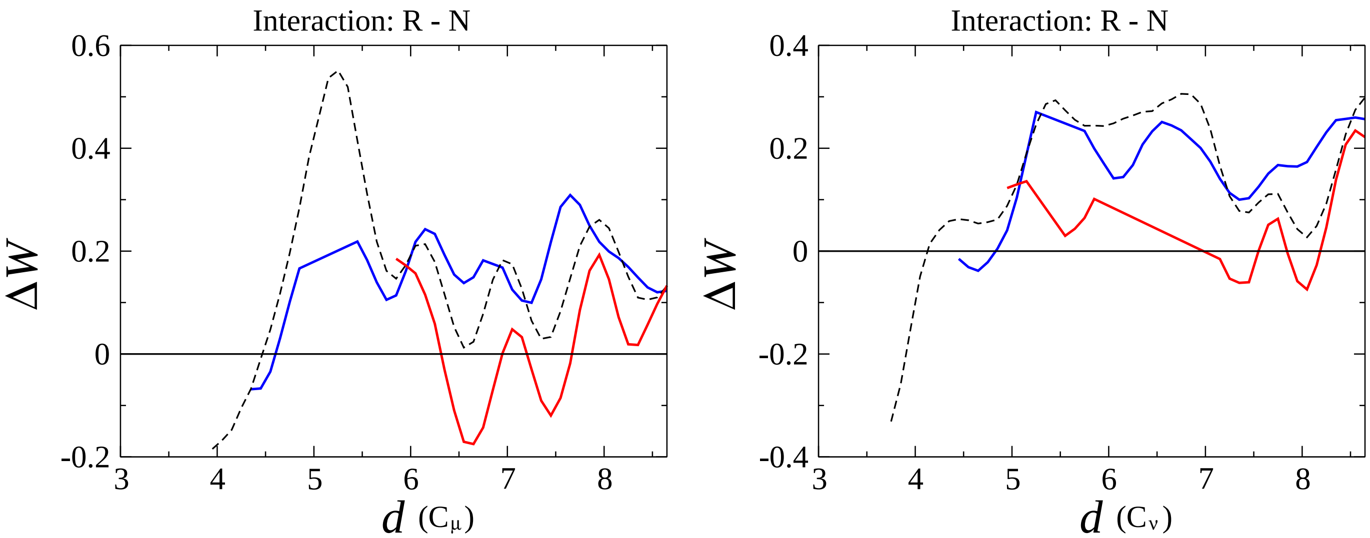
S50)


Interaction: R - KR
ΔW
d (Cμ)
Interaction: R - KR
ΔW
d (Cν)

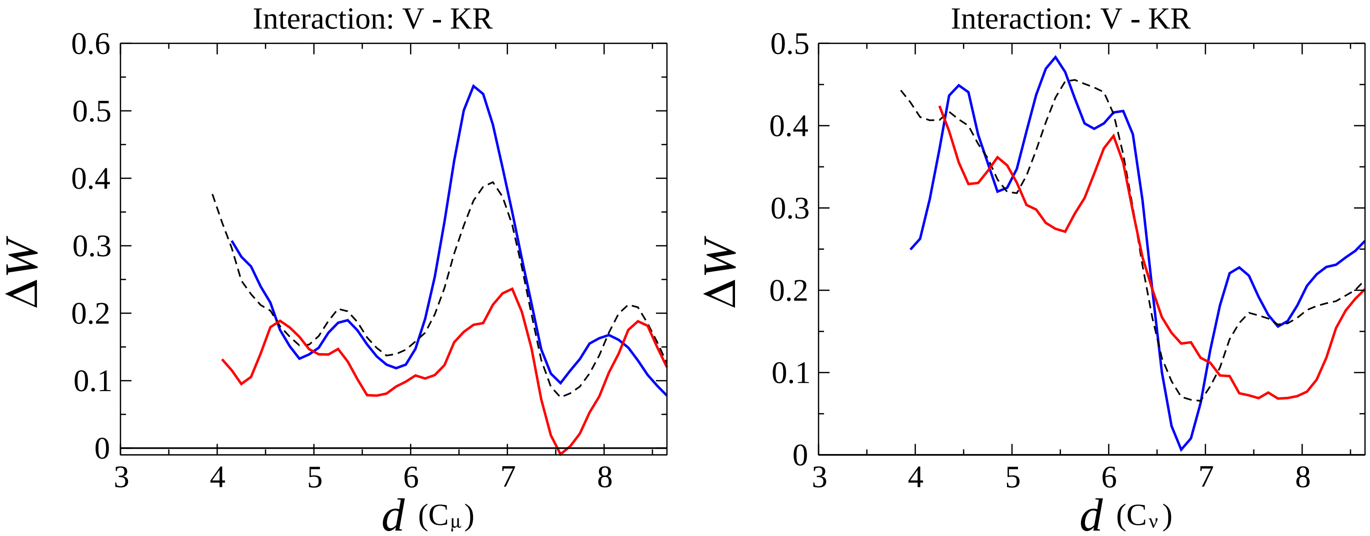
S51)


Interaction: E - N
ΔW
d (Cμ)
Interaction: E - N
ΔW
d (Cν)

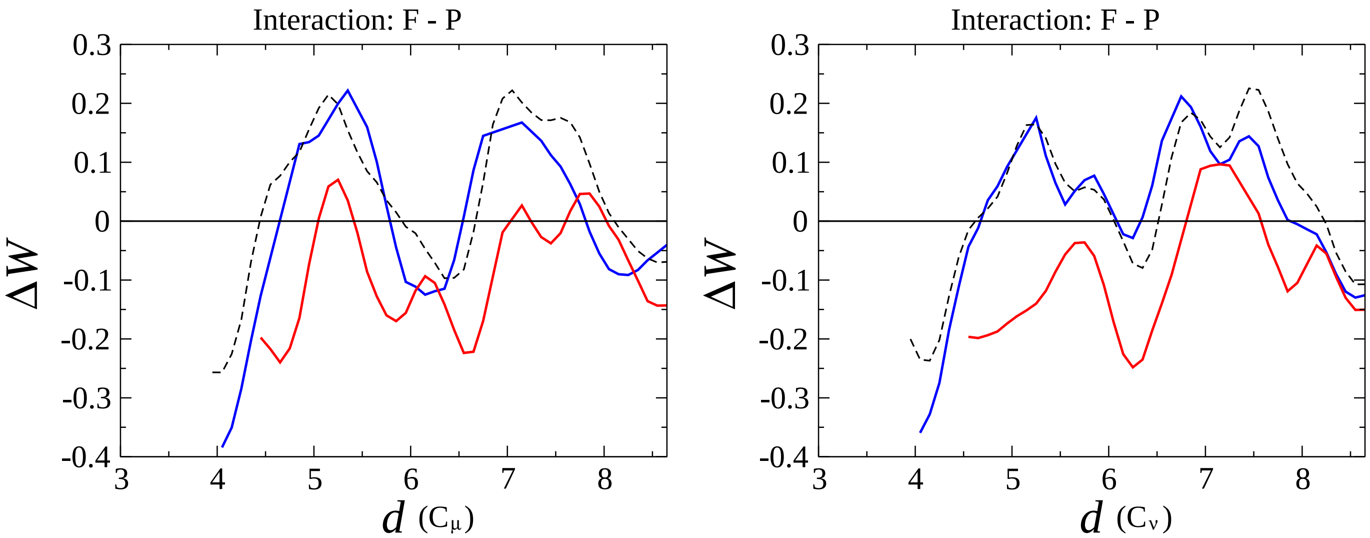
S52)


Interaction: R - N
ΔW
d (Cμ)
Interaction: R - N
ΔW
d (Cν)

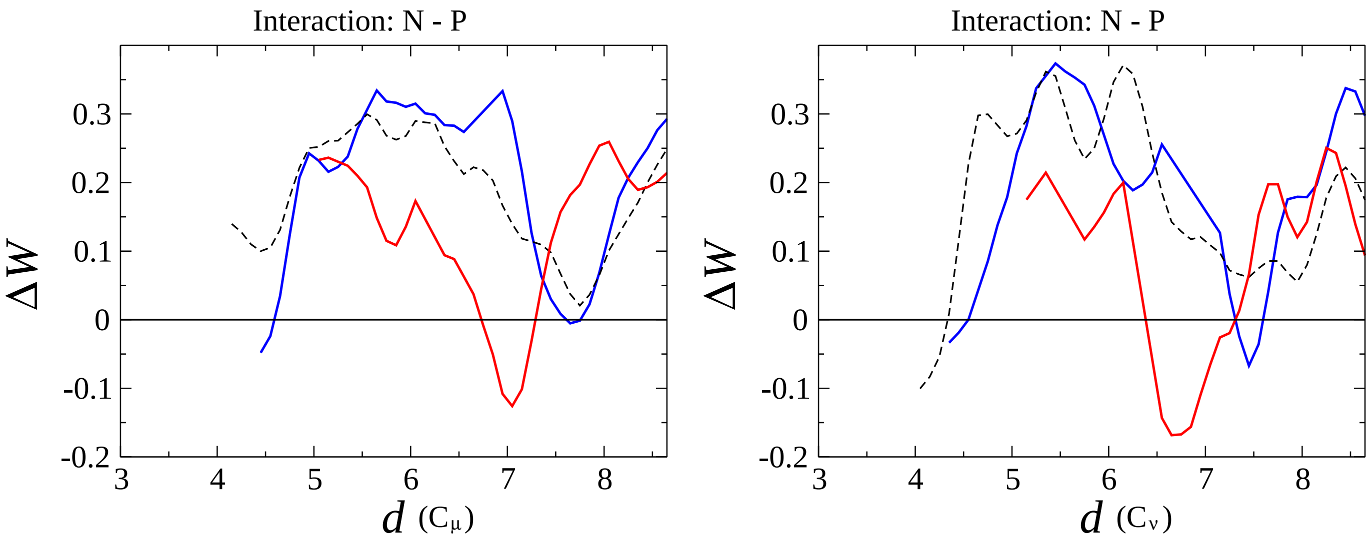
S53)


Interaction: V - KR
ΔW
d (Cμ)
Interaction: V - KR
ΔW
d (Cν)

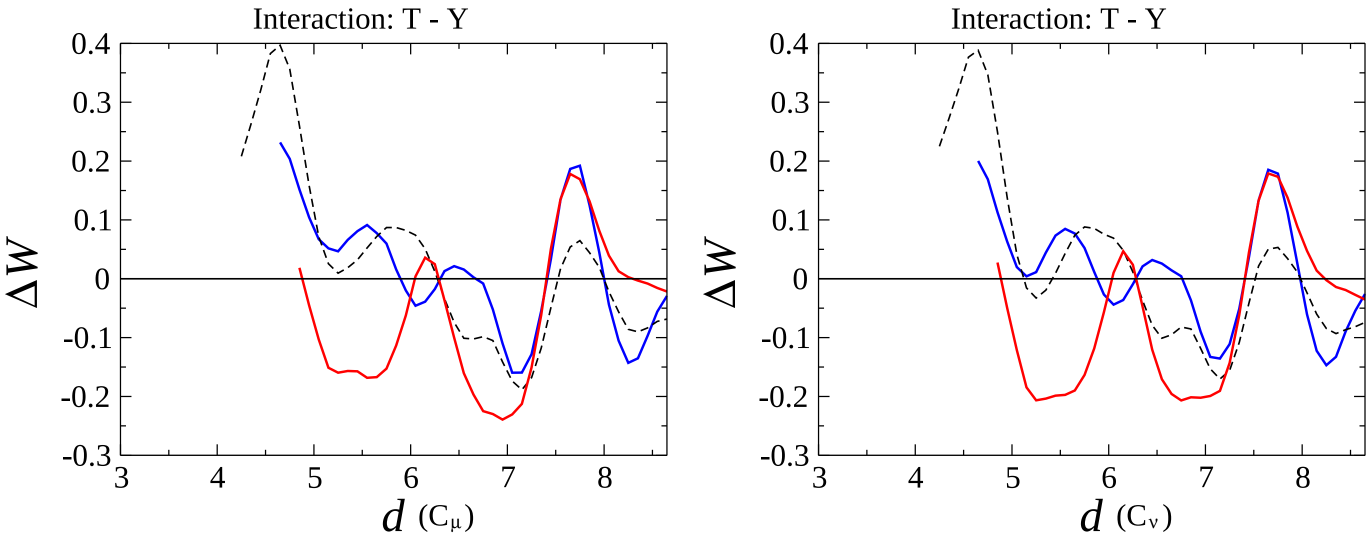
S54)


Interaction: F - P
ΔW
d (Cμ)
Interaction: F - P
ΔW
d (Cν)

S55)


Interaction: N - P
ΔW
d (Cμ)
Interaction: N - P
ΔW
d (Cν)

S56)

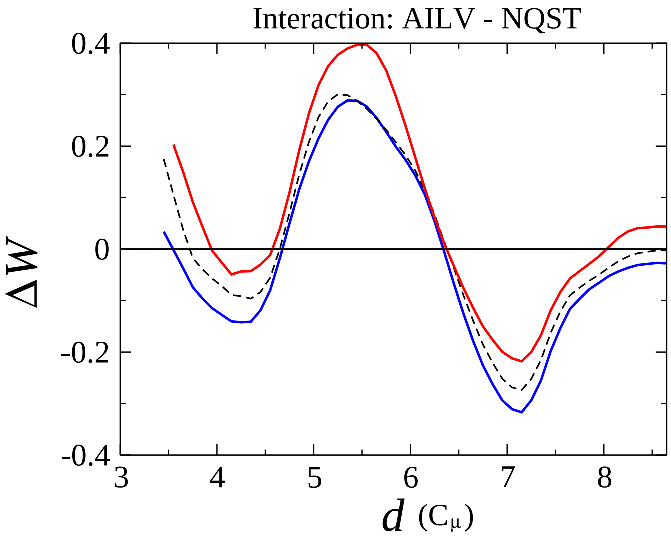


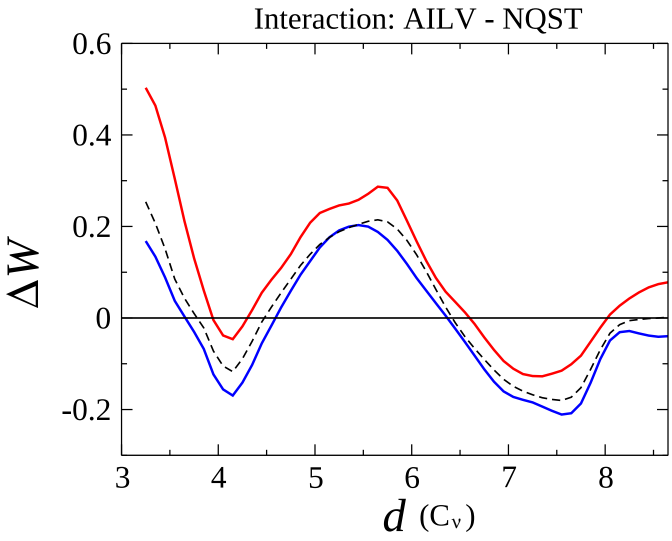
Interaction: T - Y
ΔW
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
3
4
5
6
7
8
d (Cμ)
Interaction: T - Y
ΔW
d (Cν)

Figures S57-S86 - Mesostabilizing interaction potentials

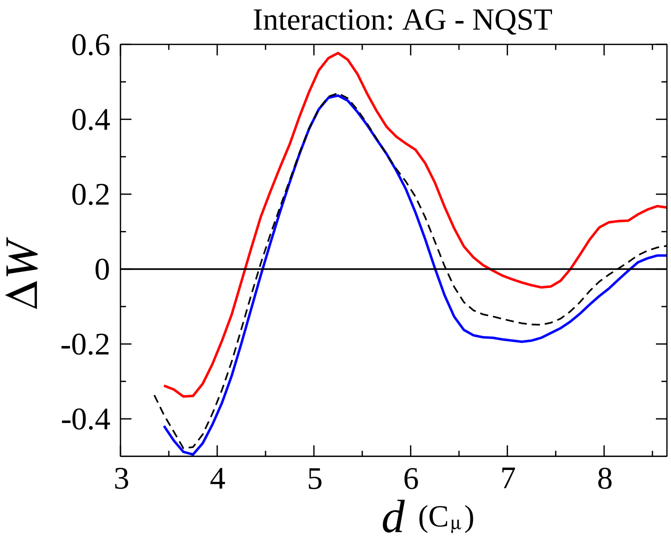
Folding free energy contribution ΔW (kcal/mol) as a function of the distance d between side chain descriptors for the thermoresistant protein subset (red), the mesoresistant one (blue), and the reference set (dashed line). Left plots are based on C_μ descriptors and right plots on the C_ν descriptors.

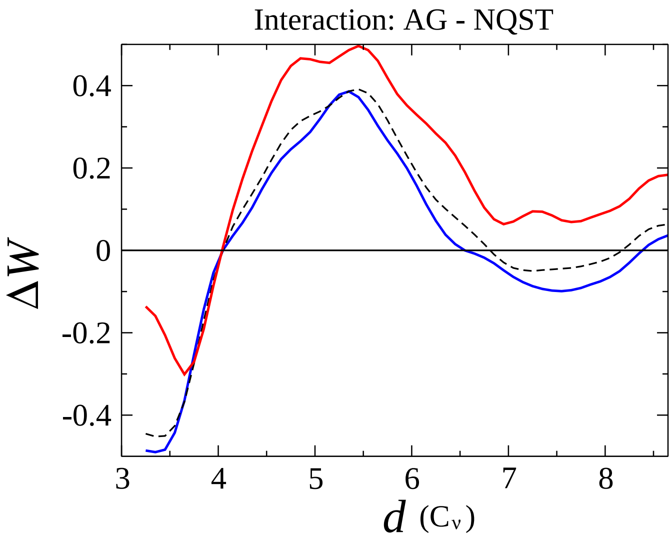
S57)



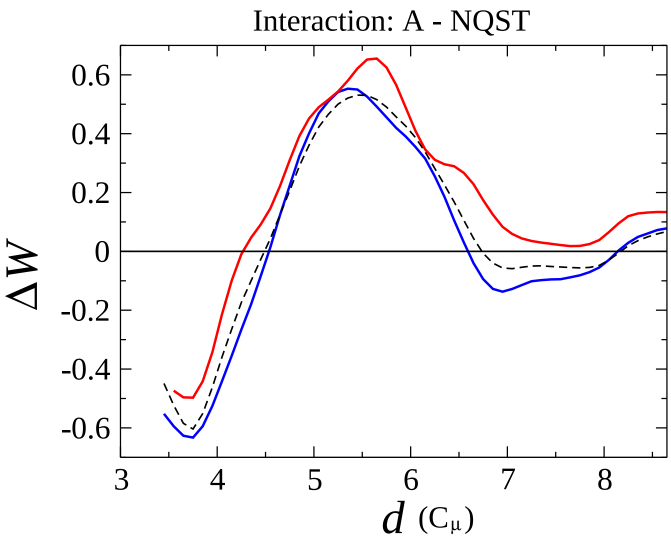


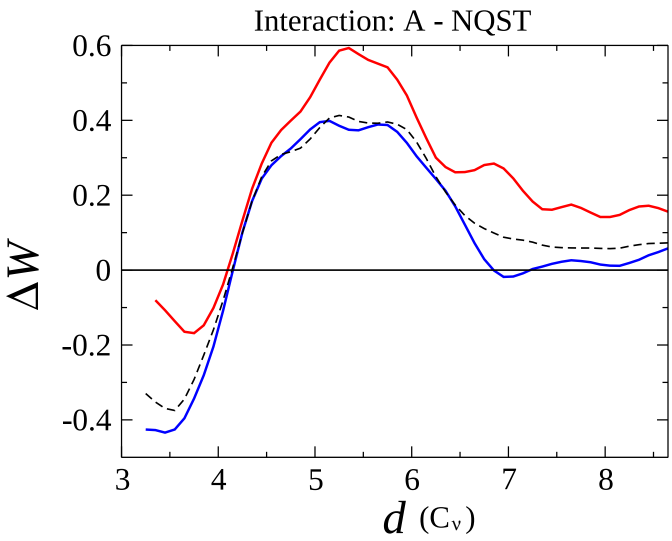
S58)



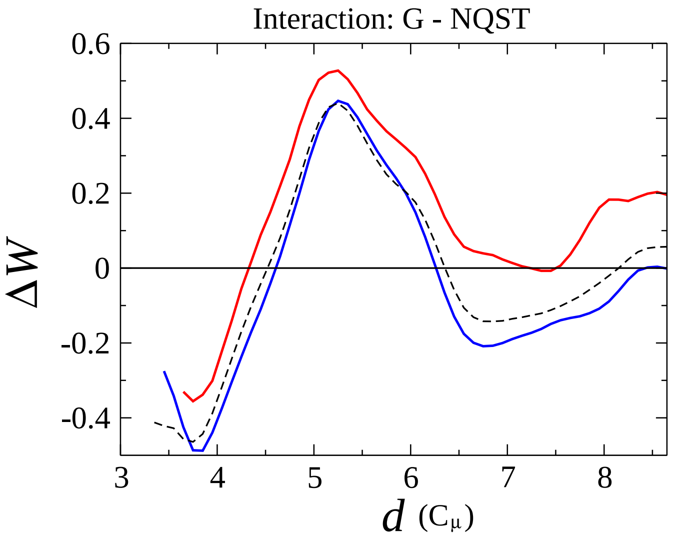


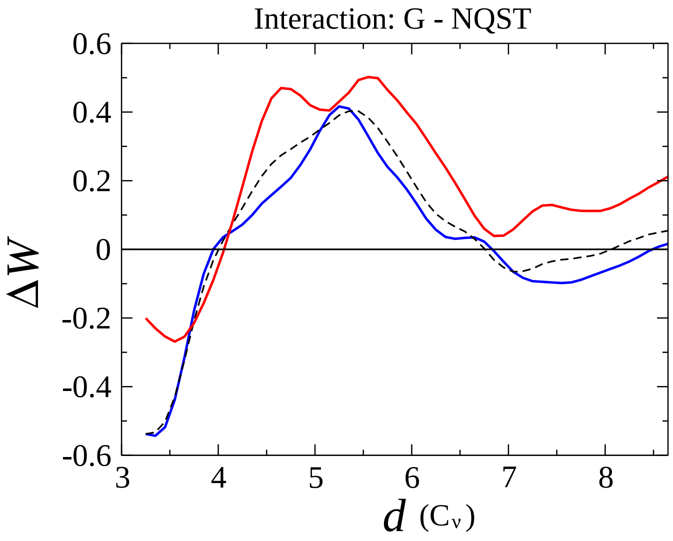
S59)



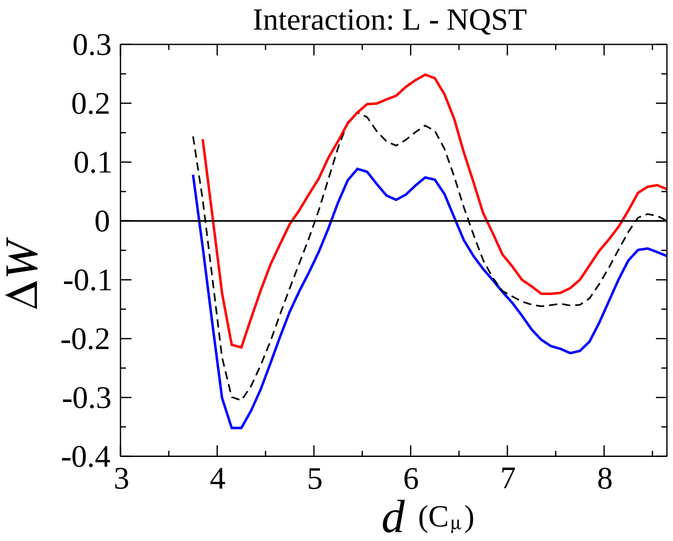


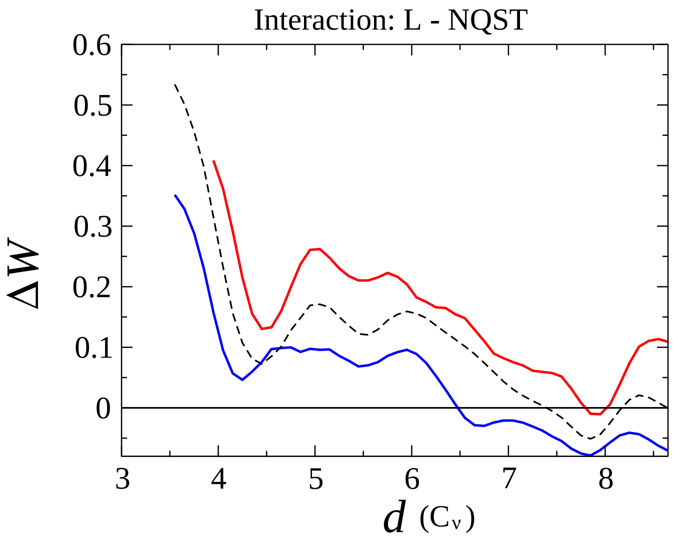
S60)


Interaction: G - NQST
ΔW
d (Cμ)

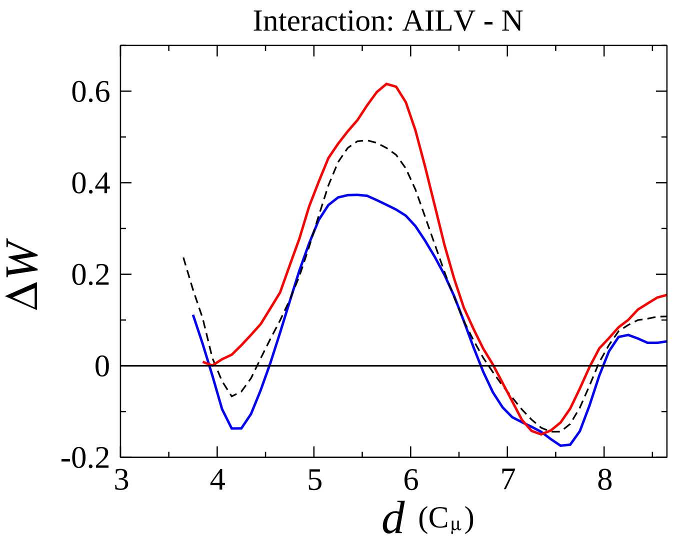

Interaction: G - NQST
ΔW
d (Cν)

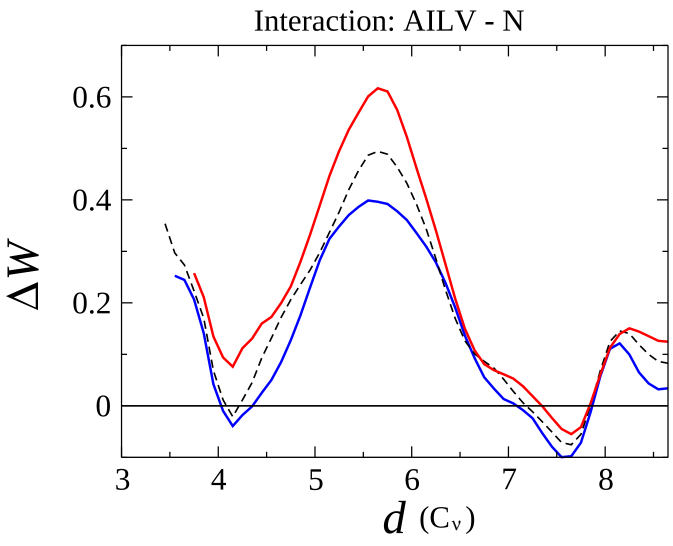
S61)


Interaction: L - NQST
ΔW
d (Cμ)

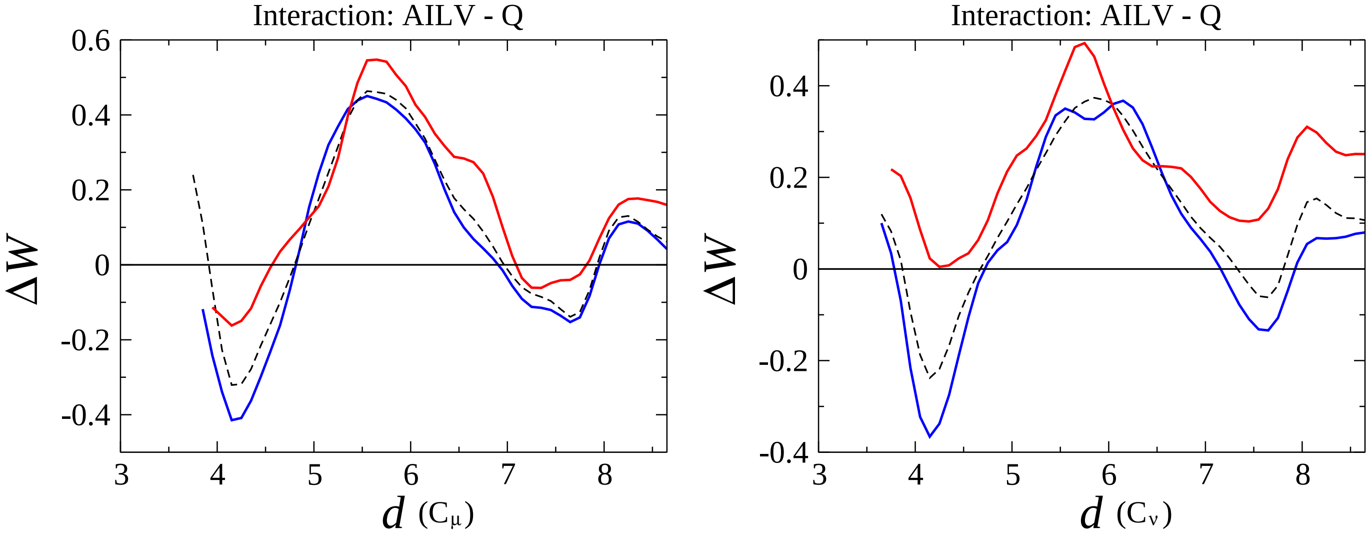

Interaction: L - NQST
ΔW
d (Cν)

S62)

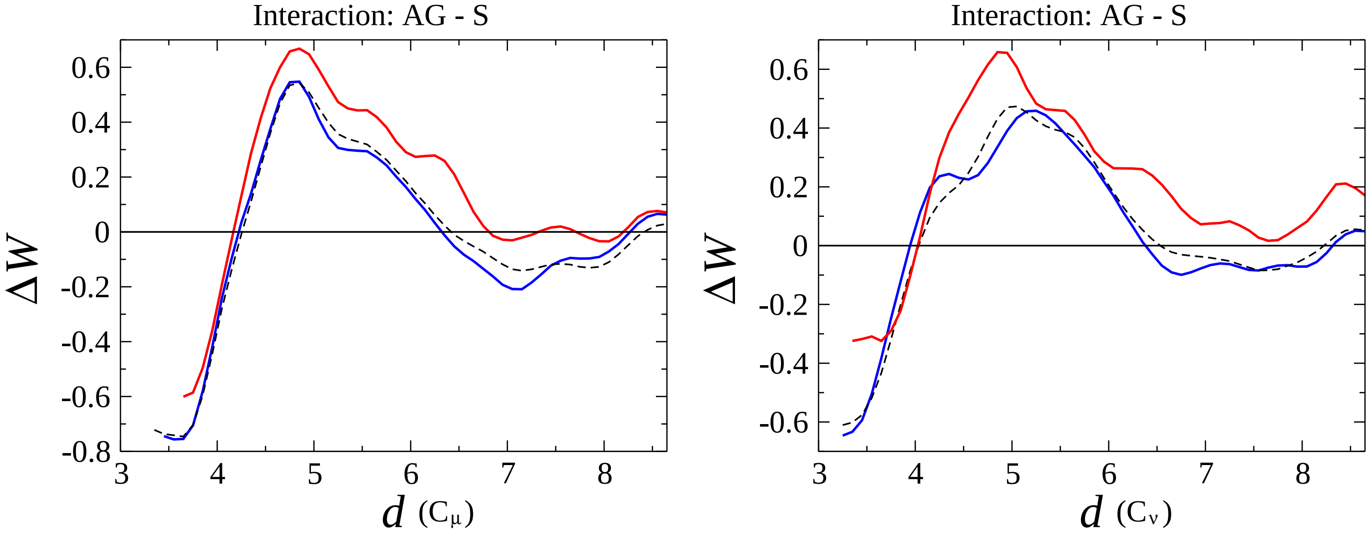

Interaction: AILV - N
ΔW
d (Cμ)


Interaction: AILV - N
ΔW
d (Cν)

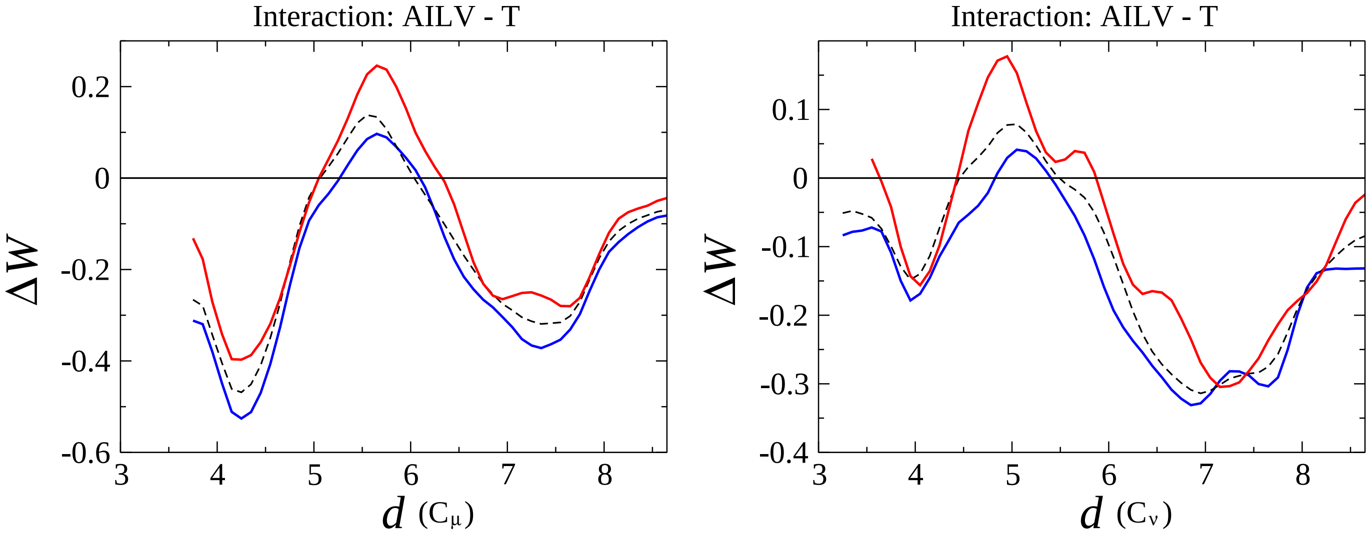
S63)


Interaction: AILV - Q
ΔW
d (Cμ)
Interaction: AILV - Q
ΔW
d (Cν)

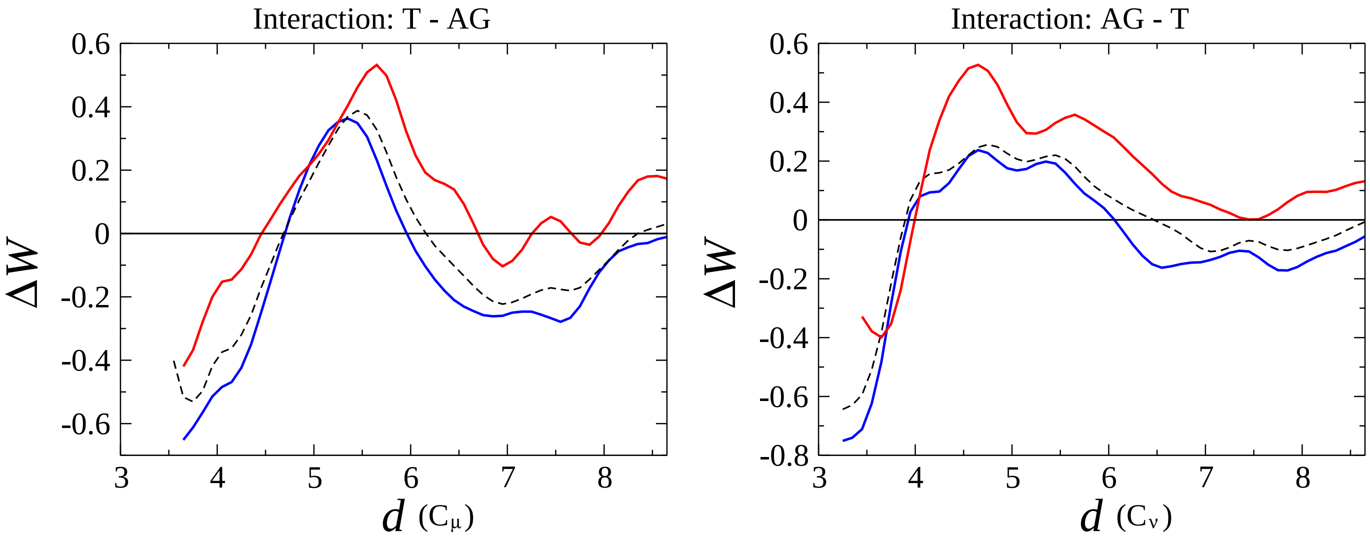
S64)


Interaction: AG - S
ΔW
d (Cμ)
Interaction: AG - S
ΔW
d (Cν)

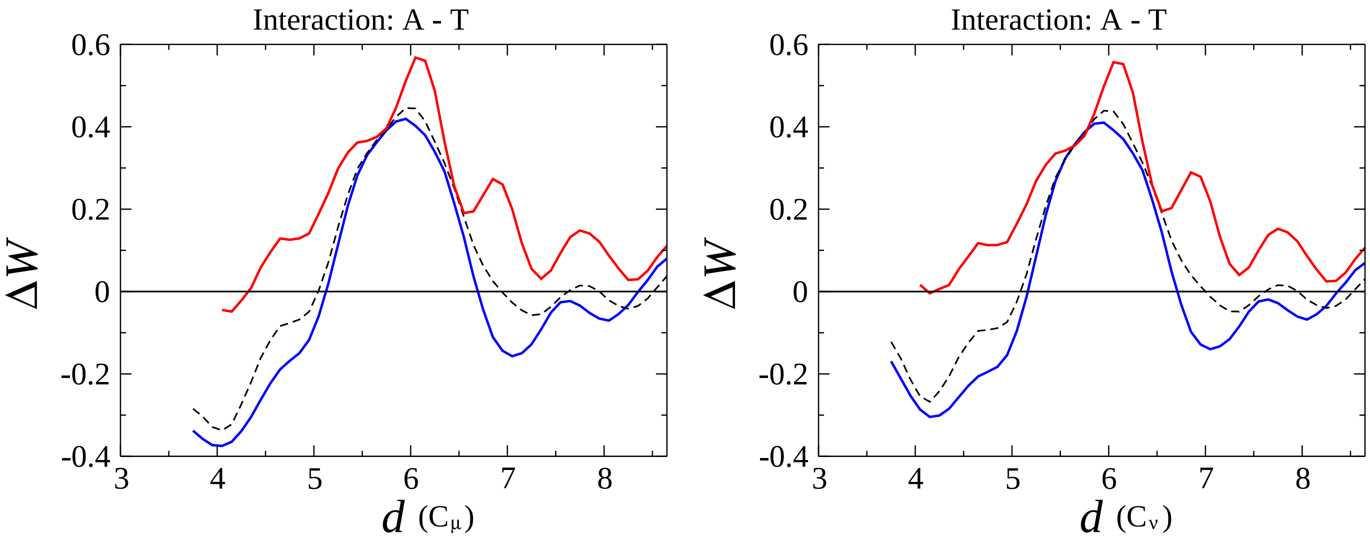
S65)


Interaction: AILV - T
ΔW
d (Cμ)
Interaction: AILV - T
ΔW
d (Cν)

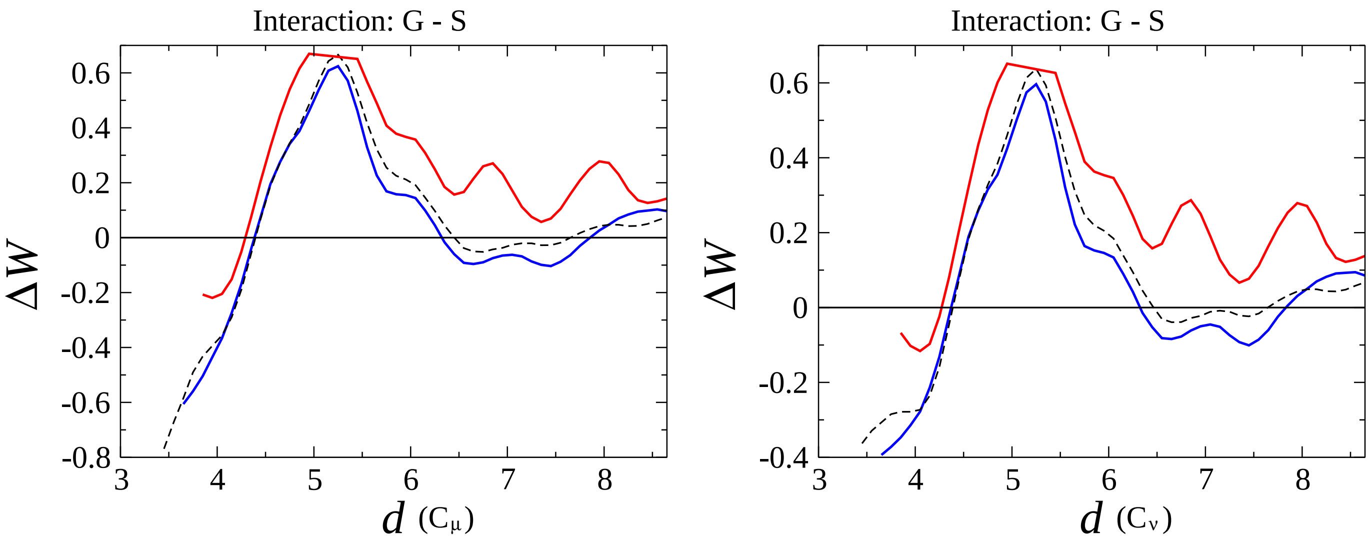
S66)


Interaction: T - AG
Interaction: AG - T
ΔW
d (Cμ)
d (Cν)

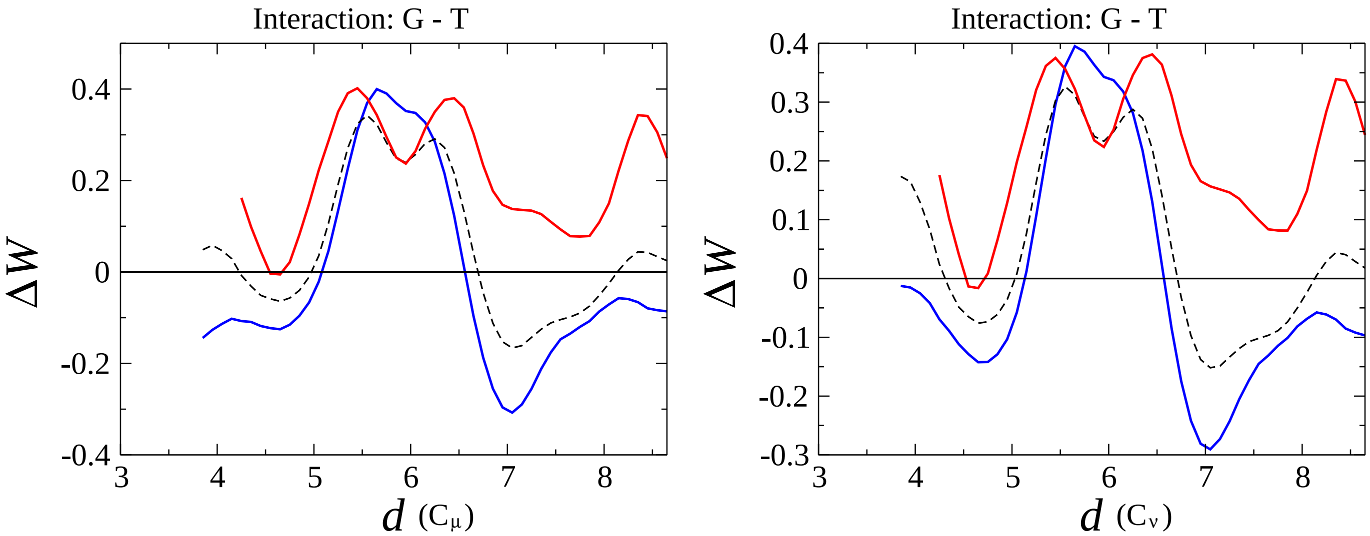
S67)


Interaction: A - T
Interaction: A - T
ΔW
d (Cμ)
d (Cν)

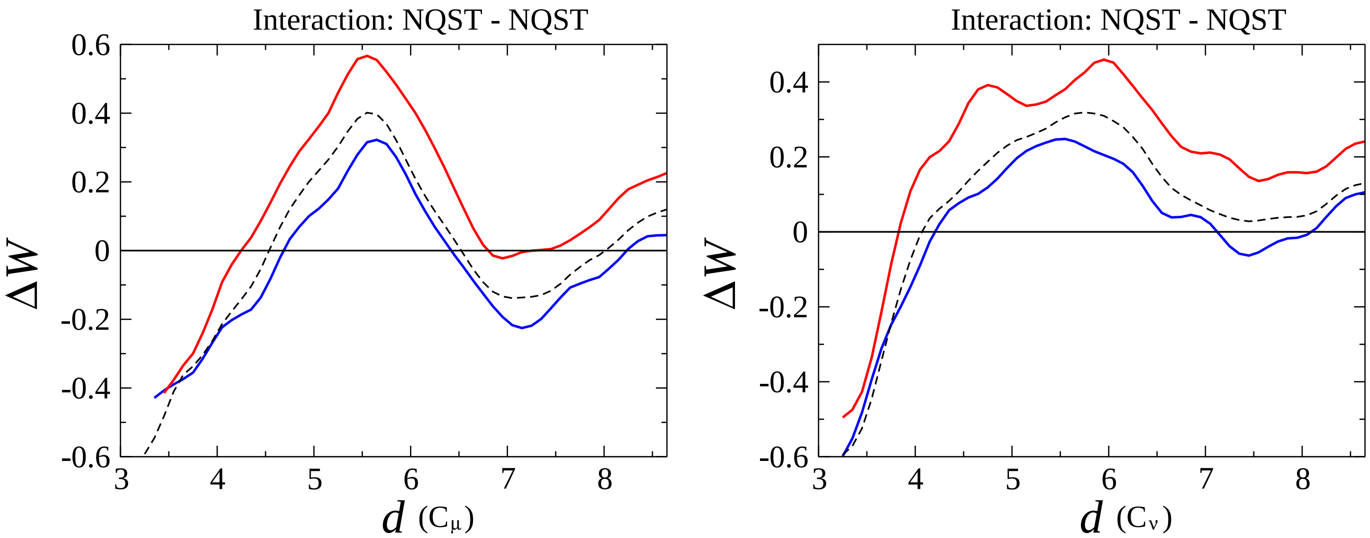
S68)


Interaction: G - S
Interaction: G - S
ΔW
d (Cμ)
d (Cν)

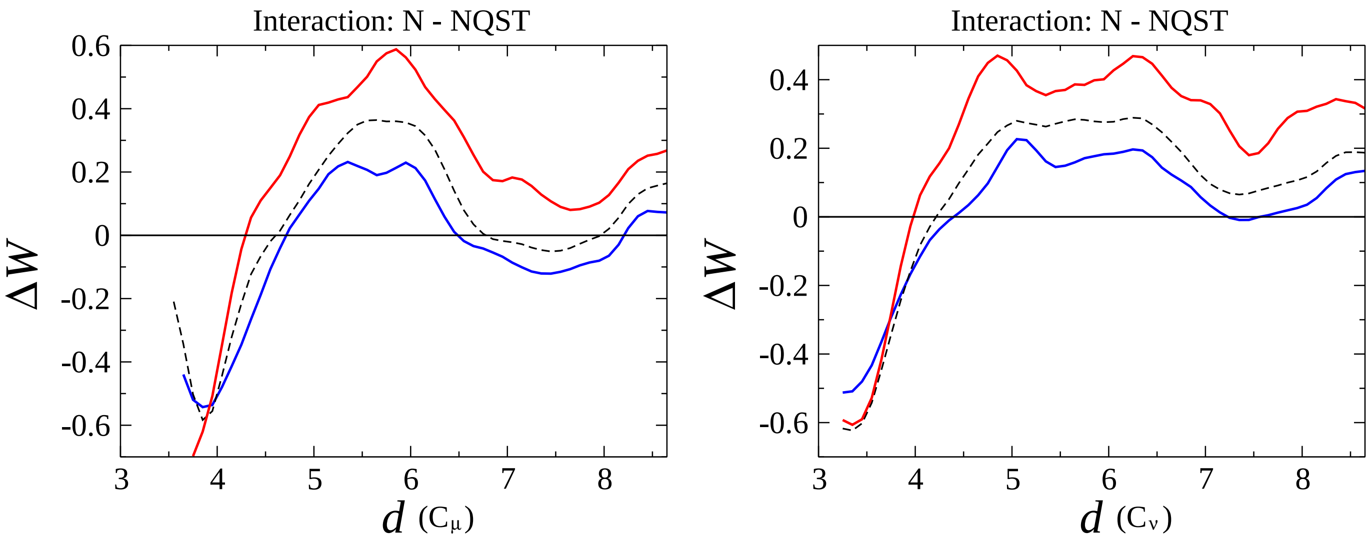
S69)


Interaction: G - T
ΔW
d (Cμ)
Interaction: G - T
ΔW
d (Cν)

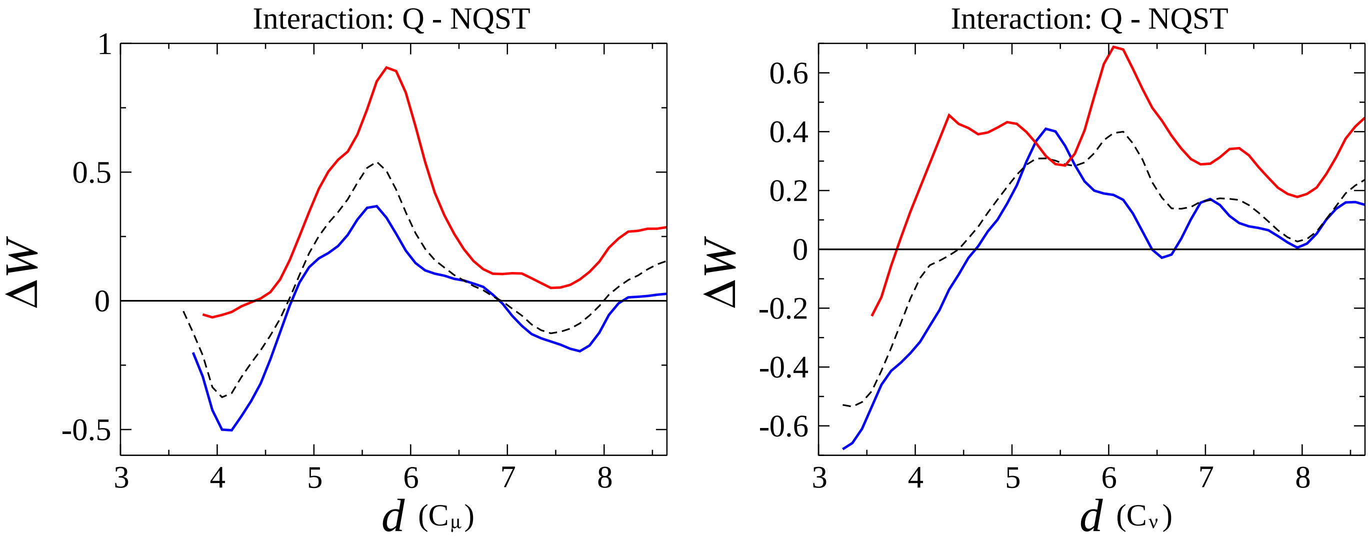
S70)


Interaction: NQST - NQST
ΔW
d (Cμ)
Interaction: NQST - NQST
ΔW
d (Cν)

S71)

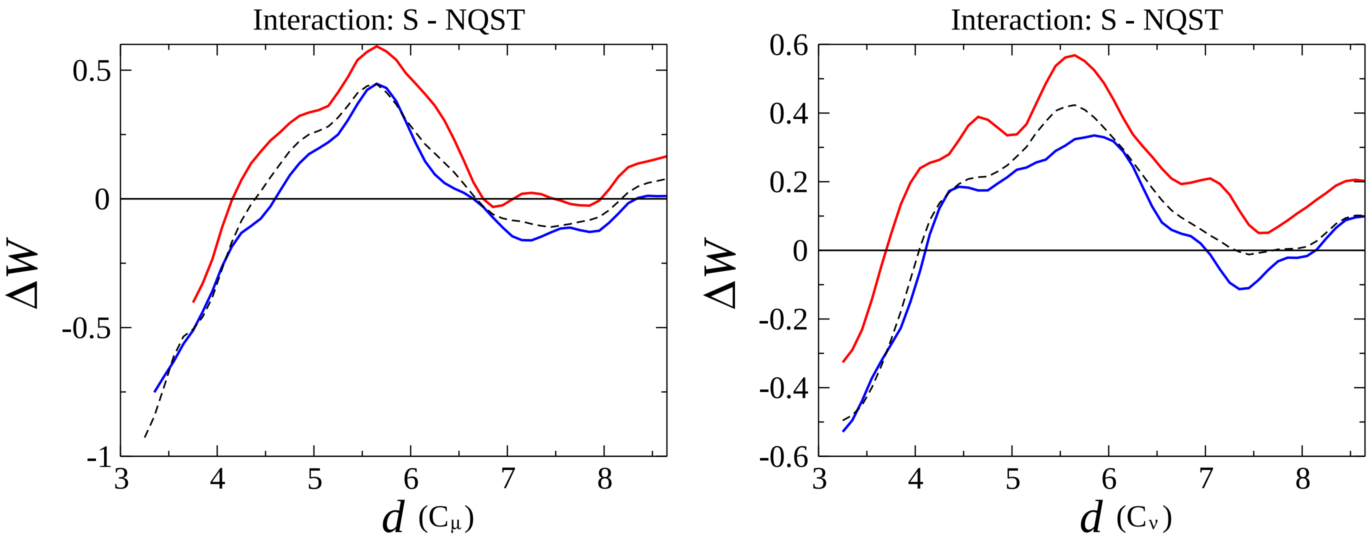

Interaction: N - NQST
ΔW
d (Cμ)
Interaction: N - NQST
ΔW
d (Cν)

S72)



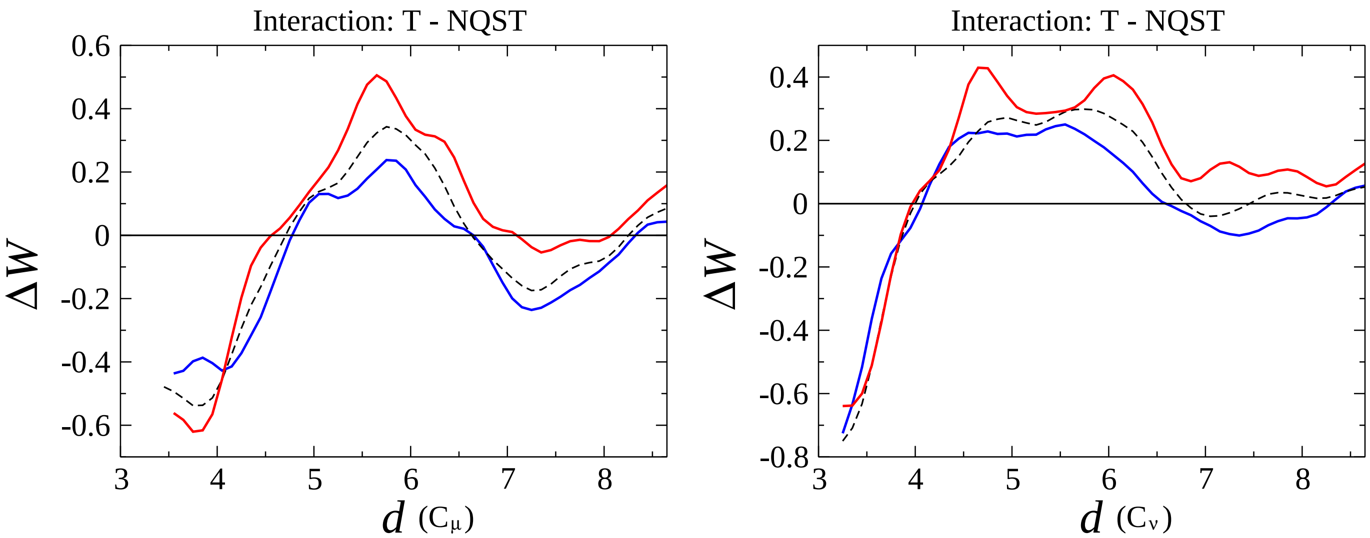
Interaction: Q - NQST
Interaction: Q - NQST
ΔW
d (Cμ)
d (Cν)

S73)



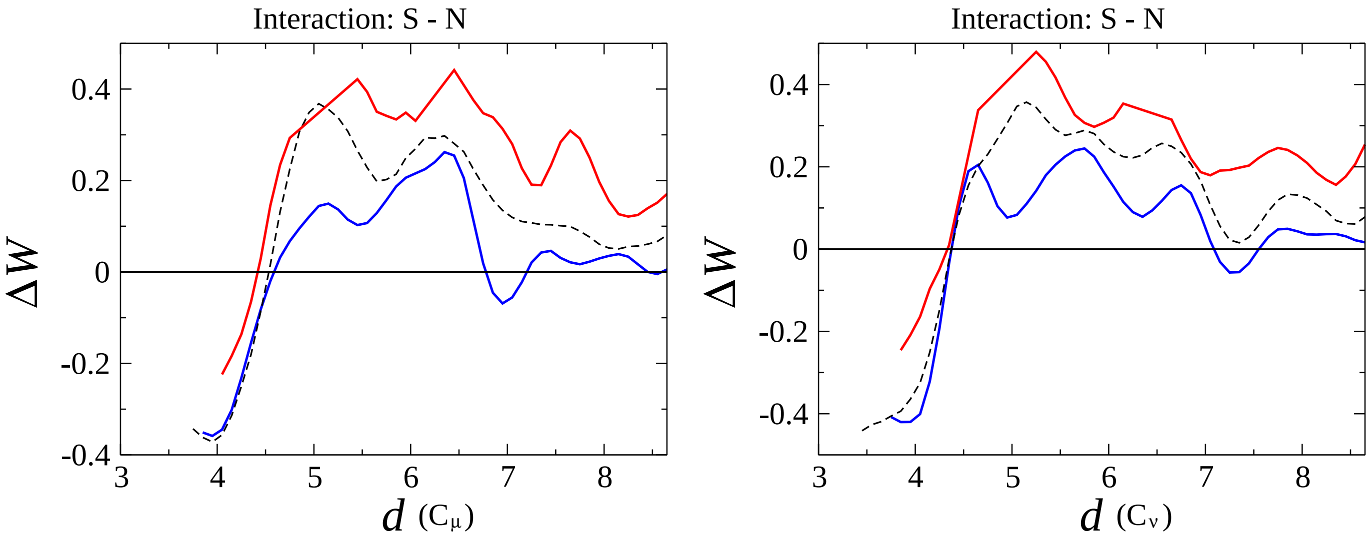
Interaction: S - NQST
Interaction: S - NQST
ΔW
d (Cμ)
d (Cν)

S74)

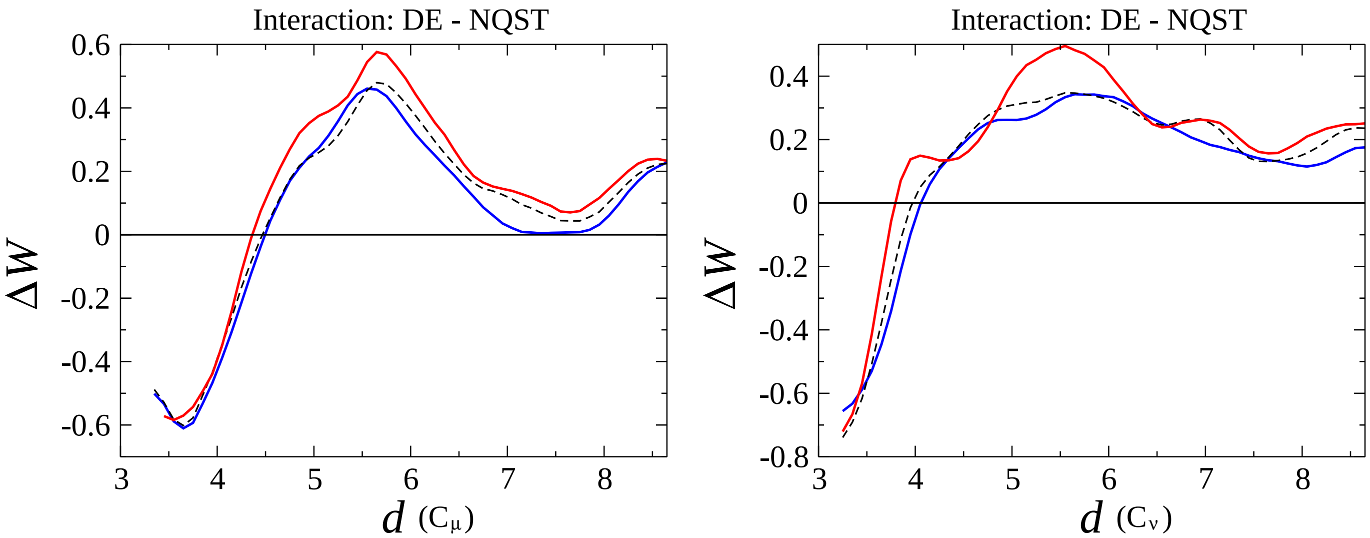


Interaction: T - NQST
Interaction: T - NQST
ΔW
d (Cμ)
d (Cν)

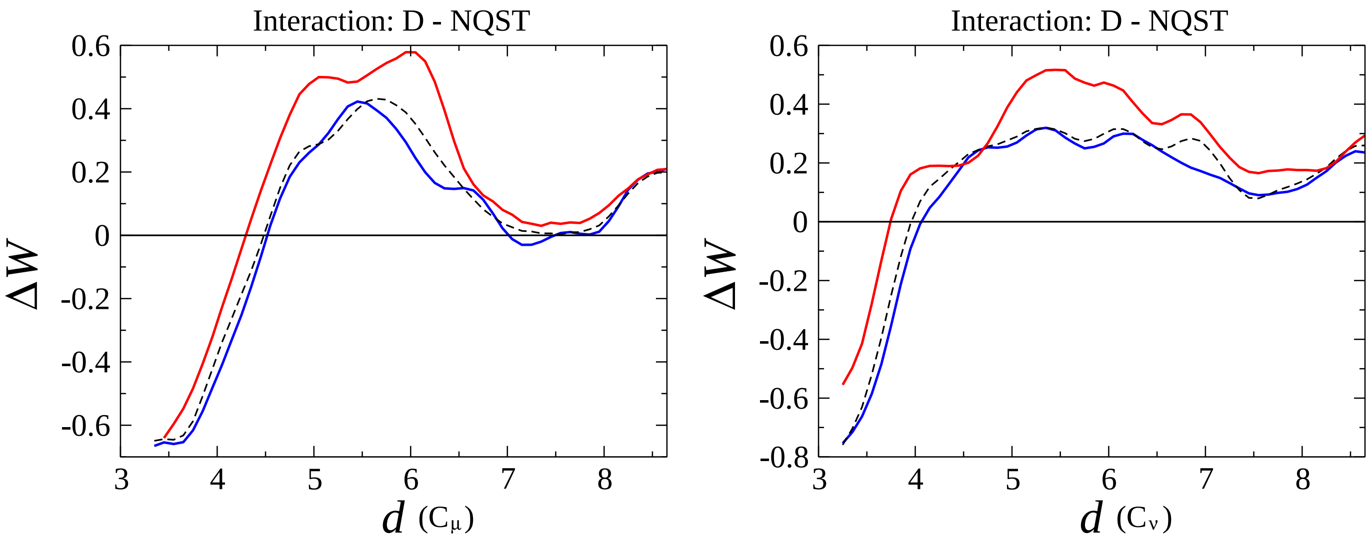
S75)


Interaction: S - N
Interaction: S - N
ΔW
ΔW
d (Cμ)
d (Cν)

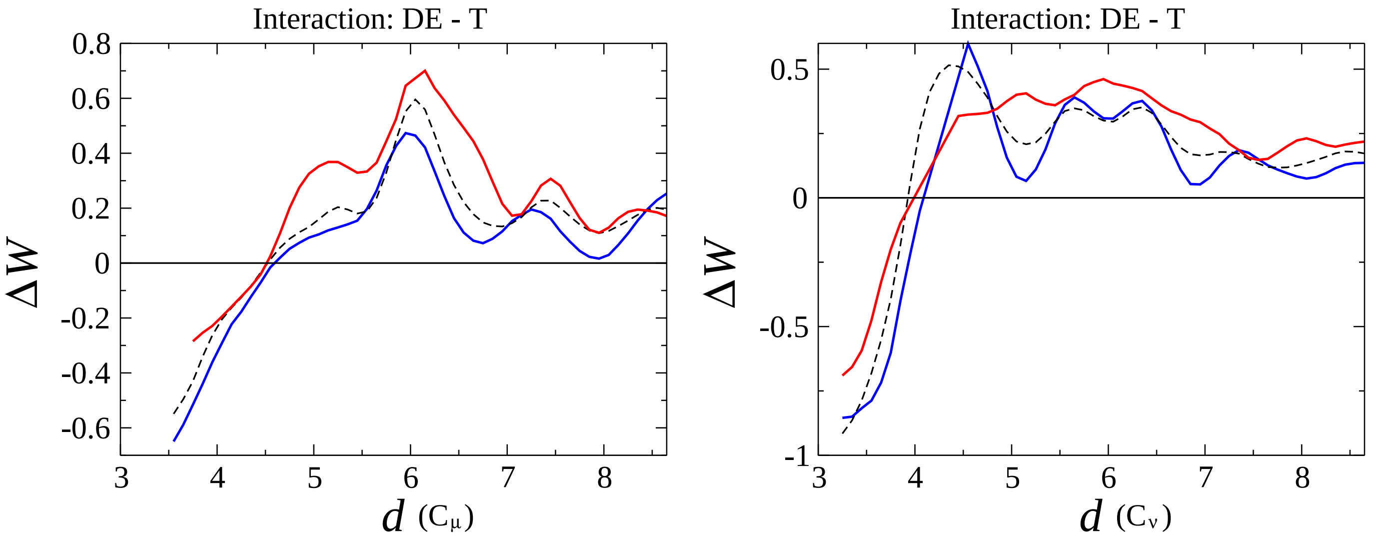
S76)


Interaction: DE - NQST
Interaction: DE - NQST
ΔW
ΔW
d (Cμ)
d (Cν)

S77)

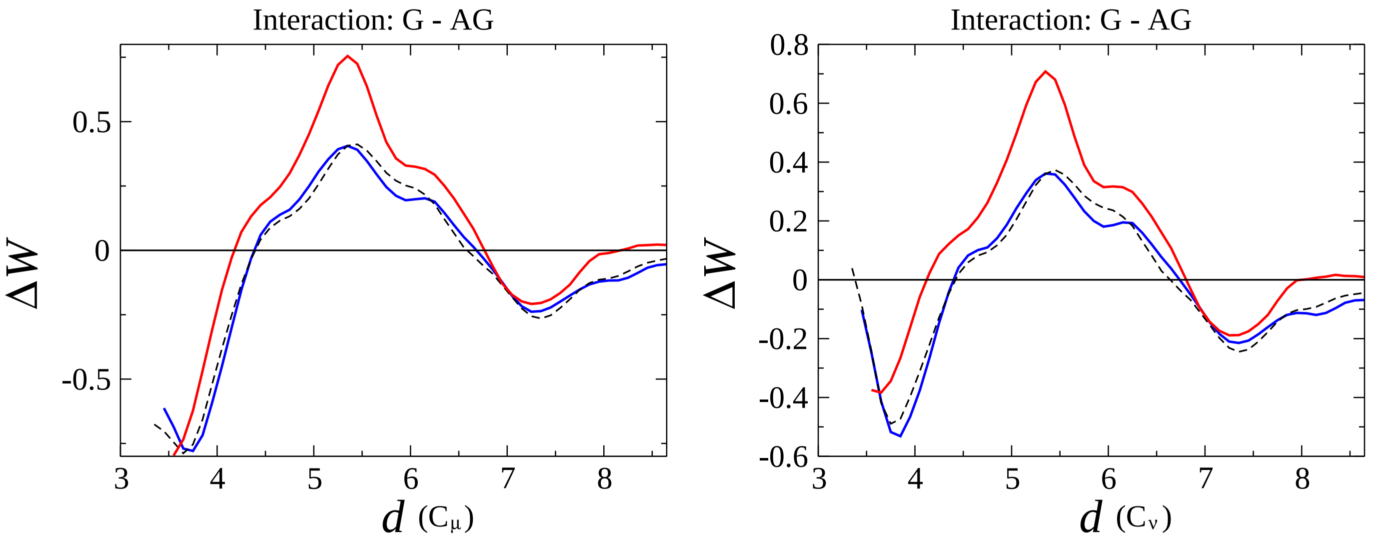

Interaction: D - NQST
Interaction: D - NQST
ΔW
ΔW
d (Cμ)
d (Cν)

S78)



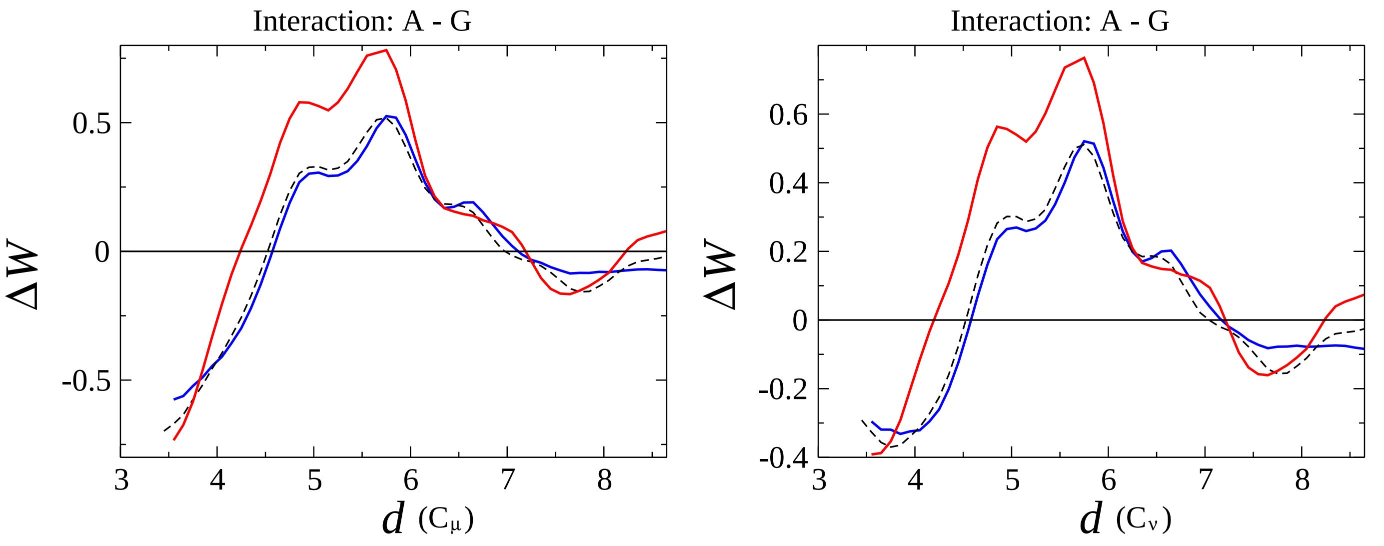
Interaction: DE - T
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
3
4
5
6
7
8
ΔW
d (Cμ)
Interaction: DE - T
0.5
0
-0.5
-1
ΔW
d (Cν)

S79)



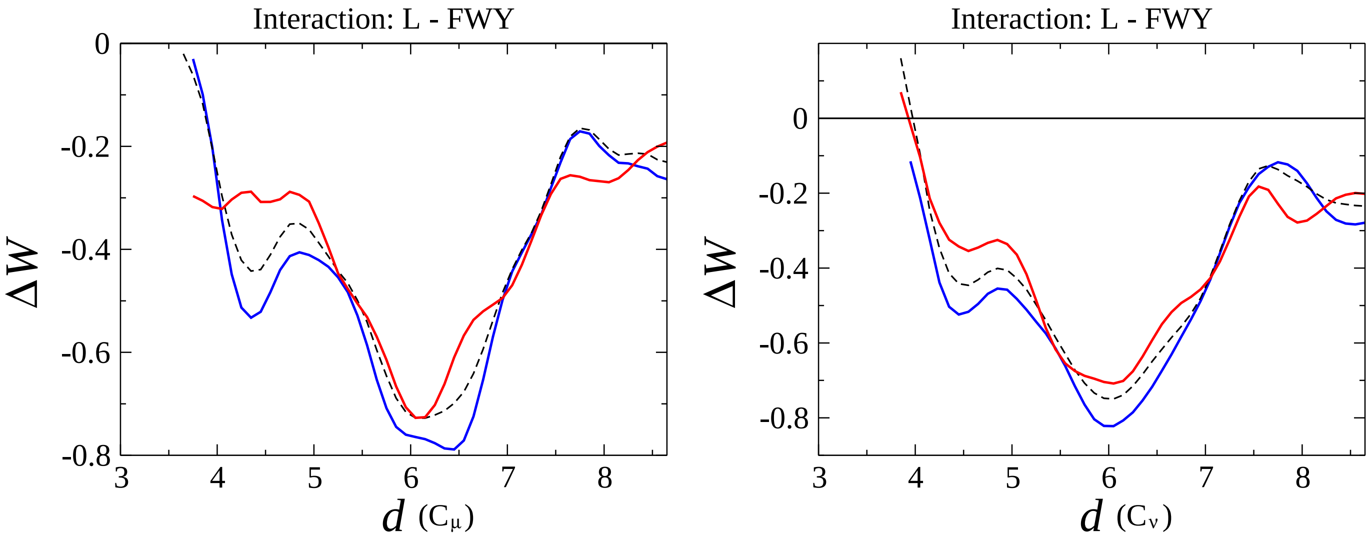
Interaction: G - AG
0.5
0
-0.5
3
4
5
6
7
8
ΔW
d (Cμ)
Interaction: G - AG
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
ΔW
d (Cν)

S80)

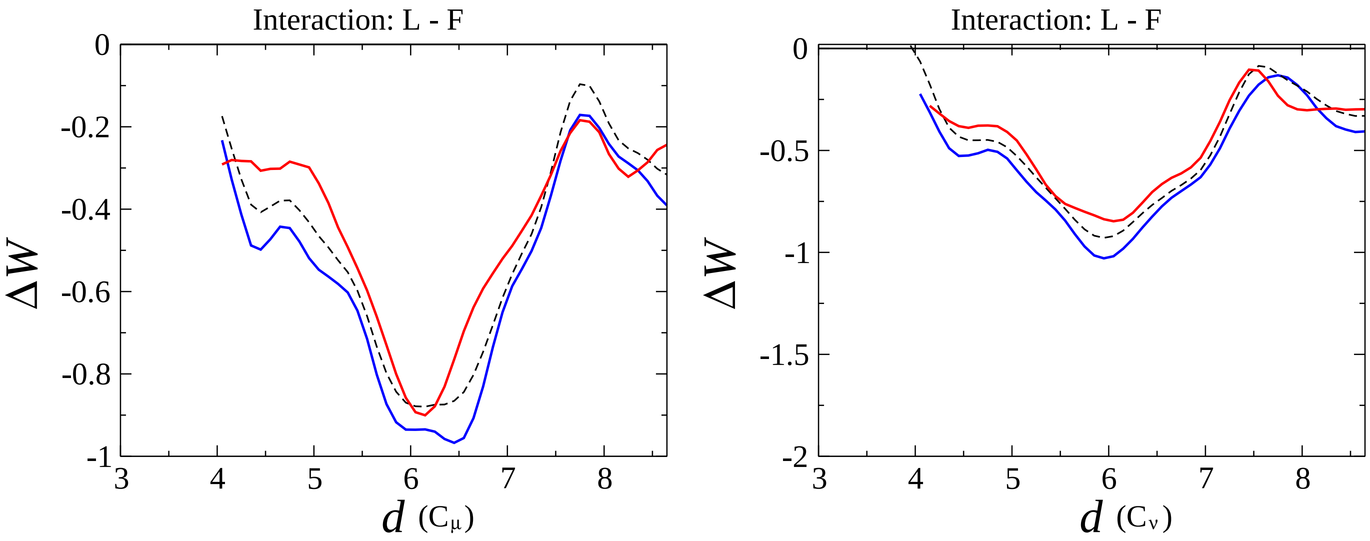


Interaction: A - G
0.5
0
-0.5
3
4
5
6
7
8
ΔW
d (Cμ)
Interaction: A - G
0.6
0.4
0.2
0
-0.2
-0.4
ΔW
d (Cν)

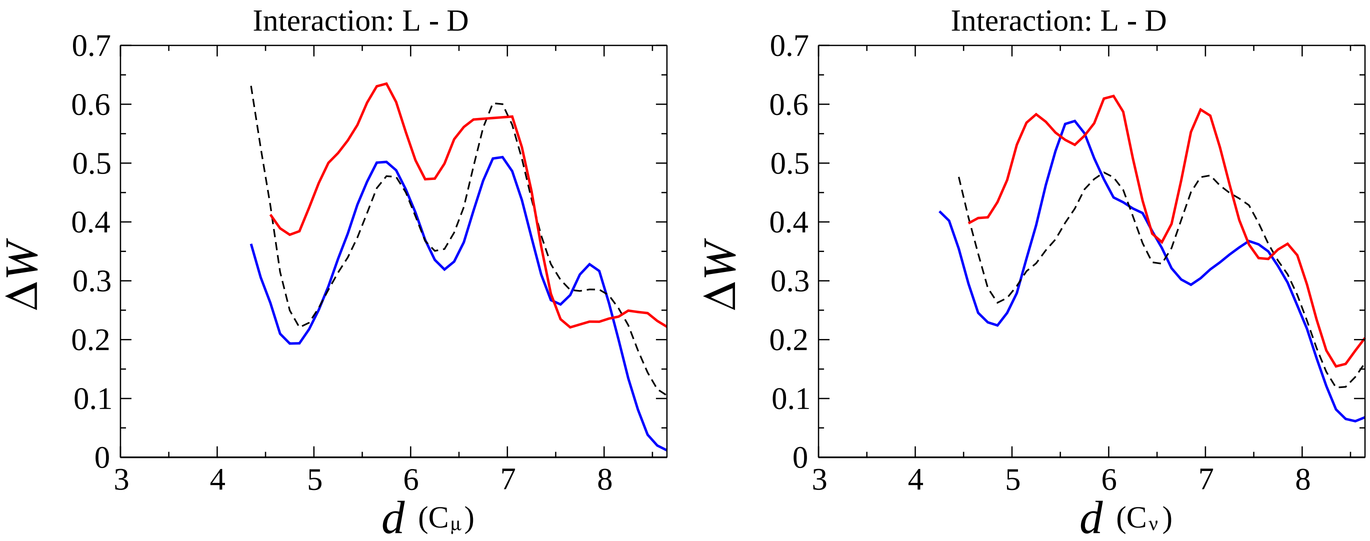
S81)


Interaction: L - FWY
ΔW
d (Cμ)
Interaction: L - FWY
ΔW
d (Cν)

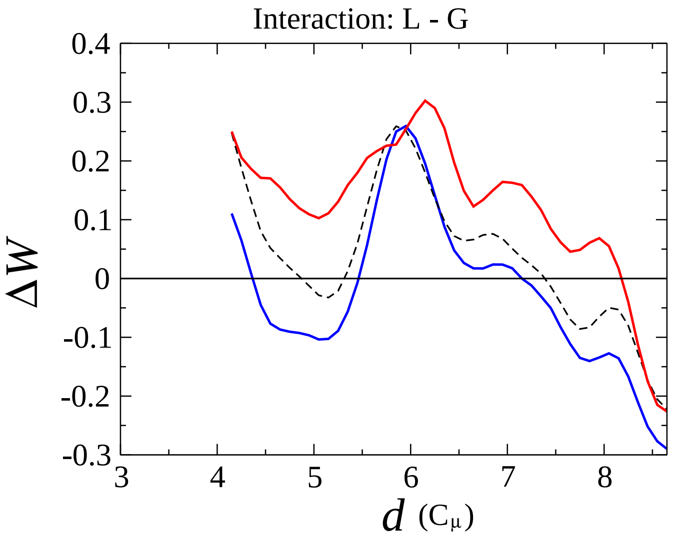
S82)

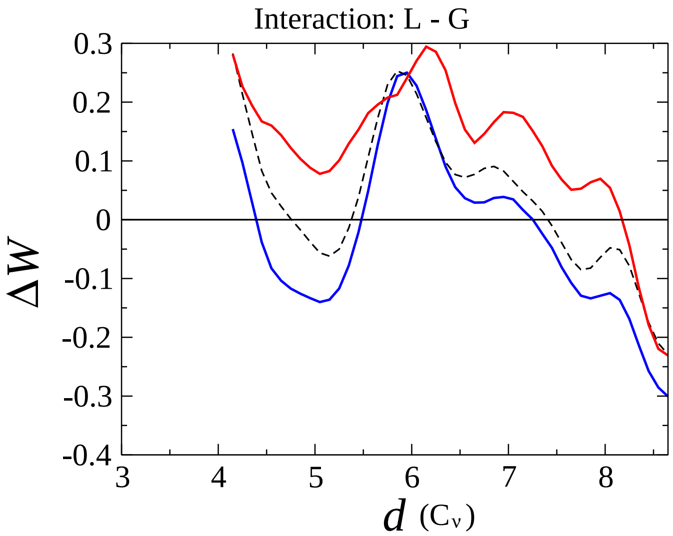

Interaction: L - F
ΔW
d (Cμ)
Interaction: L - F
ΔW
d (Cν)

S83)

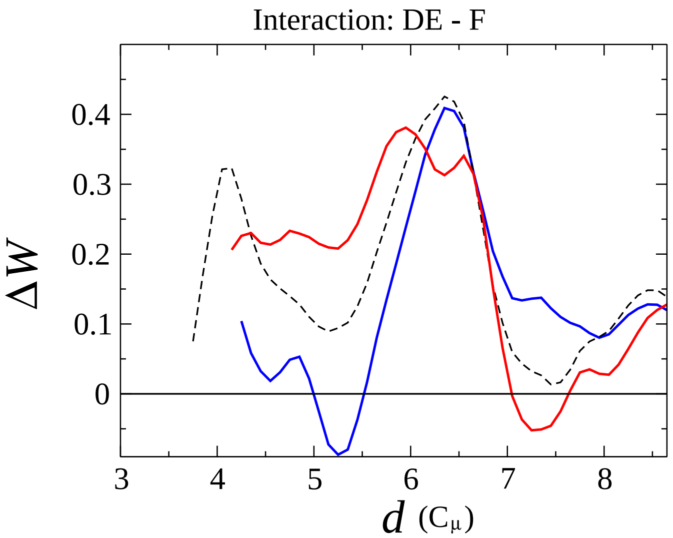

Interaction: L - D
ΔW
d (Cμ)
Interaction: L - D
ΔW
d (Cν)

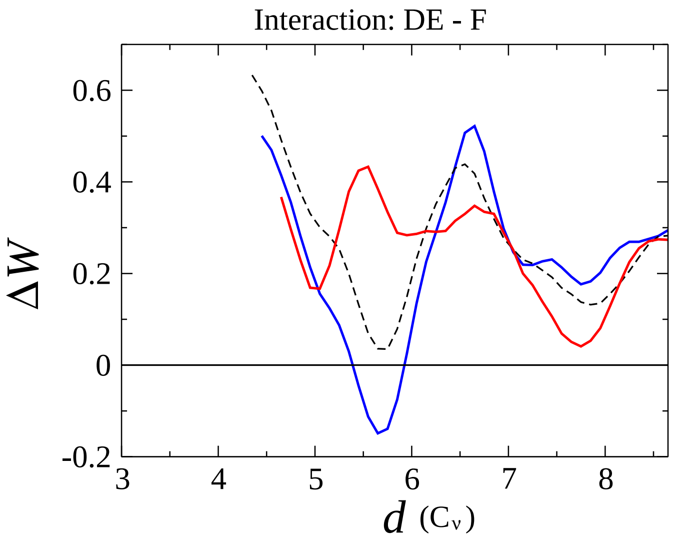
S84)


Interaction: L - G
ΔW
d (Cμ)

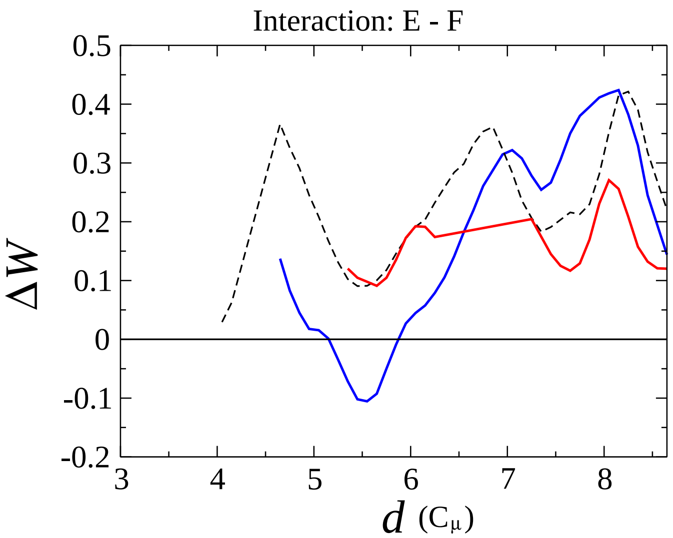

Interaction: L - G
ΔW
d (Cν)

S85)


Interaction: DE - F
ΔW
d (Cμ)


Interaction: DE - F
ΔW
d (Cν)

S86)


Interaction: E - F
ΔW
d (Cμ)

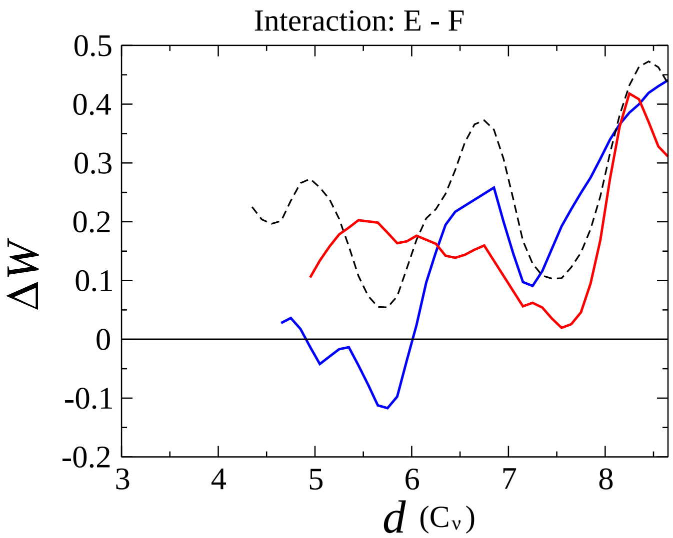

Interaction: E - F
ΔW
d (Cν)

Table S8 - Contact potentials for all thermostabilizing and mesostabilizing interactions given in Tables 1-2

Thermostabilizing interactions													
Type of interaction	Interaction	Contact distance cutoff D =6.0 Å						Contact distance cutoff D =8.0 Å					
		ΔW_{meso} *		ΔW_{thermo} †		$\Delta\Delta W$ ‡		ΔW_{meso} *		ΔW_{thermo} †		$\Delta\Delta W$ ‡	
		C_{μ}	C_{ν}	C_{μ}	C_{ν}	C_{μ}	C_{ν}	C_{μ}	C_{ν}	C_{μ}	C_{ν}	C_{μ}	C_{ν}
Salt bridges	DE - KR	-0.10	-0.16	-0.22	-0.28	0.12	0.12	0.03	-0.02	-0.08	-0.10	0.11	0.08
	D - KR	-0.13	-0.16	-0.22	-0.27	0.10	0.11	0.00	-0.04	-0.07	-0.10	0.08	0.05
	E - KR	-0.07	-0.16	-0.22	-0.28	0.15	0.12	0.07	0.00	-0.08	-0.11	0.15	0.12
	DE - R	-0.14	-0.21	-0.30	-0.36	0.16	0.15	-0.01	-0.05	-0.14	-0.16	0.13	0.11
	D - R	-0.17	-0.22	-0.32	-0.37	0.14	0.14	-0.06	-0.09	-0.15	-0.17	0.09	0.08
	E - R	-0.10	-0.20	-0.29	-0.36	0.19	0.15	0.05	-0.01	-0.13	-0.15	0.17	0.13
	E - H	-0.09	-0.10	-0.19	-0.22	0.11	0.12	0.01	-0.01	0.01	0.01	0.00	-0.02
Cation-π interactions	KR - FWY	0.05	0.14	-0.06	0.01	0.11	0.13	0.10	0.14	-0.02	0.00	0.12	0.13
	R - FWY	-0.07	-0.00	-0.16	-0.14	0.10	0.13	0.01	0.01	-0.12	-0.11	0.14	0.12
	KR - W	0.15	0.17	-0.12	0.04	0.27	0.13	0.14	0.20	-0.09	-0.04	0.24	0.25
	KR - Y	-0.01	0.05	-0.16	-0.14	0.14	0.19	0.06	0.08	-0.08	-0.09	0.14	0.17
	K - F	0.25	0.46	0.21	0.41	0.04	0.06	0.26	0.33	0.23	0.29	0.04	0.04
	K - Y	-0.01	0.11	-0.08	-0.01	0.07	0.12	0.07	0.14	0.01	0.00	0.07	0.13
	R - W	-0.08	-0.05	-0.20	-0.06	0.12	0.01	0.01	0.02	-0.14	-0.13	0.15	0.16
	R - Y	-0.02	-0.00	-0.24	-0.26	0.22	0.25	0.04	0.02	-0.18	-0.18	0.22	0.20
	H - F	0.03	0.03	-0.21	-0.29	0.24	0.32	0.06	0.07	-0.16	-0.22	0.22	0.29
Aromatic interactions	F - FWY	-0.51	-0.55	-0.61	-0.66	0.10	0.11	-0.42	-0.42	-0.53	-0.53	0.11	0.11
	F - W	-0.31	-0.36	-0.57	-0.63	0.25	0.27	-0.42	-0.41	-0.57	-0.58	0.15	0.16
Negatively charged - Y or W	DE - W	0.23	0.24	0.06	0.05	0.17	0.19	0.14	0.10	-0.01	-0.01	0.15	0.11
	DE - Y	0.00	-0.01	-0.10	-0.09	0.10	0.08	0.10	0.10	-0.05	-0.03	0.15	0.13
	D - W	0.25	0.23	0.12	0.10	0.13	0.13	0.13	0.09	-0.02	-0.02	0.15	0.10
	D - Y	0.03	0.02	-0.08	-0.07	0.12	0.10	0.13	0.13	-0.06	-0.03	0.19	0.17
Small - charged	AG - KR	0.37	0.35	0.26	0.25	0.11	0.10	0.23	0.24	0.16	0.17	0.07	0.07
	G - KR	0.30	0.27	0.19	0.14	0.11	0.13	0.20	0.19	0.08	0.09	0.11	0.10
	AG - R	0.30	0.29	0.15	0.13	0.15	0.17	0.20	0.16	0.06	0.06	0.15	0.10
	G - H	0.17	0.16	0.12	0.10	0.05	0.06	0.12	0.12	0.01	0.02	0.11	0.09
	G - R	0.23	0.21	0.03	-0.02	0.20	0.23	0.16	0.11	-0.04	-0.04	0.21	0.15
	AG - E	0.53	0.53	0.42	0.43	0.11	0.10	0.37	0.39	0.27	0.27	0.09	0.11
	A - E	0.64	0.67	0.38	0.40	0.26	0.26	0.45	0.46	0.28	0.28	0.17	0.18
Cysteine - uncharged	C - AILV	-0.18	-0.20	-0.33	-0.39	0.15	0.19	-0.18	-0.23	-0.35	-0.35	0.17	0.13
	C - AG	0.29	0.21	-0.04	-0.10	0.33	0.31	0.01	-0.02	-0.23	-0.26	0.24	0.25
	C - G	0.38	0.32	-0.11	-0.21	0.48	0.53	0.03	0.03	-0.29	-0.34	0.32	0.37
	C - FWY	-0.25	-0.29	-0.57	-0.59	0.32	0.30	-0.16	-0.17	-0.53	-0.54	0.36	0.37
	C - NQST	0.20	0.18	-0.02	0.00	0.22	0.18	0.04	0.02	-0.10	-0.10	0.14	0.12
Isoleucine - hydrophobic or small	I - AILV	-0.55	-0.55	-0.65	-0.65	0.10	0.10	-0.44	-0.44	-0.54	-0.54	0.10	0.10
	I - I	-0.61	-0.61	-0.82	-0.83	0.22	0.22	-0.50	-0.50	-0.74	-0.73	0.24	0.24
	I - AG	-0.09	-0.10	-0.25	-0.25	0.16	0.16	-0.10	-0.09	-0.22	-0.22	0.13	0.13
	I - A	-0.25	-0.25	-0.47	-0.47	0.22	0.22	-0.19	-0.18	-0.38	-0.37	0.19	0.19
	I - FWY	-0.44	-0.47	-0.53	-0.55	0.09	0.08	-0.37	-0.37	-0.47	-0.47	0.10	0.10
	I - W	-0.32	-0.38	-0.39	-0.44	0.07	0.06	-0.26	-0.26	-0.45	-0.45	0.19	0.19
	I - Y	-0.31	-0.32	-0.47	-0.48	0.16	0.15	-0.25	-0.24	-0.39	-0.38	0.14	0.14
Methionine - charged, aromatic or small	M - KR	0.15	0.33	0.07	0.32	0.09	0.00	0.29	0.29	0.17	0.19	0.12	0.10
	M - DE	0.29	0.30	0.09	0.14	0.20	0.17	0.25	0.26	0.11	0.10	0.15	0.16
	M - FWY	-0.49	-0.54	-0.64	-0.65	0.15	0.10	-0.39	-0.39	-0.51	-0.51	0.12	0.12
	M - Y	-0.34	-0.34	-0.54	-0.54	0.20	0.20	-0.18	-0.18	-0.43	-0.42	0.25	0.24
	M - A	-0.22	-0.22	-0.33	-0.34	0.11	0.11	-0.21	-0.20	-0.29	-0.29	0.09	0.09
Others	Y - AILV	-0.29	-0.30	-0.38	-0.38	0.09	0.08	-0.22	-0.21	-0.30	-0.29	0.08	0.08
	Y - V	-0.25	-0.25	-0.38	-0.39	0.13	0.14	-0.17	-0.17	-0.32	-0.31	0.15	0.15
	R - KR	0.41	0.24	0.49	0.29	-0.08	-0.05	0.32	0.21	0.29	0.20	0.03	0.01
	E - N	0.21	0.19	0.14	0.12	0.06	0.07	0.21	0.18	0.17	0.20	0.04	-0.02
	R - N	0.17	0.14	0.24	0.17	-0.07	-0.03	0.18	0.17	0.08	0.12	0.09	0.06
	V - KR	0.20	0.42	0.14	0.34	0.06	0.08	0.22	0.26	0.13	0.20	0.09	0.07
	F - P	0.02	0.03	-0.10	-0.15	0.12	0.18	0.02	0.04	-0.07	-0.06	0.09	0.10
	N - P	0.27	0.24	0.32	0.33	-0.04	-0.09	0.20	0.22	0.19	0.20	0.01	0.02
	T - Y	0.07	0.19	0.00	0.09	0.07	0.10	0.01	-0.01	-0.03	-0.01	0.04	0.00

Mesostabilizing interactions													
Type of interaction	Interaction	Contact distance cutoff D =6.0 Å						Contact distance cutoff D =8.0 Å					
		ΔW_{meso} *		ΔW_{thermo} †		$\Delta\Delta W$ ‡		ΔW_{meso} *		ΔW_{thermo} †		$\Delta\Delta W$ ‡	
		C_μ	C_ν	C_μ	C_ν	C_μ	C_ν	C_μ	C_ν	C_μ	C_ν	C_μ	C_ν
Aliphatic or small - noncharged polar	AILV - NQST	0.12	0.17	0.21	0.25	-0.09	-0.09	0.06	0.06	0.14	0.15	-0.08	-0.08
	AG - NQST	0.20	0.18	0.33	0.33	-0.13	-0.14	0.07	0.07	0.23	0.23	-0.15	-0.16
	A - NQST	0.22	0.22	0.36	0.35	-0.14	-0.13	0.11	0.11	0.24	0.26	-0.14	-0.15
	G - NQST	0.17	0.15	0.31	0.31	-0.13	-0.16	0.05	0.03	0.21	0.20	-0.17	-0.16
	L - NQST	0.04	0.10	0.17	0.24	-0.13	-0.14	0.02	0.02	0.14	0.14	-0.12	-0.12
	AILV - N	0.28	0.30	0.39	0.41	-0.11	-0.11	0.20	0.21	0.27	0.30	-0.08	-0.09
	AILV - Q	0.14	0.15	0.28	0.32	-0.15	-0.17	0.16	0.15	0.28	0.30	-0.12	-0.15
	AG - S	0.23	0.21	0.36	0.34	-0.14	-0.13	0.08	0.09	0.23	0.24	-0.15	-0.15
	AILV - T	-0.04	0.06	0.05	0.15	-0.09	-0.09	-0.08	-0.08	-0.00	-0.01	-0.08	-0.07
	AG - T	0.08	0.07	0.25	0.28	-0.18	-0.21	-0.02	-0.03	0.18	0.19	-0.21	-0.22
	A - T	0.05	0.06	0.26	0.28	-0.22	-0.22	-0.01	-0.01	0.16	0.19	-0.17	-0.20
	G - S	0.14	0.13	0.36	0.36	-0.22	-0.22	0.05	0.05	0.25	0.25	-0.20	-0.21
	G - T	0.11	0.07	0.25	0.28	-0.14	-0.21	-0.04	-0.05	0.21	0.19	-0.24	-0.24
Noncharged polar - noncharged polar	NQST - NQST	0.10	0.10	0.28	0.28	-0.18	-0.19	0.06	0.04	0.24	0.23	-0.18	-0.18
	N - NQST	0.10	0.09	0.31	0.29	-0.22	-0.20	0.07	0.07	0.30	0.29	-0.22	-0.22
	Q - NQST	0.08	0.06	0.40	0.39	-0.31	-0.33	0.08	0.07	0.36	0.33	-0.28	-0.26
	S - NQST	0.18	0.15	0.35	0.33	-0.18	-0.18	0.08	0.06	0.23	0.22	-0.15	-0.16
	T - NQST	0.07	0.09	0.16	0.21	-0.09	-0.11	0.02	0.01	0.15	0.13	-0.13	-0.12
	S - N	0.07	0.06	0.34	0.31	-0.27	-0.25	0.05	0.04	0.27	0.25	-0.22	-0.21
Negatively charged - noncharged polar	DE - NQST	0.20	0.17	0.28	0.26	-0.08	-0.09	0.18	0.16	0.26	0.24	-0.09	-0.07
	D - NQST	0.19	0.15	0.36	0.31	-0.17	-0.17	0.16	0.15	0.28	0.26	-0.12	-0.11
	DE - T	0.13	0.14	0.31	0.32	-0.18	-0.18	0.13	0.12	0.27	0.26	-0.14	-0.14
Small - small	G - AG	0.13	0.13	0.32	0.32	-0.19	-0.19	0.03	0.03	0.13	0.14	-0.10	-0.10
	A - G	0.15	0.15	0.33	0.33	-0.18	-0.18	0.05	0.05	0.12	0.13	-0.07	-0.07
Leucine - other	L - FWY	-0.57	-0.61	-0.50	-0.52	-0.07	-0.10	-0.47	-0.47	-0.42	-0.42	-0.05	-0.05
	L - F	-0.70	-0.76	-0.60	-0.62	-0.11	-0.14	-0.59	-0.59	-0.49	-0.49	-0.10	-0.10
	L - D	0.39	0.43	0.54	0.58	-0.15	-0.15	0.33	0.33	0.44	0.44	-0.11	-0.11
	L - G	0.06	0.06	0.21	0.21	-0.15	-0.15	-0.03	-0.02	0.13	0.13	-0.16	-0.16
Negatively charged - F	DE - F	0.17	0.18	0.34	0.38	-0.16	-0.20	0.23	0.22	0.26	0.26	-0.02	-0.04
	E - F	0.07	0.06	0.20	0.24	-0.13	-0.18	0.19	0.17	0.20	0.22	-0.02	-0.05

* ΔW_{meso} is the value of the contact potential (in kcal/mol) derived from the mesostable protein subset

† ΔW_{thermo} is the value of the contact potential (in kcal/mol) derived from the thermostable protein subset

‡ $\Delta\Delta W = \Delta W_{meso} - \Delta W_{thermo}$