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The sign of NMR chemical shift difference as a determinant of the origin of binding selectivity: Elucidation of the position-dependence of phosphorylation in ligands binding to Scribble PDZ1

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ABSTRACT

The use of NMR chemical shift perturbation to monitor changes taking place around the binding site of a ligand-protein interaction is a routine and widely applied methodology in the field of protein biochemistry. Shifts are often acquired by titrating various concentrations of ligand to a fixed concentration of the receptor and may serve the purposes, amongst others, to determine affinity constants, locate binding surfaces, or differentiate between binding mechanisms. Shifts are quantified by the so-called combined chemical shift difference. Although the directionality of shift changes are often used for detailed analysis of specific cases, the approach has not been adapted in standard chemical shift monitoring. This is surprising as it would not require additional efforts. Here, we demonstrate the importance of the sign of chemical shift difference induced by ligand-protein interaction. We analyze the sign of the $^{15}\text{N}/^1\text{H}$ shift changes of the PDZ1 domain of Scribble upon interaction with two pairs of phosphorylated and unphosphorylated peptides. We find that detailed differences in the molecular basis of this PDZ-ligand interaction can be obtained from our analysis to which the classical method of combined chemical shift perturbation analysis is insensitive. In addition, we find a correlation between affinity and millisecond motions. Application of the methodology to Cyclophilin, a cis-trans isomerase reveals molecular details of peptide recognition. We reckon our directionality-vector chemical shift analysis as a method of choice when distinguishing the molecular origin of binding specificities of a class of similar ligands as it is often done in drug discovery.

INTRODUCTION

Detailed understanding of intermolecular interactions requires highly localized probes. Monitoring changes of NMR chemical shifts of proteins upon ligand titration is a common and convenient tool to obtain residue- or even atom-specific information. It is well known that the chemical shift is extremely sensitive to the local electronic environment and its changes in magnitude as well as directionality can be used to infer structural and dynamic rearrangement^{1,2}. For example, if two ligands repopulate two states of a free protein in opposite ways, the chemical shift changes will also be of opposite directions, because the observed chemical shift is the population-weighted average of the values for the two individual states. This can be seen with two ligands of Pin1, fragments from Cdc25 and FFpSPR, that have opposite effects on the affinity in the catalytic site, and thus exhibit shifts in the opposite directions^{3,4}. Surprisingly, the standard procedure of chemical shift monitoring involves plotting of a combined function of absolute value change of backbone ¹H and ¹⁵N shifts¹:

$$\Delta CS = \text{SQRT} ((\Delta^1\text{H})^2 + (X(\Delta^{15}\text{N}))^2) \quad (1)$$

X is a scaling factor that regulates the weights of H and N shifts and is most commonly chosen 1, 0.1 or 0.2. This approach dismisses the sign of both shifts, which carry valuable information. The full information of a combined chemical shift change may be given in a vector in the 2-dimensional ¹H/¹⁵N space, which would retain the magnitude and sign of both shifts. Here, we propose to use this approach for chemical shift mapping, as no additional experimental effort is required as compared to the standard procedure. We present the utility of the approach with the example of the first PDZ(PSD-95/Dlg/ZO-1) domain of Scribble, where relevant differences in binding modes are hidden when using equation 1, but are recuperated when following our proposed approach. In addition, we applied the approach to Cyclophilin a, a cis-trans isomerase in an attempt to capture fine differences between the interaction of the enzyme with a peptide in a cis- or trans-conformation and the wild-type.

PDZ domains are a major class of protein-protein interacting domains present in the post synaptic density of excitatory neurons^{5,6}. They are small, globular protein domains consisting of between 90-100 amino acids and often occur as tandem repeats. PDZ domains function mainly by recognizing short C-terminal peptide motifs although some also recognize internal peptide motifs⁷.

^{8,9}. The interaction with short C-terminal peptides, also known as canonical PDZ-binding mode, has been well characterized and the last four amino acids of the peptide has been shown to be essential for recognition and the upstream residues contribute to affinity and specificity ¹⁰⁻¹². For class I PDZ-peptide interaction, peptide positions 0 and -2 (numbering from the carboxylate C-terminus) have been deemed important determinants for affinity, while other peptide positions play additional roles in selectivity and enhancement of the binding affinity. There are hundreds of known or putative C-terminal peptide ligands present in the cell¹³, which are typically shared ligands between several PDZ domains. The mechanism through which PDZ domains distinguish between respective partners is not entirely clear. It is anticipated that phosphorylation of a particular ligand can trigger alternative signaling pathways quite different from the unphosphorylated ligands through disruption or promotion of specific interactions. For example, the Tiam1 PDZ domain binds both phosphorylated and unphosphorylated syndecan 1 as well as phosphorylated syndecan 3 ligands but not other isoforms of syndecans ¹⁴. Phosphorylation of syndecan 1 promotes cell adhesion and ectodomain cleavage by disrupting the interaction between synthenin PDZ1 and phosphorylated syndecan 1 ^{15,16}. In summary, phosphorylation of PDZ ligands has been proposed as a regulatory mechanism^{17, 18, 14, 20}. A priority in the PDZ biology is to understand how a single PDZ domain can distinguish between all potential ligands. We recently found that Scribble PDZ1 domain can interact with unphosphorylated and phosphorylated ligands representing the C-termini of different biological proteins. Depending on the phosphorylation site, phosphorylation may have a switching function, increasing the affinity for Scribble PDZ1 and decreasing the affinity for other PDZ domains such as Shank1 PDZ¹⁹. Although we probed the molecular mechanism of interaction through NMR structure determination, with hindsight we realized that, the sign shift analysis provides an alternative and feasible approach for gaining deeper understanding of the molecular details of the interactions. Here, we analyze the origin of the binding specificities by probing the directionality of chemical shift perturbation with heteronuclear single quantum coherence spectra as well as slow millisecond dynamics.

RESULTS and DISCUSSION

Scribble PDZ1 domain has been shown to selectively bind to the C-terminal peptides of RPS6KA2 (MKRLTSTRL)¹⁹ and colorectal mutant cancer protein (MCC) (RPHTNETSL)²⁰, and that the

interactions are tuned in a Ser phosphorylation dependent manner¹⁹. In this study, we investigate the molecular origin of these differences by analyzing chemical shifts perturbation from heteronuclear single quantum coherence spectra as well as slow millisecond dynamics probed by NMR transverse relaxation rates (R_2). The following peptides were used for the analysis: the unphosphorylated ligands MKRLTSTRL (RPS6KA2) and RPHTNETSL (MCC) and their MKRLTpSTRL and RPHTNET(p)SL being their phosphorylated variants, respectively. The two phosphorylated ligands are probes for the phosphorylation sites at p-3 and p-1, thus allowing the study of phosphorylation in a position-dependent context. The peptides were chosen as their position dependent phosphorylation seemed to play a biological role as switches for different PDZ domains^{19,21}.

The sign rather than the magnitude of NMR chemical shift distinguishes the origin of binding affinity

In the context of protein-ligand interaction, combined chemical shifts from NMR $^1\text{H}/^{15}\text{N}$ correlation experiments are routinely used to monitor ligand interaction and ligand binding sites on proteins and receptors in solution. While the approach through which these shifts are interpreted is very powerful in localizing residues undergoing structural changes taking place upon ligand addition, it often fails to dissect the fine details associated with the binding reaction. In contrast, the directionality of the chemical shifts, which is rarely taken into consideration in this analysis, can play a vital role in distinguishing binding mechanisms, for example when two slightly different ligands that interact with the same receptor. The peptides MKRLTSTRL and MKRLTpSTRL, which differ at a single phosphorylation site, both bind to PDZ1 from Scribble but the phosphorylated variant has a higher affinity (K_D of 18 versus 4 μM from ITC)¹⁹. We reanalyzed HSQC experiments performed for free and bound Scribble PDZ1 with peptides phosphorylated at p-1, p-3 as well as unphosphorylated peptides. The shifts were then analyzed as described in the method section. The residue specific assignment is found in supplementary table 1. HSQC spectra tracing chemical shift changes of PDZ1 bound with either of the peptides show that similar residues are affected nearly to the same extent (Figure 1).

Figure 1. **Heteronuclear single quantum coherence (HSQC) ^{15}N - ^1H spectrum of free and bound PDZ1.** (A) Overlay of PDZ1 free (red), bound to unphosphorylated (green) and phosphorylated MKRLTSTRL peptide (blue). Expansions on specific regions are shown on the top-right hand corner (B) Residues exhibiting shifts of opposite sign are colored in red on a cartoon representation of PDZ1 (pdb: 6ESP). For simplicity, the residue numbers are not shown.

This is explicitly shown in Figure 2A, where the combined chemical shifts from both complexes are plotted. However, this representation does not explain the origin of the difference in binding affinity between the two peptides. In order to identify the origin of these differences we monitor the changes of the chemical shifts in a sign-sensitive manner taking into consideration the sign of

the shift. Plots of the differences (from the free protein) in ^{15}N shift (ΔN) of the Scribble PDZ1 domain bound to the phosphorylated and unphosphorylated peptides are shown in Figure 2B). A shift difference was qualified as significant if its value was above the mean (different from the mean-shift).

Figure 2. **Combined chemical shift difference and sign-sensitive chemical shift difference plots.** (A, B) ^{15}N - ^1H combined chemical shift difference between free Scribble PDZ1 and PDZ1 bound to respective peptides. (C, D) Sign-

sensitive ^{15}N chemical shift difference for respective peptides. **(E, F)** Sign-sensitive ^1H chemical shift difference for respective peptides. The combined chemical shift difference depends only on absolute values; thus, the directionality of the shift is not taken into consideration. On the other hand, the sign-sensitive chemical shift difference plot takes into consideration the directionality of shift changes. Residues contributing differently to binding may be identified.

The majority of the residues move in the same direction, while a few shifted in the opposite direction (Figures 1 and 2). Those residues that experience a difference in the ^{15}N shift include 723, 724, 733, 741-744, 747, 762, 764-765, 774, 795 and 813-814, and color coded on the structure (PDB: 6ESP) (Figure 1B)(for simplicity, the numbers are not shown on the structure). These residues are distributed throughout the PDZ domain, indicating some long-range effect. It is interesting to note that residues 741-744 in the $\beta 2$ strand that flanks the peptide binding groove seem to respond differently to binding of the p-3 phosphorylated peptide and unphosphorylated peptide ligands. These results are in agreement with our mutational analysis of R762 to alanine, where the R762A mutant showed a decrease in affinity for the p-3 phosphorylated peptide compared to the unphosphorylated peptide¹⁹. This further indicates that our analysis can identify subtle differences important for PDZ affinity. Given that the binding data for the p-1 peptide indicates only a minor affinity difference between the unphosphorylated peptide (2.6 μM vs 4.4 μM), we next verified that our analysis could also capture this. Indeed, the combined shift plots are very similar for the p-1 phosphorylated and unphosphorylated peptides (Figures 2C and D). The results indicate that perhaps both peptides interact with PDZ1 using similar residues (Figure. 2D) and phosphorylation at p-1 might not be an important switching site for Scribble PDZ1. The analysis carried out so far is based on differences in the ^{15}N direction. We observe similar trends for ^1H (Figure. 2E and F). This is further demonstrated by plotting a two-dimensional vector representation, where the change in the ^{15}N shift is plotted on the abscissa and the change in ^1H shift on the ordinate, respectively (Figure. 3).

Figure 3. Two-dimensional vector representation of ^{15}N and ^1H chemical shift change. To properly display the importance of using the sign of the shift change, we plotted the difference between the shift from the free protein and the one when bound to the respective peptides. The change in ^{15}N and ^1H shifts is plotted on the abscissa and ordinate, respectively. The plots in (A) and (B) are for the phosphorylated peptide p-3 (orange) and its unphosphorylated variant (blue) respectively, while (C) and (D) are for the phosphorylated peptide p-1 (orange) and its unphosphorylated counter-part (blue), respectively. The labeling of the subset of points shown in (B) reveals that phosphorylation at p-3 causes shift changes that are different from the unphosphorylated variant. Such a pattern is not seen when the peptide is phosphorylated at p-1.

In this representation, both the magnitude and the sign of the shift changes are retained making interpretation easy. For the p-3 case, we observe a clear spatial separation of residues (mentioned above) responsible for binding to the phosphorylated and unphosphorylated peptide (Figure 3B). Absence of a strong correlation between ^1H and ^{15}N shifts indicates that the binding mechanisms of phosphorylated and unphosphorylated peptides do not differ by simple population shifts in

opposite directions. There was no spatial separation for peptides p-1 and its unphosphorylated variant, further indicating that both peptides interact with the PDZ domain using similar residues in the same way (Figure 3D). Overall, these findings indicate that the sign of each shift carries more information for distinction of the peptide binding modes than the conventional combined representation.

To test if our analysis can capture selectivity differences in other systems, we re-analyzed chemical binding data from Cyclophilin a/peptide interaction that we previously analyzed by the combined chemical shift approach²² (Figure S1). Cyclophilin a is an enzyme that catalyzes cis-trans isomerization of X-proline peptide bonds, where X refers to any amino acid²³. We plotted and compared the sign difference plots for Cyclophilin bound to wild-type peptide (the wild type peptide exists in equilibrium mixture between the cis- and trans-isoforms), the cis- and the trans-locked peptide. While there was virtually no detectable difference between the sign difference plot of Cyclophilin-wild-type peptide complex and Cyclophilin-trans peptide complex (Figure S1 A and B), this was not the case for Cyclophilin-wild-type peptide complex and Cyclophilin-cis peptide complex (Figure S1 C and D). We observed a clear difference in shifts for residues I57, Q63, L90 and K126. As a consequence, comparison of the shift difference between the Cyclophilin-trans peptide complex and Cyclophilin-cis peptide complex showed differences for similar residues (Figure S1 e and f). In addition, we also observed that M100 has a significant sign difference between Cyclophilin bound to the cis peptide and Cyclophilin bound to the trans peptide. It seems likely that these residues that only change their shift in the Cyclophilin-cis peptide complex are responsible for cis-peptide specific recognition. It is important to note that chemical shifts are very sensitive to changes in their surroundings. Thus, the shift change of a particular residue might as well be due to an event experienced by a neighboring residue. What is the role of the residues detected in this analysis in binding and catalysis of Cyclophilin a? I57 (or its neighbor R55 and I56), Q63 and K126 (or its neighbor H125) are active site residues responsible for binding and catalysis. M100 (its neighbor S99) and L90 have all been implicated in conformational change induced by slow millisecond motion²⁴. The fact that all the residues identified in our analysis have been shown to be important for function implies our analysis can capture important molecular determinants for binding selectivity in Cyclophilin.

Millisecond dynamics in PDZ1 is responsible for the higher affinity of Scribble PDZ1 for the p-3 phosphorylated ligand

Having observed that the p-3 position phosphorylated peptide has a higher affinity than the unphosphorylated peptide and that the sign of the chemical shift can be used to explain the origin of this higher affinity, we wondered if the higher affinity is associated or can be explained by the presence of slow exchange processes between the ligand and protein (free and bound). Such dynamics associated with protein-ligand interaction typically takes place on the micro-millisecond timescales and thus can be probed by NMR relaxation dispersion²⁵. These exchange processes cause additional line broadening of the respective NMR spectral resonances and result in exchange contributions to transverse relaxation, $R_{ex} = R_2 - R_{2,0}$, where R_2 and $R_{2,0}$ are the transverse relaxation rates in the presence and absence of exchange, respectively. R_{ex} values depend on the relative populations of the exchanging states, the chemical shift difference ($\delta\omega$) between them and the rates of exchange (k_{ex})²⁶. We measured and compared backbone R_2 rates for free and bound protein with the different peptides. In addition, we deduced $R_{2,0}$ rates when contributions from exchange processes slower than 80 μ s are quenched by application of a 2 kHz spin-lock during measurement of longitudinal relaxation in the rotating frame ($R_{1\rho}$) (Figure 4). A plot of R_2 from Scribble PDZ1 bound to the different peptides shows an increase in millisecond motion when bound to the peptide phosphorylated at -3 position and no change in such motion in the complex with the other peptides (Figure 4).

Figure 4. **NMR ^{15}N transverse spin relaxation measurements.** (A, B) Transverse R_2 rates for Scribble PDZ1 bound to the phosphopeptides (blue) and unphosphopeptides (red). A protein undergoing micro- to millisecond motion often displays increased R_2 rates. These contributions from conformational exchange are quenched if spin-lattice relaxation rates are determined in the presence of a spin lock field ($R_{1\rho}$). Here, we compare the R_2 and $R_{1\rho}$ rates for the PDZ1 bound to the peptides p-3, p-1 phosphorylated peptides and their unphosphorylated variants. (A) and b) are R_2 rates and $R_{1\rho}$ rates for Scribble PDZ1 bound to the p-3 phosphorylated peptides (blue) and the unphosphorylated variant (red), respectively. C) and d) similar to (A) and (B) but for p-1 phosphorylated peptide and its unphosphorylated counter-part. $R_{1\rho}$ rates were measured with a spinlock field strength of 2 kHz. Nearly all exchange contributions disappear with $R_{1\rho}$ rates indicating that all exchange processes must be slower than 80 μs .

Application of the spin lock pulse reduces the R_2 rates to those of the free protein, indicating that these exchange processes must be slower than 80 μs .

CONCLUSION

Chemical shifts from protein-ligand binding experiments are often analyzed in an absolute value manner resulting in the loss of an important property of these shifts. Here, we re-introduce the analysis of these shifts taking into consideration their sign. Using this approach, we were able to elucidate the molecular origin of Scribble PDZ1 selectivity. We also observe that the higher affinity (5-fold increase) of PDZ1 for the p-3 phosphorylated peptide correlates with increased millisecond motion as determined from increase in R_2 spin-spin relaxation times. Further, we tested the robustness of our method by applying it to the cis-trans isomerase Cyclophilin a. We observed that our approach was able to capture previously identified molecular determinants of binding selectivity and dynamics in this class of enzyme. Our analysis clearly shows that differences in affinities or binding between slightly different ligands interacting with the same protein/enzyme can be predicted by using the sign of the chemical shift.

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ASSOCIATED CONTENT

Supporting Information

- 1) Materials and method section
- 2) Supporting Figure 1
- 3) Supporting table 1

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Supporting materials and methods

Protein expression and purification

The cDNA corresponding to residues 716 – 829 of Scribble PDZ1 in pETM-11 was used.¹ This construct encodes an N-terminal 6 x His tag Scribble PDZ1. Scribble PDZ1 was expressed in *E. coli* BL21 (DE3) pLysS cells in M9 minimal medium enriched with ¹⁵N ammonium chloride and ¹³C D-glucose. *E. coli* BL21 (DE3) pLysS cells transformed with the plasmid were grown overnight at 37°C on a plate containing 50 µg/ml of kanamycin. Single colonies were grown in 10 ml LB media containing 50 µg/ml of kanamycin over night at 30°C. On the third day, transformed cells were grown in growth media containing 50 µg/ml kanamycin final concentration at 37°C to an OD_{600nm} of 0.8. Protein expression was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside and cells were allowed to grow 4-5 h at 30°C. Finally, these cells were harvested by centrifugation and re-suspended in purification buffer (phosphate 20mM pH 7.4). The re-suspended cells were then lysed by sonication and purified using a Nickel Sepharose column (GE Healthcare). The partially pure fusion protein was dialysed to 50 mM Tris-HCl pH 8.0, 0.5 mM EDTA and 1 mM DTT along with 0.1 µg of TEV protease per mg protein sample and incubated at 8°C for overnight. The cleaved protein was passed through a second Nickle Sepharose column and unbound cleaved Scribble PDZ1 was collected as a flow through. The purity and identity of the protein was confirmed by SDS-PAGE and mass spectrometry. Pure protein was dialyzed in the experimental buffer (see below) and stored at -20°C until use. Prior to NMR experiments the lyophilized samples were dissolved in a buffer containing 10 mM NaPO₄, pH 6.5, 150 mM NaCl and dialyzed against the same buffer overnight at 25°C.

Phosphorylated and unphosphorylated peptides

The following peptides MKRLTSTRL (corresponding to residue 725-733 of RPS6KA2), RPHTNETSL (corresponding to residue 821-829 of colorectal mutant cancer protein), and their phosphorylated variants MKRLT(p)STRL, RPHTNET(p)SL were used. The peptides were obtained from GeneCust and were supplied as a lyophilized powder. The lyophilized peptides were dissolved in 10 mM sodium phosphate pH 6.5 and dialyzed against the same buffer overnight using a 300 Da cut off dialyses tubing (Biotech CE). The peptide concentrations were estimated from absorbance at 205 nm.

NMR Spectroscopy

Protein concentrations were estimated by absorbance measurement at 280 nm. The protein complexes were formed by titrating increasing amounts of either unphosphorylated peptide or phosphorylated peptide. The final concentrations of the protein-peptide complexes in the NMR tube ranged from 300-500 µM of the labeled protein and approximately a 2-3-fold excess of the peptides. To these samples, 0.01% NaN₃ and 5% D₂O were added. All NMR experiments were recorded on Bruker spectrometers (600 and 900 MHz, ¹H frequencies), equipped with triple resonance cryogenic temperature probes at 298 K. For chemical shift measurement ¹H¹⁵N-resolved TROSY-HSQC spectra² were recorded for free PDZ1 and for PDZ in complex with the different peptide ligands. Chemical shift changes were analyzed by taking the square root of the sum of the

squared ^{15}N and ^1H shifts ($\text{SQRT} (1/5 (\delta ^{15}\text{N})^2 + (\delta ^1\text{H})^2))$ as well as the simple difference, the $\Delta^{15}\text{N}$ shifts and or $\Delta^1\text{H}$ shifts ($\Delta^{15}\text{N} = ^{15}\text{N}_{\text{free}} - ^{15}\text{N}_{\text{bound}}$ and $\Delta^1\text{H} = ^1\text{H}_{\text{free}} - ^1\text{H}_{\text{bound}}$). R_1 , and $R_{1\rho}$ relaxation rates were determined in a randomized manner with different relaxation delays and a recovery delay of 3 s using TROSY-HSQC based pulse schemes². A spin lock of 2000 Hz was used for $R_{1\rho}$. $R_{2,0}$ values were then determined from $R_{1\rho}$ by correcting for the contribution of R_1 . All experiments were processed with NMRPipe³ and analyzed with CCPNmr⁴. Curve fitting was done in NMRPipe³. Errors were determined from Monte-Carlo simulations.

Figure S1. Sign-sensitive chemical shift difference plots for Cyclophilin a. The chemical shifts used for the analysis were from free Cyclophilin a, Cyclophilin a bound to the wild-type peptide, cis and trans peptide, respectively. The shift differences were obtained as described in the method

section. The respective plots were created by plotting the shift change as a function of amino acid sequence. Only the shifts from the Cyclophilin-trans and Cyclophilin-cis peptide were used for the two-dimensional vector representation. Proton (ordinate) shifts were plotted against nitrogen shifts (abscissa). (A) The ^{15}N difference plot for Cyclophilin bound to the wild type peptide (blue) and trans peptide (red). C) similar to (A) but for the wild type and cis peptide. (E) is the comparison between the Cyclophilin bound to the cis (red) and trans peptide (blue). The corresponding ^1H shifts are displayed in (B), (D) and (F). Arrows indicate residues with significant sign difference. (G) A plot of the 2D vector representation for the cis (red) and trans (blue) peptide shifts. Colored in yellow (trans peptide) and green (cis peptide) are the shifts with significant sign difference. h) is the conventional combined chemical shift plotted as a function of amino acid sequence.

Table S1

Scribble PDZ1 chemical shifts. Chemical shifts from the titration of PDZ1 with the different peptides. The respective peptides are marked at the top of the table sub-section

WT	N	H	ΔCS	ΔN	ΔH
716	119.97565	8.51248	0.007818725	0.01193	-0.00205
717	110.61147	8.39522	0.016899442	-0.02576	-0.00449
718	124.93081	8.07395	0.002111743	0.00172	-0.00181
720	124.0319	8.29584	0.008365288	-0.01231	-0.00306
722	123.71921	8.42142	0.049645384	-0.07691	-0.00993
723	126.4666	8.49626	0.005424725	-0.00609	-0.00382
724	121.0064	8.31445	0.020419064	-0.02738	0.01082
725	120.35722	8.40565	0.028888206	0.02849	-0.02258
726	120.72276	8.04572	0.03092348	0.0467	-0.00916
727	124.37397	8.83512	0.102552125	0.1607	0.01368
728	116.96324	8.45635	0.061415967	0.09696	-0.00338
729	127.96063	9.05539	0.088401412	-0.13951	0.00544
730	117.85087	8.62078	0.035348938	0.01626	-0.03382
731	126.73385	9.12388	0.132333436	0.19958	0.03974
732	128.76426	8.369	0.139811132	-0.22098	0.00378
733	123.61963	8.51343	0.025038168	0.01684	0.02266
734	121.64097	10.6883	2.749742241	0.39777	
735	114.1905	8.2973	0.258867622	-0.4093	-0.00136
736	109.4532	8.4906	0.359631905	0.56208	0.05442
737					
738	119.5151	8.6243	0.803511576	-1.24888	0.14748
739	106.06419	8.21992	0.492804468	-0.33498	0.44494
740	112.16899	7.63072	0.791856261	-1.09075	-0.38877
741	116.5943	8.14814	0.191794441	-0.29608	0.04147

742	114.62668	8.59549	0.657379863	1.03138	-0.08155
743	123.63715	9.3166	0.355392217	0.55497	-0.05574
744	107.63031	8.85482	0.221386548	-0.27513	-0.13687
745	106.59423	6.80658	0.025097021	0.03643	0.00995
746					
747	117.87168	9.37696	0.377130922	-0.59466	-0.02792
748	117.02634	8.05523	0.598183475	-0.92524	-0.12408
750					
751	124.71305	9.93801	0.050530857	0.07042	0.02387
752	113.41568	6.61183	0.146619084	-0.19738	0.0769
753	111.72109	8.50384	0.511866877	-0.80782	-0.03128
754	123.46659	8.3447	0.056000091	-0.05104	0.04576
755	122.47996	8.08516	0.092744464	-0.12313	-0.05037
756	125.88011	8.65872	0.184613309	-0.29055	0.01773
757	108.57453	9.46191	0.196963408	0.298	0.05721
758	119.97565	8.51248	0.262399226	0.41481	0.00514
759	124.20071	9.00773	0.101318214	0.04283	-0.09763
760	119.82951	8.8526	0.034342689	0.01498	-0.03301
761	124.21141	8.79221	0.344045116	-0.54398	0.00116
762	122.06448	7.51098	0.34771732	0.53557	-0.07857
763					
764	121.95551	8.47302	0.402926854	0.62955	-0.06178
765	129.81125	9.43596	0.1333631	-0.2043	0.03302
766	116.20432	8.73179	0.095584075	0.11258	0.06377
767	107.00007	7.49127	0.040788254	0.05149	0.02456
769	121.77165	7.64296	0.279729553	0.43927	-0.03264
713					
771	117.48530	7.67841	0.058233848	-0.08948	0.01373
772	119.36433	7.43592	0.059180046	-0.09205	-0.01063
773	102.93793	7.53736	0.055995786	-0.08351	-0.0186
774	121.66148	7.64074	0.116932187	0.1744	0.03882
775	125.88045	8.47806	0.10203633	0.14315	0.04706
776	120.75483	8.35385	0.059217728	-0.09339	0.00425
777	117.87181	9.74683	0.262602045	-0.3216	-0.1661
778	123.89683	7.91688	0.13535826	-0.2107	-0.02375
779	124.11706	8.88164			
780	127.76085	8.97025	0.284549397	0.44277	-0.0505
781	122.5457	8.84827	0.442591299	0.69239	-0.06423

782	118.28336	7.64377	0.223025584	-0.35139	-0.01872
783	121.87724	8.21254	0.15914481	-0.21431	-0.0834
784	128.32675	9.61046	0.160819938	-0.25117	0.02507
785	103.12185	8.58442	0.071803638	0.06193	0.06018
786	123.50414	8.12938	0.083827099	-0.07731	-0.06809
787	130.87383	8.5652	0.04187068	-0.06206	-0.01458
788	119.78094	7.95671	0.124278708	0.18822	-0.0357
789	122.7565	7.82159	0.069335446	-0.10949	-0.00349
790	115.77567	8.92123	0.170856335	0.26724	-0.025
791	120.17853	7.2806	0.123431198	0.18068	0.04666
792	121.04862	9.11203	0.35888208	-0.55693	-0.06876
793	123.51103	9.78199	0.1259149	0.16379	-0.07158
794	112.07429	8.90835	0.529601897	0.8213	-0.10327
795	119.19814	6.81205	0.124527498	0.17599	-0.05584
796	121.52166	7.17996	0.416358572	0.63509	0.10963
797	117.34247	7.53423	0.301806154	0.45776	0.08526
798					
799	122.60711	7.72837	0.046606421	0.0343	0.04125
800	116.9202	7.75976	0.40623661	-0.63769	-0.04867
801					
802	104.81444	7.78584	0.165231627	0.21208	0.09649
803	120.87622	6.94859	0.188634205	0.05328	0.1856
804	106.5059	8.20961	0.100205346	0.15811	0.00645
805	113.44232	8.26048	0.12749346	0.19289	0.03704
806	123.62276	7.76973	0.053277004	0.07963	0.01738
807	125.10997	9.10877	0.22379327	-0.3532	-0.01354
808	128.55975	9.09407	0.212468656	-0.28329	-0.1142
809	125.28026	9.37668	0.180090775	-0.27774	-0.03971
810	124.51719	8.44162	0.027880656	0.00895	-0.0273
811	119.57201	9.29284	0.089671872	-0.12466	-0.04272
812	121.70233	9.20902	0.059018938	-0.04306	-0.05236
813	129.88194	8.77595	0.186584394	-0.2766	-0.06489
814	123.74904	8.67381	0.044088896	-0.06853	-0.00808
816	120.7081	8.30669	0.019150748	-0.02977	-0.0035
817	122.83945	8.23831	0.101509552	0.16048	0.00163
818	125.72773	8.74813	0.028819	-0.04353	-0.00852
820	120.71133	8.55894	0.013530268	-0.02109	-0.00227
821	119.34961	8.36539	0.021670314	-0.0335	-0.00455

822	124.41552	8.15441	0.004216558	-0.00462	-0.00304
823	119.31737	8.07554	0.041896636	0.06621	-0.00135
824	119.05635	8.22156	0.072095864	0.11394	0.00221
825	124.27476	8.21458	0.033853781	0.0502	-0.01175
826	121.93623	8.26297	0.039842193	0.06093	0.01012
828	123.0037	8.30199	0.098836269	0.15459	0.01447
829	126.37379	7.78786	0.028812515	0.04444	0.00634

PWT	N	H	Δ CS	Δ N	Δ H
716	119.97963	8.49493	0.016295122	0.00795	0.0155
717	110.49556	8.36458	0.062726641	0.09015	0.02615
718	124.91349	8.06917	0.012402804	0.01904	0.00297
720	123.9487	8.2801	0.046593339	0.07089	0.01268
722					
723	126.35886	8.47818	0.065851626	0.10165	0.01426
724	120.84607	8.30418	0.086689498	0.13295	0.02109
725	120.31368	8.37937	0.04570578	0.07203	0.0037
726	120.58831	8.01027	0.117546983	0.18115	0.02629
727	124.32495	8.82945	0.134042582	0.20972	0.01935
728	116.96503	8.43909	0.061770429	0.09517	0.01388
729	127.90249	9.04539	0.053729176	-0.08137	0.01544
730	117.85547	8.58927	0.007727764	0.01166	-0.00231
731					
732	128.63691	8.35825	0.060973368	-0.09363	0.01453
733	123.95636	8.54753	0.20263938	-0.31989	-0.01144
734	122.24662	7.98907	0.137131609	-0.20788	-0.03898
735					
736	109.99601	8.51727	0.030308343	0.01927	0.02775
737	108.56569	8.14624	0.19966285	-0.3115	0.03244
738					
739					
740					
741	115.36537	8.17152	0.590263413	0.93285	0.01809
742	114.73456	8.48346	0.584867447	0.9235	0.03048
743	123.59109	9.20162	0.384713142	0.60103	0.05924
744	107.13338	8.67466	0.146806403	0.2218	0.04329
745	106.34787	6.79503	0.180139734	0.28279	0.0215
746					

747	117.27077	9.33599	0.013635523	0.00625	0.01305
748	116.33519	8.00444	0.165198896	-0.23409	-0.07329
750					
751	124.33422	9.86937	0.298811521	0.44925	0.09251
752	113.24785	6.66868	0.027409551	-0.02955	0.02005
753	111.12913	8.47404	0.136529873	-0.21586	-0.00148
754					
755	122.37472	8.10142	0.067583857	-0.01789	-0.06663
756	125.59805	8.6371	0.039714664	-0.00849	0.03935
757					
758	120.25079	8.51832	0.088337838	0.13967	-0.0007
759	124.13508	8.96801	0.089771915	0.10846	-0.05791
760	119.73531	8.76691	0.086852124	0.10918	0.05268
761	123.95343	8.73135	0.191219456	-0.286	0.06202
762	122.76099	7.46413	0.106615345	-0.16094	-0.03172
763	122.72798	8.46203	0.299126461	0.47078	0.02869
764	122.62651	8.38585	0.036495111	-0.04145	0.02539
765	129.57228	9.40009	0.072295475	0.03467	0.06889
766					
767	107.00205	7.48766	0.042119413	0.04951	0.02817
769	122.04155	7.67812	0.126772705	0.16937	-0.0678
713					
771	117.5102	7.69886	0.072651718	-0.11438	-0.00672
772	119.27616	7.43407	0.009116477	-0.00388	-0.00878
773	102.8802	7.52255	0.016739398	-0.02578	-0.00379
774	121.98591	7.67888	0.09488974	-0.15003	0.00068
775	125.92601	8.4942	0.069033105	0.09759	0.03092
776					
777	117.66752	9.63531	0.092106628	-0.11731	-0.05458
778	123.78545	7.89398	0.062821234	-0.09932	-0.00085
779	124.12537	8.85505			
780	127.8997	8.93468	0.192794843	0.30392	-0.01493
781	122.73797	8.82187	0.318557867	0.50012	-0.03783
782	118.11675	7.64097	0.117944503	-0.18478	-0.01592
783	121.69692	8.15784	0.035858305	-0.03399	-0.0287
784	128.23533	9.62233	0.1018934	-0.15975	0.0132
785	103.12682	8.61053	0.049583682	0.05696	0.03407
786	123.45077	8.0936	0.035681726	-0.02394	-0.03231

787	130.78238	8.54026	0.021280001	0.02939	0.01036
788	119.86336	7.92988	0.067499133	0.1058	-0.00887
789	122.65969	7.79958	0.020181758	-0.01268	0.01852
790	115.99944	8.90733	0.029649053	0.04347	-0.0111
791	120.28083	7.30419	0.054677186	0.07838	0.02307
792	120.65913	9.05536	0.106586254	-0.16744	-0.01209
793	123.63417	9.72784	0.03106081	0.04065	-0.01743
794	112.48904	8.7065	0.275374613	0.40655	0.09858
795	119.60661	6.74279	0.147644426	-0.23248	0.01342
796	121.90273	7.24541	0.166620337	0.25402	0.04418
797	117.49504	7.5752	0.198035296	0.30519	0.04429
798	117.72113	7.97664	0.480748508	0.75746	-0.04026
799	122.04155	7.68266	0.389223393	0.59986	0.08696
800	116.74175	7.76183	0.294847585	-0.45924	-0.05074
801					
802	104.89864	7.82797	0.097449101	0.12788	0.05436
803	120.89932	7.02303	0.112786872	0.03018	0.11116
804	106.55500	8.20326	0.070122122	0.10901	0.0128
805					
806	123.62503	7.76555	0.053466452	0.07736	0.02156
807	125.00005	9.09896	0.153908987	-0.24328	-0.00373
808	128.39062	9.03708	0.092119413	-0.11416	-0.05721
809	125.13972	9.34489	0.087133589	-0.1372	-0.00792
810	124.57255	8.43621	0.036615944	-0.04641	-0.02189
811	119.54420	9.2647	0.062964636	-0.09685	-0.01458
812	121.67836	9.17748	0.024067481	-0.01909	-0.02082
813	129.83671	8.70926	0.146342307	-0.23137	0.0018
814	123.56214	8.6559	0.075506368	0.11837	0.00983
816					
817	122.74914	8.21187	0.161078163	0.25079	0.02807
818	125.79363	8.7352	0.069349968	-0.10943	0.00441
820	120.68378	8.54793	0.00964781	0.00646	0.00874
821	119.24001	8.34343	0.051181951	0.0761	0.01741
822	124.37744	8.14078	0.023663828	0.03346	0.01059
823	119.33154	8.06331	0.034664666	0.05204	0.01088
824	119.07137	8.21155	0.063744764	0.09892	0.01222
825	124.24432	8.19854	0.051181324	0.08064	0.00429
826	121.87464	8.24973	0.080932995	0.12252	0.02336

828	123.17761	8.29824	0.021937943	-0.01932	0.01822
829	126.32515	7.7825	0.060020368	0.09308	0.0117

MCC	N	H	Δ CS	Δ N	Δ H
716	119.92088	8.50841	0.04223312	0.0667	0.00202
717	110.58422	8.39003	0.001173899	0.00149	0.0007
718	124.92708	8.07255	0.003471181	0.00545	-0.00041
720	124.03154	8.29311	0.007565045	-0.01195	-0.00033
722	123.69623	8.4169	0.034534708	-0.05393	-0.00541
723	126.43819	8.49206	0.014121521	0.02232	0.00038
724					
725	120.28925	8.39819	0.062852423	0.09646	-0.01512
726	120.65750	8.03346	0.070877547	0.11196	0.0031
727	124.34784	8.83292	0.119223966	0.18683	0.01588
728	116.93317	8.45073	0.080372047	0.12703	0.00224
729	127.96460	9.05201	0.091172345	-0.14348	0.00882
730	117.83186	8.61668	0.037160026	0.03527	-0.02972
731	126.74312	9.12194	0.127374962	0.19031	0.04168
732	128.74655	8.36324	0.128912718	-0.20327	0.00954
733	123.61973	8.50974	0.028397421	0.01674	0.02635
734	121.63788	10.68923	2.750847767	0.40086	
735	114.17098	8.29002	0.24658959	-0.38978	0.00592
736	109.43696	8.48807	0.370168761	0.57832	0.05695
737					
738	119.56721	8.63217	0.834578303	-1.30099	0.13961
739					
740	112.15935	7.63011	0.786249145	-1.08111	-0.38816
741	116.37785	8.13656	0.073148324	-0.07963	0.05305
742	114.65802	8.59247	0.6373374	1.00004	-0.07853
743	123.58981	9.31843	0.385259963	0.60231	-0.05757
744	107.63338	8.85298	0.221790886	-0.2782	-0.13503
745	106.54384	6.79256	0.059913653	0.08682	0.02397
746					
747	117.86133	9.36875	0.370075336	-0.58431	-0.01971
748	117.05867	8.04076	0.615459563	-0.95757	-0.10961
750	121.65641	8.39053	0.332370404	-0.5255	0.00316
751	124.63166	9.93488	0.099737207	0.15181	0.027

752	113.37453	6.60632	0.128664421	-0.15623	0.08241
753	111.74685	8.50423	0.528152663	-0.83358	-0.03167
754	123.45429	8.34193	0.054364289	-0.03874	0.04853
755	122.49549	8.08354	0.100335441	-0.13866	-0.04875
756	125.89430	8.65082	0.19443118	-0.30474	0.02563
757	108.49634	9.45559	0.246259269	0.37619	0.06353
758	119.92088	8.50841	0.297131241	0.46958	0.00921
759	124.19372	9.00205	0.097198845	0.04982	-0.09195
760	119.83804	8.85563	0.036270134	0.00645	-0.03604
761	124.17352	8.78221	0.320273915	-0.50609	0.01116
762	122.07322	7.50973	0.342050175	0.52683	-0.07732
763					
764	121.99454	8.46979	0.378039218	0.59052	-0.05855
765	129.78753	9.4322	0.119985095	-0.18058	0.03678
766	116.20454	8.72911	0.097290854	0.11236	0.06645
767	107.00029	7.48903	0.042067626	0.05127	0.0268
769					
713					
771	117.47542	7.67885	0.05206811	-0.0796	0.01329
772	119.35446	7.43356	0.052629021	-0.08218	-0.00827
773	102.93054	7.53537	0.050927339	-0.07612	-0.01661
774	121.62245	7.63501	0.142146574	0.21343	0.04455
775	125.98617	8.48074	0.05029897	0.03743	0.04438
776	120.72918	8.35205	0.043267604	-0.06774	0.00605
777	117.84968	9.74649	0.251692848	-0.29947	-0.16576
778	123.90380	7.91727	0.13976706	-0.21767	-0.02414
779	124.08856	8.88075			
780	127.78770	8.97202	0.268193832	0.41592	-0.05227
781	122.54029	8.8524	0.446590445	0.6978	-0.06836
782	118.26902	7.64275	0.213902714	-0.33705	-0.0177
783	121.85016	8.2132	0.145217467	-0.18723	-0.08406
784	128.34544	9.61321	0.172127715	-0.26986	0.02232
785	103.10525	8.58395	0.07839137	0.07853	0.06065
786	123.49871	8.12815	0.080851428	-0.07188	-0.06686
787	130.84485	8.5625	0.02405928	-0.03308	-0.01188
788	119.79098	7.95732	0.118396204	0.17818	-0.03631
789	122.73058	7.8134	0.053062868	-0.08357	0.0047
790	115.75338	8.9156	0.184136485	0.28953	-0.01937
791	120.21928	7.28592	0.097678849	0.13993	0.04134

792	121.11040	9.11606	0.398019107	-0.61871	-0.07279
793	123.43885	9.78475	0.166730837	0.23597	-0.07434
794	112.07743	8.82451	0.517814483	0.81816	-0.01943
795	119.40256	6.78451	0.033529032	-0.02843	-0.0283
796	121.61473	7.19162	0.35652825	0.54202	0.09797
797	117.33752	7.5431	0.302449417	0.46271	0.07639
798					
799	122.59811	7.72485	0.05248151	0.0433	0.04477
800	116.91484	7.75434	0.402252476	-0.63233	-0.04325
801					
802	104.79132	7.79073	0.174694522	0.2352	0.0916
803	120.85345	6.9401	0.199960919	0.07605	0.19409
804	106.39634	8.20601	0.169587423	0.26767	0.01005
805	113.43425	8.2575	0.133250025	0.20096	0.04002
806	123.62609	7.76618	0.052599818	0.0763	0.02093
807	125.14136	9.10652	0.243497949	-0.38459	-0.01129
808	128.55483	9.09537	0.210561613	-0.27837	-0.1155
809	125.29803	9.37653	0.191037843	-0.29551	-0.03956
810	124.59869	8.44611	0.055821189	-0.07255	-0.03179
811	119.59947	9.29291	0.105295688	-0.15212	-0.04279
812	121.70180	9.21299	0.062422666	-0.04253	-0.05633
813	129.93135	8.76914	0.214210864	-0.32601	-0.05808
814	123.74988	8.67492	0.044825605	-0.06937	-0.00919
816	120.72838	8.30447	0.031680268	-0.05005	-0.00128
817	122.86153	8.23607	0.087617355	0.1384	0.00387
818	125.74472	8.74488	0.038637301	-0.06052	-0.00527
820	120.70971	8.55753	0.012343904	-0.01947	-0.00086
821	119.33019	8.36138	0.008921332	-0.01408	-0.00054
822	124.38026	8.15175	0.019382163	0.03064	-0.00038
823	119.30974	8.07304	0.046714674	0.07384	0.00115
824	119.02719	8.21797	0.090690044	0.1431	0.0058
825	124.24059	8.21127	0.054023628	0.08437	-0.00844
826	121.92316	8.25929	0.048793852	0.074	0.0138
828	123.00507	8.29963	0.098355459	0.15322	0.01683
829	126.33967	7.78411	0.050699877	0.07856	0.01009

pMCC	N	H	Δ CS	Δ N	Δ H
716	119.9362	8.50782	0.032600213	0.05138	0.00261

717	110.5950	8.39009	0.005910266	-0.00929	0.00064
718	124.93741	8.07362	0.003422888	-0.00488	-0.00148
720	124.04460	8.29358	0.01583793	-0.02501	-0.0008
722	123.69147	8.41723	0.031623143	-0.04917	-0.00574
723	126.44249	8.49282	0.011403182	0.01802	-0.00038
724					
725	120.35936	8.40202	0.025235521	0.02635	-0.01895
726					
727	124.37501	8.84003	0.101357975	0.15966	0.00877
728	116.94488	8.4533	0.072935519	0.11532	-0.00033
729	127.98640	9.05121	0.104973977	-0.16528	0.00962
730	117.86578	8.62405	0.037099826	0.00135	-0.03709
731	126.84278	9.12632	0.0683978	0.09065	0.0373
732	128.79462	8.36548	0.159128904	-0.25134	0.0073
733	124.04442	8.56365	0.259478004	-0.40795	-0.02756
734	121.51436	10.78523	2.85447168	0.52438	
735	114.64318	8.26883	0.545837668	-0.86198	0.02711
736	109.58027	8.4634	0.286976139	0.43501	0.08162
737	108.72751	8.16129	0.299858535	-0.47332	0.01739
738	119.52976	8.67373	0.80512554	-1.26354	0.09805
739	106.04249	8.26253	0.448472042	-0.31328	0.40233
740	112.17347	7.61448	0.786504992	-1.09523	-0.37253
741	116.41508	8.12987	0.095033528	-0.11686	0.05974
742	114.03999	8.45311	1.025163645	1.61807	0.06083
743	123.39911	9.3011	0.503155246	0.79301	-0.04024
744	107.74879	8.85976	0.286498881	-0.39361	-0.14181
745	106.51968	6.79741	0.072747499	0.11098	0.01912
746					
747	117.91286	9.3676	0.402568598	-0.63584	-0.01856
748	117.01634	8.03948	0.588898202	-0.91524	-0.10833
750	121.66555	8.40402	0.338293779	-0.53464	-0.01033
751	124.62771	9.91936	0.107295953	0.15576	0.04252
752	113.35022	6.61033	0.114488928	-0.13192	0.0784
753	111.72195	8.50466	0.512460483	-0.80868	-0.0321
754	123.43472	8.36167	0.031238753	-0.01917	0.02879
755	122.57125	8.18122	0.199579857	-0.21442	-0.14643
756	125.90744	8.64874	0.202945613	-0.31788	0.02771
757	108.49928	9.44735	0.246732969	0.37325	0.07177

758	119.93620	8.50782	0.287466344	0.45426	0.0098
759	124.16780	8.99988	0.101759852	0.07574	-0.08978
760	119.87971	8.84727	0.035529731	-0.03522	-0.02768
761	124.12961	8.75047	0.295439589	-0.46218	0.0429
762	122.50178	7.49948	0.091439499	0.09827	-0.06707
763	123.60589	8.51229	0.258393498	-0.40713	-0.02157
764	122.11113	8.46676	0.3048382	0.47393	-0.05552
765	129.66823	9.38939	0.088524931	-0.06128	0.07959
766	116.08574	8.68292	0.184558251	0.23116	0.11264
767	106.97817	7.4815	0.057732017	0.07339	0.03433
769	121.74746	7.65033	0.295835881	0.46346	-0.04001
713					
771	117.51226	7.70404	0.074598388	-0.11644	-0.0119
772	119.36531	7.43481	0.05960254	-0.09303	-0.00952
773	102.98669	7.54554	0.087836835	-0.13227	-0.02678
774	121.65355	7.65068	0.11887702	0.18233	0.02888
775	125.80878	8.46956	0.146785444	0.21482	0.05556
776	120.74535	8.35522	0.053147433	-0.08391	0.00288
777	117.82092	9.73399	0.22978727	-0.27071	-0.15326
778	123.90406	7.91412	0.139420135	-0.21793	-0.02099
779	124.15780	8.86539			
780	127.84507	8.96917	0.232089589	0.35855	-0.04942
781	122.57221	8.85363	0.426850369	0.66588	-0.06959
782	118.21302	7.63637	0.178111716	-0.28105	-0.01132
783	121.78054	8.21733	0.115370364	-0.11761	-0.08819
784	128.45284	9.62301	0.238928427	-0.37726	0.01252
785	103.10823	8.58676	0.075023907	0.07555	0.05784
786	123.49038	8.12824	0.07808805	-0.06355	-0.06695
787	130.84560	8.56417	0.02532568	-0.03383	-0.01355
788	119.83644	7.95617	0.091005851	0.13272	-0.03516
789	122.67542	7.79937	0.025955041	-0.02841	0.01873
790	115.74125	8.9103	0.191304645	0.30166	-0.01407
791	120.27159	7.29796	0.062684893	0.08762	0.0293
792	121.11187	9.11226	0.398257345	-0.62018	-0.06899
793	123.50689	9.79389	0.135089246	0.16793	-0.08348
794	112.11281	8.90099	0.504278236	0.78278	-0.09591
795	119.22125	6.7834	0.100440101	0.15288	-0.02719
796	121.63268	7.20126	0.343018855	0.52407	0.08833

797	117.40271	7.56572	0.257099345	0.39752	0.05377
798					
799	122.41875	7.74726	0.142586675	0.22266	0.02236
800	116.96759	7.76264	0.436338452	-0.68508	-0.05155
801					
802	104.68947	7.7697	0.241094583	0.33705	0.11263
803	120.89901	6.91865	0.216400896	0.03049	0.21554
804	106.44341	8.21714	0.13952387	0.2206	-0.00108
805	113.64802	8.22588	0.072096658	-0.01281	0.07164
806	123.77990	7.75585	0.058140413	-0.07751	0.03126
807	125.18039	9.10264	0.268023264	-0.42362	-0.00741
808	128.56988	9.11043	0.22690093	-0.29342	-0.13056
809	125.39160	9.37837	0.249534083	-0.38908	-0.0414
810	124.55178	8.45123	0.040315158	-0.02564	-0.03691
811	119.65346	9.29217	0.136969834	-0.20611	-0.04205
812	121.72862	9.21912	0.076321823	-0.06935	-0.06246
813	129.92717	8.77375	0.212978533	-0.32183	-0.06269
814	123.74159	8.67434	0.03957826	-0.06108	-0.00861
816	120.72841	8.3054	0.03175038	-0.05008	-0.00221
817	122.72431	8.23075	0.174559474	0.27562	0.00919
818	125.75558	8.74559	0.045539018	-0.07138	-0.00598
820	120.69855	8.55636	0.00526484	-0.00831	0.00031
821	119.33075	8.36173	0.009301825	-0.01464	-0.00089
822	124.40210	8.15158	0.005569569	0.0088	-0.00021
823	119.32451	8.07331	0.037369511	0.05907	0.00088
824	119.04064	8.21848	0.082168322	0.12965	0.00529
825	124.26112	8.21205	0.041415295	0.06384	-0.00922
826	121.92850	8.25948	0.045507256	0.06866	0.01361
828	123.01815	8.30023	0.090106053	0.14014	0.01623
829	126.33312	7.78487	0.054630886	0.08511	0.00933

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