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# Supplemental Information

# Synergistic Allostery in Multiligand-Protein Interactions

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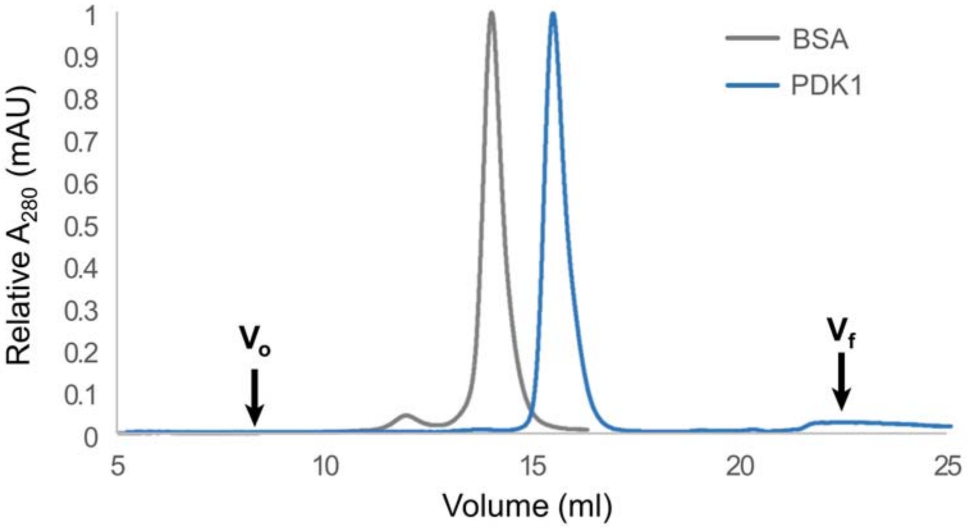

**Figure S1: Size Exclusion chromatography profile of purified PDK1 catalytic domain.** Gel-filtration chromatography profile of PDK1 catalytic domain (M.W. = 35.3 kDa, blue), obtained as described in Experimental Procedures, along with a second profile corresponding to BSA (M.W. = 66.5 kDa, grey) overlaid to show elution of PDK1 catalytic domain as a homogenous monomer.

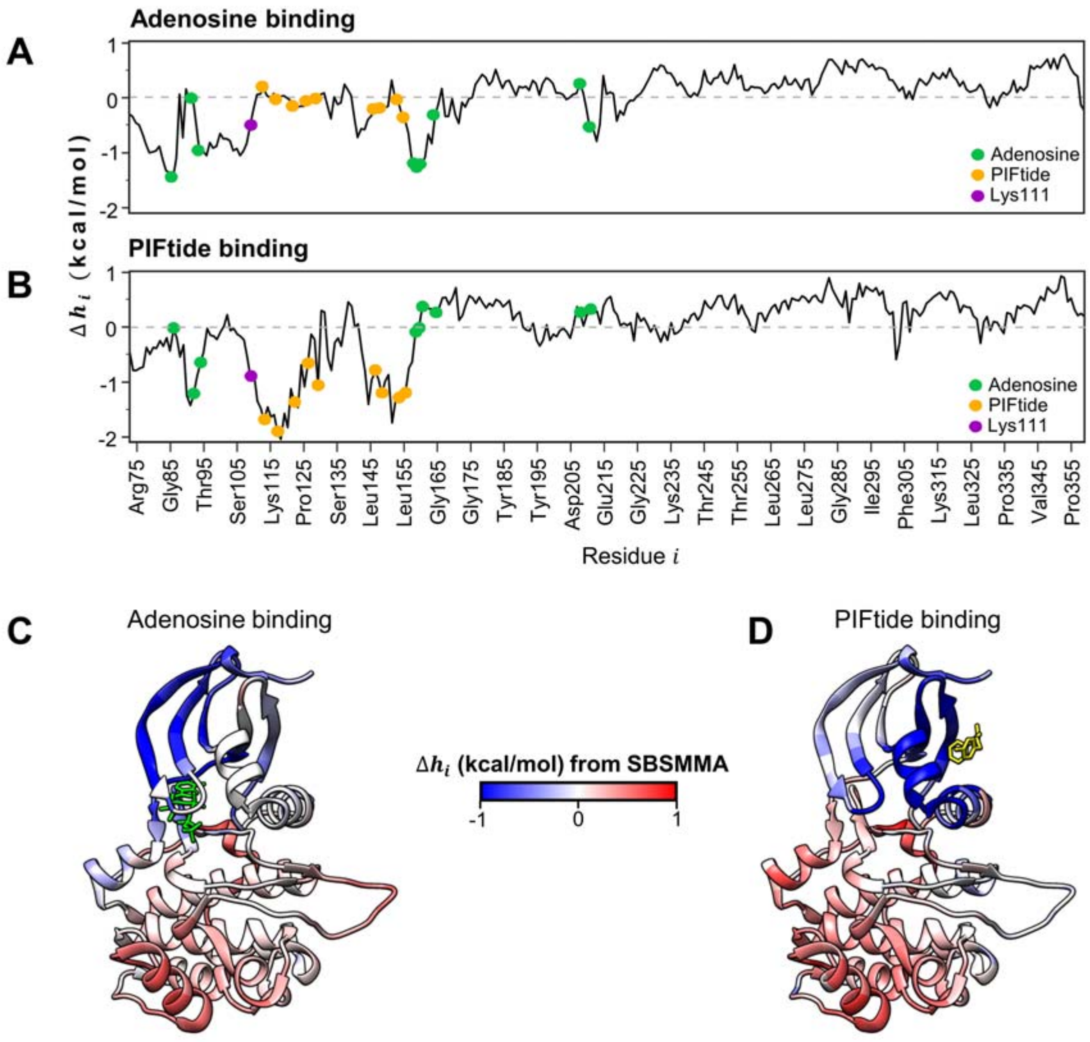

**Figure S2: SBSMMA predicts allosteric effects across PDK1 kinase domain upon binding adenosine and PIFtide. Related to Figure 2 and Figure 3.**

**(A)-(B)** Allosteric configurational work ($\Delta h_i$) for each PDK1 residue in adenosine and PIFtide bound states respectively was calculated based on structure-based statistical mechanical model of allostery (SBSMMA) using AlloSigMA server (1, 2), and plotted with residues of PDK1 arranged along x-axis. A negative $\Delta h_i$ (blue) indicates lack of configurational work (conformational stabilization), whereas a positive $\Delta h_i$ (red) is indicative of a greater propensity for allosteric conformational changes. These changes could lead to both an increase or decrease in deuterium exchange. **(C)-(D)** Heat-maps of the $\Delta h_i$ values calculated in (A)-(B) respectively, using the structure of PDK1-ATP-PS48 ternary complex (PDB: 3HRF).

**Table S1: Allosteric configurational work ($\Delta h$) from SBSMMA at key PDK1 sites for binding of adenosine and PIFtide. Related to Figure 2 and Figure 3.**

| Site[a] | Residues[b] | $\Delta h_{site}$[c] for binding of | |
|---|---|---|---|
| | | Adenosine | PIFtide |
| **αB Helix** | 115, 119 | 0.05 | - |
| **αC Helix** | 124, 128, 131 | -0.11 | - |
| **β4 strand** | 148, 150, 155, 157 | -0.23 | - |
| **β1+G-rich** | 88, 94, 96 | - | -0.65 |
| **Hinge Loop** | 160, 161, 162, 166 | - | 0.11 |
| **HRD-motif Loop** | 209, 212 | - | 0.27 |
| **β3 strand** | 108-114 | -0.50 | -0.79 |
| **Lys111** | 111 | -0.53 | -0.92 |
| **GHI-subdomain** | 286-339 | 0.29 | 0.33 |
| **αG Helix** | 286-297 | 0.56 | 0.56 |
| **αH Helix** | 306-317 | 0.24 | 0.39 |
| **αI Helix** | 332-339 | 0.06 | 0.12 |

[a] Sites of PDK1 denoted by the secondary structural elements contained in it

[b] Residues of PDK1 selected from the given site for averaging – selected based on orthosteric contacts from crystal structure and HDXMS

[c] Allosteric configurational work ($\Delta h_{site}$) calculated based on SBSMMA using AlloSigMA server, as described in Methods. These values are only reported for allosteric sites corresponding to each ligand.

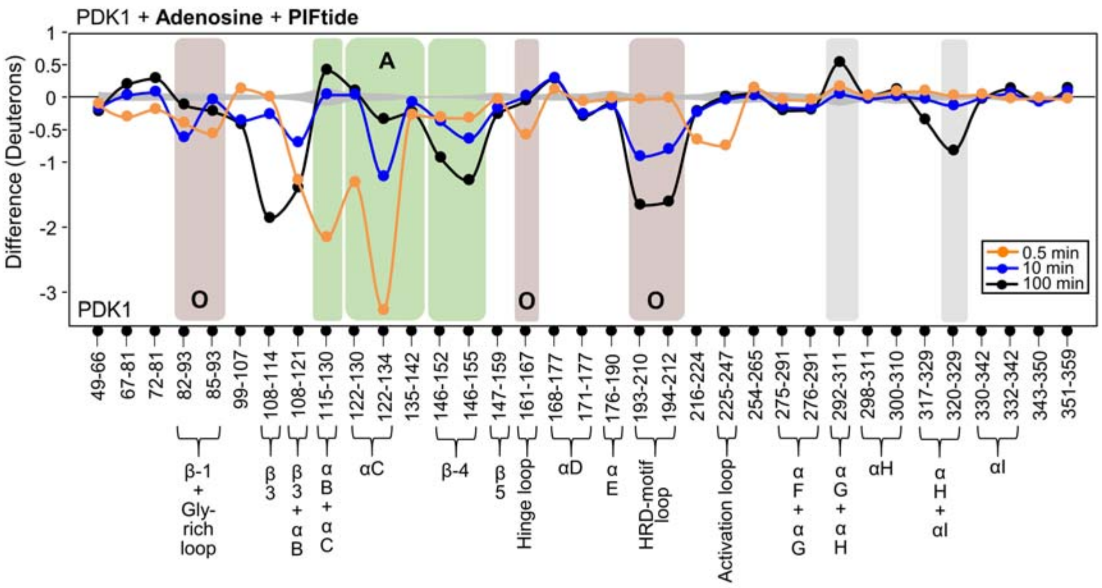

**Figure S3: Concurrent binding of adenosine and PIFtide causes reduced deuterium exchange across PDK1. Related to Figure 4.**

A deuterium exchange difference plot comparing differences in deuterium exchange between PDK1-adenosine-PIFtide ternary complex and apo PDK1. Axes, time-points and errors are similar to Figure 2A. Orthosteric and allosteric regions for each ligand are highlighted by colored boxes, and labeled according to their corresponding binding site (O and A).

**115-130**
KRHIIKENKVPYVTRE

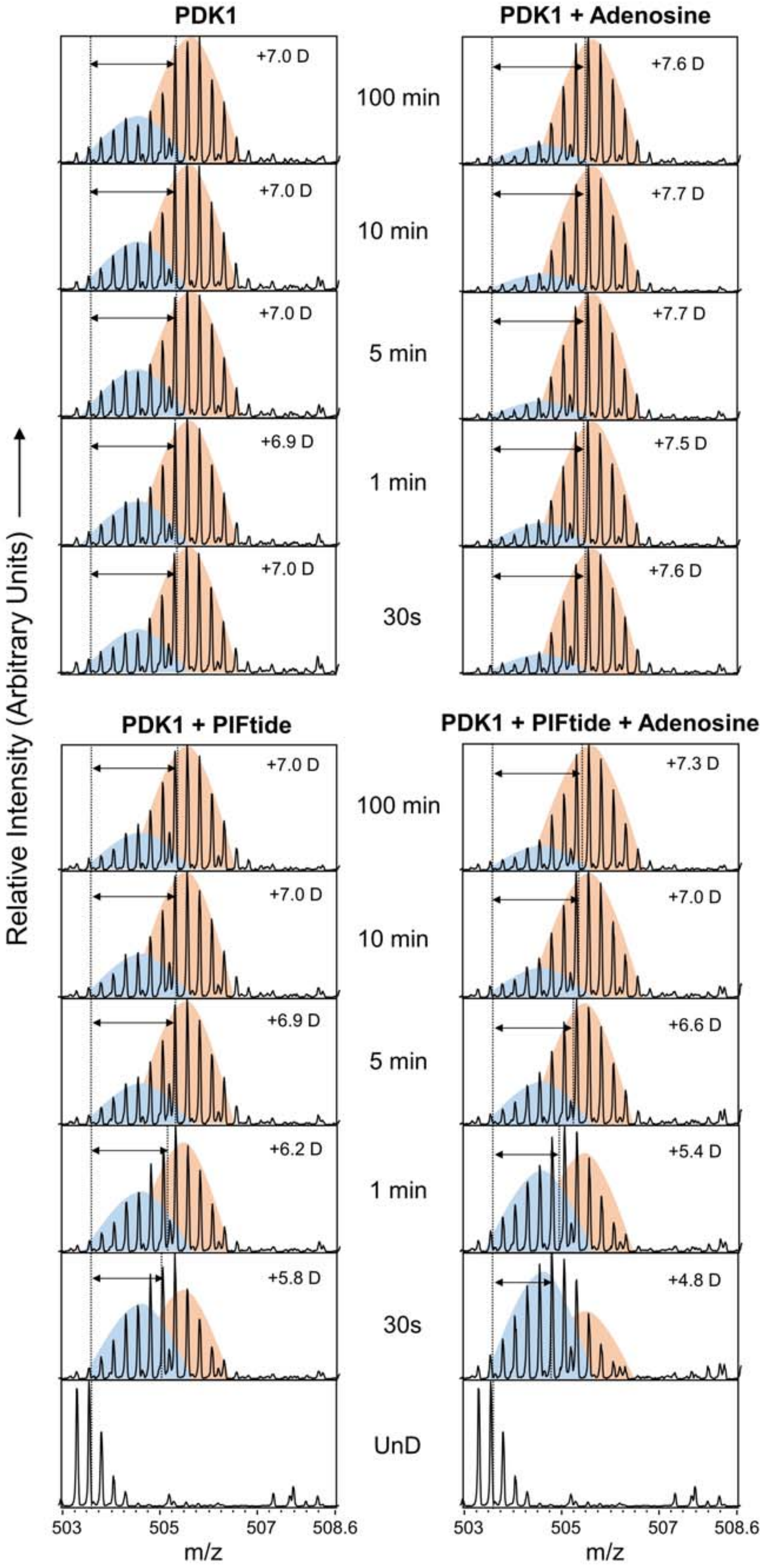

**Figure S4: Mass spectral timeseries of peptide 115-130 for all experimental states. Related to Figure 7.**

Stacked mass spectral envelope plots for peptide 115-130 spanning αB-αC helices in different states for all experimental deuterium exchange time-points. Shift in centroids are relative to the undeuterated ‘UnD’ (aqueous) peptide shown at the bottom. Bimodal isotopic distributions can be resolved into low-exchanging (blue) and high-exchanging states (orange).

**122-134**

NKVPYVTRERDVM

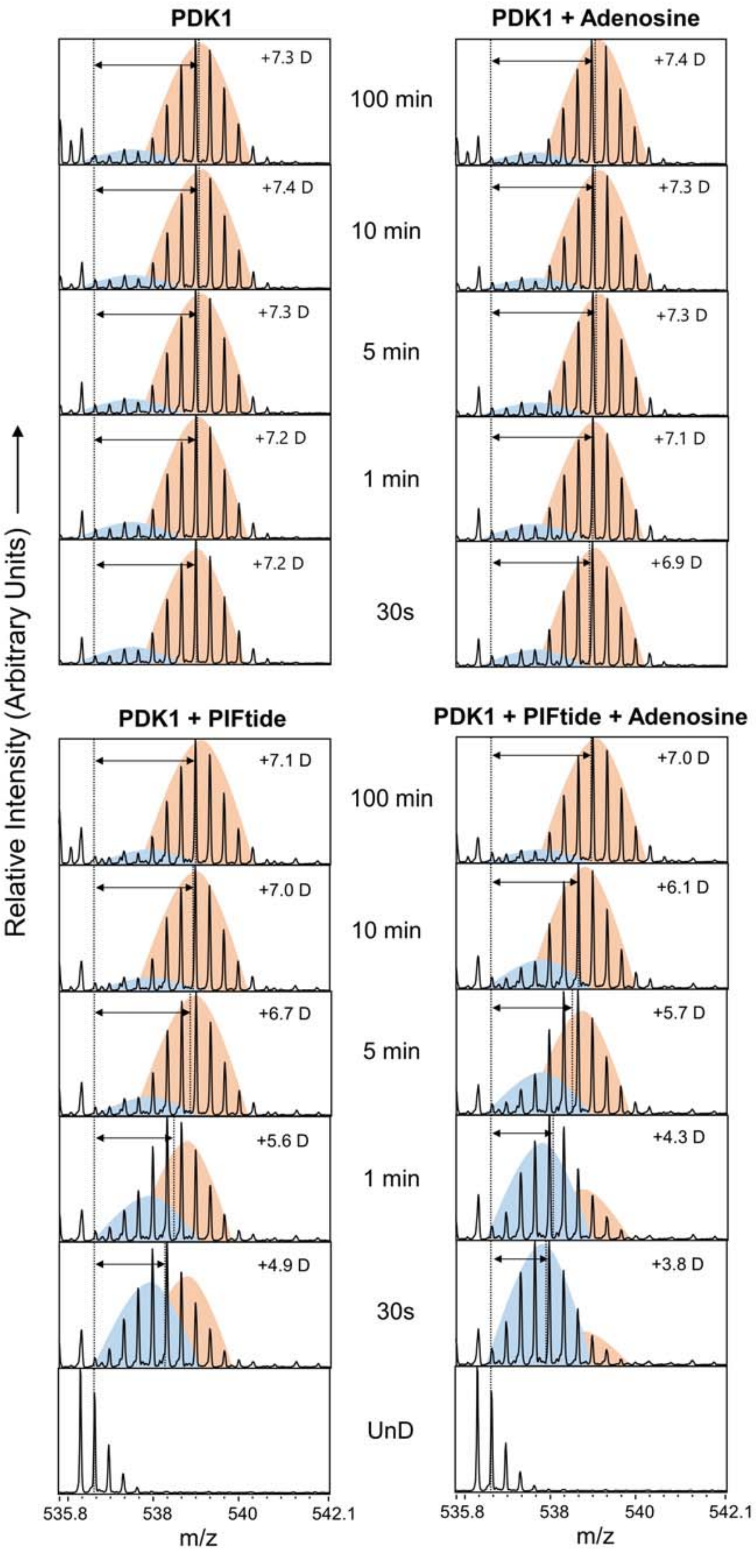

**Figure S5: Mass spectral timeseries of peptide 122-134 for all experimental states. Related to Figure 7.**

Stacked mass spectral envelope plots for peptide 122-134 spanning αC helix in different states for all experimental deuterium exchange time-points. Shift in centroids are relative to the undeuterated 'UnD' (aqueous) peptide shown at the bottom. Bimodal isotopic distributions can be resolved into low-exchanging (blue) and high-exchanging states (orange).

**Table S2: Summary of HDXMS data analysis statistics. Related to Experimental Procedures.**

| State | PDK1 Apo | PDK1 + Adenosine | PDK1 + PIFtide | PDK1 + Adenosine + PIFtide |
|---|---|---|---|---|
| **HDX Reaction Details** | Buffer B<br>(20 mM Tris-HCl pH 7.4, 500 mM NaCl, 1 mM DTT) | Buffer B +<br>1 mM adenosine | Buffer B +<br>10 µM PIFtide | Buffer B +<br>1 mM adenosine +<br>10 µM PIFtide |
| **HDX time course (min)** | 0.5, 1, 5, 10, 100 | 0.5, 1, 5, 10, 100 | 0.5, 1, 5, 10, 100 | 0.5, 1, 5, 10, 100 |
| **HDX control samples** | None | | | |
| **Back-exchange (mean / IQR)** | Not calculated | | | |
| **# of peptides** | 35 | 35 | 35 | 35 |
| **Sequence Coverage** | 89% | 89% | 89% | 89% |
| **Average peptide length / Redundancy** | 12.34 / 1.56 | 12.34 / 1.56 | 12.34 / 1.56 | 12.34 / 1.56 |
| **Replicates (biological or technical)** | 3 (technical) | 3 (technical) | 3 (technical) | 3 (technical) |
| **Repeatability (Average Standard Deviation)** | 0.027 | 0.028 | 0.025 | 0.025 |
| **Significant differences in HDX (delta HDX > X D)** | 0.106 D (99% CI) | | | |

**Table S3: Deuterium uptake in pepsin-fragment peptides of PDK1 kinase in apo-state and in complex with adenosine. Related to Figures 2, 3, 4, 5, 6 and 7; Table 1.**

| S. No. | Residues | Sequence (MH+) | MEA | z | PDK1 | | | | | PDK1 + Adenosine | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 30 s | 1 min | 5 min | 10 min | 100 min | 30 s | 1 min | 5 min | 10 min | 100 min |
| **1** | 49-66 | GAMDGTAAEPRPGAGSLQ (1685.79) | 15 | 2 | 10.0 ± 0.07 | 9.9 ± 0.05 | 10.1 ± 0.05 | 10.1 ± 0.02 | 10.1 ± 0.04 | 10.0 ± 0.06 | 10.0 ± 0.05 | 10.0 ± 0.05 | 9.9 ± 0.04 | 9.9 ± 0.01 |
| **2** | 67-81 | HAQPPPQPRKKRPED (1780.96) | 9 | 4 | 4.7 ± 0.03 | 4.8 ± 0.02 | 5.0 ± 0.03 | 5.0 ± 0.05 | 5.0 ± 0.01 | 5.0 ± 0.01 | 5.1 ± 0.03 | 5.2 ± 0.01 | 5.3 ± 0.01 | 5.3 ± 0.02 |
| **3** | 72-81 | PQPRKKRPED (1250.70) | 6 | 3 | 3.6 ± 0.03 | 3.7 ± 0.03 | 3.8 ± 0.03 | 3.8 ± 0.03 | 3.8 ± 0.03 | 3.9 ± 0.03 | 4.0 ± 0.03 | 4.1 ± 0.03 | 4.1 ± 0.02 | 4.1 ± 0.03 |
| **4** | 82-93 | FKFGKILGEGSF (1329.72) | 11 | 2 | 5.9 ± 0.04 | 6.0 ± 0.01 | 6.5 ± 0.01 | 6.6 ± 0.01 | 6.7 ± 0.01 | 5.6 ± 0.04 | 5.7 ± 0.05 | 6.1 ± 0.01 | 6.2 ± 0.01 | 6.7 ± 0.01 |
| **5** | 85-93 | GKILGEGSF (907.49) | 8 | 2 | 2.2 ± 0.03 | 2.3 ± 0.02 | 2.4 ± 0.03 | 2.6 ± 0.02 | 3.2 ± 0.05 | 1.7 ± 0.03 | 1.9 ± 0.03 | 2.4 ± 0.07 | 2.5 ± 0.05 | 3.0 ± 0.05 |
| **6** | 99-107 | ARELATSRE (1032.54) | 8 | 2 | 3.4 ± 0.02 | 3.7 ± 0.02 | 4.5 ± 0.03 | 4.7 ± 0.02 | 4.8 ± 0.03 | 3.5 ± 0.04 | 3.8 ± 0.03 | 4.4 ± 0.02 | 4.4 ± 0.02 | 4.7 ± 0.03 |
| **7** | 108-114 | YAIKILE (849.51) | 6 | 2 | 0.8 ± 0.01 | 0.8 ± 0.01 | 1.0 ± 0.01 | 1.1 ± 0.01 | 2.7 ± 0.03 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.9 ± 0.01 | 1.1 ± 0.01 |
| **8** | 108-121 | YAIKILEKRHIIKE (1754.07) | 13 | 4 | 3.8 ± 0.02 | 4.0 ± 0.02 | 4.3 ± 0.04 | 4.4 ± 0.05 | 5.6 ± 0.01 | 4.2 ± 0.02 | 4.2 ± 0.03 | 4.5 ± 0.02 | 4.4 ± 0.01 | 4.6 ± 0.01 |
| **9** | 115-130 | KRHIIKENKVPYVTRE (2010.16) | 14 | 4 | 7.0 ± 0.1 | 6.9 ± 0.05 | 7.0 ± 0.05 | 7.0 ± 0.03 | 6.9 ± 0.05 | 7.5 ± 0.13 | 7.5 ± 0.07 | 7.7 ± 0.05 | 7.6 ± 0.03 | 7.6 ± 0.02 |
| **10** | 122-130 | NKVPYVTRE (1105.60) | 7 | 2 | 4.8 ± 0.01 | 4.8 ± 0.01 | 4.8 ± 0.01 | 4.8 ± 0.02 | 4.8 ± 0.02 | 4.9 ± 0.01 | 4.9 ± 0.01 | 4.9 ± 0.02 | 4.9 ± 0.01 | 4.9 ± 0.01 |
| **11** | 122-134 | NKVPYVTRERDVM (1606.84) | 11 | 3 | 7.1 ± 0.03 | 7.2 ± 0.02 | 7.3 ± 0.02 | 7.3 ± 0.02 | 7.3 ± 0.03 | 6.9 ± 0.05 | 7.1 ± 0.04 | 7.4 ± 0.01 | 7.4 ± 0.01 | 7.4 ± 0.01 |
| **12** | 135-142 | SRLDHPFF (1018.51) | 6 | 2 | 1.3 ± 0.01 | 1.5 ± 0.01 | 1.9 ± 0.02 | 1.9 ± 0.01 | 2.2 ± 0.02 | 1.3 ± 0.04 | 1.6 ± 0.01 | 2.0 ± 0.02 | 1.9 ± 0.01 | 2.0 ± 0.01 |
| **13** | 146-152 | YFTFQDD (935.38) | 6 | 1 | 1.8 ± 0.01 | 1.8 ± 0.01 | 2.0 ± 0.01 | 2.2 ± 0.01 | 2.8 ± 0.01 | 1.8 ± 0.01 | 1.8 ± 0.01 | 1.9 ± 0.01 | 1.9 ± 0.01 | 2.2 ± 0.01 |
| **14** | 146-155 | YFTFQDDEKL (1305.60) | 9 | 2 | 1.9 ± | 2.0 ± | 2.4 ± | 2.6 ± | 3.6 ± | 2.0 ± | 2.0 ± | 2.2 ± | 2.4 ± | 3.0 ± |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.02 | 0.01 | 0.01 | 0.01 | 0.03 | 0.02 | 0.02 | 0.01 | 0.01 | 0.03 |
| **15** | 147-159 | FTFQDDEKLYFGL (1622.77) | 12 | 2 | 2.3 ± 0.02 | 2.4 ± 0.03 | 3.0 ± 0.03 | 3.2 ± 0.04 | 3.5 ± 0.04 | 2.2 ± 0.03 | 2.4 ± 0.04 | 2.9 ± 0.03 | 3.0 ± 0.03 | 3.4 ± 0.03 |
| **16** | 161-167 | YAKNGEL (794.40) | 6 | 2 | 2.3 ± 0.01 | 2.4 ± 0.01 | 2.6 ± 0.03 | 2.6 ± 0.02 | 2.6 ± 0.03 | 1.9 ± 0.03 | 2.1 ± 0.01 | 2.6 ± 0.03 | 2.6 ± 0.01 | 2.6 ± 0.01 |
| **17** | 168-177 | LKYIRKIGSF (1224.75) | 9 | 2 | 3.9 ± 0.01 | 3.9 ± 0.02 | 4.2 ± 0.05 | 4.4 ± 0.02 | 5.2 ± 0.02 | 4.2 ± 0.06 | 4.2 ± 0.08 | 4.6 ± 0.04 | 4.8 ± 0.03 | 5.6 ± 0.03 |
| **18** | 171-177 | IRKIGSF (820.50) | 6 | 1 | 3.3 ± 0.02 | 3.2 ± 0.02 | 3.4 ± 0.02 | 3.3 ± 0.01 | 3.3 ± 0.01 | 3.0 ± 0.03 | 3.1 ± 0.05 | 3.2 ± 0.03 | 3.0 ± 0.02 | 3.0 ± 0.02 |
| **19** | 176-190 | SFDETCTRFYTAEIV (1781.80) | 14 | 2 | 5.4 ± 0.05 | 5.4 ± 0.02 | 6.0 ± 0.04 | 6.3 ± 0.07 | 7.5 ± 0.04 | 5.5 ± 0.07 | 5.6 ± 0.08 | 6.0 ± 0.07 | 6.3 ± 0.04 | 7.5 ± 0.04 |
| **20** | 193-210 | LEYLHGKGIIHRDLKPEN (2132.16) | 16 | 4 | 1.5 ± 0.02 | 1.6 ± 0.01 | 2.3 ± 0.02 | 2.7 ± 0.02 | 4.3 ± 0.02 | 1.6 ± 0.01 | 1.6 ± 0.02 | 1.9 ± 0.02 | 2.1 ± 0.01 | 3.7 ± 0.02 |
| **21** | 194-212 | EYLHGKGIIHRDLKPENIL (2245.24) | 17 | 4 | 1.4 ± 0.01 | 1.5 ± 0.01 | 2.1 ± 0.01 | 2.5 ± 0.01 | 4.0 ± 0.01 | 1.5 ± 0.01 | 1.6 ± 0.01 | 1.8 ± 0.03 | 2.0 ± 0.01 | 3.4 ± 0.01 |
| **22** | 216-224 | DMHIQITDF (1119.51) | 8 | 2 | 1.4 ± 0.01 | 1.5 ± 0.02 | 1.6 ± 0.01 | 1.8 ± 0.02 | 2.3 ± 0.01 | 1.2 ± 0.01 | 1.4 ± 0.02 | 1.5 ± 0.01 | 1.6 ± 0.01 | 2.1 ± 0.01 |
| **23** | 225-247 | GTAKVLSPESKQARANSFVGTAQ (2427.20) | 21 | 3 | 13.8 ± 0.09 | 14.1 ± 0.03 | 14.4 ± 0.02 | 14.4 ± 0.02 | 14.4 ± 0.04 | 14.2 ± 0.06 | 14.4 ± 0.06 | 14.5 ± 0.02 | 14.4 ± 0.03 | 14.4 ± 0.02 |
| **24** | 254-265 | LTEKSACKSSDL (1281.64) | 11 | 3 | 4.1 ± 0.04 | 4.2 ± 0.02 | 4.6 ± 0.08 | 4.6 ± 0.06 | 4.8 ± 0.06 | 4.4 ± 0.07 | 4.5 ± 0.04 | 4.8 ± 0.05 | 4.7 ± 0.01 | 4.9 ± 0.01 |
| **25** | 275-291 | LVAGLPPFRAGNEGLIF (1770.99) | 14 | 2 | 5.5 ± 0.05 | 5.6 ± 0.01 | 6.1 ± 0.07 | 6.4 ± 0.02 | 7.7 ± 0.02 | 5.6 ± 0.06 | 5.6 ± 0.04 | 6.1 ± 0.06 | 6.4 ± 0.01 | 7.6 ± 0.03 |
| **26** | 276-291 | VAGLPPFRAGNEGLIF (1657.91) | 13 | 2 | 5.6 ± 0.04 | 5.6 ± 0.01 | 6.2 ± 0.05 | 6.4 ± 0.04 | 7.7 ± 0.01 | 5.6 ± 0.06 | 5.7 ± 0.04 | 6.1 ± 0.04 | 6.5 ± 0.01 | 7.7 ± 0.04 |
| **27** | 292-311 | AKIIKLEYDFPEKFFPKARD (2455.34) | 17 | 5 | 4.7 ± 0.09 | 4.9 ± 0.01 | 7.1 ± 0.04 | 8.0 ± 0.04 | 8.9 ± 0.04 | 5.1 ± 0.07 | 5.4 ± 0.04 | 7.3 ± 0.03 | 8.3 ± 0.03 | 9.6 ± 0.06 |
| **28** | 298-311 | EYDFPEKFFPKARD (1788.86) | 11 | 3 | 4.6 ± 0.03 | 4.6 ± 0.01 | 5.1 ± 0.03 | 5.4 ± 0.04 | 5.8 ± 0.05 | 4.8 ± 0.04 | 4.8 ± 0.02 | 5.3 ± 0.02 | 5.5 ± 0.01 | 6.0 ± 0.01 |
| **29** | 300-310 | DFPEKFFPKAR (1381.73) | 8 | 3 | 3.2 ± 0.02 | 3.3 ± 0.03 | 3.8 ± 0.08 | 4.2 ± 0.07 | 4.5 ± 0.04 | 3.4 ± 0.06 | 3.5 ± 0.08 | 4.0 ± 0.07 | 4.2 ± 0.05 | 4.6 ± 0.04 |
| **30** | 317-329 | LVLDATKRLGCEE (1446.76) | 12 | 3 | 3.2 ± 0.05 | 3.2 ± 0.02 | 3.5 ± 0.03 | 3.6 ± 0.02 | 5.0 ± 0.04 | 3.4 ± 0.06 | 3.4 ± 0.06 | 3.6 ± 0.02 | 3.7 ± 0.02 | 5.0 ± 0.04 |
| **31** | 320-329 | DATKRLGCEE (1121.53) | 9 | 2 | 2.2 ± | 2.2 ± | 2.5 ± | 2.6 ± | 4.4 ± | 2.3 ± | 2.3 ± | 2.4 ± | 2.6 ± | 4.1 ± |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.01 | 0.01 | 0.04 | 0.02 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.03 |
| **32** | 330-342 | MEGYGPLKAHPFF (1493.72) | 10 | 3 | 2.3 ± 0.01 | 2.5 ± 0.02 | 2.8 ± 0.01 | 2.8 ± 0.03 | 3.1 ± 0.02 | 2.4 ± 0.01 | 2.6 ± 0.01 | 2.8 ± 0.02 | 2.9 ± 0.01 | 3.1 ± 0.01 |
| **33** | 332-342 | GYGPLKAHPFF (1233.64) | 8 | 2 | 1.7 ± 0.02 | 1.8 ± 0.01 | 2.0 ± 0.03 | 2.1 ± 0.01 | 2.2 ± 0.04 | 1.9 ± 0.04 | 2.1 ± 0.04 | 2.2 ± 0.04 | 2.2 ± 0.04 | 2.4 ± 0.01 |
| **34** | 343-350 | ESVTWENL (977.46) | 7 | 1 | 4.0 ± 0.02 | 4.1 ± 0.01 | 4.6 ± 0.02 | 4.6 ± 0.01 | 4.7 ± 0.01 | 4.0 ± 0.01 | 4.1 ± 0.02 | 4.5 ± 0.01 | 4.6 ± 0.01 | 4.6 ± 0.01 |
| **35** | 351-359 | HQQTPPKLT (1049.57) | 6 | 2 | 2.9 ± 0.03 | 3.1 ± 0.01 | 3.3 ± 0.01 | 3.4 ± 0.01 | 3.4 ± 0.02 | 3.1 ± 0.01 | 3.2 ± 0.01 | 3.4 ± 0.01 | 3.5 ± 0.01 | 3.6 ± 0.01 |

All values are (Mean ± S.D.) from triplicates.
MH+: Mass of singly charged peptide ion
MEA: Maximum exchangeable amide hydrogens in the peptide
z: Charge-state (positive)

**Table S4: Deuterium uptake in pepsin-fragment peptides of PDK1 kinase in complex with PIFtide, and with both PIFtide and adenosine. Related to Figures 2, 3, 4, 5, 6 and 7; Table 1.**

| S. No. | Residues | Sequence (MH+) | MEA | z | PDK1 + PIFtide | | | | | PDK1 + PIFtide + Adenosine | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 30 s | 1 min | 5 min | 10 min | 100 min | 30 s | 1 min | 5 min | 10 min | 100 min |
| **1** | 49-66 | GAMDGTAAEPRPGAGSLQ (1685.79) | 15 | 2 | 10.0 ± 0.05 | 9.8 ± 0.03 | 9.8 ± 0.03 | 9.8 ± 0.02 | 9.8 ± 0.05 | 9.9 ± 0.03 | 10.0 ± 0.04 | 9.9 ± 0.06 | 9.9 ± 0.02 | 9.9 ± 0.02 |
| **2** | 67-81 | HAQPPPQPRKKRPED (1780.96) | 9 | 4 | 4.6 ± 0.02 | 4.8 ± 0.02 | 5.0 ± 0.01 | 5.1 ± 0.01 | 5.2 ± 0.04 | 4.4 ± 0.05 | 4.6 ± 0.02 | 5.0 ± 0.02 | 5.1 ± 0.01 | 5.2 ± 0.01 |
| **3** | 72-81 | PQPRKKRPED (1250.70) | 6 | 3 | 3.6 ± 0.03 | 3.7 ± 0.04 | 3.9 ± 0.03 | 3.9 ± 0.04 | 4.0 ± 0.04 | 3.4 ± 0.04 | 3.5 ± 0.03 | 3.9 ± 0.02 | 3.9 ± 0.03 | 4.0 ± 0.03 |
| **4** | 82-93 | FKFGKILGEGSF (1329.72) | 11 | 2 | 6.0 ± 0.03 | 6.1 ± 0.01 | 6.4 ± 0.01 | 6.5 ± 0.01 | 6.5 ± 0.02 | 5.5 ± 0.04 | 5.7 ± 0.01 | 5.9 ± 0.01 | 6.0 ± 0.01 | 6.6 ± 0.01 |
| **5** | 85-93 | GKILGEGSF (907.49) | 8 | 2 | 2.1 ± 0.02 | 2.2 ± 0.02 | 2.4 ± 0.04 | 2.5 ± 0.03 | 3.1 ± 0.04 | 1.6 ± 0.02 | 1.9 ± 0.02 | 2.4 ± 0.04 | 2.5 ± 0.01 | 2.9 ± 0.01 |
| **6** | 99-107 | ARELATSRE (1032.54) | 8 | 2 | 3.8 ± 0.03 | 4.1 ± 0.02 | 4.3 ± 0.02 | 4.4 ± 0.03 | 4.8 ± 0.03 | 3.5 ± 0.04 | 3.8 ± 0.02 | 4.3 ± 0.01 | 4.3 ± 0.02 | 4.4 ± 0.02 |
| **7** | 108-114 | YAIKILE (849.51) | 6 | 2 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 1.0 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 | 0.8 ± 0.01 |
| **8** | 108-121 | YAIKILEKRHIIKE (1754.07) | 13 | 4 | 3.0 ± 0.04 | 3.2 ± 0.03 | 3.8 ± 0.02 | 4.0 ± 0.02 | 4.4 ± 0.03 | 2.6 ± 0.04 | 2.8 ± 0.02 | 3.4 ± 0.01 | 3.7 ± 0.02 | 4.2 ± 0.01 |
| **9** | 115-130 | KRHIIKENKVPYVTRE (2010.16) | 14 | 4 | 5.7 ± 0.04 | 6.2 ± 0.02 | 6.8 ± 0.03 | 6.9 ± 0.01 | 7.1 ± 0.1 | 4.8 ± 0.09 | 5.4 ± 0.06 | 6.6 ± 0.03 | 7.0 ± 0.05 | 7.3 ± 0.04 |
| **10** | 122-130 | NKVPYVTRE (1105.60) | 7 | 2 | 4.3 ± 0.01 | 4.6 ± 0.01 | 4.8 ± 0.01 | 4.8 ± 0.02 | 4.9 ± 0.01 | 3.5 ± 0.02 | 3.9 ± 0.06 | 4.8 ± 0.01 | 4.9 ± 0.01 | 4.9 ± 0.03 |
| **11** | 122-134 | NKVPYVTRERDVM (1606.84) | 11 | 3 | 5.0 ± 0.09 | 5.6 ± 0.02 | 6.8 ± 0.03 | 7.1 ± 0.03 | 7.3 ± 0.03 | 3.9 ± 0.03 | 4.4 ± 0.05 | 5.8 ± 0.01 | 6.3 ± 0.02 | 7.2 ± 0.01 |
| **12** | 135-142 | SRLDHPFF (1018.51) | 6 | 2 | 1.1 ± 0.02 | 1.2 ± 0.01 | 1.6 ± 0.01 | 1.8 ± 0.01 | 1.9 ± 0.01 | 1.0 ± 0.02 | 1.2 ± 0.01 | 1.7 ± 0.01 | 1.8 ± 0.01 | 1.9 ± 0.01 |
| **13** | 146-152 | YFTFQDD (935.38) | 6 | 1 | 1.7 ± 0.01 | 1.8 ± 0.01 | 1.8 ± 0.01 | 1.8 ± 0.01 | 1.9 ± 0.01 | 1.5 ± 0.01 | 1.7 ± 0.01 | 1.8 ± 0.01 | 1.8 ± 0.01 | 1.8 ± 0.01 |
| **14** | 146-155 | YFTFQDDEKL (1305.60) | 9 | 2 | 1.8 ± | 1.9 ± | 1.9 ± | 2.0 ± | 2.4 ± | 1.6 ± | 1.8 ± | 1.9 ± | 2.0 ± | 2.3 ± |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| **15** | 147-159 | FTFQDDEKLYFGL (1622.77) | 12 | 2 | 2.3 ± 0.03 | 2.4 ± 0.03 | 2.8 ± 0.03 | 3.0 ± 0.04 | 3.3 ± 0.07 | 2.2 ± 0.06 | 2.4 ± 0.03 | 2.8 ± 0.04 | 3.0 ± 0.05 | 3.3 ± 0.04 |
| **16** | 161-167 | YAKNGEL (794.40) | 6 | 2 | 2.4 ± 0.03 | 2.5 ± 0.01 | 2.5 ± 0.01 | 2.5 ± 0.01 | 2.6 ± 0.01 | 1.7 ± 0.02 | 2.0 ± 0.01 | 2.5 ± 0.02 | 2.6 ± 0.01 | 2.6 ± 0.02 |
| **17** | 168-177 | LKYIRKIGSF (1224.75) | 9 | 2 | 3.9 ± 0.03 | 4.0 ± 0.01 | 4.2 ± 0.01 | 4.4 ± 0.01 | 5.3 ± 0.08 | 4.0 ± 0.03 | 4.1 ± 0.01 | 4.4 ± 0.03 | 4.6 ± 0.02 | 5.5 ± 0.01 |
| **18** | 171-177 | IRKIGSF (820.50) | 6 | 1 | 3.2 ± 0.01 | 3.2 ± 0.03 | 3.2 ± 0.01 | 3.2 ± 0.01 | 3.1 ± 0.01 | 3.2 ± 0.05 | 3.3 ± 0.02 | 3.1 ± 0.01 | 3.1 ± 0.02 | 3.0 ± 0.01 |
| **19** | 176-190 | SFDETCTRFYTAEIV (1781.80) | 14 | 2 | 5.5 ± 0.03 | 5.5 ± 0.06 | 5.8 ± 0.02 | 6.1 ± 0.04 | 7.4 ± 0.07 | 5.4 ± 0.08 | 5.5 ± 0.04 | 5.8 ± 0.04 | 6.1 ± 0.04 | 7.4 ± 0.02 |
| **20** | 193-210 | LEYLHGKGIIHRDLKPEN (2132.16) | 16 | 4 | 1.5 ± 0.01 | 1.6 ± 0.01 | 1.9 ± 0.02 | 2.2 ± 0.01 | 4.0 ± 0.07 | 1.4 ± 0.02 | 1.5 ± 0.01 | 1.7 ± 0.03 | 1.8 ± 0.02 | 2.7 ± 0.01 |
| **21** | 194-212 | EYLHGKGIIHRDLKPENIL (2245.24) | 17 | 4 | 1.4 ± 0.01 | 1.5 ± 0.01 | 1.7 ± 0.02 | 2.0 ± 0.01 | 3.6 ± 0.07 | 1.4 ± 0.02 | 1.4 ± 0.01 | 1.6 ± 0.03 | 1.7 ± 0.01 | 2.4 ± 0.01 |
| **22** | 216-224 | DMHIQITDF (1119.51) | 8 | 2 | 1.3 ± 0.01 | 1.5 ± 0.02 | 1.6 ± 0.02 | 1.8 ± 0.01 | 2.2 ± 0.01 | 0.8 ± 0.03 | 1.0 ± 0.04 | 1.5 ± 0.01 | 1.5 ± 0.01 | 2.1 ± 0.01 |
| **23** | 225-247 | GTAKVLSPESKQARANSFVGTAQ (2427.20) | 21 | 3 | 13.4 ± 0.08 | 13.8 ± 0.03 | 14.0 ± 0.02 | 14.1 ± 0.01 | 14.1 ± 0.05 | 13.1 ± 0.04 | 13.8 ± 0.04 | 14.3 ± 0.03 | 14.3 ± 0.03 | 14.3 ± 0.04 |
| **24** | 254-265 | LTEKSACKSSDL (1281.64) | 11 | 3 | 4.3 ± 0.06 | 4.4 ± 0.02 | 4.5 ± 0.02 | 4.6 ± 0.03 | 4.8 ± 0.01 | 4.2 ± 0.08 | 4.4 ± 0.01 | 4.6 ± 0.02 | 4.6 ± 0.01 | 4.8 ± 0.05 |
| **25** | 275-291 | LVAGLPPFRAGNEGLIF (1770.99) | 14 | 2 | 5.6 ± 0.07 | 5.6 ± 0.04 | 6.0 ± 0.02 | 6.3 ± 0.03 | 7.4 ± 0.04 | 5.5 ± 0.03 | 5.6 ± 0.01 | 6.0 ± 0.03 | 6.3 ± 0.03 | 7.5 ± 0.02 |
| **26** | 276-291 | VAGLPPFRAGNEGLIF (1657.91) | 13 | 2 | 5.6 ± 0.04 | 5.7 ± 0.04 | 5.9 ± 0.01 | 6.3 ± 0.02 | 7.4 ± 0.04 | 5.5 ± 0.01 | 5.7 ± 0.01 | 6.0 ± 0.02 | 6.3 ± 0.02 | 7.5 ± 0.01 |
| **27** | 292-311 | AKIIKLEYDFPEKFFPKARD (2455.34) | 17 | 5 | 5.0 ± 0.04 | 5.4 ± 0.01 | 7.1 ± 0.06 | 8.2 ± 0.04 | 9.3 ± 0.06 | 4.9 ± 0.1 | 5.2 ± 0.03 | 7.0 ± 0.01 | 8.1 ± 0.04 | 9.4 ± 0.03 |
| **28** | 298-311 | EYDFPEKFFPKARD (1788.86) | 11 | 3 | 4.7 ± 0.04 | 4.7 ± 0.02 | 5.1 ± 0.01 | 5.3 ± 0.03 | 5.8 ± 0.01 | 4.6 ± 0.06 | 4.6 ± 0.01 | 5.1 ± 0.02 | 5.3 ± 0.02 | 5.8 ± 0.03 |
| **29** | 300-310 | DFPEKFFPKAR (1381.73) | 8 | 3 | 3.4 ± 0.06 | 3.4 ± 0.06 | 3.9 ± 0.01 | 4.3 ± 0.06 | 4.6 ± 0.09 | 3.3 ± 0.1 | 3.3 ± 0.02 | 3.9 ± 0.07 | 4.2 ± 0.04 | 4.6 ± 0.04 |
| **30** | 317-329 | LVLDATKRLGCEE (1446.76) | 12 | 3 | 3.2 ± 0.05 | 3.4 ± 0.04 | 3.5 ± 0.01 | 3.6 ± 0.01 | 4.9 ± 0.04 | 3.3 ± 0.05 | 3.3 ± 0.02 | 3.5 ± 0.05 | 3.6 ± 0.03 | 4.6 ± 0.03 |
| **31** | 320-329 | DATKRLGCEE (1121.53) | 9 | 2 | 2.3 ± | 2.3 ± | 2.4 ± | 2.6 ± | 4.0 ± | 2.2 ± | 2.3 ± | 2.4 ± | 2.5 ± | 3.6 ± |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  | 0.01 | 0.02 | 0.02 | 0.02 | 0.07 | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 |
| **32** | 330-342 | MEGYGPLKAHPFF (1493.72) | 10 | 3 | 2.4 ± 0.01 | 2.6 ± 0.02 | 2.8 ± 0.02 | 2.8 ± 0.01 | 3.1 ± 0.02 | 2.3 ± 0.01 | 2.5 ± 0.01 | 2.8 ± 0.03 | 2.8 ± 0.02 | 3.1 ± 0.02 |
| **33** | 332-342 | GYGPLKAHPFF (1233.64) | 8 | 2 | 1.9 ± 0.05 | 2.0 ± 0.02 | 2.1 ± 0.01 | 2.1 ± 0.04 | 2.3 ± 0.04 | 1.7 ± 0.02 | 2.0 ± 0.06 | 2.1 ± 0.03 | 2.2 ± 0.03 | 2.4 ± 0.05 |
| **34** | 343-350 | ESVTWENL (977.46) | 7 | 1 | 4.1 ± 0.02 | 4.2 ± 0.01 | 4.5 ± 0.01 | 4.6 ± 0.01 | 4.6 ± 0.01 | 4.0 ± 0.01 | 4.1 ± 0.01 | 4.5 ± 0.01 | 4.6 ± 0.01 | 4.6 ± 0.01 |
| **35** | 351-359 | HQQTPPKLT (1049.57) | 6 | 2 | 3.0 ± 0.02 | 3.1 ± 0.02 | 3.2 ± 0.02 | 3.3 ± 0.01 | 3.4 ± 0.03 | 2.9 ± 0.01 | 3.0 ± 0.03 | 3.4 ± 0.02 | 3.4 ± 0.02 | 3.5 ± 0.01 |

All values are (Mean ± S.D.) from triplicates.
MH+: Mass of singly charged peptide ion
MEA: Maximum exchangeable amide hydrogens in the peptide
z: Charge-state (positive)

## SUPPORTING REFERENCES

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