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Supplemental Information

Structural Analysis of a Temperature-Induced Transition in a Viral Capsid Probed by HDX-MS

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Red (35 to 100% basal uptake)	Flat region ($m_{25 - 45°C} = m_{50 - 60°C}$)	2-18, 67-81, 71-81, 153-164, 153-172, 225-247, 296-323, 499-516.
	Saturation ($m_{25 - 45°C} > m_{50 - 60°C}$)	414-429, 422-429
Green (16 to 34% basal uptake)	Linear increase ($m_{25 - 45°C} = m_{50 - 60°C}$)	309-323, 430-446, 517-525, 534-550, 559-572, 386-413, 386-398
	Decrease deuteration after T_M ($m_{25 - 45°C} > m_{50 - 60°C}$)	*82-113*,* 178-195, 216-224, 347-385, 473-497,476-498, 482-498, 573-587, 559-587
Blue (0 to 15% basal uptake)	Linear increase ($m_{25 - 45°C} = m_{50 - 60°C}$)	106-113, 196-215, 447-455, 456-472, *99-113*,* 128-134, 526-533
	Increase deuteration After T_M ($m_{25 - 45°C} < m_{50 - 60°C}$)	60-66, 248-255

Table S1: Peptide classification according to deuterium uptake before and after the transition. *Because peptides 82-113 and 99-113 present overlapping sequence; we split their sequences in two (i.e., 82-100 and 101-113) to map them on the structure.

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1   - MSDGTSQPDSGNAVHSAARVERAADGPGGSGGGGSGGGGVGVSTGSYDNQ - 50
51  - THYRFLGDGWVEITALATRLVHLNMPKSENYCRIRVHNTTDTSVKGNMAK - 100
101 - DDAHEQIWTPWSLVDANAWGVWLQPSDWQYICNTMSQLNLVSLDQEIFNV - 150
151 - VLKTVTEQDLGGQAIKIYNNDLTACMMVAVDSNNILPYTPAANSMETLGF - 200
201 - YPWKPTIASPYRYYFCVDRDLSVTYENQEGTVEHNVMGTPKGMNSQFFTI - 250
251 - ENTQQITLLRTGDEFATGTYYFDTNSVKLTHTWQTNRQLGQPPLLSTFPE - 300
301 - ADTDAGTLTAQGSRHGTTQMGVNWVSEAIRTRPAQVGFCQPHNDFEASRA - 350
351 - GPFAAPKVPADITQGVDKEANGSVRYSYGKQHGENWASHGPAPERYTWDE - 400
401 - TSFGSGRDTKDGFIQSAPLVVPPPLNGILTNANPIGTKNDIHFSNVFNSY - 450
451 - GPLTAFSHPSPVYPQGQIWDKELDLEHKPRLHITAPFVCKNNAPGQMLVR - 500
501 - LGPNLTDQYDPNGATLSRIVTYGTFFWKGKLTMRAKLRANTTWNPVYQVS - 550
551 - AEDNGNSYMSVTKWLPTATGNMQSVPLITRPVARNTY – 587
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Figure S1: Complete sequence of the VP2 capsid protein used in this study.

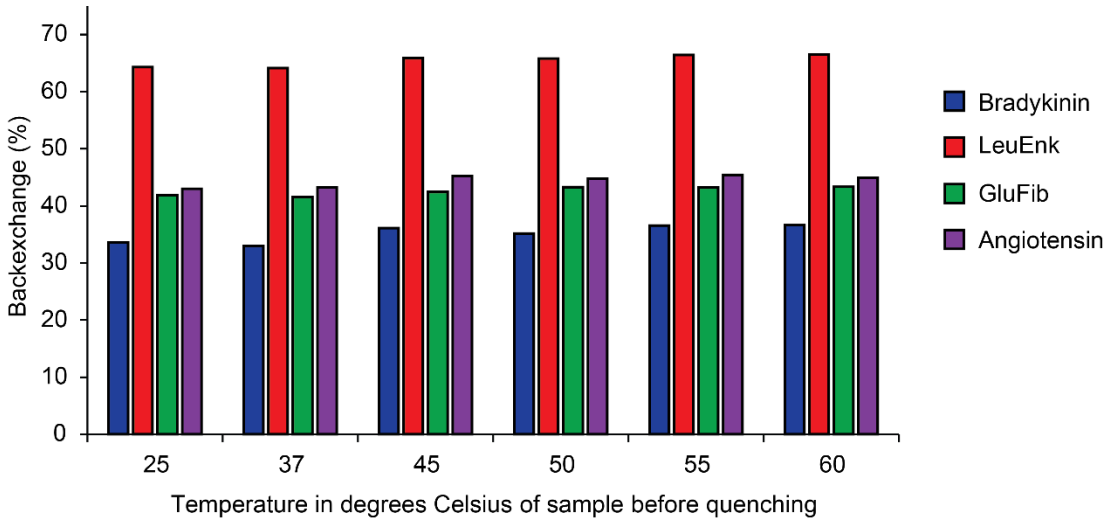

Figure S2: Dependence of back exchange on sample temperature. Back-exchange levels of four different model peptides acquired after incubation of the samples at different temperatures. Back-exchange levels did not substantially increase at higher temperatures.

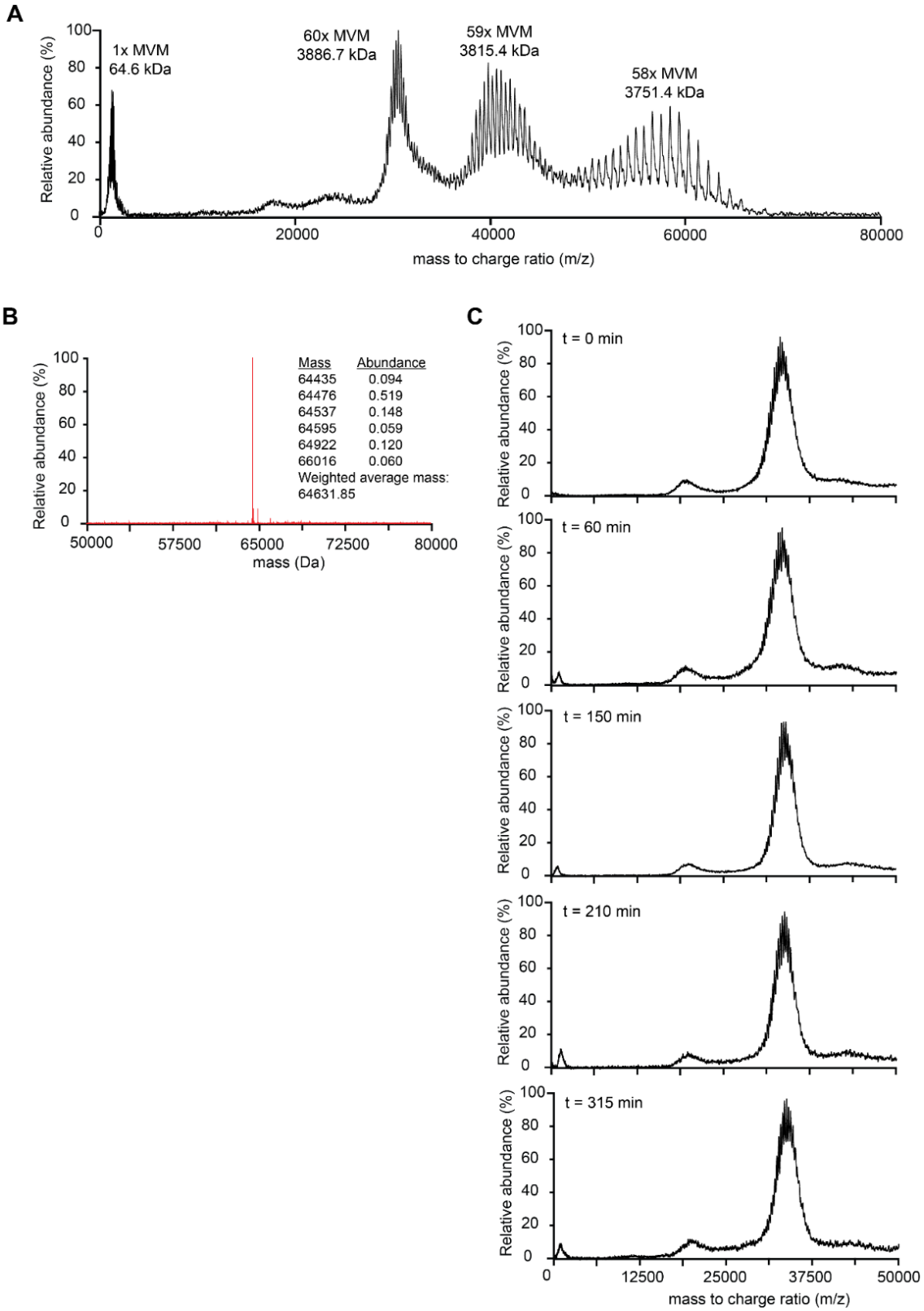

Figure S3: Mass spectrometry data on MVM VLPs used in this study. A) Tandem MS spectrum of intact VLPs (60x MVM, 3886.7 kDa) shows sequential loss of monomeric VP2 subunits (1x MVM, 64.6 kDa) resulting in VLPs missing a single copy of VP2 (59x MVM, 3815.4 kDa) and two copies of VP2 (58x MVM, 3751.4 kDa). This confirms the VLPs consist of 60 copies of VP2. B). Deconvoluted mass spectrum of denatured VP2 subunits provides a weighted averages mass of the monomeric building block. C). Native mass spectra of MVM VLPs under HDX conditions (HDX

buffer and heating at 60 ºC) shows that even for extended periods under harsh conditions the VLPs remain intact.

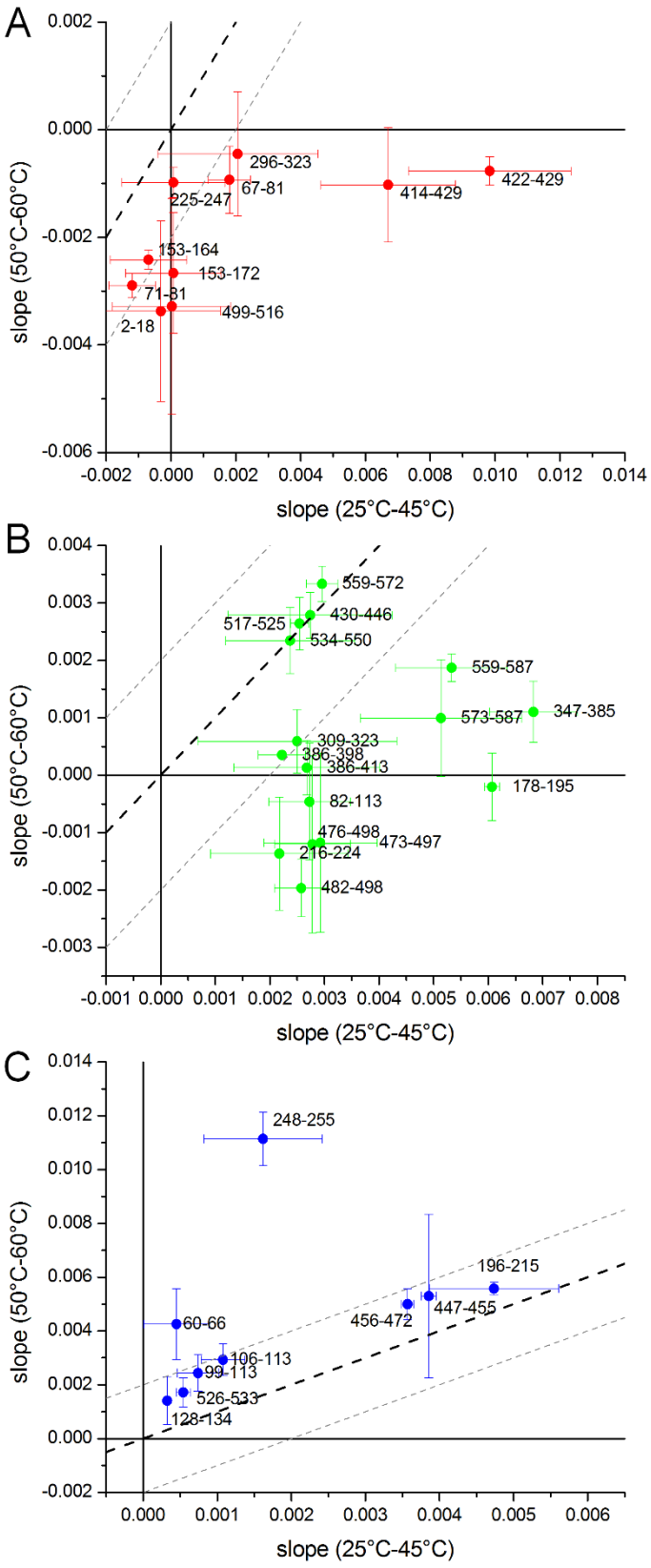

Figure S4: Average enthalpy before and after the temperature-induced transition. A) Average enthalpy from 50ºC to 60ºC versus 25ºC to 45ºC for each peptide presenting a high basal uptake (35 to 100%). Dashed line corresponds to a line with a slope equal to the unit, where peptides presenting

the same level of uptake before and after the transition should fall. B, C) The same as before but for peptides presenting medium (16 to 34%, green) and low (0 to 15%) uptake at the basal state.

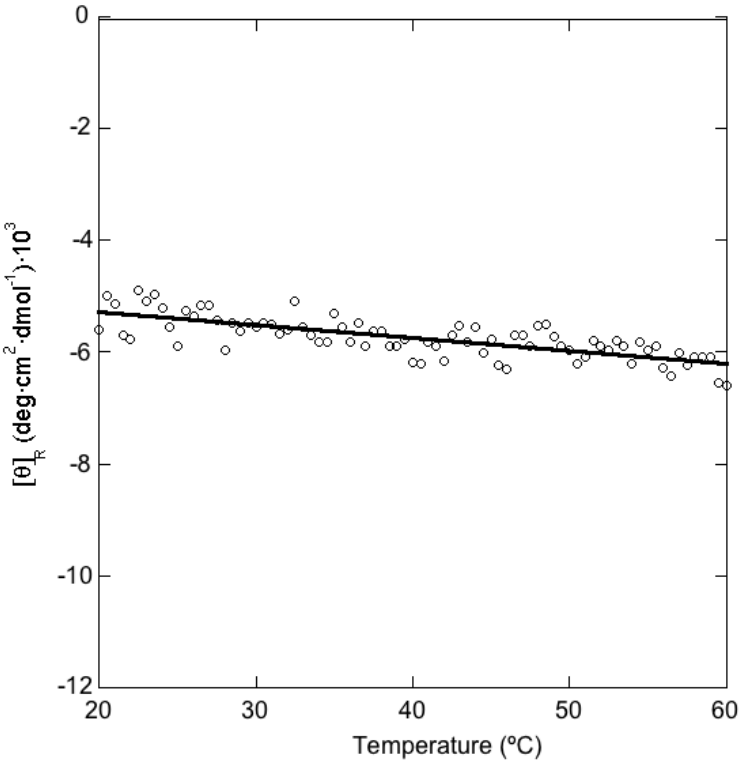

Figure S5: Circular Dichroism (CD) Spectroscopy of MVM VLPs at increasing temperatures. Molar ellipticity at 215 nm is represented as a function of temperature. No detectable change in secondary structure was observed at the transition temperature or any other temperature.

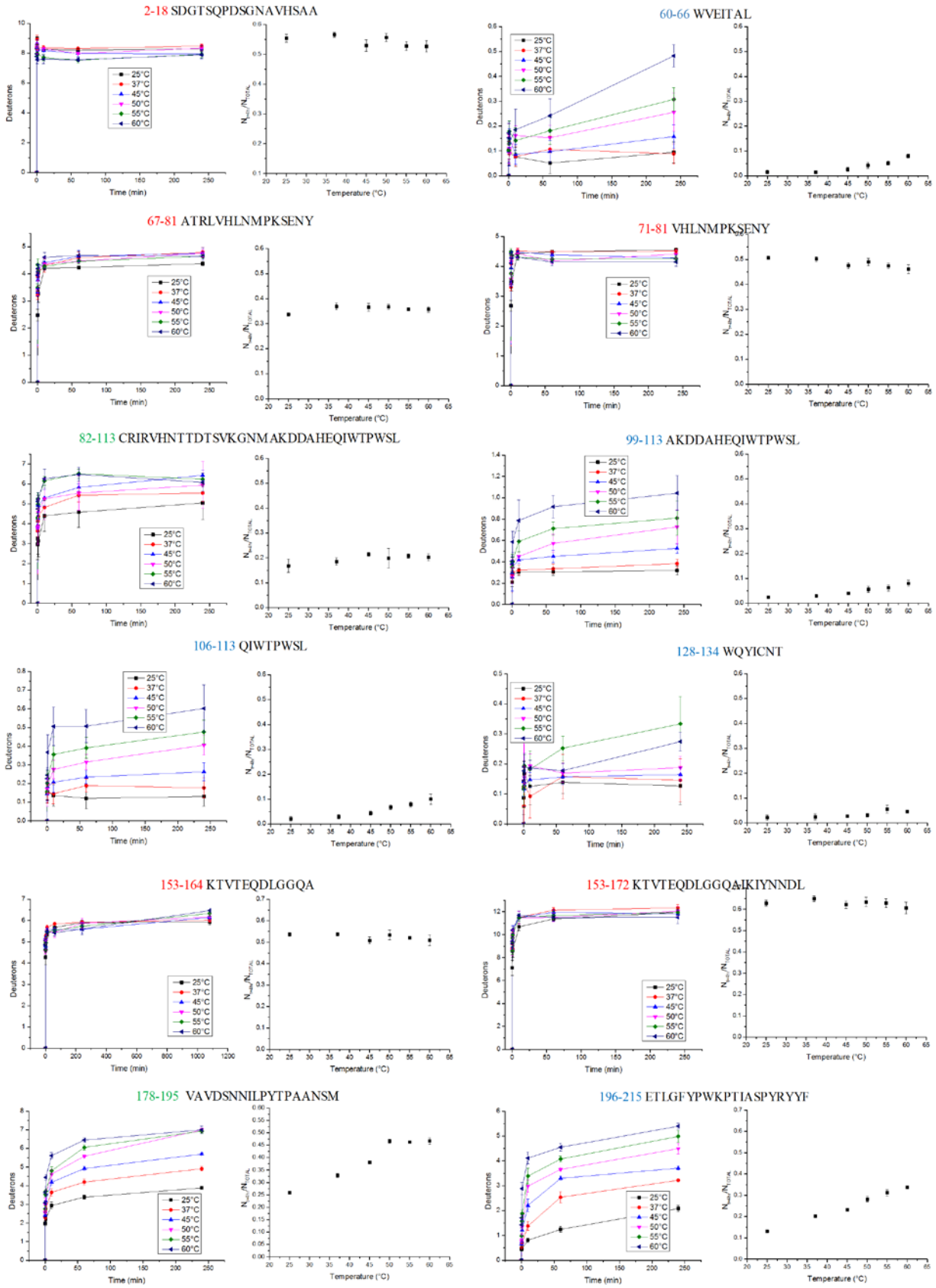

Figure S6: HDX time courses as a function of temperature are represented together with the corresponding Arrhenius plot. The peptide sequence is shown on top of each figure, with the color (red, green, and blue) indicating the level of uptake in the basal state.

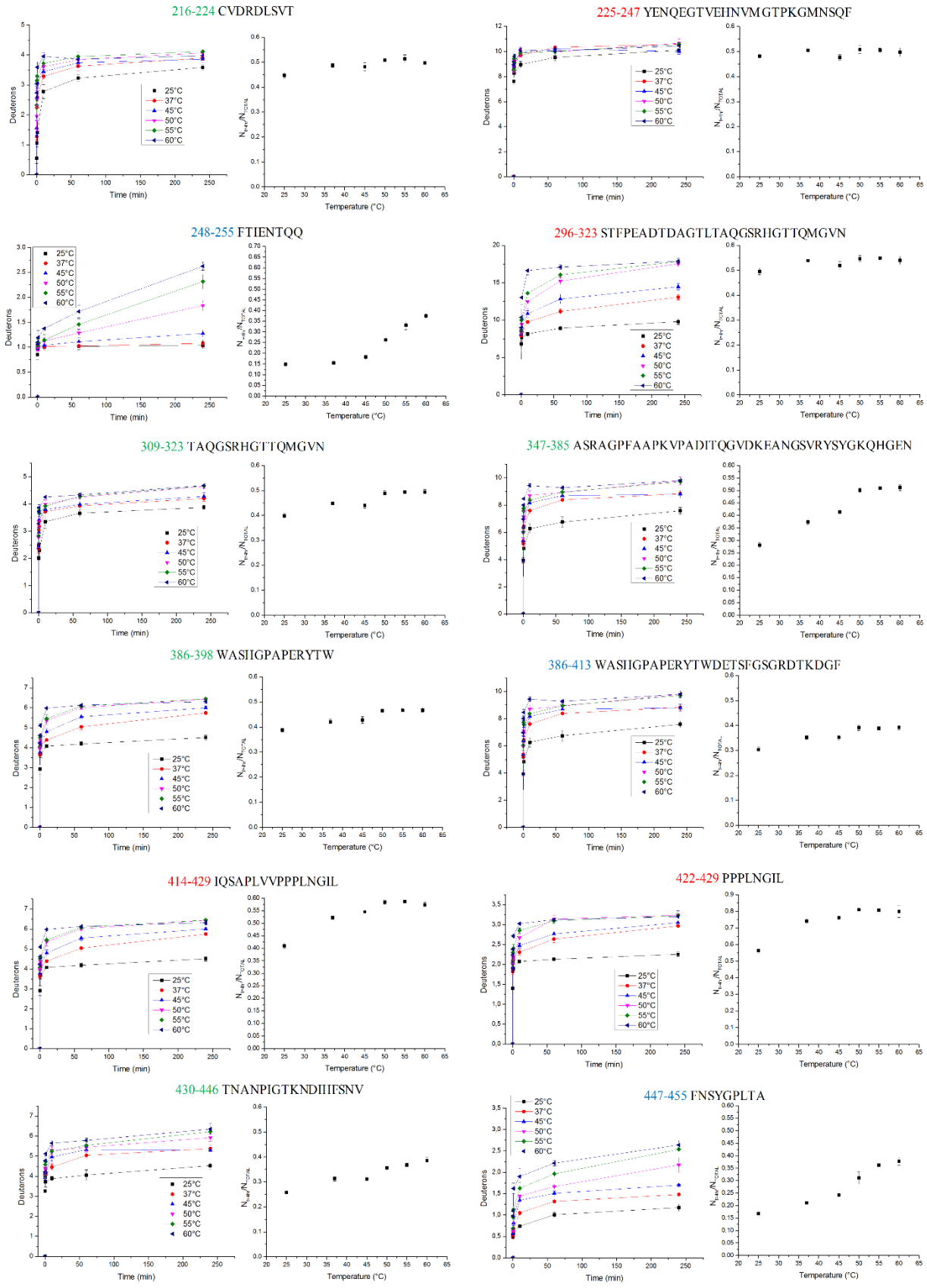

Figure S7: HDX time courses as a function of temperature are represented together with the corresponding Arrhenius plot. The peptide sequence is shown on top of each figure, with the color (red, green, and blue) indicating the level of uptake in the basal state.

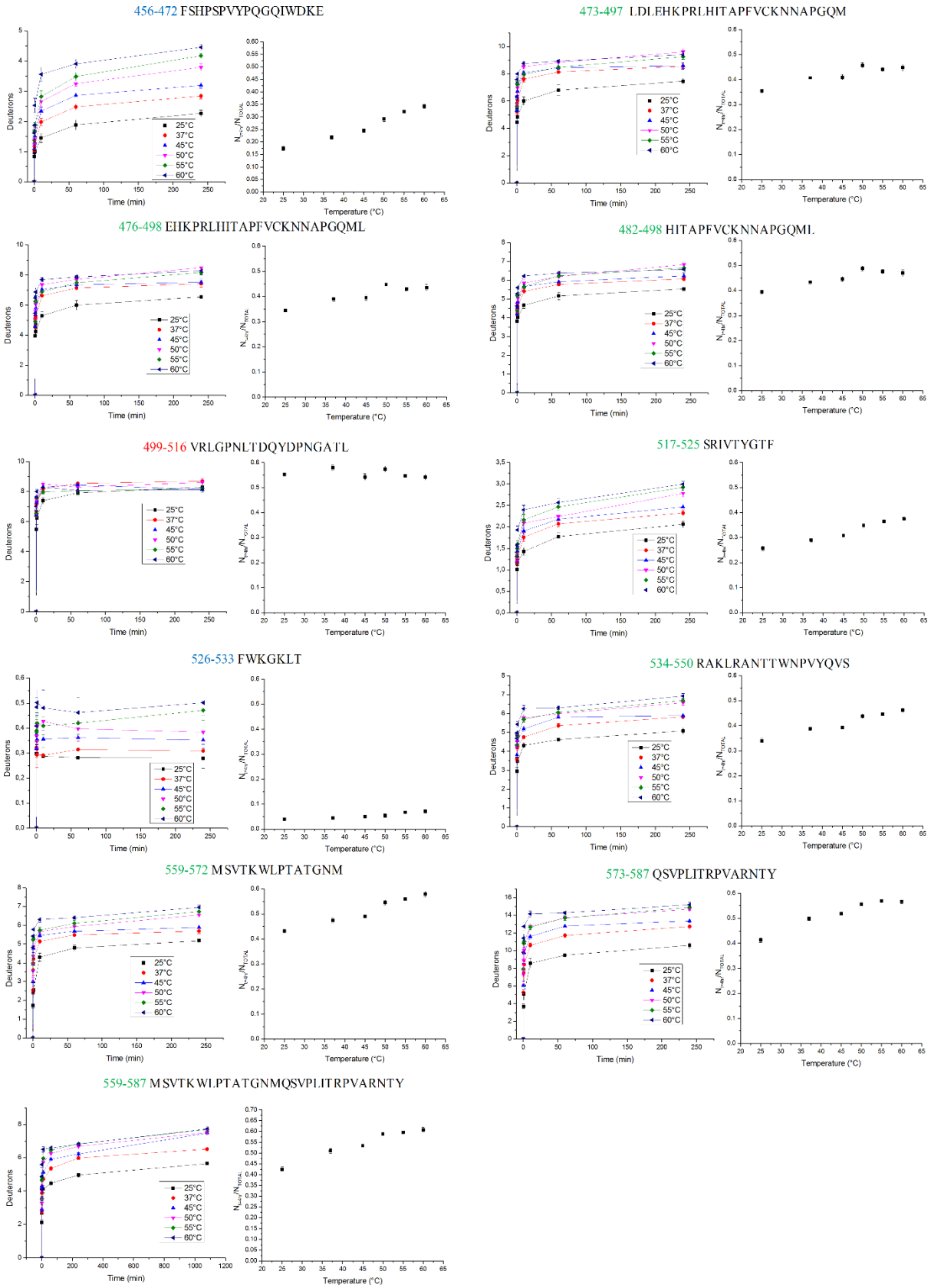

Figure S8: HDX time courses as a function of temperature are represented together with the corresponding Arrhenius plot. The peptide sequence is shown on top of each figure, with the color (red, green, and blue) indicating the level of uptake in the basal state.

NOTE 1

Data fitting to a two-state transition - The transition temperature was determined by fitting the data to a unimolecular N (native) – A (activated) two-state transition (2). Thermodynamics parameters (T_M, ΔH^{Tm}) for thermal 'unfolding' and baseline values were obtained by nonlinear fitting of the experimental number of deuterons or fluorescence intensity value D at any temperature T to the equation:

$$D = \frac{(D_{n0}+m_nT)+(D_{u0}+m_uT)e^{\frac{-\left[\Delta H^{T}{}_{M}*(1-T/T_M)+\Delta C_p*\left(T-T_M-T*ln\left(\frac{T}{T_M}\right)\right)\right]}{RT}}}{1+e^{\frac{-\left[\Delta H*(1-T/T_M)+\Delta C_p*\left(T-T_M-T*ln\left(\frac{T}{T_M}\right)\right)\right]}{RT}}}$$

where T_M is the transition temperature; ΔH^{Tm}, the enthalpy of the transition at the T_M; D_{n0} and D_{u0}, the number of deuterons or fluorescence intensity corresponding, respectively, to the native (N) or activated (A) states extrapolated to 0ºC; and m_n and m_u the linear increase in number of deuterons or fluorescence intensity as a function of temperature for the native (n) or (a) activated states, respectively. The value of ΔC_p was fixed to 1.2 kcal/mol as previously done for similar studies, although variation of it did not affect the values of ΔH^{Tm} and T_M. For most of the case the values of the transition enthalpy, ΔH^{Tm}, were subjected to large errors thus making impossible an accurate estimation. For HDX-MS experiments, all peptides showing a linear increase in uptake were fitted, and the ones showing square r values of 0.99 or higher (11 peptides) were used for the calculation of the average T_m. This is a simplified model with only two states. The model assumes that before and after the transition the linear behavior is the same. For instance, increasing the tempreature will lead to the disassociation of the capsid but the model does not account for that. Initial D_{n0}, D_{u0}, m_n and m_u values (obtained by a linear fit of the curves before and after were given for the fitting), ΔH^{Tm} and T_M were always set free. Slightly different values of D_{n0}, D_{u0}, m_n and m_u led to the same T_M value.