The membrane-associated conformation of HIV-1 Nef investigated with hydrogen exchange mass spectrometry at a Langmuir monolayer

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SUPPORTING INFORMATION

Figures S1, S2, S3 and S4

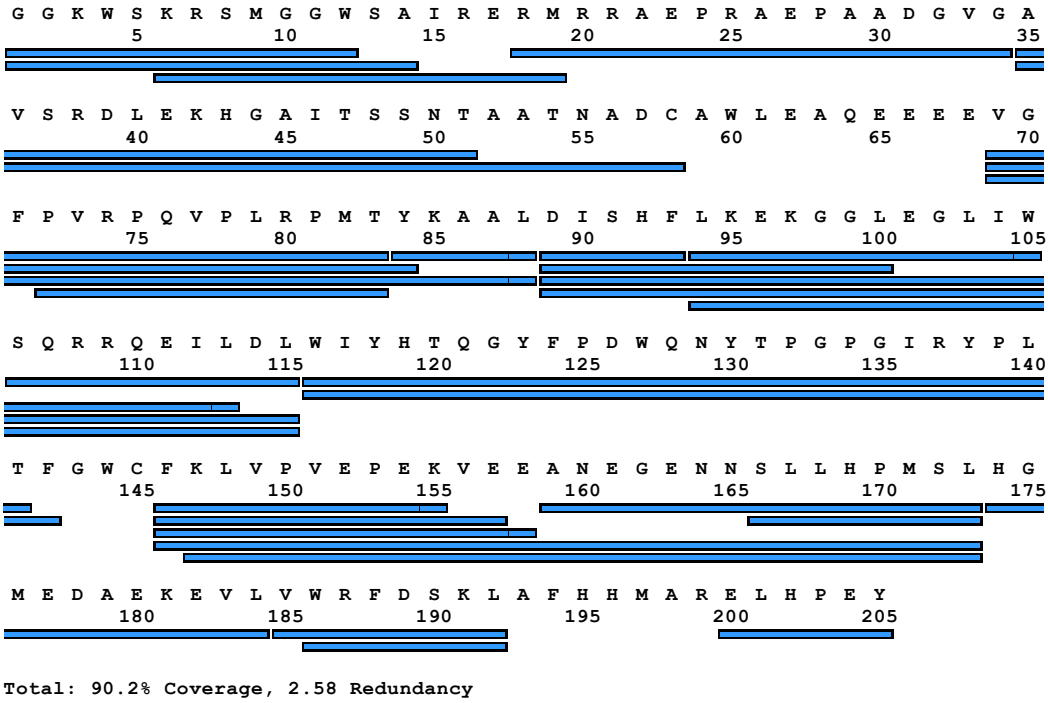

Figure S1.
Coverage map of identified peptides from myrNef generated using a mixture of pepsin and aspergillopepsin, and followed during HX MS experiments. Reproducible peptides (blue bars) were found at least three times in quadruplicate runs. The sequence coverage and redundancy are indicated below the map.

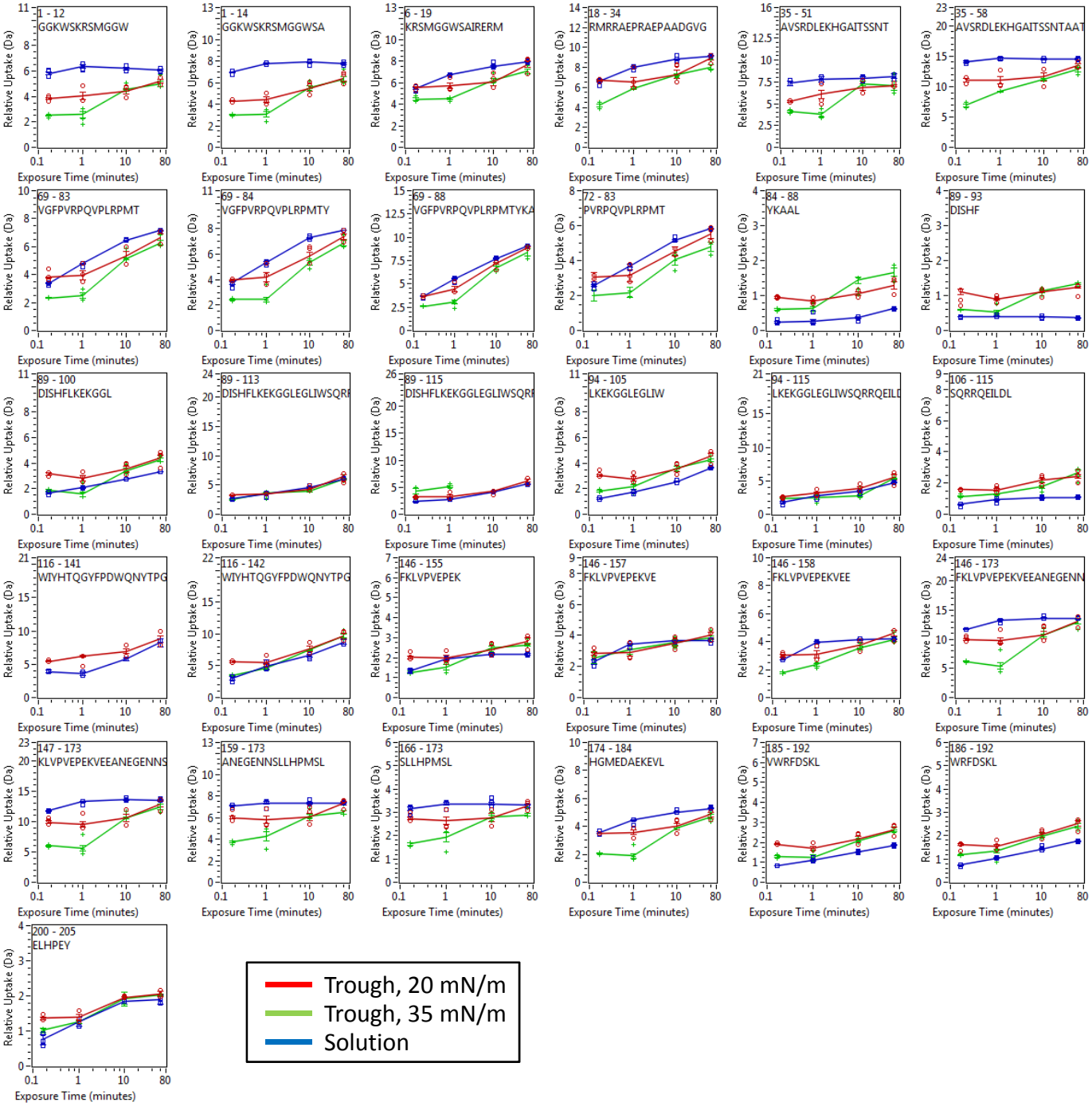

Figure S2.
Deuterium incorporation curves for myrNef comparing exchange in the trough systems with two lipid packing densities (red & green) versus exchange in solution (blue). All measurements shown were performed at pH 6.0. The average deuterium incorporation from three independent experiments is plotted with the error bars representing the spread of the measurements.

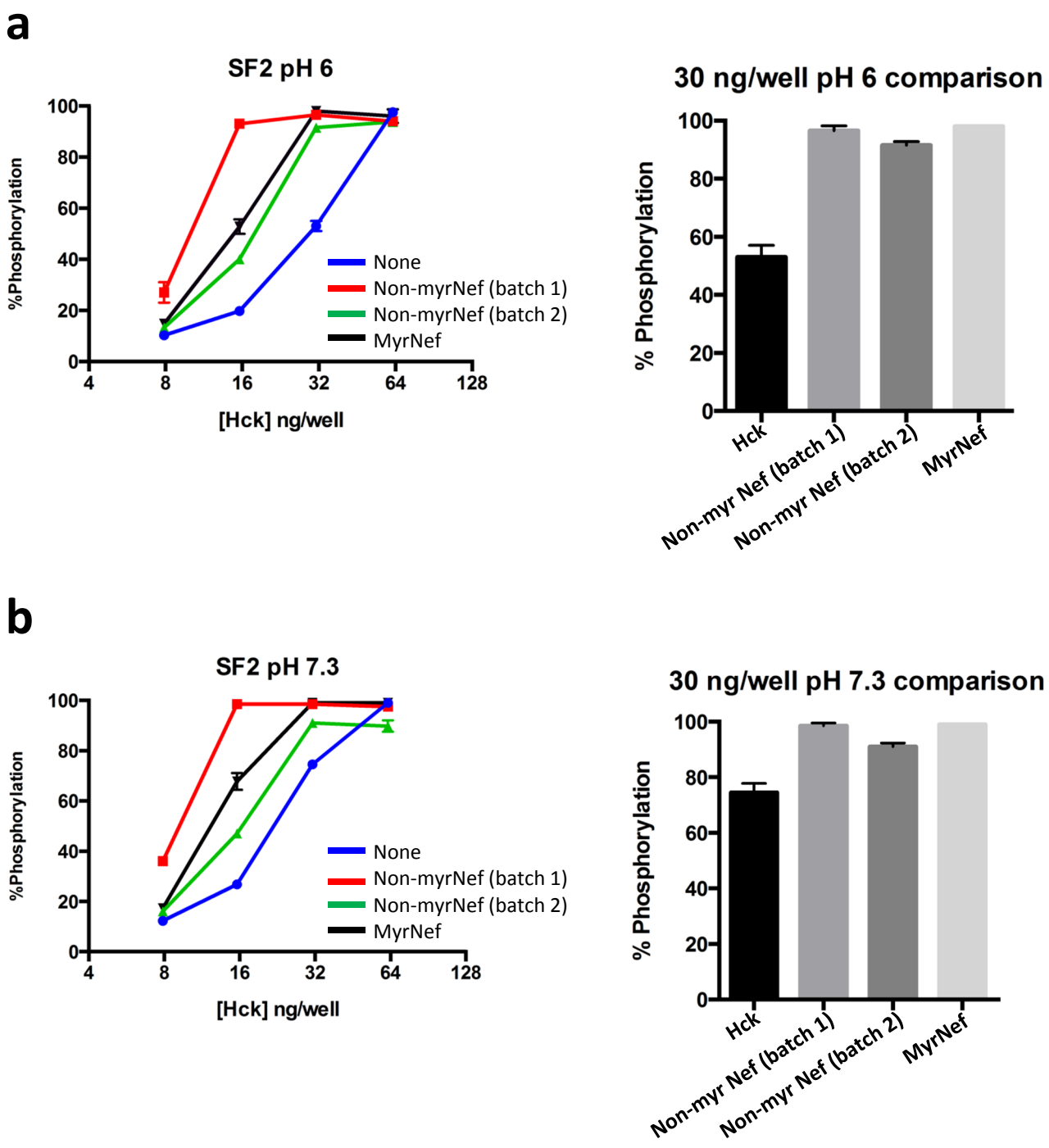

Figure S3.
Nef retains function at both pH 6.0 and pH 7.3. The activity of Hck-YEEI was measured in the Z'Lyte *in vitro* kinase assay as a reporter for Nef functionality, as described in Emert-Sedlak et al. (2009). *ACS Chem. Biol.* 4(11), 930-947. (**a**). Hck activation at pH 6.0. Hck-YEEI was titrated into the assay alone or with a ten-fold molar excess of Nef (myr or non-mry). Maximum activation was realized at 64 ng/well with both constructs. (**b**). The same assay was repeated at pH 7.3 to mimic the conditions used for some previous solution HX experiments. Maximum Hck-YEEI activation was also realized at 64 ng/well for both constructs.

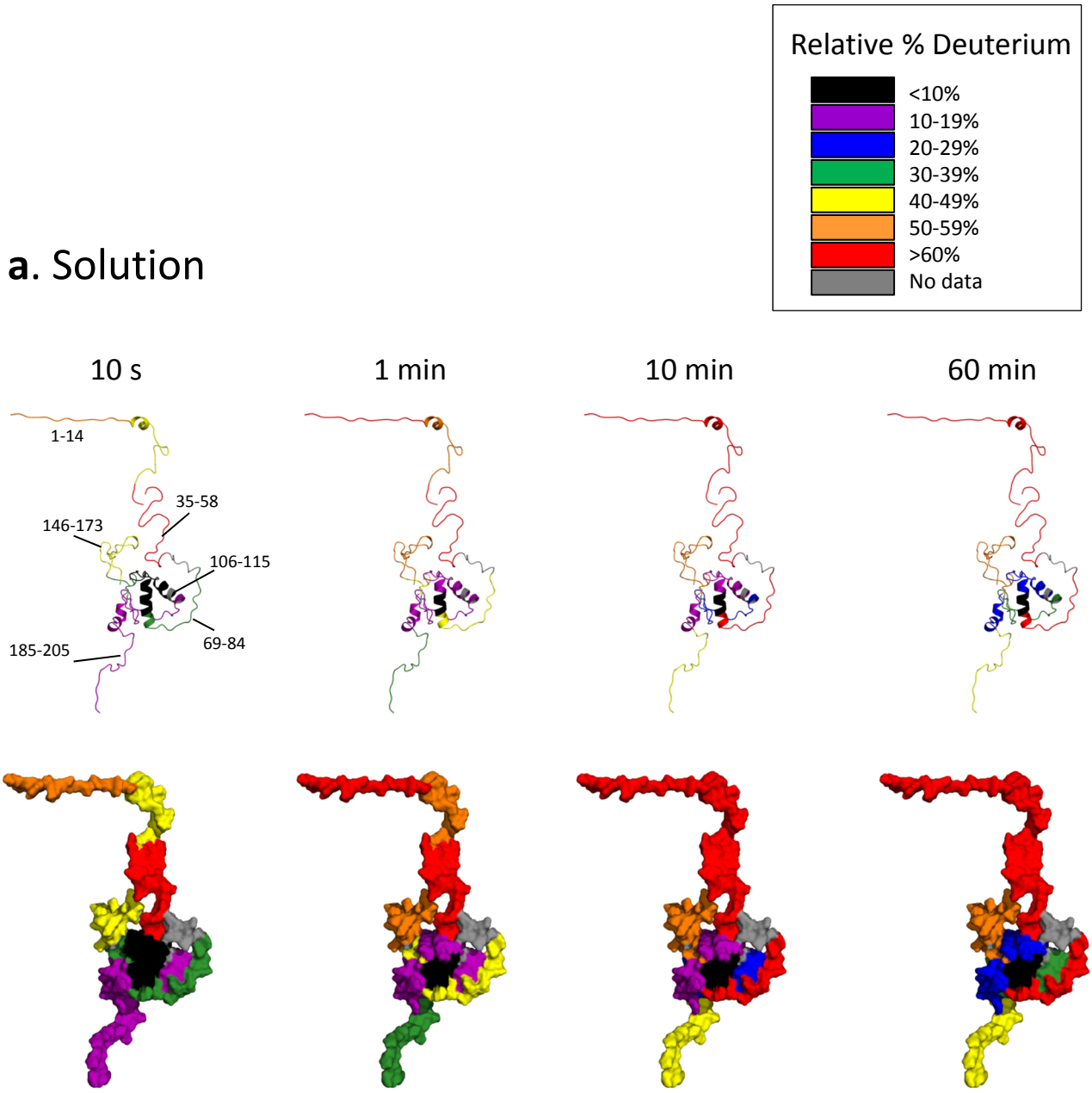

Figure S4.
Deuteration of HIV-1 myrNef in various situations mapped onto the Nef structural model [Geyer & Peterlin (2001). *FEBS Lett.* **496**, 91-95].

(**a**). The relative percent deuterium incorporation for myrNef in solution. Time in deuterium is shown at the top and % relative deuterium is colored on the structure according to the scale shown. These results are derived from the data shown in Figure S2.

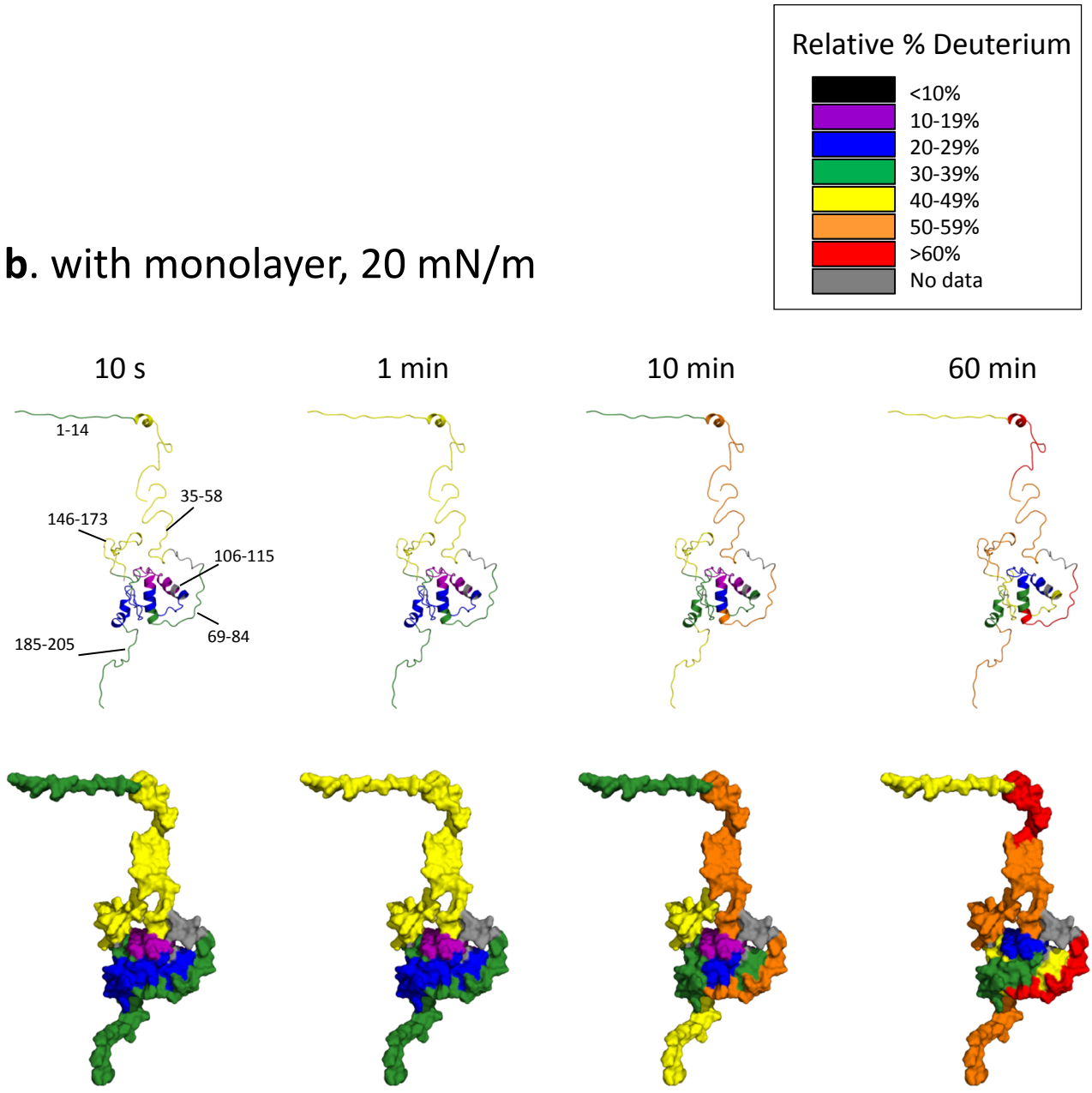

Figure S4 (continued).
(**b**). The relative percent deuterium incorporation for myrNef with a Langmuir monolayer at 20 mN/m. Time in deuterium is shown at the top and % relative deuterium is colored on the structure according to the scale shown. These results are derived from the data shown in Figure S2.

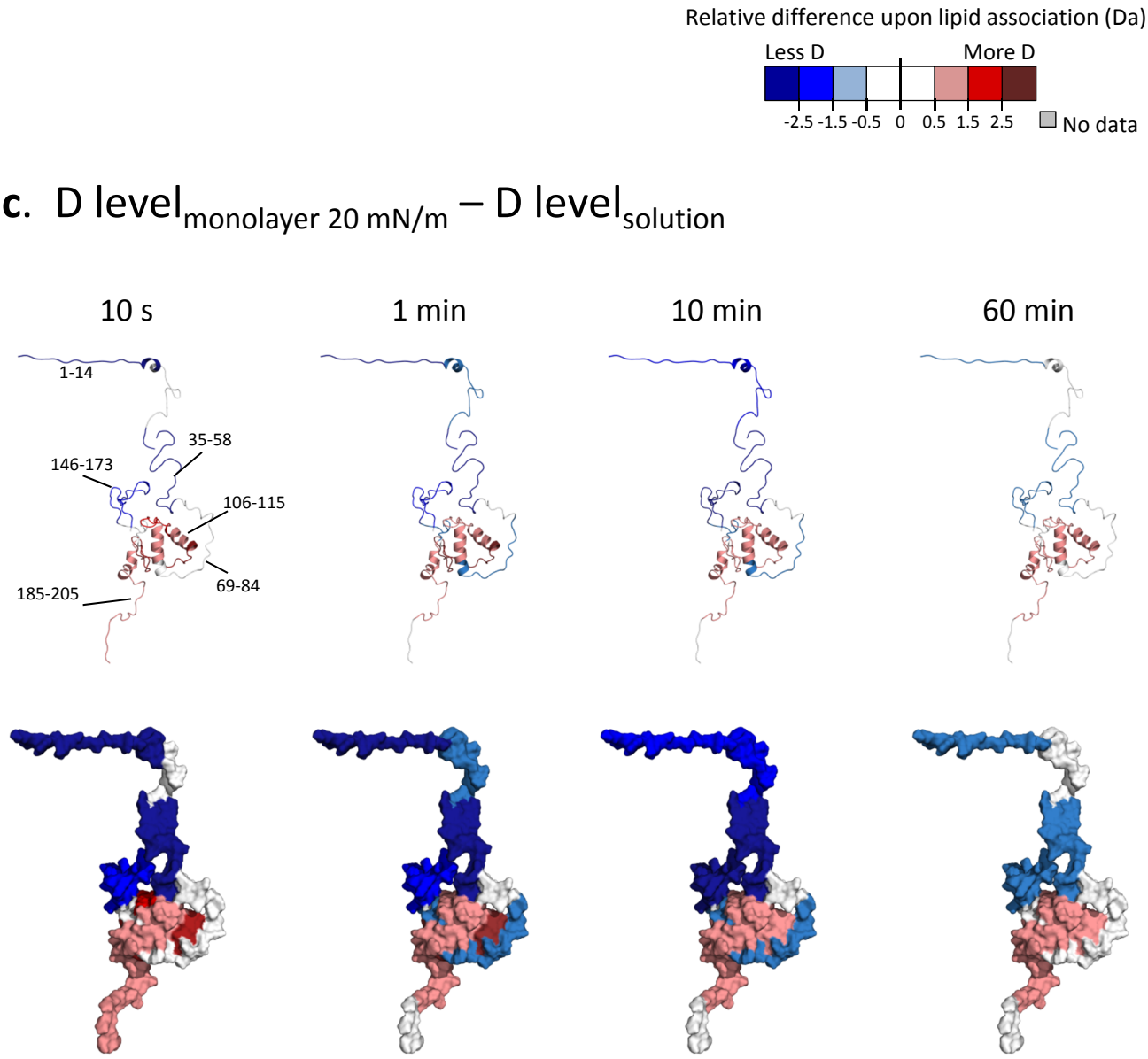

Figure S4 (continued).
(**c**). The effect of lipid association on myrNef deuteration. The average amount of deuterium (in Da) for HX in solution was subtracted from the average amount of deuterium after HX in the trough (monolayer associated 20 mN/m) and the value colored (positive values in reds, negative values in blues, as indicated – i.e. blue: less deuterium when with lipid, red: more deuterium when with lipid). Time in deuterium is shown at the top. These results are derived the data shown in Figure S2 as summarized in Figure 2a.

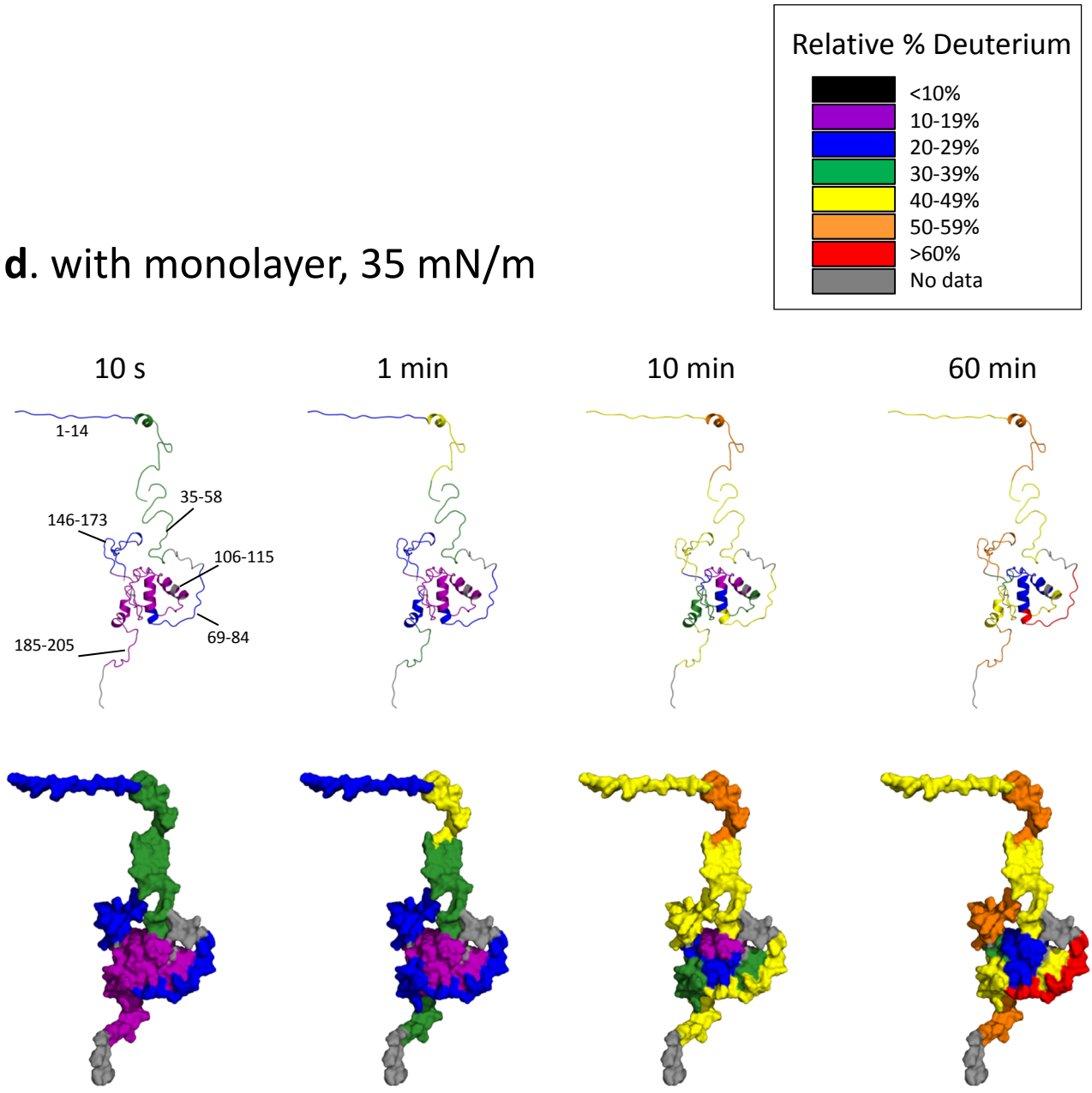

Figure S4 (continued).
(**d**). The relative percent deuterium incorporation for myrNef with a Langmuir monolayer at 35 mN/m. Time in deuterium is shown at the top and % relative deuterium is colored on the structure according to the scale shown. These results are derived from the data shown in Figure S2.

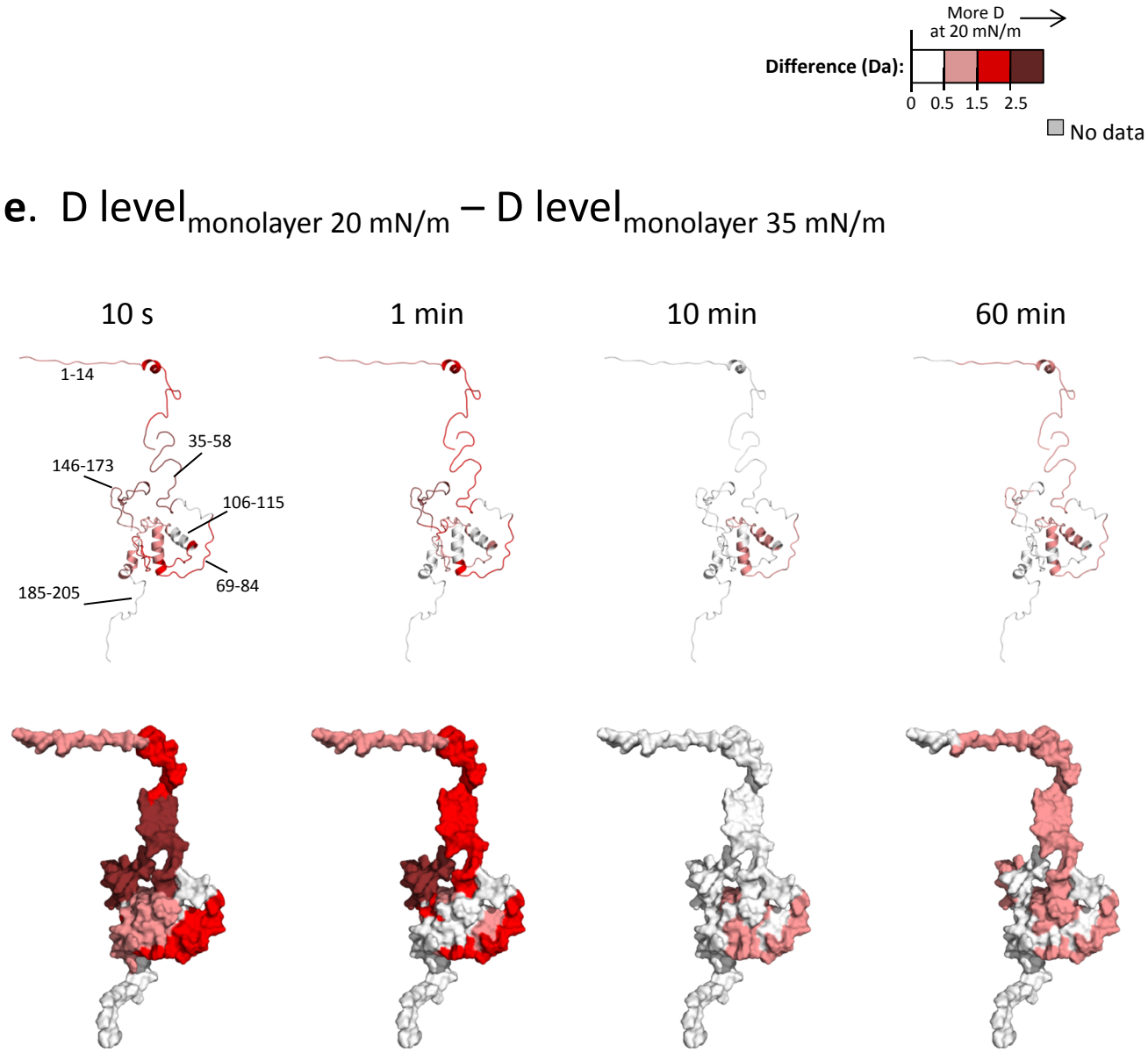

Figure S4 (continued).
(**e**). The effect of lipid packing density on myrNef deuteration. The average amount of deuterium (in Da) after HX using a monolayer pressure of 35 mN/m was subtracted from the average amount of deuterium after HX using a monolayer pressure of 20 mN/m and the value colored (positive values in reds, as indicated). Time in deuterium is shown at the top. These results are derived the data shown in Figure S2 as summarized in Figure 3b.