SUPPORTING INFORMATION

Thermodynamic and Kinetics Analysis of Peptides Derived from CapZ, NDR, p53, HDM2, and HDM4 Binding to Human S100B

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Far-UV CD

Far-UV circular dichroism spectroscopy is a valuable technique to assess the overall secondary structure composition of proteins and peptides. The helical secondary structure is characterized by minima at 222 nm and 208 nm, whereas β-sheets have a minimum around 215 nm (*114*). Intrinsically disordered proteins and unstructured polypeptides tend to have a featureless spectrum until approximately 210 nm and a minimum just below 200 nm. Far-UV CD spectra of the free peptides were recorded at 25°C to determine the secondary structure content. The helical content of the $p53^{(367-388)}$, $TRTK12^{(265-276)}$, $NDR^{(62-87)}$, and $HDM4^{(25-47)}$ peptides are estimated to be 10% or less based on fits of the CD data using the relationships expressed in Equation 5 (Figure S1A, see Methods). Interestingly, the $HDM2^{(25-47)}$ peptide is estimated to contain a higher fraction of helical structure than the other peptides, 16%. This is in contrast to the predictions of AGADIR, where the helical content of the $p53^{(367-388)}$, $TRTK12^{(265-276)}$, $HDM2^{(25-47)}$, and $HDM4^{(25-47)}$ peptides are estimated to be less than 2%, while the $NDR^{(62-87)}$ peptide was predicted to have a 15% helical content (*115*). However, these results are consistent with previous reports of $TRTK12^{(265-276)}$ and $p53^{(367-388)}$ being unstructured in solution (*34, 116*). To determine the ability of the peptides to adopt helical structure, trifluoroethanol (TFE) was added to each peptide to final concentration of 30%. It is well established that TFE promotes alpha helix formation in short peptide sequences (*117*). In the presence of TFE, all peptides show a significant amount of helical structure (Figure S1B), irrespective of their intrinsic helical propensities. TFE had a greater helix-inducing effect on the $HDM2^{(25-47)}$ and $HDM4^{(25-47)}$ peptides than on the $p53^{(367-388)}$ and $TRTK12^{(265-276)}$ peptides, suggesting they may also adopt a partially helical structure upon binding S100B.

Figure S1. Far-UV CD spectra for the TRTK12, p53, NDR, HDM2, and HDM4 peptides in both the absence (Panel A) and presence of 30% Trifluorethanol (Panel B). The NDR (spectrum 1), HDM4 (spectrum 2), HDM2 (spectrum 3), TRTK12 (spectrum 4), and p53 (spectrum 5) peptides are able to adopt a secondary helical structure in the presence of TFE.

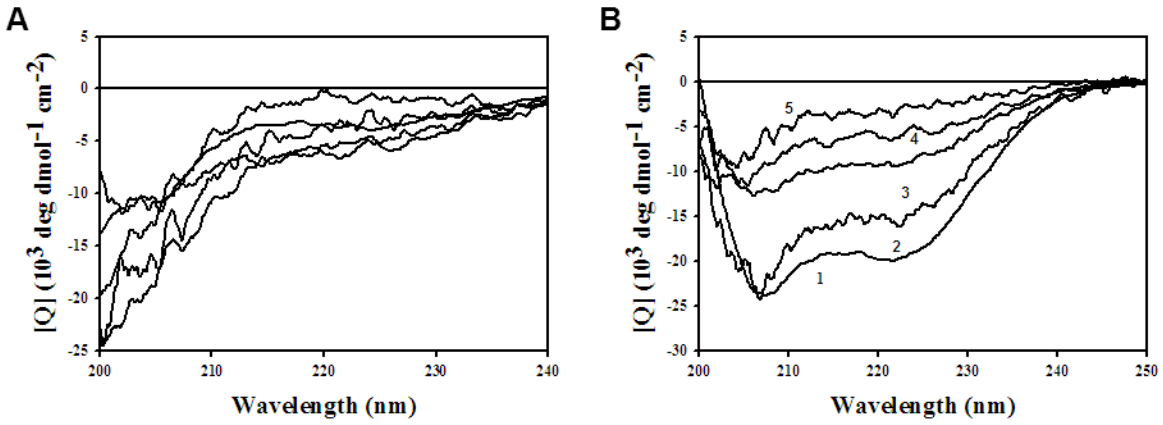

Figure S2: LineShapeKin Two-Dimensional Global Arrays. Comparison of the quality of global fits of peptide binding to S100B when the dissociation constant, K_d, and the kinetic binding constant, K_{on}, are held constant and varied over several orders of magnitude. The smallest norm corresponds to the best fit. The TRTK12, p52, and NDR peptides were fit to a two identical binding sites model, whereas the HDM2 and HDM4 peptides were fit to single binding site model.

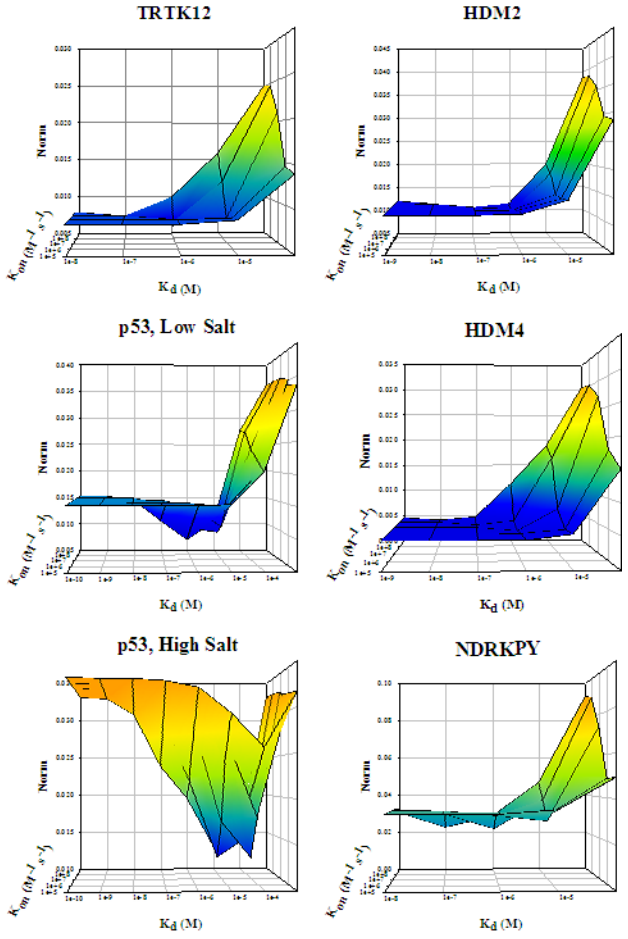

Figure S3: LineShapeKin Two-Dimensional Individual Arrays. Select fits from individual residues of S100B from the p53 peptide titration when the dissociation constant, K_d, and the kinetic binding constant, K_{on}, are held constant and varied over several orders of magnitude. The smallest norm corresponds to the best fit. Individual fits correlate well with each other and the global fit.