The repeat region of cortactin is intrinsically disordered in solution [1].

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Introduction: The multi-domain protein, cortactin, contains a 37-residue repeating motif that binds to actin filaments. This cortactin repeat region comprises 6½ similar copies of the motif and binds actin filaments. To better understand this region of cortactin, and its fold, we conducted extensive biophysical analysis. Size exclusion chromatography with multi-angle light scattering (SEC-MALS) reveals that neither constructs of the cortactin repeats alone or together with the adjacent helical region homo-oligomerize. Using circular dichroism (CD) we find that in solution the cortactin repeats resemble a coil-like intrinsically disordered protein. Small-angle X-ray scattering (SAXS) also indicates that the cortactin repeats are intrinsically unfolded, and the experimentally observed radius of gyration (R_g) is coincidental to that calculated by the program Flexible-Meccano for an unfolded peptide of this length. Finally, hydrogen-deuterium exchange mass spectrometry (HDX-MS) indicates that the domain contains limited hydrophobic core regions. These experiments therefore provide evidence that in solution the cortactin repeat region of cortactin is intrinsically disordered.

Experimental: The repeat region of cortactin, CortactinCR, was analyzed by HDX-MS at the National High Magnetic Field laboratory (NHMFL) by use of on-line LC-ESI FT-ICR methods. LC/MS data for purified cortactinCR was analyzed in triplicate after 0, 0.5, 1, 2, 4, 8, 15, 30, 60, 120, and 240 min incubation at 0.4 ºC followed by quenching. After ionization the sample was directed into a custom-built hybrid Velos Pro 14.5 T FT-ICR mass spectrometer. Approximately 350 mass spectra were collected. After the deuterium uptake profile was analyzed for each of the peptides, a deuterium uptake "heat map" was drawn as the visual representation of the localized deuteration rate for the cortactinCR, to confirm and complement structural information discovered by other experiments. The "heat map" is drawn by summarizing deuterium uptake information for all peptides from the cortactinCR. Briefly, the deuterium uptake of each residue is calculated by averaging the deuteration levels of that residue from each overlapping peptide containing it, and the deuteration level of each residue is calculated by dividing the observed deuterium uptake by the maximum possible deuterium uptake for each peptide. Although deuterium uptake for each residue could vary across the peptide, so that this calculation does not represent an accurate extent of deuteration for each residue, this approach incorporates all available information from all overlapping peptides without introducing bias by manually selecting which peptide to display in the "heat map".

Results and Discussion: We conducted an HDX-MS time-course study for cortactinCR. For each proteolytic peptide, the percentage of D-uptake (i.e., number of deuteriums divided by the number of amide hydrogens (not counting proline(s)) after each incubation period was color-coded to produce a heat map. Examination of the cortactinCR data reveals a significant correlation of solvent exposure with CD and SAXS experiments. We find most regions of cortactinCR rapidly reached HDX saturation by the first time-point (**Figure 1**), indicating that cortactinCR contains minimal hydrophobic core (unprotected) and is largely intrinsically disordered.

Conclusions: Cortactin contains 6½ cortactin repeats that form what is termed the 'cortactin repeat domain'. Whether and how the cortactin repeats domain folds in solution has been controversial, and the literature supports two possibilities, either an extended or natively unfolded, or a folded domain. To resolve the question of whether the cortactin repeats are folded in solution we conducted studies based on the orthogonal biophysical techniques of circular dichroism, small-angle X-ray scattering, and hydrogen-deuterium exchange mass spectrometry. Our studies clearly demonstrate the intrinsically disordered nature of the repeat region of cortactin.

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Fig 1. Hydrogen-deuterium exchange mass spectrometry for cortactinCR. Percentage of deuterium uptake is indicated for HDX incubation periods ranging from 30 s to 240 min. Minimal changes in deuterium uptake are observed over the time course suggesting a minimal hydrophobic core for cortactinCR, and that the protein is largely unprotected and in an unfolded state. Alternating orange and black sequences indicate cortactin repeats.