Probing Epichaperome Structure in Mouse Embryonic Stem Cells using Native Gel Electrophoresis and a Novel High-Throughput In-Gel Crosslinking Method

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INTRODUCTION

- The epichaperome is a group of long-lived protein complexes composed of chaperone proteins, co-chaperones, and other proteins, nucleated by Heat Shock Protein 90 kDa (HSP90) and Heat Shock Cognate 71 kDa Protein (HSC70).
- HSP90 in epichaperomes favors binding of inhibitor PU-H71 (PU), whereas canonical HSP90 favors geldanamycin (GA).
- The epichaperome exists in cancer, neurodegenerative diseases, and pluripotent stem cells and is involved in regulating proteotoxic stress response and cell proliferation.
- "p240" is known to form upon *in vivo* crosslinking in human cancer cell lines based on HSP90 western blot, but its composition has previously not been determined with mass spectrometry (MS).
- **We hypothesize that p240 in mESCs originates from a prokaryote-like HSP90-HSC70 binary complex, where HSC70 interacts at the middle domain of closed conformation HSP90.**

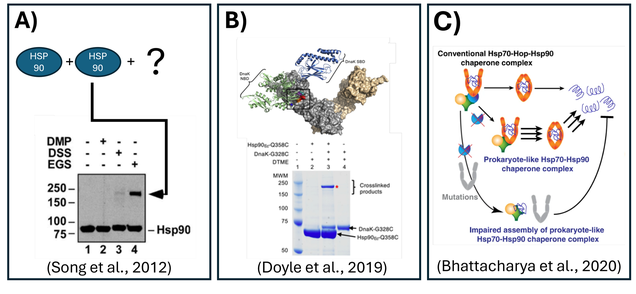

Figure 1. Multiple Sources Suggest that p240 Consists of a Prokaryote-Like HSP90-HSC70 Binary Complex with Enhanced Protein Folding Capacity. **A)** p240 is discovered in human cancer cell lines using *in vivo* crosslinking and HSP90 western blot. **B)** HSC70 interacts with the middle domain of HSP90 in the prokaryote ortholog complex. **C)** HSP90 and HSC70 form a prokaryote-like binary complex with enhanced protein folding capacity upon HSC70/HSP90 Organizing Protein (HOP) knockout in HEK293 cells.

KEY METHODS

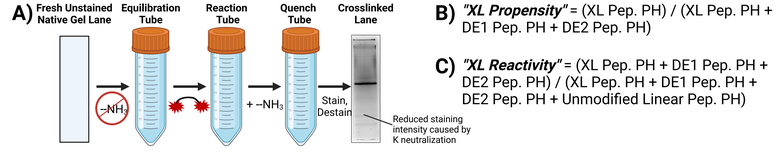

Figure 2. Key Methods Used for Crosslinking and Data Analysis. **A)** High-throughput in-gel crosslinking is performed by running a Native PAGE gel, excising an intact lane, removing primary amine containing buffers with washing, transferring the lane into the reaction tube with crosslinker, quenching with a primary amine containing buffer, then staining and destaining to visualize protein signal. **B)** "XL Propensity" provides insight into the distance between crosslinked residues in space and is calculated using the ratio of summed peak height for the crosslinked peptide to the summed peak height of the crosslinked peptide and deadends, at each charge state and accounting for missed cleavage variants. **C)** "XL Reactivity" provides insight into the surface accessibility of residues and is calculated using the ratio of the XL peptide and deadend peptides over the XL peptide and deadend peptides plus the unmodified linear peptides, at each charge state and accounting for missed cleavage variants.

RESULTS

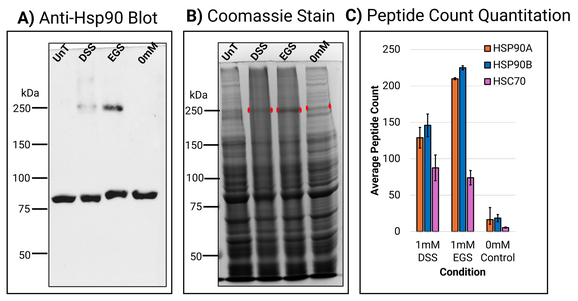

Figure 3. Characterization of p240 with Blot-Directed Band Excision and MS. **A)** Anti-HSP90 blot of crosslinked E14 mESC lysate separated on SDS-PAGE. **B)** Coomassie stain of SDS-PAGE. **C)** Peptide count quantitation of HSP90 and HSC70.

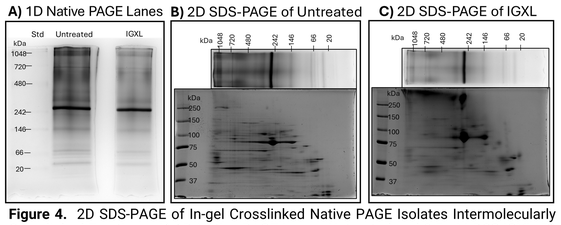

Figure 4. 2D SDS-PAGE of In-gel Crosslinked Native PAGE Isolates Intermolecularly Crosslinked Fractions. **A)** 1D Native PAGE Lanes: Untreated and 0.5mM DSS IGXL. **B)** 2D SDS-PAGE of Untreated 1D Native PAGE. **C)** 2D SDS-PAGE of 0.5mM DSS 1D Native PAGE.

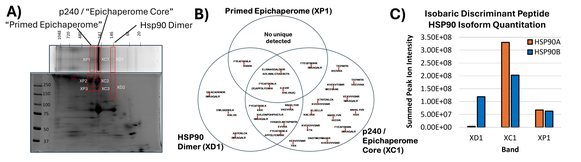

Figure 5. Detected Crosslinks in 1D Gel Bands and HSP90 Isoform Quantitation. **A)** Cut diagram of 2D SDS-PAGE of in-gel crosslinked 1D lane. **B)** Venn diagram of identified HSP90 crosslinks in each of the 1D bands. **C)** HSP90 isoform quantitation in each of the 1D bands using isobaric discriminant peptide TLTIVDTGIGMTK (α) / TLTLVDTGIGMTK (β).

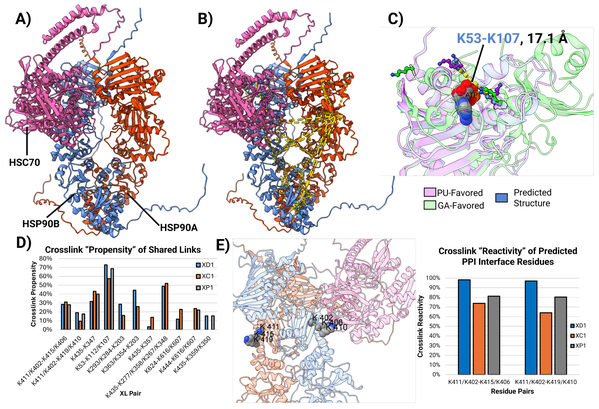

Figure 6. The Predicted HSP90-HSC70 Binary Complex Matches PU-H71 Favored HSP90 Conformation and The Previously Determined Interfaces of HSP90 and HSC70. **A)** Collabfold predicted HSP90-HSC70 binary complex. **A)** Validated crosslinks mapped to the predicted structure. **C)** Homology model of N-terminal ATP binding pocket of predicted complex with PU-favored HSP90 conformation (2CG9) and GA-favored HSP90 conformation (2IOQ). **D)** Crosslink "Propensity" of crosslinks identified in at least two of the bands. **E)** PPI interface lysines of HSP90A and HSP90B with HSC70 and calculated crosslink "reactivity" in each band.

CONCLUSION

- MS analysis of p240 reveals it is composed of HSP90 and HSC70.
- High-throughput in-gel crosslinking (IGXL) can be done with an entire Native PAGE lane.
- Whole lane IGXL allows for 2D SDS-PAGE to isolate intermolecularly linked fractions.
- The identified HSP90 crosslinks and calculated XL propensity supports an HSP90-HSC70 binary complex in closed conformation.
- Calculation of XL reactivity suggests that the predicted HSP90 PPI interface residues are occluded in the core/ p240 band by an interacting protein.
- The co-migration of two HSP90 complexes at ~242 kDa complicates data interpretation.
- Identification of p240 as epichaperome could be performed by DSS/EGS XL, followed by PU-pulldown, denaturing elution, SDS-PAGE and HSP90 blot-directed MS analysis.

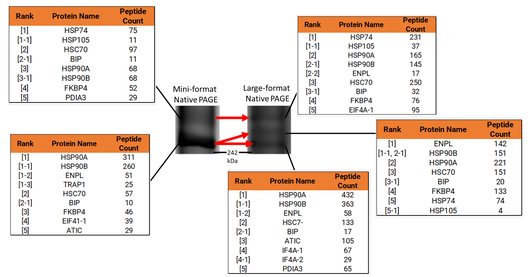

Figure 7. Large-format Naitve PAGE Reveals that Two Dominant HSP90 Complexes Co-Migrate to ~242 kDa on the Mini-Format Native PAGE.

ACKNOWLEDGEMENTS

National Science Foundation Graduate Research Fellowship (LB); NIH R01AG072599
Thank you to all of the undergrads who helped me along the way, especially Gabriel Fitzgerald and Joanna Suber.
COI Statement: The authors have none to disclose.