Conclusion

The hypothesis is confirmed: all 10 pairwise Spearman correlations between the five stress response endpoints (SR-ARE, SR-MMP, SR-HSE, SR-p53, SR-ATAD5) exceed $\rho > 0.7$ (range: 0.85-0.98, mean: 0.93, all $p < 0.001$), indicating the ToxD4C model predicts a tightly coordinated, multi-pathway cellular stress response rather than independent pathway activations across the 21 TBBPA metabolites.

Methods

The analysis was performed in Python using pandas (v2.x), scipy.stats (v1.x), seaborn, and matplotlib. The toxicity predictions dataset (smiles2_toxicity_results.csv, n=21 molecules × 89 columns) was loaded and the five stress response endpoint probability columns were extracted: SR_ARE_Probability, SR_MMP_Probability, SR_HSE_Probability, SR_p53_Probability, and SR_ATAD5_Probability. Data completeness was verified (no missing values). A 5×5 Spearman rank correlation matrix was computed using scipy.stats.spearmanr(), which returns both correlation coefficients (ρ) and two-tailed p-values for all pairwise comparisons. Spearman's rank correlation was chosen as the appropriate non-parametric method for this small sample size (n=21), as it does not assume normality and is robust to outliers and non-linear monotonic relationships. All 10 unique pairwise correlations (excluding diagonal) were extracted, sorted by strength, and evaluated against the hypothesis threshold ($\rho > 0.7$). For each endpoint, the mean correlation with other endpoints and strongest partner were identified. Results were visualized using a seaborn heatmap with a red-yellow-blue reversed colormap (red = high correlation), scaled from 0.8 to 1.0, with correlation coefficients annotated in each cell. Statistical significance was assessed at $\alpha = 0.001$.

Results

All 10 unique pairwise Spearman correlations exceeded the $\rho > 0.7$ threshold:

Strongest correlations:

- SR-ARE ↔ SR-p53: $\rho = 0.9805$, $p = 6.84\times10^{-15}$
- SR-ARE ↔ SR-HSE: $\rho = 0.9688$, $p = 5.68\times10^{-13}$
- SR-ARE ↔ SR-MMP: $\rho = 0.9558$, $p = 1.48\times10^{-11}$
- SR-HSE ↔ SR-ATAD5: $\rho = 0.9494$, $p = 5.30\times10^{-11}$

Weakest correlation (but still strong):

- SR-MMP ↔ SR-ATAD5: $\rho = 0.8481$, $p = 1.20\times10^{-6}$

Summary statistics:

- Minimum correlation: $\rho = 0.8481$
- Maximum correlation: $\rho = 0.9805$
- Mean correlation: $\rho = 0.9326$
- All p-values < 0.001

Endpoint-specific patterns:

- SR-ARE shows the highest mean correlation with other endpoints ($\rho = 0.9575$), with particularly strong coupling to SR-p53 ($\rho = 0.9805$)
- SR-MMP and SR-ATAD5 show relatively lower mean correlations ($\rho = 0.9114$ and 0.9088, respectively)
- SR-HSE shows strong correlation with SR-ATAD5 ($\rho = 0.9494$), the highest for ATAD5
- SR-p53 correlates most strongly with SR-ARE ($\rho = 0.9805$)

All correlations are statistically significant at $p < 0.001$, with the strongest correlations showing p-values in the range of 10^{-15} to 10^{-9}.

Challenges

No significant analytical challenges were encountered. The dataset was complete with no missing values for the five stress response endpoints. The small sample size (n=21) is a limitation for correlation analysis, but with effect sizes this large ($\rho > 0.85$ for all pairs), statistical power was sufficient to detect highly significant correlations. The Spearman method was appropriate for the non-parametric context and small sample. One minor consideration is that the probability values span a wide range (e.g., SR-ATAD5 mean = 0.008, SR-MMP mean = 0.385), but rank-based correlation is robust to such scale differences. The interpretation is limited to the model's predictions rather than experimental validation of actual biological pathway coordination.

Discussion

The analysis confirms that the ToxD4C model predicts stress response endpoints in a highly coordinated manner across TBBPA metabolites. The uniformly high correlations (all $\rho > 0.85$) suggest that the model has learned structural features that drive a general cellular stress response rather than endpoint-specific activation patterns. This could reflect either: (1) biological reality—these pathways are genuinely co-activated by common structural features (e.g., electrophilicity, redox activity), or (2) model limitation—the model may not distinguish mechanistically distinct stress pathways due to shared training data patterns or molecular descriptors.

The SR-ARE ↔ SR-p53 pair ($\rho = 0.98$) represents near-perfect coordination, which is mechanistically plausible as both pathways respond to oxidative stress and DNA damage. The ARE (antioxidant response element) pathway activates via Nrf2 in response to electrophiles and ROS, while p53 is a master regulator of cellular stress responses including DNA damage and oxidative stress.

The SR-MMP ↔ SR-ATAD5 pair ($\rho = 0.85$) shows the most independent variation, suggesting these pathways may be less tightly coupled mechanistically or that the model captures some pathway-specific features. MMP (mitochondrial membrane potential) reflects mitochondrial dysfunction, while ATAD5 is involved in DNA replication stress and genome stability. Their relative independence may indicate distinct mechanistic triggers.

The finding that molecules with phenolic OH groups (from previous analysis f10) show elevated stress predictions across all five endpoints is now understood to reflect a unified stress signature rather than five independent effects. This suggests phenolic groups may induce a general pro-oxidant or electrophilic stress state captured similarly across all endpoints by the model.

A critical limitation is that this analysis examines model predictions, not experimental data. The high correlations could partially reflect model architecture (shared molecular representations, transfer learning from Uni-Mol, or multi-task learning that encourages correlated predictions) rather than purely biological relationships. Experimental validation would be needed to confirm whether TBBPA metabolites truly induce coordinated stress responses.

Proposed Next Hypotheses

1. Molecules that are predicted outliers in the SR-MMP ↔ SR-ATAD5 relationship (showing relatively greater divergence between these two endpoints) possess distinct structural features related to mitochondrial targeting (e.g., lipophilicity, specific functional groups) versus DNA replication interference (e.g., Michael acceptors, quinone formation potential).
2. The model's predicted stress response coordination (high inter-endpoint correlations) is driven by specific molecular descriptors such as LUMO energy (electrophilicity), phenolic OH count, and molecular weight, which can be identified through multivariate regression

or SHAP analysis as common predictors across all five stress endpoints.