

# Comparative Target and Mechanistic Profiling of Lycopene and Primidone in Traumatic Brain Injury: A Network Pharmacology and Molecular Docking Study

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## Abstract

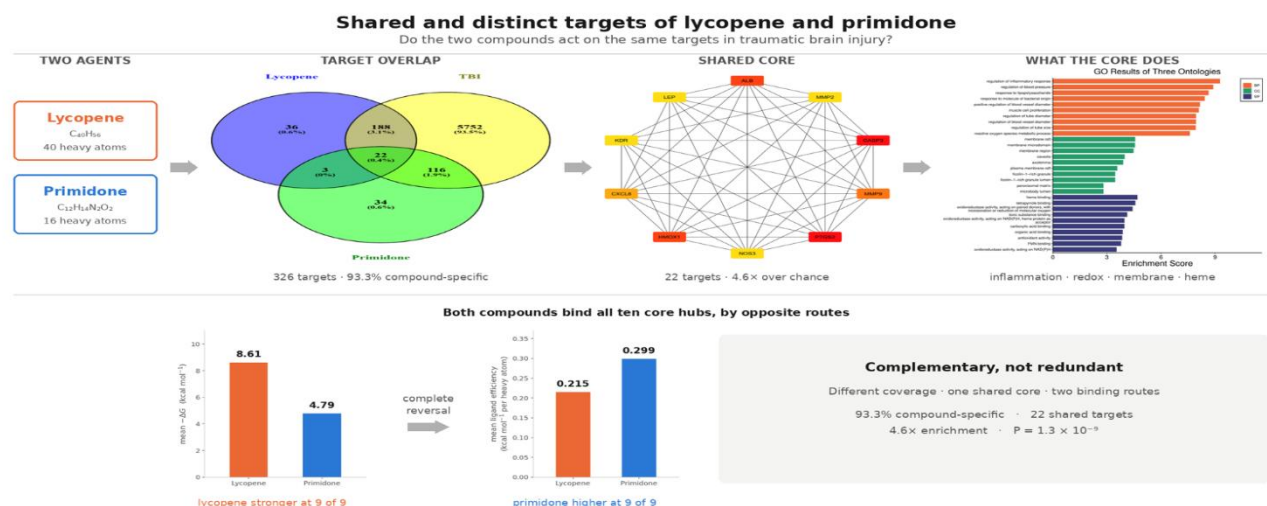
**Background:** There is no approved disease-modifying drug for traumatic brain injury (TBI). The secondary injury that follows the impact is not a single pathway but a set of linked inflammatory, oxidative and cellular processes. A drug combination is more likely to help when the two agents act on different parts of that network rather than the same part. This study compared the predicted target profiles of lycopene and primidone in TBI using network pharmacology and molecular docking.

**Methods:** Putative targets of both compounds were predicted in SwissTargetPrediction, broadened in GeneCards and standardised in UniProt. These were intersected with TBI-associated genes from GeneCards and OMIM. The observed overlap was tested against a hypergeometric null model. The shared targets were assembled into a STRING protein–protein interaction network, ranked by node degree, and analysed for functional enrichment in DAVID. Both compounds were then docked into the ten highest-ranking hub proteins using AutoDock Vina.

**Results:** Lycopene returned 269 predicted targets and primidone 175, of which 210 and 138 were TBI-associated. Of the 326 combined targets, 93.3% belonged to one compound only. The 22 shared targets were 4.6-fold more numerous than chance expectation ( $P = 1.3 \times 10^{-9}$ ), and 21 of them formed a single connected module. That module was enriched for inflammatory response, reactive oxygen species metabolism, membrane raft and axolemmal localisation, and heme binding. Both compounds showed predicted interactions with all ten hub proteins. Across nine matched pockets, lycopene gave the more negative predicted affinity at every target, while primidone gave the higher ligand efficiency at every target (0.299 against 0.215 kcal/mol per heavy atom; Wilcoxon  $P = 0.004$ ).

**Conclusion:** Lycopene and primidone act on largely different TBI-associated targets, but meet on a small inflammatory and heme–redox core. Their docking profiles differ in the same way: one compound binds more strongly, the other more efficiently. These findings support further experimental work on the two compounds alone and in combination. They also show that a docking score should not be read on its own, without ligand efficiency or other measures of molecular size.

## Graphical abstract



## 1. Introduction

Traumatic brain injury (TBI) is a major cause of death and lasting disability, with an estimated 69 million cases each year (Dewan et al., 2019). Young adults carry most of the burden, as do low- and middle-income countries (Maas et al., 2017; Naik et al., 2023). Critical care and rehabilitation have improved, but treatment is still supportive. No drug has been approved that changes the course of the disease (Maas et al., 2017). TBI has two phases. The primary injury is the mechanical damage at the moment of impact, and it cannot be reversed. The secondary injury builds over hours to weeks. It includes excitotoxicity, calcium dysregulation, mitochondrial failure, oxidative stress, neuroinflammation, blood–brain barrier breakdown and delayed neuronal death (Werner and Engelhard, 2007; Di Pietro et al., 2020). Ferroptosis, or iron-dependent lipid peroxidation, has since been added to that list, and links haemorrhagic iron and heme breakdown to late neuronal loss (Zhu et al., 2026). These processes do not run separately. They form a connected network in which oxidative, inflammatory and apoptotic events feed one another (Kumar and Loane, 2012; Loane and Kumar, 2016). Blocking one point in such a network leaves the other routes open, and those routes are often enough to keep the injury going. This

is the basic idea behind network pharmacology: in a disease with many causes, acting on one target rarely changes the system as a whole (Hopkins, 2008).

Combination therapy follows from that idea, but not every pair of drugs works well together. If two drugs hit the same targets, the second adds little, because it behaves much like a higher dose of the first. If they hit different parts of the same disease network, they can cover more of it between them. Cheng et al. (2019) tested this across 681 drug combinations and defined six topological classes linking two drug–target modules to a disease module, using the network separation measure of Menche et al. (2015). Only one class was linked to efficacy: the one where both drug modules reach the disease module but stay separate from each other. Combinations with overlapping modules did no better than single drugs. This means a candidate pair can be assessed from its two predicted target profiles before any experiment is run. Lycopene (LYC) and primidone (PRM) are very different compounds, and both have reported neuroprotective, antioxidant, anti-inflammatory or neuromodulatory activity (Lei et al., 2016; Krügel et al., 2017; Patsalos et al., 2018; Pushpakom et al., 2019; Imran et al., 2020; Khan et al., 2021).

Each has been profiled on its own, most recently PRM (Gulia et al., 2026), but the two have not been compared.

We set out to answer three questions. Are the TBI-associated predicted targets of LYC and PRM mostly different or mostly the same? Which targets do they share, and

what biological processes do those targets belong to? And how do the two compounds compare when docked into the same hub proteins (Figure 1)? Our aim was to provide a computational basis for judging whether the two compounds are mechanistically complementary, and whether they may be worth taking forward to experimental work.

## 2. Materials and Methods

### 2.1 Study design

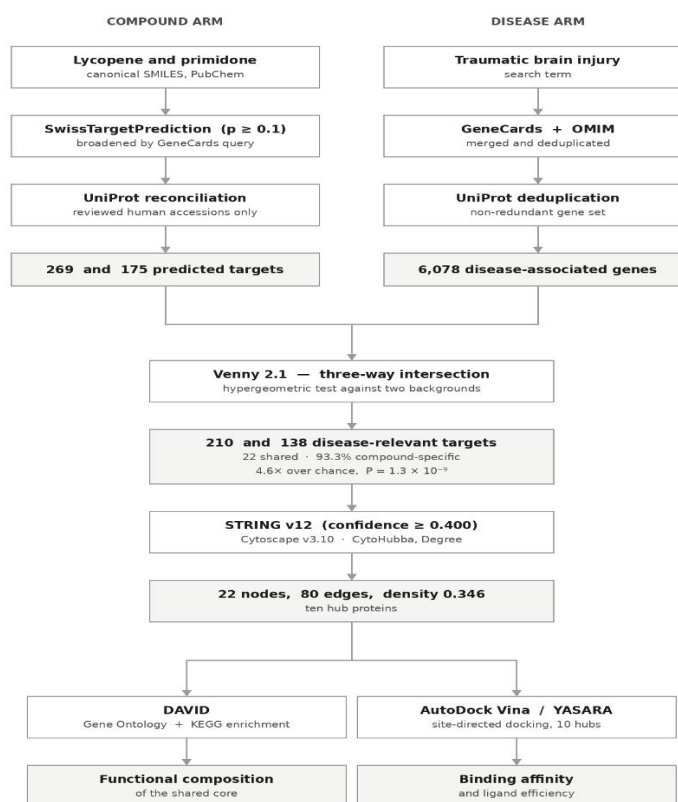


Figure 1. Flowchart of the workflow of the study.

### 2.2 Compound structure retrieval and preparation

Structures of both compounds were downloaded from PubChem (Kim et al., 2023) in SMILES and three-dimensional SDF format: LYC (CID 446925; C<sub>40</sub>H<sub>56</sub>; relative molecular mass 536.87) and PRM (CID 4909; C<sub>12</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>; 218.25). The SMILES strings used were:

|     |                                                                                                                  |
|-----|------------------------------------------------------------------------------------------------------------------|
| LYC | <chem>CC(=CCC/C(=C/C=C/C(=C/C=C/C(=C/C=C/C(/C=C/C=C(/C=C/C=C(/C</chem><br><chem>CC=C(C)C)\C)\C)\C)/C)/C)C</chem> |
| PRM | <chem>CCC1(C(=O)NCNC1=O)C2=CC=CC=C2</chem>                                                                       |

The same SMILES string was used at every prediction step, so that all downstream results refer to the same chemical entity. The LYC string encodes the all-trans (all-E) form. Heavy-atom counts were 40 for LYC and 16 for PRM. Ligand geometries were energy-minimised with the MMFF94 force field at pH 7.4 (Halgren, 1996).

### 2.3 Target prediction and TBI-associated gene collection

Protein targets were predicted from the canonical SMILES of each compound in SwissTargetPrediction (Daina et al., 2019), with the species set to *Homo sapiens* and a probability cutoff of  $\geq 0.1$ . A low cutoff was used on purpose. A target dropped at this stage cannot be recovered later, whereas a false positive is removed by the filters that follow: the intersection with the disease gene set, the network ranking and the docking. The list was broadened by a free-text search of GeneCards (Stelzer et al., 2016), then reconciled against UniProt (UniProt Consortium, 2023) to standardise gene symbols, confirm reviewed human entries and remove duplicate names. Non-human entries, pseudogenes and

uncharacterised open reading frames were removed. TBI-associated genes were collected separately from GeneCards (Stelzer et al., 2016) and OMIM (Amberger et al., 2015) using “traumatic brain injury” as the search term, with no relevance-score cutoff. The two lists were merged and deduplicated against UniProt.

## 2.4 Target intersection and overlap statistics

Overlaps were computed in Venny 2.1 (Oliveros, 2007–2015). Each compound’s predicted target list was first intersected with the TBI gene set to give its disease-relevant targets. The two resulting sets were then compared with each other and with the TBI gene set in a single three-way analysis. This splits the combined space into targets reached by LYC only, by PRM only, and by both. The three-way comparison is the main analysis of the study.

## 2.5 Statistical treatment of the observed overlap

Two measures of overlap size were used: the Jaccard index,  $|A \cap B| / |A \cup B|$ , and the

Szymkiewicz–Simpson overlap coefficient,  $|A \cap B| / \min(|A|, |B|)$ . The second is not affected by a large difference in set size. Departure from chance was tested with a hypergeometric model. For two sets of size  $a$  and  $b$  drawn from a background of  $N$  genes, the expected overlap is  $ab/N$ , and the probability of  $k$  or more shared genes is the upper tail of the hypergeometric distribution. Two backgrounds were used. The main analysis used the disease gene set itself. This is the conservative choice, because both compound sets are subsets of it, so any enrichment must come from the compounds and not from shared disease relevance. A second analysis used an approximate proteome background of 20,000 protein-coding genes. Both results are reported.

## 2.6 Protein–protein interaction (PPI) network and hub identification

The shared target set was submitted to STRING v12.0 (Szklarczyk et al., 2023) with the organism set to *Homo sapiens*, the full network of functional and physical associations selected, and a minimum interaction score of 0.4 (medium confidence). A lower score admits poorly supported edges, and a higher one breaks a network of this size into fragments. Nodes with no edge at this threshold were kept and named rather than deleted. The network

was imported into Cytoscape v3.10.2 (Shannon et al., 2003), where node count, edge count, average degree, density and clustering coefficient were recorded. Hub genes were ranked in CytoHubba (Chin et al., 2014) with the Degree algorithm, which counts how many direct partners each node has. Degree was chosen over clique-based measures because clique scores saturate in small, densely connected networks and separate the nodes poorly. The ten top-ranked genes were taken forward to docking.

## 2.7 GO and KEGG enrichment analysis

Enrichment was run in DAVID (Sherman et al., 2022). The gene list was submitted as official HGNC symbols against the full *Homo sapiens* background, and over-representation was assessed by the modified Fisher exact test, reported as the EASE score. Gene Ontology was examined across biological process, cellular component and molecular function, with KEGG pathway enrichment (Kanehisa and Goto, 2000) run alongside. Raw  $P$  values were corrected by the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) and are reported in Table 4 and Figure 4. Every term reported had an adjusted  $P$  below 0.05. Where nested or near-identical terms covered much the same genes, they were counted as one signal rather than

several. Results were plotted as bar charts and bubble plots in SRplot (Tang et al., 2023), and selected KEGG pathways were drawn with the shared targets mapped onto the reference topology in Pathview (Luo and Brouwer, 2013).

## 2.8 Protein structure selection and preparation

Structures of the ten hub proteins were downloaded from the Protein Data Bank (Berman et al., 2000). Four criteria were applied in order: human origin, X-ray diffraction, a co-crystallised ligand marking the binding site where one existed, and the best resolution consistent with the first three. The selected structures are listed in Table 5.

Receptors were prepared in AutoDockTools 1.5.7 (Morris et al., 2009). Crystallographic water and the co-crystallised reference ligand were removed, a single chain was kept where several were present, polar hydrogens were added, Gasteiger charges were assigned, and the file was converted to PDBQT format. Prosthetic groups and metal ions that form part of the binding site were kept, because removing them would destroy the pocket. These were the heme of heme oxygenase-1, the heme, tetrahydrobiopterin and interfacial zinc of endothelial nitric oxide synthase, and the catalytic zinc and

structural calcium of both matrix metalloproteinases. Missing side chains inside the binding region were modelled where needed.

## 2.9 Docking protocol and validation

Docking was directed to a defined site at every target. Blind docking over the whole receptor surface was not used, since its weakness in network pharmacology has been documented (Che and Zhang, 2025). Grid box centres, box dimensions and redocking results for every receptor are available from the corresponding author on request. The ten targets fall into three classes.

The first class covers six targets that carry a co-crystallised ligand and therefore allow redocking. Here the grid box was centred on the centroid of that ligand and sized to hold the whole pocket with room for free sampling. The protocol was checked by removing the reference ligand, docking it again under the same settings, and measuring the root-mean-square deviation from the crystal pose. A value of  $\leq 2.0$  Å was taken as acceptable. This class comprises cyclooxygenase-2 (5IKR, 2.34 Å; mefenamic acid), kinase insert domain receptor (2QU5; benzimidazole inhibitor), matrix metalloproteinase-9 (4H3X, 1.76 Å; hydroxamate inhibitor), heme oxygenase-1 (3HOK, 2.19 Å; azole inhibitor),

endothelial nitric oxide synthase (3NOS, 2.40 Å; arginine in the heme pocket) and human serum albumin (2BXD, 3.05 Å; warfarin, Sudlow site I).

The second class covers two targets whose reference ligand rules out redocking, so the box was placed without that check. At caspase-3 (2J30, 1.40 Å) the reference ligand is covalently bound to the catalytic cysteine, and AutoDock Vina docks non-covalently, so the box was centred on the peptide-binding cleft. At matrix metalloproteinase-2 (7XJO, 2.00 Å) the reference ligand is a peptide, so the box was centred on the catalytic zinc. The third class covers leptin (1AX8, 2.40 Å) and CXCL8 (5D14, 1.00 Å). Neither has a co-crystallised ligand or a well-characterised small-molecule site, so neither ligand-centred box placement nor redocking was possible. Results for these two are treated throughout as structural hypotheses rather than validated binding modes.

All calculations were run from scratch for this study and no affinity was carried over from earlier work. Docking used AutoDock Vina (Trott and Olson, 2010) through the YASARA Structure interface (Krieger and Vriend, 2014). Each of the 20 ligand–

receptor pairs, comprising the ten hub proteins docked with both compounds, was docked in three independent executions using different random seeds, giving 60 docking runs in total, and the best-scoring pose of the three was retained. Repetition was applied because the Vina search is stochastic. Docking was performed at an exhaustiveness of 24, three times the program default of 8, because LYC has thirteen rotatable bonds and is under-sampled at the default setting. Affinities were recorded in kcal/mol, and the retained pose was used for contact analysis, with complexes viewed and interactions examined in PyMOL (Schrödinger, LLC) and Discovery Studio Visualizer (Dassault Systèmes BIOVIA).

## 2.10 Ligand-efficiency analysis and statistics

A raw docking score rises with ligand size, because a bigger molecule simply makes more contacts, whatever their quality. Comparing two compounds that differ more than twofold in heavy-atom count on raw score alone is therefore not informative. Ligand efficiency was calculated for every compound–target pair as shown in Equation 1.

$$LE = -\Delta G / N \dots \dots \dots \text{Equation 1.}$$

where LE is ligand efficiency,  $\Delta G$  the predicted binding free energy in kcal/mol, and N the number of heavy atoms (Hopkins et al., 2004). N is 40 for LYC and 16 for PRM. LE is expressed in kcal/mol per heavy atom, and a larger value means the molecule uses its mass more efficiently.

At every hub target both compounds were docked into the same receptor structure, under the same protocol, and into the same grid box. Any difference between them is therefore due to the ligands and not to the receptor models. Comparative statistics were computed across the nine structure-matched pockets used in the paired comparison. Human serum albumin was left out, because its loose multi-site binding makes its score hard to compare with a

defined catalytic or allosteric pocket. Its values are reported descriptively in Table 5.

### 3. RESULTS

#### 3.1 Compound and TBI target landscapes

SwissTargetPrediction at a cutoff of  $\geq 0.1$ , broadened by GeneCards and reconciled against UniProt, returned 269 non-redundant predicted protein targets for LYC and 175 for PRM. Of the LYC set, 249 resolved to unique reviewed human UniProt accessions and entered the three-way comparison. Searching GeneCards and OMIM for “traumatic brain injury” and merging the two outputs gave us a disease gene set of 6,078 genes (Table 1).

**Table 1.** Predicted compound target sets and the TBI gene set.

| Dataset                                   | Source                                                               | <i>n</i>       |
|-------------------------------------------|----------------------------------------------------------------------|----------------|
| LYC predicted targets                     | SwissTargetPrediction ( $\geq 0.1$ ) + GeneCards, UniProt-reconciled | 269            |
| LYC targets entering three-way comparison | unique reviewed human accessions                                     | 249            |
| PRM predicted targets                     | SwissTargetPrediction ( $\geq 0.1$ ) + GeneCards, UniProt-reconciled | 175            |
| TBI-associated genes                      | GeneCards + OMIM, deduplicated                                       | 6,078          |
| LYC $\cap$ TBI                            | SwissTargetPrediction + GeneCards, UniProt-reconciled                | 210<br>(84.3%) |
| PRM $\cap$ TBI                            | SwissTargetPrediction + GeneCards, UniProt-reconciled                | 138<br>(78.9%) |

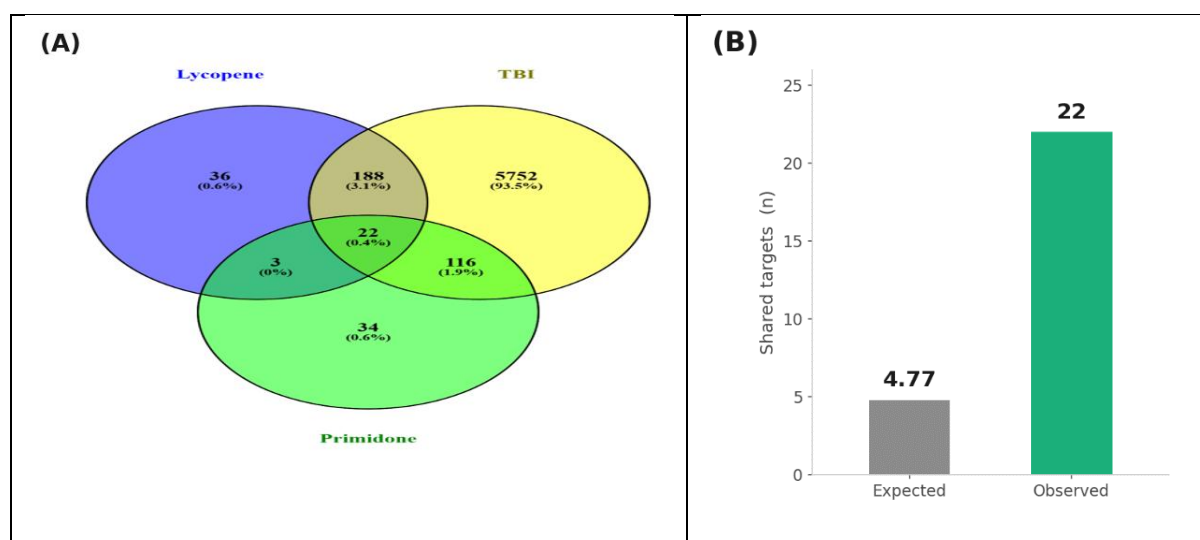
$\cap$  denotes the intersection of two sets. Percentages are of the reconciled sets entering the comparison (249 for LYC and 175 for PRM). Source: compiled by the authors from SwissTargetPrediction, GeneCards, OMIM and UniProt outputs.

The two parent target sets differ in size because the two compounds differ in chemistry. LYC is a large, highly lipophilic C<sub>40</sub> polyene, so ligand-similarity prediction returns a broad target spectrum for it, whereas PRM is a small, polar, conformationally restricted heterocycle with a correspondingly narrower predicted profile.

### 3.2 Comparative target overlap

Intersecting each compound's target list with the TBI gene set gave 210 TBI-relevant targets for LYC and 138 for PRM (Figure 2A). Together these make a union of 326 proteins. Of these, 188 (57.7%)

belong to LYC alone, 116 (35.6%) to PRM alone, and 22 (6.7%) to both (Table 2). Three further proteins are shared by the two compounds but are not in the TBI gene set, and were excluded from all later analysis. The two profiles are therefore mostly separate: 93.3% of the combined space belongs to one compound only, the Jaccard index is 0.068, and the Szymkiewicz–Simpson overlap coefficient is 0.159. Under the hypergeometric model with the 6,078-gene disease set as background, a 210-gene and a 138-gene subset drawn at random would be expected to share 4.77 proteins. The observed overlap of 22 is 4.6-fold larger ( $P = 1.26 \times 10^{-9}$ ; Figure 2B). Against a proteome background of 20,000 protein-coding genes the same overlap is 15.2-fold larger than an expectation of 1.45 ( $P = 6.01 \times 10^{-20}$ ). The two compounds therefore meet on a small core that is unlikely to arise by chance, while differing across the rest of the disease module.



**Figure 2.** Partition and enrichment of the combined disease-relevant target space. **(A)** Three-way Venn diagram of the LYC target set, the PRM target set and the 6,078-gene TBI gene set. **(B)** Observed overlap (22 proteins) against the hypergeometric expectation (4.77) under the 6,078-gene disease-set background, a 4.6-fold enrichment at  $P = 1.26 \times 10^{-9}$ .

**Table 2.** Partition of the combined TBI-relevant targets and enrichment of the shared fraction.

| Quantity                                                    | Value                  |
|-------------------------------------------------------------|------------------------|
| LYC $\cap$ TBI                                              | 210                    |
| PRM $\cap$ TBI                                              | 138                    |
| Union (combined disease-relevant space)                     | 326                    |
| LYC-specific                                                | 188 (57.7%)            |
| PRM-specific                                                | 116 (35.6%)            |
| Shared by both compounds                                    | 22 (6.7%)              |
| Compound-specific fraction                                  | 93.3%                  |
| Shared by both but not TBI-associated (excluded)            | 3                      |
| Jaccard index                                               | 0.068                  |
| Overlap coefficient (Szymkiewicz–Simpson)                   | 0.159                  |
| Expected overlap by chance, 6,078-gene TBI background       | 4.77                   |
| Fold enrichment, 6,078-gene TBI background                  | 4.6                    |
| Hypergeometric $P$ , 6,078-gene TBI background              | $1.26 \times 10^{-9}$  |
| Expected overlap by chance, 20,000-gene proteome background | 1.45                   |
| Fold enrichment, 20,000-gene proteome background            | 15.2                   |
| Hypergeometric $P$ , 20,000-gene proteome background        | $6.01 \times 10^{-20}$ |

### 3.3 Shared-target PPI network

The 22 shared TBI-relevant proteins were submitted to STRING v12 (Szklarczyk et al., 2023) at a minimum interaction score of 0.400 with all active sources enabled. The network has 22 nodes and 80 edges, an average node degree of 7.27, a density of

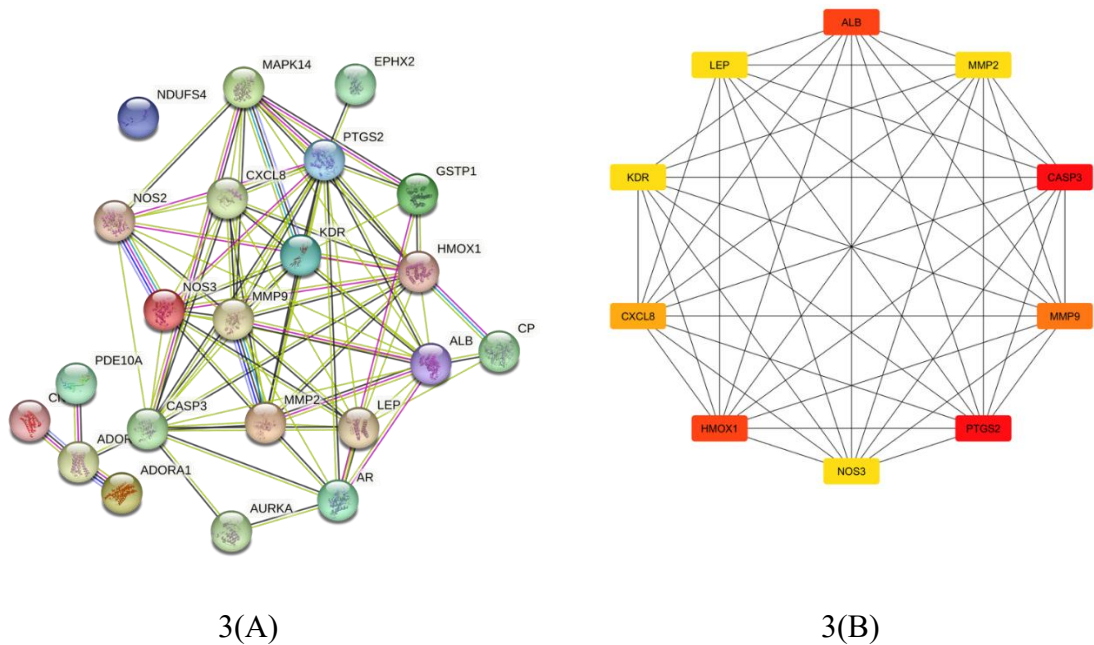
0.346 and an average local clustering coefficient of 0.581 (Figure 3A). Twenty-one of the 22 proteins sit in one connected component. Only NDUFS4 stands alone at this threshold.

A density of 0.346 means that more than one in three of all possible pairs among

these proteins is supported by evidence in STRING, and a clustering coefficient of 0.581 means the network closes into triangles locally. A set of 22 proteins assembled separately by two chemically unrelated compounds would not be expected to be this well connected.

Ranking by node degree (Chin et al., 2014) gave the ten most connected proteins (Figure 3B, Table 3). These ten carry 117 of

the 160 degree endpoints in the network. Each is a known participant in secondary injury, and between them they cover its main arms: inflammation (PTGS2, CXCL8), apoptosis (CASP3), oxidative and iron-handling stress (HMOX1), barrier proteolysis (MMP2, MMP9), and vascular and metabolic signalling (KDR, NOS3, LEP). ALB, ranked third, is both a carrier for lipophilic ligands and an established marker of barrier breakdown after injury.



**Figure 3.** Protein–protein interaction network of the 22 shared disease-relevant targets. **(A)** Full STRING network (confidence  $\geq 0.400$ ): 22 nodes, 80 edges, average degree 7.27, density 0.346, average clustering coefficient 0.581; NDUFS4 is unconnected at this threshold. **(B)** The ten highest-degree proteins, node colour scaled from red (highest degree) to yellow (lowest).

**Table 3.** The most connected proteins in the shared target network.

| Rank | Gene | Protein | Degree | Principal role in secondary injury |
|------|------|---------|--------|------------------------------------|
|      |      |         |        |                                    |

|   |       |                                           |     |    |                                                       |
|---|-------|-------------------------------------------|-----|----|-------------------------------------------------------|
| 1 | PTGS2 | Prostaglandin synthase 2 (COX-2)          | G/H | 14 | Prostanoid synthesis; neuroinflammation               |
| 1 | CASP3 | Caspase-3                                 |     | 14 | Executioner caspase; apoptotic neuronal death         |
| 3 | ALB   | Serum albumin                             |     | 13 | Lipophilic ligand transport; barrier-breakdown marker |
| 3 | HMOX1 | Heme oxygenase 1                          |     | 13 | Heme catabolism; labile iron release                  |
| 5 | MMP9  | Matrix metalloproteinase-9 (gelatinase B) |     | 12 | Basal lamina degradation; BBB opening                 |
| 6 | CXCL8 | Interleukin-8                             |     | 11 | Neutrophil chemotaxis; acute inflammatory recruitment |
| 7 | MMP2  | Matrix metalloproteinase-2 (gelatinase A) |     | 10 | Constitutive ECM proteolysis; early barrier injury    |
| 7 | KDR   | VEGF receptor 2 (VEGFR-2)                 |     | 10 | Vascular permeability; post-traumatic angiogenesis    |
| 7 | LEP   | Leptin                                    |     | 10 | Metabolic and neuroprotective signalling              |
| 7 | NOS3  | Nitric oxide synthase, endothelial (eNOS) |     | 10 | Cerebral blood flow autoregulation                    |

### 3.4 Functional and pathway enrichment

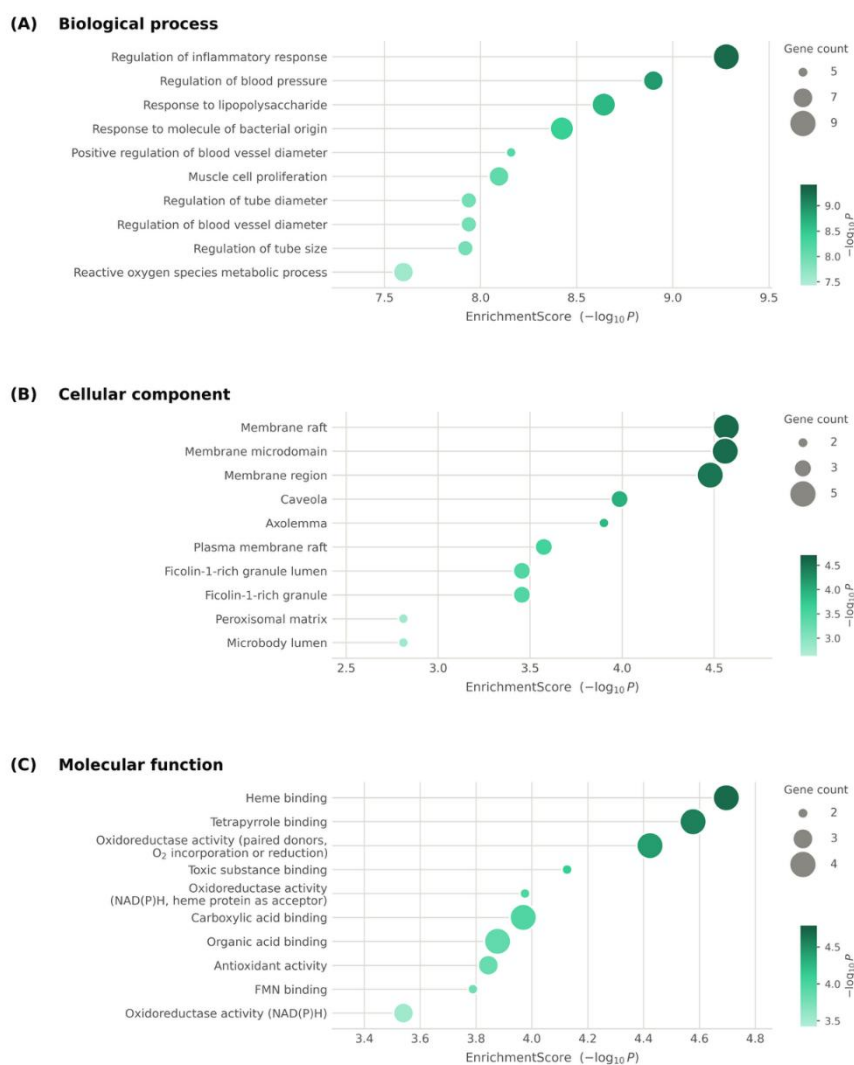
The 22 shared proteins were tested for GO over-representation in DAVID (Sherman et al., 2022) against the Homo sapiens background. The ten most significant terms in each ontology are given in Table 4 and Figure 4. Biological process was dominated by the inflammatory and vascular arms of secondary injury. Regulation of inflammatory response was the top term, drawing 9 of the 22 proteins ( $P = 5.27 \times 10^{-10}$ ). Next came regulation of blood

pressure (7 proteins), then the paired lipopolysaccharide and bacterial-origin response terms (8 proteins each). Reactive oxygen species metabolic process drew 7 proteins. Four vessel-calibre terms appeared together: positive regulation of blood vessel diameter, regulation of tube diameter, regulation of blood vessel diameter and regulation of tube size. This shows the shared core has a cerebrovascular component as well as an inflammatory one,

which fits the presence of NOS3, KDR and LEP among the hubs. Molecular function centred on heme and oxidoreductase chemistry. Heme binding (4 proteins,  $P = 2.01 \times 10^{-5}$ ) and tetrapyrrole binding led, with oxidoreductase activity appearing in three forms and antioxidant activity drawing 3 proteins. This pattern maps onto HMOX1, NOS2, NOS3 and PTGS2.

In the cellular component ontology the expected membrane raft, membrane microdomain and caveolar terms appeared,

but so did the axolemma (2 proteins,  $P = 1.26 \times 10^{-4}$ ), driven by ADORA1 and ADORA2A. The axolemma is the membrane compartment whose mechanical disruption starts diffuse axonal injury, and a general list of inflammatory proteins would not be expected to annotate there. This term may therefore mark a shared point of engagement at the site where mechanical force becomes biochemical injury, and it is the one enrichment result specific to neurotrauma rather than to tissue injury in general.



Ten most significant terms per ontology · DAVID v2021 · 22 shared disease-relevant targets

**Figure 4.** Gene Ontology enrichment of the 22 shared disease-relevant targets. The ten most significant terms in each of the three ontologies, biological process (BP), cellular component (CC) and molecular function (MF), plotted as enrichment score ( $-\log_{10} P$ ).

**Table 4.** The 10 most significantly enriched Gene Ontology terms in each ontology for the 22 shared targets.

| Ontology | Term                                | Count | EnrichmentScore ( $-\log_{10} P$ ) | $P$                    |
|----------|-------------------------------------|-------|------------------------------------|------------------------|
| BP       | Regulation of inflammatory response | 9     | 9.28                               | $5.27 \times 10^{-10}$ |
| BP       | Regulation of blood pressure        | 7     | 8.90                               | $1.26 \times 10^{-9}$  |

|    |                                                                                                       |   |      |                       |
|----|-------------------------------------------------------------------------------------------------------|---|------|-----------------------|
| BP | Response to lipopolysaccharide                                                                        | 8 | 8.64 | $2.29 \times 10^{-9}$ |
| BP | Response to molecule of bacterial origin                                                              | 8 | 8.42 | $3.77 \times 10^{-9}$ |
| BP | Positive regulation of blood vessel diameter                                                          | 5 | 8.16 | $6.93 \times 10^{-9}$ |
| BP | Muscle cell proliferation                                                                             | 7 | 8.10 | $8.02 \times 10^{-9}$ |
| BP | Regulation of tube diameter                                                                           | 6 | 7.94 | $1.15 \times 10^{-8}$ |
| BP | Regulation of blood vessel diameter                                                                   | 6 | 7.94 | $1.15 \times 10^{-8}$ |
| BP | Regulation of tube size                                                                               | 6 | 7.92 | $1.20 \times 10^{-8}$ |
| BP | Reactive oxygen species metabolic process                                                             | 7 | 7.60 | $2.52 \times 10^{-8}$ |
| CC | Membrane raft                                                                                         | 5 | 4.57 | $2.72 \times 10^{-5}$ |
| CC | Membrane microdomain                                                                                  | 5 | 4.56 | $2.76 \times 10^{-5}$ |
| CC | Membrane region                                                                                       | 5 | 4.48 | $3.32 \times 10^{-5}$ |
| CC | Caveola                                                                                               | 3 | 3.99 | $1.03 \times 10^{-4}$ |
| CC | Axolemma                                                                                              | 2 | 3.90 | $1.26 \times 10^{-4}$ |
| CC | Plasma membrane raft                                                                                  | 3 | 3.57 | $2.67 \times 10^{-4}$ |
| CC | Ficolin-1-rich granule lumen                                                                          | 3 | 3.46 | $3.50 \times 10^{-4}$ |
| CC | Ficolin-1-rich granule                                                                                | 3 | 3.46 | $3.50 \times 10^{-4}$ |
| CC | Peroxisomal matrix                                                                                    | 2 | 2.81 | $1.55 \times 10^{-3}$ |
| CC | Microbody lumen                                                                                       | 2 | 2.81 | $1.55 \times 10^{-3}$ |
| MF | Heme binding                                                                                          | 4 | 4.70 | $2.01 \times 10^{-5}$ |
| MF | Tetrapyrrole binding                                                                                  | 4 | 4.58 | $2.65 \times 10^{-5}$ |
| MF | Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | 4 | 4.42 | $3.78 \times 10^{-5}$ |
| MF | Toxic substance binding                                                                               | 2 | 4.13 | $7.49 \times 10^{-5}$ |
| MF | Oxidoreductase activity, acting on NAD(P)H, heme protein as acceptor                                  | 2 | 3.98 | $1.06 \times 10^{-4}$ |
| MF | Carboxylic acid binding                                                                               | 4 | 3.97 | $1.07 \times 10^{-4}$ |

|    |                                            |   |      |                       |
|----|--------------------------------------------|---|------|-----------------------|
| MF | Organic acid binding                       | 4 | 3.88 | $1.33 \times 10^{-4}$ |
| MF | Antioxidant activity                       | 3 | 3.84 | $1.43 \times 10^{-4}$ |
| MF | FMN binding                                | 2 | 3.79 | $1.63 \times 10^{-4}$ |
| MF | Oxidoreductase activity, acting on NAD(P)H | 3 | 3.54 | $2.89 \times 10^{-4}$ |

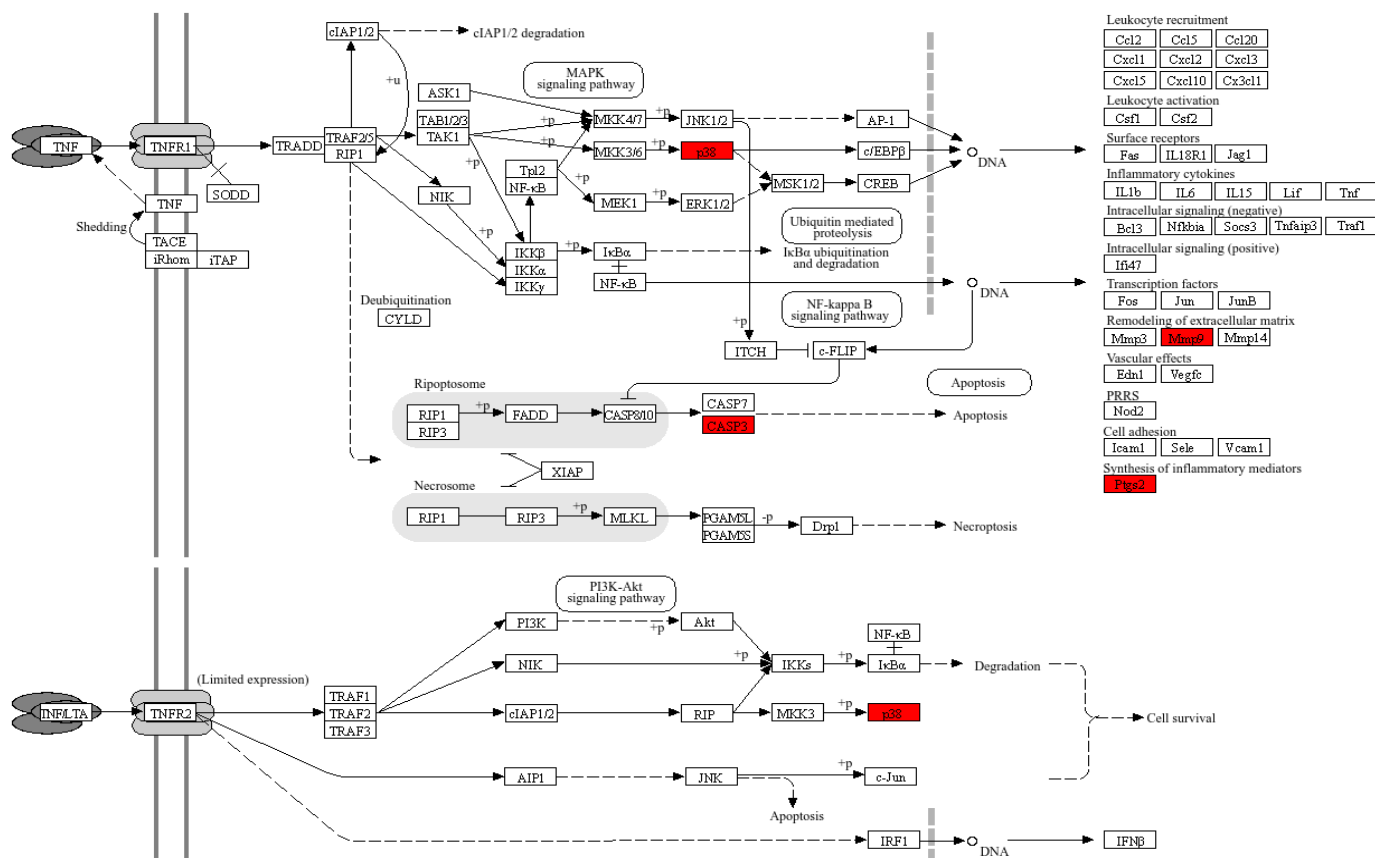
*BP, biological process; CC, cellular component; MF, molecular function.*

KEGG mapping returned pathways covering the same arms of secondary injury seen in the network analysis. Inflammatory signalling appeared as the NF- $\kappa$ B pathway, carrying CXCL8 and PTGS2 (Figure 5A). The vascular and hypoxic response appeared as HIF-1 signalling, carrying NOS2, NOS3 and HMOX1 (Figure 5B). Lipid mediator production appeared as arachidonic acid metabolism, carrying PTGS2 and the soluble epoxide hydrolase EPHX2 (Figure 5C). Second-messenger signalling appeared as the MAPK pathway, carrying KDR, CASP3 and MAPK14, and as the calcium and cAMP pathways, the latter carrying the striatal phosphodiesterase PDE10A. Nitric oxide handling appeared through arginine biosynthesis and arginine and proline metabolism. Heme and iron handling appeared through porphyrin metabolism (Figure 5D) and the peroxisome pathway,

the latter carrying EPHX2 and NOS2. Several KEGG nodes are collapsed family boxes (GPCR, RTK, NOS, ADORA, CNR) rather than single genes, and are named here by the shared target they represent.

Two points stand out. First, the two compounds converge on heme and iron chemistry: HMOX1 releases labile  $\text{Fe}^{2+}$  by breaking down heme, and CP acts as the ferroxidase needed to export that iron. This matters in TBI, where blood released by contusion and intracranial haemorrhage delivers a large iron load to brain tissue. Second, neuroactive ligand–receptor interaction draws in ADORA1, ADORA2A, CNR2 and LEP. These are the same adenosine and cannabinoid receptors that carry the axolemmal enrichment above, which places part of the shared core at neuromodulatory rather than inflammatory targets.

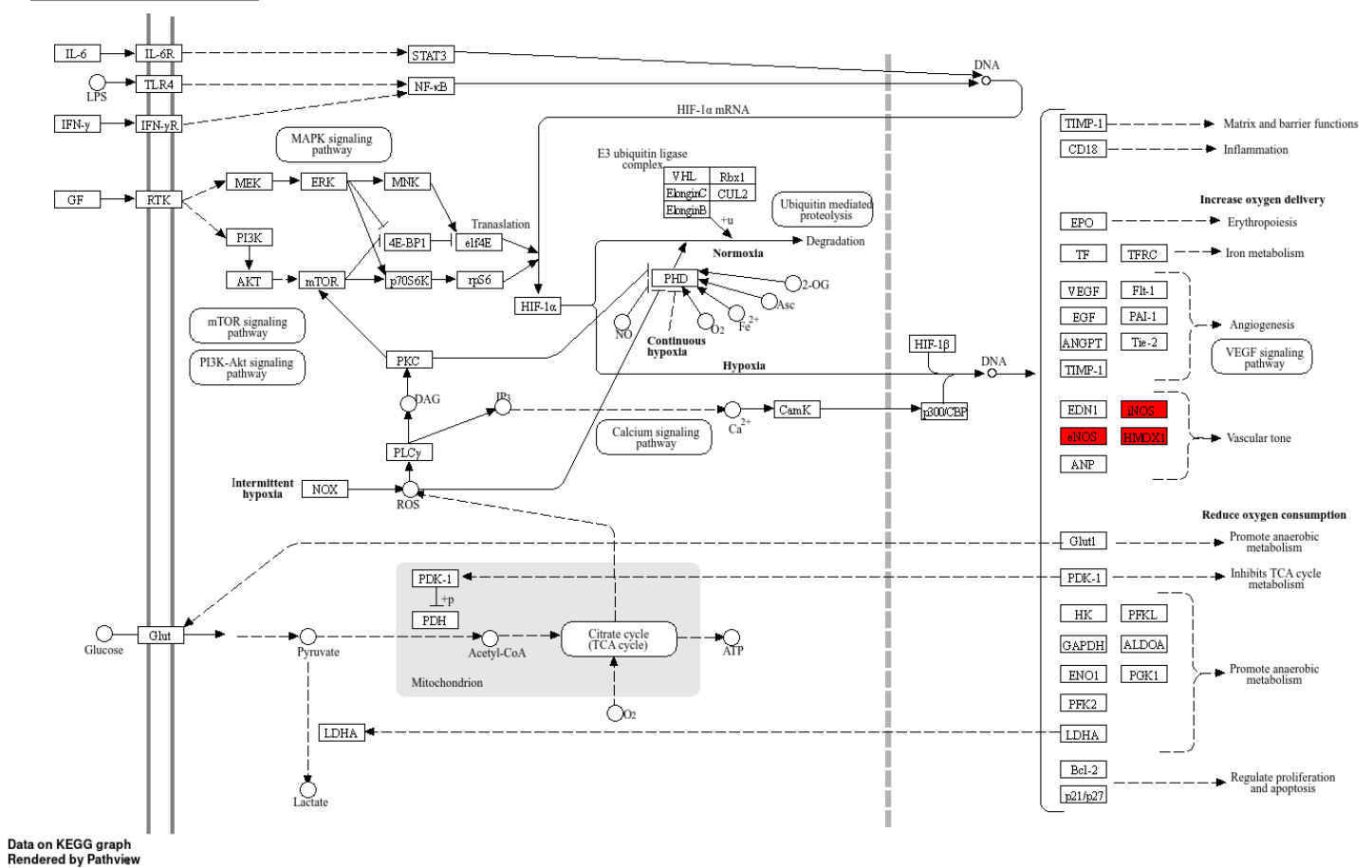
TNF SIGNALING PATHWAY



Data on KEGG graph  
Rendered by Pathview

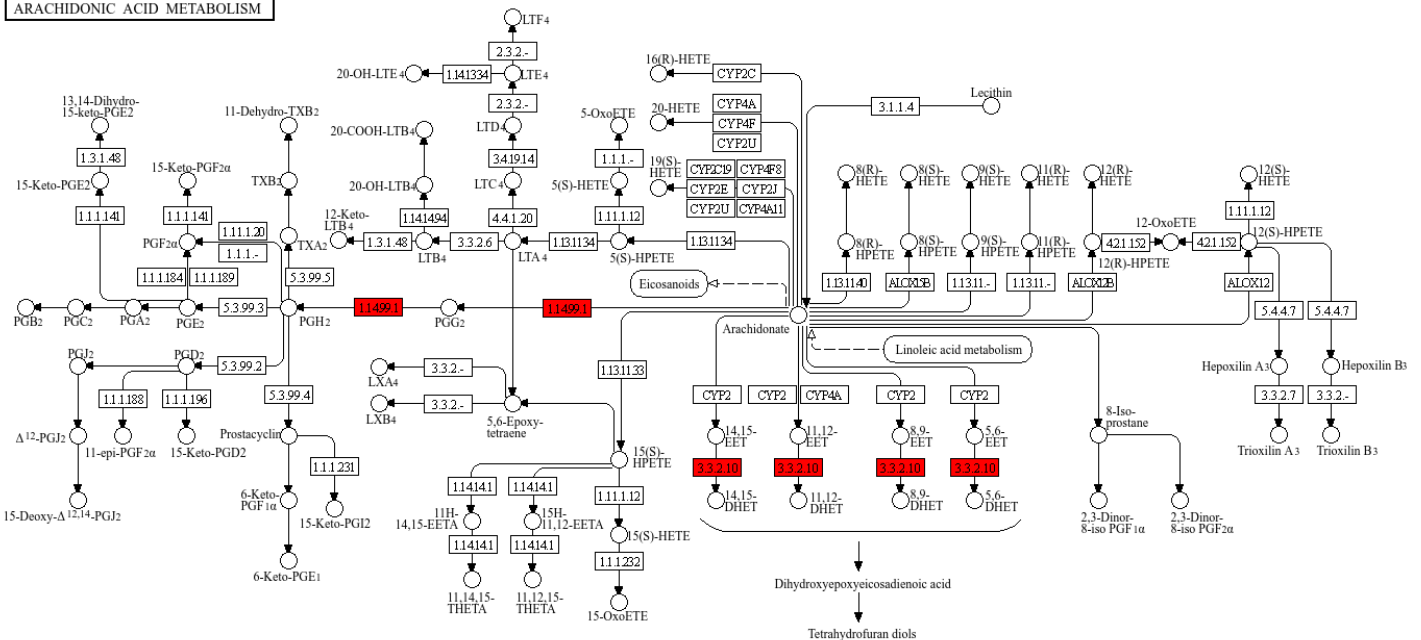
5(A)

## HIF-1 SIGNALING PATHWAY



**5(B)**

## ARACHIDONIC ACID METABOLISM





**Figure 5.** KEGG pathways mapped from the 22 shared disease-relevant targets, with shared proteins highlighted in red. **(A)** NF- $\kappa$ B signalling pathway, carrying CXCL8 and PTGS2. **(B)** HIF-1 signalling pathway, carrying NOS2, NOS3 and HMOX1. **(C)** Arachidonic acid metabolism, carrying PTGS2 (EC 1.14.99.1) and EPHX2 (EC 3.3.2.10). **(D)** Porphyrin metabolism, carrying HMOX1 (EC 1.14.14.18) at the heme-catabolic release of labile  $\text{Fe}^{2+}$  and CP (EC 1.16.3.1) at the ferroxidase step required for iron export. Source: KEGG reference pathways (Kanehisa and Goto, 2000) rendered in Pathview (Luo and Brouwer, 2013); gene mapping by the authors.

### 3.5 Structure selection and docking validation

Ten shared hub proteins were taken forward to docking. Structures were selected from the Protein Data Bank under a fixed protocol: human origin, X-ray resolution preferably at or below 2.5 Å, a resolved and unmutated binding site, and, where available, a co-crystallised reference ligand to define the site and allow redocking (5).

Docking was site-directed rather than blind. Grid boxes were centred on the co-crystallised ligand wherever one was present. Redocking of the reference ligand reproduced its crystal pose to within 2.0 Å

RMSD in every case where a suitable non-covalent, non-peptidic reference was available. Blind docking over the whole protein surface was not used at any stage, since it tends to return spurious surface minima (Che and Zhang, 2025).

### 3.6 Comparative docking and ligand efficiency

Both compounds were docked into all ten hub targets with AutoDock Vina (Trott and Olson, 2010). ALB was left out of the paired statistical comparison, because its loose multi-site binding makes its score hard to compare with a defined catalytic or allosteric pocket. This left nine matched target pairs (Table 5, Figure 6A).

LYC gave the more negative predicted binding affinity at all nine targets, with a mean of  $-8.61 \text{ kcal mol}^{-1}$  against  $-4.79 \text{ kcal mol}^{-1}$  for PRM, a difference of  $3.82 \text{ kcal mol}^{-1}$  (Table 6). LYC ranged from  $-9.40 \text{ kcal mol}^{-1}$  at PTGS2 to  $-7.30 \text{ kcal mol}^{-1}$  at LEP, and PRM from  $-5.91 \text{ kcal mol}^{-1}$  at MMP9 to  $-3.80 \text{ kcal mol}^{-1}$  at LEP. The difference is significant by the Wilcoxon signed-rank test ( $W = 0$ ,  $P = 3.91 \times 10^{-3}$ , the lowest value attainable at  $n = 9$ ) and by the paired t-test ( $t = -14.42$ ,  $P = 5.22 \times 10^{-7}$ ) (Table 7). On raw score alone, LYC would be judged the better binder at every target. That judgement does not survive normalisation, however.

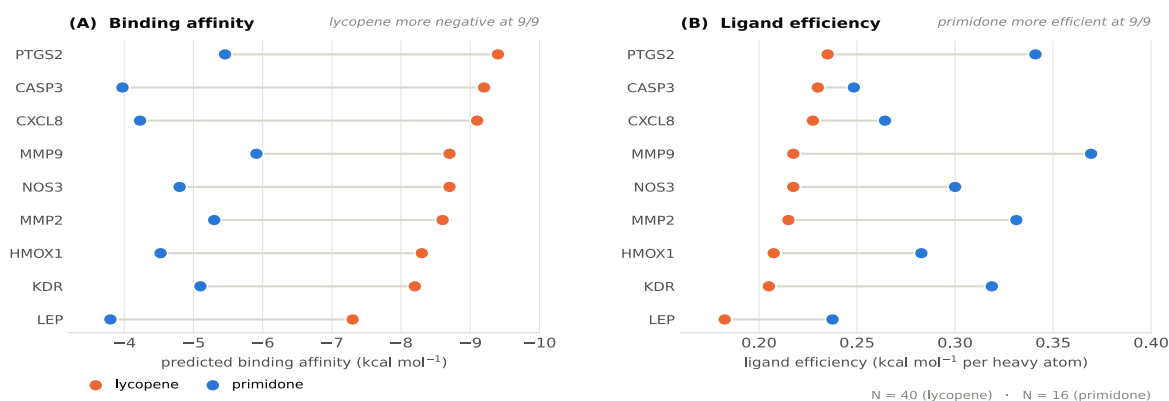
Ligand efficiency,  $LE = -\Delta G/N$  where N is the number of heavy atoms (Hopkins et al., 2004), reverses the order completely (Figure 6B). LYC's 40 heavy atoms give a mean LE of  $0.215 \text{ kcal mol}^{-1}$  per heavy atom, while PRM's 16 give 0.299. PRM is the more efficient binder at all nine targets ( $W = 0$ ,  $P = 3.91 \times 10^{-3}$ ; paired t-test  $t(8) = 5.92$ ,  $P = 3.52 \times 10^{-4}$ ). Its mean advantage is  $0.084 \text{ kcal mol}^{-1}$  per heavy atom (95% CI 0.051 to 0.117), with a mean ratio of 1.39 and a per-target range of 1.08 at CASP3 to 1.70 at MMP9. Ranked by ligand

efficiency, PRM does best at MMP9 (0.369), PTGS2 (0.341), MMP2 (0.331) and KDR (0.319), and worst at LEP (0.237). The two rank orders are unrelated: the Spearman correlation between the LYC and PRM affinity rankings is  $\rho = 0.234$  ( $P = 0.544$ ). The compounds therefore differ both in how strongly they are predicted to engage the shared core and in which members of it they prefer. The reversal is complete: 9 of 9 in favour of LYC on raw affinity, and 9 of 9 in favour of PRM on ligand efficiency.

**Table 5.** Predicted binding affinities and ligand efficiencies of LYC and PRM at the shared hub targets.

| Target              | PDB ID | LYC $\Delta G$ (kcal mol <sup>-1</sup> ) | LE (LYC, N = 40) | PRM $\Delta G$ (kcal mol <sup>-1</sup> ) | LE (PRM, N = 16) | LE ratio    |
|---------------------|--------|------------------------------------------|------------------|------------------------------------------|------------------|-------------|
| PTGS2               | 5IKR   | -9.40                                    | 0.235            | -5.456                                   | 0.341            | 1.45        |
| CASP3               | 2J30   | -9.20                                    | 0.230            | -3.974                                   | 0.248            | 1.08        |
| CXCL8               | 5D14   | -9.10                                    | 0.227            | -4.228                                   | 0.264            | 1.16        |
| MMP9                | 4H3X   | -8.70                                    | 0.217            | -5.910                                   | 0.369            | 1.70        |
| NOS3                | 3NOS   | -8.70                                    | 0.217            | -4.800                                   | 0.300            | 1.38        |
| MMP2                | 7XJO   | -8.60                                    | 0.215            | -5.300                                   | 0.331            | 1.54        |
| HMOX1               | 3HOK   | -8.30                                    | 0.208            | -4.525                                   | 0.283            | 1.36        |
| KDR                 | 2QU5   | -8.20                                    | 0.205            | -5.100                                   | 0.319            | 1.55        |
| LEP                 | 1AX8   | -7.30                                    | 0.182            | -3.800                                   | 0.237            | 1.30        |
| <b>Mean (n = 9)</b> |        | <b>-8.61</b>                             | <b>0.215</b>     | <b>-4.788</b>                            | <b>0.299</b>     | <b>1.39</b> |
| ALB*                | 2BXD   | -10.80                                   | 0.270            | -5.511                                   | 0.344            | 1.28        |

\*ALB reported descriptively and excluded from the paired comparison.  $LE = -\Delta G/N$ .



**Figure 6.** Affinity and ligand efficiency across the nine matched hub targets. **(A)** Predicted binding affinity, LYC (orange) against PRM (blue); LYC is more negative at every target. **(B)** The same comparisons after normalisation to heavy-atom count (N = 40 and N = 16); the ordering reverses completely and PRM is more efficient at every target.

**Table 6.** Descriptive statistics of LYC and PRM across the 09 structure-matched hub targets.

| Metric                                                               | LYC                              | PRM            |
|----------------------------------------------------------------------|----------------------------------|----------------|
| Heavy-atom count                                                     | 40                               | 16             |
| Predicted binding affinity (kcal mol <sup>-1</sup> ), mean ± SD      | -8.61 ± 0.63                     | -4.79 ± 0.72   |
| Predicted binding affinity, range                                    | -7.30 to -9.40                   | -3.80 to -5.91 |
| Ligand efficiency (kcal mol <sup>-1</sup> per heavy atom), mean ± SD | 0.215 ± 0.016                    | 0.299 ± 0.045  |
| Ligand efficiency, range                                             | 0.182 to 0.235                   | 0.237 to 0.369 |
| Targets at which affinity is the more negative                       | 9 of 9                           | 0 of 9         |
| Targets at which ligand efficiency is the higher                     | 0 of 9                           | 9 of 9         |
| Ligand efficiency ratio, PRM: LYC                                    | 1.39 ± 0.20 (range 1.08 to 1.70) |                |

**Table 7:** Paired comparisons of LYC and PRM across the 09 structure-matched hub targets.

| Comparison                                  | Test                         | Test statistic  | Estimate (95% CI)                                                                                   | P                     |
|---------------------------------------------|------------------------------|-----------------|-----------------------------------------------------------------------------------------------------|-----------------------|
| Binding affinity, LYC vs PRM                | Wilcoxon signed-rank (exact) | $W = 0$         | rank-biserial $r = 1.00$                                                                            | $3.91 \times 10^{-3}$ |
| Binding affinity, LYC vs PRM                | Paired $t$ -test             | $t(8) = -14.42$ | mean difference $-3.82 \text{ kcal mol}^{-1}$ ( $-4.43$ to $-3.21$ ); $dz = -4.81$                  | $5.22 \times 10^{-7}$ |
| Ligand efficiency, PRM vs LYC               | Wilcoxon signed-rank (exact) | $W = 0$         | rank-biserial $r = 1.00$                                                                            | $3.91 \times 10^{-3}$ |
| Ligand efficiency, PRM vs LYC               | Paired $t$ -test             | $t(8) = 5.92$   | mean difference $+0.084 \text{ kcal mol}^{-1}$ per heavy atom ( $+0.051$ to $+0.117$ ); $dz = 1.97$ | $3.52 \times 10^{-4}$ |
| Affinity rank concordance between compounds | Spearman rank correlation    | $\rho = 0.234$  | 95% CI $-0.51$ to $+0.78$                                                                           | 0.544                 |

$n = 9$  structure-matched pockets; human serum albumin excluded (Section 2.10). SD, standard deviation; CI, confidence interval;  $r$ , matched-pairs rank-biserial correlation;  $dz$ , Cohen's  $d$  for paired samples.  $W = 0$  with  $P = 3.91 \times 10^{-3}$  is the minimum attainable by the exact Wilcoxon test at  $n = 9$  and reflects unanimous directional agreement across all nine pairs. Normality of the paired differences was confirmed by the Shapiro–Wilk test (affinity,  $W = 0.934$ ,  $P = 0.524$ ; ligand efficiency,  $W = 0.977$ ,  $P = 0.948$ ), so parametric and

non-parametric tests are both admissible and are reported together.

### 3.7 Summary of findings

Across the three analyses, the predicted target profiles of the two compounds overlap in only 6.7% of the combined disease-relevant space, and that overlap is 4.6-fold larger than chance expectation. Twenty-one of the 22 shared targets form a single connected module with a density of 0.346, spanning inflammation, apoptosis, barrier proteolysis, cerebrovascular calibre

control, heme and iron handling, and, through the axolemmal adenosine receptors, the membrane compartment where mechanical force is first transduced. Inside that shared core the two compounds show opposite profiles of predicted affinity and ligand efficiency, and their target preferences are uncorrelated.

#### 4. DISCUSSION

We compared the predicted target profiles of LYC and PRM in TBI to establish whether the two compounds act on the same region of the disease network or on different regions. The answer was consistent at every level of the analysis. Of the 326 disease-relevant predicted targets that the two compounds address between them, 93.3% were reached by one compound and not the other, which gives a Jaccard index of only 0.068. The fraction they do share is nonetheless larger than chance would predict. The 22 shared targets were 4.6-fold more numerous than expected against the disease-set background and 15.2-fold more numerous against the proteome, and 21 of them assembled into a single dense module. The docking results followed the same pattern of difference. Both compounds were predicted to interact with every hub of that module, but they did so by opposite routes: LYC returned the more negative predicted

affinity at all nine matched pockets, while PRM returned the higher ligand efficiency at all nine. Their rank orders were uncorrelated as well. Taken together, these observations are consistent with a complementary predicted target profile rather than a duplicated one. They do not, however, establish complementarity in the formal sense defined by Cheng et al. (2019), which requires a network-separation analysis, and they say nothing about pharmacological synergy.

An overlap of only 6.7% might be read as a sign that two compounds sharing so little have little prospect of working together. That reading inverts the logic on which the comparison rests. When Cheng et al. (2019) examined 681 drug combinations, the class associated with benefit was the one in which both drug modules reached the disease module while remaining separate from one another. Combinations whose modules overlapped performed no better than the single drugs did. Within that framework, low overlap is not an objection to a candidate pair but a prerequisite for it. The distinction matters because the opposite assumption is widespread in the natural-product literature, where a long list of shared targets is often presented as evidence that two agents act together on common pathways. A profile of that kind is at least as consistent with duplication, in

which the second agent behaves much like a higher dose of the first. A comparison of two predicted target sets requires nothing beyond public data and can be repeated for any pair of annotated compounds, so it offers a cheap way of setting aside heavily overlapping pairs before any experiment begins. What our comparison provides, then, is a basis for testing possible combination effects rather than evidence that such effects occur.

Enrichment analysis in network pharmacology is often criticised because lists of broadly acting inflammatory proteins annotate to terms that do little to distinguish one disease from another. Three features of our result are harder to explain that way. The first is heme and iron handling. Heme binding emerged as the leading molecular function term, and HMOX1 ranked among the most connected proteins in the shared module. KEGG mapping then placed HMOX1 and CP at opposite ends of the same iron flux on porphyrin metabolism, with heme oxygenase releasing labile  $\text{Fe}^{2+}$  as it breaks down heme and ceruloplasmin supplying the ferroxidase activity needed to export that iron. This axis is directly relevant to the injured brain, where blood released by contusion and intracranial haemorrhage delivers a large iron load. The labile iron that results then drives the lipid

peroxidation now described as ferroptosis (Dixon et al., 2012; Zhu et al., 2026). The second feature is the axolemma, which was enriched and is carried by ADORA1 and ADORA2A. Two genes make a narrow basis for any claim, so we report the term with caution. Even so, the axolemma is the membrane compartment whose mechanical deformation initiates diffuse axonal injury (Werner and Engelhard, 2007), and a general list of inflammatory effectors would not be expected to annotate there. The third feature is the recurrence of four vessel-calibre terms alongside regulation of blood pressure. This matters because loss of cerebral autoregulation, impaired vasoreactivity and secondary ischaemia are among the principal determinants of outcome after severe injury (Maas et al., 2017). A shared core that reaches cerebrovascular control as well as inflammation therefore covers a wider portion of the clinically relevant cascade than an anti-inflammatory profile alone would.

The reversal between predicted affinity and ligand efficiency is best read as a methodological observation rather than as evidence that either compound is pharmacologically superior, since it follows arithmetically from heavy-atom counts of 40 and 16. A raw docking score rises with ligand size, because a larger molecule

makes more contacts irrespective of their quality (Hopkins et al., 2004). Any comparison that ranks a large natural product against a small synthetic drug on an unnormalised score will therefore favour the larger molecule for reasons unconnected to pharmacology. Ligand efficiency should be reported alongside affinity wherever compounds of markedly different size are compared. Beyond that corrective function, the normalised values are informative in their own right. The mean ligand efficiency of PRM, at 0.299 kcal mol<sup>-1</sup> per heavy atom, sits close to the value of approximately 0.3 conventionally applied to lead-like compounds, whereas every LYC value falls below it, with a mean of 0.215. This should not be taken to mean that LYC engages its targets poorly, but rather that its contribution is of a different kind. LYC covers a broad set of predicted targets across the oxidative and membrane-damage limbs of the cascade. That breadth fits its partitioning into membrane microdomains, where it interrupts lipid peroxidation chains (Lei et al., 2016; Khan et al., 2021), rather than a close per-atom fit to defined pockets. PRM, by contrast, engages a narrower set efficiently, and it brings established human pharmacokinetics and a characterised adverse-effect profile from decades of clinical use (Patsalos et al., 2018; Pushpakom et al., 2019). Why two such dissimilar compounds meet at all is

most likely explained by the shape of the disease network rather than by any similarity between them. The secondary cascade is densely interconnected (Kumar and Loane, 2012; Loane and Kumar, 2016), so two agents drawn into it independently will tend to converge on its most connected nodes.

Several limitations should be stated plainly. The study does not demonstrate complementary exposure in the formal sense, because Cheng et al. (2019) define their classes using the separation measures of Menche et al. (2015) computed on a curated interactome, whereas what we present here is the set-partition analogue of that condition. Nor does the study demonstrate synergy. Whether the interaction is additive, synergistic or antagonistic is a pharmacological question that only a dose–response experiment under a defined interaction model can answer. The study also leaves the excitatory limb of the cascade untouched, since no glutamate receptor, GABA-A subunit or voltage-gated ion channel appears among the 22 shared targets. This is partly an artefact of construction, because target prediction was performed on the parent compounds alone, while much of the antiseizure activity of PRM is attributed to its phenobarbital metabolite (Eadie, 1991). That omission causes the shared core to understate the

contribution of PRM, and so weakens our claim rather than inflating it. Several further constraints bear on interpretation. Target prediction rests on ligand-similarity inference (Daina et al., 2019), so a predicted target remains a hypothesis rather than an observation. The disease set of 6,078 genes is a permissive filter rather than a strict one, although both compound lists were intersected against the same set, which keeps the comparison between compounds internally controlled. Docking scores are scoring-function estimates carrying errors of the order of 2 kcal mol<sup>-1</sup>, so the analysis rests on relative rather than absolute values. Redocking could validate the protocol only where a non-covalent, non-peptidic reference ligand was available, which leaves it unvalidated at caspase-3 and at matrix metalloproteinase-2. Finally, nothing reported here has been tested experimentally, and both the bioavailability of LYC and its penetration of the blood–brain barrier remain to be established.

The programme of work that follows from this analysis is defined rather than open-ended. The first step is computational and requires no new data. Computing the separation measures of Menche et al. (2015) on a curated interactome would place the pair formally within the Cheng classification, and repeating the target prediction with phenobarbital and

phenylethylmalonamide included would bring the metabolite pharmacology of PRM into the comparison. Engagement of HMOX1 by both compounds could then be confirmed or ruled out by surface plasmon resonance or isothermal titration calorimetry, alongside an established heme oxygenase activity assay. The same approach extends readily to PTGS2 and MMP9, the two targets at which PRM shows its highest ligand efficiency. The final step is a controlled cortical impact study comparing LYC alone, PRM alone, the combination and vehicle in a factorial design, with dose–response arms sufficient to separate additivity from synergy. Its endpoints follow from the shared core rather than from convention: lesion volume and neurological severity scoring, together with heme oxygenase activity and labile iron, lipid peroxidation markers, gelatinase activity, and axonal injury assessed by amyloid precursor protein immunoreactivity. Should the combination prove additive rather than synergistic, that result would be equally informative, since it would show that topological complementarity is necessary but not sufficient for therapeutic benefit.

## 5. CONCLUSION

LYC and PRM showed largely distinct TBI-associated predicted target profiles,

with 93.3% of their combined disease-relevant predicted targets belonging to one compound only. Their 22 shared predicted targets were enriched well beyond chance, and 21 of them formed a densely connected network covering inflammatory, oxidative, vascular, apoptotic and heme-related processes. Docking showed that LYC produced more negative predicted binding scores while PRM showed greater ligand efficiency across the matched targets, which highlights how important ligand-size normalisation is in any comparative docking study. Taken together, our findings suggest potential target complementarity between the two compounds in TBI. They do not, however, establish pharmacological synergy, which would need dose–response work under a defined interaction model, and they do not place the pair formally within the Cheng classification, which needs a network-separation analysis on a curated interactome. What the analysis gives is a criterion rather than an outcome: it names the proteins at which any combination effect would have to act, and provides a basis for testing that effect experimentally. The comparison itself is cheap and works for any pair of compounds with annotated predicted target profiles. It cannot say which combinations will succeed, but it may show which are unlikely to be worth testing.

## Declarations

**Author contributions:** SG: conceptualisation, data curation, formal analysis, investigation, methodology, writing – original draft. SK: conceptualisation, supervision, validation, writing – review and editing. AL: methodology, validation, writing – review and editing. All authors read and approved the final manuscript.

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**Conflict of interest:** The authors declare that they have no competing interests.

**Ethics approval:** Not applicable

**Data availability:** All data supporting the findings of this study are included in the article. The predicted target lists, the TBI gene set, the STRING interaction export, the grid box parameters and redocking results, the DAVID enrichment output with adjusted P values, and the docking output files are available from the corresponding author on reasonable request.

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