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Conserved biological response states organize nanoparticle biosafety across neurological systems

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Abstract

Predicting biological outcomes of nanomaterials remains challenging because material properties alone do not capture context-dependent responses across exposure conditions and experimental systems. We develop a response-centred machine-learning framework that integrates heterogeneous biological responses into a Conserved Response Axis (CRA) associated with biosafety and recovery. Across 893 contexts from 599 neurological studies, integrating exposure and biological context with material properties increased ROC-AUC from 0.66 to 0.88 for biosafety and from 0.64 to 0.77 for recovery; incorporating cellular-response programmes further increased ROC-AUC to 0.90 and 0.78, respectively. Pro-inflammatory, anti-inflammatory and apoptosis-associated programmes formed coordinated patterns associated with biological outcomes. This organization was evaluated in unseen nanocomposites using 2D and 3D microglial models and independent brain, lung and liver transcriptomic datasets, supporting transferability across biological contexts. CRA identifies host-response states as a predictive layer beyond material and exposure descriptors, providing a basis for response-guided assessment and biology-informed nanomaterial design.

Keywords: Conserved response axis, biosafety, nanomaterials, heterogeneous datasets, machine learning, biological response modeling

Main

Nanoparticles offer considerable opportunities for neurological diagnosis and therapy, but their interactions with healthy and diseased biological systems remain difficult to predict. Material properties alone do not determine these interactions; exposure conditions, disease context and dynamic host responses can reshape biological outcomes. Consequently, similar nanoparticles can produce divergent responses across neurological contexts, whereas distinct nanomaterials may converge on comparable cellular response states.^{1, 2} This context dependence, compounded by heterogeneity in experimental models, exposure conditions, assays and biological readouts, limits the transferability of material-centred prediction. Current predictive frameworks, including nano-QSAR and computational nanotoxicology, have improved associations between nanoparticle descriptors and toxicity-related outcomes.^{3, 4, 5, 6, 7, 8, 9} However, many models remain context-dependent and show limited transferability across heterogeneous biological systems and experimental scales. Recent advances in nanoinformatics, adverse outcome pathway (AOP)-informed toxicology and Safe-and-Sustainable-by-Design (SSbD) frameworks increasingly incorporate mechanistic biological information, including pathway-level and transcriptomic responses.^{5,10} Nevertheless, biological responses are commonly treated as individual toxicity endpoints, mechanistic signatures or key events rather than harmonized into a common biological representation that connects heterogeneous nanoparticle perturbations with their outcomes.

Emerging evidence suggests that nanoparticle-induced effects involve recurring host-response programmes of inflammatory activation, anti-inflammatory/resolution signalling and apoptosis.^{11,12,13,14} Although these responses are individually well established in nanoparticle–host interactions, whether their coordinated organization can provide an outcome-associated biological representation across heterogeneous nanoparticle, exposure and biological contexts

remains unclear. Importantly, resolving this question could extend prediction beyond material and exposure descriptors by treating host-response organization as an additional information layer for comparing nano–bio interactions and assessing biosafety and recovery.

Here, we develop a response-centred machine learning framework to determine whether heterogeneous host responses can be harmonized into a conserved biological representation of nanoparticle biosafety and recovery across diverse experimental contexts (Fig. 1). We integrated material, exposure, biological-context and cellular-response information from 599 studies encompassing 893 experimental contexts and harmonized heterogeneous biological readouts into three programmes—pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated. We then developed a hierarchical predictive architecture progressing from material properties alone (MP), to MP with exposure conditions and biological context (MP+EC+B), and finally to the inclusion of cellular responses (MP+EC+B+CR). This architecture enabled us to quantify the contribution of successive information layers to outcome prediction. The coordinated organization of these response programmes in relation to biosafety- and recovery-associated outcomes defined the Conserved Response Axis (CRA).

We evaluated CRA-associated response organization in previously unseen nanocomposites using 2D and 3D microglial models with orthogonal cellular and molecular readouts, complemented by uptake-pathway perturbation experiments. Independent transcriptomic datasets were then used to examine programme-level organization across diseases, nanoparticle classes and *in vitro* and *in vivo* systems spanning brain, lung and liver contexts. Together, these analyses supports host-response organization as an interpretable information layer for biosafety and recovery prediction and support its conservation across distinct materials, experimental scales and biological systems.

A response-centered framework organizes heterogeneous nanoparticle perturbations through host biological states

We curated 599 original studies comprising 893 experimental contexts across six nanoparticle classes and diverse *in vitro* and *in vivo* neurological systems, including brain disease, brain injury, spinal cord injury and peripheral nerve injury (Fig. 1a). The dataset consists of different cell types, species, disease and injury settings, exposure conditions and biological readouts. Nanoparticles were characterized by types, size, morphology, agglomeration state and surface properties, together with dose, exposure route, duration, and biological context (Supplementary Fig.S1 and Supplementary Tables S1–S3). Missing values arising from inconsistent reporting were addressed during preprocessing and feature engineering, while most input variables showed weak-to-moderate pairwise correlations, indicating limited redundancy (Supplementary Fig. S2).

The framework progressively integrated material properties (MP; Step 1); exposure and biological context (MP+EC+B; Step 2); and curated biological responses (MP+EC+B+CR; Step 3) (Fig.1a). Descriptors were harmonized into biologically meaningful categories while preserving experimental context (Supplementary Tables S1–S3). Within the CR layer, heterogeneous biological readouts were mapped by functional direction onto three programmes: pro-inflammatory, anti-inflammatory (resolution-associated) and apoptosis-associated responses (Fig. 1a). Cell type, species, disease context, and exposure conditions remained explicitly represented, rather than treating distinct biological systems as equivalent. This architecture separated information describing the nanoparticle perturbation and its context from the host-response state, allowing the contribution of successive information layers to outcome prediction to be quantified.

We next tested whether host-response information provided outcome-associated information beyond that represented by material, exposure and biological-context descriptors. Multiple machine-learning models were evaluated across the three feature configurations using cross-validation and complementary performance metrics (Supplementary Fig.S3), with SHAP and

alluvial analyses used to examine feature contributions and response-outcome organization (Fig. 1a). This hierarchical design therefore tested whether harmonized host-response information provided an additional interpretable layer for outcome prediction across heterogeneous nano-bio contexts.

The coordinated relationships of pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes with biosafety- and recovery-associated outcomes defined the Conserved Response Axis (CRA). Rather than assuming identical biological responses across experimental systems, the CRA represents programme-level organization, allowing the magnitude and direction of individual responses to remain context dependent. We therefore examined whether this higher-order response organization persisted across independent materials, experimental models and biological systems.

CRA-associated response organization was subsequently evaluated in previously unseen nanocomposites using 2D and 3D microglial models with orthogonal cellular and molecular readouts, complemented by nanoparticle-uptake perturbation experiments (Fig. 1b). Independent transcriptomic datasets were used to examine programme-level organization across diseases, nanoparticle classes and *in vitro* and *in vivo* systems spanning brain, lung and liver contexts. Biosafety and recovery annotations were derived from reported phenotypic and functional outcomes, and their relationships with the harmonized response programmes were evaluated within the CRA framework (Fig. 1c). Together, this cross-scale strategy tested whether host-response organization could provide a common biological representation linking heterogeneous nanoparticle perturbations with biosafety- and recovery-associated outcomes.

Host response information provides an interpretable layer for biosafety and recovery prediction

The integrated dataset comprised 893 experimental contexts across six nanoparticle classes, more than 40 cell types or experimental models, and diverse biological readouts under *in vitro*

and *in vivo* conditions (Fig. 2a). We evaluated whether progressive integration of contextual and cellular-response information with material properties improved prediction of biosafety and recovery across this heterogeneous dataset. Models based solely on material properties (MP) showed limited predictive performance, whereas incorporating exposure conditions and biological context (MP+EC+B) substantially improved prediction across evaluation metrics (Fig. 2b–d). This context dependence is consistent with challenges in representing heterogeneous nanomaterials for predictive nanoinformatics¹⁵ with emerging approaches emphasizing integration of multiple information layers for chemical and material assessment.²⁰ Across classifiers, Random Forest (RF) showed the strongest and most consistent performance (Fig. 2b; Supplementary Fig. S3) and was therefore used for subsequent analyses. The comparative performance of classifiers further illustrates the importance of model and feature representation in machine-learning applications across biomedical and nanoscale systems.^{14, 17, 18, 19} The above features with curated cellular responses (MP+EC+B+CR) achieved the highest overall performance, with ROC analysis showing progressive discrimination across the three feature configurations (Fig. 2c). The largest performance gain occurred upon incorporation of exposure and biological context MP+EC+B descriptors, whereas addition of CR produced further, although comparatively modest, improvements (Fig. 2c,d). Specifically, biosafety ROC-AUC increased from 66% with MP to 88% with MP+EC+B and 90% with MP+EC+B+CR, whereas recovery ROC-AUC increased from 64% to 77% and 78%, respectively (Fig. 2d). Thus, the CR layer provided complementary predictive information beyond material and contextual descriptors, while its contribution differed between biosafety and recovery.^{16, 17}

To determine whether individual feature layers were retained within distinct experimental settings, we performed ablation analyses separately for the *in vitro* and *in vivo* datasets. Biosafety prediction exceeded 90% ROC-AUC in *in vitro* and *in vivo* analyses, whereas

recovery prediction reached 83% *in vitro* and 70% *in vivo* respectively. Under *in vivo* recovery conditions, MP+EC+B marginally outperformed MP+EC+B+CR, suggesting that exposure and biological context captured much of the recovery-associated signal. By contrast, curated biological-response features contributed additional information in the heterogeneous combined dataset and *in vitro* analyses, indicating that the predictive contribution of the CR layer depends on experimental context. Further, SHAP analysis identified pro-inflammatory and apoptosis-associated features as leading contributors to biosafety and recovery prediction across combined, *in vitro*, *in vivo* and analyses (Fig. 2e,f; Supplementary Fig. S4-S6). Alluvial analyses further showed that diverse nanoparticle compositions were associated with common biological-response patterns linked to distinct biosafety and recovery outcomes in combined datasets (Supplementary Fig. S7). Together, these results show that incorporating cellular-response programmes provides complementary information beyond material properties and experimental context while revealing a shared biological organization across heterogeneous nanoparticle systems.

Orthogonal experimental validation supports CRA-associated biosafety and recovery states

We next investigated whether the biological response states identified through modelling could be experimentally resolved using orthogonal molecular, cellular and functional measurements in response to previously unseen nanobiocomposites (Fig. 3). Newly designed and synthesized cellulose–cobalt nanobiocomposites with different cobalt ratios (see Supplementary Methods 2.5) were evaluated in a 2D BV2 microglial neuroinflammation model. These nanocomposites were absent from the 893-context discovery dataset and were not used during model development. LPS stimulation was used for initial cell-viability and nitric oxide (NO) screening, whereas IFN γ stimulation was used for downstream molecular and cellular

validation (Fig. 3a–c). Physicochemical characterization showed distinct morphologies among the formulations (Fig. 3b; Supplementary Fig. S8).

Cell viability and NO measurements identified material- and concentration-dependent responses in BV2 microglia (Fig. 3c). Without LPS stimulation, all four nanobiocomposites maintained relatively high viability at low-to-intermediate concentrations. At higher concentrations, viability declined with C1Co1 and C1Co5, whereas C3Co1 and C3Co5 generally maintained viability near the untreated control. LPS increased NO production to approximately 15.16 μM . Nanobiocomposite treatment reduced NO to approximately 1.01–5.25 μM across much of the tested concentration range. However, lower NO production did not always coincide with preserved viability: under LPS stimulation at 500 $\mu\text{g mL}^{-1}$, viability was approximately 29.8% with C1Co1 and 61.4% with C1Co5, compared with approximately >100% with C3Co1 and C3Co5. These results identified C3Co1 and C3Co5 as the nanocomposites that most consistently combined attenuation of the inflammatory response with maintained cell viability, supporting their selection for downstream analyses. Complementary assays in additional cell models revealed material-, concentration- and cell-type-dependent viability responses, extending the biosafety assessment beyond BV2 microglia. Hemolysis also provided an additional assessment of biocompatibility across nanocomposites (Supplementary Fig. S9 & Supplementary Fig. S10).

Independent molecular and cellular measurements further resolved these response-state changes using IFN γ as the inflammatory stimulus for downstream validation. IFN γ increased NF- κ B p65 fluorescence from approximately 189.4 to 605.2 arbitrary units (AU) and NOTCH2 fluorescence from approximately 76.5 to 783.1 AU compared to that of control (Fig. 3d,e). With IFN γ stimulation, C3Co1 and C3Co5 reduced NF- κ B p65 fluorescence to approximately 209.3 and 141.8 AU, respectively, while NOTCH2 fluorescence decreased to approximately 426.5 and 348.7 AU. The ROS signal increased to approximately 1.72-fold that of the control

after IFN γ stimulation and declined to approximately 1.22-fold with C3Co1 and 1.11-fold with C3Co5 (Fig. 3f,g). Hoechst and PI imaging provided additional measures of nuclear staining and membrane integrity, although their quantified changes were smaller than those observed for NF- κ B p65, NOTCH2 and ROS (Fig. 3h-j). Annexin V/PI flow cytometry showed that the viable population decreased from approximately 86% in the control to 38.2% after IFN γ stimulation, then increased to approximately 80.8% and 92.1% with C3Co1 and C3Co5 treatment, respectively (Fig. 3k,l). Thus, orthogonal measurements converged on coordinated attenuation of inflammatory, oxidative-stress and apoptosis-associated responses together with preservation of cell viability under inflammatory challenge.

Related response patterns were observed in SH-SY5Y neuronal cells, while organelle, gene-expression and protein-level analyses in BV2 cells further supported coordinated modulation of cellular stress, inflammatory signalling and apoptosis-associated responses (Supplementary Figs. S11 and S12). These observations are consistent with established links among inflammatory signalling, oxidative stress and apoptosis.^{20, 21, 22, 23, 24}

Integration of these measurements positioned the experimental conditions along the CRA response-state (Fig. 3m,n). IFN γ challenge was characterized by high inflammatory signalling, increased oxidative and apoptosis-associated responses and loss of viability, consistent with an injury-associated state. Nanobiocomposite treatment shifted this response organization towards states characterized by attenuated inflammatory and apoptosis-associated signals and preserved viability, consistent with biosafety- and recovery-associated profiles.^{25,26} Because these nanobiocomposites were absent from the discovery dataset, the concordance between their experimentally resolved outcome-associated states and the programme-level organization of the CRA provides independent experimental support for its application to previously unseen nanomaterials.

Endocytic perturbation reveals nanobiocomposite-specific cell-state responses within the CRA

To examine whether perturbation of nanoparticle-uptake pathways was associated with CRA-related cellular states, we combined endocytic inhibitors with Annexin V–FITC/propidium iodide flow cytometry and separately quantified cell-associated cobalt by ICP–MS (Fig. 4). BV2 microglia were treated with methyl- β -cyclodextrin (MC), which perturbs cholesterol-dependent uptake, or chlorpromazine (CH), which perturbs clathrin-mediated endocytosis. Nanobiocomposite-treated conditions were also exposed to IFN γ . Because these inhibitors can affect cellular physiology independently of nanoparticle uptake, these experiments were interpreted as pathway perturbations rather than as direct evidence that a specific uptake route caused the observed cell states.^{27, 28, 29}

The flow-cytometry distributions differed markedly between the two inhibitor conditions (Fig. 4a,b). With MC alone, approximately 54.6% of cells were classified as necrotic (Q1) and 45.4% as viable (Q3). Under IFN γ stimulation, the C3Co1 + MC and C3Co5 + MC conditions showed approximately 83.8% and 82.4% viable cells, respectively, with necrotic fractions of approximately 16.2–17.6%. CH alone was associated with approximately 85.2% viable cells. Under IFN γ stimulation, adding C3Co1 in the presence of CH shifted the distribution to approximately 37.8% viable and 39.9% late-apoptotic cells (Q2). In contrast, C3Co5 in the presence of CH retained approximately 86.7% viable cells and 8.7% late-apoptotic cells. Thus, perturbation of the same endocytic pathway produced distinct cell-state distributions between the two nanobiocomposites, with C3Co1 showing a substantially greater late-apoptotic fraction than C3Co5 under CH treatment.

Cell-associated cobalt measured by ICP–MS further differed among nanobiocomposite and perturbation conditions (Fig. 4c), indicating formulation- and condition-dependent differences in cellular nanoparticle association. Cobalt concentrations increased from approximately 0.26–

0.99 ppb in control and IFN γ -treated cells to approximately 7.7 and 6.0 ppb following C3Co1 and C3Co5 treatment, respectively. Under IFN γ challenge, cell-associated cobalt decreased to approximately 3.3 ppb with C3Co1 but remained comparatively high at approximately 6.6 ppb with C3Co5. Together, the flow-cytometry and cobalt measurements show that perturbation of nanoparticle–cell interactions was accompanied by nanocomposites-specific shifts in cellular association and cell fate. These findings further demonstrate that CRA-associated states are sensitive to the interaction between nanomaterial identity and cellular context rather than representing fixed material-specific responses.

CRA-associated response organization transfers across experimental scales and neurological contexts

We next examined whether CRA-associated response organization extended beyond the discovery framework by combining three-dimensional (3D) microglial validation with independent *in vivo* transcriptomic analyses across neurological disease contexts (Fig. 5). In BV2 spheroids, IFN γ markedly increased inflammatory signalling: NF- κ B p65 fluorescence rose from approximately 276.5 AU in control spheroids to 6878.9 AU, while NOTCH2 also increased to from 629.4 AU approximately to 9536.7 AU (Fig. 5b,d). With C3Co5 treatment under IFN γ challenge, both signals were lower, at approximately 3040.4–5633.5 AU. IFN γ also increased caspase-3 and cytochrome c signals; both were lower in the C3Co5 + IFN γ group, with the cytochrome c signal falling from approximately 9663.2 to 301 AU (Fig. 5c,e).^{28,29,30,31,32,33} These orthogonal measurements extend the coordinated inflammatory and apoptosis-associated response organization observed in 2D cultures to a 3D microglial model, supporting CRA-associated programme organization across experimental scales.

We next examined the three CRA programmes in independent *in vivo* neurological transcriptomic datasets (Fig. 5f,g). Outcome-associated profiles were assigned from experimental conditions and outcomes reported in the original studies, independently of the

gene-expression patterns used to calculate CRA programme activity. In the Alzheimer's disease-relevant dataset GSE142066, programme activity varied with APOE genotype and sex, illustrating biological heterogeneity within the disease context.³⁴ In the multiple sclerosis dataset GSE306006, the nanoparticle treatment was associated with improved outcomes in the original study, and this reported therapeutic response was used to define the recovery-associated condition before CRA analysis³⁵ The traumatic brain injury dataset GSE244966 was analysed using the same approach, with the reported improvement following treatment defining the recovery-associated condition independently of CRA programme activity.³⁶ Across these datasets, pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes occupied the same response space but exhibited disease- and treatment-dependent configurations rather than a single invariant molecular pattern (Fig. 5f,g). Network-level analysis further showed interconnected relationships among these response programmes across neurological disease and injury contexts, despite variation in their individual molecular components (Supplementary Fig. S13).

Together, the 2D and 3D experimental analyses and independent *in vivo* transcriptomic datasets support a shared higher-order organization of inflammatory, resolution and apoptosis-associated responses across neurological contexts (Fig. 5h). Injury-associated conditions were generally characterized by greater inflammatory and apoptosis-associated activity, whereas biosafety- and recovery-associated conditions occupied distinct programme configurations within the CRA response space. Thus, the CRA captures outcome-associated programme organization across experimental scales and neurological contexts while preserving disease-, treatment- and system-specific biological variation.

CRA-associated response organization generalizes across nanoparticle classes and biological systems

To test whether the CRA could organize responses beyond neurological systems, we analysed four independent transcriptomic datasets spanning *in vitro* and *in vivo* lung and liver models exposed to carbon black nanoparticles (CBNPs), multi-walled carbon nanotubes (MWCNTs), silver nanoparticles (AgNPs) and lipid nanoparticles (LNPs) (Fig. 6a–h). Across these distinct materials and models, transcriptional responses were represented through the same pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes. Injury-, exposure- and recovery-associated contexts were defined from experimental conditions and phenotypes reported in the original studies, independently of the expression values used for CRA projection.

In the *in vitro* lung dataset GSE43515, CBNP and MWCNT exposure produced time- and IL-13-dependent variation across the three programmes (Fig. 6a,e). Normalized programme scores were generally positive, spanning approximately 0.1–0.8 across the plotted conditions. Pro-inflammatory activity remained evident at 24 and 48 h, while apoptosis-associated and anti-inflammatory scores varied with material and IL-13 exposure. These patterns did not show a coordinated shift across all three programmes towards a protective profile. Thus, the injury-associated context reported in the original study was accompanied by a heterogeneous CRA programme configuration rather than a uniform change in any single programme.³⁷ The *in vivo* lung dataset GSE55286 showed a stronger temporal change after MWCNT exposure (Fig. 6b,f). At Day 3, pro-inflammatory activity reached approximately 1.3 in a plotted long-MWCNT condition, while anti-inflammatory activity reached approximately 0.7–0.8 in several conditions; apoptosis-associated scores were generally closer to zero. By Day 28, the scores were more moderate across much of the exposure series, although some inflammatory activity persisted. The response therefore changed with MWCNT length, dose and time rather than moving uniformly along a single programme.³⁸

In the *in vitro* liver dataset GSE259384, AgNP concentration was associated with distinct programme configurations (Fig. 6c,g). The plotted high-concentration condition had approximate scores of 0.1 for pro-inflammation, 0.2 for anti-inflammation and 0.3 for apoptosis, whereas the lower-concentration condition had scores near 0, -0.3 and -0.4, respectively. These concentration-dependent shifts demonstrate that exposure-associated responses in a non-neurological cellular system can be represented as coordinated changes within the same CRA response space, further supporting the ability of the CRA representation to capture exposure-associated biological states in a non-neurological system.³⁹

Finally, in the *in vivo* LAL-deficient mouse dataset GSE280933, blank LNPs showed an approximately 1.0 pro-inflammatory score and a 0.5 apoptosis-associated score. For mRNA-loaded LNPs, the corresponding scores were approximately 0.2 and -0.2, while the anti-inflammatory score remained near zero (Fig. 6d,h). The original study reported therapeutic improvement with the mRNA-LNP treatment, providing an independently defined recovery-associated context; the CRA projection describes the accompanying transcriptional configuration rather than independently establishing recovery.⁴⁰

Together, these independent datasets show that the same programme-level representation organizes transcriptional responses across lung and liver systems, four nanoparticle classes and both *in vitro* and *in vivo* experimental scales. (Fig. 6i). Injury/inflammatory, exposure-dependent and therapeutically improved conditions occupied distinct, context-dependent configurations within the common CRA response space. These findings support generalization of CRA-associated programme organization across nanoparticle classes and biological systems, while prospective outcome-labelled studies will be required to establish direct cross-organ prediction of biosafety and recovery.

Conclusion

The study establishes a response-aware architecture for organizing nanoparticle–host interactions through coordinated pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes. Progressive integration of material properties, exposure conditions, biological context and cellular responses showed that host-response information provides complementary predictive information beyond material and contextual descriptors for biosafety and recovery. Previously unseen nanobiocomposites exhibited CRA-associated biosafety and recovery states across 2D microglia and 3D spheroids using orthogonal readouts, while independent brain, lung and liver transcriptomic datasets showed shared programme-level organization across nanoparticle classes, biological systems and experimental scales. Together, these findings support the CRA as an interpretable biological representation in which heterogeneous nano–bio interactions can be compared while preserving context-specific response configurations, positioning host-response organization as a predictive layer for response-guided assessment and future biology-informed nanomaterial design.

Methods

Data Collection and Harmonization

To develop a curated dataset for modelling nanoparticle biosafety and recovery in neurological systems, we systematically collected studies covering brain diseases, traumatic, ischemic and neurovascular brain injury models, spinal cord injury models, and peripheral nerve injury models. Following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, literature searches integrating nanomaterial-, disease-, and biological-response-related keywords initially identified 987 candidate experimental datasets. Following screening, eligibility assessment, and manual verification, studies lacking sufficient biological-response information for annotation of the literature-derived response features were excluded.

This quality-control process yielded a final curated dataset comprising 893 experimental contexts derived from 599 independent studies, including 405 *in vitro* and 488 *in vivo* conditions spanning diverse nanoparticle, exposure, and biological contexts.

To systematically represent this heterogeneity, disease settings were grouped into four categories: brain diseases, brain injury, spinal cord injury, and peripheral nerve injury. *In vitro* systems were classified as neuronal, glial, primary, or other cell types. Heterogeneous cellular readouts reported in the source studies were manually curated and harmonized by functional direction into pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes to generate the cellular-response (CR) features. Biosafety and recovery outcomes were curated separately from reported phenotypic and functional evidence and were not defined from the CR features used as model inputs. This separation enabled the contribution of cellular-response information to outcome prediction to be evaluated without using the same response features to define the prediction targets. Detailed procedures for study selection, feature extraction, nanoparticle classification, exposure harmonization, cellular-response annotation, and biosafety and recovery labelling are provided in Supplementary Methods Section 1.1 & 1.2.

Nanomaterial Classification and Standardization

Nanomaterials were classified using a hierarchical framework that integrated composition, structural architecture, and formulation characteristics to generate standardized material descriptors for machine-learning analysis. At the broad material-class level, nanomaterials were annotated as inorganic nanoparticles ($n = 53$), organic and polymeric nanoparticles ($n = 58$), carbon-based nanomaterials ($n = 30$), hybrid or composite nanomaterials ($n = 40$), and hydrogel-based systems ($n = 52$). Because individual nanomaterials could incorporate characteristics of more than one material class, these annotations were non-mutually exclusive and encoded as independent binary features, allowing multi-label representation in downstream

machine-learning analyses. Detailed classification criteria and standardized annotation procedures are provided in Supplementary Methods Section 1.3.

Biological Response Feature Construction

To address heterogeneity in reported biological responses across studies, we constructed literature-derived biological response features representing conserved host-response processes associated with nanoparticle exposure. Reported biological responses were abstracted into three higher-order programmes: pro-inflammatory, anti-inflammatory (resolution-associated), and apoptosis-associated responses. These programme-level features were incorporated as cellular-response (CR) layer of the hierarchical machine-learning framework.

Feature construction was based on manual, experimental-context-level abstraction of the primary literature. Each experimental context was examined individually to identify the biological response reported by the original study and its direction, and was assigned to the corresponding programme(s) when supported by the reported experimental evidence. Literature-assisted tools, including SciSpace, were used to facilitate identification of relevant passages, while final interpretation and programme assignment were performed through manual review of the original publication. Detailed annotation criteria and handling of ambiguous or insufficiently reported responses are provided in the Supplementary Methods Section 1.4.

Because the underlying studies used heterogeneous experimental approaches and biological systems, programme-level representation was designed as a qualitative biological abstraction rather than a quantitative normalization of individual measurements. *In vitro*, *ex vivo*, and *in vivo* contexts were retained as distinct experimental contexts, while convergent biological responses were represented within the corresponding higher-order programme. Thus, the CR layer captures response identity and direction across heterogeneous systems without implying quantitative equivalence among assays, measurement platforms, or biological models.

To minimize circularity and target leakage, CR feature construction was performed independently of the biosafety and recovery outcome annotations. Biological response features were assigned solely from the reported response evidence and were not defined using the corresponding outcome labels. The resulting CR features were therefore used as predictor variables rather than directly serving as prediction targets.

The three programme-level features, encoded as pro-inflammatory, anti-inflammatory, and apoptosis-associated responses, were integrated with physicochemical properties, exposure conditions, and biological context within the hierarchical machine-learning framework. This enabled evaluation of the incremental contribution of biological response information beyond material and contextual descriptors and provided the basis for identifying the conserved biological organization termed the Conserved Response Axis (CRA).

Biosafety and Recovery Annotation Framework

Biosafety and recovery labels were assigned independently of the literature-derived biological response features used for machine-learning analyses, to prevent target leakage and circular outcome definition. Biosafety was defined according to evidence of adverse cellular, tissue, physiological, or functional effects associated with nanoparticle exposure, whereas recovery represented attenuation of an experimentally induced perturbation, improvement of an impaired phenotype or restoration of biological function within the reported experimental timeframe. Labels were based on phenotypic evidence and the biological interpretation reported in the original studies. Biosafety and recovery were each encoded as Good or Bad, with conditions lacking sufficient evidence retained as missing.

For *in vitro* datasets, Biosafety = Good was assigned when nanoparticle exposure produced no reported evidence of nanoparticle-associated cellular injury or sustained functional impairment based on an appropriately assessed phenotypic or functional endpoint. Mild or transient perturbations were considered compatible with Biosafety = Good when the original study

explicitly reported resolution without persistent cellular dysfunction. Biosafety = Bad was assigned when studies reported experimentally demonstrated cellular injury, impaired viability or proliferation, persistent functional dysfunction, or other adverse phenotypic effects attributable to nanoparticle exposure. Importantly, Biosafety = Bad was not assigned solely on the basis of biological-response programme activity or isolated molecular or signalling changes, but required supporting phenotypic evidence of cellular injury or an explicit toxicity interpretation supported by the original study. Supporting phenotypic evidence included measures of cell viability, proliferation, membrane integrity, cellular morphology, or other experimentally demonstrated functional impairment. Inflammatory or apoptosis-associated molecular measurements could provide supporting biological context but were not used alone to determine the biosafety label.

Recovery = Good was assigned when studies demonstrated attenuation of an experimentally induced adverse state, improvement of an impaired phenotype, or partial or complete restoration of cellular, tissue or physiological function within the reported experimental timeframe. Supporting evidence included recovery of viability, resolution of experimentally induced cellular or tissue injury, restoration of physiological function, or improvement towards biological homeostasis. Recovery = Bad was assigned when experimentally induced cellular, tissue, or functional impairment persisted without evidence of meaningful improvement within the reported timeframe, resulting in persistent cellular dysfunction, tissue pathology, or structural impairment.

For *in vivo* datasets, Biosafety = Good was assigned when nanoparticle exposure produced no measurable adverse physiological, histological, or behavioural effects, including preservation of tissue architecture and functional integrity. Transient alterations were considered compatible with Biosafety = Good only when their resolution was explicitly demonstrated within the reported experimental timeframe. Biosafety = Bad was assigned when studies reported tissue

injury, organ dysfunction, systemic weight loss, or behavioural abnormalities supported by histopathological, biochemical, physiological, or functional assessments. Importantly, Biosafety = Bad required evidence of structural, physiological, functional, or systemic injury rather than isolated molecular or signalling changes.

Recovery annotation in *in vivo* studies followed the same outcome-based principle. Recovery = Good required reported improvement, attenuation or reversal of an experimentally induced pathological or functional phenotype, whereas Recovery = Bad reflected persistence or progression of the impaired state within the reported experimental timeframe. Molecular or signalling changes alone were not sufficient to assign recovery without supporting phenotypic, physiological, histological or functional evidence.

All annotations were assigned exclusively from reported phenotypic or functional observations and study-level biological interpretations without inference beyond the available data. Where multiple endpoints were available, labels reflected the overall experimental evidence and the biological interpretation reported by the original authors, with conservative classification applied when evidence was ambiguous or insufficient. When the reported evidence was insufficient to determine biosafety or recovery, the corresponding outcome was retained as missing rather than assigned as Good or Bad. Annotation consistency was assessed by manual cross-checking against the original publications using the predefined criteria applied across the dataset. Detailed biosafety and recovery annotation criteria are provided in Supplementary Methods Section 1.5.

Hierarchical Machine-Learning Modelling Framework

To evaluate the progressive contribution of material properties, exposure conditions, biological context, and literature-derived biological response features, we implemented a hierarchical machine-learning framework using curated *in vitro* and *in vivo* neurological datasets. Feature sets were progressively integrated across three stages. Step 1 (MP): models were trained using

nanoparticle material properties, including size, shape, zeta potential, agglomeration state, and related physicochemical descriptors. Step 2 (MP+EC+B): exposure conditions and biological context were added to the MP feature set. Features were defined separately for *in vitro* datasets (for example, nanoparticle concentration, stimulant type and concentration, and cellular uptake) and *in vivo* datasets (for example, administered dose, dosing regimen, administration route, organism characteristics, and injury model). Step 3 (MP+EC+B+CR): literature-derived cellular responses were manually curated into pro-inflammatory, anti-inflammatory, and apoptosis-associated programme feature and added to the MP+EC+B feature set.

This hierarchical machine-learning framework enabled systematic evaluation of the predictive contribution and biological interpretability of each feature layer. Models were trained and evaluated separately for *in vitro*, *in vivo*, and combined datasets to assess performance within individual experimental settings and across the heterogeneous combined dataset. These analyses provided the computational basis for subsequent characterization of response–outcome relationships and evaluation of CRA-associated response organization using experimental and transcriptomic data. Detailed preprocessing, feature engineering and encoding procedures are provided in Supplementary Methods Section 1.6 & 1.7.

Model Development and Evaluation

All features were standardized and preprocessed before partitioning the dataset into independent training (80%) and test (20%) sets. To prevent inter-study data leakage and provide a stringent assessment of generalizability, data were partitioned using study-level stratified splitting, such that all experimental contexts derived from a given publication were assigned exclusively to either the training or independent test set. Model development within the training partition employed stratified 10-fold cross-validation for algorithm evaluation and hyperparameter optimization.

Machine-learning algorithms spanning linear, kernel-based, neural-network, and tree-based ensemble methods were evaluated. Models were initially benchmarked using standardized baseline hyperparameters without prior feature selection to enable unbiased comparison of model architectures. Final optimized models were subsequently retrained using the complete training partition and evaluated once on the locked independent test set.

Predictive performance was assessed using classification accuracy, balanced accuracy, macro-averaged F1-score, and receiver operating characteristic (ROC) and precision–recall (PR) analyses. For multi-class classification, optimization and evaluation followed a one-versus-rest strategy, with performance summarized using macro-averaged areas under the ROC (AUROC) and precision–recall (AUPRC) curves.

Model Interpretability Analysis

Model interpretability was assessed using SHapley Additive exPlanations (SHAP) to quantify the contribution of individual features to model predictions. SHAP analyses were performed using the best-performing model for each prediction task, with optimized Random Forest models used for most biosafety and recovery analyses. Global feature importance was calculated as the mean absolute SHAP value across the evaluation dataset, and feature contributions were visualized using beeswarm and force plots generated with the SHAP library (v.0.41.0).

Linear Discriminant Analysis (LDA) was additionally used as a supervised dimensionality reduction method to visualize separation of biosafety and recovery classes within the multidimensional feature space. LDA was used solely as a visualization tool and was not used to construct, define, or infer the CRA.

Experimental Validation

To experimentally evaluate the biological organization represented by the CRA, cellulose–cobalt nanocomposites that were not included in model development were synthesized

independently using solution plasma processing. Following physicochemical characterization, the nanocomposites were evaluated in BV2 microglial models subjected to IFN γ -induced inflammatory stimulation followed by nanocomposite treatment. The resulting molecular and cellular responses were examined to determine whether independently measured response patterns were consistent with the biological organization represented by the CRA.

Orthogonal molecular, cellular, and functional assays were performed to quantify nanoparticle uptake, inflammatory signalling, apoptosis-associated responses, and cellular viability using fluorescence imaging, flow cytometry, inductively coupled plasma mass spectrometry (ICP–MS), and immunofluorescence analyses. Experimental validation was further extended from 2D BV2 cultures to 2D microglial spheroids to examine CRA-associated response patterns across distinct cellular configurations. Independent transcriptomic validation was performed using datasets spanning multiple neurological disease models, followed by cross-organ evaluation using publicly available liver and lung transcriptomic datasets to assess whether similar CRA-associated response organization was observed across biological systems and nanoparticle classes. Detailed protocols for nanomaterial synthesis, physicochemical characterization, cellular assays, transcriptomic analyses, and statistical procedures are provided in the Supplementary Methods 1.8.

Statistical Analysis

Machine-learning analyses were performed using Python (v3.10), and experimental data were analysed using GraphPad Prism (v10.0). Model development employed study-level stratified data partitioning, stratified 10-fold cross-validation within the training set, and held-out test-set evaluation, as described above. Predictive performance was assessed using classification accuracy, balanced accuracy, macro-averaged F1-score, area under the receiver operating characteristic curve (AUROC), and area under the precision–recall curve (AUPRC).

Experimental data are presented as mean $\pm$ standard deviation (s.d.) from at least three independent biological replicates. Statistical comparisons were performed using one-way or two-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's multiple-comparison tests, as appropriate. Comparisons between two groups were performed using two-tailed unpaired Student's *t*-tests. Statistical significance was defined as $P < 0.05$. Exact statistical tests, sample sizes and multiple-comparison procedures are specified in the corresponding figure legends and Source Data. To ensure computational reproducibility, the curated feature matrices, literature-derived biological response features, machine-learning analysis scripts, and transcriptomic analysis code have been deposited in a public repository.

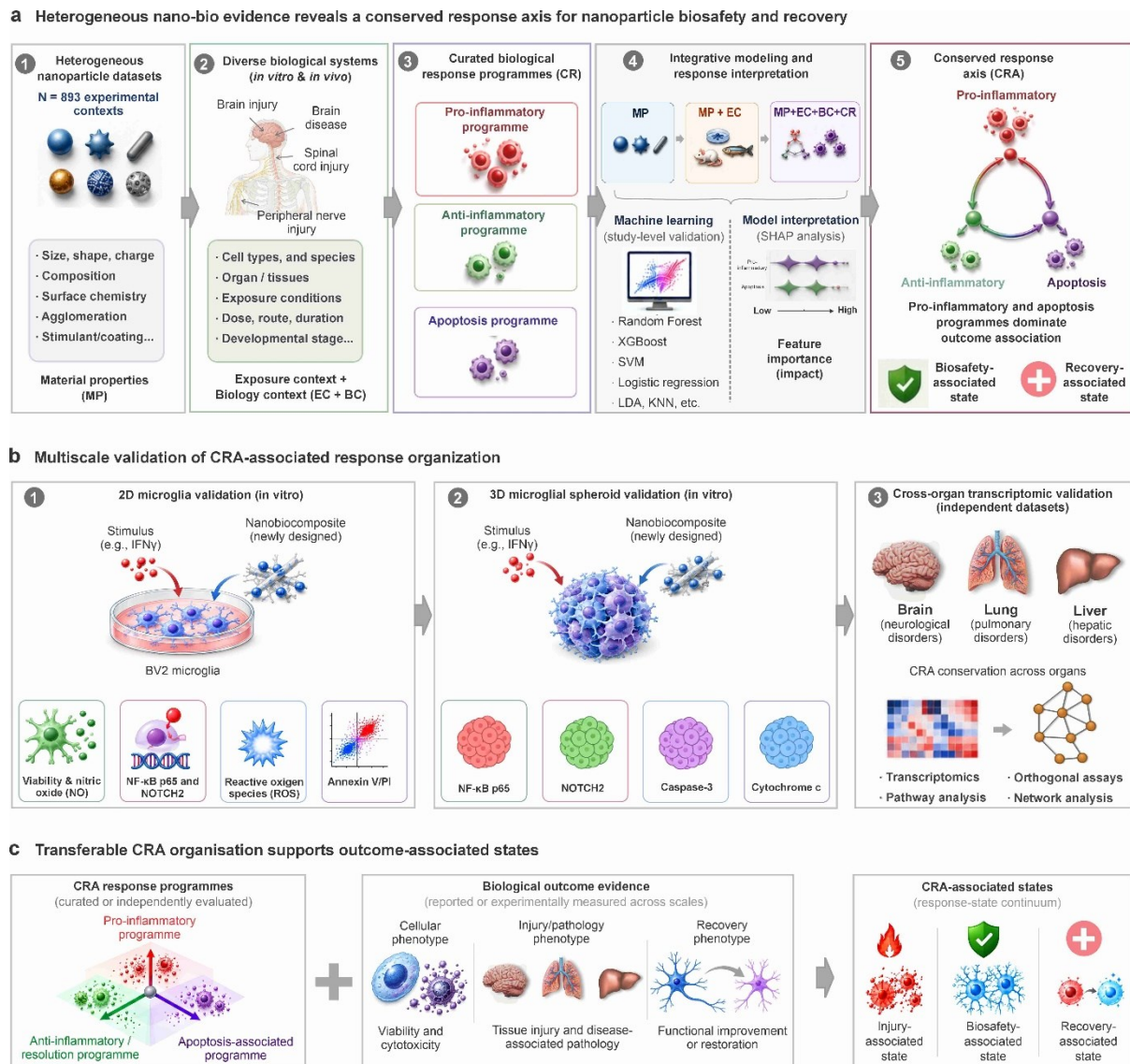


Fig. 1 | Response-centered framework for organizing nanoparticle biosafety- and recovery-associated states. **a**, Schematic of the framework integrating heterogeneous nanoparticle evidence across material properties (MP), exposure context (EC), biological context (B) and curated cellular-response programmes (CR). CR comprises pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes. Hierarchical feature integration and interpretable machine learning were used to quantify the contribution of successive information layers to biosafety- and recovery prediction. The coordinated organization of CR programmes in relation to biosafety- and recovery-associated outcomes defines the Conserved Response Axis (CRA). **b**, Multiscale evaluation of CRA-associated

response organization. Previously unseen nanocomposites were evaluated in 2D BV2 microglia and 3D microglial spheroids using orthogonal cellular and molecular readouts. Programme-level organization across biological contexts was further examined using independent transcriptomic datasets spanning neurological, lung and liver systems, diverse nanoparticle classes, and *in vitro* and *in vivo* exposures. Transferability beyond the experimental validation system was further examined using independent transcriptomic datasets spanning diverse nanoparticle classes, neurological, lung and liver models, and *in vitro* and *in vivo* exposures. **c**, Integration of CRA response programmes with biological outcome evidence. Pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes were considered together with independently derived biological outcome evidence from reported study outcomes and experimental phenotypic and functional readouts. Their coordinated organization supports interpretation along an outcome-associated response-state continuum encompassing injury-, biosafety- and recovery-associated states.

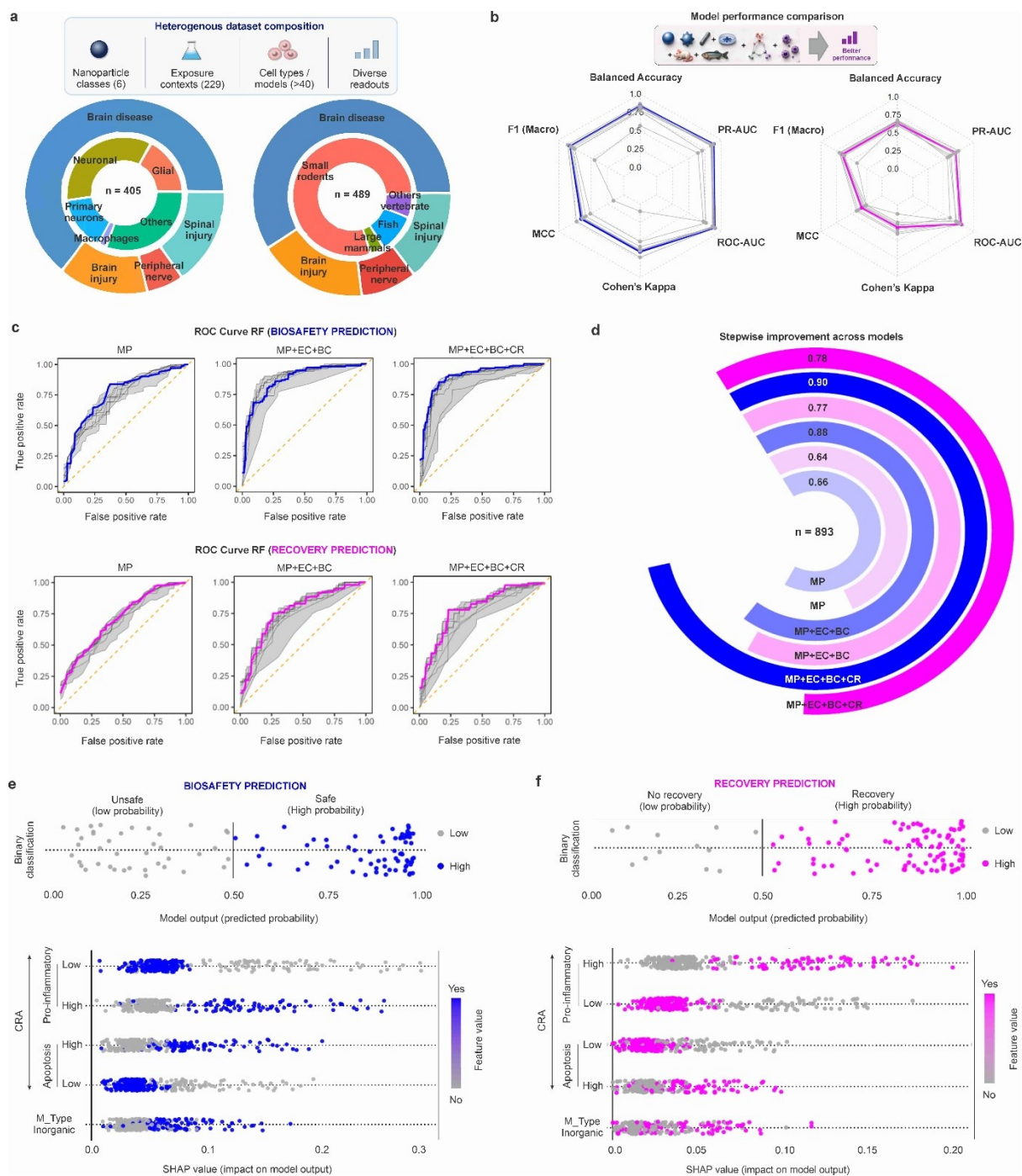


Fig. 2 | Hierarchical integration of literature-derived biological response features improves prediction of nanoparticle biosafety and recovery. a, Composition of the integrated nanoparticle dataset comprising 893 experimental contexts used for model development, spanning six nanoparticle classes, 229 exposure contexts, more than 40 cell types and biological models, and diverse experimental readouts across neurological conditions. Sunburst plots show the distribution of *in vitro* and *in vivo* contexts across disease and model

categories. **b**, Model performance across hierarchical feature representations assessed using balanced accuracy, macro-F1, PR-AUC, ROC-AUC, Cohen's kappa and Matthews correlation coefficient (MCC) for biosafety (left) and recovery (right). Feature representations comprised material properties (MP), exposure and biological context (EC + BC), and literature-derived biological response features (CR). **c**, ROC curves for biosafety (top) and recovery (bottom) using the Random Forest classifier across MP, MP + EC + BC and MP + EC + BC + CR feature representations. Grey curves represent individual cross-validation folds and coloured curves the mean ROC performance. **d**, Stepwise changes in ROC-AUC for biosafety and recovery across the three hierarchical feature representations, from MP alone to MP + EC + BC and MP + EC + BC + CR. **e,f**, Predicted probability distributions (top) and SHAP analyses (bottom) for biosafety (e) and recovery (f). Probability plots show model outputs for the binary predictions, with colour indicating feature value.outcome classes. SHAP plots show the contribution of representative features to model.

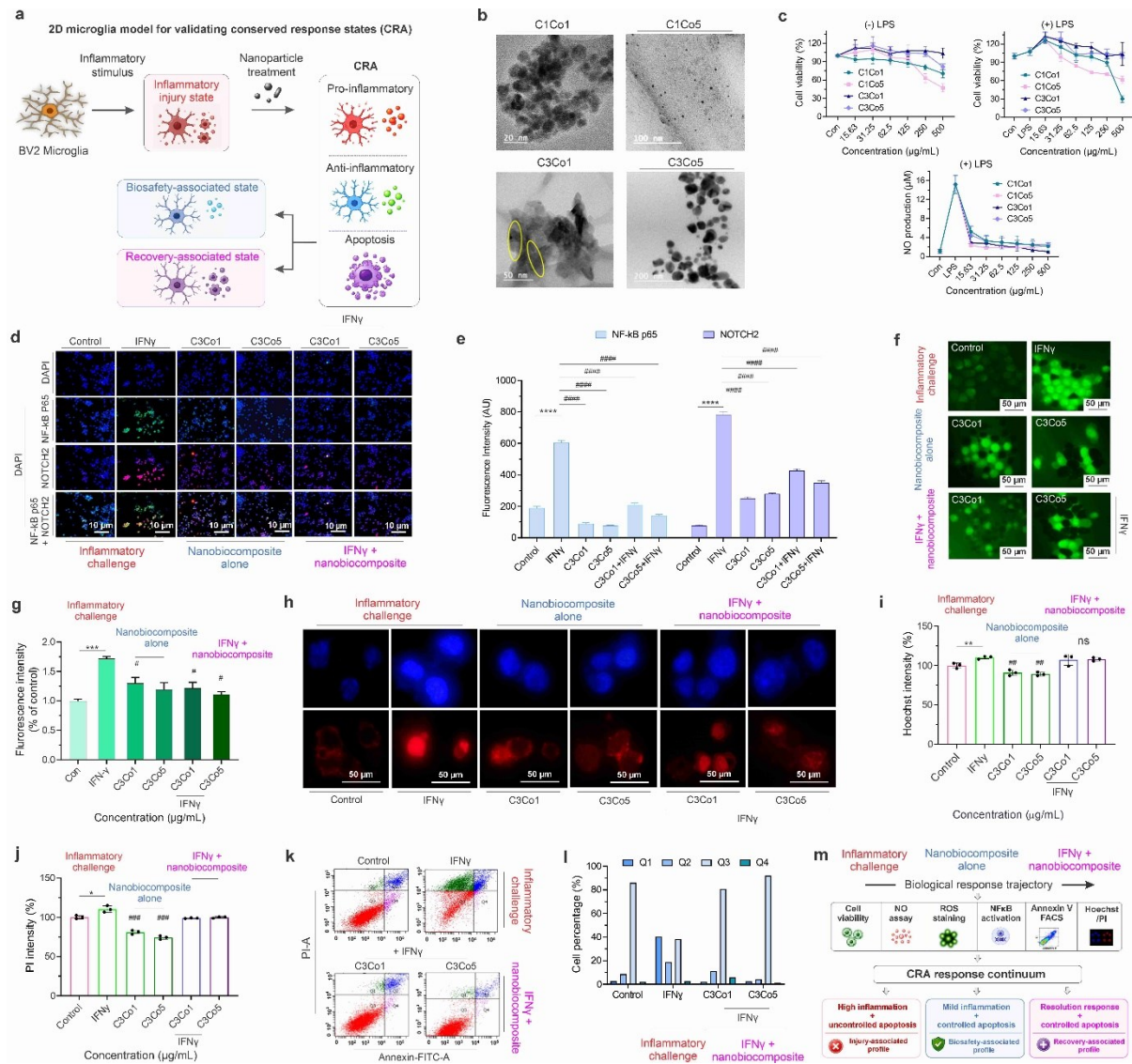


Fig. 3 | Orthogonal experimental validation of CRA-associated response states in previously unseen nanobiocomposites. a, Schematic of the experimental-validation strategy. BV2 microglia were examined under inflammatory challenge, nanobiocomposite-alone and IFN γ -plus-nanobiocomposite conditions to evaluate coordinated response modulation within the CRA framework. b, Representative transmission electron microscopy images of C1Co1, C1Co5, C3Co1 and C3Co5 nanobiocomposites. c, Dose-dependent effects of the nanobiocomposites on BV2 cell viability under basal (–LPS) and inflammatory (+LPS) conditions, together with nitric oxide production following LPS stimulation. d,e, Representative immunofluorescence images (d) and quantification (e) of NF- κ B p65 and

NOTCH2 under control, IFN γ , nanobiocomposite-alone and IFN γ -plus-nanobiocomposite conditions. f,g, Representative fluorescence images (f) and quantification (g) of intracellular reactive oxygen species. h–j, Representative Hoechst/propidium iodide fluorescence images (h) and quantification of Hoechst (i) and propidium iodide (j) fluorescence intensities. k,l, Annexin V–FITC/propidium iodide flow cytometry (k) and quantification (l) of viable, early-apoptotic, late-apoptotic and necrotic cell populations. m, Integrated summary showing coordinated changes in cell viability, nitric oxide production, reactive oxygen species, NF- κ B signalling, apoptosis and nuclear-stress readouts across the experimental conditions. The combined response patterns were interpreted as injury-, biosafety- or recovery-associated profiles within the CRA framework.. Data in c,e,g,i,j are presented as mean $\pm$ s.d. from three independent experiments. Statistical analyses were performed using GraphPad Prism 8. Differences between the indicated groups were evaluated using Student's *t*-test. $P < 0.05$, $*P < 0.01$ and $**P < 0.001$.

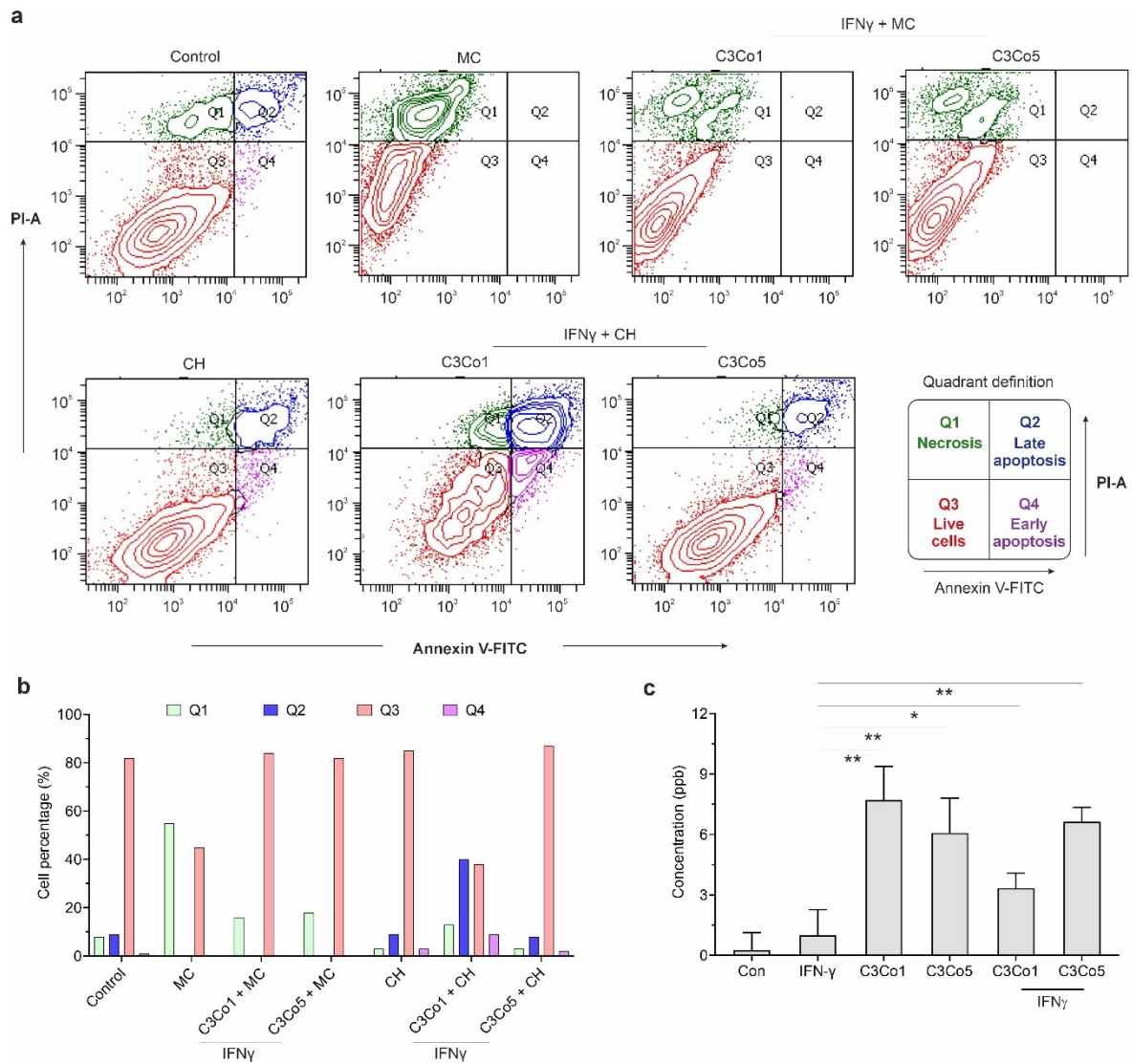


Fig. 4 | Endocytic perturbation reveals nanobiocomposite-specific cell-state responses within the Conserved Response Axis. a, Representative Annexin V–FITC/propidium iodide flow-cytometry analysis of BV2 microglia following IFN γ stimulation and nanobiocomposite treatment in the presence of methyl- β -cyclodextrin (MC), which perturbs cholesterol-dependent uptake, or chlorpromazine (CH), which perturbs clathrin-mediated endocytosis. Cell populations were classified as Q1, necrotic (PI⁺/Annexin V⁻); Q2, late apoptotic (PI⁺/Annexin V⁺); Q3, viable (PI⁻/Annexin V⁻); and Q4, early apoptotic (PI⁻/Annexin V⁺). Representative contour plots are shown for the untreated control and the indicated inhibitor and nanobiocomposite conditions under IFN γ stimulation. b, Quantification of the corresponding flow-cytometry populations, showing the percentages of necrotic (Q1), late-apoptotic (Q2),

viable (Q3) and early-apoptotic (Q4) cells across the indicated conditions. c, Cell-associated cobalt measured by inductively coupled plasma mass spectrometry following exposure to C3Co1 or C3Co5 under basal or IFN γ -stimulated conditions. Panel c represents a separate experiment performed without endocytic inhibitors. Data in c are presented as mean $\pm$ s.d. from three independent experiments. Statistical analyses were performed using GraphPad Prism 8. Differences between the indicated groups were evaluated using Student's *t*-test. $P < 0.05$, $*P < 0.01$ and $**P < 0.001$.

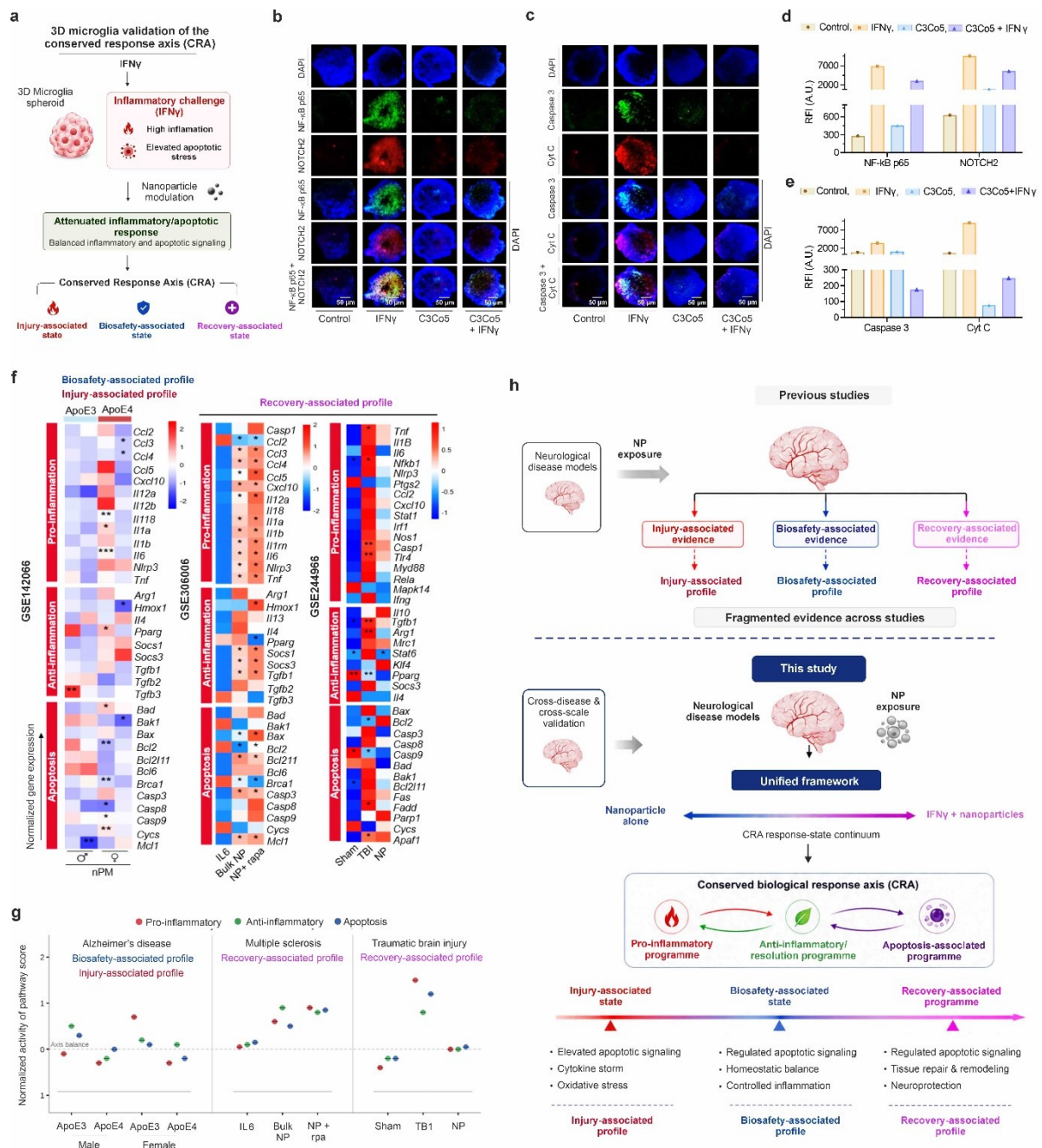


Fig. 5 | Three-dimensional and transcriptomic validation supports transferability of CRA-associated response states. **a**, Schematic of three-dimensional BV2 microglial spheroid validation examining coordinated inflammatory and apoptosis-associated response modulation within the CRA. **b,c**, Representative immunofluorescence images of NF- κ B p65 and NOTCH2 (b) and caspase-3 and cytochrome c (Cyt c; c) under the indicated experimental conditions. DAPI labels nuclei. **d,e**, Relative fluorescence intensities of NF- κ B p65 and NOTCH2 (d) and

caspase-3 and Cyt c (e). **f**, Independent transcriptomic evaluation using Alzheimer's disease (GSE142066), multiple sclerosis (GSE244966) and traumatic brain injury (GSE306006) datasets. Heatmaps show gene-wise Z-scores for pro-inflammatory, anti-inflammatory and apoptosis-associated programmes. Injury-, biosafety- and recovery-associated profiles based on the experimental conditions and biological interpretations reported in the original studies. CRA programme activity was independently evaluated from the corresponding transcriptomic patterns.. **g**, Normalized activity scores for the three CRA programmes across the independent neurological datasets, showing their organization across the injury-, biosafety- and recovery-associated profiles. **h**, Conceptual synthesis showing how fragmented injury-, biosafety- and recovery-associated evidence can be organized within a unified CRA response-state continuum comprising coordinated pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes. Data in d,e are presented as mean $\pm$ s.d. from three independent experiments. Differences between the indicated groups were evaluated using Student's *t*-test in GraphPad Prism 8. $P < 0.05$, $*P < 0.01$ and $**P < 0.001$. Dataset-specific sample sizes and transcriptomic statistical procedures are provided in the Methods and Source Data.

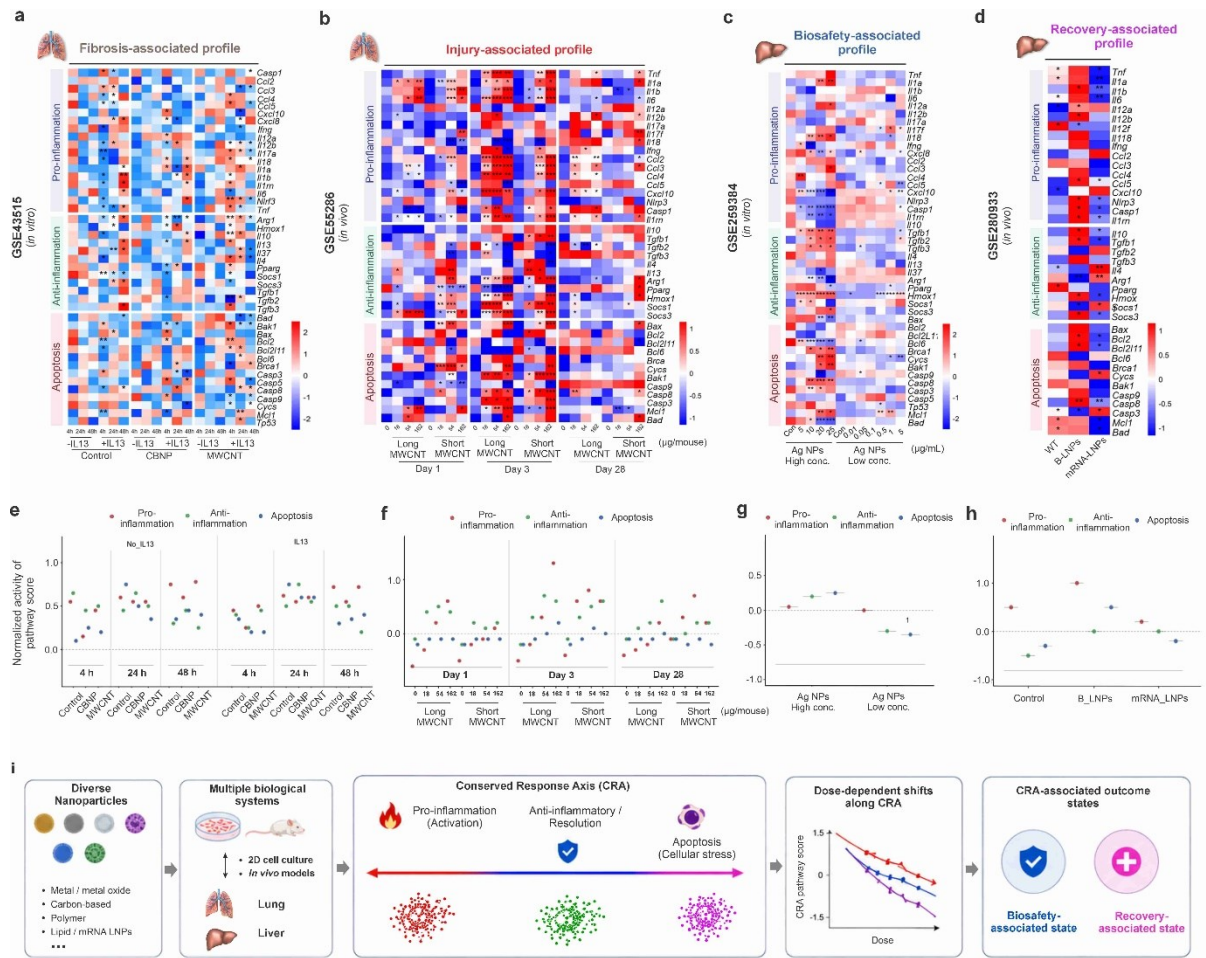


Fig. 6 | CRA-associated biological-response organization generalizes across nanoparticle classes and biological systems. **a–d**, Independent transcriptomic evaluation across lung and liver nanoparticle-exposure datasets. Heatmaps show gene-wise Z-score-normalized expression of pro-inflammatory, anti-inflammatory and apoptosis-associated programmes in a, *in vitro* lung (GSE43515); b, *in vivo* lung (GSE55286); c, *in vitro* liver (GSE259384); and d, *in vivo* liver (GSE280933) models. **e–h**, Corresponding normalized activity scores for the three CRA programmes showing responses to carbon black nanoparticles (CBNPs) and multi-walled carbon nanotubes (MWCNTs) across time and inflammatory context (e), MWCNTs across time and exposure conditions (f), silver nanoparticles (AgNPs) across concentration (g), and blank versus mRNA-loaded lipid nanoparticles (LNPs; h). Injury- and recovery-associated profile annotations were assigned according to the experimental conditions and biological interpretations reported in the original studies, whereas CRA programme activity was

independently evaluated from the corresponding transcriptomic patterns.. i, Conceptual synthesis illustrating how diverse nanoparticle classes and biological systems can be organized within a shared CRA programme-level architecture comprising coordinated pro-inflammatory, anti-inflammatory/resolution and apoptosis-associated programmes. Shifts in this programme-level organization across exposure conditions illustrate CRA-associated response states, including injury-, biosafety- and recovery-associated states.. Exact sample sizes, statistical procedures and P values are provided in the Methods and Source Data. $P < 0.05$, $*P < 0.01$ and $**P < 0.001$.

Data availability statement

The complete database and code supporting the machine-learning analyses presented in this study are publicly available through the <https://github.com/anblabs/NeuroSafeNet--CRA>.

Source data and other supplementary information are provided with this paper.

Code availability

The source code of machine learning-based model can be accessed at

<https://github.com/anblabs/NeuroSafeNet--CRA>

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Conflict of interest

The authors declare no conflict of Interest

Contributions

S.R.B. and H.P. conceived the study and designed the research. D.M.A., J.-W.K. and S.-Y.L. performed nanomaterial synthesis and characterization. S.R.B., G.B., S.A. and Y.J.K. performed the experimental work and collected the data. I.U., S.R.B. and H.P. contributed to data processing, computational analysis and interpretation. S.R.B., P.S., Sh.R., S.R., I.M., S.K., S.L. and H.P. contributed to the interpretation of the results and provided scientific guidance. Sh.R. and M.K. contributed analytical expertise and scientific insights. S.R.B., S.L. and H.P. supervised the research. S.R.B., S.K. and H.P. wrote the original manuscript. All authors reviewed and edited the manuscript and approved the final version.

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