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Methods Article

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Cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels: a prespecified baseline–edit–rescue framework for testing higher-order membrane-conditioned integration

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Abstract

Cell membranes are not mere platforms for signalling proteins; they can shape how receptor inputs are assembled into local responses. In membrane-rich microdomains, receptor identification and pathway mapping do not reveal the logic of a measured effect. That effect may arise from independent receptor activity, pairwise crosstalk or higher-order integration governed by membrane state. The membrane-encoded chemosensory system (MECS) is introduced as a conceptual framework for addressing the inferential gap between receptor co-expression mapping and mechanistic crosstalk claims in territories with cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels. Its operational method, MECS baseline–edit–rescue (MECS-BER), fixes one membrane prior, one locked proximal outcome and one three-arm candidate assembly. Eligibility gates test arm engagement and outcome competence. Combinatorial responses are analysed with κ, the third-order interaction under a pairwise-only null within a complete three-factor perturbation design, to distinguish lower-order explanation from higher-order interpretation and test edit–rescue reversibility. Deterministic matrices and simulations establish classification logic and tolerance handling; biological adjudication awaits fully compliant MECS-BER datasets. The workflow provides a prespecified and formalized methodological route, not biological proof of any specific receptor triad. It keeps nomination separate from adjudication and requires co-localization, distal phenotypes, shared downstream signals and nonlinear mixtures to be tested against a locked proximal outcome before biological interpretation. Renal micro-niches specify prospective deployment across renin, transport, barrier and flow-sensitive calcium control without claiming validated cannabinoid–olfactory–TRP assemblies. Membrane lipids may shape both the signalling vocabulary of individual receptors and the local language through which receptors communicate.

Keywords membrane prior, receptor crosstalk, cannabinoid GPCRs, ectopic olfactory GPCRs, TRP channels, kidney micro-niche

Introduction

Membrane signalling is often interpreted through a linear evidentiary sequence: a receptor is detected, a ligand is assigned and a downstream pathway is mapped. This sequence is useful, but it does not determine how a local output is produced.

The inferential gap addressed here lies between two levels of evidence that are often placed too close together. Receptor co-expression mapping can show that candidate receptors and channels are present within the same tissue, segment or micro-niche. It can nominate a candidate assembly. It cannot determine whether those arms act independently, interact pairwise or generate a membrane-conditioned higher-order regime on one locked proximal outcome. That decision requires a prespecified adjudicative route. Without such a route, receptor inventory, shared downstream biology and nonlinear response behaviour can be mistaken for mechanistic crosstalk. This distinction is consequential in membrane-dense territories, where GPCRs and ion channels operate within local physical constraints that can reshape conformational sampling, nanoscale proximity, shared-effector access and the timing of second-messenger exchange [1–13]. The central question is therefore not whether these receptors and channels are present in a tissue, but the disciplined attribution of their joint contribution to one defined local output.

For the cannabinoid arm, this problem includes both receptor pharmacology and local ligand economy. CB1 and CB2 are Gi/o-coupled GPCRs. Their signalling can vary across adenylyl cyclase,

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ion-channel, MAPK and arrestin-linked pathways [6–8]. Prolonged agonist exposure can also reweight coupling, trafficking and desensitization states. Effective endocannabinoid signalling is constrained by membrane-proximal synthesis, intracellular trafficking and enzymatic termination. FAAH is a principal determinant of anandamide persistence and of the local cannabinoid field presented to the membrane assembly [9, 10]. In the present framework, the cannabinoid arm is therefore not reduced to receptor presence. It is treated as receptor competence within the biochemical conditions that shape local endocannabinoid availability.

Within this framework, signalling denotes the capacity of a nominated arm to influence the locked proximal outcome in the defined territory. Mechanistic crosstalk denotes experimentally testable functional non-independence on that same locked proximal outcome: the contribution of one arm depends on the engaged state of another. This term does not, without separate structural evidence, assert direct receptor–receptor binding or a stable molecular complex. Pairwise crosstalk remains a lower-order explanation when pairwise terms are sufficient. Candidate higher-order integration is considered only when the residual third-order contrast lies outside the prespecified bound under the locked pairwise-only null, and when the class shift is coherent across baseline, membrane-prior edit and rescue.

The membrane-encoded chemosensory system (MECS) is proposed here as a conceptual framework for this problem. MECS does not denote a validated universal receptor assembly. It denotes a membrane-conditioned signalling logic in which cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels may remain independent, form pairwise-coupled lower-order structures or enter a candidate higher-order integration regime on one locked proximal outcome. Within this frame, membrane state is not passive context. It is a measurable constraint that can shape receptor competence, ligand access, effector proximity, channel permissiveness and local crosstalk.

In MECS, ‘membrane encoding’ does not imply a genetic or symbolic code. It denotes the capacity of a measurable membrane state—set by lipid composition, sterol accessibility, phosphoinositide topology, mechanics, trafficking history or oxidative stress—to constrain which receptor and channel behaviours become functionally expressed within a local territory. ‘Chemosensory’ is used in the broader physiological and physicochemical sense of chemical sensing at membranes, not in the restricted sensory sense of nasal olfaction. Ectopic olfactory GPCRs retain their historical receptor-class name, but outside the olfactory epithelium their relevance lies in detecting chemically informative local cues, including metabolites, lipid-derived mediators, microbial products, volatile or non-volatile small molecules, and terpenoid or cannabinoid meroterpenoid ligands. MECS therefore extends chemosensation from odour perception to local chemical interpretation in non-olfactory tissues, where cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels may convert local ligand-field information, through membrane physics and lipid-state constraints, into one locked proximal outcome. In this formulation, ligands provide the chemical field, membrane physics conditions the conversion, and the physiological output remains local, measurable and prespecified.

MECS baseline–edit–rescue (MECS-BER) converts this restricted inferential problem into a prespecified methodological sequence. Co-expression first nominates a candidate assembly within one defined territory. Eligibility and competence gates then determine whether the nominated cannabinoid GPCR, ectopic olfactory GPCR and TRP-channel arms can be engaged there and influence the same locked proximal outcome. Pairwise analysis asks whether lower-order crosstalk remains sufficient. Only after these restrictions are satisfied is κ, the third-order interaction term, interpreted under the locked pairwise-only null to test whether a residual three-arm requirement remains. Baseline, membrane-prior edit and rescue then test whether the assigned class depends on a measurable membrane state and is reversible. The gain is therefore not a new biological claim, but a stricter route for testing whether membrane-conditioned receptor integration is present, absent or only pairwise under defined conditions. MECS proposes the logic; MECS-BER adjudicates it.

Here, a membrane prior is a measurable state feature present before perturbation, such as sterol accessibility, phosphoinositide topology, trafficking state or mechanical load. The locked proximal outcome is the single local readout retained throughout the sequence. These definitions function as operational safeguards. They prevent higher-order interpretation from arising from receptor maps, verbal analogy or distal phenotype overlap alone.

The kidney is used as prospective Application 1: a constrained prospective testbed in which segment-resolved micro-niches preserve tractable proximal hinges, including renin control, vectorial transport, barrier competence and flow-sensitive calcium organization. The juxtaglomerular apparatus is retained as the principal worked deployment. The proximal tubule, podocyte barrier and distal/collecting-duct territories remain evidence-graded extensions. This application is therefore a specification exercise, not evidence that renal CB–OR–TRP assemblies have already been biologically validated.

Figure 1 frames the shift from receptor inventory to membrane-conditioned local integration regime. Figure 2 aligns renal micro-niches with nominated CB–OR–TRP assemblies and locked proximal outcomes. Figure 3 formalizes the locked analytic workflow and its adjudication classes. Detailed benchmark matrices, simulated stability templates, gate checklists, expanded renal application sheets, anchored ligand inventories, background renal olfactory-receptor candidates and auxiliary renal disease-context material are provided in Tables S1–S6. The formal MECS unit, kappa boundary and state vocabulary are defined in Supplementary Method S0.

Biological rationale for a prespecified MECS-BER test

The biological rationale for MECS-BER rests on convergent literatures rather than direct validation of a completed cannabinoid–olfactory–TRP assembly. Membrane-state studies show that phosphoinositides, sterols, sphingolipids, gangliosides, acyl-chain composition, oxidative state, trafficking history and mechanical load can alter local order, protein partitioning, channel gating and access to signalling partners. These observations justify a measurable membrane prior as a prospective state variable [1, 2, 5, 14–21].

Single-arm competence is supported asymmetrically. Cannabinoid GPCR output is shaped by receptor subtype, coupling, trafficking and local endocannabinoid metabolism, including FAAH-linked control of anandamide persistence [3, 6–10, 17]. TRP channels provide proximal calcium- and transport-facing responses to lipid, phosphoinositide, mechanical, osmotic and inflammatory states [1, 2, 5, 14–19]. Renal ectopic olfactory-GPCR evidence is more limited, but selected renal receptors, renal olfactory-transduction components and related gut-derived metabolite-context studies provide physiology-facing support sufficient for cautious nomination, not validation [11, 12, 22–30].

Renal micro-niches provide constrained prospective test spaces in which membrane state, ligand exposure and proximal physiology

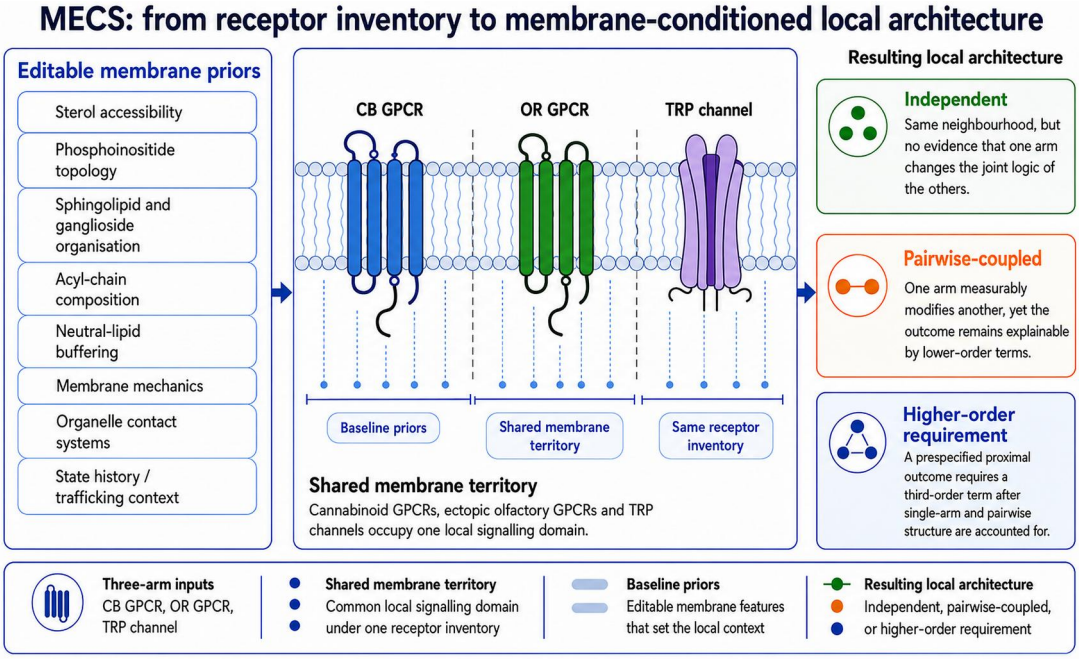

Figure 1 MECS Shifts interpretation from receptor inventory to membrane-conditioned local signalling architecture. Editable membrane priors include sterol accessibility, phosphoinositide topology, sphingolipid and ganglioside organization, acyl-chain composition, neutral-lipid buffering, membrane mechanics, organelle contact systems and state history or trafficking context. These priors may bias the local signalling environment for cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels within a shared membrane territory. Under the same nominal receptor inventory, the resulting local architecture may remain independent, become pairwise-coupled or generate a higher-order requirement on one locked proximal outcome. The figure therefore shifts emphasis from receptor presence alone to membrane-conditioned local signalling architecture.

can be aligned. The juxtaglomerular apparatus is the principal renin-facing domain; the proximal tubule and podocyte slit diaphragm remain transport- and barrier-facing extensions. These literatures justify nomination and gate design, but they do not validate a same-cell cannabinoid–olfactory–TRP assembly or establish mechanistic crosstalk without testing on the locked proximal outcome.

This rationale therefore sets the biological boundary for the method that follows: MECS-BER begins where nomination is plausible, but adjudication remains unavailable until territory, membrane prior, candidate arms and locked proximal outcome are prespecified. MECS-BER therefore provides a prespecified and formalized methodological workflow, not biological proof of any specific receptor triad.

Materials and methods

Method scope and intended use

Within MECS, MECS-BER is intended for membrane-dense territories. In such territories, one editable membrane prior and one locked proximal outcome should be prespecified before testing begins. The method is strongest when the readout remains close to the local signalling assembly. Examples include calcium organization, cyclic-nucleotide tone, renin release, transport switching and barrier-state behaviour. It should not be used to infer local mechanism from distal, multistep phenotypes alone.

Five operational units are fixed before MECS-BER interpretation begins. The membrane prior is the measurable local membrane-state feature selected for edit or rescue. The term ‘membrane prior’ is used operationally: it borrows the logical sense of a pre-existing condition from Bayesian vocabulary, but it does not imply that MECS-BER applies a formal Bayesian probabilistic model. The locked proximal outcome is the single readout on which classification is performed. The candidate assembly is the nominated cannabinoid GPCR arm, ectopic olfactory GPCR arm and TRP-channel arm within one local territory. Eligibility and competence gates determine whether those arms are biologically plausible, locally engageable and capable of influencing the locked proximal outcome. The integration class is the assigned mode of combination on that outcome after κ-based testing under the locked null. In MECS-BER, baseline is not treated as a universal healthy membrane state. It is the physiologically competent reference state for the declared territory, prior and locked proximal outcome. The reference state is admissible only when receptor and channel behaviours remain sufficiently organized, ligand fields remain bounded and the locked proximal outcome retains dynamic range. This baseline class defines the comparison point for edit and rescue; it is not a general biological label of membrane health.

The overall MECS-BER workflow is summarized in Figure 3. Representative membrane edits include sterol accessibility change, phosphoinositide depletion or redistribution, sphingolipid remodelling, acyl-chain remodelling, trafficking redistribution, inflammatory membrane stress and altered mechanical load. These edits are not interchangeable. Each changes the local constraint set in a distinct manner and can therefore bias a different route to regime change [1, 2, 5, 14–19, 31].

A membrane-prior edit is not automatically a deteriorated membrane state. It is admissible when declared in advance, biologically plausible, technically bounded and compatible with continued measurement of the locked proximal outcome. It becomes non-admissible when it causes nonspecific toxicity, calcium collapse,

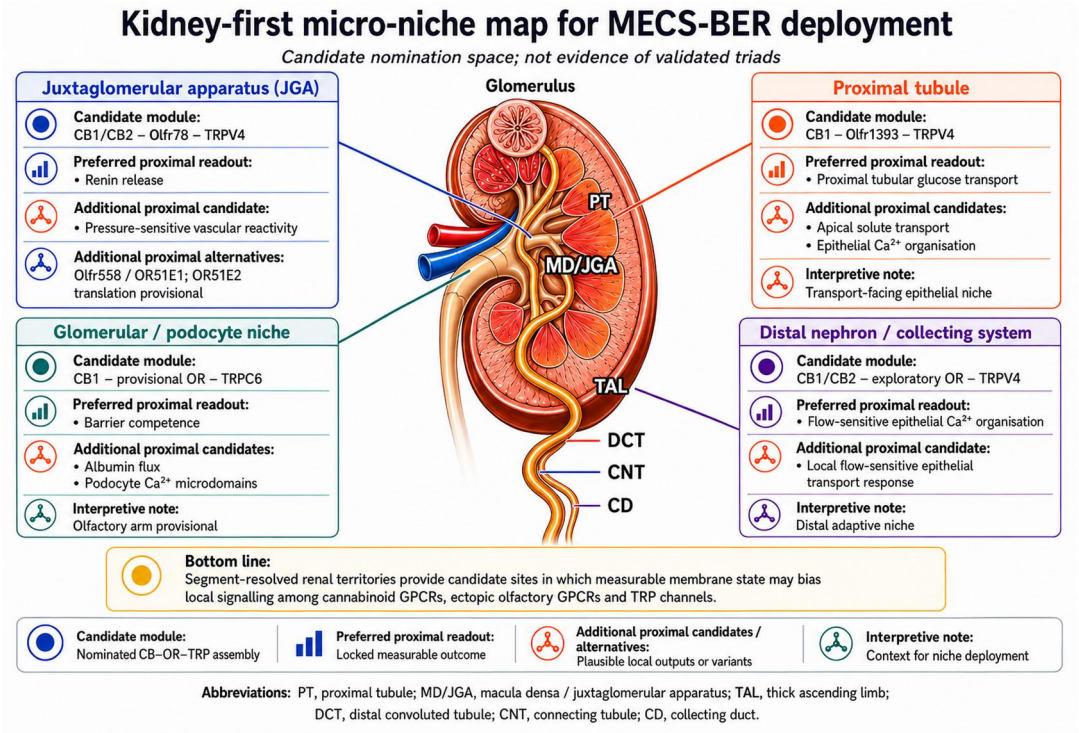

Figure 2 Kidney-first micro-niche map for prospective MECS-BER renal deployment. Renal micro-niches are aligned with nominated CB–OR–TRP candidate assemblies for prospective application 1. The juxtaglomerular apparatus is the principal worked deployment, with renin release as the preferred proximal readout. The glomerulus/podocyte niche, proximal tubule and distal nephron/collecting system are evidence-graded extension territories rather than validated CB–OR–TRP assemblies. Their preferred proximal readouts are barrier competence, proximal tubular glucose transport and flow-sensitive epithelial Ca^{2+} organization, respectively. Additional proximal candidates and local alternatives are shown where relevant, including pressure-sensitive vascular reactivity, apical solute transport, epithelial Ca^{2+} organization, albumin flux, podocyte Ca^{2+} microdomains and local flow-sensitive epithelial transport response. Segment-resolved renal anatomy is used to keep membrane-state hypotheses tied to local physiological outputs rather than distal phenotypes. The figure defines prospective renal nomination space and its guardrails; the locked analytic workflow is formalised in Fig. 3.

assay saturation, loss of compartment identity, loss of arm competence or loss of outcome dynamic range.

Table 1 translates representative membrane priors into assay-selection logic. Each edit is treated as a specific, measurable perturbation of the local constraint set rather than as a broad background condition; different priors may preferentially alter GPCR competence, TRP-channel permissiveness, receptor surface availability, mechanical coupling or access to shared effectors.

An output change after membrane editing does not by itself support higher-order interpretation. κ-based adjudication requires the locked proximal outcome, lower-order testing under the locked null and coherent behaviour across baseline, edit and rescue. The BER sequence can also record whether membrane editing changes the trajectory of the same locked proximal outcome, including delayed recovery, overshoot, rebound or mistimed re-entry after rescue or counter-editing. Such patterns remain descriptive trajectory features. They do not define a mechanism, pathway or adjudication class, and they are inadmissible when inferred from distal phenotype rebound, loss of viability, calcium collapse, nonspecific membrane disruption or assay saturation.

Required inputs and admissible study design

MECS-BER requires five prespecified inputs: one measurable membrane prior, one locked proximal outcome, and one nominated cannabinoid GPCR, ectopic olfactory GPCR and TRP-channel arm within a single biological territory. The ligand field denotes the operative local chemical environment within the defined microdomain and time window, rather than a single ligand in isolation. For the cannabinoid arm, this field includes agonist identity, membrane passage, intracellular sequestration, carrier-assisted trafficking and enzymatic termination; FAAH-linked control of anandamide persistence is therefore part of the local biochemical state [9, 10].

Admissible designs must estimate the full $2 \times 2 \times 2$ response surface on the locked proximal outcome under baseline, edited and, where feasible, rescued conditions. The workflow can be reduced to a practical gate: after territory, membrane prior, candidate assembly and locked proximal outcome are fixed, does a defined membrane-prior edit leave the outcome within lower-order explanation, or reveal a rescue-sensitive higher-order requirement?

The method is not intended for distal phenotypes, open-ended receptor cataloguing or undefined territories.

Decision outputs and classification states

The method returns one of four adjudicative outputs on the locked proximal outcome. Lower-order sufficiency indicates that single-arm and pairwise structure remain adequate under the locked null. Pairwise-coupled lower-order structure indicates non-independence without a residual third-order term. Higher-order requirement

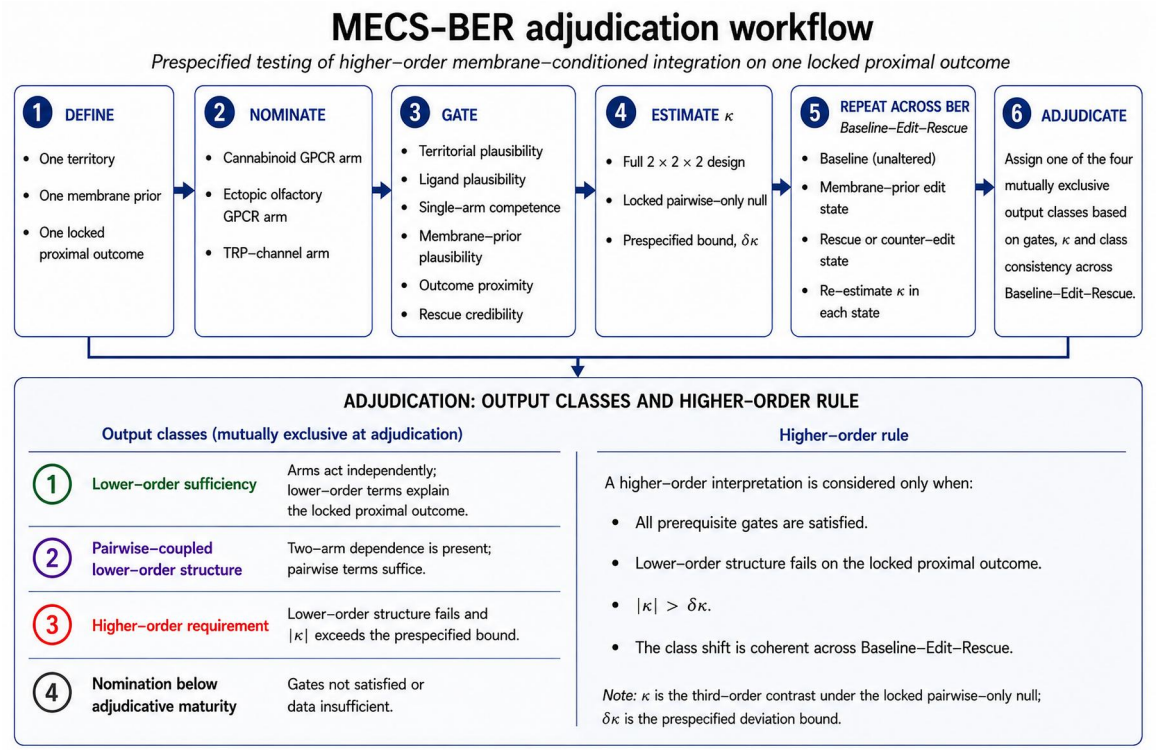

Figure 3 MECS-BER Adjudication workflow. The workflow formalises the prespecified sequence used to test higher-order membrane-conditioned integration on one locked proximal outcome. A biological territory, measurable membrane prior and locked proximal outcome are first defined. Cannabinoid GPCR, ectopic olfactory GPCR and TRP-channel arms are then nominated and passed through eligibility and competence gates, including territorial plausibility, ligand plausibility, single-arm competence, membrane-prior plausibility, outcome proximity and rescue credibility. κ is estimated under a locked pairwise-only null in a full $2\times2\times2$ design, with interpretation constrained by a prespecified bound, $\delta\kappa$. The same adjudication is repeated across baseline, membrane-prior edit and rescue or counter-edit states. Final outputs are lower-order sufficiency, pairwise-coupled lower-order structure, higher-order requirement or nomination below adjudicative maturity. A higher-order interpretation is considered only when lower-order structure fails on the locked proximal outcome, $|\kappa|$ exceeds the prespecified bound and the class shift behaves coherently across baseline–edit–rescue. Renal micro-niches are downstream worked examples, not the primary object of adjudication.

indicates that lower-order structure fails and that a residual third-order term remains after prespecification of the locked null. Nomination below adjudicative maturity indicates insufficient eligibility, competence or reversibility for disciplined interpretation. In practical terms, this class means that the assembly may be named as a candidate but should not yet be used for κ-based biological inference.

Locked null model and operational definition of κ

Adjudication proceeds under a locked null family which is the prespecified lower-order reference model against which κ is judged. The primary null is pairwise-only, with no third-order term. The outcome channel Y is measured under a full $2\times2\times2$ factorial perturbation of the three nominated arms. Here, A denotes the cannabinoid GPCR arm, B denotes the ectopic olfactory GPCR arm and C denotes the TRP-channel arm. For each arm, 0 and 1 denote the prespecified inactive/control and active/perturbed states, respectively. Here, Y denotes the response variable: the locked proximal outcome measured under each perturbation state. Thus, Yabc denotes the measured value of the same locked proximal outcome when $A=a$, $B=b$ and $C=c$, with a, b and $c\in\{0,1\}$. The third-order interaction term is then defined as:

$$\kappa=\Delta_{123}=\ Y_{111}-\ Y_{110}-\ Y_{101}-\ Y_{011}+\ Y_{100}+\ Y_{010}+\ Y_{001}-\ Y_{000}.$$

κ is not proposed as a new statistical quantity. It is the third-order interaction contrast generated by a complete three-factor, two-level factorial response surface. Classical $2\times2\times2$ (2^3) factorial-design methodology provides the statistical provenance of this alternating-sign contrast [32], while contemporary complete-factorial and multi-way-interaction literature supports the wider design and interpretive setting in which it is applied [33–35]. MECS-BER adopts this established contrast as a prespecified adjudicative quantity for testing whether a nominated three-arm response remains adequately explained by single-arm and pairwise structure under the locked pairwise-only null. The labels A, B and C therefore denote perturbation arms only; crosstalk is inferred from structured dependence in the locked proximal outcome Y, not from the arm labels themselves.

In operational terms, κ asks whether residual outcome variation remains after single-arm and pairwise structure have already been specified. A non-zero κ is biologically interpretable only under strict conditions. The outcome channel must be proximal, prespecified and attributable to the nominated territory. Alternative null families and normalizations, when used, remain secondary sensitivity analyses. They are not co-primary readouts.

Apparent non-additivity alone is insufficient for higher-order interpretation. Within the prespecified factorial design, the relevant

Table 1 Representative membrane-prior edits and predicted diagnostic consequences within the MECS-BER workflow.

Membrane edit or state history	Immediate membrane-level consequence	Expected effect on local assembly	Preferred proximal outcome channels	Predicted adjudicative consequence
Sterol accessibility change	Alters G-protein-coupled receptor (GPCR) conformational sampling, nanoscale partitioning and hydrophobic mismatch.	Can stabilise or dissolve shared receptor–channel neighbourhoods and reweight effector access.	Calcium organization; cyclic-nucleotide tone; renin output; transport switching; barrier-state behaviour.	A stable receptor inventory may shift into a different lower-order or higher-order class.
PI(4,5)P2 depletion or redistribution	Changes local phosphoinositide support for channel gating and receptor-operated signalling.	Reweights transient receptor potential (TRP)-channel permissiveness and receptor access to channel-level control.	Calcium microdomains; secretory responses; transport flux; flow-sensitive signalling.	An apparent receptor effect may instead reflect a lipid-mediated change in channel permissiveness.
Sphingolipid or ganglioside remodelling	Reorganises raft-like territories and lateral segregation of membrane proteins.	Changes which proteins occupy effective signalling proximity despite persistent expression.	Barrier integrity; inflammatory calcium responses; flow sensing; vascular reactivity.	Co-expression becomes a weaker predictor of actual crosstalk architecture.
Acyl-chain remodelling or unsaturation shift	Changes bilayer elasticity, thickness, and curvature stress.	Shifts the conformational and mechanical repertoire sampled by receptors and channels.	Flow-sensitive calcium entry; transport adaptation; contractile or secretory tone.	A membrane-state threshold may be crossed without large abundance changes.
Receptor desensitization, internalization, or endosomal redistribution	Reduces surface availability and redistributes signalling across compartments.	Changes the dominant coupling path available under repeated stimulation or chronic exposure.	cAMP or calcium tone; renin timing; acute transport or secretory behaviour.	The same nominal members can behave differently across exposure histories.
Inflammatory or oxidative membrane stress	Distorts sterol and phospholipid organization and can strengthen cytoskeletal restraint.	Destabilises an earlier regime and can amplify feedback within receptor–channel assemblies.	Barrier dysfunction; tubular transport volatility; vascular tone; injury-linked calcium states.	History-dependent recovery patterns, including constrained, delayed, rebound-like or mistimed re-entry, may become plausible under injury. These patterns remain descriptive unless they are followed on the same locked proximal outcome across baseline, edit and rescue.
Mechanical load, shear, or curvature change	Alters membrane tension and local curvature sensed by mechanosensitive proteins.	Reweights mechanically sensitive channel participation and receptor–channel alignment.	Flow sensing; distal-nephron calcium entry; pressure-sensitive secretion; transport adaptation.	Mechanical context becomes part of state definition rather than a background variable.

Entries are operational predictions for assay design, edit selection and interpretation of BER outcomes. They do not validate any specific cannabinoid–olfactory–TRP assembly.

question is whether a residual third-order contrast remains after single-arm and pairwise terms have been specified [32–35]. Pharmacological mixture effects likewise require an explicit reference model before synergy or antagonism is inferred [36].

Assay-specific indifference bound and variance handling

In analytic benchmarks, the indifference bound for κ is zero by construction. In biological or simulated deployment, κ should instead be interpreted against a prespecified assay-specific bound. That bound should reflect measurement variance, analytical precision and the dynamic range of the locked proximal outcome. A practical implementation estimates the standard error of κ from replicate measurements across the factorial grid. The bound is declared before classification. Here, $\delta\kappa$ denotes the predeclared near-zero tolerance for κ, not a fitted post hoc threshold. Values within $\pm\delta\kappa$ remain within the lower-order family; supported pairwise terms distinguish pairwise-coupled lower-order structure from lower-order sufficiency. This prespecified bound prevents trivial assay variation from being misread as higher-order integration.

Reference implementation and workflow logic

The operational sequence proceeds through seven prespecified steps. First, the biological territory, membrane prior and locked proximal outcome are defined. Second, one cannabinoid GPCR arm, one ectopic olfactory GPCR arm and one TRP-channel arm are nominated. Third, territorial plausibility, ligand plausibility, single-arm competence, membrane-prior plausibility, outcome-proximity and rescue-credibility gates are applied. Fourth, the full $2\times2\times2$ response surface is estimated, and κ is computed under the locked pairwise-only null. Fifth, the baseline class is assigned using the prespecified $\delta\kappa$. Sixth, the membrane-prior edit is introduced, and classification is repeated on the same locked proximal outcome. Seventh, rescue or counter-edit logic tests return towards the earlier class.

This sequence prevents category errors. Co-expression remains nomination. Pairwise non-independence remains lower-order when pairwise terms are sufficient. A distal phenotype remains consequence space when it is too remote from the local assembly. Higher-order interpretation is method-supported only when eligibility, competence, analysis of the locked proximal outcome, κ-based deviation and rescue-sensitive state behaviour align. The workflow therefore converts a broad membrane-crosstalk hypothesis into a

constrained test of whether one local membrane state changes the communication logic among nominated receptor and channel arms.

Eligibility and competence gates

κ is interpreted only after eligibility and competence gates have been satisfied. Territorial plausibility asks whether the nominated members can reasonably occupy one biological niche. Ligand plausibility asks whether each arm can be engaged within that niche. Competence asks whether each arm can measurably influence the locked proximal outcome. Membrane-prior plausibility asks whether the chosen membrane edit could bias access, proximity, channel gating or coupling logic within the local assembly. Outcome-channel proximity asks whether the readout is close enough to the assembly for mechanistic interpretation. Figure 4 summarizes the locked null logic, the descriptive layers that precede adjudication, the formal output classes and the gate conditions required before higher-order interpretation. The fillable gate checklist corresponding to these criteria is provided in Table S2.

For the ectopic olfactory GPCR arm, territorial plausibility should be graded by evidential tier. In many non-olfactory tissues, including kidney, RNA-level detection is firmer than condition-resolved protein localization or membrane-surface availability for specific OR candidates. A transcript-positive OR therefore supports nomination. It should not be treated as proof of stable surface participation across states unless orthogonal protein-level or live functional evidence is available [11, 12, 30]. This caution is especially relevant for olfactory receptors, where receptor-specific surface-localization evidence may remain limited by reagent validation.

Accordingly, MECS does not claim biological validation of any specific cannabinoid–olfactory–TRP assembly. It defines a restrictive inference framework that tests prospectively whether a nominated membrane-resident assembly exceeds single-arm and pairwise explanation on one locked proximal outcome. Gate failure is informative, as it identifies cases that should remain at nomination rather than proceed to adjudication.

MECS-BER deployment logic

Higher-order interpretation is tested through a BER sequence. It is not inferred from a static snapshot. A reference membrane state is first measured on the locked proximal outcome. A defined membrane-prior edit is then introduced to test whether the integration class changes despite a stable receptor inventory. Rescue or counter-edit logic then asks whether the system returns towards the earlier class when the relevant prior is restored or counter-balanced [16, 17, 31].

This state logic is important in chronic exposure, injury and metabolic stress. In these settings, receptors and channels can be redistributed without simple loss or gain of abundance. Repeated stimulation can alter surface availability, endosomal signalling and effective transducer access. Phospholipid remodelling, altered sterol balance

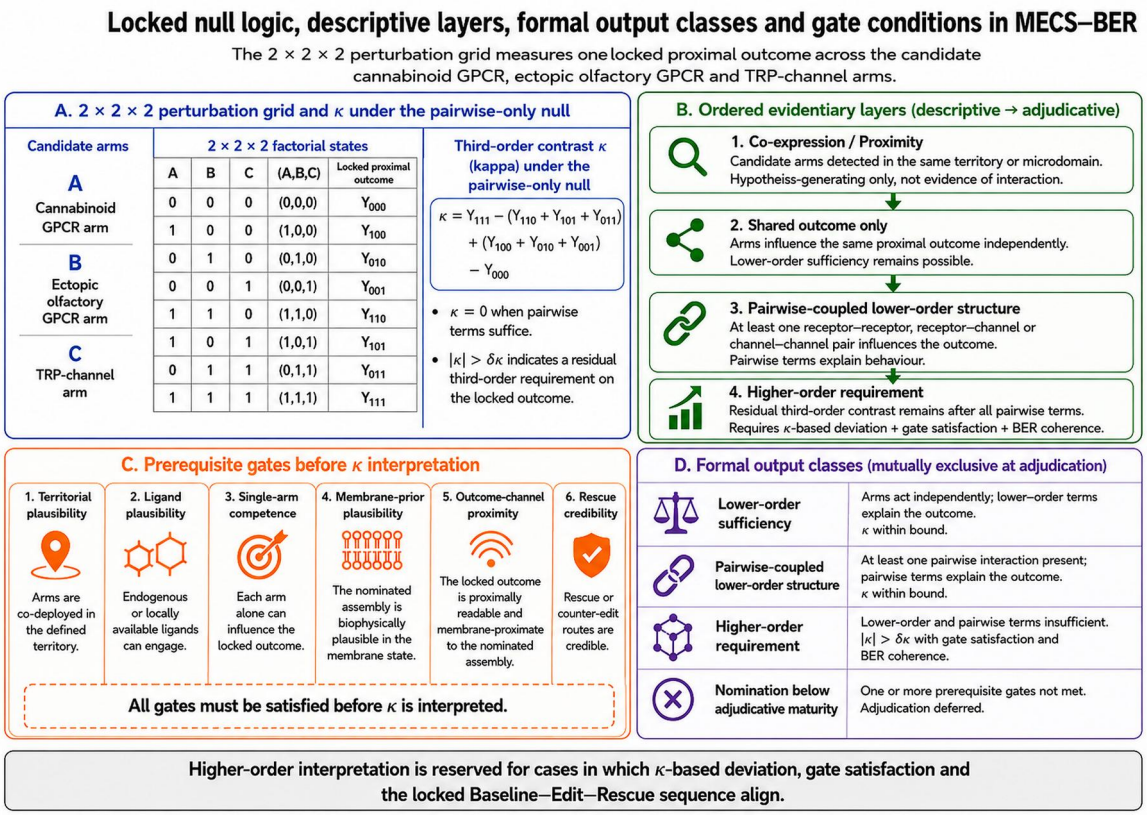

Figure 4 Locked null logic, descriptive layers, formal output classes and gate conditions in MECS-BER. The $2 \times 2 \times 2$ perturbation grid measures one locked proximal outcome across the candidate cannabinoid GPCR, ectopic olfactory GPCR and TRP-channel arms. κ is interpreted only after territorial, ligand, competence, membrane-prior plausibility, outcome-proximity and rescue-credibility gates. The figure separates descriptive evidence, lower-order sufficiency, pairwise-coupled lower-order structure and higher-order requirement. Higher-order interpretation is reserved for cases in which κ-based deviation, gate satisfaction and the locked BER sequence align.

and oxidative membrane stress can also shift the local regime without rewriting receptor expression maps [14, 16–19, 31]. Under such conditions, the same nominal input environment need not produce the same proximal response trajectory.

Positive-feedback and hysteretic control systems provide a general precedent for history-dependent state trajectories [37]. Within MECS-BER, any analogous trajectory remains descriptive unless it is established on the locked proximal outcome across baseline, edit and rescue.

One descriptive BER trajectory may be termed 'slingshot-like' when membrane-prior editing displaces the local assembly from its baseline signalling regime and rescue or counter-editing is followed by overshooting, mistimed or redirected re-entry of the same locked proximal outcome. The term describes trajectory geometry only. It does not define a mechanism, pathway or adjudication class, and it cannot substitute for gate satisfaction, κ estimation or rescue-sensitive class behaviour. It should not be inferred from a single overshoot, distal phenotype rebound, loss of viability, calcium collapse, nonspecific membrane disruption or assay saturation. Figure 5 illustrates this generic deployment logic within the same BER sequence used for formal adjudication.

Failure conditions and non-use cases

MECS-BER is not appropriate when the readout is distal, multistep or too remote from the nominated membrane territory. It should also not be used when the nominated members cannot plausibly occupy the same local territory, when one or more arms does not measurably influence the locked proximal outcome, when the membrane prior cannot be assessed or edited, or when rescue is not credible. In these settings, the framework may organize nomination, but it should not support a higher-order claim. These conditions mark the boundary between a plausible membrane-conditioned signalling hypothesis and an adjudicable local communication regime.

Methodological benchmarking design

The present analysis is methodological rather than biological. No fully compliant biological MECS-BER dataset is presented in this study. Stage 1 uses deterministic $2 \times 2 \times 2$ formal benchmark matrices representing additive independence, pairwise-only interaction, residual third-order requirement and a state-shifted BER sequence. These matrices verify the internal classification behaviour of the locked-null logic; they do not establish biological interpretability of κ in a living membrane-resolved assay.

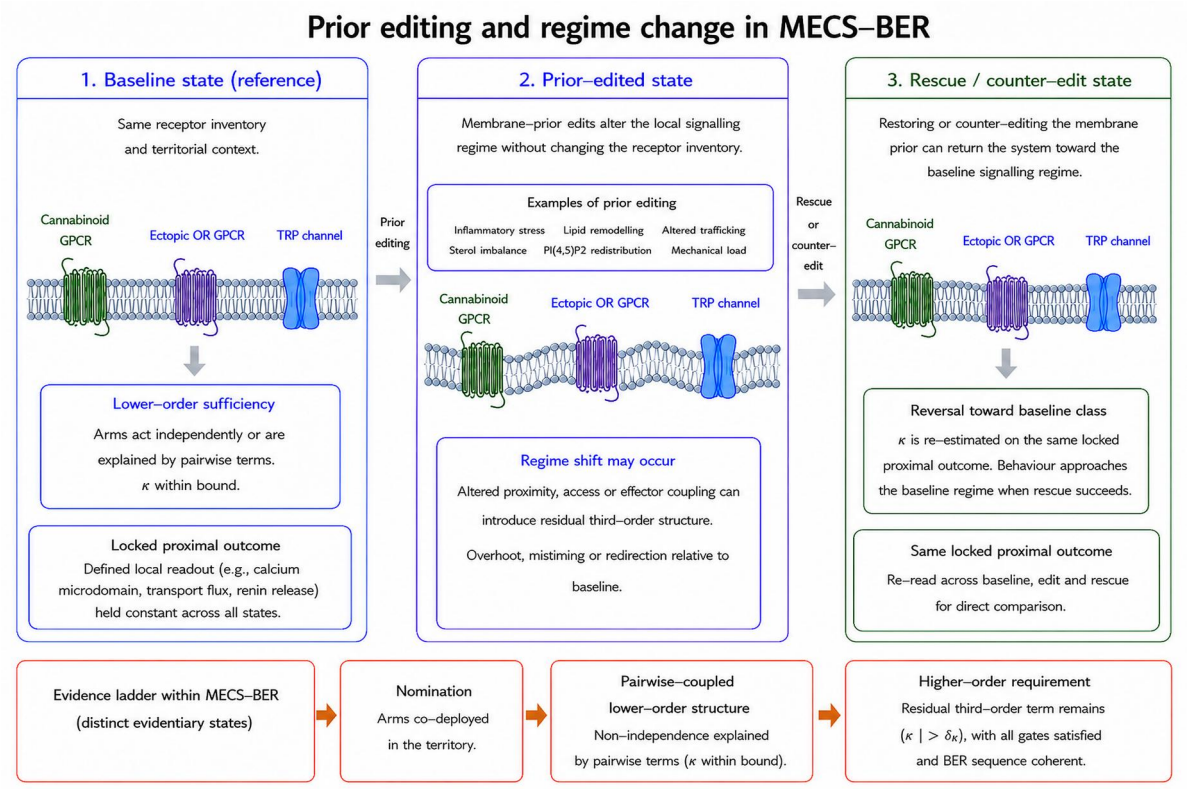

Figure 5 Prior editing and regime change in MECS-BER. Chronic exposure, inflammatory stress, lipid remodelling, altered trafficking, sterol imbalance, phosphoinositide redistribution and mechanical load can edit membrane priors. These edits may move a candidate local assembly from one integration regime to another without requiring a change in receptor inventory. In this methods figure, 'slingshot-like' denotes a history-dependent BER trajectory in which a membrane-edited assembly is displaced from its baseline signalling regime and, during rescue or counter-editing, may re-enter signalling through an overshooting, mistimed or redirected proximal response. The term is descriptive, not a claim of a distinct physical mechanism. It remains interpretable only when the same locked proximal outcome is re-read across baseline, edit and rescue. The figure distinguishes baseline, membrane-prior-edited and rescue/counter-edit states. It also separates persistence of lower-order sufficiency from emergence and rescue-sensitive reversal of a higher-order requirement. Nomination, pairwise-coupled lower-order structure and higher-order requirement remain distinct evidentiary states within MECS-BER. The figure illustrates state-dependent regime movement, not evidence of higher-order integration by overshoot alone.

Stage 2 assesses classification stability under simulated measurement noise and prespecified assay-specific bounds. It asks how formal class assignment changes as variance and tolerance are varied. These variance regimes were not derived from an executed biological assay and must not be interpreted as empirical estimates of biological performance. The deterministic matrices and arithmetic are supplied in Table S1; the simulation-based stability framework is supplied in Table S1A.

The benchmark tests the behaviour of the adjudication logic, not the biology of a receptor assembly.

Requirements for prospective biological execution

A fully compliant biological deployment would require, within one prespecified membrane territory, functional competence of the nominated cannabinoid GPCR, ectopic olfactory GPCR and TRP-channel arms on the same locked proximal outcome; one measurable membrane prior that can be selectively edited and credibly rescued or counter-edited; and complete $2 \times 2 \times 2$ factorial response surfaces under baseline, edited and rescued conditions. Full BER acquisition would therefore comprise at least 24 prespecified factorial condition combinations before biological replication, technical controls and assay-quality exclusions are added. Table S2A summarizes these minimum requirements and failure conditions.

Immediate biological acquisition is technically constrained by three linked requirements: state-resolved functional confirmation of the ectopic olfactory GPCR arm within a defined micro-niche; selective membrane-prior editing without nonspecific injury, calcium collapse or loss of outcome dynamic range; and preservation of one common proximal readout through complete factorial and rescue acquisition. The present study provides the prespecified architecture for that experiment; it does not report its biological completion.

Future biological implementation could also use reductionist membrane-biophysical systems to test the membrane-prior axis directly. Langmuir-type monolayers, surface-pressure readouts and related interfacial assays could examine how defined lipid states alter membrane organization and constrain membrane-associated proteins, whereas mutant peptides or proteins could test whether sequence-encoded structural determinants remain sensitive to membrane context. Such approaches are appropriate next tests of MECS-BER, but they are not treated here as completed validation.

Application design

Prospective application 1 was specified in renal micro-niches chosen for their membrane-dense architecture, segment-resolved geometry and candidate locked proximal outcomes. It defines deployment requirements, gate completion and output locking in a first organ-level setting; it does not report an executed biological MECS-BER experiment. Distal phenotypes, such as blood-pressure change, glucosuria, albuminuria or water-salt imbalance, remain mapped consequence space and do not substitute for the locked proximal outcome. The renal specification is a testbed for disciplined nomination, not evidence that renal assemblies are already biologically validated in vivo. Advanced CKD, dialysis exposure and transplantation may be considered future disease-state contexts in which the same MECS-BER requirements would apply; these settings are not treated here as adjudicated endpoints, deployment claims or evidence of validated renal assemblies.

Results

Analytic benchmarks separate lower-order sufficiency from higher-order requirement

The formal benchmark panel was designed to answer a narrow methodological question.

Does the locked-null workflow classify formal lower-order and higher-order cases in the expected manner?

Table 2 summarizes the benchmark set. Table S1 provides the full matrices. In these generator rules, a, b and c are formal binary arm states, not ligand concentrations or continuous pathway activities.

Additive cases yielded $\varkappa = 0$ under the locked null and were retained within lower-order sufficiency. Pairwise-only cases also yielded $\varkappa = 0$, but were classified as pairwise-coupled lower-order structure. By contrast, the formal third-order benchmark yielded $\varkappa$ outside the bound and was classified as a higher-order requirement. These matrices verify that the prespecified arithmetic distinguishes lower-order from residual third-order structure when the generating rules are known in advance. They do not demonstrate that $\varkappa$ is interpretable in a biological assay, that a candidate receptor assembly exists in vivo or that any membrane-prior edit produces rescue-sensitive higher-order behaviour in renal tissue.

BER tests state dependence rather than one-off nonlinearity

The state-shift benchmark adds a second test. A baseline matrix generated under lower-order sufficiency was paired with an edited matrix in which a prior change introduced a third-order requirement. A rescued matrix then returned the system towards the baseline class. This sequence illustrates the specific logic of MECS-BER. The method is not satisfied by a single edited state. It asks whether the classification change is recoverable when the relevant prior is counter-edited or restored.

In a future biological deployment, a stable receptor inventory could move between adjudication classes when membrane priors are altered. Rescue is therefore used to support state dependence, not to add another descriptive layer. The inferential gain lies in separating irreversible architectural claims from experimentally reversible regime shifts. The benchmark shows how the same local signalling parts may acquire different communication logic when membrane state is edited, and how rescue can test whether that logic is state-conditioned rather than fixed. This is the intended practical value of BER: it converts nonlinearity from a descriptive observation into a test of reversible state dependence.

In this context, rescue does not require return to a universal normal membrane composition. It requires recovery of interpretability on the same locked proximal outcome. A rescued or counter-edited state is most informative when the declared membrane prior is shifted back, compensated or counter-balanced, receptor-arm competence remains present, the outcome retains dynamic range and the adjudicative class moves towards its earlier assignment.

Simulation-based assessment of classification stability under noise and bound variation

A second formal benchmarking study assessed classification stability under simulated measurement noise and varying assay-specific bounds. Replicate outcome values were generated around each

Table 2 Formal benchmark panel used to verify locked-null classification behaviour in MECS-BER.

Benchmark case	Generator rule	κ under locked null	Expected adjudication or sequence behaviour	Diagnostic implication
Additive independence	$Y = a + b + c$	0	Lower-order sufficiency	Three arms influence the outcome without requiring pairwise or third-order structure.
Pairwise AB interaction	$Y = a + b + c + ab$	0	Pairwise-coupled lower-order structure	Non-independence is present, yet pairwise terms remain sufficient.
Pairwise AC interaction	$Y = a + b + c + ac$	0	Pairwise-coupled lower-order structure	A receptor–channel pair can be influential without requiring a residual third-order term.
Pairwise BC interaction	$Y = a + b + c + bc$	0	Pairwise-coupled lower-order structure	The ectopic olfactory G-protein-coupled receptor (GPCR) arm and transient receptor potential (TRP)-channel arm can be linked while the locked null still holds.
Irreducible third-order case	$Y = a + b + c + abc$	1	Higher-order requirement	Lower-order terms fail; a residual third-order term remains on the locked proximal outcome.
State-shifted sequence	Baseline: $a + b + c$; edited: $a + b + c + abc$; rescue: $a + b + c$	$0 \rightarrow 1 \rightarrow 0$	State-dependent regime change	Prior editing and rescue support reversibility of the class shift without changing nominal membership.

benchmark matrix under low-, intermediate- and high-variance conditions, and κ was re-estimated after repeated sampling under prespecified $\delta\kappa$ values. These simulations are formal stress tests rather than empirical estimates of biological performance. Their restricted purpose was to test how prospectively declared tolerance affects formal classification stability. The simulation-based outputs are provided in Table S1A.

Across the simulated benchmarking panel, class assignment remained stable in low-variance settings. It became progressively more sensitive to $\delta\kappa$ selection as variance increased. Pairwise-only structures were not systematically upgraded to higher-order requirement when the bound had been prespecified. Edited third-order cases remained distinguishable from lower-order baselines until variance approached the dynamic range of the locked proximal outcome. These results support assay-specific bounds as a required control on over-interpretation. They should be declared prospectively rather than adjusted after interpretation.

Classification stability therefore depends on declaring tolerance before classification rather than adjusting interpretation after the response is observed.

Prospective application 1: renal micro-niche deployment specification

The kidney is used here as a constrained prospective testbed: its compact membrane-rich territories link local structure, fluid mechanics and ligand exposure to candidate locked proximal outcomes. The purpose is not to validate a renal CB–OR–TRP assembly, but to ask whether a territory, membrane prior, candidate assembly and locked proximal outcome can be specified before κ-based adjudication. Distal phenotypes, including blood-pressure change, glucosuria, albuminuria or water–salt imbalance, remain mapped consequence space rather than substitutes for the locked proximal outcome.

The juxtaglomerular apparatus is prioritized as the principal worked domain. A disciplined JGA deployment would nominate cannabinoid signalling, an Olfr78-centred ectopic olfactory GPCR arm and TRPV4-centred mechanically sensitive channel signalling within the macula densa–afferent arteriole interface, then declare one membrane prior, such as sterol accessibility, and lock one renin-facing proximal outcome. Published AC3/G(olf), Olfr78 and Olfr558/OR51E1 studies support physiology-facing nomination in this region, but do not prove a completed CB–OR–TRP assembly on one locked proximal outcome [3, 22–27].

Within the JGA, slice-based or microperfused preparations that preserve native architecture can couple live Ca^{2+}, cAMP or renin-granule readouts to the macula densa–afferent arteriole interface [38–42]. GPR91/SUCNR1 and other renin-control pathways should remain comparator axes, not hidden members of the candidate assembly [41–43]. A lower-order interpretation remains preferred unless the edited state yields a residual third-order requirement on the locked proximal outcome and rescue returns the system towards its earlier class.

Three extension territories remain in the renal map. The proximal tubule is retained as a transport-facing extension through CB1, Olfr1393, TRPV4 and apical phosphoinositide topology linked to proximal tubular glucose transport. The podocyte slit diaphragm retains CB1/TRPC6 and barrier-mechanics logic, with a provisional ectopic olfactory GPCR arm. The distal nephron/collecting-duct territory remains exploratory, with TRPV4-centred flow-sensitive Ca^{2+} organization as the clearest candidate proximal outcome. Table 3 summarizes these evidence-graded territories, their first-pass membrane priors and their preferred locked proximal outcomes; additional plausible priors and candidate proximal outcomes are reserved for Table S3.

These renal territories are useful not because they validate a CB–OR–TRP assembly, but because each offers a proximal physiological

Table 3 Evidence-graded renal micro-niche deployment of MECS-BER on prespecified proximal physiological outcomes.

Niche	Cannabinoid GPCR arm	Ectopic olfactory GPCR arm	TRP-channel arm	Declared prior	Locked proximal outcome	Status
Juxtaglomerular/ afferent arteriole	CB1 or CB2 according to vascular and inflammatory context	Olfr78 anchor; Olfr558/OR51E1 vascular support; OR51E2 human translation provisional.	TRPV4	Sterol accessibility	Renin release	Principal worked domain; first prospective renal deployment.
Proximal tubule apical membrane	CB1 physiology-facing epithelial arm	Olfr1393 mouse anchor; human comparator less secure.	TRPV4	Apical PIP_2 topology	Proximal tubular glucose transport	Transport-facing extension; Olfr1393 mouse anchor.
Podocyte slit diaphragm	CB1 podocyte-relevant arm	Ectopic olfactory GPCR arm provisional	TRPC6	Sterol organization	Barrier competence	Barrier-facing extension; ectopic OR GPCR provisional.
Distal nephron/ collecting-duct extension	Context-dependent cannabinoid GPCR arm	Ectopic olfactory GPCR arm exploratory	TRPV4	Mechanical load	Flow-sensitive Ca^{2+} organization	Exploratory extension; portability testing.

The expanded renal application sheet and confidence hierarchy are provided in Table S3.

hinge that can be locked before adjudication. In the juxtaglomerular apparatus, renin-facing Ca^{2+}, cAMP or granule-release behaviour provides a tractable output close to the macula densa–afferent arteriolar interface. In the proximal tubule, apical transport behaviour links membrane polarity, phosphoinositide support and glucose-handling physiology. In the podocyte slit diaphragm, barrier competence and local Ca^{2+} microdomains remain close to cholesterol-sensitive slit-diaphragm and TRPC6 biology. In the distal nephron and collecting system, flow-sensitive epithelial Ca^{2+} organization and transport adaptation provide a membrane-proximal route into mechanical and osmotic state. These examples preserve physiology while keeping the claim restricted: each territory nominates a testable membrane-conditioned output, not a validated higher-order assembly.

Parallel renal GPCR systems, including GPER1 and GPR48/LGR4, should remain comparator axes rather than hidden members of the nominated CB–OR–TRP assembly; renin-, calcium-, salt-, acid–base- or mineralocorticoid-sensitive outputs should therefore require gate satisfaction and lower-order exclusion before any MECS-BER interpretation [44, 45].

Discussion

Position relative to existing approaches

MECS-BER sits downstream of membrane-domain theory and upstream of broad phenotype interpretation. Lipid-raft models explain why specific proteins may co-segregate. Heteromer and allostery frameworks explain how receptor partners can alter one another's pharmacology. Macromolecular assembly concepts explain how receptors, effectors and scaffolds can form signal-processing units [13, 46–49]. The present method does not replace those frameworks. MECS may provide a shared conceptual vocabulary, whereas MECS-BER provides the operational decision sequence for testing that vocabulary under defined conditions. Physiologically, this two-layer framing redirects attention from receptor presence alone to the generation of local output within a defined membrane territory. Its connection to lipid chemistry and membrane biophysics lies in treating cholesterol accessibility, phosphoinositide topology, sphingolipid organization, acyl-chain state, oxidative stress and mechanical load as measurable constraints on signalling behaviour rather than passive background composition. In receptor pharmacology, this adds a restricted adjudicative step for deciding whether cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels remain lower-order or require higher-order interpretation under declared conditions. The value for renal physiology, nephrology and medicine is one of translational restraint: separating receptor detection from receptor competence, pharmacological response from local integration, and membrane injury from potentially rescuable signalling reprogramming. MECS-BER's role is operational. It requires one locked proximal outcome, one locked null, one editable membrane prior, explicit gate conditions and a rescue-sensitive adjudication sequence before higher-order interpretation is entertained. The method therefore shifts interpretation away from a static inventory question and asks how receptors are functionally combined under defined membrane conditions.

This positioning also clarifies how MECS-BER relates to EDPS. The epigenetic dimension of protein structure (EDPS) framework defines epigenetic protein structure as the set of structural determinants not fully encoded by amino acid sequence, including membrane solvation and context-dependent stabilization [50]. EDPS provides a single-protein grammar for how lipid composition, solvation, electrostatics and local physical state shape conformational and signalling competence. MECS extends that grammar to local multi-protein communication among cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels, and MECS-BER tests whether such communication remains explainable by lower-order structure or requires higher-order interpretation on one locked proximal outcome.

In this framework, the membrane prior is the operational bridge between EDPS and MECS-BER. It is a measurable, editable and non-sequence-encoded constraint that can reshape receptor conformation, nanoscale partitioning, channel permissiveness, ligand access, receptor competence, effector proximity and access to shared effectors within a local signalling territory. Relevant physical parameters include spatial co-residence, residence time, electrostatic orientation, hydration-dependent conformational change, lipid-chain mobility,

lateral diffusion and local entropy at the membrane surface [51]. Fine-scale receptor–lipid contacts, including CH–π contacts, dispersion forces and interfacial water-mediated hydrogen-bonding geometry, may further stabilize receptor–lipid and receptor–neighbour contacts within a given membrane state [51]. Sequence-encoded motifs and membrane-conditioned realization are therefore complementary rather than competing explanations. Cholesterol-recognition motifs such as CARC and CRAC provide permissive cholesterol-interaction sites within membrane proteins [4], but their functional expression depends on cholesterol availability, leaflet organization, domain structure, electrostatic field, hydrogen-bonding geometry and aromatic lipid interactions. This is why membrane-conditioned inference cannot be reduced to sequence-derived structure prediction alone; EDPS analysis has argued that AlphaFold does not natively model the membrane-state variables that can shape stabilization, accessibility and functional expression of sequence-permitted conformations in cells [52].

This layered positioning adds inferential discipline relative to co-expression mapping, generic synergy language, pairwise crosstalk narratives and receptor pharmacology. Co-expression alone does not identify the relevant null. Apparent non-additivity alone does not show irreducible higher-order structure. Pairwise interaction alone does not establish whether a state-dependent third-order requirement emerges after a defined membrane-prior edit. Receptor pharmacology explains ligand, receptor, coupling, trafficking and exposure-history effects, but does not by itself determine whether a local output remains receptor-centred, pairwise-coupled or membrane-state-dependent within a nominated multi-arm assembly.

A further feature of MECS-BER is evidentiary restraint. The method does not assume that every receptor-rich microdomain forms a candidate assembly or that every nonlinear response reflects irreducible higher-order structure. Its available classes remain lower-order sufficiency, pairwise-coupled lower-order structure, higher-order requirement and nomination below adjudicative maturity. Shared downstream output does not by itself imply crosstalk; within MECS-BER, crosstalk is inferred only when non-independence on the locked proximal outcome exceeds the prespecified lower-order explanation.

An additional value of MECS-BER is downgrade resolution. When a nominated case fails the declared gates, the result should not be treated as an uninformative failure; it can assign the observation to receptor inventory, single-arm physiology, pairwise coupling, distal consequence biology, toxicity, species mismatch, loss of arm competence or collapse of proximal-outcome dynamic range. In this way, the framework separates phenotype movement from mechanism and preserves negative or partial results as interpretable outcomes.

The same discipline may inform membrane-active pharmacology. Broad amphiphilicity, lysosomal accumulation, global calcium collapse, mitochondrial injury and nonspecific membrane disruption are exclusion signals rather than evidence of MECS-BER specificity. A membrane-active compound becomes relevant only when its localization produces a bounded, territorial and receptor-arm-dependent change in one declared locked proximal outcome under the prespecified design. Nonspecific membrane injury, unbounded overshoot or global calcium dysregulation indicate failure of membrane-prior control.

Although developed here in a renal context, the logic is not intrinsically kidney-specific. It can be extended in principle to other GPCR classes, TRP channels and membrane signalling systems where local co-residence, shared machinery and membrane-sensitive outputs support formal testing. Adjacent literatures sharpen this boundary: odour coding across mammalian receptor repertoires is combinatorial rather than one-receptor-one-ligand; membrane protein behaviour is conditioned by the local lipid nano-environment; and membrane-associated lipid enzymology can depend on bilayer context [1, 2, 5, 19, 53, 54]. Ganglioside-rich lipid rafts, receptor-focused sphingolipid and cholesterol biology, and independent membrane-domain evidence further support the premise that local lipid state can reweight receptor competence and channel permissiveness before any higher-order claim is made [19–21, 48]. Within MECS, terpenoid-derived lipids may contribute to the physical organization of signalling membranes, whereas terpenoid or cannabinoid/meroterpenoid ligands may contribute to the local chemical field interpreted by chemosensory receptor systems.

Literature basis for the MECS-BER proposal

The present article is methodological rather than experimental. Its biological rationale therefore rests on a staged synthesis of published data. Published evidence supports MECS-BER in three linked layers. First, membrane-state studies show that phosphoinositides, sterols, sphingolipids, gangliosides, acyl-chain composition, oxidative stress, trafficking state and mechanical load can alter receptor conformation, nanoscale proximity, channel permissiveness and effector access. This evidence justifies the membrane prior as a measurable pre-existing state variable, but does not identify a three-arm assembly [1, 2, 5, 14–21].

Second, component competence is asymmetrical. Cannabinoid GPCR responses are conditioned by receptor subtype, ligand field, coupling, trafficking and local endocannabinoid metabolism, including FAAH-linked control of anandamide persistence [3, 6–10, 17]. TRP channels provide proximal calcium-, mechanics- and transport-facing outputs responsive to local lipid and mechanical conditions [1, 2, 5, 14–19]. Ectopic olfactory-GPCR evidence is more restrictive: renal receptor and olfactory-transduction examples support cautious nomination in physiology-facing territories, whereas related metabolite-context studies remain auxiliary; surface competence and shared-output participation still require direct testing [22–29]. Tables S4 and S5 retain the anchored ligand inventory and background renal olfactory-receptor candidate space.

Third, the juxtaglomerular apparatus provides the strongest prospective renal deployment space. Renin-facing physiology can be placed close to a declared membrane territory, and published studies support nomination of renal olfactory, cannabinoid and TRPV4-centred arms around tractable proximal outcomes [3, 22–29, 38–43]. Pairwise and membrane-domain literatures further justify asking whether nominated arms influence one local output through lipid-dependent partitioning, shared effectors, allosteric coupling or access to local second messengers [13, 19–21, 46–49, 53, 54]. These literatures define testable lower-order alternatives; they do not establish irreducible higher-order integration. Accordingly, the published evidence supports nomination, gate design and assay selection, not biological adjudication. It does not provide a same-cell, ligand-competent cannabinoid–olfactory–TRP assembly tested on one locked proximal outcome across baseline, membrane-prior edit and rescue. MECS-BER converts this bounded plausibility into a prospective, falsifiable test without treating receptor co-residence or shared physiology as proof of a validated three-arm assembly.

Table 4 Common inference shortcuts restricted by MECS-BER.

Inference shortcut	Why it is insufficient	MECS-BER requirement
Co-expression equals crosstalk	Same tissue or same segment detection does not establish functional dependence on one output.	Define one micro-niche, one locked proximal outcome and explicit eligibility and competence gates.
Non-additivity equals higher-order integration	Non-additive behaviour can arise from pairwise interactions, assay structure, saturation or nonspecific stress.	Test κ under the locked pairwise-only null and interpret it against a prespecified assay-specific bound.
Distal phenotype equals local mechanism	Blood pressure, glucosuria, albuminuria, survival or global injury readouts are multistep consequence spaces.	Use a proximal physiological hinge close to the nominated membrane territory.
Membrane activity equals MECS specificity	Broad amphiphilicity, lipid disruption, organelle stress or calcium collapse can mimic biological effects.	Require a bounded, territorial, receptor-arm-dependent membrane-prior edit.
Rescue equals proof	Apparent recovery may reflect nonspecific compensation or unrelated pathway correction.	Show return toward the earlier class on the same locked proximal outcome after rescue or counter-editing.
OR transcript detection equals an olfactory signalling arm	RNA-level expression supports nomination, but it does not prove protein abundance, surface localization, ligand competence, functional coupling or equivalent receptor availability across membrane states.	Grade the ectopic olfactory GPCR arm by evidence tier. Where possible, require orthogonal protein-level, surface-localization or live functional evidence before κ is interpreted biologically.
Pairwise crosstalk equals higher-order integration	A strong two-arm interaction can remain fully explainable without a residual third-order term.	Treat pairwise-coupled lower-order structure as a valid endpoint unless κ supports a higher-order requirement.
Pathology equals membrane-prior validation	Disease state can alter membranes but does not identify the relevant editable membrane prior by itself.	Declare, measure and edit the specific membrane-state variable used for adjudication.
Nominated CB–OR–TRP assembly equals the full renin-control system	Renin release is regulated by additional macula densa and juxtaglomerular inputs, including succinate–GPR91/SUCNR1 signalling, prostaglandin-linked pathways, nitric-oxide signalling and calcium-dependent mechanisms.	Treat parallel renin-control systems as contextual constraints or comparator axes, not as hidden members of the nominated assembly. A CB–OR–TRP claim should remain restricted to the locked proximal outcome and declared perturbation design.

Entries identify common interpretive shortcuts that MECS-BER is designed to restrict. The table does not add a new evidentiary claim. It summarises the stopping rules required before nomination can progress to adjudication.

Table 4 translates this evidentiary logic into practical stopping rules for biological readers. It does not deny that co-expression, non-additivity, pathology, rescue or transcript detection may be informative; it restricts what each can support before formal adjudication. A receptor map can nominate a territory but cannot prove crosstalk, a nonlinear response can justify testing but cannot prove higher-order integration, a distal phenotype can define consequence space but cannot replace a locked proximal outcome, and rescue supports state dependence only when the same locked proximal outcome returns towards the earlier class.

Current evidential status and limits

At present, MECS-BER is supported at the level of formal adjudicative behaviour and currently available literature-grounded biological nomination. The matrices and simulations establish the behaviour and tolerance sensitivity of the prespecified architecture, whereas the published component literatures identify testable membrane territories without establishing a same-cell, ligand-competent CB–OR–TRP assembly or biological interpretation of κ on one locked proximal outcome. Prospective biological execution therefore depends on stringent constraints: preservation of membrane-territory identity during measurement; functional confirmation of the ectopic olfactory GPCR arm at the relevant surface; selective membrane-prior editing and credible rescue without toxicity, calcium collapse or loss of outcome dynamic range; and complete BER acquisition with replication, integrity controls and a declared κ bound. Until these requirements are met, MECS-BER remains a prespecified route to biological adjudication rather than a completed biological demonstration. A compliant positive result would support one territory-specific MECS-type architecture, whereas a negative or gate-failing result would be equally informative by retaining the tested case within lower-order, nonspecific or nomination-level explanation. A particular limitation concerns the ectopic olfactory-GPCR arm. In kidney and other non-olfactory organs, current OR evidence often supports cautious nomination rather than definitive receptor assignment. Transcript detection and selected physiology-facing studies can identify plausible candidates, but they rarely establish receptor-specific surface localisation, ligand competence or participation in the same locked proximal output. ORx therefore marks an unresolved but testable arm that requires direct experimental resolution before κ-based biological interpretation is admissible.

Recommended use cases

The method is best suited to membrane-dense territories in which one editable membrane prior, one locked proximal outcome and one biologically coherent territory can be locked before testing begins. It is most informative when outputs remain close to membrane organization, such as calcium microdomains, cyclic-nucleotide tone, renin release, barrier-state behaviour or transport switching. It is less suitable for questions that are primarily cataloguing-based, descriptive or dominated by distal systems-level phenotypes.

MECS may offer a disciplined conceptual vocabulary, whereas MECS-BER supplies the prespecified route to testing. The benchmark studies show that the locked-null arithmetic behaves as intended, and that classification remains interpretable when variance and tolerance are handled prospectively. In practical pharmacological terms, a

candidate case requires a defined biological territory in which a cannabinoid GPCR arm, an ectopic olfactory GPCR arm and a TRP-channel arm plausibly converge on the same locked proximal outcome; a membrane-localising compound is relevant only when it performs an interpretable edit of a defined membrane state while preserving bounded receptor-arm dependence on that outcome.

Membrane-state correction provides the pharmacological form of this logic. It denotes deliberate adjustment of an editable membrane prior so that candidate CB–OR–TRP communication is tested within a defined lipid, mechanical, inflammatory or electrostatic field. In MECS-BER, such correction is not a general claim of membrane repair, cytoprotection or therapeutic benefit. The prior must be declared, edited, measured and, where feasible, rescued or counter-edited; broad amphiphilicity, lysosomal accumulation, organelle stress, global calcium collapse, mitochondrial injury and nonspecific membrane disruption should be treated as exclusion signals rather than MECS-BER engagement.

The framework is centred on cannabinoid GPCRs, ectopic olfactory GPCRs and TRP channels, but its underlying logic may extend to other GPCR–ion-channel territories where membrane state, local co-residence and shared locked proximal outcomes are likely to influence signalling behaviour. Renal portability examples include distal-nephron calcium handling involving PTH1R, CaSR and TRPV5; macula densa–juxtaglomerular renin-facing cases involving GPR91/SUCNR1 and adenosine- or prostaglandin-linked GPCR comparators; and collecting-duct cases involving AVPR2, purinergic or prostaglandin-linked GPCRs, and ENaC- or AQP2-proximal insertion or transport behaviour. These examples remain portability cases only. They preserve territorial definition, analysis of the locked proximal outcome and non-universal interpretation.

If prospectively demonstrated in a compliant biological model, a nominated MECS-BER case would remain a bounded finding rather than a universal claim. Its significance would be to instantiate one MECS-type signalling architecture in a defined membrane territory and to show that an editable membrane prior can influence whether neighbouring receptor and channel arms remain lower-order or express a rescue-sensitive higher-order requirement on one locked proximal outcome. This would add a possible membrane-state dimension to GPCR pharmacology without replacing receptor-centred analysis through ligand binding, efficacy, coupling preference, trafficking and biased signalling.

Conclusion

Receptor co-expression mapping can nominate a candidate assembly, but it cannot determine whether the nominated arms act independently, interact pairwise or form a membrane-conditioned higher-order regime on one locked proximal outcome. This limitation defines the inferential gap addressed here: pathway maps can identify plausible components, but they do not determine whether a local output is produced by independent arms, pairwise crosstalk or a membrane-state-dependent higher-order requirement. MECS-BER with a classification discipline addresses that gap as a constrained framework for testing combined receptor–channel contribution under a locked proximal outcome, locked null and BER sequence. Operationally, MECS-BER adds an adjudicative layer at the point where receptor co-expression, shared downstream biology and pairwise crosstalk can otherwise be overextended into broader architectural claims.

The framework locks the candidate assembly, null model, locked proximal outcome, membrane-prior axis and BER sequence before interpretation begins. In doing so, it provides a route from nomination towards higher-order inference only when the required territorial, functional and reversibility conditions are met.

This article establishes the formal behaviour of a methodological framework, not its completed biological execution. The benchmark and simulation panels verify classification behaviour under prespecified formal conditions, and the renal material defines where a membrane-conditioned test could be implemented. Whether membrane state reorganizes local receptor communication therefore remains an experimentally testable question for prospective biological deployment. A negative MECS-BER result would remain informative by assigning the tested case to co-expression, single-arm activity, pairwise coupling, nonspecific membrane perturbation or distal phenotype biology, rather than to MECS-type integration.

Membrane-active pharmacology would be most interpretable when an intervention corrects or counter-edits a declared membrane prior in a territorial, bounded and receptor-arm-dependent manner, and when its effect remains measurable on one locked proximal outcome. This preserves membrane state as a biological determinant of receptor communication without treating every membrane effect as evidence of integration. The conceptual value of MECS is to link membrane lipid state, local ligand fields and receptor–channel communication as one membrane-conditioned signalling problem; the operational value of MECS-BER is to test that problem by separating independent arm activity, pairwise crosstalk, nonspecific membrane perturbation and reversible membrane-state-dependent higher-order integration.

Lipids can reshape the vocabulary of single receptors. They can also reshape the conversation among receptors by regulating co-localization, access to shared machinery and the conditions under which co-expression becomes functional crosstalk.

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Author contributions

Erhan Yarar (Conceptualization [equal], Data curation [equal], Investigation [equal], Methodology [equal], Project administration [equal], Validation [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal])

Supplementary material

Supplementary material is available at *Biology Methods and Protocols* online.

Conflicts of interest

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Data availability

No new wet-lab, animal or human datasets were generated in this study, and no fully compliant biological MECS-BER dataset is presented. Deterministic formal benchmark matrices, simulation-based stability templates, the eligibility and competence checklist, the minimum biological-dataset specification, renal deployment sheets and the reference implementation logic are provided in the Supplementary Material.

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