

Backbone Extraction Reveals Structural Organization in Multicellular Functional Networks

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Abstract: Functional connectivity and network analysis provide a widely used framework for quantifying interactions in complex systems, including multicellular tissues whose coordinated activity supports normal physiological function. In this study, we construct functional networks of pancreatic β -cells within islets of Langerhans using multicellular calcium imaging data. A correlation matrix derived from experimentally recorded signals captures pairwise interactions between cells. Our primary objective is to extract functional connectivity patterns using a metric backbone approach and compare it with conventional threshold-based network construction. The backbone identifies structurally relevant connections by removing redundant links while preserving shortest-path relationships. Results obtained across six independent pancreatic islets show that both approaches yield comparable average degree and clustering, whereas backbone networks show higher efficiency, a larger connected component, and more homogeneous degree distributions, reflected by lower degree Gini coefficients and hubness values. Modular structure is nevertheless largely preserved, indicating that backbone extraction reduces redundancy without strongly distorting the underlying functional architecture. Overall, our findings support backbone extraction as a data-driven framework for constructing functional cellular networks while avoiding limitations associated with arbitrary threshold selection. This approach may improve the quantitative analysis of multicellular dynamics and be broadly applicable to other biological systems exhibiting complex interaction patterns.

Key-Words: Functional connectivity, Thresholding, Backbone extraction, Network analysis, Multicellular calcium imaging, Collective cellular dynamics, Pancreatic β -cells

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1 Introduction

Functional connectivity analysis has emerged as a powerful framework for quantifying interactions in complex systems, particularly when the underlying structural connectivity is unknown, inaccessible, or dynamically evolving. By inferring relationships from statistical dependencies between observed signals, it enables the reconstruction of interaction patterns directly from data, thereby providing access to the system's collective dynamics. Importantly, these emergent interaction patterns are not merely descriptive, but often play a decisive role in shaping the overall function of the system. This data-driven and model-agnostic nature makes functional connectivity especially suitable for studying systems composed of many interacting units, where system-level behavior arises from coordinated activity across multiple components.

The generality of this approach has enabled its successful application across a wide range of domains. In

ecology, it has been used to elucidate species interactions [1], quantify co-occurrence patterns within soil microbial communities [2], and more broadly to study the structure and dynamics of complex interaction networks underlying collective behavior [3]. In engineering and technological systems, functional connectivity analysis has been applied to electrical consumption patterns [4], transportation networks [5], and human mobility dynamics [6]. In biomedicine, particularly significant advances have been achieved in neuroscience, where functional network analysis has matured into a clinically relevant tool [7], [8]. Within the field of network neuroscience, interactions between brain regions are inferred from modalities such as fMRI, EEG, and DTI, enabling the construction of functional brain networks based on synchronization patterns and statistical interdependencies [9], [10]. These networks provide key insights into cognitive processes, sensory perception, and disease-related alterations in brain organization [11], [12],

[13].

More recently, the concept of functional connectivity has been extended to the tissue level, where it enables the characterization of coordinated activity in multicellular systems [14], [15], [16], [17]. In this context, biological tissues can be viewed as complex dynamical systems in which individual cells act as coupled nonlinear units whose interactions give rise to emergent collective behavior. Rather than operating independently, cells form coordinated ensembles, where synchronization and dynamical coupling across the population are essential for physiological function. Such coordinated activity underlies processes ranging from signal propagation to homeostatic regulation and adaptive responses to external stimuli [18]. A representative example is provided by the pancreatic islets of Langerhans, where β -cells are electrically and metabolically coupled and exhibit collective oscillatory dynamics that govern pulsatile insulin secretion in response to glucose [19], [20]. The functionality of this system critically depends on the degree of synchronization and the structure of intercellular interactions [14], [21], [22], [23].

Owing to the inherently distributed, heterogeneous, and dynamically evolving nature of these interactions, increasing attention has been directed toward analyzing functional connectivity patterns among pancreatic β -cells, often derived from multicellular calcium imaging data (for a review, see [14]). In such systems, interactions are transient and context-dependent, requiring analytical frameworks capable of capturing time-resolved coordination patterns. Network-based analyses have revealed that β -cell populations exhibit nontrivial organization, characterized by heterogeneous connectivity, small-world topology, and modular structure, rather than behaving as simple gap-junctional syncytia. They have also enabled the identification of specialized subpopulations—such as first responder cells, wave initiators, and hub cells—that play distinct roles in coordinating collective activity [21], [22], [24], [25]. Moreover, these approaches have provided insight into how intercellular interactions are reorganized in disease, highlighting alterations in synchronization and network structure associated with metabolic dysfunction [24], [26], [27].

Different approaches can be employed to construct functional networks from empirical data, each with its own advantages and limitations depending on the specific context and research question [25]. In particular, commonly used methods such as thresholding or fixed-density approaches offer simplicity and interpretability, but may rely on parameter choices and can obscure aspects of the underlying interaction structure. More generally, network sparsification aims to reduce the complexity of dense weighted

graphs while retaining the connections most relevant to the structural or dynamical properties under investigation. Existing strategies include global or fixed-density thresholding, null-model-based multiscale filtering, and backbone approaches based on structural or shortest-path criteria, which have been applied across transportation, social, biological, and brain networks [28], [29]. This issue is particularly relevant in functional-connectivity analyses, where graph-theoretical estimates may vary substantially with the selected thresholding strategy [30], [31].

In this study, we focus on metric backbone extraction as a complementary strategy that operates on the preprocessed weighted correlation network and retains direct connections that are not superseded by shorter indirect paths. While functional network approaches have been extensively employed to study pancreatic β -cell populations in previous studies [21], [22], [24], [25], the present work specifically evaluates whether a backbone-based representation can provide a sparse, shortest-path-preserving alternative to conventional threshold-based network construction. By introducing and assessing this network-sparsification framework in the context of β -cell functional connectivity, we aim to extend existing network-based analyses and explore its potential for highlighting organizational principles of multicellular activity.

2 Problem Formulation

We consider a data-driven framework for constructing functional networks from multicellular calcium imaging data (Fig. 1). Experimentally recorded signals are first extracted at the level of individual cells and preprocessed, after which pairwise statistical dependencies are quantified to obtain a correlation matrix. This matrix serves as the basis for functional network construction using both a conventional threshold-based approach and a backbone extraction procedure that identifies structurally essential connections. The resulting networks are then characterized using graph-theoretical measures to assess their organization and functional relevance.

2.1 Experimental data and signal preprocessing

We analyze multicellular calcium activity recorded from acute pancreatic tissue slices containing islets of Langerhans obtained from mice. The tissue was stimulated by a step increase in glucose concentration from a substimulatory level (6 mM) to a stimulatory level (10 mM), thereby inducing complex and coordinated activity across the β -cell population. Experiments were performed in accordance with established protocols [21] and were approved by The Administration of the Republic of Slovenia for Food

Safety, Veterinary and Plant Protection (permit numbers: U34401-35/2018-2).

Calcium dynamics were monitored using confocal fluorescence microscopy, enabling high-resolution recording of intracellular calcium signals across large populations of cells. From the acquired image sequences, regions of interest (ROIs) corresponding to individual β -cells were manually or semi-automatically identified, and the associated fluorescence intensity signals were extracted, yielding a set of time series $\{x_i(t)\}$, where $i = 1, \dots, N$ indexes individual cells.

The extracted signals were first preprocessed to remove high-frequency noise and slow baseline drifts. Specifically, a band-pass filtering procedure was applied to each time series, followed by smoothing using an adjacent averaging procedure. The resulting signals $\tilde{x}_i(t)$ represent the filtered and denoised calcium activity used in subsequent analysis.

Functional interactions between cells were quantified by computing pairwise Pearson correlation coefficients between all pairs of preprocessed signals. For a pair of cells (i, j) , the correlation coefficient is defined as

$$r_{ij} = \frac{\sum_{t=1}^T (\tilde{x}_i(t) - \mu_i)(\tilde{x}_j(t) - \mu_j)}{\sqrt{\sum_{t=1}^T (\tilde{x}_i(t) - \mu_i)^2} \sqrt{\sum_{t=1}^T (\tilde{x}_j(t) - \mu_j)^2}}, \quad (1)$$

where μ_i and μ_j denote the temporal means of the respective signals, and T is the number of time points. The resulting correlation matrix

$$R = \{r_{ij}\}, \quad i, j = 1, \dots, N, \quad (2)$$

provides a symmetric measure of pairwise statistical dependencies between cellular activity profiles. This matrix serves as the fundamental input for the construction of functional networks, as described in the following subsection.

2.2 Network construction

The correlation matrix $R = \{r_{ij}\}$ is used as the basis for constructing functional networks, where nodes represent individual cells and edges encode statistical dependencies between their activity profiles.

2.2.1 Classical threshold-based functional network

A standard approach to network construction consists of thresholding the similarity matrix in order to retain only the strongest connections. In this work, we adopt a data-driven thresholding procedure in which the threshold is chosen such that the resulting network attains a predefined average node degree [25].

Let $S = \{s_{ij}\}$ denote the non-negative similarity matrix obtained from correlations as

$$s_{ij} = \max(r_{ij}, 0), \quad (3)$$

where negative correlations are discarded. A binary adjacency matrix $A^{(1)} = \{a_{ij}^{(1)}\}$ is then defined as

$$a_{ij}^{(1)} = \begin{cases} 1, & s_{ij} \geq \theta, \\ 0, & \text{otherwise,} \end{cases} \quad (4)$$

where θ is a threshold selected such that the average degree

$$\langle k \rangle = \frac{1}{N} \sum_{i=1}^N \sum_{j=1}^N a_{ij}^{(1)} \quad (5)$$

matches a prescribed target value. In our analysis, the threshold was selected to yield networks with an average degree between 6.5 and 7.0.

The resulting weighted network is described by

$$w_{ij}^{(1)} = s_{ij} a_{ij}^{(1)}, \quad (6)$$

which retains only the strongest pairwise interactions. While this approach provides control over network density and facilitates comparisons across datasets, it remains inherently dependent on the choice of threshold and does not account for redundancy arising from indirect interactions within the network.

2.2.2 Backbone extraction via metric filtering

As an alternative, we consider a backbone-extraction approach based on the metric backbone of the network. The key idea is to identify and retain only those edges that are essential for preserving shortest-path structure, while removing edges that are redundant due to indirect connections [28], [29]. As a preprocessing step, we constructed a sparse weighted network using a k -nearest-neighbours (k -NN) scheme. The value of k was selected separately for each dataset within the range $k = 10$ –15, so as to obtain connected initial graphs while yielding metric backbones with a comparable average degree of approximately 7. Thus, the choice of k represents an initial sparsification parameter rather than an intrinsic component of the metric backbone procedure. Once the initial graph is defined, metric backbone extraction is performed without introducing an additional edge-weight threshold or prescribing a target backbone density.

For each node i , the similarity weights w_{ij} to all other nodes $j \neq i$ were ranked in descending order. Let $\mathcal{N}_k(i)$ denote the set of the k nodes associated with the largest similarity weights w_{ij} , i.e., the k strongest functional neighbours of node i . This procedure yields a directed k -nearest-neighbours adjacency matrix $A^{\text{knn}} = a_{ij}^{\text{knn}}$, defined as

$$a_{ij}^{\text{knn}} = \begin{cases} 1, & \text{if } j \in \mathcal{N}_k(i), \\ 0, & \text{otherwise,} \end{cases} \quad i \neq j. \quad (7)$$

Because neighbour selection is performed independently for each node, the resulting k -NN graph is generally directed. The graph was subsequently symmetrized using a mutual-neighbour criterion. Specifically, an undirected edge between nodes i and j was retained only if both nodes selected each other among their respective k nearest neighbours, such that

$$a_{ij,\text{sym}}^{\text{knn}} = \begin{cases} 1, & \text{if } a_{ij}^{\text{knn}} = 1 \text{ and } a_{ji}^{\text{knn}} = 1, \\ 0, & \text{otherwise.} \end{cases} \quad (8)$$

This procedure ensures that only mutually supported connections are preserved in the resulting undirected graph.

The weighted k -NN network is then transformed into a distance representation by defining

$$d_{ij} = \frac{1}{w_{ij}} - 1, \quad (9)$$

for all existing edges, where w_{ij} denotes the corresponding similarity weight. Nonexistent edges are assigned infinite distance. This transformation maps strong similarities to short distances and weak similarities to larger distances, enabling the application of shortest-path-based backbone extraction methods. Other monotonic transformations, such as logarithmic mappings or alternative reciprocal forms, could also be employed to convert similarity weights into distances. While different transformations may affect the quantitative details of the resulting backbone, the selected transformation is widely used within the distance-backbone framework and was therefore adopted throughout this study.

The shortest-path distance matrix $D^{\text{sp}} = \{d_{ij}^{\text{sp}}\}$ is computed over this weighted graph using standard graph-theoretic algorithms. An edge (i, j) is retained in the backbone if and only if it satisfies

$$d_{ij} = d_{ij}^{\text{sp}}, \quad (10)$$

i.e., if the direct connection is not superseded by a shorter indirect path.

The resulting adjacency matrix of the backbone network is thus given by

$$a_{ij}^{(2)} = \begin{cases} 1, & d_{ij} = d_{ij}^{\text{sp}}, \\ 0, & \text{otherwise,} \end{cases} \quad (11)$$

and the corresponding weighted network is

$$w_{ij}^{(2)} = w_{ij} a_{ij}^{(2)}. \quad (12)$$

This procedure yields a sparse subnetwork that preserves the shortest-path distance structure of the original graph while systematically removing edges that do not contribute to shortest-path connectivity.

2.3 Functional network analysis

To characterize the structural and functional organization of the inferred networks, we evaluated a set of standard graph-theoretical measures, focusing on their interpretation in the context of multicellular calcium dynamics [14], [32]. Node degree and its distribution quantify the number and distribution of functional connections across cells, thereby providing a first description of connectivity heterogeneity and the presence of highly connected cells. To further characterize degree heterogeneity and hub dominance, we additionally calculated the degree Gini coefficient and hubness, which quantify the unevenness of the degree distribution and the prominence of hub-like connectivity patterns, respectively. Such highly connected cells may exert a disproportionate influence on collective dynamics. Because the analyzed networks had comparable overall degree levels, these measures primarily reflect differences in their internal connectivity organization rather than gross differences in network density.

The clustering coefficient measures the tendency of nodes to form locally interconnected groups, thereby capturing the extent of local synchronization and the presence of spatially confined functional modules. In parallel, global efficiency quantifies how efficiently information can propagate across the network, reflecting the balance between local and long-range coordination. Small-worldness combines these two aspects by assessing the coexistence of high clustering and short path lengths, a property often associated with efficient yet robust communication in biological networks. The relative size of the largest connected component provides a measure of network integration, indicating the fraction of cells that participate in a globally connected structure.

To further assess mesoscale organization, we evaluated community structure using modularity, which identifies groups of cells with stronger internal connectivity and weaker external coupling. Such communities are typically associated with coordinated subpopulations and may reflect functional specialization within the islet.

At the level of individual nodes, closeness centrality was used to quantify how centrally a cell is positioned within the network, providing a proxy for its ability to rapidly interact with other cells. Finally, the spatial length of functional connections was analyzed by combining network topology with physical cell coordinates, allowing us to distinguish between predominantly local interactions and long-range func-

tional coupling.

3 Problem Solution

The workflow of the network-construction procedure, together with representative thresholded and backbone networks, is shown in Fig. 1.

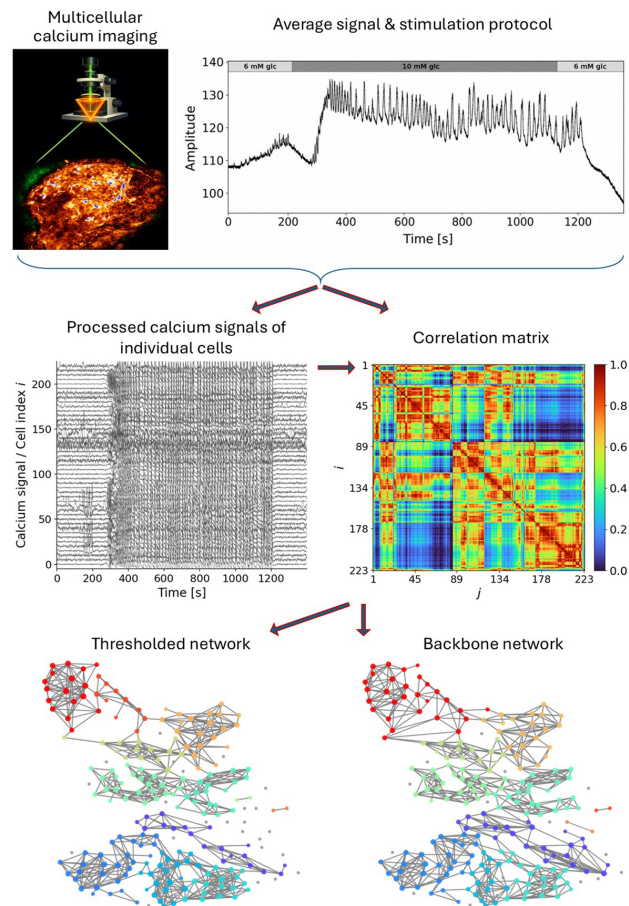


Fig. 1: Workflow for constructing functional networks from multicellular calcium imaging data of pancreatic β -cells, together with representative examples of the resulting threshold-based and metric backbone networks. Starting from multicellular calcium recordings, time series of individual cells are extracted and preprocessed to remove trends and noise. Pairwise statistical dependencies between cellular signals are then quantified to obtain a correlation matrix, which serves as the basis for subsequent network construction. The final step illustrates the two alternative network-construction strategies considered in this study: a conventional threshold-based network and a metric backbone network. Source: created by the authors.

For a consistent comparison, the two network types were constructed to exhibit comparable overall connectivity, with an average node degree of approx-

imately $\langle k \rangle \approx 6.5$. At first glance, both networks exhibit similar global organization, characterized by a relatively segregated structure and the presence of distinct communities, highlighted by node coloring. Despite these similarities, closer inspection reveals differences in local edge density, indicating that the two construction methods capture connectivity patterns with varying sparsity. Moreover, the backbone network contains substantially fewer isolated nodes and a larger connected component, despite a reduction in the total number of edges relative to the underlying k -NN graph, resulting in a more integrated structure.

To further characterize the differences between the two network-construction approaches, Fig. 2 presents node-level network properties for the same representative islet shown in Fig. 1. The node degree distribution (Fig. 2(a)) is more heterogeneous in the threshold-based network, exhibiting a broader spread and a longer tail, indicative of the presence of highly connected hub cells. In particular, some nodes exceed more than twice the average degree, whereas in the backbone network the distribution is more homogeneous, with the most connected nodes remaining below this level. This observation indicates a less pronounced dominance of hub-like nodes in the backbone network. In contrast, the distributions of clustering coefficients (Fig. 2(b)) are largely similar between the two networks, suggesting that local cohesiveness and the tendency to form clustered substructures are preserved under backbone extraction. The distribution of closeness centrality (Fig. 2(c)) is shifted toward higher values in the backbone network, indicating that nodes are, on average, more centrally positioned. This reflects the increased global integration of the backbone network, where a larger fraction of nodes participates in the connected component. Functional connection lengths (Fig. 2(d)) show comparable distributions for both approaches, with a pronounced bias toward short-range connections, consistent with the spatially localized nature of intercellular coupling in pancreatic islets. Finally, the relationship between node degrees in the two networks (Fig. 2(e)) reveals a strong positive correlation (Spearman $\rho = 0.824$), indicating that relative node importance is largely preserved despite differences in absolute degree values. This suggests that backbone extraction primarily reduces redundant connections while maintaining the fundamental organizational hierarchy of the network.

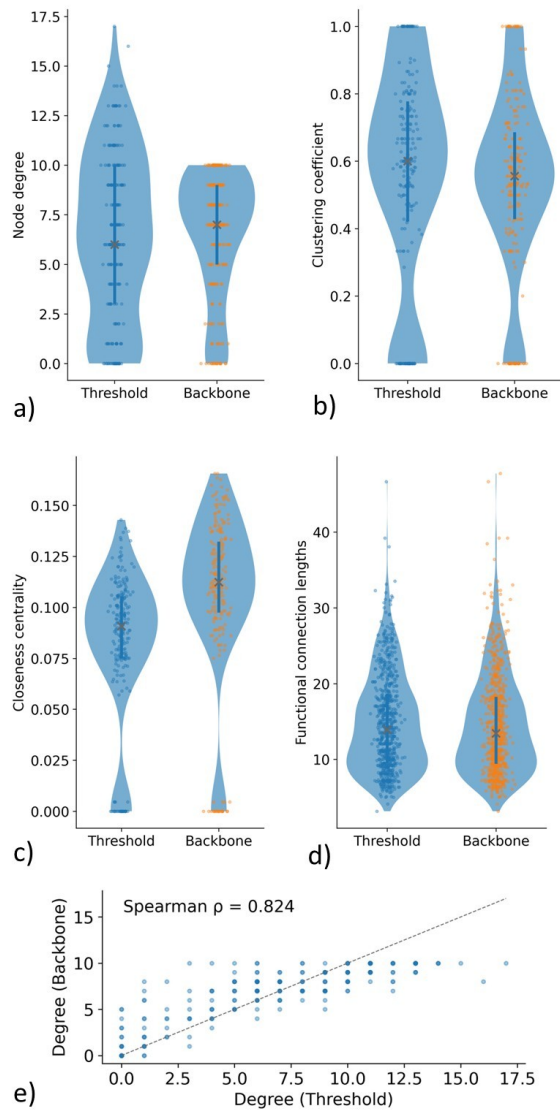


Fig. 2: Comparison of node-level network properties between the classical threshold-based network and the metric backbone network. Results are shown for a representative islet. (a) Node degree distribution, (b) clustering coefficient, (c) closeness centrality, and (d) functional connection lengths. In all panels, distributions are shown for both network construction methods. (e) Scatter plot of node degree in the threshold-based versus backbone network, with Spearman rank correlation coefficient indicated. Source: created by the authors.

To evaluate whether the differences observed in the representative example were reproducible across preparations, we extended the analysis to six independent islets subjected to the same protocol. The comparative results are summarized in Fig. 3. Although the absolute values of individual network measures varied somewhat between islets, the overall differences between the two construction approaches

were consistent. Compared with threshold-based networks, metric backbone networks exhibited significantly higher global efficiency, small-worldness, and relative size of the largest connected component.

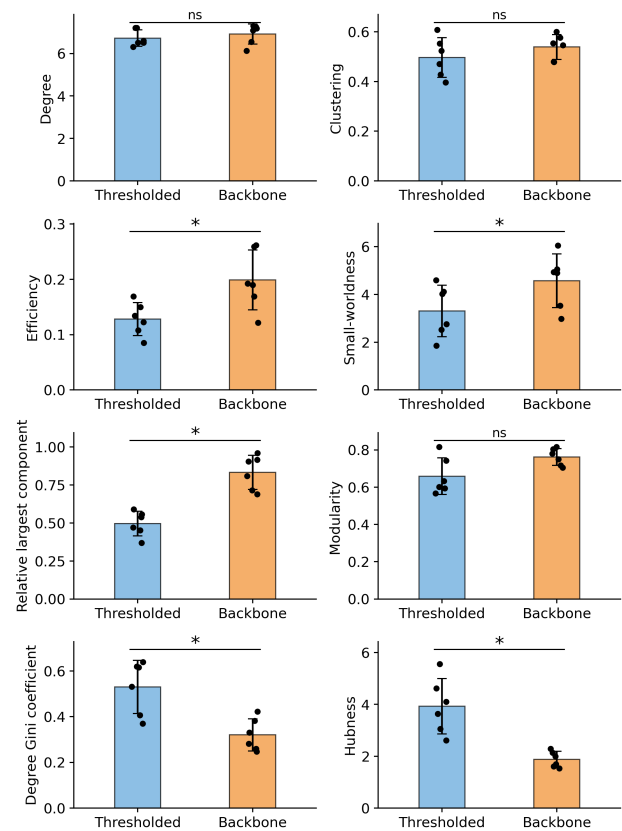


Fig. 3: Comparison of global network metrics between threshold-based (blue) and metric backbone (orange) networks across six independent islets. Metrics include mean node degree, clustering coefficient, global efficiency, small-worldness, relative size of the largest connected component, modularity, degree Gini coefficient, and hubness. Bars represent mean values and error bars denote standard deviations. Individual points correspond to measurements obtained from each islet. Statistical significance was assessed using paired Wilcoxon signed-rank tests; significant differences ($p < 0.05$) are indicated by an asterisk, whereas non-significant comparisons are denoted by "ns". Source: created by the authors.

The increases in efficiency and small-worldness were consistent with the more extensive connected component and the correspondingly smaller proportion of isolated nodes. In contrast, backbone networks showed lower degree Gini coefficients and hubness values, indicating a more homogeneous distribution of functional connectivity and a less pronounced hub structure, in agreement with the representative degree distribution shown in Fig. 2(a). Mean node degree,

clustering coefficient, and modularity did not differ significantly between the two approaches. Thus, metric backbone extraction produced more integrated network representations while maintaining comparable overall connectivity, local clustering, and modular organization.

In sum, our results indicate that the metric backbone approach preserves the fundamental organizational features of functional β -cell networks while reducing redundant connections. Despite substantial sparsification, key properties such as average degree, clustering, and modular organization remained largely preserved. At the same time, backbone networks were more integrated and less heterogeneous, exhibiting higher efficiency, greater small-worldness, and a larger connected component together with reduced hub dominance. These findings suggest that backbone extraction may provide a useful alternative to conventional threshold-based network construction while avoiding reliance on arbitrary threshold selection.

4 Conclusion

In this study, we demonstrated the application of metric backbone extraction for constructing functional networks in pancreatic β -cell collectives. To our knowledge, this represents the first application of metric backbone extraction to functional networks inferred from multicellular calcium imaging data. Compared with conventional threshold-based approaches, metric backbone extraction provides a principled reduction of the preprocessed weighted network while preserving its shortest-path structure. Across all six analysed islets, the main findings were consistent: backbone networks remained connected and exhibited a more integrated organization than their thresholded counterparts, while preserving the principal features of the underlying network architecture, including modular organization and the presence of highly connected cells. At the same time, the backbone construction led to a modest reduction in degree heterogeneity and hub prominence, consistent with the removal of redundant connections. These observations indicate that the approach captures non-redundant interactions with respect to shortest-path organization while maintaining salient structural characteristics of the original functional networks. Although functional network analysis has previously revealed heterogeneous connectivity patterns, modular organization, and specialized functional subpopulations in pancreatic islets [14], the present work establishes metric backbone extraction as an alternative network-construction strategy for this context. Importantly, while the complete workflow includes the selection of a k -nearest-neighbours preprocessing parameter, the subsequent backbone

extraction does not require an additional edge-weight threshold or a prescribed target network density. Metric backbone extraction should therefore be regarded as a complementary approach that can reduce redundancy in functional networks while retaining their shortest-path organization, rather than as a wholly parameter-free alternative to conventional network construction.

Several limitations of the present study should be acknowledged. Although the analysis was repeated across six independent islets and the principal findings were robust across datasets, the study was intended primarily as a methodological proof-of-concept rather than as a comprehensive physiological investigation. Consequently, the broader biological interpretation and generality of the observed network properties should be approached with appropriate caution. In addition, the sensitivity of the extracted backbones to preprocessing choices and sampling variability was not systematically assessed across a broader parameter space. Future studies involving larger cohorts, multiple stimulatory conditions, and pharmacological perturbations will be important for evaluating the robustness and physiological relevance of the approach, as well as for clarifying how backbone-derived network organization relates to collective cellular dynamics. More broadly, the present findings suggest that metric backbone extraction provides a useful complementary framework for investigating coordinated activity in multicellular systems. Although demonstrated here in pancreatic β -cell networks, the approach may be transferable to other biological preparations in which collective activity is inferred from weighted measures of functional association.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

The authors wrote, reviewed and edited the content as needed and they have not utilised artificial intelligence (AI) tools. The authors take full responsibility for the content of the publication.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

LK analyzed the data, prepared the figures, and co-wrote the initial draft of the manuscript. LKB and JK organized and conducted the experiments. AS contributed to the interpretation of results. MG designed the study, analyzed the data, prepared the figures and co-wrote the initial draft of the manuscript. All authors reviewed and approved the final manuscript.

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Conflicts of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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