



Figure 1. Methodological framework overview.

Throughout this figure panels the (i,j) labels refer to enhancer (i) and promoter (j) pairs.

A. Starting from a reference catalogue of enhancer and promoter regions, it is possible to quantify their respective activity status using two sets of p and q functional genomic data (e.g. ChIP-seq data for chromatin marks), respectively. Then, the Canonical Correlation Analysis (CCA) is used to investigate the synchronised activity of each enhancer-promoter (EP) pair across k cell and tissue types: the two original sets of chromatin marks are transformed through linear combinations that allow maximizing the relationship between the two sets, and the respective canonical correlation is tested. The procedure returns a p-value for each specific EP pair. **B.** For each Hi-C dataset in the selected collection, the boundaries of Topologically Associating Domains (TADs) are identified across multiple levels of resolution. The resulting ensemble of boundaries represents the hierarchical structure of TADs for a specific cell or tissue types. Considering the occurrence of each EP pair within these ensembles called from D Hi-C datasets, we can describe their broader spatial co-localization pattern through the Hierarchical Contact (HC) score. A high score is associated to pairs supported by several combinations of Hi-C datasets and hierarchical levels (e.g., E₂-P₁ pair). Conversely, a weak score is associated to pairs supported only in few combinations (e.g., E₁-P₁ pair). **C.** The 3D co-localization information encoded in the HC score is used to estimate an adaptive rejection threshold to control for FDR in the multiple testing hypothesis of EP pairs synchronisation. On similar equal nominal p-value (y-axis) a less conservative significance criterion is used for the EP pair showing higher HC score (x-axis). Namely, even if one enhancer (E₁) will exhibit a stronger synchronization with a specific promoter (P₁), being at greater 3D distance will be less likely to regulate it than the closest one (E₂).