Toward a Predictive Model of Spontaneous Clustering of VEGF Receptors

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Short Abstract — **We analyze sets of transmission electron microscopy (TEM) micrographs of the distribution of VEGF receptors on cell membrane sheets. The images show these receptors tend to localize in small clusters. From a molecular perspective, the existence of these clusters has potentially far reaching implications given the role of VEGF signaling in a range of medical conditions.**

A simple hypothesis on the proximate mechanism of cluster formation is that the clusters form in specific domains in the cell membrane, the chemical and physical properties of these domains result in a locally increased density of receptors. We use this hypothesis to build and parameterize a mathematical model that should reproduce the distribution of cluster sizes across a moderately large sample of images.

I. Background

Signal transduction provides the logical inputs a cell needs in order to perform its role within the organism. The incoming information is processed and a complex bio-molecular network formulates the response. For the vascular endothelial growth factor (VEGF), the initial step is the binding of VEGF (ligand) to its membrane bound receptors. The subsequent activation of receptors concludes signal initiation.

Modern microscopic imaging and labeling techniques reveal certain receptor types tend to co-localize in clusters, ranging from a few to hundreds [1]; consequently, the distribution of membrane receptors on the cell surface is mostly heterogeneous [2]. Our data indicates that this is also the case for VEGF receptors. VEGF mediated signaling is involved in angiogenesis, important in normal development as well as in cancers [3].

Our goal is to go beyond characterization and attempt to provide a predictive model for clustering. We rely on a simple working hypothesis that has emerged from detailed analysis of static as well as dynamic imaging data [5]. We assume that the clusters form through transient residency in membrane domains, with the potential for rapid exchange.

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II. Methods

We study TEM images of PAE-KDR cells, porcine aortic endothelial cells that express VEGFR-2 (KDR) receptors. The receptors are labeled with gold nano-particles [4]. The distribution of the labeled receptors is not uniform. We identify receptor clusters through a hierarchic distance based algorithm with a globally optimized characteristic length, and then summarize the distribution of cluster sizes.

We used the hypothesis of attractive micro-domains to build a mathematical model, which provides the probability distribution of cluster sizes. The model parameters are related to the typical size and density of the domains and the relative affinity of the receptors for them. These parameters are not directly measurable. The size of a cluster in an image depends on the number of receptors present in the imaged area, as well as on the labeling efficiency; both may vary substantially. Our approach relies on comparing the model prediction for the cluster size distribution in each image with the one derived experimentally. A Metropolis-Hastings algorithm is used to minimize the overall square distance between the model and experimental distributions.

III. Results and Outlook

We have performed the cluster analysis for a moderately sized set of micrographs, and are working on implementing the global fitting algorithm. The global fit may validate or refute our simple hypothesis. In the first case, the resulting parameters will be useful in estimating the impact of this domain structure on signaling.

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