Alzheimer’s disease is described as a progressive neurodegenerative disorder characterized by impairment in higher cognitive abilities such as memory and decision making \([4,5,6]\). One of the major neuropathological hallmarks of AD is the presence of senile plaques formed by the accumulation of a 36-43 amino acid peptide known as \(\beta\)-amyloid, i.e. \(\beta\). \(\beta\) is derived from a type 1 transmembrane protein called \(\beta\)-Amyloid Precursor Protein (APP) by sequential proteolytic processing involving multiple secretases \([5,7,8,9]\). The combination of secretases acting on APP results either in a non-amyloidogenic (\(\alpha\) and \(\gamma\)-secretases) or an amyloidogenic (\(\beta\)- and \(\gamma\)-secretases) processing. Interestingly, both these proteolytic cleavage pathways co-exist...
in the human brain. When the equilibrium shifts towards the amyloidogenic pathway, it triggers a series of signaling cascades along with the aggregation of β-amyloid monomers, resulting in senile plaques. The increase in monomers and oligomers of toxic proteoforms contribute to the decline in the health of individual neurons, resulting in synaptic dysfunction and atrophy. Rapid advances in biochemistry and molecular biology over the last two decades has focused on understanding the production and regulation of different variants of β-amyloid peptides. The primary attention was laid on 'Aβ42', a 42 amino acid variant of β-amyloid, which was found to be more neurotoxic than the other variants of Aβ. It has been identified that Aβ42 is less concentrated in the brain of a normal healthy individual in comparison to other variants such as the ‘Aβ40’, a 40 amino acid peptide. However, the modulation of local Aβ42 levels enable the formation of oligomers and senile plaques at lower concentrations when compared to other variants of Aβ. These observations and extensive biochemical and molecular characterization indicate that progression of AD can be correlated with overproduction of Aβ42 or an increase in the ratio of Aβ42 compared to other variants or due to reduced clearance of Aβ42 from the interstitial fluid in the brain. Despite more than a century of focus on AD, there exists a lack of understanding on how the equilibrium is shifted towards the amyloidogenic pathway or how the β-amyloid affects the molecular machinery involved in the basal synaptic transmission and plasticity contributing to the development and progression of AD.

In the last decade, a paradigm shift was observed towards understanding the molecular and biochemical pathways implicated in AD. It was postulated that the early onset of AD originates at the level of individual synapses, much before the manifestation of clinical symptoms. It led to a profound interest in understanding AD as a disease beginning with dysfunction of synapses in the preclinical stages of AD. In a seminal study, Wilhelm and colleagues observed that about 1% of the total presynaptic protein content is APP, a precursor to Aβ. A careful evaluation of the presynaptic indicated that around 7000 molecules of APP are distributed only on the presynaptic surface, contributing to a molarity of 40μM. It is already known that molecular changes in APP and its regulation increase the risk towards the development of AD; the presence of such high APP concentrations prompted a better evaluation of APP distribution and its instantaneous regulation at excitatory synapses. Such studies resulted in understanding the spatial heterogeneity of APP segregation at the scale of few tens of nanometers on the neuronal and synaptic membranes, as well as their regulation by lateral diffusion, which allows them to get locally immobilized in the zones of aggregation. Together, the nanoscale heterogeneity on the synaptic sub-compartments, the trafficking rates of single molecules on the membrane and the diffusional collisions with other molecules can influence the processing of APP. It is possible to evaluate how these short-term changes in the molecular level at a millisecond time scale contribute to shifting of the equilibrium to the detrimental pathway. In an excitatory synapse, APP aggregation is observed both at the postsynaptic density and at the perisynaptic region, indicating their association with distinct molecular machinery in these functional zones. However, very little is known about the effects of genetic mutations of APP (implicated in Familial AD (FAD)) on its synaptic distribution and synaptic transmission properties. Perturbation of synaptic activity is strongly correlated with cognitive decline and memory deficits in patients with early-onset AD. The increase in the monomeric or oligomeric form of β-amyloid may be critical for early synaptic failure, a signature of AD pathogenesis. Synaptic transmission and plasticity and morphological characteristics of dendritic spines are altered when the endogenous APP levels are modulated or when the neuron is exposed to an increased amount of Aβ. Super resolution microscopy imaging of excitatory synapses confirm that APP and secretases are involved in its canonical processing and major scaffolding proteins that interact with them get concentrated and immobilized in discrete functional domains (<100 nm in diameter) and not diffusely distributed in the postsynaptic density (PSD) (Figure 1, Bottom), similar to the nanomachinery involved in synaptic transmission and plasticity.

This organization of APP and secretases in nanodomains could alter the probability of collisions on the membrane, potentially affecting both local and global changes in the concentration of both protective and detrimental proteoforms. Additionally, it was recently demonstrated that the stochasticity in this organization is affected at various levels. Primarily, this changes the compositionality of molecules on the synaptic membrane, altering the accessibility of secretases to APP. Thus, a nanodomain comprising of APP and secretases can act as a nanomachine which creates an immediate increase in the concentration of proteoforms at the optimal conditions. Depending upon the combination and permutation of molecules in these nanomachines, it can either result in the complete processing of APP through protective or detrimental pathways or probably contribute to partial proteolysis of APP where they are only cleaved by any one enzyme; either α- or β-secretase resulting in intermediate proteoforms.
Though much is known and there is consensus on how these enzymatic reactions proceed in solutions [28], there is very little knowledge on the rate of formation of multiple proteoforms in the synaptic subcompartment. It is partially due to incomplete knowledge on the local compositionality and heterogeneity of APP and secretases that could yield hotspots of aggregation “nanodomains” where the local concentration can be multi-fold higher than the synaptic concentration calculated previously after generation of an average presynaptic compartment. Interestingly, current advances in electron and super resolution provide ways to calculate this heterogeneity with molecular precision, allowing us to better understand the nanoscale biochemical maps in real time, thus overcoming the major limitations of ensemble studies in vitro.

There is a recent increase in re-evaluating the heterogeneity in localization and molecular composition of nanoscale machinery, there is a big void in understanding the evolution and maintenance of such machinery comprising only a subset of molecules. This is partly due to the lack of understanding of nucleation, stabilization, and integration of these domains that are self-organized onto the plasma membrane. Large biomolecules such as proteins diffuse randomly on the plasma membrane. This movement is considered to be a random walk as a result of the inherent vibrational energy of the system. Once any molecule is inserted into the lipid bilayer, they diffuse laterally, collide with other molecules where they could be chemically modified or remain unaffected, or trapped in the chemical potential that allow them to be immobilized as a part of the molecular complex. APP and secretases are membrane molecules that interact with a plethora of extra- and intracellular molecules allowing them to be reversibly immobilized in nanodomains. APP can also undergo diffusional collisions, allowing them to be proteolytically processed and yield proteoforms. Emerging evidence indicates differing rates of lateral diffusion and immobilization for wild type, detrimental and protective variants of APP [1,3,13,26,27]. In the detrimental variant of APP (Swedish mutation), the mutation occurs proximal to the β-secretase site in APP, where two amino acids, namely, lysine (K) and methionine (M) (position 670/671) are substituted with asparagine (N) and leucine (L), respectively. On the contrary, the protective variant (Icelandic mutation) harbors a threonine instead of alanine at position 673 close to the primary β-secretase site of APP, which is retained in the Aβ peptide after proteolysis. It indicates that clustering and rates of product formation would be modulated by a set of mutations distant from the site of significant intermolecular interactions.

The novel observations of nanoscale aggregation of APP and the alteration of lateral diffusion of different APP variants provide a valuable range of regulation at the spatial scale of 10-100 nm at temporal scales of milliseconds that could control the probability of local product formation. These observations pinpoint that heterogeneity of local product formation can directly be linked to: 1. Individual diffusive collisions between APP (substrate) and secretases (enzymes) that follow unhindered random diffusion on the membrane. 2. The probability that these collisions can be controlled by reversible immobilization in nanoscale domains with varying compositionality of APP and secretases. 3. The engagement of enzyme-substrate concentrations that will vary at lower enzyme concentrations since single enzymes can process multiple substrates. 4. The existence of completely immobilized nanomachines with all the components of proteolytic machinery present that can instantaneously generate proteoforms at a higher concentration. 5. The mutations on substrate and enzymes can behave differently as they undergo free diffusion and get incorporated into individual nanodomains.

The optimal pH required for the generation of Aβ through the amyloidogenic pathway is in the acidic range (~5.5) [13]. Acidic pH is usually observed in membrane-bound compartments such as vesicles and lysosomes. Many of these compartments originate from different endocytic events on the plasma membrane of the neuronal synapses. Here, even if the molecules are transported to the membrane separately, self-organization on the membrane will control the stochasticity of molecular compositionality in each vesicle as it gets internalized. Such a stochastic arrangement would give a differing dynamic load of APP processing at the level of endocytic processes, with an extensive dynamic range across synapses. This provides an unprecedented level of stochasticity where each synapse has its local machinery and varying loads of proteoforms. In such a case, the failures of short time scale events at single synapses could take a very long time to develop and manifest before it starts affecting other synapses, creating non-uniform nucleation of plaques across brain regions and synapses. Since most of the drugs available in the market target pathways of proteolytic processing or clearance of products from differential proteolytic processing, they would be futile to revert to the natural state before the onset of disease. Thus, it becomes essential to understand the onset of AD in extensive detail when we have leeway to manipulate the compositionality of the synaptic membrane to reduce the quantum yield of detrimental product formation by controlling the rate of reaction with the aid of smart molecules that may sterically decrease the interaction of APP molecules in
pathways generating Aβ or Aβ like fragments. The alternate ways to increase APP aggregation in compartments where it may not be internalized, and controlling the lateral diffusion and retention might hold vital clues to regulate the processing of APP [28,29]. Using smart tools such as robust acid capable of dissociating β/γ secretase complexes [29], resulting in reduced amyloidogenic processing of APP and decreased Aβ production would be an example.

In short, understanding of stochastic processes in nanobiology or fine control of self-organization at the level of individual reactions offers an unprecedented level of knowledge that would allow us to engineer biology. Though the current application of single molecule and ensemble based nanoscopic approaches provides fine information at the molecular scale, we believe generating new compounds with different surface properties at nanoscale and combination of multiple paradigms involving electrophysiology, opto/pharamaco-genetics, single cell gene editing and implosion of deep learning techniques would help us customize next generation drugs that will target a pre-clinical stage of the disease. And as Prof. Richard Feynman rightly pointed out at his lecture at the annual American Physical Society meeting at Caltech on December 29, 1959, “There’s Plenty of Room at the Bottom” for understanding, manipulating, and engineering nanoscale events in biology for the improvement of human health.

Acknowledgments | This work was supported by generous grants from Department of Biotechnology (Innovative Biotechnologist Award (MJ), Early Career Research Award (SERB, India) Department of Biotechnology Genomics Engineering Taskforce (MJ), Ramalingaswami Fellowship to DN,MJ), DBT-IISc Partnership program (DN), IISC-STAR program grant (DN), Indian Institute of Science (Institute of Excellence Program) and University Grants Commission, India (DN) and Tata Trusts, India as a program grant where DN was a co-investigator. SK acknowledge graduate fellowship support from IISc (GATE-MHRD, New Delhi, India). We thank all members of nanoorganization laboratory, CNS, IISc, India for discussions. All the authors contribute equally.

REFERENCES
7. De Strooper, B. et al., Cell 164, 603 (2016).