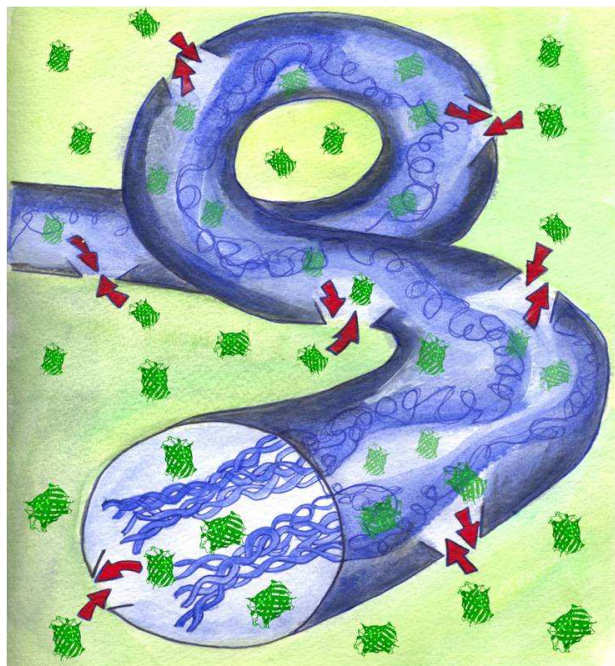


***In vivo* pair correlation analysis of enhanced green fluorescent protein (EGFP) intra nuclear diffusion**

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Intra nuclear diffusion is fundamental to enabling crucial cellular processes like gene transcription, DNA replication, DNA repair and epigenetic regulation to take place. The diffusion of molecules within the nucleus is obstructed by the steric constraints imposed by the nuclear environment. The extent to which nuclear architecture directs the diffusive route taken by these molecules is of significant interest. No methods proposed thus far have the capability to measure overall molecular flow in the nucleus of living cells. Here we apply the pair correlation function analysis (pCF) to measure molecular anisotropic diffusion in the interphase nucleus of live cells. In the pCF method we cross correlate fluctuations at several distances and locations within the nucleus, enabling us to define migration paths and barriers to diffusion. We use monomeric EGFP as a prototypical inert molecule and measure its flow in and between the different nuclear environments, marked by Hoechst 33342 as a reference of DNA density.



As schematically shown in the figure above our results suggest that there are two disconnect molecular flows throughout the nucleus, associated with high and low DNA density regions. We observe that the different density regions of DNA form a networked channel that allow EGFP to diffuse freely throughout, however with restricted ability to traverse the channel barriers. Upon more detailed analysis in time, rare bursts of EGFP molecules were detected entering and exiting the channel, with a characteristic time of approximately 300ms. The intermittent nature of this transit suggests an intrinsic localized change in chromatin structure which periodically turns on and off. Preliminary results obtained during mitosis, suggest the chromosomes to impart a markedly different mechanism toward regulating the equivalent transit. This is the first *in vivo* demonstration of the intricate chromatin network showing channel directed diffusion of an inert molecule with high spatial and temporal resolution.