the major groove of gDNA targeting the N7 atoms of guanine bases resulting in both 1,2 intrastrand (65%) and interstrand (25%) crosslinks. In the case of intrastrand crosslinking, the toxicity has been suggested to arise from bending of the DNA. Despite the success of cisplatin type drugs in treating many forms of cancer, the drug suffers from the propensity of cancer cells to relapse after the initial exposure. In addition, cisplatin type complexes also exhibit general cellular toxicity and are not specific to cancer cells. Ruthenium based metal complexes may offer an alternative to Pt based chemotherapy agents. Octahedral Ru(II)tris(2,2′-bipyridine) (RuBpy) complexes containing bidentate ligands exhibit a rich photochemistry that has been exploited in applications ranging from solar cells to drug development. Of specific interest is the excitation of Ru(II)bis(2,2′-bipyridine)(L)2 (RuBpy2L2) type complexes where L = sterically hindered bpy, primary amine, nitrile, etc which leads to the photoexchange of L with solvent molecules. Photolysis of RuBpy(L)2 complexes in aqueous solutions and in the presence of DNA results in the formation of an RuBpy2-DNA adduct providing for a potential photo-active cisPlatin analog. However, little is known on the exact mechanism through which RuBpy2 binds to DNA bases. Here we have examined the binding of nucleobases, nucleosides and nucleotides to the RuBpy2(H2O)2 complex using optical spectroscopy and computational chemistry methods. Our results demonstrate a strong affinity of the RuBpy2 toward guanine with binding taking place at the N7 position, similar to cisPlatin.

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Noise Response of P53 Oscillation against System Size Scaling

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p53 is a DNA binding protein regulating the expression of various other proteins under DNA damaging cell stress. Based on different type and extent of DNA damage, p53 up-regulates different sets of proteins and triggers cell cycle arrest, DNA repair or Apoptosis. Given the importance of p53 regarding to its role in cell fate decision, it is only reasonable for such molecules to be under delicate regulation.

One interesting observation on p53 dynamics is that when stress signal is activated, rather than staying in an activated higher level, the concentration of p53 pulses with a period of around 5.5 hours, in a rather noisy manner. mdm2, one of p53's main regulators, goes through a similar pulsing process, with a same period as the oscillation of p53 and a stable delay in phase. So far, different theories on both the origin of p53 oscillation and its variation on amplitude are explored. Yet the relation between the size of the system and the noise-driven oscillation, or rather, whether the oscillation is robust against the size variation is hardly mentioned. Given that the absolute number of molecules in a single cell being difficult to assess experimentally, along with the fact that cell size varies for different cell type in a single species, we believe a discussion on how such dynamics behaves under cell size variation being valid for both theoretical and application purposes. In our work, we adopted and modified an existing model. Based on it, we shall discuss its noise driven oscillation behaviour and explore its response against system size variation both numerically and analytically.

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The Lifetime NAD(P)H Fingerprint of Salmonella Infection

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NAD(P)H is and endogenous fluorescent coenzyme that has been used as a metabolic biomarker in recent studies using the FLIM technique. Here we describe the NAD(P)H FLIM technique that provides a label-free imaging method to monitor Salmonella enterica infection inside differentiated macrophages. We use FLIM NAD(P)H signature as a marker of bacterial metabolism. NAD(P)H in Salmonella shows a different lifetime fingerprint when the bacteria is grown in a soft agar and when the bacteria infects differentiated macrophages. Specifically, we observed that the free/bound ratio of NAD(P)H in Salmonella changes from a more bound NAD(P)H to a more free state when the bacteria is inside the host cell. During the bacterial infection we also monitored the changes of NAD(P)H surrounding the bacteria inside the macrophage. The NAD(P)H signal around the bacterium when inside the host is interpreted as a marker of the NADPH oxidase enzyme. In contrast to related organisms like Escherichia coli, Salmonella enterica is able to infect macrophages and to escape with high efficiency the attempts of the host cell to kill through the NADPH oxidase enzyme complex. In agreement with this notion, we show that Salmonella changes the state of the surrounding NAD(P)H to a more bound state while in macrophages infected with E. coli we were unable to observe the shift of the NAD(P)H signal surrounding the bacteria. This difference in the NAD(P)H signal around the bacteria corresponds to a reduced activity of the NADPH oxidase around Salmonella, thereby compromising the ability of the enzyme to destroy these types of bacteria.

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Small Cell Aggregation Method Which Enables us to Observe All Cell's Behavior Individually

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Cell aggregation is observed in various activity, for example, metastasis of cancer cells and migration of immune cells to target. But they are still unknown the mechanism to transit from random movement to aggregation state and which reaction occurs inside cells then. Dictyostelium discoideum, a kind of social amoeba, aggregate to make multicellular form and differentiate into stalks or spores when there aren't enough nutrients around them. We can easily control the transition from growing state to aggregation state by removing nutrients, so Dictyostelium discoideum is often used for study of differentiation. However, they form so huge aggregates consisted of one hundred thousand cells that we can't see whole body of them in observation fields. And more, we can't distinguish and continue to observe all of such large number cells throughout aggregation. So I came up with new technique to observe the whole process of aggregation. I trapped limited numbers of the cells in small area to keep enough cell density for aggregation. I succeeded to make small aggregates consisted of several hundreds of cells. By using this aggregation method, I can observe the behavior and position in aggregates of each target cells respectively throughout aggregation.

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GCaMP3-Derived Genetically Encoded Probes as Rapid Intracellular Signals for Calcium Dynamics

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In intracellular calcium signaling studies genetically encoded probes offer greater intracellular domain specificity than traditional fluorescent signaling molecules, with the ability to be targeted to specific organelles and microdomains. To this end we evaluated the ability of seven novel mutagenic derivatives of GCaMP3 calcium probe (EF-3, EF-4, EF-34, RS-1, RS-1 EF-3, RS-1 EF-4, and RS-1 EF-34) to rapidly detect intracellular calcium compared to the chemical calcium probe Fura-2 AM. GCaMP3 derived probes were transiently transfected into HEK293T cells which were subsequently incubated in 1 μM Fura-2 AM for 20 minutes prior to experimentation. Fura-2 and GCaMP3-derived probe fluorescence was measured at 380 nm and 488 nm respectively as HEK293T cells were rapidly exposed to 100 μM ATP for 5 s. While mutated probes did not exhibit a greater change in fluorescence than GCaMP3 precursor, mutants EF-3 and EF-4 fluorescence changes were 128.4 and 58.2 % ΔF/Fo respectively, significantly greater than Fura-2 response in the same cells. Additionally, many probes demonstrated faster rise and decay kinetics, with rise time and decay time values up to 150 % faster than Fura-2. Our results suggest that mutations in GCaMP3 can produce genetically encoded probes capable of measuring calcium with equal or larger fluorescence responses as traditional chemical dyes. Additionally, several probes such as GCaMP3 EF-4 demonstrate not only increased fluorescence but dramatically improved kinetic responses - facilitating increasingly sensitive measurements of intracellular calcium signaling.