

DISSECTING COOPERATIVE BINDING USING LIVE CELL MICROSCOPY: SEQUENTIAL BINDING OF THE LINKER HISTONE TO CHROMATIN

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Configurational cooperativity is a common strategy employed by biological molecules to efficiently target their binding sites [1]. It occurs when one part of a molecule binds a substrate such that another part of the molecule is well positioned for subsequent binding. In this sense, the molecule is pre-configured to bind. Examples span the biological spectrum, from antibodies binding to the surface of a cell to transcription factors binding to DNA. Although this form of cooperativity has previously been measured only in vitro, we have now developed a method for measuring it in living cells by light microscopy. Our assay relies on a comparison of bound to free fractions measured by either FRAP or FCS in cells transfected with wild-type and mutant molecules lacking key binding domains. The order in which the domains bind their substrate in vivo can then be determined by ranking groups of domains based on their degree of cooperation. To demonstrate, we have used our procedure to investigate the multivalent binding of the linker histone to chromatin. The linker histone is a relatively small, basic molecule that binds linker DNA. This is thought to bring neighboring nucleosomes closer together, thereby compacting DNA and generally repressing transcription [2]. How this actually occurs, however, remains unclear. Using quantitative FRAP [3] to measure the bound to free fractions required for our analysis, we find that most binding events of the linker histone to chromatin are initiated by the C terminal domain. This tethers the central globular domain and leads to cooperative binding of two key binding sites within this domain, probably as a result of a conformational change. Our live cell approach provides a systematic procedure for dissecting the cooperative binding pathways of a multivalent molecule and should thus find broad application to the many such interactions important in cell and molecular biology.

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