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volume changes, its effect on yeast ranges from growth arrest to cell death. A global genetic scan identified trp1 as a determining factor in pressure resistance in yeast. This gene is responsible for tryptophan biosynthesis in yeast. In the absence of endogenous tryptophan, yeast relies on the transportation of this amino acid for its survival. Such transport is down-regulated by pressure. This study focuses on the tryptophan uptake via its transporters tat2 under hydrostatic pressure.
The study employed 6 strains of yeast to illustrate the effect of pressure on tryptophan uptake under hydrostatic pressure: YDR228, YDR228 Δtat2, YDR228 Δtrp1, BY4741, BY4741 Δtat2 and BY4741 Δtrp1. The Δtrp1 and Δtat2 strains have trp1 and tat2 genes knocked out respectively, rendering the yeast unable to synthesize endogenous tryptophan or uptake tryptophan from the media. The strains were held at different hydrostatic pressures for various durations and the survival of the cells was determined. We show that yeast is highly reliant on tryptophan for pressure resistance. Cell death was not observed in wild type strains at pressures less than 100 MPa after 2 hr of pressurization whereas in knock out strains death was observed at pressures above 50 MPa. The wild type growth rate is independent of time of exposure to pressure whereas the growth rate of knockouts decreased with time when exposed to pressure. Thus, the Δtrp1 and Δtat2 strains are more pressure sensitive than the wild type strains. We are now investigating the effect of pressure on the interaction between the receptor, tat2, and ligands.

2964-Pos Board B119
Labeling Freedom for the Single Molecule Microscopist
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While observation of single molecule fluorescence has matured into a central tool to study biomolecular structure and dynamics, site-specific labeling of proteins with small but highly photostable fluorescent dyes remains the major bottleneck for biological applications. We have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell. Expressed proteins only differ from their natural counterparts by very few atoms, constituting a ring-strained cyclooctyne or cyclooctene functional group. We show that these completely inert and non-toxic groups can be stably incorporated into any protein and readily react with commercially available single molecule fluorophores without the need of special reagents, catalyst or non-physiological buffer conditions. In particular the fully biocompatible inverse-electron-demand Diels-Alder reaction exhibits orders of magnitude faster reaction rates than the prototypical Huisgen type click reaction. This allowed rapid and specific labeling of proteins inside E. coli and mammalian cell culture. Similarly to fluorescent proteins, the dye attachment site is genetically encoded and will thus facilitate precise labeling of proteins in vivo by only changing a single amino acid. The speed and specificity of this method holds great promise for applications of single molecule and super resolution techniques in living cells, and experimental results demonstrating this potential will be presented. Moreover, conditions were determined were the two type of click reactions are mutually orthogonal, thus providing a route to genetically encode dual-color labeling. Furthermore, we have analyzed the effects of using unnatural amino acids for protein labeling in the context of multi-parameter single molecule spectroscopy.

2965-Pos Board B120
In Vitro Thermodynamics of DNA Binding Correlate with In Vivo Transcription Repression by a Synthetic Laci/Galr Paralog
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In our ongoing studies of engineered proteins, we use a high-throughput *in vivo* assay of transcription repression to quantify functional changes for several hundred mutational variants. However, *in vivo* repression is the sum of many possible events, including: binding to the primary operator DNA, binding to auxiliary operators, nonspecific binding to genomic DNA, and protein-protein interactions. To determine which of these parameters is/are affected by mutation, we performed thermodynamic studies with purified variants of two synthetic paralogs. First, we correlated *in vivo* changes with *in vitro* measurements of DNA binding affinities for the primary operator $lacO^1$. The two techniques show a linear correlation, which indicates that repression is altered when affinity for $lacO^1$ is altered. However, estimates of *in vivo* repressor concentration suggest a $\geq$25-fold discrepancy with *in vitro* repressor concentrations. This discrepancy can be resolved by considering both the high ionic strength of the DNA environment (which has potential to lower K_d) and/or by competitive binding with nonspecific, genomic DNA. Finally, since one synthetic paralog shows evidence of *in vivo* looping (simultaneous binding of the primary and auxiliary operators), we monitored binding to a secondary operator, $lacO^2$. Surprisingly, binding to this operator was insensitive to mutation. Thus, although overall repression is strengthened by simultaneous binding to auxiliary operator, changes in *in vivo* repression are largely due to changes in $lacO^1$ binding affinity.

2966-Pos Board B121
In Vitro effects of Macromolecular Crowding on Protein Stability, Structure and Folding
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Proteins fold and function inside cell compartments that are full of other proteins, membranes and DNA. The crowded environment results in increased viscosity, excluded-volume effects and amplified opportunity for specific and non-specific inter-molecular interactions. These environmental factors are not accounted for in the mechanistic studies of protein folding and function that have been executed during the last decades. The question thus arises as for how these effects - present when polypeptides normally fold *in vivo* - modulate protein biophysical parameters? To take a step closer to understanding the *in vivo* scenario, we assess how crowded environments affect protein stability, structure and folding reactions *in vitro*. For this we use synthetic macromolecular crowding agents, which take up significant volume but do not interact with the target proteins, in combination with strategically selected proteins and a range of biophysical/spectroscopic methods. We have found that in the presence of macromolecular crowding *in vitro*, proteins become more thermodynamically stable (magnitude depends inversely on protein stability in buffer) and, protein-folded states may change both secondary structure content and overall shape. Finally, excluded volume effects may speed up folding kinetics and decrease the ruggedness of the folding energy landscape. Our findings demonstrate that excluded volume effects tune protein biophysical parameters: this is of mechanistic relevance since proteins have evolved to fold and function in crowded environments.

2967-Pos Board B122
Exploring the Kinetics of Protein Birth
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Fidelity and efficiency in protein folding are essential to sustain cell life. Overall however, very little is known about the way proteins are able to attain their native structure within the context of the cell. In addition to the ribosome's well-established role in peptide bond formation, recent studies suggest that ribosomes may have strong influence on the early stages of protein folding in the cell and may be crucial for the production of folded unaggregated proteins. The conformational changes that occur within a nascent protein during its release from the ribosome have yet to be elucidated. Here, we present a kinetic study on the release time-course of ribosome bound model proteins upon addition of the antibiotic puromycin. By time-resolved gel electrophoresis, we are able to discern that puromycin's hydrolysis of the ester bond linking nascent polypeptides to the 3' end of tRNA occurs quickly. Steady-state fluorescence anisotropy reveals the presence of two additional slower kinetic phases. Finally, time decay fluorescence anisotropy analysis complements the above results by providing insights into the local motions experienced by the nascent protein during different stages of the protein birth process.

2968-Pos Board B123
Calorimetric Determination of Desolvation Energy for a Model Binding Reaction in Dilute and Crowded Solutions
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In most thermodynamic treatments of aqueous reaction equilibria, the energetic contribution of the solvent is neglected or intentionally omitted. In the current work, water is treated as a co-reactant in the development of a general equation for binding equilibria that takes into account the unavoidable change in hydration that occurs when two solvated surfaces come into contact. The governing equation is supported by data obtained via isothermal titration calorimetry using the chelation of Ca^{2+} by EDTA as a model binding reaction. The desolvation free energy for formation of the EDTA/Ca^{2+} complex is unfavorable, as determined from the concentration dependence of the equilibrium "constant." Results for dilute and crowded solutions are compared in order to assess the role of desolvation under conditions found *in vivo*.