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Molecular dynamics simulations of firefly luciferase at elevated temperatures

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Firefly luciferase (Fluc) is a bioluminescent 62 kDa protein that finds numerous applications in cell and molecular biology research. Fluc has been a "gold standard" substrate in chaperone research because its bioluminescence is easy to measure and bioluminescence decrease and recovery is related to Fluc misfolding and chaperone-assisted refolding, respectively. Fluc is composed of a large 450 amino acid N-terminal domain and a smaller 100 amino acid C-terminal domain. Fluc is moderately stable at room temperature and at elevated temperatures above 38 C quickly loses its ability to produce light, due to alterations in its structure. Even though "thermal denaturation" is critical for using Fluc in chaperone-assisted refolding reactions, very little is known about what happens to Fluc's structure at elevated temperatures (>30 C). In this project, we use all-atom molecular dynamics simulations to systematically explore the structure of Fluc at temperatures ranging from 5 to 80 C. We find that simulations not longer than 100 ns (previous studies) may not reach an equilibrium. We therefore extended our calculations to 500 ns, for each temperature. Our results suggest that relatively small structural alterations are induced in Fluc at moderate temperatures, and confirming an earlier observation, we find that at these temperatures Fluc may populate an alternative stable state with domains rotated, which may not be functional. This second state is a possible substrate for chaperones. Thus, it is possible that the chaperone task may be simpler than assumed before, as it would involve only a moderate reorientation of the two domains, rather than the refolding of the totally denatured polypeptide chain of Fluc. This work was supported by the NSF grant number MCB 1817556 and MCB 2118357.

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Unlocking pHLIP's dynamic transformations using 2D IR spectroscopy Raiza Nara Antonelli Maia[1], Carlos R. Baiz[2].

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The pH low insertion peptide (pHLIP) is a pH-sensitive cell-penetrating peptide with potential for drug delivery and imaging of tissues linked to various pathological conditions, such as cancer, inflammation, and hypoxia. Although we have a solid grasp of pHLIP's structural transitions, shifting from an unstructured coil at pH > 7 to a transmembrane a-helix at pH < 5, the intermediate structures have not been characterized, and furthermore the relation between structure and local environments is not well understood. We use two-dimensional infrared (2D IR) spectroscopy to map out the pHinduced insertion and folding processes of pHLIP within a phospholipid bilayer (POPC vesicle). 2D IR spectroscopy offers femtosecond temporal resolution, enabling us to precisely monitor rapid molecular events. Consequently, our approach provides a comprehensive overview of pHLIP's dynamic states in the ultrafast time range, encompassing: Unstructured Coil (pH > 7): At pH levels above 7, pHLIP assumes a disordered coil conformation. This initial state is pivotal for understanding its structural evolution. Transmembrane a-Helix (pH < 5): When exposed to a lower pH environment, pHLIP undergoes folding an insertion in the lipid bilayer, adopting a transmembrane a-helix structure. This change facilitates its penetration into cell membranes. Intermediate States (pH 6.5–5.5): Our investigation sheds light on the intermediate states of pHLIP within the pH range of 6.5–5.5. These partially folded structures may stabilite pHLIP's interactions with the bilayer interface, thus could mediate interactions with intracellular components. This project characterizes the elusive intermediate structures of pHLIP, significantly enhancing our understanding of its potential in targeted drug delivery.

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Resolving chaperone action on ribosome-bound nascent chains with singlemolecule spectroscopy

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Proteins begin to fold during their synthesis by the ribosome. Specialized ribosome-binding molecular chaperones stabilize unfolded proteins that accumulate co-translationally, preventing premature folding into non-native states. Chaperones also rescue proteins from misfolded structures and protect folded domains against destabilizing interactions. However, the molecular mechanisms underpinning these functions are not well understood. Trigger factor is the first molecular chaperone encountered by nascent proteins emerging from the ribosome. We have developed a single-molecule approach for directly visualizing the interaction of trigger factor with nascent chains, using the E. coli elongation factor G protein as a model protein. We find that trigger factor dynamically engages with unfolded polypeptides on the timescale of seconds. Binding parameters change with nascent chain length and are dependent on recruitment of trigger factor to the ribosome, suggesting a multivalent mode of interaction. Experiments combining fluorescence detection with force spectroscopy indicate that trigger factor binds strongly to compact nascent chains and weakly to extended polypeptides. These results suggest that the chaperone compacts its nascent chain clients and keeps elongating protein poised for productive structure formation. Our experimental system sets the stage for exploring general molecular mechanisms of chaperone action on nascent proteins.

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Xeno interactions between MHC-I proteins and molecular chaperones enable ligand exchange on a broad repertoire of HLA allotypes Yi Sun[1], Georgia Papadaki[2], Christine Devlin[3], Julia Danon[2], Michael Young[2], Trenton Winters[1], George Burslem[1], Erik Procko[4], Nikolaos Sgourakis[5].

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Immunological chaperone TAP binding protein related (TAPBPR) plays a key role in antigenic peptide optimization and quality control of nascent major histocompatibility complex class I (MHC-I). However, the polymorphic nature of MHC-I proteins leads to various levels of allelic dependency on chaperones for their assembly, epitope selection, and antigen presentation on the cell surface, restricting the application of TAPBPR-mediated peptide exchange to a limited set of human leukocyte antigens (HLAs, human MHCIs). Here, we demonstrate that due to the divergence of the MHC-I contacting surfaces, chicken TAPBPR can catalyze peptide exchange on a different repertoire of HLA allotypes through xeno interactions, compared to human TAPBPR. Detailed biophysical characterization shows that TAPBPR orthologs recognize conserved docking sites with a preferred binding to empty than peptide-loaded MHC-I and facilitate peptide exchange by maintaining a reservoir of peptide-receptive molecules. Deep mutational scanning of human TAPBPR further identifies gain-of-function mutants, resembling the chicken sequence, which can enhance surface expression of common alleles HLA-A*01:01 and HLA-A*02:01 in situ, while promoting peptide exchange in vitro. These results highlight that polymorphic sites on TAPBPR orthologs and MHC-I can be engineered to manipulate their

interactions, expanding chaperone-mediated peptide exchange on disease-relevant HLA allotypes.

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The evolving biophysics and antigenicity of the SARS-CoV-2 Omicron spike receptor binding domain

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The SARS-CoV-2 spike (S) protein Receptor Binding Domain (RBD) binds ACE2 receptor to mediate virus entry. RBD is targeted by neutralizing antibodies making it a hotspot for immune evasive mutations. Here we characterize the stability and antigenicity of the Omicron variant RBD as it has evolved. Differential scanning fluorimetry (DSF) revealed that the melting temperature (T_m) of Omicron BA.1 RBD was 7C lower than the wild-type RBD, with stability partially recovered in Omicron BA.2 and BA.5 RBD. XBB.1.5 and XBB.1.16 showed increased T_m compared to the wild-type. An interesting correlation was observed between expression yields of the RBDs and their respective thermostability profiles, with the wild-type, XBB.1.5 and XBB.1.16 RBDs expressing at substantially higher levels than the BA.1, BA.2, and BA.5 RBD. We performed differential scanning calorimetry (DSC) on the wild-type, XBB.1.5 and XBB.1.16 RBDs, where we observed similar trends with the XBB variants showing higher T_m compared to the wild-type RBD. We used surface plasmon resonance (SPR) to measure binding of the RBDs to a panel of antibodies. Most RBD-directed antibodies tested showed dramatic decreases in binding to the Omicron RBDs. Similar results were obtained when the corresponding S protein ectodomains were