mental data. We will demonstrate use of this CG model to investigate thermody-
namics of co-translational integration at the Sec-translocon and analysis of the
minute) timescales, while retaining the level of detail required for reproducing
the Sec-facilitated membrane protein integration is difficult due to roles of long-
membrane dimension of rhodopsin. Using time-resolved UV-visible spectro-
copy, we are currently examining the photoactivity of embedded bovine rhodopsin,
and its dependency on the surface charge states and membrane moduli
Hua et al. (2011) JACS 133, 2354-2357.

2530-Plat
Coarse-Grained Modeling of Minute-Timescale Co-Translational Mem-
brane Protein Integration via the Sec-Translocon
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Integration into the cell membrane in the correctly folded structure is essential
for membrane protein function. Membrane protein integration takes place at
the Sec-translocon and typically occurs during translation of the protein
sequence. Although extensive structural data is available, study of the dynamics
of Sec-facilitated membrane protein integration is difficult due to roles of long-
timescale dynamics and ribosomal translation. We present a coarse grain simu-
lation approach that is capable of reaching the experimentally relevant (i.e.,
minute) timescales, while retaining the level of detail required for reproducibility
of available experimental data. The CG model enables direct simulation of the dy-
namics of co-translational integration at the Sec-translocon and analysis of the
resulting trajectories provides mechanistic explanations for observed experi-
mental data. We will demonstrate use of this CG model to investigate thermody-
namic and kinetic factors that govern membrane protein topology, integration
efficiency of trans-membrane domains, and hydrophilic loop translocation.

Platform: Protein Folding and Chaperones

2531-Plat
Proteomics-Level Identification of Degradation-Resistant Proteins Provide
Insight about their Potential Roles in Organismal Adaptation to Stress
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Some proteins are hyperstable, as demonstrated by their resistance to proteolysis
and detergents, and their long half-life. Some of these proteins are characterized
by having a high energy barrier toward unfolding that virtually traps them in their
native state. This property of proteins, known as “kinetic stability” (KS), ap-
ppears to be a strategy used by “mother nature” to protect certain proteins against
aggregation, and premature degradation under stress conditions. In addition to
saving organizational resources by avoiding frequent degradation-biosynthesis of
proteins, KSs could function under extreme stress to maintain vital functions
needed for survival. On the basis of our observed correlation between KS and
a protein’s resistance to the detergent SDS, we developed a diagonal two-
dimensional (D2D) SDS-PAGE method for the proteome-level identification of
KSs. We applied D2D SDS-PAGE to the lysate of Thermus thermophilus
b-D-maltoside solubilized bovine rhodopsin
2D FLCS to study the folding mechanism of B domain of protein A
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Satoshi Takahashi, Tahei Tahara1.
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Molecular-level description of the folding process of proteins is of fundamental
importance for understanding how proteins acquire their unique conformations
to show various biological functions. Elucidation of the folding mechanism re-
quires quantitative analysis with a high time-resolution. To this aim, we recently
developed two-dimensional fluorescence lifetime correlation spectroscopy
(2D FLCS). This method enables us to examine the microscopic
conformational dynamics of proteins at the single-molecule level, through the analysis of the fluorescence lifetime correlation. In this study, we applied
2D FLCS to study the folding mechanism of B domain of protein A (BdPA). The
small protein was believed to show a two-state folding process in which
the native state unfolds without exhibiting any intermediate states. However,
a recent single-molecule study suggested that the folding mechanism of
BdPA is not so simple3. To quantitatively analyze the complex folding process of
BdPA, we performed 2D FLCS for two FRET mutants of BdPA. The results
showed that the conformations of both the native and unfolded states are highly
heterogeneous and that the conformational dynamics within each conforma-
tional ensemble occurs on a timescale shorter than ten microseconds. Fur-
thermore, our results indicated that the conformational distribution in the
native and unfolded ensembles gradually change with the change of the dena-
turat concentration.