Comparison of GdmCl- and SDS-induced protein denaturation reveals massive acceleration of (un)folding kinetics of S6 protein in SDS

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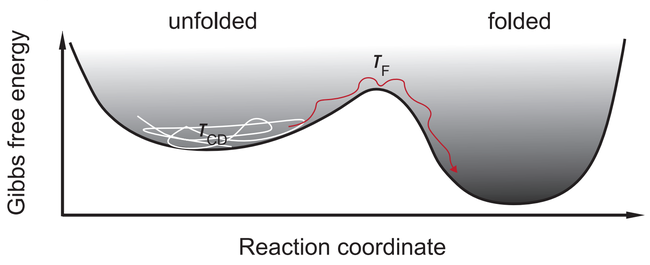
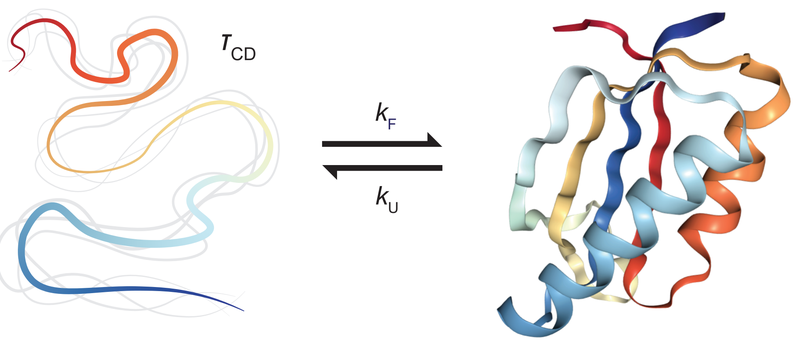
Abstract

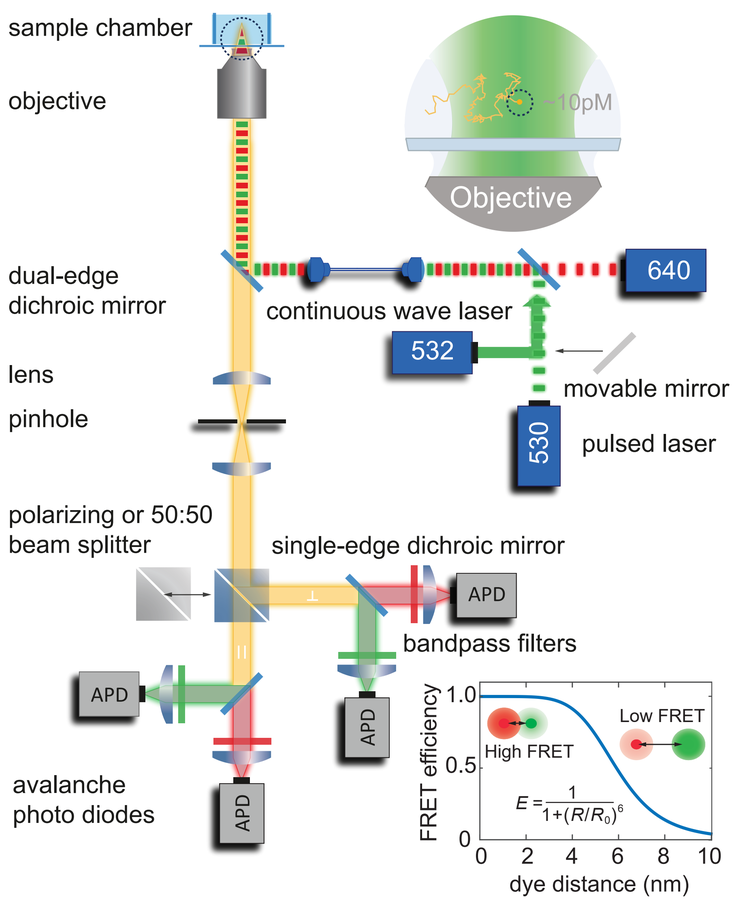
How does (un)folding of proteins in chemical denaturants compare with (un)-folding in detergents? Here, we addressed this question by scrutinizing at single molecule level the equilibrium (un)folding dynamics of the ribosomal protein S6 [1] in the presence of the chemical denaturant guanidinium chloride (GdmCl) and the surfactant sodium dodecyl sulfate (SDS). We subjected a fluorescently labeled protein variant to increasing concentrations of GdmCl or SDS and made use of single-molecule Förster resonance energy transfer (FRET) spectroscopy to probe its folding kinetics upon denaturation. We found that the protein (un)folds with greatly different kinetic rates. While S6 exhibits rather slow interconversion dynamics (> tens of milliseconds) in the presence of GdmCl, in accordance with bulk measurements of relaxation rates of 0.032 s^{-1} [2], the protein undergoes a massive increase in unfolding dynamics in SDS, with rate constants 1 ms^{-1}. Thus under strongly denaturing conditions, S6 unfolds two orders of magnitude more rapidly in SDS than in GdmCl according to single molecule measurements. This is in marked constrast to bulk measurements which suggest comparable (and slow) rates of unfolding [3]. Nanosecond fluorescence correlation (nsFCS) [4] experiments revealed that the speedup induced by SDS is paralleled by a marked speedup of polypeptide chain dynamics in the unfolded state compared with unfolded chain dynamics in GdmCl. Since chain reconfiguration times are correlated with the attempt frequency of barrier crossing, these findings may shed light on a possible mechanism for the tremendous increase of folding speed induced by SDS.





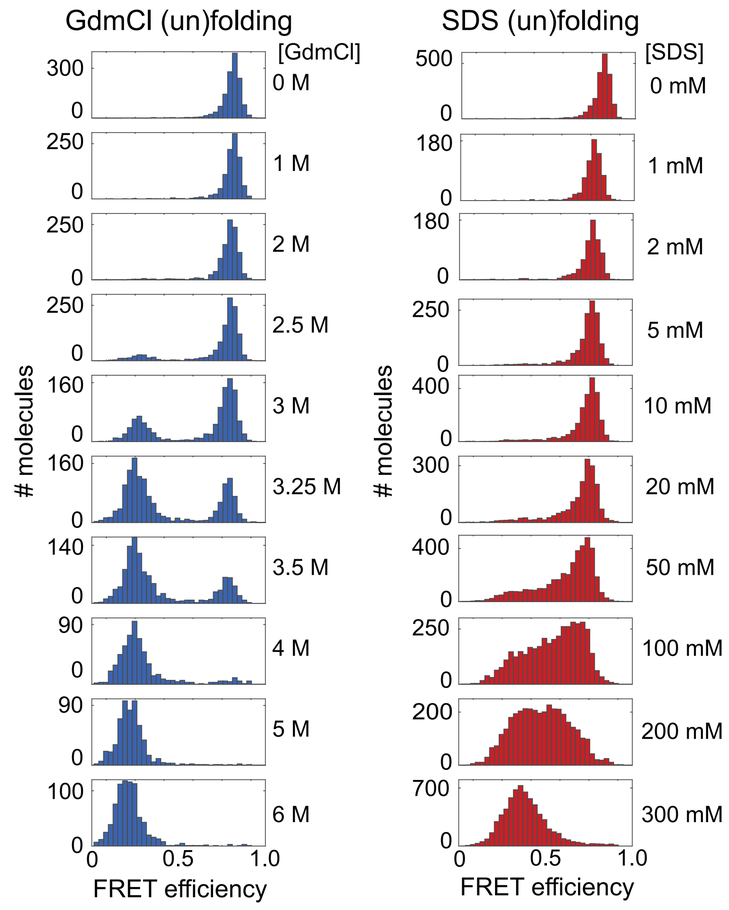
Experimental setup and method

Custom-built confocal microscope with a dual-color excitation and detection scheme [5] was implemented for all measurements. Due to Förster resonance energy transfer the detected signal in the red and green channels varied depending on the distance between the donor and acceptor dyes [6]. Investigation of the unfolded state dynamics was performed with nsFCS. For this the acceptod and donor signals were correlated yielding donor, acceptor and donor-acceptor curves. They were then fitted to obtain τ_{CD} which appears as an additional amplitude at the timescale of 10-100 ns. It should be perceived as the time over which the spacial reconfiguration of a polypeptide chain with fluorophores happens and the system looses memory about the initial conformation (*i.e.* high to low FRET transition) [4].



GdmCl and SDS unfolding series

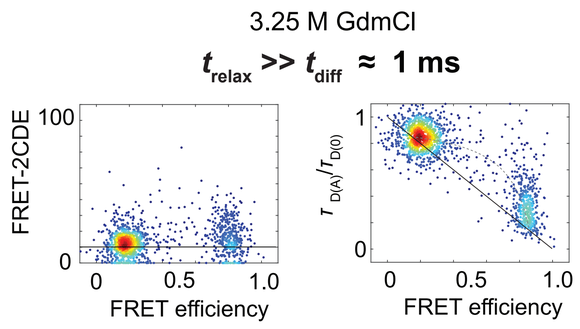
The denaturation series experiments in SDS and GdmCl provided information about the structural compactness of S6 at different detergent and denaturant concentrations in 50 mM Tris pH 7.4, 150 mM NaCl, 2 mM TCEP buffer.



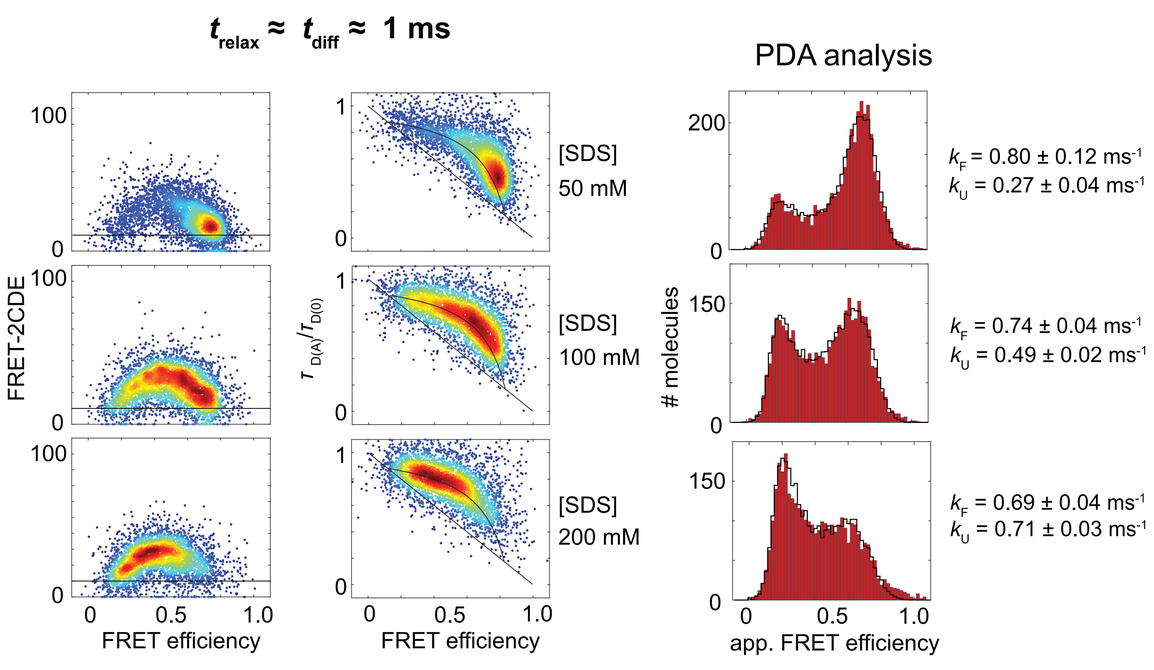
The denaturation midpoints were found to be approximately 3.2 M GdmCl and 150 mM SDS. At 5 M GdmCl and 300 mM SDS the protein was completely unfolded. Intrestingly, while in GdmCl two distinct peaks corresponding to native and unfolded states were present, in SDS this two states were connected by a bridge population. This suggested presence of dynamics on the comparable to diffusion timescale. Finally, more careful investigation of the peak shift when the SDS concentration was increased from 0 mM to 1 mM (also mentioned at [7]) hinted at a three state model with native (0 mM SDS) and two unfolded (1-20 mM and 300 mM) states.

GdmCl unfolding demostrates static FRET populations

In GdmCl populations fall on the static FRET line, indicating the folding timescale to be much slower than that of the diffusion. In contrast, in SDS their order of magnitude is similar as the arc-like distribution of $\tau_{D(A)}/\tau_{D(0)}$ with respect to E is prominent. Furthermore, photon distribution analysis (PDA) yielded rates of (un)folding that are two orders o magnitude faster in conparison to S6 in GdmCl.



SDS unfolding shows milisecon interconversion kinetics



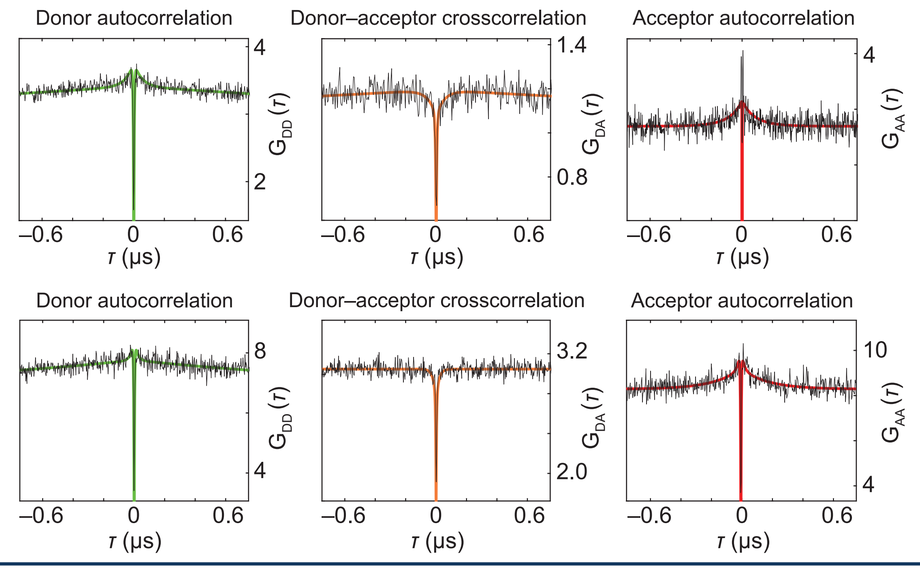
Nanosecond FCS analysis reveals a speed-up of unfolded state dynamics linked to the accelerated folding rates in SDS

From the chain dynamics in consideration of Gaussian chain model the chain reconfiguration time can be calculated if R_0 and $\langle r^2 \rangle^{1/2}$ are known [8]. From there in the simplified Kramers rates description of the folding process as the diffusion on the potential landscape with the folded and unfolded states separated by a potential barrier an inverse attmpt frequency τ_0 can be calculated.

$$\tau_0 \approx 2\pi\tau_r$$

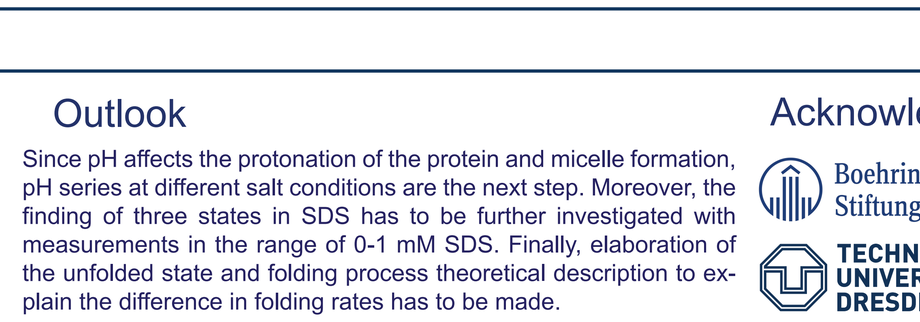
This τ_0 denotes the speed limit of folding and appears as a prefactor in the genralized transition state expression for folding.

$$\tau_F = \tau_0 \exp(\Delta G^{\ddagger}/k_B T)$$



5 M GdmCl
τ_{CD} = 50 ns
$\tau_r \approx$ 65 ns

$\tau_0 \approx$ 400 ns



300 mM SDS
τ_{CD} = 15 ns
$\tau_r \approx$ 15 ns

$\tau_0 \approx$ 95 ns

Outlook

Since pH affects the protonation of the protein and micelle formation, pH series at different salt conditions are the next step. Moreover, the finding of three states in SDS has to be further investigated with measurements in the range of 0-1 mM SDS. Finally, elaboration of the unfolded state and folding process theoretical description to explain the difference in folding rates has to be made.

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