S6 unfolds in a complex multi-pathway manner in the detergent SDS as opposed to simple two-state denaturation in GdmCl

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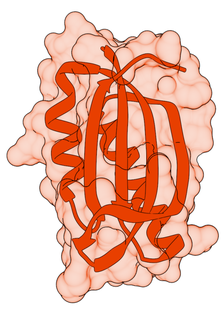
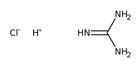
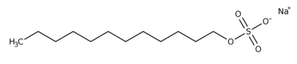


S6 - a model soluble protein for unfolding studies

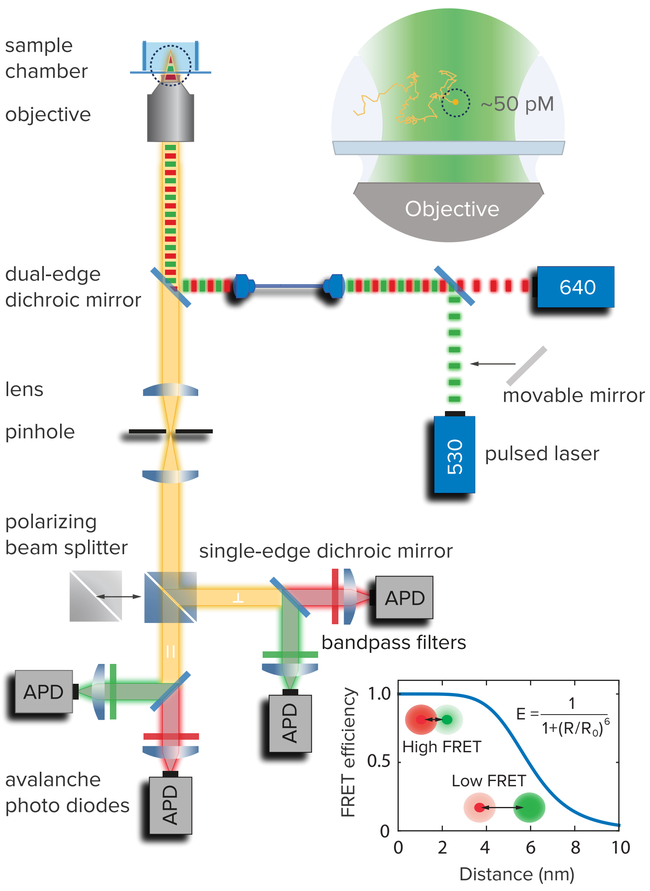
In this work, the effects of chemical denaturant **guanidinium chloride (GdmCl)** and anionic detergent **sodium dodecyl sulfate (SDS)** on a **model soluble protein S6**[1] are compared and the **unfolding pathways** are disentangled on a **single-molecule level**.

Protien denaturation by chemical denaturants and detergents

Chemical denaturants (GdmCl) and detergents (SDS) induce **drastically different denaturation modes** in proteins. The table below summarizes their main characteristics:

	GdmCl	SDS
Structure		
Affinity	Low	High (amphiphilic)
Effective concentration	~ 1 M	~ 1 mM
Denaturation mechanism	Solvation shell disruption and structure destabilization	Higlhy dependent on: Temperature, pH, ionic strength Monomer/micelle[2]
Unfolded state	Random coil[3]	Versatile micelle-protein complexes[2]

Förster resonance energy transfer (FRET): a molecular nanoruler



To this end a custom-built **multiparameter fluorescence detection (MFD)** setup[4] was utilized to disentangle the **conformational dynamics** induced in S6 during the unfolding with the **nanoscale temporal and spatial resolution**[5].

Experiments

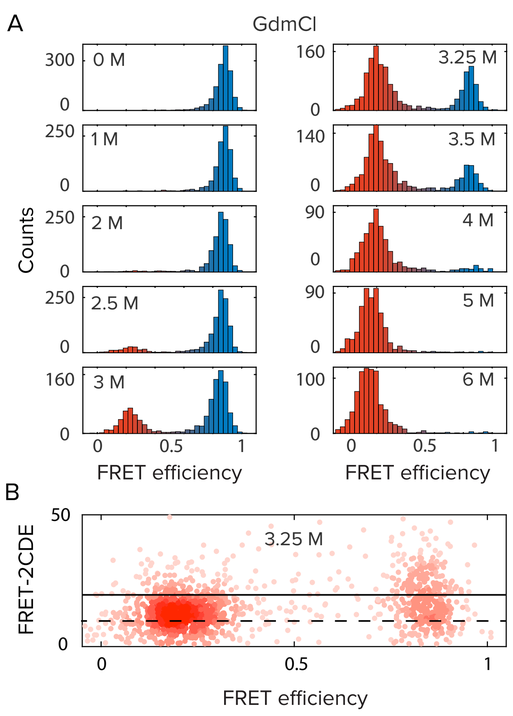
Single-molecule FRET experiments:
S6 variant M1C/F97C containing two cysteine residues at positions 1 and 97 was engineered and stochastically labeled with FRET donor Atto 532 (Atto-Tec, Germany) and acceptor Abberior Star 635P (Abberior GmbH, Germany) fluorophores. Measurements were performed at 50 pM S6 concentration;

Circular dichroism experiments:
17 µM unlabeled M1C/F97C S6 variant;

Tryptophan fluorescence:
2 µM of wt S6;

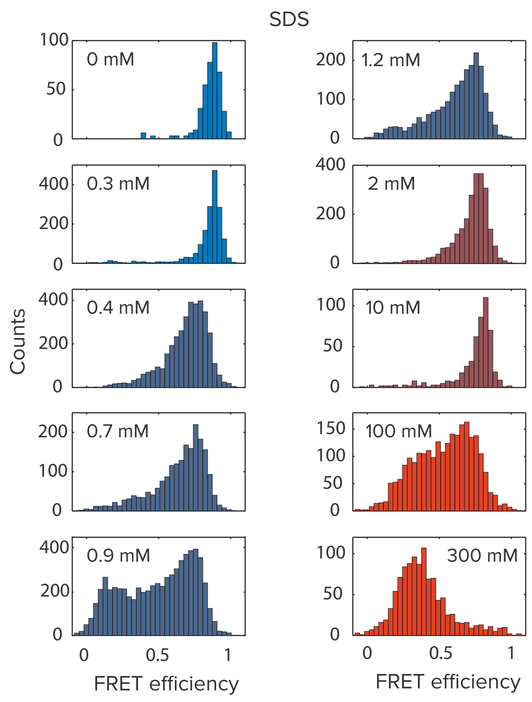
Condition:
150 NaCl, 50 mM Tris 7.4 pH, 2 mM TCEP;

Single-molecule FRET reveals two-state unfolding of S6 in GdmCl

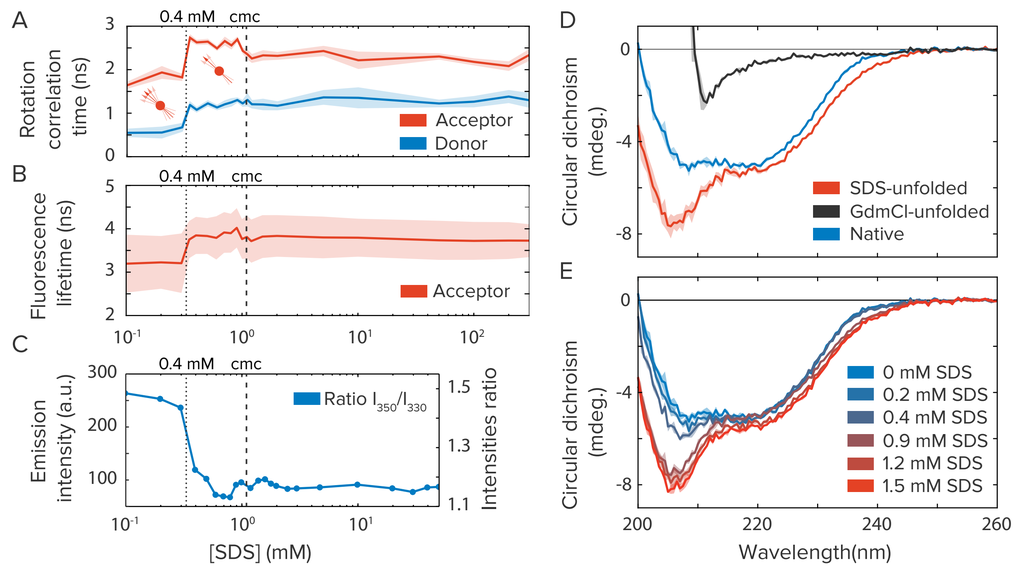


(A) Two distinct peaks correspond to the folded and unfolded states. The unfolding **midpoint** was found to be 3.2 M GdmCl
(B) FRET-2CDE analysis[6] assigned scores <20 to the majority of the observed S6 molecules. This indicated that they were **static** on the timescale of diffusion through the confocal volume (~ 1 ms).
Previously reported unfolding rate of 0.032 s^{-1} at the midpoint of 3.25 M GdmCl[7] is in great agreement with these results.

Four major stages of SDS-induced unfolding of S6 are observed with FRET

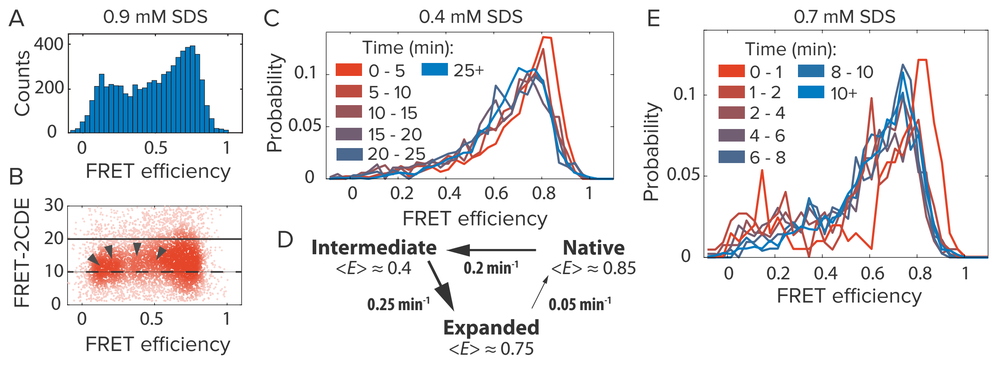


Major structural changes happen to S6 at sub-cmc SDS concentration



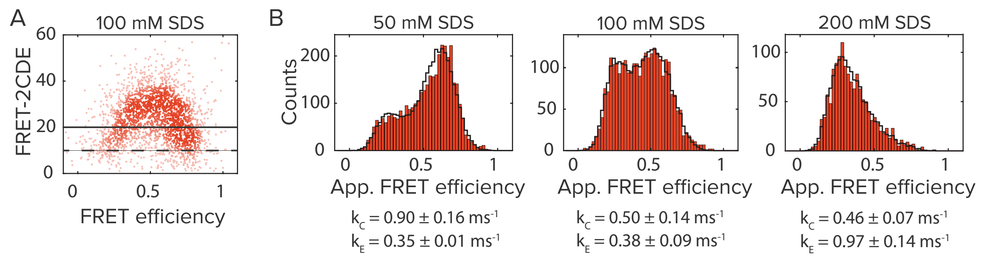
Expansion of S6 happens at 0.4 mM SDS (dotted line), **cmc** of SDS is 1.1 mM (dashed line).
(A) Hindrance of fluorophore rotation upon expansion indicated numerous SDS monomers associate with S6 and form a **hemi-micelle-protein** complex at **sub-cmc SDS**.
(B) Increase of acceptor lifetime was linked to the further spacial separation from Trp62, meaning that S6 was in a more **expanded conformation** in comparison to the native state.
(C) Blue shift of the Trp fluorescence spectra suggested a more **hydraphobic microenvironment** of the aa, i.e., the Trp is associated with alkyl tails of SDS, embeded within micelle.
Thus, the final unfolded state at hundreds mM SDS is most likely **necklace-and-beads**[8].
(D) In contrast to the random coil S6 in GdmCl, SDS promotes an **α-helice-rich state**[9-10].
(E) Major loss of β-sheets occurs at sub-cmc SDS.

Disentangling the heterogeneous populations of S6 below cmc with smFRET analysis toolkit

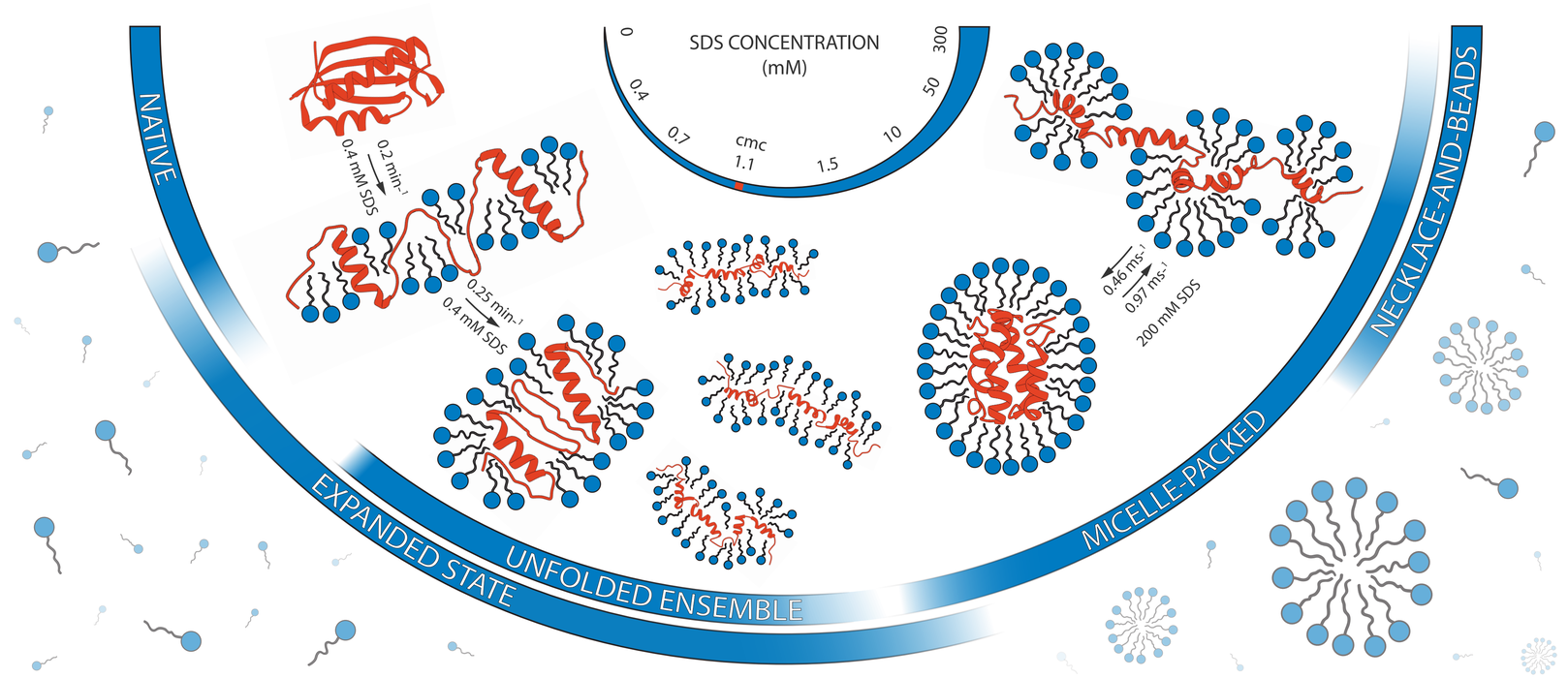


(A) Heterogeneous extended states emerged around the cmc - **unfolded ensemble**.
(B) Their reconfiguration timescale, is much slower than the observation one.
(C) FRET histograms at 0.4 mM SDS evolve over minutes.
(D) Unfolding at sub-cmc SDS occurs through **a transient intermediate state.**
(E) The **kinetic rates** of expansion are highly dependent on the SDS concentration and increase with addition of the detergent.

S6 interconverts between different protein-micelle complexes with millisecond kinetics at high micellar density



Multiple pathways of S6 unfolding in SDS



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