テラヘルツ光と蛋白質

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第2回コンパクトERLサイエンスワークショップ

2012年7月30，31日
於：　KEK 研究本館小林ホール

1. 蛋白質の動的構造

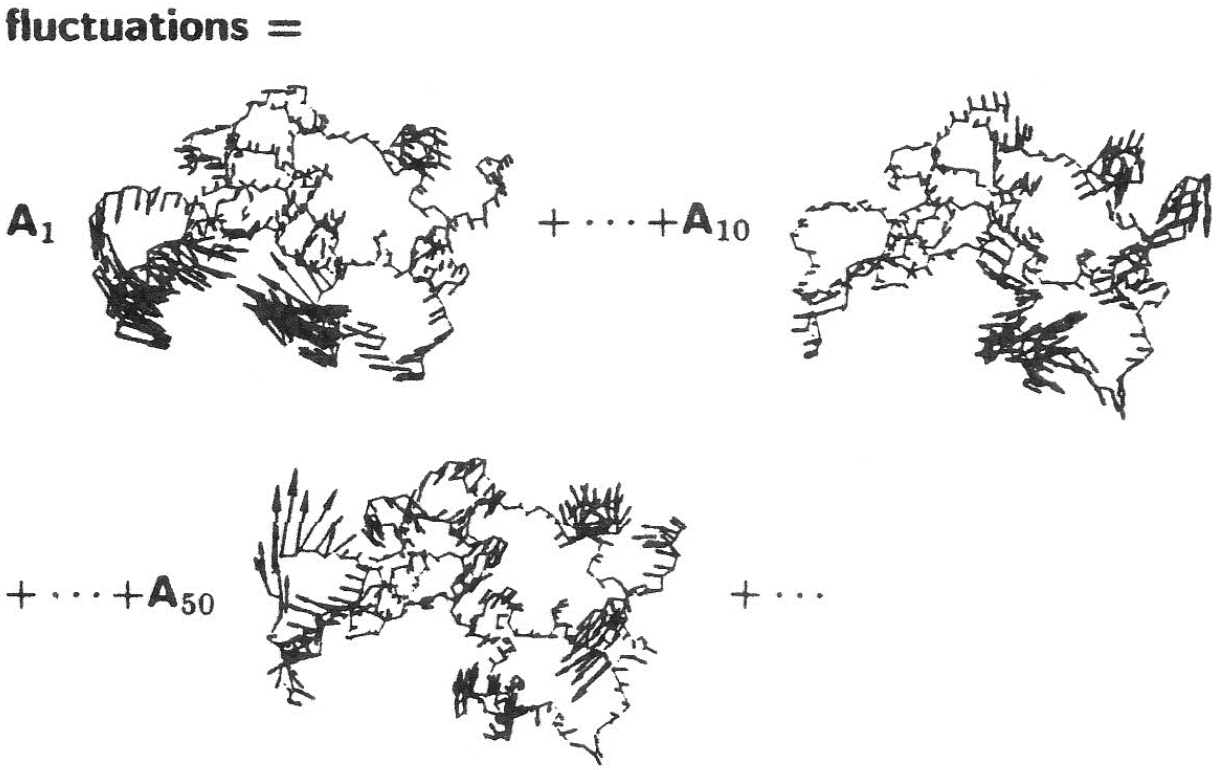
2. 蛋白質のフォールディング

3. THZ-induced dynamics

1. 蛋白質の動的構造

- 蛋白質の基準振動と生理的に意味のある振動
- 水和水の挙動

- 測定手法: 中性子非弾性散乱
  ホールバーニング
  テラヘルツ



第2図 ヒトリゾチームのゆらぎは基準振動モードの和として表現される。最低振動モードから順に1，10，50番目のモードを主鎖原子の運動を表す矢印で表示した。基準振動動的因子法でモードの振幅 A_m を変数として実験データに合うゆらぎを捜す。

木寺詔記 (1992) 日本結晶学会誌, 34, 186-191.

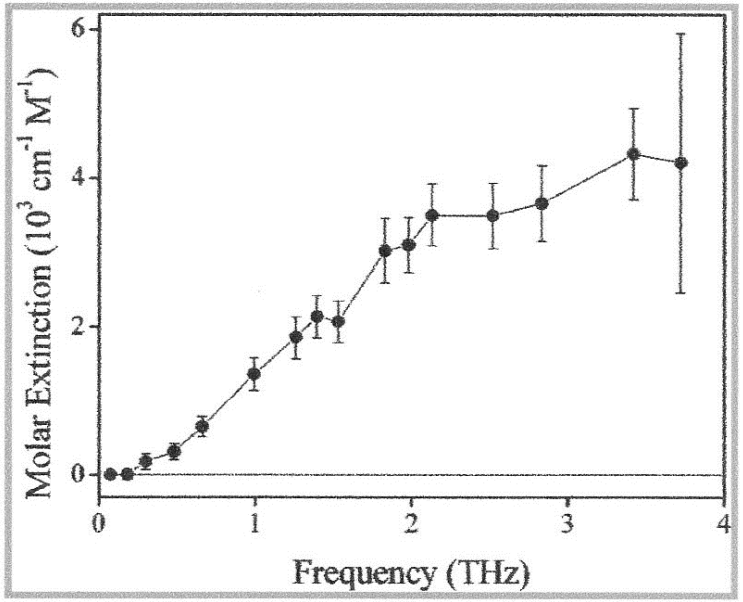


Figure 3. The terahertz molar extinctions of solvated lysozyme (along with its hydration shell) provide a measure of its low-frequency vibrational dynamics. Above ~0.2 THz, we observe a broad spectrum with an initial fast rise in absorption, and a high-frequency plateau/ saturation above ~2 THz.

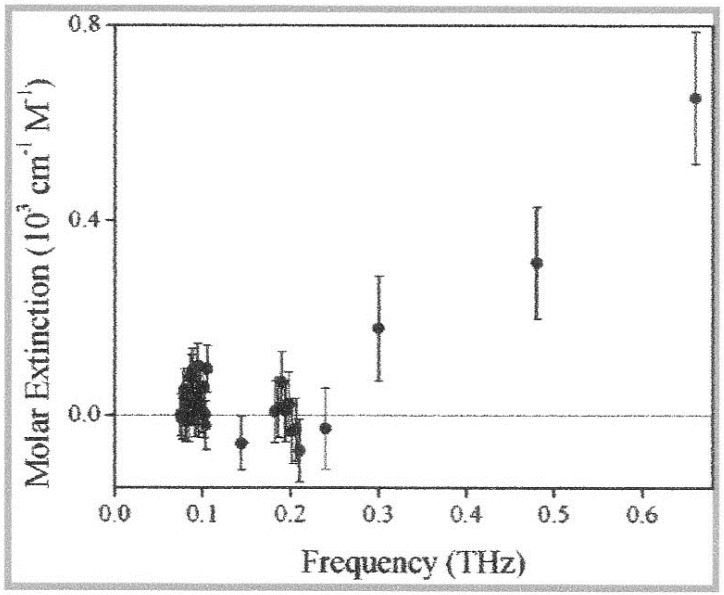


Figure 4. We see no evidence of vibrational dynamics below ~0.2 THz in solvated lysozyme. Instead, we observe a critical on-set of extinction between 0.2 and 0.3 THz. This low-frequency cutoff may simply arise from the finite size of the protein.

Xu, Plaxco & Allen (2006) J. Phys. Chem. B, 110, 24255-24259.

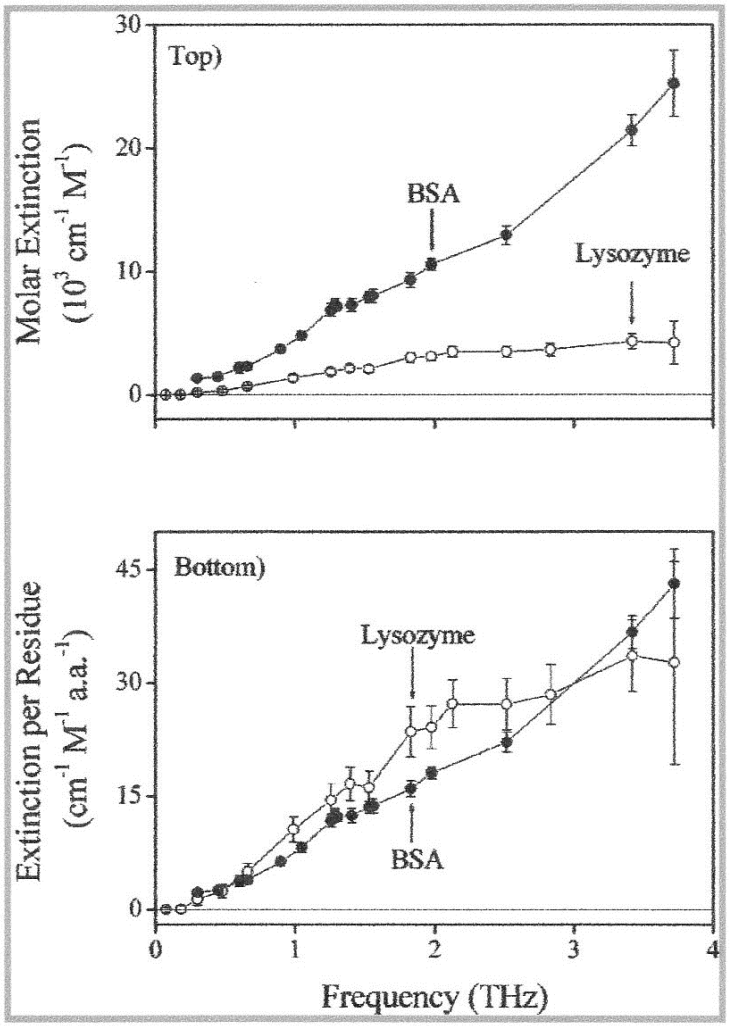


Figure 5. (Top) The absolute teraherz spectra of solvated lysozyme (○) and BSA[21] (●) differ significantly. For example, the monotonic increase in extinction observed for BSA tapers off and saturates above ~2 THz for lysozyme. (Bottom) The apparent greater overall absorption of the BSA is largely mitigated by normalizing the spectra by the number of amino acid residue.

Fig. 4 Lysozyme (0.2 mm - 0.1 mm by smoothed data)

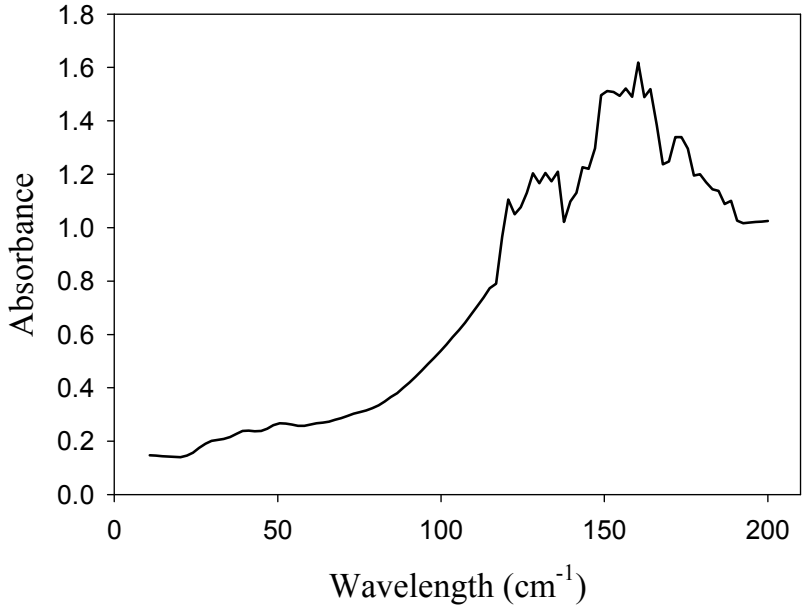


Fig. 4 BLG (0.2 mm - 0.1 mm by smoothed data)

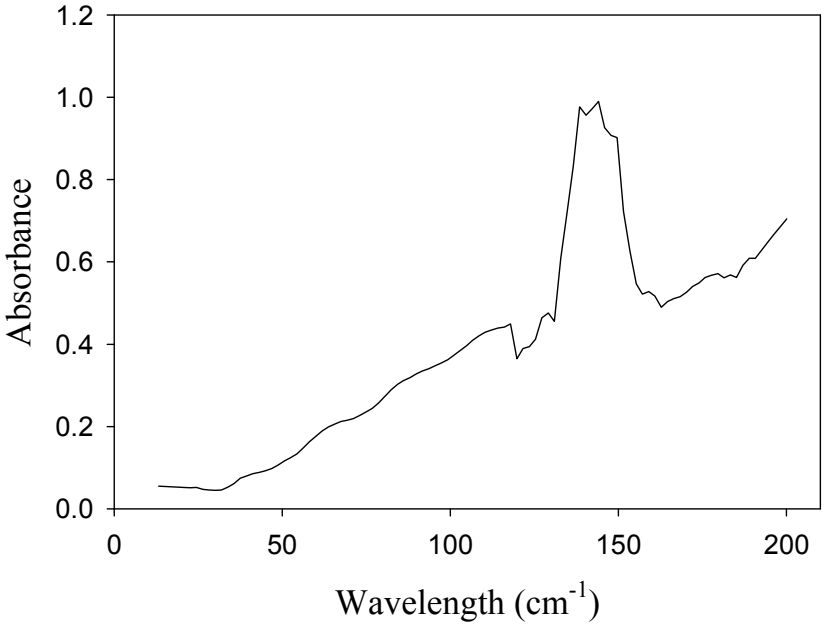


Fig. 4 Myoglobin (0.2 mm - 0.1 mm by smoothed data)

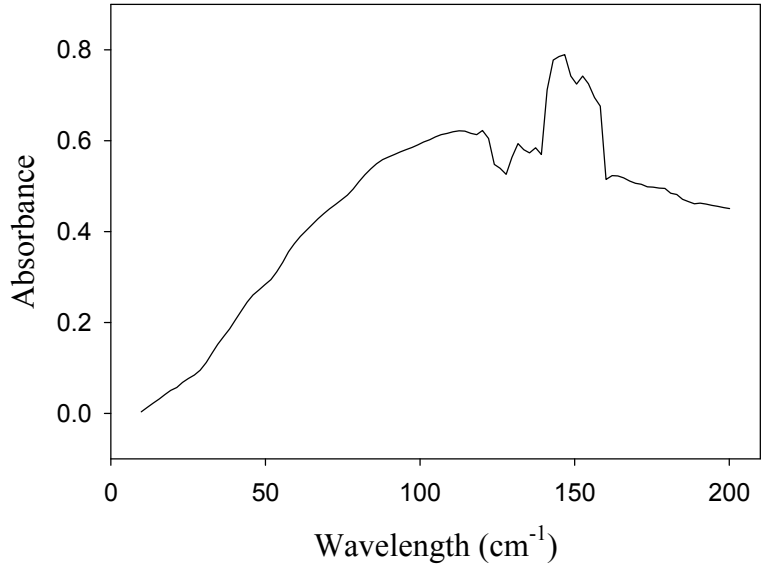


Fig. 4 α-synuclein (0.2 mm - 0.1 mm by smoothed data)

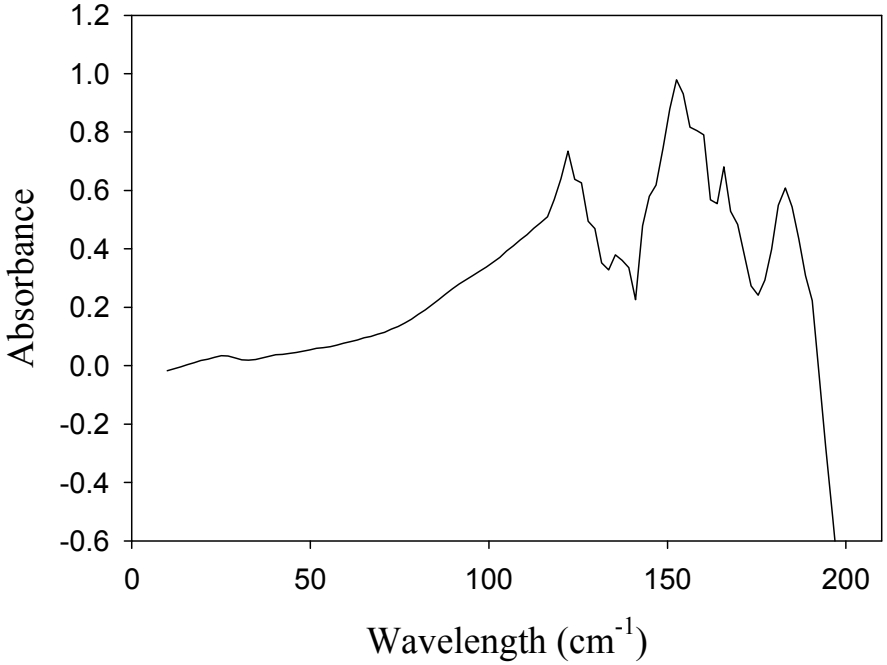
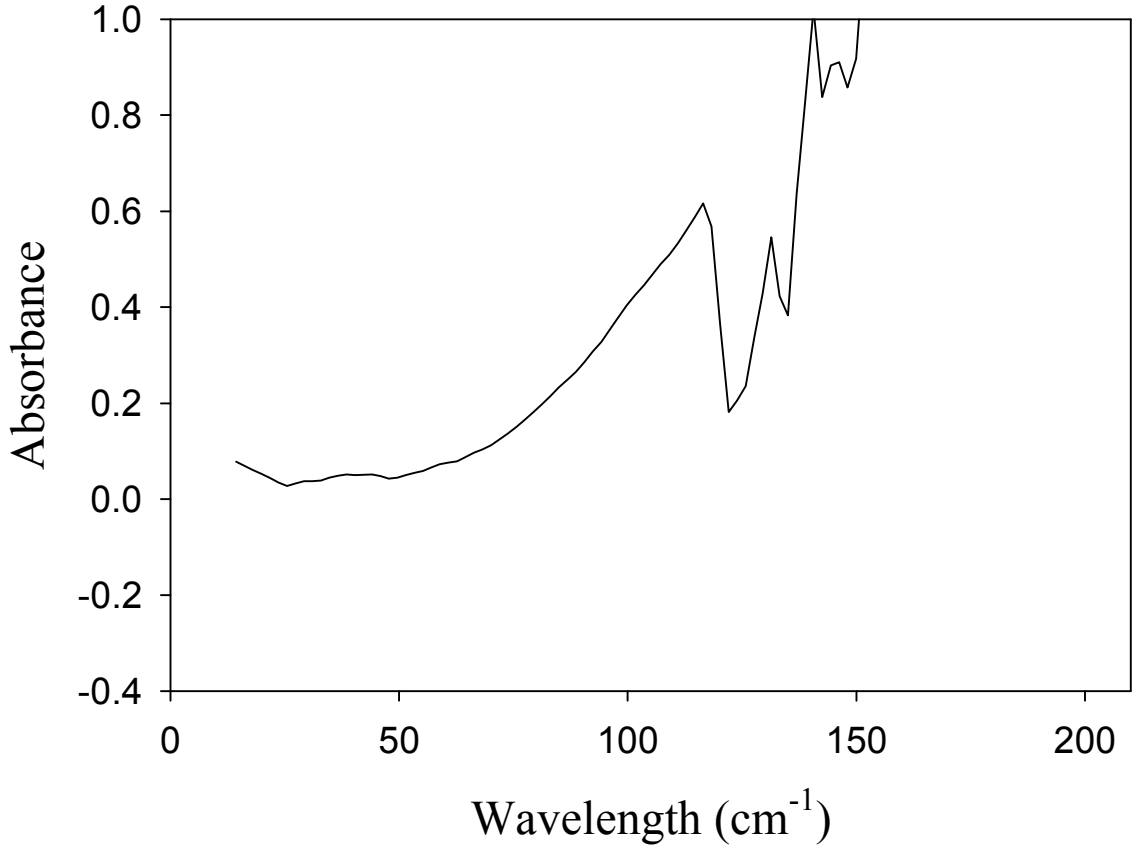


Fig. 3 src SH3 (0.2 mm - 0.1 mm by smoothed data)



An extended dynamical hydration shell around proteins λ*6–85

Simon Ebbinghaus, Seung Joong Kim, Matthias Heyden, Xin Yu, Udo Heugen, Martin Gruebele, David M. Leitner, and Martina Havenith

PNAS December 26, 2007 vol. 104 no. 52 20749–20752

$I(d) = I_0 \exp(-\alpha d) + C$, with I_0, α, d, and C corresponding to the intensity before the probe, the absorption coefficient of the probe, the layer thickness of the probe, and the detector offset, respectively.

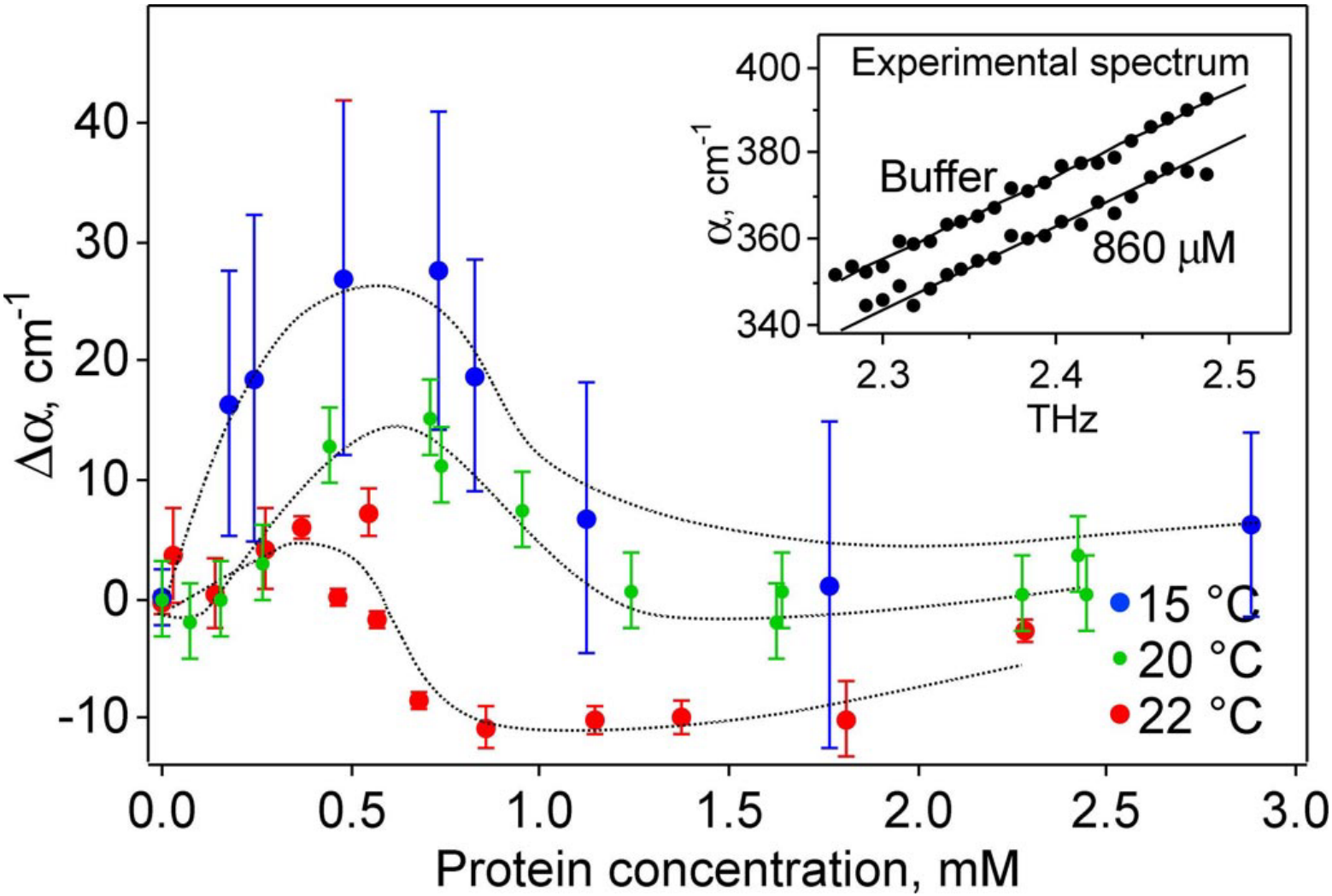
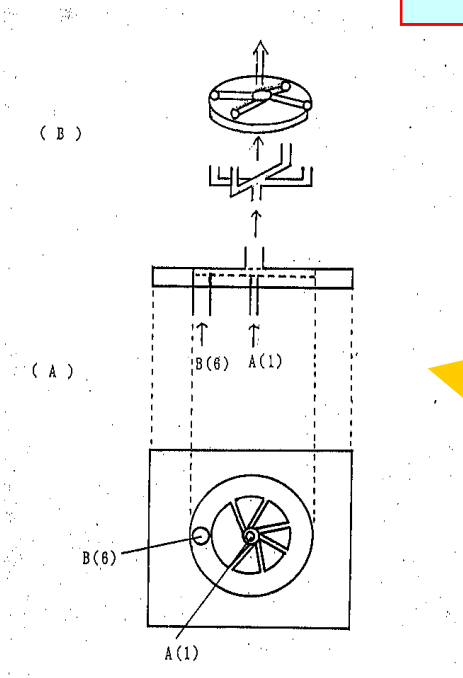
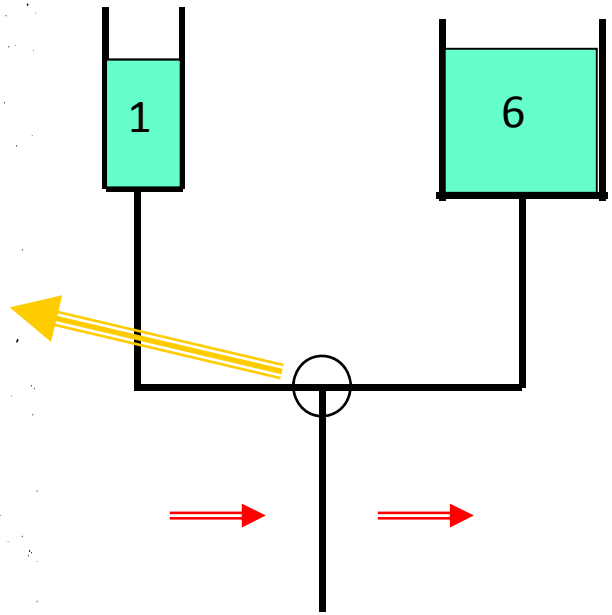


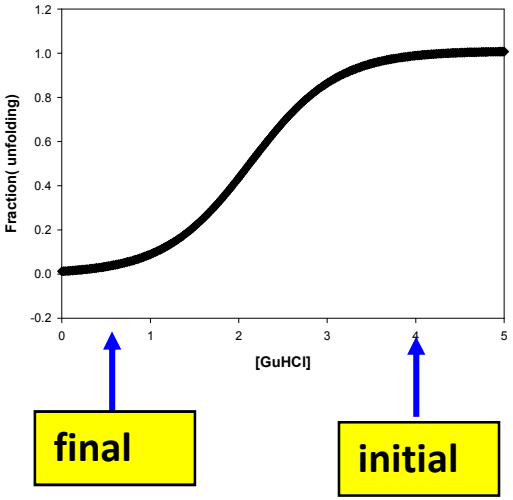
Fig. 1. Difference in the THz absorption coefficient at **2.25 THz** relative to bulk water plotted against concentration to 3 mM at 15° C, 20° C, and 22° C. The absorbance depends nonlinearly on concentration in this region. Note that the THz absorption for bulk water (zero point) increases with increasing temperature. (Inset) The frequency dependence of the absorption coefficient is linear between 2.25 and 2.55 THz (22° C: comparison of buffer and at a protein concentration of 860 M).

2. 蛋白質のフォールディング

stopped-flow apparatus







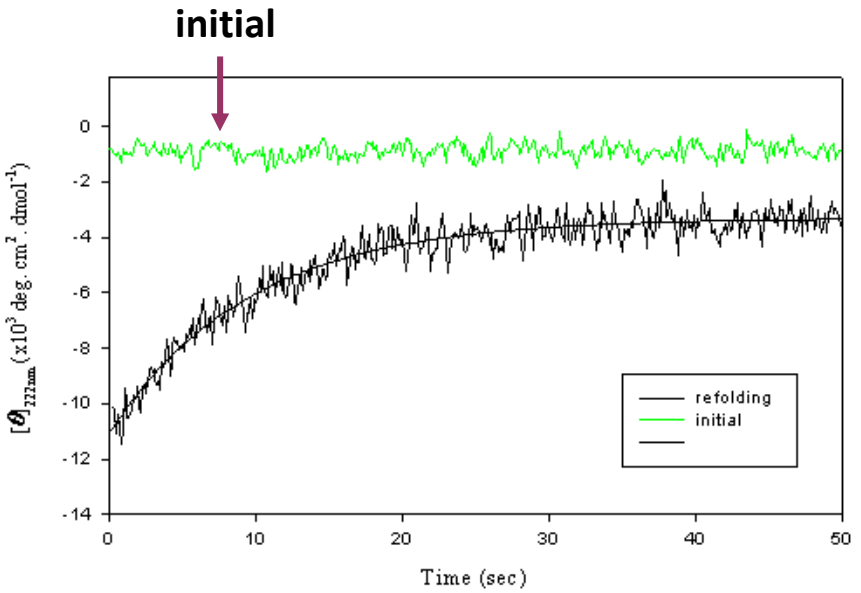
dead time 6 ms

Initial: 1: protein in unfolded buffer

6: unfolded buffer

Refolding: 1: protein in unfolded buffer

6: buffer



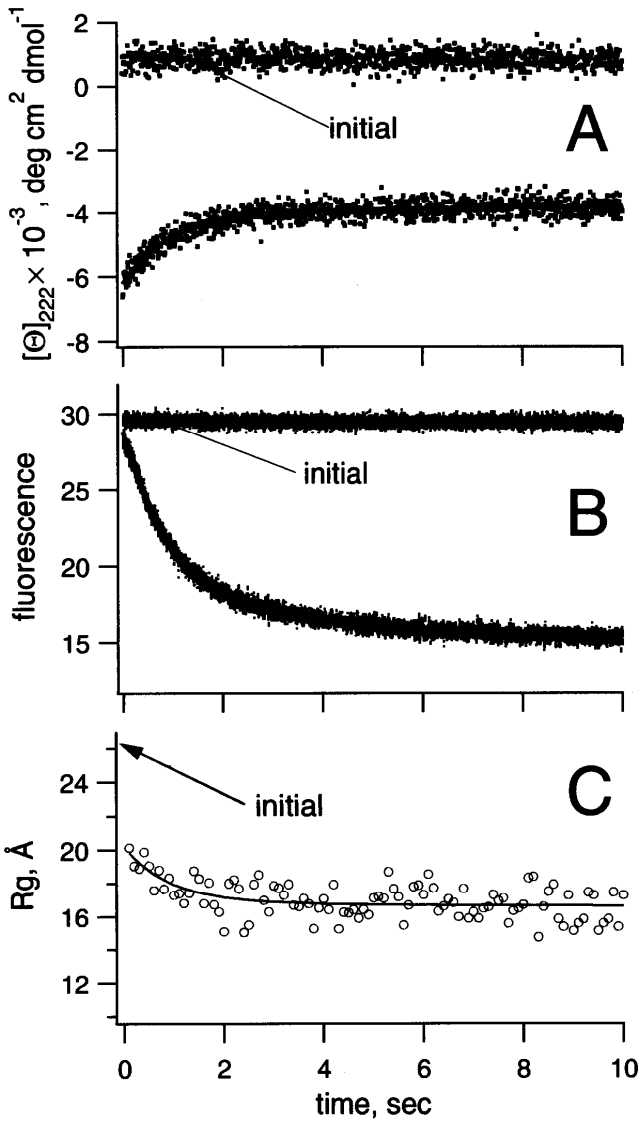
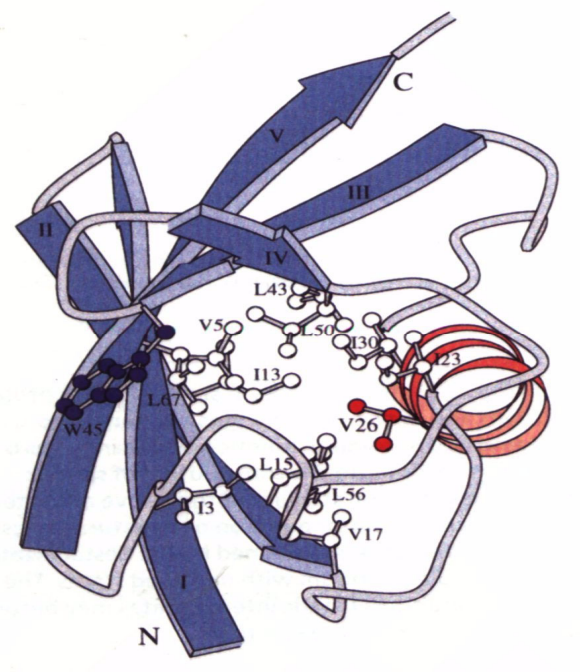


figure 3



Ub

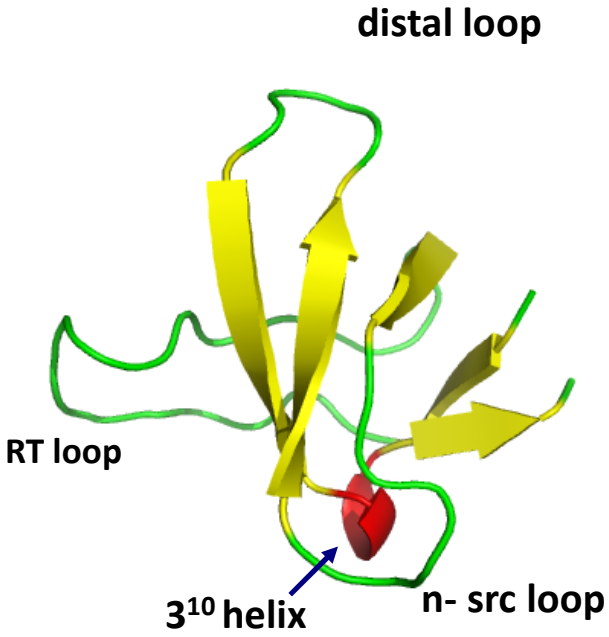
CD amplitude is indep. of T or EGOH concentration.

Ub is already compact after the burst phase.

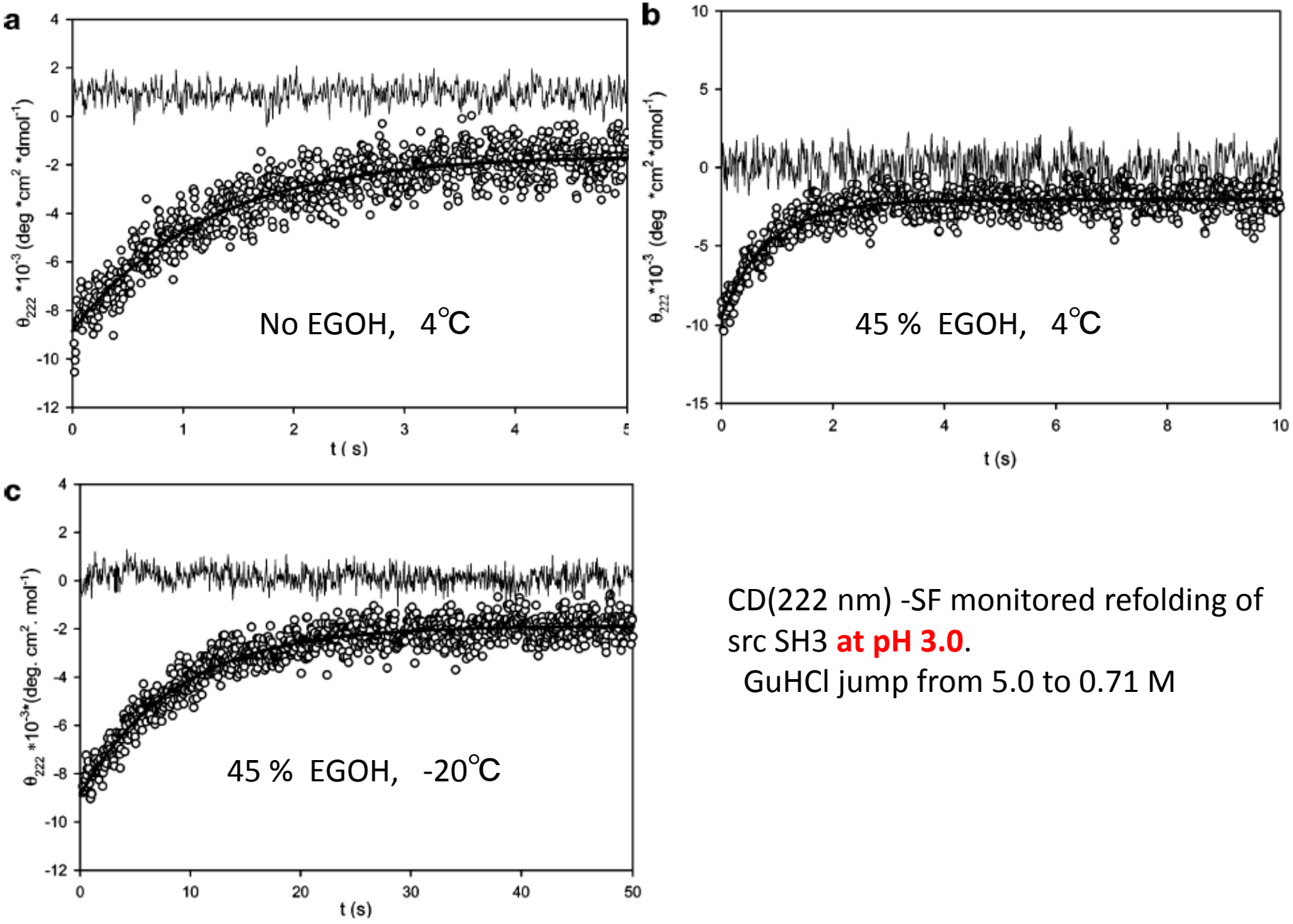
Qin et al. (2002) JPC
Larios et al. (2004) JMB

src SH3 domain

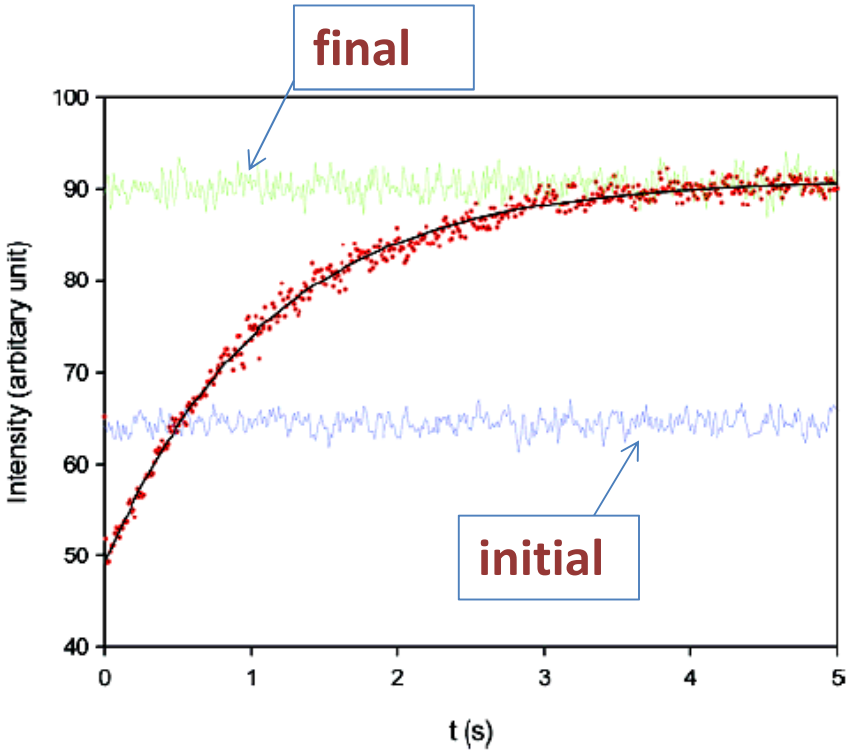
The SH3-fold consists of two small orthogonal three stranded β-sheets with an associated irregular two-stranded sheet packing against each other in a sandwich form.

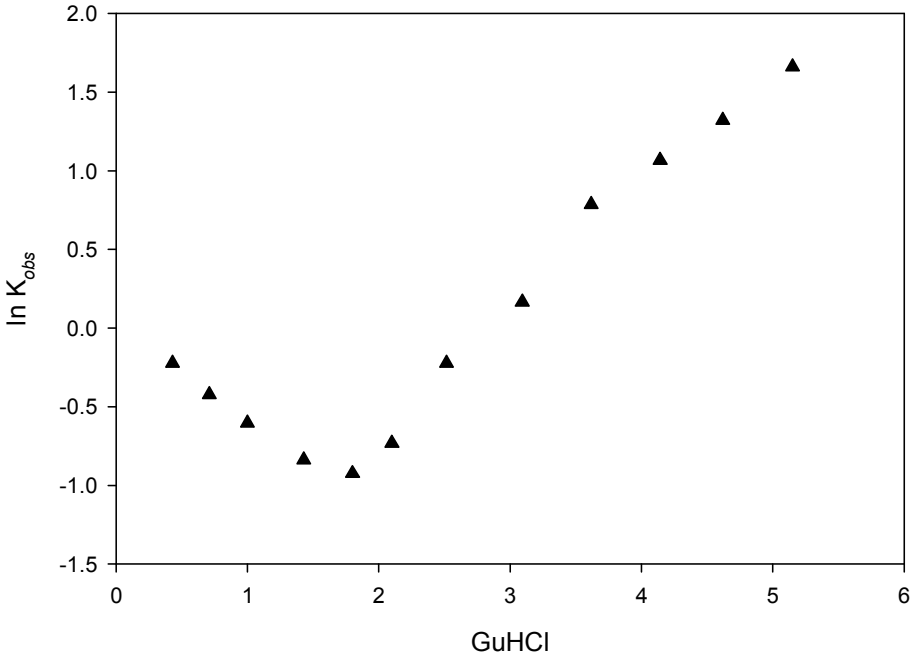


PDB ID: 1SRL



CD(222 nm) -SF monitored refolding of src SH3 **at pH 3.0**.
GuHCl jump from 5.0 to 0.71 M





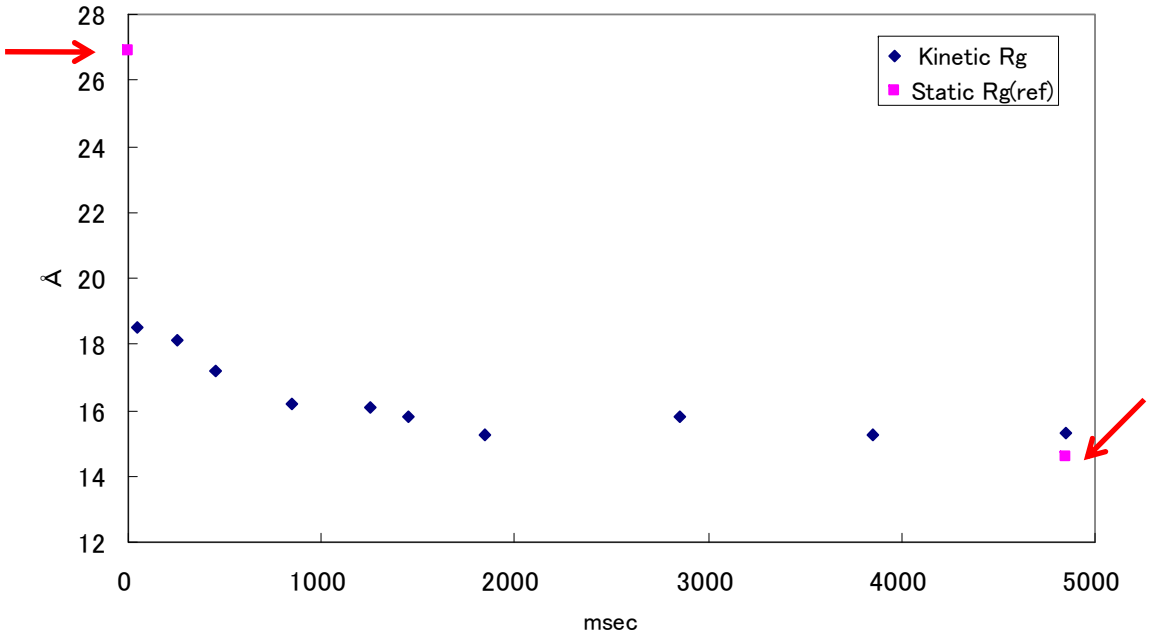
Fluorescence –monitored folding of src SH3 at pH3.0, 4 ℃
Excitation at 295 nm.
Fluorescence above 325 nm was collected.

Chevron plot of SH3 folding rate observed by fluorescence at 4 ℃ in 50mM PBS, pH 3.0

X-ray Scattering Study

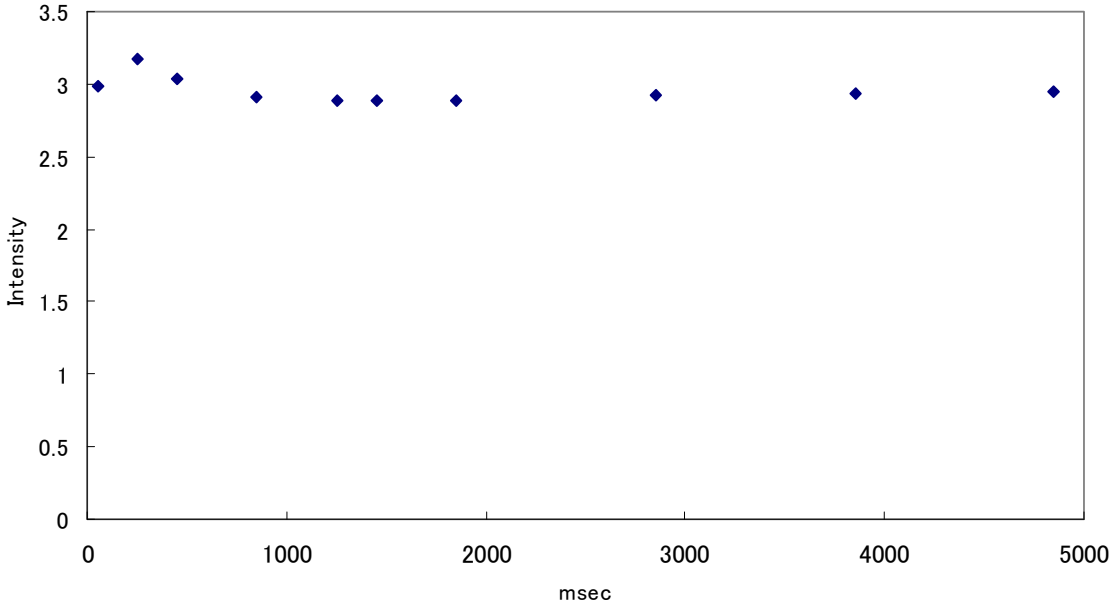
Rg value vs. time

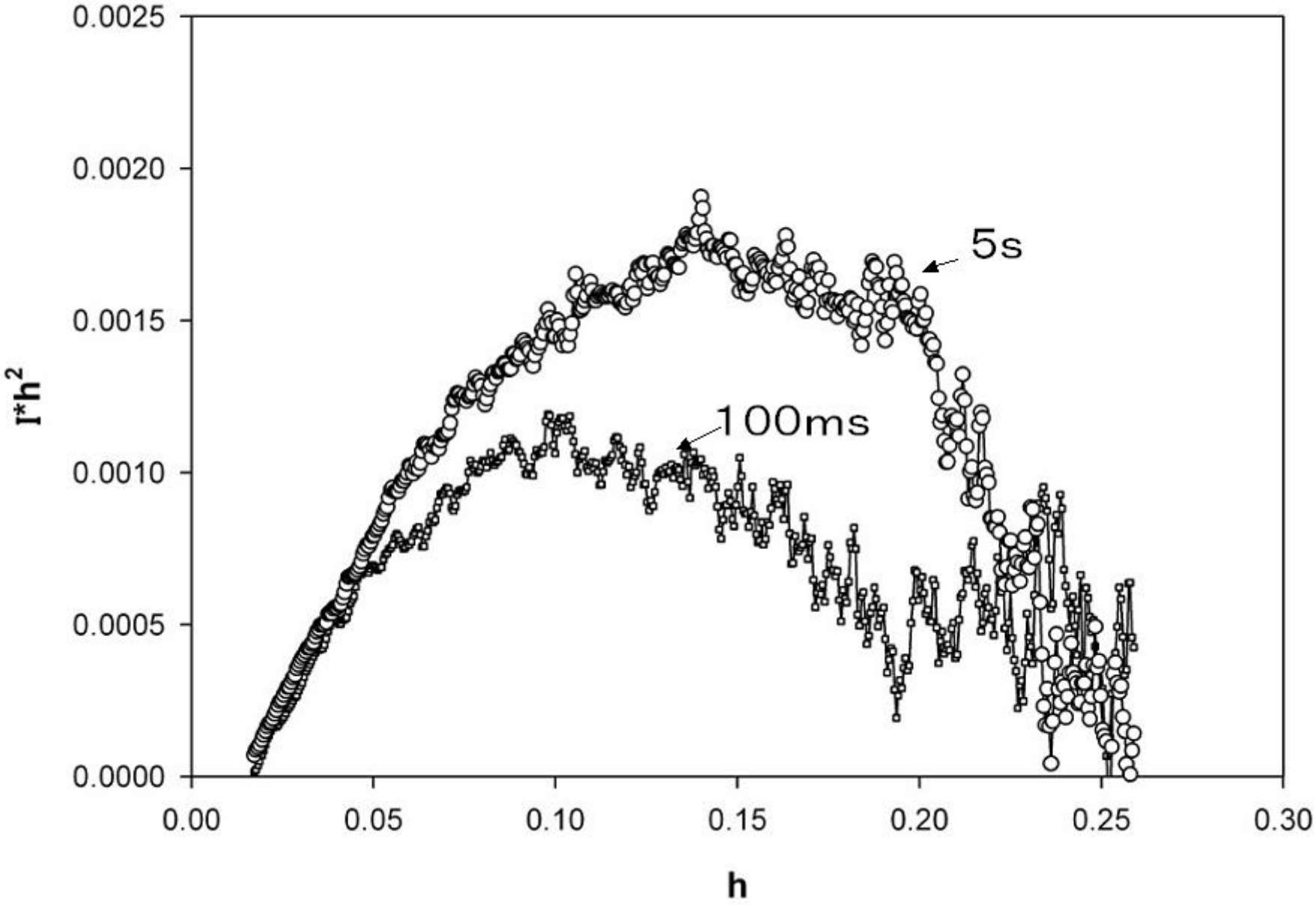
- show static unfolding and native state value.



I_0 value vs. time

I_0 show almost no change.
There are no association effect
in this refolding process.




0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
I*h²
0.00
0.05
0.10
0.15
0.20
0.25
0.30
h
5s
100ms

At which step does hydration occur?

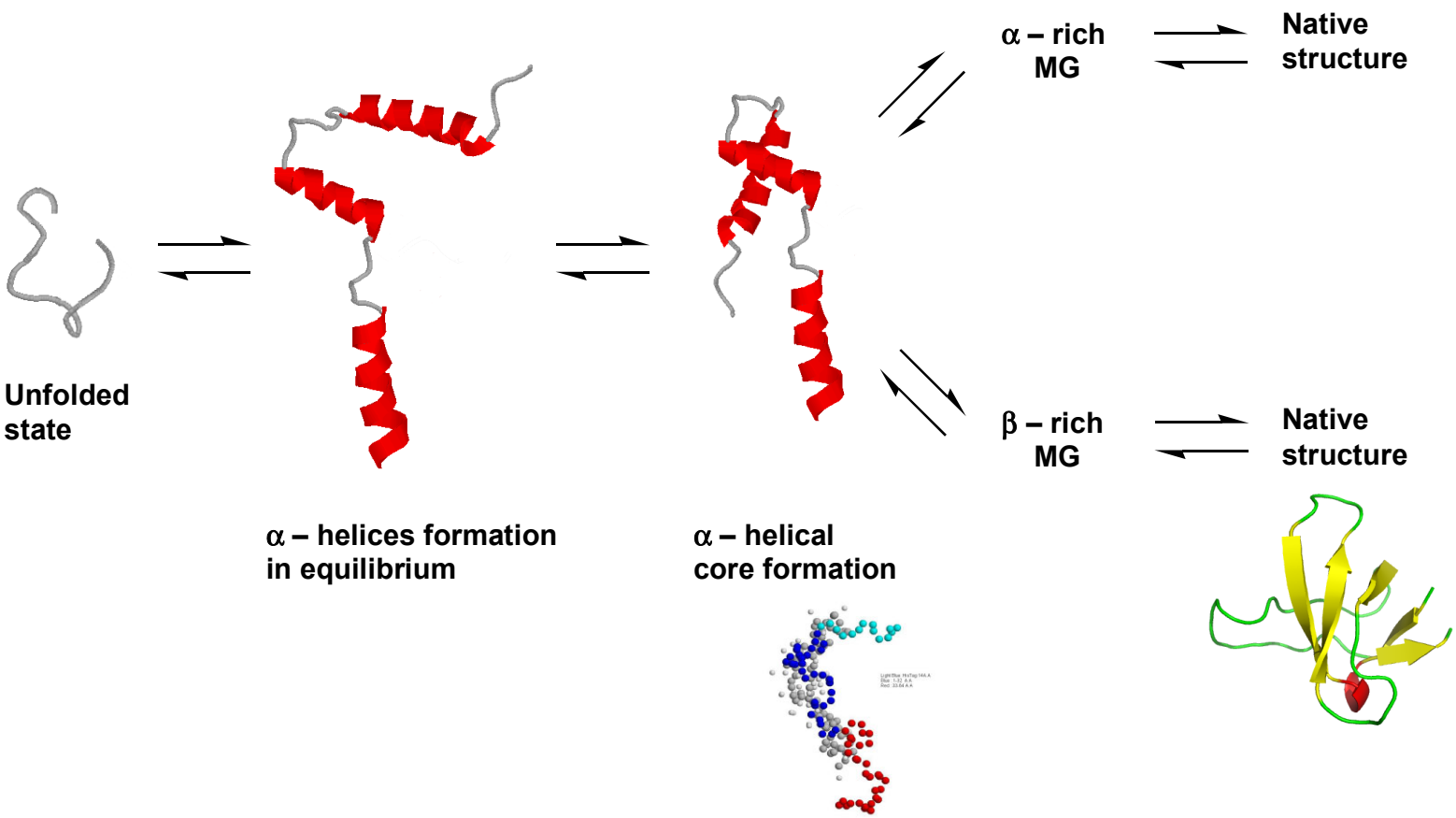


Figure 4. A proposed scheme of the initial events of protein folding, suggesting the importance of α-helical core formation in case of β-rich proteins as well as α-helical rich proteins. (Qin et al. (2001) FEBS)

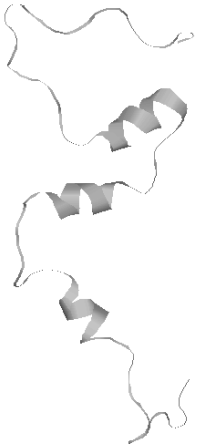
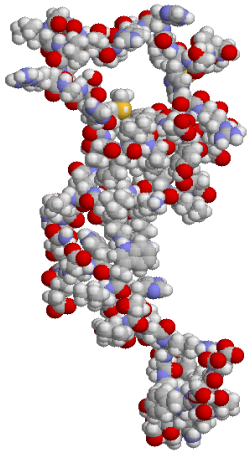


Fig.7 structure I (a) ribbon model



(b) all atom model

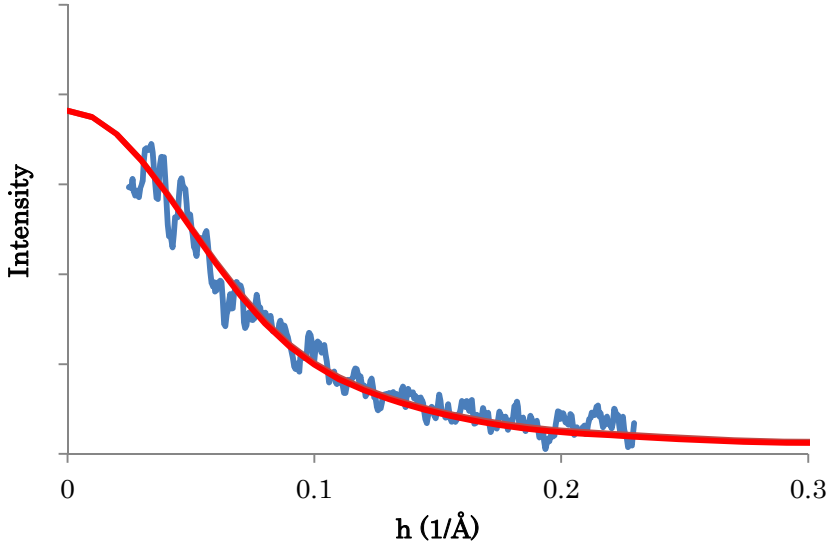


Fig.8 SAXS intensity
Blue: Experimentally obtained SAXS intensity of the intermediate
Red: Calculated SAXS intensity about structure I

Real-Time Detection of Protein–Water Dynamics upon Protein Folding by Terahertz Absorption Spectroscopy**

Seung Joong Kim, Benjamin Born, Martina Havenith, and Martin Gruebele*

Angew. Chem. Int. Ed. 2008, 47, 6486 –6489

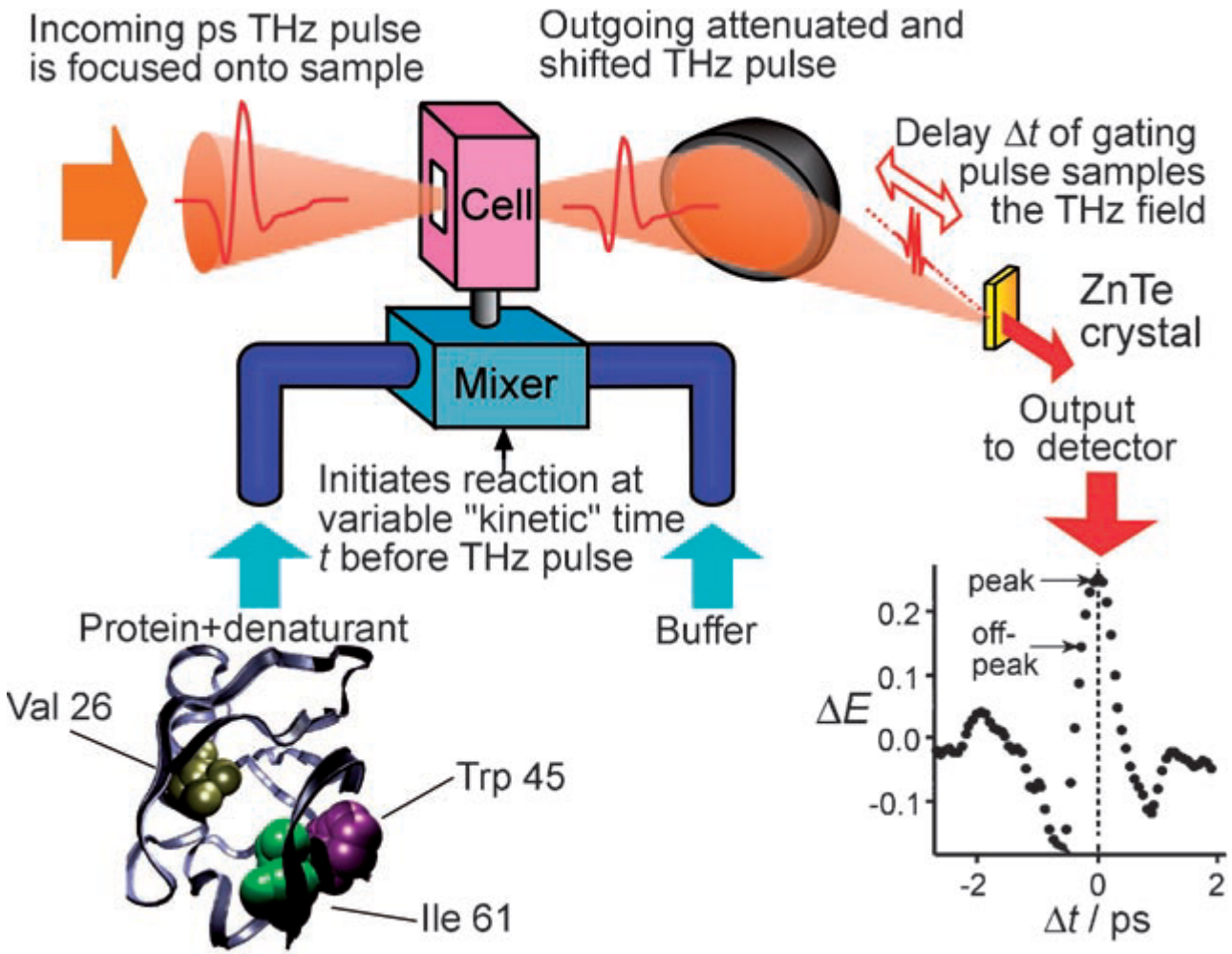


Figure 1. KITA setup: THz pulses pass through a stopped-flow cell, where a mixer combines denatured ubiquitin with denaturant-free buffer to start refolding. The shape of the transmitted THz electric field is detected using a ZnTe crystal and an 800 nm gating pulse delayed by Dt. The difference DE of the electric field between denaturant-free 1.5 mm protein solution and buffer is shown. For kinetics, the THz pulse is detected near the maximum electric field, and the mixer is scanned in time t with respect to the THz pulse.

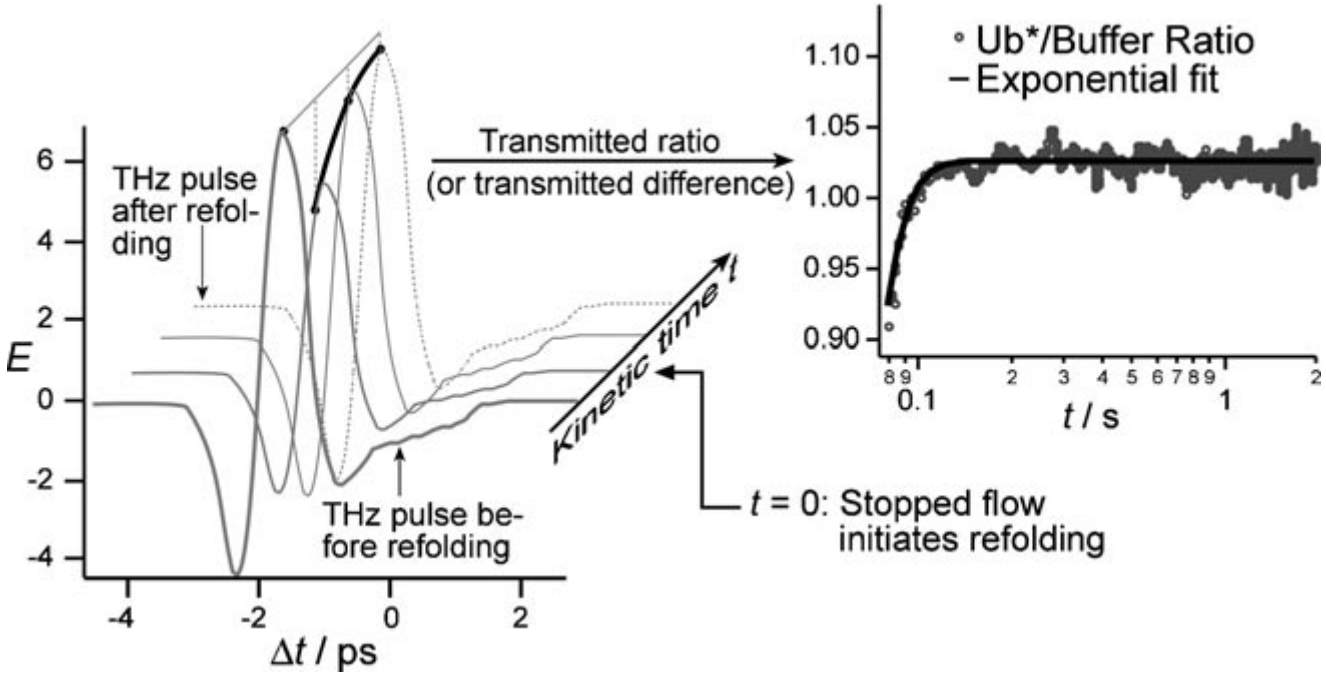


Figure 3. Left: electric field E of THz pulses. As the mixer is scanned in time t with respect to the THz pulse, the field changes because the folded protein solution has different THz absorbance and refractive index than the unfolded protein solution. Right: the ratio of protein to buffer signal reflects the refolding kinetics of Ub* (208C, water/ethylene glycol buffer).

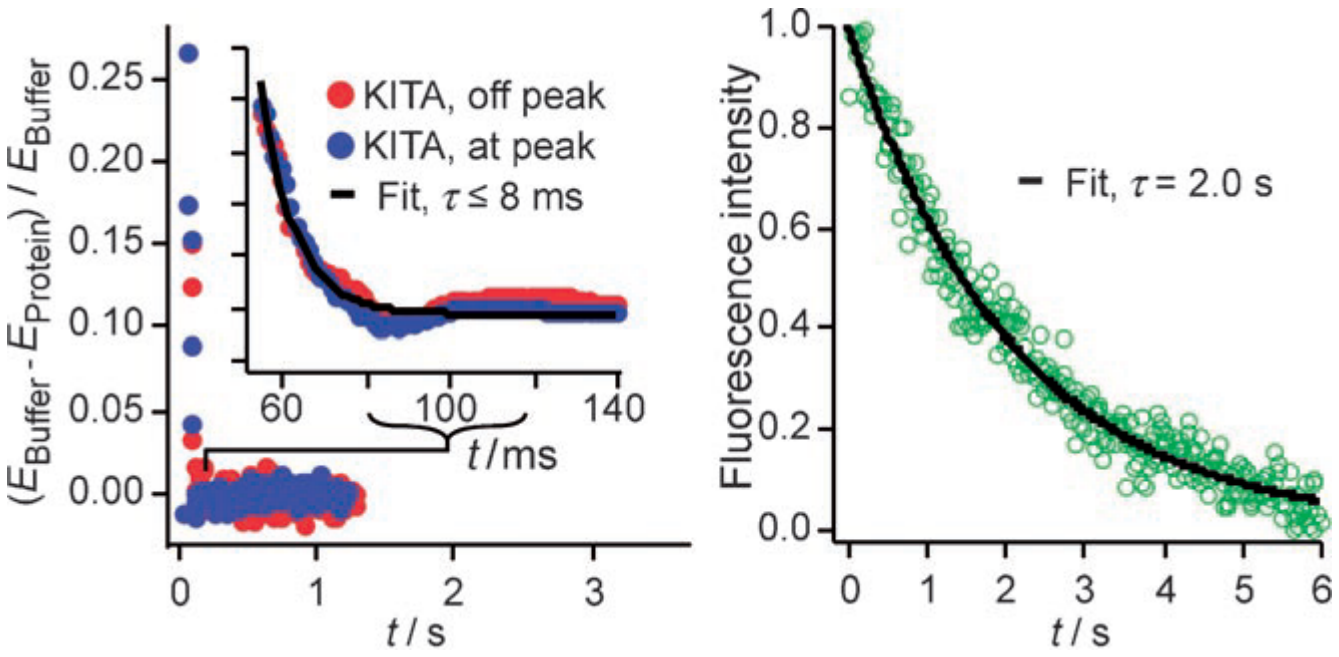


Figure 4. Ub*V26A kinetics. Left: Terahertz transmission on and off the transmitted electric field peak yields identical millisecond kinetics at 208C. Right: Fluorescence-detected kinetics are much slower.

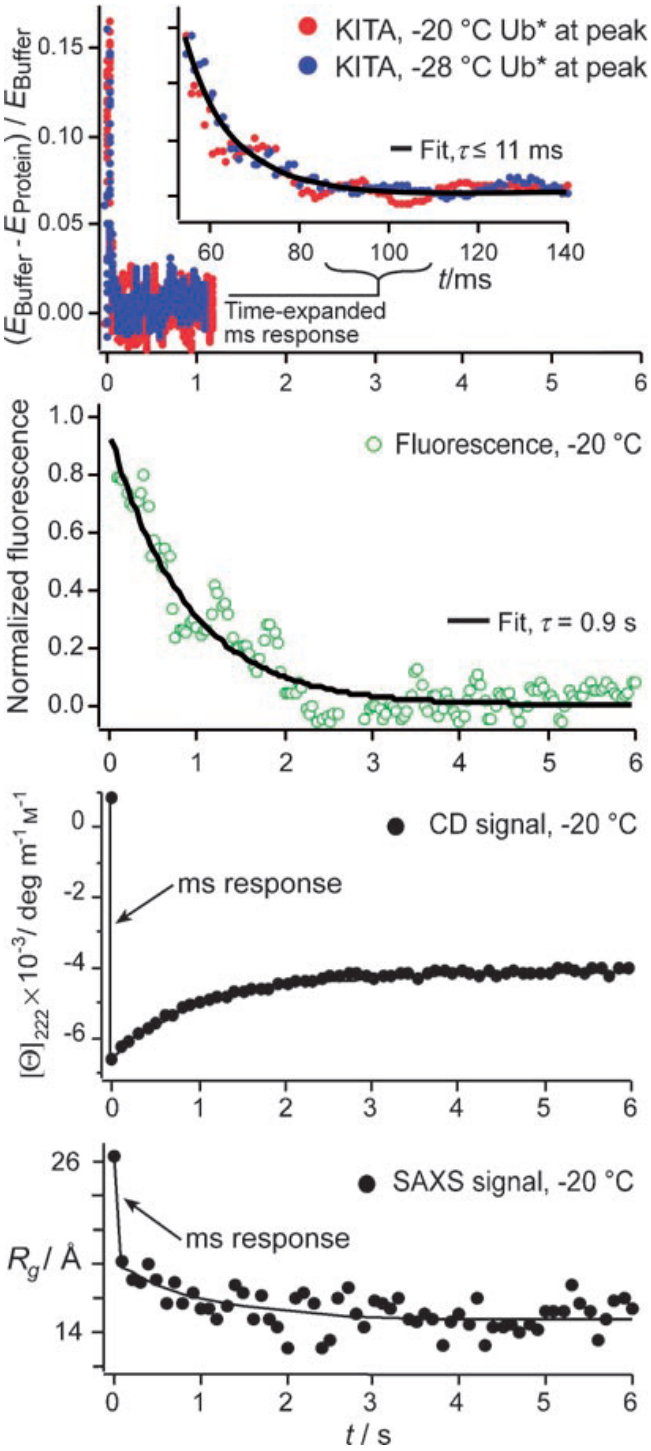
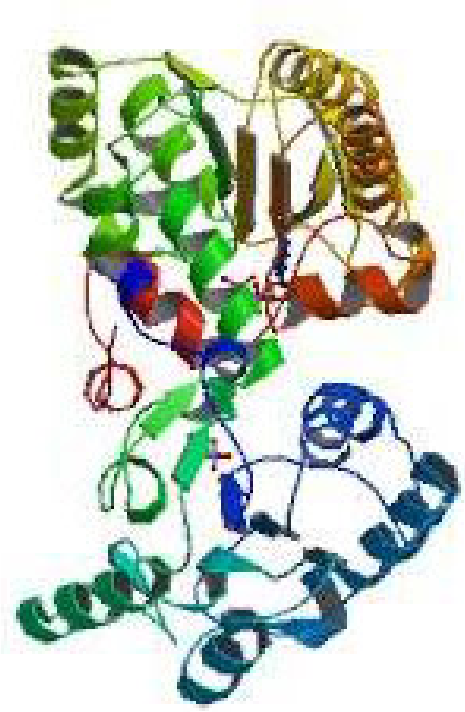


Figure 5. KITA, fluorescence, CD, and SAXS refolding kinetics of Ub*.
The bottom two panels are adapted from reference [17]. Because of the dead time, the KITA fit is an upper limit.

3. THZをトリガー・プローブとして蛋白質の動きを見る

蛋白質の柔らかさは，機能と直接関係する重要な因子であると考えられている。THZ光は，それを誘起して柔らかさと機能の因果関係を研究する重要な tool となることが期待される。



pig muscle PGK complexed with　ATP　(from PDB)

まとめ

1-1. 蛋白質の低周波内部振動は，蛋白質の生理的機能と重要な関係がある。THZ 測定は，それを測定する重要な手段である。

1-2. THZは，蛋白質の水和構造の研究に重要な手段である。

２．蛋白質のフォールディング時には，水和水がどの過程でリリースされるかが重要である。THZ はそれに重要な測定手段を与える。

３．強い THZ の光源は，蛋白質の特定の内部振動を強くさせる（共鳴）。それにより蛋白質の機能と蛋白質の柔らかさとの関係を直接関係する重要な Tool を提供できる。

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T. Matsumoto

SAXS-MD

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Stopped-flow

T. Nagamura & T. Nakagawa (UNISOKU)

src SH3 domain

S. Vyas & A.L. Fink (UCSC)

D. Baker (U. Washington)

Ub, fyn SH3

M. Gruebele, E. Larios (UIUC)

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