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多機能タンパク質の機能発現の機構解明における小角散乱の役割

Role of small-angle scattering in unraveling the recognition mechanism by a multifunctional protein

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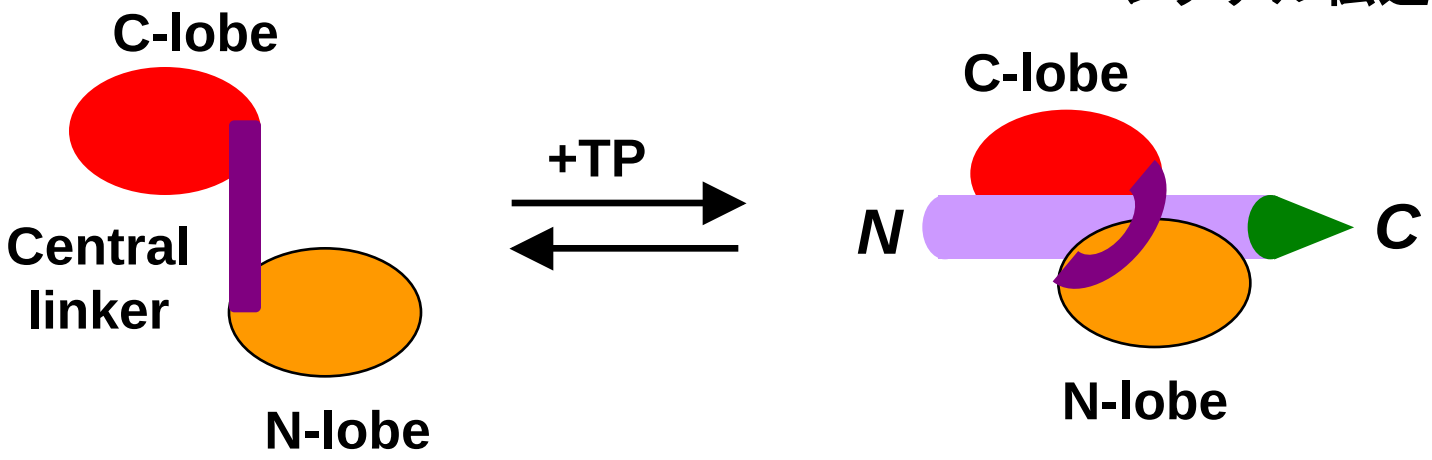
Calmodulin (CaM)

免疫細胞の生理機能
細胞の活性化からアポートーシス
シグナル伝達経路



dumbbell → **Globular**

（Hydrophobic Interaction)

Flexibility of central linker

TP: α-helix

Patches
Flexibility
⟷
Anchoring residue
Position

↓

1-(5)-8-14 : Motif

HIV-gp41:

Solution structure of Ca^{2+}/calmodulin complexed with a lentivirus lytic peptide 1 reveals a novel mode of molecular recognition,
Y.Izumi, A.Amano, T.Saito, Y.Jinbo: *J.Appl. Cryst.* (2007) **40**, S179-S183.

Peptide name	Primary sequence (1 5 8 14)	
MLCK22	KKRWKKNFIAVSAANRFKKISS	Canonical TP/α-helix

1W,5F,8V,14F:anchoring residues, if ***W*** **at 7, no globular structure**

LLP1:	1 14
HV1A2Δ10	DRVIEVVQGAYRAILHIHRR
HV1B1Δ10	DRVIEVVQGAYRAIRHIPRR
HV1H2Δ10	DRVIEVVQGACRAIRHIPRR
HV1H2WΔ10	DRVIEVVQGWCRAIRHIPRR
HV1rA2Δ10	RRHIHLIARYARQAVEIVRD
	1 5 8 14

Abbreviations for the amino acids residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y,Tyr.

解析1

Guinier Region　$(4\pi^2 R_g^2 s^2/3 \ll 1)$

$I(s,c) = I(0,c)exp\{-4\pi^2 R_g (c)^2 s^2/3\}$

In the dilute limit,

$$Kc/I(0,c) = 1/M + 2A_2 c + \ldots ,$$

$$R_g (c)^2 = R_0^2 - B_{if} c + \ldots$$

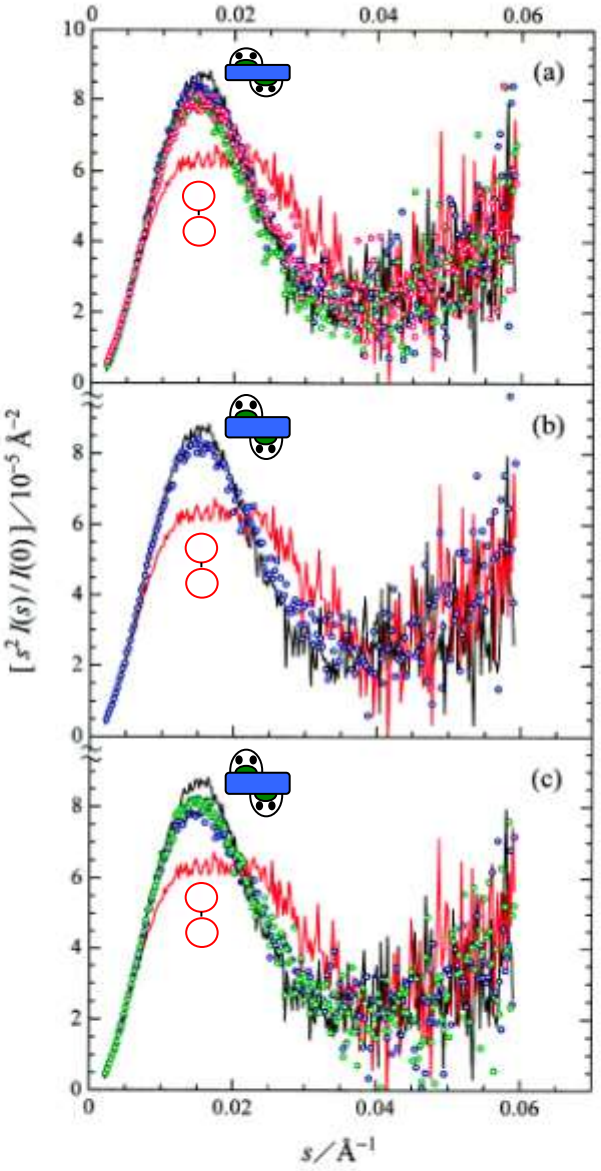
分子量(M), 回転半径(R_0), 相互作用パラメータ (A_2, B_{if})

Kratky Region

Kratky plot (KP: Shape); P(r), d_{max}

$I(s)/I(0)$ instead of $I(s)$

KP



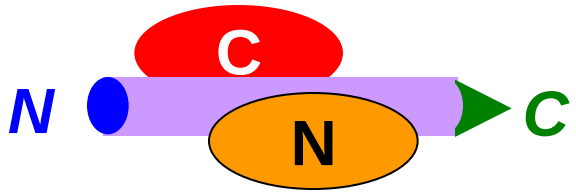
a) **CaM (red); CaM/MLCK22 (black);**
CaM/HV1AΔ3 (blue);
CaM/HV1A2Δ10 (green);
CaM/HV1rAΔ10 (magenta)

b) **CaM (red); CaM/MLCK22 (black);**
CaM/HV1B1Δ10 (blue)

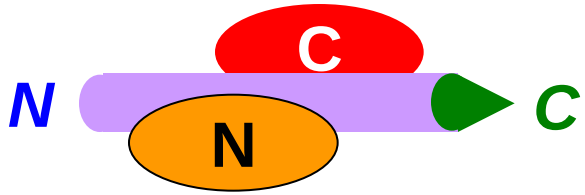
c) **CaM (red); CaM/MLCK22 (black);**
CaM/HV1H2Δ10 (blue);
CaM/HV1H2WΔ10 (green)

Ca^{2+}/CaM adopts a dumbbell-shaped structure, while the Ca^{2+}/CaM/MLCK22 complex adopts a compact globular shape. The data points for the three types of LLP complexes are almost superimposed on those for the Ca^{2+}/CaM/MLCK22 complex, indicating that these complexes adopt almost the same globular structure as that of the Ca^{2+}/CaM/MLCK22 complex, suggesting each peptide adopts an α-helical conformation in the complex.

Globular ⇨ TP: α-helix ⇨ Anchoring Residues ⇨ Motif



	1	8	14
RS20	RRK**W**QKTGH***A*****N**RAIGR**L**SSS		
MLCK22	KKR**W**KKNFI***A*****N**SAANR**F**KKISS		
HV1rA2Δ10	RRH**I**HLIAR***Y***ARQAVE**I**VRD		



LLP1:	14	8	5	1
HV1A2Δ10	DRV**I**EVVQG***A***YR**A**ILH**I**HRR			
HV1B1Δ10	DRV**I**EVVQG***A***YRAIRH**I**PRR			
HV1H2Δ10	DRV**I**EVVQG***A***CRAIRH**I**PRR			
HV1H2WΔ10	DRV**I**EVVQG***W***CRAIRH**I**PRR			

A polarity opposite!!

HIV-MA(p17)

Solution X-ray scattering Reveals a Novel Structure of Calmodulin Complexed with a Binding Domain Peptide from the HIV-1 Matrix Protein p17: Y.Izumi, H.Watanabe, N.Watanabe, A.Aoyama, Y.Jinbo, and N.Hayashi, *Biochemistry* 2008, **47**, 7158-7166.

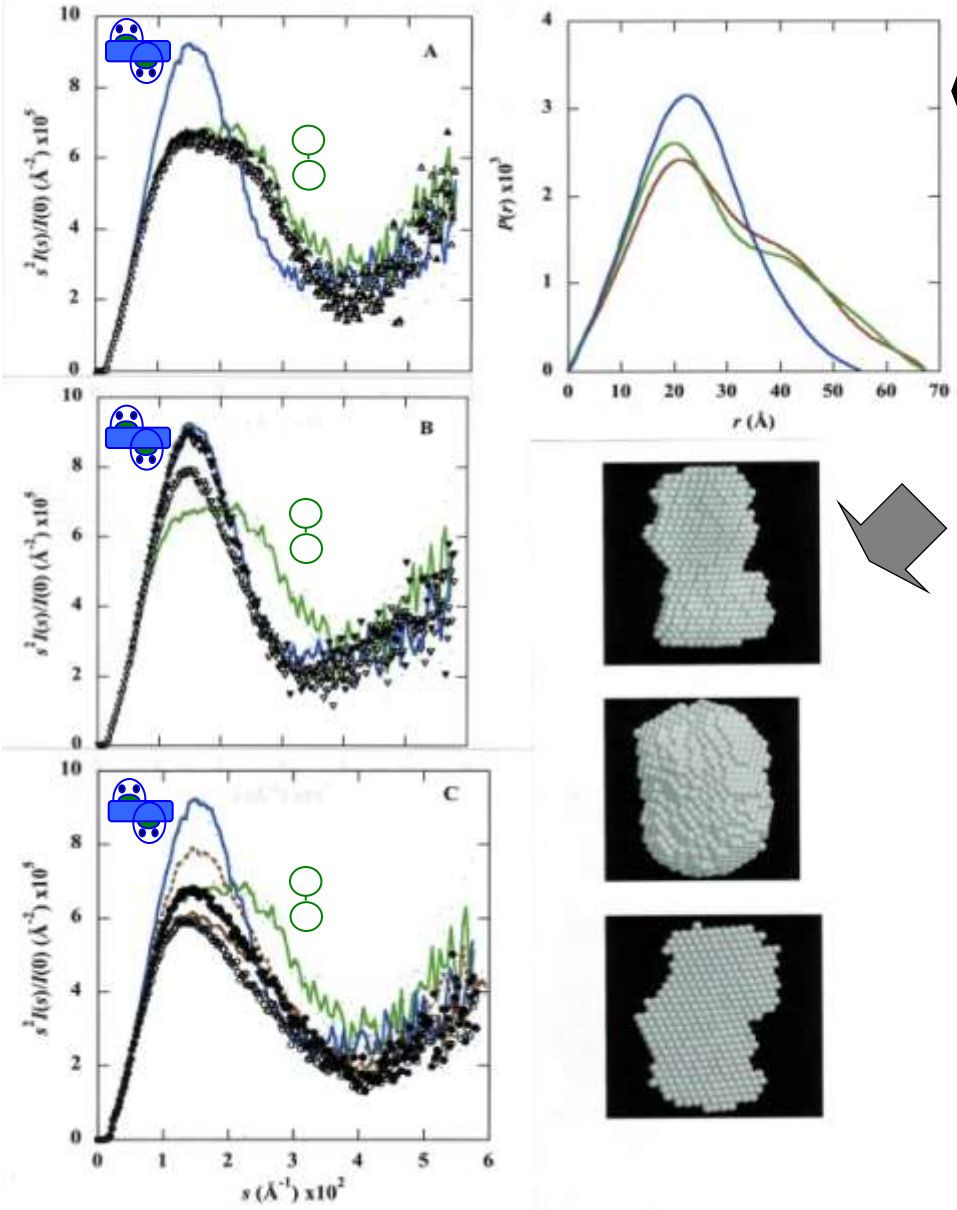
TP

Peptide name	Primary sequence
p17N	GELDRWEKIRLRPGGKKK
p17C	KKKYKLKHIVWASRELERFAVN
p17T	GELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVN

MLCK22 KKRWKKNFIAVSAANRFKKISS

1 5 8 14

KP



P(*r*) (GNOM)

Green: **A**, Blue: **B**, Red: **C**

Shape (DAMMIN)

A: Ca^{2+}/CaM/p17N at 1:2

B: Ca^{2+}/CaM/p17C at 1:1

C: Ca^{2+}/CaM/p17T at 1:1

形態の多様性を実証！

解析 2 : Tertiary structural prediction

Sequential morphs:

Ca^{2+}/CaM/RS20 (ff0, 1cdl), Ca^{2+}/CaM (ff17, 1cll) (ff11, ff13, and ff15)

Target docking: Ca^{2+}/CaM/M13 (2bbn)

p17 protein (1tam)

Discover 3 module of Insight II 2000 (Accerlys):energy min. & MD

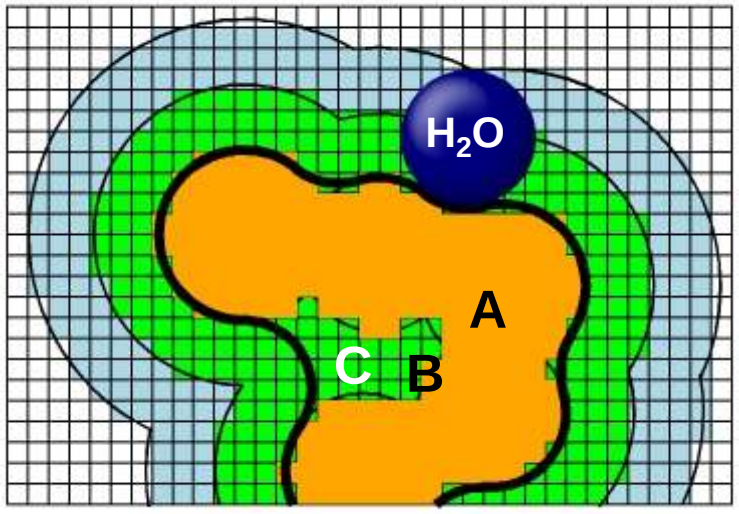
解析 3 : Calculation of SAXS profile of known tertiary structures

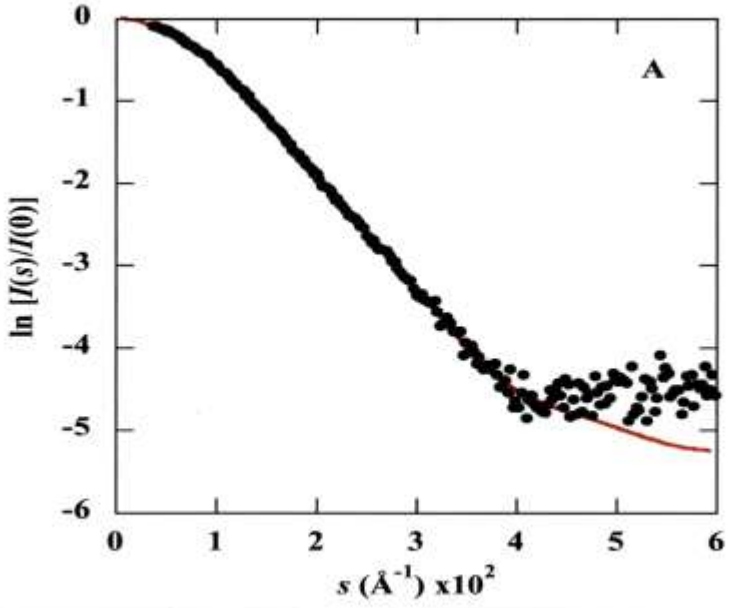
Extension of the Debye's formula

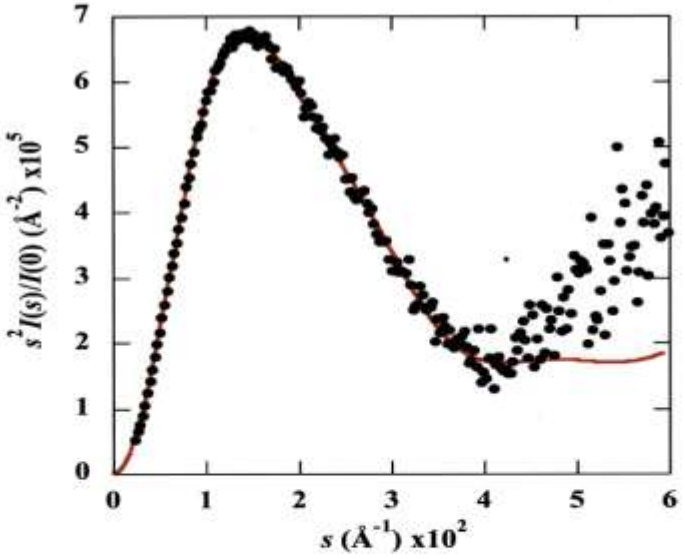
Cube size=0.5Å, r_{H2O}=1.4Å

***I*(*s*)/*I*(0) instead of *I*(*s*):**

No adjustable parameter







SAXS profile & KP of Ca^{2+}/CaM/p17T

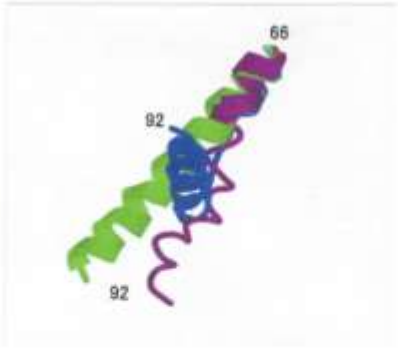
A reasonable fitting of data points except the deviation beyond s>0.043 $Å^{-1}$.

Such a deviation was also observed for both Ca^{2+}/CaM and Ca^{2+}/CaM/*sk*MLCK22 complex.

No instrumental bias!

No adjustable parameter!

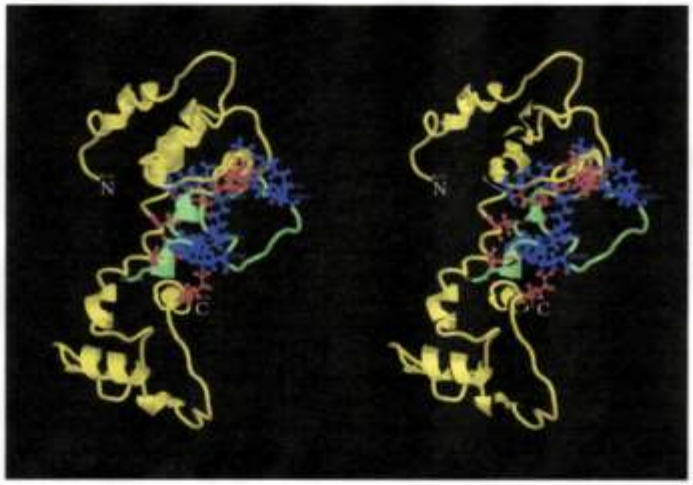
(*Biochemistry* 2008, 47, 7158-7166)



Flexibility of central linker

green:1cll, **blue**: 1cdl,
magenta: ff15

(*Biochemistry* 2008, 47, 7158-7166)



Model for the complex of Ca^{2+}/CaM/p17T

yellow: Ca^{2+}/CaM

green: p17T

C lobe of CaM

139E *139E*

p17T: GELDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVN

74R *78D* *82E* *47E* *50D* *56D* *54E* */67E*

N lobe of CaM (Electrostatic Interaction)

Ca^{2+}/CaM/HIV_LLP1

CaMはLLP1と結合し,球状構造をとる。LLP1の極性はM13, RS20複合体と逆になる:

(1) 3種の異なるHIV-1のCaM結合部位がCaMと球状の複合体を形成する,

(2) 極性を反転させたLLP1を用いても球状構造をとる,

(3) アンカー残基と隣接する7位の残基が嵩高い場合でも球状構造をとる。

CaMの結合により, gp41の3量体形成が阻害される。

Ca^{2+}/CaM/HIV_p17T

CaMの各ロブの構造は複合体形成により変化しない。複合体の全体構造は伸びた構造をとり,回転半径は20.5Åで,ドメイン間距離は34.2Åである。

CaM/p17T複合体の構造は,主として静電的相互作用により安定化されている。

CaMの疎水性パッチは, p17のN末のミリスチル基を隔離する役割を担う。

CaMは, HIV-1に対して, 抑制因子として作用している！

小角散乱は蛋白質分子の機能解析に有用である！