

赤外自由電子レーザーによるアミロイドーシス 治療法の開発に向けた基礎検討

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高エネルギー加速器研究機構
加速器研究施設



JOURNAL OF
SYNCHROTRON
RADIATION

Application study of infrared free electron laser towards development of amyloidosis therapy

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SCIENTIFIC MANUSCRIPT

For review only.

Thursday 24 February 2022

アミロイドーシス

アミロイドタンパク	全身性/局所性	主な発現臓器
免疫グロブリン軽鎖・重鎖	全身性	中枢神経系を除く全ての臓器
血清アミロイドA	全身性	中枢神経系を除く全ての臓器
トランスサイレチン	全身性	心臓、目
アポリポタンパクA	全身性	腎臓
リゾチーム	全身性	腎臓
フィブリノーゲンα	全身性	腎臓
アミロイドβ	局所性	脳組織、中枢神経系
カルシトニン	局所性	甲状腺
ラクトフェリン	局所性	角膜
ゲルソリン	全身性	角膜
シスタチンC	全身性	皮膚
ガレクチン7	局所性	皮膚

透析アミロイドーシス 一手根管症候群



Figure 1. Median nerve palmar distribution.

Reprinted with permission from LeBlanc KE, Cestia W. Carpal tunnel syndrome. Am Fam Physician. 2011;83(8):953.

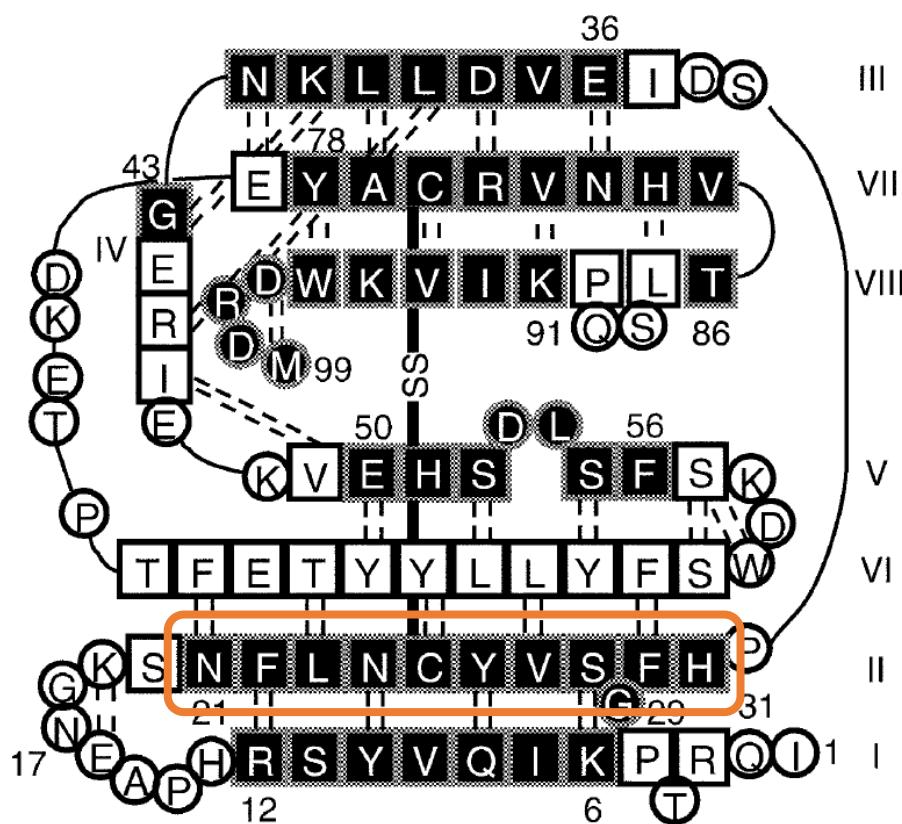
現行の治療法と診断法

Clinical recommendation	Evidence rating	References
Ultrasound measurement of a cross-sectional area of the median nerve by an experienced clinician may be used as a diagnostic test for carpal tunnel syndrome.	C	14, 15
Local corticosteroid injection is effective for more than one month in patients with mild to moderate carpal tunnel syndrome and delays the need for surgery at one year.	A	20-24
Splinting, therapeutic ultrasound, carpal bone mobilization, and nerve glide exercises are effective short-term treatments for carpal tunnel syndrome.	B	25-27
Endoscopic and open carpal tunnel release are equally effective, long-lasting treatments for carpal tunnel syndrome.	A	30

A = consistent, good-quality patient-oriented evidence; B = inconsistent or limited-quality patient-oriented evidence; C = consensus, disease-oriented evidence, usual practice, expert opinion, or case series. For information about the SORT evidence rating system, go to <http://www.aafp.org/afpsort>.

現行法以外の治療法を
患者さんに提供
⇒チョイスの機会を与える

β 2-microglobulin → 99残基からなり、アミロイド線維を形成



Kazuhiro Hasegawa et al.,
Biochem. Biophys. **304**, 102 (2003).

内部11残基ペプチドが
重合しやすい

NFLNCYVSGFH 青: 親水性

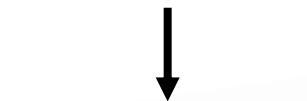
赤: 疎水性



monomer

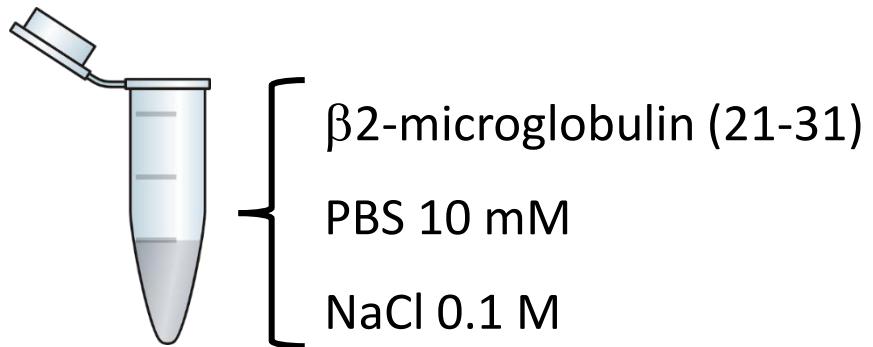


seed



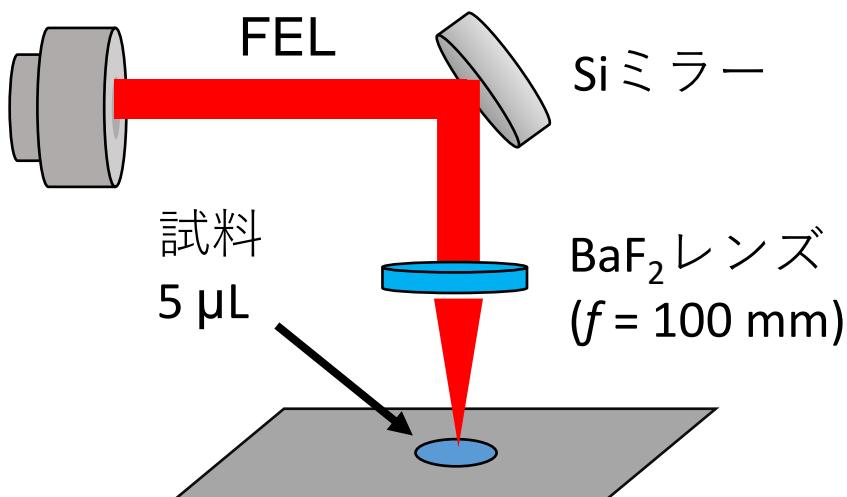
amyloid fibril

試料



37°C
4 days
線維化

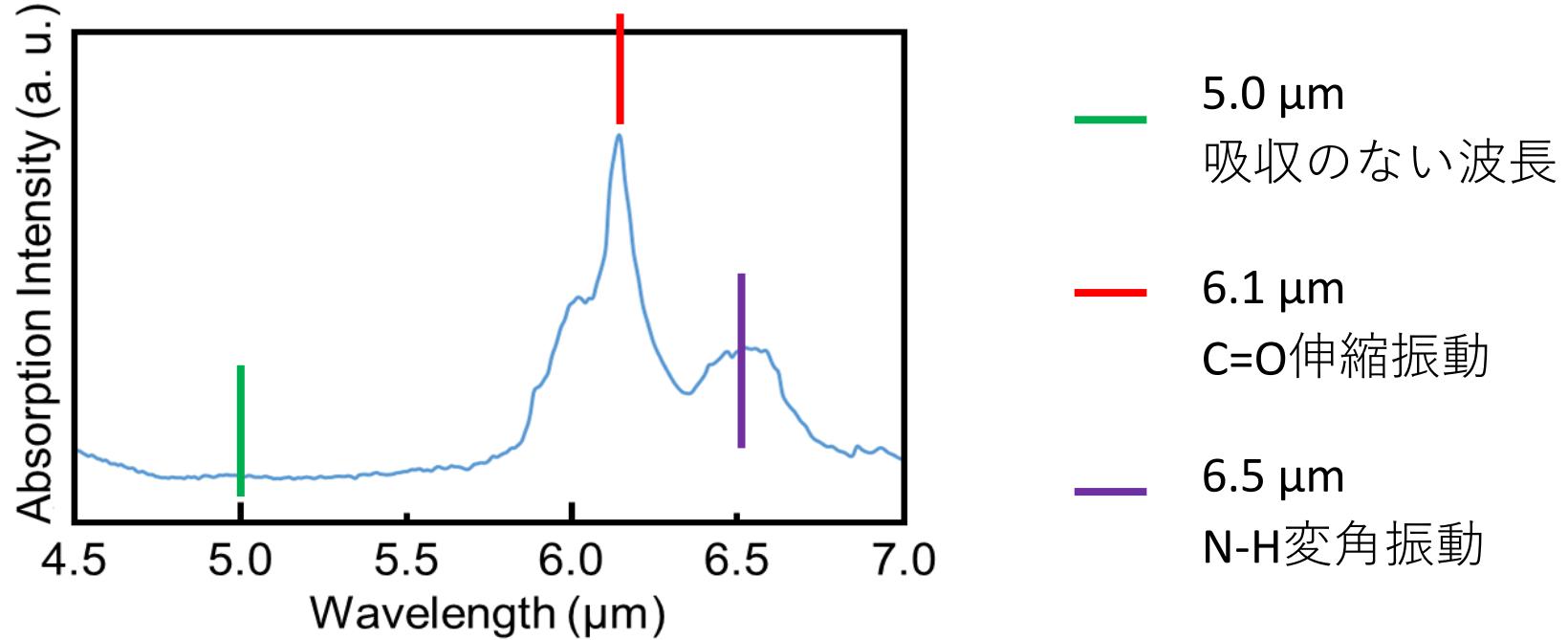
照射実験



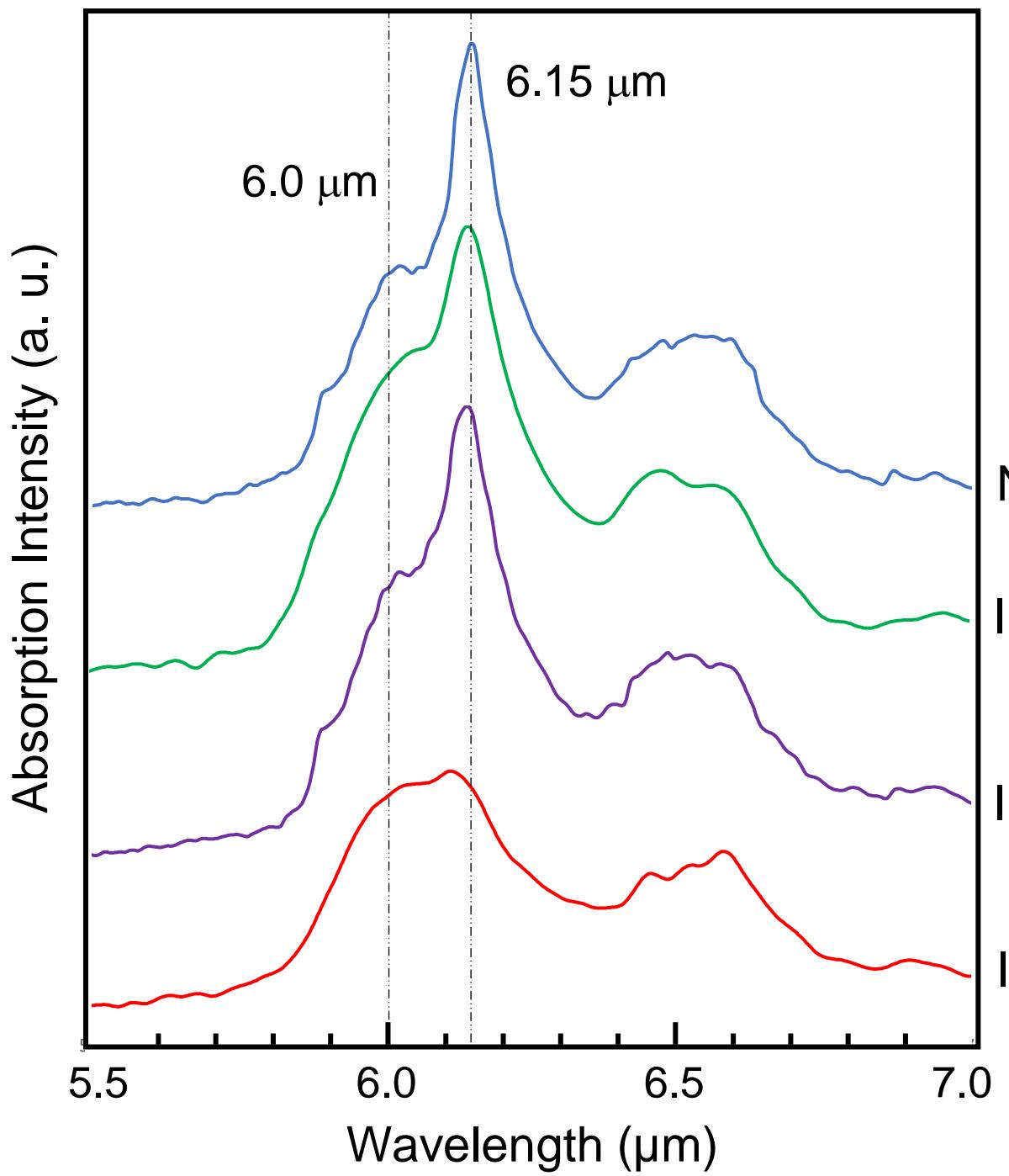
分析 → 走査型電子顕微鏡
顕微赤外分光法
比較 → 定常加熱

FEL照射波長

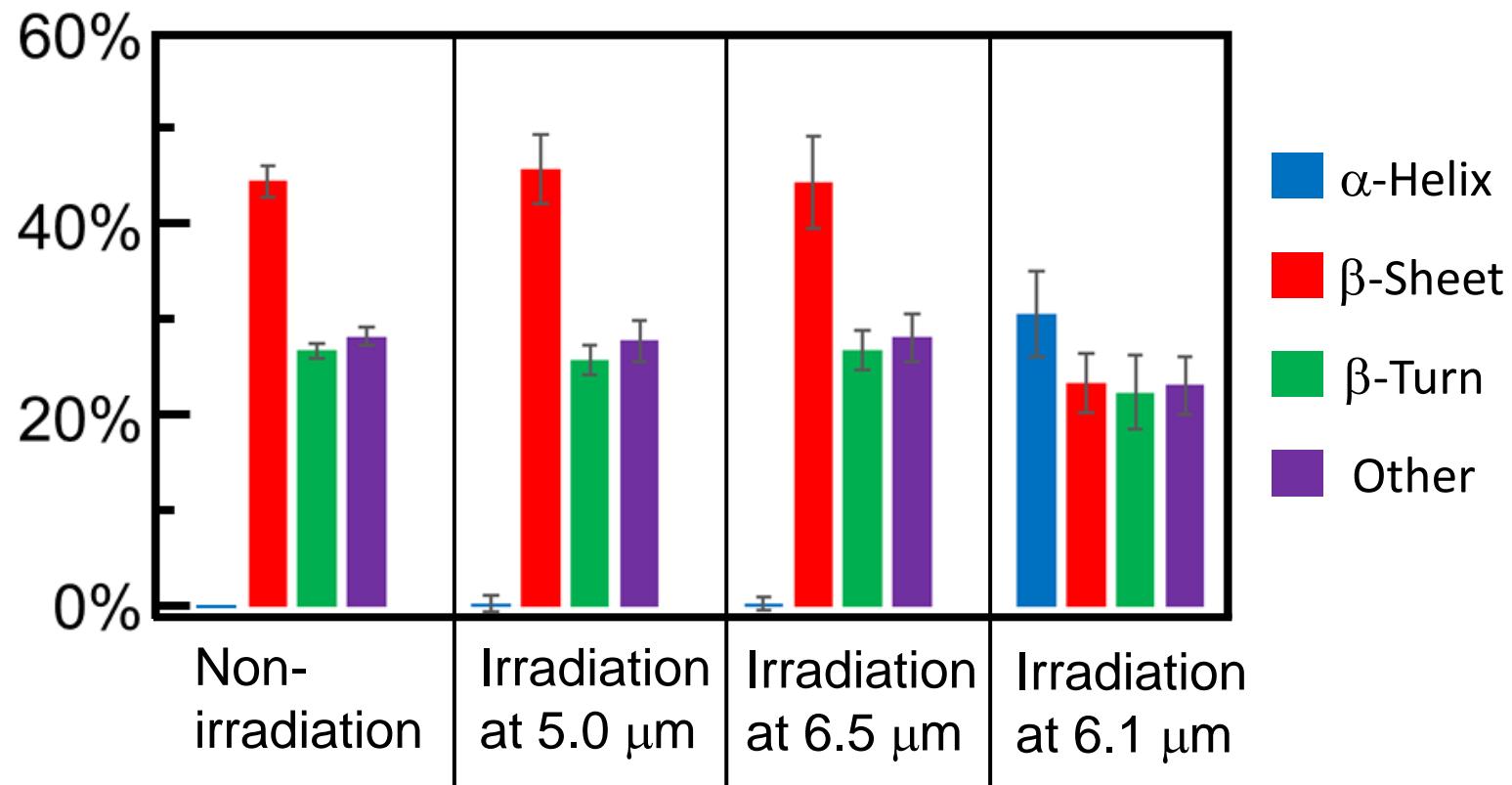
→ 線維化させた試料のIRスペクトルを測定

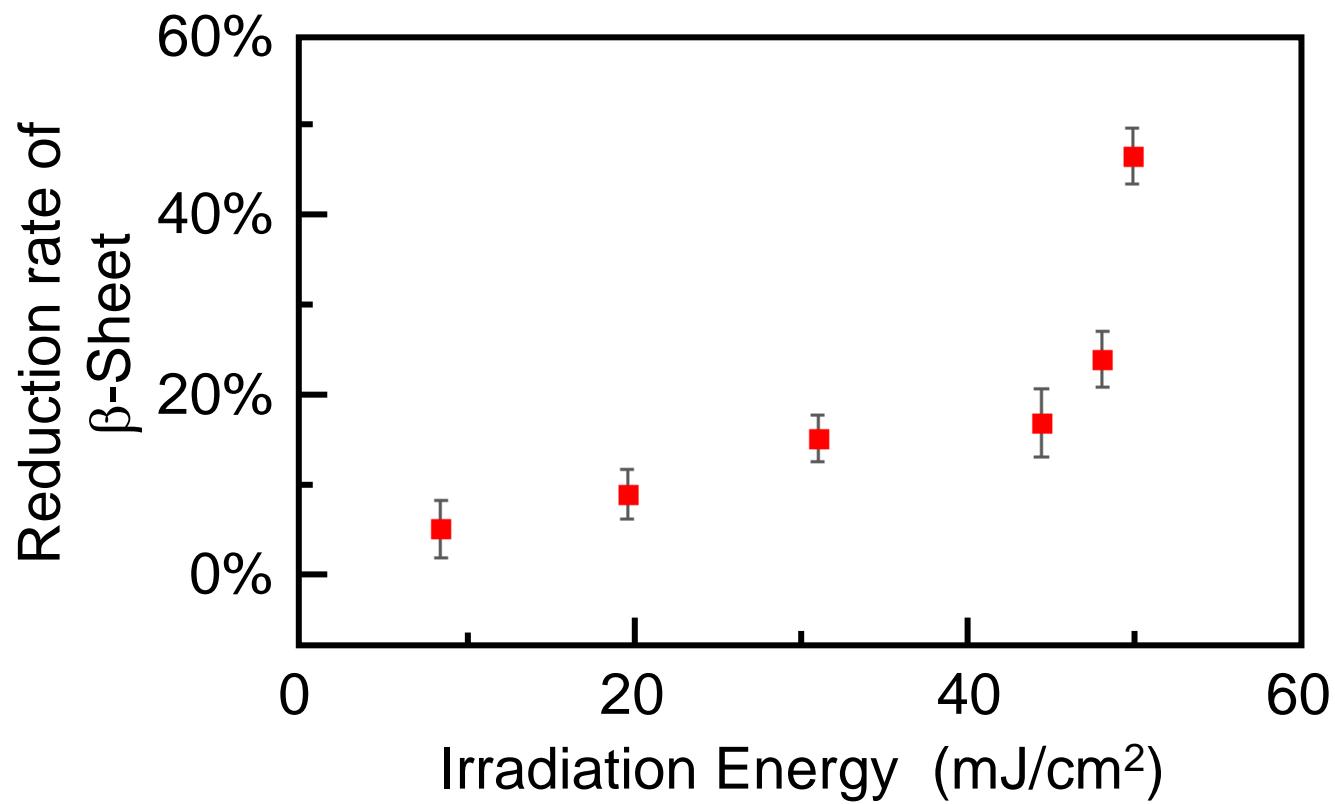


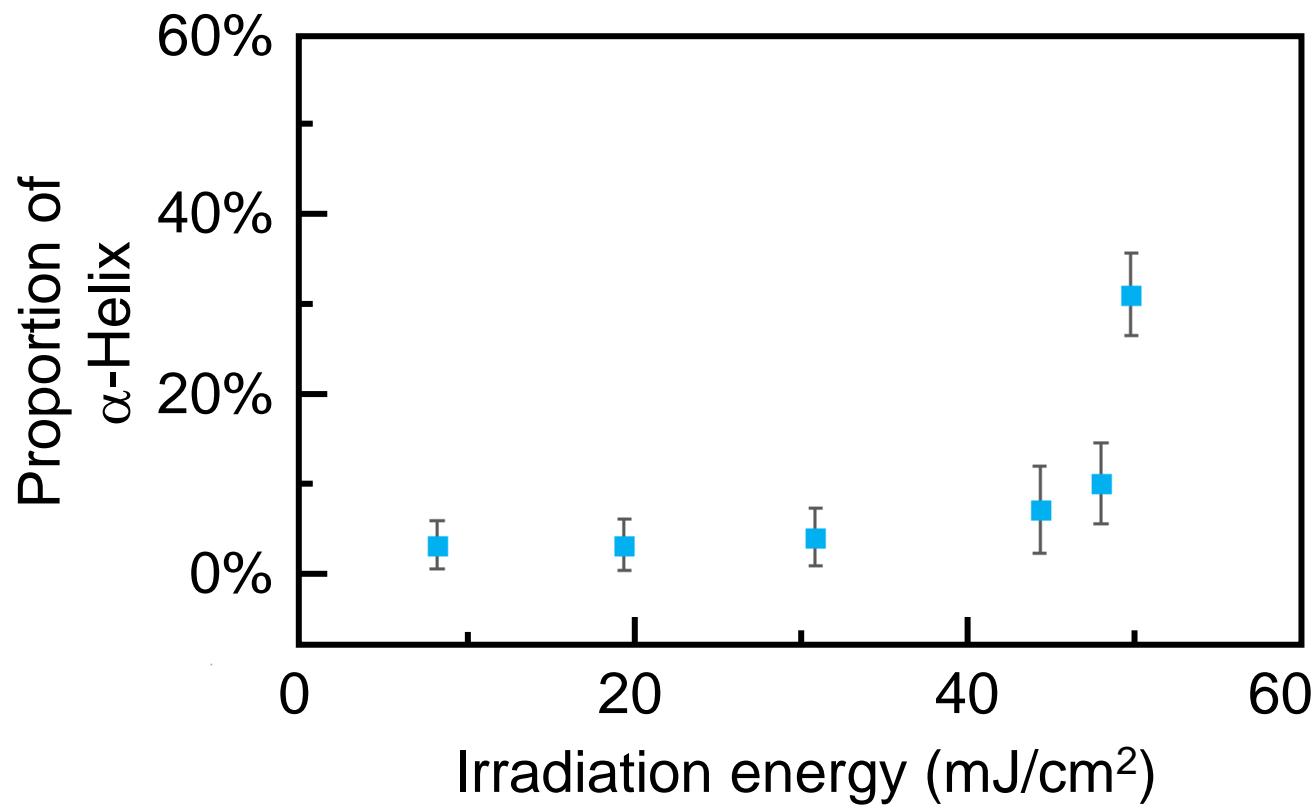
➡ 3つの波長でIR-FEL光を照射



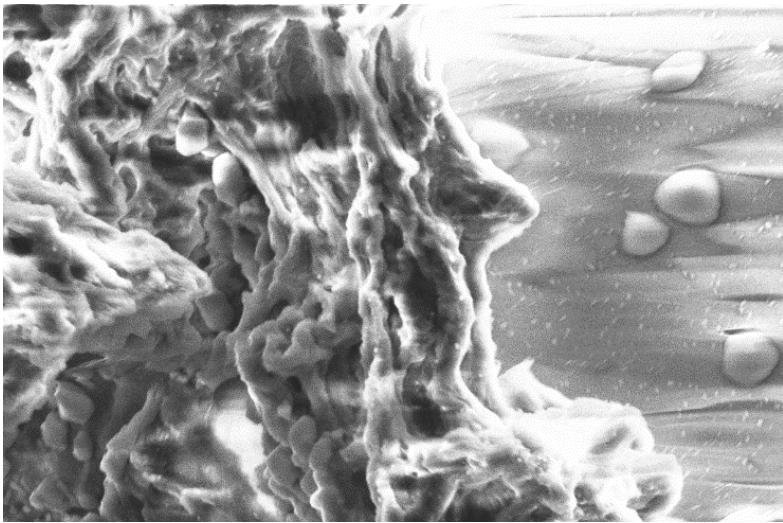
Non-irradiation
Irradiation at $5.0 \mu\text{m}$
Irradiation at $6.5 \mu\text{m}$
Irradiation at $6.1 \mu\text{m}$



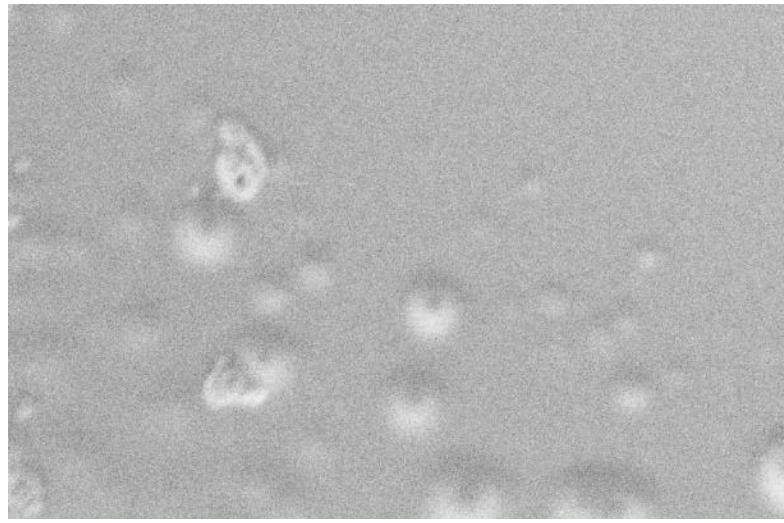




Non-irradiation

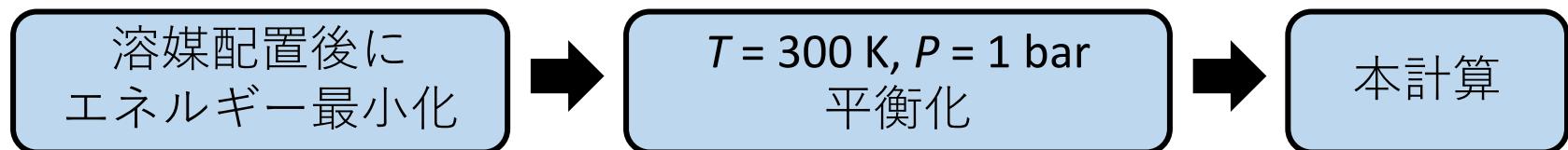
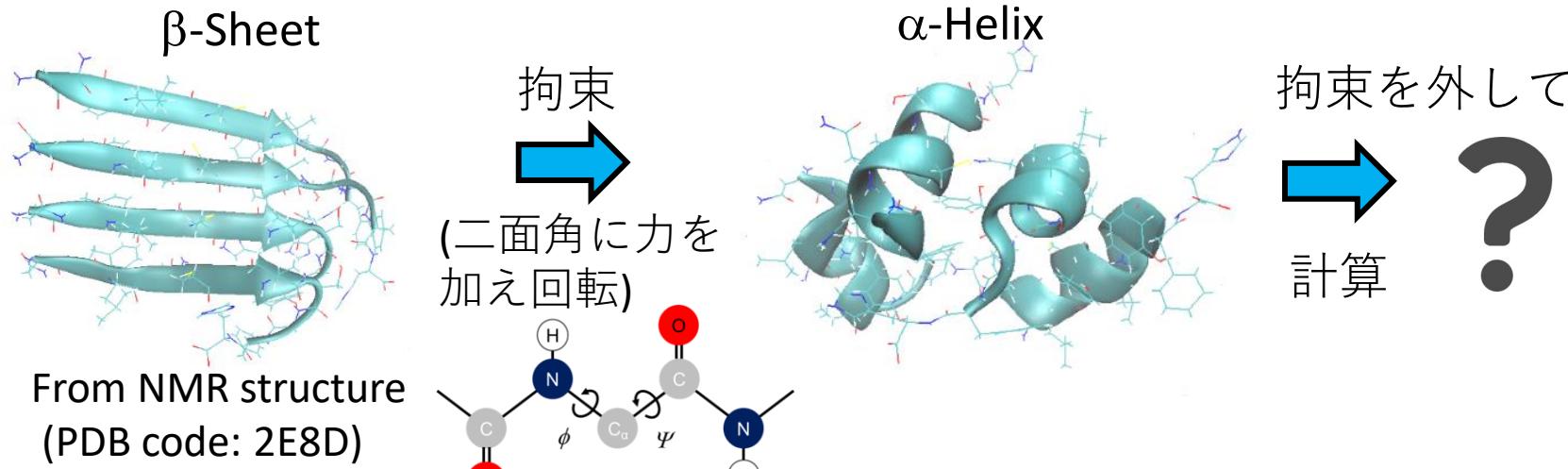


Irradiation at 6.1 μm



分子動力学計算

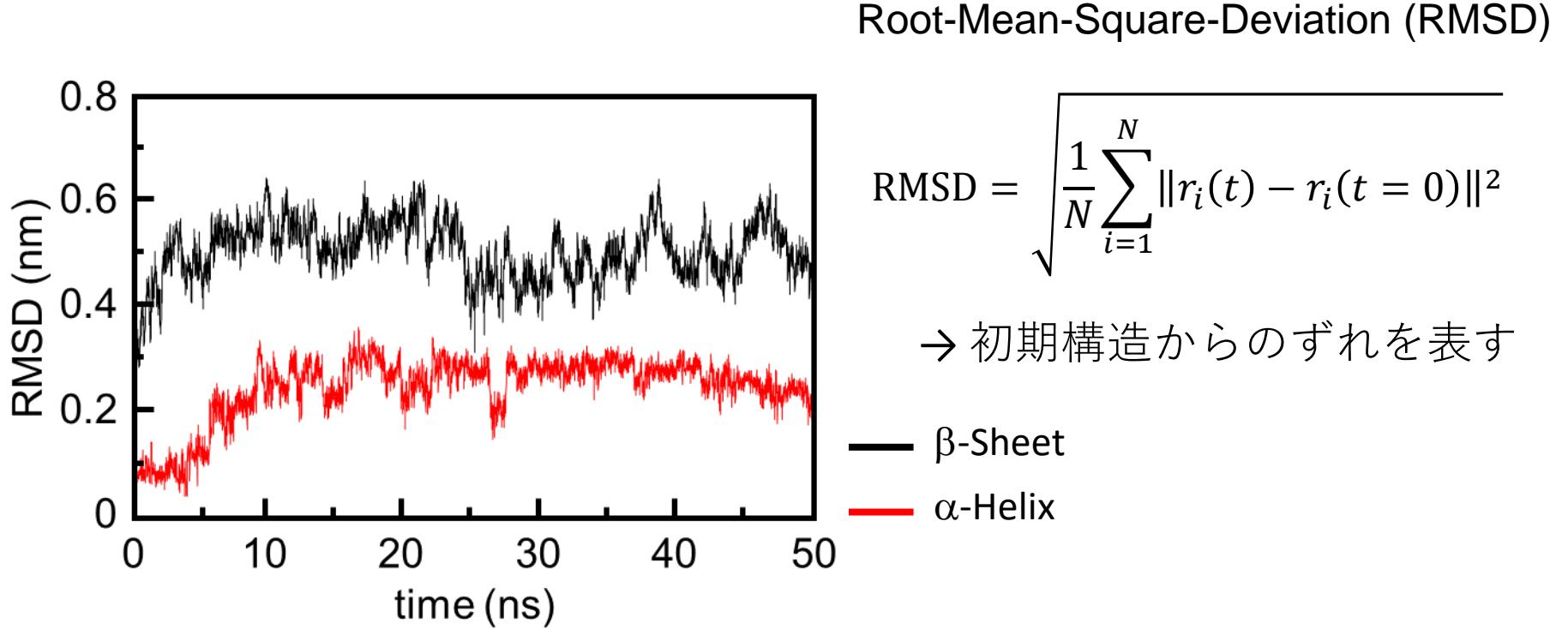
→ 6.1 μm照射時に生成した α -Helix構造の安定性について評価



0.1 M
 $\text{H}_2\text{O} \rightarrow \text{Na}^+, \text{Cl}^-$
GROMACS2019.6
AMBER96
TIP3P

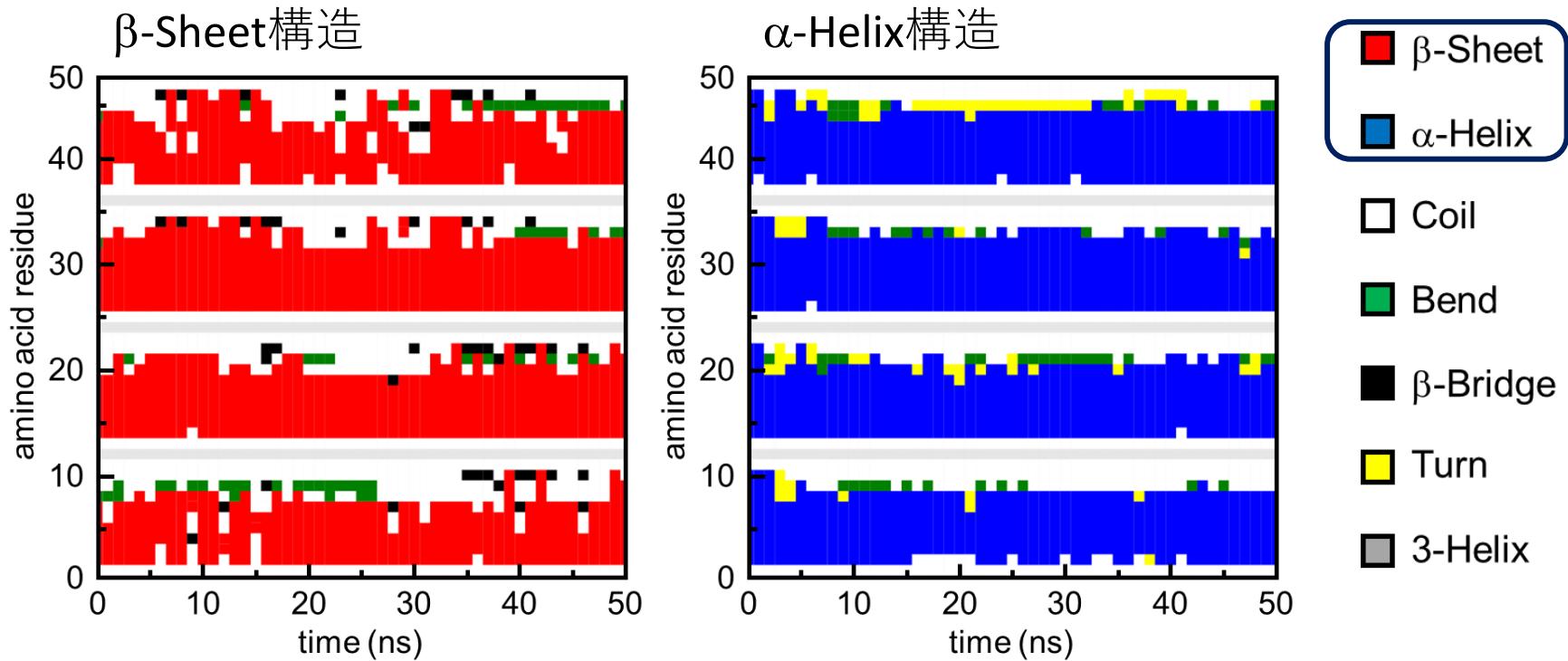
$t = 0\text{-}50 \text{ ns}$
step: $\Delta t = 2.0 \text{ fs}$

揺らぎの比較



→ α-Helix構造: RMSDの揺らぎは小さい

二次構造の安定性



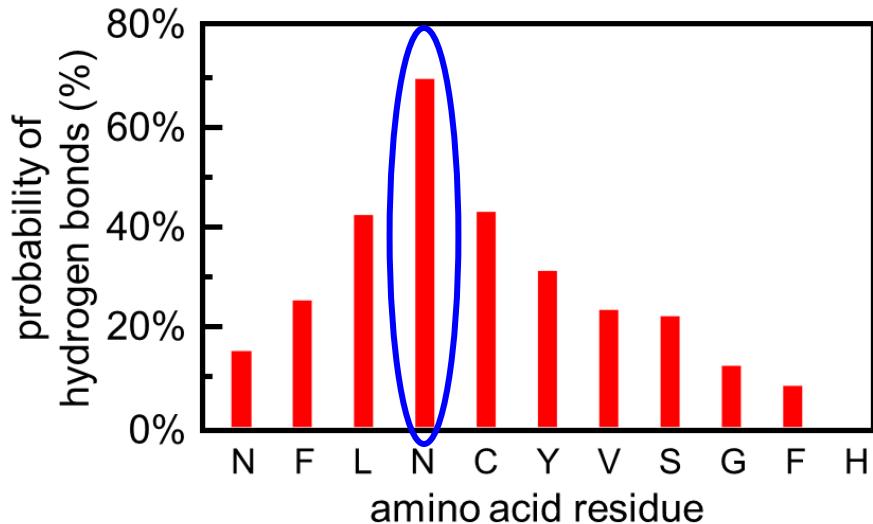
→ α-Helix構造: 二次構造の変化はほとんど生じない

→ α-Helix構造は安定している

水素結合解析

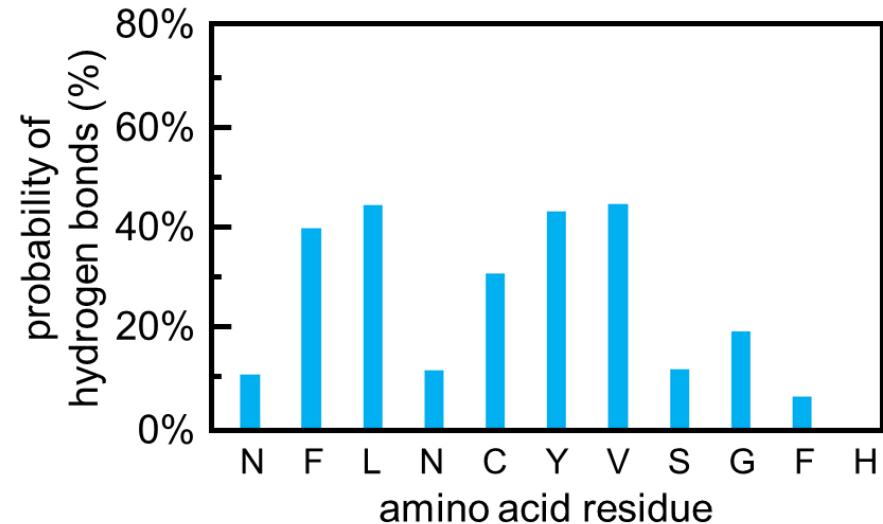
→ ペプチド主鎖間で形成される水素結合について解析

β -Sheet構造



→ N (Asn)で多くの水素結合が形成

α -Helix構造

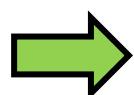
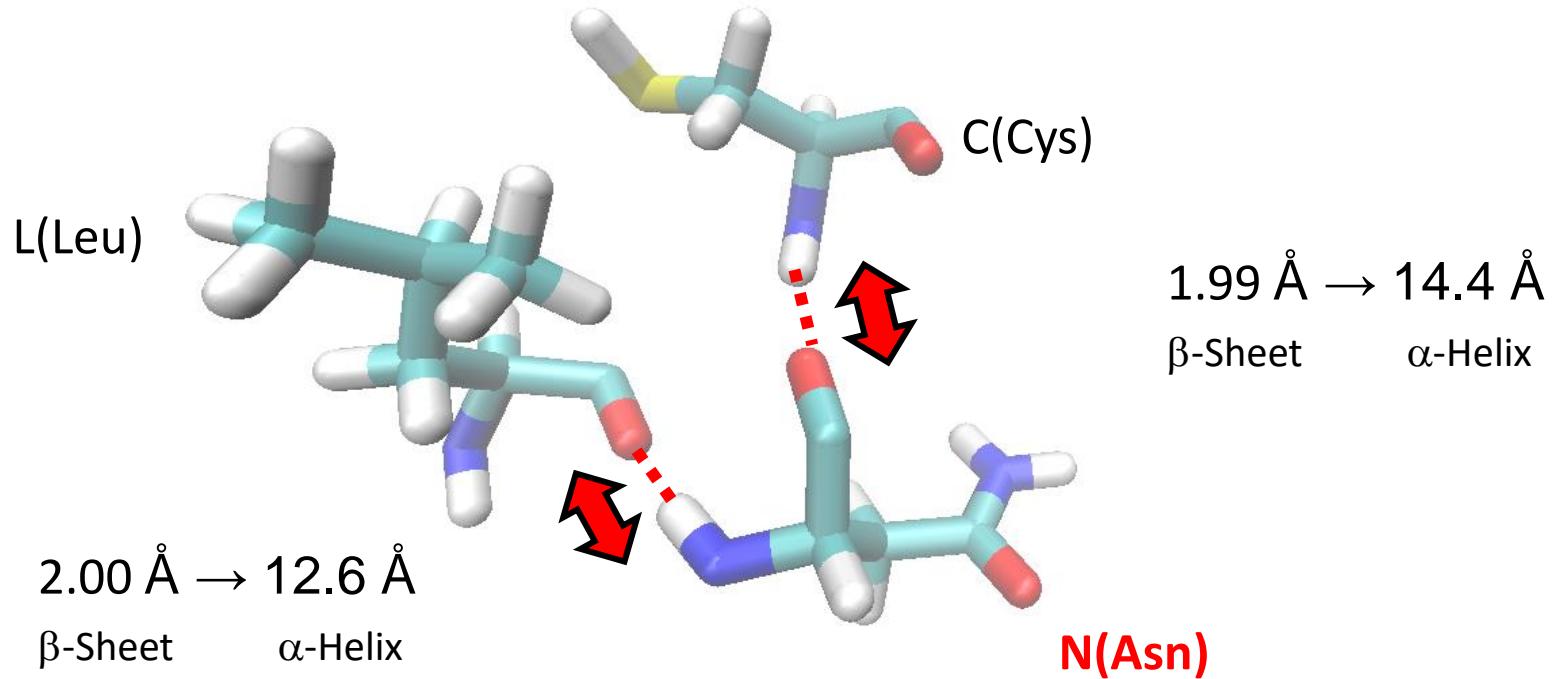


→ N (Asn)で大幅に減少

→ N(Asn)に注目し、C=O...H-Nの距離を計算

NFLNCYVSGFH

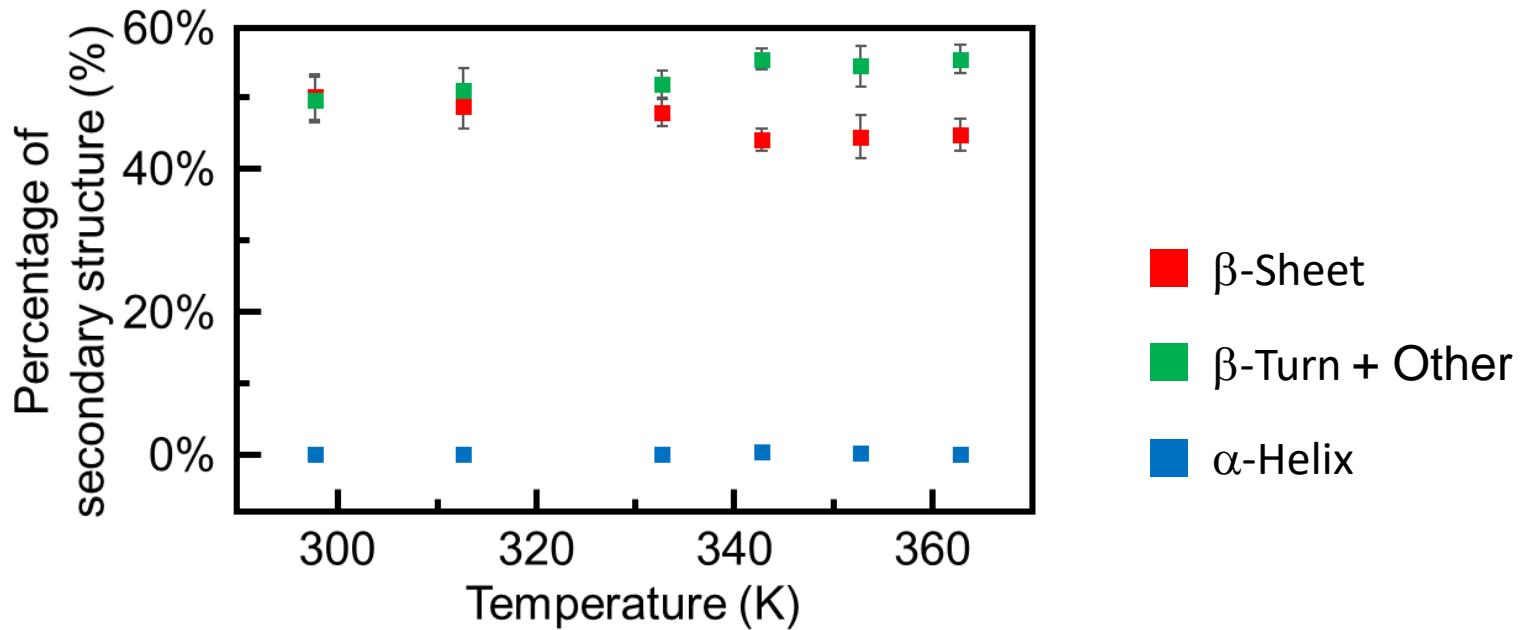
青: 親水性 赤: 疎水性



C=O伸縮振動を励起する事でペプチド主鎖間の
水素結合が弱まり周囲の水分子と安定化

定常加熱

→ IR-FEL光 $6.1\text{ }\mu\text{m}$ 照射時、局所的な温度上昇により反応が進行するのでは？

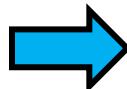
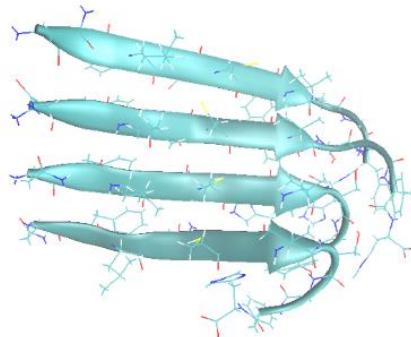


β-Sheet構造: 減少、 **α-Helix構造:** 変化なし

→ IR-FEL光照射時に起こる反応は熱反応のみではない

分子動力学計算

→ β -Sheet構造について高温での計算を実施



β -Sheet

溶媒配置後に
エネルギー最小化

0.1 M
 $\text{H}_2\text{O} \rightarrow \text{Na}^+, \text{Cl}^-$



$T = 300 \text{ K}, P = 1 \text{ bar}$
平衡化

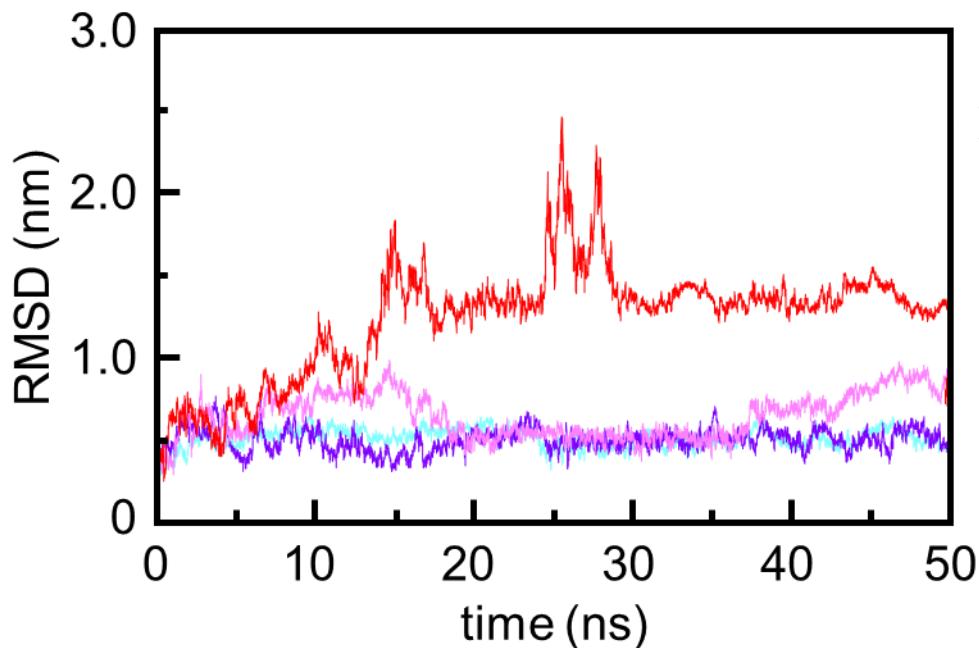
$T = 350, 370, 400 \text{ K}$ の
計算を実施



本計算

$t = 0-50 \text{ ns}$
step: $\Delta t = 2.0 \text{ fs}$

加熱による揺らぎの変化



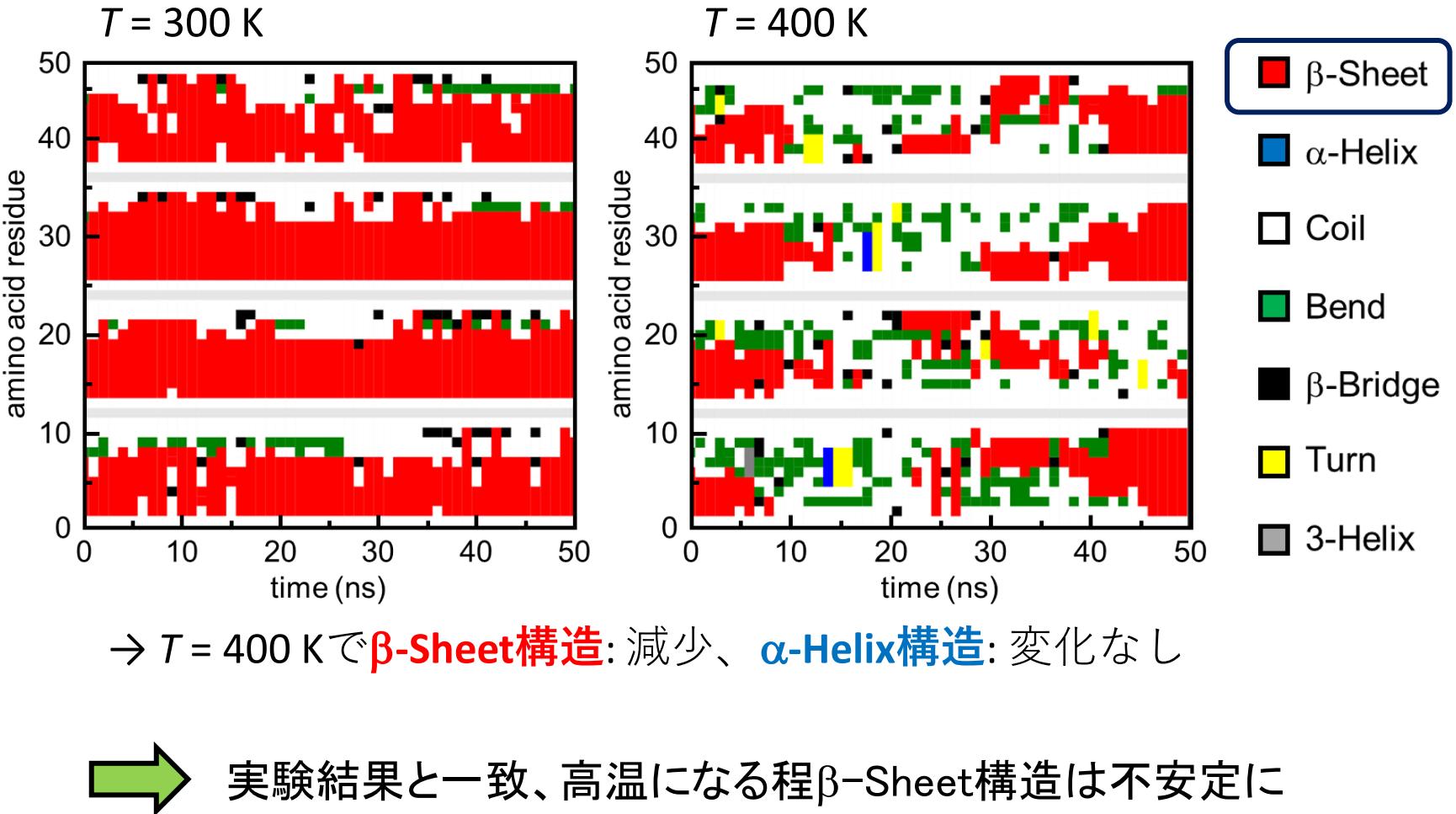
$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^N \|r_i(t) - r_i(t = 0)\|^2}$$

→ 初期構造からのずれを表す

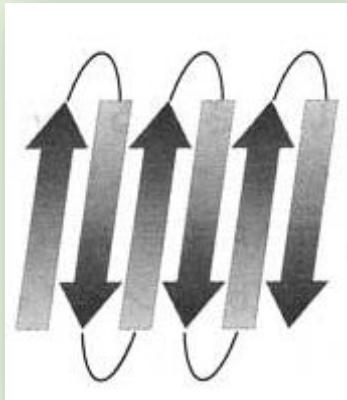
— 400 K — 370 K
— 350 K — 300 K

→ 高温になるにつれ、RMSDの揺らぎは大きくなる

二次構造変化



β -sheet



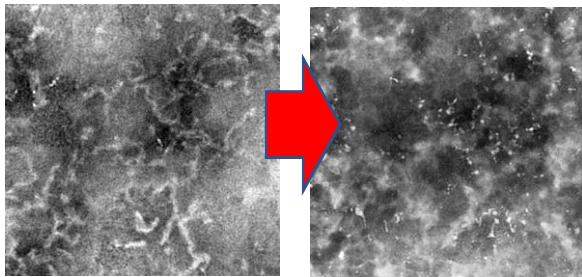
病原性

IR-FEL

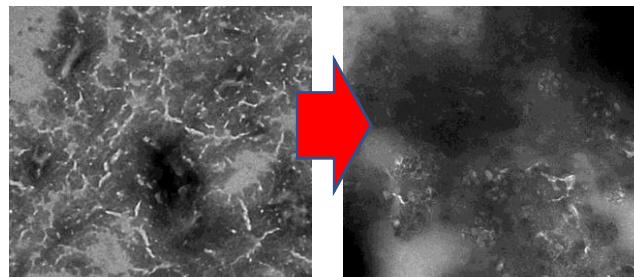


α -helix

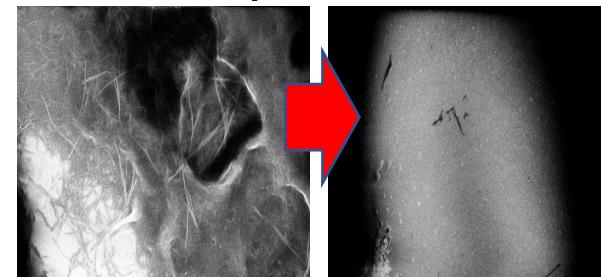
Lysozyme



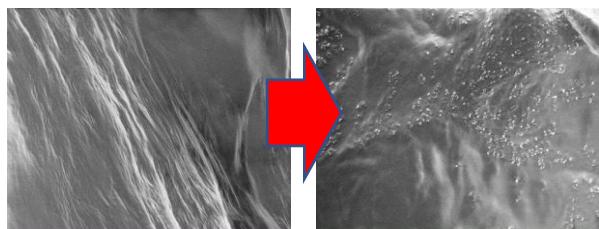
Insulin



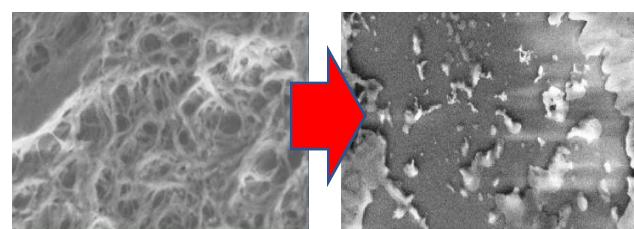
$\text{A}\beta 1-42$



Calcitonin



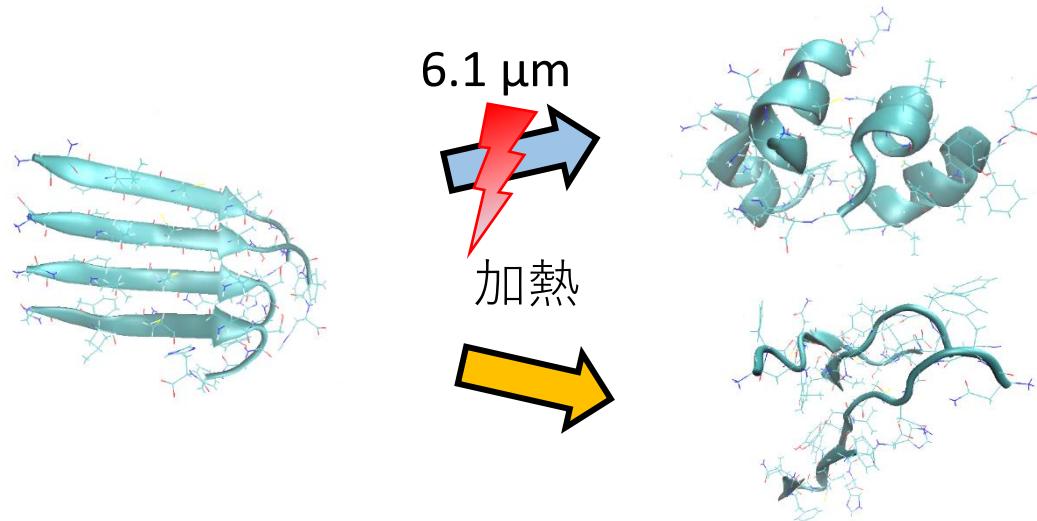
Polyglutamine



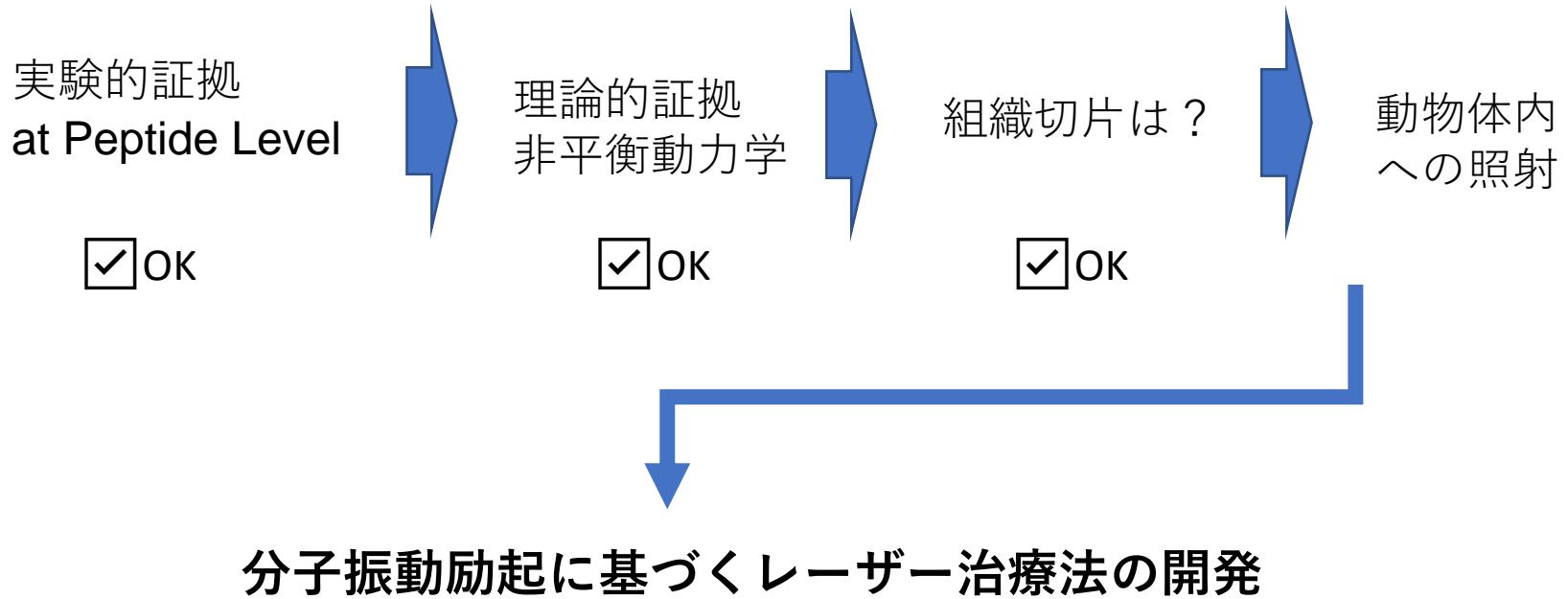
様々なアミロイドに
適用可能！

CONCLUSION

- ✓ IR-FEL光 $6.1\text{ }\mu\text{m}$ (C=O伸縮振動)照射時に β -Sheet構造が減少、 α -Helix構造が増加
- ✓ β -Sheet構造: N(Asn)で多くの分子間水素結合が形成
 α -Helix構造: 周囲の水分子と安定化
- ✓ IR-FEL光照射時に起こる反応は熱反応のみではない



Towards development of amyloidosis therapy by using IR-FEL



ご清聴ありがとうございました



FEL-TUS

