

# Kumatori Deuteration Station (KIDS) Project -Focusing on Protein-

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# Aim and Goal of KIDS Project



1. Supply for deuterated protein (for biologically interesting/important research).

Preparation of enough amount (volume) of deuterated protein for SANS

2. Development of deuteration technique.

Precise controlling of deuteration level, ligation, recycling of D<sub>2</sub>O, efficient overexpression

3. Development of SANS method attained by deuteration technique.

Crowding (concentrated system), kinetics

1. Supply for deuterated protein (for biologically interesting/important research).

# Necessity for preparation of deuterated Protein



What is the obstacle for SANS study on Protein sample?

1. Cost (D<sub>2</sub>O and deuterated carbon source)
2. Labor (Large amount of deuterated sample is needed.( $\sim 1\text{ml}$ ) (Cf.  $50\ \mu\text{l}$  for SAXS))

We will offer the service for the preparation of deuterated protein sample !!

# Planned procedure for the preparation and purification of deuterated protein

1. Expression vector



2. Preculture in deuterated media  
(Inspection of the expression condition)



3. Culture in D<sub>2</sub>O environment

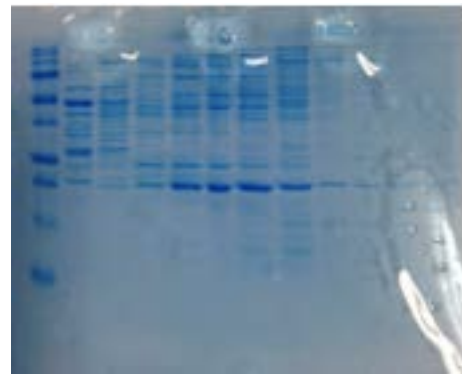


4. Purification

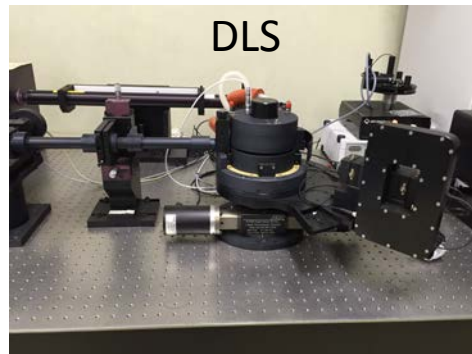


# Characterization of deuterated protein

## 5. SDS/Native PAGE



## 6. Monodispersity/Structural Check



## 7. Deuteration level



## 8. Deliver to neutron facility



# New Deuterated sample preparation room (KIDS room)

2016/2/26

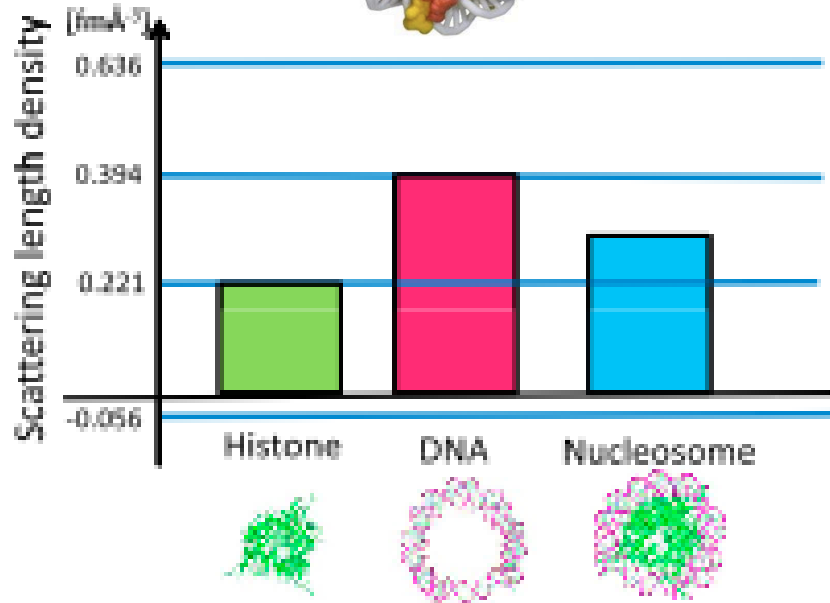
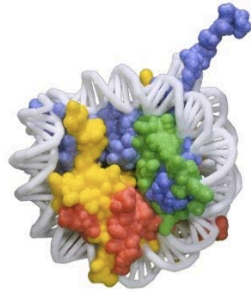


Other apparatuses are upcoming soon and new technician will join to our project next month.

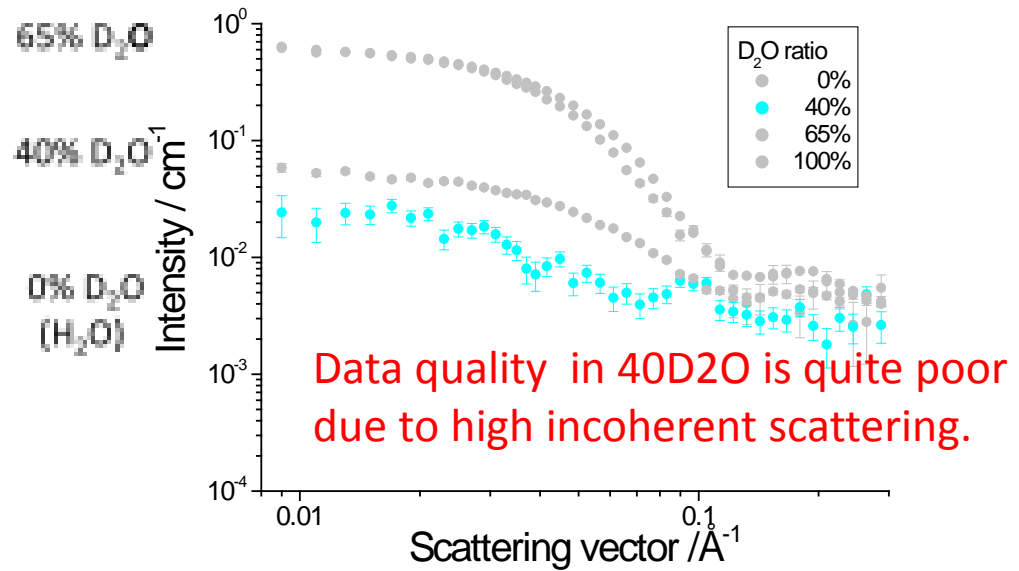
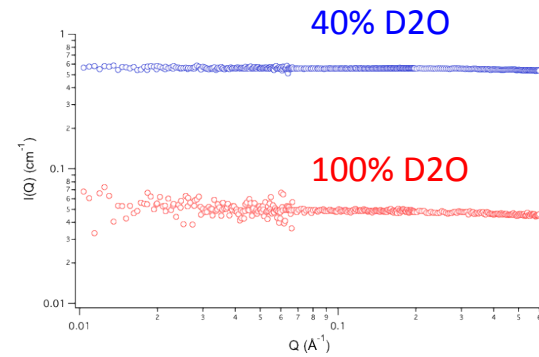
## 2. Development of deuteration technique.



# Previous studies: multi-component system



Hydrogenated protein is invisible in 40D2O.

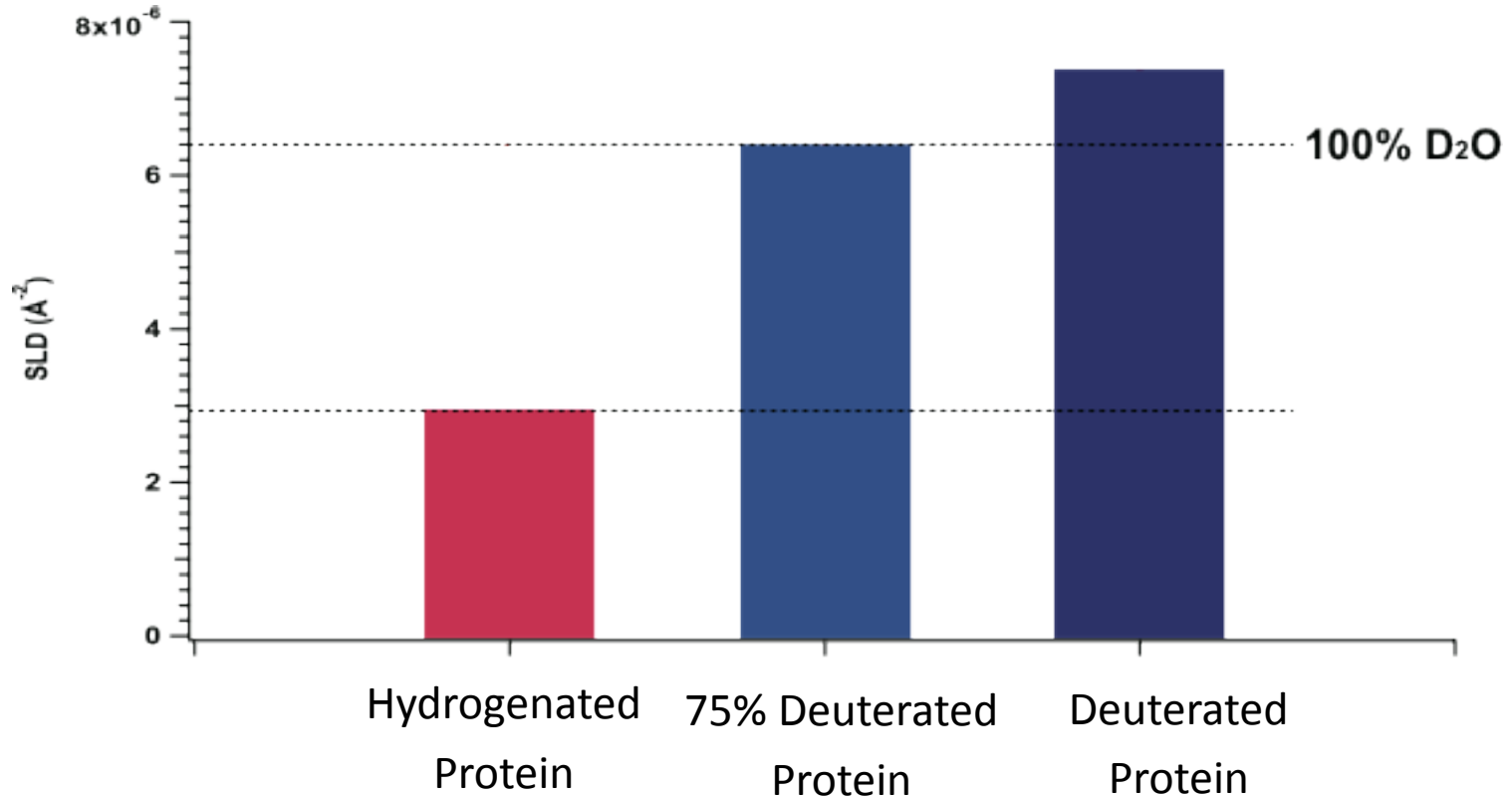


M. Sugiyama, R. Inoue et al. BBR 4, 28 (2015).  
 M. Sugiyama, R. Inoue et al. BJ 106, 2206 (2014).

Is there any good solution for realizing high S/N CV –SANS?

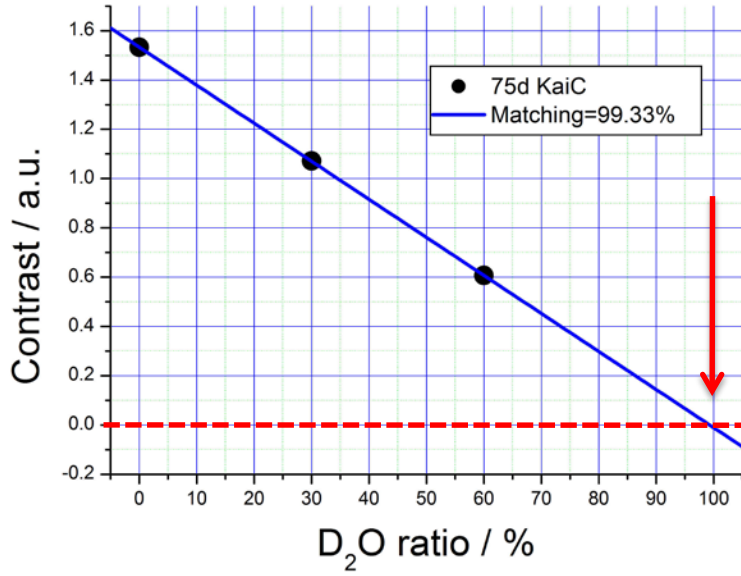
# Multi-component structural analysis

## Other strategy

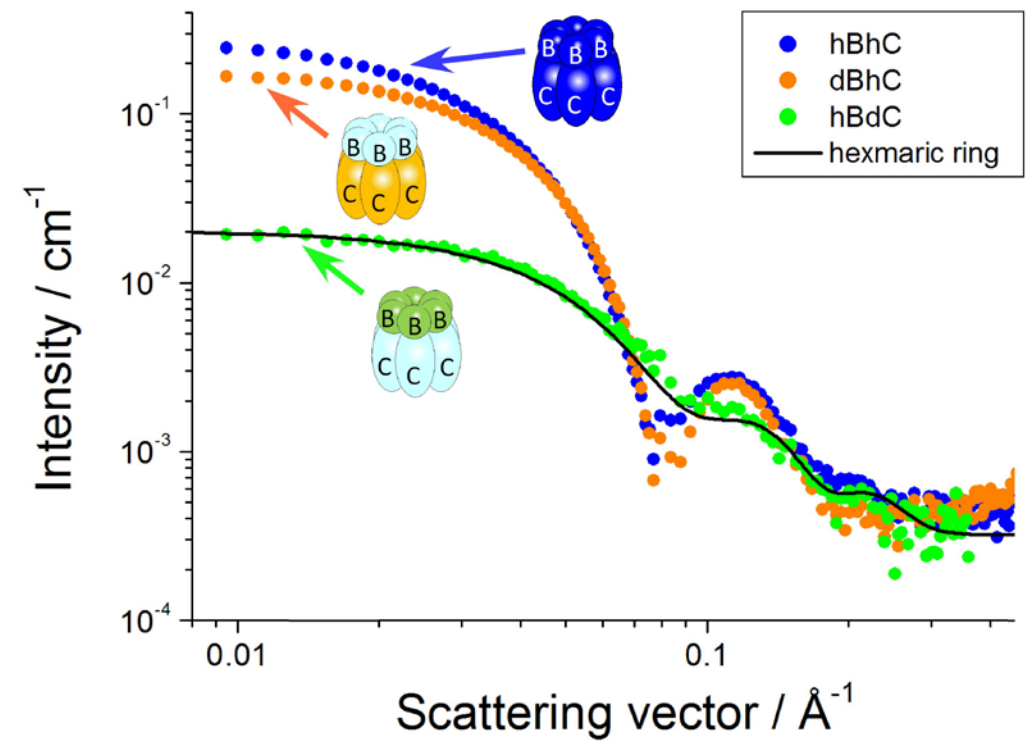


75% deuterated protein is matched out in 100% D<sub>2</sub>O.

# Powerfulness of 75% deuterated Protein



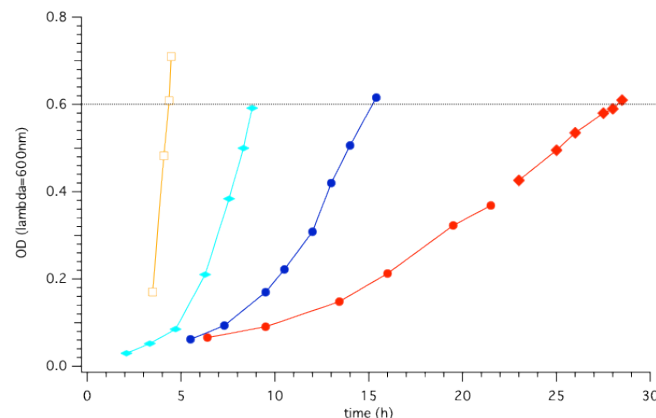
75% deuterated protein is matched out in 100% D<sub>2</sub>O.



Precise structural analysis on protein assembly is possible with 75% deuterated method.

# Precise control of deuteration level

## 1. Cultivating condition ( $D_2O$ , deuterated carbon source, media)



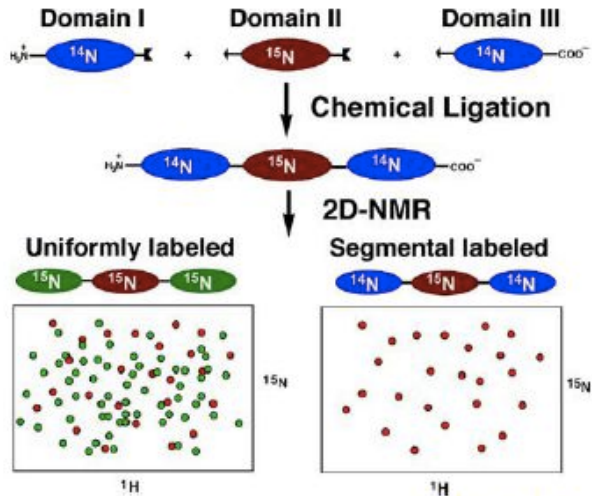
## 2. Evaluation of precise deuteration level (dual check)



Finding optimum condition from step 1 and step 2.

# Ligation and recycling system (D2O)

## 1. Ligation (selective deuteration of concerned domain)



Idea from NMR

[Muir et al. Curr Opin Biotechnol. 2002 Aug;13\(4\):297-303.](#)

## 2. Recycling of deuterated material



100% deuterated material is not necessary needed for 75% deuterated protein.

➡ **Cost down !**

# Summary

For the future of Neutron society,  
it's time for thinking about  
**Neutrons for Biology.**

