



# **Small-Angle X-ray Scattering**

#### Learn the story of your protein

Nozomi Ando @ Cornell – CHESS HP Bio Workshop 2021 – April 29 2021

### Structural biology techniques

## Crystallography



#### Cons:

- must crystallize protein
- crystallization artifacts

#### Pros:

- structure determination can be fast
- still the gold standard for atomic detail

Tinoco, A. et al. *ACS Catal* 9: 1514–1524 (2019). Davis, K. M. et al. *PNAS* 114: 10420–10425 (2017).

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SAXS



#### Cons:

- low resolution (10-20 Å)
- interpretation best done in reciprocal space **Pros:**
- insight into protein behavior in solution
- connects structural data and tells a story

Meisburger, S. P. et al. JACS 138, 6506-6516 (2016).

#### Cryo-EM



#### Cons:

- hardware and software barriers
- achieving < 3 Å resolution is not trivial **Pros:**
- game changer for large and slightly heterogeneous samples

Thomas, W. C. et al. Nature Commun 10, 2653 (2019).

### **Structural biology techniques**



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SAXS fundamentals: How to process and interpret data



**Pro tips on sample preparation:** How and more importantly, why?

Max Watkins



Advanced analysis: Extracting more information from data

Steve Meisburger



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Guinier, "30 Years of Small-Angle X-Ray Scattering", Physics Today Nov. 1969



Svergun, Barberato, & Koch, *J Appl Cryst.* 28: 768–773 (1995). Svergun, D. I. et al. *J Appl Cryst.* 30: 798–802 (1997).



Svergun, D. I. et al. J Appl Cryst. 30: 798-802 (1997).

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### Enzyme activity requires two proteins to transiently interact

#### E. coli Class la RNR



Class I RNRs require inter-subunit radical transfer.

The hunt for the active complex



#### SAXS reveals two complexes and connects structural data



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#### SAXS reveals two complexes and connects structural data



Ando, Brignole, Zimanyi, Asturias, Drennan, Nocera, Stubbe, et al. (2011) PNAS, 108: 21046–21051.



electron micrograph





1 asymmetric unit



Christina Zimanyi

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### SAXS connects conformational states and data from other techniques



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Ando, Brignole, Zimanyi, Asturias, Drennan, Nocera, Stubbe, *et al.* (2011) *PNAS*, 108: 21046–21051. Minnihan, Ando, Brignole, Asturias, Drennan, Nocera, Stubbe, *et al.* (2013) *PNAS 110: 3835–3840.* Zimanyi, Ando, Brignole, Asturias, Stubbe & Drennan (2012) *Structure* 20: 1374–1383.

#### Activity regulation in the enzyme family



# **dATP** binding to N-terminal **ATP-cone domain** promotes quaternary structures that are incompatible with catalysis.

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Ando, et al., Drennan. *PNAS* (2011). Ando, et al., Drennan.. *Biochem* (2016). Zimanyi, et al., Drennan. *Structure* (2012). Johansson, et al. *Structure* (2016).

#### **Emergence of the class lb RNRs**



Class Ib RNRs lost allosteric activity regulation.

#### Signatures of activity regulation.... in a class lb RNR?



Mac Parker JoAnne Stubbe



The catch? This is a class lb RNR.

Parker, Zhu, and Stubbe. Biochemistry (2014) 53: 766-776.

Will Thomas



Steve Meisburger



Darren Xu

## Anion exchange (AEX)-coupled SAXS



Meisburger, Taylor, Khan, Zhang, Fitzpatrick, and Ando. *JACS* (2016) 138: 6506 - 6516. Parker, Maggiolo\*, Thomas\*, Kim, Meisburger, Ando<sup>+</sup>, Boal<sup>+</sup>, and Stubbe<sup>+</sup>. *PNAS* (2018) 55: 201800356–10. Meisburger\*, Xu\*, and Ando *IUCrJ* (2020) 8: 225-237.

#### **AEX-SAXS** reveals a new oligomeric form



Endogenous ligand (dAMP) binds to truncated N-terminal domain.

Meisburger, Taylor, Khan, Zhang, Fitzpatrick, and Ando. *JACS* (2016) 138: 6506 - 6516. Parker, Maggiolo\*, Thomas\*, Kim, Meisburger, Ando<sup>†</sup>, Boal<sup>†</sup>, and Stubbe<sup>†</sup>. *PNAS* (2018) 55: 201800356–10. Meisburger\*, Xu\*, and Ando *IUCrJ* (2020) 8: 225-237.



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Thomas, Brooks, Burnim, Bacik, Stubbe, Kaelber, Chen, and Ando. Nature Comm (2019) 10: 2653.

### **Discovery of a new inhibition mechanism**



#### From SAXS, we knew:

Endogenous ligand binds 1 site per **monomer** to produce an **I-dimer**.

**dATP** converts both the monomer and I-dimer to the same filament.

∴ There are 2 binding sites for **dATP** & the I-dimer is part of the filament.



Thomas, Brooks, Burnim, Bacik, Stubbe, Kaelber, Chen, and Ando. Nature Comm (2019) 10: 2653.



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#### Take-home messages



Low-resolution but provides incredible insight into protein behavior in solution.

Easy to change solution conditions and do a comprehensive search of conformational space.

SAXS connects data from other techniques.

SAXS allows comparative study of protein homologs.

SAXS tells a story.