NREL
NATIONAL RENEWABLE ENERGY LABORATORY

FDX2 of *Chlamydomonas reinhardtii*

The crystal structure
&
probing its interaction network

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The Ferredoxins of *Chlamydomonas reinhardtii*

- six chloroplast localized 2Fe2S isoforms

- small, soluble and acidic proteins

- differentially expressed

    - FDX1 major isoform (98% of FDX transcript in TAP grown cells)

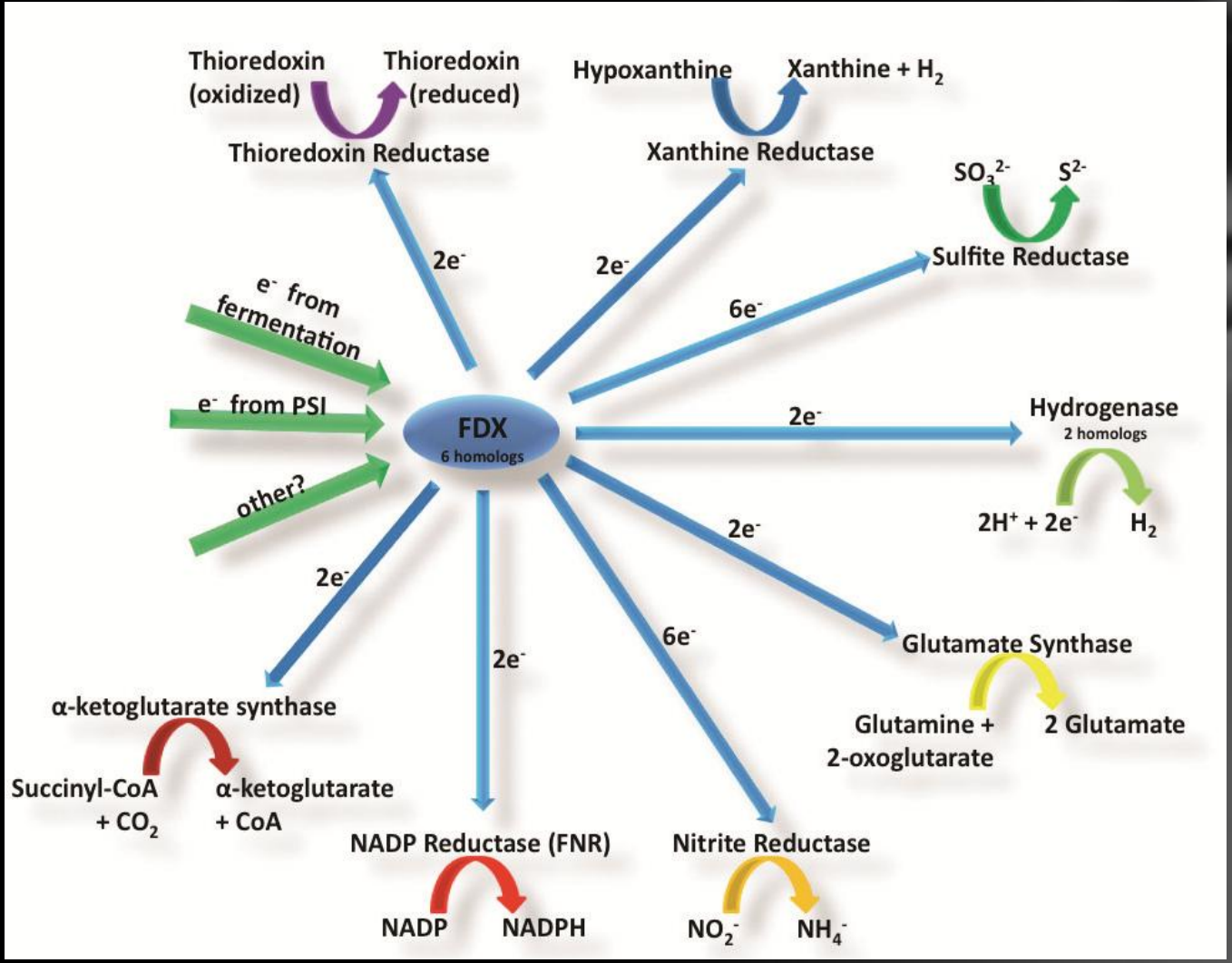
    - FDX2 induced by nitrate

    - FDX5 induced by copper stress and anaerobiosis

- function as electron shuttles to a diversity of proteins, but specific functions for each isoform remain largely unknown

The Ferredoxins of *Chlamydomonas reinhardtii*

- Key players in metabolism -



H_2 production pathways

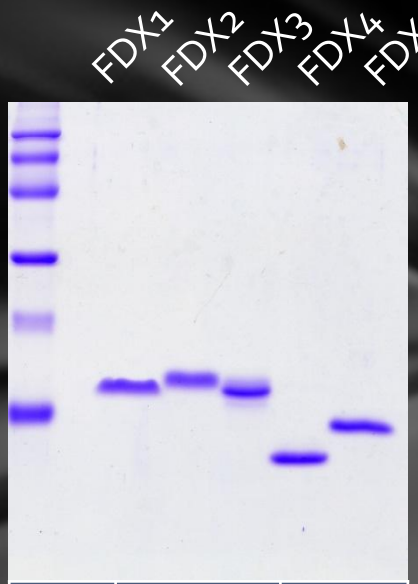
(modified from Posewitz et al. *Chlamydomonas Source Book* 2007).

Protein purification

- His-GST-TEVcs-tagged ferredoxins -

3-step purification protocol

1. GST purification (with on-column TEV cleavage)
2. HT column to remove TEV, GST-His and un-cleaved protein
3. Size exclusion chromatography



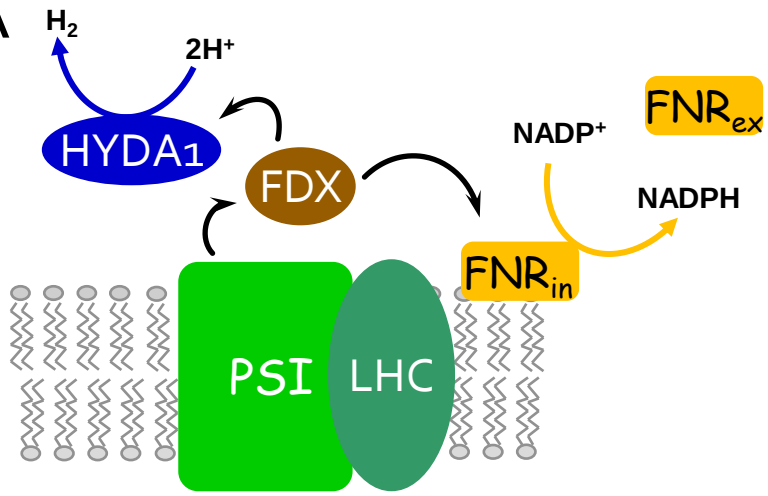
	Fe / protein	std dev
Fdx1	2.93	+/- 0.13
Fdx2	2.24	+/- 0.05
Fdx3	1.84	+/- 0.08
Fdx4	2.31	+/- 0.02
Fdx5	1.70	+/- 0.05

Project aims:

- **Interaction studies (pull-down experiments and activity assays)**
- **Characterization (spectroscopy, crystallization, etc.)**

FDX-mediated photo-hydrogen production vs NADPH photo-production

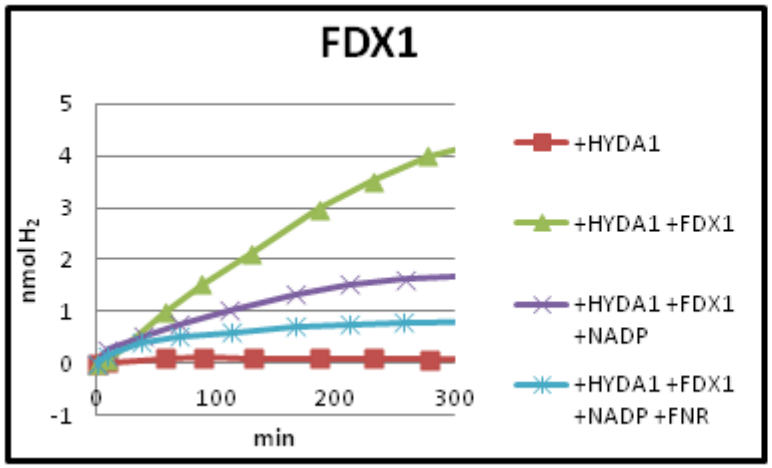
A



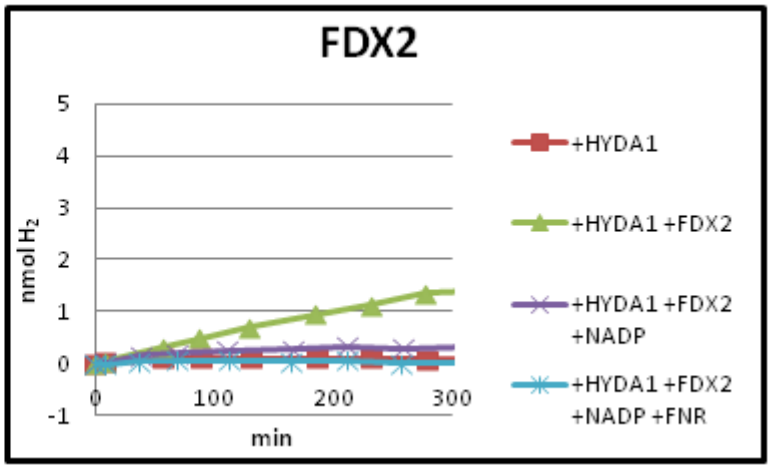
B

	H_2 Photo-production Rate (nmol hr^{-1})		
25µg Thylakoids	+	+	+
100nM HYDA1	+	+	+
2µM $NADP^+$	-	+	+
1µM FNR1	-	-	+
None	0.03951	N/D	N/D
FDX1	0.52193	0.41052	0.25175
FDX2	0.1753	0.07966	0.00899
FDX3	0	0.00211	0.00262
FDX4	0.02148	0.0791	0.05587
FDX5	0	0	0

C



D

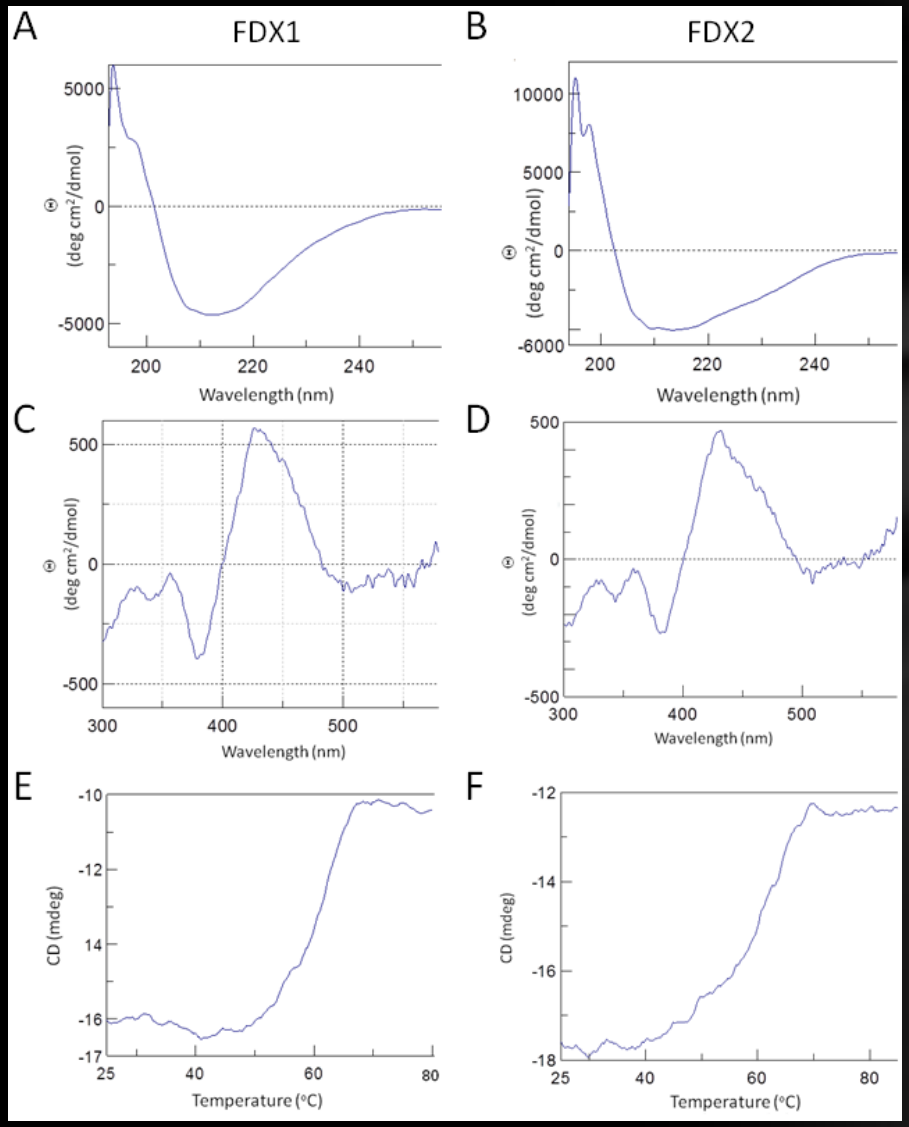


Evidence for FDX/Hydrogenase interactions

- pull-down experiments using -S cell extract -

		FDX2			FDX1		
GI #	Protein name	Sample	-Ctrl	p-value	Sample	-Ctrl	p-value
159470457 159472741	HYDA1/HYDA2 - Iron hydrogenases	2	0	0.13	2	0	0.12
159466536	HYDEF - Iron hydrogenase assembly factor	4	1	0.17	1	1	0.93
159472741	HYDA2 - Iron hydrogenase	5	2	0.25	13	2	0.001
159466244	HYDG - Hydrogenase assembly factor	15	10	0.28	18	10	0.06
159470457	HYDA1 - Iron hydrogenase	5	4	0.71	8	4	0.17
159466834	FDX5 - Apoferredoxin	8	1	0.01	7	1	0.02
	Total # of peptides detected	**3013**	**3457**		**3344**	**3457**	
	Total # of proteins detected	**924**	**1038**		**1057**	**1038**	

CD spectroscopy

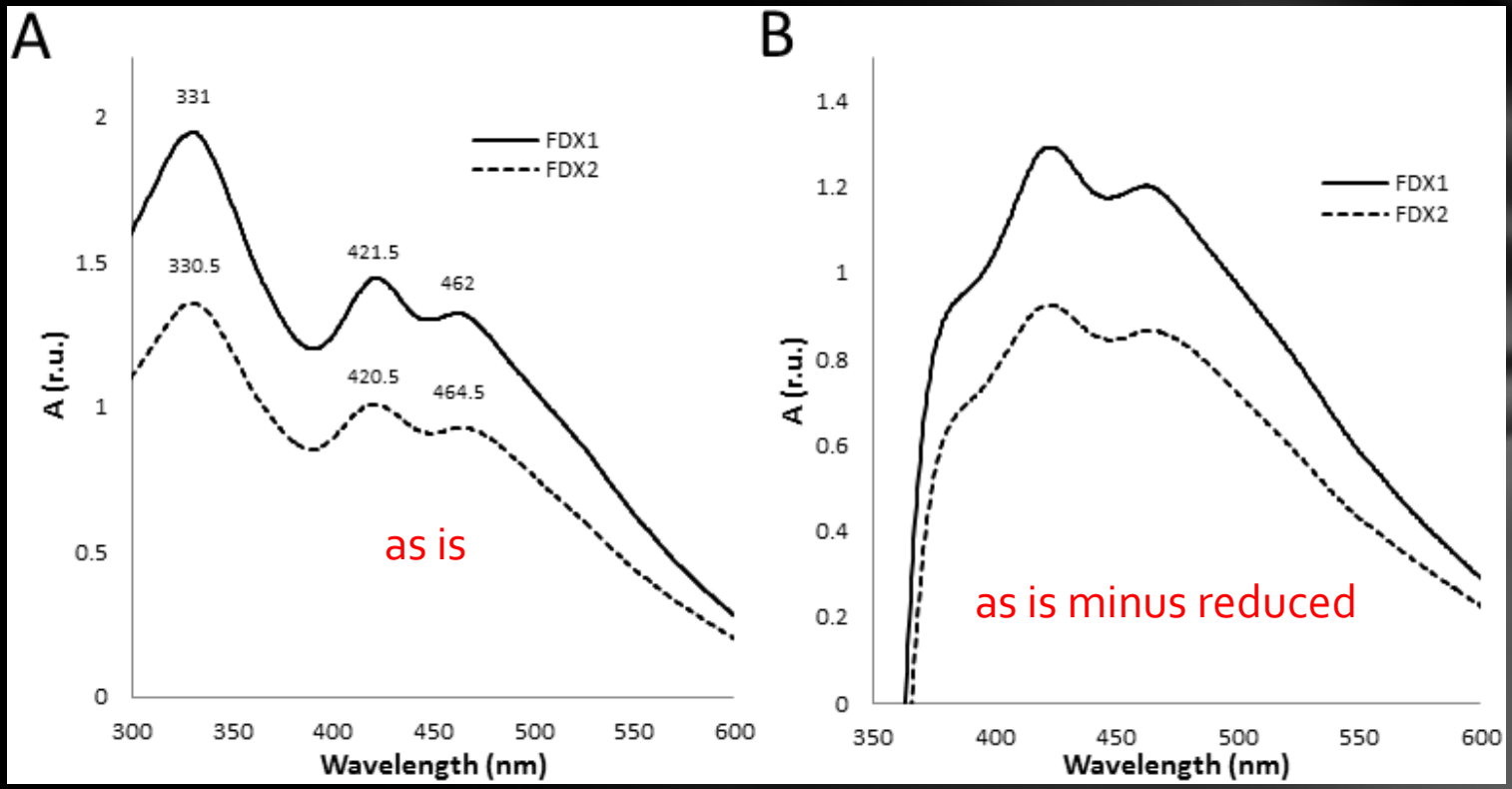


Proteins
are structured
(mix of α helices and β sheets)

Proteins
contain Fe/S cluster

Proteins are fairly
thermostable (Tm ~ 55°C)

UV/Vis spectroscopy

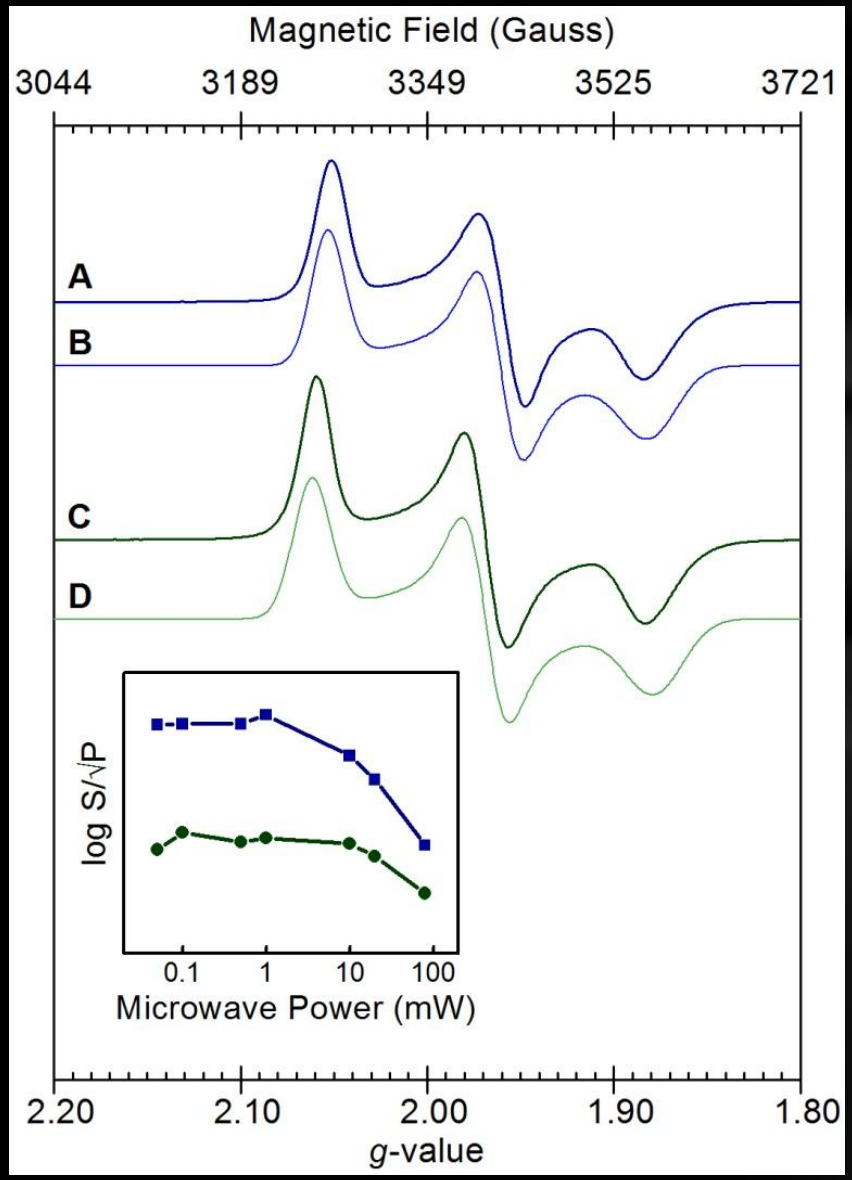


Typical FDX absorption peaks (~ 330, 420 and 460 nm).

The 420 and 460 peaks disappear after protein reduction.

High A420/A275 ratios (for FDX1 and FDX2 around 0.6) indicate a "clean" preparation.

X-band EPR spectroscopy

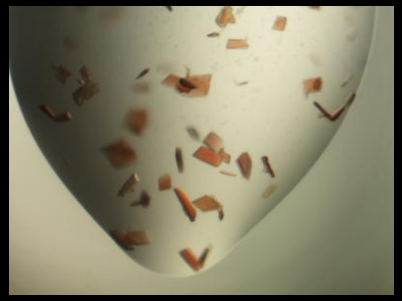


FDX1 (A), simulation (B)
FDX1: g = 2.052, 1.959, 1.879

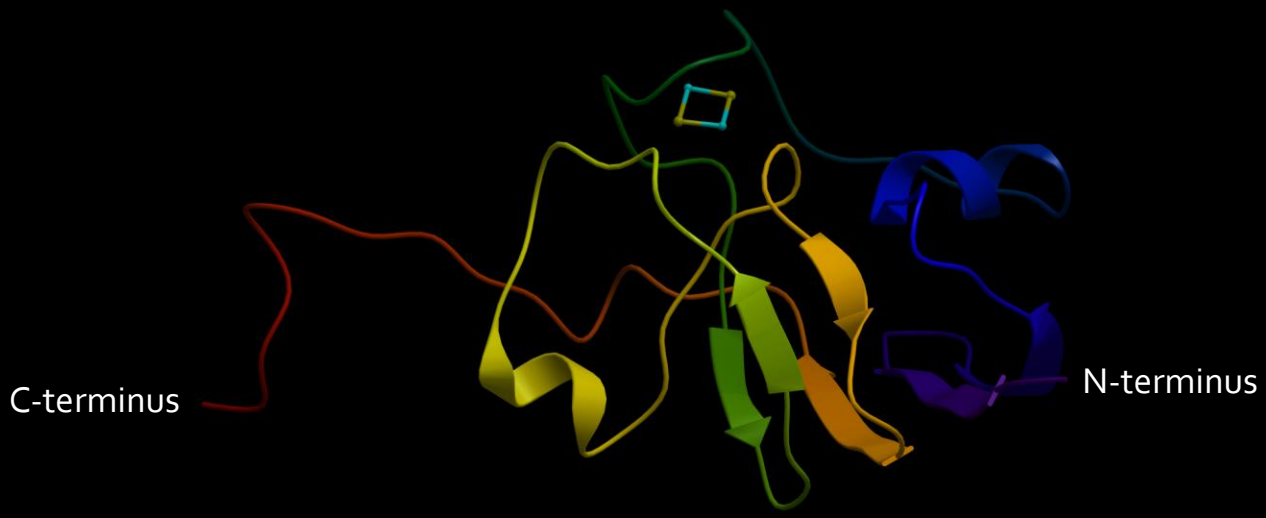
FDX2(C), simulation (D)
FDX2 g = 2.061, 1.967, 1.876

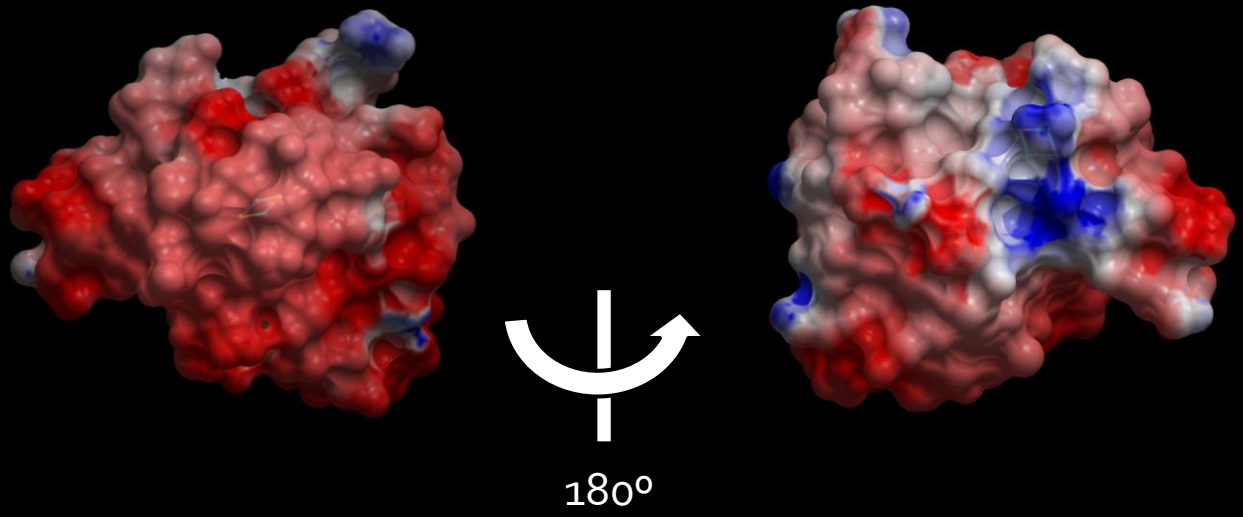
strong rhombic signal as expected for 2Fe2S proteins

Spectrometer settings:
modulation frequency: 100 kHz
modulation amplitude: 10.0 G
time constant: 327.68 ms
microwave frequency: 9.37-9.38 GHz
temperature: 23 K

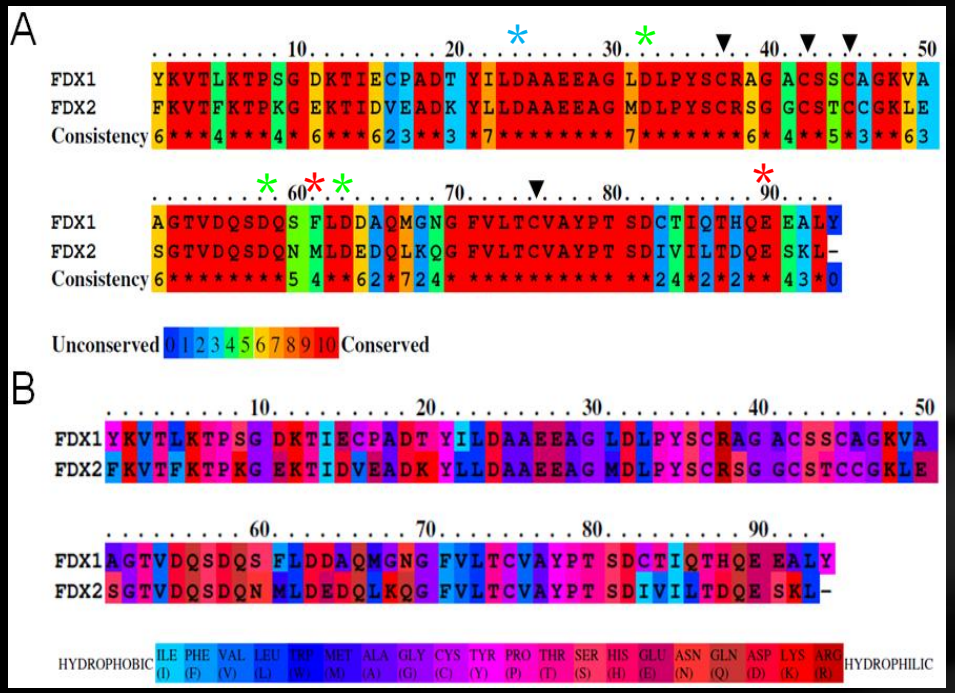


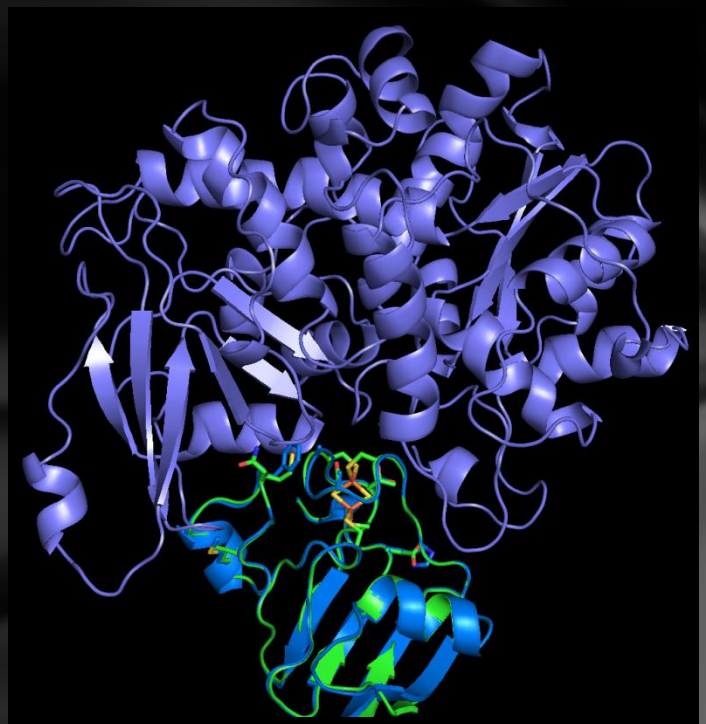
The structure of FDX2 at 1.2 Å





Structural modeling of FDX2 and FDX1 with HYDA1





HYDA1, FDX1, FDX2
(based on model by Winkler et al., 2009)

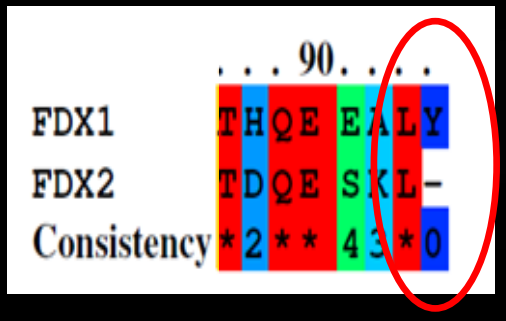
FDX1 and FDX2 share 82% sequence identity

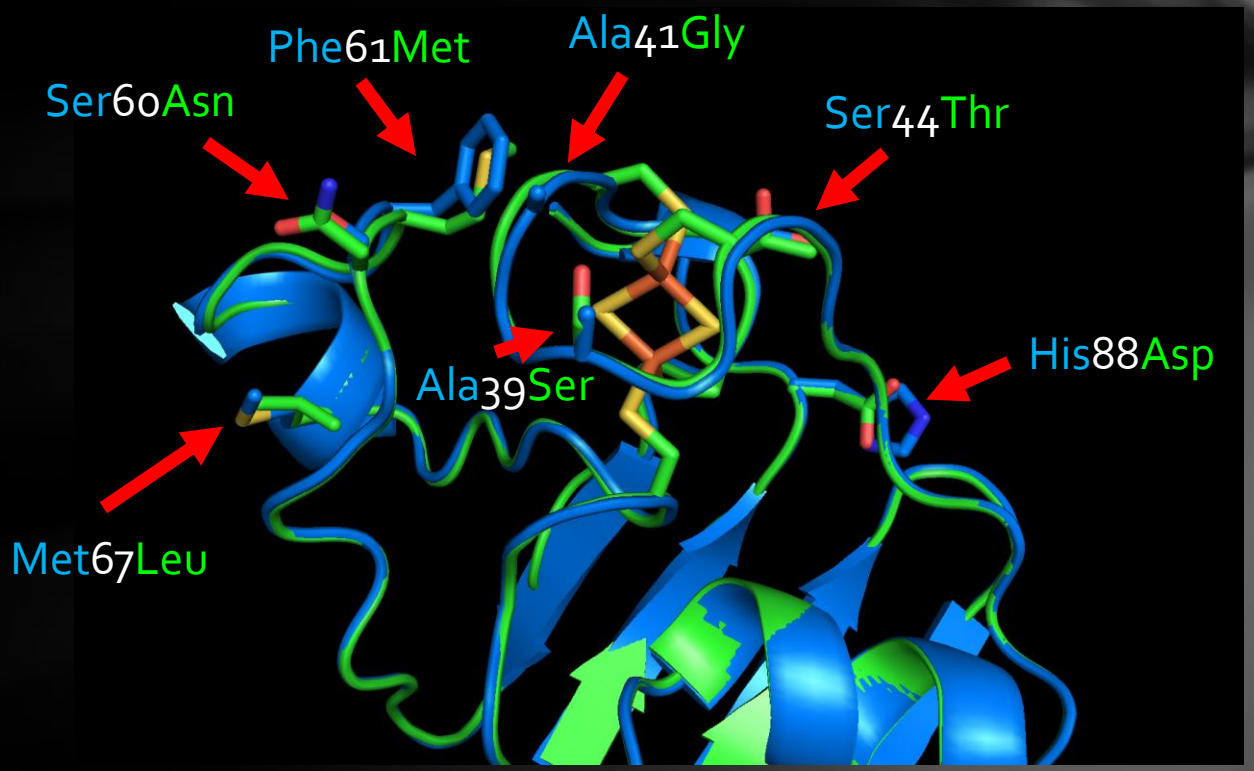
<u>Site directed mutagenesis and HYDA1 activity study (Winkler et al., 2009):</u>

- D32, D58 and D63 (*) important for intermolecular attraction and orientation in the first stages.
- D24 (*) had an effect on V_{max}
- F61 and E90 (*) most critical , i.e. strong effects on K_m and V_{max}
- Residues suggested to be involved in electron transfer: E90, Y94 and F61

Differences between FDX2 and FDX1

- close-up view of the 2Fe/2S cluster / HydA1 interaction region -





Conclusions & Future Work

- FDX1 to FDX5 were over-expressed in *E. coli* and purified.

- FDX2/1 are capable of promoting photo-hydrogen production.

- Pull-down experiments provided evidence for hydrogenase FDX2/1 interactions.

- FDX2/1 were very similar when characterized by spectroscopy.

- The FDX2 structure was solved at 1.2 Å.

- The F61M mutation and the lack of Y94 are probably the most significant differences, even though others exist on the binding surface.

Acknowledgements

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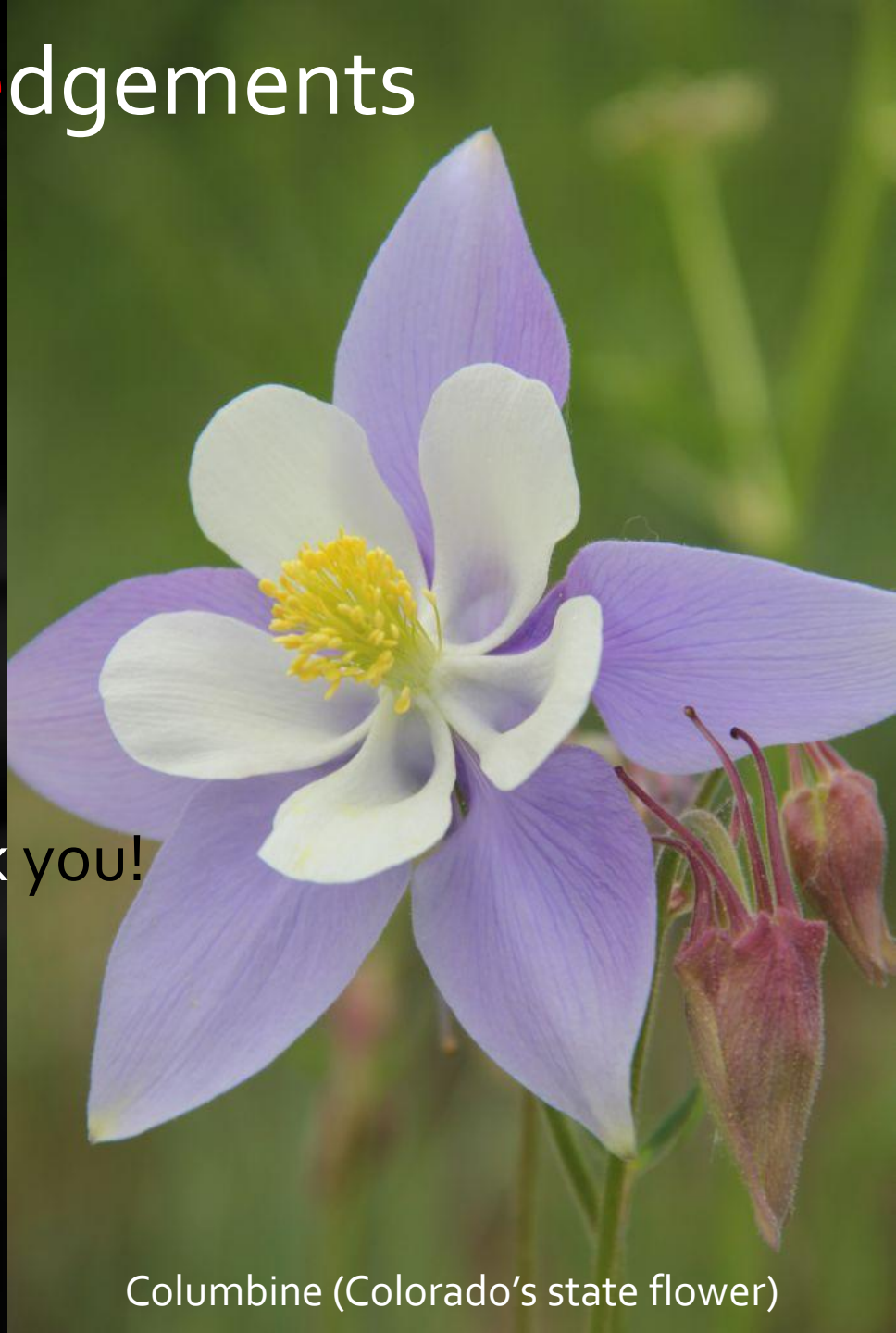
Thank you!



Columbine (Colorado's state flower)