

Crystal Structure of a Mammalian Fatty Acid Synthase

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 CON 605 Journal Club
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Sources...

- The Crystal Structure of a Mammalian Fatty Acid Synthase
 - Maier, Leibundgut & Ban
 - Science 321 (Sept 08): 1315-22
 - DOI: 10.1126/science.1161269
- An Enzyme Assembly Line
 - Smith & Sherman
 - Science 321: 1304-5
 - DOI: 10.1126/science.1163785
- Supporting online material for ~
 - DOI: 10.1126/science.1161269
- Handouts – copies of these overheads
 - <http://xtal.ohsu.edu/teaching>
 - Will be updated after Tues class.

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Agenda

Thursday

- Biological significance
- Framing the questions
- Introduction to the techniques

Tuesday

- What answers are forwarded?
- What backs these assertions
 - Dissection
 - Pointed critique
- What are the limits of the study?

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Role of Fatty Acid Synthase

- Synthesis of C₁₄ or C₁₆ fatty acids
- Lipids
 - Membrane structure
 - Energy storage
 - Messengers (prostaglandins etc.)
- Mammals: FA mostly from diet
 - Specialty synthesis
 - Cancer cells
 - Over-feeding / diabetes; inhibitors

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Biochemical Challenges

- Conceptually – reverse of β -oxidation
 - Synthesis will require energy
- New C—C bonds
 - Will need excellent leaving groups
 - “High energy priming”
- Hydrophobic intermediates

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Differences between biosynthesis & catabolism

	<u>β-oxidation</u>	<u>Biosynthesis</u>
Location	Mitochondrion	Cytoplasm
2C units	Acetyl-CoA	Malonyl-CoA (3C) (synthesized from Acetyl-CoA)
Fatty ac.	All sizes	16C (palmitate)
Cofactors	FAD ⁺ ; NAD ⁺	NADPH
Activation	CoA thioesters	Thioesters - acyl carrier prot.
Enzymes	4 distinct enzymes (all organisms)	4 enzymes (prokaryotes); 1 multi-enzyme complex (eukaryotes); polyprotein (mammals)

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Why Malonate (3C) for 2C-addition

- Principle: synthesis requires more energy than catabolic breakdown
- Condensation (C₂ w/ C_N) will be coupled to exothermic decarboxylation
- Acetyl-CoA → Malonyl-CoA requires ATP
- Transfer of 2C unit from malonyl-CoA can harvest the ATP-energy invested 1 step before

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Conversion Acetyl CoA to Malonyl CoA (step before Fatty Acid synthase)

- Malonyl CoA has additional carboxylate
- Acetyl-CoA carboxylase
 - Rate limiting in fatty acid synthesis
 - Biotin-dependent

$\text{HCO}_3^- + \text{ATP} + \text{E-biotin} \rightarrow \text{ADP} + \text{Pi} + \text{E-biotin-CO}_2^-$
 $\text{E-biotin-CO}_2^- + \text{CH}_3\text{-(CO)-SCoA} \rightarrow \text{Malonyl CoA} + \text{E-biotin}$
 $\text{Malonyl CoA: } -\text{O}_2\text{C-CH}_2\text{-(CO)-SCoA}$

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Acetyl-CoA carboxylase

- Rate-limiting on FA synthesis
- ATP hydrolysis coupled to synthesis of (high energy) carbonyl phosphate intermediate
- P_i is excellent leaving group in carboxybiotin formation
- Sets up nucleophilic attack of C on CH_2 .

Step 1: The carboxylation of biotin

Step 2: The transcarboxylation of biotin

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Acetyl-CoA Carboxylase Quaternary Structure

- Carboxylase & transcarboxylase active sites are separate
- Biotin is on a flexible leash
 - Anchored on biotin carboxyl carrier protein
 - Transfers activated carboxylate between active sites
- Bacteria – proteins are separate, assembled together
- Animals: all activities on one large 250 kDa polypeptide
 - Monomer inactive
 - Filamentous polymer active
 - ~20 subunits

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Regulation of Acetyl-CoA Carboxylase

- Rate limiting / 1st committed step – candidate for regulation
- Allosterically regulated
 - citrate is +ve effector;
 - Promotes active filamentous form
 - long fatty acids (palmitate) product -ve feedback.
 - Promotes dissociation → monomer
- Phosphorylation modulates allosteric control:
 - Phosphorylated (7 sites):
 - Amplifies [fatty acyl CoA] effect.
 - Attenuates [citrate] effect.
 - Effects of several kinases / messengers integrated.
 - Glucagon, epinephrine...
 - Dephosphorylated - opposite:
 - Insulin

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Fatty Acid Synthase, important points

- 7 catalyzed reactions
 - E. coli & plant chloroplasts - separate enzymes
 - Yeast: $\alpha_6\beta_6$ 2200 kDa protein
 - Animals α_2 534 kDa protein
 - Domains for each catalytic activity
 - Phosphopantetheine (ACP) prosthetic group may *transport substrate* between active sites

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Acyl-carrier protein (ACP)

Phosphopantetheine group of coenzyme A

Phosphopantetheine prosthetic group of ACP

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- Prosthetic group same as CoA: functional SH
 - AMP of CoA replaced by protein-Ser-OH
 - E. coli: 10 kD protein
 - Animals: part of fatty acid synthase

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Acyl Carrier Protein (2)

- Both reactants are activated w/ ACP
 - Malonyl CoA additions → Malonyl-ACP
 - Malonyl-CoA-ACP transacylase
 - Initial Acetyl CoA also → Acetyl-ACP
 - Acetyl-CoA-ACP transacylase
 - Transferred to enzyme Cys - *β ketoacyl-ACP synthase*
 - Same for extending chain

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Central steps: 1. β -ketoacyl-ACP synthase

Malonyl ACP

$\text{O}_2\text{C}-\text{CH}_2-\text{CO}-\text{S-ACP}$

CO_2

Carbanion attacks thioester

HS-ACP

$\text{CH}_3-(\text{C}_2\text{H}_4)_{0-12}-\text{CO}-\text{S-ACP}$

Product has keto + thioester

Ketoacyl-ACP, E.g. Acetoacyl-ACP (n=0)

$\text{CH}_3-(\text{C}_2\text{H}_4)_{0-12}-\text{CO}-\text{CH}_2-\text{CO}-\text{S-ACP}$

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Reduction of the 2nd keto

- Opposite of synthesis
 - Except 2nd step specific for D vs. L isomer

Ketoacyl-ACP

$\text{CH}_3-(\text{C}_2\text{H}_4)_{0-12}-\text{CO}-\text{CH}_2-\text{CO}-\text{S-ACP}$

2: Reduce this CO to CHOH (*β-ketoacyl-ACP reductase*); uses NADPH

3: Remove HO, H either side of bond (*β-hydroxyacyl-ACP dehydrase*) → double bond: --CH=CH--

4: Reduce C=C w/ NADPH (*enoyl-ACP reductase*)

Acyl-ACP, → next cycle, or...

$\text{CH}_3-(\text{C}_2\text{H}_4)_{0-12}-\text{CH}_2-\text{CH}_2-\text{CO}-\text{S-ACP}$

Palmitoyl thioesterase

Palmitate

$\text{CH}_3-(\text{C}_2\text{H}_4)_{14}-\text{COO}^-$

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Summary of the enzymes involved:

- Acetyl transacylase (AT) – initial C₂
- Malonyl tranacylase (MT) - extensions
- β-ketoacyl-ACP synthase (KS)
- β-ketoacyl-ACP reductase (KR)
- β-hydroxyacyl-ACP dehydrase (DH)
- Enoyl-ACP reductase (ER)
- Termination reaction:
 - Bacteria: Transfers to glycerol in phospholipid synthesis.
 - Fungi: Palmityl tranacylase transfers palmityl back to CoA (like MT).
 - Animals: Thioesterase releases free fatty acid.

Organization – Bacteria & Plants:

- Separate enzymes
 - Acetyl transacylase (AT)
 - Malonyl tranacylase (MT)
 - β-ketoacyl-ACP synthase (KS)
 - β-ketoacyl-ACP reductase (KR)
 - β-hydroxyacyl-ACP dehydrase (DH)
 - Enoyl-ACP reductase (ER)
 - Termination reaction

At least these 4 may form a complex

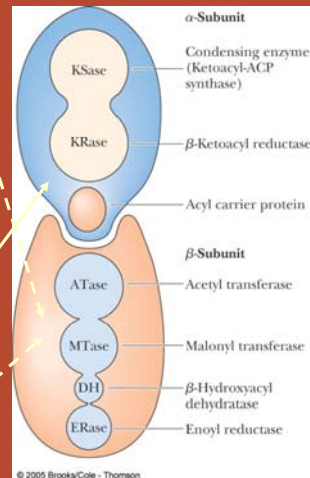
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Enzyme organization – Yeast:

- Two multifunctional subunits
 - Each ~ 200 kDa
 - Fatty acid synthase 1
 - Acetyl transacylase (AT)
 - Malonyl tranacylase (MT)
 - Fatty acid synthase 2
 - β-ketoacyl-ACP synthase (KS)
 - β-ketoacyl-ACP reductase (KR)
 - Fatty acid synthase 1
 - β-hydroxyacyl-ACP dehydrase (DH)
 - Enoyl-ACP reductase (ER)
 - α₆β₆ → 2.3 x 10⁶ Da



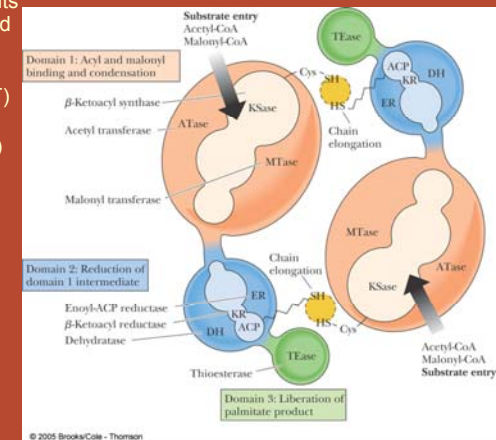
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Enzyme organization – Animals:

- Two identical subunits (270 kDa) – fatty acid synthase
 - Acetyl transacylase (AT)
 - Malonyl tranacylase (MT)
 - β-ketoacyl-ACP synthase (KS)
 - β-ketoacyl-ACP reductase (KR)
 - β-hydroxyacyl-ACP dehydrase (DH)
 - Enoyl-ACP reductase (ER)
 - Termination reaction



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Why the Organization?


- Complex:
 - Efficient transfer of substrate from one active site to the next
 - Especially important with insoluble intermediates
- Multifunctional polypeptides (- why?):
 - Gene expression – correct proportions of each enzyme are expressed automatically

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Nenad Ban - Biography



- Croatian
- Ph.D. UC Riverside
 - Alex McPherson
 - '90-'94
- Post-doc
 - Tom Steitz, Yale
 - 1994 - 2000
 - Crystal structure of Ribosome 50S
- Professor
 - structural molecular biology, Swiss Federal Institute of Technology, (ETH)
 - 2000 – current
 - Ribosome, FA synthases



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The questions... and the answers???

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Goals of the study

- Enzyme mechanism
- Previous publication at 4.5 Å
 - What more is learned at 3.5 Å resolution?
 - What is *Resolution*?
- Organization of domains
 - Relation to polyketide synthesis
 - Significance for metabolic mechanisms?
 - Relation to bacterial, fungal homologues
 - Significance for evolution?

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X-ray scattering by electron density

- $F(\mathbf{r}^*) = \sum_{j=1}^N A_j \exp 2\pi i \mathbf{r}^* \cdot \mathbf{r}_j$
 - \mathbf{r}^* - direction of scattering
 - \mathbf{r}_j – position of density (atoms)
 - $\exp\{ix\} = \cos\{x\} + i \sin\{x\}$
 - Sum of sinusoidal waves
 - Frequency depends on $|\mathbf{r}^*|$
- $\rho(\mathbf{r}) = T^{-1}[F(\mathbf{r}^*)] = V^* \int F(\mathbf{r}^*) \exp -2\pi i \mathbf{r}^* \cdot \mathbf{r} d\mathbf{r}^*$
 - High resolution – include high freq waves
 - Experimental limitations on measuring scattered waves.

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Scattering by elements of electron density

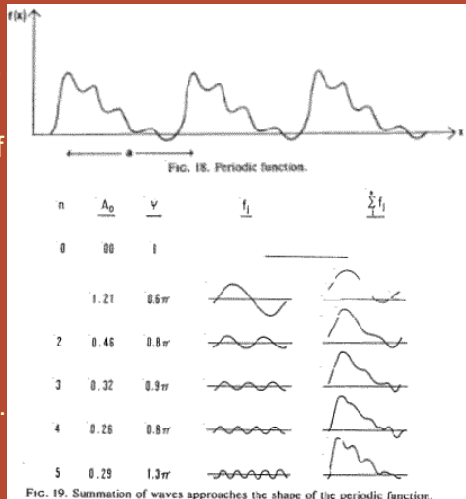
- Structure determination:
 - measure amplitude
 - determine phase throughout (continuous) function, $F(\mathbf{r}^*)$
 - compute inverse $\mathcal{FT} \rightarrow$ electron density:
- $\rho(\mathbf{r}) = T^{-1}[F(\mathbf{r}^*)] = V^* \int F(\mathbf{r}^*) \exp -2\pi i \mathbf{r}^* \cdot \mathbf{r} d\mathbf{r}^*$

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Fourier Series

Detail depends on frequency of waves included.

“Technical” definition of resolution in crystallography.

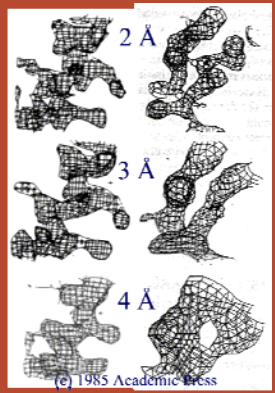


n	A_n	ν	f_1	$\sum_{j=1}^n f_j$
0	00	1		
1	1.21	0.6 π		
2	0.46	0.8 π		
3	0.32	0.9 π		
4	0.26	0.8 π		
5	0.25	1.3 π		

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Resolution – impact

- 2 Å – infer individual atoms
- 3 Å – infer side chain locations, backbone path
- 4 Å – locations of helices, sheets.
 - Infer domain homologies



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Methods - questions

- Quality of the data (table supplement p.2)
 - What do the statistics tell us?
- Heavy atom data sets?
- (Density modification?)
- What is TLS / TLSMD?
- Bottom line
 - R / R_{free} – definition; good values?
 - Density – Fig S14 – how to interpret?

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Completeness of the model

- How much is missing?
- Why?
- What parts?
- How does this impact the interpretation?

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Structure superposition

- Role in structure determination?
 - Structure initially not fully resolvable.
 - Use of homology.
- Role in interpretation?
 - Structural similarity.
 - More than sequence.
 - Evolutionary implications.
- How is it done?
- Sounds easy.
- What are the issues?

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Interpretation - methods

- Interfaces
- How strongly are subunits associated?
- How do we estimate?
- Supplement p.4 & tables S2 & S3.

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Data Quality

Max resolution	R _{merge}	R _{pim}	I/σ(I)
Native			
3.40 Å	66.4%	26.9%	2.5
3.35 Å	74.1%	30.2%	2.2
3.30 Å	84.3%	35.3%	1.9

- R_{merge}
 - What is it?
 - What is a good value?

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$$R = \frac{\sum |y - f(x)|}{\sum |y|}$$

2) Make model more flexible:
 a) Add parameters:
 $y = ax + c \rightarrow y = ax^2 + bx + c$
 b) Adding H₂O, Bs etc.
 c) Relaxing stereochemistry

3) Discard data
 Easier to fit, but worse model

1) Improve the model
 (change the line)

Cross-validation w/ "free" data $\rightarrow R^{free}$

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Density

Experimental

Model

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Structural Homology Searches

(From table S4.)
 What do the statistics mean?

RMSD	Nalign	%seq	%sse	Match PDB	Match title
MAT					
1.13	385	79	96	2jfd:A	Human FAS MAT Domain
2.65	343	21	70	2uv8: H,B	Type 1 FAS Yeast...

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Subunit association energies

- Lee & Richards algorithm
- Enhancements
 - Atomic solvation
 - H-bonding; Salt bridges

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