Firefly Luciferin

Down the Rabbit Hole of Quirality

(no Quantum Madness today…)

Pathway inside Photinus pyralis

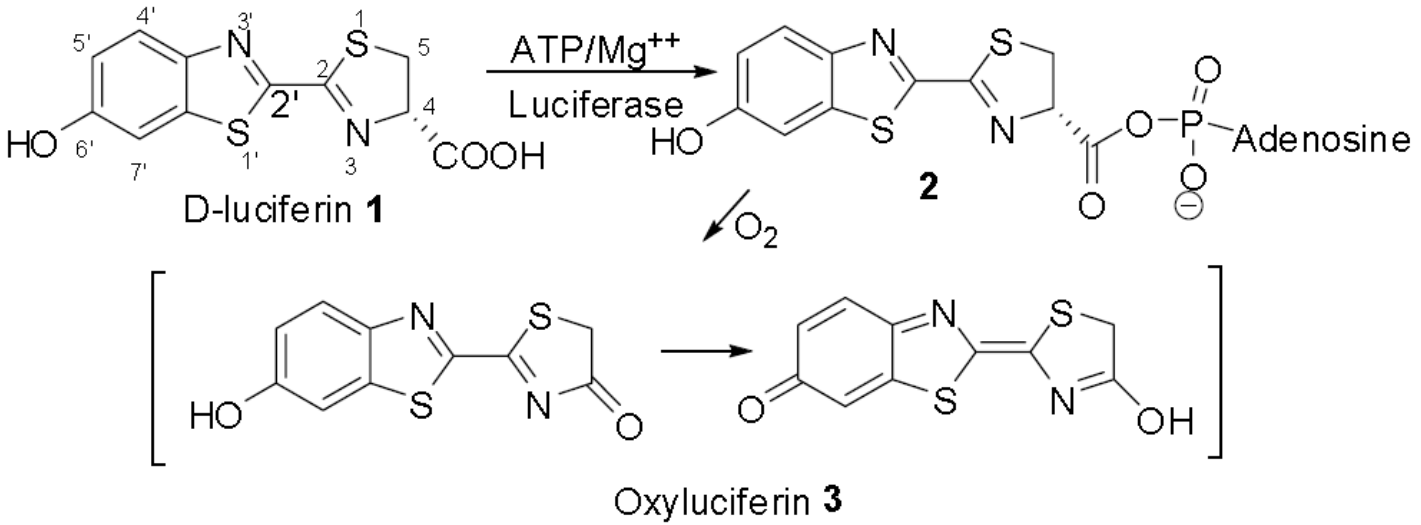


Figure 1. Luciferase-catalyzed transformation of D-luciferin (**1**) into oxyluciferin (**3**).

Notice that while luciferin is a quiral protein,
oxyluciferin is not.

How to Recycle oxyluciferin: pathway

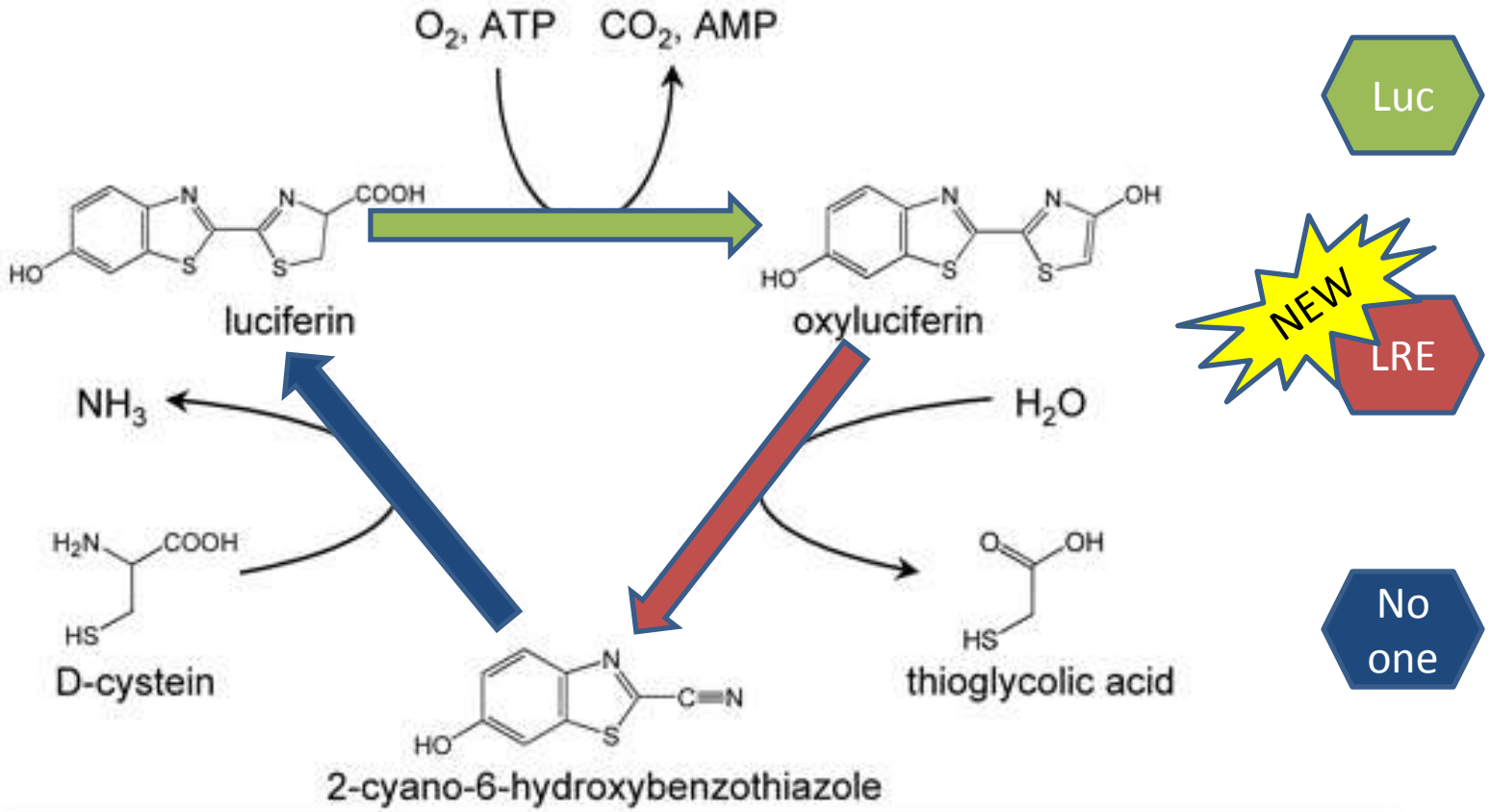


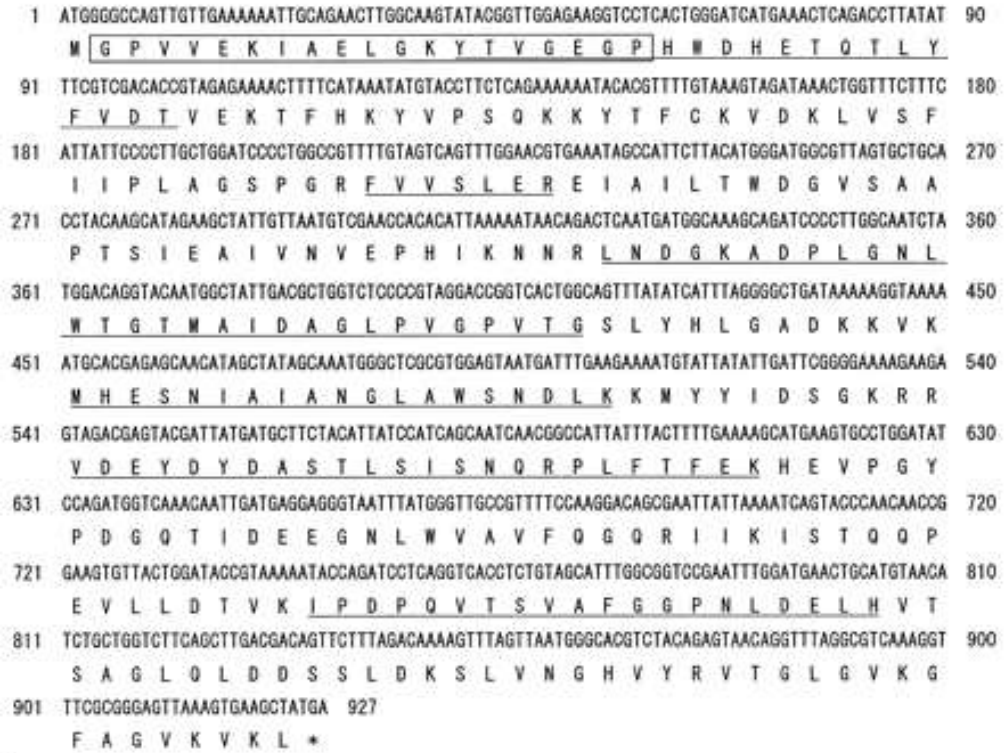
Figure 1

Luciferin recycling in the firefly.

How to Recycle oxyluciferin: enzyme

Meet:

Luciferine Regenerating Enzyme



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1   ATGGGGCCAGTTGTTGAAAAAATTGCAGAACTTGGCAAGTATACGGTTGGAGAAGGTCCTCACTGGGATCATGAAACTCAGACCTTATAT  90
    M  G  P  V  V  E  K  I  A  E  L  G  K  Y  T  V  G  E  G  P  H  W  D  H  E  T  Q  T  L  Y
91  TTCGTCGACACCGTAGAGAAAACTTTTCATAAATATGTACCTTCTCAGAAAAAATACACGTTTTGTAAAGTAGATAAACTGGTTTCTTTC  180
    F  V  D  T  V  E  K  T  F  H  K  Y  V  P  S  Q  K  K  Y  T  F  C  K  V  D  K  L  V  S  F
181 ATTATTCCCCTTGCTGGATCCCCTGGCCGTTTTGTAGTCAGTTTGGAACGTGAAATAGCCATTCTTACATGGGATGGCGTTAGTGCTGCA  270
    I  I  P  L  A  G  S  P  G  R  F  V  V  S  L  E  R  E  I  A  I  L  T  W  D  G  V  S  A  A
271 CCTACAAGCATAGAAGCTATTGTTAATGTCGAACCACACATTAAAAATAACAGACTCAATGATGGCAAAGCAGATCCCCTTGGCAATCTA  360
    P  T  S  I  E  A  I  V  N  V  E  P  H  I  K  N  N  R  L  N  D  G  K  A  D  P  L  G  N  L
361 TGGACAGGTACAATGGCTATTGACGCTGGTCTCCCCGTAGGACCGGTCACTGGCAGTTTATATCATTTAGGGGCTGATAAAAAGGTAAAA  450
    W  T  G  T  M  A  I  D  A  G  L  P  V  G  P  V  T  G  S  L  Y  H  L  G  A  D  K  K  V  K
451 ATGCACGAGAGCAACATAGCTATAGCAAATGGGCTCGCGTGGAGTAATGATTTGAAGAAAATGTATTATATTGATTCGGGGAAAAGAAGA  540
    M  H  E  S  N  I  A  I  A  N  G  L  A  W  S  N  D  L  K  K  M  Y  Y  I  D  S  G  K  R  R
541 GTAGACGAGTACGATTATGATGCTTCTACATTATCCATCAGCAATCAACGGCCATTATTTACTTTTGAAAAGCATGAAGTGCCTGGATAT  630
    V  D  E  Y  D  Y  D  A  S  T  L  S  I  S  N  Q  R  P  L  F  T  F  E  K  H  E  V  P  G  Y
631 CCAGATGGTCAAACAATTGATGAGGAGGGTAATTTATGGGTTGCCGTTTTCCAAGGACAGCGAATTATTAAAATCAGTACCCAACAACCG  720
    P  D  G  Q  T  I  D  E  E  G  N  L  W  V  A  V  F  Q  G  Q  R  I  I  K  I  S  T  Q  Q  P
721 GAAGTGTTACTGGATACCGTAAAAATACCAGATCCTCAGGTCACCTCTGTAGCATTTGGCGGTCCGAATTTGGATGAACTGCATGTAACA  810
    E  V  L  L  D  T  V  K  I  P  D  P  Q  V  T  S  V  A  F  G  G  P  N  L  D  E  L  H  V  T
811 TCTGCTGGTCTTCAGCTTGACGACAGTTCTTTAGACAAAAGTTTAGTTAATGGGCACGTCTACAGAGTAACAGGTTTAGGCGTCAAAGGT  900
    S  A  G  L  Q  L  D  D  S  S  L  D  K  S  L  V  N  G  H  V  Y  R  V  T  G  L  G  V  K  G
901 TTCGCGGGAGTTAAAGTGAAGCTATGA  927
    F  A  G  V  K  V  K  L  *
```

Figure 6

Nucleotide sequence of A-LRE cDNA and deduced amino acid sequence. The N-terminal and partial amino acid sequences determined by Edman degradation are *boxed* and *underlined*, respectively.

in Photinus pyralis ergo:

- Sustainable reconversion reaction of oxyluciferin into D-luciferin.
- Quirality of D-luciferin donated by D-cysteine.
- With L-cysteine, oxyluciferin becomes L-luciferine.
- However, only D-luciferin has luminescent properties!
- Ergo: we need either D-cysteine or D-luciferin

Enter Magic Enzyme

- Magic Enzyme will stereoisomericaly bio-inverse L-luciferin into D-luciferin
- Magic Enzyme will be simple and cheap
- Magic Enzyme will be coded by a few genes
- Magic Enzyme will be expressable in E. coli
- Magic Enzyme won't have complexities…
- Does Magic Enzyme exist?

A Voice from Beyond

- *“We previously reported that in the presence of ATP, Mg2+ and CoA, firefly luciferase exhibits coenzyme A ligase (CoA-ligase) activity on L-luciferin in vitro, but not on D-luciferin, to give luciferyl-CoA”*

Magic Quirality Conversion

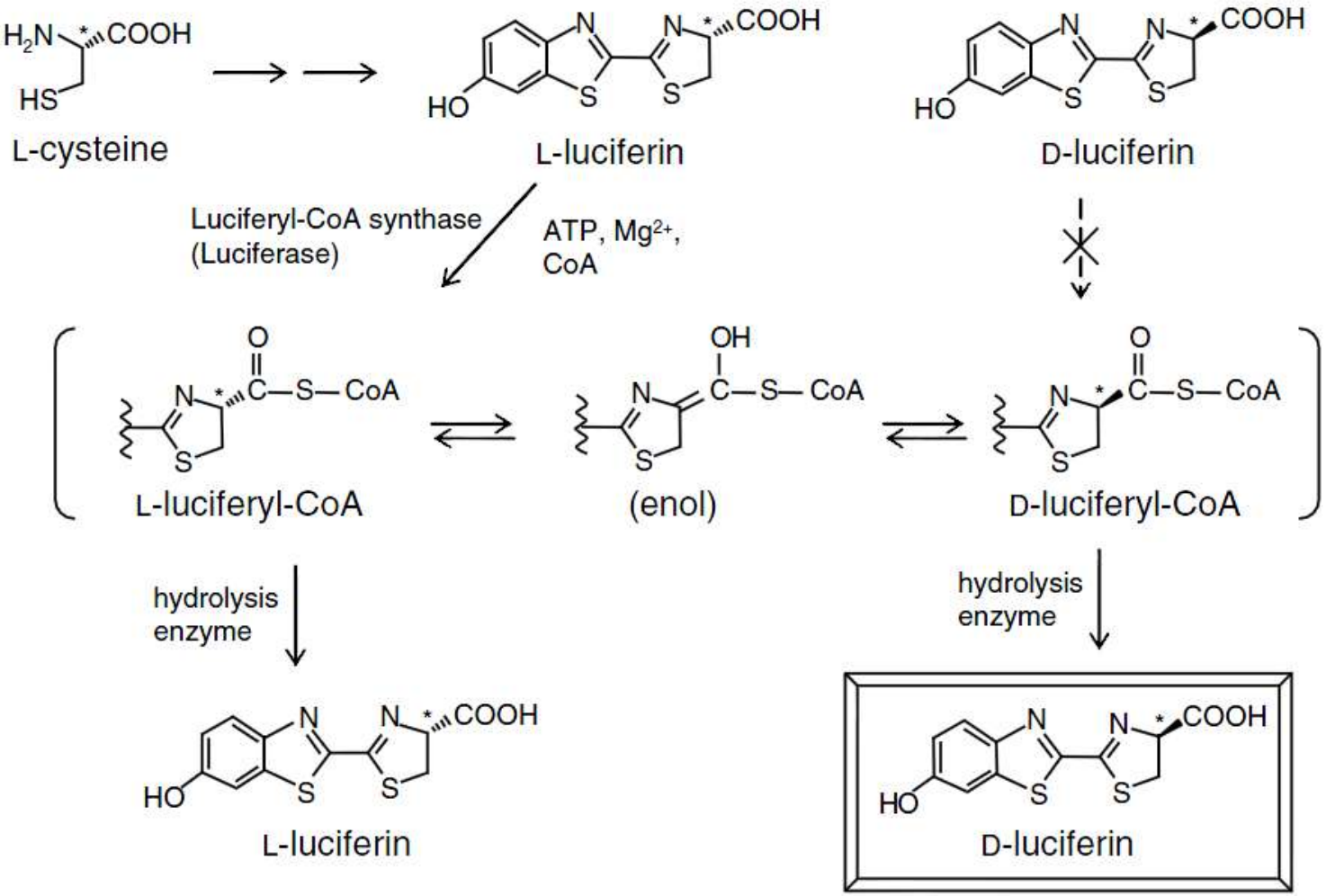

Fig. 5. Proposed biosynthetic pathway of D-luciferin. L-luciferin is produced from natural L-cysteine. L-Luciferin is converted into L-luciferyl-CoA that is easy to racemize by enolization. Hydrolysis of D-luciferyl-CoA gives the bioluminescent substrate, D-luciferin.

The Prestige

- Magic enzyme is Luciferase!
- *“Lembert first reported the light production from L-luciferin and proposed that L-luciferin was racemized to give D-luciferin and it was effectively stimulated by the addition of pyrophosphate. This racemization is an in vitro reaction of luciferase in the absence of CoA. In the presence of CoA, L-luciferin is readily converted into luciferyl-CoA.”*

Huh?!

- Initial medium inoculation with D-luciferin
- Luciferase takes D-luciferin, produces oxyluciferin
- Oxyluciferin is tranformed into L-luciferin via action of LRE with L-cysteine
- L-luciferin is transformed into D-luciferin through Luciferase action

So:

- We CAN use Photinus pyralis luciferase
- Suggested red-shifted mutant: Ser284Thr shines at 615nm

Sources

Special Issue Reviews and Accounts ARKIVOC 2009 (i) 265-288

D-Luciferin, derivatives and analogues: synthesis and *in vitro/in vivo* luciferase-catalyzed bioluminescent activity

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Oxyluciferin, a Luminescence Product of Firefly Luciferase, Is Enzymatically Regenerated into Luciferin*

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A set of multicolored *Photinus pyralis* luciferase mutants for *in vivo* bioluminescence applications

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Stereoisomeric bio-inversion key to biosynthesis of firefly D-luciferin

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Red- and green-emitting firefly luciferase mutants for bioluminescent reporter applications

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