Metabolic Engineering

Metabolic Engineering for Biomedical Applications

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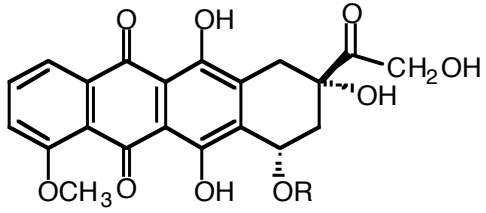
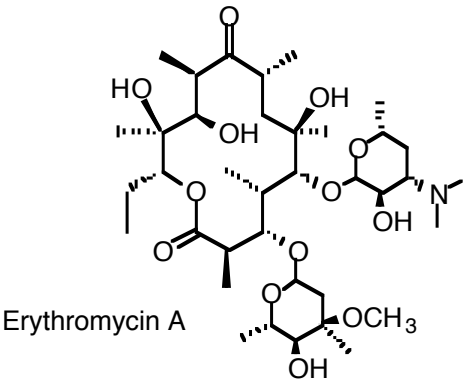
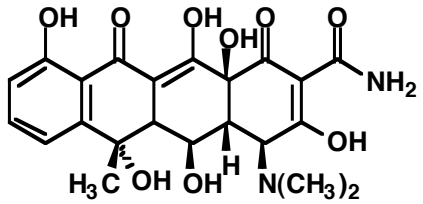
Polyketide Natural Products



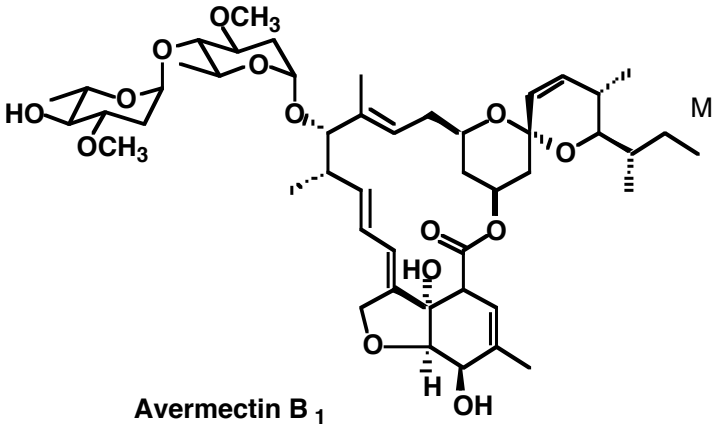
Doxorubicin



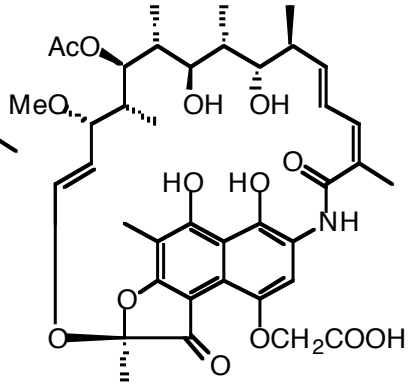
Erythromycin A



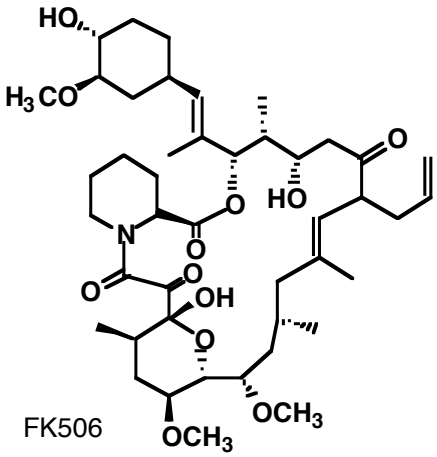
Tetracycline



Avermectin B 1



Rifamycin B

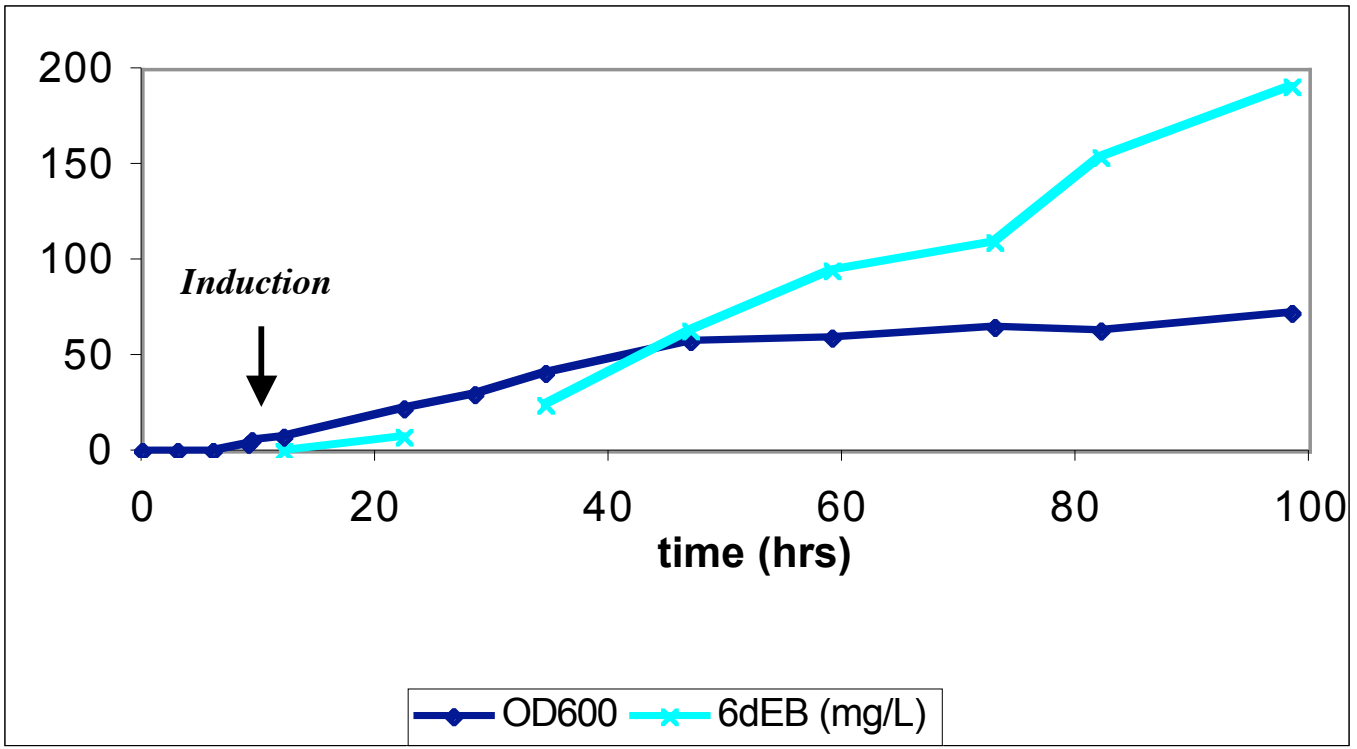


FK506

Problems

- **Polyketides are expensive**
- **Polyketides are difficult to modify**

Metabolic Engineering: Production of a Complex Polyketide in Heterologous Hosts



McDaniel et al *Science* (*1993*)
Kao, et al *Science (1994)*
Pfeifer, et al *Science (2001)*

Epothilones: A Practical Challenge

Epothilones

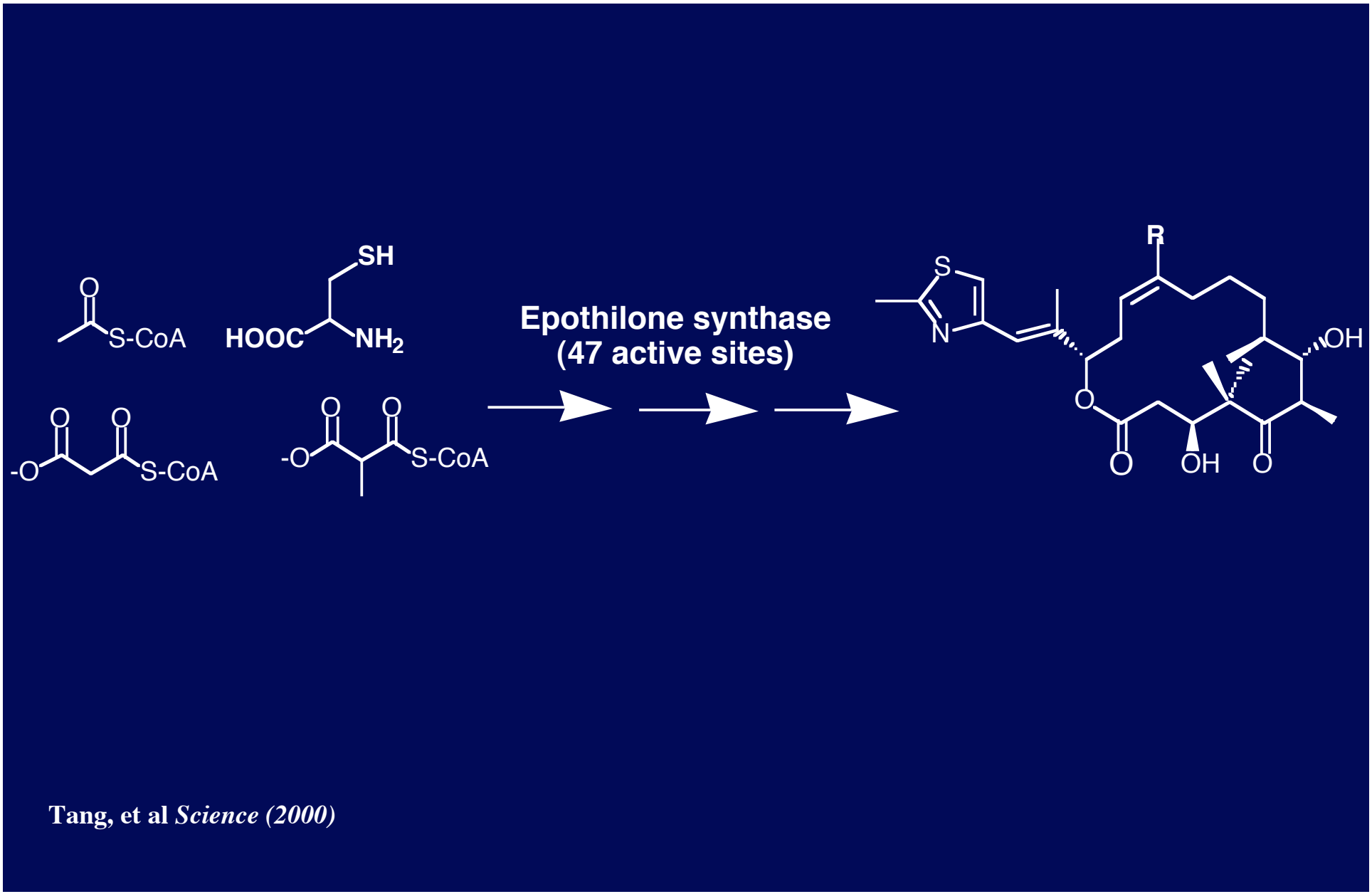
	R
A	H
B	CH_3

Sorangium cellulosum

Epothilone D

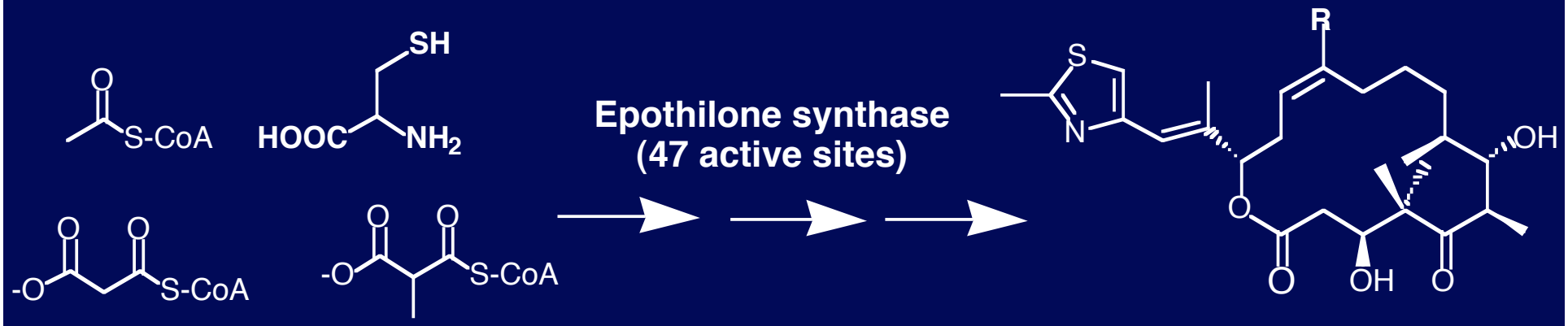
Hofle & coworkers, 1994
Bollag & coworkers, 1995
Danishefsky & coworkers, 1998

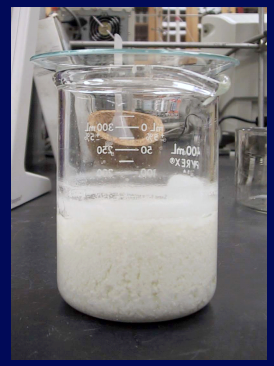
Heterologous Production of Epothilone D



Tang, et al *Science (2000)*

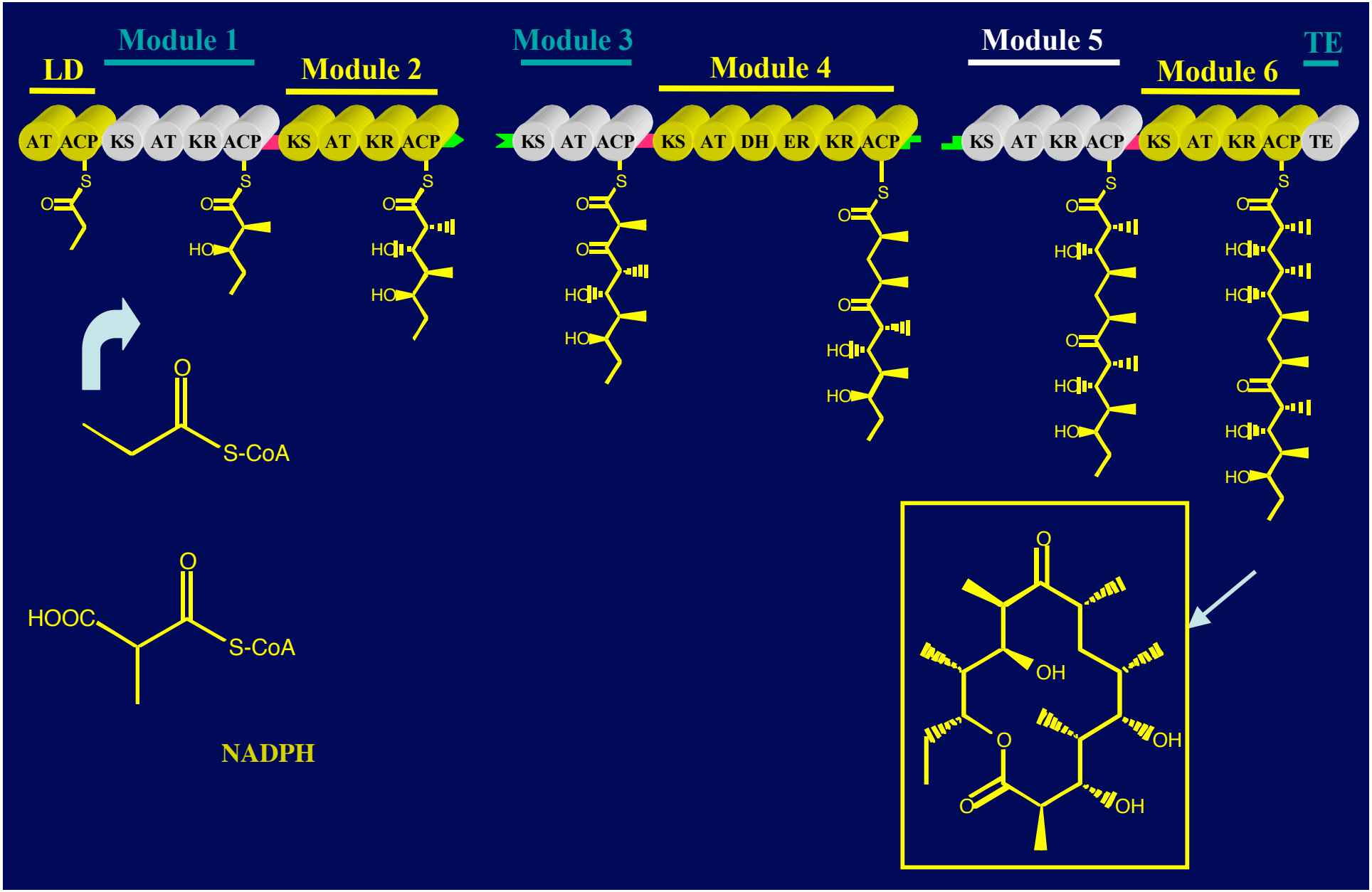
Heterologous Production of Epothilone D



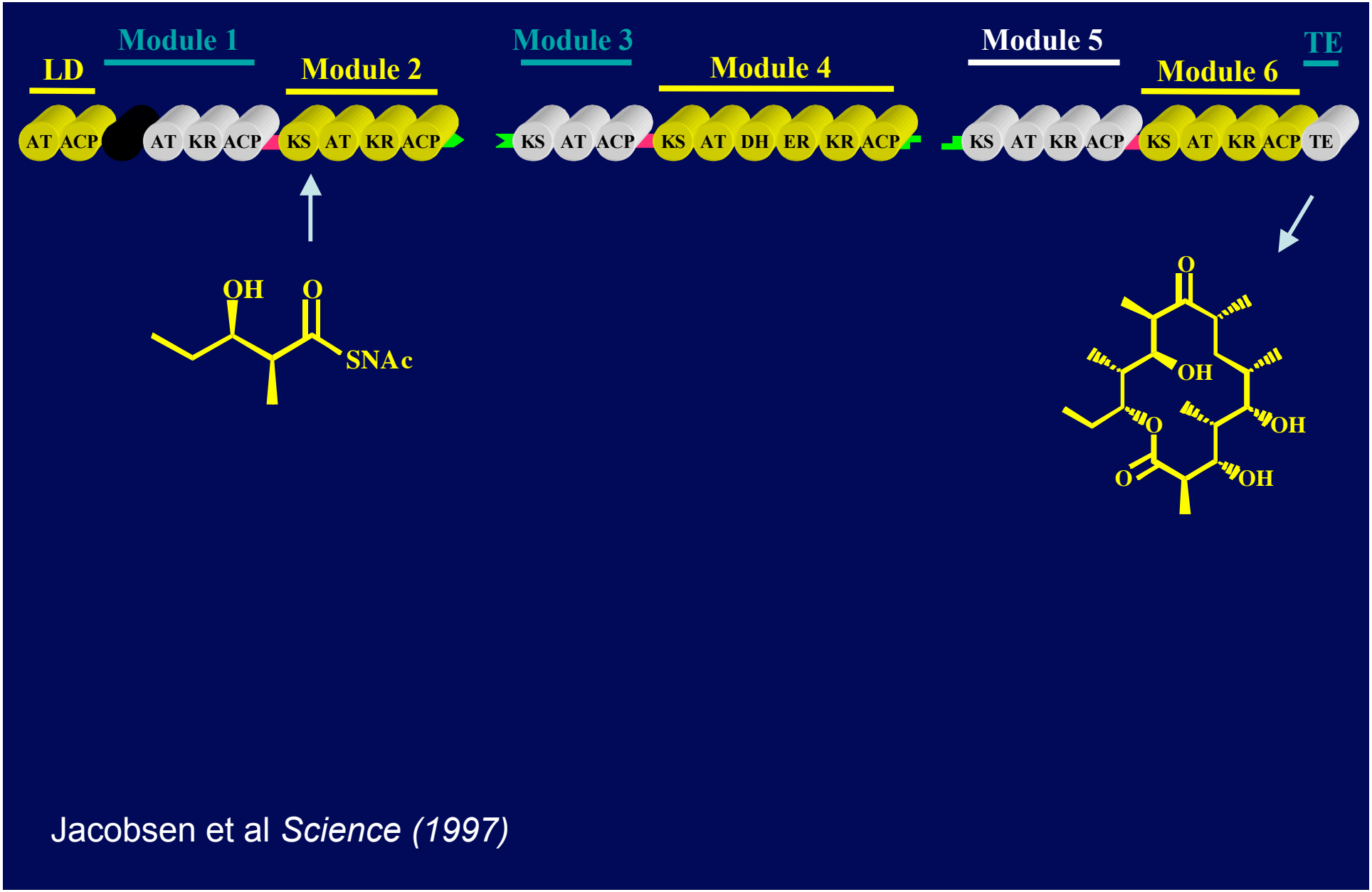


Tang, et al *Science (2000)*

Biosynthesis of the Erythromycin Polyketide

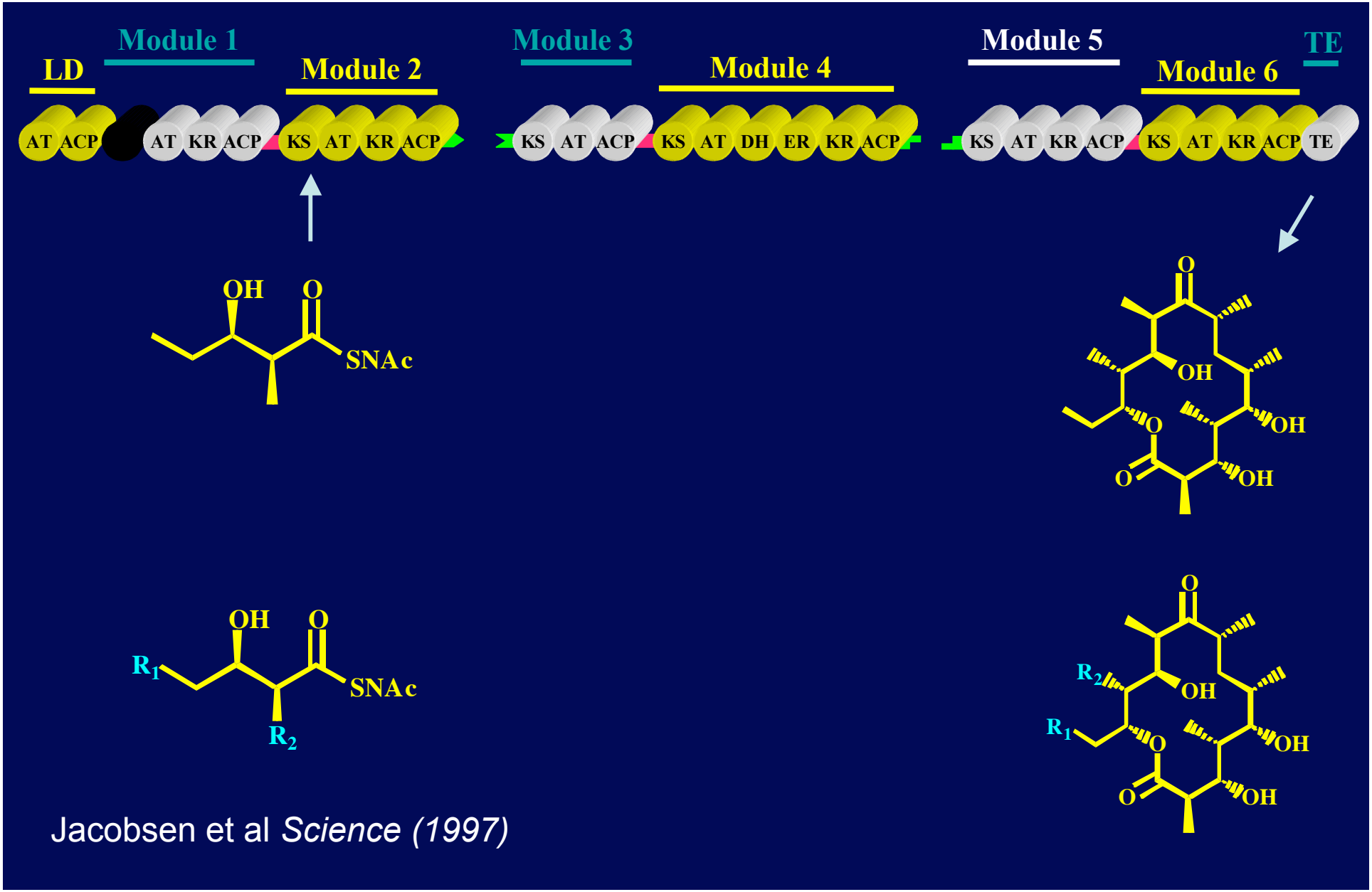


Metabolic Engineering of New Erythromycins



Jacobsen et al *Science (1997)*

Metabolic Engineering of New Erythromycins

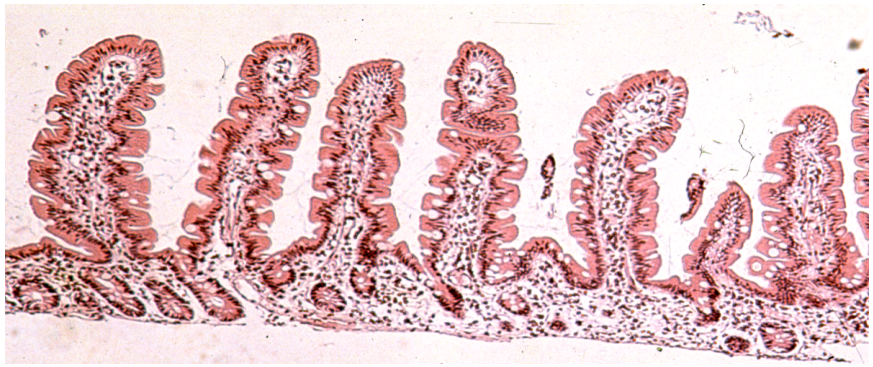


Jacobsen et al *Science (1997)*

Outlook

- **Polyketides are expensive**
  - **Production in metabolically engineered heterologous hosts**
- **Polyketides are difficult to modify**
  - **Genetic & chemical modification of assembly-line metabolism**

Engineering Gluten Metabolism to Treat Celiac Sprue

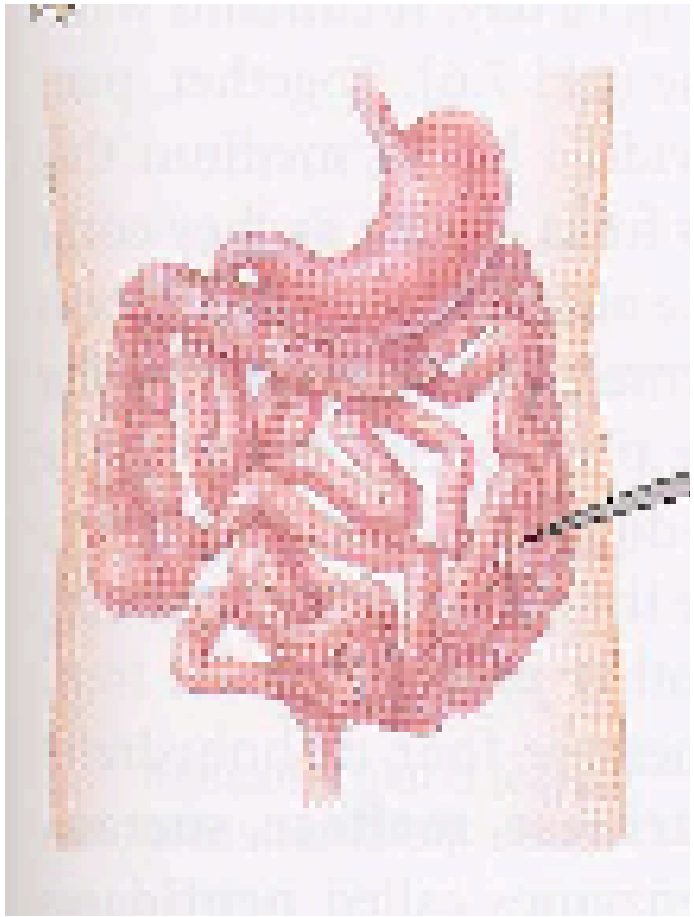


- **Widespread, inheritable immune disease of small intestine**
- **Induced by gluten**
- **Severe symptoms in early childhood**
  - malnutrition, failure to thrive
- **Adult onset of disease associated with diffuse symptoms**
  - fatigue, diarrhea, weight loss, anemia, neurological symptoms
- **Severe complications of untreated disease**
  - infertility, osteoporosis, cancer
- **No therapeutic option**

Approach

- **Understand how gluten is metabolized in the gastrointestinal tract**
- **Alter gluten metabolism in Celiac patients to evade immune response**

Simulating Gluten Digestion



Pepsin

⬇

Trypsin
Chymotrypsin
Elastase
Carboxypeptidase

⬇

Intestinal brush border membrane preparations

⬇

???

Key Findings

- Gluten proteins are difficult to digest

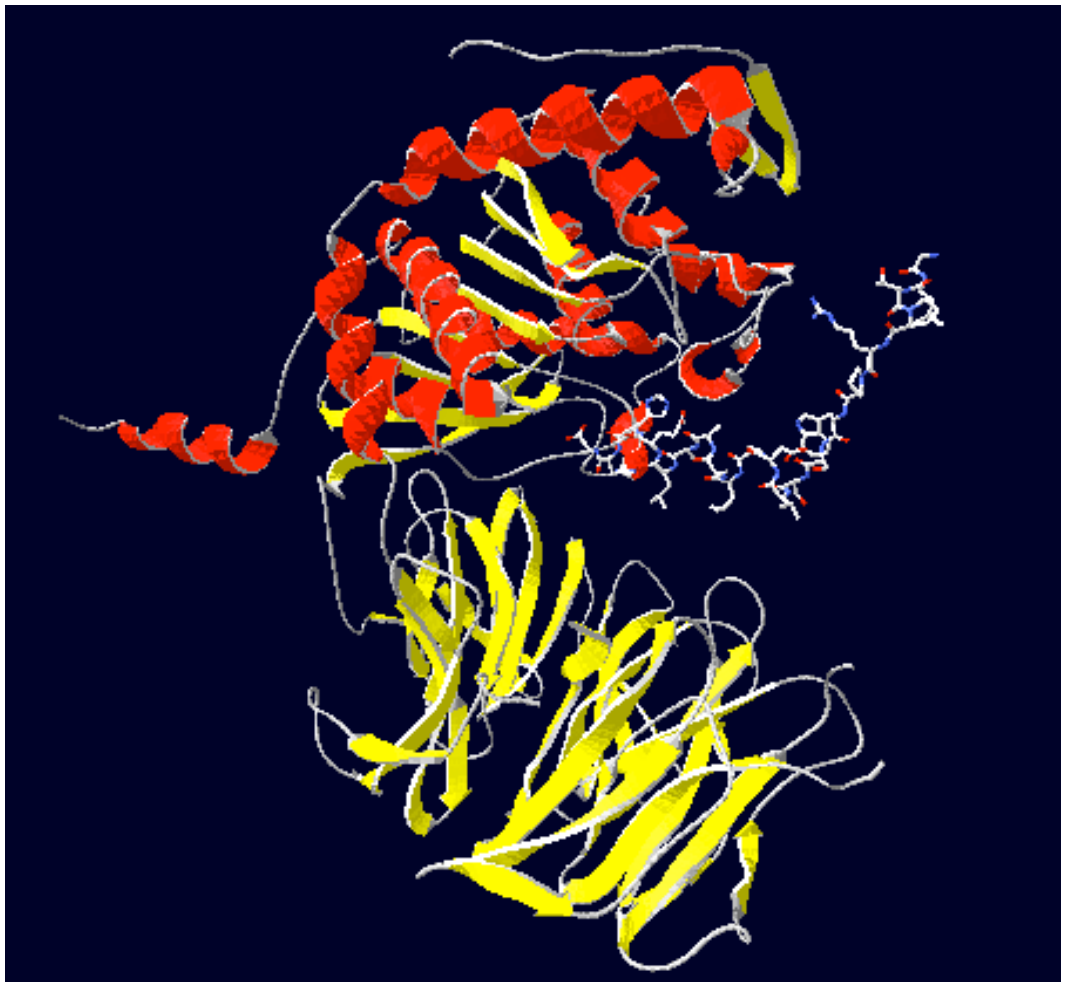
- The most difficult-to-digest parts of gluten are also the most immunotoxic

- Metabolic bottleneck due to inability of the mammalian gut to cleave gluten at internal proline residues

Hypothesis: **Supplement gluten digestion in the Celiac gut with a prolyl endopeptidase (PEP)**

Shan et al *Science* (2002)

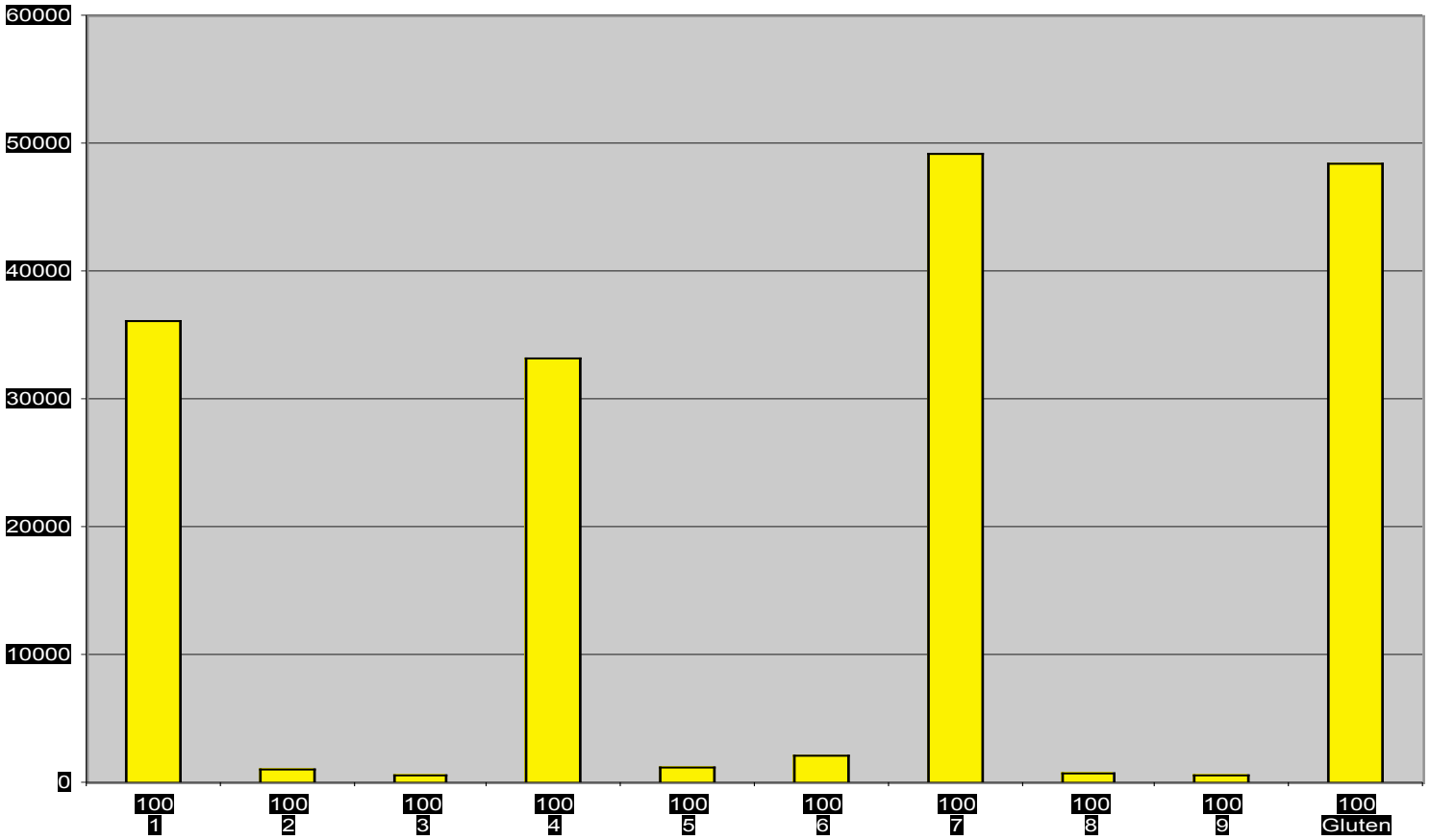
A Recombinant Bacterial PEP



Scaled up fermentation process to 1000 L: Produced 100 g

Shan, Irimpan, et al + Celiac Sprue Research Foundation (unpublished)

Immunogenicity of Proteolyzed Gluten Against Patient-Derived T Cells



- 1, 4, 7: no PEP
- 2, 5, 8: 60' PEP
- 3, 6, 9: 240' PEP
- 1-3: no BBM
- 4-6: 60' BBM
- 7-9: 240' BBM

Celiac Sprue Research Foundation + Sollid & coworkers (unpublished)

Gluten Detoxification in Celiac Patients?

- Clinical Trial Format:
  - Open label PEP-gluten safety study in 4 healthy volunteers
  - Double-blind, placebo controlled crossover trial
  - 14 days dosing - 2 months washout - 14 days dosing
- Gluten Dose: 5 gluten/day OR 5 g PEP-gluten/day
- PEP dose: 1000 units/day
- Subjects: 12-20 biopsy-proven Celiacs in remission
- Tests: xylose, fat, collect PBMCs and serum

Celiac Sprue Research Foundation (unpublished)

Conclusions

- **Metabolic engineering has broad applicability to medicine:**
  - **New bioprocesses**
  - **New bioproducts**
  - **New approaches to treat diseases**

Acknowledgements

- Stanford University
- Kosan Biosciences, Inc.
- Celiac Sprue Research Foundation
- Rikhospitalet, University of Oslo

- NSF => NIH