

AnTigone

A living biotherapeutic to manage hyperandrogenism in PMOS

iGEM European Meet Up Málaga'26

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iGEM Lund 2026; Lund University, LTH

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The Problem

Polyendocrine **M**etabolic **O**varian **S**yndrome

The Problem – PMOS

Polyendocrine **M**etabolic **O**varian **S**yndrome (formerly PCOS) is a common hormonal & metabolic disorder



~10–13% of women worldwide are affected; up to 70% undiagnosed

Current treatments are symptom-based and not specifically designed for treating PMOS.



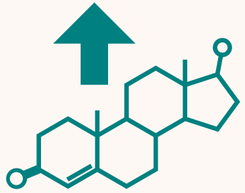
Lifestyle changes



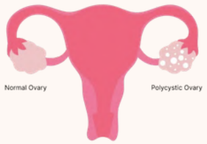
Medication
(COC, Metformin, anti-androgens)

→ need for targeted strategies

Rotterdam Criteria



Hyperandrogenism



Polycystic ovarian morphology



Ovulatory dysfunction

Hyperandrogenism as central, clinically relevant driver of PMOS symptoms:



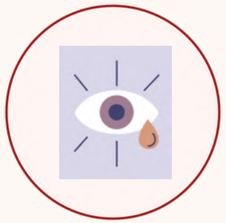
anovulatory infertility



Irregular periods, obesity



acne, hirsutism, alopecia



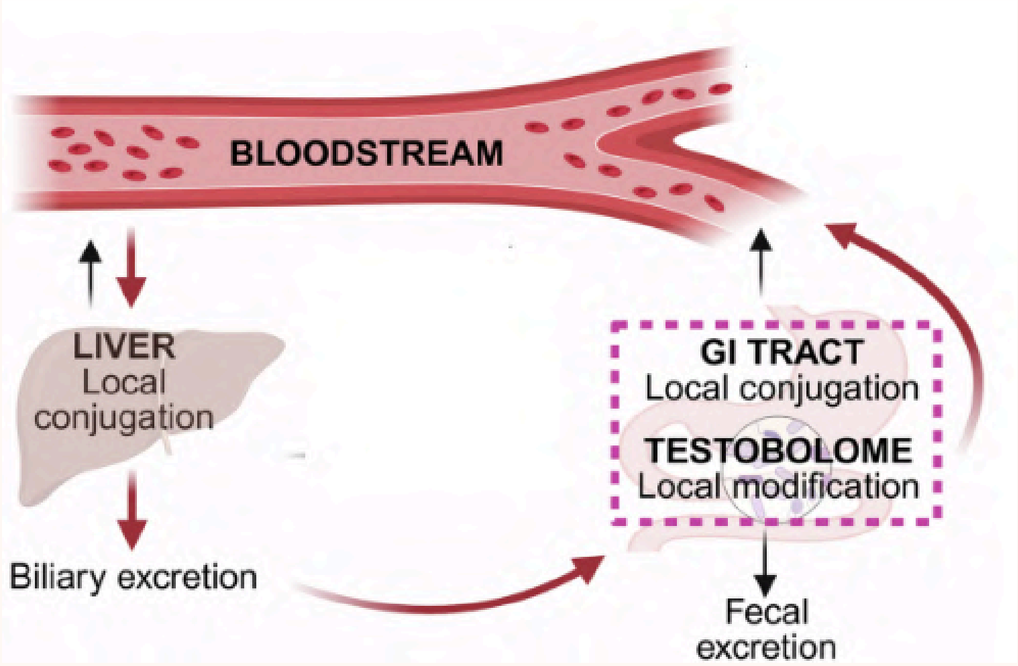
Low mood, anxiety, depression

Our question: can we lower excess androgen levels (more specifically testosterone) locally to have an effect on the entire body?

The Testobolome

Gut microbes may reshape **systemic** androgen levels

- Bile delivers conjugated androgens to the gut
- Microbial enzymes deconjugate & reduce them → free, active testosterone
- Reabsorbed into blood stream



→ targeting the gut microbiome for **local** testosterone modulation, possibly altering **systemic** testosterone levels (local→systemic).

The Solution (Design)

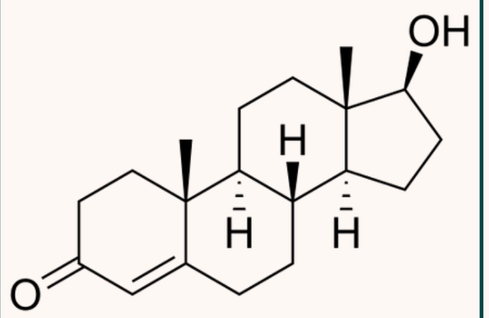
Gut-gated 5β-reduction & A-ring cleavage

The Solution: Gut-gated 5β-reduction & A-ring cleavage

A gut-gated system expressing an effector that converts testosterone into a less-androgenic form

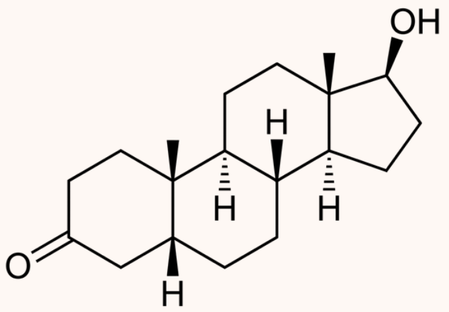
Two mechanisms: **5β-reduction**

Testosterone



5β-reductase

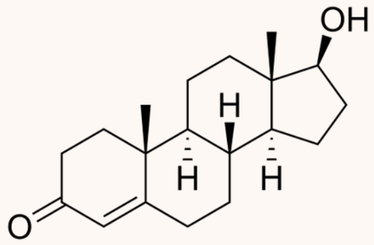
5β-DHT
less-androgenic



**DHT = Dihydrotestosterone*

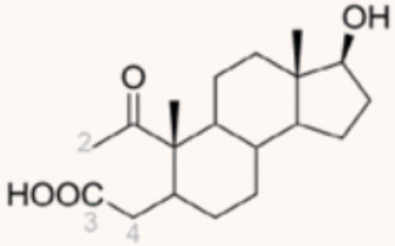
A-ring–cleavage

Testosterone



2,3-seco pathway
(multi-step)

2,3-SAOA



Total loss of androgen receptor affinity

Effector Candidates

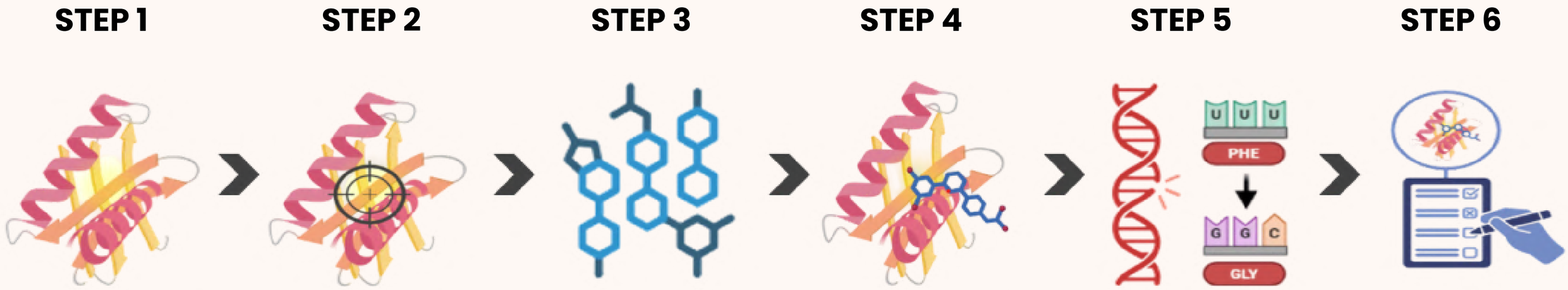
Three enzymes, different trade-offs.

Gene	Source	Mechanism	Challenge
atcABC	Bacterial (*Thauera sp. GDN1*)	A-ring cleavage (2,3-seco)	Complex; 3 subunits; cofactor needed (molybdopterin)
ci2350	Bacterial *(C. innocuum)*	5β-reduction (NADH dependent)	Poorly characterised; progesterone affinity
AKR1D1	Human	5β-reduction (NADPH dependent)	Not testosterone-specific; forms inclusion bodies

Modelling

Rational Mutagenesis Protocol

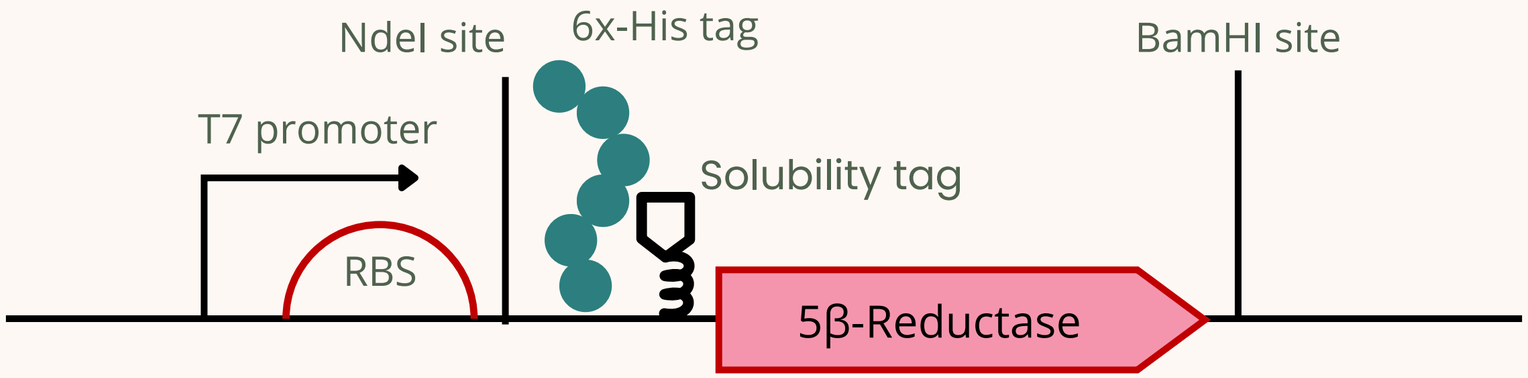
Aim: effector specificity for testosterone
Targeted candidates: AKR1D1 & ci2350



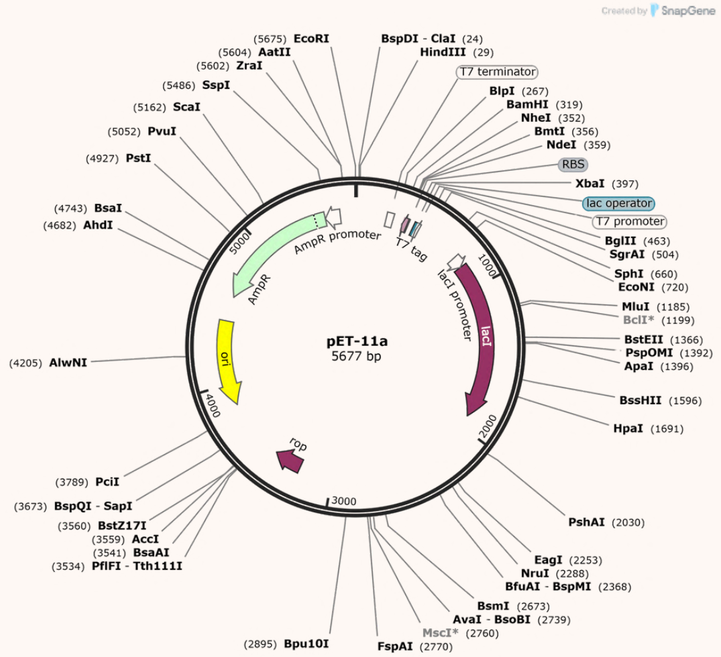
The Approach (Build, Test)

Experimental validation

Gene Construct for 5β-Reductases



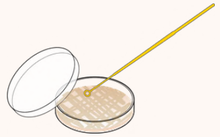
Vector: pET-11a



Different fusion tags:

- **Solubility**: SUMO, TrxA, MBP
- **Purification**: 6xHis

Experimental Workflow



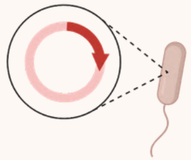
Colony PCR + Sequencing

Cloning & Expression

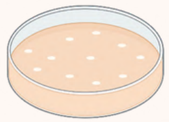
1 Cloning



2 Transformation (first TG1, then BL21)



3 Selection



4 Cultivation



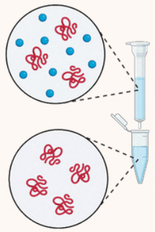
5 Cell lysis



E.coli TG1 for cloning and amplification; E.coli BL21(DE3) for expression (IPTG induced)

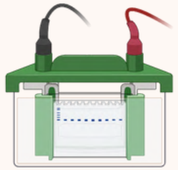
Purification

6 His-tag IMAC (Ni2+)



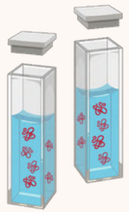
Characterization

7 Analyze purification with SDS-PAGE



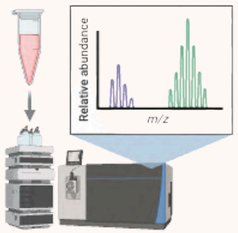
8 Assays:

- Purified protein
- Whole cell
- Androgen receptor



Confirmation

9 LC-MS



**IMAC = Immobilized metal affinity chromatography*

Human Practices, Biosafety & Outlook

Human Practices



Anna Benrick
PMOS expert



Trevor Penning
Wet lab,
AKR1D1 expert



Simon Ekström
Dry lab



Suman Rice
PMOS expert



Anton Sellerberg
Dry lab



Katia Palombino
Clinician

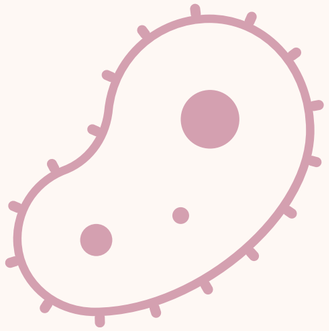


Ana Nordberg
Legislative and ethics

Biosafety

not implemented (yet)

- **Gut-gate:** targeted effector activity (e.g. hypoxia-/bile-responsive promoter)
- **kill-switch/auxotrophy**: avoiding biocontamination
- GMO Regulations



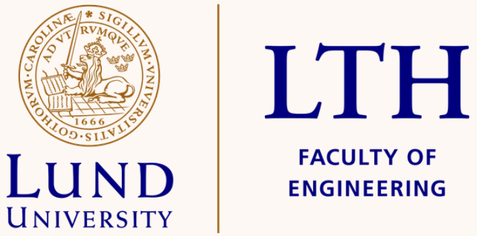
Future outlook

- Testosterone-specificity for ci2350 / AKR1D1 variants
- Chassis: E. coli proof-of-concept → gut-relevant probiotic (e.g. *E. coli Nissle 1917*), including genome integration
- **Adapting this proof-of-concept to more hyperandrogenism driving androgens (e.g. DHT)**

THANK YOU!

To our **sponsors** and collaborators







Foundation for
Interdisciplinary
Scientific Research



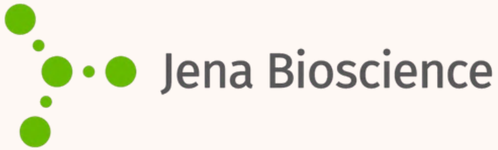




























Thank You!

Questions?

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Sources

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