Designing allosteric control into enzymes

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Sensors: protein can be chosen to suit application, identity of small molecule matters

Switches: identity of protein matters, small molecule can be chosen to suit application

Goal: devise a general way to modulate protein function with a small molecule

Current approaches:
fusion of a naturally allosteric domain
DeGrado enzyme design

Approach - “indole rescue”

Traditional chemical rescue

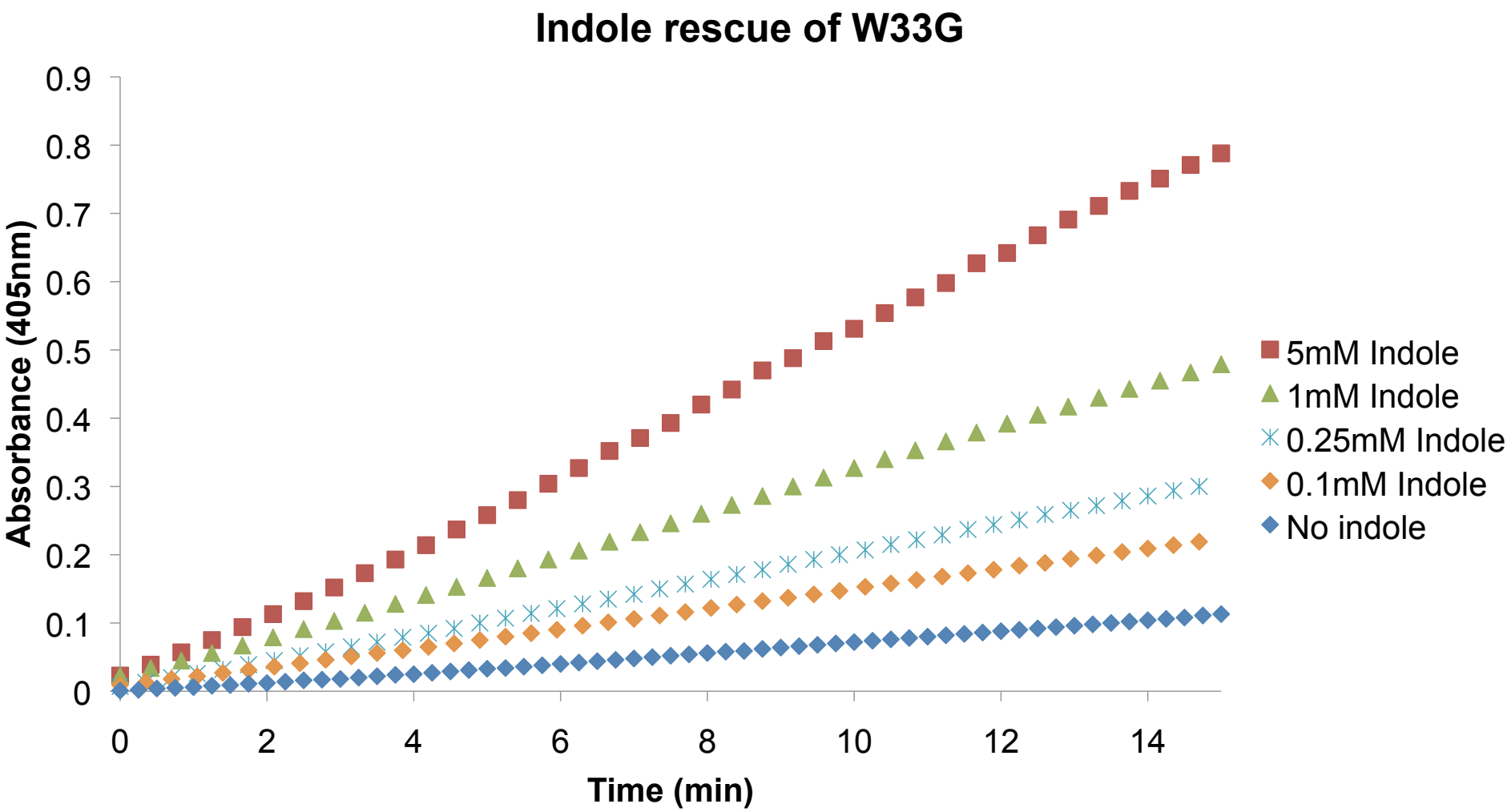
- An active-site sidechain is deleted leading to loss of function; exogenous addition of the cognate moiety restores function

- Active-site H→A mutations rescued by imidazole are popular

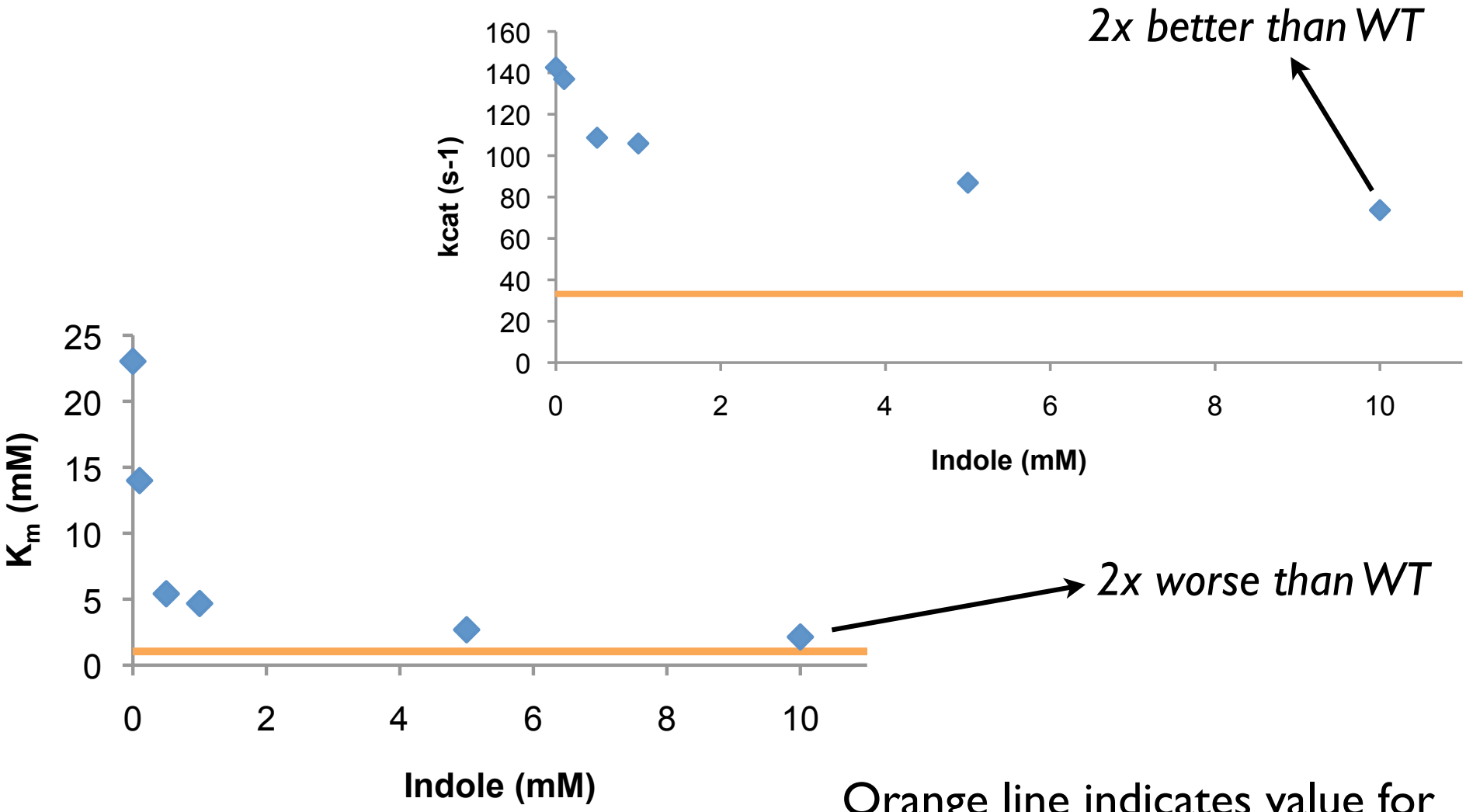
Advantage of rescuing structure

- Not limited to enzymes
- More hydrophobic activator ⇒ better affinity
- Buried ligand ⇒ better selectivity
- Opportunity for *allosteric* activation

Model system: β-glycosidase

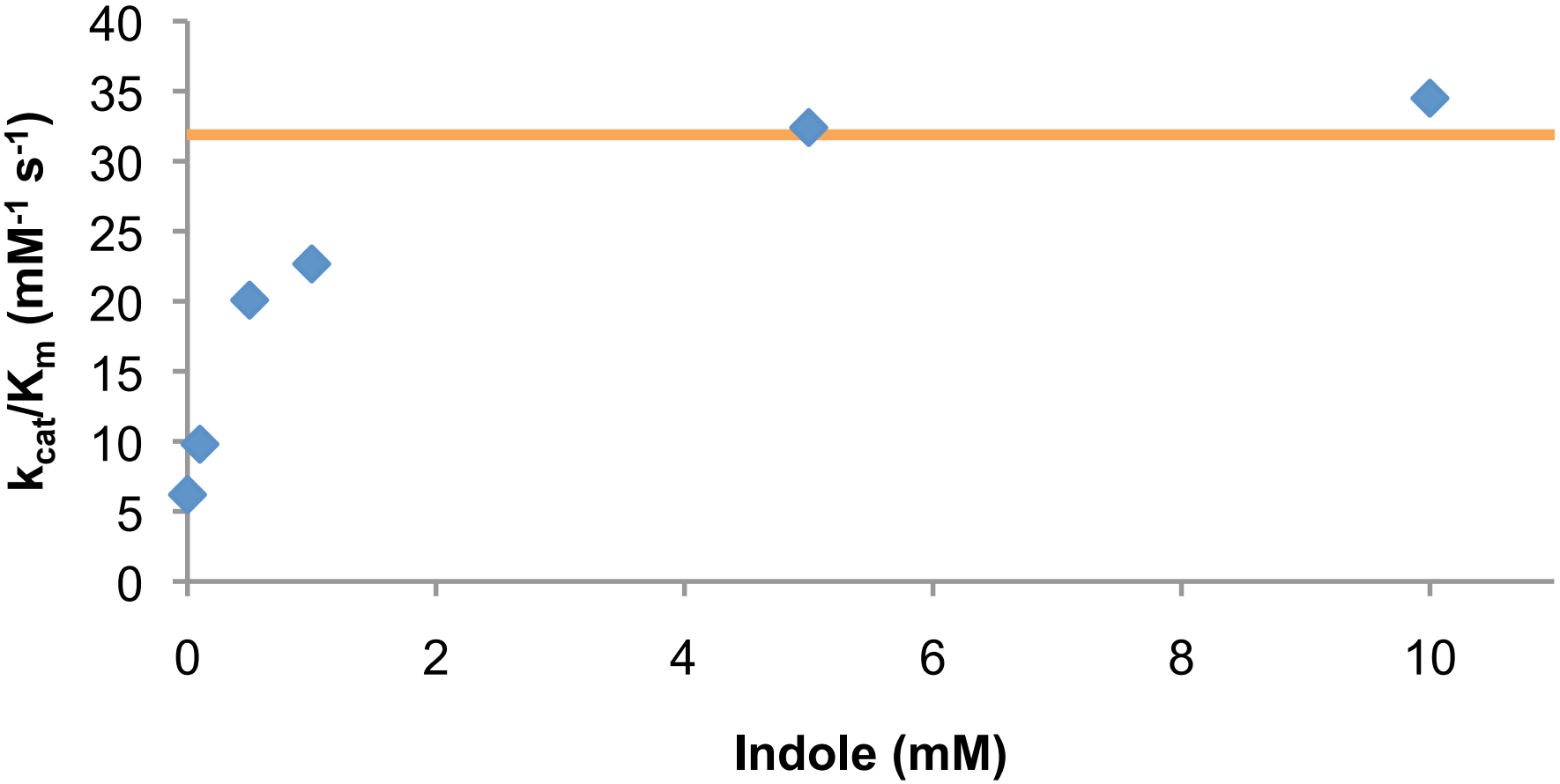


Mechanism of rescue



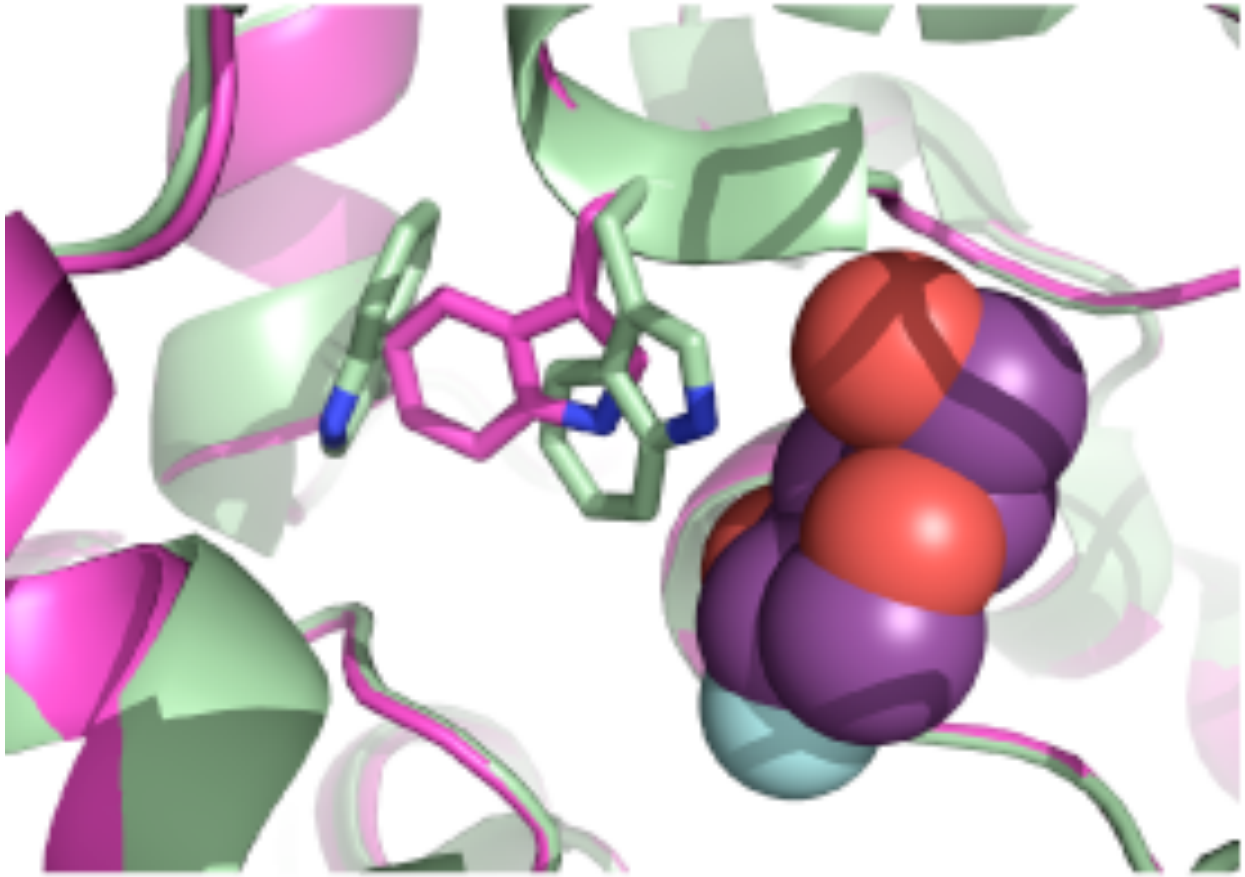
Orange line indicates value for WT enzyme (indole independent)

Catalytic efficiency upon rescue



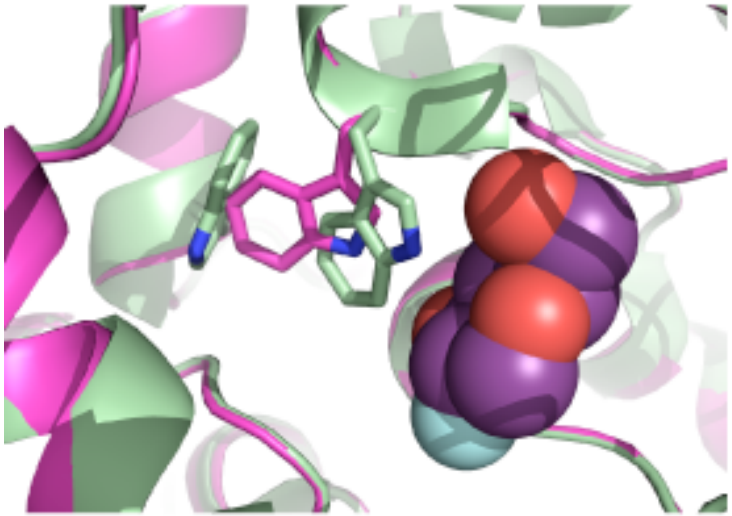
Orange line indicates value for WT enzyme (indole independent)

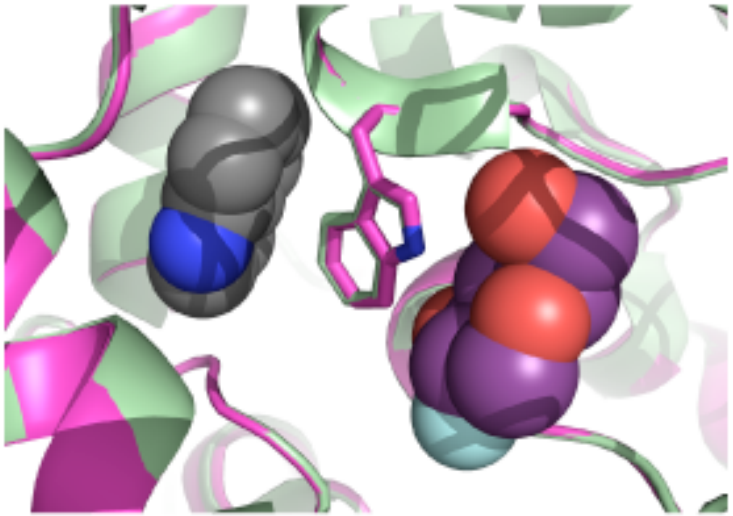
Structural basis for inactivation

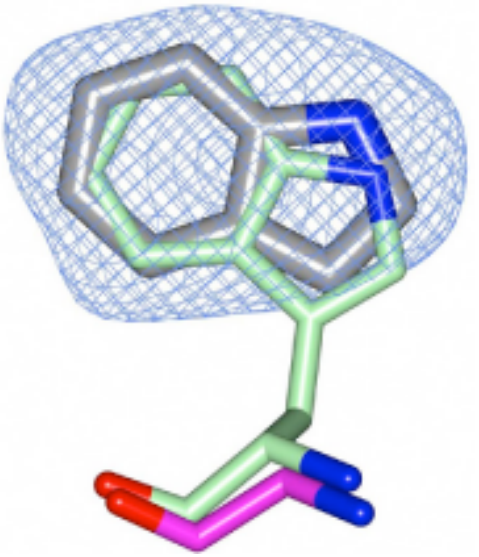


Wild-type
W33G

Structural basis for activation





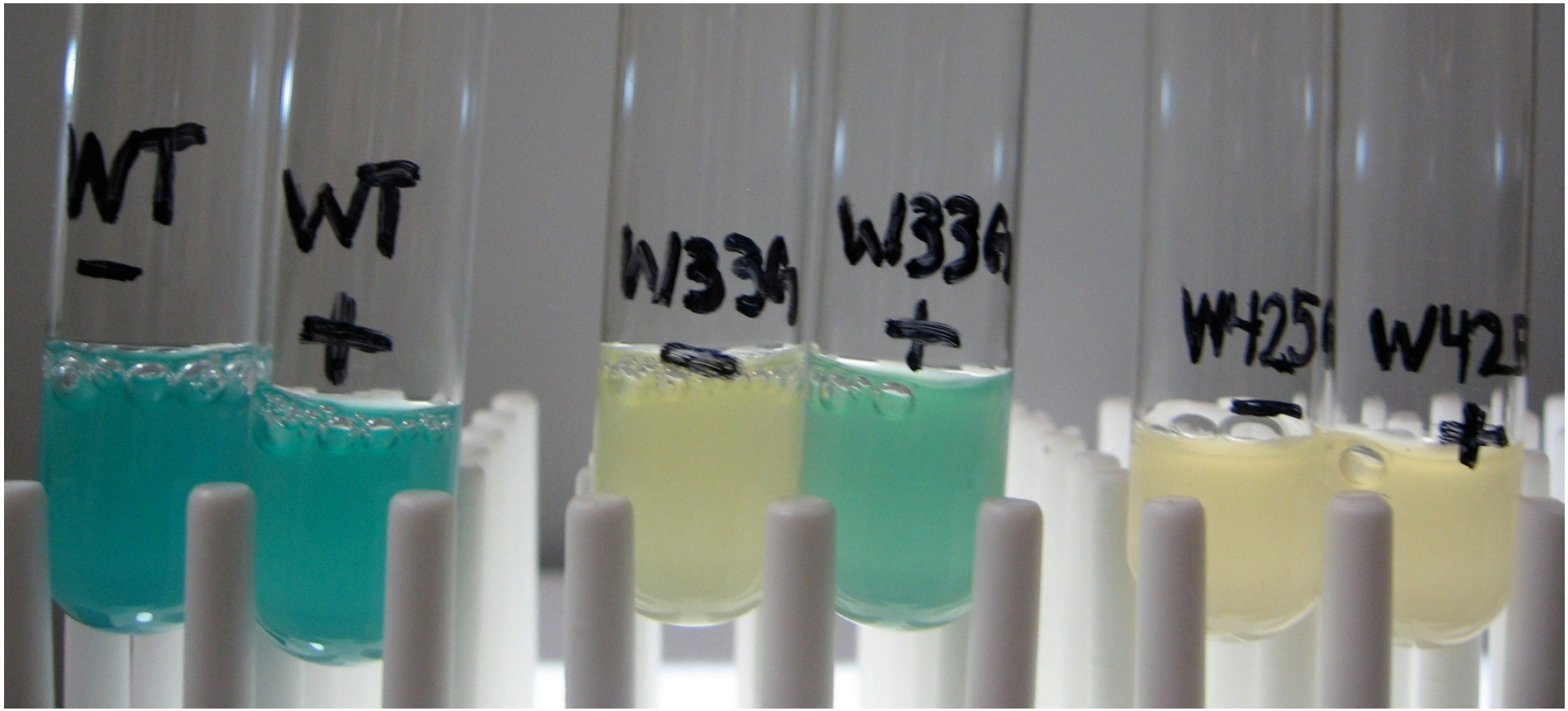


Wild-type
W33G (apo)

Wild-type
W33G (holo)
Indole

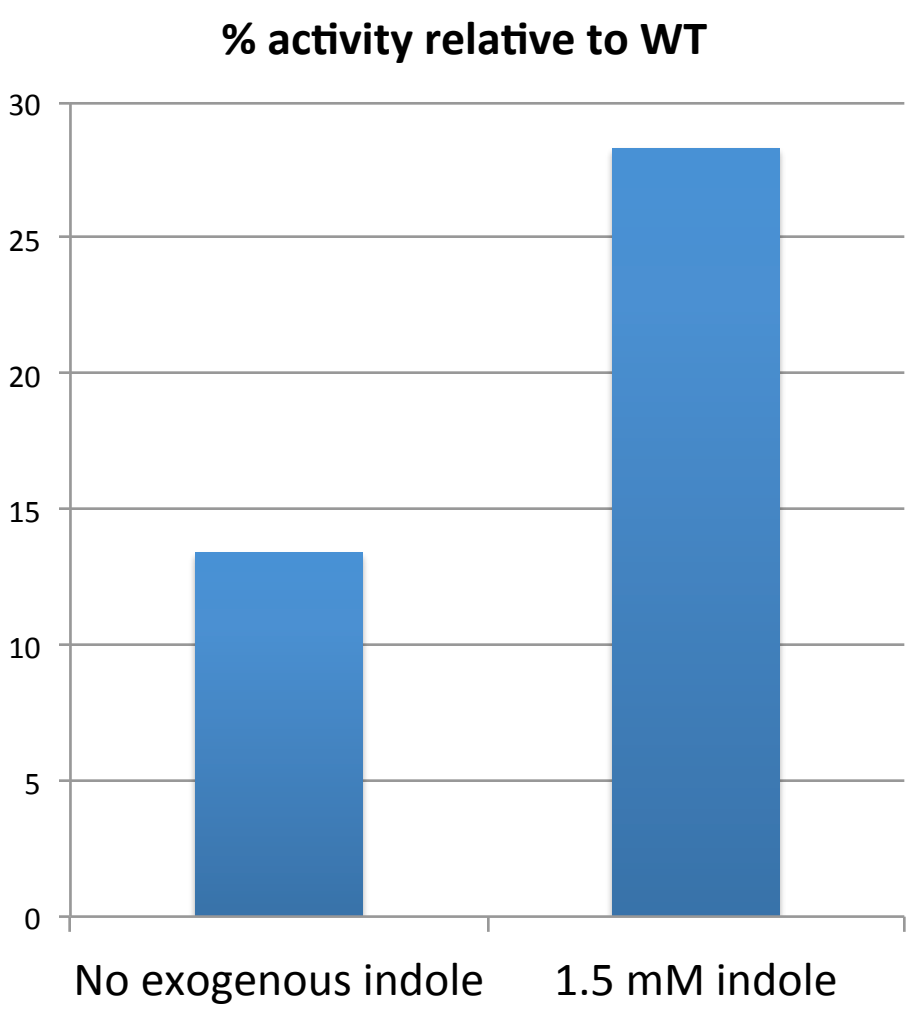
Fo-Fc map
contoured at 4σ
Note: indole orientation ambiguous at 2.25 Å resolution.

Rescue in living cells

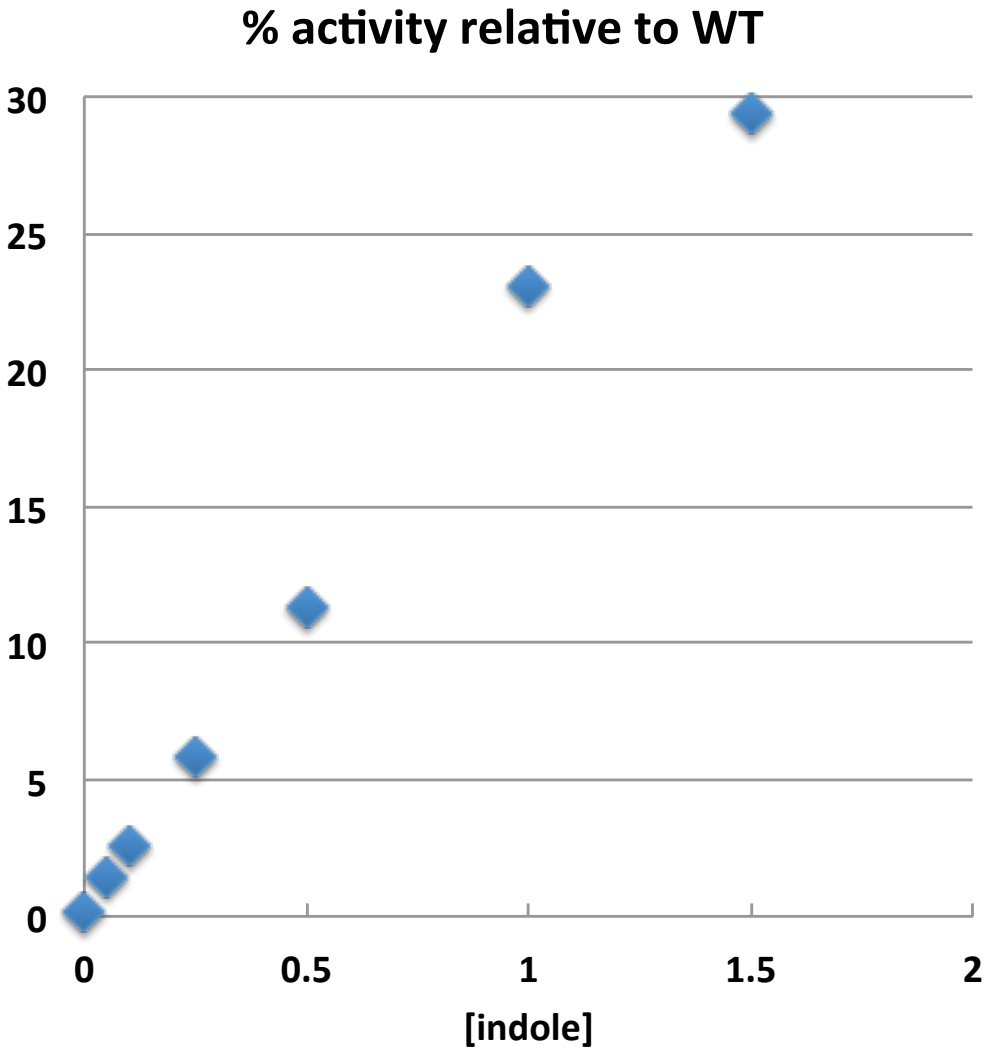


All tubes contain 1 mM IPTG and 0.2 mM X-gal,
those marked "+" additionally contain 2 mM indole.

Quantification in living cells (FDG)

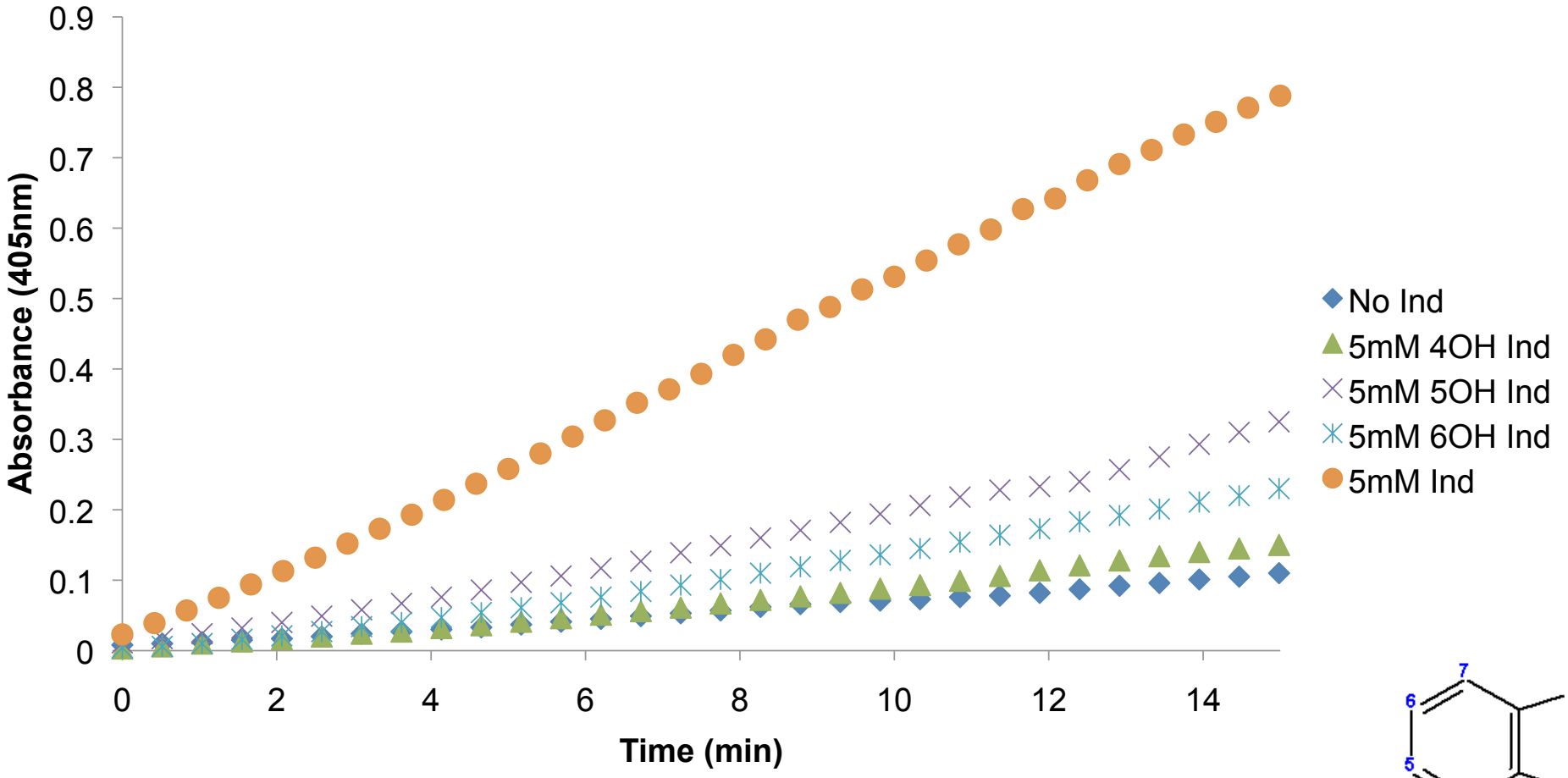


Activity *in cells*

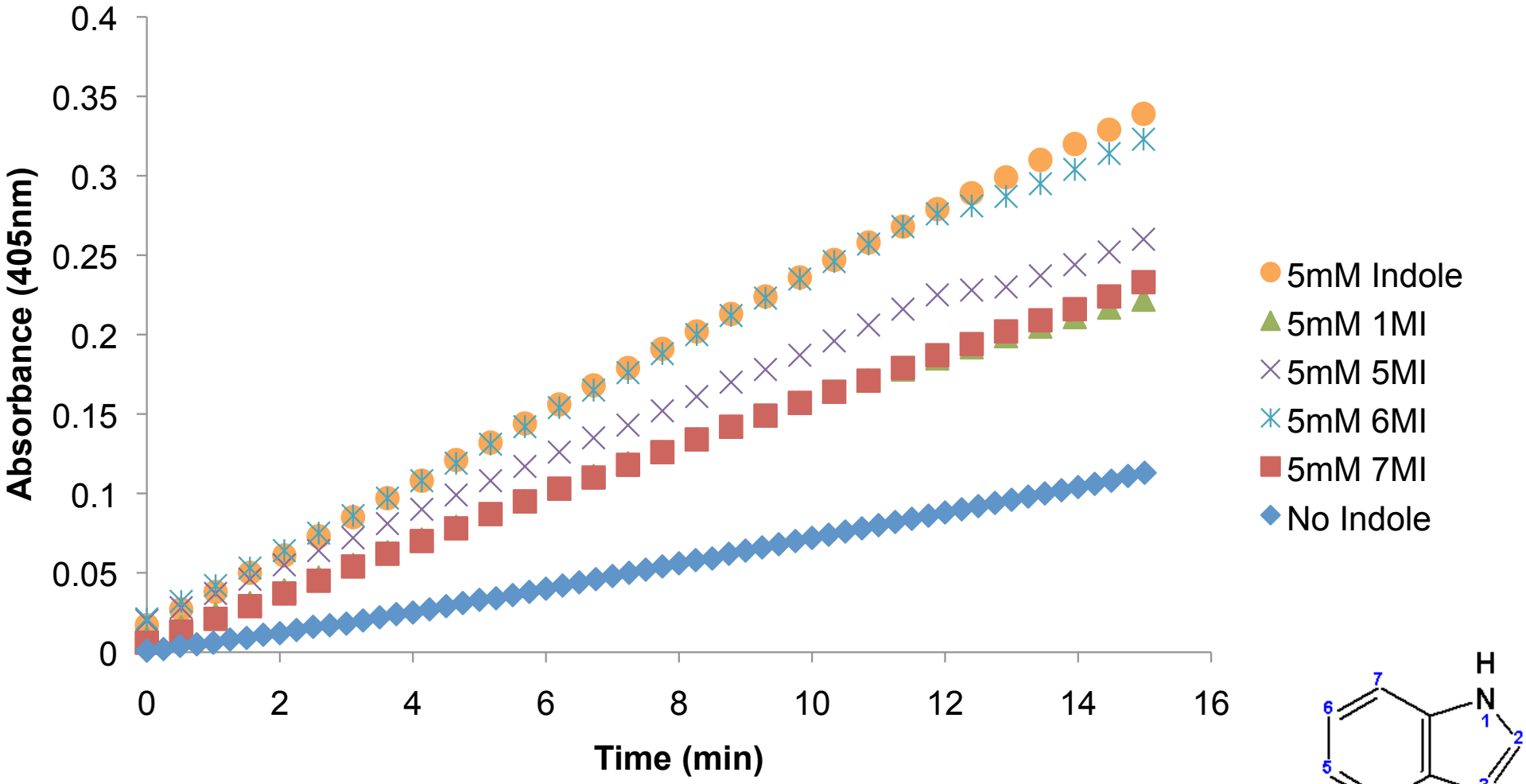


Activity *in vitro*

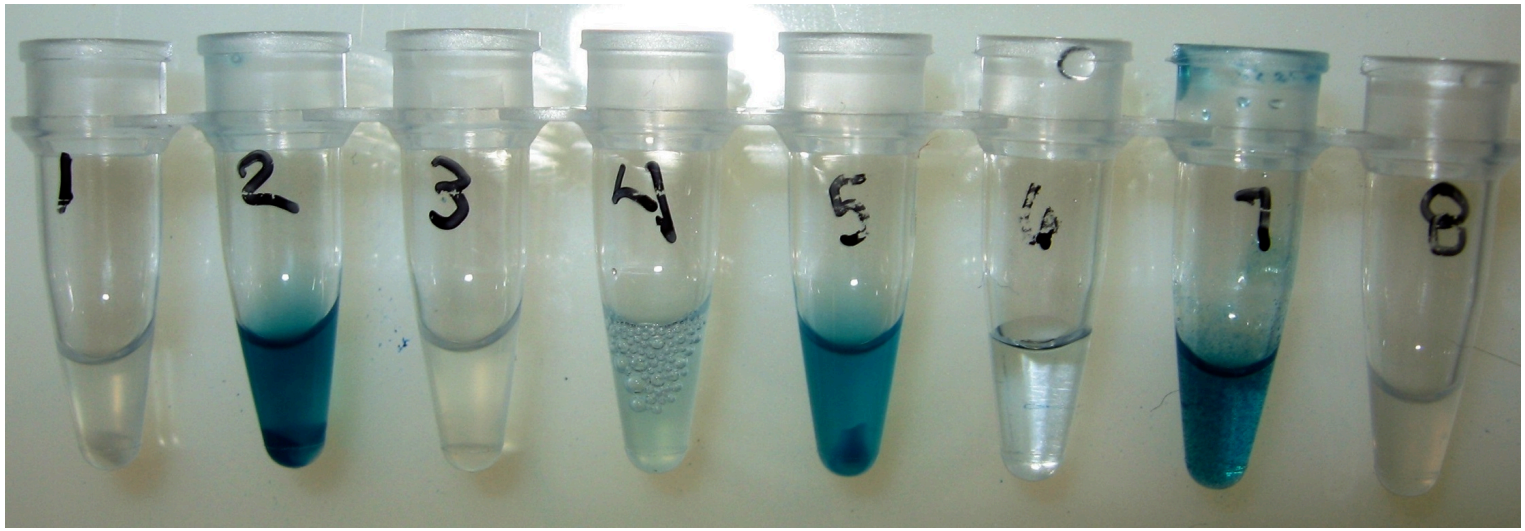
Selectivity against hydroxyindoles



Selectivity profile of methylindoles



Activation by cell extracts



#1: cell extracts + X-gal

#2: cell extracts + X-gal + wild-type β-gly

#3: cell extracts + X-gal + β-gly W425G

#4: cell extracts + X-gal + β-gly W33G

#5: cell extracts + X-gal + β-gly W33G + indole

#6: X-gal + β-gly W33G

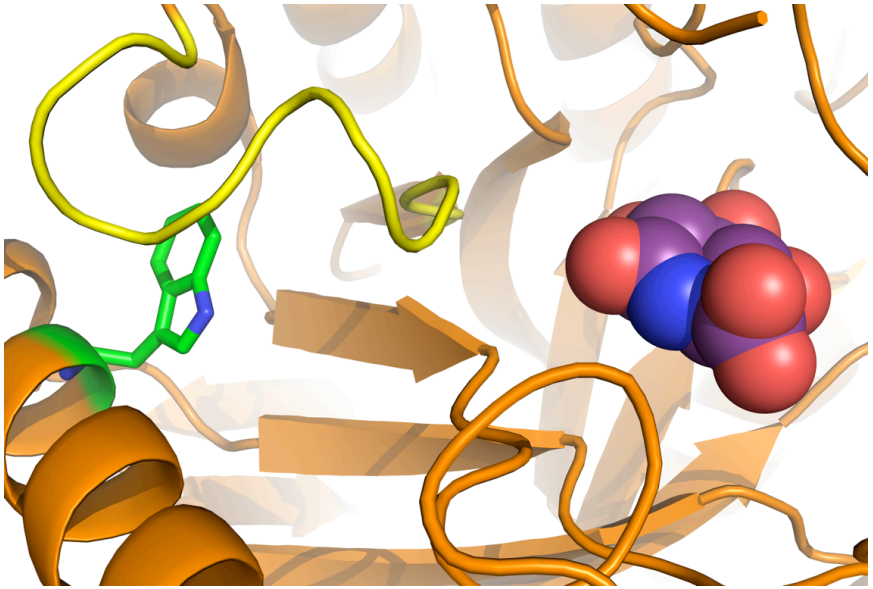
#7: X-gal + β-gly W33G + indole

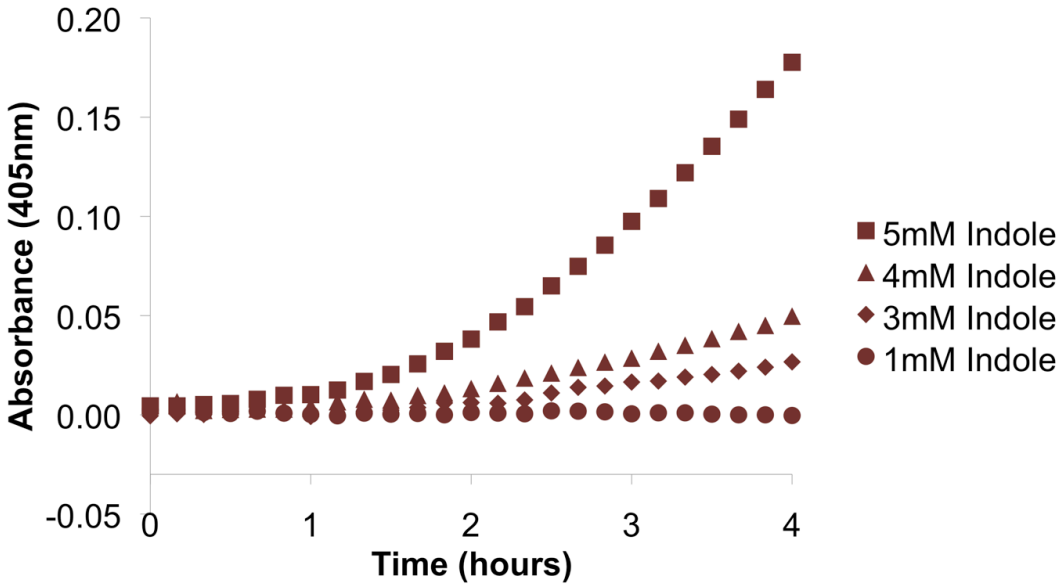
#8: cell extracts + β-gly W33G

Open questions

- Generality : what range of functions can we modulate? by what allosteric mechanisms?
- Selectivity : what endogenous activating/inhibitory effectors should we be concerned with?
- Malleability : what range of effector ligands can we recognize? Bio-orthogonal ligands?

A second example





E. coli β-gluc W492G
(13 Å from active site)

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