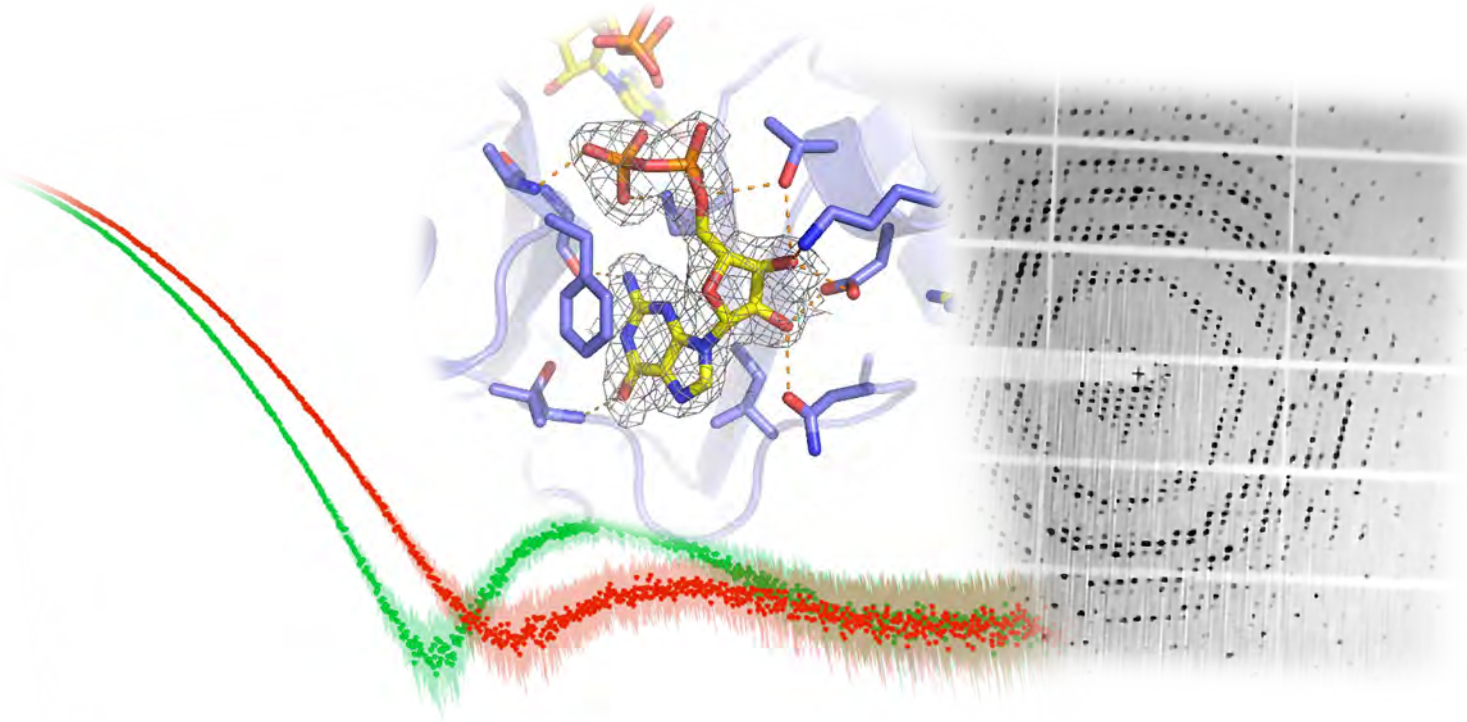
STRUCTURAL APPROACHES TO IDENTIFY NOVEL IMP DEHYDROGENASE INHIBITORS

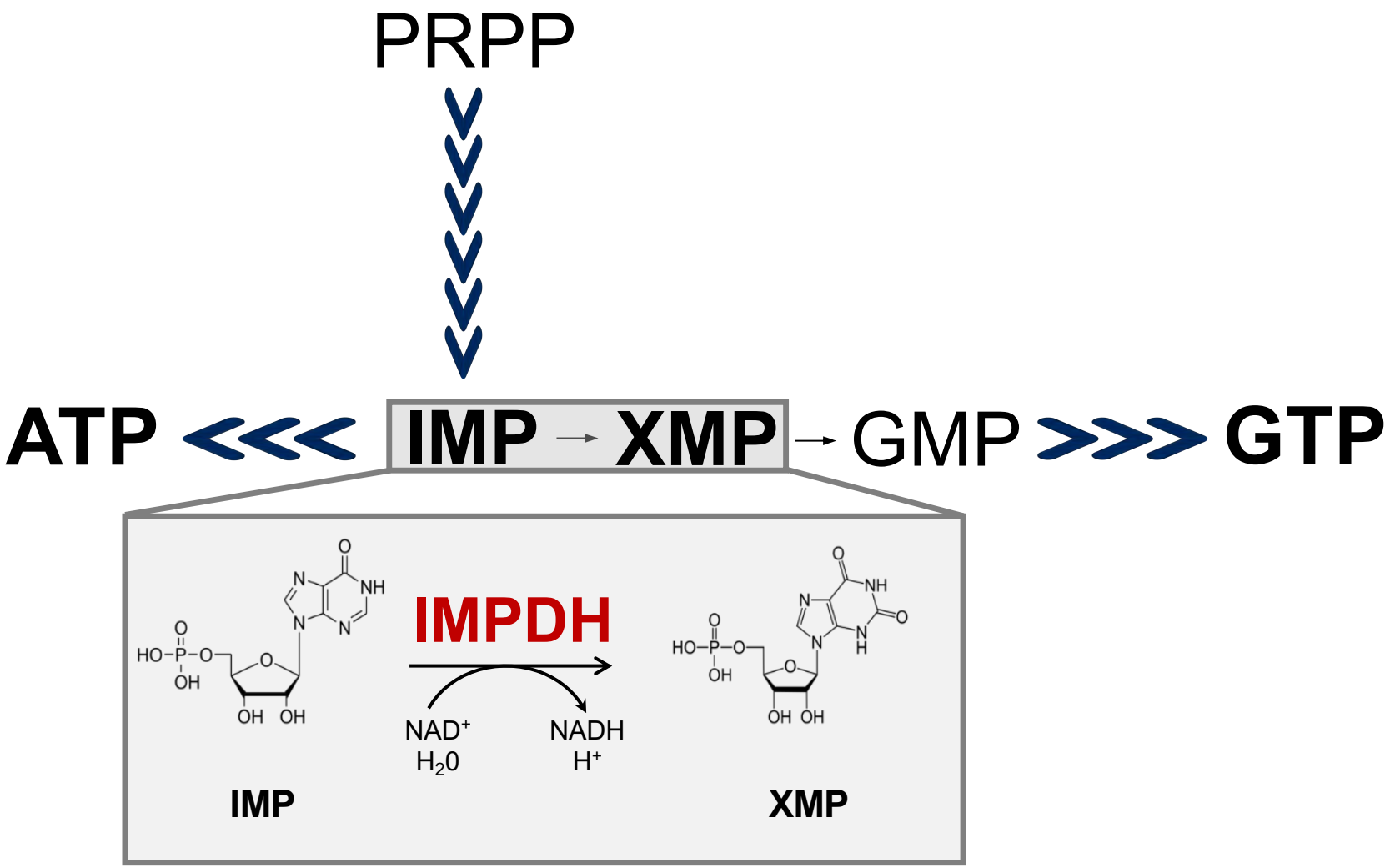


Rubén Martínez-Buey

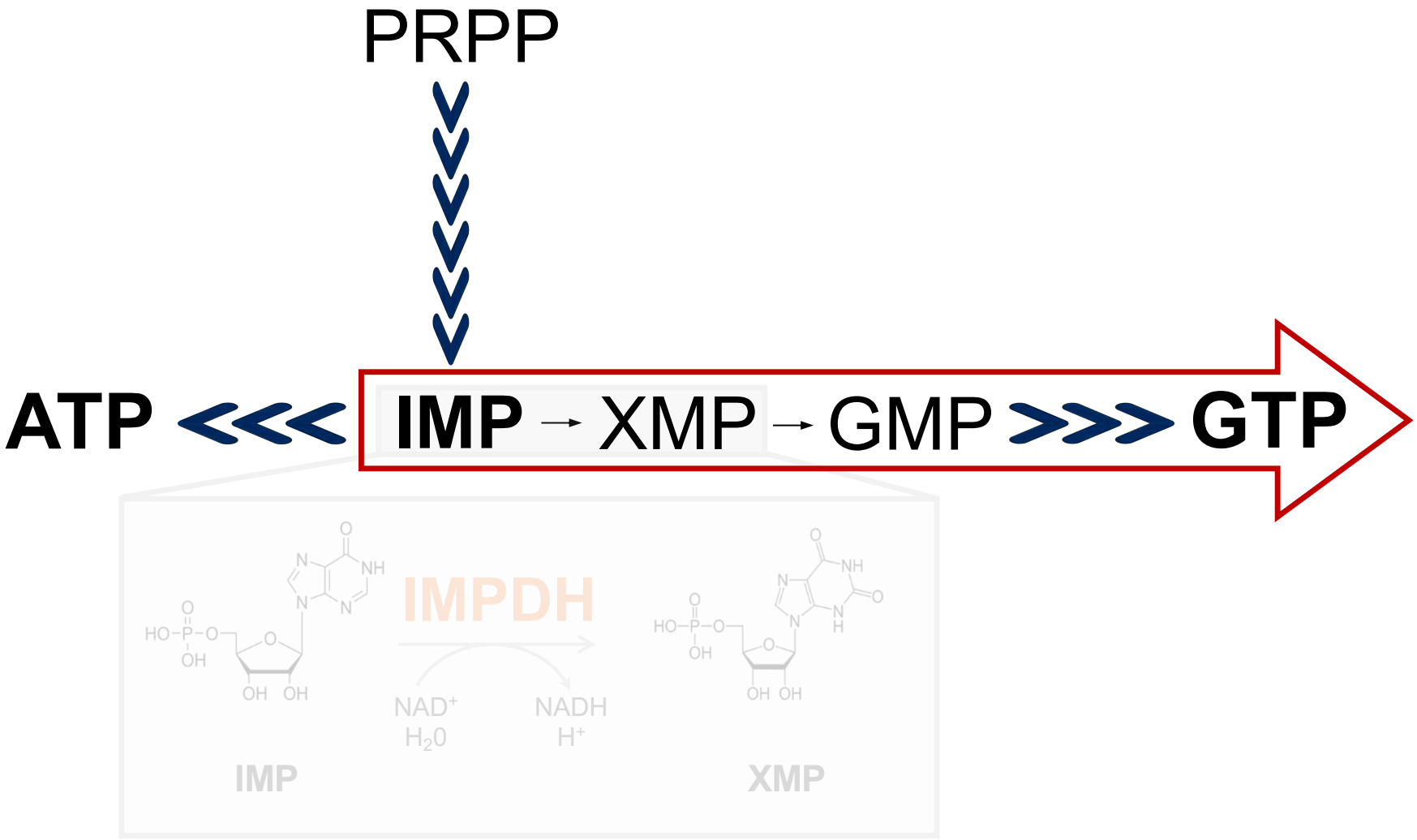
METABOLIC ENGINEERING GROUP

VNiVERSiDAD D SALAMANCA

1st COMMITTED STEP IN GTP SYNTHESIS



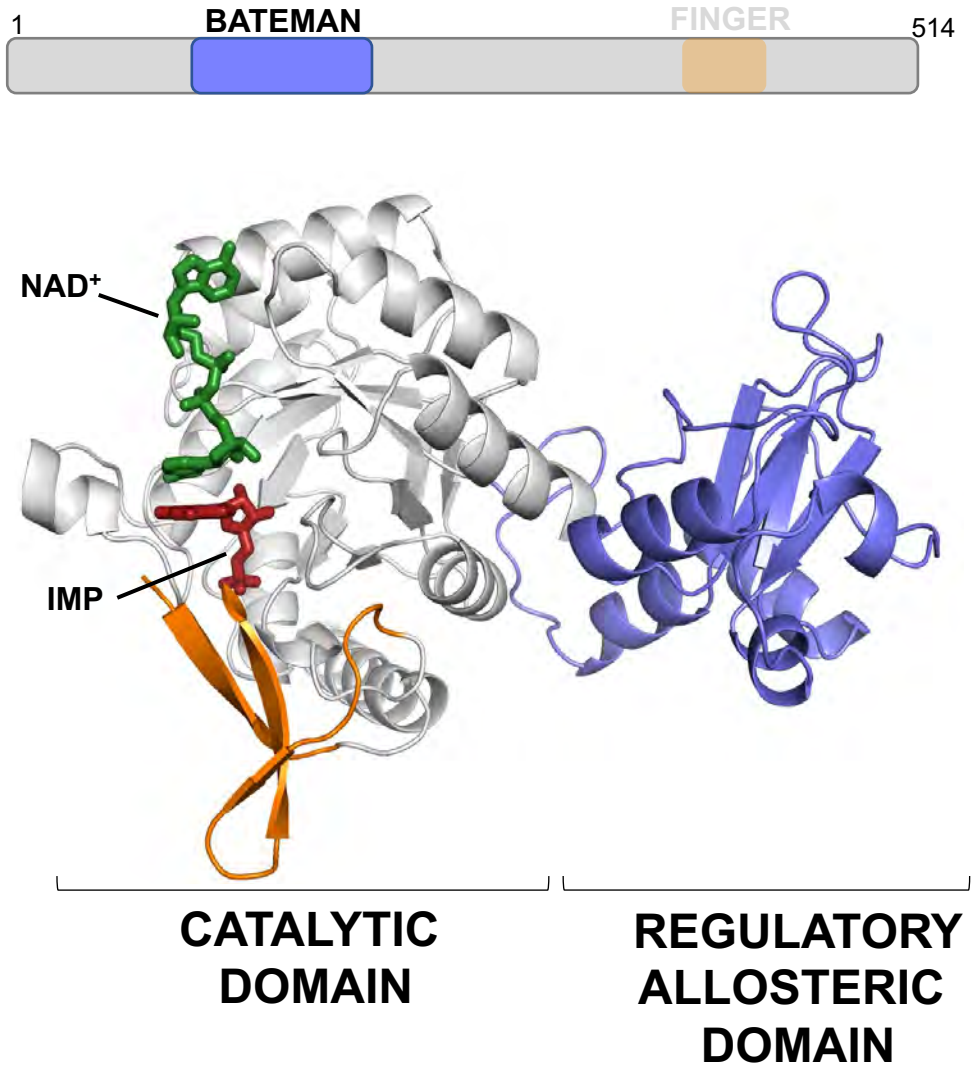
IMPDH CONTROLS GTP BIOSYNTHESIS



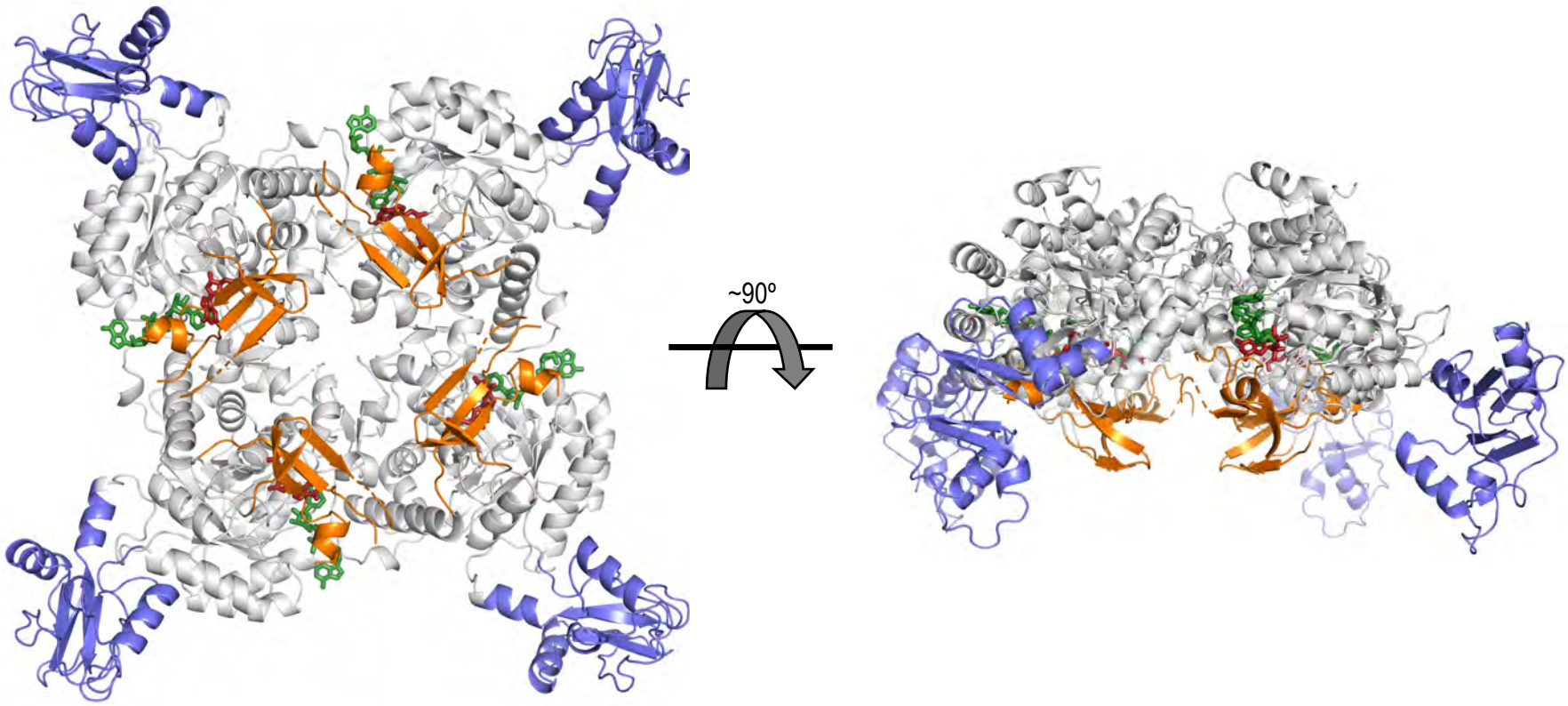
IMPDH IS A DRUG TARGET

ATP <<< **IMP** → XMP → GMP >>> **GTP**

IMPDH MONOMERIC STRUCTURE

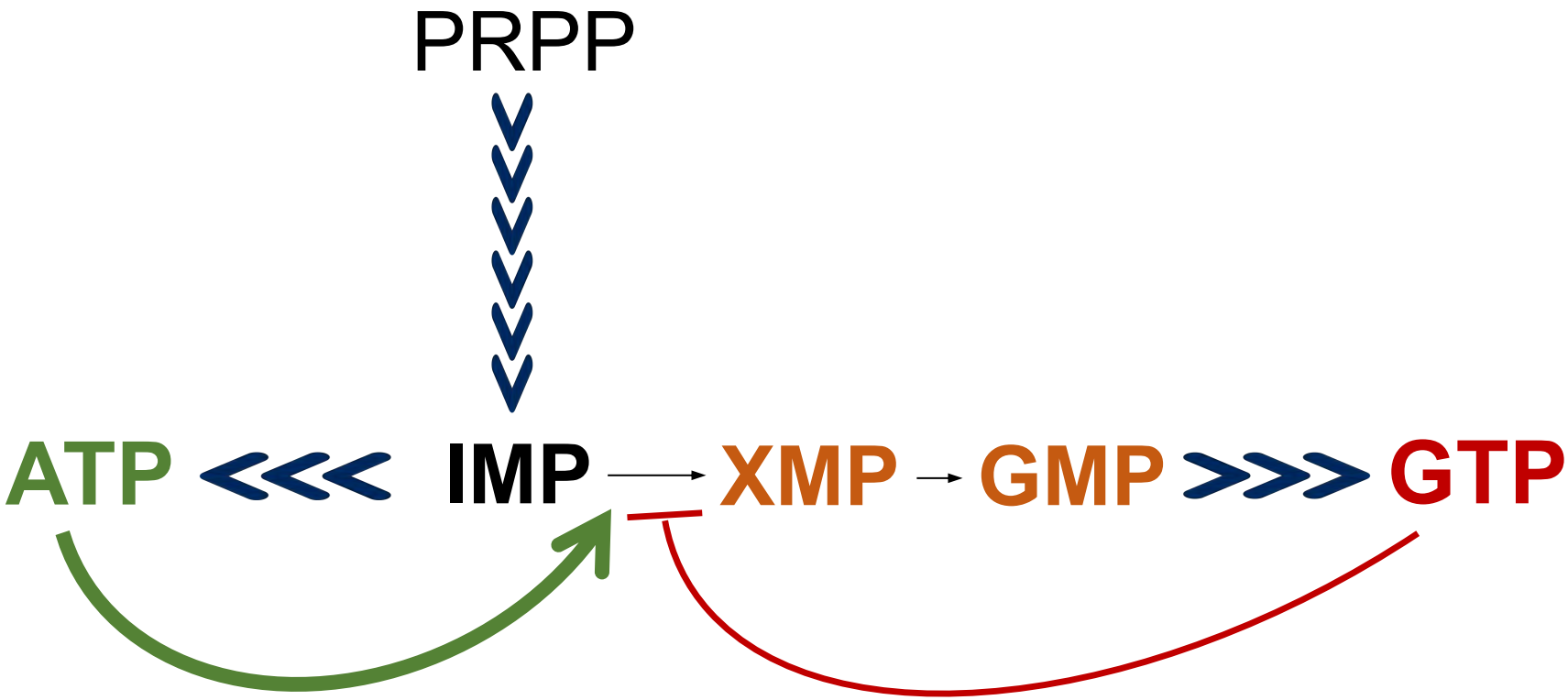


IMPDH FORMS TETRAMERS IN SOLUTION

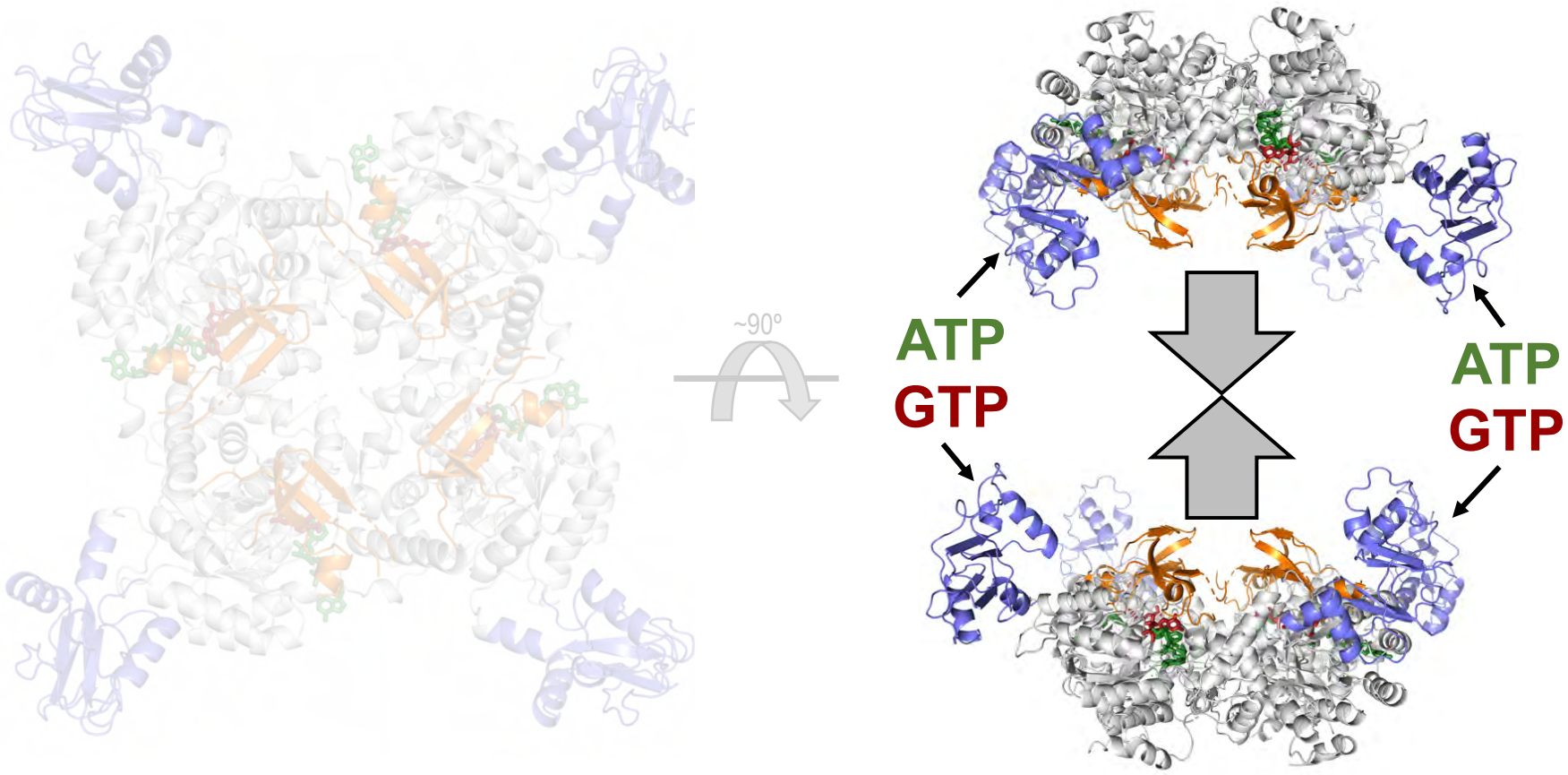


HOW IS IMPDH REGULATED?

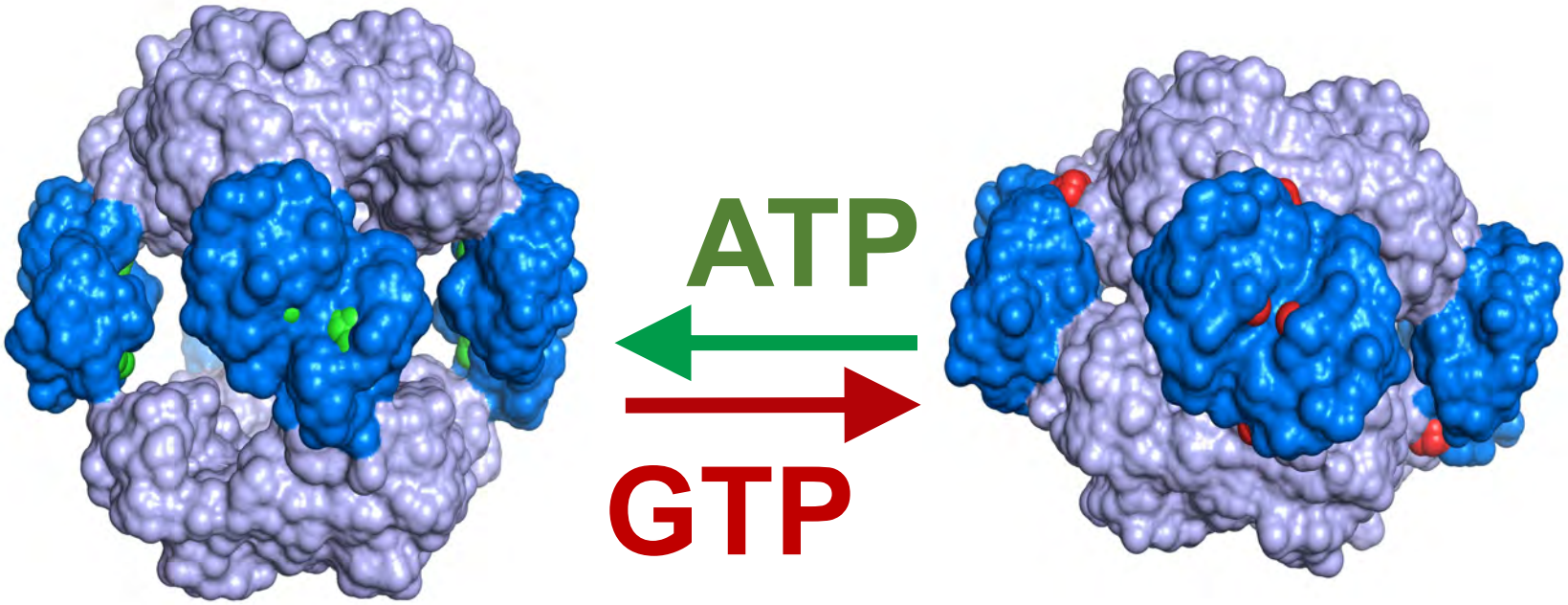
ATP & GTP ARE ALLOSTERIC MODULATORS



ATP & GTP INDUCE OCTAMERS

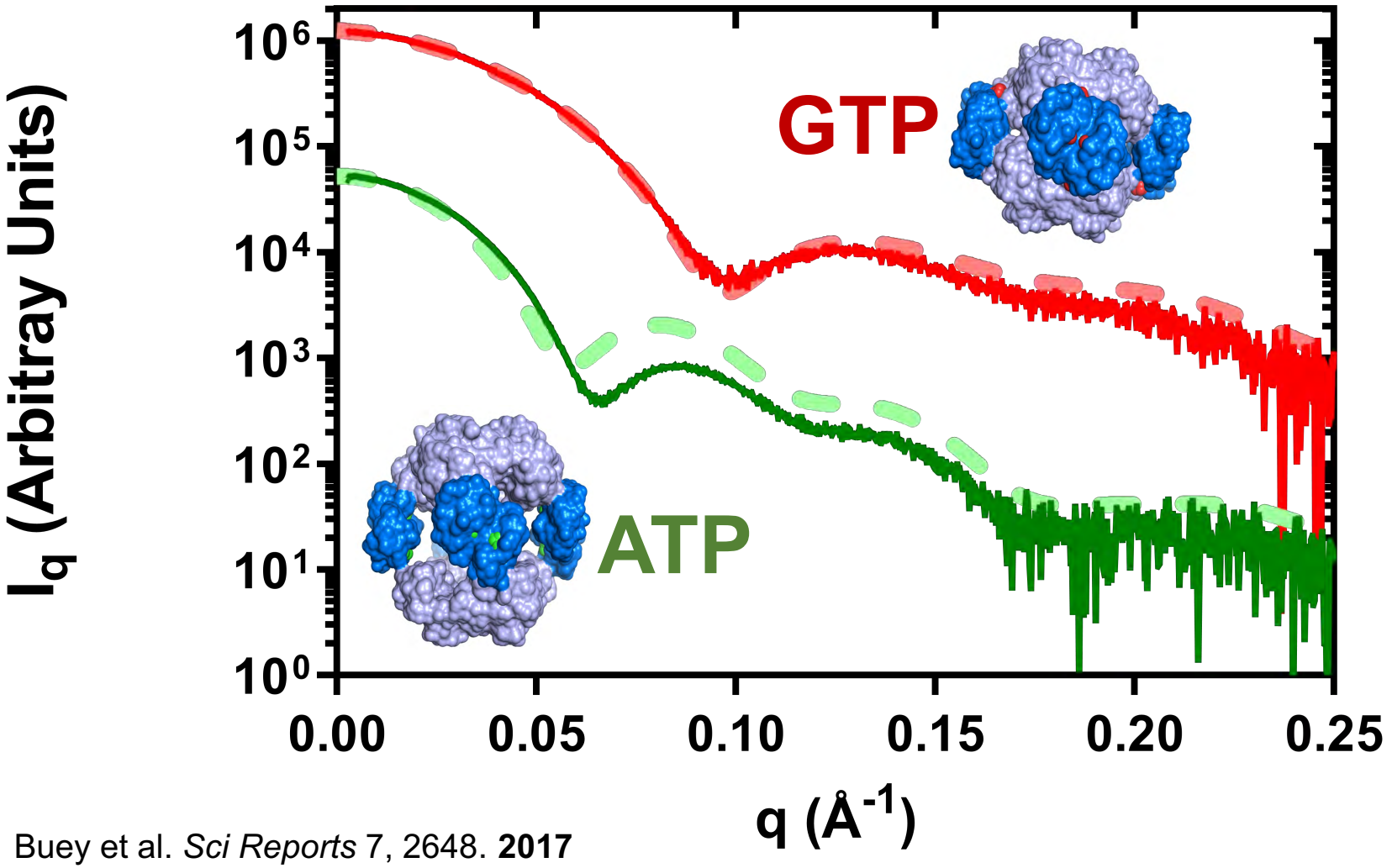


CONFORMATIONAL SWITCH



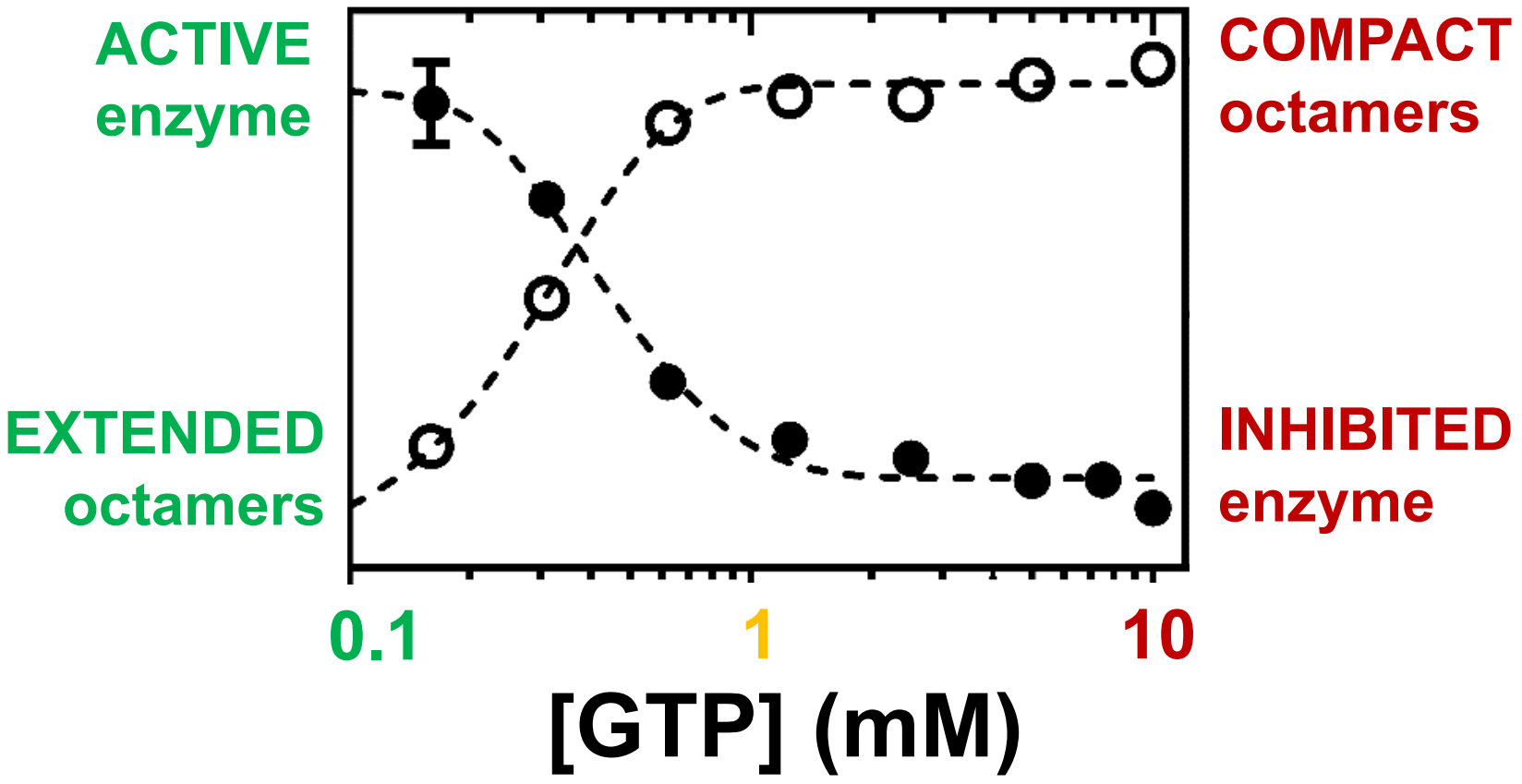
PDB-ID **4Z87**; Buey et al. *Nat Commun* 6, 8923. **2015**
PDB-ID **5MCP**; Buey et al. *Sci Reports* 7, 2648. **2017**

CONFORMATIONAL SWITCH BY SAXS



Buey et al. *Sci Reports* 7, 2648. **2017**

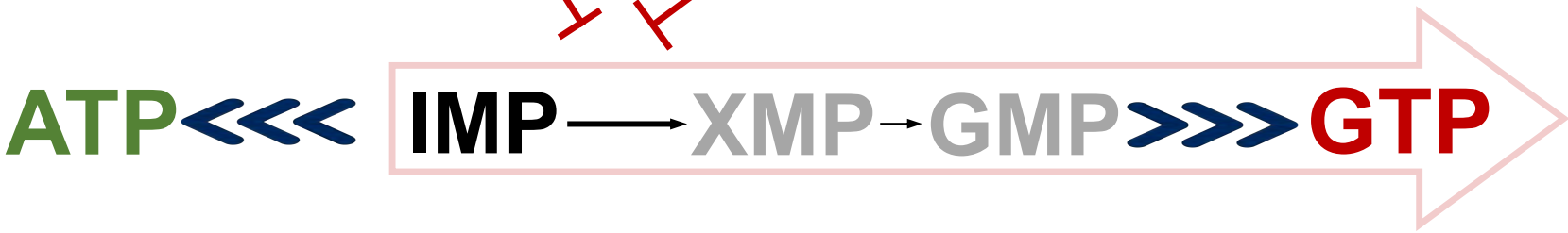
THE CONFORMATIONAL SWITCH CONTROLS ENZYME ACTIVITY



Buey et al. *Sci Reports* 7, 2648. **2017**

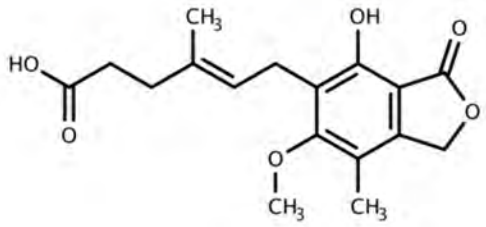
HOW IS IMPDH REGULATED?

IMPDH IS A DRUG TARGET

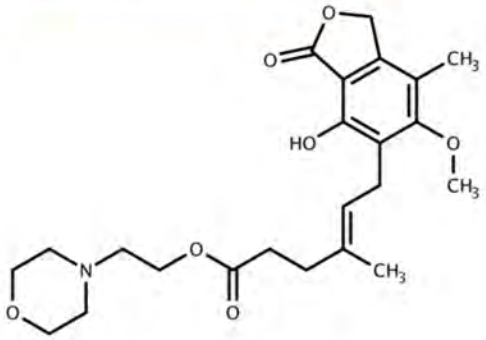


ALL KNOWN IMPDH INHIBITORS ARE ORTHOSTERIC

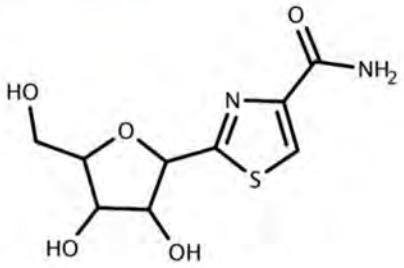
Mycophenolic Acid (MPA)



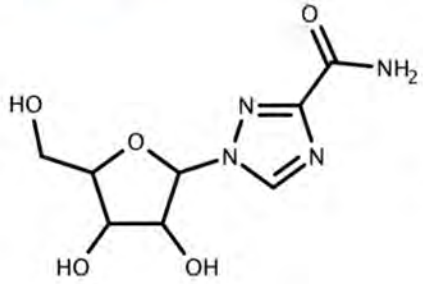
Mycophenolate Mofetil (MMF)



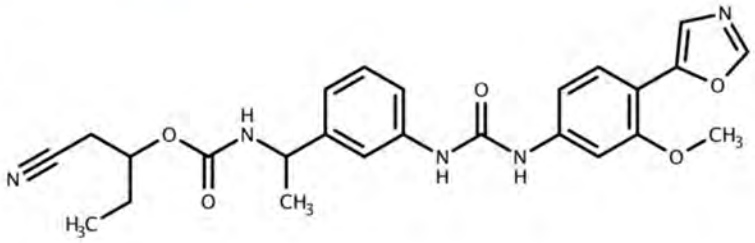
Tiazofurin



Ribavirin



VX-944



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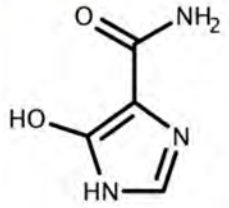
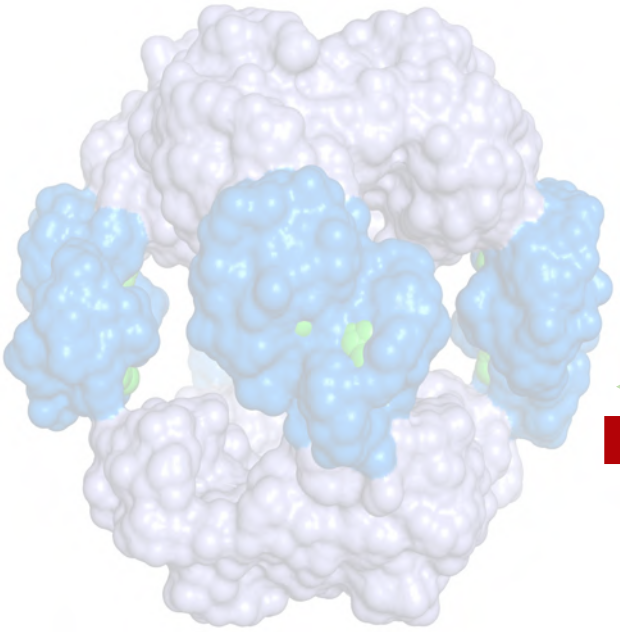


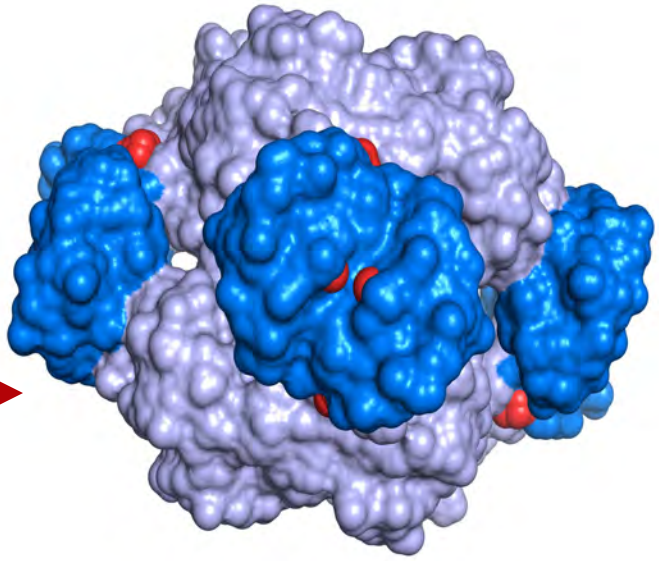
CAN WE OBTAIN ALLOSTERIC IMPDH INHIBITORS ?

BENEFITS OF ALLOSTERIC INHIBITORS

BENEFITS OF ALLOSTERIC INHIBITORS

WHAT ARE WE SEARCHING FOR?

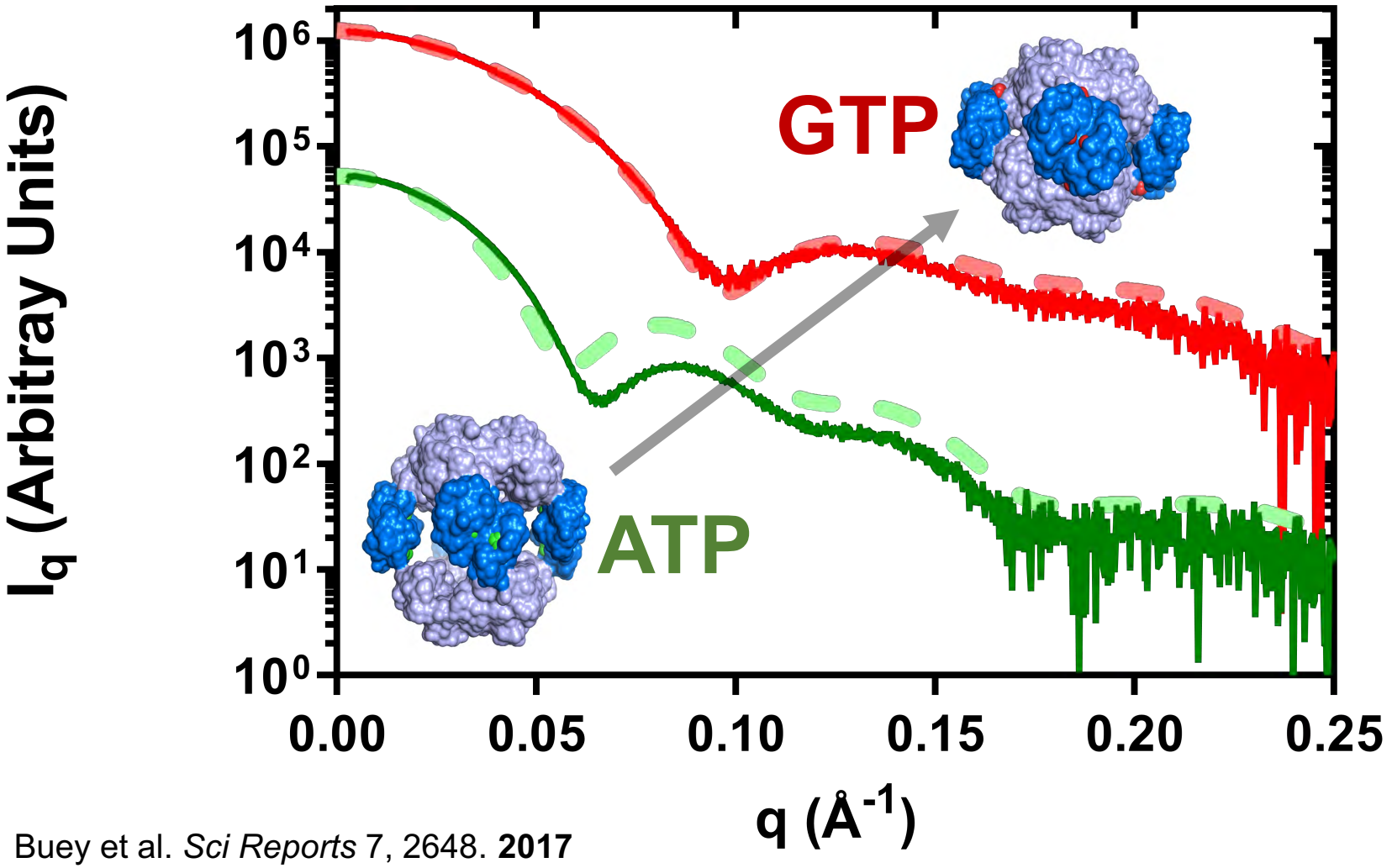




ACTIVE

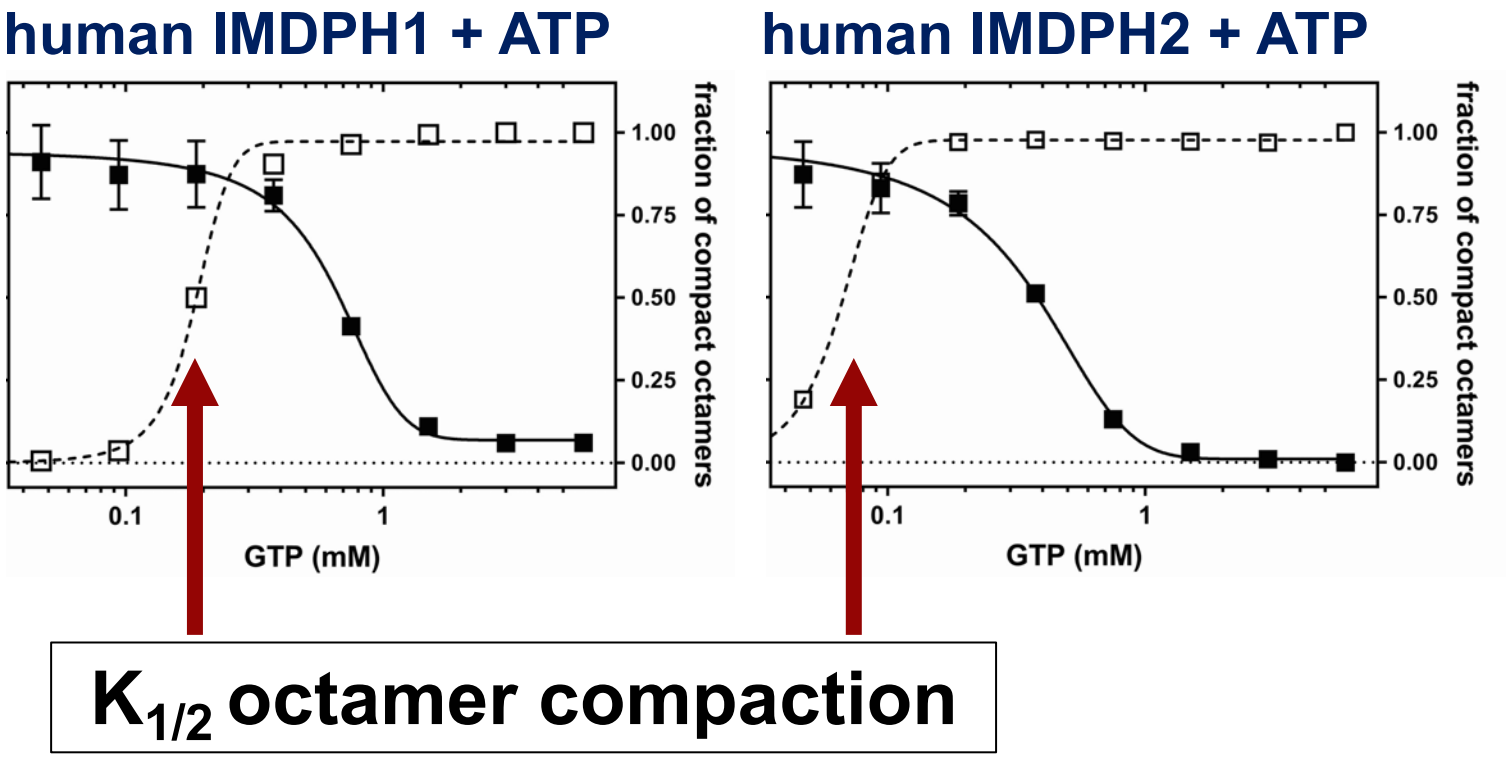
INHIBITED

IMPDH CONFORMATIONS BY SAXS



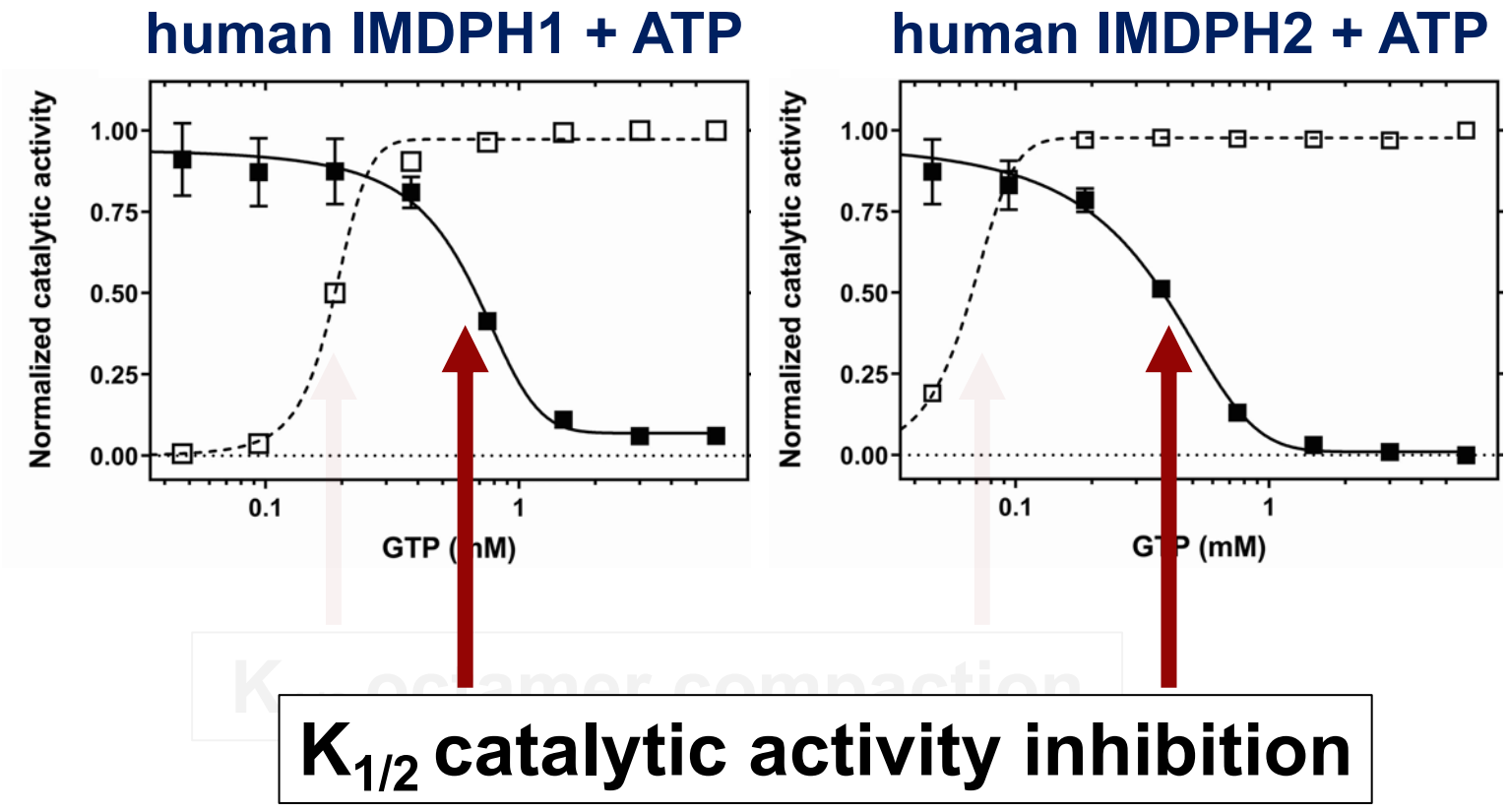
Buey et al. *Sci Reports* 7, 2648. **2017**

CONFORMATION SHIFTS AT SUB-INHIBITORY CONCENTRATIONS



Fernández-Justel et al. *J Mol Biol 431*, 956-969. **2019**

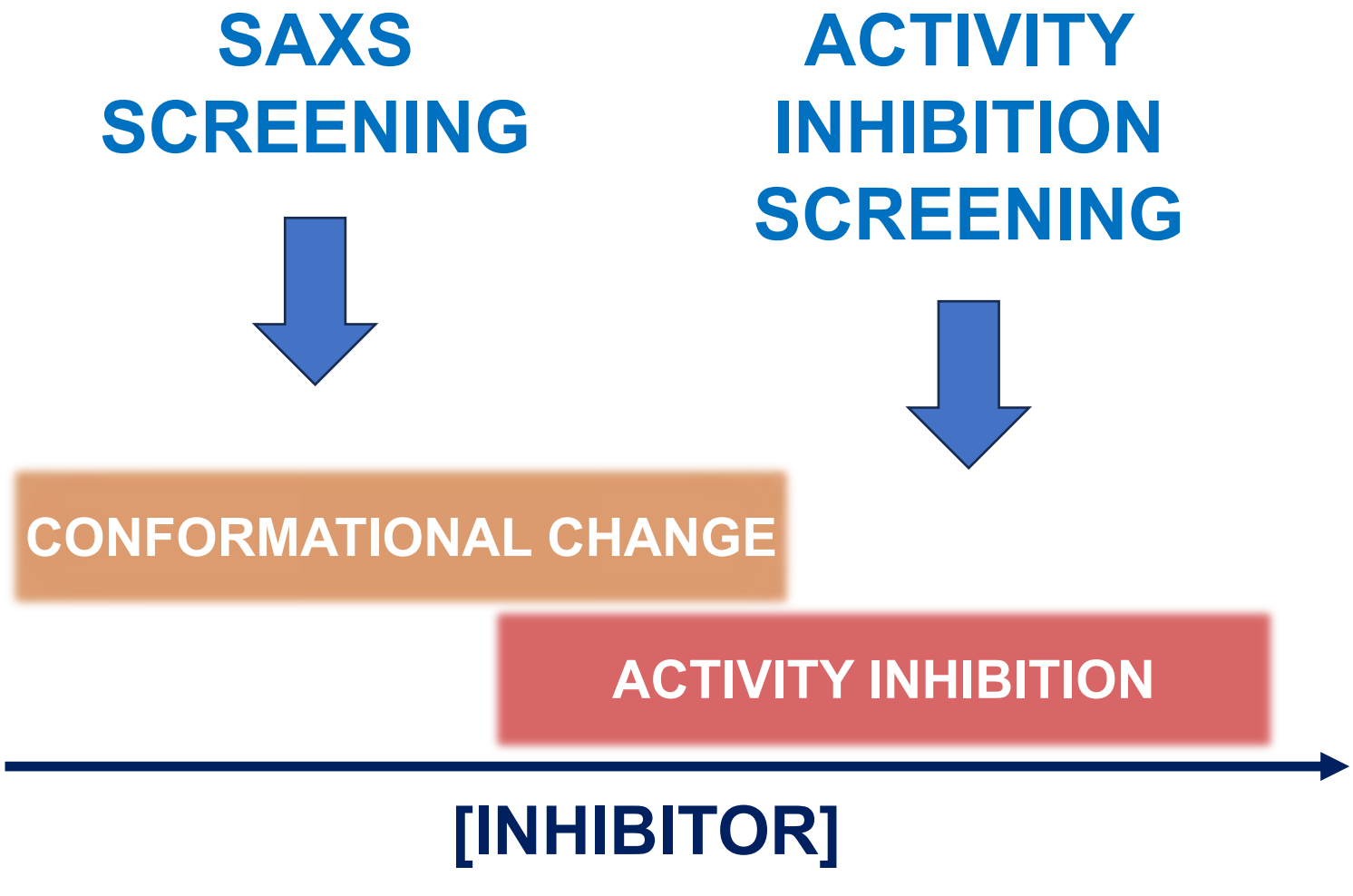
CONFORMATION SHIFTS AT SUB-INHIBITORY CONCENTRATIONS



$K_{1/2}$ catalytic activity inhibition

Fernández-Justel et al. *J Mol Biol 431*, 956-969. **2019**

SAXS IS MORE SENSITIVE THAN FUNCTIONAL ASSAYS

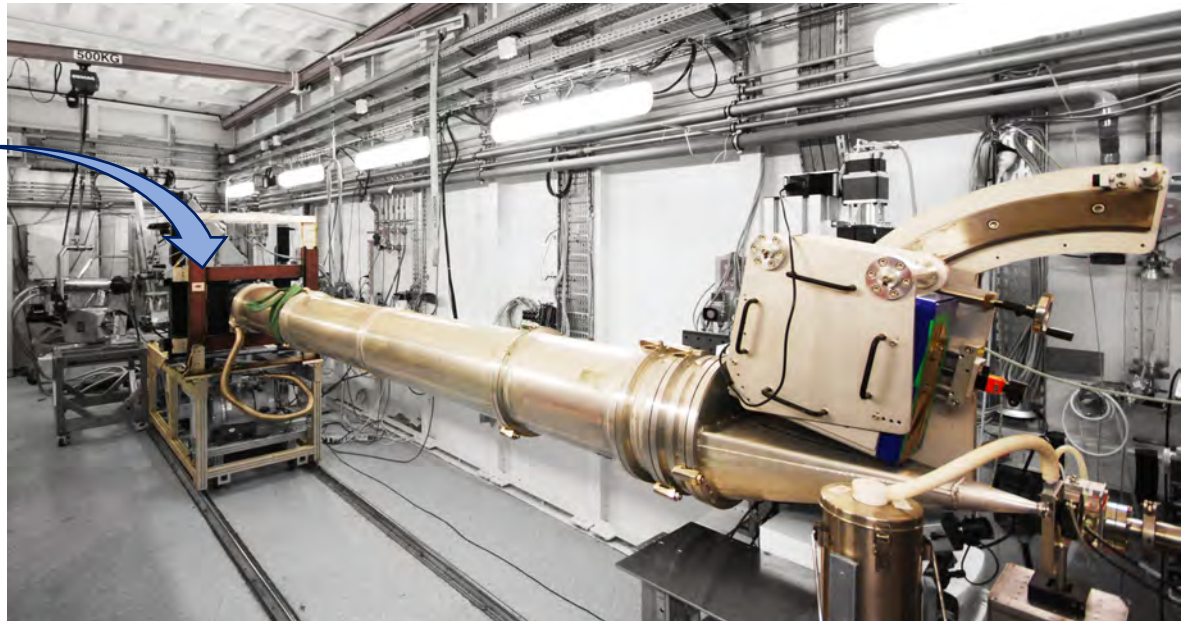


SAXS AS A PRIMARY SCREENING METHOD?

IDENTIFYING ALLOSTERIC INHIBITORS BY HT-SAXS

Guanosine Nucleotide Library

Jena Bioscience

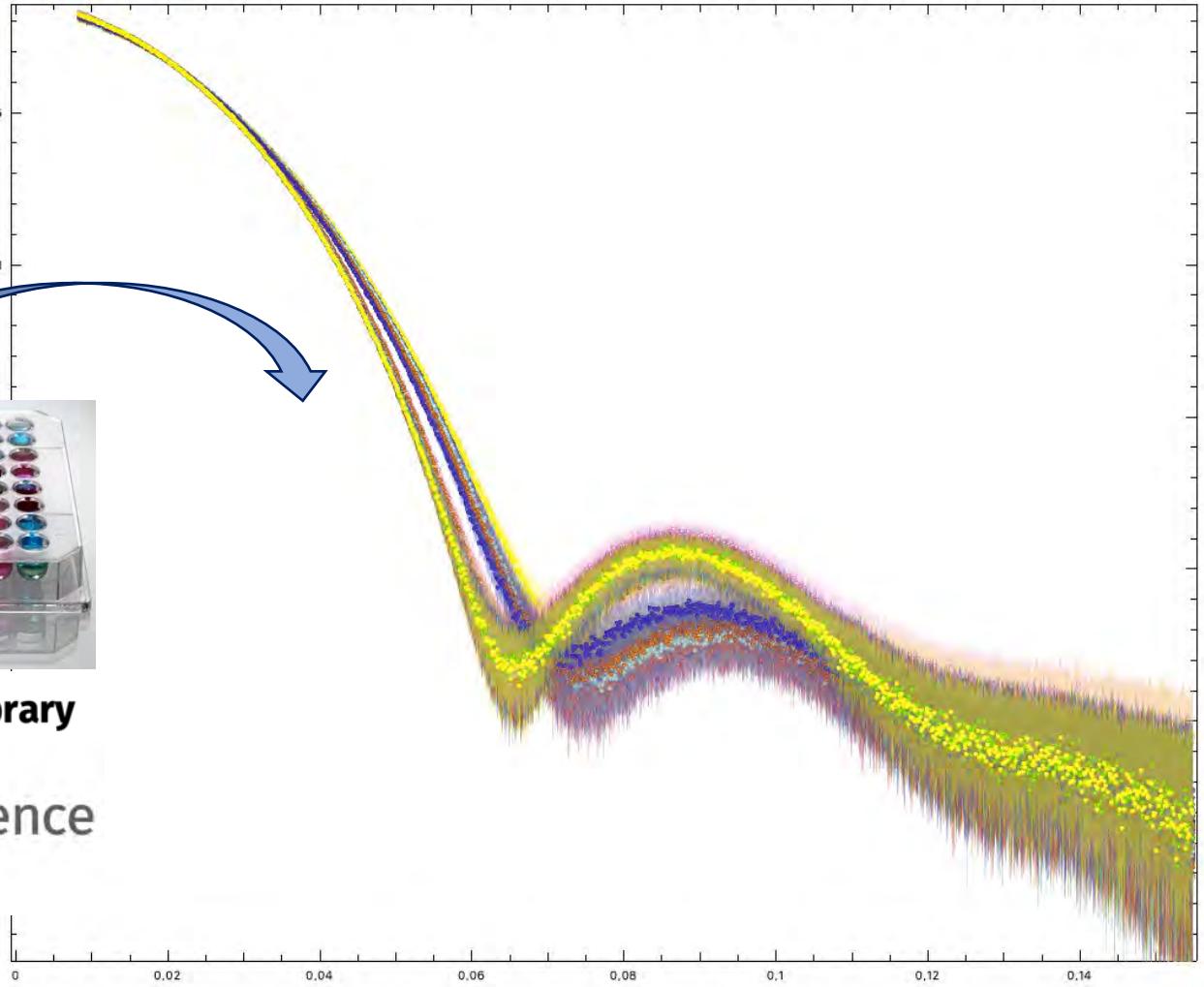


B21 BEAMLINE @ diamond

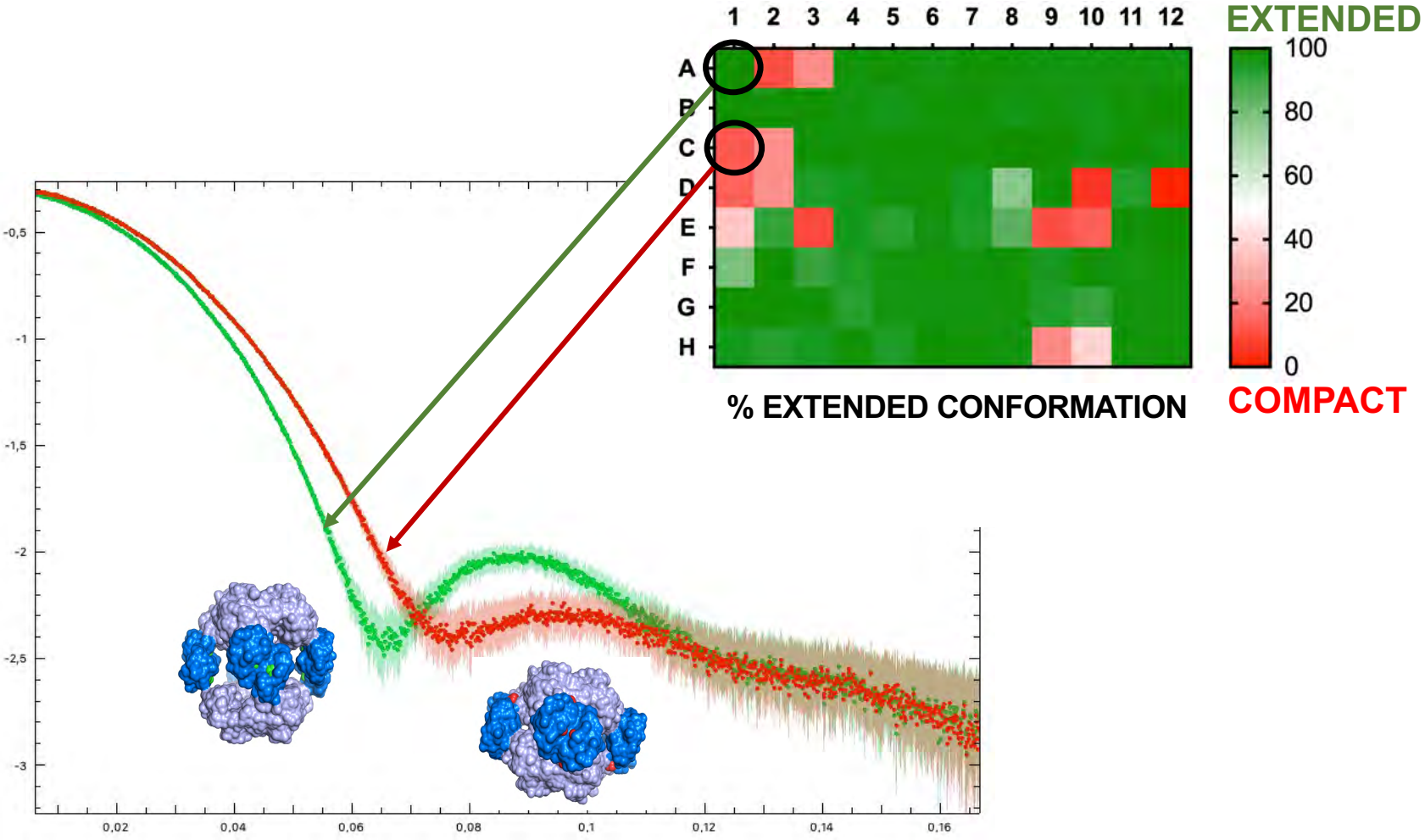
SCATTERING PROFILES

Guanosine Nucleotide Library

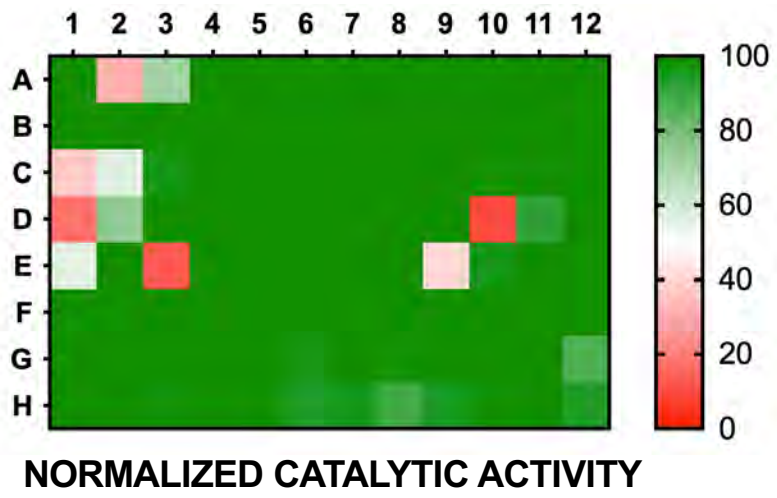
Jena Bioscience



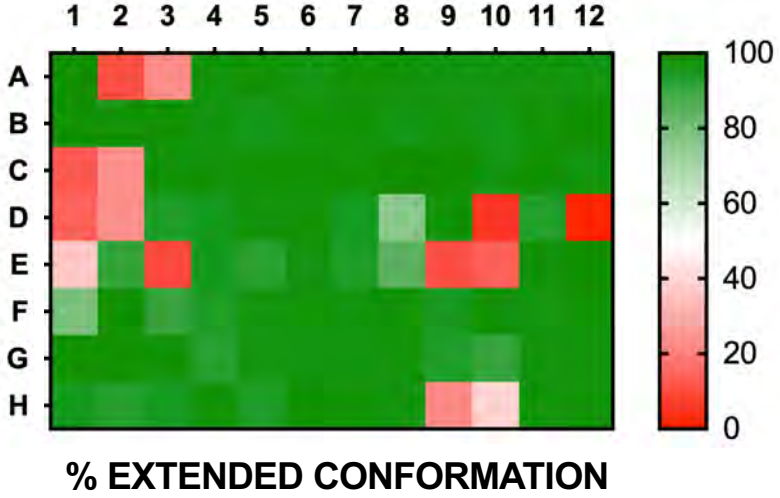
EXTENDED *vs* COMPACT OCTAMERS



FUNCTIONAL *vs* STRUCTURAL ASSAY



NORMALIZED CATALYTIC ACTIVITY

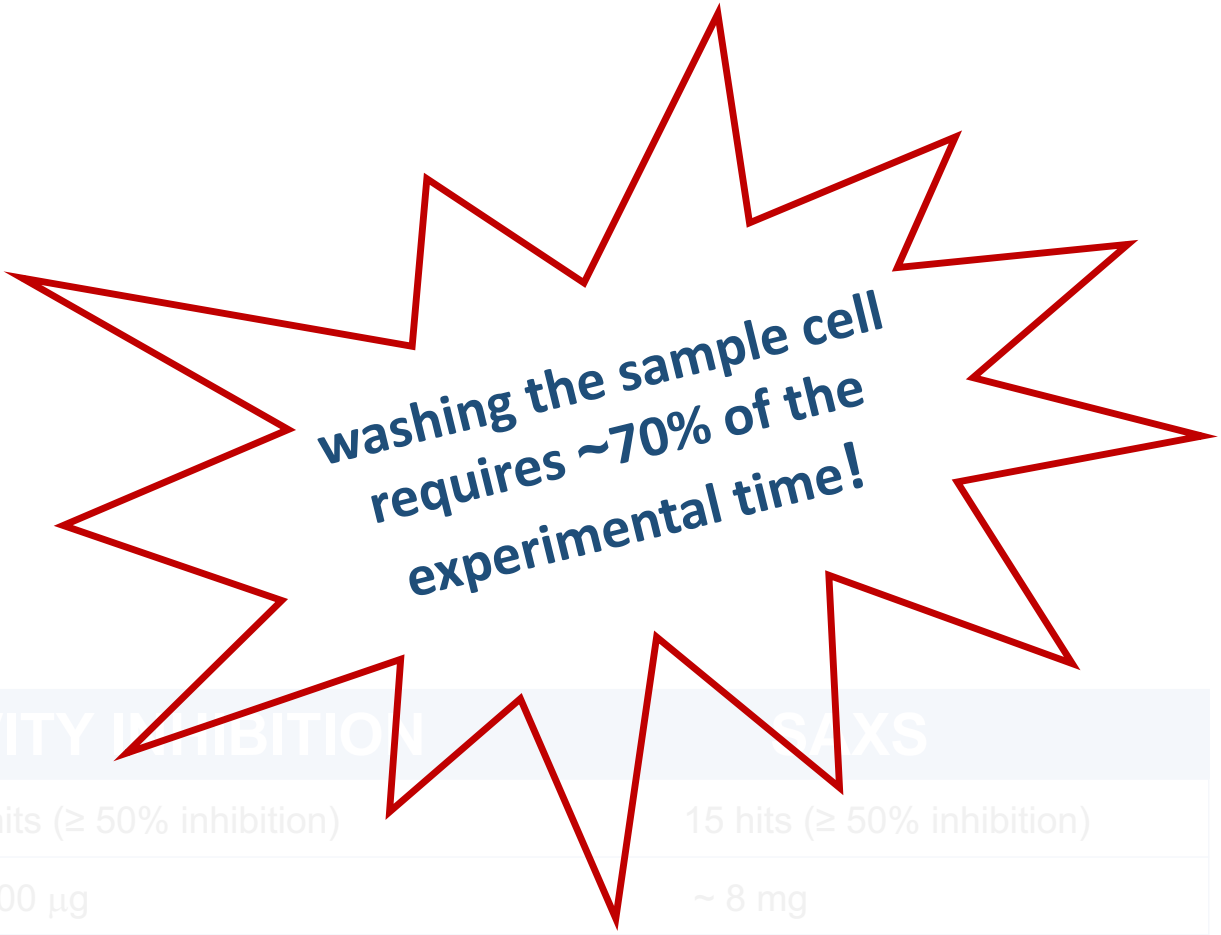


% EXTENDED CONFORMATION

FUNCTIONAL *vs* STRUCTURAL ASSAY

	ACTIVITY INHIBITION	SAXS
Sensitivity	10 hits (≥ 50% inhibition)	15 hits (≥ 50% inhibition)
[Protein]	~ 100 μg	~ 8 mg
Time	~ 30 min	~ 3 h 20 min

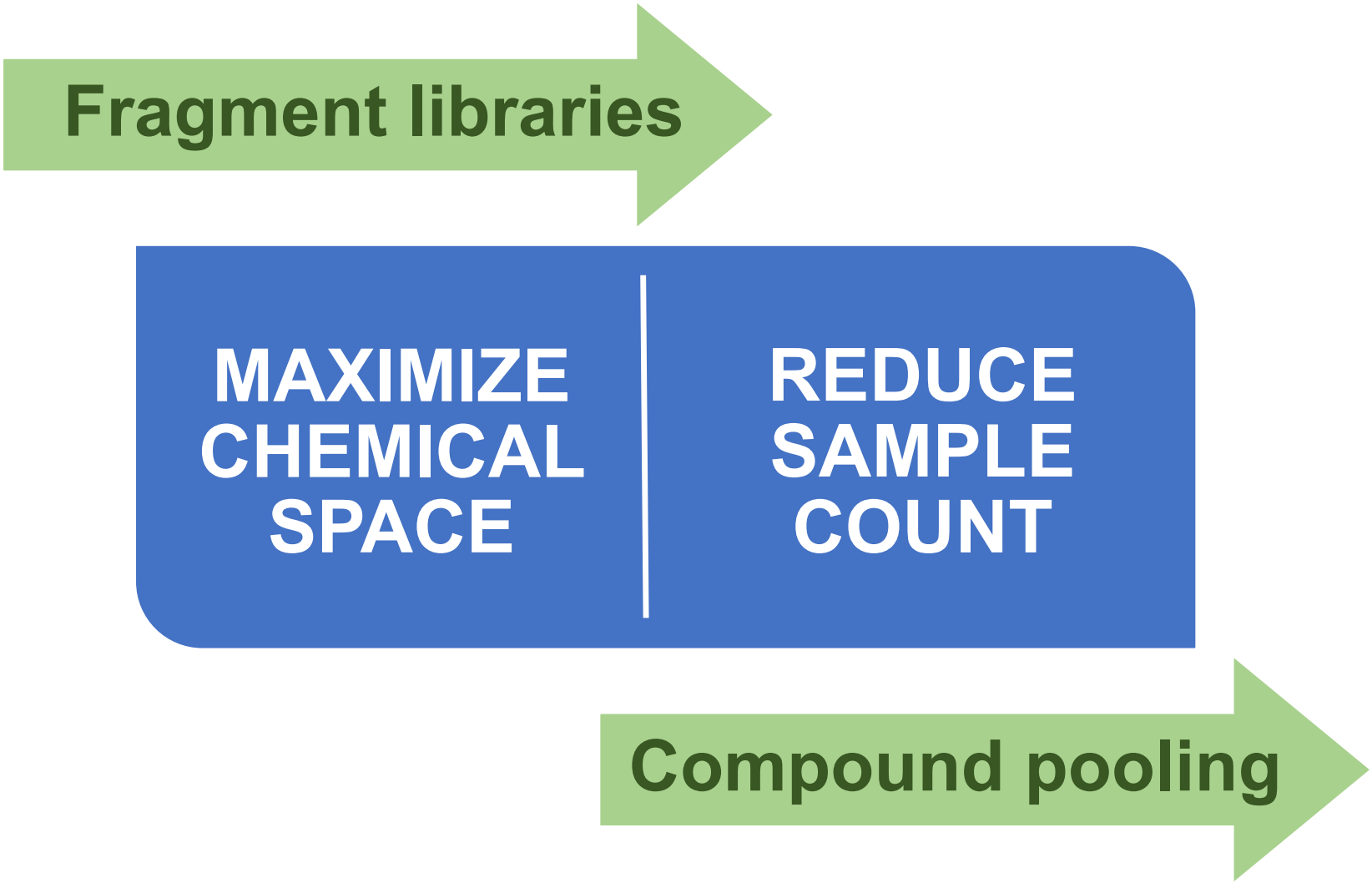
BIO-SAXS BEAMLINE OPTIMIZATION



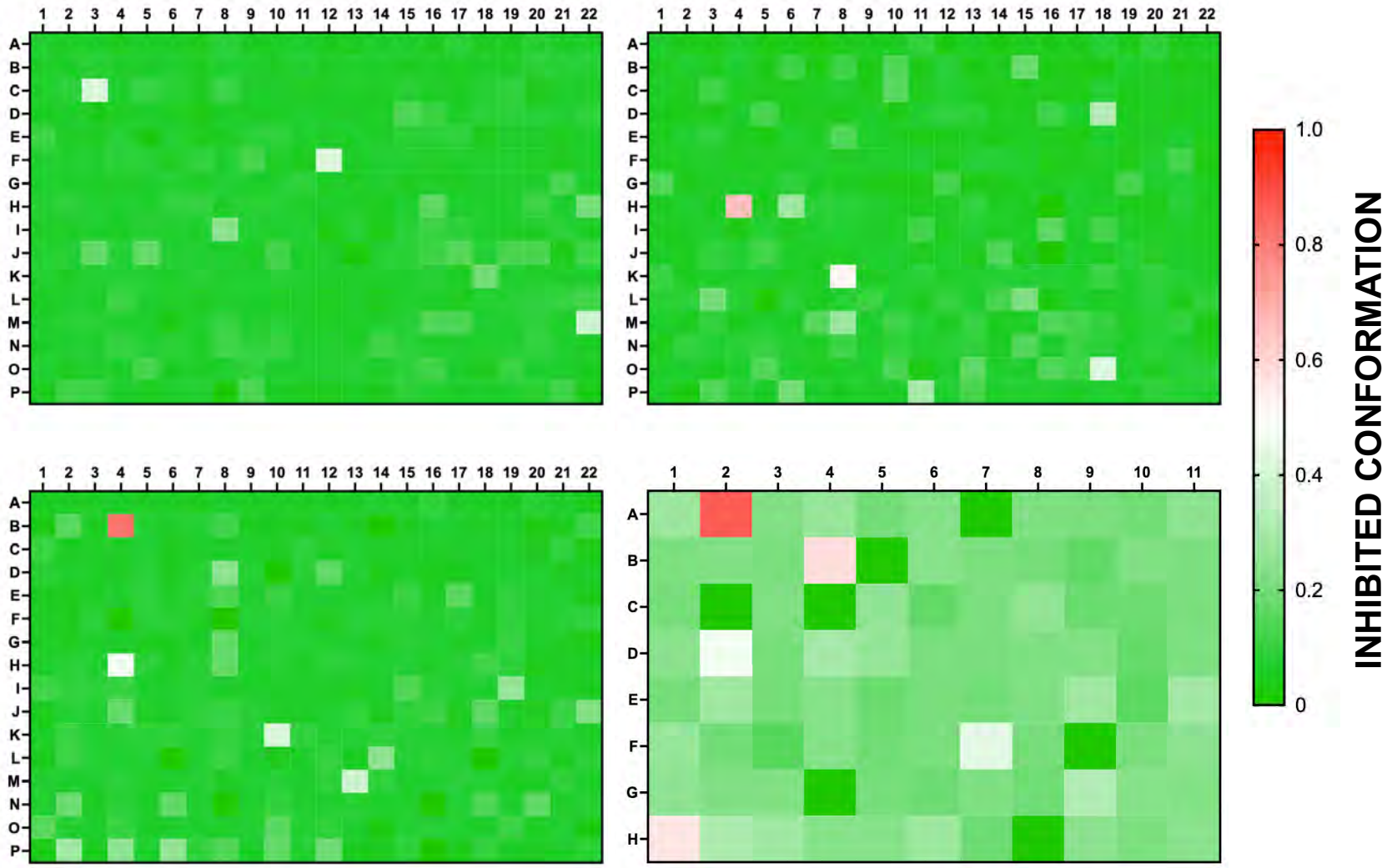
	ACTIVITY INHIBITION	SAXS
Sensitivity	10 hits (≥ 50% inhibition)	15 hits (≥ 50% inhibition)
[Protein]	~ 100 μg	~ 8 mg
Time	~ 30 min	~ 3 h 20 min

SCREENING LARGE CHEMICAL LIBRARIES USING SAXS?

REDUCING SAXS EXPERIMENTAL TIME



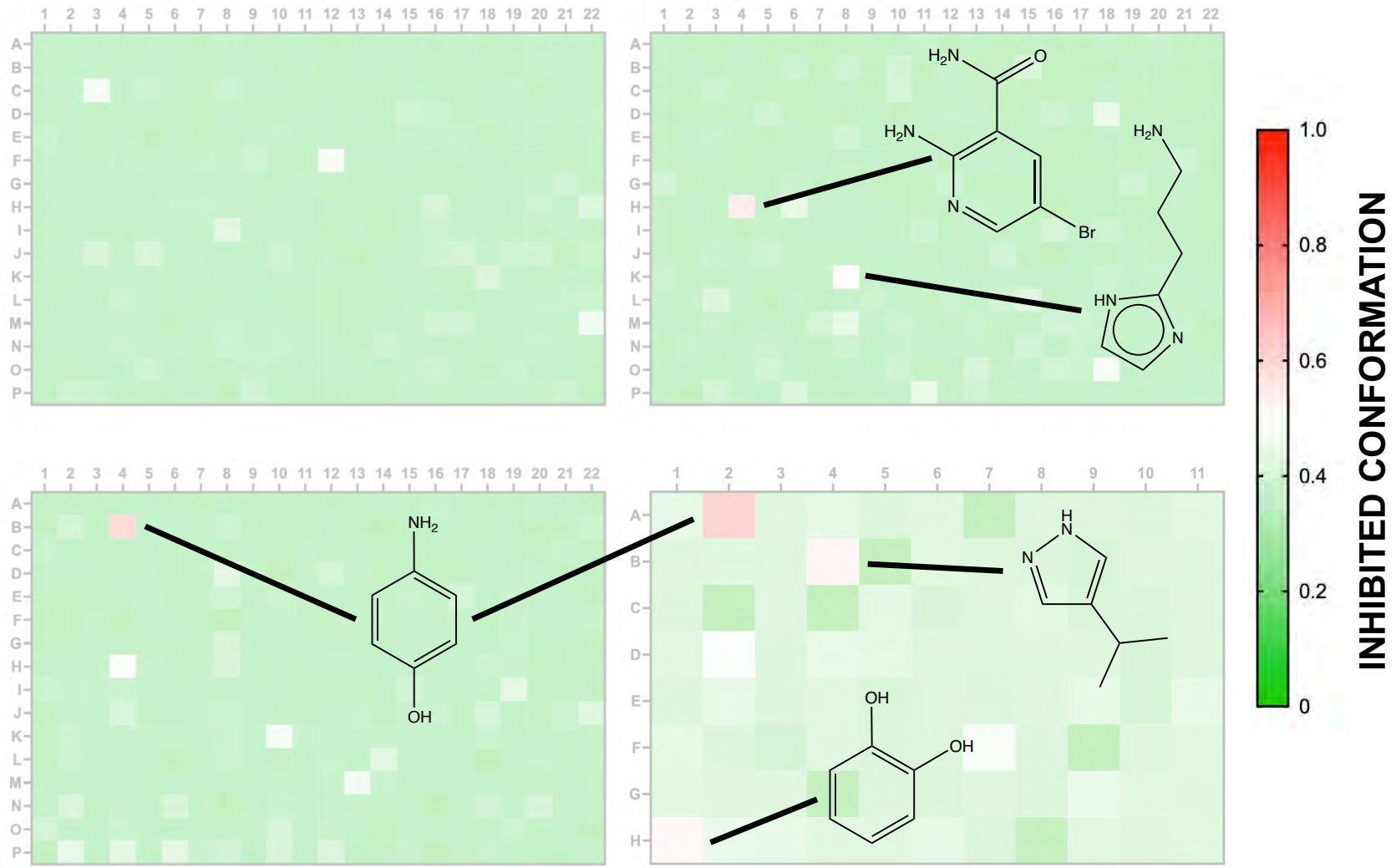
EU-OPENSCREEN FRAGMENT LIBRARY



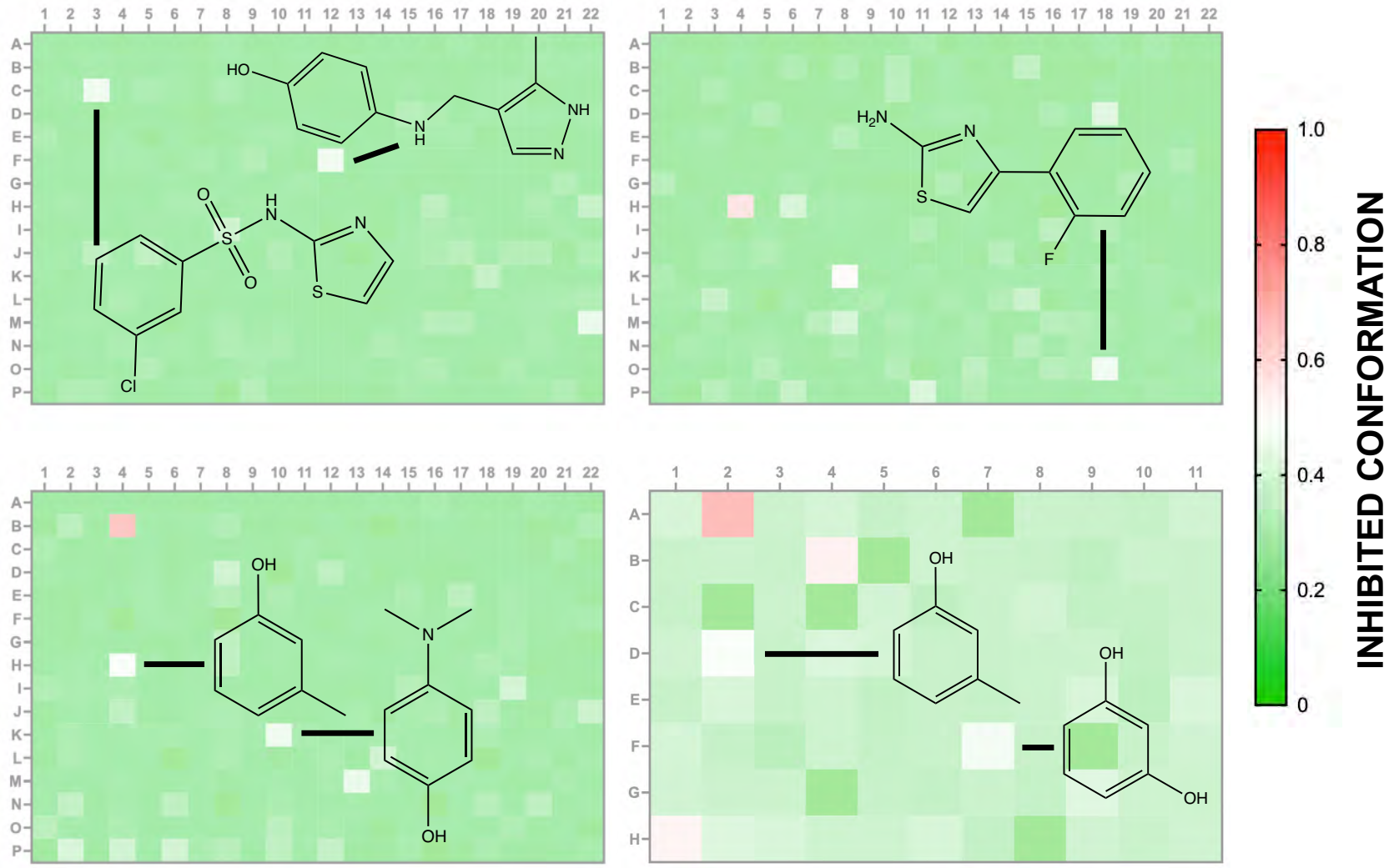
~ 42 HOURS @ B21

diamond

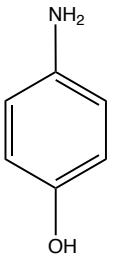
INHIBITED CONFORMATION ≥ 50%



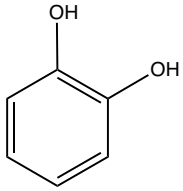
50% ≥ INHIBITED CONFORMATION ≥ 40%



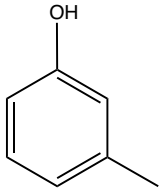
2 MAJORITARY FRAGMENT CHEMOTYPES



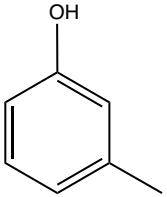
(87%)



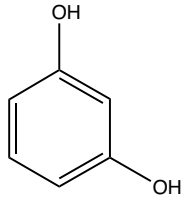
(56%)



(47%)



(47%)



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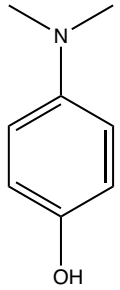
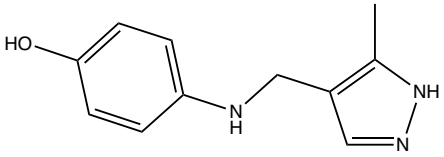


(41%)



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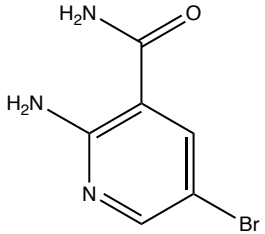

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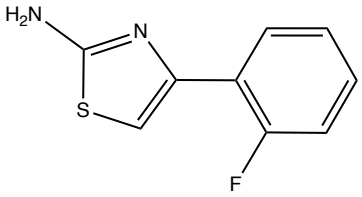

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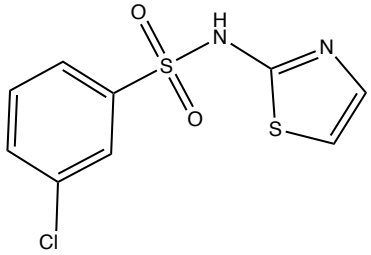

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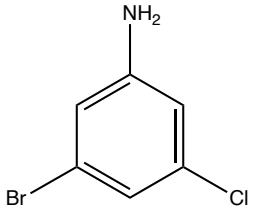

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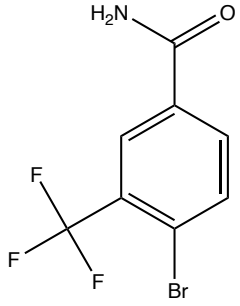

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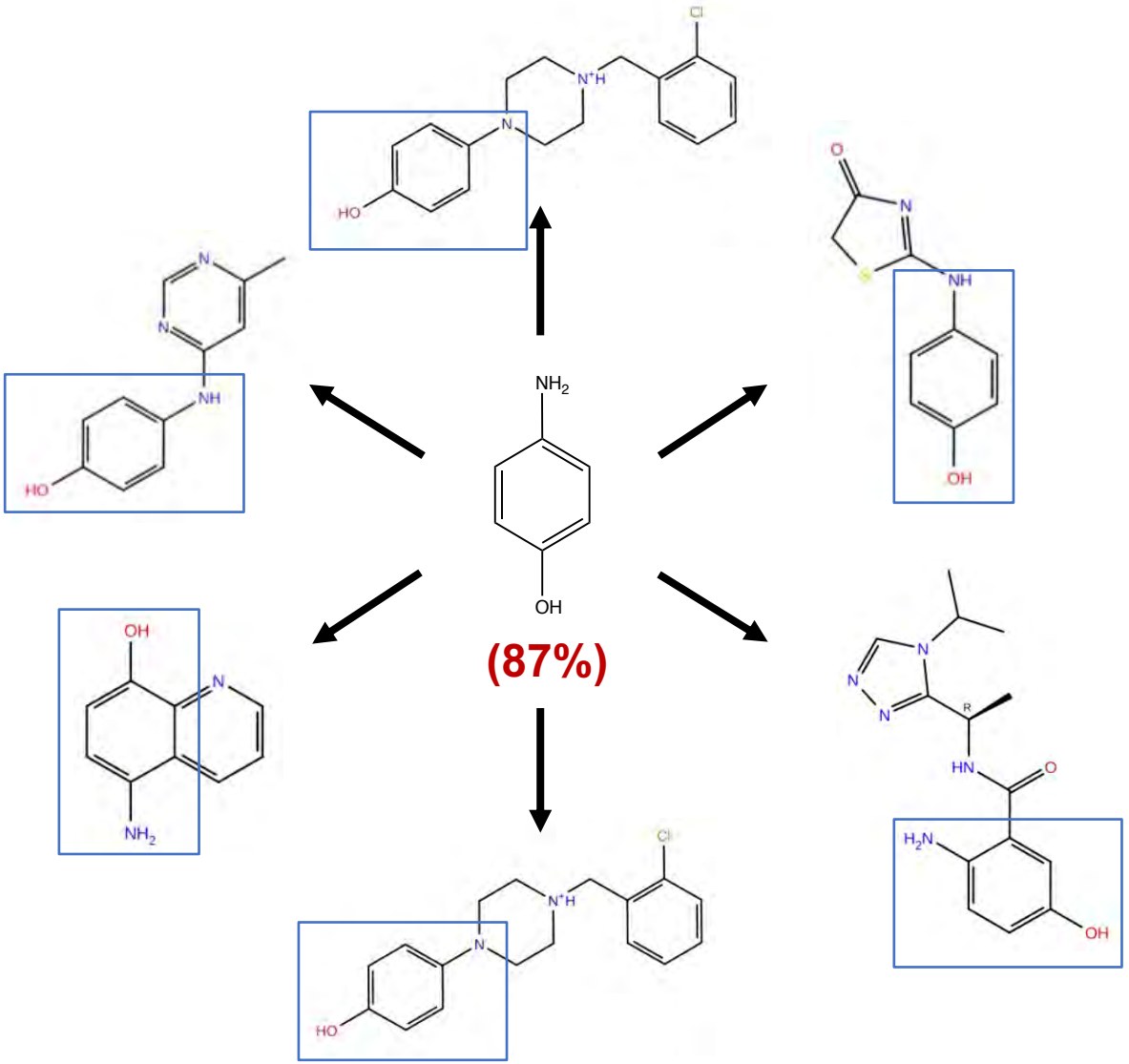
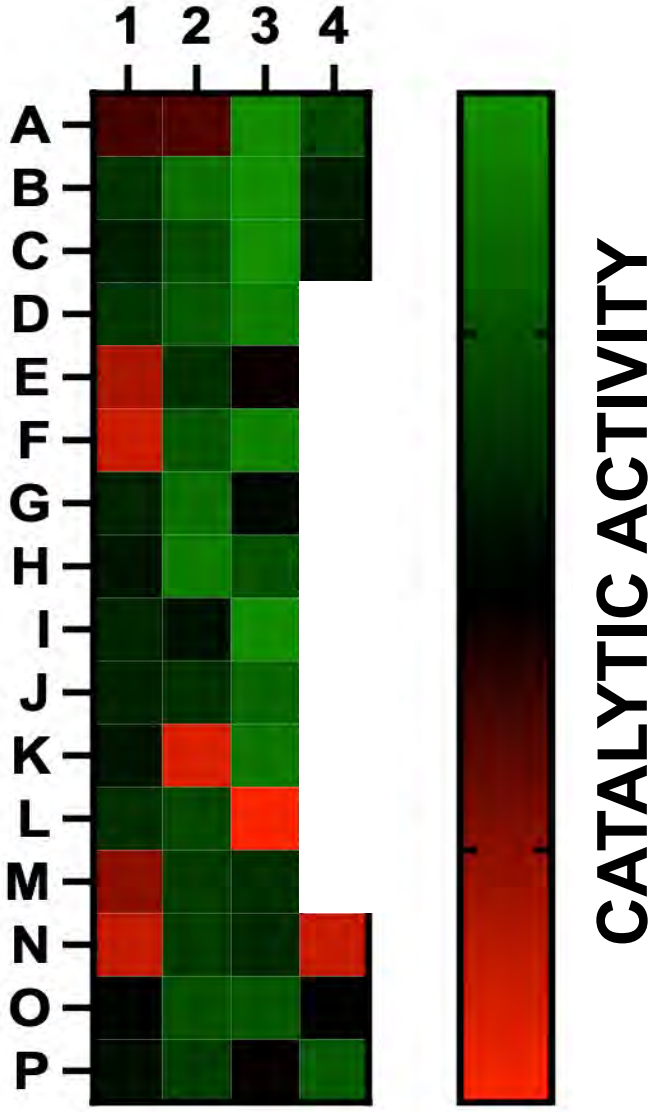
ENHANCED SUB-STRUCTURE SEARCH IN THE EU-OPENSCREEN LIBRARY



EFFECT ON CATALYTIC ACTIVITY



in progress…

COLLABORATORS

diamond

NATE COWIESON

ANA MARTÍNEZ

VNiVERSiDAD D SALAMANCA

RAFAEL PELÁEZ