*GRC Diffraction Methods, July 27 2022*

# Divining Ligands with Confidence


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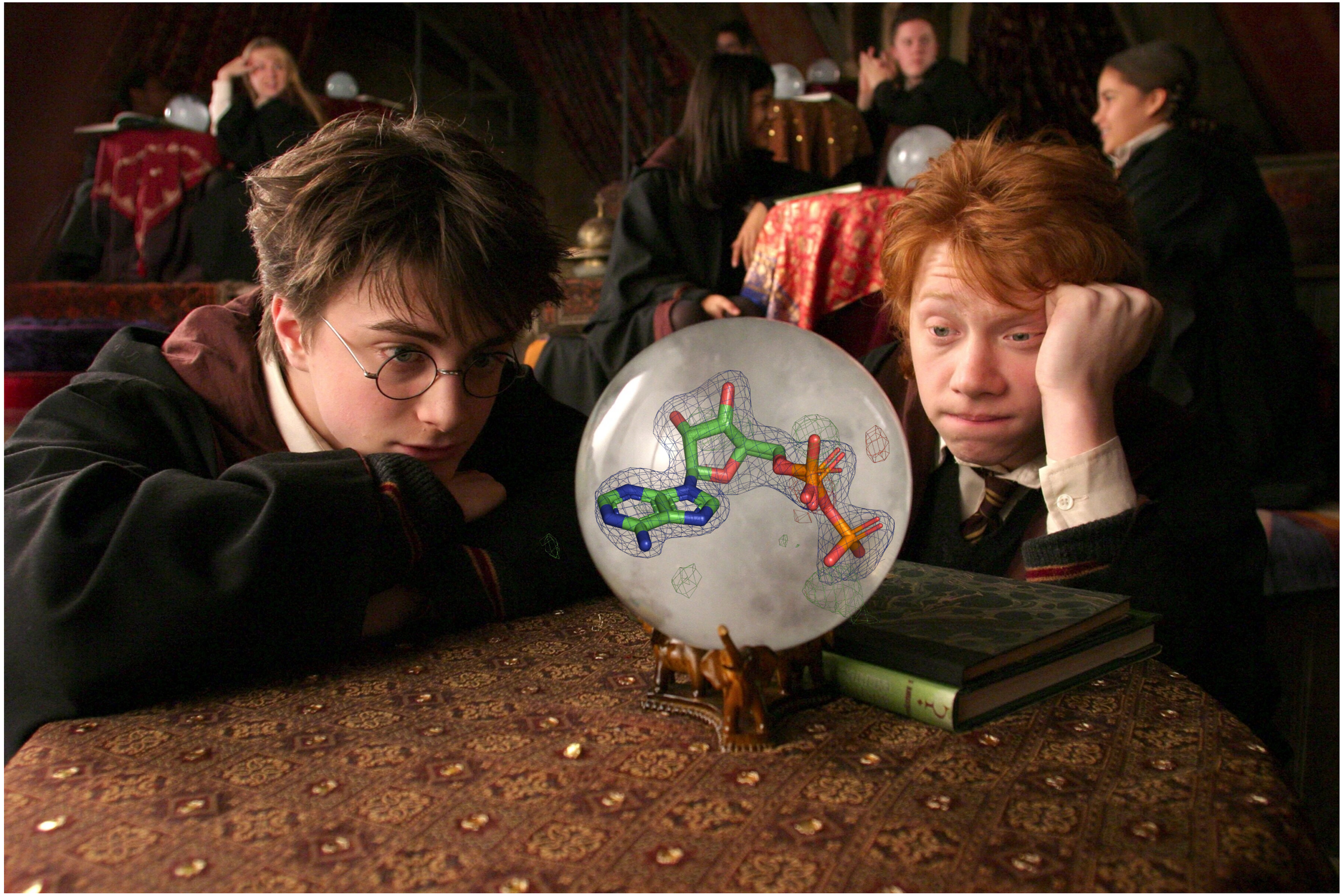
# Divination



# Divining rod

# Semantics

## divine verb

**divined**; **divining** di·vine | \ də-ˈvīn

***transitive verb***

**1** **:** to discover by intuition or insight **:** INFER
*// **divine** the truth*

**2** **:** to discover or locate (something, such as underground water or minerals) usually by means of a divining rod

**Definition of *determine***

**4** **:** to find out or come to a decision about by investigation, reasoning, or calculation
*// determine the answer to the problem*
*// determine a position at sea*

# Semantics

intuition

vs.

reasoning

Is there a way to rationalize better how we find/place/validate ligands?

# Fun Facts about ligands


(July 2022)

193k models in the PDB

146k have at least one ligand (“non-polymer entity”)

↓ 75 %

↓ 37k different ligands

Everyone has to deal with ligands.

# Fun Facts about ligands

Everyone has to deal with ligands.

→ “What-is-in-my-blob” questions on ccp4bb

| Subject | From | Date ▼ |
|---|---|---|
| CryoEM staff microscopist and scientist positions available - SEMC, NYC, USA | Ed Eng | Thu, 30 Jun 2022 21:30:48 +0000 |
| qRTView 1.16 ccp48.0.002 | Krishnan Raman | Thu, 30 Jun 2022 15:38:47 +0000 |
| Re: CCPBioSim Industry Talk | Nigel Moriarty | Thu, 30 Jun 2022 06:22:32 -0700 |
| Re: CCPBioSim Industry Talk | Nigel Moriarty | Thu, 30 Jun 2022 06:10:19 -0700 |
| Re: Unidentified electron density | Mark J. van Raaij | Thu, 30 Jun 2022 14:59:54 +0200 |
| Re: Unidentified electron density | David Briggs | Thu, 30 Jun 2022 08:55:51 +0000 |
| Re: Unidentified electron density | Harmer, Nicholas | Thu, 30 Jun 2022 07:58:01 +0000 |
| Unidentified electron density | Sayan Saha | Thu, 30 Jun 2022 12:33:17 +0530 |

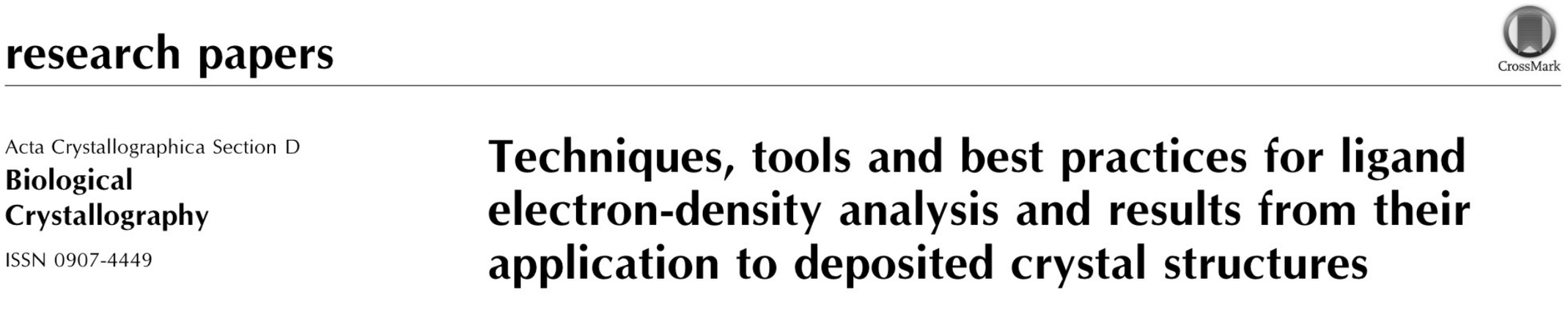
# Less fun facts about ligands

”There are far too many protein–ligand structures in the PDB which either

(i) clearly do not contain the purported ligand,

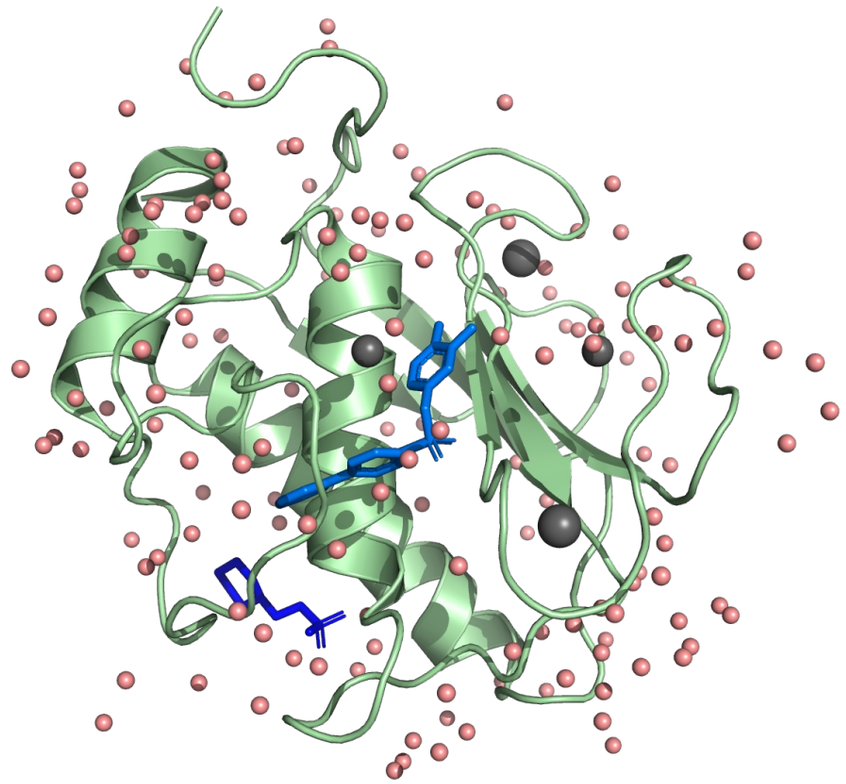
(ii) provide only insufficient crystallographic evidence that such a ligand might be present or

(iii) present an incorrect description of the ligand.”




Pozharski, E., Weichenberger, C. X. & Rupp, B. (2013). *Acta Crystallogr. D Biol. Crystallogr.* **69**, 150–167.

# Why are ligands difficult?



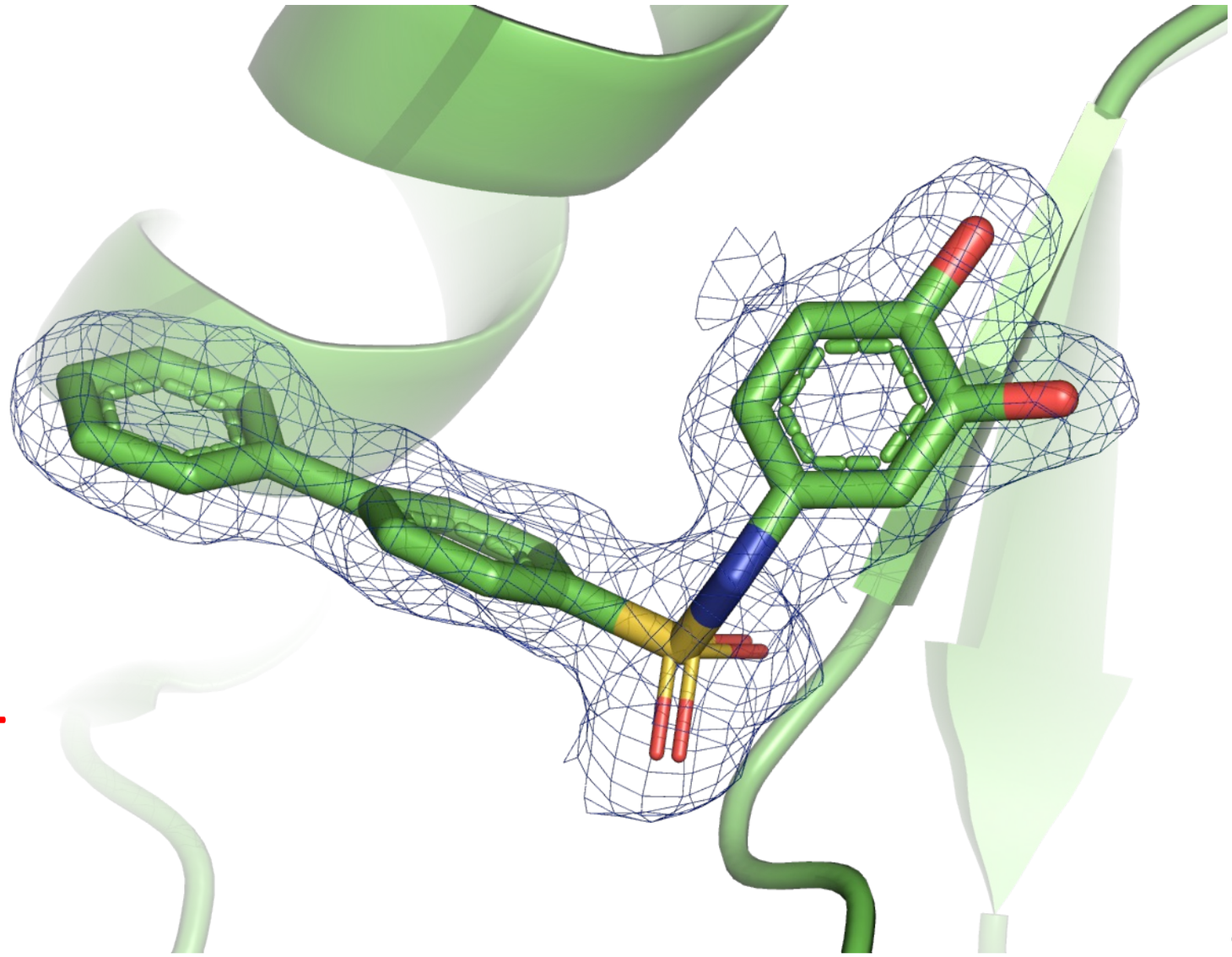
A ligand contributes to 1/100 to 1/1000 of total scattering

→ will not affect the absolute values of global quality measures (crystallographic R-factors)

# Why are ligands difficult?

Ligand binding depends on binding affinity and ligand concentration.

→ It is likely that ligand does not have full occupancy



occ = 1

Fmodel map
2Å

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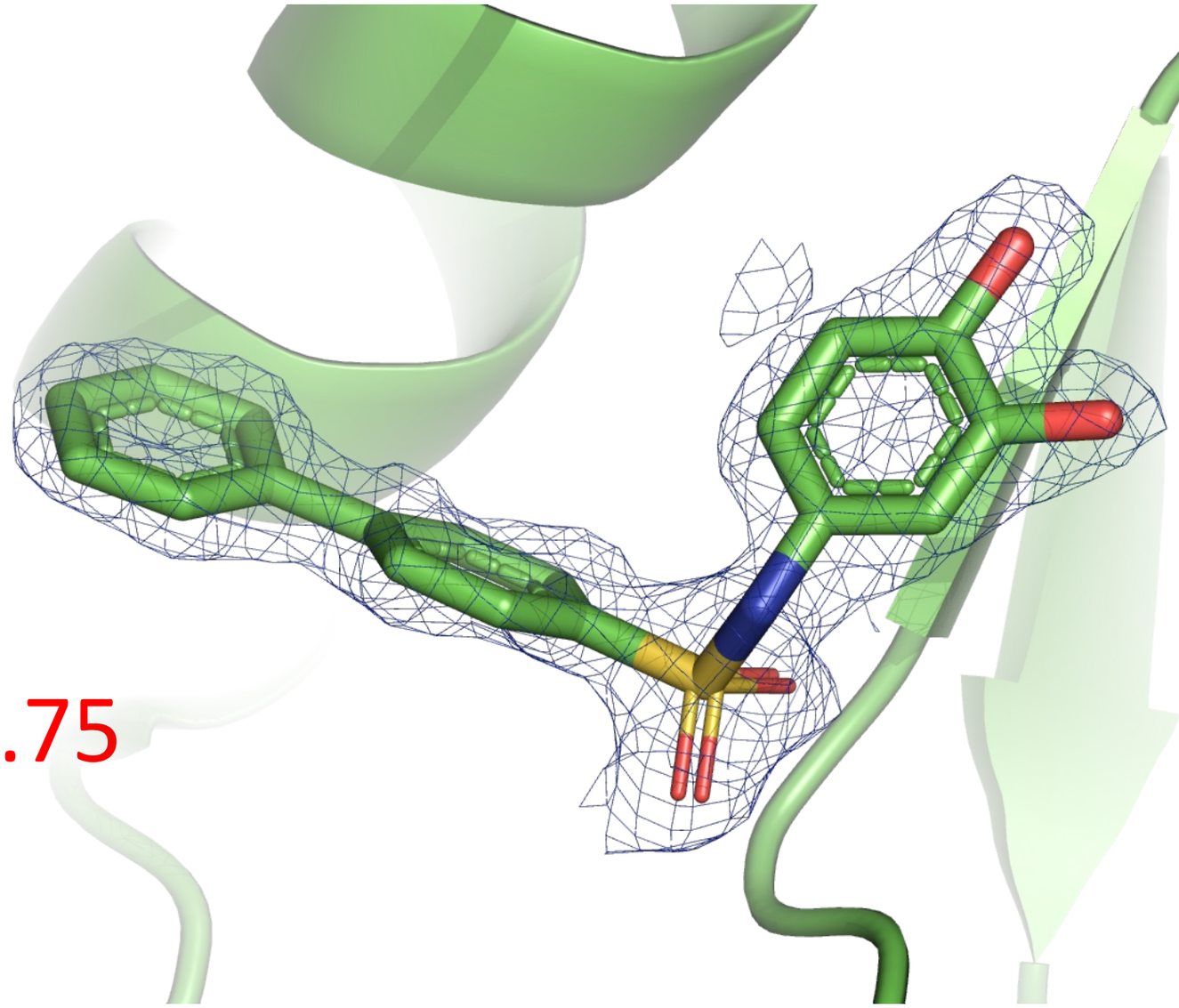

occ = 0.75

Fmodel map
2Å

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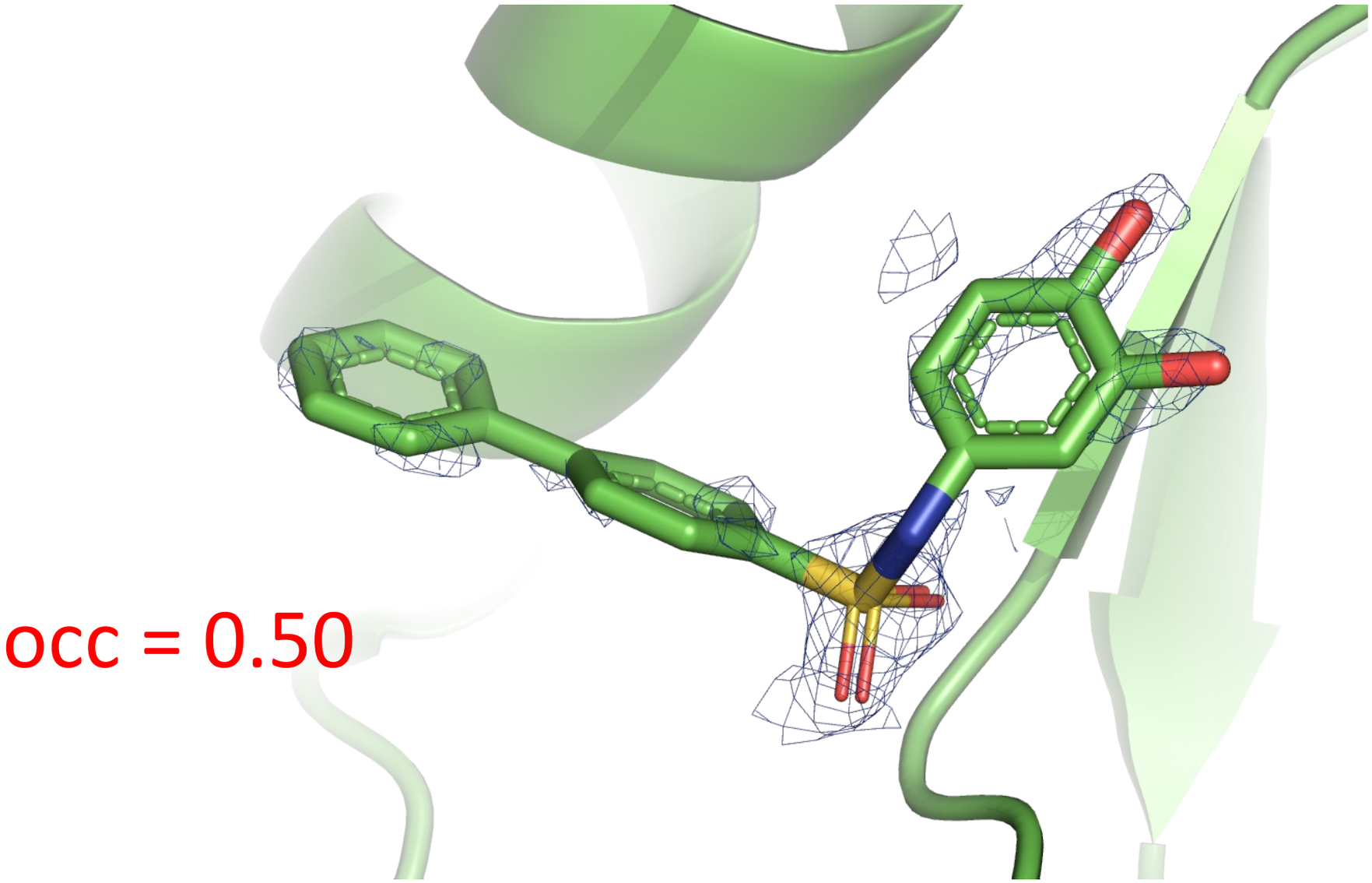


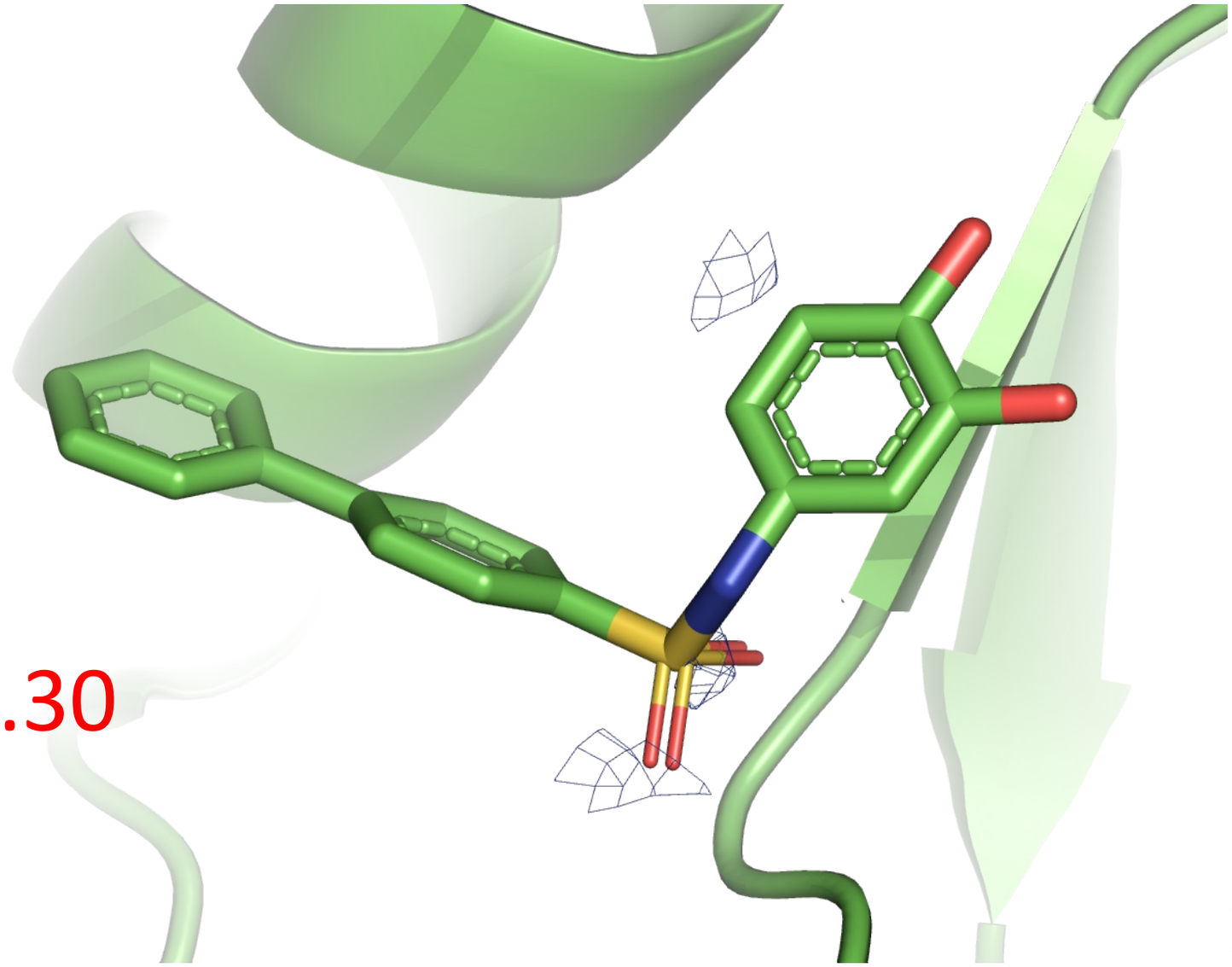
occ = 0.50

Fmodel map
2Å

# Why are ligands difficult?

Ligand binding depends on binding affinity and ligand concentration.

→ It is likely that ligand does not have full occupancy



occ = 0.30

Fmodel map
2Å

# Consequence of low binding affinity

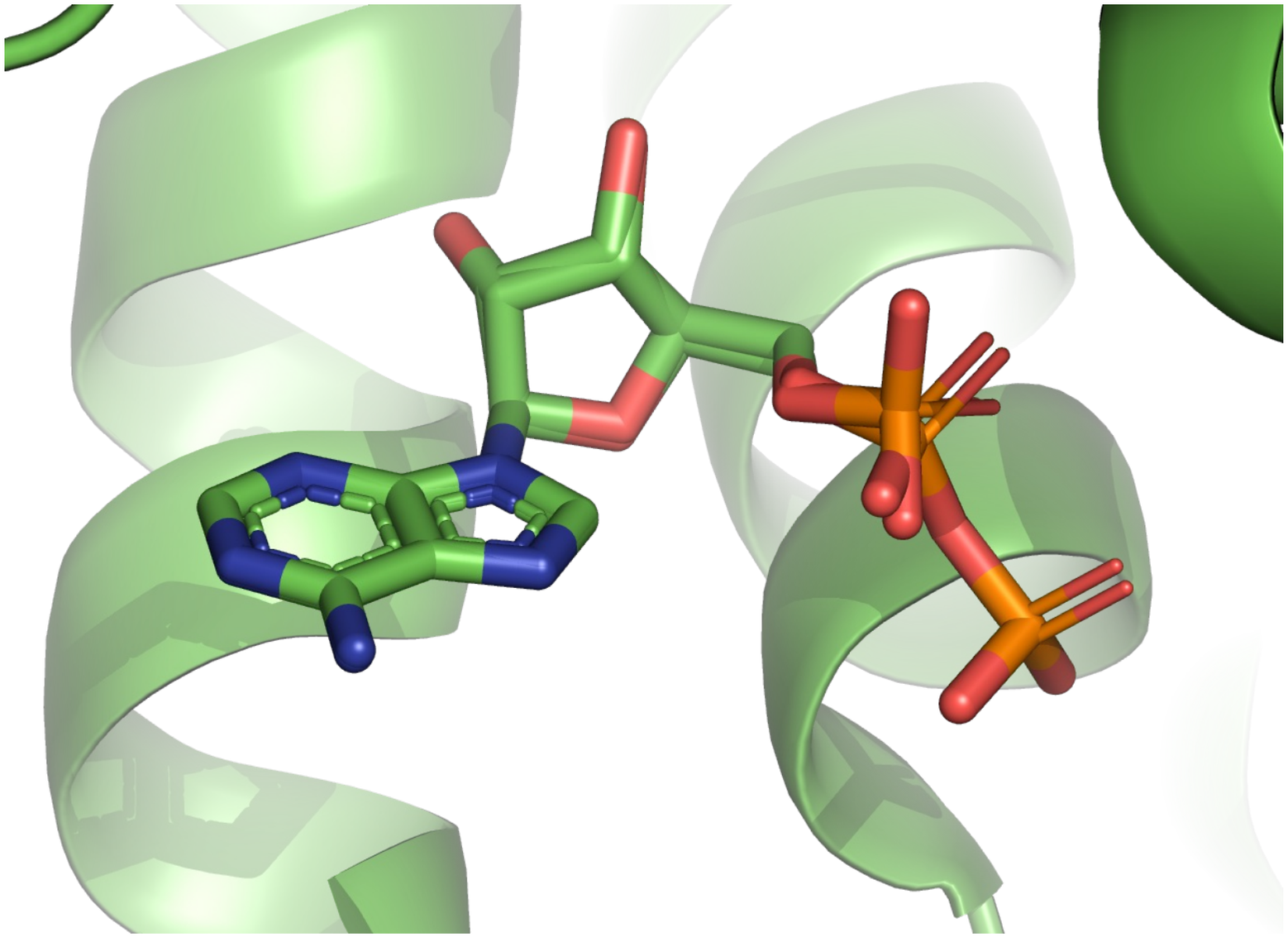
If the ligand does not have full occupancy…

…what is in the binding site if the ligand is not there?

- Water
- Bulk solvent
- Other molecules
- ?
- Combination of all of the above

# Toy example

Search the PDB for a site with two different ligands.



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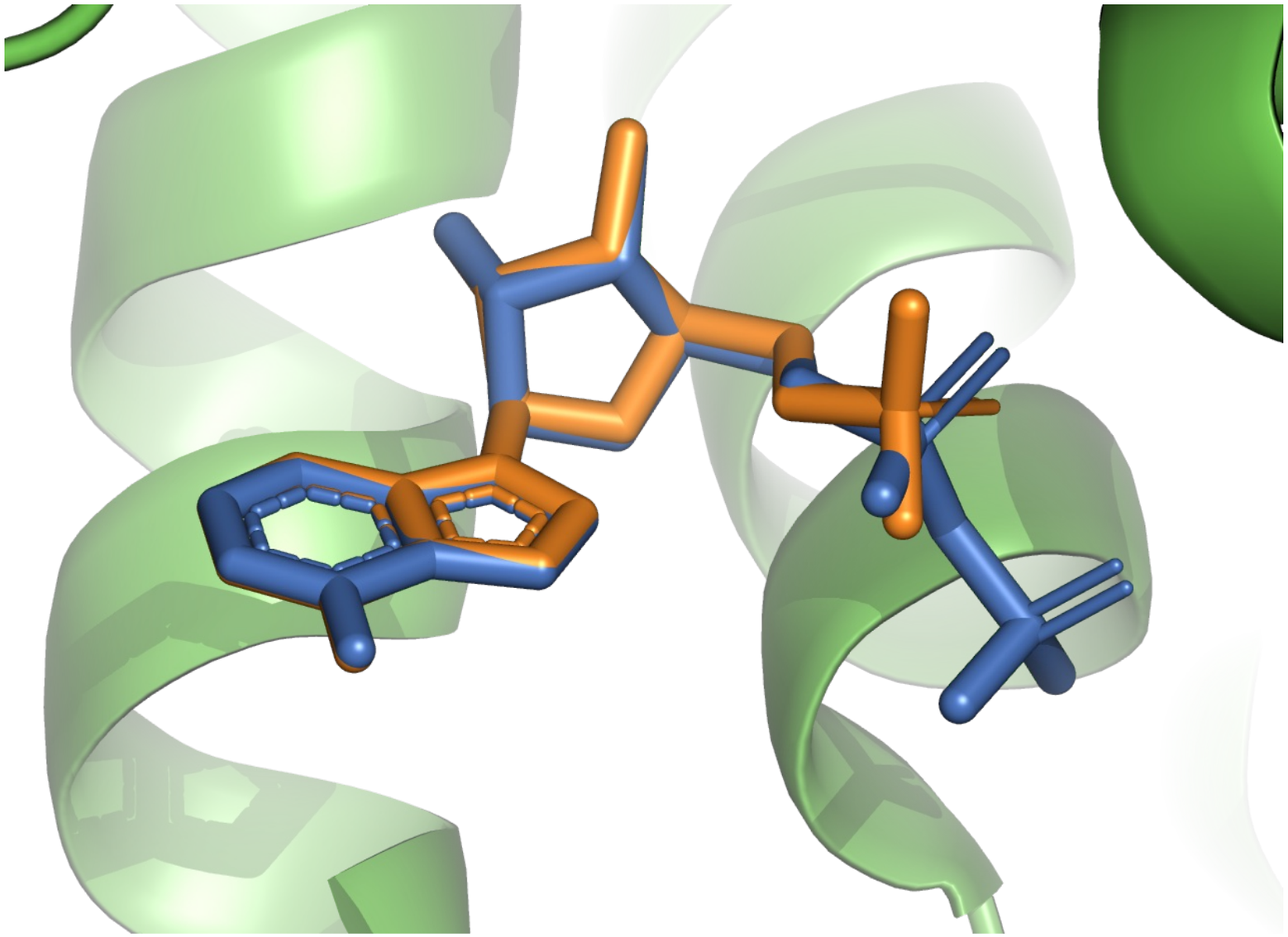
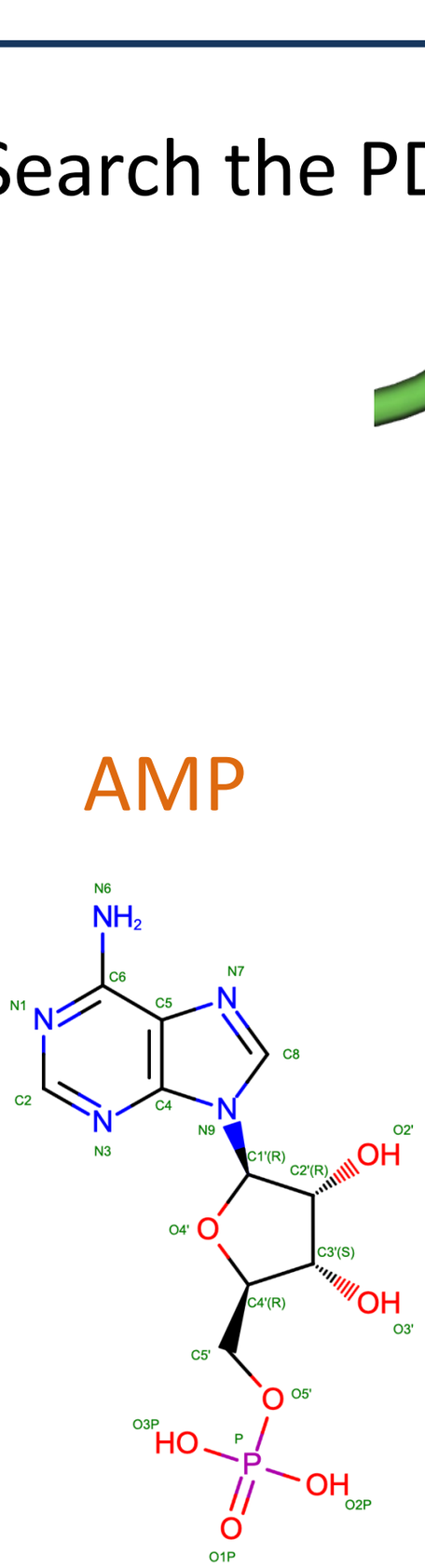
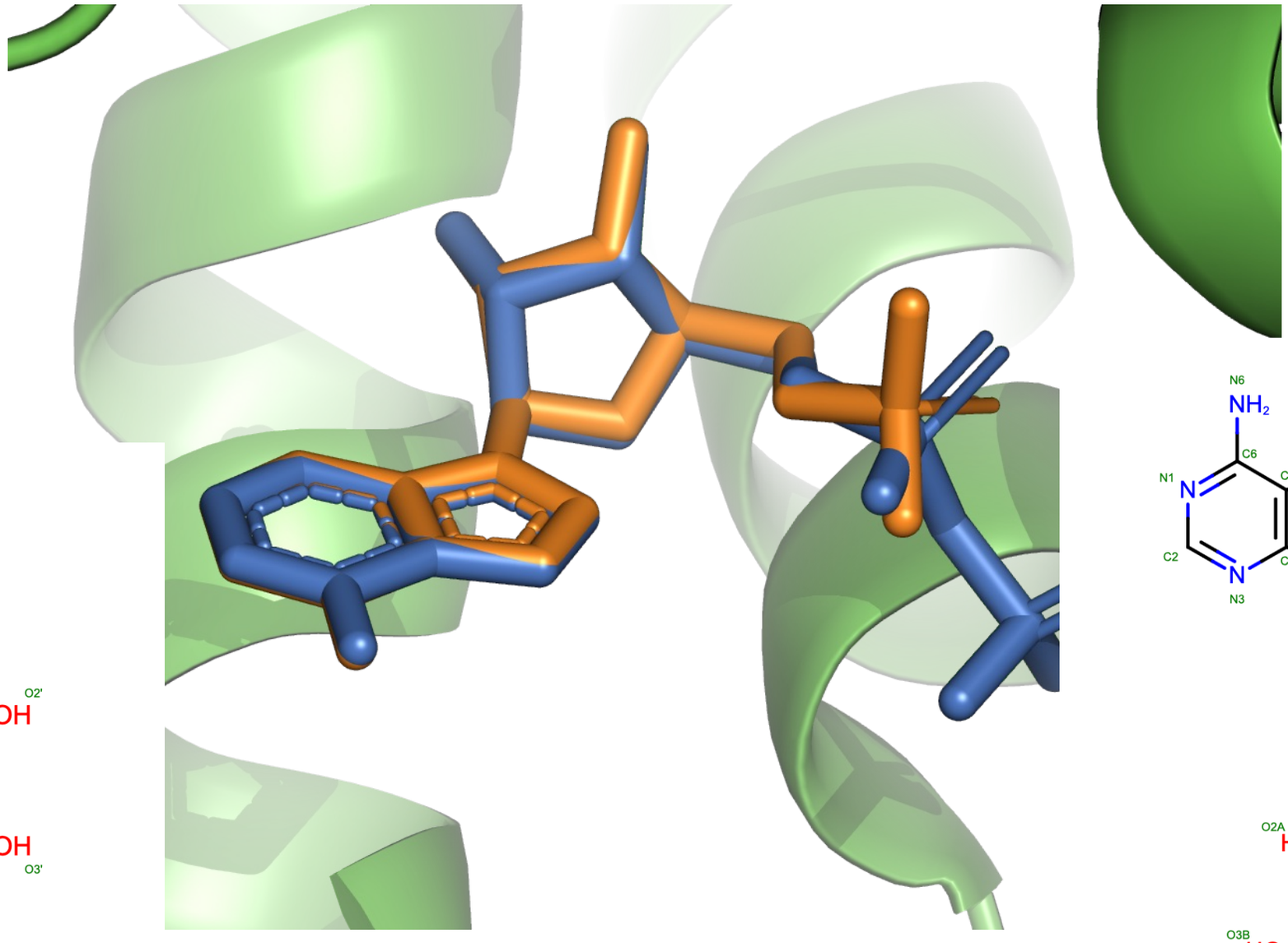


# Toy example

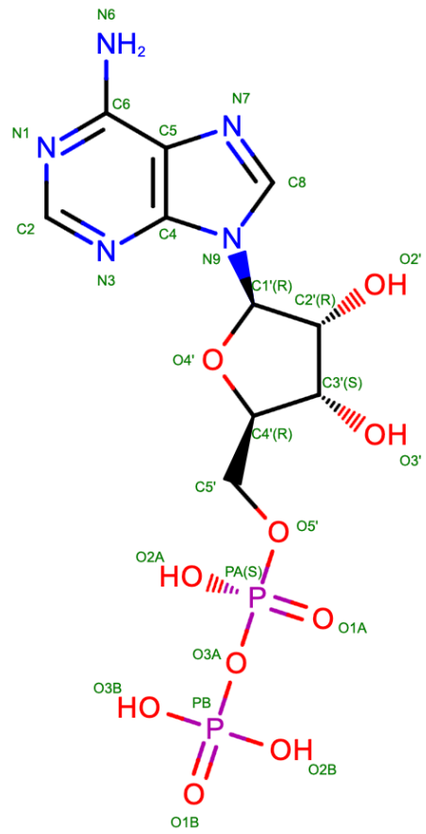
Search the PDB for a site with two different ligands.

AMP



ADP



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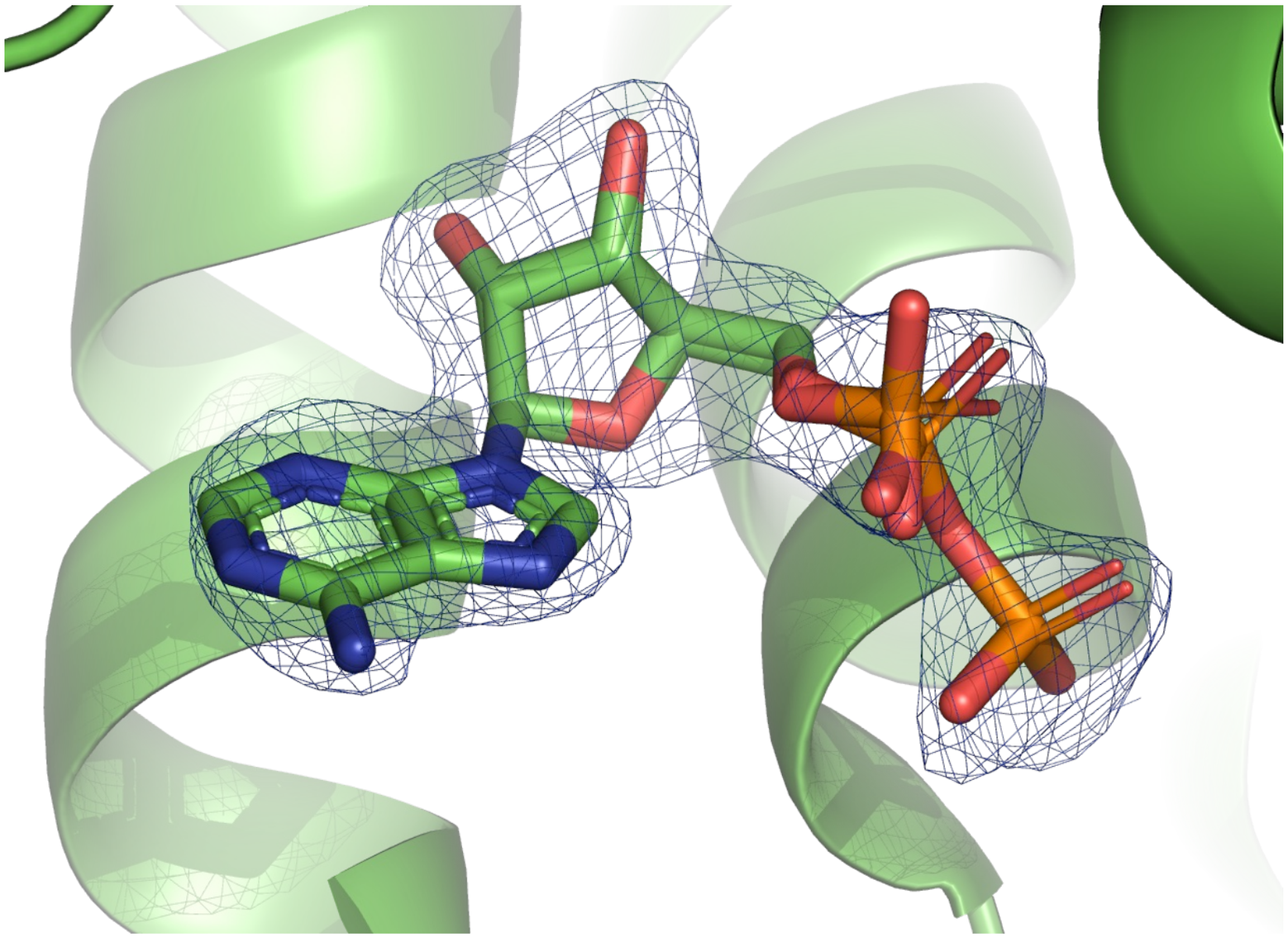
Search the PDB for a site with two different ligands.



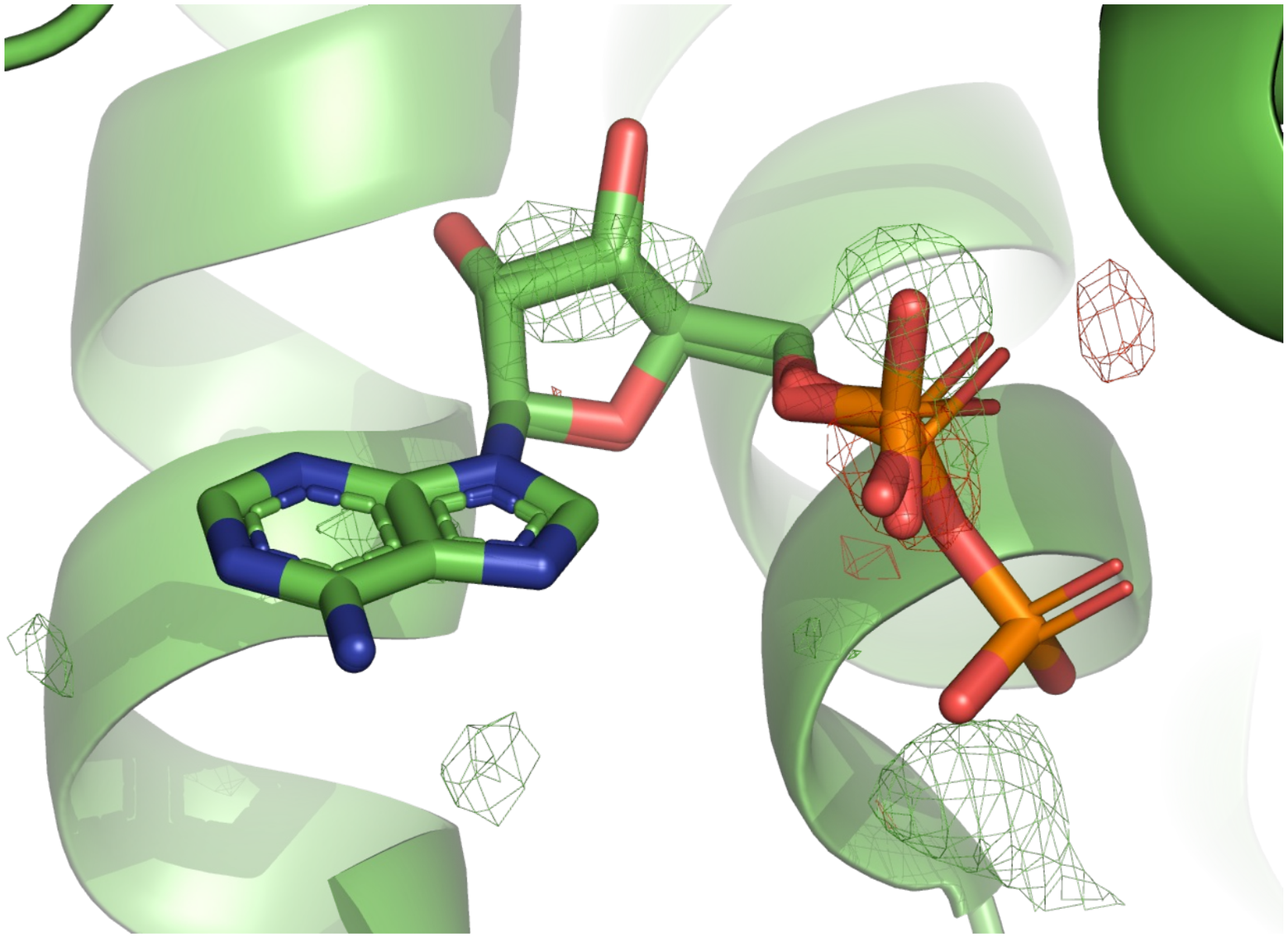
$2mF_{obs}$-$DF_{model}$
contour: 1.0 rms

# Toy example

Search the PDB for a site with two different ligands.

AMP

occ = 0.22



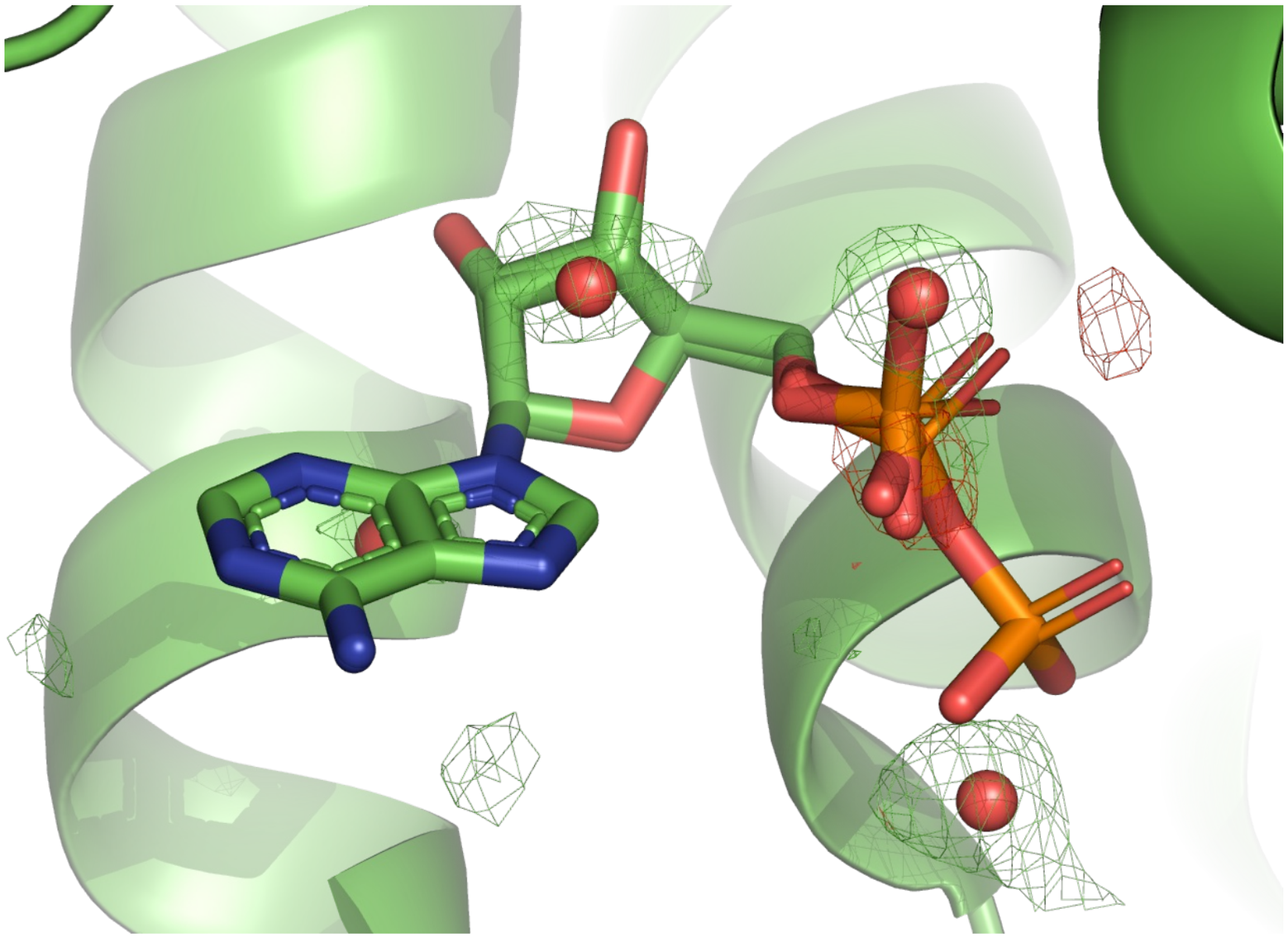
ADP

occ = 0.5

$mF_{obs}$-$DF_{model}$
contour: +/- 3 rms

# Toy example

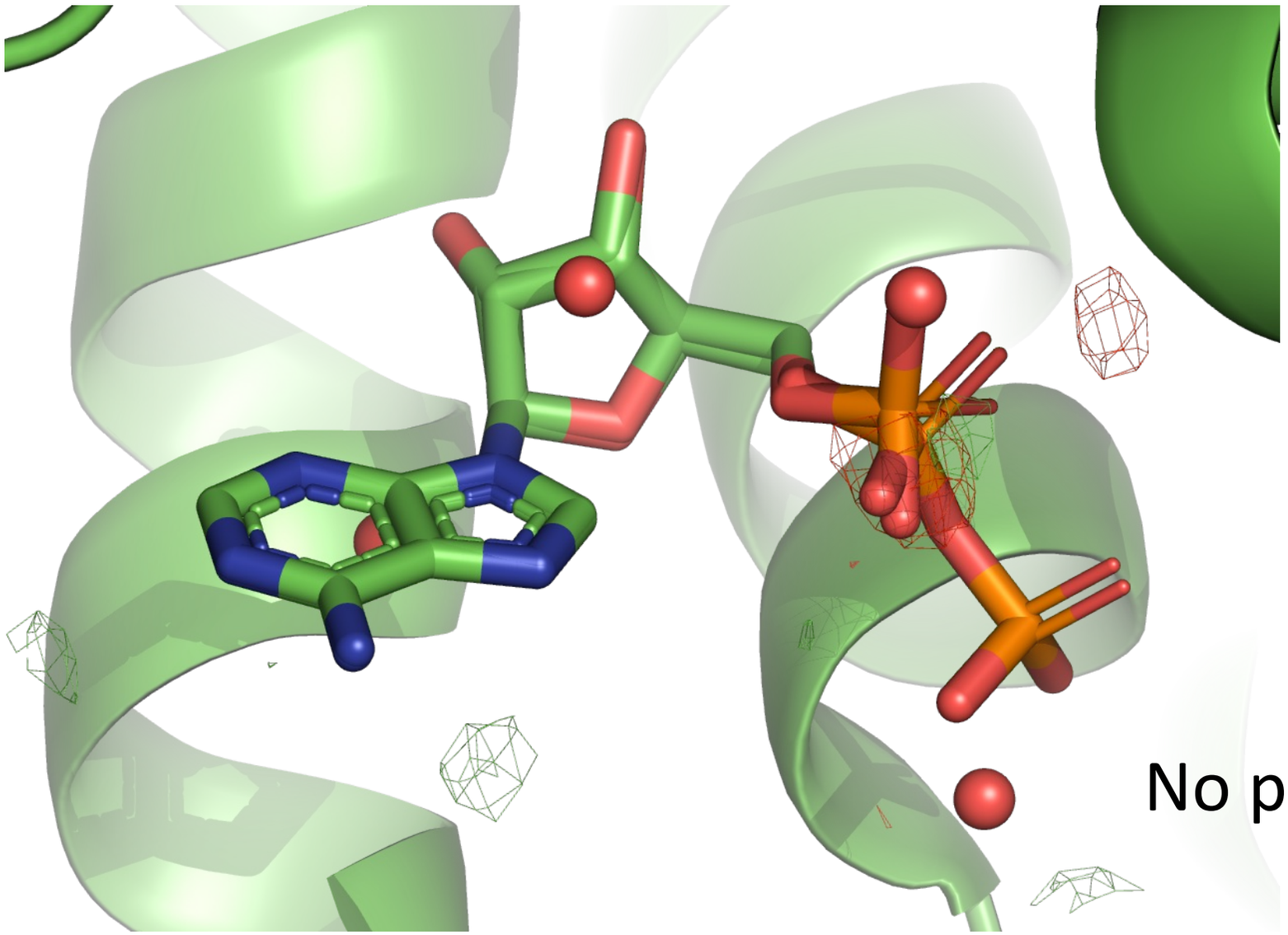
Place water molecules into the positive difference density



$mF_{obs}$-$DF_{model}$
contour: +/- 3 rms

# Toy example

Place water molecules into the positive difference density



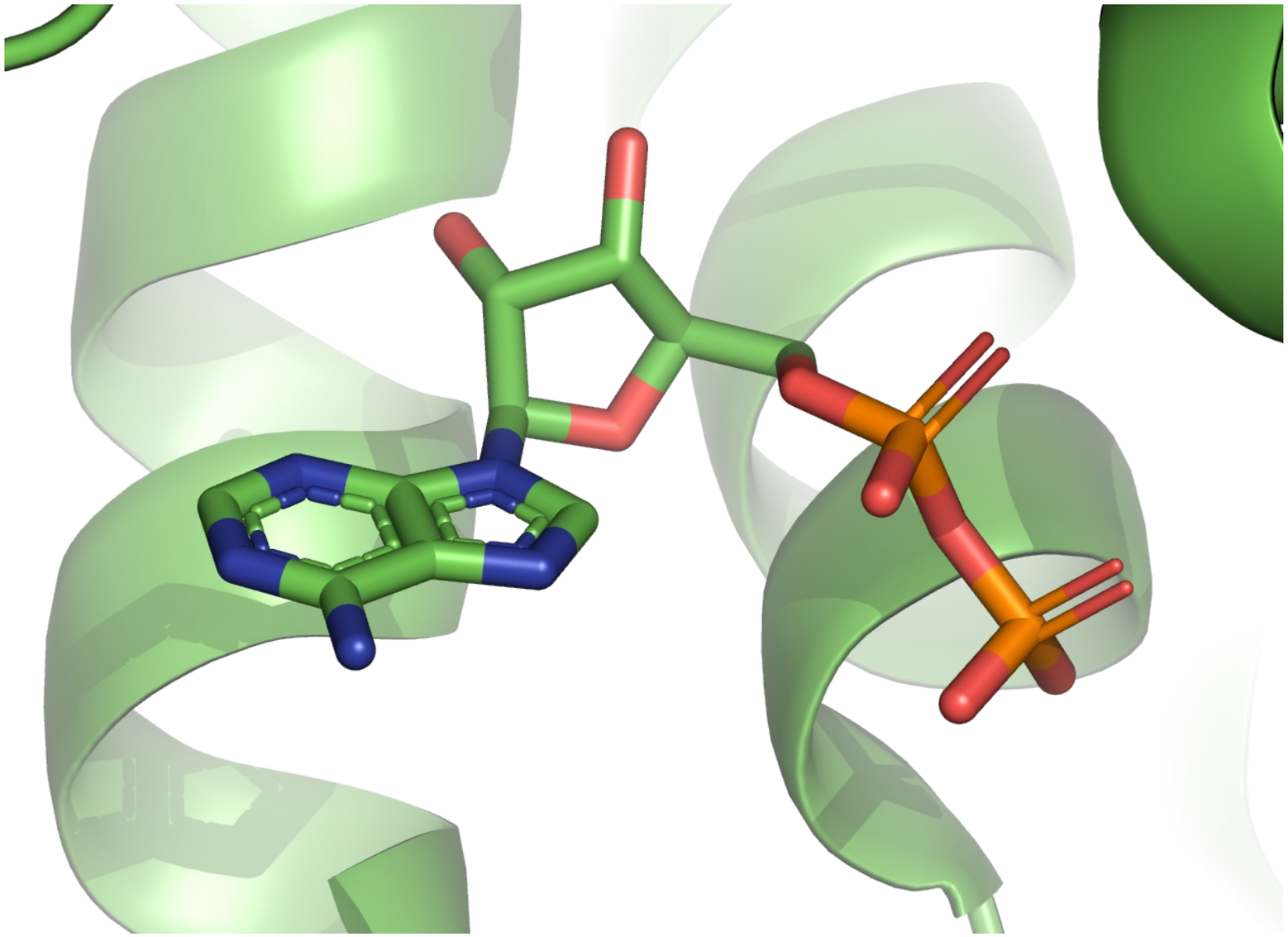
No peaks left…

…but…

$mF_{obs}$-$DF_{model}$
contour: +/- 3 rms

# Toy example

Use one single conformation of ADP (occ = 1.0)



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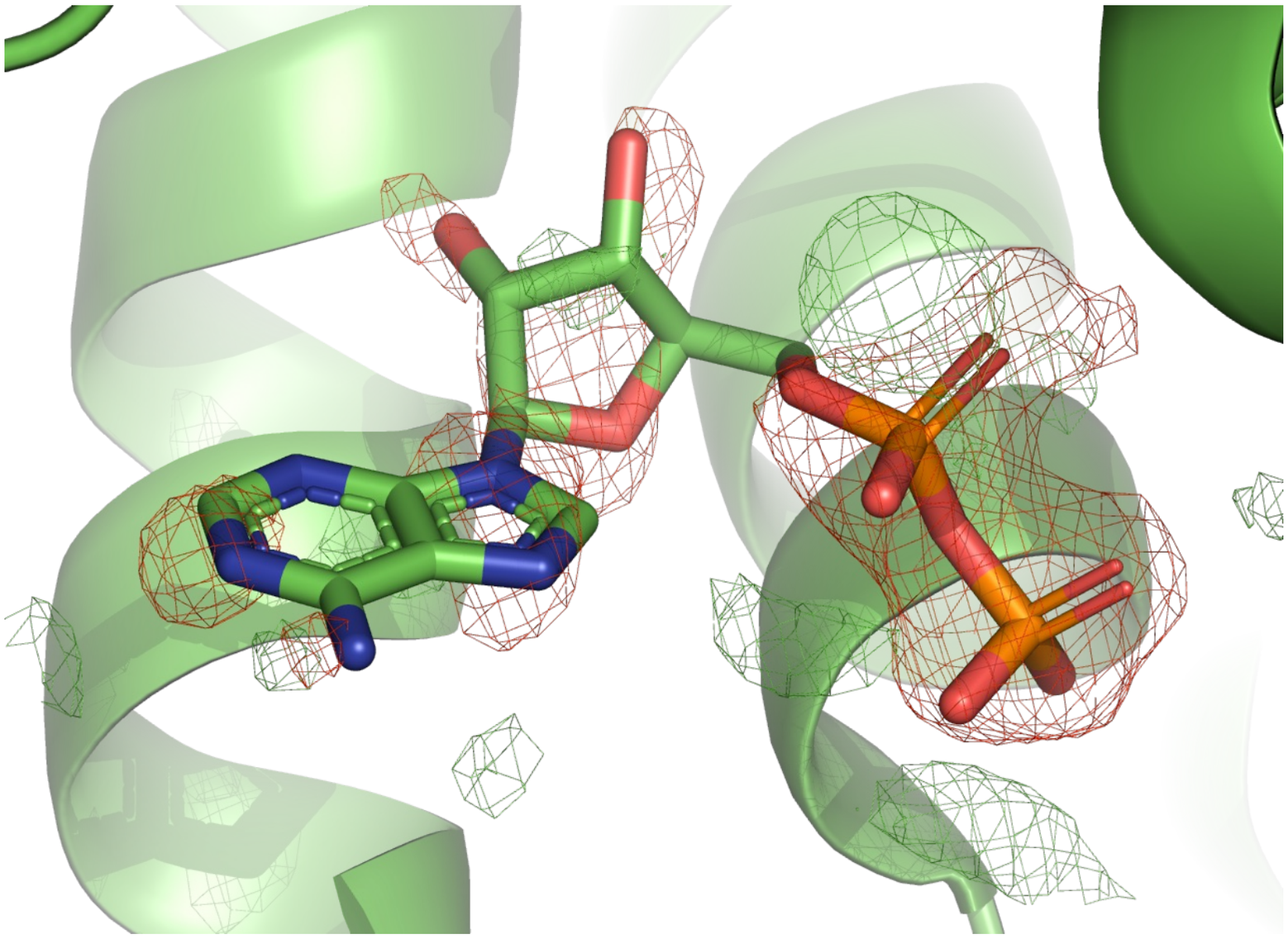

$mF_{obs}$-$DF_{model}$
contour: +/- 3 rms

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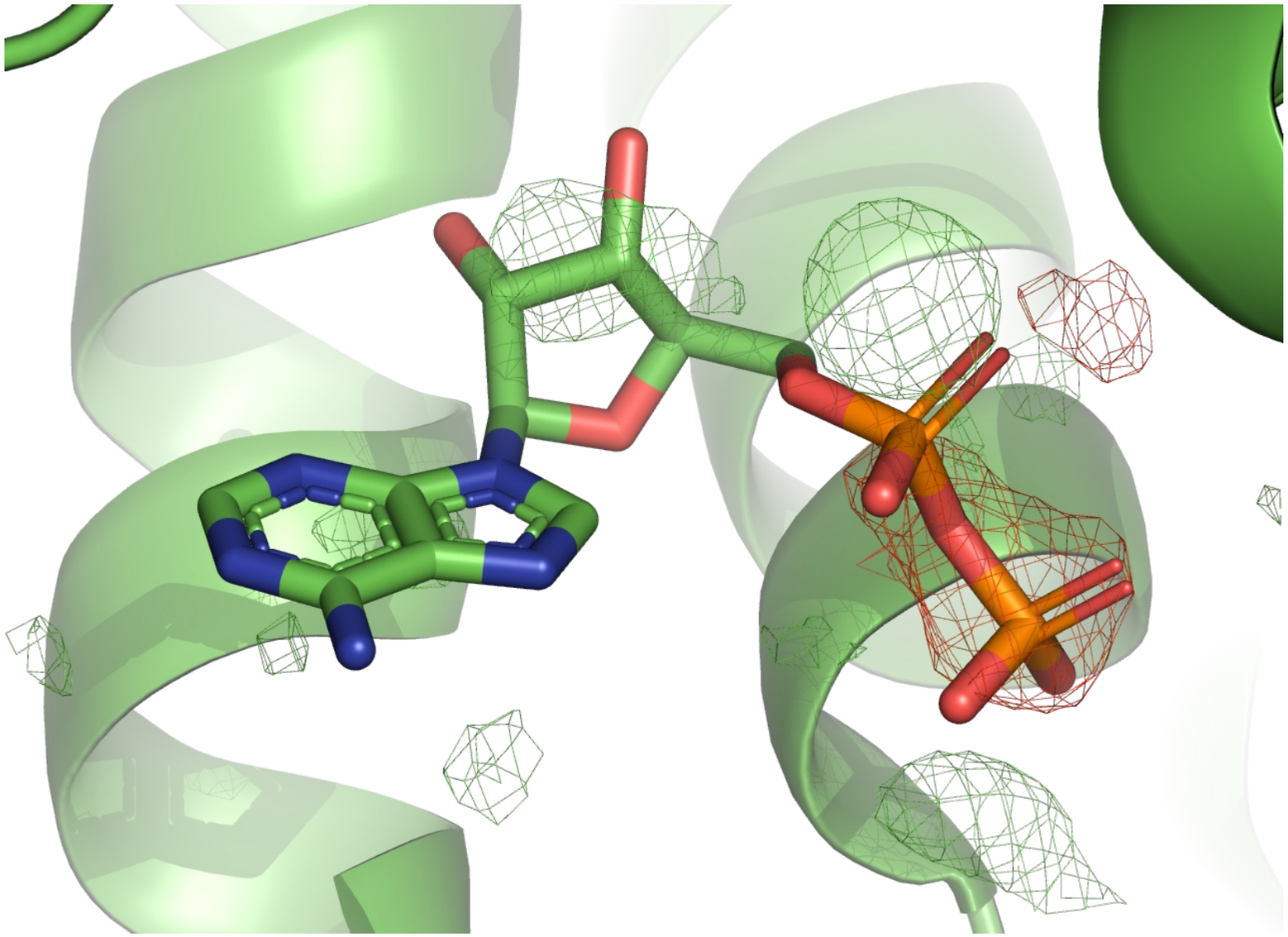
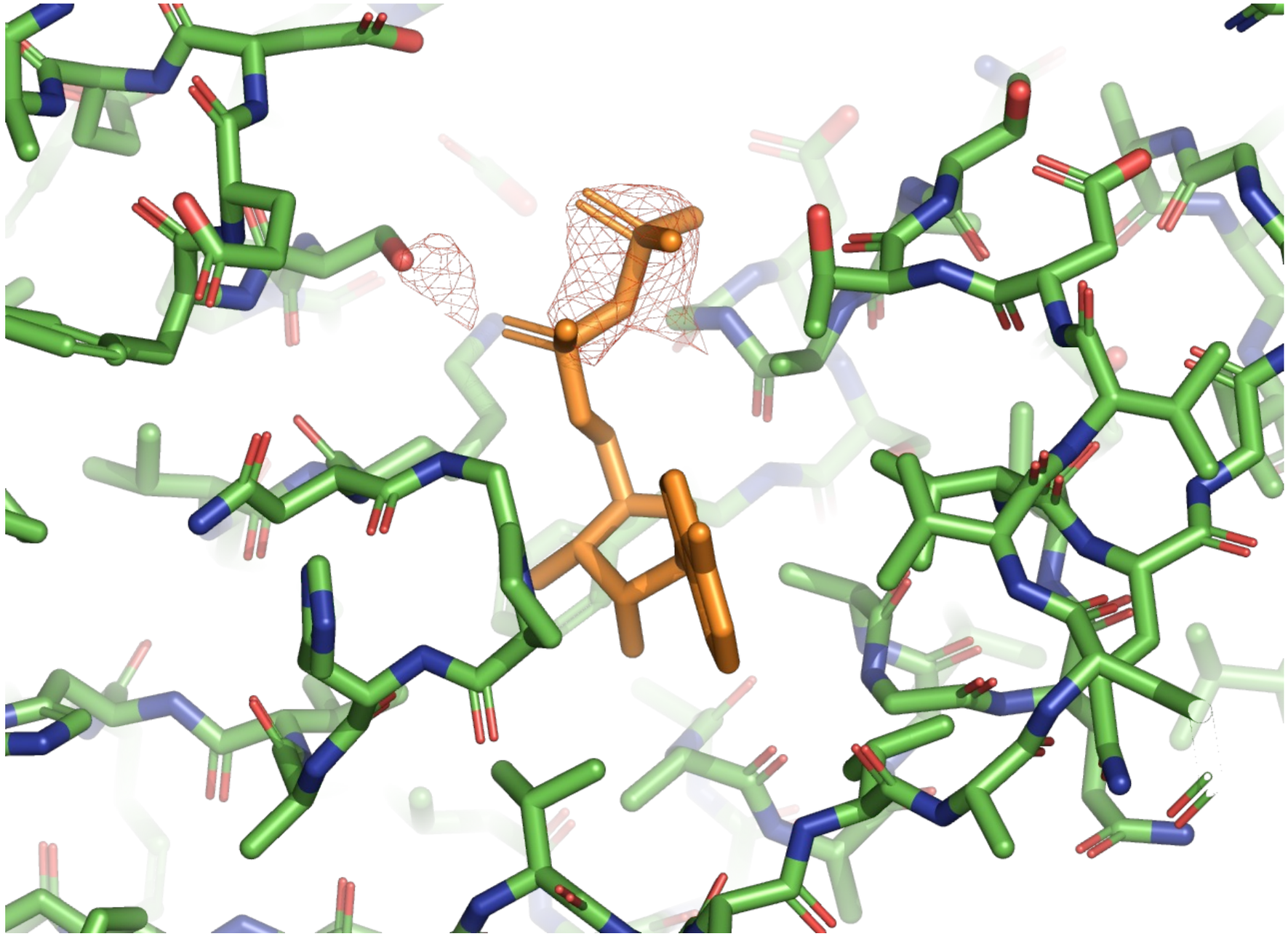
Use one single conformation of ADP (occ = 0.75)



$mF_{obs}$-$DF_{model}$
contour: +/- 3 rms

# Toy example

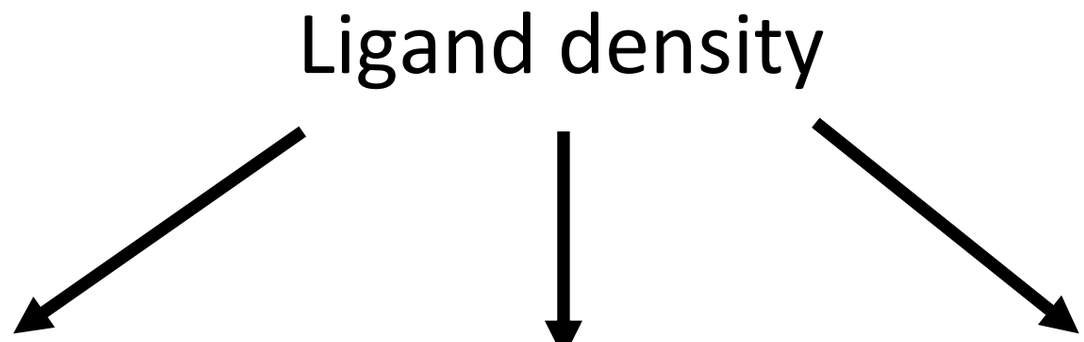
Use one single conformation of ADP (occ = 0.75)



$mF_{obs}$-$Df_{model}$
contour: -3 rms

Maybe part of the molecule is disordered

# Toy example




**Hypothesis 1**
Clean maps, but frivolous

**Hypothesis 2**
Complicated (ADP and AMP)

**Hypothesis 3**
Simple, but messy maps

# Discussion prompts

**Is there a way to rationalize better how we find/place/validate ligands?**

**Better education?**

- Ligands as topic at crystallographic schools?
- Promote technical knowledge?
- Ethical aspects: expectation bias and responsibility to the community?

**Methods development?**

- Better tools to handle ligands (restraints, placement, try out hypothesis)?
- Better approaches?
- Comprehensive validation?

Other?

# Divining Ligands with Confidence

**Tom Peat** (UNSW, Australia)
"Is it There? Or Not?"

**Ying Zhang** (Ambagon Therapeutics, United States)
"Industrial Perspective"

**Discussion**