Identifying Cryptic Pockets in FGFRs with Enhanced Sampling Simulations

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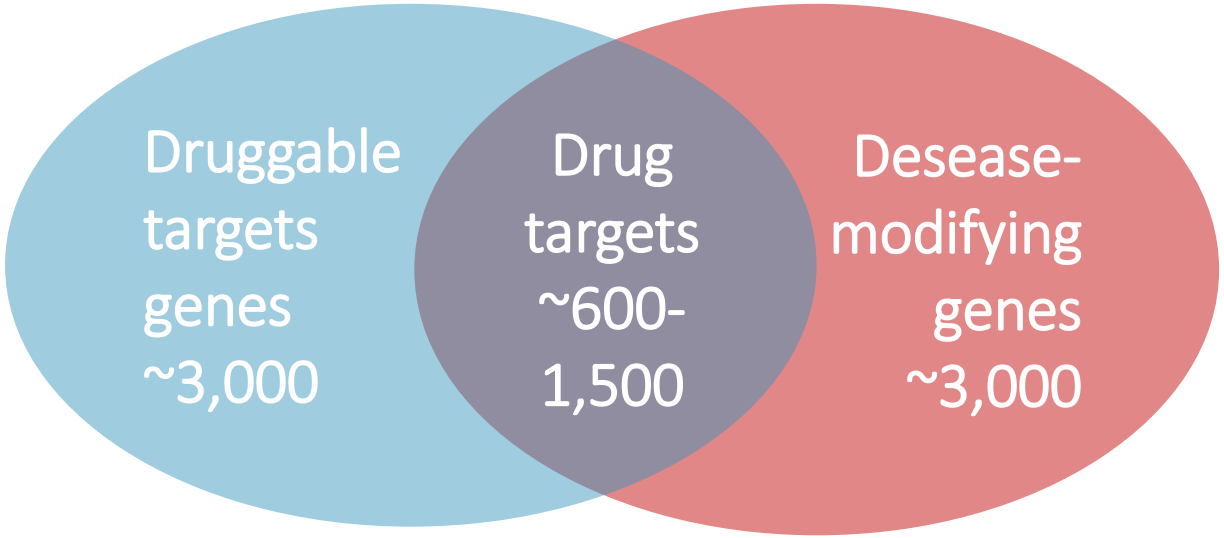
Cryptic Pockets

- Binding sites that are **not identifiable** in the **apo** crystal structure, but become **apparent** when **bound by a ligand**



- Naïve definitions of druggability exclude proteins without apparent pockets in the apo structure

A. L. Hopkins and C. R. Groom *Nat Rev Drug Discov*, 1 (9), 2002

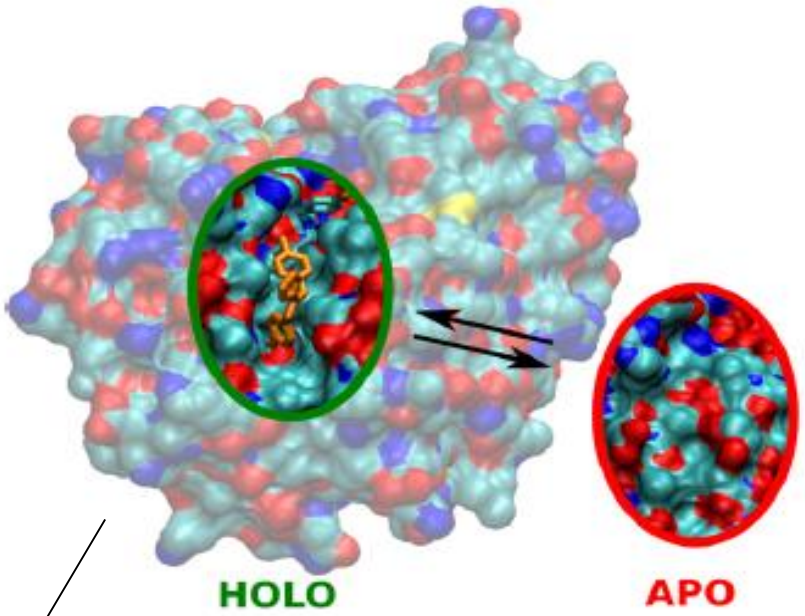
Cryptic Pockets II

- The protein undergoes conformational changes to accomodate the ligand, the **dynamical properties** cannot be ignored

- So far most cryptic pockets have been discovered **serendipitously**

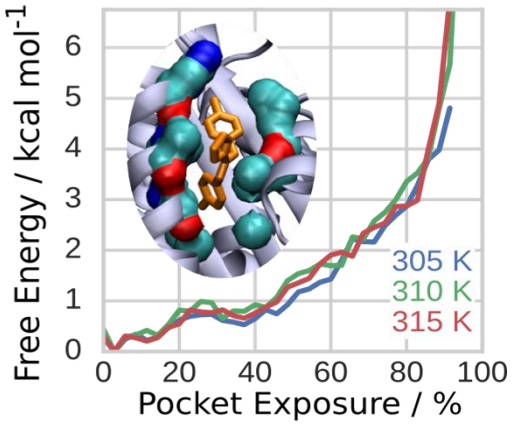
  ↳ Develop an efficient approach **to identify druggable hidden pockets** with **MD simulations**

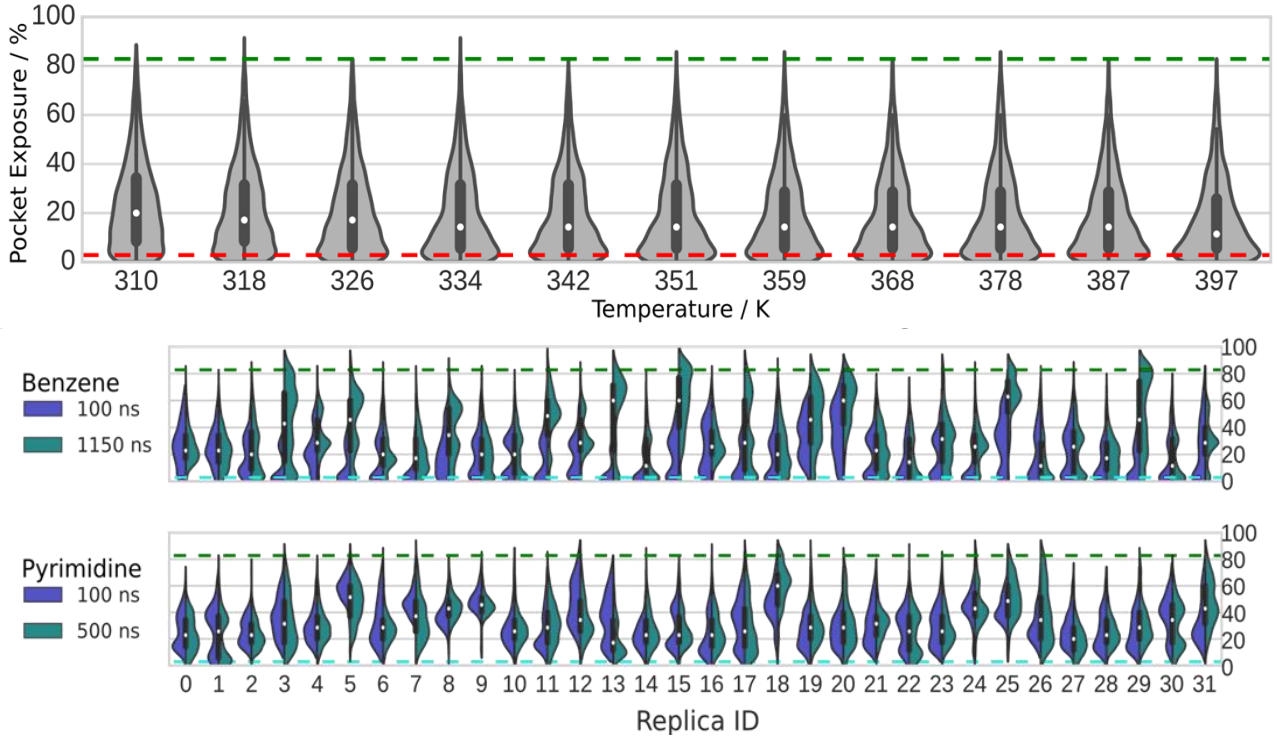
Parallel Tempering MD?
Mixed solvent with ligand fragments?



TEM1 β-lactamase: Brute Force Approach

- Plain MD: **hydrophobic** pocket, closes immediately
- PTMD: the temperature does not have a relevant effect, risk of increasing **entropic barrier**
- Mixed Solvent: not reliable





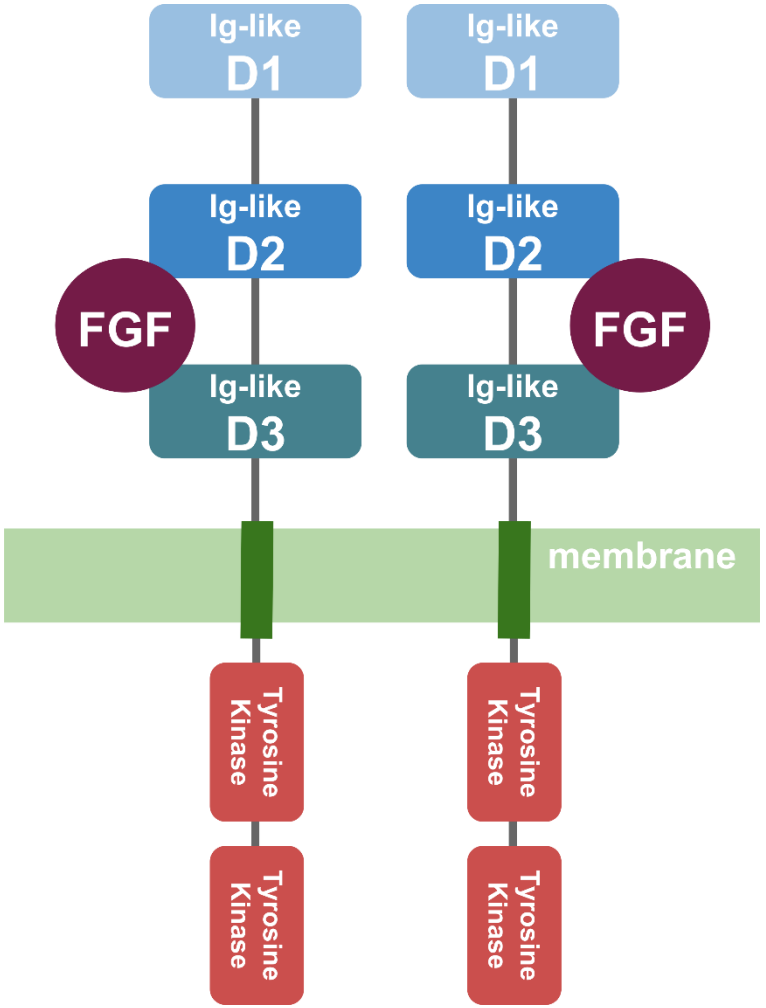
Fibroblast Growth Factor Receptors (FGFR)

- **FGFs**: growth factors, mitogens, neural development, angiogenesis, wound healing…
  - 23 promiscuous ligands – 4 (5) receptors

- (2-) **3 extracellular domains** (ECD): FGF/heparin binding
  single-helix transmembrane domain (TMD)
  tyrosine kinase intracellular domains (ICD)

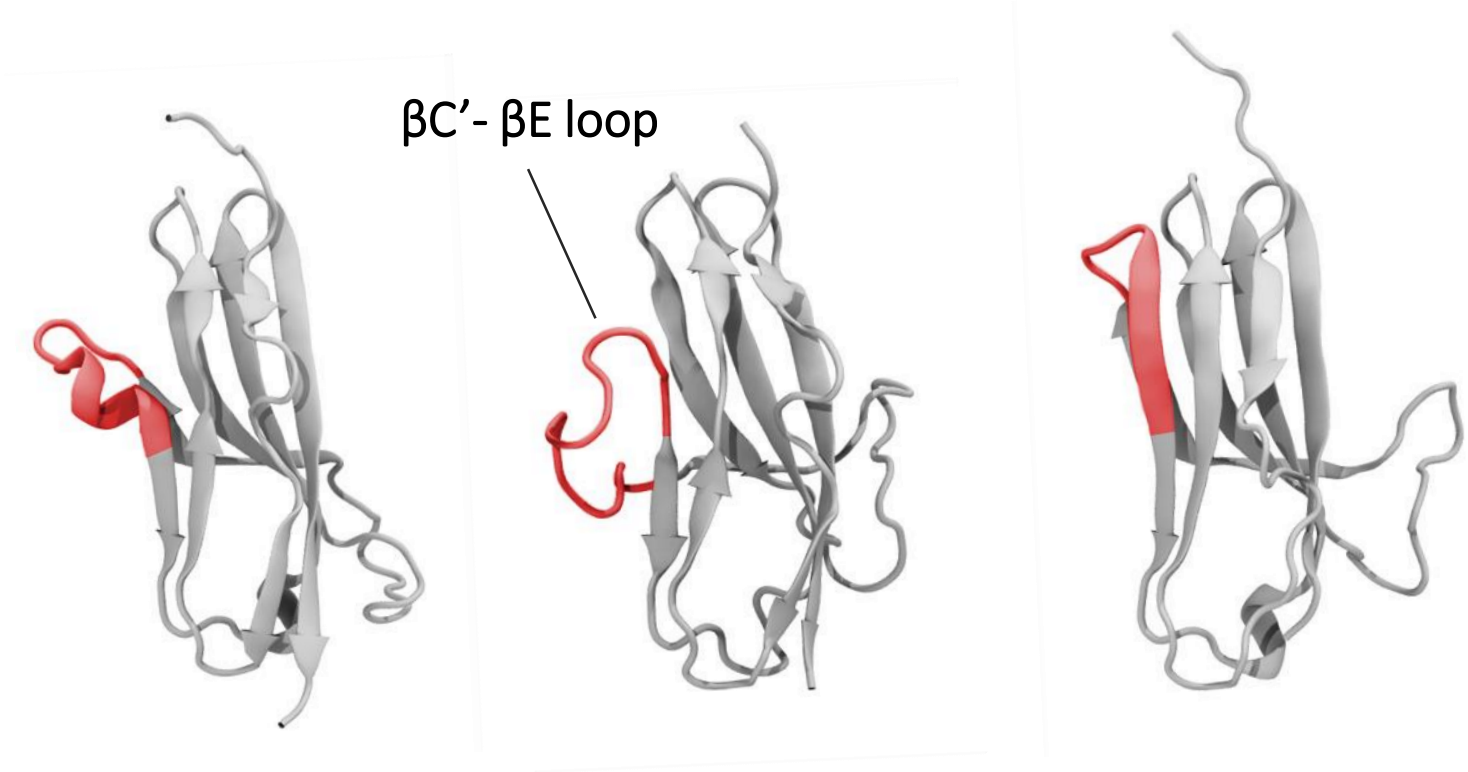
- Binding of FGF leads to **dimerization** -> trans-phosphorylation of tyrosines (ICD)
  - **internalization**

- **D3** intrinsically disordered, molten globule



FGFR D3 isoforms differences

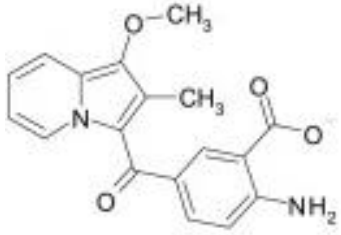
FGFR2c (1FQ9)	FGFR3c (1RY7)	FGFR3b (3GRW)
NT*TDKEIEVL*YIRN	NTTDKELEVLSLHN	ESVEADV-RLRLAN
helix **βE**	**βE**	extended **βE**



- The presence or absence of helix in the βC'- βE loop of FGFR2/3c may be dependent on the presence of FGF bound in the crystal structure
- The flexibility of the βC'- βE modulates ligand binding specificity or promiscuity for FGF.

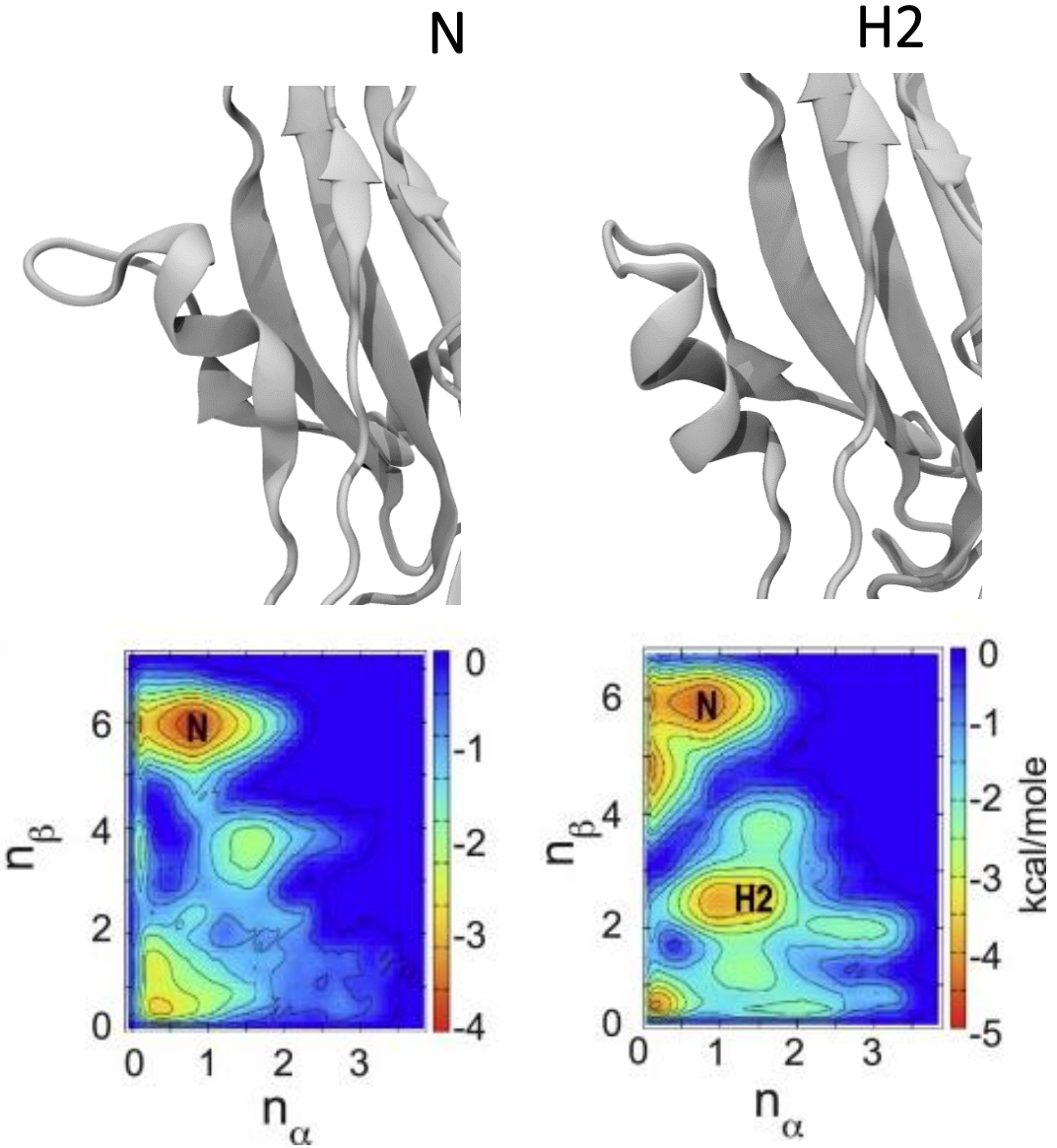
Evotec FGFR Collaboration

- FGFR inhibitor, SSR128129E (**SSR**), binds to the ECD D3 domain and prevents internalisation **allosterically**



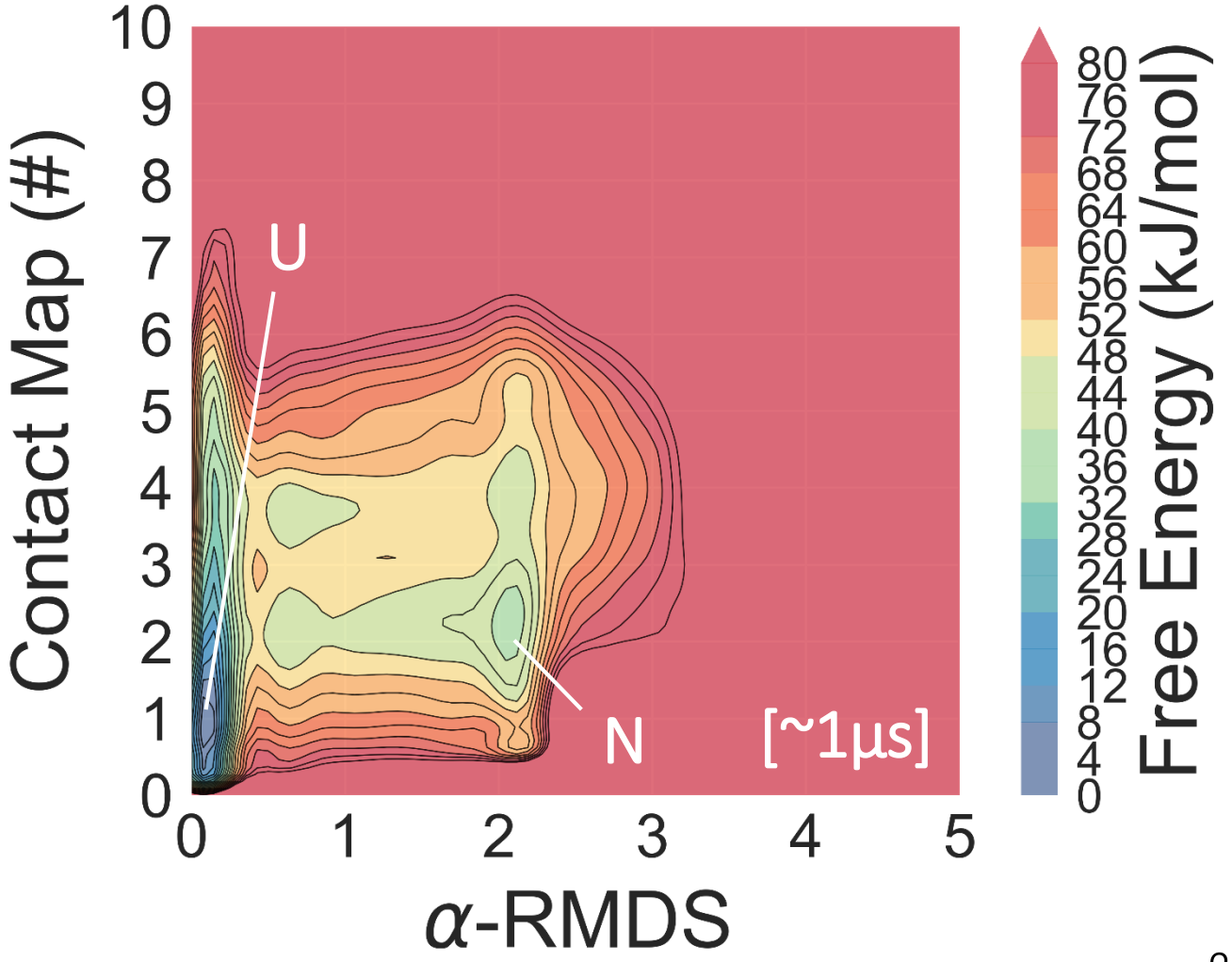
- Initial low-affinity binding step -> stabilise helical conformation of βC'- βE loop (H2)
  ~10 kcal/mol BFE

- Mutations of Y328 reduces helical formation and affects SSR binding, but does not influence FGF binding

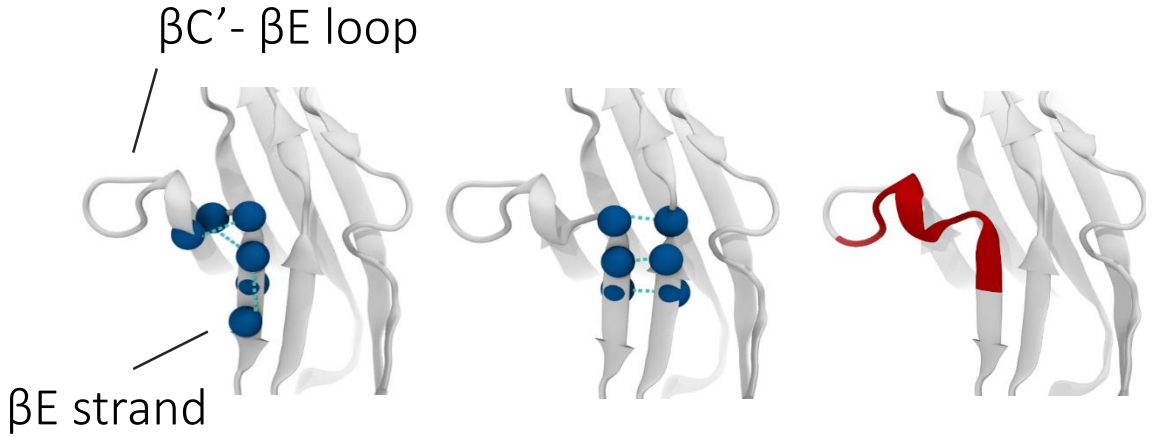


[1] C. Herbert, … F. L. Gervasio, et al. *Cancer Cell*, 23 (4), 2013

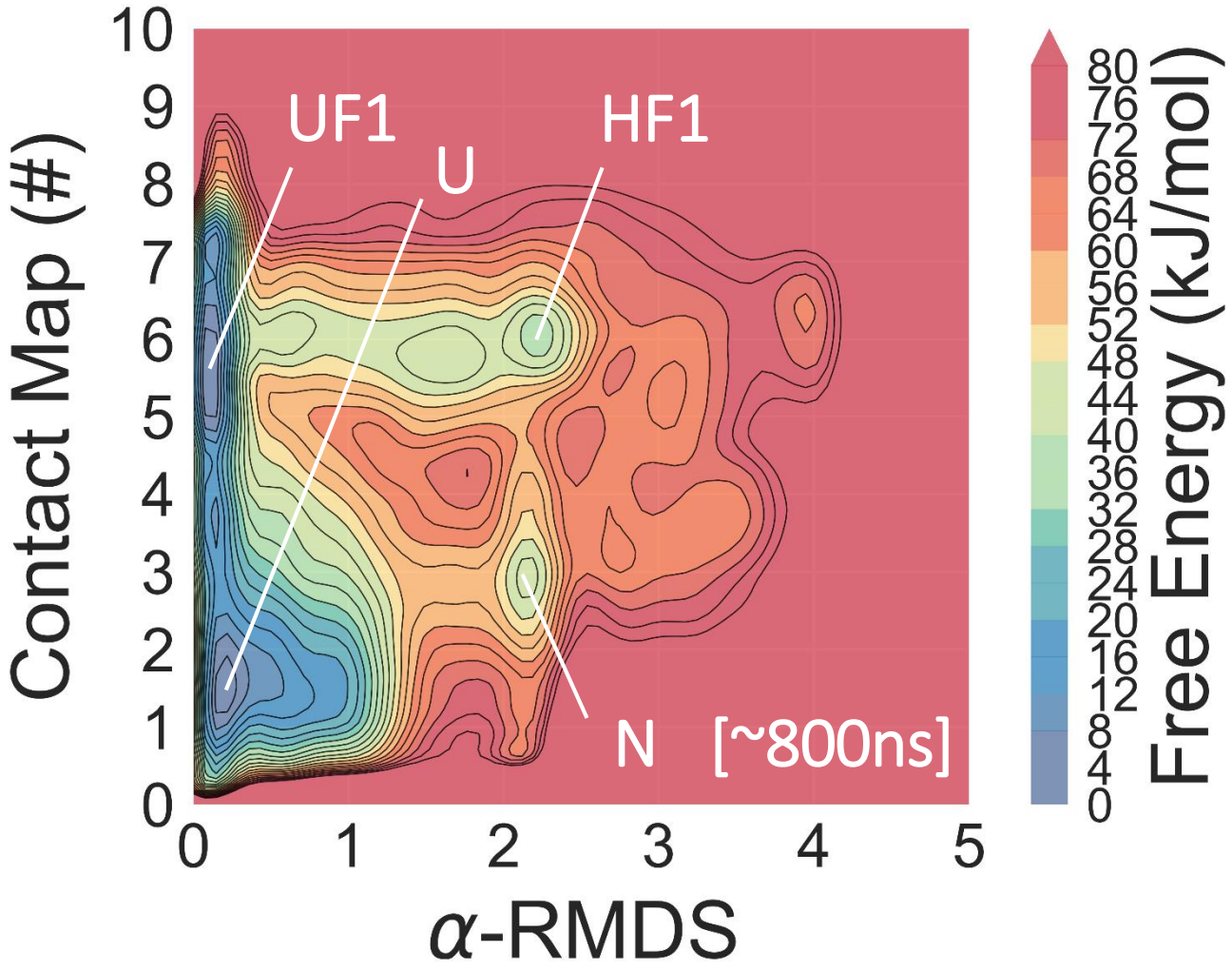
FGFR3c Conformational Free Energy

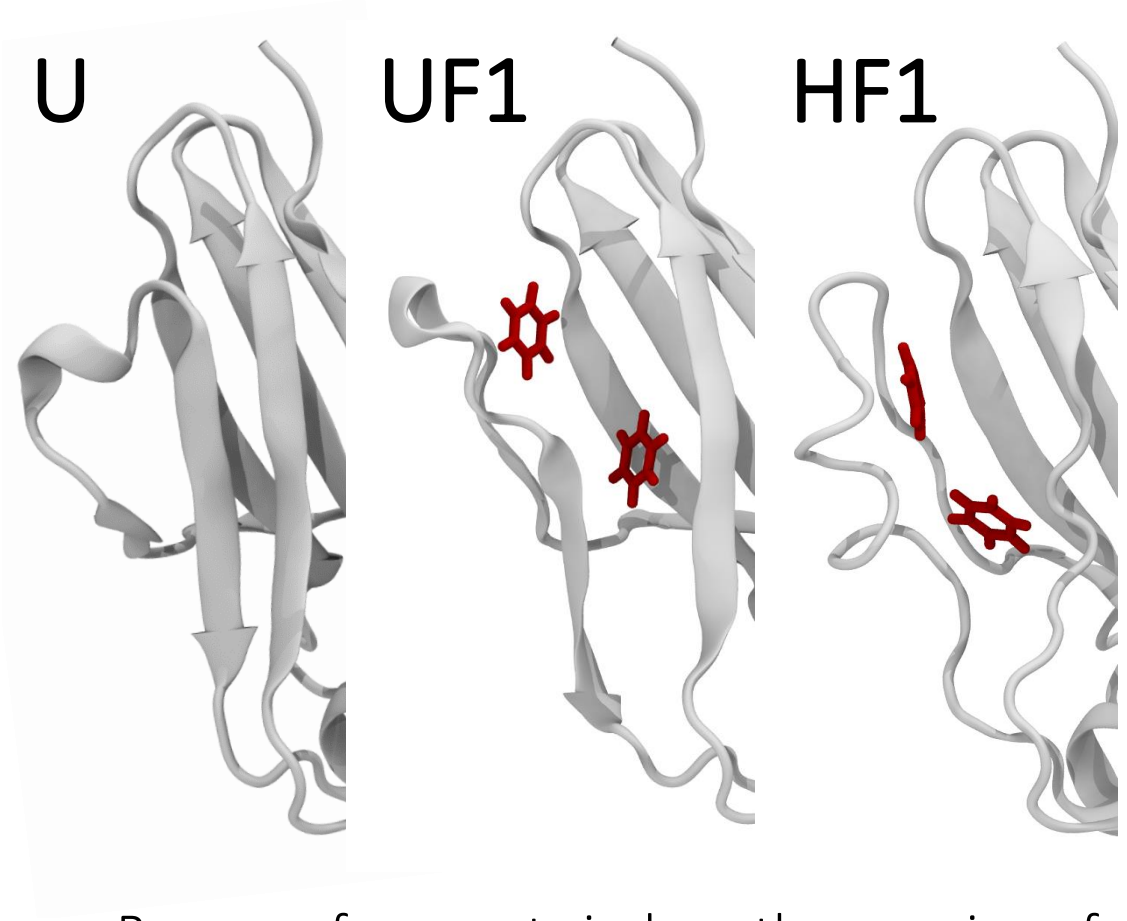


- PTMetaD 5 rex 300-310K Amber FF14SB
- **Collective Variables**:
  - α-helix on βC'- βE loop
  - contact map [Cαs, N, O] in proximity of the SSR binding site (βC'- βE loop, βE strand), X-ray crystal structure as reference



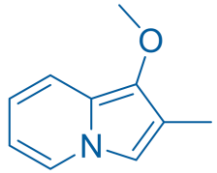
FGFR3c with Benzene

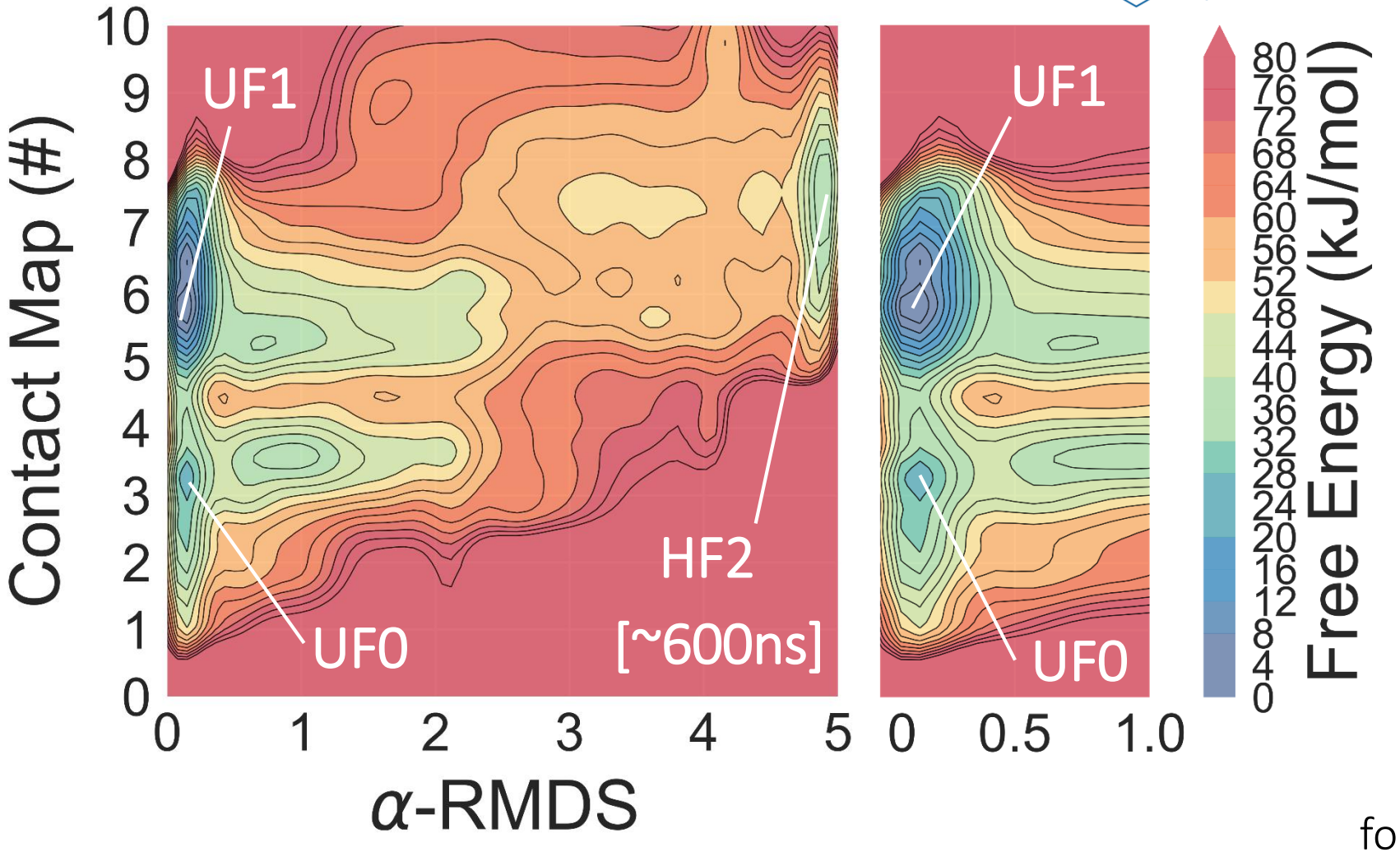




Benzene fragments induce the opening of the pocket but do not seem to increase the the helical content

FGFR3c with SSR-derived Fragments





SSR fragments speed up the opening of the pocket and allow for the formation of longer helices

SWISH

Sampling Water Interfaces through Scaled Hamiltonians

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Understanding Cryptic Pocket Formation in Protein Targets by Enhanced Sampling Simulations

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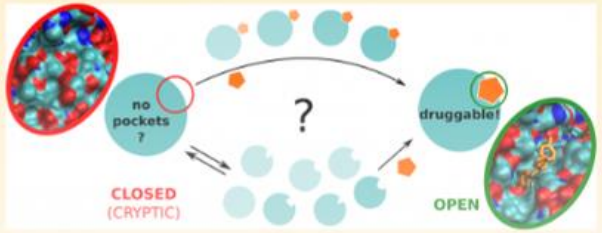
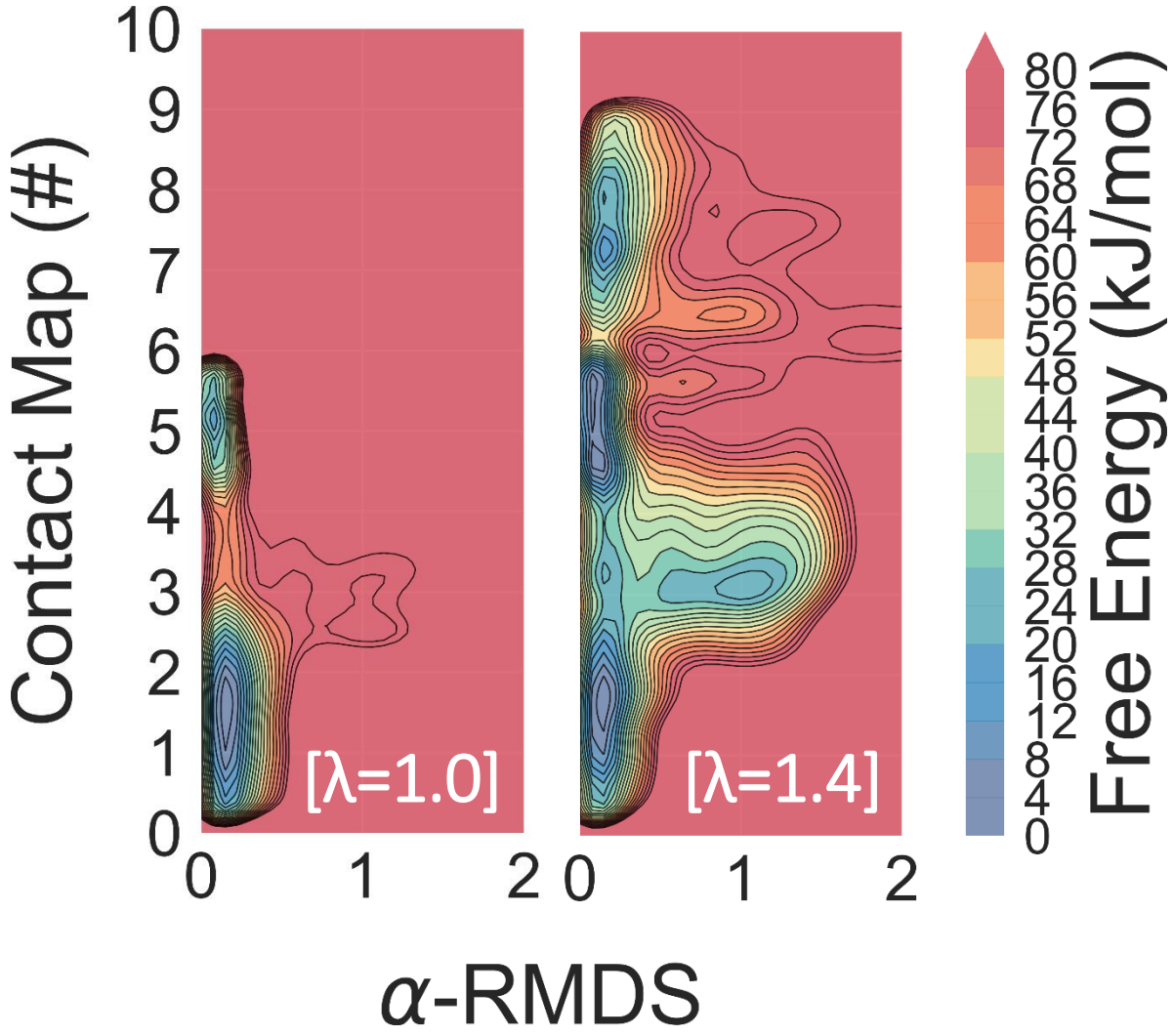
Supporting Information

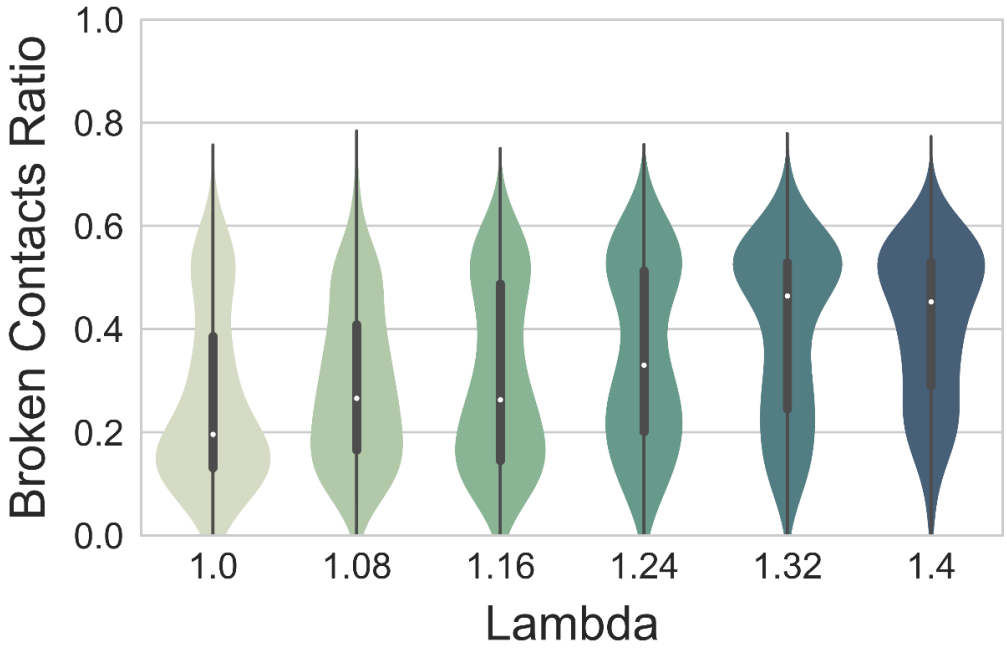
ABSTRACT: Cryptic pockets, that is, sites on protein targets that only become apparent when drugs bind, provide a promising alternative to classical binding sites for drug development. Here, we investigate the nature and dynamical properties of cryptic sites in four pharmacologically relevant targets, while comparing the efficacy of various simulation-based approaches in discovering them. We find that the studied cryptic sites do not correspond to local minima in the computed conformational free energy landscape of the unliganded proteins. They thus promptly close in all of the molecular dynamics simulations performed, irrespective of the force-field used. Temperature-based enhanced sampling approaches, such as Parallel Tempering, do not improve the situation, as the entropic term does not help in the opening of the sites. The use of fragment probes helps, as in long simulations occasionally it leads to the opening and binding to the cryptic sites. Our observed mechanism of cryptic site formation is suggestive of an interplay between two classical mechanisms: induced-fit and conformational selection. Employing this insight, we developed a novel Hamiltonian Replica Exchange-based method "SWISH" (Sampling Water Interfaces through Scaled Hamiltonians), which combined with probes resulted in a promising general approach for cryptic site discovery. We also addressed the issue of "false-positives" and propose a simple approach to distinguish them from druggable cryptic pockets. Our simulations, whose cumulative sampling time was more than 200 μs, help



SWISH and FGFR3c



- **HREX**, scale the non-bonded interactions of solvent with protein apolar Cα and S, **increase** the **water affinity** to **apolar surfaces**

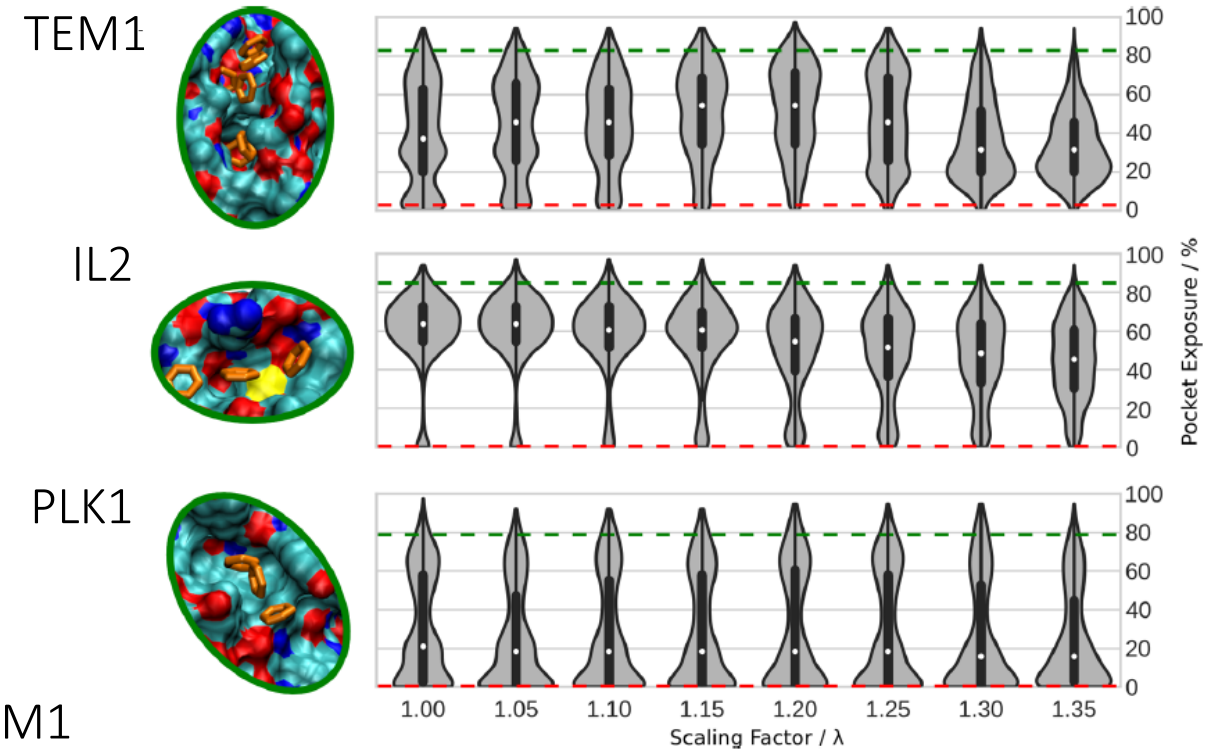


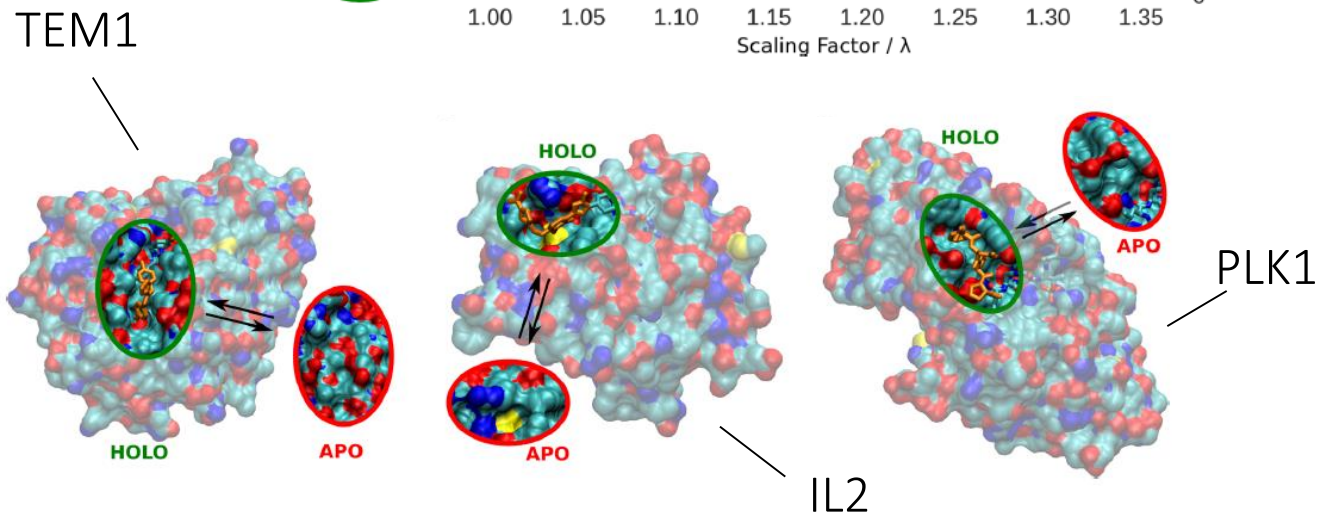
SWISH with Fragments

- **SWISH** sampling helps **exposing** the cryptic sites
  - → **fragments stabilise** them

- Efficient, especially when **induced-fit** effects are present
- **General method**, no need to know where the pocket is a priori
- High λ may lead to **protein unfolding**, ligands could be **out-competed** by water

Codes: Amber, Gromacs + PLUMED





Conclusions

- Cryptic pockets are an attractive opportunity to **expand the number of druggable targets**, and improve on the crystal structure-based techniques. They are more selective (allosteric vs orthosteric)

- Tempering methods and mixed-solvent alone are **not reliable**:
  ↳ **enhanced sampling methods** (Metadynamics, SWISH)

- **SWISH** + suitable **probe molecules** proved to be an efficient strategy and no priori knowledge of the binding site is needed

Aknowledgments

EPSRC
Pioneering research and skills

evotec

PRACE

PLUMED

G. Saladino F. L. Gervasio V. Oleinikovas

Thank you for your attention