Biophysical tools for drug discovery: infectious and conformational diseases, and cancer

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FUNDACIÓN AGENCIA ARAGONESA PARA LA INVESTIGACIÓN Y EL DESARROLLO

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Centro de Investigación Biomédica en Red
Enfermedades Hepáticas y Digestivas

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New diseases (infectious, genetic…)

Neglected and rare diseases

Aggravated known diseases (resistances, low efficacy)

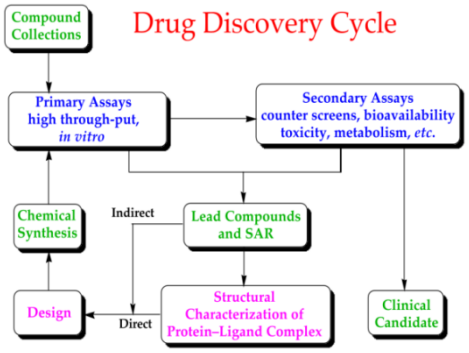
↓

Need for new drugs

↓

Drug Discovery ⟶ Drug Development

- Identification
- Preclinical
- Clinical



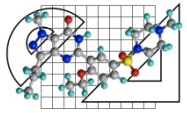
Drug Discovery and Development

Disease

Target Molecule

in vitro Assays

Experimental and Computational Screening



Optimization

Preclinical Assays

Clinical Trials

Molecular Screening

Biophysical information
Structural stability
Biological interactions
Functional information

Selection of target variant

Selection of experimental conditions

Selection of chemical library

Specific assay vs. general assay

Design of screening procedure (assay development)

Validation of screening procedure

A molecule inducing an effect must bind to the target
Methods for detecting binding are required

A molecule inducing an effect must alter target properties (structure, function, behavior)
Methods for detecting structure, function, or behavior are required

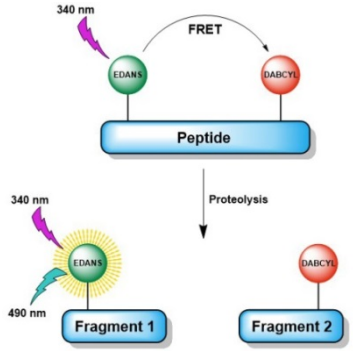
Ligand binding to a protein is a prerequisite for the ligand to modulate the biological activity of that protein. However, binding is not necessarily linked to having a modulatory effect.

Phenotypic assays are needed in order to assess the potential bioactivity of selected compounds

Enzymatic Activity Assay

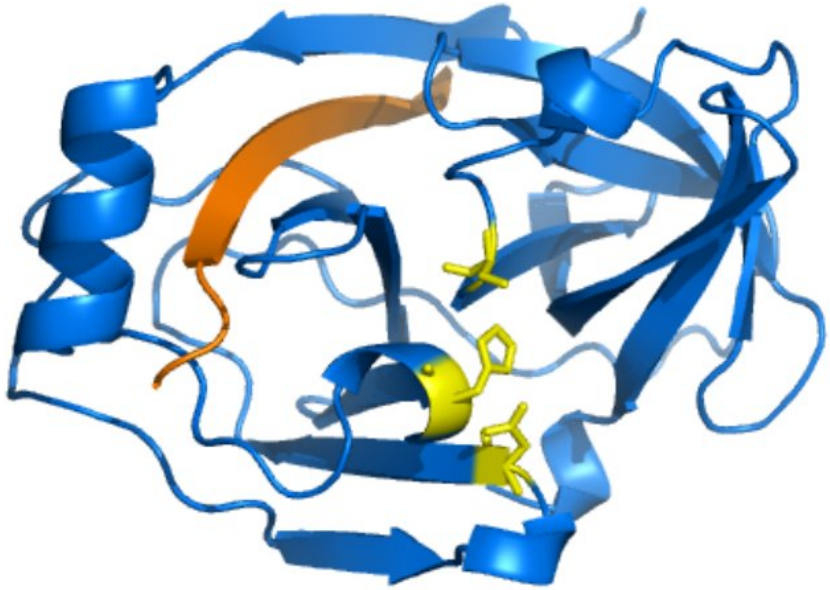
Equipment	**Plate reader, real-time qPCR**
Measurement	**Extrinsic probe (e.g., FRET)**
Output	**Enzymatic activity (continuous/end-point)**
Caveats	**Influence of target concentration** **Influence of substrate concentration** **Influence of substrate affinity** **Influence of inhibitor type** **Aggregation-prone ligand** **Optically active ligand** **Ligand interacting unspecifically**

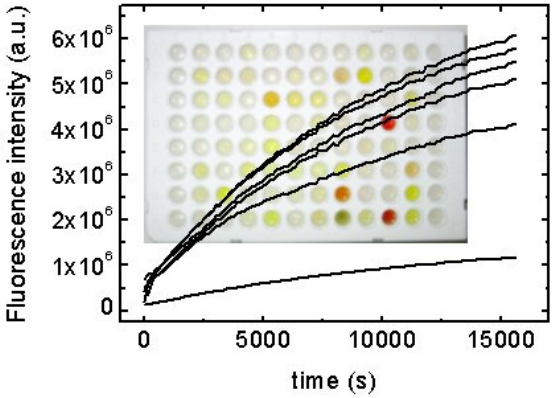
HTS by Enzymatic Activity

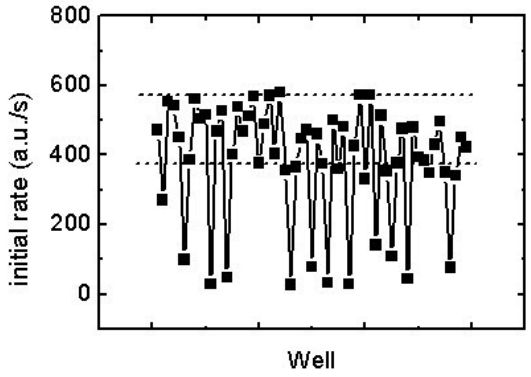


FRET Protease Substrate

Hepatitis C NS3 Protease

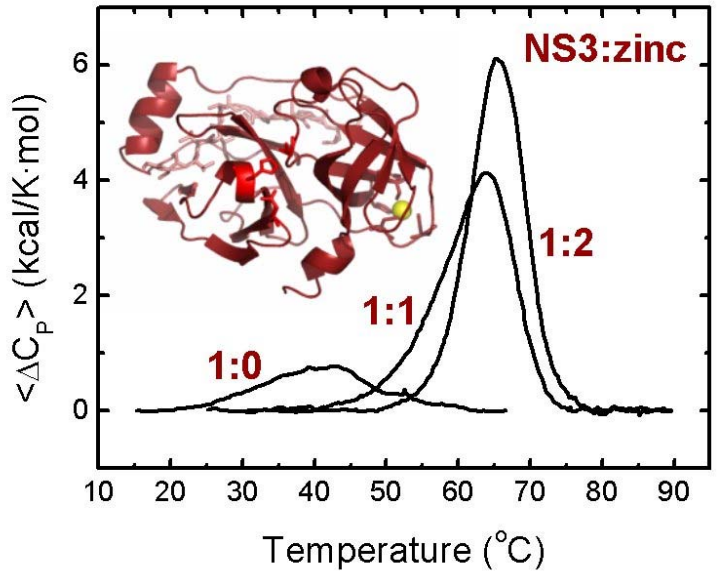






If a ligand binds preferentially to a certain protein conformational state, it stabilizes such conformational state

Therefore, we can find ligands for a given protein by searching for molecules that increase the conformational stability of the protein



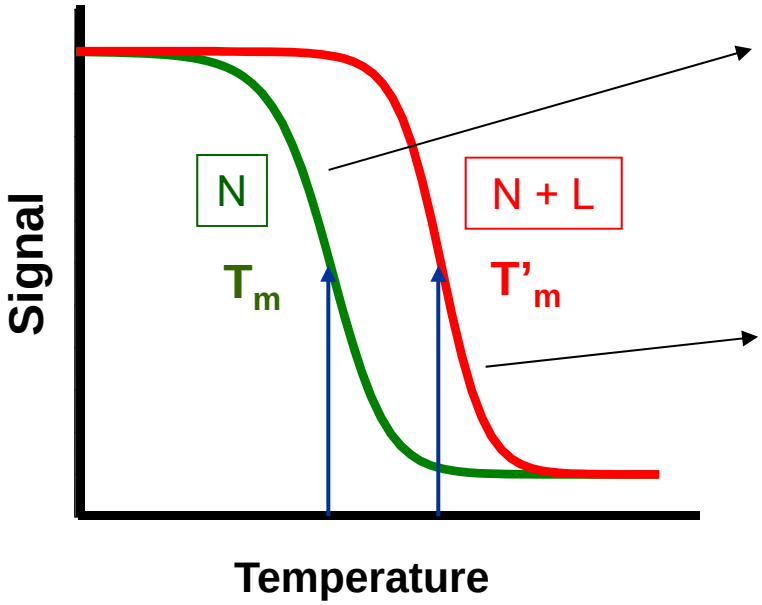
Thermal-Shift Assay (TSA)

Equipment	**Plate reader, real-time qPCR**
Measurement	**Intrinsic fluorescent probe (Trp, cofactor)** **Extrinsic fluorescent probe (ANS, SyproOrange)**
Output	**Mid-denaturation temperature**
Caveats	**Ligand stabilizing protein unfolded state** **No affinity ranking** **Ligand interacting unspecifically** **Aggregation-prone ligand** **Fluorescent ligand**

Velazquez-Campoy et al. Current Drug Targets 2016 17:1492-1505

Thermal-Shift Assay (TSA)

$$N + L \overset{K_L}{\longleftrightarrow} NL \overset{K}{\longleftrightarrow} U$$



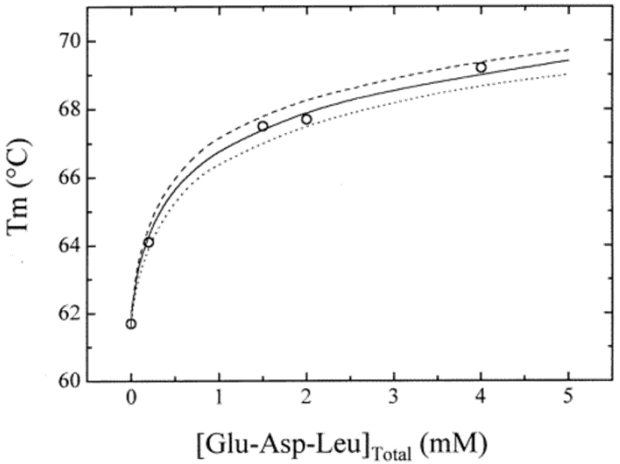
$$K = \frac{[U]}{[N]}$$

$$\Delta G = -RT\ln K$$

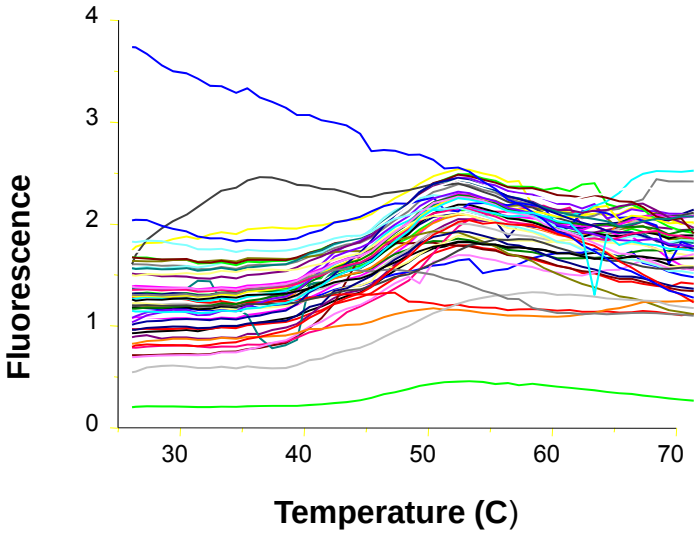
$$K^{app} = \frac{[U]}{[N]+[NL]} = \frac{K}{1+K_L[L]}$$

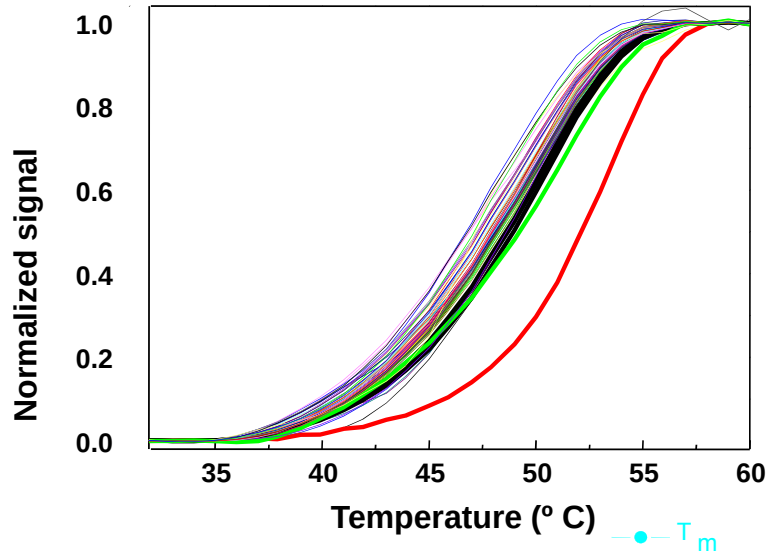
$$\Delta G^{app} = -RT\ln K^{app} = \Delta G + RT\ln(1+K_L[L])$$

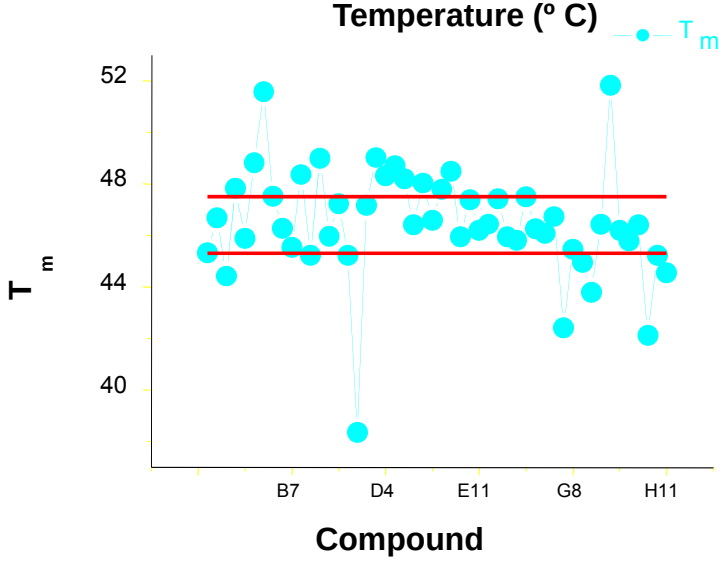
$$\Delta T_m = \frac{RT_m^2}{\Delta H(T_m)}\ln(1+K_L[L])$$



HTS by Thermal-Shift Assay (TSA)



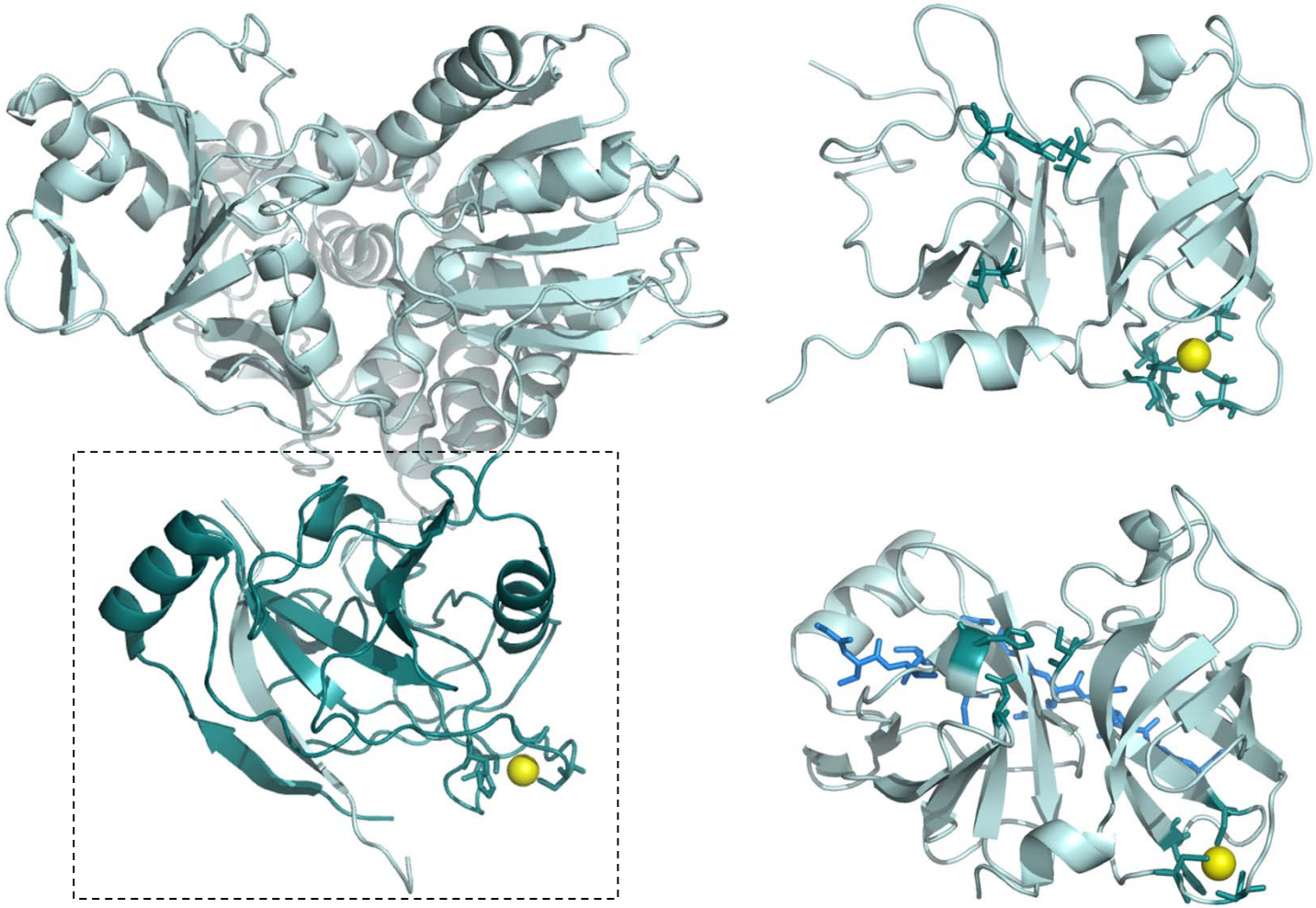




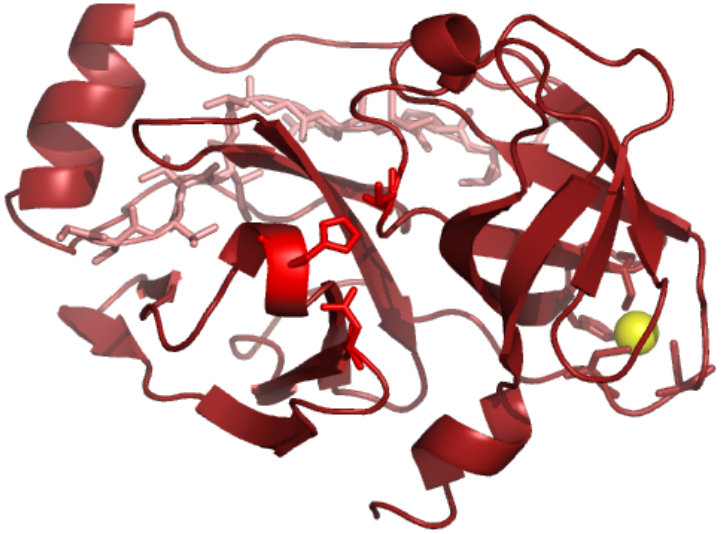
Pantoliano et al. *Journal of Biomolecular Screening* 2001 6:429-440

Niesen et al. *Nature Protocols* 2007 2:2212-2221

NS3 Protein from Hepatitis C Virus



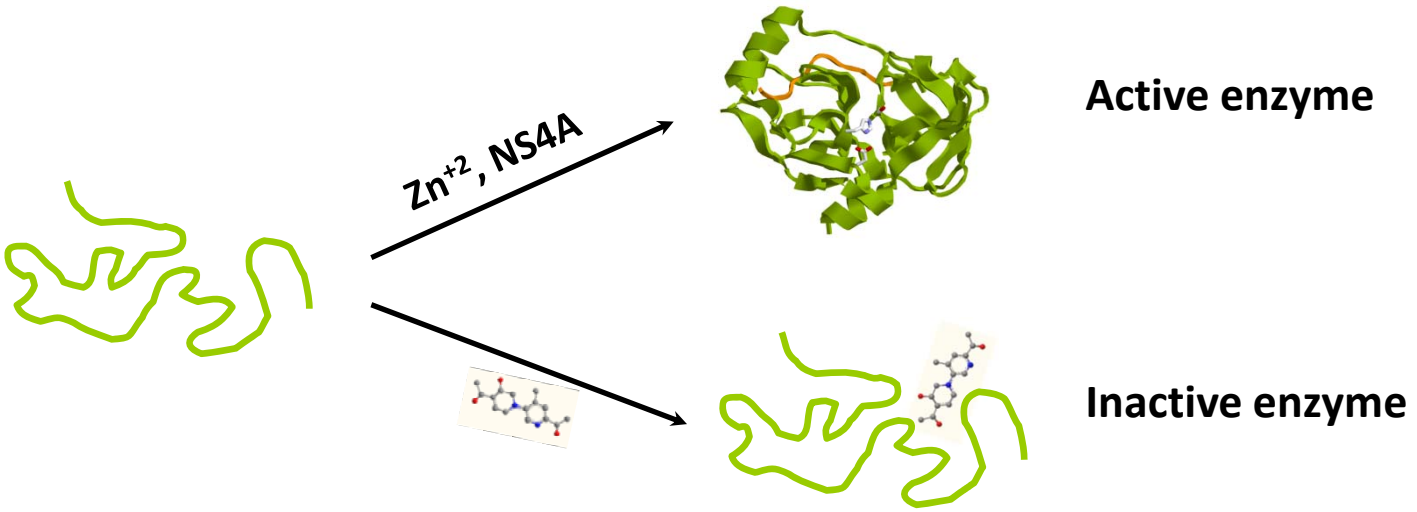
Structural Zn^{+2} Binding Site in NS3 Protease



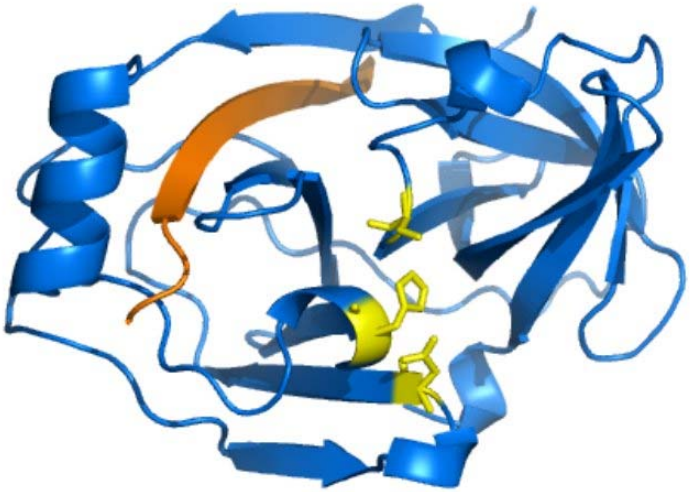
Structural role (CCCH)

Tetra-coordinated CXC/CXXXH

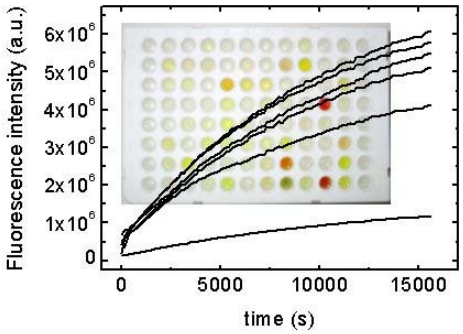
Stabilization of both domains

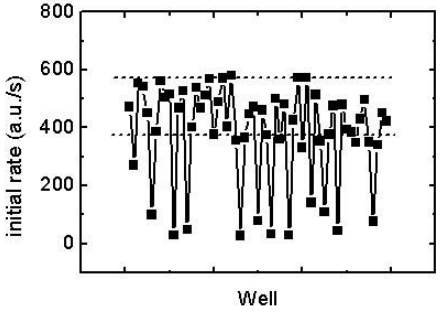

Zn^{+2}, NS4A
Active enzyme
Inactive enzyme

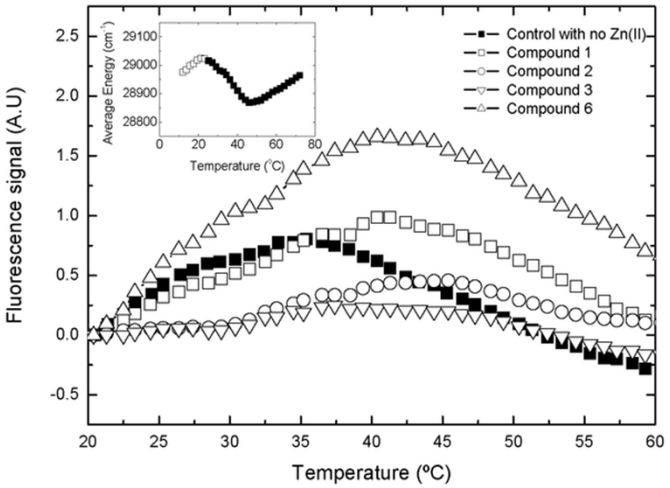
NS3 Protease



FRET Protease Substrate

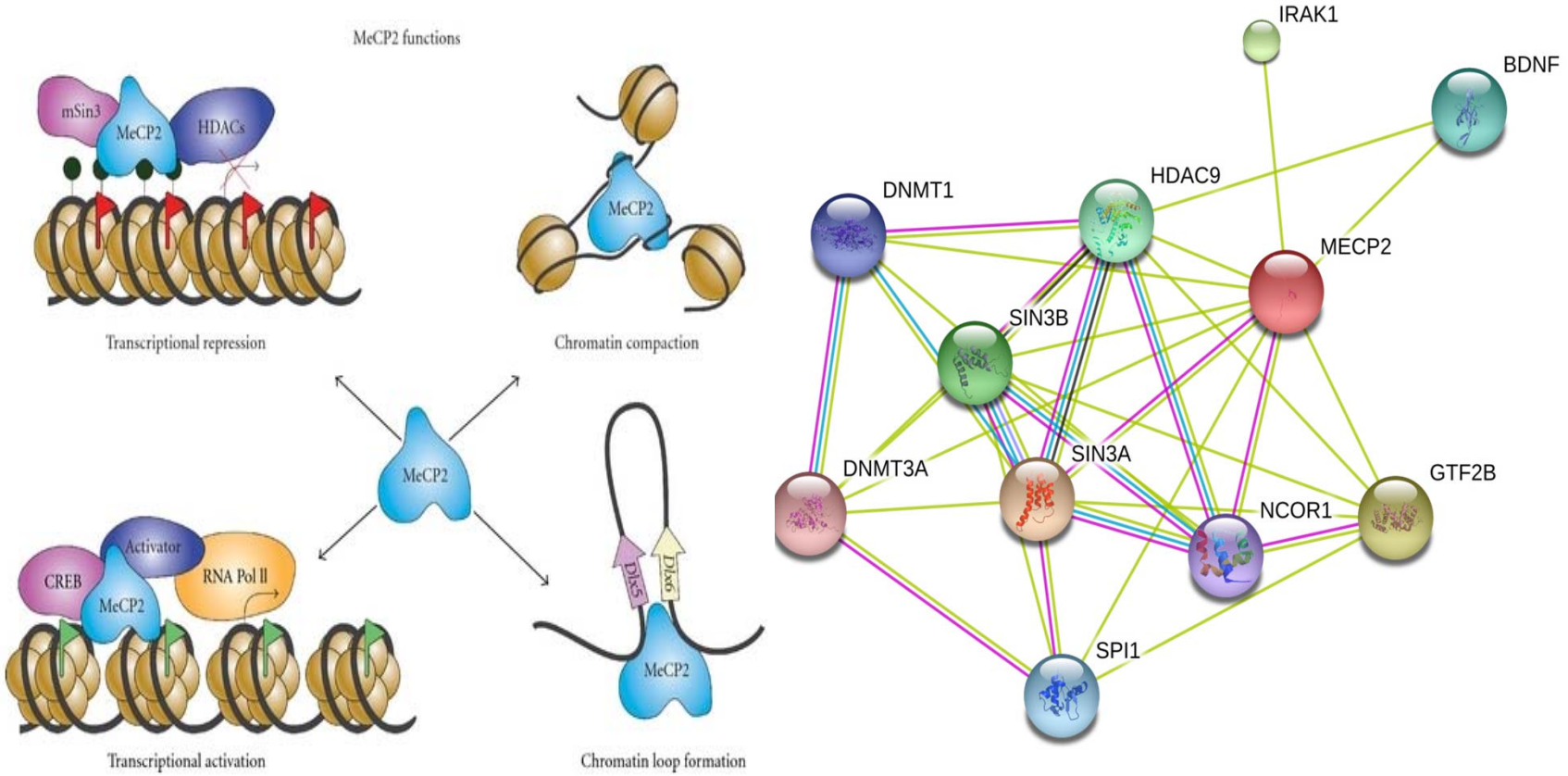




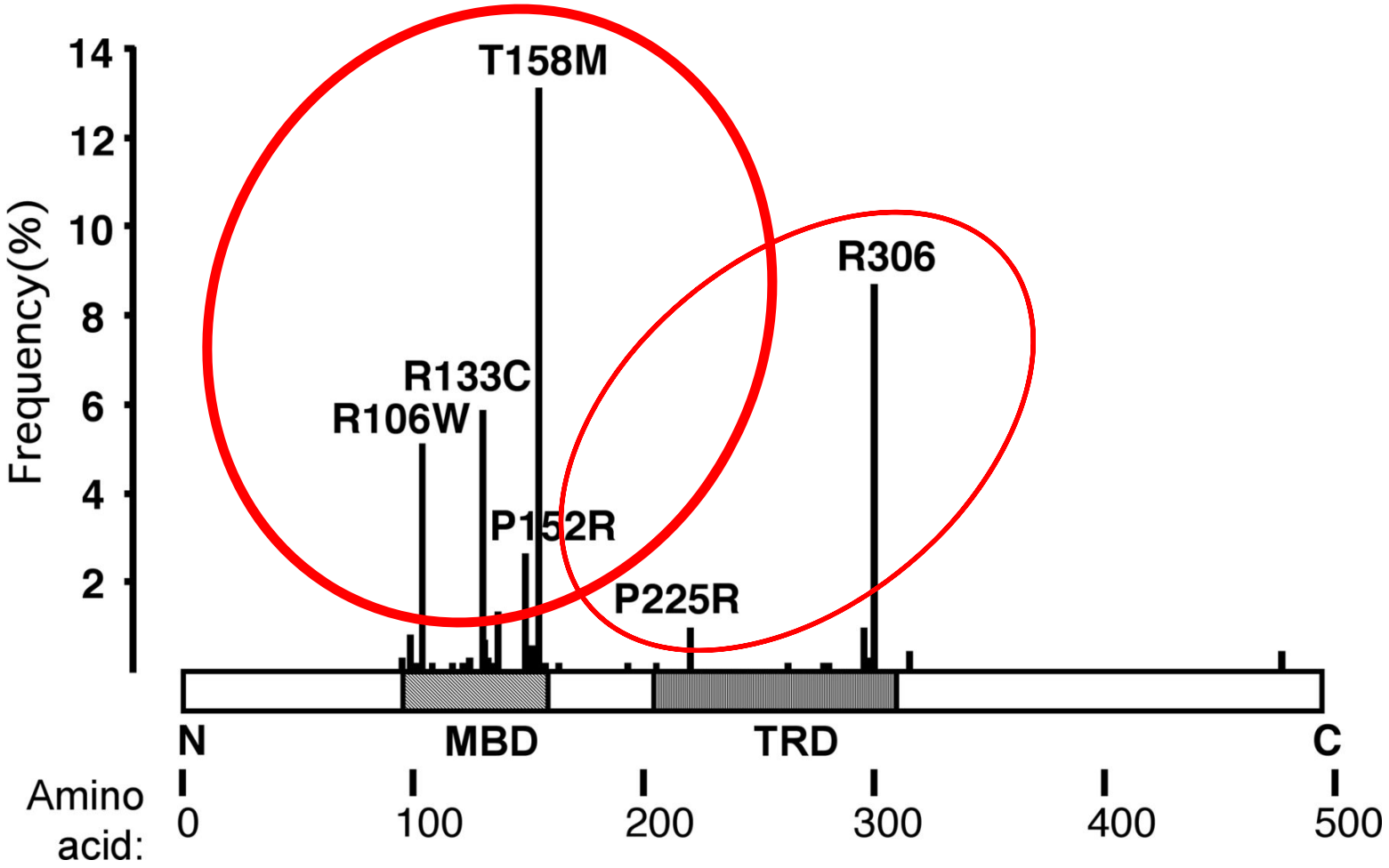


Abian et al. *PLoS ONE* 2013 8:e69773

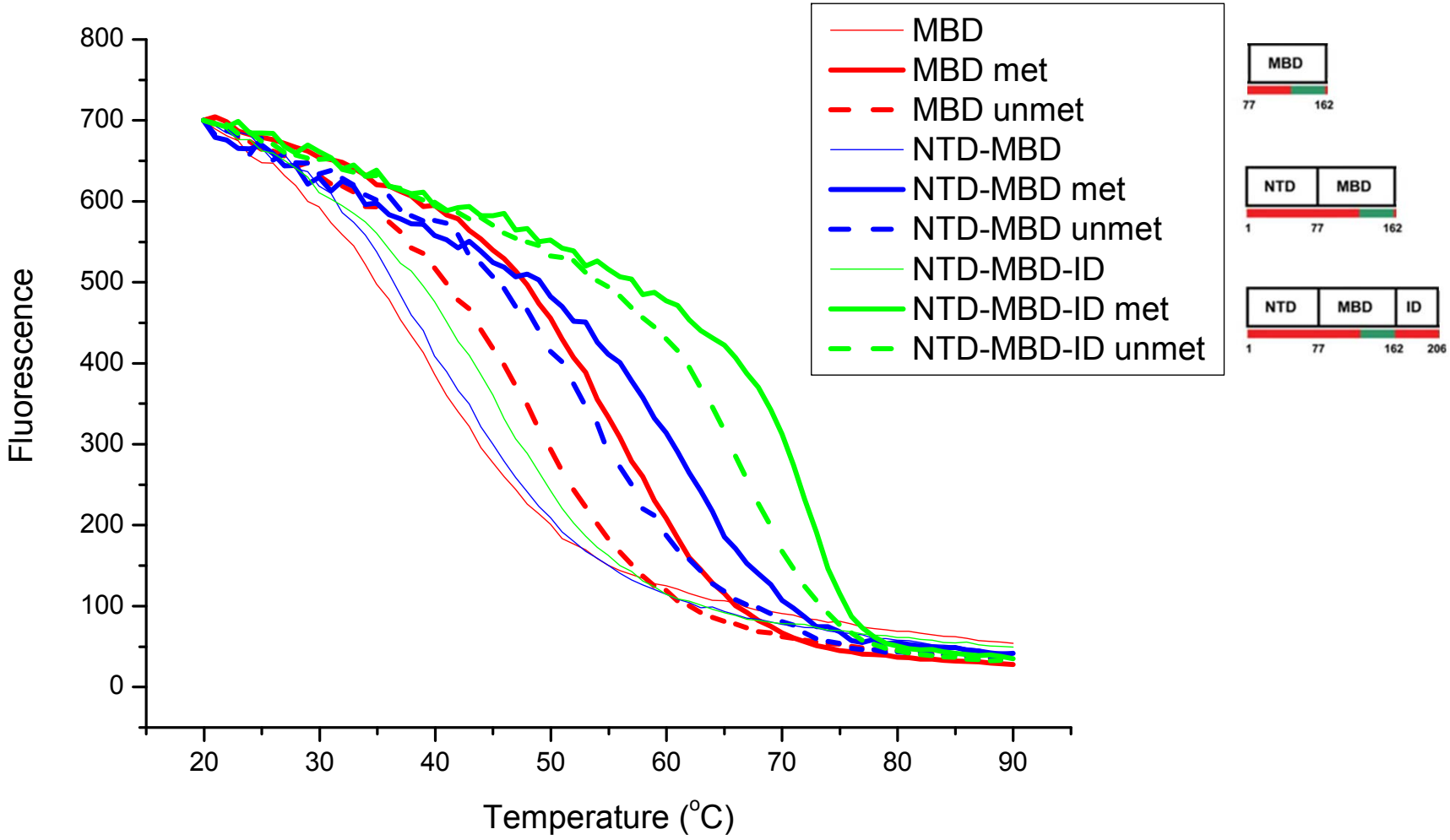
MeCP2: Methyl-CpG Binding Protein 2



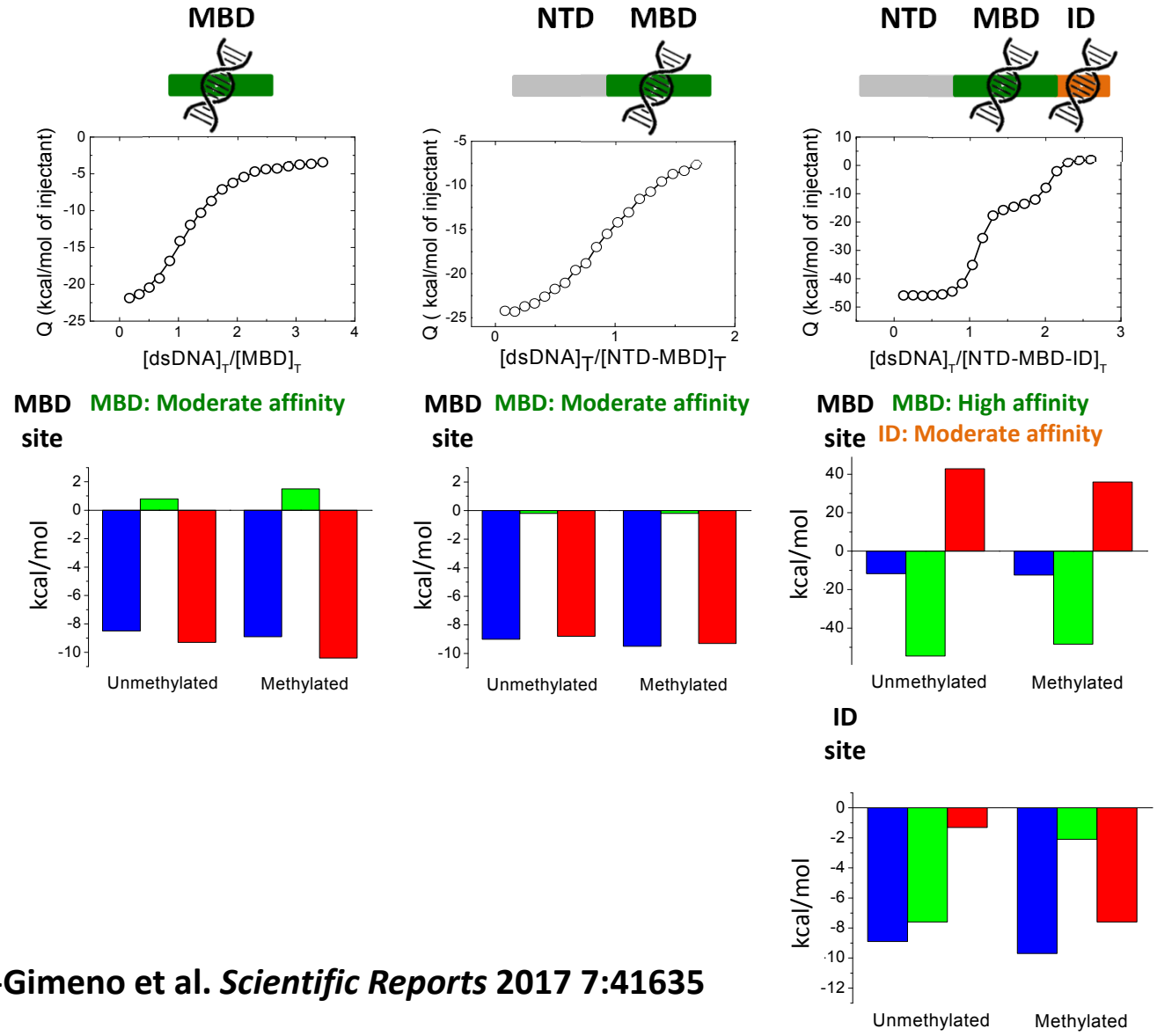
MeCP2 Mutations



MeCP2 Stability

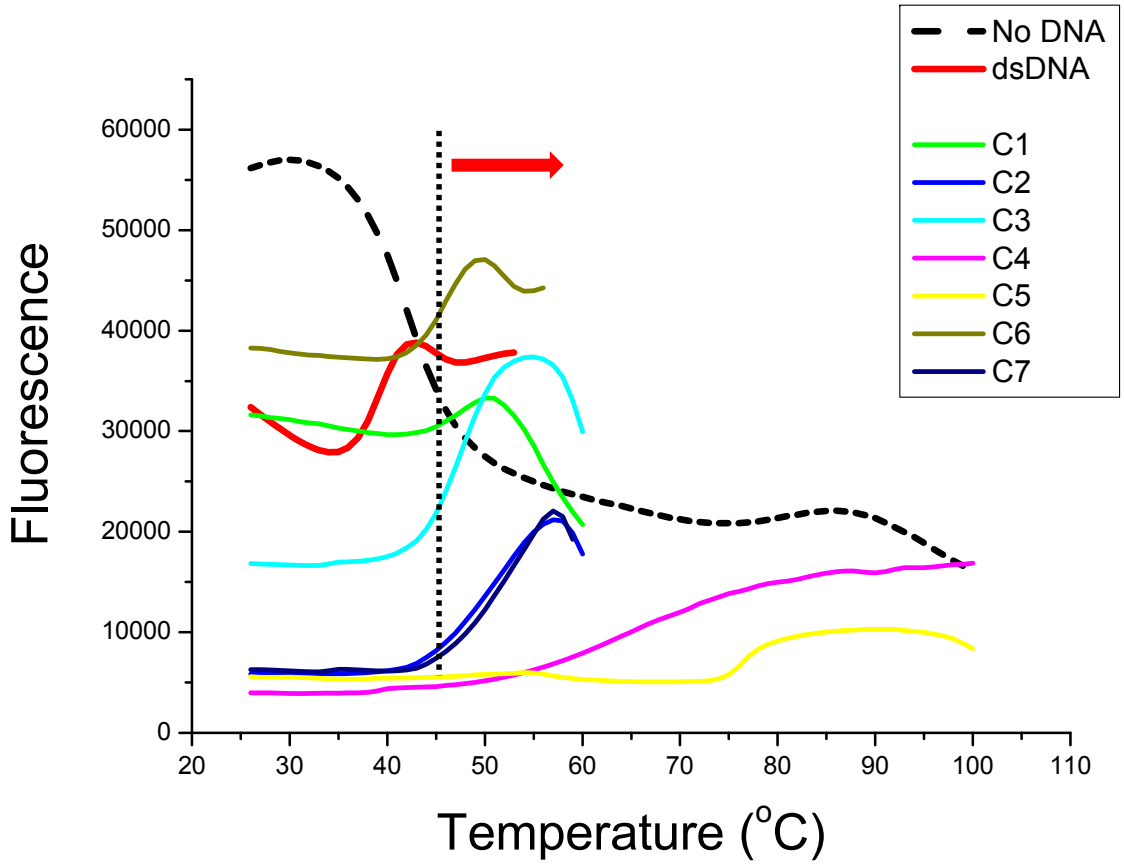


Protein-DNA interaction



Claveria-Gimeno et al. *Scientific Reports* 2017 7:41635

Compound Identification



Pancreatic cancer is the most lethal of all types of cancer

- 6% 5-year survival rate
- 6 months median survival
- 4th leading cause of cancer-related deaths in the US

Pancreatic ductal adenocarcinoma (PDAC)

- Most common (>95%) type of pancreatic malignancy
- Therapeutic interventions include: surgical resection, radiation therapy, chemotherapy, immunotherapy
- Lack of specific symptoms and limitations in diagnostic methods enable cancer progression

Nuclear protein 1 (NUPR1) is a protein over-expressed and involved in PDAC development

- NUPR1 overexpression during acute pancreatitis and precancerous lesions
- NUPR1 expression controls pancreatic cancer cell migration, invasion, and adhesion
- NUPR1 involved in apoptosis, DNA-damage response, and chemoresistance (stress-protecting effect)

NUPR1 is a potential target for drug discovery

Goruppi & Iovanna. *Journal of Biological Chemistry* 2010, 285:1577-1581
Sandi et al. *Journal of Cellular Physiology* 2011, 226:3442-3451
Cano et al. *Journal of Cellular Physiology* 2011, 226:1439-1443
Hamidi et al. *Journal of Clinical Investigation* 2012, 122:2092-2103

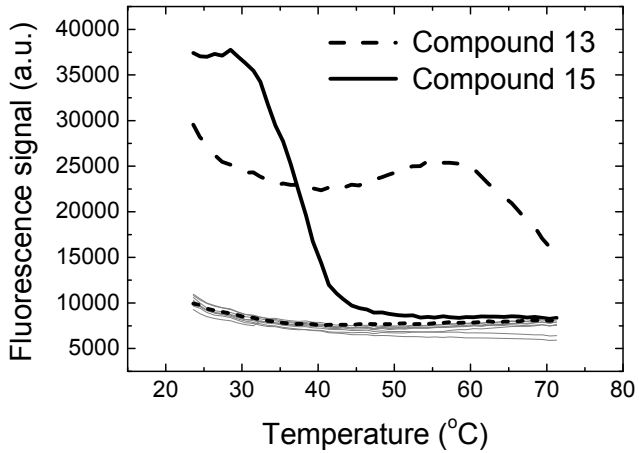
Challenges

- PPIs are difficult to block with small molecules
- NUPR1 lacks a well-defined structure
- NUPR1 is a multifunctional protein

Molecular Screening

Ligand-induced stabilization against thermal denaturation (TSA)

Velazquez-Campoy et al. *Current Drug Targets* 2016, 17:1492-505

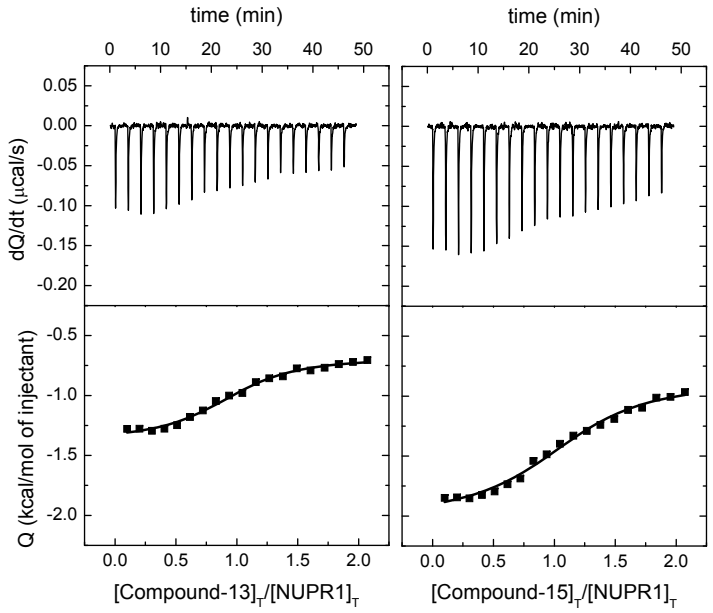


Compounds interacting with NUPR1 were selected as those altering NUPR1 thermal denaturation profile

15 compounds have been identified as NUPR1 binding candidates

Biophysical Assays (Target Engagement)

Direct interaction of selected compounds with NUPR1 was assessed experimentally (calorimetry, spectroscopy, nuclear magnetic resonance)

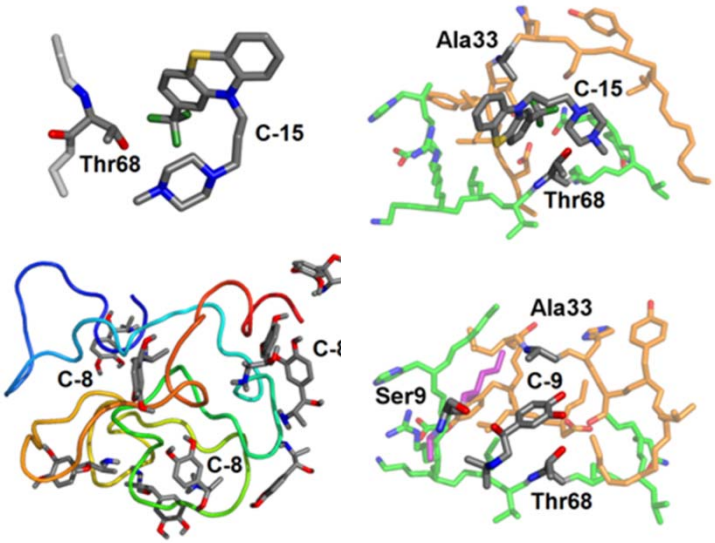


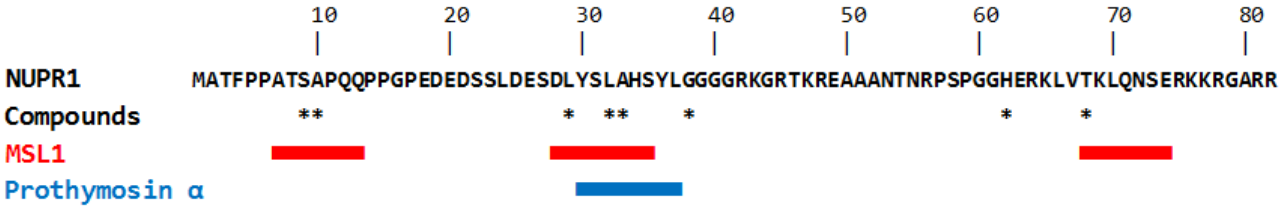
	Compound	K_d (µM)
1	Terfenadine	5.0
2	Fluphenazine	2.0
3	Caffeic acid	2.0
4	Reserpine	3.2
5	(-)-Isoproterenol	3.9
6	Flunarizine	3.1
7	Halofantrine	3.3
8	Levonordefrin	1.5
9	(+)-Isoproterenol	4.0
10	Pheniramine maleate	4.3
11	Terconazole	n.d.
12	Dihydroergotoxine mesylate	4.0
13	Benzethonium	3.6
14	Chlortetracycline	1.5
15	Trifluoperazine	5.2

NMR and Computational Simulations

Direct interaction of selected compounds with NUPR1 was assessed computationally (molecular dynamics simulations)

Selected compounds interact with key NUPR1 regions interacting with protein binding partners



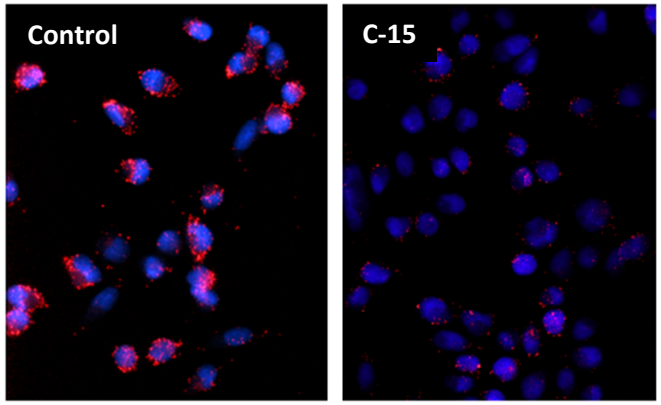


Cell-Based Assays

Direct interaction of selected compounds with NUPR1 was assessed in cell-based assays

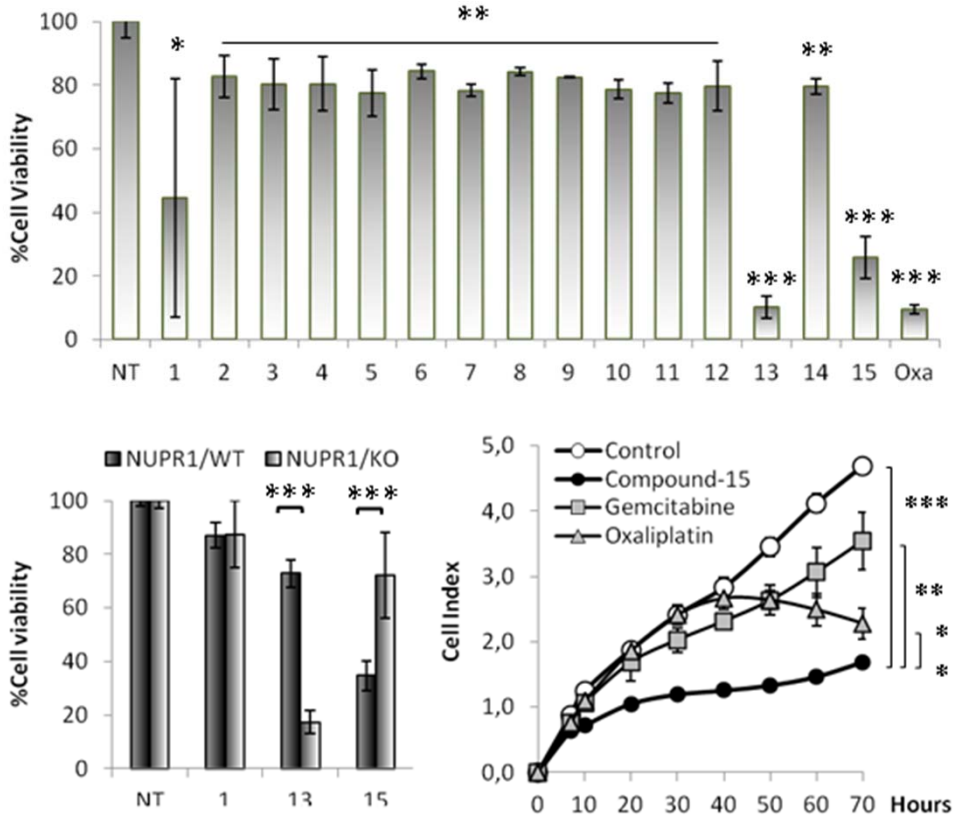
Colony formation, wound healing, interaction with protein partners, and compound efficacy was determined in PDAC-derived cell-based assays

Proximity Ligation Assay was employed for assessing NUPR1-MSL1 interaction and evaluating the inhibitory effect of selected Compounds



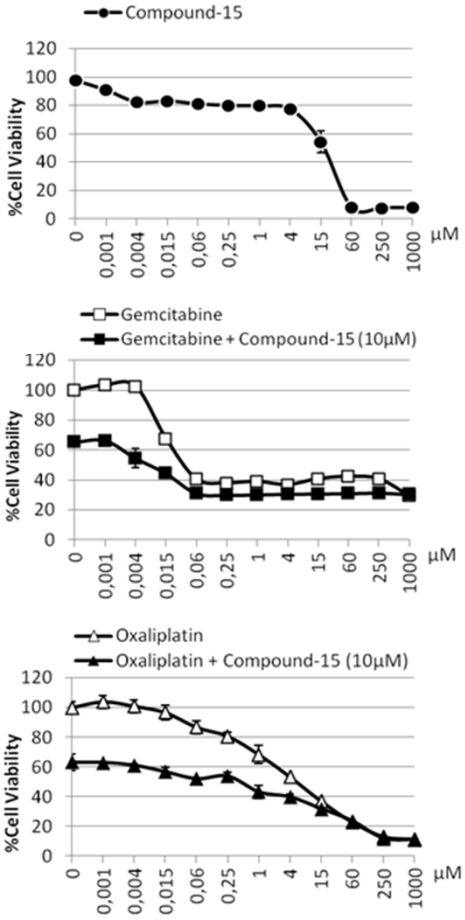
Cell-Based Assays

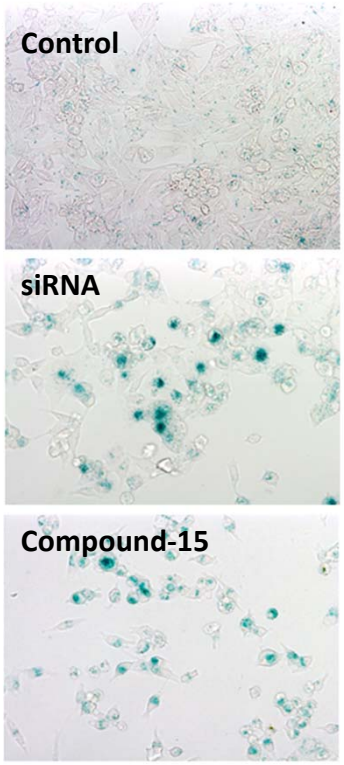
Compound-15 inhibits cell viability in a NUPR1-dependent manner



Cell-Based Assays

Compound-15 decreases chemo-resistance in pancreatic cancer cell line

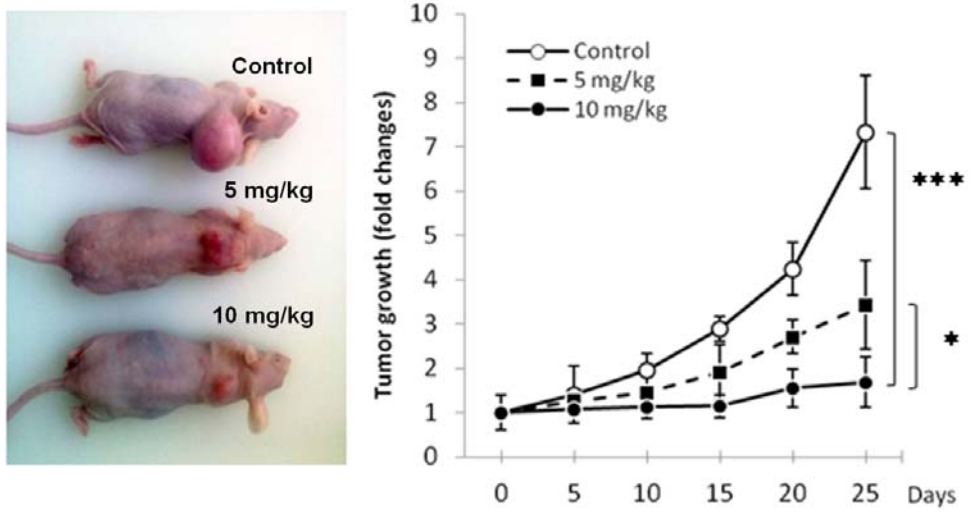




Compound-15 induces senescence, counteracting cell adaptive responses triggered by NUPR1

In Vivo Assays

Compound efficacy was determined in *in vivo* assays on xenografted PDAC-derived human cells in mice



Compound-15 promotes complete arrest of tumor development

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Thanks for your attention!!!

"Una manera de hacer Europa"