

Spectroscopic and Structural Characterization of FXR Agonists and Antagonists

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Farnesoid X Receptor (FXR)

Metabolic Syndrome

- Central Obesity
- High blood pressure
- High triglycerides
- Low HDL-cholesterol
- Insulin resistance



FXR

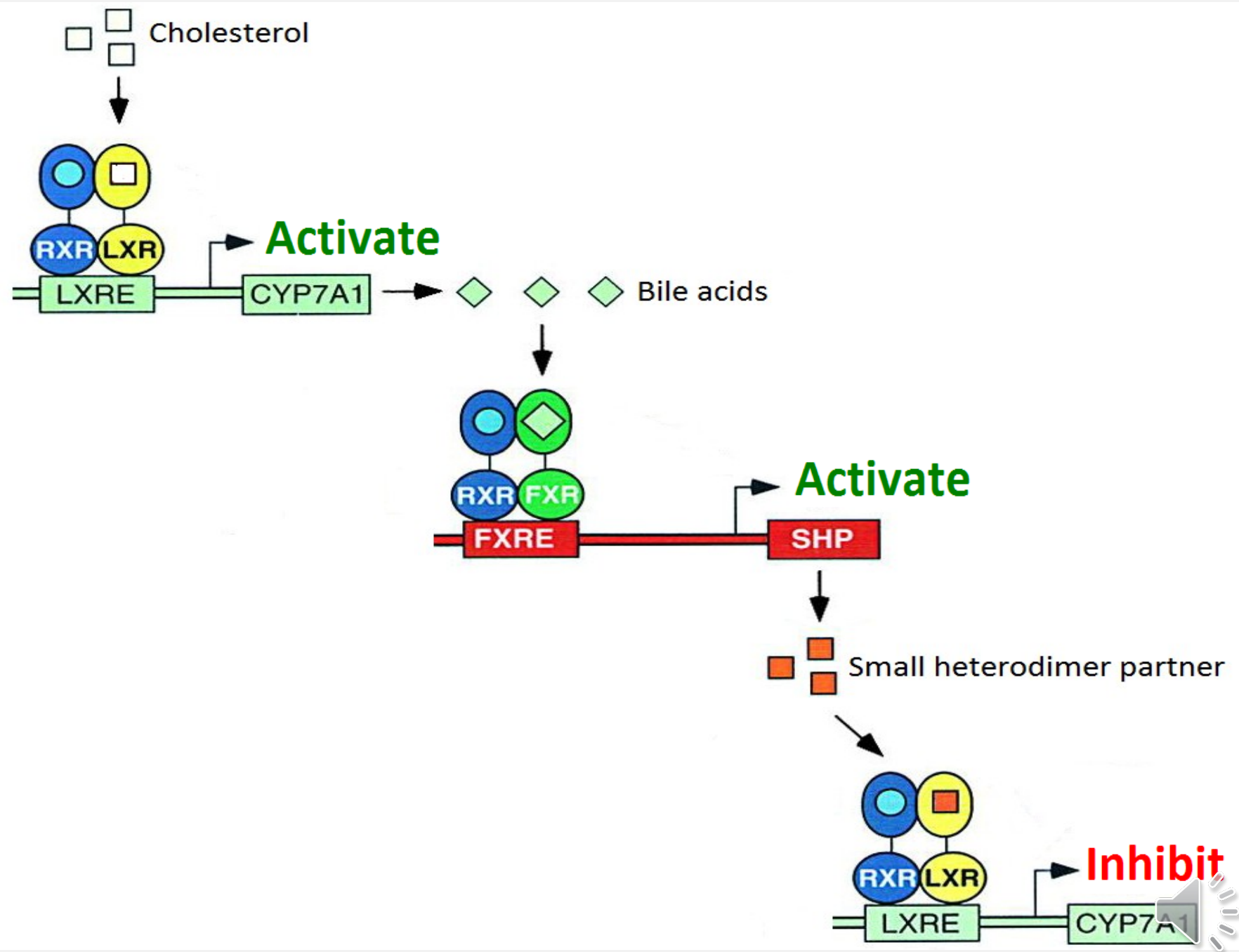
- Glucose homeostasis
- Lipoprotein metabolism
- Bile acid homeostasis
- Liver regeneration



FXR Gene Regulations

<u>Organ</u>	<u>Function</u>	<u>Gene Product</u>	<u>Regulatory Effect</u>
Liver	Bile Acid Metabolism	BSEP	Induced
		MRP2	Induced
		MDR3	Induced
		SHP	Induced
		BAT	Induced
		BACS	Induced
		NTCP	Repressed
		CYP7A1	Regulated
		CYP8B1	Repressed
		Mrp4	Induced
	Lipid Metabolism	SREBP-1c	Repressed
		ApoAI	Repressed
		ApoB	Repressed
		ApoCII	Repressed
		ApoCIII	Repressed
		PDK4	Induced
	Glucose Metabolism	PEPCK	Induced or Repressed
		Glucose-6-phosphatase	Induced
Small Intestine	Bile Acid Transport	IBABP	Induced
		FGF15	Induced
		ASBT	Induced
Kidney	Bile Acid Transport	OST	Induced
		MRP2	Induced
		ASBT	Repressed
Heart and Blood Vessels	Vascular Function	Endothelin1	Repressed
		ICAM1	Induced
		VCAM1	Induced
		DDAH1	Induced





Purpose

Development of a biophysical assay that
can be used to facilitate the direct
identification of agonist and antagonist
ligands of FXR



Hypothesis

Ligands will have spectroscopic characteristics consistent with whether they exhibit agonist or antagonist properties

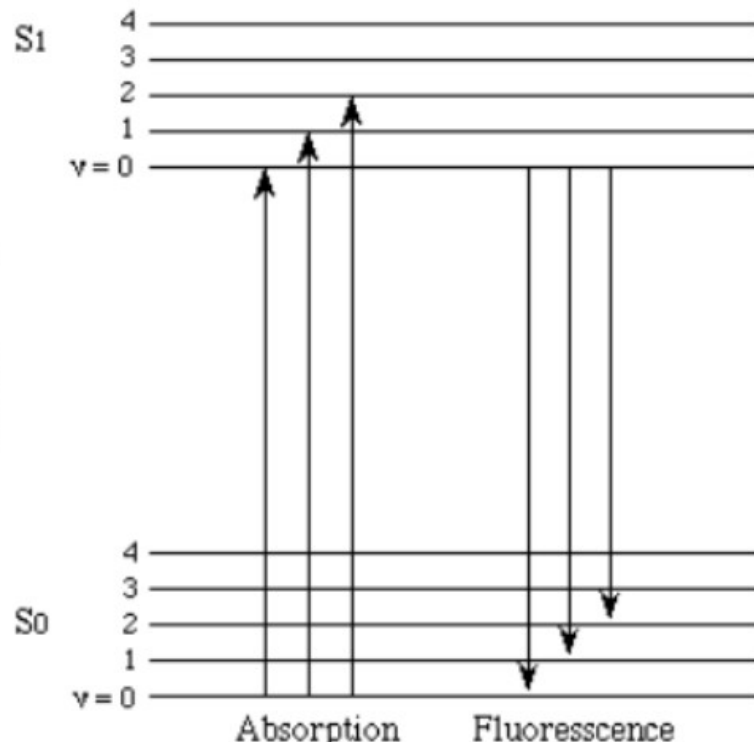
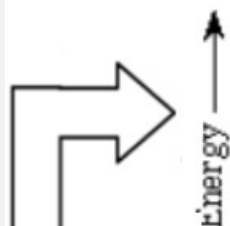


Method: Fluorescence Spectroscopy



Solution:

- Buffer
- FXR
- Ligand
 - GW4064
 - CDCA
 - GG
 - XN



Acrylamide Titration

- Acrylamide is a quenching molecule
- FXR is made up of 227 amino acids, two of which are tryptophan (Trp)

In the core of
the protein



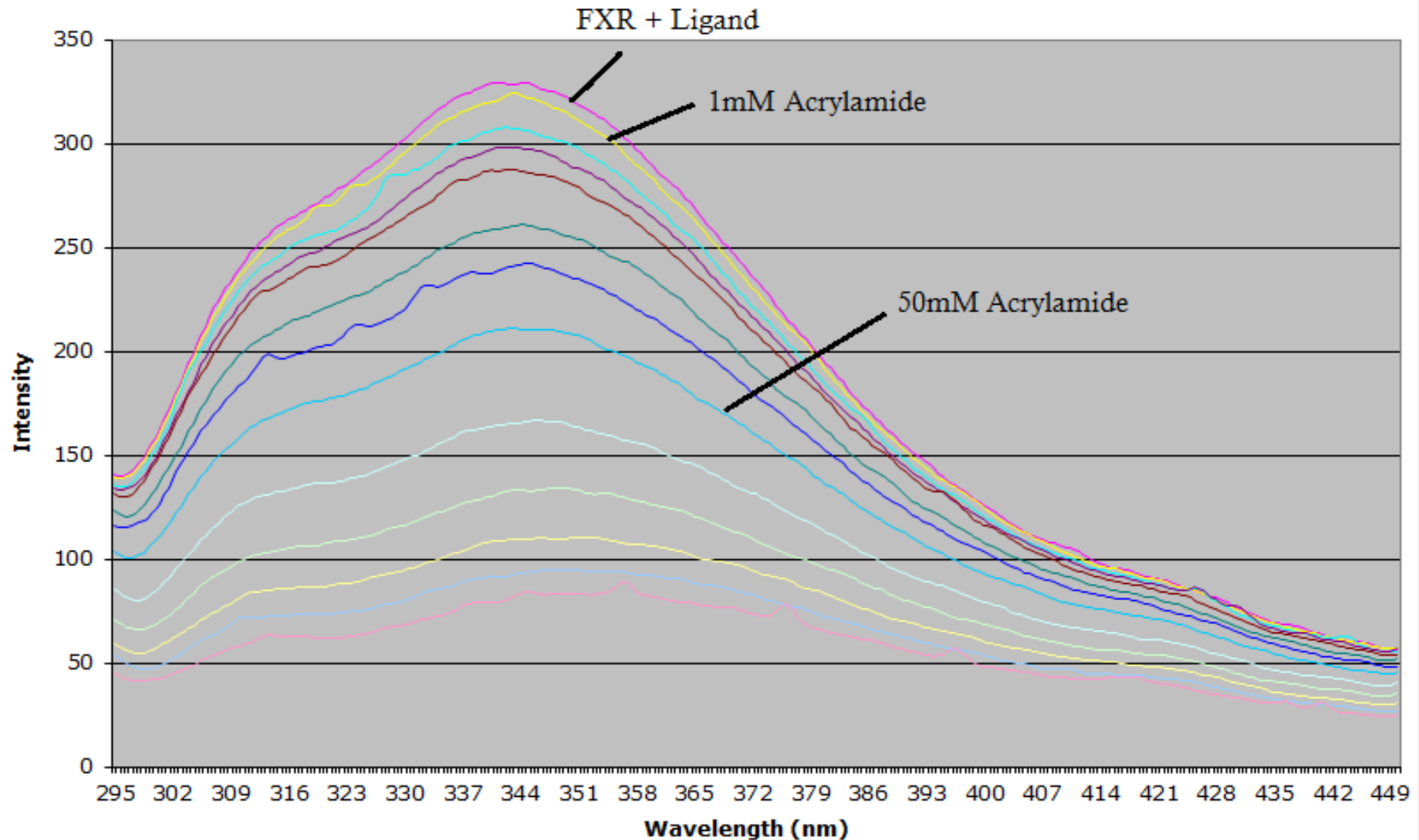
In the
binding site



External to the
binding site



Fluorescence Spectroscopy



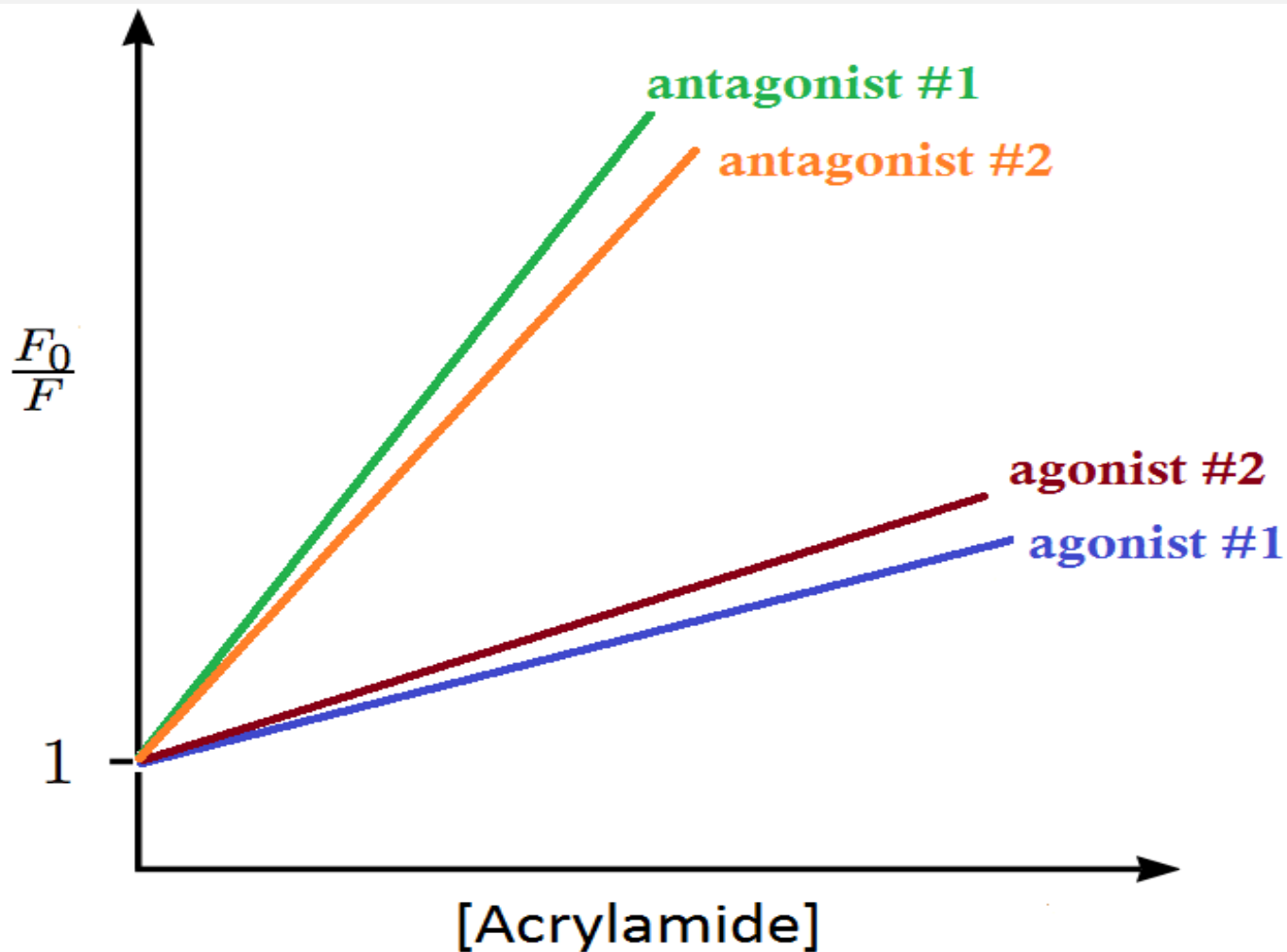
Method: Stern-Volmer Relationship

$$\frac{F_0}{F} = 1 + K_{sv}[Q]$$

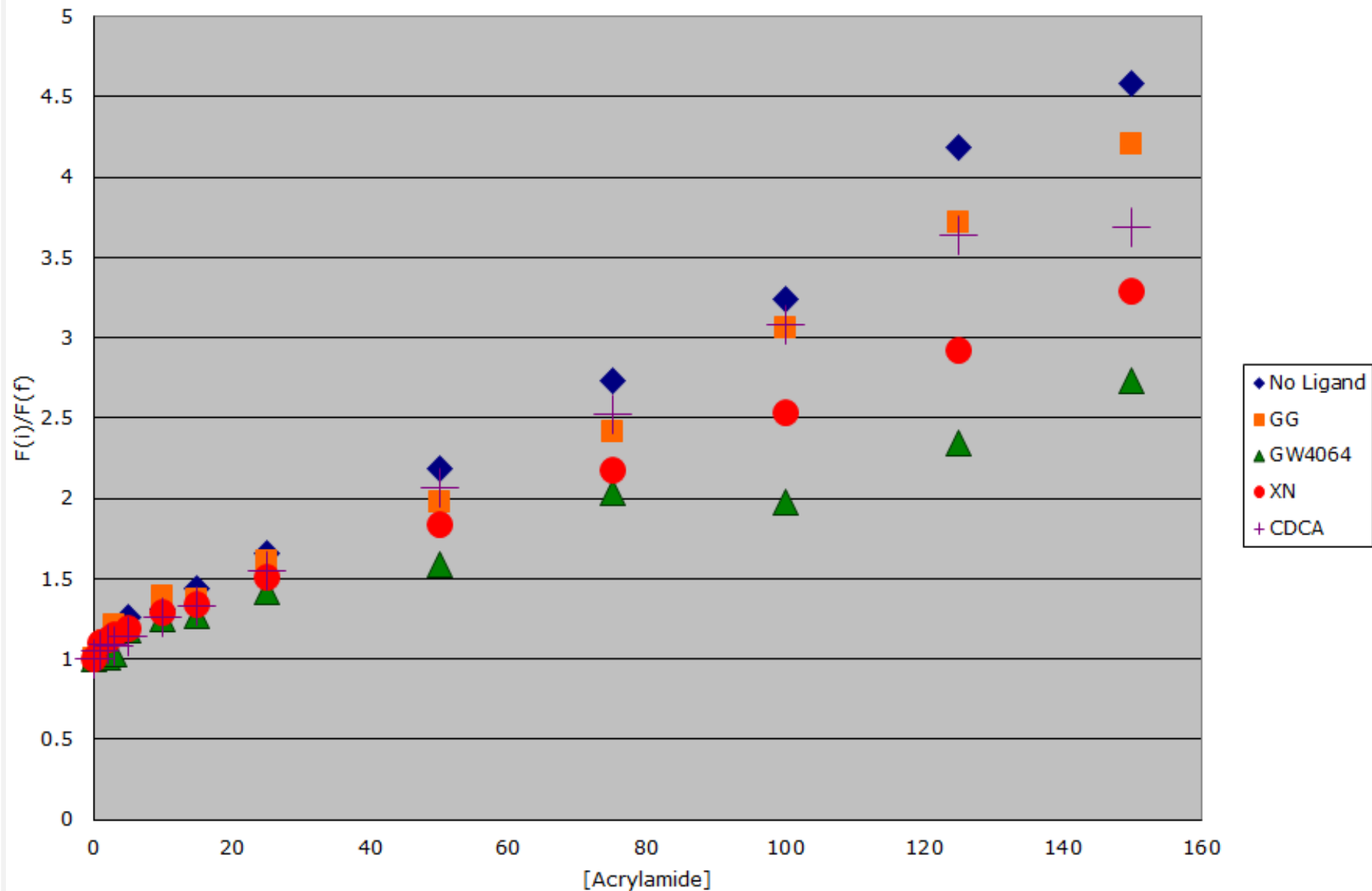
- F_0 is the fluorescence without quenching
- F is the fluorescence after quenching with given amounts of acrylamide
- K_{sv} is the Stern-Volmer quenching constant
- $[Q]$ is the concentration of the quenching molecule



Prediction: Stern-Volmer Plot



Stern-Volmer Relationship of Ligands and FXR



Further Research

- Additional acrylamide titrations to better confirm the observed data
- Experiment with other known agonist and antagonist ligands to refine our assay
- Analyzing the structure of known FXR ligands to predict the general chemical and structural property of possible agonist vs. antagonist ligands to later test the assay

Acknowledgment

Dr. Victor Hsu
Dr. Kevin Ahern
Liping Yang
Stefan Lucchini

The Howard Hughes Medical Institute

Undergraduate Research, Innovation,
Scholarship Creativity (URISC)

Cripps scholarships