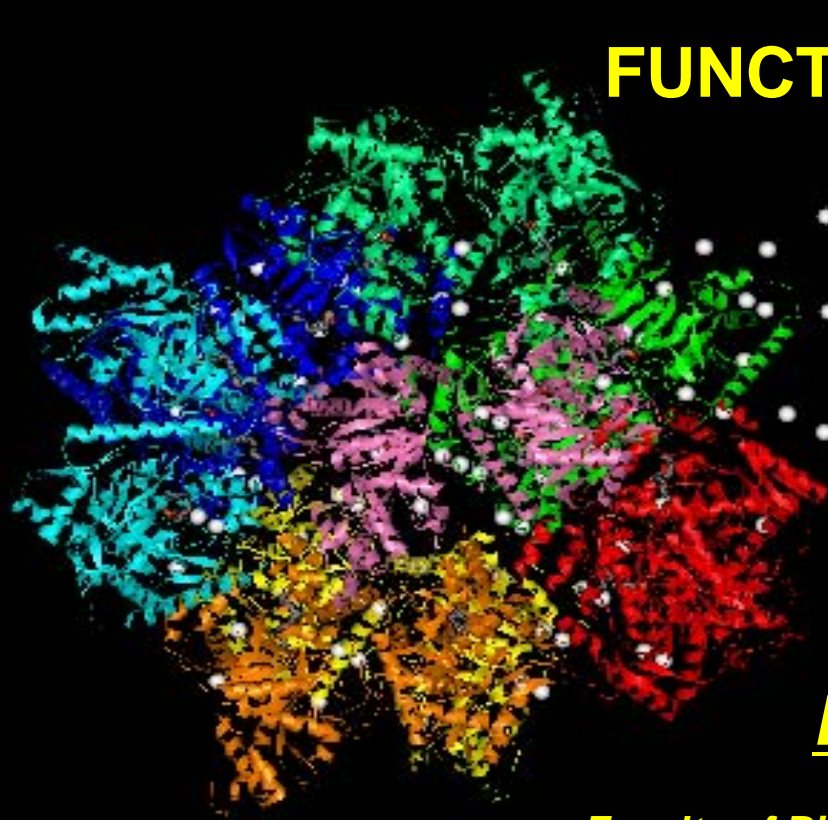


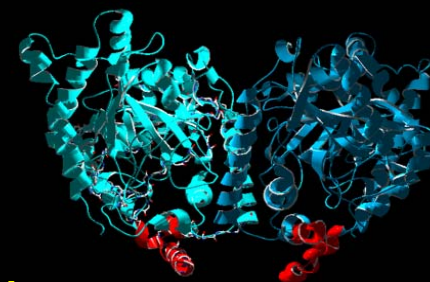
NEW EXPERIMENTAL APPROACHES FOR STUDYING FUNCTIONAL INTERACTIONS



Enrico Dainese

*Faculty of Biosciences, University of Teramo, Italy
&*

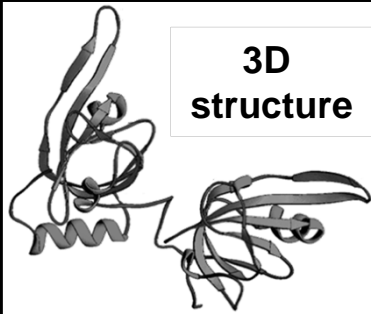
*Laboratory of Lipid Neurochemistry, CERC (European Center for Brain
Research)/IRCCS Santa Lucia Foundation, Rome, Italy*



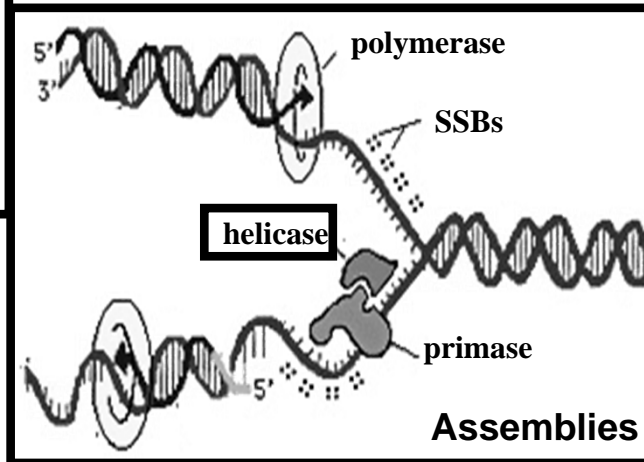
Sequence

MESDAMESETMESSRSMYN
AMEISWALTERYALLKINCAL
LMEWALLYIPREFERDREVIL
MYSELFIMACENTERDIRATV
ANDYINTENNESSEEILIKENM
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WILLWINITALL

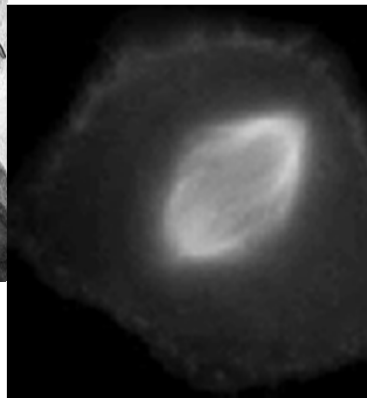
3D structure



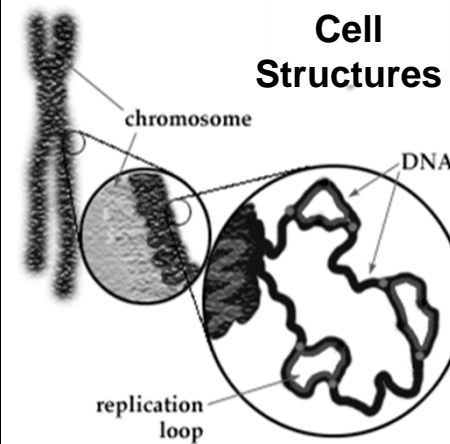
Structural Scales



Organism

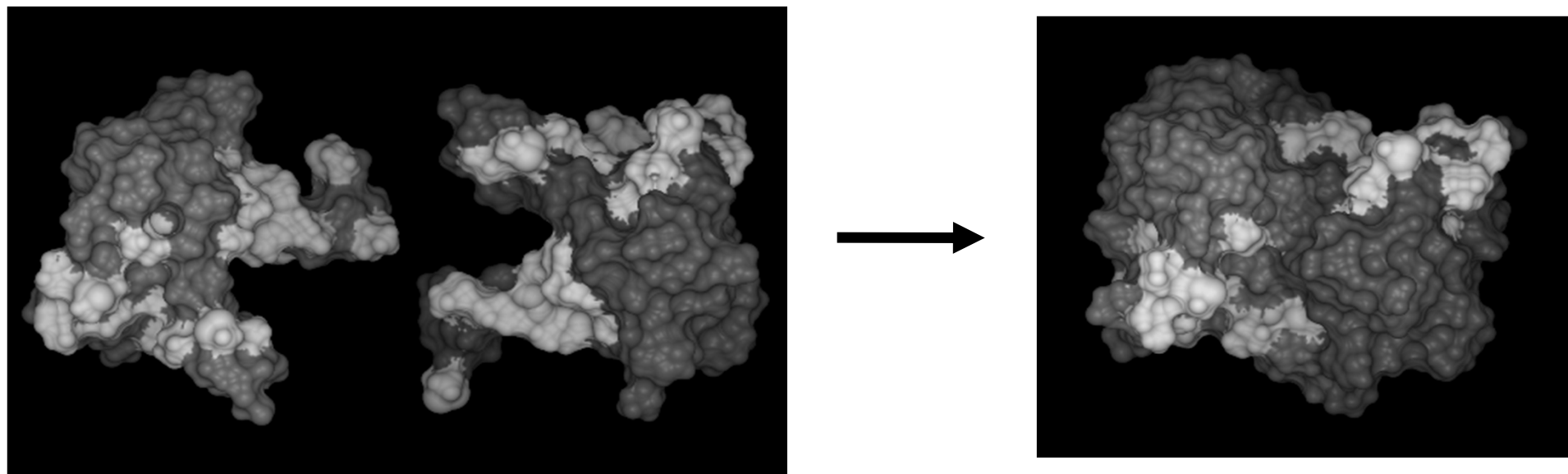


Cell Structures



System Dynamics

High Resolution Structural Biology



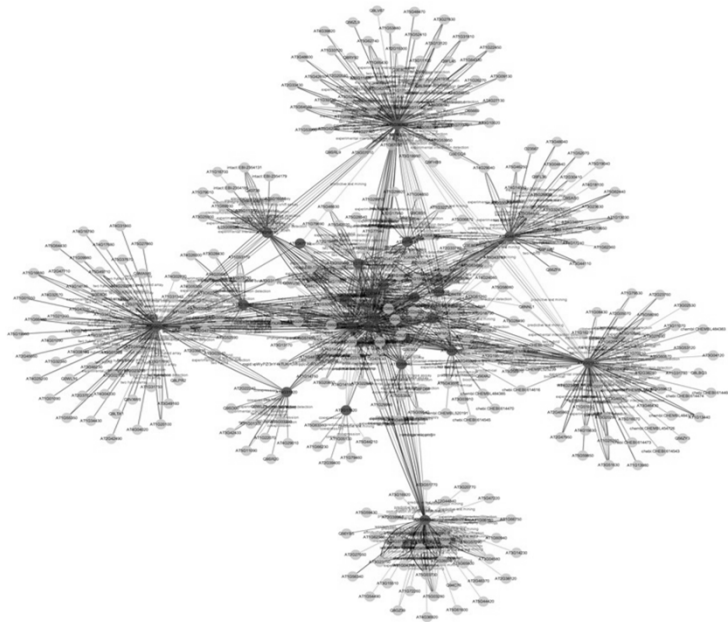
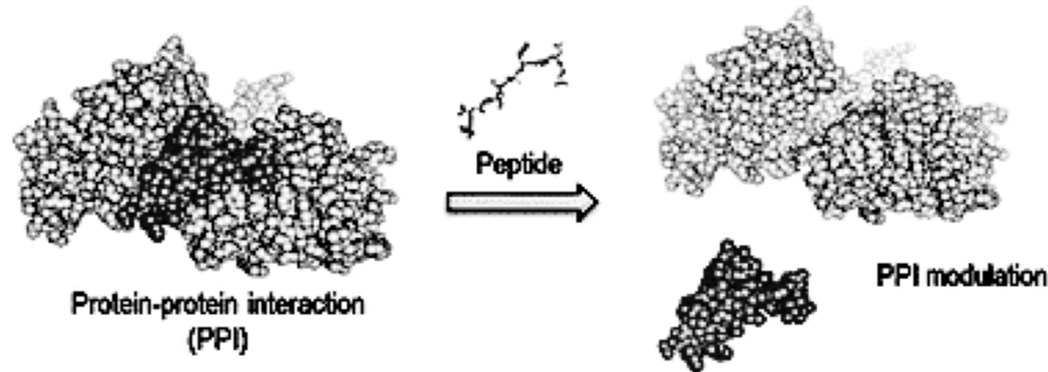
Determine atomic structure
Analyze why molecules interact

High Resolution Structural Biology

Organ → Tissue → Cell → Molecule → Atoms

- A cell is an organization of millions of molecules
- Proper communication between these molecules is essential to the normal functioning of the cell
- To understand communication:
 - *Determine the Organization of INTERACTIONS**

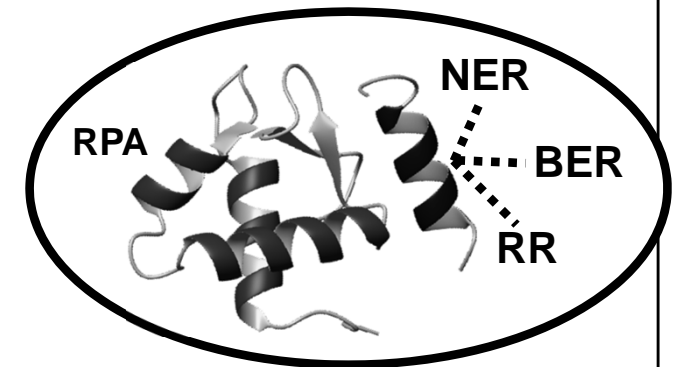
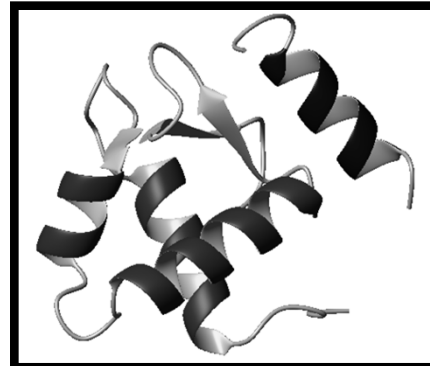
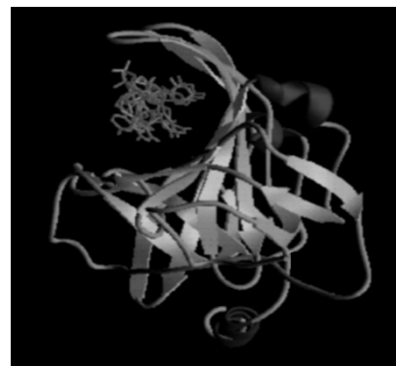
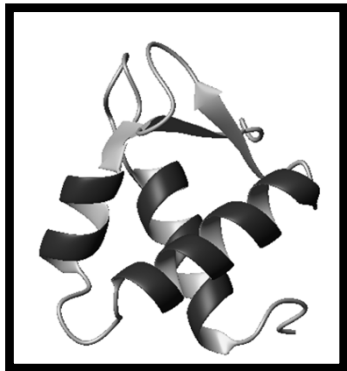
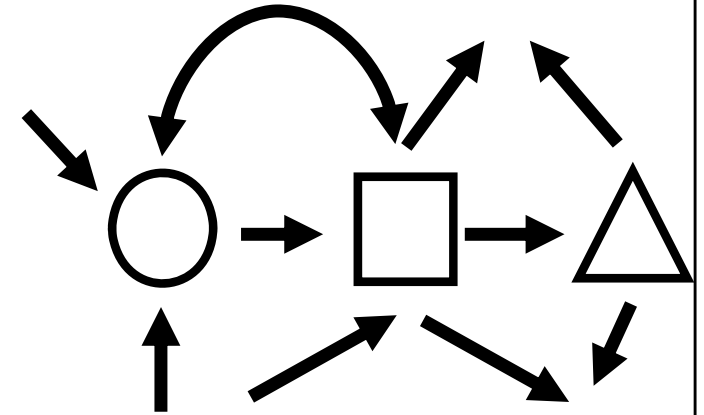
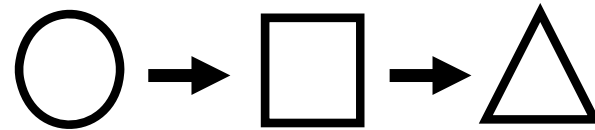
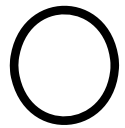
Protein-Protein Interaction (PPI)



Information stored in specific PPIs databases that can be used for:

- 1) Prediction of interacting sites of proteins and design of specific interacting molecules;
- 2) Reliability Assessment of Protein-Protein Interactions;
- 3) Establishment of interaction networks for specific collections of proteins, and involved with pathological/physiological processing.

How Atomic Structure Fits In



Molecule

Structural Genomics

Pathway

Functional Proteomics

Activity

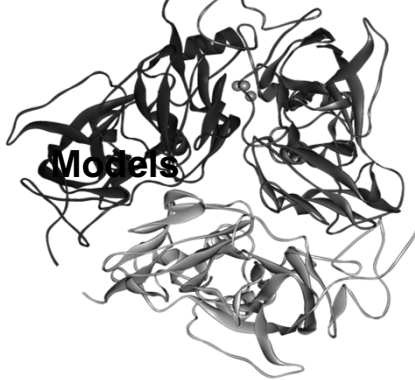
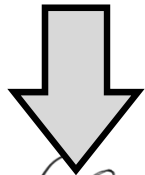
Drug design

Structural Biology

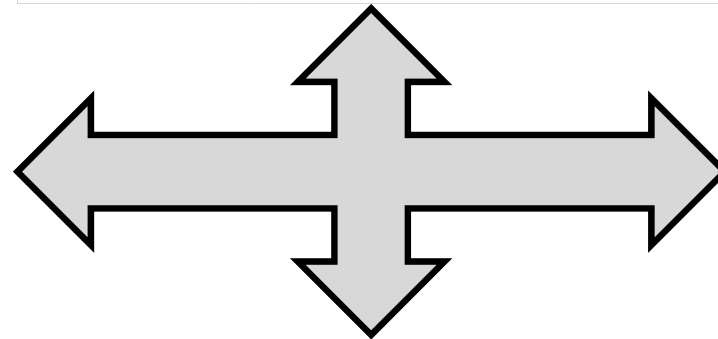
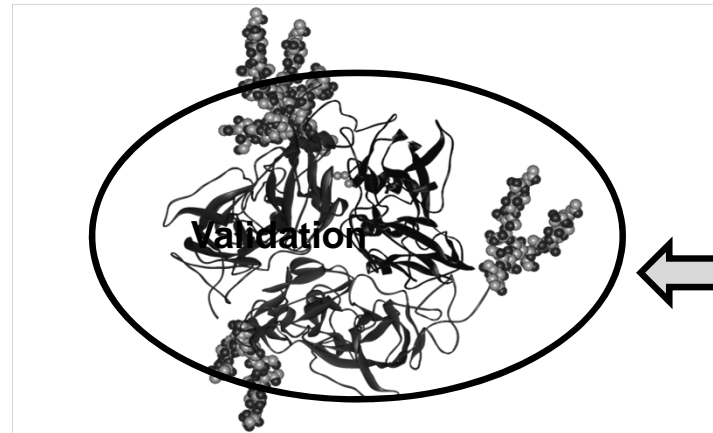
In silico approaches

Experimental approaches

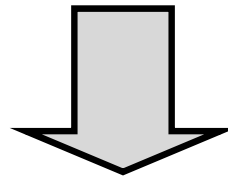
SEQUENCE
(gene or protein)



Ab initio models
Homology models



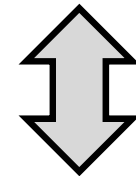
**MOLECULAR
DOCKING**



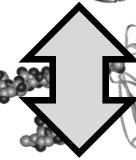
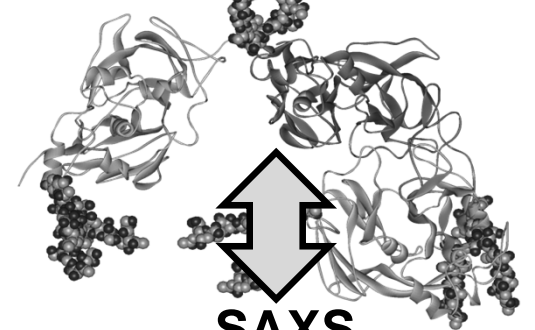
DRUG DESIGN

Activity assays
(e.g. ENSPIRE)

X-ray crystallography
NMR



Protein Data Bank

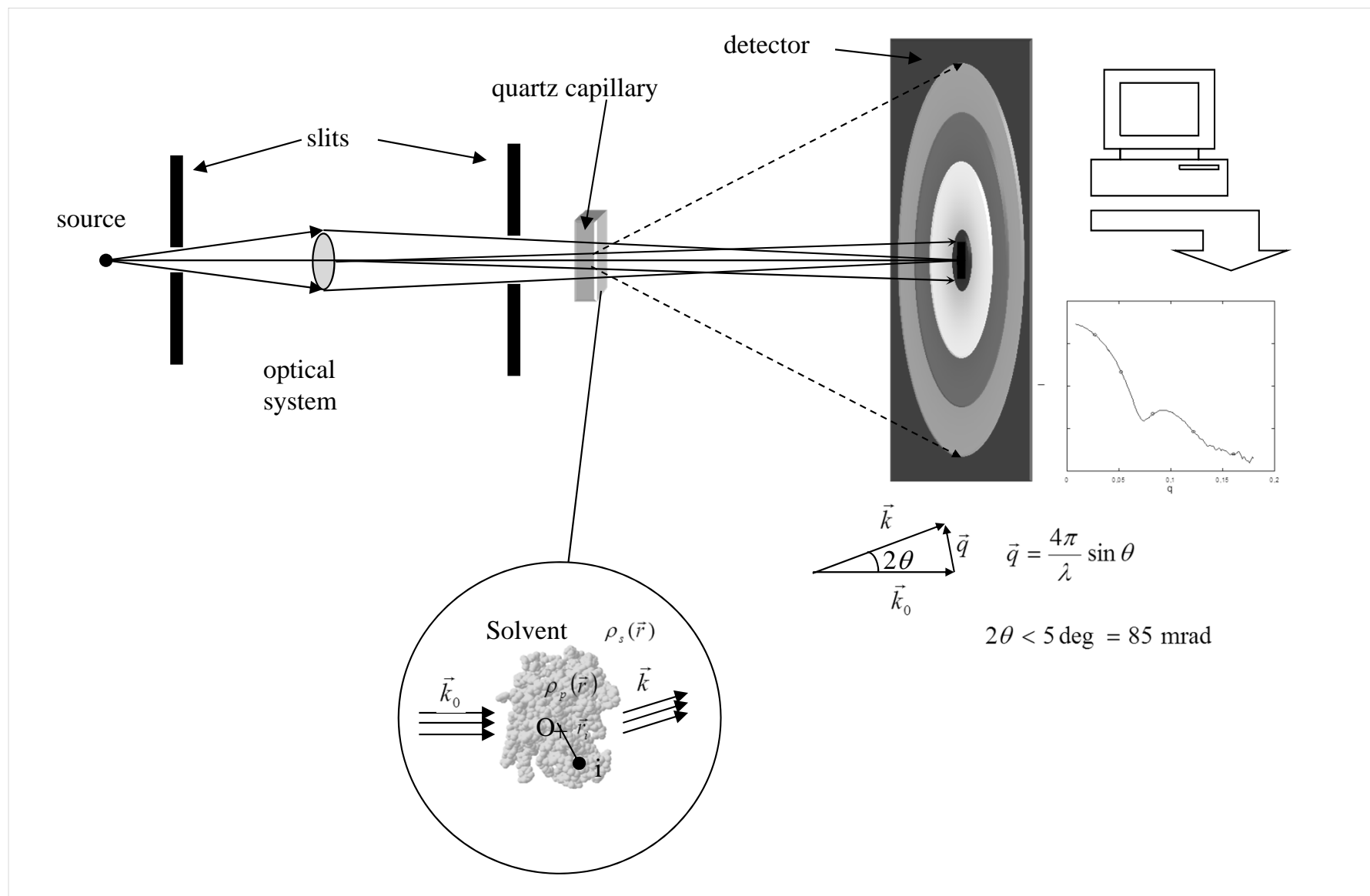


SAXS



Cryo-EM

SMALL ANGLE X-RAY SCATTERING

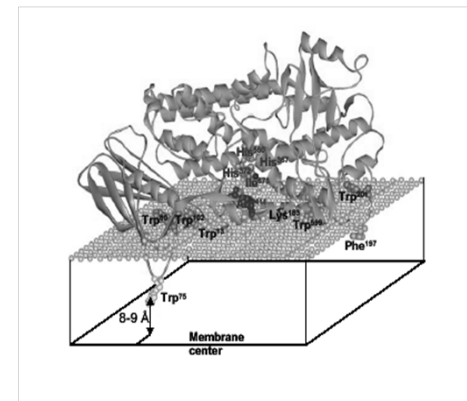


Studies by SAXS: interactions with Biological macromolecules

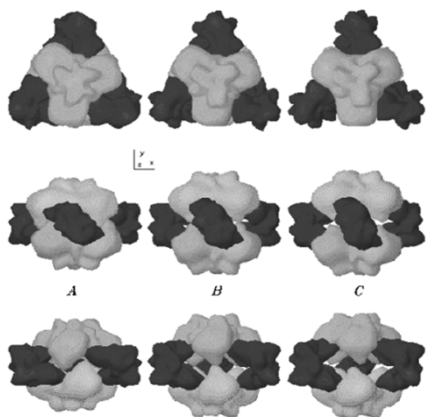
Applications to
proteins



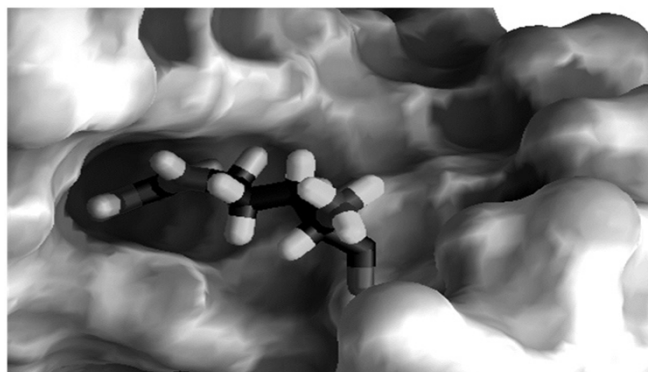
folding-unfolding



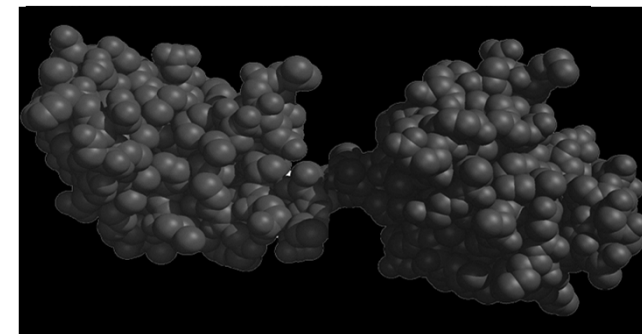
Protein-lipids interactions



Oligomeric organization

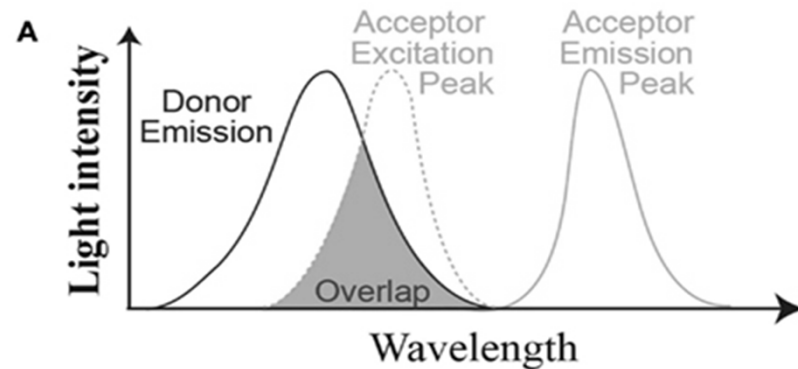


Protein-ligand interactions

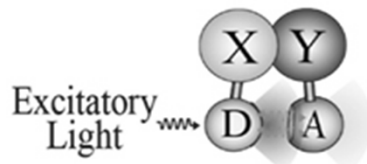


Protein-protein interactions

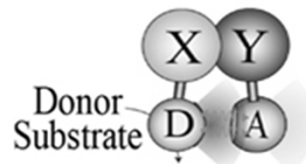
Additional experimental approaches



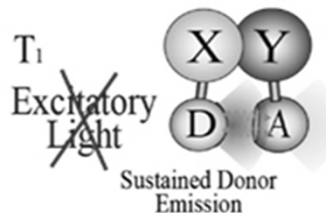
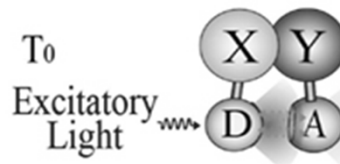
B
FRET



C
BRET

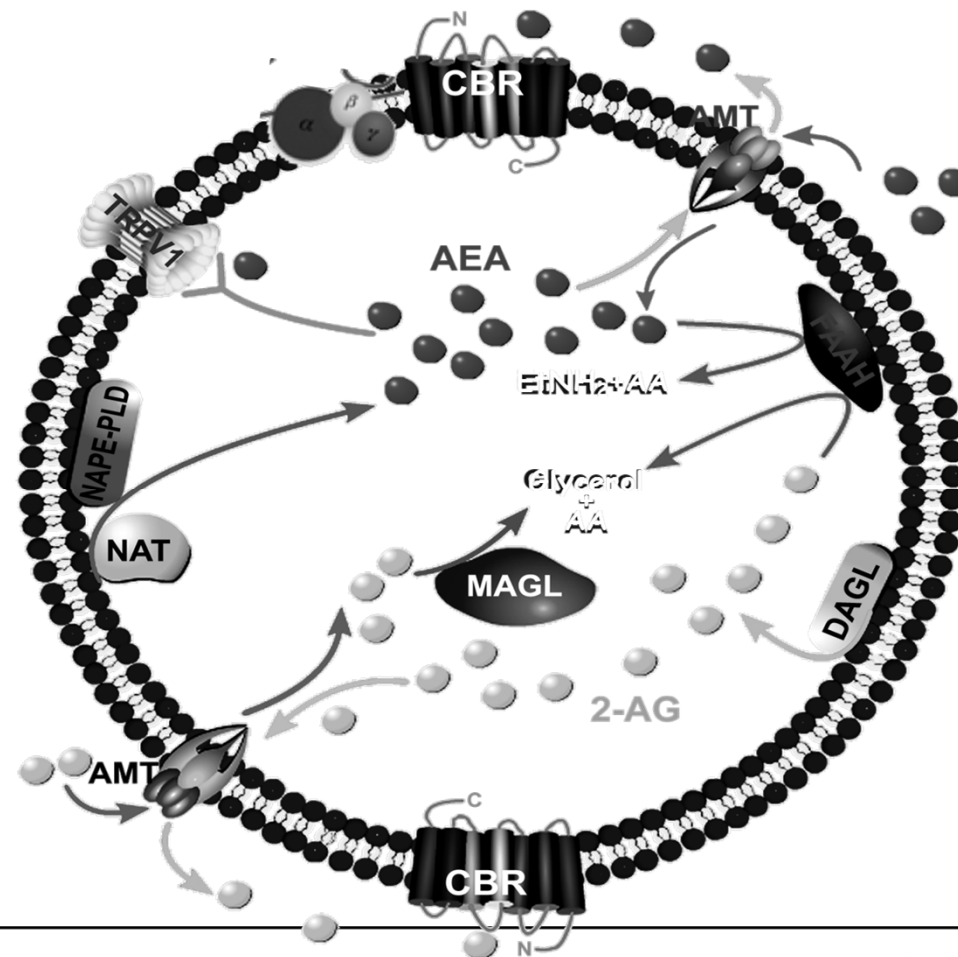


D
HTRF

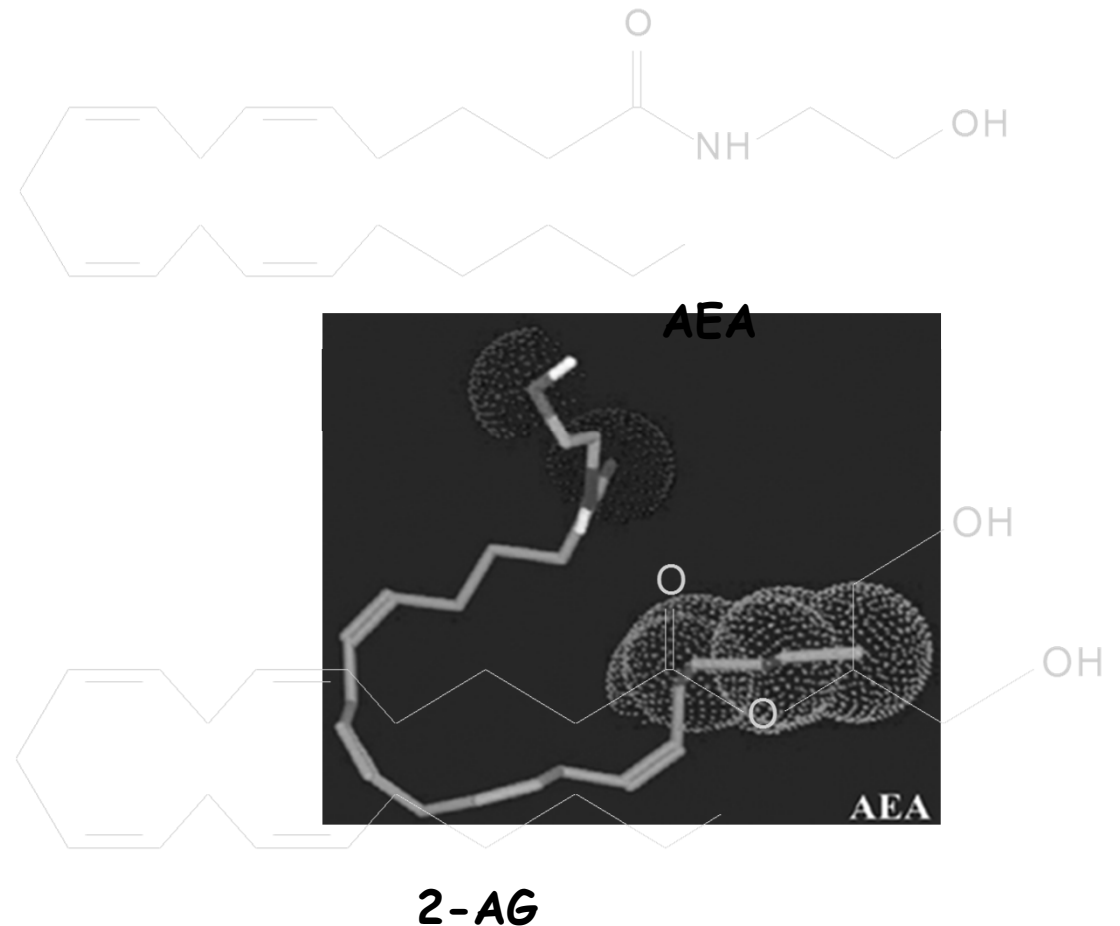
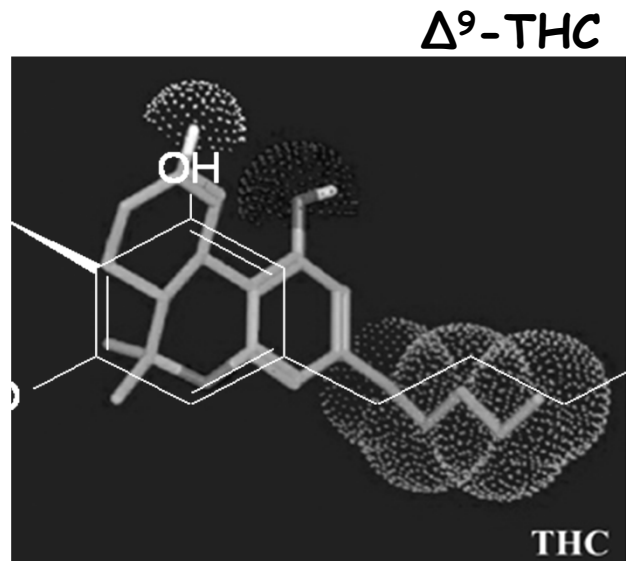


- Label Transfer Protein Interaction Analysis (FRET-HTRF-BRET-FCS)
- Co-immunoprecipitation (co-IP)
- Pull-Down Assay
- Crosslinking Protein Interaction Analysis
- Far-Western Blot Analysis

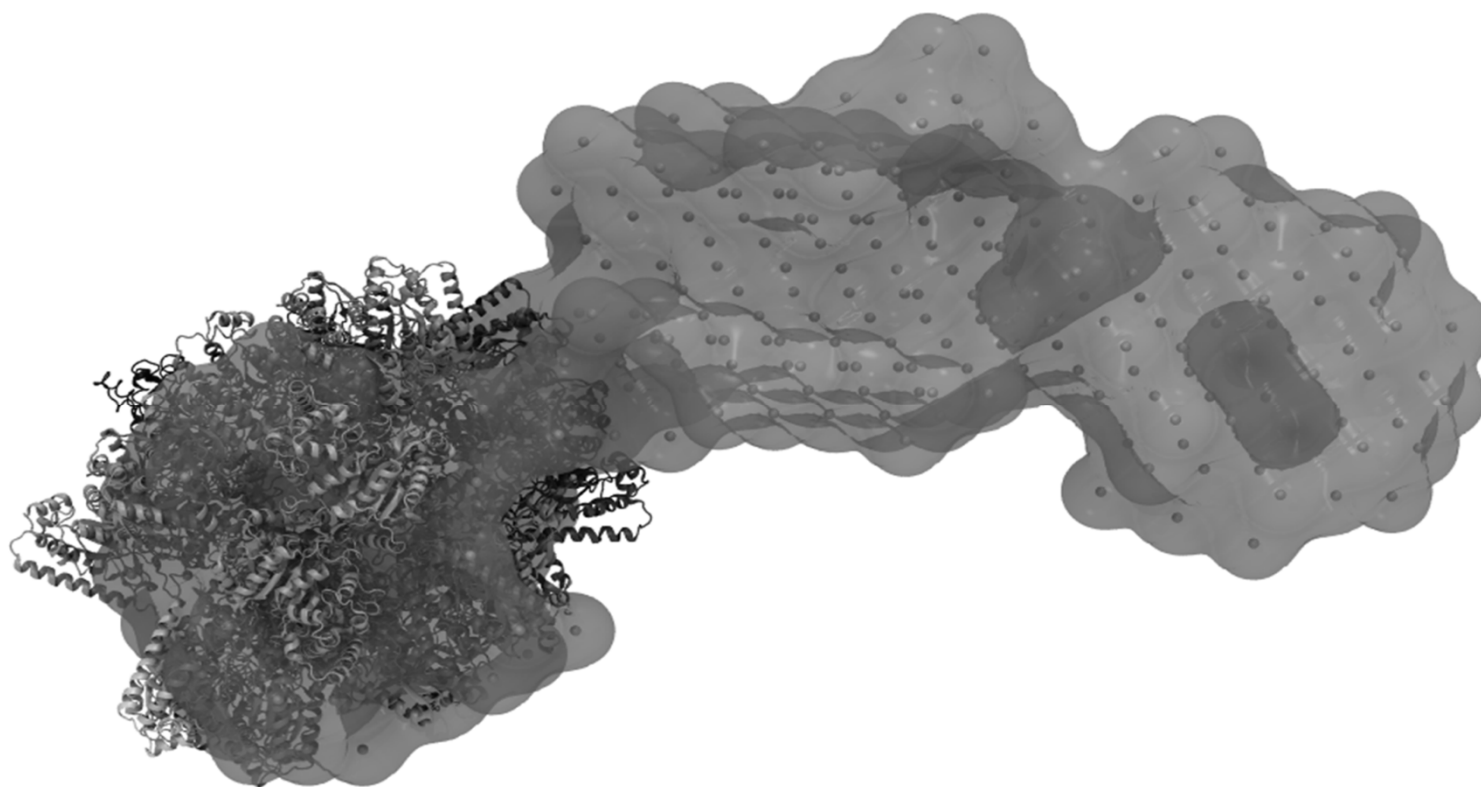
An example: developing drugs modulating the endocannabinoid system



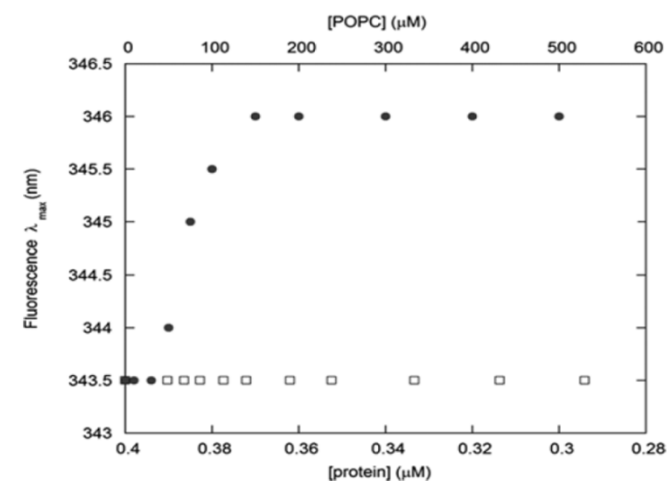
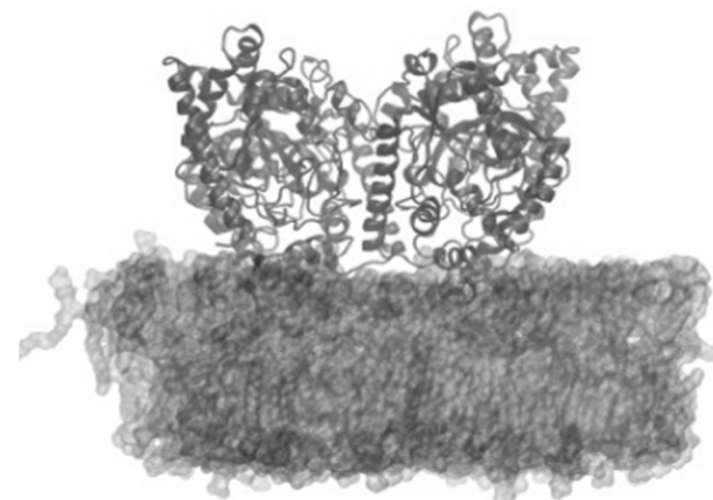
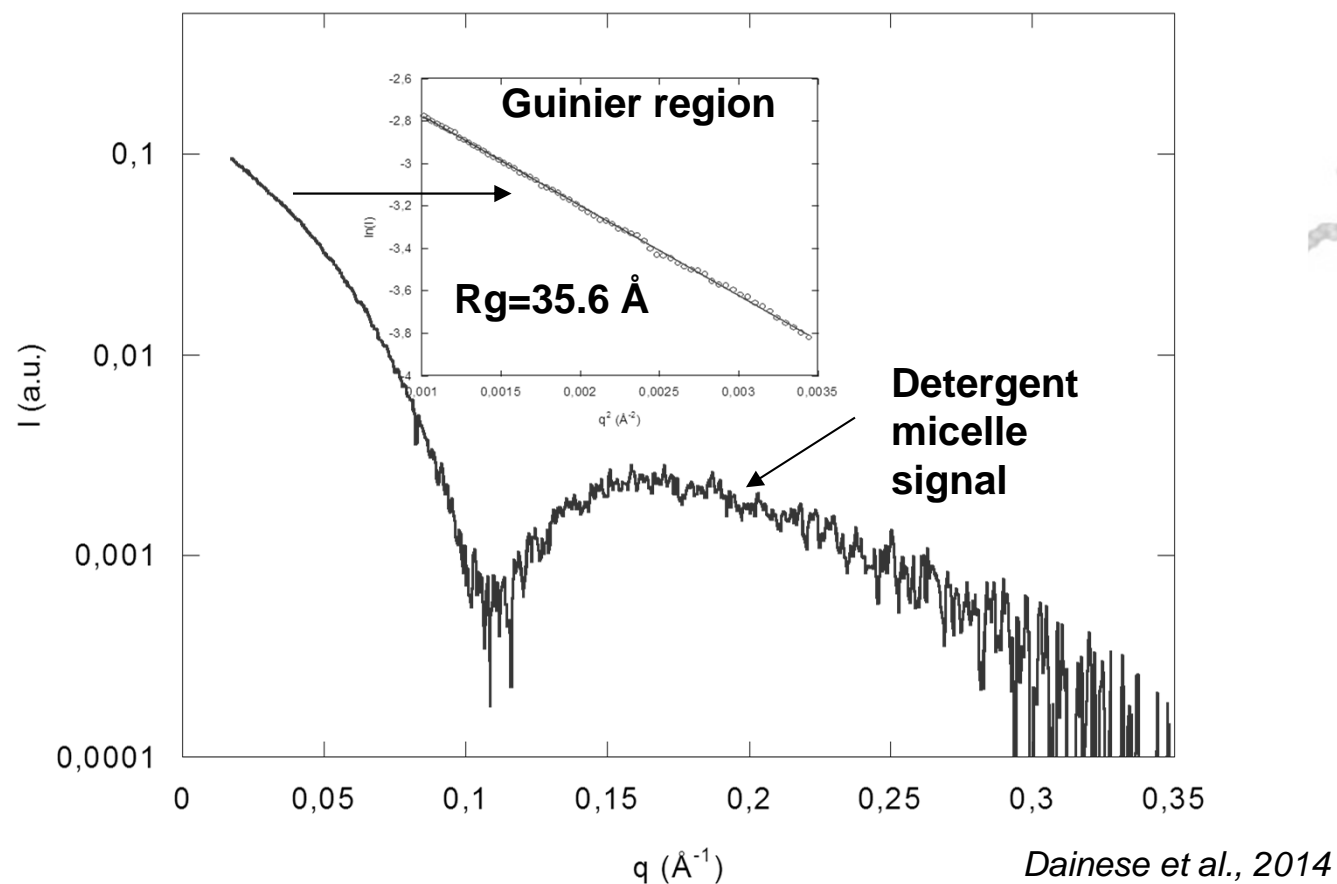
An example: the endocannabinoids



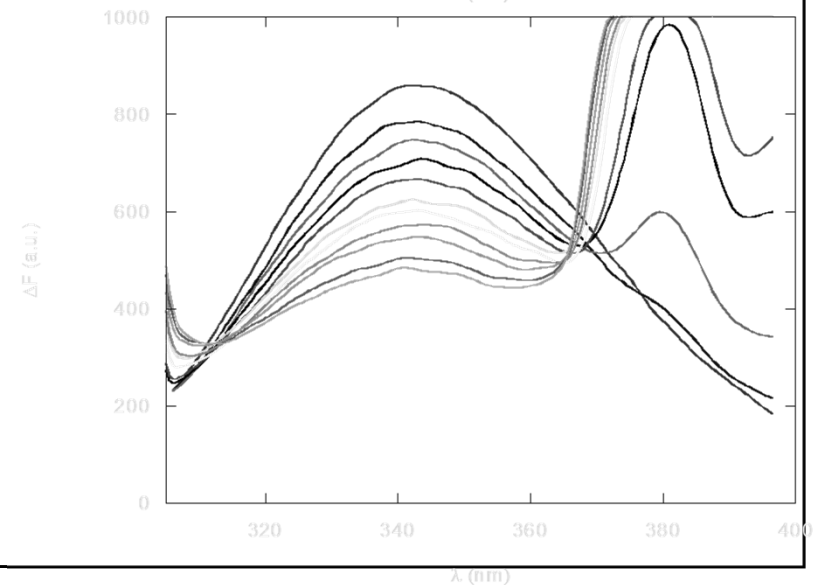
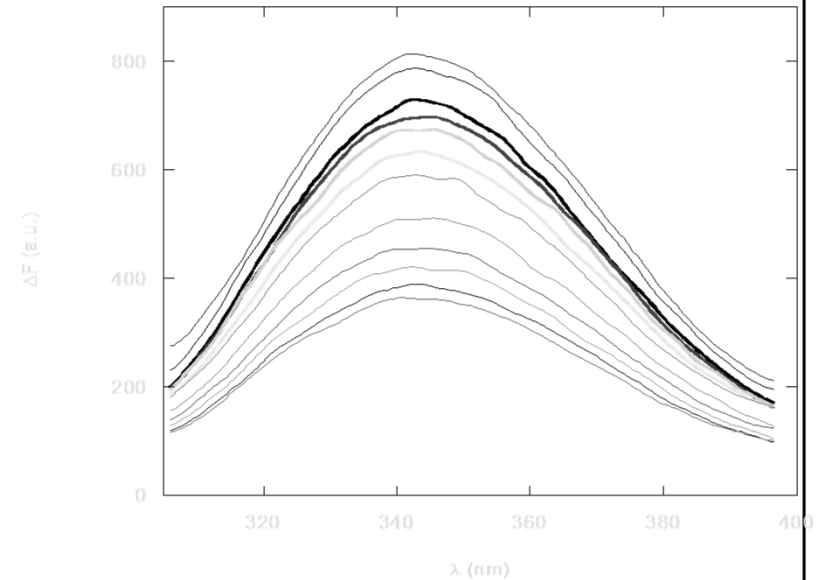
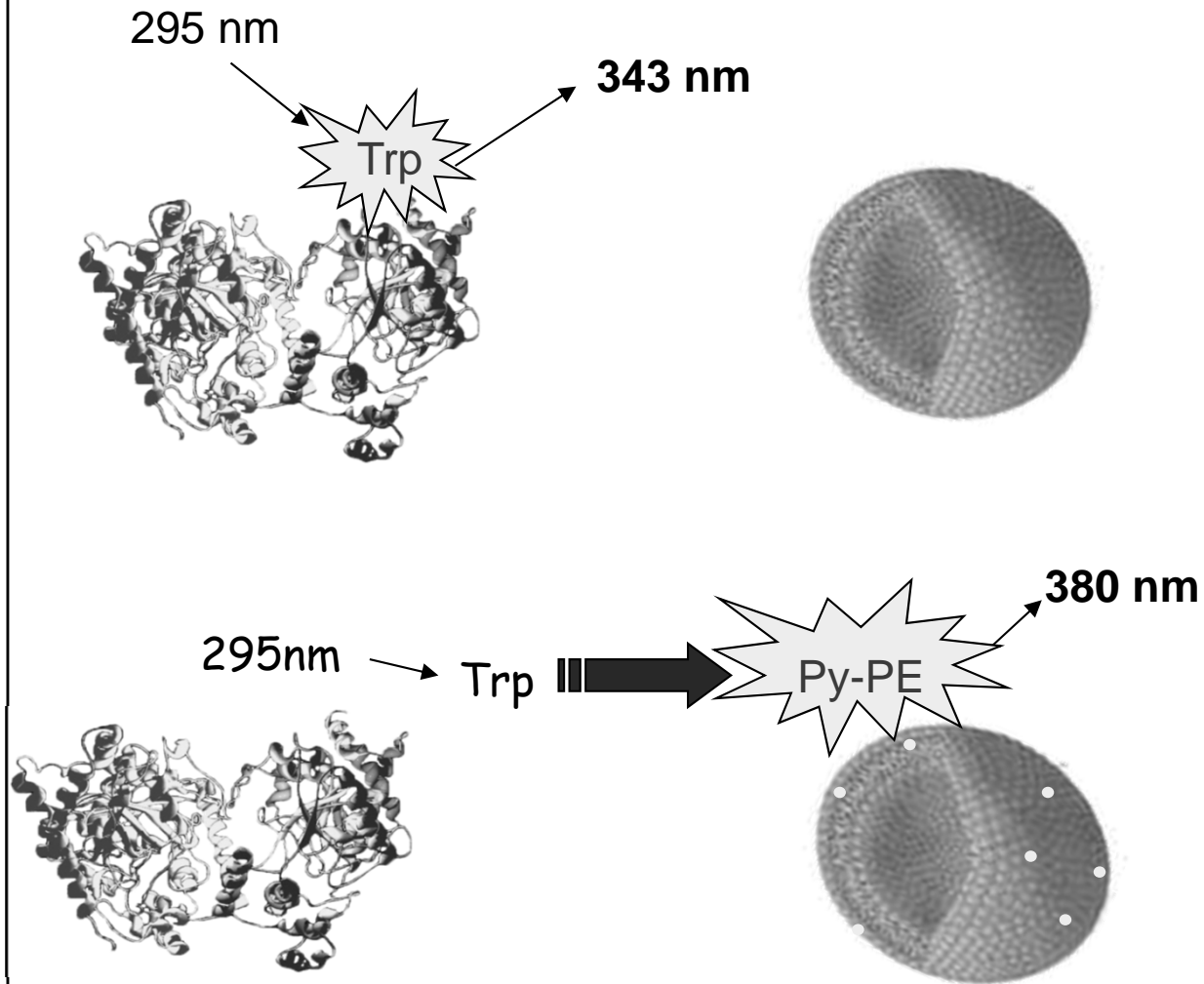
Determination of the oligomerization state of FAAH in solution by small angle X-ray scattering (SAXS)



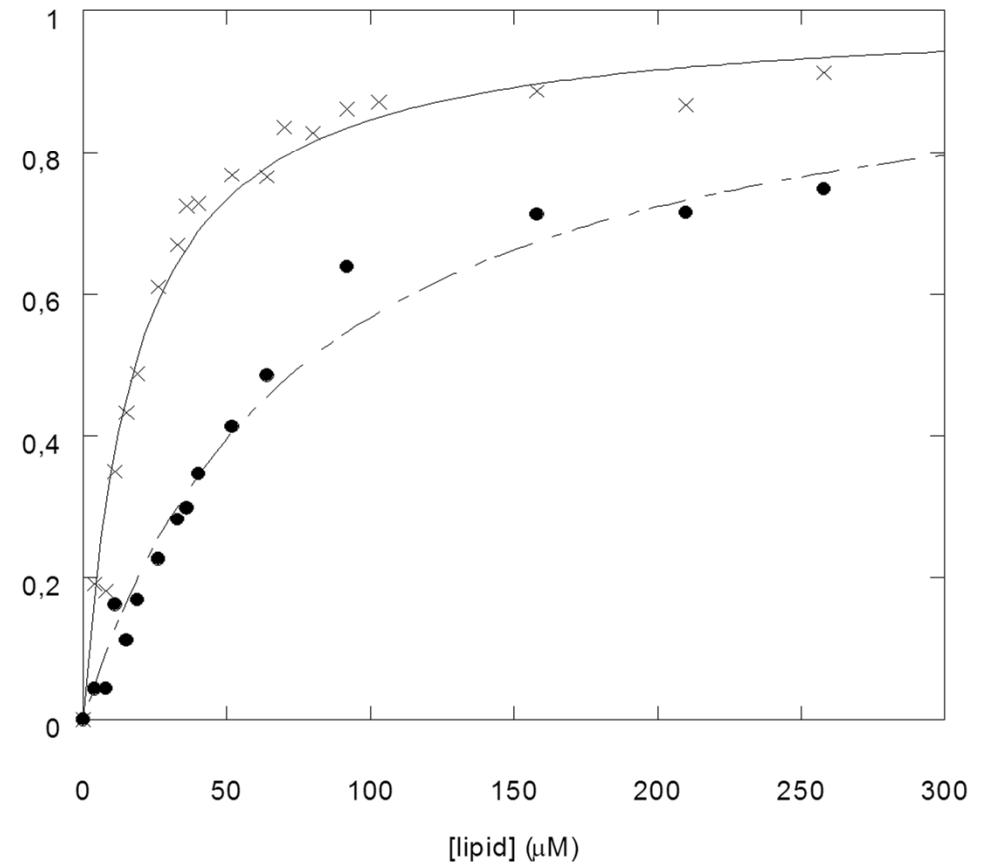
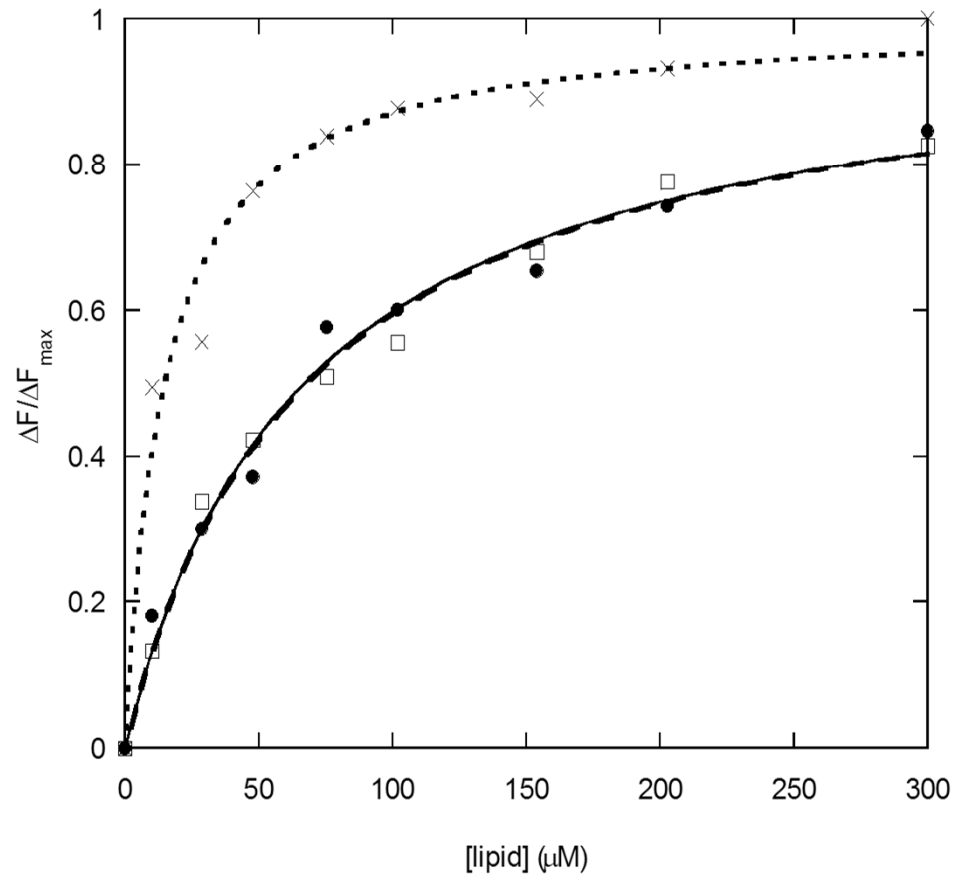
Membrane lipids dissociate these oligomers and stabilize FAAH dimer



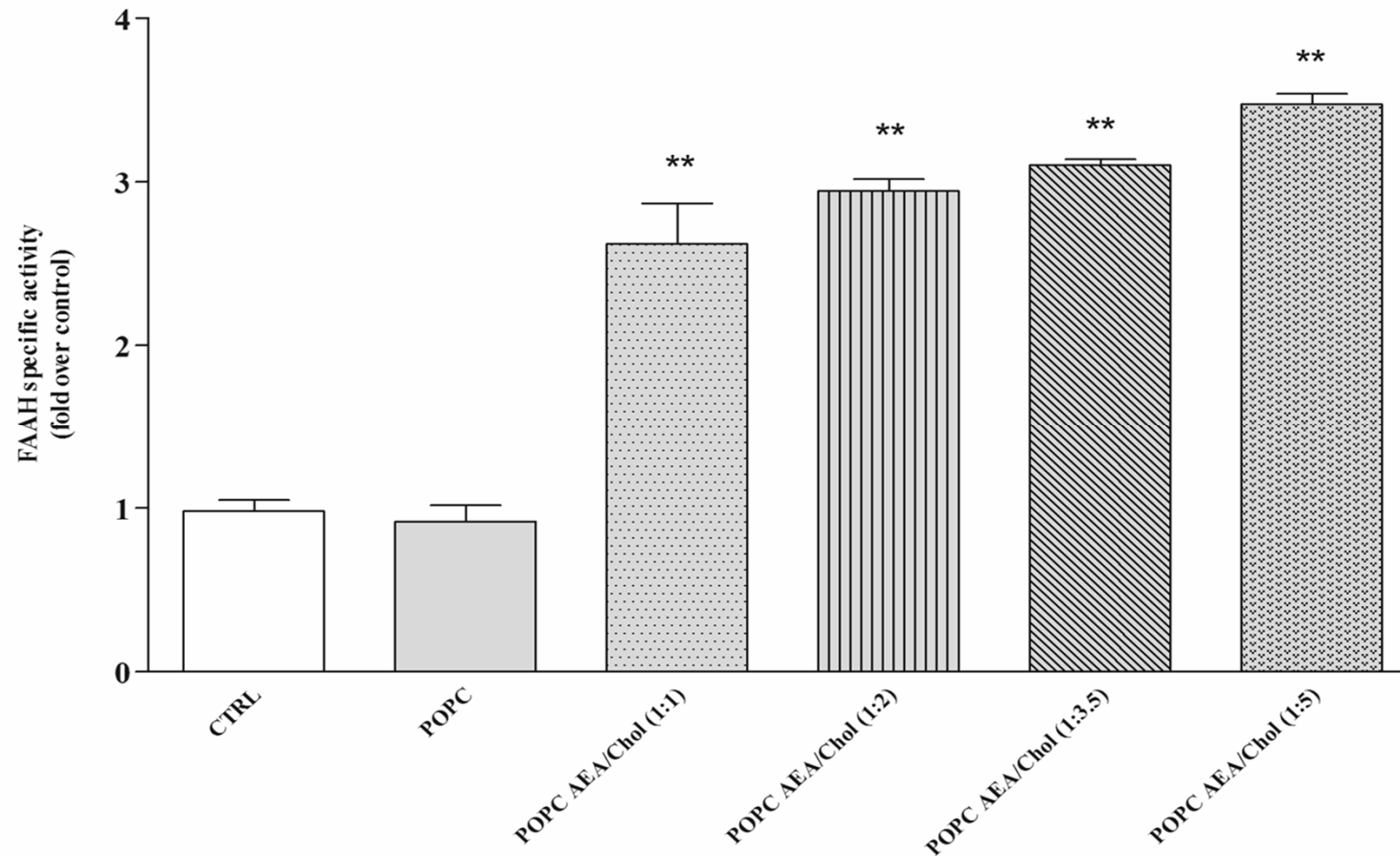
Study of FAAH/membrane interaction by FRET



Higher membrane affinity of FAAH to ER membranes containing AEA and cholesterol



FAAH activity is strongly increased by membranes containing AEA and cholesterol



Dainese et al., 2014