BSc Pharmacology and Translational Medicine

Module: Principles of Pharmacodynamics and Pharmacokinetics
Lecture: P-Glycoprotein and the Removal of Drugs from Cells
Date: Tuesday 11th October 2011

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Diffusion and Active Transport

Lecture Outline

- ATP-binding cassette (ABC) transport proteins
  - P-glycoprotein
    - Structure
    - Substrates and modulators
    - Role in drug transport
    - Mechanism of drug transport
    - Regulation
  - MRP
    - Outline
    - Structure
  - BRCP
    - Outline
    - Structure
- Summary

ATP-Binding Cassette (ABC) Transporters

Drug Transporters

ABC Transporters

Transporter	Isoform	Typical Substrates	Sites in the Body
ABC Superfamily			
P-glycoprotein / MDR1[a]	ABCB1	Hydrophobic and cationic (basic) molecules; numerous drugs, including anticancer drugs	Apical surface of epithelial cell membranes. Liver, kidney, lung, intestine, BBB, testes, placenta,
MRP1[a]	ABCC1	Numerous molecules, including anticancer drugs, glucuronide and glutathione conjugates	Basolateral surface in most cell types. Kidney, lung, testes, blood:tissue barriers.
MRP2[a]	ABCC2	Numerous molecules, including anticancer drugs, glucuronide and glutathione conjugates	Apical surface of membranes. Liver, kidney, intestine
BRCP	ABCG2	Anticancer, antiviral drugs, fluoroquinolones, flavinoids	Apical surface of breast ducts and lobules. Intestine, colon, liver, placenta, BBB, lungs

From Waller D. G. et al. Medical Pharmacology and Therapeutics 3rd Edition, Saunders / Elsevier Ltd. 2010.

P-Glycoprotein

ABC Transporters

P-Glycoprotein (PgP) – a.k.a. MDR1

- Adenosine triphosphate (ATP)-binding cassette transporter
- ATP-driven drug efflux pump
- Encoded by the ABCB1 gene
- Expressed in most tissues
- Far higher expression in certain epithelial cells related to excretory functions such as:
  - Colon
  - Small intestine
  - Proximal tubules in the kidney
  - Pancreas
  - Bile ducts
- Also found in endothelial cells in the blood brain barrier (BBB)

ABC Transporters

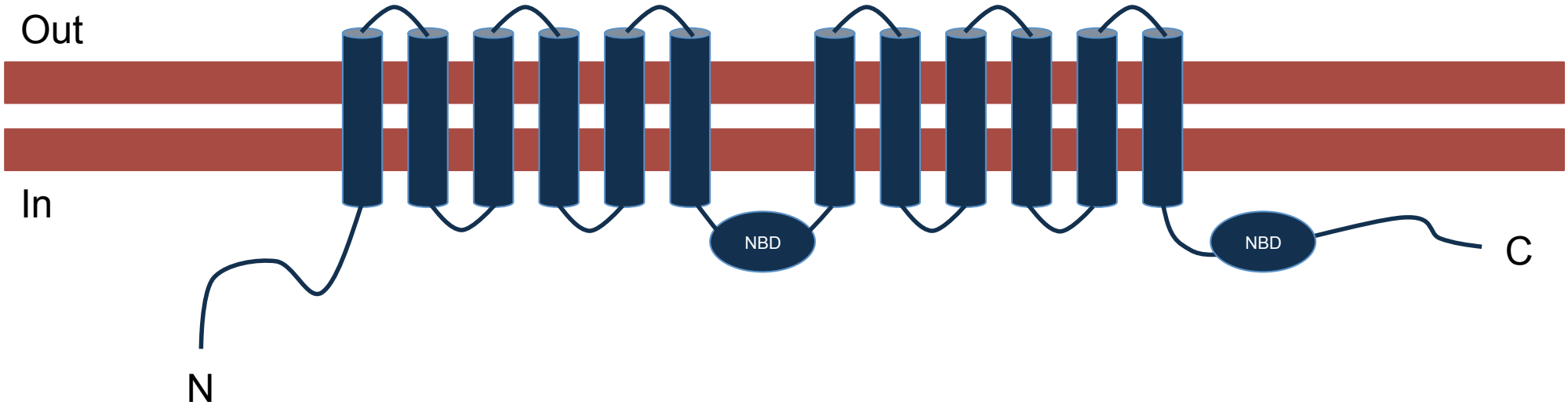
Structure of P-Glycoprotein in Tissues

- Transmembrane ABC transport protein
- Size:
  - 1280 amino acid residues
  - MW= 170 kDa
- Total of 12 transmembrane domains
- Contains *N*-terminal glycosylated residues
- Two ATP binding sites
- Well conserved sequences for ATP binding
  - Walker A and Walker B motifs

ABC Transporters

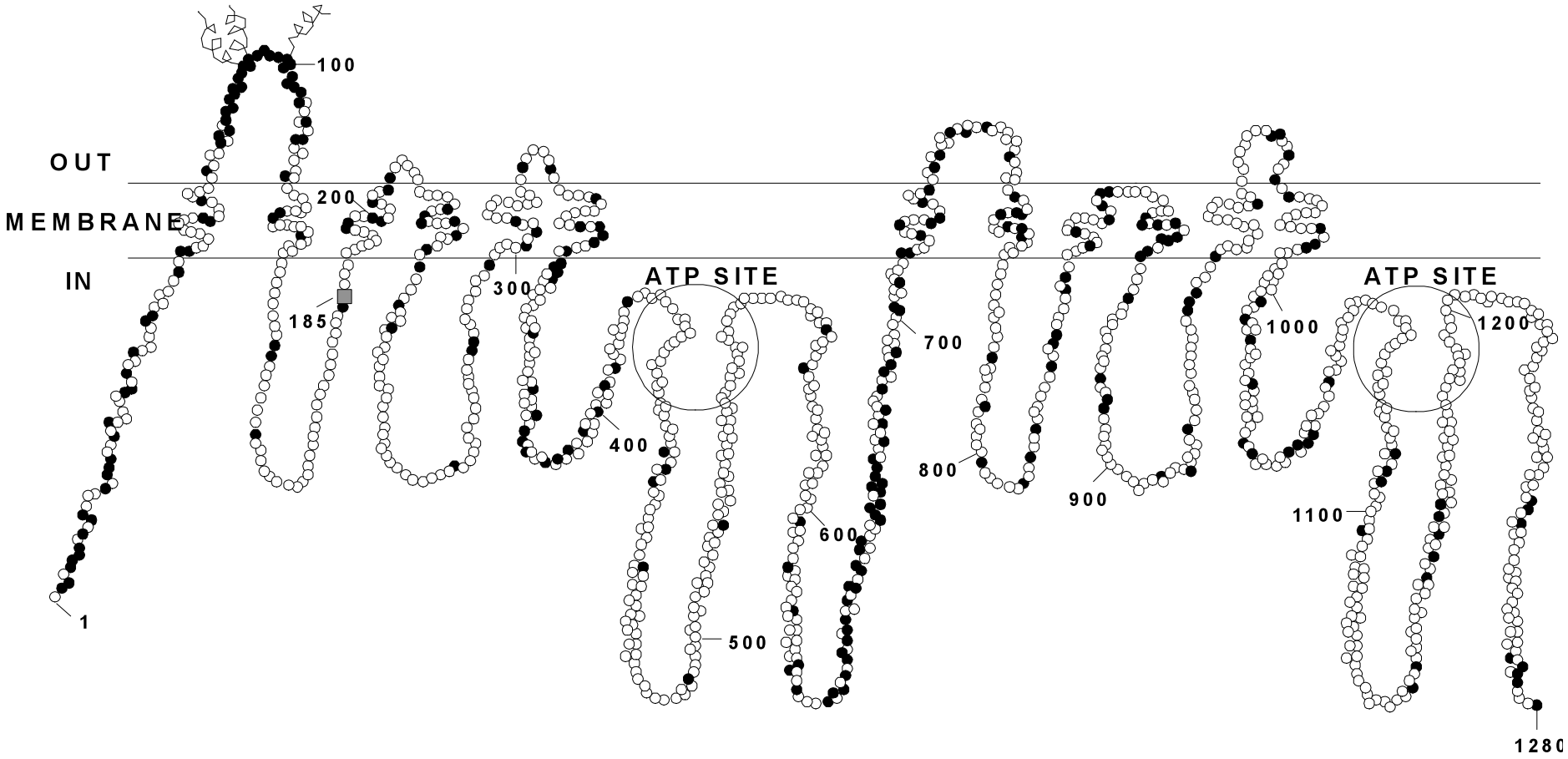
Multidrug Resistance-Associated Proteins

- Structure - PgP



Structure of P-Glycoprotein

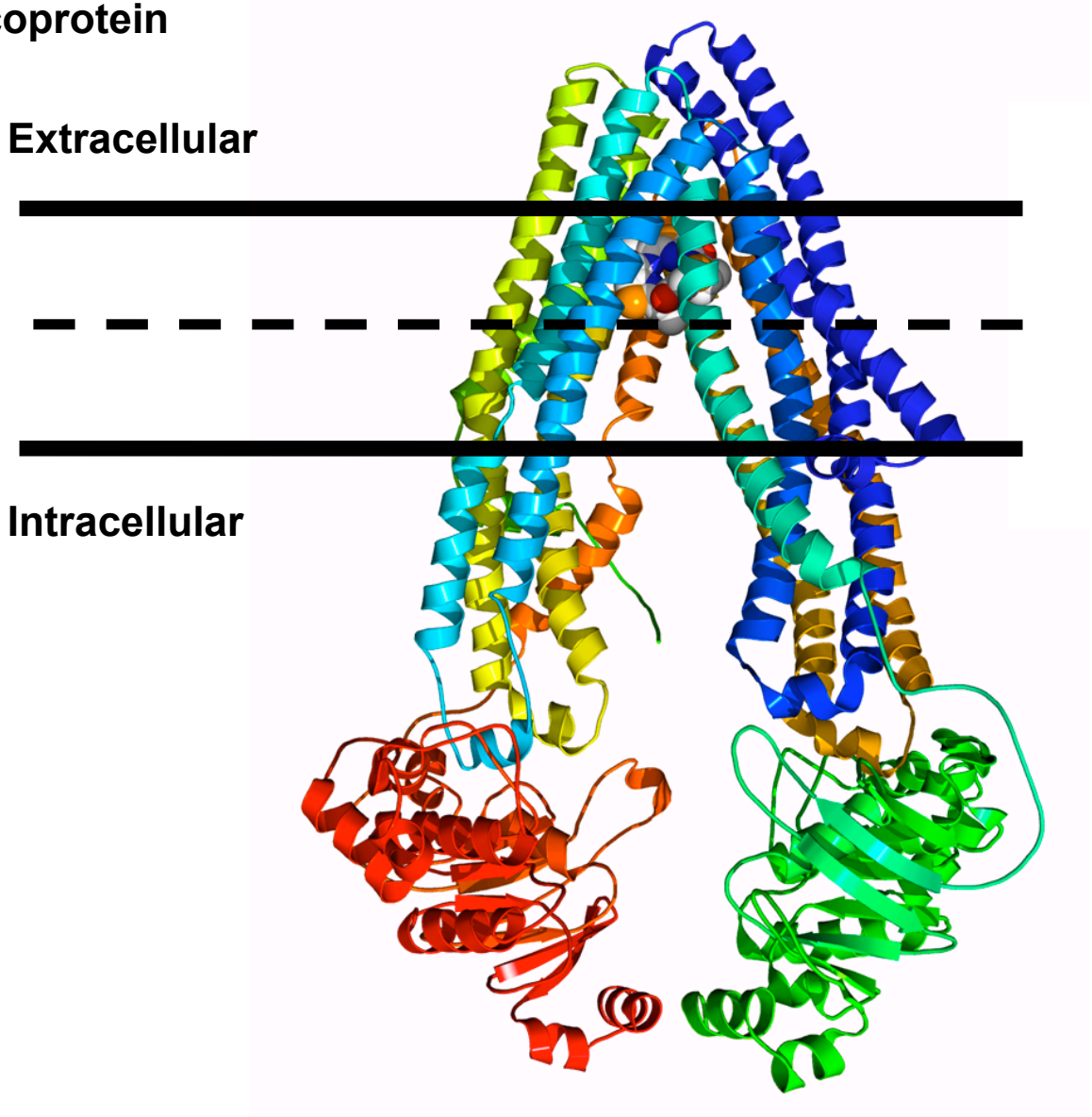
- 2D representation



Adapted from Gottesman M.M. and Pastan I.1988. The multidrug transporter: a double-edged sword. *J. Biol. Chem.* 263, 25, 12163-12166.

ABC Transporters

Structure of P-Glycoprotein



ABC Transporters

Substrates and Modulators of P-Glycoprotein

- Substrates
  - PgP exhibits an affinity for an unusually diverse range of substrates
  - These substrates are largely:
    - nonpolar or weakly amphipathic (containing both hydrophilic and lipophilic groups)
    - include a wide range of compounds:
      - drugs used in therapy
      - compounds used as tracers
      - detergents
      - natural products

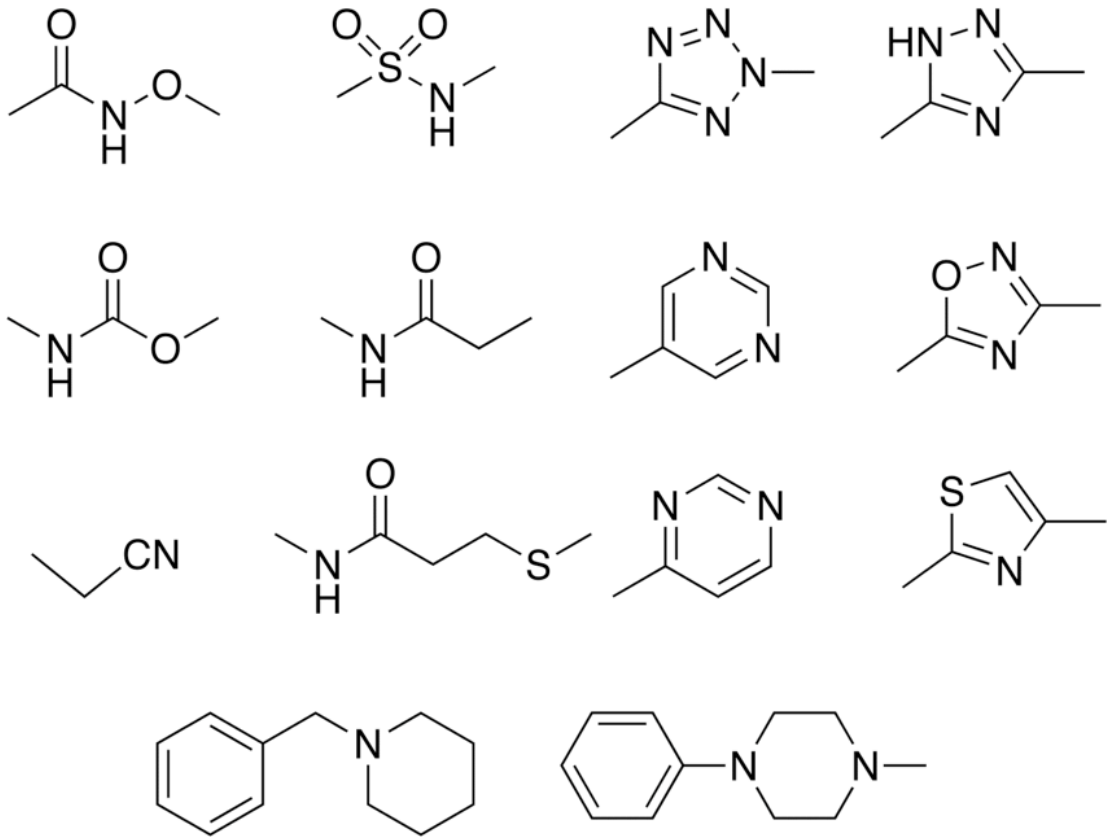
- Modulators
  - A number of compounds alter the activity and/or specificity of PgP
  - Can interact with various regions of the protein e.g.
    - Substrate binding site
    - ATP-binding site

- Some substances can act as both substrates *and* modulators of PgP

What is a Drug?

Substrates of P-Glycoprotein

- Example functional groups that have a high affinity with PgP:



From You and Morris. Drug Transporters: molecular charaterixation an role in drug transport. Wiley, NJ, USA. 2007.

ABC Transporters

Example Substrates and Modulators of P-Glycoprotein

Substrates		Modulators
vinblastine	rhodamine 123	verapimil
doxorubicin	gramicidin D	trifluoperazine
paclitaxel	colchicine	cyclosporin A
etoposide	cimetidine	tamoxifen
dexamethasone	imatnib	disulfiram
indinavir	gemtuzamab	chloroquine
morphine		reserpine
digoxin		terfenadine
antihelminthics		

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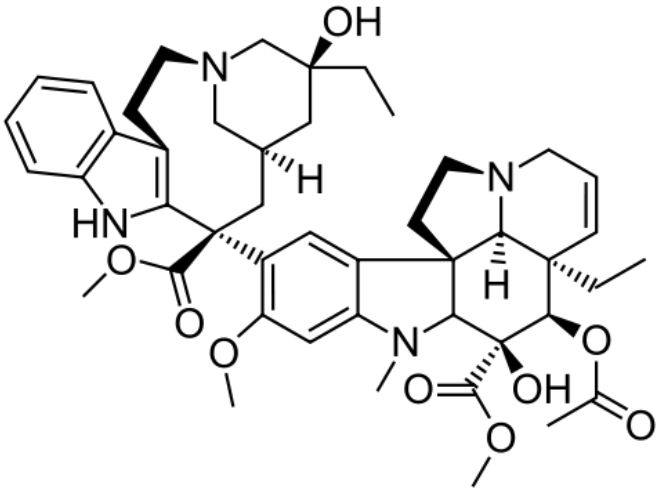
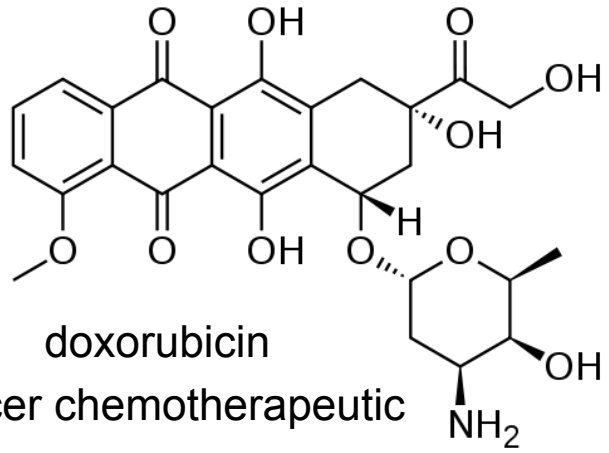
What is a Drug?

Substrates of P-Glycoprotein

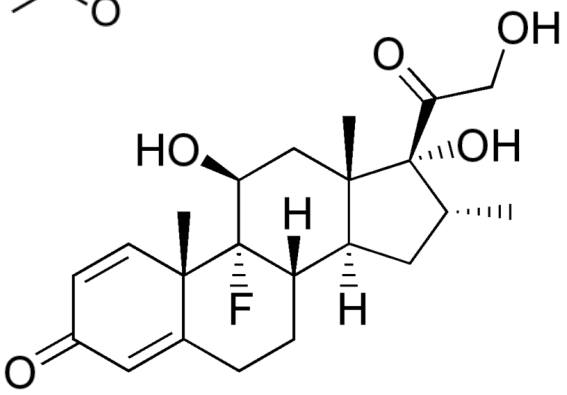
- Examples of PgP substrates



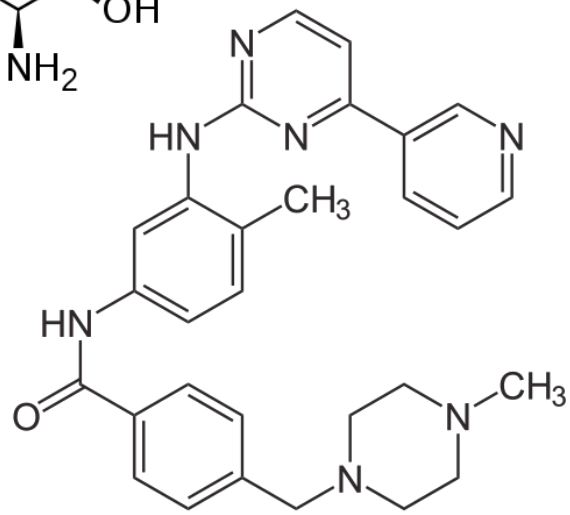
vinblastine
antimicrotubule agent



doxorubicin
cancer chemotherapeutic



dexamethasone
steroidal anti-inflammatory



imatinib
cancer chemotherapeutic

ABC Transporters

Role of P-Glycoprotein

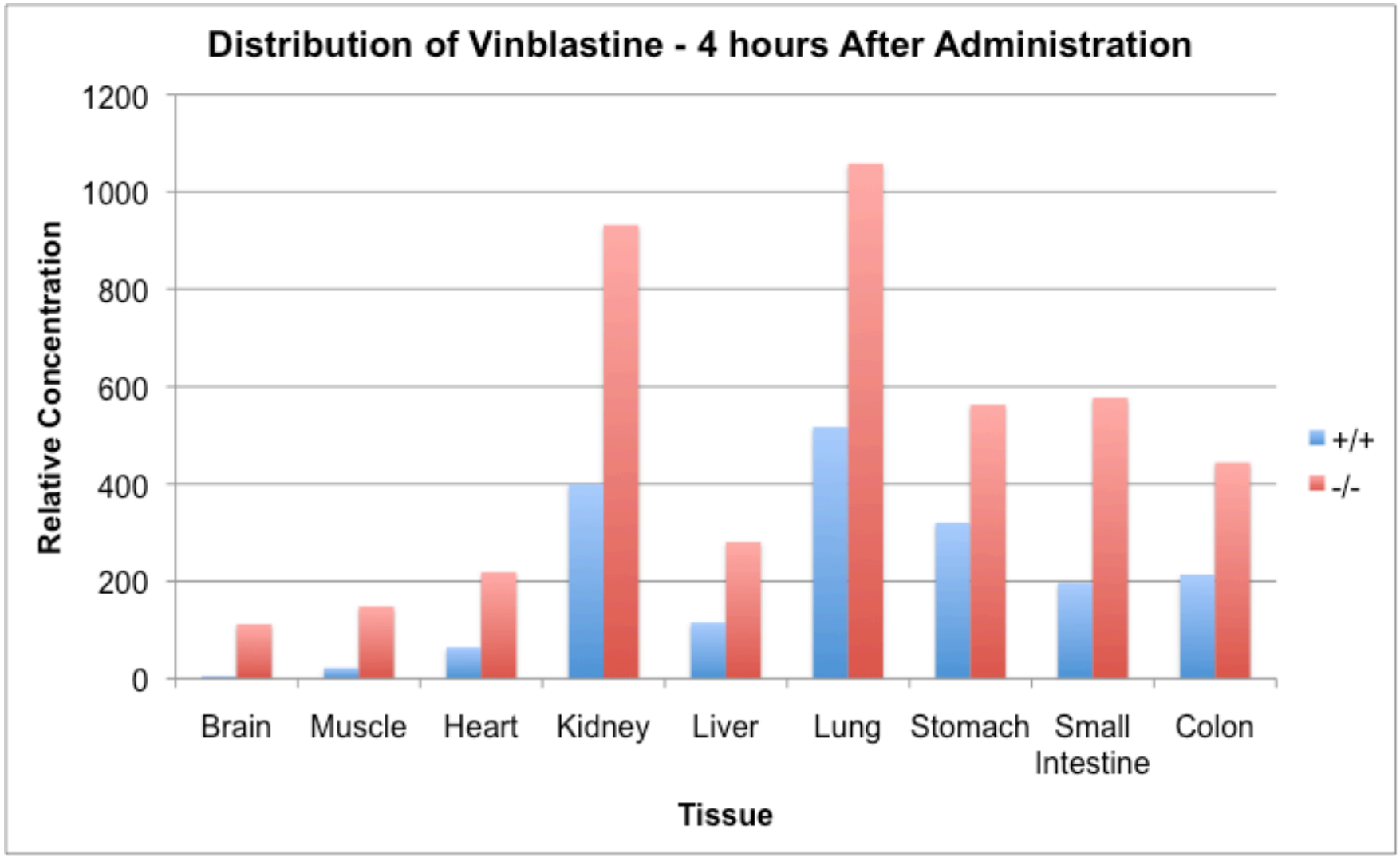
- PgP is expressed highly in the BBB and other barrier regions (blood-testis barrier, placenta)
  - This distribution of PgP suggests that it provides a protective role for sensitive organs

- Does not appear to fulfill any essential physiological role (under normal conditions)
  - Seemingly not essential for transport of endogenous substrates
  - Double knockout mice have been shown to be be phenotypically normal

- However, absence of PgP has a dramatic impact on the effect of drugs
  - Double knockout mice have been shown to have an increased sensitivity to a variety of drugs

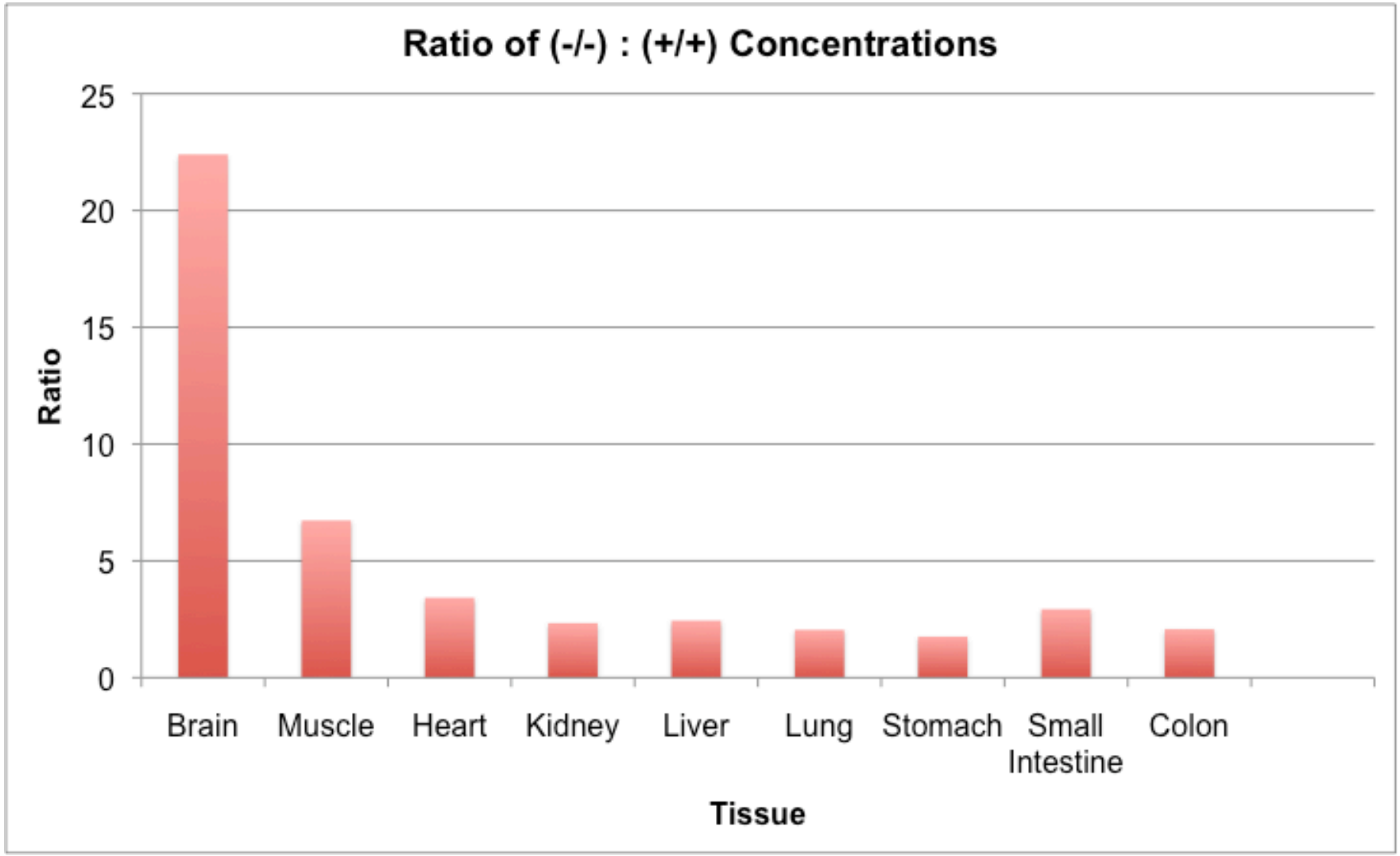
- PgP found in intestinal epithelial cells
  - Transport drugs from the blood and into the gastrointestinal tract
  - Prevent drug in the epithelium from entering the blood
  - Dramatically change (reduce) the rate and extent of absorption from some orally delivered drugs
  - Affects bioavailability (circulating, available drug)

Role of P-Glycoprotein



Data from Schinkel *et al.* 1994. Disruption of the mouse mdr1a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell.* 77, 491-502.

Role of P-Glycoprotein



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ABC Transporters

Mechanism of Drug Transport by PgP

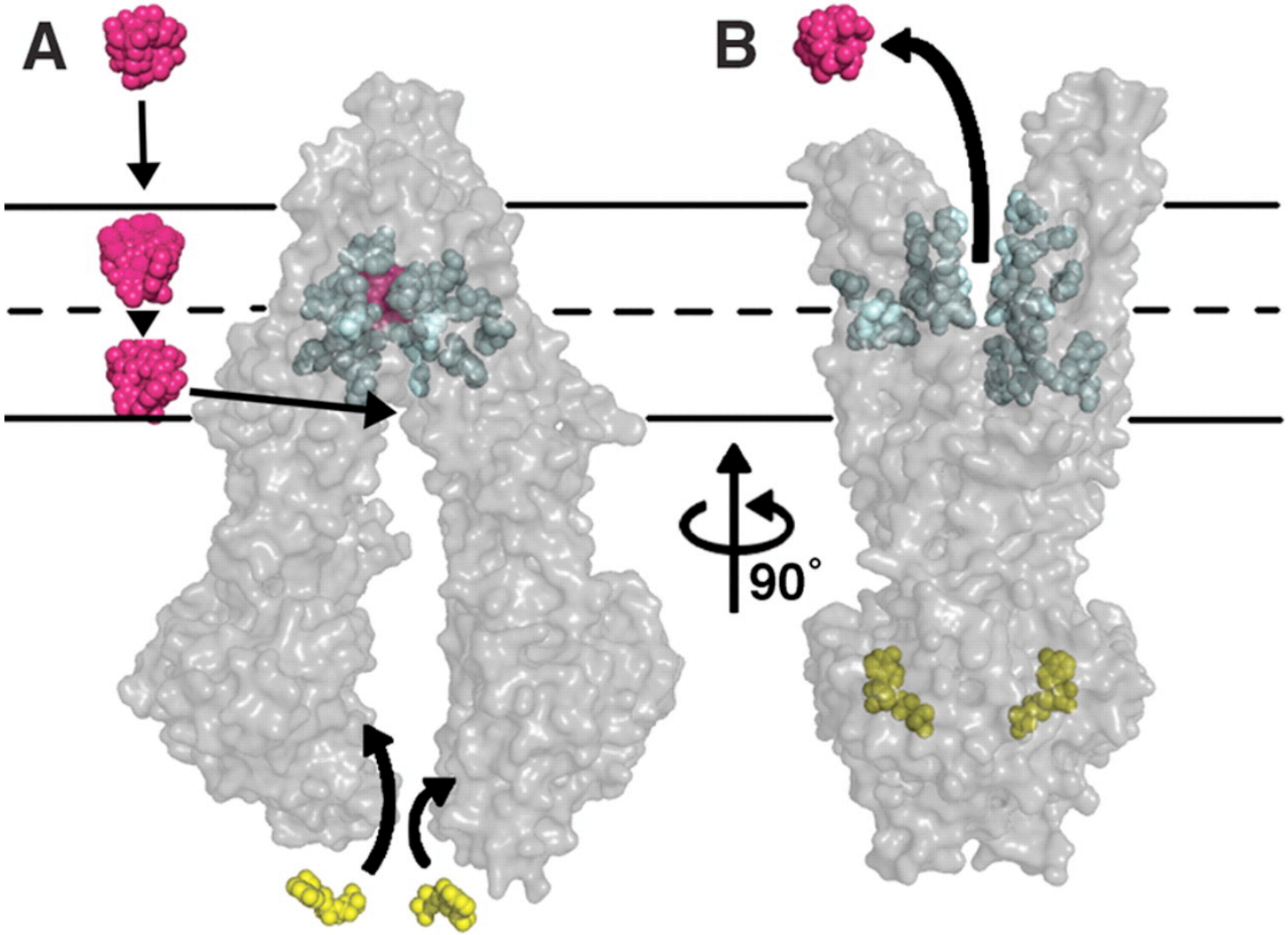
- Substrates of PgP are transported across cell membranes and thereby removed from cells in an ATP-dependent mechanism

- Process:
  - Substrates diffusing into the cell partition into the lipid bilayer of the membrane
  - Upon reaching the inner (cytoplasmic) leaflet of the membrane, the substrate will interact with an available PgP molecule
  - The substrate enters the internal drug-binding site of the PgP through a portal region
  - The substrate binds to the drug-binding site
  - ATP binds to the ATP binding sites of the PgP
  - Hydrolysis of ATP to ADP + Pi provides the energy for causing a conformational change
  - The substrate in the drug-binding site is exposed and becomes unbound from PgP
  - Hydroylsis of a further ATP to ADP + Pi returns the protein to the initial conformation

- *Note:* The exact process/order in which ATP is utilised and the substrate ejects from the cell is still under investigation

ABC Transporters

Mechanism of Drug Transport by PgP



ABC Transporters

Regulation of PgP Expression

- PgP coded by the ABCB1 gene

- Upregulated in response to xenobiotic (foreign compound) exposure
- Upregulated in cancer cells that develop resistance to chemotherapy

- PgP Gene Polymorphisms
  - Around 30 different single nucleotide polymorphisms (SNPs) have been identified by large scale sequencing efforts
  - Polymorphisms affect the expression and function of PgP
  - Polymorphisms may affect individual susceptibility to disease of response to drug treatment
    - Variation in ability to remove harmful toxins e.g. bacterial toxins
    - Variation in ability to remove administered drugs e,g. cancer chemotherapeutics
  - Relevant to personalised healthcare strategies (right drug for the right patient)

ABC Transporters

P-Glycoprotein as a Drug Target

- The presence of PgP in cell membrane is of benefit as it protects cells from harmful exposure to deleterious effects of xenobiotic, toxic compounds

- The wide range of PgP substrates means that it significantly affects the transport of drugs to cellular targets

- PgP expression is often upregulated in cancer cells, which provides an additional barrier to chemotherapy aimed at target in cancerous tissues

- Upregulation of PgP expression correlates with increased resistance to chemotherapy
  - Mechanism for avoiding cell death from toxicity
  - Reduced exposure of cellular targets to drug

- Therefore, modulators that affect drug transport by PgP have clinical relevance
  - Can help reduce the effect of PgP in multidrug resistance (MDR)
  - May be used in combination with other drugs to improve passage of drug to target

Multidrug Resistance-Associated Proteins

ABC Transporters

Multidrug Resistance-Associated Proteins (MRPs)

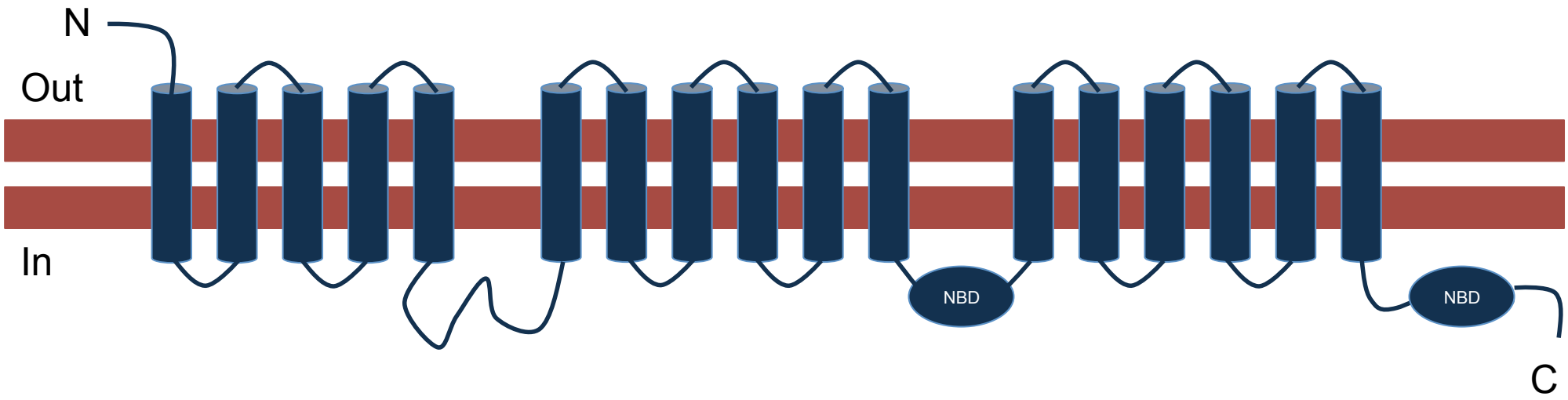
- Family of ABC transporter proteins
- Widely expressed in multiple tissues types
- Encoded by the ABC genes:
- MRP1 and MRP2
  - Transport of both suitable endogenous and xenobiotic compounds that are conjugated:
    - Glutathione conjugates
    - Glucuronide conjugates
    - Sulfate conjugates
- In epithelial cells:
  - MRP1 localised in the basolateral membrane
  - MRP2 localised in the apical membrane

Gene	Protein
ABCC1	MRP1
ABCC2	MRP2
ABCC3	MRP3
ABCC4	MRP4
ABCC5	MRP5
ABCC6	MRP6
ABCC10	MRP7
ABCC11	MRP8
Etc…	>12 now known

ABC Transporters

Multidrug Resistance-Associated Proteins

- Structure – MRP1



ABC Transporters

Multidrug Resistance-Associated Proteins

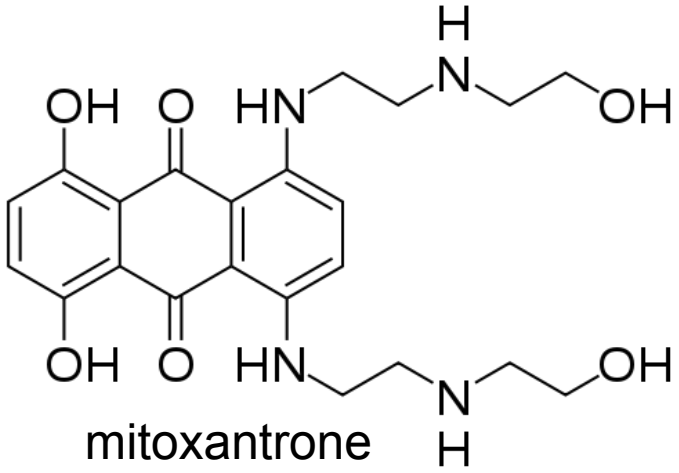
- MRP1 fulfill a similar role to PgP in that it exports xenobiotic substrates that may be toxic
- MRP1 also transports endogenous molecules
- Typically, substrates are conjugated with glutathione, glucuronide or sulfate
- MRP1 is linked with multidrug resistance and is upregulated in some cancers and contributes to multidrug resistance (MDR) in chemotherapy
- The other MRP proteins have similar functions, with varying specificity and selectivity of substrates

Breast Cancer Resistance Protein

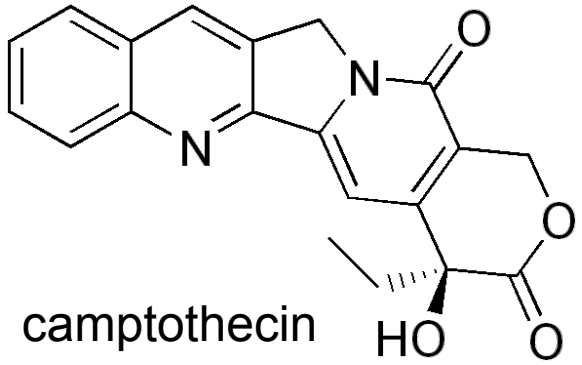
ABC Transporters

Breast Cancer Resistance Proteins (BRCP)

- Family of ABC transporter proteins
- Expressed in several tissue types especially:
  - breast duct lobules
  - placenta
  - intestine
- Encoded by the ABCG2 gene
- BRCP related to drugs resistance in cancer chemotheraputivcs. E.g.:
  - mitoxantrone
  - camptothecin
  - …and analogs of these drugs
- Not modulated by most PgP modulators (e.g. Verapamil, cyclosporin A).



mitoxantrone

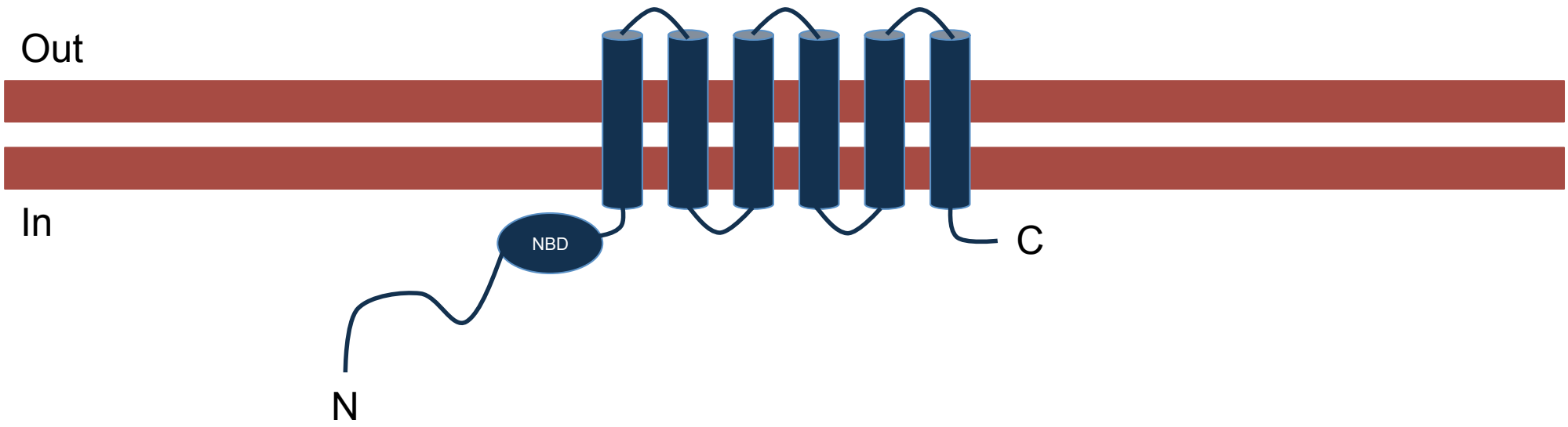


camptothecin

ABC Transporters

Multidrug Resistance-Associated Proteins

- Structure - BRCP



Lecture Summary

Main Points

- ATP-binding cassette (ABC) transporters are a superfamily of proteins that transport substrates out across cellular membranes

- They are seemingly non-essential in normal conditions but dramatically alter the transport of drugs through tissues, having an effect of the pharmacological effects observed

- They are capable of exporting a diverse range of substrates that cover nonpolar and amphipathic solutes as well as conjugates. Many of the chemical groups that have a high affinity for ABC transporters are found in drugs.

- P-glycoprotein is the most studied of these transporters and has been shown to transport substrates using a process that requires ATP

- ABC transporters are expressed in most tissues but expression is higher in those tissues that protect sensitive organs in the body. Expression increases when cells are exposed to xenobiotics

- ABC transporters play a key role in multidrug resistance (MDR), with particular relevance to cancer therapy. Targeting these proteins with modulating compounds is a strategy for overcoming MDR.

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