"*In Silico* Design and QMMM Testing of New Cancer Chemotherapy Compounds"

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Acknowledgements

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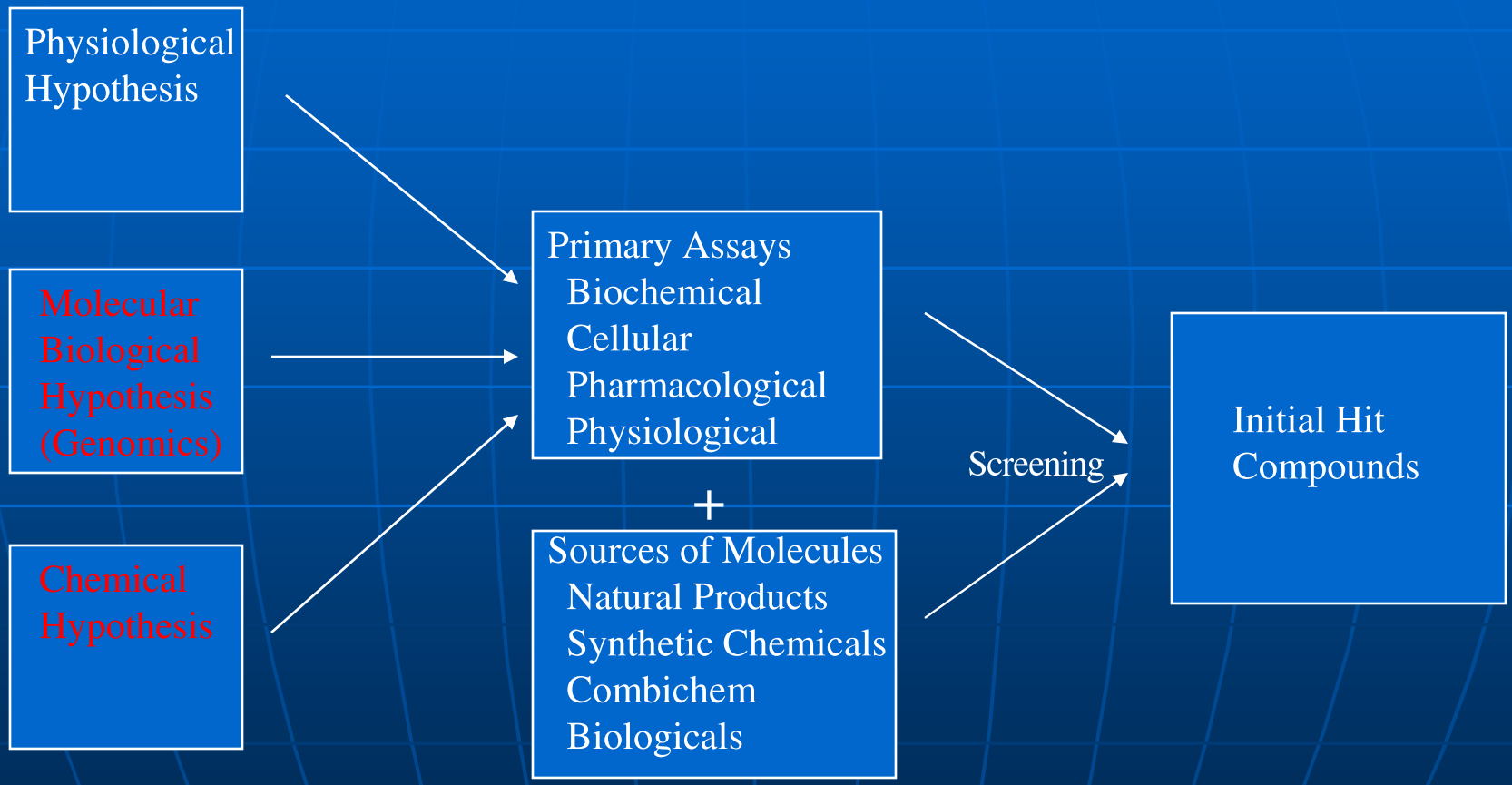
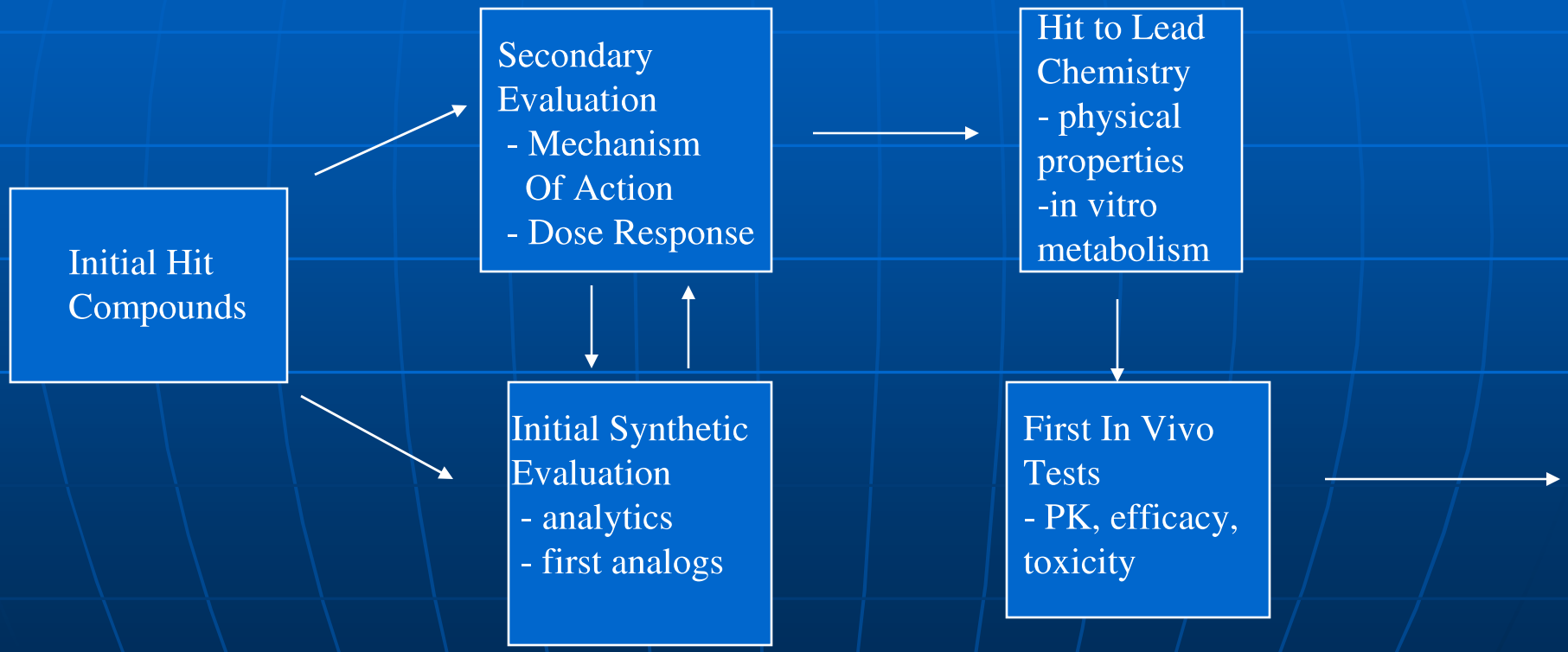
Discovery and Development

- The time from conception to approval of a new drug is typically 10-15 years
- The vast majority of molecules fail along the way (>100,000)
- The estimated cost to bring to market a successful drug is now $800 million (Dimasi, 2000)

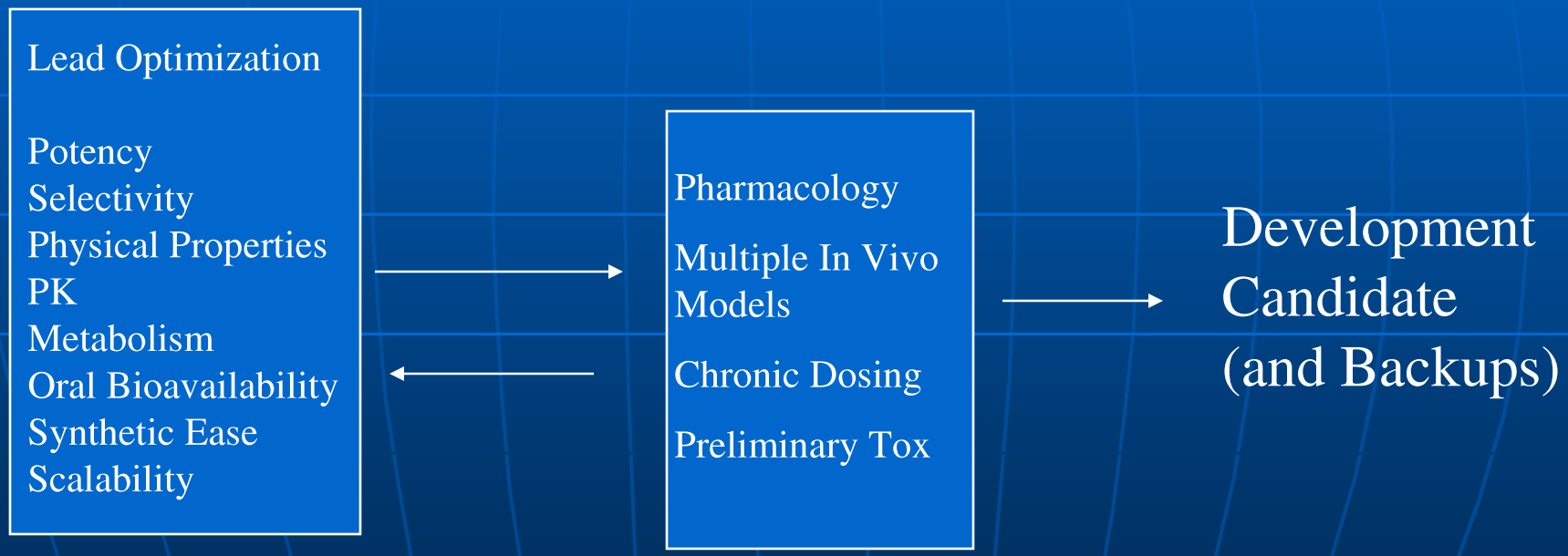
Drug Discovery Processes Today



Drug Discovery Processes - II



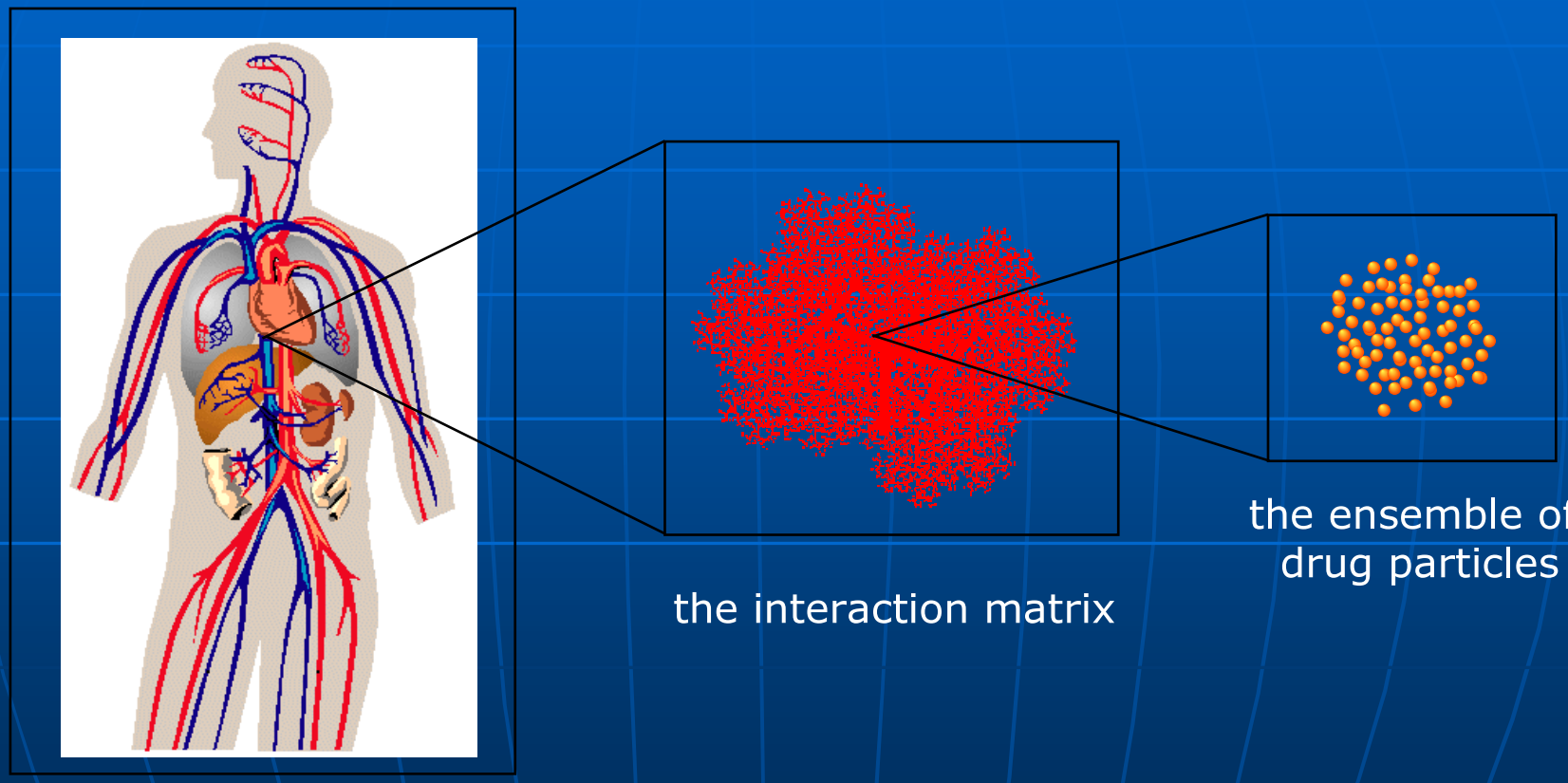
Drug Discovery Processes - III



Issues in Drug Discovery

- Hits and Leads - Is it a "Druggable" target?
- Resistance
- Pharmacodynamics and pharmacokinetics
- Delivery - oral and otherwise
- Metabolism
- Solubility, toxicity
- Patentability

The Pharmacokinetic System



the medium

the interaction matrix

the ensemble of drug particles

Chemotherapeutic agents' interactions with targets

Target : a molecule whose interaction with an anticancer agent will induce a cytotoxic effect

Targets are key molecules involved or required for cell mitosis and/or survival

Conventional chemotherapy acts on dividing cells only, but does not distinguish normal and abnormal dividing cells

Targeted agents are designed to act on targets which are specific to tumor cells

Relating druggable targets to disease...

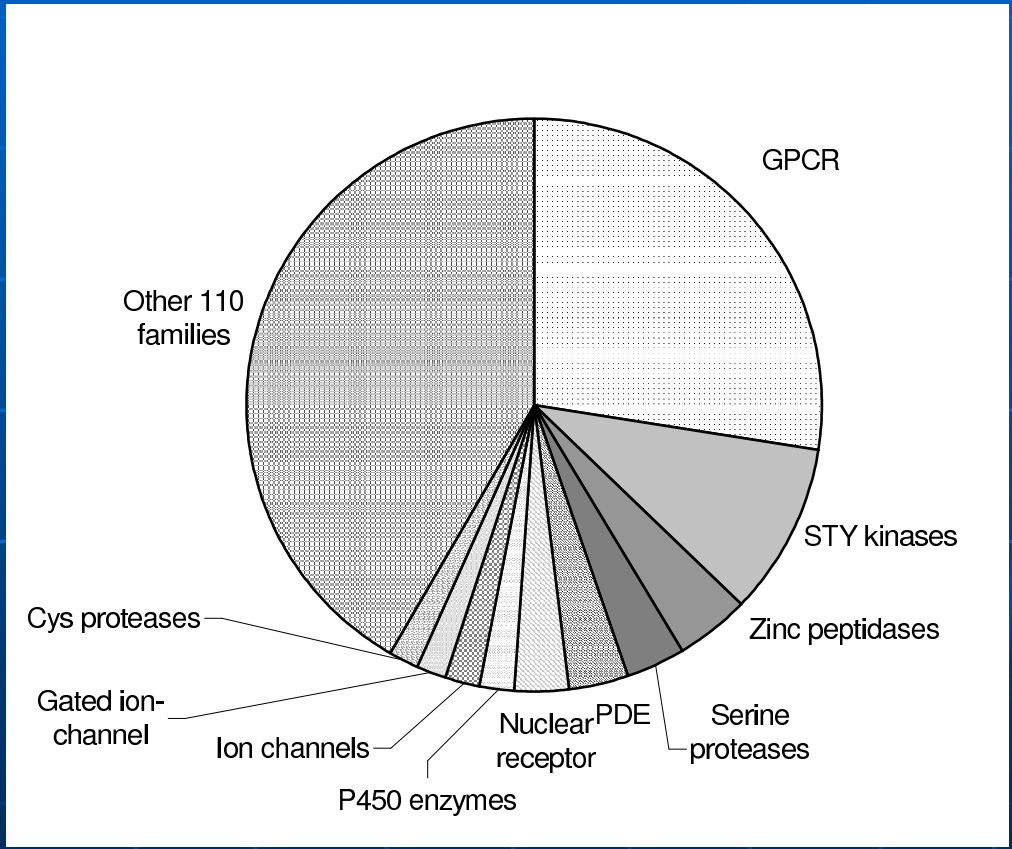
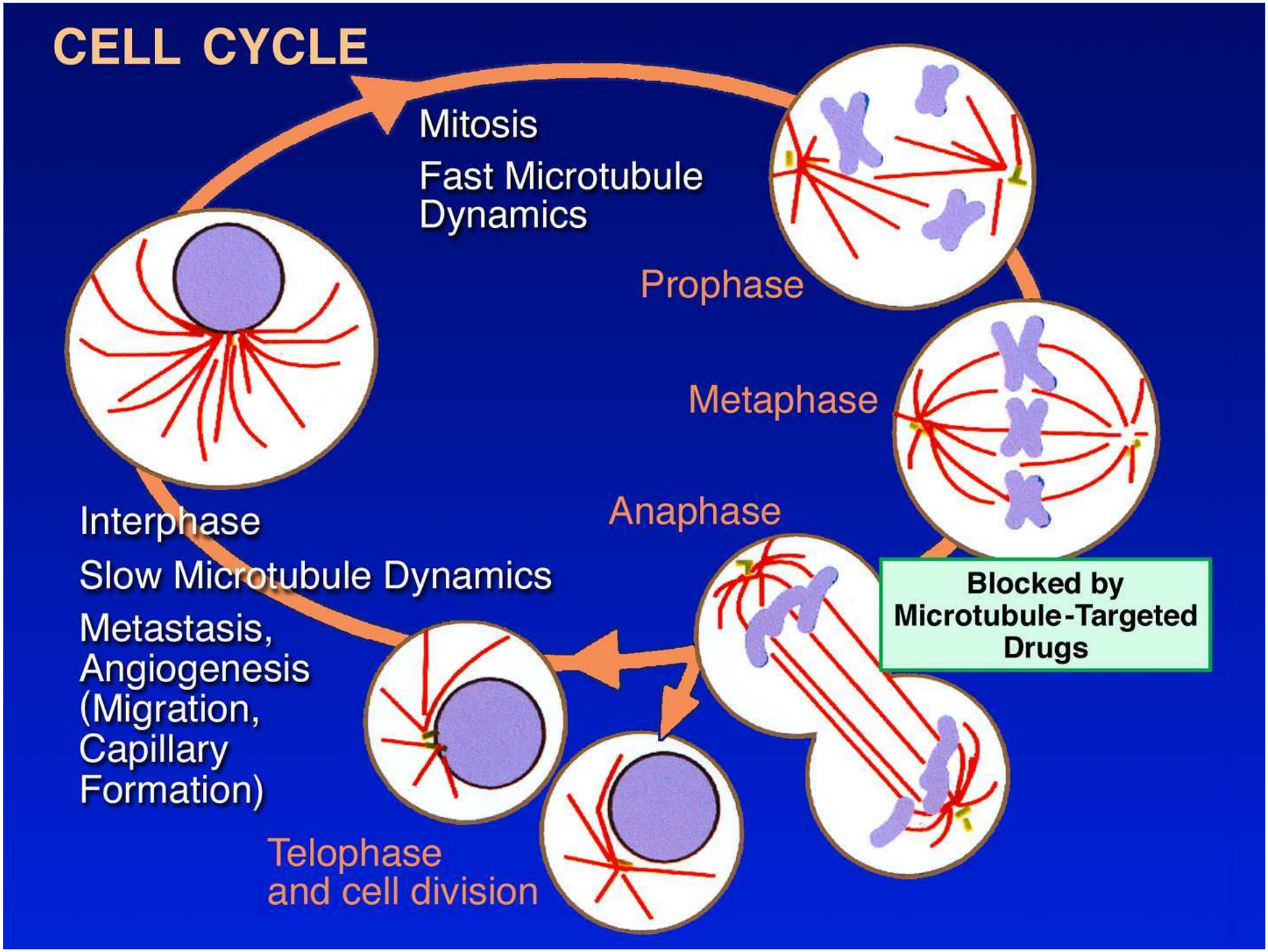
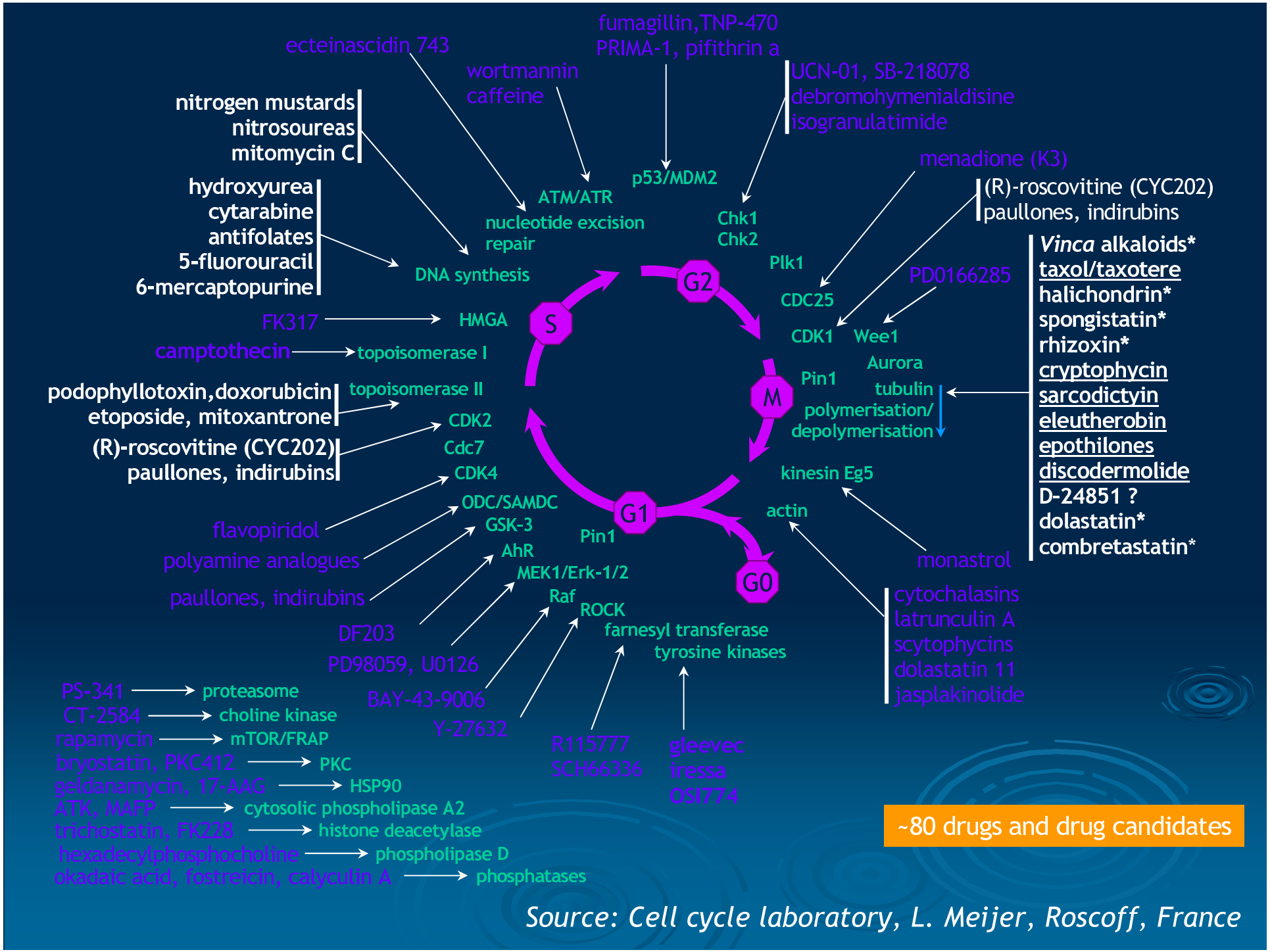


Fig. 3, Fauman et al.

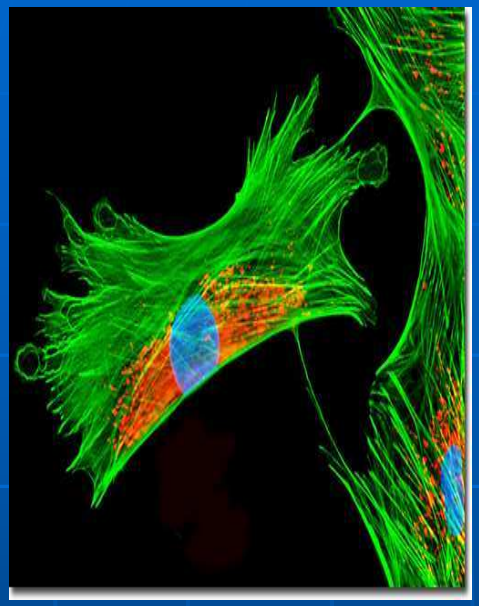
Analysis of pharma industry reveals:

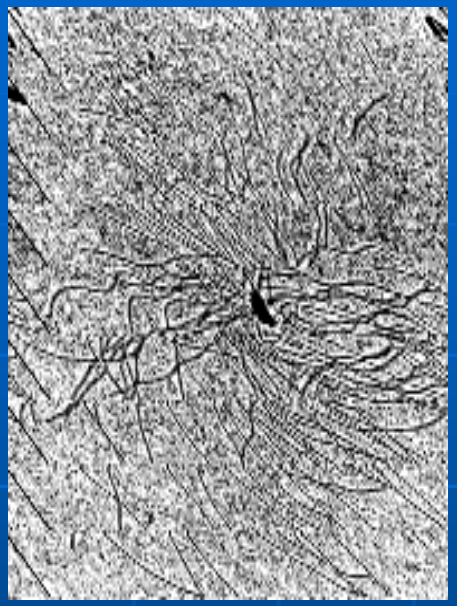
- Over 400 proteins used as drug targets
- Sequence analysis of these proteins shows that most targets fall within a few major gene families (GPCRs, kinases, proteases and peptidases)

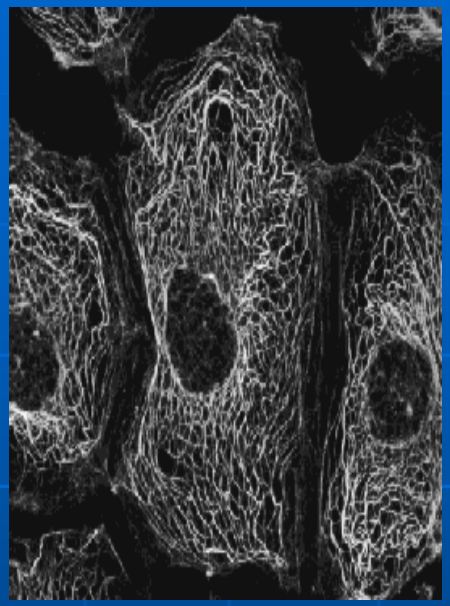

CELL CYCLE
Mitosis
Fast Microtubule Dynamics
Prophase
Metaphase
Anaphase
Blocked by Microtubule-Targeted Drugs
Telophase and cell division
Interphase
Slow Microtubule Dynamics
Metastasis, Angiogenesis (Migration, Capillary Formation)


ecteinascidin 743
fumagillin,TNP-470
PRIMA-1, pifithrin a
wortmannin
caffeine
UCN-01, SB-218078
debromohymenialdisine
isogranulatimide
nitrogen mustards
nitrosoureas
mitomycin C
menadione (K3)
(R)-roscovitine (CYC202)
paullones, indirubins
p53/MDM2
ATM/ATR
nucleotide excision
repair
Chk1
Chk2
hydroxyurea
cytarabine
antifolates
5-fluorouracil
6-mercaptopurine
DNA synthesis
Plk1
PD0166285
CDC25
CDK1
Wee1
Vinca alkaloids*
taxol/taxotere
halichondrin*
spongistatin*
rhizoxin*
cryptophycin
sarcodictyin
eleutherobin
epothilones
discodermolide
D-24851 ?
dolastatin*
combretastatin*
FK317
HMGA
camptothecin
topoisomerase I
podophyllotoxin,doxorubicin
etoposide, mitoxantrone
topoisomerase II
(R)-roscovitine (CYC202)
paullones, indirubins
CDK2
Cdc7
CDK4
Aurora
Pin1
tubulin
polymerisation/
depolymerisation
kinesin Eg5
actin
monastrol
flavopiridol
polyamine analogues
paullones, indirubins
ODC/SAMDC
GSK-3
AhR
MEK1/Erk-1/2
Raf
ROCK
farnesyl transferase
tyrosine kinases
DF203
PD98059, U0126
BAY-43-9006
Y-27632
R115777
SCH66336
gleevec
iressa
OSI774
cytochalasins
latrunculin A
scytophycins
dolastatin 11
jasplakinolide
PS-341 → proteasome
CT-2584 → choline kinase
rapamycin → mTOR/FRAP
bryostatin, PKC412 → PKC
geldanamycin, 17-AAG → HSP90
ATK, MAFP → cytosolic phospholipase A2
trichostatin, FK228 → histone deacetylase
hexadecylphosphocholine → phospholipase D
okadaic acid, fostreicin, calyculin A → phosphatases
S
G2
M
G1
G0
Pin1
~80 drugs and drug candidates
Source: Cell cycle laboratory, L. Meijer, Roscoff, France

Properties and Functions of Cytoskeleton







	Actin Filaments	Intermediate Filaments	Microtubules
Structure	Self-assembling protein, definite directionality	Tough, rope-like fibers	Stiff, hollow tubes of tubulin
Outer diameter	7nm	10nm	24 nm
Tensegrity structure	Tensional component Creates pulling forces	Tension-resistant component, hardens under strain Doesn't generated forces	Compression-resistant component Creates pushing forces
Inhibition by drugs	Cytochalasin B	Acrylamide	Colchicine, nocadzole

Conventional chemotherapy compounds

- ***DNA*** — *alkylating agents, platinum compounds, nucleotide analogues*
- ***DNA processing enzymes*** — *topoisomerase 1 and 2 inhibitors*
- ***Microtubules*** — *vinca alkaloids, taxanes*

Targeted agents

Tyrosine kinases — *imatinib, sunatinib, …*

Membrane antigens — *monoclonal antibodies: trastuzumab*

Anti-angiogenesis compounds: **bevacizumab**

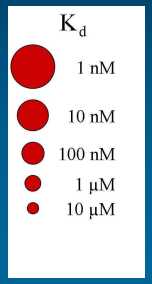
Targetted … really ?

Imatinib

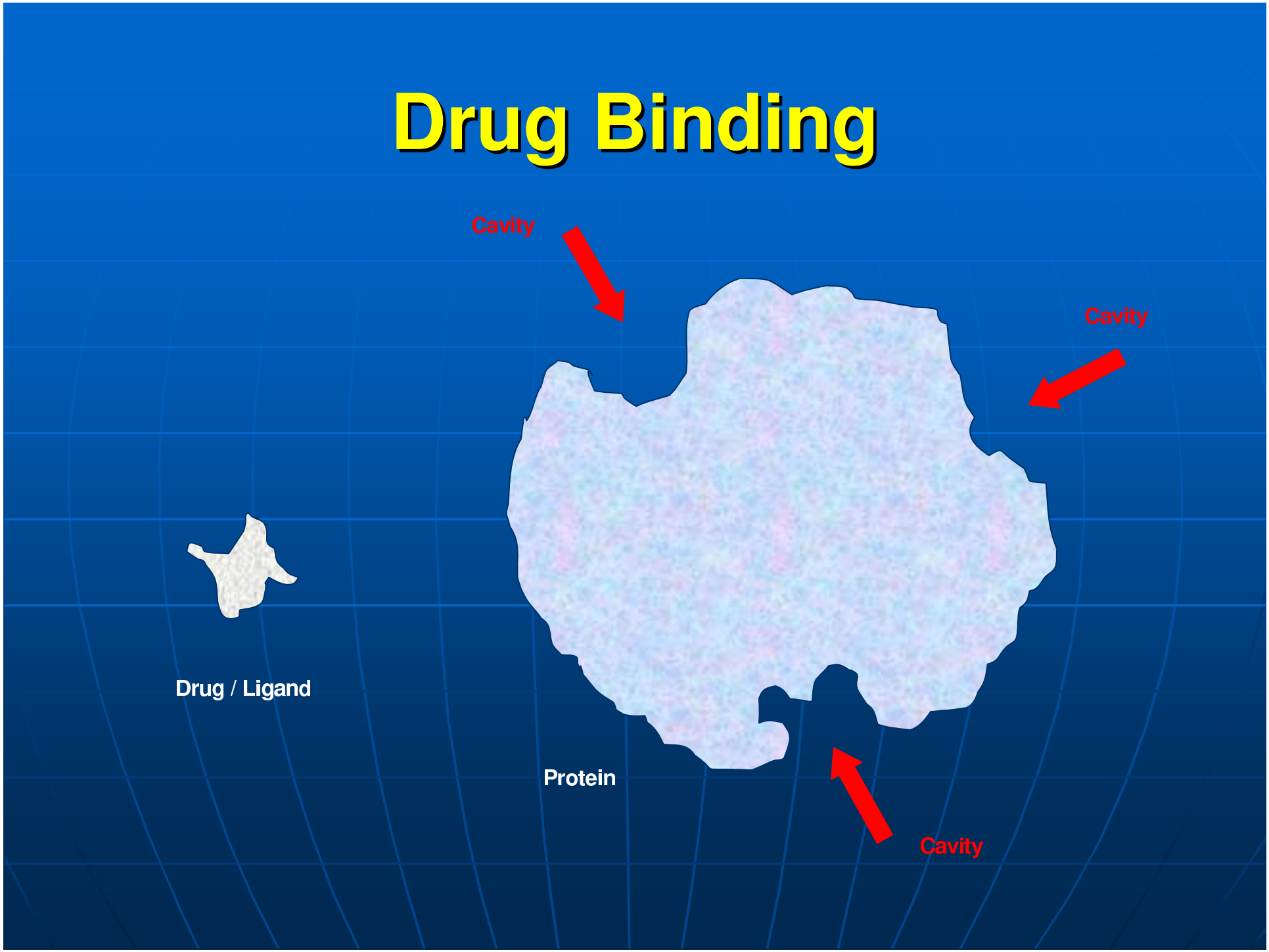
Sorafenib
(BAY43-9006)

Valatanib
(PTK787)

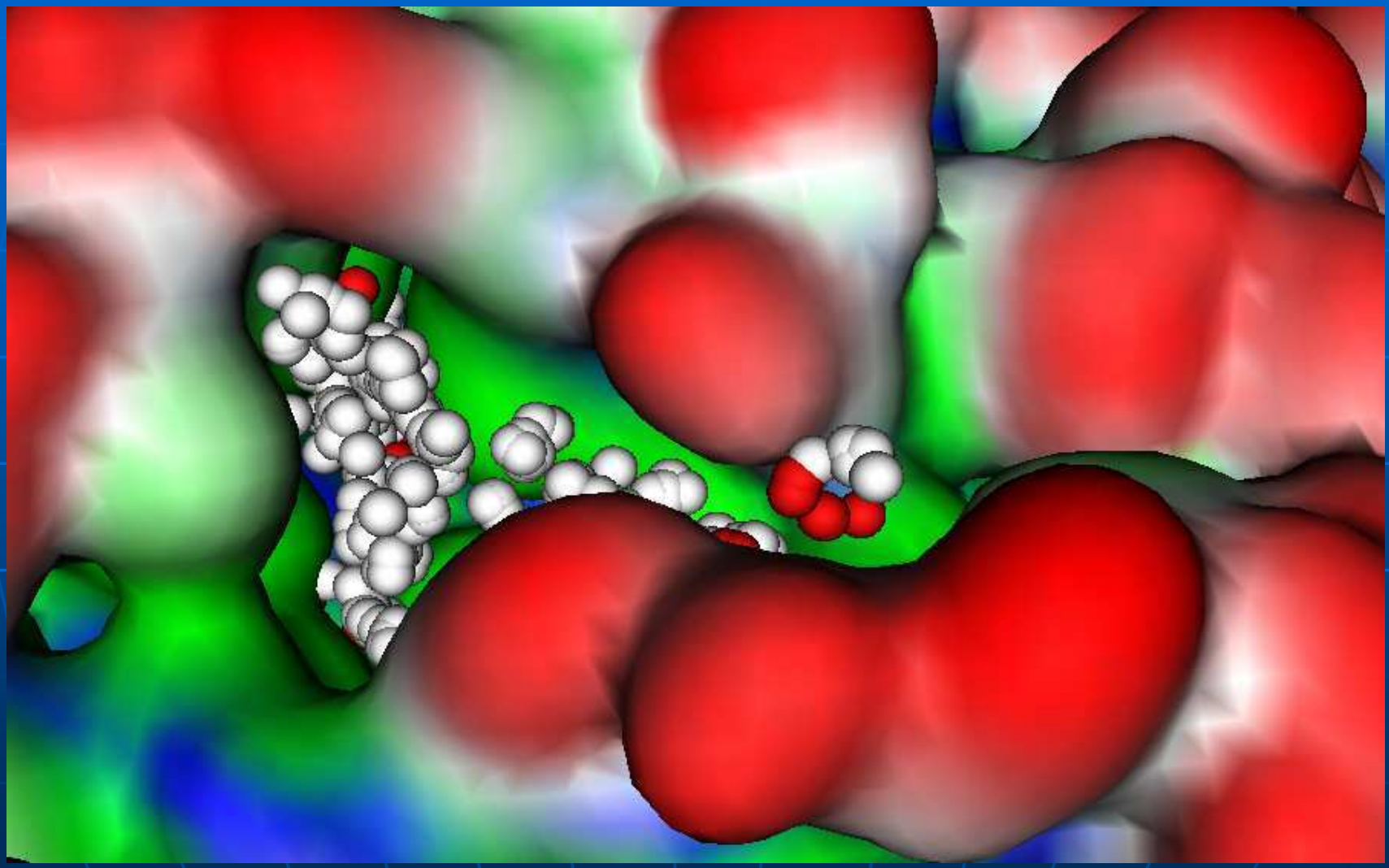
Sunitinib
(SU11248)



Drug Binding



Idea of a pocketome



Growing complexity

PDB : 48,000 proteins + homologues
Several pockets per protein

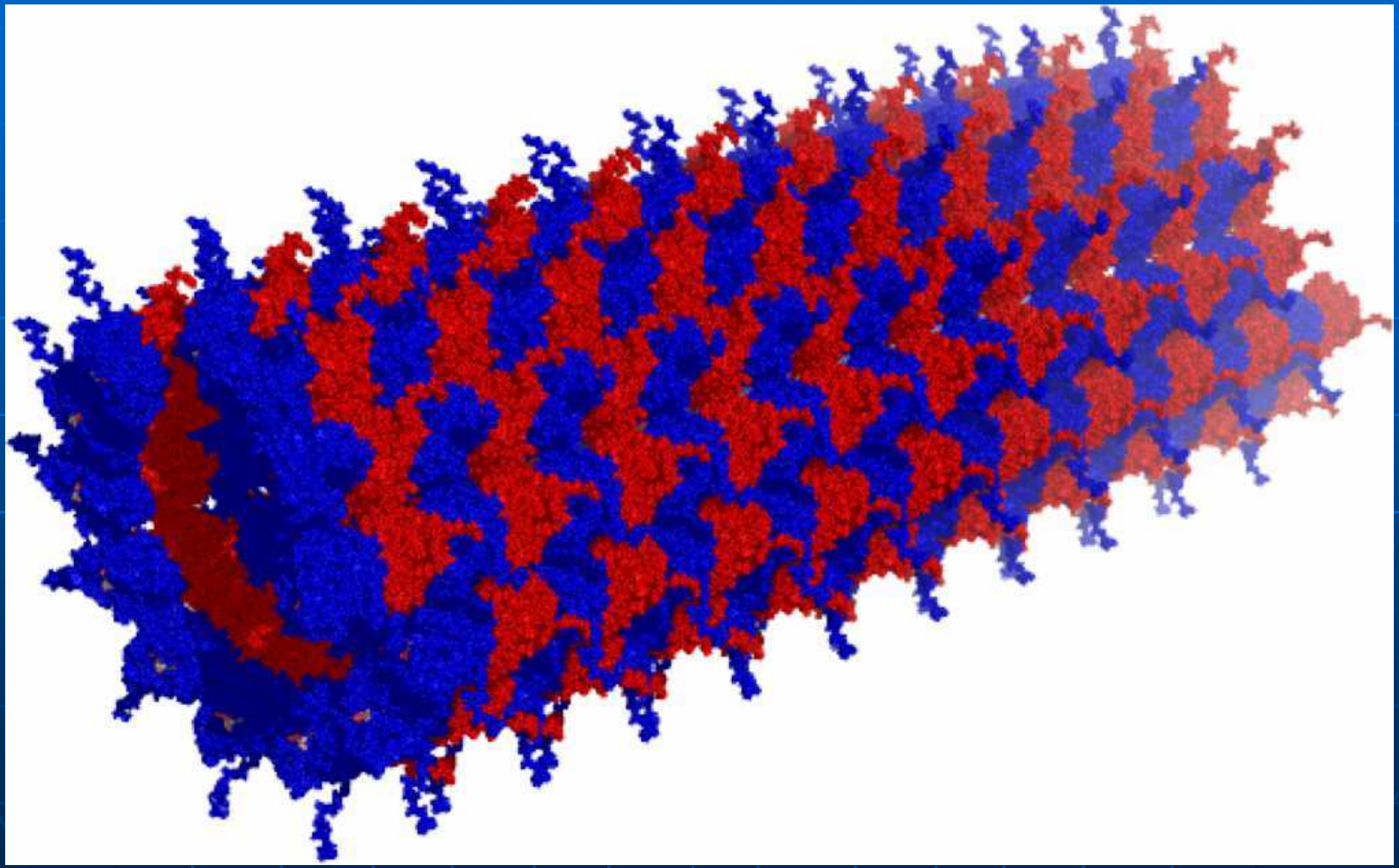
1500 potential targets (human proteins)
Approx. 400 (80 in cancer) utilized

Orange Book: 1800 medicinal drugs
Wishart's Drug Bank: 4900 drugs
Cancer chemotherapy drugs: 103

Combinatorial possibilities: infinite

Protein-drug interactions but also
Protein-protein inetractions

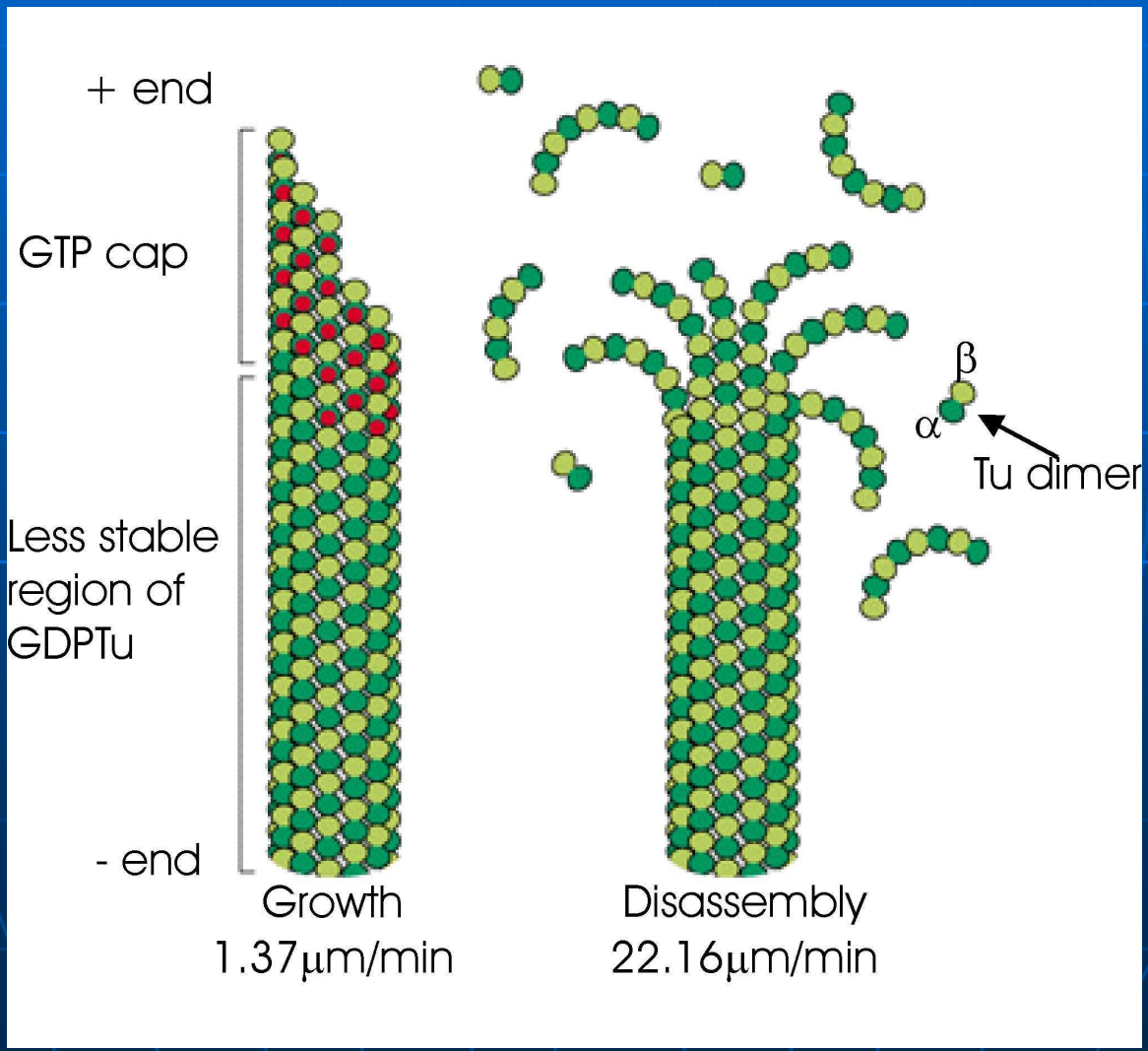
Microtubule Reconstruction



MT's Exhibit Dynamic Instability

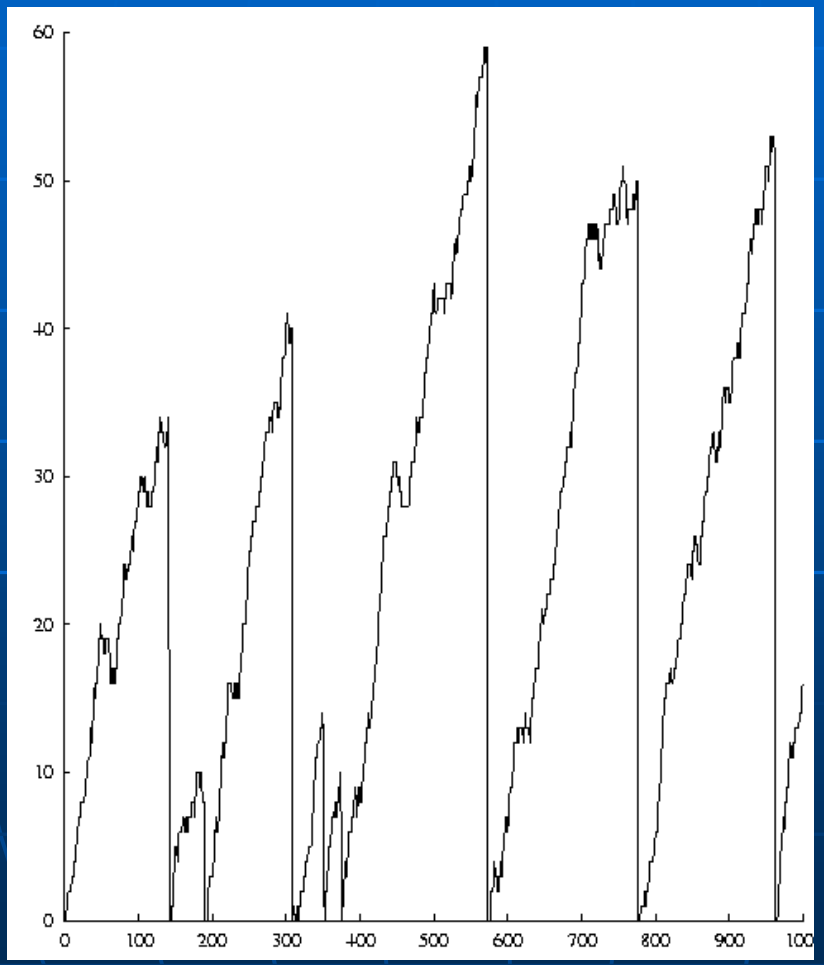
Mechanism:

- Stochastic
- Non-equilibrium
- Enigmatic

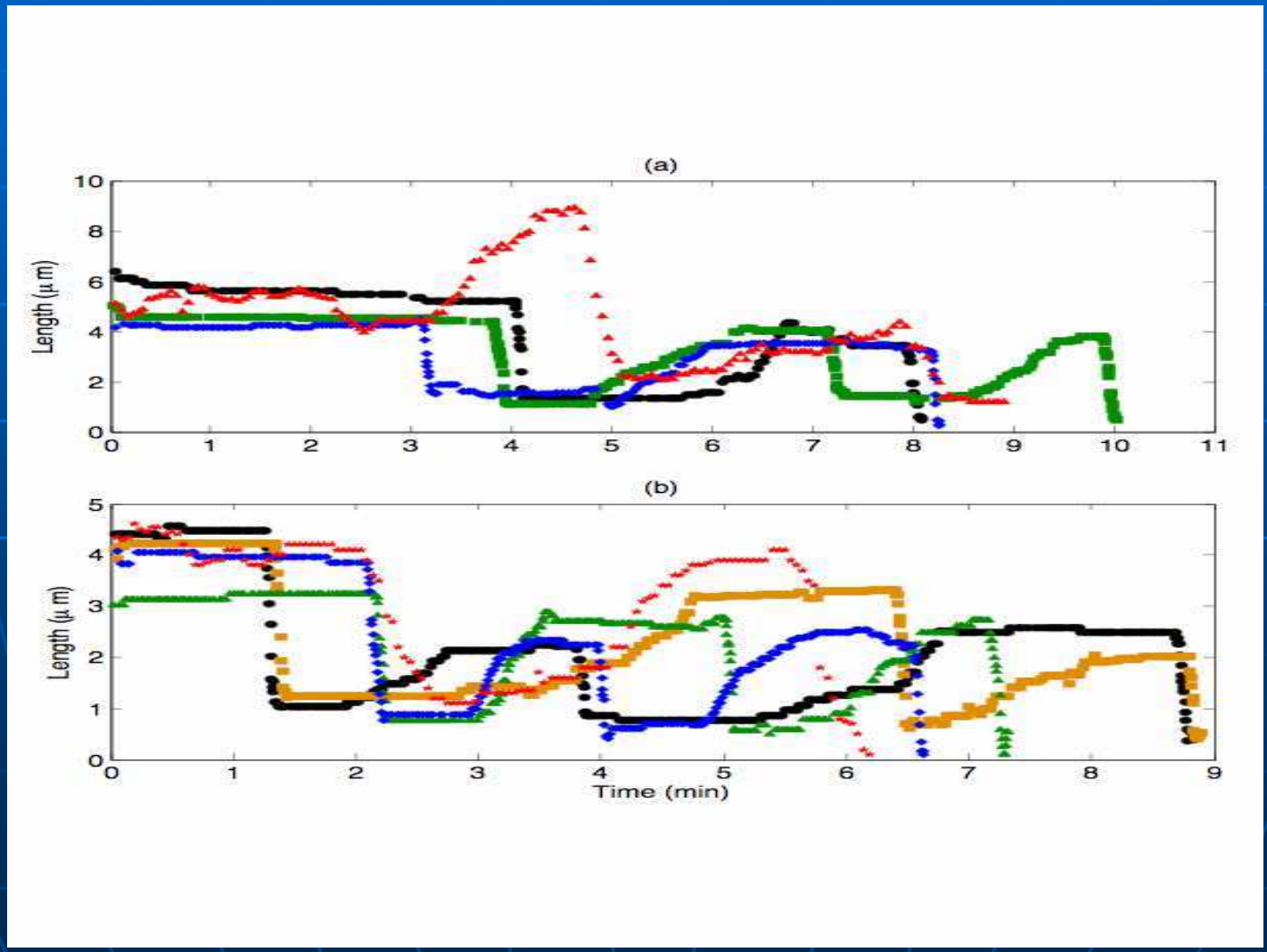


Individual MT life story: dynamic instability

- Catastrophes
- Rescues
- Growth phase
- Shrinking phase



MT polymerization for different isotypes of tubulin appears to differ



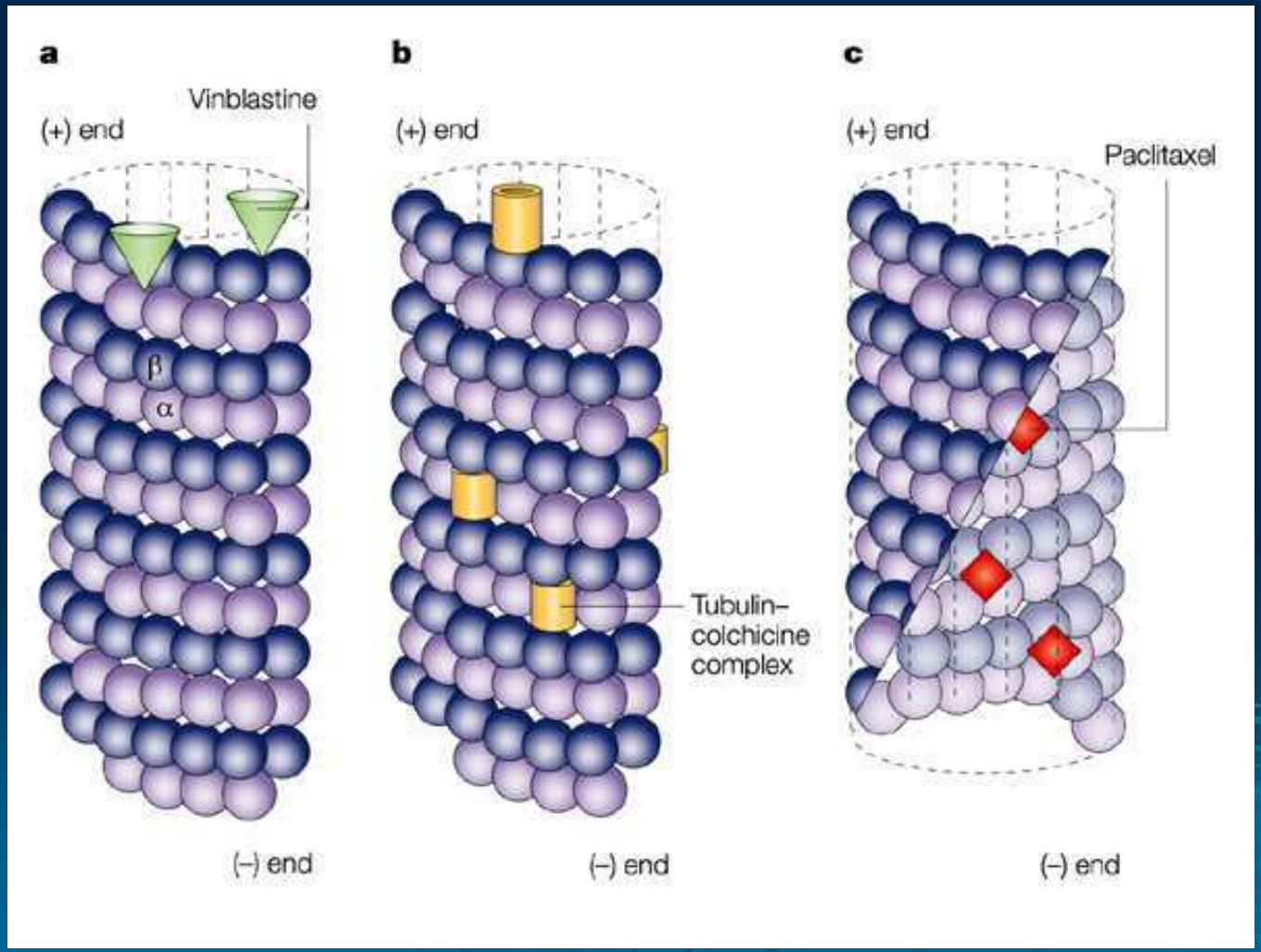
Microtubule Dynamics is Exquisitely Regulated in Cells

- **Regulated differentially in different *regions* of a single cell**
- **Regulated differentially during different *cell activities***
- **Speed up dramatically in *mitosis***
- ***Endogenous* cellular proteins regulate MT dynamics**
- **Tubulin isotypes and post-translational modifications regulate interactions with regulatory proteins**

Microtubule-Targeted Drugs Mimic Endogenous Regulators

Regulatory Protein	Location on MT	Mechanism
Tau, MAP 2, MAP4	Surfaces	↓ dynamics, enhance G-rate
XMAP215	Surfaces	Enhance dynamicity
MCAK	+ ends	↑ catastrophe
EB1	+ ends	↓ catastrophe, ↑ rescue
CLASP 1	+ ends	Enhance dynamicity
CLIP 170	+ ends	↑ rescue
Dynactin 1 (p150Glued)	+ ends	Nucleation, recruit dynein-cargo
LIS 1	+ ends	↓ catastrophe, recruit dynein?
NudA (dynein homolog)	ends	Cat, rescue frequencies, S-rate
stathmin	- ends, + ends, surfaces	↑ catastrophe, sequester tubulin
Γ-tuRC	- ends	Nucleation
ninein	- ends	Nucleation, anchorage

Drugs Bind Differently to Microtubules

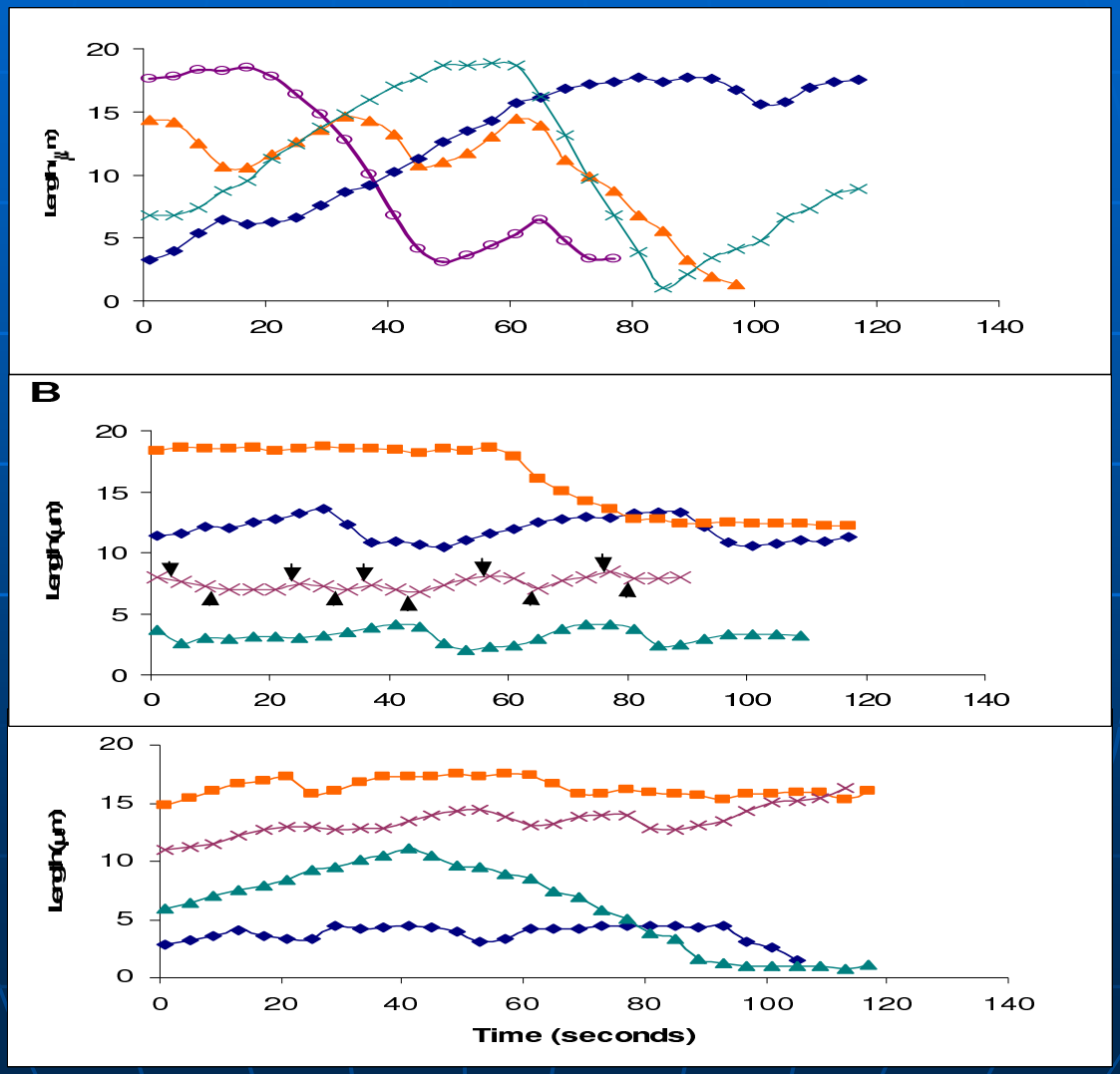


Microtubule Life Histories in Live Cells: Taxol and Epothilone Suppress Dynamic Instability

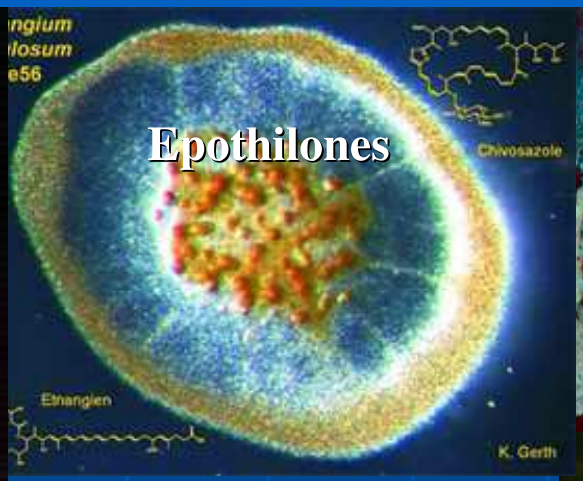
Control

3.5 nM epothilone B

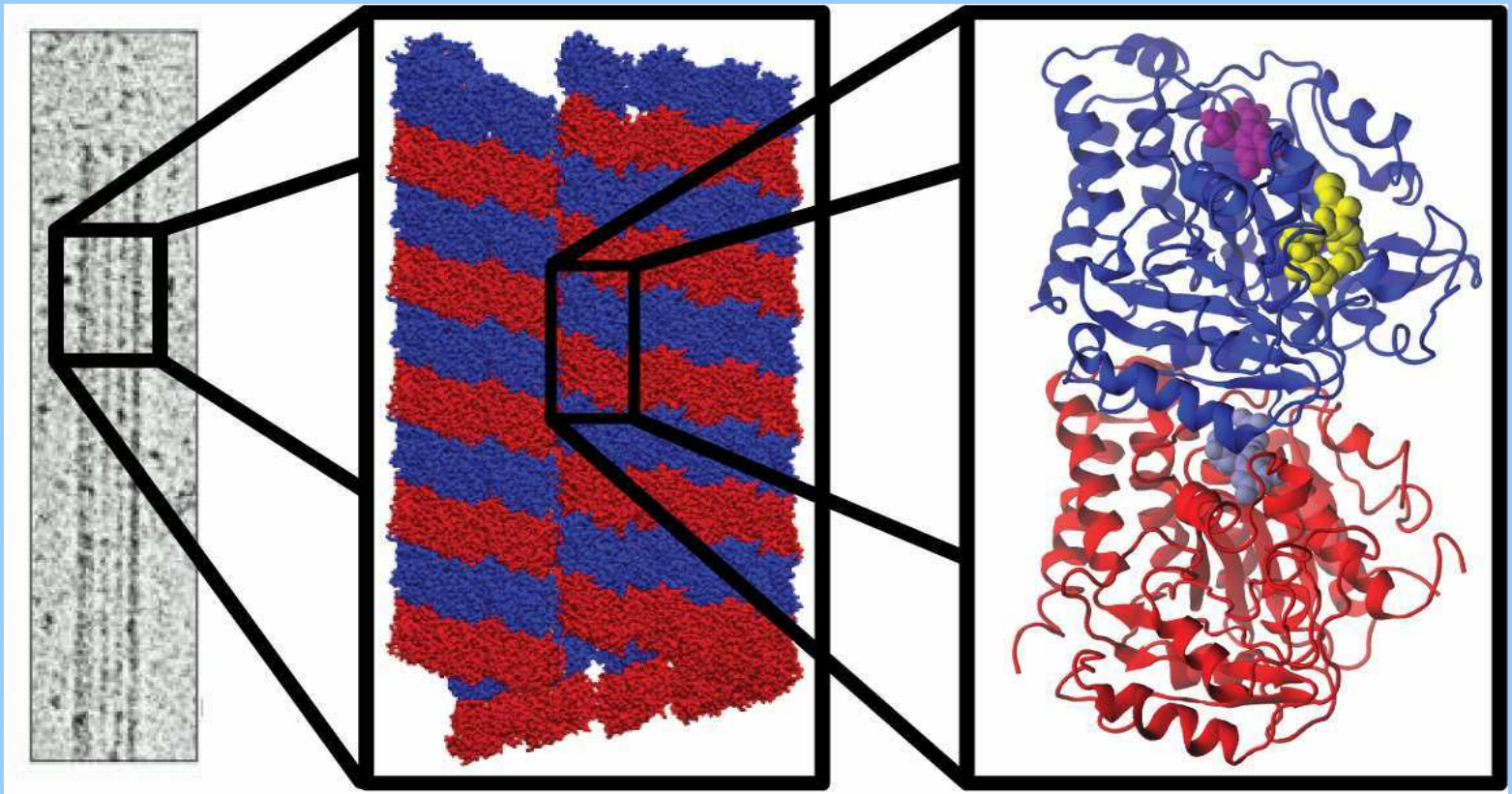
7.5 nM paclitaxel



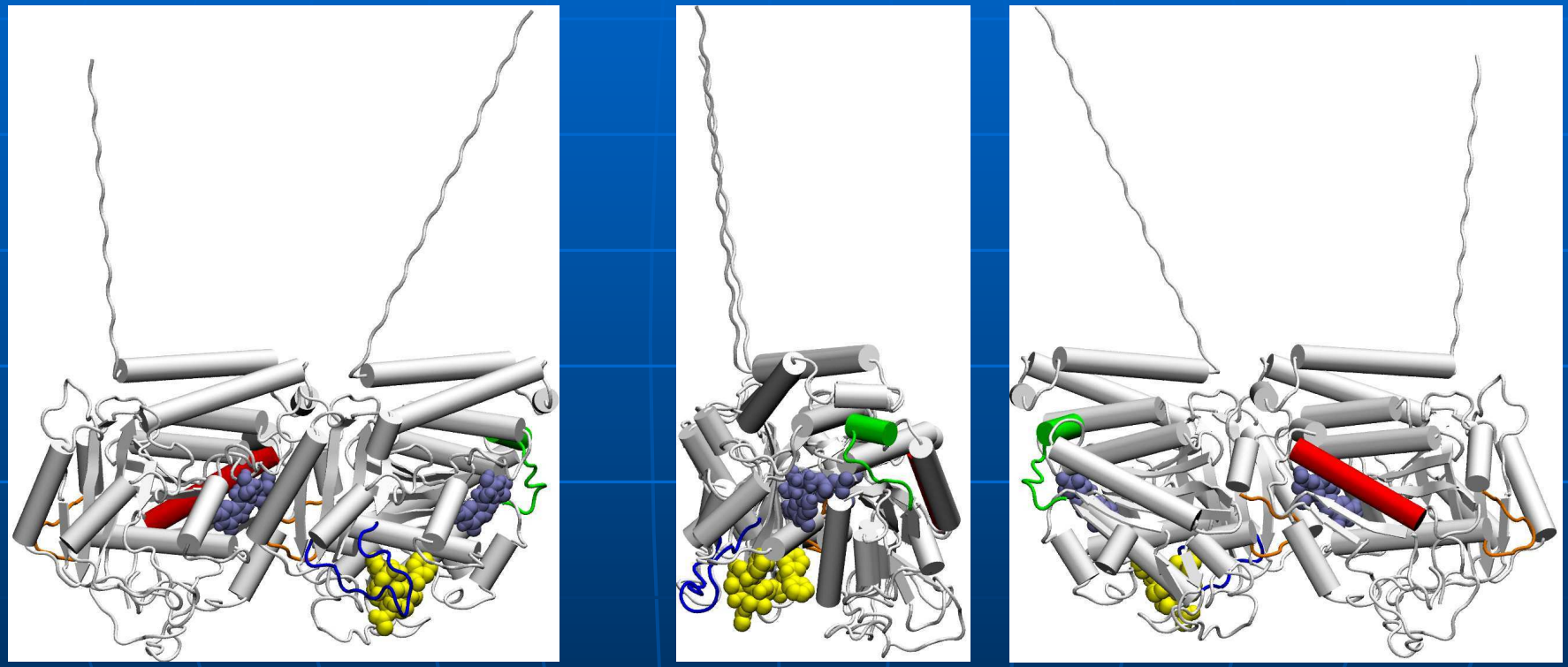
Natural Products have Yielded Potent Microtubule-Targeted Drugs



Zeroing in on the target



Tubulin Structural Motifs

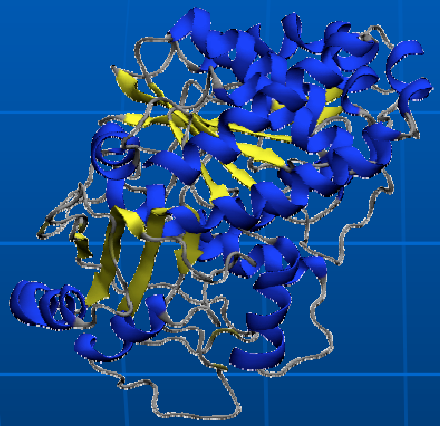


GTP/GDP M Loop H3 Helix T3 Loop T7 Loop Taxol

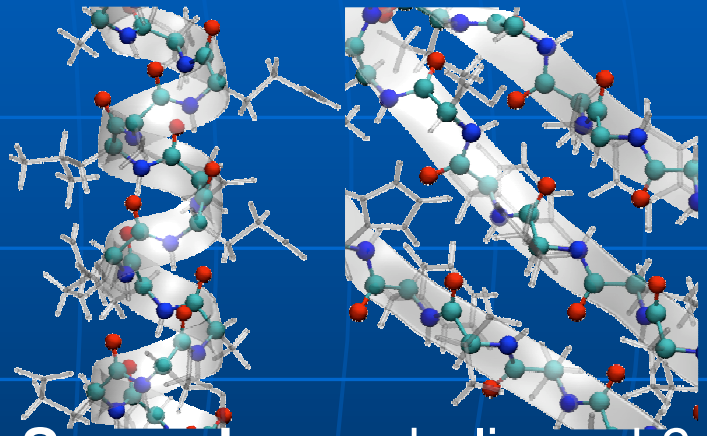
Target-Protein Structure

MRECISIHVGQAGVQIGNACWELYCLEHGIQPDGQMPSDKTIGGGDDSFNTFFSETGAGKHVPRAVFVDLEPTVIDEVRTGTYR
QLFHPEQLITGKEDAANNYARGHYTIGKEIIDLVLDRIRKLADQCTGLQGFSVFHSFGGGTGSGFTSLLMERLSVDYGKKSKLEF
SIYPAPQVSTAVVEPYNSILTTHTTLEHSDCAFMVDNEAIYDICRRNLDIERPTYTNLNRLIGQIVSSITASLRFDGALNVDLTEFQT
NLVPYPRGHFPLATYAPVISAEKAYHEQLSVAEITNACFEPANQMVKCDPRHGKYMACCLLYRGDVVPKDVNAAIATIKTKRTIQ
FVDWCPTGFKVGINYEPPTVVPGGDLAKVQRAVCMLSNTTAIAEAWARLDHKFDLMYAKRAFVHWYVGEGMEEGEFSEARED
MAALEKDYEEVGVDSVEGEGEEEGEEY

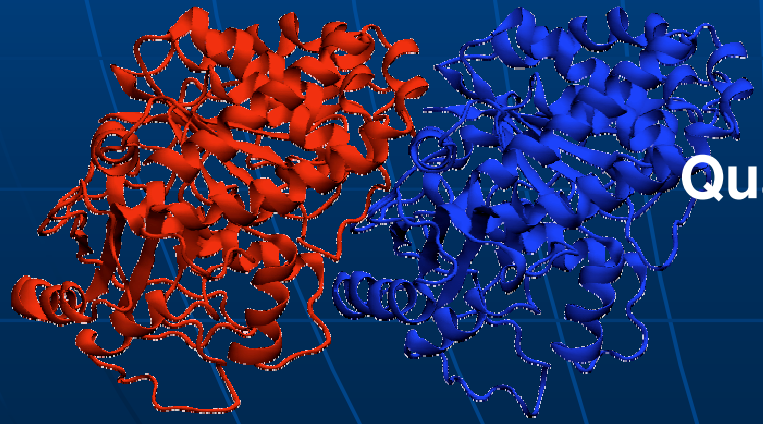
Primary: amino acid sequence



Tertiary: 3D-folding



Secondary: α-helix and β-sheet



Quaternary: multimeric arrangement