Considerations for bioequivalence evaluation of nano-particulate/molecular medicine

Jessie L.-S. Au, Pharm.D., Ph.D.
Institute of Quantitative Systems Pharmacology
University of Oklahoma College of Pharmacy
Taipei Medical University College of Pharmacy
Ohio State University
Optimum Therapeutics LLC

Outline

- **Definition of nanotechnology and molecular medicines**
- **FDA paradigm for equivalence recommendation**
- **Dimension-dependent and nanomaterial-dependent issues**
  - **Transport: whole organism, organ, extracellular matrix**
  - **Biointerfaces (interactions with biological materials)**
  - **Internalization, intracellular trafficking, recycling/exocytosis**
- **Quantitative multiscale modeling to address**
  - **Systemic/blood BE vs. target sites BE**
  - **Product-specific critical quality attributes**

Nanotechnology medicine (FDA Guidance for industry: Considering whether an FDA-regulated product involves the application of nanotechnology, June 2014)

- **Engineered to have (a) ~1-100 nm dimension, or (b) dimension-dependent effects, up to 1000 nm**
- **Exclude products that are not engineered to be the above**
- **8 approved drug-loaded intravenous products: 5 liposomal preparations, 1 nanoparticle, 1 lipid-drug complex); 45-150 nm**

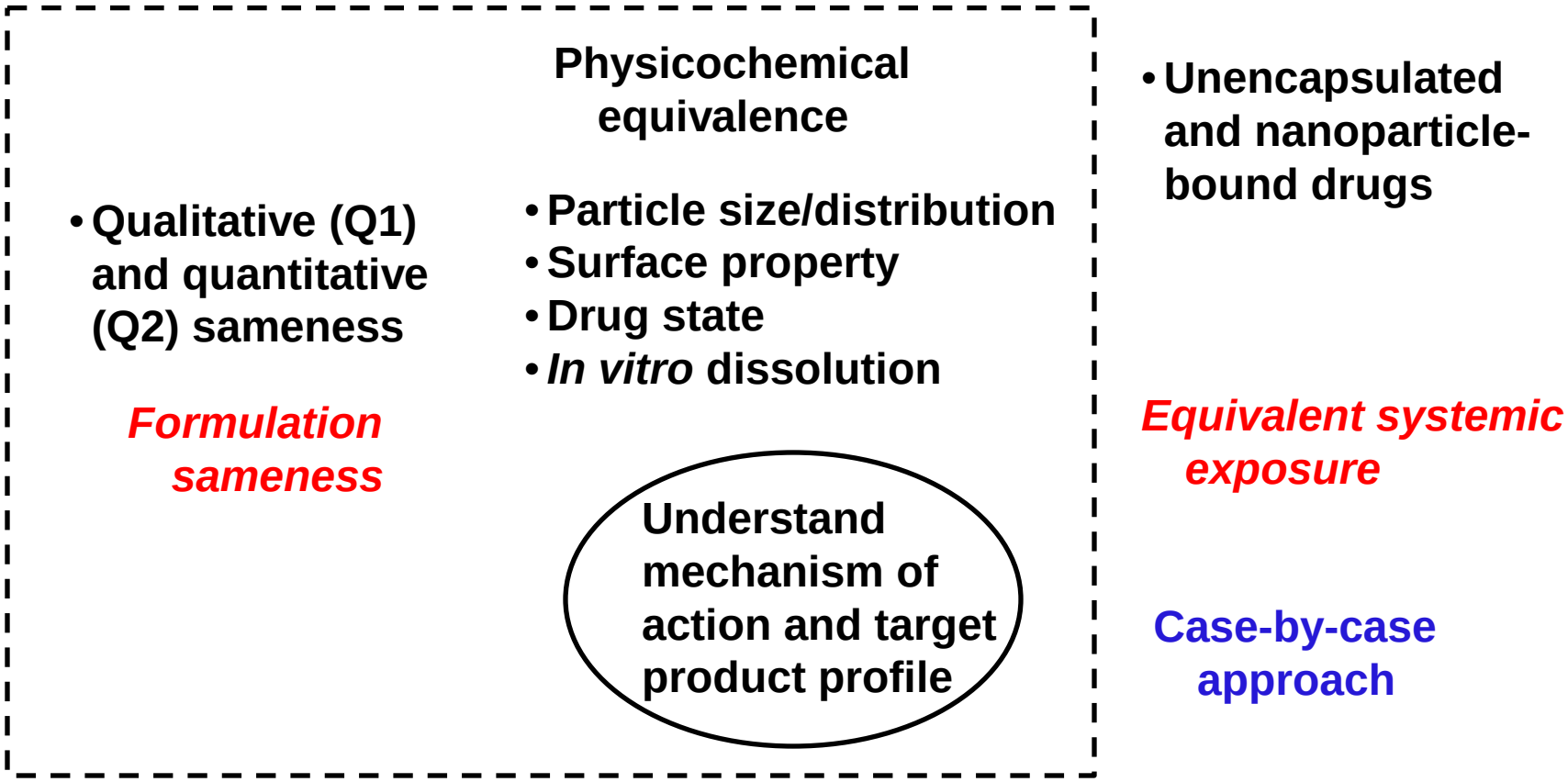
Molecular medicine

- **Agents that target extra-, peri- and intra-cellular molecules, are of nm dimension, share similar dimension- and biomaterial-dependent considerations as nanotechnology**
- **Approved products:**
  - **239 Proteins and peptides, >2 kDa, most >1 nm**
  - **72 Antibodies, >40 kDa, >5 nm**
  - **4 Antibody-drug conjugates, IgG-based, 149-160 kDa, ~15 nm**

Zheng et al., *AAPS J*. 19:619, 2017 Usmani et al., *PLoS One.* 12:e0181748, 2017 https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm

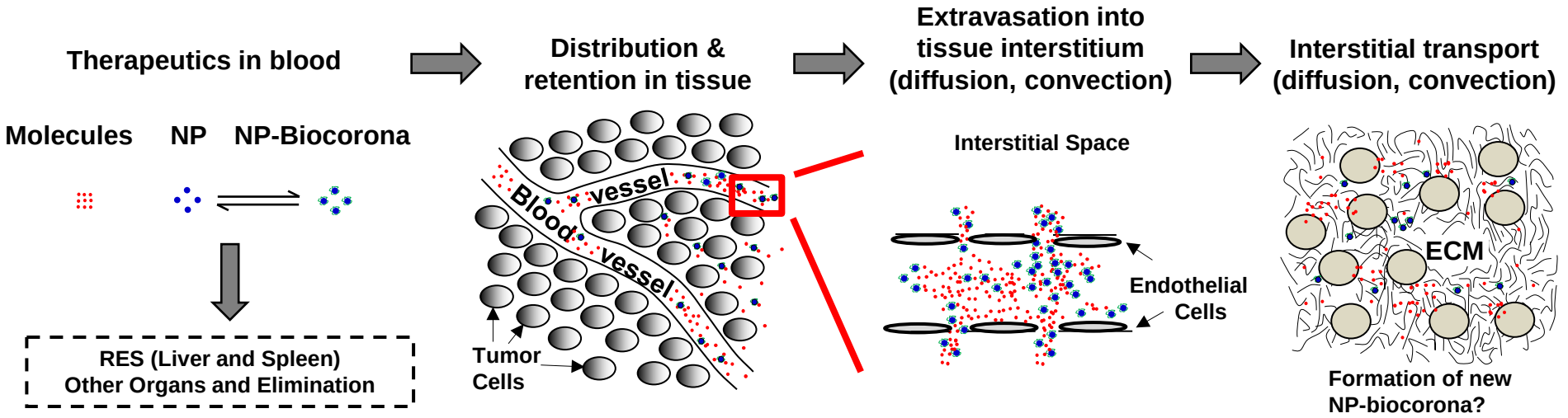
Zheng, N., *et al*., Scientific and regulatory considerations for generic complex drug products containing nanomaterials, AAPS J., 19:619, 2017

Fig. 1 Schematic illustration of the paradigm for equivalence recommendation of parenteral nanomaterials



- Dimension- & nanomaterial-dependent determinants of target site exposure/BE
- Quantitative methods to identify critical quality attributes?

Factors/variables affecting transport and biointerfaces of nano-therapeutics



- **Variables: Binding to serum proteins, immunogenicity, RES entrapment, transport in blood, transport across vessels, interstitial transport, binding to extracellular matrix, biocorona evolution**
- **These variables determine access of nanotherapeutics/API to and retention at extracellular, pericellular and intracellular targets**
- **All are dimension-dependent and/or nanomaterial-dependent**

Au *et al*., ADDR, 97:280, 2016

Delivery to target site is dimension- and nanomaterial-dependent

Extravasation from blood vessel

$$\theta = Lp_v \cdot (1-\sigma) \cdot (P_v - P_{int} - \sigma_p \cdot (\pi_v - \pi_{int})) \cdot \frac{S}{V} \cdot C_{blood} + P_d \cdot \frac{S}{V} \cdot (C_{blood} - C_{int}) \frac{Pe_v}{exp(Pe_v) - 1}$$

Convective transport (pressure gradient)

Diffusive transport (concentration gradient)

Blood-to-tissue

Accumulation in tissue interstitium

$$\frac{\partial C_{int}}{\partial t} = \theta + D \cdot \nabla^2 C_{int} - \nabla(\vec{u} \cdot C_{int}) - \varphi$$

Extravasation | Diffusive | Convective | Lymphatic drainage

Interstitium-to-cell

$$\frac{dC_{bound}}{dt} = k_{on} C_{int} \cdot (B_{max} - C_{bound}) - k_{off} C_{bound}$$

Factors determined by only tissue properties (7)

- Hydraulic conductivity of microvessel walls Lp_v
- Osmotic pressure in blood π_v & in interstitial fluid π_{int}
- Protein reflection coefficient across vascular wall σ_p
- Maximum NP binding sites in interstitium B_{max}
- Pressure in blood P_v and in interstitium fluid P_{int}
- Interstitial fluid flow velocity $\vec{u}$
- Blood vessel surface area per unit tissue volume $\frac{S}{V}$

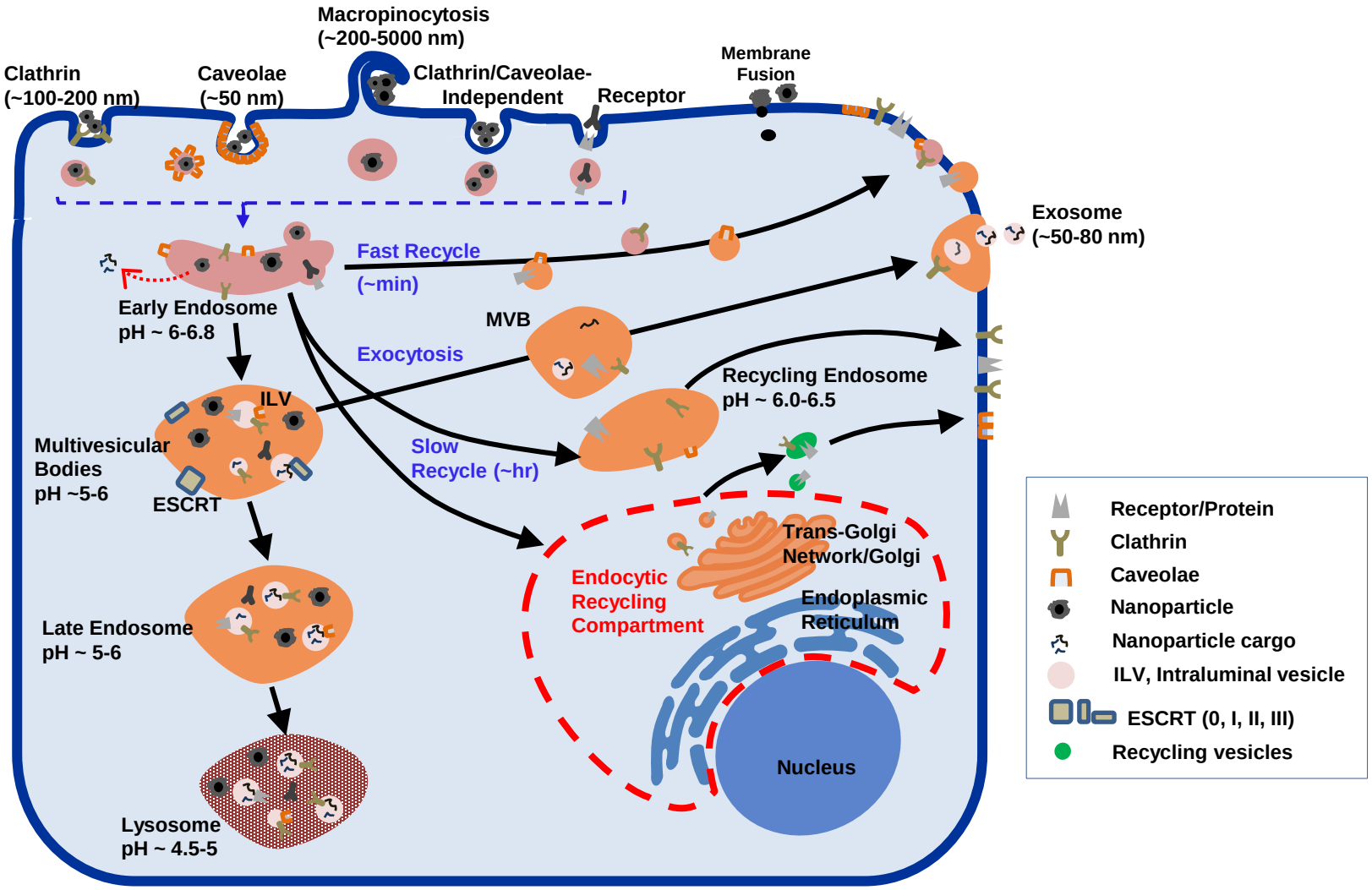
Factors determined by both NP & tissue properties and by NP-tissue interactions (>10)

- Diffusive permeability P_d: NP size, vessel wall thickness
- NP interstitial diffusion coeff *D:* NP size, interactions with ECM/cells, media viscosity, tissue tortuosity
- Concentration in blood C_{blood}: NP-host interactions affecting ADME
- Concentration in interstitium C_{int}: NP interactions with ECM/cells affecting interstitial transport & retention
- Reflection coefficient σ: NP size relative to vessel pore size
- Rate constants of NP association and dissociation to cells, k_{on} and k_{off}, affect C_{int} and internalization

******Pe*: Ratio of convection flux to diffusion flux

Au et al., *AAPS J,* 16, 424-439, 2014

Internalization and intracellular trafficking of nanotherapeutics



All processes are dimension- and/or nanomaterial-dependent

Stan, Microsc Res Tech, 57:350, 2002
Grant, Nature Rev Mol Cell Bio, 10:597, 2009
Huotari and Helenius, EMBO Journal, 30:3481, 2011

Machen et al., Am J Physiology, 285:C205, 2003
McMahon and Boucrot, Nat Rev Mol Cell Biol, 12:517, 2011
Xie et al., Mol Bio Cell, 27:108, 2016

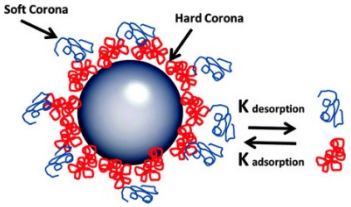
Ang et al., J Cell Bio, 167:531, 2004
Lim and Gleeson, Immunol Cell Biol, 89:836, 2011

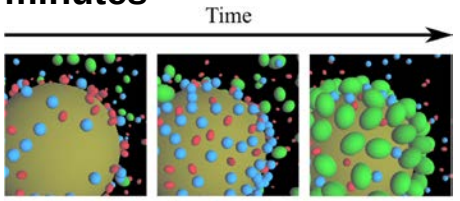
NP interactions with biological materials and target site exposure are dependent on dimension and nanomaterial

Property	Outcome/Effect (Examples)
Size	• Reduced opsonization and RES uptake at <200 nm • Affects transport (transvascular & interstitial) & retention (EPR in tumors at 50-200 nm) • Internalization of inorganic NP and liposomes (maximum at 30–50 nm) • Intracellular trafficking/processing
Surface charge	• Affect opsonization, e.g., rapid RES clearance of cationic liposomes • Affect electrostatic interaction with vessel pore • Promote interactions with ECM components, reduces interstitial transport • Increase binding to cell membrane and internalization, e.g., positively charged NP
Bio-material & Surface modifi-cation	• PEGylation reduces opsonization and RES uptake • Coating with hyaluronic acid reduces immunogenicity • Cationic cell penetrating peptide promotes NP internalization & perinuclear localization • Collagenase & hyaluronidase alters ECM, promotes interstitial transport • Ligands for targeting (e.g., folate, transferrin, CD19, CD20, uPAR, HER2) • pH-sensitive fusogenic polymers/peptides/lipids enhance cargo release in endosomes

All properties affect biocorona formation due to NP (inorganic/organic) interactions with proteins (hundreds) in serum and microenvironment (proteins coating the NP)

- **Publications with NP and corona as key words: 9 in 2004 and 134 in 2014**
- **van der Waals forces & electrostatic interactions, completed within minutes**
- **Hard corona covered by soft corona**





Kinetics of protein adsorption onto NPs.
Red: Albumin, blue: Transferrin, green: Fibrinogen.

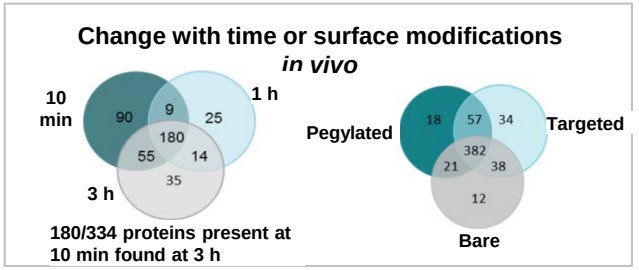
Shang et al., J Nanobiotech, 12:5, 2014
Li et al., Adv Drug Deliv Rev, 64:29, 2012
Rahman et al., Series in Biophysics 15, 2013

Andar et al., Pharm Res, 31:401, 2014
Paliwal et al., Drug deliv, 22:231, 2015
Villanova et al., ACS Nano, 10:10842, 2016

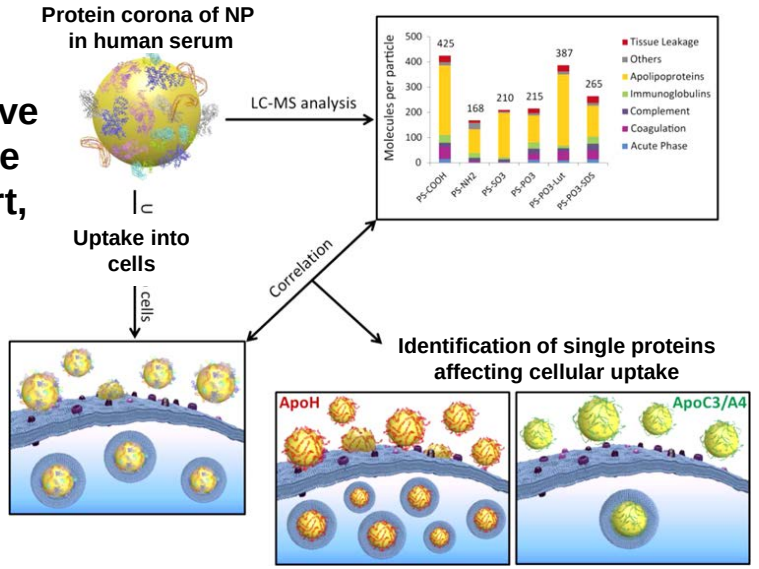
Wang et al. AAPS J, 12:492, 2010
Treuel et al., Beilstein J. Nanotechnol *6*:857, 2015
Au et al., Adv Drug Del Rev 97:280, 2016

Biocorona: Evolution with time and environment

- **Protein selectivity unclear**
  - Only a few dozen of thousands of serum proteins in biocorona
  - Hard corona proteins not the most abundant proteins in plasma or have highest binding affinity
  - Depends on NP properties (material, surface properties, size, charge, shape) and environment (ECM composition, pH, temperature, shear stress)
- **Evolution due to reversible binding**
  - Replacement by proteins with high affinity or abundance
  - Change with exposure time (no change in total amount)
  - Change with microenvironment, e.g., blood *vs.* cytosolic fluid, serum from healthy vs. diseased subjects



- **Effects**
  - Increase size (up to 150% for polystyrene & silica NP)
  - Change surface charge from positive to neutral/negative
  - Surface modifications elicit opsonization & RES uptake and alter ADME, transvascular and interstitial transport, internalization and intracellular processing
  - Pathobiology (hemolysis, endothelial cell death)
  - Destabilize nucleic acid-lipoplex/polyplex
- **Many unknowns for regulation purpose, e.g.,**
  - species difference (relevance of preclinical results)
  - Healthy *vs.* diseased subject



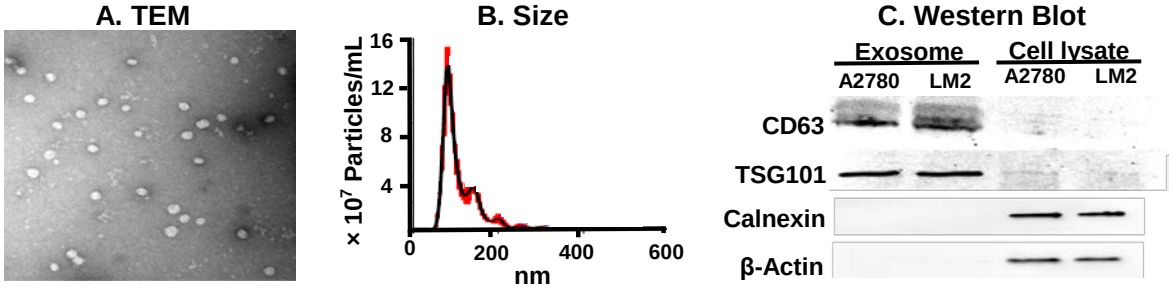
Hadjidemetriou et al., ACS Nano, 9:8142, 2015
Monopoli et al., Nature Nanotechnol., 7:779, 2012
Au et al., Adv Drug Del Rev 97:280, 2016

Hadjidemetriou et al., Nanoscale, 8:6948, 2016
Tenzer et al., Nature Nanotech 8:772, 2013
Nguyen & Lee, Int J Nanomed 12:3137, 2017

Lundquist et al., ACS Nano 5:7503, 2011
Ritz et al., Biomacromol 16:1311, 2015

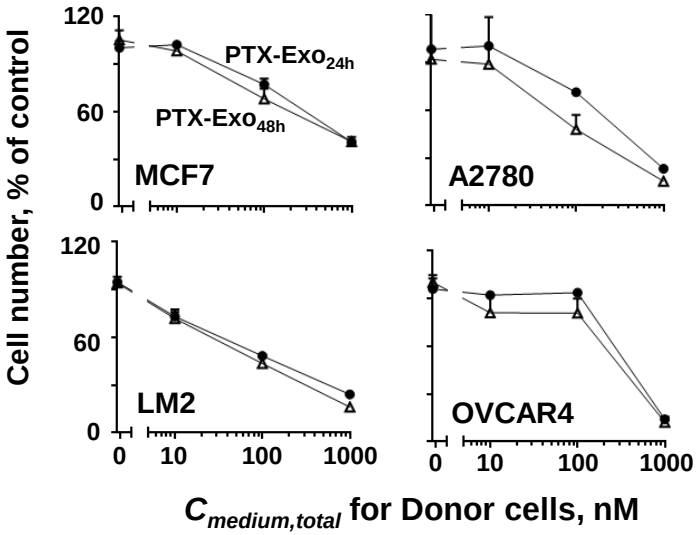
Exosomes is an intercellular drug transfer mechanism with pharmacological consequences

- **Cells treated with clinically relevant drug concentrations produce exosomes**

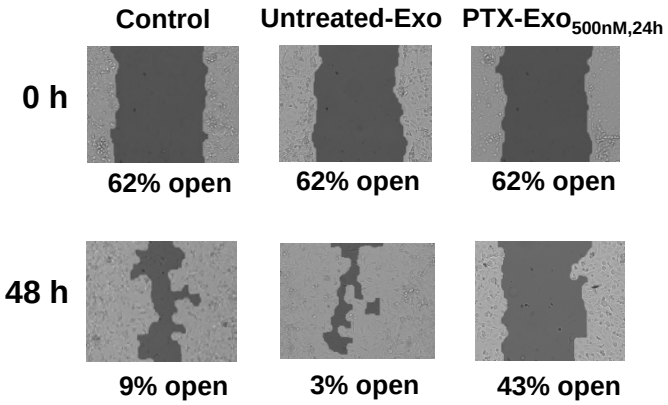


- **Exosomes collected after paclitaxel treatment (PTX-Exo) exhibit cytotoxicity and anti-migration effects in drug-naïve recipient cells**

***Cytotoxicity*$_{recipient}$ of PTX-Exo**



***Anti-migration*$_{recipient}$ of PTX-Exo**



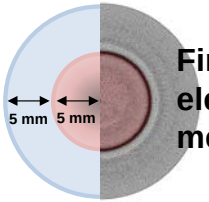
Wang, J., *et al*., Exosome is a mechanism of intercellular drug transfer: Application of quantitative pharmacology. Accepted with revisions, JCR, 2017

FDA Guidance for Industry-Statistical Approaches to Establishing (Systemic) BE:

Calculated confidence interval for ratio of the averages (population geometric means) of measures for Test and Reference products should fall within a limit, usually 80-125%

When does Systemic BE of nanotherapeutics not equal Target Site BE?
Examples: Simulations using computational fluid dynamics

Tumor embedded in normal tissue: Model assumptions



Finite element mesh

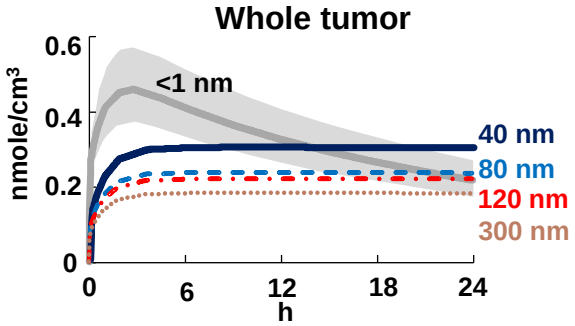
Tumor
- Necrotic core
- High interstitial pressure
- Irregular blood vessels
- No lymphatic vessels

Normal tissue
- Regular blood vessels
- Normal interstitial pressure
- Lymphatic vessels

Simulated *C-T* profiles in whole tumor, or tumor interstitium/tumor cells
- Controls: Systemic BE (80-125%) as for (a) small molecules (<1 nm), or (b) no binding to cells
- Effects of 3 variables

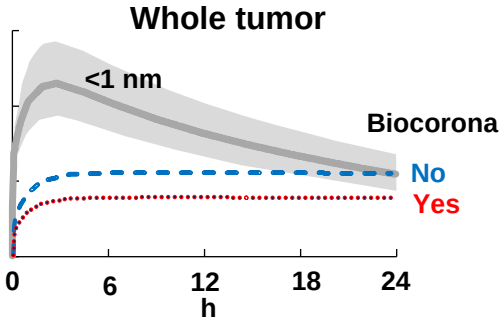
Effect of NP diameter

σ (reflection coefficient across blood vessel) increased from 0.45 to 0.67 when cationic NP size increased from 40 to 300 nm, for 400 nm vessel pores (Stylianopoulos et al., 2013, Ann Biomed Eng, 41:68, 2013)



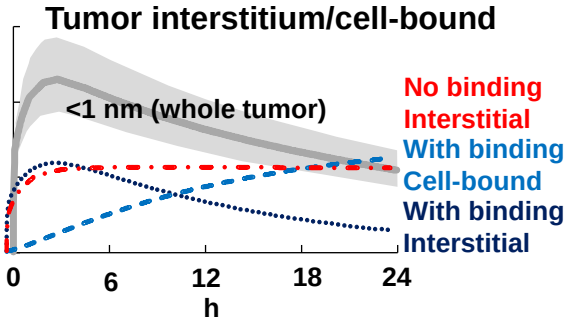
Effect of biocorona

Increased average diameter from 100 nm (with 100% below 200 nm) to 250 nm (with 30%>200 nm), simulated using σ of 0.6, 120 nm NP & tumor vessel pore of 200 nm



Effect of cell binding

No binding to cells *vs.* moderate cell binding, simulated using σ of 0.6, 120 nm NP & tumor vessel pore of 200 nm



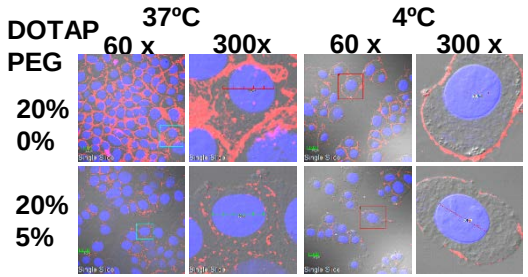
Need to determine equivalence in transvascular transport, interstitial transport, transcellular transport, intracellular trafficking and exocytosis

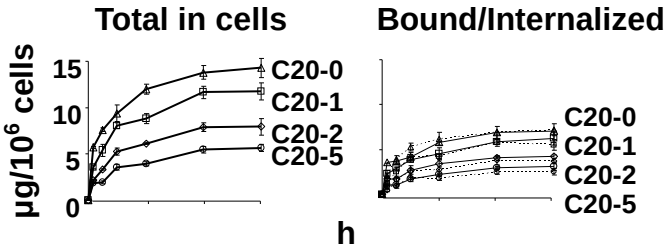
A quantitative method to determine target site exposure

- **Differences between Systemic and Target Site Exposures of nanotherapeutics are primarily due to differences in**
  - **diffusive transport as convective transport is determined by pressure gradient, not dependent on NP properties**
  - **interactions with biological materials, leading to differences in transport, binding to cell membrane, internalization, intracellular trafficking/processing**
- **Supplement Systemic BE data with**
  - **Use *in vitro* studies to compare Test and Reference products for (a) interactions with cells/extracellular matrix, (b) diffusive transport in 2D and 3D systems, (c) pharmacodynamics at multiple *C* and *T***
  - **Use multiscale modeling and computational tools to combine (a) systemic *C-T* profiles, (b) blood-to-organ transvascular transport, (c) interstitial transport to target cells, (d) intracellular processing to molecular targets**
  - **Identify product-specific critical quality attributes and the range of acceptable deviations**
- **Some examples from our own work**

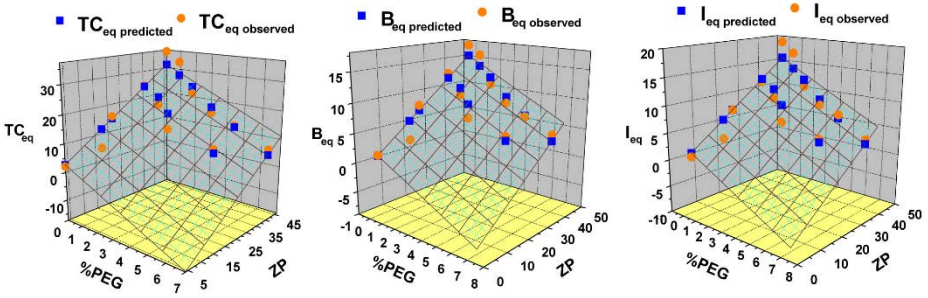
Predicting NP internalization and retention in cells

- Variables: surface charge, pegylation
- Quantified membrane-bound conc, total cell-associated, and internalized conc





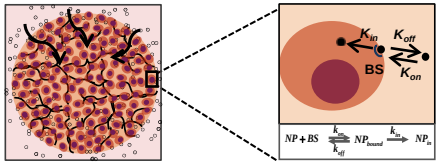
- Used data to define model relating ZP and PEG to NP conc
- Model-prediction vs. experimental data



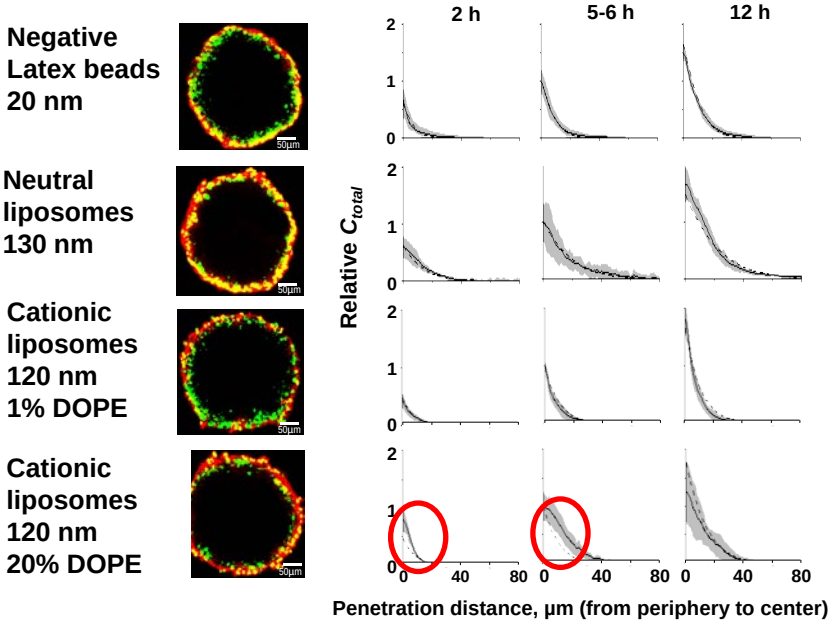
Li et al., AAPS J, 13:585, 2011

Predicting diffusive transport of NP in 3D tumor spheroids

Model of NP diffusive transport, based on calculated *D* & experimentally measured NP-cell binding data



Model-prediction (dashed) *vs.* experimental data (95% CI): Effect of surface charge and treatment time



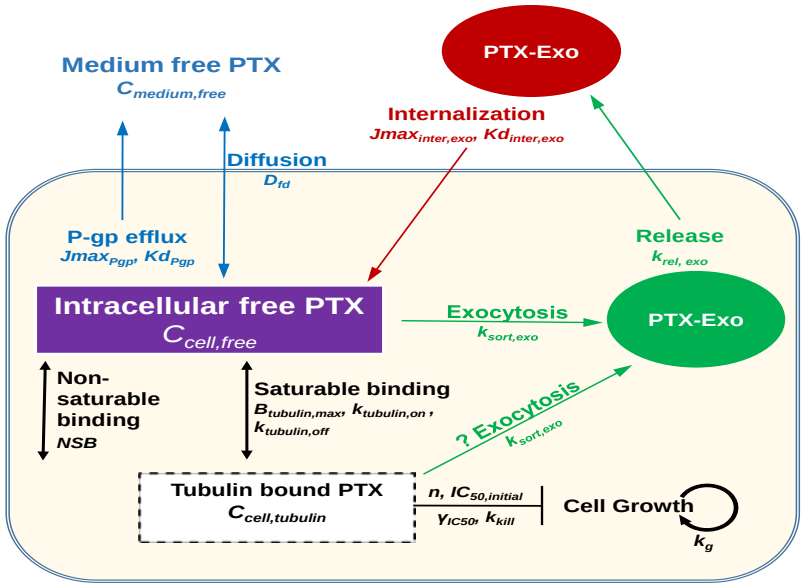
- Can predict diffusive transport of neutral and negative NP, and positive NP with low fusogenic lipid content
- Positive liposomes with 20% DOPE formed aggregates in presence of cells

Gao et al., AAPS J, 15:816, 2013; Wientjes et al., JCR, 192:10-18, 2014

Predicting pharmacological activities of exosomes

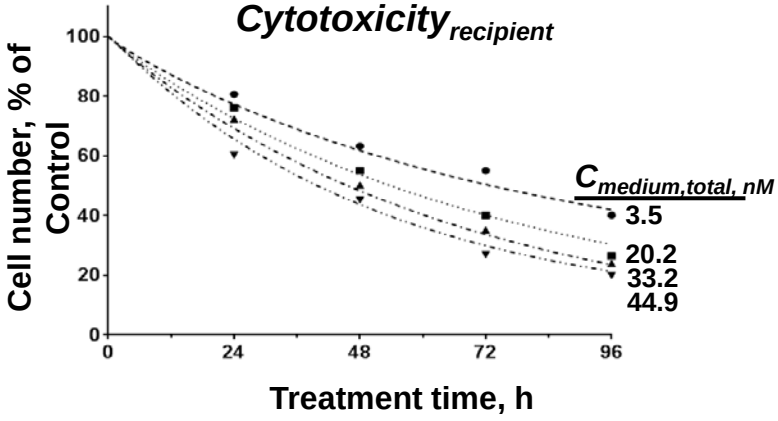
Cellular PK/PD Models:

- Paclitaxel cellular transport kinetics
- Paclitaxel concentration-cytotoxicity

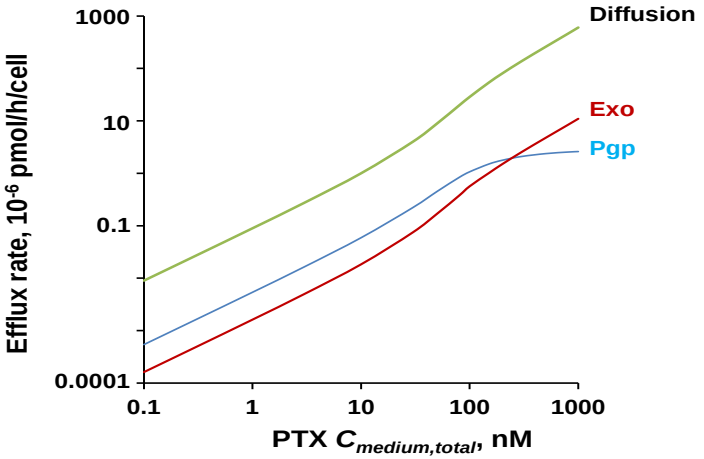


Model predictions

- Cytotoxicity in drug-naïve recipient cells (symbols: experimental results)



- Exosomes, by reducing intracellular drug retention, is also a mechanism of resistance at clinically relevant concentrations



Kuh, H.J., et al., JPET, 293:761, 2000 Jang, S.H., et al., JPET, 298:1236, 2001 Jang, S.H., et al., JPET, 304: 773, 2003

Wang, J., *et al.*, Exosome is a mechanism of intercellular transfer mechanism: Application of quantitative pharmacology. Accepted with revisions, JCR, 2017

Conclusions

- **Nanoparticulate and molecular medicines are subjected to dimension- and material-dependent effects on transport and residence, and biointerfaces**
- **These properties can result in differences in target site PK/PD that can be predicted by systemic BE**
- **Therapeutic equivalence (TE) for nanotherapeutics requires additional considerations, such as equivalence in**
  - **transvascular transport (blood-to-organ)**
  - **interstitial transport (organ-to-extra-/peri-cellular targets)**
  - **transcellular transport, intracellular trafficking, exocytosis (from interstitium to intracellular targets)**
- **Potential use of *in vitro* studies & computational multiscale modeling tools to supplement Systemic BE results, to demonstrate Target Site BE and TE**

Acknowledgements

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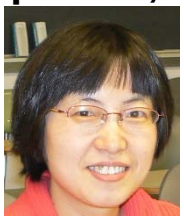

Yue Gao, PhD

Bertrand Yeung, PhD

Jin Wang, PhD

Roberto Abbiati, PhD

Minjian Cui

Michael Wientjes

Ze Lu, PhD

Guill Wientjes, PhD

Collaborators

OUHSC (Oklahoma)
Sukyung Woo, PhD

NCI
Cody Peer, PhD

NCI
William Figg Sr., PharmD

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