

Nanoscale Constructs for Therapy

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Examples from the Northwestern CCNE
molecular diagnostics—PSA
nano-flares for mRNA detection
gold nanoparticles for gene regulation
validating targets in acetylation biology

Prostate Cancer and PSA: CCNE Past Success

Background:

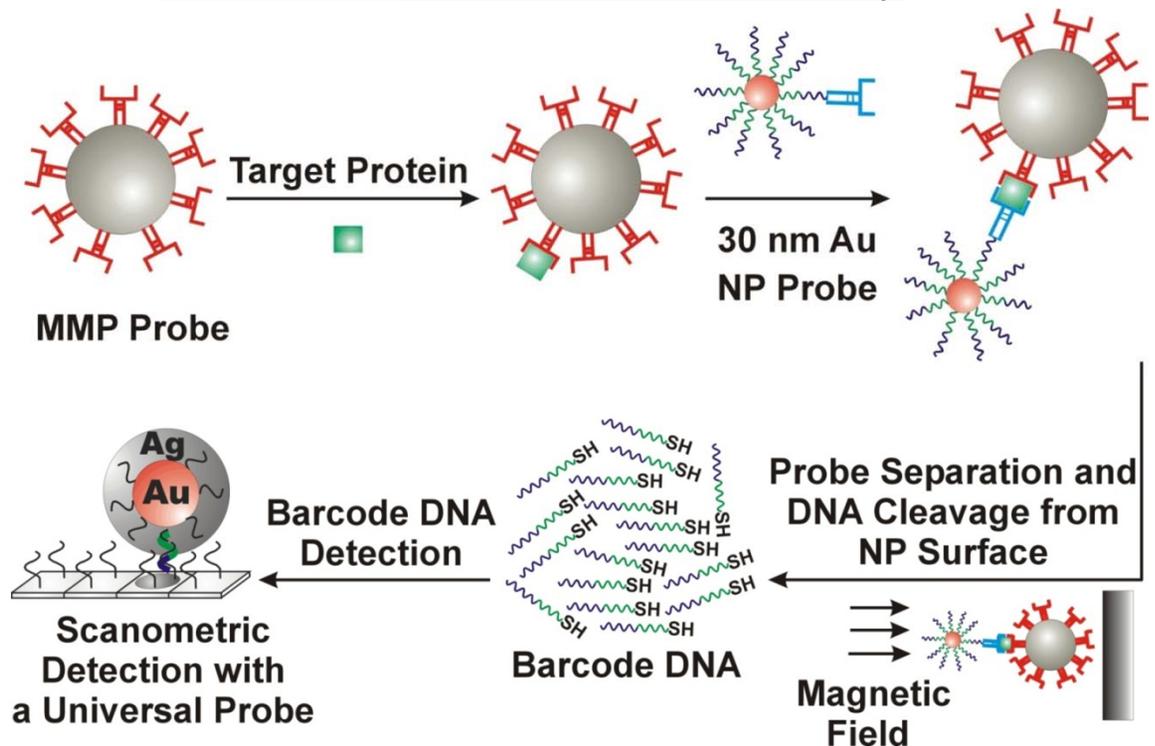
- Prostate cancer is the most common (200,000 dx/year) and 2nd most deadly cancer among American men (30,000 deaths/year)
- 70,000 men / year experience PSA failure after undergoing surgery for prostate cancer (40%), remaining 60% don't objectively know they are cured!

Problem:

Conventional PSA immunoassays (LOD 0.1 ng/ mL) do not have the requisite sensitivity to:

- Define disease cure (modify/define surveillance protocols)
- Diagnose recurrence early for enrollment in clinical trials
- Assess biological response to adjuvant/ salvage therapy

Innovation: The Bio-Barcode Assay



LOD 0.3 pg/ mL = >300X Increase

Retrospective Clinical Validation of Post-Operative PSA Kinetics

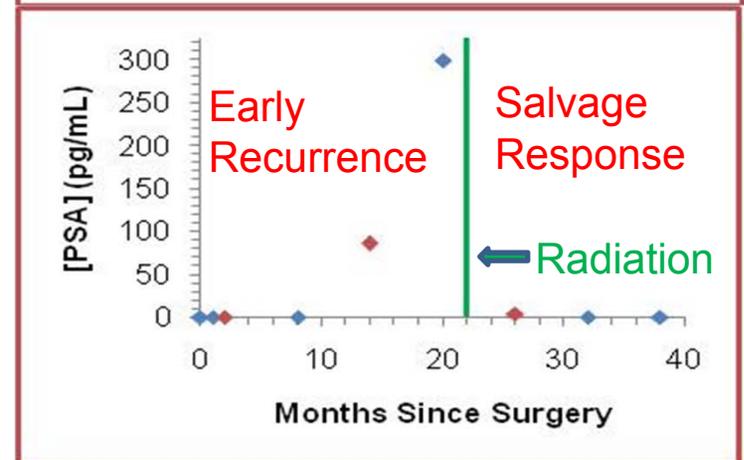
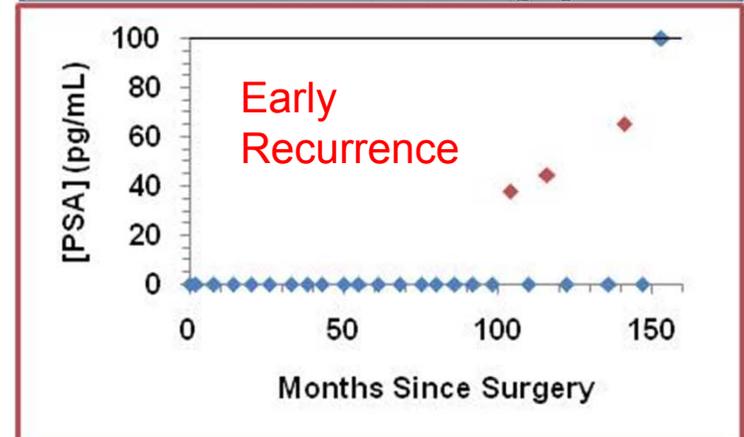
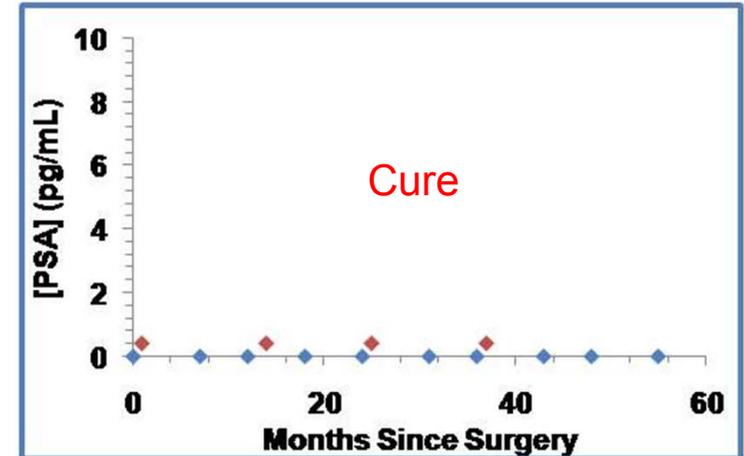
Translation:

Retrospective Trial (ongoing)

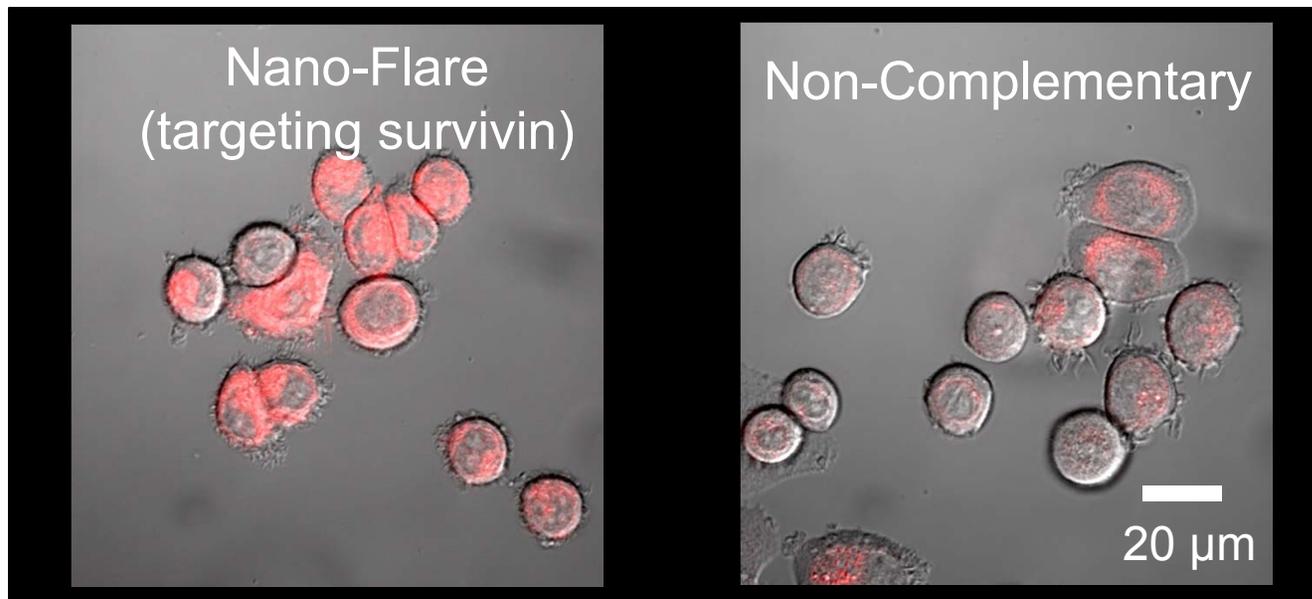
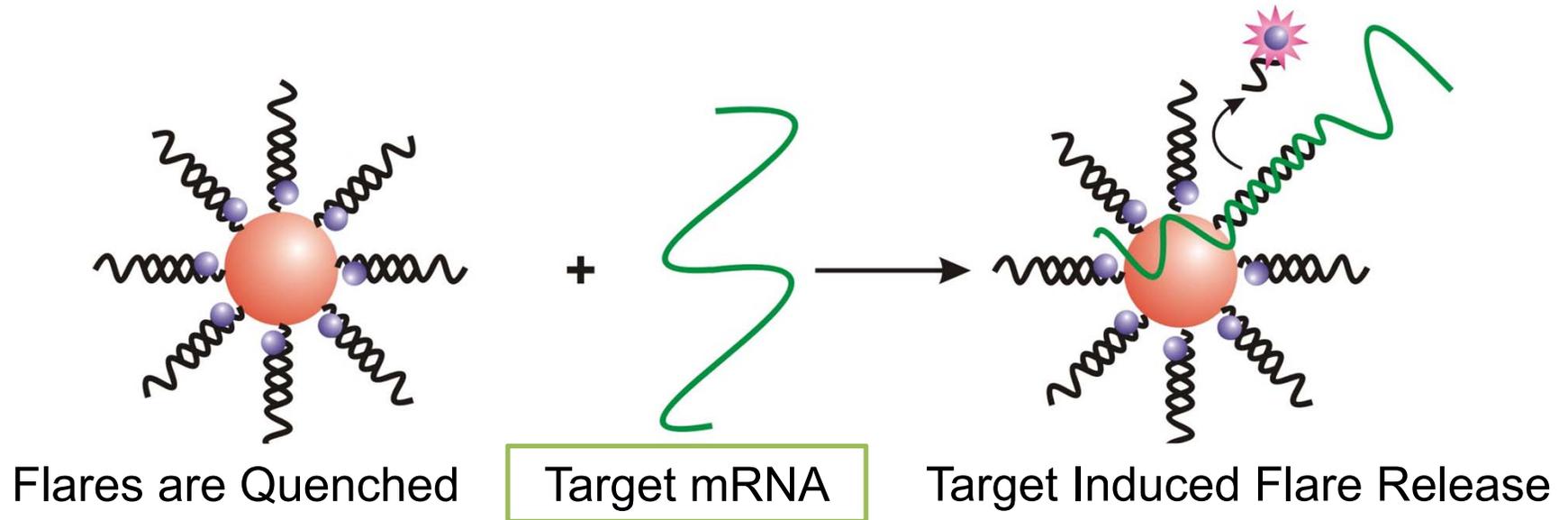
- ~410 patients following surgery (T=0)
- ~750 samples
- Patients w and w/o recurrence
- All conventional PSA ≤ 0.1 ng/mL
- NanoPSA (red)
- Up to 15 years follow-up
- Multi-Institution (NU, Wash U)



 Nanosphere

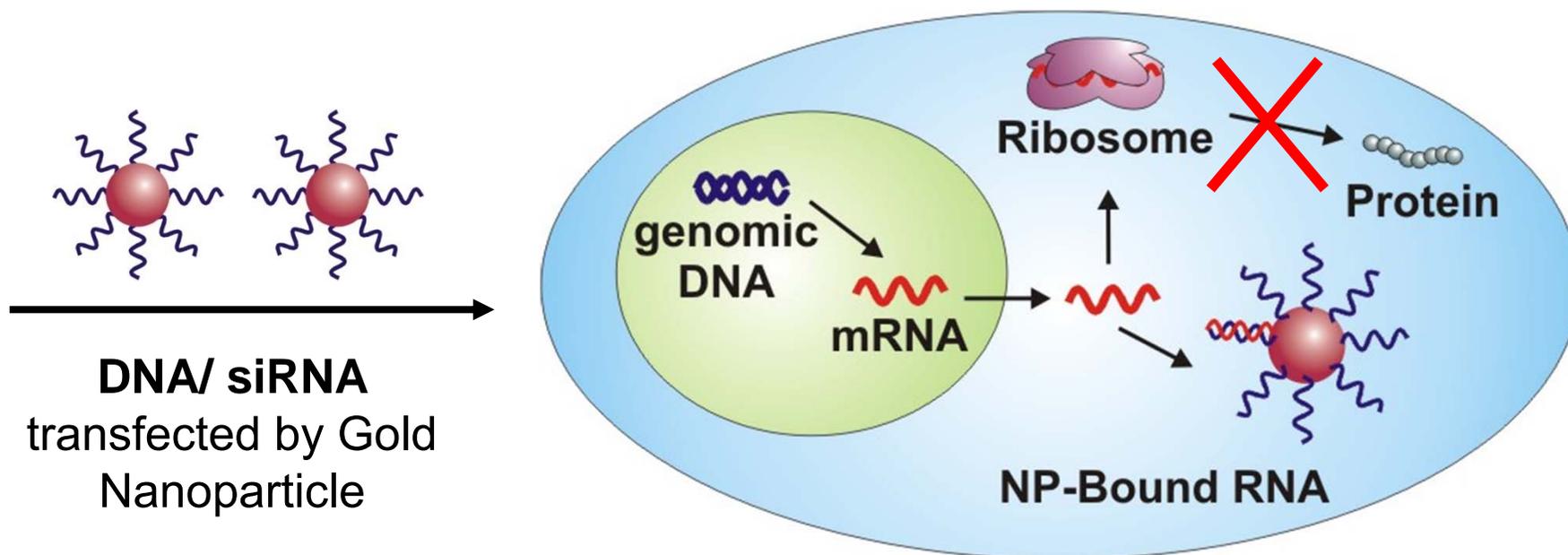


Nano-Flares for Intracellular mRNA Detection

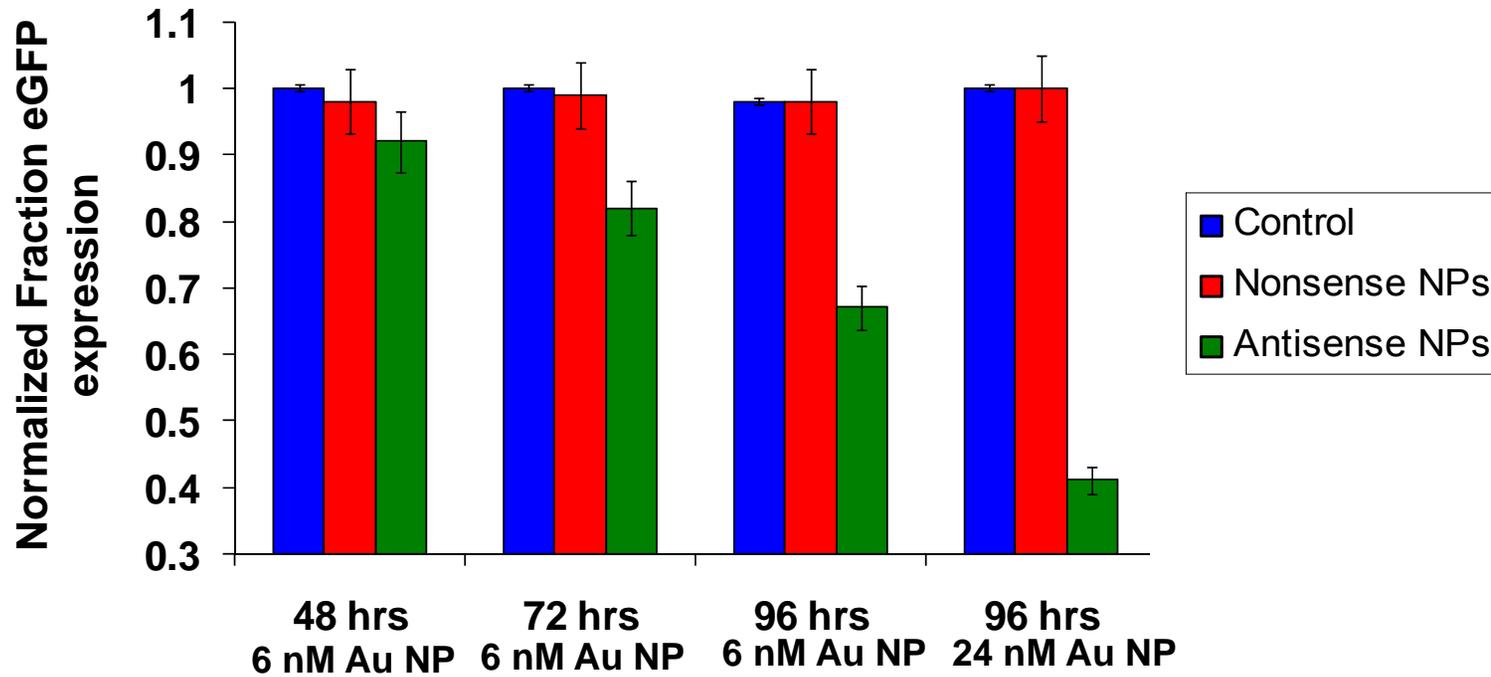
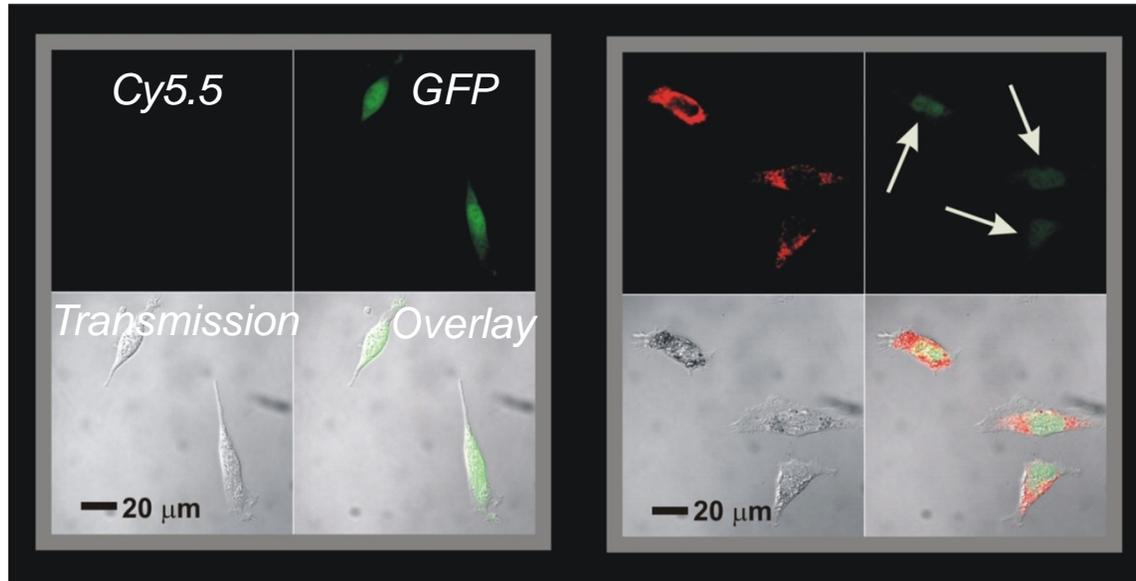


Patient prognostication, treatment response measurement, stem cell isolation

Gold Nanoparticle Agents for Gene Regulation



Antisense-DNA Knockdown of GFP



Translation of Gene Regulating DNA/ siRNA AuNPs

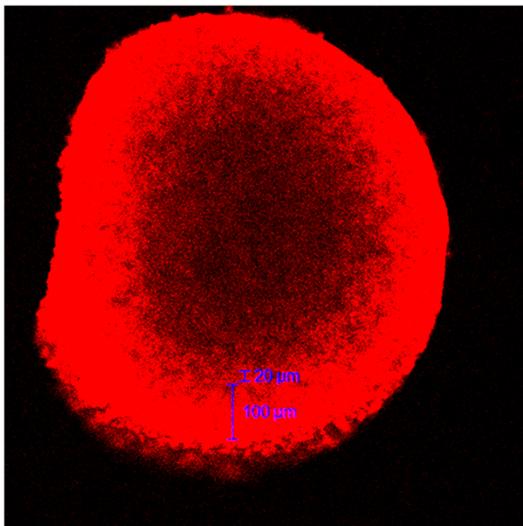
Indications

Glioblastoma (Brain Tumors)
Local Delivery Following Tumor Removal—
Clean Up

Pancreatic Cancer
Local Delivery Following Tumor
Resection—Clean Up

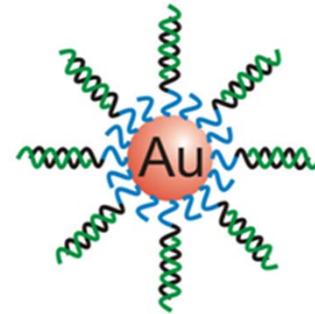
New Properties/ New Opportunities Beyond Cells

Deep siRNA AuNP Penetration Into Target
Organs (Red)



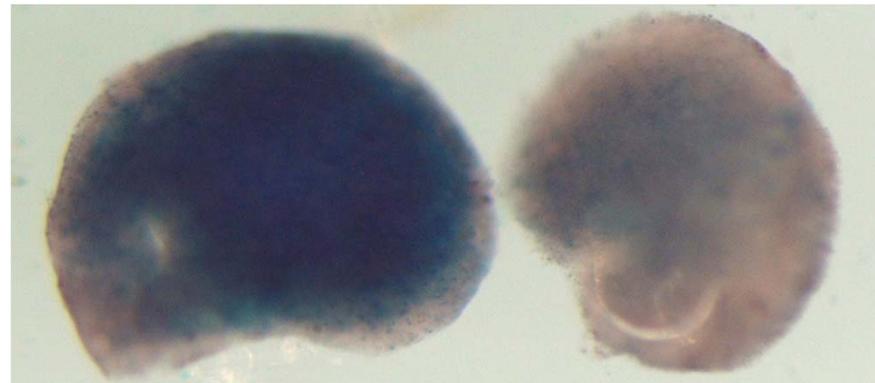
Innovation

Indication-Specific
Targeted siRNA AuNP



Organ Level Efficacy

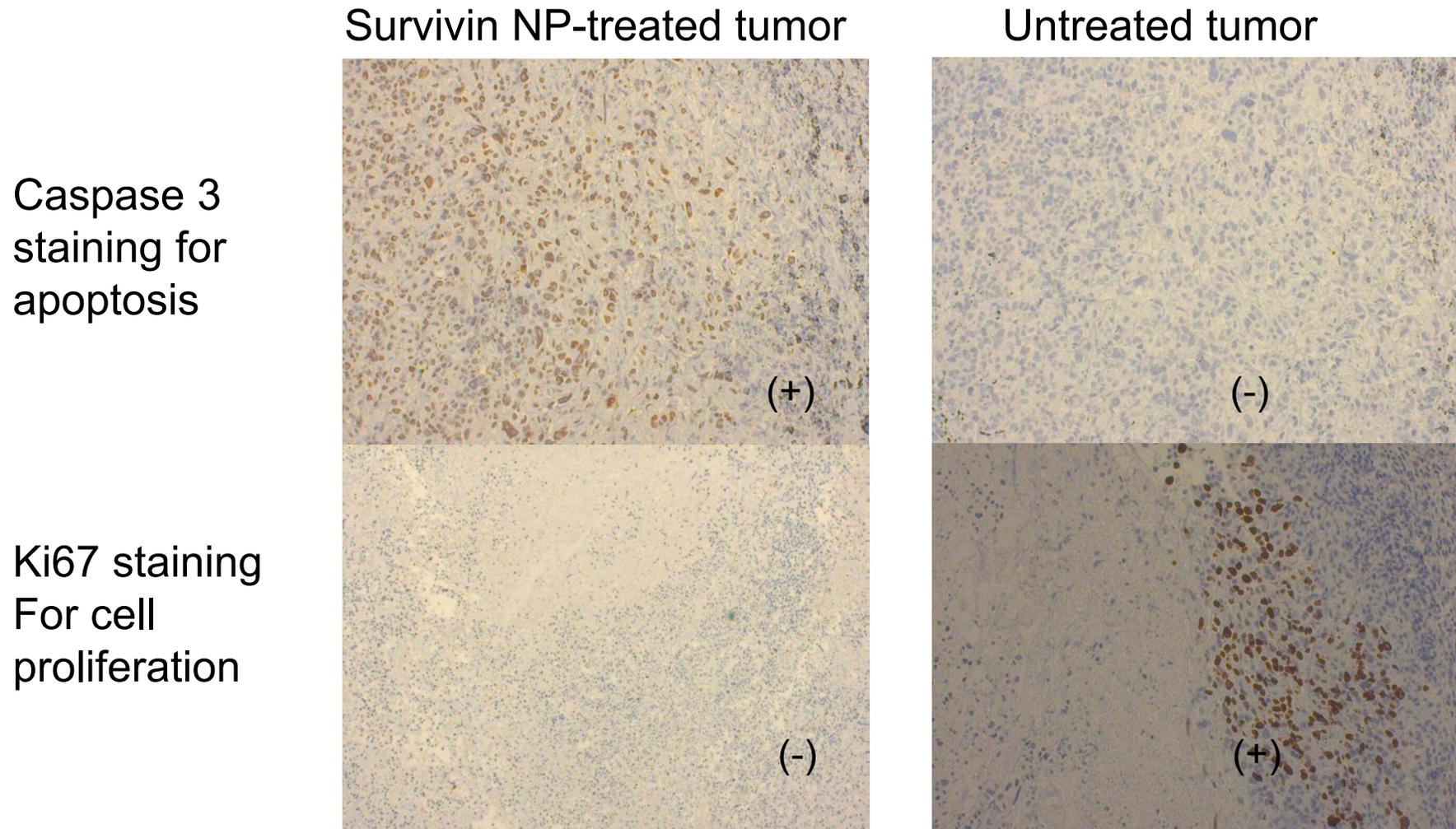
Organ Level β gal Knockdown (right) in ovaries
using anti- β gal siRNA AuNPs



In Vivo Efficacy Demonstrated:

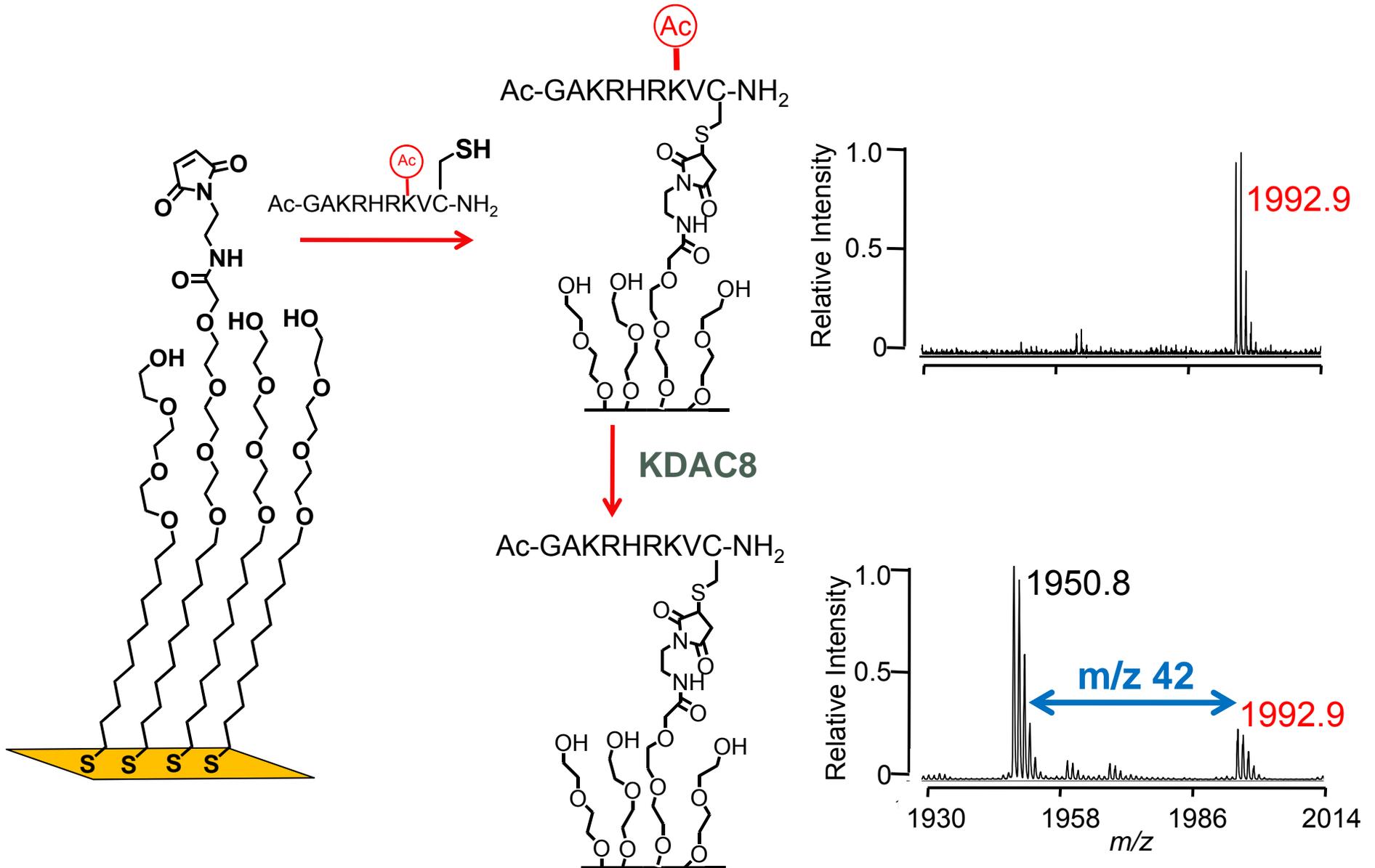
Breast cancer, skin disorders (15 current
collaborations, 6 in animal models)

Translational Efficacy of siRNA AuNPs in Breast Cancer

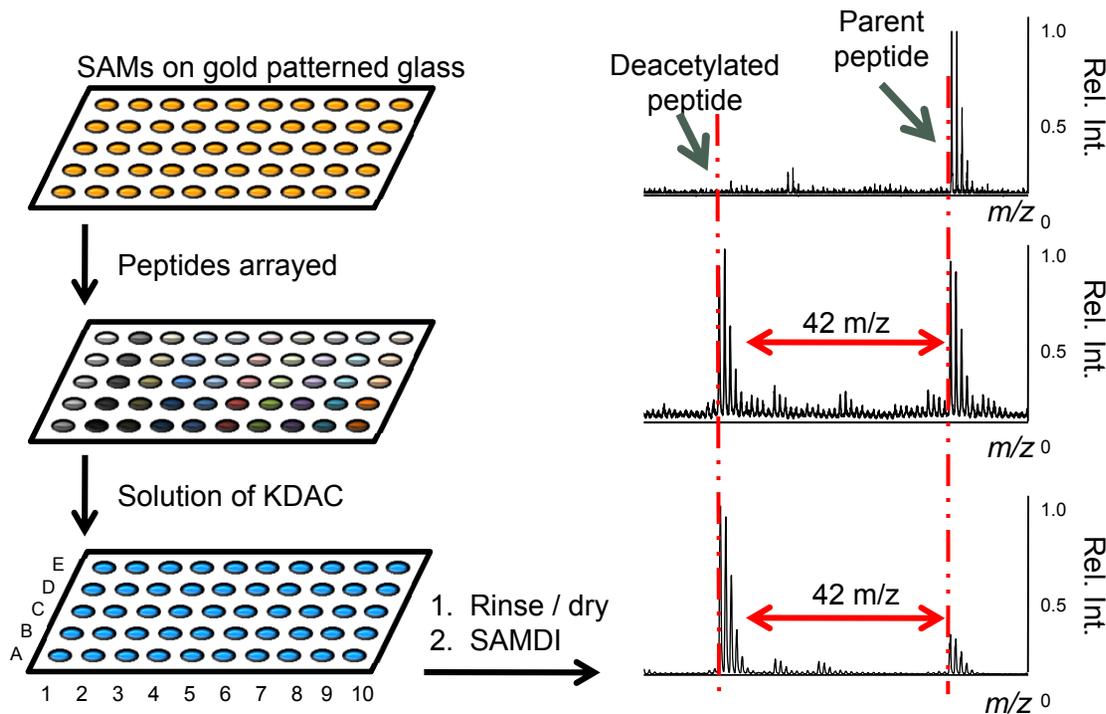
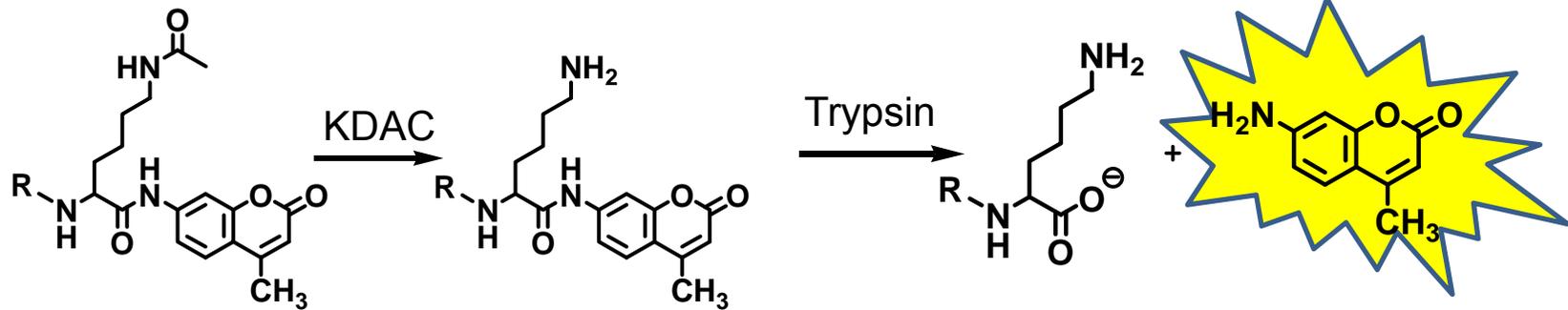


In Targeted siRNA- AuNP Treated Tumors, and Increase in Apoptosis and Decrease in Proliferation is observed

Label-Free Deacetylase Assay

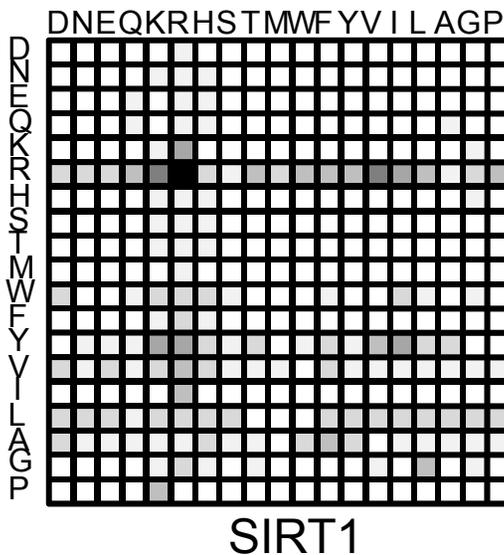
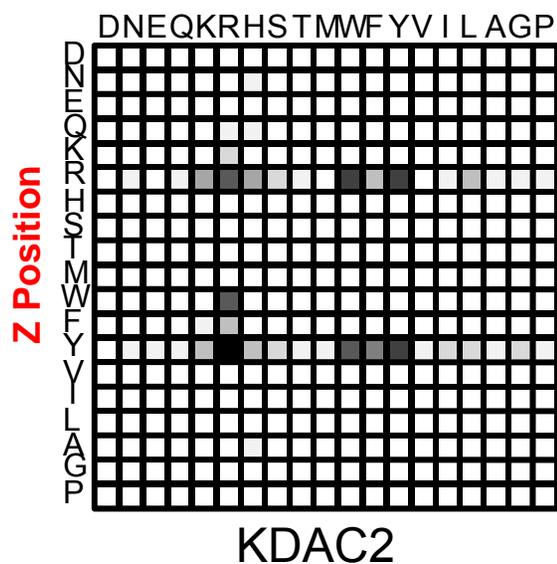
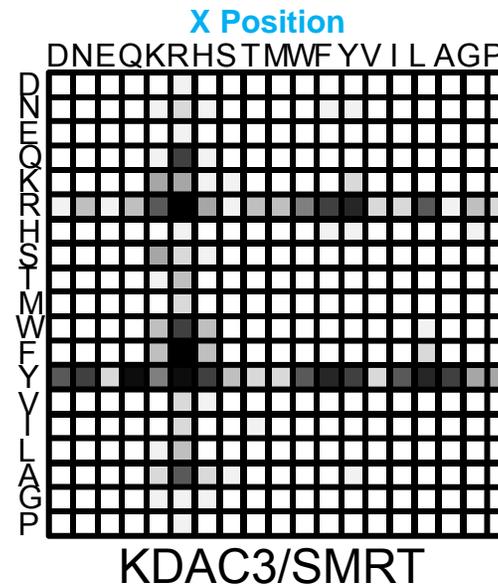
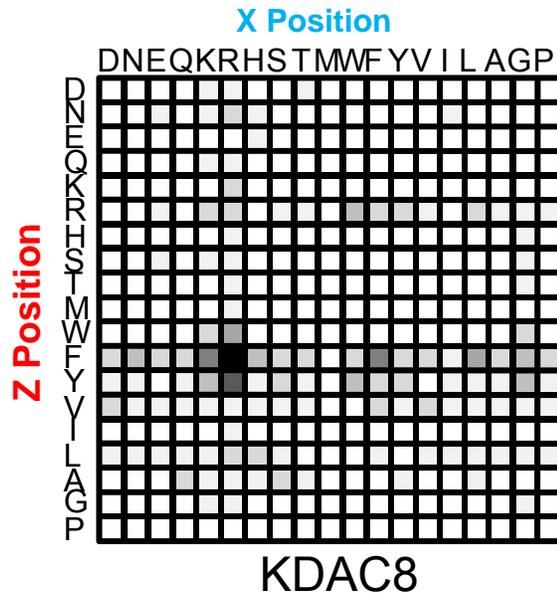


Lack of Suitable KDAC Activity Assays



Applied Biosystems 4800
MALDI TOF/TOF

Specificity Profiles for Deacetylases



KDAC8, SIRT1 specific for
RKF and RKR,
respectively

Overlapping activity on
active substrates

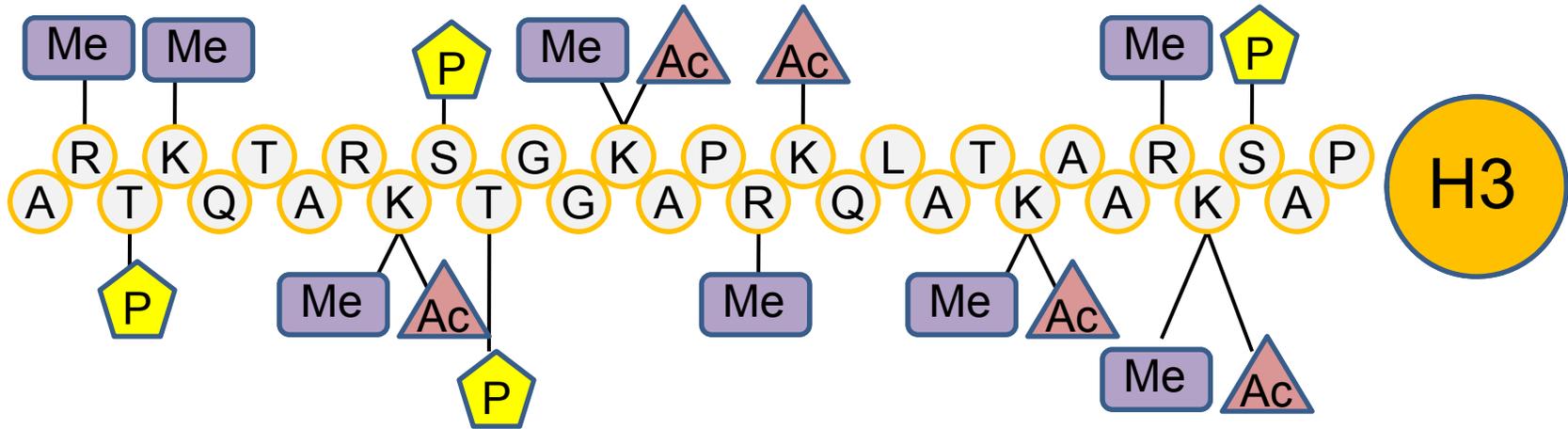
Non-selective substrates

*KDAC1 and 3 were not active
on any peptides in this array,
despite showing activity on
fluorescently-labeled
substrates.*

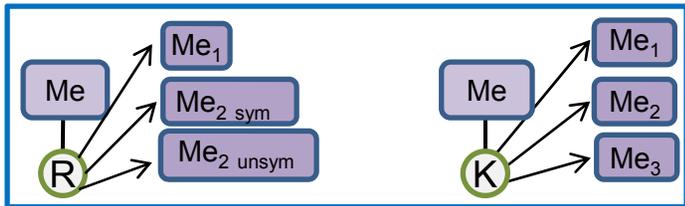
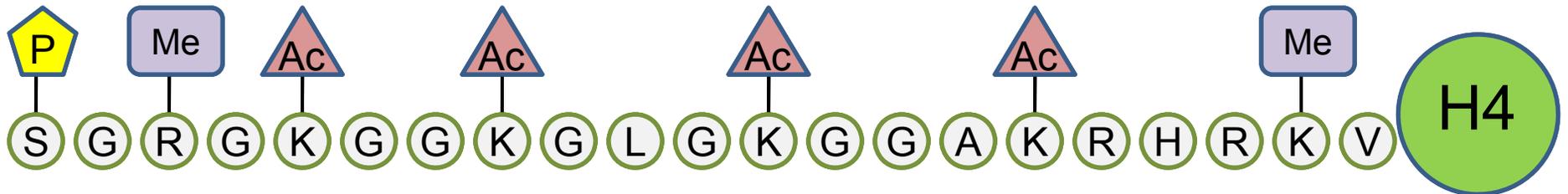


Many Possible Combinations, Few Observed

H3 Tail: 13 Modified Residues = 25,600,000 Possible Combinations!



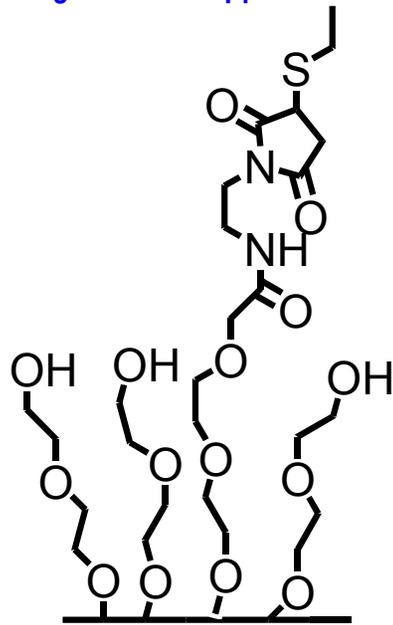
H4: 7 Modified Residues = 512 Possible Combinations!



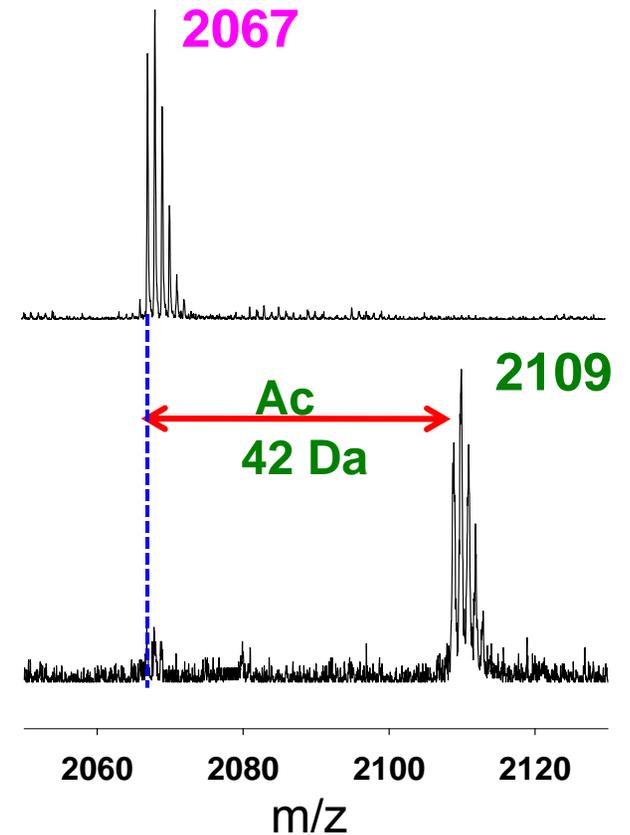
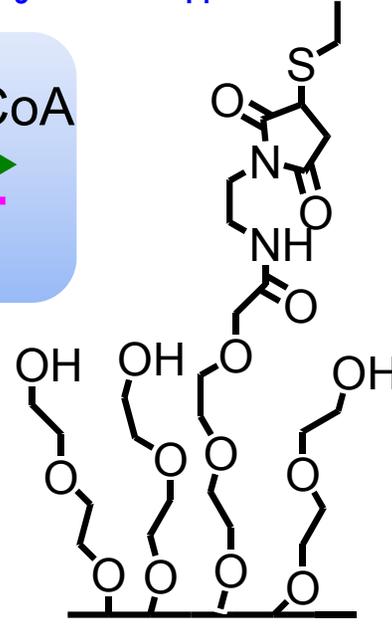
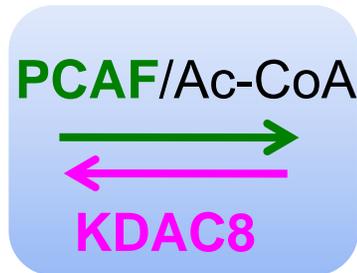
Histone Code: Modifications act sequentially or in combination to create a 'code' read by other proteins to regulate cellular events.

Acetylation of a H3-Derived Peptide

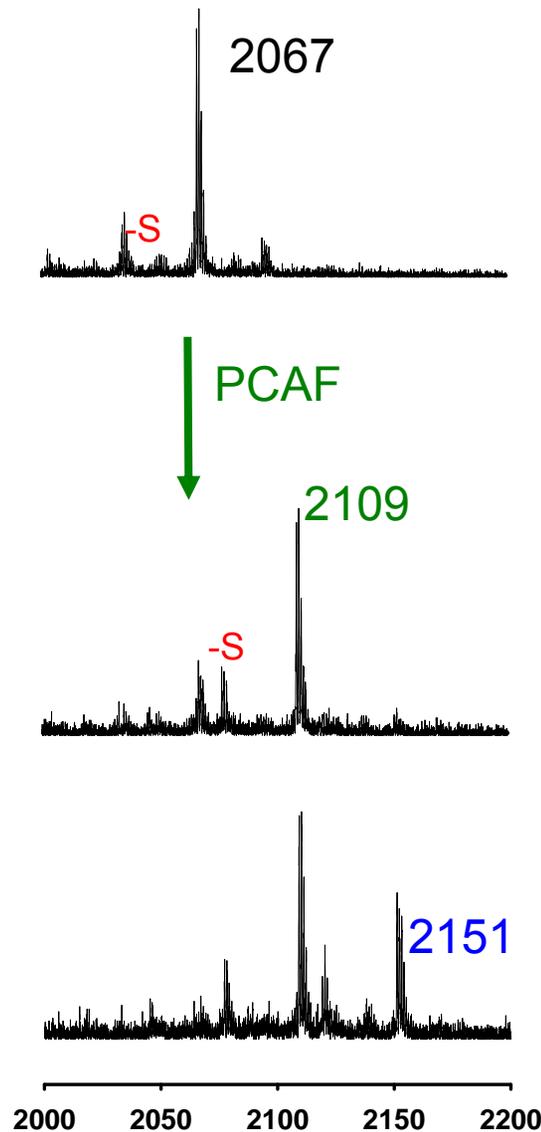
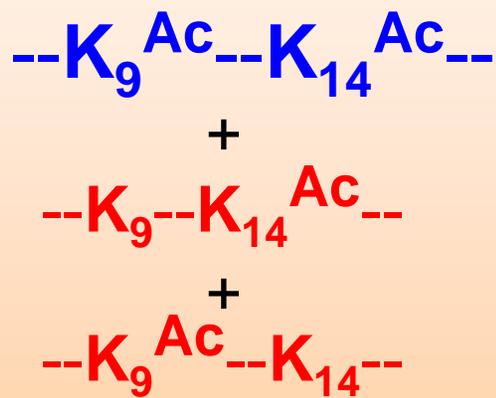
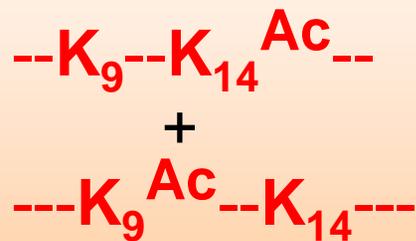
Ac-TARK₉STGGK₁₄APC



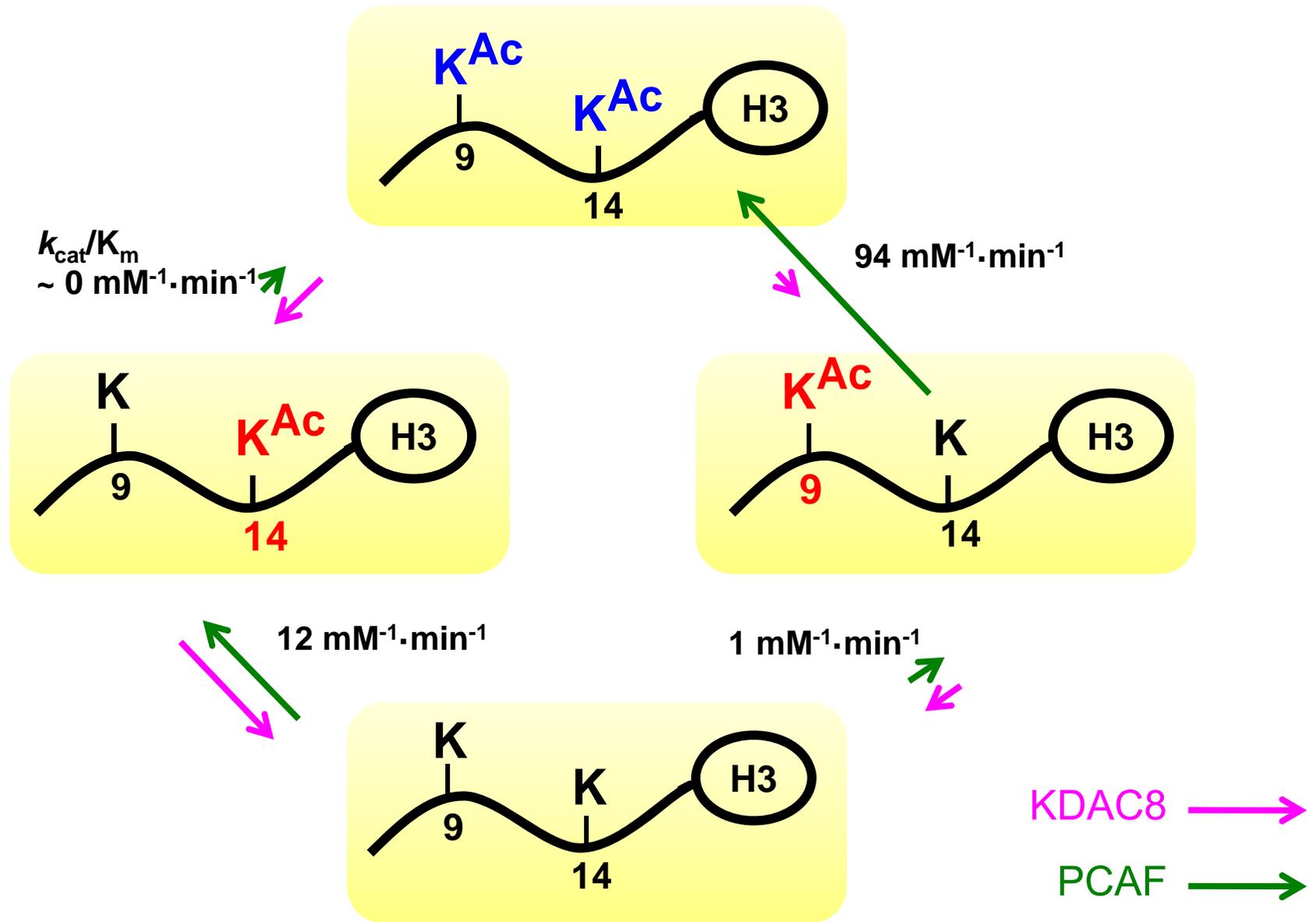
Ac-TARK₉STGGK₁₄^{Ac}APC



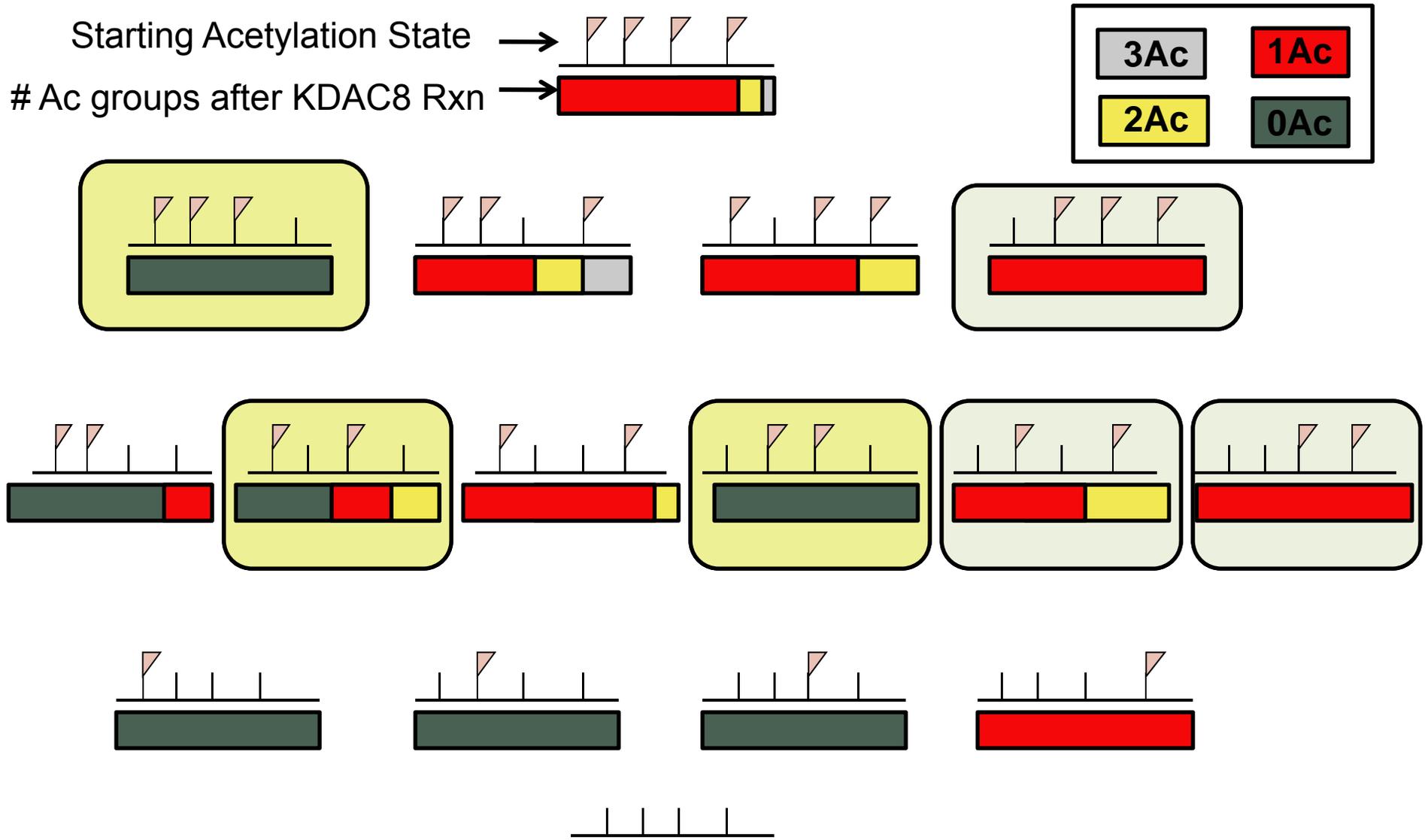
Addition of KDAC Gives MORE Acetylation



Kinetic Model for Increased Acetylation

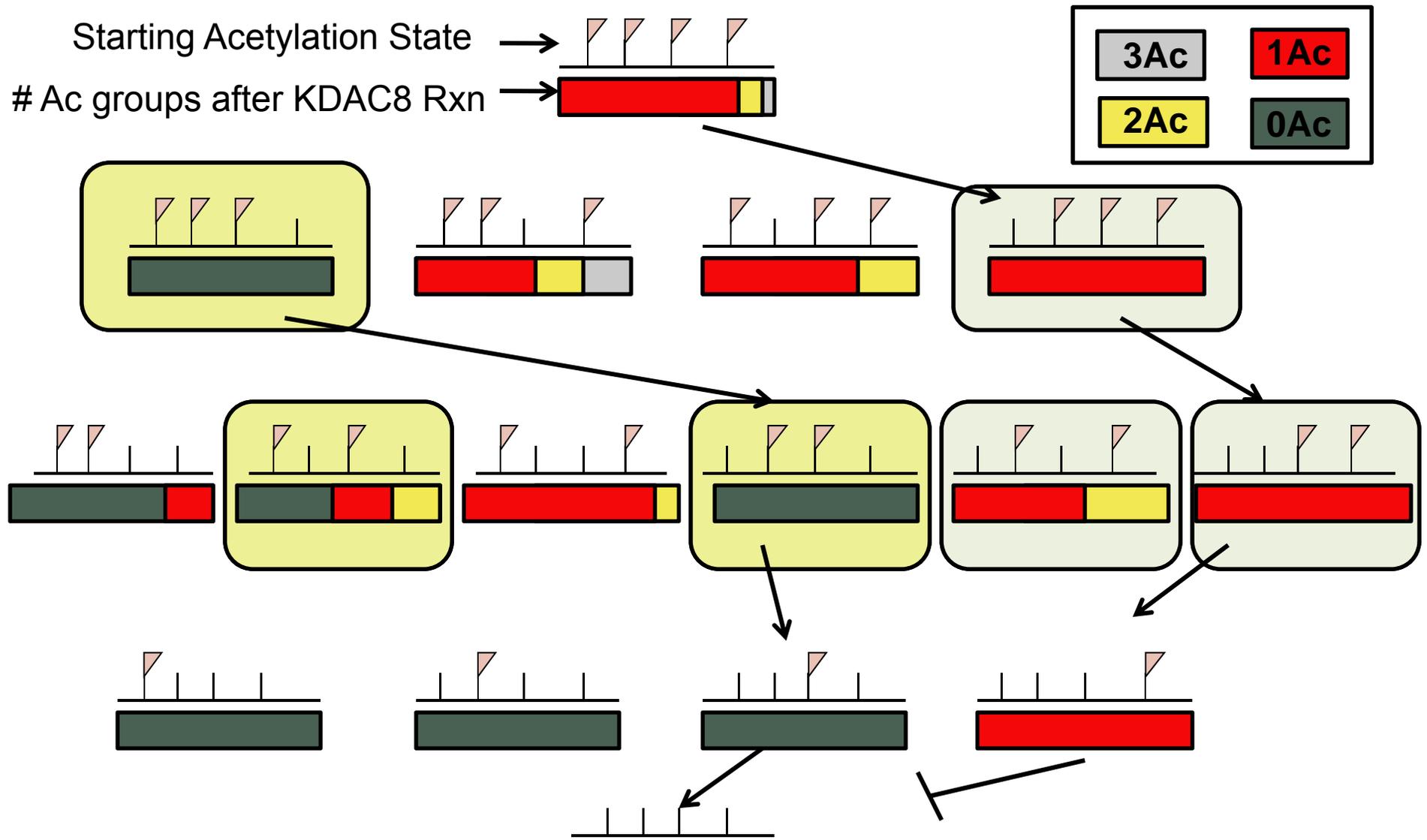


Relative Kinetics Dictates Deacetylation Pathway



H4 Histone "Tail" Sequence: SGRG**K**GG**K**GLG**K**GGAKRHRC

Relative Kinetics Dictates Deacetylation Pathway



H4 Histone "Tail" Sequence: SGRG**K**GG**K**GLG**K**GGAKRHRC

Role of Centers in Translational Research

NORTHWESTERN UNIVERSITY

CENTER *of* CANCER NANOTECHNOLOGY EXCELLENCE NU-CCNE

A strongly integrated partnership between the
International Institute for Nanotechnology and the
Robert H. Lurie Comprehensive Cancer Center

Funded by the NCI Alliance for Nanotechnology in Cancer U54-CA119341

- **Established in 2005**
- **Mission**
 - To advance discovery and knowledge within the field of nanotechnology through focused and collaborative research leading to the design and testing of nanomaterials and nanodevices for their translational application into the clinic
- **Supports**
 - **6** interdisciplinary translational research projects
 - **2** seed projects each year with university funds
 - **37** nano-scientists, cancer biologists, engineers, and clinicians
 - **26** postdoctoral associates
 - **10** undergraduate researchers

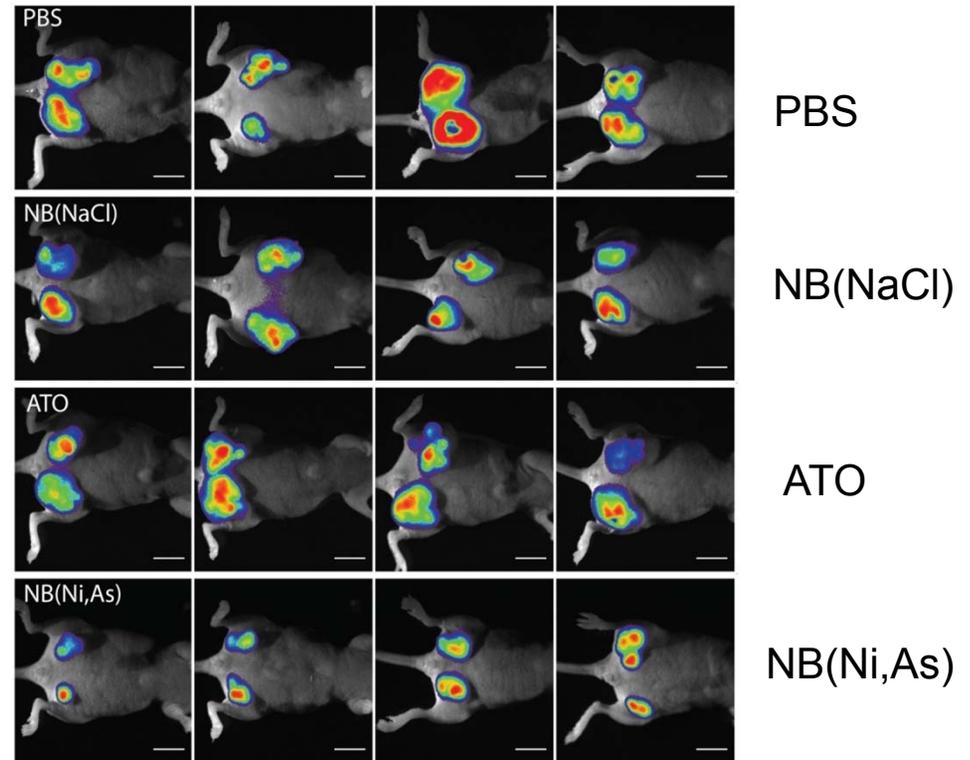
Translation Through Industrial Partnerships

- **Nanosphere, Inc.**
 - Retrospective study for development of ultra-sensitive PSA detection
- **Takara Biosciences**
 - Scanometric detection and nanoparticle-based electrical and optical transduction mechanisms
- **NanoInk, Inc.**
 - Commercialization of DPN technology portfolio
- **PreDx, Inc.**
 - Transform conventional diagnostic imaging techniques, e.g. MRI, from their current role as *anatomical* imaging tools into metabolic and theranostic probes
- **Bracco Corp and Siemens Inc.**
 - New probe development
- **Neopharm Pharmaceuticals**
 - Polymer coated liposome (PCL) technology
- **Altor Biosciences**
 - Targeting technology
- **Integrated DNA Technologies**
 - Studies of DNA thermodynamics
- **Nanotope**
 - Multiplatform therapies in medicine
- **Baxter**
 - Assays for the quantification of cell “polarizability” independent of the cell asymmetry

Example of Technology Heading to the Clinic

ATO nanobin inhibits breast tumor growth

- **Summer 2009** - NCI/NCL
Anticipated Completion of
Preclinical Work
- **August 1, 2009** Submission to
Rapid Access to Intervention
Development (NCI-RAID)
(GMP scale up, toxicity and
pharmacokinetic testing)
- **Winter 2010** - Pre IND meeting with
FDA
- **Mid Summer 2011** - Investigational
New Drug Application
(Enables Phase 1 Clinical Trials)



***At completion of NCI-RAID IND will be filed
Anticipated entry into clinical trials Summer 2011***

Nanoscale Constructs for Diagnostics and Therapy

Activity depends on shape, size, composition
knock-down with gold NPs

Nanomaterials have novel, and tunable properties
enable label-free assays

Centers critical to translational work
nanoassemblies in the clinic
partnerships with other Centers, Industry