Sāpidyne
INSTRUMENTS

# Newsletter 2021

## Sections

1| Cell Pellets & the Effects on Equilibrium

2| Improved Flow Cells

3| LED Lamps

4| Ask the Inventor

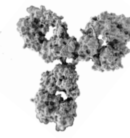
5| Geek Corner

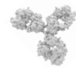
6| Employee Spotlight

7| Cleaner Water with KinExA

Since 1995

# 1 Cell Pellets & the Effects on Equilibrium





KinExA affinity measurements for a specific binding target to cell expressed receptors require the reaction to be at equilibrium. After cells equilibrate with the target, they are pelleted and the supernatant is retained for subsequent experiments (**Figure 1**).

During the centrifugation process, which takes a few minutes, the cells become concentrated into a pellet at the bottom of the sample tube. This process will not effect the equilibrium of the samples and can be demonstrated by following the equations.

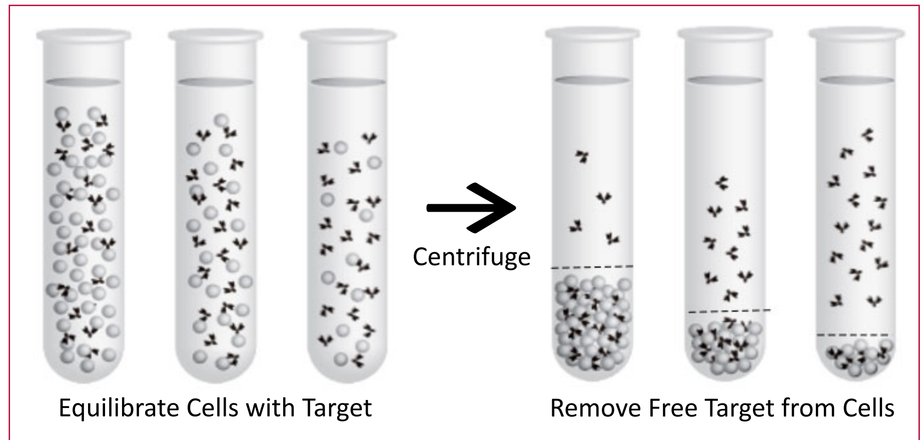



***Figure 1.*** *Experimental set up for cell based measurements. Once cells have been centrifuged the free target is removed above the dashed line to run in an equilibrium experiment.*

**Equation 1** shows a simple algebraic observation.

Where:
[R]= Unbound Cell Receptor
[T]= Free Target
[RT]= Bound Cell Receptor-Target Complex

$$K_d = \frac{[R][T]}{[RT]} = [T] \cdot \left( \frac{[R]}{[RT]} \right)$$

***Equation 1.***

At equilibrium the ratio [R]/[RT] is constant, and is determined by the $K_d$ and the free target concentration [T], as expressed in **Equation 2**.

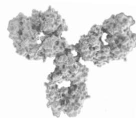
$$\frac{K_d}{[T]} = \frac{[R]}{[RT]}$$

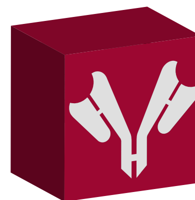
***Equation 2.***

When the cells are pelleted, their concentration is increased in a local area, but the free target concentration did not change. Since both the bound and unbound cell concentrations are increased by the same amount in the pellet, the ratio [R]/[RT] stays the same. Looking again at **Equation 2**, you can see that although the local concentration of the cells changed, the overall equation does not. Therefore, during the pelleting process, you can be sure the equilibrium does not shift.

In fact, after the cells are pelleted, you can remove the supernatant and the equilibrium is still not affected. Although you did remove some of the volume, you did not change the free target concentration therefore everything in **Equation 2** remains the same.

Kinetic Exclusion Assay (KinExA)
Now On Wikipedia
en.wikipedia.org/wiki/Kinetic_exclusion_assay





## Advanced Flow Cells For Sticky Sytems | 2

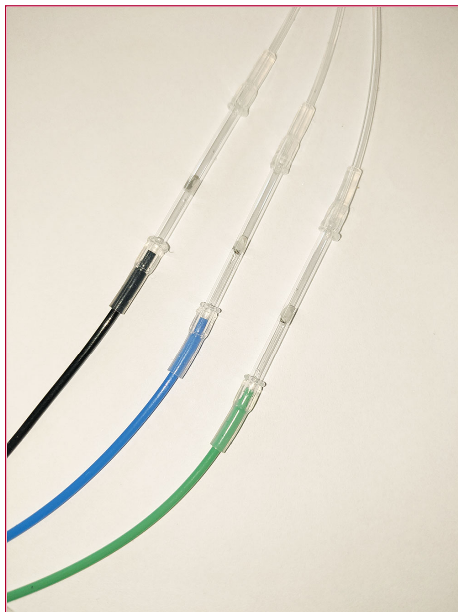


***Figure 2.*** *(Black) Siliconized Flow Cell (Blue) Non-treated Flow Cell (Green) Hydrophilic Flow Cell*

At Sapidyne, our R&D team is continuously working to improve our measurement capabilities and overcome the issues that interfere with getting consistent, clean data. One of these issues is measuring "sticky" systems that cause baseline creep and titrant related non-specific binding. See *Tech Note TN216 Baseline Creep* and *Tech Note TN210 Titrant Related NSB* for more information. In our *2013 Newsletter* we introduced our siliconized Flow Cells to help fight these sticky systems. We now have a new, improved, flow cell that does the job better.

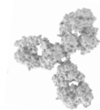
The inner surface of the new flow cell has been chemically modified by attaching a PEG-Silane molecule. This makes a hydrophilic surface thereby reducing the ability of proteins to stick. We tested a particularly sticky system of antibodies extracted from chicken yolks that were run through the Hydrophilic Flow Cell. The Hydrophilic Flow cell resulted in only 0.30 V of baseline creep after 10 runs compared to the 0.77 V increase observed in the Siliconized Flow Cell.

| Part Number | Description | Line Color |
|---|---|---|
| 392534 | 3200 Non-Treated Flow Cell Pack of 3 | Blue |
| 392360 | 4000 Non-Treated Flow Cell Pack of 3 | Blue |
| 392151 | 3200 Siliconized Flow Cell Single | Black |
| 392170 | 4000 Siliconized Flow Cell Single | Black |
| 392534 | 3200 Hydrophilic Flow Cell Pack of 3 | Green |
| 392544 | 4000 Hydrophilic Flow Cell Pack of 3 | Green |

***Table 1.*** *Flow Cells*

Available Flow Cells can be seen in **Figure 2** and differentiated by exit line color. See **Table 1** for more information and contact Sapidyne for ordering information.

## LED Lamp Replacement KinExA 3200 | 3



We are now offering an LED lamp replacement with a much longer life span and a 10 year warranty (**Figure 3**). The LED lamp is a direct replacement for the halogen lamp (**Figure 4**).

Advantages to using the LED lamp are: fewer lamp replacements, lower baseline signal, and low risk of lamp burnout while an experiment is running. LED lamps also use 10% of the electricity that halogen lamps use.

Replacing your old lamp with the new LED lamp is straight forward and can be completed in 5 minutes with 3 easy steps:

***Figure 3.*** *LED Lamp for KinExA 3200*

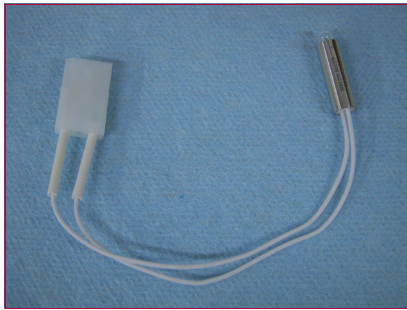
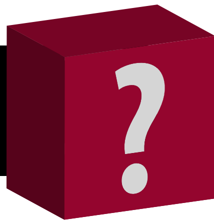


*Figure 4. Halogen Lamp for KinExA 3200*

1. Turn the old lamp off and remove it from the instrument.
2. Plug in the new LED lamp and turn it on.
3. Adjust the new LED lamp to ensure proper focus and alignment.

An in-depth How to Guide can be found on our website under the resources tab titled *HG204 Lamp Replacement and Alignment*, which walks you through the alignment process in detail with helpful pictures to guide you. There are specific LED lamps depending on the filter set being used. Refer to **Table 2** for LED lamp specifications.



| Part Number | Description | Filter Set |
|---|---|---|
| 511100 | 3200 LED Lamp, Red | Red Filter |
| 511101 | 3200 LED Lamp, Blue | Blue Filter |
| 511102 | 3200 LED Lamp, Green | Green Filter |

***Table 2.*** *LED Lamp Filter Sets*

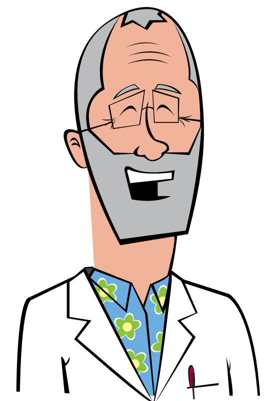
## 4 Ask the Inventor ?

# Q:What is the difference between avidity and cooperativity?

A. I'll start with two definitions.

Cooperativity: (noun) specifically, biochemistry: the molecular interaction between a ligand and macromolecule (e.g. protein) having two or more binding sites in which binding of a ligand to one site positively or negatively influences subsequent ligand binding at other sites by either increasing or decreasing the affinity of other binding sites for a ligand.

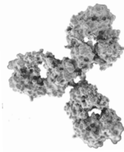
Avidity: (noun) In proteins, avidity describes the binding intensity of multiple bond interactions between proteins.

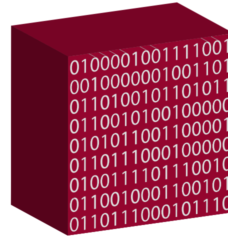


The difference is in the biochemistry definition. Cooperativity refers to a change in a binding site's affinity caused by binding at an alternative binding site on the same molecule. The molecule exhibiting cooperativity must be multivalent but the ligand causing the change does not. Avidity, on the other hand, does not include a change in binding site affinity but refers instead to the tighter binding that can result from multiple simultaneous binding events between two multivalent molecules.

I believe the confusion arises because cooperativity suggests cooperation (the process of working together to the same end) and in avidity the binding sites do work together to produce a stronger overall binding. Biochemically speaking, the difference is significant. Cooperativity changes the shape of the binding curve making it steeper than normal 1:1 binding and avidity does not. A well known example of cooperativity is with hemoglobin and oxygen. The steeper cooperative curve significantly increases hemoglobin's oxygen transporting efficiency and is also important in signaling cascades[1]. Please see *Technology Note TN213 Cooperativity* for more information.

[1] Ferrell, J.E., Jr. (2009). "Q&A: Cooperativity." J Biol **8**(6): 53.






Geek Corner 5

**21 CFR Part 11 Compliance**

Data integrity and safety for electronic records is important for FDA submissions, patent applications, and scientific publications. In the early 1990's, computerized systems were increasingly being used in place of paper records and signatures. Because of this, the scientific community wanted guidelines for proper electronic records and signatures. In 1997, the 21 CFR Part 11 Final Rule went into effect[1]. There have been revisions and clarifications over the years but the main theme still exists; Data must be trustworthy and accurate.

The benefits to part 11 compliance are as follows[2]:

- Protection and retrieval of electronic records
- Operational consistency
- Improved productivity and efficiency through automation
- Minimized or eliminated management of paper documentation
- Faster data-related searches
- Electronic submission to the FDA

All KinExA instruments are compliant with 21 CFR Part 11. The instruments are a passive system that create an unalterable read-only copy of every experiment that is run. If you look in the upper left hand corner on the "Experiment" tab of any experiment that was run, there is an ID number. During management review and internal or external audits, any experiment can be verified by checking the unalterable backup copy on the instrument.

Refer to Part 11, Electronic Records; Electronics Signature – Scope and Application 1 for guidance when keeping electronic records. If paper records are kept, then Part 11 would not apply.

[1] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/part-11-electronic-records-electronic-signatures-scope-and-application

[2] https://validationcenter.com/category/data-integrity/

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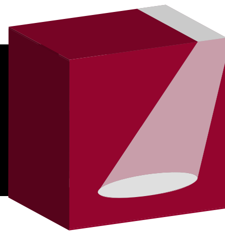
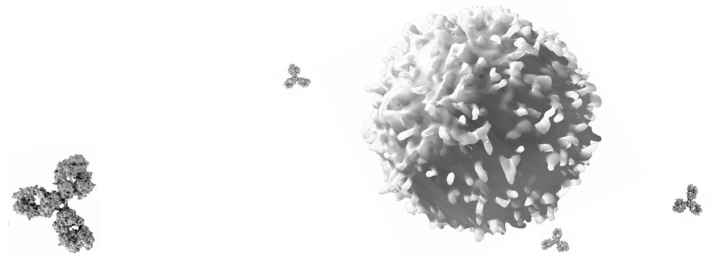
# 6 | Employee Spotlight





At Sapidyne we are proud to work with a highly qualified and experienced team to further develop our KinExA instruments and to serve the scientific community. The tradition of our company is to build relationships with our customers in order to give them the best possible assistance for their projects. Therefore, we are including an additional section in our Newsletter to introduce our employees more personally by showcasing their background and experience.

In this Newsletter edition, we introduce Frank Hamacher who started his work for the European branch of Sapidyne Instruments in 2014. He was born in Western Germany and studied human medicine in Hannover before completing his diploma in biochemistry at the Freie Universität in Berlin. His main focus was immunobiology and, after finishing his diploma thesis, he worked for the Institute of Pathology at the Charité Hospital in Berlin. His research at the Institute was on signal transduction pathways in cancer. Several years later he began working for the Laboratory of Immunological and Molecular Cancer Research, a division of the Oncology Department of Landesklinikum in Salzburg, Austria. There he helped to build and establish the Gene Expression Facility within the department. His research was focused on finding new drugs and biomarkers for blood cell, breast, and pancreatic cancers. During his tenure he published numerous scientifific papers, gave talks at international cancer conferences, and received several awards.

In 2014, Frank ventured to the private sector by starting his work for Sapidyne Instruments. He helped to build and establish the European branch of Sapidyne in Germany to better serve those customers directly. Frank performs contract research measurements, service visits, demonstrations, trainings, and supplies consumable/part shipments. Additionally he is always ready to give instant support for any open questions regarding the KinExA technology. His goal is to make KinExA Technology more popular in Europe and to convey the excellence of the technology to our potenial users.

Frank‘s hobbies include traveling, cycling, tennis, and anything that promotes a happy, healthy, lifestyle.

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# Evaluation of a rapid biosensor tool for measuring PAH availability in petroleum-impacted sediment

## Highlights

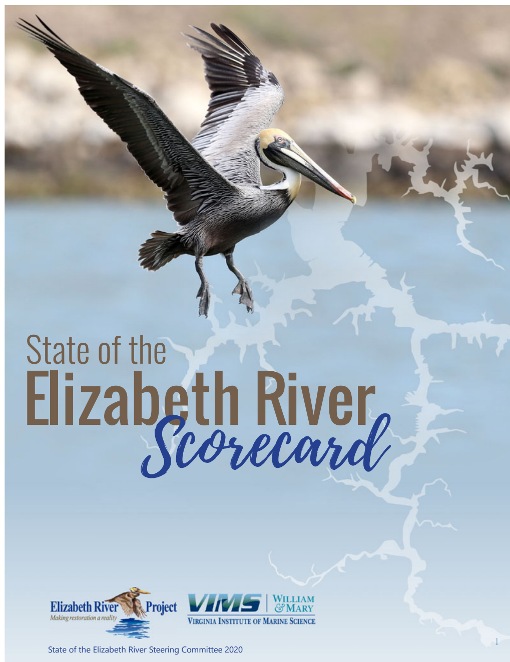
- Freely dissolved PAHs (Cfree) in sediment correlates with bioavailability.
- A rapid and field-deployable biosensor Cfree measurement tool was evaluated.
- Biosensor results for test sediments correlated with passive sampler Cfree results.
- The biosensor is a promising screening tool to optimize future investigations.

"The same day of porewater collection and filtration, the samples were analyzed with a KinExA Inline instrument (Sapidyne Instruments, Boise ID) to determine the ΣPAH using the antibody-based biosensor method...

The biosensor offers flexible field- or laboratory-based rapid assessment capabilities for generating real-time data that can be used to prioritize sediment for additional evaluations such as Cfree determination with passive samplers, toxicological testing, or other ecological evaluations. These capabilities are particularly attractive for evaluating sediments in remote locations and during a variety of investigation and remediation monitoring phases of typical sediment sites."

**Jason Conder, Mehregan Jalalizadeh, Hong Luo, Amanda Bass, Steven Sande, MIchael Healey, Michael A. Unger, 2021. Evaluation of a rapid biosensor tool for measuring PAH availability in petroleum-impacted sediment . Envionmental Advances 10.1016/j.envadv.2021.100032**

**https://www.sciencedirect.com/science/article/pii/S266676572100003X**




Page 15

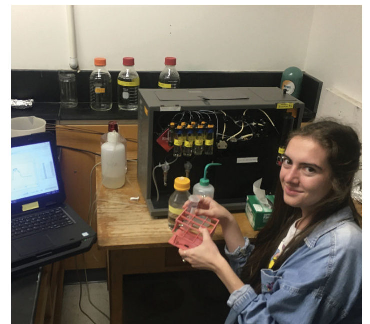
## Give her an oyster and 7 minutes

### She can say if there's a possible PAH trouble spot

**In the past, identifying river hotspots** for contamination with PAH—associated with cancer in fish—was a long, arduous and expensive task. Now Kristen Prossner, a Ph.D. student at VIMS, has developed a way to target areas of concern for the contaminant in just 7 minutes, using oysters.

Using biosensor technology, Prossner has shown that the fluid around the oyster mantle ("oyster liquor") can be analyzed for PAHs in a few minutes to predict the tissue concentrations in the whole animal, a task that traditionally takes weeks. She says the novel method should prove an economical way to "help us target areas of concern where remediation should continue," while also tracking the recovery of remediated sites.

Her recent work confirms previously identified PAH hotspots on the Southern Branch, in the vicinity of sites known as Money Point III and Republic Creosote, but also flags potential problems at the mouth of the Western Branch and Eastern Branch of the Elizabeth (see map). Funder: NIEHS-SRP grant RO1ES024245.



*Kristen Prossner measures oysters for PAH contamination.*

*This will determine PAH concentration in the oyster by how much antibody binds in sample.*

**The KinExA Inline biosensor is pictured on page 15 of the *State of the Elizabeth River Scorecard* 2020. The KinExA Inline biosensor has been deployed to aid in remediation and recovery efforts in the Elizabeth River, coastal Virginia, and Galveston Bay.**

# Contract KînExA Research

## www.sapidyne.com/cro

With over 25 years of experience you can trust Sapidyne scientists to accurately characterize a wide variety of binding interactions such as antibody-antigen, protein-protein, antibody-cell surface receptors, and more. Our research services are ideal for organizations that require third-party verification, measurement for a special project, or have high affinity interactions and need the most accurate affinity and kinetics data available in the industry. Our research services are also available through Science Exchange or Scientist.com. Standard measurement services include full characterization of a given binding pair using GLP/GMP guidelines, with a detailed formal report provided.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