**Notes for Discussion: PAINS**

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* **What are PAINS?**
  + **P**an-**A**ssay **IN**terference compound**S** (frequent hitters, false positives, non-specifics etc.)
  + Use multiple, different sites on enzyme
  + Widespread in biochemical HI enzyme activity assays
  + Can be the test compound and/or its impurities
  + Often not recognised as non-specific ⇨ Confusion, wasted time and money
* **Many Mechanisms**
  + Technology interference
* Generate reactive species
  + Reactive
* Redox
  + Form molecular aggregates
  + Insolubility
* Promote denaturation by binding unfolded protein
* **Identification Guide**
  + IC50 > 3µM
  + IC50 shift with [E]
  + Steep concentration-inhibition curve   
    (super-stoichiometric)
  + Non-competitive
  + Irreversible
  + Time-dependent
  + Active in unrelated screens
* Sensitive to detergent (aggregators)
* No comparable activity in orthogonal assay (assay with different physical basis)
* A known or predicted PAINS structure
* Flat SAR within similar compounds
* A PAINS is not necessarily a PAINS in all assays
* **Avoidance**
  + Don’t remove from screening set
  + Chelators
  + Ensure enzyme is assay-stable
* Reductant
* Detergent
* Share new techniques and PAINS

* **Position Statement**
  + Aim to identify and avoid PAINS in early drug discovery and publications
  + Keep PAINS in screening sets and flagged (computational and historical data)
  + Develop a robust PAINS assay cascade specific to target
  + Determine and strengthen hit SAR early
  + MOI only when hit structure and SAR demonstrated
  + Share PAINS management best practice & learning
* **Discussion points**
  + PAINS property guidelines - What’s the most important and why?
  + Early HI screening – What makes an efficient screening cascade to avoid PAINS?