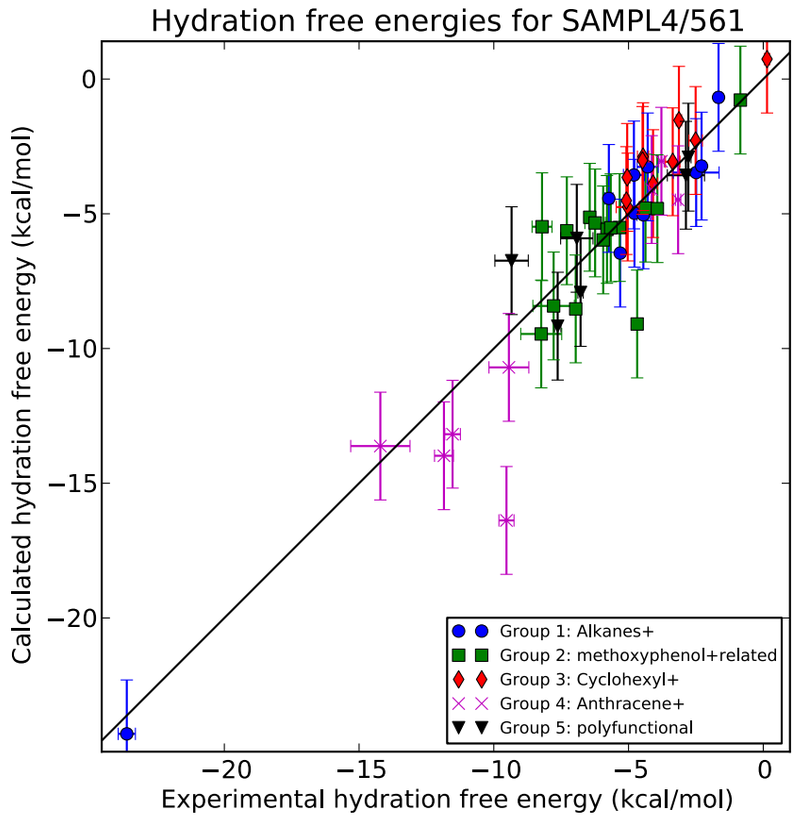
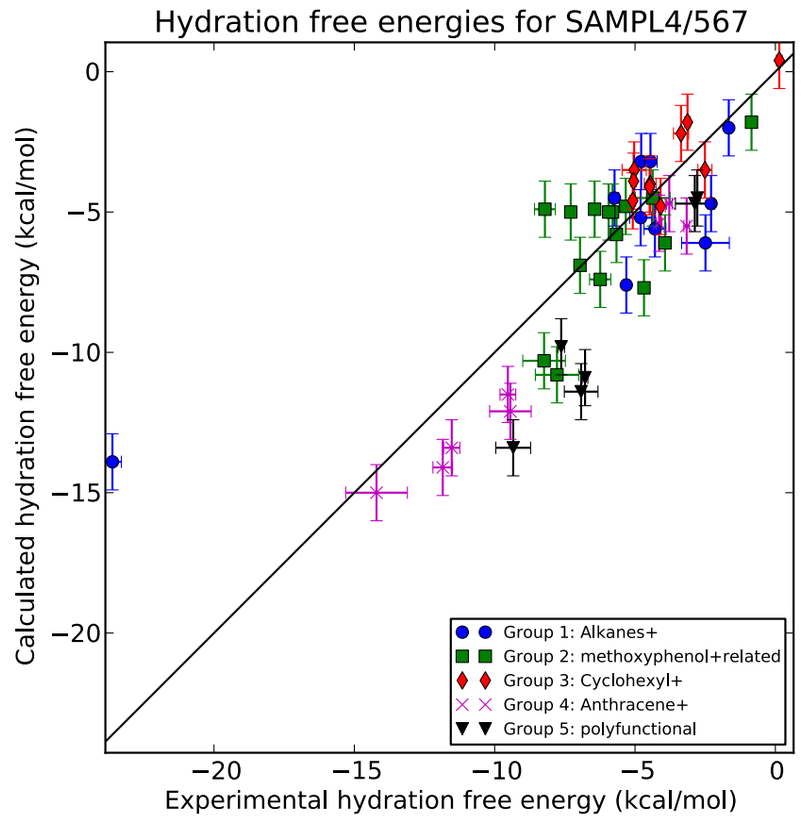
Error analysis may not seem that important for drug discovery -- but poor understanding of error is a huge reason modeling doesn't see more use in discovery settings

A method's usefulness is partly determined by how well it can estimate error





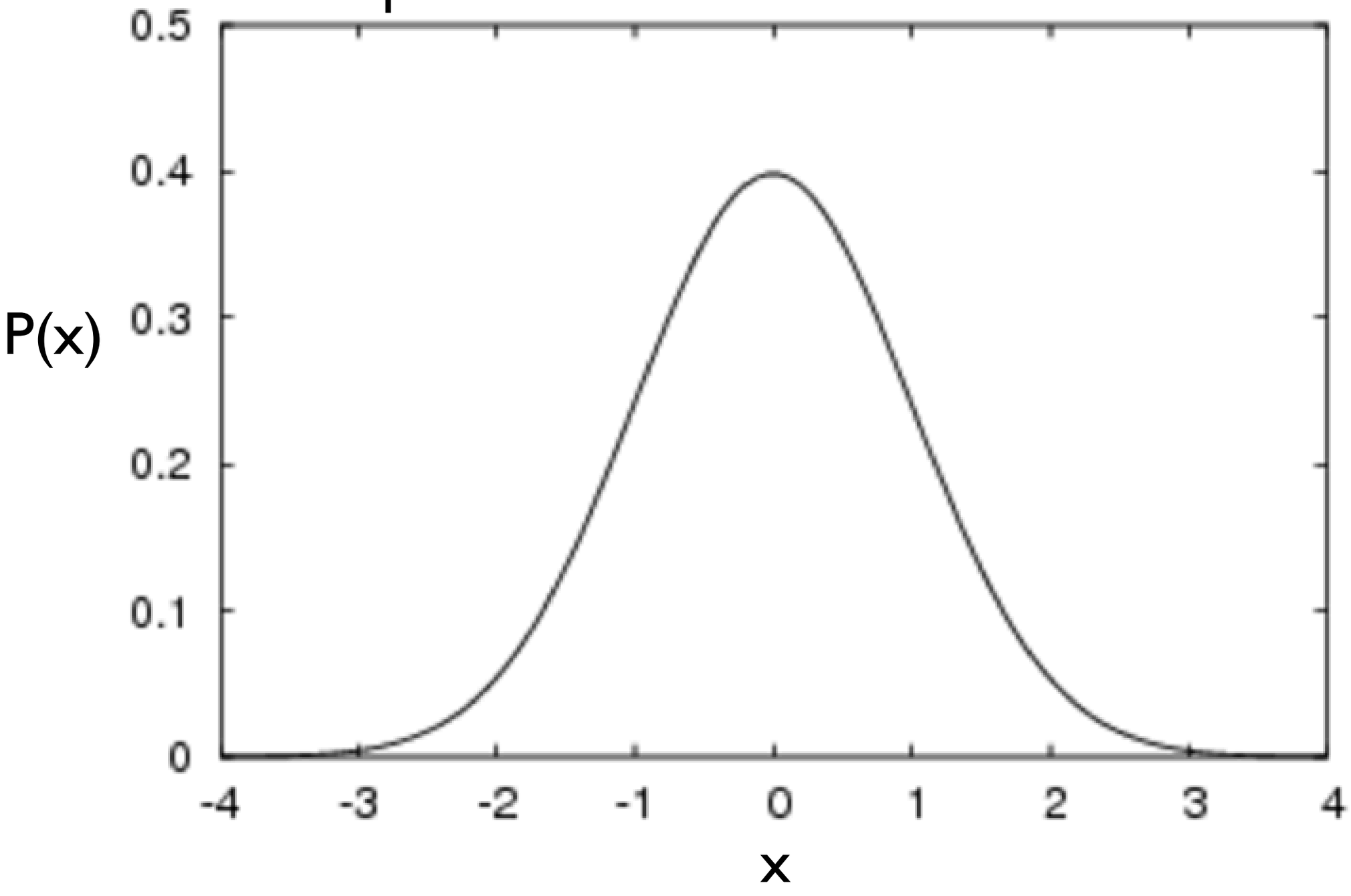
• Average error	-0.12+/-0.24	-0.55+/-0.33
• RMS error	1.61+/-0.32	2.38+/-0.40
• Average unsigned error	1.13+/-0.17	1.78+/-0.23
• Kendall tau	0.73+/-0.05	0.58+/-0.08
• Pearson R	0.93+/-0.04	0.81+/-0.05

A couple of key references to save for your records

- Simulation-oriented: “Best practices for quantification of uncertainty and sampling quality in molecular simulations”: https://github.com/dmzuckerman/Sampling-Uncertainty
- General/screening oriented: “Confidence limits, error bars and method comparison in molecular modeling by A. Nicholls. Part 1 (http://dx.doi.org//10.1007/s10822-014-9753-z) , Part 2 (http://dx.doi.org/10.1007/s10822-016-9904-5) .

We also have to deal with distributions of continuous variables

Example: Gaussian or "normal" distribution



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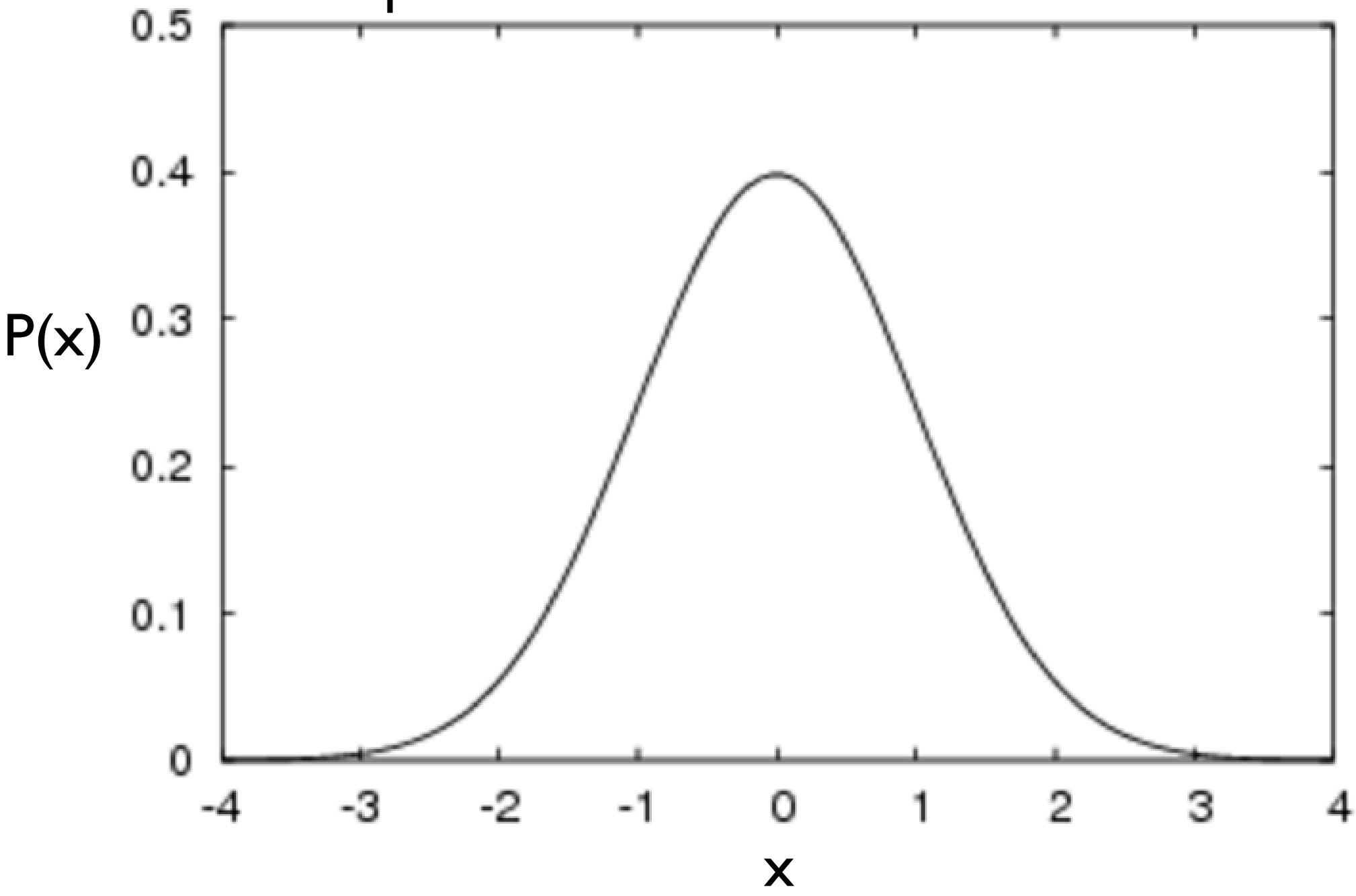
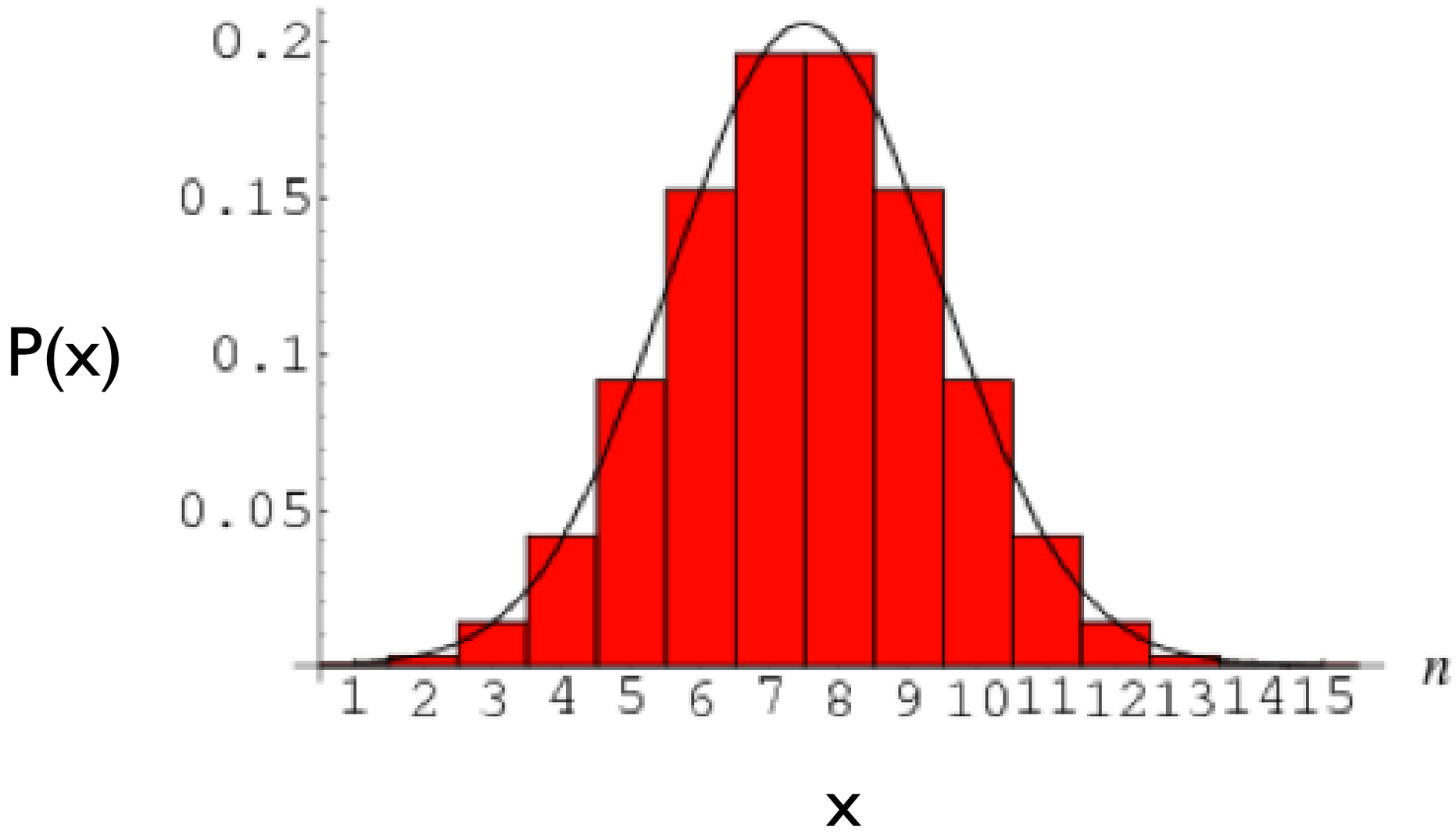


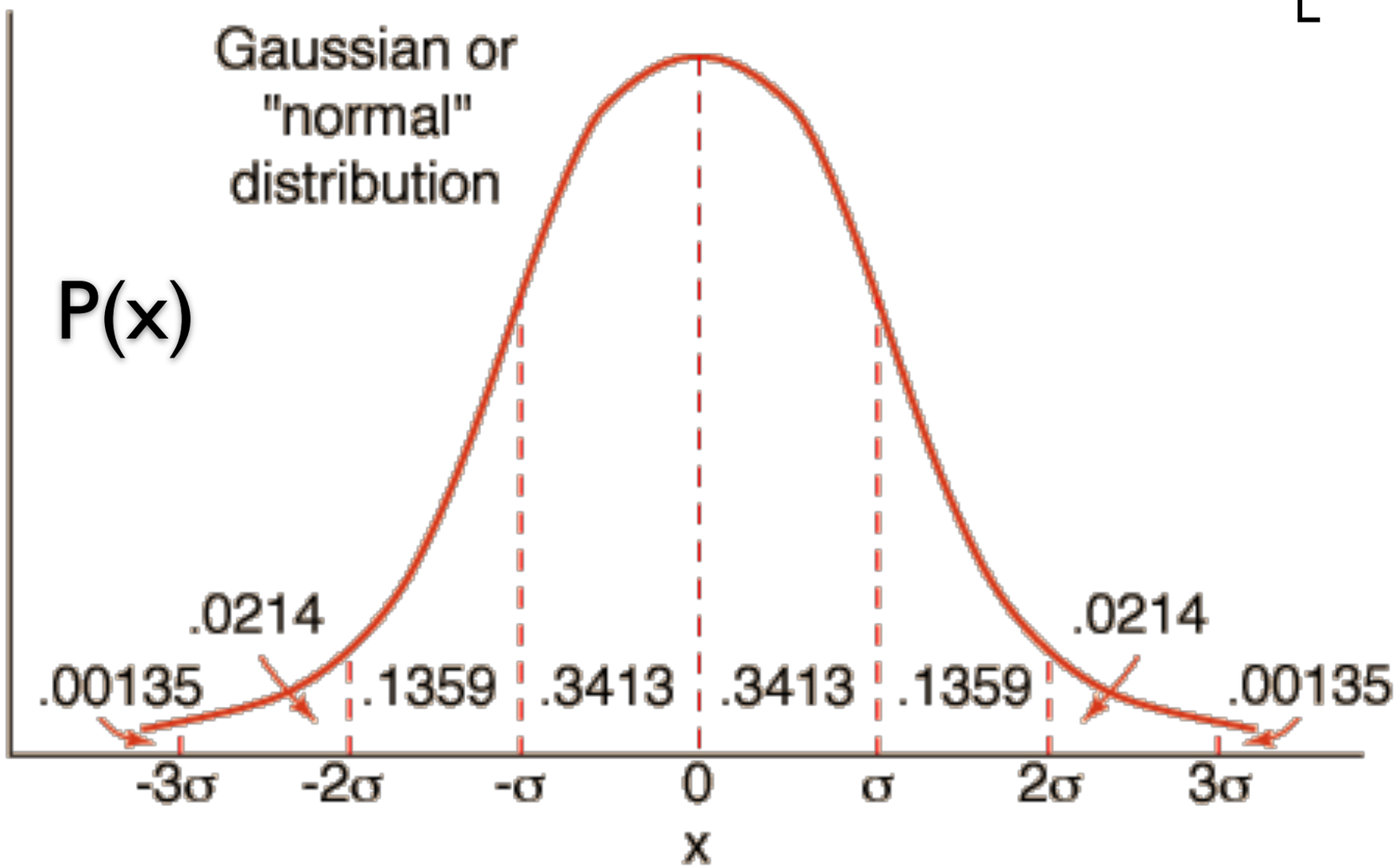
Probability density function

One can also "discretize" continuous distributions



Distributions have a characteristic width, σ

$$p(x) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left[-\frac{x - \langle x \rangle^2}{2\sigma^2}\right]$$



http://hyperphysics.phy-astr.gsu.edu/hbase/math/immath/gauds.gif

Averages are computed by integrating over the probability density function

$$\langle f(x) \rangle = \langle f \rangle = \int dx f(x) p(x)$$

Example, average x. For uniform distribution from 0 to 1 -- that is, p(x) = 1:

$$\langle x \rangle = \int dx \cdot x = 1/2$$

For distribution p(x) = 2x on range 0 to 1:

$$\langle x \rangle = \int dx \cdot x \cdot 2x = 2/3$$

Many experiments amount to averaging over probability distributions

- For example we make many observations
- Or we effectively average over many observations:
  - Observe the same property averaged over many molecules (~Avogadro's number)
  - Average over "long" times (seconds or more)

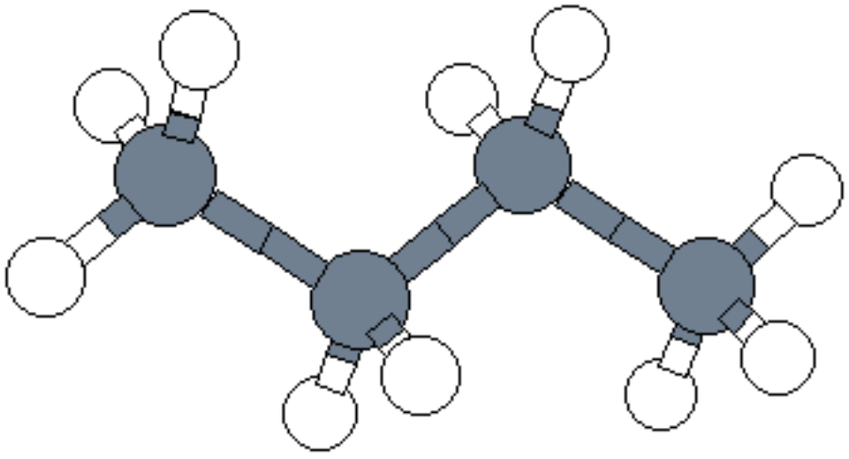
Often, we perform repeated observations of some property to estimate its average

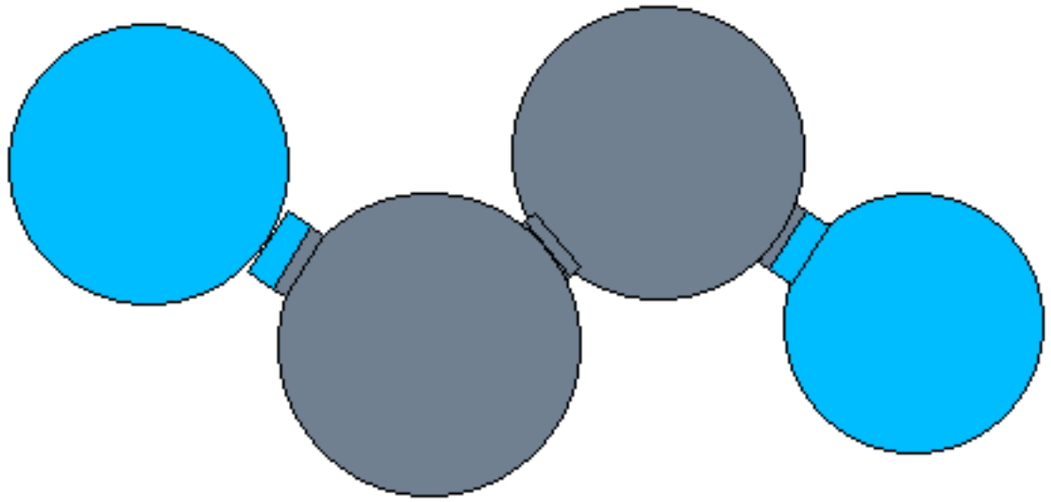
$$\langle f \rangle \doteq \frac{1}{N} \sum_{i=1}^{N} f(x_i)$$

Many observations i=1 to N of some property f of our observed values x_i

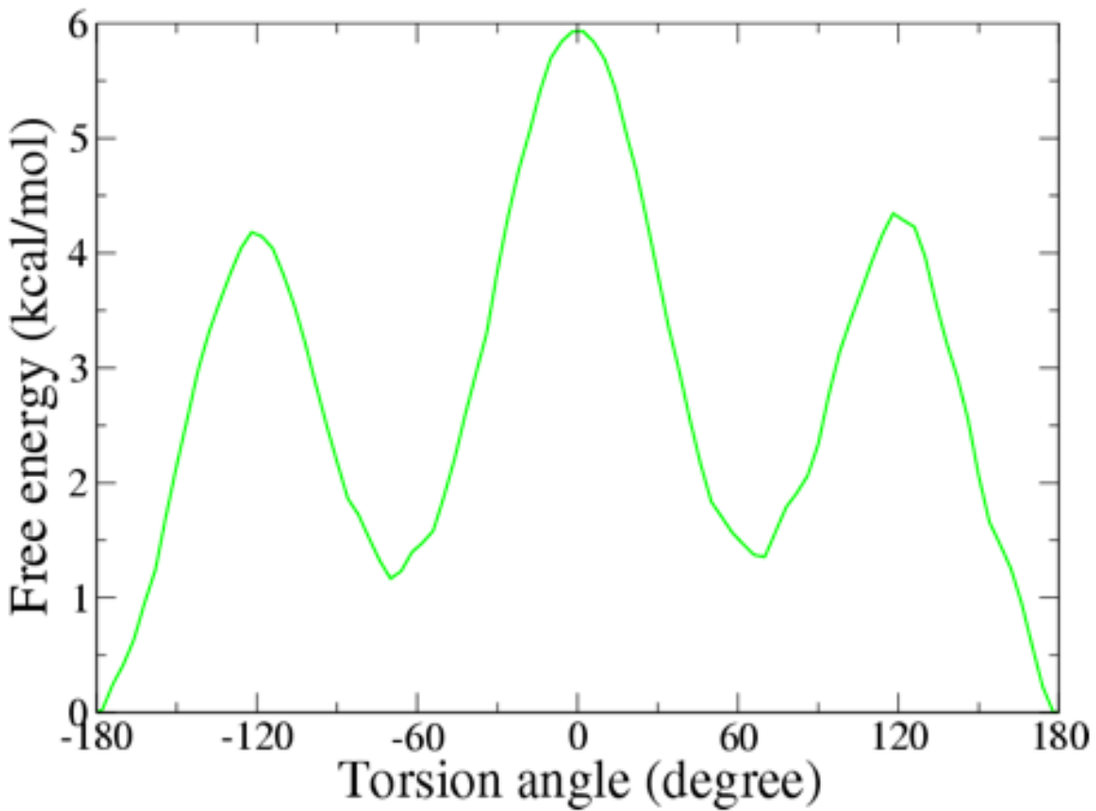
The dot indicates we approximate the true average -- only equal as $N \to \infty$

Example: Butane





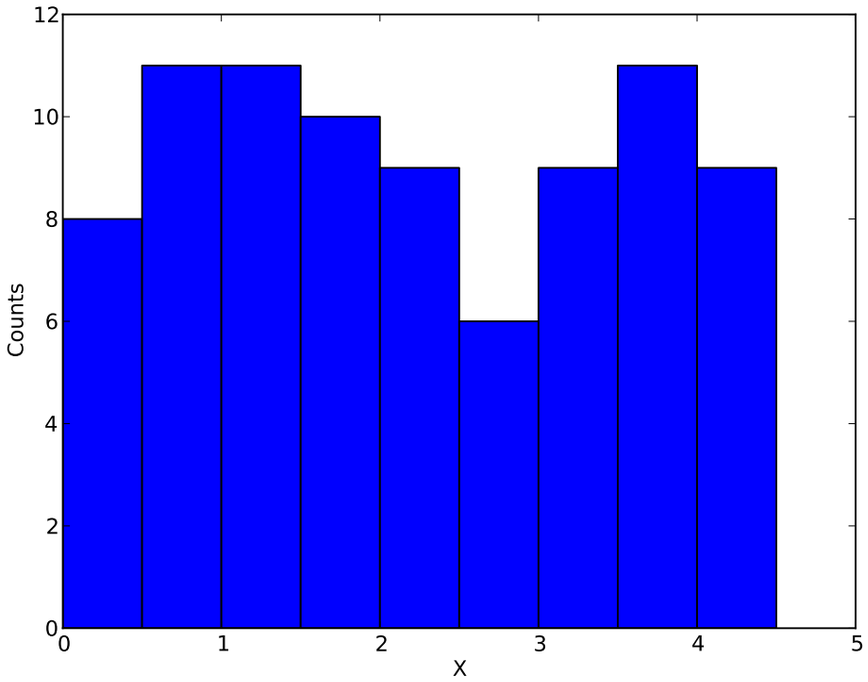
http://www.theo.chemie.tu-darmstadt.de/group/services/yaspdoc/kurs/butan/index.html

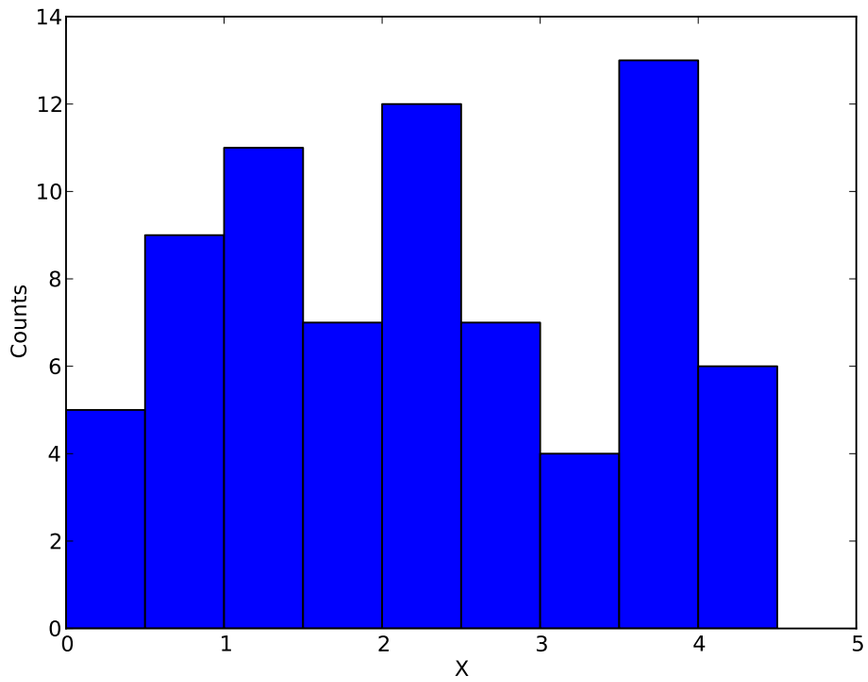


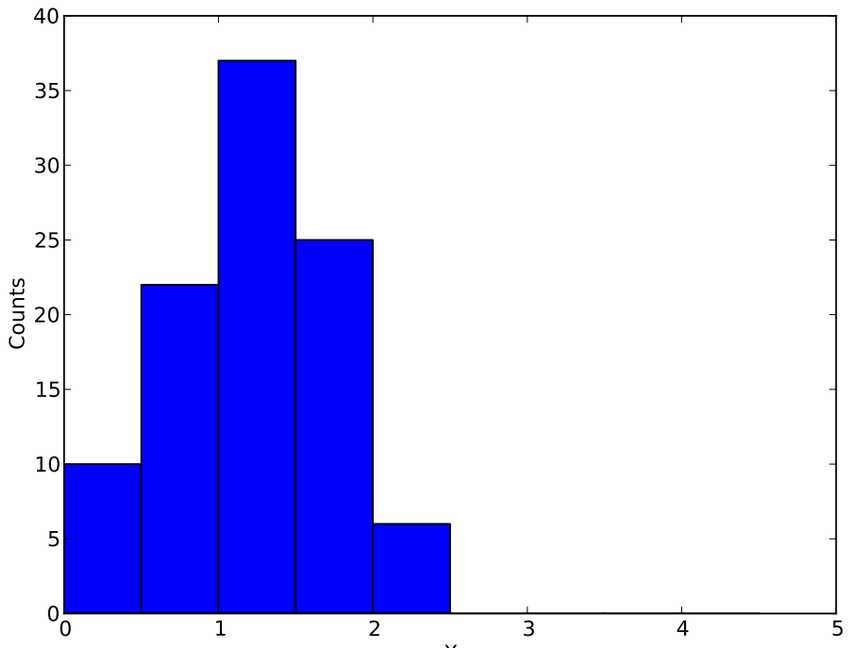
http://projects.eml.org/mcm/people/wang/PMF/tutorial.html

Observations are taken from distributions; distinguishing distributions is nontrivial

100 trials







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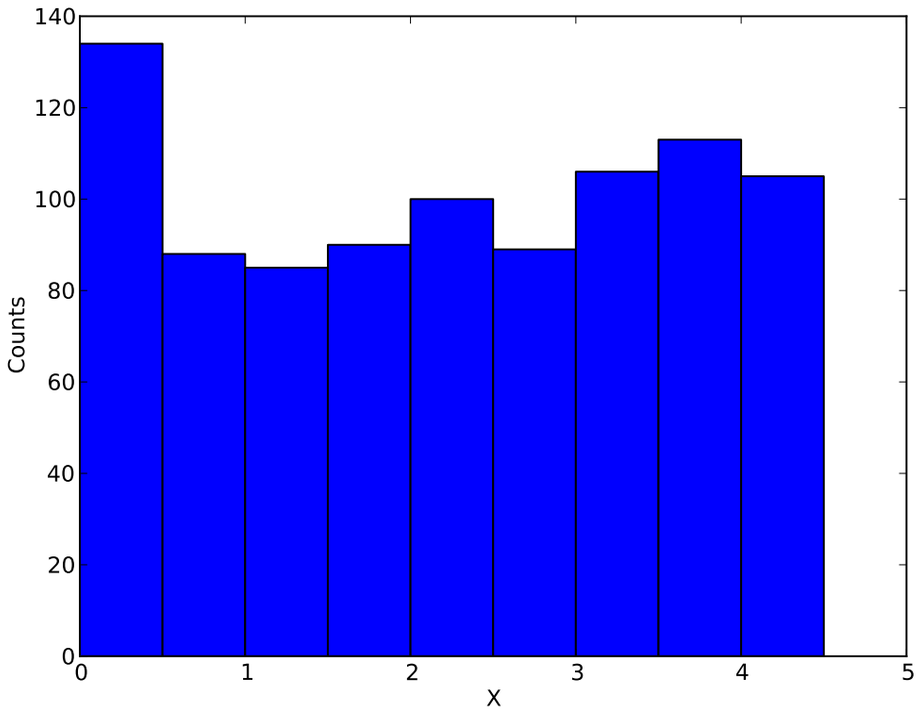
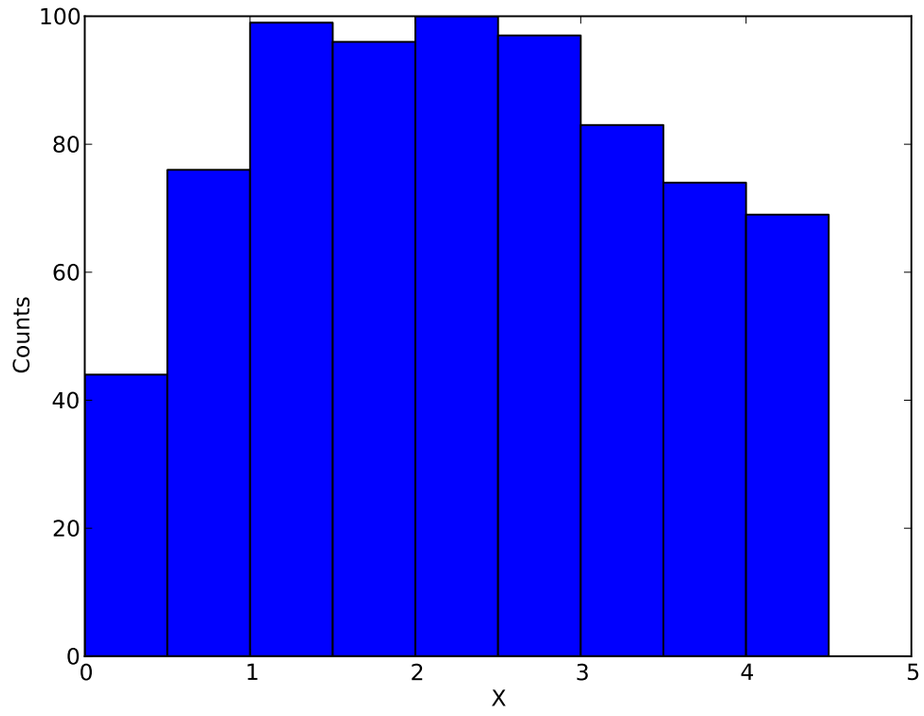
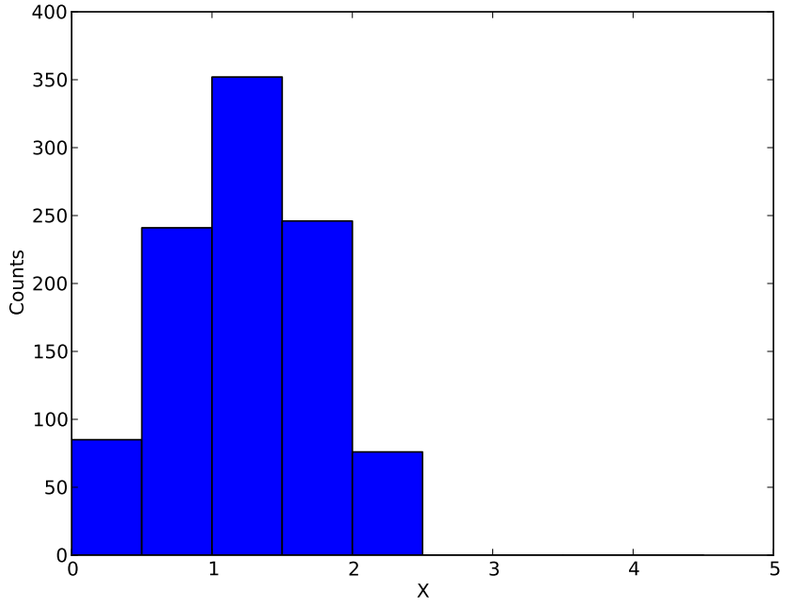


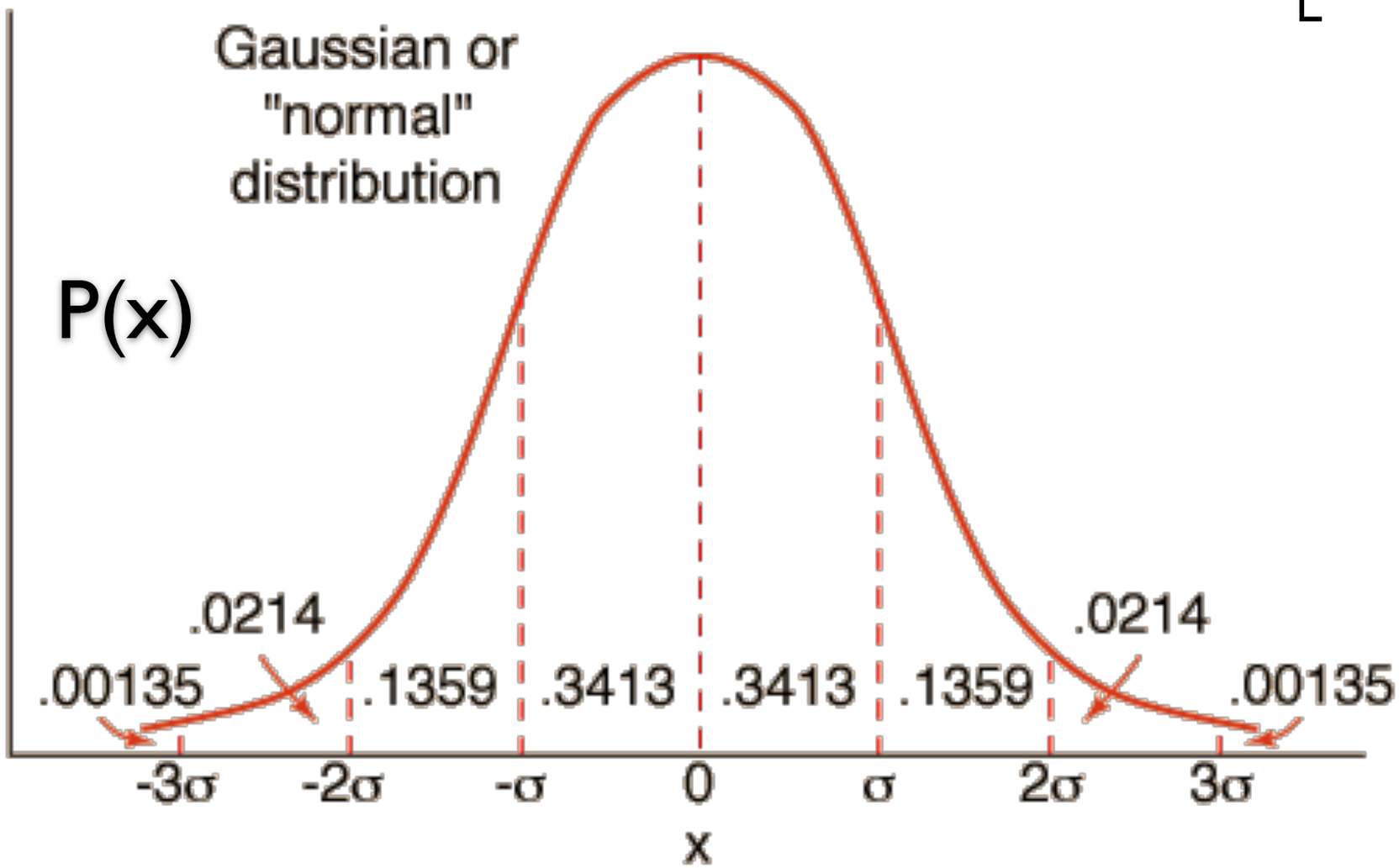




Distributions have a characteristic width, σ

which is called the standard deviation

$$p(x) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left[-\frac{x - \langle x \rangle^2}{2\sigma^2}\right]$$



http://hyperphysics.phy-astr.gsu.edu/hbase/math/immath/gauds.gif

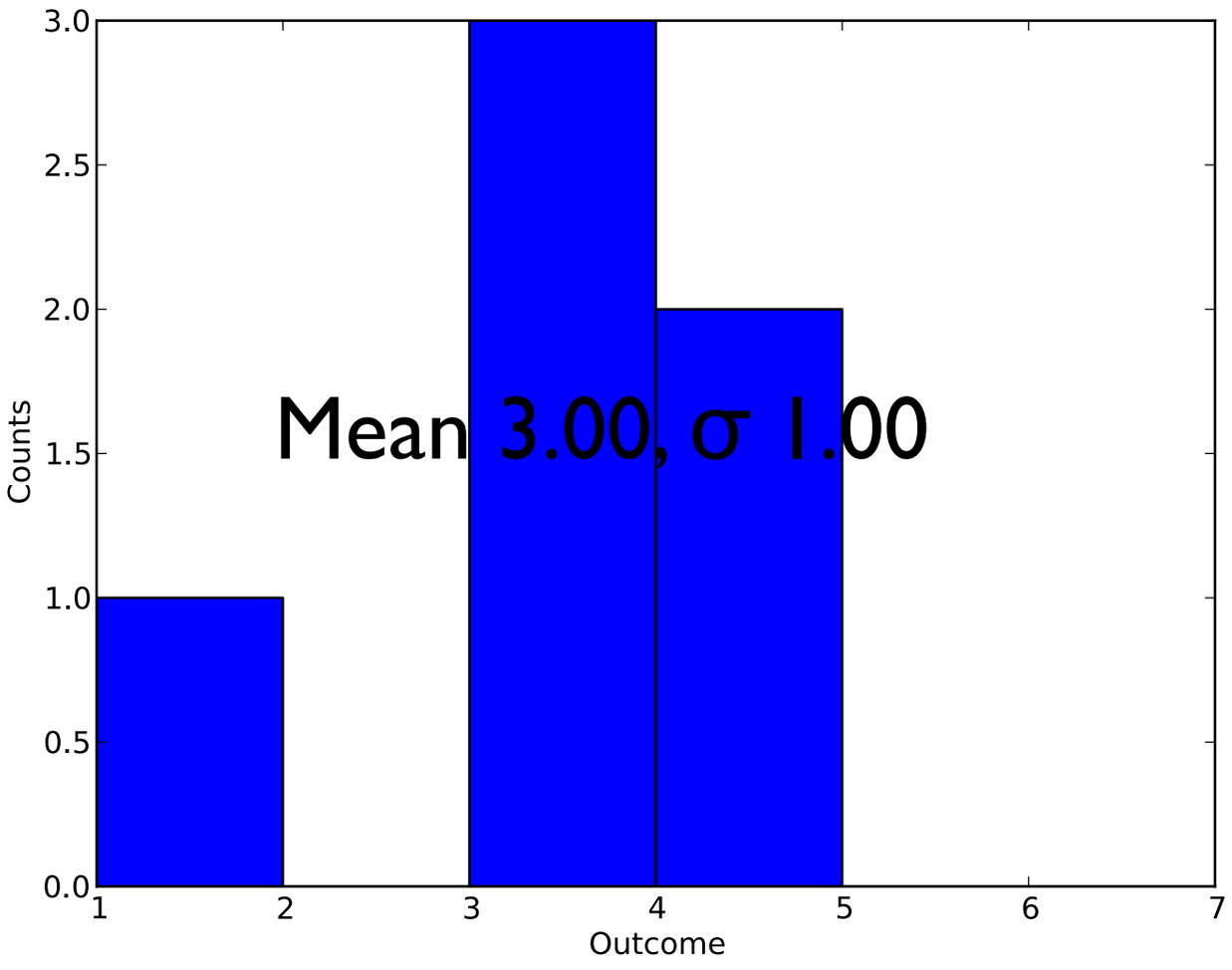
The standard deviation is computed the same way regardless of the shape of the distribution

$$\sigma = \sqrt{\sum_i (x_i - \bar{x})^2} \quad \text{with measurements } x_i$$

and the mean x is: $\bar{x} = \frac{1}{N} \sum_{i=1}^{n} x_i$

To do a good job calculating the mean and the standard deviation, we need lots of data

Example: Throwing dice - try 6 tosses, three times



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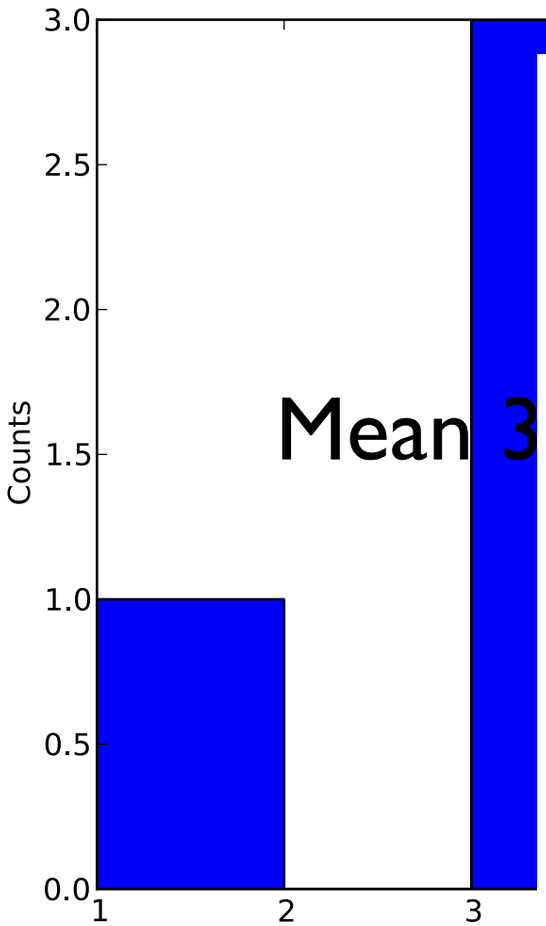

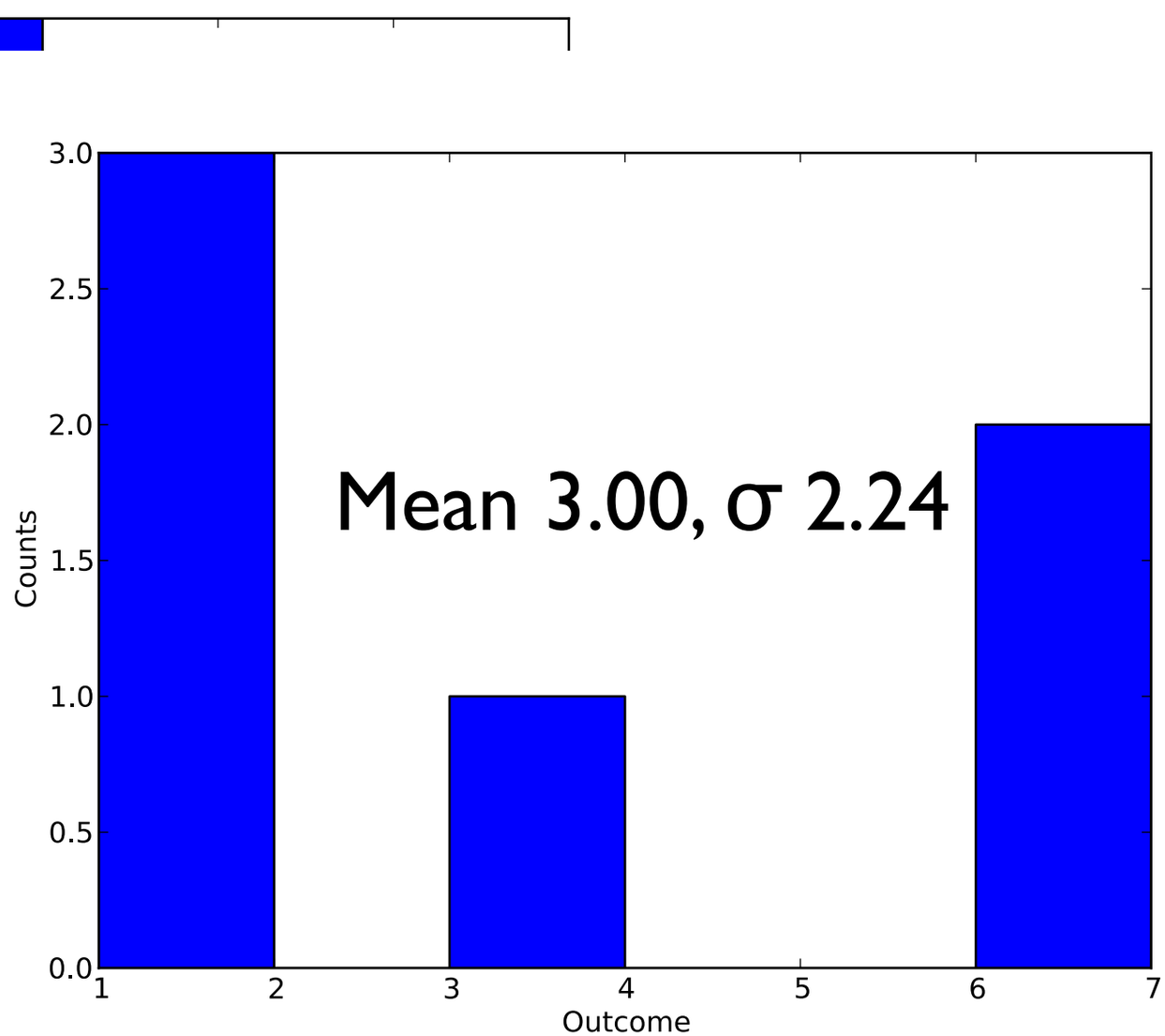

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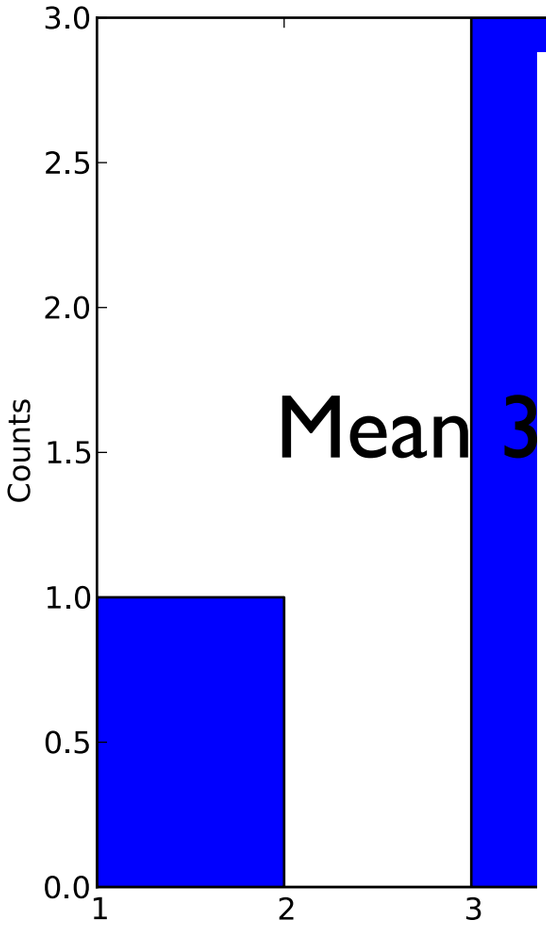

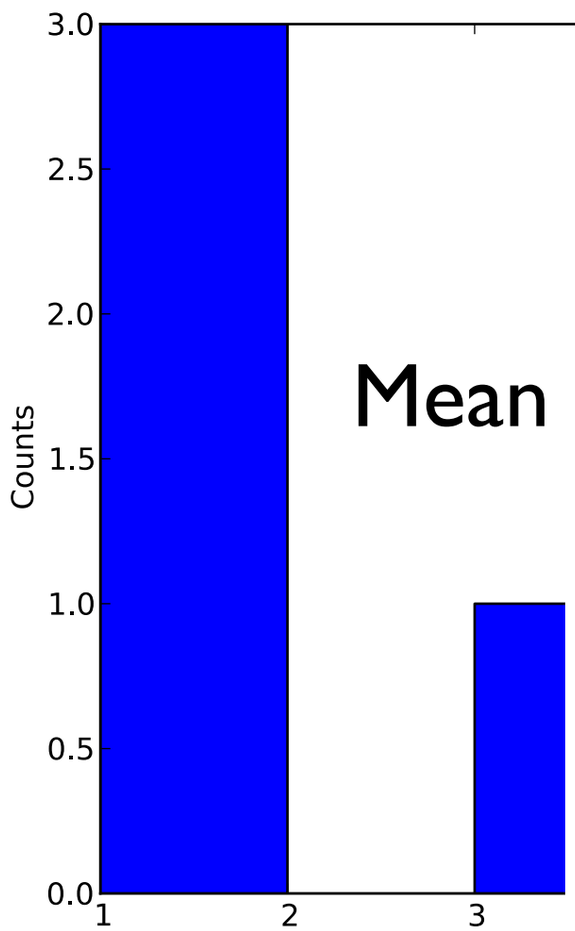

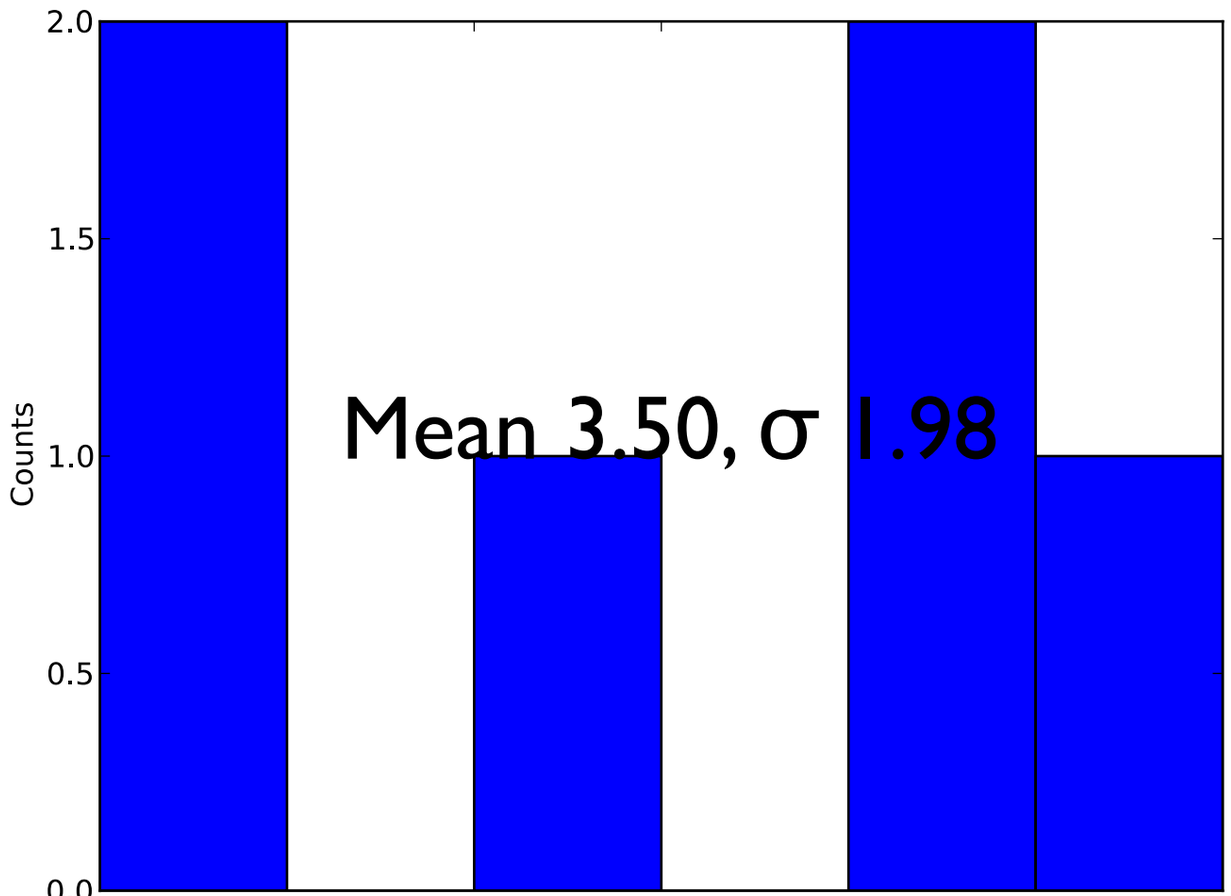

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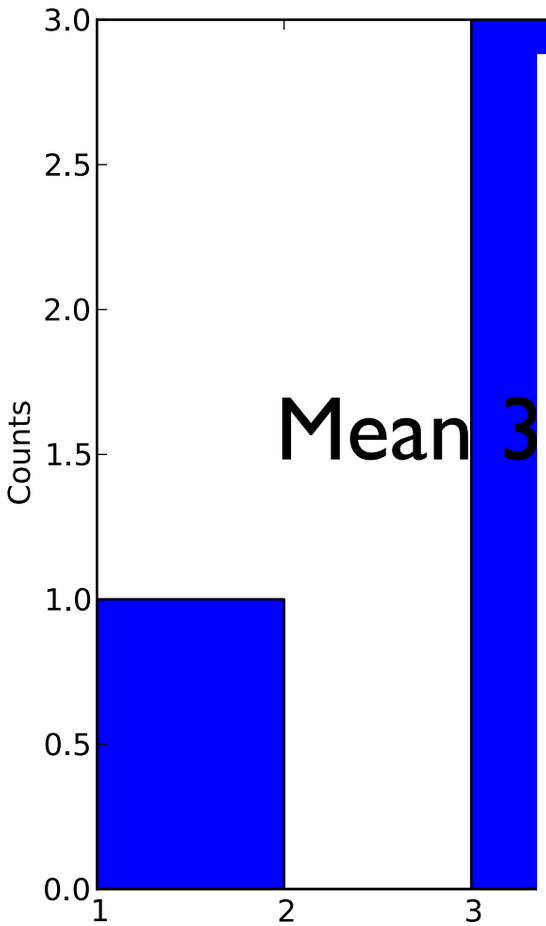
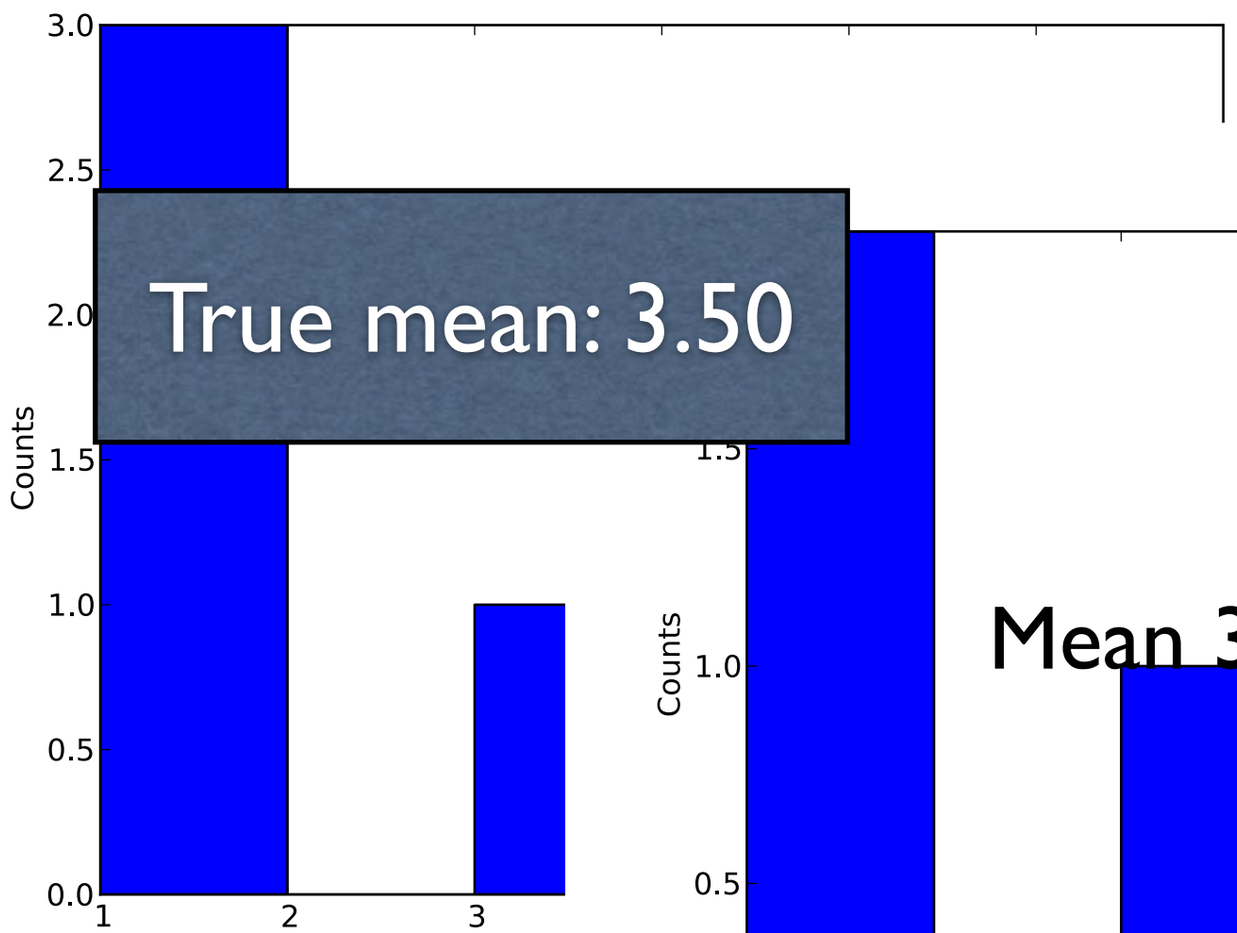
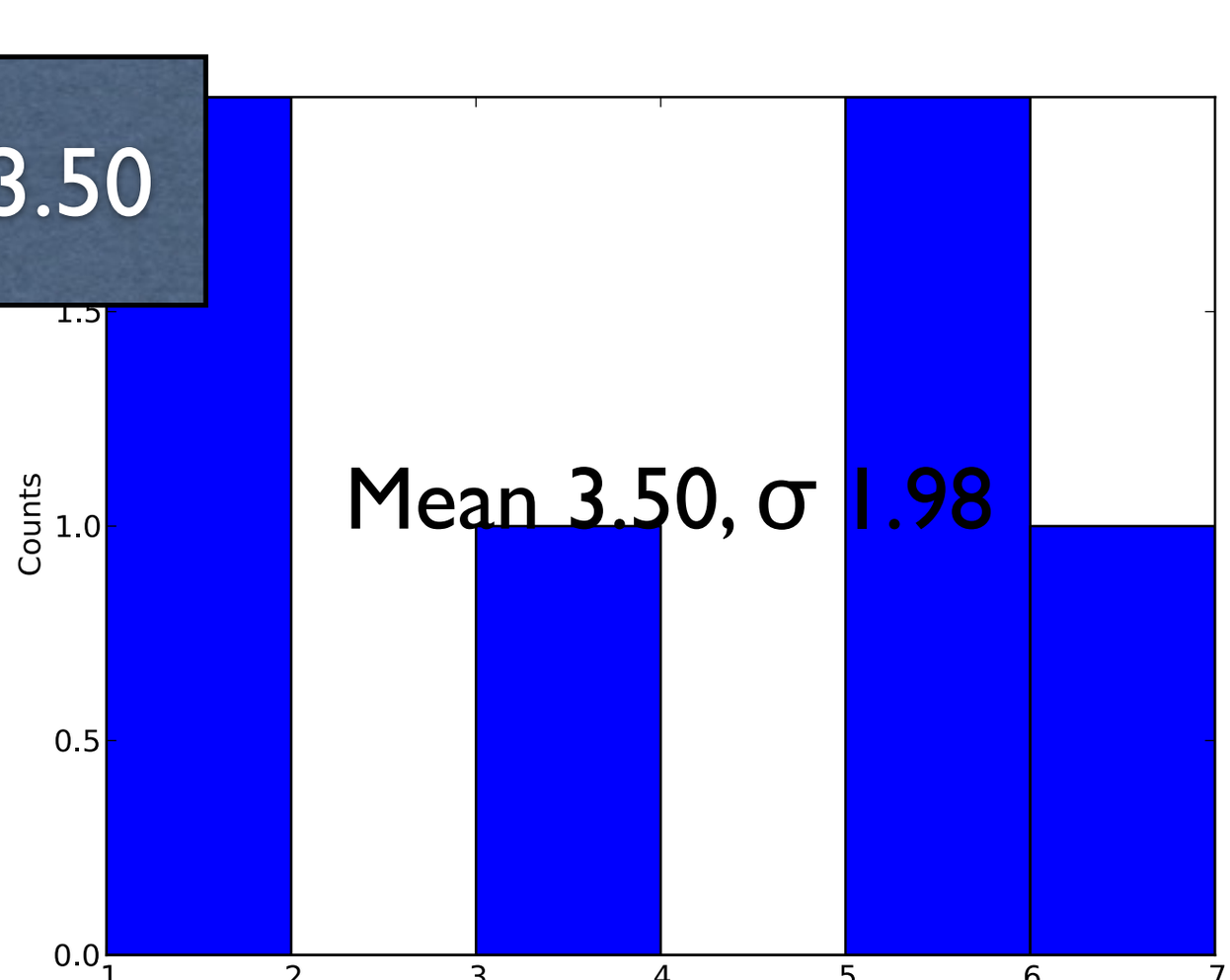
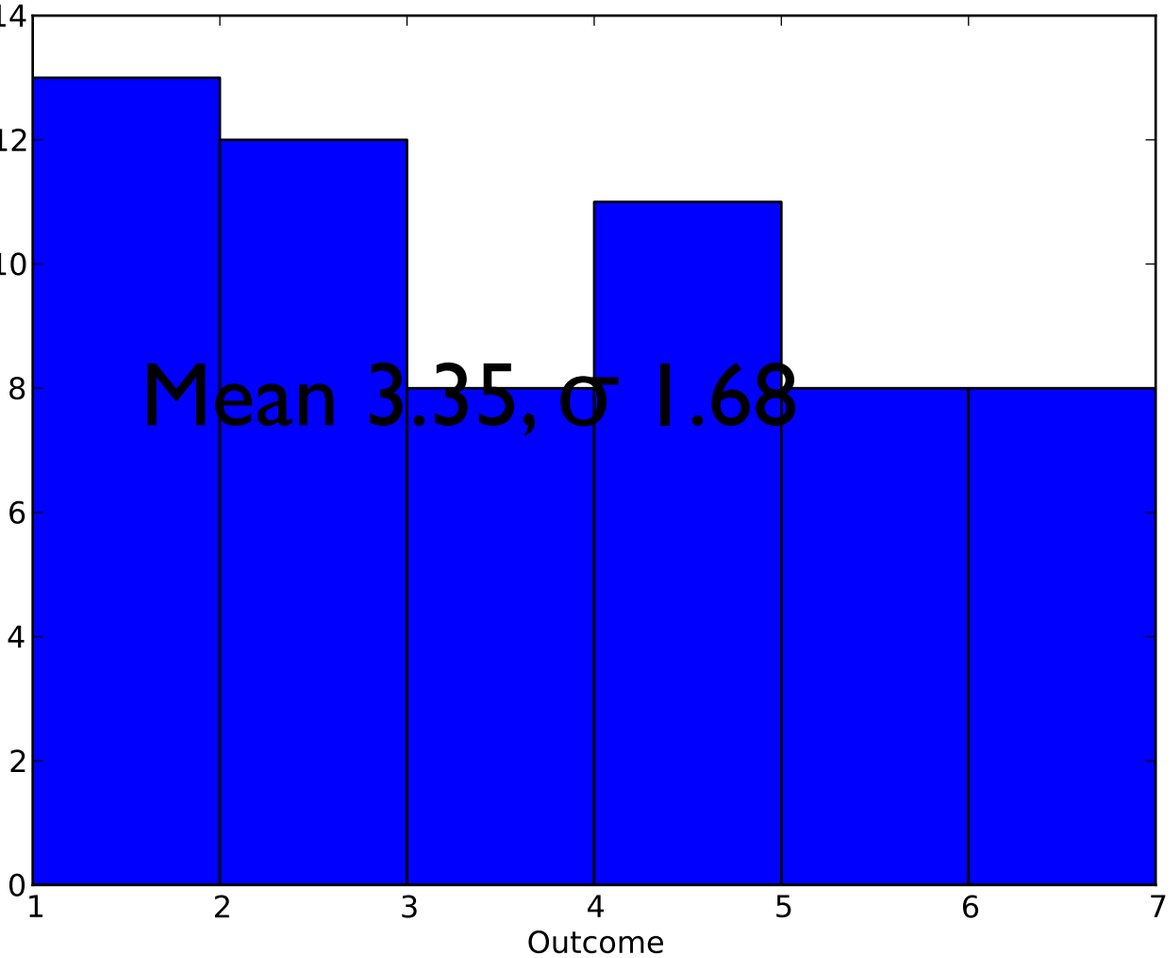






To do a good job calculating the mean and the standard deviation, we need lots of data

Example: Throwing dice - try 60 tosses, three times



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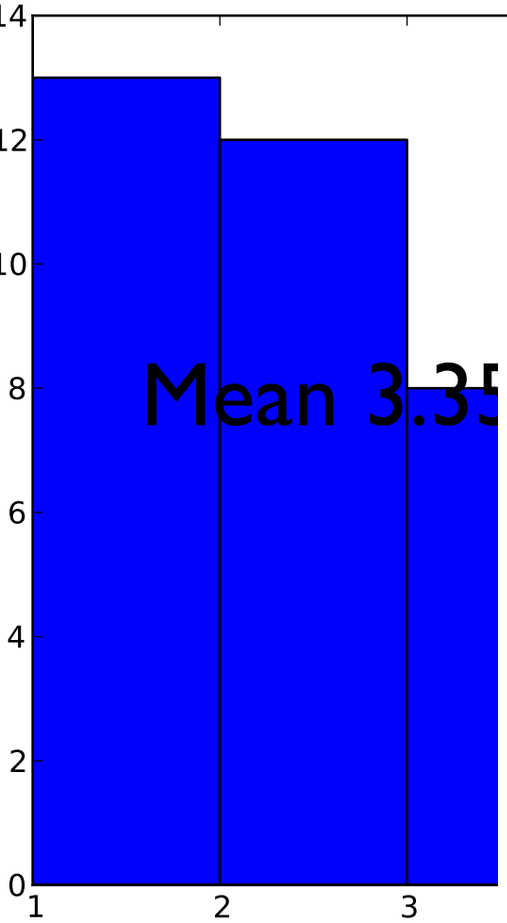

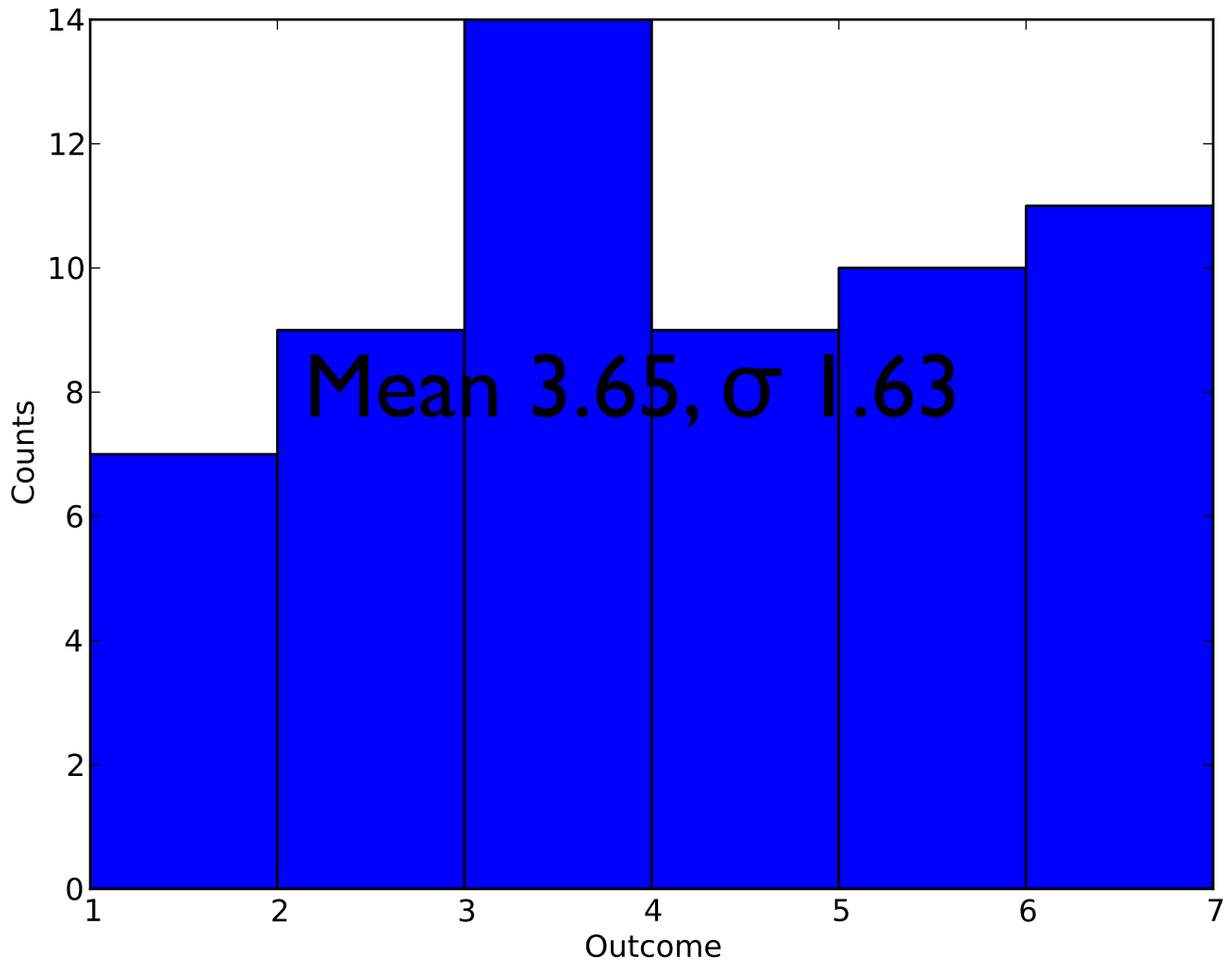

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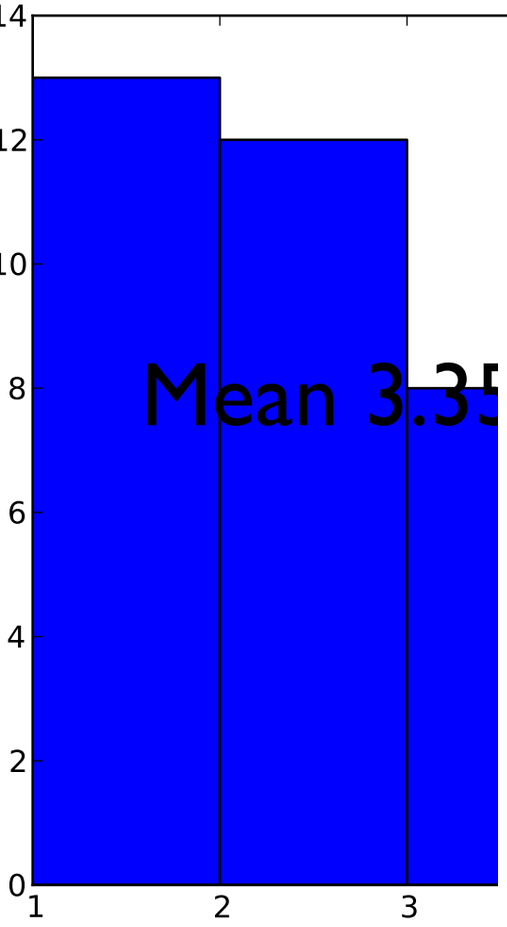

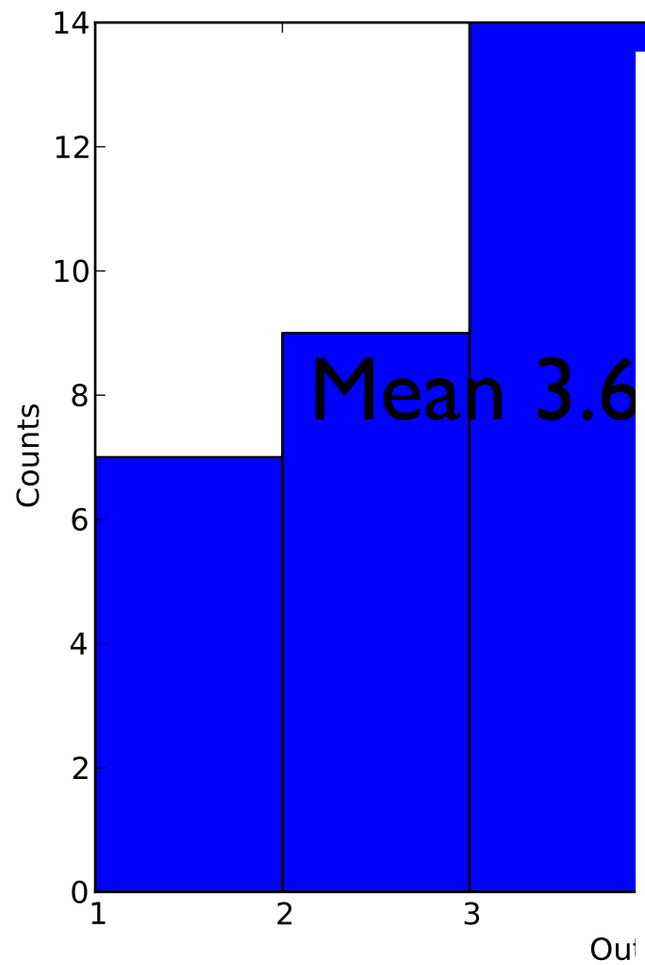

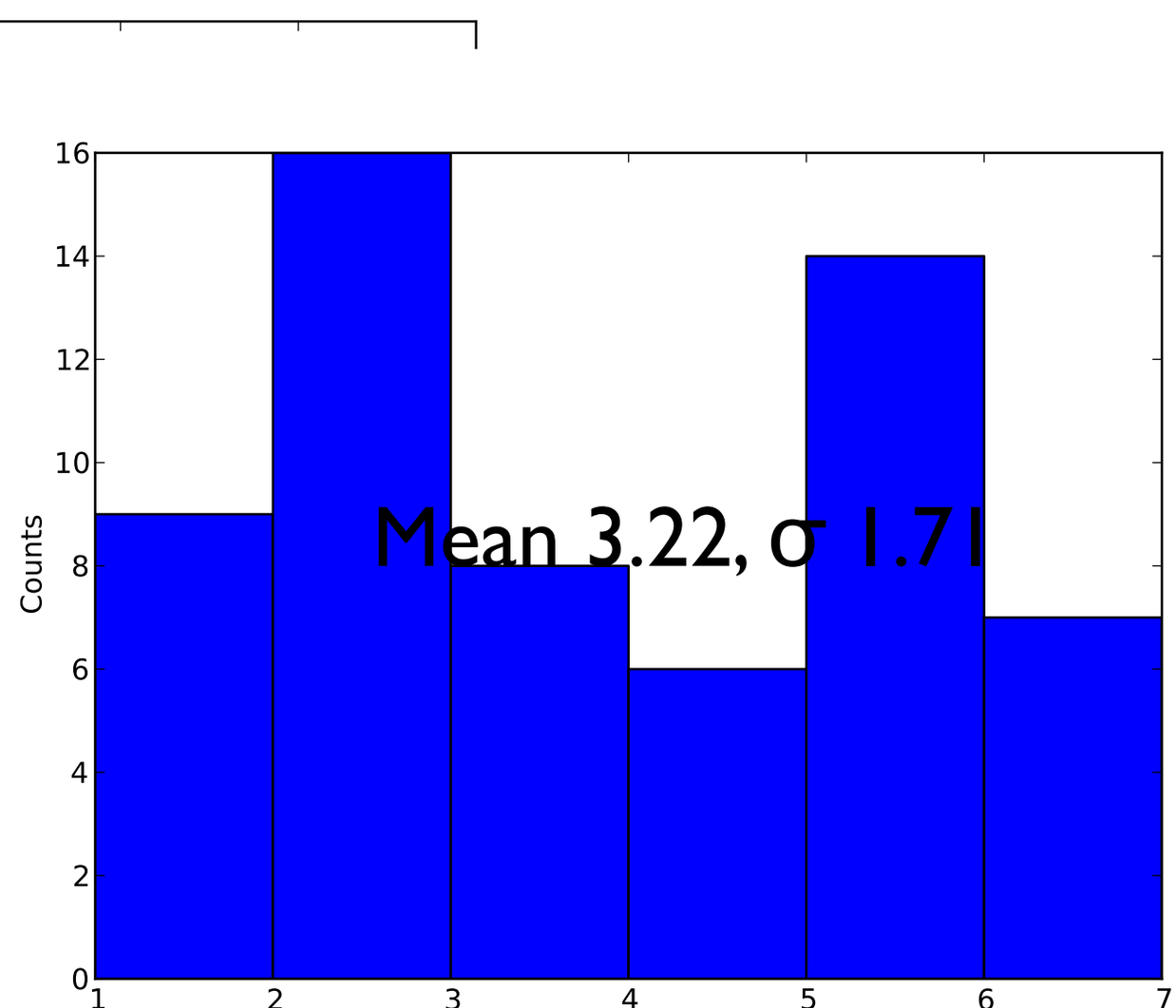

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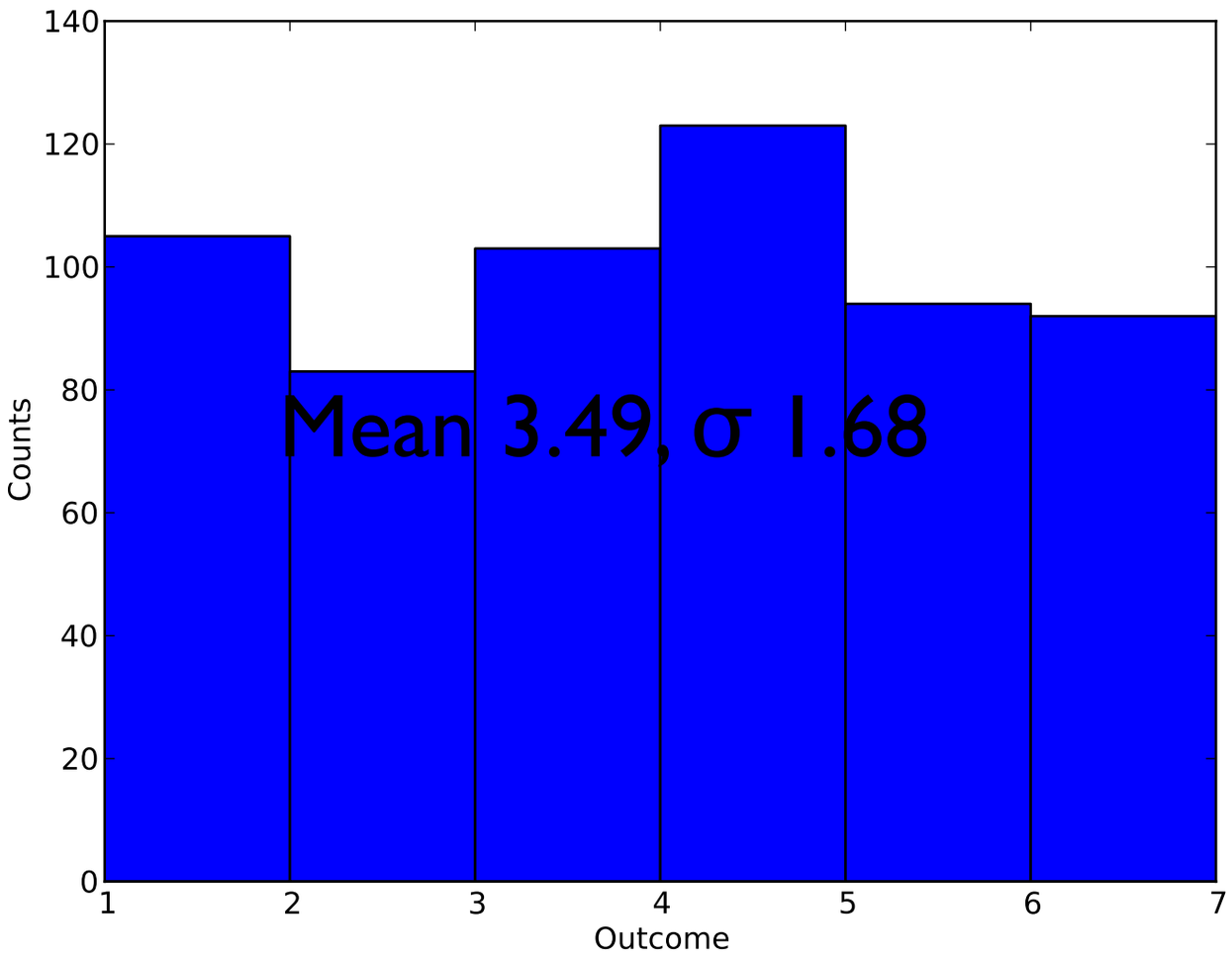
Example: Throwing dice - try 600 tosses, three times



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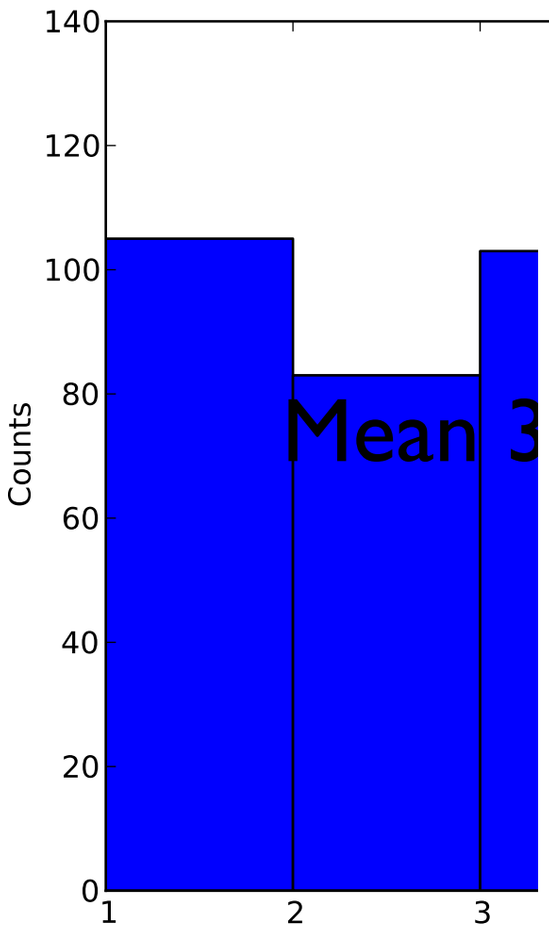

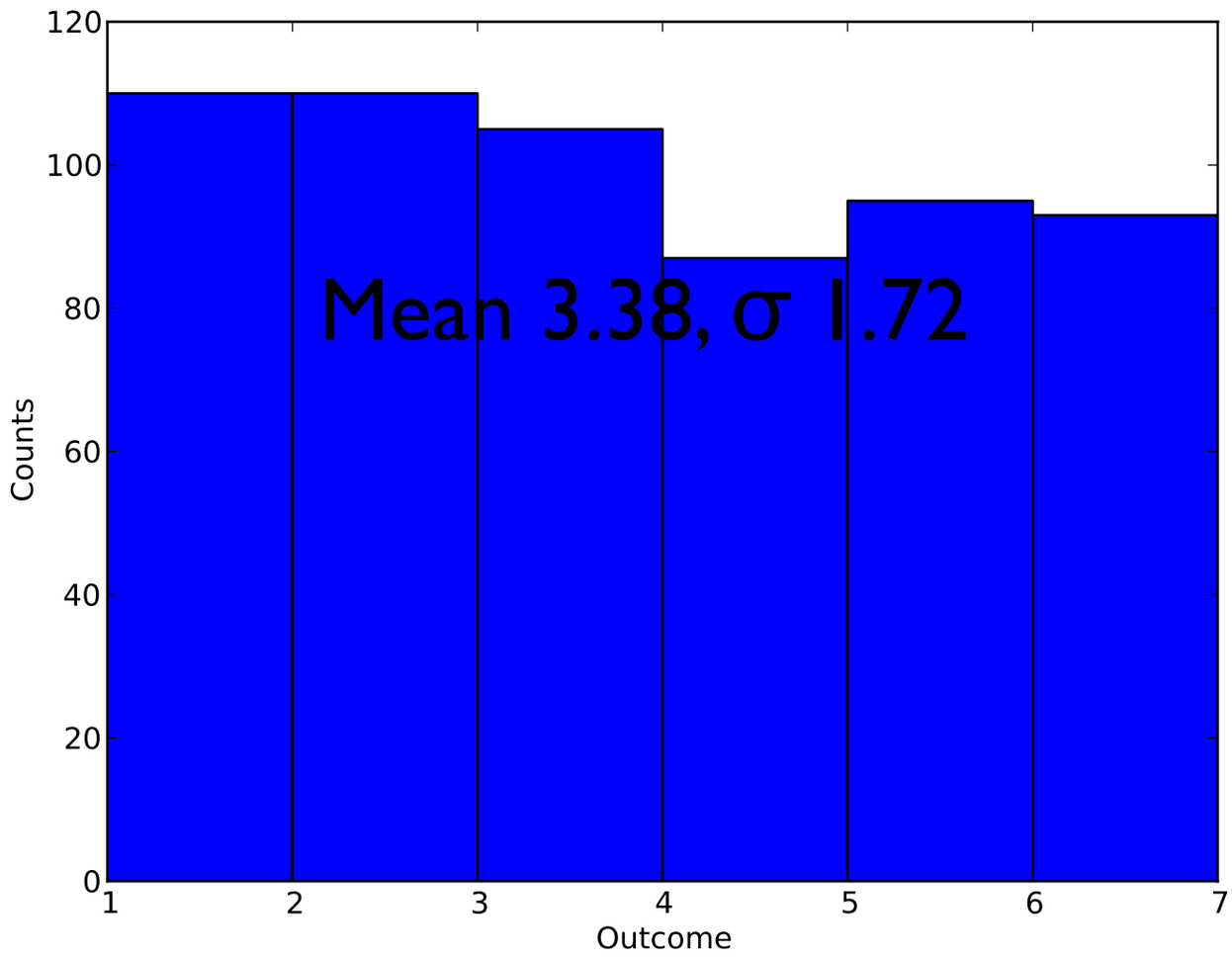

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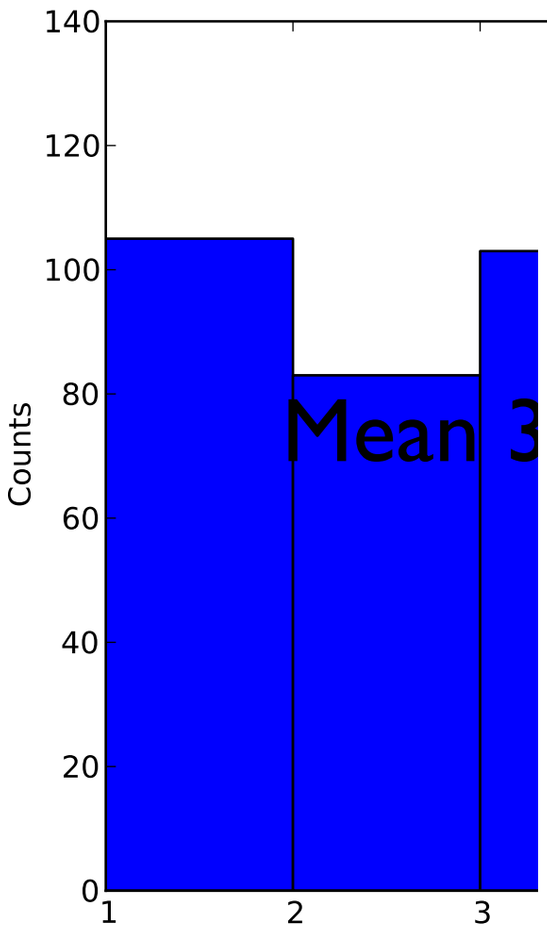
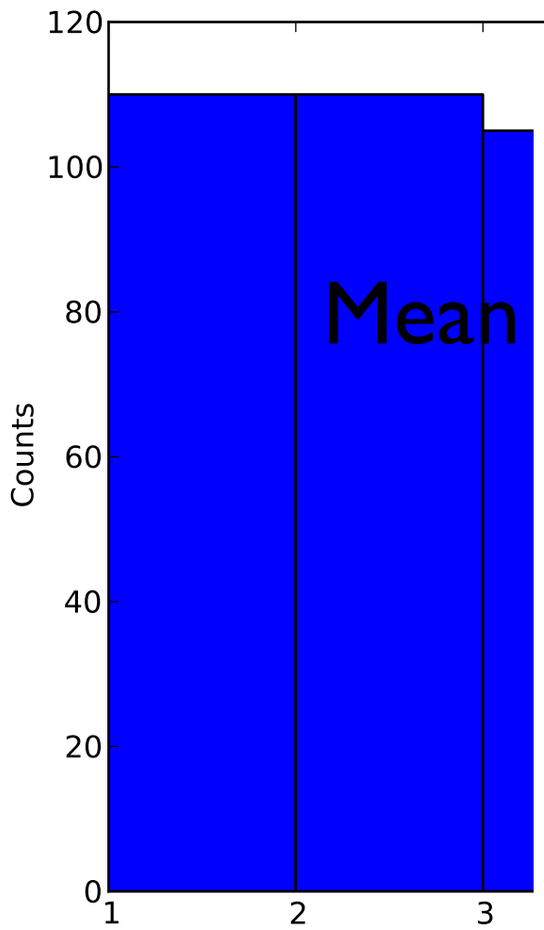
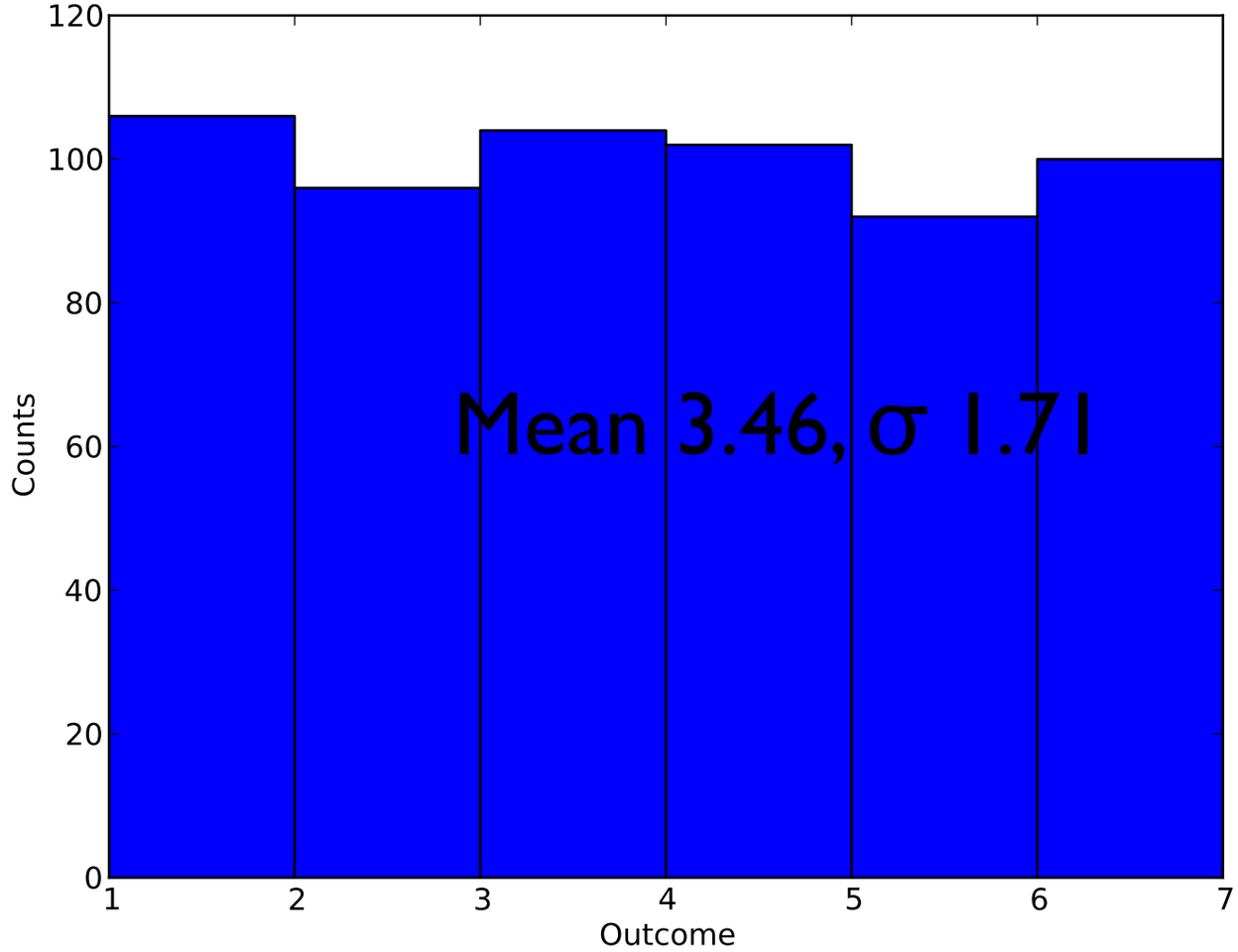






Many problems involve multiplying multiple probability distributions

Simple example: Flipping a coin twice

P(two heads) = P(heads) * P(heads) = 1/4
P(two tails) = P(tails) * P(tails) = 1/4
P(heads then tails) = P(heads) * P(tails) = 1/4
P(tails then heads) = P(tails) * P(heads) = 1/4

But:
P(one tails, one heads) = P(tails) * P(heads) + P(heads)*P(tails)

More ways -- higher entropy

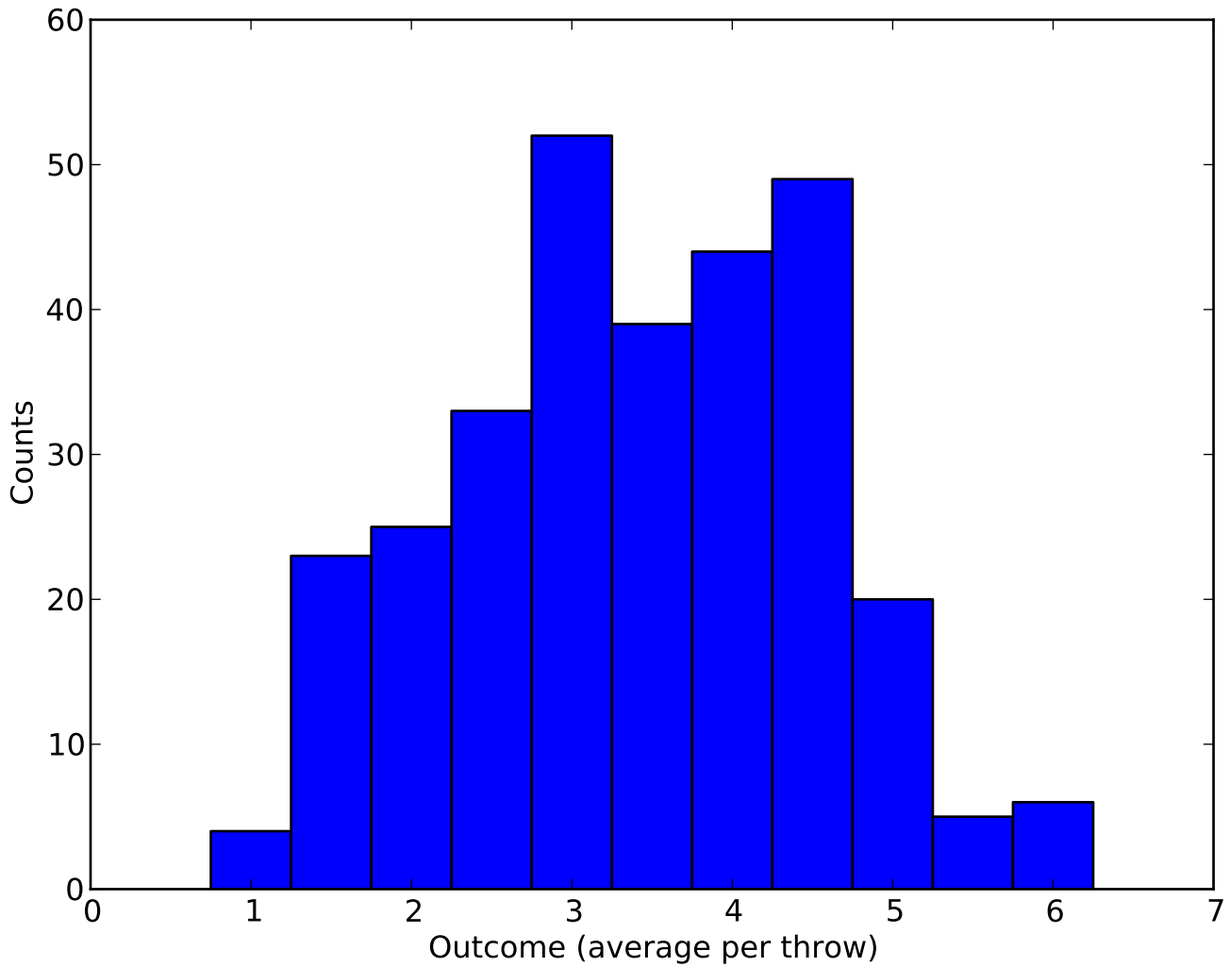
Many problems involve multiplying multiple probability distributions

Rolling two dice:

Outcome	Probability	Ways	Outcome	Probability	Ways
			7	6/36	1+6, 2+5, 3+4
2	1/36	1+1	8	5/36	2+6, 3+5, 4+4
3	2/36	1+2	9	4/36	3+6, 4+5
4	3/36	1+3, 2+2	10	3/36	4+6, 5+5
5	4/36	1+4, 2+3	11	2/36	5+6
6	5/36	1+5, 2+4, 3+3	12	1/36	6+6

Let's test this numerically -- two dice

300 throws of two dice



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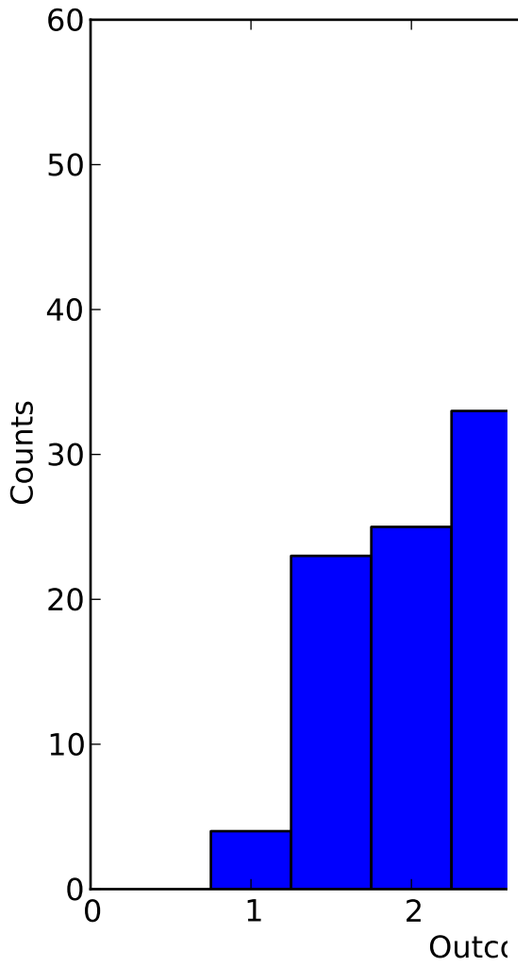
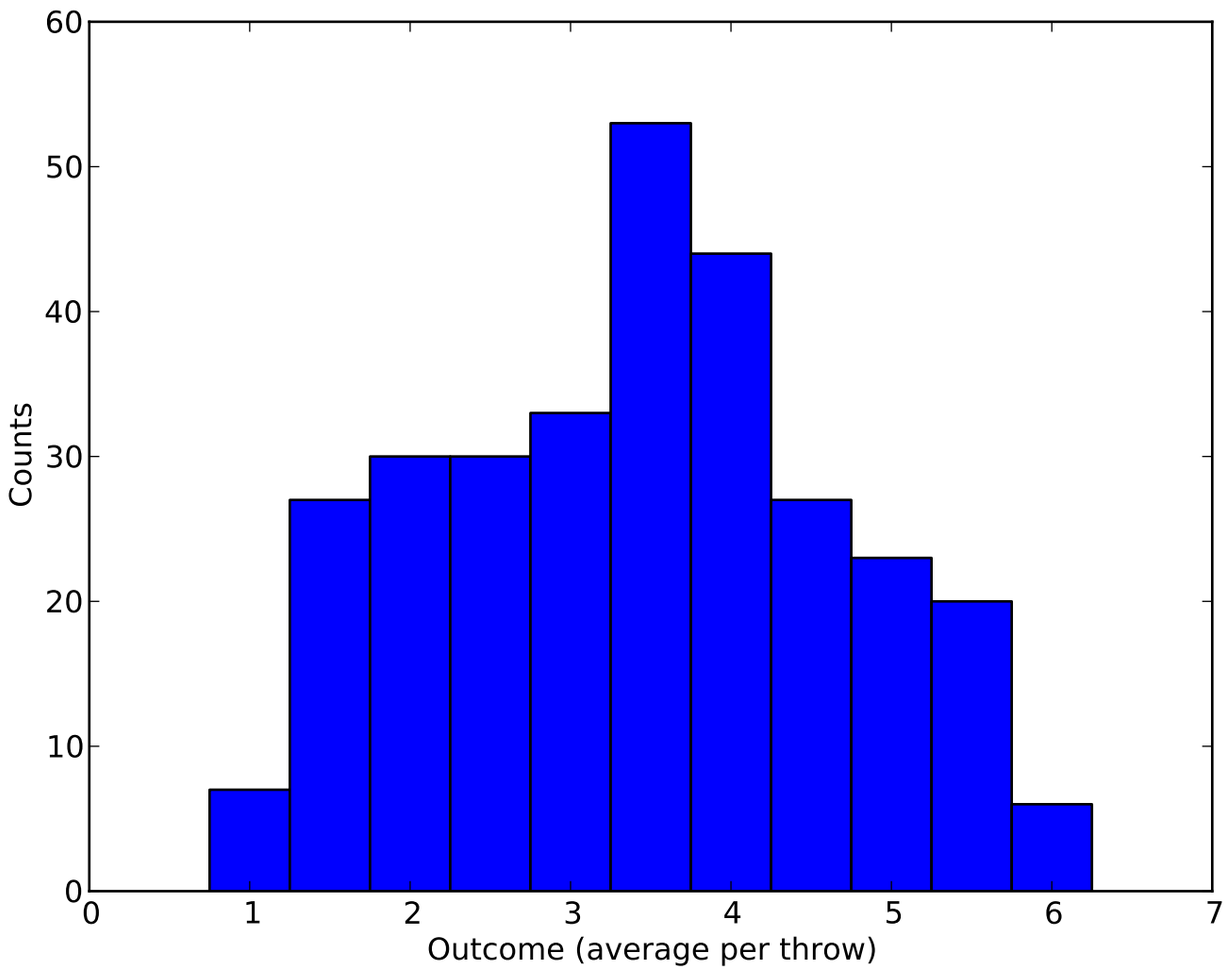
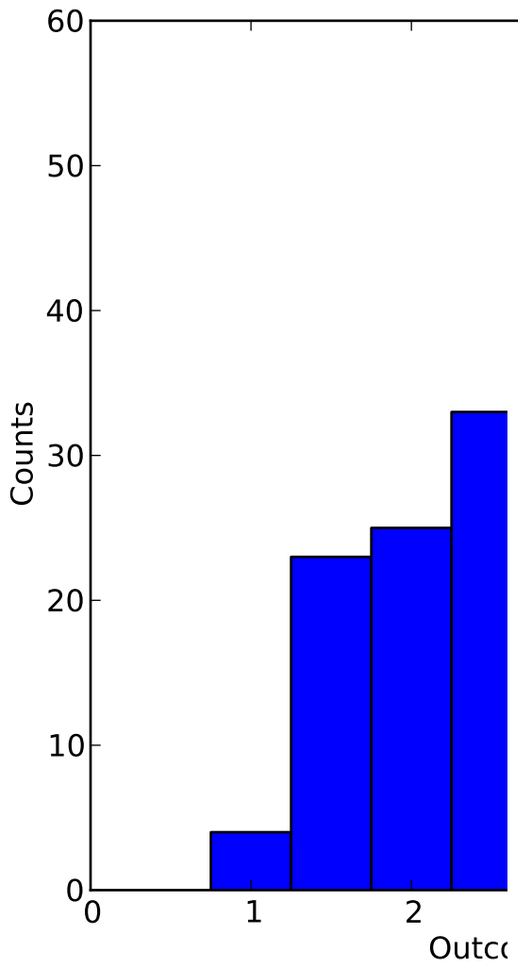
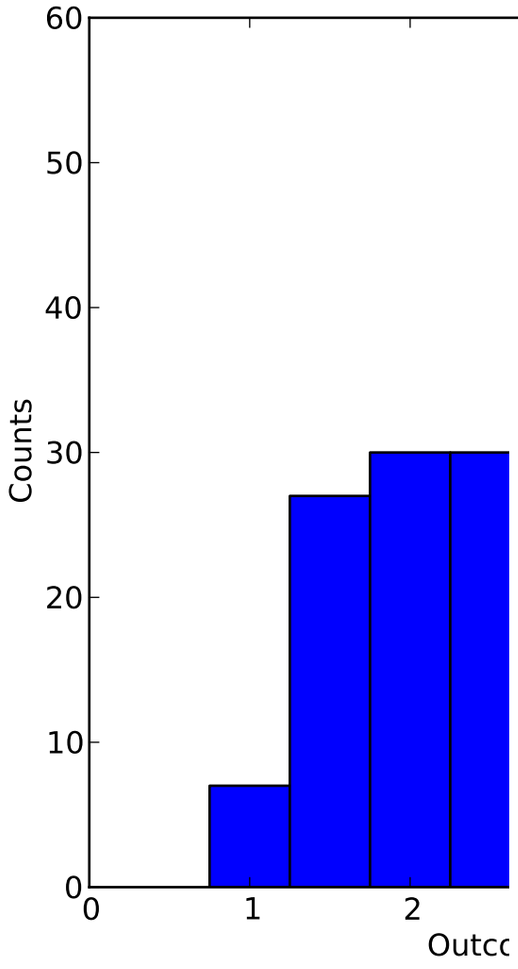


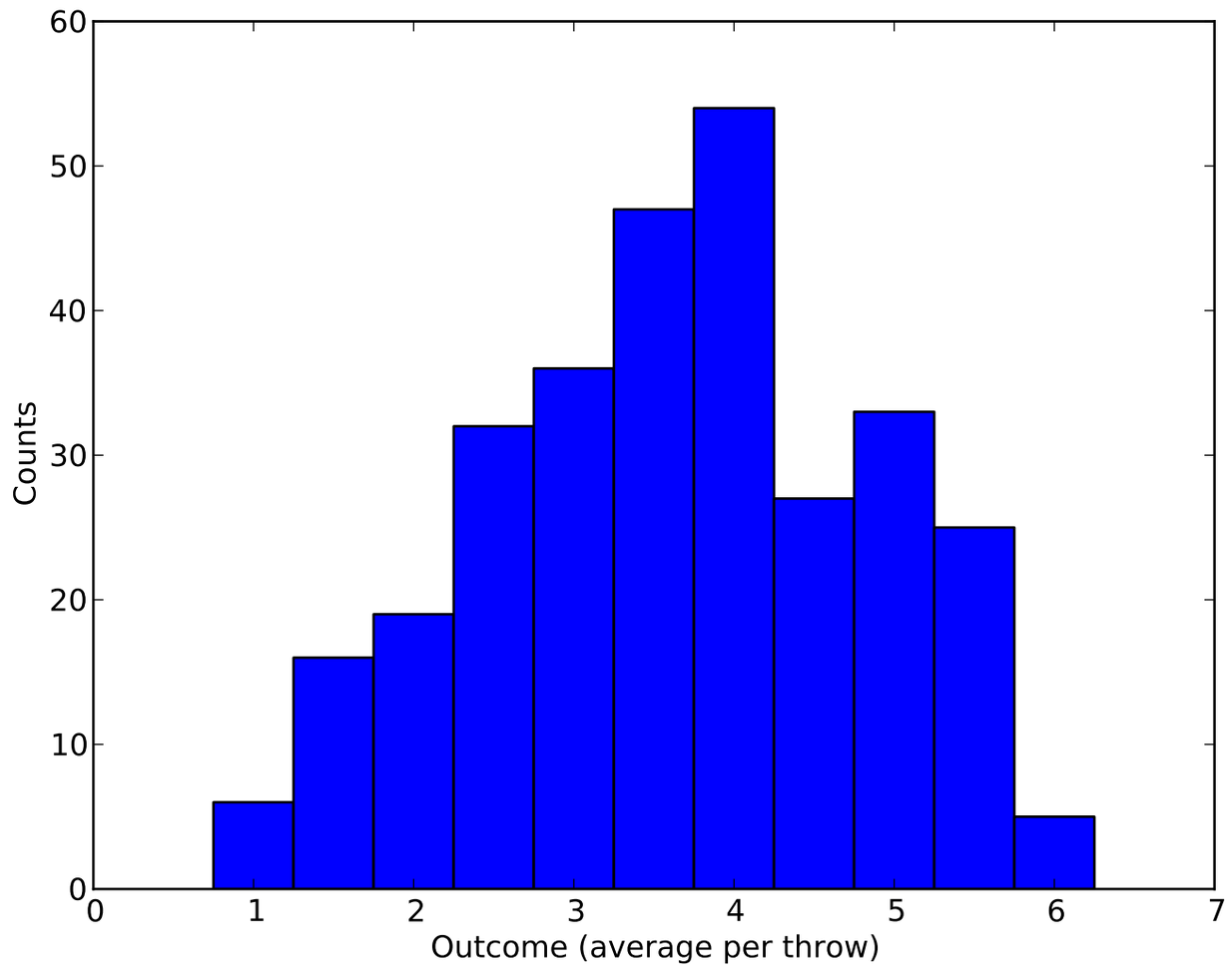


Let's test this numerically -- two dice

300 throws of two dice

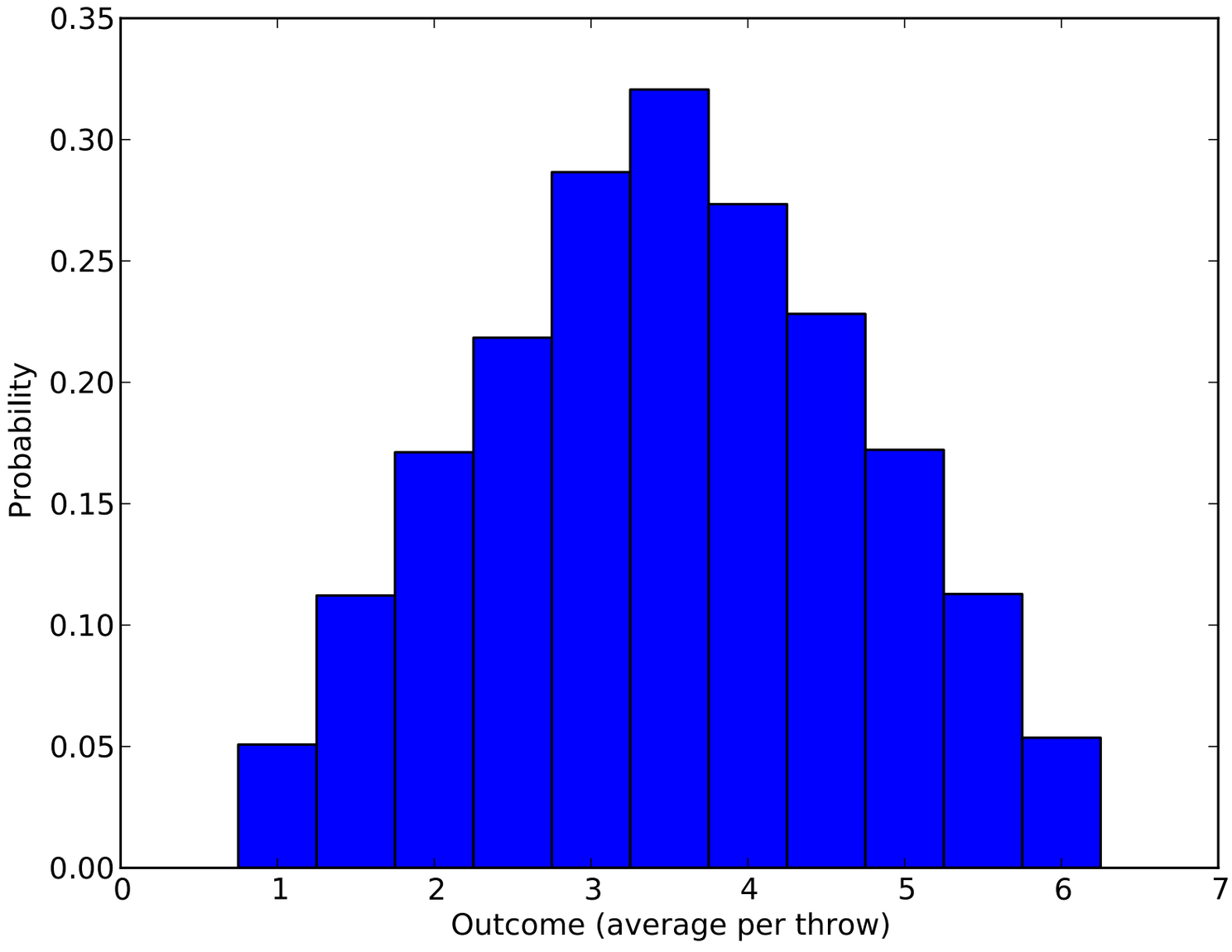






It's hard to verify our predicted probabilities without doing more trials

10000 throws of two dice



Mean 3.5, σ 1.2

Take-aways:

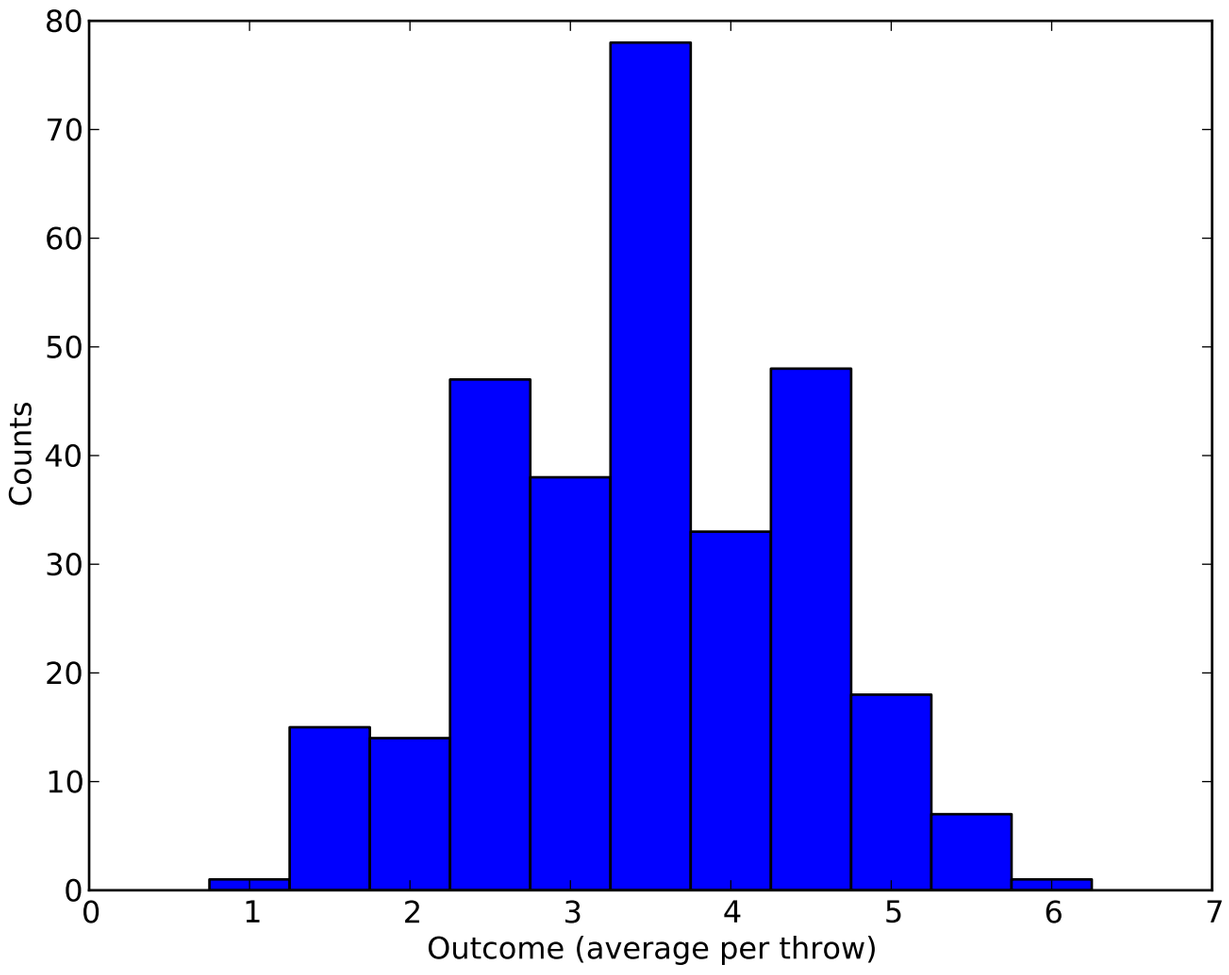
Combining uniform distributions can give non-uniform things

To really get the correct average takes a lot of trials

What about more dice throws?

Three dice -- more sharply peaked

300 throws of three dice



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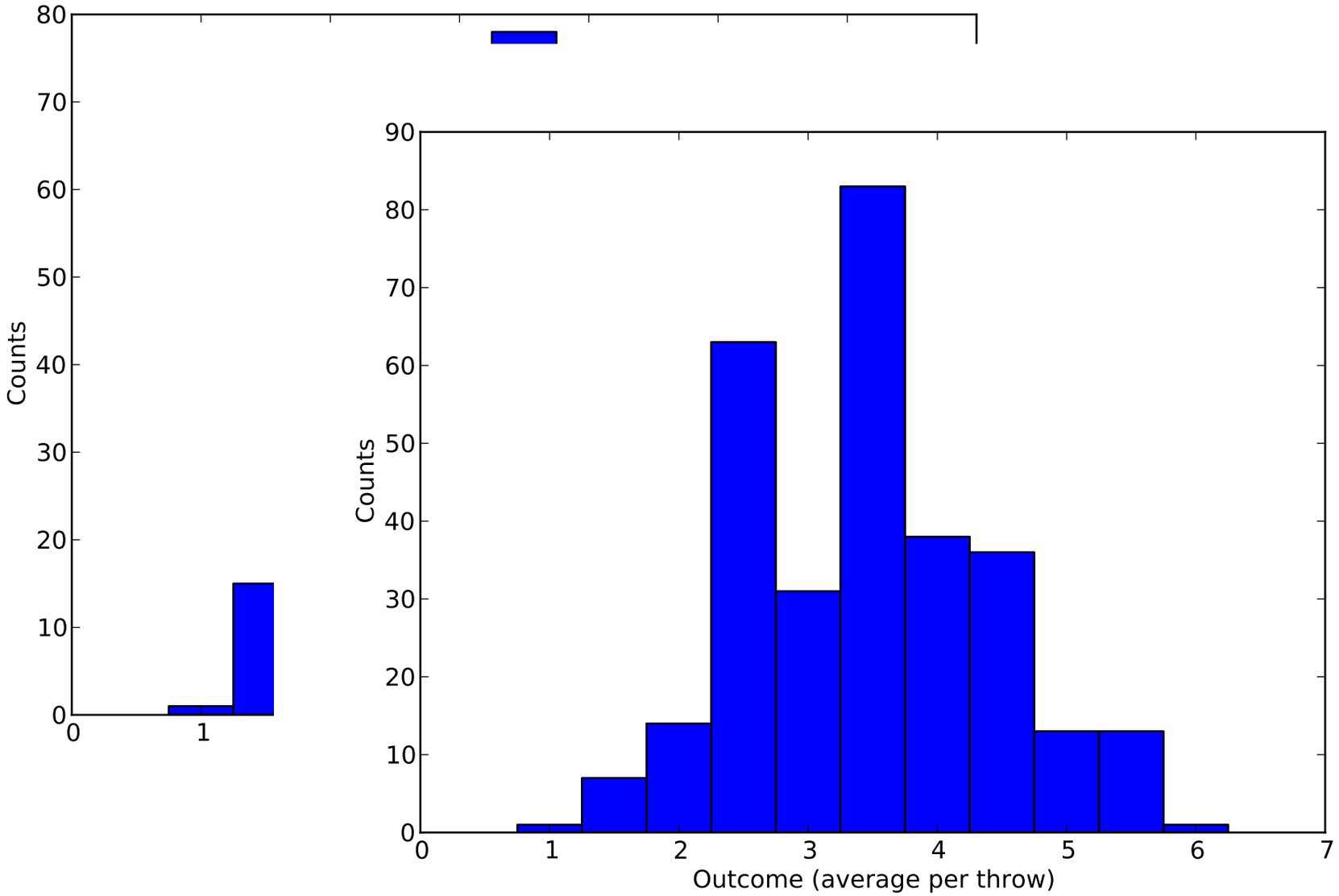

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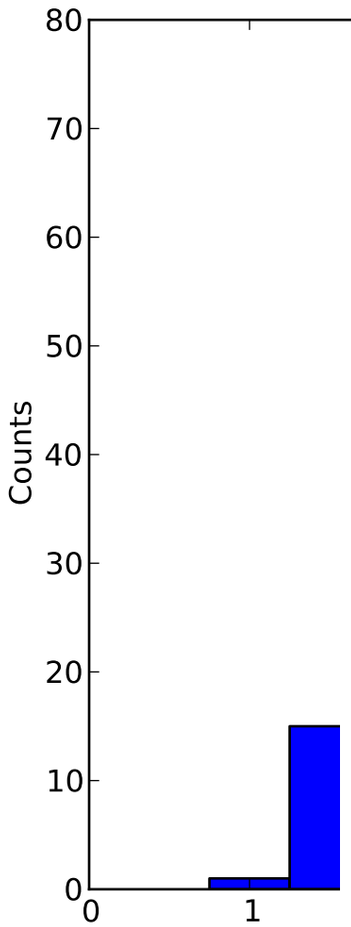
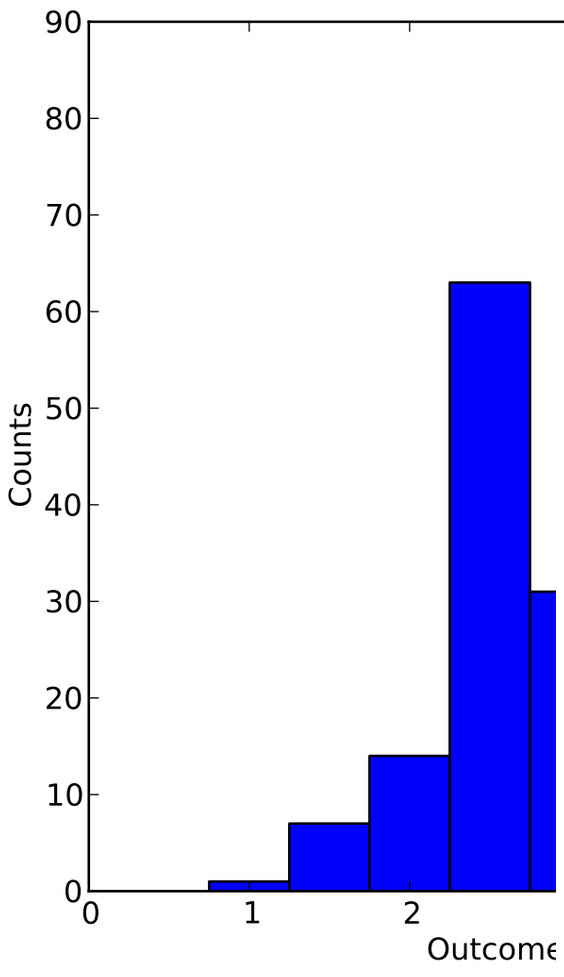
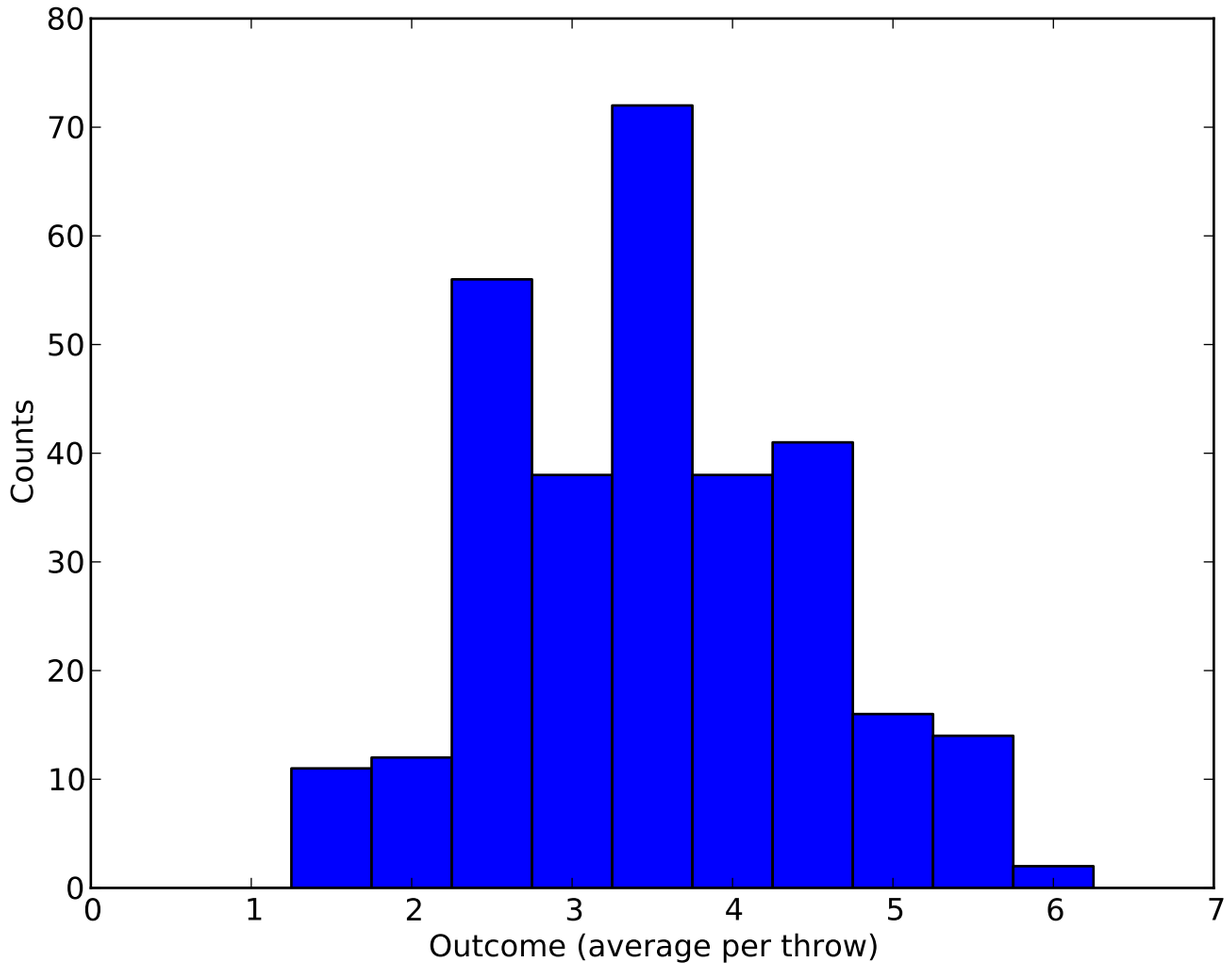
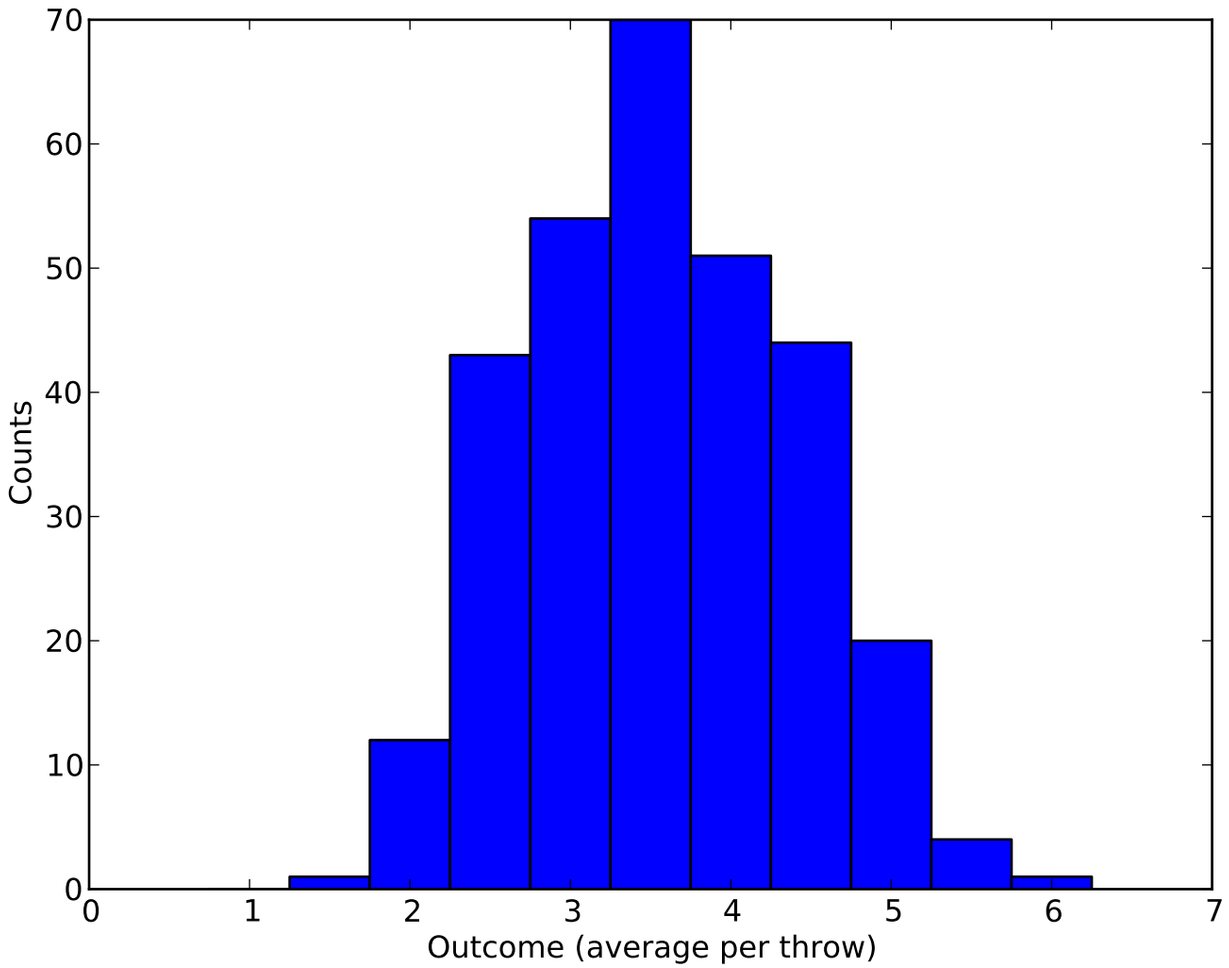






Four dice -- even more sharply peaked

300 throws of four dice



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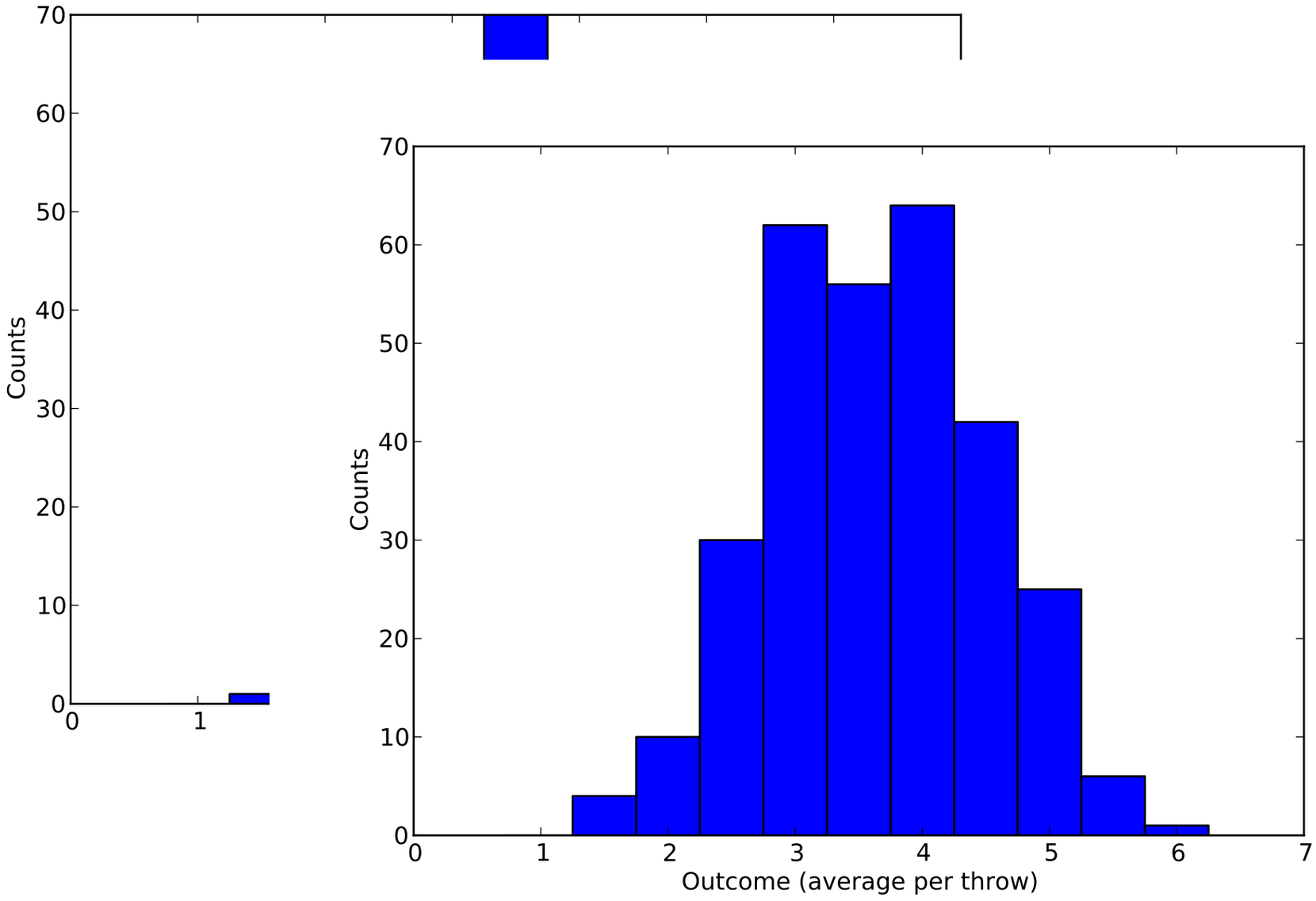

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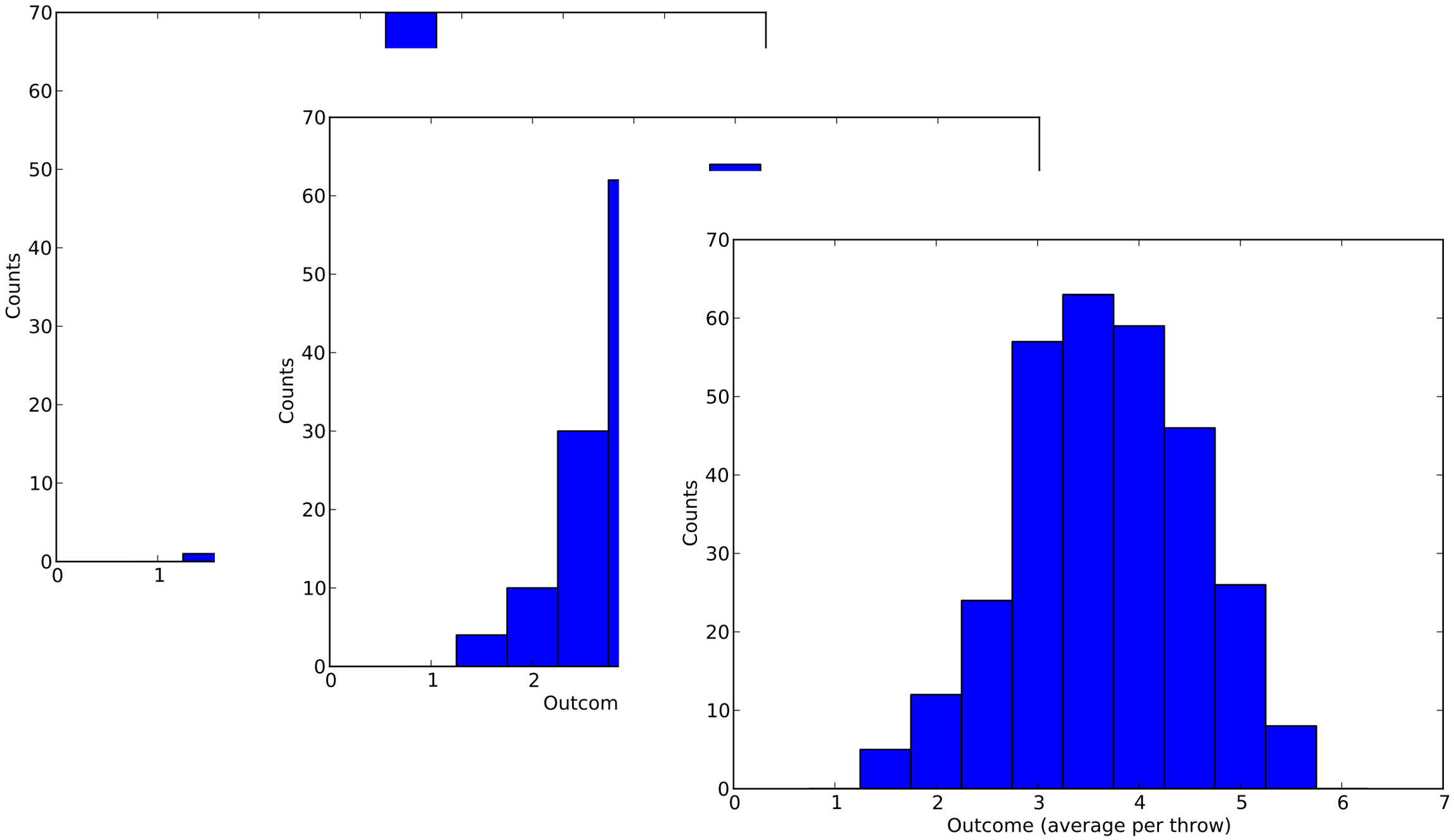
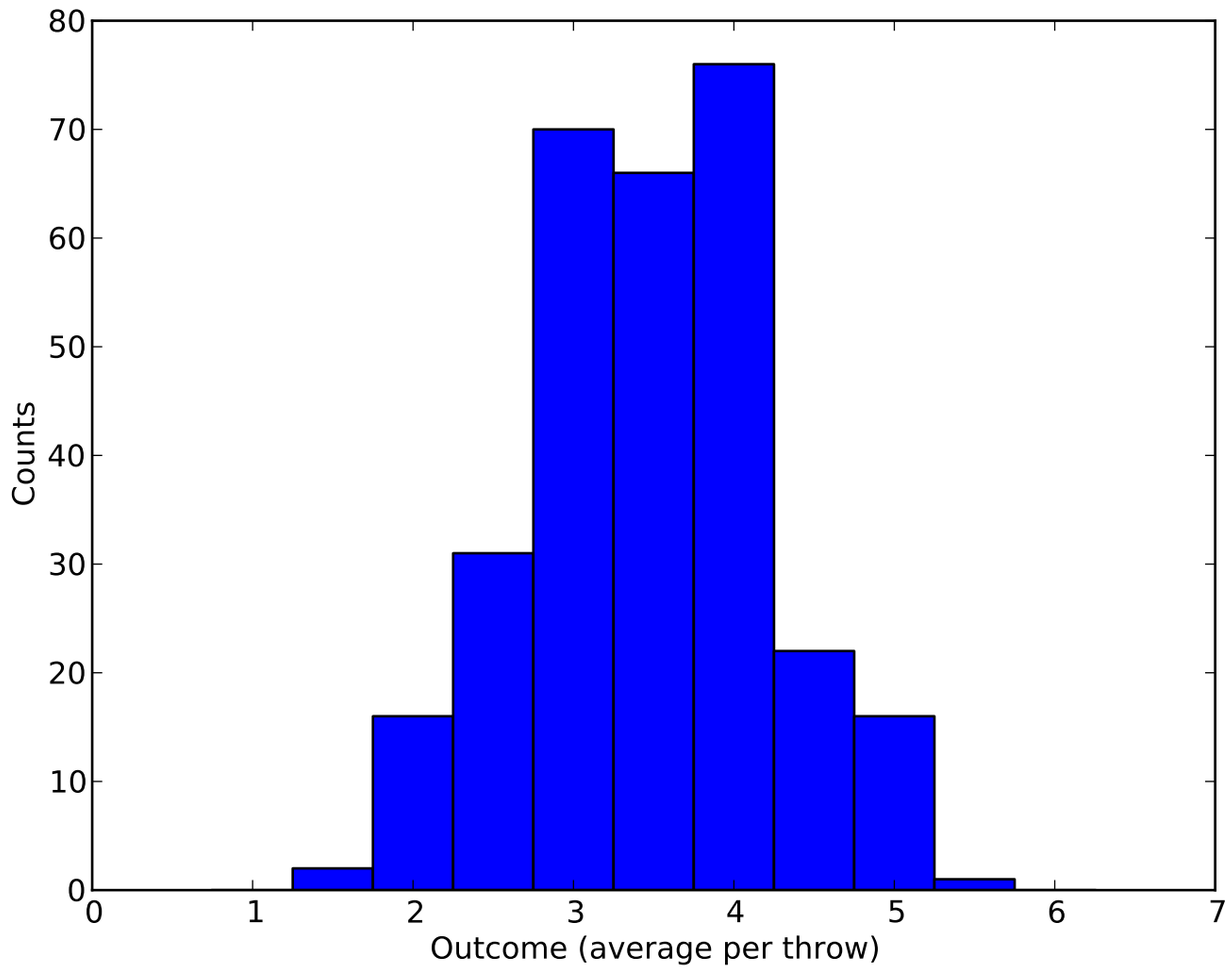


Five dice -- is this starting to look familiar?

300 throws of five dice



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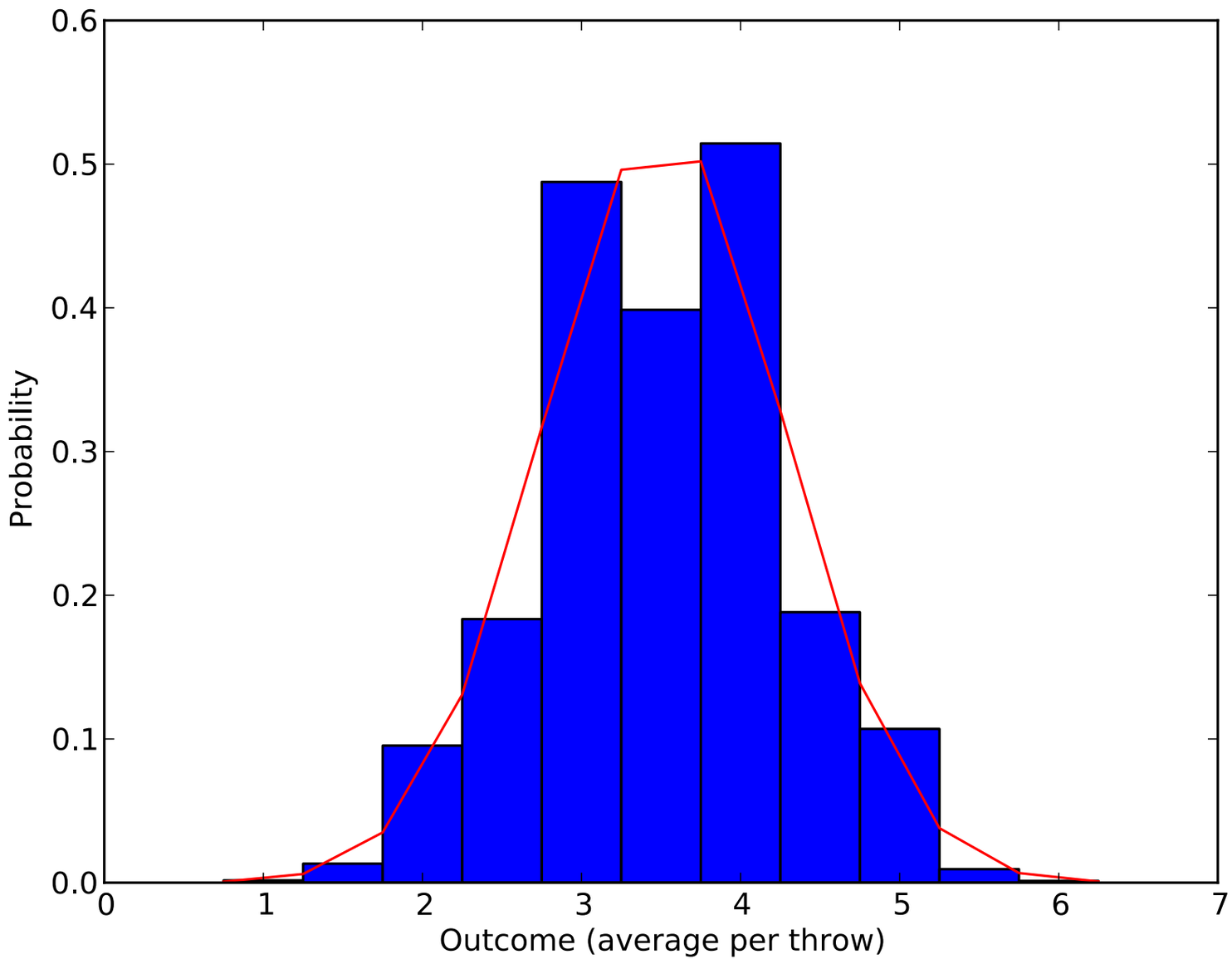
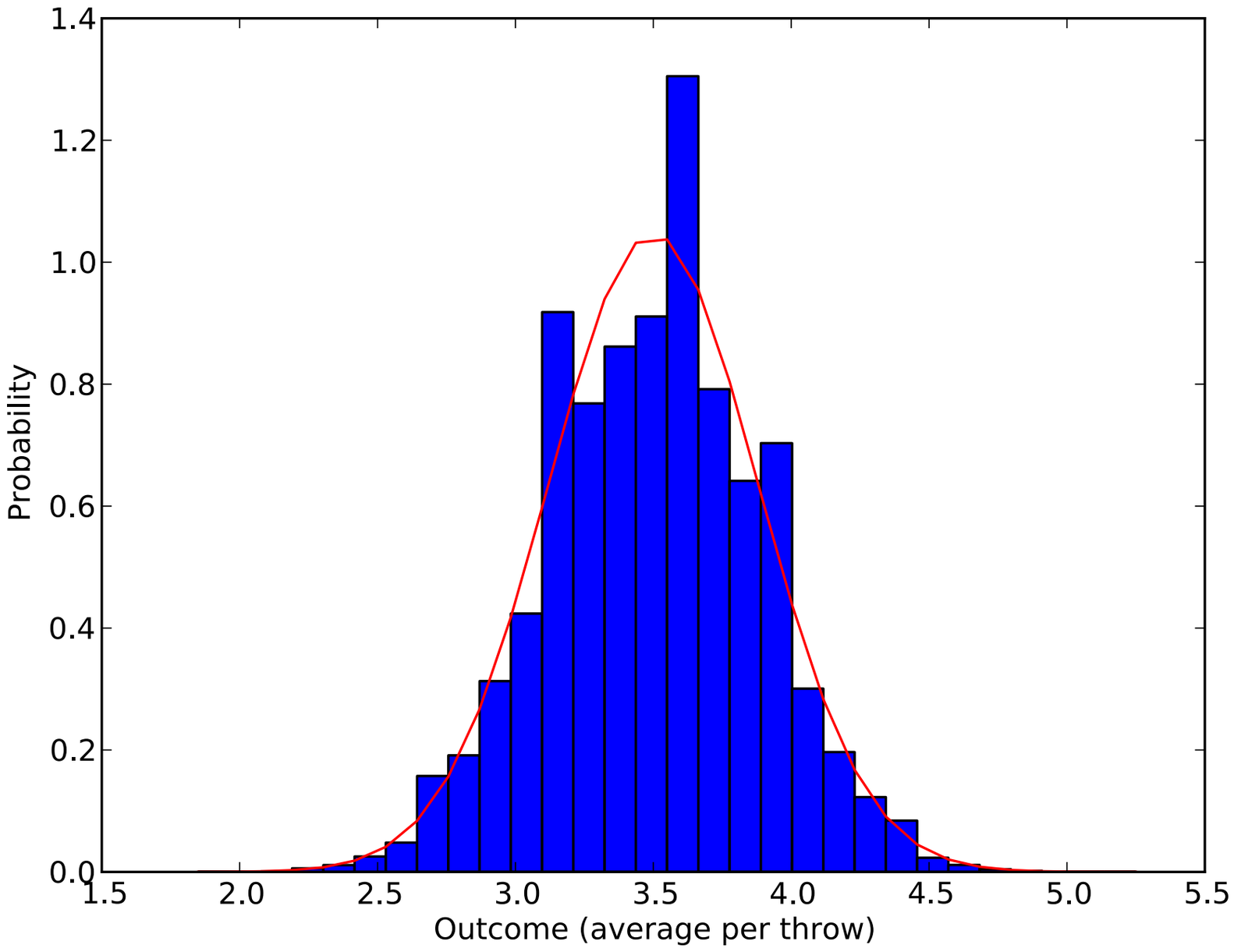
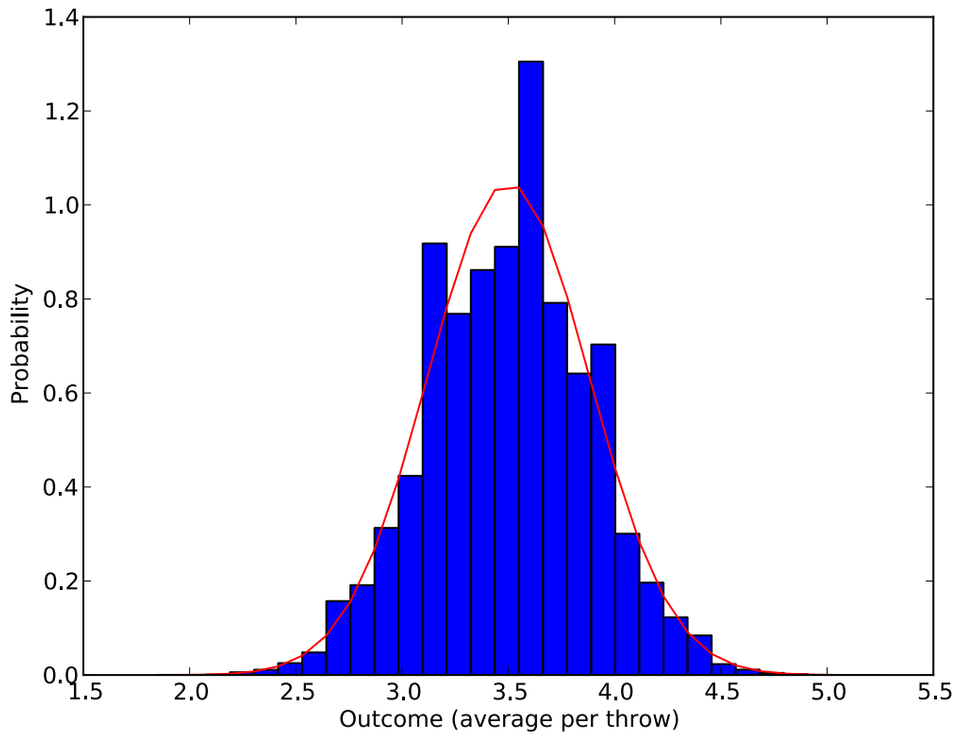


What about 20 dice?

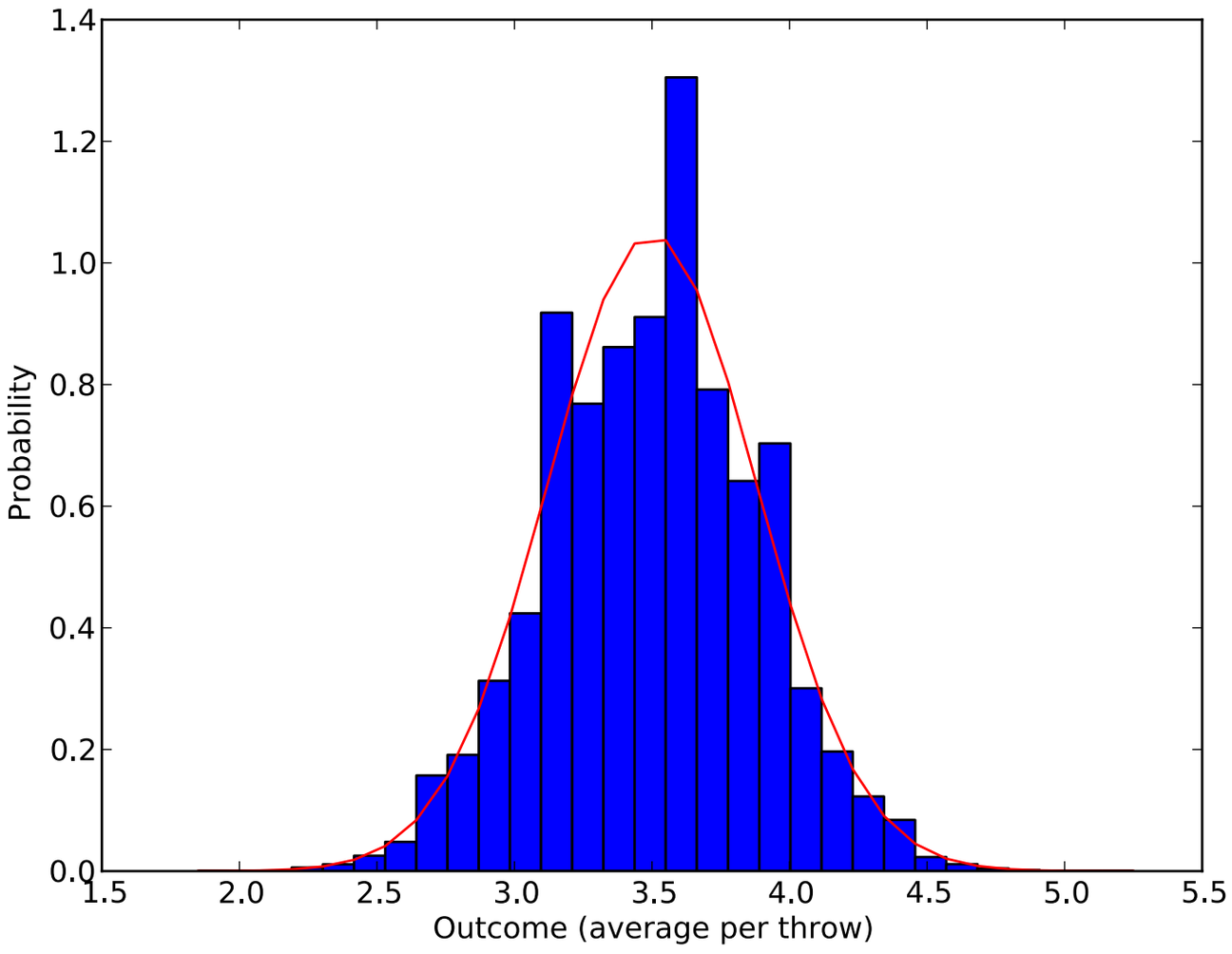


It turns out there is something pretty profound behind this -- the "central limit theorem"



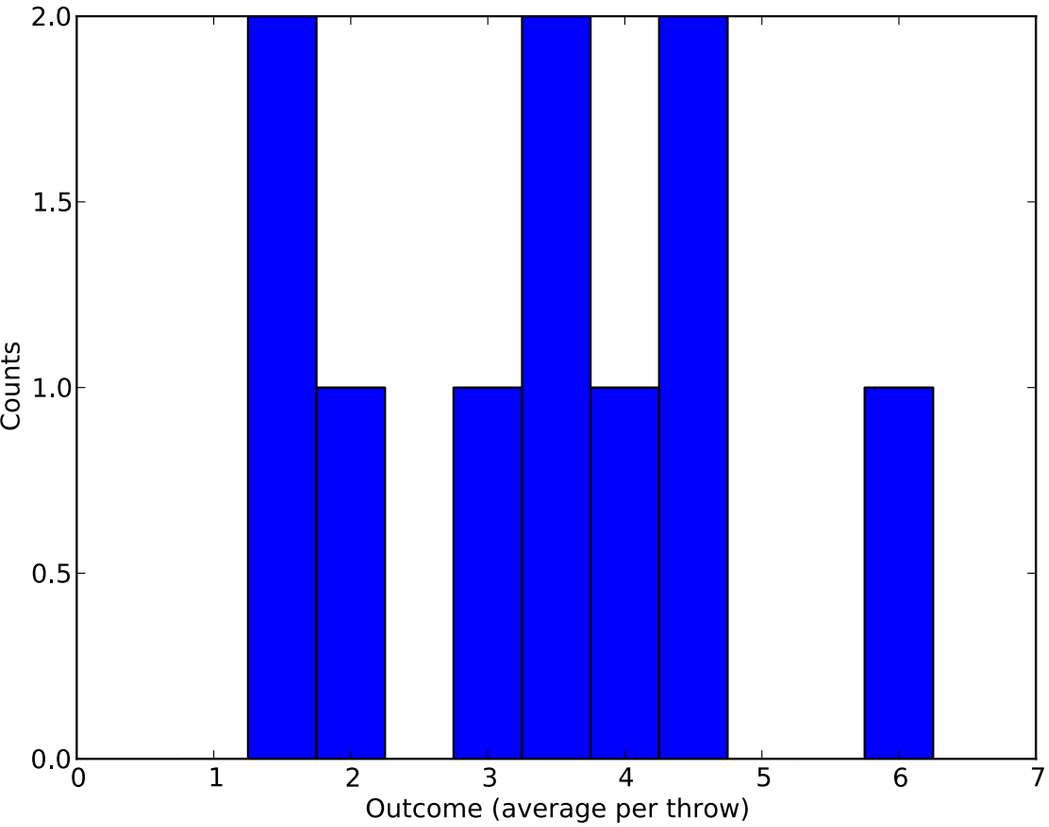
The distribution of a sum of many small values will follow a Gaussian (normal) distribution, *regardless* of the starting distribution

When we compute observables from simulations, we usually expect property estimates to follow the central limit theorem



$$\langle f \rangle \doteq \frac{1}{N} \sum_{i=1}^{N} f(x_i)$$

If we make a measurement from some distribution of possible results, how do we assess reliability?



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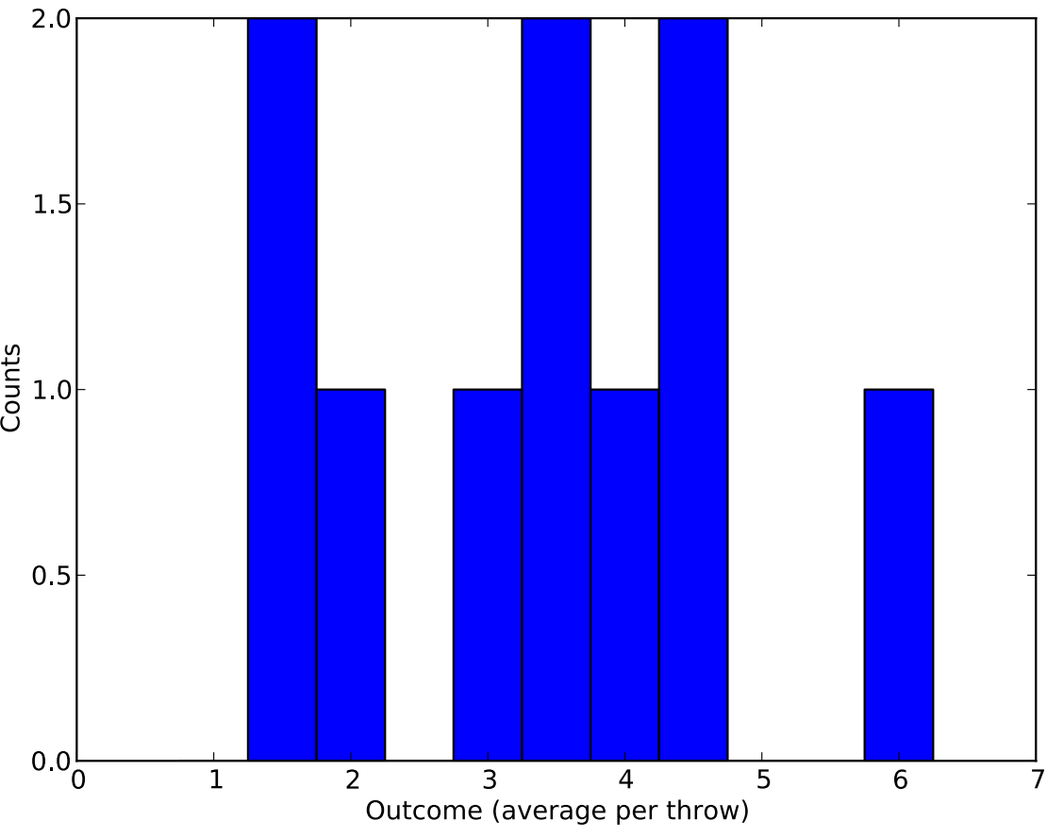

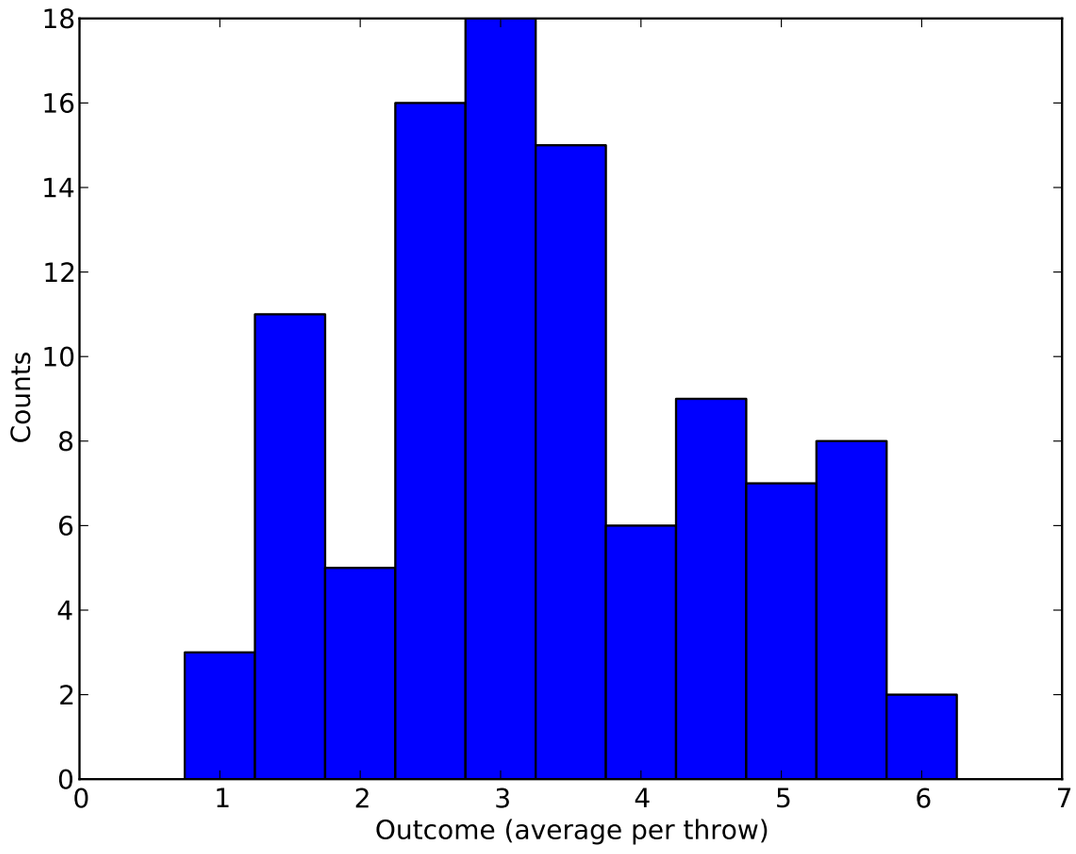

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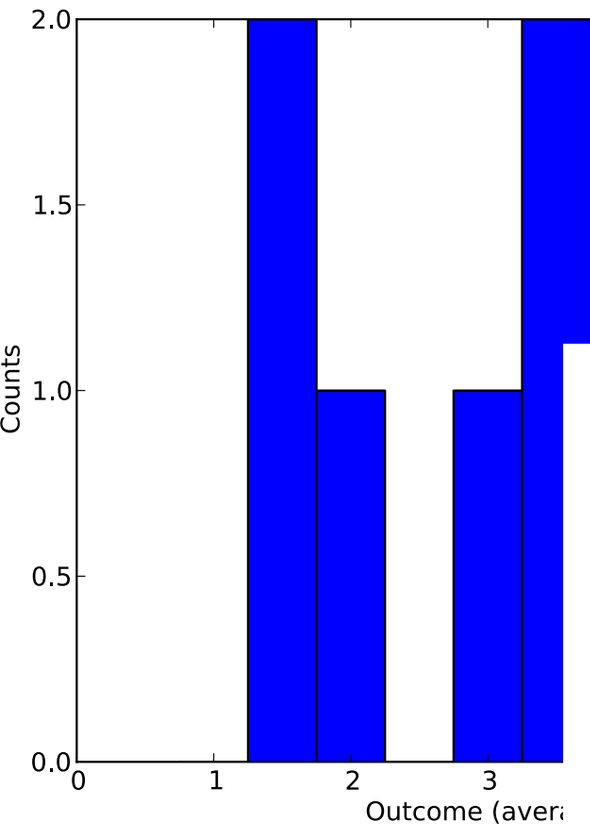

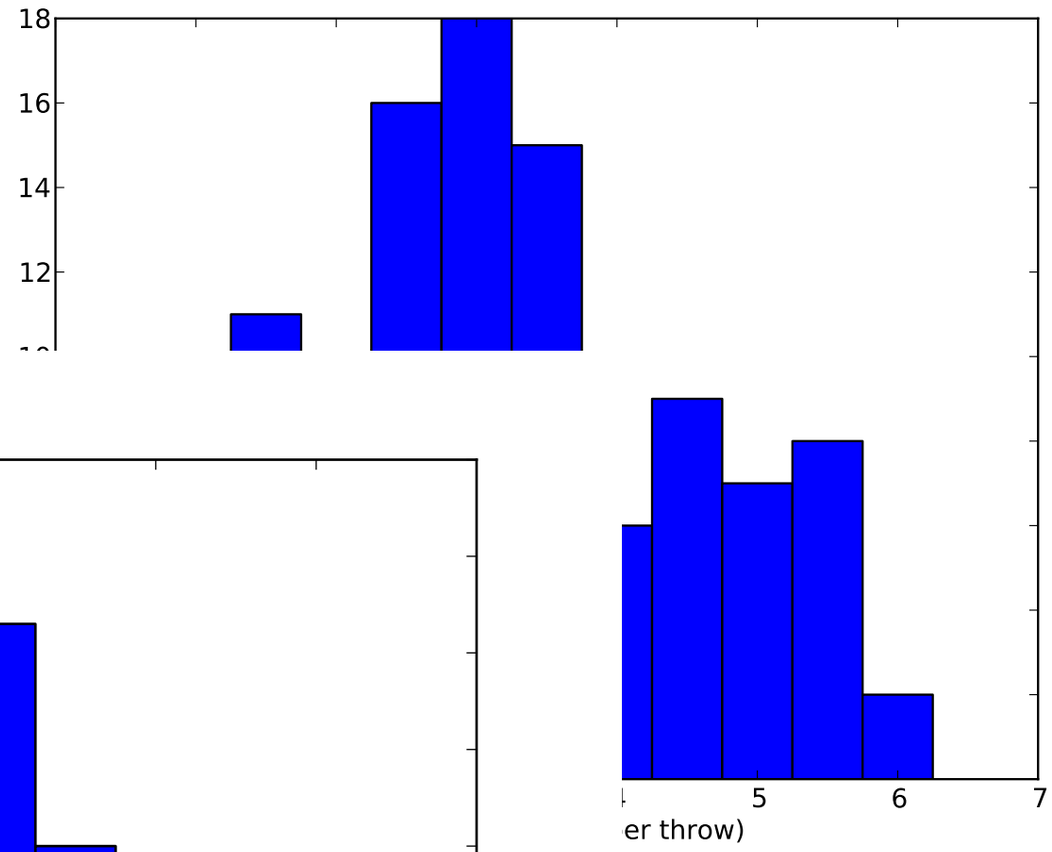

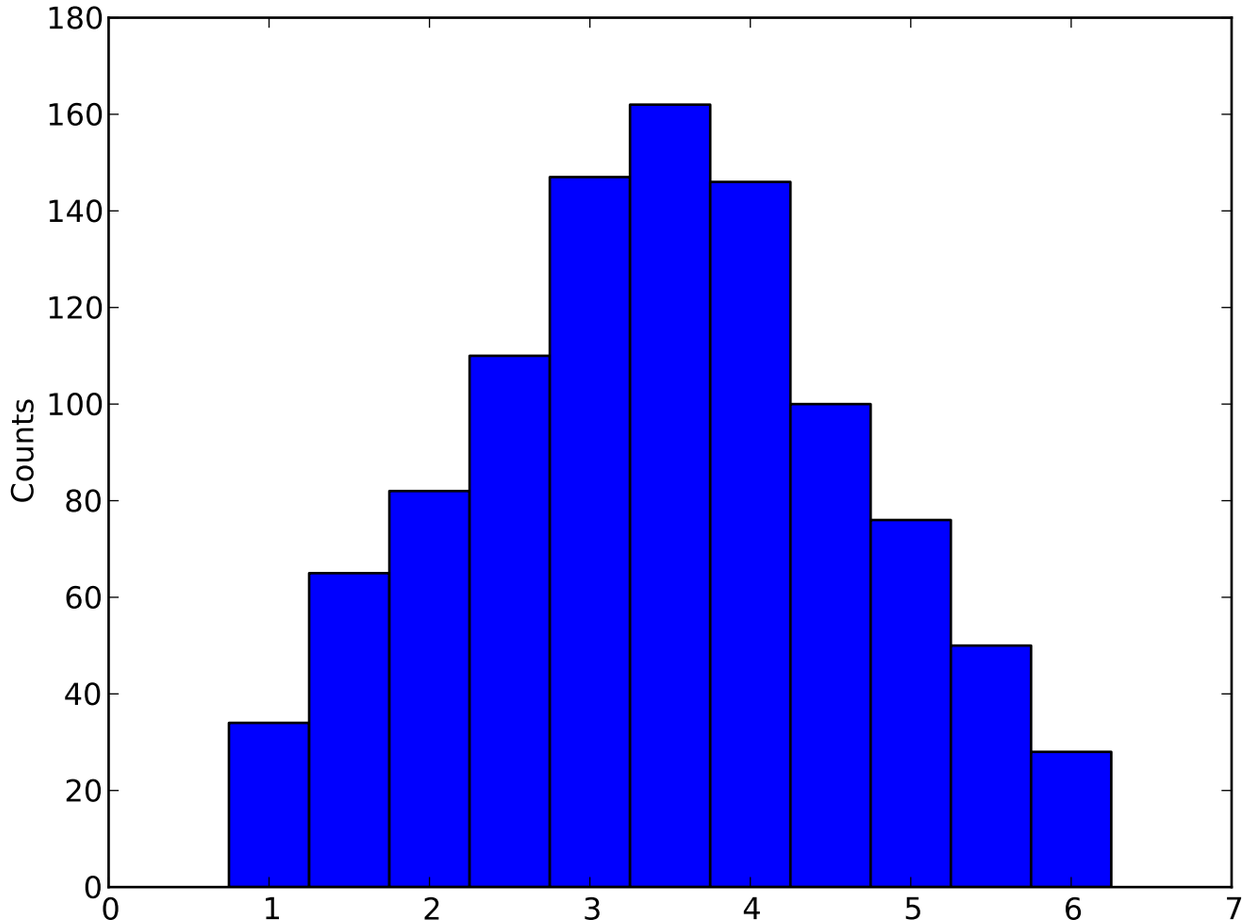

We need more information than σ since collecting more data doesn't change σ (just our estimate of it)

Particularly, we want the "standard error of the mean" -- "std-err" $\sigma_\theta = \frac{\sigma}{\sqrt{N}}$

where N is the number of samples

sometimes called the uncertainty

(Assumes a symmetric distribution

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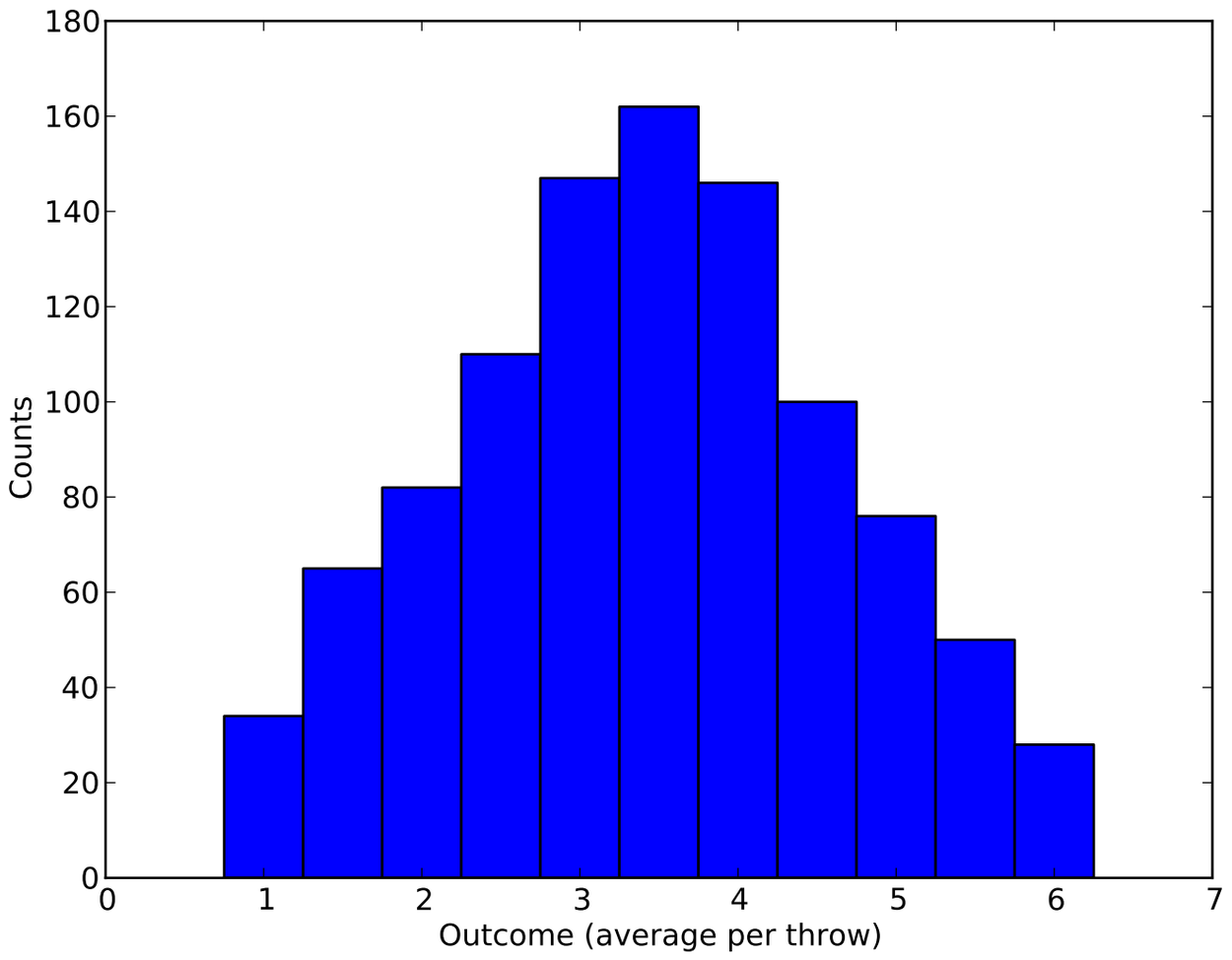

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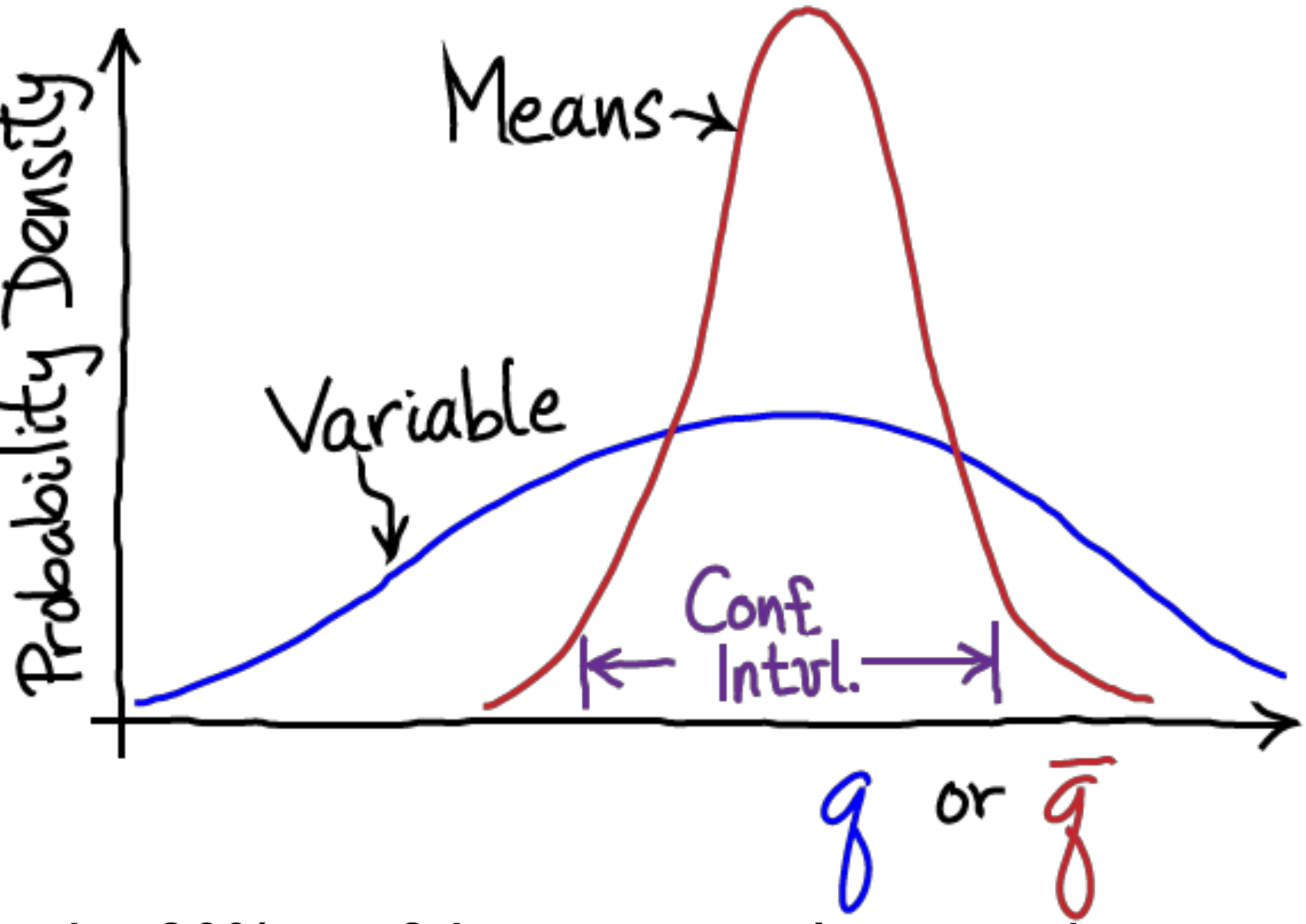
Confidence intervals are another way of thinking about uncertainty

Such as, "90% chance that the true value of our observable falls between X and Y", i.e. "A 90% chance that the mean age of graduate students is between 25 and 26".

Turns out for a unimodal Gaussian distribution, 2σ(err) is essentially a 90% confidence interval

But confidence intervals work for asymmetric distributions

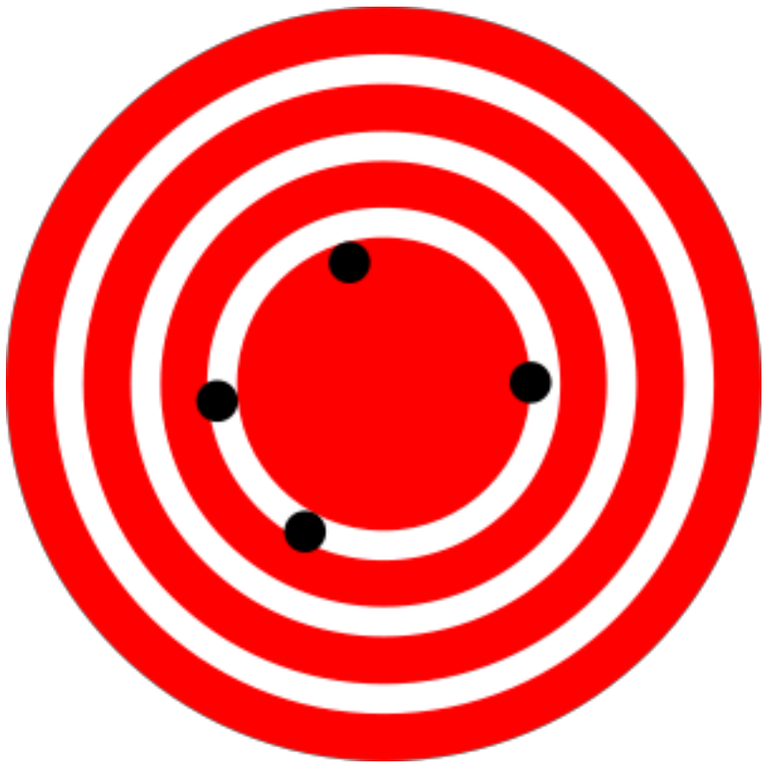
Confidence intervals are very relevant for us



In this case the 90% confidence interval means there is a 90% chance any future (equivalent) simulation would fall within that range

Image from Dan Zuckerman (with permission), from the great blog post at http://statisticalbiophysicsblog.org/?p=180

Confidence intervals and standard error assess precision of results



Accurate, not precise

Precise, not accurate

To assess accuracy, you have to calibrate/compare to other methods or experiment

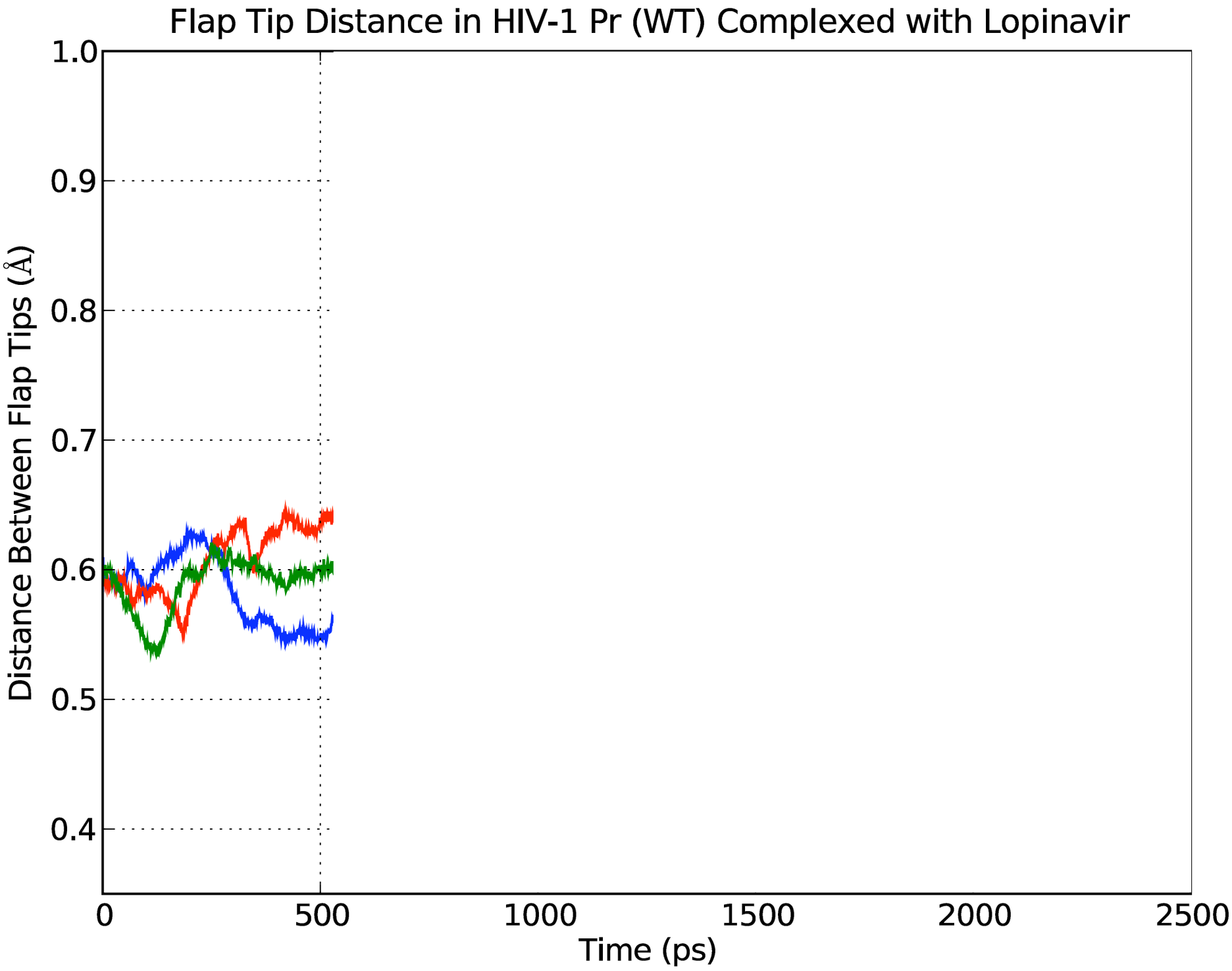
It's important to remember the difference between accuracy and precision

- Precision - a computed observable (usually a mean over observations) is very precise if
  - We are very certain of the value we are reporting, that is:
    - Our error bars/uncertainties are very small
- A value is accurate if it agrees well with the true value
- A result may be accurate and not precise, or precise and not accurate, both, or neither

Keeping standard errors/CIs in mind can avoid wild goose chases

- i.e. experimental value for some observable is 3.5, and we compute 4.3. Difference is 0.7.
  - How precise are the values?
  - 3.5+/-1.5, 4.3+/-1.0; averaging these gives 3.9+/-1.8; both expt. and calculated values fall within uncertainty of this, so the results **agree**
    - (Note that uncertainties combine, for a sum, by sqrt($\sigma_1^2+\sigma_2^2$))
  - But, if the values were 3.5+/-0.1 and 4.3+/-0.2, they definitely **disagree**
- Warnings:
  - Don't waste time tracking down differences between numbers when your precision is too low!
  - Don't come up with explanations for differences that aren't significant
  - ALWAYS know your precision before making comparisons

There is another problem with real data -- it can have correlations



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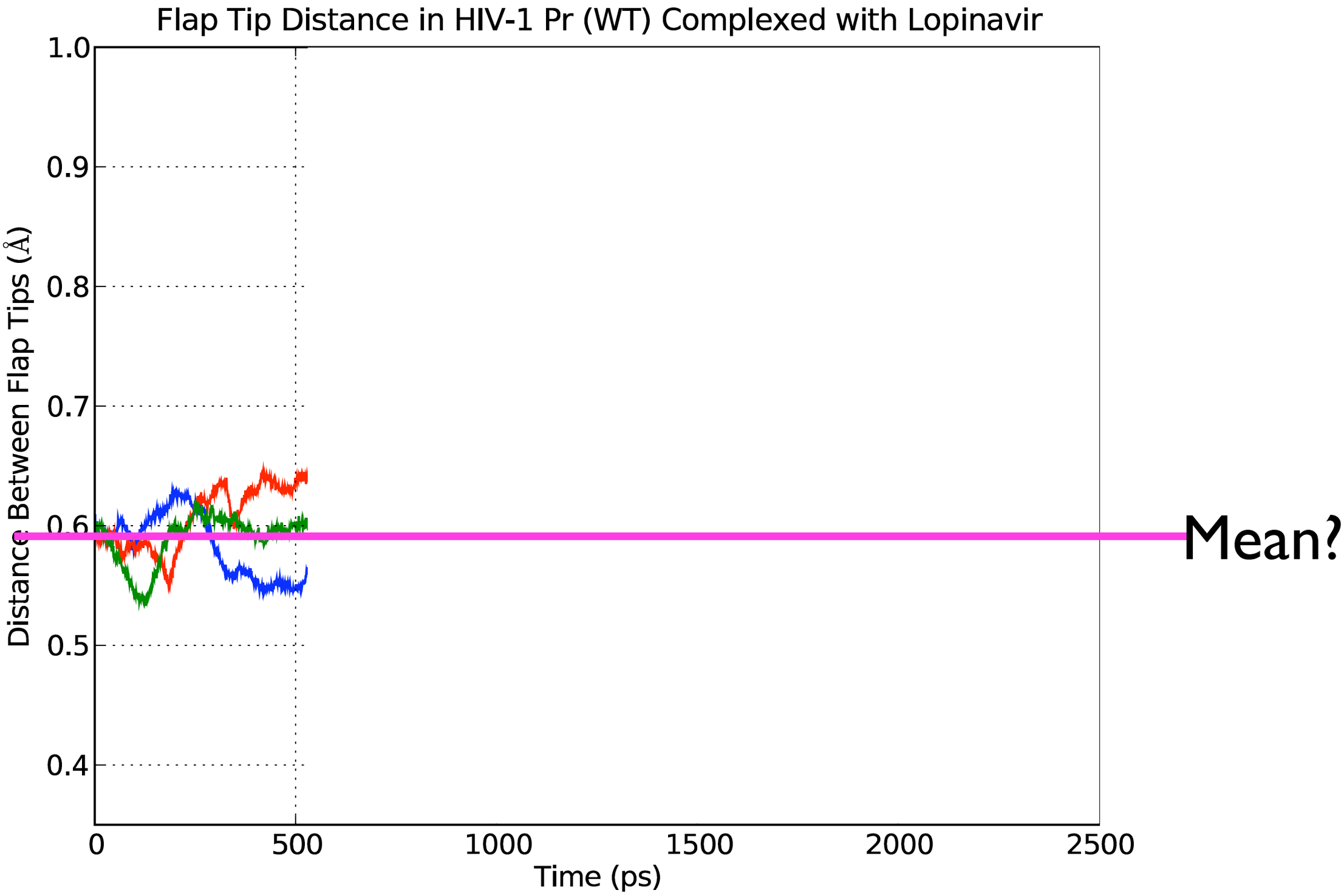

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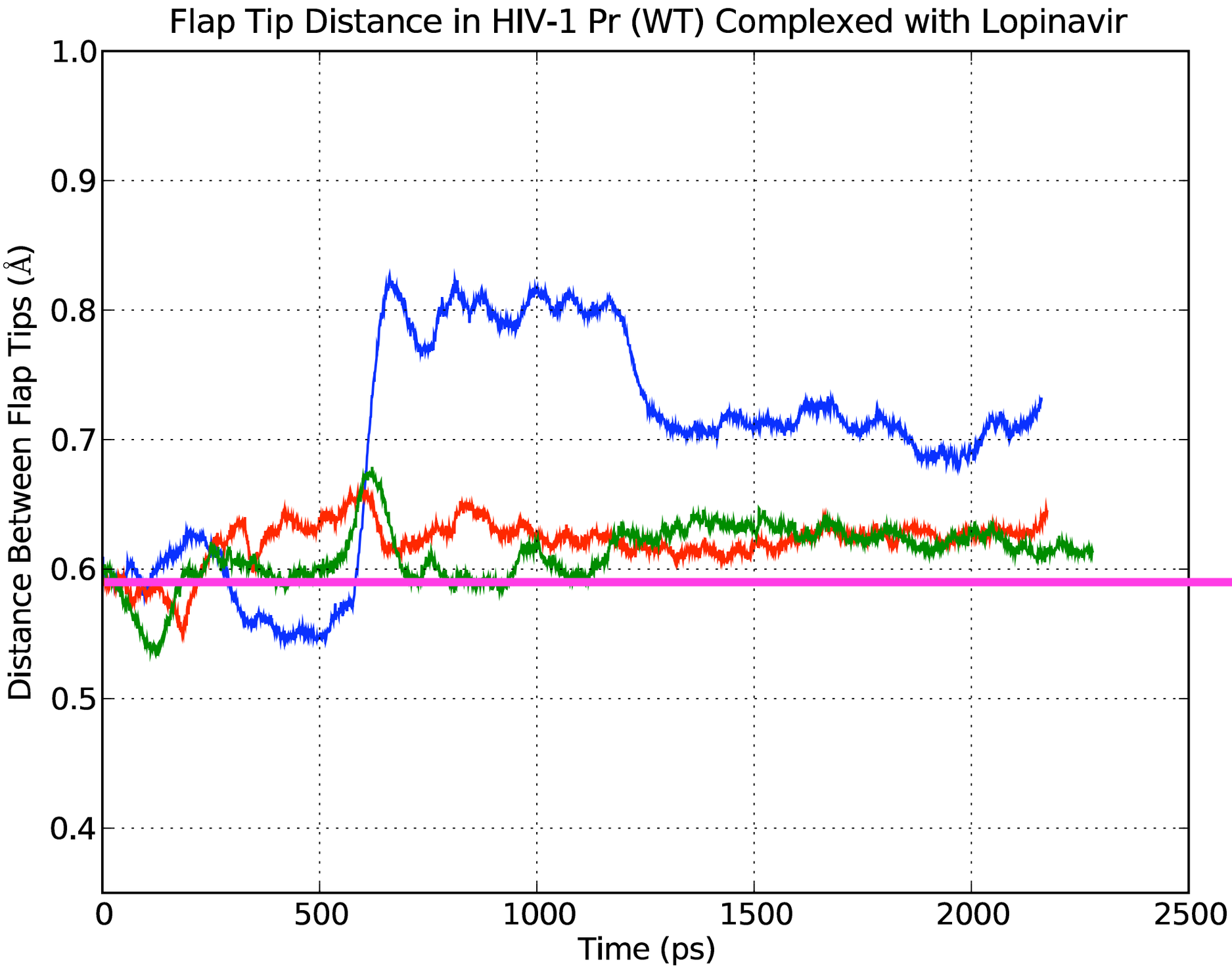

Mean?

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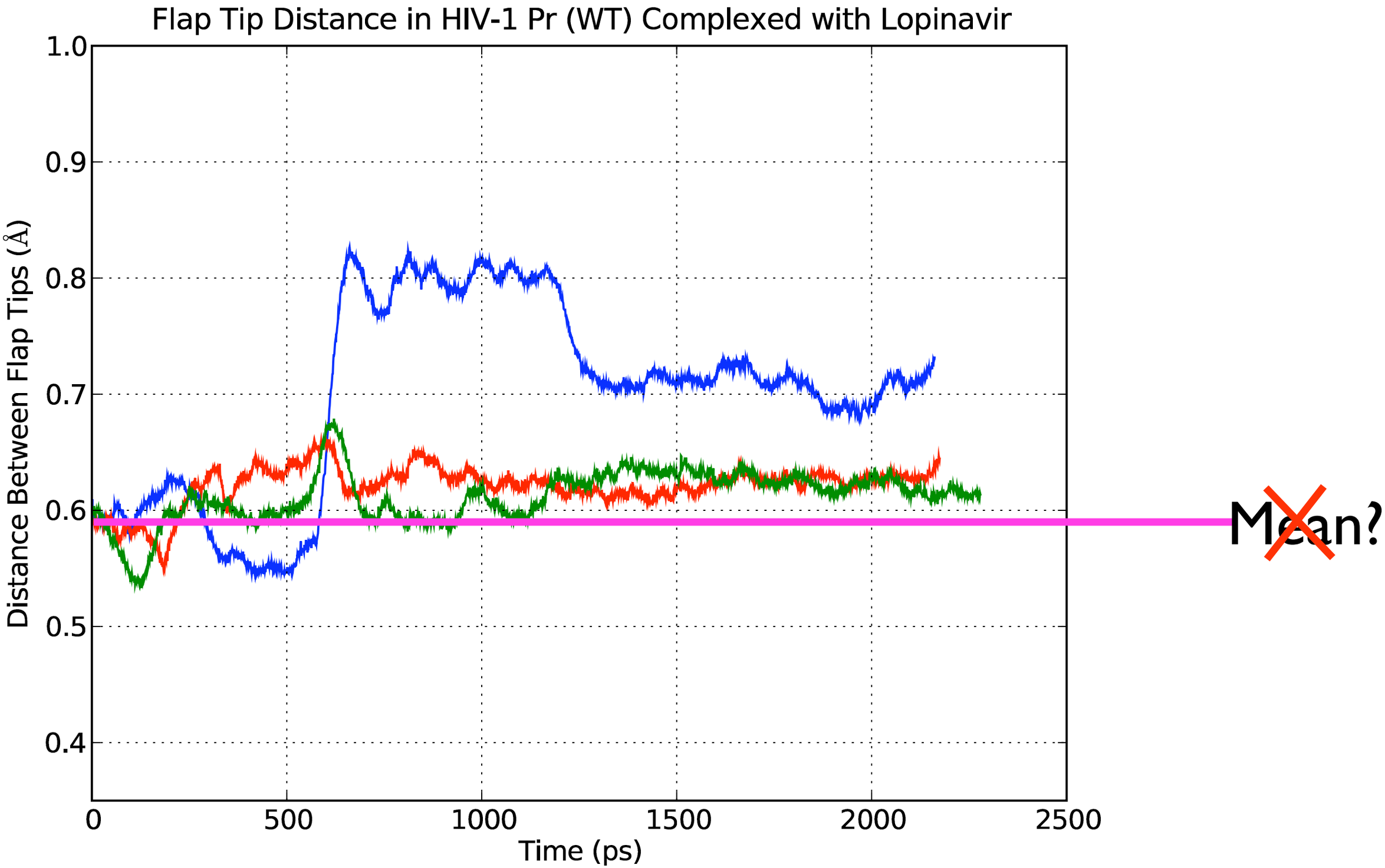

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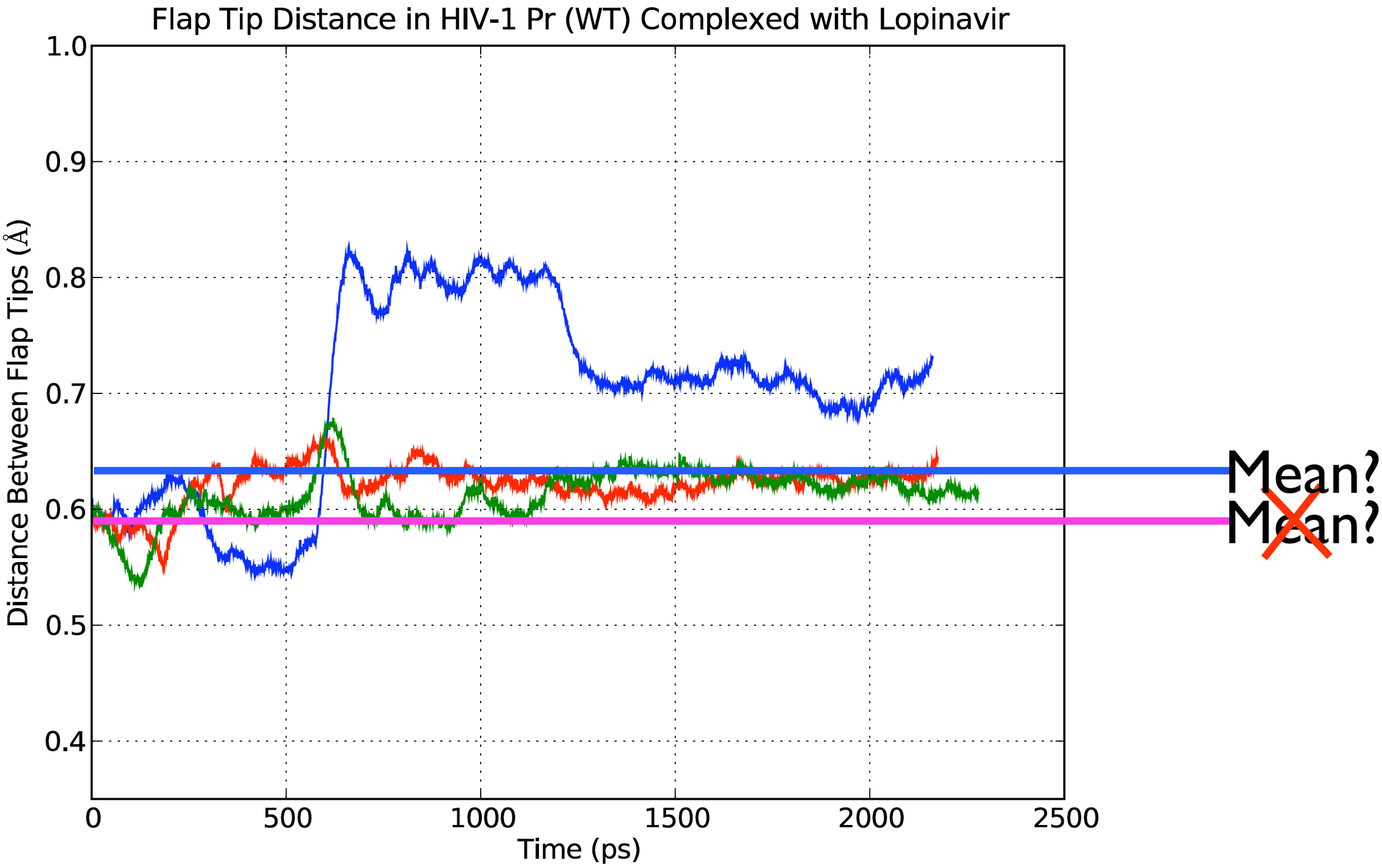

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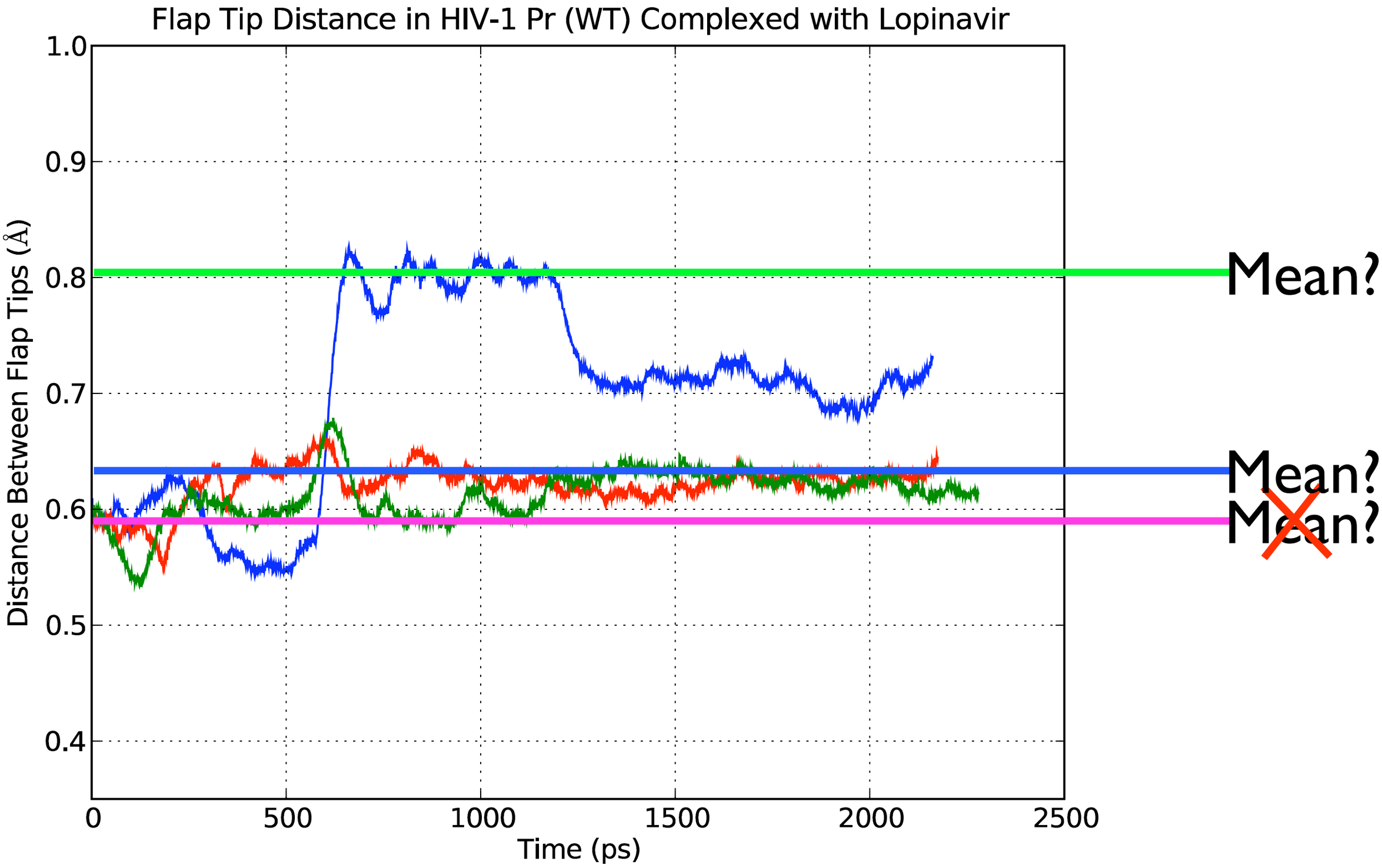

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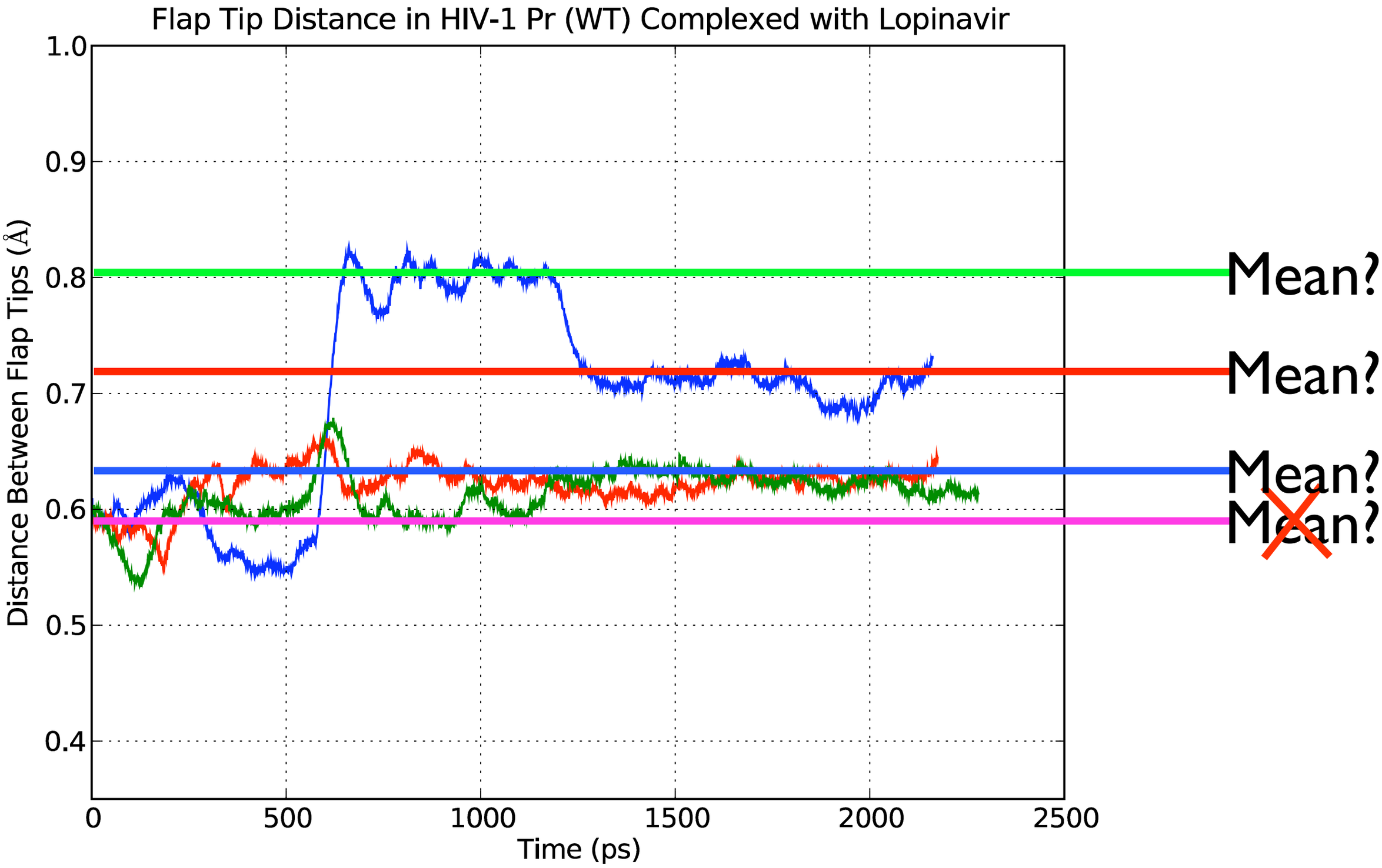
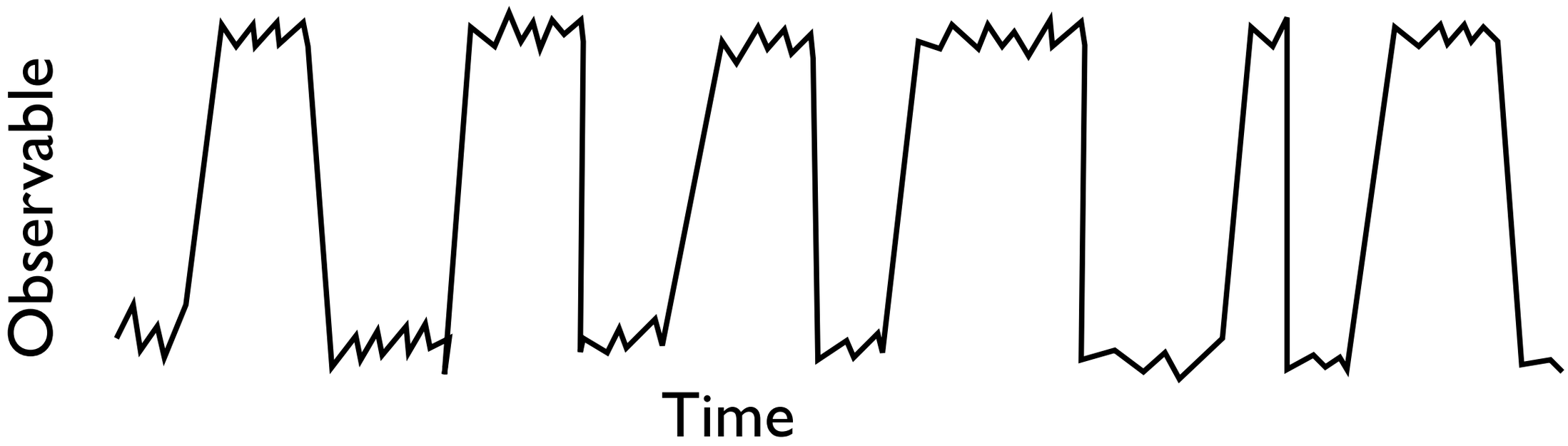
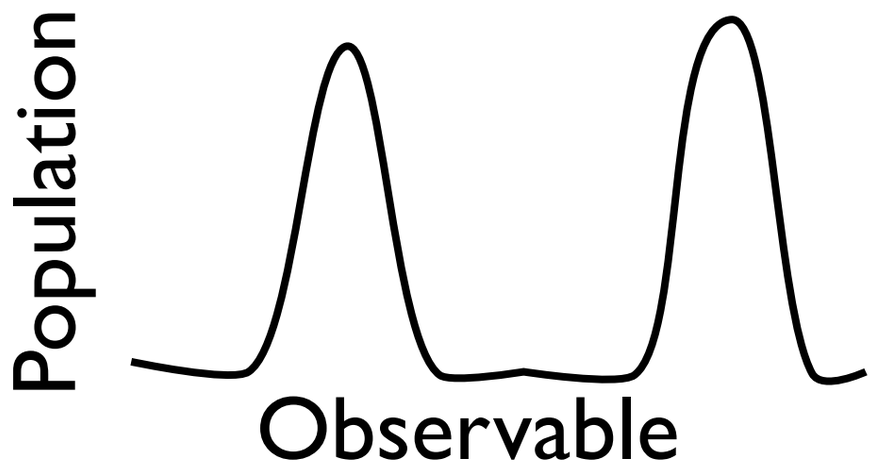


To get an accurate value of an observable with transitions, we need to see enough transitions to get the populations correct





Remember,

$$\langle f(x) \rangle = \langle f \rangle = \int dx f(x) p(x)$$

So we need correct p(x)

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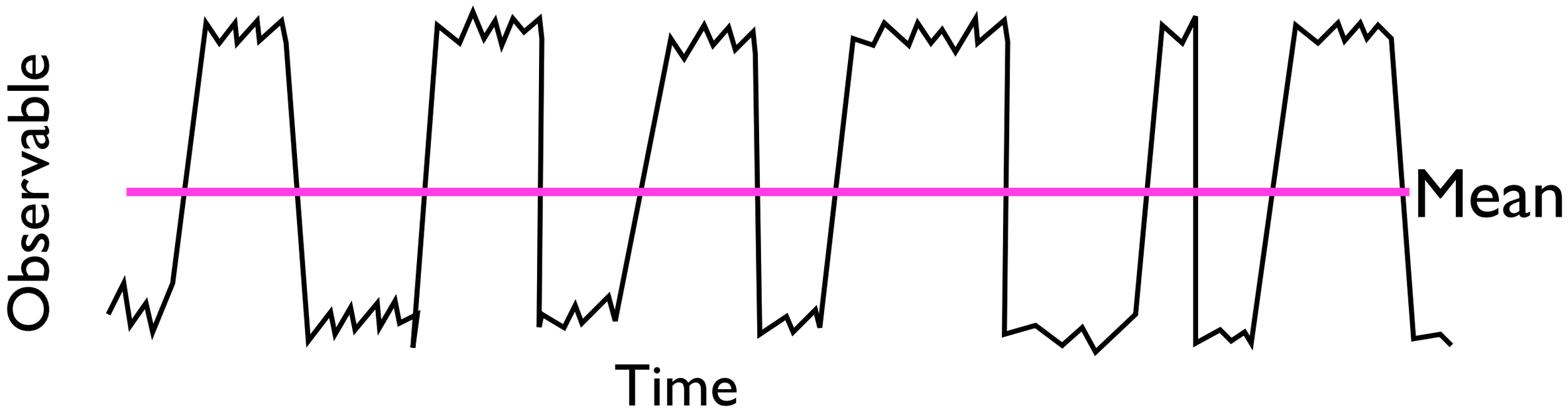

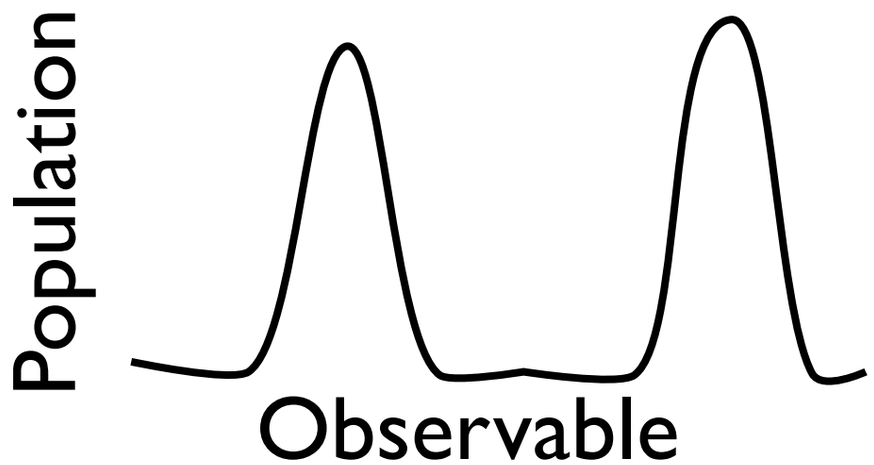

Remember,

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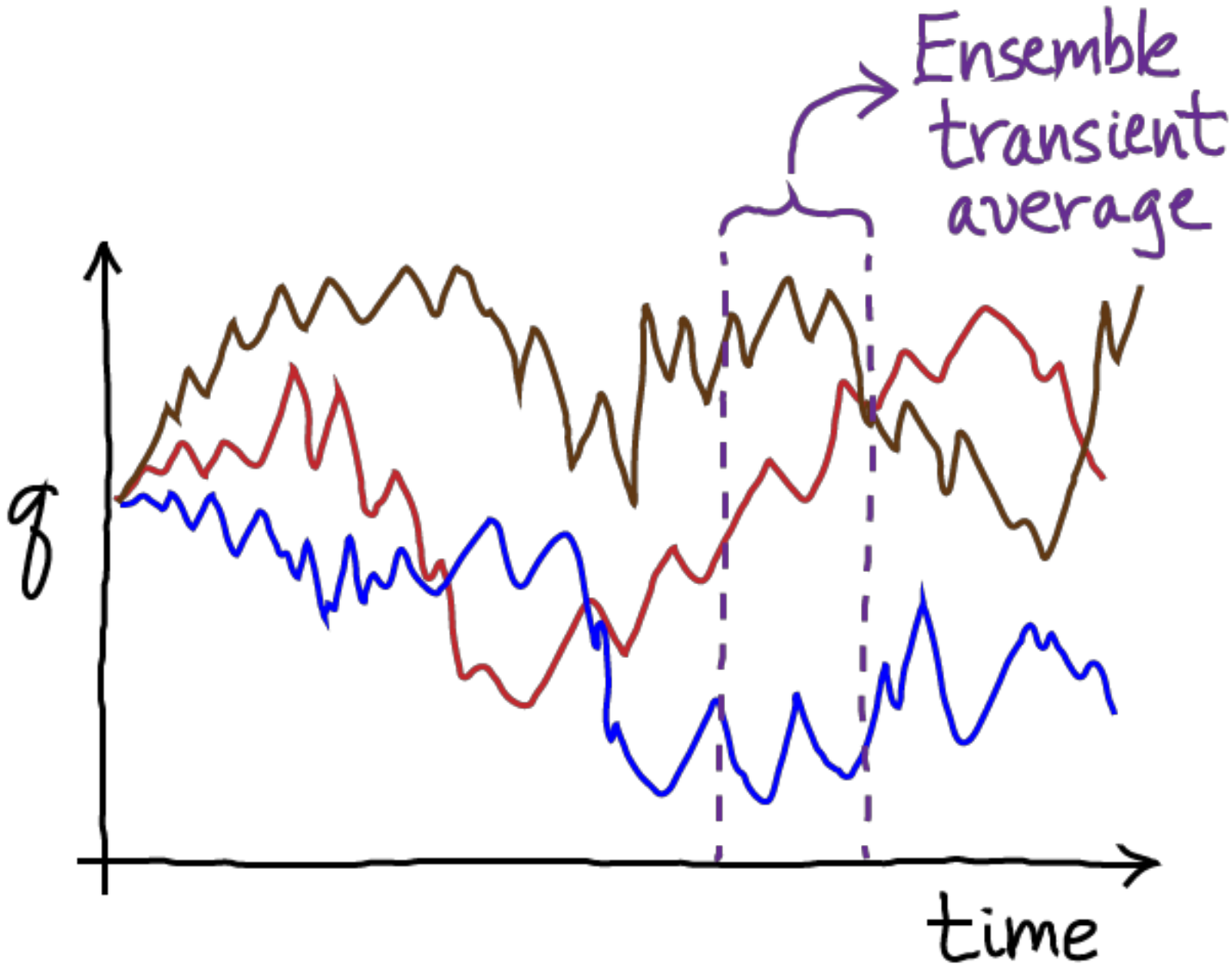
For uncertainty with "too short" trajectories, perhaps use an ensemble transient average



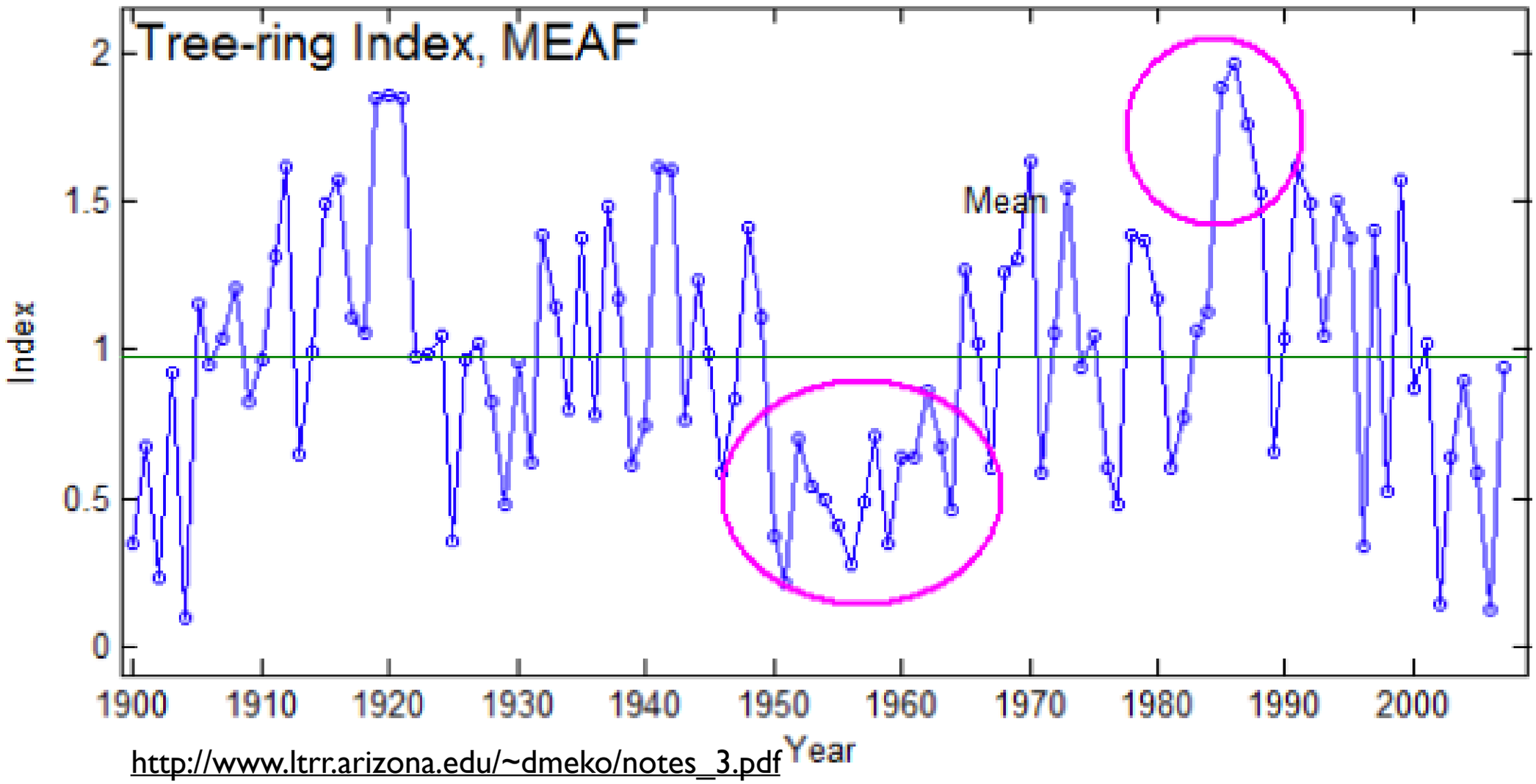
Having several "too short" trajectories generated independently, we can at least estimate a conditional uncertainty (given starting state and time elapsed), Zuckerman points out

Image from Dan Zuckerman (with permission), from the great blog post at http://statisticalbiophysicsblog.org/?p=180

For correlated data, we have to deal with the number of *effective* samples

- Compute the standard error of the mean, using error analysis
  - Standard error: $\sigma_\theta^2 = \frac{\sigma_x^2}{n}$
  - But if we take frequent samples, many may be correlated $N_{eff} = n/g$ $\sigma_\theta^2 = \frac{\sigma_x^2}{N_{eff}}$
  - For correlated samples, we have fewer "effective" samples:
    - g is the "statistical inefficiency" and is related to how correlated the measurements are; we will come back to this
- Error analysis should always be done, but also useful to test that separate trials agree
  - Some use standard error over trials as estimate of uncertainty, but probably not adequate

Correlation detection can be done using autocorrelation analysis



http://www.ltrr.arizona.edu/~dmeko/notes_3.pdf

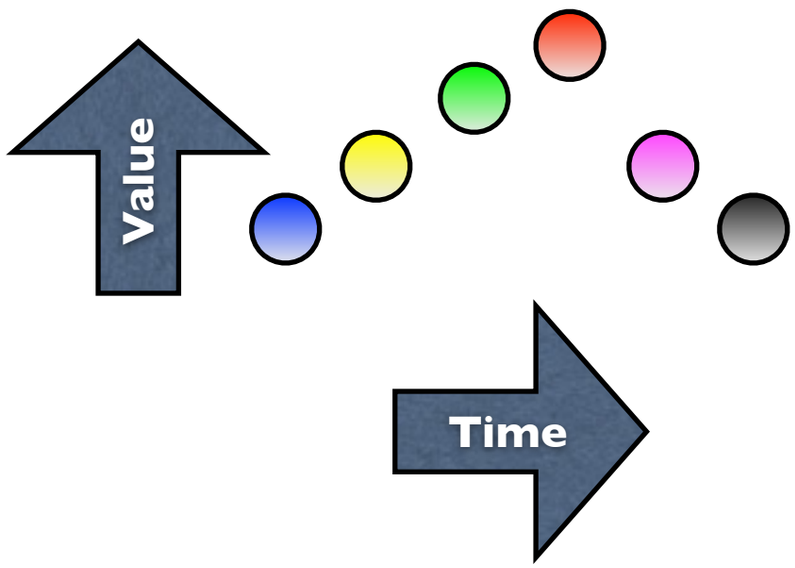
Autocorrelation function $$\rho_i = \sum_{j=0}^{N-1} a_j a_{j+i}$$

The autocorrelation time measures the timescale for correlations

- An easy way to factor this in:
  - conda install pymbar
  - pymbar.statisticalInefficiency(timeseries) gives the statistical inefficiency g
  - $N_{eff} = N/g$

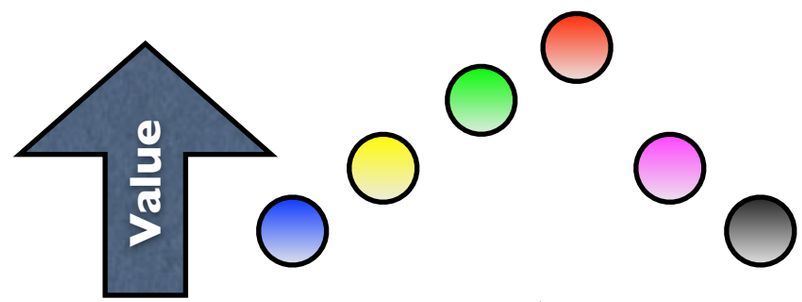
Bootstrapping provides another way to analyze correlated data

Original data

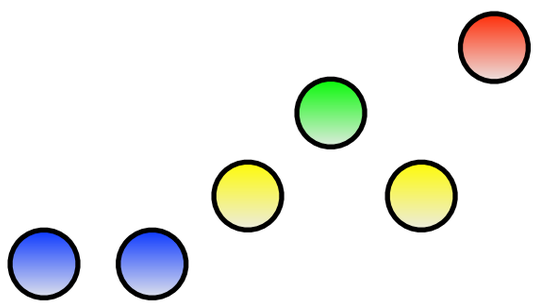


Bootstrapping provides another way to analyze correlated data

Original data



Bootstrap set 1



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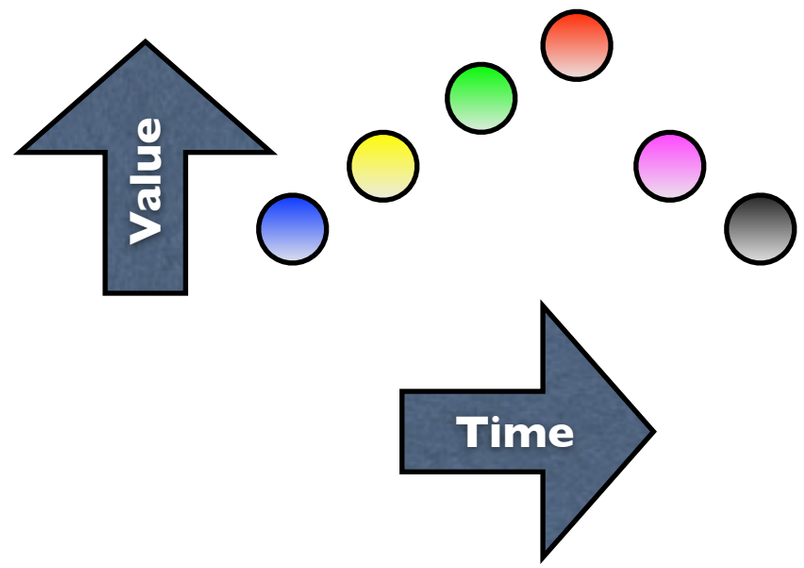
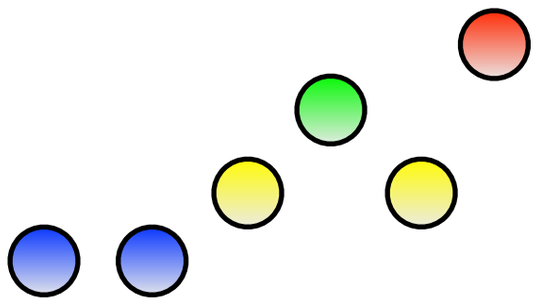
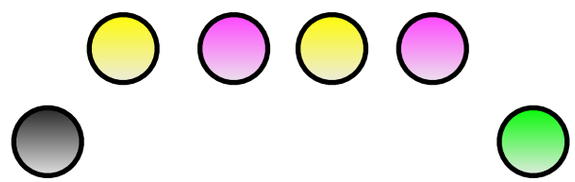
Original data



Bootstrap set 1

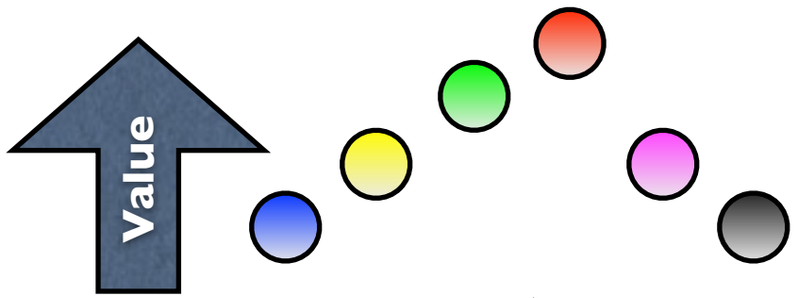


Bootstrap set 2

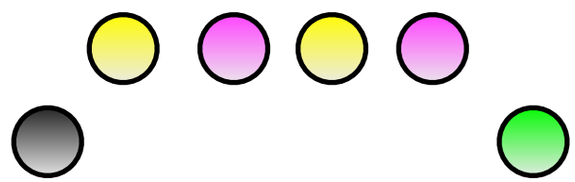


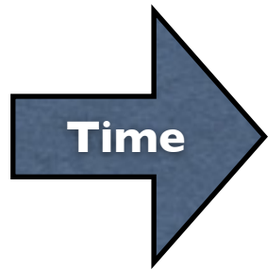
Bootstrapping provides another way to analyze correlated data

Original data

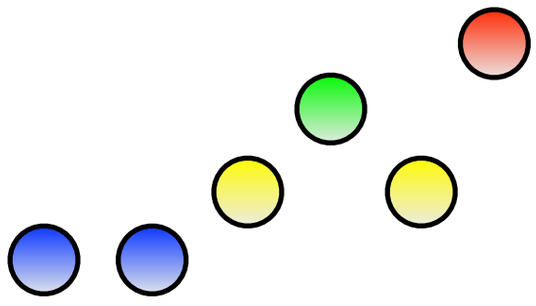


Bootstrap set 2

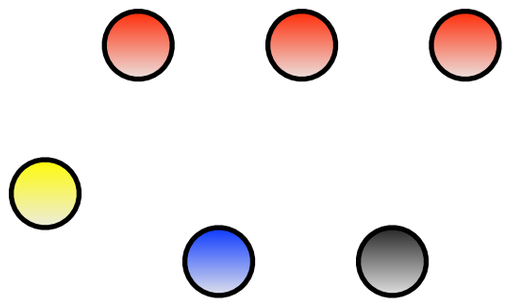




Bootstrap set 1



Bootstrap set 3



Time correlations may not be that important in much experimental data -- but know when to check

- What is the timescale of your measurement compared the timescales of the system?
- Are there conditions which could be changing with time (hr, day) in your lab?
- etc.

Error propagates when doing math -- propagation is easy for addition and subtraction

$$\sigma^2_{\theta,z} = \sigma^2_{\theta,x} + \sigma^2_{\theta,y}$$

for $z = x + y$ or $z = x - y$

that is

$$\sigma_{\theta,z} = \sqrt{\sigma^2_{\theta,x} + \sigma^2_{\theta,y}}$$

Error propagation is harder when mathematical expressions are more involved

- In general for f(x,y):

$$\sigma_{\theta,f}^2 = \left(\frac{\partial f(x,y)}{\partial x}\right)^2 \sigma_{\theta,x}^2 + \left(\frac{\partial f(x,y)}{\partial y}\right)^2 \sigma_{\theta,y}^2$$

- For example for multiplication, z = x*y:

$$\frac{\partial f(x,y)}{\partial x} = y \qquad \frac{\partial f(x,y)}{\partial y} = x$$

$$\sigma_{\theta,f}^2 = y^2\sigma_{\theta,x}^2 + x^2\sigma_{\theta,y}^2 \quad \text{or} \quad \frac{\sigma_{\theta,f}}{xy} = \sqrt{\left[\frac{\sigma_{\theta,x}}{x}\right]^2 + \left[\frac{\sigma_{\theta,y}}{y}\right]^2}$$

The "null hypothesis" is vitally important in experimental design and analysis

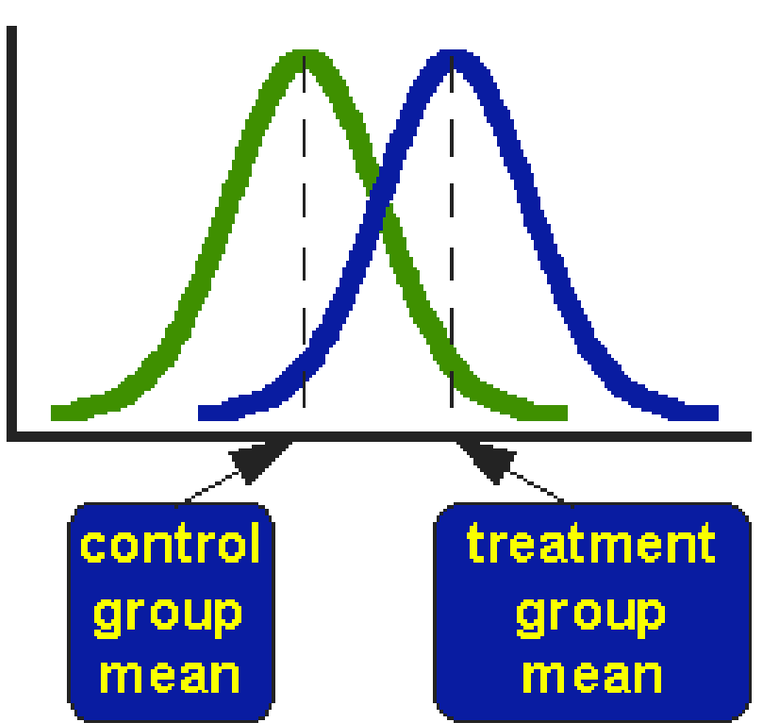
- Null hypothesis: An uninteresting outcome for your experiment
  - i.e. what you are changing makes no difference in the outcome
  - or you are observing only noise/random fluctuations
  - or you observe no binding of your compound
  - or...

The null hypothesis can never be proven, but it can be rejected or not rejected

- An experiment can reject or fail to reject the null hypothesis
- Example: Testing new treatment on control vs test groups
  - With enough data you may be able to reject the null hypothesis
  - Without enough data, there MAY still be a difference, but not one you can see

The t-test is a commonly used approach to assess significance of results (rejection of the null hypothesis)

- The t-value relates the difference in measured means to the standard error in the mean



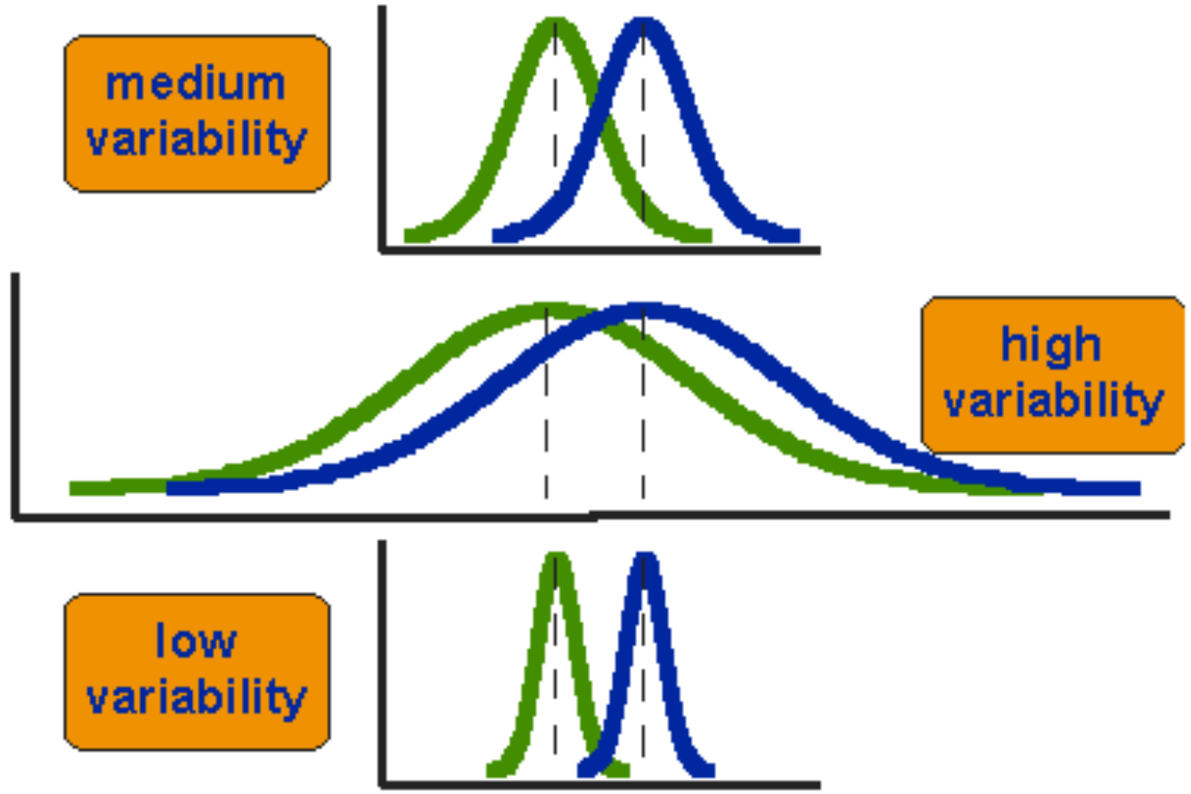


Figure 2. Three scenarios for differences between means.

http://www.socialresearchmethods.net/kb/stat_t.php

The t-value is easy to calculate (though there are different forms for different cases)

- The t-value relates the difference in measured means to the standard error in the mean

$$t = \frac{\mu_c - \mu_t}{\sqrt{\sigma^2_{\theta,c} + \sigma^2_{\theta,t}}}$$

http://www.socialresearchmethods.net/kb/stat_t.php

The t-value is easy to calculate (though there are different forms for different cases)

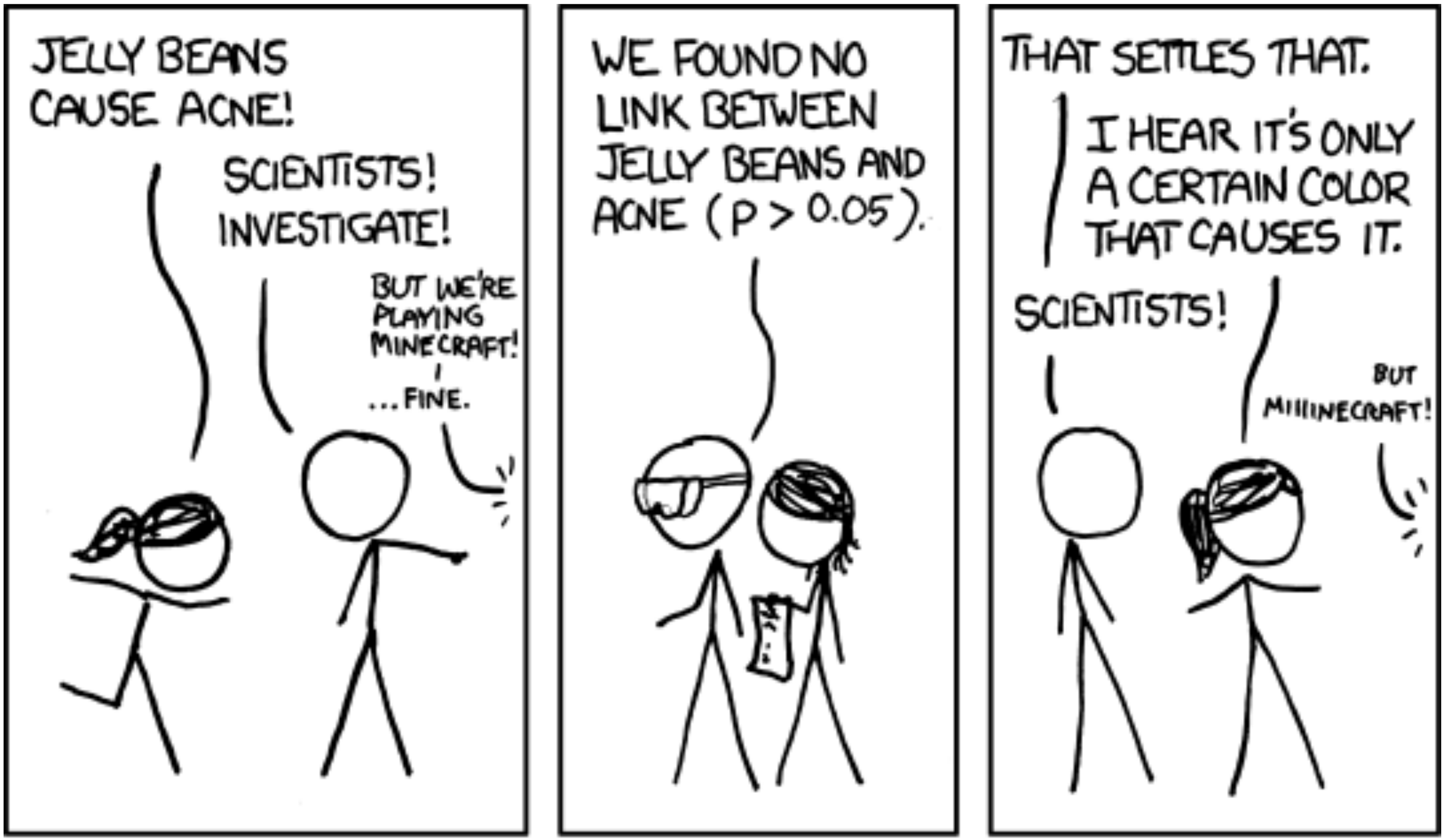
- The t-value relates the difference in measured means to the standard error in the mean

$$t = \frac{\mu_c - \mu_t}{\sqrt{\sigma^2_{\theta,c} + \sigma^2_{\theta,t}}}$$

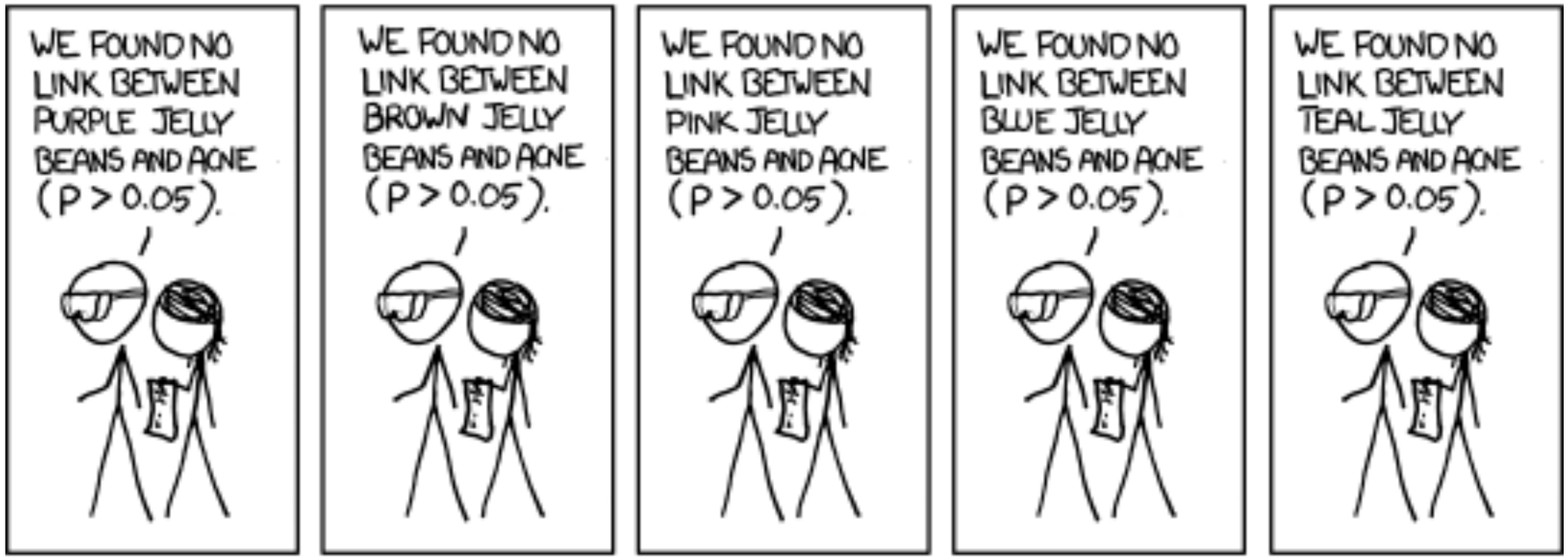
Degrees of Freedom	Probability, p			
	0.1	0.05	0.01	0.001
1	6.31	12.71	63.66	636.62
2	2.92	4.30	9.93	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96

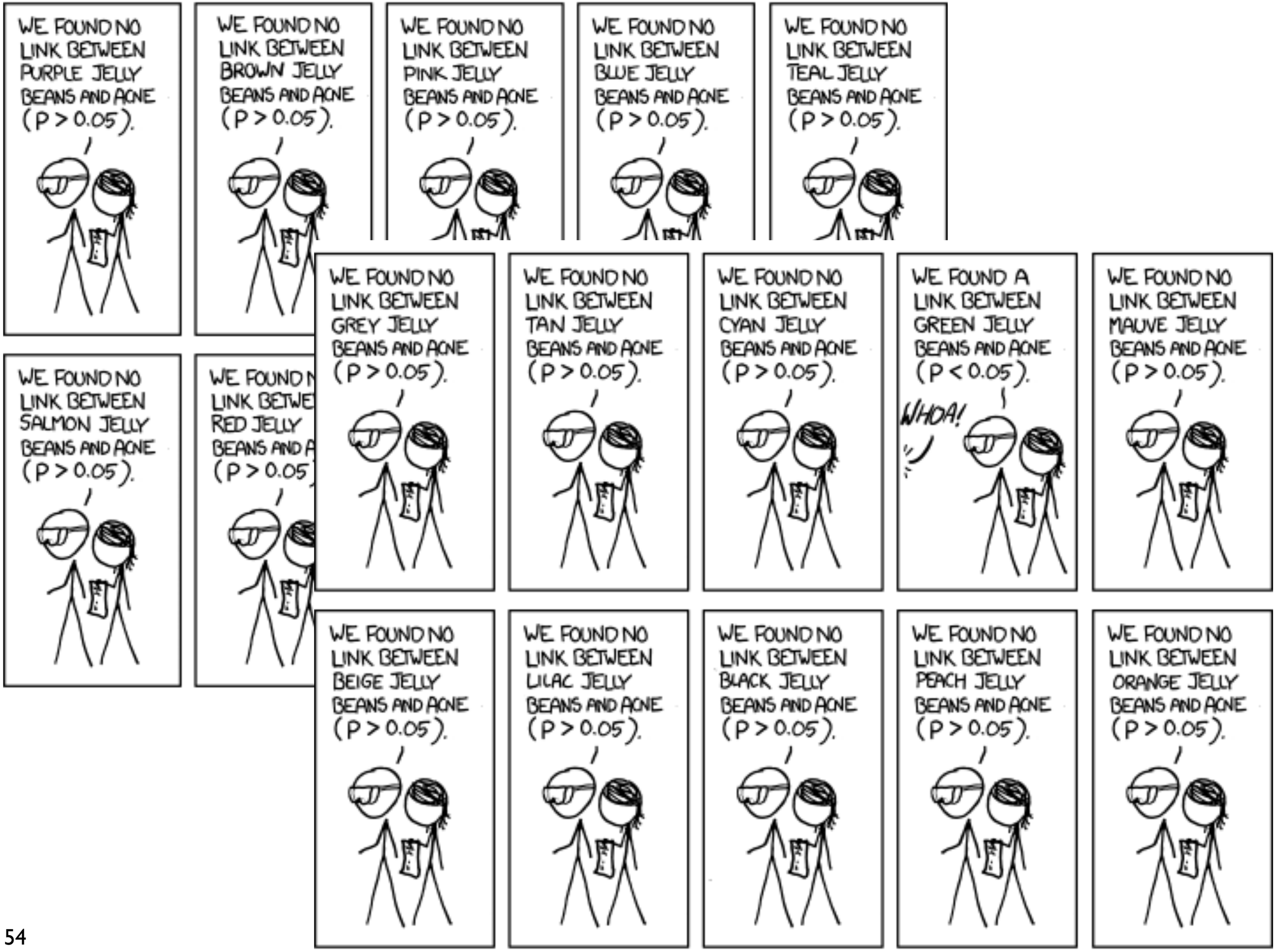
Tabulated t values for the confidence with which you can reject the null hypothesis

http://www.socialresearchmethods.net/kb/stat_t.php



https://xkcd.com/882/


WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TURQUOISE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN MAGENTA JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN YELLOW JELLY BEANS AND ACNE (P > 0.05).


WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE (P > 0.05).
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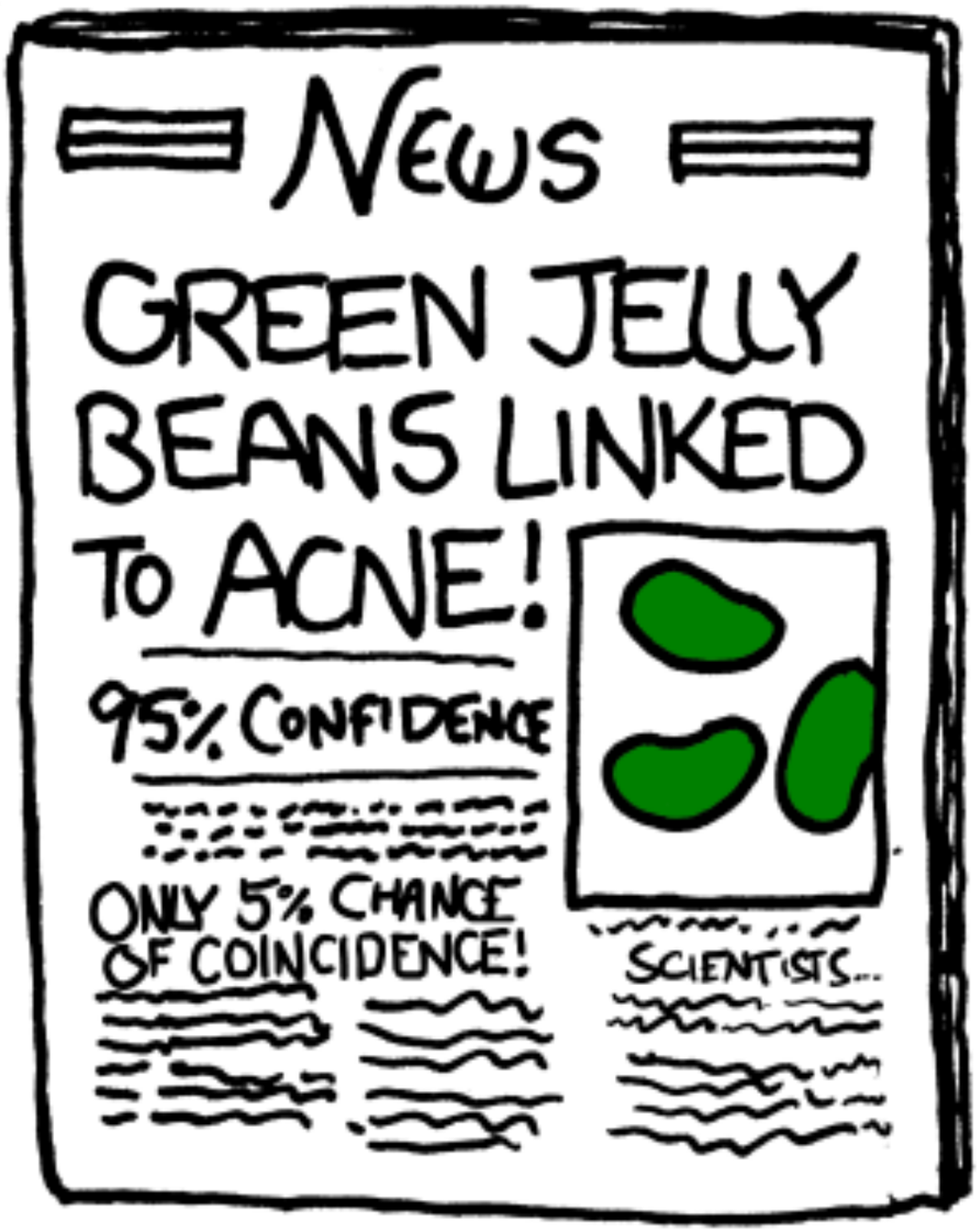
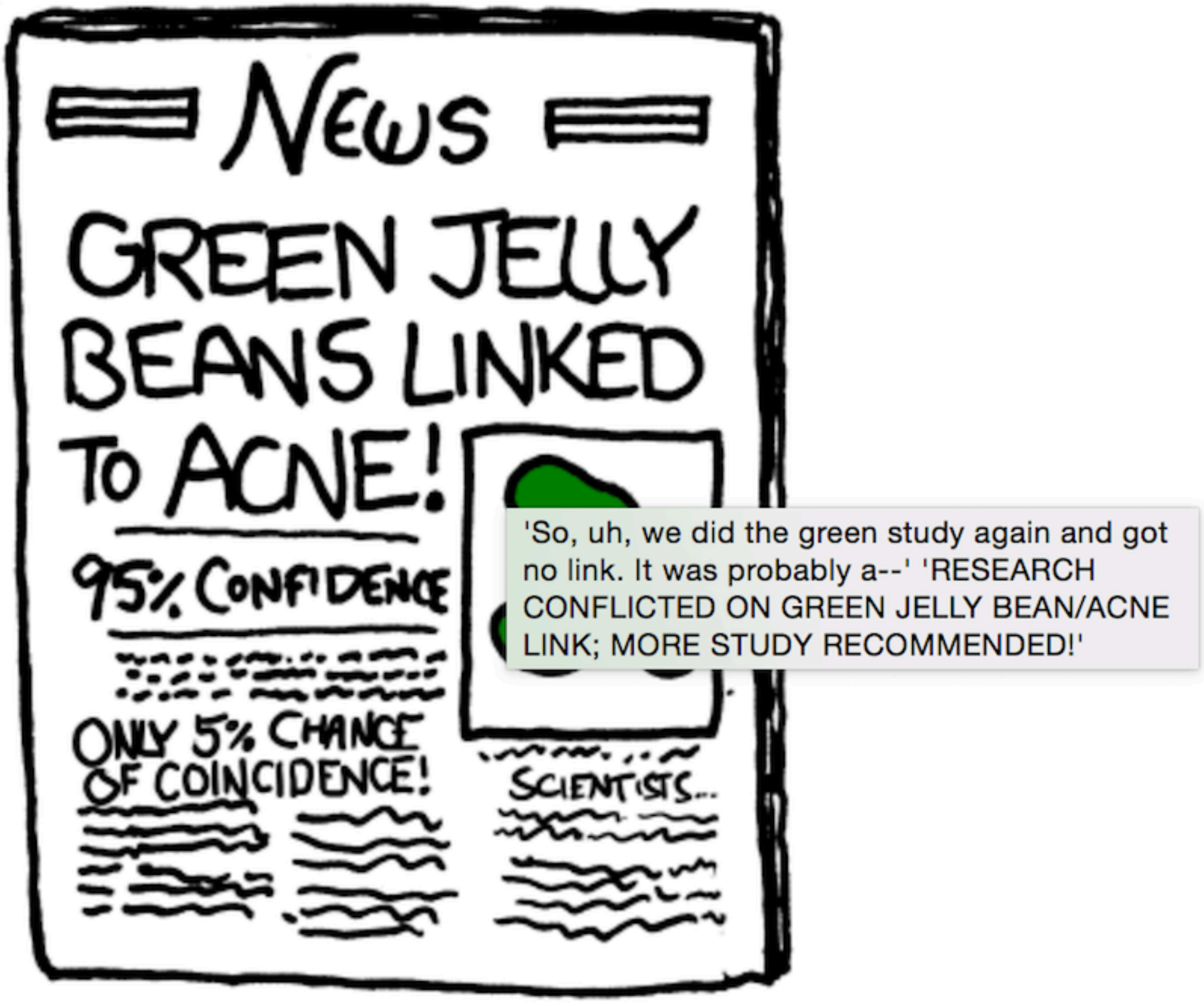
WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN GREY JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN CYAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND A LINK BETWEEN GREEN JELLY BEANS AND ACNE (P < 0.05).
WHOA!
WE FOUND NO LINK BETWEEN MAUVE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BEIGE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN LILAC JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLACK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PEACH JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN ORANGE JELLY BEANS AND ACNE (P > 0.05).


NEWS
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...


NEWS
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...
'So, uh, we did the green study again and got no link. It was probably a--' 'RESEARCH CONFLICTED ON GREEN JELLY BEAN/ACNE LINK; MORE STUDY RECOMMENDED!'

Who wants to spend their career analyzing statistical fluctuations?

- Tests for significance prevent wasted time
- Examples:
  - My lab: HIV protease, hydration work, ...
  - Presidential polling
  - From the class?

Note, though, there are other kinds of uncertainty such as model uncertainty