

Publication Quality Graphics

Biochemistry Boot Camp 2022

Session #7

Nick Fitzkee

nfitzkee@chemistry.msstate.edu

Why Quality Graphics?

- Clarity of Presentation
 - Sharp-looking Posters, Presentations, Papers
 - Make your message easier to understand
- Requirement of Many Journals: Print Standards
- Enable Close Inspection in Digital Format
 - Blurry, pixelated graphics are not professional

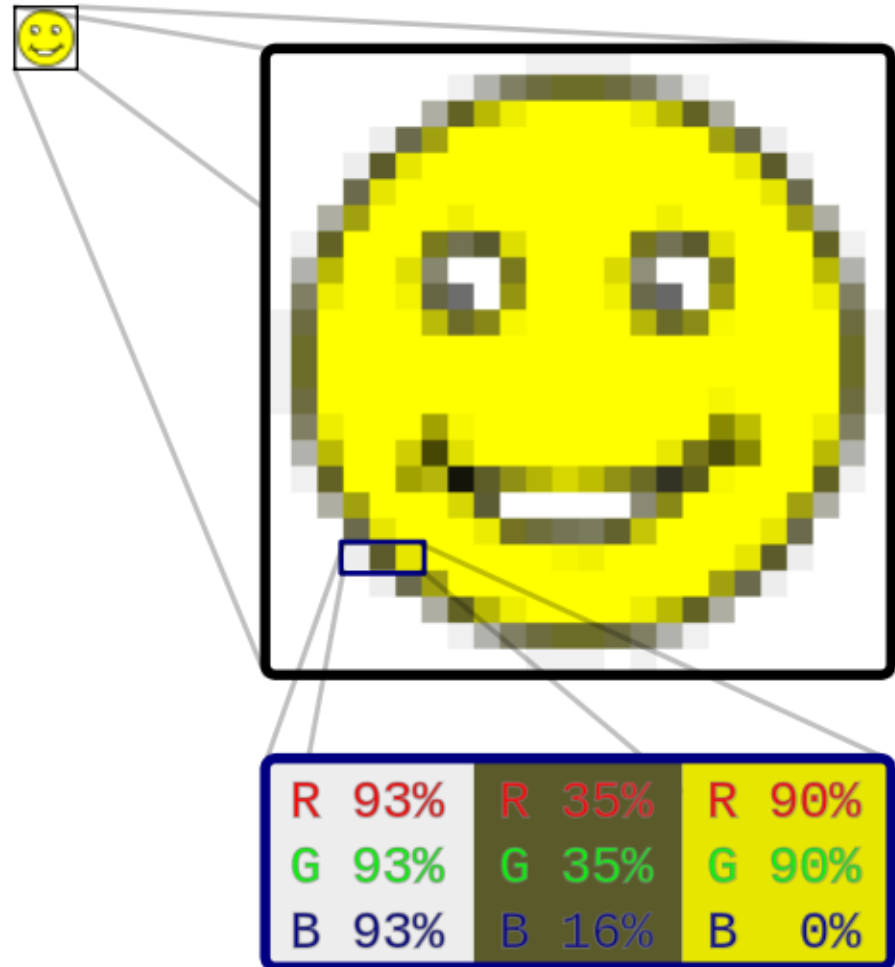
Graphics with Bully

(try zooming in)



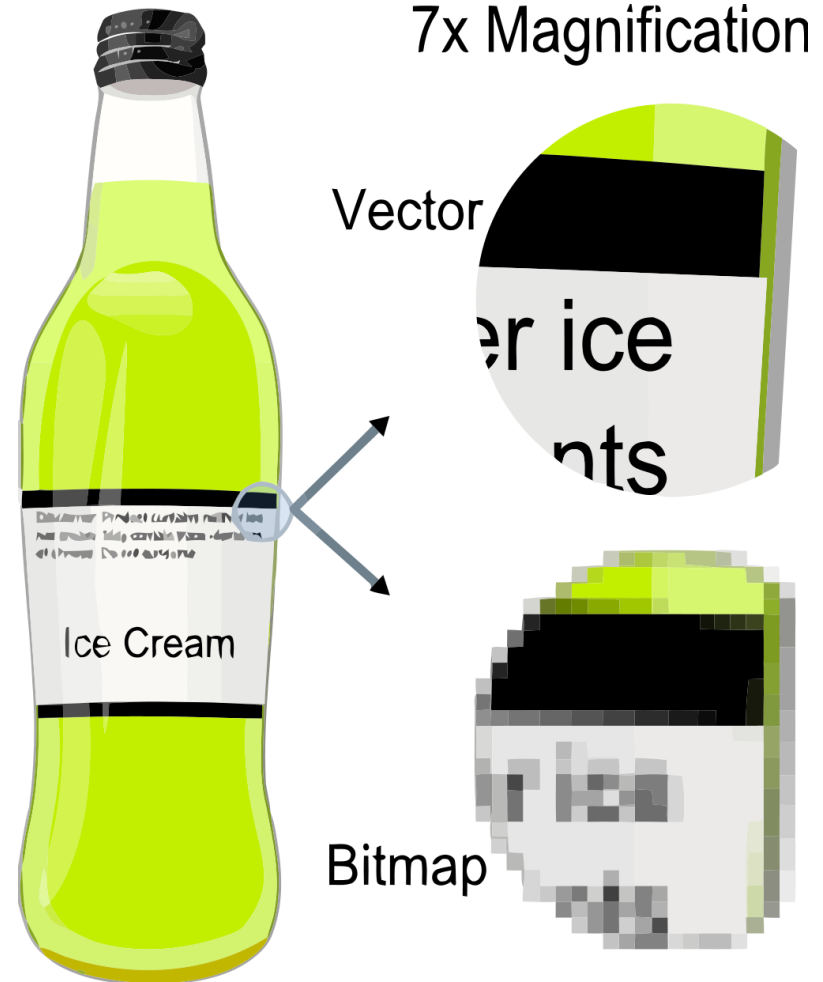
How to Store Graphics

- *Raster* (or bitmap) graphics
- Pixels stored in pre-determined x, y order
- Can be grainy when zoomed
- Color must be stored with each pixel
- **Good for:** photos, artwork, graphs, diagrams
- **Software:** Photoshop, Gimp



How to Store Graphics

- *Vector* graphics
- Shapes, text drawing effects are stored mathematically
- Arbitrary zoom without graininess
- Color is stored with each shape/line
- **Good for:** graphs, diagrams, but *not* photographs
- **Software:** Illustrator, Inkscape



Raster vs. Vector File Formats

Raster Formats

- GIF
- BMP
- PNG – Portable network graphics
- JPEG – Joint photographic experts group
- TIF – Tagged image file

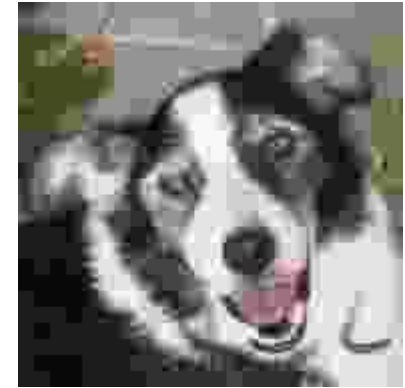
Vector Formats*

- PDF
- SVG – Scalable vector graphics
- WMF – Windows Metafile
- PS – Postscript
- EPS – Encapsulated Postscript
- DOCX, PPTX – Office tools

* Most vector formats can also store rasterized graphics as a subset of artwork.

Image Compression

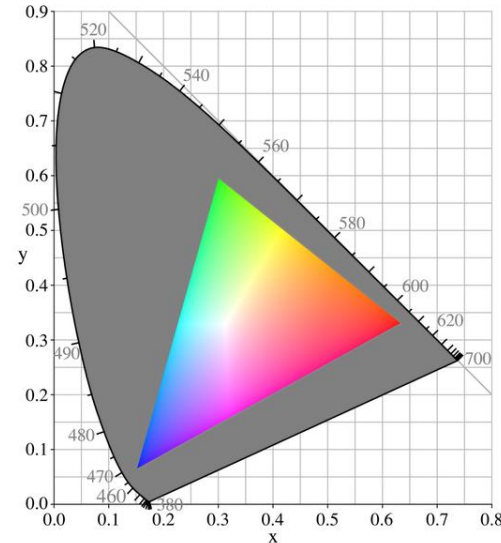
(only applies to raster graphics)



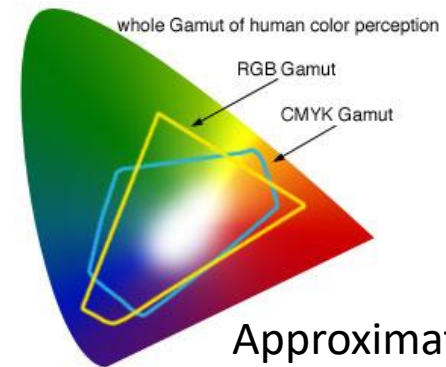
- **Lossy:** Image is made smaller by “losing” data; approximations are made to maintain image
 - Generally can adjust quality (see above)
- **Lossless:** No image data is lost; quality is the same
 - File sizes will be bigger than lossy

Color: RGB and CMYK

- **Computer monitors:** additive color (red, green, blue)
- **Printed material:** subtractive color (inks; cyan, magenta, yellow, black)
- Different technologies are limited to different colors!



Actual representation of RGB color gamut (all visible colors would be in the grey area)



Approximation!

Best Practices For Publication

- Size (dimensions)
 - One column is 8 cm wide; two columns are 17 cm
 - Most journal pages are 28-30 cm tall
 - Poster figures can vary widely, but should be bigger
- Resolution
 - 300 dpi minimum, some publishers want 1,200 dpi for graphs and diagrams (overkill)
- Color Mode
 - RGB or CMYK (frequently doesn't matter, but used to be more important; check requirements)

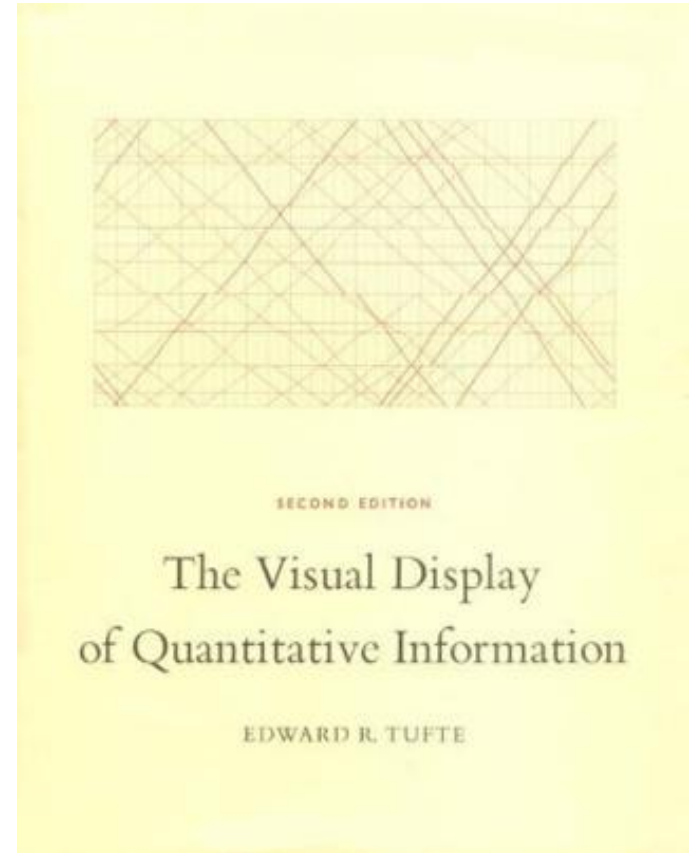
Best Practices For Publication

(continued)

- Fonts
 - 5 pt minimum, 8-11 pt for most labels
 - Panels (A, B, C) vs (a, b, c) → Check recently published papers
- Always read author guidelines carefully (format, dpi, color requirements)
- **Be Observant!**
 - Pay attention to what is acceptable in papers
 - Notice what works in posters/presentations

Tips and Tricks

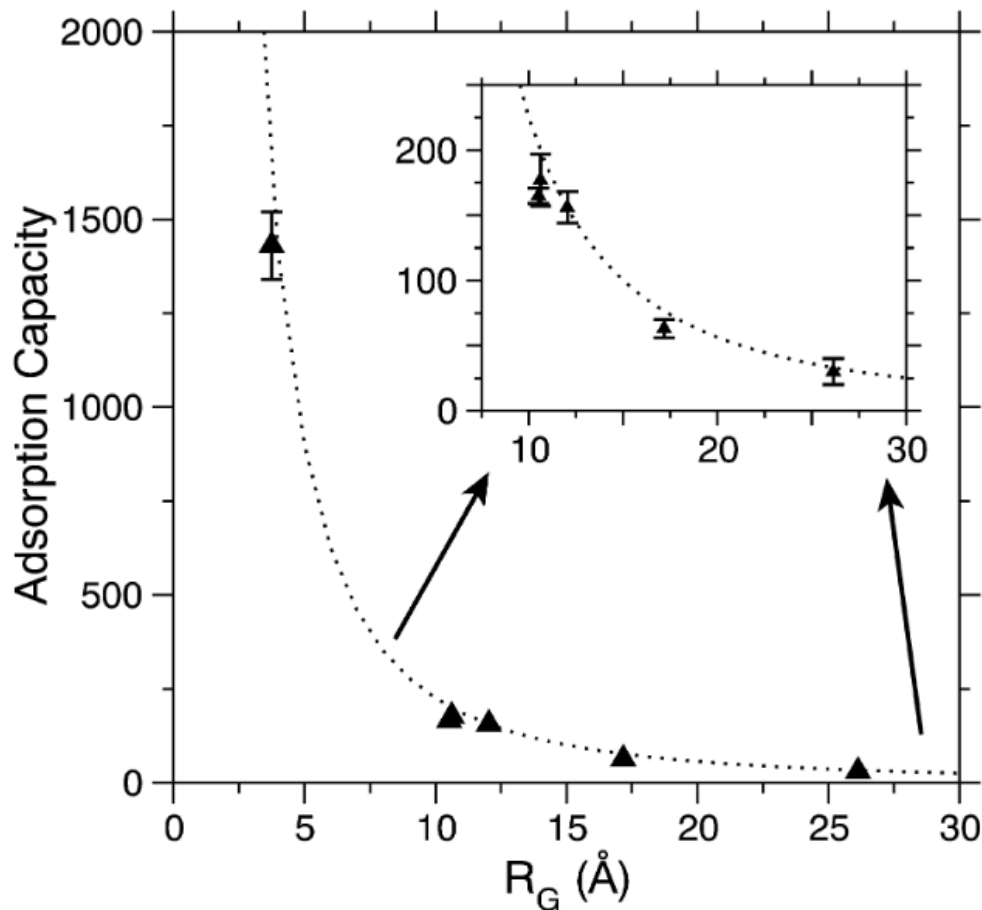
- Think carefully about layout and display: one column or two? How many panels?
- Layout figures in PowerPoint to get a rough estimate, then use other software to make a final version
 - PowerPoint is not a print publication tool!
- Learn your adviser's preferences and model them (read papers, talk to other students)
- Pay attention to good design and effective communication!



The Software

- **Graphs:** Origin, Graphpad/Prism, Grace, Matlab, Gnuplot, Mathematica, Excel (?)
- **Touch-Up:**
 - Raster: Photoshop, GIMP
 - Vector: Illustrator, Inkscape
- Never (EVER) doctor an image or graph to make it look better
 - If there is any doubt about image manipulation, ask your adviser
 - An interesting afternoon: <https://retractionwatch.com/>

Doing it With Excel: The Target



The Journal of Physical Chemistry C

Article

with AuNPs is highly intriguing, and work is ongoing to identify the source of this discrepancy.

Are Proteins Compact on AuNPs? Using simple geometric considerations, it is possible to predict the adsorption capacity of a spherical AuNP. This approach has been applied to quantum dots and polymer-based nanoparticles.^{30,39} We have adopted a similar approach, using the radius of gyration (R_G) calculated from the protein structure. While the R_G does not directly capture the longest dimension of the protein, and it cannot account for nonspherical shapes, it roughly represents the occluded surface on the AuNP itself. Using this metric, the predicted maximum adsorption capacity (N_{max}) is given by

$$N_{max} = \frac{4R_{AuNP}^2}{R_G^2} \quad (4)$$

For AuNPs of 15 nm diameter, the agreement between the predicted and measured adsorption capacity is very good (Figure 4 and Table 1). No parameters are adjusted to create the curve in Figure 4, yet the Pearson correlation coefficient is 0.9998 ($p < 7.5 \times 10^{-11}$).

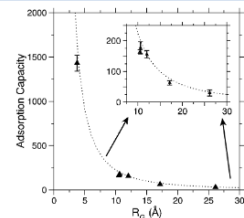


Figure 4. Adsorption capacity of AuNPs for differently sized proteins. The dotted line represents the predicted maximum adsorption capacity assuming a folded protein R_G . Triangles indicate experimental measurements. The same data are plotted in the inset to illustrate error bars, which are too small to visualize when GSH is included.

MBI Competition. Previously, we used MBI as a probe to examine the stability of protein–AuNP complexes.⁴² Wild-type (WT) GB3 does not have any cysteine residues, and SERS experiments suggested that it could be displaced by the addition of MBI. By monitoring the NMR signals of GB3 and MBI, we are able to examine this displacement directly (Figure 5). AuNP and GB3 were mixed and allowed to incubate at room temperature for 2 days. Then, MBI was added to test whether GB3 could be removed from AuNPs. To test the importance of surface Cys residues, we used both WT and K19C GB3. Initially, both WT and K19C GB3 were observed to bind to AuNPs similarly (Figure 5a), but after addition of MBI, the concentration of unbound GB3 is observed to increase, indicating that WT GB3 can be displaced by MBI. This reaction occurs within 15 min after adding MBI, and it does not change significantly after 24 h. K19C GB3 behaves differently; MBI cannot displace this variant from the AuNP (Figure 5a,

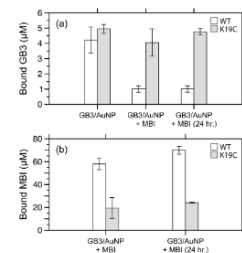


Figure 5. Competition of MBI with wild-type (WT) and K19C GB3. The concentration of GB3 (a) and MBI (b) bound to AuNPs was monitored after mixing GB3 with AuNPs and then adding MBI. Samples were prepared containing WT (no cysteines) and K19C GB3. Error bars represent the standard deviation from three independently prepared samples. MBI can effectively displace WT GB3, but the Cys residue in K19C prevents displacement. After 24 h, samples are essentially unchanged relative to initial preparation, suggesting that competition is relatively fast.

gray bars), and even after 24 h most of the protein remains bound to the AuNP surface.

The same effect can be observed by examining the MBI signal (Figure 5b). MBI can bind in the WT GB3 sample (white bars), but it cannot bind in the K19C sample (gray bars). Ostensibly, this is because K19C cannot be displaced from AuNPs. Therefore, in the absence of surface cysteine residues, the protein can associate and dissociate reversibly from the AuNP. However, a single surface cysteine is enough to make protein association effectively irreversible over time.

A Model for Protein–AuNP Adsorption. Together, our data support a three-step model for protein–AuNP adsorption. In the first step, proteins experience reversible association to the AuNP surface (Figure 6). Given that no chemical shift perturbations are observed in our experiments, the average lifetime on the AuNP surface is on the order of 10–100 ms (or longer) so that exchange is slow on the NMR time scale. Once on the surface, the protein remains fairly globular, if not native. It is likely that the structure of some proteins is perturbed more than others, as this would account for the different enzymatic activities observed.^{19,21} However, the overall shape is likely maintained; otherwise, the folded structure would not reliably predict the adsorption capacity. Similarly, the proteins are likely able to reorient on the AuNP to a small degree. This reorientation would ensure that a near-maximal number of proteins are able to bind. Finally, over time most proteins will become irreversibly attached to the AuNP. This hardening process will occur in proteins containing surface cysteine residues, since these residues can irreversibly oxidize on the AuNP surface, as demonstrated by the K19C GB3–MBI competition experiments. Our experiments with WT GB3 indicate that proteins can be displaced by organothiol, and they appear to resist irreversible hardening. Hardening could occur if it is mediated by amines or other moieties, and this is

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Problems With Excel

(these must be overcome)

- Bad font choice
 - No labels
 - Layout
 - Grey text (seriously, why?)
 - Extra “chart junk” like gridlines
 - Etc., etc., etc...
-
- **Let's try it!**

Excel Tips

- Save templates (right click on graph) to make changing dumb defaults easier
- Use Excel's own grid to help with alignment (hold down alt while dragging a graph)
 - Take a look at Page Layout → Align for more
 - Panel labels (A, B, C) can go in cells behind graphs
- Use the Print Area to control what is saved to a PDF
 - Photoshop and GIMP can read PDFs and convert to high-resolution TIF/PNG file
 - Illustrator/Inkscape can read PDFs as vector images

Summary

- Clear, professional images make it easier to communicate your science.
 - Make it easy for your audience to think you're smart!
- As a scientist, you will need to learn not only about science, but about publishing and graphics, too.
- PowerPoint and Excel can be useful tools in the publication repertoire, but some know-how is needed.
- Best skill is observation: What works? What doesn't?