

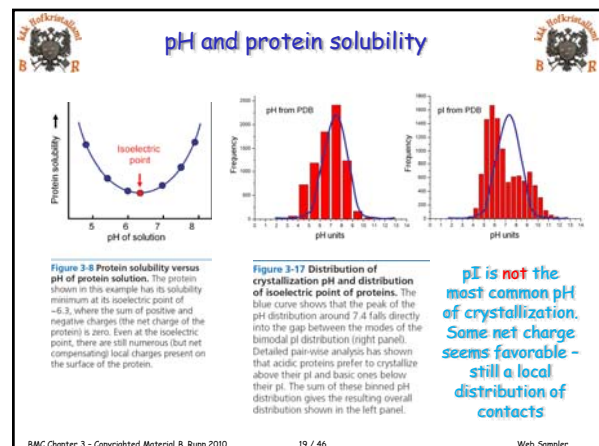
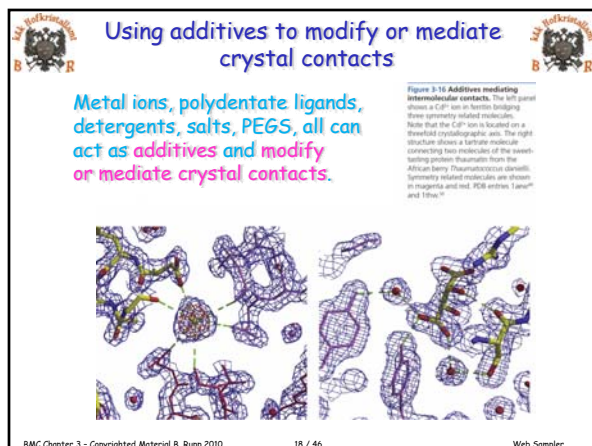
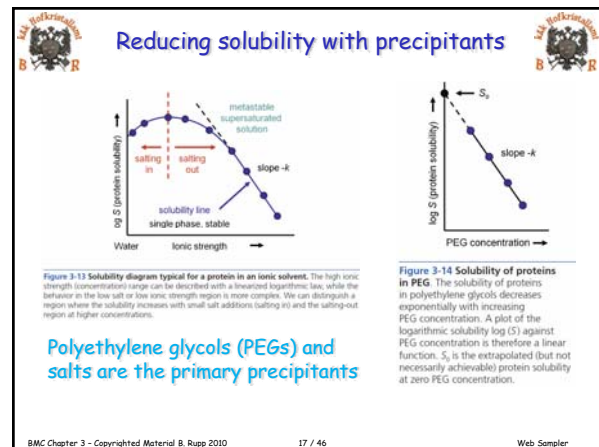
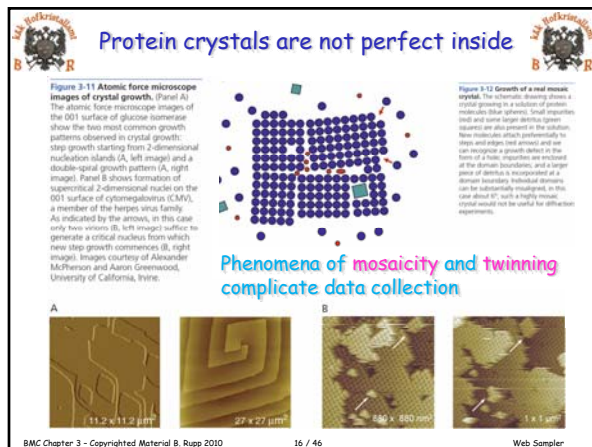
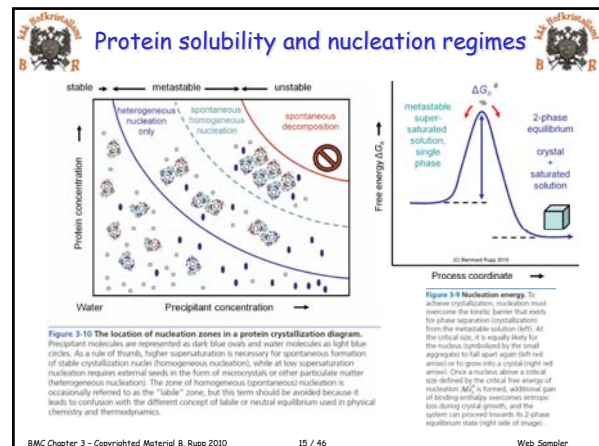
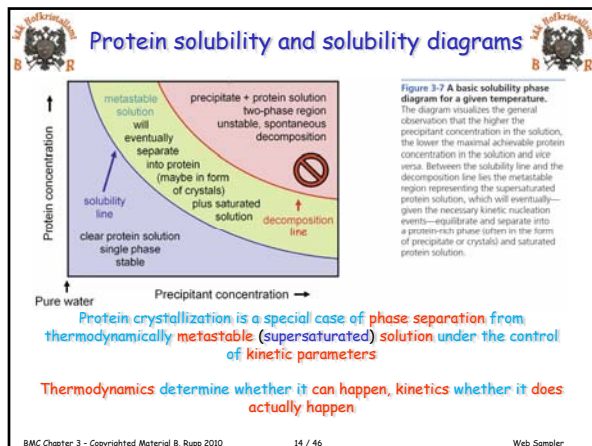


Figure 2-28 Logarithmic acid dissociation constant (pK_a) of charged amino acids. The figure provides a quick overview of the normal range of pK_a values for charged amino acid side chains and of amino- and carboxy-terminal groups. Unusual and extreme local environments can further shift these normal ranges to atypical values.

note the highly variable protonation state of His at physiological pH

change of pH shifts the surface charge distribution and thus the intermolecular interactions - pH is therefore a most significant factor for protein crystallization

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Summary of physical chemistry of crystallization

A) The solution must be in or move into a composition region where phase separation is thermodynamically possible.

Necessary requirement: supersaturation

B) The free energy of formation is driven by competition between entropic terms and not the enthalpic terms:

$$\Delta G_c = \Delta H_c - T(\Delta S_{\text{protein}} + \Delta S_{\text{solvent}})$$

Necessary requirement: Phase stability

c) The kinetics must allow that the thermodynamically stable state - hopefully a crystal - can be reached.

Necessary requirement: nucleation

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How to: crystallization techniques

How do different crystallization methods traverse the crystallization phase space?

Figure 3-19 Schematic sketches of popular crystallization techniques. Hanging-drop vapor diffusion is a common method used in small-scale manual setups while sitting-drop vapor diffusion is preferred with robotic setups. The absence of additional sealing requirements and the ease of miniaturization factors added microbatch screening under oil, although hanging tends to be more difficult. Use of silicone oils in microbatch wells allows partial exchange of solvent vapor (indicated by the broken arrow). Microdialysis is hard to miniaturize but can be used to grow very large crystals. Miniaturized free-interface diffusion screening chips are gaining popularity, but automation and harvesting issues remain to be resolved. Each method traverses the crystallization phase space in a different path and the same chemical screening conditions can produce widely varying results.

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Figure 3-20 Path of a successful vapor diffusion experiment through the crystallization diagram. The diagram on the left illustrates the principle of the hanging-drop setup. Equal amounts of protein solution (green) and precipitant (black) are mixed, forming a supersaturated solution (blue). As water vapor diffuses into the precipitant, the precipitant becomes saltier and both protein and precipitant concentrations in the drop increase. Once the region of spontaneous nucleation is reached, crystallization can occur. In this case, a crystal may start to grow. While the crystallization is in progress, the solution depletes in protein, and a few of the initial crystals continue to grow large in the growth region of the phase diagram. Once the crystals are in equilibrium with the saturated protein solution (d), they have reached their final size and will grow large. A phase of 24-well hanging-drop plate is shown in Figure 3-21.

Figure 3-21 Microtome of a successful vapor diffusion experiment. From the starting drop (a) the system moves deep into the supersaturation, and spontaneous nucleation of many microcrystals occurs. As much of the material is consumed in the formation of a shower of microcrystals, no significant crystal growth occurs and the very crystals containing all the protein material are in equilibrium with the saturated protein solution.

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