The Importance of Protein Charge Measurements[1]

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With the emergence of high concentration protein therapeutics, accurate protein charge measurements have become necessary. High concentration biologics are prone to unfavorable solution behaviors such as aggregation, phase separation and high viscosity. Protein charge measurements provide insight into the colloidal properties of macromolecules in solution, allowing for improved molecular characterization.

Protein charge is a fundamental property in biological systems that is directly linked to stability, solubility, and electrostatic interactions. [2-9] Charged amino acids are not only found at the active sites of proteins, enabling ligands, substrates and transition states to bind, but are found over the entire protein surface.[6] It is the influence of these charged residues on the physical properties of proteins that has renewed interest in charge measurements, especially with the emergence of high concentration protein formulations.[10, 11] The focus of this paper is on how protein charge can be used as a diagnostic to optimize formulations and reduce costs in the pharmaceutical development of therapeutics.

Intravenous administration remains the conventional drug delivery method for high concentration therapeutics that must be administered chronically and frequently (e.g. cancer therapies). However, delivering the same drugs subcutaneously is more appealing for at least two reasons:

a. Subcutaneous (SC) administration provides a convenient alternative to patients, allowing for in-home use of therapeutics, and
b. SC administration is less expensive in clinical, development and bulk storage costs.

Because SC administration is volume restricted (< 1.5 ml), biologics must be formulated at high concentrations (> 100 mg/ml) to be effective. [12, 13] At these concentrations the distance between molecules is on the same order of magnitude as the size of the molecules. When intermolecular distances become short (< 1 nm), the collision frequency increases (10^9-10^{10} collisions per second) as does the protein surface-area-to-volume ratio. [14, 15] Consequently, high concentration protein solutions are prone to aggregation, insolubility and high viscosity making drug delivery, manufacturability, efficacy and safety difficult. [14-16]

These unfavorable solution behaviors are due to the colloidal properties of proteins, which in turn are governed by proximity energies. Proximity

Type	X_1	X_2	n	Dependence[a]
Charge-charge	Q	Q	1	Γ
Charge-dipole	Q	μ	2	Γ, Θ
Dipole-dipole	μ	μ	3	Γ, Θ
Charge-induced dipole	Q		4	Θ
Hydrogen bond	E	E	4	Θ
Dipole-induced dipole	μ	α	5	Θ
Dispersion	α	α	6	Not D
van der Waals	-	-	12	Not D

Table 1. Electrostatic properties of proximity energies. n is the distance dependence of the proximity energy, where the potential energy, U is proportional to 1/rn . Most proximity energies are dependent on solvent conditions; [a]Γ = ionic strength dependent, Θ = orientation dependent, Not D = not dependent on dielectric constant of solvent.

energies are interactions, electrostatic in nature, that occur at the surface of molecules and become significant when the distance between molecules becomes short (0-1 nm). Table 1 displays the different proximity energies and their electrostatic properties. [14, 15]

All proximity energies follow the general formula:

$$U = \frac{X_1 X_2}{D r^n} \quad (1)$$

Where U is the potential energy (kJ/mol), X_1 and X_2 are the charge terms, D is the dielectric constant of the solvent, and r^n is the distance dependence of the proximity energy. A positive potential energy corresponds to repulsive interactions while a negative potential energy corresponds to attractive interactions. A van der Waals interaction is the only proximity energy that is always repulsive, becoming significant *only* when the surfaces of adjacent molecules are touching. Charge-charge interactions can be either repulsive (like signed) or attractive (oppositely charged). All remaining proximity energies result in attractive interactions. All interactions listed in Table 1 are distance dependent and weaken significantly with increased distance (> 1 nm). Thus, proximity energies must be considered when developing high concentration protein solutions as the intermolecular distances are typically between 0-1 nm. [14, 15]

Figure 1 is an example of the potential energy profile of two approaching molecules, each with a net charge of +10. The X-axis is the distance of approach between two molecules ranging from 0-1 nm, where 0 nm is the collision distance and 1 nm is the longest distance in which proximity energies are significant. It is also important to note that the proximity energy will be the sum of the contributions from the various potential energies at any point along the axis of approach. [15] The positive potential energy peak ($G^{\ddagger}$) at 0.12 nm is the activation energy barrier that the two approaching molecules have to surmount before protein association occurs (denoted by the attractive well at 0.05 nm). Charge-charge repulsion is the longest range proximity energy and if strong enough can keep macromolecules apart, maintain protein solubility and overcome the short-range attractive energies that lead to unfavorable solution behaviors. [14-16] Proteins with sufficient charge (e.g. for mAbs, z_{DHH} > 15) will repel each other at a distance of 2-3 Å. They are much less likely to form aggregates and gels and more likely (than proteins with a lower net charge) to remain in solution. [15]

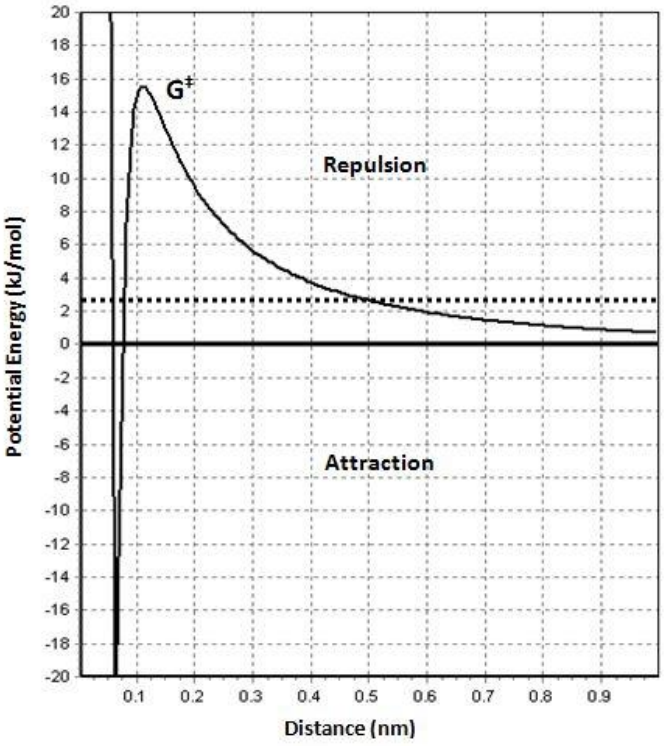

Figure 1. Potential energy profile of two approaching molecules, each with a net charge of 10. $G^{\ddagger}$ is the activation energy (16 kJ/mol) at 0.12 nm that must be surmounted for protein association to occur. The horizontal bold line shows the reference energy of pure solvent. The horizontal dotted line represents the thermal background energy, 2.5 kJ/mol (RT).

Charge measurements can be used as a diagnostic to help identify good candidate molecules from poor candidates early in the drug formulation and development process. This can be done by advancing biologics with a significant net charge (> 15) through the development process while eliminating those with little to zero net charge. Using charge measurements in this manner can help pharmaceutical companies reduce development, clinical, and storage costs significantly by focusing resources on only good-candidate molecules.

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