# Analysis of dextran hydrodynamic volume using the Fluidity One-W

Fina Biosolutions provides amino-modified dextrans in a wide range of relatively well-defined molecular weights and amine ratios. The hydrodynamic radius (Rh) of dextran polymers in solution is typically determined using dynamic light scattering. An alternative method is to use the Fluidity One-W instrument from Fluidic Analytics.

# Analysis of dextran hydrodynamic volume using the Fluidity One-W

Dextran, a linear hydrophilic polymer of glucose of predominantly α–1,6 glycosidic linkages, is used in a wide variety of applications, including plasma extenders, molecular weight tracers, and surface passivation agents, as well as for the modification of surfaces, beads and molecules. Dextran polymers are sometimes modified to improve functionality.

The Fluidity One-W measures the hydrodynamic radius of molecules once they are fluorescently labeled. To measure the unmodified and amino-modified dextrans, these polymers were labeled with a single fluorescent probe (Alexa Fluor 488) at the reducing end. The hydrodynamic radius of the unmodified dextrans and the amino-modified dextrans of MW 1.5 – 500 kDa with varying amino ratios (or charge density) were evaluated using the Fluidity One-W. The measured hydrodynamic radius values were then compared with the calculated radius for folded globular proteins or “unfolded” dextran polymer.

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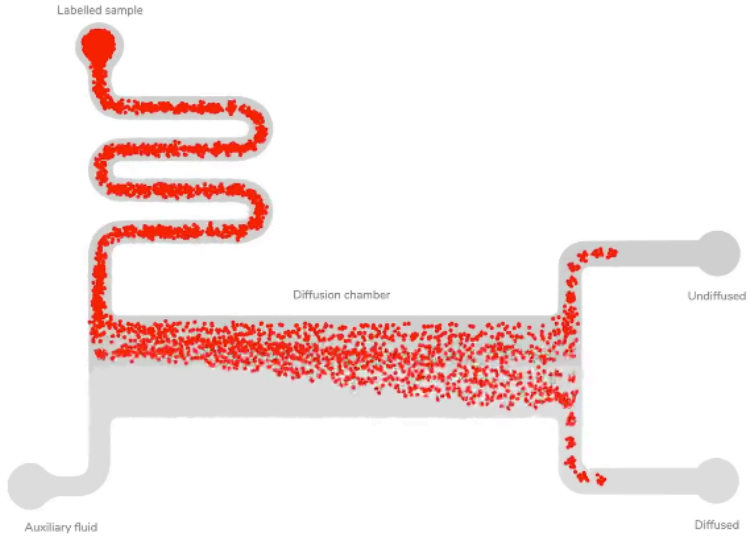
## HOW DOES IT WORK?

# Fluidity One-W

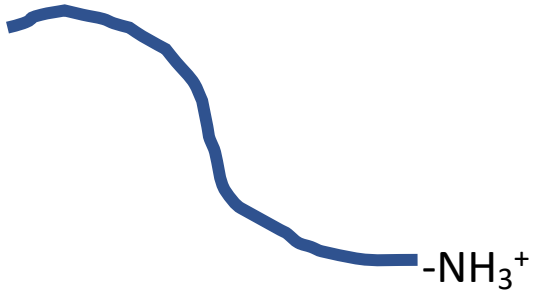
The Fluidity One-W measures the rate of diffusion of proteins under steady state laminar flow in a microfluidic chip — a technique known as microfluidic diffusional sizing (MDS).

1. To do this, a stream of fluorescently labelled protein is introduced alongside an auxiliary stream.
2. These streams flow in parallel and because there is no convective mixing the only way protein can migrate into the auxiliary stream is by diffusion, the rate of which depends on the size of the protein. Small proteins will diffuse rapidly, and large proteins and aggregates more slowly.
3. At the end, the streams are re-split, and at this point the degree of diffusion is fixed. The quantity of protein in each stream is then determined by the fluorescence from the label. The ratio of the fluorescence between the two streams gives the protein's hydrodynamic radius ($R_h$).

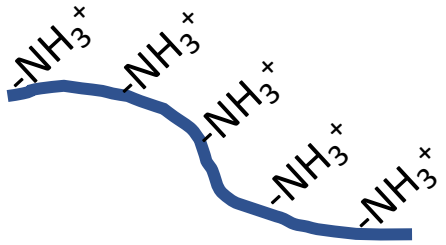

How does the Fluidity One-W work?

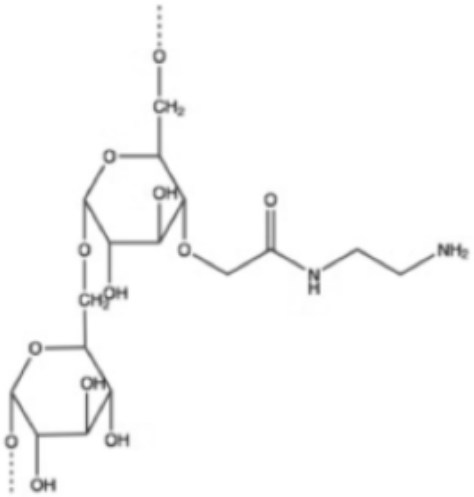


# Amino-Modified Dextrans




Monoamine dextran



Amino dextran

A single amino group is installed on the reducing end of the dextran polymer. For analysis with Fluidics One-W, the amine is labeled with a fluorescent dye. The terminal dye has a negative charge, but the rest of the polymer remains uncharged.

Amino dextran is made by converting a limited number of glucose hydroxyls to (2-aminoethylcarbamoyl)methoxy groups (right). For analysis with Fluidics One-W, a limited number of amines are labeled with a fluorescent dye. The polymer remains positively charged (cationic).

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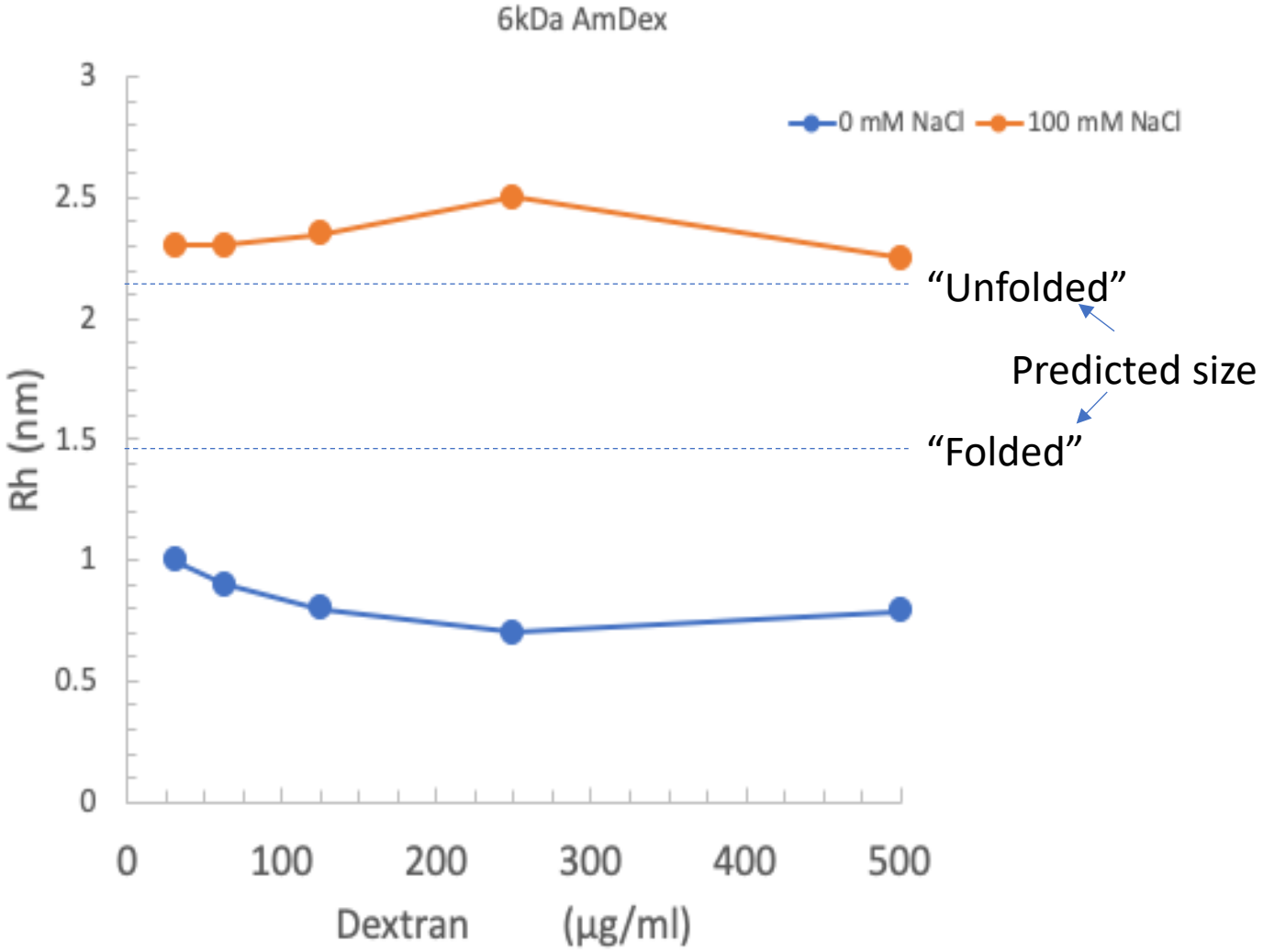
# The hydrodynamic volume of cationic dextran is dependent on ionic strength

The importance of ionic strength was first determined. A cationic 6 kDa dextran with an average of 5 amines per polymer (AD6x5) was run on the Fluidity One-W at 20-500µg/ml in the absence or presence of 100 mM NaCl. The measured radii of the dextran in the presence of salt was very close to the expected values for unfolded, fully hydrated polymer chains, while in the absence of salt much smaller sizes were determined. The size was not heavily dependent on concentration.

The observed apparent size reduction in the absence of salt is attributed to an absence of charge screening between dextran molecules. This leads to intermolecular repulsive forces which act in addition to passive diffusion, leading molecules to distribute more rapidly than would be expected based solely on size. When measured on the Fluidity One-W this is seen as a reduction in the apparent hydrodynamic radius. To prevent this, all subsequent samples were run in the presence of 100 mM NaCl.



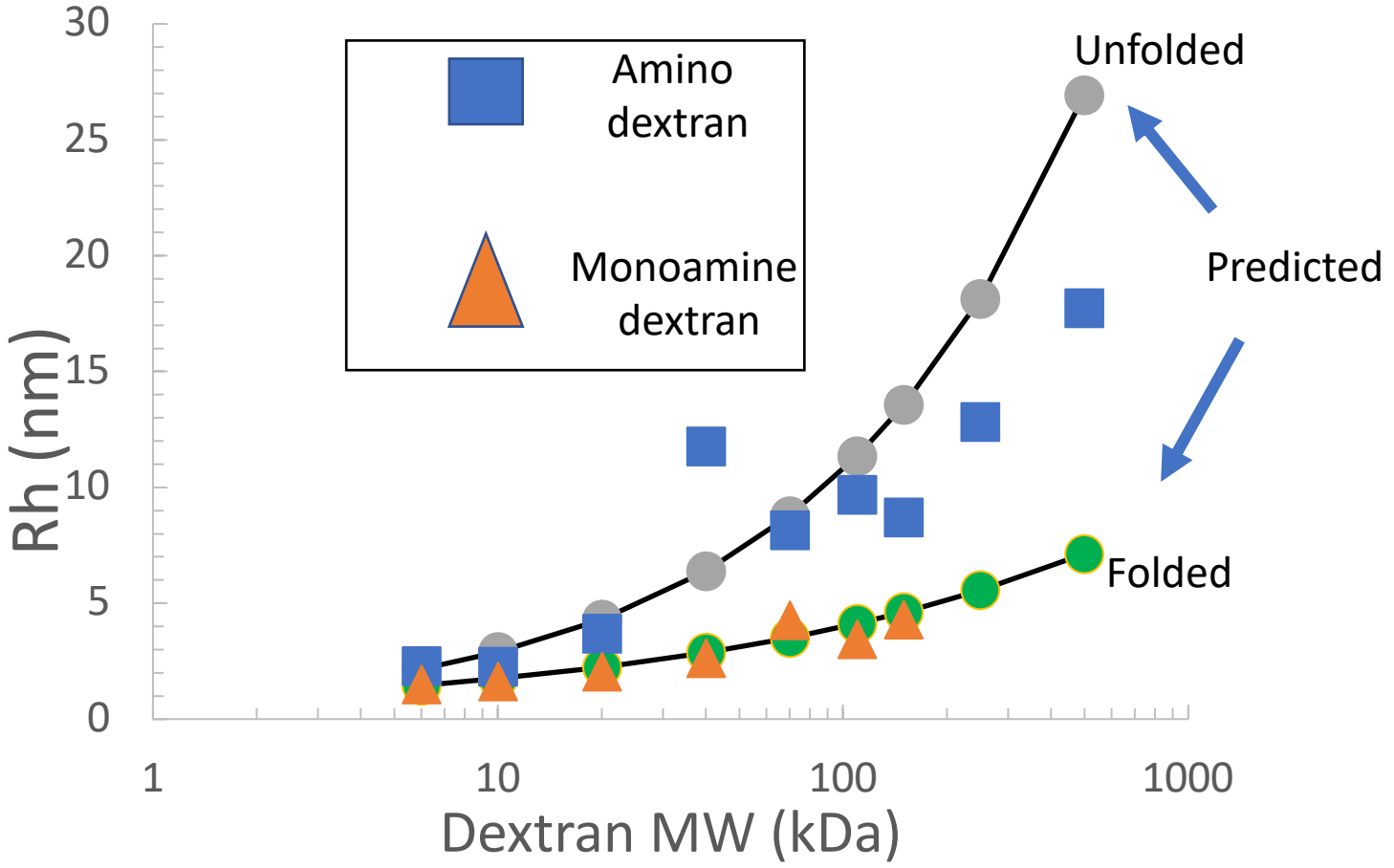

Thus, the lower Rh in the absence of salt is an artifact of the measurement technique.

# Unmodified dextran has a radius of a globular ball. Increasing cationic charge causes the polymer to “unfold” due to charge repulsion

Dextran with a single terminal amine has the same radius as is predicted for a folded, globular protein.

In contrast, high ratio amino dextran more closely tracked the size predicted for “unfolded’ dextran. The cationic dextrans had one amine per 5-10 glucose units.

This difference between the molecules can be attributed to intramolecular electrostatic repulsion in the poly-amino dextrans forcing the polymers into an open, extended conformation.

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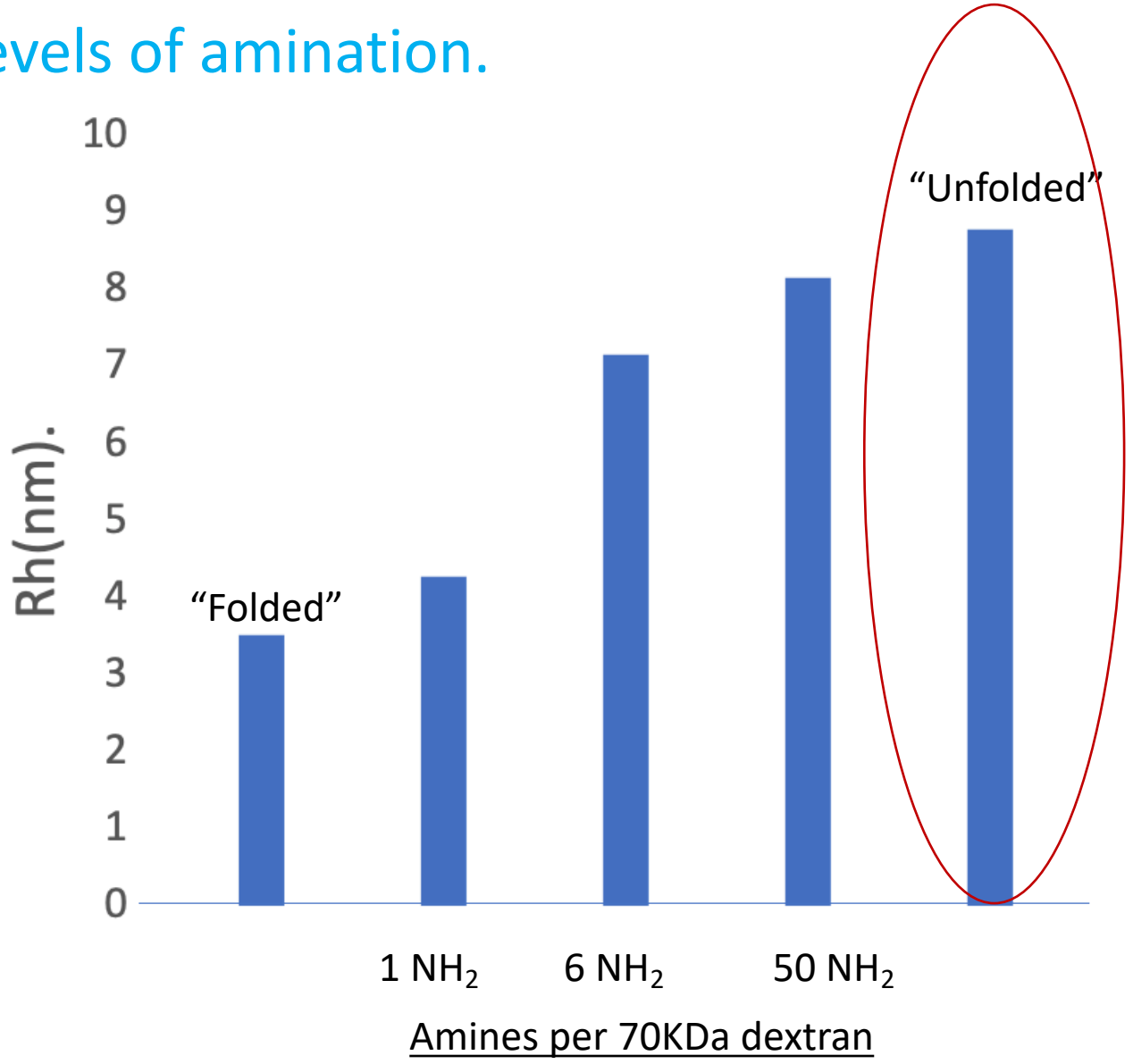
# The dextran hydrodynamic radius increases with increasing levels of amination.

The effect of charge repulsion on the hydrodynamic radius can be seen with Rh increases with charge density. Charge repulsion causes the hydrodynamic radius to increase. With increasing charge, the dextran progressively unfolded. Even a low level of amination causes the polymer to unfold.

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# Conclusions

The Fluidity One-W instrument determines the hydrodynamic radius for the the average size of species in solution. As it uses fluorescence rather than light scattering, the average is mass-based without a bias towards larger species, which occurs when light scattering methods are used.

Electrostatic repulsion is an important consideration in solution behaviour of molecules. The absence of salt can lead to intermolecular forces while high charge densities induce intramolecular forces. For the amino dextrans studied here the forces are consistently repulsive due to the polymer bearing only positive charges.

**Fina Biosolutions** provides amino dextrans in a wide range of molecular weights and amine ratios.
FinaBio also manufactures other modified dextrans for research and diagnostic use. Learn more at **FinaBio.com**

**Fluidic Analytics** is a biotech company originating from the University of Cambridge, established in 2013, and headquartered in Cambridge, UK. Fluidic Analytics provides a unique technology, Microfluidic Diffusional Sizing (MDS), which enables the rapid characterization of proteins based on the physical properties that determine their function. Specifically, MDS allows characterization of size, concentration, stoichiometry, as well as binding affinity of any protein interaction - even in complex backgrounds, even with challenging targets. And because proteins are characterized in solution and in their natural state – without the need for surfaces, matrices, or ionization – this platform gives our customers access to unique quantitative insights into protein behaviour that are not accessible using other approaches. Learn more at **Fluidic.com/**