The molecular weight of $NaYF_4$:RE photonic up-conversion nanoparticles

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Abstract

Luminescence up-conversion nanoparticles (UCNPs) consisting of a $NaYF_4$ crystal lattice doped with rare earth (RE) ions have found widespread application in bio-sensing, bio-imaging, and therapeutics; yet the molecular weight of UCNPs is not known. Lack of knowledge of molecular weight of UCNPs results in sub-optimal functionalisation and dosages of UCNPs. We present a simple method for calculating the molecular weight of $NaYF_4$:RE UCNPs from arbitrary crystal lattice parameters and UCNP diameter measurements, and we apply this method to estimate the molecular weight of various $NaYF_4$:RE UCNPs from the literature. UCNP molecular weight scales exponentially with UCNP volume (i.e. diameter cubed). UCNPs of 10 nm diameter are estimated to be a molecular weight of ~ 1 MDa, and 45 nm UCNPs are estimated to be ~100 MDa. Hexagonal lattice UCNPs were found to have a greater molecular weight than their cubic lattice UCNP counterparts. A Gaussian distribution of nanoparticle diameters was found to produce a lognormal distribution of nanoparticle molecular weights. We provide stand-alone graphic user interfaces to calculate UCNP:RE molecular weight. This approach can be generalised to estimate the molecular weight of crystalline nanoparticles of arbitrary size, geometry, and elemental composition where nanoparticle unit cell parameters are known.

1. Introduction

Up-converting nanoparticles (UCNPs) consisting of a low phonon-energy crystal lattice host (typically $NaYF_4$) co-doped with rare earth (RE) ions (typically Yb^{3+} with Er^{3+} and/or Gd^{3+}) have recently generated widespread scientific interest as optical platforms for numerous applications, including: imaging contrast labels in cells and animals *in vivo* and *ex vivo*[1–10]; the detection of antibiotics[11] or toxins in food[12–14]; aggressive targeting of cancer cells[15,16]; imaging thermometry[17,18]; enhancement of photovoltaic technology,[19,20] and the measurement of biomarker molecules in biological fluids.[21–26]

$NaYF_4$:RE UCNPs have garnered intense interest due to their unique optical luminescence excitation and emission properties, which are highly advantageous for bio-sensing and bio-imaging applications. $NaYF_4$:RE UCNPs absorb multiple near infra-red (NIR) photons (typically ~ 980 nm) and emit luminesce at visible wavelengths via photonic up-conversion.[27] NIR excitation is highly advantageous for biomedical applications because: (1) NIR light can penetrate several centimetres in tissue[28]; (2) NIR light is not phototoxic, and (3) NIR excitation results in no auto-fluorescence from tissue or endogenous proteins. The wavelength and intensity of UCNP luminescence emission can be tuned by altering UCNP composition,[29] the concentrations of RE dopants,[30] altering the UCNP crystal lattice phase,[5,29] and altering nanoparticle surface area to volume ratio.[31] It has become common practice to add protective inert outer shells - typically made of silica - to shield UCNPs from quenching effects by solution and enhance luminescence emission intensity.[32,33] Thus, the optical properties of UCNPs can be tailored to suit individual applications. The surface of $NaYF_4$:RE UCNPs are typically functionalised with antibodies[22,24–26] or oligonucleotides[34] to enable specific binding for labelling, bio-sensing, and therapeutic applications. Close proximity of molecules (e.g. a target analyte protein) to the surface of UCNPs results in luminescence energy resonance transfer (LRET) between the UCNP (donor) and a proximal molecule (acceptor), resulting in altered UCNP luminescence emission. The resulting change in luminescence emission depends on the particular physical configuration and optical properties of the UCNP and acceptor molecule.[2,24–26,35,36]

Despite the widespread applications of $NaYF_4$:RE UCNPs, the molecular weight of these nanoparticles has not been reported. Consequently, the concentration of UCNPs is typically reported in terms of UCNP weight per volume of aqueous media.[37] This relatively crude measure does not allow the number of UCNPs in a given sample to be known and consequently may result in sub-optimal functionalisation of UCNPs or inaccurate estimation of UCNP dosage for bio-imaging and therapeutic applications. Further, knowing the molecular weight of UCNPs would be helpful in

understanding UCNP behaviour when trapped with optical tweezers[38] and understanding the uptake of UCNPs by cell membranes.[3,5,39]

This study presents the theory required to calculate the molecular weight of $NaYF_4$:RE UCNPs of arbitrary size and RE dopant composition, assuming that crystal lattice parameters are known. The molecular weight of $NaYF_4$:RE UCNPs can be calculated from first principles if the lattice parameters and elemental composition of $NaYF_4$ crystal unit cells are known from X-ray diffraction (XRD) experiments; and if the geometry and diameter of UCNPs are known from transmission electron microscopy (TEM) experiments.[5,6,40] The molecular weight of a UCNP can be estimated by simply dividing the volume of a UCNP by the volume of an individual unit cell to get the number of unit cells in a UCNP. From this, the total molecular weight of a UCNP is calculated summing the atomic weight of all atoms within a single unit cell, and multiplying by the total number of unit cells within the UCNP. From this theory, we estimate the molecular weight of various $NaYF_4$:RE UCNPs reported in the literature. Of particular note, we show that a Gaussian distribution of $NaYF_4$:RE UCNP diameters will result in a lognormal (i.e. non-normal) distribution of $NaYF_4$:RE UCNP molecular weights.

Additionally, we provide two stand-alone graphical user interfaces (GUIs) to enable others to easily calculate the molecular weight of $NaYF_4$:RE UCNPs with arbitrary size, lattice, and RE dopant parameters.

2. Theory

2.1 Structure and photonic up-conversion of $NaYF_4$:RE UCNPs

In $NaYF_4$:RE UCNPs, photonic up-conversion is enabled by the sequential absorption of two or more near-infrared photons, which, via excitation of several long-lived metastable electron states, and subsequent non-radiative multi-phonon and radiative relaxation, produces luminescence emission at visible wavelengths. Up-conversion requires a crystalline host lattice, which is doped with multiple different lanthanide ions (typically Yb^{3+} and Er^{3+}), where one lanthanide ion (typically Yb^{3+}) acts as a photo-sensitizer and the other (typically Er^{3+}) emits photons.[33] The up-conversion process is sensitive to the distance between ions and unit cell geometry. Although many different combinations of lattice and RE dopants have been explored,[41] the combination of Yb^{3+} and Er^{3+} ions in a $NaYF_4$ host lattice has been found to provide high up-conversion efficiency, and as such is commonly used for UCNPs.[27,42]

$NaYF_4$:RE unit cells are typically a cubic crystal lattice arrangement or a hexagonal crystal lattice arrangement. The arrangement of unit cells influences the crystal lattice parameters, consequently changing photonic properties and density of $NaYF_4$:RE UCNPs. Thus, to calculate the molecular

weight of a $NaYF_4$:RE UCNP for a given size, the atomic weight of a single $NaYF_4$:RE UCNP unit cell must be determined.

2.2. $NaYF_4$:RE unit cells reported in the literature

Several studies have reported crystal lattice parameters for cubic and hexagonal $NaYF_4$:RE UCNPs obtained via x-ray diffraction studies (see Table 1). Wang et al., (2010)[40] report unit cell parameters for cubic (α phase) and hexagonal (β phase) unit $NaYF_4$:RE unit cell configurations (see Figure 1). In the face-centred cubic lattice arrangement, high-symmetry cation sites are formed, which are randomly occupied by either Na or RE ions (see Figure 1a). Cubic unit cells follow the formula $Na_2Y_2F_8$, with Y ions substituted for other RE ions depending on doping parameters. Hexagonal unit cells follow the formula $Na_{1.5}Y_{1.5}F_6$.[40] In the hexagonal lattice arrangement, there are two low-cation symmetry sites, which contain either Na or RE ions (see Figure 1b).

2.3. $NaYF_4$:RE size distributions

Synthesising $NaYF_4$:RE UCNPs typically creates spherical UCNPs with a range of diameters. For example, Sikora et al., (2013) report a range of UCNP Gaussian distribution of diameters ranging between 15 – 70 nm for a single batch of $NaYF_4$:Er,Yb UCNPs,[5] and Haro-González et al. (2013)[38] report $NaYF_4$:Er,Yb (2% Er, 18% Yb) UCNPs with diameters ranging between ~10 – 50 nm, following a Gaussian distribution.

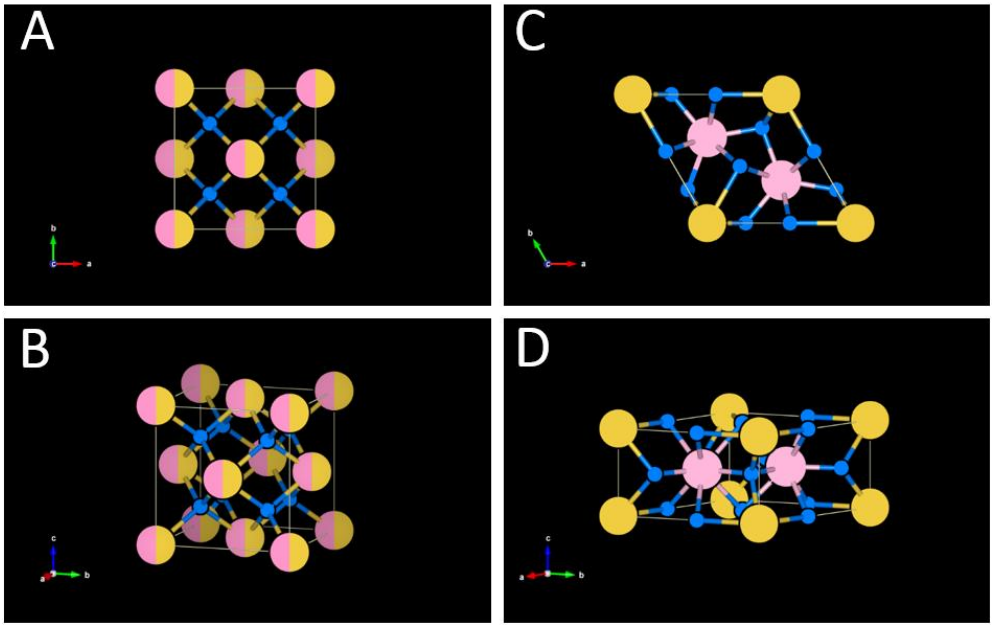

Figure 1. Unit cell structures of $NaYF_4$:RE UCNPs. Colour key: Na ions are yellow; Y and RE dopant ions are pink; and F ions are blue. **(A and B)** Cubic lattice unit cell; Na/Y/RE ions are distributed randomly between the cation sites that are depicted as both pink and yellow. **(C and D)** Hexagonal lattice unit cell. Figures based upon data from Kramer et al., (2004)[30] and Wang et al., (2010).[40] Diagrams created with VESTA (open-source software).[43]

Table 1. Crystal lattice parameters of $NaYF_4$:RE UCNPs reported in the literature.

Study	$NaYF_4$ RE dopant composition	UCNP lattice structure	a (Å)	c (Å)	Mean UCNP diameter (nm)	UCNP diameter range (nm)
Sikora et al., (2013).[5]	30% Yb, 2% Er	Cubic	5.51	-	~ 30	15 -70
Cao et al., 2010.[6]	20% Yb, 2% Er	Hexagonal	5.960	3.510	33 ± 1 nm	32 - 34 nm
Wang et al., 2010.[40]	18% Yb , 2% Er	Hexagonal	5.96	3.53	Not reported	Not reported
Wang et al., 2010.[40]	18% Yb, 2% Er, 60% Gd,	Hexagonal	6.02	3.60	Not reported	Not reported

2.4. Number of $NaYF_4$ unit cells in a UCNP

The volume of a spherical nanoparticle (V_{UCNP}) is given by:

$$V_{UCNP} = \frac{4}{3}\,\pi\, r^3, \tag{1}$$

where r is the radius of the UCNP. NB: Equation 1 can be changed to calculate arbitrary non-spherical nanoparticle geometries if desired. If the cubic lattice parameter a_c is known, then the volume of a cubic unit cell (uV_{cubic}) is calculated by:

$$uV_{cubic} = a_c^3. \tag{2}$$

If the hexagonal lattice parameters a_h and c_h are known, then the volume of a hexagonal unit cell $(uV_{hexagonal})$is calculated by:

$$uV_{hexagonal} = \frac{2\sqrt{3}}{4}\, a_h^2 c_h. \tag{3}$$

The number of unit cells in a given UCNP ($uN_{cubic}\ or\ uN_{hexagonal}$) can then be calculated by:

$$uN_{cubic} = V_{UCNP}/uV_{cubic}, \tag{4}$$

$$uN_{hexagonal} = V_{UCNP}/uV_{hexagonal}. \tag{5}$$

2.5. Atomic weight of $NaYF_4$ unit cell

Assuming no RE dopants, the atomic weight of a single cubic $NaYF_4$ (uAW_{cubic}) or hexagonal $NaYF_4$ unit cell (uW_{hex}) are described by:

$$uAW_{cubic} = (2 \times AW_{Na}) + (2 \times AW_Y) + (8 \times AW_F), \tag{6}$$

$$uAW_{hex} = (1.5 \times AW_{Na}) + (1.5 \times AW_Y) + (6 \times AW_F). \tag{7}$$

Where AW_{Na}, AW_Y, and AW_F are the atomic weight of Sodium, Yttrium, and Fluorine respectively (see Table 2). If RE dopant ions are added, a fraction of Y^{3+} ions are substituted for RE^{3+} dopant ions, and thus the average atomic mass of unit cells within an UCNP is altered. Thus, RE doping can be accounted for by defining an additive-factor (af):

$$af = fRE_{d1} + fRE_{d2} \ldots . + fRE_{dn}, \tag{8}$$

where fRE_{d1} to fRE_{dn} is the fractional percentage of up to n RE dopants. The additive factor is a numeric value between 0 and 1, representing 0% and 100% Y substitution, respectively. The atomic mass of a single cubic or hexagonal unit cell whilst accounting for RE dopants can thus be calculated by:

$$uAW_{cubic}\, RE_{Doped} = (2 \times AW_{Na}) + (8 \times AW_F) + (2(1 - af) \times AW_Y) + \left(2 \times fRE_{d1} \times AW_{RE_{d1}}\right) + \left(2 \times fRE_{d2} \times AW_{RE_{d2}}\right) + \cdots + \left(2 \times fRE_{dn} \times AW_{RE_{dn}}\right), \tag{9}$$

$$uAW_{hexagonal}RE_{Doped} = (1.5 \times AW_{Na}) + (6 \times AW_F) + (1.5(1-af) \times W) + \left(1.5 \times fRE_{d1} \times AW_{RE_{d1}}\right) + \left(1.5 \times fRE_{d2} \times AW_{RE_{d2}}\right) + \cdots + \left(1.5 \times fRE_{dn} \times AW_{RE_{dn}}\right). \quad (10)$$

Where $uAW_{cubic}\,RE_{Doped}$ and $uAW_{hexagonal}RE_{Doped}$ are the average atomic weight of RE doped cubic and hexagonal unit cells, respectively.

Table 2: Standard atomic weight of elements typically utilised in $NaYF_4$:RE UCNPs. Data from Meija et al., (2013).[44]

Element	Standard atomic weight
Na	22.989
F	18.998
Y	88.905
Yb	173.054
Er	167.259
Gd	157.25

2.6. Molecular weight of a $NaYF_4$ UCNP

Assuming that unit cells are distributed uniformly throughout the UCNP, the molecular weight of a cubic lattice UCNP ($MWcubic$) is calculated from Equations 4 and 9 as:

$$MW_{cubic} = uAW_{cubic}\,RE_{Doped} \times uN_{cubic}. \quad (11)$$

From Equations 5, the molecular weight of a hexagonal lattice UCNP ($MW_{hexagonal}$) is calculated by:

$$MW_{hexagonal} = uAW_{hexagonal}\,RE_{Doped} \times uN_{hexagonal}. \quad (12)$$

From Equations *4*, *5*, *11*, and *12*, it can be seen that the molecular weight of UCNPs scales with UCNP volume: i.e. diameter cubed.

3. Methods

3.1. Molecular weight of cubic and hexagonal $NaYF_4$ UCNPs

Using the theory presented in Sections 2.4 – 2.6, the molecular weight of $NaYF_4$ hexagonal and cubic lattice UCNPs were calculated, assuming the following generic lattice parameters: cubic lattice parameter a = 5.51 Å; hexagonal lattice parameters a = 5.91 Å, c = 3.53 Å.

3.2. The effect of RE doping

The effect of RE doping was investigated by using the theory presented in Sections 2.4 – 2.6 to calculate the molecular weight of $NaYF_4$:RE UCNPs with various concentrations of Yb^{3+} and Er^{3+} dopant ions, up to 30 M% of total RE ions.

3.3 Molecular weight of $NaYF_4$:RE UCNPs from the literature

The molecular weight of $NaYF_4$:RE UCNPs reported in the literature was calculated by incorporating various lattice parameters and dopant M% from the literature into the theory presented in Sections 2.4 – 2.6.

3.4. UCNP diameter distribution vs. molecular weight distribution

The size distribution of a single batch of $NaYF_4$:YbEr UCNPs was reproduced from data presented in Sikora et al.[5] A Gaussian fit was applied to this size distribution data by using non-linear least squares fitting in MATLAB (MATLAB 2016a, MathWorks) and the corresponding molecular weight distribution was calculated by the theory presented in Sections 2.4 – 2.6.

3.5. Stand-alone GUIs for calculation of nanoparticle molecular weight

Two graphic user interfaces (GUIs) were written in MATLAB, each incorporating different features. The first simple GUI was developed to enable other researchers to calculate the molecular weight of spherical $NaYF_4$:RE UCNPs for a user-defined nanoparticle size range. The second, more powerful, GUI was designed to enable users to estimate the molecular weight of nanoparticles with arbitrary nanoparticle geometry; arbitrary lattice parameters; and arbitrary elemental composition, across a user-defined range of characteristic nanoparticle sizes. Additional technical information for both GUIs is provided in the supplementary material section. The stand-alone GUIs developed are shown in supplemental Figures S1 and S2.

4. Results

4.1. Molecular weight of cubic and hexagonal $NaYF_4$:RE UCNPs

As expected, the molecular weight of UCNPs scaled proportionally to the volume of the UCNPs, resulting in a large increase of UCNP molecular weight for a comparatively small increase in UCNP diameter. Hexagonal lattice UCNPs were found to have a considerably greater molecular weight than cubic lattice UCNPs due to the lower volume of hexagonal unit cells (see Figure 2).

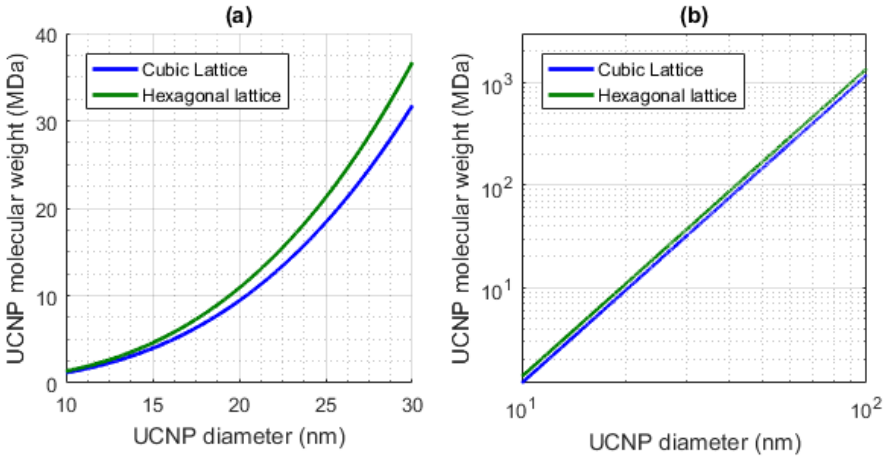

Figure 2. UCNP diameter vs. UCNP molecular weight for hexagonal and cubic $NaYF_4$ UCNPs. Lattice parameters are assumed to be: a = 5.51 Å for cubic UCNPs; a, c = 5.91 Å and 3.53 Å for hexagonal UCNPs. **(a)** The exponential increase of molecular weight with diameter is apparent. **(b)** The same data on a logarithmic scale.

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4.2. The effect of RE doping

The effect of RE ion doping on molecular weight of UCNPs was investigated by varying the percentage of RE dopants. Increasing Yb^{3+} or Er^{3+} was found to increase the molecular weight of UCNPs (see Figure 3). The increased UCNP mass is because Yb^{3+} and Er^{3+} have a greater atomic mass than Y^{3+}. The difference in molecular weight of UCNPs doped with Yb^{3+} and Er^{3+} was relatively small to the small difference between the atomic weight of $Yb^{3+}$and Er^{3+} (see Table 2). Hexagonal lattice UCNPs show a slightly higher increase in molecular weight for a given dopant concentration than cubic lattice UCNPs due to the smaller unit cell volume of hexagonal lattices compared to cubic lattices.

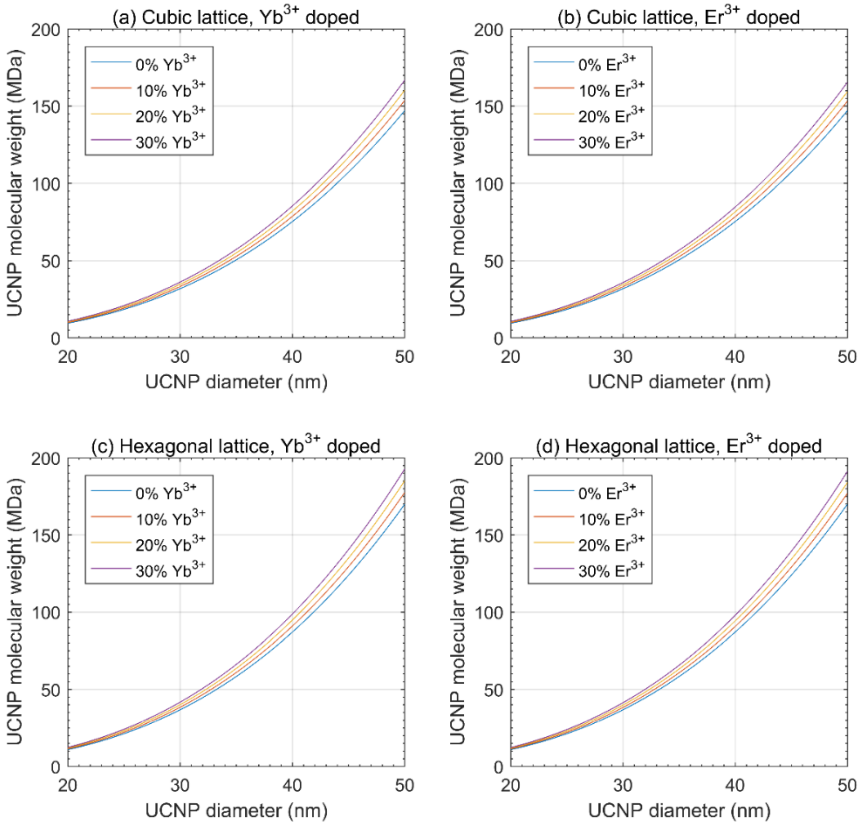

Figure 3. The effect of RE doping on UCNP molecular weight. (a, b) The molecular weight of cubic lattice $NaYF_4$:RE UCNPs (a = 5.51 Å) for increasing dopant percentage of Yb^{3+} and Er^{3+}. **(c - d)** The molecular weight of hexagonal lattice $NaYF_4$ UCNPs (a = 5.91 Å; c = 3.53 Å) for increasing dopant percentage of Yb^{3+} and Er^{3+}. NB: calculations assume that lattice parameters remain constant.

4.3. Molecular weight of $NaYF_4$:RE UCNPs reported in the literature

220 The estimated molecular weight of various $NaYF_4$:RE UCNPs calculated from values reported in the literature are shown in Figure 4.

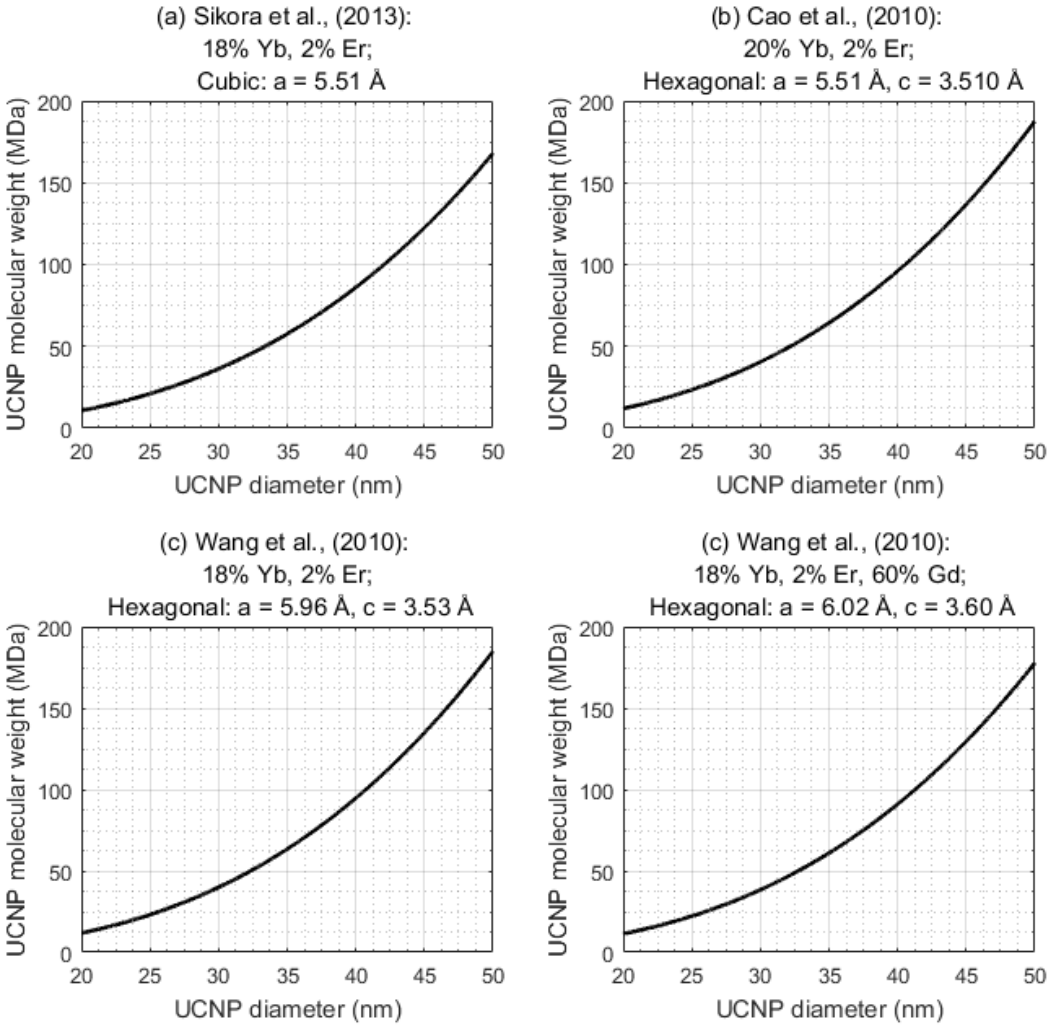

Figure 4. Estimated molecular weight of various UCNPs reported in the literature. **(a)** UCNPs from Sikora et al., (2013).[5] **(b)** UCNPs from Cao et al., (2010).[6] **(c - d)** UCNPs from Wang et al., (2010).[40]

4.4. UCNP diameter distribution vs. molecular weight distribution

UCNP diameter distribution data was reproduced from Sikora et al., (2013) (see Figure 5a) and was well-fitted by a Gaussian distribution (R^2 = 0.96). The corresponding molecular weight distribution was then calculated for each UCNP diameter. The resultant molecular weight is shown in Figure 5b. A Gaussian fit to the molecular weight distribution plotted on a logarithmic scale produces an excellent fit (R^2 = 0.98), establishing that a Gaussian diameter distribution of nanoparticles follows lognormal distribution of molecular weight (see Figure 5c).

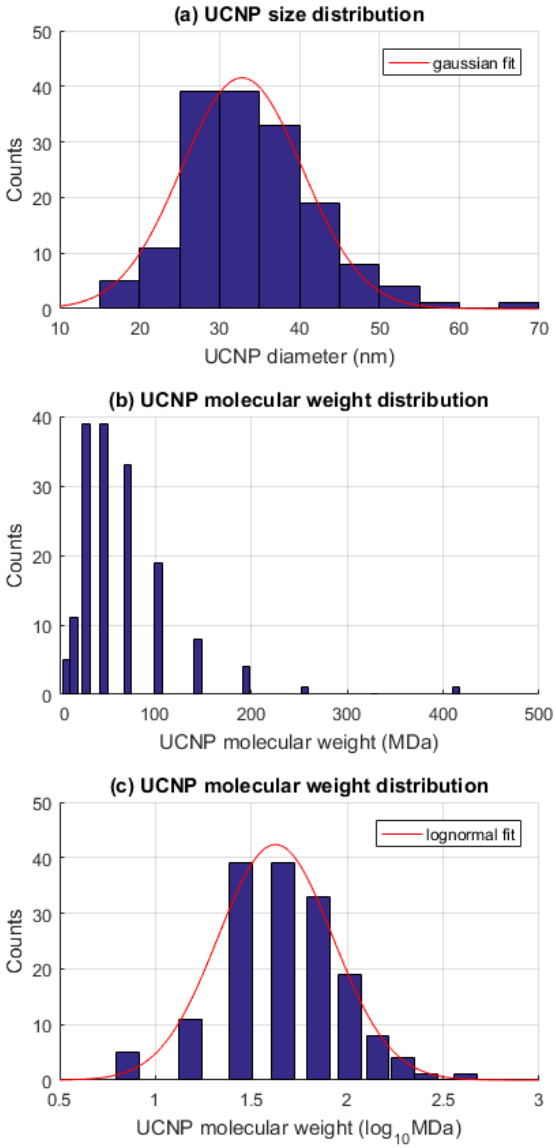

Figure 5. Gaussian size distributions give arise to lognormal molecular weight distributions. (a) Gaussian size distribution of cubic $NaYF_4$ UCNPs (data as reported by Sikora et al., (2013)[5]) is well described by a normal distribution (R^2 = 0.96). **(b)** The corresponding molecular weight distribution of UCNPs on a linear molecular weight scale and **(c)** on a lognormal scale. The molecular weight distribution in **(c)** is well fitted by a log normal distribution (R^2 = 0.98).

Discussion

Our method to estimate the molecular weight of $NaYF_4$:RE UCNPs relies on two basic assumptions: 1. that UCNPs are of homogenous composition, and 2. that the lattice parameters are accurate. The first assumption can be tested by TEM and XRD, and the second assumption can be experimentally verified via XRD. Ensuring accurate lattice parameters is particularly important when estimating the molecular weight of UCNPs with arbitrarily large dopant concentrations. For example, Wang et al., (2010)[40] experimentally demonstrated that by doping a hexagonal phase $NaYF_4$:Yb,Er UCNP (18% Yb, 2% Er) with increasing concentrations of Gd^{3+} increases the lattice parameters of the UCNP significantly, resulting in an increased unit cell volume. Thus, because of this dependence of lattice parameter on RE dopant percentage, our estimations of UCNP molecular weight in Figure 3 may be an over-estimation on true values if lattice parameters are not independently verified for each RE dopant concentration of interest. We assumed a spherical geometry here, but our method could be trivially adapted for arbitrary nanoparticle geometries; e.g. rods,[40,45] triangular,[46] or prism-shaped[47] nanoparticles. The extension of our technique to arbitrary geometries, arbitrary crystal lattice parameters, and arbitrary elemental composition is demonstrated by the development and application of an advanced GUI incorporating all of these variables (see Figure S2).Further, our method does not account for any surface functionalisation or enhancement. Thus the molecular weight of UCNPs modified by addition of a silica[24,32,33] or calcium fluoride[48] shell coating will be somewhat different from the molecular weight estimated by our technique. Our method could be augmented by estimating the molecular weight of UCNP shell coating layers and thus provide enhanced molecular weight information for surface functionalisation applications. The method presented here could be applied to nanoparticles of varying composition (e.g. silica or gold nanoparticles) with known crystal lattice parameters, known unit cell volume, and known nanoparticle size/geometry. It should be noted that a simple theory for estimation of the molecular weight of a single homogenous gold nanoparticle was proposed by Lewis et al. (2006),[49] but this theory did not account for unit cell parameters or elemental doping. Further, it did not describe the molecular weight distribution of a population of nanoparticles. In future, it would be ideal to test the accuracy of our molecular weight estimation against an experimental method for determining molecular weight such as size exclusion chromatography,[50] mass spectrometry,[50] or sedimentation-velocity analytical ultracentrifugation.[51,52]

It is of note that for a given population of UCNPs with a Gaussian diameter distribution the corresponding molecular weight distribution will be lognormal (see Figure 5). This lognormal distribution arises from the fact that molecular weight scales exponentially with diameter. This

lognormal distribution will be important to consider, in that a large fraction of UCNPs will be of a significantly lower or higher molecular weight than the arithmetic mean UCNP.

Estimation of molecular weight of $NaYF_4$:RE UCNPs could be of use in multiple applications. Knowledge of UCNP molecular weight will likely be of great utility in studies where UCNP surfaces are functionalised with additional molecules such as antibodies [22,24–26] or oligonucleotides.[34] If the molecular weight of UCNPs is known, then the molar concentrations of substances in the functionalisation processes can be altered to reach desired parameters. When combined with estimation of UCNP surface area, this could inform the optimisation of functionalisation for a given application. Knowing the molecular weight of UCNPs would also be beneficial in the processing of particles for downstream applications. In particular, steps taken to functionalise the nanoparticles may require separation procedures to remove unreacted moieties or unwanted reactants. If the molecular weight of UCNPs were known, then it may be beneficial for the optimisation of conjugation stoichiometry, which can be concentration dependant. The reaction rates of UCNPs will be heavily influenced by their molecular weight; thus a greater understanding of their molecular weight may increase the knowledge of thermodynamic properties of the UCNP System. This is particularly important when considering the use of bio-receptors with UCNPs where the mass of the particle may affect the binding kinetics of the UCNP-receptor construct.

The molecular weight of UCNPs will also be of interest in the study of cytotoxicity, bio-distribution, cellular uptake, and clearance of UCNPs in biological systems.[3,5] Currently, it is difficult to compare the results from various imaging and therapeutic studies because UCNP concentration is reported as weight of UCNPs per volume of aqueous media (i.e. mg/mL or similar).[3] This is a crude measure which does not quantify number of UCNPs in a given sample. For example, nanoparticles can induce membrane damage[39] and initiate apoptosis (programmed cell-death).[53,54] Thus, understanding the molar concentration of UCNPs would help assessment of cytotoxicity effects. A standardised protocol based on molecular weight of UCNPs would help assessment of where UCNPs accumulate *in vivo* and what time is required to clear the UCNPs from the various organs (e.g., lung, liver, kidney, heart)[4] or tumours.[48] Reporting the molar concentration of UCNP composites may also help to develop highly-localised targeted delivery of therapeutic drugs to the required sites in the body, leading to better controlled targeted photodynamic therapy,[15] and for improved targeted drug delivery.[16]

Conclusions

We have presented a theoretical method to estimate the molecular weight of hexagonal and cubic crystal lattice phase $NaYF_4$:RE UCNPs from experimentally measured UCNP crystal lattice parameters and nanoparticle diameters. Our calculations assume spherical $NaYF_4$:RE UCNP geometry, but our technique can easily be extended to estimate the molecular weight for nanoparticles with arbitrary geometries (e.g. cubes, rods, triangles, or tetrahedrons) and with arbitrary crystal lattice (e.g. cubic, hexagonal, orthorhombic), and arbitrary elemental compositions. It should be noted that our method does not account for any surface modification of nanoparticles, only the molecular weight of the $NaYF_4$:RE lattice itself. We expect this method will be highly accurate in estimation of the molecular weight of $NaYF_4$:RE UCNPs where the crystal lattice parameters of non-surface modified UCNPs have been experimentally verified. Direct experimental validation of our technique is desirable.

UCNP molecular weight was found to increase exponentially with UCNP diameter. Thus a small increase in UCNP diameter results in a large increase in UCNP molecular weight. For example, the molecular weight of $NaYF_4$ UCNPs were estimated to be ~ 1 MDa for a UCNP of 10 nm diameter, and ~ 100 MDa for a UCNP of 45 nm diameter. From this relation, we demonstrated that a population of UCNPs with a Gaussian diameter distribution will have a lognormal molecular weight distribution.

Our method to estimate the molecular of $NaYF_4$:RE UCNP populations will be of utility in a variety of applications, including the study of UCNP uptake by cells; separation of UCNPs by molecular weight, optical tweezing of UCNPs, and UCNP surface functionalisation for bio-imaging, bio-sensing, and therapeutic applications. Additionally we provide a stand-alone GUI to allow others to calculate molecular weight of spherical $NaYF_4$:RE UCNPs of arbitrary crystal lattice parameters, arbitrary diameters, and arbitrary RE dopant compositions.

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Supplementary material:

MATLAB Executable GUIs

Two stand-alone GUIs were created to enable calculation of UCNP molecular weight (the simple GUI, Figure S1), and the molecular weight of nanoparticles with arbitrary parameters (the advanced GUI, Figure S2). These GUIs are shown Figures S1 and S2. [Download Link]. NB: the final peer-reviewed manuscript will link to a data repository with a DOI.

These GUIs were developed as stand-alone applications for Windows PCs (Windows 7 or newer) and are not compatible with Macintosh or Linux PCs. The GUIs were compiled into stand-alone 64-bit executable files by using the MATLAB complier toolbox *deploytool* functionality. The GUIs were tested on 64-bit PCs running Windows 10. The first installation of a GUI was found to take around 45 minutes: this is primarily due to the automated download and installation of the required MATLAB runtime. After MATLAB runtime installation, the GUIs start-up time is typically < 10 seconds. Note that a MATLAB runtime compatible with MATLAB 2016a or newer is required to run these GUIs, and that MATLAB runtime versions older than 2016a may need to be removed from PCs prior to GUI installation.

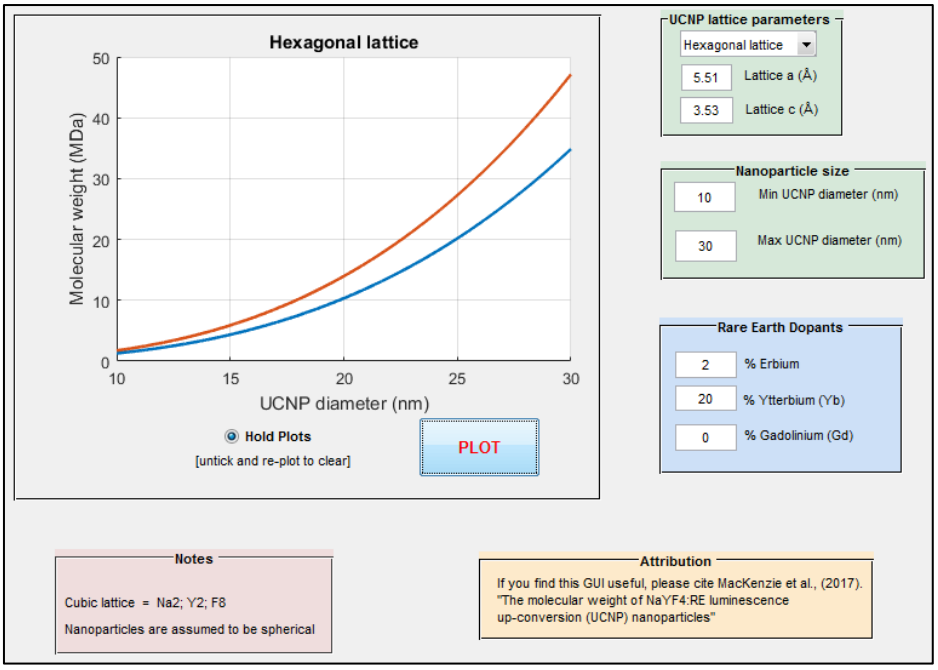

Figure S1. The basic GUI developed to estimate the molecular weight of NaYF4:RE UCNPs of spherical geometry and cubic or hexagonal lattice parameters.

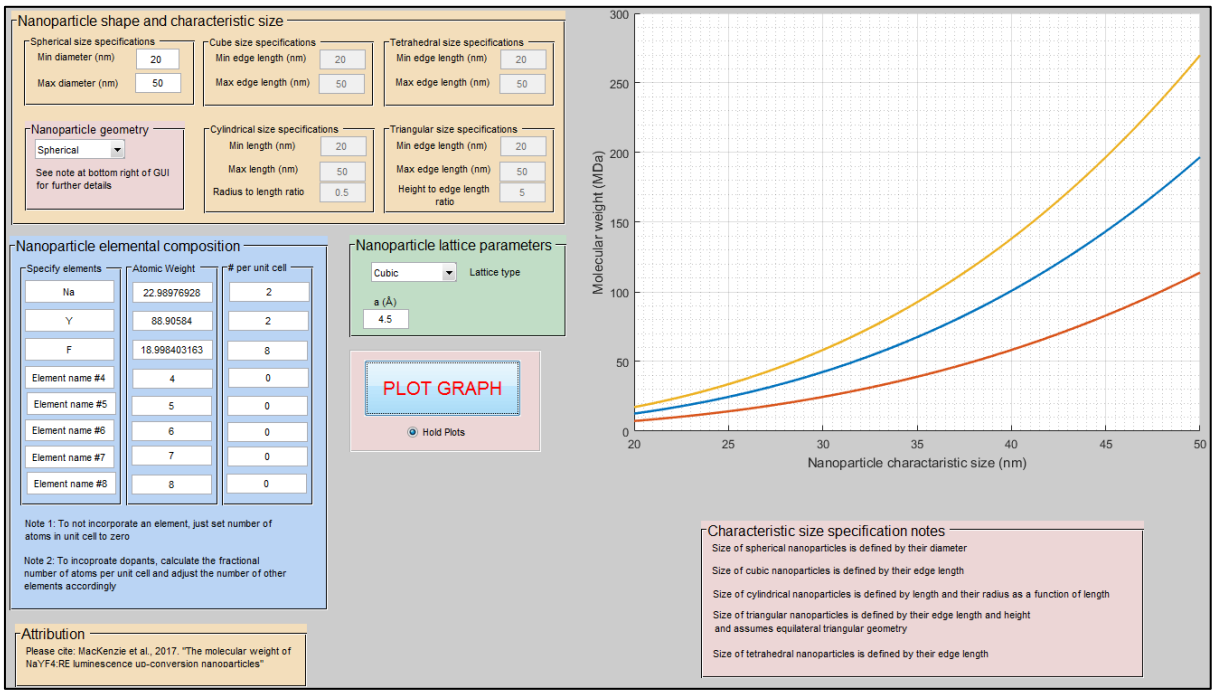

Figure S2. The advanced GUI developed to estimate the molecular weight of nanoparticles with arbitrary elemental constituents; arbitrary cubic or hexagonal lattice spacing; and arbitrary nanoparticle geometry.

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6. Author contributions

- L.E.M and J.A.G. conceived the research concept and wrote the manuscript.
- L.E.M. performed all calculations, provided Figures 2, 3, 4, 5, 6, and created the stand-alone graphic user interface.
- J.A.G. provided Figure 1.
- All authors contributed to the manuscript and reviewed the manuscript.

7. Competing financial interests

The authors declare no competing financial interests