# IWA Wastewater

Water and Resource Recovery Conference
10 – 13 April 2022, Poznan, Poland

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# A Thermodynamic Analysis of Intermediary Metabolic Steps and Nitrous Oxide Production in Ammonium-Oxidizing Bacteria

**Michelle N. Young,** Joshua P. Boltz, Andrew Marcus, Jose A. Jimenez, Ahmed Al-Omari, Imre Takács, and Bruce E. Rittmann

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# ACKNOWLEDGEMENTS

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- Dr. Bruce E. Rittmann
- Dr. Joshua P. Boltz
- Dr. Andrew Marcus

- Dr. Jose A. Jimenez
- Ahmed Al-Omari

- Dr. Imre Takács

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# PROJECT GOAL: WORK TOWARDS A BETTER PREDICTIVE MODEL OF AMMONIUM OXIDIZING BACTERIA (AOB) PERFORMANCE

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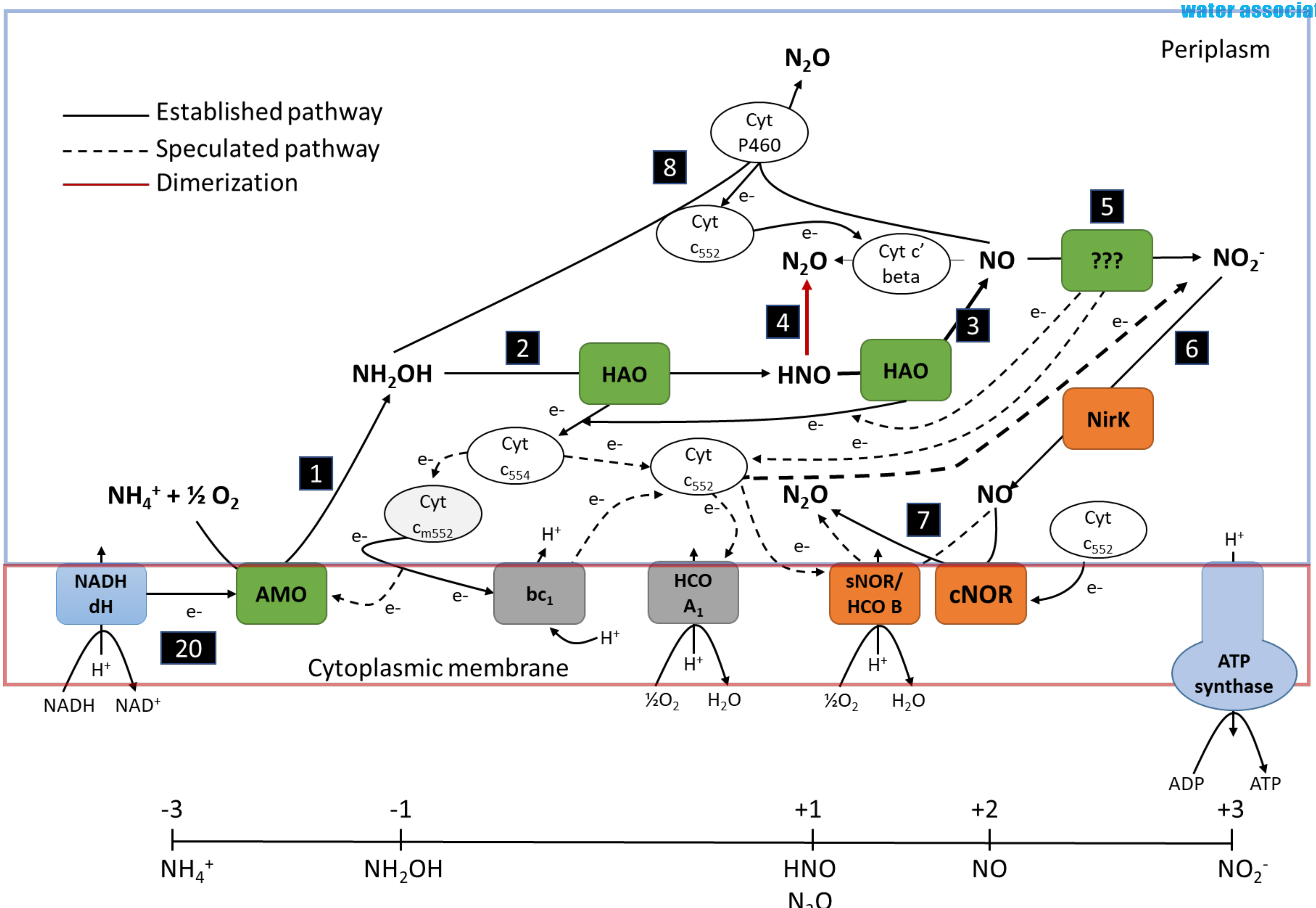
- Gain a better understanding of AOB metabolism to develop a more-accurate model of their performance
- Use metabolic information for better estimates of AOB energetics and kinetics

# LATEST UNDERSTANDING OF AOB METABOLIC PATHWAY



# THREE MISUNDERSTOOD/IGNORED MECHANISMS

- **$N_2O$ can be produced at a variety of steps**
- Ammonium monooxygenation (AMO) is an electron sink
- Respiration and biomass synthesis are not associated with all steps in nitrification or reduction

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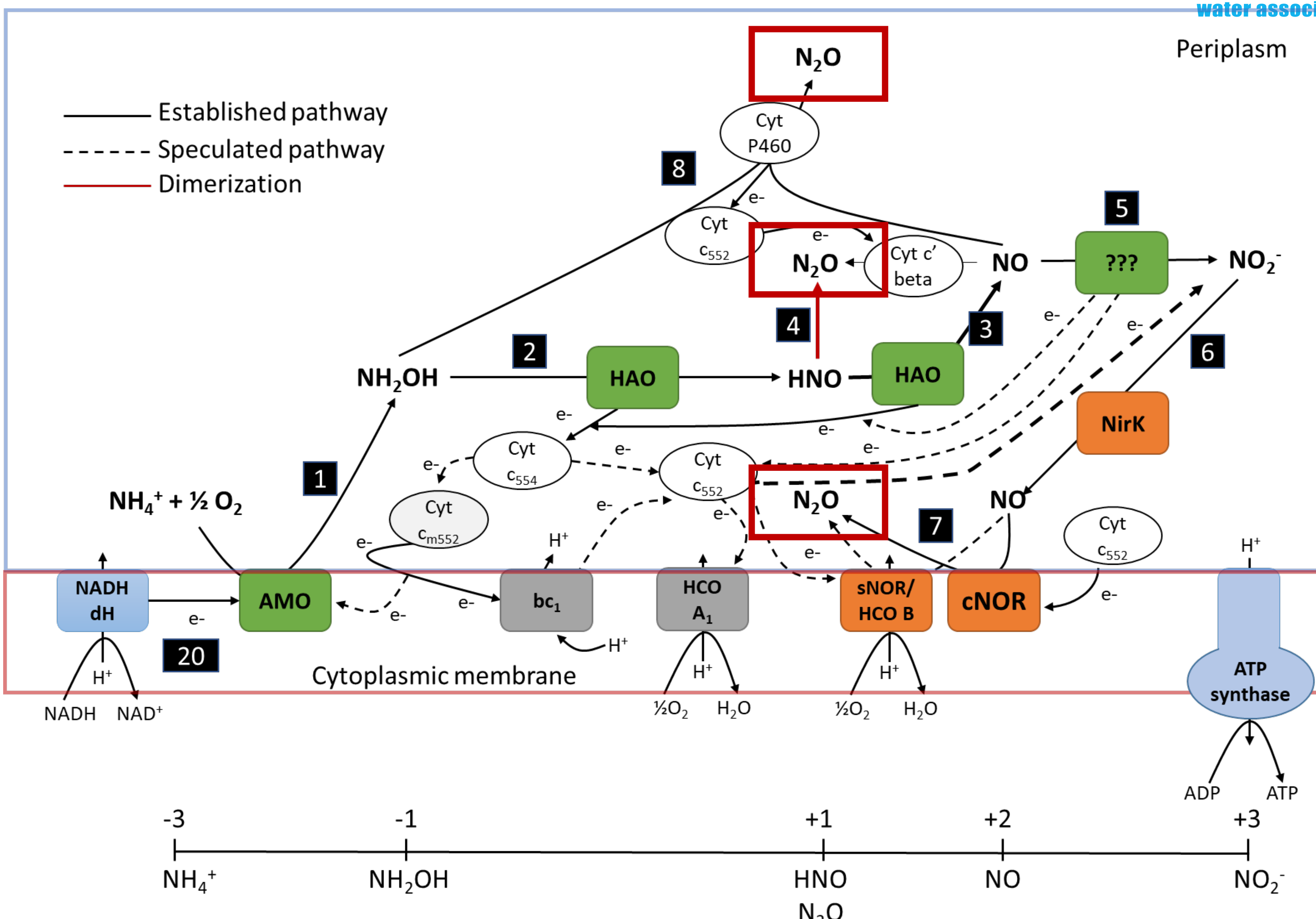

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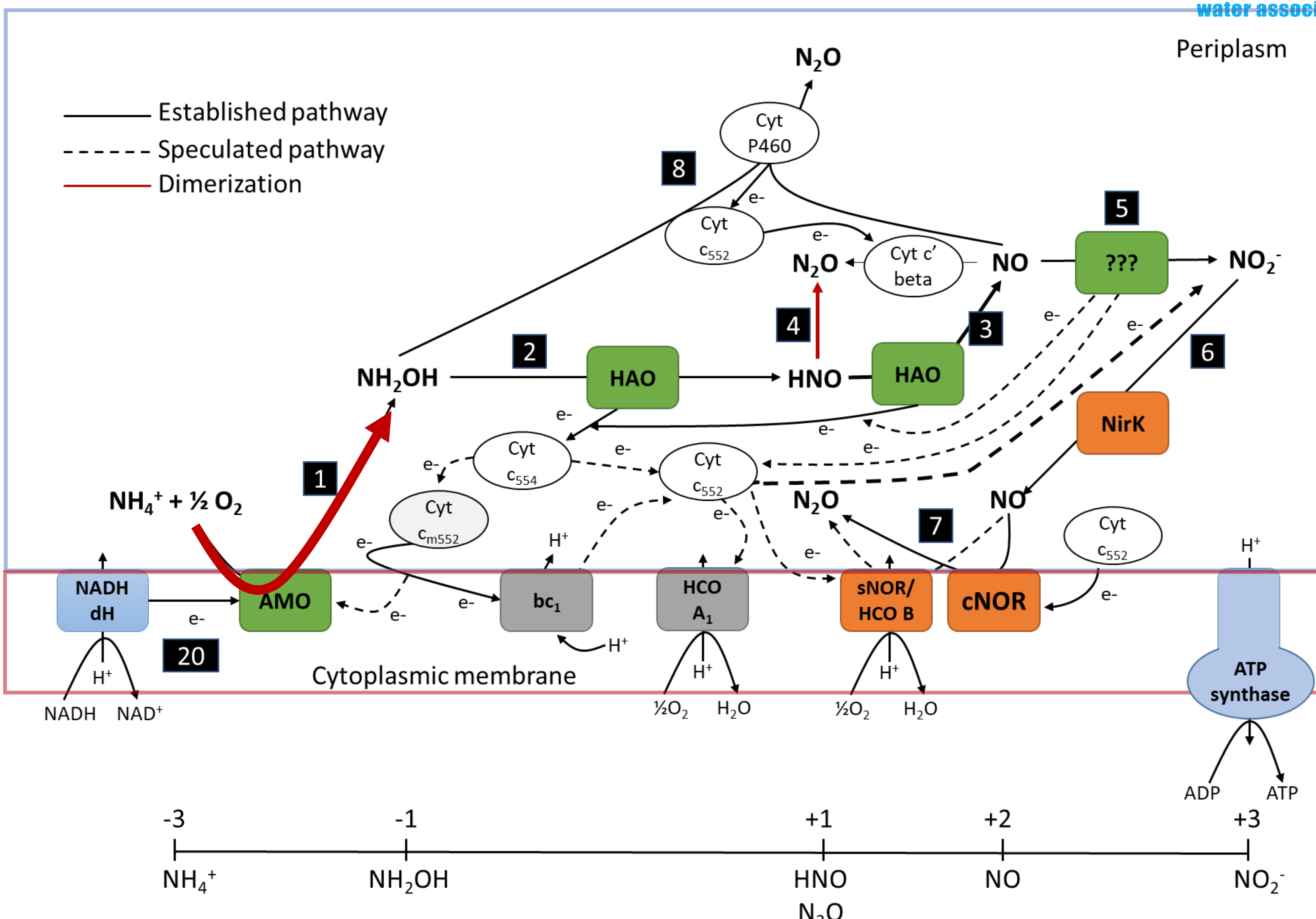

# THREE MISUNDERSTOOD/IGNORED MECHANISMS

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$$NH_4^+ + O_2 + 2[H] \rightarrow NH_2OH + H_2O + H^+$$

**where [H] = electron equivalent**

- 4 out of 6 electrons produced during nitrification are invested in this step!
- 2 additional electrons are from downstream products
- Those electrons do not produce energy or biomass synthesis

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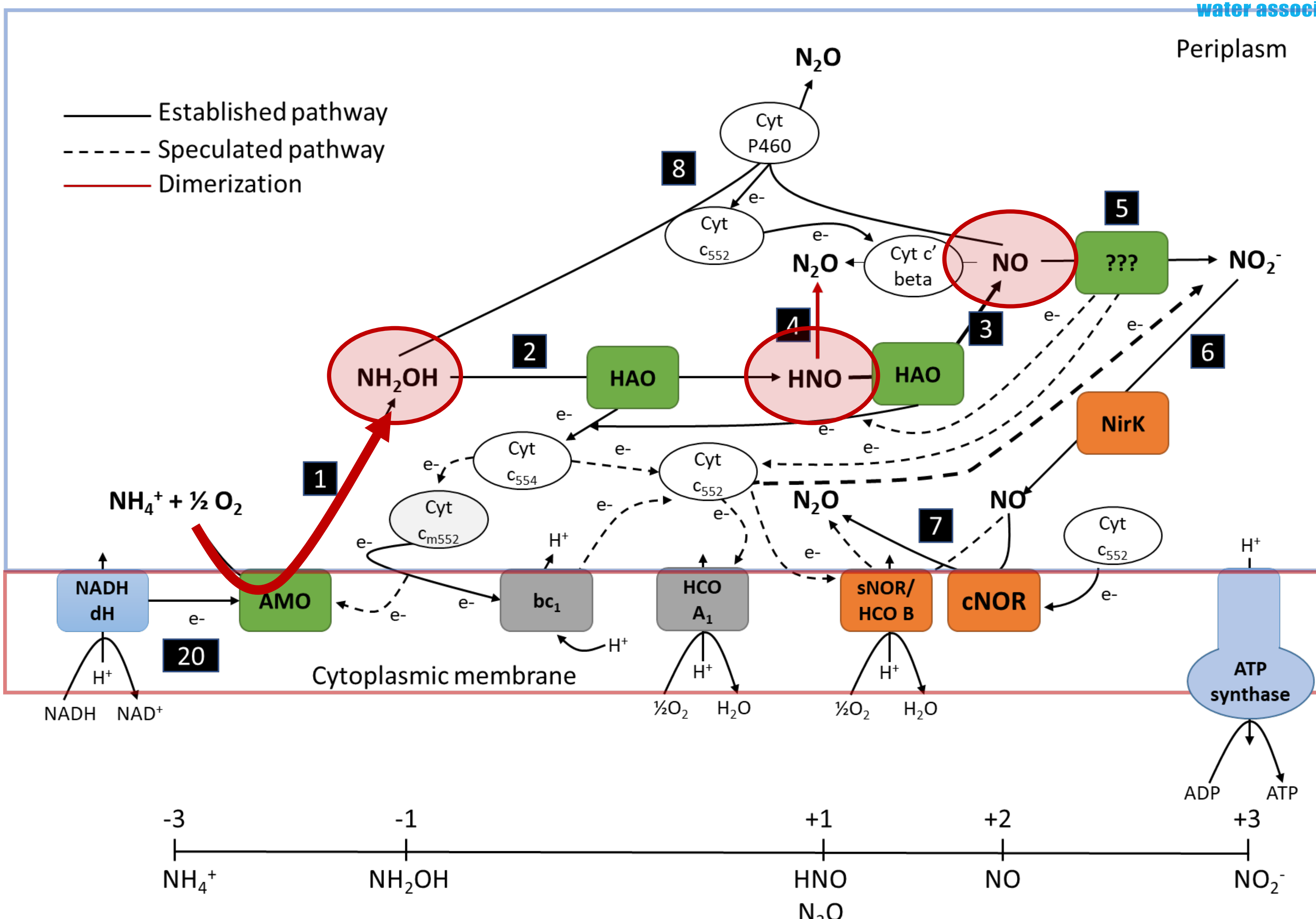

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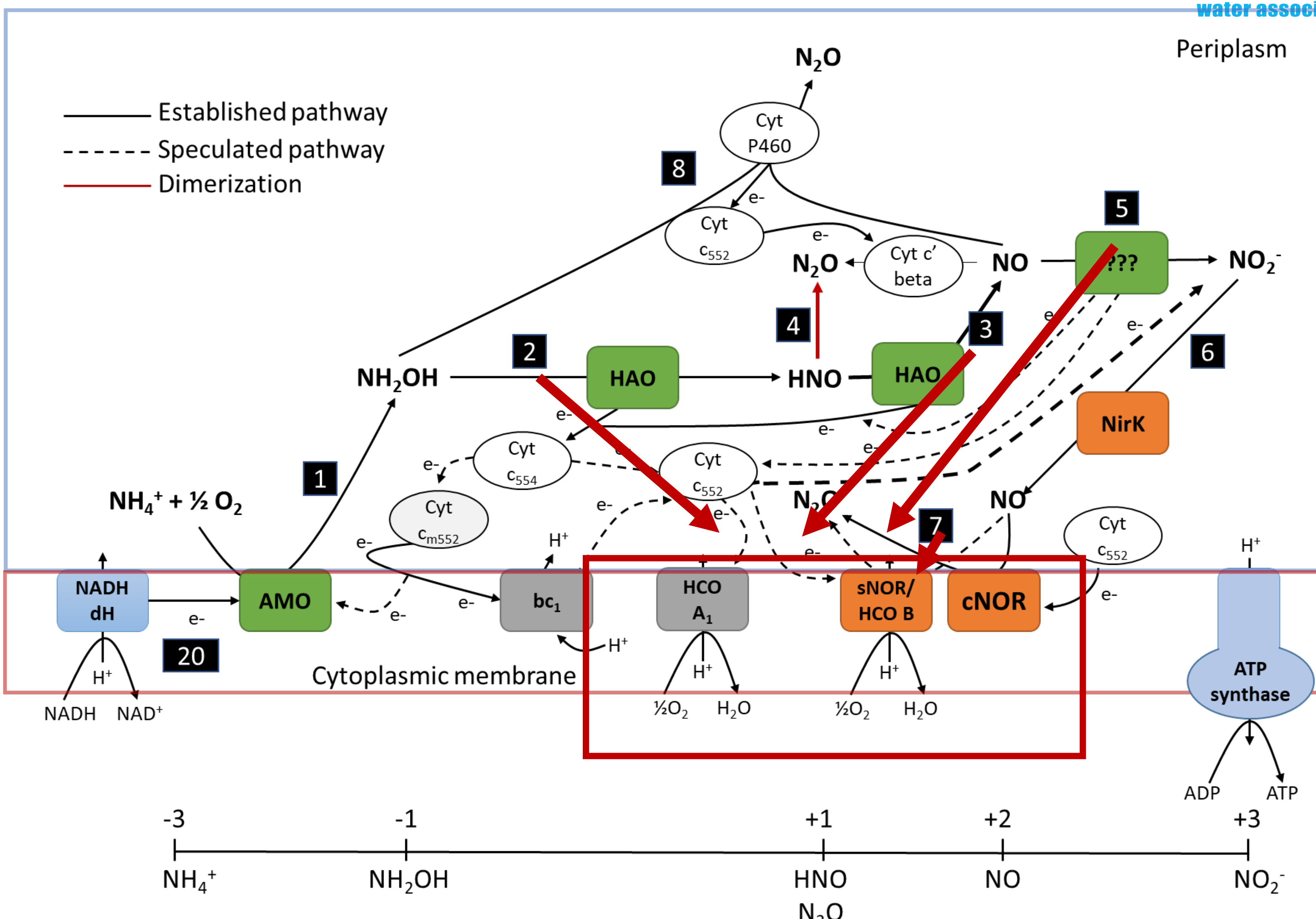

# ENERGETICS, STOICHIOMETRY, AND KINETICS

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- Use the Thermodynamic Electron Equivalents Model (TEEM) to estimate the stoichiometry and kinetics of respiration/biomass growth steps
- Using Gibbs free energies of donor and acceptor half reactions and carbon assimilation energetics, calculate fraction of electrons used for biomass synthesis ($f_s^0$)
- Using $f_s^0$, calculate yield (Y), maximum substrate utilization rate ($\hat{q}$), maximum specific growth rate ($\hat{\mu}$)
- Described in McCarty (2008) and Sections 5.4 and 6.2 of Rittmann and McCarty (2020)

# HYBRID TEEM PATHWAY ANALYSIS

- Calculate the stoichiometry/kinetics for each individual pathway that can produce biomass
- Normalize the individual pathway value based on the number of electrons contributed to overall biomass growth for the whole cell
- Compare whole cell values to literature

# INDIVIDUAL PATHWAYS IN NITRIFICATION

| Reaction # | Electron donor for respiration and biomass synthesis | Pathway fraction of electron to cell synthesis, $f_s^0$ | Yield, Y (gVSS/gN) | Maximum substrate utilization rate, $\hat{q}$ (g N/gVSS/d) | Maximum specific growth rate, $\hat{\mu}$ (1/d) |
|---|---|---|---|---|---|
| 1a-d | $NH_4^+$ to $NH_2OH$ | 0 | N/A | N/A | N/A |
| 2 | $NH_2OH$ to HNO | 0.17 | 0.14 | 8.4 | 1.2 |
| 3 | HNO to NO | 0.23 | 0.19 | 18.2 | 1.8 |
| 5 | NO to $NO_2^-$ | 0.24 | 0.19 | 18.4 | 1.8 |

# WHOLE CELL KINETICS FOR NITRIFICATION BASED ON INDIVIDUAL PATHWAY KINETICS

| Electron source for the AMO reaction | | | $f^0_{s,tot}$ | $Y_{tot}$ | $\hat{q}_{tot}$ | $\hat{\mu}_{tot}$ |
|---|---|---|---|---|---|---|
| $NH_2OH$ | HNO | NO | eeq cells/eeq donor | mg $X_a$ /mg N | mg N/ mg $X_a$/d | 1/d |
| 0 | 1 | 1 | 0.057 | 0.14 | 3.7 | 0.70 |
| 1 | 1 | 0 | 0.067 | 0.16 | 5.0 | 0.94 |
| 1 | 0 | 1 | 0.067 | 0.16 | 5.0 | 0.94 |
| 2 | 0 | 0 | 0.078 | 0.19 | 7.5 | 1.42 |

**For comparison, literature values are: 0.15-0.44 3.7-7.5 0.7-1.4**

# WHOLE CELL KINETICS FOR REDUCTION BASED ON INDIVIDUAL PATHWAY KINETICS

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| Electron source for the NOR reaction | $f^{0}_{s,tot}$ eeq cells/eeq donor | $Y_{tot}$ mg $X_a$ /mg N | $\hat{q}_{tot}$ mg N/ mg $X_a$/d | $\hat{\mu}_{tot}$ 1/d |
|---|---|---|---|---|
| $NH_2OH$ | 0.12 | 0.10 | 8.0 | 0.77 |
| HNO | 0.15 | 0.12 | 8.2 | 0.96 |
| **Literature values based on mathematical modeling only are:** | | **0.02** | **1.3** | **0.15-0.28** |

# CONCLUSIONS

- Provide an up-to-date synopsis of AOB metabolism
- Which pathways contribute to biomass growth
- Determine stoichiometry and kinetics based on thermodynamic electron equivalents modeling for the individual pathways
- Reconcile individual pathway values vs whole cell values
- Gained insights into which electron donors are likely preferrable under different conditions

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